

## RIBOSOMAL GRANULES ASSOCIATED WITH OUTER MITOCHONDRIAL MEMBRANE IN AEROBIC YEAST CELLS

EZZATOLLAH KEYHANI. From the Johnson Research Foundation, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19174

### INTRODUCTION

It is well established that mitochondria possess the ability to synthesize proteins (2, 4, 12, 25). Recent investigations show that mitochondrial protein synthesis depends, at least in part, on nuclear genes, cytoplasmic ribosomes, and nuclear RNA polymerase (3). Biochemical studies of Kellems and Butow (13) show that 80S cytoplasmic ribosomes (26) bind to purified mitochondria of yeast (13). These ribosomes differ from intramitochondrial ribosomes (1, 27, 28, 29, 31) by insensitivity to chloramphenicol and sensitivity to cycloheximide. Thus their observations suggest the presence in yeast of 80S ribosomal particles attached to the cytoplasmic side of the outer mitochondrial membrane. A continuity between endoplasmic reticulum and outer mitochondrial membranes has been observed in rat liver and onion stem (17), fungi (5), and *Tetrahymena pyriformis* (10); it was postulated that this continuity played a key role in transferring proteins to the inside of the mitochondria. In yeast cells, however, a continuity between endoplasmic reticulum and outer mitochon-

drial membranes has not been reported. Hence, a different mechanism may operate to get the protein into the mitochondria.

It is well known that yeast cells are difficult to prepare for ultrastructural observation (7, 31, 32). Recently, however, several alternatives for better preservation of ultrastructure in this organism have become available (20, 31, for review see reference 7). In the present study, double fixation in glutaraldehyde-osmium tetroxide (23) has made it possible to observe ribosome-like granules structurally associated with mitochondrial membranes in a carotenoid-containing aerobic yeast cell *Rhodotorula rubra*. Electron microscope examination of thin sections reveals ribosome-like granules on the cytoplasmic side of the outer mitochondrial membrane and inner face (matrix side) of the inner mitochondrial membrane. A preliminary report of this work was presented previously (14).

### MATERIALS AND METHODS

Yeast cells, strain *R. rubra*, were grown in Galzi and Slonimski (11) medium with 2% glucose as

the carbon source. The cells were harvested at an early stationary phase (after 18 h) and washed twice with distilled water. Protoplasts were prepared according to the methods of Darling et al. (8). The protoplasts were fixed 2 h in 2.5% glutaraldehyde buffered to pH 7.2 with 0.1 M phosphate. After primary fixation, the cells were washed in 0.1 M phosphate buffer containing 10% sucrose and were postfixed for 2 h in 1% osmium tetroxide buffered to pH 7.2 with 0.1 M phosphate. The cells were dehydrated in ethanol and embedded in Epon (16). Sections were cut on a Porter-Blum MT-2 ultramicrotome equipped with a diamond knife and were collected on 400-mesh grids. They were doubly stained with uranyl acetate and lead citrate (22). Sections were examined in a JEOL (type JEM T8) electron microscope at an accelerating voltage of 60 kV with a 50  $\mu$ m objective aperture.

## RESULTS

Ribosome-like granules are observed at the surfaces of the outer membrane (cytoplasmic side) and inner membrane (matrix side) (Figs. 1, 2).

The ribosome-like granules inside the mitochondria appear either as monomers (Fig. 2 *d, e, g, h, i*), although they could be a section through a ribosomal aggregate, or as ribosomal aggregates composed of seven to eight ribosomal units (Fig. 2 *a, c*). They are usually located at the periphery of the inner membrane (Figs. 1, 2). The average size of the mitochondrial ribosome is 130  $\text{Å}$  in width and 160  $\text{Å}$  in length. These measurements can be subject to variation, due to the thickness of the section, the effect of the fixation, embedding and staining, and the lack of assessment of the orientation of the particles within the section.

Although ribosomes located inside the mitochondrion are relatively few in number, ribosomes located at the cytoplasmic surface of the outer membrane are numerous. Fig. 2 shows several mitochondria selected in different cells to illustrate the frequency of ribosome granules associated with mitochondrial membranes. The ribosome granules cover the cytoplasmic side of the outer membrane. This is not always the case, however, and, in some mitochondria, portions of the outer membrane are free of ribosomes (Fig. 2 *a, b*). The average diam-

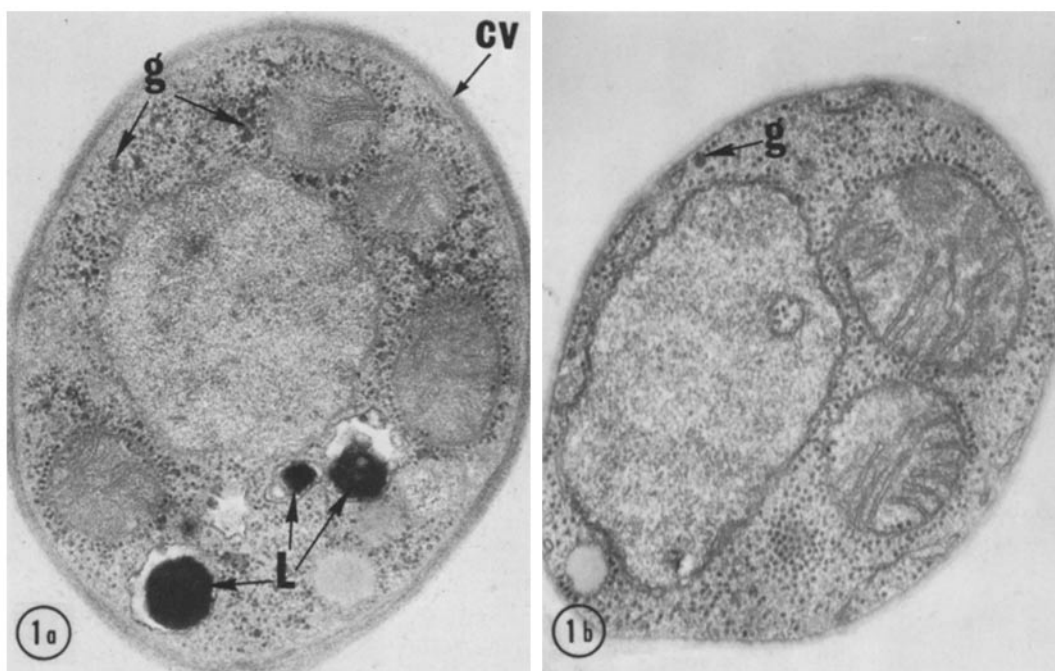


FIGURE 1 (a) Electron micrograph of the *R. rubra* cell showing the mitochondria which are well preserved and have moderately developed cristae. Lysosome-like organelles (*L*) are surrounded by a "unit membrane." Note the numerous glycogen particles (*g*).  $\times 35,000$ . (b) This electron micrograph shows a section of the yeast cell with a few glycogen (*g*) particles (see text). Around the mitochondria numerous ribosome-like granules in contact with outer membrane are visible.  $\times 42,000$ .

eter of these ribosomes is 160–200 Å, a value identical to that for the cytoplasmic ribosomes. Although many intra- and extramitochondrial ribosome-like granules appear to be contiguous with the mitochondrial membrane, the contact is not as tight as that observed in rough endoplasmic reticulum. A similar observation has been reported for the mitochondrial ribosomes in chicken and rat embryonic liver (1). The distinction between the ribosomal particles and glycogen granules should be stressed. The glycogen particle distribution in *Rhodotorula* is variable. In some cells (Fig. 1 *b*) few glycogen granules are observed whereas in other cells, glycogen granules are abundant (Fig. 1 *a*). They show both  $\alpha$ -form (1,300 Å, Fig. 2 *i*) and  $\beta$ -form (300–350 Å  $\pm$  20 Å, Fig. 2 *b*). In either form they are larger than ribosomal granules (160–200 Å). The  $\alpha$ -particles in yeast (Fig. 2 *i*) are loosely organized in contrast with the tight organization of such particles in liver (9).

#### DISCUSSION

The results presented in this work indicate that *Rhodotorula rubra* mitochondria possess two types of ribosome-like granules: one intramitochondrial, located on the matrix side of the inner membrane, and another, extramitochondrial, close to the cytoplasmic side of the outer membrane. The outer membrane bearing ribosome-like granules could operate as a rough endoplasmic reticulum. A vectorial transfer of protein has been shown for microsomal fractions of rat and guinea pig liver (21) which may take place by way of a channel in the large subunit (15, 18, 24) and a corresponding discontinuity in the membrane of endoplasmic reticulum (24). Such a relationship allows the nascent polypeptide chain to be directed toward the cisternal space of the growing peptide chain (24). On the basis of biochemical information Parsons et al. (19) conclude that the endoplasmic reticulum might be continuous with the outer mitochondrial membrane. Electron microscope ex-

amination further indicated (5, 10, 17) a continuity between endoplasmic reticulum and outer membrane. Similar to those on the endoplasmic reticulum, the ribosomes associated with the outer mitochondrial membrane in yeast presumably synthesize proteins which may be vectorially transferred to the inside of the mitochondrion. The present investigation does not allow an unequivocal statement as to whether the ribosomes are bound to the outer mitochondrial membrane under physiological conditions or whether the attachment results from an ability of ribosomes to bind to existing binding sites of the membrane under the conditions used for fixation (such as low ionic strength).

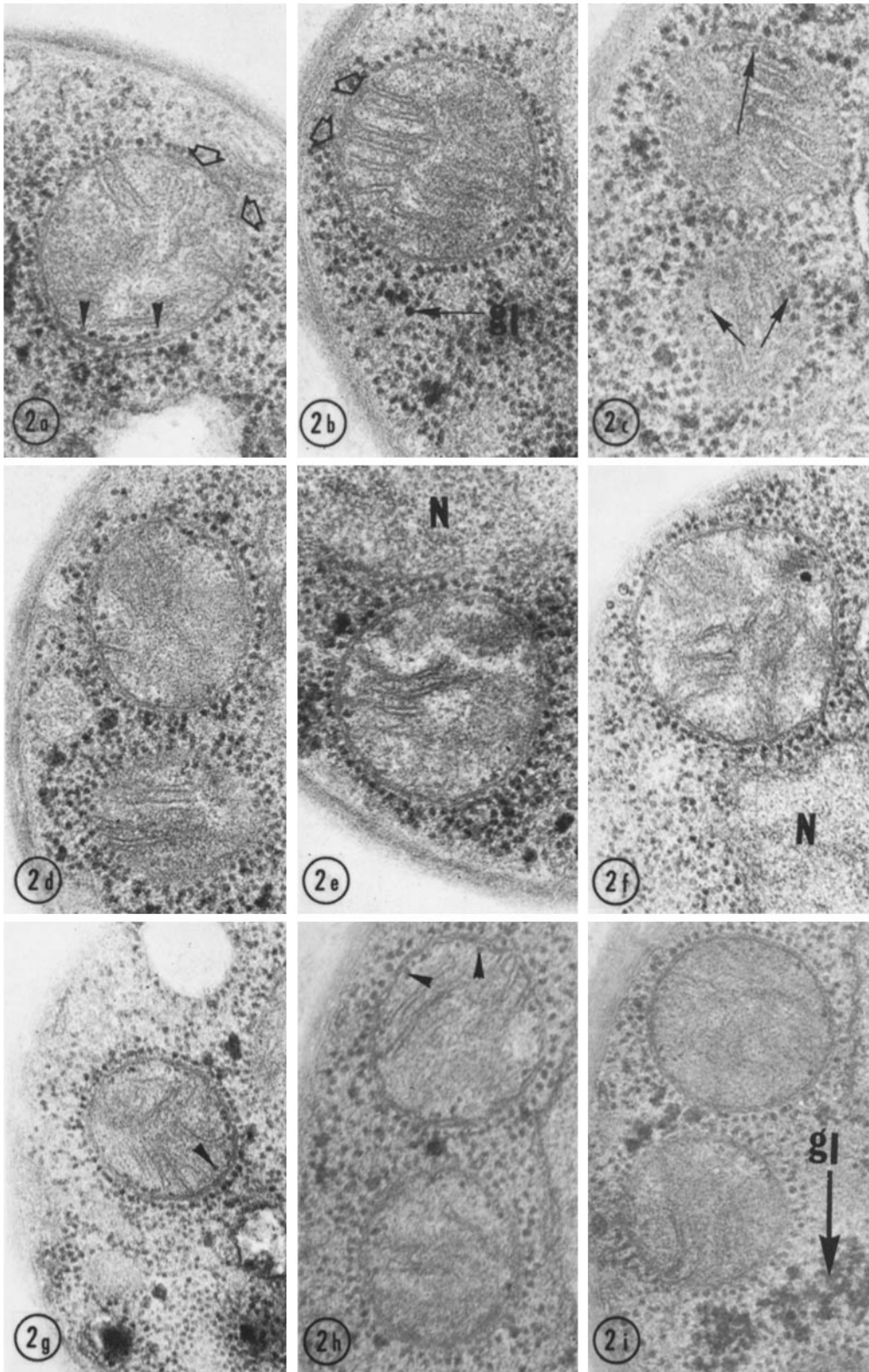
Intramitochondrial ribosomes are smaller (130–160 Å) than cytoplasmic ribosomes (160–200 Å), although variability does exist in the size of mitochondrial ribosomes from various sources (27, 29, 30). Vignais et al. (30) found no difference in the sedimentation properties of mitochondrial and cytoplasmic ribosomes.

The preferential localization of intramitochondrial ribosomes at the periphery of the organelles has been previously reported (6, 31). It has also been shown that some of the ribosomes are in contact with the inner mitochondrial membrane (1), although the contact is not as intimate as that seen in the endoplasmic reticulum (1). The attachment of ribosomes to the inner membrane brings up a point concerning the destination of proteins synthesized by such ribosomes. It might be interesting to speculate that (*a*) the protein may be released to the intermembrane space and subsequently incorporated into the mitochondrial membrane and (*b*) like those on the endoplasmic reticulum, these ribosomes could possibly synthesize the proteins which are exported to the cytoplasm.

Outer mitochondrial membrane ribosomes as seen in *R. rubra* are also seen in *Candida utilis* (Keyhani, unpublished data). However, the ribosomes are extremely numerous in the cytoplasm of

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FIGURE 2 These electron micrographs represent selected areas from several yeast cells showing the distribution of ribosome-like granules inside and those located at the surface of the outer membrane of mitochondria. The ribosome granules inside the mitochondria are generally located at the periphery of the inner membrane (small arrows and arrowheads). They are either polysome-like (*a*, *c*) or are monomers (*d*, *e*, *g*, *h*). Ribosomes are clearly seen around the mitochondria in contact with the outer membrane. They either completely cover (*d*, *e*, *f*, *g*, *h*, *i*) or partially cover (*a*, *b*, space between open arrowheads) the cytoplasmic side of the outer membrane. Glycogen, *gl*. (*a*)  $\times$  64,000, (*b*)  $\times$  60,000, (*c*)  $\times$  70,000, (*d*)  $\times$  62,000, (*e*)  $\times$  64,000, (*f*)  $\times$  58,000, (*g*)  $\times$  58,000, (*h*)  $\times$  70,000, (*i*)  $\times$  60,000.



this latter species and the distinction between bound ribosomes and simple contact is impossible. In *R. rubra* the cytoplasmic ribosomes are less numerous and a clear limit is visible between ribosomes bound to the outer membrane and surrounding cytoplasm. In this paper we suggest that the particles in contact with outer membranes are ribosomes rather than glycogen. This distinction is based upon the fact that the size of the glycogen granules is larger than that of ribosomes. Nevertheless, biochemical data are necessary to give a definitive answer to the ribosomal nature of these particles.

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