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ADP/ATP translocator is essential only for anaerobic growth of yeast *Saccharomyces cerevisiae*

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All three genes (AAC1,AAC2 and AAC3) encoding the mitochondrial ADP/ATP translocator, were inactivated in a haploid yeast strain by a gene disruption technique. The triple mutant was still able to grow on fermentable carbon sources but only in the presence of oxygen. Under aerobic conditions neither translocator-protein nor carrier-mediated transport was detected in all mutants in which the AAC2 gene was disrupted. It was further shown that a functional AAC genes product is essential only for anaerobic growth of *Saccharomyces cerevisiae* but not for growth under derepressed conditions. Under anaerobic conditions a non-detectable amount of AAC3 gene product is sufficient to ensure the cell growth and multiplication.

Yeast; Mitochondrion; ADP/ATP translocator; Gene disruption

1. INTRODUCTION

The ADP/ATP translocator is a nuclear-encoded integral protein of the inner mitochondrial membrane. It exchanges ADP with ATP between the cytosol and mitochondrial matrix, a process that links the energy transduction between the two compartments. It is therefore essential for the aerobic energy metabolism of the cell. When respiration is inhibited, ATP is taken up into the mitochondrial matrix, exchanging internal ADP. Earlier studies in yeast showed that the continual presence of ATP inside mitochondria is essential for eukaryotic cell [1,2]. Among other processes protein uptake into mitochondria requires energy [3]. ATP-dependent protein folding catalyzed by mitochondrial heat-shock proteins seems to be one of the vital intramitochondrial ATP-dependent reactions [4,5]. It is therefore anticipated that the abolishment of all ATP and ADP transport across the mitochondrial membrane will have a lethal effect on the yeast cell. To eliminate the ADP/ATP translocator in yeast it is necessary to disrupt three highly homologous genes, AAC1 [6], AAC2 [7] and AAC3 [8]. Recently it has been shown that one of these genes, AAC2, encodes the majority of the translocator in yeast [9,10], but it is not clear yet to what extent the products of the other two genes participate in the ATP transport.

In the present study several AAC deletion mutants including a triple mutant were prepared and the ADP/ATP translocator properties were studied in mitochondria isolated from the mutant strains. The results show that the ADP/ATP translocator is essential only for anaerobic growth of yeast indicating that an alternative mitochondrial ATP uptake may take place during aerobic growth of these cells.

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2. MATERIALS AND METHODS

Saccharomyces cerevisiae strains were used as previously described [8]: W303-1B (MATα, ade2, leu2, his3, ura3, trp1) provided by B.L. Trumpower (Dartmouth Medical School) [8], JLY-73 (MATα, HIS::aac2, ade2, trp1, leu2, ura3, his3) [7] provided by M. Douglas (University of North Carolina, NC), WB-3 (MATα, URA::aac3, ade2, his3, leu2, trp1, ura3) and JL-3 (MATα, HIS::aac2,URA::aac3, ade2, leu2, trp1, his3, ura3) prepared as in [8]. Respiratory deficient (ρ^-) mutants were prepared by ethidium bromide mutagenesis. Yeast cells were grown on YPD media containing glucose (0.5 or 2%) or galactose (2%). Cells were grown anaerobically in sealed jars containing a Gas-Pak anaerobic system (BBL Microbiology Systems) as previously described in [8]. Rabbit antiserum to the purified yeast ADP/ATP translocator [11] was kindly provided by I. Hapala (Institute of Animal Physiology, Ivanka pri Dunaji). The AAC1 gene [6] was cloned as described in [8], and a deletion was introduced into the reading frame by the overlap extension method exactly as described in [12]. The oligonucleotides used for constructing the deletion also introduced *Xho*I and *Sal*I sites at the 5' and 3' ends of the deleted sequence, respectively. The 2.2 kb *Xho*I-*Sal*I fragment containing the LEU-2 gene was then inserted into the deleted AAC1 gene and the linearized construct was used to transform the strains described above to give a LEU$^+$ phenotype. The procedures for isolation of mitochondria from respiratory-deficient yeast strains as well as for measurements of [^{14}C]ADP specific binding and exchange were performed by published procedures [13,14]. Recombinant DNA methods were performed as described [15].

3. RESULTS

The aac3 mutant and the aac2, aac3 double mutant constructed previously [8] were used for the preparation

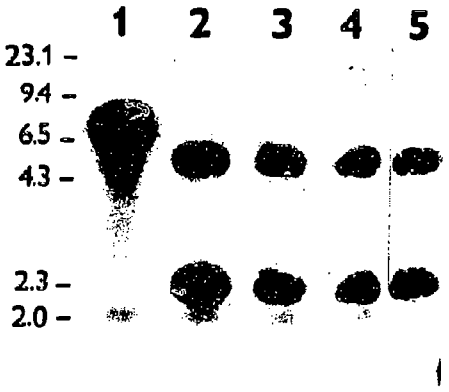

Fig. 1. Disruption of the AAC1 gene in various yeast strains. Genomic DNA was prepared from the wild-type and transformed haploid strains, digested with *Eco*RI and separated on a gel. Southern blots were probed with a 1.35 kb fragment [8] of the AAC1 gene. (1) wild-type W303-1B; (2) AAC1 disruptants prepared from wild-type; (3) AAC1 disruptants prepared from JLY-73 (aac2), (4) AAC1 disruptants prepared from WB-3 (aac3) and (5) AAC1 disruptants prepared from JL-3 (aac2, aac3).

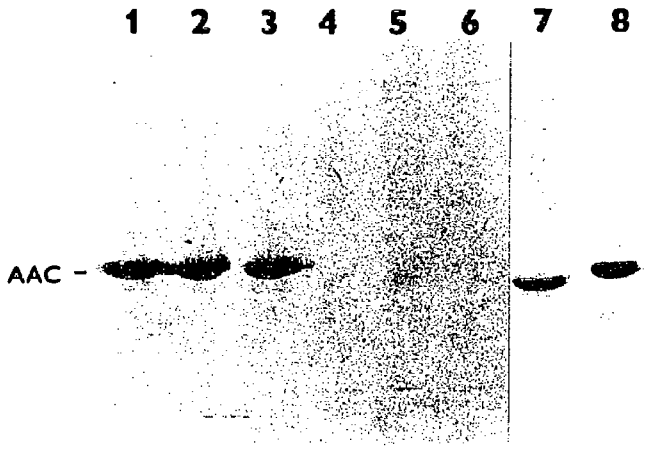

Fig. 2. ADP/ATP translocator content in mitochondria from AAC mutant cells. Mitochondrial proteins (75 μg) isolated from the wild-type and mutants described in Fig. 1 were electrophoresed on polyacrylamide gels, blotted on nitrocellulose and decorated for ADP/ATP translocator protein with a translocator specific antibody [11]. (1 and 8) wild-type; (2) WB-1 (aac1); (3) WB-1-3 (aac1, aac3); (4) JLY-73 (aac2); (5) JL-1 (aac1, aac2); (6) JL-1-3 (aac1, aac2, aac3); (7) JL-1-3 (aac1, aac2, aac3) in which the AAC1 gene was expressed on a multicopy plasmid YPN2 [8].

of aac1, aac3 double and aac1, aac2, aac3 triple mutants. For this purpose a 1.35 kb fragment from AAC1 was cloned and 0.5 kb from the coding region of the gene was deleted using two consequent PCR reactions as described in section 2. The *Xho*I-*Sal*I 2 kb fragment containing the LEU-2 gene was introduced into the deleted region of the AAC1 gene and the linearized construct was used to transform the wild-type and AAC deletion mutants to the LEU$^+$ phenotype. A Southern blot of genomic DNA digested with *Eco*RI and probed with a 1.35 kb fragment containing the AAC1 gene [8] demonstrated the presence of two bands in the strains with the disrupted AAC1 gene (Fig. 1). The disruptions of the AAC3 and AAC2 genes in the mutants were verified in a similar way (not shown). The disruption of both aac1 and aac3 together yielded no distinct phenotype. The aac1, aac3 mutant cells were able to grow on respiratory carbon sources, to form viable mitochondrial respiratory-deficient (ρ^-) mutants and to grow in the absence of oxygen.

Surprisingly the triple mutant in which all three AAC genes were disrupted was still able to grow on fermentable carbon sources. The mutant cells grow on both glucose and galactose to a cell density corresponding to respiratory deficient cells with a growth rate slightly slower than that of the double deletion mutants aac2, aac3 from which it has been constructed. In agreement with results obtained earlier [8] for the double deletion mutant aac2, aac3, the triple mutant was not able to grow under anaerobic conditions.

Various antisera have been used recently to determine the quantity of the translocator protein in mutant with deletions in AAC1 and AAC2 genes [7,9]. These experiments were extended here using a specific antibody prepared against wild-type yeast translocator [11] and mutants in all AAC genes. The antibody crossreacted not only with the AAC2 protein but also with the product of the AAC1 (Fig. 2, line 7) and AAC3 (not shown) genes when expressed from a multicopy plasmid. Nonetheless, this antiserum fails to detect the ADP/ATP translocator protein in the mitochondrial membrane of mutants with the disrupted AAC2 gene. This also holds for mitochondrial membranes of the aac1, aac2 double deletion mutant with the intact AAC3 gene grown under anaerobic conditions (not shown).

The disruption of the AAC1 and AAC3 genes either separately or together did not influence the content of the ADP/ATP translocator measured by this technique. To examine directly whether AAC1 and AAC3 gene products could participate in the transport of ADP and ATP across the mitochondrial membrane of yeast cells under derepressed conditions the kinetic properties of the translocator in mutant mitochondria were measured. Fig. 3 shows the carrier specific C^{14}-ADP binding (Fig. 3A) and exchange transport (Fig. 3B) to the mitochondria isolated from mutants with disrupted AAC genes. For comparison the same properties in mitochondria isolated from a respiratory-deficient (ρ^-) mutant are shown. The results demonstrate that neither specific C^{14}-ADP binding nor exchange transport could be detected in mitochondria isolated from strains with the disrupted AAC2 gene (Fig. 3). Mitochondrial fractions isolated from both the double and triple mutants exhibited the highest level of unspecific [^{14}C]ADP binding and exchange. This most probably reflects the low quality of the mitochondria isolated from these mu-

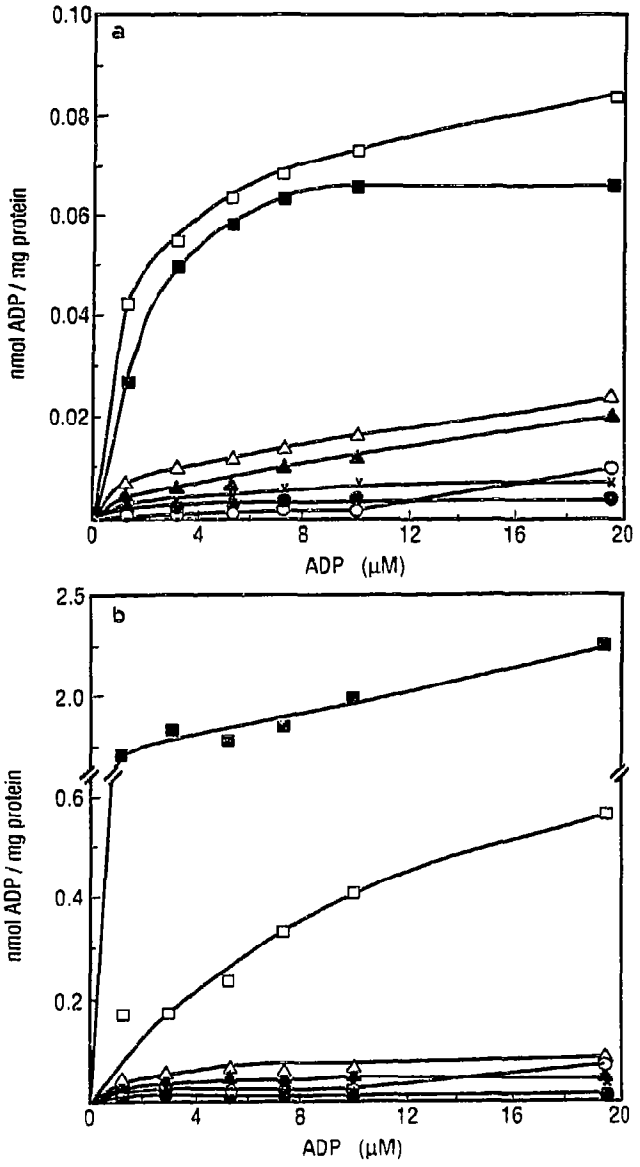

Fig. 3. Concentration dependence of [^{14}C]ADP binding and exchange by mitochondria isolated from different mutants. (A) Binding of [^{14}C]ADP. (B) Exchange of [^{14}C]ADP. Mitochondria (1–3 mg/ml) were incubated at 0°C for 1.5 min in a medium containing 0.6 M sorbitol, 2 mM EDTA, 10 mM MOPS, pH 6.4 and [^{14}C]ADP at concentrations indicated on the abscissa. Carboxyatractylate (20 μM) was either omitted from the medium or added before or 1.5 min after [^{14}C]ADP. The binding to the carrier specific sites (A) and the exchange (B) were evaluated as described in section 2. (■) W303-1B (ρ^+); (□) W303-1B (ρ^-); (○) JL-1 (aac1, aac2) grown anaerobically; (X) JLY-73 (aac2); (▲) JL-1 (aac1, aac2); (●) JL-3 (aac2, aac3); (△) JL-1-3 (aac1, aac2, aac3).

tants. The results of these experiments in addition to those obtained by immunoblotting indicate that the products of both AAC1 and AAC3 genes are not substantially participating in mitochondrial ADP/ATP transport under normal growth conditions. Even under anaerobic conditions when AAC3 gene transcription is specifically induced [8] we were not able to detect the AAC3 gene product in the aac1, aac2 double mutant by the same techniques.

4. DISCUSSION

In this work we report on the construction and characterization of haploid yeast strains in which the three AAC genes were disrupted simultaneously. The mutant can grow slowly on a fermentable carbon source under aerobic conditions. Under anaerobic conditions the triple mutation is lethal. The reason for this is not clear. One of the possibilities is that ATP inside the mitochondria is necessary for growth, and in the complete absence of ATP translocator and inhibition of electron transport by anaerobiosis, ATP could no longer be synthesized or transported into the mitochondria [1–5]. If this is correct, a small leak of nucleotides may support growth under aerobic conditions where ATP is generated by oxidative phosphorylation. The protein responsible for this nucleotide transport is not known and it is expected that in its absence the mutation will be lethal. The disruption mutants described in this study support this idea. Each one of the three AAC gene products can support growth on an oxidative carbon source when expressed from a multicopy plasmid. AAC2 gene products are exclusively involved in ADP/ATP transport in aerobically grown cells. AAC2 and AAC3 genes can support growth on a fermentable carbon source under anaerobic conditions. In a mutant in which the AAC2 gene was interrupted the ATP/ADP translocator could not be detected by specific antibody or transport measurements. It is suggested that low amounts of AAC3 gene product which is below the sensitivity of both detection methods if present in the mitochondrial membrane under anaerobic conditions. This low amount of translocator is sufficient to support anaerobic growth.

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