Intranuclear Microtubules

Abstract. *Intranuclear microtubules are a regular feature of spermatocyte meiosis in a crane fly* (Nephrotoma suturalis *Loew*).

Microtubules (*1*) have been observed in many cell types, especially since the introduction of glutaraldehyde fixation (*2*). They are currently recognized as ubiquitous components of the cytoplasm and as components of the mitotic apparatus (*3*).

Intranuclear microtubules have previously been seen only in cells in which the entire division apparatus is intranuclear (*4*), and, in one other case, in a crystalline array of microtubules in nondividing insect epidermal cells (*5*). We herein report the presence of spindle microtubules in telophase nuclei as a regular feature of the meiotic divisions in crane fly spermatocytes.

Testes of last-instar crane fly larvae (*Nephrotoma suturalis* Loew) were fixed in buffered 2 percent glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and were examined with an electron microscope (*6*).

Microtubules were regularly seen enclosed within meiotic telophase nuclei, in all stages of nuclear membrane formation. Microtubules were regularly spaced within the nuclei (Figs. 1 and 3), and as many as 70 have been observed in individual nuclei.

The intranuclear microtubules were 240 to 260 Å in diameter with electron dense walls and less dense cores (Figs. 1 and 2), and they were morphologically identical to the spindle microtubules of metaphase and anaphase. Cross sections often showed indication of substructures in the microtubule walls (Fig. 2), such as those seen in cytoplasmic microtubules (*7*), in axostyle microtubules (*8*), and in an intranuclear crystalline array of microtubules (*5*).

The intranuclear microtubules were centrally placed within less dense nuclear regions of approximately 1000 Å in diameter which separated the microtubules from the chromatin (Fig. 1). These peritubular regions contained a material of low density which seemed to connect the microtubules with the chromatin (Figs. 1, 2, and 3). In cross sections of microtubules this "bridging" material was often radially oriented, reminiscent of the "spokes" in cilia and flagella which connect the central pair of microtubules with the peripheral doublets (*9*).

In metaphase and anaphase, microtubules pass through less dense chromosome regions which are similar to the less dense regions found in telophase nuclei, and there is bridging material between the microtubules and the chromosomes similar to that found in telophase nuclei (*6*). Because the bridging material is associated with the chromosomes during cell division, we suggest that this material plays a role in movement of the chromosomes.

The significance of the intranuclear microtubules in crane fly spermatocytes is unknown. A few spindle microtubules may occasionally be observed within telophase nuclei of mammalian meiotic and mitotic cells (*10*), but this is observed only occasionally, and only before the nuclear membrane is completely formed. In crane flies, however, microtubules are regularly included within telophase nuclei in a manner which does not seem accidental. This does not necessarily imply an intranuclear function, however, for they may be present in the nuclei only as remnants of a function during division.

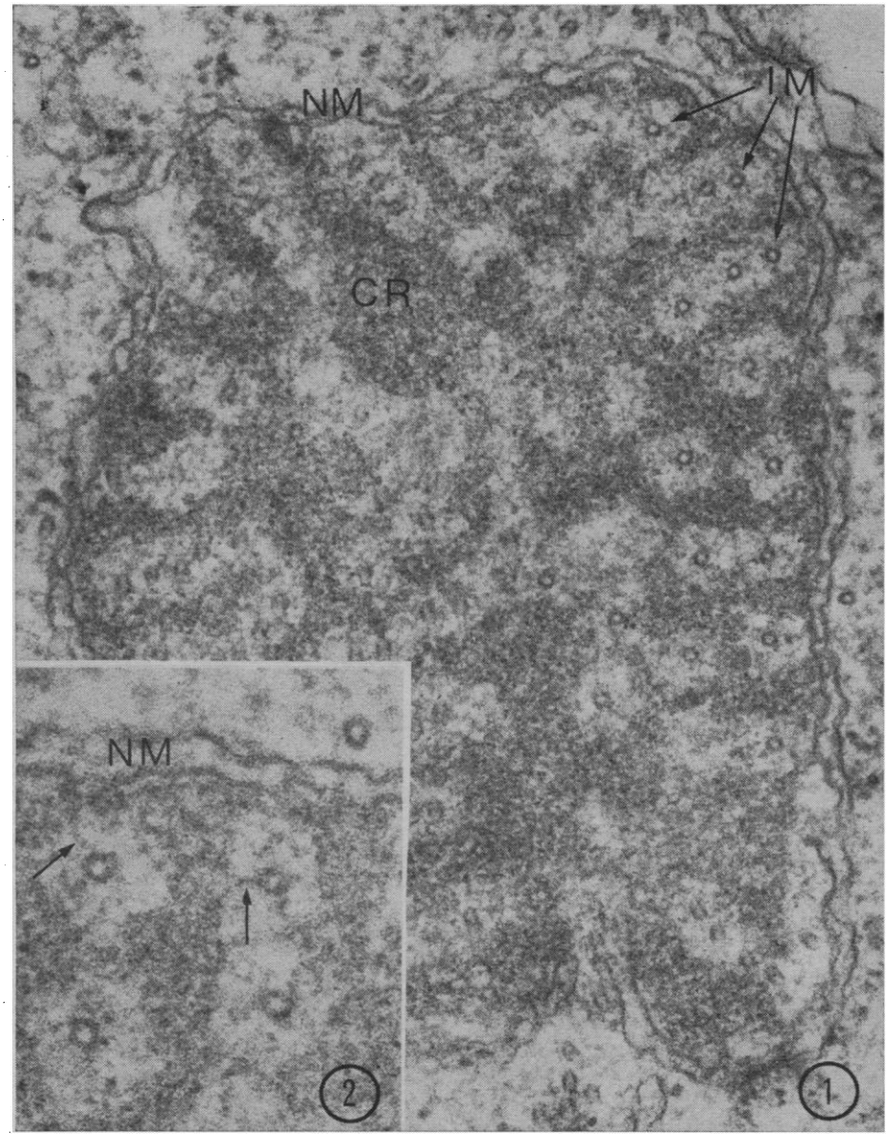

Fig. 1. Part of a telophase nucleus, with intranuclear microtubules (*IM*), in cross-section, separated from the chromatin (*CR*) by less dense peritubular regions. *NM*, nuclear membrane. Note the regular spacing of the intranuclear microtubules (× 85,000).

Fig. 2. Part of a telophase nucleus. Material in the peritubular regions seems to connect intranuclear microtubules with the chromatin (arrows). *NM*, nuclear membrane. The intranuclear microtubules are morphologically similar to the microtubule seen in the cytoplasm (× 150,000).

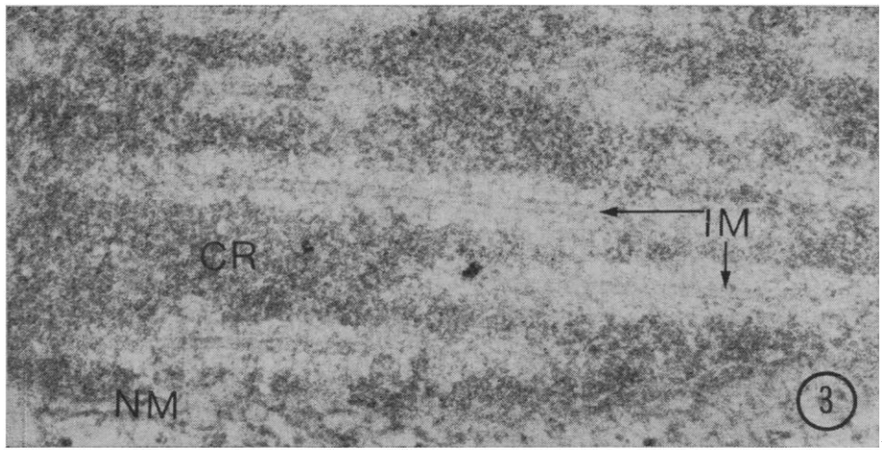

Fig. 3. Part of a telophase nucleus, showing intranuclear microtubules (*IM*), in longitudinal section, surrounded by less dense peritubular regions. *NM*, nuclear membrane. *CR*, chromatin (× 73,000).

The ultimate fate of the intranuclear microtubules is unknown. In late telophase they are not found, presumably because they have been depolymerized into morphologically unidentified subunits. Whether these hypothetical subunits really exist, and whether they remain in the nucleus, are open questions.

Thus, meiotic nuclei are added to the ever-increasing list of places where microtubules are found (*1, 3–9, 11*), and microtubules are added to the list of structures seen within nuclei (*5, 12*).

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Nature of Seed Dormancy in Phacelia tanacetifolia

Abstract. *The inhibiting effect of light on germination of* Phacelia tanacetifolia *seed is overcome by removing the tip of the endosperm; however, immersion in solutions of high osmotic pressure reinstates the light sensitivity. Inhibition of germination by high temperature behaves similarly. Dormancy is ascribed to balance between mechanical constraint by the endosperm and "expansive force" of the embryo.*

Germination of the seed of *Phacelia tanacetifolia* (family Hydrophyllaceae) is strongly inhibited by light (*1, 2, 3*). However, if that part of the seed-covering structures (endosperm plus remnants of seedcoats) which directly cover the radicle is removed, full germination occurs in light. Removal of other parts of the seedcoat has no effect on normal germination, although a deep cut at the cotyledonary end allows the embryo to grow out abnormally from the cut surface (*4*). The isolated embryos grow just as well in light as in darkness. These facts have led us to conclude (*3*) that light does not prevent germination either by inhibiting growth of the embryo or by giving rise to an inhibiting substance in the seed (since such a substance should diffuse out through the cuts). Instead, control of germination rests in the balance between internal expansive forces developed by the embryo and mechanical restraint exerted by the tough endosperm, and this balance is modified by light. The same situation exists in light-requiring lettuce seed, in which full germination in darkness is made possible by appropriate cuts into the seed. Gibberellin induces germination of both types of seed, irrespective of illumination. In consequence, Ikuma and Thimann (*5*) deduced that the limiting factor in germination is not elongation of the radicle but the mechanical properties of the endosperm layer. A number of experiments with both species of seed have supported this explanation and even the effect of kinetin has been brought into line (*6*). Thus the earlier view that seed dormancy is due to inhibiting substances has been made increasingly untenable.

In general the mechanical restraint can be overcome by either or both of the following means: (i) softening of the endosperm, presumably by enzy-