

layer, perhaps even from the inner plexiform layer to the receptors and horizontal cells. The meaning of such a hypothetical feedback system cannot be defined at this time. In any case, their presence in the dolphin as well as terrestrial mammals suggests that they may be a general feature of the mammalian retina.

The most unusual structures we have seen in the dolphin retina are the giant ganglion cells and their giant appendages. Although a few giant cells may be found in other mammals, such as dog and cat, they comprise only a small percentage of the ganglion cell population. Furthermore, there is no data to show that the axons produced by them attain the diameters which we have found in the dolphin retina. In the central 30° to 40°, the giant cells occupy a large percentage of the space between the inner nuclear layer and the inner limiting membrane. Functionally, such large dimensions must lead to rapid communication between the retina and the brain. Since very large ganglion cells have been reported in other cetaceans (2, 3), it is possible that they play some role in the maintenance of function when conversion to anaerobic metabolism is required by long periods under water.

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11. We thank Dr. and Mrs. David Caldwell and the management and personnel at Marineland of Florida for their contributions to these experiments. Supported in part by NSF grant GB27562.

21 December 1972

24 AUGUST 1973

Mitochondrion of Yeast: Ultrastructural Evidence for One Giant, Branched Organelle per Cell

Abstract. Three-dimensional models constructed from 80 to 150 consecutive serial sections of entire yeast cells showed that all the separate mitochondrial profiles were cross sections through a single, branching, tubular structure about 50 to 60 micrometers in length and 200 to 600 nanometers in diameter. The data are contrary to conventional notions of mitochondrial size, form, and number per cell and should lead to a reassessment of mitochondrial genetics and biogenesis.

Mitochondria are energy-producing organelles in eukaryotic cells. Except for the unicellular alga *Micromonas*, which is reported to contain a single mitochondrion (1), all eukaryotic species have been presumed to carry numerous mitochondria per cell. This presumption is based on observations of whole cells and sections by various microscopic methods, and on data from centrifugation experiments (2). We now propose a different model of the mitochondrion. Serial sections of entire cells revealed only one huge, branched, cristate mitochondrion per cell in yeast, regardless of the physiological state of the aerobic culture at the time of collection and processing. This folded, branched organelle occupied a substantial portion of the extranuclear space but was not closely appressed to

the cell membrane. Data from budding cells in synchronous cultures further revealed that the single mitochondrion was continuous between mother and bud portions through 80 to 90 percent of the cell cycle and remained thus until the new cell wall was completed.

Cells of the diploid strain iso-N of *Saccharomyces cerevisiae* were grown synchronously and asynchronously in liquid nutrient which included glucose or ethanol as energy sources (3). Samples of cell suspensions were fixed in 3 percent glutaraldehyde and then were sedimented and resuspended in fresh fixative solution containing 5 percent glutaraldehyde and 8 percent formaldehyde in neutral cacodylate buffer (4). Subsequent treatment included either postfixation with 5 percent sodium permanganate or digestion of the cell wall

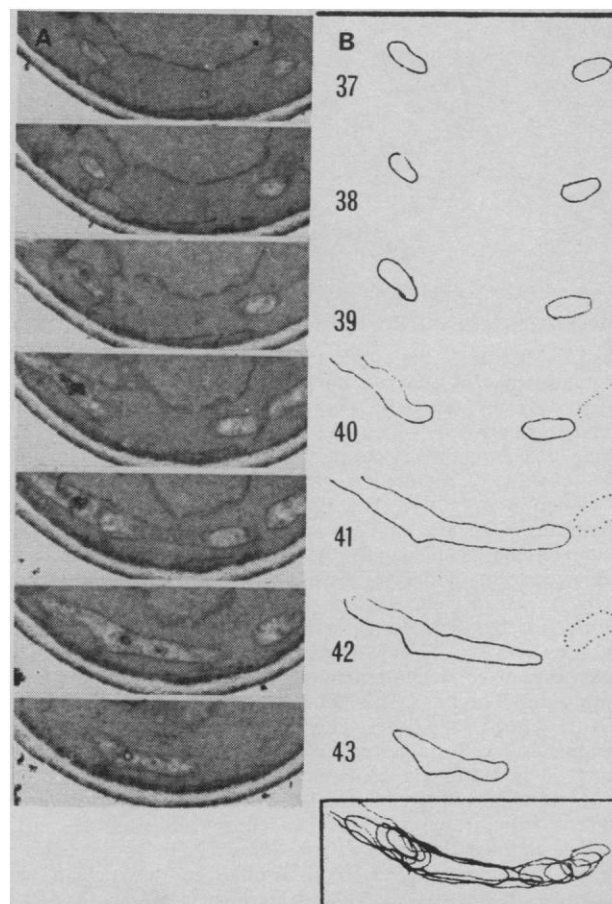


Fig. 1. (A) Electron micrographs of thin sections of a consecutive series through a budding yeast cell are shown in part. The separate mitochondrial profiles in sections 37 to 40 are connected to one another in sections 41 to 43. This single entity in turn was connected to the rest of the giant, complex mitochondrion in this cell. (B) A cumulative view of the superimposed tracings of the mitochondrial profiles aids in showing the continuity ($\times 12,000$).

with snail-gut enzyme (Glusulase, Endo Labs, Garden City, N.Y.) and post-fixation with 2 percent osmium tetroxide at pH 6.8. We embedded the sedimented cells and poststained them with 2 percent uranyl acetate (4). Adapting conventional techniques of ultrathin serial sectioning (5), we transferred intact ribbons of 80 to 150 consecutive sections, 60 to 75 nm thick, onto single-hole nickel or copper grids covered with layers of Parlodion and carbon. Complete series were examined and photographed with a Philips 300 or RCA 3G electron microscope. Mitochondrial profiles were traced onto graph paper and three-dimensional models were assembled to scale from three of the nine cells which were serially complete. We superimposed square-ruled transparencies to ascertain the steric correlation of each cell section with the next and of the mitochondrial profiles within each section.

Analysis of nine serially sectioned cells showed that the classical ovoid

or ellipsoid mitochondrial profiles were actually cross sections of a single, large, branching mitochondrion (Fig. 1). The giant mitochondrion consisted of a continuous network of tubules that varied in cross-sectional diameter from 200 to 600 nm (Fig. 2). The average diameter apparently differs according to the physiological state of the cell; exponentially growing cells showed an average mitochondrial diameter of about 200 nm, whereas the slower growing cells of midstationary phase showed an average mitochondrial diameter of about 400 nm. Models of either mitochondrial type had an approximate overall length of about 50 to 60 μ m. The limits of microscopy prevented us from viewing the mitochondrion in its entirety within the cell. Problems of resolution, depth of focus, and maximum section thickness permitted us to observe only portions of the mitochondrion in situ.

Our model of a giant branched mitochondrion implies that the relative

homogeneity in size and shape of mitochondria isolated from cell homogenates (6) are a consequence of the procedures used in centrifugation studies. The production of mitochondrial vesicular fragments during extraction should not be surprising since similar effects are induced in extracting and processing the continuous endoplasmic reticulum or Golgi apparatus of the cell (7). The microsomal vesicles are familiar artifacts of preparation and not at all representative of the in situ subcellular membranous network of endoplasmic reticulum.

The concept of a single mitochondrion per yeast cell should lead to a reassessment of a number of current problems, especially of mitochondrial biogenesis and of the genetics of the organelle. Experiments which were designed and interpreted according to the notion of intracellular populations of mitochondria should be reevaluated (8). The concept of growth and division of numerous small mitochondria is qualitatively and quantitatively different from one based on a giant mitochondrion that grows continuously and subdivides passively at cytokinesis. Similarly, interpretations of segregation and recombination of nonchromosomal alleles, cloning analyses, and postmeiotic segregation patterns in zygote lineages may be more comprehensible in terms of multiple populations of genomes within a single mitochondrion (9).

The remarkable ultrastructural and biochemical similarities among mitochondria of eukaryotic cells suggest that one or a few giant mitochondria will be found in other species if cells are properly examined. Several recent reports (10) on the mitochondria of green algae, parasitic fungi, and insect spermatocytes showed branching, tubular structures which were fewer in number per cell than had been expected. Although Keddie and Barajas (10) found only one mitochondrion in one of three series of sections of the fungus *Pityrosporum orbiculare*, they attributed this to the resting phase of the cell. While there may be special differences in subcompartmentation of the mitochondrion of more complex cell types, our observations of mammalian cells also suggest an apparently similar mitochondrial organization to the structure which we propose for yeast.

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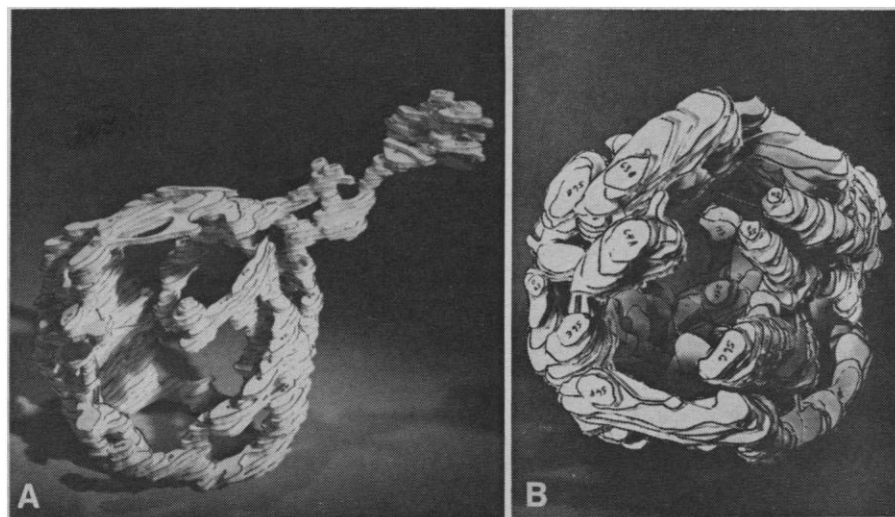


Fig. 2. Models of the single mitochondrion of the yeast cell were constructed to scale from tracings of electron micrographs. The thickness and size of each piece of foam board was in scale with the average section thickness and size of the photographic enlargements from which the profiles were traced. (A) The giant mitochondrion was assembled from 305 separate mitochondrial profiles in the 58 consecutive sections of the total of 72 required to include the entire budding cell. The average thickness of a section was 75 nm. The smaller protuberant portion occurred in the bud, but the continuity with the mother-cell mitochondrial structure is apparent. About three to four sections included only the cell wall; another three to four sections passed through the peripheral cytoplasm before the first mitochondrial profile of the series became evident. Six to eight sections were lacking in mitochondrial profiles in the concluding portion of the consecutive series. The budding cells were harvested from synchronous cultures growing in liquid medium containing 3 percent glucose, at 25°C (3). The cells were fixed in glutaraldehyde, treated with sodium permanganate, and poststained with uranyl acetate. This model was reconstructed from the same cell described in Fig. 1 ($\times 11,000$). (B) This model was reconstructed for the mitochondrion of a nonbudding cell harvested from a stationary phase culture grown for 20 hours at 25°C in liquid media containing 2 percent ethanol. The cells were fixed in a mixture of glutaraldehyde and formaldehyde, treated with sodium permanganate, and poststained with uranyl acetate. The entire cell was cut into 72 consecutive sections of 60 nm average thickness. There were one to two profiles visible at the mitochondrial periphery and about 15 to 18 profiles in the median sections, with a total of 443 separate profiles in the 58 thin sections in which the organelle occurred ($\times 12,000$).

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11. Supported by AEC contract AT(11-1)-3516. We thank Dr. M. W. Rancourt and L. Hollenbeck of Ortho Research Foundation for help in parts of this study and for the use of the Philips 300 electron microscope. We thank B. Mangold and C. Wood for technical assistance.

20 April 1973; revised 29 May 1973

Differences in Collagen Metabolism between Normal and Osteoarthritic Human Articular Cartilage

Abstract. Normal human articular cartilage synthesizes only one type of α chain, which exhibits the chromatographic behavior of the $\alpha_1(\text{II})$ chains described for chick and bovine cartilage. Osteoarthritic cartilage, on the other hand, synthesizes in addition a collagen containing α_2 chains and β components. The different structural features of the two types of collagen may account for some of the functional defects of osteoarthritic cartilage.

Osteoarthritis is a degenerative disease affecting articular cartilage. The early changes are associated with a loss of proteoglycans, while the collagen concentration remains constant (1). Cartilage destruction seems to begin in areas exposed to maximum mechanical stress leading to eburnation or complete loss of the hyaline tissue. Many investigators have focused on the metabolic changes that accompany osteoarthritis, particularly on the synthesis and turnover of glycosaminoglycans.

Recently it has been shown that collagen from chick cartilage (2, 3) and mammalian cartilage (4) differ significantly from that of other tissues. Cartilage collagen is assembled from three identical α chains identified as $\alpha_1(\text{II})$ to distinguish them from the homologous chain of skin, bone, and other adult tissues or $\alpha_1(\text{I})$. The most significant differences between these two types of collagens reside in hydroxylysine and glycosidically bound carbohydrates, since the $\alpha_1(\text{II})$ chains contain four- to fivefold more of these residues than the $\alpha_1(\text{I})$.

The fact that this unique type of collagen is found in articular cartilage

suggests that its particular configuration may be essential for the structural integrity of this tissue. This highly glycosylated structure may be the most suitable in establishing an adequate collagen-proteoglycan complex. An alteration of the fibrous network may affect this interaction and lead to a poor functional unit unable to resist stress.

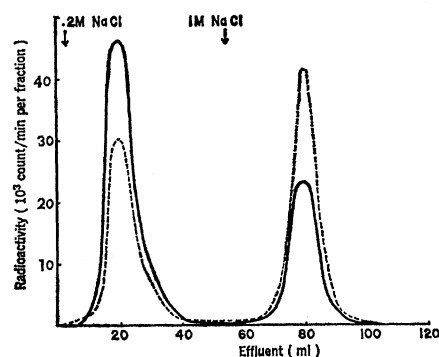


Fig. 1. Profiles of biosynthetically labeled cartilage extracts as they are eluted from a DEAE-cellulose column. After 50 ml of 0.05M tris buffer, pH 7.5, containing 0.2M NaCl was circulated, the salt concentration was raised to 1M to elute the proteoglycans. The solid line corresponds to a normal specimen, and the dotted line corresponds to osteoarthritic cartilage.

In an attempt to begin looking at this problem, we have compared the nature of the collagen synthesized by normal human cartilage to that synthesized by cartilaginous tissue proximal to areas of osteoarthritic degeneration.

Osteoarthritic cartilage was obtained primarily from human subjects undergoing surgical resection of the femoral head during replacement with prosthetic devices. Samples were taken from the areas surrounding the denuded area where cartilage was being actively eroded. Normal cartilage was obtained from autopsy or after corrective surgery not complicated by osteoarthritis.

Cartilage slices were incubated in 10 ml of Dulbecco's phosphate-buffered solution (GIBCO) containing 30 μC of L-[2,3- ^3H]proline (specific activity, 29.8 c/mmole). Incubations were carried out at 37°C for 4 hours with gentle shaking under air. At the end of this period, the tissue slices were removed, rinsed with saline, and homogenized with a VirTis-45 homogenizer in 0.45M NaCl adjusted to pH 7.0 with 0.02M sodium phosphate. The homogenate was kept in a shaker at 4°C for 24 hours and then centrifuged. The supernatant was dialyzed first against distilled water and then 0.05M tris buffer, pH 7.5, in 0.2M NaCl.

The dialyzed extracts were chromatographed on a diethylaminoethyl (DEAE)-cellulose column (0.6 by 30 cm) and eluted with 0.05M tris · HCl buffer in 0.2M NaCl (3). After 50 to 60 ml of effluent volume was collected, the NaCl concentration was raised to 1M, and the elution was continued. The flow rate was kept at 25 ml/hour, and 2-ml fractions were collected.

The radioactive material that eluted with the initial buffer was collected, lyophilized, dissolved in 0.06M sodium acetate buffer, pH 4.8, and dialyzed exhaustively against the same buffer.

The radioactive fraction recovered from the DEAE-cellulose column was mixed with 2 to 3 mg of purified acid-soluble collagen from rat skin, heated at 40°C for 15 minutes, and chromatographed on carboxymethyl (CM) cellulose with the use of a linear gradient between 0 and 1.0M NaCl (50 ml each) (5). A small column (0.8 by 5 cm) equilibrated with 0.06M sodium acetate buffer, pH 4.8 at 40°C, was used. The flow rate was maintained at 10 to 12 ml/hour and 1-ml fractions were collected, read at 230 nm, and counted in a Beckman liquid scintillation counter.

Figure 1 shows the elution profiles from a DEAE-cellulose column of the