NONKINETOCHORE ASSOCIATION OF CHROMATIN AND MICROTUBULES

A Preliminary Note

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INTRODUCTION

During the process of gametogenesis the developing sperm cells of many species have been observed to undergo various morphological changes. These changes often include condensation of chromatin within the nucleus which is concurrent with elongation of the cell itself. The role of microtubules in elongation of spermatid nuclei has been suggested in several studies of spermiogenesis (Kessel, 1966, 1967; Clark, 1967; McIntosh and Porter, 1967), as well as critically questioned (Fawcett et al., 1971). In fact, the close spatial relationship of microtubules to the nuclear membrane in *Tubifex* and *Lymnodulus* spermiogenesis has implied the possible inductive effect of microtubules on actual chromatin condensation (Ferraguti and Lanzavecchia, 1971).

Studies have illustrated not only condensation patterns of chromatin in sperm but also, in many cases, the specific arrangement of the hereditary material within the male germ cells. Evidence for such precise alignment of DNA molecules in sperm cells of the squid *Loligo pealei* was shown by Wilkins and Randall (1953), and Sato and Inoué (1964). The current ultrastructural study of sperm differentiation in *Loligo* presents evidence of a close association between chromatin strands and microtubules during nuclear condensation in these cells.

MATERIALS AND METHODS

Immature specimens of *L. pealei* (average mantle length, 120 mm) were obtained from the Marine Biological Laboratory in Woods Hole, Massachusetts. Segments of the testes were dissected and immediately fixed in collidine-buffered 2% glutaraldehyde in seawater (pH 7.2) for 1 h at room temperature. The pieces of gonad were then washed in buffered seawater and postfixed for 1 h in buffered 1% OsO_4 in seawater. The tissues were washed again in buffer, dehydrated through a graded series of alcohols, transferred to propylene oxide, and embedded in Epon. Thin sections were stained with a saturated solution of uranyl acetate in methanol followed by lead citrate, and observed on a JEM 100-B electron microscope.

OBSERVATIONS

During spermiogenesis in *L. pealei*, a jacket of microtubules, which is termed a manchette, envelops the elongating nucleus. The microtubules are of considerable length and run parallel to the long axis of the cell (Fig. 1). Cross sections through the spermatids show that the microtubules actually lie singly or in pairs in "grooves" or longitudinal indentations of the nuclear envelope. This results in an almost scalloped appearance of the nuclear envelope (Fig. 2).

Within the nucleus, strands of chromatin parallel the nuclear long axis and thus parallel the manchette. Favorable glancing longitudinal sections through the spermatid reveal an exact alignment of microtubules and peripheral chromatin strands (Fig. 1, arrows). In cross sections of the spermatid nucleus, a condensation of the chromatin at the periphery of the nucleus is located directly across the nuclear envelope from a microtubule (Fig. 2). These chromatin strands often appear in contact with the inner nuclear leaflet. The microtubules opposite appear in contact with the outer leaflet of the nuclear envelope by means of a small arm or beadlike extension (Fig. 2). The cisterna of the nuclear envelope between the microtubules and condensing chromatin is collapsed. In fact, the leaflets are brought into such close proximity that examination at higher magnifica-

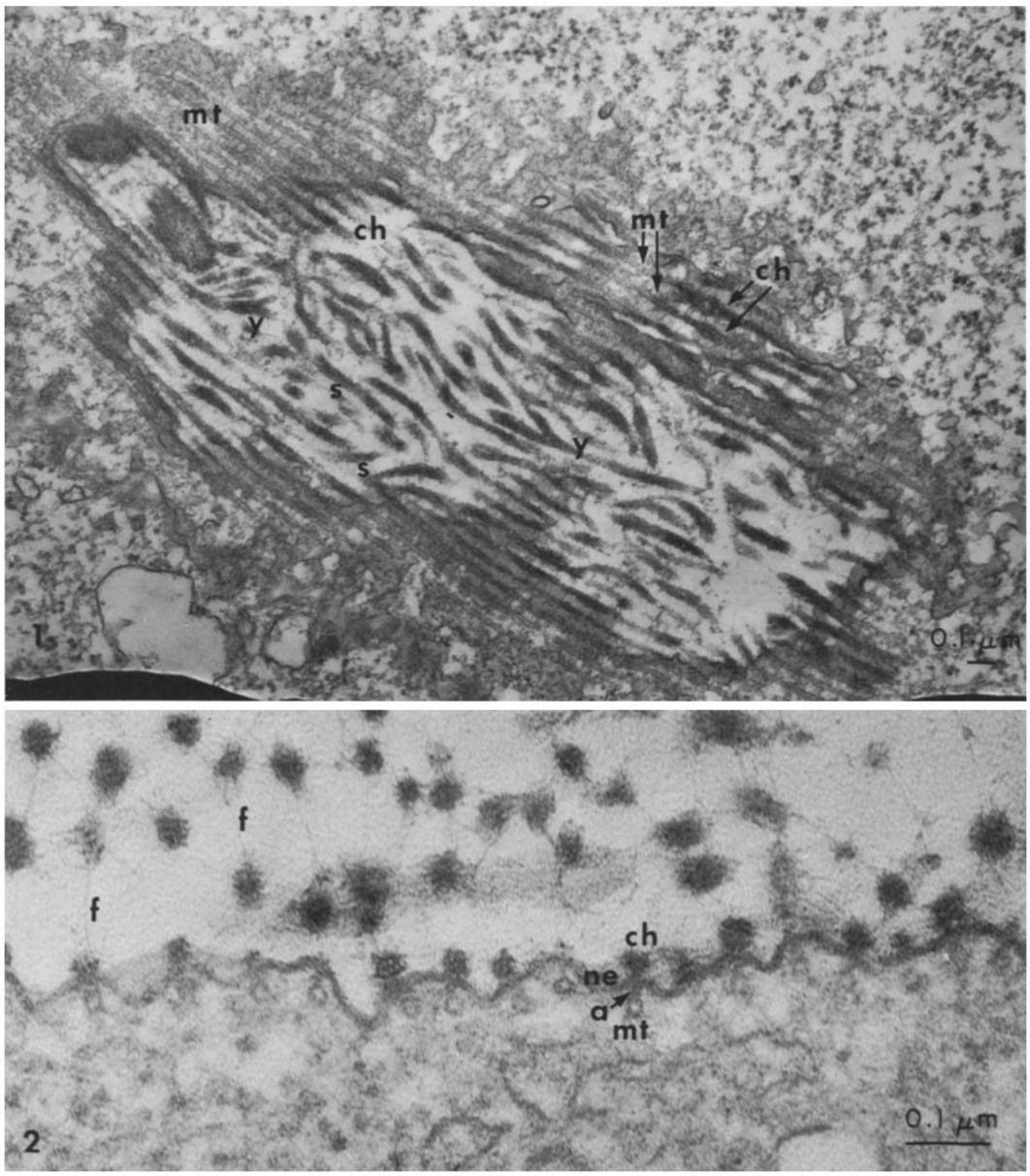

FIGURE 1 Glancing section of the nucleus of a maturing spermatid showing the microtubules (*mt*) of the manchette aligned with the peripheral chromatin condensations (*ch*) inside the nuclear envelope (*arrows*). The cisterna of the nuclear envelope is collapsed so that the microtubules are directly outside the peripheral chromatin cords. The inner chromatin cords appear to be composed of multiple strands (*s*) and branch and interconnect (*y*). Ca. × 34,000.

FIGURE 2 Cross section of the maturing spermatid nucleus showing the microtubule-nuclear envelope-chromatin complex. Note that the cisterna of the nuclear envelope is collapsed and the inner and outer leaflets are in direct contact. In some cases there is a short arm or bead (*a*) between the microtubules (*mt*) and the nuclear envelope (*ne*). The cords of condensing chromatin (*ch*) are interconnected by fibers (*f*) and appear to have a multiple-stranded structure. Ca. × 115,000.

tion is often required to verify the consistent double nature of the envelope.

Chromatin at the peripheral border of the nucleus attaches to inner chromatin strands by small fibers (Fig. 2). Almost all the chromatin strands are connected by fibrous bridges to at least one other neighboring strand. The diameter of the strands varies, and in longitudinal sections the chromatin can be seen to branch and interconnect (Fig. 1). Thicker condensations of chromatin, which could be termed "chromatin cords," appear to be composed of several thinner strands wound together (Fig. 1). Only where strands or "chromatin cords" come in contact with the inner nuclear leaflet can the structural complex of chromatin-nuclear envelope-microtubule be seen.

DISCUSSION

Although previously mentioned studies of spermiogenesis have implied that the sole function of manchette microtubules is in applying external pressure to the elongating sperm head, Fawcett et al. (1971) proposed recently that the shape of the sperm head may be determined from within by a genetically controlled pattern of nucleoprotein aggregation. The data presented here lend support to a hypothesis of the latter type. However, the process of condensation may involve genetic control as well as microtubule influence. This mechanism of condensation would require an interaction between condensing chromatin and microtubules. The complex of chromatin-nuclear envelope-microtubule in *Loligo* spermatids supplies the structural basis to suggest such a mechanism. Condensation of chromatin appears directly opposite the microtubule-nuclear envelope complex.

During spermiogenesis in the dragonfly, microtubules also connect with the nuclear envelope (Kessel, 1966). The inner and outer leaflets of the nuclear envelope are in close contact in areas of microtubule association. The double nuclear leaflets of the spermatids in *Loligo* are similarly closely applied to each other, suggesting a possible interaction of the microtubules on the structure of the nuclear envelope, often to an extent that masks the dual nature of the membrane.

Peripheral condensation of chromatin as expressed in *Loligo* spermatids is also illustrated in gametogenesis in *Lumbricus* (Anderson et al., 1967; Lanzavecchia and Donin, 1972), and *Drosophila* (Stanley et al., 1972). In *Lumbricus*, condensation of chromatin is initiated in regions of the nucleus nearest the manchette microtubules. In *Drosophila*, chromatin ridges in the periphery of the nucleus are in direct alignment with ridges of dense material located between manchette microtubules. All these instances illustrate the possibility that the site of initiation of chromatin condensation may be at a microtubule-nuclear envelope complex. Chromatin condensation in grasshopper spermatids also shows nucleoprotein alignment with microtubules that surround the elongating nucleus (Kessel, 1967).

Previous descriptions of chromatin-microtubule association have concerned primarily the kinetochore. Kinetochores are the points of attachment of spindle microtubules and condensed chromosomes during mitosis and meiosis. The ultrastructure of kinetochores is discussed at length in two recent reviews (Brinkley and Stubblefield, 1970; Bajer and Molè-Bajer, 1971). In some kinetochores, (for example, in Chinese hamster cells [Brinkley and Stubblefield, 1966]) a double-layered structure is observed between the microtubules and the chromatin. This may be comparable to the microtubule-nuclear envelope-chromatin complex reported here. Kinetochores have been reported to be located in close proximity with, or as a part of the nuclear envelope in mouse spermatocytes (Woollam et al., 1967).

Although the structural association between microtubules and chromatin in kinetochores and the morphology of the microtubule-nuclear envelope-chromatin complex described here seem quite different, perhaps a basic interaction between microtubules and chromatin can be suggested. If manchette microtubules influence the positioning of the chromatin at the onset of condensation in spermatids, and spindle microtubules position condensed chromosomes during cell division, a basic similarity of chromatin-microtubule interaction might be proposed.

This communication is simply a preliminary note to further investigations now in progress, and it is not the authors' intent to imply that the sole function of these microtubules in *Loligo* spermiogenesis is the initiation of chromatin condensation. Details of additional chronological events in the condensation process, as well as consideration of the assembly, location, and permanence of all the microtubules in the system will be further elaborated in future publications.

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