Chapter 8
Organization of Chromosomes in Human Sperm Nucleus

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Abstract Chromosomes in the human sperm nucleus adopt a hierarchy of structures starting from protamine toroids, the elementary units of DNA packaging, and up to the higher-order organization of chromosome territories. Nonrandom intranuclear positioning of individual chromosomes, with centromeres aggregated in the internally located chromocenter and preferential location of telomeres at the nuclear periphery, suggests a highly defined architecture of the sperm nucleus at the level of genome. Such an ordered chromatin organization in the sperm nucleus may have a functional significance and determine the onset of paternal gene activity at early stages of embryonic development. This chapter describes relevant experimental data with primary attention to studies of human spermatozoa and discusses the implications of sperm chromosome organization for male reproductive health.

Abbreviations

CHR	Chromosome
CT	Chromosome territory
FISH	Fluorescence in situ hybridization
ICSI	Intracytoplasmic sperm injection
MSCI	Meiotic sex chromosome inactivation
MSUC	Meiotic silencing of unsynapsed chromatin
WCP	Whole chromosome painting probe

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Introduction

Our understanding of higher-order chromatin arrangement in somatic cell nucleus (referred to as nuclear architecture, genome architecture, or chromosome architecture) has progressed considerably in recent decades, mainly as the result of the development of a wide variety of hybridization probes for specific genomic sequences and advances in fluorescence imaging techniques. It has been established that chromosomes are compartmentalized into distinct subvolumes—chromosome territories—that are nonrandomly distributed in the nucleus. The organization of chromosome territories and chromosome domains, chromatin structure, and their dynamics inside the interphase nucleus have functional consequences for gene expression regulation (reviewed in Cremer and Cremer 2001; Lanctôt et al. 2007; Misteli 2007; Pai and Engelke 2010; Meister et al. 2011).

Spermatozoa are the specialized cells that differentiate from spermatogonia precursor cells in the course of spermatogenesis. Spermatogenesis includes two major stages: meiosis and spermiogenesis. Spermatogonia are divided mitotically and differentiate into spermatocytes that undergo meiotic divisions which result in formation of haploid spermatids. During spermiogenesis, the maturation of spermatids into motile spermatozoa occurs, which is accompanied by the formation of the acrosome, the loss of the excess cytoplasm, and the formation of a flagellum. The maturation is also characterized by chromatin reorganization: the replacement of somatic histones with sperm-specific proteins (protamines), supercondensation of DNA, and inhibition of transcriptional activity. For a long time, the compact hydrodynamically shaped sperm cell with inert DNA was considered as a "sack" of genes unlikely to bear any information beyond its genomic load. However, a complex nonrandom organization of the genome was demonstrated for mammalian sperm cells (reviewed by Mudrak et al. 2011). An emerging concept is that in addition to the paternal genome per se, the sperm nucleus provides a structural context that is essential for proper genome activation upon fertilization.

This chapter summarizes the current state of knowledge on human sperm chromosome organization at different structural levels, starting from the packaging of the DNA with protamines to the higher-order chromosome configuration and intranuclear chromosome positioning. Possible implications of sperm chromosome architecture for male factor infertility are discussed.

DNA Packaging in Spermatozoa

The chromatin of the sperm nucleus is characterized by extensive protamination that leads to the tight packaging of chromatin, which is necessary for the protection of parental genome during movement of the spermatozoa through the female reproductive tract.

There are two types of protamines in humans, P1 and P2. The P1/P2 ratio ranges from 0.54 to 1.43 in healthy individuals (Nanassy et al. 2011). Protamines are highly basic proteins that are characterized by an arginine-rich core and cysteine residues.

Positively charged arginine residues electrostatically neutralize DNA, leading to a strong protamine–DNA binding. The cysteine residues of protamines mediate the formation of multiple inter- and intramolecular disulfide bonds so that sperm DNA is organized into a nucleoprotamine complex with extreme compactness (Balhorn 1982, 2007).

Protamines coil sperm DNA into toroidal structures approximately 50–70 nm in outer diameter and 25 nm thick, a form of packaging that inactivates the sperm genome (Brewer et al. 1999; Balhorn 2007). Each toroid contains 50 kb DNA and is linked to the neighboring ones by nuclease-sensitive histone-bound DNA fragments, toroid linkers (Sotolongo et al. 2005; Ward 2010). Stacking of these nucleoprotamine toroids results in DNA compaction approximately tenfold more efficient as compared with nucleohistone packaging. Toroids and core particles remain in decondensed human sperm nuclei, as was demonstrated by the method of atomic force microscopy (Hud et al. 1993; Allen et al. 1996; Joshi et al. 2000; Nazarov et al. 2008).

According to the "donut-loop" model of chromatin organization in mammalian sperm suggested by S.W. Ward (reviewed by Ward and Ward 2004), during spermiogenesis, individual DNA loop domains condense into toroidal (donut-like) structures, so that each toroid contains a single DNA loop domain that is attached to the sperm nuclear matrix via toroid linkers (Ward 2010).

Mudrak et al. (2005) reported 500-nm chromatin beads in the partially decondensed human sperm nuclei and suggested that they consisted of a number of toroid stacks, thus representing the next hierarchical level of sperm chromatin organization. Earlier, Haaf and Ward, using fluorescence in situ hybridization (FISH) on sperm chromatin spreads, observed beaded fibers with basic package units of 180, 360, and 600 nm (Haaf and Ward 1995). The relationship of such beads to nucleoprotamine toroids remains to be elucidated. At the next level, two rows of 500-nm chromatin beads interconnected by thinner and less dense chromatin filaments organize the chromosome-arm fiber with thickness about 1,000 nm (Mudrak et al. 2005).

It has been demonstrated that replacement of somatic histones by protamines during spermiogenesis occurs in a stepwise manner. First (during meiosis), somatic histones are replaced with testis-specific histone variants; then (during chromatin condensation at the elongating spermatid stage of spermiogenesis), the latter are replaced by transition proteins, which, in turn, are replaced by protamines (Meistrich et al. 2003; Churikov et al. 2004; reviewed in Mudrak et al. 2011). As a result, in mature spermatozoa, histones are not completely replaced by protamines; 1–2 % of mouse sperm DNA (Balhorn 1982; Pittoggi et al. 1999; Brykczynska et al. 2010) and as much as 15 % of human sperm DNA remains histone bound and packed into nucleosomes (Churikov et al. 2004; Gineitis et al. 2000; Hammoud et al. 2009).

Mature human spermatozoa contain core histones (H2A, H2B, H3, H4) (Gatewood et al. 1990); histone variants such as CENP-A (Zalensky et al. 1993), TSH2B (Zalensky et al. 2002); H3.1, 3.2, 3.3 (van der Heijden et al. 2008), H2A.Z (Hammoud et al. 2009), and H2AX (Li et al. 2006); and the modified histones H3K4 Me2, Me3, and H3K9 Me3 (Hammoud et al. 2009).

Histone hTSH2B (human testis/sperm-specific H2B variant), which is transcribed exclusively in the testis, is also found in spermatogonia, spermatids, and in a subpopulation of mature sperm cells (Zalensky et al. 2002), where it is involved in nuclear decondensation during fertilization (Singleton et al. 2007).

Phosphosphorylated histone H2AX (γH2AX) plays a crucial role in the process known as meiotic sex chromosome inactivation (MSCI), a form of X-chromosome inactivation present in male germ cells. γH2AX marks entire sex chromosomes at the onset of MSCI (Mahadevaiah et al. 2001).

H3.1/H3.2 histone variants originated from male germ cells were found in paternal pronuclei in pre-S-phase human zygotes (van der Heijden et al. 2008).

Several research groups addressed the question whether histone-bound DNA distributed randomly throughout the sperm genome or the histones were associated with certain sequences. Earlier work by Gatewood et al. (1987) suggested that histone binding was restricted to specific sequences (Gatewood et al. 1987). Later, analysis of the chromatin structure of members of β-globin gene family expressed at different times during development showed that embryo-specific ε- and γ-globin genes were histone enriched, whereas the postnatal-expressed β-globin gene was protamine enriched in human sperm cells (Gardiner-Garden et al. 1998). It was suggested that histones in sperm chromatin might mark sets of genes that would be preferentially activated during early development. It was shown in subsequent studies that histone-associated DNA included telomeres (Zalenskaya et al. 2000), centromeres (Zalensky et al. 1993; Hammoud et al. 2009), paternally imprinted regions (Banerjee and Smallwood 1995; Hammoud et al. 2009), specific gene loci (Wykes and Krawetz 2003; Hammoud et al. 2009), and regulatory sequences of developmentally important genes (Arpanahi et al. 2009). Recent data from Arpanahi et al. (2009) and Hammoud et al. (2009) have demonstrated that histone-bound DNA is distributed in paternal genome in two ways: in relatively large tracts, ranging from 10 to 100 kb, and in smaller tracts interspersed throughout the genome. Smaller tracts of histone-associated DNA distributed evenly throughout the paternal genome were suggested to represent a repeating unit of sperm chromatin structure, such as linker regions between protamine toroids in the chromatin fiber (Ward 2010).

Together, these findings suggest that there are at least two types of sperm chromatin: nucleoprotamine-containing domains and nucleohistone-containing domains. The nucleohistone domains are structurally more open as compared to the nucleoprotamine domains and are nuclease sensitive in contrast to nuclease-resistant nucleoprotamine domains.

Histone-associated chromatin might represent the residual active chromatin that persists through chromatin condensation and therefore reveals the transcriptional history of spermatogenesis (Johnson et al. 2011).

The transmission of core histones together with modified histones and histone variants to the egg leaves the door open for DNA and histone-based epigenetic signals that may be important for consequent embryonic development (Miller et al. 2010).

It has been speculated that sperm genome is frozen in a dynamic configuration that reflects its pending introduction to the ooplasm (Miller et al. 2010). Deviation from this configuration caused by DNA-packaging anomalies may lead to infertile phenotypes. It has been shown that abnormally high P1/P2 ratios are associated with decreased fertilization rates and embryo quality (Simon et al. 2011). In line with this observation, an aberrant expression of P2 induced by oxidative stress caused by cigarette smoking leads to abnormal elevation of the P1/P2 level and improper sperm chromatin condensation (Hammadeh et al. 2010). Antioxidant therapy after oxidative stress resulted in significant improvements in sperm chromatin integrity and protamine packaging (Tunc et al. 2009).

Defects in DNA methylation at imprinted loci have been found in oligozoospermic patients and patients with improper histone replacement by protamines, indicating the existence of a tight link between epigenetic chromatin alterations and male infertility (Carrell and Hammoud 2010; Hammoud et al. 2010).

Therefore, similar to somatic cells, the structural organization of chromatin in the sperm nucleus apparently has a functional significance, and its alterations can affect spermatogenesis and early developmental processes.

Higher-Order Chromatin Organization in the Sperm Nucleus

In the following sections we describe elements of sperm genome architecture above the level of nucleoprotamine/nucleohistone complexes, revealed by methods of FISH and microscopy.

Application of FISH to Chromosome Positioning Studies in Spermatozoa

Almost 40 years ago, Geraedts and Pearson (1975) demonstrated that in human spermatozoa, chromosome 1 (CHR 1) is frequently located adjacent to CHR Y, which suggested a nonrandom chromosome arrangement in the human sperm nucleus. Later, Luetjens et al. (1999) showed that CHR 18 had a tendency to locate in the basal area of the nucleus, near the sperm tail, whereas CHR X was preferentially located in the apical area (Luetjens et al. 1999).

FISH studies using whole chromosome painting (WCP) probes demonstrated that, similarly to somatic cells, individual chromosomes occupy distinct territories in human spermatozoa (Haaf and Ward 1995; Zalensky et al. 1995; Hazzouri et al. 2000). In the following years, data on the defined chromosome positioning in human sperm began to appear (Hazzouri et al. 2000, Tilgen et al. 2001; Sbracia et al. 2002; Zalenskaya and Zalensky 2004; Mudrak et al. 2005; Wiland et al. 2008; Manvelyan

et al. 2008). These studies provided a strong basis for the current view of nonrandom chromosome arrangement in human spermatozoa.

By now, information on the location of all 23 chromosomes in the haploid human male gamete is available. However, it is not entirely conclusive, because application of different protocols resulted in inconsistent data on chromosome positioning coming from different laboratories.

Centromeres are traditionally used in somatic cells as indicators of intranuclear chromosome location (Nagele et al. 1999; Sun and Yokota 1999). Along with WCP, chromosome-specific centromeric probes were employed in chromosome positioning studies on sperm cells (Sbracia et al. 2002; Zalenskaya and Zalensky 2004; Finch et al. 2008; Wiland et al. 2008). The WCP probe, covering the entire length of the chromosome, yields the large FISH signal; the intranuclear position of the chromosome territory (CT), in this case, is often determined by the position of the hybridization signal center (Foster et al. 2005).

FISH studies on sperm cells have some peculiarities originating from sperm cell characteristics. For example, the mature spermatozoon has almost no cytoplasm. Its nucleus occupies almost the whole of the sperm head. The amount of cytoplasm is diminished during spermiogenesis to reduce the cell weight and achieve a more hydrodynamic shape, improving sperm motility in the female reproductive tract. Therefore, no pretreatment is needed to remove the cytoplasm before denaturation to enable efficient hybridization of the probe to the target.

Although removal of cytoplasmic debris can be omitted, the decondensation of densely packed sperm chromatin is a prerequisite for performing FISH. Because of its extensive protamination, sperm DNA is the most condensed DNA in eukaryotes. Therefore, to enable penetration of painting probes and antibodies during FISH, sperm chromatin needs to be relaxed. Sperm chromatin decondensing buffers slightly differ from each other, but all include dithiothreitol (DTT), the thiol reducing agent, which reduces disulfide bonds between protamines (Zalensky et al. 1995, 1997; Luetjens et al. 1999; Hazzouri et al. 2000; Zalenskaya and Zalensky 2004; Mudrak et al. 2005; Finch et al. 2008; Wiland et al. 2008).

Longitudinal and Radial Chromosome Positioning in Spermatozoa

Asymmetry of the sperm nucleus and the presence of a spatial reference point, the place of tail attachment, facilitate chromosome positioning studies. The elongated shape of the human sperm nucleus allows the assessment of not only radial but also longitudinal chromosome position.

To determine the longitudinal position, that is, the location of the chromosome along the anterior–posterior axis, the length of the sperm nucleus can be divided into equal sectors: “subacrosomal, equatorial, basal” (Sbracia et al. 2002), “regions (I–IV)” (Zalenskaya and Zalensky 2004), and “apical, medial, basal” (Wiland et al. 2008), and the number of FISH signals in each sector is calculated. Alternatively, the normalized distance from the center of the FISH signal to the tail attachment spot

can be computed and used as the indicator of longitudinal chromosome location (Zalenskaya and Zalensky 2004).

The preferential location in the anterior part of the human sperm nucleus has been demonstrated for CHR X (Luetjens et al. 1999; Hazzouri et al. 2000; Sbracia et al. 2002; Zalenskaya and Zalensky 2004) and CHR 1 (Zalenskaya and Zalensky 2004; Mudrak et al. 2005), whereas CHR 18 has been found preferentially in the posterior part (Luetjens et al. 1999; Sbracia et al. 2002; Mudrak et al. 2012). A random distribution along the long nuclear axis has been demonstrated for CHR 13 (Hazzouri et al. 2000).

Radial position can be defined as the position of chromosomes in relationship to the nuclear periphery or the nuclear interior. Some authors divided sperm nuclei into concentric zones, "central and peripheral" (Manvelyan et al. 2008), or enumerated "shells" (Finch et al. 2008), and scored the number of FISH signals in each zone. Other authors ascertained the distance from FISH signals either to the nearest peripheral edge (Foster et al. 2005) or to the long axis of the nucleus (Zalenskaya and Zalensky 2004). It has been shown that CHR 6 (Zalenskaya and Zalensky 2004), CHR 7 (Manvelyan et al. 2008), and CHR 18 (Manvelyan et al. 2008; Mudrak et al. 2012) are mostly peripheral in location, whereas CHR X (Zalenskaya and Zalensky 2004; Mudrak et al. 2012) and CHR 19 (Manvelyan et al. 2008; Mudrak et al. 2012) are internal. Figure 8.1a–c demonstrates different approaches to chromosome position determination. Some disagreements in data on chromosome localization between different research groups can result from different methods of cell treatment and FISH data analysis. For instance, the internal location of CHR X and peripheral location of CHR 6 (Zalenskaya and Zalensky 2004) were reported, as compared to the peripheral position of CHR X and central position of CHR 6 found in another study (Manvelyan et al. 2008).

Analysis of three-dimensional (3D) chromosome arrangement in human spermatozoa (Manvelyan et al. 2008) has shown the correlation of radial positioning with chromosome size and gene content. Large chromosomes occupied mostly the periphery and small chromosomes the nuclear interior: gene-rich chromosomes were more centrally located in comparison with gene-poor chromosomes.

A similar tendency in the radial intranuclear positioning of chromosomes of different size and gene content was observed in human somatic cells (Croft et al. 1999; Bickmore and Chubb 2003; Parada et al. 2004). However, detailed study of chromosome positioning in spermatozoa from three breeds of pig (Foster et al. 2005), although showing the association between position and gene density, did not demonstrate any correlation between position and chromosome size.

Interestingly, a nonrandom chromosome arrangement has been reported in sperm cells of other mammals: rat (Meyer-Ficca et al. 1998), mouse (Garagna et al. 2001), pig (Foster et al. 2005), cow (Mudrak et al., unpublished data), and mammals of the earliest groups, such as marsupials (Greaves et al. 2001) and monotremes (Watson et al. 1996; Greaves et al. 2003; Tsend-Ayush et al. 2009).

The observed evolutionary conservation of nonrandom chromosome arrangement in mammalian sperm cells implies its functional importance.

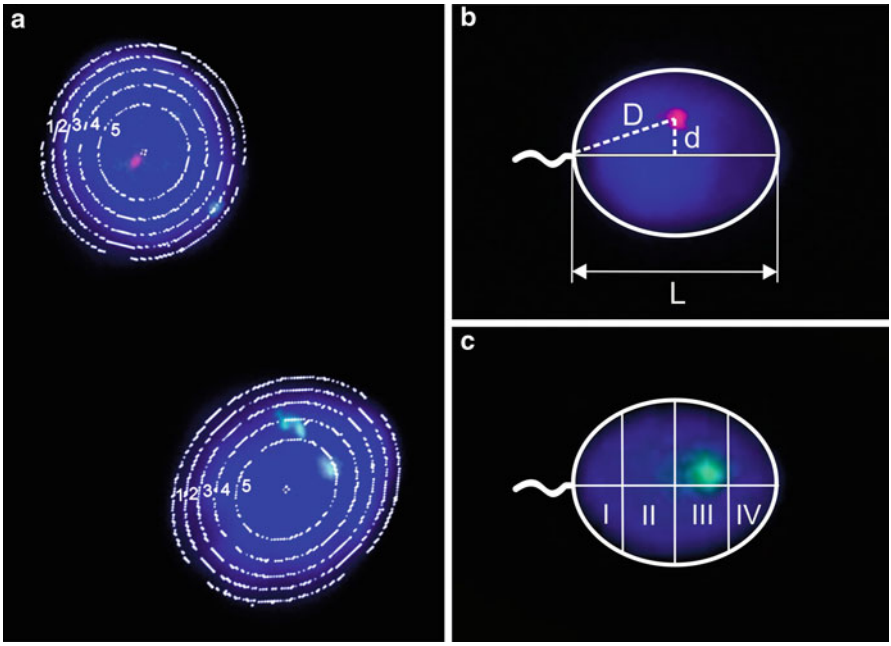

Fig. 8.1 Determination of chromosome position in human sperm nuclei using fluorescent in situ hybridization (FISH) with centromere-specific and whole chromosome painting (WCP) probes. (**a**) Centromere-specific probes with five shell template overlaid are used to determine the radial chromosome location. FISH signals are scored according to which of the five shells they appeared in; if a probe is spanned more than one shell, it is scored based on the location of the majority of its signal. Upper sperm nucleus: the chromosome 18 (*aqua*) is the outermost, the chromosome Y (*red*) is innermost; lower sperm nucleus: chromosomes X (*green*) and 18 (*aqua*) occupy intermediate positions. (From Finch et al. 2008, with kind permission from Oxford University Press.) (**b**) Centromere-specific probe (*red*) and measurements of normalized distances from FISH signal to the tail attachment point and to the long nuclear axis (D/L and d/L) are used to describe the intranuclear chromosome position. (**c**) Chromosome painting probe is used to visualize the chromosome territory (CT) of a chromosome (*green*). To determine the longitudinal position along the anterior–posterior axis (shown as a *horizontal line*), the sperm nucleus is divided into four regions, I–IV, starting from the basal side that is determined by the tail attachment site. Methods in (**b** and **c**) suggested by Zalenskaya and Zalensky (2004). Nuclear DNA (**a**–**c**) is counterstained with DAPI (*blue*)

Clustering of Centromeres into Compact Chromocenter in the Interior of the Sperm Nucleus

Immunofluorescent detection of the centromere-specific histone CENP-A and in situ hybridization with alphoid pan-centromeric DNA probes followed by confocal microscopy analysis revealed the localization of centromeres in mature human sperm nuclei. It was shown that centromeres of all nonhomologous chromosomes demonstrate a strong clustering that leads to the formation of a compact chromocenter localized to the nuclear interior (Zalensky et al. 1993, 1995). Individual centromeres

within the chromocenter of the decondensed mature human sperm nucleus form different structural elements: dimers, tetramers, linear arrays, and V-shaped structures (Zalensky et al. 1993). Later on, the existence of the chromocenter in human sperm nucleus was supported (Hazzouri et al. 2000; Gurevitch et al. 2001; Finch et al. 2008; Wiland et al. 2008). Chromocenters were also found in bovine (Powell et al. 1990), rat (Meyer-Ficca et al. 1998), and mouse (Haaf and Ward 1995; Hoyer-Fender et al. 2000; Dolnik et al. 2007) sperm nuclei, suggesting similar principles of overall nuclear architecture in mammalian spermatozoa.

Peripheral Location of Telomeres in the Sperm Nucleus

The position of telomeres in human sperm cells was examined using FISH with the telomere-specific (TTAGGG)n probe. It was shown that telomeres are localized at the periphery of human sperm nuclei, and it was suggested that they form associations with the nuclear membrane (Zalensky et al. 1995). Using minimal nuclear swelling of the sperm cells pretreated with heparin in combination with DTT, it was found that in human sperm, telomeres associate into tetrameres and dimers (Zalensky et al. 1997). Telomere dimers are observed not only in humans but in five other mammals: rat, mouse, pig, horse, and cow (Zalensky et al. 1997; Meyer-Ficca et al. 1998). The progress in the studies on telomere positioning has been done after human subtelomeric chromosome- and arm-specific probes for FISH became available (Knight et al. 1997, Kingsley et al. 1997, Knight and Flint 2000). Interesting results were obtained with the use of two-color FISH with pairs of DNA probes that correspond to p- and q-arms of seven human chromosomes (metacentric and submetacentric, large and small) on sperm cells after heparin-DTT nuclear swelling (Solov'eva et al. 2004). These studies have revealed that the telomere dimer appears as the result of specific interaction of chromosome ends of the same chromosome, indicating that human sperm chromosomes are looped. It has been proposed that these interactions depend on chromosome arm-specific subtelomeric sequences and may involve protein complexes specifically binding these sequences that are not established yet.

Hairpin Configuration of Chromosomes in Human Sperm Nucleus

Using two-color FISH with microdissected probes for the whole p- and q-arms of human metacentric chromosomes 1 and 2 and submetacentric chromosome 5, the looped configuration of human sperm chromosomes proposed earlier (Zalensky et al. 1995; Solov'eva et al. 2004) was confirmed (Mudrak et al. 2005). In sperm nuclei mildly decondensed with heparin/DTT, FISH signals from p- and q-arms of the chromosomes overlapped or were located closely to each other in the antiparallel fashion inside the compact CT, while the chromosome was bent at almost 180° in the centromere region. The antiparallel position of chromosome arms produced

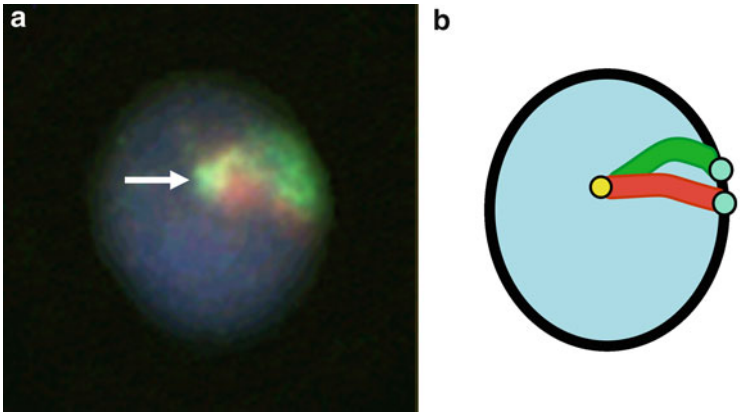

Fig. 8.2 Hairpin configuration of the chromosome in human sperm nucleus revealed by two-color FISH with arm-specific chromosome paints. (**a**) Typical pattern of hybridization: chromosome fiber is bent at 180° at the centromere (*arrow*) so that p-arm (*red*) and q-arm (*green*) of the chromosome appear aligned in the antiparallel way. This arrangement produces a looped shape of chromosomes with telomeres located close to each other. (**b**) Schematic representation of the hairpin chromosome configuration presented in (**a**). p- and q-chromosome arms are shown in *red* and *green*; the centromere, in *yellow*, and telomeres located at the nuclear periphery, in *light green*

the hairpin configuration (Fig. 8.2) of chromosomes (Mudrak et al. 2005). The hairpin shape of chromosomes was preserved in nuclei swollen to a higher degree. The hairpin conformation is most probably characteristic to the sperm chromosomes of other mammals where telomeric dimers located at the nuclear periphery were observed (Zalensky et al. 1997; Meyer-Ficca et al. 1998).

In summary, chromosome organization in human spermatozoa has the following features: (1) chromosomes are nonrandomly located in the nucleus and occupy distinct CTs; (2) centromeres of all chromosomes are joined into the chromocenter in the nuclear interior; (3) telomeres are located at the periphery of the nucleus, forming dimers and tetramers; (4) each CT appears to be stretched between its internally located centromere with p- and q-arm telomeres interacting at the periphery, so that overall chromosome configuration resembles a hairpin; (5) chromosome arms represent chromatin fibers 1,000 nm wide; each 1,000-nm fiber is composed of two rows of chromatin globules 500 nm in diameter interconnected with thinner chromatin strands (illustrated in Fig. 8.3a–c).

Chromosome Positioning in Spermatozoa and Early Embryonic Development

Complex and dynamic organization of the genome in somatic cells contributes to the regulation of nuclear processes such as DNA replication and repair, gene transcription, and RNA processing (for review, see Schneider and Grosschedl 2007).

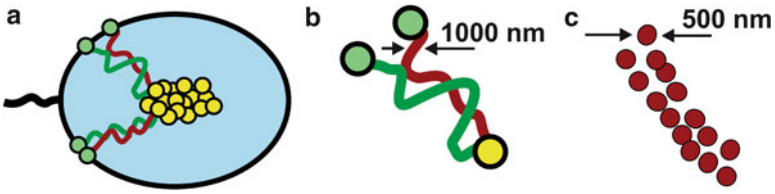

Fig. 8.3 Organization of chromosomes in human sperm nucleus. (**a**) Chromosomes (only two ones are shown) stretch between the sperm chromocenter formed by an aggregation of centromeres (*yellow circles*) in the nuclear interior and peripherally located telomeres (*light green circles*), associated into dimers. Closely located p- and q-arms produce a characteristic hairpin configuration of the sperm chromosome. (**b**) Enlarged chromosome hairpin: an individual arm presents a 1,000-nm chromatin fiber. (**c**) A 1,000-nm arm fiber consists of two rows of 500-nm chromatin globules seen in a conventional epifluorescent microscope. These globules presumably are formed by stacked nucleoprotamine toroids. Each toroid consists of a packed 50-kb DNA loop. (Overviewed in Section "DNA Packaging in Spermatozoa")

It is not entirely clear whether global genome organization in the genetically silent sperm nucleus is of any functional importance or if it arises in the process of efficient packaging of chromatin into the miniature sperm head.

Intranuclear location of CHR X was studied in spermatozoa of several mammalian species including monotremes and marsupials—nonplacental mammals evolutionarily diverged from placentals 170 and 130 million years ago, respectively. In fibrillar spermatozoa of two monotreme species—platypus and echidna—sex chromosomes are located apically near the acrosome, the place of the first contact with the egg during fertilization (Watson et al. 1996). Therefore, it was suggested that sex chromosomes were possibly the first chromosomes to be exposed to the egg cytoplasm, decondensed and remodeled by ooplasmic factors (Greaves et al. 2003). Anterior (subacrosomal) location of X chromosomes in humans (Luetjens et al. 1999; Hazzouri et al. 2000; Sbracia et al. 2002) supported this view. Although in sperm cells of two Australian marsupials, the fat-tailed dunnart and southern hairy-nosed wombat, CHR X occupied the medial position (Greaves et al. 2001, 2003), during maturation, dunnart and wombat sperm cells undergo the morphological transition to T-shape (Breed 1994), so that their medially located CHR X appears to be located in the place of spermatozoon first contact to the egg during fertilization.

The opposite point of view is that sex chromosomes are the last ones to be activated by ooplasm during fertilization (Foster et al. 2005); this is based on the analysis of sex chromosome location in spermatozoa of humans and pigs. Although the longitudinal position of CHR X in these two species (human and pig) could be defined as anterior (subacrosomal), its radial position is strictly central (Luetjens et al. 1999; Hazzouri et al. 2000; Sbracia et al. 2002; Zalenskaya and Zalensky 2004). It has been suggested that in oval-shaped porcine and human nuclei, radial positioning is more relevant, and therefore chromosomes X and Y, located deeply in the nuclear interior, are the last ones to be exposed to the ooplasm during fertilization. Similar CHR X location was observed in bovine sperm nuclei (Mudrak et al., unpublished data). Despite the diametrical points of view on timing of sex chromosome

activation during fertilization, both groups of authors (Greaves et al. 2003; Foster et al. 2005) are in agreement on functional importance of intranuclear sex chromosome positioning.

Global rearrangement of nuclear architecture takes place in spermatogenesis. Individual centromeres come together to form a chromocenter in humans (Zalensky et al. 1993, 1995) and mice (Namekawa et al. 2006) by the round spermatid stage. At the same stage, repositioning of the X chromosome from the periphery to the center of the nucleus was observed also in spermatogenesis of mice (Namekawa et al. 2006) and marsupials (Namekawa et al. 2007). Similarly, in the spermatogenesis of the pig (Foster et al. 2005), relocation of CHR X from the periphery to the center occurs, when primary spermatocytes become secondary spermatocytes and then differentiate into spermatids, and this relocation seems to be important for proper sperm functioning. In sperm cell precursors such as primary spermatocytes, CHR X is located at the nuclear periphery (reviewed in Handel 2004; Foster et al. 2005).

Migration of the X chromosome may be associated with MSCI, another form of X-chromosome inactivation characteristic for male germ cells. Male MSCI is Xist independent (McCarrey et al. 2002; Turner et al. 2002), as compared to female X-inactivation, well known for placental mammals, when a noncoding RNA (Xist) decorates the entire X chromosome to initiate chromosome-wide gene silencing (Chow and Heard 2010; Lee 2010). MSCI is a special case of the more general mechanism of meiotic silencing of unsynapsed chromatin (MSUC), which silences chromosomes that fail to pair with their homologous partners and is thought to prevent the aneuploidy resulting from synaptic errors (Turner 2007).

MSCI begins during the first meiotic prophase of spermatogenesis when homologous pairing and synapsis take place. Sex chromosomes lack homology, except for a tiny pseudoautosomal region. Although autosomes undergo synapsis, sex chromosomes remain unpaired. They start condensing and eventually form a transcriptionally inactive XY-body by the mid-pachytene stage, at the nuclear periphery of primary spermatocytes (Hoyer-Fender 2003; Namekawa et al. 2006). Transcriptional silencing is achieved by a number of chromatin modifications, including histone ubiquitination, phosphorylation, methylation, and acetylation (Baarends et al. 2007). It was demonstrated that the silencing persists throughout meiosis II and spermiogenesis into mature sperm, long after dissolution of the XY-body. Inactive X and Y chromosomes, showing epigenetic similarity to the XY-body, were termed postmeiotic sex chromatin (Namekawa et al. 2006).

In postmeiotic haploid round spermatids of mice, postmeiotic sex chromatin occupies a novel compartment in the center of the nucleus, juxtaposed to the chromocenter, and adopts a distinctive chromosome configuration as compared to autosomes. Although autosomes are organized radially around the chromocenter in a Rabl configuration (Leitch 2000), the X and Y occupy compact domains that do not extend to the nuclear periphery (Namekawa et al. 2006). Similar CHR X configuration was observed in mature spermatozoa of pigs (Foster et al. 2005), bulls, and humans (Mudrak et al., unpublished data). Thus, the X-chromosome position may be critical for establishing X-chromosome inactivation.

Polarity of sperm nuclei together with the nonrandom chromosome/chromatin domain arrangement in sperm cells may lead to the sequential manner of sperm chromatin remodelling during fertilization (Foster et al. 2005). Following penetration into the oocyte, the sperm nuclear envelope starts to disperse, and sperm chromatin becomes exposed to the ooplasm. It is imaginable that chromatin bordering the disassembling nuclear envelope is decondensed and remodeled by ooplasmic factors in the first turn. Chromosomes that are closer to the dispersing nuclear envelope should be remodeled earlier than others.

During early mammalian embryogenesis, male and female genomes remain topologically separated up to the four-cell embryo stage, at the time when chromatin remodeling (Mayer et al. 2000) and programming of the appropriate patterns of parent-specific developmental gene expression occur (Fundele and Surani 1994).

Thus, the well-organized and conserved nuclear architecture in sperm may provide the mechanism for differential exposure of chromatin domains to the ooplasmic factors and the controlled activation of the male genome following fertilization.

Genome Architecture Abnormalities and Male Infertility

Male factor infertility is a heterogeneous disorder including abnormal sperm morphology, low motility and sperm count, chromosome abnormalities, deficiencies in basic chromosomal proteins, and chromatin condensation defects.

If the genome architecture is functionally important, its change is expected to be associated with an abnormal phenotype. Indeed, in somatic cells, alterations in genome architecture are correlated with epilepsy (Borden and Manuelidis 1988), some laminopathies (Misteli 2004), and cancer (Meaburn et al. 2005).

In sperm cells, deviations from normal genome architecture may affect fertilization or development. There are a few studies of genome architecture in infertile males. Finch et al. (2008) studied the radial distribution of three centromeric loci (in CHRs X, Y, and 18) in spermatozoa from infertile men diagnosed as having impaired semen parameters such as oligozoospermia, asthenozoospermia, and teratozoospermia. They found that the sex chromosome distribution pattern in these patients was altered in comparison with a control group of normozoospermic males. The distribution of X- and Y-centromeres in infertile males was close to random, whereas in the control group, centromeres were located in the center of the nucleus, which is characteristic of normal human sperm genome architecture (Zalensky et al. 1993, 1995; Zalenskaya and Zalensky 2004). The authors suggested that the distorted genome architecture in infertile males could be the consequence of impaired spermatogenic regulation. X-chromosome migration to the center of the nucleus may be related to the MSCI/MSUC process, whereas compromised semen parameters are often connected with the increase of aneuploidy level (reviewed by Tempest and Griffin 2004). Hence, Finch and coauthors speculated that the altered pattern of genome architecture in infertile males might reflect failure in sex chromosome

migration from the periphery to the center and defects in MSCI/MSUC mechanisms (Finch et al. 2008).

A distorted centromere distribution pattern in spermatozoa was reported to be correlated with infertility (Olszewska et al. 2008). The authors demonstrated that although in fertile individuals the localization of centromeres (CHRs 15, 18, X, and Y) was restricted to a small area in the nuclear interior, some disturbances in the centromere area existed in sperm cells of infertile patients. In disomic sperm cells ($n+1$), centromeres of CHRs 15,15 or 18,18, or Y,Y (but not X,X) had a shifted average longitudinal position in comparison with normal sperm cells ($n=23$) (Olszewska et al. 2008).

Aberrations of genome architecture in spermatozoa do not necessarily cause infertility. A study of longitudinal and radial localization of centromeres (CHRs 7, 9, X, Y) in fertile normozoospermic males that were carriers of the reciprocal chromosome translocations t(1;7), t(7;2), t(7;13), t(7;9), t(9;14), and t(4;13) demonstrated deviations in normal chromosome positioning: the centromeres of chromosomes with translocations had a shifted intranuclear localization, which influenced the localization of other chromosomes (X and Y). The chromocenter in sperm nuclei of translocation carriers was widened toward the apical end of the nucleus in comparison with the chromocenter in control males (Wiland et al. 2008).

An increase of aneuploidy level and the degree of mosaicism in the embryo derived from parents—carriers of a balanced translocation—was reported previously (Iwarsson et al. 2000). Based on this observation, Wiland and coauthors hypothesized that distorted chromosome positioning in spermatozoa might affect the position of chromosomes during the first mitotic division of the zygote and thus could lead to aneuploidy.

Therefore, no deterministic link has been established between aberrant sperm genome architecture and male fertility. Further studies are needed to evaluate the contribution of altered sperm genome architecture to male infertility.

Sex Chromosome Positioning in Male Gametes and the Increased Rate of Sex Chromosome Aneuploidy After ICSI

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technology that is used to treat sperm-related infertility. In this technique, a single spermatozoon is injected into a mature egg, thus bypassing all the steps of normal gamete interaction. The injected spermatozoon does not undergo the acrosome reaction and preserves the intact perinuclear theca, which can cause delay in decondensation of subacrosomal chromatin.

An increased occurrence of chromosomal abnormalities in newborn children conceived through ICSI was reported (Bonduelle et al. 1998; In't Veld et al. 1995; Liebaers et al. 1995). It was suggested that one of these abnormalities, the increase in sex chromosome aneuploidy of paternal origin, might arise de novo and be a consequence of the ICSI procedure itself (Luetjens et al. 1999).

An impaired chromatin decondensation pattern was also observed in monkey (Sutovsky et al. 1996; Hewitson et al. 1999; Ramalho-Santos et al. 2000), porcine (Katayama et al. 2002), mouse (Ajduk et al. 2006), and heterologous human–hamster ICSI (Terada et al. 2000; Jones et al. 2010).

Human sex chromosomes have been shown to be preferentially located in subacrosomal areas of the sperm nucleus (Luetjens et al. 1999; Hazzouri et al. 2000; Sbracia et al. 2002; Zalenskaya and Zalensky 2004; Mudrak et al. 2012). Atypical decondensation of the subacrosomal region may lead to unbalanced remodeling of sex chromosomes (for instance, delaying their entry into S-phase) and eventually lead to errors during the first mitotic division of the zygote and chromosome loss (Terada et al. 2000).

An alternative explanation of the increased sex chromosome aneuploidy after ICSI may be an increased rate of sex chromosome aneuploidy in sperm cells. A statistically significant increase of the fraction of sperm cells with sex chromosome abnormalities was found in the semen of oligozoospermic men (Shi and Martin 2001; Sbracia et al. 2002). It was shown that normal morphology was not an absolute indicator for the selection of genetically normal sperm, and therefore the observed pregnancy failures among ICSI patients might in part be caused by the selection of aneuploid spermatozoa (Ryu et al. 2001). Prospective sperm aneuploidy testing would be beneficial for understanding the basis of chromosome anomalies and for improving the efficiency and safety of infertility therapies.

Concluding Remarks

Chromatin remodeling in the nucleus in differentiating male germ cells results in complete suppression of gene expression and supercompact packaging of the male genome into a volume of about 5 % of that of somatic cells in mature spermatozoa. Because of this, for a long time, the sperm function was considered to be limited only to the delivery of paternal genetic material to the oocyte. However, data that have emerged in the past two decades suggest that, in addition to the paternal genome, the mammalian sperm cell may bear epigenetic information important for early embryogenesis.

Human sperm chromatin adopts complex spatial organization on multiple levels starting from the packaging of DNA by protamines up to the higher-order structure of CTs. However, protamination of sperm chromatin is not complete, and it contains somatic-like domains organized in nucleosomes located at imprinted genes, developmentally transcribed genes, and genes of signaling factors (Miller et al. 2010). Similarly to chromatin in somatic cells, histone-containing domains can be marked by histone modifications (Hammoud et al. 2009; Brykczynska et al. 2010), suggesting potential paternal contribution to the epigenetic reprogramming of the zygote following fertilization.

Large-scale chromosome and nuclear architecture in sperm cells may have a functional significance as well. Individual chromosomes demonstrate nonrandom intranuclear positions, with their centromeric regions aggregated in the internal

chromocenter, and specific pairing of the telomeric domains at the nuclear periphery. Such well-defined nuclear architecture can potentially meditate the order of unpacking and activation of the male genome upon fertilization, thus transmitting epigenetic information to descendant cells (Zalensky and Zalenskaya 2007).

Emerging evidence suggests that spermatozoa are not just vehicles for genome delivery, but that, similarly to somatic cells, sperm chromatin transmits epigenetic information.

However, our understanding of the functional significance of the structural organization of sperm chromatin is far from being complete. Further studies of chromatin organization in spermatozoa are essential for revealing mechanisms involved in early stages of embryogenesis.

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