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Headpiece-Stalk Particles Lining Membranes of Mitochondria Isolated from Normal and Oligomycin-Resistant Mutants of *Saccharomyces cerevisiae*

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Abstract

Mitochondria isolated from the yeast *Saccharomyces cerevisiae* were negatively stained with ammonium molybdate. Extensive headpiece-stalk projections were observed lining the disrupted mitochondrial membranes. These observations represent the first clear demonstration of headpiece-stalk particles on yeast mitochondrial membranes. Phosphotungstic acid was somewhat less satisfactory than ammonium molybdate in the visualization of the headpiece-stalk particles. Mitochondria isolated from glucose-repressed cells and oligomycin-resistant mutant cells were also examined by negative staining and found to show numerous headpiece-stalk elements. Gross differences in the morphology of mitochondria from normal, glucose-repressed and oligomycin-resistant cells, as examined by negative staining, were not apparent in the present studies. The nature and expression of the oligomycin mutation is discussed in terms of possible changes in membrane protein and phospholipid.

Introduction

The morphological description of the oligomycin-sensitive ATPase as shown by negative staining of mitochondrial membranes is the tripartite repeating unit first described by Fernandez-Moran *et al.* [1]. Morphological and biochemical studies, primarily using beef heart mitochondria, have identified the headpiece as the oligomycin-insensitive ATPase (complex F_1) [2] and the basepiece as consisting of components of the respiratory chain [3]. The attachment of headpiece to the stalk component comprises the complex identified as the oligomycin-sensitive

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ATPase [4]. A protein conferring oligomycin-sensitivity to F_1 (OSCP), has been purified from beef heart [5, 6] and yeast mitochondria [7]; Electron microscopy studies of MacLennan and Asai [8] have suggested that the OSCP of beef heart is localized in the stalk sector of the mitochondrial inner membrane subunit.

Yeast mitochondrial ATPase has been reported as being similar in many of its properties but not identical to that of the corresponding enzyme from beef heart [9]. Thus, the yeast enzyme is only inhibited by relatively high levels of oligomycin as compared with liver mitochondria [10]. Moreover, although the biochemical properties of yeast mitochondrial ATPase have been well characterized [7, 9, 11, 12] the presence of stalked particles on yeast mitochondrial membranes has not been clearly demonstrated morphologically.

There have been recent reports on the isolation of oligomycin-resistant mutants of yeast some of which were shown to be nuclear and others cytoplasmically inherited [13-16]. In a preliminary communication the mitochondrial ATPase activity of oligomycin-resistant mutants has been reported by Avner and Griffiths [16] to be also insensitive *in vitro* to high concentrations of oligomycin. On the other hand, Wakabayashi and Gunge [15] have reported oligomycin-resistant mutants in which the mitochondrial ATPase activity was relatively sensitive *in vitro* to oligomycin.

This laboratory has recently reported the isolation and some biochemical properties of oligomycin-resistant mutants of yeast [17]. As part of a programme of investigation into yeast mitochondrial membrane morphology under different physiological conditions, we have examined by negative staining the fine structure of mitochondria isolated from normal and oligomycin-resistant mutants of yeast.

This communication presents electron micrographs of negatively stained mitochondria, isolated from normal yeast cells, which clearly show the presence of headpiece-stalk particles on the mitochondrial membranes. Furthermore, these headpiece-stalk particles are also observed in mitochondria isolated from oligomycin-resistant mutant and glucose-repressed cells.

Methods

Strains. Three strains of *Saccharomyces cerevisiae* were used for the present studies. One was a haploid strain, L410, and the other two, denoted L3000 and L4000, were oligomycin-resistant haploid strains. The procedure for the isolation of the oligomycin-resistant strains was essentially as described previously [18]. Strain L410 was unable to grow on ethanol as substrate in the presence of 0.3 μg oligomycin/ml of medium while strains L3000 and L4000 were able to grow on ethanol in the presence of 20 μg oligomycin/ml of medium.

Growth of Cells

Cells were grown aerobically on 1% ethanol, 1% yeast extract-salts medium in 2 litre fluted conical flasks and shaken on a New Brunswick Gyrotory Shaker at 250 revs/min. Cells were harvested in early stationary phase, corresponding to 3-4 mg dry weight/ml medium, and mitochondria isolated and purified as described previously [19].

Electron Microscopy

Mitochondria were negatively stained as follows. One drop of a concentrated suspension of mitochondria (10-20 mg protein/ml) in 0.5 M sorbitol-1 mM EDTA, pH 7.2, was mixed with 3-4 drops of ice-cold H_2O followed by a volume of ice-cold 2% phosphotungstic acid or 1-4% ammonium molybdate until only a faint turbidity was observed. Alternatively, the mitochondrial suspension was briefly sonicated prior to negative staining. Using a fine Pasteur pipette, one drop of suspension was placed on to a carboned Parlodion coated grid. After approximately

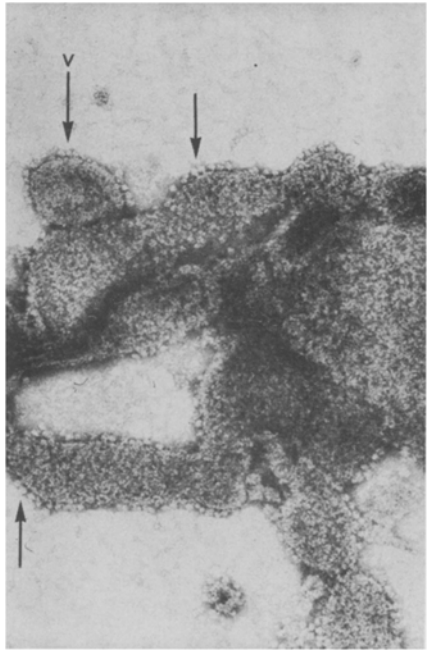


Figure 1. Electron micrograph of mitochondria isolated from the normal strain L410. Negatively stained with 2% ammonium molybdate. The knob-like projections are seen attached to the mitochondrial membranes via a narrow stalk sector (arrows). Note the vesicle with headpiece-stalk projections (v-arrow). x200,000.

10-20 sec the excess of liquid was drawn off using the edge of a piece of Whatman No. 1 filter paper. The grid was allowed to air dry before examination in a Hitach-11A electron microscope. Specimens were examined at an accelerating voltage of 75 kV and plate magnifications of between 50-80,000.

Results and Discussion

Purified mitochondria isolated from the wild type strain L410 were negatively stained as described in Methods. Electron micrographs of the negatively stained mitochondrial membranes are shown in Figs. 1 and 2. Extensive knob-like particles are observed lining the disrupted mitochondrial membranes. The headpiece varied in diameter from 80-90 Å and could be seen to be connected via a narrow stalk sector, approximately 40 Å long by 30 Å wide, to the mitochondrial membranes. The attached knob-like structures were frequently observed encircling disrupted mitochondrial membrane vesicles (Fig. see also

Figure 2. Mitochondrial membranes isolated from strain L410. Negatively stained with 2% ammonium molybdate. Narrow strips of membrane are seen which are lined on both sides by headpiece-stalk particles. x250,000.

Fig. 3,5). Less frequently, narrow strips of membrane, presumably cristae, are observed which are lined on both sides by stalked particles (Fig. 2).

Although Shinagawa *et al.* [20] presented some evidence for the presence of knob-like structures in yeast mitochondrial membranes, the quality of the micrographs did not allow a clear recognition of the stalk structure. More recently, the absence of knob-like structures in mitochondria isolated from glucose-repressed yeast cells has been reported [11]. However, in our hands, using the negative staining techniques described in the Methods section, knob-like structures can be recognized in mitochondria isolated from yeast cells grown on high glucose concentrations. Figure 3 shows an electron micrograph of mitochondria isolated from cells grown on high glucose and negatively stained with ammonium molybdate. Knob-like projections are clearly observed lining the mitochondrial membranes.

We have routinely employed phosphotungstic acid and ammonium molybdate as negative stains and although both stains gave essentially

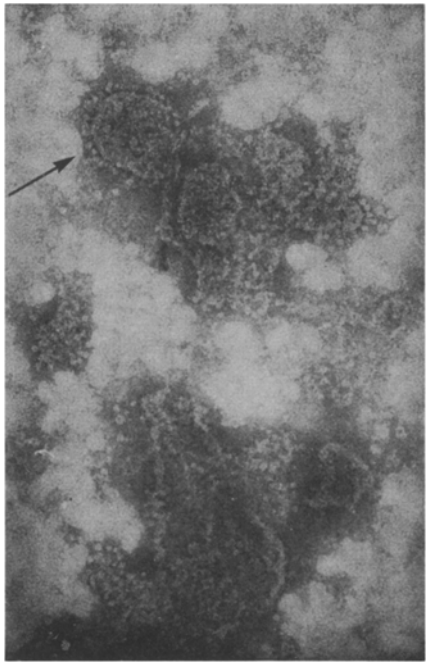


Figure 3. Mitochondrial membranes isolated from strain L410 grown on 10% glucose as substrate. Negatively stained with 2% ammonium molybdate. Note the headpiece-stalk particles encircling part of a disrupted mitochondrial membrane. x200,000.

similar results, phosphotungstic acid was in general less satisfactory. As shown in Fig. 4, yeast mitochondria negatively stained with 2% phosphotungstic acid show knob-like projections but the projections appear less frequent and morphologically less distinct as compared to mitochondria negatively stained with ammonium molybdate (cf. Figs. 1, 3 and 5).

The difficulties of previous authors to visualize unequivocally the presence of knob-like structures in yeast mitochondria may therefore have been due to the use of phosphotungstate. Recent reports have shown that aqueous solutions (2-4%) of ammonium molybdate, apart from being isotonic with media commonly employed in the isolation of mitochondria, have a less destructive effect on mitochondrial membranes than solutions of phosphotungstate [21, 22].

The electron micrographs presented in this communication allow, for the first time, a clear description of the nature of the stalked particles of yeast mitochondrial membranes and demonstrate that the morphology of these particles are similar or identical to that reported for other mitochondrial membrane systems.

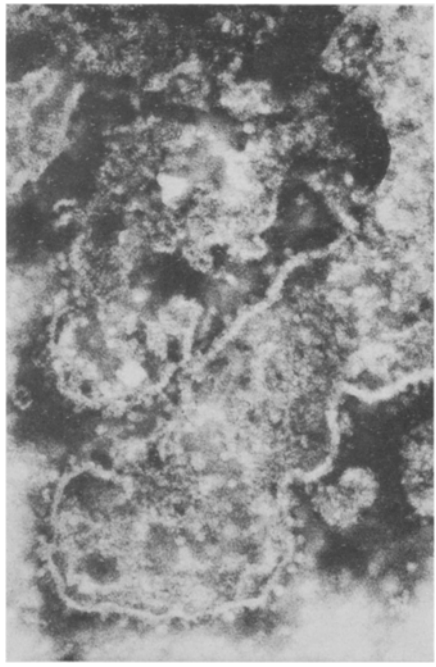


Figure 4. Mitochondria isolated from strain L410 and negatively stained with 2% phosphotungstic acid. The headpiece-stalk particles are not as distinct as compared to staining with ammonium molybdate (cf. Fig. 1, 5). x230,000.

It was of interest to examine the morphology of mitochondria isolated from oligomycin-resistant mutants of yeast since the headpiece-stalk attachment to the mitochondrial membranes is associated with the oligomycin-sensitive ATPase complex [4]. Electron micrographs of negatively stained mitochondria isolated from the oligomycin-resistant mutant strains L3000 and L4000 are shown in Fig. 4A, B). As in the case of mitochondria from the normal, oligomycin-sensitive strain L410, knob-like projections are seen lining the disrupted mitochondrial membranes in which the stalk sector can be recognized as forming the bridge for the attachment of the knobs to the mitochondrial membranes (Fig. 5A, B, C).

Gross morphological differences in the headpiece-stalked particles of mitochondria from normal and oligomycin-resistant cells were not apparent in the present studies.

Although the mutant cells were resistant to oligomycin *in vivo,* the mitochondrial ATPase has been shown to be sensitive *in vitro* to the

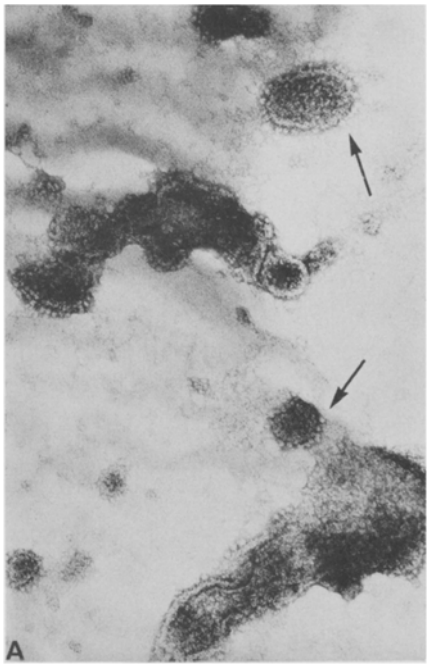
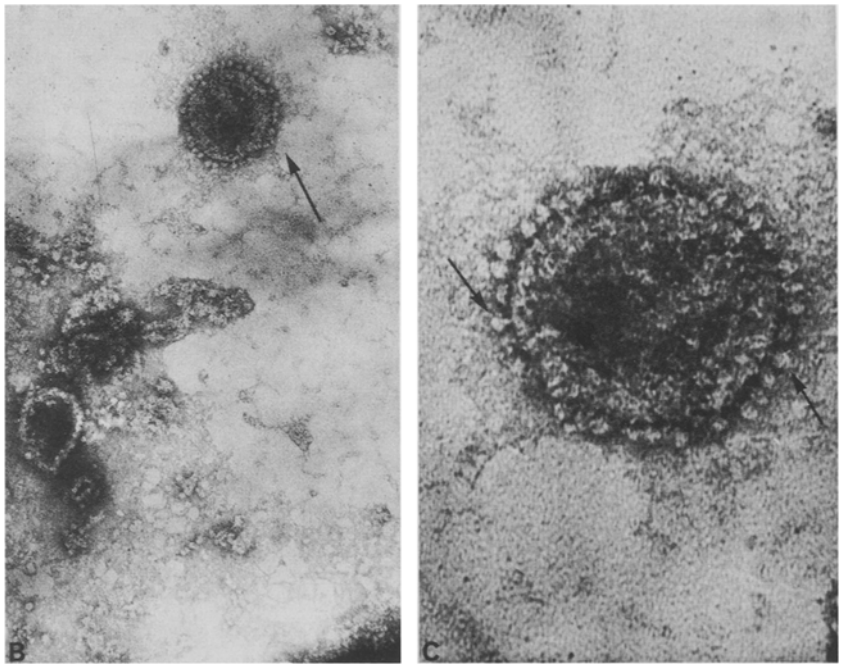


Figure 5. Negatively stained (1% ammonium molybdate) mitochondria isolated from the oligomycin-resistant strains L3000 and L4000. Numerous headpiece-stalk projections are seen encircling membrane vesicles (arrows). A: Strain L3000. x200,000. B: Strain L4000. x200,000. C: High magnification of Fig. 5B showing the attachment of the headpiece via a narrow stalk sector to the mitochondrial membranes. x560,000.



Figures 5B and 5C.

antibiotic [17]. This sensitivity of the mitochondrial ATPase to oligomycin *in vitro* supports the morphological observations presented in this communication that the stalk sector, which has been proposed to contain the OSCP [8], is not missing in mitochondria isolated from these oligomycin-resistant mutants. These observations do not exclude other possibilities as to the nature of the oligomycin resistance, such as alterations in stalk protein or other membrane proteins. The suggestion that the oligomycin-resistant mutants may be the result of a membrane mutation is supported for the strain L3000 by its extensive cross-resistance to other antibiotics such as chloramphenicol and mikamycin [18].

Recent studies in this laboratory have shown that the oligomycin-resistant mutants may be rendered sensitive *in vivo* by manipulation of growth conditions which result in membrane disorganization of both protein and lipid [17]. The importance of phospholipid for high ATPase activity has been well documented [4, 23] and more recently Palatini and Bruni [24] have underlined the role of phospholipids in the determination of ATPase sensitivity to oligomycin. Thus, the observation that mitochondria from oligomycin-resistant mutants become sensitive on isolation could be interpreted as disorganization of membrane

proteins, lipids or both. However, until more biochemical and genetic data is accumulated, the nature of the mutation is still uncertain and further work is currently in progress in this laboratory.

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