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Homologous chromosome pairing and recombination during meiosis in wild type and synaptic mutants of tomato

Francis W.J. Havekes

Homologous chromosome pairing and recombination during meiosis in wild type and synaptic mutants of tomato

Homologe chromosoomparing en recombinatie tijdens de meiose van wild type en synaptische mutanten van de tomaat

Proefschrift
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Stellingen

1 Initiatie van synapsis wordt bevorderd door crossing-over. (Dit proefschrift)

2 In de review van Dawe (1998) wordt het interferentie model van Storlazzi et al. (1996) op het belangrijkste punt (de rol van het SC) volledig verkeerd geïnterpreteerd.
Dawe *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49: 371-395
Storlazzi *et al. Proc. Natl. Acad. Sci. USA* 93: *9043-9048*

3 Wetenschap zal nooit de rol van (bij)geloof overnemen.

4 Het gebrek aan diplomatie van Nederlandse politici maakt de nietige plaats van Nederland op de wereldbol nog kleiner.

5 Stadsmensen verwarren tolerantie vaak met stedelijke arrogantie.

6 Het gezamenlijk schrijven van een artikel is te vergelijken met een autorit: men koestert een ijdel genoegen in de eigen stijl, voorziet elkaar daarom graag van commentaar en vergeet ondertussen op de weg te letten.

7 De "spijspot" (cadeau voor alle personeelsleden ter gelegenheid van het 75-jarig jubileum van de LUW) is een treffend symbool voor de manier waarop de Wageningse universiteit haar zittend personeel schijnbaar onvoorwaardelijk onderhoudt en symboliseert tevens de dood-in-de-pot uitwerking van dit beleid op jonge onderzoekers.

8 Gezondheidsfanatisme is schadelijker voor het welzijn dan genotzucht.

9 Innerlijke rijkdom c.q. armoede weerspiegelen bewustzijnstoestanden van respectievelijk geloof en ongeloof in de eigen waarde.

10 Het verschil tussen menselijke en kunstmatige intelligentie is dat mensen in tegenstelling tot computers de vrijheid hebben in te gaan tegen hun 'programmering'.

Stellingen behorende bij het proefschrift 'Homologous chromosome pairing and recombination during meiosis in wild type and synaptic mutants of tomato'.
Francis Havekes, Wageningen, 8 januari 1999.

Content

1

General introduction

DNA (deoxyribonucleic acid) molecules represent the biological blueprints for the hereditary processes by which cells produce their proteins and reproduce themselves. DNA molecules are coiled and folded, together with histones and non–histone proteins into complex, well organised packages, the chromosomes. The number and morphology of chromosomes are constant in each undifferentiated somatic cell of an individual, and are unique for each species. In a diploid organism there are two copies of each chromosome, the two homologues, one of maternal and one of paternal origin. The chromosomal complement remains unchanged after each mitotic cycle. The DNA molecule in each chromosome is reproduced during the S–phase of the mitotic interphase by semi–conservative replication, so that daughter cells get identical DNA–copies as their mothers. At the chromosomal level, the two identical sister–chromatids, which remain attached to each other, become apparent at late prophase. At metaphase, when chromosomes are highly condensed, the chromosomes orient in the equatorial plane of the spindle with the sister–centromeres facing opposite poles. This ensures proper equational division of chromosomes at anaphase (Figure 1).

In the sexual life cycle, haploid and diploid generations of cells alternate. The transition from the diploid to the haploid phase takes place during meiosis. Meiosis consists of two successive divisions (meiosis I and II) without an intermediate round of DNA replication (Figure 1). The homologous chromosomes disjoin during meiosis I (reductional division), whereas the sister chromatids are separated during meiosis II (equational division). The diploid phase is restored shortly after fertilisation when two haploid gametes fuse.

The products of meiosis not only have a reduced, haploid, chromosome complement, they also carry new combinations of parental alleles. Both effects are the consequence of specific events during the unique first meiotic division. During prophase I, homologous chromosomes pair along their entire length, and non–sister chromatids of homologues cross–over. These crossovers provide physical links between the homologous chromosomes (chiasmata), which enable the homologues to orient towards opposite poles at metaphase I. When the chiasmata are released at anaphase I, the recombined homologues move to opposite poles, and thus disjoin (Figure 1).

The fertility of sexually reproducing higher eukaryotes depends on their ability to correctly reduce the chromosome number during the two meiotic divisions. This can be accomplished only when parental chromosomes are capable of pairing and recombining at meiotic prophase I. These

inter–homologue processes are almost always accompanied by the formation of a meiosis–specific structure, the synaptonemal complex (SC) along the axes of the chromosomes. For long, the SC was thought to be a prerequisite for crossing–over (von Wettstein *et al.* 1984), but more recently, the opposite has also been proposed (Kleckner 1996).

This PhD project aims at obtaining further insight in the relation between synapsis and recombination. We chose tomato for reasons mentioned below, and compared chromosome pairing and recombination in normal wild type tomato with the same processes in meiotic mutants that were disturbed in chromosome pairing or recombination. Electron microscopy was used for the ultrastructural analysis of the SCs at meiotic prophase I, whereas light microscopic studies were helpful in elucidating chromosome behaviour in later stages of male and female meiosis. First I will describe the general aspects of synapsis and recombination, and then will focus on more specific aspects of meiosis in tomato.

Synapsis

The synaptonemal complex (SC) is a tripartite proteinaceous structure which is formed between homologous chromosomes as they pair during meiotic prophase I. The SC was first described by Moses (1956) and Fawcett (1956). Moses described the SC in an ultrastructural study on sections of meiotic prophase spermatocytes of crayfish and correctly interpreted its relation with chromosome pairing. Since then the structure was described for numerous species and was soon considered as a universal phenomenon of meiotic prophase I (reviewed in Westergaard and von Wettstein 1972, von Wettstein *et al.* 1984). Especially the development of various SC spreading techniques (Counce and Meyer 1973, Moses 1977, Gillies 1981, Stack 1982) advanced our knowledge on SC formation, and the synaptic process is now well documented for many organisms (review: Gillies 1984).

Figure 2 shows a schematic representation of the morphological changes of the SC through prophase I of meiosis. At the onset of prophase I, shortly after DNA replication during premeiotic interphase, chromosomes begin to condense. During this leptotene stage, a core structure known as axial core is deposited along the fused sister chromatids of each chromosome. Homologous axial elements move to each other by a still unknown mechanism and when they are at a distance of about 100 nm, a third longitudinal element is assembled between them. This is the central element, which, together with the axes of the homologues makes up the tripartite structure of the SC. Thus, SCs consist of two relatively thick lateral elements (called axial elements when unsynapsed) with a thinner central element between them, in the so called central region (the region between the lateral elements). In some organisms transverse filaments, crossing the central region, have been observed (Schmekel *et al. 1995).*

SC formation continues in a zipper–like fashion along the chromosomes until they are synapsed from telomere to telomere, at the end of zygotene. In several organisms, synapsis is preceded by presynaptic alignment, which is a rough, parallel alignment of homologous axial elements at a larger

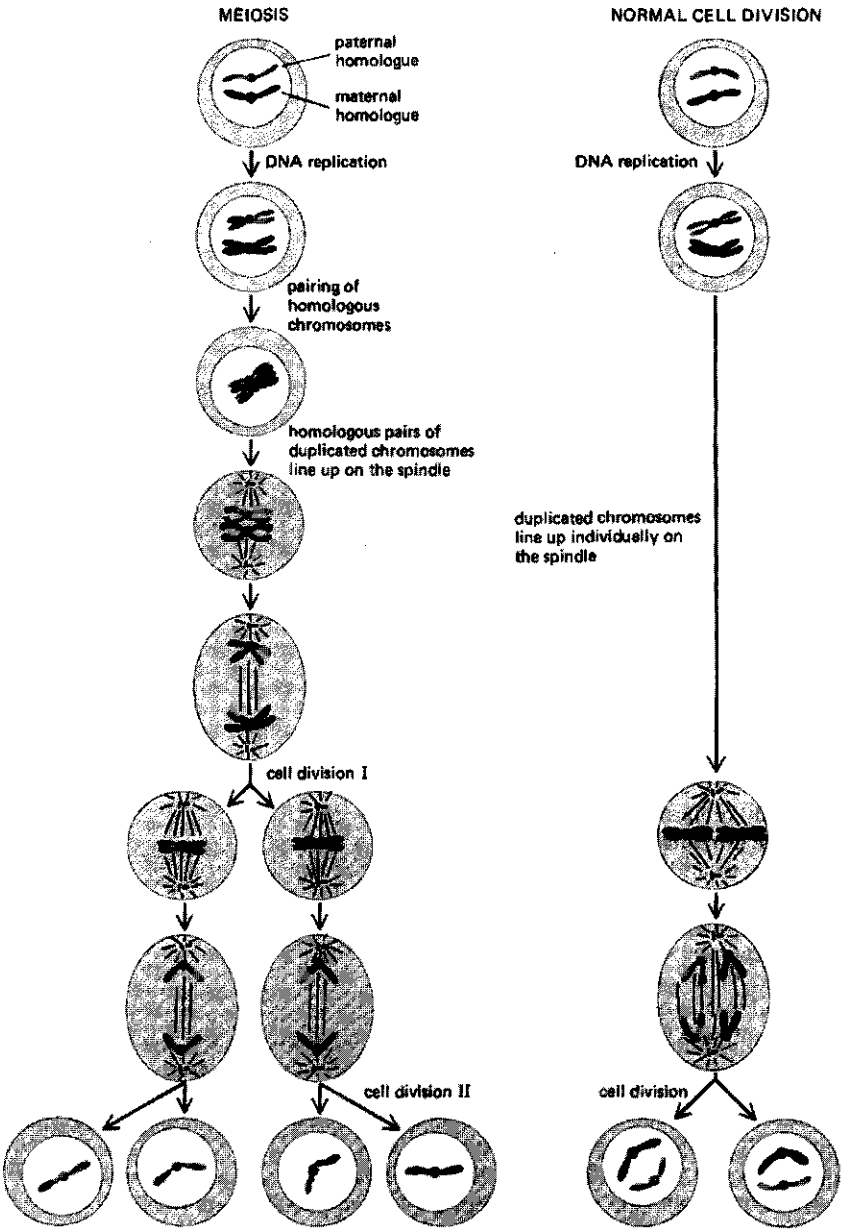


Figure 1: The course of meiosis compared to that of mitosis. Only one set of homologous chromosomes is shown. The pairing of the replicated homologous chromosomes is unique to meiosis. Because meiosis consists of two successive nuclear divisions, each diploid cell entering meiosis produces four haploid cells (from Alberts *et al.* 1989).

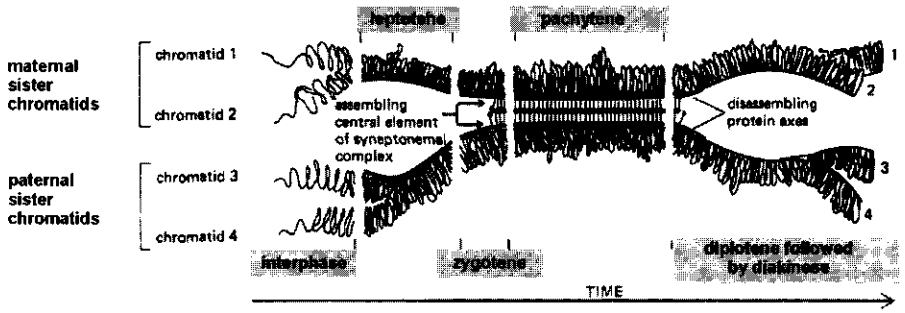


Figure 2: A schematic figure showing the successive stages of meiotic prophase as defined on the basis of morphological changes of the SC: leptotene (proteinaceous axes start to form along the chromosomes), zygotene (start of synapsis of the homologues), pachytene (synapsis is completed and the SC extends from telomere to telomere), diplotene (SC disassembles) and diakinesis (SCs are completely disassembled) (from Alberts *et al.* 1989).

distance than the width of the tripartite SC–structure (review in Loidl 1990). Presynaptic alignment is probably a common feature of meiosis, although it can not normally be observed in species in which the assembly and alignment of axial elements is rapidly followed by synapsis (see Loidl 1994). Presynaptic alignment and synapsis are distinct events; the former occurs only between homologous axes, whereas the latter may occur between non–homologous axes as well. The presynaptic association sites probably provide or maintain homologous contacts from which SC initiation may (but does not have to) ensue. The preference for homologous chromosome synapsis would thus be ensured by homologous alignment (Loidl and Jones 1986). Mechanisms that have been suggested to enhance homologous encounters are non–random premeiotic chromosome disposition, attachment and clustering of telomeres to the inner nuclear membrane (bouquet configuration), and aggregation of (heterochromatic) chromosome segments in small areas of the nucleus (synizetic knot, Loidl 1990).

Possible roles of the SC, apart from its putative function in holding homologues together (von Wettstein 1984), include the direction and maintenance recombinational interactions. There is evidence that the SC directs initiated recombination events towards the homologue rather than the sister–chromatid (Schwacha and Kleckner 1994). Intact SCs may also be required for crossover interference (the repression of crossing–over in the vicinity of a crossover), because (1) the fungi *Schizosaccharomyces pombe* (Kohli and Bahler 1994) and *Aspergillus nidulans* (Egel–Mitani *et al.* 1982), which lack crossover interference, have no SC and (2) the *zip1* mutation in yeast abolishes interference and lacks SC (Sym and Roeder 1994). A third suggested role for the SC concerns chiasma maintenance, because pieces of SC persist at chiasma sites in some organisms (Westergaard and von Wettstein 1972).

Chiasma formation

Chiasmata, the physical connections between homologous chromosomes that mark the sites of crossing–over during late diplotene–metaphase I, are necessary for proper reductional division. Only homologues that are connected by at least one chiasma are able to orient to opposite poles in the metaphase I plate. The bivalent attains stable bipolar attachment because of tension resulting from spindle forces from the two poles counteracted by attachment of the homologues to each other (review: Miyazaki and Orr–Weaver 1994).

Crossovers are by themselves inadequate for persistent chiasmate association, so that additional functions are required to maintain the connections until anaphase I (Maguire 1995). Darlington (1932) first considered that chiasmata can be stabilised by the association of segments of the sister chromatids that lie distal of the crossover site. Two possible mechanisms that can be envisaged to explain this cohesion of sister–chromatids are: (1) sister chromatids may be linked (catenated) through virtue of DNA structure or chromatin topology, or (2) cohesion may be directly conferred by chromatid–linking proteins (Miyazaki and Orr–Weaver 1994). An alternative model for chiasma maintenance is that chiasmata are stabilized by a binder substance which is positioned only at crossover locations and which may be a product or a remnant of the SC (reviewed in Carpenter 1994a,b and Maguire 1995). The release of chiasmata at anaphase I would be triggered by the sudden loss of either or both of these bonds.

Chiasmata have been studied in many species since the earliest investigations in meiosis. They provide a direct, rapid and technically straightforward approach to the analysis of recombination events. In few organisms, generally with long chromosomes, they give detailed information on the total amount of recombination and on distribution of recombination events throughout the genome (Jones 1986). However, there are also drawbacks to chiasma studies: in species with small chromosomes, chiasmata are imprecise indicators of crossover position. Chiasma frequencies may be underestimated in such species because adjacent crossovers can not be resolved by light microscopy (Sybenga 1996). In addition, chiasmata represent only the reciprocal recombination events, not the non–reciprocal gene conversion events (Jones 1986). A welcome alternative to the analysis of recombination by the study of chiasmata came in the 1970s when specific structures involved in recombination were found along pachytene SCs: the recombination nodules (see Carpenter 1994 for overview).

Recombination nodules

Recombination nodules (RNs) where first described in sectioned pachytene cells of female *Drosophila* (Carpenter 1975). The roughly spherical and densely staining nodules were located adjacent to the central element and spanned the width of the SC. Their number and distribution showed a strikingly good agreement with the distribution of exchange events as determined from conventional recombination analysis (Carpenter 1975). Shortly after their discovery, RNs were also described in many other organisms, where they were studied in relation to chiasmata (Zickler 1977;

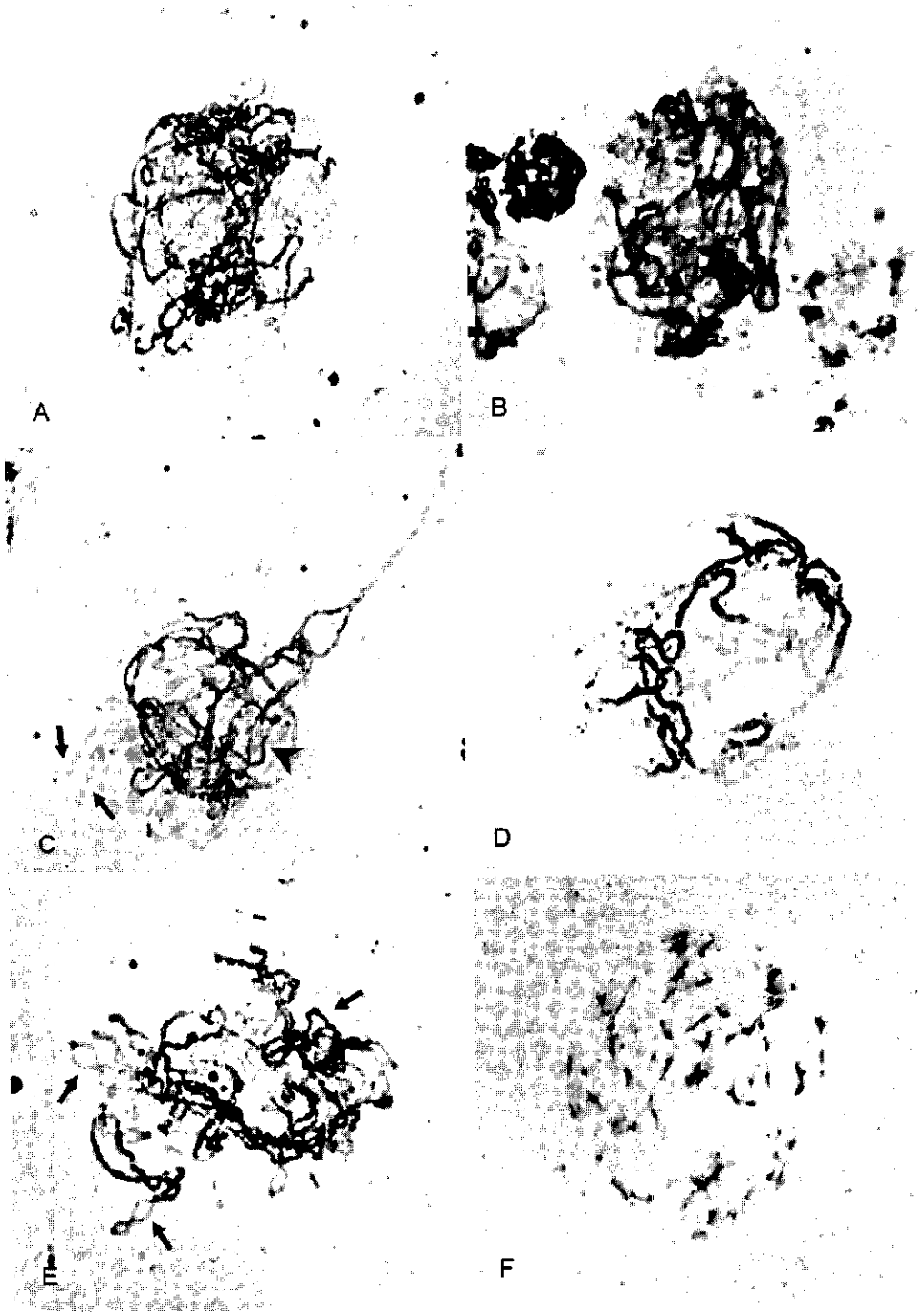
Bernelot–Moens and Moens 1986; Albini and Jones 1988; Stack *et al* 1989). In all these organisms the frequency and distribution of RNs in mid–late pachytene corresponded well to chiasma frequency and distribution, so it is now generally accepted that RNs in mid to late pachytene SCs (late RNs) are structures that mark the sites of crossing–over. In several species, nodules were also found in earlier stages of meiosis. Such early recombination nodules, however, occurred in far larger numbers than late RNs and differed from late RNs by their distribution along the chromosomes and their morphology. It was suggested that the early RNs might mark the sites of gene conversion, the non–crossover recombination event (Carpenter 1987). Recently the RecA–like proteins Rad51 and/or DMC1, which are known to play a role in early steps of meiotic recombination, were localised immunocytochemically in early meiotic nodules (Anderson *et al.* 1997). This indicates that these early nodules are indeed involved in recombinational activities.

Molecular aspects of recombination

The molecular aspects of recombination have been studied mainly in budding yeast (*Saccharomyces cerevisiae*). The double strand break (DSB) repair model of meiotic recombination (Szostak *et al.* 1983) is generally accepted, at least for yeast. According to this model, meiotic recombination is initiated by double–strand DNA–scission. The ends of the broken molecule are resected by 5' to 3' exonucleolytic activity. The single stranded tails invade into an uncut homologous DNA duplex where they promote repair synthesis and branch migration to produce a double Holliday junction. Depending on the direction in which these junctions are resolved, the result will be either a non-crossover (gene conversion) or a reciprocal crossover.

In timing studies it was found that DSB formation precedes initiation of synapsis, SCs are formed approximately concomitantly with double Holliday junctions, and crossovers and non– crossovers appear at the end of pachytene, immediately before or concomitant with (but not dependent upon) SC disappearance (Schwacha and Kleckner 1994). Invasion of a single stranded DNA tail into an intact DNA duplex would provide a way of testing for homology between two DNA molecules. It seems unlikely however, that homology is tested in this way, because yeast mutants that lack invasion of single stranded tails nevertheless display a significant level of meiotic chromosome pairing as detected by chromosome painting (reviews in Loidl et al. 1994; Weiner and Kleckner 1994; Nag et al. 1995).

Figure 3. Meiotic stages in wild type tomato as observed by light microscopy in spread preparations. **A** and **B.** Leptotene – early zygotene nuclei with long, thin, almost exclusively unpaired chromosomes. The heterochromatic regions are darkly stained, the euchromatic regions are lightly stained. **C.** Zygotene nucleus with paired euchromatic regions at the periphery (arrows) and unpaired heterochromatic regions in the centre (arrow head). **D.** Pachytene nucleus: complete chromosome pairing. **E.** Early diplotene nucleus. Chromosomes have desynapsed over most of their lengths (arrows), but remain associated at the telomeres and at some distal euchromatic regions. **F.** Diffuse diplotene: chromosome structure has completely disappeared. Only blocks of heterochromatin can be seen.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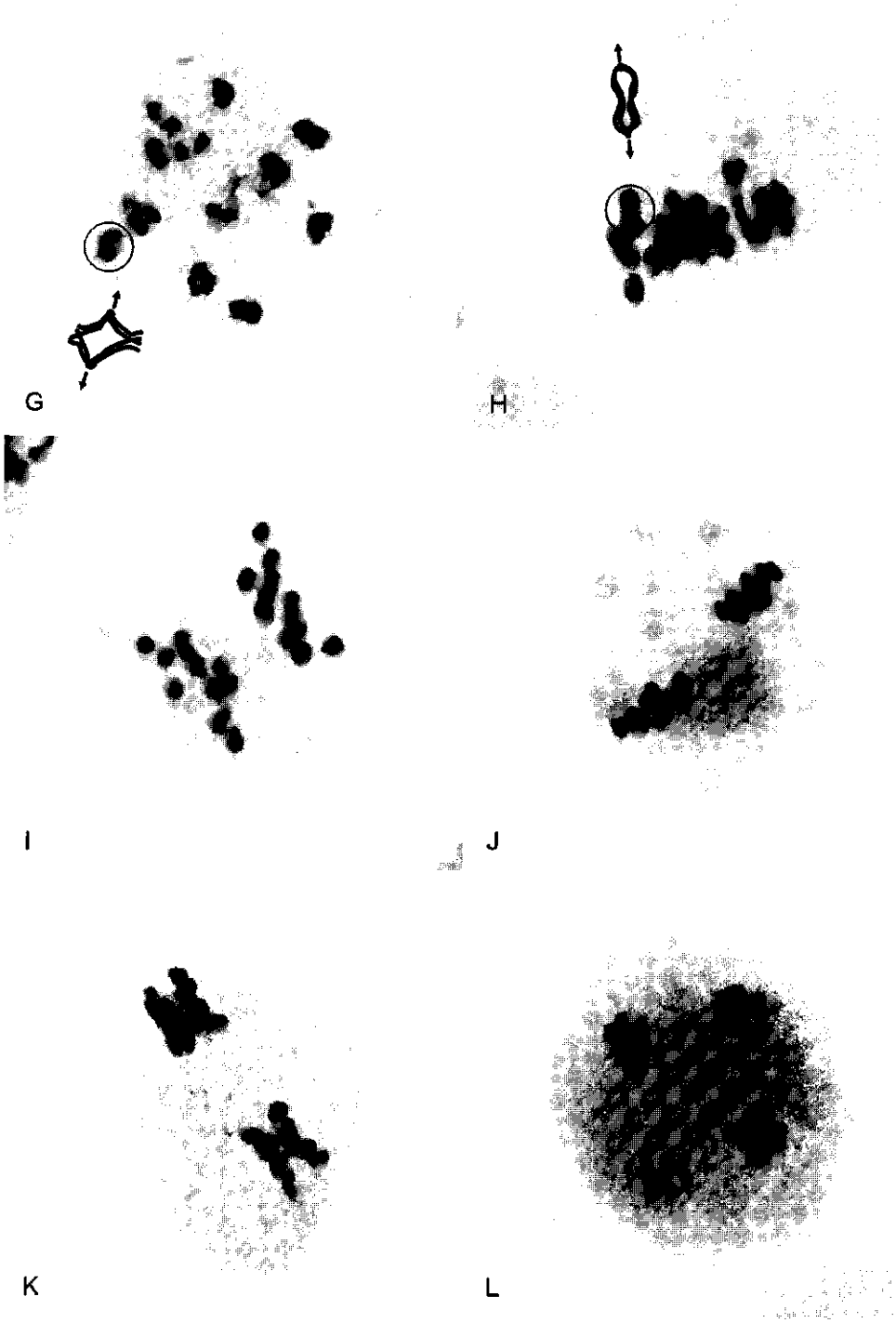
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Homology search interactions would rather be guided by direct DNA–DNA contacts between intact duplexes, with searching facilitated by appropriate proteins (discussed in Weiner and Kleckner 1994).

The principles of meiosis

One major effect of meiosis is the reduction of the chromosome complement, usually from the diploid to the haploid number. How to achieve this? By separating the homologous (parental) chromosomes in advance of the normal (mitotic) separation of the two chromatids of replicated chromosomes. Meiosis is usually regarded as a two–step process with the division of homologues as step one and the separation of sister chromatids as step two. In my opinion, it is just as appropriate to consider the following two phases. In the first phase homologous chromosomes undergo the elaborate processes of pairing and recombination, culminating in at least one crossover between non–sister chromatids of homologues. This phase begins at early leptotene and ends at diplotene. The second phase in which the chromosomes undergo the two meiotic divisions rather quickly, begins at diakinesis and ends at telophase II. At the transition between these two phases, in some organisms defined as the diffuse diplotene stage, chromatin is reorganised in preparation of the two divisions. The main subject of this thesis is the analysis of processes of the first phase, involving homology search, homologue pairing and recombination, and their effect on chromosome behaviour in the second phase.

Meiosis in tomato

The tomato (*Lycopersicon esculentum*, 2n=24) was the research object in this study. As will become clear in the following sections, all the characteristics of normal meiotic chromosome behaviour such as presynaptic alignment, synapsis, formation of two types of recombination nodules and chiasmate bivalent formation can be observed in tomato. In addition, there are several mutants with abnormal pairing or crossing–over, as well as numerous structural (deletions, translocations) and numerical variants (haploids, polyploids, trisomics) of tomato, which allow the analysis of meiotic processes under certain restraints. I will first give a view of tomato meiosis as observed by light microscopy, and then outline the electron microscopic studies of synapsis and recombination nodules.

Figure 3 (*continue*). **G.** Diakinesis: twelve bivalents. An interpretation drawing with centromeres and chiasmata is shown. **H.** Metaphase I with twelve bivalents lined up in the equator. The drawing shows the positions of centromeres and chiasmata. **I.** Anaphase I: separation of homologous chromosomes. **J.** Metaphase II with two spindles each with twelve chromosomes. **K.** Anaphase II: simultaneous equational division of chromosomes in both spindles. **L.** Telophase II: Four (haploid) sets of twelve chromosomes (each consisting of one chromatid).

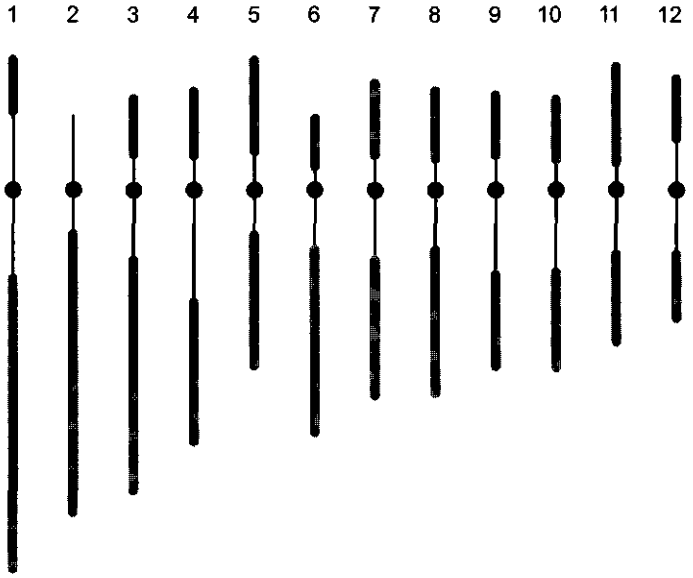

Figure 4: Ideogram of the twelve tomato chromosomes. The diagrams represent SC lengths according to Sherman and Stack (1991, 1992). Thick gray lines represent the euchromatic regions; thin lines are heterochromatic regions. The solid spheres are the kinetochores.

Because tomato chromosomes are rather small, most cytogenetic studies focus on the pachytene chromosomes. At this meiotic prophase stage, chromosomes are on average fifteen times longer than their counterparts at mitotic metaphase and depict a well differentiated pattern of heterochromatic segments, which makes them particularly appropriate for cytogenetic analysis. Pachytene chromosomes have been used for identification of the 12 tomato chromosomes (Ramanna and Prakken 1967), for mapping of morphological markers in deletion studies (Khush and Rick (1968), and for physical mapping of repetitive and unique sequences by means of FISH (Zhong 1998, and references therein).

The meiotic stages of tomato as observed by light microscopy in spread preparations are shown in Figure 3. Leptotene (Figure 3A+B) and zygotene (Figure 3C) stages are difficult to analyse because the chromosomes are tightly bunched in a so–called synizetic knot and resist flattening (Moens 1964, Cawood and Jones 1980). The maximum contraction of the synizetic knot is reached at or just before the time of complete pairing (Cawood and Jones 1980).

At pachytene (Figure 3D), pairing is complete, the knot begins to loosen, the bivalents become dispersed, and chromosome morphology is now at its clearest (Cawood and Jones 1980). Ramanna and Prakken (1967) were able to identify all 12 pachytene bivalents of tomato by differences in total chromosome length, arm ratio, proportion of heterochromatin on both arms, characteristic

chromomeres in euchromatic regions and characteristic gaps in heterochromatic regions. A schematic representation of the 12 tomato chromosomes based on SC lengths is shown in Figure 4.

Diplotene begins when homologues start to separate along their arms (Figure 3E). At this time, the centromeres and telomeres frequently remain paired (Moens 1964). The nucleus becomes filled with a network of fine threads as separation progresses (diffuse diplotene). The chromatin then rapidly contracts and individual bivalents become discernible again. The homologous centromere regions appear to repel each other, whereas distal (euchromatic) chromosome arms are still connected by chiasmata. Bivalents condense further until diakinesis (Figure 3G) and congress to the centre of the cell where they become oriented in the equatorial plane of the spindle at metaphase I (Figure 3H). The chiasmate bonds between the homologous pairs are released and the forces of the microtubule apparatus pull the homologues towards opposite poles at anaphase I (Figure 3I). The second meiotic division quickly follows the first one; chromosomes orient at metaphase II (Figure 3J), and the sister chromatids are now pulled towards opposite poles (Figure 3K). This results in four sets of twelve chromosomes (each consisting of one chromatid) at telophase II (Figure 3L).

Tomato LM studies are useful for the analysis of chiasma formation and of the two meiotic divisions. LM studies are less practical for detailed analysis of homologous chromosome pairing in early prophase I. For this purpose, EM analyses of synaptonemal complex formation are more appropriate.

Tomato SCs

Tomato SCs were first analysed and described by Menzel and Price (1966) in EM sections. Stack (1982) developed a technique for hypotonically bursting primary microsporocytes of tomato, which allowed the analysis of SCs in two–dimensional spreads. Stack and Anderson (1986a) described the synaptic process in tomato: in nuclei at leptotene axial elements are formed along each chromosome. Images of spread cells at this stage are difficult to obtain and in no case completely interpretable. Pictures of zygotene through diplotene cells are more easily obtained. At zygotene, axial elements show presynaptic alignment and start to synapse. Synapsis can be initiated at multiple sites along the chromosomes, but usually it starts at or near the ends of chromosomes and it always proceeds to completion through centromeres and most heterochromatin. Synapsis proceeds rather synchronously along the bivalents of a cell. The heterochromatin around the nucleolus organiser near the end of the short arm of chromosome 2 behaves differently: the axial elements remain unsynapsed and in spreads they are often interrupted or broken. Kinetochores are not visible on zygotene and early pachytene SCs but become increasingly prominent as pachytene proceeds. Kinetochore staining is therefore a reliable indicator of the stage. The length of SCs does not change significantly between early to late pachytene. The end of pachytene is marked by the initiation of desynapsis, which usually occurs near the ends of SCs or adjacent to kinetochores; progression of desynapsis is retarded by telomeres, kinetochores and RNs. During diplotene, SCs often become irregularly coated by associ-

ated material, at least in spreads, and then fragment until SC remnants are no longer visible in spread cells.

Tomato SCs can be identified by their length, arm ratio and heterochromatin/euchromatin distribution. The tomato SC ideogram as described in Sherman and Stack (1992) is shown in Figure 4. It is similar to the ideogram of Ramanna and Prakken (1967), which was based on light microscopic observations of carmine stained pachytene chromosomes.

Tomato RNs

Stack and Anderson (1986b) analysed RN formation in tomato. During early and mid–zygotene, numerous ellipsoidal to spherical RNs associate with the axial elements and central element of the SC as it forms. Relatively few RNs associate with SC in the pericentric heterochromatin. During pachytene the number of RNs drops 4–fold. Stack and Anderson (1986b) propose that the numerous early nodules represent sites of recombinational interaction; only those nodules that persist into late pachytene would resolve recombination intermediates as crossover. It is possible that positive crossover interference is caused by the loss of RNs during early pachytene, because the first successful reciprocal recombination event transmits a signal in either direction down the SC which would result in the loss of nearby RNs. Such a mechanism would guarantee at least one, but not too many crossovers per SC (Mortimer and Fogel 1974).

Silver staining experiments indicate that there exist two types of nodules during zygotene and early pachytene (Sherman *et al.* 1992), one type that is stainable under silver nitrate at 50 °C and another that is less stainable under these conditions. Sherman *et al.* (1992) suggest that early nodules that are stainable at 50° C are normally retained into mid–pachytene through early diplotene as late nodules, whereas the less stainable type is normally lost during early pachytene. An extensive and as yet unequalled analysis of the distribution of late pachytene RNs was undertaken in tomato by Sherman and Stack (1995). This study revealed that; (1) every SC has at least one RN, (2) there are no RNs at the ends of SCs, (3) there are no RNs in kinetochores, (4) single RNs are found in the long arms more often than would be expected on the basis of arm length, (5) patterns of multiple RNs on SCs indicate crossover interference, (6) RNs may occur anywhere along SCs in euchromatin if telomeres and euchromatin/heterochromatin borders are not considered. Sherman and Stack (1995) were able to construct an RN map for all twelve tomato SCs.

Correspondence between RNs, chiasmata and genetic recombination

Sherman and Stack (1995) compared the frequency of RNs with the frequency of chiasmata at diakinesis–metaphase I. Chiasmata were counted by interpreting rod bivalents as having one chiasma and ring bivalents as having two chiasmata. The average number of RNs was not in close agreement

with the average number of chiasmata; 21 or 22 versus 17. But because the method used to count chiasmata ignores the possibility of more than one chiasma per chromosome arm, they also compared the predicted frequency of rod and ring bivalents if each RN would form a chiasma with the observed frequency or rods and rings. Rod bivalents would then result from one or more RNs in one arm, ring bivalents from one or more RNs in both arms. The differences between the observed and predicted frequencies were not significant, and it appears that a chiasma forms at the site of each RN (Sherman and Stack 1995).

Sherman and Stack (1995) also compared the (male meiosis derived) RN map with the tomato linkage maps (based on female and male meiosis). Tomato has one of the best linkage maps available (Tanksley *et al.* 1992), with many morphological markers, isozyme markers and molecular (RFLP, RAPD) markers. The molecular map (1275.9 map units) must be near saturation with 1030 molecular markers, whereas the classical map (1063 map units) is probably not (Tanksley *et al.* 1992).

Sherman and Stack (1995) interpreted the average of 21.89 RNs per complement to be equal to a map length of 1094.5 map units (21.89 RNs x 50 map units/RN), which is less than the molecular map. The shorter RN map length was probably not caused by loss of RNs during preparation, but rather, it might be due to higher recombination rates in female than in male meiosis, inaccuracies in RFLP mapping or differences in crossover rate between the materials studied (Sherman and Stack 1995).

Outline of this thesis

The aim of this thesis was to analyse the relationship between chromosome pairing (synapsis), recombination and chiasma formation in wild type tomato and in tomato meiotic mutants and haploid tomato.

The following questions were addressed:

1. How are synapsis and chiasma formation related to each other in synaptic mutants of tomato? Several meiotic mutants of tomato are available and some have already been studied light microscopically (Soost 1951, Moens 1969). These light microscopic analyses have not given satisfactory answers as to whether these mutants are asynaptic (reduced or abolished chromosome pairing) or desynaptic (complete chromosome pairing but reduced chiasma frequencies). Chapter 2 describes the synaptic pattern in four meiotic mutants of tomato, and relates this pattern to chiasma formation.
2. Are there differences in chromosome behaviour between female and male meiosis? Genetic studies indicate higher female–derived recombination frequencies (de Vicente and Tanksley 1991). This could not be related to cytological data because female meiosis had not yet been studied in tomato. Chapter 3 describes the analysis of female meiosis in wild type and in three meiotic mutants of tomato.

3. How is synapsis initiated in wild type and mutant tomato. In Chapter 4, the initiation of presynaptic alignment and synapsis are analysed in different bivalent classes of tomato.
4. How are synapsis and recombination related in mutants with reduced synapsis? The work of Moens (1969) shows that recombination frequencies may be higher in meiotic mutants than in wild type. Chapter 5 shows data on synapsis and recombination nodules in an asynaptic and a desynaptic mutant, and gives possible explanations for the higher crossover frequencies found by Moens.
5. How do synapsis and recombination proceed in the absence of homologous chromosomes? Humphrey (1933) found no evidence for chromosome pairing in haploid tomato but Ecochard *et al.* (1969) observed some chromosome pairing and occasional chiasmata in their haploid tomato material. Menzel and Price (1966) studied sections of haploid tomato anthers in the electron microscope and found occasional nonhomologous synapsis. Chapter 6 shows the results of an EM search for synapsis and RNs in spread nuclei of haploid tomato.

2

Synapsis and chiasma formation in four meiotic mutants of tomato (*Lycopersicon esculentum*)

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Synapsis and chiasma formation were studied in pollen mother cells of four meiotic mutants of tomato. The four mutants displayed defects in the assembly of the synaptonemal complex (SC) covering the whole range from almost complete absence of synapsis to complete synapsis at pachytene. In three mutants, we found a good correlation between the number of bivalents connected by at least one tripartite SC segment at pachytene and the number of chiasmatic bivalents at metaphase I. We suggest that in tomato functional chiasmata are only formed in the context of the tripartite SC.

Introduction

Meiotic chromosome pairing, recombination and segregation are accompanied by the assembly and disassembly of the synaptonemal complex (SC) (review: von Wettstein *et al.* 1984). During leptotene, axial elements are formed along each homologue; subsequently, at zygotene, the axial elements of homologous chromosomes are connected by transverse filaments, and a third longitudinal element, the central element is formed on the transverse filaments. The axial elements together with the central element make up the tripartite structure of the SC. Within the tripartite structure, axial elements are called lateral elements. At pachytene, the homologous chromosomes are connected (synapsed) by the tripartite structure along their entire length. At diplotene, the SCs are disassembled, and chromosomes condense further in preparation of metaphase I. During zygotene and pachytene, electron-dense structures, called recombination nodules (RNs), associate with the axial elements or with the tripartite structure (Carpenter 1975). In several species, two types of RNs (early and late) can be distinguished by differences in morphology, number, distribution and time of appearance (Carpenter 1979, Stack & Anderson 1986a,b, Albini & Jones 1987, 1988, Bojko 1989). Early RNs occur in large numbers at zygotene and early pachytene, and decrease in number at late pachytene. Late RNs appear somewhat later than early RNs; at late pachytene their number and localization correlates with the number and localization of crossover events (Carpenter 1987). Late RNs are therefore assumed to have a function in the formation of crossovers. The function of early RNs is still a matter of debate: it is possible that they have a function in detecting homologies, and that they leave conversion events as 'footprints' of their activity (Carpenter 1987).

The functions fulfilled by SCs and the regulation of their assembly and disassembly are still enigmatic. An important source of information about the regulation of SC assembly is the analysis of meiotic mutants. Several meiotic mutants of yeast have been identified which do not assemble a normal SC (Alani *et al.* 1990, Rockmill & Roeder 1990, Engebrecht & Roeder 1990, Hollingsworth *et al.* 1990, Bishop *et al.* 1992). From the analysis of these mutants it appears that synapsis and recombination are intimately related (Alani *et al.* 1990, Kleckner *et al.* 1991), but also that an intact SC is not absolutely required for meiotic levels of recombination (Rockmill & Roeder 1990, Engebrecht *et al.* 1990 , Sym *et al.* 1993). However, an important limitation of yeast as an object for the analysis of SC assembly is its poor cytology. The substructures of yeast SCs are ill-defined, RNs cannot be observed in spread preparations of meiotic prophase nuclei (Dresser & Giroux 1988, Loidl *et al.* 1991) and chiasmata cannot be observed directly. In contrast, tomato presents several advantages for detailed analysis of synapsis. The morphology of the SCs of this species is excellent, so that defects in SC assembly have a better chance of being detected and can be well defined. Furthermore, all 12 tomato SCs can be identified (Sherman & Stack 1992), early and late RNs can be distinguished (Sherman *et al.* 1992) and chiasmata can be visualized.

In this paper we describe the ultrastructural analysis of SC assembly in four meiotic mutants of the tomato that displayed defects in SC assembly covering the whole spectrum from (almost) com-

plete absence of synapsis to complete synapsis at pachytene. In three mutants, we found a good correlation between the number of bivalents connected by at least one tripartite SC segment at pachytene and the number of chiasmatic bivalents at metaphase I.

Materials and methods

Plant material

The three meiotic mutants of tomato, *asb, as1* and *as5,* were obtained from Dr P. B. Moens, Department of Biology, York University, Toronto, Canada. They are described in Soost (1951) and Moens (1969). A fourth synaptic mutant of tomato, *as6,* was isolated by one of us (M. S. Ramanna, manuscript in preparation). All four mutations are monogenic recessive and cause the formation of variable numbers of univalents at metaphase I, and high levels of pollen and ovule sterility (Soost 1951, Ramanna, manuscript in preparation).

Plants were grown under greenhouse conditions. We collected flower buds from 10-20 plants of each mutant for microscopical and ultrastructural analysis.

Cytological analysis

SCs were spread by means of the hypotonic bursting technique and stained with silver or uranyl acetate-lead citrate as described by Stack (1982) and Sherman *et al.* (1992). Because synapsis is disturbed in three of the mutants that are studied in this paper, we identified pollen mother cells (PMCs) at a stage comparable to pachytene by the following criteria: 1) PMCs had to be released from the. pollen sac and 2) chromosomes had to be resolved from their synizetic knot and distributed all over the nucleus after squashing in 1% aceto–carmine. Because of the high level of synchronization between anthers of the same bud (Stack & Anderson 1986a), we used one anther for monitoring the stage of development, and the remaining anthers for EM preparations of spread SCs. Spread nuclei were examined and photographed in a transmission electron microscope at a magnification of 1000-2000x. Axial elements and SCs were measured on prints by means of a digitizer, and total cell complement length values (TCC: the summed length of all axial and lateral elements in a PMC) were determined. The percentage synapsis was calculated as the total length of all tripartite SC segments divided by half the value of TCC x 100.

Flower buds containing anthers with PMCs at metaphase I were fixed and stored at room temperature in a solution of 1 part propionic acid saturated with iron acetate and 3 parts 96% ethanol. When anthers had become black (after about 1 week) they were cut into four or five small pieces. Each segment was squashed in a drop of 1% aceto–carmine. Slides were heated before squashing to allow better spreading of the chromosomes (Ramanna & Prakken 1967).

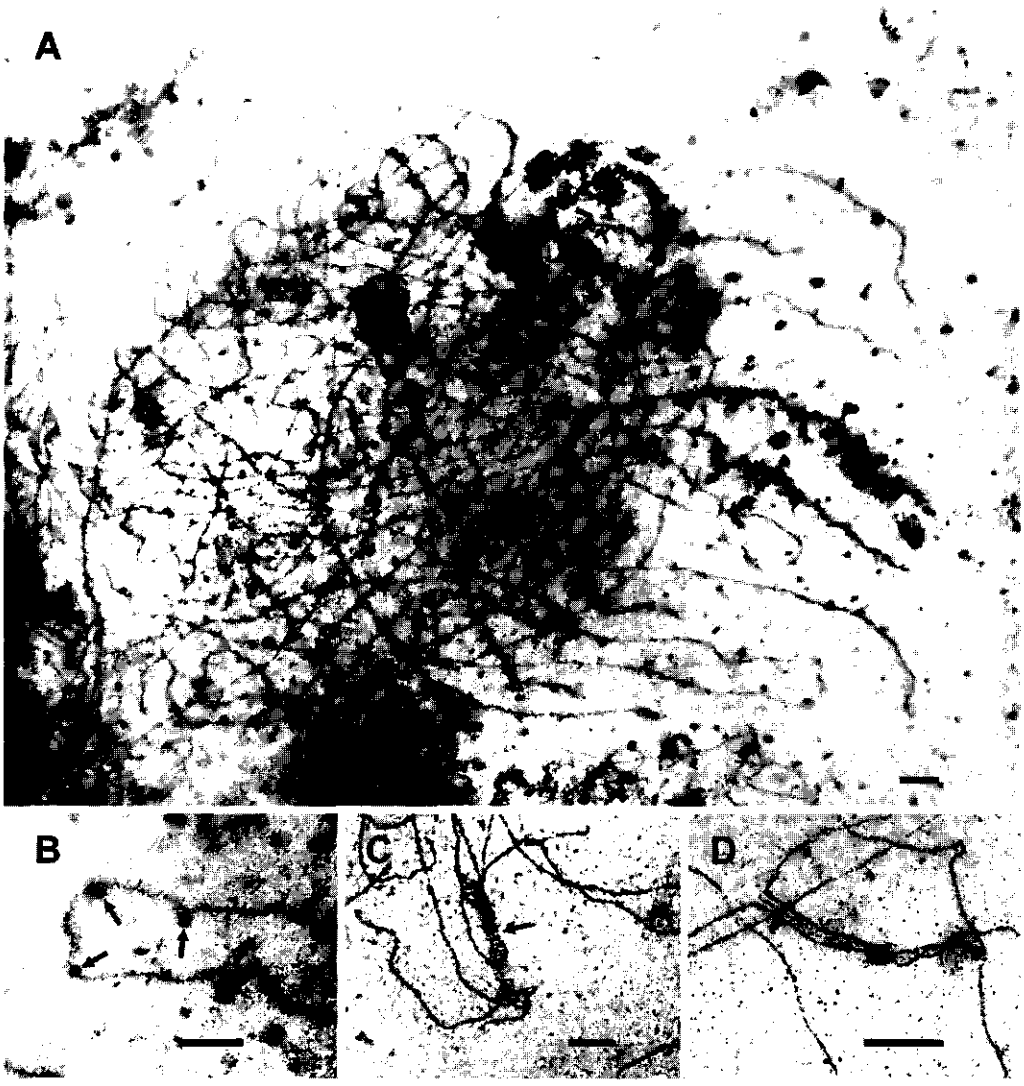

Figure 1. Electron micrographs of hypotonically burst PMCs of *as6*. **A.** Completely asynaptic nucleus with only axial elements. Stained with uranyl acetate–lead citrate (UP). **B.** Detail of axial element with early RNs (arrows) attached to it. UP stained. **C.** Detail of UP stained stacked SC material (arrow). **D.** Detail of an intercalary tripartite segment in a silver stained nucleus. Bars = 1 µm.

Results

In this study synapsis and chiasma formation were analysed in four meiotic mutants of tomato. The light microscopic and genetic analysis of three of these mutants, *asb*, *as1* and *as5* have been described

earlier (Soost 1951, Moens 1969). The mutant *as6* was recently isolated by one of us (Ramanna, manuscript in preparation).

The *as6* mutation is not allelic to either *asb, as1*or *as5*, since crosses of homozygous recessive *asb, as1* and *as5* plants with heterozygous *As6as6* plants yielded only progeny with wild-type phenotype (13, 15 and 13 plants checked respectively).

as6 mutant

The 32 spread nuclei of *as6* that were analysed showed normal development of axial elements. Chromosome complements were completely asynaptic in 29 nuclei (Figure 1A). In each of the remaining three nuclei we found a single short intercalary tripartite segment, 2.8-4.5 μm long (Figure 1D). We could not determine whether these segments involved homologous or non-homologous chromosomes. Synapsis ranged from 0 to 1.3%, with an average of 0.1%. Numerous round (early) RNs were associated with the axial elements (Figure 1B), but these did not connect two axial elements as is often seen in early prophase of wild type and of the other three mutants (Figure 2B). TCC values were measured in 11 nuclei and averaged 658 μm (Table 1). In about 30% of the nuclei, most of the axial elements were running parallel to other axial elements at distances of 50-650 nm, and in some nuclei the telomeres were arranged in a bouquet (Figure 1A). We suppose that this type of rough alignment was nonhomologous, because one axial element was often running parallel to several other axial elements and other segments of the same axial element. The typical presynaptic alignment of homologous segments that is observed during early prophase of wild type tomato and of the other mutants (Figure 2) was not observed in *as6*. In most of the *as6* nuclei however, the bouquet arrangement and alignment of axial elements were not obvious. In four nuclei we observed stacks of presumed SC material (Figure 1C). These stacks contained alternating thin and slightly thicker layers; it is possible that they contain transverse filament and/or central element material. The distance between two thin or two thicker layers was approximately 120 nm, which corresponds well to the distance between lateral elements in normal SCs from tomato.

In all four mutants, we compared the number of bivalents connected by at least one tripartite SC segment at pachytene, to the number of bivalents observed at metaphase I. In *as6*, an average of 0.1 bivalent was formed per cell at pachytene (Table 2). Analysis of bivalent frequencies at metaphase I was not straightforward in *as6* because bivalents could not always be discriminated from bivalent-like structures that consisted of two closely associated univalents, and because the orientation of structures in the equatorial plate could not be used as a criterion for a bivalent since, in most cases, not more than one such structure was present in a PMC. We therefore classified the bivalents into two groups. Group 1 contained bivalents with a clear chromatin thread that connected the two chromosomes; these threads may represent the terminalizing chiasmata (Figure 3A, B). We interpret these bivalents as chiasmatic bivalents. Group 2 contained paired structures without stretched chromatin connections; these structures are probably non-chiasmatic associations. The average frequency of

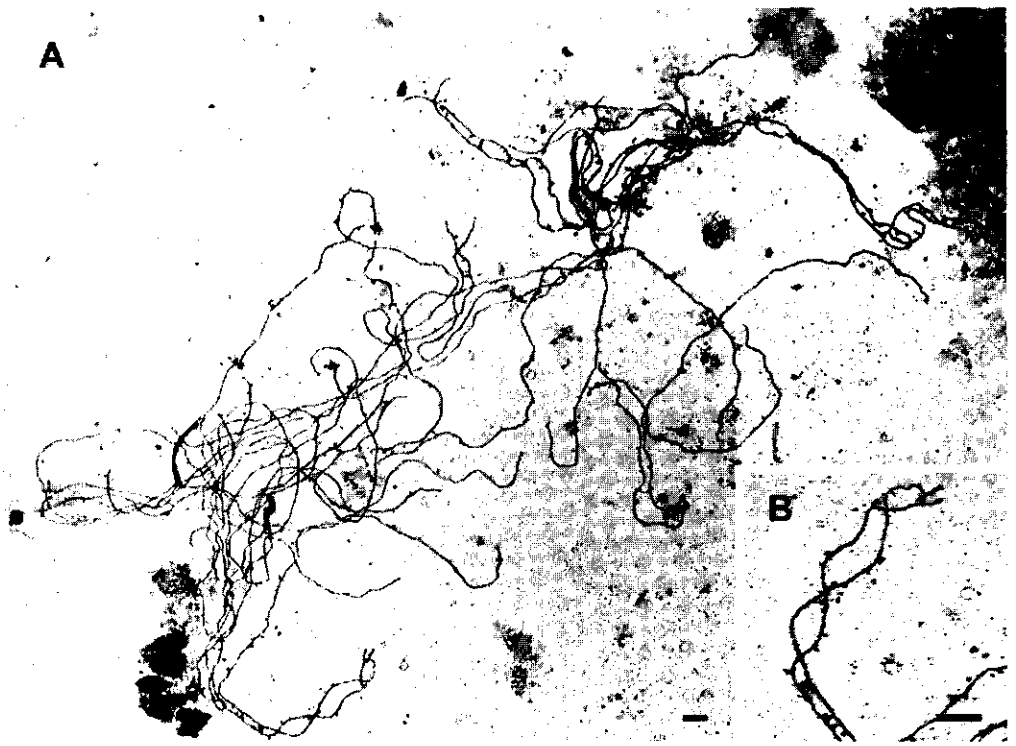

Figure 2. Electron micrographs of hypotonically burst PMCs of *asb*. **A.** Partially asynaptic nucleus with 4% synapsis. Aligned axial elements are connected by RNs. Silver stained. **B.** Detail of aligned axial elements with RNs connecting both axial elements. Bars = 1 μm.

group 1 bivalents was 0.2 ± 0.4 per cell, and the average frequency of group 1 and group 2 bivalents was 0.9 ± 0.9 per cell (Table 2).

asb mutant

We analysed 17 pachytene nuclei of *asb*. Three of these had no tripartite segments, although some axial elements showed presynaptic alignment at a distance of approximately 300 nm. In the other nuclei we observed both presynaptic alignment of axial elements and synapsis, predominantly at or near the telomeres (Figure 2). Synapsis was probably homologous, since chromosome axes of about the same length were involved. The aligned axial elements were connected by RNs (Figure 2B). The percentage synapsis ranged from 0 to 17% (average of 6.1%; Table 1). The TCC, measured in eight nuclei, was on average 620 μm, compared with 486 μm in wild type nuclei. In the other nine nuclei only the SC segments could be measured. The average TCC of 620 μm was used to calculate the percentage synapsis in these nine nuclei (Table 1). The mean number of tripartite SC segments per nucleus was 4.7; their length ranged from 0.3 μm to 16.0 μm (average 4.0 μm; Table 1).

Table 1. Average total cell complement (TCC), % synapsis, number of SC segments and length of SC segments in pachytene nuclei of four meiotic mutants.

Mutant	number of nuclei	Average TCC (µm)	Average % synapsis	Average no. SC segments	Average length SC segments (µm)
as6	32	658±98[a]	0.1±0.3	0.1±0.3	3.4±1.0
asb	17	620±59[b]	6.1±5.8	4.7±3.5	4.0±3.4
as1	19	703±129[c]	25.0±18.9	9.8±5.8	8.7±8.2
as5	12	549±63	100	12	22.9
Wild-type	7	486±71	100	12	20.3

[a]) 11 nuclei measured; [b]) 8 nuclei measured; [c]) 17 nuclei measured.

Table 2. Bivalent frequencies at pachytene[a] and metaphase I in nuclei of four meiotic mutants.

Mutant	pachytene		metaphase I	
	number of nuclei	Average number of bivalents	number of nuclei	Average number of bivalents
as6	32	0.1±0.3	250	0.2±0.4[b] 0.9±0.9[c]
asb	16	3.2±2.4	258	3.2±1.4
as1	18	6.4±3.4	120	7.3±1.7
as5	12	12.0±0	111	7.7±1.5
Wild-type	7	12.0±0	100	12.0±0

[a]) A pachytene bivalent is a pair of homologous chromosomes, connected by at least one tripartite SC segment; [b]) Minimum number of metaphase I bivalents (group 1); [c]) Maximum number of metaphase I bivalents (group 1 and 2).

At pachytene we found an average of 3.2 bivalents with at least one tripartite segment per cell (Table 2). At metaphase I, we could easily distinguish chiasmatic bivalents from non-chiasmatic associations by their clear orientation in the equatorial plate (Figure 3C). We found an average of 3.2 chiasmatic bivalents per cell ($n = 258$), which corresponds well to the average of 3.2 bivalents per cell ($n = 16$) observed at pachytene (Table 2).

as1 mutant

The nineteen pachytene nuclei of *as1* that were analysed showed presynaptic alignment and synapsis (Figure 4A). RNs were found between the aligned axial elements and on the tripartite SC segments (Figure 4A). The percentage synapsis ranged from 4 to 70% (mean 25.0%; Table 1). The TCC was

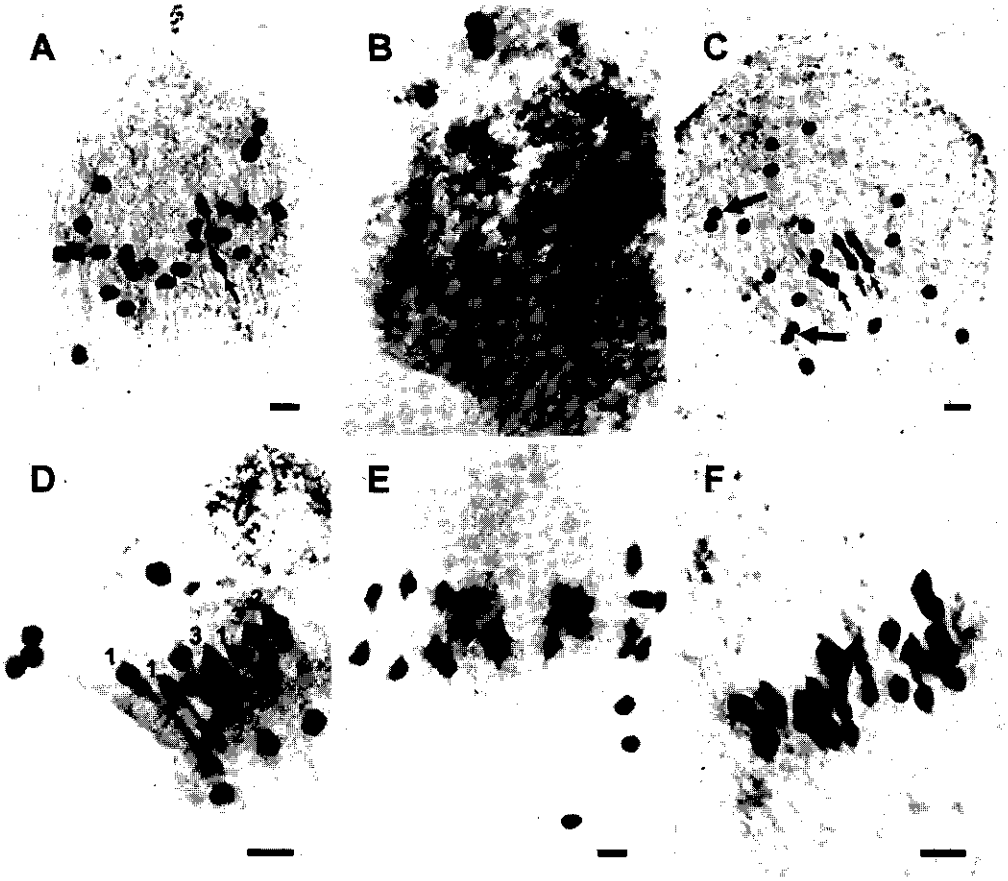

Figure 3. Squash preparations of aceto carmine stained PMCs at metaphase 1. **A, B.** Metaphase 1 in *as6*. Chiasmatic bivalents are indicated by arrow. **C.** Metaphase 1 in *asb* with three bivalents oriented in the equatorial plate (small arrows) and two nonchiasmatic associations of univalent pairs (big arrows). **D.** Metaphase I in *as1* with eight bivalents oriented in the equatorial plate. Bivalents indicated with 1 are supposed to have chiasmata in only one chromosome arm. Bivalents indicated with 2 and 3 are supposed to have chiasmata in both chromosome arms. **E.** Metaphase 1 in *as5* with ten bivalents and four univalents. **F.** Metaphase 1 in wild type: twelve bivalents. Bars = 1 μm.

measured in 17 cells and averaged 703 μm. The average number of SC segments per nucleus was 9.8. The length of the SC segments ranged from 0.3 μm to 36.9 μm (average 8.7 μm; Table 2).

At pachytene, the average number of bivalents with at least one tripartite segment was 6.5. At metaphase I, a mean of 7.3 chiasmatic bivalents per cell was found (Table 2). Soost (1951) reported averages of 5.19–8.40 bivalents per cell at metaphase I of *as1* mutants.

The spread nuclei of *as1* had a better SC morphology than the *asb* nuclei. A larger fraction of the axial elements remained intact after spreading, and less chromatin was connected to the lateral/ axial

Table 3. Configurations of pachytene[a] bivalents in ten *as1* nuclei.

Nucleus	number of bivalents	Configuration				
		 (1)	 (2)	 (3)	 (4)	 (5)
2883.1.1	7	0	2	5	0	5
2883.1.2	9	1	3	5	2	1
2883.1.3	12	4	3	5	0	0
2883.5.1	12	5	4	3	0	0
2883.6.1	2	0	0	2	4	6
2883.7.1	9	2	3	4	0	3
2883.10.1	6	0	2	4	1	5
2928.3.3	6	0	1	5	0	6
3016.1.1	5	0	3	5	1	6
3016.2.1	4	0	0	4	0	8
Average	7.2	1.2	2.1	3.9	0.8	4.0

[a]) Pachytene bivalent is a pair of homologous chromosome condensation by at least one tripartite SC segment. (1) completely synapsed bivalent; (2) bivalent with synapsis in both arms; (3) bivalent with synapsis in one arm; (4) bivalent with alignment; (5) two univalents.

elements. Synapsis could therefore be studied in more detail in *as1*. In a nucleus with 1.4% synapsis, we found stacked SC material, similar to that seen in *as6* (Figure 4B). In a nucleus with 33.8% synapsis, we observed stacked material of a different type (Figure 4C, D), consisting of several layers of central element-like material, which ran parallel to one or two lateral/axial element-like layers. In ten *as1* nuclei, we classified the configurations of all bivalents into five groups (Table 3): group 1 bivalents had synapsed completely, group 2 bivalents had synapsed segments in both arms, group 3 bivalents had synapsed segments in only one arm, group 4 contained univalent pairs with alignment of axial elements and group 5 contained univalent pairs without alignment of axial elements. In these ten nuclei, 3.3 bivalents showed synapsis in both arms, 3.9 showed synapsis in one arm and 4.8 pairs of univalents were formed on average. An average of 0.8 of the univalent pairs showed presynaptic alignment in one or both arms. The average number of bivalents was 7.2.

At metaphase 1 we analysed the frequencies of different bivalent configurations in 55 cells of *as1* (Table 4). The different configurations are shown in Figure 3D. Bivalents with the chromosomes connected by a thin stretched chromatin thread (type 1) were interpreted as having one or more chiasmata in only one arm (rod bivalents). Bivalents with a thickening in the middle (type 2) and bivalents that were evenly thick over their entire length (type 3) were interpreted as having at least one chiasma in each arm (ring bivalents). The average number of bivalents interpreted as ring bivalents

Table 4. Absolute frequencies and frequencies per cell of six different configurations in 55 *as1* nuclei at metaphase I.

	Bivalent configurations						sum type 1	sum type 2+3	sum type 1+2+3
	 (1a)	 (1b)	 (2a)	 (2b)	 (3a)	 (3b)			
Absolute frequency	161	22	82	19	19	68	183	188	371
Freq./cell (mean±SD)	2.9±1.2	0.4±0.7	1.5±1.1	0.3±0.6	0.3±0.6	1.2±1.1	3.3±1.2	3.4±1.6	6.7±1.7

(1) bivalent with thin thread in the middle; (2) bivalent with a thickening in the middle; (3) evenly thick bivalents. Configuration 1 is interpreted to have a chiasma in only one arm; configurations 2 and 3 are interpreted to have chiasmata in both arms.

was 3.4, compared to an average of 3.3 bivalents interpreted as rod bivalents. The number of rod bivalents may be underestimated, because some of the bivalents of configuration 2a (Table 4) may actually represent rod bivalents rather than ring bivalents. The average number of bivalents per cell was 6.7 in this sample of 55 metaphase I cells of *as1*.

The average number of ring bivalents versus rod bivalents at metaphase 1 (3.4: 3.3, Table 4) correlates well with the average number of bivalents with synapsis in both arms versus bivalents with synapsis in only one arm at pachytene (3.3: 3.9, Table 3). This suggests that the 10 pachytene nuclei represent end-points of SC development.

as5 mutants

In all 12 pachytene nuclei analysed of *as5*, 12 completely synapsed sets were observed (Table 1 and Figure 5A). The average length of a complete set of 12 SCs was 274.5 μm (TCC = 549 μm) compared with 243 μm (TCC = 486 μm) in wild-type plants (Table 1). RNs were associated with the SCs (Figure 5B). Between pachytene and metaphase 1, many bivalents separated precociously, resulting in the appearance of univalents at metaphase 1 (Figure 3E). An average of 7.7 bivalents per PMC was maintained until metaphase I (Table 2).

Discussion

Synapsis and chiasma formation

The four tomato mutants studied here displayed different degrees of synapsis at pachytene, namely 0.1%, 6.1%, 25.0% and 100%, in *as6*, *asb*, *as1* and *as5* respectively. In *as6*, *asb* and *as1* we found a close correlation between the number of (partially) synapsed bivalents per nucleus at pachytene and the

Figure 4. Electron micrographs of hypotonically burst PMCs of *as1*. **A.** Partially asynaptic nucleus with 33% synapsis. RNs can be observed on aligned and synapsed segments (arrows). Silver stained. **B.** Detail of stacked SC material (arrow) in a UP stained nucleus. **C, D.** Details of central element like layers (arrows), formed parallel to one or between two lateral elements. UP stained. Bars= 1 µm.

Figure 5. Electron micrographs of hypotonically burst PMCs of *as5*. **A.** Late pachytene nucleus with twelve completely synapsed sets of SCs. Centromere structures are indicated by big arrows, and each of the twelve SCs is identified (numbers). Late RNs are indicated by small arrows. UP stained. **B.** Detail of UP stained SC with late RN. Bar = 1 μm.

number of bivalents per nucleus at metaphase 1. In one of the mutants, *as1*, we could even establish that the pattern of synapsis in pachytene bivalents corresponds with the pattern of chiasma formation in metaphase I cells. This suggests that synapsis and chiasma formation are intimately related.

The relationship between synapsis, recombination and chiasma formation has been the subject of a large number of studies (reviewed in Stack *et al.* 1989). Good correlations between synapsis and chiasma formation were observed in meiotic mutants of *Sordaria macrospora* (Zickler *et al.* 1992), and in a variety of research objects with structural chromosomal rearrangements (Maguire 1977, Stack & Soullierre 1984, Herickhoff *et al.* 1993). However, there are also various examples where synapsis and chiasma formation do not correlate. In female *Bombyx mori* (Sturtevant 1915, Rasmussen 1976), haploid organisms (de Jong *et al.* 1991, Menzel & Price 1966), interspecific hybrids (reviewed in Von Wettstein *et al.* 1984), certain meiotic mutants in yeast (Engebrecht *et al.* 1990) and the *as5* mutant of tomato (this study), synapsis is not always followed by the formation of chiasmata. Furthermore, at least two species, *Aspergillus nidulans* (Egel-Mitani *et al.* 1982) and *Schizosaccharomyces pombe* (Olson *et al.* 1978) display a high frequency of meiotic recombination in the total absence of a detectable tripartite SC. Although chiasmata cannot be observed in these species, we consider the meiotic recombination events functionally equivalent to chiasmata. Thus, in some spe-

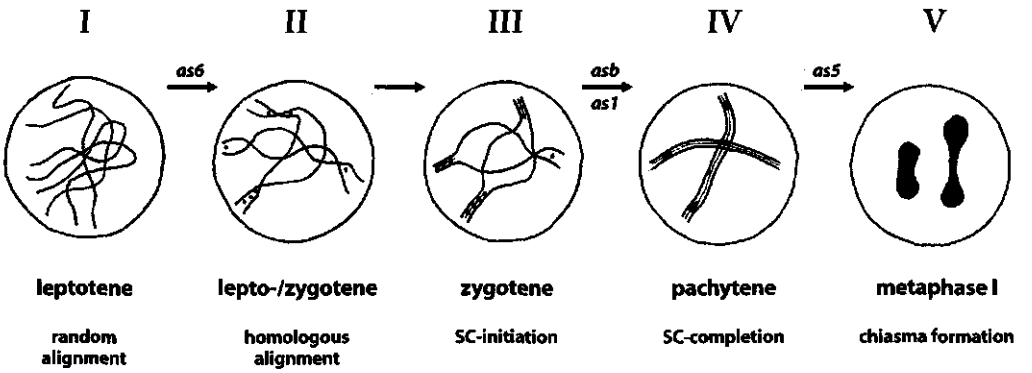

Figure 6. Schematic representation of SC assembly and chiasma formation in wild type pollen mother cells. The position of the meiotic arrest of the mutants is indicated.

cies the tripartite SC is not required for chiasma formation. However, on the basis of studies in other species, particularly in *Sordaria macrospora* (Zickler *et al.* 1992), maize (Maguire 1977) and tomato (this study), we propose that in most eukaryotes functional chiasmata are only formed in the context of a tripartite SC.

Possible nature of the defects in the analysed mutants

For discussion of the nature of the meiotic defect in the analysed mutants we refer to Figure 6, which represents the successive steps in SC assembly and chiasma formation in wild type pollen mother cells.

The phenotypic effect of the *as6* mutation becomes apparent at the transition from random alignment to homologous (presynaptic) alignment. As far as we could tell, the *as6* mutant did not display the typical presynaptic alignment observed during zygotene in wild-type tomato. Non- homologous alignment of axial elements was extensive in some as6 nuclei. These observations suggest that the homologues are unable to find their counterpart in this mutant. Several models have been proposed with respect to homology recognition during early prophase I (reviewed by Loidl 1990). According to most models, homology is tested by DNA-DNA interactions (Smithies & Powers 1986, Stern & Hotta 1987, Carpenter 1987, Kleckner *et al.* 1991). Smithies & Powers (1986) and Carpenter (1987) propose that most simple gene conversion events result from searches for homology. Carpenter (1987) also proposed that these searches are mediated by early RNs. In several *as6* nuclei we found numerous early RNs all along the axial elements (Figure 1): the synaptic defect is therefore not caused by a total absence of early RNs. It is possible, however, that the early RNs in *as6* have a defect that is not morphologically recognizable. Another possible cause for the synaptic defect in *as6* is a defect in the assembly of the central region. There are two (non-conclusive) arguments against this explanation, however: (1) the defect in alignment of axial elements is not explained, and (2) the occurrence of stacks of central element-like material suggests that the components of the central region are synthe-

sized and can assemble. Yeast mutants *rad50* and *meil04* have a similar meiotic phenotype (Alani *et al. 1990*, Menees *et al. 1992*), and the defect in the alignment of axial elements is also accompanied by defects in meiotic recombination and chromosome synapsis.

In *asb* and *as1* nuclei synapsis is initiated but not completed, and chiasma formation is reduced. Nuclei of *asb* have fewer and shorter SC segments than *as1* nuclei (Table 1). Some nuclei show a relatively large amount of presynaptic alignment (Figure *2*). It seems as if, after recognition of homology, SC initiation is delayed in these nuclei. Mutants with a similar meiotic phenotype have been found in yeast (*rad50S* and *dmcl* mutants; Alani *et al. 1990*, Bishop *et al. 1993*). These mutants accumulate supposed recombination intermediates, i.e. molecules with DNA double-strand breaks. It is not known how this defect in meiotic DNA metabolism is related to the defect in SC assembly.

Mutant *as5* displays complete synapsis at pachytene, but at metaphase I the number of chiasmata and chiasmatic bivalents is reduced. Similar mutants have been described in maize (mutant *dy*, Maguire *et al. 1991*), potato (mutant *ds-1*, Jongedijk & Ramanna *1988*), and *Sordaria* (mutant *asy2-17*, Zickler *et al.* 1992). A defect in the SC is suggested to cause the loss of chiasma maintenance in the *dy* mutant of maize (Maguire *et al. 1991*). The *ds-1* mutant of potato shows a reduction in chiasma frequency and recombination frequency, whereas in the *asy2-17* mutant of *Sordaria* the number of late RNs is reduced. In *as5*, recombination frequencies have not yet been determined, and it is not known whether the number of late RNs is reduced.

3

Comparative analysis of female and male meiosis in three meiotic mutants of tomato

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Female meiosis was analysed in squash preparations of ovules from three meiotic mutants and wild-type plants of tomato. In the completely asynaptic mutant *as6*, chromosome pairing and chiasma formation were virtually absent in both sexes. In the partially asynaptic mutant *asb*, with intermediate levels of chromosome pairing at pachytene, there were a higher number of chiasmate chromosome arms in female meiosis than in male meiosis, whereas in the desynaptic mutant *as5* there were normal levels of chromosome pairing at pachytene and a similar reduction in chiasma frequency in the two sexes. In wildtype tomato, we found slightly higher numbers of chiasmate chromosome arms in female meiosis than in male meiosis. We propose that the higher female chiasma frequencies in mutant *asb* and wild-type tomato result from a longer duration of female meiotic prophase. This would allow chromosomes more time to pair and recombine. It is possible that a longer duration of prophase I does not affect mutants *as5* and *as6*, either because the meiotic defect acts before the pairing process begins (in *as6*) or because it acts at a later stage and involves chiasma maintenance (in *as5*).

Introduction

Cytogenetic studies of female meiosis have long been neglected in plants. The main reasons have been the technical difficulty of locating and handling the solitary embryo sac mother cell (EMC) and the small chance of finding an EMC at the correct meiotic stage. Chromosomes were studied mostly in the easily accessible pollen mother cells (PMCs), and their behaviour in EMCs and PMCs was generally assumed to be the same. Some authors recognised the need for additional analyses of female meiosis. Darlington and La Cour (1940) and Fogwill (1958) studied female meiosis in *Lilium* and *Fritillaria*. Fogwill (1958) found a higher chiasma frequency in female meiosis in both species and, in *Fritillaria*, apparently larger bivalents in EMCs than in PMCs. The higher female chiasma frequencies were explained by the larger female nucleus, which might facilitate chromosome pairing, and by differences in two variables of meiosis mentioned earlier by Darlington (1940): time limit and torsion. Fogwill (1958) proposed that a longer period of time is allowed for pairing in EMCs. Furthermore, she suggested that chromosome coiling proceeds further and lasts longer in EMCs and that this contributes to higher crossover frequencies. Similar results and conclusions were reported in studies of four *Tulbaghia* species (Vosa 1972) and *Allium* (Ved Brat 1966). Gohil and Kaul (1980), however, obtained contrary results in their study of four *Allium* species, which exhibited higher chiasma frequencies in male meiosis.

Bennett *et al.* (1973) were the first to report on the duration of female and male meioses, and they also studied the relationship between meiotic duration and chiasma frequency. In the cereals *Hordeum vulgare* (barley) and *Triticum aestivum* (wheat), the duration of female and male meioses was very similar (Bennett *et al.* 1973). Chiasma frequencies were studied only in *H. vulgare* and turned out to be similar in female and male meioses. Bennett *et al.* (1973) concluded that this result was not proof of a relationship between meiotic duration and chiasma frequency, but that it might be interpreted as lending support to such a relationship. To test Fogwill's (1958) assertion that in *Lilium* meiosis lasts longer in EMCs than in PMCs, Bennett and Stern (1975) estimated the duration of meiosis in plants of two *Lilium* hybrids. Their results clearly showed that this assertion was correct.

Mogensen (1977) described the first, and so far the only, analysis of synaptonemal complexes (SCs) in female meiosis of a plant, maize. He found that the short arm of chromosome 9 was shorter in female meiosis than in male meiosis, which correlates well with the reduced female crossing-over value in the short arm of chromosome 9 (Rhoades 1978). Mogensen concluded that in maize, sex differences in recombination may reflect differences in SC length.

Davies and Jones (1974) stressed the importance of studying both female and male meioses in a range of genetically different types, such as meiotic mutants or inbred lines. In their analysis of five distinct inbred lines of rye, they found similar chiasma frequencies in female and male meioses. They concluded that, in rye, chiasma formation in female and male meioses is governed by a single control mechanism.

The effects of meiotic mutations on female meiosis have been analysed in only very few plant species. A synaptic mutant of potato showed the same severe reduction in chiasma frequency in both sexes (Jongedijk and Ramanna 1989). In maize, Golubovskaya *et al.* (1992) reported the effects of five meiotic mutations on megasporogenesis, but this study did not include data on chiasma frequency in female meiosis.

As a cytogenetic model, tomato would be an outstanding plant in which to study female meiosis; chromosome behaviour has been studied in detail in male meiosis (Stack and Anderson 1986a, 1986b) and sexual differences in genetic recombination have been reported (de Vicente and Tanksley 1991; van Ooijen *et al.* 1994). Meiotic mutants are available and have been extensively analysed in tomato (Soost 1951; Moens 1969; Havekes *et al.* 1994), and electron microscope studies have provided information on the extent of SC formation (synapsis) in male meiosis of these mutants (Havekes *et al.* 1994).

Synaptonemal complex spreading is practically impossible in female meiosis of tomato, but light microscope studies on chromosome pairing and chiasma formation are feasible in EMCs. We have undertaken such a light microscope study in EMCs of three meiotic mutants of tomato. As SC formation was not studied in female meiosis, we refer to the association of homologous chromosomes at pachytene as chromosome pairing. Herein, synapsis is used exclusively to indicate the formation of a SC.

In this paper we describe the effect of three different meiotic mutations on chiasma frequencies in female and male meioses of tomato. Since female meiosis has not been studied before in tomato, we included a comparison of meiotic chromosome behaviour in female and male meioses of wild-type tomato. Here we report sex differences in chiasma frequency in meiosis for one of the mutants and for wild-type tomato. Possible explanations for these differences are discussed.

Materials and methods

Plant material

The mutants *asb* and *as5* were obtained from Dr. P.B. Moens, Department of Biology, York University, Toronto, Canada. They were described by Soost (1951) and Moens (1969). A third mutant, *as6*, was obtained from Dr. M.S. Ramanna, Laboratory of Plant Breeding, Wageningen Agricultural University, The Netherlands. All mutations are monogenic recessive and cause high levels of pollen and ovule sterility. Male meiosis in these mutants was analysed in a previous study (Havekes *et al.* 1994).

Methods

Squash technique for EMCs

The squashing technique developed by Jongedijk (1987) for potato EMCs was slightly modified for our preparations of tomato ovules. Flower buds with style lengths of approximately 1.5 mm were

fixed in a mixture of 1 part propionic acid and 3 parts ethanol at room temperature for about 1 week. Ovary walls were peeled off with needles under a dissecting microscope. After a ten minutes hydrolysis in 1 N HCl at room temperature, the two placentas were divided and squashed separately in a drop of 1 % aceto–carmine. The cover glass was sealed with nail polish and the preparations were screened under a phase contrast microscope. Each preparation yielded 5-20 EMCs at various stages of meiosis I. Cell complements at late diplotene-diakinesis were drawn, chiasma numbers were established, and representative examples of all stages of prophase I were photographed.

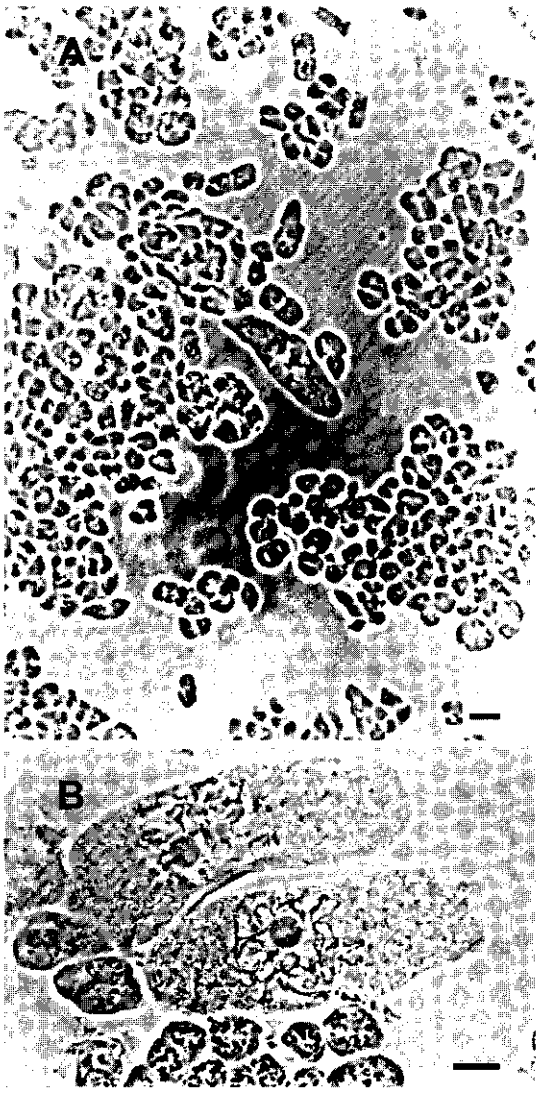


Figure 1. EMCs of tomato. **A.** A single EMC in a squash preparation of tomato ovules. The EMC (indicated by an arrow) can be recognised by its large and elongated shape. **B.** An example of two "twin" EMCs of the same meiotic stage: early diplotene. Scale bars = 10 µm.

Spreading technique for PMCs

Flower buds fixed in Carnoy's fluid (1 part acetic acid and 3 parts ethanol) and stored at 4° C were digested for 1 h at 37° C in an enzyme solution consisting of 0.1 % cellulase, 0.1 % pectolyase, and 0.1 % cytohelicase in 10 mM citrate buffer (pH 4.5), and anthers were then spread as follows. One anther was placed on a slide and divided into little pieces. First a drop of 60% acetic acid was added, and after this had mixed with the anther material, a drop of Carnoy's fluid was added. Excessive fixative was drained off, the slide was dipped in ethanol, and finally, air–dried. Slides were Giemsa stained. PMCs at diakinesis and metaphase I were used for chiasma counts.

Results

Female meiosis in wild-type tomato

Female meiosis was analysed in stained squash preparations of 37 wild-type tomato ovaries. Each ovary had two placentas, both covered

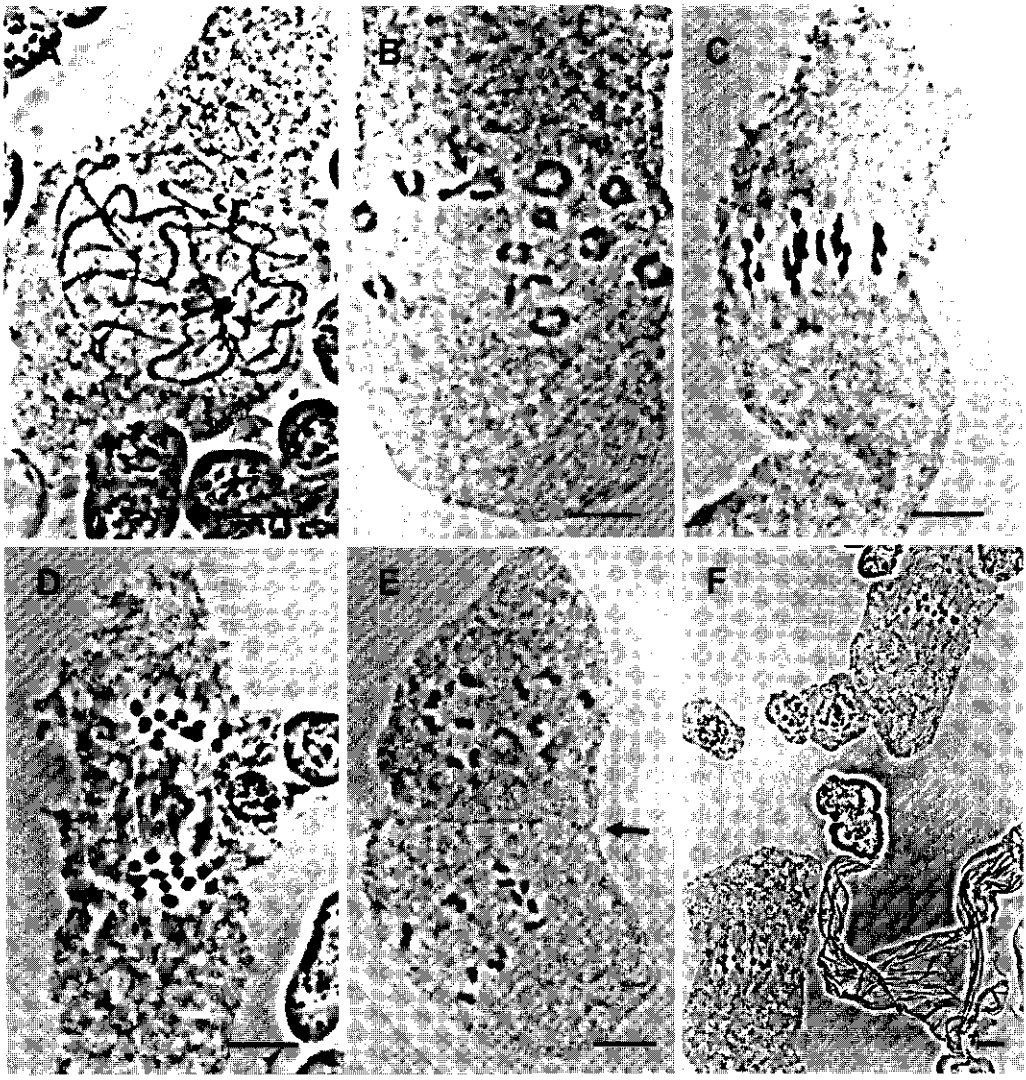
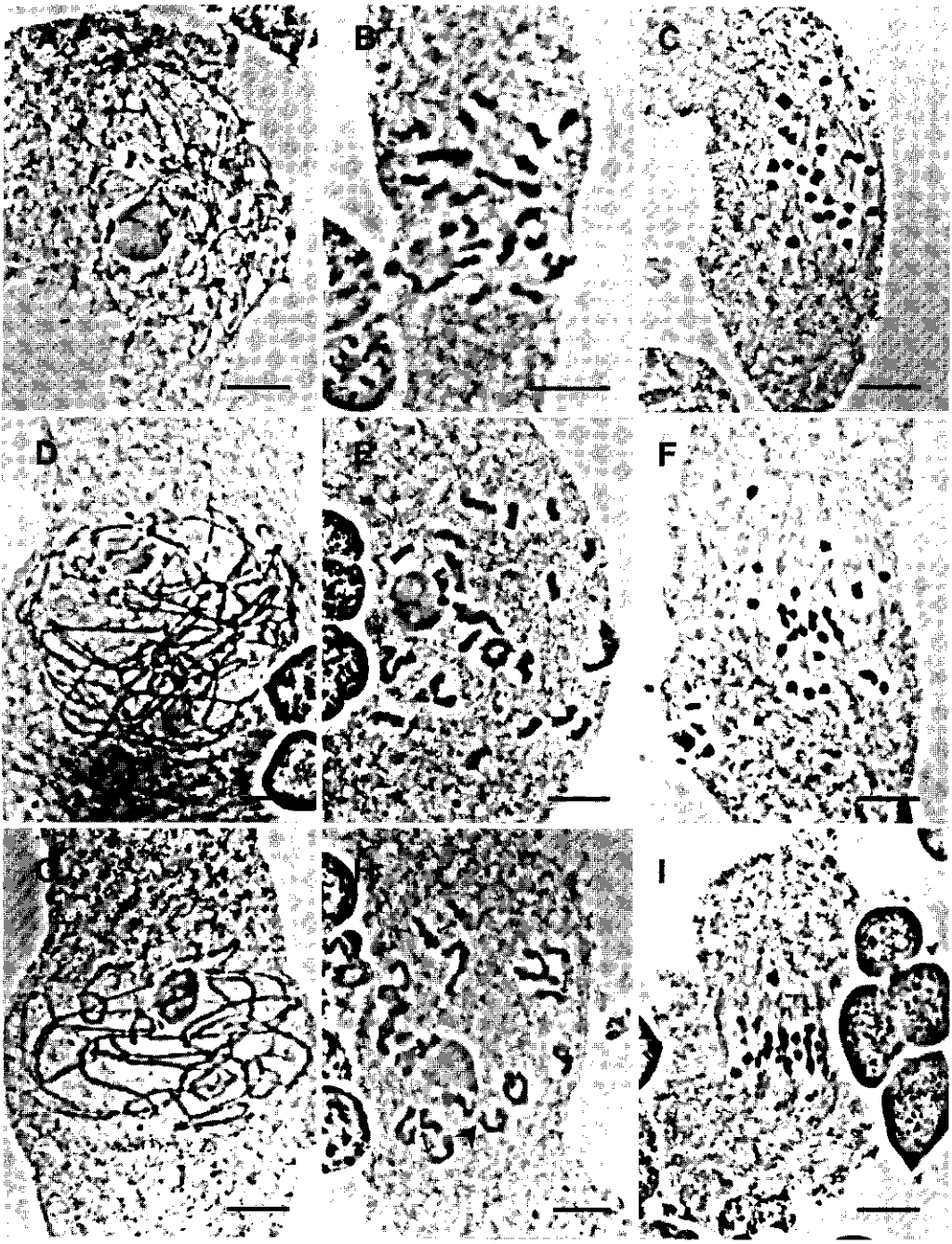


Figure 2. Female meiotic stages of wild-type tomato. **A.** Pachytene. The twelve tomato chromosomes have paired along their entire length. Darkly staining regions represent the pericentric heterochromatin and lightly staining regions represent distal euchromatic arm segments. **B.** An EMC at diakinesis with ten ring bivalents and two rod bivalents. The dark centromeric regions and light euchromatic (chiasmate) arms are clearly visible. Chromosome 2, the nucleolar chromosome, is indicated by an arrow. **C.** Metaphase I with twelve bivalents oriented in the equator. **D.** Anaphase I. **E.** Telophase I. A membrane (phragmoplast) is formed between the two daughter cells (arrow). **F.** Metaphase II. The daughter cells have separated as a result of the squashing technique. Scale bars = 10 µm.


A
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with 50-70 ovules. Ovules normally contained only one EMC, recognisable in the preparations as single cells of large and elongated shape compared with the cells of the surrounding ovule tissue (Fig. 1A). On average, an EMC measured 35 μm wide and 96 μm long. We sometimes observed two adjacent "twin" EMCs instead of a single one (Fig. 1B). EMCs from one placenta did not develop synchronously (except for the "twin" EMCs), revealing cells at prophase I through to anaphase I in the same preparation. Owing to the assembly of a phragmoplast immediately upon onset of anaphase I (Fig. 2E), cells at meiosis II could not be identified, and the two daughter cells easily separated during the squash procedure (Fig. 2F) and were lost between the ovule tissue cells.

Since we were primarily interested in chromosome pairing and chiasma formation, our study on EMCs in pachytene, diakinesis, and metaphase I. At pachytene, the 12 tomato chromosomes were paired along their entire length and showed the differential staining of proximal heterochromatin and distal euchromatin (Fig. 2A). Homologues disjoined at late diplotene–diakinesis and remained connected by chiasmata in the euchromatic segments (Fig. 2B). The lighter euchromatic parts of the bivalents could be traced very well in EMCs at diakinesis, much better than in PMCs at the same meiotic stage. Ring bivalents (chiasmata in both chromosome arms) were more frequent than rod bivalents (chiasmata in only one arm) in the female diakinesis cells that we studied. The 12 tomato chromosomes could not be distinguished individually at diakinesis, except for chromosome 2, the nucleolar chromosome, which was often visibly attached to the nucleolus. Chromosome 2 always appeared as a rod bivalent, owing to failure of recombination in its completely heterochromatic short arm. At metaphase I, the condensed bivalents were oriented in the equator (Fig. 2C). Rod and ring bivalents could not be distinguished unambiguously at metaphase I, because the bivalents often overlapped and had lost their differentiated morphology.

Female meiosis in meiotic mutants

We analysed female meiosis in preparations from 28 ovaries of mutant *as6*, 43 ovaries of mutant *asb*, and 57 ovaries of mutant *as5*. In all three homozygous mutants, EMCs had the same long and elongated shape as in the wild type, and were also single, with exceptional cases of double ("twin") EMCs.

Figure 3 shows examples of EMCs at pachytene, diakinesis, and metaphase I. At pachytene, the extent of chromosome pairing could not be determined as exactly as in electron micrographs of SC spreads. In *as6*, the homologues appeared not to pair at all (Fig. 3A), and 24 univalents were observed in most diakinesis and metaphase I cells (Figs. 3B-3C). Only eight bivalents (all rods) were found in a

Figure 3. Stage of female meiosis in mutants of tomato. **A-C.** Mutant *as6*. **A.** Unpaired chromosomes at a stage comparable to pachytene. **B.** Diakinesis with twenty-four univalents. **C.** Metaphase I with twenty-four univalents not oriented in the equator. **D-F.** Mutant *asb*. **D.** Incomplete pairing at a stage comparable to pachytene. **E.** Diakinesis with mostly univalents. **F.** Metaphase I with five bivalents in the equator. **G-I.** Mutant *as5*. **G.** Normal chromosome pairing at pachytene. **H.** Diakinesis with parallel arrangement of identical univalents (indicated by arrows). Chiasmate bonds are sometimes very thin (arrowheads). **I.** Metaphase I with bivalents in the equator and some univalents elsewhere in the cell. Scale bars = 10 μm.

Table 1. Average number of chiasmate chromosome arms per cell in female and male meioses of three meiotic mutants of tomato and the wild type.

Mutant	Number of cells		Average number of chiasmate number arms per cell		Significance[a]
	Female	Male	Female	Male	
as6	31	50	0.3±0.5	0.1±0.3	
asb	34	73	9.5±4.3	3.6±1.9	$P << 0.001$
as5	29	33	9.9±5.2	10.8±3.0	
Wild type	28	40	20.5±2.2	19.0±1.6	$0.001<P<0.01$

[a]) The numbers of chiasmate chromosome arms per cell were compared in female and male meioses by Welch's approximate *t* test of equality of two sample means with unequal variances.

total of 34 *as6* cells. Of these, three could be identified as chromosome 2 (nucleolar chromosome), one was certainly not chromosome 2, and of the remaining four, we could not determine whether they concerned chromosome 2.

In female meiosis of mutant *asb*, we found incomplete chromosome pairing at a stage morphologically comparable to pachytene (Fig. 3D). Although the extent of chromosome pairing could not be determined accurately at the light-microscope level, it was likely more than 10% on the average. At diakinesis, EMCs of *asb* showed varying mixtures of univalents, rod bivalents, and ring bivalents (Fig. 3E). The bivalents were oriented in the equatorial plane and the univalents were scattered over the cell at metaphase I (Fig. 3F).

In mutant *as5*, chromosome pairing at pachytene was complete (Fig. 3G), but both univalents and bivalents were observed at diakinesis and metaphase 1 (Figs. 3H-3I). The connections between the homologues could be very thin in *as5* (Fig. 3H). The univalents of this mutant showed pairwise parallel alignment (Fig. 3H, arrows), and the pattern of staining (heterochromatin–euchromatin) was similar within each observed pair. This type of arrangement was unique to *as5* and did not occur in the other two mutants.

Chiasma formation compared in female and male meioses

Chiasma formation was compared in the two sexes by means of numbers of chiasmate chromosome arms, because we could not discriminate between one or more chiasmata within chromosome arms in male tomato. In each mutant and in wild-type tomato, the numbers of univalents, rod bivalents, and ring bivalents were counted in samples of approximately 30 EMCs at diakinesis. For each cell, we calculated the number of chiasmate arms and compared the sample average with the equivalent average in male meiosis (Table 1). In mutant *asb*, the average number of chiasmate arms was 2.6 times higher in female meiosis than in male meiosis. Also, the higher number of chiasmate arms in female meiosis was correlated with a 2.6 times higher number of bivalents per cell, whereas the average num-

ber of chiasmate arms per bivalent was the same in both sexes. In *as6* and *as5*, the number of chiasmate arms in female and male meioses did not differ significantly (Table 1). In wild type, however, the slightly higher number of chiasmate arms in female meiosis was significantly different from the number of chiasmate arms in male meiosis. In all mutants and in wild type, the variance of the number of chiasmate arms was higher in female than in male mcioses (Table 1).

There were also striking differences in chromatin morphology between male and female meiotic prophase nuclei. In EMCs at diakinesis, the euchromatic chromosome regions were clearly visible (Figs. 2 and 3); in contrast, PMCs at a comparable stage depicted mainly the proximal heterochromatic regions of the chromosomes. In female meiosis, differences in the size and shape of the euchromatic regions gave additional information on the number of chiasmata in such a region: we could observe the difference between bound chromosome arms with only one chiasma and arms with more than one chiasma. Assuming a maximum of two chiasmata per arm, we could distinguish rod bivalents with one chiasma from rod bivalents with two chiasmata, and ring bivalents with two chiasmata from ring bivalents with three or with four chiasmata. The average number of chiasmata per EMC was 0.4 ± 0.8 for *as6*, 11.8 ± 5.2 for *asb*, 11.2 ± 5.5 for *as5*, and 23.6 ± 3.0 for the wild type. In male meiosis, we could not carry out such an analysis of chiasma frequency. But if we assume the ratio between the average number of chiasmate arms (data in Table 1) and the average number of chiasmata to be the same in both sexes, average chiasma frequencies in PMCs would be approximately 0. 1 (1.33 x 0. 1) in *as6*, 4.5 (1.24 x 3.6) in *asb*, 12.2 (1.13 x 10.8) in *as5*, and 21.9 (1.15 x 19.0) in the wild type.

Discussion

In mutant *asb* and wild-type tomato, we found a higher number of chiasmate arms in female meiosis than in male meiosis. This difference was evident for *asb* and less obvious, but also significant, for the wild-type.

Wild-type tomato

The number of chiasmate arms in female meiosis of wild-type tomato was slightly but significantly higher than in male meiosis. The variance of the number of chiasmata per cell was also higher in female meiosis than in male meiosis. Chiasmate bonds were scored in EMCs at diakinesis and in PMCs at both diakinesis and metaphase I. That chiasmata were scored at different meiotic stages may explain the slight difference found in the number of chiasmate arms in the two sexes of the wild type. The greater variation in the number of chiasmate chromosome arms in female meiosis may have been caused by the higher number of flower buds used in the analysis of female meiosis. Although the differences in meiotic stage and in number of flowers used may have influenced the averages given in Table 1, the higher number of chiasmata in female meiosis is in agreement with genetic recombination studies of tomato. In a cross between *Lycopersicon esculentum* and *Lycopersicon pennellii*, a genome-wide reduction in recombination was found in the male-derived progeny compared with

the female derived progeny (de Vicente and Tanksley 1991). The same result was found in a cross of *L. esculentum* with *Lycopersicon peruvianum* (van Ooijen *et al.* 1994). Possible explanations for higher female chiasma frequencies are discussed below, in the section on meiotic mutants.

In female meiosis, we estimated the average number of chiasmata to be 23.6±3.0 per EMC. In male meiosis, we could not establish exact chiasma counts, but extrapolation from female data suggests a frequency of 21.9 chiasmata per PMC. This frequency corresponds well to the number of recombination nodules (RNs) reported for PMCs of tomato. An average number of 21.25 RNs was counted in 278 complete sets of mid- to late-pachytene SCs; if SCs from incomplete cells were included in the calculation, an even higher frequency was found, namely 21.89 (Sherman and Stack 1995). These results support our view that chiasma frequencies in tomato anthers are underestimated when rod bivalents are supposed to have one chiasma and ring bivalents to have two.

Meiotic mutants

Although pachytene pairing could not be studied in detail in EMCs and a quantitative analysis of SC formation in female cells is practically impossible, we were able to confirm that all three mutants fitted their classification on the basis of synapsis in male meiosis (Havekes *et al.* 1994). Female meiosis was completely asynaptic in *as6*, partially asynaptic in *asb*, and desynaptic in *as5*. In mutant *asb*, we could not determine whether the intermediate extent of pachytene pairing in female meiosis was comparable to the extent of synapsis in male meiosis. In *asb*, 6% of the complement was synapsed in male meiosis on the average (Havekes *et al.* 1994), whereas in female meiosis this may have been more than 6%.

One striking result of this study was the significantly higher number of chiasmate chromosome arms found in female meiosis compared with male meioses in mutant *asb*; in the other two mutants, similar numbers of chiasmate arms were found in the two sexes. The higher female chiasma frequency in *asb* reflected an increased number of bivalents per cell, not an increased number of chiasmate arms per bivalent. We wondered why the sex difference, already present to a certain degree in wild-type tomato, is so pronounced in this mutant specifically. A possible explanation is that prophase I in tomato lasts longer in female meiosis, providing chromosomes with more time for pairing, a situation that will be most effective in mutants with impaired chromosome pairing. Unfortunately, we do not have any data on the duration of female meiosis compared with male meiosis in tomato. Bennett *et al.* (1973) tested Darlington's (1940) theory that meiotic duration and chiasma frequency are related. Although they did not find direct proof, they interpreted their results in *H. vulgare* as support for such a relationship. Bennett and Stern (1975) showed that in *Lilium*, where the chiasma frequency is higher in EMCs than in PMCs, meiosis took longer in EMCs than in PMCs. They stressed that such a correlation does not necessarily indicate the existence of a causal relation between time in meiotic stages and chiasma frequency, but that they might both be controlled by some other nuclear character.

Another explanation for sex differences in chiasma formation may be a larger female meiotic nucleus, which could facilitate the pairing process (Fogwill 1958). The tomato EMC is about three times larger than the PMC, and pachytene chromosomes may occupy more space in female meiosis. However, in a mutant with disturbed pairing, a larger nucleus might hamper pairing just as well as facilitate it.

Mogensen (1977) and Fogwill (1958) mentioned that difference in chromosome lengths might be correlated with differences in crossing-over or chiasma frequency. Mogensen's (1977) study referred to the length of pachytene chromosomes, the SCs, whereas Fogwill (1958) referred to metaphase I chromosomes. We do not have data on the length of pachytene chromosomes in female meiosis so we cannot judge its validity for tomato.

In EMCs at diakinesis, euchromatic chromosome regions are clearly visible, which is not the case in PMCs, where bivalents seem smaller and more condensed. Recent fluorescence in situ hybridization experiments with telomere and telomere-associated repeats as probes revealed highly decondensed euchromatic areas at diakinesis in male tomato (Zhong *et al.* 1998). These findings suggest that in female cells at diakinesis, chromosome condensation progresses further than in male cells at a comparable stage. If such increased condensation results in higher chiasma frequencies, as proposed by Fogwill (1958), then higher within-bivalent chiasma frequencies might be expected in EMCs. We did not find higher numbers of chiasmate arms per bivalent in female meiosis of mutant *asb* however.

In meiotic mutants, chiasma distribution is sometimes affected (Jones 1967; Jongedijk and Ramanna 1989). In our study, we concentrated on differences in the number of chiasmate chromosome arms between female and male meioses. The higher number of chiasmate arms found in mutant *asb* was correlated with a higher number of bivalents per cell, whereas the overall number of chiasmate arms per bivalent was the same.

In the mutants *as5* and *as6*, there was no significant sex difference in the number of chiasmate arms. Mutant *as6* is likely defective in homology recognition and apparently the defect is equally expressed in both sexes. In female meiosis, we found that a relatively high number of the bivalents involved chromosome 2. Soost (1951) also reported higher chiasma frequencies for the nucleolar chromosome in male meiosis of meiotic mutants and wild-type tomato. The two homologues of chromosome 2 are associated with the nucleolus and therefore they always occupy a small nuclear domain. This may facilitate chromosome pairing and recombination in chromosome 2 compared with the other chromosomes, especially in a mutant defective in homology recognition like *as6*. Scherthan *et al.* (1992) also reported that, in yeast, NOR chromosomes are not representative of the pairing behaviour of other chromosomes.

In mutant *as5*, the homologous univalents were still arranged in pairs at diakinesis, probably as a result of synapsis at earlier stages of meiosis. Desynaptic mutants are supposed to be disturbed in making crossovers or in maintaining the resulting chiasmata. In the desynaptic (*dy*) mutant of maize, Maguire (1978) showed that crossing-over between the centromere and a distal knob was normal. The loss of chiasmata was caused by a failure in chiasma maintenance rather than by reduced recom-

bination levels. In the potato desynaptic mutant *ds-1* however, crossing-over was severely reduced for two genetic loci but slightly increased for a third (Jongedijk *et al.* 1991). The reduction in overall chiasma frequency of this mutant (Jongedijk and Ramanna 1989) was explained by reduced overall recombination frequencies and a differentially altered chiasma distribution along individual chromosomes.

The desynaptic mutant *as5* of tomato is probably disturbed in chiasma maintenance. Preliminary results on RNs indicate that in mutant *as5*, every SC has at least one RN in male meiosis. The observation that chiasmate connections are sometimes very thin in EMCs at late diplotene-diakinesis also suggests that chiasmata are initially present but that part of them are lost at some time before the diakinesis stage.

To summarise, we analysed chiasma formation in female meiosis of wild-type and mutant tomato. Chiasma frequencies were slightly higher in female meiosis than in male meiosis of the wild type and considerably higher in female meiosis than in male meiosis of the asynaptic mutant *asb*. As a possible explanation, we propose a longer duration of prophase I, the stage in which homologous chromosomes pair, in EMCs than in PMCs of tomato. This would explain the slightly higher female chiasma frequencies in wild-type tomato, but particularly the much higher number of chiasmate arms in the mutant with pairing problems, *asb*. We furthermore propose that the completely asynaptic mutant *as6* and the desynaptic mutant *as5* show no sexual differences because the first is probably disturbed before pairing, in homology recognition, and the second possibly has a defect that acts after pairing, in chiasma maintenance.

4

Chromosome pairing in wild type and synaptic mutants of tomato (*Lycopersicon esculentum*) I. Initiation of synapsis

F.W.J. Havekes, J.H. de Jong and C. Heyting

Initiation of meiotic chromosome pairing was studied in wild type and mutant tomato (*Lycopersicon esculentum*). We performed a detailed electron microscopic analysis of synaptonemal complex (SC) formation in pollen mother cells, which were spread by a hypotonic bursting technique. General features of pairing initiation in wild type meiosis were successively: (1) a complex network of axial cores with telomeres at the periphery (bouquet); (2) loosening of the AC network as homologues become involved in presynaptic alignment and synapsis; (3) up to four pairing initiation sites along a homologous pair; (4) predominantly distal SC initiation and extension; (5) fairly synchronous synapsis in bivalents of all lengths.

The asynaptic mutant *asb* differed from wild type in that synapsis was almost exclusive to longer acrocentric bivalents, whereas short, especially metacentric, chromosomes often failed to synapse. In addition, SC formation was mostly confined to the long arm of (sub)telocentric chromosomes. These observations suggest regulation of synapsis at the bivalent level. Our data also suggest the existence of some minimum requirement for synapsis, which in the mutant is hard to fulfil in the small metacentric chromosomes. The possible relation of synaptic initiation with aspects of very early meiotic chromosome behaviour (telomere clustering, distant alignment and presynaptic alignment) and with recombination are discussed.

Introduction

Prophase I of the first meiotic division is featured by unique nuclear processes including homology search, chromosome pairing and crossing over. The communication between DNA strands involved in homology search is believed to commence at leptotene and early zygotene, though recent studies claim associations between homologues at pre–meiotic interphase (Schwarzacher 1997, Aragón–Alcaide *et al.* 1997, Mikhailova *et al. 1998).*

The cytology of leptotene / early zygotene stages is difficult to interpret, if possible at all. In most species, nuclei at these stages appear in light microscopic preparations as highly condensed, amorphous structures. Electron microscopic images of very early prophase I nuclei reveal the onset of the formation of proteinaceous axial cores (ACs), but because the long ACs are often entangled, broken or discontinuous, reliable interpretation of early interactions between homologues is hampered (Albini & Jones 1987, Holm 1986, Gillies 1985). Most studies on synaptic initiation present information from zygotene nuclei with considerable extents of synapsis. However, synapsis is not the initial step in chromosome pairing.

When two homologous ACs come into close contact at early zygotene, they become connected to a third longitudinal structure (the central element), which forms between them. The tripartite structure that is thus generated, the synaptonemal complex (SC), extends as a zipper along the ACs until they become completely connected (synapsed) by the end of zygotene. In several species, synapsis is preceded by presynaptic alignment of homologous AC regions (Stack & Anderson 1986a, Albini & Jones 1987, Anderson & Stack 1988). Presynaptic alignment and synapsis are distinct pairing mechanisms, because the former normally occurs between homologues only whereas the latter may occur between non–homologous cores as well (Loidl 1990).

Tomato (*Lycopersicon esculentum*, 2n=2x=24) belongs to one of the best model species in plant cytogenetics and is in several respects appropriate for detailed analyses of early prophase I. Its chromosomes are sufficiently small so that they can be traced at pachytene, and well–differentiated patterns of proximal heterochromatin and diagnostic chromomeres on most chromosome arms allow identification of individual pachytene chromosomes in light microscopic preparations. In ultrastructural preparations, SCs display an excellent morphology and their lateral elements show obvious density differences in heterochromatic and euchromatic segments. The twelve different chromosomes or the twelve SCs can thus be identified in LM and EM preparation on the basis of length, centromere position and heterochromatin pattern (Ramanna & Prakken 1967, Sherman & Stack 1992). In addition, presynaptic alignment and early and late recombination nodules can be unequivocally demonstrated (Stack & Anderson 1986a+b). Meiotic mutants of tomato, with defects in different phases of chromosome pairing and chiasma formation have been described by Soost (1951) and Moens (1969). Havekes *et al.* (1994) assessed their phenotypes with respect to SC–formation, and identified a completely asynaptic mutant (*as6*), two partially asynaptic (*asb, as1*), and a desynaptic mutant (*as5*).

This report describes presynaptic alignment and synaptic initiation in early prophase I nuclei in wild type tomato and in two partially asynaptic mutants, *asb* and *as1*. In these mutants, early prophase I nuclei can be more easily spread and their ACs can be better traced than in wild type tomato. Moreover, kinetochores can sometimes be stained, which allows the identification of individual chromosome pairs in mutant nuclei.

One other synaptic mutant was considered for this analysis as well. The completely asynaptic *as6* (Havekes *et al.* 1994), in which pairing is arrested at a stage preceding presynaptic alignment, is potentially interesting for determining whether homologous ACs are already in a rough parallel arrangement before presynaptic alignment and synapsis take place. It was impossible though, to trace individual ACs and discern putative sites of alignment in the network of unpaired ACs in this mutant.

Material and methods

The asynaptic mutants *asb* and *as1* of tomato (*Lycopersicon esculentum*) were obtained from Dr P.B. Moens, Department of Biology, York University, Toronto, Canada. However, the wt plants heterozyogous for the *asb* or *as1* mutation had a completely regular meiosis and were used as wild type control plants.

Synaptonemal complex preparations were made by spreading microsporocytes by the hypotonic bursting technique of Stack (1982), with slight modifications as described by Havekes *et al.* (1994). Preparations were stained with either silver nitrate at 40° C, or uranyl acetate–lead citrate at room temperature (Sherman *et al. 1992).*

Results

Synaptic initiation in wild type tomato

Six nuclei, covering the whole range from 0% synapsis (transition from leptotene to zygotene) to 92% synapsis (late zygotene) were selected. Table 1 shows the main features of these nuclei with respect to presynaptic alignment, as characterised by close parallel alignment of axial cores at a distance of approx. 300 nm, and true synapsis (SC), as defined by the presence of a tripartite synaptonemal complex. We will use the term chromosome pairing if we consider both presynaptic alignment and synapsis. The distal part was recorded as a distal 1.5 μm end of the SC and the other parts were recorded as intercalary segments.

Nuclei with little or no synapsis, like wt1, were hard to spread and showed a dense mass of chromatin. The long stretches of ACs in wt1 had numerous discontinuities. Presynaptic alignment was observed in five chromosome arms and comprised 17.4 μm together. There was one short SC segment. In nucleus wt2, most ACs were close together in a similar dense mass of chromatin as shown in Figure 1. At the periphery of the dense mass, ACs appeared more loosened and could be traced. Most

Table 1. Overview of pre–synaptic alignment and SC formation in six zygotene nuclei of wild type and synaptic mutants *as1* and *asb* of tomato.

Nucleus	staining[a]	TCC[b]	aligned segments					SC segments					total number of paired segments
			total %	interstitial[c]		distal[c]		total %	interstitial[c]		distal[c]		
				number	length	number	length		number	length	number	length	
wt1	AG40	?[d]	>4[e]	2	2.5–3.0	3	4.0±2.4	0	1	2.3	0	–	6
wt2	UP	?	>15	3	3.5±2.3	5	5.4±3.4	±7	2	4.1–6.0	4	4.6±1.8	14
wt3	AG40	430	6	2	5.8–9.6	4	2.4±1.2	10	2	0.9–1.7	10	3.8±1.7	18
wt4	AG40	360	2	2	0.9–4.7	1	2.0	53	6	1.8±0.6	17	10.5±7.0	26
wt5	AG40	364	0	0	0	0	0	67	7	2.3±1.1	22	10.4±6.0	29
wt6	AG40	376	0	0	0	0	0	92	1	4.4	18	19.0±8.3	19
(all wt)				**9**	**4.1±2.8**	**13**	**3.9±2.9**		**19**	**2.4±1.4**	**71**	**11.4±8.1**	**92**
as1–1	AG40	404	5	2	1.4–2.0	3	5.2±1.0	4	1	2.7	2	1.7–4.3	8
as1–2	UP	385	6	4	2.5±1.4	5	2.2±1.7	5	3	4.5±2.7	3	1.9±1.5	15
as1–3	UP	347	0.2	0	–	1	0.6	17	2	1.2–8.7	8	6.5±6.2	11
as1–4	AG40	338	3	1	2.2	2	2.7–4.2	23	1	1.4	7	11.2±7.0	11
as1–5	AG40	410	3	0	–	3	3.6±3.9	33	1	5.8	11	11.8±7.4	15
(all *as1*)				**7**	**2.3±1.1**	**14**	**3.2±2.3**		**8**	**4.2±2.8**	**31**	**8.7±7.1**	**60**
asb–1	UP	?	~15	5	5.4±3.4	5	2.9±1.0	~7	4	3.3±2.8	2	2.1–6.7	16
asb–2	UP	?	~8	5	4.3±3.3	5	8.0±7.0	~7	1	15.2	3	3.4±2.4	14
asb–3	UP	?	~13	4	3.6±2.1	2	6.4–16.1	~8	3	7.4±3.0	1	1.3	10
asb–4	UP	?	~5	4	4.0±2.6	1	1.4	~9	0	–	5	5.5±4.7	10
asb–5	UP	?	~10	0	–	6	4.9±2.0	~13	4	3.1±2.2	2	2.1–23.5	12
asb–6	UP	?	~4	5	1.5±1.1	2	1.0–1.1	~20	2	8.0–15.0	4	8.3±7.2	13
asb–7	UP	?	~6	1	7.1	4	2.2±1.3	~23	1	10.2	7	8.1±8.7	13
asb–8	UP	283	13	1	6.8	6	5.0±2.6	24	5	3.2±1.9	7	7.2±6.6	19
(all *asb*)				**25**	**4.0±2.8**	**31**	**4.8±4.2**		**20**	**5.6±4.4**	**31**	**6.9±6.9**	**107**

a) AG40 = Silver nitrate staining at 40° C; UP = Uranyl acetate / lead citrate staining at room temperature; b) TCC = half the summed lengths (μm) of all axial cores or the total length of all SCs in a cell. c) distal regions of the ACs / SCs are the 1.5 μm end segments, the remaining segments are considered as interstitial d) ? = TCC values could not accurately be determined; e) rough estimates.

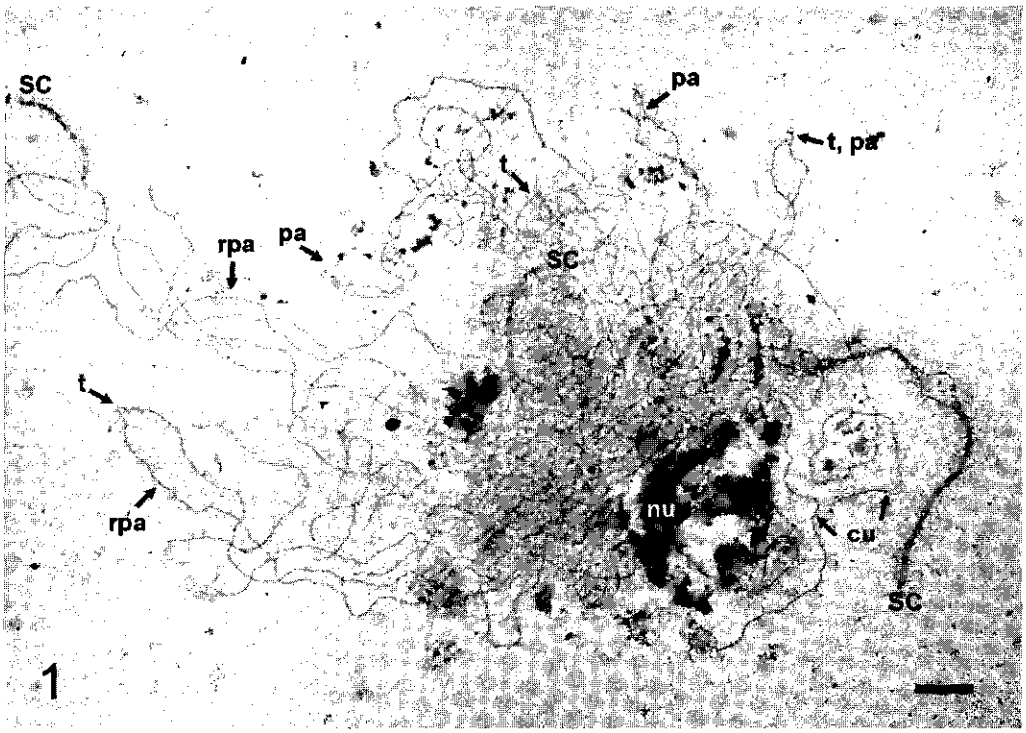

Figure 1: Uranyl acetate stained nucleus at early zygotene from wild type tomato (wt2). Most unpaired axial cores did not spread out well and were still in the dense chromatin mass. Most distal regions were released from the chromatin cluster showing telomere association (t), presynaptic alignment (pa), synapsis (SC), curved ACs (cu) and rough parallel arrangement ACs (rpa). The nucleolus (nu) is degenerating. The bar equals 2 µm.

telomeric regions were found here, and several of these appeared in close two–by–two associations: four were associated by (sub)telomeric synapsis, five by distal presynaptic alignment and three by association only at the telomeres. Some peripheral AC regions were curved and folded, whereas other AC regions were more straight and ran roughly in parallel to other cores (Figure 1). In the remaining nuclei (wt3–6), ACs were well– spread so that all chromosomes in the complement could be traced. In wt3 with 6% alignment and 10% synapsis, 11 of the 12 chromosome pairs had started SC formation. The ACs showed several discontinuities.

Figure 2 shows an electron photomicrograph of nucleus wt4, with 2% alignment and 53% synapsis. Extensive synapsis occurred in most distal chromosome segments. In addition, short intercalary SC segments were found in several bivalents. One such segment, flanked at both sides by dissimilar ACs, was considered non–homologous. Only two short segments of presynaptic alignment were observed; a distal and an interstitial one. In the longest bivalent (#1), unpaired homologous ACs were running distantly in parallel. In another chromosome, which presumably was the nucleolar

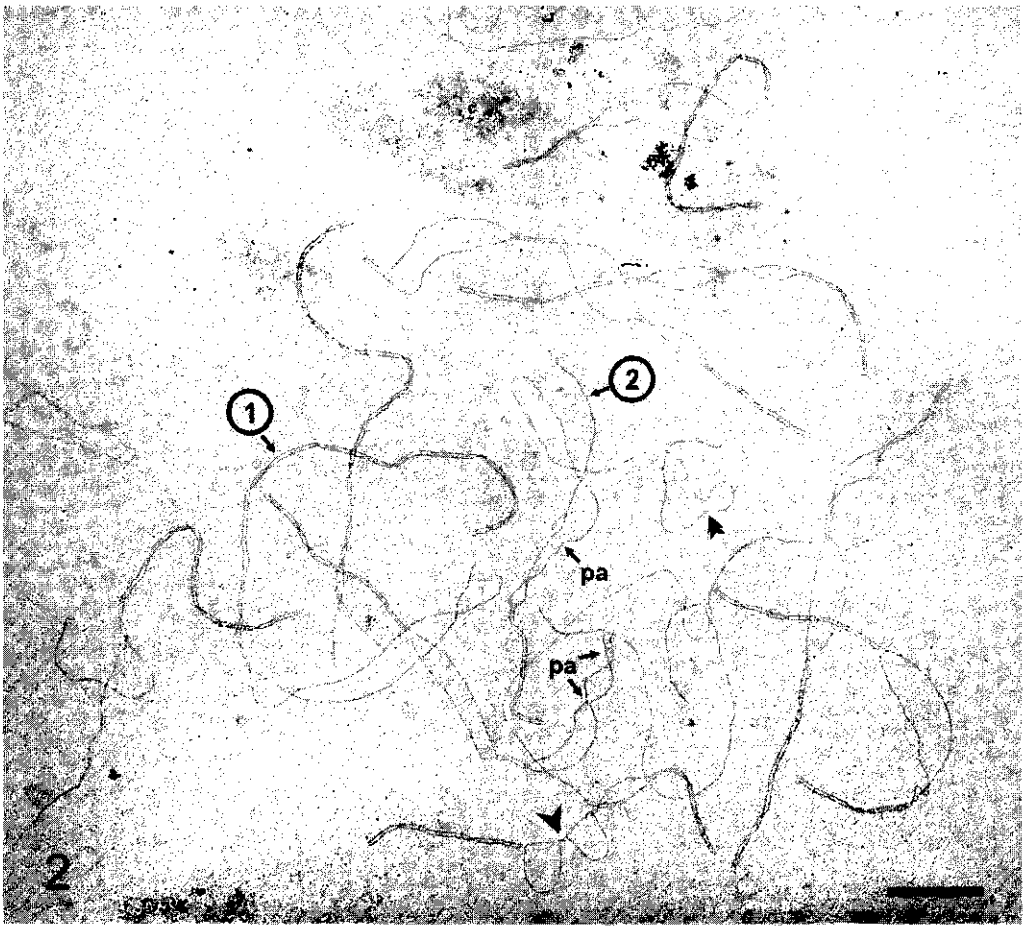

Figure 2: Silver stained zygotene nucleus (wt 4) from wild type tomato with 53% synapsis. The SCs of chromosome 1 and chromosome 2 could be discerned. Examples of presynaptic alignments are given (pa). The double arrow head indicates intrachromosomal foldback association. The single arrowhead shows a non–homologous intercalary SC segment. The bar equals 5 µm.

chromosome (#2), unpaired arms were involved in non–homologous foldback association. In the nuclei wt5 and wt6 with 67 and 92% synapsis respectively, only one or two chromosome ends and several intercalary regions were still unsynapsed.

Schematic representations of presynaptic alignment and synapsis in the bivalents of wt3–6 are shown in Figure 3A. As kinetochores were not visible, we could not distinguish long and short chromosome arms. Therefore we could only identify chromosome #1, which is by far the longest in the complement, and chromosome #2 which can be recognised because the short arm with the nucleolar organiser region remains unsynapsed at late zygotene. Paired segments were common at or near the telomeres and most of them involved synapsis. Aligned segments were obvious in nucleus wt3, but

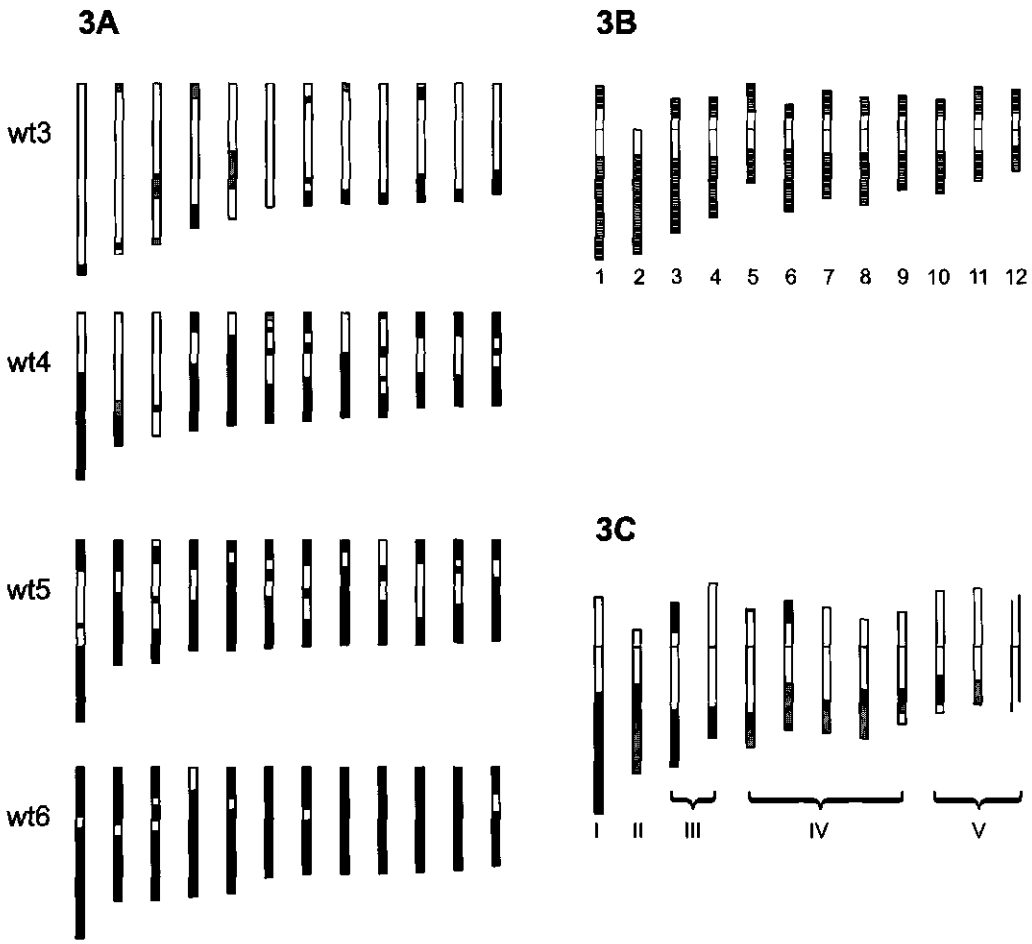

Figure 3: Schematic representations of synapsis and alignment in four wild type nuclei and in nucleus asb–8 of the synaptic mutant a*sb* of tomato. **3A.** The chromosome complements in the zygotene nuclei wt3, wt4, wt5 and wt6. Chromosomes were arranged in sequence of decreasing length. Kinetochores were not visible. The solid blocks represent synaptic segments; the gray blocks represent presynaptic alignment, whereas the white blocks represent unpaired regions. **3B.** Ideogram of the pachytene complement of tomato according to Sherman and Stack (1992). Chromosomes are arranged according to number (chromosome 1 *left* to chromosome 12 *right*) with their kinetochores (bars) in one line. Blocks with the horizontally striped textures represent euchromatin and the white blocks are heterochromatin regions. **3C.** Schematic representation of nucleus asb–8. Chromosomes were ordered into five different classes according to length and kinetochore position. The black, gray and white blocks are as in fig. 3A.

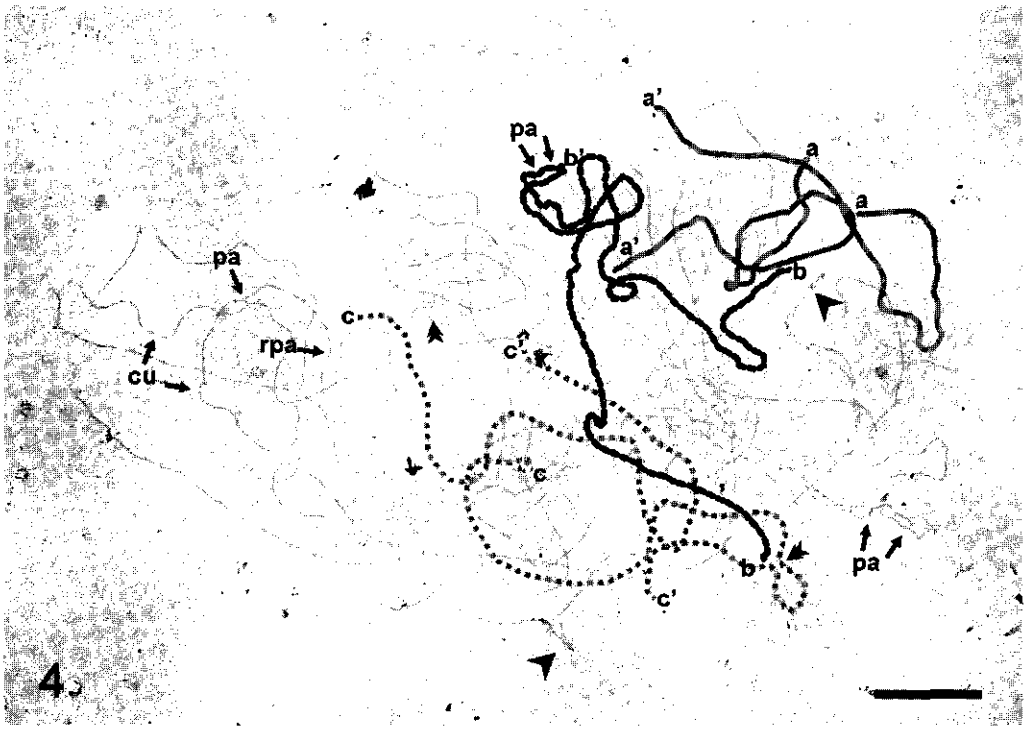

Figure 4: Uranyl acetate stained early prophase nucleus (~ early zygotene) from mutant *as1* (as1–1) with mostly unpaired ACs. Examples of presynaptic alignment (pa), AC curving (cu) and rough parallel arrangement (rpa) of ACs are indicated. Two short distal SC segments are indicated by arrowheads. Most telomeres occur in a small regions at the periphery. The double arrowheads indicate intra– chromosomal foldback associations. Three putative homologous AC pairs are highlighted. The a–a' and c–c' AC pairs were considered as putative homologues on the basis of most similar length and similarities in the bending patterns of the two ACs. The bar equals 5 µm.

disappeared almost entirely in wt4. The small number of SC segments in nucleus wt6 is likely due to zipping up of unsynapsed segments (Table 1).

In the wt3–6 nuclei we compared bivalent core lengths (ACs or lateral elements) with the percentage synapsis within the bivalent. In wt3 and wt4, we also analysed the relation of bivalent length with the percentage of presynaptic alignment. Computation of the Pearson product– moment correlation coefficient revealed that in none of the nuclei these correlations were significant at the 5% level (Table 2), which indicates that chromosome length does not contribute significantly to the variation in pairing extent. This means that within these nuclei bivalents of all lengths paired fairly synchronously. However, when all bivalents of the four wt nuclei were pooled, a slight negative correlation between bivalent length and percentage synapsis was found (Table 2).

Table 2: Correlation between bivalent length and percentage synapsis, presynaptic alignment and pairing in wild type and mutant tomato. NA = no data available. *, **, significant at α=0.05 or 0.01, respectively.

nucleus	number of bivalants	correlation between bivalent length and:		
		% SC	% alignment	% pairing
wt3	12	−0.45	+0.43	−0.30
wt4	12	−0.23	+0.21	−0.21
wt5	12	−0.13	NA	0.13
wt6	12	−0.16	NA	−0.16
all wt	48	**−0.35***	NA	**−0.33***
wt3+4	24	NA	+0.30	NA
as1–1	11	−0.14	−0.05	−0.11
as1–2	10	+0.45	−0.22	+0.16
as1–3	7	−0.09	−0.08	−0.11
as1–4	10	−0.06	+0.51	+0.01
as1–5	12	−0.59	−0.04	**−0.62***
all *as1*	50	−0.24	+0.04	−0.25
asb–1	8	+0.67	+0.51	**+0.83***
asb–2	7	**+0.77***	−0.02	+0.44
asb–3	8	**+0.77***	+0.01	+0.48
asb–4	4	+0.84	+0.95	+0.95
asb–5	5	+0.60	−0.36	+0.39
asb–6	6	+0.71	−0.48	+0.75
asb–7	9	**+0.74***	−0.54	+0.62
asb–8	12	**+0.85****	−0.31	**+0.58***
all asb	59	**+0.55****	−0.02	**+0.53***

Synaptic initiation in as1

Nuclei of the asynaptic mutant *as1* were more easily spread than wild type nuclei with comparable extents of synapsis, and showed few discontinuities of ACs. Five *as1* nuclei, with synapsis ranging from 4 to 33%, were selected. The data on pairing are summarised in Table 1.

Figure 4 shows the photomicrograph of nucleus as1–1, with 5% alignment and 4% synapsis. All ACs were together in a complex mass, though less tight and less obscured by chromatin than in wild type nuclei. In this figure we highlighted the positions of three putative homologous AC pairs. The

Figure 5. Part of the nucleus asb–8. Characteristic features of this mutant are the clear kinetochores (indicated by ki in the figure) and the transition of presynaptic alignment into true synapsis (arrow heads). The bar equals 5 µm.

bending patterns of these homologous AC pairs was roughly similar. The few segments of presynaptic alignment and SCs were found at the periphery of the nucleus.

In the five *as1* nuclei we detected 50 homologous AC pairs, which were either aligned, synapsed, or still unpaired. Completely unpaired ACs were considered homologous when they had similar lengths and centromere positions (if visible), and these often showed the rough parallel orientation as indicated in Figure 4. Most of the distal chromosome segments were present in a small area in the periphery of the nucleus (bouquet, see upper left corner of Figure 4). We did not find a clear relation between chromosome length and percentage pairing (Table 2). Only in nucleus as1–5 we found a significant negative correlation between bivalent length and percentage of pairing, which means that in this nucleus pairing had progressed further in the shorter chromosomes.

Table 3. Synapsis and presynaptic alignment in the bivalent groups of early prophase I cells of*asb*. The observed values were tested against: 1) Expected values, based on total chromosome lengths; 2) Expected values, based on lengths of euchromatin; 3) Expected values, based on lengths euchromatin segments in longest chromosome arm.

class	µm synapsis				µm alignment				µm pairing			
	Obs.	Exp[1]	Exp[2]	Exp[3]	Obs.	Exp[1]	Exp[2]	Exp[3]	Obs.	Exp[1]	Exp[2]	Exp[3]
I	67	19.6	22.1	23.6	18	19.5	21.9	23.4	85	39.1	44.0	47.0
II	17	13.9	16.8	22.6	22	13.8	16.7	22.5	39	27.7	33.5	45.1
III	38	28.7	29.1	30.7	26	28.5	28.9	30.5	64	57.2	58.0	61.2
IV	23	57.4	55.3	54.4	64	57.1	54.8	54.1	87	114.5	110.0	108.5
V	5	30.3	26.9	18.7	19	30.1	26.7	18.5	24	60.4	53.5	37.2
total	150	149.9	150.2	150	149	150	149	149	299	299	299	299
χ^2		160.1	130.6	111.1		10.1	6.4	3.8		87.8	60.8	40.6
$P_{df=4}$		<.001	<.001	<.001		<.038	<.169	<.441		<.001	<.001	<.001

Synaptic initiation in asb

We selected eight cells of the asynaptic mutant *asb*, with percentages synapsis ranging from about 7% in nucleus asb–1 to 24% in nucleus asb–8 (Table 1). The zygotene nuclei of this mutant were denser and more difficult to spread than those of *as1* and apart from nucleus asb–8 no complements could be fully analysed. Figure 5 shows the electron photomicrograph of part of nucleus asb–8, and Figure 3C presents the schematic drawing of the full complement of this nucleus. In all bivalents the pericentromeric region was not paired. The bivalents in nucleus asb–8 were shorter than those in wild type zygotene nuclei. Most bivalents contained paired segments with adjacent aligned and synapsed stretches (Figure 3C+5). Such transitions between presynaptic alignment and synapsis occurred far more frequently in the nuclei of *asb* than in those of *as1* and wild type (cf. nucleus wt4, Figure 2). The aligned and synapsed parts of such segments were registered separately. The data on number and extent of paired segments in the eight cells of *asb* are summarised in Table 1.

The correlations between chromosome length and percentages synapsis, presynaptic alignment and pairing in the eight nuclei of *asb* are shown in Table 2. In mutant *asb*, chromosome length and percentage synapsis were positively correlated (significant for four of the eight analysed nuclei), whereas this was not found in wild type and mutant *as1*. Thus in mutant *asb* longer chromosomes synapse more extensively than shorter chromosomes. We found no correlation between chromosome length and the percentage of presynaptic alignment.

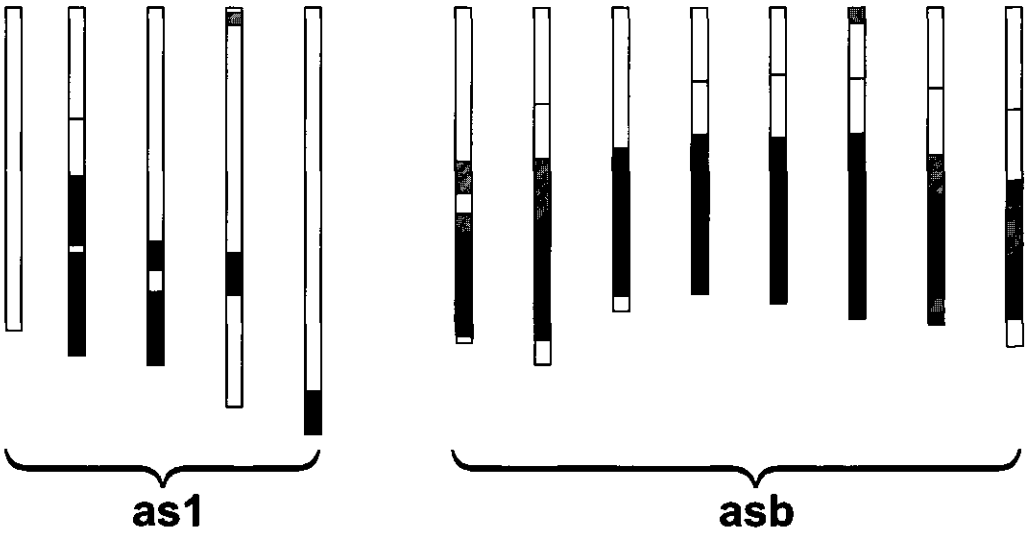

Figure 6: Schematic representations of the chromosome 1 SCs of the five *as1* nuclei and seven asb nuclei. The solid blocks represent synaptic segments; the gray blocks represent presynaptic alignment, whereas the white blocks represent unpaired regions. Bars are the kinetochores.

Kinetochores were generally clearly detectable in *asb* (Figure 5); in the asb–1, asb–3, asb–5 and asb–8 nuclei, all twelve kinetochores could be discerned. Kinetochores allowed us to distinguish chromosome arms, which is helpful for the identification of individual bivalents. We measured arm lengths in these four nuclei and classified the bivalents (nomenclature according to Levan *et al.* (1966): i) Class I, the largest submetacentric SC 1; class II, the nucleolar chromosome SC 2; class III, two large submetacentric SCs 3 and 4; class IV the five smaller subtelocentric SCs 6–10 and class V the three small metacentric SCs 5, 11 and 12. The SCs were identified on the basis of total SC length and centromere position (Figure 3B). In table 3 we analyse more in detail the relation between the length of chromosomes or chromosome arms and the extent of synapsis or presynaptic alignment. We tested the following possible relations: 1) the extent of pairing is proportional to total chromosome length, 2) the extent of pairing is proportional to chromosome length in euchromatin, and 3) the extent of pairing is proportional to the length of the longest chromosome arm in euchromatin. The relative lengths of euchromatin and total SC length per chromosome class were estimated according to Sherman and Stack (1995; see their Tables 5 and 7). The observed extents in synapsis and in pairing differed significantly from all theoretical values (Table 3). This was mainly due to an excess of synapsis in class I bivalents and a lack of synapsis in class IV and V bivalents. However, the extents of presynaptic alignment fitted most expected values; Table 3 suggests that in mutant *asb* presynaptic alignment is proportional to total euchromatin length, or to the length of euchromatin in the long chromosome arms whereas synapsis is not.

Table 4. Numbers of bivalents in four early and four late prophase nuclei of mutant *asb*. The observed values were tested against expected numbers by the χ^2 test. The expected numbers were based on the following assumptions: **Exp**[1]: Chromosomes in all classes have an equal chance of bivalent association; **Exp**[2]: Probability of bivalent association is proportional to total chromosome length; **Exp**[3]: Probability of bivalent association is proportional to length in euchromatin; **Exp**[4]: Probability of bivalent association is proportional to long arm length in euchromatin; **Exp**[5]: Number of chromosome pairs with synaptic associations in early nuclei are comparable to the number of bivalents in late nuclei; **Exp**[6]: Number of chromosome pairs with pairing (alignment or SC) associations in early nuclei are comparable to the number of bivalents in late nuclei.

	Observed numbers of bivalents						χ^2 values for several expectations					
	total	I	II	III	IV	V	Exp[1]	Exp[2]	Exp[3]	Exp[4]	Exp[5]	Exp[6]
four early nuclei: paired bivalents (alignment or SC)	30	4	3	5	12	6	1.32	0.14	0.35	2.33		
synapsed bivalents (only SC)	20	4	3	5	7	1	8.58	4.15	2.85	1.33		4.83
four late nuclei: synapsed bivalents	21	3	4	5	8	1	7.93	4.91	3.41	1.37	0.64	

None of the χ^2 values were significant

Our next question was: Are the different percentages of synapsis in chromosomes of different classes accompanied by non–random bivalent formation in mutant *asb*? We analysed the number of bivalents per class in the same four (early prophase I) nuclei as mentioned above, and in another four nuclei, which were probably in a later stage of meiotic prophase because they lacked presynaptic alignment and had strongly reduced numbers of recombination nodules. (For further analysis of recombination nodules, see Chapter 5 of this thesis).

In Table 4, we compared the observed distribution of bivalents over the five chromosome classes with the distribution expected on the basis of various assumptions (indicated by Exp.[1] to Exp.[6]). In the first model (Exp[1]) we assume that each chromosome pair has an equal probability of initiating pairing, so the five classes are expected to contain 1/12, 1/12, 2/12, 5/12, and 3/12 of the bivalents respectively. In the remaining three models, we assume that pairing initiation is proportional to total chromosome length, to length in euchromatin or to length in euchromatin of the longest arm. In addition, we compared the bivalent distribution in the late nuclei with both the paired (Exp[6]) and the synapsed (Exp[5]) bivalent distributions of the early nuclei. Table 4 shows that in the early nuclei, half of the bivalents in class IV and the majority of the bivalents in class V are connected by alignment only, and thereby account for the difference between the paired bivalent distribution and synapsed bivalent

distribution. The paired bivalent distribution fits rather well with all proposed models, whereas the synapsed bivalent distribution does less so, because bivalents of class IV and class V chromosomes are underrepresented. The bivalent distribution of the late nuclei fits best with the synapsed bivalent distribution in the early nuclei. However, non of these differences is significant.

Pairing behaviour of chromosome 1

Chromosome 1 was the only chromosome that we could always recognise in the complements, even in wild type and *as1* nuclei in which other chromosomes could not be identified because the kinetochores could not be stained. In the four fully analysable early *asb* nuclei chromosome 1 (class I) was overrepresented among the bivalents. In the remaining four *asb* nuclei and in four out of the five *as1* nuclei, the chromosomes 1 formed a bivalent as well. Figure 6 shows a compilation of the thirteen chromosome 1 SCs of *as1* and *asb*. We could establish centromere positions in one *as1* bivalent and in six of the eight *asb* bivalents, and in these bivalents synapsis was confined to the long arms. The (short) unsynapsed arms were far apart in seven bivalents, had distal presynaptic alignment in two bivalents, and showed distant parallel arrangement in two bivalents (similar to the wt–4 chromosome 1 in Figure 2). The eight bivalents 1 of *asb* were on average shorter than those of *as1* and wild type tomato (40.4±2.9 μm, 47.0±4.7 μm and 46.9±2.6 μm in *asb*, *as1* and wild type, respectively), which was significant at $P < 0.05$.

Discussion

Chromosome pairing in tomato essentially follows the same pattern as described for several other plant species like maize (Gillies 1975), *Lilium* (Holm 1977), *Tradescantia* (Hasenkampf 1984), rye (Gillies 1985), wheat (Holm 1986) and *Allium* (Albini & Jones 1987). In short, synapsis predominantly initiates in distal regions (with telomeres clustered in a bouquet), extends into proximal regions, and overtakes the intercalary initiations.

Tomato was more appropriate for the analysis of entire zygotene nuclei than the plant species mentioned above. First, tomato chromosomes are far shorter than those of wheat, rye, *Lilium*, *Tradescantia* and *Allium*, and therefore tomato complements at early prophase are more simply to unravel. Second, the unique partially asynaptic mutants of tomato allow pairing studies in nuclei with only little synapsis. In these mutants, ACs remained intact during spreading so that entire chromosome complements could be traced, whereas visible kinetochores allowed identification of individual bivalents in part of the nuclei.

Chromosome pairing in tomato

In early prophase I of tomato, ACs are in a tight chromatin mass (knot). In spreads, the ACs are curved and bended at this stage, and two or more axial cores can be observed to run roughly in parallel at a considerable distance, generally more than 300 nm. In mutant *as1*, we found that homologues

may have similar bending patterns. Rough parallel arrangements was not only apparent between putative homologous AC regions but occurred between non–homologous regions as well. Extensive non–homologous parallel arrangement of ACs has been found in the completely asynaptic mutant *as6* of tomato (Havekes *et al.* 1994). Possibly, this type of distant alignment reflects weak reversible contacts between the peripheral chromatin loops. Such contacts could precede the typical presynaptic alignment. In contrast to rough parallel arrangement, presynaptic alignment only involved homologous ACs in our meterial. This is in agreement with observations in other species (review: Loidl 1990). Presynaptic alignment therefore seems to be the first cytological sign of relatively stable homologous contacts.

Presynaptic alignment

It is possible that presynaptic alignment is common in early prophase I nuclei of wild type tomato, but the tight associations of ACs in the chromatin knot do not allow reliable observations. In wild type tomato we observed that shortly after this synizetic association, when the chromosomes release from the knot and individual AC pairs become easily traceable, synapsis had progressed considerably and had presumably covered most of the original stretches of presynaptic alignment. It was therefore difficult to determine whether there was a certain pattern in the initiation and progression of presynaptic alignment in the wild type. In contrast, the partially asynaptic mutants in our study displayed large–scale presynaptic alignment. The data from these mutants (Table 2 + 3) suggest that presynaptic alignment is an overall nuclear process in which all euchromatic chromosome regions participate equally. The limited data on presynaptic alignment in wild type tomato are in agreement with this.

Synapsis

In wild type tomato and in mutant *as1*, bivalents of different lengths synapsed rather synchronously (Table 2), although shorter chromosomes appeared to be slightly ahead. In contrast, in mutant *asb* we found a clear positive correlation between chromosome length and the percentage of synapsis. The long chromosomes, especially #1 and #2, displayed a higher percentage of synapsis than the shorter ones, in particular the short metacentric chromosomes #5, #11 and #12, which often failed to initiate synapsis at all. Thus, unlike presynaptic alignment, synapsis is influenced somehow by the size of individual chromosomes or chromosome arms in *asb*. We think that mechanical factors may have contributed to this effect in *asb*. For instance, it is possible that (extension of) synapsis is hampered by aberrant chromosome condensation in this mutant, and that aberrant condensation has more effect on synapsis in short chromosome arms than in long chromosomes or chromosome arms. The relatively short chromosome complement lengths and the intense kinetochore staining in mutant *asb* suggest that chromosome condensation may not be normal in the mutant.

Another aspect of synapsis that was not random in mutants *asb* and *as1* (and possibly also in the wild type) is the distribution of synapsis over long and short arms. In none of the chromosomes 1 that

we have analysed we found initiation of synapsis in the short arms (Figure 6), although presynaptic alignment has been found in the short arm of one chromosome 1. Apparently the length of a chromosome arm influences synapsis or synaptic initiation.

A related observation has been made by Sherman and Stack (1995), who remarked that in wild type tomato single late RNs never occur in the short arms of chromosome 1. If sites of crossing over are normally the sites of synaptic initiation, then the preferential positioning of the first RN on the long chromosome 1 arm could reflect the same mechanism as the preferential synaptic initiation in the long arm. One possibility is that this mechanism is torsion or tension. Darlington (1940) proposed that torsion is an important factor for establishment of a crossover. Kleckner (1996) suggested that tension along the chromosome influences the resolution of recombination intermediates in crossovers. She postulated that tension is built up at the chromosome – axial core border by chromosome condensation. It is possible that short chromosome (arms) have difficulties in building up sufficient torsion/tension for generating a crossover. If chromosome condensation is abnormal (as we suspect for mutant *asb*), then it may not be possible at all for the mutant to form crossovers in short chromosome arms.

5

Chromosome pairing in wild type and synaptic mutants of tomato (*Lycopersicon esculentum*) II. Recombination nodules in mutant tomato

Francis W.J. Havekes, J. Hans de Jong, Christa Heyting

This report describes the manifestation of early and late recombination nodules (RNs) in meiotic prophase I, as observed in electron micrographs of hypotonically burst pollen mother cells of the partially asynaptic mutant *asb* and the desynaptic mutant *as5* of tomato. In *asb*, early RNs were numerous in segments of presynaptic alignment and SC during early prophase I. Their number dropped sharply in stages comparable to early zygotene and early pachytene, and only few RNs remained on the SCs until mid prophase I. In chromosome regions with presynaptically aligned segments most if not all early RNs disappeared. At a stage comparable to pachytene in wild type tomato, we observed that bivalents with two or more SC segments do not have late RNs in all segments. The genetic analyses of *asb* (Moens 1969), which demonstrated increased crossover frequencies in chromosome 2, is discussed.

The desynaptic mutant *as5* with normal synapsis and reduced chiasma formation had morphologically normal SCs, which each carried at least one late RN. The reduced chiasma frequencies in the mutant therefore probably result from a defect in the formation of maintenance of chiasmata rather than a defect in homology recognition or synapsis.

Introduction

A prerequisite for the successful orientation and segregation of parental chromosomes at metaphase I / anaphase I of meiosis is the presence of at least one chiasma, which connects the two homologues in a bivalent. This assures that bivalents properly congress to and orient in the equatorial plane of the metaphase I spindle, with the still undivided centromeres of the two homologues facing opposite poles. The distribution of chiasmata along the chromosomes is not random. First, all bivalents, including the smallest ones, always have at least one chiasma; second, chiasmata on the same bivalent are not randomly positioned, because the presence of a chiasma decreases the chance of another chiasma nearby on the same bivalent, a phenomenon called chiasma interference (reviewed by Jones 1984). Third, in many organisms, chiasmata are preferentially formed in certain regions within bivalents, most often in distal euchromatic regions (Jones 1967).

Chiasmata represent reciprocal recombination events (crossing–over) between homologous chromosomes. The non–random distribution of chiasmata reflects a non–random distribution of reciprocal recombination events along the bivalent. Recombination takes place during the lengthy prophase I of meiosis and is closely related to homologous chromosome synapsis: the formation of the synaptonemal complex (SC) between homologues (von Wettstein *et al.* 1984). Specific structures called recombination nodules (RNs), have been found along SCs of several species (reviewed in Carpenter 1994). The numbers and distribution of RNs along the bivalents during mid–late pachytene correspond well to the frequencies and distributions of crossing–over and chiasmata (Carpenter 1975, Zickler 1977, Bernelot–Moens and Moens 1986, Albini and Jones 1988, Stack *et al.* 1989, Sherman and Stack 1995). Therefore, these mid– or late pachytene RNs are supposed to mark the sites of crossing–over. Like crossovers and chiasmata, late RNs display in most cases positive interference with respect to their position along the bivalents.

In most species, including tomato, RNs are also present during earlier stages of meiosis (Stack and Anderson 1986b, Albini and Jones 1987, Anderson and Stack 1988). These so–called early RNs occur along axial cores (ACs) and between lateral elements (LEs) of SCs in leptotene – zygotene – early pachytene. They are more numerous than late RNs, and in contrast to late RNs, they are randomly distributed along SCs. Their shape can be (slightly) different from late RNs. In tomato, they are smaller and more spherical than late RNs. It has been proposed that early RNs mark the sites of all recombinational interactions, the gene conversions and crossovers (Carpenter 1987). During pachytene, early RNs are gradually lost, whereas late RNs persist until the end of pachytene. This loss of early RNs during early pachytene renewed the attention for existing models for crossover interference (Mather 1937, Stam 1979) and lead to the formulation of new models (King and Mortimer 1990, Foss *et al.* 1993). King and Mortimer (1990) proposed that the assembly of a hypothetical polymer along the central element of the SC was nucleated at the sites of crossing over. Extension of the polymer from this site would prevent the establishment of additional crossovers nearby, and would thus cause positive chiasma interference. The fact that the *zip1* mutation in yeast abolishes both interfer-

ence and synapsis also indicates that SCs have a role in interference (Sym and Roeder 1994). Furthermore, at least two organisms, namely *Aspergillus nidulans* (a filamentous fungus) and *Schizosaccharomyces pombe* (fission yeast) lack both interference and SCs (Egel–Mitani *et al.* 1982, Kohli and Bahler 1994, Munz *et al.* 1994).

Moens (1969) reported a strong reduction of genetic interference in the meiotic mutants *as1* and *asb* of tomato; these mutants have reduced levels of synapsis (Havekes *et al.* 1994, chapter 2). In this paper we describe early and late RNs in one of these partially asynaptic mutants, *asb*, and consider the relation between synapsis and loss of RNs. In addition, we analyse late RNs in the desynaptic mutant *as5*, which has wild type levels of synapsis, but forms reduced numbers of chiasmata (Havekes *et al.* 1994).

Materials and Methods

Plant material

The partially asynaptic mutant *asb* and the desynaptic mutant *as5* were obtained from Dr P.B. Moens, Department of Biology, York University, Toronto, Canada. They were described by Soost (1951), Moens (1969), Havekes *et al.* (1994) and chapter 4 of this thesis. The sib plants in backcross families had a completely regular meiosis and were used as wild type control plants.

Methods

Synaptonemal complexes were spread according to Stack (1982), with slight modifications as described by Havekes *et al.* (1994). Late pachytene cells were stained either with 33% silver nitrate at 40° C or with uranyl acetate–lead citrate (UP); both methods stain late RNs in tomato (Sherman *et al.* 1992).

Results

Mutant asb

In *asb*, the wild type criteria for sub–staging prophase I (extents of synapsis and kinetochore staining) did not apply, because synapsis never reaches completion and kinetochores can be prominent in nuclei with otherwise early prophase characteristics such as presynaptic alignment and the occurrence of many early nodules. We use the terms early and late nuclei to refer to stages comparable to early and late pachytene nuclei in wild type tomato. Discrimination between these stages is based on the presence of presynaptic alignment (only in early nuclei) and by the number of RNs, which is far higher in early nuclei than in late nuclei.

Figure 1: Uranyl acetate stained mid-prophase nucleus asb-8. The large arrowheads indicate kinetochores. The double arrowhead shows the position of four fused (non-homologous) kinetochores. Different manifestations of early recombination nodules are shown by numbered arrows: 1. Single spherical RNs at unsynapsed ACs; 2. Aggregates of several RNs at ACs; 3. Pairs of RNs between converging ACs in a presynaptic aligned regions; 4. Merging RNs between ACs in presynaptic aligned segment. 5. (Early?) RN on SC segments; 6. (late?) RN on SC segment. The bar equals 5 µm.

Table 1. Correlation between the numbers of early RNs per µm SC, per µm presynaptic alignment and per µm single AC, and the length of chromosome 1, the percentage of SC and the percentage alignment in eight early prophase I nuclei of mutant *asb*. Bold figures are significant at the 0.05 level (critical value is 0.70).

			The number of early RNs per µm SC		
	% SC	% presynaptic alignment	SC	presynaptic alignment	unpaired ACs
Length chr. 1	**–0.86**	+0.60	+0.66	–0.41	+0.44
% SC	–	–0.47	**–0.77**	–0.07	**–0.70**
% pres. align.	–	–	+0.49	+0.49	+0.63

Early RNs in mutant asb

Early RNs in wild type tomato are small spherical bodies and occur in large numbers along the axial cores and lateral elements of the synaptonemal complex (An extensive description of their morphology is given by Stack and Anderson 1986a, 1986b and Sherman *et al.* 1992). The same type of RNs was detected in mutant *asb*. Figure 1 shows a detail of one of the early cells of *asb*. Details of the different RN types are also shown in Figure 4. Nodules were located between presynaptically aligned ACs (Figures 1 and 4a), in the central region of Scs and along single unpaired ACs. Nodules associated with ACs were spherical and nodules on SCs were spherical to ellipsoid. Figure 1 also shows examples of twin nodules located between converging ACs, and aggregates of more than two nodules associated with ACs. Nodule–pairs were rather common and occurred three, two, five, and five times, respectively, in the four *asb* nuclei that we studied in detail. Merging nodules with a shape intermediate to a pair and a single one were also observed.

We determined the numbers of early RNs in eight early prophase I nuclei of *asb*, with synapsis ranging from 7 to 24 percent. We found 1.36±0.38 RNs per µm SC, 1.42±0.27 RNs per µm presynaptic aligned ACs, and 0.16±0.12 RNs per µm of single AC. Within the eight nuclei, we compared the number of early RNs per µm SC, per µm presynaptic alignment and per µm unpaired AC, with the length of chromosome 1 (the chromosome that could be identified in all nuclei), the percentage of synapsis and presynaptic alignment. The data are given in Table 1 and show that the length of chromosome 1 is negatively correlated with the percentage of synapsis. We found a significant negative correlation between the percentage of the complement involved in synapsis (SCs) and the number of RNs per µm SC and per µm unpaired AC. The numbers of RNs in presynaptic alignment were not negatively correlated with the percentage of synapsis.

In four of the nuclei with prominent kinetochores we were able to identify bivalents, and thus to analyse the numbers of nodules per bivalent class as defined in Chapter 4 (Table 2). We did not find a clear chromosome–specific pattern in RN frequency in this small sample of bivalents.


2
2

Table 2. Number of nodules per µm synapsis, presynaptic alignment and axial element in the different bivalent groups in four early prophase I cells of mutant *asb*.

		Numbers of RNs (early + late) per µm SC		
Bivalent group	Numbers of bivalents	SC	Presynaptic alignment	unpaired AC
I	4	1.57±0.39	1.93±0.50	0.16±0.19
II	3	1.75±0.71	1.41±0.14	0.06±0.06
III	5	1.56±0.81	1.36±0.78	0.17±0.10
IV	12	1.30±0.27	1.84±0.87	0.22±0.16
V	6	1.06±0.23	1.28±0.54	0.19±0.21

Late RNs in mutant asb

Late RNs were analysed in sixteen late nuclei. Identification was based on the ellipsoid morphology as was described for wild type tomato (Stack and Anderson 1968a, 1968b, Sherman *et al.* 1992). Examples of late nuclei of *asb* are shown in the Figures 2, 4b and 4c. Fig. 3 shows diagrams of synapsis with late RNs from four *asb* nuclei in which the kinetochores were visible and bivalents could be classified (see Chapter 4). Twenty–eight RNs were stained intensely in the late nuclei and were interpreted as late RNs, whereas only eight nodules, which were relatively small and/or more faintly stained, were considered as early RNs.

In tomato, late RNs occur almost exclusively in euchromatic regions (Sherman and Stack 1995). We therefore compared the number of late RNs per µm SC in euchromatin (Table 3), right column) in the two mutants and in wild type. Mutant *as5* and wild type tomato had about the same number of late RNs per µm SC in euchromatin, but in mutant *asb* the number of late RNs per µm SC in euchromatin was two–fold higher than in wild type.

The four late nuclei of *asb* represented in Figure 3, illustrate the following aspects of SC formation and late RNs in *asb*: 1) most bivalents have only one SC segment, which often extends from a chromosome end; 2) all bivalents with one SC segment have at least one RN in this segment; 3) bivalents with more than one SC segment have an RN in at least one of the segments; 4) almost all late RNs are located in the middle of an SC segment; they are rarely seen at the pairing fork and never at the telomeres; 5) long SC segments have more RNs per µm SC than short SC segments.

These aspects of SC formation and distribution of late RNs also applied to the bivalents that we studied in twelve other late prophase I cells of *asb*. These cells were analysed in addition to the four

Figure 2. An example of a UP stained late nucleus (comparable to late pachytene in wild type tomato) of the partially asynaptic mutant asb. Arrow heads indicate the positions of the kinetochores; small arrows show examples of late RNs. A large arrow indicate a small RN. The NOR chromosome 2 with three late RNs is shown in the figure. The bar equals 5 µm.

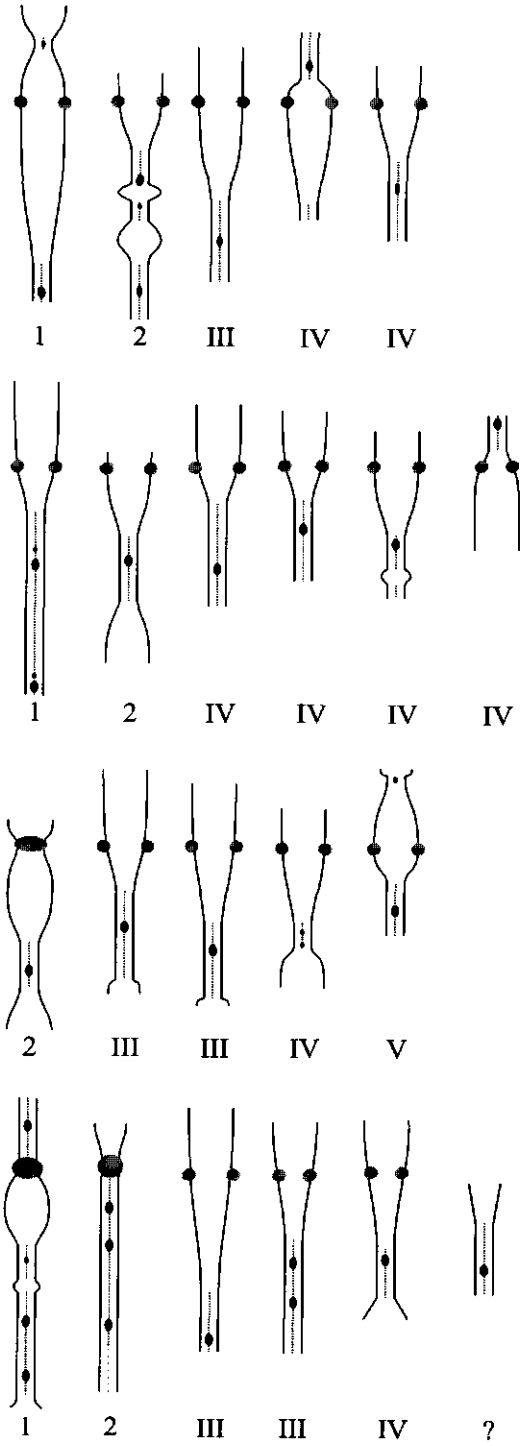

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Table 3. Comparison of numbers of bivalents and numbers of late RNs of nuclei at (comparable to) pachytene of the mutants *asb* and *as5* and in wild type tomato.

mutant	number of cells	number of bivalent	μm SC	# RNs	# RNs/SC	number of RNs/μm SC	number of RNs/ μmSC euchrom.[a]
asb	16	68	625	86	1.26	0.165±0.101	0.165±0.101
as5	10	116	2532	152	1.31	0.061±0.010	0.092±0.03
wild type	6	59	1367	75	1.27	0.055±0.005	0.083±0.002

a) Number of RNs per μm SC in euchromatin. We assume that 2/3 of the total SC-length in wt and *as5* in euchromatic regions; in *asb*, SC formation is virtually restricted to euchromatin.

cells represented in Figure 3. Out of 68 bivalents studied, 58 had only one SC segment, seven had two SC segments and three had three SC segments. All 58 bivalents with a single SC segment had one or more RNs in this segment. In six of these bivalents, only small/faint RNs were found. These small and faint RNs were unique for *asb* and were not found in the wild type. Of the seven bivalents with two SC segments, three had RNs in both segments, two had RNs in one segment and two other bivalents had only one small/faint RN in one segment. The three bivalents with three SC segments all had RNs on two of the segments and a small RN in the third segment. The thirteen SC segments without an RN (or in some cases with a small RN) were all short segments, namely between 0.93 and 5.92 μm long, and with an average of 2.90±1.43 μm, whereas the overall average SC segment length in mutant *asb* was 7.35±4.87 μm.

Thus, the SC segment length was positively correlated with the number of late RNs ($r=0.69$, $P<0.01$).

Late RNs in mutant as5

In mutant *as5*, the number of late RNs per μm SC, or per μm SC in euchromatin did not differ significantly from from the corresponding numbers of RNs in wild type (Table 3). All 116 analysed SCs in mutant *as5* carried at least one RN. We did not observe any morphological difference between RNs in *as5* and wild type tomato.

Figure 3. Diagrammatic representation of axial cores, SC segments and RNs in four late *asb* nuclei (comparable to late pachytene in wild type tomato). Early and late RNs are represented by the small and large black spots on the central element, respectively. Kinetochores are the large gray spherical structures.

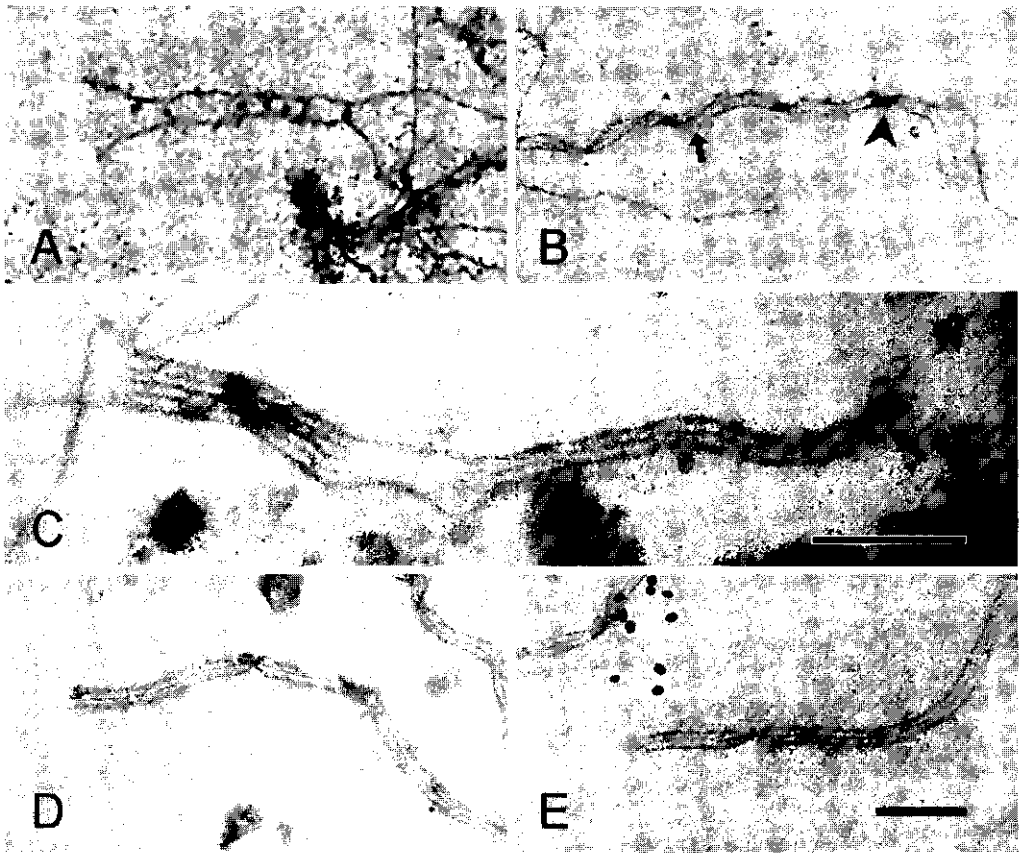


Figure 4: Details of recombination nodules in the partially asynaptic mutant *asb*, the desynaptic mutant *as5* and wild type tomato. **A.** Presynaptic aligned segment in *asb*. Early RNs are formed between the two axial cores. **B.** SC of a late *asb* nucleus with a large bar–like RN (arrow head) and a small RN (arrow). **C.** Double synapsis (polycomplex) of a late nucleus of *asb*. **D.** SC of a late pachytene *as5* nucleus with a late RN at a twist of the lateral elements. **E.** Late RN in a late pachytene SC of the wild type tomato. The dark particles outside the SCs are likely staining artefacts. The bars equal 2 μm.

Discussion

Distribution and morphology of RNs in the partially asynaptic mutant asb

Mutant *asb* was capable of assembling both early and late RNs. Early RNs had a similar frequency and distribution along SC segments, and aligned and unpaired ACs as in wild type tomato. With respect to the late RNs, however, there were some differences: 1) there were twice as many late RNs per μm SC (in euchromatic region) in *asb* than in wild type, and 2) part of the late RNs were abnormally small or

faintly stained in *asb*. The average value of 1.27 RNs/SC for wild type in our study was significantly lower than the 1.79 RNs/SC as observed by Sherman and Stack 1992). This discrepancy may be attributable to differences in wild type genotypes and staining conditions.

The larger number of late RNs per μm SC in *asb* is of interest, because Moens (1969) found higher crossover frequencies between linked genes on chromosome 2 in *asb*. He also found a lower crossover interference for the markers than in wild type. It is possible that the same mechanism underlies these various observations in *asb*: positive crossover interference may not extend beyond the boundaries of an SC segment, so that interruptions in the SC (as they are found in *asb*) will result in a decreased crossover interference.

However, two observations do fit nicely with this proposal, firstly the absence of late RNs in several short SC segments in *asb*. If short SC segments carried RNs, they were small and/or faint. One possible explanation is, that a minimum SC length is required for the assembly or maintenance of late RNs, or, alternatively, that non–crossover events are not capable of initiating extensive synapsis. Second, the relatively high number of RNs in long SC segments suggests that interference has decreased with the SC. If interference would act, in contrast to what we suggested before, beyond interruptions in SCs the virtual absence of late RNs on short SC fragments adjacent to longer SC segments would be explained, but not the increased frequency of late RNs in the longer SC segments.

In short, we cannot explain the number and distribution of late RNs in mutant *asb* on the basis of a simple model according to which crossover interference cannot extend beyond the boundaries of an SC segment. For the interpretation of the *asb* phenotype more information is required about the relation between late RNs, synapsis and crossover formation formation in this mutant.

The RNs in mutant *asb* displayed a few intriguing features. We observed twin nodules, and even aggregates of three and more early RNs in *asb* nuclei comparable to early pachytene in wild type. Similar appearances of such RNs can be seen in other reports on plant RNs (*e.g.* Albini and Jones 1987: Figures 10-12, Sherman *et al.* 1992: Figure 1, Stack and Roelofs 1996: Figure 10), but none of these papers gave a clear explanantion for that phenomenon. It is tempting to believe that twin and merging early RNs are intermediate structures that may give rise to late RNs, but more observations and experimental evidence will be necessary for find a relation between aberrant morphology and function of these groups of early RNs.

The desynaptic mutant as5

We observed RNs with normal morphology in all SCs of *as5*. We concluded therefore that this mutant has probably a defect in the maintenance of chiasmata, rather than in crossing–over. Maguire (1978) described a similar desynaptic mutant in maize.

6

Synapsis, recombination nodules, chiasmata and chromosome disjunction in meiosis of a haploid tomato

Francis W.J. Havekes, J. Hans de Jong, Christa Heyting

An ultrastructural analysis of meiotic prophase I in haploid tomato revealed low levels of synaptonemal complex (SC) formation, despite the lack of homologous chromosomes. All fourteen analysed pollen mother cells had axial cores, and ten of them contained tripartite SC segments. We found faint, spherical "clouds" associated with axial cores. These structures possibly represent morphologically abnormal early RNs. In addition, part of the SC segments carried one or two recombination nodules (RNs), which, on the basis of their morphology, were classified as late RNs. Light microscopic analysis of (pro)metaphase pollen mother cells revealed few examples of bivalents and trivalents, which indicates that chiasmata were occasionally formed. The number of tripartite SC segments per nucleus was more than ten times higher than the number of chiasmata per nucleus. Apparently, SC–formation in the haploid does not necessarily have to contain a crossover event (RN/chiasma) in the synapsed segments.

Chromosome transmission was established in pollen mother cells at stages from diakinesis to telophase II. In most cells at (pro)metaphase I only univalents were found, whereas only a minority of the haploid PMCs underwent both meiotic divisions. At the tetrad stage we observed both dyads (53%), triads (23%), tetrads (13%), monads (8%) and few polyads (3%). In the few embryo-sac mother cells, which we studied, we observed that equational division of all chromosomes was synchronised and took place only after all chromosomes had oriented in the equatorial plane, which had one or more spindles.

Introduction

The main processes of the first meiotic division are pairing and crossover between homologues at prophase I, and orientation and segregation of bivalents at metaphase and anaphase I. In a regular meiosis in a diploid organism, these processes enable the proper disjunction of homologous chromosomes. Our knowledge on the cytology and molecular genetics of the meiotic processes is largely based on the analysis of meiotic mutants. Numerous meiotic mutants have been described for yeast (Roeder *et al.* 1995), *Drosophila* (Orr-Weaver 1995, and references therein) and several other genetic model species. These mutants displayed defective chromosome synapsis (asynaptic mutants), lack of chiasma formation (desynaptic mutants), abnormal chromosome morphology and unbalanced transmission. A second source of information on meiotic processes is provided by numerical chromosome variants, including haploids and polyploids.

Haploids – plant geneticists prefer the term mono(ha)ploid – contain only one set of chromosomes in each undifferentiated somatic cell. At meiotic prophase I chromosomes lack their homologous partners for proper pairing and recombination. Haploids are therefore ideal for studying pairing and recombination between non–homologous chromosome segments and establishing the consequences of the lack of homology for orientation and segregation of chromosomes at metaphase I.

Haploids have been described for most crop species. They can easily be isolated and are in general viable. In spite of the absence of homologues, extensive pairing and synaptonemal complex (SC) formation was reported in haploids of several species (Gillies 1974, Wang 1988, de Jong *et al.* 1991, Loidl 1991). However, crossing–over is strongly reduced in haploids; chiasma frequencies were found to be low in haploid wheat (Wang 1988) and rye (de Jong *et al.* 1991), and exchanged chromosomes were rare in the products of haploid meiosis in yeast (Loidl *et al.* 1997).

Tomato (2n=2x=24) is most appropriate for the cytological studies of meiotic processes (Moens 1964, Cawood and Jones 1980, Stack and Anderson 1986a, 1986b). The chromosomes are well– differentiated at pachytene, and show a unique pattern of heterochromatin blocks in the pericentromeric and telomeric regions. All twelve chromosomes can thus be identified on the basis of arm lengths and diagnostic heterochromatic regions. In addition, the SCs of hypotonically burst pollen mother cells exhibit a comparable distinctive morphology, with clear kinetochores and an obvious difference in staining intensity between heterochromatin and euchromatin SC segments (Sherman and Stack 1992). Tomato also shows early and late recombination nodules (Sherman and Stack 1995).

In this paper, we present a light and electron microscopic study of meiosis in a haploid of tomato. Emphasis is put on the occurrence and morphology of non–homologous SC segments and the presence of early and late recombination nodules. We also estimate chiasma frequencies in diakinesis / metaphase I and compare chromosome behaviour between microsporocytes and megasporocytes.

Materials and Methods

Material

The haploid tomato plant analysed in this study was found among the seedlings from a cross between *Lycopersicon esculentum* genotype LA 291 and *L. esculentum* Moneymaker by Koornneef *et al.* (1989). LA 291 is homozygous recessive for the genes *a* (anthocyaninless), *hl* (hairless) and *ms–2* (male–sterile). The haploid seedling had the recessive phenotype and was therefore named LA 291–H (Koornneef *et al.* 1989).

Methods

We used for our electron microscopic analysis Stack's hypotonic bursting technique for meiotic prophase pollen mother cells (Stack 1982). One anther per bud was squashed in acetocarmine to assess the meiotic stage of the pollen mother cells in that flower bud (PMCs). Anthers containing PMCs in a stage comparable to pachytene (*i.e.* PMCs easily released from the pollen sac and mid–prophase chromosomes released from the synizetic knot) were used for the SC spreading technique. Preparations were stained either by silver nitrate at 40° C (Ag) or by uranyl acetate/lead citrate (UP). Silver nitrate staining at 40° C and 33% w/v (Sherman *et al.* 1992) was favourite for distinct axial cores and SC, and revealed part of the RNs. UP gave a weaker contrast of the axial cores and SCs than Ag, but it is a reliable stain for RNs. LM preparations of haploid tomato anthers and ovules were made as described in Havekes *et al.* (1997).

Results

The morphology of axial cores, synaptonemal complexes and recombination nodules were analysed in microsporocytes only. For the light microscopic characterisation of meiotic processes and the comparison of male and female meiosis we studied spread preparations of both microsporocytes and megasporocytes.

EM observations

In the SC preparations of prophase I nuclei, the morphology of SCs and RNs was less distinct than in diploid tomato, and the chromosome axes were often covered with faintly staining material. Most nuclei were incomplete and contained broken or interrupted axial cores or SC segments. We selected ten silverstained and four UP–stained nuclei at a stage comparable to pachytene.

Axial core formation

All nuclei formed axial cores. It was not possible to trace the full complement with the twelve ACs, as most of them were broken, interrupted or entangled. In seven of the Ag–stained nuclei we found

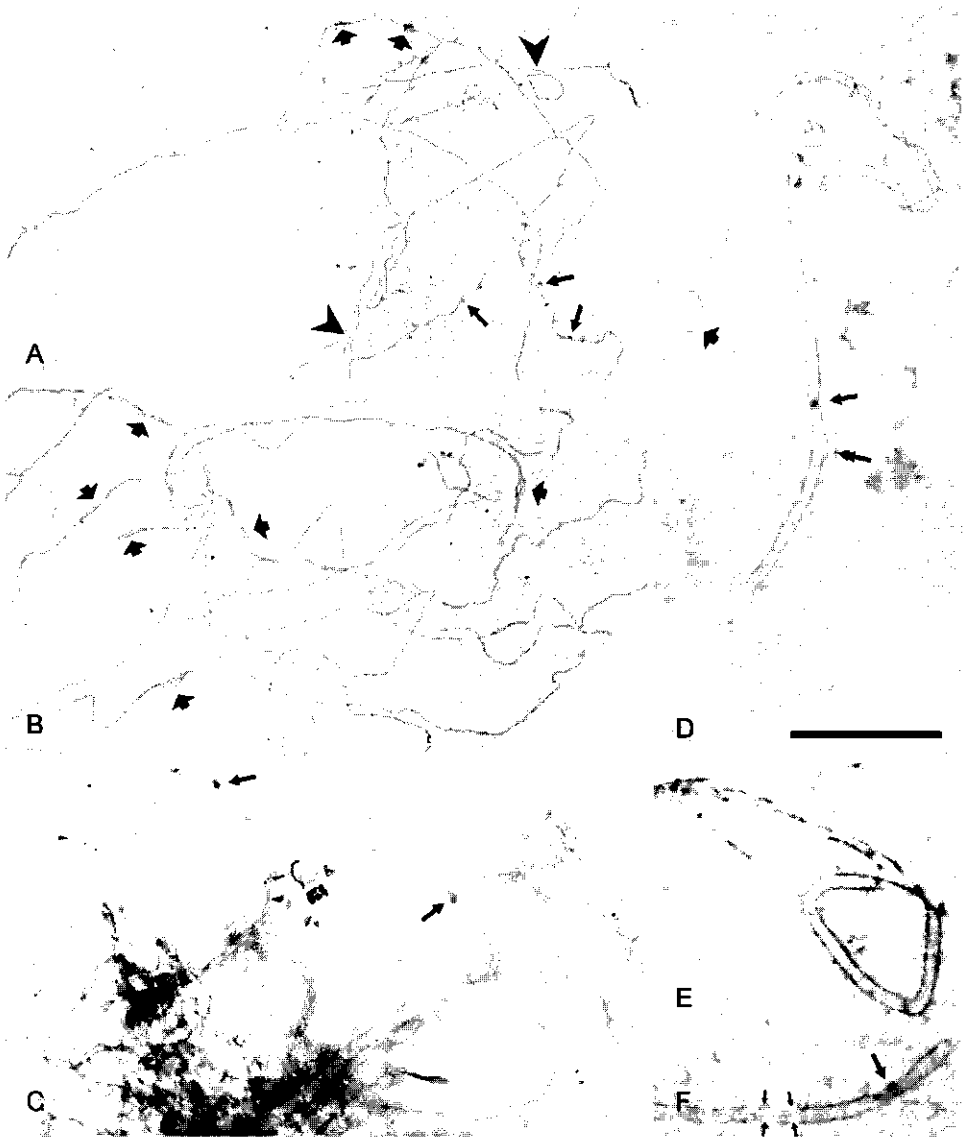


Figure 1: Electron micrographs of hypotonically burst PMCs of haploid tomato. **A.** Part of a Ag–stained nucleus. Arrowheads indicate parallel arrangement of ACs, large arrows indicate split or thickened AC segments, and small arrows indicate cloud–like structures along ACs. **B.** Part of an Ag–stained nucleus with extensive AC splitting (arrows) and some thickened AC segments. **C.** Part of a UP stained nucleus with synapsis and RNs (arrows). **D.** SC with two non–homologous chromosomes of different lengths: telomeres do not match at one end (arrow) and one core bulges slightly (small arrow). An RN is indicated by the small arrow. Ag–stained. **E.** Detail of loop with intrachromosomal non–homologous synapsis. UP stained. **F.** Detail of a foldback SC segment with a RN at a twist (large arrow). The lateral elements are unevenly stained, only thin threads are visible at the left side (small arrows). UP stained. Bar represents 5 μm.

short segments of thickened or longitudinally split ACs (Figure 1A+B), both on distal and intercalary positions. Within the split AC segments, we always observed two substructures, which remained aligned at less than 50 nm. The diameter of the individual substructures was similar to that of an intact AC. We also observed AC thickenings, which were on average shorter than the split AC segments (Table 1) and had a diameter of 100 to 200 nm. The thickenings were either solid or showed longitudinal striations (Figure 1B). None of the UP–stained nuclei revealed split AC segments or AC thickenings.

Alignment and synapsis of non–homologous core segments

Typical presynaptic alignment of ACs, which often precedes synapsis in diploid tomato (Stack and Anderson 1986a, Havekes *et al.* 1994), was not found in the haploid tomato, although AC regions sometimes ran parallel at distances of 50 to 250 nm (Figure 1A+B). This parallel arrangement of ACs in the haploid differed from presynaptic alignment in a normal diploid in the following respects: (i) the distance between the parallel ACs of the haploid was often less than the 300 nm that is usually seen between presynaptically aligned ACs (Havekes *et al.* 1994); (2) there were no clear early RNs between the parallel ACs in the haploid, whereas in diploid tomato, early RNs normally connect presynaptically aligned ACs (Stack and Anderson 1986a, Havekes *et al.* 1994).

Segments of synaptonemal complex were found in only ten of the fourteen nuclei (Table 1; Figure 1C). Their morphology, though essentially similar to that of diploid tomato, appeared more fragile in the haploid: the lateral elements appeared irregularly shaped and thin (Figure 1E,F). Non–homologous synapsis was less than 20% and measured 5–10 μm in most cases. We also observed intrachromosomal foldback synapsis (Figure 1F) and other aberrant configurations (Figure 1E). Some SC segments show buckles with a wide central region and with two layers of central element (Figure 1D). Of the synapsed segments (n=46), 18% occurred distally with matched telomeres and another 18% occurred as intrachromosomal foldback loops.

Table 1. Ultrastructural features of ten Ag–stained and four UP–stained prophase I nuclei in haploid tomato.

		split AC segments		AC thickenings		SC segments		late RNs
		nr./cell	length (μm)	nr./cell	length (μm)	nr./cell	length (μm)	nr./cell
Ag	average	2.8	0.88	1.4	0.58	2.9	5.18	0.4
	range	0–11	0.18–3.51	0–7	0.33–0.76	0–8	0.39–14.08	0–2
UP	average	–	–	–	–	4.25	5.11	1.5
	range					1-9	0.66–11.93	0–3
total	average	2.8	0.88	1.4	0.58	3.29	5.16	0.7
	range	0–11	0.18–3.51	0–7	0.33–0.76	0–9	0.39–14.08	0–3

Recombination nodules

In UP stained nuclei, we observed recombination nodules (Figure 1C) in six of the seventeen SC segments: five of these SC segments contained a single RN (see Figure 1D), and only one SC segment contained two RNs. The RNs were about 150 nm long and 100 nm wide, which is similar to the dimensions of late RNs that in normal diploid nuclei. In the silver stained nuclei, RNs were not as obvious as in the UP stained nuclei, and faint RNs showed up in only four of the twentynine SC segments. Late RNs occurred both in interchromosomal and intrachromosomal synapsed segments, mostly in the middle of these segments. The average number of SC segments per cell was 3.3 (Table 1) and there were 0.7 late RNs per cell. We also observed faint "spherical" clouds associated with unsynapsed ACs (Fig. 1A); possibly, they represent abnormal, early RNs.

LM observations

Male haploid meiosis

Pachytene pairing

The light microscopic preparations of spread pollen mother cells at pachytene revealed low levels of chromosome pairing, both between and within chromosomes (Figure 2), which is in agreement with the electron microscopic observations. heterochromatic regions could be detected in the centromere regions and at few chromosome ends (Figure 2B, C) as was observed in the diploid pachytene nuclei. Chromosome pairing occurred mostly between two heterochromatic or between two euchromatic regions, but we also found some examples of pairing between a heterochromatic and a euchromatic region.

Chiasma formation

We analysed chiasma formation in 200 pollen mother cells at (pro)metaphase I. These cells were characterised by randomly positioned univalents and few bivalents or trivalents, which were positioned in the equatorial plane. In 79% of the nuclei, we observed twelve small highly condensed univalents (Figure 3A). In 17.5% of the nuclei, one rod bivalent was found in the equatorial plane, together with ten scattered univalents (Figure 3B). Most bivalents were stretched, and in some bivalents the two non–homologous chromosomes were only connected by a thin, presumably, chiasmate connection. In the remaining 3.5% of the nuclei, we found a trivalent, two or three bivalents or combinations of bivalents and/or trivalents (Figure 3C). The average chiasma frequency was 0.255 per nucleus, which is significantly (0.01 level) lower than the 0.7 RNs per cell (see above, and Table 1).

In six of the 200 analysed (pro)metaphase I cells, we detected small fragments, which were either connected to one chromosome by a thin thread (Figure 3C) or were lying apart. Most fragments were less densely stained than the intact chromosomes. Fragments are likely the result of recombination in inverted chromosome regions or inverted loops.

Chromosome behaviour at later meiotic stages

In haploid meiosis, chromosome orientation and segregation is less synchronous than the diploid, where homologues disjoin simultaneously at anaphase I, and chromatids at anaphase II. In the majority of the haploid (pro)metaphase cells, we found twelve randomly distributed chromosomes. We also found some anaphase I / telophase I like configurations (Figure 3D), which probably originated from a pseudo–reductional division, in which univalents moved individually to one of the two poles.

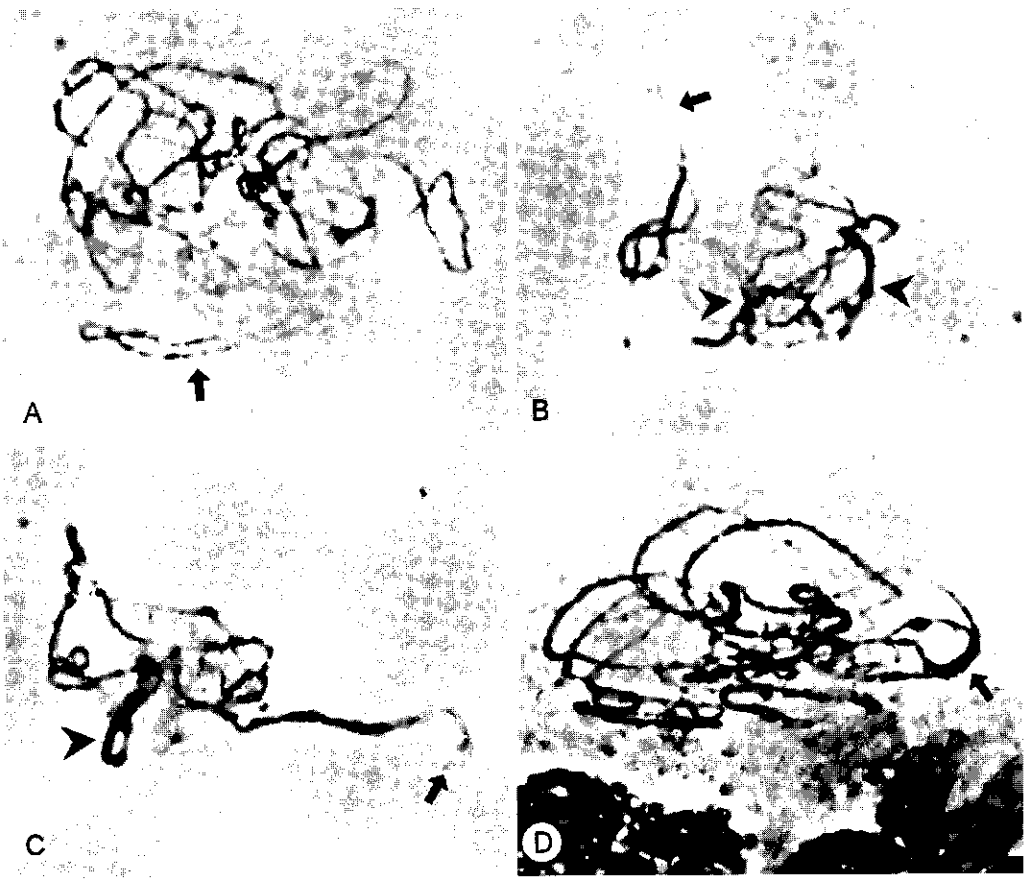


Figure 2. Light micrographs of spread 'pachytene' PMCs of haploid tomato. Dark regions represent heterochromatin, light regions are euchromatic. **A.** Nucleus without visible chromosome pairing. Intrachromosomal parallel association is indicated by the arrow. **B.** Nucleus with distal chromosome pairing with matched telomeres (arrow) and pairing between proximal heterochromatin (large arrows). **C.** Nucleus with extensive pairing between two non–homologous chromosomes with ends, which do not match (arrow). The large arrow indicate pairing between two or three heterochromatic regions. **D.** Nucleus with pairing in heterochromatic regions (arrows).

We assume that bivalents would divide reductionally in such divisions because bivalents were always oriented in the metaphase I plate (Figure 3B+C).

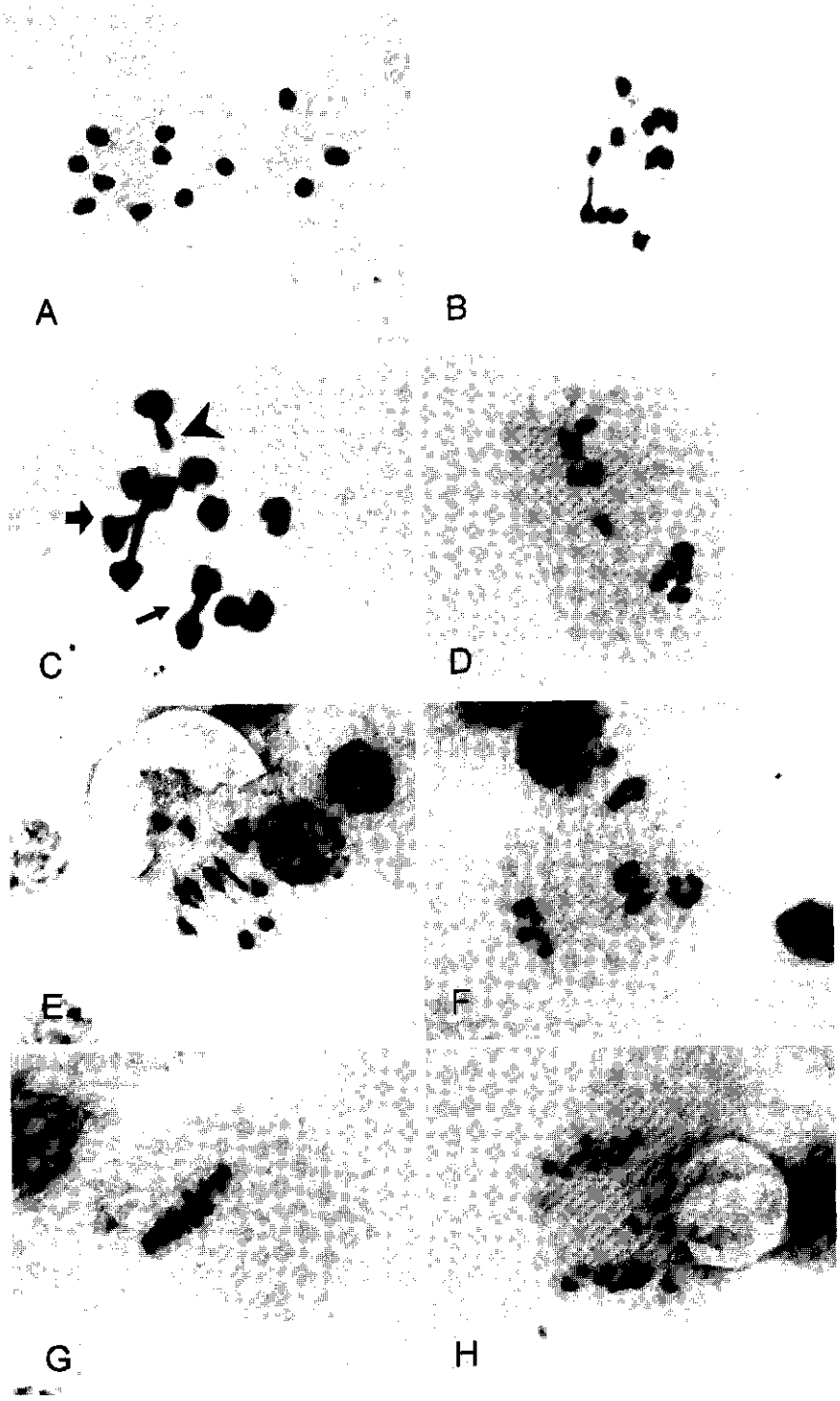
The asynchrony of chromosome movement in haploid meiosis I was obvious in several nuclei with lagging chromosomes. These laggards often oriented equationally while the other chromosomes moved to one of the poles (Figure 3E). Even though the laggards stretched considerably, the two sister chromatids did not separate precociously at this stage. Chromatids intermingled with chromosomes were only occasionally observed in telophase I. In nuclei undergoing equational division, all chromosomes divided synchronously. In those cells that had previously undergone pseudo–reductional chromosome segregation, two spindles were formed (Figure 3F). In many equational divisions, however, all twelve chromosomes were arranged in one spindle (Figure 3G) and divided together (Figure 3H).

Pollen fertility could not be determined, because the haploid tomato line contained the ms–2 gene, which causes complete male sterility (Koornneef *et al.* 1989). The tetrad stage was the last stage that we could study before *ms–2* induced degeneration. In the tetrad cells that we analysed we observed 53% dyads, 23% triads, 13% tetrads, 8% monads and 3% polyads. As to the dyads, the two microspores usually were of equal size, but we also found dyads with differently sized microspores. In the tetrads, the microspores were either similarly sized or they had one pair of bigger and one pair of smaller microspores. We also observed very small microspores, which indicates that even one or two individual chromosomes can become encapsulated into a spore. The variety in meiotic products suggests that many different combinations of reductional and equational division can occur in haploid meiosis.

Female haploid meiosis

The timing of equational division was determined in female haploid meiosis. Embryo sac mother cells (EMCs) in haploid tomato had roughly the same appearance as those described for the diploid tomato (Havekes *et al.* 1997). EMCs were easily identified in the spread preparations of female organs by their large and elongated shape. Because EMCs form a phragmoplast immediately after the first meiotic division, the two daughter cells of meiosis I easily separate during the squash procedure, and cannot be recovered together. Figure 4 shows examples of nuclei at different stages of female meiosis. Like in male meiosis, pachytene chromosomes were mostly unpaired (Figure 4A) but some paired

Figure 3. Light micrographs of spread PMCs from metaphase I to telophase II in haploid tomato. **A.** Twelve univalents spread over the nucleus. **B.** Nucleus with one centrally located bivalent and ten univalents. **C.** Nucleus with a bivalent (small arrow) and a trivalent (large arrow). A chromosomal fragment is associated to one of the univalents (arrowhead). **D.** Anaphase with pseudo-reductionally segregating univalents. **E.** Nucleus in which some univalents have moved to a pole while the rest is equationally oriented in the metaphase plate. **F.** Telophase II resulting from a pseudo-reductional division (7-5 distribution) followed by equational division at anaphase II. **G.** 12 equationally oriented univalents. **H.** Anaphase with twelve equationally divided chromosomes.


A
B
C
D
E
F
G
H

chromosome regions were also observed. A quantitative comparison of pairing in the male and female cells was impossible due to the indistinct morphology of the chromosomes in light microscopic preparations of female haploid meiosis.

In nine of the ten analysed diakinesis–metaphase I cells we found twelve univalents, which were randomly distributed through the cell (Figure 4B+C). In only one nucleus we detected a bivalent. By the time the phragmoplast was about to be formed, the chromosomes had either been distributed over the two poles (Figure 4D), or they were all located at one side of the nucleus (Figure 4E). Equational orientations and divisions were observed in the few metaphase II – anaphase II EMCs that we could find. Figure 4F shows an example of metaphase II orientation in a daughter cell that has captured all twelve chromosomes. Our observations indicate that equational division does not or rarely take place in female haploid meiocytes at metaphase I, but rather occurs at a stage past phragmoplast formation (*i.e.*, in meiosis II).

Discussion

During meiosis, the chromosomes in haploid tomato, although lacking a homologous partner, can nevertheless (1) be involved in synapsis, (2) have early and late RNs along the SC, and (3) form occasional chiasmata. However, synapsis and RNs were abnormal in various respects. In contrast to diploid tomato, haploid tomato did not display typical presynaptic alignment (see chapter 4). Furthermore, the axial cores and the lateral elements of the SCs showed morphological abnormalities like short stretches that were split into two substructures and local thickenings with a dense or striated structure, whereas SCs were often irregular without any difference between euchromatin and heterochromatic regions.

Presynaptic alignment

Although ACs showed some parallel arrangement in the haploid, they did not display the typical presynaptic alignment that normally occurs at leptotene / zygotene in the diploid tomato (Stack and Anderson 1986a) and that accumulates in some partially asynaptic mutants of tomato (Havekes *et al.* 1994, and the Chapters 2+4). In the haploid, parallel ACs were often closer than the 300 nm that is typical for presynaptic alignment, and in some instances even closer than the 100 nm that is specific for SCs. In contrast to the numerous early RNs between presynaptically aligned ACs in the normal diploid zygotene nuclei, we did not detect early RNs between the parallel arranged ACs in the haploid. We assume, therefore, that presynaptic alignment is confined to true homologous chromosome regions only.

Synapsis

We found low levels of synapsis (about 17 μm SC per cell) both between and within chromosomes in haploid tomato. Menzel and Price (1966) also observed occasional formation of non–homologous

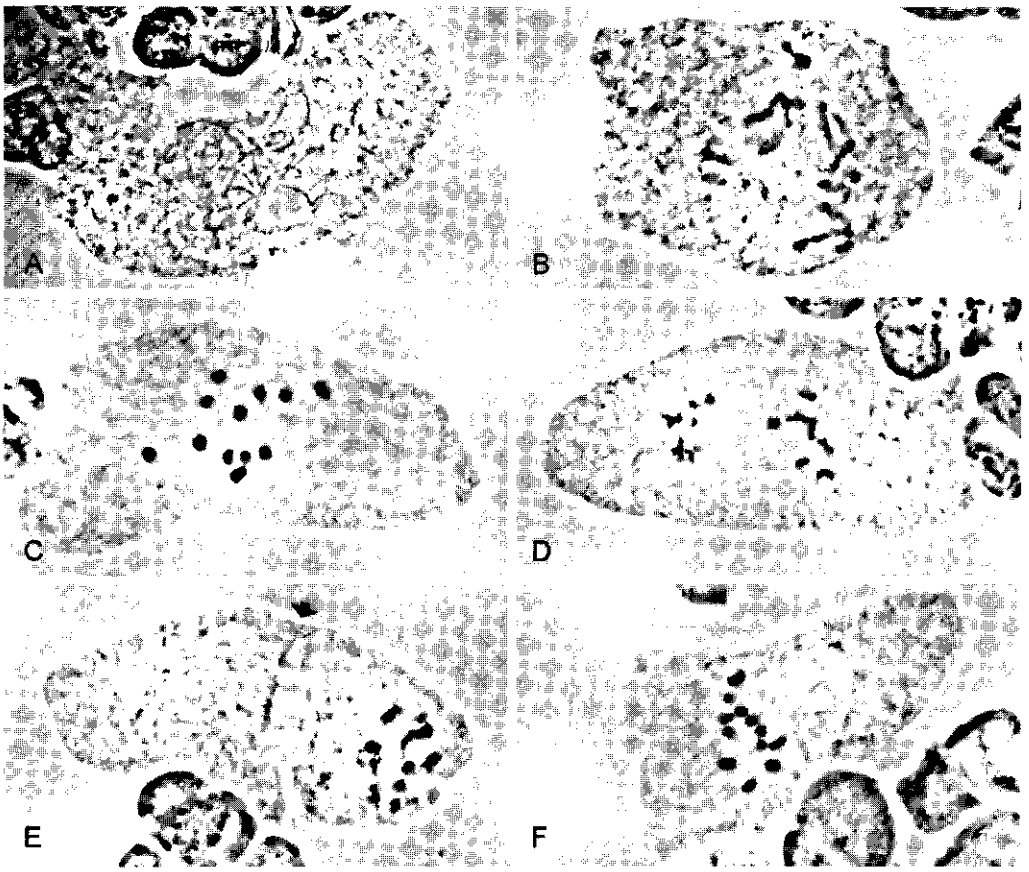


Figure 4: Light micrographs of squashed EMCs from haploid tomato. **A.** Pachytene nucleus with no visible chromosome pairing. **B.** Nucleus at diakinesis: twelve univalents. **C.** Metaphase I EMC with random distribution of univalents. **D.** Telophase I with a 7–5 distribution. **E.** Telophase I with all twelve univalents captured in one daughter nucleus. Arrow indicates phragmoplast. **F.** Equational orientation of all univalents in a daughter cell of an EMC at metaphase II.

SC in thin sections of haploid tomato. Synapsis has also been observed in haploids from several other plant species (barley: Gillies 1974, rye: de Jong *et al.* 1991, wheat: Wang 1988) and in yeast (Loidl *et al.* 1991).

The question is as to whether synaptic initiation in these haploids is related to recombinational activity at sites with regional ectopic homology or small duplicated segments, or whether synapsis occurs independently of the recombination pathway. Our data are in favour of the hypothesis that synaptic initiation is not necessarily related to crossover events. First, most of the SC segments did not contain RNs. Second, the average number of SC segments found in the fourteen prophase I nuclei was thirteen times higher than the average number of chiasmata found in our sample of 200

(pro)metaphase I nuclei. Comparable discrepancies have been found between synapsis and chiasma formation in haploid cereals (Gillies 1974, Wang 1988, de Jong *et al.* 1991), and between synapsis and chromosome exchange in haploid yeast (Loidl and Nairz 1997). That synapsis can extend across long non–homologous segments without crossovers is also documented by observations of Moses (1982) on synaptic adjustment of paracentric loops in mouse.

In diploid yeast the initiation of recombination takes place by the formation of double strand DNA breaks (DSBs). In haploid yeast, DSBs have similar distributions and occur at similar frequencies as in diploid meiosis, which indicated that initiation of recombination is not dependent on the presence of a homologue (de Massy *et al.* 1994, Gilbertson and Stahl 1994). Is synaptic initiation in haploids then related to gene conversion events? Generally, early RNs which are supposed to be involved in gene conversion (Carpenter 1987), were not abundant in SCs of haploid tomato. It is also questionable if gene conversion would occur relatively frequently between non–homologous chromosomal regions in haploids. The meiotic DSBs in haploid yeast are thought to be repaired by using the sister chromatid instead of the homologue as a template (Gilbertson and Stahl 1994). The timing of such repair is probably delayed compared to interhomologue recombination in diploid yeast (de Massy *et al.* 1994).

We postulate that in haploid tomato initiation of synapsis depends on incidental contacts between chromosome segments rather than on homology and recombination. Distal synapsis might then result from intimate associations of chromosome ends brought together by the clustering of telomeres in the bouquet at early prophase, whereas it is possible that interstitial and proximal synapsis result from association of heterochromatic regions in the synizetic knot. Although we have not found a bouquet in haploid tomato, the presence of telomere associations could be unequivocally demonstrated for haploid wheat (Wang 1988) and rye (de Jong *et al.* 1991). In light microscopic preparations, pairing was mainly found between two (proximal) heterochromatic regions or between two (distal) euchromatic regions, which could be the direct effect of the clustering of telomeres or heterochromatic centromere regions. Heterochromatic regions are known to be persistently connected when euchromatic regions are separate (Dernberg *et al.* 1996, Loidl 1987). In the haploid, the absence of primary homology might allow initiation of synapsis at chromosome regions held together by association of heterochromatic regions. This synapsis is probably delayed compared to synapsis in diploid tomato.

Recombination nodules

We found RNs in part of the synapsed regions in haploid tomato. To our knowledge this is the first report of RNs in a haploid plant. Only in one haploid nucleus of the fungus *Physarum polycephalum*, Lie and Laane (1982) show a possible example of a RN. Although the low frequency of RNs per SC segment and the ellipsoidal shape of RNs suggest that the RNs in the haploid are comparable to late RNs in diploid meiosis (cf. Stack and Anderson 1986a, 1986b, Sherman and Stack 1995), the average of 1.5 RNs per UP–stained nucleus is much higher than the average of 0.255 chiasma per metaphase I

cell. This difference may be explained by the fact that RNs in foldbacks will not result in chiasmate bivalents. Furthermore, the frequent occurrence of presumed early RNs with aberrant morphology ("clouds") along the ACs suggests that not all RNs in the haploid were entirely normal and functional. It is possible that some abnormal late RNs in the haploid are not stained with silver. That would explain the relatively low number of Ag–stained RNs in the haploid.

Meiotic divisions in haploid tomato

A normal reductional division, which depends on chiasmate connections between pairs of homologous chromosomes, could not take place in the haploid tomato. In the majority of cells, only univalents were present at (pro)metaphase. As a consequence, most nuclei went through only one division (53% of the meiotic products were dyads): an equational division of all twelve chromosomes in one spindle. Haploid nuclei seemed to be temporarily stalled at prometaphase I, and then continue with an equational division, skipping division I. The nuclei consisting of partly pseudo– reductionally separating chromosomes, and partly equationally orienting laggards, may very well have contributed to the 23% triads resulting from haploid meiosis. Laggards with equational orientation stretched considerably, more than is observed in a normal metaphase II, but seemed unable to separate the sister chromatids in spite of the apparently strong pulling forces. In female haploid meiosis, the equational division occurred after phragmoplast formation, i.e. at about the same time as the equational division (Meiosis II) in the diploid, and thus later than metaphase I would have occurred.

A minority of the male haploid nuclei underwent both meiotic divisions and are represented by the 13% of tetrads in the meiotic products. It is likely that these tetrads resulted from a pseudo–reductional, random segregation of chromosomes, followed by a synchronous separation of sister chromatids in an equational division.

Abnormalities of SCs in haploid tomato

The ACs in haploid tomato showed split and thickened segments, whereas these abnormalities have never been observed in wild type tomato (Havekes *et al.* 1994, Stack and Anderson 1986a). AC thickenings have been found in diploid maize (Gillies 1981), rye (Fedotova *et al.* 1989) and Tradescantia (Hasenkampf 1984). The shape and size of the thickenings is very similar in all reports, and they occur in cores involved in synapsis and in unpaired ACs. The origin of the thickenings is not known. Occasional examples of split AC segments were also observed in haploid rye (de Jong *et al.* 1991) and in haploid wheat (Wang 1988). The split segments in haploid rye resemble those found in the tomato haploid, i.e. they occur in unpaired ACs and the two substructures remain closely parallel. In contrast, the split segments in wheat are different because they occur in synapsed regions and the substructures, which are far apart, are uneven in length and thickness.

Although the split and thickened AC segments could be a characteristic of the haploid tomato line (the diploid line from which it was derived was not studied), it is also possible that these abnormalities reflect chromosome behaviour that under normal diploid conditions would not take place. ACs

assembly may be aberrant in the haploid and cause defective sister chromatid cohesion (splitting of AC segments into sister cores).

The occasional chromosomal fragments that we observed in metaphase I nuclei of the haploid tomato are probably caused by crossovers between two different inverted non–homologous chromosome segments or a crossover in an inversion loop. This phenomenon has also been reported in haploid maize (McClintock 1933).

7

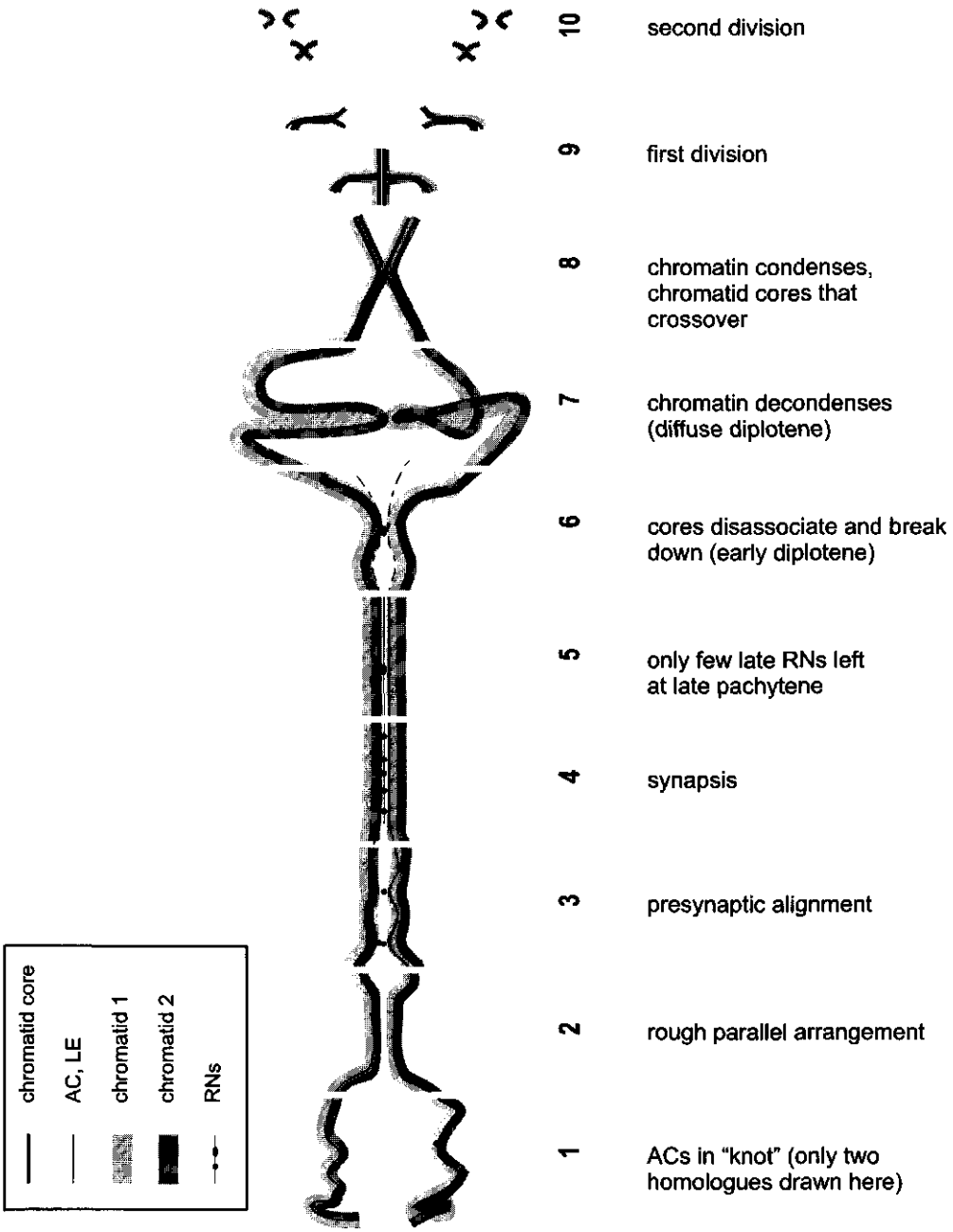
General discussion: A walk through meiosis

The previous chapters of this thesis provide the results of a research project performed by the author at the Laboratory of Genetics, Wageningen University and Research Centre. The text furnishes you, the reader, with a bulk of information on chromosome morphology and behaviour at different meiotic stages in four synaptic mutants, a haploid and wild type tomato. More facts and details are added to the already extensive knowledge on meiotic chromosomes. But does it all fit, is there a pattern, are we able to perceive the rationale of the meiotic processes?

In this final chapter, I will guide you through the different steps of meiosis, incorporating parts of my own research of tomato into known theories of meiotic pairing, recombination and segregation. For clarity, I will first give a rough description of all stages, a sort of general overview of meiosis. Meiotic chromosomes consist of chromatin (folded DNA plus associated histones and non–histones), which, at some stages, is organised in loops that are attached to a proteinaceous core (reviews in Rattner *et al.* 1981, Moens and Pearlman 1988). Based on the appearance of chromatin of higher eukaryotes in the light microscope, two general classes can be distinguished: 1) heterochromatin, permanently condensed chromatin and 2) euchromatin, chromatin that is only condensed during prophase–telophase and decondense during interphase. In most organisms, including tomato, heterochromatic regions is mainly located around the centromeres.

Prophase and metaphase chromosomes can also display different types of cores along their axes. One of them is the *chromosome scaffold* as seen in histone depleted metaphase chromosomes (Earnshaw and Laemmli, 1984). The second type is observed only in chromosomes that are involved in meiotic pairing. At leptotene, proteinaceous structures are assembled along the chromosome axes. These structures, which connect the sister chromatids, are called *axial cores* (ACs). Once the chromosomes start pairing, the cores become closely aligned at a distance of c. 100 nm and contribute to the formation of the *synaptonemal complex* (SC). Within this tripartite complex, the cores are called *lateral elements* (LE), separated by a third *central element* between them. A third type of cores, the *chromatid cores*, has been found in chromosomes at diplotene to anaphase II of some species (Stack 1991, Rufas *et al.* 1992) and during the mitotic division (Giménez–Abian *et al.* 1995).

Figure 1 illustrates the characteristics of chromatin and cores during meiosis. As I have mentioned in the General Introduction, meiosis can be divided in two phases. A first phase with ongoing inter-homologue processes culminating in the establishment of at least one crossover per bivalent,


chromatid core
AC, LE
chromatid 1
chromatid 2
RNs
10
second division
9
first division
8
chromatin condenses, chromatid cores that crossover
7
chromatin decondenses (diffuse diplotene)
6
cores disassociate and break down (early diplotene)
5
only few late RNs left at late pachytene
4
synapsis
3
presynaptic alignment
2
rough parallel arrangement
1
ACs in "knot" (only two homologues drawn here)

and a second phase in which the two meiotic divisions take place. At the transition of the two phases is the diffuse diplotene stage. I will use Figure 1 as a guide during my walk through meiosis.

The establishment of homologous contacts (Figure 1: interval 1–3)

In this section I will discuss the following electron microscopic observations in wild type, mutant and haploid tomato:

1. In early prophase I ACs are tightly held in a cluster that will not spread after hypotonic bursting of nuclei (Chapter 4).
2. This tight 'knot' loosens as homologous ACs undergo presynaptic alignment and synapsis (Chapter 4).
3. The spreading area, which is a rough measure for the compactness of the nuclear volume, increases from leptotene to pachytene in wild type tomato, but not in asynaptic mutants or haploid tomato (Table 1).

The AC clusters

Within the complex dense mass of long chromosomes typical of early prophase I, ACs keep a certain distance from one another (Chapter 2, Figure 1 and 2; Chapter 4, Figure 1 and 4). It is conceivable, though, that the peripheral parts of chromatin loops from different chromosomes, which emanate radially from the core (von Wettstein *et al.* 1984), may already have encountered. Von Wettstain and colleagues supposed that the chromatin of the two sister–chromatids is arranged in two separate, opposite domains along the axial core. Here, I assume that the resistance of ACs against spreading is caused by association of peripheral chromatin loops, possibly with the aid of certain proteins. Such an association can occur between regions of different chromosomes or between intrachromosomal regions in folded chromosomes. Interstitial and proximal (heterochromatin) regions are predominantly captured in the cluster, whereas distal regions often spread out from the dense chromatin mass into the periphery of the nucleus.

AC clustering disappears concurrently with the progression of presynaptic alignment, the first phase in chromosome pairing. Is the release of paired ACs from the chromatin mass induced at the bivalent level by changes in AC/chromatin organisation during pairing, or is it a regulated at the cell level, independent of pairing? The comparison of areas of spread prophase I nuclei in the mutants and wild type tomato (Table 1) revealed that in nuclei that cannot complete pairing, the tight association of cores (small surface spreading) is maintained. This indicates that the release of ACs from the chromatic mass is probably regulated at the bivalent level and that only completely synapsed

Figure 1. Characteristics of chromatin and cores during meiosis. **1–3.** Leptotene – zygotene; **4.** Pachytene; **5–7.** Diplotene; **8.** Diakinesis – Metaphase I; **9.** Anaphase I – Telophase I; **10.** Anaphase II – Telophase II.

chromosomes allow full spreading. However, it is not known yet, as to whether the chemical and physical conditions in our spreading technique change the *in vivo* interaction between the chromatin domains.

Towards homologous DNA contacts

How are chromosome associations directed from the rather non–specific associations postulated above towards homologous ones? Possibly, homologous chromosomes have similar folding patterns (see Chapter 4), which favour coalignment of a chromosome with its homologue over coalignment with others. Furthermore, chromosome movement should allow homologues to meet (Dawe *et al.* 1994). One way in which such movements are accomplished is the clustering of telomeres at very early prophase I (Bass *et al.* 1997). This phenomenon, known as bouquet stage, is directed by contractile elements in the cytoskeleton, which pull the telomeres together along their nuclear attachments sites (Sheldon *et al.* 1988). Once the meeting of chromosomes is established, homology should be sensed, and the homologous sites should become more stably associated. The first obvious manifestation of stable homologous connections is when ACs start to converge and align at approximately 300 nm. As the homologous AC regions come into closer contact, RNs and/or other bridging materials become visible between them (Albini and Jones 1987, Anderson and Stack 1988). Possibly, the proteins that form these structures are able to contract and pull the ACs towards each other (see review: Dawe 1998). Such movement of ACs towards one another changes their location from centrally in the chromatin to a lateral position (Westergaard and von Wettstein 1972). The sites of primary homologous contact and the associated RNs are now in the vicinity of the cores. The bulk of chromatin, however, has to be reorganised along the ACs, which may be accomplished by rotation of the two sister chromatids relative to the single AC (Westergaard and von Wettstein 1972). In maize, such a phase of chromatin reorganisation preceding chromosome synapsis has been observed, and sister chromatids

Table 1: Average spreading area of hypotonically burst nuclei of wild type, mutant and haploid tomato. Mutant as6 is completely asynaptic, *asb* and *as1* are partially asynaptic and *as5* is desynaptic. The number of nuclei is indicated between brackets.

material	average spreading area of nuclei		
	early zygotene	late zygotene	pachytene
haploid	298±111 µm		
as6	665±256 µm		
asb	658±272 µm	481±119 µm	
as1	651±205 µm	787±230 µm	
as5			1245±525 µm
wild type	570±131 µm	2004±484 µm	2139±284 µm

are temporarily visible during this procedure (Dawe *et al.* 1994). It is still unknown, if such a reorganisation of chromatin relative to the core has any affect on other processes. For example, it might induce the release of any remaining non–homologous associations from the reorganised region. Thus, a bivalent could gradually be released from the network as it pairs and the cluster would unravel co–ordinately with homologous pairing. Table 1 shows the spreading areas of meiotic prophase I nuclei of the synaptic mutants, the haploid and the wild type tomato. It demonstrates clear differences in spreading ability of the clustered ACs between the mutants, haploid and diploid tomato.

The aforementioned hypothesis of unravelling tight chromatin clusters, does not agree with the light microscopic observations of early prophase clustering of (proximal) heterochromatin, a phenomenon that is known as synizetic knot. In tomato, this knot is most discrete at the end of zygotene, which suggests that chromosomes cluster more tightly with ongoing synapsis (Cawood and Jones 1980). A direct comparison between light and electron microscopic images is difficult to make, because chromosomes preparations made according to a squash and cell spreading technique, in which the acetic acid treatment for cell fixation and spreading will have a completely different effect on chromatin conformation and interactions than the hypotonic bursting technique for EM preparations, which includes an alkaline formaldehyde fixation of air–dried chromatin.

Synapsis and crossover control (Figure 1: interval 4–5)

In wild type tomato, presynaptic alignment is probably rapidly followed by synapsis in all bivalents. However, in Chapter 4 we found evidence for bivalent specific potentials for synapsis in mutant *asb*, which indicates that requirements other than presynaptic alignment must be fulfilled before synapsis can take place.

Synapsis requires additional steps before it can ensue between cores that are already homologously associated. In this section I will discuss the relation between synaptic initiation and crossing–over, with special reference to the following pairs of observations:

1a) In tomato mutant *asb*, smaller chromosomes synapse poorly, whereas longer ones have less difficulty in doing so (Chapter 4).

1b) Chiasma frequencies are much higher in female than in male meiosis of mutant *asb* (Chapter 3).

2a) Chromosome 1 synapses easily in the long, but hardly in the short arm in mutant *asb* (Chapter 4).

2b) Single RNs are never found only in the short arm of long subacrocentric chromosomes of wild type tomato (Sherman and Stack 1995).

Ad 1a+b

In mutant *asb*, chiasma frequencies are 2.6–fold higher in female than in male meiosis (Chapter 3). The higher chiasma frequency in the female meiosis was due to participation of more chromosome pairs in crossing over, rather than to higher crossover frequencies within bivalents. Other studies comparing chiasma / recombination frequencies in male and female meiosis have revealed that chromosome length plays a role. In a study of synapsis in female meiocytes of maize, Mogensen (1977) established a relation between SC length and crossing over. Fogwill (1958) established a similar relation between metaphase chromosome length and crossing–over in *Fritillaria*. The fact that *asb* is the mutant with the largest difference in recombination between male and female meiosis corresponds well to the fact that synaptic initiation is strongly influenced by chromosome length in male meiosis of this mutant.

Ad 2a+b

Sherman and Stack (1995) analysed large numbers of RNs in spread pachytene nuclei of wild type tomato. They never observed single RNs in the short arms of the subacrocentric SCs 1, 2, 3, 4, and 6. Similarly, in the submetacentric SCs 7, 8, 9 and 10, fewer RNs occurred in short arms than would be predicted by their relative euchromatic lengths. Even in the metacentric SCs 5, 11 and 12, which have little or no difference in the euchromatic length of their two arms, again a single RN occurred more often in one (not necessarily the slightly longer) arm. Sherman and Stack concluded that crossing over may occur preferentially near the synaptic initiation sites, which then should always occur first in the long arms of the subacrocentric bivalents, usually occur first in the long arms of the submetcentric bivalents, and usually occur first in one specific arm of the metacentric bivalents. In the tomato mutants where synaptic initiation can be easily established, we noticed that such an order of synapses within bivalents may indeed exist (Chapter 4). Presynaptic alignment however, did not occur preferentially in long chromosomes or long chromosome arms.

These observations suggest both a relation between synaptic initiation and crossing–over, and an effect of a chromosome–dependent factor, *e.g.* a structural feature of chromosomes or chromosome arms. But at which stage exactly is the decision for a crossover or a non–crossover made? Among the following four possible sequences of events, I will try to point out the most likely one.

1. presynaptic alignment > synaptic initiation > SC completion > crossover decision
2. presynaptic alignment > synaptic initiation > crossover decision > SC completion
3. presynaptic alignment > crossover decision > synaptic initiation > SC completion
4. crossover decision > presynaptic alignment > synaptic initiation > SC completion

Possibility 1 holds the classical view that synapsis has to be completed before the crossing–over decision is made. It is a tempting model because RNs show interference during the mid–late pachytene stage when synapsis is completed (Stack and Anderson 1986b). In yeast, however, the recombination pathway is already far on its way before synapsis is complete (Padmore et al. 1991), and nodules may therefore reflect the maturation of events that have already been initiated earlier on.

However, this pathway still is the most favourable to explain the discrepancy between synaptic initiation and RN and chiasma localisation in *Allium fistulosum* (Albini and Jones 1987, 1988).

Both possibilities 2 and 3 are capable of explaining the obvious correspondence between synaptic initiation and RN or chiasma localisation (Zickler and Sage 1991, Maguire 1995), and may therefore be interesting to consider its validity in our material. Which of the two should be preferred? Sherman and Stack (1995) chose possibility 2, with presynaptic alignment > synaptic initiation > crossover decision > SC completion, whereas I am more inclined towards possibility 3 with presynaptic alignment > crossover decision > synaptic initiation > SC completion. In the tomato mutant *asb* it seems that a certain requirement has to be fulfilled before synapsis can be initiated between homologous ACs that are already in presynaptic alignment. Given the ease of the synaptic process in normal plants, where synapsis simply initiates at homologous regions in all chromosome pairs, mutant *asb* should also be able to do so in all chromosome pairs that are able to align, but this is not the case. If synapsis is reduced in this mutant because of limiting amounts of central region proteins, synapsis should be expected to occur according to length, but that does not occur either.

If crossing–over were the step required for synapsis to initiate between homologous chromosomes, the observed chromosomal differences in synaptic initiation might be explained. Some crossover models involve tension which may or may not be high (or low) enough to initiate crossing–over (reviews in Darlington 1940 and Kleckner 1996). Kleckner (1996) suggests that stress on the axial core–chromatin connection determines crossing over, that this stress is driven by compaction and acts at an early stage (prior to synapsis). If I make the third possibility my favourite, which means that the decision for the first crossover between homologues induces synaptic initiation, can this event be given a place in the process of unravelling of ACs that I postulated? Crossing–over may be necessary to drive synapsis into the condensed proximal heterochromatic region and thus allow a chromosome pair to release from the clustered ACs (synizetic knot).

The non–crossover recombination events in presynaptic alignment may have a comparable effect on releasing (distal) euchromatic regions, but will not be able to induce the release of ACs within the condensed heterochromatin, which then remain in the knot. If crossing over is necessary to initiate synapsis, it does so only in the diploid organism where chromosomes have their homologous counterparts. Chapter 6 on the synapsis and recombination in the haploid tomato made clear that synapsis can be initiated and extended without a visible proof of crossing over.

The obscure diplotene stage (Figure 1: interval 6–7)

Diplotene is a transition stage between early meiotic prophase I with ongoing homologous interactions that culminate in crossovers, and the diakinesis stage in which the crossovers become visible as chiasmata. In tomato and many other organisms, the diffuse diplotene marks a phase in which chromosomes decondense and probably reorganise in advance of the meiosis I division. At the same time the SC disassembles. The difference between pre– and post– diffuse diplotene is obvious when core

formation is taken as an example. During pre–diplotene chromosomes shed their lateral elements except for the sites with late RNs, which become converted into chiasmata. At post–diplotene chromatid–cores appear along the axes of all chromatids and these cores do cross–over at the sites of chiasmata (Stack 1991, Rufas *et al.* 1992).

The first (reductional) division of meiosis (Figure 1: interval 8–9)

In the first meiotic division, homologous chromosomes orient to opposite poles and separate reductionally. This is achieved only when the two homologues are connected by an exchange between non–sister chromatids (chiasma). Congression of bivalents to the metaphase I plate depends on tension created by the pulling forces of microtubules from opposing poles, counteracted by the chiasmate connections between the homologues. When such tension is lacking, as is the case with univalents, unbalanced segregation or even chromosome loss may arise at metaphase I – anaphase I. For this reason meiotic mutants and haploids, lacking crossovers or the mechanism to stabilise them, fail to go through meiosis I properly.

The most important requirements for reductional division are: (1) The exchanges between non–sister chromatids must be stabilised in order to be maintained until anaphase I; (2) Large parts of non–sister chromatids must be exchanged as the homologues divide at anaphase I; and (3) Sister centromeres must remain together when homologues separate.

These three requirements are fulfilled by the formation of a core along each chromatid, and the connection of sister–chromatid cores. At diakinesis / metaphase I such cores are observed in grasshoppers (Rufas *et al.* 1992) and in lily (Stack 1991). Sister cores are connected possibly because they are linked to catenated DNA. When these DNA regions are decatenated, the cores can separate. An important aspect is that the cores crossover, and are therefore (re)formed along crossed–over non–sister chromatids. Chiasmate bonds then depend on the cohesion of sister chromatids distal to such crossovers. As this cohesion is lost, homologues can separate, and the cores drag along the exchanged parts of chromatids. In the meantime, cohesion between sister chromatids should not be lost entirely, because this would result in the separation of chromatids at metaphase I. The centromeric regions seem to be protected from decatenation, because the sister chromatids remain associated in these regions, so that precocious segregation of sister chromatids is avoided. The different localisation of the Cor1 core protein, which is localised to centromere regions but largely disappears from arm regions at diakinesis (Dobson *et al.* 1994, Moens and Spyropoulos 1995), suggest that Cor1 may have a function in sister–centromere cohesion.

The second (equational) division of meiosis (Figure 1: interval 10)

During the second meiotic division, the sisterchromatids of each chromosome orient their centromeres towards opposite poles and disjoin at anaphase II. This division is quickly performed in a

strikingly orderly fashion. Tomato meiotic mutants and haploids have various numbers of univalent chromosomes at meiosis I. Irrespective of the causes for their formation, univalents in tomato always seem capable of orienting equationally. Actual separation of sister chromatids however, is observed only in cells which have all chromosomes synchronously oriented as in metaphase II (Chapter 6). In cells in which only part of the chromosomes obtain the equational orientation, despite severe stretching, sister chromatids will not let go (Chapter 6). Similar observations are reported in meiotic mutants of maize (Maguire 1990) and grasshopper (Giménez–Abián *et al.* 1997).

In the long chromosomes of lily, the connection between sister chromatids is at the sister centromeres while arms are widely separate from anaphase I on to metaphase II (Stack 1991). Giménez–Abián *et al.* (1997) determined that the ultimate link that keeps sister centromeres together until the metaphase II / anaphase II transition is a silver–positive strand connecting the two kinetochores. Immunocytological experiments in mouse have revealed that the Cor 1 core protein accumulates at sister centromeres during metaphase I and dissociates from these regions as soon as sister centromeres separate at anaphase II (Dobson *et al.* 1994; Moens and Spyropoulos 1995). The signal for dissociation of this bond between sister centromeres is probably given only when all chromosomes have oriented in the metaphase II plate.

8

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9

Summary

This thesis presents the results of a cytogenetic investigation of meiotic prophase I stages in tomato (*Lycopersicon esculentum*). The aim of this study was to analyse the relationships between chromosome pairing, and formation of recombination nudules and chiasmata. Tomato was chosen as study material for the following reasons: 1) the species features an extensive genetic map, a pachytene chromosome map and a recombination map based on the distribution of recombination nodules along late pachytene synaptonemal complexes. These three maps together allow a direct comparison between genetic data on the one hand and meiotic chromosome organisation and behaviour on the other; 2) both light and electron microscope observations of spread pachytene nuclei depict detailed chromosomal substructures including centromeres/kinetochores and heterochromatic blocks in the centromere regions, which enables identification of individual chromosomes; 3) a collection of four meiotic mutants, which are disturbed at different phases of chromosome pairing and crossing over (formation of RNs and chiasmata), and a haploid are the basis of this study on the relationship of chromosome pairing and crossing over.

Chapters 2 and 4 deal with various ultrastructural aspects of homologous chromosome pairing in wild type and mutant tomato. In wild type tomato, the first unmistakable ultrastructural manifestation of homologous chromosome associations was the alignment of homologous axial cores (ACs) at a distance of approximately 300 nm. Shortly later, the aligned ACs converged at several sites, often to centrally positioned recombination nodules (RNs). This phase of pairing initiation is known as presynaptic alignment. Subsequently, homologous ACs approached each other to within 100 nm distance were then incorporated into the tripartite structure of the synaptonemal complex (SC), which was then assembled along each pair of homologous chromosomes.

During the processes of pairing initiation and SC formation (letotene - late zygotene) numerous RNs appeared at and between the lateral elements of the SC. Most of these RNs disappeared during early pachytene, so that only one or a few RNs per SC persisted in late pachytene.

Light and electron microscopic preparations of spread pollen mother cells from the meiotic mutants displayed the following characteristics (Chapter 2): mutant *as6* was completely asynaptic, virtually without any presynaptic alignment or SC. Mutants *asb* and *as1* were partially asynaptic, with average values of 6.1% and 25 % synapsis, respectively. The desynaptic mutant *as5* had normal, wild type levels of synapsis and about the same number of late pachytene RNs as wildtype, but showed

strongly reduced chiasma frequencies at metaphase I. This suggests that this mutant is disturbed in crossing–over and/or the formation of functional chiasmata.

The three asynaptic mutants *asb*, *as6* and *as1* showed a good correlation between the number of bivalents connected by at least one tripartite SC segment at pachytene and the number of chiasmate bivalents at metaphase I (Chapter 2). In mutant *asb*, morphologically normal late RNs were only observed on SCs, and not on presynaptically aligned segments (Chapter 5). This indicates that in tomato functional late RNs and chiasmata are only formed in the context of the tripartite SC.

In mutant *asb*, long chromosomes participated more frequently in synapsis, and displayed a higher percentage of synapsis than short chromosomes. Presynaptic alignment, however, occurred equally frequently in all chromosome pairs, and the length of synapsed segments was proportional to chomosome length (Chapter 4). This indicates that synaptic initiation, and probably crossing-over, are under constraints that act differentially on chromosomes of different length.

Chapter 3 gives a comparative light microscopic analysis of male and female meiosis in wild type tomato and in the meiotic mutants *as6*, *asb* and *as5*. Only mutant *asb* showed significant differences between female and male meiosis. We found 2.6 fold more chiasmate bonds between chromosome arms in embryosac mother cells (EMCs) than in pollen mother cells (PMCs). In wild type tomato, the percentage of chromosome arms there was bound by a chiasma was only 7% higher in EMCs than in PMCs. We ascribe this sex effect on chiasmate bonds to the comparatively long duration of female prophase I, particularly of the substage when chromosomes synapse, rather than to higher cross–over frequencies in bivalents of EMCs.

We also analysed how meiosis proceeds in haploid tomato, where chromosomes do not have homologous counterparts (Chapter 6). In the absence of a homologue, synapsis still occurred, though at the low average frequency of 3.3 SC segments per cell. Presynaptic alignment, though, was absent. Furthermore, the SCs in haploid tomato showed various aberrations. The ACs were locally split at several sites, and we found spherical cloud-like structures along the ACs, which we interpreted as morphologically abnormal early RNs. In the haploid, we also found thirteenfold more SC–segments in prophase I than chiasmata in metaphase I. Apparently, SC–formation in the haploid is not necessarily accompanied by the formation of a chiasma.

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Samenvatting

Dit proefschrift geeft de resultaten weer van een cytogenetisch onderzoek van stadia van de meiotische profase I in de tomaat (*Lycopersicon esculentum*). Het doel van deze studie was om de relaties tussen chromosoomparing en de vorming van recombination nodules en chiasmata te analyseren. Wij kozen tomaat als studieobject om de volgende redenen: 1) de soort heeft een uitgebreide genetische kaart, een pachyteenchromosoomkaart, en een recombinatiekaart die gebaseerd is op de verspreiding van recombinatie nodules (RNs) langs synaptonemale complexen in laat–pachyteencellen. De drie kaarten tesamen staan een directe vergelijking toe tussen genetische data enerzijds, en de organisatie en het gedrag van meiotische chromosomen anderzijds; 2) zowel licht– als elektronenmicroscopische waarnemingen van gespreide pachyteenkernen laten gedetailleerde chromosomale substructuren zien, zoals centromeren/kinetochoren en heterochromatische blokken in de centromeergebieden, waarmee chromosoomgebieden kunnen worden geïdentificeerd; 3) een collectie van vier meiotische mutanten, die in verschillende fases van de chromosoomparing en crossing over (vorming van RNs en chiasmata) zijn gestoord, en een haploïd vormen de basis van deze studie naar de relatie tussen chromosoomparing en crossing over.

De hoofdstukken 2 en 4 gaan over diverse ultrastructurele aspecten van de homologe chromosoomparing in de wildtype en mutante tomaat. In de wildtype tomaat was de eerste onmiskenbare ultrastructurele manifestatie van homologe chromosoomassociaties de parallelle rangschikking van homologe axiale elementen (ACs) op een afstand van ongeveer 300 nm. Kort daarna naderen ACs elkaar op verschillende plaatsen, waar zich vaak vroege RNs tussen de ACs bevinden. Deze fase van paringsvoorbereiding staat bekend als presynaptic alignment. Vervolgens gaan de homologe ACs verder naar elkaar toe tot op een afstand van 100 nm, en worden vervolgens geïncorporeerd in de tripartiete structuur van het synaptonemale complex, die aldus langs elk paar homologe chromosomen werd aangelegd.

Gedurende de processen van paringsinitiatie en vorming van SCs (leptoteen en zygoteen) verschenen grote aantallen RNs in en bij de axiale elementen van het SC. De meeste ervan verdwijnen weer in de loop van laat–zygoteen - vroeg pachyteen tot er in het laat–pachyteen uiteindelijk nog maar een of enkele RNs per SC overblijven.

De analyse van licht- en elektronenmicroscopische preparaten van gespreide pollenmoedercellen van de meiotische mutanten leverde de volgende informatie op (Hoofdstuk 2): mutant *as6* was volle-

dig asynaptisch, wat betekent dat er bijna geen presynaptic alignment of SC–vorming plaatsvond. De mutanten *asb* en *as1* waren partieel asynaptisch met gemiddeld respectievelijk 6,1% en 25% synapsis. De desynaptische mutant *as5* toonde weliswaar een normale, wildtype synapsis en normale aantallen RNs in het laat–pachyteen, maar toonde sterk verminderde aantallen chiasmata in de metafase I. We concludeerden hieruit dat deze mutant gestoord was in crossing over en/of de vorming van functionele chiasmata.

De drie asynaptische mutanten *asb*, *as1* and *as6* lieten een goede correlatie zien tussen de aantallen bivalenten, die in het pachyteen met elkaar verbinden door ten minste een SC–segment, en de aantallen (chiasmatische) bivalenten per cel tijdens de metafase I. In mutant *asb* werden morfologisch normale late RNs slechts waargenomen op de SCs, en niet op segmenten die presynaptic alignment vertoonden (Hoofdstuk 5). Dit duidt er op dat functionele late RNs en chiasmata slechts worden gevormd in de context van een tripartiete SC.

In mutant *asb* vonden we dat lange chromosomen vaker dan korte chromosomen deelnamen aan synapsis, en tevens dat lange chromosomen een relatief hoger percentage synapsis lieten zien. Zo'n verschil vonden we echter niet in het geval van presynaptische alignement, waar alle chromosomen in gelijke mate aan deelnamen en waar de lengtes van de synaptische segmenten evenredig was met de chromosoomlengte (Hoofdstuk 4). Deze waarnemingen maken aannemelijk dat synaptische initiatie, en mogelijk crossing–over, onder controle staan van mechanismen die onderscheidend werken voor chromosomen van verschillende lengtes.

Hoofdstuk 3 geeft een vergelijkende lichtmicroscopische analyse van de mannelijke en vrouwelijke meiose voor zowel het wildtype als de meiotische mutanten *as6*, *asb* en *as5*. We vonden alleen voor mutant *asb* significante verschillen tussen de vrouwelijke en mannelijke meiose. Zo bleken er 2,6 keer zoveel chiasmatische bindingen tussen chromosoomarmen in embryozakmoedercellen (EMCs) op te treden als in pollenmoedercellen (PMCs). In de wildtype tomaat was het percentage chromosoomarmen dat door een chiasma was gebonden slechts 7% hoger in de EMCs dan in de PMCs. We zijn meer geneigd om dit effect op chiasmatische bindingen bij *asb* en de wildtype tomaat eerder toe te schrijven aan de relatief lange duur van de vrouwelijke meiotische profase I, met name van het stadium waarin chromosoomsynapsis optreedt, dan aan een hogere cross–over frequentie in bivalenten van EMCs.

Ten slotte analyseerden we hoe de meiose zich voltrok in de haploïde tomaat (Hoofdstuk 6). Ondanks de afwezigheid van een homologe paringspartner treedt er toch synapsis op, zij het met gemiddeld maar 3,3 SC segmenten per cel. Presynaptische alignement, zoals in detail beschreven voor de wildtype tomaat en de mutanten *asb* en *as5*, werd niet gevonden. Verder namen we in de SCs van de haploïde tomaat verschillende afwijkingen waar. Zo vonden we op diverse plaatsen gespleten ACs en zagen we ronde wolkerige structuren langs de ACs, die we interpreteerden als morfologisch abnormale vroege RNs. Tevens vonden we in de haploïd dertien keer zoveel SC segmenten in de profase I dan chiasmata in de metafase I. Kennelijk gaat SC-vorming in de haploïd niet altijd gepaard met de vorming van chiasmata.

Nawoord

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Curiculum vitae

Francisca Wilhelmina Jozefina Havekes werd op 6 maart 1965 geboren in de buurtschap Linde van het Achterhoekse dorp Vorden. Na het behalen van het VWO diploma aan het Stedelijk Lyceum te Zutphen begon ze in 1984 een studie Plantenveredeling aan de Landbouwuniversiteit te Wageningen. De docteraalfase omvatte afstudeervakken in de landbouwplantenteelt, plantenveredeling en erfelijkheidsleer (cytogenetica). De stageperiode benutte ze om praktijkervaring op te doen in de veredeling van zonnebloemen, waarvoor ze 6 maanden doorbracht aan het Northern Crop Science Laboratory te Fargo, North Dakota, USA. In 1990 studeerde ze af en werkte daarna tijdelijk voor Cebeco-Zaden in Elst (GLD) en nabij Bourges, Frankrijk. Haar interesse in erfelijkheidsleer, aangewakkerd tijdens het afstudeervak cytogenetica bij Dr. Hans de Jong, leidde haar terug naar Wageningen, waar ze eind 1991 als onderzoeker in opleiding in dienst trad bij de vakgroep Erfelijkheidsleer. De resultaten van het onderzoek dat ze in de daaropvolgende jaren uitvoerde zijn in dit proefschrift weergegeven.