OBSERVATIONS ON CERTAIN PLASTIDS OF THE OVULE OF *GINKYO BILOBA* L.

AMBRETTA CECCHI FIORDI and ELENA MAUGINI

Botany Institute, University of Florence, Florence, Italy

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It appears that the localization of elements of endoplasmic reticulum (ER), so as to form a periplastidial sheath around certain plastids, is fairly frequent in the ovule of *Ginkyo biloba* L. In the sections this sheath completely or partially envelopes the plastids. This phenomenon can be observed in the endosperm (Fig. 1), as described by CAMEFORT and SCHAEFFER (1965), in the tapetum (Fig. 2) (but only rarely), in the central cell of the archegonium (Fig. 3)(1), and in the oosphere (CAMEFORT 1965).

A search of the literature relevant to the relationships between ER and plastids showed that in several groups of algae there exists a periplastidial sheath of ER (GIBBS 1962; BERKALOFF 1963; BOUCK 1965; DODGE 1969; MASSALSKI and LEEDALE 1969; LUCAS 1970; KIERMAYER 1970). In some cases this sheath is smooth and in others it bears ribosomes on the cytoplasmic side of the surface. Moreover, since it is very often continuous with the nuclear membrane, it was thought that such an association would allow a direct passage of substances between nucleus and chloroplasts (GIBBS 1962). In certain brown algae (BOUCK 1965) the « chloroplast ER » can be continuous either with the nuclear membrane or with the cytoplasmic ER. Vesicles would be formed both from the nuclear membrane and from the cytoplasmic ER and would then be incorporated in the Golgi apparatus. According to BOUCK (1965), at least some of the photosynthesis products, after having passed into the « chloroplast ER » lumen, would perhaps thereafter be conveyed to the dictyosomes for the elaboration of certain components of the cell wall. Analogously, the « periplastidial cisterna » described in *Micrasterias*

(1) For Material and Methods see MAUGINI and CECCHI FIORDI (1970).

denticulata Bréb. (*Desmidiaceae*) would probably be used to transport the products synthesized in the plastids to the Golgi apparatus (KIERMAYER 1970). BERKALOFF (1963) hypothesizes that the constant localization of an ER element around the young plastids of the meristematic cells of *Himanthalia Lorea* (L.) S. F. Gray is perhaps important for the differentiation of the plastids themselves.

Although such an association is typical of algae (LUCAS 1970), it has also been observed in some higher plants. In the callus from *Pinus pinea* leaf, grown on solid agar medium, and in the sieve tubes of *Acer pseudoplatanus,* an element of ER with ribosomes on the cytoplasmic side of the surface surrounds the plastids during certain stages of their development, and could take part in certain transformations of the plastids themselves (WOODING and NORTHCOTE 1965*b*; NORTHCOTE and WOODING 1966). A periplastidial sheath having the same characteristics has also been observed in the phloem companion cells of *Acer pseudoplatanus* and in the cells of the resin canals of *Pinus pinea.* It has been hypothesized that in *Acer pseudoplatanus* it would be part of a sucrose transport system between the sieve tubes and the plastids of the companion cells, and in *Pinus pinea* the sheath would be involved in the synthesis of the resin and its transfer to the lumen of the canals (WOODING and NORTHCOTE 1965*a*, 1965*b*, 1965*c*). A participation of the smooth periplastidial ER in the secretory activity is also admitted by SCHNEPF in the gland hairs of *Calceolaria* (1969*a*) and in the oil channels of certain *Umbelliferae* (1969*b*).

As far as concerns the ovule of *Ginkyo biloba*, it might be supposed that the elements of ER localized around the plastids of the endosperm provide a particularly efficient conduction of carbohydrates from the endosperm to the interior of the archegonium — a situation partially analogous to that formulated by WOODING and NORTHCOTE (1965*a*, 1965*b*) in the phloem of *Acer pseudoplatanus.* In the tapetum the association between ER and plastids would be more infrequent perhaps because this endospermic layer is preferentially reserved for the passage of nutritive substances rather than for their synthesis and accumulation. In fact, the plastids of the tapetum cells differentiate only a few stromatic lamellae and contain scanty grains of

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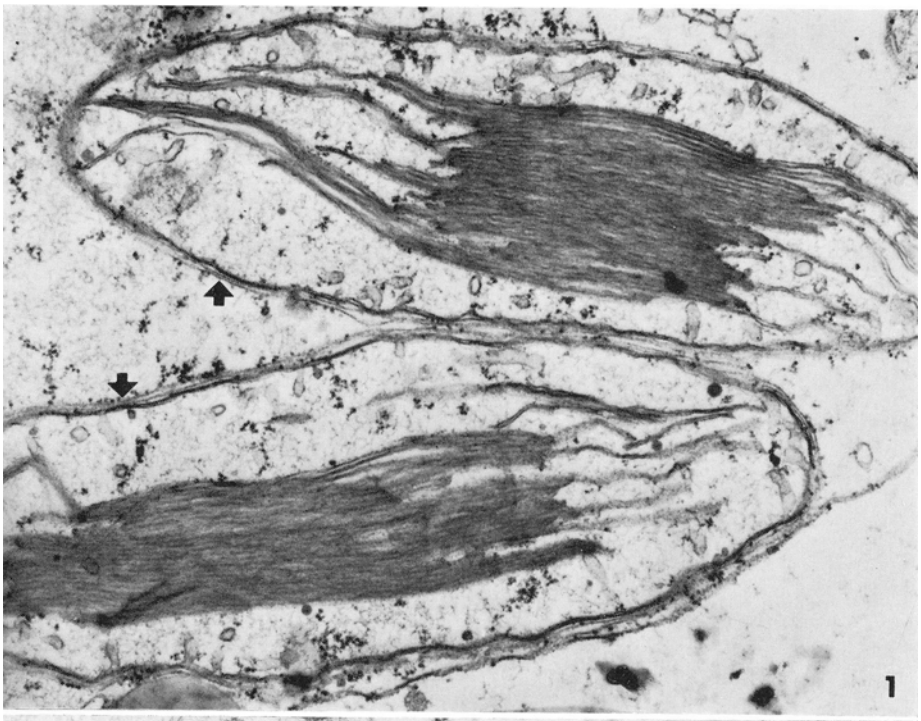
Fig. 1. — Two endosperm plastids surrounded by an element of ER (arrows) (x 27,950 ca.).
Fig. 2. — Plastid of a cell of the tapetum surrounded by an element of ER (arrow) (x 35,900 ca.).

Fig. 1. — Due plastidi dell'endosperma, di cui ognuno è circondato da un elemento di reticolo endoplasmico (r.e.) (frecce) (x 27.950 ca.).
Fig. 2. — Plastidio di una cellula del tappeto circondato da un elemento di r.e. (freccia) (x 35.910 ca.).



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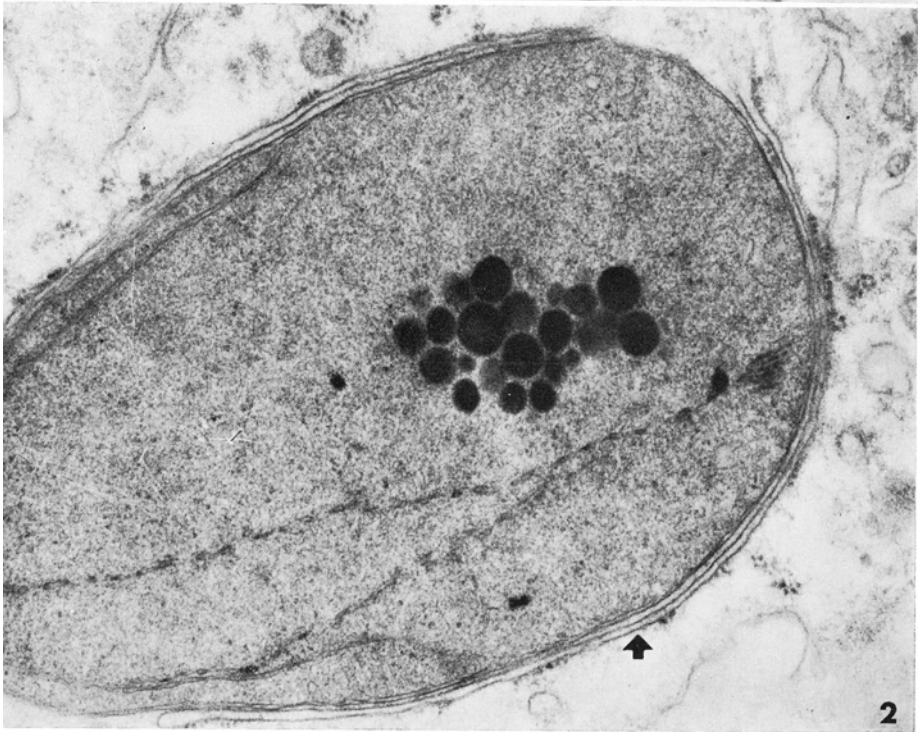

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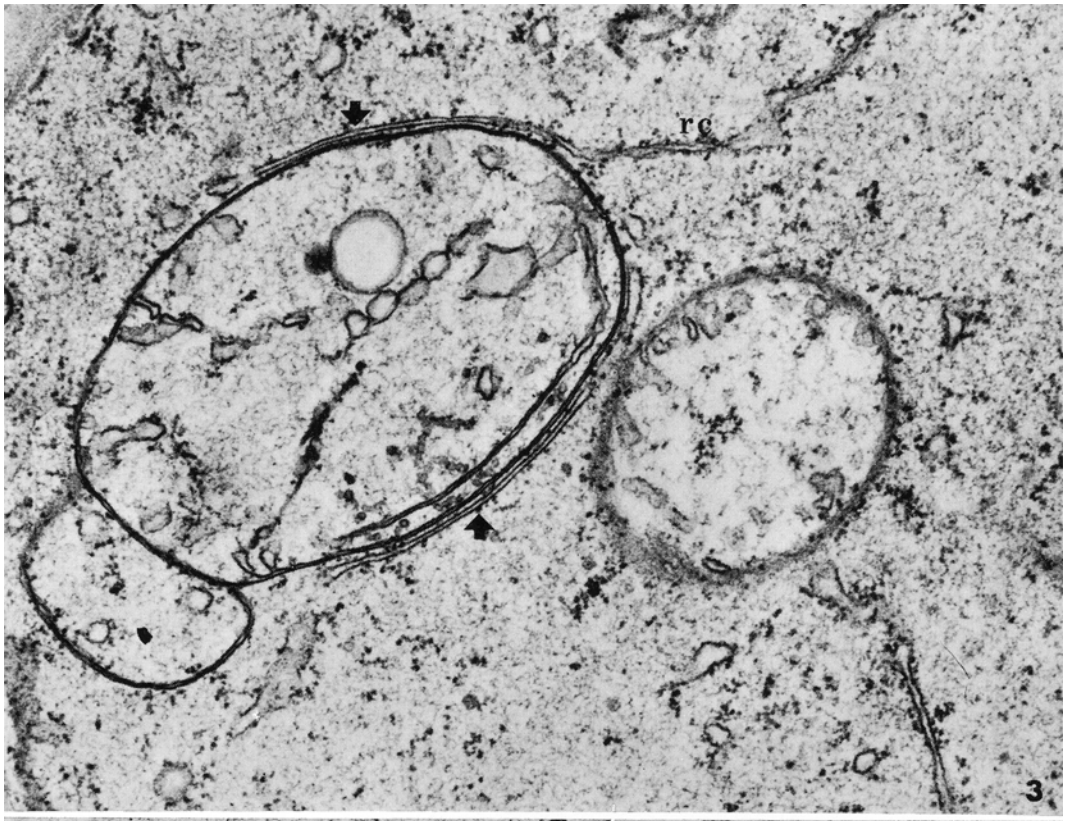
starch. The ER that partially or totally surrounds the plastids of the central cell contains very few ribosomes and can be continuous with the plasma membrane (Fig. 6 in MAUGINI and CECCHI FIORDI 1970) or with the rough-surfaced cytoplasmic ER (Fig. 3). The plastids, moreover, often appear to be undergoing scission (Fig. 4). We therefore feel that the ER associated with these organelles could convey to them, both from the extracellular space and from the cytoplasm, not only carbohydrates, which would be deposited as a few scanty starch grains, but perhaps also other substances necessary for plastid growth and multiplication. On the other hand it is presumable that such processes require rather large quantities of plastic and energetic material, given that it is often observed that a plastid will give rise, simultaneously, to three new organelles (Fig. 4). The possibility of some relationships between periplastidial ER and plastidial transformation processes has been previously formulated, as was mentioned above, both in vascular plants (WOODING and NORTHCOTE 1965*b*; NORTHCOTE and WOODING 1966) and in algae (BERKALOFF 1963).

Concerning the plastids of the central cell, it was mentioned in a previous paper on the ovule of *Ginkyo biloba* (MAUGINI and CECCHI FIORDI 1970) that, in certain cases, profiles of ER appear to be continuous with the outer layer of the plastidial wall. A more accurate examination of the same material led us to interpret these profiles not as elements of ER but rather as expansions of the outer layer of the double membranes of the plastids (Fig. 5), since we observed that these membranes are more similar, in thickness and in density to the electrons, to the two elements of the plastidial wall than to both cytoplasmic and periplastidial membranes of the ER. The functional significance of these evaginations of the wall of the plastids is unknown to us. Their origin could perhaps be related to an unusual multiplication process involving scission of the plastids themselves.

Fig. 3. — Plastid of the central cell of the archegonium partially surrounded by the ER (arrows); one of the two elements of the ER associated with the plastid is continuous with the cytoplasmic ER (r.c.) (x 31,920 ca.).
Fig. 4. — Plastid of the central cell of the archegonium that splits into three new organelles (x 14,880 ca.).
Fig. 5. — Plastid of the central cell of the archegonium whose outer membrane bears an evagination (arrow) (x 23,260 ca.).

Fig. 3. — Plastidio della cellula centrale dell'archegonio parzialmente circondato dal r.e. (frecce); uno dei due elementi di r.e. associati al plastidio è continuo con il r.e. citoplasmatico (r.c.) (x 31.920 ca.).
Fig. 4. — Plastidio della cellula centrale dell'archegonio che si divide in tre nuovi organuli (x 14.800 ca.).
Fig. 5. — Plastidio della cellula centrale dell'archegonio la cui membrana esterna della parete mostra una evaginazione (freccia) (x 23.260 ca.).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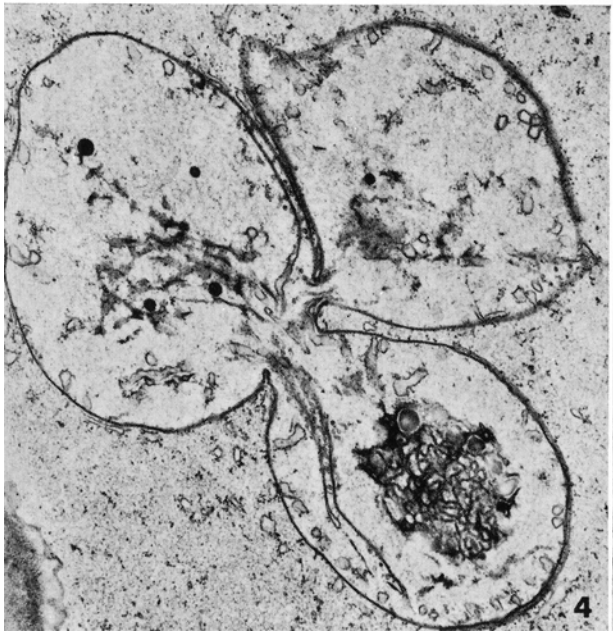
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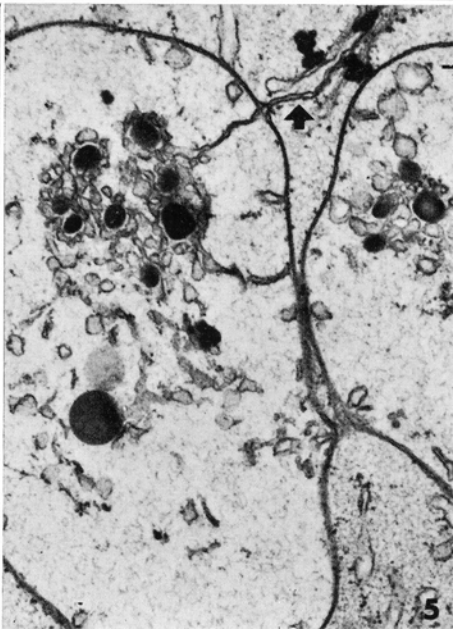
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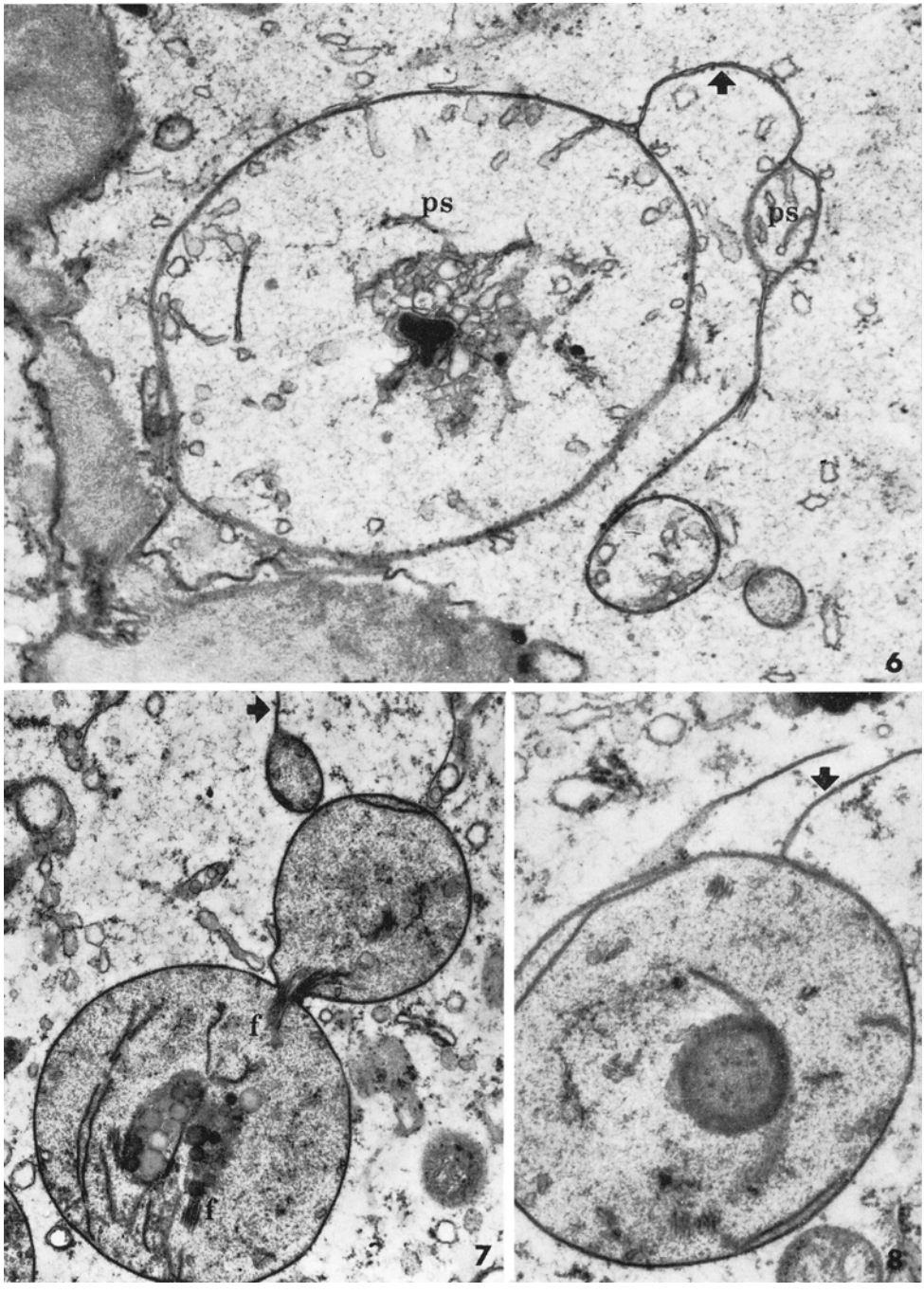
In fact, two of these organelles often appear to be connected to one another by an expansion of their outer membrane, probably in the form of a tubule or a flattened saccule. These images could be interpreted as the result of the fact that the division of a plastid into new organelles initially involves only the plastidial stroma and the inner membrane of the wall, whereas the outer membranes of the newly-formed plastids would remain continuous with each other for a certain time by means of a more or less long evagination. As a consequence, the periplastidial spaces would also remain temporarily intercommunicating. The new plastids that are formed would not be, therefore, completely independent even from their first characterization. The morphological continuity, even if limited to the outer membranes and to the periplastidial spaces, probably reflects a temporary physiological interdependence.

The plastids of the coenocytic pro-embryo show the peculiarity of having little fibrils bundles of about 165 Å thickness in their stroma. Little fibrils bundles have often also been observed in the plastids of the subsidiary cells of the stomata of *Opuntia* sp. (THOMSON and DE JOURNETT 1970); however, these fibrils have a diameter of only about 60 Å. The plastids of the coenocytic pro-embryo of *Ginkyo biloba*, like those of the central cell, are often in the process of division (Fig. 7) and their outer membrane bears several evaginations that extend into the cytoplasm (Fig 8). The origin of these latter could be the same as that discussed when we considered the plastids of the central cell. In the oosphere of *Ginkyo biloba* a particular plastid fragmentation process has been observed (CAMEFORT 1965), which, however, is completely different from the one hypothesized by us in the central cell and in the coenocytic pro-embryo, and which, indeed, probably leads to a complete disorganization of the plastids themselves.

Fig. 6. — Two plastids (ps) of the central cell of the archegonium connected by an evagination (arrow) of the outer membrane (x 22,500 ca.).
Fig. 7. — Plastid of the coenocytic pro-embryo during scission; little bundles of fibrils (f) can be seen in the stroma. In the upper portion of the figure the superficial section of a plastid whose outer membrane bears an evagination (arrow) can be noted (x 15,390 ca.).
Fig. 8. — Plastid of the coenocytic pro-embryo whose outer membrane bears an evagination (arrow) (x 26,150 ca.).

Fig. 6. — Due plastidi (ps) della cellula centrale dell'archegonio collegati da una evaginazione (freccia) della membrana esterna (x 22.500 ca.).
Fig. 7. — Plastidio del proembrione cenocitico in corso di scissione; nello stroma si osservano fascetti di fibrille (f). Nella parte alta della figura si nota la sezione superficiale di un plastidio la cui membrana esterna presenta una evaginazione (freccia) (x 15.390 ca.).
Fig. 8. — Plastidio del proembrione cenocitico la cui membrana esterna presenta una evaginazione (freccia) (x 26.150 ca.).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SUMMARY

As reported in the literature for several groups of algae and for certain cells of higher plants, among which the endosperm (CAMEFORT and SCHAEFFER 1965) and the oosphere of *Ginkyo biloba* L. (CAMEFORT 1965), the authors observe that there is also often a contiguity between endoplasmic reticulum and plastids in the tapetum and in the central cell of the archegonium of this plant. The functional significance of this relationships is hypothesized. Moreover, the authors note that in the central cell and in the coenocytic pro-embryo the outer membrane of the plastidial wall bears some evaginations that extend into the cytoplasm. These evaginations seem, in certain cases, to connect two plastids in the central cell, for which it is thought that their formation is due to a particular scission process of the plastids themselves.

RIASSUNTO

Come riportato dalla bibliografia per diversi gruppi di alghe e per certe cellule di piante superiori, fra cui l'endosperma (CAMEFORT e SCHAEFFER 1965) e l'oosfera di *Ginkyo biloba* L. (CAMEFORT 1965), gli AA. osservano che anche nel tappeto e nella cellula centrale dell'archegonio di questa stessa pianta vi è spesso un rapporto di contiguità tra reticolo endoplasmico e plastidi; di tale rapporto viene prospettato il probabile significato funzionale. Gli AA. inoltre segnalano che nella cellula centrale e nel proembrione cenocitico la membrana esterna della parete plastidiale presenta delle evaginazioni che si protendono nel citoplasma. Queste, nella cellula centrale, appaiono, in alcuni casi, collegare due plastidi, per cui si pensa che la loro formazione sia dovuta ad un particolare andamento del processo di scissione dei plastidi stessi.