PARTICIPATION OF A MITOCHONDRIAL PROTEINASE IN THE BREAKDOWN OF MITOCHONDRIAL TRANSLATION PRODUCTS IN YEAST

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Received 28 February 1979

1. Introduction

An essential aspect of mitochondriogenesis is the assembly of catalytic complexes of the mitochondrial inner membrane. An organizing role in this process has been ascribed to mitochondrial translation products [1,2]; on the other hand, very little is known as yet about the turnover characteristics of these proteins. Polypeptides synthesized in yeast in the presence of an inhibitor of cytoplasmic translation, cycloheximide, were shown [3] to be subject to proteolysis with a half-life of ~60 min at the end of the exponential growth phase. However, the identity of proteolytic enzymes involved remained unknown. The results in [4] provided in vitro evidence for the existence of a yeast intramitochondrial proteinase digesting mitochondrial translation products. In an attempt to assess the in vivo role of the mitochondrial proteinase, this study reports data on the efficiency of the proteolysis of mitochondrial translation products in *Saccharomyces cerevisiae* cells and on the inhibitor sensitivity of this process at different phases of yeast growth.

2. Materials and methods

Saccharomyces cerevisiae was grown batchwise as in [5] and the breakdown of mitochondrial translation products was studied in exponential and early stationary cells as in [3]. This method is based on a double-labelling pulse-chase technique and uses inhibitors of cytoplasmic and mitochondrial protein synthesis. The values of $^{3}H_{m,\,synth}$ depicted here reflect the amount of [^{3}H]leucine incorporated into mitochondrial translation products during the pulse which is retained in the mitochondrial protein to the given moment of the chase. Analogously, $^{3}H_{m,\,degr}$ indicates the amount of [^{3}H]leucine accumulated in the acid-soluble fraction of mitochondria owing to the breakdown of the pulse-labelled mitochondrial translation products [3].

All experimental conditions were the same as in [3], except that counting of the radioactivities of both protein and acid-soluble fractions was performed in a toluene/Triton X-100 scintillator solution, and a Nuclear-Chicago Mark III spectrometer system was used.

In experiments with proteinase inhibitors, pepstatin (1 mg/l), leupeptin (2 mg/l) or phenylmethyl sulfonyl fluoride (50 μmol/l) were added 10 min before terminating the pulse and at zero time of the chase. The indicated amount of phenylmethyl sulfonyl fluoride was also added at 10 min of the chase. The washing medium contained the above concentrations of the respective inhibitors.

Activities of yeast proteinases A and B were assayed according to [6].

Pepstatin and leupeptin were generously provided by Professor H. Umezawa. Phenylmethyl sulfonyl fluoride was from Sigma Chem. Co.

3. Results

As follows from fig.1A, in exponentially growing yeast the pulse-labelled mitochondrial translation products degrade with a half-life of ~20 min. Concomitantly, the label initially contained in these proteins is accumulated in the acid-soluble fraction of mitochondria (fig.1B), suggesting a proteolytic process. The breakdown of mitochondrial translation

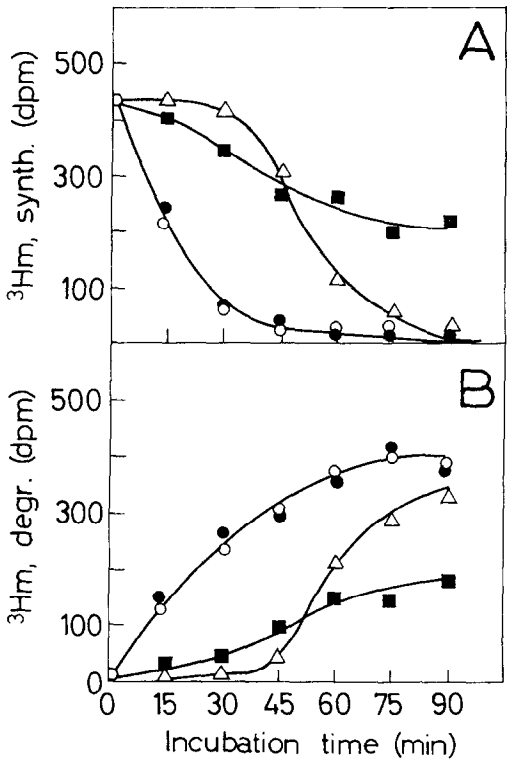

Fig.1. Breakdown of mitochondrial translation products in the cells of exponentially growing yeast. (A) Decrease in the content of pulse-labelled mitochondrial proteins. (B) Accumulation of the acid-soluble products of proteolysis. Additions: none (○); pepstatin (●); phenylmethyl sulfonyl fluoride (△); leupeptin (■). For details see section 2.

products is completely suppressed in the presence of phenylmethyl sulfonyl fluoride, an inhibitor of serine proteinases (fig.1). However, the inhibition only lasts for 30–40 min, and in the subsequent 60 min the mitochondrially synthesized polypeptides are completely degraded. It is noteworthy that in this case degradation proceeds under conditions when the activity of the vacuolar proteinase B is inhibited by $> 95\%$, as evidenced by the assay of this enzyme in cell homogenates.

An inhibitor of trypsin-like proteinases, leupeptin [7], when added to the yeast growth medium produces a partial but prolonged suppression of the proteolysis, so that only 50% of the pulse-labelled mitochondrial translation products are broken down during the 1.5 h chase (fig.1).

The presence of pepstatin, an inhibitor of the yeast vacuolar proteinase A [8], in the incubation medium does not affect the degradation of mitochondrial translation products (fig.1). Since the assay of proteinase A according to [6] demonstrates that in such conditions the enzyme is markedly ($\geqslant 90\%$) inhibited, proteinase A is not likely to participate in the in vivo proteolysis of polypeptides synthesized on mitoribosomes.

Experiments with the early stationary culture (fig.2) also reveal the breakdown of mitochondrial translation products, though it is substantially slower (a half-life of 60 min) than in exponential cells. It is also apparent that phenylmethyl sulfonyl fluoride and leupeptin affect the proteolysis in the same way as in exponentially growing yeast, while pepstatin is equally ineffective (cf. fig.1,2).

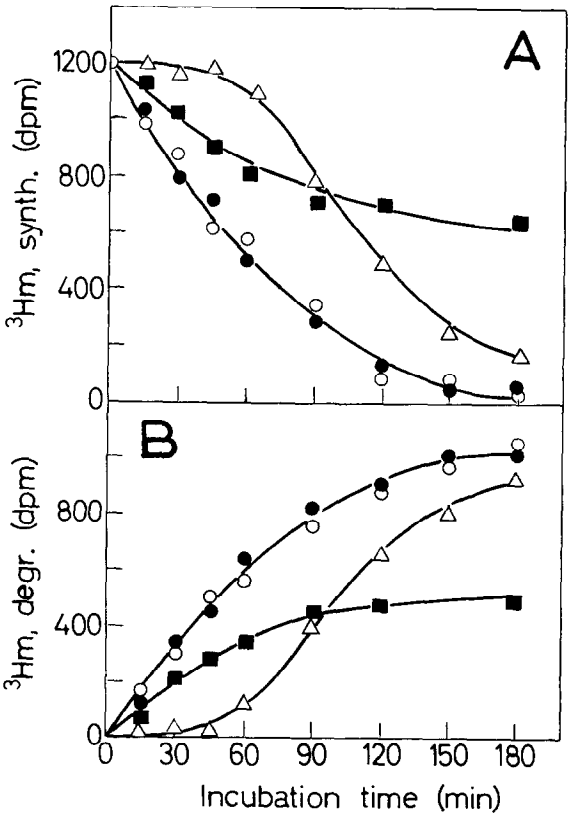

Fig.2. Breakdown of mitochondrial translation products in early stationary yeast cells. Conditions and symbols as in fig.1.

4. Discussion

The possible role of mitochondrial proteinases in the biogenesis of mitochondria was discussed [9–11]. Several works [12–15] have demonstrated proteolytic enzymes in animal cell mitochondria, and some of these were obtained in a purified state [12,13].

Recent studies from this laboratory [4] on the in vitro degradation of mitochondrial translation products revealed that mitochondria of *S. cerevisiae* possess a proteinase which can be completely inhibited by phenylmethyl sulfonyl fluoride, antipain and chymostatin. A characteristic feature of the mitochondrial enzyme is its sensitivity to leupeptin which is ineffective towards cytoplasmic proteinases A, B and C [6].

The data reported here demonstrate that the in vivo breakdown of mitochondrial translation products is also suppressed by phenylmethyl sulfonyl fluoride and leupeptin, suggesting the involvement of the mitochondrial proteinase.

As can be seen from fig.1,2, inhibition by phenylmethyl sulfonyl fluoride has a peculiar transient character. This could be explained by the fact that phenylmethyl sulfonyl fluoride is prone to be rapidly hydrolyzed under certain conditions [16]; then, if the mitochondrial proteinase has a high turnover rate (see e.g., [17]), newly synthesized molecules would be uninhibited. It should be emphasized that in the present experiments the proteolysis of mitochondrial translation products was observed under conditions when vacuolar proteinases B and A were markedly suppressed by phenylmethyl sulfonyl fluoride and pepstatin. Further, the extent of inhibition by leupeptin (~50%) is in good correspondence with its in vitro effectiveness towards the mitochondrial enzyme [4]. Thus, the above strongly argues in favour of the participation of the intramitochondrial proteinase in the degradation of mitochondrial translation products in yeast cells.

No unequivocal interpretation can at present be given to the difference in the half-lives of mitochondrial translation products at different culture growth phases (cf. fig.1,2). It is hardly probable that the set of enzymes responsible for the breakdown of these proteins is altered during yeast growth, since the patterns of inhibition by antiproteolytic agents remain essentially the same. However, biochemical and morphological data from this laboratory [18] show that differentiation of mitochondria during yeast aerobic growth is accompanied by their increasing stability. In this particular case the latter might manifest itself as increasing resistance of mitochondrial translation products to the endogenous proteolysis. Other explanations, however, are not excluded. On the whole, the body of evidence obtained in this and other works [3,4,19] support the idea of an important role played by a mitochondrial proteinase in the biogenesis of mitochondria.

Acknowledgement

Thanks are due to Dr A. V. Galkin for fruitful discussions and rendering this paper into English.

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