Meiotic sister chromatid cohesion and recombination in two filamentous fungi

Meiotische zuster-chromatiden cohesie en recombinatie in twee filamenteuze schimmels

Promotor: dr. C. Heyting
hoogleraar in de moleculaire en celgenetica

Co-promotor: dr. ir. H.W.J. van den Broek
universitair hoofddocent bij het Departement Plantenwetenschappen

NN08201, 2908

Stellingen

1. Het is mogelijk dat eiwitten als Spo76p/BIMD net als Mre11 vele raakvlakken hebben: het kan daarom moeilijk zijn een aspect van chromosoom metabolisme te vinden waar deze eiwitten niet bij betrokken zijn.
   Haber (1998) Cell 95: 583-586; dit proefschrift
2. De lange, zure staart van Spo76p/BIMD zou een rol kunnen spelen in veranderingen van het chromatine, bijvoorbeeld door interactie met histonen.
3. De argumentaties voor synthesis-dependent strand annealing als mechanisme voor genconversie berusten deels op het toenemende inzicht in de onderlinge afhankelijkheid van homologe recombinatie en DNA replicatie.
   Haber (2000) Current Opinion in Cell Biology 12: 286-292.
4. Eiwitten die functioneren in het herstel van DNA schade via homologe recombinatie kunnen een essentiële rol hebben in S-phase progressie.
   Petrini (2000) Current Opinion in Cell Biology 12: 293-296.
5. Nu de volledige sequentie van het menselijke genoom bekend is zullen vakgebieden als biochemie en cytogenetica opnieuw opbloeien.
6. Dat men specifieke toepassingen voor menselijke genen wil patenteren is volkomen begrijpelijk, dat men echter op voorhand alle mogelijk interessante menselijke genen wil patenteren betekent broodroof voor het onderzoek.
7. De wind mee hebben is in vele opzichten voordelig, behalve als je de andere kant op wil.
8. Promoveren is net als het "echte" leven: als een bepaald stadium eenmaal is bereikt lijkt alles voor de hand liggend, ordelijk en zorgvuldig ingepland; de belangrijke beslissingen zijn echter onderweg genomen op een moment dat hun uitkomst nog in hoge mate onzeker was.
9. Een ieder die kiest voor zekerheid heeft veel te verliezen; de keuze voor het aangaan van nieuwe uitdagingen en het avontuur levert daarentegen altijd iets op.
10. In de meeste culturen spelen rituelen een belangrijke rol bij de overgang van een lid van de gemeenschap naar een nieuwe positie: een promotieceremonie is dus meer dan alleen een poppenkast.

Stellingen behorende bij het proefschrift "Meiotic sister chromatid cohesion and recombination in two filamentous fungi" van Diana van Heemst.

Wageningen, 11 december 2000

Diana van Heemst

Meiotic sister chromatid cohesion and recombination in two filamentous fungi

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr. ir. L. Speelman,
in het openbaar te verdedigen
op maandag 11 december 2000
des namiddags te vier uur in de Aula.

van Heemst, Diana

Meiotic sister chromatid cohesion and recombination in two filamentous fungi / Diana van Heemst [S.I.: s.n.]
Thesis Wageningen. - With summary in Dutch and French
ISBN 90-5808-318-7.
Subject headings: meiosis/sister chromatid cohesion/recombination/filamentous fungi

The work presented in this thesis was performed at Wageningen University, Laboratory of Genetics, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands, and Institut de Génétique et Microbiologie, UMR 8621, Université Paris Sud, 91405 Orsay Cedex, France.
This research was financially supported by grants from the Dutch Organization for Scientific Research (NWO, contract 805-18.262), the EEC (contract CHRX-CT94-0511) and the NIH (R37 GM25326).

Voor mijn ouders

Contents

Chapter 1

General introduction and aim of the thesis

GENERAL INTRODUCTION AND AIM OF THE THESIS

The mitotic versus the meiotic cycle

In sexually reproducing eukaryotes, haploid and diploid generations of cells alternate. In the haploid phase of the life cycle, cells have a single set of chromosomes. The transition from the haploid to the diploid phase takes place by fusion of two haploid cells (gametes), and subsequently two haploid nuclei. These events (fertilization and karyogamy) yield a diploid cell, which has two sets of chromosomes per nucleus: one set from each parental cell. The two sets of chromosomes are equivalent but not necessarily identical, because, depending on the species, the two gametes usually originate from different individuals.

Haploid and diploid cells increase in number by mitotic divisions, by which mother cells divide into two daughter cells which are genetically identical to the mother cell with respect to both chromosome number and combination of alleles. Before a mitotic division, DNA is replicated, and each chromosome is duplicated into two identical sister chromatids. Concomitantly with DNA-replication, cohesion is established between sister chromatids. This cohesion persists while the chromosomes orient themselves in the mitotic spindle, and is not released until all chromosomes are lined up in the equatorial plane. Then, cohesion is suddenly lost along the chromosome arms and in the centromeric regions, so that the chromosomes split into their constituting sister chromatids (=equatorial division), which move to opposite poles. Each of the two progeny nuclei will thus receive one copy (sister chromatid) of each of the chromosomes (Fig. 1A), and normally no changes in ploidy level will occur during a mitotic division.

The transition from the diploid to the haploid phase takes place during meiosis by a second type of division, the reductional division. Meiosis consists of two successive divisions, by which one diploid cell produces four haploid daughter cells, with new combinations of alleles. The first meiotic division (meiosis I or MI) is reductional, and brings about the transition from the diploid to the haploid phase of the life cycle, whereas the second meiotic division (meiosis II or MII) is equational and similar to a mitotic division. A single round of DNA-replication and establishment of sister chromatid cohesion precede the two meiotic divisions. During prophase of meiosis I, replicated homologous chromosomes pair, and non-sister chromatids of homologous chromosomes exchange precisely corresponding segments (crossing over). The sites of reciprocal exchange show up as chiasmata when chromosomes

condense in preparation of metaphase I. Crossovers (chiasmata), in concert with cohesion between sister chromatids distal to chiasmata, keep homologous chromosomes connected during meiosis I, so that chromosome pairs (bivalents) rather than chromosomes orient themselves in the metaphase I spindle. When all bivalents are lined up, cohesion between sister chromatid arms is lost, whereas centromere cohesion is maintained. Because the kinetochores still act as a single unit at metaphase I, (recombined) homologous chromosomes rather than sister chromatids disjoin (Fig. 1B). At the metaphase/anaphase transition of meiosis II, centromere cohesion is lost, and sister chromatids disjoin, like in mitosis (Fig. 1B). Thus, sister chromatid cohesion is essential for the proper segregation of homologous chromosomes at meiosis I, and for the proper segregation of sister chromatids in meiosis II and in mitosis.

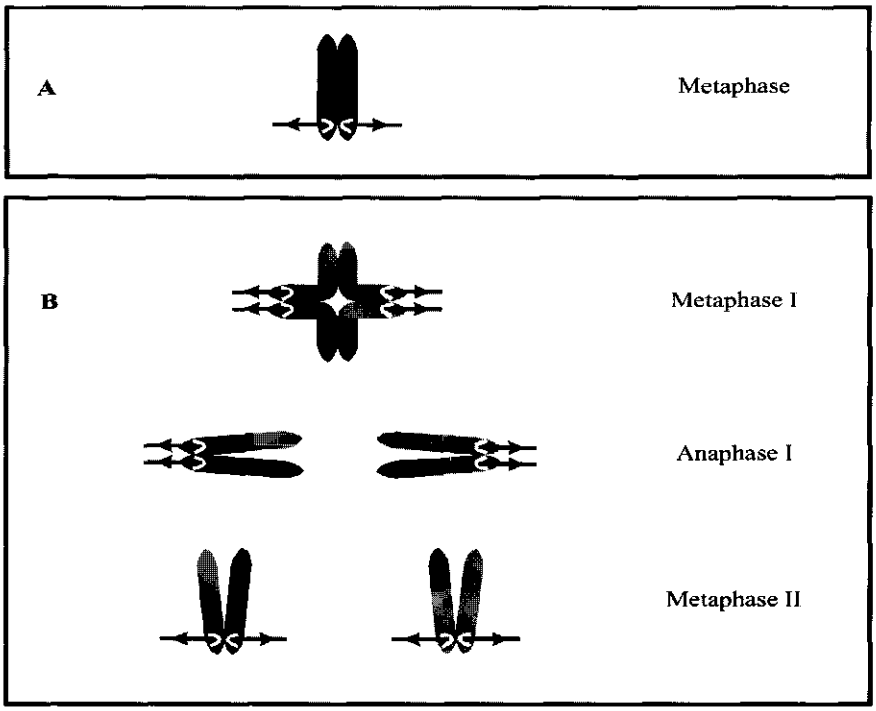


Figure 1: Cohesion, kinetochore orientation, and chromosome segregation in mitosis (A) and meiosis (B). Kinetochores are depicted as white, cup-shaped entities and non-sister chromatids are differently colored. The arrowed lines represent spindle-microtubules; the arrow points towards the pole to which the microtubule is attached.

In most eukaryotes, meiotic recombination is accompanied by the assembly and disassembly of a prominent proteinaceous structure: the synaptonemal complex or SC (reviewed in Heyting, 1996). In meiotic prophase, the sister chromatids of each chromosome develop a common proteinaceous axis, the axial element. Transverse filaments then connect the axial elements of homologous chromosomes, and a third longitudinal structure, the central element, appears between the two axial elements, to form the tripartite structure of a SC. Although SC-formation is a prominent landmark in meiotic prophase of almost all sexually reproducing eukaryotes, its functions remain largely unknown. In budding yeast, a component of the transverse filaments is essential for positive crossover interference (i.e., a crossover reduces the probability of a crossover in an adjacent chromosomal region) (Sym and Roeder, 1994). The filamentous fungus *Aspergillus nidulans* is exceptional in that it does not assemble SCs during meiosis, and does not display crossover interference. Possible functions of the axial elements are the promotion of recombination between non-sister chromatids of homologous chromosomes rather than between sister chromatids, and maintenance of sister chromatid cohesion (discussed in chapter 6).

As outlined above and discussed in more detail in chapter 6, sister chromatid cohesion and recombination play important roles in meiotic chromosome segregation. At the start of this Ph.D. project, very little was known about meiotic sister chromatid cohesion at the molecular level. More was known about meiotic recombination, but studies at the molecular level had almost exclusively been performed in budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizzosaccharomyces pombe*). It was unknown to what extent these studies would apply to other eukaryotes. Furthermore, little was known about the role of SCs (or components thereof) in meiotic sister chromatid cohesion and recombination. We therefore initiated a molecular analysis of meiotic sister chromatid cohesion and recombination in filamentous fungi. These organisms are generally amenable to molecular genetic techniques, and are phylogenetically closer to higher eukaryotes than is *S. cerevisiae* (Smith, 1989). In order to analyze to what extent molecular genetic studies of meiosis in yeasts would apply to filamentous fungi, I isolated and characterized fungal genes involved in meiotic recombination and sister chromatid cohesion, and compared their roles with those of corresponding genes in yeast.

Two filamentous fungi: Aspergillus nidulans and Sordaria macrospora

Table 1 summarizes the most important features for meiotic studies in some fungi that have commonly been used as experimental organism for this purpose. None of these fungi meets all requirements for the unraveling of meiosis, albeit that budding yeast (*S. cerevisiae*) is by far the most useful organism for molecular genetic studies of meiosis. Some filamentous fungi are also amenable to molecular genetic manipulations; a powerful method for cloning genes in these organisms is complementation of mutant phenotypes by transformation. Ideally, a filamentous fungus should fulfil the following requirements for the cloning and characterization of meiotic genes:

- Meiotic mutants must be available (or easily obtainable).
- In fungi, the haploid phase of the life cycle predominates; this greatly facilitates the recovery of meiotic recessive mutations (see also chapter 5).
- It should be easy to screen large numbers of transformants for complementation of meiotic defects, and to recover transformants showing complementation (chapter 2). Tests for complementation of meiotic defects are easier in self-fertile fungi, where a crossing partner is not needed. Three different types of mating system exist among fungi (reviewed in Nelson, 1996): homothallism (self-fertile, homokaryotic strains), heterothallism (self-sterile, homokaryotic strains) and pseudohomothallism (self-fertile, heterokaryotic strains). For our purpose, homothallism is thus the most useful mating system.
- Duplications introduced by transformation should be stably maintained. Fungi that inactivate duplicated sequences during the sexual cycle by MIP (methylation induced premeiotically) or RIP (repeat-induced point mutation) are not suitable for gene cloning by transformation complementation. *Neurospora crassa*, which shows RIP, therefore dropped out as candidate experimental organism.
- Molecular genetic tools such as a genomic cosmid library and a transformation system must be available.
- For analysis of mutant defects in meiotic sister chromatid cohesion or SC-formation, LM analysis of meiotic chromosomes and EM analysis of SC-formation should be possible.
- The formation of ordered tetrads is advantageous for analysis of meiotic recombination, because the arrangement in the ascus of spores carrying specific alleles provides information about the segregation of alleles during each of the two meiotic divisions (reviewed by Perkins, 1992).

- If a parasexual cycle exists besides the sexual cycle, mitotic recombination can be compared in detail with meiotic recombination (reviewed by Bos, 1996). In the parasexual cycle, diploid, heterokaryotic nuclei arise at a low frequency (10^{-5} - 10^{-6}) by somatic karyogamy. The chromosomes of these nuclei undergo mitotic crossing over at a frequency of about 10^{-3} per nucleus (Käfer, 1961). Diploid nuclei can haploidize by gradual loss of chromosomes, and in the resulting haploid cells, the products of mitotic recombination can be analyzed (Pritchard, 1955; see also chapter 3).

Table 1. Most important features of fungi that are commonly used for meiosis research

organism	*Saccharomyces cerevisiae*	*Schizo-saccharomyces pombe*	*Neurospora crassa*	*Aspergillus nidulans*	*Sordaria macrospora*	*Coprinus cinereus*
phylum	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes	Basidiomycetes
growth mode	yeast	yeast	filamentous fungus	filamentous fungus	filamentous fungus	mushroom
meiotic mutants	+	+	+	+/-	+	+/-
meiotic chromosomes in LM	+/-	+/-	+	+/-	+	+
SCs in EM	+	+/-	+	-	+	+
ordered tetrads	-	-	+	-	+	-
parasexual cycle	-	-	-	+	-	-
mating system	pseudo-homothallic	pseudo-homothallic	heterothallic	homothallic	homothallic	heterothallic
RIP/MIP	-	-	+	-	-	+
molecular biological tools	+	+	+	+	+/-	+/-

It is clear from Table I that no fungal system meets all our requirements. We therefore chose two different filamentous fungi, namely *Sordaria macrospora* and *Aspergillus nidulans*. The major advantages of *S. macrospora* were the well-defined meiotic chromosomes and SCs, the availability of various mutants with defects in sister chromatid cohesion and/or meiotic recombination, and its homothallism. *A. nidulans* had the advantage of well-developed molecular genetic tools (reviewed by Goosen *et al.* 1992), a good genetic system (many genetic markers available) and the presence of a parasexual cycle (for the exploitation of this feature, see thesis of H. Thijs, 1998). *A. nidulans* is furthermore of interest for meiosis research because it is one of the two known organisms that do not assemble SCs during meiotic prophase and do not display positive crossover interference (reviewed by Egel, 1995; the other organism is *S. pombe*). The choice for *S. macrospora* and *A. nidulans* would

provide the possibility to compare the role(s) of genes involved in meiotic sister chromatid cohesion and/or recombination in a fungus with and one without SCs.

Outline of this thesis

One of the available mutants of *S. macrospora*, *spo76-1*, was defective in meiotic sister chromatid cohesion, SC-formation, mitotic DNA-repair (Moreau et al., 1986) and meiotic recombination (Zickler et al., 1992). In chapter 2, we describe the cloning of the *SPO76* gene of *S. macrospora*, the localization of the encoded protein during the successive stages of the mitotic and meiotic cell cycles, and further analyses of the *spo76-1* mutant defects. Spo76p shows significant sequence homology to BIMD of *A. nidulans*, which suggested to us that the two proteins might be functional homologues. In chapter 3, we test for functional homology by heterologous complementation and further analysis of the defects of *bimD6* mutants. Furthermore, we compare the localization of BIMD in *A. nidulans* during the successive stages of meiosis and mitosis with that of Spo76p in *S. macrospora*. The results presented in chapter 2 and 3 reveal a link between diverse aspects of chromosome structure and metabolism, such as sister chromatid cohesion, chromosome compaction, DNA repair and recombination. In addition, these results also reveal differences between the two fungi in meiotic chromosome structure.

For *A. nidulans*, no meiotic mutants had been described. However, many mutants with a mitotic repair phenotype were also defective in meiosis. An example is the *uvsC114* mutant (Jansen, 1970a). We cloned the *uvsC* gene, and found that it was homologous to yeast *RAD51*, which is also involved in DNA-repair and meiotic recombination. Thus, we identified one gene with a similar role in meiosis in yeast and in a filamentous fungus (chapter 4). In addition, as a first step towards further analyses of the meiotic process in *A. nidulans*, we isolated a series of new meiotic mutants in this fungus (chapter 5). Some of these mutants show phenotypes that could be caused by defects in meiotic sister chromatid cohesion and/or recombination.

In chapter 6, we speculate upon the possible links between sister chromatid cohesion and recombination in meiosis.

Chapter 2

Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programs

D. van Heemst [1], F. James [1], S. Pöggeler [2], V. Berteaux-Lecellier [1] and D. Zickler [1]

[1] Institut de Génétique et Microbiologie, UMR 8621, Université Paris Sud, 91405 Orsay Cedex, France; [2] Lehrstuhl für Allgemeine Botanik, Ruhr Universität, D-44780 Bochum, Germany

Published in Cell 98: 261-271 (1999)

Cell, Vol. 98, 261–271, July 23, 1999, Copyright ©1999 by Cell Press

Spo76p Is a Conserved Chromosome Morphogenesis Protein that Links the Mitotic and Meiotic Programs

Diana van Heemst,*§ Françoise James,* Stefanie Pöggeler,† Véronique Berteaux-Lecellier,* and Denise Zickler*‡
*Institut de Génétique et Microbiologie
UMR 8621
Université Paris-Sud
91405 Orsay Cedex
France
†Lehrstuhl für Allgemeine Botanik
Ruhr Universität
D-44780 Bochum
Germany

Summary

Spo76p is conserved and related to the fungal proteins Pds5p and BIMD and the human AS3 prostate proliferative shutoff-associated protein. Spo76p localizes to mitotic and meiotic chromosomes, except at metaphase(s) and anaphase(s). During meiotic prophase, Spo76p assembles into strong lines in correlation with axial element formation. As inferred from *spo76-1* mutant phenotypes, Spo76p is required for sister chromatid cohesiveness, chromosome axis morphogenesis, and chromatin condensation during critical transitions at mitotic prometaphase and meiotic midprophase. Spo76p is also required for meiotic interhomolog recombination, likely at postinitiation stage(s). We propose that a disruptive force coordinately promotes chromosomal axial compaction and destabilization of sister connections and that Spo76p restrains and channels the effects of this force into appropriate morphogenetic mitotic and meiotic outcomes.

Introduction

Chromosome morphogenesis involves a number of basic stages, elucidated for both the mitotic cycle and meiosis. Chromosomes replicate, with concomitant establishment of sister cohesion (e.g., Uhlmann and Nasmyth, 1998). At early–mid prophase, chromosomes are still relatively extended; from late prophase and into metaphase, higher order axial condensation (coiling/folding) arises, yielding chromosomes compact enough for separation at the ensuing division (reviewed in Koshland and Strunnikov, 1996).

The meiotic version of this program involves several specialized differentiations. First, at early–mid prophase, chromosomes are highly ordered, with sister chromatids organized into parallel, cooriented linear arrays of loops, closely conjoined at their bases by the axial element (AE) (e.g., Moens and Pearlman, 1988). Second, chromosomes remain in this extended configuration for a long time, presumably to accommodate the complex events of meiotic interhomolog interactions (pairing, recombination, and synaptonemal complex [SC] formation). Third, meiotic prophase includes the structurally differentiated attachment of chromosome ends to the nuclear envelope plus programmed migration of those ends into and out of the bouquet configuration (review in Zickler and Kleckner, 1998). These events are all completed by the end of pachytene. The meiosis-specific structures (AE/SC) then disintegrate, and after a transient diffuse stage, chromosomes compact progressively into their metaphase conformation just as during the corresponding mitotic stage (review in von Wettstein et al., 1984). Notably, also, meiotic prophase is followed by two rounds of chromosome segregation. During mitosis, sister chromatid connections lapse first along the chromatid arms at the metaphase/anaphase transition and then, at the onset of anaphase, in centric regions. The same two phases occur during meiosis except that arm connections lapse at the first meiotic division while centric connections lapse only at the second division (review in Nicklas et al., 1995; Moore and Orr-Weaver, 1998).

Mechanisms responsible for the basic processes of chromosome morphogenesis and for specializations during the meiotic program are not yet fully understood. Insights into mitotic chromosome metabolism, and especially the essential process of sister chromatid cohesion, have emerged recently from genetic and cytogenetic studies in budding and fission yeasts and by biochemical approaches in *Xenopus* egg extracts (reviewed by Hirano, 1999). Analysis of molecules like Mcd1/Scc1p (related to Rad21p), Scc3p, Smc1p, and Smc3p, currently proposed as cohesion proteins per se (Guacci et al., 1997; Michaelis et al., 1997; Toth et al., 1999 and references therein), showed that sister chromatid cohesion is functionally related to other aspects of chromosome metabolism. The *mcd1* mutant is also defective in chromosome compaction (Guacci et al., 1997), and several cohesion mutants display hypersensitivity to DNA-damaging agents, suggesting a link between chromosome structure and DNA repair (review in Hirano, 1999).

Genetic and cytogenetic analyses have also been powerful tools for dissection of meiotic chromosome morphogenesis (e.g., Moore and Orr-Weaver, 1998). All known mutants defective in meiotic chromatid cohesion exhibit premature loss of sister centromeric connections during meiosis I with subsequent abnormal chromosome segregation. The *Drosophila mei-S322* mutant appears specifically defective in maintaining centric connections at meiosis I (e.g., Tang et al., 1998). Other mutants (e.g., *Sordaria spo76-1*, fission yeast *rec8*, and budding yeast *red1*) exhibit, additionally, cohesion defects along the arms as early as prophase plus defects in AEs (Moreau et al., 1985; Molnar et al., 1995; Smith and Roeder, 1997; Bailis and Roeder, 1998). The maize *dy1* and *dsy1* mutants make SCs but lose sister cohesion during the pachytene-to-diplotene transition (Maguire

‡To whom correspondence should be addressed (e-mail: zickler@igmors.u-psud.fr).
§Present address: Department of Genetics, Agricultural University, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands.

et al., 1993). Finally, the *Drosophila ord* mutant exhibits early prophase defects and absence of sister cohesion prior to metaphase I (Bickel et al., 1997). Correspondingly, MEI-S332 protein localizes to centromere regions from prometaphase I to anaphase II (Moore et al., 1998), while Red1p localizes along chromosomes throughout prophase (Smith and Roeder, 1997). Notably, except for *meiS322*, these mutants are also defective in meiotic recombination, suggesting that critical aspects of meiotic interhomolog interactions are functionally related to the intersister interaction process (e.g., Kleckner, 1996; Roeder, 1997).

To shed light on the relationships among these diverse processes, we have further analyzed the roles of the *Sordaria SPO76* gene. Previous studies have shown that the *spo76-1* mutant is defective in meiotic sister chromatid cohesion and mitotic DNA repair (Moreau et al., 1985; Huynh et al., 1986). Here, we report cloning of *SPO76*, Spo76p localization throughout mitosis and meiosis, and further analysis of *spo76-1* phenotypes. Such analysis is facilitated by the fact that *Sordaria* chromosomes are large enough to be directly visualized in all stages of both programs. Spo76p is an evolutionarily conserved protein with homologs in fungi and in human. Spo76p is chromosome associated in both mitosis and meiosis except at metaphase(s) and anaphase(s). During meiotic prophase, Spo76p is mostly axis associated. A specific *spo76-1* defect occurs at mitotic prometaphase, with cohesion and compaction coordinately affected on a regional basis. This phenotype points to the existence of a critical chromosomal transition point, corresponding to the onset of higher order chromosome compaction, where cohesion and chromosome condensation are functionally linked and where Spo76p plays a crucial role. The meiotic defects of *spo76-1* can be explained analogously, with the addition of a program-specific difference that correlates with meiosis-specific features of Spo76p chromosome localization. These and other results suggest that Spo76p is a conserved component of basic chromosome structure that has been recruited from the mitotic cycle and functionally adapted for use in the meiotic program.

Results

Spo76p Is Evolutionarily Conserved from Fungi to Human

The *SPO76* gene was cloned from a *Sordaria macrospora* genomic cosmid library by transformation and SIB selection (Akins and Lambowitz, 1985). The *SPO76* gene was mapped to the only major open reading frame (ORF) present on a complementing 6.4 kb PstI-SmaI fragment by the strategy of Turcq et al. (1990). The *SPO76* ORF comprises 4791 bp, interrupted by two small introns (of 62 bp and 73 bp), whose presence was confirmed by sequencing of corresponding cDNAs. To verify that we had cloned the *SPO76* gene and not a suppressor, the *spo76-1* allele and the wild-type allele from the isogenic parent strain were also sequenced. The *spo76-1* allele differs from the isogenic wild-type by two deletions: a 1 bp deletion causes a frameshift, but the reading frame is subsequently restored by the second deletion of 11 bp. The two mutations together change a region of 60 aa in the Spo76 protein (Figure 1). *spo76-1* is unlikely to be a null allele (see Discussion).

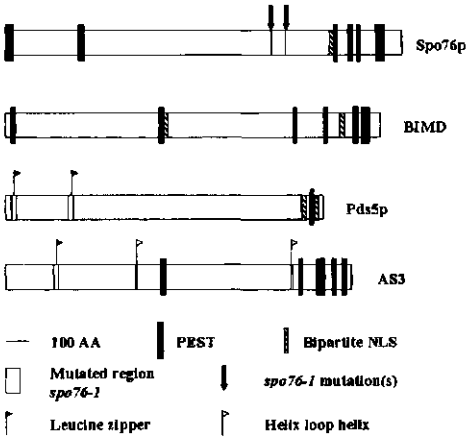


Figure 1. Spo76p and Its Three Cognate Proteins

For sequence alignments, see the website <http://www.cell.com/cgi/content/full/98/2/261/DC1>.

The *SPO76* ORF encodes a potential protein of 1579 amino acids (aa) with a predicted molecular mass of 176.9 kDa and an average pI of 5.4. Database searches plus evaluation of the quality of alignment expressed by the Z parameter (Slonimsky and Brouillet, 1993) show that the predicted Spo76p shares significant homology with three proteins and the putative products encoded by two cDNA fragments. The three proteins are BIMD of *Aspergillus nidulans* (Denison et al., 1992), Pds5p of *Saccharomyces cerevisiae* (accession Q04264), and an androgen-induced prostate proliferative shutoff-associated protein encoded by the human *AS3* gene (Geck et al., 1999). Percentages of identity are, respectively, 44%, 30%, and 22%; Z scores are 84, 38, and 24. The two incomplete proteins are a 390 aa fragment from *Schizosaccharomyces pombe* (accession AF049529) and an 851 aa fragment of the human KIAA0648 protein (accession AB014548), with percentages of identity of 32% and 23% and Z scores of 31 and 18. Notable features of the four complete proteins (Figure 1) include numerous potential PEST sequences, proposed to target proteins for proteolysis (Rechsteiner and Rogers, 1996); bipartite nuclear localization signals (bipartite NLS in Spo76p, BIMD, and Pds5p); and putative DNA-binding motifs (leucine zipper in Pds5p and AS3; helix loop helix in AS3).

Spo76p Localizes to Foci on Chromosomes at All Stages of the Mitotic Cycle Except Metaphase and Anaphase

The cytological localization of Spo76p was determined using epitope- and reporter-tagged versions of the protein, carrying carboxy-terminal additions of, respectively, a triple hemagglutinin (HA) epitope and green fluorescent protein (GFP). Each construct, when introduced into a *spo76-1* strain, complemented the *spo76-1* meiotic and sporulation defects with exactly the same

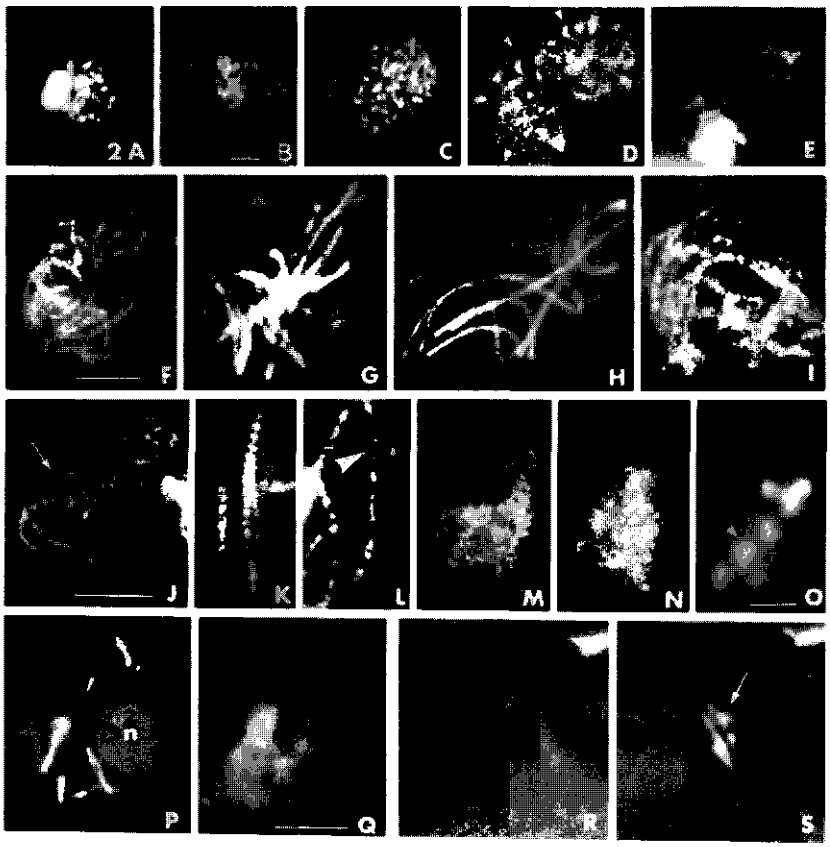


Figure 2. Spo76p Localization during Mitosis and Meiosis

(A–C) Mitotic prophase nuclei stained with (A) Spo76-GFPp (the nucleolus is visible left of the foci) and (B) corresponding DAPI; (C) Spo76-HAp. (D–S) Meiosis. (D) Karyogamy nucleus with short Spo76-GFPp lines at chromosome ends (arrowheads). (E) Corresponding DAPI. Spo76-GFPp stains as continuous lines along the chromosomes of leptotene (F), early zygotene (G), and pachytene (H) nuclei. (I–O) Nuclei stained with Spo76-HAp. (I) Leptotene. (J) Spread early zygotene; the arrow points to homologous nonsynapsed regions. Pachytene bivalents show clear punctuate pattern with either matching (K) or in staggered (L) rows of spots (indicated by the arrowhead). (M) Diffuse stage. (N) Diplotene with corresponding (O) DAPI (arrowhead points to one of the seven bivalents). (P) Spo76-GFPp in diffuse stage and corresponding DAPI (Q) at corresponding focus level. (R) Metaphase I and corresponding DAPI (S) with arrow indicating three bivalents. Bars, 5 μm.

efficiency as the 6.4 kb complementing subclone: the average number of complemented transformants per transformation (4 in total) was 10.5 for -HA, 9.8 for -GFP and 9.5 for the 6.4 kb subclone.

Spo76p staining is exclusively observed in nuclei. Spo76p localizes as foci in nuclei of all mitotic stages except metaphase and anaphase. The disappearance of Spo76p at prometaphase precedes any discernible separation of sisters (the metaphase/anaphase transition). Foci are best defined in nuclei from cells of the sexual cycle, which are larger (Figures 2A and 2B). Such nuclei exhibited ~27 Spo76-GFPp foci (range 19–36 among 100 nuclei representing all relevant cell cycle stages). Foci were usually seen located along the chromosomes, but in nondividing nuclei they sometimes also occurred outside of the main chromosome area. Spo76-HAp gave the same results except that foci were both smaller and more numerous (range 40–70; compare Figures 2C and 2A). In all cases, foci were much brighter in telophase and prophase nuclei than in interphase nuclei.

Spo76p Localizes along Meiotic Chromosomes as Foci at Early and Late Prophase and as Lines at Midprophase

By both approaches, meiotic prophase nuclei are more brightly stained than any other nuclei of either vegetative or sexual cycles. Meiotic stages can be defined independently of chromosome status by three criteria: the ascus length, which increases 10-fold from leptotene to metaphase I; the nucleolar shape and localization when compared to the chromosome mass; and the nuclear volume, which increases progressively (5-fold) during prophase.

Before karyogamy, Spo76-GFPp is observed in foci. Concomitant with nuclear fusion, these foci become organized into short lines, a change that initiates preferentially at the chromosome ends (Figures 2D and 2E). Thereafter, Spo76-GFPp stains as continuous lines along the lengths of all unsynapsed (leptotene), synapsing (zygotene), and synapsed (pachytene) chromosomes (Figures 2F–2H).

Spo76-HAp exhibits this same staining pattern except that finer details can be seen. At early leptotene, each chromosome has a single continuous line of regularly spaced dots plus some fainter foci in what we infer to be the peripheral chromatin (Figure 2I). From late leptotene to midpachytene, continuous lines are observed, though vigorously spread chromosomes exhibit contiguous regularly sized dots at these stages as well (illustrated for early zygotene in Figure 2J). At late pachytene, however, a punctuate pattern of staining is always seen, with linear rows of dots (2.07 ± 0.2 foci per μm along 50 bivalents) that are more prominent and less regular in spacing and size than earlier (Figures 2K and 2L). Interestingly, foci on homologs do not always occur at matching positions (arrowhead in Figure 2L).

Spo76p was shed from the bivalents at diplotene by both approaches, but with slightly different patterns. For Spo76-HAp, the bright pachytene punctuate pattern (Figure 2L) is rapidly lost when cells enter the diffuse stage preceding diplotene (Figure 2M), and diplotene nuclei contain only small foci spread over the entire chromatin mass (Figures 2N and 2O). For Spo76-GFPp, in contrast, early diffuse-stage nuclei still contain seven bright lines, but these are no longer axes associated, as deduced from the fact that they are seen in a different focal plane from the chromatin mass of the bivalents (Figures 2P and 2Q). These lines subsequently break into smaller spots until, finally, GFP fluorescence is lost after middiplotene. With respect to external staging criteria (above), assembly and disassembly of Spo76p into and out of linear arrays during meiotic prophase I correspond closely to the formation and disassembly of AEs. Furthermore, the earliest segments of AE, like the earliest linear arrays of Spo76p foci, tend to occur at chromosome ends. Finally, by both approaches, Spo76p is not detected on metaphases or anaphases I (Figures 2R and 2S) and II but reappears along chromosomes as foci at telophase I, prophase II, and telophase II. Thus, as during mitosis, Spo76p is seen on meiotic chromosomes at all stages except metaphase(s) and anaphase(s).

Overall, the two tagged Spo76p give similar staining patterns, though Spo76-GFPp tends to give more uniform or continuous staining than Spo76-HAp. While we cannot exclude a true difference between the two situations, it is equally possible that the underlying distribution of Spo76p is the same for both versions of the protein. Regions of greater abundance may alternate with regions of lesser abundance, with peculiarities of the two detection systems accentuating or minimizing this variation. For example, the Spo76-HAp signal might be attenuated in regions of lower protein abundance due to absence of specific epitopes. Oppositely, the Spo76-GFPp fluorescence might give a strong signal at low protein level but saturate at high protein level to give an apparently smoother distribution.

The *SPO76*GFP-tagged sequence exhibited the same staining pattern of both the mitotic and meiotic nuclei when present in a wild-type strain background as when present in a *spo76-1* background (data not shown). Thus, protein localization is not affected by the presence of the mutant protein or by an extra amount of wild-type protein.

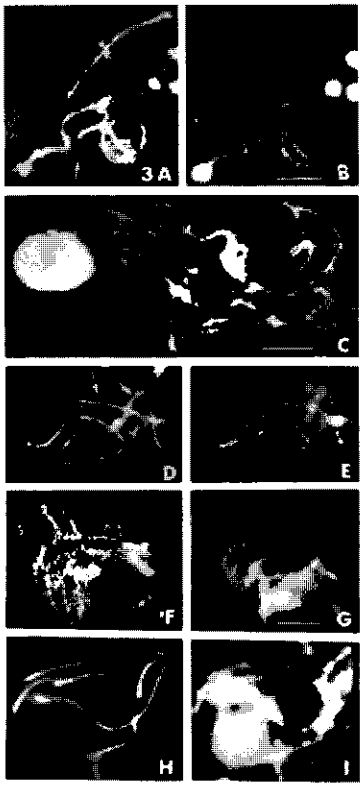


Figure 3. MPM-2 Staining of Meiotic Prophase Nuclei

(A) Spread wild-type late zygotene; arrow points to two synapsing homologs. (B) Corresponding DAPI. Lines of fine dots seen along each chromosome are similar in wild type (A) and *spo76-1* (C) zygotene nuclei; the nucleolus is stained in both strains. (D) Pachytene wild-type nucleus with faint chromatin staining (arrow); MPM-2 signal is clearly narrower than the DAPI staining (E). Double-stained pachytene nucleus with MPM-2 antibody (F) and Spo76-GFPp (G). The GFP signal is less bright than in (H) due to the longer fixation needed for the double labeling. Spo76-GFPp pachytene nucleus (H) and corresponding DAPI (I). Bars, 5 μm.

MPM-2 Defines a Narrower Staining Pattern along Meiotic Chromosomes Than Does Spo76p

The monoclonal phosphoprotein antibody MPM-2 (Davies et al., 1983) recognizes topoisomerase IIα in mitotic chromosome scaffolds (e.g., Taagepera et al., 1993). In *Sordaria*, this antibody stains the chromosomal axes during meiotic prophase (Figures 3A and 3B). Rows of discrete and almost contiguous foci are seen along the lengths of leptotene, zygotene, and early pachytene

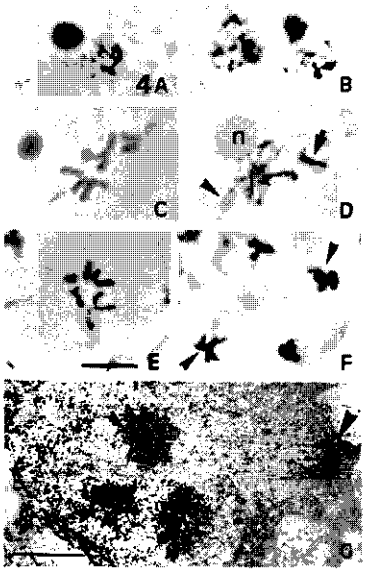


Figure 4. Mitotic Phenotypes

(A–F) Hematoxylin-stained mitotic nuclei (LM). Protoplast wild-type (A) and *spo76-1* (B) prophase nuclei. (C) Wild-type prometaphase. (D) Chromosome arms with either discernibly separated (arrowhead) or conjoined kinky chromatids (arrow) in an early *spo76-1* prometaphase nucleus. (E–G) *spo76-1*. (E) Metaphase. (F) Two synchronous anaphases (arrowheads). (G) Electron micrograph of a metaphase spindle; small arrowheads indicate chromosomes; large arrowhead points to the SPB. n, nucleolus. Bars, 5 μm (LM) and 0.5 μm (EM).

chromosomes in both wild type (Figure 3A) and *spo76-1* (Figure 3C). The MPM-2 signal is much narrower than the DAPI signal at all times and, at pachytene, forms a single line running down the middle of the bivalent (Figures 3D and 3E). However, some peripheral chromatin staining can be observed, for example, as tiny, faint foci in a halo around the axial signal (e.g., arrow in Figure 3D). Staining is no longer detectable after pachytene.

MPM-2 and Spo76p staining (above) are contemporaneous. Interestingly, the MPM-2 signal is narrower than the Spo76-GFPp signal (Figures 3F and 3G). In fully synapsed bivalents, the Spo76-GFPp lines define a narrower region of each bivalent than that of DAPI-stained chromatin, implying preferential localization of Spo76p in the vicinity of the chromosome axes (Figures 3H and 3I). Thus, while both signals are axis associated, the MPM-2 epitopes appear to be more tightly localized to the bases of the chromatin loops than is Spo76p. This result suggests that Spo76p may establish sister cohesion in relationship with, but immediately above, the AEs (i.e., in a "supraaxial" position).

spo76-1 Exhibits Defective Mitotic Chromosome Morphogenesis at Prometaphase but Not at Later Stages

The effect of *spo76-1* on mitosis was examined by light and electron microscopy. Prophase was normal (compare Figures 4A and 4B), but striking effects of the *spo76-1* mutation were seen at prometaphase. First, while prometaphase nuclei are rare in wild-type mycelia and protoplasts (5/100 nuclei), they were frequently observed in *spo76-1* (60/100), implying prolongation of this stage in the mutant. Second, mutant chromosome morphology and morphogenesis are significantly altered. In wild-type mitoses, chromosome compaction proceeds smoothly from modest chromosome individuality at prophase to a fully compacted metaphase condition; moreover, sister chromatids are never discernibly distinct (Figure 4C) until anaphase. In *spo76-1* prometaphases, in contrast, (a) chromosomes exhibit segments in which sister chromatids are distinctly separated from one another (arrowhead in Figure 4D); (b) chromatin has a markedly fuzzy appearance (compare Figures 4D and 4C); (c) chromosomes are longer than normal for this stage; and (d) some chromosome segments or arms exhibit a kinky appearance, indicative of higher order coiling/folding (arrow in Figure 4D), again a morphology never observed in the 100 analyzed wild-type nuclei. Interestingly, the sister chromatid cohesion and the axial compaction phenotypes vary in concert along the chromosome arms (Figure 4D; Discussion).

However, despite these differences at prometaphase, mutant and wild-type nuclei are essentially indistinguishable at later stages. During the metaphase/anaphase transition, *spo76-1* chromosomes are as compact as wild-type chromosomes, and sisters are no longer separated (Figure 4E). Furthermore, chromosome segregation at anaphase (Figure 4F) is qualitatively normal. First, *spo76-1* protoplasts examined at regular intervals for 24 hr after their preparation do not produce dead cells, and their nuclei exhibit the same size—both strong indications that chromosome segregation is regular (absence of aneuploidy). Second, anti-tubulin immunofluorescence revealed that mitotic spindles showed no gross abnormalities. Third, electron microscopy confirmed that *spo76-1* chromosomes, microtubules, and SPBs were similar to wild type, at least in premeiotic mitoses (Figure 4G). Finally, despite a prometaphase pause, *spo76-1* exhibits an essentially normal growth rate at a wide range of temperatures (13°C, 23°C, 30°C, and 34°C).

spo76-1 Exhibits Axial Element Splitting plus Coordinate Absence of Synaptonemal Complex Formation

The ultrastructural hallmark phenotype of the *spo76-1* mutant was splitting and discontinuity of AEs at midprophase (Moreau et al., 1985). Serial reconstructions of 20 prophase nuclei, nine available from previous work and eleven more made for the current study, revealed additional *spo76-1* defects. In wild type, AEs are formed as small stretches just after karyogamy and are complete at leptotene for all chromosomes. The same is true for *spo76-1* except that AEs were clearly less dense and more irregular in shape than in wild type (compare Figures 5A and 5B). Since wild-type AEs are less regular at early than at late leptotene, the *spo76-1* mutation may "exaggerate" these early phenotypes.

Wild-type zygotene and pachytene nuclei always show, respectively, seven synapsing and regularly synapsed SCs. Mutant nuclei, in contrast, were all abnormal, in several respects. Most AEs showed split segments, with all split AEs being half as wide as unsplit

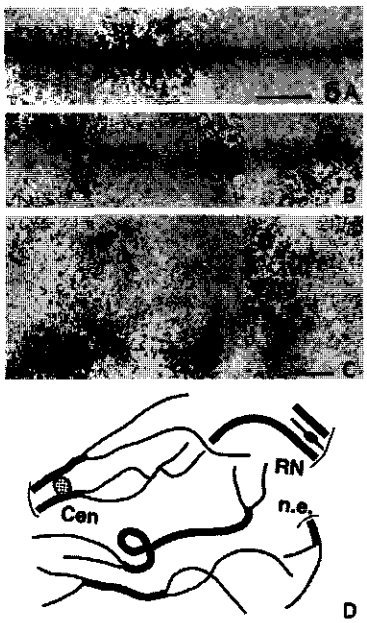


Figure 5. Electron Micrographs of Sectioned AEs

AEs from (A) wild-type and (B) *spo76-1* leptotene nuclei. (C) Section in a *spo76-1* nucleus showing both split AEs (arrows) and a half AE (arrowhead). (D) Reconstruction of four *spo76-1* chromosomes illustrative of the different mutant phenotypes; thick lines indicate unsplit AEs and thin lines, split AEs. Cen, centromere; RN, recombination nodule; n.e., nuclear envelope. Bars, 0.5 μm.

ones (arrowhead in Figure 5C). Overall, few AEs could be followed from telomere to telomere because split regions, whose two "half AEs" extended widely from one another (Figure 5D), were often broken. The total AE length (split plus unsplit) per nucleus was, however, approximately normal (79–86 μm versus 86–96 μm in wild type), suggesting that discontinuities reflect primarily breaking, rather than disassembly of AE components. Nuclei contained only 2–11 SC pieces per nucleus, representing 10% of the wild-type SC length. Interestingly, split AE and SC morphologies varied coordinately along the chromosomes; when SC was present, AEs were intact, and in regions with split AEs, SCs were absent, though a few segments exhibited unsynapsed but unsplit AEs. Also, virtually all centromeric regions (identifiable as fuzzy densities irrespective of AE/SC status) remained connected by a piece of SC or two intact AEs (Figure 5D).

spo76-1 Exhibits Diffuse, Kinked, and Partially Split Chromosomes at Meiotic Midprophase plus Full Sister Separation and Delayed Chromosome Compaction at Diplotene/Prometaphase

A striking feature of *spo76-1* meiosis is that, by LM, 28 chromosomes are regularly observed at metaphase I rather than the 7 bivalents seen in wild type (n = 7), implying that sister chromatids fully separate prematurely (Moreau et al., 1985). To understand this phenotype, we performed a detailed LM study of *spo76-1* meiosis.

The mutant shows normal fruiting-body development, implying that premeiotic stages including karyogamy are occurring normally. In wild-type prophase, chromosomes are straight and clearly individualized from leptotene (Figure 6A) through pachytene (Figures 6B and 6C). The corresponding *spo76-1* nuclei, in contrast, showed mainly kinky and diffuse chromosomes (compare respectively Figures 6D, 6E, and 6F). The presence of kinkiness (arrowheads in Figures 6D and 6E) implies some tendency for folding/coiling of mutant chromosomes, a feature never observed during wild-type meiotic prophase (Figures 6A–6C). In addition, *spo76-1* chromosomes exhibited coordinate regional defects in sister cohesion and homologous synapsis from late leptotene onward (e.g., arrows in Figure 6F; Discussion). Thus, the AE and SC defects are also reflected at the level of bulk chromatin.

Wild-type chromosomes exit pachytene into the diffuse stage, where chromosome individualization is lost, and then reemerge into diplotene, where sister chromatids are closely juxtaposed but homologs are separated except at chiasmata. While the diffuse stage was grossly similar in both strains (Figures 6G and 6H), the onset of diplotene in the mutant showed important differences. In wild-type nuclei, chromatin condenses progressively, and all bivalents show chiasmata (Figure 6I). In *spo76-1*, chromosomes come out of the diffuse stage less compacted than in wild type (compare Figure 6J with 6I). Furthermore, when diplotene condensation starts, 28 chromosomes emerge (Figure 6K and compare with 6L). Since the mutant still exhibited seven bivalents at pachytene, albeit with a tendency for sister splitting, additional loss of cohesion must occur specifically during the diffuse stage–diplotene transition.

At prometaphase and metaphase I, the final degree of chromosome compaction (Figures 6K and 6L) and spindle formation (Figures 6M and 6N) is similar in both strains. At anaphase I, the 28 chromosomes of the mutant segregate randomly on an elongated spindle (Figure 6N). The mutant also undergoes a cell cycle arrest at this stage: 2-week-old asci still exhibit elongated spindles, whereas wild-type asci form eight ascospores after 4 days. Presumably, since individualized sister chromatids cannot undergo bipolar alignment, absence of tension activates the spindle attachment checkpoint (see Nicklas et al., 1995). Only 1%–5% of the asci go through meiotic second division, and even fewer form ascospores. None of the 200 tested ascospores ever germinated (versus 99% germination in wild type).

spo76-1 Is More Defective for Late Recombination Nodules Than for Formation of Dmc1p and Rad51p Foci

Since a *spo76-1* homozygote produces no viable ascospores, recombination levels cannot be measured. Moreover, since chromatid cohesion is lost before diplotene, recombination cannot be evaluated by counting chiasmata. A role for *SPO76* in recombination can, however, be inferred by analyzing recombination nodules. In *Sordaria*, as in other organisms, SC-associated late

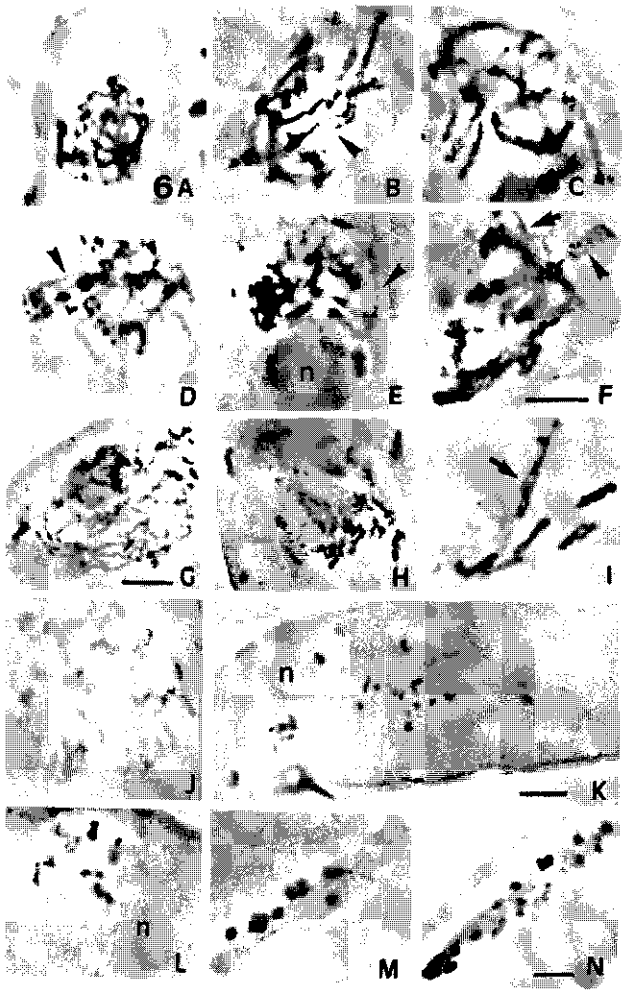


Figure 6. Meiotic Phenotypes

Wild-type leptotene (A), zygotene (B), and pachytene (C) nuclei. The *spo76-1* chromosomes are kinky (arrowheads) in all leptotene (D) and zygotene (E) nuclei. (F) Mutant pachytene nucleus in which chromosome regions show either widely (arrow) or slightly (arrowhead) separated chromatid regions. Wild-type (G) and mutant (H) diffuse stage. (I) Wild-type diplotene nucleus with clear chiasmata (arrow). (J) Mutant diplotene. Although mutant chromatids are separated (K), condensation is similar in mutant (K) and wild-type (L) prometaphase chromosomes. Wild-type (M) and mutant (N) metaphases l, n, nucleolus. All nuclei are stained by hematoxylin. Bars, 5 μm.

recombination nodules (LNs) correspond to crossovers (Zickler et al., 1992). The mean number of LNs (Figure 7A) in homozygous *spo76-1* is about one-sixth the wild-type level: 4.1 (range 2–6 in 16 pachytene nuclei) versus ~25 (range 23–26 in 100 pachytene nuclei) in wild type, suggestive of a reduction in crossovers. Since mutant SCs are discontinuous, one cannot rule out that additional LNs might occur in regions lacking SCs, but the reliability of the LN findings is further supported by the fact that the +/*spo76-1* heterozygote, which has normal SCs, exhibits a ~40% reduction in both LNs and crossovers (Zickler et al., 1992).

In yeast, where most or all meiotic recombination events are initiated by meiosis-specific double-strand breaks (DSBs), immunostaining foci of Dmc1p and Rad51p appear only when DSBs have occurred (e.g., Bishop, 1994). Thus, analysis of Dmc1p or Rad51p foci should provide insight into the efficiency with which meiotic recombination is initiated. In wild-type *Sordaria* nuclei, as in yeast, each of the two antibodies localized to foci only on leptotene and early zygotene chromosomes (Figures 7B and 7C), with ~25–35 foci per nucleus (50 analyzed). Precise counting of foci along each bivalent is impossible, as spreading of nuclei at these stages is difficult.

In homozygote *spo76-1* nuclei (Figures 7D and 7E), a 30% reduction was observed in the number of Dmc1p and Rad51p foci (18–24 in 50 leptotene/early zygotene nuclei). Also, contrary to wild type, all mutant nuclei (50 observed) showed 3–8 bright Rad51p foci at early pachytene (according to external staging criteria). The high level of Rad51/Dmc1p foci in *spo76-1* suggests that recombination initiation is nearly normal. The contrast with the low level of LNs suggests a strong defect specifically in the later stages of recombination, at least for events associated with crossovers.

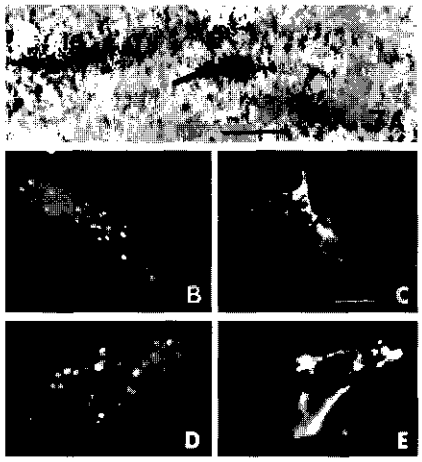


Figure 7. LN and Rad51p Localization

(A) Electron micrograph of a mutant SC piece with associated LN (arrow). Rad51p foci in squashed early zygotene wild-type (B) and mutant (D) nuclei and their corresponding DAPI (C and D). Bars, 0.5 μm in (A) and 5 μm in (B–E).

Discussion

Spo76p Is a Conserved Chromosome Morphogenesis Protein

Spo76p is evolutionarily conserved from yeast to human. Fungal homologs are BIMD (*A. nidulans*) and Pds5p (*S. cerevisiae*). The *Sordaria SPO76* gene can complement the temperature-sensitive lethality conferred by a *bimD6* mutation (D. v. H., unpublished results), implying direct functional homology between the cognate proteins. Accordingly, existing mutants of *SPO76, BIMD,* and *PDS5* show interrelated defects in chromosome morphogenesis during the mitotic cycle. The *spo76-1* mutant is defective in sister chromatid cohesiveness and chromosome compaction at prometaphase and is hypersensitive to DNA damage (above). At nonpermissive temperature, the *bimD* mutants show random chromosome localization along the spindle and anaphase arrest, phenotypes consistent with a cohesion defect; at permissive temperature they exhibit DNA damage hypersensitivity (Denison et al., 1992). The *pds5* mutant exhibits precocious dissociation of sister chromatids (SGDID S0004681). Since *BIMD* is an essential gene (Denison et al., 1992), the same is likely true of *SPO76,* with *spo76-1* being a specialized, non-null allele.

Interestingly, Spo76p is also significantly homologous to the products encoded by two human sequences. Since the *BIMD* gene occurs as a single copy in the *Aspergillus* genome (Denison et al., 1992), a gene duplication might have occurred during evolution, possibly with concomitant functional specialization of the two genes. Two facts suggest that *SPO76,* while required for both mitotic and meiotic divisions, may also play a crucial role during the G1-S interval. First, Spo76p is present in all nondividing nuclei. Second, overexpression of the *BIMD* gene is lethal because it blocks cells in the G1-S interval (Denison et al., 1992). The fact that proliferative shutoff of human prostate cells requires the androgen-induced expression of the *AS3* gene (one of the two potential human homologs of *SPO76*) (Geck et al., 1999) could be explained analogously; in this case, hormone-induced (over)expression of AS3p might prevent entry into mitosis. It would also be interesting to know whether the two roles of the fungal protein, in the divisions and at G1-S, might be implemented by the two different human Spo76 homologs.

Spo76p is unrelated in sequence to any of the known yeast or *Xenopus* cohesins (review in Hirano, 1999). Interestingly, however, the conditional lethal phenotype conferred by the *bimD6* mutation can be suppressed by a mutation in the gene encoding SUDA, the *Aspergillus* homolog of the cohesin protein Smc3p (Holt and May, 1996). Furthermore, the timing of Spo76p localization is similar to that observed for *Xenopus* cohesins, which also are depleted from chromosomes at or before metaphase (Losada et al, 1998). Thus, the possibility remains open that Spo76p is an additional member of the cohesins complex. In any case, while one cannot exclude very low levels of Spo76p remaining on chromosomes, particularly since it reappears at telophase, the timing of Spo76p localization points to roles other than maintenance of sister cohesion at the metaphase/anaphase transition.

Spo76p is also required for normal meiotic chromosome morphogenesis. The *spo76-1* mutation confers pronounced defects in intersister cohesion and chromosome compaction during prophase. Those defects are closely related, but not identical, to those seen at mitotic prometaphase. Meiotic recombination is also defective. Meiotic phenotypes of *pds5* have not been reported, but *bimD* mutants are sterile (D. v. H., unpublished results). Spo76p is found associated with chromosomes in the meiotic program similarly as during the mitotic program except for increased continuity at meiotic prophase, a difference concordant with meiosis-specific aspects of this period. These findings (below) suggest that Spo76p is a component of basic chromosome structure recruited and functionally adapted for the meiotic program.

A Critical Role for Spo76p at the Transition from Prophase to Prometaphase in the Mitotic Cycle

The *spo76-1* chromosomes are morphologically normal at all stages of the mitotic cycle except at prometaphase. This stage corresponds to the transition between the extended prophase chromosome configuration and the more compact metaphase configuration. The *spo76-1* phenotype fits with the notion that the onset of higher order compaction represents a discrete transition point in chromosome morphogenesis, in which Spo76p may play a critical role. Interestingly, also, mutant nuclei accumulate at the point of the defect, implying that exit from prometaphase is delayed for structural and/or regulatory reasons.

The prometaphase mutant phenotype includes defects in sister chromatid cohesion, axial shortening, and chromatin compactness, which points to functional coordination among these three different aspects of chromosome morphogenesis. A mutant situation in which

sister cohesion and chromosome compaction are coordinately disrupted was first described for yeast *mcd1* (Guacci et al., 1997). Interestingly, the *spo76-1* defects vary in concert, from region to region along the chromosome arms: a split and loose morphology alternates with a conjoined and tight morphology that includes pronounced kinking. This pattern provides strong evidence that the three different aspects of chromosome morphology not only involve common components but are directly coupled at the mechanistic level. A similar situation has also been observed for Indian muntjack mitotic chromosomes (Gimenez-Abian et al., 1995). In that system, prometaphase corresponds to a transition between prophase chromosomes having a single conjoined linear core (silver stained) and metaphase/anaphase chromosomes having two cores that are split and kinked. Chromosomes progress through intermediate morphologies, and the first of these is essentially identical to the *spo76-1* prometaphase: along the chromosomes, regions of conjoined kinked cores alternate with regions of straight, less well-defined, and separated cores.

On the basis of these data, we could suggest that the prophase to metaphase transition involves the imposition of special forces on the chromosomes as required for initiation of higher order compaction and that Spo76p is required for chomosome integrity during this transition, as part of the force transduction mechanism. In *spo76-1*, the force or stress is imposed normally but then relieved, on a region by region basis, either by loss of both cohesion and chromatin compactness or by a relatively normal process in which cohesion and compactness are maintained and axial coiling/folding occurs but in an attenuated form. The presence of Spo76p on prometaphase chromosomes, but not at metaphase, would further suggest that Spo76p normally leaves the chromosomes either concomitant with, or as a consequence of, these events.

Critical Roles for Spo76p during Recombination and at Two Transition Points during Meiosis

A strong meiotic defect in *spo76-1* chromosome morphogenesis is first observed at late leptotene, after AEs are fully formed. This defect includes general chromosome diffuseness plus splitting of AEs. Since nuclei can be found in which splitting of AEs (EM) or bulk sister chromatin (LM) have occurred in the absence of SC formation (EM) or homologous synapsis (LM), AE splitting appears to precede SC formation (i.e., zygotene).

The fact that SC forms only in regions of unsplit AEs could reflect an intrinsic inability of SC to form between "half AEs." SCs can, however, form perfectly normally in such a situation: in the *Coprinus* mutant *spo22*, which skips premeiotic S phase, single chromatids form AEs and these "half AEs" synapse into regular SCs (Pukkila et al., 1995). Thus, we favor the alternative interpretation for *spo76-1* that AE splitting and formation of SCs with unsplit AEs comprise two alternative responses, implemented on a region-by-region basis, to a single underlying problem that arises at the leptotene/zygotene transition.

The meiotic midprophase *spo76-1* phenotype is strikingly similar to the mitotic prometaphase phenotype. Both include chromosomal diffuseness; each involves a prominent defect at a specific transitional stage; and in both cases, chromosome arms exhibit a binary response in which some regions exhibit split sisters and a less ordered conformation, while other regions exhibit unsplit sisters and a more ordered conformation. Indeed, in a precise analogy, wide separation of split AEs could imply local chromatin bulkiness, which precludes intimate juxtaposition of nonsister axes into the SC, while unsplit regions might have more compact chromatin and thus be able to make SCs. Also, in both cases, centromeres remain largely unaffected. It is therefore tempting to envision that meiotic leptotene/zygotene transition is, in fact, the meiotic equivalent of the mitotic prophase/prometaphase transition, with Spo76p playing analogous roles in both situations.

There is, however, one obvious difference between the two transition points: the mitotic prophase/prometaphase transition signals the onset of higher order chromosome folding/coiling, while during meiosis this phase does occur much later, after the diffuse stage. This difference can be explained by saying that the same triggering chromosomal transition occurs in both cases but in meiotic midprophase does not provoke chromosome coiling/folding due to the extra stiffness/resistance from the chromosomes, which have structurally prominent, very straight axes (AEs) as compared to their mitotic prophase counterparts. Such a scenario is supported directly by the fact that *spo76-1* exhibits chromosome kinking from late leptotene through pachytene. This finding implies that some sort of force for axial compaction is exerted at the appropriate stage and, moreover, that Spo76p is required for the normal block to such compaction. Furthermore, the fact that Spo76p exhibits more continuous staining along the chromosome axes during meiotic than during mitotic prophase provides a direct structural correlate for such a meiosis-specific role. In further support of the occurrence of a disruptive force as a normal feature of leptotene/zygotene, there is also clear evidence for a tendency for individualization of sister chromatids at this transition in other organisms as exemplified by 3D analyses of maize meiosis (Dawe et al., 1994 and references therein).

The *spo76-1* mutant is also defective in meiotic recombination. In budding yeast this process occurs progressively during prophase with DSBs at leptotene, strand exchange intermediates at zygotene/early pachytene, and recombinant products appearing at mid/late pachytene (Padmore et al., 1991; Schwacha and Kleckner, 1997). The finding that *spo76-1* exhibits high levels of Dmc1p and Rad51p foci at leptotene, but a strong deficit of LNs at midzygotene/pachytene, points to a defect in the recombination process between these two stages (i.e., at the same midprophase period where chromosome morphogenesis is especially aberrant). Since many or all stages of meiotic recombination occur in spatial association with the chromosome axes (review in Kleckner, 1996; Roeder, 1997), it is tempting to link the recombination defect with an underlying defect in axial chromosome morphogenesis, specifically one that is related to intersister cohesion. Thus, during meiotic prophase, we could suggest that Spo76p again serves as a transducer of a disruptive chromosomal transition, as during mitotic prometaphase, except that now the

disruptive force is met by the resistance of axial stiffness and is channeled into another type of outcome, one related to recombination. Kleckner (1996) has proposed that the decision as to whether recombinational interactions will yield crossover or noncrossover products is determined at the leptotene/zygotene transition (e.g., at the transition from DSBs to strand exchange products) by the imposition and relief of tension/stress along the chromosomes. It was further proposed that this process has evolved by modification of an analogous progression that normally induces chromosome coiling/folding along mitotic chromosomes at prometaphase so as to minimize intersister connections. This model accounts for all of our results.

Once meiotic chromosomes exit pachytene and pass through the diffuse stage, an additional defect becomes apparent: when chromosomes reemerge at diplotene, sister chromatids are fully separated. Mitotic chromosomes, in contrast, do not exhibit this feature at any stage. Perhaps the additional disruptive forces of the diffuse stage (during which strong chromatin decondensation takes place), which does not occur during the mitotic cycle, acts upon the already compromised *spo76-1* chromosomes to further exaggerate the defect; alternatively, or in addition, Spo76p might play a second, meiosis-specific role at this point (e.g., via effects in the peripheral chromatin where Spo76-GFPp is seen localized).

Experimental Procedures

Strains, Cosmids, and Plasmids

Isolation of *spo76-1* is described in Moreau et al. (1985). Culture conditions and both growth M1Gx and regeneration media RG7 were described in Huynh et al. (1986). Subcloning and plasmid preparations utilized pUC19 and *E. coli* strain DH5a (Hanahan, 1983). The HA epitope from plasmid BFG1 (kindly provided by J. Camonis; Chardin et al., 1993) and EGFP from plasmid pEGFP-1 (Clontech) were inserted at a 3′ NsiI site. Details available upon request. All cotransformations were performed with pANscosI (Osiewacz, 1994).

Transformation of *Sordaria*

Protoplasts were prepared as in Pöggeler et al. (1997) except for the following: glucanex was used instead of novozym; protoplastation buffer was 13 mM Na_2HPO_4, 45 mM KH_2PO_4, 600 mM KCl (pH 6.0); transformation buffer was 1 M Sorbitol, 80 mM $CaCl_2$, 10 mM Tris-HCl (pH 7.5). After an overnight regeneration in RG7, protoplasts were added to 7 ml RG7 top agar (45°C) with hygromycin (Boehringer-Mannheim) at a final concentration of 0.4 mg/ml and plated on RG7 plates. After 2–3 days of growth at 23°C, growing colonies were recovered from the selective top layer and individually transferred to fresh M1Gx plates with hygromycin (0.125 mg/ml final concentration). After 14 days at 23°C, the cover plates of the Petri dishes were screened for projections of black spores, and transformants were purified according to Huynh et al. (1986).

Sequencing

The 6.4 kb complementing fragment was sequenced on both strands using pUC primers (Stratagene) and gene-specific primers (Genosys). Genomic DNA from *spo76-1* and its isogenic wild type was isolated according to Lecellier and Silar (1994) with PCR products sequenced directly (Rosenthal et al., 1993). Transcription start and polyadenylation sites were determined by sequencing the products obtained by, respectively, 5′ and 3′ RACE (Frohman et al., 1988) on poly(A$^+$) RNA from the isogenic wild-type strain using kits (GIBCO–BRL). Total RNA was isolated essentially following Pöggeler et al. (1997): mycelia were ground in 4 M guanidine thiocyanate, 50 mM Tris-HCl (pH 7.8), 10 mM EDTA, 2% sarkosyl, 1 vol % β-mercaptoethanol; RNA was precipitated with LiCl and purified with a RNeasy Plant Mini Kit (Qiagen). Poly(A$^+$) RNA was isolated by oligotex mRNA Mini Kit (Qiagen). All sequence reactions used the DyeDeoxy Terminator, Cycle Sequencing Kit (Applied) and were analyzed on a 373 DNASequencer (Applied).

Cytology

Cells were processed for immunofluorescence as described in Thompson-Coffe and Zickler (1994). Primary antibodies used were anti-HA 3F10 (Boehringer Mannheim) at 1:4000; MPM-2 (kindly provided by P. N. Rao) at 1:750; and anti-Dmc1 and anti-Rad51 (Bishop, 1994) at 1:1000. Secondary antibodies were Jackson FITC anti-rat (Interchim) or CyTM3 anti-rabbit (Jackson) at a dilution of 1:100 and 1:4000, respectively. Controls included the use of primary or secondary antibodies alone. EGFP was visualized using the Zeiss filter set for FITC and for GFP. In total, four HA and five GFP transformants were analyzed. All cells were observed on a Zeiss Axioplan microscope and images captured by a CCD Princeton camera, or on T-Max 400 film.

For LM, cells were fixed in fresh Lu's fixative (butanol, proprionic acid, and 10% aqueous chromic acid, 9:6:2 v/v). After 10 min of hydrolysis at 70°C, cells were stained in two drops of 2% hematoxylin mixed on the slide with one drop of ferric acetate solution.

For EM, asci were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3 hr, postfixed in phosphate-buffered 2% osmium tetroxide for 1 hr, and dehydrated through an alcohol series. Asci were embedded in Epon 812 at 60°C for 24 hr. Serial sections were mounted on Formvar-coated single hole grids and stained in aqueous uranyl acetate for 30 min, followed by lead citrate for 10 min.

Acknowledgments

We thank Douglas Bishop and Akira Shinohara for anti-Dmc1p and anti-RAD51p antibodies, respectively. We are grateful to Nancy Kleckner for fruitful discussions and comments on the manuscript. We also thank David Perkins for his sustaining interest. This work was supported by the Centre National de la Recherche Scientifique (UMR 8621) and the European Economic Community (contract CHRX-CT94–0511) to D. Z.; D. v. H. was supported by grants from the EEC (contract CHRX-CT94–0511), the NIH (R37 GM25326), and the Netherlands Organization of Scientific Research (NWO, contract 805–18.262).

Received April 22, 1999; revised June 17, 1999.

Chapter 3

The BIMD protein of *Aspergillus nidulans* is involved in chromosome structure, sister chromatid cohesion, DNA repair and recombination

D. van Heemst [1,2], E. Kafer [3], T. John [3], C. Heyting [1], M. van Aalderen [1] and D. Zickler [2]

[1] Department of Genetics, Wageningen University, 6703 HA Wageningen, The Netherlands; [2] Institut de Génétique et Microbiologie, UMR 8621, Université Paris Sud, 91405 Orsay Cedex, France; [3] Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

Manuscript in preparation

ABSTRACT

The *Aspergillus nidulans bimD6* mutation confers a pleiotrophic phenotype, including a mitotic chromosome segregation defect at high temperature and increased sensitivities to UV and MMS (methyl-methane sulfonate) at low temperature. Here we show that *bimD6* causes other abnormalities at low temperature: premeiotic development is disturbed and meiosis blocked at metaphase I. We found that BIMD is chromosome-associated at all stages of mitosis and meiosis, except when chromosomes are highly compacted. BIMD shows significant sequence homology to Spo76p of *Sordaria macrospora*, and *SPO76* can complement the temperature and MMS sensitivities of *bimD6*. However, *SPO76* cannot rescue the sexual sporulation defects of *bimD6*, nor can *bimD* rescue the sexual sporulation defects of *spo76-1*. In addition, whereas the mitotic localization of the two proteins was roughly similar, the meiotic localization showed some important differences. These differences may be related to the absence of synaptonemal complexes and crossover interference in *A. nidulans*. Like *spo76-1* of *S. macrospora* and rec⁻ mutants of *A. nidulans*, *bimD6* is sensitive to X-rays and UV. Spontaneous allelic recombination in vegetative diploids was as much reduced in *bimD6* as found for *uvsC114* (a homologue of *RAD51*). In contrast, conversion in the interrupted duplication of *benA22*/+ was at wild-type levels in *bimD6*, but almost absent in *uvsC114*. BIMD thus plays a role in some but not all types of recombination in addition to its roles in sister chromatid cohesion and chromosome structure. We speculate that the recombinational repair machinery needs to cooperate with sister chromatid cohesion complexes flanking a DNA-lesion if the search for a homologous template has to extend outside an intrachromatid loop domain.

INTRODUCTION

The *Aspergillus nidulans bimD6* (blocked in mitosis) mutant was isolated as a conditional lethal mutant with a mitotic arrest phenotype at the restrictive temperature (Morris 1976). During the metaphase/anaphase transition in wild type cells, two discrete, condensed chromatin masses are pulled towards the opposite poles as the mitotic spindle elongates. In contrast, in *bimD6*, a chromosome segregation defect was demonstrated; the condensed chromatin lies randomly in more than two discrete masses along the mitotic spindle, which never fully elongates (Denison *et al.* 1992). In addition, at the permissive temperature, the *bimD6* mutant was found to exhibit increased sensitivity to DNA damaging agents (UV and MMS), a phenotype consistent with an additional role for *bimD* in mitotic DNA repair. As many other DNA repair mutants in *A. nidulans* (reviewed in Kafer and May 1998), *bimD6* is sterile in selfings and homozygous crosses (unpublished observations). The *bimD* gene does however not seem to have a checkpoint function in delaying cell cycle progression in response to DNA damage (Denison and May 1994). One gene carrying suppressor mutations of *bimD6* is *sudA,* which encodes the *A. nidulans* homologue of Smc3p (Holt and May 1996), a protein implicated in sister chromatid cohesion and/or recombination in a variety of organisms (reviewed by Hirano 1999).

In addition, BIMD shows a significant degree (44% identity) of amino acid sequence homology to Spo76p of *S. macrospora* (van Heemst *et al.* 1999). The *spo76-1* mutant of *S. macrospora* was identified as a mutant defective in meiotic sister chromatid cohesion and mitotic DNA repair (Moreau *et al.* 1985; Hyunh *et al* 1986). Furthermore, in a *spo76-1*/+ heterozygote, the *spo76-1* mutation has a dominant-negative effect on meiotic recombination (Zickler *et al.* 1992). Detailed cytological analyses of *spo76-1* mutant phenotypes combined with protein localization studies pointed to crucial roles of Spo76p during three transitions in chromosome morphogenesis: the mitotic prophase/metaphase transition, the meiotic leptotene/zygotene transition and the meiotic diffuse stage/diplotene transition (van Heemst *et al.* 1999). It was furthermore suggested that Spo76p is required for maintenance of sister chromatid cohesion against disruptive forces, such as those applied during crossover interference (see below) and higher order chromosome compaction (van Heemst *et al.* 1999). Thus, both BIMD and Spo76p are required for chromosome segregation, recombination and/or DNA repair, possibly by influencing aspects of higher order chromosome architecture such as sister chromatid cohesion and chromosome compaction.

Information about the molecular composition of chromosomes and the processes that alter the physical properties of chromosomes remains scarce (review in Koshland and Guacci, 2000). In eukaryotic cells, nuclear DNA is progressively compacted into chromatin in independent successive stages. The first level of compaction is the nucleosome, which forces DNA in ordered, solenoidal supercoils (Luger *et al.* 1997). At the second level, the 30 nm fiber is formed (Widom 1998). The third level of compaction likely involves the formation of topologically constrained loop domains on a central axis (Saitoh and Laemmli 1993). It has been argued that these loops can be formed by coalescence of adjacent sister chromatid cohesion sites along the length of paired sister chromatids (Guacci *et al.* 1997). Higher levels of compaction might involve folding of the loops and coiling of the axis (review in Koshland and Strunnikov 1996). When properly condensed, the chromosomes orient themselves on the metaphase plate, until all sister kinetochores have captured spindle microtubuli emanating from opposite poles. When all sister chromatids are under bipolar tension, sister chromatid cohesion is resolved in a highly regulated manner (review in Nasmyth *et al.* 2000).

During meiotic prophase, interactions between homologues occur. First, recombination between homologous regions on non-sister chromatids is initiated at high frequency by the formation of DNA double strand breaks (DSBs). The DSBs give rise to recombination intermediates which are resolved into either "crossovers" or "noncrossovers". Positive crossover interference affects crossovers in such a way that these are limited in number and well spaced. Most species form on average about two crossovers per bivalent. Second, almost all sexually reproducing eukaryotes analyzed to date assemble tripartite synaptonemal complexes (SCs). In yeast, SCs are assembled concomitantly with intermediate stages of recombination (reviewed by von Wettstein *et al.* 1984; Heyting, 1996; Zickler and Kleckner 1999). The two sister chromatids first assemble a single, common axial element along their length; subsequently, transverse elements connect the axial elements of homologous chromosomes to form the tripartite SC structure. At pachytene SCs become full length; in diplotene the SCs disappear concomitantly with or immediately following completion of recombination. This occurs in a transition phase which is accompanied by the onset of higher order chromosome compaction and which is approximately analogous to mid-prophase of the mitotic cycle. The crossovers, in concert with sister chromatid cohesion distal to the crossover sites, enable bivalents to align in the metaphase I spindle, and allow homologs to disjoin properly at the first meiotic division (reviewed in Moore and Orr-Weaver 1998).

In the *S. macrospora spo76-1* mutant, lateral elements split during the leptotene/zygotene transition, while in wild type *S. macrospora*, the localization of Spo76p close to lateral elements exhibits the same temporal pattern as their assembly and disassembly (van Heemst *et al.*1999). Interestingly, *A. nidulans* is claimed to have neither SCs nor lateral elements (Egel-Mitani *et al.* 1982) and to lack positive crossover interference (Strickland 1958). An interesting question is therefore how meiotic chromosomes are organized and function in the absence of SCs and crossover interference and which role BIMD may play is these processes. We have therefore analyzed the role of BIMD in mitotic and meiotic chromosome organization and metabolism. We have cytologically compared meiotic cell cycle progression in wild-type and *bimD6* strains at the permissive temperature. We also determined the cytological localization of the BIMD protein during the various stages of mitosis and meiosis in wild-type and *bimD6* strains. We determined the nature of the *bimD6* mutation and tested whether *SPO76* could functionally complement three aspects of the phenotype of *bimD6*: heat-sensitivity, sensitivity to MMS and the meiotic defect. In addition, we have analyzed the effects of *bimD6* on mitotic recombination in diploids and duplication strains.

MATERIALS AND METHODS

Strains

Genotypes and origin of all haploid, duplication and diploid strains of *A.nidulans* used in this study are shown in Table 1. *A. nidulans* is homothallic and all laboratory strains are derived from a single nucleus, and therefore are isogenic barring induced mutations (Pontecorvo *et al* 1953). Wild type and many derived strains can self, but sterility is conferred by many mutations, including nutritional markers. Any two haploid strains can be crossed and individual hybrid cleistothecia can be identified by segregation of color markers. For all *bimD6* strains, 25° or 30° was used as the permissive temperature, and 42° as the restrictive temperature. The recipient strain for transformation of *S. macrospora* was *spo76-1* (Moreau *et al.* 1985).

Table 1: *A. nidulans* strains used in this study

Strain No. (FGSC)	Genotype	Source or our isolation No.
(A773)	*pyrG89; wA3; pyroA4*	Morris NR; GR5
D6.9	*riboA1; sC12; bimD6 pyroA4*	Denison *et al.* (1992)
	Strains for cytology	
WG539	*pyroA4*	A773 x 3692
WG540	*wA3; pyroA4*	A773 x 3692
WG541	*pyroA4 bimD6*	A773 x 3692
WG542	*wA3; pyroA4 bimD6*	A773 x 3692
	Duplication strains	
IS88	*pyrG89 (/+) pabaA1; fwA1 Dp[benA22/+ - pyr-4] uaY9*	Dunne and Oakley
IS89	*pyrG89 (/+) pabaA1; fwA1 Dp[benA22/+ - pyr-4] uaY9*	(1988)
4304	*pyrG89 (/+) pabaA1; bimD6 pyroA4; Dp[benA22/+ - pyr-4] (±uaY9) riboB2 chaA1*	3291.4.23
4334	*pyrG89 (/+) pabaA1; bimD6 pyroA4; fwA1 Dp[benA22/+ - pyr-4] (±uaY9) riboB2*	3291.3.13
4296	*pyrG89 (/+); choA1; uvsC114 fwA1 Dp[benA22/+ -pyr-4] (±uaY) riboB2 (±chaA)*	3294.4.14
4337	*pyrG89 (/+) pabaA1; choA1; uvsC114 Dp[benA22/+ - pyr-4] (±uaY9) riboB2*	3294.3.12
	Transformation recipients	
WG544	*pyrG89; wA3; pyroA4*	A773 x 3692
WG546	*pyrG89; wA3; pyroA4 bimD6*	A773 x 3692
3104 (A771)	*pyrG89 riboA1 yA2; wA3; pyroA4*	2781.1.15
4078 (A1061)	*pyrG89 pabaA1; wA3; pyroA4 bimD6; chaA1*	3303.5.7
4247	*pyrG89; bimD6 pyroA4; riboB2 chaA1*	3281.3.9
	Diploids	
3456	*pabaA6 yA2 adE8;AcrA1; nicA2; malA1; uvsC114 fwA2*	2770.7.13
2749 (2n)		
3455	*adE20 biA1; OliC2 pantoB100; uvsC114 riboB2*	2769.4.8
4120	*sulA1 anA1 adE20 biA1; wA2 cnxE16; sC12; bimD6 pyroA4; sbA3; fwA2*	3207.10.1
3223 (2n)		
4122	*pabaA6 yA2 adE8; bimD6; riboB2 chaA1*	3208.5.5

Media and genetic procedures

Standard *A. nidulans* media and general genetic techniques were used, especially for random ascospore analysis of crosses, as developed by Pontecorvo *et al.* (1953). Required modifications of the media (e.g., addition of trace elements to minimal medium, MM) and methods of mitotic mapping and congenic strain construction were those developed for *A. nidulans* (Kafer 1977a, Appendix; available on request from the author; Scott and Kafer 1982). Procedures for genetic analysis of DNA repair mutants and of their effects on mitotic recombination in diploids were as described in detail previously (Kafer and Mayor 1986; Zhao and Kafer 1992). For tests of allelic recombination, the frequencies of selected ad^+ recombinants were determined taking into account the survival observed in low density platings on CM. While the control was plated at the standard density of 10^5 or 10^6 per plate (Zhao and Kafer 1992), *bimD6* and *uvsC114* were plated at a higher density (of 10^6 or 10^7 per plate). Clones occasionally can occur (and were observed for *uvsC114*). The values presented were averages (±SE) combined from both densities (and corrected for clonal distribution). Even though the frequencies determined do not fit exactly a normal distribution, the statistics used are considered appropriate approximation (Sarkar 1991). For tests of intrachromosomal recombination, we used the *benA* interrupted duplication system (Dunne and Oakley 1988), which was adapted for visual assessment of recombination frequencies (details to be published elsewhere). The two original *benA22*/+ duplication strains had been constructed by transformation of a benomyl-resistant (*pyrG89; benA22*) recipient with a plasmid containing the *benA* gene, and the *Neurospora crassa pyr-4* gene as a selectable marker. To obtain the corresponding *bimD6* and *uvsC114* duplication strains, the original duplications were crossed with these mutant strains (containing *pyrG89*; Table 1).

Transformation

The *A. nidulans* protocols for mycelial protoplasting and transformation using *pyrG89* as the selective marker were modifications of methods devised previously (Debets and Bos 1986; Osmani *et al.* 1987). Recipient *pyrG89* strains were grown in liquid YG medium (May *et al.* 1985; 0.5% yeast extract, 2% glucose, trace elements and vitamin supplements) to which 10 mM uracil and 5 mM uridine were added for optimal growth. Selective platings of putative pyr^+ transformants were made onto the corresponding YAG medium (2% agar in bottom layers, 1% in overlays, and 1 M sucrose replacing glucose, without uridine, uracil).

Transformation procedures for *S. macrospora* were as described recently (van Heemst *et al.* 1999).

Plasmids

The plasmids used were: pANscos1 (*HygR*; Osiewacz 1994); pPL6 (=pPyrG) (Oakley *et al.* 1987); pRG3 (*pyr-4*; Waring *et al.* 1989); pBimD1 (*bimD*; Denison *et al.* 1992); pDH1(*SPO76*) and pDH13 (*SPO76-GFP*; van Heemst *et al.* 1999). For the construction of pGW1454 a 6.7 kb *Nco*I-*Sma*I fragment from pBimD1 was cloned into pBR328 *Eco*RV-*Nco*I; for pGW1460, EGFP was amplified from pEGFP-1 (Clonetech) with Vent polymerase and the primers EGFPB-up (tctactcgagccatggtgagcaagggcg) and EGFPB-do (tctactcgagacttgtacagctcgtcca) and inserted into the *Xho*I site of pGW1454; for pGW1463, a 1.4 kb 3'-*Bgl*II/*Xho*I *bimD* cDNA fragment was cloned into the expression vector pQE32 (Qiagen) *Bam*HI-*Sal*I. The *E. coli* strain DH5α was used as a host in plasmid propagation (Hanahan 1983).

Sequencing

An 1.2 kb fragment spanning the location of *bimD6* was amplified by PCR from genomic DNA isolated from *bimD6* and wild type strains (WG542 and WG540; Table 1). Genomic DNA was isolated as described before (Thijs *et al.* 1995). PCR products were sequenced directly (Rosenthal *et al.* 1993) with gene-specific primers. For DNA-sequencing, we used the Dyedeoxy Terminator, Cycle Sequencing kit (Applied) and a 373 DNASequencer (Applied).

Cytological methods

For cytological analysis of *A. nidulans* meiosis, fruiting bodies of different stages were used, as judged by the color of the fruiting body wall, which changes from yellow/pink (early meiotic stages) towards purple (ripe ascospores). A typical fruiting body of the color most frequently used for cytological examination (pink) contains thousands of asci in the various stages of meiotic development (Elliot 1960). For all cytological analyses, fruiting bodies were mechanically squashed with a blunted metal needle and stained with the DNA-specific dye 4,6-diamidino-2-phenylindole (DAPI).

Cells were processed for immunofluorescence following the procedure described by Thompson-Coffe and Zickler (1984) for *S. macrospora*. For the preparation of an antiserum against the C-terminus of BIMD, *E. coli* SG13009 cells (Qiagen) were transformed with plasmid pGW1463. The fusion protein was expressed after induction with isopropyl-β-D-thiogalactopyranoside (IPTG), purified from the bacterial cell lysate by affinity chromatography on a nickel column (Qiagen), and dialyzed against PBS (140 mM NaCl, 10 mM sodium phosphate pH 7.3). We raised an antiserum (serum 560) against the fusion protein in a rabbit and affinity-purified the anti-BIMD antibodies using Western blot strips containing the fusion protein, as described (Lammers *et al.* 1994). The affinity-purified antibodies were used in a dilution corresponding to 1: 200 diluted serum. As secondary antibody we used antirabbit IgG conjugated to CyTM3 (Jackson), diluted 1:4000. As controls we incubated cells in primary or secondary antibodies alone.

For BIMD localization with GFP, three fully complemented cotransformants, obtained by transformation of strain WG546 (*pyrG89; bimD6*) with pGW1460 (*bimD-GFP*) and pPL6 (*pyrG*), were analyzed in detail using the Zeiss filter set for FITC. All cells were observed on a Zeiss Axioplan microscope and images captured by a CCD Princeton camera.

Western Blot Analysis

For crude protein extracts of *A. nidulans*, mycelium was powdered in liquid nitrogen in the presence of the complete mini protease inhibitor cocktail (Boehringer, cat. No. 1836153), boiled in electrophoresis sample buffer and centrifuged. Proteins extracted from 7 mg mycelium were loaded per 3.5 cm wide slot of a 10% polyacrylamide gel and electrophoresed (Laemmli 1970) in parallel with molecular weight markers (BioRad HMW). Of each lane, 0.5 cm was stained with Coomassie blue, and the remainder was transferred to nitrocellulose (Dunn 1986). The resulting blot was stained with Ponceau S to determine the position of the markers and the lanes containing the mycelial proteins. 0.3 cm wide strips were cut from these lanes and probed with anti-BIMD antibodies. Binding of antibodies to the strips was detected by goat-anti-rabbit secondary antibodies conjugated to alkaline phosphatase and incubation in NBT/BCIP, as described (Lammers *et al* 1994).

RESULTS

Nature of the bimD6 mutation

The *bimD* gene of *A. nidulans* has been cloned and sequenced (Denison et al. 1992). It is an essential gene, and the *bimD6* mutation is thus a non-null mutation. The *bimD6* mutation had been mapped to the 5' region of the *bimD* gene and we have sequenced a region spanning this mutation on genomic DNA from the *bimD6* mutant and from an isogenic *bimD* parent strain. Unexpectedly, we found a T to A mutation at position 420 in the ORF, which turns a TAT codon (Tyr) into a TAA stop-codon. At the protein level, the most straight-forward outcome of this mutation would be the production of only a short, truncated protein (~ 15 kD), that would comprise the first 140 N-terminal AA. However at the posttranscriptional level, ribosome-mRNA interactions could result in other outcomes (reviewed by McCarthy 1998), such as read-through of the stop-codon and/or translation reinitiaton. To analyze this, we performed Western blot analysis of proteins from wild type and *bimD6* strains of *A. nidulans* (Figure 1), using affinity-purified antibodies directed against the C-terminus of BIMD. On the blot carrying proteins from wild type, these antibodies recognized a protein of about 170 kDa, which corresponds to the predicted molecular mass of BIMD (166 kDa). On the *bimD6* blot, the antibodies did not recognize any protein. Thus, if re-initiation and/or read-through occur at all in *bimD6*, it should happen infrequently, so that no detectable BIMD protein is produced.

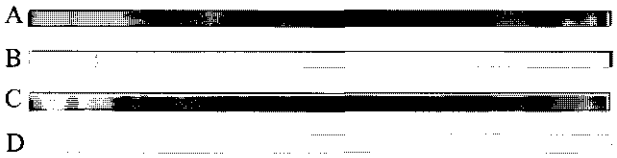


Figure 1: Immunoblot analysis of proteins extracted from wild type and *bimD6* strains. Mycelial proteins of wild-type (lanes A and B) and *bimD6* (lanes C and D) strains were incubated with antibodies against the C-terminus of the BIMD protein. (A) and (C) Coomassie-blue stained gels; (B) and (D) Corresponding immunoblots probed with affinity-purified anti-BIMD antibodies from serum 560. Molecular size standards are indicated below lane D in kilodaltons.

Mitotic localization of BIMD in wild type A. nidulans

The cytological localization of the BIMD protein during wild-type mitosis was determined in 100 nuclei representing all relevant mitotic stages by detection of a BIMD-GFP fusion protein. The construct encoding the BIMD-GFP fusion protein (pGW1460) complemented both the mitotic lethality at the restrictive temperature, and the meiotic defect at the permissive temperature (see below) of *bimD6* as well as a plasmid with the *bimD* gene. Furthermore, the staining patterns that we observed for BIMD-GFP were fully comparable between transformants in *bimD6* and *bimD*$^+$ strain backgrounds. The BIMD-GFP fusion protein was exclusively localized in nuclei and could be detected on the chromosomes in all stages except metaphase and anaphase (Figure 2). The latest stage at which BIMD-GFP was still localized to the chromosomes was prometaphase when the individual chromosomes were visible but not yet fully condensed (Figure 2, A and B). At metaphase, when the chromosomes had reached their highest degree of compaction, BIMD-GFP was no longer detectable on the chromosomes, although it was still present in the nucleus (Figure 2, C and D). At this stage, the protein seemed to fill the nuclear volume, and to be preferentially present in the spaces that were not occupied by the condensed chromosomes. A similar localization was observed at anaphase when the condensed chromatin masses move to the poles (Figure 2, E and F). At anaphase, the strongest BIMD-GFP staining was consistently observed between the two separating chromatin masses.

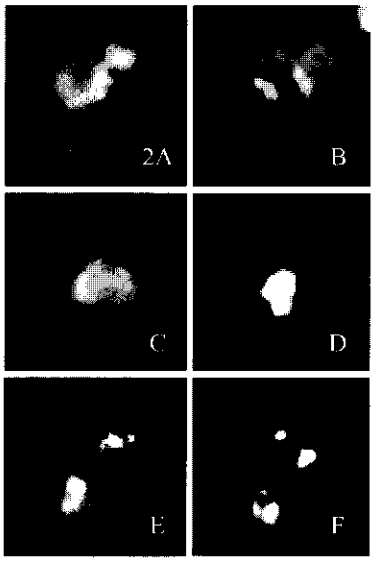


Figure 2: Localization of the BIMD-GFP fusion protein during wild type mitosis. (A) Prometaphase nucleus, GFP image; (B) DAPI image. (C) Metaphase nucleus, GFP image; (D) DAPI image. Note that the chromosome area clearly seen in (D) is not stained in (C) but that in (C) BIMD-GFP is localized in the rest of the nucleus and also predominantly at the poles. (E) Anaphase nucleus, GFP image; (F) DAPI image. Bar, 5 μm.

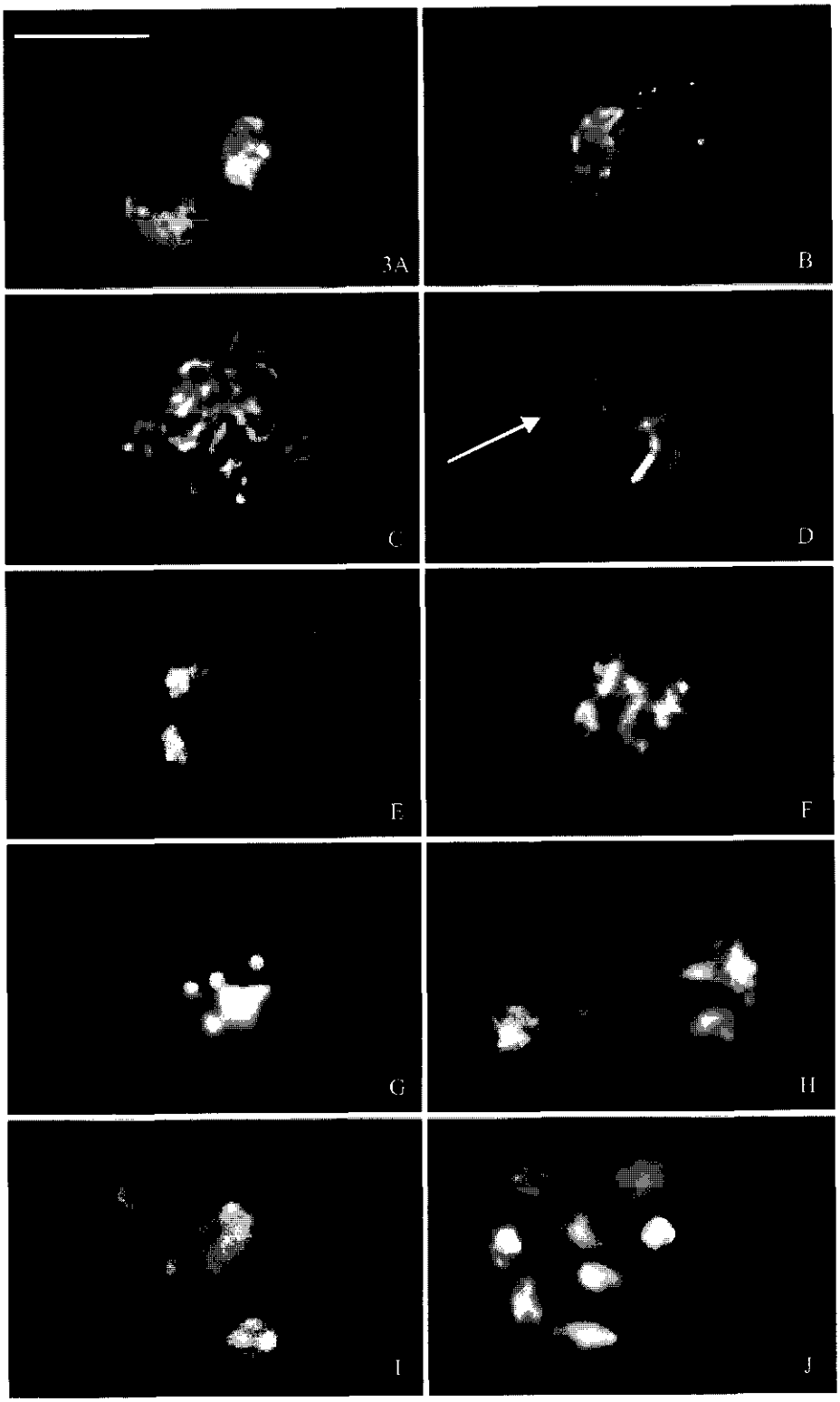
We also localized BIMD in mitotic cells by immuno-fluorescence, using affinity-purified anti-BIMD antibodies from serum 560, and obtained corresponding results (data not shown). The mitotic localization of BIMD in *A. nidulans* thus strongly resembles the mitotic localization found for the homologous protein Spo76p of *S. macrospora* (van Heemst *et al.* 1999).

Meiotic progression in wild type and bimD6 strains at the permissive temperature

An important feature of the meiotic phenotype of the *spo76-1* mutant of *S. macrospora* is a block at the first metaphase/anaphase transition due to a complete and precocious separation of all sister chromatids (Moreau *et al.* 1985; van Heemst *et al.* 1999). In the *bimD6* mutant of *A. nidulans* the meiotic phenotype hadn't yet been described at the cytological level.

In order to identify abnormalities in (pre)meiotic development of *bimD6,* we first analyzed meiosis in cells of a wild type strain. In *A. nidulans*, individual chromosomes are difficult to discern by light microscopy, and cytological analysis of meiosis in this organism has been limited to one study (Elliot 1960). We have used the DNA-specific dye DAPI to elucidate the different stages of meiosis in wild type *A. nidulans* (Figure 3).

Figure 3: Progression of meiosis in wild type *A. nidulans* meiocytes. Wild type meiotic cells stained with DAPI (LM). (A) Binucleate penultimate cell before karyogamy. The dark area in the two nuclei corresponds to the position of the nucleolus. Note that DAPI also stains the mitochondria in the cytoplasm. (B) Leptotene: individual chromosomes become visible, but remain intertwined. (C) Zygotene: chromosomes start to pair. (D) Pachytene: the eight homologous chromosomes are paired over their entire length. The arrow indicates either the nucleolus or a vegetative nucleus. (E) After a transient diffuse stage, in which chromosomes can not be discerned, they reemerge in early diplotene and the eight bivalents compact further. (The two nuclei seen left of the chromosomes correspond to an adjacent binucleate cell). (F) Late diplotene. (G) Metaphase I: four bivalents are clearly separated from the mass made by the other four bivalents. (H) Late anaphase I with one late segregating chromosome. (I) Telophase II. (J) Telophase of postmeiotic mitosis: the ascus contains eight immature ascospores. Bar, 5 µm.


3A
B
C
D
E
F
G
H
I
J

Although the initiating event is unknown, it is assumed that the fruiting body is built around a specialized cell from which ascogeneous hyphae develop (Champe and Simon 1992). As in other ascomycetes, meiotic asci develop from dikaryotic croziers in *A. nidulans* (Elliot 1960). The two nuclei in a crozier undergo coordinated mitosis. After septa deposition on each side of the crook, a binucleate penultimate cell is formed, flanked by two uninucleate cells, the tip cell and the basal cell. The penultimate cell (Figure 3, A) enlarges to form the ascus; its two nuclei fuse (karyogamy) to give the diploid zygote, which immediately enters meiosis. During meiotic prophase, the diploid nucleus enlarges and the chromosomes progressively condense from leptotene to pachytene. A typical pachytene stage with fully paired homologous chromosomes (Figure 3, D) was however difficult to find, possibly due to the relatively short duration of this stage. As the chromosomes reemerge from the diffuse stage, the eight bivalents gradually compact further into their metaphase I configurations. After the first meiotic division, the two haploid nuclei form a characteristic dyad and after the second meiotic division, a tetrad is formed. The nuclei in the tetrad divide mitotically, which gives rise to eight nuclei, which separate into eight individual immature ascospores. Finally, each nucleus again divides mitotically such that each mature ascospore is binucleate. Although *bimD6* strains are completely self-sterile, under optimal conditions, fruiting bodies were formed in numbers and with a morphology that is comparable to the fruiting bodies of a wild type strain. However, when the contents of mutant fruiting bodies were compared to those of wild type fruiting bodies several defects became apparent.

First, in the mutant fruiting bodies, non-meiotic, sterile paraphysae outnumber the number of asci. The paraphysae have relatively little cytoplasm, small, dense nuclei and are very irregular in shape. In wild type fruiting bodies of comparable stages this situation is reversed: meiotic asci outnumber the number of sterile paraphysae. While wild type fruiting bodies contain thousands of asci, mutant fruiting bodies contained on average less than ten asci. This result strongly indicates that premeiotic stages do not occur normally in *bimD6*.

Moreover, those asci present were abnormal in several respects (Figure 4). Among 200 meiotic nuclei analyzed, 65 were in metaphase I, a stage that is rarely observed in wild type cells (probably due to its short duration). Most of these metaphase I configurations contained more than eight units (Figure 4, D). These units probably correspond to prematurely separated bivalents or to prematurely separated sister chromatids. Because of their small sizes (arrows in Figure 4, D) it seems most likely that some sister chromatids have separated precociously. Importantly however, sister chromatid separation in *bimD6* never seemed complete: in all mutant metaphase I configurations regularly sized bivalents were also

detected (Figure 4, D). Very few (five among 200 analyzed) meiotic nuclei progressed beyond metaphase I, even in older fruiting bodies, and ascospores were never detected. The cytology of the stages preceding metaphase I was abnormal in *bimD6*: in prophase nuclei (n=120), the chromatin consistently appeared less ordered and more diffuse than in wild type nuclei in comparable stages (compare Figure 4, A with Figure 3, B and C). In addition, a typical pachytene stage with orderly paired homologous chromosomes was never observed in the mutant (compare Figure 4, B with Figure 3, D).

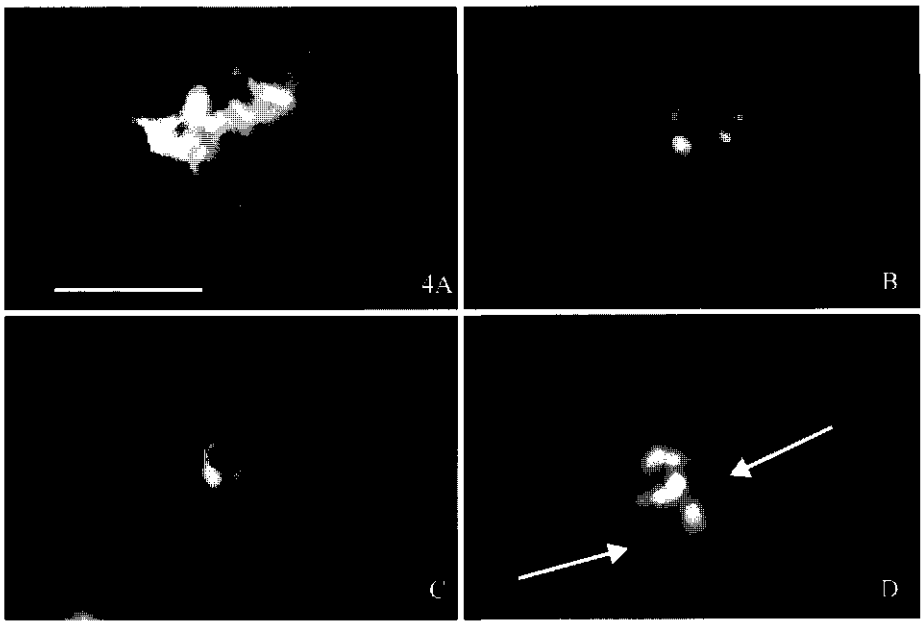


Figure 4: Progression of meiosis in *bimD6* mutant cells. Meiotic cells of *bimD6* stained with DAPI (LM). (A-B) Prophases: when compared to wild type (Figure 3, B-D) the chromatin appears more fuzzy, even at pachytene (B). (C) Diplotene. (D) Metaphase I: note that the number of units on the metaphase I plate is greater than eight and note the small sizes (arrows) of some of these units. Bar, 5 μm.

Localization of the BIMD protein during wild-type meiosis

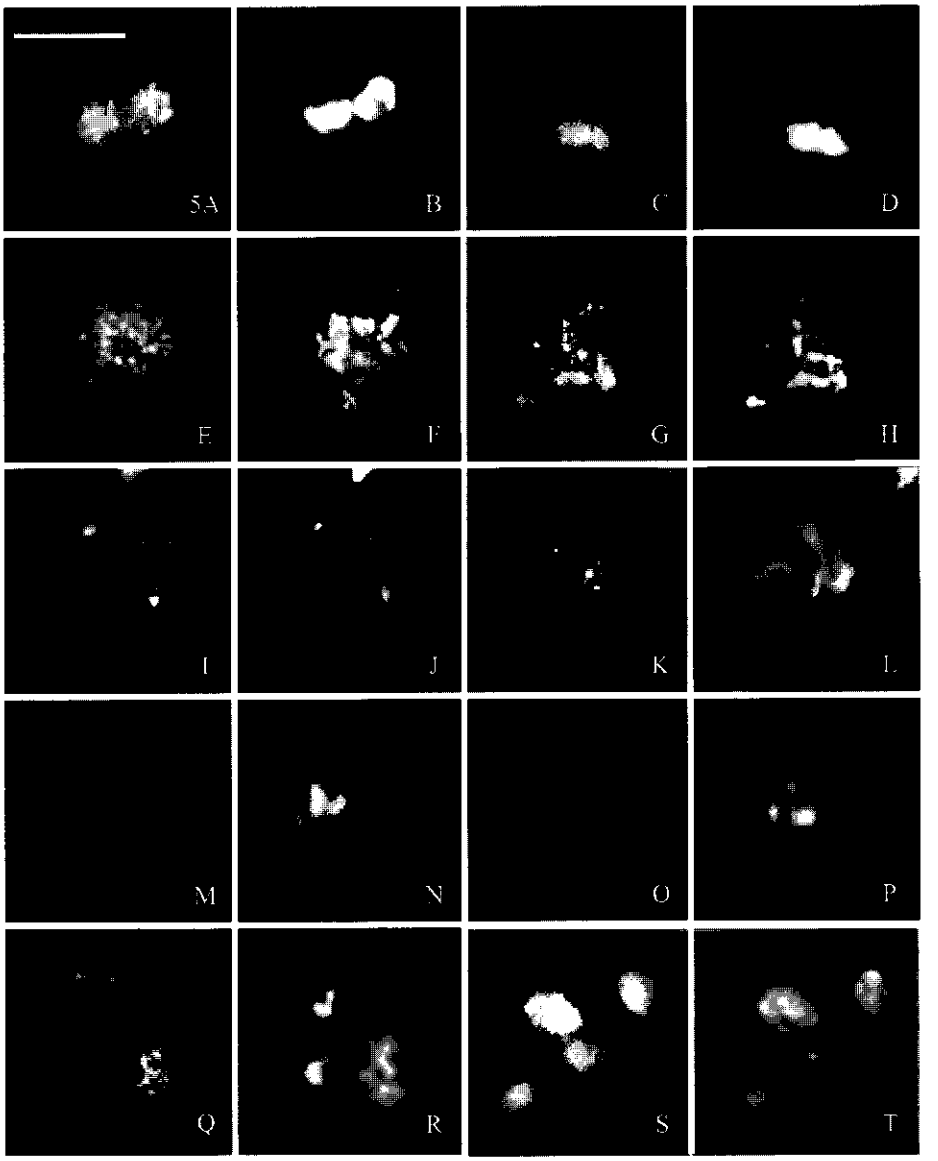
The cytological localization of the BIMD protein in wild-type meiosis was determined by two approaches: by immuno-localization with BIMD-specific antibodies, and by detection of a BIMD-GFP fusion protein. Although both approaches gave essentially similar results, we detected numerous, tiny foci with the antibody-staining, while GFP-staining seemed more continuous (compare in example Figure 5, E and G). These minor differences might be explained by differences in sensitivity between both detection techniques. By both approaches the cytological localization of BIMD was exclusively nuclear and revealed a similar staining pattern with respect to cell cycle stages (Figure 5).

BIMD was found to be chromosome-associated in all stages of meiotic prophase and was displaced from the chromosomes as they emerged from the diffuse stage into diplotene. BIMD could no longer be detected at late diplotene, metaphase I through anaphase I by either approach. BIMD staining was again visible on the chromosomes after they had moved to the poles. During the second meiotic division, BIMD was shed from the chromosomes at metaphase II. However, although BIMD was no longer localized on the condensed chromosomes, it was still detectable in the nucleus, mainly forming two polar caps (Figure 5, Q and R). Finally, BIMD reappeared on the chromosomes at telophase II. The chromosomal association of BIMD in meiosis thus exhibits the same temporal pattern as had been described for the localization of Spo76p (van Heemst *et al.* 1999). However, in contrast to the bright

---

Figure 5: Localization of the BIMD protein during the successive stages of wild type meiosis. BIMD was detected by affinity-purified antibodies against the C-terminus of the BIMD protein (serum 560) and by fusion of BIMD with GFP. (A-D) Binucleate penultimate cells before karyogamy. (A) Stained with serum 560; (C) GFP image; (B and D) corresponding DAPI images. (E-H) Early meiotic prophases (leptotene/zygotene). (E) Stained with serum 560; (G) GFP image; (F and H) corresponding DAPI images. (I-J) Pachytene nucleus with (I) GFP image and (J) DAPI image. (K-L) Diffuse stage-early diplotene nucleus stained with (K) serum 560 and (L) DAPI. (M-N) Late diplotene-metaphase I nucleus stained with (M) serum 560 and (N) DAPI. (O-P) Metaphase I nucleus with (O) GFP image and (P) DAPI. (Q-R) Late anaphase II nuclei stained with (Q) serum 560 and (R) DAPI. (S-T) Tetrad with (S) GFP image (note that, compared to Q, in resting nuclei, the entire nucleus is uniformly stained) and (T) DAPI. Bar, 5 μm.

axial localization of Spo76p in *S. macrospora* (van Heemst *et al.* 1999), the BIMD-staining of meiotic prophase chromosomes in *A. nidulans* was not as obviously brighter than the BIMD-staining in nuclei of other stages. Moreover, although the BIMD-staining was relatively intense in early prophase nuclei, BIMD-staining faded as the chromosomes compacted into pachytene (compare Figure 5, E and G with Figure 5, I). Compared to the staining observed



with DAPI, the BIMD-staining was always more fuzzy. It thus appeared that BIMD was not preferentially localized on the chromosome axes during meiotic prophase, suggesting differences between the localization of BIMD and Spo76p, even though temporal patterns are the same (van Heemst *et al.* 1999).

The S. macrospora SPO76 gene can complement the heat and MMS sensitivities of A. nidulans bimD6

To determine whether the *S. macropora SPO76* gene can complement defects conferred by *bimD6* in *A. nidulans*, we introduced the *SPO76* gene in *pyrG89; bimD6* double mutants by cotransformation with plasmids carrying the selectable marker pyr^+. As controls, we cotransformed the *bimD* gene with pyr^+ plasmids into the same strains, and we also transformed these strains with pyr^+ plasmids only (Table 2). We selected pyr^+ transformants at 42° and 30°, and after purification determined the percentage of stable transformants rescued for heat sensitivity.

Table 2: Frequencies of cotransformants in *pyrG89;bimD6* (ts) strains complemented by heterologous *SPO76* vs. homologous *bimD* DNA in addition to pyr^+ plasmids[a].

transforming plasmid	purified (pyr^+) transformants selected at:				
	42°	30° showing growth at			
	confirmed	42°	30° only	total	-> % complemented
SPO76	30/30	26	51	77	34 %
bimD	31/31	34	20	54	63 %
pyr^+ plasmids only	0/0	0	49	49	0 %

[a] All transformants were selected for pyr^+. Two pyr^+ plasmids used contained either the *A. nidulans pyrG* (pPyrG) or the *N. crassa pyr-4* (pRG3) gene.

Ts^+ transformants were obtained with the *SPO76* as well as the *bimD* gene, not only when selected at 42 °, but also as a considerable fraction among non-selected cases (30°). Moreover, all stable ts^+ transformants were fully complemented for MMS sensitivity. Subtle differences were however observed between the results obtained for *SPO76* and *bimD*.

The overall proportion of (non-purified) complemented transformants was lower for *SPO76* (data not shown). Furthermore, initially many ts^+ transformants with *SPO76* grew less vigorously than those with *bimD*. In early tests such transformants (about 20%) gave ambiguous results, and only the *bimD* types became stably complemented after further purification, while *SPO76* transformants reverted to ts^- and MMS sensitivity (while remaining pyr^+). Importantly however, these data show that the *S. macrospora SPO76* gene is able to complement two aspects of the mitotic phenotype of *bimD6* in *A. nidulans*: mitotic lethality at 42 ° and MMS sensitivity at 30 °.

SPO76 can not rescue the meiotic defects of bimD6 in A. nidulans, and bimD can not rescue the meiotic defects of spo76-1 in S. macrospora

To determine whether the *S. macropora SPO76* gene can complement the meiotic defects conferred by *bimD6* in *A. nidulans*, several of the ts^+ transformants (Table 2) were tested for rescue of the meiotic defect by selfing and/or intercrossses. While we did find fertile transformants among those obtained by transformation with *bimD*, we did not find any fertile transformants among those obtained by transformation with *SPO76* with either approach (Table 3).

For the reciprocal test, i.e. complementation of the meiotic defects of *spo76-1 in S. macrospora*, we introduced the *bimD* gene in the *spo76-1* mutant by cotransformation with a plasmid carrying the selectable marker *HygR*. As a positive control, we cotransformed the *SPO76* gene with the *HygR* gene into the same *spo76-1* strain. Among the hyg^r transformants obtained with *bimD*, none produced any sexual spores, while among the hyg^r transformants obtained with *SPO76*, about half produced viable ascospores (Table 3).

These data indicate that *SPO76* can not complement the meiotic defects conferred by *bimD6* in *A. nidulans*, and *vice versa*, that *bimD* can not complement the meiotic defects in the *S. macrospora spo76-1* strain.

Table 3: Tests for heterologous complementation of meiotic defects in transformants of *A. nidulans bimD6* and *S. macrospora spo76-1*.

	No. of Tfs tested	No. of fertile hybrid cleistothecia/ total analyzed	No. of selfed cleistothecia/ total analyzed
Tf in *bimD6* [a]			
SPO76 x *SPO76*	16	0 / 55	0 / 75
SPO76 x *bimD*	7	18 / 27	0 / 27
bimD x *SPO76*	6	17 / 36	16 / 36
bimD x *bimD*	6	14 / 24	5 / 24
Tf in *spo76-1*			
bimD	786	N.D.	0 / 786
SPO76	51	N.D.	25 / 51

[a] The number of *A. nidulans* transformants (Tfs) indicated were crossed in various combinations. In the crosses listed above, the first parent was able to self, whereas the second parent was not able to self due to self-sterility markers.

The bimD6 mutation confers sensitivity to X-rays

In the mitotic cell cycle, *A. nidulans bimD6* mutants show increased sensitivities to MMS and UV, but only in dividing cells (Denison *et al.* 1992). Similarly, *S. macrospora spo76-1* mutants show increased sensitivities to X-rays and UV (Huynh *et al.* 1986). To compare these phenotypes more precisely, we have tested *bimD6* for X-ray sensitivity. We found that germinating, but not quiescent conidia of *bimD6* are also more sensitive to X-rays than the *bimD* (+) control (data not shown). Thus, *bimD6* not only closely resembles *spo76-1* of *S. macrospora*, but also rec$^-$ mutants of *A. nidulans*, especially *uvsC114* (Jansen 1970b) and *uvsE182* (Fortuin 1971) which also are sensitive to radiation and MMS only when dividing cells are exposed (Chae and Kafer 1997; reviewed in Kafer and May 1998).

Considering the resemblance for mutagen sensitivities of *bimD6* to rec$^-$ mutants (above), it was of considerable interest to examine *bimD6* for defects in mitotic recombination. Since the spontaneous incidence of crossing over in *A. nidulans* is low, selective methods are used for detection (Pontecorvo and Kafer 1958). The most informative system that tests for allelic recombination in the *adE* gene was chosen (Pritchard 1955). Rare ad$^+$ recombinants were selected from diploids heterozygous for the two distinguishable alleles *adE8* and *adE20* (Table 1; Zhao and Kafer 1992). Because of the unusually close linkage to markers on chromosome I, the relative frequencies of gene conversion versus crossing over can be determined by genetic analysis of haploid segregants which reveal the arrangement of markers on the two homologues of chromosome I. As a "positive" control to *bimD6* diploids, corresponding tester diploids homozygous for the hyporec mutant *uvsC114* and a repair-proficient control were analyzed in parallel. As shown in Table 4, *bimD6* reduced the frequencies of ad$^+$ recombinants in *adE8/adE20* diploids to a similarly low level as found for *uvsC114* (Zhao and Kafer 1992).

However, even though the absolute frequencies of ad$^+$ recombinants are highly reduced in the two mutants, the relative frequencies of conversion versus crossover types resemble those found for wild type (Table 4).

Table 4: Absolute frequencies of selected ad$^+$ recombinants from *adE8/adE20* diploids homozygous for *bimD6* compared to repair-proficient and rec$^-$ (*uvsC114*) controls, and relative frequencies of conversion vs. crossing over as deduced from the genotypes.

	Control (+)	*bimD6*	*uvsC114*
Absolute frequencies of ad$^+$, x 10^6	11.43 +/- 1.33	1.22 +/- 0.21	1.20 +/- 0.35
Class[a] Convertants/Crossovers	**Relative frequencies of recombination types**		
I Single conversions[b]	80%	78%	82%
II Single crossovers	12%	15%	11%
III Two events: Conversion and/or CO	6%	5%	5%
IV Multiple events	2%	2%	2%
[Total of ad$^+$ recombinants analyzed]	[97]	[53]	[44]

[a] For classification of ad$^+$ recombinants, based on genotypes identified by haploidization, see Zhao and Kafer (1992). [b] *adE8->+* or *adE20 ->+*

Conversion in an interrupted duplication of benA

To characterize the effect on other types of recombination for *bimD6* as well as *uvsC114*, we used the *benA22/+* duplication system of Dunne and Oakley (1988; Materials and Methods). Heterozygous *benA22/+* duplication strains show intermediate resistance to benomyl (a spindle poison) and are quite stable, but only under selection for pyr^+. Because crossing-over and single strand annealing can eliminate the integrated plasmid on CM supplemented with uridine, we used selective conditions (CM without uridine supplement), on which only conversion can result in benomyl-resistant homozygous sectors. When replica plated to benomyl, the recombination-proficient control (+) gave many sectors (Figure 6, A), and *uvsC114* only very few (Figure 6, C), although their overall growth characteristics on benomyl plates were comparable.

In contrast, *bimD6* was less resistant to benomyl and grew very poorly (Figure 6, B). However, even though growth was less vigorous, *bimD6* benomyl-resistant sectors appeared with frequencies similar to those of the repair proficient control (Figure 6, B). Thus, while *uvsC114* reduces conversion between interrupted *benA22/+* repeats, *bimD6* has no influence on this type of mitotic recombination.

DISCUSSION

BIMD and mitotic sister chromatid cohesion

The elevated sensitivity of *bimD6* to benomyl at the permissive temperature (Figure 6, B), the chromosome segregation defects of *bimD6* at the restrictive temperature (Denison *et al.* 1992) and the genetic interaction between *bimD6* and *sudA/smc3* mutants (Holt and May 1996) imply *bimD* in mitotic sister chromatid cohesion. In addition, we demonstrate that BIMD and Spo76p are functional homologues with respect to their roles in mitotic chromosome metabolism: *SPO76* can complement both the mitotic arrest phenotype of *bimD6* at the restrictive temperature (Table 2) and the MMS sensitivity of *bimD6* at the permissive temperature. Interestingly, in *spo76-1*, mitotic sister chromatid cohesion and chromosome compaction are coordinately affected on a regional basis (van Heemst *et al.* 1999). One possible mode of action for proteins like BIMD/Spo76p is therefor mediating the coalescence of adjacent sister chromatid cohesion complexes and thus stimulating chromosomes to adapt an axial-loop structure. Thus, in contrast to components like

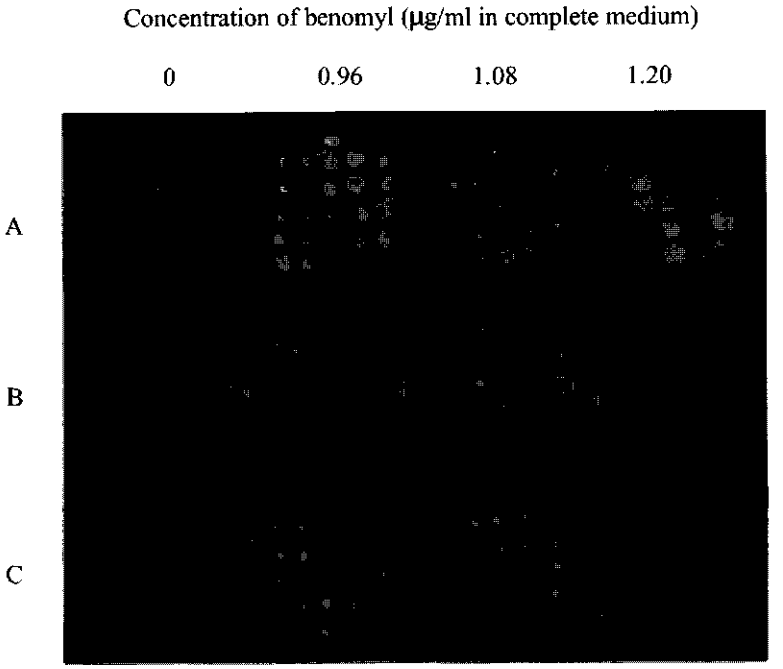


Figure 6: Reduced growth and/or recombination of rec⁻ *benA22/+* duplication strains and control. Three different strains were replicated to different concentrations of benomyl as indicated. Upper panel (A) Recombination proficient control (+). Middle panel (B) *bimD6*. Lower panel (C) *uvsC114*.

Scc1/Mcd1, which are thought to cross-link cohesion complexes located on the two sister chromatids (review in Nasmyth *et al.* 2000), BIMD might (directly or indirectly) stimulate cross-links between cohesion complexes (or sub-components thereof) along the length of sister chromatids. This could either be achieved directly by cross-linking proteins between cohesin complexes, or through stimulation of cooperative interactions between cohesin subunits. The supra-axial localization of Spo76p in meiotic prophase (when all chromosomes have adopted an axial-loop configuration) (van Heemst *et al.* 1999) is consistent with the latter possibility. In mitosis, BIMD and Spo76p could thus reinforce sister chromatid cohesion, which might be necessary for maintenance of cohesion during subsequent stages of higher order chromosome compaction. It is however to be expected that not all cohesion sites will be reinforced in this way, because the stabilization of too many connections between sister chromatids might hinder further chromosome compaction and chromosome disjunction.

According to this scenario, the amount and/or activity of BIMD must remain limiting and/or be strictly regulated. BIMD disappears from the chromosomes before the sister chromatids separate (Figure 1). Possibly, a subset of cohesin-complexes have than been made less vulnerable to (non-cleavage mediated) dissociation. The biochemical mechanism through which proteins as BIMD work remains elusive. A domain of a human homologue (AS3) is associated with kinase activity, but it is unclear which substrates are phosphorylated and whether the AS3 domain possesses the kinase activity itself (Geck *et al* 1999). It is also not yet known whether AS3 presents a true functional homologue of BIMD/Spo76p. Importantly however, one of the extragenic suppressor mutations of *bimD6* was located in a gene, *sudD*, encoding a protein with a high serine plus threonine content, which suggest that its function might be regulated through phosphorylation (Anaya *et al* 1998).

BIMD and recombinational repair

We found that BIMD plays a role in recombinational repair in addition to its role sister chromatid cohesion. Interestingly however, BIMD does not seem to be required for all types of recombinational repair. When assayed for allelic recombination, *bimD6* resembled *uvsC114*. In these two mutants, the absolute frequencies of allelic recombination were strongly reduced (Table 4). Also, in both *uvsC114* and *bimD6*, the relative frequencies of all classes of recombinants were comparable to those obtained in wild type (Table 4). This contrasts with results obtained with other mutants, such as *musL222*, in which case crossovers seemed less reduced than gene conversions (Zhao and Kafer 1992). However, when assaying intrachromosomal conversions, we found a difference between *bimD6* and *uvsC114*. Intrachromosomal conversions between interrupted duplicated sequences were significantly reduced in *uvsC114* (Figure 6, C). Because *uvsC* encodes a Rad51 homologue (van Heemst *et al.* 1997), the requirement for *uvsC* in this type of recombination suggests that it involves strand invasion/exchange on a homologous template. Interestingly, *bimD6* was not defective for this type of repair (Figure 6, B). Thus, *bimD* and *uvsC* might not participate in a similar step during repair, a conclusion supported by tests for epistatic relationships (E.K., unpublished results). Our results obtained with two different recombination assays, and the observed sensitivity of *bimD6* to various DNA damaging agents (Denison *et al.* 1992), suggest that BIMD is required for homologous recombination in case the template is located on another (sister or non-sister) chromatid, but not when the template is located on the same sister chromatid (loop). Several other mutants that are detective in some aspect of sister

chromatid cohesion are also radiation sensitive at the permissive temperature and/or defective in DNA double strand break (DSB) repair (reviewed by van Heemst and Heyting 2000). Efficient recombinational repair apparently requires sister chromatid cohesion. Possibly, scaffold-associated cohesin complexes serve as nucleation sites for DNA-repair complexes, and assist these complexes in homology search on the corresponding segment of the sister chromatid (van Heemst and Heyting 2000). They would thus play an important role in maintenance of genome stability (van Heemst and Heyting 2000). In the tandem array of rDNA repeats of budding yeast all repeats have a single cohesin-binding site and most sites are occupied (reviewed by Koshland and Guacci 2000). Possibly, sister chromatid cohesion proteins are required to keep repeats in register and to avoid unequal sister chromatid exchange (van Heemst and Heyting 2000). Our results show that BIMD is required for homologous recombination when the template is located on another (sister or non-sister) chromatid, but not when the template is available in close proximity on the same sister chromatid (loop). A similar result was obtained for the *rad21-45* mutant of *S. pombe*: although the *rad21-45* mutant was highly sensitive to DNA damaging agents (Subramani 1991), the mutation had no effect on recombination between interrupted direct repeats (Fortuno *et al* 1996). Rad21 of *S. pombe* is homologous to cohesin Mcd1/Scc1 of *S. cerevisiae* (Guacci *et al.* 1997; Michaelis *et al.* 1997). As cohesion complexes are located at the basis of the loops (Blat and Kleckner 1999), it is tempting to speculate that the repair machinery is obliged to cooperate with these guardians in its search for a homologous template outside an intrachromatid loop domain.

BIMD and meiosis

Although BIMD and SPO76p play functionally analogous roles in mitosis (Table 2), their meiotic roles probably differ in a species-specific manner (Table 3). We also found some important differences between the meiotic localization of the two proteins. In *S. macrospora*, the Spo76p is most abundant during meiotic prophase and preferentially localizes in the vicinity of the chromosome axes during all stages of meiotic prophase (van Heemst *et al.* 1999). In *A. nidulans*, the BIMD protein is only slightly more abundant in meiotic prophase than in the mitotic cycle, and it was not localized preferentially close to the chromosome axes during the pairing of homologous chromosomes (Figure 5). Instead, the meiotic localization of BIMD resembled the mitotic localization of the protein, both in the intensity of staining and in the diffuse distribution of BIMD on the chromatin as detected by DAPI. The

differences between the meiotic localization of Spo76p and BIMD can best be explained by a difference in meiotic chromosome structure. In contrast to *S. macrospora* (Zickler 1977), *A. nidulans* is claimed not to form SCs (Egel-Mitani *et al.* 1982) and to lack positive crossover interference (Egel 1995). It was proposed that crossover interference involves the imposition and relief of stress along the chromosome axes (Kleckner 1996). Based on this proposition, and on both the mutant phenotypes conferred by *spo76-1* and the Spo76p localization during meiotic prophase, it was argued that Spo76p might be important as a transducer of the disruptive forces that are applied during crossover interference (van Heemst *et al.* 1999). If this proposition is true and if BIMD and Spo76p are indeed functional homologues, one might expect that such a stress-transducing protein is more abundant in an organism that exhibits crossover interference than in an organism in which interference is absent. Our results concerning the localization of both Spo76p (van Heemst *et al.* 1999) and BIMD (Figure 5) are in full agreement with such a hypothesis. Furthermore, the extent of meiotic sister chromatid separation seems less severe in *bimD6*, where variable amounts of univalents were observed at metaphase I (Figure 5, D), than in *spo76-1*, where all sister chromatids were found to be fully separated prior to metaphase I (Moreau *et al.* 1985; van Heemst *et al.* 1999). Possibly, the tendency for sister chromatid separation is greater in an organism that exhibits crossover interference than in an organism in which this process is absent. It is however also possible that these differences are caused by the different nature of the *bimD6* and *spo76-1* mutations. The differences in meiotic chromosome structure between the two fungi might furthermore explain why the *SPO76* gene could not complement the meiotic phenotype of *bimD6* in *A. nidulans* and likewise, why the *bimD* gene could not complement the meiotic phenotype of *spo76-1* in *S. macrospora* (Table 3).

ACKNOWLEDGMENTS

We thank Françoise James for expert technical assistance. We are grateful for the donation of strains and plamids given by Greg May and Berl Oakley. This work was financially supported by the Netherlands Organization for Scientific Research (NWO), grant 805 18 262, by the EEC contract CHRXCT 94 0511 (to C.H. and D.Z.), by a research grant from the Natural Science and Engineering Research Council of Canada (to E.K.), and by the Centre National de la Recherche Scientifique, contract UMR 8621 (to D.Z.).

Chapter 4

Cloning, sequencing, disruption and phenotypic analysis of *uvsC*, an *Aspergillus nidulans* homologue of yeast *RAD51*

D. van Heemst, K. Swart, E. F. Holub, R. van Dijk, H. H. Offenberg, T. Goosen, H. W. J. van den Broek and C. Heyting

Department of Genetics, Wageningen University, 6703 HA Wageningen, The Netherlands

Published in Molecular and General Genetics (MGG) 254: 654-664 (1997)

Mol Gen Genet (1997) 254:654–664

ORIGINAL PAPER

D. van Heemst · K. Swart · E. F. Holub
R. van Dijk · H. H. Offenberg · T. Goosen
H. W. J. van den Broek · C. Heyting

Cloning, sequencing, disruption and phenotypic analysis of *uvsC*, an *Aspergillus nidulans* homologue of yeast *RAD51*

Received: 23 September 1996 / Accepted: 2 December 1996

Abstract We have cloned the *uvsC* gene of *Aspergillus nidulans* by complementation of the *A. nidulans uvsC114* mutant. The predicted protein UVSC shows 67.4% sequence identity to the *Saccharomyces cerevisiae* Rad51 protein and 27.4% sequence identity to the *Escherichia coli* RecA protein. Transcription of *uvsC* is induced by methyl-methane sulphonate (MMS), as is transcription of *RAD51* of yeast. Similar levels of *uvsC* transcription were observed after MMS induction in a $uvsC^+$ strain and the *uvsC114* mutant. The coding sequence of the *uvsC114* allele has a deletion of 6 bp, which results in deletion of two amino acids and replacement of one amino acid in the translation product. In order to gain more insight into the biological function of the *uvsC* gene, a *uvsC* null mutant was constructed, in which the entire *uvsC* coding sequence was replaced by a selectable marker gene. Meiotic and mitotic phenotypes of a $uvsC^+$ strain, the *uvsC114* mutant and the *uvsC* null mutant were compared. The *uvsC* null mutant was more sensitive to both UV and MMS than the *uvsC114* mutant. The *uvsC114* mutant arrested in meiotic prophase-I. The *uvsC* null mutant arrested at an earlier stage, before the onset of meiosis. One possible interpretation of these meiotic phenotypes is that the *A. nidulans* homologue of Rad51 of yeast has a role both in the specialized processes preceding meiosis and in meiotic prophase I.

Key words Meiotic mutants · Recombination repair · *RAD51* · *uvsC* · Aspergillus nidulans

Communicated by C. A. M. J. J. van den Hondel

D. van Heemst · K. Swart · E. F. Holub · R. van Dijk
H. H. Offenberg · T. Goosen · H. W. J. van den Broek (✉)
C. Heyting
Department of Genetics, Agricultural University Wageningen,
Dreyenlaan 2, 6703 HA Wageningen, The Netherlands

Introduction

The sexual life cycle of most eukaryotes is characterized by an alternation of haploid and diploid phases. The transition from the diploid to the haploid phase is accomplished at meiosis, when a single S phase is followed by two successive nuclear divisions. During meiosis I, the homologous chromosomes recombine, pair and disjoin. During meiosis II, the sister chromatids of each chromosome are separated. In most organisms, the chromatin rearrangements that take place during prophase I are accompanied by the assembly and disassembly of synaptonemal complexes or SCs (Moses 1966). *Aspergillus nidulans* (Egel-Mitani et al. 1982) and *Schizosaccharomyces pombe* (Olson et al. 1978) are exceptional in that they fail to assemble detectable SCs during prophase I. Interestingly, these organisms are also known to lack cross-over interference (Snow 1979; Strickland 1958). We are interested in a detailed comparison of meiosis in *A. nidulans* with meiosis in other organisms that do assemble SCs. For this purpose we have started to clone genes that are involved in meiosis in *A. nidulans*. As a first step, we isolated a set of mutants of *A. nidulans* that are specifically defective in the production of sexual spores (unpublished results, manuscript in preparation). Furthermore, we have started to analyze previously isolated mutants that are defective in both mitotic DNA repair and ascospore formation. The *uvsC114* mutant (Jansen 1970a) is an example of such a mutant. Resting conidia of the *A. nidulans uvsC114* mutant exhibit the same UV sensitivity as $uvsC^+$ strains, but the UV sensitivity of conidia of the *uvsC114* mutant strongly increases during germination, whereas the UV sensitivity of $uvsC^+$ conidia fluctuates only slightly during germination (Jansen 1970a). The *uvsC114* mutant is highly sensitive to the DNA alkylating agent methyl methane sulphonate (MMS) (Kafer and Mayor 1986). The *uvsC114* mutation enhances the frequency of spontaneous mutation in chromosomal genes significantly (Jansen 1972; Chae and

Kafer 1997, following paper) but practically abolishes UV-induced mutation (Kafer and Mayor 1986; Chae and Kafer 1997). The frequency of spontaneous mitotic recombination in *uvsC114* diploids is abnormally low and sexual crosses between *uvsC114* haploids are sterile (Jansen 1970b). In this article we describe the cloning, sequence and gene disruption of *uvsC*, and we identify the mutation site in the *uvsC114* allele. The predicted protein encoded by the *uvsC* gene turns out to be homologous to Rad51 of yeast. We found that the phenotype of the *uvsC* null mutant was more extreme than that of the *uvsC114* mutant, both in mitotic and in meiotic cells.

Materials and methods

Strains and media

The genotypes of the *A. nidulans* strains used in these studies are listed in Table 1. All strains were derived from Glasgow stocks (Clutterbuck 1986). The media and techniques for growing and handling *A. nidulans* have been described by Pontecorvo et al. (1953). *E. coli* DH5α was used as a host in plasmid propagation.

Genomic library

A wild-type genomic library (Brody et al. 1991), constructed in the cosmid vector pWE15 (Evans and Wahl 1987) was obtained from the Fungal Genetic Stock Center (University of Kansas Medical Center, Kansas City, Kan., USA).

Transformation of *A. nidulans*

Protoplasts were prepared from mycelium as described in Coenen et al. (1996). For transformation experiments, 2×10^7 protoplasts in 200 µl STC (10 mM TRIS-HCl pH 7.5, 1.0 M sorbitol, 50 mM $CaCl_2$) were incubated for 20 min on ice with up to 20 µg of DNA (in maximally 20 µl) and 50 µl PEG solution (25% polyethyleneglycol 6000, 10 mM TRIS-HCl pH7.5, 50 mM $CaCl_2$). After addition of 2 ml PEG solution, the mixture was incubated at room temperature for 5 min and diluted by addition of 4 ml STC. Aliquots of 1 ml were diluted in 5 ml of melted (43° C) selective medium containing 1.5% agar, stabilized with 1 M sucrose and plated onto plates of the same composition. For the selection of MMS-resistant transformants, the selective medium contained 0.01% MMS.

Table 1 *Aspergillus nidulans* strains used

Strain	Genotype
WG352	*yA2 pabaA1; amdS320 amdI18; uvsC114*
WG518	*yA2; argB2; uvsC114*
WG519	*yA2; argB2*
WG505	*yA2 pyrG89*
WG044	*ornB7 fwnA1 VeA*$^+$
WG518TF1[a]	WG518 with a single-copy integration of pGW1446 at the *argB* locus
WG505Δ1[a]	WG505 with a single-copy replacement of the 1.3 kb *uvsC* coding region by the 3.7 kb *Bam*HI fragment of pAOPYRG-Not; *uvsC* and *pyrG* promoters in same orientation
WG505Δ2[a]	WG505 with a single-copy replacement of the 1.3 kb *uvsC* coding region by the 3.7 kb *Bam*HI fragment of pAOPYRG-Not; *uvsC* and *pyrG* promoters in opposite orientations

[a] for details of the construction of these strains, see Materials and methods

For an explanation of symbols, see Clutterbuck (1993)

Isolation of DNA and RNA

Small-scale DNA isolations from fungal mycelium for Southern hybridization and PCR were carried out as described previously (Thijs et al. 1995). For RNA isolations 1–2 g of frozen, ground mycelium was homogenized in 33 ml of denaturation buffer (42 mM sodium citrate pH 4.0, 0.83% N-lauryl sarcosinate, 0.2 mM β-mercaptoethanol). After addition of 1.2 ml 2 M sodium acetate (pH 4.0), the lysate was mixed with 12 ml phenol:chloroform:isoamylalcohol (25:24:1), incubated on ice for 15 min, and centrifuged for 20 min at 10 000 × *g* (4° C). RNA was precipitated by mixing the aquous phase with an equal volume of isopropanol, incubation at −20° C for 30 min and centrifugation for 15 min at 10 000 × *g* (4° C). The pellet was resuspended in 5 ml denaturation buffer and precipitated with isopropanol as before. After washing with (at least 10 ml) 75% ethanol, the pellet was dried and resuspended in 3–5 ml diethyl pyrocarbonate (DEPC)-treated deionized water. After precipitation with isopropanol as before, the pellet was resuspended in 3 ml 1 mM EDTA, and again precipitated with isopropanol. The final pellet was washed with 75% ethanol, dried and resuspended in DEPC-treated water. Poly(A$^+$) RNA was isolated from total RNA using the PolyATract mRNA isolation system IV (Promega), according to the recommendations of the manufacturer.

(Sub)cloning and sequencing

The cosmid that complements the *uvsC114* mutation, 3D11, was subcloned as follows. A 5 kb *Kpn*I-*Sma*I fragment was cloned in both orientations in the filled-in *Not*I site of pBluescript KS$^-$. This resulted in plasmids pGW1418 and pGW1419. For the generation of overlapping deletion subclones derived from pGW1418 and pGW1419, the Erase-a-Base system (Promega) was used, according to the recommendations of the manufacturer.

A 5′ RACE (Frohman et al., 1988) experiment was performed to confirm the positions of introns in the genomic sequence of the *uvsC*$^+$ allele. For the synthesis of *uvsC*-specific cDNA, the oligonucleotide (5′-TGTCGAGTTGATAGTTCACCG-3′), Superscript reverse transcriptase, and poly(A$^+$) RNA isolated from strain WG505 were used. The cDNA was tailed with dCTP by means of terminal transferase, and amplified by Vent polymerase by means of PCR with a (2.5:1:2.5) mixture of the oligonucleotides (5′-GATTGAGCAGTTGCAGTTGG-3′, a nested *uvsC* specific primer): (5′-AAATGGATCCTTCTAGATGGGGGGGGGGGG-GGGG-GGG-3′, an anchor primer, which can anneal to the homopolymer tail): (5′-AAATGGATCCTTCTAGATGC-3′, an adaptor primer). The final 800 bp PCR product was cloned and sequenced.

We obtained the coding sequences of the *uvsC114* allele by means of PCR using genomic DNA of strain WG352 as a template, Vent polymerase, and two oligonucleotides which just flank the coding sequence, namely (5′-CGCGTCTTTGTTGAAGTG-3′) and (5′-GTCGGAAACAGCATTGTG-3′). The resulting 1.3 kb PCR fragment was cloned and sequenced.

We determined the sequences of both strands using a Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and an automatic DNA sequencer (Applied Biosystems Model 373). The sequence was assembled by means of the Winconsin GCG sequence analysis package. Sequence similarity searches in protein data bases were performed by means of the FASTA-X program (Altschul et al. 1990). The translated sequences were aligned with other RecA-homologous proteins by means of PILEUP from the GCG suite of programs.

uvsC gene targeting and gene disruption

pGW1446, the plasmid used for gene targeting, was derived from pGW1409, which consists of a 3.6 kb *Kpn*I-*Not*I fragment of cosmid 3D11 in pBluescript KS$^-$. pGW1446 was constructed by cloning the *argB112* allele, located on a 3.2 kb *Xba*I fragment, in the filled-in *Not*I site of pGW1409. To obtain the *argB112* mutation, a subclone containing a 3.2 kb *Xba*I fragment, originally derived from plasmid pBB116 (Berse 1981), was digested with *Bgl*II, treated with Klenow polymerase, and religated. pGW1446 was used to transform the *A. nidulans* recipient WG518 and a transformant containing a single-copy integration at the *argB* locus was identified by Southern hybridization (strain WG518TF1).

To obtain a construct for gene disruption, pGW1409 was digested with *Hind*III and vector and *uvsC*-flanking sequences were amplified by PCR. For (inverse) PCR two oligonucleotides which just flank the coding sequences were used, namely (5′-TGAAAGAGCACAATGCTG-3′) and (5′-TTTGATCAGGTACTGGAGG-3′). The PCR product was religated in the presence of a *Bam*HI linker (5′-CGGGATCCCG-3′) yielding pGW1415. A 3.7 kb *Bam*HI fragment, containing the *A. oryzae pyrG* gene, was isolated from pAOPYRG-Not and cloned into the *Bam*HI site of pGW1415. This resulted in plasmids pGW1416 and pGW1417. A 5.5 kb *EcoR*I fragment containing only the *pyrG* gene of *A. oryzae* and *uvsC*-flanking sequences was derived from pGW1416 and pGW1417 and used to transform the *A. nidulans* recipient WG505. Transformants containing a single-copy disruption with the *pyrG* and *uvsC* promoters in the same (type 1, WG505Δ1 is a typical example) and in opposite orientation (type 2, WG505Δ2 is a typical example) were identified by Southern hybridization.

Northern blot analysis

For Northern hybridization, 10 μg of total RNA was electrophoresed in 1.2% agarose formaldehyde gels as described in Sambrook et al. (1989), and transferred onto a Hybond N$^+$ membrane (Amersham) by capillary blotting. Hybridizations were carried out at 65° C for 16 h in a solution of 0.5 M sodium phosphate buffer pH 7.2, 7% SDS, 1 mM EDTA. A 0.6 kb *Sph*I fragment was used as an *uvsC*-specific probe, and a 1.2 kb *Bam*HI-*Eco*RI fragment was used as a glyceraldehyde-3-phosphate dehydrogenase (*gpdA*)-specific probe (Punt et al. 1988).

UV irradiation and MMS treatment

UV survival was determined as described by Fortuin (1970), with minor modifications: spores were plated (in triplicate) on CM in three concentrations (between 10^6 and 10^2 spores/plate). After preincubation for 6 h at 37° C, the plates were UV irradiated (18 erg/mm^2/s) for various periods of time (up to 90 s). After incubation for two to three days at 37° C, the colonies on plates with surviving colony numbers between 50 and 100, were counted.

MMS survival curves were determined essentially as described by Kafer and Mayor (1986).

Cytology

The cytological analyses were performed with cleistothecia in different stages of development (varying from slightly translucent to purple). For analysis of the *uvsC* null mutant, cleistothecia were harvested after growth on medium with (10 mM) uridine, and on medium without uridine. Iron-haematoxylin staining of cytological preparations of *A. nidulans* cleistothecia was performed as described in Zickler (1977) and Lu and Galeazzi (1978): isolated cleistothecia were fixed in freshly prepared Lu's (1996) fixative (ethanol: propionic acid: 10% aqueous chromic acid (9:6:2)) for 24 h at 25° C. Fixed cleistothecia were stored at −20° C. The fixative was pipetted off and the cells were hydrolyzed in 1 M HCl by heating quickly to 70° C and then slowly to 72–74° C. The total hydrolysis time was 8 min. Hydrolysis was stopped by placing the cells on ice. A few drops of the hydrolysis mixture were pipetted on a microscopic slide and excess HCl was carefully removed by means of filter paper. A drop of ferric citrate (saturated solution in 50% propionic acid) was added and the cleistothecia were crushed in this solution with a glass rod. Two or three drops of haematoxylin (2% solution in 50% propionic acid) were added and quickly mixed with the cells by means of the glass rod. A cover slip was placed on top of the slide and the cells were squashed by pressing the cover slip. The cytological preparations were examined by light microscopy.

Nucleotide sequence accession number

The nucleotide sequence presented in this paper will appear in the EMBL Nucleotide Sequence Database under accession number Z80341.

Results

Isolation of cosmid clones that complement the *uvsC114* mutation

A *uvsC114* strain, WG352, was transformed with an *Aspergillus* wild-type genomic cosmid library, by means of a three-step sib selection procedure. The first set of transformations was performed with 31 DNA pools, each consisting of 96 cosmids. The 96 cosmids of each of the two pools that could complement the *uvsC114* mutation with respect to growth on medium containing 0.01% MMS, were in the second step subdivided in 12 pools, which each consisted of 8 cosmids. In the third step, in which individual cosmids were used for transformation, two *uvsC114*-complementing cosmids were identified (3D11 and 14F12). Upon transformation with restriction fragments derived from these cosmids, the complementing activity was assigned to a 3.6 kb *Not*I-*Kpn*I fragment which was present in both these cosmids. Plasmid pGW1446 contains this 3.6 kb *Not*I-*Kpn*I fragment together with the *argB112* allele. An *argB2*, *uvsC114* strain, WG518, was transformed with pGW1446, and among the ArgB transformants, a transformant with a single-copy integration of pGW1446 at the *argB* locus (WG518TF1) was identified by Southern hybridization. WG518TF1 was tested for UV and MMS sensitivity (Fig. 1). WG518TF1 was as resistant to UV as *uvsC*$^+$, but showed survival rates on MMS intermediate between those observed for the *uvsC*$^+$ strain and the *uvsC114* mutant. In contrast to the *uvsC114* mutant, WG518TF1 formed viable ascospores.

Sequence analysis of the *uvsC* gene and the *uvsC114* mutation

Deletion subclones of pWG1418 and pGW1419, covering almost the complete 3.6 kb *Not*I-*Kpn*I fragment from the cosmid clone 3D11, were sequenced. Potential introns were found by comparing the sequence with consensus sequences for introns in *A. nidulans* (Rambosek and Leach 1987). To confirm their positions, a 5′ RACE

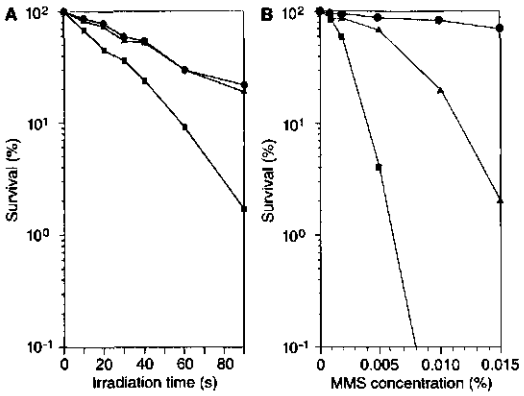


Fig. 1A–B UV and MMS survival curves of wild-type and mutant *A. nidulans* strains. A Germinating (6 h) conidiospores were irradiated for various periods of time with UV (18 erg/mm^2/s). **B** Conidiospores were plated on media containing various concentrations of MMS. ●– WG519 (*uvsC*$^+$); ■– WG518 (*uvsC114*); ▲– WG518TF1 (WG518 transformed with pGW1446)

experiment was performed (see Materials and methods). The sequence of the PCR generated-cDNA was identical to the genomic sequence from −216 to +797, except that it lacked the predicted introns. The *uvsC* gene (Fig. 2) has an open reading frame (ORF) of 1044 bp, interrupted by three introns of 52 bp, 62 bp and 64 bp, respectively. A transcript of approximately 1.4 kb was detected by Northern blot analysis in RNA from both the *uvsC*$^+$ strain and the *uvsC114* mutant (see below). In order to identify the mutation site and to confirm that the cloned gene is truly *uvsC*, a 1.3 kb fragment containing the coding sequence of the *uvsC114* allele was cloned and sequenced (see Materials and methods). Compared to the *uvsC*$^+$ allele, the coding sequence of the *uvsC114* lacked 6 bp. As a consequence, the predicted UVSC114 protein has a two-amino acid deletion (L^{76} and V^{77}) and a one-amino acid replacement (E^{78} changed to Q) compared to the predicted UVSC protein (see Fig. 3). The predicted UVSC protein shares a high sequence identity with all known Rad51-homologous proteins, with a higher sequence identity with Rad51 (61%) than with Dmc1 (47%) of yeast (see Fig. 3), and a moderate degree of sequence identity to the bacterial strand exchange protein RecA (27.4%). The region of identity with RecA is confined to the so-called homologous core (Ogawa et al. 1993a).

Expression of the *uvsC* gene in MMS-treated cells

The level of transcription of *uvsC* after treatment with MMS was analyzed by Northern hybridization. As shown in Fig. 4, *uvsC* transcripts were detectable in untreated cells of both the *uvsC*$^+$ strain (WG505) and the *uvsC114* mutant (WG352), but the level of *uvsC* transcript was higher in untreated cells of WG352 than in untreated cells of WG505. After treatment with MMS, the level of *uvsC* transcript increased strongly and to similar levels in both WG505 and WG352 cells.

UV and MMS sensitivity of the *uvsC* null mutant

Two sets of ten MMS-sensitive transformants, were obtained after transformation of recipient WG505, with each of the plasmids pGW1446 and pGW1447. After Southern analysis, we identified, respectively, 4/10 and 7/10 single-copy *uvsC* null mutants with the *uvsC* and *pyrG* promoters in the same (type 1) and in opposite orientation (type 2). The phenotypes within the groups were identical, whereas between the groups significant differences were apparent: mitotic and meiotic development was seemingly normal in type 1 transformants (except for the production of mature ascospores, see next section), whereas type 2 transformants exhibited retarded mitotic growth and formed small cleistothecia. One typical representative of type 1, called WG505Δ1, was used for a detailed characterization of the *uvsC* null phenotype (see also Discussion). Figure 5 shows that disruption of *uvsC* causes a higher sensitivity to UV and MMS than the *uvsC114* mutation.

Meiotic phenotype of the *uvsC* mutants

Cleistothecia of a *uvsC*$^+$ strain, the *uvsC114* mutant and the *uvsC* null mutant were dissected and analyzed cytologically. In wild-type *A. nidulans*, asci arise in developing cleistothecia from so-called croziers (Elliot 1958). Primary crozier formation starts with a last conjugate mitosis of the two nuclei in the terminal cell of an ascogenous hypha. Septum formation generates a uninucleate tip cell, a binucleate penultimate cell and a uninucleate basal cell (Fig. 6). The penultimate cell then enlarges to form the ascus. The two nuclei in the penultimate cell fuse (karyogamy) and immediately thereafter, the diploid zygotic nucleus enters meiosis. Successive croziers can arise from binucleate cells that originate from fusion of basal cells with tip cells; it is possible that they can also arise from other binucleate cells (Raju 1980). As a consequence, meiosis is normally asynchronous in *A. nidulans* cleistothecia, and a single wild-type cleistothecium will contain meiotic asci in various stages of meiosis (Fig. 6). In cleistothecia of the *uvsC114* mutant however, the asci arrested at meiotic prophase I (Fig. 7). In contrast, the *uvsC* null mutant was blocked before the onset of meiosis, because we very rarely (three cells out of ten cleistothecia) found cells that might correspond to meiotic (prophase) cells (Fig. 8B). Young cleistothecia of the *uvsC* null mutant contained some croziers (Fig. 8A) like those found in wild-type cleistothecia, but occasionally we also observed abnormal croziers with four nuclei in the penultimate cell (Fig. 8C). Older cleistothecia of the *uvsC* null

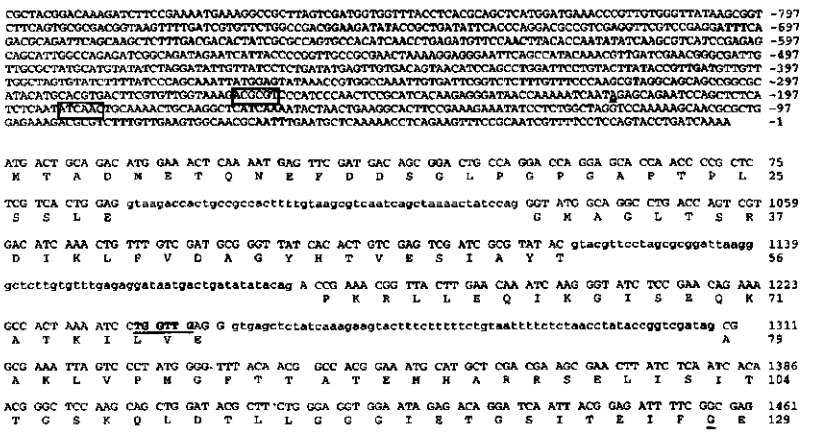


Fig. 2 Sequence of the *uvsC* locus. The predicted amino acid sequence is shown *below* the nucleotide sequence. Two putative *MluI* cell cycle sequences are *boxed*. The three introns are shown in *lower case letters*. The putative transcription start point is *double underlined*. The 6 bp deleted in *uvsC114* are represented in *underlined bold characters*. A- and B- type nucleotide-binding sequences (Walker et al., 1982) are *underlined*

mutant contained many large multinucleate cells (Fig. 8D). No differences were found between the cleistothecia of the *uvsC* null mutant after growth on medium with (10 mM) and without uridine.

Discussion

The *uvsC* gene of *A. nidulans* is homologous to *RAD51* of yeast

This paper describes the isolation and characterization of the *uvsC* gene of *A. nidulans* by complementation of the MMS sensitivity of the *uvsC114* mutant with a wild-type genomic cosmid library. Cloning of the *uvsC* gene was confirmed by identification of a mutation within the *uvsC114* allele. The predicted sequence of the UVSC protein shows moderate sequence identity to the bacterial strand exchange protein RecA and greater sequence identity to two RecA homologues of *S. cerevisiae*: Rad51 (Shinohara et al. 1992, Aboussekhra et al. 1992, Basile et al. 1992) and Dmc1 (Bishop et al. 1992). The strongest sequence identity was found with Rad51-homologous proteins of yeast and various other organisms (for summary see Stassen et al. 1996) and we conclude therefore that the *A. nidulans uvsC* gene is homologous to *RAD51* of yeast. The predicted UVSC protein shares the two major conserved domains, core domains I and II (Ogawa et al. 1993a), with the predicted Rad51 proteins from various other organisms. The UVSC114 mutation is located in core domain I. In both Rad51 and RecA the region corresponding to the site of the UVSC114 mutation is thought to form a flexible structure which protrudes from the body of the protein and which is

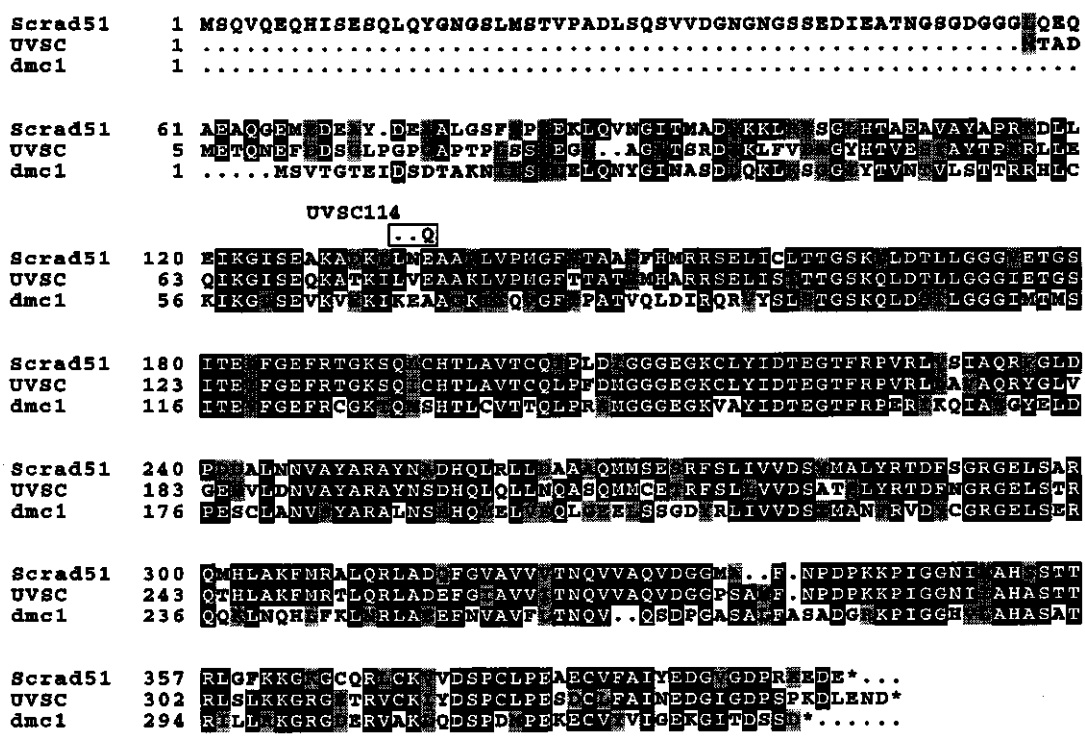


Fig. 3 Comparison of the amino acid sequences of *A. nidulans* UVSC, *S. cerevisiae* Rad51 and *S. cerevisiae* Dmc1. The amino acid sequences were aligned using the program PILEUP. Identical amino acid residues are indicated as *black boxes*, similar residues are indicated as *shaded boxes*. The aminoacid changes in UVSC114 are depicted in an *open box*

involved in protein-protein interactions (Story et al. 1992, 1993; Ogawa et al. 1993b).

Transcription of the *uvsC* gene

Transcription of the *uvsC* gene is inducible by MMS, as is transcription of the *RAD51*-homologous genes of various other organisms (Shinohara et al. 1992, Basile et al. 1992; Jang et al. 1996; Hatakeyama et al. 1995). In the absence of DNA-damaging agents, the *uvsC* gene appears to be expressed constitutively at a low level, whereas transcription increases considerably after treatment with MMS (Fig. 4). *Mlu*I cell cycle boxes are found in the upstream regions of *RAD51* of *S. cerevisiae* (Basile et al. 1992) and *rph51*$^+$ of *Schizosaccharomyces pombe* (Jang et al. 1996), but not in the upstream region of *mei-3* of *Neurospora crassa* (Hatakeyama et al. 1995). In yeast, *Mlu*I boxes are associated with cell cycle-regulated expression of genes (Pizzagalli et al. 1988, Gordon and Campbell 1991). The upstream region of the *uvsC* gene contains two putative *Mlu*I cell cycle boxes, one of which however is located downstream of the transcription start as determined by the 5′RACE experiment (Fig. 2), and is therefore not likely to be functional. However, none of the direct repeat sequences or damage responsive elements (DREs) which are present in the upstream region of *RAD51* of *S. cerevisiae* and *rph51*$^+$ *of S. pombe*, are obvious in the upstream region of the *uvsC* gene of *A. nidulans* and the *mei-3* gene of *N. crassa*. Mutation analyses of the *uvsC* upstream sequences will be required to identify the sequences necessary for induction of *uvsC* gene expression by MMS. In untreated cells of the *uvsC114* mutant, we found a higher level of *uvsC* transcription than in untreated cells of the *uvsC*$^+$ strain. One explanation for this result is that, because recombination repair is not fully functional in the *uvsC114* mutant, spontaneous damage accumulates to a level that induces increased transcription of the *uvsC* gene.

Mitotic phenotypes associated with different alleles of the *uvsC* gene

In WG518TF1, an *uvsC114* mutant with a single-copy integration of the 3.6 kb *Not*I-*Kpn*I fragment at the *argB* locus, UV sensitivity was restored to wild-type levels,

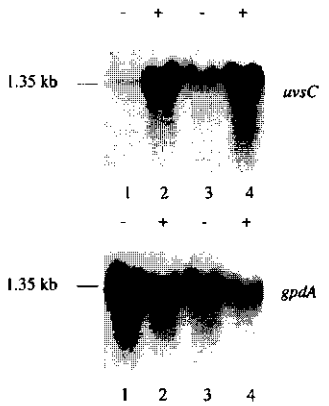


Fig. 4 Effect of MMS on the level of *uvsC* transcription in *uvsC*$^+$ and *uvsC114* strains, analyzed by Northern blot hybridization. Ten micrograms of RNA, isolated from untreated cells (−) or cells that were exposed to (50 μg/100 ml) MMS for 1 h (+), was loaded per slot, electrophoresed, blotted and hybridized with probes specific for *uvsC* (upper panel) and *gdpA* (lower panel). Lanes 1 and 2, RNA from strain WG505 (*uvsC*$^+$), lanes 3 and 4, RNA from strain WG352 (*uvsC114*)

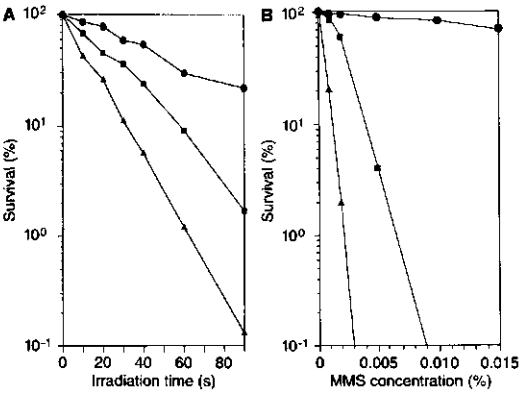


Fig. 5A–B UV and MMS survival curves of wild-type and mutant *A. nidulans* strains. **A** Germinating (6 h) conidiospores were irradiated for various periods of time with UV (18 erg/mm^2/s). **B** Conidiospores were plated on media containing various concentrations of MMS. ●—● WG519 (*uvsC*$^+$); ■—■ WG518 (*uvsC114*); ▲—▲ WG505Δ1 (*uvsC* null)

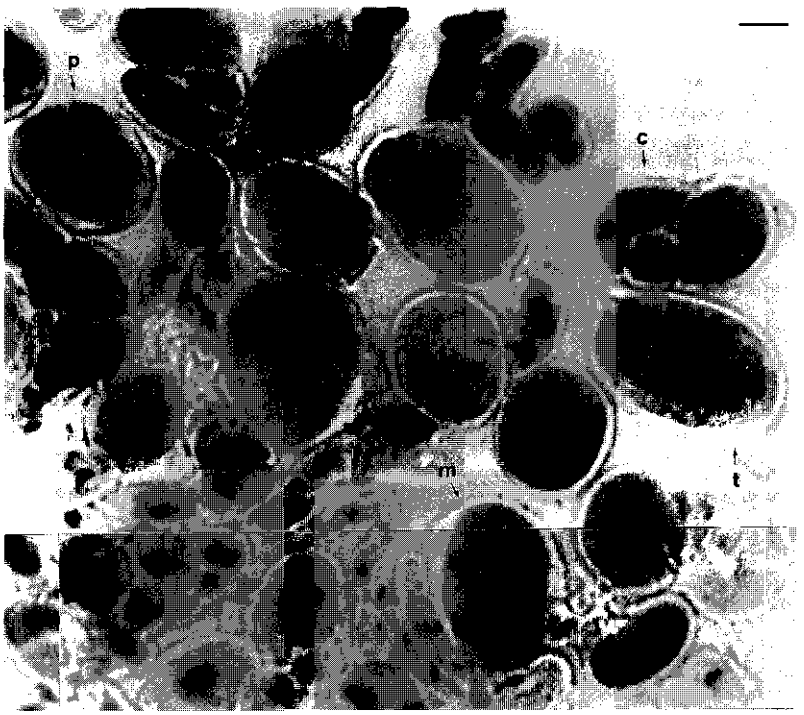


Fig. 6 Iron-haematoxylin staining of the contents of cleistothecia isolated from a *uvsC*$^+$ strain (WG044), showing various stages of (pre)meiotic development. Examples are indicated of a crozier with enlarged nucleoli in the two nuclei of the penultimate cell (**c**), an ascus in prophase I (**p**), an ascus in metaphase-I (**m**), and an ascus in telophase I (**t**). Scale bar, 10 μm

but MMS sensitivity was restored to a level intermediate between that seen in *uvsC*$^+$ and *uvsC114* strains. It is possible that the *uvsC* allele at its ectopic position in this tranformant is transcribed at a lower level than in wild type, so that the wild-type level of MMS sensitivity is not completely restored. It is also possible that the *uvsC*$^+$ allele and the *uvsC114* allele in this transformant compete for transcription factors, or that the *uvsC114*

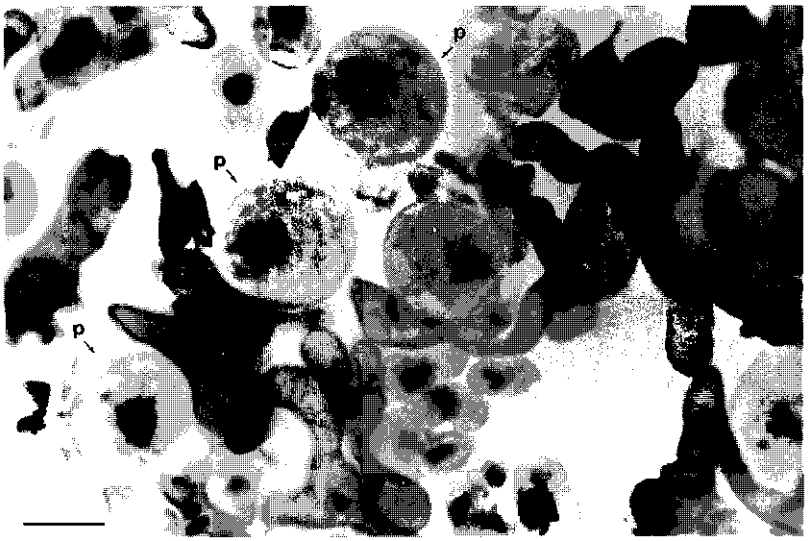


Fig. 7 Iron-haematoxylin staining of the contents of cleistothecia isolated from the *uvsC114* mutant (WG352). Several examples are indicated of asci in prophase I (p). Scale bar, 20 μm

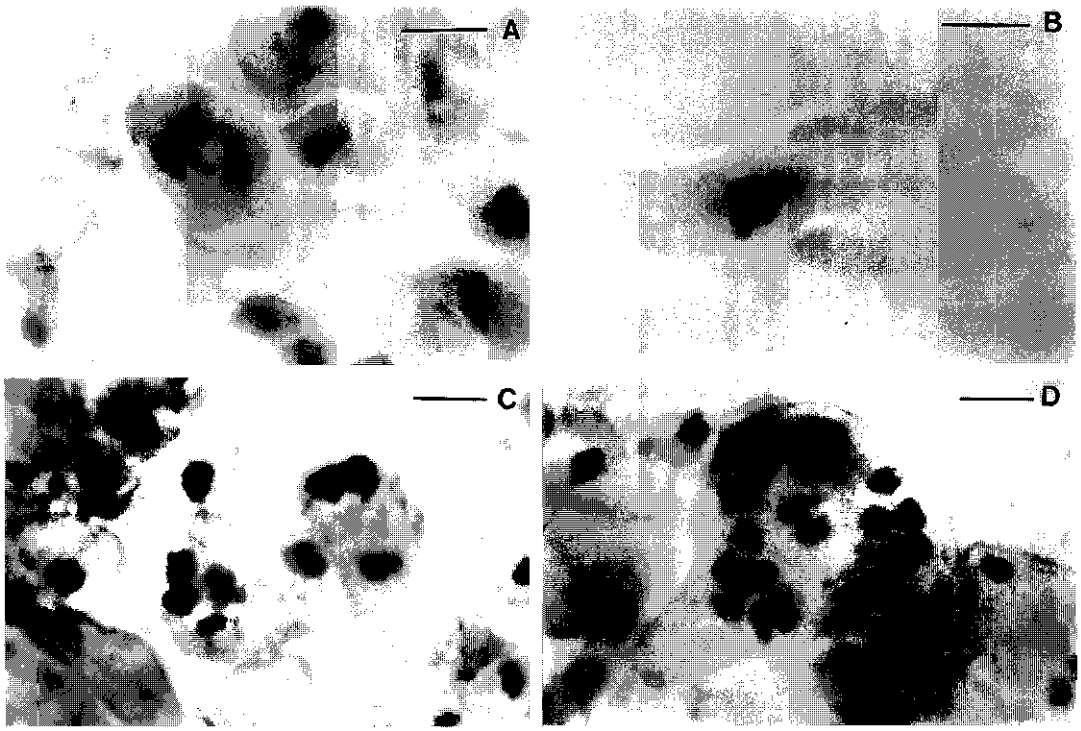


Fig. 8A–D Iron-haematoxylin staining of the contents of cleistothecia isolated from the *uvsC* null mutant (WG505Δ1). **A** Crozier with enlarged nucleoli in the two nuclei of the penultimate cell. **B** Putative meiotic ascus in prophase I. **C** Crozier with four nuclei in the penultimate cell. **D** Abnormal, large, multinucleate cells. Scale bar, 10 μm

mutation has a semidominant effect at the protein level. Meiosis in this transformant seemed to proceed normally, since viable sexual spores were formed.

Unexpectedly, we found different phenotypes for the two types of *uvsC* null mutants which are represented by WG505Δ1 and WG505Δ2, respectively. These two transformants only differ in the orientation of the replacing sequence at the *uvsC* locus. Possibly, transcription of the integrated *pyrG* gene can be initiated both at the integrated *pyrG* promoter and at the *uvsC* promoter. Because the two promoters are oriented oppositely in WG505Δ2, it is possible that transcription of the *pyrG* gene is affected or that antisense RNA of the *pyrG* gene is produced by transcription from the *uvsC* promoter. Another explanation for the phenotypic differences between the two *uvsC* null mutants is differential activity of the integrated *pyrG* promoters in the two transformants. Alternatively, one might assume a cryptic, additional mutation in WG505Δ2, giving this transformant a deviant phenotype. This however, is highly unlikely, because all type 2 transformants had the same phenotype.

The mitotic phenotypes of *rad51* mutants of yeast (for review see Friedberg et al. 1991; Petes et al. 1991) correspond with those reported for the *uvsC114* mutant (for review see Kafer and Mayor 1986) in most respects. Mutations in these genes are known to act as mutators, which can be explained by a block in the recombination repair pathway which causes channeling of spontaneous lesions to an error-prone repair pathway (Hastings et al. 1976). However, in contrast to Rad51 of yeast but like RecA of *E. coli*, UVSC is also required for postreplication repair of DNA damage induced by UV and bleomycin (Chae and Kafer 1997). Mutants in both *uvsC* and *RAD51* show a slight increase in UV sensitivity, and a strong increase in sensitivity to the radiomimetic drug MMS. When compared to the *uvsC114* mutant, the *uvsC* null mutant had a more severe phenotype with respect to MMS and UV sensitivity than the *uvsC114* mutant. Apparently, the *uvsC114* mutation is not a null mutation.

Meiotic phenotypes of the *uvsC114* mutant and the *uvsC* null mutant

The non-null mutant *uvsC114* arrests in meiotic prophase I, which corresponds well with the meiotic phenotype described for the N289 mutation in the *N. crassa mei-3* gene (Raju and Perkins 1978). The *uvsC* null mutant, however, showed a different meiotic phenotype: sexual development was blocked before the onset of meiosis. We observed this early block in cleistothecia isolated after growth on medium with and without uridine. Since the recipient WG505 strain was able to form mature ascospores after addition of uridine to the medium, we consider it unlikely that the early block we observe in the *uvsC* null mutant is caused by self-sterility effects of the resident *pyrG89* marker. Instead, we think that the abnormalities which we observe in the *uvsC* null mutant are the result of the *uvsC* disruption. Young cleistothecia of the *uvsC* null mutant contained several croziers with binucleate penultimate cells. The two nuclei in these cells had expanded chromatin and large nucleoli, like the nuclei in wild-type penultimate cells just before karyogamy (Fig. 6). The enlarged nucleoli are a characteristic feature of this developmental stage (Lu 1996). Very rarely, we observed cells with nuclei that were similar to meiotic prophase nuclei of wild type (compare Fig. 8B with Fig. 6); however, these cells had an abnormal, elongated shape (Fig. 8B) rather than the characteristic rounded shape of a wild-type meiotic prophase cell (Fig. 6). Older cleistothecia of the *uvsC* null mutant contained large, multinucleate cells which were not present in wild-type cleistothecia. Occasionally, we observed abnormal penultimate cells with four nuclei. We think that these cells originally contained two nuclei, but that these nuclei entered mitosis rather than karyogamy. It is possible that the large multinucleate cells (Fig. 8D) that we observed in older cleistothecia of the *uvsC* null mutant originate from penultimate cells in which the two nuclei have undergone a number of successive mitotic divisions. Alternatively, these multinucleate cells could originate from arrested and degenerated croziers. Similar multinucleate cells were also found in certain *N. crassa* mutants that arrest at the crozier stage at the time of karyogamy (Raju and Perkins 1978). Following this line of reasoning, these results would indicate that in the *uvsC* null mutant, premeiotic development is blocked at karyogamy, and that this block is followed by mitotic divisions and/or crozier degeneration. In most fungi, premeiotic S-phase takes place just before karyogamy (Iyengar et al., 1977, Bell and Therrien, 1978). Premeiotic S-phase lasts longer (Callan, 1972) and possibly also differs in other respects (Stern and Hotta 1987) from mitotic S-phase. It is possible that Rad51 has a role in premeiotic S-phase. In mouse spermatocytes, the Rad51-homologous protein was found to associate with a subset of chromatin sequences during premeiotic S-phase (Plug et al. 1996). Another interpretation of the meiotic defect of the *uvsC* null mutant is that crozier formation itself is affected. In *A. nidulans*, two nuclei are thought to be responsible for the formation of all sexual spores within a cleistothecium (Pontecorvo et al. 1953; Zonneveld 1988). It is possible that the conjugate mitotic divisions in crozier and precrozier cells depend in an as yet unknown manner on UVSC (= Rad51) functions, and that they differ in this respect from the mitotic divisions in vegetative nuclei. The meiotic phenotype of the *uvsC* null mutant differs from the meiotic phenotype of *S. cerevisiae rad51* disruption mutants (Shinohara et al. 1992; Basile et al. 1992) and also from the meiotic phenotype of the *N. crassa mei-3* mutant N289 (Raju and Perkins 1978), which both arrest at meiotic prophase I. It is not known, however, whether the N289 mutant is a null mutant at the *mei-3* locus.

Disruption of *RAD51*-homologous genes in different organisms

Disruption of *RAD51*-homologous genes has different effects in different organisms. In *S. cerevisiae*, disruption of *RAD51* has no effect on mitotic growth (Shinohara et al. 1992; Aboussekhra et al., 1992; Basile et al. 1992), whereas in *S. pombe*, disruption of *rph51*$^+$ affects mitotic growth rate but not cell viability (Muris et al. 1993). In the mouse, disruption of the *Rad51* gene results in cell lethality (Tsuzuki et al. 1996). In *A. nidulans*, disruption of *uvsC* has no detectable consequences for mitotic growth. In *S. cerevisiae* disruption of *RAD51* causes arrest at meiotic prophase I, whereas in *A. nidulans*, disruption of *uvsC* causes arrest at an earlier (premeiotic) stage. Taken together, these results indicate that Rad51 either has a different role in different organisms, or that some organisms possess other systems which can supplement one or more of the functions of Rad51.

Acknowledgements We thank G.J.O. Jansen (Institute of Genetics, State University, Utrecht, The Netherlands) for the gift of the *A. nidulans uvsC114* mutant. We thank J.H.M. Lammers (Agricultural University Wageningen, The Netherlands) for sharing with us his adaptation of the original RACE protocol. We thank P.J. Punt (TNO, Nutrition Food Research Institute, Rijswijk, The Netherlands) for the gift of plasmid pAOPYRG-Not.

Chapter 5

Isolation and characterization of sexual sporulation mutants of *Aspergillus nidulans*

K. Swart [1], D. van Heemst [1,2], M. Slakhorst, F. Debets and C. Heyting

Department of Genetics, Wageningen University, 6703 HA Wageningen, The Netherlands
[1] These authors contributed equally to this work; [2] Present address: Lab. of Cell Biology and Genetics, Erasmus University, PO box 1738, 3000 DR, Rotterdam, The Netherlands

Submitted for publication in Fungal Genetics and Biology

ABSTRACT

For the genetic dissection of sexual sporulation in *Aspergillus nidulans*, we started a collection of ascosporeless mutants. After mutagenization of conidiospores with high doses of UV, we isolated 20 mutants with defects in ascospore formation. We crossed these mutants in two successive rounds with the wildtype strain. Eighteen of the 20 isolated mutants produced progeny with the original mutant phenotype in these crosses, and these mutants were further analyzed. All 18 analyzed mutations were recessive to wildtype. We assigned them to 15 complementation groups, based on crosses between mutants. The mutants could be classified as follows according to their cytological phenotype: (1) no croziers; (2) arrest at pre-karyogamy; (3) arrest in early meiotic prophase; (4) arrest in late meiotic prophase; (5) arrest in meiotic metaphase I; (6) defective post meiotic mitosis and/or deliniation of ascospores; and (7) slow progression through the post meiotic stages of ascospore formation. A large proportion of the mutants, namely 11 out of 18, arrested in meiotic prophase or metaphase I. We discuss a possible approach for isolating the wildtype alleles of the genes that carry the sexual sporulation mutations.

INTRODUCTION

The life cycle of the homothallic filamentous ascomycete *Aspergillus nidulans* consists of distinct generative (sexual) and vegetative (parasexual) phases (reviewed in Elliott, 1994). Growth, propagation and recombinational repair can all occur in the context of the vegetative, parasexual phase, and therefore, *A. nidulans* does not depend on the sexual phase of the life cycle for survival, at least in the laboratory.

We took advantage of these features for the isolation of mutants that are specifically disturbed in sexual sporulation. We treated (vegetative) conidiospores with high doses of UV, which allowed only 1-2% survival, and plated the irradiated spores on minimal medium. We expected that a large fraction of the survivors would carry mutations in genes that are not essential for vegetative growth, including genes specifically required for the sexual phase of the life cycle. Because *A. nidulans* is homothallic, we could examine the surviving colonies directly for defects in the formation of cleisthothecia (fruiting bodies), asci or ascospores by visual inspection.

Mutants of *A. nidulans* disturbed in sexual sporulation are important for the genetic dissection of the sexual developmental pathway. This pathway starts in *A. nidulans* without any sign of differentiated female and/or male sexual structures (Benjamin, 1955; Zonneveld, 1988). The first morphologically detectable developmental step consists of the formation of cleistothecial primordia. These are surrounded by globular hülle cells, which possibly support the hyphae of the developing cleistothecium. The fruiting body is supposedly built around a specialized cell from which dikaryotic cells develop, which bend to form croziers (Champe and Simon, 1992). The two nuclei in a crozier undergo a coordinated mitosis. By formation of septa at both sides of the crook, a binuclear penultimate cell is formed, which is flanked by two uninuclear cells, the tip cell and the basal cell (Elliott, 1960; see below). The penultimate cell develops into an ascus with sexual spores (ascospores). Its two nuclei fuse (karyogamy) to produce a diploid zygotic nucleus, which immediately undergoes meiosis. The resulting four haploid nuclei each undergo a mitotic division, so that eight nuclei are formed, which differentiate into eight ascospores. The nuclei of maturing ascospores undergo one additional mitosis, so that a mature ascus contains eight binuclear ascospores.

We were particularly interested in genes involved in meiosis in *A. nidulans*, because in this fungus, meiosis is atypical in some respects. Contrary to almost all analyzed sexually reproducing eukaryotes, *A. nidulans* does not develop detectable synaptonemal complexes (SCs) between homologous chromosomes during meiotic prophase (Egel-Mitani *et al.*, 1982)

and does not display positive interference of meiotic crossovers (Strickland, 1958). The only other eukaryote known to lack SCs and crossover interference is *Schizzosaccharomyces pombe* (Bähler *et al.*, 1993; Munz, 1994; reviewed by Egel, 1995). Although meiotic recombination has been extensively studied in *A. nidulans* (reviewed in Pontecorvo *et al.*, 1953; Whitehouse, 1982), the meiotic process has been analyzed only to a limited extent in this species, and thus far no screens for meiotic mutants have been undertaken. Previous screens for DNA-damage repair mutants of *A. nidulans* yielded various mutants defective in both DNA repair and meiotic recombination (reviewed in Käfer and May, 1997). In this paper, we focus on mutations that specifically affect meiosis or ascospore formation by searching for mutants that grow on supplemented minimal medium and form cleistothecia, but do not form mature asci and/or ascospores. This approach appeared favorable because most of the naturally occurring sexual developmental mutants of *Neurospora crassa* form perithecia, but arrest at some stage in ascospore development (Leslie and Raju, 1985). Thus, perithecial and ascospore development can be separated by mutation, and the chances of finding sexual sporulation mutants by inspecting perithecial/cleistothecial contents of surviving colonies appeared good. A large proportion of the mutants that we found was blocked in meiotic prophase and/or metaphase I.

MATERIALS AND METHODS

Strains and media

For mutagenization and crosses, we used *A. nidulans* strain WG044 (*ornB7 fwA1* VeA+/VIII) and WG337 (*biA1*/I; *methG1*/IV) respectively. For parasexual linkage analysis, we used a tester strain with a marker for each individual chromosome (linkage group), namely WG129 (*pabaA1*/I; *wA3*/II; *galA1*/III; *pyroA4*/IV; *facA303*/V; *sB3*/VI; *nicB8*/VII; *riboB2*/VIII). These strains are derived from the Glasgow stocks (Clutterbuck, 1986); their genetic markers have been described (Clutterbuck, 1984).

The media and procedures for genetic manipulations of *A. nidulans* have been described by Pontecorvo *et al.* (1953). BM (basal medium) is a balanced salt solution for *A. nidulans*. MM (minimal medium) is sufficient for growth of *A. nidulans* strains without auxotrophic mutations, and consists of BM supplemented with 1% glucose and 10 mM $NaNO_3$. SM (supplemented medium) is MM supplemented with all nutrients required by a given strain.

Mutagenesis

We UV-irradiated conidial suspensions of strain WG044 (10^7 spores/ml) in petridishes at 40 erg/sec/mm^2 for 0, 90, 120, 150, 180 or 210 seconds. For each time point, we plated various dilutions of the conidial suspensions on SM (+ornithine) to determine the percentage of surviving spores (data not shown). After irradiation for 150 sec. at 40 erg/sec/mm^2, 1.5% of the conidiospores survived. These conidiospores were plated at appropriate dilutions on SM (+ornithine) and incubated at 40 °C to form single colonies. Subsequently, we selected by visual inspection 1250 single colonies with apparently normal vegetative growth and fruiting body (cleistothecium) development. Of each of these colonies, we examined visually the contents of ten ripe fruiting bodies. Those colonies that did not form normal asci and/or ascospores at 40 °C were selected for further characterization. To determine whether the defects in ascospore formation depended on the temperature, we analyzed the colonies that displayed this defect at 40 °C also at 30 °C.

We removed possible background mutations by crossing the mutants in two successive rounds with wildtype strain WG337 and selecting progeny with defects in ascospore formation.

Complementation analysis and mapping

We classified the selected mutants, which showed impaired ascospore formation at 40 °C or at 30 °C and 40 °C, into complementation groups by performing crosses between them in all possible pairwise combinations, and determining restoration of the sexual sporulation defect among the progeny at 30 °C and at 40 °C. Based on the numbers of complementation groups that were represented by 1, 2 or 3 mutants (Table 1), and using Poisson's formula, we calculated as a first estimation that the average number (μ) of mutants per complementation group was 0.281. By non-linear regression to the Poisson equation, using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, California, USA) and assuming 0.28 as initial value for μ, we found 0.275 as best-fit value for μ, with a 95% confidence interval from 0.1765 to 0.3736.

We mapped three mutations by parasexual linkage analysis (Pontecorvo and Käfer, 1958), using procedures reviewed by Bos (1996).

Cytological analysis

Of each mutant, we collected fruiting bodies in various stages of development (from slightly pink to purple) and cleaned them from adhering hülle cells by rolling them on a 3% water-agar plate. Subsequently, these fruiting bodies were stained as described for *Neurospora crassa* (Raju, 1986): they were treated with 4N HCl for 20-30 min at 30 °C, rinsed once in water, and stained with acriflavin (100-200 mg/ml acriflavin in a solution of 5 mg/ml $K_2S_2O_5$ in 0.1 N HCl) for 20-30 min at 30 °C. The cleistothecia were washed three times (3-5 min per wash) at 30 °C in a mixture of 37% HCl- 70% ethanol (2:98 v/v), and twice in water. Subsequently, they were placed on a microscope slide in a drop of 25% glycerol, and the cleistothecial walls were removed by dissection. The contents of the cleistothecia were squashed under a cover slip so that the cells were sufficiently flattened for observation of the structure of the nuclei. We examined the stained preparations using a fluorescence microscope (Nikon UFX-II) equipped with a HBO 100 Hg-lamp and dichroic mirror DM510, which allows excitation at 450-490 nm and emission at > 520 nm, and photographed selected images on a Kodak TMAX 400 film. We scanned the negatives at high resolution and processed their computer images using the Corel Photopaint 9 software package.

RESULTS

Rationale of the procedure

Figure 1 provides an overview of our mutant screen for sexual sporulation mutants in *A. nidulans*. Essential features of this screen were the high dose of UV, which allowed only 1.5% survival of the irradiated conidiospores, the plating of the irradiated conidiospores on supplemented minimal medium (SM) rather than rich medium, and the elimination of all colonies from the mutant screen that showed poor or aberrant vegetative growth.

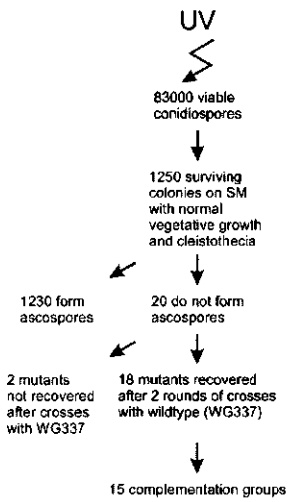


Figure 1: Overview of the mutant screen

We chose UV as mutagen because it can induce a broad spectrum of mutations in any gene, and we applied a high dose because the mutant yield increases linearly with the dose of UV up to high doses (Nevalainen, 1981). High doses of UV (0.1-4% survival) applied to *Coprinus cineraeus* yielded 1.3% basidiosporeless mutants among survivors (Kanda *et al.*, 1989). A high yield of mutants was essential because we had to search for sexual sporulation mutants by inspecting the contents of the cleistothecia of each individual surviving colony.

We plated the UV-irradiated conidiospores on SM in order to avoid auxotrophic mutants. Rich medium would have allowed survival of such mutants; auxotrophs for certain amino acids are self-sterile in *A. nidulans* (Käfer, 1977b; Eckert *et al.*, 1999). We also discarded all mutants that displayed abnormal vegetative growth, because we were primarily interested in mutations in genes that are specifically involved in ascospore formation, and not in genes that also have a role in vegetative growth, for instance in DNA repair (cf. van Heemst *et al.*, 1997). Collections of mutants in such genes already exist in *A. nidulans* (Osman *et al.*, 1993).

The high UV dose had the disadvantage of increasing the risk of chromosomal aberrations (Käfer, 1977a) and undesired background mutations. Therefore, we crossed the sexual sporulation mutants in two successive rounds with a wildtype strain before we analyzed them further. All crosses with wildtype yielded crossed fruiting bodies with normal amounts of ascospores, which indicates that all analyzed mutations were recessive. Eighteen of the 20 analyzed mutants produced two phenotypically homogeneous classes of progeny upon crosses with the wildtype strain, namely the original mutant phenotype and wildtype. This indicates that these mutants did not carry background mutations that influence the analyzed characteristics.

Two of the 20 analyzed mutants did not yield progeny with the original mutant phenotype upon crosses with the wildtype strain, but only wildtype progeny. Possibly, the sexual sporulation defect in these mutants resulted from a combination of multiple, separable mutations. We did not analyze these two mutants further.

To verify that individual mutations could be assigned to single chromosomes (linkage groups) or chromosomal locations, we mapped three mutations by parasexual linkage analysis, namely D9, H15 and B26. D9 was assigned to chromosome VII because of linkage to *nicB8*, and absence of linkage to genetic markers on all other chromosomes. H15 was assigned to chromosome I because of exclusive linkage to *pabaA1*, and B26 was linked to *galA1* (on chromosome III) and *sB3* (on chromosome VI). We think that mutation B26 either coincides with a translocation breakpoint between chromosomes III and VI, or is closely

linked to such a translocation breakpoint. First, the mutant phenotype of B26 is consistently transmitted to the progeny upon crosses with wildtype or other strains, and is therefore probably not due to mutations on two different chromosomes. Second, the progeny from crosses of B26 with other strains comprised many clones with morphological abnormalities that are characteristic of disomic strains (Pollard *et al.*, 1968; Käfer and Upshall, 1973); such disomics may arise as a result of nondisjunction in meiotic metaphase I in crosses that are heterozygous for a translocation.

Complementation analysis

Based on crosses between mutants in all possible pairwise combinations, we assigned the 18 selected mutants to 15 complementation groups; the results are shown in Table 1. Thirteen complementation groups were represented by a single mutant; one was represented by two mutants, and one by three mutants. If the complementation groups correspond with individual genes (see Discussion), we estimate from these numbers that the genes that were target in our screen received on average 0.275 mutagenic hit (95% confidence interval: 0.177-0.374; see Materials and Methods), and that the fraction of these genes that was hit in our screen was 0.245. Because this fraction corresponds to the 15 genes (complementation groups) in Table 1, the total number of target genes was 61 (95% confidence interval: 48-92). Thus, we estimate that about 50 - 100 genes of *A. nidulans* are specifically involved in ascosporogenesis, without having a major effect on vegetative growth under standard growth conditions on SM (see also Discussion).

Cytological characterization of the mutants

All 18 selected mutants were cytologically characterized and compared with wildtype (Table 1 and Figure 2).

Figure 2A-C shows stages in ascospore formation in wildtype *A. nidulans*. Cleistothecial primordia develop around a specialized cell, which at some stage produces dikaryotic hyphae, which bend to form croziers. After a coordinated mitosis of the two nuclei and formation of septa, a binucleate penultimate cell is formed which is flanked by two uninucleate cells, the tip cell and the basal cell (Figure 2A; Elliott, 1960). The penultimate cell enlarges to form an ascus. Its two nuclei fuse (karyogamy, Figure 2A) to give a diploid zygotic nucleus, which immediately enters meiosis (Figure 2B and C). The two meiotic

divisions yield four haploid nuclei (Figure 2C), which divide mitotically, to produce eight haploid nuclei, which separate into eight individual spores. Within each spore, each nucleus undergoes one mitotic division, so that a mature ascus contains eight binucleate spores (Champe and Simon, 1992).

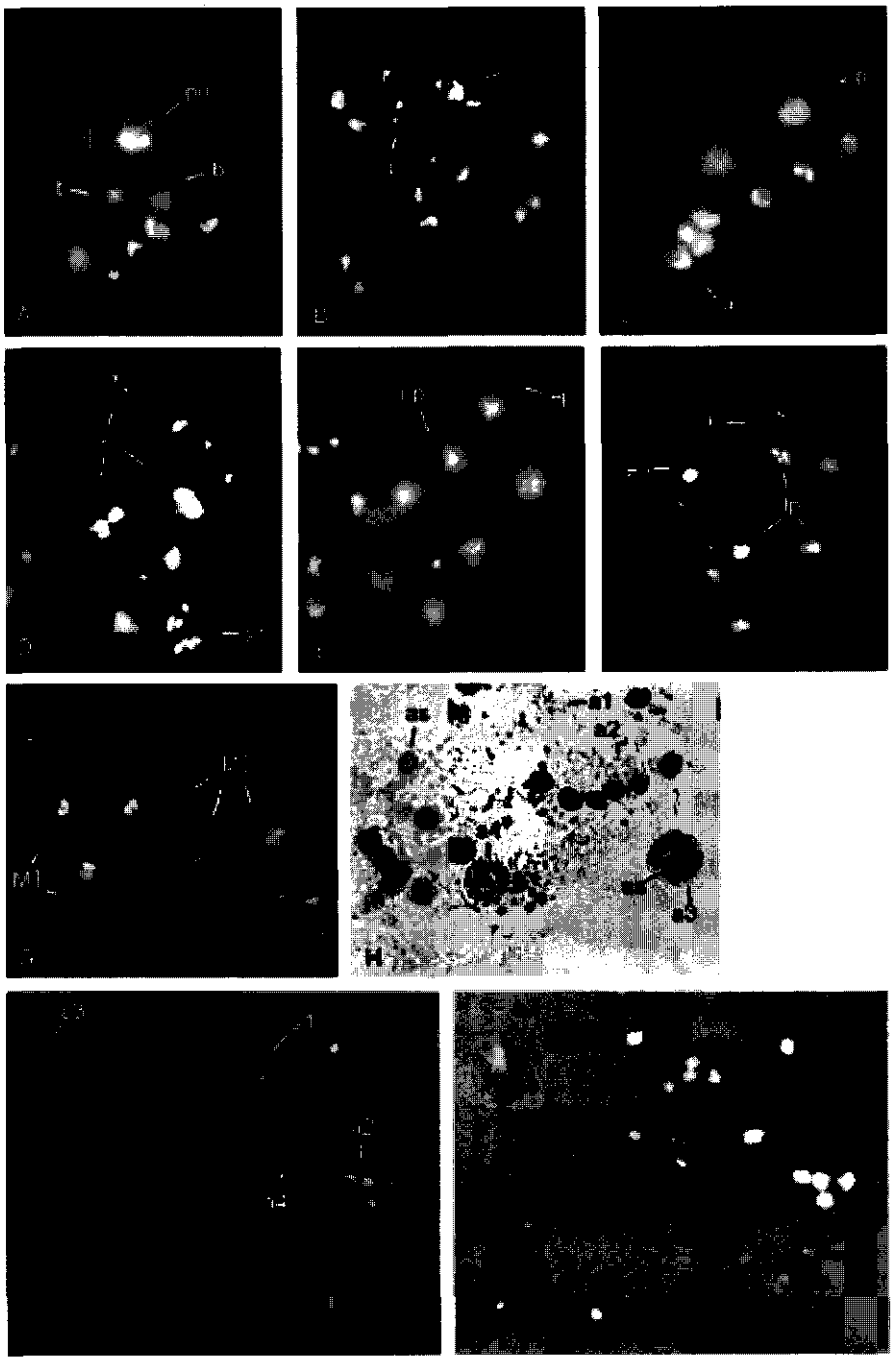
Table 1 shows that most of the analyzed mutants display a clear block in this developmental pathway, and that members of the same complementation group are usually blocked at the same developmental step.

Two mutants in different complementation groups, G12 and S24, did not form croziers, and developed empty fruiting bodies with undifferentiated hyphae; in mutant G12 we found occasionally an ascus-like cell. One mutant, I16, arrested just before karyogamy, with the two nuclei closely together. The three mutants in complementation group H all accumulated asci in early meiotic prophase, although H13 and to a lesser extent H14

Table 1: Cytological phenotype of mutants of *A. nidulans* defective in ascospore formation[a]

Mutant[b]	Developmental stage								Remarks
	crozier	pre-karyo-gamy	early pro-phase	late pro-phase	meta-phase I	MII, tetrad	post-meiotic mitosis	asco-spore	
G12	#								
S24 (ts)	#								
I16	*	****	#						
H13	*	*	****	L	#				
H14	*	*	****	?	#				
H15	*	*	****	#					
C8	*	*	*	****	#				
K17 (ts)	*	*	*	****	L	L	#		leaky?
O21	*	*	*	****	L	#			
B27 (ts)	*	*	*	****	L?	#			
B26	*	*	*	*	****	#			
E10 (ts)	*	*	*	*	****				
L18	*	*	*	*	****	#			
R23	*	*	*	*	****	#			
D9	*	*	*	*	*	*	***	#	
M19	*	*	*	*	*	*	**	L	
P22	*	*	*	*	*	*	**	L	
F11 (ts)	*	*	*	*	*	*	*	*	slow

[a] (ts), mutant phenotype is temperature-dependent; [b] first character indicates complemetation group;
*: stage present; **: some accumulation of this stage; ***: moderate accumulation;
****: accumulation; L: leakage to this stage; #: first stage not observed.



displayed some leakage to late meiotic prophase. Representatives of four complementation groups, C, B, K and O, arrested in late meiotic prophase. The phenotypes of the two mutants in complementation group B differed from one another: mutant B27 proceeded to late meiotic prophase and mutant B26 to metaphase I. Members of four of the 15 complementation groups, namely B, E, L and R, arrested in metaphase I (Table 1 and Figure 2G). Interestingly, mutants in three complementation groups, D, M and P, showed at least partial arrest after meiosis, in the 4-nuclei stage (Figure 2 I and J), although they were not affected in vegetative growth. As is shown in Figure 2H-J for mutant P22, these mutants develop apparently normal tetranucleate asci (Figure 2 I and J), but do not form normal asci with eight nuclei or eight spores. Although mutant P22 formed some morphologically normal ascospores (Figure 2H), these spores were not viable. Instead of asci with eight morphologically normal spores, we only found asci containing one or two immature spores (Figure 2H, asci a1 and a2), numerous mini-ascospores (Figure 2H, ascus a3), or a mixture of one or two immature or apparently normal mature ascospores and numerous mini-ascospores (Figure 2H ascus a3).

Figure 2: Ascospore formation in wildtype and mutant *Aspergillus nidulans*. (A-C) Various stages in ascospore development in wildtype, strain WG044. (A) Crozier with pu, penultimate cell (ascus mother cell) just after karyogamy; the domains of the two fusing nuclei are still visible; t, tip cell; b, basal cell. (B) Ascus mother cell in lp, late meiotic prophase (chromatin expanded). (C) Ascus mother cells in a, 4-nuclei stage and p, midprophase. (D) Mutant I16, which accumulates the karyogamy stage (k); k* could represent a karyogamy stage with the nuclei in metaphase-like configuration. (E) Mutant H15, which accumulates early meiotic prophase (ep; chromatin condensed). (F) Mutant C8, which accumulates late meiotic prophase (lp); ep, early prophase; k, karyogamy. (G) Mutant L18, which accumulates metaphase I (MI; chromatin condensed and contours of individual chromosomes partly visile). (H-J) Mutant P22, which is defective in postmeiotic mitosis and/or ascosopore formation. (H) Phase contrast image showing apparently normal ascospores (as) and asci containing less than 8 normal ascospores (a1, a2 and a3) and/or numerous mini-ascospores (a3 and a4). (I) Acriflavin fluorescence. (J) The same fluorescence image as shown in I, merged with the phase contrast image; a1 and a2, apparently normal ascus mother cells in the 4-nuclei stage; a3, ascus mother cell presumably in late meiotic prophase; a4, ascus mother cell in mid meiotic prophase; u, uninucleate ascospore.

One mutant, F11, which we originally identified as ascosporeless, turned out to make viable ascospores, albeit delayed: whereas wildtype strain WG044 formed the first ascospores at 40 °C on day eight after plating, mutant F11 did not form ascospores at 40 °C before day 11, although vegetative growth is not grossly retarded in F11.

DISCUSSION

Mutant yield

Treatment with high doses of UV and selection of surviving colonies with normal vegetative growth and externally normal but empty cleistothecia proved to be an effective strategy for isolating sexual sporulation mutants of *A. nidulans*. Colonies with empty ("barren"; Raju and Perkins, 1978) cleistothecia occurred sufficiently frequently among the survivors (1.6%, see Figure 1) to allow their identification by visual inspection of cleistothecial contents. Despite the high UV-dose, 18 out of our 20 mutants appeared to carry a single mutation that affected ascospore formation. This fits with the results of Kanda *et al.* (1989), who applied similar doses of UV (0.1-4% survival) to *Coprinus cineraeus* as we applied to *A. nidulans*, and found that 25 of their 29 basidiosporeless mutants were likely due to a single mutation. One of the mutations that we mapped, B26, was most probably a translocation, or closely linked to a translocation breakpoint. This confirms earlier observations in *N. crassa* (Perkins *et al.*, 1962) and *A. nidulans* (Käfer, 1965) that UV can induce structural chromosomal aberrations, albeit less effectively than ionizing radiation.

Complementation analysis

Based on complementation analysis, we estimated that 50-100 genes were target in our mutant screen, i.e., were involved in ascospore formation without having a major role in vegetative growth. This estimation is based on the equation of complementation groups with genes. Intragenic complementation will therefore lead to overestimation of the number of target genes, and failure of complementation of two mutations in different genes to underestimation. The latter situation can occur if the two mutated genes encode proteins that interact *in vivo*, or participate in the same protein complex or pathway. In our screen, it is furthermore conceivable that structural chromosomal aberrations apparently fail to complement other mutations, because chromosomal aberrations could interfere with meiotic chromosome pairing and chromosome disjunction, and thereby cause meiotic failure in

heterozygous crosses. However, all mutations analyzed by us, including the supposed translocation B26, were recessive in heterozygous crosses with wildtype and other strains, and therefore we do not think that structural chromosomal aberrations have interfered with our complementation tests. Furthermore, Perkins and Barry (1977) and Raju and Perkins (1978) considered this problem in detail for sexual sporulation mutants of *N. crassa*. Over 150 carriers of reciprocal chromosomal rearrangements went through meiosis and produced ascospores in heterozygous crosses, albeit that not all spores were viable, most likely because of deficiencies and duplications. Thus, in *Neurospora*, reciprocal chromosomal rearrangements do not not cause meiotic failure because of pairing and disjunction problems in heterozygous crosses. *N. crassa* carriers of chromosomal duplications usually fail to form ascospores, but they fail to do so in both heterozygous and homozygous crosses, and this effect is thus not due to meiotic chromosome pairing problems because of structural chromosomal aberrations (Raju and Perkins, 1978). In *A. nidulans* also, most translocation carriers are fertile in heterozygous crosses, although they may cause meiotic nondisjunction, and produce disomic progeny clones (Pollard *et al.*, 1968; Käfer and Upshall, 1973).

Despite these uncertainties, our estimation of the number of target genes is of the same order as estimations of the number of genes that are specifically required for ascospore formation in yeast. Burns *et al.* (1994) performed large-scale gene disruption experiments in yeast, using a transposon construct carrying the *E. coli lacZ* reporter gene. They estimated that 93-135 genes are specifically expressed during meiosis and ascospore formation, and that transposon insertions in about 36% of these genes (i.e. 33-48 genes) negatively affect sporulation efficiency and/or spore viability. Other studies suggest that a larger number of genes are involved in ascospore formation. Micro array experiments in yeast (reviewed by Vershon and Pierce, 2000) identified more than 500 genes that are induced above background levels during meiosis and sporulation (Chu *et al.*, 1998). However, it remains to be established how many of these genes are specifically required for sporulation without having a major role in vegetative growth.

Mutant phenotypes

Most mutants obtained in this screen arrest at some stage in meiosis (Table 1). Three mutants belonging to one complementation group arrest in early meiotic prophase, three mutants in three different complementation groups arrest in late prophase, and five mutants in five different complementation groups arrest in metaphase I. The predominance of meiotic

mutants probably reflects the large proportion of sexual sporulation genes that are specifically involved in meiosis (Esposito and Klapholz, 1981). Part of the mutants arresting in late meiotic prophase possibly respond to a checkpoint that detects meiotic DNA-breaks. Such a checkpoint has been identified in yeast (Lydall *et al.*, 1996), and mutations in several genes involved in meiotic recombination will result in persistence of DNA-breaks (reviewed by Smith and Nicolas, 1998) and arrest in late pachytene. Mutants arresting in metaphase I possibly respond to a checkpoint at the metaphase I- anaphase I transition that ensures the alignment of all bivalents in the spindle before anaphase I onset, by monitoring tension across the kinetochores (reviewed by Allshire, 1997).

Similar screens for sexual sporulation mutants in other fungi often yielded other spectra of mutant phenotypes than the screen described here, albeit that mutants arresting in meiosis were usually frequent. Kanda *et al.* (1989), who searched for basidiosporeless mutants of *C. cineraeus* after UV-mutagenization, isolated predominantly mutants arresting in postmeiotic stages of development, although 6 of their 25 mutants arrested in metaphase I - anaphase I. Most of the natural ascosporeless mutants of *N. crassa* that were analyzed by Leslie and Raju (1985) formed perithecia, and were blocked in postmeiotic stages of ascospore development. We ascribe these differences in mutant phenotypes partly to coincidence, because the number of mutants analyzed by us is small compared to the total number of target genes, and to differences in mutant selection conditions in the various screens.

Prospects for isolating sexual sporulation genes

In *A. nidulans*, it is in principle possible to isolate wildtype alleles of mutant genes by complementation and sib-selection. By this method, mutants are transformed with successively smaller pools of cosmids containing genomic DNA-fragments. After each transformation, the complemented phenotype is detected, and transformation is repeated with the complementing cosmid pool until a single complementing cosmid is identified (Vollmer and Yanofsky, 1986). This approach depends on the easy identification or selection of the complemented phenotype. However, complementation of a sexual sporulation defect is not easily detectable in *A. nidulans*, and requires dissection of mature fruiting bodies of each individual transformant. This problem can be circumvented by reducing the number of transformants to be screened, and detecting the restored sexual sporulation indirectly, by selecting for meiotic reassortment of alleles (Figure 3). In *A. nidulans*, mutations can be

assigned relatively easily to chromosomes (linkage groups) by parasexual linkage analysis (Pontecorvo and Käfer, 1958), as we have shown in this paper for D9, H15 and B26. Complementation of meiotic mutations can then be performed with chromosome-specific (Brody *et al.*, 1991), ordered (Xiong *et al.*, 1996) genomic libraries, which will greatly reduce the number of transformants to be screened. Furthermore, many suitable auxotrophic markers of *A.nidulans* are available (Clutterbuck, 1984), so that for each meiotic mutation two haploid strains can be constructed, which differ from one another by auxotrophic markers (Käfer, 1977b). One strain is transformed with a pool of genomic cosmids, and the transformants are subsequently crossed *en masse* with the second strain. The contents of the fruiting bodies that result from this cross can then be plated on minimal medium. If complementation has occurred, meiosis will take place and ascospores will be produced. Some of these spores will grow on minimal medium because reassortment of auxotrophic markers during meiosis will result in the production of prototrophic progeny (see Figure 3). The time-consuming dissection of fruiting bodies is thus be replaced by a simple test for prototrophic progeny from a limited number of crosses. By this approach, it should be feasible to clone the wildtype alleles of sexual sporulation mutations, if these mutations are stable and not leaky.

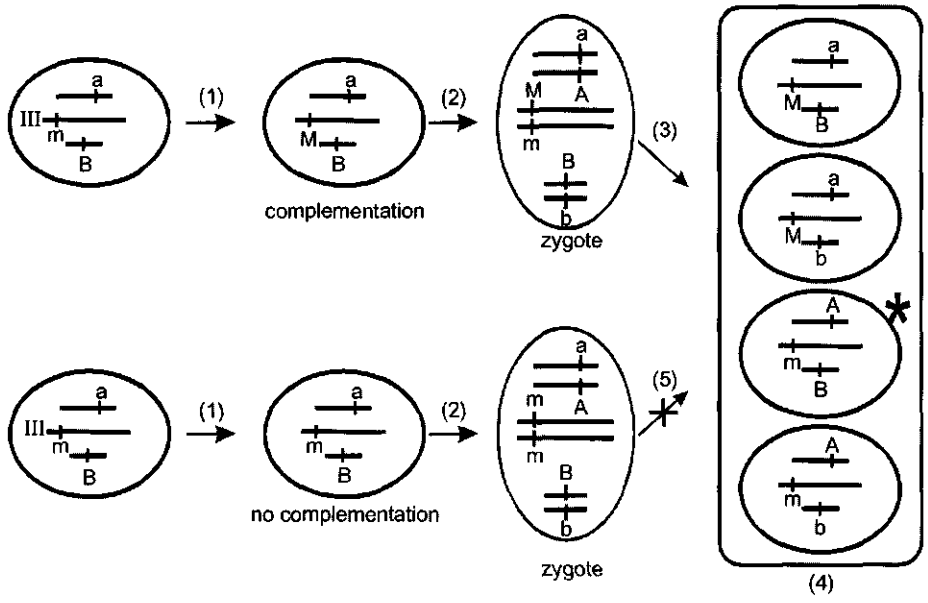


Figure 3: Indirect test for complementation of sexual sporulation defects. From a mutant strain carrying meiotic mutation m on chromosome III, two strains have been derived, each with an auxotrophic mutation in another gene (indicated by a and b; A and B represent the wildtype alleles of these mutations). (1) One of the two strains, with auxotrophic marker a, is transformed with a chromosome-specific library for chromosome III; in some transformed cells, m is complemented (indicated by M). (2) All transformed cells are crossed with the other of the two strains, which carries auxotrophic marker b. (3) In the zygotes that stem from complemented cells, meiosis can proceed, and ascospores are formed. (4) Some of the ascospores (indicated by asterisk) are prototrophic, as a result of reassortment of alleles during meiosis, and will give rise to colonies on minimal medium. (5) In most transformants, the meiotic mutation will not be complemented, so that meiosis and ascospore formation do not take place upon crossing; these transformants will not give rise to prototrophic colonies.

ACKNOWLEDGEMENTS

We thank Edu Holub and Gerda Kobus for expert technical assistance, and Jaap Lelie for performing parasexual linkage analyses during a traineeship at our laboratory.

Chapter 6

General discussion: sister chromatid cohesion and recombination in meiosis

D. van Heemst and C. Heyting

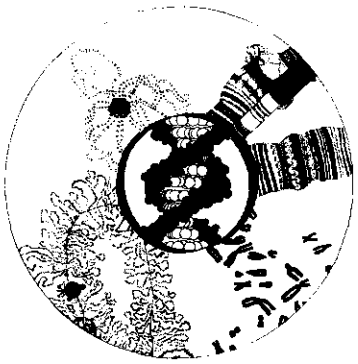
Department of Genetics, Wageningen University, 6703 HA Wageningen, The Netherlands

Published in Chromosoma 109: 10-26 (2000)

Chromosoma (2000) 109:10–26

CHROMOSOMA

Chromosoma Focus



Sister chromatid cohesion and recombination in meiosis

Diana van Heemst, Christa Heyting

Laboratory of Genetics,
Wageningen University and Research Centre,
Dreijenlaan 2, 6703 HA Wageningen, The Netherlands

Received: 11 October 1999; in revised form: 3 December 1999 /
Accepted: 6 December 1999

Abstract. Sister chromatids are associated from their formation until their disjunction. Cohesion between sister chromatids is provided by protein complexes, of which some components are conserved across the kingdoms and between the mitotic and meiotic cell cycles. Sister chromatid cohesion is intimately linked to other aspects of chromosome behaviour and metabolism, in particular chromosome condensation, recombination and segregation. Recombination, sister chromatid cohesion and the relation between the two processes must be regulated differently in mitosis and meiosis. In meiosis, cohesion and recombination are modified in such a way that reciprocal exchange and reductional segregation of homologous chromosomes are ensured.

Mitosis

The mechanism of sister chromatid cohesion has been analysed in most detail in mitosis. We will therefore summarise the roles of sister chromatid cohesion in the mitotic cycle before we turn to meiosis.

Mitotic sister chromatid cohesion and chromosome disjunction

Sister chromatid cohesion is essential for the faithful segregation of chromosomes during mitosis. In the mitotic cycle, sister chromatids are associated from S-phase until anaphase along their arms and in their centromeric regions (Selig et al. 1992; Guacci et al. 1994). In higher eukaryotes, cohesion is most persistent in heterochromatic domains containing large blocks of repetitive DNA (Lica et al. 1986; Cooke et al. 1987; Sumner 1991; Carmena et al. 1993; Warburton and Cooke 1997). Possible sources of cohesion that have been proposed are: DNA catenations that persist from replication (Murray and Szostak 1985), proteins that glue sister chromatids together, or a combination of these factors. Whereas catenation is dispensable for cohesion, at least in yeast (Koshland and Hartwell 1987), several proteins have been identified during the last few years that are essential for mitotic sister chromatid cohesion and/or its regulation. Most of these proteins are conserved among eukaryotes, and have been analysed in detail in budding yeast (*Saccharomyces cerevisiae*) (Table 1). In yeast, at least four proteins contribute stoichiometrically to a multi-subunit complex, called cohesin, which is essential for sister chromatid cohesion: Mcd1/Scc1, Scc3, Smc1 and Smc3 (Guacci et al. 1997; Michaelis et al. 1997; Toth et al. 1999). The cohesin complex binds to chromosomes from late G1 onwards (Toth et al. 1999), and cohesin-mediated links between sister chromatids are established after passage of the replication forks, with the catalytic help of Eco1/Ctf7 (Skibbens et al. 1999; Toth et al. 1999; Uhlmann et al. 1999). The cohesin complex probably binds to chromosomes by direct interaction with specific DNA sequences. Smc1 and Smc3 belong to an ancient family of modulators of chromosome structure; they form heterodimers and are capable of binding to chromatids, possibly

Correspondence to: C. Heyting
e-mail: Christa.Heyting@molcelgen.el.wau.nl

Table 1. Budding yeast proteins with a role in mitotic sister chromatid cohesion are conserved among eukaryotes

Protein (*S. cerevisiae*)	Role in cohesion	Homologous proteins (species)	References
Mcd1/Scc1	Cohesin		Guacci et al. (1997); Michaelis et al. (1997)
		Rad21 (*S. pombe*)	Birkenbihl and Subramani (1992)
		hHR21 (*Homo sapiens*)	McKay et al. (1996)
		XRAD21 (*Xenopus laevis*)	Losada et al. (1998)
		PW29 (*Mus musculus*)	Darwiche et al. (1999)
Scc3/Irr1	Cohesin		Toth et al. (1999)
		Rec11 (*S. pombe*)	de Veaux and Smith (1994)
Smc1	Cohesin		Michaelis et al. (1997)
		bSMC1 (*Bos bovis*)	Stursberg et al. (1999)
		XSMC1 (*X. laevis*)	Losada et al. (1998)
		hSMC1 (*H. sapiens*)	Schmiesing et al. (1998)
		mSMCB (*M. musculus*)	Darwiche et al. (1999)
Smc3	Cohesin		Michaelis et al. (1997)
		SUDA (*A. nidulans*)	Holt and May (1996)
		DCAP (*D. melanogaster*)	Hong and Ganetzky (1996)
		bSMC3 (*B. bovis*)	Stursberg et al. (1999)
		XSMC3 (*X. laevis*)	Losada et al. (1998)
		hSMC3 (*H. sapiens*)	Schmiesing et al. (1998)
		mSMCD (*M. musculus*)	Darwiche et al. (1999)
Scc2	Adherin		Michaelis et al. (1997); Toth et al. (1999)
		Mis4 (*S. pombe*)	Furuya et al. (1998)
		Rad9 (*C. cinereus*)	Seitz et al. (1996)
		Nipped-B (*D. melanogaster*)	Rollins et al. (1999)
Eco1/Ctf7	Establishment	–	Toth et al. (1999); Skibbens et al. (1999)
Pds1	Securin		Yamamoto et al. (1996);
		PTTG (*X. laevis*): functional homologue?	Cohen-Fix et al. (1996; Ciosk et al. (1998) Funabiki et al. (1996)
		Cut2 (*S. pombe*): functional homologue?	Zou et al. (1999)
Esp1	Separin		Ciosk et al. (1998; Uhlmann et al. (1999)
		BIMB (*A. nidulans*)	May et al (1992)
		Cut1 (*S. pombe*): functional homologue?	Funabiki et al. (1996)

through transient interaction with the adherin Scc2 (Toth et al. 1999). In vitro, Smc1 binds to DNA, in particular to double-strand DNA with AT-rich sequences and to sequences with a tendency to form secondary structures (Akhmedov et al. 1998). In yeast, Smc1 and Mcd1/Scc1 bind predominantly to AT-rich regions (Blat and Kleckner 1999; Tanaka et al. 1999). In yeast centromeres, a highly specialised chromatin structure is required for binding of Mcd1/Scc1 (Tanaka et al. 1999). Proteins that determine chromatin conformation may thus influence cohesion. Mcd1/Scc1 and Scc3 possibly provide intermolecular links between Smc3/Smc1 heterodimers that are bound to different sister chromatids (Toth et al. 1999). In short, as we will discuss in more detail below, most available evidence suggests that cohesins physically connect sister chromatids, but direct evidence for this is lacking.

Dissolution of sister chromatid cohesion depends on the anaphase-promoting complex APC, which ligates ubiquitin to proteins and thus targets them for proteolysis (Irniger et al. 1995). APC can associate with so-called activator proteins, which specify which protein(s) will be ubiquinated, and when. In the mitotic cycle, Pds1 becomes an APC target (Cohen-Fix et al. 1996) upon association of the activator protein Cdc20 with APC (Visintin et al. 1997). Pds1 inhibits anaphase by capturing the separin protein Esp1 (Ciosk et al. 1998). Upon liberation from Pds1, Esp1 promotes cleavage of Mcd1/Scc1 (Uhlmann et al. 1999). This probably destabilises the cohesin complex, so that Mcd1/Scc1 and Scc3 dissociate from the chromatin (Michaelis et al. 1997; Toth et al. 1999). Cohesin, in particular Mcd1/Scc1, is thus a target for the regulation of cohesion, but it is possible that other proteins are responsible for the mechanical strength of cohesion.

In yeast, dissociation of Mcd1/Scc1 from the chromatin coincides with the actual separation of sister chromatids at the onset of anaphase (Michaelis et al. 1997; Toth et al. 1999). Before chromatid disjunction, between S-phase and G2/M, the ratio of Mcd1/Scc1 and Smc1 in centromeres to Mcd1/Scc1 and Smc1 in the arms increases (Blat and Kleckner 1999). The relative amount of cohesin in centromeric regions possibly increases during G2/M-phase because higher order chromosome condensation causes some loss of cohesin from the arms, although it cannot be excluded formally that centromeres bind more cohesin during G2/M. Even so, minimal yeast centromeres by themselves cannot withstand the forces exerted by the spindle microtubules; they also need some flanking (arm) cohesion for this (Tanaka et al.

1999). Although cohesin is more abundant in centromeric regions than in the arms of yeast G2/M chromosomes, there are no indications that release of arm and centromeric cohesion is differentially regulated. In yeast cells arrested in G2/M-phase by a microtubule-depolymerising agent (nocodazole), sister chromatids remain associated along their arms and centromeres (Guacci et al. 1994).

In higher eukaryotes, differentiation of cohesion along mitotic metaphase chromosomes is more pronounced than in yeast. In contrast to yeast chromosomes eukaryotic chromosomes visibly lose arm cohesion before centromeric cohesion during mitosis in some animal cells (Sumner 1991). Moreover, mammalian cells arrested in metaphase by a microtubule-depolymerising agent (colchicine) separate sister chromatid arms but maintain centromeric cohesion (reviewed in Rieder and Cole 1999). Such a differentiation in cohesion along the chromosome might be mediated by local differences in the affinity or concentration of residual cohesin-binding sites, and/or through association of specific proteins (reviewed in Miyazaki and Orr-Weaver 1994; Tanaka et al. 1999). In *Drosophila*, the Mei-S332 protein localises to the centromeres during prometaphase before microtubules attach to the kinetochores (Tang et al. 1998) and dissociates from the centromeres at anaphase when sister chromatids disjoin (Moore et al. 1998). Mei-S332 thus possibly contributes to maintenance of centromeric cohesion when chromosomes align on the metaphase plate (congression).

It is uncertain whether cohesins fulfil similar roles in mitotic chromosome segregation of higher eukaryotes (reviewed by Nasmyth 1999). In *Xenopus* extracts, most cohesin dissociates already from the chromosomes in mitotic prometaphase (Losada et al. 1998). Possibly, a small fraction of cohesin links persists and contributes to proper chromosome segregation in animal cells, or cohesin complexes have no role in chromosome segregation in animal mitosis but function only in G2, for instance in DNA repair. Other factors would then maintain cohesion until anaphase in higher eukaryotes. Catenation and association of heterochromatic domains have been considered as such factors. Sister chromatids are catenated after DNA replication, and require topoisomerase II for separation (reviewed by Holm 1995), in particular of the arms, and not of the centromeres (Funabiki et al. 1993; reviewed by Rieder and Cole 1999). However, there is no positive evidence that catenation is required for maintenance of cohesion in higher eukaryotes. Association of heterochromatic domains contributes to cohesion of sister chromatids throughout their length but in particular to centromeric cohesion (reviewed by Allshire 1997). However, it is possible that such associations are due to the ability of heterochromatin of the appropriate conformation to bind Mcd1/Scc1 (Tanaka et al. 1999).

In many higher eukaryotes mitotic centromeric and arm cohesion are differentially regulated (Bardhan 1997; Rieder and Cole 1999). In *Drosophila*, at least two genes, *pim* (*pimples*) and *thr* (*three rows*), are specifically required for release of centromeric cohesion (Stratmann and Lehner 1996). Furthermore, sister chromatid separation depends on the presence of tension on all kinetochores (Nicklas et al. 1995). Bipolar tension leads to loss of some phosphoepitope(s) from kinetochores in mammalian cells (Gorbsky and Ricketts 1993). These epitope(s) are not found on chromatid arms; they probably emit a signal that prevents release of centromeric cohesion (Campbell and Gorbsky 1995).

Mitotic sister chromatid cohesion and recombinational repair

While centromeric cohesion primarily serves disjunction, arm cohesion plays additional roles in recombinational repair. Various mutants with defects in sister chromatid cohesion are also radiation sensitive and/or defective in DNA double-strand break (DSB) repair (Table 2). Furthermore, two cohesin components, Smc1 and Smc3, are part of the recombinational repair complex RC1 (Jessberger et al. 1996; Stursberg et al. 1999), and Smc1/3 heterodimers promote re-annealing of complementary DNA strands (Jessberger et al. 1996).

Sister chromatid cohesion and recombination are linked in various ways: at the level of cell cycle regulation, overall chromosome organisation and individual protein complexes or proteins (Table 2).

Cohesins provide links between recombination and cohesion at all these levels (reviewed in Strunnikov and Jessberger 1999). At the chromosomal level, they provide the proximity of an undamaged template for recombinational repair and possibly enable communication between sister chromatids: if one chromatid is damaged, the sister has to be prepared for repair. The presence of common components in cohesin and recombination complexes furthermore indicates that sister chromatid cohesion and recombinational repair make use of similar mechanisms. For example, stabilisation of recombination intermediates might require a mechanism resembling that used for sister chromatid cohesion. Some non-cohesin proteins involved in recombinational repair, including Rad50, share structural features with Smc proteins (reviewed in Jessberger et al. 1998; Strunnikov and Jessberger 1999) and possibly recognise similar DNA structures and/or fulfil similar steps.

Individual cohesin complexes may furthermore function as nucleation sites for the assembly of DNA repair complexes, and this in turn may have resulted in a sisterbiased recombinational repair pathway. The Rad50/Xrs2/Mre11 complex in yeast (see Fig. 1) has a role in various mitotic DSB repair pathways, including recombinational repair (reviewed in Pâques and Haber 1999). *rad50* mutants of budding yeast are radiation sensitive and hyperrec (Malone and Hoekstra 1984; Malone et al. 1990): they display more interchromosomal mitotic recombination than wild type. Possibly, certain DNA damage cannot be repaired on the sister chromatid in these mutants and is therefore channelled into interchromosomal repair pathways (Pâques and Haber 1999). Interestingly, mutants of *RAD21* (the *MCD1/SCC1*-homologous gene of fission yeast) are also hyperrec (Grossenbacher-Grunder and Thuriaux

Table 2. Links between cohesion and recombinational repair in mitosis

Protein (species)	Link with cohesion	Link with recombinational repair	References
Pds1 (*S. cerevisiae*)	Separin	*pds1-1* mutant: no inhibition of cell cycle progression in response to DNA damage; γ-radiation sensitive	Yamamoto et al. (1996)
Mcd1/Scc1 (*S. cerevisiae*)	Cohesin component	*mcd1-1* mutant: radiation sensitive	Guacci et al. (1997)
Rad21 (*S. pombe*)	Homologous to Mcd1/Scc1	*rad21–45* mutant: γ-radiation sensitive, defective in DSB repair; *rad21-45* mutant: hyperrec	Birkenbihl and Subramani (1992) Grossenbacher-Grunder and Thuriaux (1981)
bSMC1 and bSMC3 (*B. bovis*)	Homologous Smc1 and Smc3	Hetero-dimer promotes re-annealing of complementary DNA strands in vitro; components of recombinational repair complex RC1	Jessberger et al. (1996) Stursberg et al. (1999)
Rad9 (*C. cinereus*)	Homologous to Scc2	*rad9-1* mutant: γ-radiation sensitive; *Rad9* mRNA: induced after γ-radiation	Zolan et al. (1988) Seitz et al. (1996)
Spo76[a] (*S. macrospora*)	*spo76-1* mutant: transient cohesion defect at prometaphase	*spo76-1* mutant: UV- and X-ray sensitive	van Heemst et al. (1999) Moreau et al. (1985; Huynh et al. (1986)
BimD[a] (*A. nidulans*)	*bimD5*, *bimD6* mutants: blocked at metaphase/anaphase; *sudA* (homologous to *Smc3*): suppressor of segregation defect of *bimD6*	*bimD5*, *bimD6* mutants: UV and MMS sensitive	Denison et al. (1993) Holt and May (1996)

[a] Spo76 and BimD are homologous proteins

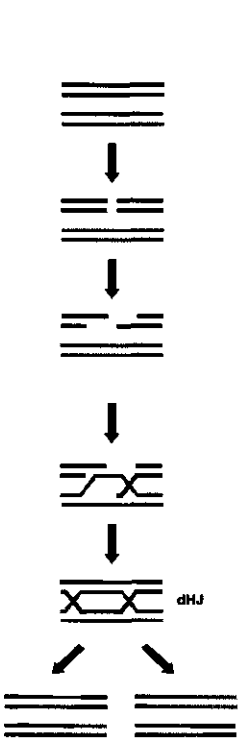


Fig. 1. The double-strand break repair model of meiotic recombination (Szostak et al. 1983). For each step it has been indicated which proteins of budding yeast are most likely involved. Inactivation of each of the *underlined* proteins causes coordinated defects in recombination and sister chromatid cohesion. In the last step of this pathway, resolution of the double Holliday junction (*dHJ*) can yield recombinant molecules with exchange of flanking markers (crossover) (*right*) or without exchange of flanking markers (*left*). For details and discussion, see reviews by Smith and Nicolas (1998) and Pâques and Haber (1999)

1981). Possibly, Mcd1/Scc1 (Rad21) contributes to the preference for the sister chromatid as template in Rad50-mediated recombinational repair.

Spo76/BimD provides an example of a link between recombination and cohesion at the level of chromosome organisation (Table 2). The *Sordaria* Spo76 protein is chromosome associated, except in mitotic and meiotic metaphase and anaphase (van Heemst et al. 1999). In mitotic prometaphase, the non-null allele *spo76-1* causes regional, co-ordinate defects in chromosome condensation and sister chromatid cohesion (van Heemst et al. 1999). In *Aspergillus nidulans* two non-null alleles, *bimD5* and *bimD6*, cause a block at the mitotic metaphase/anaphase transition (Denison et al. 1993). These results suggests that Spo76/BimD influence the strength of cohesion. Because *SUDA* (orthologous to *SMC3*) carries an extragenic suppressor mutation for *bimD6* (Holt and May 1996), Spo76/BimD probably influences cohesion through interaction with cohesin. Importantly, the defects in cohesion and chromosome organisation are accompanied by radiation sensitivity (Table 2). Because cohesion is important for recombinational DNA repair (see above), radiation sensitivity could be a straightforward effect of defective cohesion. It is also possible that repair of radiation-induced DNA damage causes local alterations in chromatin conformation that put cohesion to the test and aggravate the cohesion defect in these mutants. Chromosome condensation might also put a strain on cohesion and result in local loss of cohesion in mitotic prometaphase of *spo76-1* mutants.

Cohesion and recombination are also linked through the cell cycle. The cell monitors DNA damage and progression of recombination and repair and relates it to cell cycle progression, whereas cohesin is an important target for the cell cycle regulatory machinery (reviewed by Nasmyth 1996, 1999). Pds1 is not only important for inhibiting the release of cohesion until the metaphase/anaphase transition but is also directly involved in the mitotic G2 DNA damage cell cycle checkpoint. The temperature-sensitive *pds1-1* mutant of budding yeast (Table 2) is unable to inhibit anaphase and other aspects of cell cycle progression (cytokinesis, DNA replication and bud formation) in response to DNA damage. *pds1-1* mutants are therefore γ-irradiation sensitive (Yamamoto et al. 1996).

The link between cohesion and recombination manifests itself by the preference of all analysed eukaryotes for the sister chromatid above the homologous chromosome as template for mitotic DSB repair (Latt 1981; Kadyk and Hartwell 1993; Richardson et al. 1998). In yeast, the major recombination repair pathway in diploid G2 utilises the sister chromatid and requires Rad54 (Arbel et al. 1999), whereas a minor pathway is homologue oriented and depends on Rad54 and/or a paralogous protein, Tid1/Rhd54 (Klein 1997; Arbel et al. 1999).

A bias for the sister chromatid in recombinational repair prevents various problems for the mitotic cell. It precludes ectopic recombination, and obviates segregation problems. Interhomologue crossovers will give rise to a bivalent configuration (compare Fig. 2A), which will probably hamper the sister kinetochores of the crossover chromosomes in capturing microtubules from opposite poles of the spindle (Chua and Jinks-Robertson 1991). A bias for the sister chromatid will furthermore prevent loss of heterozygosity distal to the crossover. Loss of heterozygosity is a problem if homologous chromosomes are not entirely equivalent because of heterozygosity or genomic imprinting (Moulton et al. 1996). These disadvantages of mitotic crossing over probably not only favoured the development of an intersister bias in mitotic recombinational repair but also enhanced the development of specialised mechanisms for dealing with the interhomologue interactions that nevertheless occur. In mammalian cells, almost all interhomologue interactions result in gene conversions rather than crossovers (Richardson et al. 1998), possibly because repair mechanisms predominate that primarily yield conversions, such as synthesis-dependent strand annealing (SDSA; reviewed in Pâques and Haber 1999). In yeast, the *RAD9* gene prevents homology-directed reciprocal translocations (Fasullo et al. 1998), presumably by activating enzymes of the non-homologous end-joining pathway (Mills et al. 1999). If, despite these precautions, mitotic crossovers are formed, the weakness of mitotic arm cohesion or the release of arm cohesion before centromeric cohesion may facilitate the resolution of mitotic bivalents at the mitotic metaphase/anaphase transition.

To summarise, the role of cohesins in recombinational repair is conserved throughout eukaryotes and includes the direction of recombinational repair towards the sister chromatid in mitotically dividing cells.

Meiosis

Meiotic recombination and chromosome behaviour

Meiosis is responsible for two essential features of the sexual life cycle: the transition from the diploid to the haploid state and the generation of new combinations of alleles.

Meiosis has probably evolved from a mitosis-like process by adaptation of the cell cycle and chromosome behaviour (see discussion in Kleckner 1996). In the mitotic cycle, one round of DNA replication is followed by one nuclear division, whereas in meiosis, a single S-phase is followed by two successive nuclear divisions, meiosis I and II. Most differences between chromosome behaviour in mitosis and meiosis I concern recombination and the relation between sister chromatids. Recombination occurs at a 100-to 1000-fold higher frequency in meiosis than in mitosis. The meiotic prophase cell actively initiates recombination, and it does so preferentially at certain chromosomal loci called hotspots. In meiosis, recombination is directed preferentially towards the homologous chromosome rather than the sister chromatid. A large proportion of meiotic recombination events are resolved as crossovers, at least in yeast. The distribution of crossovers along the chromosomes is controlled in such a way that there is at least one crossover (obligate crossover) per pair of homologues (biva-

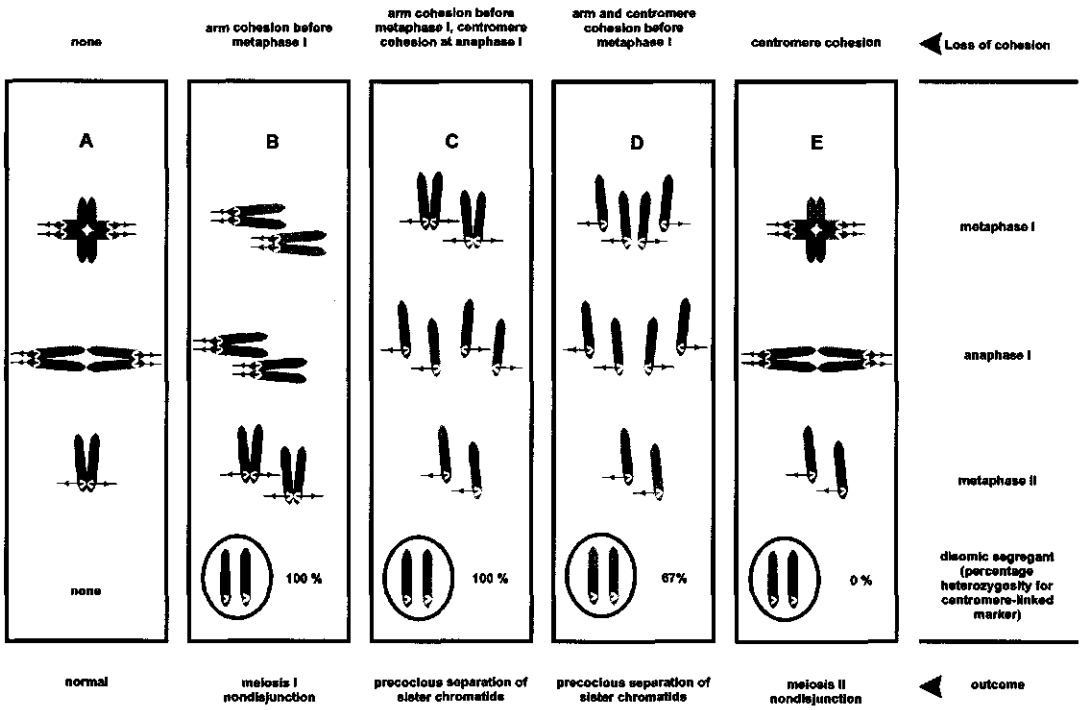


Fig. 2A–E. Loss of arm cohesion and/or centromere cohesion and the effects on chromosome disjunction and segregation of centromere-linked markers in meiosis. At the *top* of the figure is indicated where and when cohesion is lost. Panel **A** shows chromosome segregation if cohesion is normal. Panels **B–E** show aberrant segregation patterns resulting from various types of cohesion loss. Each of these panels shows only one of the possible segregation patterns that result from loss of cohesion and will yield a disomic ascospore. Kinetochores are indicated as *white, cup-shaped entities*, and non-sister chromatids are differently *shaded*. The *ovals* at the bottom of panels **B–E** represent disomic ascospores that can arise from aberrant segregation; beside these ovals is indicated the percentage of all disomic ascospores that will be heterozygous for a centromere-linked marker. **A** If cohesion is normal, recombined homologous chromosomes disjoin at meiosis I and sister chromatids at meiosis II to form haploid segregants. **B** If arm cohesion, but not centromeric cohesion, is lost before metaphase I, homologous chromosomes can nondisjoin at meiosis I, whereas chromatids will separate normally (equationally) at meiosis II; this can lead to disomic ascospores, of which 100% will be heterozygous for centromere-linked markers of the nondisjoined chromosome. Panels **C–E** show chromosome segregation patterns resulting from other types of cohesion loss. The four types of aberrant chromosome segregation that can occur are: **B** meiosis I nondisjunction of homologous chromosomes; **C, D** precocious separation of sister chromatids; panel **C** shows precocious separation of sister chromatids at meiosis I, with equational sister segregation, and panel **D** shows random segregation of sister chromatids at meiosis I. Note that disomics resulting from meiosis I segregation errors (panels **B–D**) are predominantly heterozygous for centromere-linked markers, whereas disomics resulting from meiosis II nondisjunction (panel **E**) are 100% homozygous for centromere-linked markers

lent); if multiple crossovers occur, they are maximally spaced (crossover interference). Finally, most meiotic recombination events occur in the context of a prominent proteinaceous structure, the synaptonemal complex (SC) (reviewed by Heyting 1996). Synaptonemal complexes are assembled between homologous chromosomes during meiotic prophase. First, a single axial element (AE), which is shared by the two sister chromatids, is assembled along each chromosome; subsequently, the AEs of homologous chromosomes are connected by numerous transverse filaments to form the structure of an SC. As is discussed in more detail below, SCs (or components thereof) have a role in steering meiotic recombination in the right direction (the homologous chromosome) and in regulating the number and distribution of crossovers along the bivalents.

Most species form on average about two crossovers per bivalent. The crossovers serve a dual role: they yield new combinations of alleles, and they have a mechanical role in the most specific feature of meiotic chromosome behaviour: the disjunction of homologous chromosomes at meiosis I.

Meiotic sister chromatid cohesion and chromosome disjunction

Disjunction of homologous chromosomes in meiotic anaphase I requires that bivalents rather than individual chro-

mosomes line up on the metaphase I spindle (Fig. 2A; reviewed in Moore and Orr-Weaver 1998). The two sister kinetochores of a meiotic metaphase I chromosome do not separate, as in mitosis, but they act as a single unit and retain the same orientation, so that they attract microtubules from only one pole of the metaphase I spindle. The bivalent orients itself because the still united sister kinetochores of the homologous chromosome catch microtubules from the opposite pole. The crossover(s) and the cohesion between sister chromatid arms distal to the crossover(s) (reviewed by Maguire 1990) prevent a bivalent falling apart when it experiences the opposite poleward pulling forces of the microtubules during congression (Fig. 2A). At anaphase I, arm cohesion is released, so that homologous chromosomes disjoin (reductional division). Centromeric cohesion is maintained until anaphase II. Between anaphase I and metaphase II, the orientation of the sister kinetochores changes so that they can catch microtubules from opposite poles of the metaphase II spindle. Finally, at anaphase II, cohesion at the centromeres is lost so that sister chromatids segregate (equational division).

Thus, as in mitosis of higher eukaryotes, in meiosis sister chromatid cohesion is released in two steps. However, in meiosis, loss of cohesion is spread over two divisions: arm cohesion distal to crossovers is released in meiosis I and centromeric cohesion in meiosis II. Furthermore, sister kinetochores retain the same orientation and act as a single unit at meiosis I, and lose co-orientation between anaphase I and metaphase II (Suja et al. 1999). In budding yeast, the *SPO13* gene plays a pivotal role in these meiotic adaptations of chromosome behaviour through modification of the cell cycle (see below).

Meiotic recombination

Meiotic recombination is initiated by double-strand DNA scission, and probably proceeds largely according to the DSB repair model (Fig. 1) of Szostak et al. (1983) (reviewed by Smith and Nicolas 1998; Pâques and Haber 1999).

The endonuclease that makes meiotic DSBs is almost certainly Spo11, a topoisomerase II-like enzyme, which is thought to cleave double-stranded DNA by a transesterification reaction (Bergerat et al. 1997; Keeney et al. 1997) and remains covalently attached to the 5′ ends of the break (de Massy et al. 1995; Keeney and Kleckner 1995; Liu et al. 1995). Several additional proteins,which probably create the right preconditions and context for meiotic DSB, are involved in this step.

Spo11 is subsequently removed, and the 5′ ends are resected. According to the model of Szostak et al. (1983), one of the resulting 3′ tails (Fig. 1) then invades a homologous region of a donor DNA duplex, displacing a small D-loop. This D-loop would then be enlarged by repair synthesis primed from the invading 3′ end; the enlarged D-loop would eventually contain sequences complementary to the 3′ end from the other side of the gap, and anneal. A second round of repair synthesis would then follow from this 3′ end (Szostak et al. 1983). Some or all DSBs are ultimately converted to a form of joint molecule (JM) that consists of two DNA duplexes connected by a double-Holliday junction (Fig. 1, dHJ). In suitable experiments, JMs between sister chromatids (intersister JMs) can be distinguished from interhomologue JMs. The preference for the homologous chromosome above the sister chromatid could therefore be demonstrated at this step in meiotic recombination (Schwacha and Kleckner 1994, 1997): in wild-type yeast meiosis, the interhomologue JMs are several-fold more frequent than intersister JMs (Schwacha and Kleckner 1997). The JMs are finally resolved into mature recombinant DNA molecules.

Some observations on meiotic recombination are not easily explained by the Szostak model; for the later steps in meiotic recombination alternative mechanisms have been considered in detail, in particular SDSA (see review by Pâques and Haber 1999 and references therein).

Meiotic sister chromatid cohesion and recombination

Most genes involved in mitotic recombinational repair of DSBs are also required for meiotic recombination (Fig. 1). However, recombinational repair of DSBs is heavily modified and adapted in meiosis, as is evident from the requirement of meiosis-specific genes for many steps in the recombination process (Fig. 1). These adaptations include an altered relationship between recombination and sister chromatid cohesion. We have argued that in mitosis cohesin complexes are involved in both cohesion and sister chromatid-based recombinational repair. In meiosis, recombination has to be directed towards the homologue but, at the same time, arm cohesion has to be maintained and possibly even reinforced to ensure correct reductional segregation of chromosomes at meiosis I (Fig. 2A). One possible solution to this problem would have been to unlink meiotic recombination from sister chromatid cohesion, but this has not happened: several genes have a role in both meiotic recombination and sister chromatid cohesion (Table 3), including genes involved in mitotic cohesion and DNA repair (compare Tables 1, 2 and 3).

Recently, it has turned out that not only the recombination machinery but also the cohesin complex has been modified in meiosis. In budding and fission yeast, a meiotic paralogue of Mcd1/Scc1, called Rec8, is required for meiotic sister chromatid cohesion and recombination. Fission yeast (but not budding yeast) also has a meiosis-specific paralogue of Scc3, called Rec11, which participates in similar functions to Rec8, but also in distinct functions (Krawchuk et al. 1999). Furthermore, a mammalian, testis-specific paralogue of Smc1, called Smc1β, has been identified, which could represent a meiosis-specific variant (Revenkova et al., personal communication).

Role of cohesin and related proteins in meiosis

REC8 was identified in fission yeast as a meiosis-specific gene involved in recombination (de Veaux et al. 1992),

Table 3. Examples of meiotic cohesion mutants: comparison of defects in meiotic sister chromatid cohesion, meiotic recombination and axial element/synaptonemal complex formation. (AI anaphase I, MI, metaphase I, N.D., not determined, RN recombination nodule)

Mutation (species)	Meiotic cohesion[a]	Meiotic recombination[a]	AE/SC morphology[a]
red1Δ (*S. cerevisiae*)	Missegregation of recombined chromosomes (1);) loss of arm cohesion before AI (2)	Reduction in DSB initiation (3) and crossovers and gene conversions (4)	No AEs, no SC (1)
mek1Δ (*S. cerevisiae*)	Loss of arm cohesion before AI (2)	Reduction in DSB initiation (3) and crossovers and gene conversions (5, 6)	Full-length AEs, but multiple, short stretches of SC (5)
mer1 (*S. cerevisiae*)	Missegregation of recombined chromosomes (7)	Reduction in DSB initiation (8) and crossovers and gene conversions (7, 8)[b]	Full-length AEs, but no SC (9)
med1-1 (*S. cerevisiae*)[c]	Missegregation of recombined chromosomes (10)	Reduction in crossovers and gene conversions (10)	Apparently normal SC (10)
dis1-1 and dis1-2 (*S. cerevisiae*)	Missegregation of recombined chromosomes (11); sister centromeres probably segregate equationally at AI (11)	Not defective (11)	N.D.
tam1/ndj1 (*S. cerevisiae*)	Missegregation of recombined chromosomes (12)	Defective crossover interference on small (12) but not larger (13) chromosomes	Delay in the formation of AEs (13) and SC (12, 13)
spo13-1 (*S. cerevisiae*)	Sister centromeres segregate equationally in a one division meiosis (14)	Not defective (14)	SC present in *spo12-1, spo13-1* double mutant (15, 16)
smc3-42 (*S. cerevisiae*)	Loss of centromeric cohesion before MI (17)	DSBs: normally formed but hyperresected and probably not repaired at all (17)	No AEs, no SC (17)
rec8Δ (*S. cerevisiae*)	Loss of arm and centromeric cohesion before MI (17)	DSBs: normally formed but hyperresected and probably not repaired at all (17)	No AEs, no SC (17)
rec8-110 and rec8-Δ (*S. pombe*)	Loss of arm cohesion before MI (18); sister centromeres segregate equationally at AI (19)	Reduction in crossovers (20, 21, 22)	Linear elements: short stretches and aggregates (18, 22)
dy1 (*Zea mays*)	Male meiosis: loss of arm cohesion before MI; sister centromeres segregate equationally at AI (23, 24)	Not defective (23)	SC central region: slightly wider (25), prematurely disassembled (23)
dsy1 (*Z. mays*)	Loss of arm cohesion before MI (26)	Crossovers occur, frequency N.D. (26)	SC: incomplete; SC central region: slightly wider, less robust, and prematurely disassembled (26)
ord (alleles 1-12) (*D. melanogaster*)	Loss of arm and centromeric cohesion before MI (27, 28, 29, 30)	Reduction in crossovers (27, 29, 30)	N.D.
meiS332 (alleles 1-10) (*D. melanogaster*)	Loss of centromeric cohesion (28, 31, 32, 33)	Not defective (31, 32)	N.D.
spo76-1 (*Sordaria macrospora*)	Loss of arm and centromeric cohesion before MI (34, 35)	Strong reduction in late RNs (35, 36), but only slight reduction in Rad51/ Dmc1 foci (35)	AEs: some segments thin and split, others of normal width and synapsed (34, 35)

[a] References: 1, Rockmill and Roeder (1990); 2, Bailis and Roeder (1998); 3, Xu et al. (1997); 4, reviewed by Mao-Draayer et al. (1996); 5, Rockmill and Roeder (1991); 6, Leem and Ogawa (1992); 7, Engebrecht et al. (1990); 8, Storlazzi et al. (1995); 9, Engebrecht and Roeder (1990); 10, Rockmill and Roeder (1994); 11, Rockmill and Fogel (1988); 12, Chua and Roeder (1997); 13, Conrad et al. (1997); 14, Klapholz and Esposito (1980); 15, Moens (1974); 16, Moens et al. (1977); 17, Klein et al. (1999); 18, Molnar et al. (1995); 19, Watanabe and Nurse (1999); 20, Ponticelli and Smith (1989); 21, de Veaux and Smith (1994); 22, Parisi et al. (1999); 23, Maguire (1978); 24, Maguire (1990); 25, Maguire et al. (1991); 26, Maguire et al. (1993); 27, Mason (1976); 28, Goldstein (1980); 29, Miyazaki and Orr-Weaver (1992); 30, Bickel et al. (1997); 31, Sandler et al. (1968); 32, Davies (1971); 33, Kerrebrock et al. (1992); 34, Moreau et al. (1985); 35, van Heemst et al. (1999); 36, Zickler et al. (1992)

[b] The gene conversion defect but not the crossing over defect of the *mer1* mutant can be completely restored by overexpression of *MER2* (7, 8)

[c] *med1-1* is an allele of the *DMC1* gene of *S. cerevisiae* (see Fig. 1)

assembly of linear elements (structures equivalent to axial elements of the SC), and sister chromatid cohesion (Molnar et al. 1995; Watanabe and Nurse 1999). An orthologous gene with similar functions was later discovered in budding yeast (Klein et al. 1999).

Rec8 is probably loaded onto the chromatin during premeiotic S-phase (Watanabe and Nurse 1999); in meiotic prophase of *Schizosaccharomyces pombe*, Rec8 is localised in foci all over the chromatin (Parisi et al. 1999), with the highest concentration around the centromeres (Watanabe and Nurse 1999). As meiosis I proceeds, the protein is gradually lost from the arms, but it persists at the centromeres until metaphase II. In *S. cerevisiae* (Klein et al. 1999), Rec8 lines meiotic prophase chromosomes along their length. At the end of prophase, most Rec8 is lost from the chromosome arms, while it persists at the centromeres until anaphase II, as in *S. pombe*. In *rec8Δ* mutants of *S. cerevisiae*, sister chromatids separate prematurely (i.e. before meiosis I) and then segregate randomly (not equationally). In budding yeast, Rec8 is thus required both for arm and centromeric cohesion during meiosis (compare Fig. 2D). In *rec8* mutants of *S. pombe*, sister chromatids also separate prematurely but then segregate equationally (Watanabe and Nurse 1999). In this species, Rec8 is thus required for maintenance of arm cohesion and the co-orientation of kinetochores at metaphase I but, in the absence of Rec8, sufficient cohesion is retained at the centromeres to allow the chromosomes to align in the spindle and undergo equational segregation (compare Fig. 2C). In budding yeast, Spo13 is responsible for the persistence of Rec8 at the centromeres until the metaphase/anaphase II transition (Klein et al. 1999), as we will discuss in more detail below.

Furthermore, *rec8* mutants of *S. pombe* and *S. cerevisiae* show profound defects in meiotic recombination (Table 3). In *rec8Δ* mutants of *S. cerevisiae*, meiotic DSBs appear with normal kinetics, but they persist and become more extensively resected than in wild type; mature recombinant DNA molecules are not formed (Klein et al. 1999). Rec8 is thus not required for initiation but for some later step in meiotic recombination. Because cohesins have a role in chromosome organisation and in recombination, the question remains to be answered whether *rec8* mutants are incapable of creating the right meiotic context for DSB repair, or whether they are unable to perform some step in the repair process itself, or both.

rec8 mutants are also deficient in linear element/AE formation (Table 3). In *rec8Δ* mutants of budding yeast (Klein et al. 1999) no AEs are formed, and Red1, an AE component, remains dispersed in numerous dots throughout the meiotic prophase nucleus without forming linear structures. In fission yeast, *rec8* mutants make only very short stretches of linear elements, while most linear element material is found in aggregates (Molnar et al. 1995; Parisi et al. 1999).

Rad21 and Mcd1/Scc1, the mitotic paralogues of Rec8 in fission and budding yeast, play less important roles in meiosis. In budding yeast (Klein et al. 1999), Mcd1/Scc1 is expressed in meiosis, but at much lower levels than Rec8. Unlike Rec8, Mcd1/Scc1 does not localise to chromosome cores. In *mcd1/scc1* mutants, meiotic chromosomes segregate with an almost wild-type level of fidelity. However, Mcd1/Scc1 still has some role in meiosis because spore viability is only 50% in *mcd1/scc1* mutants (Klein et al. 1999). In fission yeast (Watanabe and Nurse 1999), overexpression of Rad21 cannot restore the meiotic defects of *rec8* mutants. On the other hand, mitotically expressed Rec8 complements most defects of Rad21-deficient cells of *S. pombe*, albeit that mitotic chromosomes display a slight tendency to segregate reductionally in these cells. Apparently, Rec8 functions in a similar way to Rad21 but has acquired additional roles in meiosis that Rad21 cannot fulfil.

Other components of the mitotic cohesin complex have no meiotic paralogue and participate both in mitosis and meiosis (Tables 2, 3). Smc3, for instance, is involved in the same meiotic functions as Rec8, at least in budding yeast (Klein et al. 1999). Proteins that interact functionally with cohesins may also have a role in both mitosis and meiosis. An example is Spo76 of *Sordaria* and its *Aspergillus* homologue BimD, which are involved in mitotic chromosome disjunction and DNA repair (see above). In *Sordaria*, Spo76 is required for meiotic homologous recombination and AE formation (Moreau et al. 1985; Zickler et al. 1992; van Heemst et al. 1999). The *spo76-1* mutant, which shows regional defects in mitotic sister chromatid cohesion, displays partially split AEs in meiotic prophase (Table 3). The resemblance of the mitotic and meiotic defects of *spo76-1* mutants suggests that the Spo76 protein fulfils similar roles in both types of cell cycle by influencing the strength of cohesion. The same could be true for BimD, which is required for ascospore formation in *Aspergillus* (van Heemst, unpublished observations), and is thus possibly involved in meiosis.

In summary, the cohesin complex is active in meiosis in a modified form. In *S. cerevisiae*, at least one component (Mcd1/Scc1) is partially replaced by a meiosis-specific paralogue (Rec8), whereas in *S. pombe* two components are replaced, Rad21 (by Rec8) and Scc3 (by Rec11). In this modified condition, the cohesin complex functions in linear element/AE formation, meiotic recombination, arm cohesion, centromeric cohesion, and the reductional orientation of kinetochores (at least in fission yeast) at meiosis I.

It remains to be investigated to what extent these conclusions can be extrapolated to higher eukaryotes, in particular with respect to chromosome disjunction. There are some indications that cohesins may not persist along the chromosomes of higher eukaryotes during mitotic metaphase (Losada et al. 1998) (see above), and it remains to be established whether they persist along the arms of meiotic metaphase I chromosomes of these organisms, or whether other factors are responsible for cohesion. This could have implications for the regulation of disjunction. However, there is little reason to doubt that the role of cohesins in mitotic G2-phase (cohesion for recombination) is recruited in meiotic prophase (cohesion for homologous recombination) (see below).

Meiotic sister chromatid cohesion and axial element formation

At first sight, it seems unlikely that cohesin complexes would provide the basis for AE formation. Axial elements are single axial structures that are shared by both sister chromatids. In the mitotic cycle, such shared chromatid axes are not normally observed, and cohesin complexes are not normally seen in mitotic axial structures. However, under certain experimental conditions (cell cycle drugs in combination with a topoisomerase II inhibitor), single chromosomal axes supporting both sister chromatids appear in G2 of mitotically dividing mammalian cells (Gimenez-Abian et al. 1995). We propose that these single intersister axes contain cohesins and that similar single axes arise in meiotic prophase and provide the basis for AEs. There are various indications for this. Grasshopper spermatocytes in early diakinesis (i.e. immediately after AE disintegration) have one single silver-stainable core per homologue, which probably represents the still unseparated chromatid axes (Rufas et al. 1992). This single core differentiates into two cores (one per chromatid) during metaphase I. Furthermore, Smc1 and Smc3 are localised in dots along the AEs of rat SCs, and Smc1 interacts in vivo and in vitro with two AE components of the rat, Scp2 and Scp3 (Eijpe et al. 2000). Spo76 of *Sordaria*, which probably interacts functionally with cohesins (Table 2), is also localised along the AEs during meiotic prophase (van Heemst et al. 1999); *spo76–1* mutants assemble abnormal, partially split AEs (Table 3).

Axial element components, sister chromatid cohesion and recombination

In budding yeast, three meiotic proteins have been identified that localise along AEs: Red1, Mek1 (see Table 3) and Hop1. Red1 localises along meiotic chromosomes wherever AEs are present (Smith and Roeder 1997), whereas Mek1 is a functional kinase that also localises along meiotic prophase chromosomes and can phosphorylate Red1 (Bailis and Roeder 1998; de los Santos and Hollingsworth 1999). Two-hybrid analyses, studies of genetic interactions and co-immunoprecipitations provide ample evidence that these three proteins interact in vivo (see Hollingsworth and Ponte 1997; Smith and Roeder 1997; Bailis and Roeder 1998; de los Santos and Hollingsworth 1999; and references therein), and it seems likely that they cooperate in AE formation and functioning.

red1 mutants (see also Table 3) show a combination of defects that is very informative for the link between meiotic recombination, sister chromatid association and AE formation:

AEs are not detectable (Rockmill and Roeder 1990)

red1 mutants induce meiotic DSBs, albeit at reduced levels (about 25% of the wild-type level; Xu et al. 1997). Apparently, Red1 assists Spo11 in cleaving DNA without being absolutely necessary (Xu et al. 1997).

Double-strand breaks that are induced in *red1* mutants are converted into intersister JMs and interhomologue JMs, but the bias for the formation of interhomologue JMs has turned into an intersister bias; probably, the *red1* mutation abolishes an interhomologue-specific pathway, leaving behind a pathway in which interhomologue bias is absent (Schwacha and Kleckner 1997).

Part of the interhomologue JMs in *red1* mutants are resolved into crossovers, but these do not ensure proper disjunction of homologues at meiosis I (Rockmill and Roeder 1990). Nondisjunction of crossover chromosomes is indicative of a lack of arm cohesion at meiosis I (see Fig. 2). A defect in arm cohesion was also evident in fluorescent in situ hybridization experiments performed on pachytene nuclei of *red1* mutants (Bailis and Roeder 1998).

Centromeric cohesion is probably not affected in *red1* mutants because the spore inviability of *red1* mutants is rescued by the *spo13-1* mutation (Rockmill and Roeder 1988). If the *red1* mutation abolishes centromeric cohesion, one would expect that chromatids would segregate randomly in *spo13 red1* mutants. However, it is possible that Red1 is important for co-orientation of kinetochores in meiosis I.

red1 mutants bypass the so-called pachytene arrest, which is triggered by persistence of certain recombination intermediates and requires Mek1 as well as Red1 (Xu et al. 1997).

Red1 thus plays a pivotal role in meiotic chromosome behaviour. Probably, it forms the physical link between the protein complex that forms the DSBs, the (meiotic) cohesin complex and the AE, and co-ordinates recombination with chromosome behaviour and meiotic cell cycle progression.

Mek1 is a protein kinase that phosphorylates Red1 and depends for its chromosomal localisation on Red1 and Hop1 (Bailis and Roeder 1998). A small amount of Mek1 persists along the chromosomes until metaphase/anaphase I. *mek1* mutants show arm cohesion defects and bypass the pachytene arrest. These effects may arise through defective phosphorylation of Red1.

The *HOP1* gene was identified in a screen for mutants that can perform meiotic intrachromosomal recombination and are defective in interhomologue recombination (Hollingsworth and Byers 1989). *hop1* mutants induce meiotic DSBs, albeit at reduced levels (about 10% of the wild-type level) (Schwacha and Kleckner 1994). However, they form neither detectable heteroduplex DNA (Nag et al. 1995) nor interhomologue JMs and crossovers. They do form intersister JMs, but this is considerably delayed, as if a block on intersister interactions had to be relieved before intersister JMs could be formed (Schwacha and Kleckner 1994). In short, Hop1 functions in the context of the AE and is indispensable for meiotic interhomologue recombinational interactions.

Cohesion and recombination: meiosis versus mitosis

In Fig. 3 and below, we present a partially hypothetical comparison of the roles of cohesins in mitotic and mei-

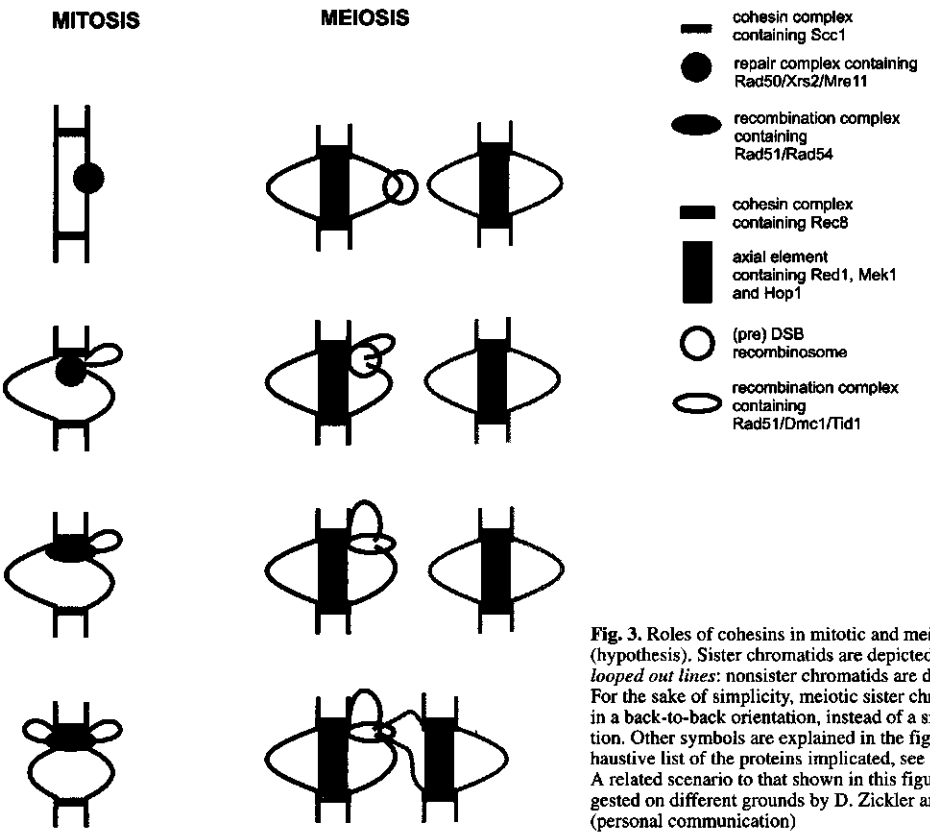


Fig. 3. Roles of cohesins in mitotic and meiotic recombination (hypothesis). Sister chromatids are depicted as *straight or looped out lines*: nonsister chromatids are differently *shaded*. For the sake of simplicity, meiotic sister chromatids are drawn in a back-to-back orientation, instead of a side-by-side orientation. Other symbols are explained in the figure. For a more exhaustive list of the proteins implicated, see Fig. 1 and the text. A related scenario to that shown in this figure has been suggested on different grounds by D. Zickler and N. Kleckner (personal communication)

otic cohesion and recombination, in order to provide an overview of the many gene functions involved.

Meiotic crossover formation versus mitotic recombinational repair: an hypothesis

We suppose that in mitotic G2 cohesins keep sister chromatids precisely aligned. If accidentally a DSB occurs, DNA repair proteins, including the Rad50/Xrs2/Mre11 complex, associate with the DNA ends and prepare them for repair. The protein complex containing the DSB is then transferred to the nearest cohesin complex, possibly through contact between Rad50 and Mcd1/Scc1. Cohesin plus the initial repair complex then attract other proteins of the DSB recombinational repair pathway (Fig. 1). Possibly, cohesin components assist these proteins in searching for homology in the corresponding segment of the sister chromatid and completing recombinational repair. Such a procedure would simplify homology search, avoid unequal sister chromatid exchanges and prevent all the problems of mitotic crossing over discussed above.

Meiotic prophase corresponds (roughly) to mitotic G2. We suppose that cohesin complexes ensure maintenance of sister chromatid cohesion, as in mitosis (see Fig. 3), but that in most complexes Mcd1/Scc1 has been replaced by Rec8. Presumably, the Rec8-containing cohesin complexes then serve as a basis for AE formation, whereas one possible difference between Rec8 and Mcd1/Scc1 function could be that Mcd1/Scc1-containing complexes cannot. Spread meiotic prophase nuclei of yeast *red1* mutants contain long "thin stained structures" with a stretched appearance (Rockmill and Roeder 1990). These could represent the still unseparated sister chromatid axes and contain cohesin complexes. In *red1* mutants of yeast (which do not assemble AEs), Rec8 still localises in rows of dots or thin elongated structures (Klein, personal communication); presumably, these correspond to the stretched, unseparated chromatid axes. Possibly, these Rec8-containing structures attract AE components (in budding yeast: Red1), so that short AE fragments arise (Padmore et al. 1991; Schwarzacher 1997), which later fuse to form full-length AEs. Meiotic recombination is not required for this because yeast *spo11* mutants can form full-length AEs (Loidl et al. 1994). DSBs are induced concomitantly with AE assembly (Padmore et al. 1991). The Rad50/Xrs2/Mre11 complex, together with other factors (Fig. 1), prepares the DSB site for double-strand DNA scission (Ohta et al. 1994) and possibly es-

tablishes contact with the nearest cohesin complex, which in most cases will contain Rec8 and will thus be associated with the AE. Cohesin plus (pre)DSB complex then attract additional proteins for homologous recombination as we supposed for mitotic recombinational repair. In higher eukaryotes, this has been beautifully visualised, first by demonstration of ultrastructurally recognisable protein complexes along AEs (early recombination nodules; reviewed in Carpenter (1988) and later by immunocytochemical localisation of various recombination proteins in early recombination nodules (Anderson et al. 1997) and along AEs (Bishop 1994; Terasawa et al. 1995; Ashley and Plug 1998). If the cohesin complex plus (pre)DSB complex contains Rec8 and is associated with an AE component (in budding yeast: Red1), homology search on the sister chromatid will be blocked, and the already assembled recombination complex has to search for another target. How contact is made with the homologous chromosome is not understood. In yeast, homologous chromosomes are already paired to some extent at the beginning of meiotic prophase (Weiner and Kleckner 1994); various mechanisms, including paranemic joints and alignment of heterochromatic blocks, might help roughly to align homologous chromosomes before DSBs are induced. For the interhomologue recombinational interactions, the yeast proteins Hop1 (see above) and Dmc1 (Schwacha and Kleckner 1997) are indispensable. Hop1 possibly stabilises initial recombination intermediates with the homologous chromosome, and limits resection of the 5′ ends (Kironmai et al. 1998). *hop1* mutants store meiotic DSBs in an unknown form and repair them later on the sister chromatid, presumably after Red1 has disappeared (Schwacha and Kleckner 1994). Once Hop1 has fulfilled its task (which still has to be defined), Mek1 regulates dissociation of Hop1 (Bailis and Roeder 1998), so that the recombination process can proceed and synapsis can follow.

To summarise the possible effects of cohesins on meiotic recombination (hypothesis): cohesin complexes containing Rec8 initiate AE formation by attracting Red1, Hop1 and Mek1. The cohesin complex can associate with the pre-DSB complex and attract proteins for further steps in homologous recombination, but homology search on the sister chromatid is blocked in the presence of Red1. Hop1 and Dmc1 are required for recombination with the homologue, and Mek1, in association with Red1, monitors progression of the recombination process (Xu et al. 1997) and relates this to the cell cycle through phosphorylation of Red1 (and possibly other proteins).

We propose that Mcd1/Scc1-containing cohesin complexes participate in a minor meiotic recombination pathway, which is more similar to mitotic recombinational repair. If, as seems likely (Klein et al. 1999), Mcd1/Scc1-containing complexes do not associate with Red1, this pathway should allow recombination with the sister chromatid. The Mcd1/Scc1-pathway could correspond to the "less differentiated pathway for meiotic recombination" postulated by Schwacha and Kleckner (1997). Defects in such a minor pathway could account for the reduced spore viability (50% of wild type) of *mcd1/scc1* mutants of budding yeast (Klein et al. 1999).

In higher eukaryotes, the relation between cohesion and meiotic recombination could be similar to that in yeast (see also Tables 2 and 3). A human orthologue of Rec8 has been identified and is expressed at high levels in the testis (Parisi et al. 1999). A possible homologue of Hop1 has been found in *Arabidopsis thaliana* (Caryl et al. 2000), and in *Caenorhabditis elegans*, where it localises along chromosome cores during meiotic prophase and meiosis I until the metaphase I-anaphase I transition (Zetka et al. 1999). A (functional) homologue of yeast Red1 has not yet been identified in other eukaryotes but Scp2 of the rat is a candidate (Offenberg et al. 1998), and phosphorylation of an AE component of the rat (Scp3) has been found (Lammers et al. 1995).

Meiotic versus mitotic chromosome disjunction

In meiosis, sister chromatid cohesion and recombinational repair have not only been recruited for crossover formation, but also for chromosome disjunction. This required adaptations at the chromosome level and the cell cycle level.

At the chromosome level, reciprocal recombination events have to be converted into crossovers of chromatid axes: chiasmata (Jones 1987). It is not known how this is accomplished. Probably, interhomologue recombination proceeds not only in association with the axis of the broken chromatid (as we suggest in Fig. 3), but also of the template chromatid. Breakage and reunion of DNA can then be linked directly to breakage and reunion of chromatid axes.

Furthermore, chromosome orientation depends in meiosis I on cohesion of chromatid arms only (Fig. 2A), whereas in mitosis, arm cohesion is relatively weak, and centromeric cohesion is far more important. Cohesion between meiosis I sister chromatids is mediated at least in part by cohesin containing Rec8 (Klein et al. 1999). In budding yeast, Mek1 persists on the chromosomes until the metaphase/anaphase I transition (Bailis and Roeder 1998), whereas in rat two AE components, Scp2 (Schalk 1999) and Scp3 (Moens and Spyropoulos 1995) persist in small amounts between the sister chromatids during meiosis I and possibly reinforce cohesion. Other proteins that might contribute to arm cohesion during meiosis I are the products of maize *DSY1* and *DY1* (Table 3) and as yet unidentified phosphoepitopes between metaphase I sister chromatids of grasshopper (Suja et al. 1999). The importance of arm cohesion distal to chiasmata is evident from the effect of crossover position on meiosis I chromosome disjunction: nondisjunction of human chromosome 21 at meiosis I is correlated with a more distal position of crossovers (Lamb et al. 1997).

At the cell cycle level, the timing of several events has changed to allow utilisation of cohesion and recombination for meiotic chromosome disjunction. In particular, the timing of sister kinetochore orientation, release of arm and centromeric cohesion and DNA synthesis have been adjusted to the two meiotic divisions (see above). In budding yeast, the *SPO13* gene plays a pivotal role in these adaptations.

Meiotic arm and centromeric cohesion depend on Rec8, which is related to Mcd1/Scc1 (see above; Klein et al. 1999). It therefore seems likely that release of arm cohesion in anaphase I and of centromeric cohesion in anaphase II requires Cdc20-activated APC, and involves scission of Rec8 and probably also Mcd1/Scc1. Other factors such as chromosome condensation and loss of phosphoepitopes between sister chromatids (Suja et al. 1999) possibly affect arm cohesion and enhance (but not trigger) its release in anaphase I.

Spo13 probably indirectly influences meiotic centromeric cohesion. In mitosis, overexpression of *SPO13* causes a block in G2-M. The arrested cells degrade Pds1 but not Mcd1/Scc1, and Spo13 therefore acts downstream of Pds1 in the regulatory pathway for release of cohesion (S. Prinz and A. Amon, personal communication). In meiosis I, Spo13 may have a similar effect and indirectly and temporarily protect Rec8 from cleavage by Esp1. The special conformation of the centromere (Tanaka et al. 1999) or the presence of specific centromeric factors such as *Drosophila* Mei-S332 and/or the absence of factors that affect arm cohesion (see above) might explain why protection is confined to the centromeric region. Another factor that may contribute to maintenance of centromeric cohesion during meiosis I is the co-orientation of sister kinetochores: this precludes one factor required for the release of centromeric cohesion, namely bipolar tension (Nicklas et al. 1995) between all sister kinetochores. However, co-orientation alone cannot ensure maintenance of centromeric cohesion through meiosis I: in *Drosophila*, the Mei-S332 protein appears on the centromeres at metaphase I (Moore et al. 1998) and is thus not involved in establishment of sister kinetochore co-orientation. In *mei-S332* mutants, centromeres fall apart immediately after meiosis I (Table 3), and Mei-S332 is thus required for protection of centromeric cohesion against effects that occur from metaphase I onwards (compare Fig. 2E). The yeast *DIS1* gene possibly also contributes to maintenance of centromeric cohesion through meiosis and mitosis (Rockmill and Fogel 1988) (Table 3).

Spo13 furthermore influences cell cycle progression. In mitosis, *SPO13* overexpression causes an arrest in G2-M by interfering with the M-phase promoting factor (MPF) pathway (McCarroll and Esposito 1994). In meiosis, *spo13-1* mutants perform a single division, as if meiosis I is skipped (Klapholz and Esposito 1980). In this single division, chromosomes segregate from almost exclusively equationally (Klapholz and Esposito 1980) to mixed reductionally and equationally (Hugerat and Simchen 1993). In wild type, Spo13 causes a delay in entering meiosis I, which possibly allows the cells to prepare their chromosomes for a reductional division. After meiosis I, Spo13 prevents full return to interphase, so that the cells enter a second division without an intervening S-phase. It has been suggested (McCarroll and Esposito 1994) that Spo13 achieves these effects by decreasing the rate at which transitions between different states of p34 kinase (which forms part of MPF) occur.

It is not known how Spo13 contributes to kinetochore co-orientation. Possibly, the Spo13-mediated delay in entering meiosis I allows the cells to co-orient the sister kinetochores. Analysis of a recently identified allele of *SPO13*, *spo13-23*, revealed that recombination can partially substitute for this aspect of Spo13 function. Recombination-proficient *spo13-23/spo13-23* diploid yeast cells perform two meiotic divisions and produce tetrads, like *SPO13/–* cells. In contrast, (recombination-deficient) *spo11/spo11 spo13-23/spo13-23* diploids perform one equational division, like *spo13-1/spo13-1* cells. Even recombination on a single chromosome affects the segregational behaviour of other chromosomes. Apparently, recombination events produce a diffusable signal that substitutes for the *spo13-23* defect with respect to centromeric cohesion and kinetochore co-orientation during meiosis I (L.H. Rutkowski and R.E. Esposito, personal communication). This would represent another aspect of the altered relationship between sister chromatid cohesion and recombination in meiosis.

Spo13 homologues have not been identified in other organisms. However, McCarroll and Esposito (1994) have pointed out that the vertebrate *c-MOS* oncogene might fulfil similar functions in meiosis to the yeast *SPO13* gene, albeit that the analogy is not perfect: c-Mos inhibits APC (Vorlaufer and Peters 1998), whereas Spo13 appears not to (S. Prinz and A. Amon, personal communication). Because meiosis is always embedded in a cellular developmental pathway (gametogenesis, sporogenesis), it is to be expected that many species-specific variations of the regulation of the meiotic cell cycle exist.

Conclusions

The discovery of the role of cohesins in meiotic recombination and sister chromatid cohesion reveals important connections between large diverse fields of research, covering recombination, DNA repair, mitosis and meiosis. It furthermore demonstrates the pivotal position of cohesins in the regulation of chromosome behaviour and DNA metabolism. Important topics to be investigated include the interactions engaged by cohesins and associated proteins in various states of the cell cycle and under various conditions of genotoxic stress. This will further elucidate the relation between the mitotic cycle and meiosis, and will provide numerous opportunities for connecting cytological observations with intracellular events at the DNA level and vice versa.

Acknowledgements. This work was financially supported by the Netherlands Organisation for Scientific Research (NWO), grants 901 01 094 and 805 18 262, and the EEC, contracts CHRXCT 94 0511 and BI04 CT97 2028. The discussions with the participants of the 4th European Meiosis Meeting have been very stimulating to us in writing this review. We thank several participants for permission to discuss the unpublished results that they presented at this meeting.

References

Aboussekhra A, Chanet R, Adjiri A, Fabre F (1992) Semidominant suppressors of Srs2 helicase mutations of *Sacchromyces cerevisiae* map in the *RAD51* gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins. Mol Cell Biol 12:3224-3234

Akhmedov AT, Frei C, Tsai-Pflugfelder M, Kemper B, Gasser SM, Jessberger R (1998) Structural maintenance of chromosomes protein C-terminal domains bind preferentially to DNA with secondary structure. J Biol Chem 273:24088-24094

Akins RA, Lambowitz AH (1985) General method for cloning *Neurospora crassa* nuclear genes by complementation of mutants. Mol Cell Biol 5:2272-2278

Allshire RC (1997) Centromeres, checkpoints and chromatid cohesion. Curr Opin Genet Dev 7:264-273

Altschul SF, Gish W, Miller W, Myer EW, Lipman DJ (1990) Basic local alignment search tool. Mol Biol 215:403-410

Anaya P, Evans SC, Dai C, Lozano V, May GS (1998) Isolation of the *Aspergillus nidulans sudD* gene and its human homologue. Gene 211:323-329.

Anderson LK, Offenberg HH, Verkuijlen WC, Heyting C (1997) RecA-like proteins are components of early meiotic nodules in lily. Proc Natl Acad Sci USA 94:6868-6873

Arbel A, Zenvirth D, Simchen G (1999) Sister chromatid-based DNA repair is mediated by *RAD54*, not by *DMC1* or *TID1*. EMBO J 18:2648-2658

Ashley T, Plug AW (1998) Caught in the act: deducing meiotic function from protein immunolocalization. In: Handel MA (ed) Meiosis and gametogenesis. Academic Press, San Diego, pp 201-239

Bähler J, Wyler T, Loidl J, Kohli J (1993) Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. J Cell Biol 121:241-256

Bailis JM, Roeder GS (1998) Synaptonemal complex morphogenesis and sister-chromatid cohesion require Mek1-dependent phosphorylation of a meiotic chromosomal protein. Genes Dev 12:3551-3563

Bardhan A (1997) Sister chromatid cohesion during meiosis: Differential mechanisms for arms and centromere coherence. Curr Sci 72:376-382

Basile G, Aker M, Mortimer RK (1992) Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51*. Mol Cell Biol 12:3235-3246

Bell WR, Therrien CD (1977) A cytophotometric investigation of the relationship of DNA and RNA synthesis to ascus development in *Sordaria fimicola*. Can J Genet Cytol 19:359-370

Benjamin CR (1955) Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47:669-687

Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P (1997) An atypical topoisomerase II from archaea with implications for meiotic recombination. Nature 386:414-417

Berse B (1981) Cloning of *Aspergillus* DNA. Selection of a plasmid capable of complementing the *arg3* mutation in *Saccharomyces cerevisiae*. *Aspergillus* Newslett 15:24-26

Bickel SE, Wyman DW, Orr-Weaver TL (1997) Mutational analysis of the *Drosophila* sister-chromatid cohesion protein ORD and its role in the maintenance of centromeric cohesion. Genetics 146:1319-1331

Birkenbihl RP, Subramani S (1992) Cloning and characterization of *rad21* an essential gene of *Schizosaccharomyces pombe* involved in DNA double-strand-break repair. Nucleic Acids Res 20:6605-6611

Bishop DK (1994) RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell 79:1081-1092

Bishop DK, Park D, Xu L, Kleckner N (1992) *DMC1*: A meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell 69:439-456

Blat Y, Kleckner N (1999) Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. Cell 98:249-259

Bos CJ (1996) Somatic recombination. In: Bos CJ (ed) Fungal Genetics; principles and practice. Marcel Dekker Inc, New York, pp 73-95

Brody H, Griffith J, Cuticchia AJ, Arnold J, Timberlake WE (1991) Chromosome-specific recombinant DNA libraries from the fungus *Aspergillus nidulans*. Nucleic Acids Res 19:3105-3109

Callan HG (1972) Replication of DNA in the chromosomes of eukaryotes. Proc R Soc London Ser B 181:19-41

Campbell MS, Gorbsky GJ (1995) Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase. J Cell Biol 129:1195-1204

Carmena M, Abad JP, Villasante A, Gonzalez C (1993) The *Drosophila melanogaster* dodeca-satellite sequence is closely linked to the centromere and can form connections between sister chromatids during mitosis. J Cell Sci 105:41-50

Carpenter ATC (1988) Thoughts on recombination nodules, meiotic recombination, and chiasmata. In: Kucherlapati R, Smith GR (eds) Genetic recombination. Am Soc Microbiol Washington, DC, pp 529-548

Caryl AP, Armstrong SJ, Jones GH, Franklin CH (2000) A homolog of the yeast *HOP1* gene is inactivated in the *Arabidopsis* meiotic mutant *asy1*. Chromosoma 109:62-71

Chae SK, Kafer E (1997) Two *uvs* genes of *Aspergillus nidulans* with different functions in error-prone repair: *uvsI* active in mutation-specific reversion and *uvsC*, a *recA* homolog, required for all UV mutagenesis. Mol Gen Genet 254:643-653

Champe SP, Simon LD (1992) Cellular differentiation and tissue formation in the fungus *Aspergillus nidulans*. In: Rossomando EF, Alexander S (eds) Morphogenesis; an analysis of the development of biological form. Marcel Dekker Inc, New York, pp 63-91

Chardin P, Camonis JH, Gale NW, Van Aelst L, Schlessinger J, Wigler MH, Bar-Sagi D (1993) Human Sos1: A guanine nucleotide exchange factor for Ras that binds to GRB2. Science 260:1338-1343

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. Science 282:699-705

Chua P, Jinks-Robertson S (1991) Segregation of recombinant chromatids following mitotic crossing over in yeast. Genetics 129:359-370

Chua PR, Roeder GS (1997) Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference. Genes Dev 11:1786-1800

Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K (1998) An *ESP1/PDS1* complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell 93:1067-1076

Clutterbuck AJ (1984) Loci and linkage map of the filamentous fungus *Aspergillus nidulans* (Eidam) Winter (n=8). In: O'Brien SJ (ed) Genetic Maps 1984. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 265-273

Clutterbuck AJ (1986) Glasgow stock list of *Aspergillus nidulans* strains 1986. Fungal Genet Newslett 33:59-69

Clutterbuck AJ (1987) *Aspergillus nidulans*, nuclear genes. In: O'Brien SJ (ed) Genetic Maps, Vol. 4. Cold Spring Harbor Laboratory Press, New York, pp 325-335

Clutterbuck AJ (1993) *Aspergillus nidulans* genetic loci (n = 8). In: O'Brien SJ (ed) Genetic maps, Sixth edition. Cold Spring Harbor Laboratory Press, New York, pp 3.71-3.84

Coenen A, Croft JH, Slakhorst M, Debets F, Hoekstra R (1996) Mitochondrial inheritance in *Aspergillus nidulans*. Genet Res Camb 67:93-100

Cohen-Fix O, Peters JM, Kirschner MW, Koshland D (1996) Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev 10:3081-3093

Conrad MN, Dominguez AM, Dresser ME (1997) Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. Science 276:1252-1255

Cooke CA, Heck MM, Earnshaw WC (1987) The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis. J Cell Biol 105:2053-2067

Darwiche N, Freeman LA, Strunnikov A (1999) Characterization of the components of the putative mammalian sister chromatid cohesion complex. Gene 233:39-47

Davies RW (1971) The genetic relationship of two quantitative characters in *Drosophila melanogaster*. II. Location of the effects. Genetics 69:363-375

Davies FM, Tsao TY, Fowler SK, Rao PN (1983) Monoclonal antibodies to mitotic cells. Proc Natl Acad Sci USA 80:2926-2930

Dawe RK, Sedat JW, Agard DA, Cande WZ (1994) Meiotic chromosome pairing in maize is associated with a novel chromatin organization. Cell 76:901-912

de los Santos T, Hollingsworth NM (1999) Red1p, a *MEK1*-dependent phosphoprotein that physically interacts with Hop1p during meiosis in yeast. J Biol Chem 274:1783-1790

de Massy B, Rocco V, Nicolas A (1995) The nucleotide mapping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *Saccharomyces cerevisiae*. EMBO J 14:4589-4598

de Veaux LC, Hoagland NA, Smith GR (1992) Seventeen complementation groups of mutations decreasing meiotic recombination in *Schizosaccharomyces pombe*. Genetics 130:251-262

de Veaux LC, Smith GR (1994) Region-specific activators of meiotic recombination in *Schizosaccharomyces pombe*. Genes Dev 8:203-210

Debets F, Bos K (1986) Isolation of small protoplasts from *Aspergillus niger*. Fungal Genet Newslett 33:24

Denison SH, Kafer E, May GS (1993) Mutation in the *bimD* gene of *Aspergillus nidulans* confers a conditional mitotic block and sensitivity to DNA damaging agents. Genetics 134:1085-1096

Denison SH, May GS (1994) Mitotic catastrophe is the mechanism of lethality for mutations that confer mutagen sensitivity in *Aspergillus nidulans*. Mutat Res 304:193-202

Dunn SD (1986) Effects of the modification of transfer buffer composition and the renaturation of proteins in gels on the recognition of proteins on Western blots by monoclonal antibodies. Anal Biochem 157:144-153

Dunne PW, Oakley BR (1988) Mitotic gene conversion, reciprocal recombination and gene replacement at the *benA*, β-tubulin, locus of *Aspergillus nidulans*. Mol Gen Genet 213:339-345.

Eckert SE, Hoffmann B, Wanke C, Braus GH (1999) Sexual development of *Aspergillus nidulans* in tryptophan auxotrophic strains. Arch Microbiol 172:157-166

Egel R (1995) The synaptonemal complex and the distribution of meiotic recombination events. Trends Genet 11:206-208.

Egel-Mitani M, Olson LW, Egel R (1982) Meiosis in *Aspergillus nidulans*: another example for lacking synaptonemal complexes in the absence of crossover interference. Hereditas 97:179-187

Eijpe M, Heyting C, Gross B, Jessberger R (2000) Association of mammalian SMC1 and SMC3 proteins with meiotic chromosomes and synaptonemal complexes. J Cell Sci 113:673-682

Elliot CG (1960) The cytology of *Aspergillus nidulans*. Genet Res 1:463-476

Elliott CG (1994) Reproduction in fungi; genetical and physiological aspects. Chapman & Hall, London, pp 122-125

Engebrecht J, Hirsch J, Roeder GS (1990) Meiotic gene conversion and crossing over: Their relationship to each other and to chromosome synapsis and segregation. Cell 62:927-938

Engebrecht J, Roeder GS (1990) *MER1*, a yeast gene required for chromosome pairing and genetic recombination, is induced in meiosis. Mol Cell Biol 10:2379-2389

Esposito RE, Klapholz S (1981) Meiosis and ascospore development. In: Stathern JN, Jones EW, Broach JR (eds) The molecular biology of the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor Laboratory Press, New York, pp 211-287

Evans GA, Wahl GM (1987) Cosmid vectors for genomic walking and rapid restriction mapping. Methods Enzymol 152:604-610

Fasullo M, Bennett T, AhChing P, Koudelik J (1998) The *Saccharomyces cerevisiae RAD9* checkpoint reduces the DNA damage-associated stimulation of directed translocations. Mol Cell Biol 18:1190-1200

Fortuin JJH (1970) Another two genes controlling mitotic intragenic recombination and recovery from UV damage in *Aspergillus nidulans* 1. UV sensitivity, complementation and location of six mutants. Mutat Res 11:149-162

Fortuin JJH (1971) Another two genes controlling mitotic intragenic recombination and recovery from UV damage in *Aspergillus nidulans* II. Recombination behavior and X-ray sensitivity of *uvsD* and *uvsE* mutants. Mutat Res 11:265-277

Fortuno EA, Osman F, Subramani S (1996) Analysis of spontaneous and double-strand break induced recombination in *rad* mutants of *S. pombe*. Mutat Res 364:147-160

Friedberg EC, Siede W, Cooper AJ (1991) Cellular responses to DNA damage in yeast. In: Broac JR, Pringle JR, Jones EW (eds) The Molecular and Cellular Biology of the Yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Plainview, New York, pp 147-192

Frohman MA, Dush MK, Martin GR (1988) Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer. Proc Natl Acad Sci USA 85:8998-9002

Funabiki H, Hagan I, Uzawa S, Yanagida M (1993) Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. J Cell Biol 121:961-976

Funabiki H, Kumada K, Yanagida M (1996) Fission yeast cut1 and cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes. EMBO J 15:6617-6628

Furuya K, Takahashi K, Yanagida M (1998) Faithful anaphase is ensured by Mis4, a sister chromatid cohesion molecule required in S phase and not destroyed in G(1) phase. Genes Dev 12:3408-3418

Geck P, Szelei J, Jimenez J, Sonnenschein C, Soto AM (1999) Early gene expression during androgen-induced inhibition of proliferation of prostate cancer cells: a new suppressor candidate on chromosome 13, in the *BRCA2-Rb1* locus. J Steroid Biochem Mol Biol 68:41-45

Gimenez-Abian JF, Clarke DJ, Mullinger AM, Downes CS, Johnson RT (1995) A postprophase topoisomerase II-dependent chromatid core separation step in the formation of metaphase chromosomes. J Cell Biol 131:7-17

Goldstein LS (1980) Mechanisms of chromosome orientation revealed by two meiotic mutants in *Drosophila melanogaster*. Chromosoma 78:79-111

Goosen T, Bos CJ, van den Broek HWJ (1992) Transformation and gene manipulation in filamentous fungi: an overview. In: Arora DK, Elander RP, Mukerji KG (eds) Handbook of applied mycology, Vol. 4. Marcel Dekker Inc, New York, pp 151-195

Gorbsky GJ, Ricketts WA (1993) Differential expression of a phosphoepitope at the kinetochores of moving chromosomes. J Cell Biol 122:1311-1321

Gordon CB and Campbell JL (1991) A cell cycle-responsive transcriptional control element and a negative control element in the gene encoding DNA polymerases in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 88: 6058-6062

Grossenbacher-Grunder AM, Thuriaux P (1981) Spontaneous and UV-induced recombination in radiation-sensitive mutants of *Schizosaccharomyces pombe*. Mutat Res 81:37-48

Guacci V, Hogan E, Koshland D (1994) Chromosome condensation and sister chromatid pairing in budding yeast. J Cell Biol 517-530

Guacci V, Koshland D, Strunnikov A (1997) A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *MCD1* in *Saccharomyces cerevisiae*. Cell 91:47-57

Hanahan D (1983). Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol 166:557-580

Hastings PJ, Quah SK, von Borstel RC (1976) Spontaneous mutations by mutagenic repair of spontaneous lesions in DNA. Nature 264:719-722

Hatakeyma S, Ishii C, Inoue H (1995) Identification and expression of the *Neurospora crassa mei-3* gene which encodes a protein homologous to Rad51 of *Saccharomyces cerevisiae*. Mol Gen Genet 249:439-446

Heyting C (1996) Synaptonemal complexes: Structure and function. Curr Opin Cell Biol 8:389-396

Hirano T (1999). SMC-mediated chromosome mechanics: a conserved scheme from bacteria to vertebrates? Genes Dev 13:11-19

Hollingsworth NM, Byers B (1989) *HOP1*: A yeast meiotic pairing gene. Genetics 121:445-462

Hollingsworth NM, Ponte L (1997) Genetic interactions between *HOP1*, *RED1* and *MEK1* suggest that *MEK1* regulates assembly of axial element components during meiosis in the yeast *Saccharomyces cerevisiae*. Genetics 147:33-42

Holm C (1995) Coming undone: how to untangle a chromosome. Cell 77:955-958

Holt CL, May GS (1996) An extragenic suppressor of the mitosis-defective *bimD6* mutation of *Aspergillus nidulans* codes for a chromosome scaffold protein. Genetics 142:777-787

Hong CS, Ganetzky B (1996) Molecular characterization of neurally expressing genes in the para sodium channel gene cluster of *Drosophila*. Genetics 142:879-892

Hugerat Y, Simchen G (1993) Mixed segregation and recombination of chromosomes and YACs during single-division meiosis in *spo13* strains of *Saccharomyces cerevisiae*. Genetics 135:297-308

Huynh AD, Leblon G, Zickler D (1986) Indirect intergenic suppression of a radiosensitive mutant of *Sordaria macrospora* defective in sister-chromatid cohesiveness. Curr Genet 10:545-555

Irniger S, Piatti S, Michaelis C, Nasmyth K (1995) Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. Cell 81:269-277

Iyengar GAS, Deka PC, Kundu SC, Sen SK (1977) DNA syntheses in course of meiotic development in *Neurospora crassa*. Genet Res Camb 29:1-8

Jang YK, Jin YH, Shim YS, Kim MJ, Yoo EJ, Choi IS, Lee JS, Seong RH, Hong SH, Park SD (1996) Identification of the DNA damage-responsive elements of the *rph51*$^{+}$ gene, a *recA* and *RAD51* homolog from the fission yeast *Schizosaccharomyces pombe*. Mol Gen Genet 251:167-175

Jansen GJO (1970a) Survival of *uvsB* and *uvsC* mutants of *Aspergillus nidulans* after UV-irradiation. Mutat Res 10:21-32

Jansen GJO (1970b) Abnormal frequencies of spontaneous mitotic recombination in *uvsB* and *uvsC* mutants of *Aspergillus nidulans*. Mutat Res 10:33-41

Jansen GJO (1972) Mutator activity in *uvs* mutants of *Aspergillus nidulans*. Mol Gen Genet 116:47-50

Jessberger R, Riwar B, Baechtold H, Akhmedov AT (1996) SMC proteins constitute two subunits of the mammalian recombination complex RC-1. EMBO J 15:4061-4068

Jessberger R, Frei C, Gasser SM (1998) Chromosome dynamics: the SMC protein family. Curr Opin Genet Dev 8:254-259

Jones GH (1987) Chiasmata. In: Moens PB (ed) Meiosis. Academic Press, Orlando, pp 213-244

Kadyk LC, Hartwell LH (1993) Replication-dependent sister chromatid recombination in *rad1* mutants of *Saccharomyces cerevisiae*. Genetics 133:469-487

Käfer E (1961) The process of spontaneous recombination in vegetative nuclei of *Aspergillus nidulans*. Genetics 46:1581

Käfer E (1977a) Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv Genet 19:33-131

Käfer E (1977b) The anthranilate synthetase enzyme complex and the trifunctional *trpC* gene of *Aspergillus*. Can J Genet Cytol 19:723-728

Käfer E, Upshall A (1973) The phenotypes of the eight disomics and trisomics of *Aspergillus nidulans*. J Hered 64:35-38 Kafer E, Mayor O (1986) Genetic analysis of DNA repair in *Aspergillus*: evidence for different types of MMS-sensitive hyperrec mutants. Mutat Res 161: 119-134

Kafer E, May GS (1997) The *uvsF* gene of *Aspergillus nidulans* codes for a protein with homology to DNA replication factor C. Gene 191:155-159

Kafer E, May GS (1998) Toward repair pathways in *Aspergillus nidulans*. In: Nickoloff JA, Hoekstra MF (eds) DNA Damage and Repair, Vol. 1: DNA repair in prokaryotes and lower eukaryotes. Humana Press Inc, Totowa, NJ, pp 477-502

Kanda T, Goto A, Sawa K, Arakawa H, Yasuda Y, Takemaru T (1989) Isolation and characterization of recessive sporeless mutants in the basiodiomycete *Coprinus cineraeus*. Mol Gen Genet 216:526-529

Keeney S, Kleckner N (1995) Covalent protein-DNA complexes at the 5' strand termini of meiosis-specific double-strand breaks in yeast. Proc Natl Acad Sci USA 92:11274-11278

Keeney S, Giroux CN, Kleckner N (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell 88:375-384

Kerrebrock AW, Miyazaki WY, Birnby D, Orr-Weaver TL (1992) The *Drosophila mei-S332* gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. Genetics 130:827-841

Kironmai KM, Muniyappa K, Friedman DB, Hollingsworth NM, Byers B (1998) DNA-binding activities of Hop1 protein, a synaptonemal complex component from *Saccharomyces cerevisiae*. Mol Cell Biol 18:1424-1435

Klapholz S, Esposito RE (1980) Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics 96:589-611

Kleckner N (1996) Meiosis: How could it work? Proc Natl Acad Sci USA 93:8167-8174

Klein F, Mahr P, Galova M, Buonomo SC, Michaelis C, Nairz K, Nasmyth K (1999) A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98:91-103

Klein HL (1997) *RDH54*, a *RAD54* homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. Genetics 147:1533-1543

Koshland D, Hartwell LH (1987) The structure of sister minichromosome DNA before anaphase in *Saccharomyces cerevisiae*. Science 238:1713-1716

Koshland D, Strunnikov A (1996) Mitotic chromosome condensation. Ann Rev Cell Dev Biol 12:305-333

Koshland D, Guacci V (2000) Sister chromatid cohesion: the beginning of a long and beautiful relationship. Curr Opin Cell Biol 12:297-301

Krawchuk MD, de Veaux LC, Wahls WP (1999) Meiotic chromosome dynamics depends upon the *rec8+*, *rec10+* and *rec11+* genes of the fission yeast *Schizosaccharomyces pombe*. Genetics 153:57-68

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685

Lamb NE, Feingold E, Savage A, Avramopoulos D, Freeman S, Gu Y, Hallberg A, Hersey J, Karadima G, Pettay D, Saker D, Shen J, Taft L, Mikkelsen M, Petersen MB, Hassold T, Sherman SL (1997) Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum Mol Genet 6:1391-1399

Lammers JHM, Offenberg HH, van Aalderen M, Vink ACG, Dietrich AJJ, Heyting C (1994) The gene encoding a major component of the lateral elements of synaptonemal complexes of the rat is related to X-linked lymphocyte regulated genes. Mol Cell Biol 14:1137-1146

Lammers JHM, van Aalderen M, Peters AHFM, van Pelt AAM, Gaemers IC, de Rooij DG, de Boer P, Offenberg HH, Dietrich AJJ, Heyting C (1995) A change in phosphorylation pattern of the 30000-33000 Mr synaptonemal complex proteins of the rat between early and mid-pachytene. Chromosoma 104:154-163

Latt SA (1981) Sister chromatid exchange formation. Annu Rev Genet 15:11-55

Lecellier G, Silar P (1994) Rapid methods for nucleic acids extraction from Petri dish-grown mycelia. Curr Genet 25:122-123

Leem SH, Ogawa H (1992) The *MRE4* gene encodes a novel protein kinase homologue required for meiotic recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res 20:449-457

Leslie JF, Raju NB (1985) Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. Genetics 111: 759-777

Lica LM, Narayanswami S, Hamkalo BA (1986) Mouse satellite DNA, centromere structure, and sister chromatid pairing. J Cell Biol 103:1145-1151

Liu J, Wu TC, Lichten M (1995) The location and structure of double-strand DNA breaks induced during yeast meiosis: Evidence for a covalently linked DNA-protein intermediate. EMBO J 14:4599-4608

Loidl J, Klein F, Scherthan H (1994) Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. J Cell Biol 125:1191-1200

Losada A, Hirano M, Hirano T (1998) Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. Genes Dev 12:1986-1997

Lu BCK, Galeazzi DR (1978) Light and electron microscope observations of a meiotic mutant of *Neurospora crassa* Can J Bot 56: 2694-2706

Lu BCK (1996) Chromosomes, mitosis, and meiosis. In: Bos CJ (ed.) Fungal genetics: principles and practice. Marcel Dekker Inc, NewYork, pp 119-176

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 389:251-260

Lydall D, Nikolsky Y, Bishop DK, Weinert T (1996) A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature 383: 840-843

Maguire MP (1978) Evidence for separate genetic control of crossing over and chiasma maintenance in Maize. Chromosoma 65:173-183

Maguire MP (1990) Sister chromatid cohesiveness: Vital function, obscure mechanism. Biochem Cell Biol 68:1231-1242

Maguire MP, Paredes AM, Riess RW (1991) The desynaptic mutant of maize as a combined defect of synaptonemal complex and chiasma maintenance. Genome 34:879-887

Maguire MP, Riess RW, Paredes AM (1993) Evidence from a maize desynaptic mutant points to a probable role of synaptonemal complex central region components in provision for subsequent chiasma maintenance. Genome 36:797-807

Malone RE, Hoekstra MF (1984) Relationships between a hyper-rec mutation (*REM1*) and other recombination and repair genes in yeast. Genetics 107:33-48

Malone RE, Ward T. Lin S, Waring J (1990) The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. Curr Genet 18:111-116

Mao-Draayer Y, Galbraith AM, Pittman DL, Cool M, Malone RE (1996) Analysis of meiotic recombination pathways in the yeast *Saccharomyces cerevisiae*. Genetics 144:71-86

Mason JM (1976) Orientation disruptor (*ord*): a recombination-defective and disjunction-defective meiotic mutant in *Drosophila melanogaster*. Genetics 84:545-572

May GS, Gambino J, Weatherbee JA, Morris NR (1985) Identification and functional analysis of Beta-tubulin genes by site-specific integrative transformation in *Aspergillus nidulans*. J Cell Biol 101:712-719

May GS, McGoldrick CA, Holt CL, Denison SH (1992) The *bimB3* mutation of *Aspergillus nidulans* uncouples DNA replication from the completion of mitosis. J Biol Chem 267:15737-15743

McCarroll RM, Esposito RE (1994) *SPO13* negatively regulates the progression of mitotic and meiotic nuclear division in *Saccharomyces cerevisiae*. Genetics 138:47-60

McCarthy JEG (1998) Posttranscriptional control of gene expression in yeast. Microbiol Mol Biol Rev 62:1496-1553

McKay MJ, Troelstra C, van der Spek P, Kanaar R, Smit B, Hagemeijer A, Bootsma D, Hoeijmakers J (1996) Sequence conservation of the *rad21 Schizosaccharomyces pombe* DNA double-strand break repair gene in human and mouse. Genomics 36:305-315

Michaelis C, Ciosk R, Nasmyth K (1997) Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. Cell 91:35-45

Mills KD, Sinclair DA, Guarente L (1999) *MEC1*-dependent redistribution of the Sir3 silencing protein from telomeres to DNA-double-strand breaks. Cell 97:609-620

Miyazaki WY, Orr-Weaver TL (1992) Sister-chromatid misbehavior in *Drosophila ord* mutants. Genetics 132:1047-1061

Miyazaki WY, Orr-Weaver TL (1994) Sister-chromatid cohesion in mitosis and meiosis. Annu Rev Genet 28:167-187

Moens PB (1974) Modification of sporulation in yeast strains with two-spored asci (*Saccharomyces*, Ascomycetes). J Cell Sci 16:519-527

Moens PB, Mowat M, Esposito MS, Esposito RE (1977) Meiosis in a temperature-sensitive DNA-synthesis mutant and in an apomictic yeast strain (*Saccharomyces cerevisiae*). Philos Trans R Soc Lond B Biol Sci 277:351-358

Moens PB, Pearlman RE (1988) Chromatin organization at meiosis. Bioessays 9:151-53

Moens PB, Spyropoulos B (1995) Immunocytology of chiasmata and chromosomal disjunction at mouse meiosis. Chromosoma 104:175-182

Molnar M, Bahler J, Sipiczki M, Kohli J (1995) The *rec8* gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. Genetics 141:61-73

Moore DP, Orr-Weaver TL (1998) Chromosome segregation during meiosis: building an unambivalent bivalent. Curr Top Dev Biol 37:263-299

Moore DP, Page AW, Tang TL, Kerrebrock AW, Orr-Weaver TL (1998) The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. J Cell Biol 140:1003-1012

Moreau PJF, Zickler D, Leblon G (1985) One class of mutants with disturbed centromere cleavage and chromosome pairing in *Sordaria macrospora*. Mol Gen Genet 198:189-197

Morris NR (1976) Mitotic mutants of *Aspergillus nidulans*. Genet Res 26:237-254

Moses MJ (1966) Synaptonemal complex. Annu Rev Genet 2:363-412

Moulton T, Chung WY, Yuan LW, Hensle T, Waber P, Nisen P, Tycko B (1996) Genomic imprinting and Wilms' tumor. Med Pediatr Oncol 27:476-483

Munz P (1994) An analysis of interference in the fission yeast *Schizosaccharomyces pombe*. Genetics 137:701-707

Muris DFR, Vreeken K, Carr AM, Broughton BC, Lehmann AR, Lohman PHM, Pastink A (1993) Cloning the *RAD51* homologue of *Schizosaccharomyces pombe*. Nucleic Acids Res 21:4586-4591

Murray AW, Szostak JW (1985) Chromosome segregation in mitosis and meiosis. Annu Rev Cell Biol 1:289-315

Nag DK, Scherthan H, Rockmill B, Bhargava J, Roeder GS (1995) Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. Genetics 141:75-86

Nasmyth K (1996) Viewpoint: putting the cell cycle in order. Science 274:1643-1645

Nasmyth K (1999) Separating sister chromatids. Trends Biochem Sci 24:98-104

Nasmyth K, Peters JM, Uhlmann F (2000) Splitting the chromosome: cutting the ties that bind sister chromatids. Science 288:1379-1384

Nelson MA (1996) Mating types in ascomycetes: a romp in the sac. Trends Genet 12:69-74

Nevalainen KM (1981) Induction, isolation, and characterization of *Aspergillus niger* mutant strains producing elevated levels of beta-galactosidase. Appl Environ Microbiol 41:593-596

Nicklas RB, Ward SC, Gorbsky GJ (1995) Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. J Cell Biol 130:929-939

Oakley BR, Rinehart JE, Mitchell BL, Oakley CE, Carmona C, Gray GL, May GS (1987) Cloning, mapping and molecular analysis of the *pyrG* (orotidine-5'-phosphate decarboxylase) gene in *Aspergillus nidulans*. Gene 61:385-399

Offenberg HH, Schalk JAC, Meuwissen RLJ, van Aalderen M, Kester HA, Dietrich AJJ, Heyting C (1998) SCP2: a major protein component of the axial elements of synaptonemal complexes of the rat. Nucleic Acids Res 26:2572-2579

Ogawa T, Shinohara A, Nabetani A, Ikeya T, Yu X, Egelman EH, Ogawa H (1993a) RecA-like recombination proteins in eukaryotes: functions and structures of *RAD51* genes. Cold Spring Harbor Symp 58:567-576

Ogawa T, Yu X, Shinohara A, Egelman EH (1993b) Similarities of the yeast Rad51 filament to the bacterial RecA filament. Science 259:1896-1899

Ohta K, Shibata T, Nicolas A (1994) Changes in chromatin structure at recombination initiation sites during yeast meiosis. EMBO J 13:5754-5763

Olson LW, Eden U, Egel-Mitani M, Egel R (1978) Asynaptic meiosis in fission yeast? Hereditas 89:189-199

Osiewacz HD (1994) A versatile shuttle cosmid vector for the efficient construction of genomic cosmid libraries and for the cloning of fungal genes. Curr Genet 26: 87-90

Osman F, Tomsett B, Strike P (1993) The isolation of mutagen-sensitive *nuv* mutants of *Aspergillus nidulans* and their effect on mitotic recombination. Genetics 134:445-454

Osmani SA, May GS, Morris NR (1987) Regulation of the mRNA levels of *nimA*, a gene required for the G2-M transition in *Aspergillus nidulans*. J Cell Biol 104:1495-1504

Padmore R, Cao L, Kleckner N (1991) Temporal comparison of recombination and synaptonemal complex formation during meiosis in *Saccharomyces cerevisiae*. Cell 66:1239-1256

Paques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 63:349-404

Parisi S, McKay MJ, Molnar M, Thompson MA, van der Spek PJ, van Drunen-Schoenmaker E, Kanaar R, Lehmann E, Hoeijmakers JHJ, Kohli J (1999) Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. Mol Cell Biol 19:3515-3528

Perkins DD 1992 *Neurospora*: the organism behind the molecular revolution. Genetics 130:687-701.

Perkins DD, Barry EG (1977) The cytogenetics of *Neurospora*. Adv Genet 19:133-285

Petes TD, Malone RE, Symington LS (1991) Recombination in yeast. In: Broach JR, Pringle JR, Jones EW (eds) The molecular and cellular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Plainview, New York, pp 407-521

Pizzagalli A, Valsasnini P, Plevani P, Lucchini G (1988) DNA polymerase I gene of *Saccharomyces cerevisiae*: nucleotide sequence, mapping of a temperature sensitive mutation, and protein homology with other DNA polymerases. Proc Natl Acad Sci USA 85:3772-3776

Plug AW, Xu J, Reddy G, Golub EI, Ashley T (1996) Presynaptic association of Rad51 protein with selected sites in meiotic chromatin. Proc Natl Acad Sci USA 93:5920-5924

Pöggeler S, Risch S, Kuck U, Osiewacz HD (1997) Mating-type genes from the homothallic fungus *Sordaria macrospora* are functionally expressed in a heterothallic ascomycete. Genetics 147:567-580

Pollard DR, Käfer E, Johnston MT (1968) Influence of chromosomal aberrations on meiotic and mitotic nondisjunction in *Aspergillus nidulans*. Genetics 60:743-757

Pontecorvo G, Roper JA, Hemmons LM, MacDonalds KD, Bufton AWJ (1953) The genetics of *Aspergillus nidulans*. Adv Genet 5:141-238

Pontecorvo G, Käfer E (1958) Genetic analysis based on mitotic recombination. Adv Genet 9:71-104

Ponticelli AS, Smith GR (1989) Meiotic recombination-deficient mutants of *Schizosaccharomyces pombe*. Genetics 123:45-54

Pritchard RH (1955) The linear arrangement of a series of alleles of *Aspergillus nidulans*. Heredity 9:343-371

Pukkila PJ, Shannon KB, Skrzynia C (1995) Independent synaptic behavior of sister chromatids in *Coprinus cinereus*. Can J Bot 73 (Suppl.1):S215-S220

Punt PJ, Dingemanse MA, Jacobs-Meijsing BJM, Pouwels PH, van den Hondel CAMJJ (1988) Isolation and characterization of the glyceraldehyde-3-phosphate gene of *Aspergillus nidulans*. Gene 69:49-57

Raju NB (1980) Meiosis and ascospore genesis in *Neurospora*. Eur J Cell Biol 23:208-233

Raju NB (1986) Ascus development in two temperature-sensitive four-spore mutants of *Neurospora crassa*. Can J Genet Cytol 28:982-990

Raju NB, Perkins DD (1978) Barren perithecia in *Neurospora crassa*. Can J Genet Cytol 20:41-59

Rambosec J, Leach J (1987) Recombinant DNA in filamentous fungi: progress and prospects. Crit Rev Biotechnol 6:357-393

Rechsteiner M, Rogers SW (1996) PEST sequences and regulation by proteolysis. Trends Biochem Sci 21:267-271

Richardson C, Moynahan ME, Jasin M (1998) Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. Genes Dev 12:3831-3842

Rieder CL, Cole R (1999) Chromatid cohesion during mitosis: lessons from meiosis. J Cell Sci 112:2607-2613

Rockmill B, Fogel S (1988) *DIS1*: a yeast gene required for proper meiotic chromosome disjunction. Genetics 119:261-272

Rockmill B, Roeder GS (1988) *RED1*: a yeast gene required for the segregation of chromosomes during the reductional division of meiosis. Proc Natl Acad Sci USA 85:6057-6061

Rockmill B, Roeder GS (1990) Meiosis in asynaptic yeast. Genetics 126:563-574

Rockmill B, Roeder GS (1991) A meiosis-specific protein kinase homolog required for chromosome synapsis and recombination. Genes Dev 5:2392-2404

Rockmill B, Roeder GS (1994) The yeast *med1* mutant undergoes both meiotic homolog nondisjunction and precocious separation of sister chromatids. Genetics 136:65-74

Rollins RA, Morcillo P, Dorsett D (1999) Nipped-B, a *Drosophila* homologue of chromosomal adherins, participates in activation by remote enhancers in the *cut* and *Ultrabithorax* genes. Genetics 152:577-593

Rosenthal A, Coutelle O, Craxton M (1993) Large-scale production of DNA sequencing templates by microtitre format PCR. Nucleic Acids Res 21:173-174

Rufas JS, Santos JL, Diez M, Suja JA (1992) Meiotic chromosome structure: Relationship between the synaptonemal complex and the chromatid cores. Genome 35:1054-1061

Saitoh Y, Laemmli UK (1993) From the chromosomal loops and the scaffold to the classic bands of metaphase chromosomes. Cold Spring Harbor Symp Quant Biol 58:755-765

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sandler L, Lindsley DL, Nicoletti B, Trippa G (1968) Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics 60:525-558

Sarkar S (1991) Haldane's solution of the Luria-Delbrück distribution. Genetics 127:257-261

Schalk JAC (1999) SCP2, a major protein component of the axial elements of synaptonemal complexes. PhD Thesis. Wageningen Agricultural University, The Netherlands

Schmiesing JA, Ball AR, Gregson HC, Alderton JM, Zhou SL, Yokomori K (1998) Identification of two distinct human SMC protein complexes involved in mitotic chromosome dynamics. Proc Natl Acad Sci USA 95:12906-12911

Schwacha A, Kleckner N (1994) Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell 76:51-63

Schwacha A, Kleckner N (1997) Interhomolog bias during meiotic recombination: Meiotic functions promote a highly differentiated interhomolog-only pathway. Cell 90:1123-1135

Schwarzacher T (1997) Three stages of meiotic homologous chromosome pairing in wheat: recognition, alignment and synapsis. Sexual Plant Reprod 10:324-331

Scott BR, Kafer E (1982) *Aspergillus nidulans* - An organism for detecting a range of genetic damage. In: de Serres FJ, Hollaender A (eds) Chemical Mutagens, vol.7. Plenum Press, New York and London, pp 447-479

Seitz LC, Tang KL, Cummings WJ, Zolan ME (1996) The *rad9* gene of *Coprinus cinereus* encodes a proline-rich protein required for meiotic chromosome condensation and synapsis. Genetics 142:1105-1117

Selig S, Okumura K, Ward DC, Cedar H (1992) Delineation of DNA replication time zones by fluorescence in situ hybridization. EMBO J 11:1217-1225

Shinohara A, Ogawa H, Ogawa T (1992) Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell 69:457-470

Skibbens RV, Corson LB, Koshland D, Hieter P (1999) Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev 13:307-319

Slonimsky PP, Brouillet S (1993) A data-base of chromosome III of *Saccharomyces cerevisiae*. Yeast 9:941-1029

Smith TL (1989) Disparate evolution of yeasts and filamentous fungi indicated by phylogenetic analysis of glyceraldehyde-3-phosphate dehydrogenase genes. Proc Natl Acad Sci USA 86:7063-7066

Smith AV, Roeder GS (1997) The yeast Red1 protein localizes to the cores of meiotic chromosomes. J Cell Biol 136:957-967

Smith KN, Nicolas A (1998) Recombination at work for meiosis. Curr Opin Genet Dev 8:200-211

Snow R (1979) Maximum likelihood estimation of linkage and interference from tetrad data. Genetics 92:231-245

Stassen Y, Logsdon J, Offenberg HH, Vora GJ, Palmer JD, Zolan ME (1997) Isolation and characterization of *RAD51* orthologs from *Coprinus cinereus* and *Lycopersicon esculentum*, and phylogenetic analysis of eukaryotic *recA* homologs. Curr Genet 31:144-157

Stern H, Hotta Y (1987) The Biochemistry of meiosis. In: Moens PB (ed) Meiosis. Academic Press, New York, pp 303-331

Storlazzi A, Xu L, Cao L, Kleckner N (1995) Crossover and noncrossover recombination during meiosis: Timing and pathway relationships. Proc Natl Acad Sci USA 92:8512-8516

Story RM, Weber IT, Steitz T (1992) The structure of the *E. coli* recA protein monomer and polymer. Nature 355:318-325

Story RM, Bishop DK, Kleckner N, Steitz T (1993) Structural relationship of bacterial RecA proteins to recombination proteins from bacteriophage T4 and yeast. Science 259:1892-1899

Stratmann R, Lehner CF (1996) Separation of sister chromatids in mitosis requires the *Drosophila* pimples product, a protein degraded after the metaphase-anaphase transition. Cell 84:25-35

Strickland WN (1958) An analysis of interference in *Aspergillus nidulans*. Proc R Soc B 149: 82-101

Strunnikov AV, Jessberger R (1999) Structural maintenance of chromosomes (SMC) proteins - Conserved molecular properties for multiple biological functions. Eur J Biochem 263:6-13

Stursberg S, Riwar B, Jessberger R (1999) Cloning and characterization of mammalian *SMC1* and *SMC3* genes and proteins, components of the DNA recombination complexes RC-1. Gene 228:1-12

Subramani S (1991) Radiation resistance in *Schizosaccharomyces pombe*. Mol Microbiol 5:2311-2314

Suja JA, Antonio C, Debec A, Rufas JS (1999) Phosphorylated proteins are involved in sister chromatid arm cohesion during meiosis I. J Cell Sci 112:2957-2969

Sumner AT (1991) Scanning electron microscopy of mammalian chromosomes from prophase to telophase. Chromosoma 100:410-418

Sym M, Roeder GS (1994) Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell 79: 283-292

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983) The double-strand-break repair model for recombination. Cell 33:25-35

Taagepera S, Rao PN, Drake FH, Gorbsky GJ (1993) DNA topoisomerase IIα is the major chromosome protein recognized by the mitotic phosphoprotein antibody MPM-2. Proc Natl Acad Sci USA 90:8407-8411

Tanaka T, Cosma MP, Wirth K, Nasmyth K (1999) Identification of cohesion association sites at centromeres and along chromosome arms. Cell 98:847-858

Tang TTL, Bickel SE, Young LM, Orr-Weaver TL (1998) Maintenance of sister-chromatid cohesion at the centromere by the Drosophila MEI-S332 protein. Genes Dev 12:3843-3856

Terasawa M, Shinohara A, Hotta Y, Ogawa H, Ogawa T (1995) Localization of RecA-like recombination proteins on chromosomes of the lily at various meiotic stages. Genes Dev 9:925-934

Thijs H (1998) On homologous recombination in *Aspergillus nidulans*. PhD Thesis. Wageningen Agricultural University, The Netherlands

Thijs H, Garde J, Goosen T, Tomsett B, Swart K, Heyting C, van den Broek HWJ (1995) Polarity of meiotic gene conversion is 5' to 3' within the *niaD* gene of *Aspergillus nidulans*. Mol Gen Genet 247:343-350

Thompson-Coffe C, Zickler D (1994) How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. Dev Biol 165:257-271

Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K (1999) Yeast Cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev 13:320-333

Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro Y, Morita T (1996) Targeted disruption of the *Rad51* gene leads to lethality in embryonic mice. Proc Natl Acad Sci USA 93:6236-6240

Turcq B, Denayrolles M, Begueret J (1990) Isolation of the two allelic incompatibility genes s and S of the fungus *Podospora anserina*. Curr Genet 17:297-303

Uhlmann F, Nasmyth K (1998) Cohesion between sister chromatids must be established during DNA replication. Curr Biol 8:1095-1101

Uhlmann F, Lottspeich F, Nasmyth K (1999) Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature 400:37-42

van Heemst D, Swart K, Holub EF, van Dijk R, Offenberg HH, Goosen T, van den Broek HWJ, Heyting C (1997) Cloning, sequence, gene disruption and mitotic and meiotic phenotypes of *uvsC*, an *Aspergillus nidulans* homolog of yeast *RAD51*. Mol Gen Genet 254:654-664

van Heemst D, James F, Pöggeler S, Berteaux-Lecellier V, Zickler D (1999) Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programs. Cell 98:261-271

van Heemst D, Heyting C (2000) Sister chromatid cohesion and recombination in meiosis. Chromosoma 109:10-26

Vershon AK, Pierce M (2000) Transcriptional regulation of meiosis in yeast. Curr Opin Cell Biol 12:334-339

Visintin R, Prinz S, Amon A (1997) *CDC20* and *CDH1*: A family of substrate-specific activators of APC-dependent proteolysis. Science 278:460-463

Von Wettstein D, Rasmussen SW, Holm PB (1984) The synaptonemal complex in genetic segregation. Annu Rev Genet 18:331-413

Vorlaufer E, Peters JM (1998) Regulation of the cyclin B degradation system by an inhibitor of mitotic proteolysis. Mol Biol Cell 9:1817-1832

Walker JE, Saraste M, Runswick MJ, Gay NJ (1982) Distantly related sequences in the α and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J 1:945-951

Warburton PE, Cooke HJ (1997) Hamster chromosomes containing amplified human alpha-satellite DNA show delayed sister chromatid separation in the absence of de novo kinetochore formation. Chromosoma 106:149-159

Waring RB, May GS, Morris NR (1989) Characterization of an inducible expression system in *Aspergillus nidulans* using alcA and tubulin-coding genes. Gene 79:119-130

Watanabe Y, Nurse P (1999) Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature 400:461-464

Weiner BM, Kleckner N (1994) Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. Cell 77:977-991

Whitehouse H (1982) Genetic recombination; understanding the mechanism. John Wiley & Sons, Chichester.

Widom J (1998) Structure, dynamics, and function of chromatin in vitro. Annu Rev Biophys Biomol Struc 27:285-327

Xiong M, Chen HJ, Prade RA, Wang Y, Griffith J, Timberlake WE, Arnold J (1996) On the consistency of a physical mapping method to reconstruct a chromosome in vitro. Genetics 142:267-284

Xu LH, Weiner BM, Kleckner N (1997) Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev 11:106-118

Yamamoto A, Guacci V, Koshland D (1996) Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). J Cell Biol 133:99-110

Zetka MC, Kawasaki I, Strome S, Muller F (1999). Synapsis and chiasma formation in *Caenorhabditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. Genes Dev 13:2258-2270

Zhao P, Kafer E (1992) Effects of mutagen-sensitive *mus* mutations on spontaneous mitotic recombination in *Aspergillus*. Genetics 130:717-728

Zickler D (1977) Development of the synaptonemal complex and the "recombination nodules" during meiotic prophase in the seven bivalents of the fungus *Sordaria macrospora* Auersw. Chromosoma 61:289-316

Zickler D, Moreau PJF, Huynh AD, Slezec AM (1992) Correlation between pairing initiation sites, recombination nodules and meiotic recombination in *Sordaria macrospora*. Genetics 132:135-148

Zickler D, Kleckner N (1998) The leptotene-zygotene transition of meiosis. Annu Rev Genet 32:619-697

Zickler D, Kleckner N (1999) Meiotic chromosomes: integrating structure and function. Annu Rev Genet 33:603-754

Zolan ME, Tremel CJ, Pukkila PJ (1988) Production and characterization of radiation-sensitive meiotic mutants of *Coprinus cinereus*. Genetics 120:379-387

Zonneveld BJ (1988) Morphology of initials and number of nuclei initiating cleistothecia in *Aspergillus nidulans*. Trans Br mycol Soc 90:369-373

Zou H, McGarry TJ, Bernal T, Kirschner MW (1999) Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. Science 285:418-422

Summary

Homologous recombination and sister chromatid cohesion play important roles in the maintenance of genome integrity and the fidelity of chromosome segregation in mitosis and meiosis. Within the living cell, the integrity of the DNA is threatened by various factors that cause DNA-lesions, of which DNA double-strand breaks (DSBs) are considered particularly deleterious. The causative agents can be of endogenous origin, such as metabolically produced free radicals, and of exogenous origin, such as ultraviolet light and ionizing radiation. The accurate repair of DSBs is important to prevent chromosomal fragmentation, translocations and deletions. Of the sophisticated (networks of) DNA repair pathways that have evolved, homologous recombination, which repairs the DSB by copying information from an intact homologous DNA-template, is considered one of the most accurate. In mitotic G2, the sister chromatid is preferentially used as a template for recombinational repair of DSBs.

DSBs can also arise as normal intermediates in several DNA repair and recombination pathways, including meiotic recombination. In meiotic recombination (in yeast), the cells actively induce large numbers of DSBs, and channel their search for a homologous template towards a non-sister chromatid of the homologous chromosome. In each pair of homologous chromosomes, at least one DSB is repaired by reciprocal exchange of precisely corresponding segments of non-sister chromatids (crossing over), whereas additional DSBs in the same chromosome pair are repaired by either reciprocal or non-reciprocal exchange. The reciprocal exchanges between non-sister chromatids (visible as chiasmata) are essential for the proper disjunction of homologous chromosomes during the first meiotic division (meiosis I).

When I started my investigations for this thesis, it was recognized that reciprocal exchanges as such could not direct the proper segregation of homologous chromosomes at meiosis I; some "glue" should keep the chiasmata in place, either by binding to the chiasmata, or by maintaining cohesion between the sister chromatids distal to the chiasmata. Although mutants existed that appeared to be defective in the production of this glue, its nature remained unknown. In this thesis, I have tried to identify components involved in meiotic sister chromatid cohesion and recombination, to analyze the interplay between these two processes in meiosis and to gain insight into their relationship with mitotic DNA repair and recombination.

In **chapter 1**, I explain the choice of the experimental model systems that I used for the research described in this thesis. All investigations were performed in two filamentous fungi, namely *Sordaria macrospora* and *Aspergillus nidulans*. The most important reason for the choice of these two fungi was that mutants were available (or easily obtainable) that were defective in meiotic sister chromatid cohesion and/or recombination, and that it should be feasible to clone the corresponding wild-type genes by means of transformation complementation of the mutant defects. *S. macrospora* had the additional advantage of a well-developed cytology and *A. nidulans* had the additional advantages of well-developed molecular genetic tools and the presence of a parasexual cycle in addition to the sexual cycle. This latter feature offers the possibility of analyzing mitotic allelic recombination. Moreover, *A. nidulans* is one of the two known organism that do not assemble synaptonemal complexes (SCs) during meiotic prophase and that do not display positive crossover interference. The choice for both *S. macrospora* and *A. nidulans* would thus make it possible to compare the role(s) of genes involved in meiotic sister chromatid cohesion and/or recombination in a organism with and one without SCs.

In **chapter 2**, we describe the cloning of the *SPO76* gene of *S. macrospora* by transformation complementation of the meiotic defects of the *spo76-1* (non-null) mutant. It was known that this mutant displayed defects in meiotic sister chromatid cohesion, meiotic recombination and mitotic DNA-repair. Furthermore, we analyzed the localization of the Spo76 protein throughout wild-type mitosis and meiosis and performed a detailed analysis of the *spo76-1* mutant phenotype. We show that Spo76p is chromosome-associated during all stages of mitosis and meiosis, except at metaphase(s) and anaphase(s). During mitosis, Spo76p disappears from the chromosomes at prometaphase. During meiotic prophase I, Spo76p is more abundant than during any other cell cycle stage, and localizes preferentially close to the chromosome axes. Spo76p disappears from the chromosomes at diplotene. In the *spo76-1* mutant, we observed a transient defect in chromosome organization at (mitotic) prometaphase: the duration of this stage was prolonged and chromosome morphology was abnormal. Strikingly, chromosomal regions with defects in both sister chromatid cohesion and chromosome compaction alternated with regions with apparently normal cohesion and compaction. We speculate that the mitotic prophase to metaphase transition involves forces that tend to disrupt cohesion and that Spo76p promotes the maintenance of a minimum of cohesion in combination with chromosome compaction. Likewise, we observed in meiotic prophase of *spo76-1*, from late leptotene on, that sister chromatid cohesion and chromosome compaction were coordinately affected on a regional basis. Regions with widely split axial

elements alternated with regions with unsplit segments of axial elements. These unsplit segments could form stretches of SC, which contained rare late recombination nodules (late RNs: ultrastructurally recognizable enzyme-complexes involved in the later steps of meiotic recombination). Whereas the number of late RNs was strongly reduced *in spo76-1*, early RNs (as recognized by immunocytochemical labelling of Rad51 and Dmc1) occurred at only slightly reduced levels in the mutant and persisted longer. This suggest that *spo76-1* is deficient in some intermediate step of meiotic recombination. The role of Spo76p in the meiotic leptotene/zygotene transition may be related to its role during the mitotic prophase/metaphase transition, in that the leptotene/zygotene transition might also bring along forces that tend to disrupt cohesion, and that Spo76p is also needed for maintenance of cohesion during this meiotic transition. Spo76p may have an additional role in late meiotic prophase because in *spo76-1*, meiotic sister chromatids separated completely from diplotene on, whereas in wild type this does not occur before anaphase II.

The predicted protein encoded by the *SPO76* gene is evolutionary conserved from fungi to man; the highest percentage of amino acid identity (44%) was found with the BIMD protein of *A. nidulans*. The *A. nidulans bimD6* mutant was previously identified as a conditional lethal mutant with a (lethal) mitotic chromosome segregation defect at high temperature and a (non-lethal) DNA repair deficiency phenotype at low temperature.

In **chapter 3**, we demonstrate by heterologous complementation that the *SPO76* gene of *S. macrospora* can complement both the temperature and the MMS (methyl methane sulphonate) sensitivities of *bimD6* in *A. nidulans*, implying direct functional homology between the mitotic roles of two proteins. We also show that, like *spo76-1*, *bimD6* mutants do not form sexual spores (ascospores). However, *bimD6* mutants display, unlike *spo76-1*, disturbances in premeiotic development and form only few asci. The few asci that entered meiosis were blocked at metaphase I with prematurely separated sister chromatids, but the extent of meiotic sister chromatid separation was less severe in *bimD6* than in *spo76-1*. In addition, whereas the mitotic localization of the two proteins was roughly similar, the meiotic localization of the two proteins showed some important differences. Unlike Spo76p in *S. macrospora*, BIMD in *A. nidulans* was not more abundant during meiotic prophase than in the mitotic cycle, and did not localize preferentially close to the chromosome axes during the pairing of homologous chromosomes. Moreover, *SPO76* could not complement the sexual sporulation defects of *bimD6* in *A. nidulans,* and *vice versa*, *bimD* could not restore the sexual sporulation defects of the *S. macrospora spo76-1* mutant. These results indicate that Spo76p and BIMD may differ in a species-specific manner with respect to their meiotic roles. The

species-specific aspects of the roles of Spo76p and BIMD in meiosis are possibly related to the differences in meiotic chromosome organization between the two fungi (see above; **chapter 1**): in contrast to *S. macrospora*, *A. nidulans* does not form SCs and does not display positive interference of meiotic crossovers.

We also show that *bimD6* mutants are hypersensitive to X-rays in addition to their elevated sensitivities to UV and MMS, but only when dividing cells are exposed to these agents. The *bimD6* mutant thus closely resembles recombination-deficient mutants of *A. nidulans*, such as *uvsC114* (the *uvsC* gene of *A. nidulans* is homologous to *RAD51* of *Saccharomyces cerevisiae*; **chapter 4**). We have therefore compared *bimD6* with *uvsC114* regarding defects in mitotic recombination. When assayed for allelic recombination, *bimD6* and *uvsC114* yielded similar results. In both mutants, the absolute frequencies of allelic recombination were strongly reduced, although the distribution of recombinants among the various classes was comparable to wild type. However, when assayed for intrachromosomal conversions, *bimD6* and *uvsC114* produced different results. Intrachromosomal conversions between interrupted duplications were strongly reduced in *uvsC114*, but occurred at wild-type frequencies in *bimD6*. We propose that BIMD is required for homologous recombination when the template is located on another (sister or non-sister) chromatid, but not when the template is available in close proximity on the same sister chromatid or the same chromatin domain/loop. The repair machinery may thus be obliged to cooperate with cohesion complexes (which are probably located at the borders between chromatin domains/loops) if the homologous template lies outside an intrachromatid loop domain. In contrast, *uvsC* was required for both types of repair.

In **chapter 4**, we describe the cloning of the *uvsC* gene of *A. nidulans* by transformation complementation of the mitotic repair defects of a *uvsC114* mutant. Furthermore, we disrupted the entire *uvsC* gene, and we compared the phenotypic effects of the resulting null mutation with those of *uvsC114*. The predicted UVSC protein shows 67% amino acid identity with Rad51p of *S. cerevisiae* and 27% amino acid identity with the RecA protein of *Escherichia coli*. These proteins are involved in strand invasion and exchange during homologous recombination. We found that in the absence of DNA-damaging agents, the *uvsC* gene was transcribed at a higher level in the *uvsC114* mutant than in wild-type. Transcription of *uvsC* was inducible by MMS in wild-type and *uvsC114* mutant strains. We compared the mitotic and meiotic phenotypes of mutants carrying the *uvsC114* point mutation (a deletion of 6 bp in core domain I) with those of the *uvsC* null mutant. The *uvsC* null mutant was more sensitive to UV and MMS than *uvsC114*, indicating that *uvsC114* is not

a null mutation. The sexual developmental phenotypes of the two mutants also differed. In the *uvsC* null mutant, sexual development was disturbed before the onset of meiosis, whereas in *uvsC114* it was blocked in meiotic prophase. We observed large, multi-nucleated cells in older cleistothecia of the *uvsC* null mutant. These cells possibly represent degenerated croziers, which might have been blocked at premeiotic S-phase. In *S. cerevisiae,* disruption of *RAD51* has no effect on mitotic growth and causes arrest at meiotic prophase I, whereas in mouse, disruption of *Rad51* results in embryonic lethality. In *A. nidulans*, disruption of *uvsC* had no effect on mitotic growth, and caused an arrest at a premeiotic stage of sexual development. Disruption of *RAD51*-homologous genes thus has different effects in different organisms.

In **chapter 5**, we describe the isolation and characterization of sexual sporulation mutants of *A. nidulans*. Vegetative spores were treated with a high dose of UV (1.5 % survival) and surviving colonies were plated on supplemented minimal medium. Colonies that did not display aberrant vegetative growth were visually screened for the appearance of "barren" fruiting bodies (=fruiting bodies without or with only few ascosores). This screen (1250 colonies analyzed) yielded 20 mutants with the desired phenotype. After two successive rounds of backcrosses with wildtype, two mutants yielded no longer progeny with the original mutant phenotype; these mutants were not analyzed further. The remaining 18 mutants were all recessive and were assigned to 15 complementation groups. Based on these numbers, we estimate that, under the growth conditions tested, about 50-100 genes are specifically involved in ascospore formation in *A. nidulans*. Three mutations were mapped by parasexual analysis: two mutations could be assigned to a specific chromosome, and one was associated with a translocation breakpoint. For all 18 mutants, the contents of the "barren" fruiting bodies were cytologically analyzed. A large proportion of the mutants, namely 11 out of 18, arrested in meiotic prophase I (like *uvsC114*; **chapter 4**) or metaphase I (like *bimD6*; **chapter 3**). It is thus possible that this new collection contains mutants that are specifically affected in meiotic recombination and/or sister chromatid cohesion. We suggest a strategy to clone the corresponding wild-type genes, by selecting for the appearance of prototrophic progeny clones that result from meiotic reassortment of auxotrophic markers.

In the General Discussion (**chapter 6**), we speculate upon the possible links between sister chromatid cohesion and recombination in mitosis and meiosis. Whereas in mitosis centromeric cohesion primarily serves chromosome disjunction, arm cohesion may play additional roles in repair by recombination. Cohesion complexes at the basis of the chromatin domains/loops may function as nucleation sites for the assembly of recombinational repair

complexes and assist these complexes in finding a homologous template in a precisely corresponding segment of the undamaged sister chromatid. They may thus be responsible for the observed bias for the sister chromatid as a template for recombinational repair during mitotic G2. In meiosis I, recombination has to be directed towards a non-sister chromatid of the homologous chromosome and, at the same time, arm cohesion has to be maintained and possibly even reinforced to ensure correct reductional chromosome segregation. Consequently, both the recombinational repair machinery and the cohesion complexes function in meiosis in a modified form. In this modified form, cohesion complexes may serve as a basis for axial element formation. DSBs are probably produced concomitantly with axial element assembly. As we proposed for mitotic recombinational repair, we hypothesize that meiotic DSBs are transferred to the basis of chromatin loops/domains, where they are brought into contact with the meiotic cohesion complexes and additional proteins required for recombinational repair. However, we speculate that homology search on the sister chromatid will now be blocked by linear element components so that another template for homologous recombination has to be found. Furthermore, axial element components, in concert with the modified recombination complex, will possibly establish DNA-DNA contacts with a non-sister chromatid of the homologous chromosome. Thus, by providing the basis for linear element formation and assembly of a meiosis-specific recombination complex, cohesion complexes may contribute to the preference for a non-sister chromatid of the homologous chromosome as a template for homologous recombination in meiosis.

Samenvatting

Homologe recombinatie en zuster-chromatiden cohesie zijn belangrijk voor het behoud van de integriteit van het genoom en de betrouwbaarheid van de chromosoomsegregatie in mitose en meiose. In de levende cel wordt de integriteit van het DNA bedreigd door diverse factoren die kunnen leiden tot DNA-beschadigingen, waarvan dubbelstrengs DNA-breuken (DSB's) als bijzonder schadelijk worden beschouwd. Sommige van deze factoren zijn endogeen, bijvoorbeeld vrije radicalen die ontstaan bij het metabolisme in de cel, en andere zijn exogeen, zoals ultraviolet licht en ioniserende straling. Het is van groot belang dat DSB's zorgvuldig hersteld worden, om deleties, translocaties en fragmentatie van chromosomen te voorkomen. Er hebben zich dan ook over diverse uitgekiende (netwerken van) DNA-herstelroutes ontwikkeld, waarvan homologe recombinatie, waarbij DBS's worden hersteld door intacte, homologe DNA-moleculen als matrijs te gebruiken, wordt beschouwd als één van de nauwkeurigste. Bij recombinatie-herstel tijdens de mitotische G2 wordt de zuster-chromatide preferentieel als matrijs gebruikt.

DSB's kunnen ook ontstaan als normale intermediairen in verschillende DNA-herstel- en recombinatieprocessen, inclusief de meiotische recombinatie. Bij de meiotische recombinatie (in gist) induceert de cel zelf grote aantallen DSB's, en zorgt ervoor dat deze bij voorkeur hersteld worden op een niet-zuster-chromatide van het homologe chromosoom als matrijs. In elk paar homologe chromosomen wordt tenminste één DSB hersteld door reciproke uitwisseling (overkruising) van precies overeenkomstige stukken niet-zuster-chromatide, terwijl de overige DSB's in hetzelfde chromosomenpaar door reciproke of niet-reciproke recombinatie kunnen worden hersteld. De reciproke uitwisselingen tussen niet-zuster-chromatiden (zichtbaar als chiasmata) zijn essentieel voor de correcte segregatie van homologe chromosomen tijdens de eerste meiotische deling (meiose I).

Toen ik mijn promotieonderzoek begon, realiseerde men zich dat chiasmata als zodanig niet de correcte segregatie van homologe chromosomen tijdens de meiose I konden bewerkstelligen. Er moest een soort "lijm" zijn die de chiasmata op hun plaats hield, hetzij door aan de chiasmata te binden, hetzij door handhaving van de zuster-chromatiden cohesie distaal van de chiasmata. Hoewel er mutanten bestonden die deze lijm niet leken te kunnen maken, bleef de aard van de lijm onbekend. Ik heb in het kader van dit promotieonderzoek geprobeerd om componenten te identificeren die een rol spelen in de meiotische zuster-chromatiden

cohesie en recombinatie, om het samenspel tussen deze twee processen in de meiose te analyseren, en om inzicht te verwerven in de relatie tussen deze processen en DNA-herstel en recombinatie in de mitose.

In **hoofdstuk 1** leg ik uit welke modelorganismen ik heb gebruikt voor dit onderzoek. Alle experimenten zijn uitgevoerd met twee filamenteuze schimmels, *Sordaria macrospora* en *Aspergillus nidulans*. Mijn belangrijkste motivatie voor deze keuze was, dat er mutanten van deze schimmels bestonden (of makkelijk te verkrijgen waren) met een defect in de meiotische zuster-chromatiden cohesie en/of recombinatie, en dat de klonering van de betreffende wildtype genen door transformatie complementatie van het defect in de mutanten haalbaar leek. *S. macrospora* had als bijkomend voordeel dat dit organisme bijzonder geschikt is voor cytologische analyses, en *A. nidulans* had als bijkomend voordeel dat er voor dit organisme goede moleculair-genetische analysemethoden waren ontwikkeld, en dat er naast de seksuele cyclus een paraseksuele cyclus was. Dat laatste opende de mogelijkheid om ook de mitotische allelische recombinatie te analyseren. Bovendien zijn er slechts twee organismen bekend (*A. nidulans* en *Schizosaccharomyces pombe*) die geen synaptonemale complexen (SC's) vormen tijdens de meiotische profase, en geen positieve interferentie van meiotische overkruisingen vertonen. De keuze voor zowel *S. macrospora* als *A. nidulans* zou dus de mogelijkheid bieden om de functie(s) te vergelijken van genen die betrokken zijn bij meiotische recombinatie en/of zuster-chromatiden cohesie in een organisme met SC's en één zonder SC's.

Hoofdstuk 2 beschrijft hoe we het *SPO76* gen van *S. macrospora* gekloneerd hebben door transformatie complementatie van de meiotische defecten van de *spo76-1* (non-nul) mutant. Het was reeds bekend dat de meiotische recombinatie en zuster-chromatiden cohesie, en het mitotische DNA-herstel verstoord waren in deze mutant. Ook lokaliseerden we het Spo76 eiwit (Spo76p) in de opeenvolgende stadia van de mitose en meiose in wildtype *Sordaria*, en we voerden een gedetailleerde fenotypische analyse uit van de *spo76-1* mutant. We laten zien dat Spo76p met de chromosomen is geassocieerd is in alle stadia van de mitose en de meiose, behalve de metafase(s) en anafase(s). In de mitose verdwijnt Spo76p van de chromosomen tijdens de prometafase. In de profase I van de meiose is er meer Spo76p op de chromosomen aanwezig dan in enig ander stadium van de celcyclus, en het eiwit ligt dan vooral vlak langs de chromosomale assen. Spo76p dissocieert van de chromosomen tijdens het diploteen. In de *spo76-1* mutant vonden we een tijdelijke verstoring van de chromosoomorganisatie in de mitotische prometafase: het stadium duurde langer dan in het wildtype, en het was opmerkelijk dat zich op de chromosomen stukken met een gebrekkige zuster-chromatiden cohesie en condensatie afwisselden met ogenschijnlijk normale stukken. We veron-

derstellen dat bij de overgang van profase naar metafase krachten optreden die de cohesie tussen de zuster-chromatiden op de proef stellen, en dat Spo76 een minimum aan cohesie helpt handhaven als de chromosomen condenseren. Evenzo zagen we dat in de meiotische profase van *spo76-1* vanaf laat-leptoteen de zuster-chromatiden cohesie en chromosoomcondensatie gezamenlijk waren verstoord, op een regionale schaal. Stukken chromosoom met gespleten axiaal element wisselden af met regio's waar de axiale elementen niet gespleten waren. De niet gespleten segmenten konden stukjes SC vormen met hier en daar een late recombinatie nodule (late RN: ultrastructureel herkenbaar enzymcomplex dat betrokken is bij de latere stappen in de meiotische recombinatie). Terwijl het aantal late RN's sterk verminderd was in *spo76-1*, kwamen vroege RN's (die door immuuncytochemische kleuring van Rad51 en Dmc1 te herkennen waren) in bijna normale aantallen voor, maar ze bleven langer dan normaal aanwezig. Mogelijk is in de *spo76-1* mutant een tussenstap van de meiotische recombinatie verstoord. Misschien speelt Spo76p in de meiose bij de overgang van leptoteen naar zygoteen een vergelijkbare rol als in de mitotische profase-metafase overgang: ook tijdens de leptoteen-zygoteen overgang zouden er krachten kunnen optreden die de cohesie zouden kunnen verbreken, en Spo76p zou ook dan kunnen bijdragen aan het behoud van cohesie. Spo76p zou ook nog een rol kunnen spelen tijdens de late meiotische profase, omdat in *spo76-1* de zuster-chromatiden vanaf het diploteen geheel uiteenwijken, terwijl dit in wildtype niet gebeurt vóór anafase II.

Het voorspelde eiwitproduct van *SPO76* (Spo76p) is evolutionair geconserveerd van schimmels tot de mens; het hoogste percentage identieke aminozuren (44%) werd gevonden met het BIMD eiwit van *A. nidulans*. Er was al een voorwaardelijk letale mutatie in het *bimD* gen van *A. nidulans* bekend, namelijk *bimD6*. Bij hoge temperatuur vertoont de *bimD6* mutant (letale) defecten in de mitotische chromosoomsegregatie, en bij lage temperatuur (niet letale) DNA-herstel defecten.

In **hoofdstuk 3** tonen we door heterologe complementatie aan dat het *SPO76* gen van *S. macrospora* zowel de temperatuur- als MMS (methyl methaan sulfonaat) gevoeligheid van *bimD6* in *A. nidulans* kan complementeren; dat betekent dat Spo76p en BIMD in de mitose functioneel overeenkomstige rollen spelen. We laten ook zien dat de *bimD6* mutant, net als *spo76-1*, geen seksuele sporen (ascosporen) vormt. Echter, in tegenstelling tot *spo76-1* is *bimD6* verstoord in de premeiotische ontwikkeling, en vormt nauwlijks asci. In de paar asci die in *bimD6* ontstaan en de meiose ingaan, wijken de zuster-chromatiden voortijdig uiteen, maar dit defect lijkt in *bimD6* minder ernstig te zijn dan in *spo76-1*. In tegenstelling tot de mitotische eiwitlokalisatie, vertoonde de meiotische lokalisatie van Spo76p en BIMD boven-

dien een paar belangrijke verschillen. Anders dan Spo76p in *Sordaria*, was BIMD in *A. nidulans* tijdens de meiotische profase niet in grotere hoeveelheden aanwezig dan in de mitotische cyclus, en ook was BIMD niet preferentieel langs de chromosomale assen gelokaliseerd tijdens de chromosoomparing. Bovendien kon *SPO76* niet de seksuele sporulatie defecten van *bimD6* in *A. nidulans* complementeren, en omgekeerd kon *bimD* ook niet de seksuele sporulatie defecten van *spo76-1* verhelpen. Deze resultaten duiden op soortspecifieke verschillen tussen de functies van Spo76p en BIMD in de meiose. Deze verschillen houden mogelijkerwijze verband met de eerder besproken (**hoofdstuk 1**) verschillen in de meiotische chromosoomorganisatie in beide schimmels: in tegenstelling tot *S. macrospora* vormt *A. nidulans* geen SC's en vertoont het geen positieve interferentie van meiotische overkruisingen.

Verder laten we zien dat *bimD6* behalve voor UV en MMS ook verhoogd gevoelig is voor ioniserende röntgenstraling, echter alleen als delende cellen aan deze agentia worden blootgesteld. De *bimD6* mutant lijkt in dit opzicht sterk op recombinatie-deficiënte *A. nidulans* mutanten, zoals *uvsC114* (het *uvsC* gen van *A. nidulans* is homoloog aan *RAD51* van *Saccharomyces cerevisiae*; **hoofdstuk 4**). We hebben *bimD6* daarom met *uvsC114* vergeleken wat betreft defecten in de mitotische recombinatie. In tests voor allelische recombinatie leverden *bimD6* en *uvsC114* vergelijkbare resultaten op. In beide mutanten waren de absolute frequenties voor allelische recombinatie sterk verlaagd, hoewel de verdeling van de recombinanten over de verschillende klassen ongeveer hetzelfde was als in wildtype. De testresultaten voor intrachromosomale conversies waren daarentegen verschillend voor *bimD6* en *uvsC114*. De frequentie van intrachromosomale conversies tussen onderbroken duplicaties was sterk verlaagd in *uvsC114*, en op wildtype niveau in *bimD6*. Wij veronderstellen daarom dat BIMD nodig is voor homologe recombinatie als de matrijs zich bevindt op een ander (zuster- of niet-zuster-) chromatide, maar niet als de matrijs zich dichtbij bevindt op dezelfde zuster-chromatide of dezelfde chromatine domein/loop. De DNA-herstel machinerie moet dus mogelijk samenwerken met zuster-chromatiden cohesie complexen (die zich waarschijnlijk op de grens tussen de domeinen/loops bevinden) om toegang te krijgen tot een matrijs in een ander chromatide of in een andere loop/domein van hetzelfde chromatide. *uvsC* is daarentegen in beide situaties nodig voor recombinatie-herstel.

Hoofdstuk 4 beschrijft de klonering van het *uvsC* gen van *A. nidulans* door transformatie complementatie van de mitotische DNA-herstel defecten van de *uvsC114* mutant. We hebben het *uvsC* gen ook geheel uitgeschakeld en we hebben de fenotypische effecten van deze *uvsC* nulmutatie vergeleken met die van de *uvsC114* mutatie. Het *uvsC* gen codeert voor een eiwit waarvan 67% van de aminozuren hetzelfde is als in Rad51p van *S. cerevisiae* en

27% hetzelfde is als in het RecA eiwit van *Escherichia coli*. Deze eiwitten zijn betrokken bij homologe recombinatie: ze helpen enkelstrengs DNA om homoloog dubbelstrengs DNA binnen te dringen en de plaats in te nemen van één van de twee strengen. In afwezigheid van DNA-beschadigende agentia was de transcriptie van het *uvsC* gen hoger in de *uvsC114* mutant dan in het wildtype. In zowel wildtype als *uvsC114* stammen was de transcriptie van het *uvsC* gen induceerbaar met MMS. We hebben de mitotische en meiotische fenotypes van de *uvsC114* mutant (die een deletie van zes baseparen heeft in core domain I) vergeleken met die van de *uvsC* nulmutant. De nulmutant was gevoeliger voor UV en MMS dan *uvsC114*, dus *uvsC114* is kennelijk geen nulmutatie. Het effect van deze *uvsC* mutaties op de seksuele sporulatie was ook verschillend. In de *uvsC* nulmutant was de seksuele ontwikkeling geblokkeerd vóór de meiose, terwijl de *uvsC114* mutant geblokkeerd was in de meiotische profase. Oudere cleistothecia van de *uvsC* nulmutant bevatten grote, meerkernige cellen. Deze komen mogelijk overeen met gedegenereerde croziers die geblokkeerd zijn in de premeiotische S-fase. Uitschakeling van *RAD51* heeft in *S. cerevisiae* geen gevolgen voor de vegetatieve groei en veroorzaakt een blokkade in de meiotische profase. Uitschakeling van *Rad51* in de muis is daarentegen embryonaal-letaal. Uitschakeling van *uvsC* had in *A. nidulans* geen effect op de vegetatieve groei, en veroorzaakte een blokkade in een stadium voorafgaand aan de meiose. Blijkbaar heeft uitschakeling van *RAD51* in verschillende organismen een ander effect.

Hoofdstuk 5 beschrijft de isolatie en karakterisering van seksuele sporulatiemutanten in *A. nidulans*. We hebben vegetatieve sporen bestraald met een hoge dosis UV (1.5% overleving) en de overlevende kolonies uitgeplaat op gesupplementeerd minimaal medium. Vervolgens hebben we de ascosporevorming bekeken in rijpe vruchtlichamen van 1250 ogenschijnlijk normaal groeiende kolonies. Twintig van de 1250 kolonies vormden vruchtlichamen die niet of nauwelijks seksuele sporen bevatten. Nadat deze mutanten in twee opeenvolgende rondes waren gekruist met het wildtype, leverden twee mutanten geen nakomelingschap meer op met het oorspronkelijke mutante fenotype; deze mutanten hebben we niet verder geanalyseerd. De overige 18 mutanten waren alle recessief en konden worden ingedeeld in 15 complementatie groepen. Op grond van deze getallen schatten we dat ongeveer 50-100 genen onder de geteste groeicondities specifiek betrokken zijn bij de vorming van seksuele sporen in *A. nidulans*. We hebben de chromosomale locatie van drie van de 18 mutaties bepaald door middel van paraseksuele analyse: twee mutaties bleken elk met een bepaald chromosoom te zijn geassocieerd, terwijl de derde mutatie geassocieerd was met een translocatie breekpunt. Van alle 18 mutanten hebben we de inhoud van de vruchtlichamen cytologisch geanalyseerd. Een groot deel van de mutanten, namelijk 11 van de 18, was geblokkeerd was in de meioti-

sche profase (zoals *uvsC114*; **hoofdstuk 4**) of in metafase I (zoals *bimD6*; **hoofdstuk 3**). Deze nieuwe collectie mutanten bevat dus mogelijk mutanten die specifiek verstoord zijn in meiotische recombinatie en/of zuster-chromatiden cohesie. We suggereren een strategie om de betreffende wildtype genen te kloneren, namelijk door te selecteren op het ontstaan van prototrofe nakomelingen als gevolg van de herverdeling van auxotrofe markers tijdens de meiose.

In de Algemene Discussie (**hoofdstuk 6**) speculeren we over de mogelijke raakvlakken tussen zuster-chromatiden cohesie en recombinatie in mitose en meiose. Terwijl cohesie tussen zuster centromeren in de mitose voornamelijk in dienst staat van de chromosoomdisjunctie, zou cohesie tussen de armen van zuster-chromatiden in de mitose een additionele rol kunnen spelen bij DNA-herstel door recombinatie. De cohesiecomplexen aan de basis van chromatine domeinen/loops zouden kunnen fungeren als een basis voor de assemblage van recombinatie-herstel complexen. Wij denken dat in mitotische cellen de cohesie complexen de recombinatie-machinerie helpen een homologe matrijs te vinden in een exact overeenkomstig segment van de onbeschadigde zuster-chromatide. Ze zouden zo verantwoordelijk kunnen zijn voor de voorkeur voor de zuster-chromatide als matrijs bij recombinatie-herstel tijdens de mitotische G2. In de meiose I moet die voorkeur omgebogen worden naar het homologe chromosoom, maar dit mag niet gepaard gaan met een verlies aan arm cohesie, omdat arm cohesie in de meiose I mogelijk nog belangrijker is voor de correcte chromosoomsegregatie dan in de mitose. Daarom functioneren de recombinatie machinerie en het cohesie complex in de meiose in een gewijzigde vorm. De gemodificeerde cohesie complexen zouden als basis kunnen dienen voor de assemblage van axiale elementen. Waarschijnlijk vindt de inductie van meiotische DSB's gelijktijdig met de vorming van axiale elementen plaats. Net zoals we veronderstelden voor mitotisch recombinatie-herstel, nemen we aan dat de meiotische DSB's worden overgebracht naar de basis van de chromatine loops, waar ze in contact komen met de cohesie complexen en met additionele componenten van de recombinatie machinerie. We denken echter dat de nu aanwezige axiale elementen de zuster-chromatide blokkeren als matrijs voor recombinatie-herstel van de DSB's. Bovendien zouden componenten van de axiale elementen samen met de gemodificeerde meiotische recombinatie machinerie DNA-DNA contacten met een niet-zuster-chromatide van het homologe chromosoom tot stand kunnen brengen. Dus, door de basis te vormen voor de assemblage van axiale elementen en een meiose-specifiek recombinatie complex, zouden de cohesie complexen kunnen bijdragen aan de voorkeur voor de niet-zuster-chromatide van het homologe chromosoom als matrijs voor de homologe recombinatie tijdens de meiose.

Résumé

La cohésion des chromatides soeurs et la recombinaison homologue jouent un rôle essentiel dans la maintien de l'intégrité du génome et la fidèlité de la ségrégation des chromosomes durant la mitose et la méiose. Divers facteurs causant des lésions dans l'ADN peuvent menacer l'intégrité du génome. Parmis ces lésions, les cassures des deux brins (CDBs) de l'ADN sont considérées comme particulièrement néfastes. L'origine de ces CDBs peut être endogène, comme les radicaux libres produits par le métabolisme cellulaire, ou exogène, comme les radiations ionisantes et la lumière ultraviolette. La réparation fidèle des CDBs est importante car elle empêche la fragmentation des chromosomes qui souvent engendre des translocations ou des délétions délétères. Parmi les voies complexes de réparation de l'ADN, la recombinaison homologue, qui utilise comme moule une copie intacte et homologue de l'ADN détérioré pour effectuer la réparation des CDBs, est considérée comme la plus fidèle.

Les CDBs de l'ADN peuvent aussi être générées dans la cellule comme intermédiaires normaux lors de processus de réarrangement génomique dont, par exemple, la recombinaison méiotique. Au début de la prophase de méiose, les cellules (de levure) induisent activement un très grand nombre de CDBs tout le long de leur ADN chromosomique. Ces cassures sont ensuites rapidement réparées par un processus de recombinaison homologue qui utilise préférentiellement comme moule les chromatides non-soeurs des chromosomes homologues. Pour chaque paire de chromosomes homologues, il y au moins une CDB qui est réparée avec un échange réciproque d'un bras chromosomique (crossing over) tandis que la majorité des CDBs, le long de cette même paire chromosomique, sont réparées sans échange de bras chromosomiques. Ces sites d'échanges réciproques entre chromatides non-soeurs (chiasmata) jouent un rôle important dans la ségrégation des chromosomes homologues lors de la première division méiotique.

Lorsque j'ai commencé mon travail de thèse, on savait que ces échanges réciproques en tant que tels n'étaient pas suffisants pour assurer la ségrégation correcte des chromosomes homologues mais qu'il fallait également pouvoir stabiliser les chiasmata en place. On pensait que cette cohésion permettait soit de maintenir directement les chiasmata, soit de renforcer le lien entre les chromatides soeurs à distance des chiasmata. Bien que des mutants déficients dans leur abilité de produire cette force adhésive avaient été identifiés, la nature de celle-ci

restait encore énigmatique. Durant ma thèse, j'ai essayé d'identifier des composants impliqués dans la cohésion des chromatides soeurs et/ou dans la recombinaison méiotique, de façon à analyser la relation entre ces deux processus pendant la méiose mais aussi pendant la réparation mitotique de l'ADN et le métabolisme des chromosomes.

Dans le **chapitre 1**, je présente les systèmes expérimentaux modèles utilisés pour la recherche décrite dans cette thèse. Toutes mes investigations ont été réalisées avec deux champignons filamenteux, à savoir *Sordaria macrospora* et *Aspergillus nidulans*. Les raisons les plus importantes qui m'ont conduite au choix de ces deux organismes, étaient la disponibilité (ou la facilité d'obtention) de souches mutantes défectives dans les processus de cohésion des chromatides soeurs et/ou de la recombinaison, mais aussi l'existence de techniques de clonage par transformation et complémentation fonctionelle des phénotypes mutants pour isoler les gènes impliqués dans ces processus. Par ailleurs, des techniques performantes d'observation cytologique avaient été développées pour *S. macrospora*, ainsi que des outils de génétique moléculaire dans le cas de *A. nidulans*. Ce dernier possède une phase parasexuelle dans son cycle de vie qui offre la possibilité d'étudier la recombinaison allélique durant la mitose. De plus, pendant la prophase méiotique, *S. macrospora* assemble des complexes synaptonémaux (CSs) tandis que *A. nidulans* est un des rares organismes qui ne forme pas de CSs et qui ne présente pas de mécanisme de régulation des crossing over appelé interférence positive. Le choix de ces deux organismes offre donc la possibilité exceptionelle de comparer le ou les rôles des gènes impliqués dans la cohésion des chromatides soeurs et/ou la recombinaison dans des méioses avec CSs et sans CSs.

Dans le **chapitre 2**, nous décrivons le clonage du gène *SPO76* de *S. macrospora* par transformation et complémentation fonctionelle des défauts méiotiques de la souche mutante (hypomorphe) *spo76-1*. L'analyse de cette souche mutante avait mis en évidence des défauts de cohésion des chromatides soeurs et de recombinaison au cours de la méiose, ainsi que dans le processus mitotique de réparation de l'ADN. Nous avons analysé la localisation de la protéine Spo76p (taggée par HA et GFP) au cours des cycles mitotiques et méiotiques de la souche sauvage, et nous présentons une analyse détaillée des phénotypes de la souche mutante *spo76-1*. Nous montrons que Spo76p est associée aux chromosomes durant toutes les phases de la méiose et de la mitose excepté pendant les métaphases et les anaphases. En mitose, Spo76p disparait des chromosomes durant la prométaphase. Pendant la première prophase méiotique, Spo76p est plus abondante que durant toutes les autres phases du cycle cellulaire, et est préférentiellement localisée au niveau de l'axe des chromosomes; elle disparait des chromosomes au diplotène. Dans la souche mutante *spo76-1*, nous observons un défaut

transitoire dans l'organisation des chromosomes de prométaphase de mitose: la durée de cette phase est prolongée et la morphologie des chromosomes est anormale. On observe notamment, le long de chaque chromosome, une alternance de régions normales et de régions où la cohésion des chromatides soeurs ainsi que la compaction de la chromatine sont perturbées. Nous proposons que la transition de la prophase à la métaphase mitotique met en jeu des forces qui ont tendance à rompre la cohésion, et que Spo76p pourvoit au maintien d'un minimum de cohésion en relation avec la compaction des chromosomes. De même, pendant la prophase de méiose de la souche mutante *spo76-1*, nous observons, à partir de la fin du leptotène, que la cohésion des chromatides soeurs et la compaction des chromosomes sont également perturbées de façon coordonnée. Les régions chromosomiques où les éléments axiaux des CSs sont séparés, alternent avec les régions où ces éléments sont associés. Seules ces dernières régions forment des CSs. Ces rares morceaux de CSs contiennent néanmoins des nodules de recombination tardifs (NRs: complexes enzymatiques reconnaissables ultrastructurellement et impliqués dans les dernières étapes de la recombinaison méiotique). Alors que le nombre de NRs tardifs de *spo76-1* est fortement réduit par rapport à celui de la souche sauvage, la fréquence des NRs précoces (identifiés par marquage avec des anticorps anti- Rad51p et Dmc1p) n'est que légèrement inférieure à celle de la souche sauvage; par contre, ils persistent plus longtemps. Le rôle de Spo76p durant la transition leptotène/zygotène de la prophase méiotique pourrait être similaire à son rôle durant la transition prophase/métaphase de mitose, car la transition leptotène/zygotène pourrait aussi mettre en jeu des forces qui tendent à rompre la cohésion des chromatides soeurs. Spo76p pourrait également avoir un rôle en fin de prophase méiotique puisque dans la souche mutante *spo76-1*, les chromatides soeurs se séparent complètement dès le diplotène, tandis que dans la souche sauvage, cette séparation ne débute pas avant l'anaphase de la deuxième division méiotique.

L'analyse de la structure primaire de la protéine Spo76p montre qu'elle appartient à une famille de protéines bien conservées durant l'évolution et qui est présente aussi bien chez les champignons que chez l'homme. Spo76p est homologue à la protéine BIMD d' *A. nidulans* (44% d'identité au niveau de la séquence des acides aminés). La souche mutante *bimD6* d'*A. nidulans* avait été identifiée préalablement grâce à l'expression de son phénotype conditionel de léthalité avec un défaut dans la ségrégation des chromosomes lorsque la souche est maintenue à haute température et un défaut dans la réparation de l' ADN lorsqu'elle est cultivée à basse température.

Dans le **chapitre 3**, nous démontrons par complémentation fonctionelle hétérologue que le gène sauvage *SPO76* de *S. macrospora* peut corriger la sensitivité à la température ainsi

qu'au méthyle sulfonate de méthane (MSM) de la souche mutante *bimD6* d'*A. nidulans* et par conséquence nous inférons qu'il y a homologie fonctionelle directe du rôle des deux protéines durant la mitose. Nous montrons aussi que, tout comme *spo76-1*, les souches mutantes *bimD6* ne forment pas de spores sexuelles. Cependant, contrairement à *spo76-1*, les souches mutantes *bimD6*, sont perturbées dans leur développement préméiotique et ne forment que de rares asques qui sont bloqués en métaphase de première division. Les chromatides soeurs se séparent également prématurément, mais le défaut de séparation est moins sévère dans *bimD6* que dans *spo76-1*. De plus, tandis que la localisation mitotique des deux protéines est très semblable, leur localisation méiotique présente d'importantes différences. Contrairement à Spo76p chez *S. macrospora*, BIMD n'est pas plus abondante pendant la prophase méiotique que durant la mitose, et ne se localise pas préférentiellement près de l'axe des chromosomes pendant l'appariement des chromosomes homologues. *SPO76* n'est pas capable de complémenter les défauts de sporulation sexuée de la souche mutante *bimD6* d'*A. nidulans*, et *vice versa*, *bimD* ne corrige pas les défauts de sporulation sexuée de la souche mutante *spo76-1* de *S. macrospora*. Ces résultats suggèrent des rôles différents pour Spo76p et de BIMD durant la méiose. Ces différences sont probablement liées aux différences d'organisation méiotique des chromosomes chez ces deux champignons, notamment l'absence de CSs et d'interférence positive des crossing overs chez *A. nidulans* (voir ci-dessus; **chapitre 1**).

Nous montrons aussi que les mutants *bimD6* sont hypersensibles aux rayons X en plus de leur sensibilité élevée à la lumière ultraviolette et au MSM, mais ceci uniquement quand les cellules exposées sont en division. Le mutant *bimD6* ressemble donc très fort aux mutants rec$^-$ d'*A. nidulans*, tel que *uvsC114* (le gène *uvsC* d'*A. nidulans* est homologue au gène *RAD51* de *Saccharomyces cerevisiae*; voir ci-dessous, **chapitre 4**). Nous avons donc comparé la recombinaison mitotique dans les souches *bimD6* à *uvsC114*. L' analyse de la recombinaison allélique de *bimD6* et de *uvsC114* donne des résultats similaires. Chez les deux mutants, les fréquences absolues de recombinaison allélique sont fortement diminuées bien que la distribution relative des différentes classes de recombinants (crossing over et conversion génique) reste comparable à celle mesurée dans les souches sauvages. Cependant les taux de conversion intrachromosomique sont différents pour *bimD6* et *uvsC114*. La conversion intrachromosomique entre des duplications interrompues est significativement réduite chez *uvsC114*, mais pas chez *bimD6*. Nous proposons que *bimD* est requis pour la recombinaison homologue lorsque la copie intacte et homologue d'ADN servant de moule, se trouve sur une autre chromatide (soeur ou non-soeur), mais ne l'est pas lorsque celle-ci se trouve à proximité sur la même chromatide soeur (boucle chromatidique). Nous suggérons

que la machinerie de réparation coopère avec les complexes de cohésion (associés à la matrice des chromosomes) lorsque la copie homologue servant de moule pour la réparation se trouve en dehors du domaine de la boucle intrachromatidique. Au contraire, *uvsC* est requis pour les deux types de réparation.

Dans le **chapitre 4**, nous décrivons le clonage du gène *uvsC* d'*A. nidulans* par transformation et complémentation fonctionelle des défauts de réparation mitotique de la souche mutante *uvsC114*. De plus, nous avons interrompu le gène *uvsC* et comparé le phénotype de cet allèle amorphe à celui de l'allèle hypomorphe *uvsC114*. La protéine préduite par le gène *uvsC* est identique à 67% à la protéine Rad51p de *S. cerevisiae* et à 27% à la protéine RecA de *Escherichia coli*. Ces protéines sont impliquées dans les processus d'invasion et d'échange de brin d'ADN durant la recombinaison homologue. Nous avons observé que les transcripts du gène *uvsC* sont plus abondants dans la souche mutante *uvsC114* que dans la souche sauvage lorsque les cellules ne sont pas exposées à des agents capable d'endommager l'ADN. Le MSM stimule la transcription du gène *uvsC* de la souche mutante *uvsC114* et de la souche sauvage. Nous avons comparé les phénotypes mitotiques et méiotiques de souches contenant la mutation *uvsC114* (une délétion de 6 paires de bases dans le domaine central I) à ceux de souches mutantes *uvsC* amorphes. Le mutant amorphe *uvsC* est plus sensible à la lumière ultraviolette que le mutant *uvsC114*, indiquant que ce dernier est un allèle hypomorphe. Les phénotypes de différentiation sexuelle diffèrent aussi chez les deux mutants. Dans le mutant amorphe *uvsC*, la différentiation sexuelle est perturbée avant le début de la méiose, tandis qu'elle est bloquée durant la prophase méiotique chez le mutant *uvsC114*. Nous avons observé la formation de grandes cellules plurinucléées dans les cleistothecia agés de la souche mutante amorphe *uvsC*. Ces cellules sont probablement des crochets dégénérés ayant été bloqués pendant la phase S préméiotique. Chez *S. cerevisiae*, l'interruption du gène *RAD51* n'a pas d'effets sur la multiplication cellulaire durant le cycle mitotique mais induit un arrêt de la différentiation sexuelle au stade de la prophase de la première division méiotique. Chez *Mus musculus*, l'interruption du gène *RAD51* est léthale pour l'embryon. Chez *A. nidulans*, l'interruption du gène *uvsC* n'a pas d'effets sur la multiplication cellulaire par mitose mais induit un arrêt de la différentiation sexuelle à un stade préméiotique. Les conséquences de la disruption des gènes homologues à *RAD51* sont donc différentes d'une espèce à l'autre.

Dans le **chapitre 5**, nous décrivons la sélection et la caractérisation de mutants de la sporulation sexuelle d'*A. nidulans*. Pour obtenir ces mutants, nous irradié les spores végétatives avec une forte dose de lumière ultraviolette (survie de 1.5%), puis étalé les

colonies survivantes sur milieu minimum supplémenté. Les colonies capables de croître normalement furent criblées visuellement de manière à identifier celles qui forment des organes fructifères "stériles" (avec quelques ou sans ascopores). Vingt mutants présentant le phénotype attendu furent selectionnés parmi 1250 colonies analysées. Deux de ces mutants ne présentant pas de ségrégation mendélienne furent rejetés. Les 18 mutants restants contenaient tous un alléle récessif et furent classés en 15 groupe de complémentation. Nous estimons que sous les conditions testées, 50 à 100 gènes sont impliqués spécifiquement dans la formation des ascospores chez *A. nidulans*. Trois mutations furent cartographiées par analyse parasexuelle: deux mutations purent être assignées à un chromosome spécifique et une autre à un site de cassure d'une translocation. L'analyse cytologique des organes fructifères "stériles" de ces 18 mutants a montré que dans la majorité d'entre eux (11/18), la différentiation sexuelle était arrêtée soit à la prophase de la première division méiotique (comme *uvsC114;* **chapitre 4**) soit à la métaphase de la première division méiotique (comme *bimD6;* **chapitre 3**). Il est donc possible que cette nouvelle collection contienne des mutants spécifiquement affectés dans les processus de recombinaison homologue et/ou de cohésion des chromatides soeurs. Nous présentons une stratégie de clonage des gènes sauvages correspondants, par sélection de la progéniture prototrophique issue du réassortiment méiotique de marqueurs auxotrophiques.

Dans la discussion générale (**chapitre 6**), nous spéculons sur les liens possibles entre la cohésion des chromatides soeurs et la recombinaison mitotique et méiotique. Tandis que le rôle premier de la cohésion centromérique pendant la mitose est d'assurer la disjonction correcte des chromosomes, la cohésion des bras chromosomiques pourrait jouer des rôles additionels durant la réparation de l'ADN par recombinaison. Les complexes de cohésion, situés à la base des boucles chromatidiques, pourraient fonctionner comme sites de nucléation pour l'assemblage des complexes de réparation de l'ADN, et pourraient aussi aider ces complexes à trouver un moule homologue dans le segment équivalent de la chromatide soeur intacte. Un tel rôle des complexes de cohésions permettrait d'expliquer pourquoi, durant la phase mitotique G2, le processus de réparation de l'ADN par recombinaison utilise de façon fortement biaisée les chromatides soeurs comme moule pour la réparation. Durant la recombinaison méiotique ce sont les chromatides non-soeurs qui sont utilisées comme moule pour la réparation, mais en même temps, la cohésion des chromatides soeurs doit être maintenue et vraisemblablement renforcée pour garantir la ségrégation réductionelle correcte des chromosomes homologues. Par conséquence, la machinerie de réparation par recombinaison et celle de la cohésion fonctionnent de concert de façon spécifiques à la méiose. Durant cette collaboration, les complexes de cohésion pourraient servir de fondement

pour l'assemblage des élements axiaux et latéraux des CSs lors de la différentiation méiotique des chromosomes. Les CDBs sont formées en même temps que les éléments axiaux et latéraux, et sont amenées en contact avec d'autres protéines requises pour la réparation par recombinaison, à la base des boucles chromatidiques. Nous proposons que la recherche d'homologie sur la chromatide soeur sera alors bloquée par la formation des éléments axiaux et latéraux, de façon à forcer l'utilisation d'un autre moule pour la réparation par recombinaison homologue. Avec les composants des éléments axiaux, les complexes de recombinaison vont établir des contacts ADN-ADN avec la chromatide non-soeur du chromosome homologue. Les complexes de cohésion contribuent donc, par le rôle de fondement qu'ils apportent à la formation des éléments axiaux et des complexes de recombinaison spécifiques à la méiose, au choix préférentiel de la chromatide non-soeur du chromosome homologue comme moule de réparation durant la recombinaison homologue de la méiose.

Dankwoord

Het onderzoek dat in dit proefschrift is beschreven is het resultaat van vele jaren werk van diverse personen. Ik wil hier dan ook graag van de gelegenheid gebruik maken om iedereen te bedanken die, op wat voor wijze dan ook, heeft meegeholpen aan de totstandkoming van dit proefschrift. Een aantal personen wil ik graag met naam noemen.

In de eerste plaats de twee personen die over de gehele periode bij dit werk betrokken zijn geweest: Christa Heyting (promotor) en Henk van den Broek (co-promotor). Christa, ik ben jou dankbaar voor je hulp bij het structureren van gedachten en resultaten. Daarnaast wil ik je bedanken voor je inzet en gedrevenheid, je vermogen om altijd kritisch te blijven, en je bereidheid om tot op het laatste moment de puntjes op de i te blijven zetten. Ik heb veel geleerd van (en veel plezier beleefd aan) het gezamenlijk bediscussiëren en vormen van wetenschappelijke ideeën! Henk, ik ben jou dankbaar voor je loyaliteit. Ik heb in de loop van deze promotie op diverse plekken aan diverse onderwerpen gewerkt. Ik ben je dankbaar voor de vrijheid die je me hierin gegeven hebt, en voor het vertrouwen dat de diverse eindjes ooit tot een logisch geheel zouden worden samengeknoopt.

Het grootste deel van dit onderzoek heeft zich afgespeeld op de vakgroep Erfelijkheidsleer in Wageningen, waar ik een bench had binnen de secties Moleculaire Genetica en Schimmel Genetica. Van de sectie Moleculaire Genetica een speciaal woord van dank voor Theo Goosen. Theo, de start van dit onderzoek werd gemaakt onder jouw leiding, en ik ben je dankbaar voor de bevlogenheid en inzet waarmee je me de fijne kneepjes van het vakgebied Moleculaire Genetica hebt bijgebracht. Ik wil je er ook voor bedanken dat je me hebt aangespoord het uiterste uit mezelf te halen en een eigen weg in te slaan. Nog iemand die betrokken was bij mijn wetenschappelijke start in Wageningen is Bert Wennekes. Bert, wat hadden we gemoeten zonder jouw vermogen tot relativeren! Ik ben jou en Jannie (Wennekes) dankbaar voor jullie vriendschap. Verder wil ik Hans Thijs en Sandor Snoeiers bedanken voor het samen delen van de ups and downs van onze promoties. Hans en Sandor: heel erg bedankt voor jullie betrokkenheid! Sandor, ook bedankt voor het feit dat je straks op de grote dag als paranimf aan mijn zijde wilt staan! Er zijn diverse studenten geweest die voor hun stage of afstudeervak Moleculaire Genetica een steen(tje) aan mijn promotieonderzoek hebben bijgedragen. Marjola van der Weide, Ralf van Dijk, Jens Pilling, Helmut Kessels, Boris Bartholdy en Kristin Steger: allemaal van harte bedankt voor jullie inzet en bijdrages. Ik wil ook alle medewerkers van de sectie Schimmel Genetica bedanken, in het bijzonder Klaas

Swart, Edu Holub, Marijke Slakhorst en Fons Detbets. Klaas, bedankt voor de cytologische analyses van *Aspergillus* mutanten en voor de belangstelling en inzet waarmee je alle onderdelen van mijn promotieonderzoek gevolgd hebt! Edu, heel erg bedankt voor je hulpvaardigheid, adviezen en je vriendschap! Marijke en Fons: bedankt voor jullie input in de mutagenese experimenten en voor de vele praktische adviezen. Van de sectie Moleculaire Celgenetica ben ik Mirjam van Aalderen erg dankbaar voor haar hulp bij het antibodywerk, en wil ik Hildo Offenberg bedanken voor zijn hulp bij computerprogramma's en sequentieanalyses. Verder wil ik natuurlijk alle andere medewerkers van de vakgroep Erfelijkheidsleer die hier niet met naam genoemd zijn bedanken. Bedankt voor jullie bijdrage aan de werksfeer en voor het feit dat ik altijd op jullie terug kon vallen voor materiele ondersteuning, handige, technische tips en voor het uitwisselen van ideeën!

Une partie importante de cette thèse a été faite à Orsay, France, dans le laboratoire de Meiose et Morphogénèse dirigé par Denise Zickler. Denise: un grand merci pour l'hospitalité et la générosité que vous m'avez montrées depuis le début de ce travail. La première semaine vous m'avez accueillie sans hésitation dans votre laboratoire et dans votre maison, et je n'oublierai jamais les soirées sympathiques passées avec Dinu autour d'un bon repas et de bons verres de vin (dans cette semaine et aussi pendant les années qui ont suivi). Dans le travail, j'admire beaucoup votre force et votre persévérance, votre esprit ouvert et surtout votre plaisir contagieux de faire et de parler science. Je vous remercie pour la confiance avec laquelle vous m'avez laissé la liberté de me débrouiller quand vous pensiez que j'en étais capable, et avec laquelle vous m'avez présenté aux collègues (nationaux et internationaux). Grâce à votre investissement total dans notre travail, je n'ai jamais eu le sentiment de me débrouiller toute seule. J'ai été très heureuse de travailler avec vous et les autres membres de votre laboratoire. Françoise (James): merci beaucoup pour toutes les belles photos que vous avez tirées pour nous. Merci aussi d'avoir tapé les cleistothecia d'*Aspergillus* avec une force telle que nous ayons pu enfin observer les chromosomes d'*Aspergillus* (bien que le prix à payer était des ampoules)! Sophie (Tessé): merci et bonne chance pour la continuation de la chasse aux gènes impliqués dans la cohésion des chromatides soeurs! Anne (Levasseur): merci pour la bonne volonté et le courage avec lesquels tu nous a coulé d'innombrables boites de milieu! Véronique (Berteaux-Lecellier): merci pour l'aide avec HA et les analyses de séquences! Marie-Claire (Fontyne) et Christiane (Rayssiguier): merci d'avoir partagé la paillasse et le bureau avec moi. Je tiens aussi à remercier tous les autres membres de l'Institut de Génétique et Microbiologie pour leur esprit ouvert et la volonté de partager du materiel et de donner des conseils. Je dois beaucoup à Chantal (Bohn) et à Annie (Grégoire) pour l'aide

avec les commandes et aussi pour m'avoir encouragée à apprendre la culture et la langue française. Annie, il me semble bien que le premier mot de français que tu m'avais appris c'était le mot 'emprunter': je crains que plein de personnes peuvent témoigner que je m'en suis servi souvent depuis! Je ne peux nommer tous ceux que je voudrais remercier. Je souhaite néanmoins remercier particulièrement les membres des équipes de Jean-Luc Rossignol, Marguerite Picard, Philipe Bouloc, Gilles Vergnaud, Marie-José Daboussi, Claudio Scazzocchio, Betty Fellenbok et Michel Duguet d'avoir partagé votre matériel et votre connaissance avec moi. Je veux spécialement remercier Christophe Goyon et Fabienne Malagnac d'avoir partagé autre chose en plus: merci pour votre amitié. Je veux exprimer aussi ma reconnaissance à Almuth et Jean Collard: vous m'avez donné un vrai sens de la famille en dehors du labo. Merci beaucoup pour votre hospitalité, l'amitié et pour l'intérêt avec lequel vous avez suivi mon travail. Almuth, merci aussi pour être venue au labo (bien après ta retraite), à des moments "de bourre", pour m'aider à repiquer des transformants ou à préparer des lames. Finalement, je veux remercier tous les collègues et aussi tous mes amis parisiens pour les bons moments que nous avons passés ensemble, et je veux exprimer ma reconnaissance à Marie-Noëlle Berthelot pour avoir accepté d'être ma marraine du grand jour.

There are three other colleagues from abroad that I would like to thank for their collaborations: Etta Kafer (Simon Fraser University, Canada), Nancy Kleckner (Harvard University, USA) and Stefanie Pöggeler (Ruhr Universität, Germany). Etta, it was a real honor for me to talk and collaborate with somebody who has been in the field for such a long time as you have been: you made me feel the excitement of the early days over things that we now take for granted! Many thanks for the accuracy, open-mindedness, helpfulness and expertise with which you contributed to this thesis! Nancy, I am grateful for the financial and scientific support that you gave to the Spo76 project. I liked my stay in Boston very much and I would like to thank you and the other members of your lab for your hospitality. Stefanie, I would like to thank you very much for your willingness to collaborate and to share materials. I am grateful to you, to Prof. Kück and to the other members of the lab for the hospitality I enjoyed during my visits.

Verder wil ik mijn collega's van de afdeling Celbiologie en Genetica van de Erasmus Universiteit Rotterdam bedanken. De uiteindelijke afronding van dit proefschrift heeft plaats gevonden nadat ik als postdoc in Rotterdam begonnen was. Ik ben met name Dik van Gent zeer erkentelijk voor de vrijheid die hij mij heeft gegeven deze promotie af te ronden. I also would like to thank Mauro Modesti for the many discussions we had about meiosis and for his very nice translation of the summary of this thesis into French.

Voor het welslagen van deze universitaire onderneming zijn het vertrouwen en de ondersteuning van het thuisfront heel belangrijk voor mij geweest. Ik wil mijn familie en schoonfamilie en mijn vrienden bedanken voor hun ondersteuning en interesse. Een speciaal woord van dank gaat hierbij uit naar mijn ouders voor het niet aflatende geduld en de belangstelling waarmee zij de vele (vaak met technische details overgoten) verhalen over mijn vorderingen aangehoord hebben. Francis: een goed voorbeeld doet goed volgen! Nu we beiden onze promoties afgerond hebben, kunnen we eindelijk .....

Curriculum Vitae

Naam	Diana van Heemst
Geboren	5 oktober 1964 te Wageningen
1977-1983	VWO-β aan het Wagenings Lyceum te Wageningen
1983-1989	Studie Biologie (cum laude) aan de Landbouw Universiteit Wageningen, met de volgende combinatie van afstudeervakken en stages: (1) Moleculaire Genetica: afstudeervak op de vakgroep Erfelijkheidsleer in Wageningen (begeleiding: Dr. T. Goosen); stage aan de vakgroep Moleculaire Carcinogenese van de Rijks Universiteit Leiden (begeleiding: Drs. M. Timmers); (2) Immunologie: afstudeervak en stage op de afdeling Immunologie van het Centraal Diergeneeskundig Instituut in Lelystad (begeleiding: Dr. P. van der Heyden); (3) Endocrinologie: afstudeervak op de vakgroep Dierfysiologie in Wageningen (begeleiding: Dr. J. Mattheij).
1989-1991	Toegevoegd onderzoeker aan de Landbouw Universiteit Wageningen, vakgroep Erfelijkheidsleer, sectie Moleculaire Genetica.
1991-1993	Wetenschappelijk onderzoeksmedewerker in dienst van het Centraal Diergeneeskundig Instituut te Lelystad, afdeling Immunologie.
1993-1999	Promotie-onderzoek (Promotor: Prof. Dr. C. Heyting; Co-promotor: Dr. H. W. J. van den Broek), uitgevoerd in de volgende laboratoria:
1993-1996	Landbouw Universiteit Wageningen, vakgroep Erfelijkheidsleer (Dr. T. Goosen, Dr. K. Swart);
1996-1997	Institut de Génétique et Microbiologie, Université Paris-Sud, Orsay, France (Prof. D. Zickler);
1997	Harvard University, Department of Molecular and Cellular Biology, Boston, USA (Prof. N. Kleckner);
1997-1998	Institut de Génétique et Microbiologie, Université Paris-Sud, Orsay, France (Prof. D. Zickler);
1998-1999	Landbouw Universiteit Wageningen, vakgroep Erfelijkheidsleer (Dr. H. W. J. van den Broek, Dr. K. Swart).
1999-heden	Post-doc in tijdelijke dienst van de vakgroep Celbiologie en Genetica aan de Erasmus Universiteit Rotterdam.

Foto omslag: met dank aan de zusjes Laura en Marijn Miessen