Structure of Mitochondria and Vacuoles of *Candida utilis* and *Schizosaccharomyces pombe* Studied by Electron Microscopy of Serial Thin Sections and Model Building

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SUMMARY

The structure of mitochondria and of vacuoles in *Candida utilis* and *Schizosaccharomyces pombe* has been studied by electron microscopy of serial thin sections and subsequent model building. The models of the two cells of *C. utilis* which were studied confirmed our earlier findings, made by high voltage electron microscopy of thick sections, that there is a single, branched and continuous mitochondrial network in the cell (Davison & Garland, 1975). A model of a *S. pombe* cell showed that the mitochondrial structure was far more continuous than expected from inspection of thin sections, there being but two large and two small mitochondria. The models demonstrated that the few large vacuoles in *C. utilis* were interconnected into a single cluster, whereas in *S. pombe* there were two separate complexes of interconnected vacuoles towards each pole of the cell.

INTRODUCTION

Electron microscopy of random thin sections of eukaryotic cells such as yeasts and algae gives inadequate visualization of the three-dimensional arrangement of intracellular contents. Two methods are available for describing three-dimensional intracellular structure. One method involves reconstruction of models from serial thin sections and has been applied to *Pityrosporum orbiculare* (Keddie & Barajas, 1969), *Chlamydomonas reinhardtii* (Arnold *et al.*, 1972), *Saccharomyces cerevisiae* (Hoffmann & Avers, 1973; Grimes, Mahler & Perlman, 1974), *Euglena gracilis* (Osafune, 1973), *Chlorella fusca* (Atkinson, John & Gunning, 1974) and *Polytomella agilis* (Burton & Moore, 1974). The other method directly visualizes most or all of the cell up to a thickness of 1 to 3 μm by use of high voltage electron microscopy (h.v.e.m.) (Davison & Garland, 1974, 1975; Paulin, 1975). These two methods are complementary because serial sectioning examines a few cells in great detail for a given expenditure of time and effort whereas h.v.e.m. of thick sections examines a great number of cells but in less detail because overlapping structures obscure each other.

The purpose of this work was to confirm and extend by serial sectioning and model building our earlier description of mitochondrial structure in the budding yeast *C. utilis* obtained by h.v.e.m. of thick sections (Davison & Garland, 1974, 1975). A comparative study of the fission yeast *Schizosaccharomyces pombe* also seemed desirable, particularly in view of the interest in this yeast as a model for studying the cell division cycle of eukaryotic cells. Unfortunately, our attempts to study *S. pombe* by h.v.e.m. of thick sections have so far been frustrated by poor contrast. We have therefore made a model of one cell from

serial thin sections of *S. pombe*, and find that the numerous mitochondrial profiles seen in these sections are largely continuous with each other. We also describe continuity of vacuolar structures.

METHODS

Candida utilis strain 193, from the National Collection of Yeast Cultures, Nutfield, Surrey, and *Schizosaccharomyces pombe* strain 972h⁻, from Dr David Lloyd, Department of Microbiology, University College, Cardiff, were maintained as stock cultures on solid media at 4 °C and periodically subcultured. Initial cultures for inoculation of larger cultures were grown from the stock cultures in 100 ml glucose-yeast medium (Conti & Naylor, 1959) at 30 °C for 24 h, aerobically, with shaking. Larger scale cultures were made aerobically at 30 °C in 500 ml 0.5 % (v/v) glycerol medium (Light & Garland, 1971) for *C. utilis* and 1 % (w/v) glucose medium (Mitchison, 1970) for *S. pombe*. The cells of *C. utilis* were harvested, by centrifuging, from the exponential phase of batch culture and they were then separated into size classes corresponding to stages of their life-cycle by the rate zonal centrifugation procedure of Poole, Lloyd & Kemp (1973). *Schizosaccharomyces pombe* was harvested, by centrifuging, from the early stationary growth phase (25 h culture), when the glucose concentration of the culture medium had dropped to less than 0.01 % (w/v) and the cytochrome concentration of the cells, as determined spectrophotometrically (Haddock & Garland, 1971), showed that mitochondrial synthesis was derepressed.

For electron microscopy, the cells were fixed with 2.5 % (w/v) glutaraldehyde in 0.1 M-sodium cacodylate buffer pH 7.0 for 2 h, and then treated with 2 % (w/v) KMnO₄ as described for lead citrate staining by Davison & Garland (1975).

After fixation the cells were embedded in either Spurr's resin (Spurr, 1969) or araldite (Luft, 1961) modified for serial sectioning (H. Eichelberger, personal communication) and sectioned with a Reichert OMU3 ultramicrotome using a diamond knife. Serial sections, each about 80 nm thick, were collected as described by Sjöstrand (1967) and stained with lead citrate (Reynolds, 1963). Electron micrographs were recorded with an AEI 801 electron microscope operated at 60 or 80 kV accelerating voltage. High voltage electron micrographs of thick sections of *C. utilis* were taken as described by Davison & Garland (1975).

Three-dimensional models were constructed from serial thin sections essentially as described by Atkinson *et al.* (1974), using 11.5 mm thick expanded polystyrene sheeting. The models for *C. utilis* were made at 150000 × actual cell size, and that for *S. pombe* at 136000 ×. To make these models we assigned the larger and vesicular intracellular profiles of membranous structures into one of three categories: nuclear, mitochondrial and vacuolar. The vacuolar category was subdivided into electron-opaque and electron-transparent vacuoles in *S. pombe*. Recognition of the nuclear membrane was unambiguous. Mitochondrial profiles were identified as such by their cristae. The vacuoles in *C. utilis* were readily distinguished from mitochondria, although in *S. pombe* the distinction was sometimes less obvious and where a serial run of mitochondrial profiles appeared to be separated by a vacuolar profile, it was assumed that the assignment to the vacuolar profile was incorrect and it was therefore revised.

RESULTS

The three-dimensional structure of mitochondria and vacuoles in C. utilis

Models were made from serial thin sections of two cells. One cell was from the fraction of smallest cells collected by rate zonal centrifugation and was representative of young,

Fig. 1. Polystyrene reconstruction from serial thin sections (75 nm) of batch-grown *C. utilis* at an early stage in the life-cycle. Mitochondria (M) form a single branched network overlying the nucleus (N) and vacuoles (V).

Fig. 2. Polystyrene reconstruction from serial thin sections (75 nm) of batch-grown *C. utilis* at a late stage in the life-cycle. Mitochondria (M) form a single branched interconnecting network overlying the nucleus (N) and vacuoles (V) in the mother cell (right) and daughter cell (left).

Fig. 3. Thick section (1 µm) of a chemostat-cultured, glycerol-limited *C. utilis* cell, stained with lead citrate. The section shows a cluster of rounded vacuoles (V) above the nucleus (N) and a number of apparently unconnected mitochondria (M) around the periphery of the cell. Accelerating voltage 1000 kV. Bar marker represents 1.0 µm.

Fig. 4. Thin section (85 nm) of a batch-cultured *S. pombe* cell at approximately 0.6 of the cell cycle, harvested in early stationary phase. This is one of 34 sections from which the model was constructed. Mitochondrial profiles (M) containing cristae are seen at either end of the cell together with electron-dense vacuoles (EDV) and electron-transparent vacuoles (ETV). The nucleus (N) is placed centrally. Accelerating voltage 80 kV. Bar marker represents 1.0 μm .

Fig. 5. Polystyrene reconstruction from serial thin sections (85 nm) of a *S. pombe* cell at approximately 0.6 of the cell cycle, harvested in stationary phase (glucose derepressed).

Fig. 6. Graphic representation of the model described in Fig. 5, showing two multi-lobed vacuolar complexes (V) sited either side of the nucleus (N). Mitochondria (M) form two continuous reticula except for two small pieces (m).

newly budded cells. The other cell was from the fraction of largest cells and was representative of cells with buds. In both cases the models (Figs 1 and 2) showed that there was only one mitochondrion per cell, and that the mitochondrion forms a peripherally placed reticulum. Even in the budding cell (Fig. 2) only one mitochondrion was present, extending from the mother into the daughter cell and preceding the appearance of a nucleus in the latter.

Vacuoles seen as separate structures in thin sections were found to be connected into a cluster in the models. The vacuolar cluster was clearly demonstrated by h.v.e.m. study of thick sections, as shown in Fig. 3. The appearance of the vacuole cluster suggests that there is a single continuous multi-lobed structure, rather than a clump of otherwise separate vacuoles.

*The three-dimensional structure of mitochondria and vacuoles in *S. pombe**

A typical electron micrograph of a thin section of an early stationary phase cell is shown in Fig. 4. There is little in the appearance of the mitochondrial or vacuolar structures to suggest that they might form part of larger continuous structures. However, construction of a model from serial thin sections showed that there is an extensive and largely continuous mitochondrial reticulum which forms two large separate parts centred at opposite ends of the cell (Figs 5 and 6). Two further separate, but very much smaller mitochondria were also found.

The vacuoles formed two multi-lobed complexes, one towards each end of the cell. Both electron-transparent and electron-opaque vacuoles contributed to the complexes. The lumina of the vacuoles were continuous throughout the complex irrespective of whether these contents were electron-opaque or electron-transparent. Two or three smaller vacuoles were unconnected with, and lay separately from, the main complexes. As noted by Heslot, Goffeau & Louis (1970), the vacuoles touched the nuclear membrane in places. The length of the sectioned cell was about $9\text{ }\mu\text{m}$, and this, with the presence of a centrally placed spherical nucleus, indicates that the cell was about 0.6 of the way through the cell division cycle, by which time mitochondrial growth is essentially complete (Osumi & Sando, 1969).

DISCUSSION

Mitochondrial structure

The models of *C. utilis* confirm our earlier conclusion that there is a mitochondrial reticulum in this species of yeast, and identify a single continuous mitochondrial reticulum per cell. In *S. pombe*, at least in the cell studied, we again found a mitochondrial reticulum though not as continuous as in *C. utilis*, there being two large reticular structures and two small rounded mitochondria. The finding of a mitochondrial reticulum in the fission yeast *S. pombe* poses questions about the timing of mitochondrial division, and the fact that there were two separate mitochondrial reticula at opposite ends of the cell could be interpreted as indicating that mitochondrial division had preceded nuclear division in the cell cycle.

The functional significance of a mitochondrial reticulum is obscure, particularly in view of the fact that the reticulum is not constantly present in all yeast species (Grimes *et al.*, 1974; Rosen *et al.*, 1974). A possible consequence of a mitochondrial reticulum concerns intracellular energy transmission, given that the separate oxidative and phosphorylative enzymes of oxidative phosphorylation are coupled to each other by an energized state capable of spreading laterally throughout the inner mitochondrial membrane. It is immaterial whether the energized state is a proton-motive force (Mitchell, 1966), an intra-membrane

charge separation (Williams, 1961) or a conformational state (Boyer, 1975), provided that it is sufficiently mobile. In each case, the mechanism of oxidative phosphorylation would permit respiratory chain activity at one part of the mitochondrial reticulum to drive ATP synthesis at another.

Vacuolar structure

The finding that vacuoles were in multi-lobed clusters or complexes was unexpected. The appearance of a continuous membrane with distensions into vacuoles of differing sizes suggests that new vacuolar membrane arises from the extension of pre-existing vacuole. The two complexes of vacuoles at opposite ends of the *S. pombe* cell would then provide a mechanism for apportioning vacuoles between the two cells formed at division. Presumably a different mechanism operates in the budding yeast *C. utilis* where distinctive cell poles and a dual location for vacuolar clusters are absent.

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