- 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

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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

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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