REVIEW

Sex Matters in Meiosis

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In mammals, fertilization typically involves the ovulation of one or a few eggs at one end of the female reproductive tract and the entry of millions of sperm at the other. Given this disparity in numbers, it might be expected that the more precious commodity—eggs—would be subject to more stringent quality-control mechanisms. However, information from engineered mutations of meiotic genes suggests just the opposite. Specifically, the available mutants demonstrate striking sexual dimorphism in response to meiotic disruption; for example, faced with adversity, male meiosis grinds to a halt, whereas female meiosis soldiers on. This female "robustness" comes with a cost, however, because aneuploidy appears to be increased in the resultant oocytes.

Anyone who has watched couples in video stores knows that men and women respond differently to the same stimulus. In meiosis, as in movie preference, sexual dimorphism is the rule; mammalian males and females use different strategies, transit meiosis with different levels of success, and exit with different end products. In this review, we briefly summarize recent data suggesting that the response to meiotic disturbances is also sex-specific.

The basic features of meiosis—two cell divisions with no intervening DNA replication, resulting in a halving of the chromosome complement—are conserved throughout evolution. Thus it is not surprising that the general outline applies to both mammalian males and females. However, the details are remarkably different. The mammalian oocyte begins meiosis during fetal development but arrests part-way through meiosis I (MI) and does not complete the first division until ovulation; the second division (MII) is completed only if the egg is fertilized. Thus oogenesis requires several start and stop signals and, in some species (e.g., human), may last for several decades. In contrast, male meiosis is less complicated. It begins at puberty and is a continuous process, with spermatocytes progressing from prophase I through the second division in little more than a week.

Perhaps the most striking male-female meiotic difference is in the error rate in humans. At least 10 to 25% of all human fetuses have the "wrong" number of chromosomes (*1*). Studies of the commonest classes of abnormality (trisomies and monosomies) indicate that approximately 80 to 90% result from nondisjunction at maternal MI (*1*). Although it is formally possible that paternally derived aneuploidies are preferentially eliminated, there is no evidence that selection in utero discriminates on the basis of parental origin. Thus, the difference seems likely to originate in meiosis, an interpretation consistent with direct studies of human gametes, where as many as 20% of oocytes, but only 3 to 4% of sperm, are chromosomally abnormal (*2*). Hence, female meiosis in general, and MI in particular, appears extraordinarily error-prone.

The basis for this female MI "vulnerability" is not yet clear but presumably arises in one of two ways; either more errors occur during oogenesis or mechanisms for recognizing and correcting or eliminating cells with errors are more efficient in spermatogenesis. Direct measurements of error rates at MI are virtually impossible to obtain; however, there is increasing evidence that males and females do, indeed, respond differently to meiotic disturbances. Specifically, abnormalities in male meiosis that elicit arrest phenotypes, either at the metaphase-anaphase transition or during prophase, frequently appear to escape detection in the female. Thus, the same precipitating event may lead to meiotic arrest and infertility in males, whereas in females, the outcome may be a chromosomally abnormal gamete. The remainder of this review discusses the evidence leading to this conclusion.

In somatic cells, a spindle assembly checkpoint that monitors chromosome alignment and spindle integrity during cell division is well characterized (*3, 4*). In the absence of proper chromosome alignment, anaphase is delayed, allowing the cell to correct errors that might otherwise produce aneuploid progeny. Also, there is evidence that this checkpoint is operational in mammalian male germ cells. For example, in early studies of infertile human males, Chandley *et al.* (*5*) demonstrated a negative correlation between the presence of unpaired (univalent) chromosomes at metaphase I and progression to metaphase II. Similarly, in the male mouse, numerical or structural chromosome abnormalities and single gene defects that generate univalent chromosomes lead to metaphase I arrest and subsequent death of spermatocytes. Thus, evidence from both human and mouse suggests that stringent quality controls operate during the male meiotic divisions.

Surprisingly, there is growing evidence that this checkpoint is missing or less stringent in mammalian oogenesis. Gross disturbances in alignment of chromosomes on the MI spindle, as a result of either environmental exposures (*6*) or mutations that disrupt folliculogenesis (*7*), are not associated with meiotic arrest or a delay in anaphase onset. Nevertheless, cells that proceed to MII exhibit a striking increase in aneuploidy, indicating that the cost of relaxed cell-cycle control in females is a reduction in the genetic quality of gametes.

In addition to the spindle assembly checkpoint that monitors the metaphase-anaphase transition, an earlier acting control mechanism operates during prophase (*8*). In lower eukaryotes and mammals alike, there is compelling evidence that this so-called pachytene checkpoint is activated by mutations in genes whose products play an integral role in processing the double-strand breaks (DSBs) that initiate meiotic recombination (*8*). Thus, this control is thought to be analogous to the DNA damage checkpoint that operates in somatic cells and to be activated by unresolved DSBs or other recombination intermediates.

Is there any reason to suspect that this prophase control mechanism—like the spindle assembly checkpoint—exhibits sexual dimorphism? Further, could sex-specific differences in response to disturbances during prophase contribute to the disparity in error rates observed between mammalian spermatogenesis and oogenesis? It has been difficult to address these questions, owing to the absence of naturally occurring mammalian meiotic mutants. However, the meiotic "road map" available from lower eukaryotes has made it possible to generate mammalian mutants, typically through targeted disruption of mammalian orthologs of yeast meiotic genes. Most such mutants exhibit meiotic abnormalities, but it is noteworthy that spermatogenesis frequently appears more severely compromised than oogenesis (Fig. 1). This suggests sex-specific differences in cell-cycle control during prophase as well as metaphase, but there are caveats to this simple interpretation: First, for most mutations the male has been studied in detail, but the comparatively complex task of analyzing the female has limited all analyses of oogenesis. Thus, reliable information on the timing of female germ cell loss and on the proportion of oocytes that escape checkpoint detection is not always avail-

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able. Second, differences in the kinetics and tempo of meiosis make direct comparisons inherently difficult. Because germ cells in the ovary initiate meiosis within a period of days (mouse) or weeks (human), the events of meiotic prophase occur in a semisynchronous population of cells. Moreover, the first group of oocytes is recruited for growth in the juvenile ovary, even if this completely depletes the oocyte pool. Thus, even a small population of germ cells that escape the actions of a checkpoint mechanism is readily detectable. In contrast, the seminiferous tubules contain cells in various stages of spermatogenesis, thus complicating efforts to identify and quantify cells that escape checkpoint mechanisms. Moreover, the cytoplasmic bridges retained between spermatocytes as a result of incomplete cytokinesis create a common environment that may conspire to eliminate normal cells in the toxic atmosphere created by the demise of neighboring cells.

These concerns notwithstanding, it seems likely that the male-female differences observed in the mouse mutants are real. For genes involved in sex-specific aspects of gametogenesis (e.g., follicle growth, spermiogenesis), this is not surprising. However, genes involved in the prophase I events of synapsis and recombination are conserved throughout evolution; thus, mutations in these genes might be expected to cause similar defects in oogenesis and spermatogenesis. This is not the case, however, as is apparent from a consideration of 12 mutations on which at least some information is available for both sexes (Fig. 1). Loci thus far represented encode proteins involved in the initiation [SPO11 (*9, 10*)] or early processing [DMC1 (*11, 12*)] of DSBs, mismatch repair proteins that participate in meiotic recombination [MLH1 (*13, 14*), PMS2 (*15*), MSH4 (*16*), and MSH5 (*17, 18*)], a component of the synaptonemal complex [SCP3 (*19*)], proteins with roles in meiotic cell-cycle control or DNA repair activity [Cyclin A1 (*20*), ATM (*21, 22*)], and three proteins whose meiotic functions are not yet clear [the mammalian homolog of VASA, MVH (*23*); the cytoplasmic polyadenylation element–binding protein, CPEB (*24*); and a gene whose function is not yet known, *Mei 1* (*25*)].

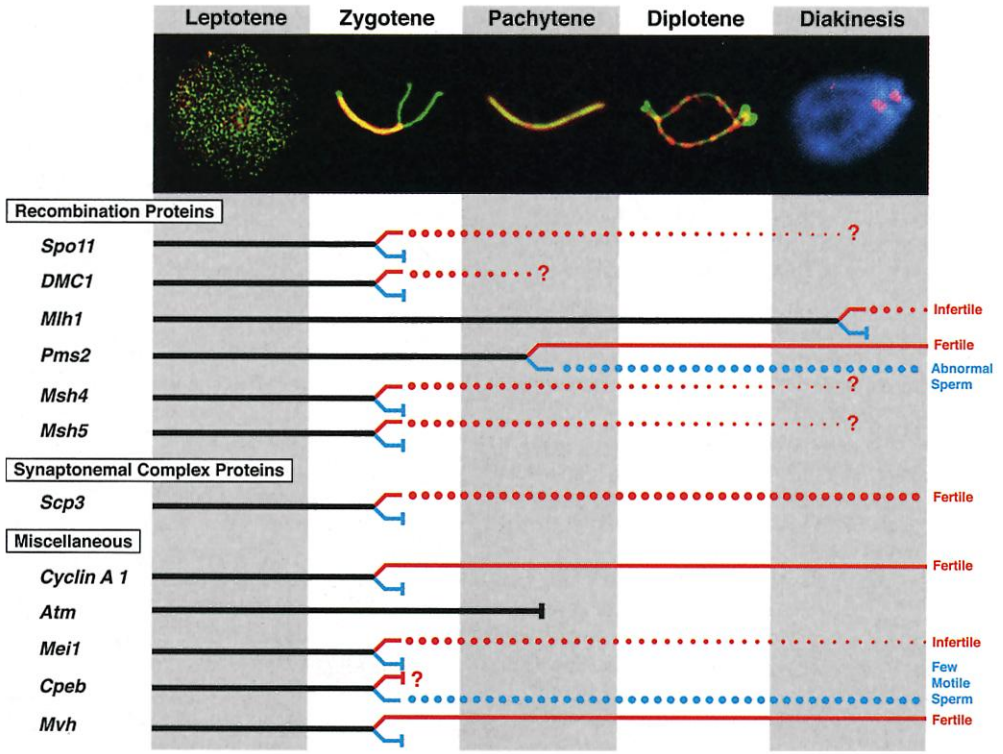

Fig. 1. Meiotic progression and arrest points in mice homozygous for induced mutations. Only mutations with information on both sexes and in which sterility is a feature of at least one are considered. **(Top)** Photographic representations of MI prophase: presynapsis (leptotene), partial synapsis (zygotene), full synapsis (pachytene), desynapsis (diplotene), and chiasmate configuration (diakinesis or MI). SC components are visualized in the first four panels (SCP1, red; SCP3, green); chromatin staining (blue) and kinetochore localization (red) are included only for the condensed pair of homologs in the last panel. **(Bottom)** Meiotic phenotypes for induced mutations. A solid black line denotes stages during which male and female meiosis are similar; sex-specific phenotypes are indicated by a switch to red (female) and blue (male) lines. A vertical bar indicates meiotic arrest, and a switch from a solid to dotted line denotes continued survival of only a proportion of cells.

Of the 12 mutations, all but one (*Atm*) display apparent sex-specific differences. Moreover, in seven, spermatogenesis grinds to a halt in early prophase, whereas the female mutant either retains fertility (*Scp3*, *Cyclin A1*, *Mvh*) or has at least a few growing follicles in the postnatal ovary (*Spo11*, *Msh4*, *Msh5*, *Mei1*). As indicated above, the detection of female germ cells that escape death during prophase is easy, as growing oocytes are hard to miss. However, for two of the best-characterized mutants, *Pms2* and *Scp3*, the sex-specific difference cannot simply be one of detection. The PMS2 null male is infertile, exhibiting synaptic defects and apoptosis of most spermatocytes during prophase, with production of only a few morphologically abnormal, nonmotile sperm (*15*). In contrast, females exhibit apparently normal fertility, although closer inspection reveals an increase in aneuploid eggs (*26*). As found in PMS2 deficiency, males deficient for the synaptonemal complex protein SCP3 are infertile, with prophase arrest attributable to failure to form a normal synaptonemal complex (*19*). Remarkably, SCP3 null females are somehow able to surmount this abnormality, exhibiting nearly normal levels of recombination. More important, they are fertile, albeit with reduced litter size and an increased likelihood of aneuploid progeny (*27*).

Clearly, these interpretations about male-female differences must be viewed with caution, because varying amounts of data are available for the different mutations; for some, there is detailed information on pachytene-stage chromosome configurations, whereas for others, only histological analyses are available. Nevertheless, one general conclusion seems inescapable—faced with adversity, oogenesis is more robust than spermatogenesis. However, this "robustness" comes with a cost, because preliminary observations on PMS2-deficient and on SCP3-deficient females indicate an increase in aneuploid gametes.

Thus, data from mouse mutants suggest more stringent control mechanisms in the male. However, one exception that defies the general rule has already emerged. A null mutation of CPEB (*24*), an RNA binding protein that regulates translation, causes arrest at prophase in females, but males produce a few motile sperm! No doubt further surprises await, and, although it is tempting to suggest that differences in control during prophase and metaphase conspire to make oogenesis error-prone, at this juncture only one conclusion is certain: Focusing our attention on mutant phenotypes in only one sex is analogous to providing an alien anthropologist with a film archive prepared ex-

clusively by men. The conclusions, whether with respect to mutations or civilization, are likely to be inaccurate.

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REVIEW

Penetration, Adhesion, and Fusion in Mammalian Sperm-Egg Interaction

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Fertilization is the sum of the cellular mechanisms that pass the genome from one generation to the next and initiate development of a new organism. A typical, ovulated mammalian egg is enclosed by two layers: an outer layer of ~5000 cumulus cells and an inner, thick extracellular matrix, the zona pellucida. To reach the egg plasma membrane, sperm must penetrate both layers in steps requiring sperm motility, sperm surface enzymes, and probably sperm-secreted enzymes. Sperm also bind transiently to the egg zona pellucida and the egg plasma membrane and then fuse. Signaling in the sperm is induced by sperm adhesion to the zona pellucida, and signaling in the egg by gamete fusion. The gamete molecules and molecular interactions with essential roles in these events are gradually being discovered.

In mammals, fertilization is completed by the direct interaction of sperm and egg, a process mediated primarily by gamete surface proteins. Therefore, an essential task in the study of sperm-egg interaction is an exploration of the capabilities of a distinct set of surface proteins, some gamete specific and others more widely expressed. On gametes, these proteins act in a sequential pattern to orchestrate the close approach and ultimate fusion of the two cells.

Sperm penetration of the cumulus. To penetrate the substantial cumulus cell barrier surrounding ovulated eggs of most mammalian species, sperm use hyperactivated motility (*1*) and a glycosylphosphatidylinositol (GPI)-anchored surface hyaluronidase, named PH-20 (Fig. 1A) (*2*). The motility and surface hyaluronidase are necessary, and perhaps sufficient, to digest a path through the extracellular matrix of the cumulus cells; no proteases have yet been implicated in this process.

Sperm interaction with the zona pellucida. The egg's zona pellucida is a cell type–specific extracellular matrix or coat composed of three glycoproteins termed ZP1, ZP2, and ZP3. Sperm that reach and bind to the zona pellucida receive a signal to acrosome react, i.e., release by exocytosis the contents of their large secretory granule, the acrosome (Fig. 1B).

The currently favored model is that sperm bind to O-linked carbohydrate on ZP3. Sperm preincubation with ZP3 strongly inhibits sperm binding to the zona, whereas preincubation with ZP1 or ZP2 has no effect (*3*). Other studies show that sperm binding can be blocked by O-linked oligosaccharides of ZP3, present on Ser^{332} and Ser^{334} near the ZP3 COOH-terminus (*4*, *5*). Thus, sperm adhesion to the zona is a carbohydrate-mediated event. A requirement for

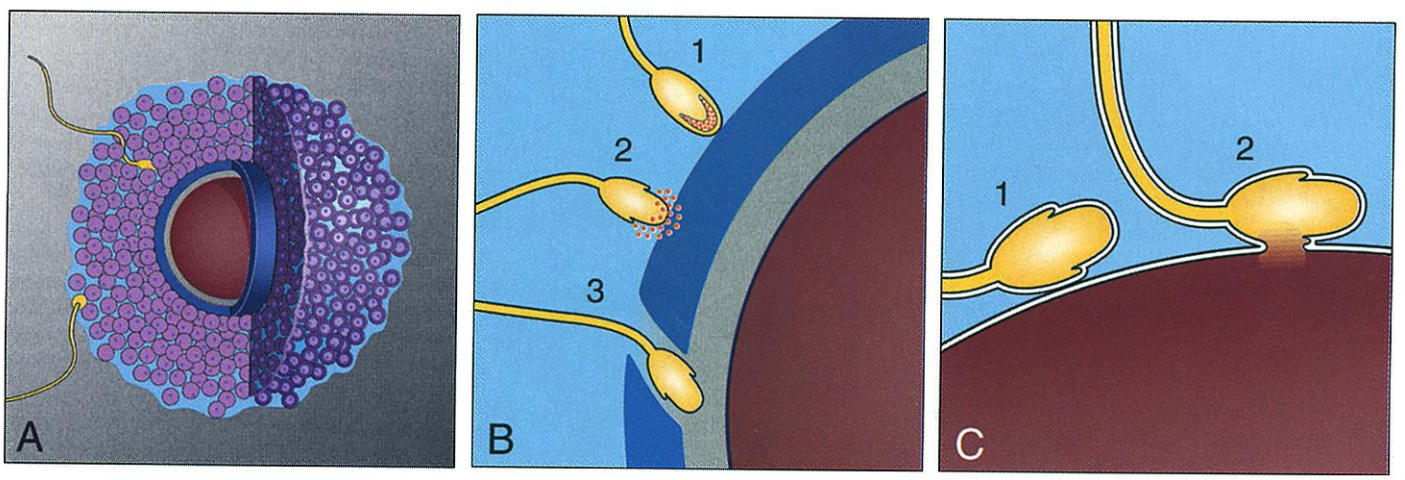

Fig. 1. (**A**) Sperm penetration of cumulus cells (purple) to reach zona (navy blue). (**B**) Egg depicted with cumulus cells removed; sperm 1 binds to the zona pellucida (navy blue); sperm 2 undergoes exocytosis, releasing acrosomal contents (orange-red); sperm 3 penetrates the zona pellucida and begins entry into perivitelline space (gray). (**C**) Sperm 1 binds to the egg plasma membrane by the side of its head, in a central region (equatorial region); sperm 2 fuses with the egg plasma membrane.