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**SEGONA EDICIÓ DE SABADELL UNIVERSITAT
ESTIU DE 2003**

**SIMPOSI SOBRE INFERTILITAT
MASCULINA: GENÈTICA I AMBIENT**

BIOLOGIA MOLECULAR DE LA MEIOSIS

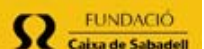
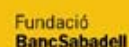
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Sabadell, setembre de 2003

Organitzadors:



Patrocinadors:



Meiosis creates haploid daughter cells from a diploid parental cell. While diploid cells carry two sets of chromosomes, these haploid cells differentiate into gametes that contain just one full set. The faithful separation of the two sets of homologs at the first division of meiosis is critical because each resulting gamete must carry only one copy of each chromosome.

Therefore the most important, and most intricate, phase of meiosis is the events leading up to and including the first nuclear division, collectively known as meiosis I. The events of meiosis I can be summarized in what I like to call the “central dogma of meiosis”: Pairing to exchange to segregation. At the chromosomal level, the mechanics of meiosis are generally conserved. Chromosomes are paired, held together by a variety of mechanisms and then separated from each other.

Initially, the two sets of chromosomes pair up such that each chromosome aligns with its corresponding partner, or homolog, during the initial stages of meiosis. Each pair of chromosomes is then held together by means that are varied between species. Two of the most common mechanisms are the building of a protein lattice between the homologs known as the synaptonemal complex, or SC, and the production of exchanges, or crossovers, between the two chromosomes through homologous recombination.

The pairs of chromosomes are ultimately pulled to opposite poles of the meiosis I spindle. This is the cellular basis for Mendel's laws of segregation and independent assortment and is the division responsible for reducing the chromosome number from diploid to haploid. Meiosis II is essentially a haploid mitosis in which sister chromatids separate from each other.

Defects in meiosis are a considerable concern in the field of reproductive biology. aneuploidy, or an abnormal chromosome number, is a factor in ~35% of spontaneous pregnancy losses, and is the most common recognized cause of mental retardation. The frequency of aneuploidy among oocytes can reach 20 to 25%, and increases among aging women. In contrast, chromosome abnormalities are detected in only 3-4% of sperm. This low rate of aneuploidy in male meiosis may result from more stringent surveillance mechanisms during spermatogenesis that direct aneuploid spermatogonial cells to undergo apoptosis. It has been proposed that errors in meiosis could result in infertility and the production of aneuploid sperm. In support of this, several studies have demonstrated an increased frequency of aneuploid sperm from men with low sperm concentrations.

What I am mainly interested in are the molecular mechanisms that underlie meiosis. Most of what is known about meiosis on the molecular level has been learned from studies in model organisms. This work has been done in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the

nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the mouse. While my own work on meiosis is predominantly uses *Drosophila* as a model system, today I will also mention studies done by other researchers that have broadened our knowledge of the molecular biology of meiosis. While the time is not available for a comprehensive review, I intend to provide a look into a number of the key molecular processes that occur during meiosis.

Pairing

Homologous chromosomes are brought together by a number of chromosome pairing and alignment mechanisms, which can generally be divided into mechanisms that require DNA double strand break (DSB) formation and those that are DSB-independent. The DSB-dependent class of mechanisms likely involves a homology search that is directly based on DNA sequence. In *S. cerevisiae*, for example, DSB formation and early recombination intermediates are required to establish a stable pairing interaction between homologs. In contrast, DSB-independent processes might include mechanisms such as the maintenance of premeiotic pairings or the pairing of homologs based on the aggregation of sequence-specific DNA binding proteins. Both of these mechanisms can, and usually do, lead to synapsis, the stage at which the SC connects the two homologs that comprise the mature bivalent along their lengths.

The Synaptonemal Complex (SC)

An almost universal feature of meiosis is the building of a structure called the synaptonemal complex, or SC, between homologous chromosomes during prophase. The SC has been described in a large number of species of plants, animals, and fungi using electron microscopy of meiotic chromosomes.

The SC is composed of two lateral elements (LEs) that are derived from the axial cores, or axial elements (AEs), of meiotic chromosomes. The mature LEs lie adjacent to chromatin along the length of each chromosome and are connected to each other by transverse filaments. A central element is often observed midway between the lateral elements, running down the central portion of the transverse filaments.

In at least some organisms the SC serves to hold homologs together during the processes of chromosome compaction and condensation that occur during the transition from leptotene to zygotene. Components of the transverse filaments and/or central element may well play an important role in promoting interhomolog exchanges. The ablation of SC proteins leads to a reduction or elimination of meiotic exchange in most organisms.

The cohesin complex, which mediates cohesion between sister chromatids, plays a major role in the assembly of the LEs. The cohesin complexes present on mammalian meiotic chromosomes are usually composed of the SMC proteins SMC1 and SMC3, along with meiosis-specific proteins called REC8 and STAG3.

Work from Christa Heyting's lab and others has shown that REC8 is initially present on short axial structures in the absence of other cohesin components. Other cohesin complex proteins appear to associate with these REC8-containing AE fragments simultaneously during leptotene. Cohesin remains associated with these AE fibers as they coalesce to run along the entire length of the chromosomes at pachytene, suggesting that the cohesins form part of the LEs of the SC. Similar results have been observed for *Drosophila*, *S. cerevisiae*, and *S. pombe*, suggesting that cohesin complexes play similar roles in the SC of many species.

Proteins that form the transverse filaments, which stretch between lateral elements, have now been identified in several species. These include Zip1p in *S. cerevisiae*, SCP1 in mammalian species, C(3)G in *Drosophila melanogaster*, and SYP-1 in *C. elegans*. These proteins play similar roles in the construction of the SC, and although their primary amino acid sequences differ greatly, certain structural characteristics are shared among these proteins. The most notable

characteristic in common is the presence of an extended coiled-coil rich segment located in the center of the protein, flanked by largely globular domains.

This conserved structure helped lead to the identification of the *c(3)G* gene in *Drosophila*. The *c(3)G* mutant was one of the first meiotic mutations discovered, dating from the early 20th century. The mutant phenotype included the elimination of the SC, as well as the loss of all meiotic exchange, suggesting that in *Drosophila*, at least, exchange is dependent on the SC.

The *c(3)G* gene was the focus of a mapping project by Paul Szauter, who mapped the mutant to within 15 kilobases and identified a candidate gene. This candidate was of interest because the predicted protein possessed a central coiled-coil domain flanked by globular domains, similar to the previously cloned Zip1 and SCP1 proteins. We were able to demonstrate that the candidate gene is *c(3)G* in two ways. First, I identified mutations in the two extant alleles of *c(3)G*. One is a point mutation, and the other is a transposon insertion, and both introduce stop codons near the beginning of the open reading frame. Second, two genomic rescue constructs were introduced into *Drosophila* by P-element mediated transformation. The first contains a wild-type copy of the candidate gene, and the second bears a deletion within the putative *c(3)G* gene. I was then able to show that the wild-type construct fully rescues the *c(3)G* mutant phenotype, whereas the deleted construct only partially rescues, indicating that the candidate gene is *c(3)G*.

Using an antibody generated against C(3)G, we then showed that C(3)G localizes to the *Drosophila* SC. In the *Drosophila* ovary, meiotic prophase begins in a structure called the germarium. Normally, a small number of germline cells enter meiosis and build SC. C(3)G protein localization matched this pattern in wild type, and also matched the expected pattern in the egalitarian mutant, in which all germline cells transiently enter meiosis and form SC.

Under higher magnification, C(3)G can be seen to localize along thread-like structures adjacent to chromatin. This can be observed using either anti-C(3)G or a C(3)G-GFP fusion protein. The thread-like signals appear between paired chromosomes, as shown by DAPI staining of DNA and by in situ hybridization to a locus on the X chromosome. Furthermore, I was able to trace the linear C(3)G signals and determine that they form five major threads, which most likely correspond to the five major chromosome arms in *Drosophila*. These lines of evidence all support the idea that C(3)G is present within the SC, and could form the transverse filament of the SC.

Immunolocalization of SCP1 and Zip1p by electron microscopy has elucidated the organization of these proteins within the SC. These results support a model for transverse filaments in which the proteins form parallel dimers through the coiled-coil regions and then align between the chromosomes with the

C-termini along the lateral elements, and the N-termini from opposing dimers interacting in an antiparallel fashion down the center of the SC.

In collaboration with Lorinda Anderson, we have localized the C(3)G protein on wild-type *Drosophila* SC by immunoelectron microscopy. Using monoclonal antibodies specific to the C terminus of the C(3)G protein, we observe immunogold staining along transverse filaments near the lateral elements. This suggests that C(3)G is likely to be part of the transverse filament of the SC, and fits the model established by Zip1 and SCP1 in which the C-termini of the transverse filament protein lies along the lateral elements. We are continuing this work with antibodies to the N terminus of C(3)G, which we expect to localize along the central element of the SC.

Exchange

The pathway for meiotic recombination has largely been defined through genetic and biochemical studies in yeast, and several of the proteins involved in recombination have been identified.

In a large number of organisms the initiation of synapsis requires the creation of double strand breaks (DSBs) in chromosomal DNA by the topoisomerase-like protein Spo11. DSBs are essential for synapsis in *S.*

cerevisiae, and similar observations have been made in *Arabidopsis* and in mammalian spermatocytes. That there is indeed a requirement for DSB formation to execute synapsis is suggested by the observation in both *Coprinus* and in mice that the synapsis defect observed in the absence of a functional Spo11 protein can be rescued by experimentally induced DSBs.

Several other proteins, including Rad50, Mre11, and Xrs2, are also required for forming DSBs and for the subsequent processing of DSBs into substrates for homologous recombination. After DSB formation, the broken DNA ends are resected to expose single stranded 3' tails that are used for homology recognition and invasion of a homologous DNA molecule. In meiosis, the homologous chromosome is the preferred partner for homologous recombination. Joint molecules are formed between the DNA duplexes, allowing new DNA synthesis to repair gaps. Finally the Holliday junctions thus formed must then be resolved into separate double helices. They may be resolved in one of two ways to produce either a crossover or noncrossover, either of which may be associated with gene conversion.

What are the consequences if some part of the meiotic recombination process goes wrong? The failure to repair DNA breaks is an obvious problem for a cell, while the lack of exchanges can result in chromosome nondisjunction and aneuploidy, which is also deleterious. Several studies using mouse mutants for

meiotic recombination proteins have shown that spermatogenesis is quite sensitive to disruptions in meiotic recombination.

Spermatogenesis arrests in early prophase when any one of a number of genes involved in meiotic recombination are mutated in the mouse. These include mutations in genes necessary for early events in meiotic recombination, such as Spo11, and Dmc1, and late events, such as Msh4 and Msh5.

Similarly, Christer Höög's lab has shown that disruption of the SC protein SCP3 in the mouse leads to prophase arrest during spermatogenesis. In fact, among these mouse studies, the most common consequence of the genetic disruption of meiosis in males is a shutdown of spermatogenesis. In some instances a small number of sperm are produced.

Although the basic events of meiotic recombination are basically the same in male and female mice, the effects of these mutants on gametogenesis can be quite different between the sexes. In contrast to spermatogenesis, oogenesis in the presence of these mutants can proceed past meiotic prophase and produce developing follicles, and for some, lead to normal fertility in females, although the frequency of aneuploid oocytes is increased.

Segregation

The key to meiosis is the separation of homologous chromosomes at the first division of meiosis. Chromosome segregation is accomplished through the building of a microtubule-based spindle that mediates the travel of chromosomes to opposite poles of the cell. In males, the meiosis I spindle is often assembled in the presence of centrioles, similar to the mitotic spindle. Females usually form a spindle in the absence of centrioles, and one still unsolved question is how the meiotic chromosomes direct the formation of a bipolar spindle without centrioles or centrosomes.

In *Drosophila* females, rather than congressing to the metaphase plate, the chromosomes enter metaphase in a condensed mass called the karyosome, and the spindle microtubules appear to nucleate from the chromosomes. Several proteins that are involved in this process have been identified. Two microtubule motor proteins, Ncd and Sub, have been implicated in bundling microtubules to taper the ends of the spindle, while D-Tacc, Msps, and Asp appear to localize to the poles of the meiotic spindle, raising the possibility that they may function similarly to centrosomes in the formation of the meiosis I spindle in females. Finally, Axs is a transmembrane protein that is also necessary for the tapering and lengthening of the female meiotic spindle. Recent findings by Joe Kramer have suggested the possibility that Axs may function as a component of a

previously undescribed structure that surrounds the meiotic spindle and mediates its assembly.

Many meiotic systems possess checkpoint or surveillance mechanisms to ensure that chromosomes are attached to the proper poles, as assessed by the presence of tension on the kinetochores. An improperly attached kinetochore, or pair of kinetochores, will not be under tension and thus trigger a meiotic arrest, or even apoptosis. Moreover, mutations in the spindle checkpoint genes lead to high frequencies of chromosome nondisjunction at anaphase I. There is a much stronger system for error-detection in male meiosis that detects misaligned or unpaired chromosomes prior to the first meiotic division and having done so directs the cell towards death. Although oocytes appear to lack such a tension-sensitive checkpoint, at least some workers have proposed that age-dependent defects in the ability of oocytes to monitor at least some aspects of spindle assembly and/or chromosome position may well be a factor in the maternal effect on meiotic nondisjunction in humans.

Chiasmata, the physical manifestation of reciprocal meiotic exchange, lock the homologs together. Once the two homologous centromeres are attached to opposite poles of the meiotic spindle, the chiasmata prevent premature progression of the centromeres to the poles by balancing the poleward forces localized mainly at the kinetochore. Sister chromatid cohesion distal to crossovers maintains chiasmata at their initial position until anaphase I. For

example, mutants of the *desynaptic* gene in maize display both precocious sister chromatid separation, suggesting a defect in sister centromere cohesion, and the early separation of chiasmate bivalents.

In *Drosophila*, chiasmata fail to conjoin homologous chromosomes in the presence of mutations at the *ord* locus, which disrupt sister chromatid cohesion during meiotic prophase. Similarly, in yeast the cleavage of Rec8p, a protein required for meiotic sister chromatid cohesion, is required for chiasma resolution, and homolog separation is blocked in the absence of this cleavage.

The release of sister chromatid cohesion distal to chiasmata allows homologs to separate. In mitotic cells, a protein called separase is necessary for the proteolytic cleavage of cohesin complexes around the centromere (and both centromeres and arms in yeast), allowing chromosome segregation at anaphase. In a similar manner, cleavage of cohesin by separase is necessary for chromosome segregation during anaphase I. Although arm cohesion must be maintained through prophase in order to maintain chiasmata, the release of cohesion is essential to allow the segregation of homologs to opposite poles at anaphase I. However, this creates a 'Catch-22' situation: dissolution of sister chromatid cohesion in the arms would release the chiasmata that hold the homologs together, yet the centromeres of sister chromatids have to remain together to ensure that the homologs segregate correctly and prevent sister chromatid missegregation.

Maintenance of cohesion at the centromeres and release of sister chromatid cohesion along the arms is achieved through the differential removal of cohesin complexes from the arms and centromeres of the chromosomes. Immunolocalization of cohesin components during mammalian meiosis indicates that the cohesin complex is removed from chromosome arms at metaphase I, but is retained at the centromere until the second meiotic division. Maintenance of cohesin at the centromeres depends on the presence of the meiosis-specific Scc1 homolog Rec8, which replaces Scc1 in the meiotic cohesin complex in most systems. Scc1 is sufficient for sister chromatid cohesion in yeast meiosis in the absence of Rec8, but is not protected from cleavage at the first meiotic division.

Errors in chromosome segregation during meiosis I may result from a combination of failures in sister chromatid cohesion and achiasmate segregation mechanisms. In general, chiasmata are not positioned randomly along chromosome arms, but rather tend to cluster in the middle of the arms. Studies of spontaneous nondisjunction in both humans and *Drosophila* have linked meiosis I nondisjunction to a reduction or absence of exchange along the missegregating chromosome. When an exchange is present, it is usually located near the distal, or telomeric, end of the chromosome. If sister chromatid cohesion distal to the chiasma is insufficient to maintain the chiasma, or is released prematurely, the homologous chromosome pair may not be held together properly. In this

situation, or in the case where no exchange has occurred at all, it is the job of achiasmate segregation systems to make sure the chromosomes segregate properly.

Several achiasmate segregation systems have been described. In *Drosophila*, the Nod protein is able to perform the role of chiasmata in their absence. Chromosomes that do not undergo exchange in *Drosophila* normally pair, and Nod keeps them together at the metaphase plate. When Nod is absent, the achiasmate chromosomes may come apart and segregate at random. Although it isn't clear how human achiasmate segregation systems might function, the finding of reduced recombination in both maternal and paternal meiosis I nondisjunction for chromosome 21 raises the possibility that similar defects in sister chromatid cohesion or achiasmate segregation may predispose certain individuals toward aneuploidy. This possibility has already been raised as one possible cause for the maternal age effect, in which increases in aneuploidy rates occur with advanced maternal age.

In conclusion, we have seen many recent advances in our knowledge of the molecular biology underlying meiosis. The contribution of research on model organisms has played a large part in these advances, which are hoped to contribute to the understanding, diagnosis and treatment of human disorders of reproduction.