tase, 40%; NADH-ferricyanide oxidoreductase, 15%; NADPH-ferricyanide oxidoreductase, 27%. Acid phosphatase was 27% sedimentable.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970). *Biochem. J.* **116**, 40 P.

Changes in Subcellular Distribution of Enzymes during Respiratory Adaptation of *Saccharomyces carlsbergensis*

By D. LLOYD, LYNDA HOWELLS and T. G. CARTLEDGE.
(Department of Microbiology, University College, Cathays Park, Cardiff CF1 3NR, U.K.)

Anaerobically grown cultures of *Saccharomyces carlsbergensis* [containing glucose (10%, w/v), ergosterol and Tween 80] were allowed to adapt in the growth medium by sparging the fermenters with air. Adaptation was arrested by the addition of cycloheximide and chloramphenicol. Homogenates prepared anaerobically from sphaeroplasts of yeast cells after respiratory adaptation were analysed by isopycnic zonal centrifugation as described by Cartledge, Howells & Lloyd (1970a).

After adaptation for 10 min, both cytochrome *c* oxidase and catalase were detectable (4.6 and 4.9 nmol/min per mg of protein respectively). Cytochrome *c* oxidase was found mainly (66%) at ρ 1.225, whereas catalase was virtually all non-sedimentable (95%) although some enzyme was detected at ρ 1.19. The distribution of NADH- and NADPH-cytochrome *c* oxidoreductase and NADH- and NADPH-ferricyanide oxidoreductase was similar to that found in anaerobically grown non-adapted cells (Cartledge, Howells & Lloyd, 1970b). Thus the distribution of newly synthesized cytochrome *c* oxidase was similar to, but not identical with, that of those enzymes of particles of population B found in anaerobically grown cells.

After 30 min adaptation the specific activities of cytochrome *c* oxidase and catalase had increased (26.3 and 8.6 nmol/min per mg of protein respectively) and a marked change in distribution of cytochrome *c* oxidase, adenosine triphosphatase and NADH-cytochrome *c* oxidoreductase had occurred. Although NADH-cytochrome *c* oxidoreductase and cytochrome *c* oxidase were still detectable (6.3% and 8.3% of the total respectively) at ρ 1.21–1.22, both enzymes also showed a broad distribution up to ρ 1.15. Although a portion of both enzymes corresponded with the peak of soluble protein the heterogeneous density distribution suggested that both enzymes (and also adenosine triphosphatase) were associated with either slowly sedimenting particles or particles of $\rho < 1.15$.

After adaptation for 1 h the specific activities of cytochrome *c* oxidase and catalase were 37.8 and

8.9 nmol/min per mg of protein respectively; succinate-cytochrome *c* oxidoreductase was also now detectable (6.2 nmol/min per mg of protein). Again most of the cytochrome *c* oxidase (83.4%) and NADPH-cytochrome *c* oxidoreductase (92%) showed $\rho < 1.15$; three other peaks of activity of cytochrome *c* oxidase were present at ρ 1.13, 1.18 and 1.225. Succinate-cytochrome *c* oxidoreductase had a broad distribution in the soluble region and at ρ 1.18. The sedimentable oligomycin-sensitive adenosine triphosphatase showed a broad distribution between ρ 1.12 and ρ 1.20 (median ρ 1.16).

'Soluble' adenosine triphosphatase (50% of the total) was not oligomycin-sensitive.

Distribution of acid phosphatase did not alter during adaptation, although the specific activity of whole homogenates showed a threefold increase over the anaerobic level of activity after 1 h adaptation.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970a). *Biochem. J.* **116**, 24 P.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970b). *Biochem. J.* **116**, 40 P.

The Functional Characterization of Mitochondria from Rat Liver and Krebs II Ascites-Tumour-Cell Mitochondria

By MARGARET ASHWELL and T. S. WORK. (National Institute for Medical Research, Mill Hill, London N.W.7, U.K.)

Mitochondria synthesize proteins both in intact cells and after isolation (Work, Coote & Ashwell, 1968). Reports have appeared that mitochondria from animal cells contain a 55S ribosome-like particle (O'Brien & Kalf, 1967a,b). We find that washed sterile mitochondria from liver or Krebs cells can be pulse-labelled with [14 C]leucine or [35 S]methionine. After short pulses (up to 5 min) most of the radioactivity is found attached to a particle sedimenting with *Escherichia coli* ribosome fraction in sucrose gradients containing 10 mM-Mg²⁺. After longer periods of incubation or after a 'chase' of unlabelled leucine there is transfer of radioactivity to membrane proteins.

The incorporation of [14 C]leucine into the radioactive peak is not inhibited by the presence of cycloheximide (100 μ g/ml) or ribonuclease (20 μ g/ml) during the pulse, but the presence of D-threo-chloramphenicol (100 μ g/ml) will cause 80% inhibition of incorporation. The radioactivity attached to the ribosomal particles is presumed to be in the form of incomplete peptide chains, since exposure of mitochondria to puromycin for 30 s or longer under conditions favouring protein synthesis results in complete removal of radioactivity from the peak; moreover this effect of puromycin is partially blocked by chloramphenicol. The

radioactive peak was most readily obtained when Nonidet (NP 40) or BRIJ 58 was used to disrupt the mitochondria after pulse-labelling, but similar ribosome-like particles were also obtained by use of sodium deoxycholate or Triton X-100. Mitochondria disrupted in very low Mg^{2+} concentrations showed radioactivity to about 50S. The results are entirely consistent with the assumption that animal mitochondria contain mitoribosomes sedimenting at less than 80S. Experiments in which radioactive mitoribosomes were mixed with unlabelled *E. coli* ribosomes and sedimented in a sucrose density gradient containing 10mM- Mg^{2+} showed that the two peaks (radioactivity and extinction) were close together whereas *E. coli* ribosomes were readily separable under these conditions from rat liver cytoribosomes and Krebs-cell cytoribosomes. These results reinforce the suggestion that in protein synthesis mitochondria resemble bacteria much more closely than they do the eukaryotic cytoplasmic ribosomes.

O'Brien, T. W. & Kalf, G. W. (1967a). *J. biol. Chem.* **242**, 2172.

O'Brien, T. W. & Kalf, G. W. (1967b). *J. biol. Chem.* **242**, 2180.

Work, T. S., Coote, J. L. & Ashwell, M. A. (1968). *Fedn Proc. Fedn Am. Soc. exp. Biol.* **27**, 1174.

Factors Affecting Protein Synthesis in Yeast Mitochondria

By L. A. GRIVELL and D. B. ROODYN. (*Department of Biochemistry, University College of London, London W.C.1, U.K.*)

Mitochondria isolated essentially free of micro-organisms from protoplast lysates of *Saccharomyces carlsbergensis* have been found to incorporate [^{14}C]leucine into protein during incubation *in vitro* (Grivell, 1967). Incorporation takes place mainly into an RNA-rich fraction, possibly located within the inner mitochondrial membrane, and is sensitive to chloramphenicol and erythromycin, but resistant to cycloheximide and ribonuclease. Activity requires K^+ , Mg^{2+} and phosphate and has a broad optimum at pH 6.7; Li^+ and Na^+ are inhibitory. Response to Mg^{2+} is dependent on the phosphate concentrations and the adenine nucleotide present. The system requires ATP, which may be exogenous or generated by respiratory phosphorylation in the presence of oxidizable substrate and ADP; the phosphoenolpyruvate-pyruvate kinase ATP-regenerating system is fully effective only when succinate is present. Experiments with oligomycin suggest that there is not an obligatory participation of the hypothetical high-energy intermediates of oxidative phosphorylation.

Transport of [^{14}C]leucine has also been measured by a centrifugation-filtration technique; there is approximately twofold concentration of amino acid in a process that appears to be complete within 20s of addition of mitochondria to the incubation medium and that is not markedly affected by the addition of oxidizable substrate, ATP, oligomycin or antimycin. It is concluded that there is not a simple relationship between the ability of the mitochondrion to accumulate amino acids and to synthesize protein.

Direct measurement of the changes occurring in the concentrations of mitochondrial amino acids during incubation reveal discrepancies between radioactive and chemical methods of estimation of concentration ratios; equilibration between [^{14}C]leucine and the endogenous pool is not complete even after 30min. Although the size of the endogenous leucine pool does not increase during incubation, there is an approximately threefold increase in exogenous leucine. The difficulties of assessing true rates of protein synthesis in a system where the amino acid pool is not homogeneous and increases during incubation are to be stressed.

The authors are grateful to the Science Research Council for financial support and for the award of a Research Scholarship to L. A. G.

Grivell, L. A. (1967). *Biochem. J.* **105**, 44c.

Ribosome Particles in Yeast Mitochondria

By P. V. VIGNAIS and J. HUET. (*Biochimie, Faculté de Médecine et Centre d'Études Nucléaires, 38-Grenoble, France*) and J. ANDRÉ (introduced by D. E. GRIFFITHS). (*Biologie Cellulaire 4, Faculté des Sciences, 91-Orsay, France*)

Evidence for the existence of ribosomes within mitochondria from yeast cells (*Candida utilis*, C.B.S. 1516) has been obtained from examination of electron micrographs of isolated mitochondria, and also from the characterization of the mitoribosomes after their isolation from mitochondria.

Electron micrographs of thin sections of yeast mitochondria stained with uranyl acetate and lead citrate showed rows of particles with diameters of 180 Å attached along the matrix side of the inner membrane and of the cristae. Free mitoribosomes were obtained by disruption of mitochondria with deoxycholate and purified by centrifugation on a linear 5–20% (w/v) sucrose density gradient in 5mM- $MgCl_2$ –50mM-KCl–10mM-tris–HCl buffer, pH 7.6. The sedimentation profile of mitoribosomes obtained under the above conditions is characterized by a major peak at 53–55S and a smaller one at 76–78S. Increasing the $MgCl_2$ concentration in the sucrose density gradient above 20mM results in an increase of the 76–78S peak with a slight shift to