Ultrastructure of Tumor Cells during Mitosis

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SUMMARY

The fine structure of the mitotic process of solid hepatoma cells of rat has been described. The replication and migration of centrioles, appearance and disappearance of spindle tubules and kinetochores, and movement and replication of chromosomes resemble those reported in other cell systems.

It has been observed that the nucleoli persist throughout all stages of cell division. The Golgi apparatus retains its discrete entity of lamellar and vesicular components during the entire mitotic cycle. Remnants of the nuclear envelopes have been observed in all phases, even in telophase, after the reconstruction of the nuclear membrane of the daughter cells is completed. Penetration of rough endoplasmic reticula into the mitotic apparatus may appear in metaphase and anaphase. In telophase, cytokinesis is initiated by symmetrical invagination of the plasma membrane and, at certain stages, mitochondria may move freely in the region of the intercellular bridge which connects the daughter cells.

The mitotic activities of these tumor cells represent the behavior of the cellular organelles in natural and unaltered nutritional environment.

INTRODUCTION

The fine structure of mitosis and meiosis has been studied in a wide variety of plant and animal cells, including onion root tip (33), spermatocytes (16, 24, 31), meristem of wheat (32), developing embryos (1, 18, 19, 20), protozoans (15, 38-40), and several types of tissue culture cells (6-8, 17, 26, 36). Reports of investigations of mammalian cell division have been limited in number (10, 14, 30). Electron microscopic observations of the mitotic cycle in tumor cells have been scanty and offer only fragmentary information. Buck (9) described the lamellae in the spindle of mitotic cells of Walker tumor. Yasuzumi (47) studied the nuclear membrane in prophase and telophase of Yoshida sarcoma cells. Selby (42) published electron micrographs of low magnification (× 3300-4200) of mitotic figures of cultured Ehrlich ascites cells. Due to lack of adequate technic at that time, important cellular organelles such as mitochondria, Golgi bodies, nucleolus, and centrioles could not be identified in these micrographs. It appears that systematic study of fine structure of mitotic activity of solid tumor cells in their natural environment is lacking. This research was thus undertaken to fill the need. Data from this investigation would be of special value and interest when compared with those from cultured cells.

MATERIALS AND METHODS

Induction of primary hepatoma by feeding rats the carcinogen, 3'-methyl-4-dimethylaminoazobenzene, and the establishment of transplantable hepatomas from this tumor have been described elsewhere (12). In the present study, tissues of the transplantable tumor were fixed in 3% glutaraldehyde (41) or in a glutaraldehyde-acrolein mixture at 4°C or at 25°C, and postfixed in 1% osmium tetroxide. All fixatives were buffered with Millinogis's (29) phosphate buffer at pH 7.4. After fixation for 90 minutes, the tissues were washed in tap water containing a trace of calcium chloride and were dehydrated rapidly with ethyl alcohol by the drop-wise replacement method. Replacement of the ethyl alcohol by propylene oxide preceded embedding in Epox 812 (28). Thick sections (0.5-1 μ) stained with methylene blue and azure II (35) were used to locate mitotic cells and to identify the tumor type as well as the exact mitotic stage at the level of the light microscope. The tumor cells studied were carcinomas. Thin silver or gray sections were stained with uranyl acetate (45) and lead citrate (34) and examined with an Hitachi HU-11A electron microscope.

OBSERVATIONS

Interphase

The fine structure of the interphase cell in the primary tumor and in the transplants has been described in a previous paper (11). The nuclear pattern and the extent of the cytoplasm vary considerably; general features, however, are illustrated in Fig. 1. The nuclei are round or irregular in shape and may occupy a large portion of the cell. The chromatin and the nuclear envelope are closely associated. Nucleoli are large, irregular in shape, and are located centrally or peripherally. They are composed of the usual granular and fibrillar elements (the pars granulosa and the pars fibrosa). The cytoplasm, which is of moderate amount, contains scattered mitochondria, well-developed Golgi areas, and a heavy population of free ribosomes. Microbodies are rarely observed, yet lysosomes are abundant, usually near the Golgi region. Elements of rough
endoplasmic reticulum are scattered through the cytoplasm; usually, they are not stacked in parallel fashion, as are those in normal hepatocytes. Glycogen particles have not been positively identified in any sections thus far examined. Centrioles are often found in the tumor cells in the vicinity of the Golgi complex. Each centriole is composed of nine radiating groups of tubules in triplets, arranged to form a cylinder (Fig. 1 inset) as previously described (4, 36, 44). At interphase, four centrioles are located in a small hilus in one side of the nucleus.

**Prophase**

Condensations of chromatin into discrete chromosomes along the nuclear membrane characterize prophase (Figs. 2, 3). The association of the nuclear envelope and the chromatin becomes less pronounced as prophase is approached. On some sections, the kinetochore may be visible (Fig. 2). As cell division proceeds, the nuclear membrane gradually breaks into pieces. These remnants have also been observed in other stages of mitosis. Often the two smooth sides of remnants come in contact with each other (Fig. 3). This feature corresponds to the duplication of the nuclear envelope in rat lymphocytes (30) and the “stacking” of the envelope reported by others (2).

In early prophase, migration of the centrioles toward the opposite poles takes place in the usual manner. On rare occasions, more than two centrioles have been seen at one polar region in a prometaphase (Fig. 4). This phenomenon resembles the observation on colcemid-treated Chinese hamster cells (8). The behavior of the nucleolus in prophase is similar to that of the Chinese hamster cells (6). In essence, the organelles gradually undergo morphologic transformation and lose their fibril element, and the granular elements become more uniformly and distantly dispersed. Eventually, they break into smaller masses; usually these masses become attached to condensed chromosomes, although they have been found free in the cytoplasm. This condition persists throughout metaphase and anaphase.

The Golgi systems are well defined in prophase. They exhibit both laminar and vesicular components (Fig. 3). Also, they tend to remain in the polar regions (Fig. 3), although occasionally they are observed in other locations.

The spindle tubules are not detected until late prophase. The centrioles are then firmly situated in the polar region, while the chromosomes have moved toward the equatorial plate.

**Metaphase**

In this stage the chromosomes are aligned on the equatorial plate and the spindle tubules form a cup-shaped umbrella radiating from the poles where the centrioles are located. The tubules, varying in number, may attach to or penetrate between the chromosomes (Figs. 5, 6). During this stage segments of proliferating endoplasmic reticulum may lie parallel to the spindle tubules. The two organelles can be identified easily: the former have a varied diameter, a somewhat undulating profile, and attached ribosomes, whereas the latter are always uniform in width, have a rigid profile, and lack ribosomes. Figure 8 shows a number of the endoplasmic reticula radiating from the pole toward the chromosomes. It demonstrates one endoplasmic reticulum in contact with the tubule; the latter, though poorly sectioned, is identifiable for a short distance and is attached to a kinetochore. The proliferation of endoplasmic reticulum into mitotic apparatus of root-tip cells of onion has been reported (33). Smooth-surfaced membrane, resembling the cisternae of the endoplasmic reticulum, has also been observed in metaphase of HeLa cells (36).

Remnants of nuclear envelope are scattered in the cytoplasm. A few mitochondria are apparent in the mitotic apparatus, at times near the chromosomes (Figs. 6, 7). The Golgi apparatus retain their identity in metaphase and are usually localized in the two polar regions (Fig. 5). Both the laminar and vesicular forms are often detected in different planes of section.

Finally, the nucleolar material always maintains its identity as small masses attached to the chromosomes (Figs. 5–7). This phenomenon is clearly demonstrated in Fig. 7, which shows a polar view of a section cut through the equatorial region. The morphologic characteristics of the nucleolus are similar to those previously described (6).

**Anaphase**

The ultrastructural features of early anaphase closely resemble those of late metaphase. In ultrathin sections, separation of these phases into two distinct stages is extremely difficult (3). Figures 9 and 10 show the relationship between the centriole and the chromosomes, the arrangements of spindle tubules and proliferating endoplasmic reticula, and mitochondria near the anaphase chromosome. The spindle tubule may be seen attached to the kinetochore. In Fig. 10 the Golgi apparatus is clearly apparent in the polar region. As cell division progresses, the chromosomes move toward the centrioles, and the nucleolar masses become more prominent and more electron opaque. A well-organized Golgi system and occasional stacked nuclear envelopes are also observed. When the two masses of chromosomes separate still farther, the reconstruction of nuclear membrane becomes obvious. At times, a number of membrane-bound electron-opaque droplets are discovered scattered through the cytoplasm (Fig. 11). These bodies, presumably lipid in nature, correspond to the membrane-bound osmophilic bodies described by Robbins and Gonatas (36); as a rule, however, they do not form clusters in the tumor cells and are rarely found in prophase. In many sections they become more numerous in anaphase.

**Telophase**

The typical telophase is illustrated in Figs. 12 and 13. The chromosomes condense into highly electron-opaque masses at each pole. The newly formed nuclear membrane with nuclear pores gradually surrounds the chromosome. The nucleolus may be seen embedded in the mass of chromosomes; usually it is located at the periphery of the nucleus. At the late stage, the granular and fibrillar components of the nucleolus may be observed (Fig. 16). The Golgi apparatus with both laminar and vesicular elements generally are well developed; they are located between the daughter nuclei and the equatorial plate (Fig. 13).

Cytokinesis takes place by symmetrical invagination of plasma membrane in the late telophase of these tumor cells.
The cytoplasm forms an intercellular bridge between the two daughter cells just before they separate (Figs. 13–15). As a rule, numerous spindle tubules converge into a dense zone, the midbody, as they pass through the bridge (Fig. 15). Numerous electron-dense granules aggregate outside these tubules. Occasionally, mitochondria may be found in the region of the midbody where there are few spindle tubules (Fig. 14). No information has been obtained to indicate how the midbody is eliminated when the daughter cells finally separate.

DISCUSSION

The authors have encountered many difficulties in conducting this research because of the scarcity of suitable mitotic figures in a given section and the variable incidence of different phases of cell division. Our efforts were worthwhile, since data obtained from the study revealed the cellular activities of tumor cells in a natural and unaltered nutritional environment. Thus far, the results indicate that the replication and migration of centrioles, the formation and dissolution of spindle fibers and kinetochores, and the movement and replication of chromosomes resemble those in other cell systems, although inconsistent variations are observed. Certain organelles in the tumor cells, however, exhibit a characteristic behavior during mitosis.

Hay (21) stated that persistent nucleoli are common in mitotic cells of plants, though normally the nucleoli do not persist during mitosis in animal cells. Heneen and Nichols (22) believed that the nucleoli persisted in mitotic cells but were not included in daughter nuclei of mammalian cells; they either degenerated in the cytoplasm or were eliminated from the cell. Lafontaine and Choninard (27) reported that in Vicia faba the nucleolus dissolves completely during late prophase and becomes indistinguishable from other nuclear and cytoplasmic material; further, the fibrillar and granular forms of the nucleolus reappear along the arms of anaphase chromosomes. Similar observations in grasshopper were published by Stevens (43). In autoradiographic, cytochemical, fluorescent, electron microscopic, and ribonuclease digestion examinations of a number of mammalian cells, Hsu et al. (23) observed that persistent nucleoli, having the same characteristics as the nucleolus in interphase, could be identified in all the stages of mitosis. In a detailed ultrastructural study of Chinese hamster cells, Brinkley (6) found that in prophase, nucleoli disperse into smaller masses. These masses appear as a loose, predominantly fibrous structure with widely scattered granules. This structure persists throughout mitosis; in telophase it becomes included in the daughter nucleolus.

In the present study of the tumor cells, although nucleolar particles were dispersed and discrete fibrillar areas were lost during the mitotic cycle, nucleolar material was found during each stage (Figs. 2, 5–7, 9, 16). Figure 16 demonstrates a nucleolar mass containing both granular and fibrillar material in a separating daughter nucleus. This indicates that the persistent nucleolus enters the daughter cell. One cannot, however, disregard the possibility that the nucleolus is newly organized in telophase. The functional significance of persistent nucleoli in mammalian cells is not clear. It was thought (6) that they serve either as a primer for new nucleolar material or as a source of nucleolar RNA for the daughter cell during the formation of a new nucleolus.

The exact disposition of the nuclear envelope during mitosis is still an open question. In the amoeba (39) the membranes of the nuclear envelope are present in all stages but are not continuous in metaphase. In rat thymocytes a complete ring of nuclear envelope has been observed in late prophase and early metaphase; even at anaphase large segments have been recognizable (30). Although large segments of the nuclear envelope are not visible in solid tumor cells, remnants of it are clearly present in all phases of the mitotic cycle (Figs. 3, 5, 13). Buck (9) detected lamellar structures in the spindle area of the mitotic cells of Walker 256 carcinosarcoma. He believed that these structures were similar to the endoplasmic reticulum and represented a newly forming nuclear membrane. Barer et al. (2) also suggested that the new nuclear membrane might be formed by a fusion of remnants of the endoplasmic reticulum. In human lymphocytes, the nuclear membrane is believed to reform from a fusion of vesicles derived from the old nuclear membrane (25). It is possible, therefore, that the new envelopes are reassembled either from the remnants of the old nuclear envelope or from endoplasmic reticulum, or from both. The findings in the present study do not strongly support any of the theories in particular, since both the stacked remnants of the nuclear envelope (Figs. 3, 5) and the proliferating endoplasmic reticulum into the spindle (Figs. 8, 9) were often observed. In late telophase, after the reconstruction of nuclear membrane is completed, an abundant amount of the stacked nuclear membrane is occasionally present in the cytoplasm (Fig. 13). This stacked material will probably either degenerate or transform into normal endoplasmic reticulum in interphase.

The fate and function of the Golgi apparatus in mitotic cells is another enigma. In a review of the Golgi complex, Bourne and Tewari (5) stated, "In mitosis the Golgi apparatus usually breaks up into small particles or granules which are distributed more or less evenly through the cytoplasm." Dalton (13) also reported the absence of Golgi elements in several tumor cells during mitosis. In HeLa cells, Robbins and Gonatas (36, 37) presented histochemical and fine structural evidence that the Golgi disappears during metaphase and appears rapidly in telophase. Conversely, Dougherty (14) observed the persistence of the Golgi complex during mitosis of hepatic cells in rats. Roth et al. (40) reported unmistakable evidence that typical Golgi bodies appear in metaphase and anaphase in both the spermatocytes of European corn borer and the giant amoebae. In our study, the Golgi apparatus did not break into small vesicular clusters, but retained its laminar and vesicular components throughout different phases of the mitotic cycle (Figs. 3, 5, 10, 13).

The separation of the daughter cells is evidently initiated by a symmetrical invagination of the cytoplasmic membranes. No visible signs of specific morphologic changes of cellular organelles in the cytoplasm are detected in this process. The phenomenon described by Whaley et al. (46), that the Golgi apparatus is clearly involved in the formation of cell plate during cytokinesis, does not apply to the hepatoma cells. Evidently, a difference in species explains the differences in be-
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behavior of these organelles. At the end of telophase, the spindle tubules forming the midbody are condensed in the region of the intercellular bridge (Fig. 15). The structure of the midbody is similar to that of the HeLa cells (36). However, the disposition of the midbody in the hepatoma cells is not clear. The pincer-like invagination of the plasma membrane (36) or the enclosure by the furrow membrane and its subsequent abandonment (1) have not been observed in our system. On occasion, mitochondria appear within the intercellular bridge (Fig. 14) where no midbody is formed. This could represent a different type of cytokinesis, or could be caused by sectioning at a different plane or at different stage of the development of the midbody.

It may be concluded that rat hepatoma cells possess some fundamental features of mitotic division similar to those of other species. Data obtained so far indicate the persistence of nucleoli and Golgi systems throughout the mitotic cycle. In addition, proliferation of endoplasmic reticulum into the mitotic apparatus, dissolution and reconstruction of nuclear membrane, and initiation of cytokinesis and formation of midbody in telophase are observed in the hepatoma cells. It is not known whether manifestations of these characteristics are controlled by genetic or nutritional factors. Further elucidation of these problems awaits the completion of other studies now in progress.

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REFERENCES


Figs. 1–16. Tissues were fixed in glutaraldehyde-acrolein mixture (unless stated otherwise) and postfixed in osmium tetroxide. All sections were stained with uranyl acetate and lead citrate.

Fig. 1. Interphase cell. Showing irregularly shaped nucleus (N) and nucleolus (n) with fibrillar (f) and granular (g) components, elements of the endoplasmic reticulum, and a number of mitochondria. Note the well developed Golgi (G). × 37,000. Insertion is a cross section through a centriole. × 1,255,600.

Fig. 2. Prophase. Showing condensation of chromatin into discrete chromosomes (Ch) along the nuclear membrane (Nm). Note the kinetochore (K) and the homogeneous nucleolus (n). × 18,270.

Fig. 3. Advanced prophase. Showing the breakage of the nuclear membrane and pieces of its remnants (arrows) having their smooth sides joining. Laminar and vesicular elements of the Golgi (G) apparatus are shown. A similar mass of the Golgi (not shown) is found on the opposite side of the nucleus. Note the lysosome granules in the Golgi area. × 21,420.

Fig. 4. Prometaphase. Showing three centrioles (arrows) at the polar region and masses of chromatin material. × 32,900.

Fig. 5. Metaphase. Tissue fixed in glutaraldehyde showing the complete mitotic apparatus. Laminar and vesicular forms of the Golgi complex (G) are located at the poles. Chromosomes (Ch) are aligned on the equatorial plate. Note the nucleolar mass (n) the remnants of the nuclear membrane (Nm), and the dense granules near the Golgi. × 24,380.

Fig. 6. Metaphase. Showing the chromosomes (Ch) aligned on the equatorial plate, the nucleolus (n), kinetochore (K), etc. × 24,480.

Fig. 7. Polar view of metaphase. Showing the chromosomes (Ch) aligned on the equatorial plate, the nucleolus (n), kinetochore (K), etc. × 24,480.

Fig. 8. Late metaphase. Showing endoplasmic reticulum (ER) in the area of the mitotic apparatus. A spindle tube (St) appears with an ER and attached to a kinetochore (K). × 44,880.

Fig. 9. Anaphase. Showing chromosomes moving to the poles. Radiating from the centriole (C) are spindle tubules (St) and endoplasmic reticulum (ER). × 35,520.

Fig. 10. Anaphase. Showing the relationship between the centriole (C), the chromosomes, and the spindle tubules. The Golgi apparatus (G) is evident in the polar region. × 42,300.

Fig. 11. Late anaphase. Showing chromosomes (Ch) situated at their respective poles. A number of membrane-bound electron-opaque droplets (arrows) are scattered through the cytoplasm. × 20,060.

Fig. 12. Early telophase. Showing at each pole, the condensed chromosomes (Ch) with newly formed nuclear membrane (Nm). Cytokinesis is evident (arrows). A symmetrical invagination (not shown) appears at the opposite side of this cell. × 19,470.

Fig. 13. Late telophase. Showing daughter nuclei with complete envelopes. Remnants of the excess nuclear membrane (Nm) remain in the cytoplasm. Note the midbody (Mb), the intercellular bridge (Ib), and the Golgi (G). × 15,120.

Fig. 14. Late telophase. Showing mitochondria (M) moving freely in the intercellular bridge where few spindle tubules (arrows) exist. × 33,120.

Fig. 15. Higher magnification of a midbody of another cell showing numerous microtubules. × 30,100.

Fig. 16. Late telophase. Showing the centriole (C), the newly formed nuclear membrane, and the nucleolus (n) which contains material suggesting fibrillar (f) and granular (g) components. × 29,240.
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