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Membrane Continuities Involving Chloroplasts and Other Organelles in Plant Cells

Abstract. *Plant cell membranes were examined in thin sections by electron microscopy. Numerous membrane continuities appeared between chloroplasts and other organelles of the fern* Pteris vittata *L., and occasionally in the leaves of the tomato* Lycopersicon esculentum *Mill. A number of these continuities had not been reported earlier or had been seen only infrequently.*

Although many continuities between different organelles and membrane systems have been reported, some possibilities, especially those involving the chloroplast, have not yet been documented (*1*). In this report we describe at least 14 different combinations of continuities between organelles and membrane systems in the fern gametophyte of *Pteris vittata* L., and describe several that involve the outer membrane of the chloroplast. We also present evidence from higher plants which suggests that such continuities may be more common than is generally recognized.

The observations on *Pteris* were made on the cells of two different prothallia (6- and 12-cell specimens), which were grown as reported (*2*), fixed in 3 percent glutaraldehyde in cacodylate buffer, postfixed in osmium tetroxide, dehydrated in acetone, and embedded in an epoxy plastic. Leaves of tomato (*Lycopersicon esculentum* Mill.) were similarly processed.

Continuities were observed in *Pteris* between the outer membrane of the chloroplast and each of the following: (i) the rough endoplasmic reticulum (Figs. 1 and 4), (ii) the plasma membrane (Fig. 2), (iii) the outer membrane of the mitochondrion (Fig. 4), (iv) the tonoplast, (v) the dictyosome vesicle, and (vi) the microbody (the latter three are not shown here). If a junction between the outer membranes of the nucleus and chloroplast were seen in this organism, it would complete the possible combinations between the chloroplast and other major membranous organelles.

Continuities observed between other organelles in *Pteris* (most not illustrated here) include conjoinings between the outer mitochondrial membrane and (i) the plasma membrane and (ii) the tonoplast; between the dictyosome vesicles and (i) the plasma membrane and (ii) the tonoplast; and between the endoplasmic reticulum and (i) the outer nuclear membrane, (ii) the dictyosome cisterna, (iii) the plasma membrane, and (iv) the outer mitochondrial membrane (Fig. 4). The interconnections between the endoplasmic reticulum and these organelles have been noted in several organisms (*3*).

Several multiple conjunctions have been observed in *Pteris*. A somewhat complicated situation is shown in Fig. 4: The perichloroplast and perimitochondrial spaces are in continuity through a cisternum of the rough endoplasmic reticulum; this is in addition to the chloroplast-mitochondrion continuity at another site in the same photomicrograph. Serial sections reveal that the two mitochondrial profiles in Fig.

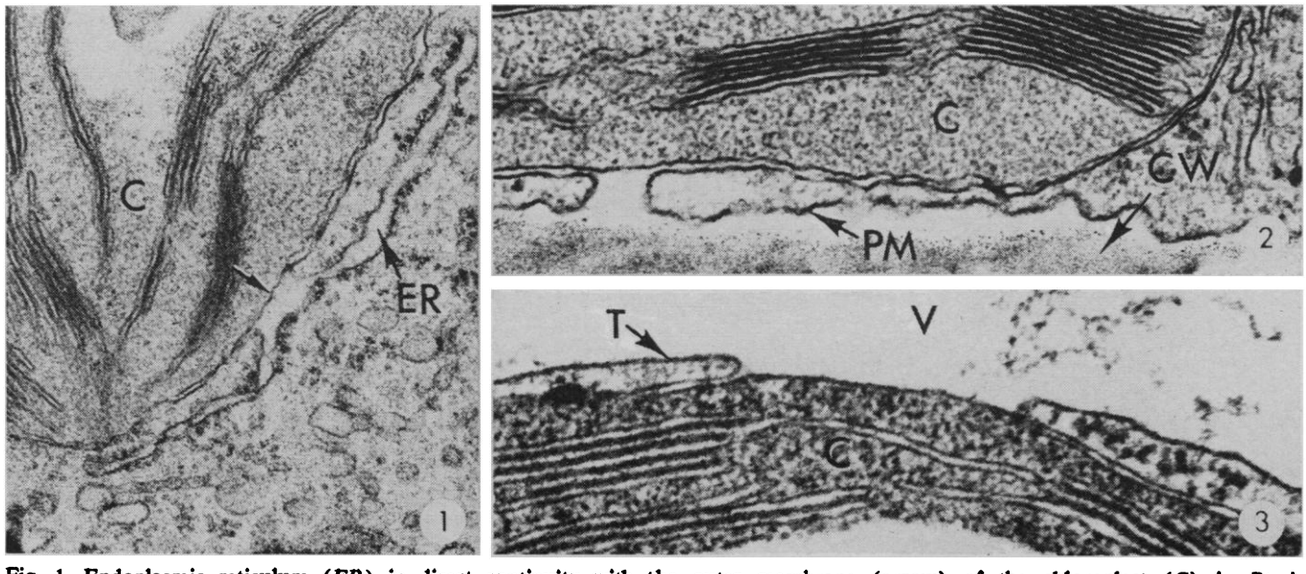

Fig. 1. Endoplasmic reticulum (*ER*) in direct continuity with the outer membrane (arrow) of the chloroplast (*C*) in *Pteris vittata* L. (× 40,000). Fig. 2. Outer membrane of the chloroplast (*C*) in continuity with the plasma membrane (*PM*) near the cell wall (*CW*) of *P. vittata* (× 62,000). Fig. 3. Outer membrane of the chloroplast (*C*) in continuity with the tonoplast (*T*) of the vacuole (*V*) in a leaf cell of tomato, *Lycopersicon esculentum* Mill. At another location, the same chloroplast outer membrane is in continuity with the plasma membrane (as in Fig. 2), so that the perichloroplast space is open to the vacuole and to the outside of the protoplast simultaneously (× 76,000).

4 belong to a single organelle. A review of the literature indicates that in *Pteris* there are an unusually large number of different types of membranous organelles involved in the conjoinings as well as unusually high frequencies of particular types of continuities.

In addition to those in the fern gametophyte, clear continuities were also observed between the outer membrane of the chloroplast and the tonoplast in the tomato (Fig. 3); in another region of this chloroplast, the outer membrane is in continuity with the plasma membrane, as shown for *Pteris* in Fig. 2. A continuous channel therefore exists between the vacuole and the outside of the protoplast via the perichloroplast space. The same situation can be demonstrated in *Pteris*. Although no continuities between the outer nuclear membrane and other organelles (except for the endoplasmic reticulum) were seen in *Pteris*, we observed such a connection between the nuclear membrane and outer mitochondrial membrane in the flower of wheat (*Triticum aestivum* L.); connections involving the nucleus have been reported for other plants (*4*).

Although close association between the chloroplast and other organelles such as the nucleus or the endoplasmic reticulum has been reported in electron micrographs (*5*), an actual continuity with the chloroplast was first reported by Diers (*6*). In a careful study of archegonium and egg development in the liverwort *Sphaerocarpus donnellii* Aust., he only found a single instance of membrane continuity between the outer membrane of a plastid and the smooth endoplasmic reticulum. Diers felt that, because of the low frequency, an explanation invoking accidental coalescence was more likely than one involving a functional relationship. If the observations reported for *Pteris* can be confirmed as having more than limited significance, then the frequency with which endoplasmic reticulum appears in continuity with the outer chloroplast membrane in these specimens would suggest (i) that there may be, at certain times, direct functional channels between the perichloroplast space and the cisterna of the endoplasmic reticulum, and substances may pass in either direction between the chloroplast and the endoplasmic reticulum; and (ii) that the chloroplast may actually give rise to some of the plant cell endoplasmic reticulum in the same manner sometimes ascribed to the nucleus. The same considerations and suggestions apply to other conjoinings. These suggestions would require considerable experimental verification. A study by Cran and Dyer (*7*) of the gametophyte of the fern *Dryopteris borreri* provides additional evidence of direct joinings between the outer chloroplast membrane and the smooth endoplasmic reticulum. These authors also show a clear connection between the outer chloroplast membrane and the plasma membrane, as we had found (*1*).

Evidence based on phase cinephotomicrography at light microscope magnification indicates that bodies indistinguishable from mitochondria are derived from chloroplasts and that these bodies can fuse with each other, and with chloroplasts (*8*). Although some supporting evidence for the possible origin of mitochondria-like structures from chloroplasts has been presented by Öpik (*9*), others (*10*) have reservations. No electron microscopic evidence has been presented to clarify the nature of the conjoinings seen by cinephotomicrography. The observations reported here suggest at least one type of membrane connection that might explain the temporary adhesions between these bodies.

Membrane continuities have often been cited as evidence of the origin of one organelle from another. Other investigators, however, have pointed out the danger of making such interpretations based only on static electron micrographs. We share the latter reservation, and emphasize that the observations reported here cannot be used per se as evidence of organelle derivation. Indeed, before questions of membrane transformation and interaction can be pursued in the organisms described here, it will be necessary to explore the possibility that these confluencies have been induced by some unspecified variable in the handling and preparation of the material. It is also possible that the phenomena are normal, but are related to a peculiar physiological condition, or to a developmental situation atypical of more mature stages.

Some confusion and skepticism about membrane continuities have developed because continuities have often been cited as evidence favoring one model of membrane architecture over others. Although Robertson (*11*) proposed a modified Danielli-Davson structure when he formalized the notion of the unit membrane and the idea of membrane homology, the phenomenon of continuities does not, of itself, demand acceptance of one model of membrane architecture over any other. As interest in mecha-

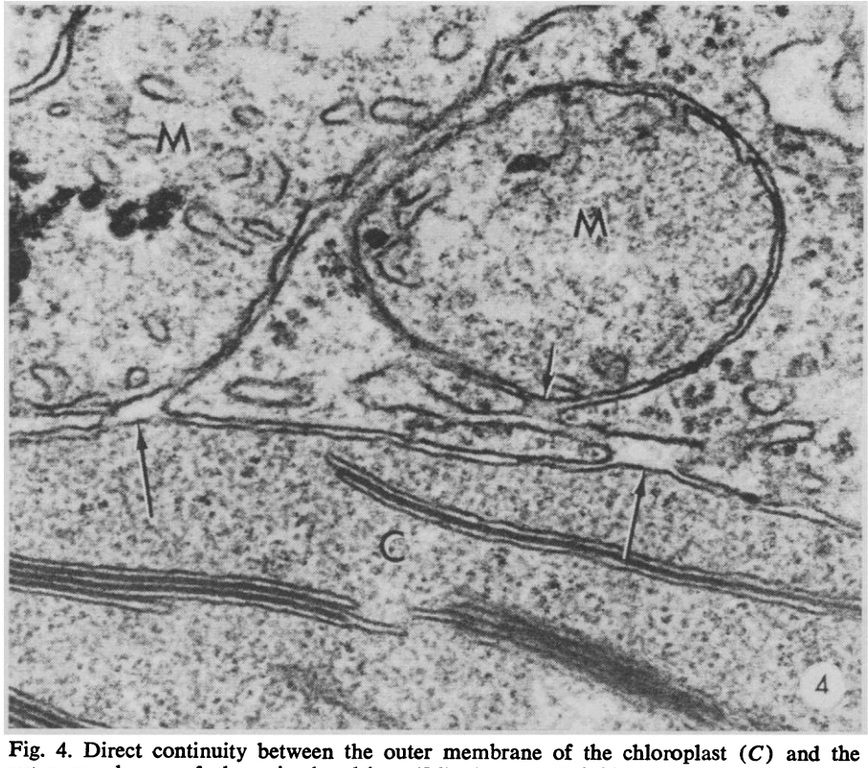

Fig. 4. Direct continuity between the outer membrane of the chloroplast (*C*) and the outer membrane of the mitochondrion (*M*) (arrow at left), and a more complex interconnection between the outer chloroplast membrane, the endoplasmic reticulum, and the outer mitochondrial membrane (arrows at right). Both mitochondrial profiles in *P. vittata* are from the same organelle, as revealed by serial sections (× 88,000).

nisms of membrane fusions has grown, it has become clearer that explanations of membrane fusions can start from different models of membrane architecture (*12*). One of the fruitful results of such model building is that ideas will be generated that can be tested experimentally, and this may help to explain the observations reported here.

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Porphyrin-Heme Pathway: Regulation by Intermediates in Bile Acid Synthesis

Abstract. *Dihydroxycoprostane and trihydroxycoprostane, intermediates in normal bile acid synthesis in the liver, enhanced the rate of porphyrin synthesis in cultured liver cells by induction of δ-aminolevulinate synthetase, the rate-limiting enzyme for this pathway. Other 5β-cholestane derivatives and cholest-5-ene derivatives were ineffective. The selectivity of the induction may indicate that the above-mentioned coprostanes have a physiological role in porphyrin synthesis.*

We have found that two normal intermediates in bile acid synthesis, dihydroxycoprostane (*1*) and trihydroxycoprostane stimulate porphyrin formation in cultured liver cells by induction of δ-aminolevulinic acid synthetase (ALAS), the rate limiting enzyme for this biosynthetic pathway. These compounds are precursors in the formation of bile acids from cholesterol (*2*), and their rate of production in man is estimated to be 200 to 400 mg/day. Their accumulation in liver cells can therefore be a potent endogenous stimulus for porphyrin overproduction in liver disease.

Dihydroxycoprostane and trihydroxycoprostane were prepared by electrolytic reduction and condensation of isovaleric acid with either chenodeoxycholic acid or cholic acid (*3*). The reaction mixture was fractionated by alumina chromatography and the final product was recrystallized from aqueous acetone (*3*). Other intermediates in bile acid synthesis such as 7α-hydroxycholesterol and 26-hydroxycholesterol were prepared as described (*4*, *5*). Related compounds were obtained from commercial sources and were either recrystallized or purified by thin-layer chromatography prior to use.

Chick embryo liver cells were grown in culture according to the method described by Granick (*6*). Briefly, livers from 14- to 15-day chick embryos were minced, trypsinized, and suspended in Earle's basic salt solution. Aliquots of 5×10^5 cells were added to glass vials, each of which contained a 16-mm cover slip. To each vial, 1 ml of Eagle's basal medium supplemented with amino acids and antibiotics was added, and the cells were grown at 37°C in 5 percent CO_2 in air for 24 hours. The medium was then changed, and additions were made to the vials. The compounds to be tested were added to the cultures in 5 μl of propylene glycol, with at least four replicate vials at each dose. In each assay, controls included 10 to 15 vials to which 5 μl of propylene glycol and 5 to 14 vials to which 20 μg of allylisopropylacetamide (AIA), a compound known to be a potent inducer of porphyrin formation, were added. After incubation for an additional 20 to 24 hours, the vials were frozen, and their contents were lyophilized. Porphyrins were extracted and quantified as described (*7*) in a spectrophotofluorimeter (Hitachi model MPF 2A), at an excitation wavelength of 400 nm and an emission wavelength of 650 nm. Coproporphyrin III was used as a standard (Fig. 1).

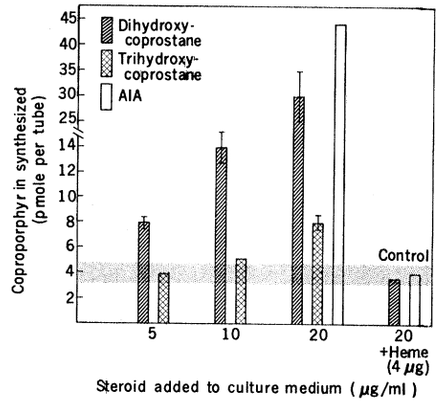

Fig. 1. Induction of porphyrin synthesis in liver cell culture by di- and trihydroxycoprostanes (see text).

Dihydroxycoprostane and trihydroxycoprostane caused significant stimulation of porphyrin synthesis (Fig. 1). The effect was dose-related and consistent throughout the dose ranges studied. Dihydroxycoprostane always induced significantly more porphyrin synthesis than trihydroxycoprostane. Further delineation of the mechanism of coprostane induction of porphyrin synthesis was obtained by studying the effect of hemin on this process (Fig. 1) and by directly determining the activity of mitochondrial ALAS in coprostane-treated 17-day-old chick embryos (*8*). Hemin completely suppressed the porphyrinogenesis induced by dihydroxycoprostane in cultures (Fig. 1), while a 20-fold increase in hepatic ALAS activity was produced in vivo in the chick embryo. These findings indicate that the coprostane compounds act on the porphyrin-heme pathway in a manner analogous to that of drugs, foreign chemicals, and neutral 5β-steroid hormone metabolites (*6*, *8*, *9*).

In contrast 7α-hydroxycholesterol and 26-hydroxycholesterol, bile acid intermediates that do not have a 5β nucleus, failed to stimulate porphyrin production. Similarly, the dihydroxy- and trihydroxy-5β-cholanoic acids and their taurine conjugates were also inactive in cell culture. Although the monohydroxy