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Yeast ADP/ATP carrier (AAC) proteins exhibit similar enzymatic properties but their deletion produces different phenotypes

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Saccharomyces cerevisiae strains expressing a single type of ADP/ATP carrier (AAC) protein were prepared from a mutant in which all AAC genes were disrupted, by transformation with plasmids containing a chosen AAC gene. As demonstrated by measurements of [^{3}C]ADP specific binding and transport, all three translocator proteins, AAC1, AAC2 and AAC3 when present in the mitochondrial membrane, exhibited similar translocation properties. The disruption of some AAC genes, however, resulted in phenotypes indicating that the function of these proteins in whole cells can be quite different. Specifically, we found that the disruption of AAC1 gene, but not AAC2 and AAC3, resulted in a change in colony phenotype.

ADP/ATP transport; Mitochondria; AAC gene; *Saccharomyces cerevisiae*

1. INTRODUCTION

The ADP/ATP translocator is a protein of the inner mitochondrial membrane that catalyses the exchange of cytosolic and mitochondrial ADP and ATP. In the yeast *Saccharomyces cerevisiae* three separate genes, AAC1, AAC2, and AAC3 [1–3], encode this protein and their expression is under the control of environmental factors [3]. While AAC1 and AAC2 are expressed preferentially under derepressed conditions, the AAC3 gene is expressed only under anaerobic conditions [3]. Among these three genes only AAC2 encodes a translocator protein functioning in the ADP/ATP transport during oxidative phosphorylation [2–6]. On the other hand the AAC1 and AAC3 genes are not essential for growth on respiratory carbon sources and their products cannot be detected in the mitochondria of cells grown under different conditions [4]. Thus, the cellular function of the products of the AAC1 and AAC3 genes remains unclear.

All three AAC proteins exhibit similar primary structures with several fully conserved regions. In general AAC2 and AAC3 proteins are more like each other (90% identity) than AAC1 and AAC2 (75% identity) or AAC1 and AAC3 (73% identity). The C-terminal ends of all three proteins contain only a few conservative changes while the N-terminus of AAC2 differs markedly from those of the AAC1 and AAC3 proteins [3]. In order to understand the functions of these different AAC proteins in yeast cells we took advantage of the null mutants prepared by disruption of the AAC genes [2–4]. A triple mutant with disruptions in all AAC genes was used to prepare mitochondria containing only a single type of AAC protein and to study the ADP/ATP translocation properties of these proteins. The results obtained show that AAC1 and AAC3 proteins, when present in the mitochondrial membrane, exhibit translocation properties similar to those of AAC2 protein. Several properties of the *aac* deletion mutants, however, indicate that the function of these proteins in whole cells can be quite different.

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2. EXPERIMENTAL

All *aac* deletion mutants used in this study were prepared as described [3,4] by gene disruptions in the following *S. cerevisiae* strains: W303-1B (*MATα, ade2,leu2,his3,trp1,ura3*), and JLY 73 (*MATα, HIS::aac2,ade2,his3,trp1,ura3,leu2,can1*), provided by M. Douglas, University of North Carolina, NC. The triple *aac* mutant JL-1-3 (*MATα,LEU::aac1,HIS::aac2,URA::aac3,ade2,trp1,leu2,ura3, his3*) [4] was transformed with a YPN2 shuttle vector containing a TRP1 selectable marker [7] and a genomic fragment with the desired AAC gene [3,4]. The procedures for yeast cell transformation [8], for isolation of yeast mitochondria [9], for measurements of [^{3}C]ADP specific binding and exchange [10], and for immunoblot analysis of AAC proteins [4] have been described previously. The color of the colonies formed by *ade2* mutants was estimated after several days of growth on solid synthetic media containing the appropriate auxotrophic requirements and a limiting amount (5–10 μg/ml) of adenine [11–13].

3. RESULTS

To obtain yeast mitochondria with a single specific type of ADP/ATP translocator protein, JL-1-3 strain in which all AAC genes were interrupted was transformed with shuttle vector YPN2 [7] bearing a single AAC gene and the TRP1 gene as a selectable marker. Colonies

which grew on minimal plates lacking tryptophan were transferred to the media containing nonfermentable carbon sources. All three genes, AAC1, AAC2 and AAC3, promoted growth of the JL-1-3 triple mutant in glycerol-containing media to a similar extent (data not shown). This is in agreement with the results obtained earlier [2,3,6] with other *aac* mutants. An antibody which recognized all three AAC proteins [4] was used to test for the presence of a translocator protein in mitochondria isolated from transformants grown on glycerol. It was shown previously [4] that a carrier protein cannot be detected in the mitochondria of the JL-1-3 mutant. Western blot analysis (Fig. 1) showed that all three AAC proteins were present in nearly equal amounts in the respective mitochondria.

Translocation properties of mitochondria containing a single type of carrier protein were estimated by a procedure enabling discrimination between exchange, nonspecific binding, and specific binding of ADP to the nucleotide carrier [10]. All three types of mitochondria exhibited functional ADP/ATP transport with similar properties (Fig. 2). This was even more evident when carrier-specific binding of [^{14}C]ADP to AAC1, AAC2 and AAC3 proteins was compared directly (Fig. 3). Only minor differences in both the number of carrier-specific binding sites and the dissociation constants were found with mitochondria containing different AAC proteins. Moreover, both values were comparable to those obtained with wild-type yeast mitochondria [9].

Disruption of AAC2 results in oxidative phosphorylation-deficient cells unable to grow on respiratory carbon sources [2]. This is due to a complete absence of ADP/ATP translocator proteins in mitochondria of the *aac2* mutants [4]. The disruption of AAC1 and AAC3

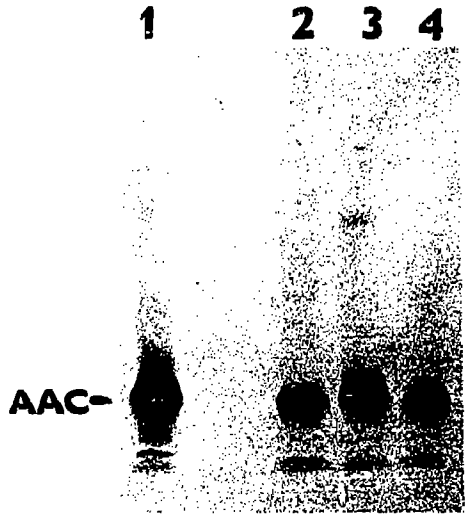

Fig. 1. Immunoblot analysis of mitochondria containing a single type of AAC protein. Mitochondrial proteins (100 μg) isolated from the wild-type strain W303-1B (1), and from JL-1-3 (*aac1,aac2,aac3*) mutant transformed with YPN2 shutle vector bearing AAC1 (2), AAC2 (3) and AAC3 (4) genes, were solubilized, electrophoresed on SDS-polyacrylamide gels, blotted onto nitrocellulose, and AAC proteins were detected with a translocator-specific antibody as in [4].

genes, either separately or together in the same cell does not affect the mitochondrial ADP/ATP translocation and no distinct phenotype of the mutant cells is observed [2–4]. In the present study, however, we found that if the AAC1 gene was disrupted in cells bearing an additional *ade2* mutation, a clearly visible change in the accumulation of an endogenous red pigment was observed. As shown in Table I, the colony phenotype of the *aac1,ade2* mutant was different from that of the AAC1,*ade2*. Furthermore the expression of this pheno-

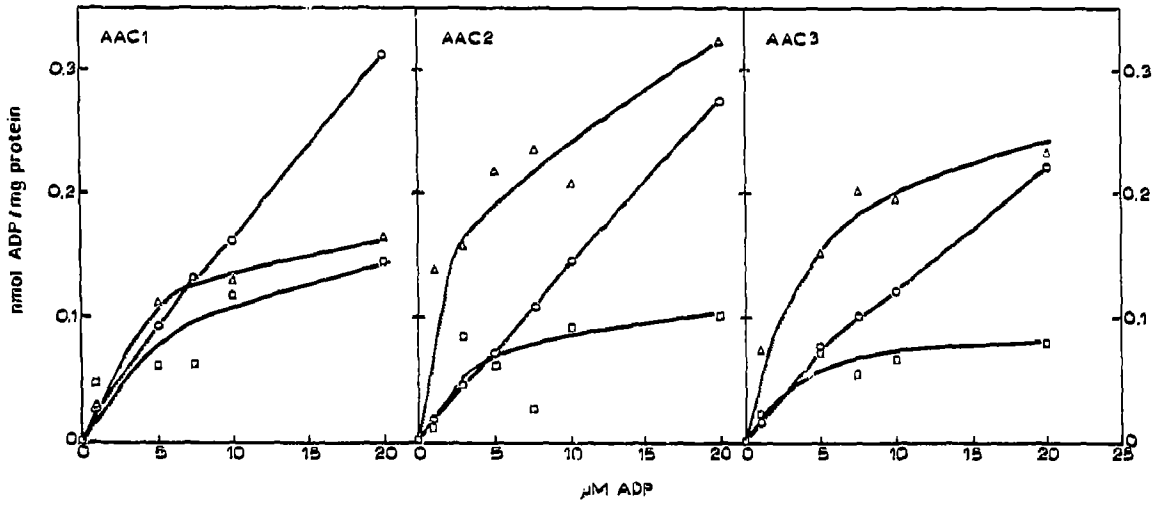

Fig. 2. [^{14}C]ADP binding and exchange by mitochondria containing a single type of AAC protein. Mitochondria (1 mg/ml) were incubated at 0°C for 2 min in a medium containing 0.6 M sorbitol, 2 mM EDTA, 10 mM MOPS, pH 7.4, and [^{14}C]ADP at concentrations indicated on the abscissa. Atractylate (70 μM) was either omitted from the medium or added before or 1.5 min after [^{14}C]ADP. The nonspecific binding (○) as well as the binding to the carrier specific sites (□), and exchange (△) were evaluated as described in [9]. Mitochondria were isolated from the JL-1-3 (*aac1,aac2,aac3*) cells transformed with the AAC1 (left), AAC2 (middle) and AAC3 (right) gene in the YPN2 yeast shutle plasmid and grown for 40 h in liquid YPG media with 3% glycerol.

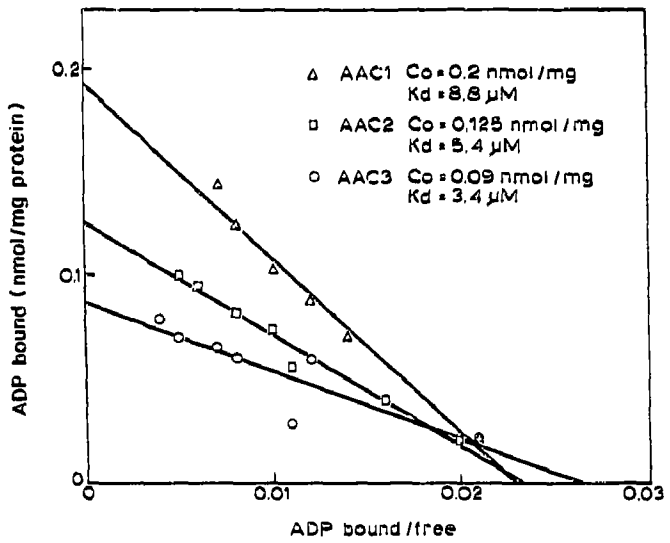

Fig. 3. Scatchard plot of the binding of [^{14}C]ADP to atractylate-removable binding sites. The values obtained for carrier-specific binding were plotted against the individual bound/free ratios of [^{14}C]ADP.

type did not depend on the respiration efficiency of the cells or upon the mutations in the other AAC genes. The effect of AAC1 disruption on the red pigment accumulation in *ade2* cells was partially reversed by shifting the pH of the growth media from acidic to neutral.

4. DISCUSSION

In *S. cerevisiae* three independent genes, AAC1, AAC2 and AAC3, encode mitochondrial ADP/ATP carriers [1–3]. The mutation of only one of them, AAC2, results in impaired ADP/ATP exchange across the inner mitochondrial membrane and consequently in cells defective in oxidative phosphorylation [2–4]. This defect cannot be repaired by single genomic copies of AAC1 or AAC3, but multiple copies of both genes are able to restore the growth of *aac2* cells on respiratory carbon sources. Moreover, the disruption of AAC1 and/or AAC3 in the genome does not produce a distinct phenotype [2-6]. Therefore, it is concluded that the AAC2 protein is the major ADP/ATP carrier involved in mitochondrial adenine nucleotide transport.

The cellular functions of the AAC1 and AAC3 gene products, however, remain obscure. In order to understand the functions of AAC proteins in the cell, we first compared their functional properties. As shown in this work, mitochondria containing a single type of AAC protein exhibited atractylate-sensitive [^{14}C]ADP binding and exchange transport. There were no substantial differences in the number of binding sites and affinity towards substrate among the different AAC proteins. From this we conclude that AAC1, AAC2 and AAC3 genes encode functionally equivalent proteins and that the differences among their primary structures are not reflected in their enzymatic properties. This, however, does not answer the question concerning the functions of these AAC proteins in the whole cell. Therefore, the phenotypes of *aac* mutants were further characterized under different growth conditions and within different genetic backgrounds.

The results obtained earlier [2–4] indicated that under normal growth conditions the products of AAC1 and AAC3 genes do not participate in mitochondrial ADP/ATP transport. This is confirmed also by the fact that the mutations in these two genes (but not in the AAC2 gene) can be combined with a mitochondrial ρ^- mutation without loss of cell viability (Table I). The inability of the double *aac2,aac3* mutant to grow under anaerobic conditions as well as the derepression of AAC3 gene under anaerobic conditions [3] suggests that the latter gene may have a specific function in cells grown in the absence of oxygen. Conversely, the disruption of the AAC1 gene either in the wild-type yeast, or in the other *aac* mutants grown under different conditions yielded no distinct phenotype. But if AAC1 was disrupted in *ade2* cells a clear phenotype connected with the accumulation of an endogenous red pigment was observed. The *ade2* defect is a block in adenine biosynthesis such that an intermediate accumulates in the vacuole which is apparently the source of the red colouration [11,12]. Different colony phenotypes can be subsequently observed depending on the adenine concentration in the medium. Defects in the accumulation of this pigment have been used to identify mutants in vacuolar morphogenesis and function [13]. The results presented in Table I show that AAC1 disruption influenced the accumulation of the red pigment in *aac1,ade2* cells indicating that the product of AAC1 gene participates in vacuolar metabolism. Like other vacuolar defects [13], the one we observed with the *aac1,ade2* mutant was also pH dependent. The phenotype connected with AAC1 mutation now provides an important tool necessary to initiate a detailed study of the role of AAC1 gene product in cellular metabolism.

Table I

Colony color of *ade2,aac* mutants

Strain	Relevant genotype ade2, aac	Colony phenotype of [ρ^+]/[ρ^-] cells
W303-1B	*ade2*	white/white
WB-1	*ade2,aac1*	red/red
WB-3	*ade2,aac3*	white/white
WB-1-3	*ade2,aac1,aac3*	red/red
JLY-73	*ade2,aac2*	white/*
JL-1	*ade2,aac1,aac2*	red/*
JL-3	*ade2,aac2,aac3*	white/*
JL-1-3	*ade2,aac1,aac2,aac3*	red/*

*The combination of mitochondrial (ρ^-) mutation with AAC2 mutation is lethal [4,8].

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