1 Sperm Chromatin: An Overview

Rod Balhorn

Abstract

The dramatic changes in the structure and function of sperm chromatin that occur during spermatogenesis have continued to intrigue researchers for more than a century. In addition to wanting to understand how these changes in chromatin organization affect genome function, many of the studies conducted in placental mammals have been driven by a desire to understand the relationship between sperm chromatin organization and sperm function (fertility) or dysfunction (subfertility or infertility). While we have learned a great deal, many important questions still remain unanswered. Major technological advances in imaging techniques, transgenic animal production, gene function disruption, molecular and compositional analysis at the single cell and subcellular level as well as the development of many new molecular probes now make it possible to design and carry out studies that examine structure and function at the level of the individual cell in ways that have not been previously possible.

Keywords

Sperm chromatin • Spermatogenesis • Chromatin remodeling • DNA–protamine complex • Chromatin reorganization

Origins of Sperm Chromatin Research

The first research conducted on sperm chromatin, which dates back almost 150 years, began with the discovery of its two primary molecular components – DNA and protamine. Only a year after Gregor Mendel reported his work on the laws of heredity in 1865 [1], Ernst Haeckel suggested that the nuclei of cells must contain the material responsible for the transmission of genetic traits [2]. Friedrich Miescher, working in Felix Hoppe Seyler's laboratory in Germany, had become intrigued by cells and began conducting experiments to determine their chemical composition. Working initially with lymphocytes obtained from blood and later enriched populations

R. Balhorn (✉)
Department of Applied Science,
University of California, Davis, CA, USA
e-mail: rodbalhorn@hughes.net

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of leukocytes he obtained from hospital bandages, Miescher noticed a precipitate that formed when he added acid to extracts of cells he was using to isolate proteins [3]. While he and the rest of the scientific community were unaware that this material, which he called nuclein, was the genetic material Mendel and Haeckel had referred to, he became fascinated by and continued to study its properties [4]. Walther Flemming's work over the next decade introduced the scientific community to the cellular substructures called chromosomes and the concept of mitosis, and Flemming was the first to introduce the term *chromatin* [5]. It took the next 30 years, however, before cellular biologists began to realize the importance of individual chromosomes as the carriers of genetic information.

Miescher, who began his research career isolating and characterizing proteins, spent the majority of his time investigating nuclein (DNA). When he discovered he could not obtain enough of the nuclein from human cells to properly examine its properties, he turned to working with fish sperm. Salmon provided an abundance of sperm, and the sperm cells were considered ideal because they had almost no cytoplasm to contaminate his nuclear preparations with protein. In addition to being the first to isolate DNA, Miescher was also the first to isolate protamine, which he called protamin, and to discover its highly basic nature [6]. He discovered that nuclein and protamin made up the majority of the mass of the sperm head, and he also provided the first insight into the fundamental interaction that bound these two components together inside the sperm nucleus – that nuclein was bound in a salt-like state to protamin. As the interest in DNA and protamine grew, other researchers began to examine the molecules present in sperm. The majority of the initial work characterizing the composition of protamine molecules was carried out by Kossel and his group, not Miescher, over several decades spanning from about 1890–1920 [7–10]. The proteins bound to DNA in sperm were distinguished from those found in other cells very early on, but the real significance of this difference was not appreciated until almost half a century later when more detailed studies of spermatogenesis and spermiogenesis revealed significant differences in DNA packaging and sperm chromatin compaction. Up until this time, sperm chromatin was considered by many to be similar to the chromatin found in somatic cells.

Spermatogenesis: A Special Form of Terminal Differentiation

In species that reproduce sexually, testicular cells undergo a radical transformation as they progress through a process of differentiation called spermatogenesis. Diploid somatic cells that contain two complements of the genome divide in meiosis to produce haploid cells containing only a single copy of each chromosome. The nuclei and chromatin inside these haploid cells also undergo a series of structural and functional changes. In mammals, specific genes within the male genome are imprinted to identify their "parent of origin" [11, 12], and the chromatin is transformed from a highly functional, genetically active state characteristic of the somatic testis cell it was derived from to a quiescent or completely inactive state found in the fully mature sperm cell.

One might think of this transformation as the testicular cell embarking on a path of terminal differentiation similar to the differentiation of a stem cell into a liver, kidney or brain cell. The final cell not only differs structurally from the stem cell but also performs very different functions. Unlike the genome in most stem cells, however, the genome of most maturing vertebrate spermatids undergo an additional step in the process, a transient stage in which the entire genome is deprogrammed and shut down. This genome-wide inactivation bears some similarity to processes of heterochromatinization that have been observed to occur with one X-chromosome in vertebrates [13, 14], the entire genome in avian erythrocytes [15], and one set of chromosomes in mealy bugs [16]. These changes, which are induced by modifying or replacing the proteins that bind to and package DNA, enable the male

genome of the sperm to be deprogrammed and maintained in a quiescent state until it enters the oocyte and is ready to be combined with the genome of the female to create a diploid embryonic cell. The process provides a mechanism by which the genes contributed by the male can be reactivated in the proper combinations to ensure the first cells function as embryonic stem cells, subpopulations of which later redifferentiate into the other types of cells that are required for the development of a fully functional organism.

Variability in the Composition of Sperm Chromatin

Both Miescher's and Kossel's studies of sperm focused on the morphological and compositional differences they observed between sperm and other cells. Kossel examined the proteins found in the sperm head, using the properties and composition of the proteins as indicators of the differences or similarities that might distinguish these cells in different species. The majority of the fish protamines analyzed by Kossel and others were found to be small proteins with unusually high contents of the two amino acids arginine and lysine. While these two amino acids were known to be present in all proteins at a low level (typically ~5%), the arginine-rich fish protamines were found to contain 50–90% arginine and the lysine-rich fish protamines contained as much as 28% lysine. Because the fish protamines appeared to be comprised mostly of arginine and lysine, Kossel proposed that the protamines might be one of the simplest proteins.

As researchers began examining the sperm chromatin proteins of other species, it became clear that there was a great deal of variability in the types of proteins used to package DNA in sperm. Sea urchins also proved to be an easy source from which sperm could be obtained in large numbers, and analyses of sea urchin sperm revealed that protamines were not present in the sperm chromatin of this organism. Instead, the DNA was found to be packaged by histones [17, 18]. Each of the five histones is larger (by a factor of two) than protamines and significantly less basic. In contrast to the protamines, the histones contain a great deal less arginine (2–10% of the total amino acids) and more lysine (13–28%). Subsequent analyses of sperm chromatin proteins isolated from the sperm of other invertebrates and vertebrates have shown that the size and amino acid sequences of the proteins used to package sperm DNA vary considerably [19]. Many of these proteins are smaller and substantially more basic than the histones and larger and less basic than protamines.

Amphibian and fish sperm provide one of the best examples of this variability. Sperm produced by frogs in the genus *Rana*, for example, have their DNA packaged entirely by histones [20]. Both histones and protamine-like intermediate proteins are found in the sperm chromatin of the clawed African frog (*Xenopus*) [21], while histones and protamines package the DNA in toad (*Bufo*) sperm [22]. Similar observations have been made in studies of fish sperm. Different species of fish, even within the same order, have been shown to use histones, protamine-like proteins, or protamines to condense their sperm chromatin, demonstrating that these differences do not correspond strictly with phylogeny. In addition, the particular type of protein used to package sperm DNA does not appear to be linked to mode of fertilization, as had been suggested based on the studies conducted with amphibian sperm. While several internally fertilizing fish such as *Xiphophorus helleri guentheri* (swordtail), *Xiphophorus maculatus* (platyfish), *Poecilia reticulata* (guppy), *Poecilia picta* (guppy), and *Cymatogaster aggregata* (shiner perch) all produce sperm containing protamines [23], several externally fertilizing species such as the grass carp (*Ctenopharyngodon idella*) [24], tub gurnard (*Trigla lucerna*) [25], and sea bream (*Sparus aurata*) [26] produce sperm containing DNA packaged by histones. However, this relationship between the mode of fertilization and type of protein used to package DNA in sperm does not extend to all species of fish. The sperm produced by salmon, herring, and many other species of fish that spawn and fertilize externally contain DNA that is packaged by protamines.

What these studies and those of chromatin in the sperm of other vertebrates and invertebrates have demonstrated is an evolutionary pattern in which the sperm chromatin proteins transition from histones to protamine-like proteins to protamines [27]. The variation observed in amphibians show that sporadic reversions are possible [28], and the fish studies [29] are consistent with this idea and provide additional examples that show the change from protamine to histone (or alternatively histone to protamine) has occurred independently several times during evolution.

Spermatid Differentiation and Chromatin Remodeling

Prior to meiosis, the chromatin in the spermatocyte nucleus is diffusely organized and appears structurally similar to that found in the nuclei of all other somatic cells. The predominant chromatin proteins are the somatic histones and a wide variety of other proteins that interact with DNA to regulate gene activity, anchor the genome to the nuclear matrix, and contribute to chromatin function. As the cell proceeds through meiosis and enters the early stages of spermiogenesis, several new DNA-binding proteins are synthesized that bind to DNA and initiate a series of subtle transformations in the organization and activity of the spermatid's chromatin. The nature of these proteins and their impact on chromatin organization and function differ widely among species.

The changes that have been characterized in the greatest detail are those that occur in placental mammals. The first new proteins to appear are four histone variants that replace some or the majority of their somatic H2B, H3, H2A, and H1 histone counterparts [30]. These proteins were originally referred to as testis specific histones with a "T" designation being added to the histone's name. More recently, the same histone variants have been referred to as sperm-specific histones because they are frequently retained at some level in mature sperm. TH3 histone appears very early in spermatogenesis in spermatogonia. TH2B and TH2A histone variants are synthesized and integrated into the chromatin of pachytene spermatocytes just prior to meiosis, and a new H1 histone variant, H1t or TH1, appears near the end of meiotic prophase. Up to 90% of H2B is replaced by TH2B. The proportion of replacement for H3 and H2A is unknown. Seven H1 variants or subtypes have been identified in mice and men. In the case of the spermatid H1 variant, H1t, it replaces approximately half of the other H1 subtypes. However, some of these subtypes, such as H1a, actually increase in abundance and are not replaced. While these sperm histone variants are thought to play some role in altering the functionality of the chromatin, the basic structural subunit of chromatin organization, the nucleosome, is retained.

Electron microscopy studies have shown that the first noticeable change in chromatin structure occurs when the sperm specific histone H1t variant is deposited in spermatid chromatin. Prior to H1t deposition, the chromatin appears more diffuse and contains regions that are more clumped than others. When H1t appears, the chromatin is transformed into a more uniform and granular state. H1t remains bound to DNA for a relatively short period of time and then begins to disappear in elongating spermatids. Following its loss, the chromatin takes on a more filamentous organization [31].

In mammals, the majority of the histones are replaced after meiosis by three smaller, more basic proteins that have been designated "transition proteins" because they only remain associated with DNA for a relatively short period of time. The mammalian transition proteins TP1, TP2, and TP4 appear in the chromatin of mid-stage spermatids at the same time the majority of the histones are removed from the chromatin. Studies in human and rat spermatids have shown that TP2 synthesis and deposition in spermatid chromatin precedes that of TP1 [32, 33]. With the appearance of TP1 and TP2, the chromatin begins to condense somewhat with condensation progressing in the nucleus from an apical to caudal direction [31, 34]. Very little is currently known about TP4. While a great deal remains to be learned about the function of these proteins, it is clear

that they play important roles in replacing histones (TP1 has been reported to destabilize nucleosomes by preventing DNA bending [35]), initiating the termination of gene transcription by TP2 binding to CpG sites [35], enabling or facilitating the repair of DNA strand breaks [36], and contributing to chromatin condensation. By the time TP1, TP2, and TP4 deposition are completed, the chromatin becomes uniformly condensed and no longer appears to retain the subunit structure characteristic of nucleosomes. A fourth protein, TP3, was also considered to be a member of this group when it was first observed in spermatid chromatin. Once the protein was sequenced, however, TP3 was identified to be the precursor form of protamine 2 [37]. Instead of being displaced from late-spermatid DNA, the protein is simply processed to a smaller form (protamine 2) that remains bound to DNA throughout the remainder of spermiogenesis.

These transition proteins are replaced by a set of positively charged proteins called protamine in late-step spermatids as the chromatin is reorganized one final time before the sperm becomes fully mature. The mammalian protamines are small proteins rich in cysteine and the basic amino acids arginine, lysine, and histidine. Considerable variation in amino acid sequence has been observed within the protamines of mammals [38–41], but all the proteins examined fall into one of two protamine families, protamine P1 or protamine P2. The nature of protamine binding to DNA and the consequences of the synthesis and incorporation of the protamines into spermatid chromatin suggest that these proteins may perform a number of functions. These include protecting the DNA from physical and chemical damage while the chromatin is in a state in which it cannot repair DNA damage and compacting the genomic material to produce a smaller, more hydrodynamically shaped cell. The compaction of the genome that occurs when protamine binds to DNA also ensures the entire genome is retained in a genetically inactive state until fertilization, and it may even aid in the shaping of the sperm head by generating the forces needed to shape the nucleus from within [42].

Higher Ordered Organization of Chromatin in Mature Sperm

In contrast to the variability that has been observed in the composition of sperm chromatin in many vertebrates and invertebrates, there appears to be remarkably little variation in the final modes of DNA packaging that have been observed in sperm produced by different species of mammals. The sperm of all mammals examined to date, including monotremes, marsupials, and placental mammals, use protamines to package the majority of their DNA into the sperm head. In several mammalian species, a small fraction of the sperm genome has been observed to retain its histone packaging. This histone-containing fraction, which is currently thought to be present in all mammalian sperm, is small, comprising not more than a fraction to 1% of the genome. In human sperm, however, the fraction of DNA bound by histones is significantly larger, possibly as high as 10–15% [43–47].

Recent studies have identified a number of DNA sequences or genes that remain associated with histones in mammalian sperm. These include telomeric DNA [48], genes for epsilon and gamma globin [49], a paternally imprinted IGF-2 gene [50], microRNA clusters, the promoters of a number of genes expressing signaling proteins important for early embryonic development, and genes that produce transcription factors such as those in the Hox family [51]. Based on the types of genes that have been identified in histone associated sperm chromatin, it has been suggested that one function for the retention of these histones may be to maintain a subset of genes contributed by the male in a quiescent but accessible state so they can be activated immediately after fertilization and prior to the removal of the protamines. The histone-associated genes were also found to be highly enriched in a variety of imprinted genes, indicating another function of these histones may also be to play a role in epigenetic programming.

The chromatin in monotreme and marsupial spermatids is condensed during spermiogenesis in a fashion similar to that observed in other

species that use only protamines to package their DNA, but the nature of the nuclear protein–DNA interactions that lead to this condensation in monotreme sperm have not yet been characterized. Chromatin condensation in platypus sperm is initiated by the formation of a layer of electron dense chromatin granules under the nucleolemma [52]. As the spermatids continue to mature, foci of condensing chromatin are observed throughout the nucleus. These studies have not, however, provided much information about either the organization or subunit structure of mature sperm chromatin in monotremes. A combination of EM and AFM studies of sperm chromatin in two marsupials, the fat tailed dunnart (*Sminthopsis crassicaudata*) and brush-tailed possum (*Trichosurus vulpecula*), has indicated the DNA is organized in nodular subunits [53]. Those regions of the chromatin that appear to be packaged by protamines have nodules with diameters of 50–80 nm, while other regions believed to contain histones bound to DNA contained much larger clusters (120–160 nm) of smaller nodules.

Chromatin reorganization and compaction occurs in a similar manner in placental mammals. The chromatin is transformed from the diffuse, genetically active state to a highly electron dense, compact form of chromatin that is completely inactive. Both electron and atomic force microscopy studies of spermatid chromatin and partially decondensed sperm chromatin have provided insight into the higher ordered structure of sperm chromatin in placental mammals. EM images of the chromatin in differentiating late-step spermatids have shown that the DNA starts off organized with features characteristic of somatic chromatin (~11 nm nodules and 30 nm fibers [54]), which are subsequently transformed into nodular structures or fibers with diameters (50–100 nm) much larger than individual nucleosomes. As chromatin condensation progresses, these nodules coalesce into increasingly larger masses or fibers that eventually become so electron dense and tightly packed that they can no longer be distinguished.

Similar structural information has been derived from high resolution microscopy studies of sperm chromatin that has been partially decondensed by treatment with polyanions, reducing agents, or high ionic strength or by partial digestion by nucleases [55–62]. Analyses of partially decondensed sperm chromatin by electron microscopy have shown that at least two different sized structural units are present, small nodules similar in size to nucleosomes and much larger globular structures. Atomic force microscopy images of decondensed human sperm also revealed the presence of two types of structures: small subunits similar in diameter (~10 nm) and thickness (~5 nm) to somatic nucleosomes and lifesaver shaped larger structures approximately 60–100 nm in diameter and 20 nm thick with a hole or depression in the center [56]. Toroids with lifesaver-like features and similar dimensions have also been generated in vitro when protamine or other polycations were added to dilute solutions of DNA or to individual DNA molecules [63–65]. These toroids, which contain approximately 50,000 bp of DNA complexed with protamine, are spontaneously generated when protamine binds to and neutralize the phosphodiester backbone of double-stranded DNA [56, 66]. Closely packed beads with diameters similar to these toroids were found by Koehler to comprise the lamellar sheets of chromatin packed inside rat, rabbit, bull, and human sperm [59, 60, 67].

Mammalian Protamines

While the unusually high arginine content of protamine was recognized by both Miescher and Kossel to be a unique feature of fish sperm nuclear proteins more than 100 years ago, it took more than 50 years for researchers to begin to understand and appreciate the structural and functional differences between the protamines and histones. Structurally, the two families of DNA-binding proteins are very different. The four core histones interact with each other to form a well-defined octamer core of protein around which almost two turns of DNA are wrapped [68]. The DNA bound to the histones remains accessible to polymerases and other proteins and the genes packaged by histone remain active or can be readily activated. By marked contrast, the protamines contain so

many positively charged amino-acid side chains that when protamine binds to DNA, it wraps around the DNA helix, neutralizing the negatively charged phosphodiester backbone of DNA and creating a maximally compact form of chromatin [56]. This prevents the genes packaged by protamines from being accessed by other proteins and modified, transcribed or repaired.

Two different types of protamines package DNA in mammalian sperm, P1 and P2. The smaller protein, protamine P1, is found in the sperm of all mammals [69]. The P1 protamine of placental mammals is a single peptide chain containing only 50 amino acids [70]. The one known exception is stallion P1, which contains 51 amino acids. The P1 protamines in marsupials and monotremes are larger (57–70 residues). The platypus and echidna protamines also differ from the P1 protamines of placental mammals in that they do not contain any cysteine residues [71]. This is also the case for most marsupial protamines [41]. One exception has been reported, however, in the family of Dasyuridae. Shrew-like marsupials in the genus *Planigales* produce protamines that containing 5–6 cysteines [72], a number similar to the number of cysteines that are typically found in the P1 protamines of placental mammals.

The P1 protamine of placental mammals is unstructured in solution and only adopts a specific conformation when bound to DNA [73]. Protamine P1 sequences are typically divided into three small domains, a central DNA-binding domain comprised of a series of $(Arg)_n$ DNA-binding domains interspersed with one or two uncharged amino acids and two short N- and C-terminal peptide domains that do not bind to DNA [70, 74]. Only the DNA-binding domain appears to be present in monotreme and marsupial P1 molecules [41, 71]. The two short terminal peptide domains in placental mammal P1 molecules contain serine and threonine residues that are phosphorylated shortly after the protein is synthesized, and this modification is thought to facilitate the protein's binding correctly to DNA. Similar phosphorylatable residues appear to be distributed throughout the monotreme and marsupial P1 sequences. These domains in placental mammal P1 molecules also contain multiple cysteine residues that form inter- and intraprotamine disulfide bonds and link each protamine molecule to its neighbor when the maturing spermatid passes through the epididymis [74].

Protamine P2, which is slightly larger than P1 (63 amino acids in mouse) is only expressed in the differentiating spermatids of a subset of placental mammals. These include primates, most rodents, lagomorphs, and perissodactyls [69]. Unlike protamine P1, P2 is synthesized as a larger precursor protein (106 residues in mouse) that is deposited onto DNA and subsequently shortened over a period of several days [75]. This processing of the precursor protein occurs by progressive and sequential cleavage of short peptide fragments from the amino terminus of the precursor [76–78]. The function of this processing remains unknown. P2 also appears to be phosphorylated transiently. How the final processed form of P2 interacts with DNA has not yet been determined, but studies of P1 and P2 in several species suggest the majority of the length of the P2 molecule binds to DNA. The "footprint" of P1 when bound to DNA is 10–11 base pairs, or one full turn of DNA, while the "footprint" of P2 appears to be larger (15 bp) [43]. The final processed form of P2 also appears to use a series of $(Arg)_n$ anchoring peptide segments to bind to DNA. These segments are shorter and less well defined than those found in the DNA-binding domain of P1, and they are distributed throughout the entire length of the P2 sequence. P2 also contains multiple cysteine residues that participate in the formation of the disulfide bonds that interconnect all the protamines late in spermiogenesis.

Structure of the DNA–Protamine Complex

While the relative proportion of the two protamines in sperm chromatin varies widely between mammalian genera, the proportion appears to be conserved among the species within a genus [69]. P2 is believed to bind to DNA in a manner similar to P1, but the evidence for this is

limited and primarily circumstantial. Beyond the knowledge that both protamines P1 and P2 bind along the DNA in some manner that allows the two proteins to be cross-linked together by disulfide bridges during the final stage of sperm maturation, very little is known about the details of P2 binding to DNA or the distribution of the two protamines along a segment of DNA.

Because it has not been possible to determine the structure of a native or artificial protamine–DNA complex by X-ray crystallography or NMR spectroscopy, most of the information that has been learned about how the protamines interact with DNA has been determined using lower resolution techniques. Low-angle X-ray scattering experiments performed on intact sperm heads confirmed the close packing of the DNA within sperm chromatin, showing the center to center distance between adjacent DNA molecules is approximately 2.7 nm [79]. To achieve this tight packing, the molecules must be organized in a hexagonal arrangement with only 7 Å distance of separation between the surfaces of adjacent molecules. High-resolution EM studies of individual toroidal subunits [80] have shown that the individual DNA molecules coiled into the toroid are tightly packed in a hexagonal arrangement, consistent with what has been observed by low-angle X-ray scattering. Such a packing arrangement for DNA is also consistent with the microscopy data obtained from stallion sperm heads [81], particularly if the toroidal structures are stacked tightly together as lifesavers and organized in layers similar to the lamellae reported by Koehler [59, 60, 67].

At the molecular level, the protamines bind to duplex DNA in a manner that is independent of base sequence [66, 82]. The primary interactions are electrostatic and involve the binding of the positively charged guanidinium groups in the arginine residues present in the DNA anchoring domains of protamine to the negatively charged phosphates that comprise the DNA phosphodiester backbone. The high affinity of binding is derived from two aspects of these interactions, the formation of a salt bridge and hydrogen bond between the guanidinium group and the phosphate and the binding of every arginine residue in the DNA-binding domain of protamine to every phosphate group in one turn of DNA. Both computer modeling and X-ray scattering and other experimental studies [73, 83–85] have shown that the DNA-binding domain of protamine P1 wraps in an extended conformation around the DNA helix, partially filling the major groove. By interacting in this way, adjacent arginine residues in the $(Arg)_n$ anchoring domains would be expected to bind to phosphates on opposite strands of the duplex DNA molecule, interlocking the relative positions of the bases together and preventing strand separation or changes in DNA conformation throughout the period that the protamines remain bound to DNA. This would result in the production of a neutral, highly insoluble complex that allows the DNA strands to be packed tightly together without charge repulsion.

Chromosome Territories, Loop Domains, and Matrix Attachment Regions

Three important structural features of somatic chromatin organization appear to be retained by mammalian sperm chromatin even after all the nuclear protein transitions and condensation have been completed. Confocal microscopy of somatic cells hybridized to fluorochrome-tagged DNA probes have shown that the DNA of individual chromosomes are not randomly distributed throughout the nucleus, but each is confined to a specific domain or territory inside the interphase nucleus [86–90]. Not only is there evidence that the chromosomal DNA molecules occupy a reproducible position, but there is also evidence that the domains are folded into shapes characteristic of a particular chromosome [91]. Similar observations have been made regarding the distribution of chromosomal DNA in mammalian sperm nuclei. Fluorescence in situ hybridization has been used to demonstrate that the DNA of individual chromosomes are also localized to specific domains inside the heads of human, bull, mouse, echidna, and platypus sperm [48, 91–94].

While these studies have not provided strong evidence that the chromosomes are arranged in any particular order relative to each other in the sperm heads of placental mammals, there is some evidence for a particular arrangement in echidna and platypus sperm.

Two other organizational features that are retained in sperm cell nuclei are the chromatin loop domains and the attachment of the chromatin to a nuclear protein scaffold or nuclear matrix [95–98]. The protein content of the nuclear matrix changes as the spermatid differentiates [95], but the DNA remains bound to the matrix at a very large number of sites (~50,000). This matrix appears by EM to be a network of dense protein filaments filling the interior of the head of the spermatid and sperm bounded by a peripheral structure, the lamina. The DNA in between the sites of attachment to the matrix appears to retain the loop organization present in somatic cells [99, 100]. These loops, which contain 40,000–50,000 bp of DNA in both the somatic and sperm nucleus, are anchored to a matrix through specific chromatin domains, called nuclear scaffold/matrix attachment regions (SARs/MARs). The retention of the matrix and its associations with DNA in sperm are important to maintain because their presence would facilitate and speed up the process of genome reactivation following fertilization and the initiation of the first cycle of DNA replication in the male pronucleus [101, 102]. The loop domains are believed to play an essential role in transcriptional regulation, DNA replication, and chromosome organization both prior to spermiogenesis and after fertilization. In sperm, these loops may also aid in the packing of the DNA by protamines into toroids, which also contain ~50,000 bp of DNA.

The retention of these particular features of chromosome and chromatin organization appears to preserve important genome organizational information critical to both germinal and somatic cell function. Clearly, the primary function of spermiogenesis is to produce a package of genomic information, the sperm cell, that will facilitate the transport of one complement of the male's chromosomes to and into the oocyte for the purpose of generating an embryo containing genomic contributions from both the male and female of the species. Once this is accomplished, the genome must be quickly reactivated so that it can begin functioning as a somatic cell, with subsets of genes being turned on and off as the cells are transformed from embryonic stem cells into the cells of the various tissues and organs.

Reorganization of Sperm Chromatin Following Fertilization

The formation of the male pronucleus and other processes associated with early embryonic development that occur immediately after fertilization have been well characterized by light microscopy. However, remarkably little is known at the molecular level about the early events that contribute to the unpackaging of sperm chromatin following fertilization. The current hypothesis is that the protamines are actively removed from the DNA by a histone chaperone similar to the nucleoplasmin first identified in frogs [103–105]. This protein chaperone has been shown to bind and carry core histones and, in the presence of DNA, is able to load the histones onto the DNA and generate nucleosomes. Sequence analyses of the frog and related mammalian proteins have shown that these proteins contain a series of polyglutamic acid sequences. Experiments conducted with sperm chromatin have also shown that the protein is able to remove protamine from the DNA prior to loading it with histones [106]. One possible mechanism of protamine removal may involve these segments of polyglutamic acid. The polyglutamic acid regions in nucleoplasmin-like proteins could form a series of salt bridges with the $(Arg)_n$ DNA-binding domains of the protamines and remove the protamines from DNA intact prior to depositing the histones and reestablishing the nucleosomal organization required to reactivate the new embryo's genome.

Another early event associated with the unpacking of the sperm chromatin that occurs almost immediately after removing the protamines is the initiation of a period of DNA synthesis

associated with DNA damage repair [107–110]. This repair synthesis is required to repair DNA strand breaks and remove DNA adducts or other damage that is acquired during spermiogenesis and epididymal transit and storage when repair activities could not be performed due to the packaging of the genome by protamines. Studies have shown that the majority of the damage brought into the oocyte by the sperm is repaired during this period of DNA synthesis, and this process is considered to be critical for maintaining the integrity of the male genome and for ensuring normal embryonic development.

Consequences of Disrupting Sperm Chromatin Remodeling

Several changes associated with the reorganization of spermatid chromatin have been shown to be important for male fertility. One involves the removal of the majority of the histones and their replacement by protamines. Numerous studies have suggested that there is a positive correlation between male subfertility or infertility and elevated levels of histone in mature human sperm [77, 111–117]. It is not known, however, whether the problems encountered relate to the lack of removal of somatic histones from genes that need to be packaged by protamines, deficiencies in expression and incorporation of the sperm specific histone variants into subsets of nucleosomes, or errors in imprinting that may involve histone packaging.

Alterations in the expression and/or translation of the protamine genes have also been linked to infertility. Changes in the proportion of the P1 or P2 proteins present in sperm chromatin have been shown to not only be linked to infertility [118–124] but also adversely impact in vitro fertilization outcome and early embryonic development [125–129]. The observed differences in protamine content ranged from having very little protamine, to having too little protamine P1 or too little protamine P2. By contrast, analyses of sperm obtained from fertile human males have shown repeatedly that the sperm contain a specific proportion (1:1) of P1 and P2 [118–120, 130]. The primary cause for the observed changes in sperm protamine content appears to involve errors in gene expression, although incomplete processing of the P2 precursor may also contribute to decreased levels of the mature P2 protein.

Other studies have shown that the timely formation of the protamine disulfide cross-links that occur during the final stages of sperm maturation are important. In mammals, both protamines P1 and P2 contain multiple cysteine residues. The thiol groups of these cysteines are in the reduced form (free thiols) when the protamines are synthesized and deposited onto DNA, and they remain reduced until the final stage of spermiogenesis when they participate in the formation of both inter-and intramolecular protamine disulfides as the sperm pass through the epididymis [74, 131–134]. Cases of human, stallion, and bull infertility have been correlated with what appear to be errors in disulfide cross-linking among the protamines. What role these disulfide bonds play is still not known, but one theory is that the formation of interprotamine disulfide bonding stabilizes the chromatin and protects it from physical damage. An equally feasible possibility is that these disulfide bonds not only stabilize the chromatin but also prevent the thiol groups from being oxidized or alkylated during the long period of time required for spermatid maturation and sperm storage prior to fertilization. This might be important if the cysteine residues in mammalian protamine also play some other role in sperm chromatin, such as participating in protamine removal from DNA after fertilization. If the thiols were required for efficient protamine removal, the oxidation or alkylation of even a few cysteines could potentially complicate or prevent the efficient removal of the modified protamine from the male genome, and its retention would block the gene it was bound to from being transcribed or replicated later in development. Mice exposed to alkylating agents such as methyl methanesulfonate and ethylene oxide at a time prior to protamine disulfide bond formation have been shown to produce sperm with alkylated protamine thiols [135–137]. Matings conducted with the treated males resulted in the production of

embryos that died early in development from dominant lethal mutations [136]. The sperm containing the protamines with alkylated cysteines succeeded in fertilizing oocytes and inducing embryonic development, but at some point after fertilization the embryo died when a key gene could not be turned on.

Male infertility has also been linked to deficiencies in sperm chromatin-associated zinc. Zinc is known to be essential for several aspects of sperm development, ranging from contributions to structural elements in the tail to roles in chromatin organization and protamine structure and function [138]. A deficiency in zinc can affect the developing sperm directly, or it can impact the function of other testicular cells that contribute to or play a role in spermatid maturation, such as sertoli cells. Because zinc plays multiple roles in spermatogenesis and testicular function, it has been difficult to decipher how sperm chromatin bound zinc impacts the functionality of the sperm cell. Chromatin associated zinc is almost exclusively bound to protamine P2 in mammals [139]. In human, bull, mouse, and hamster sperm, a single zinc atom is bound to each P2 molecule. Zinc does not appear to bind to protamine P1. Zinc ion coordination by P2 occurs sometime after the synthesis of P2 and its deposition onto DNA, long before the sperm cell enters the seminal fluid and the sperm chromatin can be impacted by seminal fluid zinc. Where the zinc binds in P2 has not been determined, but the amino acids in protamine P2 that coordinate the zinc appear to change during sperm maturation. In sonication resistant spermatids, the zinc is coordinated only by cysteines, while in mature sperm, both histidine and cysteine residues participate in the coordination (unpublished results). The function of this P2 bound zinc is not known, but it has been suggested that the coordination of the zinc by protamine may influence the binding of the protamine to DNA [140, 141] or to other protamines [138]. An alternative possibility is that zinc coordination by cysteine residues in protamine might also protect the thiol groups and prevent their oxidation until it is time for the cysteines to form inter- and intramolecular disulfide bonds. Several studies have also suggested that exposures to other metals, such as copper and lead, may result in these metals binding to the cysteines in protamine in place of zinc (or prior to disulfide bond formation) and their being transported into the oocyte upon fertilization [133, 142, 143]. In addition to potentially disrupting the function of sperm by altering chromatin decondensation or protamine P2 function, the delivery of these and other toxic metals into the oocyte would also be expected to have an adverse impact on early embryonic development.

Future Research and Practical Applications

The dramatic changes in the structure and function of sperm chromatin that occur during spermatogenesis have continued to intrigue researchers for more than a century. In addition to wanting to understand how these changes in chromatin organization affect genome function, many of the studies conducted in placental mammals have been driven by a desire to understand the relationship between sperm chromatin organization and sperm function (fertility) or dysfunction (subfertility or infertility). While we have learned a great deal, many important questions still remain unanswered. Major technological advances in imaging techniques, transgenic animal production, gene function disruption, molecular and compositional analysis at the single cell and sub-cellular level as well as the development of many new molecular probes now make it possible to design and carry out studies that examine structure and function at the level of the individual cell in ways that have not been previously possible. Studies to be conducted in the next decade using these tools should advance our understanding of sperm chromatin structure and function quickly while providing new information that can be used to diagnose and treat male infertility, develop new male contraceptives, and contribute to other unrelated areas of research such as improving the efficiency of creating transgenic animals or targeted genome silencing for cancer therapy.

References

1. Mendel G. Experiment in plant hybribization. Paper presented at: Brunn Natural History Society; March, 1865, 1865; Brunn, Czechoslovakia.
2. Haeckel E. Generelle Morphologie der Organismen. Berlin: Reimer; 1866.
3. Miescher F. Letter I to Wilhelm His; Tubingen, February 26th, 1869. In: His W, ed. Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher – Aus dem sissenschaft – lichen Briefwechsel von F. Miescher. Vol 1. Liepzig: F. C. W. Vogel; 1869:pp. 33–8.
4. Miescher F. Uber die chemische Zusammensetzung der Eiter – zellen. Med Chem Unters. 1871;4: 441–60.
5. Flemming W. Uber das Verhalten des Kern bei der Zellltheilung und uber dei Bedeutung mekrkerniger Zellen. Arch Pathol Anat Physiol. 1879;77:1–29.
6. Miescher F. Das Protamin – Eine neue organishe Basis aus den Samenssden des Rheinlachses. Ber Dtesch Chem Ges. 1874;7:376.
7. Kossel A. Ueber die Constitution der einfachsten Eiweissstoffe. Z Pysiologische Chemie. 1898;25: 165–89.
8. Kossel A, Dakin HD. Uber Salmin und Clupein. Z Pysiologische Chemie. 1904;41:407–15.
9. Kossel A, Dakin HD. Weitere Beitrage zum System der einfachsten Eiweisskorper. Z Pysiologische Chemie. 1905;44:342–6.
10. Kossel A, Edlbacher F. Uber einige Spaltungsprodukte des Thynnins und Pereins. Z Pysiologische Chemie. 1913;88:186–9.
11. Reik W, Walter J. Genomic imprinting: parental influence on the genome. Nat Rev Genet. 2001;2(1): 21–32.
12. Solter D. Differential imprinting and expression of maternal and paternal genomes. Annu Rev Genet. 1988;22:127–46.
13. Gartler SM, Goldman MA. X-chromosome inactivation, Encyclopedia of life. New York: Wiley Interscience; 2005. p. 1–6.
14. Heard E, Clerc P, Avner P. X-chromosome inactivation in mammals. Annu Rev Genet. 1997;31: 571–610.
15. Ney PA. Gene expression during terminal erythroid differentiation. Curr Opin Hematol. 2006;13(4): 203–8.
16. Berlowitz L. Chromosomal inactivation and reactivation in mealy bugs. Genetics. 1974;78(1):311–22.
17. Bloch D. Handbook of Genetics, vol. 5. New York: Plenum Press; 1976.
18. Palau J, Ruiz-Carrillo A, Subirana JA. Histones from sperm of the sea urchin *Arbacia lixula*. Eur J Biochem. 1969;7(2):209–13.
19. Eirin-Lopez JM, Ausio J. Origin and evolution of chromosomal sperm proteins. Bioessays. 2009; 31(10):1062–70.
20. Kasinsky HE, Huang SY, Mann M, Roca J, Subirana JA. On the diversity of sperm histones in the vertebrates: IV. Cytochemical and amino acid analysis in Anura. J Exp Zool. 1985;234(1):33–46.
21. Mann M, Risley MS, Eckhardt RA, Kasinsky HE. Characterization of spermatid/sperm basic chromosomal proteins in the genus Xenopus (Anura, Pipidae). J Exp Zool. 1982;222(2):173–86.
22. Takamune K, Nishida H, Takai M, Katagiri C. Primary structure of toad sperm protamines and nucleotide sequence of their cDNAs. Eur J Biochem. 1991;196(2):401–6.
23. Su H. Characterization of nuclear basic proteins in sperm and erythrocytes of vertebrates. Vancouver: Department of Zoology, University of British Columbia; 2004.
24. Kadura SN, Khrapunov SN, Chabanny VN, Berdyshev GD. Changes in chromatin basic proteins during male gametogenesis of grass carp. Comp Biochem Physiol B. 1983;74(2):343–50.
25. Saperas N, Lloris D, Chiva M. Sporadic appearance of histones, histone-like proteins, and protamines in sperm chromatin of bony fish. J Exp Zool. 2005; 265(5):575–86.
26. Kurtz K, Saperas N, Ausio J, Chiva M. Spermiogenic nuclear protein transitions and chromatin condensation. Proposal for an ancestral model of nuclear spermiogenesis. J Exp Zool B Mol Dev Evol. 2009;312B(3):149–63.
27. Ausio J. Histone H1 and evolution of sperm nuclear basic proteins. J Biol Chem. 1999;274(44):31115–8.
28. Kasinsky HE, Gutovich L, Kulak D, et al. Protamine-like sperm nuclear basic proteins in the primitive frog *Ascaphus truei* and histone reversions among more advanced frogs. J Exp Zool. 1999;284(7):717–28.
29. Saperas N, Chiva M, Pfeiffer DC, Kasinsky HE, Ausio J. Sperm nuclear basic proteins (SNBPs) of agnathans and chondrichthyans: variability and evolution of sperm proteins in fish. J Mol Evol. 1997; 44(4):422–31.
30. Churikov D, Zalenskaya IA, Zalensky AO. Male germline-specific histones in mouse and man. Cytogenet Genome Res. 2004;105(2–4):203–14.
31. Oko RJ, Jando V, Wagner CL, Kistler WS, Hermo LS. Chromatin reorganization in rat spermatids during the disappearance of testis-specific histone, H1t, and the appearance of transition proteins TP1 and TP2. Biol Reprod. 1996;54(5):1141–57.
32. Kistler WS, Henriksen K, Mali P, Parvinen M. Sequential expression of nucleoproteins during rat spermiogenesis. Exp Cell Res. 1996;225(2):374–81.
33. Steger K, Klonisch T, Gavenis K, Drabent B, Doenecke D, Bergmann M. Expression of mRNA and protein of nucleoproteins during human spermiogenesis. Mol Hum Reprod. 1998;4(10):939–45.
34. Alfonso P, Kistler WS. Immunohistochemical localization of spermatid nuclear transition protein 2 in the testes of rats and mice. Biol Reprod. 1993;48(3): 522–9.

35. Pradeepa MM, Rao MR. Chromatin remodeling during mammalian spermatogenesis: role of testis specific histone variants and transition proteins. Soc Reprod Fertil Suppl. 2007;63:1–10.
36. Caron N, Veilleux S, Boissonneault G. Stimulation of DNA repair by the spermatidal TP1 protein. Mol Reprod Dev. 2001;58(4):437–43.
37. Unni E, Zhang Y, Meistrich ML, Balhorn R. Rat spermatid basic nuclear protein Tp3 is the precursor of protamine 2. Exp Cell Res. 1994;210(1):39–45.
38. Queralt R, Adroer R, Oliva R, Winkfein RJ, Retief JD, Dixon GH. Evolution of protamine P1 genes in mammals. J Mol Evol. 1995;40(6):601–7.
39. Retief JD, Dixon GH. Evolution of pro-protamine P2 genes in primates. Eur J Biochem. 1993;214(2): 609–15.
40. Retief JD, Krajewski C, Westerman M, Dixon GH. The evolution of protamine P1 genes in dasyurid marsupials. J Mol Evol. 1995;41(5):549–55.
41. Retief JD, Krajewski C, Westerman M, Winkfein RJ, Dixon GH. Molecular phylogeny and evolution of marsupial protamine P1 genes. Proc Biol Sci. 1995; 259(1354):7–14.
42. Cree LH, Balhorn R, Brewer LR. Single molecule studies of DNA-protamine interactions. Protein Pept Lett. 2011;18(8):802–10.
43. Bench GS, Friz AM, Corzett MH, Morse DH, Balhorn R. DNA and total protamine masses in individual sperm from fertile mammalian subjects. Cytometry. 1996;23(4):263–71.
44. Gatewood JM, Cook GR, Balhorn R, Schmid CW, Bradbury EM. Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. J Biol Chem. 1990;265(33): 20662–6.
45. Gusse M, Sautière P, Bélaiche D, et al. Purification and characterization of nuclear basic proteins of human sperm. Biochim Biophys Acta. 1986;884(1): 124–34.
46. Tanphaichitr N, Sobhon P, Taluppeth N, Chalermisarachai P. Basic nuclear proteins in testicular cells and ejaculated spermatozoa in man. Exp Cell Res. 1978;117(2):347–56.
47. Wykes SM, Krawetz SA. The structural organization of sperm chromatin. J Biol Chem. 2003;278(32): 29471–7.
48. Zalenskaya IA, Zalensky AO. Non-random positioning of chromosomes in human sperm nuclei. Chromosome Res. 2004;12(2):163–73.
49. Gardiner-Garden M, Ballesteros M, Gordon M, Tam PP. Histone- and protamine-DNA association: conservation of different patterns within the beta-globin domain in human sperm. Mol Cell Biol. 1998; 18(6):3350–6.
50. Banerjee S, Smallwood A. Chromatin modification of imprinted H19 gene in mammalian spermatozoa. Mol Reprod Dev. 1998;50(4):474–84.
51. 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(7254):473–8.
52. Lin M, Jones RC. Spermiogenesis and spermiation in a monotreme mammal, the platypus, Ornithorhynchus anatinus. J Anat. 2000;196(Pt 2):217–32.
53. Soon LL, Bottema C, Breed WG. Atomic force microscopy and cytochemistry of chromatin from marsupial spermatozoa with special reference to *Sminthopsis crassicaudata*. Mol Reprod Dev. 1997;48(3):367–74.
54. Horowitz RA, Agard DA, Sedat JW, Woodcock CL. The three-dimensional architecture of chromatin in situ: electron tomography reveals fibers composed of a continuously variable zig-zag nucleosomal ribbon. J Cell Biol. 1994;125(1):1–10.
55. Allen MJ, Lee C, Lee JDt, et al. Atomic force microscopy of mammalian sperm chromatin. Chromosoma. 1993;102(9):623–30.
56. Balhorn R, Cosman M, Thornton K, et al. Protamine mediated condensation of DNA in mammalian sperm. In: Gagnon C, editor. The male gamete: from basic knowledge to clinical applications: Proceedings of the 8th International Symposium of Spermatology. Vienna, IL: Cache River; 1999.
57. Evenson DP, Witkin SS, de Harven E, Bendich A. Ultrastructure of partially decondensed human spermatozoal chromatin. J Ultrastruct Res. 1978; 63(2):178–87.
58. Koehler JK. Fine structure observations in frozen-etched bovine spermatozoa. J Ultrastruct Res. 1966;16(3):359–75.
59. Koehler JK. A freeze-etching study of rabbit spermatozoa with particular reference to head structures. J Ultrastruct Res. 1970;33(5):598–614.
60. Koehler JK, Wurschmidt U, Larsen MP. Nuclear and chromatin structure in rat spermatozoa. Gamate Res. 1983;8:357–77.
61. Sobhon P, Chutatape C, Chalermisarachai P, Vongpayabal P, Tanphaichitr N. Transmission and scanning electron microscopic studies of the human sperm chromatin decondensed by micrococcal nuclease and salt. J Exp Zool. 1982;221(1): 61–79.
62. Wagner TE, Yun JS. Fine structure of human sperm chromatin. Arch Androl. 1979;2(4):291–4.
63. Allen MJ, Bradbury EM, Balhorn R. AFM analysis of DNA-protamine complexes bound to mica. Nucleic Acids Res. 1997;25(11):2221–6.
64. Bloomfield VA. Condensation of DNA by multivalent cations: considerations on mechanism. Biopolymers. 1991;31(13):1471–81.
65. Marquet R, Wyart A, Houssier C. Influence of DNA length on spermine-induced condensation. Importance of the bending and stiffening of DNA. Biochim Biophys Acta. 1987;909(3):165–72.
66. Brewer LR, Corzett M, Balhorn R. Protamine-induced condensation and decondensation of the same DNA molecule. Science. 1999;286(5437): 120–3.

67. Koehler JK. Human sperm head ultrastructure: a freeze-etching study. J Ultrastruct Res. 1972;39(5): 520–39.
68. Finch JT, Lutter LC, Rhodes D, et al. Structure of nucleosome core particles of chromatin. Nature. 1977;269(5623):29–36.
69. Corzett M, Mazrimas J, Balhorn R. Protamine 1: protamine 2 stoichiometry in the sperm of eutherian mammals. Mol Reprod Dev. 2002;61(4):519–27.
70. Balhorn R. The protamine family of sperm nuclear proteins. Genome Biol. 2007;8(9):227.
71. Retief JD, Winkfein RJ, Dixon GH. Evolution of the monotremes. The sequences of the protamine P1 genes of platypus and echidna. Eur J Biochem. 1993;218(2):457–61.
72. Retief JD, Rees JS, Westerman M, Dixon GH. Convergent evolution of cysteine residues in sperm protamines of one genus of marsupials, the Planigales. Mol Biol Evol. 1995;12(4):708–12.
73. Hud NV, Milanovich FP, Balhorn R. Evidence of novel secondary structure in DNA-bound protamine is revealed by raman spectroscopy. Biochemistry. 1994;33(24):7528–35.
74. Balhorn R. Mammalian protamines: structure and molecular interactions. In: Adolph KW, editor. Molecular biology of chromosome function. New York: Springer; 1989. p. 366–95.
75. Yelick PC, Balhorn R, Johnson PA, et al. Mouse protamine 2 is synthesized as a precursor whereas mouse protamine 1 is not. Mol Cell Biol. 1987; 7(6):2173–9.
76. Carré-Eusèbe D, Lederer F, Lê KH, Elsevier SM. Processing of the precursor of protamine P2 in mouse. Peptide mapping and N-terminal sequence analysis of intermediates. Biochem J. 1991;277 (Pt 1):39–45.
77. Chauviere M, Martinage A, Debarle M, Sautiere P, Chevaillier P. Molecular characterization of six intermediate proteins in the processing of mouse protamine P2 precursor. Eur J Biochem. 1992; 204(2):759–65.
78. Elsevier SM, Noiran J, Carre-Eusebe D. Processing of the precursor of protamine P2 in mouse. Identification of intermediates by their insolubility in the presence of sodium dodecyl sulfate. Eur J Biochem. 1991;196(1):167–75.
79. Schellman JA, Parthasarathy N. X-ray diffraction studies on cation-collapsed DNA. J Mol Biol. 1984; 175(3):313–29.
80. Hud NV, Vilfan ID. Toroidal DNA condensates: unraveling the fine structure and the role of nucleation in determining size. Annu Rev Biophys Biomol Struct. 2005;34:295–318.
81. Livolant F. Cholesteric organization of DNA in the stallion sperm head. Tissue Cell. 1984;16(4): 535–55.
82. Bianchi F, Rousseaux-Prevost R, Bailly C, Rousseaux J. Interaction of human P1 and P2 protamines with DNA. Biochem Biophys Res Commun. 1994; 201(3):1197–204.
83. Feughelman M, Langridge R, Seeds WE, et al. Molecular structure of deoxyriboncleic acid and nucleoprotein. Nature. 1955;175:834–8.
84. Prieto MC, Maki AH, Balhorn R. Analysis of DNA-protamine interactions by optical detection of magnetic resonance. Biochemistry. 1997;36(39): 11944–51.
85. Wilkins MFH. Physical studies of the molecular structure of deoxyribonucleic acid and nucleoprotein. Cold Spring Harb Symp Quant Biol. 1956; 21:75–90.
86. Cremer T, Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet. 2001;2(4):292–301.
87. Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC. Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet. 1988;80(3):224–34.
88. Savage JR. Interchange and intra-nuclear architecture. Environ Mol Mutagen. 1993;22(4):234–44.
89. Schardin M, Cremer T, Hager HD, Lang M. Specific staining of human chromosomes in Chinese hamster × man hybrid cell lines demonstrates interphase chromosome territories. Hum Genet. 1985;71(4): 281–7.
90. Weierich C, Brero A, Stein S, et al. Three-dimensional arrangements of centromeres and telomeres in nuclei of human and murine lymphocytes. Chromosome Res. 2003;11(5):485–502.
91. Manuelidis L. Individual interphase chromosome domains revealed by in situ hybridization. Hum Genet. 1985;71(4):288–93.
92. Manvelyan M, Hunstig F, Bhatt S, et al. Chromosome distribution in human sperm – a 3D multicolor banding-study. Mol Cytogenet. 2008;1:25.
93. Mudrak O, Tomilin N, Zalensky A. Chromosome architecture in the decondensing human sperm nucleus. J Cell Sci. 2005;118(Pt 19):4541–50.
94. Zalensky A, Zalenskaya I. Organization of chromosomes in spermatozoa: an additional layer of epigenetic information? Biochem Soc Trans. 2007;35 (Pt 3):609–11.
95. Chen JL, Guo SH, Gao FH. Nuclear matrix in developing rat spermatogenic cells. Mol Reprod Dev. 2001;59(3):314–21.
96. Santi S, Rubbini S, Cinti C, et al. Ultrastructural organization of the sperm nuclear matrix. Ital J Anat Embryol. 1995;100 Suppl 1:39–46.
97. Ward WS, Coffey DS. DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod. 1991;44(4): 569–74.
98. Yaron Y, Kramer JA, Gyi K, et al. Centromere sequences localize to the nuclear halo of human spermatozoa. Int J Androl. 1998;21(1):13–8.
99. Heng HH, Goetze S, Ye CJ, et al. Chromatin loops are selectively anchored using scaffold/matrix-attachment regions. J Cell Sci. 2004;117(Pt 7): 999–1008.

100. Heng HH, Krawetz SA, Lu W, Bremer S, Liu G, Ye CJ. Re-defining the chromatin loop domain. Cytogenet Cell Genet. 2001;93(3–4):155–61.
101. Shaman JA, Yamauchi Y, Ward WS. Function of the sperm nuclear matrix. Arch Androl. 2007;53(3): 135–40.
102. Shaman JA, Yamauchi Y, Ward WS. The sperm nuclear matrix is required for paternal DNA replication. J Cell Biochem. 2007;102(3):680–8.
103. Frehlick LJ, Eirin-Lopez JM, Jeffery ED, Hunt DF, Ausio J. The characterization of amphibian nucleoplasmins yields new insight into their role in sperm chromatin remodeling. BMC Genomics. 2006;7:99.
104. McLay DW, Clarke HJ. Remodelling the paternal chromatin at fertilization in mammals. Reproduction. 2003;125(5):625–33.
105. Philpott A, Leno GH. Nucleoplasmin remodels sperm chromatin in Xenopus egg extracts. Cell. 1992;69(5):759–67.
106. Katagiri C, Ohsumi K. Remodeling of sperm chromatin induced in egg extracts of amphibians. Int J Dev Biol. 1994;38(2):209–16.
107. Derijck A, van der Heijden G, Giele M, Philippens M, de Boer P. DNA double-strand break repair in parental chromatin of mouse zygotes, the first cell cycle as an origin of de novo mutation. Hum Mol Genet. 2008;17(13):1922–37.
108. Generoso WM, Cain KT, Krishna M, Huff SW. Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg. Proc Natl Acad Sci USA. 1979;76(1):435–7.
109. Matsuda Y, Seki N, Utsugi-Takeuchi T, Tobari I. Changes in X-ray sensitivity of mouse eggs from fertilization to the early pronuclear stage, and their repair capacity. Int J Radiat Biol. 1989;55(2): 233–56.
110. Matsuda Y, Yamada T, Tobari I. Studies on chromosome aberrations in the eggs of mice fertilized in vitro after irradiation. I. Chromosome aberrations induced in sperm after X-irradiation. Mutat Res. 1985;148(1–2):113–7.
111. Blanchard Y, Lescoat D, Le Lannou D. Anomalous distribution of nuclear basic proteins in round-headed human spermatozoa. Andrologia. 1990; 22(6):549–55.
112. de Yebra L, Ballesca JL, Vanrell JA, Bassas L, Oliva R. Complete selective absence of protamine-P2 in humans. J Biol Chem. 1993;268(14):10553–7.
113. Foresta C, Zorzi M, Rossato M, Varotto A. Sperm nuclear instability and staining with aniline blue: abnormal persistence of histones in spermatozoa in infertile men. Int J Androl. 1992;15(4):330–7.
114. Hofmann N, Hilscher B. Use of aniline blue to assess chromatin condensation in morphologically normal spermatozoa in normal and infertile men. Hum Reprod. 1991;6(7):979–82.
115. Terquem A, Dadoune J. Aniline bule staining of human spermatozoa chromatin: evaluation of nuclear maturation. The Hague: Martinus Nijhoff; 1983.
116. van Roijen HJ, Ooms MP, Spaargaren MC, et al. Immunoexpression of testis-specific histone 2B in human spermatozoa and testis tissue. Hum Reprod. 1998;13(6):1559–66.
117. Zhang X, SanGabriel M, Zini A. Sperm nuclear histone to protamine ratio in fertile and infertile men: evidence of heterogeneous subpopulations of spermatozoa in the ejaculate. J Androl. 2006;27(3):414–20.
118. Aoki VW, Liu L, Carrell DT. Identification and evaluation of a novel sperm protamine abnormality in a population of infertile males. Hum Reprod. 2005; 20(5):1298–306.
119. Balhorn R, Reed S, Tanphaichitr N. Aberrant protamine 1/protamine 2 ratios in sperm of infertile human males. Experientia. 1988;44(1):52–5.
120. Belokopytova IA, Kostyleva EI, Tomilin AN, Vorobev VI. Human male infertility may be due to a decrease of the protamine-P2 content in sperm chromatin. Mol Reprod Dev. 1993;34(1):53–7.
121. Carrell DT, Emery BR, Hammoud S. Altered protamine expression and diminished spermatogenesis: what is the link? Hum Reprod Update. 2007; 13(3):313–27.
122. Carrell DT, Liu L. Altered protamine 2 expression is uncommon in donors of known fertility, but common among men with poor fertilizing capacity, and may reflect other abnormalities of spermiogenesis. J Androl. 2001;22(4):604–10.
123. Chevaillier P, Mauro N, Feneux D, Jouannet P, David G. Anomalous protein complement of sperm nuclei in some infertile men. Lancet. 1987;2(8562):806–7.
124. Oliva R. Protamines and male infertility. Hum Reprod Update. 2006;12(4):417–35.
125. Aoki VW, Christensen GL, Atkins JF, Carrell DT. Identification of novel polymorphisms in the nuclear protein genes and their relationship with human sperm protamine deficiency and severe male infertility. Fertil Steril. 2006;86(5):1416–22.
126. Aoki VW, Emery BR, Liu L, Carrell DT. Protamine levels vary between individual sperm cells of infertile human males and correlate with viability and DNA integrity. J Androl. 2006;27(6):890–8.
127. Aoki VW, Liu L, Jones KP, et al. Sperm protamine 1/ protamine 2 ratios are related to in vitro fertilization pregnancy rates and predictive of fertilization ability. Fertil Steril. 2006;86(5):1408–15.
128. Cho C, Jung-Ha H, Willis WD, et al. Protamine 2 deficiency leads to sperm DNA damage and embryo death in mice. Biol Reprod. 2003;69(1):211–7.
129. Depa-Martynow M, Kempisty B, Lianeri M, Jagodzinski PP, Jedrzejczak P. Association between fertilin beta, protamines 1 and 2 and spermatid-specific linker histone H1-like protein mRNA levels, fertilization ability of human spermatozoa, and quality of preimplantation embryos. Folia Histochem Cytobiol. 2007;45 Suppl 1:S79–85.
130. Mengual L, Ballesca JL, Ascaso C, Oliva R. Marked differences in protamine content and P1/P2 ratios in sperm cells from percoll fractions between patients and controls. J Androl. 2003;24(3):438–47.

131. Bedford JM, Calvin HI. The occurrence and possible functional significance of -S-S- crosslinks in sperm heads, with particular reference to eutherian mammals. J Exp Zool. 1974;188(2):137–55.
132. Calvin HI, Bedford JM. Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. J Reprod Fertil Suppl. 1971;13 Suppl 13:65–75.
133. Calvin HI, Yu CC, Bedford JM. Effects of epididymal maturation, zinc (II) and copper (II) on the reactive sulfhydryl content of structural elements in rat spermatozoa. Exp Cell Res. 1973;81(2):333–41.
134. Saowaros W, Panyim S. The formation of disulfide bonds in human protamines during sperm maturation. Experientia. 1979;35(2):191–2.
135. Sega GA, Generoso EE. Measurement of DNA breakage in spermiogenic germ-cell stages of mice exposed to ethylene oxide, using an alkaline elution procedure. Mutat Res. 1988;197(1):93–9.
136. Sega GA, Owens JG. Methylation of DNA and protamine by methyl methanesulfonate in the germ cells of male mice. Mutat Res. 1983;111(2):227–44.
137. Sega GA, Owens JG. Binding of ethylene oxide in spermiogenic germ cell stages of the mouse after low-level inhalation exposure. Environ Mol Mutagen. 1987;10(2):119–27.
138. Bjorndahl L, Kvist U. Human sperm chromatin stabilization: a proposed model including zinc bridges. Mol Hum Reprod. 2010;16(1):23–9.
139. Bench G, Corzett MH, Kramer CE, Grant PG, Balhorn R. Zinc is sufficiently abundant within mammalian sperm nuclei to bind stoichiometrically with protamine 2. Mol Reprod Dev. 2000;56(4):512–9.
140. Bianchi F, Rousseaux-Prevost R, Sautiere P, Rousseaux J. P2 protamines from human sperm are zinc -finger proteins with one CYS2/HIS2 motif. Biochem Biophys Res Commun. 1992;182(2):540–7.
141. Gatewood JM, Schroth GP, Schmid CW, Bradbury EM. Zinc-induced secondary structure transitions in human sperm protamines. J Biol Chem. 1990;265(33):20667–72.
142. Hernandez-Ochoa I, Sanchez-Gutierrez M, Solis-Heredia MJ, Quintanilla-Vega B. Spermatozoa nucleus takes up lead during the epididymal maturation altering chromatin condensation. Reprod Toxicol. 2006;21(2):171–8.
143. Johansson L, Pellicciari CE. Lead-induced changes in the stabilization of the mouse sperm chromatin. Toxicology. 1988;51(1):11–24.