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Chromatin and chromosomal fine structure in spermatogenesis of some species of amphibians

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Summary

Spermatogenesis is a complex differentiation process which is characterised, among other features, by conspicuous stage-specific nuclear events such as the pairing of homologous chromosomes coupled with the formation of synaptonemal complexes, the replacement of histones with sperm-specific proteins during spermiogenesis and, as a result, chromatin condensation and its inactivation in sperm cells. The chromatin of spermatogenic cells undergoes dramatic conformational changes upon differentiation from spermatogonia to mature spermatozoa. During the haploid stage of spermatogenesis, histones are gradually replaced, firstly by transition proteins and later by sperm-specific proteins. As a result of the high degree of condensation and inactivation of spermatid and sperm chromatin, Sertoli cells are responsible for the nourishment of germ cells with ribosomal RNA and nutritive substances.

Keywords: Nuclear sperm-specific proteins, Polytenisation, Sertoli cells, Spermatogenesis, Synaptonemal complex

Introduction

The study of nuclear molecular architecture and chromosomal phenotype during gametogenesis represents one approach towards deciphering the molecular organisation of the eukaryotic chromosome.

Spermatozoa are among the most highly specialised cell types. Such specialisation is designed to allow the spermatozoon to reach the egg and to fuse with it. The testes are very efficient 'sperm factories', which produce vast numbers of these elaborate cells.

Spermatogenesis is a process of division and differentiation by which spermatozoa are produced in seminiferous tubules. Seminiferous tubules are composed of somatic cells (myoid cells and Sertoli cells) and germ cells (spermatogonia, spermatocytes and spermatids). Considering the activity of these three types of germ cells, spermatogenesis can be divided into spermatocytogenesis, meiosis and spermiogenesis, respectively. Spermatocytogenesis involves mitotic cell divisions, which lead to an increase in the yield of spermatogonia associated with production of stem cells and primary spermatocytes. Meiosis is performed in two steps (meiosis I and meiosis II), ensuring genetic recombination (independent disjunction of the chromosomal pairs and crossing-over) as well as the reduction of chromosome number from 2n to n and DNA quantity from 4C to 2C in the first meiotic division, and from 2C to 1C in the second meiotic division, with the generation of four haploid cells (n) with 1C DNA. Spermiogenesis is a complex process in which spherical spermatids become mature spermatids, which are released at the luminal free surface as spermatozoa.

During spermatogenesis, chromatin undergoes several dynamic transitions, which are often associated with important changes not only in its physical conformation but even in its composition and structure. In most instances, the composition of the chromosomal proteins at the onset and in the final stages of spermatogenesis is quite different. These compositional changes also alter the structure of chromatin. As a result, chromatin becomes highly compacted and gene expression is completely shut down in the spermatozoon – a situation that is totally different from that encountered in oocytes, in which intense synthetic processes such as extra replication, transcription and RNP synthesis take place. The ways in which all these molecular events are achieved can be mediated by a wide spectrum of apparently diverse chromosomal proteins (Bloch, 1969), which are mirrored by the morphological metamorphoses which accompany the

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formation of the mature sperm cell. The structure of chromatin conformation as a result of protein–DNA interactions in each particular situation is poorly understood in most instances, and the evolutionary relationships amongst these proteins remain obscure (Ausio, 1995).

In a wide range of vertebrate organisms, the DNA within the sperm nucleus is organised into a genuine three-dimensional conformation due to replacement of histones with another class of nuclear proteins, namely nuclear sperm-specific proteins. During the last 10 years, important progress has been made in the chemical and physical characterisation of these proteins, in a wide range of organisms. The data emerging from these studies indicate that the nuclear sperm-specific proteins are not as heterogeneous as originally thought. Indeed, from the compositional point of view, they can be arranged in a discrete number of basic types: H type (histone); P type (protamine); and PL type (protamine-like), consisting of proteins with an intermediate composition between histones and protamines (Bloch, 1969).

Comparing the nucleosomal binding pattern of somatic histones with the binding pattern of nuclear sperm-specific proteins (that are localised in the minor groove of the DNA double helix) one can notice that nuclear sperm-specific proteins ensure an almost complete covering of the DNA, with very few uncovered areas. Thus, in contrast to the somatic cell nucleus, in the sperm nucleus the nucleosomal structure as well as the negative superhelicity are lost, the nucleoprotein complex acquiring a special physical conformation with very important functional consequences (Nishi *et al*., 1994).

Our research was focused on chromatin conformational dynamics during spermatogenesis in *Triturus* and *Xenopus*, evidenced by transmission electron microscopy (TEM).

Materials and methods

Male spermatogenesis in *Triturus cristatus* and *Xenopus laevis* was studied. Testes were dissected and fixed in 2.5–5% glutaraldehyde in 0.1 M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide, in the same buffer. Samples were dehydrated in gradually reducing concentrations of an ethyl alcohol series and embedded in Epon 812. They were then sectioned using an ultarmicrotome, stained in 4% aqueous uranyl acetate, post-stained with lead citrate and examined with a Philips 201 electron microscope.

Results and discussion

Using TEM analysis, some unusual aspects of chromatin and chromosome organisation and evolution, including synaptonemal complexes in spermatogenesis of the amphibians *Triturus cristatus* and *Xenopus laevis*, were evidenced.

The relationship between the Sertoli nourishing cell and spermatids, which becomes spermatozoa, is particularly interesting. The Sertoli cell appears as a highly differentiated cell, with a hierarchically structured chromatin architecture in which several condensed chromatin levels can be noticed: decondensed chromatin; chromatin with an intermediate level of condensation, randomly distributed within the nucleus: and hypercondensed chromatin located on the internal membrane of the nuclear envelope (Fig. 1). The Sertoli cell chromatin architecture thus reveals its functional specialisation in gene process regulation through its condensation/decondensation.

The connections between Sertoli cells and spermatids are sometimes obvious. Through some cytoplasmic bridges, the synthesised substances from the Sertoli cell are transferred to spermatids. Such substances are involved in the differentiation of spermatids into spermatozoa.

The structural aspects of Sertoli cells reveal that these cells have a high metabolic activity, expressed as nucleolus hypertrophy; the existence of fibrillar structures which might represent genetic transcripts is frequently encountered.

The nucleosomal structure of chromatin fibrils in Sertoli cells, as well as transcript storage on the loop structure of the chromatin fibrils, can easily be evidenced in TEM analyses (Fig. 2).

It is possible that Sertoli cells achieve high polyploidy levels, the extra cycles of replication being followed by polytenisation. In the nucleus, the organisation of chromatin in a series of darkly stained transverse bands which alternate with interband (lightly stained) regions could occasionally be evidenced (Fig. 3).

In the successive stages from spermatogonia to spermatozoa, we have deciphered the fine-structural elements of cytoplasm and nuclear components. The chromatids at the level of spermatids and spermatozoa achieve the highest condensation state of chromatin encountered in nature, which represents an adaptive process enabling spermatozoa to fulfil their function of transporting genetic material (Figs. 4–6). Chromatin condensation helps to streamline the spermatozoon by reducing volume. It also serves a protective function, reducing the susceptibility of the DNA to mutation or physical damage.

Condensation is facilitated by the formation of specific DNA–protein complexes. Proteins that may be involved include protamines (small, highly basic, argi-

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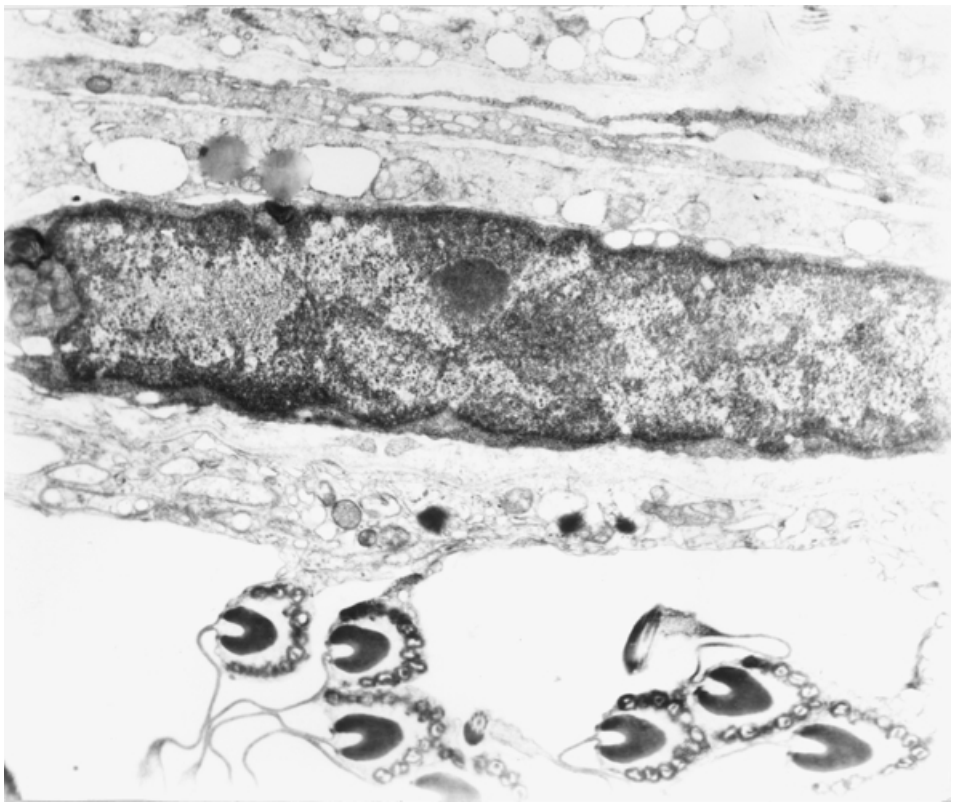

Figure 1 *Triturus cristatus.* The structural relationship between the Sertoli nourishing cells and spermatids. The Sertoli cell appears as a highly differentiated cell, with a hierarchically structured chromatin architecture. Under the Sertoli cell are two spermatids directly connected to its cytoplasm via cytoplasmic bridges (× 23 214).

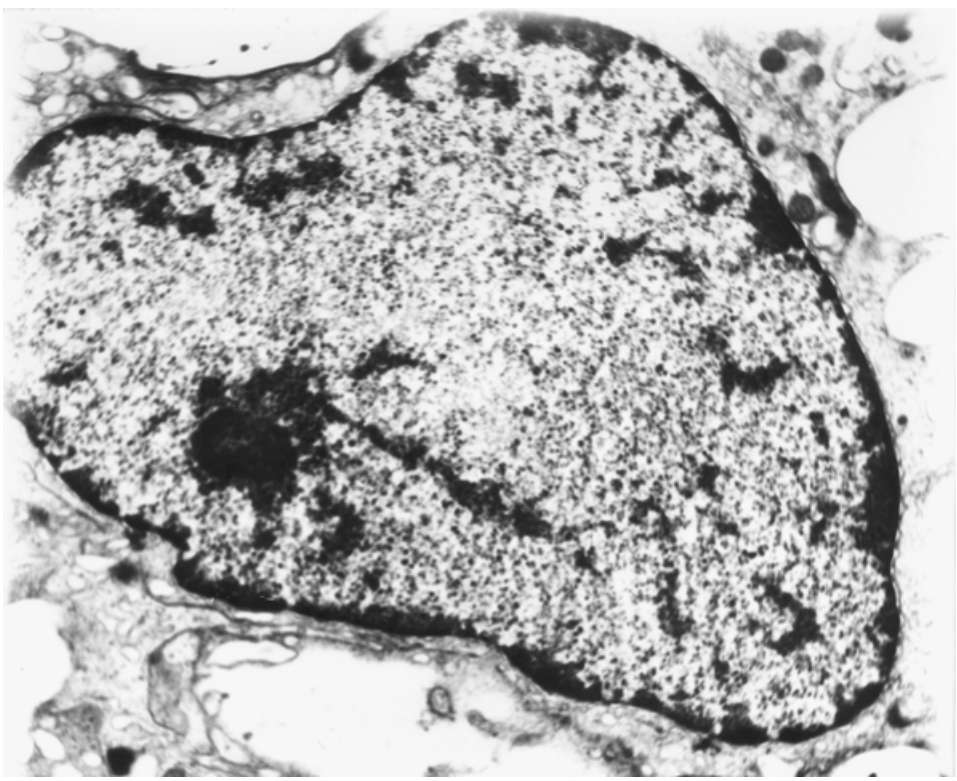

Figure 2 *Triturus cristatus.* Nucleosomal structure of chromatin fibres in Sertoli cells (× 11 872).

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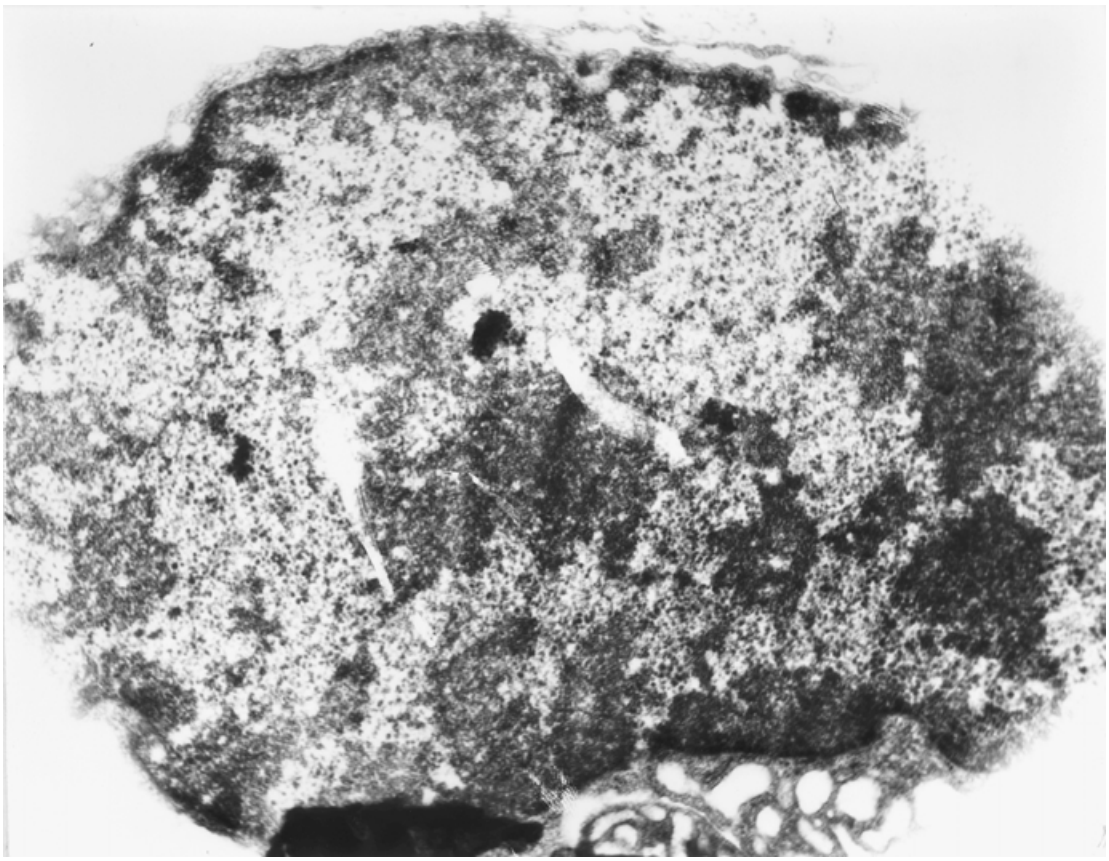

Figure 3 Sertoli cell nucleus. Chromatin is organised in a series of darkly stained transverse bands which alternate with interband (lightly stained) regions, a peculiar feature of polytenic chromosomes (× 16 960).

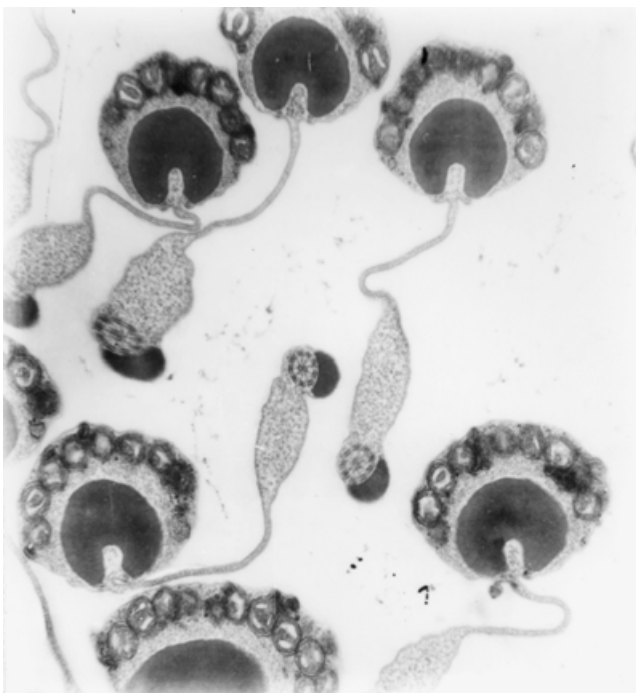

Figure 4 *Triturus cristatus.* Transverse sections through a sperm head showing urodele features of a longitudinal juxtaaxonemal fibre, a long undulating membrane, and mitochondria (× 23 214).

nine-rich proteins), protamine-like proteins or other sperm-specific proteins. The replacement of histones with sperm-specific proteins may be gradual, involving discrete steps in which transitional proteins interact with the DNA after the histones are removed and before the protamines or protamine-like proteins are added. In salmonid fishes, somatic histones become hyperacetylated or undergo similar modifications that reduce histone binding to DNA. In mammals, somatic histones are removed by protease digestion. Chromatin remodelling in mammals is a two-step process. In the first step, the histones are replaced by small, highly basic transition proteins. This process leads to elimination of the nucleosomal structure of chromatin and stops transcription. The replacement of the transition proteins by protamine stabilises and further compacts the chromatin via the formation of disulphide cross-bridges. The replacement of histones by sperm-specific proteins results in transcription blockage. This aspect has important consequences for gene expression during spermiogenesis. It means that all molecular events after this point must rely upon post-transcriptional processes only (Hecht, 1995).

How, then, are proteins synthesised that are needed to complete spermiogenesis? For example, the synthesis of sperm-specific proteins occurs after transcription

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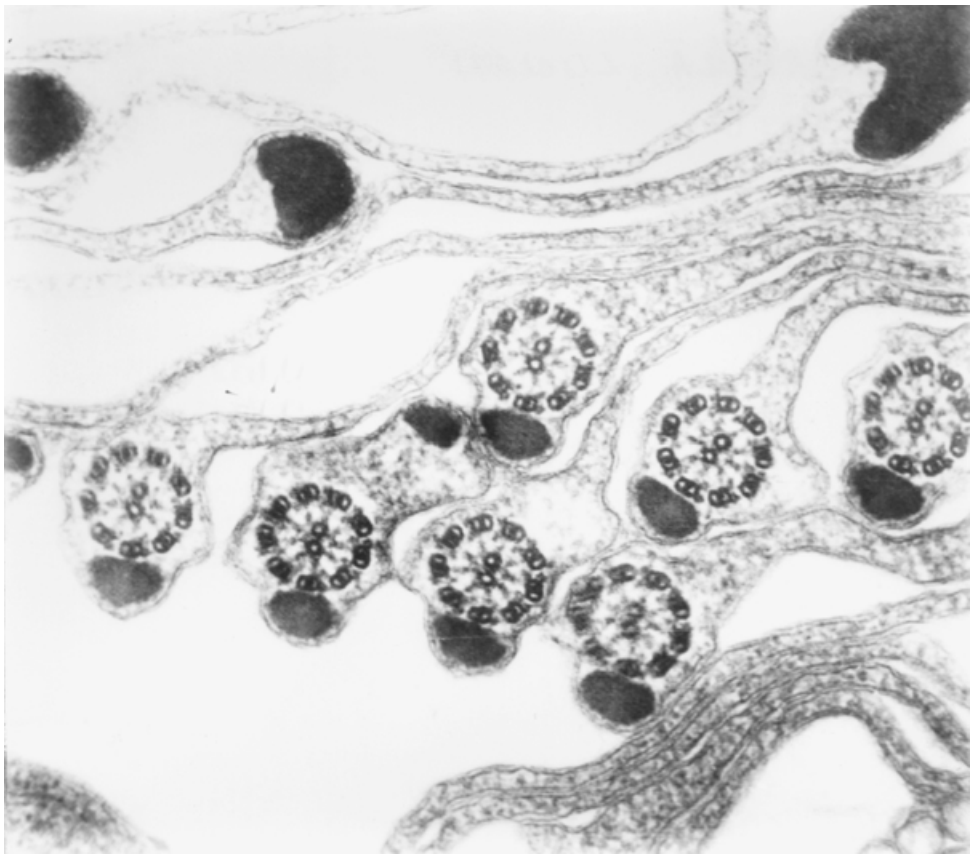

Figure 5 *Triturus cristatus.* Detail of a sperm tail axoneme (× 73 670).

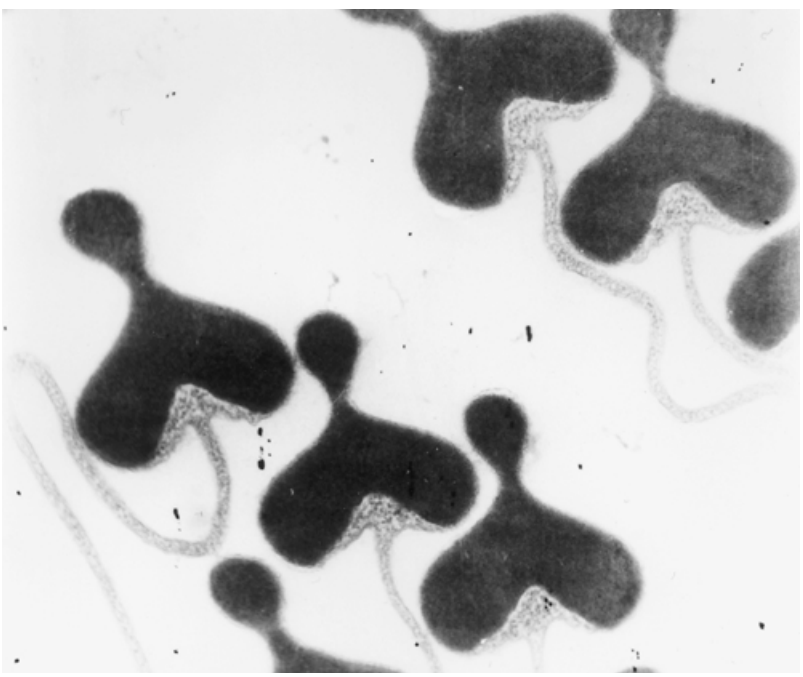

Figure 6 *Triturus cristatus.* Transverse sections through sperm tails showing the urodele feature of an axial major fibre (× 23 214).

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has been terminated by replacement of somatic histones with transcriptional proteins. The sperm-specific proteins are synthesised on transcripts that were themselves synthesised before the loss of somatic histones. These transcripts are stored in the cytoplasm of developing spermatids (Hecht, 1995).

How are they maintained in a repressed state and subsequently de-repressed and translated? The 3′ untranslated regions (UTRs) of sperm-specific messengers appear to determine when they are translated (Hecht, 1995). Translational regulation mediated by 3′UTR sequences is a common mechanism of translational control. Another mechanism that regulates translation during spermiogenesis may function by controlling the level of polyadenylation of mRNA. Several of these transcripts are stored with poly(A) tails of about 160 nucleotides and become partially deadenylated to about 30 nucleotides when they are translated. Polyadenylation is a common strategy used in translational regulation during development (Browder *et al*., 1991).

In the cells formed at the end of the second division, we could see intermediate stages of chromatin condensation in *Xenopus laevis*, preparing such meiotic products to become spermatids (Fig. 7).

The type of sperm-specific protein in *Xenopus* is a protamine-like (PL) protein (Bloch, 1969). PL proteins usually coexist with a small quantity of histones in the sperm nucleus (Avramova *et al*., 1984; Ausio, 1988; Gatewood, 1987). The structure of the nucleoprotein complexes arising from the interaction of these proteins with DNA lacks the nucleosomal organisation of the somatic chromatin type, as can be visualised by the X-ray diffraction analysis of such complexes (Ausio & Subirana, 1982; Ausio & Suan, 1983). The overall negative superhelicity of DNA is lost, most likely as a result of the topoisomerase II activity associated with the histone displacement/replacement by these PL proteins. Thus, the nucleohistone-nucleoprotamine-like transition leads to a complete reorganisation of chromatin, while maintaining the specific three-dimensional organisation of DNA as well as its DNA loop domain structure (McPherson & Longo, 1993).

The detailed molecular structures of the nucleoprotein (protamine (P) and protamine-like (PL) complexes) are still controversial. Both PL and P proteins interact electrostatically with DNA (which basically retains a B conformation) to form fully saturated complexes, unlike the somatic nucleohistone (Ando *et al*., 1973; Ausio & Suan, 1983; Ausio & Van Holde, 1987). In

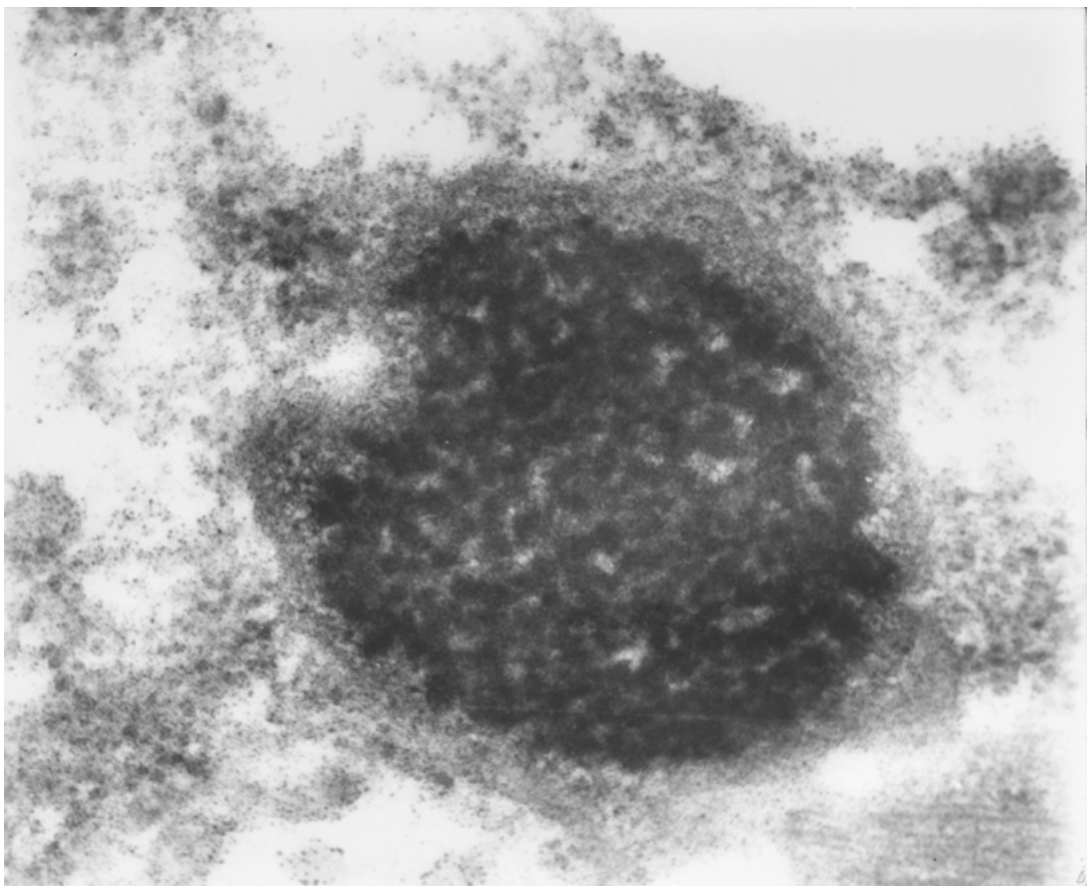

Figure 7 Intermediate stages of chromatin condensation in the transition from telophase II to spermatid, in *Xenopus laevis* (× 74 370).

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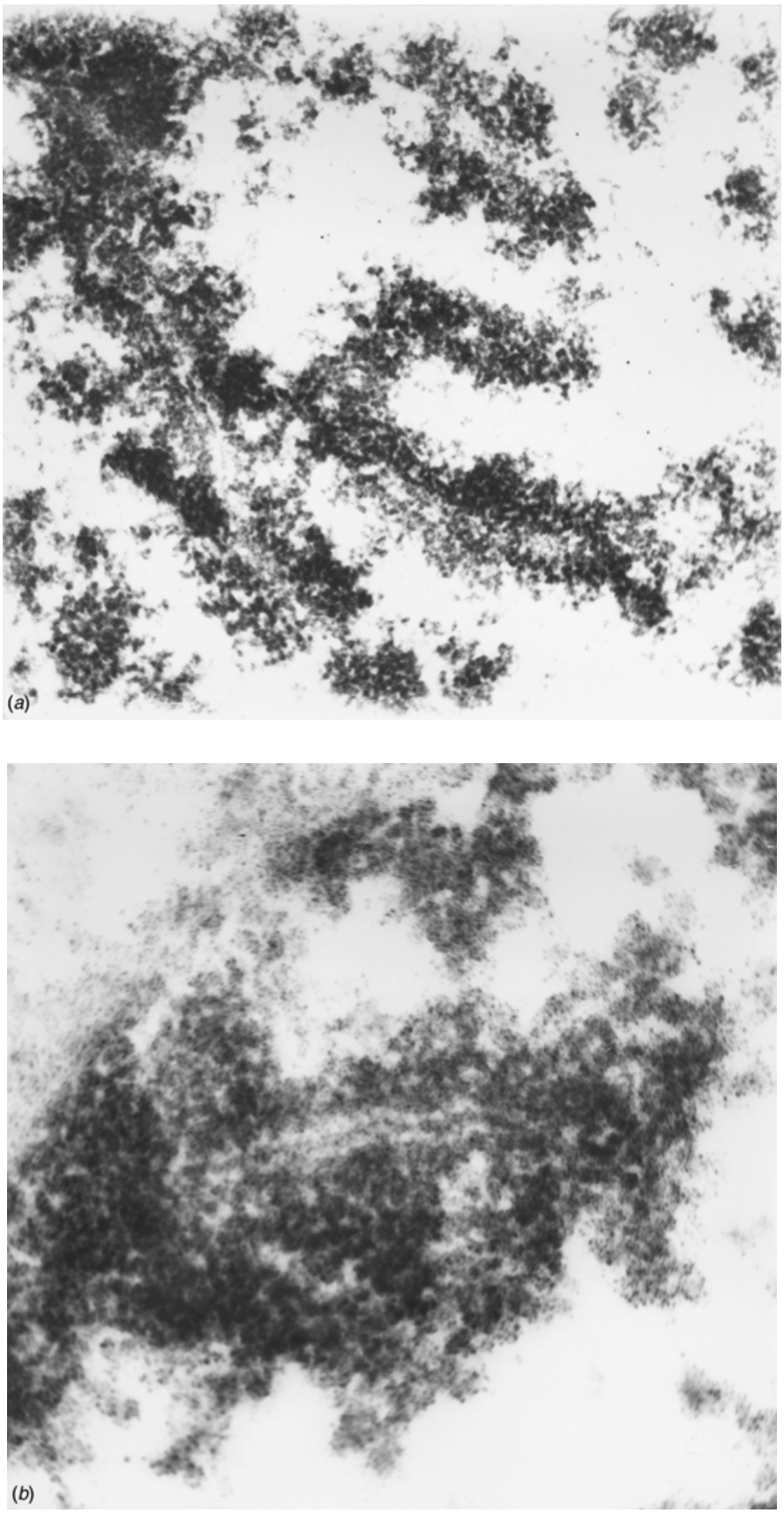

Figure 8 (*a*), (*b*) Spermatocyte nucleus of *Xenopus laevis*: bivalents with homologus chromosomes held together by synaptonemal complexes (*a*, × 49 764; *b*, × 99 160).

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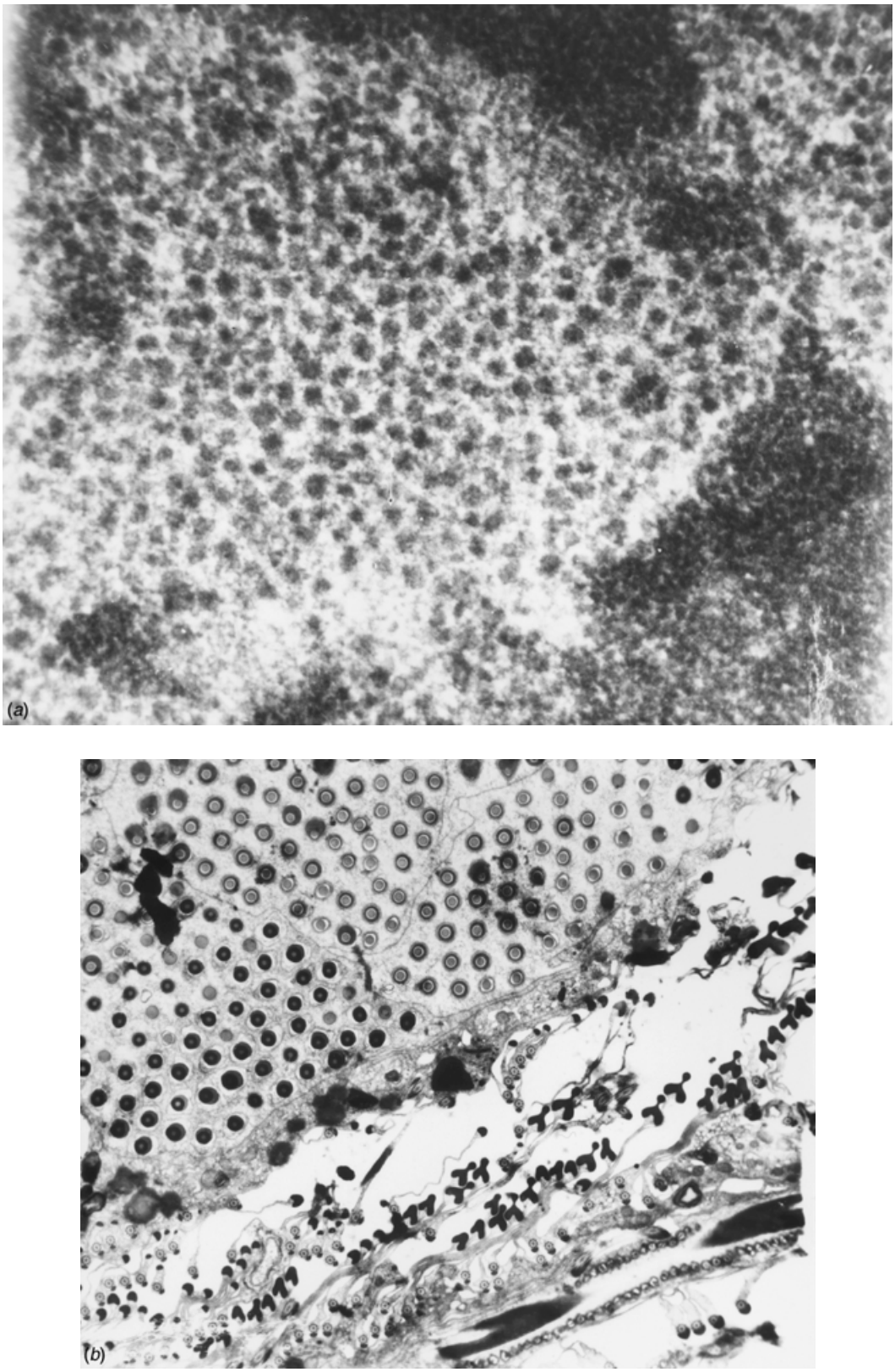

Figure 9 (*a*)–(*c*) Transverse sections through *Triturus cristatus* testes. Developmental stages of some presumed endoparasites, with a specific location in the testes, and with an ordered structure, like a paracrystalline network (*a*, × 73 670; *b*, × 7879; *c*, × 73 670).

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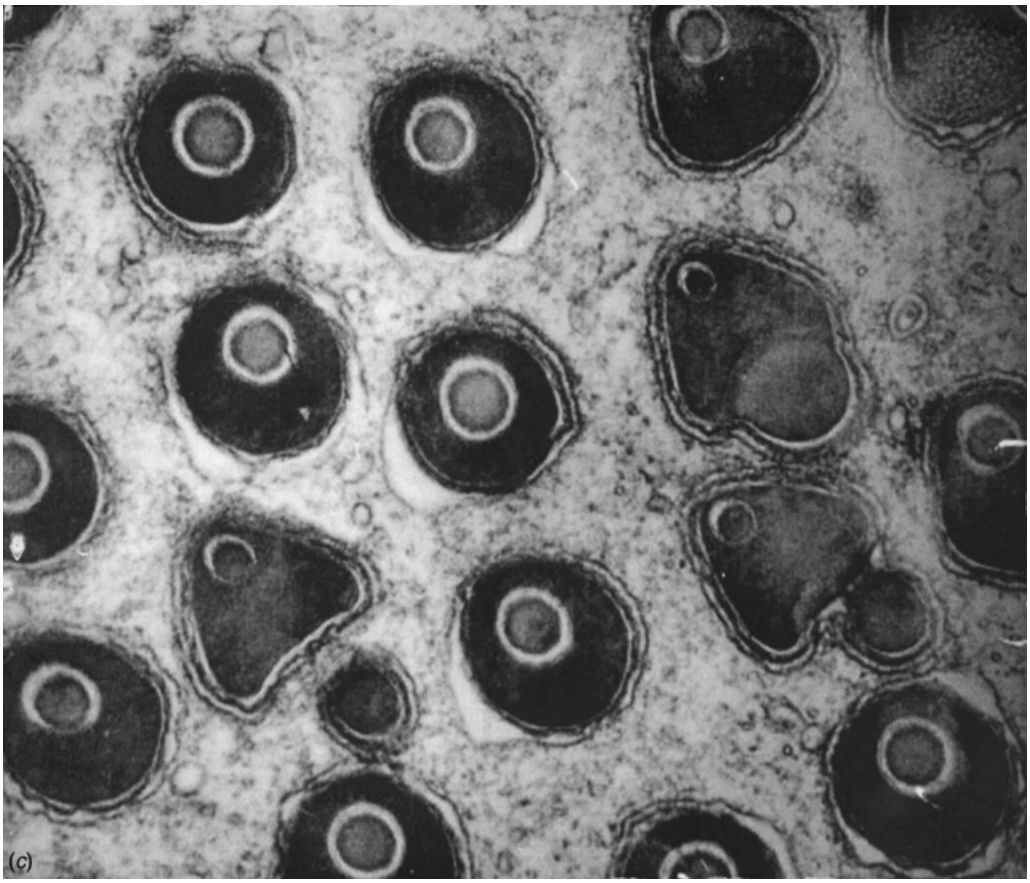

Figure 9 *Continued*

these complexes, the PL and P proteins have been postulated to adopt an α-helical like configuration (Nishi *et al*., 1994).

At the higher level of chromatin conformational organisation, it has recently been shown (Casas *et al*., 1993) that PL proteins, like H type proteins, can organise the nucleoprotein complexes into 250–500 Å fibres, regardless of the particular PL composition and the absence of nucleosome-like structures. This is an important finding, because it indicates that the higher-order structures of the nucleoprotein complexes are mainly determined by the ionic nature of interaction involved (Gatewood *et al*., 1987), rather than the particular structure of the proteins themselves.

In many sections through spermatocyte nuclei, the presence of bivalents consisting of homologous chromosomes joined by synaptonemal complexes could be evidenced (Fig. 8). Fine structural analysis of synaptonemal complexes reveals a 'classical' appearance of these *ad hoc* meiotic structures, exhibiting two lateral elements and the central element. Our investigations revealed some puzzling features in *Triturus cristatus* testes that were represented by an ordered structure, like a paracrystalline network (Fig. 9*a*, *b*), as well as peculiar spherical structure with a lightly electron-dense area in the centre, bounded by a white circle (halo) and a cortical area with a highly electron-dense appearance inside a porous structure, the whole being enclosed in a multilayered membranous envelope (Fig. 9*c*). At present we can only speculate that these structures are the developmental stages of an endoparasite, with a specific location in the testes, since to our knowledge they have not previously been described.

Conclusions

The germinal cells show a peculiar architecture of genetic material, with functional implications. The major events in nuclear modification in sperm cells are chromatin condensation and morphological changes.

Gene amplification occurs in both oogenesis and spermatogenesis, the difference consisting in the ribosomal gene amplification mechanism. In male germinal cells, the Sertoli cells are responsible for the nourishment of germ cells with ribosomal RNA and nutritive substances, because the nucleus of the male germ cells is very condensed and, as a result, inactive. In the Sertoli cell nucleus the ribosomal gene amplification process takes place by polytenisation, such cells being afterwards directed towards self-destruction (apoptosis). Polytene chromosomes are transient,

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irreversible genetic functional structures, and they were found in Sertoli cell nuclei. On the other hand, ribosomal gene amplification in oogenesis is performed in cells with genetic continuity, while the bivalents are in a lampbrush phenotype state, through a mechanism of rDNA extra replication.

The syncytial pattern of spermatids and spermatozoa and the cytoplasmic bridges between germ cells and Sertoli cells allow each germ cell to be supplied with all the products required for its growth and cell differentiation.

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