THE PRESENCE OF THE ADENINE NUCLEOTIDE TRANSLOCATOR IN ρ^- YEAST MITOCHONDRIA

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1. Introduction

The contribution of the mitochondrial genetic system to the biosynthesis of the mitochondrial inner membrane can be evaluated by the use of the respiratory-deficient yeast ρ^- mutants [1]. In these mutants the mtDNA is either physically or functionally absent and ρ^- mitochondria do not carry out protein synthesis [1–3]. Therefore, enzymes or enzyme systems that are still present in ρ^- mitochondria must be coded for in the nucleus and synthesized on cytoplasmic ribosomes.

Kolarov et al. [4,5] have reported that isolated yeast ρ^- mitochondria catalyse an atractyloside- and bongkrekic acid-sensitive exchange of adenine nucleotides. Moreover, ρ^- yeast cells cannot grow in the presence of bongkrekic acid, a specific inhibitor of the adenine nucleotide translocator, indicating that also in vivo in ρ^- cells an active adenine nucleotide translocator is necessary for viability [6]. Haslam et al. [7], however, were unable to detect the presence of an atractyloside-sensitive adenine nucleotide translocator in ρ^- mitochondria and they concluded that products of mitochondrial protein synthesis probably coded for by mtDNA are required for the normal function of the adenine nucleotide translocator.

In order to solve this discrepancy, we have reinvestigated this problem and found that ρ^- mitochondria do have an atractyloside- and bongkrekic acid-sensitive adenine nucleotide translocator, but due to the variable content of endogenous adenine nucleotides this activity sometimes appears to be absent in freshly-isolated ρ^- mitochondria. Incubation of the mitochondria in the presence of a high concentration of ATP increases the amount of intramitochondrial adenine nucleotides and subsequently an inhibitor-sensitive adenine nucleotide translocation can be observed.

2. Materials and methods

The respiration-deficient ρ^- yeast *Saccharomyces cerevisiae*, strain RD1A, was used. This strain possesses a highly repetitive, non-functional mtDNA containing 97 mole percent (A+T) [8]. ρ^- Mitochondria were prepared as described before [9]. The adenine nucleotide translocator activity was measured as described by Kolarov et al. [4] at 0°C. Endogenous adenine nucleotides were determined after extraction with 15% $HClO_4$ as described previously [10]. Mitochondria were loaded with adenine nucleotides by incubating 29 mg mitochondrial protein in 0.6 M sorbitol, 1 mM EDTA, 100 mM ATP (pH 6.8) for 30 min at 25°C in 2.6 ml. Subsequently, the mitochondria were washed 4 times with 0.6 M sorbitol, 1 mM EDTA (pH 6.8) at 0°C.

3. Results and discussion

Fig. 1 shows the results of an experiment in which we measured the 'back exchange' of adenine nucleotides (see [11]). Mitochondria preincubated with [^{3}H]ATP lose their radioactivity very quickly after the addition of 200 μM unlabelled ADP. At 0°C the $t_{1/2}$ of the reaction is about 1 min. The reaction is inhibited by 75% by the addition of 125 μM atractyloside (fig. 1B). These results are in complete agreement with Kolarov et al. [4], who also found that the adenine

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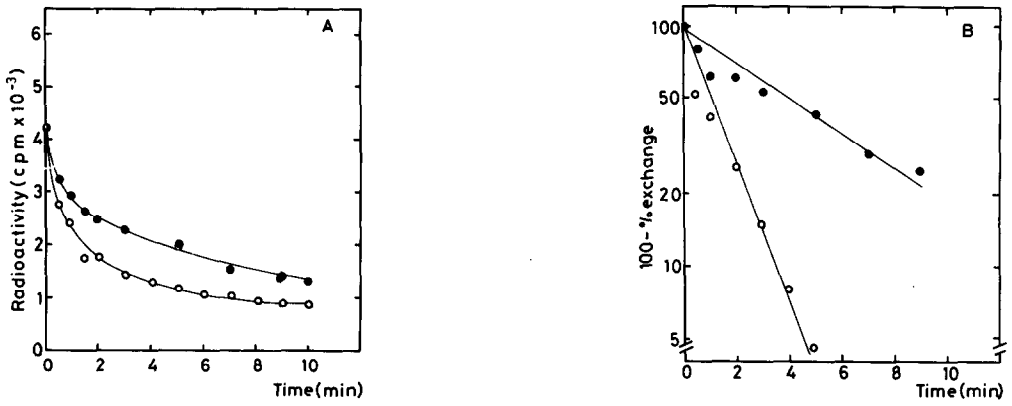

Fig. 1. Time course of the 'back exchange' in ρ^- yeast mitochondria. ρ^- yeast mitochondria were preincubated with [^{3}H]ATP as described by Kolarov et al. [4] to a specific activity of 20 000 cpm/mg protein. The back exchange was started by the addition of 200 μM ADP in the presence or absence of 125 μM atractyloside. The reaction was terminated by quick filtration on Millipore filters, using the inhibitor-stop method [4]. (o–o–o) Control; (•–•–•) 125 μM atractyloside added. The ordinate gives the radioactivity remaining in the mitochondria after filtration.

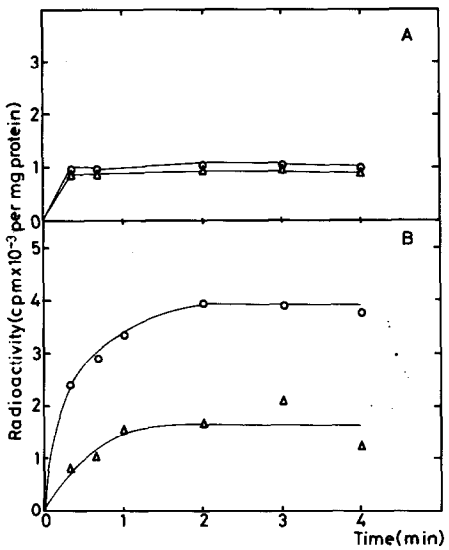

Fig. 2. Time course of the 'forward exchange' in ρ^- yeast mitochondria. The reaction was started by the addition of 200 μM [^{3}H]ADP (specific activity 2.07 × 10^6 cpm/μmol). When bongkrekic acid was added (27.5 μM) the mitochondria were preincubated with the inhibitor for 10 min. (o–o–o) Control; (Δ–Δ–Δ) 27.5 μM bongkrekic acid added. A) Freshly-isolated mitochondria; B) mitochondria loaded with adenine nucleotides.

nucleotide translocation in ρ^- yeast mitochondria, even at 0°C, is a very fast process.

However, with some preparations we were not able to show inhibitor-sensitive uptake of adenine nucleotides (fig. 2A). In this experiment, where we measured the 'forward exchange', the ρ^- mitochondria show a small uptake of [^{3}H]ADP, but this process is insensitive to bongkrekic acid. However, when the mitochondria were loaded with adenine nucleotides in the presence of ATP and subsequently washed to remove exogenous ATP, the adenine nucleotide content of the mitochondria increased significantly (table 1). Simultaneously, the appearance of a bongkrekic acid-sensitive uptake of [^{3}H]ADP is clearly visible (fig. 2B).

Table 1
Adenine nucleotide content of ρ^- yeast mitochondria

Preparation	ATP	ADP	AMP	AdN translocator activity
	nmol/mg protein			
A	1.7	2.3	–	+
B(1)	0.1	1.1	1.2	–
B(2) (after loading)	4.4	11.0	1.5	+

The preparations B(1) and B(2) are the same as used in fig. 2.

Our results show clearly, in agreement with Kolarov et al. [4,5], that the adenine nucleotide translocator is present in ρ^- mitochondria. Since the expected sensitivity to both atractyloside and bongkrekic acid is found it can be concluded that all components necessary for this enzymatic activity are coded for by nuclear DNA and synthesized on cell-sap ribosomes and that the mitochondrial genetic system does not contribute to its synthesis. It seems very likely that the failure of Haslam et al. [7] to detect the adenine nucleotide translocator is due to the fact that freshly-isolated ρ^- mitochondria contain a very low amount of endogenous adenine nucleotides, the presence of which is a necessary prerequisite to detect the adenine nucleotide translocator.

We have found that also in other preparations (like beef-heart mitochondria or submitochondrial particles) in which the presence of an inhibitor-sensitive adenine nucleotide translocator is obscured due to the low endogenous adenine nucleotide content, this activity can be restored by loading the preparation with adenine nucleotides [12].

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