

Utah State University

DigitalCommons@USU

UAES Publications

Agricultural Experiment Station

12-1-2017

Heritable Sperm Chromatin Epigenetics: A Break to Remember

Ralph G. Meyer

Utah State University, Ralph.Meyer@usu.edu


Chelsea C. Ketchum

Utah State University

Mirella L. Meyer-Ficca

Utah State University, Mirella.Meyer@usu.edu

Follow this and additional works at: https://digitalcommons.usu.edu/uaes_pubs

 Part of the [Biology Commons](#), and the [Genetics and Genomics Commons](#)

Recommended Citation

Meyer, Ralph G.; Ketchum, Chelsea C.; and Meyer-Ficca, Mirella L., "Heritable Sperm Chromatin Epigenetics: A Break to Remember" (2017). *UAES Publications*. Paper 3.

https://digitalcommons.usu.edu/uaes_pubs/3

This Article is brought to you for free and open access by the Agricultural Experiment Station at DigitalCommons@USU. It has been accepted for inclusion in UAES Publications by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



Heritable Sperm Chromatin Epigenetics: A Break to Remember

Running Title:

DDR signaling forms sperm chromatin epigenetics

5

Summary Sentence:

DNA repair pathways are involved in normal establishment of the mature sperm chromatin structure as a carrier of heritable epigenetic information to the next generation.

Keywords:

Histone modifications, topoisomerase, epigenetics, PARP, ADP-ribose, spermiogenesis, sperm,
10 chromatin, gametogenesis, male infertility, reprogramming, spermatid, spermatogenesis, testis.

Ralph G. Meyer^{1,2,3}, Chelsea Ketchum^{1,2}, Mirella L. Meyer-Ficca¹,

¹ Department of Animal, Dairy and Veterinary Sciences, College of Agriculture and Applied
Sciences, ² Utah Experimental Station, Utah State University, Logan, UT, USA

15

Grant support:

This work was supported by a grant from the Utah Agricultural Experiment Station, Utah State
University (UTA01166) to RGM.

20

³Correspondence:

Ralph G. Meyer, PhD (Ralph.Meyer@usu.edu)

Tel: 435-797-1774

ABSTRACT

25 Sperm chromatin not only has a unique structure to condense and protect the paternal DNA in transit, but also provides epigenetic information that supports embryonic development. Most of the unique sperm nuclear architecture is formed during the sweeping postmeiotic chromatin remodeling events in spermiogenesis, where the majority of nucleosomes are removed and replaced by protamines. The remaining histones and other chromatin proteins are located in
30 structurally and transcriptionally relevant positions in the genome and carry diverse posttranslational modifications relevant to the control of embryonic gene expression. How such postmeiotic chromatin-based programming of sperm epigenetic information proceeds, and how susceptible the process is to modulation by exogenous factors are key questions for understanding the inheritance of acquired epigenetic marks through the male germ line. We
35 propose that transient DNA strand breaks mediated by topoisomerase 2 beta and the subsequent activation of DNA damage response pathways result in defined posttranslational modifications of histones in spermiogenesis. These pathways, likely along with others, may contribute to chromatin remodeling in elongating spermatids, influence chromatin-based intergenerational inheritance of epigenetic information, and may be defective in pathologies of abnormal male
40 gametogenesis and infertility.

1. INTRODUCTION

There is now accumulating evidence of non-genetic (epigenetic) inheritance through the male germ line allowing individual ancestors to transfer information about their environment to their
45 progeny [1–7].

Four distinct but interconnected molecular pathways that permit epigenetic information transfer from sperm to progeny have been discovered so far: (i) Coding and non-coding RNA, as well as tsRNA [8–13] in sperm, (ii) DNA methylation of the sperm genome ([14], reviewed in [15]), (iii) gene positioning and sperm nuclear architecture [16–18], and (iv) sperm chromatin
50 components including histones and other DNA binding proteins [19–22].

How exactly germ cells obtain clues to environmental factors and encode them in some form of epigenetic information remains enigmatic at this time. However, the influence of environmental factors on the epigenome of male germ cells appears to be most impactful if it happens during a developmental phase when these cells are epigenetically reprogrammed. It has
55 been established that male germ cells undergo at least two of such major epigenetic programming phases, namely during the prenatal differentiation of primordial germ cells, and during differentiation of germ cells into spermatogonia for the execution of spermatogenesis [3,23–25]. An additional window of opportunity likely also exists in the adult male, where continuous spermatogenesis is a life-long continuous process that could provide sensing of
60 environmental cues and translation into epigenetic messages carried by the mature sperm to the next generation. [26,27]. Focusing on the emerging role of sperm chromatin proteins in intergenerational epigenetic inheritance, a review of recent data yields overwhelming evidence that certain chromatin-borne epigenetic signatures in mature sperm are important to embryonic development. How these signatures are established during spermatogenesis is poorly understood,

65 in part because they depend to an unknown extent on complex and not yet well characterized
chromatin remodeling events [28]. Particularly the postmeiotic chromatin remodeling events,
where spermatids develop into sperm, are probably among the most dramatic ones in cell biology
and the question arises how sperm epigenetic information survives these events or may even be
dependent on them. Here, we review emerging concepts and vehicles of sperm chromatin-based
70 epigenetic inheritance and explore the potential influence of DNA damage repair (DDR)
pathways on epigenetic programming of sperm in the context of postmeiotic chromatin
remodeling events.

2. SPERM CHROMATIN AS A CARRIER OF EPIGENETIC INFORMATION

75 2.1 Spermiogenesis and the origin of sperm nucleosomes

Sperm are both, highly adapted to ensure safe transport of the paternal genome complement to
the egg as well as epigenetically configured to support embryonic programming and totipotency
of the zygote. To accommodate both of these functions, the protamine-based chromatin and
compact nuclear architecture of sperm is uniquely organized and very different from the
80 nucleosomal nuclear organization of the archetypal cell type [29]. Sperm DNA is not
superspiralized but relaxed and associated with protamines that are cross-linked by disulfide
bonds to form comparatively large toroid-like loops [17,30], which are physically very stable. To
accomplish that chromatin change, the majority of histones are temporarily replaced by transition
proteins (TP1 and TP2) and then finally by protamines (PRM1, PRM2) during the postmeiotic
85 phase of spermatogenesis, termed spermiogenesis [31]. Only a small portion of the genome
remains nucleosomal (approximately 2 % to 15 % in mice and humans, respectively [32,33]).
Initial investigations revealed that at least a portion of the remaining nucleosomes are retained in

defined telomeric and pericentromeric chromosome regions of mammalian sperm, where they have important structural roles [33,34]. Besides their structural functions, histones often also carry posttranslational modifications (PTM) that are able to control gene activity, and therefore have the potential to transmit epigenetic information to the offspring [35]. Until recently, sperm were not thought to provide much histone-mediated epigenetic information because the majority of histones have been replaced by protamines during spermatid differentiation and the remaining ones were thought to be restricted to non-coding repetitive sequences in pericentromeric and telomeric heterochromatin.

Newer studies then found that another portion of the remaining sperm histones are located also elsewhere in the genome, but their exact positions, their functions, and their posttranslational modifications have been controversially discussed. Results of individual studies varied considerably, which may in part be due to differences in experimental details of the general technical approach used to map histones to the sperm genome [36,37], which is based on sequence analysis of DNA bound to nucleosomes separated from protaminated chromatin by limited micrococcal nuclease (MNase) digestion of sperm nuclei followed by chromatin immunoprecipitation (ChIP-seq) [38]. Some studies using this approach found sperm histones to be enriched in intergenic regions rather than in promoter regions [36,37,39] while other reports place histones at gene promoters, imprinted gene clusters, microRNA, HOX gene clusters and binding sites of the chromatin insulator protein CCCTC-binding factor (CTCF) [19–21,37]. There is some consensus that a portion of the sperm histones maps to DNA in GC-rich, hypomethylated promoter regions and first exons in most genes in human and murine sperm [36,37,40–42]. The potential association of residual sperm histones with promoters of specific genes and other genomic regions relevant to embryonic development gave rise to the concept

that such sperm histones may regulate gene expression in the early embryo [19–21,43–45,37,41]. Supporting this hypothesis, a direct link between the location and number of histones in sperm gene promoter regions and the expression of these genes in resulting 2-cell embryos could be shown using two mouse models with altered poly(ADP-ribose) metabolism [41]. Further supporting the concept that sperm chromatin carries a rich source of gene regulatory information, Jung and colleagues recently found that most promoter regions in mouse sperm are flanked by active histone modifications when they used transposase Tn5 instead of MNase processing to map accessible open chromatin states. They also used ChIP-seq analyses of MNase-digested sperm chromatin to demonstrate that adult enhancer regions carrying gene activating histone marks are already specified in sperm, and that CTCF and cohesin are important factors that organize the three-dimensional (3D) structure in sperm [22]. CTCF and cohesin are also involved in the formation of topologically associating domains (TADs) of chromosomes that form a 3D structure of mammalian nuclei, including sperm [46,47,16] and of nuclear lamina-associated domains (LADs) [48]. TADs in sperm are unique in that they are characterized by extra-long-range interactions and frequent inter-chromosomal interactions in addition to having conserved TADs found in somatic cells [16,49]. Pioneering, microscopy-based investigations have long shown that sperm exhibit a nuclear architecture that is very distinct from that found in somatic cells. In human and murine sperm, pericentromeric heterochromatin is clustered in the center of the nucleus, and telomeres with their subtelomeric repeats are located near its surface [50,51]. These heterochromatic sequences appear to be important for the specific and systematic arrangement of chromosomes during meiosis to form the specific sperm nuclear structure which has long been considered to constitute an additional layer of epigenetic information in itself [18,50,52–55]. The unique nuclear structure may therefore explain a subset of the unique TADs

found in sperm. The extent to which the sperm TAD structure is inherited by the embryo is
135 unclear as the high-order structures of both the paternal and maternal genomes in zygotes and
two-cell embryos are not well defined but are later gradually re-established through development
[49].

Given the important roles of heterochromatic, nucleosomal regions in sperm nuclei, it is not
surprising that genome-wide nucleosome mapping also suggests that a large portion of sperm
140 nucleosomes are preferentially located at repetitive sequences throughout the genome, e.g. SINE,
LINE, and retrotransposons [39]. These findings, which appear also to be in line with other
studies [56,57] have been disputed [58], because histone enrichment in repetitive sequences may
be overestimated by certain computational approaches to map high-throughput sequencing reads
to the genome. While this may be true, the overall result that sperm nucleosomes are
145 preferentially retained in repetitive sequences [59] is consistent with results from other groups
who used histone immunostaining or fluorescence in situ hybridization (FISH) of sperm nuclei.
These investigations support the hypothesis that sperm histones tend to co-localize with repeat-
enriched constitutive heterochromatin blocks such as found in pericentromeric regions
[37,57,60]. All of these findings are, however, not inconsistent with the overwhelming evidence
150 that at least a portion of sperm histones occupy gene regulatory and other genomic regions in a
way that is expected to direct early embryonic gene expression, because these regions represent
only a comparatively small fraction of the genome.

In addition to histones and CTCF, other DNA binding proteins capable of genome
regulation and transcriptional regulation, such as transcription factors and the transactive
155 response DNA binding protein (TDP-43) have been detected in mature sperm [19,22,37,61,62].
The roles of these proteins in sperm mediated epigenetic inheritance have yet to be determined.

2. 2. Testicular histone variants as carriers of epigenetic information.

Canonical histone genes (H2A, H2B, H3 and H4) are clustered in the mammalian genome and expressed from these clusters solely in S-phase to permit histone incorporation into newly replicated DNA. In addition, non-canonical variants of all canonical histones except for H4 are present in mammals, where they are expressed from individual genes throughout the cell cycle [7,63]. Male germ cells express a large number of non-canonical histone variants, including H2AFX (also known as H2A.X) [64], H2A.Z, TH2A [65], H3.3 [66–68], H3T [69], three H2AL and two H2BL variants [57], TH2B [70], as well as macroH2A.1, macroH2A.2 and their subtypes [71,72] (reviewed in [28,63]). Histone H1 has 11 variants, of which H1t [73,74], H1T2 [75] and HILS1 [76] are selectively expressed in the testis (reviewed in [77]). Expression of testicular histone variants is regulated temporally and spatially during germ cell development. They fulfill various structural and gene regulatory functions during spermatogenesis, and may contribute to epigenetic mark formation in response to environmental clues [63].

Structurally, histone variants deviate from canonical histones by small protein sequence alterations that changes the stability of the nucleosomes they are incorporated in. A change in nucleosomal stability goes along with a changed degree of chromatin condensation and a changed accessibility of the DNA for binding factors, which has consequences for transcription and chromatin reorganization during later phases of spermatogenesis. Some functions of testicular histone variants appear to be limited to germ cell development, without directly affecting subsequent embryo development. For example, H2AL1 and H2AL2 disappear rapidly from the paternal genome after fertilization [78], and it is unknown whether they convey any epigenetic information to the embryo. A recent report proposed functions of H2AL2 in the

180 loading of transition proteins onto the DNA in elongating spermatids where protamines are recruited subsequently to facilitate histone eviction from the DNA [79].

TH2B is a testis-specific histone variant that replaces most of the canonical H2B histones during the meiotic prophase of spermatogenesis [80]. TH2B destabilizes nucleosomes in spermatids because its carboxyterminal surface reduces nucleosomal binding affinity to DNA.

185 As a consequence, incorporation of TH2B is thought to facilitate the transition of nucleosomes to transition proteins and protamines [80,81]. This raises the question whether selective placement of TH2B could be involved in epigenetic memory formation. Some TH2B is found in mature sperm [82] and an interesting open question is whether TH2B is mostly eliminated alongside with canonical H2B from the elongating spermatid nucleus or whether it is able to selectively
190 escape nucleosome removal during spermiogenesis, with possible epigenetic consequences.

In sperm, histone variant H3.3 is enriched at promoter regions (CpG islands, CGI) of genes that are highly transcribed during spermiogenesis, while canonical histones H3.2 and H3.1 are less enriched at these regions, suggesting that they were replaced by H3.3 during gene transcription events in round spermatids [40,83].

195 In summary, DNA replication-independent expression of non-canonical histone variants and their insertion into the chromatin of actively transcribed genes, where they substitute canonical histones, could be one of the mechanisms of sperm chromatin mark formation [67]. If that is the case, then a form of histone variant-based epigenetic information could exist in sperm chromatin to represent a memory of gene activity that occurred during spermatogenesis and that
200 could be transmitted to the next generation to inform progeny. However, mechanisms underlying the sensing of environmental influences (diets, toxicant exposures) by germ cells and how gene expression could be altered in response to such environmental cues are currently still unclear.

2.3. Posttranslational modifications (PTM) of sperm histones with the potential to control

205 **gene expression.**

Sperm histones carry various PTMs that can regulate chromatin functions and gene expression. PTM in the testis include mono-, di- and trimethylation of lysine residues, acetylation, phosphorylation, ubiquitination, ADP-ribosylation [84], crotonylation [85] and others (reviewed in [28,62]). Most of these histone PTM are regulated by a complex interplay of enzymes either adding or removing defined molecular groups to histone N-terminal domains. The acetylation of histones is generally associated with gene activation and transcription [86]. Constitutive heterochromatin, e.g. as it is found in pericentromeric, telomeric and repeat-rich genomic regions, is marked by trimethylation of lysine 9 on histone H3 (H3K9me3), a silencing histone modification, and by DNA methylation. Both marks persist in heterochromatic regions during fertilization [87,88]. Di- and trimethylated lysine 4 on histone H3 (H3K4me2 and H3K4me3), both generally considered activating histone marks, are enriched at certain developmental promoters and paternally expressed loci [20]. Genes important for developmental regulation and cellular homeostasis concurrently carry both, H3K4me2 and H3K27me3 chromatin marks [21]. The simultaneous presence of markings with apparently contradictory function in the same region, e.g. silencing H3K27me3, in addition to activating H3K4me3, marks “bivalent” promoters of developmental genes expressed in embryonic development [20,21]. Both markings can occur on the same nucleosome, but typically not on the same histone N-terminal tail within one histone [20,89].

225 During *Xenopus* spermiogenesis, the activating H3K4me2 and H3K4me3 marks are removed from a subset of developmentally important genes to prevent their promiscuous expression in

early embryogenesis [90], further highlighting the importance of sperm chromatin epigenetics for embryo development. Generally, H3K4me2 is an activating mark that is found on meiotic sex chromosomes and elsewhere throughout the spermatid genome and that has been suggested as an important mediator of heritable epigenetic memory in mammals [20,21,91]. H3K27me3 marks are mediated by the polycomb repressor protein complex PRC2 [92]. This mark, which is recognized and bound by a second complex, PRC1, is important for Trithorax- and Polycomb-mediated gene silencing, and paternal diet affecting H3K27 methylation in sperm may represent one mechanism by which an epigenetic signature forms due to environmental factors [93].

Dietary methyl (C1) donors, such as folate, methionine and choline, are known players important for methylation of DNA in spermatogenesis [94] and variations in the availability of those metabolites change the DNA methylation component of the sperm epigenome. How deficient or excessive uptake of these methyl donor changes body-wide and importantly germ cell-specific methylation of histones is not understood [95–99]. Targeted research needs to address the question to what extent dietary C1 donors affect the sperm chromatin-borne epigenome, specifically the methylation of histones, in addition to the sperm DNA methylome [95]. The importance of sperm histone methylation patterns for the regulation of embryonic development was recently demonstrated in male transgenic mice with H3K4 demethylase KDM1A overexpression. Progeny of such animals with reduced sperm H3K4 dimethylation were developmentally impaired and had reduced survival rates [100]. Similar results were obtained from a different group who used a heat-inducible testicular KDM1A overexpressing mouse model [101]. In summary, while the exact mechanism remains unclear, pathways that modulate the activity of H3K4me3 or H3K27me3 demethylating enzymes during spermatogenesis in response to environmental cues may be able to affect the sperm epigenetic

program and consequently embryonic gene expression. The activity of poly(ADP-ribose) (PAR)
250 polymerase (PARP) enzymes in response to DNA strand breaks is an example for a DDR
pathway with a modulating influence on the establishment of histone methylation marks that can
also respond to environmental cues (see also 3.1. below). Given the apparent importance of
H3K4 and H4K27 methylation in sperm, it is puzzling that the paternal genome, but not the
maternal genome, appears to become depleted of H3K4me3 peaks in zygotes [102]. In addition,
255 Zheng et al. observed global erasure of H3K27me3 marks from sperm after fertilization,
accompanied by inheritance of distal H3K27me3 from oocytes downstream of gene transcription
start sites [103]. More investigations are needed to understand the roles of H3K4 and H3K27
methylation in sperm and after fertilization. An attractive, but untested hypothesis is that these
histone modifications could be involved in directing the rapid DNA de- and remethylation events
260 that occur during and shortly after fertilization to direct gene expression later in development.
Besides sperm histones, which provide potential epigenetic information in the form of at least 26
different modifications, protamines have recently also been shown to bear at least 11 PTM,
which may add a whole new layer of sperm chromatin-borne epigenetic information [35]. The
exact functions of these remain to be elucidated.

265

3. DNA DAMAGE RESPONSE PATHWAYS IN SPERMATID CHROMATIN

REMODELING

Mouse embryos derived from injection of round spermatids into oocytes develop into adult mice
at much lower frequencies than embryos generated by the injection of sperm, which highlights
270 how functionally important the mature sperm chromatin structure is [104,105]. The process that
leads to the typical protamine-based chromatin structure during spermiogenesis in mammals

requires extensive nucleoprotein exchange (see 2.1) and controlled DNA strand breaks. These breaks accommodate the DNA conformational change from the superspiralized, histone-based nucleosomal conformation to a linear, relaxed one stabilized by protamines. [7,63,82,106–112].

275 DDR pathways that are associated with controlled DNA strand breaks are therefore emerging as major factors driving the necessary nucleoprotein exchange during chromatin remodeling in the elongating spermatid (see also Figure 1).

3. 1. Topoisomerase II beta (TOP2B) provides DNA relaxation and triggers a DNA damage 280 response

Because homologous recombination is not an option for repairing the DNA double strand breaks (DSBs) that occur during chromatin condensation in haploid spermatids, free DNA breaks could theoretically only be repaired by the alternative, more error-prone pathway of non-homologous end joining (NHEJ), or remain unrepaired until after fertilization. Classical NHEJ repair is not
285 functional in spermatids, and the effectiveness of the alternative NHEJ pathway in repairing DSBs in spermatids is unclear [113].

While such DNA DSBs may exist as rare events in spermatids, the majority of them seem to occur as a result of type II topoisomerase activity to allow for DNA relaxation and reorganization of chromatin loops [114–117]. In this process, which in spermiogenesis is
290 mediated by topoisomerases, including the topoisomerase variant 2 β (TOP2B), DNA ends remain covalently bound to the enzyme during the decatenation reaction (Figure 1a-c) [107,114,115,118]. It is unclear whether TOP2B is able to perform DNA relaxation in a sequence-specific way. In somatic cells, the enzyme preferentially binds to DNA sites that are supercoiled or arranged in a four-way junction, which is a conformation of DNA in torsional

295 stress, such as during transcription [119]. Such sites are mostly AT- rich and occur throughout
the mammalian genome, particularly in nuclear matrix- and scaffold- attachment sites [120]. In
elongating spermatids, binding of TOP2B to a given genomic locus therefore could be driven by
torsional stress in supercoiled DNA during or prior to the histone-to-protamine exchange and
occur near nuclear matrix attachment sites [107,121]. An interesting observation is that
300 elongating spermatids become positive in the Terminal deoxynucleotidyl transferase (TdT)
dUTP Nick-End Labeling (TUNEL) assay, which recognizes only free, but not protein-bound
DNA ends, suggesting that a large portion of these breaks are not the result of normal TOP2B
activity [122]. Both single and double strand breaks are present in elongating spermatids, raising
the possibilities that TOP2B activity may either be abortive at times, and that a tyrosyl-DNA
305 phosphodiesterase (likely TDP1) may remove TOP2B from the DNA, forming a true DNA break
[107,108,121]. The excision of stalled TOP2B by TDP1 from the DNA, followed by NHEJ as a
potential alternative repair pathway, has been shown in somatic cells, but not in spermatids so far
[108] (Figure 1c). Alternatively, SPO11, an endonuclease responsible for creating breaks during
the meiotic recombination process in spermatocytes, may also form DNA strand breaks in
310 elongating spermatids [121]. In somatic cells, TOP2B creates DNA single strand breaks in silent
promoters to activate poly(ADP-ribose) polymerase 1 (PARP1), which facilitates the
replacement of the silencing histone H1 by high mobility group B protein, which then allows
transcription to proceed [123,124]. Whether this mode of TOP2B/PARP1 interaction also exists
in elongating spermatids is not clear, but the activation of PARP enzymes in elongating
315 spermatids has been shown [125,126].

In somatic cells PARP1 and PARP2 enzymes are rapidly recruited to DNA single and
double strand breaks, where their catalytic domains become immediately activated. Activated

PARP enzymes cleave NAD^+ to synthesize poly(ADP-ribose) (PAR) as a first signal that DNA strand breaks are present (black arrow in Figure 1a) [127] (reviewed in [128]). In somatic cells, PAR formation mediates subsequent recruitment of MRE11, NBS1 and ATM to the DNA lesion (see further below) [129]. PAR is a highly electronegative biopolymer that is attached to target proteins like PARP enzymes themselves, histones and other chromatin proteins as a large posttranslational modification. The strong electronegative charge of PAR reduces or fully neutralizes the ability of target proteins to bind DNA and leads to a profound local chromatin decondensation required for DNA repair and transcription [130,131]. Subsequently, PAR is degraded by the catabolic enzyme PAR glycohydrolase (PARG), which is also expressed in spermatids [109,132]. The concerted activity of PARP and PARG enzymes thus results in a rapid local consumption of NAD^+ and turnover of PAR near DNA strand breaks that facilitates chromatin decondensation as well as histone displacement, which has been studied extensively in somatic and germ cells such as spermatocytes [133–136] (Figure 1a, 1b). The DNA strand breaks that activate PARP in elongating spermatids could also stem from other sources than TOP2B, such as SPO11, however, it could be demonstrated that PAR formation by PARP negatively regulates TOP2B activity both in vitro and in elongating mouse spermatids in vivo. The activities of PARP1 and PARP2 therefore seem to curb TOP2B activity by providing a negative feedback loop [125]. PAR degradation by PARG is necessary to remobilize TOP2B to complete its DNA unknotting activity and the combined activity of PARP1, PARP2, PARG and PAR appears to be important for the regulation of TOP2B activity [125]. After completion of TOP2B-mediated DNA decatenation that is necessary to achieve the chromatin structure in mature sperm (Figure 1d), TOP2B may remain bound to loops of nucleosomal DNA between toroids of protaminated DNA where it could play a role in sperm chromatin fragmentation and unpacking of the paternal

genome after fertilization [17,137,138]. Altering PAR metabolism in elongating spermatids using genetic or pharmacologic intervention results in abnormal histone retention in sperm, deviant sperm head shaping and altered epigenetic regulation of embryonic gene expression in 2-cell embryos, highlighting the importance of PARP-mediated chromatin remodeling in the establishment of sperm chromatin [41,60,82,109,126,139–141]. PARP activity is modulated by various endogenous and exogenous substances and pathways, e.g. the presence of endogenous PARP inhibitors (e.g. vitamin D3 metabolites, certain other vitamins, and unsaturated fatty acids [142,143], reviewed in [142]), and by the availability of NAD^+ as the substrate. In humans, blood NAD^+ levels vary according to nutritional vitamin B3 (niacin, nicotinic acid and nicotinamide) intake [145–148], since vitamin B3 is the main dietary precursor of NAD^+ . Whether a niacin uptake-dependent modulation of PARP activity in spermiogenesis occurs is an open question. Furthermore, in somatic cells, ADP-ribosylation of histone N-terminal tails influences other histone modifications such as H4K16ac and H3K27me3 on the same nucleosomes in somatic cells either by direct competition for the same amino acid or indirectly by steric hindrance [84,139,141,149,150] (see Figure 2). Further research is necessary to determine whether PARP activity has any influence on histone modifications in spermiogenesis as well.

Interestingly, PARP is also directly involved in the regulation of the activity of the protein demethylases KDM5B and KDM4D. These enzymes remove methylation marks at H3K4me3 and H3K9me3, respectively. In somatic cells, PARP activity in the vicinity of DNA strand breaks inhibits these demethylases [151–153]. According to Krishnakumar and Kraus [154], PARP1 binds to promoter regions of active promoters where KDM5B binds as well, where it ADP-ribosylates KDM5B and thus inhibits KDM5B from removing H3K4me3 in somatic cells. By inhibiting this demethylase, PARP therefore maintains the active H3K4me3 mark to keep

genes active in these cells. The regulation of other KDM enzymes by PARP1 may be different as
365 Gong and colleagues reported recently that PARP1 recruits KDM5A to DNA DSBs in somatic
cells to help removing H3K4me3 in the vicinity and to silence genes affected by DNA strand
breakage [155]. While interactions of PARP with any of these histone-modifying enzymes in
spermatogenesis are likely, they are hypothetical at this time. In addition, PARP1 in somatic
cells also directly ADP-ribosylates H3K27 as a posttranslational modification that competes with
370 methylation of this lysine residue [84,149], but to-date no comparable insights are available for
male germ cells. In summary, DNA strand breaks that occur naturally in elongating spermatids
trigger DNA damage response signaling, including PARP activation.

3. 2. Activation of the ATM/ATR pathway by TOP2B to phosphorylate H2AFX

375 The ataxia telangiectasia mutated (ATM) signaling network is an important part of DNA repair
pathways that are activated by TOP2B activity in spermatids, as well as by other sources of DNA
double strand breaks [108,113,156]. As discussed above, the classical NHEJ pathway is likely
not present in spermatids, but the alternative Parp1/XRCC1 dependent NHEJ pathway is active
in these cells to recruit ATM and Rad3-related protein (ATR) [113,156]. It was shown in both
380 somatic and testicular cells that the serine/threonine-protein kinase ATM and ATR interact to
phosphorylate the histone variant H2AFX (H2A.X, in phosphorylated form also known as
gamma-H2AX) in response to DNA DSBs (Figure 1b) [157]. The ATM/ATR-mediated H2AFX
phosphorylation spreads along the DNA for hundreds of thousands of base pairs surrounding the
break, where the modification is bound by MDC1 (mediator of DNA damage checkpoint 1)
385 [158]. Phosphorylated H2AFX as well as the histone variant H2A.Z (H2AFZ) are also essential
in DNA damage response signaling in somatic cells [159–161]. Transient PAR/NAD⁺ turnover

mediated by PARP1/2 and PARG activity after DNA strand breakage is critical for activation of the ATM signaling pathway, and absence of PARP activity impairs ATM/ATR phosphorylation [162,163] (Figure 1b).

390 Interestingly, the activities of several proteins involved in DDR pathways that are discussed here are also essential for the progression of meiosis, inactivation and epigenetic programming of the sex chromosomes (meiotic sex chromosome inactivation, MSCI) and chromatin remodeling that takes place during male meiosis [91,164]. For example, SPO11, an enzyme that creates DNA double strand breaks for genetic recombination, activates the ATM pathway in pachytene spermatocytes. ATM provides a negative feedback regulation to limit SPO11 activity, and thus 395 the number of DNA strand breaks formed by SPO11 during meiosis, which renders *Atm*^{-/-} testes sterile due to meiotic recombination defects [165]. Phosphorylation of H2AFX by ATR, and to a lesser extent by ATM, is a hallmark of XY body formation as part of the MSCI where several DDR enzymes colocalize to silence sex chromosomal gene expression ([166] reviewed in [167]).

400 The interplay of ATR with other proteins such as ATM, BRCA1, MDC1 and the topoisomerase II-binding protein (TOPBP1) is a central mechanism that promotes meiotic sex chromosome silencing by H2AFX phosphorylation. In mice, genetic disruption of several DDR proteins therefore mostly leads to a halt of meiotic progression, which illustrates their importance in meiotic surveillance [166–168]. H2AFX phosphorylation is also involved in H2AFZ deposition, 405 as outlined in the next section, which could have an influence on embryonic gene expression.

3. 3. Functions of H2AFZ in the DNA damage response in spermatids

In mammalian somatic cells, spermatocytes, and round spermatids H2AFZ is inserted into the chromatin to replace H2A and macroH2A in nucleosomes. The canonical H2A is exchanged

410 after its ubiquitination by RNF8 in an interaction with MDC1. In male meiosis, H2AFZ is
enriched in facultative heterochromatin of inactivated sex chromosomes [169], where it is
involved in the activation of genes that escape transcriptional silencing due to MSCI [91].
Insertion of H2AFZ depends at least partially on the binding of phosphorylated H2AFX by
MDC1 [158] (Figure 1b) but the precise mechanism has not been elucidated in spermatids. In
415 somatic cells, MDC1 recruits the NuA4 complex with its TIP60 (Tat-interacting protein of 60
kDa) and p400 motor ATPase subunits to the DNA strand break [170] (Figure 1c, panel i). The
p400 motor ATPase exchanges canonical H2A/H2B dimers for H2AFZ/H2B dimers in
nucleosomes in the vicinity of the DNA strand break, where H2AFZ and H4 subsequently
become acetylated by the TIP60 histone acetyl transferase subunit. Whether the scenario is the
420 same in spermatids is not known. Comparable to PAR-mediated local chromatin decondensation,
incorporation of H2AFZ into nucleosomes and the subsequent acetylation step by TIP60 and the
interacting protein EPC1 locally opens the chromatin structure [7,63,170,171] (Figure 1c, panel
i). In somatic cells, H2AFZ-containing nucleosomes are located directly at transcriptional start
sites and where they are involved in transcriptional control [172]. H2AFZ also promotes
425 recruitment of DNA repair factors and ubiquitination of chromatin components by the RNF8
(RING finger protein 8) ubiquitin ligase [170].

In line with expectations extrapolated from somatic cells, recent analyses of knock-out
animals with functionally disrupted TIP60 function showed reduced histone acetylation in
spermatids as well as impaired elongation and condensation of spermatids [171]. Therefore,
430 certain protein factors involved in DDR in somatic cells also fulfill important functions in
epigenetic remodeling during meiosis and the subsequent spermatid development. The interplay

of TIP60 and EPC1 is essential for histone acetylation, particularly of H4, which is a prerequisite for the exchange of nucleosomes for protamine [171], as outlined in the next section.

435 **3. 4. Histone H4 Acetylation**

Global lysine residue hyperacetylation of core histones, including H4, is an important, but not completely understood step preceding nucleosome eviction during spermiogenesis in various species, e.g. flies, roosters and mammals [173–180]. The proteins MDC1, TIP60 and EPC1 seem to account for most of the observed global histone acetylation observed in round spermatids
440 [171]. Besides the acetylation of H3 (H3K122 and H3K64) and other histones, the increasing acetylation of H4 in lysine residues K5, K8, K12 and K16 adds negative charges to the histone, which destabilizes the nucleosome and weakens its binding to DNA [181]. While the net result is likely a general chromatin decondensation, the individual acetylation marks of H4 also serve specific functions in spermatid gene expression. In addition to histone acetylation, the acylation
445 of histones with different types of lysine posttranslational modifications other than acetylation, such as propionylation, butyrylation, crotonylation and others, have been linked the regulation of gene expression and the metabolism of acyl-CoA in somatic cells [182,183]. Spermatid gene expression is regulated by both, histone acetylation and butyrylation marks [184]. H4 marked with both H4K5ac and H4K8ac are bound by the first bromodomain (BD1) of the testis-specific
450 bromodomain protein BRDT [185–187], a protein that stimulates the transcription of certain spermatogenesis-specific genes by binding directly to their transcriptional starts sites. While histone butyrylation generally directly stimulates transcription, butyrylation of H4K5 prevents the binding of BRDT, which leads to a dynamic regulation of genes marked with both, acetylation and butyrylation of H4 in their promoter regions [184]. BRDT also functions in

455 transcriptional repression during spermatogenesis, where it interacts with HDAC1, PRMT5 and TRIM28 [188] and in RNA processing [189].

The interaction of BRDT with acetylated H4K5 and H4K8 is essential for successful histone removal and sperm development as deletion of BRDT results in deformed sperm with excessive histone retention [190]. How BRDT binding of H3K5ac and H3K8ac contributes
460 mechanistically to histone eviction is still unclear, but a direct mechanical ‘squeezing’ of chromatin fibers by polymerizing BRDT proteins has been proposed [191] (Figure 1c, panel ii). Dhar and colleagues reported that this process involves binding of other proteins including beta actin and SMARCE1, which is a subunit of the SWI/SNF family of ATP-dependent chromatin remodelers [192]. In humans, strong H4 acetylation already appears in early round spermatids
465 long before nucleosome eviction [193], indicating that additional events besides H4K5ac/H4K8ac binding may be required for BRDT-mediated histone eviction.

The bromodomain protein BRD4 and likely other proteins, have also been proposed to have important roles in the removal of nucleosomes from spermatid chromatin [194] (Figure 1c, panel iii). Other H4 acetylation events include H4K12 acetylation, but its involvement in histone
470 removal for protamine deposition is unlikely. Genome-wide mapping showed that H4K12ac remains present at least in part in human mature sperm, where it is enriched in binding sites of the chromatin insulator CTCF (CCCTC-binding factor) and in the promoter regions of genes that are later expressed in 8-cell embryos and blastocysts, suggesting a role of the histone mark in epigenetic inheritance [195].

475 In contrast, H4K16 acetylation is an event in elongating spermatids that is common to all mammals and that occurs just prior to histone eviction. The H4K16ac mark is formed independently from H4K5 and H4K8 acetylation specifically in response to DNA strand breaks

[91]. H4K16ac is created by the acetyltransferase KAT8 (MYST1, MOF), which is phosphorylated by the DNA DSB-activated ATM/ATR pathway. All protein factors of this pathway, which also involves the kinase TSSK6 [196] colocalize with phosphorylated H2AFX in the vicinity of DNA strand breaks in somatic cells [197] (Figure 1b and 1c, panel ii). As discussed above (3.2), the TOP2B-mediated DNA torsional relaxation activates PARP and ATM/ATR signaling, which provides a hypothetical but plausible link between DDR signaling and histone H4K16 acetylation in elongating spermatids.

Although MDC1, TIP60 and EPC1 seem to account for most of the observed H4 acetylation, not all histone acetyl transferases (HAT) that are responsible for H4 hyperacetylation may have been conclusively identified. Deletion of Chd5 (chromodomain helicase DNA binding protein 5) in mice resulted in reduced expression of the GCN5-related N-acetyltransferase 8 (GCN5-related) family member 5 (Nat8f4 in mice, 1700019G17Rik) and in reduced H4 acetylation in elongating spermatids [111,198], indicating that NAT8F4 may also be involved in the acetylation process. Chd5 binds to unmodified H3 and H3K27me3 nucleosomes [199–201] and may act as a multifunctional key orchestrator of nucleosome acetylation and eviction prior to transition protein and protamine deposition (Figure 1c, panel iii) [111].

After highly acetylated histones, which also carry the H4K16ac mark, have been removed from chromatin, they are eliminated by a large and unique proteasome complex (“spermatoproteasome”) [202]. A spermatoproteasome comprises a spermatid-specific alpha subunit (PSMA8), the catalytic beta subunits of immunoproteasomes and an activator protein PA200 (Figure 1c). The activator protein binds acetylated histones with a bromodomain-like domain, and mice lacking PA200 have defects in both, acetylation-dependent degradation of

500 canonical histones and histone disappearance during sperm head condensation [202,203] (Figure 1c, panel ii).

Overall, the DNA strand break-dependent H4K16ac mark may be emerging as the possible final and crucial negative charge needed to tip the balance towards histone binding of a remodeling complex via BRDT or another bromodomain protein, and the eviction of
505 nucleosomes, underscoring the importance of DNA damage response pathways in the chromatin remodeling process in elongating spermatids. The observation that assisted reproduction in humans by round spermatid injection into oocytes (ROSI, ROSNI) is possible, but inefficient, likely because of the immature epigenetic information in round spermatids, illustrates that the defined eviction of histones is important for correct epigenetic programming of sperm,
510 [204,205].

3. 5. Epigenetic marks of genotoxic agent exposure in sperm

External mutagens that elicit DNA repair in germ cells have an impact on the epigenome of sperm [63,206–208] and on testicular gene expression of progeny [209]. Besides purely
515 mutagenic effects of DNA damaging agents that are considered rare events based on unsuccessful DNA repair, DNA repair events themselves leave epigenetic marks in chromatin domains and gene loci even after successful repair. This has been demonstrated in rodents treated with bleomycin, etoposide and cis-platinum (BEP) to emulate a testicular cancer chemotherapy regimen. In that study, BEP treatment led to DNA hyper- and hypo-methylation, partly in
520 preferred loci, suggesting either an interference with normal methylation patterning or abnormal repair of damaged patterns during spermatogenesis [207]. Furthermore, epimutations, i.e.

epigenetically abnormal gene regulation, can cause genetic mutations if such epimutations affect DNA repair enzymes [210], likely contributing to transgenerational epigenetic inheritance.

Nutritional parameters and genotoxic insults are closely interconnected. For example, metabolic syndrome is associated with hyperglycemia, hyperinsulinism and hyperlipidemia causing elevated oxidative stress (reviewed by Rani et al. [211]). Interesting but unknown is whether oxidative stress and resulting DNA damage caused by metabolic syndrome affects selective chromatin regions in spermatids, e.g. by triggering DNA repair events in Dad's germ cells during spermatogenesis, similar to the ones shown in Figure 1, with epigenetic consequences to the sperm chromatin, and ultimately the offspring.

4. AFTER FERTILIZATION

The significance of sperm epigenetic information hinges on its potential to exert long-term consequences on the offspring. After fusion of sperm and oocyte, the sperm nucleus is rapidly unpacked, protamines are efficiently removed from the DNA and replaced by maternally provided histones that are hypomethylated or acetylated and lack silencing marks [87]. At the same time, there is genome-wide DNA demethylation in the male, but not in the female pronucleus. Differentially methylated regions of paternally imprinted genes are the exception as they remain methylated in the male pronucleus [212]. Persisting sperm-associated histones apparently remain at their original loci in the male PN throughout this extensive process [213]. Such persistence is important because core histones present in sperm retain their posttranslational modifications [19–21], similar to differentially methylated DNA regions of imprinted genes that survive the global DNA demethylation in the 1-cell stage. In *Xenopus*, paternal H3K4me2/3 and H3K27me3 influence embryonic gene expression, further supporting this theory [90].

545 In somatic cells, gene silencing histone PTM (such as H3K9me2, H3K9me3) and DNA
methylation were found to promote each other [214]. In contrast, activating histone PTM (such
as H3K4 methylation) generally prevent *de novo* DNA methylation and preserve an
unmethylated DNA state. This seems highly relevant prior to the first cell division in mammals
when the DNA of the paternal PN becomes largely demethylated except for some regions, e.g.
550 imprinted genes and regions associated with H3K9me2. The maternal genome in contrast is
protected from this active DNA demethylation step in the zygote by PGC7/STELLA [215,216].
As the preimplantation embryo develops differentiated tissues, the DNA of both chromosome sets
is methylated to program cells for pluripotency and for differentiation. In view of this extensive
de- and re-methylation of the paternal DNA during the zygotic chromatin remodeling, it is a
555 plausible hypothesis that residual sperm histones bound to gene regulatory sequences may direct
embryonic DNA methylation patterns according to the nature of their PTM and position in the
genome, which would have delayed effects on embryonic transcription.

Preimplantation embryos are initially transcriptionally silent and use maternally provided
RNA until their genome becomes activated during the maternal-to-embryonic transition (e.g. in
560 mid-late 2-cell stage in murine embryos [217]). It is therefore also theoretically possible that the
retention or elimination of histones in the sperm nucleus, which upon fertilization becomes the
paternal pronucleus, could also immediately affect embryonic gene expression based on their
activating or silencing PTM. Experimental evidence obtained in mice shows that changing the
numbers and positions of sperm nucleosomes in gene promoters correlates highly significantly
565 with altered expression of corresponding genes in 2-cell embryos sired by such sperm, providing
some support of this hypothesis [41].

5. CONCLUSIONS

While other mechanisms that were not discussed here may exist as well, current evidence
570 supports the idea that DNA damage signaling and DNA repair events in elongating spermatids,
as well as transcription in round spermatids, are essential steps in sperm epigenetic programming
to support embryonic development. Similar to other phases of the mammalian germline life
cycle, in which major epigenetic programming occur, namely during primordial germ cell (PGC)
development and after fertilization in the early embryo (reviewed in [218,219]), chromatin
575 remodeling in spermiogenesis represents a potential window of opportunity for the acquisition of
epigenetic marks in response to the environment. Interestingly, and comparable to
spermiogenesis, successful epigenetic reprogramming in PGC development and programming of
the zygote also depend on DNA repair pathways involving strand breaks and the resulting DNA
damage response signaling [220–223]. A major difference between the reprogramming events in
580 PGC, the zygote and the spermatid is that there is no known DNA demethylation in
spermiogenesis but, on the other hand, spermatid maturation is the time of one the most dramatic
chromatin protein reorganization events in mammalian biology.

ACKNOWLEDGEMENTS

585 We realize that there is a large body of excellent relevant work that was not discussed in this
review due to space limitations and we apologize to all those whose work could not be cited here
for this reason.

590

REFERENCES

- [1] Wei Y, Schatten H, Sun Q-Y. Environmental epigenetic inheritance through gametes and implications for human reproduction. *Hum Reprod Update* 2015; 21:194–208.
- 595 [2] Guerrero-Bosagna C, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of phenotype and disease. *Mol Cell Endocrinol* 2012; 354:3–8.
- [3] Wu H, Hauser R, Krawetz SA, Pilsner JR. Environmental Susceptibility of the Sperm Epigenome During Windows of Male Germ Cell Development. *Curr Environ Health Rep* 2015; 2:356–366.
- [4] Migicovsky Z, Kovalchuk I. Epigenetic Memory in Mammals. *Front Genet* 2011; 2.
- 600 [5] Ozanne SE. Epigenetic signatures of obesity. *N Engl J Med* 2015; 372:973–974.
- [6] Aiken CE, Ozanne SE. Transgenerational developmental programming. *Hum Reprod Update* 2014; 20:63–75.
- [7] Henikoff S, Smith MM. Histone variants and epigenetics. *Cold Spring Harb Perspect Biol* 2015; 7:a019364.
- 605 [8] Kramer JA, Krawetz SA. RNA in spermatozoa: implications for the alternative haploid genome. *Mol Hum Reprod* 1997; 3:473–478.
- [9] Ostermeier GC, Miller D, Huntriss JD, Diamond MP, Krawetz SA. Reproductive biology: delivering spermatozoan RNA to the oocyte. *Nature* 2004; 429:154.
- [10] Yan W, Morozumi K, Zhang J, Ro S, Park C, Yanagimachi R. Birth of mice after intracytoplasmic injection of single purified sperm nuclei and detection of messenger RNAs and MicroRNAs in the sperm nuclei. *Biol Reprod* 2008; 78:896–902.
- 610 [11] Yan W. Potential roles of noncoding RNAs in environmental epigenetic transgenerational inheritance. *Mol Cell Endocrinol* 2014.
- [12] Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng G, Peng H, Zhang X, Zhang Y, Qian J, Duan E, et al. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* 2016; 351:397–400.
- 615 [13] Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY, Belleannee C, Kucukural A, Serra RW, Sun F, Song L, Carone BR, et al. Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science* 2016; 351:391–396.
- 620 [14] Ly L, Chan D, Aarabi M, Landry M, Behan NA, MacFarlane AJ, Trasler J. Intergenerational impact of paternal lifetime exposures to both folic acid deficiency and supplementation on reproductive outcomes and imprinted gene methylation. *Mol Hum Reprod* 2017; 23:461–477.
- 625 [15] Tillo D, Mukherjee S, Vinson C. Inheritance of Cytosine Methylation. *J Cell Physiol* 2016; 231:2346–2352.
- [16] Battulin N, Fishman VS, Mazur AM, Pomaznoy M, Khabarova AA, Afonnikov DA, Prokhortchouk EB, Serov OL. Comparison of the three-dimensional organization of sperm and fibroblast genomes using the Hi-C approach. *Genome Biol* 2015; 16:77.
- 630 [17] Ward WS, Zalensky AO. The unique, complex organization of the transcriptionally silent sperm chromatin. *Crit Rev Eukaryot Gene Expr* 1996; 6:139–147.
- [18] Zalensky A, Zalenskaya I. Organization of chromosomes in spermatozoa: an additional layer of epigenetic information? *Biochem Soc Trans* 2007; 35:609–611.
- 635 [19] Arpanahi A, Brinkworth M, Iles D, Krawetz SA, Paradowska A, Platts AE, Saida M, Steger K, Tedder P, Miller D. Endonuclease-sensitive regions of human spermatozoal

- chromatin are highly enriched in promoter and CTCF binding sequences. *Genome Res* 2009; 19:1338–1349.
- [20] Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. *Nature* 2009; 460:473–478.
- 640 [21] Brykczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC, Beisel C, Schubeler D, Stadler MB, Peters AH. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nat Struct Mol Biol* 2010; 17:679–687.
- [22] Jung YH, Sauria MEG, Lyu X, Cheema MS, Ausio J, Taylor J, Corces VG. Chromatin States in Mouse Sperm Correlate with Embryonic and Adult Regulatory Landscapes. *Cell Rep* 2017; 18:1366–1382.
- 645 [23] Schagdarsurengin U, Steger K. Epigenetics in male reproduction: effect of paternal diet on sperm quality and offspring health. *Nat Rev Urol* 2016; 13:584–595.
- [24] Ly L, Chan D, Trasler JM. Developmental windows of susceptibility for epigenetic inheritance through the male germline. *Semin Cell Dev Biol* 2015; 43:96–105.
- 650 [25] Fernandez-Twinn DS, Constância M, Ozanne SE. Intergenerational epigenetic inheritance in models of developmental programming of adult disease. *Semin Cell Dev Biol* 2015; 43:85–95.
- [26] Rando OJ. Daddy issues: paternal effects on phenotype. *Cell* 2012; 151:702–708.
- 655 [27] Rando OJ. Intergenerational Transfer of Epigenetic Information in Sperm. *Cold Spring Harb Perspect Med* 2016.
- [28] Rathke C, Baarends WM, Awe S, Renkawitz-Pohl R. Chromatin dynamics during spermiogenesis. *Biochim Biophys Acta* 2014; 1839:155–168.
- [29] Ward WS, Coffey DS. DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. *Biol Reprod* 1991; 44:569–574.
- 660 [30] Balhorn R. A model for the structure of chromatin in mammalian sperm. *J Cell Biol* 1982; 93:298–305.
- [31] Grimes SR, Meistrich ML, Platz RD, Hnilica LS. Nuclear protein transitions in rat testis spermatids. *Exp Cell Res* 1977; 110:31–39.
- 665 [32] Balhorn R, Gledhill BL, Wyrobek AJ. Mouse sperm chromatin proteins: quantitative isolation and partial characterization. *Biochemistry (Mosc)* 1977; 16:4074–4080.
- [33] Gatewood JM, Cook GR, Balhorn R, Bradbury EM, Schmid CW. Sequence-specific packaging of DNA in human sperm chromatin. *Science* 1987; 236:962–964.
- [34] Zalenskaya IA, Bradbury EM, Zalensky AO. Chromatin structure of telomere domain in human sperm. *Biochem Biophys Res Commun* 2000; 279:213–218.
- 670 [35] Brunner AM, Nanni P, Mansuy IM. Epigenetic marking of sperm by post-translational modification of histones and protamines. *Epigenetics Chromatin* 2014; 7:2.
- [36] Saitou M, Kurimoto K. Paternal nucleosomes: are they retained in developmental promoters or gene deserts? *Dev Cell* 2014; 30:6–8.
- 675 [37] Carone BR, Hung J-H, Hainer SJ, Chou M-T, Carone DM, Weng Z, Fazzio TG, Rando OJ. High-resolution mapping of chromatin packaging in mouse embryonic stem cells and sperm. *Dev Cell* 2014; 30:11–22.
- [38] Nazarov IB, Shlyakhtenko LS, Lyubchenko YL, Zalenskaya IA, Zalensky AO. Sperm chromatin released by nucleases. *Syst Biol Reprod Med* 2008; 54:37–46.
- 680 [39] Samans B, Yang Y, Krebs S, Sarode GV, Blum H, Reichenbach M, Wolf E, Steger K, Dansranjav T, Schagdarsurengin U. Uniformity of nucleosome preservation pattern in

- Mammalian sperm and its connection to repetitive DNA elements. *Dev Cell* 2014; 30:23–35.
- 685 [40] Erkek S, Hisano M, Liang C-Y, Gill M, Murr R, Dieker J, Schübeler D, van der Vlag J, Stadler MB, Peters AHFM. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nat Struct Mol Biol* 2013; 20:868–875.
- [41] Ihara M, Meyer-Ficca ML, Leu NA, Rao S, Li F, Gregory BD, Zalenskaya IA, Schultz RM, Meyer RG. Paternal poly (ADP-ribose) metabolism modulates retention of inheritable sperm histones and early embryonic gene expression. *PLoS Genet* 2014; 690 10:e1004317.
- [42] Vavouri T, Lehner B. Human genes with CpG island promoters have a distinct transcription-associated chromatin organization. *Genome Biol* 2012; 13:R110.
- [43] Hammoud SS, Nix DA, Hammoud AO, Gibson M, Cairns BR, Carrell DT. Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. *Hum Reprod Oxf Engl* 2011; 26:2558–2569.
- 695 [44] Schagdarsurengin U, Paradowska A, Steger K. Analysing the sperm epigenome: roles in early embryogenesis and assisted reproduction. *Nat Rev Urol* 2012; 9:609–619.
- [45] Erkek S, Hisano M, Liang C-Y, Gill M, Murr R, Dieker J, Schübeler D, van der Vlag J, 700 Stadler MB, Peters AHFM. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nat Struct Mol Biol* 2013; 20:1236.
- [46] Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 2012; 485:376–380.
- 705 [47] Pombo A, Dillon N. Three-dimensional genome architecture: players and mechanisms. *Nat Rev Mol Cell Biol* 2015; 16:245–257.
- [48] Gonzalez-Sandoval A, Gasser SM. On TADs and LADs: Spatial Control Over Gene Expression. *Trends Genet TIG* 2016; 32:485–495.
- [49] Ke Y, Xu Y, Chen X, Feng S, Liu Z, Sun Y, Yao X, Li F, Zhu W, Gao L, Chen H, Du Z, 710 et al. 3D Chromatin Structures of Mature Gametes and Structural Reprogramming during Mammalian Embryogenesis. *Cell* 2017; 170:367–381.e20.
- [50] Zalensky AO, Allen MJ, Kobayashi A, Zalenskaya IA, Balhorn R, Bradbury EM. Well-defined genome architecture in the human sperm nucleus. *Chromosoma* 1995; 103:577–590.
- 715 [51] Zalensky AO, Breneman JW, Zalenskaya IA, Brinkley BR, Bradbury EM. Organization of centromeres in the decondensed nuclei of mature human sperm. *Chromosoma* 1993; 102:509–518.
- [52] Meyer-Ficca M, Muller-Navia J, Scherthan H. Clustering of pericentromeres initiates in step 9 of spermiogenesis of the rat (*Rattus norvegicus*) and contributes to a well defined genome architecture in the sperm nucleus. *J Cell Sci* 1998; 111 (Pt 10):1363–1370.
- 720 [53] Zalenskaya IA, Zalensky AO. Non-random positioning of chromosomes in human sperm nuclei. *Chromosome Res Int J Mol Supramol Evol Asp Chromosome Biol* 2004; 12:163–173.
- [54] Scherthan H. Analysis of telomere dynamics in mouse spermatogenesis. *Methods Mol Biol Clifton NJ* 2009; 558:383–399.
- 725 [55] Scherthan H. A bouquet makes ends meet. *Nat Rev Cell Biol* 2001; 2:621–627.

- 730 [56] Pittoggi C, Renzi L, Zaccagnini G, Cimini D, Degrassi F, Giordano R, Magnano AR, Lorenzini R, Lavia P, Spadafora C. A fraction of mouse sperm chromatin is organized in nucleosomal hypersensitive domains enriched in retroposon DNA. *J Cell Sci* 1999; 112 (Pt 20):3537–3548.
- [57] Govin J, Escoffier E, Rousseaux S, Kuhn L, Ferro M, Thevenon J, Catena R, Davidson I, Garin J, Khochbin S, Caron C. Pericentric heterochromatin reprogramming by new histone variants during mouse spermiogenesis. *J Cell Biol* 2007; 176:283–294.
- 735 [58] Royo H, Stadler MB, Peters AHFM. Alternative Computational Analysis Shows No Evidence for Nucleosome Enrichment at Repetitive Sequences in Mammalian Spermatozoa. *Dev Cell* 2016; 37:98–104.
- [59] Dansranjav T, Schagdarsurengin U. The Rationale of the Inevitable, or Why Is the Consideration of Repetitive DNA Elements Indispensable in Studies of Sperm Nucleosomes. *Dev Cell* 2016; 37:13–14.
- 740 [60] Meyer-Ficca ML, Lonchar JD, Ihara M, Bader JJ, Meyer RG. Alteration of poly(ADP-ribose) metabolism affects murine sperm nuclear architecture by impairing pericentric heterochromatin condensation. *Chromosoma* 2013; 122:319–335.
- [61] Varghese DS, Chandran U, Soumya A, Pillai SM, Jayakrishnan K, Reddi PP, Kumar PG. Aberrant expression of TAR DNA binding protein-43 is associated with spermatogenic disorders in men. *Reprod Fertil Dev* 2014.
- 745 [62] Castillo J, Amaral A, Oliva R. Sperm nuclear proteome and its epigenetic potential. *Andrology* 2014; 2:326–338.
- [63] Talbert PB, Henikoff S. Environmental responses mediated by histone variants. *Trends Cell Biol* 2014; 24:642–650.
- 750 [64] Fillingham J, Keogh M-C, Krogan NJ. GammaH2AX and its role in DNA double-strand break repair. *Biochem Cell Biol Biochim Biol Cell* 2006; 84:568–577.
- [65] Trostle-Weige PK, Meistrich ML, Brock WA, Nishioka K, Bremer JW. Isolation and characterization of TH2A, a germ cell-specific variant of histone 2A in rat testis. *J Biol Chem* 1982; 257:5560–5567.
- 755 [66] Franklin SG, Zweidler A. Non-allelic variants of histones 2a, 2b and 3 in mammals. *Nature* 1977; 266:273–275.
- [67] Bramlage B, Kosciessa U, Doenecke D. Differential expression of the murine histone genes H3.3A and H3.3B. *Differ Res Biol Divers* 1997; 62:13–20.
- 760 [68] Witt O, Albig W, Doenecke D. Transcriptional regulation of the human replacement histone gene H3.3B. *FEBS Lett* 1997; 408:255–260.
- [69] Ueda J, Harada A, Urahama T, Machida S, Maehara K, Hada M, Makino Y, Nogami J, Horikoshi N, Osakabe A, Taguchi H, Tanaka H, et al. Testis-Specific Histone Variant H3t Gene Is Essential for Entry into Spermatogenesis. *Cell Rep* 2017; 18:593–600.
- 765 [70] Unni E, Mayerhofer A, Zhang Y, Bhatnagar YM, Russell LD, Meistrich ML. Increased accessibility of the N-terminus of testis-specific histone TH2B to antibodies in elongating spermatids. *Mol Reprod Dev* 1995; 42:210–219.
- [71] Pehrson JR, Fried VA. MacroH2A, a core histone containing a large nonhistone region. *Science* 1992; 257:1398–1400.
- 770 [72] Costanzi C, Pehrson JR. MACROH2A2, a new member of the MARCOH2A core histone family. *J Biol Chem* 2001; 276:21776–21784.
- [73] Drabent B, Kardalidou E, Doenecke D. Structure and expression of the human gene encoding testicular H1 histone (H1t). *Gene* 1991; 103:263–268.

- [74] Drabent B, Benavente R, Hoyer-Fender S. Histone H1t is not replaced by H1.1 or H1.2 in pachytene spermatocytes or spermatids of H1t-deficient mice. *Cytogenet Genome Res* 2003; 103:307–313.
- [75] Martianov I, Brancorsini S, Catena R, Gansmuller A, Kotaja N, Parvinen M, Sassone-Corsi P, Davidson I. Polar nuclear localization of H1T2, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis. *Proc Natl Acad Sci U S A* 2005; 102:2808–2813.
- [76] Yan W, Ma L, Burns KH, Matzuk MM. HILS1 is a spermatid-specific linker histone H1-like protein implicated in chromatin remodeling during mammalian spermiogenesis. *Proc Natl Acad Sci U S A* 2003; 100:10546–10551.
- [77] Happel N, Doenecke D. Histone H1 and its isoforms: contribution to chromatin structure and function. *Gene* 2009; 431:1–12.
- [78] Wu F, Caron C, De Robertis C, Khochbin S, Rousseaux S. Testis-specific histone variants H2AL1/2 rapidly disappear from paternal heterochromatin after fertilization. *J Reprod Dev* 2008; 54:413–417.
- [79] Barral S, Morozumi Y, Tanaka H, Montellier E, Govin J, de Dieuleveult M, Charbonnier G, Couté Y, Puthier D, Buchou T, Boussouar F, Urahama T, et al. Histone Variant H2A.L.2 Guides Transition Protein-Dependent Protamine Assembly in Male Germ Cells. *Mol Cell* 2017; 66:89–101.e8.
- [80] Montellier E, Boussouar F, Rousseaux S, Zhang K, Buchou T, Fenaille F, Shiota H, Debernardi A, Hery P, Curtet S, Jamshidikia M, Barral S, et al. Chromatin-to-nucleoprotamine transition is controlled by the histone H2B variant TH2B. *Genes Dev* 2013; 27:1680–1692.
- [81] Shinagawa T, Huynh LM, Takagi T, Tsukamoto D, Tomaru C, Kwak H-G, Dohmae N, Noguchi J, Ishii S. Disruption of Th2a and Th2b genes causes defects in spermatogenesis. *Dev Camb Engl* 2015; 142:1287–1292.
- [82] Meyer-Ficca ML, Ihara M, Lonchar JD, Meistrich ML, Austin CA, Min W, Wang ZQ, Meyer RG. Poly(ADP-ribose) Metabolism Is Essential for Proper Nucleoprotein Exchange During Mouse Spermiogenesis. *Biol Reprod* 2011; 84:218–228.
- [83] Yuen BTK, Bush KM, Barrilleaux BL, Cotterman R, Knoepfler PS. Histone H3.3 regulates dynamic chromatin states during spermatogenesis. *Dev Camb Engl* 2014; 141:3483–3494.
- [84] Messner S, Altmeyer M, Zhao H, Pozivil A, Roschitzki B, Gehrig P, Rutishauser D, Huang D, Caflisch A, Hottiger MO. PARP1 ADP-ribosylates lysine residues of the core histone tails. *Nucleic Acids Res* 2010.
- [85] Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N, Lu Z, Ye Z, et al. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* 2011; 146:1016–1028.
- [86] Turner BM. Cellular memory and the histone code. *Cell* 2002; 111:285–291.
- [87] van der Heijden GW, Ramos L, Baart EB, van den Berg IM, Derijck AA, van der Vlag J, Martini E, de Boer P. Sperm-derived histones contribute to zygotic chromatin in humans. *BMC Dev Biol* 2008; 8:34.
- [88] van de Werken C, van der Heijden GW, Eleveld C, Teeuwssen M, Albert M, Baarends WM, Laven JSE, Peters AHFM, Baart EB. Paternal heterochromatin formation in human embryos is H3K9/HP1 directed and primed by sperm-derived histone modifications. *Nat Commun* 2014; 5:5868.

- 820 [89] Voigt P, LeRoy G, Drury WJ, Zee BM, Son J, Beck DB, Young NL, Garcia BA, Reinberg D. Asymmetrically Modified Nucleosomes. *Cell* 2012; 151:181–193.
- [90] Teperek M, Simeone A, Gaggioli V, Miyamoto K, Allen G, Erkek S, Peters A, Kwon T, Marcotte E, Zegerman P, Bradshaw C, Gurdon J, et al. Sperm is epigenetically programmed to regulate gene transcription in embryos. *Genome Res* 2016.
- 825 [91] Sin H-S, Barski A, Zhang F, Kartashov AV, Nussenzweig A, Chen J, Andreassen PR, Namekawa SH. RNF8 regulates active epigenetic modifications and escape gene activation from inactive sex chromosomes in post-meiotic spermatids. *Genes Dev* 2012; 26:2737–2748.
- [92] Campos EI, Stafford JM, Reinberg D. Epigenetic inheritance: histone bookmarks across generations. *Trends Cell Biol* 2014; 24:664–674.
- 830 [93] Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, et al. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* 2010; 143:1084–1096.
- [94] Lambrot R, Xu C, Saint-Phar S, Chountalos G, Cohen T, Paquet M, Suderman M, Hallett M, Kimmins S. Low paternal dietary folate alters the mouse sperm epigenome and is associated with negative pregnancy outcomes. *Nat Commun* 2013; 4:2889.
- 835 [95] Singh K, Jaiswal D. One-carbon metabolism, spermatogenesis, and male infertility. *Reprod Sci Thousand Oaks Calif* 2013; 20:622–630.
- [96] Zeisel SH. Dietary choline deficiency causes DNA strand breaks and alters epigenetic marks on DNA and histones. *Mutat Res* 2012; 733:34–38.
- 840 [97] Dobosy JR, Fu VX, Desotelle JA, Srinivasan R, Kenowski ML, Almassi N, Weindruch R, Svaren J, Jarrard DF. A methyl-deficient diet modifies histone methylation and alters Igf2 and H19 repression in the prostate. *The Prostate* 2008; 68:1187–1195.
- [98] Smith AD, Kim Y-I, Refsum H. Is folic acid good for everyone? *Am J Clin Nutr* 2008; 87:517–533.
- 845 [99] Garcia BA, Luka Z, Loukachevitch LV, Bhanu NV, Wagner C. Folate deficiency affects histone methylation. *Med Hypotheses* 2016; 88:63–67.
- [100] Siklenka K, Erkek S, Godmann M, Lambrot R, McGraw S, Lafleur C, Cohen T, Xia J, Suderman M, Hallett M, Trasler J, Peters AHFM, et al. Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science* 2015; 350:aab2006.
- 850 [101] Perez-Cerezales S, Ramos-Ibeas P, Lopez-Cardona A, Pericuesta E, Fernandez-Gonzalez R, Pintado B, Gutierrez-Adan A. Elimination of methylation marks at lysines 4 and 9 of histone 3 (H3K4 and H3K9) of spermatozoa alters offspring phenotype. *Reprod Fertil Dev* 2015.
- 855 [102] Zhang B, Zheng H, Huang B, Li W, Xiang Y, Peng X, Ming J, Wu X, Zhang Y, Xu Q, Liu W, Kou X, et al. Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. *Nature* 2016; 537:553–557.
- [103] Zheng H, Huang B, Zhang B, Xiang Y, Du Z, Xu Q, Li Y, Wang Q, Ma J, Peng X, Xu F, Xie W. Resetting Epigenetic Memory by Reprogramming of Histone Modifications in Mammals. *Mol Cell* 2016; 63:1066–1079.
- 860 [104] Kimura Y, Yanagimachi R. Mouse oocytes injected with testicular spermatozoa or round spermatids can develop into normal offspring. *Dev Camb Engl* 1995; 121:2397–2405.

- [105] Kishigami S, Van Thuan N, Hikichi T, Ohta H, Wakayama S, Mizutani E, Wakayama T. Epigenetic abnormalities of the mouse paternal zygotic genome associated with microinsemination of round spermatids. *Dev Biol* 2006; 289:195–205.
- 865 [106] Laberge RM, Boissonneault G. Chromatin remodeling in spermatids: a sensitive step for the genetic integrity of the male gamete. *Arch Androl* 2005; 51:125–133.
- [107] Laberge RM, Boissonneault G. On the nature and origin of DNA strand breaks in elongating spermatids. *Biol Reprod* 2005; 73:289–296.
- 870 [108] Leduc F, Maquennehan V, Nkoma GB, Boissonneault G. DNA damage response during chromatin remodeling in elongating spermatids of mice. *Biol Reprod* 2008; 78:324–332.
- [109] Meyer-Ficca ML, Lonchar J, Credidio C, Ihara M, Li Y, Wang ZQ, Meyer RG. Disruption of Poly(ADP-Ribose) Homeostasis Affects Spermiogenesis and Sperm Chromatin Integrity in Mice. *Biol Reprod* 2009; 81:46–55.
- 875 [110] Leduc F, Nkoma GB, Boissonneault G. Spermiogenesis and DNA repair: a possible etiology of human infertility and genetic disorders. *Syst Biol Reprod Med* 2008; 54:3–10.
- [111] Li W, Wu J, Kim S-Y, Zhao M, Hearn SA, Zhang MQ, Meistrich ML, Mills AA. Chd5 orchestrates chromatin remodelling during sperm development. *Nat Commun* 2014; 5:3812.
- 880 [112] Smith A, Haaf T. DNA nicks and increased sensitivity of DNA to fluorescence in situ end labeling during functional spermiogenesis. *BioTechniques* 1998; 25:496–502.
- [113] Ahmed EA, Scherthan H, de Rooij DG. DNA Double Strand Break Response and Limited Repair Capacity in Mouse Elongated Spermatids. *Int J Mol Sci* 2015; 16:29923–29935.
- [114] Roca J, Mezquita C. DNA topoisomerase II activity in nonreplicating, transcriptionally inactive, chicken late spermatids. *EMBO J* 1989; 8:1855–1860.
- 885 [115] McPherson SM, Longo FJ. Nicking of rat spermatid and spermatozoa DNA: possible involvement of DNA topoisomerase II. *Dev Biol* 1993; 158:122–130.
- [116] Chen JL, Longo FJ. Expression and localization of DNA topoisomerase II during rat spermatogenesis. *Mol Reprod Dev* 1996; 45:61–71.
- 890 [117] Cobb J, Reddy RK, Park C, Handel MA. Analysis of expression and function of topoisomerase I and II during meiosis in male mice. *Mol Reprod Dev* 1997; 46:489–498.
- [118] Shaman JA, Prisztoka R, Ward WS. Topoisomerase IIB and an extracellular nuclease interact to digest sperm DNA in an apoptotic-like manner. *Biol Reprod* 2006; 75:741–748.
- [119] West KL, Austin CA. Human DNA topoisomerase IIBeta binds and cleaves four-way junction DNA in vitro. *Nucleic Acids Res* 1999; 27:984–992.
- 895 [120] Masliah G, René B, Zargarian L, Fermandjian S, Mauffret O. Identification of intrinsic dynamics in a DNA sequence preferentially cleaved by topoisomerase II enzyme. *J Mol Biol* 2008; 381:692–706.
- [121] Gouraud A, Brazeau M-A, Grégoire M-C, Simard O, Massonneau J, Arguin M, Boissonneault G. ‘Breaking news’ from spermatids. *Basic Clin Androl* 2013; 23:11.
- 900 [122] Marcon L, Boissonneault G. Transient DNA strand breaks during mouse and human spermiogenesis new insights in stage specificity and link to chromatin remodeling. *Biol Reprod* 2004; 70:910–918.
- [123] Ju BG, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, Rosenfeld MG. A topoisomerase IIBeta-mediated dsDNA break required for regulated transcription. *Science* 2006; 312:1798–1802.
- 905 [124] Ju BG, Rosenfeld MG. A breaking strategy for topoisomerase IIBeta/PARP-1-dependent regulated transcription. *Cell Cycle Georget Tex* 2006; 5:2557–2560.

- 910 [125] Meyer-Ficca ML, Lonchar JD, Ihara M, Meistrich ML, Austin CA, Meyer RG. Poly(ADP-ribose) polymerases PARP1 and PARP2 modulate topoisomerase II beta (TOP2B) function during chromatin condensation in mouse spermiogenesis. *Biol Reprod* 2011; 84:900–909.
- [126] Meyer-Ficca ML, Scherthan H, Burkle A, Meyer RG. Poly(ADP-ribosyl)ation during chromatin remodeling steps in rat spermiogenesis. *Chromosoma* 2005; 114:67–74.
- 915 [127] Mortusewicz O, Ame JC, Schreiber V, Leonhardt H. Feedback-regulated poly(ADP-ribosyl)ation by PARP-1 is required for rapid response to DNA damage in living cells. *Nucleic Acids Res* 2007; 35:7665–7675.
- [128] Burkle A, Virag L. Poly(ADP-ribose): PARadigms and PARadoxes. *Mol Aspects Med* 2013.
- 920 [129] Haince JF, McDonald D, Rodrigue A, Dery U, Masson JY, Hendzel MJ, Poirier GG. PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *J Biol Chem* 2008; 283:1197–1208.
- [130] Tulin A, Spradling A. Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at *Drosophila* puff loci. *Science* 2003; 299:560–562.
- 925 [131] Tulin A, Chinenov Y, Spradling A. Regulation of chromatin structure and gene activity by poly(ADP-ribose) polymerases. *Curr Top Dev Biol* 2003; 56:55–83.
- [132] Meyer-Ficca ML, Meyer RG, Jacobson EL, Jacobson MK. Poly(ADP-ribose) polymerases: managing genome stability. *Int J Biochem Cell Biol* 2005; 37:920–926.
- 930 [133] Strickfaden H, McDonald D, Kruhlak MJ, Haince J-F, Th'ng JPH, Rouleau M, Ishibashi T, Corry GN, Ausio J, Underhill DA, Poirier GG, Hendzel MJ. Poly(ADP-ribosyl)ation-dependent Transient Chromatin Decondensation and Histone Displacement following Laser Microirradiation. *J Biol Chem* 2016; 291:1789–1802.
- [134] Althaus FR. Poly ADP-ribosylation: a histone shuttle mechanism in DNA excision repair. *J Cell Sci* 1992; 102 (Pt 4):663–670.
- 935 [135] Tramontano F, Malanga M, Quesada P. Differential contribution of poly(ADP-ribose)polymerase-1 and -2 (PARP-1 and -2) to the poly(ADP-ribosyl)ation reaction in rat primary spermatocytes. *Mol Hum Reprod* 2007; 13:821–828.
- [136] Di Meglio S, Tramontano F, Cimmino G, Jones R, Quesada P. Dual role for poly(ADP-ribose)polymerase-1 and -2 and poly(ADP-ribose)glycohydrolase as DNA-repair and pro-apoptotic factors in rat germinal cells exposed to nitric oxide donors. *Biochim Biophys Acta* 2004; 1692:35–44.
- 940 [137] Ward WS. Regulating DNA Supercoiling: Sperm Points the Way. *Biol Reprod* 2011.
- [138] Gawecka JE, Ribas-Maynou J, Benet J, Ward WS. A model for the control of DNA integrity by the sperm nuclear matrix. *Asian J Androl* 2015; 17:610–615.
- 945 [139] Dantzer F, Mark M, Quenet D, Scherthan H, Huber A, Liebe B, Monaco L, Chicheportiche A, Sassone-Corsi P, de Murcia G, Menissier-de Murcia J. Poly(ADP-ribose) polymerase-2 contributes to the fidelity of male meiosis I and spermiogenesis. *Proc Natl Acad Sci U S A* 2006; 103:14854–14859.
- 950 [140] Quenet D, Mark M, Govin J, van Dorsselaar A, Schreiber V, Khochbin S, Dantzer F. Parp2 is required for the differentiation of post-meiotic germ cells: identification of a spermatid-specific complex containing Parp1, Parp2, TP2 and HSPA2. *Exp Cell Res* 2009; 315:2824–2834.
- [141] Quenet D, Gasser V, Fouillen L, Cammas F, Sanglier-Cianferani S, Losson R, Dantzer F. The histone subcode: poly(ADP-ribose) polymerase-1 (Parp-1) and Parp-2 control cell

- 955 differentiation by regulating the transcriptional intermediary factor TIF1beta and the heterochromatin protein HP1alpha. *FASEB J Off Publ Fed Am Soc Exp Biol* 2008; 22:3853–3865.
- [142] Banasik M, Stedeford T, Strosznajder RP. Natural inhibitors of poly(ADP-ribose) polymerase-1. *Mol Neurobiol* 2012; 46:55–63.
- 960 [143] Mabley JG, Wallace R, Pacher P, Murphy K, Szabó C. Inhibition of poly(adenosine diphosphate-ribose) polymerase by the active form of vitamin D. *Int J Mol Med* 2007; 19:947–952.
- [144] Banasik M, Komura H, Ueda K. Inhibition of poly(ADP-ribose) synthetase by unsaturated fatty acids, vitamins and vitamin-like substances. *FEBS Lett* 1990; 263:222–224.
- 965 [145] Kirkland JB. Niacin status, NAD distribution and ADP-ribose metabolism. *Curr Pharm Des* 2009; 15:3–11.
- [146] Kirkland JB. Niacin status and genomic instability in bone marrow cells; mechanisms favoring the progression of leukemogenesis. *Subcell Biochem* 2012; 56:21–36.
- [147] Hageman G., Stierum R. Niacin, poly(ADP-ribose) polymerase-1 and genomic stability. *Mutat Res Mol Mech Mutagen* 2001; 475:45–56.
- 970 [148] Rawling JM, Jackson TM, Driscoll ER, Kirkland JB. Dietary niacin deficiency lowers tissue poly(ADP-ribose) and NAD⁺ concentrations in Fischer-344 rats. *J Nutr* 1994; 124:1597–1603.
- [149] Hottiger MO. Nuclear ADP-Ribosylation and Its Role in Chromatin Plasticity, Cell Differentiation, and Epigenetics. *Annu Rev Biochem* 2015; 84:227–263.
- 975 [150] Malik N, Smulson M. A relationship between nuclear poly(adenosine diphosphate ribosylation) and acetylation posttranslational modifications. 1. Nucleosome studies. *Biochemistry (Mosc)* 1984; 23:3721–3725.
- [151] Le May N, Iltis I, Amé J-C, Zhovmer A, Biard D, Egly J-M, Schreiber V, Coin F. Poly (ADP-ribose) glycohydrolase regulates retinoic acid receptor-mediated gene expression. *Mol Cell* 2012; 48:785–798.
- 980 [152] Krishnakumar R, Kraus WL. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol Cell* 2010; 39:8–24.
- [153] Goldberg M. KDM4D crosstalks with PARP1 and RNA at DNA DSBs. *Cell Cycle* 2015; 14:1495–1495.
- 985 [154] Krishnakumar R, Kraus WL. PARP-1 regulates chromatin structure and transcription through a KDM5B-dependent pathway. *Mol Cell* 2010; 39:736–749.
- [155] Gong F, Clouaire T, Aguirrebengoa M, Legube G, Miller KM. Histone demethylase KDM5A regulates the ZMYND8-NuRD chromatin remodeler to promote DNA repair. *J Cell Biol* 2017.
- 990 [156] Ahmed EA, de Boer P, Philippens MEP, Kal HB, de Rooij DG. Parp1-XRCC1 and the repair of DNA double strand breaks in mouse round spermatids. *Mutat Res* 2010; 683:84–90.
- [157] Hamer G, Roepers-Gajadien HL, van Duyn-Goedhart A, Gademan IS, Kal HB, van Buul PPW, de Rooij DG. DNA double-strand breaks and gamma-H2AX signaling in the testis. *Biol Reprod* 2003; 68:628–634.
- 995 [158] Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 Directly Binds Phosphorylated Histone H2AX to Regulate Cellular Responses to DNA Double-Strand Breaks. *Cell* 2005; 123:1213–1226.

- 1000 [159] Cimprich KA, Shin TB, Keith CT, Schreiber SL. cDNA cloning and gene mapping of a candidate human cell cycle checkpoint protein. *Proc Natl Acad Sci* 1996; 93:2850–2855.
- [160] Lee J-H, Paull TT. Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. *Oncogene* 2007; 26:7741–7748.
- 1005 [161] Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, Shiloh Y, Gygi SP, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 2007; 316:1160–1166.
- [162] Haince JF, Kozlov S, Dawson VL, Dawson TM, Hendzel MJ, Lavin MF, Poirier GG. Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. *J Biol Chem* 2007; 282:16441–16453.
- 1010 [163] Guleria A, Chandna S. ATM kinase: Much more than a DNA damage responsive protein. *DNA Repair* 2016; 39:1–20.
- [164] Alavattam KG, Kato Y, Sin H-S, Maezawa S, Kowalski IJ, Zhang F, Pang Q, Andreassen PR, Namekawa SH. Elucidation of the Fanconi Anemia Protein Network in Meiosis and Its Function in the Regulation of Histone Modifications. *Cell Rep* 2016; 17:1141–1157.
- 1015 [165] Lange J, Pan J, Cole F, Thelen MP, Jasin M, Keeney S. ATM controls meiotic double-strand-break formation. *Nature* 2011; 479:237–240.
- [166] Royo H, Prosser H, Ruzankina Y, Mahadevaiah SK, Cloutier JM, Baumann M, Fukuda T, Hoog C, Toth A, de Rooij DG, Bradley A, Brown EJ, et al. ATR acts stage specifically to regulate multiple aspects of mammalian meiotic silencing. *Genes Dev* 2013; 27:1484–1494.
- 1020 [167] Ichijima Y, Sin H-S, Namekawa SH. Sex chromosome inactivation in germ cells: emerging roles of DNA damage response pathways. *Cell Mol Life Sci CMLS* 2012; 69:2559–2572.
- 1025 [168] Fedoriw AM, Menon D, Kim Y, Mu W, Magnuson T. Key mediators of somatic ATR signaling localize to unpaired chromosomes in spermatocytes. *Development* 2015; 142:2972–2980.
- [169] Greaves IK, Rangasamy D, Devoy M, Marshall Graves JA, Tremethick DJ. The X and Y chromosomes assemble into H2A.Z-containing [corrected] facultative heterochromatin [corrected] following meiosis. *Mol Cell Biol* 2006; 26:5394–5405.
- 1030 [170] Xu Y, Ayrapetov MK, Xu C, Gursoy-Yuzugullu O, Hu Y, Price BD. Histone H2A.Z controls a critical chromatin remodeling step required for DNA double-strand break repair. *Mol Cell* 2012; 48:723–733.
- 1035 [171] Dong Y, Isono K-I, Ohbo K, Endo TA, Ohara O, Maekawa M, Toyama Y, Ito C, Toshimori K, Helin K, Ogonuki N, Inoue K, et al. EPC1/TIP60-mediated histone acetylation facilitates spermiogenesis in mice. *Mol Cell Biol* 2017.
- [172] Weber CM, Ramachandran S, Henikoff S. Nucleosomes Are Context-Specific, H2A.Z-Modulated Barriers to RNA Polymerase. *Mol Cell* 2014; 53:819–830.
- 1040 [173] Awe S, Renkawitz-Pohl R. Histone H4 acetylation is essential to proceed from a histone- to a protamine-based chromatin structure in spermatid nuclei of *Drosophila melanogaster*. *Syst Biol Reprod Med* 2010; 56:44–61.
- [174] Oliva R, Mezquita C. Histone H4 hyperacetylation and rapid turnover of its acetyl groups in transcriptionally inactive rooster testis spermatids. *Nucleic Acids Res* 1982; 10:8049–8059.
- 1045

- [175] Sonnack V, Failing K, Bergmann M, Steger K. Expression of hyperacetylated histone H4 during normal and impaired human spermatogenesis. *Andrologia* 2002; 34:384–390.
- [176] Rousseaux S, Reynoird N, Escoffier E, Thevenon J, Caron C, Khochbin S. Epigenetic reprogramming of the male genome during gametogenesis and in the zygote. *Reprod Biomed Online* 2008; 16:492–503.
- 1050 [177] Hazzouri M, Pivot-Pajot C, Faure AK, Usson Y, Pelletier R, Sele B, Khochbin S, Rousseaux S. Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. *Eur J Cell Biol* 2000; 79:950–960.
- [178] Meistrich ML, Trostle-Weige PK, Lin R, Bhatnagar YM, Allis CD. Highly acetylated H4 is associated with histone displacement in rat spermatids. *Mol Reprod Dev* 1992; 31:170–181.
- 1055 [179] Grimes SR, Henderson N. Hyperacetylation of histone H4 in rat testis spermatids. *Exp Cell Res* 1984; 152:91–97.
- [180] Dai L, Endo D, Akiyama N, Yamamoto-Fukuda T, Koji T. Aberrant levels of histone H3 acetylation induce spermatid anomaly in mouse testis. *Histochem Cell Biol* 2015; 143:209–224.
- 1060 [181] Goudarzi A, Shiota H, Rousseaux S, Khochbin S. Genome-scale acetylation-dependent histone eviction during spermatogenesis. *J Mol Biol* 2014; 426:3342–3349.
- [182] Dai L, Peng C, Montellier E, Lu Z, Chen Y, Ishii H, Debernardi A, Buchou T, Rousseaux S, Jin F, Sabari BR, Deng Z, et al. Lysine 2-hydroxyisobutyrylation is a widely distributed active histone mark. *Nat Chem Biol* 2014; 10:365–370.
- 1065 [183] Sabari BR, Zhang D, Allis CD, Zhao Y. Metabolic regulation of gene expression through histone acylations. *Nat Rev Mol Cell Biol* 2017; 18:90–101.
- [184] Goudarzi A, Zhang D, Huang H, Barral S, Kwon OK, Qi S, Tang Z, Buchou T, Vitte A-L, He T, Cheng Z, Montellier E, et al. Dynamic Competing Histone H4 K5K8 Acetylation and Butyrylation Are Hallmarks of Highly Active Gene Promoters. *Mol Cell* 2016; 62:169–180.
- 1070 [185] Pivot-Pajot C, Caron C, Govin J, Vion A, Rousseaux S, Khochbin S. Acetylation-dependent chromatin reorganization by BRDT, a testis-specific bromodomain-containing protein. *Mol Cell Biol* 2003; 23:5354–5365.
- 1075 [186] Moriniere J, Rousseaux S, Steuerwald U, Soler-Lopez M, Curtet S, Vitte AL, Govin J, Gaucher J, Sadoul K, Hart DJ, Krijgsveld J, Khochbin S, et al. Cooperative binding of two acetylation marks on a histone tail by a single bromodomain. *Nature* 2009; 461:664–668.
- [187] Miller TCR, Simon B, Rybin V, Grötsch H, Curtet S, Khochbin S, Carlomagno T, Müller CW. A bromodomain-DNA interaction facilitates acetylation-dependent bivalent nucleosome recognition by the BET protein BRDT. *Nat Commun* 2016; 7:13855.
- 1080 [188] Wang L, Wolgemuth DJ. BET Protein BRDT Complexes With HDAC1, PRMT5, and TRIM28 and Functions in Transcriptional Repression During Spermatogenesis. *J Cell Biochem* 2016; 117:1429–1438.
- 1085 [189] Berkovits BD, Wang L, Guarnieri P, Wolgemuth DJ. The testis-specific double bromodomain-containing protein BRDT forms a complex with multiple spliceosome components and is required for mRNA splicing and 3'-UTR truncation in round spermatids. *Nucleic Acids Res* 2012; 40:7162–7175.
- 1090 [190] Shang E, Nickerson HD, Wen D, Wang X, Wolgemuth DJ. The first bromodomain of Brdt, a testis-specific member of the BET sub-family of double-bromodomain-containing

- proteins, is essential for male germ cell differentiation. *Dev Camb Engl* 2007; 134:3507–3515.
- 1095 [191] Gaucher J, Boussouar F, Montellier E, Curtet S, Buchou T, Bertrand S, Hery P, Jounier S, Depaux A, Vitte AL, Guardiola P, Pernet K, et al. Bromodomain-dependent stage-specific male genome programming by Brdt. *EMBO J* 2012; 31:3809–3820.
- [192] Dhar S, Thota A, Rao MRS. Insights into role of bromodomain, testis-specific (Brdt) in acetylated histone H4-dependent chromatin remodeling in mammalian spermiogenesis. *J Biol Chem* 2012; 287:6387–6405.
- 1100 [193] Sonnack V, Failing K, Bergmann M, Steger K. Expression of hyperacetylated histone H4 during normal and impaired human spermatogenesis. *Andrologia* 2002; 34:384–390.
- [194] Bryant JM, Donahue G, Wang X, Meyer-Ficca M, Luense LJ, Weller AH, Bartolomei MS, Blobel GA, Meyer RG, Garcia BA, Berger SL. Characterization of BRD4 during mammalian postmeiotic sperm development. *Mol Cell Biol* 2015; 35:1433–1448.
- 1105 [195] Paradowska AS, Miller D, Spiess A-N, Vieweg M, Cerna M, Dvorakova-Hortova K, Bartkuhn M, Schuppe H-C, Weidner W, Steger K. Genome wide identification of promoter binding sites for H4K12ac in human sperm and its relevance for early embryonic development. *Epigenetics Off J DNA Methylation Soc* 2012; 7:1057–1070.
- [196] Jha KN, Tripurani SK, Johnson GR. TSSK6 is required for γ H2AX formation and the histone-to-protamine transition during spermiogenesis. *J Cell Sci* 2017; 130:1835–1844.
- 1110 [197] Gupta A, Hunt CR, Hegde ML, Chakraborty S, Chakraborty S, Udayakumar D, Horikoshi N, Singh M, Ramnarain DB, Hittelman WN, Namjoshi S, Asaithamby A, et al. MOF phosphorylation by ATM regulates 53BP1-mediated double-strand break repair pathway choice. *Cell Rep* 2014; 8:177–189.
- 1115 [198] Zhuang T, Hess RA, Kolla V, Higashi M, Raabe TD, Brodeur GM. CHD5 is required for spermiogenesis and chromatin condensation. *Mech Dev* 2014; 131:35–46.
- [199] Paul S, Kuo A, Schalch T, Vogel H, Joshua-Tor L, McCombie WR, Gozani O, Hammell M, Mills AA. Chd5 requires PHD-mediated histone 3 binding for tumor suppression. *Cell Rep* 2013; 3:92–102.
- 1120 [200] Oliver SS, Musselman CA, Srinivasan R, Svaren JP, Kutateladze TG, Denu JM. Multivalent recognition of histone tails by the PHD fingers of CHD5. *Biochemistry (Mosc)* 2012; 51:6534–6544.
- 1125 [201] Egan CM, Nyman U, Skotte J, Streubel G, Turner S, O’Connell DJ, Rraklli V, Dolan MJ, Chadderton N, Hansen K, Farrar GJ, Helin K, et al. CHD5 is required for neurogenesis and has a dual role in facilitating gene expression and polycomb gene repression. *Dev Cell* 2013; 26:223–236.
- [202] Qian M-X, Pang Y, Liu CH, Haratake K, Du B-Y, Ji D-Y, Wang G-F, Zhu Q-Q, Song W, Yu Y, Zhang X-X, Huang H-T, et al. Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. *Cell* 2013; 153:1012–1024.
- 1130 [203] Khor B, Bredemeyer AL, Huang C-Y, Turnbull IR, Evans R, Maggi LB, White JM, Walker LM, Carnes K, Hess RA, Sleckman BP. Proteasome activator PA200 is required for normal spermatogenesis. *Mol Cell Biol* 2006; 26:2999–3007.
- [204] Practice Committee of American Society for Reproductive Medicine, Practice Committee of Society for Assisted Reproductive Technology. Round spermatid nucleus injection (ROSNI). *Fertil Steril* 2008; 90:S199-201.

- 1135 [205] Tanaka A, Nagayoshi M, Takemoto Y, Tanaka I, Kusunoki H, Watanabe S, Kuroda K, Takeda S, Ito M, Yanagimachi R. Fourteen babies born after round spermatid injection into human oocytes. *Proc Natl Acad Sci U S A* 2015; 112:14629–14634.
- [206] de Boer P, Ramos L, de Vries M, Gochhait S. Memoirs of an insult: sperm as a possible source of transgenerational epimutations and genetic instability. *Mol Hum Reprod* 2010; 16:48–56.
- 1140 [207] Chan D, Delbès G, Landry M, Robaire B, Trasler JM. Epigenetic alterations in sperm DNA associated with testicular cancer treatment. *Toxicol Sci Off J Soc Toxicol* 2012; 125:532–543.
- [208] Bagheri-Sereshki N, Hales BF, Robaire B. The Effects of Chemotherapeutic Agents, Bleomycin, Etoposide, and Cisplatin, on Chromatin Remodeling in Male Rat Germ Cells. *Biol Reprod* 2016; 94:81.
- 1145 [209] Maselli J, Hales BF, Robaire B. The effects of chemotherapy with bleomycin, etoposide, and cis-platinum (BEP) on rat sperm chromatin remodeling, fecundity and testicular gene expression in the progeny. *Biol Reprod* 2013; 89:85.
- 1150 [210] Skinner MK, Guerrero-Bosagna C, Haque MM. Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics* 2015; 10:762–771.
- [211] Rani V, Deep G, Singh RK, Palle K, Yadav UCS. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sci* 2016; 148:183–193.
- 1155 [212] Oswald J, Engemann S, Lane N, Mayer W, Olek A, Fundele R, Dean W, Reik W, Walter J. Active demethylation of the paternal genome in the mouse zygote. *Curr Biol CB* 2000; 10:475–478.
- [213] van der Heijden GW, Derijck AA, Ramos L, Giele M, van der Vlag J, de Boer P. Transmission of modified nucleosomes from the mouse male germline to the zygote and subsequent remodeling of paternal chromatin. *Dev Biol* 2006; 298:458–469.
- 1160 [214] Fuks F. DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev* 2005; 15:490–495.
- [215] Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, Sekimoto T, Ikawa M, Yoneda Y, Okabe M, Tanaka S, Shiota K, et al. PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nat Cell Biol* 2007; 9:64–71.
- 1165 [216] Nakamura T, Liu Y-J, Nakashima H, Umehara H, Inoue K, Matoba S, Tachibana M, Ogura A, Shinkai Y, Nakano T. PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* 2012; 486:415–419.
- [217] Zeng F, Schultz RM. RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. *Dev Biol* 2005; 283:40–57.
- 1170 [218] Reik W, Surani MA. Germline and Pluripotent Stem Cells. *Cold Spring Harb Perspect Biol* 2015; 7.
- [219] Hackett JA, Zyllicz JJ, Surani MA. Parallel mechanisms of epigenetic reprogramming in the germline. *Trends Genet TIG* 2012; 28:164–174.
- 1175 [220] Hajkova P, Jeffries SJ, Lee C, Miller N, Jackson SP, Surani MA. Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. *Science* 2010; 329:78–82.
- [221] Ladstätter S, Tachibana-Konwalski K. A Surveillance Mechanism Ensures Repair of DNA Lesions during Zygotic Reprogramming. *Cell* 2016; 167:1774–1787.e13.

- 1180 [222] Wossidlo M, Arand J, Sebastiano V, Lepikhov K, Boiani M, Reinhardt R, Schöler H, Walter J. Dynamic link of DNA demethylation, DNA strand breaks and repair in mouse zygotes. *EMBO J* 2010; 29:1877–1888.
- [223] Wossidlo M, Nakamura T, Lepikhov K, Marques CJ, Zakhartchenko V, Boiani M, Arand J, Nakano T, Reik W, Walter J. 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. *Nat Commun* 2011; 2:241.
- 1185 [224] Quesada P, Tramontano F, Faraone-Mennella MR, Farina B. The analysis of the poly(ADPR) polymerase mode of action in rat testis nuclear fractions defines a specific poly(ADP-ribosylation) system associated with the nuclear matrix. *Mol Cell Biochem* 2000; 205:91–99.
- 1190 [225] Altmeyer M, Messner S, Hassa PO, Fey M, Hottiger MO. Molecular mechanism of poly(ADP-ribosylation) by PARP1 and identification of lysine residues as ADP-ribose acceptor sites. *Nucleic Acids Res* 2009; 37:3723–3738.

1195

FIGURE LEGENDS

Fig. 1: Proposed model of DNA damage repair (DDR) signaling involvement in chromatin remodeling and nucleoprotein exchange in elongating spermatids.

- 1200 Please see also detailed discussion of this figure in the main body of the text. Processes shown in panels (a)-(d) may be successive or concurrent events. Events that have been demonstrated only in somatic cells and are considered hypothetical in spermatids are marked with an asterisk* (a) TOP2B activity initiated by DNA torsional stress or other, currently unknown factors, potentially including the formation of DNA strand breaks by nucleases like SPO11, results in
- 1205 PARP1/PARP2 activation. Modification of PARP enzymes and histones with PAR results in partial core histone and H1 removal from the site [224] and PAR recruits diverse DNA repair factors including ATM. After recruitment by PARP, KDM5A removes H3K4me3 in somatic cells; whether this also occurs in spermatids has not been investigated. Histones may be already partly acetylated such as in H4K5ac and H4K8ac. (b) During and after TOP2B-facilitated DNA

1210 decatenation, PARP-mediated activation of the ATM pathway results in KAT8 and H2AFX
phosphorylation and H4K16ac formation as the last step of histone H4 hyperacetylation
necessary for histone eviction and replacement by transition proteins. The deacetylase SIRT1,
which normally counteracts H4 acetylation in somatic and earlier germ cells, is likely no longer
present at this time. PARP, auto-modified with PAR, is gradually removed from the site of
1215 TOP2B activity. MDC1 binds to phospho-H2AFX and recruits RNF8 to ubiquitinate H2A
histones. (c) Panel i: MDC1 recruits the TIP60 (KAT5) histone acetyltransferase complex that
also replaces H2A/H2B for H2AFZ/H2B in nucleosomes. This is an event that was also taking
place earlier in spermatocytes. The TIP60 complex also acetylates ATM to enhance
phosphorylation of histones including H2AFX and H2AFZ. Histones acetylated at H4K16 by
1220 MYST1/KAT8 are removed from the DNA by a complex containing BRDT, or a different
bromodomain protein like BRD4, replaced by transition proteins or protamines, and degraded
(panel ii). Alternatively, or additionally, histones are bound by CHD5 and acetylated by NAT8F4
for removal (panel iii). TDP1 may remove stalled TOP2B covalently bound to DNA, creating a
strand break that could be repaired by a non-homologous end joining pathway or remain
1225 unrepaired. (d) The final sperm chromatin structure is based on protamine-based toroid structures
with nucleosomal linker chromatin domains carrying epigenetic information.

Figure 2) A model of epigenetic modifier regulation by DNA strand break-dependent poly(ADP-ribose) metabolism.

1230 TOP2B activity relaxes DNA in elongating spermatids in a decatenation reaction and for
reorganization of chromatin loops. This step triggers PARP1/2 activation and resulting
poly(ADP-ribose) (PAR) formation provides a feedback loop to regulate TOP2B activation

[123–125]. PARP activation is necessary for activation of the ATM/ATR kinase pathway [162,163], leading to phosphorylation of H2AFX [157] and H4K16 acetyltransferase MYST1 (KAT8, MOF) [197]. Direct ADP-ribosylation by PARP1 in H3K27 position (H3K27-ADPR) 1235 may compete with H3K27 methylation [84,225] but PARP1 also interacts with histone demethylases of the KDM family either through PARP enzyme-bound PAR chains or through direct protein interaction at PARP binding sites in the genome. For instance, KDM5A is recruited by PARP1 and activated to remove H3K4me3 from promoter sites as part of DNA 1240 damage-mediated gene silencing [155]. In contrast, KDM5B has been reported to be inhibited by PARP1 and excluded from DNA strand breaks to maintain H3K4me3 and transcription near sites of DNA damage repair [154]. These may be mutually exclusive pathways or they could depend on the activity of the PAR digesting activity of poly(ADP-ribose) glycohydrolase (PARG). KDM4D, which removes H3K9me2 associated with promoters of retinoic acid receptor- 1245 dependent genes heterochromatin, is inhibited by PARP1, unless PARG is present to allow for retinoic acid-dependent gene expression [151]. Events that have been demonstrated only in somatic cells are considered hypothetical in spermatids and are marked with an asterisk*.

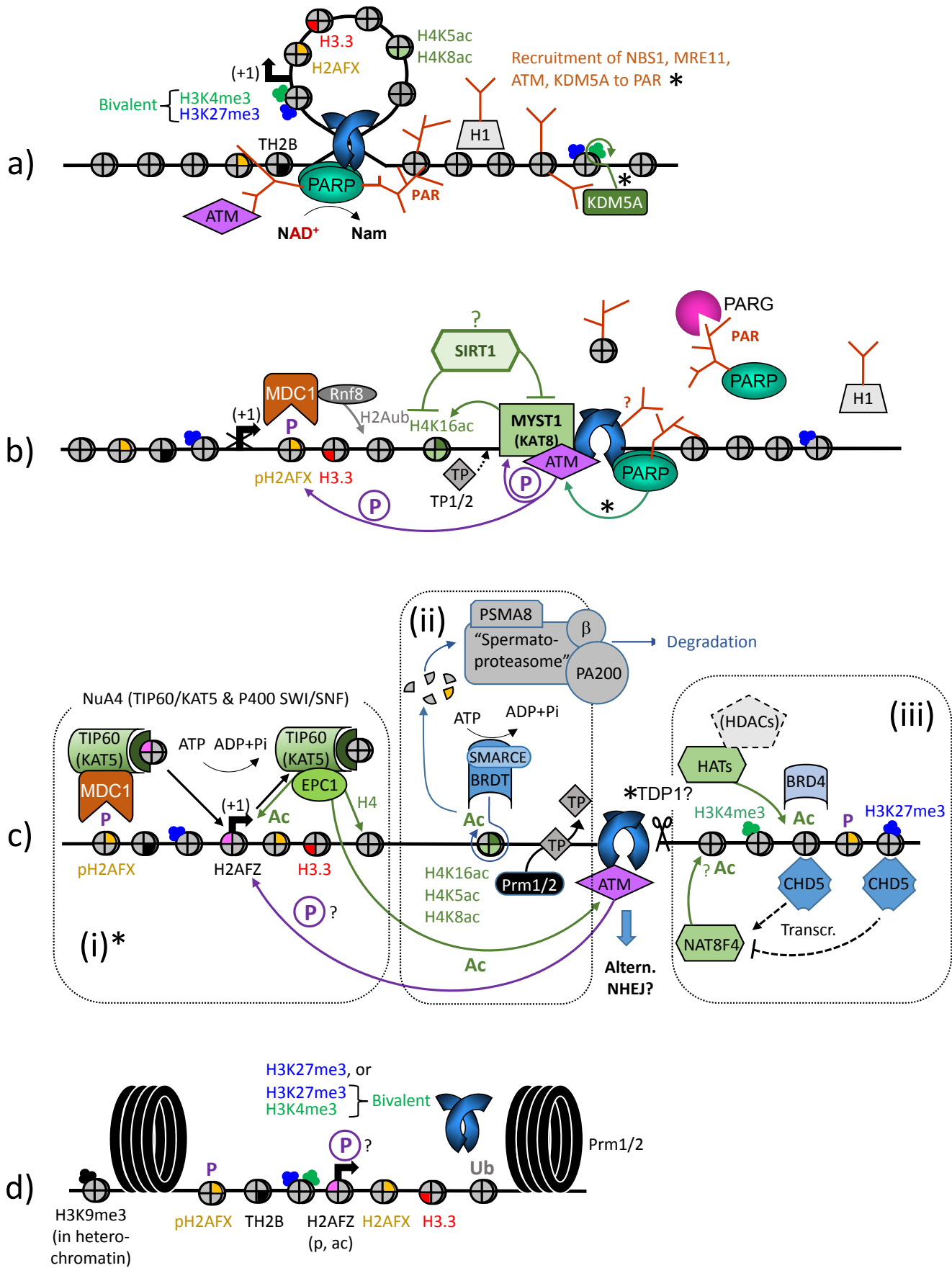


Figure 1

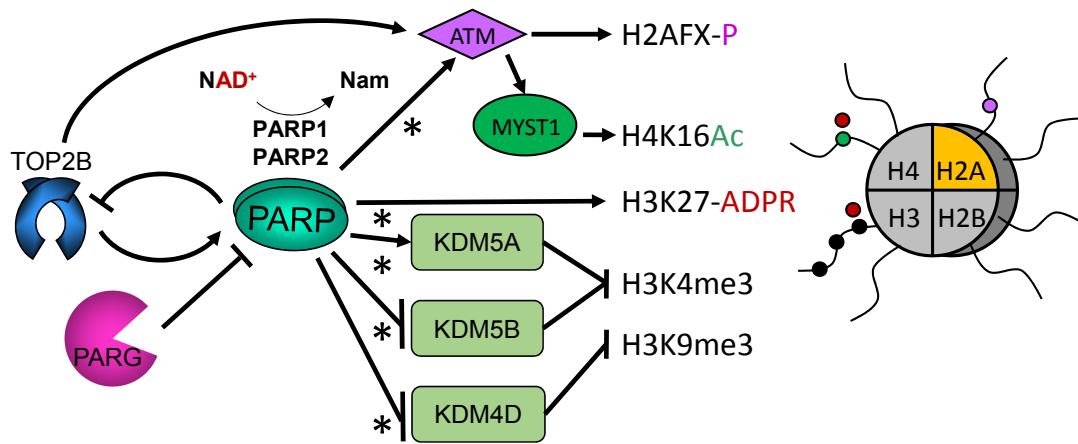


Figure 2