SPERMATOGENESIS AND CHROMATIN ORGANIZATION IN
THE MALE GERM CELLS OF RANA TIGERINA

SIRIKUL MANOCHANTR

With compliments
of

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The aims of this investigation are: (1) to classify the male germ cells in R. tigerina and study their chromatin organization and condensation by light and electron microscopy, chromatin decondensation, and digestion with micrococcal nuclease; (2) to study the basic nuclear protein profile in these germ cells by acid/urea/triton X gel electrophoresis; (3) to determine the sequence of changes of basic nuclear proteins by immunoelectron microscopy.

R. tigerina testis is composed of numerous seminiferous tubules surrounded by basement membranes. Each tubule contains various stages of developing male germ cells within a spermatocyst surrounded by processes of follicular cells. Cells in each cyst may be derived from a single spermatogonium. Male germ cells of R. tigerina, as studied by light and transmission electron microscopy, can be classified into 14 stages based on the pattern and degree of chromatin condensation. There are two stages of spermatogonia (Sg1, Sg2) containing large spherical nucleus with mostly euchromatin (Sg1), and increasing number of small heterochromatin blocks (Sg2). Primary spermatocytes are divided into 6 stages. Leptotene spermatocyte (LSc) contains fine chromatin threads about 30 nm that start to fold around a single electron dense line, the condensation axis, into loosely packed chromatin blocks. Heterochromatin blocks increase in length and thickness in zygotene spermatocyte (ZSc) which are joined together by synaptonemal complexes. Pachytene spermatocyte (PSc) shows long and thick intertwined heterochromatin blocks or cords. These chromatin blocks are distributed in a cartwheel pattern in diploctene spermatocyte (DSc). Long and large heterochromatin blocks are separating from each other during diakinesis stage and become aligned along the equatorial region in metaphase spermatocyte (MSc). Throughout the transformation of spermatogonia to primary spermatocytes, 30 nm chromatin fibers become increasing condensed into heterochromatin blocks of various sizes, while 10 nm fibers are decreasing in quantity until absent entirely in metaphase spermatocyte. Secondary spermatocyte has a nucleus that contains 4-6 large blocks of heterochromatin along the inner face of the nuclear envelope, whose 30 nm fibers are loosened up and 10 nm fibers start to reappear. Spermatids can be divided into 4 stages. During the transition of secondary spermatocyte to spermatid I (St1) the dense chromosomes are reorganized into evenly distributed 30 and 10 nm fibers. Thereafter, chromatin fibers (30 nm) are packed together and become increasingly condensed in spermatid II (S12), while its nucleus is decreased in size and becomes oval shape. In spermatid III (St3), the nucleus becomes elongated and its contains 30 nm chromatin fibers which are aggregated more tightly and evenly together, while 10 nm fibers disappear. In spermatid IV (St4), the nucleus becomes highly elongated into cylindrical shape, with a small acrosome covering the anterior pole; and the chromatin is highly condensed but the outline of 30 nm fibers could still be observed. The spermatids (S2) had elongated cylindrical shape head that contains completely electron opaque chromatin. The head is covered by a small acrosome. The midpiece is composed of centriolar complex surrounded by striated cylindrical fibrous sheath and non-helical mitochondrial sheath. The rest of the tail consists of only the axoneme complex surrounded by plasma membrane. The condensation of chromosomes in Rana spermatid is, therefore, similar to the process of heterochromatization in somatic cells, where 30 nm fibers are coalesced together without changing the initial size and nucleosomal organization. This conclusion is supported by the finding that the full set of core histones (H2A, H2B, H3, H4) are still present in sperm chromatin, but with H1 variants replacing H1. Rabbit anti sera were raised against histone H3, H1, H1V, and H5. Anti-histone H1 antiserum cross-reacts with histone H1V which may have a common epitope with H1. Anti-histone H1V and H5 also show cross-reaction with each other but not with histone H1 which may be due to the presence of common epitope not shared by histone H1. Immunolocalization of histones in LR White-embedded testis sections indicated that histone H3, H1 are present in all stages of male germ cells including Sertoli cells, Leydig cells and red blood cells. Histone H1V and H5 are detected in spermatids (St1-St4) and spermatid whose chromatin are undergoing increasing condensation. They are also detected in metaphase spermatocytes and red blood cells. Thus the nucleosomal organization of chromatin persists until spermatid and the complete condensation may be brought about by H1V. Both micrococcal nuclease digestion and electron microscopy of sperm chromatin decondensed by egg cytoplasmic extract and distilled water also provided further supporting evidence that sperm chromatin consists of nucleosomal organization. Chromatin condensation in spermatid probably use a similar mechanism as occurring in the process of heterochromatization in fully differentiated somatic cells, such as, in frog and chick erythrocytes, which H5 (another form of H1 variant) initiates the final and complete condensation of 30 nm nucleosomal type fibers.