Chapter 10
How do sperm get the correct number of chromosomes?

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Germ cells use meiosis to ensure gametic haploidy. Meiosis is a unique and defining event of gametogenesis serving at least two functions in the reproductive life cycle: it reduces chromosome number to a perfect haploid state in the gamete (thus allowing diploidy to be restored at fertilization), and it shuffles gene allele combinations, giving rise to genetic diversity. Meiosis is accomplished in two remarkably coordinated divisions, without an intervening S phase (Fig. 1). The first division, a reductional division, segregates the homologous chromosomes into separate cells, and the second division, an equational division, reduces the DNA and chromosomal content to haploidy. The success of these divisions depends on the unique dynamics of chromosomes during the extended meiotic prophase in primary spermatocytes.

Meiosis is initiated after mitotic proliferation of spermatogonia by DNA synthesis that accomplishes precise replication of each chromosome to form two chromatids. Thus, the DNA content ("C" value) has doubled from 2C to 4C, but the chromosome number ("N" value) of the germ cell is not changed – it is still the 2N diploid value, e.g., 46 chromosomes in humans (note: spermatocytes are not tetraploid). During meiosis I prophase, homologous chromosomes pair, forming bivalents, and undergo recombination – these are defining events of meiosis and key features that distinguish meiosis from mitosis. Meiotic prophase I is divided into substages that mark the dynamics of chromosome behavior. During the leptotene stage, chromosomes are subject to endogenous double-strand DNA breaks, mediated by the SPO11 enzyme, that initiate the molecular events of meiotic recombination. Also during the leptotene phase, homologous chromosomes find each other by homology searching mechanisms that are not well understood, but may be facilitated by telomere clustering into a "bouquet" on the nuclear envelope and/or the DNA breaks and subsequent formation of short single-stranded ends. During the zygotene stage, chromosomes pair and homologs

initiate their intimate association by synapsis, a process mediated by the synaptonemal complex (SC). The SC is a protein complex comprised of lateral elements that form the scaffold, or axes, of each homolog, and a central element that is structural "glue" mediating complete synapsis. The completion of synapsis marks the beginning of the pachytene stage, which is lengthy (approximately 16 days in the human) and characterized by considerable growth of the spermatocyte as well as by important chromosome dynamics. Now the chromosomes can be visualized as homologous pairs, called bivalents, e.g., 23 bivalents in humans. Notably, however, the non-homologous X and Y chromosomes are synapsed only in a small region of homology (the pseudo-autosomal region) and are sequestered in a heterochromatic nuclear domain known as the XY body (or sex body). During the pachytene stage, molecular events of homologous recombination unfold. Interestingly, the number of recombination-initiating double-strand breaks is in approximately ten-fold excess to the number of final reciprocal recombinations (crossovers), which is always at least one per chromosome, but can be up to two or three in longer chromosomes. The excess DNA double-strand breaks are chromosomes thought to be repaired by a recombination mechanism that involves non-crossover gene conversion, or site-specific exchange of information without exchange of surrounding chromosomal regions.

The completion of recombination marks the passage of the germ cell into the final, diplotene, stage of meiosis I prophase, when the chromosomes undergo desynapsis and condense. At this stage, the homologs are still held together by the recombination sites (crossovers), visibly manifest as chiasmata. The chiasmata serve the essential function of maintaining the homologous pairs in a bi-polar orientation as they line up on the spindle apparatus at metaphase of the first meiotic division, maternal homolog oriented to one pole and paternal homolog to the other. The first meiotic division is reductional, separating the members of each homologous pair. The result is two cells, secondary spermatocytes, each with the haploid chromosome content (e.g., 23 chromosomes in humans), but with each chromosome still comprised of two chromatids. The meiosis II division ensues rapidly and is an equational division much like mitosis, separating the chromatids to separate cells, each of which now contains the haploid 1N chromosome number (e.g., 23 chromosomes in humans) and 1C DNA content. As is the case with the mitotic divisions of differentiated spermatogonia, the two meiotic divisions are characterized by incomplete cytokinesis and the

persistence of intercellular bridges. Thus, when meiosis is completed, the haploid round spermatids are conjoined in a syncytium as they commence the differentiation process of spermiogenesis.

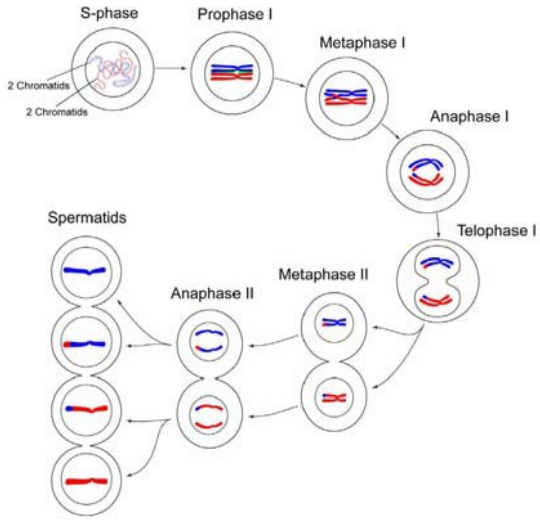

Figure 1. This diagram illustrates the sequence of key events of meiosis in male germ cells. Red and blue depict the two homologs of an autosomal chromosome, one maternally derived and the other paternally derived. At the completion of S-phase, each chromosome consists of two chromatids. At the pachytene stage of prophase I, homologs are synapsed to form a bivalent, an event mediated by the synaptonemal complex (green, seen in Prophase I). By metaphase I, chiasmata, the visible manifestation of recombination events, are seen. In anaphase I and telophase I, the homologs separate from each other, reducing the chromosome number to the haploid content. Spermatocytes rapidly progress to metaphase II, and subsequently the chromatids are separated in anaphase II, to form the 4 haploid spermatids.

What do we know about molecular regulation of these remarkable and precise chromosomal dynamics, how do we know it, and is this information useful in the clinic? We know that the processes of creating and repairing DNA double-strand breaks (DSBs) drive much of the chromosome interactions during meiotic prophase. Mutations impairing function of any of the many DSB-

related proteins cause arrest of male germ-cell development in meiotic prophase with subsequent infertility. Much of this information has derived from programs exploiting deliberate chemical mutagenesis or CRISPR gene targeting of the mouse genome. These endeavors have identified a plethora of genes required for spermatogenesis, many of which control meiosis, a seemingly particularly vulnerable stage. More recently, single-cell RNA sequencing is identifying the transcriptomes of cells throughout spermatogenesis and this too is leading to the discovery of genes that control the progress and tempo of meiosis, ensure gametic haploidy, and lay the foundation for post-meiotic spermiogenic differentiation.

With the identification of molecular mechanisms of meiosis, much attention has been directed to how the meiotic choreography of chromosome movement can go wrong, and whether these errors can explain any cases of human infertility. The penalty of meiotic error can be germ-cell arrest or generation of aneuploid gametes and offspring bearing the wrong number of chromosomes. Either failure of recombination and chiasmata formation or abnormal persistence of chiasmata can negatively impact fidelity of chromosome segregation during the first meiotic division by causing nondisjunction (failure of homologs to separate to the two spindle poles). Likewise, absence or persistence of sister chromatid cohesion can cause abnormalities in chromosome segregation. Screening infertile men for gametic aneuploidy by monitoring sperm chromosome content using fluorescent in situ hybridization (FISH) has failed to find strongly significant correlations between infertility and production of aneuploid sperm, and, indeed, meiotic nondisjunction is less frequent in the human male than in the female, where it is prevalent and age-related. It appears that in males meiotic errors are detected and defective germ cells are eliminated. Infertility due to arrested spermatogenesis and germ cell death ("maturation arrest") is found in some human male infertilities. Germ-cell death is a common phenotype in mouse models lacking proteins involved in meiotic recombination. Although similar mutations have been found among infertile males, causality has been difficult to establish. Indeed, it is even the case that when modeled in mice, some human mutations predicted to be deleterious do not cause meiotic arrest or infertility. Interestingly, some mouse meiotic mutations that cause germ-cell arrest and infertility on one genetic background do not on other genetic backgrounds. Lines of evidence such as this strongly suggest considerable inter-individual

genetic diversity in the phenotypic expression of mutations affecting meiosis and other aspects of spermatogenesis, a cautionary note warning against over interpretations of male meiotic arrest infertility in the clinic.

In a summary of the essentials, meiosis is a defining event of spermatogenesis and is comprised of two divisions (Fig. 1). Driving progress of meiosis are the events of synapsis of homologous chromosomes and reciprocal recombination, resulting in new combinations of gene alleles. The first reductional division separates homologous chromosomes and reduces chromosome number from 2N to 1N. The second division is equational, separating chromatids. The products of these meiotic divisions are spermatids, each now with the genetically correct haploid 1N chromosome number and 1C DNA content.

Suggested reading

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