not specific for Mg^{2+} or ATP, and are likely to be composites of several enzyme systems.

O'Donnell, J. M. & Ellory, J. C. (1970) *Experientia* **26**, 20
 Quinn, P. J. & White, I. G. (1967) *Biochem. J.* **104**, 238
 Quinn, P. J. & White, I. G. (1968) *J. Reprod. Fert.* **15**, 449
 Uesugi, S. & Yamazoe, S. (1966) *Nature (London)* **209**, 403

An Electron-Microscopic Technique for Measuring Ribosomal Profiles from Cardiac Muscle

By KENNETH HEARN, KEITH GIBSON and PETER HARRIS (*Institute of Cardiology, University of London, 2 Beaumont Street, London W1N 2DX, U.K.*)

Current methods of studying ribosomal profiles from cardiac tissue involve the use of sucrose density gradients and measurements of the absorbance. Such methods produce results that are difficult to quantify. An electron-microscopic technique has therefore been developed that attempts to describe ribosomal profiles in numerical terms.

Ribosomes and polyribosomes are separated from cardiac tissue by the method of Earl & Morgan (1968). One drop of a suspension of ribosomes is layered on to a carbon-coated Formvar-backed copper grid. The grids are positively stained with a 1% uranyl acetate solution in the manner described by Burghouts *et al.* (1907). They are then ready for electron microscopy.

Randomly selected fields are photographed with a magnification of 63000 and the subsequent print is enlarged twice. A differential count is performed on each photograph, the results being expressed as a percentage of the total number of ribosomes and polyribosomes per photograph.

Three studies were performed to determine the degree of variation in distribution of the ribosomal populations prepared in this way. In the first, ten micrographs were taken at regular intervals across an individual square of the grid. In the second, 30 micrographs were taken diametrically across an entire grid. In the third, five grids were spread with the same ribosomal preparation and four micrographs taken from each.

Differential counts were performed on each micrograph and the mean percentage of monoribosomes in each study was 42.1 (S.D. ± 4.8), 45.6 (S.D. ± 7.3) and 42.5 (S.D. ± 5.1) respectively. These results show that there is a random distribution of ribosomes between grids, within grids and within squares, and that the results are reproducible.

To examine the sensitivity of the method, it has been used to study the effect of actinomycin D on cardiac and hepatic ribosome profiles. Treatment with this drug produces an increase in the percentage of monoribosomes whereas the percentage of

polyribosomes containing six or more ribosomes decreases. This effect is more noticeable in the liver.

Burghouts, J. T. M., Stolls, A. L. H. & Bloemendal, H. (1970) *Biochem. J.* **119**, 749
 Earl, D. C. N. & Morgan, H. E. (1968) *Arch. Biochem. Biophys.* **128**, 460

Giant Mitochondria in Iron- or Copper-Deficient *Candida utilis*

By MICHAEL J. DAVISON, J. ALLAN DOWNIE and PETER B. GARLAND (*Department of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, U.K.*)

Dallman & Goodman (1970) demonstrated that rats fed on an iron- or copper-deficient diet developed enlarged mitochondria in their livers and hearts. These effects were more marked with copper deficiency. It was decided to investigate the possible generality of these phenomena, and we have therefore made an electron-microscopic study of *Candida utilis* cells grown in continuous culture under conditions of glycerol, iron or copper limitation (Light & Garland, 1971; Light, 1972; Downie & Garland, 1972). Sections of glycerol-limited cells were of typical appearance for aerobically grown yeast, showing up to 10–15 oval mitochondrial profiles per cell. The larger dimension of these mitochondria was about 0.4–0.5 μm . Sections of copper-limited cells exhibited in many instances as few as one mitochondrial profile per cell, the mitochondria being elongated to a length of 3.0 μm or more. Similar but less marked effects were observed with iron-limited cells. These observations provide strong evidence that the morphological changes described by Dallman & Goodman (1970) are due to abnormal composition of the mitochondria rather than to any more generalized whole-animal effect. It is of significance that deficiency of riboflavin (in mice) also causes mitochondrial enlargement (Tandler *et al.*, 1969). The mechanisms and controls that effect mitochondrial division are unknown; nevertheless it seems possible that one signal for mitochondrial division could be the presence of an inner mitochondrial membrane containing appropriate concentrations of cytochrome oxidase, other iron proteins and flavoproteins. Deficiency of any one appears to inhibit mitochondrial division.

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Dallman, P. R. & Goodman, J. R. (1970) *Blood* **35**, 496–505

Downie, J. A. & Garland, P. B. (1972) *Biochem. J.* **129**, 47 P

- Light, P. A. (1972) *FEBS Lett.* **19**, 319–322
Light, P. A. & Garland, P. B. (1971) *Biochem. J.* **124**, 123–134
Tandler, B., Erlandson, R. A., Smith, A. L. & Wynder, E. L. (1969) *J. Cell Biol.* **41**, 477–493

A Mutant of *Candida utilis* with a Novel Terminal Oxidase

By J. ALLAN DOWNIE and PETER B. GARLAND
(Department of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, U.K.)

During continuous culture of *Candida utilis* under conditions of copper limitation with glycerol as the carbon and energy source, as described by Light (1972), we observed that the culture spontaneously changed to give an approximately fourfold increase of cell concentration. At the same time the concentration of unused glycerol in the culture fell to almost zero. In view of the highly selective environment of a chemostat, we tentatively concluded that a mutant had established itself in the culture through its ability to by-pass a copper-requiring process that was previously rate-limiting for growth. Studies of the mutant cells and their isolated mitochondria showed that (i) the content of cytochrome *aa*₃ was decreased to less than 1% of that for the wild-type grown under conditions of glycerol limitation, (ii) respiration rates with natural substrates were unaffected, (iii) mitochondrial respiration was insensitive to cyanide or antimycin A but not to rotenone or piericidin A, (iv) the P/O ratio for isolated mitochondria was 1.0 for NAD-linked substrates and zero for others, (v) growth parameters (Clegg & Light, 1971) indicated a single site of mitochondrial ATP synthesis, rather than three, (vi) the normal antimycin A-sensitive respiratory chain from cytochrome *b* to cytochrome *c* was present but non-functional owing to the absence of cytochrome *c* oxidase, and (vii) the apparent *K_m* of the mitochondrial oxidase for O₂ was less than 10⁻⁶M. From these results we conclude that an alternative oxidase was present and communicated with the respiratory chain at about the level of cytochrome *b*.

Aerobic but non-growing incubation of the cells with or without copper salts did not restore cytochrome *aa*₃, in contrast with the behaviour of the copper-limited wild-type (Light, 1972). Growth of the mutant for 20 generations with added copper resulted in the return of cytochrome *aa*₃ but not the loss of the alternative oxidase. It seems likely that an alternative oxidase, previously shown to be inducible in *C. utilis* by Haddock & Garland (1971), had become constitutive. The copper-limited cells possessed giant mitochondria (Davison *et al.*, 1972).

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- Clegg, R. A. & Light, P. A. (1971) *Biochem. J.* **124**, 152–154
Davison, M. J., Downie, J. A. & Garland, P. B. (1972) *Biochem. J.* **129**, 46P
Haddock, B. A. & Garland, P. B. (1971) *Biochem. J.* **124**, 155–170
Light, P. A. (1972) *FEBS Lett.* **19**, 319–322

Proton Translocation by Rat Liver Mitochondria with Quinones as Terminal Acceptors

By HUGH G. LAWFORD and PETER B. GARLAND
(Department of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, U.K.)

In attempting to provide a sufficient number (three) of hydrogen carriers in the respiratory chain to meet the demands of the chemiosmotic hypothesis, Mitchell (1966) placed ubiquinone as the donor for cytochrome *c*. A consequence of this arrangement is that the predicted ratio of protons translocated per pair of electrons ($\rightarrow H^+/2e$) is 4.0 for the oxidation of NADH by ubiquinone. We have made direct measurements of this ratio by using ubiquinone analogues as acceptors for the oxidation of L-3-hydroxybutyrate by rat liver mitochondria, the underlying assumption being that the behaviour of the analogues of ubiquinone (duroquinone, ubiquinone-0, ubiquinone-1 and ubiquinone-2) is representative of that of endogenous ubiquinone (ubiquinone-10).

Measurements of the pH of mitochondrial suspensions were made essentially as described by Mitchell & Moyle (1967), except that the incubation medium included antimycin A and had a total volume of 1.0 ml. Parallel investigations of the rate of NADH oxidation by the ubiquinone analogues with sub-mitochondrial particles, and of the kinetics of oxidation of intramitochondrial NADH, showed that the ubiquinone analogues reacted rapidly at low concentration ($<5 \mu M$) with the respiratory chain. These analogues were therefore suitable for use as electron acceptors in pulse experiments of the type performed by Mitchell & Moyle (1967) with O₂.

With pulses of duroquinone, ubiquinone-0, ubiquinone-1 and ubiquinone-2 over the range 0.5–5.0 nmol of quinone/mg of mitochondrial protein, $\rightarrow H^+/2e$ ratios 1.0–1.5 were observed in the presence of antimycin and valinomycin. The lower values were obtained at the higher concentrations of quinone. These pH changes were abolished by