Endoplasmic reticulum hypertrophy and nuclear envelope formation—a postulate

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Abstract

Dividing endosperm cells of *Haemanthus katherinae* Bak., treated with 0.025 per cent aqueous solution of a mixture of glycosides from *Nerium oleander* were examined *in vitro* in the light and in the electron microscope. A high hypertrophy of endoplasmic reticulum was noted. In prometaphase and metaphase, after treatment for about 1 h 45 min there appeared very narrow cisternae forming various configurations, frequently in parallel and concentric arrangement. On the membranes of these cisternae there are formed dark areas interpreted as pores characteristic for nuclear envelopes, this indicating that at least part of the two-membrane structures transforms to the nuclear envelope. The formation of the new nuclear envelope prematurely and apparently in excess is discussed.

INTRODUCTION

The role of endoplasmic reticulum (ER) in the cell is well known and documented. It results from some studies on cell division that ER cisternae are a component of the mitotic apparatus, however, their role in the latter is not clear.

The suggestion of some authors (e.g. Porter and Machado, 1960; Harris and Bajer, 1965; Pickett-Heaps and Northcote, 1966; Sakai, 1969; Wilson, 1970; Hepler and Palevitz, 1974) that the accumulation in prophase, on the future cell poles, of membranous ER structures may constitute a "support" for the forming mitotic spindle seems probable. Part of the ER vesicles are a component of the clear zone. These vesicles after disintegration of the nuclear envelope (NE) penetrate with part of the membranes from the poles, between the chromosomes and the microtubules of the mitotic spindle.

and also into the later developing fragmoplast (Porter and Machado, 1960; Pickett-Heaps and Northcote, 1966; Sakai, 1968, 1969). The ER membranes are sometimes considered as a permanent component of the spindle. In some papers the distribution of ER in the successive mitosis phases and its probable role in this process were analysed in detail.

Porter and Machado (1960) observed in normal dividing apical meristem cells of *Allium cepa* and *A. sativum* roots, after the breaking of the NE, an invasion of ER membranes from the polar regions into the mitotic spindle between the microtubules, and their in general parallel arrangement to the latter. These relations between the usually tubular, but also vesicular, endoplasmic reticulum and the microtubules last throughout mitosis and are most pronounced in anaphase, when the tubular ER extends between the microtubules from the poles to the equatorial part of the cell. On cross sections some authors found that the ER membranes surround the particular chromosomes and also lie between them. It is not excluded, according to Porter and Machado (1960), that some of these membranes participate in the formation of a new nuclear envelope.

The invasion of ER membranes from the poles into the spindle and their usually parallel arrangement to the microtubules were also described by Pickett-Heaps and Northcote (1966) in root apical meristems and wheat coleoptile as well by Bajer and Molè-Bajer (1969) in dividing normal *Haemanthus katherinae* endosperm cells. Exposure to certain chemical compounds causes usually in the cell high hypertrophy of the membraneous structures within the whole cytoplasm, with formation of various figures, sometimes even concentric. This was observed among other authors by Molè-Bajer (1969) in all mitosis phases after treatment with chloral hydrate on *Haemanthus* endosperm cells. Similar effects were obtained by the present author after application of an aqueous solution of an oleander glycoside mixture. This intensive development of ER membranes in experimental conditions seems to have some particular significance.

MATERIAL AND METHODS

The object of the studies were endosperm cells of *Haemanthus katherinae* Baker pressed out onto nutrient medium. They were treated with a 0.025 per cent aqueous solution of a glycoside mixture from *Nerium oleander* L. (Oleander Gesamtglycoside, Laborchemikalien Carl Roth OHG, Karlsruhe-West). The material for electron microscopic studies was prepared according to the technique of Molè-Bajer and Bajer (1968) and applied with success by Tarkowska (1978b). Cells in various

division phases (from early prophase to anaphase) were treated with the glycosides for various time periods from 40 min to 2 h 30 min. For fixation of the cells (first inspected *in vitro* in phase contrast) 3.1 per cent glutaraldehyde and further 1 per cent osmium tetroxide (pH 6.9, phosphate buffer) were used. Sections of mean thickness 100 nm were prepared with LKB ultramicrotome, and stained with uranyl acetate, and lead citrate. Observations were noted and photographs were taken with a Siemens Elmiskop IA operated at 80 KV.

RESULTS

The water-soluble oleander glycosides cause, beside mitosis disturbances (Tarkowska, 1976), a very strong hypertrophy of endoplasmic reticulum in the whole cell. The presumable role of narrow ER cisternae present between microtubules in the kinetochore bundle has been suggested in earlier papers (Tarkowska, 1978a, b).

Hypertrophy of the endoplasmic reticulum within the entire cytoplasm is the first effect of glycoside treatment on endosperm cells. In prophase numerous, at first a small vesicles appear as early as after 40 min of treatment both in the clear zone and within the entire cytoplasm. After 1 h 45 min, beside vesicles, tubular ER are formed, frequently branched and usually rough. The number of ribosomes on the surface of these membranes varies: they are the less numerous the more the ER shape resembles a spherical cisterna. During treatment with glycosides for 2 h (or more) the number of variously-shaped cisternae increases still more and sporadically profiles of very narrow cisternae appear of unequal diametre. It results from all observations of prophase cells that the number, size and diversity of ER shapes increases with prolongation of glycoside treatment. Cytoplasm vacuolisation is noticeable at this time, the vacuoles arising mainly in the ER (Plate II Fig. 7). It cannot, however, be ruled out that some of the vacuoles form *de novo* within the ground cytoplasm.

Particularly noteworthy are the membraneous structures forming in prometaphase, still more numerous in metaphase after about 1 h 45 min of glycoside treatment. Beside the varied vesicles and cisternae here described, there occur around the area occupied by the chromosomes and between them two-membrane structures: narrow tubules or greatly flattened cisternae 40–80 nm in diametre (Plate I, Fig. 1). These two-membrane structures do not enclose the chromosome area, as is the case with the nuclear envelope. There may be 2, 3 and even 7 profiles arranged parallelly, and in other places there may be none at all (Plate I, Fig. 1 and Plate II Fig. 5 enlargement). On the other hand, between the chromosomes, beside the variously-shaped figures, also concentric closed systems

PLATE I

Metaphase, 1 h 45 min of oleander glycoside application. At the upper right corner: a light micrograph of the cell in plastic.

Fig. 1 — ER hypertrophy: very narrow cisternae, outside the area occupied by the chromosomes, form parallel patterns (arrows), between chromosomes various configurations, some concentric (asterisk). Magn. ca 900×

Fig. 2 — Fragment of concentric arrangement of two-membrane structures (denoted with asterisk in Fig. 1). Constrictions and dark areas are visible (arrows), inside vesicular ER. Magn. ca 22 000×

Fig. 3 — Endings of two-membrane structure, constrictions form "beads" which can become detached. Magn. ca 15 000×

Fig. 4 — Two-membrane structure (very narrow ER cisterna) with distinct dark areas (arrows), forming pores characteristic for NE. Magn. ca 24 000×

Figs 2, 3 and 4 are enlarged fragments of Fig. 1

PLATE II

Fig. 5 — Fragment of 5 parallel narrow ER cisternae surrounding chromosomes. Arrows indicate sites of pore formation. At the end of one cisterna vesicles detach. Prometaphase, 1 h 45 min of exposure to glycosides. Magn. ca 15 000×. At the upper corner — the cell in plastic.

Fig. 6 — Fragment of narrow ER cisterna with forming pores (arrows). The same cell as in Fig. 5. Magn. ca 15 000×

Fig. 7 — Vacuole arising from ER. Dark areas are visible. Fragment of Fig. 1, Plate I. Magn. ca 15 000×

Fig. 8 — In upper left corner fragment of broken NE with distinct pores (thick arrow). Thin arrows point to ER cisterna with forming pores. Anaphase, 2 h 20 min of exposure to glycosides. Magn. ca 15 000×

of 3 or 4 such flattened cisternae are formed, within vesicular ER may be present (Plate I, Fig. 1, asterisk and Fig. 2 enlargement). The width of these cisternae is not uniform: in some places they are occasionally greatly constricted, probably when they contact one another, in other places they resemble beads. At the ends of these structures, independently of their situation in the cell, numerous vesicles form which become detached and extend (Plate I, Fig. 3 and Plate II, Fig. 5). On the membranes some few ribosomes are visible and some distinct dark areas. These areas have a fine granular structure resembling seemingly ribosomes. These unidentified dark areas have no regular localisation, they are not associated with the constrictions characteristic for the described two-membrane structures, but they appear on them (Plate I, Fig. 2, Plate II, Figs 5—7, arrows).

The action of oleander glycosides on anaphase does not produce such drastic disturbances in cell division as it does in earlier phases (Tarkowska, 1976), the ER hypertrophy is also less pronounced. The different-shaped cisternae, mostly of smooth ER occur both in the peripheral parts of the cell and between the chromosomes. The narrow ER profiles characteristic for prometaphase and metaphase, with dark areas are much less numerous in anaphase (2 h 20 min of glycoside treatment) and do not form parallel or concentric patterns.

Towards the end of prophase, after breaking of the nuclear envelope, fragments of the latter with distinct pores may remain over the entire period of nucleus division (to anaphase inclusively). Plate II, Fig. 8 shows part of a cell in anaphase. In it is visible fragment of the old NE with several pores and a part of tubular ER on which pores form (unfortunately the picture is rather blurred). The possibility of NE fragments remaining until the formation of a new nuclear envelopes of sister nuclei was indicated by Bajer and Molè-Bajer (1969). These authors described in detail the behaviour of the nuclear envelope (using the same methods as in the present work) in normally dividing *Haemanthus katherinae* endosperm cells.

DISCUSSION AND CONCLUSIONS

Oleander glycoside provoke within the whole cell almost at once the appearance of membraneous structures during the whole period of mitosis. After about 2 h of treatment, in prometaphase and metaphase, there form, moreover, the above described very narrow cisternae between the chromosomes and outside the area occupied by the latter. The appearances of the structures described and the presence of ribosomes (not numerous) on their surface indicate that they are very narrow ER cisternae. A

characteristic feature of the membranes of these cisternae is the occurrence of dark areas in random places.

Membranes similar in structures with dark areas have been described by Bajer and Molè-Bajer (1969) in normal cell division in the endosperm of *Haemanthus*, in the endosperm of the same plant after application of chloral hydrate (Molè-Bajer, 1969) and in rat Kangaroo cells (Roos, 1973). The authors interpret the dark areas as pores forming in the ER, thus they postulate that at least part of these membranous structures transform to the nuclear envelope.

Detailed observation of Roos (1973) revealed that, almost simultaneously with the breaking of the NE, its pores disappear in prometaphase, and the distance between the outer and inner membrane becomes irregular. In metaphase and early anaphase these membranes are not distinguishable from ER (the same reported by Sakai, 1969), but in telophase there again reappear pores on part of these membranes and a new NE arises. A necessary condition for pore formation according to the author is the direct contact of the membranes with chromatin. Therefore, the above mentioned bead-like cisternae would be characteristic for the stage following immediately the disappearance and preceding the formation of pores in the nuclear envelope.

Molè-Bajer (1969) also observed a tendency of part of these two-membrane structures to surround, or even adhere to, chromosome arms. Owing to pore formation a new NE formed surrounding the particular chromosomes or their groups (e.g. micronuclei forming after chloral hydrate treatment). The pores were also formed in membranes not connected with the chromosomes. The author noted that the NE formed in excess.

The submicroscopic pictures obtained in the present study favour the conclusion that the numerous two-membrane structures are ER. Such an interpretation is supported both by the presence of ribosomes (although scarce), their endings in the form of distinct vesicles and the formation of vacuoles in random places. The dark areas form on all the above described membranes independently of their position in the cell and always in random places. The membranes were never found to adhere to the chromosomes, however, submicroscopic analysis of the dark areas leads to the conclusion that they are forming pores and their appearance is analogous to the pictures obtained by Molè-Bajer (1969) after chloral hydrate and by Roos (1973) in normally dividing cells. The present author did not succeed with the use of oleander glycosides to follow the fate of the broken NE during mitosis as did Roos, since the cells showed a strong tendency to inhibition of division and to restitution transformations. It is noteworthy that these very narrow profiles appear most abundantly in prometaphase and metaphase, thus when there is a strong tendency to formation of restitution nuclei (Tarkowska, 1976 and in

preparation). If we assume that the dark areas are forming NE pores, it would mean that at least part of the so abundant ER transforms to ER. They would represent the nuclear envelope forming as if in excess and prematurely. Its surprisingly large amount especially in prometaphase and metaphase may be justified by the "higher requirements" since glycosides in this period of cell division cause the formation of multinuclear cells (Tarkowska, 1971a, b), sometimes with as many as 10—13 nuclei (Tarkowska, unpublished). On the other hand in anaphase, when the probability of multinucleate cell formation is much smaller than in the earlier phases, the number of narrow cisternae with pores forming on their surface is strikingly lower. Although these observations concern cells dividing under experimental conditions, it is not excluded, that NE may form analogously in a normally dividing cell as was observed by Bajer and Molè-Bajer (1969).

The tubular and usually rough ER with its characteristic arrangement, forming concentric configurations has been described in the literature in various types of cells. The role of this ER is as yet an open question (Esau and Gill, 1971) and this phenomenon is interpreted in various ways. Some authors associate its presence with a definite active phase of the cell life cycle (Duckett, 1972), others with the loss of activity (Dexheimer, 1966; Bergfeld and Falk, 1968; Robert, 1969). Under experimental conditions the occurrence of these figures was interpreted as a reaction of the cell to energy deficit and the vigorously growing rough ER would be the site of intensified metabolic activity (Podbielkowska et al., 1975; Podbielkowska and Kupidłowska, 1976). The latter interpretation is noteworthy. Although after the application of glycosides no distinct signs of cell hypoxia were noted, the cells are in the unnormal environment and the numerous areas separated by membranes might, at least sometimes, fulfill "defence" functions, and later part of these membranes could transform to the nuclear envelope.

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Hypertrofia endoplazmatycznego retikulum a powstawanie błony jądrowej — postulat

Streszczenie

Przedmiotem badań były wyciśnięte na pożywkę, dzielące się komórki bielma *Haemanthus katherinae* Bak. poddane działaniu 0,025% wodnego roztworu mieszaniny glikozydów z *Nerium oleander* L. Preparaty przygotowano i obserwacje przeprowadzono analogicznie jak w poprzedniej pracy (Tarkowska 1978b). W mikroskopie świetlnym i elektronowym badano te same komórki.

Na terenie całej komórki stwierdzono silną hipertrofię endoplazmatycznego retikulum (ER): są to różnokształtne i różnej wielkości pęcherzyki i kanaliki, których ilość wzrasta wraz z przedłużaniem czasu działania glikozydami. Po około 1 godz. 45 min. w prometafazie i metafazie powstają ponadto struktury dwumembranowe w postaci bardzo wąskich kanalików czy bardzo silnie spłaszczonych cystern tworzących różne konfiguracje, często układy paralelne i koncentryczne (fig. 1, tabl. I). Średnica cystern nie jest jednakowa; w niektórych (przypadkowych) miejscach są silnie zwężone, wpukłone, a ich błony prawdopodobnie stykają się ze sobą, co daje obraz koralików. Cysterny te zakończone są zwykle pęcherzykami, które mogą się odrywać i powiększać. Na błonach opisywanych struktur, w przypadkowych miejscach, występują ciemne utwory określone jako pory, odpowiadające porom błony jądrowej (NE). Podobne budową błony opisali Bajer i Molè-Bajer (1969), oraz Roos (1973) w normalnie dzielących się komórkach, a także Molè-Bajer (1969) po działaniu chloralhydratem.

Analiza obrazów submikroskopowych skłania mnie do przyjęcia wniosku, że te bardzo wąskie dwumembranowe struktury to endoplazmatyczne retikulum, a ciemne utwory — to tworzące się pory, co oznacza, że przynajmniej część tak liczego ER przekształca się w NE. Ta powstająca pozornie wcześniej i w nadmiarze ilość NE związana jest, jak się wydaje, z większym zapotrzebowaniem, bowiem w zaawansowanej prometafazie i metafazie w komórkach poddanych działaniu glikozydów oleandra istnieje silna tendencja do powstawania licznych jąder (Tarkowska, 1971a, b).