

Influence of testis-specific proteins on chromosome behaviour during mammalian spermatogenesis

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Abstract

Several new testes-specific nuclear proteins are expressed at different stages of mammalian germ cell development and differentiation. Our laboratory has been interested over the last several years in studying DNA-protein interactions to gain an insight into their possible biological functions. This review summarizes our recent findings.

Key words: Testis-specific proteins, DNA-protein interactions.

1. Introduction

Spermatogenesis is a complex process of differentiation during which the spermatogonial stem cells are transformed into haploid and highly condensed spermatozoa. The chromatin structure of the mammalian germ cells undergoes extensive reorganization at two important steps of spermatogenesis, namely, meiotic division and spermiogenesis. During the meiotic prophase, several testis-specific histones are synthesized which replace the somatic histones while during spermiogenesis, more than 90 per cent of the histones are replaced by highly basic protamines¹. Our laboratory has been engaged over the last several years in understanding the organization of meiotic chromosomes and also the DNA-binding properties of two transition proteins, TP1 and TP2, which are expressed during spermiogenesis. Our major findings of these studies are summarized below.

2. Testis-specific histones

Histone variant forms that are found in rat testes include (i) the germ cell-enriched variants H1a and H2Ax, (ii) germ cell-specific variants H1t, TH2A, TH2B and TH3². Among these variants, H1a, H2Ax and TH3 are present and actively synthesized in spermatogonia while TH2A, TH2B and H1t are actively synthesized in primary

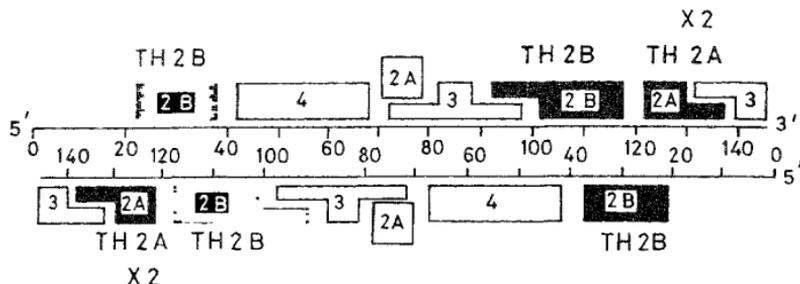


FIG. 1. The organisation of core histones on core particle DNA.

spermatocytes. The levels of TH2A and TH2B are relatively constant throughout meiotic prophase while that of H1t is very low in early primary spermatocytes and reaches maximum levels in late pachytene.

Although the functional importance of these histone variants is not clear, it is generally believed that they facilitate the meiotic events. The major event that takes place during meiotic prophase of the germ cells (pachytene stage) is the pairing of homologous chromosomes and genetic recombination³. Based on the premise that whatever roles these histone variants do have in the meiotic events, they should be mediated through subtle structural alterations at the chromatin level, we have carried out a detailed study of rat pachytene spermatocyte chromatin using biochemical and biophysical techniques. For comparative purposes, liver chromatin containing only the somatic histone subtypes served as control. Our very early studies on the circular dichroic properties of nucleosome core particles indicated that pachytene core particles are less compact than the liver core particles⁴. Studies on the thermal denaturation properties of the two types of core particles revealed that the fraction of the pachytene core DNA melting at the premelting temperature region of 55–60°C was significantly higher than that of the liver-core DNA. Subsequently, we have shown by comparing the DNaseI digestion products of the ³²P 5'-end-labelled pachytene nucleosome core particles that the cleavage sites that are 30, 40 and 110 nucleotides away from the 5'-end are significantly more accessible in the pachytene core particles⁵. These cleavage sites exactly correlate to the region wherein histone H2B interacts with the core DNA as shown in Fig. 1. From these results we have concluded that the histone-DNA interaction at these sites in the pachytene core particles is weaker possibly because of the presence of the histone-variant TH2B interacting at similar topological positions in the nucleosome core as that of its somatic counterpart H2B. By analysing the rate of generation of subnucleosome particles following digestion of native pachytene chromatin with micrococcal nuclease we have obtained further evidence that such loosened structure is maintained not only in isolated nucleosome core particle but also in native pachytene chromatin. According to the present accepted model for the nucleosome core particle, the two H2A-H2B dimers add

on to the two sides of the exposed face of the H3-H4 tetramer, binding to the DNA of the last half turn of the superhelix. Since the two H2B binding sites are 80 nucleotides apart, which actually spans one superhelical turn, it can be visualized that the H2A-H2B dimers stabilize the inner folding of the core DNA induced by histones H3 and H4. Thus, it is quite obvious that the replacement of H2B by TH2B destabilizes this inner folding of core DNA in pachytene chromatin. Since the DNA in the eukaryotic cell is packaged in the form of nucleohistone complex, it is quite likely that such a loosened histone-DNA interaction may facilitate 'disentanglement' of the DNA strand of one of the parental chromosomes to undergo exchange with the DNA strand of the other homologue. Since the replacement of H2B by TH2B is quite substantial ($\approx 80\%$) we have speculated that the entire length of pachytene chromatin is competent to undergo genetic recombination.

3. Histone H1t

Histone H1 is the most divergent histone among all the five histone types. Histone H1 is implicated in the maintenance of chromatin structure at different levels. It is involved in the locking of the chromatosome core particle by interacting with 10 bp each of incoming and outgoing DNA. It is also required for the formation of intermediate higher order structures as well as the compact chromatin fibers. Proteolytic digestion studies and physicochemical studies have defined three structural domains in histone H1 and they are: (1) basic and random-coiled N-terminal tail, (2) a central globular structure involving approximately 80 amino acids, and (3) a long basic and random-coiled C-terminal half. While the globular domain is implicated in the interaction with the chromatosome particle, as well as maintenance of higher order structure, the C-terminal half which is the strong DNA-binding region of histone H1, and the basic N-terminal tail are implicated in the maintenance of higher order structure. Since H1t is expressed only in pachytene spermatocytes, it would be very interesting to see the influence of H1t on chromatin structure. Towards this direction, we carried out a comparative study of the immunological properties of histone H1t in comparison with other histone H1 subtypes⁶. These experiments have shown that polyclonal anti-histone H1t-IgG reacted specifically with histone H1t but not with other histone subtypes H1a, b, c, d, e, and H1^o. The anti-histone H1t-IgG also did not react with chicken erythrocyte H5. Immunoblotting studies also revealed that the epitopes were localized both in the globular domain and the C-terminal fragment. From these results we had concluded that histone H1t is antigenically distinct among all the histone subtypes. Subsequent analysis of the cDNA-derived amino sequence of H1t has revealed that the C-terminal sequence is highly divergent from that of somatic H1s. Our very recent unpublished observations have shown that histone H1t does not condense DNA as effectively as somatic H1s although the arginine content is more in the C-terminal region of H1t.

Several views have been put forward on the possible functions of histone variants during mammalian spermatogenesis. As already mentioned, one of the views has been that they modulate the chromatin structure to facilitate chromosome pairing and genetic recombination. But, as the chromosome pairing is already initiated during

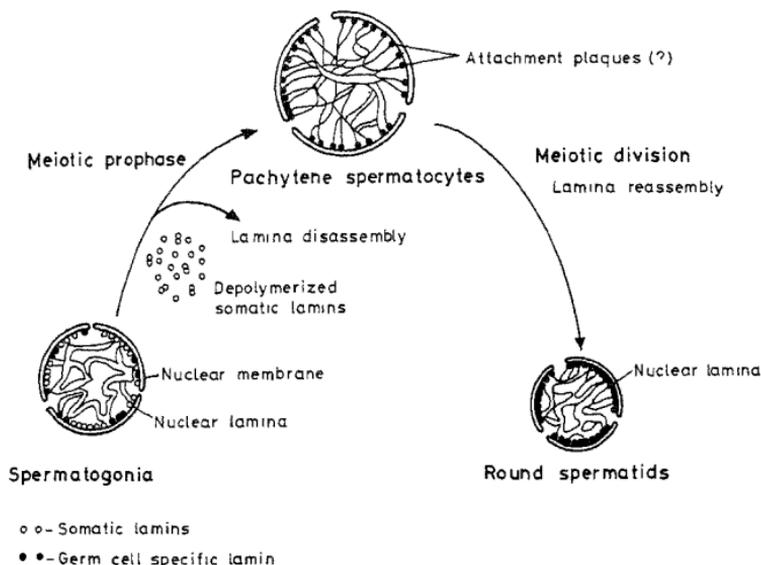


FIG. 2. The fate of nuclear lamina during spermatogenesis of rat.

the leptotene interval and is completed at the zygotene interval, at least the role of H1t and TH2B in the pairing process can be ruled out. However, the possibility still exists that they may be involved in the recombination process. Another view held envisages that they may facilitate the histone replacement process during spermiogenesis. However, it is difficult to visualize this possibility in view of the fact that after recombination the homologous chromosomes undergo condensation and extensive morphological changes during metaphase I and metaphase II of meiotic division.

4. Behaviour of nuclear lamina during spermatogenesis

The nuclear lamina is a supramolecular protein assembly containing 1 to 3 major polypeptides in the molecular weight range of 60 to 80 kDa⁷. One of the most distinct and well-characterized features of the nuclear lamins is their depolymerization before the onset of mitotic and meiotic cell divisions. During the mitotic division, following disassembly of the lamina structure, lamins A and C are dispersed as soluble 4.5S particles while lamin B is found as oligomers of a similar size but associated with membrane vesicles. The status of nuclear lamina during meiosis differs in two respects from the reversible depolymerization seen in mitosis. First, the nuclear lamina disassembles during meiotic prophase without affecting the structure of nuclear

membranes. Second, the proteins of the dissociated lamina are undetectable by immunological methods in pachytene cells. These conclusions were based on experiments using antibodies against somatic lamins^{8,9}.

Recently, we have shown that there exists a germ cell-specific lamin which is expressed in both male and female germ cells¹⁰. This lamin is retained in the pachytene nucleus and is reutilized to form the lamina structure in the haploid round spermatids. Interestingly enough this germ cell-specific lamin is antigenically conserved in the germ cells of all the eukaryotic kingdom¹¹. We have also shown that this germ cell-specific lamin is related but not identical to somatic lamin B. Based on these studies, we have proposed a model for the stage-dependent changes in the localization of this germ cell-specific lamin during spermatogenesis (Fig. 2).

5. Postmeiotic nuclear protein transitions—Involvement of TP1 and TP2

During the later phases of spermiogenesis, the nucleosomal organization of chromatin disappears and is replaced by smooth fibers¹². Condensation of chromatin and loss of transcriptional activity are the main biochemical events that occur during this period. The replacement of histones by protamines during mammalian spermatogenesis is brought about in two steps. In the stages between 9 and 12, the histones are replaced by testis-specific nuclear proteins (transition proteins). The transition proteins and residual histones are further replaced by arginine- and cysteine-rich proteins, called protamines.

Transition proteins (TP1, TP2 and TP3) are a group of small molecular weight basic proteins. They have a molecular weight of less than 20 kDa. TP1 is the smallest of these and its amino acid and cDNA sequences have been determined¹³. Rat TP1 has 54 amino acids and contains 50 mole per cent of arginine and lysine. Its mRNA appears postmeiotically and is inactive for 3–4 days before it is translated. By studying the *in vitro* DNA-binding properties of TP1, Singh and Rao¹⁴ have inferred the possible functions of the protein. Applying a variety of physicochemical techniques such as fluorescence quenching, uv absorption and thermal melting, to study the nature of protein–DNA interactions, it was inferred that TP1 has DNA melting property which is probably mediated through the two tyrosine residues both of which are flanked by basic amino acids. By employing circular dichroism studies it was indeed shown that TP1 causes destabilization of nucleosome-core particle DNA. Because of its random and extended conformation and also a uniform distribution of positively charged amino acids with an overall hydrophilic character, TP1 has been implicated to interact all along the length of the DNA occupying the minor groove of the double helix. Based on these observations, we have speculated that TP1 may be involved in aiding the displacement of the histones from nucleosomes¹⁵.

The second transition protein TP2 of 13 kDa was initially thought to be a variant of histone H4. The cDNA-derived amino acid sequence of mouse TP2 reveals two in-phase initiation codons both of which may be used in generating two polypeptides which differ in length at the amino terminus¹⁶. This probably explains the migration of TP2 as a doublet in acid-urea gels. The cDNA-derived amino acid sequence of

6. Meiotic recombination in pachytene spermatocytes

As mentioned earlier, the most significant molecular event that takes place in pachytene spermatocytes is genetic recombination. It is known that specific nicks are introduced in the pachytene DNA. It is generally believed that these sites might be related to initiation sites of recombination. Our laboratory has been currently engaged in characterizing these nicked and repaired sites. Furthermore, we are characterizing the enzymatic machinery involved in the recombination process.

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