ASYNCHRONOUS DEVELOPMENT OF DIFFERENT FUNCTIONS IN MITOCHONDRIA OF *SACCHAROMYCES CEREVISIAE*

G. LENAZ, G. P. LITTARRU and A. CASTELLI
Istituto di Chimica Biologica dell'Università Cattolica, Rome, Italy

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Yeasts have been considered very suitable organisms for the study of mitochondrial biogenesis. Irrespective of the genetic origin of the mitochondrial proteins [1,2], little is known about how the mitochondrial membranes and their lipoprotein components are assembled together. Anaerobic yeasts have been shown to lack mitochondria [3]; some mitochondrial activities are nevertheless present [4]; and the presence of mitochondrial membranes in these yeasts is determined by the nature of the growth medium [5]. When anaerobically grown yeasts are allowed to grow in aerobiosis, mitochondria are formed [6], and there is parallel synthesis of the cytochromes [3].

The recognition by Green and his coworkers that membranes are made up of repeating units, containing fixed and detachable sectors [7], and the finding that the known functional activities of the mitochondrion are located within the sectors of the repeating units of the two membranes [8], open the way to a more precise study by biochemical means of the order by which the various mitochondrial components are synthesized and assembled.

The present study deals with the development of some mitochondrial activities in yeast under controlled experimental conditions in which mitochondria pass from a repressed to an active stage. The results of this investigation demonstrate that membrane biosynthesis is a stepwise process in which the different components are independently formed, and that the assembly of these components precedes the emergence of integrated processes.

Saccharomyces cerevisiae, strain ATCC 7754, was grown for 16 hr in a medium similar to that described by Klein [9] but containing 5% glucose, and then inoculated into a fermentor containing a rich medium [3] with glucose at a final concentration of 0.3%. At different time intervals the cells were harvested by centrifugation and a mitochondrial fraction was prepared according to Schatz [10]. Oxidative phosphorylation, ATPase activity, and the activity of Krebs cycle enzymes were assayed as described elsewhere [11,12].

A mitochondrial fraction was obtained at all the time intervals investigated, but the yield was lower between zero time and the 6th hour; at this time under our conditions, glucose is completely consumed. Table 1 shows the specific activities of different enzymes of the Krebs cycle which have been localized in the outer membrane [12]. The four enzymes tested

Table 1
Specific activities of malic, isocitric, pyruvic and α-ketoglutaric dehydrogenase during the growth phases of *S. cerevisiae*.

Time (hr)	Malic dehydrogenase [a]	Pyruvic dehydrogenase [a]	α-keto-glutaric dehydrogenase [a]	Isocitric dehydrogenase [b]
0	0.193	0.119	0.043	0.078
4	0.017	0.013	0.007	0.007
5	0.275	0.206	0.068	0.041
6	0.293	0.320	0.050	0.040
8	0.436	0.304	0.114	0.064
10	0.715	0.392	0.148	0.070
12	0.770	0.385	0.120	0.067
18	0.613	0.294	0.157	0.092
24	0.580	0.095	0.120	0.047

[a] Specific activities are expressed as μmoles of NAD reduced per min per mg of mitochondrial protein.
[b] Specific activity is expressed as μmoles of NADP reduced per min per mg of mitochondrial protein.

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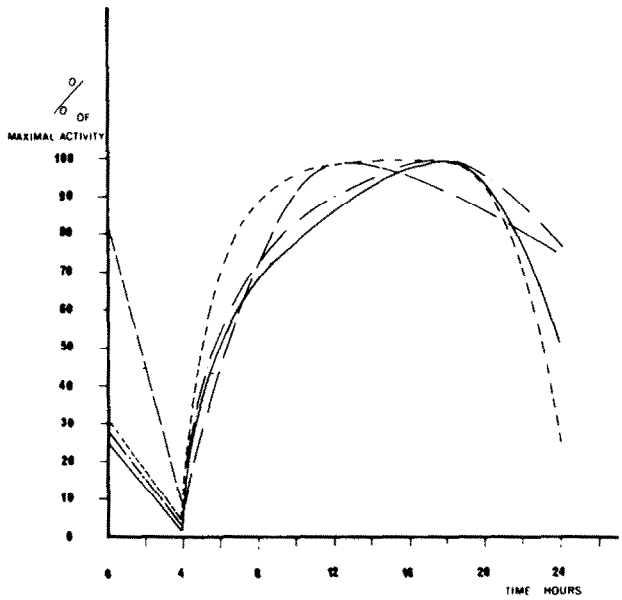

Fig. 1. Specific activities of certain representative Krebs cycle enzymes during the phases of growth of *S. cerevisiae*. – – – – malic dehydrogenase; ——— isocitric dehydrogenase; ------- pyruvic dehydrogenase; – · – · – α-ketoglutaric dehydrogenase.

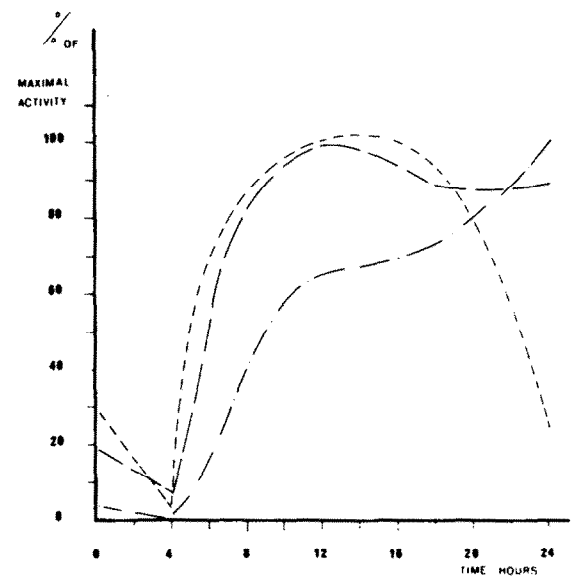

Fig. 2. Synoptic curve of specific activities of pyruvic dehydrogenase, ATPase, and succinoxidase during the phases of growth of *S. cerevisiae*. ------- pyruvic dehydrogenase; – – – – ATPase; – · – · – succinoxidase.

follow similar patterns (cf. fig. 1); they have some activity at "zero time", but all the activities fall to very low values at the fourth hour of growth; nevertheless in the following hour there is a dramatic increase in activity which continues until a maximum is reached; then a more or less marked fall in activity sets in. ATPase activity (table 2) has a similar pattern, but remains constant after the 10th hour. Oxygen uptake with succinate as the substrate (table 3) is not measurable at the 4th hour, then increases slowly to a maximum at the last hour examined (the 24th). P:O

Table 2
ATPase activity of mitochondria of *S. cerevisiae* at different times of growth and extent of inhibition of this activity by oligomycin [a].

Time (hr)	Specific activity (μmoles P_i/min/mg protein)	Oligomycin sensitivity (% inhibition)
0	0.611	82
4	0.227	79
5	0.745	82
6	1.676	88
8	2.870	89
10	3.078	90
12	3.256	90
18	2.870	85
24	2.878	87

[a] Oligomycin was added at levels of 5 μg per ml of assay medium. The amount of mitochondrial protein per assay was 0.2–0.4 mg.

Table 3
Oxidative phosphorylation in mitochondria of *S. cerevisiae* at different times of growth.

Time (hr)	Succinoxidase activity (μatoms O_2/min/mg protein)	P:O ratio
0	0.016	–
4	0.000	–
5	0.028	0.4
6	0.085	0.6
8	0.160	0.7
10	0.260	0.9
12	0.285	1.0
18	0.295	0.9
24	0.440	1.0

ratios are low at the 5th hour, then increase to near 1 after the 10th hour.

In fig. 2 the activities, as percentages of the highest values reached, are shown for pyruvic dehydrogenase (a representative Krebs cycle enzyme), for ATPase, and for succinoxidase. Direct comparison shows asynchrony in the development of the three activities; for example, at the 5th hour Krebs cycle enzymes already have reached about 50% of their maximal activities, ATPase 25%, whereas the rate of oxygen uptake is still only 6% of its highest activity. The sequence of formation of the activities considered appears to be the following: while glucose is consumed, the activities of Krebs cycle enzymes and of ATPase increase sharply, but electron transfer remains low; when the activities of Krebs cycle enzymes and ATPase reach a maximum, electron transfer is still low but slowly continues to increase, subsequently electron transfer activity increases as the activity of the citric cycle enzymes declines, whereas the level of ATPase activity remains constant. Oxidative phosphorylation, which may be considered as the integration of the electron transfer chain with the ATPase system [13] (i.e., of the base-pieces with the headpieces of the repeating units of the inner membrane) [14], is not in phase with either electron transfer or ATPase, since P:O ratios are not constant during the experimental period but increase to a maximum at the 10th hour.

These findings, when related to the structures to which the above-mentioned activities belong, suggest a possible scheme of membrane morphogenesis. The outer membrane is formed first (as suggested by the electron microscopic observation of empty mitochondrial profiles) (unpublished observations), and is soon filled by "soluble" enzymes (ATPase and Krebs cycle enzymes); then the Krebs cycle enzymes may be attached to the outer membrane (although we do not have any direct proof for this phenomenon), and ATPase is contained inside the mitochondrion as an organized system, although not yet attached to a complete inner membrane; in fact the basepieces which make up the cristae are not yet formed as shown by absence of oxygen uptake. This stage may correspond to that shown by Wallace and Linnane [6] in the aerobic development of anaerobically repressed yeast in which mitochondrial profiles are found to be filled with amorphous material. Oligomycin sensitivity of ATPase is always high, and is not markedly changed in our conditions during growth. Schatz [15] on the other hand has found that the mitochondrial ATPase of "petite" mutants is oligomycin-insensitive – a property which is incompatible with it being membrane-bound. The final stage in the formation of mitochondria is the assembly of the cristae as reflected in the rate of oxygen uptake: this stage is accompanied by coupling of the electron transfer chain (basepieces) to the previously formed oligomycin-sensitive ATPase system (stalk–headpieces–phospholipids) [14] – a coupling which leads to the emergence of oxidative phosphorylation. This coupling is probably a limiting step in the assembly of mitochondria. The increase in the rate of oxygen uptake which parallels formation of cristae results in diminution of the other activities.

References

[1] M.E.Pullman and G.Schatz, Ann. Rev. Biochem. 36 (1967) 359.
[2] A.W.Linnane, D.R.Biggs, M.Huang and G.O.Clark-Walker, in: Aspects of Yeast Metabolism, ed. R.K.Mills (Blackwell, Oxford, 1967).
[3] A.W.Linnane, in: Oxidases and Related Redox Systems, eds. T.E.King, H.S.Mason and M.Morrison (Wiley, New York, 1965) vol. 2, p. 1102.
[4] G.Schatz, Biochim. Biophys. Acta 96 (1965) 342.
[5] P.G.Wallace, M.Huang and A.W.Linnane, J. Cell Biol. 37 (1968) 207.
[6] P.G.Wallace and W.A.Linnane, Nature 201 (1964) 1191.
[7] D.E.Green and J.F.Perdue, Proc. Natl. Acad. Sci. US 55 (1966) 1295.
[8] D.E.Green, Israel J. Med. Sci. 1 (1965) 1187.
[9] H.P.Klein, J. Bacteriol. 69 (1955) 620.
[10] G.Schatz, in: Methods in Enzymology, vol. 10, eds. R.W.Estabrook and M.E.Pullman (Academic Press, New York, 1967) p. 197.
[11] E.Bachmann, G.Lenaz, J.F.Perdue, N.Orme-Johnson and D.E.Green, Arch. Biochem. Biophys. 121 (1967) 73.
[12] E.Bachmann, D.W.Allmann and D.E.Green, Arch. Biochem. Biophys. 115 (1966) 153.
[13] Y.Kagawa and E.Racker, J. Biol. Chem. 241 (1966) 461.
[14] K.Kopaczyk, J.Asai, D.W.Allmann, T.Oda and D.E. Green, Arch. Biochem. Biophys. 123 (1968) 602.
[15] G.Schatz, J. Biol. Chem. 243 (1968) 2192.