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Perspective

Meiotic transverse filament proteins: essential for crossing over

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Abstract

Meiosis is a specialized set of two nuclear divisions, meiosis I and II, by which a diploid cell produces four haploid daughters. After premeiotic DNA replication, homologous chromosomes pair and recombine, and then disjoin at meiosis I. Subsequently, at meiosis II, the sister chromatids of each chromosome segregate. In nearly all eukaryotes, meiotic chromosome pairing culminates in the formation of a ladderlike supramolecular protein structure, the synaptonemal complex (SC) (Page and Hawley, 2004). The rungs of the ladder are known as transverse filaments (TFs). Genes encoding TF proteins have been identified in a limited number of organisms, and their function has been studied by mutational analysis. Although TF proteins show little amino acid sequence conservation, their structure and function are largely conserved. In all analyzed species, TF proteins are required for meiotic reciprocal recombination (crossing over).

Introduction

The sexual reproduction cycle of eukaryotes is characterized by the alternation of haploid and diploid phases. At fertilization, two haploid gametes fuse to form a diploid zygote, whereas at meiosis, a single diploid cell produces four haploid daughter cells. Meiosis consists of a specialized set of two nuclear divisions, meisosis I and II, which follow a single round of DNA replication. The transition from the diploid to the haploid phase occurs at meiosis I, when homologous chromosomes (homologs) disjoin. This event requires extensive preparations during the prophase of this division. In particular, homologs have to recognize each other and form pairs of stably connected homologs, called bivalents. In metaphase I, bivalents (rather than individual chromosomes) line up in the equatorial plane of the spindle, because the two connected homologs attach to microtubules from opposite poles of the spindle. At anaphase I the connection between homologs is lost, and the homologs move to opposite poles (Figure 1).

Recombinational interactions between homologs fulfill essential roles in meiosis

In most analyzed species (but not in *Drosophila* and *Caenorhabditis*) the recognition and initial pairing of homologs requires homologous recombinational interactions. In addition, the stable connection of homologs until anaphase I requires reciprocal recombination between homologs (crossing over) in nearly all analyzed eukaryotes, including *Caenorhabditis* and female *Drosophila*. Crossing over between non-sister chromatids of homologs ultimately results in cytologically visible chiasmata, which, together with cohesion between sister chromatids, ensure the stable connection of homologs during metaphase I (Figure 1c). In anaphase I, sister chromatid cohesion in the chromosome arms is lost, so that the homologs

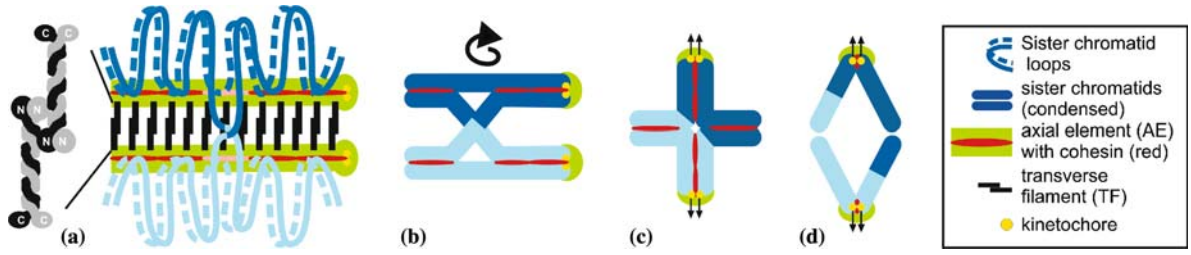

Figure 1. A pair of homologous chromosomes (bivalent) during successive steps of the first meiotic division. a. After homologous chromosomes have recognized each other, they are closely apposed by a synaptonemal complex (SC), which consists of two lateral elements (LEs) (one along each chromosome), connected by numerous transverse filaments (TFs). A detail of TF protein molecules within a TF is shown to the left. Reciprocal exchange between two non-sister chromatids (crossing over) has already occurred in the stage shown here, but this is cytologically not yet visible. b. In a later stage, the SC structure is disassembled, and the crossover becomes cytologically detectable as a chiasma. The sister chromatids of each homolog are kept together by a protein complex called cohesin (red), and the homologs remain connected to each other by the chiasma and cohesion between the sister chromatids. c. At metaphase I, the sister kinetochores of each chromosome capture microtubules from the same pole of the spindle (small black arrows); the kinetochores of the homologous chromosome capture microtubules from the opposite pole. The bivalent now lines up in the equatorial plane of the spindle. d. At anaphaseI, the cohesion between the sister chromatid arms is lost, and the homologs disjoin.

can disjoin (Figure 1d). Thus, meiotic crossing over is of dual importance: it ensures the proper disjunction of homologs at meiosis I, and it contributes to the genetic diversity of the products of meiosis, the (precursors of) gametes. The mechanisms that control the number and distribution of crossovers along meiotic chromosomes are therefore of fundamental importance for eukaryotic genetics.

In nearly all analyzed species, homolog recognition and crossing over are accompanied by the assembly of a synaptonemal complex (SC) between the homologs (Fig. 1a). The two sister chromatids of each chromosome first develop a common proteinaceous axis called axial element (AE). Then, following homolog recognition and alignment, the AEs of homologs are closely apposed by numerous transverse filaments (TFs), a process called synapsis. The SC is the ladderlike structure formed by the two homologous AEs (named lateral elements (LEs) in the context of the SC) and the TFs.

Transverse filament proteins are essential for crossing over

Whereas the ultrastructural appearance of the SC is highly conserved, many of its components, including TF proteins, are ill-conserved at the amino acid sequence level. TF proteins, have therefore been identified independently in only a limited number of species, namely mammalian species (SYCP1) (Meuwissen et al., 1992), budding yeast (Zip1) (Sym et al., 1993), *Drosophila* (C(3)G) (Page and Hawley, 2001) and *Caenorhabditis* (Syp-1 and Syp-2) (Colaiácovo et al., 2003; MacQueen et al., 2002). Despite their lack of amino acid sequence similarity, SYCP1, Zip1 and C(3)G have a similar structure: they are long coiled coil proteins with globular domains at both ends. Within SCs, they form parallel coiled-coil homodimers, which are embedded with their C-termini in the AEs, whereas the N-termini of TF protein molecules from opposite AEs overlap in the narrow region (called central region) between the AEs of the two homologs (Dong and Roeder, 2000; Yuan et al., 1996; Liu et al., 1996; Schmekel et al., 1996; Sym and Roeder, 1995) (Fig. 1a). *Caenorhabditis* Syp-1 and Syp-2 are two short coiled coil proteins, which possibly take the place of a single longer coiled coil protein in other species (Colaiácovo et al., 2003; MacQueen et al., 2002).

TF- deficient mutants have now been generated and characterized in yeast, *Drosophila* and *Caenorhabditis* and, most recently, in the mouse (de Vries et al., 2005). These mutants still initiate meiotic recombination (by induction of DNA double-strand breaks) (de Vries et al., 2005; Colaiácovo et al., 2003; Jang et al., 2003; Storlazzi et al., 1996), and align homologous chromosomes (de Vries et al., 2005; reviewed by Page and Hawley, 2003). However, in all four analyzed species crossover formation is affected in TF-deficient mutants (reviewed by Page and

Hawley, 2004 and Zickler and Kleckner, 1999). In *Caenorhabditis* and *Drosophila* meiotic crossing over is completely abolished (Colaiácovo et al., 2003; MacQueen et al., 2002; Page and Hawley, 2001), and in mouse almost completely (de Vries et al., 2005). Yeast *zip1* null mutants on the other hand, still display about 30% of the wildtype level of crossing over (Sym et al., 1993).

TF-dependent crossovers display interference

In nearly all analyzed species, meiotic crossovers display interference: the presence of a crossover at a given chromosomal position reduces the probability of another crossover nearby. Because the crossovers that still occur in yeast *zip1* null mutants do not display interference, Zip1 is specifically required for a class of crossovers that are sensitive to/can exert interference. In worms, flies and mice, virtually all meiotic crossovers belong to this category, whereas in yeast, two types of meiotic crossovers have been postulated (de los Santos et al., 2003): non-interfering crossovers, which do not depend on TF proteins or SCs, and interfering crossovers, which depend on TF proteins.

What do TF proteins do?

Given their essential role in meiotic crossing over, it is important to find out what TF proteins do. How do TF proteins contribute to crossover formation, and what causes interference between the crossovers that depend on TF proteins?

Because TF proteins are ill-conserved, it seems more likely that they fulfill a structural role than that they participate directly in recombination. On the other hand, TF-proteins do not require intact SCs for contributing to crossover formation (Storlazzi et al., 1996). Perhaps only a few TF protein molecules (rather than an intact SC) are sufficient to closely appose homologs locally, and stabilize a recombination intermediate in such a way that it is resolved as a crossover; if so, it remains to be sorted out how they do this, however.

Even more enigmatic is the phenomenon of crossover interference between TF-dependent crossovers. In some species, e.g. *Caenorhabditis*, interference may cover an entire chromosome, so that only a single crossover can occur per chromosome (Hillers, 2004). How does interference spread along the chromosome, and are TF proteins required for this? In *Caenorhabditis*, interference extends along chromosomal regions that can assemble a continuous SC structure (Hillers and Villeneuve, 2003). That might suggest that intact SCs, and thus TF proteins, are required for interference in *Ceanorhabditis*, but it cannot be ruled out that interference spreads along the axial elements, provided that these are capable of assembling a continuous SC structure.

Unfortunately, the role of TF-proteins in interference (if any) cannot be analyzed by measuring crossover interference in TF-deficient mutants, because interfering crossovers are abolished if TF proteins are knocked out. However, Fung et al. (2004), following an immunocytological approach, analyzed the positions of protein complexes that mark precursors of crossovers in wildtype yeast. *zip1* mutants also assemble such protein complexes, and Fung et al. found interference between these complexes both in wildtype yeast and in *zip1* mutants. Apparently, interference can occur in the absence of TFs, and therefore also in the absence of intact SCs. That might suggest that in yeast interference spreads along the axial elements or the chromosomes. However, this does not yet rule out a role of TF proteins or intact SCs in interference, because there may be more than one interference mechanism (Fung et al., 2004), one that spreads along the axial elements or chromosomes, and one that requires TFs or SC.

The recently described *Sycp1* knockout mouse (de Vries et al., 2005) provides new opportunities for analyzing the role of TF proteins in crossover interference, because the cytology of mouse meiosis is very well developed, and various recombination-related proteins have been identified. The positions along the chromosomes of protein complexes that mark recombination intermediates can therefore be analyzed in great detail in the mouse, so that the strength of interference between such protein complexes can be assessed. It will be of interest to find out whether interference between recombination-related protein complexes is equally strong in wildtype and *Sycp1*-deficient mice. It should also be possible to determine the strength of interference between recombination-related complexes during successive stages of mouse meiosis, to find out whether interference is imposed in more than one step.

The mechanisms that control the number and distribution of crossovers along meiotic chromsomes are not only important for eukaryotic genetics, but have also major implications for animal and plant breeding, and for our insight in human inborn and hereditary diseases that result from defects in meiotic recombination and chromosome disjunction. We are therefore eagerly looking forward to more detailed studies of the role(s) of TF proteins in the formation and control of meiotic crossovers.

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