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Degradation of Mitochondrial Translation Products in Neurospora crassa

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Several mitochondrial membrane proteins (Cytochrome oxidase, Cytochrome b, ATPase) (1) are composed of polypeptides synthesized at cytoplasmic and at mitochonrial ribosomes. The formation of these proteins, therefore, requires a coordinated supply of both types of translation products. If one of the translation systems is blocked, the unblocked system continues to form polypeptides (2). What happens to these precursors, which cannot be assembled to integral complexes of the mitochondrial inner membrane? This question was studied in the case of the translation products formed in the mitochondria.

Two experimental procedures are widely used to study the mitochondrial translation process: (a) Incorporation of amino acids into protein of isolated mitochondria; (b) Incorporation of amino acids into mitochondrial protein in whole cells, in which cytoplasmic translation is inhibited by cycloheximide. In both cases cytoplasmic translation products are either totally absent, or are present until a certain pool is exhausted.

1. Translation in isolated mitochondria

Incorporation of radioactive leucine into TCA precipitable protein of isolated Neurospora mitochondria occurs for about 30-40 min. Following this period a decrease of incorporated radioactivity is observed (Fig. 1). Also, after a chase of unlabelled leucine which effects that no more labelled polypeptide chains are formed, a continuous decrease of TCA precipitable radioactivity originally present is noticed. This reaction can be triggered regardless whether incorporation proceeds or

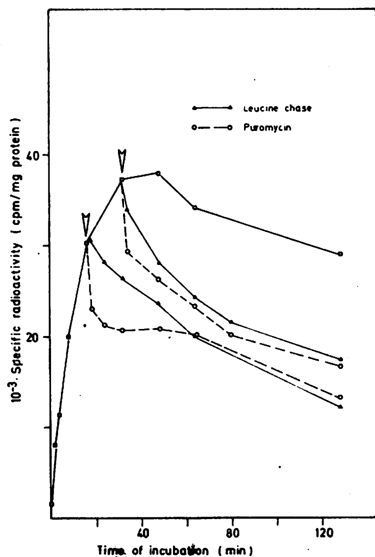

Fig. 1

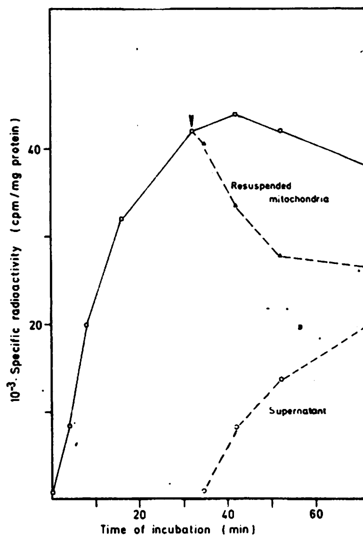

Fig. 2

Fig. 1. Incorporation of radioactive leucine into isolated Neurospora mitochondria.

Isolated mitochondria were incubated in a medium described by Sebald et al. (6) with 3-H-L-leucine (32 uM, 70 uCi/ml) at 25°C. Mitochondria were precipitated with TCA and washed as described (6). Radioactivity was determined on glas fibre filters with a counting efficiency of 20%.

At the points indicated either unlabelled leucine (final concn. 3 mM) or puromycin (final concn. 0.4 mM) were added.

Fig. 2. Degradation of mitochondrial translation products after labeling of isolated mitochondria with radioactive leucine.

Isolated mitochondria were incubated with 3-H-L-leucine under the same conditions described for Fig. 1. At the time point indicated a portion of the incubation mixture was cooled to 0°C. The mitochondria were repeatedly washed in isolation medium (4) and resuspended in incubation medium at 25°C. Aliquots were successively withdrawn, the mitochondria were spun down after immediate cooling to 0°C and radioactivity was measured in the supernatant and in the TCA precipitate of the mitochondrial pellet.

has ceased (Fig. 1). Practically the same effect is observed, if protein synthesis is blocked by adding puromycin (Fig. 1) or chloramphenicol. Similar observations were made by Wheeldon and Lehninger with rat liver mitochondria (3).

In a further experiment, incorporation was stopped after 30 min, free leucine was removed by repeated washing of the mitochondria. Then the mitochondria were resuspended in incubation medium not containing leucine. Upon further incubation at 25°C the same decrease of TCA precipitable radioactivity as described in Fig. 1 can be seen. The radioactivity lost from the mitochondria is found in the supernatant (Fig. 2). Double labelling experiments show that only newly formed material is released but not breakdown products of preexisting protein components.

These results strongly suggest that mitochondrial translation products are broken down by proteolytic activity. It appears that in isolated mitochondria a simultaneous formation and breakdown of polypeptide chains takes place.

2. Translation in whole cells in the presence of cycloheximide

If mitochondrial translation is studied in cycloheximide treated cells, it is observed that the amount of radioactive products appearing in the mitochondrial membrane is highly dependent on the time period, for which these cells were preincubated with the inhibitor. After preincubation of the cells for one hr with cycloheximide, the amount of radioactive leucine which is incorporated into TCA precipitable material of the mitochondria is greatly diminished (Fig. 3). This decline can have two different reasons: (a) Mitochondrial translation is blocked by some regulatory mechanism, or (b) Mitochondrial translation continues, but the products are not any longer integrated into the membrane and are degraded by proteolytic enzymes after release from the ribosomes.

The following results are in agreement with the second assumption: (a) Mitochondria isolated from cells which were kept in the presence of cycloheximide for 4 hrs, incorporate amino acids to an extent comparable to that of cells not treated with the inhibitor (Fig. 4). (b) Mitochondrial ribosomes isolated from cells which were kept for one hr in the presence of

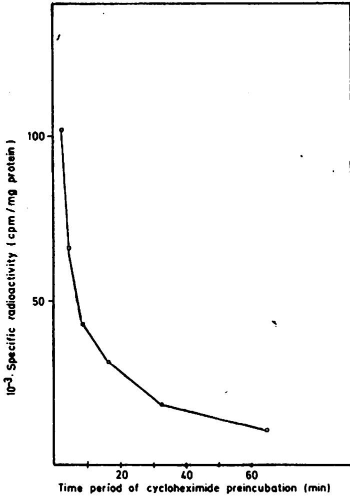

Fig. 3

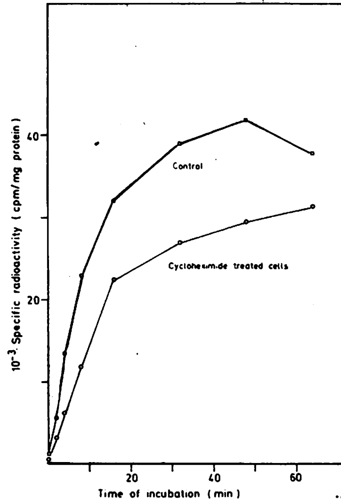

Fig. 4

Fig. 3. Effect of the time period of cycloheximide incubation of Neurospora cells on the incorporation of radioactive leucine into mitochondria in vivo

Neurospora cells were preincubated with cycloheximide (0.1 mg/ml) for the time periods indicated. Then 3-H-L-leucine was added (0.4 uCi/ml). After 4 min the cells were rapidly cooled to $0^{o}C$, mitochondria were isolated and the incorporated radioactivity was determined.

Fig. 4. Incorporation of radioactive leucine into isolated mitochondria from normal and cycloheximide preincubated Neurospora cells.

A culture of Neurospora cells was divided into two equal portions. From one portion mitochondria were immediately isolated. To the other portion cycloheximide was added and after 4 hrs mitochondria were isolated. 3-H-L-leucine was incorporated into mitochondria under the conditions described in Fig. 1.

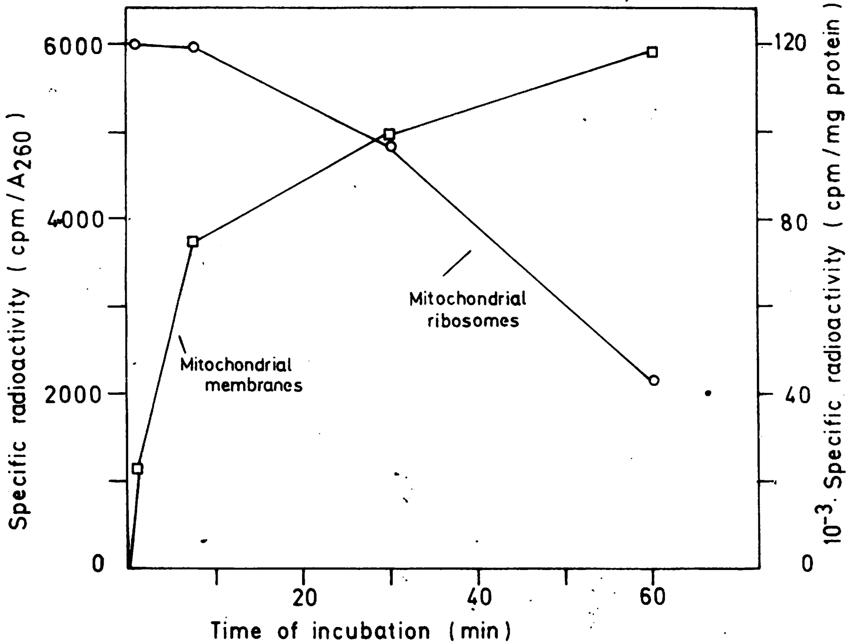

Fig. 5. Incorporation of radioactive leucine into mitochondrial membranes and into translation products on mitochondrial ribosomes in cycloheximide treated Neurospora cells.
To a culture of Neurospora cells cycloheximide and after 2.5 min 3-H-L-leucine (1 uCi/ml) were added. After the time periods indicated, aliquots of the culture were withdrawn. Mitochondrial membranes and mitochondrial ribosomes were isolated and radioactivity was measured.

cycloheximide and of radioactive leucine, still contain about one third to one half of the nascent radioactive translation products (4) of the zero time control (Fig. 5). Since the cellular pool of free leucine is increased to about three times its normal size by cycloheximide incubation (5), it can be concluded that the ribosomes are active in translation to an extent similar to that after short cycloheximide treatment.

These observations suggest that mitochondria contain proteolytic enzyme activity which may have the following functions:

(a) Removal of incomplete or aberrant mitochondrial translation products.

(b) A regulatory function to remove excess translation products which cannot be integrated into the membrane and assembled to functional complexes, because translation counterparts of cytoplasmic origin are missing.

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