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Review

Epigenetic Transitions in Germ Cell Development and Meiosis

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Germ cell development is controlled by unique gene expression programs and involves epigenetic reprogramming of histone modifications and DNA methylation. The central event is meiosis, during which homologous chromosomes pair and recombine, processes that involve histone alterations. At unpaired regions, chromatin is repressed by meiotic silencing. After meiosis, male germ cells undergo chromatin remodeling, including histone-to-protamine replacement. Male and female germ cells are also differentially marked by parental imprints, which contribute to sex determination in insects and mediate genomic imprinting in mammals. Here, we review epigenetic transitions during gametogenesis and discuss novel insights from animal and human studies.

Introduction

Meiosis is unique to sexually reproducing organisms. During gametogenesis, this exceptional cell cycle generates the gametes and is essential for the survival and evolution of species. It enables genetic exchange between parental genomes through a process called meiotic recombination, in which homologous chromosomes become aligned in pairs (synapsis) during prophase-I, and DNA double-strand breaks are made and repaired, to form crossover exchanges between homologous chromosomes. This process is associated with specific chromatin changes (Figure 1). Chromosomes and chromosomal regions that are present on only one of the two homologous chromosomes cannot become paired and are inactivated by a mechanism called meiotic silencing. Germ cell development and meiotic division eventually lead to the conversion of diploid cells to haploid gametes, which will give rise to a new diploid organism that is genetically different from its parents.

Before they undergo meiosis, germ cells are subject to extensive chromatin reorganization (Reik, 2007; Sasaki and Matsui, 2008). During these early stages of germ cell differentiation, genome-wide chromatin changes are similar in male and female germ cells and contribute to the suppression of somatic cell differentiation. In the early germ cells, called primordial germ cells (PGCs; Figure 1), the genomes are wiped clean of most of their DNA methylation and of other covalent chromatin modifications that are associated with somatic gene regulation, so that germ cells can acquire the capacity to support postfertilization development. This process also prepares the germ cells for meiosis, during which homologous chromosomes become aligned to allow synapsis and recombination. Recent studies suggest that besides specific DNA sequence motifs, histone modifications contribute to synapsis formation and recombination and enhance recombination at preferential regions called ''hot spots of recombination'' (Baudat et al., 2010; Myers et al., 2010; Parvanov et al., 2010). Chromatin features are also thought to contribute to desynapsis and the subsequent separation of the homologous chromosomes.

During postmeiotic spermatogenesis, again major chromatin changes take place (Gaucher et al., 2010). In many species, this includes the removal of nucleosomal histone proteins and their replacement by basic proteins called protamines. This histone-to-protamine exchange contributes to the extreme compaction of the genome in mature sperm. Recent studies show that this remarkable chromatin remodeling process might also contribute to the regulation of the embryonic transcriptional program after fertilization. In some animal species, there is a less extensive histone-to-protamine exchange, but the reason(s) behind these differences among species have not been elucidated yet. Little is known about chromatin regulation during the final stages of oogenesis. However, the genome maintains its nucleosomal histones throughout as opposed to mature sperm. The maturing oocyte undergoes various gene regulational changes that prepare its essential contribution to early embryonic development. This process is of particular importance in flies and worms, in which maternal determinants have a major impact on embryonic development, including the specification of germ cells.

Another fascinating aspect of gametogenesis is the differential marking of genes and chromosomes in male and female germ cells, observed in different groups of insects and in mammals. These chromatin imprints are truly epigenetic as shown by the fact that they are transmitted to the developing embryo and mediate expression depending on the gene inheritance from the sperm or the oocyte. In insects, this epigenetic marking contributes to differences between the sexes in the offspring. In placental mammals it does not have this function, but plays an important role in the control of fetal growth and development. Imprint establishment is intricately linked to the differentiation of male and female germ cells. Like other key events in gametogenesis, it involves covalent modifications that are put onto DNA and histones. Together with imprinted X chromosome inactivation in mammals, genomic imprinting provides an excellent paradigm to address the differential marking of the oocyte and the sperm genomes and to explore its developmental consequences.

Here, we review epigenetic events occurring in germ cells before, during, and after meiosis, comparing the male and the female germline. Novel insights from studies on mammalian species are presented and selected examples from

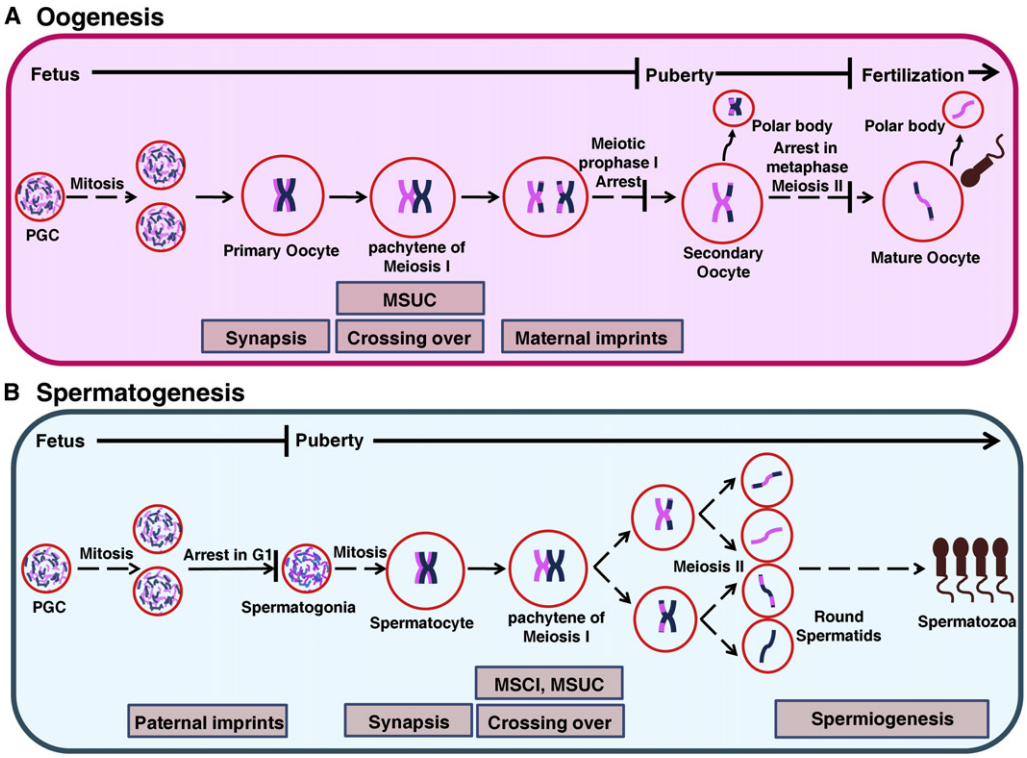

Figure 1. Developmental Transitions in Female and Male Gametogenesis

Primordial germ cells (PGCs) form during embryonic development.

(A) In the developing ovaries, they undergo mitotic divisions before entering meiosis I after which primary oocytes arrest in prophase I until ovulation. Upon onset of sexual maturation, oocytes complete meiosis I and arrest in metaphase of meiosis II, until fertilization occurs.

(B) Male PGCs defer meiosis and undergo mitotic proliferation in the developing male gonad until arresting in G1. From sexual maturity onward, spermatogonia resume mitotic proliferation to form spermatocytes, which then activate meiotic differentiation and form four haploid spermatids each, which in turn develop into spermatozoa. For simplicity, meiosis is shown for one pair of homologous chromosomes (in blue and pink). The timing of meiotic recombination, maternal and paternal imprint acquisition, meiotic silencing of unpaired chromatin (MSUC), meiotic sex chromosome inactivation (MSCI), and spermiogenesis are indicated.

nonvertebrate species are discussed as well. Chromatin changes during gametophyte development and gamete formation in flowering plants remain less well characterized (Zhang and Ogas, 2009) and are not included here. The emerging picture is that animal germ cells have a specific fate that is linked to a sequence of epigenetic events unique to this particular cell type. Chromatin reprogramming ensures the appropriate expression of germline-specific genes, contributes to the maintenance of chromosome integrity and meiosis, and prepares the genome for gene expression in the embryo. Although not the emphasis of this text, exploring epigenetic mechanisms in germ cell development is essential in relation to fertility, assisted reproduction, and human health.

Chromatin Remodeling in Developing Primordial Germ Cells

Mammalian germ cells arise relatively late during embryo development. They derive from a small population of extraembryonic mesoderm cells of the epiblast, as a consequence of signaling from adjacent tissues. This process involves epigenetic reprogramming of the somatic cell state, and some of the earliest markers of germ cell differentiation are linked to factors that modify histones. In the mouse, the transcriptional repressor BLIMP1 (B lymphocyte induced maturation protein 1) is expressed specifically in the group of precursor cells in which PGC specification occurs, at around embryonic day 7.25 (E7.25), and contributes significantly to the suppression of the somatic gene expression program (Kurimoto et al., 2008). *BLIMP1*-deficient embryos, for instance, show aberrant expression of *Hox* genes in PGC-like cells (Ohinata et al., 2005). BLIMP1 associates through its PR domain with the arginine methyltransferase PRMT5 to control global levels of histone H2A and H4 arginine-3 symmetrical dimethylation (H2A/H4R3me2s), which might be involved in the developmental transition from somatic to germ cell fate (Ancelin et al., 2006).

Cells from this PGC founder population, about 40 initially, proliferate and migrate toward the genital ridges which they colonize (at around E10.5 in the mouse), after which the gonads form. During and after migration, PGCs undergo global epigenetic changes in chromatin organization (Sasaki and Matsui, 2008). Although genome-wide loss of DNA methylation appears to initiate in migrating PGCs (Seki et al., 2005), it largely occurs after colonization of the genital ridges and affects about 80% to 90% of the genome (Popp et al., 2010). The process includes the loss of methylation marks that control somatic gene expression and also removes DNA methylation imprints from imprinted gene loci (see below).

Beside DNA methylation, extensive changes in histone methylation have also been documented in murine PGCs, particularly gradual loss of H3 lysine-9 dimethylation (H3K9me2) from E7.5

onward. This epigenetic transition coincides with loss of expression of the lysine methyltransferase (KMT) KMT1D, also called GLP (Seki et al., 2007). A global gain of H3 lysine-27 trimethylation (H3K27me3) occurs from E8.25 onward. Furthermore, there is expression of the histone chaperone proteins HIRA and NAP1, which could be important for the developmentally regulated incorporation of histone variants (Hajkova et al., 2008).

Because nucleosomes and histones undergo gradual epigenetic changes at around the time DNA demethylation occurs, these transitions could precede or be a consequence of DNA demethylation. Mechanistically, the DNA demethylation process remains poorly understood. In developing PGCs, it depends in part on the expression of the cytidine deaminase AID (Popp et al., 2010), which converts 5-methyl-cytosine to thymine, suggesting involvement of a DNA repair process. An initial insight into such a mechanism came from a zebrafish study on MBD4, a methyl binding domain protein with mismatch-specific thymine glycosylase activity. In zebrafish embryos, MBD4, together with AID, was found to be involved in DNA demethylation (Rai et al., 2008). Besides the possible involvement of methyl-cytosine deamination, recent evidence indicates that DNA demethylation in PGCs is mechanistically linked to the occurrence of single-strand DNA breaks and the activation of the base excision repair (BER) pathway (Hajkova et al., 2010). In mammals, a comparable mechanism seems to be involved in the DNA demethylation that occurs in the paternal pronucleus of the zygote (Wossidlo et al., 2010).

Intriguingly, some sequences, such as IAP (Intracisternal A Particle) retrotransposons, are partially resistant to DNA demethylation in murine PGCs (Hajkova et al., 2002; Popp et al., 2010). In principle, this could allow transmission of specific DNA methylation patterns to the next generation. It would be interesting to explore whether these exceptional sequence elements present particular chromatin features that protect them against DNA demethylation.

In worm and fly species, germ cell specification occurs earlier during embryonic development and involves maternal determinants that are present in the oocyte and get sequestered into the future primordial germ cells (Strome and Lehmann, 2007). As in mammals, however, the early PGCs of these species are transcriptionally inactive, possibly to avert activation of the somatic program. The unrelated transcriptional regulators PIE1 in worms and PGC in flies suppress transcriptional elongation by preventing phosphorylation of RNA polymerase II in the early germ cells (Strome and Lehmann, 2007). In *Caenorhabditis elegans*, the later stages of female germ cell development are controlled by the *MES* (maternal-effect sterile) genes, whose function is to maintain gene repression. MES-2 comprises a SET methyltransferase domain and methylates H3K27; MES-4 specifically methylates H3K36 (Bender et al., 2004, 2006). Similarly, in *Drosophila melanogaster,* germ cell development is also dependent on lysine methyltransferases. Moreover, both in *C. elegans* and *D. melanogaster*, proteins in cytoplasmic structures called P granules are implicated in chromatin repression during germ cell development, although their precise action is still poorly understood. The RNA-binding proteins PIWI and AUBERGINE, for instance, play important roles in silencing of repeat elements through histone methylation, and this function is conserved in mice (see below). P granules also comprise proteins involved in translational repression, which are essential for germ cell development in flies and worms (Thomson and Lin, 2009).

Epigenetic Transitions Guide Meiotic Progression

In mammals, after sex determination has initiated—in the mouse this occurs at around E12.5—DNA methylation patterns are re-established progressively in germ cells. Although its extent and precise timing remain unclear, this de novo methylation is important for meiosis. In male germ cells deficient for the DNA methyltransferase-like protein DNMT3L—a protein that guides the acquisition of de novo methylation—retrotransposons become unmethylated and transcribed and this severely affects meiosis (Bourc'his and Bestor, 2004). Moreover, absence of DNMT3L leads to widespread nonhomologous synapsis at the pachytene stage, possibly because of inappropriate alignment of unmethylated retrotransposon elements, chromosome breaks induced by aberrant retrotransposition events, or altered gene expression. Meiosis in male germ cells is affected also by loss of proteins that control small RNA regulatory pathways (Sasaki and Matsui, 2008). For instance, absence of MILI, a PIWI-like member of the Argonaute family of RNA binding proteins, leads to reduced DNA methylation and activation of LINE1 retrotransposons (Aravin et al., 2007). The similarity between this phenotype and the one caused by DNMT3L deficiency emphasizes the importance of DNA methylation in meiotic progression. Similarly, targeted deletion in the mouse of Heterochromatin Protein 1 gamma (HP1γ) activates LINE1 retrotransposons and is essential for spermatogenesis (Brown et al., 2010), but its link with DNA and histone methylation is unknown.

A functional link between DNA methylation and meiosis was also discovered in female germ cells. Specifically, ablation of the ATP-dependent chromatin remodeling factor *Lsh* (lymphoid specific helicase) gives rise to DNA demethylation and activation of transposable elements in mouse female germ cells. Reduced methylation was seen at tandem repeats and pericentric heterochromatin as well. These changes are associated with incomplete synapsis of homologous chromosomes and developmental arrest at the pachytene stage (De La Fuente et al., 2006). In the mutant female germ cells, double-strand breaks (DSBs) are initially established as in wild-type germ cells. However, the subsequent crossover foci persist in an aberrant manner and this is associated with severe loss of oocytes.

Major histone posttranslational modifications during meiosis have been documented in *C. elegans* and in mice (Nottke et al., 2009; Sasaki and Matsui, 2008). In both species, levels of H3 lysine-4 mono-, di-, and trimethylation and H3K9me2 undergo global changes at meiotic prophase-I and during chromosome pairing and DSB formation and progression. The precise importance of these epigenetic modifications is not known yet. However, several factors that control histone methylation are essential for meiotic transitions, particularly in the male germline (Nottke et al., 2009). Loss of KMT1C (also called G9A, EHMT2), which controls H3K9me1/2 and acts together with KMT1D (GLP), negatively affects pairing of the homologous chromosomes during meiotic prophase in both germlines and hampers gametogenesis progression (Tachibana et al., 2007). A similar phenotype was reported in double knockout mice for the H3K9 methyltransferases KMT1A and KMT1B (SUV39H1 and SUV39H2), which display delayed synapsis and impaired

development to post-pachytene spermatocytes (Peters et al., 2001). MLL2, a H3K4-specific KMT, is also important for the formation and progression of pachytene cells in male mice and affects female gametogenesis as well (Glaser et al., 2009). PRDM9 (also called MEISETZ) is another KMT that mediates H3K4 trimethylation. It is recruited to specific target sequences (see below), and its ablation leads to deficient pairing of homologous chromosomes, meiotic arrest at the pachytene stage, and male and female sterility (Hayashi et al., 2005). These selected examples suggest important roles of H3K4 and H3K9 methylation in meiotic progression, but it remains difficult to distinguish between direct effects and indirect consequences, for instance, resulting from altered gene expression.

Specific lysine demethylases (KDMs) are also expressed during gametogenesis and some are important for meiotic progression (Nottke et al., 2009). In *Drosophila*, the suppressor of variegation SUVAR3.3 (KDM1) demethylates lysine 4 on histone H3. Its absence leads to aberrant H3K4me2 in early germ cells and consequently decreased global levels of H3K9me2/3. This leads to spermatogenic defects in male and absence of oocytes in female flies (Rudolph et al., 2007, and references therein). How increased H3K4me2, mostly at euchromatic regions, induces sterility is not known, but this could be through aberrant expression of essential regulatory genes. In *C. elegans*, targeting of this demethylase leads to progressive male and female sterility over many generations, resulting from failure to erase H3K4me2 in primordial germ cells. This defect causes misregulation of spermatogenesis-expressed genes (Katz et al., 2009). In the mouse, the role of KDM1 (also called LSD1, OAF2) could not be assessed by gene targeting resulting from early embryonic death (Wang et al., 2009). KDM3A (also called JHDM2A) demethylates H3K9 and is important for the organization of postmeiotic chromatin (see below). Undoubtedly, future targeting studies in the mouse will reveal yet other KDMs and KMTs involved in germ cell development and meiosis. A challenge will be to unravel whether phenotypic effects arise because of altered histone methylation or whether alterations of nonhistone target proteins contribute as well. Key insights are also derived from human studies. Some cases of male infertility, for instance, are linked to loss of the lysine demethylase KDM5D (also called SMCY, JARID1D), a demethylase of H3K4me2/3 (Nakahori et al., 1996). Its requirement could be linked to its observed association with MSH5, a meiosis-specific protein that controls synapsis and crossing over (Akimoto et al., 2008).

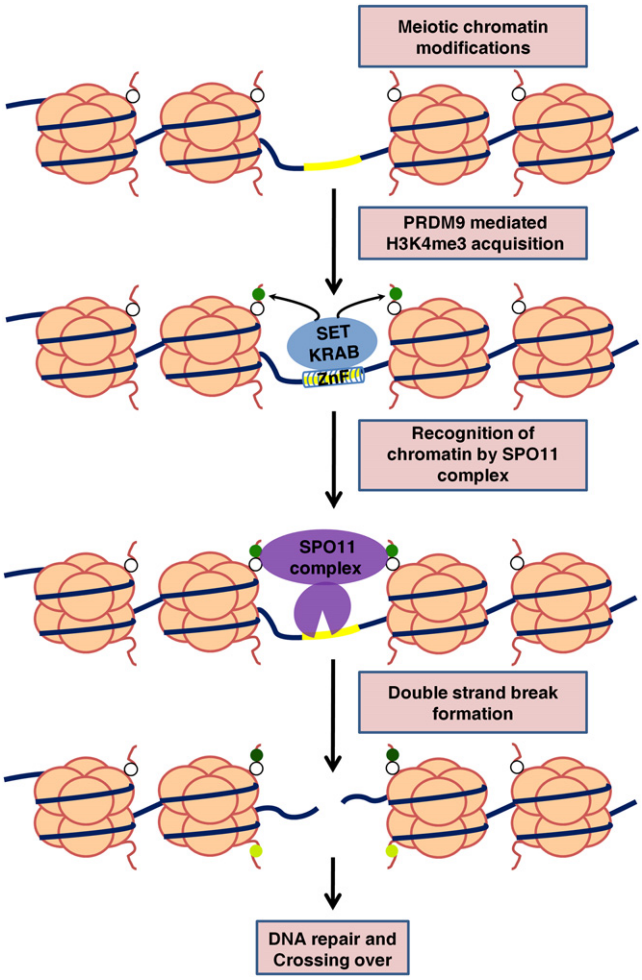

Figure 2. A Histone Methyltransferase Mediates Meiotic Recombination

Through its zinc finger domain (ZnF), the mammalian histone methyltransferase PRDM9 is recruited to recombination hotspots (yellow rectangle), a process that is facilitated by specific histone modifications (white circles) on close-by nucleosomes (large orange composite circles). PRDM9 then catalyzes H3K4me3 (green circles) through its SET domain (blue oval). Possibly together with modifications brought about by the PRDM9 KRAB domain, the newly acquired H3K4me3 mediates recruitment of SPO11 and other proteins of the recombination machinery. This induces the formation of a double-strand break, which, through DNA repair, can be converted into a crossover. Additional acquired histone modifications are indicated by light green circles.

Histone Methylation at Recombination Hotspots

The first division in meiosis is a reductional one, unique to germ cells, during which homologous chromosomes segregate into daughter cells (Figure 1). At the onset of meiosis, during meiotic prophase, homologous chromosomes pair and become stably associated and synapsed through a structure visible as the synaptonemal complex. Recombination events between homologous chromosomes, or crossovers, occur in this context. Crossing over involves repair of double-strand breaks (DSBs), which map to preferred chromosomal locations in budding and fission yeast (meiotic recombination hotspots). Although in mammals DSBs have not been mapped directly, crossovers also cluster at preferential sites. In humans, a high proportion of the recombination hotspots comprise a degenerate, 13-mer sequence. Recent studies in mice show that the initiation sites of recombination are enriched in H3K4me3 and in H3K9 acetylation (H3K9ac) (Buard et al., 2009). This chromatin configuration could guide recombination initiation, a process that involves recruitment of SPO11, a topoisomerase II-like protein that catalyzes DSBs but has no apparent DNA sequence specificity (Figure 2). Concordantly, in *S. cerevisiae* deletion of the H3K4me3 methyltransferase, SET1 strongly reduces recombination at most of the recombination hotspots (Borde et al., 2009). Several other enzymes that control histone modifications influence the initiation of meiotic DSBs as well (Buard et al., 2009), and some modifications appear to diminish the frequency

of recombination. For instance, loss of the transcription factor Ying Yang 1 (YY1) leads to a global decrease in H3K9me3 in mouse spermatocytes, associated with increase in DSBs, which could however be due to deficient repair (Wu et al., 2009). It is unclear to which extent histone alterations associated with meiotic recombination and DSB repair are comparable to those required for DNA damage repair in somatic cells (van Attikum and Gasser, 2009). Specific modifications could, however, facilitate access to the recombination machinery and, perhaps, make it easier to produce long DNA resection intermediates.

Exciting recent papers have revealed how specific sequence elements are recognized during meiosis, leading to local H3K4 hypermethylation of the chromatin and thus specifying the recombination hotspots. This newly discovered mechanism involves PRDM9, a meiosis-specific KRAB-domain zinc finger protein that comprises a SET domain that trimethylates lysine 4 of histone H3 (Baudat et al., 2010; Myers et al., 2010; Parvanov et al., 2010). In humans, PRDM9 is the only known zinc-finger protein that can recognize and bind, through its C2H2 zinc finger domain, to the 13-mer motif at recombination hotspots, depositing H3K4me3 on the adjacent chromatin. H3K4me3 then triggers SPO11-mediated DSB formation (Figure 2), possibly together with other histone modifications mediated by the KRAB domain of PRDM9. Other histone modifications, including histone H4 acetylation, are acquired after DSB formation. Human populations are characterized not only by DNA sequence polymorphism at hotspots, but also by amino acid polymorphism in the PRDM9 protein (Myers et al., 2010). Unfavorable combinations of these two kinds of genetic variation could affect meiotic progression and the formation of mature germ cells (Miyamoto et al., 2008).

Meiotic Silencing of Sex Chromosomes in Male Germ Cells

Chromosomes and chromosomal regions that do not become synapsed during meiosis undergo transcriptional silencing (Kelly and Aramayo, 2007). This silencing mechanism called "meiotic silencing of unpaired chromatin" (MSUC; Figure 1) prevents illegitimate recombination and crossing over at unpaired sites and between nonhomologous chromosomes, events that can trigger apoptosis. MSUC plays an important role in genome defense as well. Chromatin silencing of asynapsed chromosomal regions at the pachytene stage was originally described in the fungus *Neurospora crassa* (Shiu et al., 2001), where it is part of this species' rigorous genome defense system against foreign genetic elements. In *C. elegans* XO males, the single, unpaired X chromosome forms a heterochromatic body in which histone modifications that are normally associated with gene activity are absent, whereas repressive histone modifications, such as H3K9me2, are enriched. Meiotic silencing in *C. elegans* affects unpaired chromosomal regions other than the sex chromosome as well (Bean et al., 2004).

MSUC in *N. crassa* and *C. elegans* requires RNA interference (RNAi) pathways (Kelly and Aramayo, 2007). In mammals, other mechanisms are involved (Baarends et al., 2005; Turner et al., 2005). The best-studied example of MSUC in mammals is the condensation and inactivation of the sex chromosomes during male meiosis, which is cytologically visible as the sex body. This process is referred to as meiotic sex chromosome inactivation (MSCI; Figure 1; Burgoyne et al., 2009). It involves the "coating" of the large, unpaired X-Y bivalent by BRCA1 and then recruitment of the kinase ATR to phosphorylate the histone variant H2AX at serine-139 (γH2AX). The accumulation of γH2AX is an important signal for triggering MSCI (Turner et al., 2004), after which other histone changes occur, including the substitution of the canonical histone H3 by the variant H3.3 (van der Heijden et al., 2007). The sex body is also enriched in the H2A variant macro-H2A1 and in HP1β (Kimmins and Sassone-Corsi, 2005). As a consequence of the histone replacements, certain modifications are lost from the chromatin. MSCI involves acquisition of other histone modifications, including H2A and H2B ubiquitination, the precise function of which remains unclear (Lu et al., 2010). Interestingly, ablation of the ubiquitin conjugating enzyme HR6B gives rise to male infertility resulting from meiotic defects, but female gametogenesis is unaffected (Roest et al., 1996). Small ubiquitin-like modifier (SUMO) proteins have been shown to localize to the sex body as well, but their role is unknown.

As a consequence of MSCI, the X chromosome becomes transcriptionally inactive and depleted of RNA polymerase-II. It was hypothesized, therefore, that MSCI in mammals could be linked to imprinted X inactivation, a process in which the paternally inherited X chromosome becomes inactive in the early embryo (Huynh and Lee, 2003). However, recent studies in the mouse and in marsupials indicate that these two epigenetic phenomena are not linked (see below).

Postmeiotic Chromatin Remodeling in Male Germ Cells

After meiosis II, male germ cells will develop into mature spermatozoa (Figure 3A). This final step is called spermiogenesis and during this remarkable remodeling process, histone proteins are replaced by protamines (Gaucher et al., 2010; Kimmins and Sassone-Corsi, 2005). In mammals, nucleosomal histones are first replaced by small basic proteins called transition nuclear proteins (TNPs) and later by protamine proteins. The transcriptional activation of the genes encoding TNPs and protamines is tightly regulated and involves local chromatin changes, including H3K9 demethylation by KDM3A (also called JHDM2A). Specifically, loss of *Kdma3a* is associated with reduced transcription of *Transition Nuclear Protein 1* (TNP1) and *Protamine 1* (PRM1), both essential for postmeiotic chromatin remodeling in male germ cells (Okada et al., 2007). However, TNPs seem not to be an absolute requirement for histone-to-protamine exchange because in many vertebrate and invertebrate species, this transition occurs in the absence of canonical TNPs. Interestingly, male mice that do not express the two known transition proteins TNP1 and TNP2 still show some replacement of histones by protamines, albeit with aberrant effects on chromatin condensation (Zhao et al., 2004).

During the exchange process, or as a consequence, histone proteins are replaced and eventually might be degraded. In *Drosophila*, histone exchange was found to depend on a testis-specific subunit of the proteasome (Zhong and Belote, 2007).

What orchestrates histone replacement and could this process be linked to the observed incorporation of specific histone variants? Of the known variants of histones H1, H2A, and H2B, the majority are expressed specifically in the testis

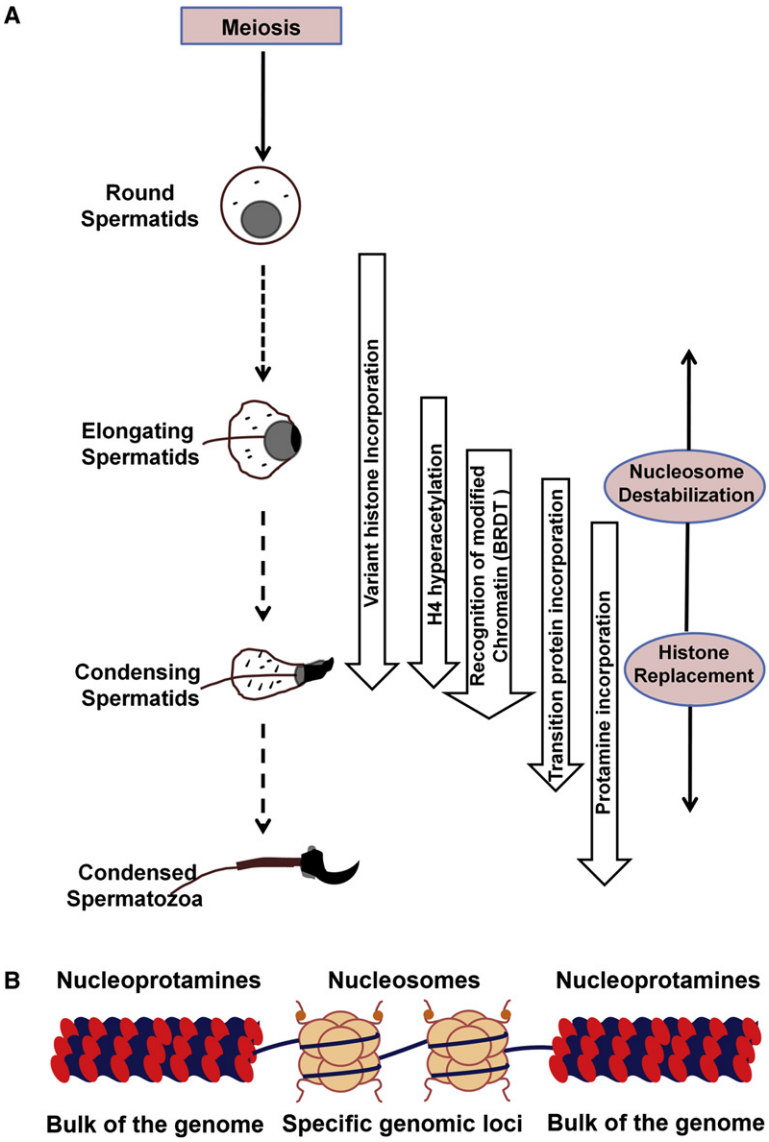

Figure 3. Global Chromatin Remodeling during Spermiogenesis

(A) Postmeiotic male germ cells undergo extensive chromatin remodeling. This involves histone replacement and packaging of the DNA with protamine proteins. The main epigenetic events during spermiogenesis are indicated relative to the morphological transformation during this developmental process. In different mammalian species, these sequential events do not remodel the entire genome and some genes remain associated with histones in mature sperm. The layout of this figure was inspired by Gaucher et al. (2010).

(B) The bulk of the mammalian genome undergoes histone-to-protamine exchange during spermiogenesis and is tightly packaged with protamines (blue strands with red ovals) in mature sperm. However, studies on human and mouse sperm show that DNA at specific gene loci still retains nucleosomes. Histones at these regions are marked by specific covalent modifications (maroon lines with small brown circles). After fertilization, these modifications could be maintained and may mediate specific expression states.

(Gaucher et al., 2010). In vitro studies have shown that incorporation of one or more of these histone variants makes nucleosomes less stable. Therefore, histone variants could facilitate chromatin decondensation and histone removal (or degradation), a hypothesis that needs testing in vivo. Chromatin rdecondensation is thought to be influenced also by covalent modifications on the incorporated variant histones.

Cells no longer divide during spermiogenesis, and thus, the incorporation of histone variants is replication independent. Possibly, this process involves specialized testis-specific chaperone proteins. After and partly overlapping with the incorporation of histone variants, there is acquisition of acetylation on the N-terminal lysines of histone H4 (Hazzouri et al., 2000; Awe and Renkawitz-Pohl, 2010). This hyperacetylation and other changes, including histone ubiquitination, are thought to facilitate the histone-to-protamine exchange, possibly by making the chromatin more accessible. The E3 ubiquitin ligase RNF8 mediates H2A and H2B ubiquitination in elongating spermatids. *Rnf8* targeting in the mouse established that ubiquitination is essential for the acquisition of H4 hyperacetylation and for nucleosome removal (Lu et al., 2010). H4 acetylation could then be recognized by specific nuclear proteins triggering subsequent steps of chromatin remodeling. Proteins comprising bromo domains can bind to acetylated histone tails. BRDT (Bromo domain testis) is specifically expressed in male germ cells and can remodel chromatin in vitro (Pivot-Pajot et al., 2003). Elegant structural and biochemical studies have shown that one of its two bromo domains recognizes the histone H4 tail acetylated on both lysines 5 and 8, an epigenetic state that occurs in hyperacetylated H4 (Morinière et al., 2009). Deletion of this unusual bromo domain in the mouse leads to aberrant spermiogenesis from the elongating spermatid stage onward and, consequently, infertility (Shang et al., 2007). It remains to be discovered how precisely BRDT binding to hyperacetylated H4 contributes to spermiogenesis and histone-to-protamine exchange.

Histone-to-protamine exchange does not occur to the same extent in different species (Gaucher et al., 2010). In humans and mice, protamines are clearly essential for postmeiotic germ cell differentiation. In contrast, deletion of both protamine genes in *Drosophila* still allowed the development of motile and fertile spermatozoa (Rathke et al., 2010). However, sperm cells had an unusual nuclear organization and were susceptible to radiation-induced DNA breaks. This phenotype supports the hypothesis that histone-to-protamine exchange helps protect the sperm genome against damage induced by mutagens (Rathke et al., 2010). Another intriguing question is whether the entire sperm genome undergoes histone-to-protamine exchange and whether this could be linked to histone variants. In the mouse, incorporation of the histone variants H2AL1 and H2AL2 occurs in part of the genome. Their incorporation and retention gives rise to unusual nucleosome-like structures, and these might play a role in conferring paternal epigenetic

information to the zygote (Govin et al., 2007). Recent studies on human and mouse spermatozoa show that many regions (together accounting for several percent of the genome) retain nucleosomal histones. This includes loci comprising key developmental genes, imprinted genes (see below), microRNAs, and homeotic genes (Hammoud et al., 2009; Brykczynska et al., 2010). The retained histones were enriched in H3K4me3 and the DNA was unmethylated. Repressive H3K27me3 was found at many of the histone-retaining gene loci as well. After fertilization, these epigenetic modifications could be transmitted to the embryo and may contribute to appropriate developmental gene expression (Figure 3B). The recent studies extend earlier observations on the overall and locus-specific presence of histones in sperm and their transmission to the zygote (Gardiner-Garden et al., 1998; Wykes and Krawetz, 2003; Govin et al., 2007; van der Heijden et al., 2008). Whether genetic variation between humans influences the extent of the histone-to-protamine exchange in the sperm genome is not well known. This is a relevant question to explore in relation to sperm development and male fertility. For instance, in sperm samples from subfertile men with sperm abnormalities, abnormally high histone contents are frequently detected and this could have an effect on the transmission of epigenetic information (Ramos et al., 2008).

Epigenetic Germ Cell Marks that Control Genomic Imprinting

Genomic imprinting was first discovered in sciarid flies (Diptera, Sciaridae). During the early cleavage stages, all somatic cells eliminate one or both of the paternally inherited X chromosomes, depending on the sex of the fly embryos. Additionally, in male flies, the whole paternal chromosome complement is extruded from the cells at the onset of meiosis. The underlying epigenetic mechanism remains unclear, but immunocytological work in *Sciara ocellaris* suggests involvement of histone modifications. In the early somatic cells, the paternally inherited chromosomes are decondensed and have much higher levels of H3 and H4 acetylation than the maternally inherited chromosomes. Conversely, H3K4me3 is detected on the maternal but not on the paternal chromosomes (Greciano and Goday, 2006). The role of these differential modifications, and whether they originate from the germ cells, is unclear. Also in mealybugs, which are coccid insects, the sperm-inherited genome is imprinted (Khosla et al., 2006). In one group of mealybugs, the entire set of paternal chromosomes becomes heterochromatic and repressed during male development, whereas in females both of the parental genomes remain active. DNA methylation seems not to represent the paternal silencing mark, as shown by the fact that the paternal chromosomes are hypomethylated compared to the maternal ones. On the other hand, recent research on *Planococcus citri* (Bongiorni et al., 2009) shows that H3K9me2 and H3K9me3 become globally enriched toward the end of spermatogenesis (but not during oogenesis) and are still detectable on the paternal genome in the zygote. Histone methylation could thus be the paternal signal involved in insect imprinting.

In mammals, genomic imprinting is known to lead to the parental allele-specific expression of more than a hundred autosomal genes in the embryo. A recent study of mouse brain (Gregg et al., 2010) suggests that detailed tissue-specific analysis could lead to the discovery of many more imprinted genes.

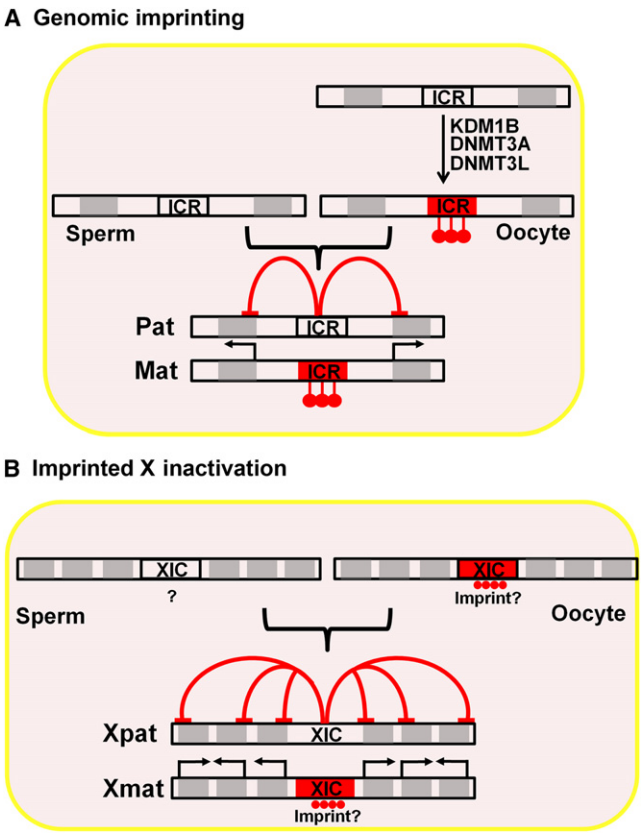

Figure 4. Gamete-Specific Epigenetic Marks Control Imprinting and X Inactivation

(A) Imprinted gene expression in mammals is controlled by "imprinting control regions" (ICRs). Most ICRs acquire their parental allele-specific DNA methylation during oogenesis, as in the shown example. This involves DNMT3A, which is recruited by DNMT3L to the chromatin. The latter requires prior demethylation of H3K4 by KDM1B at several ICRs. After fertilization, the methylation imprint (lollipops) is somatically maintained and inactivates the ICR on the maternal copy. On its unmethylated paternal copy, the ICR mediates repression of close-by genes (gray boxes). The chosen example depicts the situation at some imprinted domains, at which allelic silencing is mediated by a noncoding RNA (ncRNA) transcribed from the ICR. At other domains, ICRs mediate imprinted expression differently (Koerner et al., 2009).

(B) Imprinted X inactivation in the mouse arises during preimplantation development and persists in the extraembryonic tissues. It is probably controlled by an oocyte-inherited epigenetic imprint (red circles), which prevents the "X inactivation center" (XIC) to silence genes in *cis* on the maternal chromosome. Although its nature is unknown, in the mouse the imprint prevents expression of *Xist*, the ncRNA that mediates gene silencing on the X chromosome.

The process also influences X chromosome inactivation in female embryos. In the extraembryonic tissues of female embryos, the paternally inherited X is silenced (Figure 4; Okamoto et al., 2004). In contrast, in the embryo proper, there is random X inactivation. Both kinds of X inactivation depend on the X inactivation center (XIC). This genetic locus comprises the *Xist* gene, which transcribes a noncoding RNA that mediates chromatin silencing along the X chromosome (Senner and Brockdorff, 2009). In marsupials there is imprinted X inactivation as well, but in this group of animals it occurs in both the embryo and the extraembryonic membranes. As in the mouse, it is the paternally inherited X that is inactivated. Because in marsupials

there is no equivalent of the *Xist* gene (Duret et al., 2006), the underlying mechanism could be different. It is not excluded, however, that an "Xist-like" noncoding RNA confers X inactivation in the metatherian lineage.

An appealing hypothesis implied that imprinted X inactivation involved inheritance of an already repressed X chromosome from the sperm and that the process was initiated through MSCI (Huynh and Lee, 2003). However, recent studies in mice and in the marsupial *Monodelphis domestica* show that, after MSCI, many of the X chromosome genes become reactivated and that in the early embryo the paternal X is then silenced again (Mahadevaiah et al., 2009; Okamoto et al., 2004). In experiments in which *Xist* was transgenically inserted into autosomes, these transgenes did not undergo meiotic silencing, but upon paternal inheritance, they nevertheless gave rise to imprinted silencing in early female embryos (Okamoto et al., 2005). An alternative proposal is that imprinted X inactivation is mediated by an epigenetic mark that keeps the maternal X chromosome active by preventing X inactivation to occur. Indeed, in Robertsonian translocations in which zygotes with two maternal X chromosomes were generated, both remained active in the early embryo and the trophoblast (Goto and Takagi, 1998). The nature of this maternal imprint is unknown, but it was shown not to require acquisition of de novo DNA methylation (Chiba et al., 2008). In mice, the epigenetic maternal imprint could possibly activate expression of *Tsix*, an antisense transcript that prevents expression of *Xist* RNA. Conversely, the early embryonic expression of *Xist* from the paternal chromosome could be facilitated by its promoter being nucleosomally organized in mature sperm (Hammoud et al., 2009). Although a recent study suggests that the initiation of paternal X inactivation in the early mouse embryo would not depend on *Xist* RNA expression (Kalantry et al., 2009), the process as such clearly does. The exciting recent studies reveal unexpected similarities between mice and marsupials but leave unanswered the intriguing question of which gametic epigenetic mark(s) controls imprinted X inactivation in different species.

More is known about the epigenetic marks that control imprinted gene expression on autosomal chromosomes. Imprinted mammalian genes are expressed from either the maternally or the paternally inherited copy and are clustered in domains comprising up to several megabases of DNA. Imprinted expression along each of these gene clusters is mediated by an "imprinting control region" (ICR). These CpG-rich regulatory elements (most correspond to CpG islands) are marked by DNA methylation and repressive histone methylation on one of the two parental alleles (Delaval et al., 2007). At most ICRs, the allelic DNA methylation comes from the mother, and the establishment of these maternal imprints occurs in meiotic, growing oocytes (Figure 4). At only a few ICRs, the DNA methylation imprint originates from sperm and is established before meiosis, during fetal development. In both germlines, the acquisition of the imprints at ICRs involves the de novo DNA methyltransferase DNMT3A (Kaneda et al., 2004). Recruitment of DNMT3A to its ICR target sequences is mediated through binding to DNMT3L, a DNMT-like protein without catalytic activity (Bourc'his et al., 2001; Kato et al., 2007).

How are DNMT3A-DNMT3L complexes recruited to specific ICRs in only one of the two germlines? Could chromatin features be involved in this process? DNMT3L can bind to H3 but not when this histone is methylated at lysine-4 (Ooi et al., 2007). Accordingly, ICRs that do not gain DNA methylation in the male germline were found to be enriched in H3K4 methylation at pre- and postmeiotic stages of spermatogenesis (Delaval et al., 2007; Hammoud et al., 2009). In the female germline, recent work shows that the H3K4-specific lysine demethylase KDM1B (also called OAF1) is essential for the acquisition of DNA methylation imprints at several ICRs (Ciccone et al., 2009). It remains to be explored whether other histone modifications facilitate the acquisition of methylation imprints. Furthermore, at least for one ICR in the female germline, there is evidence that transcription is involved in imprint acquisition (Chotalia et al., 2009).

It is becoming clear that DNA methylation is not the sole feature of gametic imprints. *DNMT3L*-deficient female mice do not establish DNA methylation imprints in their oocytes. However, in some of their offspring, the maternal methylation imprints were found to be normally present at the *Snrpn* ICR and several other ICRs (Henckel et al., 2009). Somehow, the DNA methylation machinery had recognized the right parental allele in the early embryo, possibly through an oocyte-derived chromatin mark. This is interesting relative to studies indicating that the human *SNRPN* ICR acquires its maternal DNA methylation after fertilization only (El-Maarri et al., 2001; Kaufman et al., 2009). In one other study, however, the human *SNRPN* ICR was found to become methylated during late oogenesis (Geuns et al., 2003), as it does in the mouse. In mouse studies on the ICR controlling the *Igf2* gene, insertion into ectopic sites often led to the expected paternal allele-specific DNA methylation. Intriguingly, the methylation was acquired after fertilization only (Matsuzaki et al., 2009), indicating that another epigenetic signal was inherited from the sperm. One future challenge will be to unravel which chromatin features other than DNA methylation constitute the germ cell imprints in genomic imprinting and in imprinted X inactivation and how these are regulated.

Outlook

In this review, we discussed chromatin transitions during gametogenesis and emphasized the importance of posttranslational histone modifications and the essential role of DNA methylation. Many histone methyltransferases and histone demethylases have been identified during the last years, several of which are expressed at specific stages of germ cell development. Gene targeting studies on these factors have provided important insights into the roles of histone methylation in gametogenesis and meiosis. Among many examples in mammals (Table 1), we discussed the essential involvement of PRDM9 in meiotic recombination and that of KDM1B in imprint establishment in oocytes. Given their specific developmental patterns of expression and their enrichment on certain chromosomal regions, histone variants are likely to be key players in gametogenesis as well. As for covalent histone modifications, histone variants could be team players, because it has been observed during spermiogenesis, when different histone variants become incorporated into the chromatin. Such concerted action may complicate the interpretation of functional studies that explore individual variants. The ultimate challenge will be to understand how changes in histone modifications and variants, together with

Table 1. Epigenetic Regulators of Mammalian Gametogenesis and Meiosis

Protein	Alternative Name(s)	Substrate/ Interactant	Mouse Knockout Phenotype	Reference
KMT1A /KMT1B	SUV39H1/SUV39H2	H3K9	meiotic defects, male >and female infertility	Peters et al., 2001
KMT1C	G9A	H3K9	meiotic defects, male and female infertility	Tachibana et al., 2007
KMT2B	MLL2	H3K4	male and female infertility	Glaser et al., 2009
PRDM9	MEISETZ	H3K4	meiotic arrest, male and female infertility	Hayashi et al., 2005
KDM1B	LSD2	methylated H3K4	defects in establishment of maternal imprints	Ciccone et al., 2009
KDM3A	JHDM2A	methylated H3K9	defects in postmeiotic chromatin condensation, male infertility	Okada et al., 2007
UBR2	-	HR6B-H2A	meiotic defects, male infertility	An et al., 2010
RNF8	-	γ-H2AX	defects in histone-to-protamine exchange, male infertility	Lu et al., 2010
HR6B	UBE2B	H2A	male infertility	Roest et al., 1996
LSH	HELLS	chromatin	meiotic defects, female infertility	De La Fuente et al., 2006
DNMT3A	-	DNA	lack of imprint establishment, male and female infertility	Kaneda et al., 2004
DNMT3L	-	DNA	lack of imprint establishment, male and female infertility	Bourc'his et al., 2001; Kato et al., 2007
MILI	PIWIL2	piRNA	meiotic arrest, male infertility	Aravin et al., 2007
MIWI	PIWIL1	piRNA	defects in sperm development, male infertility	Deng and Lin, 2002
MIWI2	PIWIL4	piRNA	meiotic arrest, male infertility	Carmell et al., 2007
BRDT	BRD6	acetylated H4	abnormal sperm, male infertility	Shang et al., 2007

other chromatin features such as binding of nonhistone proteins, contribute to key transitions during spermatogenesis and meiosis, at single loci and genome wide.

Developmental transitions in histone modifications, histone variants, and DNA methylation have been detected often through elegant immuno-fluorescence approaches. Although in many cases, observed global transitions agreed with locus-specific studies, each of these approaches has its limitations. Given the difficulty in obtaining enough germ cells of high purity at different developmental stages, particularly during oogenesis, it has been a challenge to perform high-throughput chromatin studies. Improvements in germ cell purification and chromatin immunoprecipitation (ChIP) procedures are being proposed, including "carrier ChIP" and ChIP on limited numbers of cells. Together with genome-wide amplification strategies and the use of next generation sequencing approaches, they will facilitate the generation of epigenetic maps of histone modifications and chromatin regulatory factors. The recent work on histone-retaining regions in mature sperm provides a nice example of the use of high-throughput sequencing approaches (Hammoud et al., 2009), but for many stages of gametogenesis, cell numbers seem still too limited for the current technologies. Single-molecule high-throughput sequencing, which is already coming into use, could provide an answer when looking at very small amounts of starting material. For the male germline, the in vitro culture of spermatogonial stem cells (Kanatsu-Shinohara et al., 2004) and methodologies that aim at differentiating ESCs into sperm (Nayernia et al., 2006) could provide alternative means to explore chromatin in higher numbers of cells.

Chromatin transitions in germ cells are still poorly understood compared to those in somatic cells, and many important questions remain to be addressed. Given the growing interest in this field, during the coming years undoubtedly many new insights will emerge on the role of epigenetic modifications and the machineries that control key transitions in female and male gametogenesis. Novel data from different model systems will facilitate research on pathologies that are linked to aberrant gametogenesis and infertility in humans.

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