REGULATION OF MITOCHONDRIAL BIOGENESIS: FURTHER EVIDENCE FOR PROTEINASE INVOLVEMENT

A. V. GALKIN, T. V. TSOI and V. N. LUZIKOV

Department of Subcellular Biogenesis, Belozersky Laboratory of Molecular Biology and *Bioorganic Chemistry, Lomonosov State University, Moscow 117234, USSR*

Received 18 July 1979

1. Introduction

In this laboratory we are concerned with elucidation of the mechanisms regulating mitochondrial biogenesis. It has been hypothesized [1,2] that mitochondrial assembly is controlled by lytic enzymes through selective elimination of components that fail to perform properly. Our recent report [3] has provided evidence that mitochondrial formation during yeast growth in a galactose medium is indeed accompanied by appearance of respiratory chain components which prove to be inactive (most probably due to improper alignment within the membrane) and are proteolytically eliminated. It, however, remained uncertain whether such phenomena are general or only a unique property of the particular experimental conditions.

The present work demonstrates that such non-functioning components of the mitochondrial respiratory chain are also retained during yeast growth in a glucose medium in the presence of proteinase inhibitors.

2. Materials and methods

Saccharomyces cerevisiae were grown batchwise as described [4] in a medium containing 2.5 g glucose, 1 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4$ and 2 g yeast extract (Serva, Heidelberg) per litre. Phenylmethyl sulfonyl fluoride (PMSF) was added to the cell suspension in portions of 50 μmol/l at intervals of 90–120 min, beginning from 6–7 h of growth, to a cumulative 2.5×10^{-4} M. Pepstatin was added twice, at 6 and 9 h of growth, at 0.5 mg/l suspension (cumulative 1.5×10^{-6} M).

Membrane fractions containing the entire mitochondrial populations were isolated as in [3] from early stationary (16 h of growth) control and PMSF-grown cells.

3. Results and discussion

The development of yeast respiratory system was initially studied by measuring the changes in cell respiration and in the overall content of mitochondrial cytochromes in intact cells during growth in culture with and without proteinase inhibitors, PMSF and pepstatin.

The growth rate was only slightly and transiently inhibited by PMSF or pepstatin, and the culture completely recovered within 2 h from the first addition. By the early stationary phase identical cell numbers ($\sim 6 \times 10^7$ cells/ml) and biomass yields (0.88 g dry wt cells/l suspension) were obtained with control, PMSF- and pepstatin-grown batches. These results are in complete accord with our earlier observations [5] which have been confirmed [6].

Cell respiration, which is evidently respiratory chain-determined since it is completely inhibited by 1 mM KCN or 2×10^{-7} M antimycin and considerably stimulated by CCCP, is not affected by proteinase inhibitors. At the same time, during yeast growth in the presence of antiproteolytic agents cytochromes *c*, c_1, *b* and aa_3 gradually accumulate above the corresponding control levels, so that by the early stationary phase the cytochrome content in

Table 1
Characteristics of mitochondrial populations from control and PMSF-grown yeast cells

Source of preparation	Cytochrome contents (nmol/g dry wt. cells)			Succinate oxidase (μg-atoms O/min .g dry wt cells)	
	cyt. $c + c_1$	cyt. b	cyt. aa_3		+ 5 μM cyt. c
Control	41.7	57.8	14.4	46.0	61.2
PMSF-grown	52.0	72.0	18.5	46.5	60.4

	Ascorbate oxidase, (μg-atoms O/min .g dry wt cells)	Ubiquinol oxidase (μmol CoQ_2 oxidized/ min .g dry wt cells)	Succinate : cyt. c reductase (μmol cyt. c reduced/min .g dry wt cells)
Control	112	44.0	81.0
PMSF-grown	115	45.0	79.5

Mitochondrial populations were obtained from stationary cells as 70 000 × g membrane fractions [3]. Cytochrome contents were calculated from the difference (reduced minus oxidized) room-temperature spectra using the following wavelength pairs and extinction coefficients: cyt. $c + c_1$ (551–540 nm) 20 mM^{-1} .cm^{-1}, (cyt. b (562–575 nm) 19.1 mM^{-1} cm^{-1}, cyt. aa_3 (605–630 nm) 24 mM^{-1} .cm^{-1} [7]. Activities were measured in 50 mM potassium phosphate (pH 7.4), 1 mM EDTA. Values presented are the maximal ones which were attained at the following substrate concentrations: succinate oxidase, 20 mM succinate; ascorbate oxidase, 20 mM ascorbate; TMPD, 1 mM; ubiquinol oxidase, 11 μM coenzyme Q_2 reduced according to [8]; succinate : cytochrome c reductase, 20 mM succinate, 15 μM cytochrome c

PMSF-grown cells exceeds the control by 25–30%; the effect of pepstatin is only slightly less.

Thus, growth in a glucose medium in the presence of proteinase inhibitors induces a discrepancy between cell respiration and cytochrome content similar to that observed with galactose-grown yeast [5].

This is confirmed by the characteristics of mitochondrial populations isolated from the stationary control and PMSF-grown cells (table 1). As can be seen, preparations from inhibitor-treated cells contain 25–30% more cytochromes $c + c_1$, b and aa_3 than the control ones, in accord with whole-cell determinations; succinate-reducible flavoproteins were also found to be 20–30% higher in mitochondrial populations from PMSF-grown cells. Nevertheless, succinate oxidase and partial activities of the succinate oxidase chain prove to be identical in control and inhibitor-grown preparations, regardless of the concentration of substrates.

In order to evaluate the potential catalytic capacity of the individual respiratory chain complexes (i.e., succinate : ubiquinone reductase, ubiquinol : cytochrome c reductase and cytochrome c oxidase), these enzymes were activated maximally by freezing–thawing the membrane preparations in the presence of Lubrol WX [3]. As exemplified in table 2, under such conditions all the above activities in preparations from PMSF-grown cells exceed the control ones. Moreover, the activities are nearly proportional to the spectrally determined contents of electron carriers comprising the corresponding complexes and display similar app. K_m values (e.g., 3 μM CoQ_2 at 20 mM succinate for complex II, 4.7 μM ferrocytochrome c at 0.24 mM dissolved oxygen for complex IV). Thus it seems that the 'excess' components retained in the presence of proteinase inhibitors, although possessing normal catalytic properties, do not participate in electron flow mainly because their improper alignment within the membrane hinders interaction with adjacent carriers. All this appears to be in perfect analogy to the results obtained with galactose-grown yeast [3].

Reconciliation of the data on glucose- and galactose-grown cells gives rise to several issues.

(1) The reported phenomena can hardly be ascribed to any side effects of proteinase inhibitors like suppression or distortion of the synthesis and/or assembly of mitochondrial constituents, in view of the essential normality of the 'excess' respiratory chain complexes. Some questions remain

Table 2
Activities of the individual complexes of the respiratory chain in solubilized membrane preparations

Preparation	Succinate: CoQ reductase (μmol CoQ reduced/min)	$CoQH_2$: cyt. *c* reductase (μmol cyt. *c* reduced/min)	Cytochrome oxidase (μmol cyt. *c* oxidized/min)
Control	52.2	77.0	51.5
PMSF-grown	63.4	98.3	65.1

Activation was achieved by freezing–thawing of the membrane preparations from control and PMSF-grown cells in the isolation medium with 0.05% Lubrol WX; the detergent was also present in the assay medium (see table 1) in concentrations producing maximal activation. Activities presented in table 2 pertain to the following conditions: succinate : ubiquinone reductase, 20 mM succinate, 6.7 μM coenzyme Q_2, 0.01% Lubrol; ubiquinol : cytochrome *c* reductase, 15 μM reduced coenzyme Q_2, 11 μM cytochrome *c*, 0.03% Lubrol; cytochrome oxidase, 7 μM ferrocytochrome *c*, ~0.24 mM dissolved oxygen, 0.06% Lubrol. Activities are given per g dry wt cells

obscure such as whether a precursor-processing machinery is operative in yeast respiratory chain assembly and, if so, whether it is affected by these antiproteolytic compounds. However, features very similar to those of the mitochondria of inhibitor-treated cells are found in the preparations from control galactose-grown yeast at the earlier stages of respiratory development [3], which strongly argues in favour of such aberrations in respiratory chain arrangement being indeed naturally occurring.

(2) As neither integral nor partial respiratory chain activities are affected by elimination, retention (in galactose cultures [3]) or accumulation (in glucose cultures) of a portion of respiratory chain components, it is most reasonable to conclude that it is the inactive entities that are preferentially eliminated in normal conditions and preserved by proteinase inhibitors. Otherwise it has to be presumed that the changes in the amount of respiratory chains are somehow exactly compensated by activation or deactivation of the whole mitochondrial population, which is hardly likely.

(3) The body of data obtained testifies that the appearance and selective elimination of non-functioning components are not the unique property of yeast growth on galactose, making it plausible that the principle of performance control by lytic enzymes is widely if not universally applicable to mitochondrial biogenesis.

References

[1] Luzikov, V. N. (1973) Sub-Cell. Biochem. 2, 1–31.
[2] Luzikov, V. N. (1979) in: Processing and Turnover of Proteins and Organelles in the Cell, FEBS Symp. no. 53, pp. 133–142.
[3] Galkin, A. V., Tsoi, T. V. and Luzikov, V. N. (1979) FEBS Lett. 103, 111–113.
[4] Luzikov, V. N., Zubatov, A. S., Rainina, E. I. and Bakeyeva, L. E. (1971) Biochim. Biophys. Acta 245, 321–334.
[5] Luzikov, V. N., Makhlis, T. A. and Galkin, A. V. (1976) FEBS Lett. 69, 108–110.
[6] Ryrie, I. J. and Gallagher, A. (1979) Biochim. Biophys. Acta 545, 1–14.
[7] Lee, C. P., Estabrook, R. W. and Chance, B. (1965) Biochim. Biophys. Acta 99, 32–45.
[8] Lester, R. L., Hatefi, Y., Widmer, C. and Crane, F. L. (1959) Biochim. Biophys. Acta 33, 169–176.