Vol. 85, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 29, 1978 Pages 519-525

# ULTRASTRUCTURAL AND FUNCTIONAL DIFFERENCES IN MITOCHONDRIA ISOLATED FROM PARAMECIUM AURELIA GROWN AXENICALLY AND MONOXENICALLY

Deborah J. Prince* and Ian Gibson
School of Biological Sciences,
University of East Anglia, Norwich, England

*Department of Microbiology, Medical Center,
University of Colorado, Denver, Colorado 80262, U.S.A.

Received September 7, 1977

SUMMARY: Differences in the ultrastructure of mitochondria in P. aurelia grown axenically and monoxenically have been observed. Functional mitochondria have been isolated from both cultures and various metabolic parameters have been tested; respiration rates, cytochrome content, oligomycin sensitive ATPase activity, and an attempt has been made to relate the observed structure and metabolic activity of the mitochondria to the growth conditions.

Paramecium aurelia is generally maintained in the laboratory under axenic or monoxenic conditions. Observations on the general morphology and growth characteristics have shown distinct morphological and biochemical differences between the same stock of Paramecium grown under the two culture conditions (1,2). The present study was initiated to see if these observations could be correlated with changes in organelle morphology and function.

There are numerous reports of variations in the environment affecting the structure of mitochondria (3,4). Structural variability has also been related to the energy state of the mitochondria (5) and more recently to the degree of ADP binding to, or translocation in the inner mitochondrial membrane (6,7). The latter has been taken to indicate that ultrastructural changes can occur without concomitant changes in the respiration rate. Changes in the environment have also been reported to cause alterations in the metabolic activity of mitochondria with regard to respiration rates (8), cytochrome content (9,10) and respiratory sensitivity to blocking agents, e.g., cyanide (10).

The present study has isolated functional mitochondria from both culture situations and has examined various parameters in an attempt to correlate physiological differences with ultrastructural.

0006-291X/78/0852-0519$01.00/0

MATERIALS AND METHODS: Growth of Cells. The cells used in this study were Paramecium biaurelia stock 562s. The monoxenic cells were derived from the axenic cells by careful, sterile, additions of Klebsiella pneumonia (2). The axenic cells were maintained in Soldo's 1966 axenic medium at 26°C (11). Monoxenic cells were also maintained in grass medium previously innoculated with Klebsiella pneumonia, at 26°C (12). Cells were harvested in the late stationary phase; which correspond to eight days after innoculation for axenic cells, fourteen days after innoculation for monoxenic cells (to eliminate possibilities of bacterial contamination). Large culture volumes were harvested by the method of Cullis (13) which prevents cell damage.

Electron microscopy. Cell pellets were fixed following the method of Herrman and Kowallik (14). The cells were stained in 1% osmium tetroxide and then stained with uranyl acetate and lead citrate (15). The sections were viewed on a Philips 200 electron microscope.

Preparation of mitochondria. The extraction of mitochondria from P. aurelia in a raffinose buffer has been described.(16). The methodology here was the same except no EDTA was included in the raffinose extraction buffer. Protein was estimated by the Biuret assay (17).

Assays

Oxygen consumption: This was done using an oxygen electrode following the method described by Stockdale (18). The reaction chamber contained 5mM pyruvate, 5mM malate, 100mM $KNO_3$ in 5mM HEPES pH 7.6, 10mM $KPO_4$ and 100 µg mitochondrial protein. The reaction was started by addition of 0.2mM ADP. Oxygen consumption was calculated from the recorder traces (18).

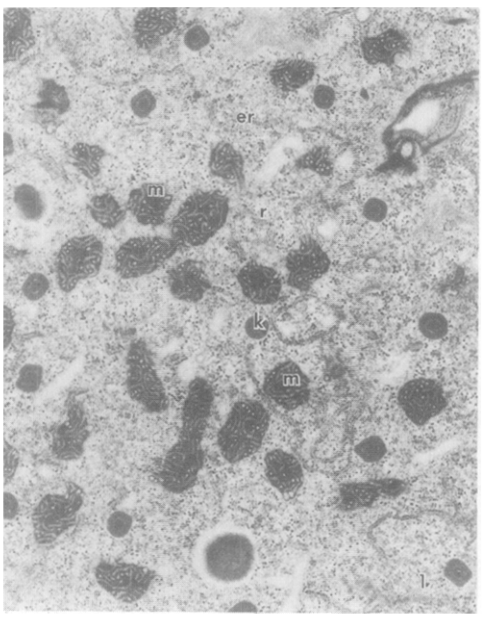
Oligomycin sensitive ATPase activity: This is the reaction system described by Selwyn (19). The ATPase activity is monitored by the release of $PO_4^{\equiv}$/min/mg mitochondrial protein.

Dual beam spectrophotometry: This procedure provides information on cytochrome content and wavelength maxima. Two, one ml mitochondrial suspensions were scanned from 400nm to 620nm after the reduction of one sample with sodium dithionite. The cytochrome content may be calculated using the molar extinction co-efficients of the cytochrome (5).

RESULTS: Electron micrographs. Figures 1 and 2 show respectively two characteristi types of mitochondria of axenic and monoxenic culture. The axenic mitochondria can be described as in Hackenbrock's condensed configuration and the monoxenic mitochondria as being in the expanded, or orthodox state (20).

Oxygen consumption. The respiration rates obtained for both mitochondrial preparations are shown in Table 1. Controls using whole cell preparations of K. pneumonia (>$10^{10}$ cells/ml) gave minimal respiration rates (less than 1% of monoxenic rate).

ATPase activity. The levels of oligomycin-sensitive ATPase activity for the two mitochondrial types are shown in Table 1. Controls using K. pneumonia gave no ATPase activity under similar conditions. Any possible cellular ATPases that might contaminate the mitochondrial preparations are excluded by the addition of oligomycin to the assay system.



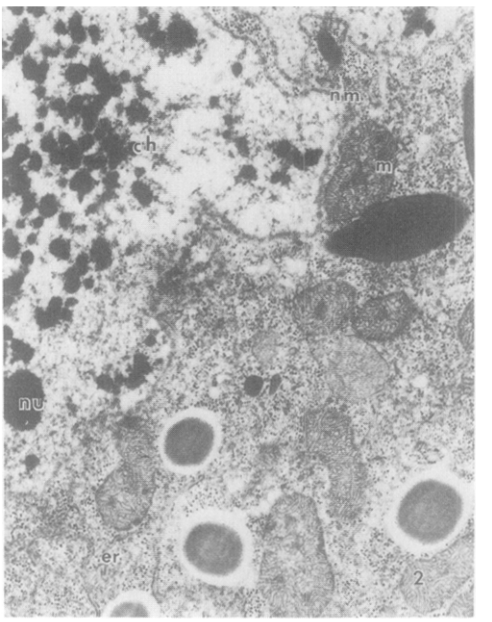




Figure 1 Axenically cultured P. aurelia cells showing mitochondria x 25600. m = mitochondrion, k = kinetosome, r = ribosomes, E.R. = endoplasmic reticulum.

Figure 2 Monoxenically cultured P. aurelia cells showing mitochondria x 25600. m = mitochondrion, nu = nucleolus, ch = chromatin body, nm = nuclear membrane, E.R. = endoplasmic reticulum.

Table 1 Oxygen consumption and oligomycin sensitive ATPase activity in mitochondria from monoxenically and axenically cultured P. aurelia

| | Axenic mitochondria | Monoxenic mitochondria |
|---|---|---|
| Oxygen consumption (nmoles $O_2$/min/mg protein) | 44.6±5 | 1079.9±100 |
| Oligomycin sensitive ATPase (μmoles $PO_4^=$ released/ min/mg protein) | 2.23±0.2 x $10^{-3}$ | 2.78±0.2 x $10^{-3}$ |

The results are the average of at least six assays. The mitochondria results are the average of at least six assays. The mitochondria used were extracted from cells in the late stationary phase of growth. Mitochondria from both preparations not affected by uncouplets.

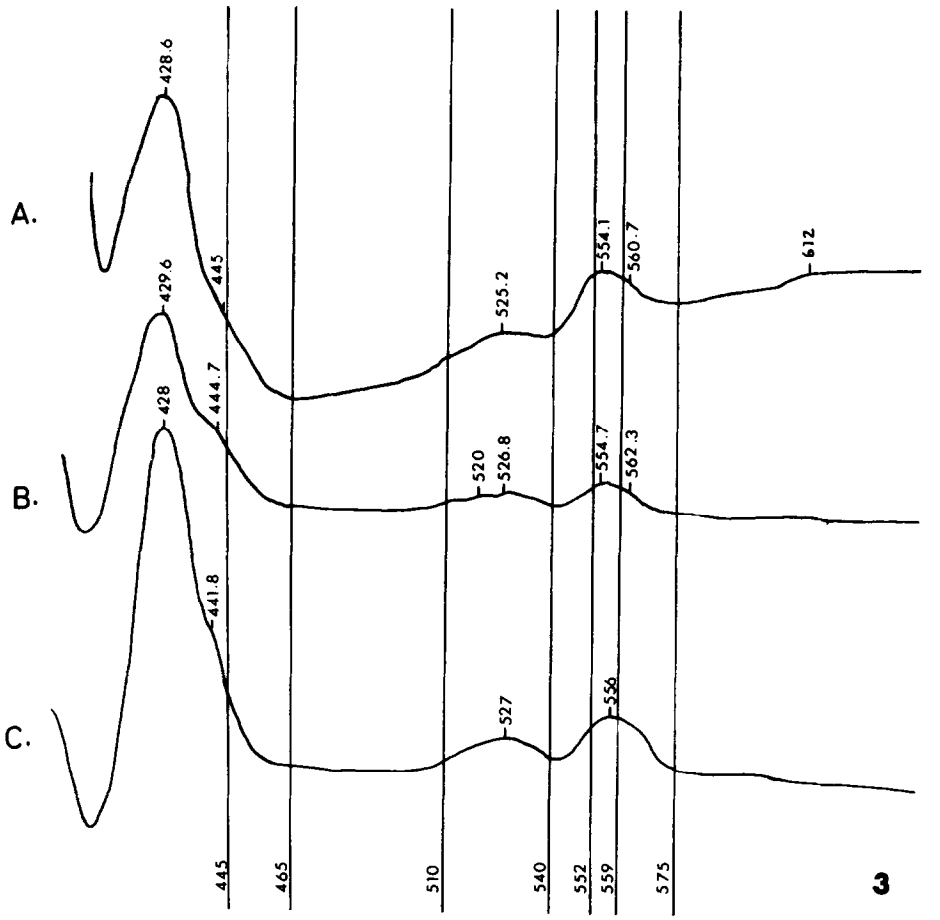



Figure 3 Abscissa: Wavelength in nm. Ordinate: Extinction increment. Dual beam spectrophotometry. Scan A 'monoxenic' mitochondria, protein concentration: 10 mg. (Scale 16cm = 0.1 ext.) Scan B 'axenic' mitochondria, protein concentration: 35 mg. (Scale 4cm = 0.1 ext.) Scan C K. pneumonia, protein concentration: 12 mg. The reference cell must be aerated before any spectrum can be obtained (Scale 8cm = 0.1 ext.)

The scans are representative of at least three separate mitochondria preparations and were carried out at 26°C.

Cytochrome content. Figure 3 shows the dual beam spectra obtained for isolated axenic and monoxenic mitochondria, and whole cells of K. pneumonia. Table 2 shows the values obtained for the total cytochrome content of the axenic and monoxenic mitochondrial preparations. Bacterial contamination of the monoxenic scan is nil, (or negligible) under the conditions used.

Table 2 Cytochrome content of monoxenically and axenically grown P. aurelia

| | | Axenic mitochondria | Monoxenic mitochondria |
|---|---|---|---|
| Cytochrome content (mM/mg protein) | b | $1.8 \times 10^{-5}$ | $0.45 \times 10^{-5}$ |
| | c | $1.9 \times 10^{-5}$ | $5.5 \times 10^{-5}$ |
| | a | $1.3 \times 10^{-5}$ | $1 \times 10^{-5}$ |
| | NHI | $0.6 \times 10^{-5}$ | $11 \times 10^{-5}$ |
| Percentage composition of cytochromes | b | 32.1 | 2.4 |
| | c | 33.7 | 29.7 |
| | a | 22.3 | 5.3 |
| | NHI | 11.7 | 62.5 |

The cytochrome content was determined from the dual beam spectrophotometer scans (see Fig. 3) using the following data:

Molar extinction co-efficients (3)

Cyt b ($\Delta$ ext. 559nm - 575nm) = $20 \times 10^{-3} cm^{-1} M^{-1}$

Cyt c ($\Delta$ ext. 552nm - 540nm) = $19 \times 10^{-3} cm^{-1} M^{-1}$

Cyt a ($\Delta$ ext. 445nm - 465nm) = $91 cm^{-1} mM^{-1}$

NHI ($\Delta$ ext. 465nm - 510nm) = $11 \times 10^{-3} cm^{-1} M^{-1}$

The results above are representative of several scans.

The wavelength maxima between the two culture situations are similar, except that the monoxenic mitochondrial show an extra broad shelf at 612nm not present in the axenic spectrum. This band may correspond to a similar unusual band found in Tetrahymena pyriformis which Lloyd (4) designates as an $a_2$ band. Also there is a difference in the $b_1$ band which peaks at 560.9nm in the monoxenic preparations and 562.3nm in the axenic. The preparations show that the a, b and c type chtochromes are present in P. aurelia, and the spectra compare well with other reported results from whole cells (1,22).

DISCUSSION: The results presented here suggest that there is a metabolic and physiological divergence between the mitochondria isolated from cells grown axenically and monoxenically. The possibilities of such a divergence were seen in the

EM study and are borne out by the biochemical assays. The monoxenic mitochondria (seen in the electron microscope) are in an expanded condition generally associated with a more energized state and this would appear to be the case. The respiration rates, levels of ATPase activity and cytochrome content are all significantly greater than seen in axenically derived mitochondria.

The cytochrome absorption spectra obtained agrees with other reports (1,21) with the exception that neither Kung or William's report a peak at 612nm, and Kung obtains a peak at 588nm which is not observed here, or in Williams' study. The variations may be due to stock differences, the use of whole cells rather than isolated mitochondria, and the techniques employed for obtaining the spectra.

It is interesting to note that the ultrastructure of the axenic mitochondria resembles mitochondria of ageing monoxenic cells (A-M cells) (16). Micrographs of A-M cells (175-180 fissions) show the condensed mitochondria with densely staining matrix and few tubules. Other ageing characteristics are not seen in axenically grown Paramecium. This might suggest that either (a) the axenic mitochondria are in the same physiological state as A-M mitochondria, i.e., the metabolic demands are similar, or (b) the axenic mitochondria is experiencing the same type of environment as the A-M mitochondria, which may be determining its structure.

Considering (a): Possibly axenic mitochondria have the same metabolic activity a A-M,which may explain the peculiar death phase observed under axenic growth conditions (2,1) where the cell population disappears in less than two days compared with the monoxenic death phase which can last fifteen days.

Considering (b): Possibly the cytoplasmic environment of an ageing cell is basically "toxic" to itself and the mitochondrial structure reflects this. It is possible that axenic mitochondria may too be experiencing a "hostile" cytoplasmic environment resulting in the observed ultrastructure and low metabolic activity. The 'hostile' environment may be in part due to a build-up of cellular ADP (6,7), possibly due to a lack of the phosphorylating enzyme or phosphate cations.

The present study raises the question of the suitability of the available axenic medium for the growth of P. aurelia. It does not refute the necessity of

a sterile axenic medium, but perhaps underlines the need for an improved axenic medium. We would suggest that research on axenic cells to date, although valid in context, may not fully stand up to extrapolation to the "wild" state.

This work was carried out whilst D. J. Prince was in receipt of an S.R.C. studentship. We would like to thank Drs. Selwyn and Dawson and B. Barrett for their advice and assistance. Thanks also to G. Bedingfield and H. Baker for their help with the electron micrographs.

REFERENCES:

1. Williams J. (1971). Studies on killer particles of Paramecium. Ph.D. thesis, University of East Anglia, Norwich, England.
2. Prince D.J. (1976). The biochemistry of Paramecium aurelia. Ph.D. thesis, University of East Anglia, Norwich, England.
3. Munn E A. (1974). The Structure of Mitochondria. Academic Press. London.
4. Lloyd D. (1974). The mitochondria of microorganisms. Academic Press. London.
5. Chance B, Williams G R. (1955). J. Biol. Chem. 217. 409-29.
6. Sherer B, Klingenberg M. (1974). Biochemistry. 13. 161-70.
7. Innis M A, Beers T R, Craig S P. (1976). Exp. Cell Res. 98. 47-56.
8. Light P A, Garland P B. (1971). Biochem. J. 124. 123-34.
9. Cartledge T G, Lloyd D, Erecińska M, Chance B. (1972). Biochem. J. 130. 739-47.
10. Bohringer S, Hecker H. (1975). J. Protozool. 22. 463-67.
11. Soldo A T, Godoy G A, van Wagtendonk W T. (1966). J. Protozool. 13. 492-97.
12. Sonneborn T M. (1950). J. Exp. Zool. 113. 87-147.
13. Cullis C. (1971). Cell Transformation in Paramecium. Ph.D. thesis, University of East Anglia, Norwich, England.
14. Herrman R G, Kowallik K V. (1970). Cell Biol. 45. 198-202.
15. Gibbons I H, Grimstone A V. (1960). J. Biophy. Biochem. Cytol. 7. 697-715.
16. Suyama Y, Preer J R. (1965). Genetics. 52. 1051-58.
17. Gornal A E, Bardawill C S, David M M. (1949). J. Biol. Chem. 177. 751-66.
18. Stockdale M, Dawson A P, Selwyn M J. (1970). Eu. J. Biochem. 15. 342-51.
19. Selwyn M J. (1967). Biochem. J. 105. 279-88.
20. Hackenbrock C R. (1966). J. Cell Biol. 30. 269-97.
21. Kung C. (1970). J. Gen. Micro. 61. 371-78.