Chromatin remodelling and epigenetic features of germ cells

Sarah Kimmins & Paolo Sassone-Corsi

Institut de Génétique et de Biologie Moléculaire et Cellulaire, B.P. 10142, 67404 Illkirch, Strasbourg, France

Germ cells have the unique capacity to start a new life upon fertilization. They are generated during a sex-specific differentiation programme called gametogenesis. Maturation of germ cells is characterized by an impressive degree of cellular restructuring and gene regulation that involves remarkable genomic reorganization. These events are finely tuned, but are also susceptible to the introduction of various types of error. Because stable genetic transmission to future generations is essential for life, understanding the control of these processes has far-reaching implications for human health and reproduction.

Cells transmit information to the next generation via two distinct routes, genetic and epigenetic. While genetic inheritance is based on the DNA code, epigenetic information comprises modifications occurring directly on DNA or on the chromatin, a proteinaceous complex associated with DNA. Whereas the major type of DNA modification is the methylation at cytosines, there are multiple modifications associated with chromatin. Their inheritability has been demonstrated only in rare cases[1,2].

The basic unit of chromatin, the nucleosome, is composed of a histone octamer that includes the histones H2A, H2B, H3 and H4. Adjacent nucleosomes are connected by histones of the H1 linker class. Various covalent modifications occur on specific residues of histones, including methylation, phosphorylation, acetylation and ubiquitination. Combinations of these are thought to contribute in various ways to chromatin organization and gene expression. Several excellent review articles on the molecular mechanisms of epigenetic control constitute useful further reading[3–6].

In the lifetime of a mammal, two periods are characterized by epigenetic reprogramming—gametogenesis and early embryonic development. During spermatogenesis, global sex-specific changes to the epigenome occur as a wave of DNA demethylation, followed by DNA methylation and chromatin modifications[7,8]. This may contribute to a unique feature of the germ line—the control of gene function from one generation of an organism to the next.

The epigenetic control of gene function in germ cells follows highly specialized programmes. In particular, there are striking sex-specific differences in the development of male and female gametes, and in the epigenetic state of their genome[7,8]. During gametogenesis the male- and female-specific epigenetic programmes are 'reset', a remodelling of the epigenome, to allow the gametes to fuse at fertilization and give rise to a zygote that is totipotent and thus able to give rise to any cell type. The fidelity of the resetting process is important for preventing aberrant epigenetic modifications that can be passed on to the next generation. Hence, the implications for human health are profound. Male and female germ cells from parents who had a failure in epigenetic reprogramming—from either undergoing assisted reproductive manipulations[9] or exposure to harmful environmental or chemical factors—produce offspring with a greater susceptibility to disease[10].

Germ cells differ from somatic cells in that they exhibit a number of specialized regulatory pathways, either by expressing specific transcription factors or isoforms. These might form unique regulatory complexes that may involve specific chromatin components unique to germ cells. Does the highly dynamic, sex-specific nature of chromatin remodelling in germ cells contribute to these differences?

Indeed, germ cells possess a remarkably diverse set of histone variants[11]—highly abundant proteins thought to be important for chromatin organization. One interesting sex-specific difference is that in the male many variants are expressed in a highly regulated temporal manner, underscoring that spermatogenesis has evolved in a different fashion from oogenesis. In addition, maturing sperm cells undergo an extraordinary process of chromatin remodelling called the histone-to-protamine transition, which reshapes the nucleus and compacts chromatin in an unparalleled architecture. These events prepare germ cells for fertilization and thereby constitute essential regulatory processes. In this review we bring into focus the molecular players and regulatory pathways involved in chromatin remodelling and gene regulation in germ cells. The epigenetic programme of male germ cells is especially emphasized because it shows characteristics that are highly distinct from somatic cells.

Sex-specific routes to haploidy

Sex-specific differences in the development and cellular organization of male and female gametes begin as early as meiosis, the reductive cell division through which haploidy is achieved. In the male, early spermatocytes undergo the S (synthesis) phase of the cell cycle, giving rise to tetraploid spermatocytes, marking the beginning of meiotic prophase. At this point, the homologous chromosomes pair up and become joined by a proteinaceous scaffold called the synaptonemal complex[12]. At this time, genetic recombination occurs and paired chromosomes align on the metaphase plate for segregation of sister chromatids into two daughter cells.

After meiosis, spermatogenesis follows a tightly regulated programme during which, under the control of the hypothalamic–pituitary–gonadal axis, major morphological and biochemical changes occur, including cytoplasm elimination and nuclear reshaping. Chromatin remodelling in late spermiogenesis is particularly impressive. During this process, most somatic histones are replaced by DNA packaging proteins that are unique to male germ cells—namely the transition proteins, which are later replaced by the protamines. The incorporation of protamines into sperm chromatin induces DNA compaction, and is followed by cytoplasmic ejection, and acrosome and flagellar formation[8].

The female gamete needs to produce factors that are involved in metabolism and early development and therefore, in contrast to the sperm, which ejects most of its cytoplasm, has evolved an enriched cytoplasm. At the first cellular division of the primary oocyte the metaphase spindle is polarized at one end of the cell, resulting in the production of two daughter cells, one large with much cytoplasm (secondary oocyte), and the other with very little cytoplasm (the polar body) (Box 1). In meiosis II, similar mechanisms operate to ensure that the mature egg conserves a large cytoplasm[12].

© 2005 Nature Publishing Group

Unique chromatin architecture in male germ cells

Spermatogenesis is characterized by a particularly spectacular chromatin remodelling process, in which somatic linker histones are sequentially replaced by testis-specific variants, followed by the replacement of most histones with protamines. Are the germ cell-specific histones and special chromatin organizing proteins that are required for these events unique to meiosis? Are they involved in the ability of sperm to fertilize the egg and do they thereby give rise to embryos? In this section we explore the unusual chromatin composition of the mammalian male germ cell and consider its role in sperm development and function.

The transition proteins

Transition proteins are thought to prepare the chromatin for association with protamines, possibly by influencing DNA condensation[13]. During development of the post-meiotic haploid spermatids, transition proteins become a significant chromatin component. After histone removal and before protamine deposition, transition proteins constitute 90% of all chromatin basic proteins. The best characterized of these proteins, transition protein 1 and transition protein 2, represent about 55% and 40% of spermatid total nuclear proteins, respectively[13]. Transition protein 1 is a small basic protein of 54 residues, rich in arginine, lysine and serine. Twice the size of transition protein 1, transition protein 2 is enriched in basic residues in its carboxy terminus and contains two putative zinc fingers in the amino-terminal region. Mice mutants for transition proteins 1 and 2 are able to produce offspring, although with reduced fertility, suggesting overlapping roles of these proteins[14,15].

What are the signals that trigger the incorporation of transition proteins into chromatin? How is the timing of this event coordinated with histone replacement? Transition proteins become phosphorylated in their basic domain at synthesis, followed by dephosphorylation that seems to facilitate DNA binding and chromatin condensation. In transition protein 2, both Ser 109 and Thr 101 are potential phosphoacceptor sites for the cAMP-dependent PKA (protein kinase A)[16]. Notably, phosphorylated transition protein 2 is associated with less condensed DNA, which might facilitate protamine entry[16]. It is believed that histone H4 hyperacetylation facilitates the transition to protamines[17]. While it is unclear how H4 hyperacetylation may elicit this function, it is notable that CDY, a Y-chromosome encoded histone acetyltransferase protein, is testis-specific and readily acetylates H4 (ref. 18).

The protamines

Protamines are small proteins (relative molecular mass 4,000–12,000) that are evolutionarily related to histone H1 (refs 19, 20), but have significantly different biochemical properties. Somatic histone H1s have lysine-rich N- and C-terminal tails, and very low arginine content. In contrast, protamines have very low lysine content, and more than 50% of their residues are arginine, which is probably responsible for their high DNA-binding affinity. This can be attributed to the fact that arginine has a greater flexibility in the formation of hydrogen bonds with the DNA backbone owing to its complex guanidinium group[21]. It seems likely that during the evolution of protamines from H1 histone, protamines acquired higher arginine content, which may have resulted in their improved chromatin condensing properties.

Most mammals express only protamine 1, whereas mice and humans express two protamines. Their structures differ and disruption of either gene in the mouse results in male infertility[22]. Similar to transition proteins, protamine phosphorylation seems to lead to incorporation into DNA[19]. Protamine 2 undergoes phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase IV (CamK4), and disruption of the *CamK4* gene in the mouse results in failure to exchange transition protein 2 for protamine 2, and subsequently blocks germ cell differentiation[23]. However, CamK4 may not be solely responsible. Another candidate kinase is the

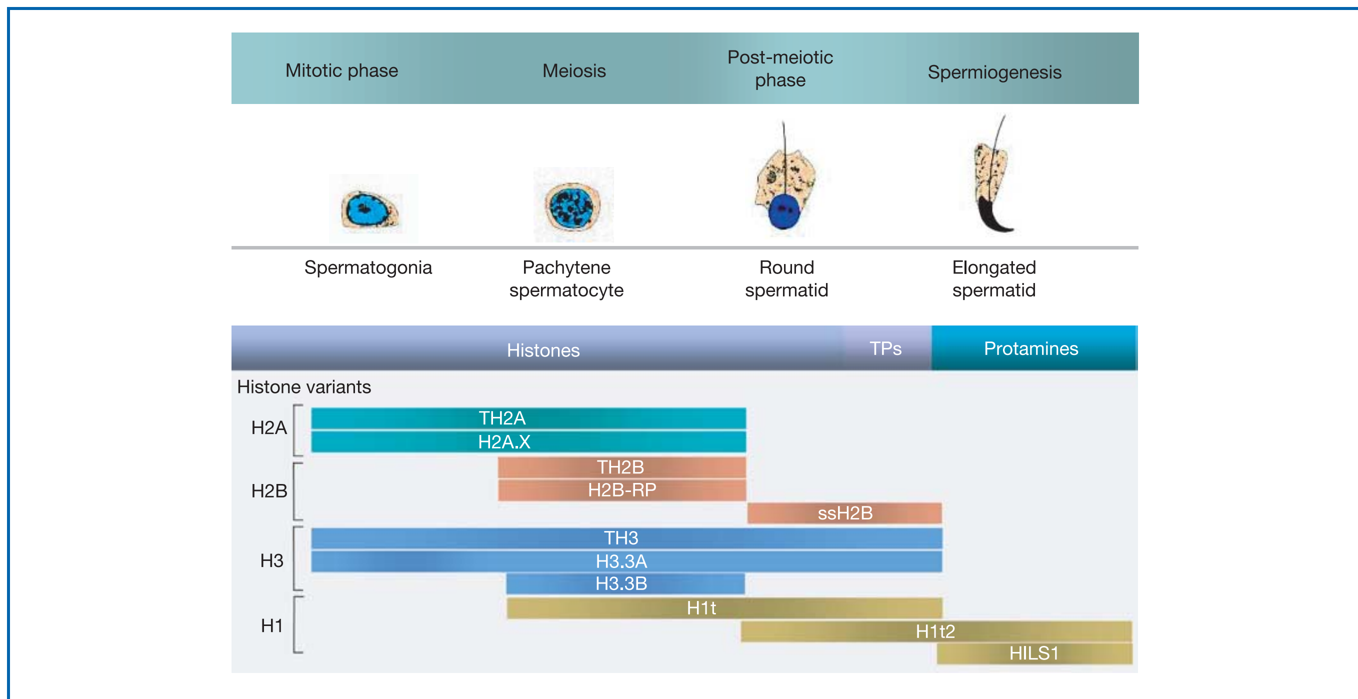


Figure 1 Unique chromatin remodelling during the development of male germ cells. Spermatogonia generate preleptotene spermatocytes, which enter meiosis. Next, round spermatids enter the spermiogenic phase. Germ cells contain many histone variants, which are expressed at different stages of male germ cell development. During the last phase of spermiogenesis, chromatin is highly compacted, a process that includes the replacement of most histones with sperm-specific transition proteins (TPs), which are subsequently replaced by protamines. Coincident with chromatin remodelling and reshaping of the sperm head is the formation of acrosome and tail. For details of histones ssH2B see ref. 78, and for H2B-RP see GenBank accession number AAP57490.

© 2005 Nature Publishing Group

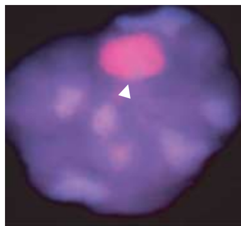
cAMP- and Ca^{2+}-independent casein kinase II (CK2). The CK2α′ catalytic subunit isoform is preferentially expressed in male germ cells and its ablation in the mouse results in spermatogenic defects, including morphologically abnormal sperm[24]. Whatever the signalling pathways involved, the timing of the histone-to-protamine transition is a finely tuned event. Indeed, premature expression of protamine 1 in transgenic mice leads to precocious chromatin condensation[25].

A multitude of histone variants

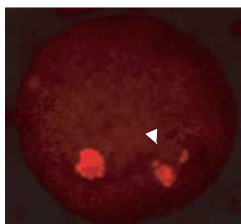
Male germ cells have an unusually high number of histone variants in comparison to somatic cells (Figs 1–3). There are two types of variants: those like the testis-specific histone H4, with minor or no amino-acid differences from the somatic H4 (ref. 26); and variants with different amino-acid sequences and structure, such as H2A.X (ref. 27). Several characteristics distinguish variants from their somatic counterparts. First, their messenger RNA synthesis is often uncoupled from DNA replication, and accordingly they lack a stem-loop in the transcript 3′ end, a structure classically required for cell-cycle-regulated degradation[28]. Instead, transcripts encoding variants carry longer poly-A tails, which increases their stability[29]. Second, histone variant genes are not organized in clusters, but are solitary and may contain introns[30].

Incorporation of variants into the nucleosome could influence gene regulation. Indeed, H2A.Z and H3.3 participate in distinct nucleosome assembly pathways, possibly specific for DNA-synthesis-independent chromatin deposition. H2A.Z is deposited by the SWR1 complex[31], whereas H3.3 is deposited by the HIRA complex[32]. This specificity implies that 'variant' nucleosomal octamers could be deposited in a highly regulated fashion, so that their position would impose inheritable boundaries of active or inactive chromatin.

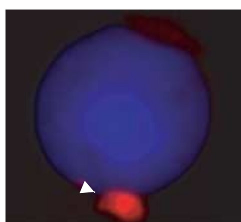
Box 1



Sex (XY) body
The sex chromosomes of mammalian spermatocytes form a specialized nuclear territory known as the XY (or sex) body, where both transcription and homologous recombination are restricted. Proteins assembled into the XY body are typical of heterochromatin. Here the XY body is marked in a pachytene spermatocyte using an antibody against methylated histone H3-Lys 9 (arrowhead).



Polar body
The polar body is a minute structure that separates by karyokinesis from the ovum during its maturation. In the maturation of ordinary ova two polar bodies are formed. The first is usually larger than the second one, and often divides into two after its separation from the ovum. Each of the polar bodies removes maternal chromatin from the ovum to make room for the chromatin of the fertilizing spermatozoa. The polar body exhibits phosphorylation of histone H3 on Ser 10 (P-H3), a hallmark of chromosome condensation during mitosis and meiosis. Pictured is an oocyte collected 16 h after ovulation and immunolabelled for P-H3 (arrowhead).



Chromatoid body
The chromatoid body is a germ cell-specific cytoplasmic organelle that closely interacts with the nucleus and contains compacted mRNA. Its localization is shown in a spermatid that has been immunolabelled for the mouse *VASA* homologue, MVH, an RNA helicase (arrowhead). Some intriguing observations suggest that the chromatoid body could function to monitor mRNA localization.

Histone H3 variants

Germ-cell-specific histones appear in spermatogonia[33], and later in spermatids[34,35], but the majority are synthesized and incorporated into chromatin during meiosis (Fig. 1). H3.3A is an interesting variant that is incorporated into the germ line, and is present during phases of development from spermatogonia to spermatid[33]. By prophase I, histone H3 is largely replaced by H3.3A and H3.3B, which apparently associate with euchromatin[33,36], suggesting a role in the massive transcription programme in spermatocytes[8]. Indeed, H3.3 is relatively enriched in modifications associated with transcriptional activation and is deficient in dimethyl Lys 9 (ref. 37), a modification associated with silencing and heterochromatin formation[3]. Finally, as H3 phosphorylation at Ser 10 is coupled to mitotic chromosome condensation[38], it is appealing to speculate a role for phosphorylated H3 variants at meiosis.

Of all the histone variants, CENP-A (centromere protein A) possesses features suggestive of a critically important role in epigenetic function. Located at the centromere, it is a divergent paralogue of H3, with which it shares very little homology in the N-terminal tail (Fig. 2). CENP-A is not displaced during the histone-to-protamine transition and thus behaves as an inherited element, conserved in the two daughter chromatids during S phase. Interestingly, clear differences can be spotted in N-terminal tails between somatic histones and germ-cell-specific variants (Fig. 2). Despite the divergences with H3, human CENP-A undergoes phosphorylation by Aurora-B at Ser 7 (ref. 39), a residue absent in mouse CENP-A, which may instead become phosphorylated at Ser 15. The inheritability of CENP-A hints at tantalizing roles in fertilization.

Histone H2A and H2B variants

The H2A and H2B variants possess notable differences in their N-terminal tails. For example TH2B, a testis-specific variant of H2B (ref. 40), differs by the addition of three potential phosphorylation sites (Ser 12, Thr 23 and Thr 34) and the repositioning of two others (Ser 5 and Ser 6), resulting in a different 'phosphorylation map' of the N-terminal tail (Fig. 2). Germ cell kinases, such as testis-specific isoforms of protein kinase C[41] and Aurora-C[42], could potentially target TH2B through these sites. In addition, in the context of the binary switch hypothesis of combined histone modifications[3], the insertion of specific phosphoacceptor sites in TH2B generates combinatorial associations of lysine, serine and threonine residues, which could impart unique patterns of acetylation and/or methylation (Fig. 2). TH2B nuclear distribution seems unequal, suggestive of association with specific chromatin domains[40].

In H3.3, the Thr–Lys and Lys–Ser binary sites are perfectly conserved with respect to H3, although an Ala > Ser change (Ser 31) in H3.3 generates a putative phosphoacceptor site for cell-cycle-regulated kinases (Fig. 2). In H3.3, a specialized distribution of methylation versus acetylation modification is indicative of association with transcriptionally active chromatin[37], and suggests that it functions in post-meiotic gene expression.

The case of H2A.X is particularly interesting. A single Glu > Thr change (Thr 7) generates a potential binary site that might be targeted by a testis-specific kinase. In addition, the Ser 139 phosphoacceptor site in H2A.X is a target for kinases of the PI3 family, including DNA-PK[43], ATM (ataxia telengiectasia mutated)[44] and the ATM-related protein, ATR[45]. H2A.X is implicated in meiosis and is thought to be involved in chiasmata formation because of its

© 2005 Nature Publishing Group

function at sites of double-strand-breakage[46]. Importantly, H2A.X deficiency results in arrested spermatogenesis at the pachytene stage[46], a phenotype reminiscent of the ATM-null mice, which show severe meiotic disruption[47]. ATM and ATR kinases are involved in the cellular response to ionizing radiation and DNA double-strand break-inducing events. ATM also targets the cohesin SMC1, p53 and the checkpoint kinase Chk2 (ref. 48), stressing its implicated role in meiotic chromosome dynamics. ATM is present in the spermatocyte cellular compartment, known as the XY body or sex body, where the X and Y chromosomes are unsynapsed (Box 1).

The sex body

The sex body is a nuclear compartment in germ cells where RNA polymerase II is absent[49], and where selective inactivation of the sex chromosomes occurs. In male meiosis, the heteromorphic X and Y chromosomes undergo the condensation process of heterochromatinization, accompanied by transcriptional silencing. It is believed that the sex body excludes promiscuous pairing or recombination between nonhomologous chromosomes, thereby reducing the risk of aneuploidy[49].

The sex body contains a number of proteins that are implicated in heterochromatinization, including the H2A histone variant, macroH2A1, and heterochromatin protein 1 (HP1β)[50]. Both are known to be involved in meiotic sex chromosome inactivation (MSCI) and formation of the XY body. Another feature of the sex body is ubiquitination of H2A, which suggests it has a role in gene silencing[51].

The special case of the histone H1 family

H1 linker histones influence the degree to which chromatin folds, by virtue of their association with the DNA-connecting nucleosomes. Proteins of the H1 family greatly diverge in their structure, and several are germ-cell-specific variants (Fig. 3). The C termini of the variants are highly different, which may influence the degree to which they bind chromatin[52]. Additional α-helices are predicted to form in the C terminus of H1t2, HILS1 and H1Foo (Fig. 3), which could influence interaction with nuclear components.

The two testis-specific H1 variants, H1t (ref. 53) and H1t2 (ref. 35), show a high degree of identity. The restructuring of sperm chromatin begins during meiotic prophase when the somatic linker histones, H1A and H1B, are displaced by the variant H1t (ref. 54). Targeted deletion of H1t or H1.1 does not affect fertility, suggesting functional redundancy[53,55].

Another testis-specific linker histone is HILS1 (histone H1-like protein in spermatids 1), which is restricted to elongating spermatids[56]. The nuclear distribution of HILS1, transition protein 2 and protamine 1 (ref. 56) is identical, suggesting that HILS1 is intimately linked to chromatin condensation—a process that may involve histone kinases[3] for which there are multiple potential sites in HILS1. Importantly, of the five Ser/Thr cyclin/CDK sites in somatic histone H1 that seem to be important for dynamic nuclear mobility, several are absent in the variants, particularly H1t and H1t2 (ref. 57).

Methylation of somatic H1 by the lysine methyltransferase Ezh2 (human Enhancer of Zeste homologue) at Lys 26 seems to be important for transcriptional repression[58]. This site is markedly conserved among somatic H1 isoforms and shares a striking similarity to the Lys 27 site in histone H3, which is targeted by a Ezh2-containing complex with a different histone lysine methyltransferase specificity. Testis H1 variants have glycine or alanine residues in the place of Lys 26, which makes them unlikely Ezh2 targets, and suggests that testis-specific H1 variants are engaged in more dynamic and regulatory functions in germ cells.

The recently described H1t2 seems to play a highly specialized role in establishing cell polarity and, similar to protamines, in directing chromatin condensation[35]. H1t2 appears in round spermatids, in a territory underneath the nuclear membrane and basal to the presumptive acrosome, where chromatin condensation initiates. H1t2 may be a component of the chromatin organizing centre, as indicated by the impaired chromatin condensation in the sperm nucleus of H1t2-null mice[35]. Like other variants, the H1t2 C terminus is enriched in putative phosphorylation sites (Fig. 3). H1t2 seems to be as important as protamines in chromatin reorganization and constitutes a notable example of a histone with highly selective intranuclear distribution.

Figure 2 Modifications of histone variants. N-terminal tails of generic histones and some testis-specific variants, with red residues indicating differences. The indicated modifications (red, phosphorylation; green, acetylation; blue, methylation) are either demonstrated[3,5] (filled circles) or putative (open circles). Shaded boxes indicate binary-switch sites (blue for Thr/Lys associations, red with Ser). Various differences exist in H2A.X and TH2B. The Ser 31 change in H3.3 generates a putative phosphorylation site. The N-terminal tail of CENP-A contains several putative phosphorylation sites (Ser 7 is demonstrated as such). There are nine prolines (H3 has only two) and one lysine as putative sites for modifications (H3 has eight lysines).

The female side of chromatin remodelling

In the oocyte, acetylation is the most dominant form of histone modification. The different patterns of lysine acetylation observed on histones H3 and H4 in meiotic oocytes and preimplantation embryos, suggests that acetylation is important in epigenetic reprogramming and possibly in chromosome dynamics. H3 and H4 lysines (with the exception of H4 Lys 5) are deacetylated during meiosis in mouse oocytes, as well as in somatic nuclei that have been transferred into enucleated oocytes[59]. The global reduction in acetylation levels that has been observed in maturing oocytes is thought to help reprogramming by erasing information on active genes. Indeed, histones H3 and H4 are highly acetylated in regions of active gene expression and underacetylated on silent genes[2].

Furthermore, histone deacetylation in meiotic oocytes facilitates the binding of ATRX, a member of the SWI/SNF helicase family of chromatin-modifying proteins[60]. ATRX localizes to centromeric heterochromatin, and alignment of chromosomes on the metaphase plate is abnormal when ATRX's function is blocked[60]. As ATRX is dependent upon histone deacetylation, removal of this mark seems critical for oocyte differentiation and chromosome segregation. In contrast to the rapid deacetylation occurring at germinal vesicle breakdown, histone methylation remains throughout meiosis and is probably required for HP1 binding to the centromeric domain[60].

During oocyte development, chromatin composition is altered by the replacement of somatic histone H1 with a specialized maternal

© 2005 Nature Publishing Group

variant, H1Foo (H1 histone family, oocyte-specific; formerly named H1oo). H1Foo is distinct from its various male counterparts in that its mRNA is heavily polyadenylated and the protein itself is highly rich in lysines, which are potential sites for methylation or acetylation. The N terminus contains ten potential phosphorylation sites, whereas the extended C terminus (Fig. 3a) could facilitate chromatin condensation or influence mobility. H1 subtypes are known to play a role in gene silencing in *Caenorhabditis elegans*[61], so H1Foo may serve a similar purpose in mammals. H1Foo appears first in secondary follicles and disappears by the four-cell embryonic stage in the mouse[62]. Transcription decreases during oocyte development, as the chromosomes condense[63]. It is possible that H1Foo acts as a transcriptional repressor by altering chromatin structure. There is correlative data to suggest this is the case—H1Foo is present during the time that oocyte transcription is low, and is rapidly removed from the chromatin when zygotic gene activation occurs[64].

Thus, whereas male germ cells incorporate a multitude of H1, H2A, H2B and H3 variants—and undergo extraordinary chromatin remodelling—the oocyte maintains a chromatin structure much like somatic cells. In this scenario, the incorporation of H1Foo could constitute an important exception because it may help define the active/inactive transcription borders that are likely to be established upon fertilization.

Specialized transcription rules in male germ cells

The impressive wave of transcription that occurs post-meiotically in male germ cells is achieved through assembly of transcription complexes and specialized chromatin remodelling enzymes. Studies on the nuclear factors CREM, TLF, TAF7L and related co-activators, have revealed a number of interesting germ-line-specific features discussed elsewhere[8]. For example, CREM-dependent transcriptional activation in somatic cells requires CREM phosphorylation and subsequent CBP recruiting. In contrast, in germ cells the requirements of phosphorylation and histone acetyltransferase function[8] is bypassed by a specific coactivator ACT (activator of CREM in testis).

Transcriptional regulation also plays an important role early in spermatogenesis, when spermatogonial cells face the choice of self-

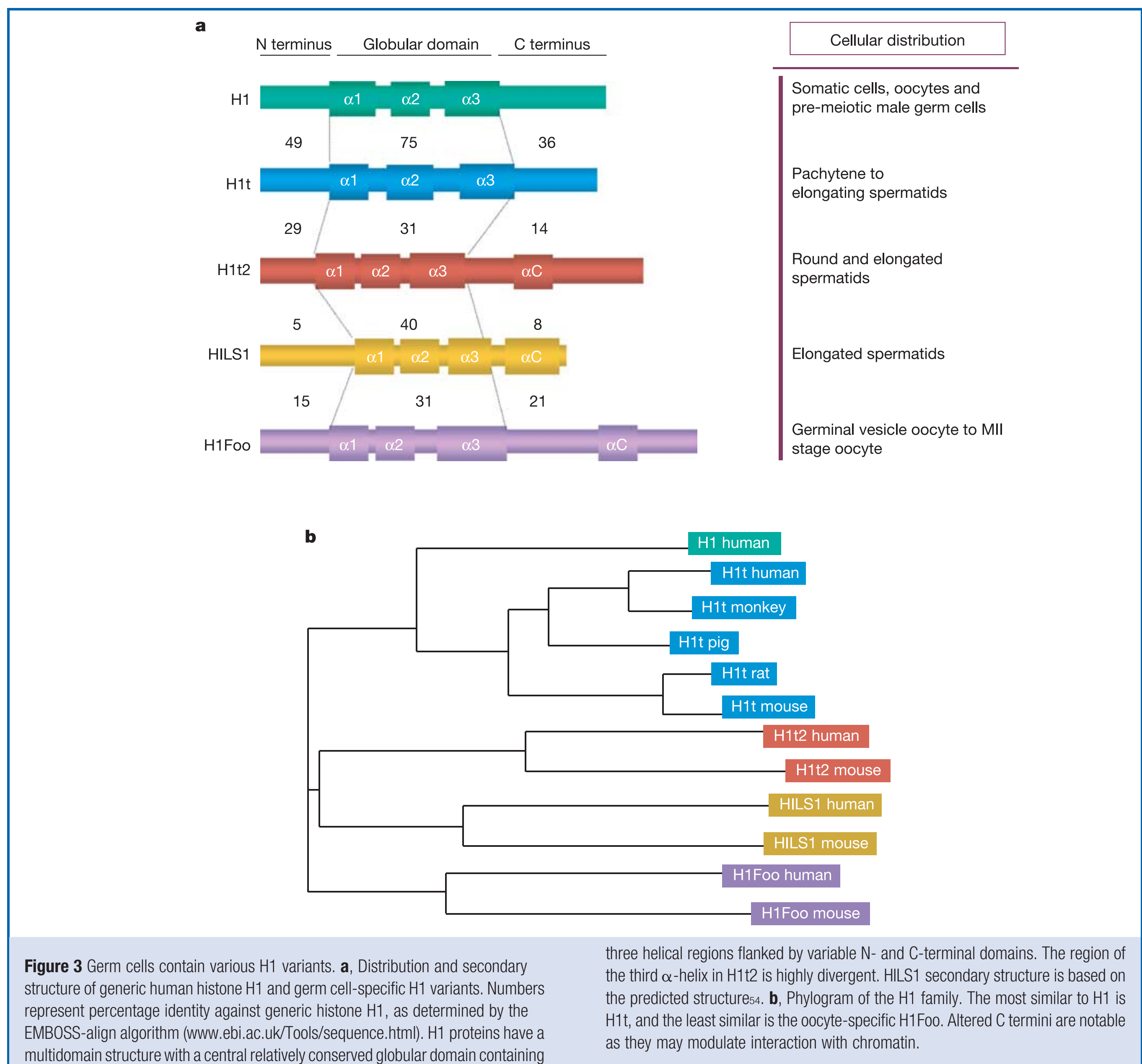


Figure 3 Germ cells contain various H1 variants. **a**, Distribution and secondary structure of generic human histone H1 and germ cell-specific H1 variants. Numbers represent percentage identity against generic histone H1, as determined by the EMBOSS-align algorithm (www.ebi.ac.uk/Tools/sequence.html). H1 proteins have a multidomain structure with a central relatively conserved globular domain containing three helical regions flanked by variable N- and C-terminal domains. The region of the third α-helix in H1t2 is highly divergent. HILS1 secondary structure is based on the predicted structure[54]. **b**, Phylogram of the H1 family. The most similar to H1 is H1t, and the least similar is the oocyte-specific H1Foo. Altered C termini are notable as they may modulate interaction with chromatin.

© 2005 Nature Publishing Group

renewal or differentiation. One of the players in this decision-making process is Plzf (promyelocytic leukaemia zinc finger), a nuclear factor of the POK (POZ and Krüppel) family of transcriptional repressors. The POZ domain of Plzf recruits Polycomb proteins such as BMI1, which subsequently recruits histone deacetylases. The role of BMI1 and other Polycomb proteins is to maintain stable and heritable repression of specific developmental genes; thus it is possible that through this mechanism, Plzf-dependent histone deacetylases regulate chromatin remodelling in defining spermatogonial cell fate. Importantly, Plzf-null mice show progressive loss of spermatogonial cells and increased apoptosis[65,66].

Because DNA methylation plays a central role in male germ cell differentiation, its potential influence on histone methyltransferases may have far-reaching consequences. Indeed, there are several histone methyltransferases, which show hallmarks of being important regulators of germ cell transcription. One is the mammalian H3 Lys 9 histone methyltransferase (Suv39), which is enriched at heterochromatin and seems to be involved in gene repression and chromosome pairing. In addition, there are two mouse SET-domain-containing Suv39 histone methyltransferases, Suv39h1 and Suv39h2. Suv39h2 is preferentially expressed in the testis, and accumulates with chromatin of the sex body (Box 1). Indeed, mice mutated for both Suv39h1 and Suv39h2 are infertile owing to spermatogenetic arrest[67]. Finally, although the relationship between DNA methylation and DNA repair is not fully understood[68], HR6B-deficient animals show a phenotype that is similar to Suv39-null mice[69]. HR6B is a ubiquitin-conjugating DNA repair enzyme that is involved in meiosis.

A fascinating possibility is that non-coding RNAs may have a function in spermatogenesis. For instance, naturally occurring RNAi constitute a powerful route to dynamically silence specific gene expression, so it is conceivable that such mechanisms may induce silencing initiation ahead of the more classical heterochromatization process that is mediated by histone methyltransferase-mediated Lys 9 histone H3 methylation[70]. It will be interesting to know how much specific RNAi is made in germ cells, and how the levels are controlled during differentiation and meiosis. Are there types of RNAi that can specifically target a whole chromosome? While these remain open questions, it is worth mentioning that germ cells contain an intriguing organelle, the chromatoid body (Box 1), which is mostly constituted by RNA. This cytoplasmic structure is conserved throughout evolution (the *Drosophila* equivalent is called nuage[71]). Its proteinaceous composition is still mostly unexplored. One component, however, is known and noteworthy: MVH (mouse homologue of VASA), an RNA helicase that is involved in RNA silencing[72].

Histone modifications are recognized by bromodomain-containing proteins that specifically bind to acetylated lysines, and chromodomains that contain methylated Lys 9 on H3 ref. 73). One such protein is a testis-specific bromodomain protein called BRDT (bromodomain testis-specific), which induces chromatin remodelling in the presence of histone hyperacetylation[74]. BRDT may mediate nuclear reorganization in spermiogenesis at the stage where histone hyperacetylation precedes the replacement of the testis-specific nuclear proteins[75].

What's coming?

Germ cell development is unique in the way that it generates the haploid cells that are responsible for the maintenance of the species. Understanding how epigenetic patterning occurs in the germ line may eventually help in the prevention of heritable diseases, improvement of assisted reproductive technologies, and stem cell therapy[76]. The way is now paved for exciting new discoveries of the modifying enzymes that are responsible for these epigenetic modifications, the signalling pathways involved and the downstream effects on transcription, DNA repair and replication. Given that in males the spermatogenic process is continuous throughout life, it is essential to elucidate how genetic and epigenetic processes are influenced by environmental cues. Finally, the long-standing link between metabolism and reproduction might also be based on epigenetic regulation, suggesting new research directions. New evidence suggests that embryonic DNA methylation patterns are influenced by maternal nutrition, and that epigenetic mutations induced by malnutrition could lead to development of diseases such as cancer and diabetes in adult life[77]. □

doi:10.1038/nature03368.

1. Jaenisch, R. & Bird, A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genet.* **33** (suppl.), 245–254 (2003).
2. Cheung, P., Allis, C. D. & Sassone-Corsi, P. Signaling to chromatin through histone modifications. *Cell* **103,** 263–271 (2000).
3. Fischle, W., Wang, Y. & Allis, C. D. Binary switches and modification cassettes in histone biology and beyond. *Nature* **425,** 475–479 (2003).
4. Felsenfeld, G. & Groudine, M. Controlling the double helix. *Nature* **421,** 448–453 (2003).
5. Sims, R. J. III, Nishioka, K. & Reinberg, D. Histone lysine methylation: a signature for chromatin function. *Trends Genet.* **19,** 629–639 (2003).
6. Bannister, A. J., Schneider, R. & Kouzarides, T. Histone methylation: dynamic or static? *Cell* **109,** 801–806 (2002).
7. Holliday, R. DNA methylation and epigenetic mechanisms. *Cell Biophys.* **15,** 15–20 (1989).
8. Sassone-Corsi, P. Unique chromatin remodeling and transcriptional regulation in spermatogenesis. *Science* **296,** 2176–2178 (2002).
9. DeBaun, M. R., Niemitz, E. L. & Feinberg, A. P. Association of *in vitro* fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am. J. Hum. Genet.* **72,** 156–160 (2003).
10. Rhind, S. M. *et al.* Human cloning: can it be made safe? *Nature Rev. Genet.* **4,** 855–864 (2003).
11. Sarma, K. & Reinberg, D. Histone variants meet their match. *Nature Rev. Mol. Cell Biol.* **6,** 1–11 (2005).
12. Marston, A. L. & Amon, A. Meiosis: cell-cycle controls shuffle and deal. *Nature Rev. Mol. Cell Biol.* **5,** 983–997 (2004).
13. Meistrich, M. L., Mohapatra, B., Shirley, C. R. & Zhao, M. Roles of transition nuclear proteins in spermiogenesis. *Chromosoma* **111,** 483–488 (2003).
14. Yu, Y. E. *et al.* Abnormal spermatogenesis and reduced fertility in transition nuclear protein 1-deficient mice. *Proc. Natl Acad. Sci. USA* **97,** 4683–4688 (2000).
15. Zhao, M. *et al.* Targeted disruption of the transition protein 2 gene affects sperm chromatin structure and reduces fertility in mice. *Mol. Cell. Biol.* **21,** 7243–7255 (2001).
16. Meetei, A. R., Ullas, K. S., Vasupradha, V. & Rao, M. R. Involvement of protein kinase A in the phosphorylation of spermatidal protein TP2 and its effect on DNA condensation. *Biochemistry* **41,** 185–195 (2002).
17. Sung, M. T. & Dixon, G. H. Modification of histones during spermiogenesis in trout: a molecular mechanism for altering histone binding to DNA. *Proc. Natl Acad. Sci. USA* **67,** 1616–1623 (1970).
18. Lahn, B. T. *et al.* Previously uncharacterized histone acetyltransferases implicated in mammalian spermatogenesis. *Proc. Natl Acad. Sci. USA* **99,** 8707–8712 (2002).
19. Oliva, R. & Dixon, G. H. Vertebrate protamine genes and the histone-to-protamine replacement reaction. *Progr. Nucleic Acid Res. Mol. Biol.* **40,** 25–94 (1991).
20. Lewis, J. D. *et al.* Histone H1 and the origin of protamines. *Proc. Natl Acad. Sci. USA* **101,** 4148–4152 (2004).
21. Pogany, G. C., Corzett, M., Weston, S. & Balhorn, R. DNA and protein content of mouse sperm. Implications regarding sperm chromatin structure. *Exp. Cell Res.* **136,** 127–136 (1981).
22. Cho, C. *et al.* Haploinsufficiency of protamine-1 or -2 causes infertility in mice. *Nature Genet.* **28,** 82–86 (2001).
23. Wu, J. Y. *et al.* Spermiogenesis and exchange of basic nuclear proteins are impaired in male germ cells lacking Camk4. *Nature Genet.* **25,** 448–452 (2000).
24. Xu, X., Toselli, P. A., Russell, L. D. & Seldin, D. C. Globozoospermia in mice lacking the casein kinase IIα' catalytic subunit. *Nature Genet.* **23,** 118–121 (1999).
25. Lee, K., Haugen, H. S., Clegg, C. H. & Braun, R. E. Premature translation of protamine 1 mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice. *Proc. Natl Acad. Sci. USA* **92,** 12451–12455 (1995).
26. Grimes, S. *et al.* A rat histone H4 gene closely associated with the testis-specific H1t gene. *Exp. Cell Res.* **173,** 534–545 (1987).
27. Redon, C. *et al.* Histone H2A variants H2AX and H2AZ. *Curr. Opin. Genet. Dev.* **12,** 162–169 (2002).
28. Pandey, N. B. & Marzluff, W. F. The stem-loop structure at the 3′ end of histone mRNA is necessary and sufficient for regulation of histone mRNA stability. *Mol. Cell. Biol.* **7,** 4557–4559 (1987).
29. Moss, S. B., Challoner, P. B. & Groudine, M. Expression of a novel histone 2B during mouse spermiogenesis. *Dev. Biol.* **133,** 83–92 (1989).
30. Albig, W. *et al.* All known human H1 histone genes except the H1(0) gene are clustered on chromosome 6. *Genomics* **16,** 649–654 (1993).
31. Kobor, M. S. *et al.* Protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol.* **2,** E131 (2004).
32. Tagami, H., Ray-Gallet, D., Almouzni, G. & Nakatani, Y. Histone H3.1 and H3.3 complexes mediat nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* **116,** 51–61 (2004).
33. Bramlage, B., Kosciessa, U. & Doenecke, D. Differential expression of the murine histone genes H3.3A and H3.3B. *Differentiation* **62,** 13–20 (1997).
34. Zalensky, A. O. *et al.* Human testis/sperm-specific histone H2B (hTSH2B). Molecular cloning and characterization. *J. Biol. Chem.* **277,** 43474–43480 (2002).
35. Martianov, I. *et al.* Polar nuclear localization of H1T2, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis. *Proc. Natl Acad. Sci. USA* **102,** 2808–2813 (2005).
36. Henikoff, S., Ahmad, K., Platero, J. S. & van Steensel, B. Heterochromatic deposition of centromeric histone H3-like proteins. *Proc. Natl Acad. Sci. USA* **97,** 716–721 (2000).

© 2005 Nature Publishing Group

37. McKittrick, E., Gafken, P. R., Ahmad, K. & Henikoff, S. Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proc. Natl Acad. Sci. USA* **101,** 1525–1530 (2004).
38. Crosio, C. *et al.* Mitotic phosphorylation of histone H3: spatio-temporal regulation by mammalian Aurora kinases. *Mol. Cell. Biol.* **22,** 874–885 (2002).
39. Zeitlin, S. G., Shelby, R. D. & Sullivan, K. F. CENP-A is phosphorylated by Aurora B kinase and plays an unexpected role in completion of cytokinesis. *J. Cell Biol.* **155,** 1147–1157 (2001).
40. Zalensky, A. O. *et al.* Human testis/sperm-specific histone H2B (hTSH2B). Molecular cloning and characterization. *J. Biol. Chem.* **277,** 43474–43480 (2002).
41. Niino, Y. S. *et al.* PKCϕ II, a new isoform of protein kinase C specifically expressed in the seminiferous tubules of mouse testis. *J. Biol. Chem.* **276,** 36711–36717 (2001).
42. Tseng, T. C., Chen, S. H., Hsu, Y. P. & Tang, T. K. Protein kinase profile of sperm and eggs: cloning and characterization of two novel testis-specific protein kinases (AIE1, AIE2) related to yeast and fly chromosome segregation regulators. *DNA Cell Biol.* **17,** 823–833 (1998).
43. Park, E. J., Chan, D. W., Park, J. H., Oettinger, M. A. & Kwon, J. DNA-PK is activated by nucleosomes and phosphorylates H2AX within the nucleosomes in an acetylation-dependent manner. *Nucleic Acids Res.* **31,** 6819–6827 (2003).
44. Burma, S., Chen, B. P., Murphy, M., Kurimasa, A. & Chen, D. J. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J. Biol. Chem.* **276,** 42462–42467 (2001).
45. Shroff, R. *et al.* Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Curr. Biol.* **14,** 1703–1711 (2004).
46. Celeste, A. *et al.* Genomic instability in mice lacking histone H2AX. *Science* **296,** 922–927 (2002).
47. Barlow, C. *et al.* Atm deficiency results in severe meiotic disruption as early as leptonema of prophase I. *Development* **125,** 4007–4017 (1998).
48. Abraham, R. T. Checkpoint signaling: epigenetic events sound the DNA strand-breaks alarm to the ATM protein kinase. *Bioessays* **25,** 627–630 (2003).
49. McKee, B. D. & Handel, M. A. Sex chromosomes, recombination, and chromatin conformation. *Chromosoma* **102,** 71–80 (1993).
50. Chadwick, B. P. & Willard, H. F. Histone H2A variants and the inactive X chromosome: identification of a second macroH2A variant. *Hum. Mol. Genet.* **10,** 1101–1113 (2001).
51. Baarends, W. M., Roest, H. P. & Grootegoed, J. A. The ubiquitin system in gametogenesis. *Mol. Cell. Endocrinol.* **151,** 5–16 (1999).
52. Hendzel, M. J., Lever, M. A., Crawford, E. & Th'ng, J. P. The C-terminal domain is the primary determinant of histone H1 binding to chromatin *in vivo. J. Biol. Chem.* **279,** 20028–20034 (2004).
53. Fantz, D. A. *et al.* Mice with a targeted disruption of the H1t gene are fertile and undergo normal changes in structural chromosomal proteins during spermiogenesis. *Biol. Reprod.* **64,** 425–431 (2001).
54. Doenecke, D. *et al.* Histone gene expression and chromatin structure during spermatogenesis. *Adv. Exp. Med. Biol.* **424,** 37–48 (1997).
55. Nayernia, K. *et al.* Male mice lacking three germ cell expressed genes are fertile. *Biol. Reprod.* **69,** 1973–1978 (2003).
56. Yan, W., Ma, L., Burns, K. H. & Matzuk, M. M. HILS1 is a spermatid-specific linker histone H1-like protein implicated in chromatin remodeling during mammalian spermiogenesis. *Proc. Natl Acad. Sci. USA* **100,** 10546–10551 (2003).
57. Contreras, A. *et al.* The dynamic mobility of histone H1 is regulated by cyclin/CDK phosphorylation. *Mol. Cell. Biol.* **23,** 8626–8636 (2003).
58. Kuzmichev, A., Jenuwein, T., Tempst, P. & Reinberg, D. Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol. Cell* **14,** 183–193 (2004).
59. Kim, J. M., Liu, H., Tazaki, M., Nagata, M. & Aoki, F. Changes in histone acetylation during mouse oocyte meiosis. *J. Cell Biol.* **7,** 37–46 (2003).
60. De La Fuente, R., Viveiros, M. M., Wigglesworth, K. & Eppig, J. J. ATRX, a member of the SNF2 family of helicase/ATPases, is required for chromosome alignment and meiotic spindle organization in metaphase II stage mouse oocytes. *Dev. Biol.* **272,** 1–14 (2004).
61. Jedrusik, M. A. & Schulze, E. A single histone H1 isoform (H1.1) is essential for chromatin silencing and germline development in *Caenorhabditis elegans*. *Development* **128,** 1069–1080 (2001).
62. Clarke, H. J., Bustin, M. & Oblin, C. Chromatin modifications during oogenesis in the mouse: removal of somatic subtypes of histone H1 from oocyte chromatin occurs post-natally through a post-transcriptional mechanism. *J. Cell Sci.* **110,** 477–487 (1997).
63. Debey, P. *et al.* Competent mouse oocytes isolated from antral follicles exhibit different chromatin organization and follow different maturation dynamics. *Mol. Reprod. Dev.* **36,** 59–74 (1993).
64. Teranishi, T. *et al.* Rapid replacement of somatic linker histones with the oocyte-specific linker histone H1Foo in nuclear transfer. *Dev. Biol.* **266,** 76–86 (2004).
65. Buaas, F. W. *et al.* Plzf is required in adult male germ cells for stem cell self-renewal. *Nature Genet.* **36,** 647–652 (2004).
66. Costoya, J. A. *et al.* Essential role of Plzf in maintenance of spermatogonial stem cells. *Nature Genet.* **36,** 653–659 (2004).
67. Peters, A. H. *et al.* Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107,** 323–337 (2001).
68. Sedgwick, B. Repairing DNA-methylation damage. *Nature Rev. Mol. Cell Biol.* **5,** 148–157 (2004).
69. Roest, H. P. *et al.* Inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. *Cell* **86,** 799–810 (1996).
70. Verdel, A. *et al.* RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* **303,** 672–676 (2004).
71. Hay, B., Ackerman, L., Barbel, S., Jan, L. Y. & Jan, Y. N. Identification of a component of *Drosophila* polar granules. *Development* **103,** 625–640 (1988).
72. Toyooka, Y. *et al.* Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mech. Dev.* **93,** 139–149 (2000).
73. Bannister, A. J. *et al.* Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410,** 120–124 (2001).
74. Pivot-Pajot, C. *et al.* Acetylation-dependent chromatin reorganization by BRDT, a testis-specific bromodomain-containing protein. *Mol. Cell. Biol.* **23,** 5354–5365 (2003).
75. Hazzouri, M. *et al.* Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. *Eur. J. Cell Biol.* **79,** 950–960 (2000).
76. Brinster, R. L. Germline stem cell transplantation and transgenesis. *Science* **296,** 2174–2176 (2002).
77. Waterland, R. A. & Jirtle, R. L. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition* **20,** 63–68 (2004).
78. Moss, S. B. & Orth, J. M. Localization of a spermatid-specific histone 2B protein in mouse spermiogenic cells. *Biol. Reprod.* **48,** 1047–1056 (1993).

Acknowledgements We were unfortunately unable to include all the relevant references owing to space constraints. We are grateful to C. D. Allis, D. Reinberg, R. Jaenisch, E. Borrelli, I. Davidson, N. Kotaja, U. Kolthur, G. Fienga, K. Hogeveen, C. Krausz, M. Parvinen, S. Henikoff, R. L. Brinster and all members of the Sassone-Corsi laboratory for critical reading of the manuscript, advice and stimulating discussions. S.K. is supported by fellowships from the Fondation pour la Recherche Médicale and the Marie Curie Programme.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to P.S.-C. (paolosc@igbmc.u-strasbg.fr).

© 2005 Nature Publishing Group