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Outer Mitochondrial Membrane Continuous with Endoplasmic Reticulum

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With 7 Figures

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Summary

Continuity of outer mitochondrial membrane with tubular profiles of endoplasmic reticulum (ER) character is found in rat liver hepatocytes and in the ciliate *Tetrahymena pyriformis*. Such membrane continuity is evident from thin sections through intact cells and isolated fractions as well as with negatively stained isolated mitochondria. The ER-profiles continuous with the outer mitochondrial membrane appear predominantly "smooth", whereas in some cases they showed a few ribosomes associated with them. The observations are discussed as another indication of the close structural and chemical relationship between the outer mitochondrial membrane and the ER. In addition, it is hypothesized that this cisternal continuity provides a route for transfer of special proteins (and possibly lipid components, too) from the rough ER into the mitochondria.

1. Introduction

In many cell types, mitochondria are located very close to cisternae of the endoplasmic reticulum (ER) and the nuclear envelope (reviews in ROODYN and WILKIE 1968, STANG-VOSS and STAUBESAND 1970). This structural relationship has brought about a number of speculations on how ER-like cytomembranes, especially the nuclear envelope, could take part in mitochondrial formation. During work with cell types in which mitochondrial "enjacketing" by ER-cisternae is especially frequent, such as mammalian hepatocytes and the ciliate *Tetrahymena pyriformis*, we repeatedly found situations in which the outer mitochondrial membrane appeared continuous with cisternal membranes of ER-character.

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2. Materials and Methods

Tetrahymena pyriformis (amicronucleate strain GL) cultures were kept, grown into exponential phase, fixed and prepared for thin section investigation as described elsewhere (FRANKE *et al.* 1971). For negative staining (2% phospho-tungstic acid, adjusted to pH 7.0), cells were disrupted either in distilled water or in isolation medium proper for isolating macronuclei and mitochondria from this organism (*cf., e.g.*, FRANKE 1967) with a few strokes of a glass-teflon Potter-Elvehjem homogenizer. Drops of this "homogenate" were immediately placed onto formvar-film coated copper-grids, staining solution was added, and the whole rapidly sucked off.

Rat liver tissue was fixed and prepared for thin section electron microscopy as previously outlined (KARTENBECK and FRANKE 1971). Fractions enriched in microsomes or mitochondria were prepared from freshly obtained rat liver tissue using the isolation medium given in a previous article (FRANKE *et al.* 1970).

A Siemens electron microscope IA was used, and the sections were made with a Reichert ultramicrotome OmU 2.

3. Results and Discussion

In thin sections through *Tetrahymena* cells (Figs. 2 and 3), as well as through rat hepatocytes (Fig. 5) and also through mitochondria isolated therefrom (Fig. 4), membranous continuities between the outer mitochondrial membrane and distinct cisternal or tubular profiles suggesting an ER-character could be found. The tubules and sacs which are in such a direct continuity with the outer mitochondrial membrane appear predominantly smooth, although, in some instances (Figs. 2–4), an attachment of ribosomes was recognized. Corresponding continuities of the outer mitochondrial membrane with ER-like tubular or tubulo-vesicular membrane profiles were also routinely observed with the negatively stained mitochondria isolated from *Tetrahymena* cells (Fig. 1). The maximum number of such continuities counted for an isolated *Tetrahymena* mitochondrion was 5, whereas 2 to 3 such connections per mitochondrion were relatively more frequent (*e.g.*, Fig. 1). It cannot be totally excluded at the moment that such profiles might represent tubular myeliniza-

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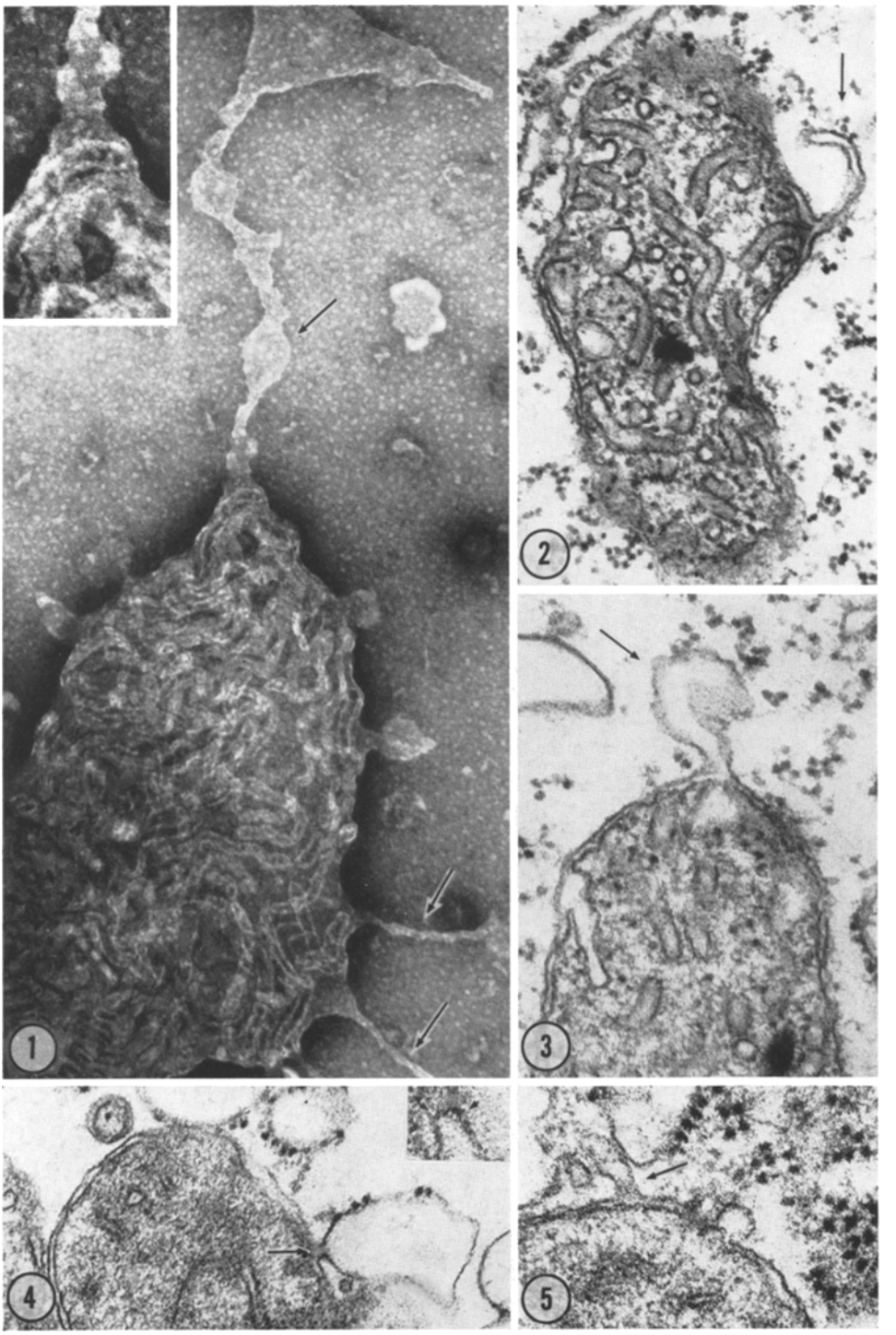
Fig. 1. Isolated mitochondrion of *Tetrahymena pyriformis* GL showing continuities of the outer membrane with tubules and sacs of ER-character (arrows). The very site of such a continuity is detailed in the inset. Note also the dense arrangement of inner membrane tubules within the mitochondrion. Magnification ×50,000; inset ×100,000

Figs. 2 and 3. Thin sections through *Tetrahymena* which show a continuity of the outer mitochondrial membrane with tubule- (Fig. 2) or sac-like (Fig. 3) cisternal membranes. These profiles occasionally appear studded with ribosomes (arrows). Fig. 2 ×55,000; Fig. 3 ×75,000

Fig. 4. Section through isolated rat liver mitochondria showing continuity of the outer mitochondrial membrane with ribosome-bearing profiles of ER-character (arrow). This membrane continuity is shown at higher magnification in the inset. Magnification ×60,000; inset ×150,000

Fig. 5. Section through a rat hepatocyte fixed in situ. Continuity of outer mitochondrial membrane with ER-like profiles is recognized (arrow). Magnification ×120,000



Figs. 1–5

tion artifacts such as are known to occur, especially under hypotonic conditions, with diverse types of membranes (though relatively infrequent with the outer mitochondrial membrane; compare, *e.g.*, MUNN 1968, PARSONS *et al.* 1966), or that they are extensions of the outer membrane pulled away from the inner membranes plus mitochondrial matrix. However, the general resemblance of these structures to the many "true" ER-tubules lying next by as well as their occurrence in the thin sectioned samples reduces this type of argument. Tight associations of ER-elements with isolated mitochondria

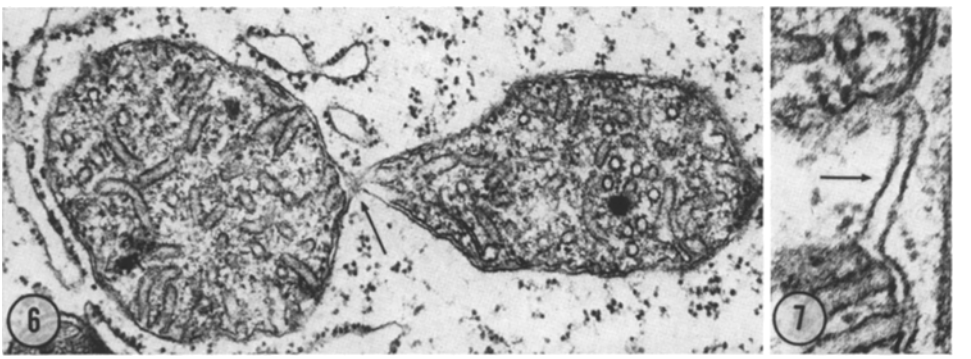


Fig. 6. Mitochondrial configuration in *Tetrahymena* which suggests a late stage in mitochondrial fission. The two "daughter mitochondria" appear connected by an isthmus (arrow) of the outer membrane. Magnification ×42,000

Fig. 7. A slender about 300 Å wide tubule is continuous with the outer membrane of either of two neighboured mitochondria. This may suggest an outer mitochondrial membrane connection persisting after mitochondrial fission for a while. Magnification ×80,000

have also been mentioned by various authors, usually in the sense as a somewhat "inevitable contamination". For the fractions, it could be argued that such a continuity might have been artificially caused by membrane breakage and subsequent re-fusion processes. This argument, however, seems very unlikely for thin sections through the whole cells and also for the negative stainings. Another argument, namely that the outer mitochondrial membrane-connected tubules might represent just extensions of this membrane residual from the mitochondrial fission process (SWIFT 1965) seems to receive some support from the observation of relatively frequent situations suggesting mitochondrial fission in the exponentially growing *Tetrahymena* cells (*e.g.*, Fig. 6). During such stages, in fact, a tubular connection of the outer membrane appears to persist and to connect the two daughter mitochondria for a certain time span as this is suggested, *e.g.*, from Fig. 7. However, these "post-division-tubules" are unlikely to be the only explanation for the structural connection described in view of the fact that more than one such tubule can be distinguished per mitochondrion. It is also difficult to conceive that

the tubular profiles represent merely accidental local extensions of the outer mitochondrial membrane, since with the negative stained mitochondria they could be traced for more than 5 micra. The fact that the continuities are rather rarely observed and occupy only a very small area of the total mitochondrial surface might indicate that they are either temporary or very infrequent. Similar continuities with ER-like profiles have hitherto been mentioned sporadically from various cell types (*e.g.*, ROBERTSON 1960, SANDBORN *et al.* 1964), especially with vertebrate oocytes (RUBY *et al.* 1969, KESSEL 1971). SPRIGGS *et al.* (1967) reported continuities of the outer mitochondrial membrane with the membranes of the terminal vesicles of adrenergic axons. In two other articles of this issue, BRACKER and GROVE (1971) describe such continuities of the outer mitochondrial membrane with ER-like tubules for the oomycetous fungus, *Pythium aphanidermatum*, and MORRÉ and associates (1971) elucidate the nature of the particular ER being continuous with the outer mitochondrial membrane in the rat hepatocytes. Taken together, these descriptions with the present study, it is obvious that ER-outer mitochondrial membrane connections occur throughout very different cell types and apparently are a general structural feature of the cellular cytomembrane (= endomembrane) system. Such structural continuities between the two membrane systems are especially interesting in the light of the well-known similarities of ER and outer mitochondrial membrane with respect to structure, lipid and protein composition including some common enzymes (*e.g.*, PARSONS *et al.* 1966, LEVY *et al.* 1967, PARSONS and YANO 1967, SOTTOCASA *et al.* 1967, BRUNNER and BYGRAVE 1969, ERNSTER and KUYLENSTIERNA 1970, PARKES and THOMPSON 1970) and certain turnover kinetics (*e.g.*, BYGRAVE 1969, DRUYAN *et al.* 1969, DE BERNHARD *et al.* 1969). They could also indicate routes for the transfer of mitochondrial proteins from the ER, the site of their synthesis, into the mitochondria (*e.g.*, GONZALEZ-CADAVID and CAMPBELL 1967, KADENBACH 1966 and 1970). Similar transfer kinetics have also been shown for mitochondrial lipid constituents (MCMURRAY and DAWSON 1969, WIRTZ and ZILVERSMIT 1969, JUNGALWALA and DAWSON 1970). Thus, it seems conceivable that such transfer does not take the route through the cytoplasmic sap but rather might be carried out by "membrane drift" (*i.e.*, "membrane flow" in its original definition by BENNETT 1956) or by intracisternal translocations. The latter intracisternal process would provide then the most direct route from the rough ER to the inner mitochondrial membrane. Another interesting possibility suggested from this finding is that, at least temporarily, all cisternal cytomembrane components are one continuous channeling system, including the nuclear envelope, the annulate lamellae, the rough and smooth ER, the Golgi cisternae, and the space between outer and inner mitochondrial membrane. Although a close enjacketing with ER-elements is well-known for plastids, too, and distinct relationships of certain algae with special ER-cisternae (periplastidal cisterna and peri-

plastidal reticulum; reviewed, *e.g.*, by Falk and Kleinig 1968) have been described, a comparable membrane continuity has not been demonstrated so far, at least to the authors' knowledge, for the outer membrane of plastids.

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