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Review

Chromatin dynamics during spermiogenesis ☆,☆☆

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ABSTRACT

The function of sperm is to safely transport the haploid paternal genome to the egg containing the maternal genome. The subsequent fertilization leads to transmission of a new unique diploid genome to the next generation. Before the sperm can set out on its adventurous journey, remarkable arrangements need to be made during the post-meiotic stages of spermatogenesis. Haploid spermatids undergo extensive morphological changes, including a striking reorganization and compaction of their chromatin. Thereby, the nucleosomal, histone-based structure is nearly completely substituted by a protamine-based structure. This replacement is likely facilitated by incorporation of histone variants, post-translational histone modifications, chromatin-remodeling complexes, as well as transient DNA strand breaks. The consequences of mutations have revealed that a protamine-based chromatin is essential for fertility in mice but not in *Drosophila*. Nevertheless, loss of protamines in *Drosophila* increases the sensitivity to X-rays and thus supports the hypothesis that protamines are necessary to protect the paternal genome. Pharmaceutical approaches have provided the first mechanistic insights and have shown that hyperacetylation of histones just before their displacement is vital for progress in chromatin reorganization but is clearly not the sole inducer. In this review, we highlight the current knowledge on post-meiotic chromatin reorganization and reveal for the first time intriguing parallels in this process in *Drosophila* and mammals. We conclude with a model that illustrates the possible mechanisms that lead from a histone-based chromatin to a mainly protamine-based structure during spermatid differentiation. This article is part of a Special Issue entitled: Chromatin and epigenetic regulation of animal development.

1. Introduction

Germ cells mediate the transfer of genetic information from generation to generation and are thus pivotal for maintenance of life. Spermatogenesis is a continuous and precisely controlled process that leads to the formation of haploid sperm capable of fertilization.

The process of spermatogenesis is highly conserved among many organisms and can be subdivided into three crucial phases: a mitotic amplification phase, a meiotic phase, and a post-meiotic phase also known as spermiogenesis (Fig. 1A). The meiotic phase ensures haploidization of the genome as well as an independent assortment of genetic information within individual germ cells. Germ cells in the post-meiotic phase can be subdivided into early spermatids with round nuclei, intermediate spermatids with elongating nuclei, and spermatids with condensed nuclei [1]. In many organisms (e.g., humans, mice, *Drosophila*), male germ cells undergo a series of morphological transformations during spermiogenesis to build a sperm with its typical species-specific shape from an initially round cell [2–6]. A common feature of spermatogenesis is that transcription stops at a defined point during germ cell differentiation. Thus, translational repression and storage of mRNAs, such as those encoding protamines, are crucial for completion of spermiogenesis [7,8] (Fig. 1A). Within the scope of this review, we will mainly focus on chromatin reconstruction during spermiogenesis.

Already in the last third of the 19th century, Miescher and Kossel described salt-like linkages of nucleic acid with two different complexing substances: protamines and histones [9–12]. In addition, they identified histones as the major chromatin proteins in somatic cells, and either histones (e.g., in carp) or protamines (e.g., in salmon) as the chromatin proteins of sperm cells [9–12]. We now know that in many organisms spermiogenesis is accompanied by a dramatic reorganization of chromatin from a nucleosomal histone-based structure to a structure largely based on protamines [1,7,13–15]. The replacement of histones by protamines is gradual [16]. First, some of the canonical histones are replaced by testis-specific histone variants. Subsequently, so-called transition proteins are incorporated as nucleosomes are removed, and finally,

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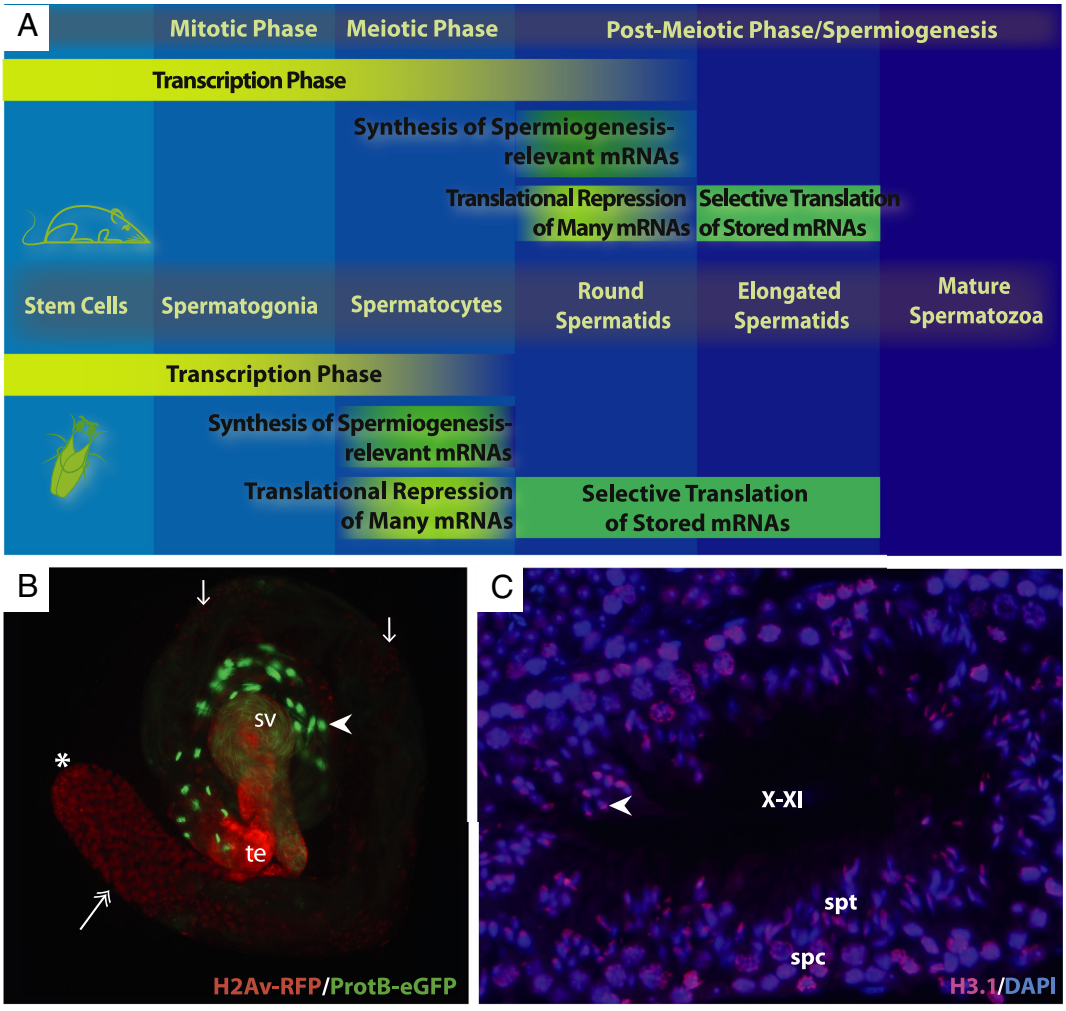

Fig. 1. Comparison of spermatogenesis in mice and flies. (A) Schematic drawing of the different stages of spermatogenesis. Spermatogenesis is characterized by a mitotic proliferation phase, a meiotic phase, and a post-meiotic phase known as spermiogenesis. In mice, global transcription in male germ cells is repressed by the time round spermatids start to elongate. In *Drosophila*, hardly any gene activity is detectable during spermiogenesis. Thus, post-meiotic spermatid differentiation is programmed by translationally repressed mRNAs synthesized in spermatocytes (in flies) or in round spermatids (in mice). During spermiogenesis, translationally repressed mRNAs are gradually released and spermatid differentiation-relevant proteins are synthesized. (B) Testis of a double transgenic fly that expresses the fusion proteins H2Av-RFP and ProtB-eGFP. The asterisk marks the tip of the testis tube, where stem cells localize. Expression of H2Av-RFP is visible in mitotic spermatogonia and meiotic spermatocytes (double arrow) up to early post-meiotic spermatids (arrows). During spermiogenesis, H2Av-RFP vanishes and ProtB-eGFP becomes detectable (arrowhead). Somatic cells of the terminal epithelia (te) and the seminal vesicle (sv) also express H2Av-RFP. (C) Cross-section of a mouse seminiferous tubule at stages X–XI, stained with DAPI (blue) and with an antibody that recognizes histone H3.1 (red). All cells except condensed spermatids express H3.1. Spermatids in the process of removing the histones are partially positive for H3.1 (arrowhead). The areas containing spermatocytes (spc) and elongating and condensing spermatids (spt) are indicated.

protamines generate the tightly packaged sperm nucleus [17,18]. In both flies and mammals, specific histone modifications and transient formation of DNA breaks precede or accompany protamine deposition [19–24]. Over the past two decades, many chromatin components that specifically function during spermiogenesis have been described. However, the exact regulatory cascade of events of chromatin reorganization in developing sperm as well as the reason why sperm possess such an unusual chromatin composition are still poorly understood.

Here, we highlight the current knowledge on mammalian and *Drosophila* spermiogenesis. In Section 2 we provide an overview of the histone-to-protamine transition. In Section 3, we briefly discuss the potential function of protamines. Sections 4–6 discuss the different chromatin components of spermatids during post-meiotic chromatin remodeling in more detail. We then present current mechanistic insights into this process in Sections 7 and 8. In Sections 9 and 10, we briefly discuss the possible relevance of residual histones within sperm nuclei as well as protamine removal after fertilization. We conclude with a model that integrates the current knowledge and highlight the questions to be addressed in future research.

2. From histones to protamines — an overview

The process of spermatogenesis in mammals and in *Drosophila* species is similar (Fig. 1A), although the size and shape of mature sperm as well as the time span for spermatogenesis differ considerably (Fig. 2) [25]. The testes of *Drosophila* and all mammalian species contain all stages of spermatogenesis, from stem cells to mature sperm, and spermatogenesis occurs within a tubular structure (Fig. 1B, C). Germ cells develop in close contact with the surrounding somatic cells, known as cyst cells in *Drosophila* and Sertoli cells in mammals. In *Drosophila*, germ cells move from the apical tip to the basal end of the tubule during differentiation, while in mammals, germ cells move from the periphery to the lumen, followed by transport to the epididymis, a long tubule that stores the mature sperm [3,26]. Thus, in *Drosophila*, the process of spermatogenesis can be followed from proximal to distal along a single testis tubule (Fig. 1B), whereas in mammalian testis, many tubules are present and specific associations of cell types can be found together in a single cross-section (Fig. 1C). In mammals, the number of stages that can be observed varies among species [26]. For a general overview on fly

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