A PROBABLE ROLE OF CELL PROTEINASES IN THE BIOGENESIS OF MITOCHONDRIA IN YEAST

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

Department of Biokinetics, Laboratory of Bioorganic Chemistry, Lomonosov State University, Moscow 117234, USSR

Received 15 July 1976

1. Introduction

An interesting aspect of mitochondriogenesis is the possible role of proteolytic enzymes in the regulation of the development of the organelle. Several authors have given evidence for the existence of mitochondrial proteinases in mammalian cells [1–5]. It has been suggested that these proteinases control the quantity of mitochondrial material in the cell [3] and the rates and synchrony of the turnover of mitochondrial proteins [4]. According to Wheeldon et al. [2], a regulatory function in membrane assembly can be ascribed to an intramitochondrial proteinase attacking polypeptides released from mitochondrial ribosomes.

However, the physiological and biochemical bases for the suggested regulatory role of proteinases in mitochondriogenesis remain obscure, particularly how their activity is regulated. Our studies on the stability of the oxidative phosphorylation system both in vitro and in vivo, reviewed in [6], allowed Luzikov to formulate the principle that the viability of mitochondria in the cell is determined by the efficiency of their functioning. Any failure in performance, due to external factors (e.g. anaerobiosis, inhibition of respiration or uncoupling [7,8]) or to hypothetical 'errors' in the organelle composition or structure, would therefore result in the degradation of mitochondria or parts thereof, presumably by lytic enzymes [6]. In other words, mitochondrial differentiation and persistence imply selection of the most efficient (and hence the most viable) organelles.

Previously [9] we encountered an interesting system which probably demonstrates this principle in operation. During aerobic growth of *Saccharomyces cerevisiae* in a galactose-containing medium the cell cytochrome content reaches its maximal level in the early exponential phase and then decreases through the rest of the exponential phase by a factor of 1.7–2; meanwhile cell respiration remains constant [9].

Here we report that the decrease in cell cytochrome content during the exponential phase of yeast growth in culture is blocked by the presence of the specific inhibitors of yeast cell proteinases, phenylmethyl sulfonylfluoride and pepstatin, while cell respiration is unaffected. We consider these results indicate that yeast proteinases participate in the process of mitochondrial development, apparently by eliminating the 'excess' cytochromes that are probably non-functioning.

2. Materials and methods

S. cerevisiae was grown under the same conditions as before [9]. Phenylmethyl sulfonylfluoride (PMSF, Sigma Chemical Co.) and pepstatin (a hexapeptide inhibitor of acid proteinases isolated form a streptomycete [10], a generous gift from Professor H. Umezawa) were administered to the cell suspension as solutions in 70% ethanol prepared before use. The changes in the cytochrome content of the intact yeast cells were determined by low-temperature (77°K) spectrophotometry as previously described [9]. Cell respiration was measured with a PO 4 Radiometer polarograph in 50 mM potassium phosphate, pH 7.4, at 30°C.

North-Holland Publishing Company – Amsterdam

3. Results and discussion

The decrease in cell cytochrome content during exponential growth could be due either to cytochrome breakdown by proteolytic enzymes, or to a decreased rate of their synthesis [9]. We decided to test the first suggestion directly using proteinase inhibitors.

It is known that *S. cerevisiae* possesses two cellular endopeptidases, proteinase A and proteinase B, which are specifically inhibited in vitro by pepstatin and PMSF, respectively [11]; this has been confirmed for our strain of *S. cerevisiae* (unpublished data). In the present work these inhibitors were added to the yeast culture growing under standard conditions [9] in order to induce in vivo inhibition of the cell proteinases. Culture growth, cell respiration and cell cytochrome content were followed. PMSF was administered either at the moment of growth corresponding to the peak cytochrome concentration (8.5 h of growth, 2×10^{-4} M PMSF) or in a 'stepwise' mode (50 μmol PMSF per 1 cell suspension four times, at 2, 4, 6 and 8 h of growth); pepstatin was added at 8.5 h of growth in a concentration of 1 mg/l (approx. 1.5×10^{-6} M, assuming a molecular weight of 670).

Figure 1B–E demonstrates the changes in the cell cytochrome content during growth in culture in the presence of PMSF or pepstatin and in the control suspension. As can be seen, the pattern of the cytochrome behaviour is dramatically altered in the presence of proteinase inhibitors. The fall in the amount of cytochrome *c* is significantly attenuated (fig.1B), and the decrease in the contents of cytochromes c_1, *b* and aa_3 (fig.1C–E) is completely blocked (in fact, a certain rise in the amounts of these cytochromes can be observed at 10–12.5 h of growth,

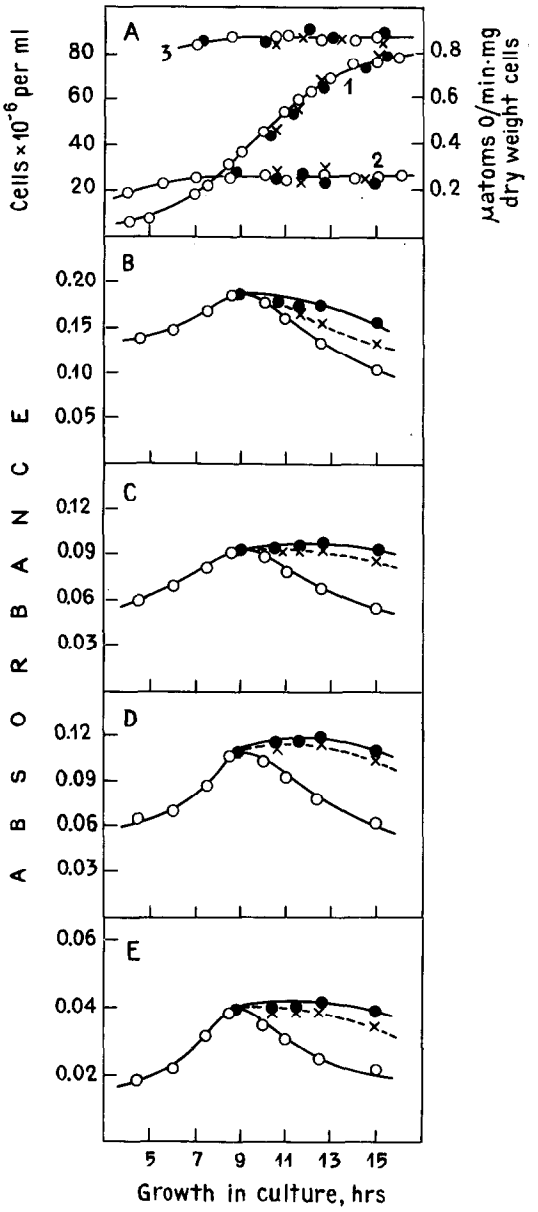

Fig.1. Effect of proteinase inhibitors on aerobic growth of *S. cerevisiae* in galactose-containing medium and on cell cytochrome content. -o-o-, control suspension (no additions); -•-•-, in the presence of PMSF (cumulative 2×10^{-4} M, see text); -X-X-, in the presence of pepstatin (1 mg/l cell suspension). A, growth in culture; curve 1, concentration of cell suspension, 10^6 cells/ml; curves 2 and 3, cell respiration, μatoms O/min·mg dry weight cells; 2 – non-stimulated respiration on 0.25% galactose, 3 – maximally stimulated respiration on 3% (v/v) ethanol, 50 μM carbonyl cyanide, *m*-chlorophenyl hydrazone. B–E, cytochrome content of the intact cells. The data are presented in the form of absorbance of each cytochrome in the difference (reduced minus oxidized) spectra recorded as previously described [9] with cell suspensions containing 30 mg dry weight/ml (1.2×10^9 cells/ml). No attempt has been made to express the cytochrome contents in molarities because no reliable extinction coefficients are available for these experimental conditions. B, cytochrome *c* (548–533 nm); C, cytochrome c_1, (553–533 nm). D, cytochrome *b* (559–570 nm); E, cytochrome aa_3 (603–630 nm).

which is succeeded by a decrease to the 8.5 h level at the end of the exponential phase). The two modes of PMSF administration produce quantitatively similar effects. The effect of pepstatin is only slightly less pronounced. The specificity of inhibitor action is evidenced by the effectiveness of low concentrations of PMSF and pepstatin (comparable with those used for selective in vitro inhibition [11]). No spectral shifts or appearance of new bands were observed during growth in culture either in the presence or absence of the proteinase inhibitors.

Under the indicated experimental conditions inhibition of culture growth in the presence of pepstatin and PMSF never exceeded 15–20%. Moreover, no suppression of growth and biomass yield could be observed at the end of the exponential phase (fig.1A). The decrease in the cell cytochrome content reported by Galkin et al. [9] could be attributed to a decrease in the rate of cytochrome formation, resulting in the dilution of cytochrome due to cell divisions, the fall in the amount of cytochromes per cell being determined by the increase of the biomass during growth. Since it is hardly probable that the proteinase inhibitors selectively stimulate cytochrome formation, and since the increase in the biomass is unaffected and the diminution of the cytochrome content is blocked in the presence of PMSF and pepstatin, the cytochrome dilution possibility seems unlikely. The most plausible explanation, therefore, is that some of the cell cytochromes are eliminated during the exponential phase of growth. This process evidently requires participation of the cell proteinases, since it is suppressed by the specific proteinase inhibitors.

Further, an important point must be emphasized. PMSF and pepstatin, while preventing the decrease in the cell cytochrome content (fig.1B–E), fail to produce any detectable effect on cell respiration (either non-stimulated or stimulated by an uncoupler) when compared with the control (fig.1A). Thus, if the development of the respiratory apparatus of facultatively anaerobic yeast can in the absence of inhibitors be described in terms of 'intensification' (i.e. increase of the ratio of the respiratory activity to the cytochrome content) [9], no 'improvement' of the respiratory machinery is observed when the cellular proteolytic factors are out of action.

In conclusion, it can be claimed that the yeast cell proteinases participate in the differentiation of the cell respiratory apparatus, apparently by eliminating some 'excess' cytochrome material synthesized in the early exponential phase. The nature and the fine mechanism of this phenomenon are still not clear. As respiration per unit weight of cells, irrespective of the cell cytochrome content, is constant through the exponential phase (compare fig.1A and fig.1B–E), it may well be that some cytochromes at the early stages of mitochondrial development cannot function normally and they are likely to be preferentially degraded by proteolytic enzymes [6]. It should be remembered that the postulated selection by the criterion of efficiency [6] may operate at both molecular and subcellular levels. Thus, local 'errors' (e.g. abnormal or wrongly oriented components) may be eliminated locally with subsequent replacement and/or repair; large-scale abnormalities would result in destruction of significant membrane regions or the whole organelle. It is quite possible that cell proteinases A and B are not the only factors involved. For example, they may perform the final stages of degradation, while the primary destabilization may be due to the action of intramitochondrial enzymes. A promising field is open for further investigation.

References

[1] Alberty, K. G. M. M. and Bartley, W. (1969) Biochem. J. 111, 763–774.
[2] Wheeldon, L. W., Dianaux, A., Bof, M. and Vignais, P. V. (1974) Eur. J. Biochem. 46, 189–199.
[3] Gear, A. R. L., Albert, A. D. and Bednarek, J. M. (1974) J. Biol. Chem. 249, 6495–6504.
[4] Rajwade, M. S., Katyare, S. S., Fatterpaker, P. and Sreenivasan, A. (1975) Biochem. J. 152, 379–387.
[5] Aoki, Y., Urata, G., Takaku, F. and Katunuma, N. (1975) Biochem. Biophys. Res. Commun. 65, 567–574.
[6] Luzikov, V. N. (1973) Sub-Cell. Biochem. 2, 1–31.
[7] Luzikov, V. N., Zubatov, A. S. and Rainina, E. I. (1970) FEBS Lett. 11, 233–236.
[8] Luzikov, V. N., Zubatov, A. S., Rainina, E. I. and Bakeyeva, L. E. (1971) Biochim. Biophys. Acta 245, 321–334.
[9] Galkin, A. V., Makhlis, T. A., Zubatov, A. S. and Luzikov, V. N. (1975) FEBS Lett. 55, 42–45.
[10] Umezawa, H. (1973) Pure Appl. Chem. 33, 129–144.
[11] Saheki, T. and Holzer, H. (1975) Biochim. Biophys. Acta 384, 203–214.