A carbon-source-responsive element is required for regulation of the hypoxic ADP/ATP carrier (AAC3) isoform in *Saccharomyces cerevisiae*

Barbora SOKOLÍKOVÁ*, L’udmila SABOVÁ*, Ingrid KLÍŠOVÁ† and Jordan KOLAROV†

*Department of Molecular Biology, Cancer Research Institute, Slovak Academy of Sciences, Vlarska 7, 833 91 Bratislava, Slovakia, and †Department of Biochemistry, Faculty of Sciences, Comenius University, Mlynska dolina CH-1, 842 15 Bratislava, Slovakia

The mitochondrial ADP/ATP carrier in *Saccharomyces cerevisiae* is encoded by three genes that are differentially expressed under different physiological conditions. We investigated the transcriptional control of AAC3, an oxygen-repressed isoform. By deletion analysis, DNA electrophoretic mobility-shift assays, DNase I footprinting and site-directed mutagenesis, we identified a promoter region (upstream repressing sequence 1, URS1) involved in a carbon-source-dependent repression of AAC3. It is different from the previously characterized oxygen-dependent ROX1 (regulation by oxygen 1) repressor-binding region (URS5). The complex character of URS1 includes the presence of two different cis-acting sequences: (i) a RAP1 (repressor activator protein 1)-binding site that is capable of binding the RAP1 protein *in vitro* and (ii) two putative ethanol-repression sequences, the modification of which derepresses the AAC3 gene. These findings demonstrate that the hypoxic AAC3 gene is regulated by two upstream repressor sites; one controlled by oxygen and haem, the other by the carbon source. Both sites function to completely switch off the expression of the AAC3 isoform when ATP is made by oxidative phosphorylation, and they modulate AAC3 expression when import of glycolytic ATP into mitochondria is required.

Key words: anaerobiosis, gene expression, hypoxic promoter.

INTRODUCTION

Genome-wide transcriptional analysis of aerobic and anaerobic cultures of *Saccharomyces cerevisiae* demonstrated the presence of more than 100 genes exhibiting several-fold higher expression under anaerobic than under aerobic conditions [1]. Transcriptional regulation of several such ‘hypoxic’ genes has been investigated intensively (reviewed in [2–4]), and two general pathways, one involving a ROX1 (regulation by oxygen 1) factor and the other ROX1-independent, were delineated [5–9]. Apart from ROX1, little is known about the other ROX1-independent factors participating in the hypoxic gene regulation. Previous studies have characterized the AAC3 gene, which encodes an ADP/ATP carrier of mitochondria, as a hypoxic isogene expressed under anaerobic conditions [10,11]. A DNA sequence between −225 and −180 bp in the 5′ flanking region of the AAC3 gene (termed upstream repressing sequence 2, or URS2), consisting of a ROX1 repressor-binding site (YYYATT-GTTCCT) and a long poly-dT segment, was found to be essential for the repression of AAC3 by oxygen [11]. The 28-fold higher expression of AAC3 under anaerobiosis [1] most probably reflects the requirement of intramitochondrial ATP under these conditions [12]. Transcriptional regulation, similar to that exerted by ROX1, has been identified recently within the promoter of the human ADP/ATP carrier isoform, *ANT2* [13]. A new motif, termed GRBOX (glycolysis-regulated box) has been characterized and it was proposed that the GRBOX element may represent a link to the glycolytic metabolism of the highly proliferative cells.

The yeast *S. cerevisiae* possesses a high fermentative capacity even in the presence of oxygen. The variation in ATP production in *S. cerevisiae* under different growth conditions, ranging from full respiratory competent aerobic to strict anaerobic conditions, would require precise adjustment of the amount and type of ADP/ATP-carrier isoform. In this study we provide evidence that an additional upstream repressor sequence (URS3) in the AAC3 promoter is involved in carbon-source-dependent regulation. This URS3 is different from the ROX1 site that has been implicated in the regulation by oxygen. The function of this new regulatory site is to completely exclude AAC3 from mitochondria during oxidative phosphorylation and to modulate its level during high aerobic glycolysis.

MATERIALS AND METHODS

Strains and growth conditions

The following *S. cerevisiae* strains were used: RZ53-6 (*MATa, leu2-3, leu2-112, ura3-52, ade1-106, trp1-289*) and RZ53-b, which contains a rox1::LEU2 allele, were provided by Dr Ch. Lowry (State University of New York at Albany, NY, U.S.A.). Yeast cells were grown aerobically at 30 °C in YP medium (1% yeast extract/2% bactopeptone) supplemented with the indicated carbon sources, or under selective conditions in SM medium (minimal synthetic growth medium containing 0.67% yeast nitrogen base) containing the proper nutritional requirements. Anaerobic cultures were grown in glucose-rich (8%) or raffinose (2%) SM media, supplemented with 12 µg/ml ergosterol, 0.2% Tween 80 and nutritional requirements. The solid media contained 2% Bacto-Agar (Difco).

The construction of the YEPA4 plasmid containing 1150 bp of the AAC3 promoter (*EcoR1/HindIII*, −1150/+269) fused to the *lacZ* gene was described previously [11]. This plasmid was

**Abbreviations used:** URS, upstream repressing sequence; ERA, ethanol-repression autoregulation; EMSA, electrophoretic mobility-shift assay; ROX1, regulation by oxygen 1; RAP1, repressor activator protein 1; SM medium, minimal synthetic growth medium.

1 To whom correspondence should be addressed (e-mail exonsab@savba.sk).
used to prepare all other deletion mutants used in this study, including the test plasmid, YEpXa. This plasmid contained the basal promoter of the \textit{AAC3} gene (−158/+269) fused to the \textit{lacZ} gene. Deletions in the \textit{AAC3} promoter were made using unique restriction sites and \textit{Bal}31 nuclease. End points of all deletions and the mutated sequences were verified by sequencing using the T7 polymerase kit (Amersham Pharmacia Biotech).

\textbf{Site-directed mutagenesis}

The promoter fragment (\textit{EcoRI/HindIII}, −1150/+269) from YEpA4 was cloned into the pGEM3Zf− vector and the resulting plasmid (pZA4) served as a template for mutagenesis. The following primers were used to introduce mutations into the RAPI (repressor activator protein 1)-binding site (mutated nucleotides are in lower case and the respective binding sites are underlined): \textit{R}_{EcoRI}^5-5'TGCAAAAATGCAGcGTcTAAAAAAA-\textit{GGAAAC\textit{US}}-3', and \textit{R}_{HindIII}^5-5'TTTCCTTTTGAGApCcTGcATT-\textit{TGTCAG\textit{US}}-3', where US and LS refer to upper and lower strand, respectively.

To specifically alter the putative ethanol-repression auto-regulation (ERA)-binding sequences at −301/−293 (ERA\textsubscript{1}) and −324/−316 (ERA\textsubscript{2}), the following pairs of primers were used: \textit{E}_{i} \textit{A}_{j}^5-5'GTCATCCAGACAT\textit{US}TGGTGGTGA-AAA\textit{US}-3', \textit{E}_{i} \textit{A}_{j}^5-5'TTTTTTACACC\textit{US}GTCATCCAGACAT\textit{US}-3', \textit{E}_{i} \textit{A}_{j}^5-5'CGGACcAaGgGTATGcTGGTGGTGA-AAA\textit{US}-3', and \textit{E}_{i} \textit{A}_{j}^5-5'GTGATCCAGACATACcTggTCC\textit{US}-3'. The pZA4 plasmids carrying the described mutations were constructed by overlap-extension PCR [14] in which the above-mentioned nucleotides and SP6 and T7 primers were used.

\textbf{Electrophoretic mobility-shift assay (EMSA)}

DNA EMSAs were performed as described in [15,16]. Protein extracts (5–20 \(\mu\)g of protein) were added to 10 \(\mu\)l of incubation mixture containing 20 mM Tris/HCl, pH 8.0, 5 mM MgCl\(_2\), 0.5 mM dithiothreitol, 0.5 mM CaCl\(_2\), 0.1 mM EDTA, 1 \(\mu\)g of calf thymus carrier DNA (fragmented by sonication) and 0.2–0.5 ng (10000 c.p.m.) of \(\alpha\)\(^{32}\)P-end-labelled DNA fragments. The DNA–protein binding reactions were incubated on ice for 15 min and the resulting complexes were separated on 4% polyacrylamide gels in 0.5 TBE (45 mM Tris/borate/1 mM EDTA, pH 8.0). The anti-RAPI serum used in the EMSAs was provided by Dr S. Johnaton (University of Minnesota, St. Paul, MN, U.S.A.).

\textbf{DNase I protection assay}

For DNase I protection assays, the upper-strand (−378/−244) and lower-strand (−474/−158) fragments were labelled at their 5’ ends. Reaction mixtures (50 \(\mu\)l) contained 50 mM KCl, 10 mM MgCl\(_2\), 20 mM Tris/HCl, pH 8.0, 0.1 mM EDTA, 2.5–30 \(\mu\)g of protein extract and 1 \(\mu\)g of 5’-end-labelled probe. After 15 min incubation on ice, the samples were digested partly by DNase I (Boehringer Mannheim) and reactions were stopped by addition of 0.1 M EDTA/0.6 M ammonium acetate containing 20 \(\mu\)g/ml sheared single-stranded DNA from calf thymus. The labelled DNA was extracted by phenol/chloroform (1:1), precipitated, dissolved in loading buffer and separated on 6% polyacrylamide sequencing gels. Maxam–Gilbert sequencing reactions for purines (A+G) were performed on the same fragments.

\textbf{Miscellaneous}

Published procedures were used for isolation, manipulation, labelling and sequencing of DNA [17]. Yeast cells were transformed by lithium acetate treatment [18]. Protein extracts were prepared as in [19]. For analysis of \(\beta\)-galactosidase expression, transformants were grown overnight in SM medium, diluted to the desired density in fresh medium and grown to \(D_{600}\) of approx. 1.0. For preparation of crude extracts, transformants were broken by vortex-mixing with glass beads and \(\beta\)-galactosidase activity was assayed as described in [20]. A minimum of five transformants was assayed in duplicate and each experiment was repeated three to five times. The values from each experiment were normalized for variation between experiments and the means of at least three experiments are reported.

\section*{RESULTS}

\textbf{Identification of carbon-source-dependent regulatory elements in the \textit{AAC3} promoter}

The \textit{AAC3} gene was identified as a hypoxic gene that is regulated negatively by oxygen, haem and the ROX1 factor [10,11]. To determine other factors involved in its regulation, \textit{AAC3} gene expression was analysed further in the \textit{Δrox1} strain (RZ53-b). The \(\beta\)-galactosidase activity expressed from the YEpA4 plasmid carrying full-length promoter was very low in cells grown on either ethanol or lactate and was induced 3–20-fold in cells grown on raffinose or glucose (Table 1). Higher expression of the \textit{AAC3} gene on glucose, as compared with the non-fermentable carbon sources, was also apparent in the wild-type (RZ53-6) strain in spite of the repression induced by the ROX1 factor.

To delineate the promoter domains involved in the carbon-source-dependent regulation of the \textit{AAC3} gene, a number of promoter deletion constructs were prepared. All constructs were fused to \textit{lacZ}, transformed into the \textit{Δrox1} strain and \(\beta\)-galactosidase activity expressed from each deletion construct was determined as the response to growth on glucose or lactate. As shown in Figure 1, deletion of the upstream sequences between −1150 and −474 bp did not affect the expression of \(\beta\)-galactosidase in cells grown on fermentable or non-fermentable carbon sources. Deletion of the sequences downstream of −474 bp, however, resulted in a several-fold increase of the reporter gene expression in cells grown on lactate. Under repressing conditions (8% glucose), none of the deletion constructs resulted in a more than 2-fold increase in reporter gene expression. The presence of a carbon-source-dependent negative regulatory element in the \textit{AAC3} promoter was confirmed further by two internal deletions, −474/−244 bp and −314/−244 bp (Figure 1). Comparison of \(\beta\)-galactosidase expression from

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Carbon source} & \textbf{Galactosidase activity} (mmol ONPG hydrolysed/min per mg of protein) \\
\hline
\textbf{Glucose} & \textbf{Raffinose} & \textbf{Ethanol} & \textbf{Lactate} \\
\hline
\textbf{Strain} & \textbf{+O\textsubscript{2}} & \textbf{-O\textsubscript{2}} & \textbf{+O\textsubscript{2}} & \textbf{-O\textsubscript{2}} & \textbf{+O\textsubscript{2}} & \textbf{-O\textsubscript{2}} \\
\hline
RZ53-b (\textit{Δrox1}) & 7.00 & 89.0 & 12.0 & 18.0 & 4.0 & 5.0 \\
RZ53-6 (\textit{rox1}) & 2.0 & 34 & 2.0 & 12.0 & 0.1 & 0.1 \\
\hline
\end{tabular}
\caption{Expression of \textit{AAC3}–\textit{lacZ} reporter constructs in the \textit{Δrox1} mutant and wild-type cells cultured under different conditions}
\end{table}

The YEpA4 plasmid used for transformation contains the \textit{EcoRI}/\textit{HindIII} (−1150/+269) fragment of \textit{AAC3} fused to \textit{lacZ}. Transformants were grown in SM media containing the indicated carbon sources under either aerobic (+O\textsubscript{2}) or anaerobic (−O\textsubscript{2}) conditions, as described in the Materials and methods section. ONPG, o-nitrophenyl \(\beta\)-d-galactopyranoside.
Transcriptional regulation of hypoxic AAC3 gene

Figure 1 Deletion analysis of the AAC3 upstream region

Deletions were constructed in the YEpA4 plasmid and then introduced into the Δrox1 (RZ53-b) deletion strain. Nucleotide positions refer to the translational start codon. Transformants were grown in synthetic media supplemented with 8% glucose or 2% lactate as a carbon source and the expression of β-galactosidase was measured. All values represent the means from at least three independent experiments. S.D. were less than 20% of the means. ONPG, o-nitrophenyl β-D-galactopyranoside.

Constructs carrying these internal deletions also indicated that a part of the repression region may be located upstream of −314 bp. The expression from the remaining internal deletion constructs showed that the repression of AAC3 did not require the presence of the previously characterized negative site (−201/−179 bp) responsible for oxygen repression [11]. The data in Figure 1 thus indicate that the sequence between −322 and −244 bp might be involved in the repression of the AAC3 gene by a respiratory carbon source.

The function of the carbon-source-dependent regulatory elements present in the −474/−244 bp region were determined by cloning fragments in front of the basal AAC3 promoter (−158/+269 bp) fused to the lacZ gene (plasmid YEpXa). The YEpXa plasmid does not contain a ROX1-binding site and expresses β-galactosidase at a relatively high level. The ability of the individual fragments to repress the AAC3 gene was tested in the wild-type strain (ROX1+) grown in either the presence or absence of oxygen (Figure 2). Two AAC3 promoter sequences, −474/−244 bp and −323/−244 bp, were equally effective in suppressing AAC3–lacZ expression in cells grown both aerobically and anaerobically. The −474/−295 bp fragment resulted in less efficient repression only in aerobically grown cells, whereas under anaerobiosis a weak activation, rather than repression, was observed. These results suggest that distinct parts of the −474/−244 bp region may bind different regulatory proteins possessing different activities under either aerobic or anaerobic conditions.

Specific protein–DNA interactions within URS

To test for DNA–protein interactions within the URS, region of the AAC3 promoter the −378/−244 bp fragment was isolated, end-labelled and used in DNA EMSA. Figure 3(A) shows protein binding to the −378/−244 bp fragment (lane 1) that was inhibited competitively by unlabelled probe (lanes 2–5). Figure 3(B) shows that the binding of protein or proteins is not subject to carbon-source regulation. The formation of a DNA–protein complex was observed with protein extracts from cells grown on different carbon sources (glucose, raffinose and lactate). These results suggest that the protein (or proteins) binding to the −378/−244 bp fragment of the AAC3 promoter is not affected specifically by the carbon source.

To identify the cis-acting sequences on the −378/−244 bp fragment of the AAC3 promoter, we employed DNase I foot-printing analysis using cell extract from raffinose-grown wild-

| β-galactosidase activity (nmolONPG hydrolysed/min/mg protein) |
|------------------|------------------|------------------|
| Lactate(+O2) | Glucose(-O2) | Raffinose(-O2) |
| -158 | 80 | 10 | 16 |
| -474 | -244 | -158 | 7 | 1.5 | 0.5 |
| -474 | -295 | -158 | 14 | 19 | 13 |
| -323 | -244 | -158 | 2 | 0.5 | 0.2 |

Figure 2 Repression induced by insertion of URS, fragments into the ΔURS, control plasmid

The indicated DNA sequences of the AAC3 promoter were inserted in front of the basal promoter as specified in the Materials and methods section. Resulting plasmids were transformed into the wild-type strain (RZ53-6) and transformants were grown in media containing the indicated carbon sources under either aerobic (+O2), or anaerobic (−O2) conditions. ONPG, o-nitrophenyl β-D-galactopyranoside.

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Figure 3 DNA mobility-shift analysis of the AAC3 URS1 region

(A) The 134 bp 32P-end-labelled DNA fragment (−378/−244) of the AAC3 promoter was incubated with protein extracts (10 μg) from the wild-type (RZ53-6) cells grown on 2% raffinose (lane 1). Lanes 2–5, 2, 10, 25 and 100 times molar excesses of unlabelled fragment were added to the binding reactions. (B) Protein extracts used (10 μg) were prepared from cells grown on 2% raffinose (lane 2), 2% lactate (lane 3) and 8% glucose (lane 4). The control (lane 1) contains the labelled fragment incubated with 10 μg of BSA. C, complex.

Figure 4 DNase I footprint protection assay

DNase I protection analysis was performed with both the upper (A) and lower (B) strands of the 32P-end-labelled AAC3 URS1 fragment. (A) Lane 1, Maxam–Gilbert sequencing reaction for A+G; lane 2, 10 μg of BSA added to the binding reaction; lanes 3–9, increasing amounts of protein extract from the wild-type strain (RZ53-6) grown on raffinose were added to the binding reactions. (B) Lane 1, Maxam–Gilbert sequencing for A+G; lanes 2–5, increasing amounts of protein extract from the RZ 53-6 strain grown on raffinose were added to the binding reactions. The protected sequences are indicated. hs, hypersensitive site.

type cells (Figure 4). A relatively short sequence between −302 and −285 bp was protected on both strands (Figure 4A, lanes 3–9). In addition, a DNase I-hypersensitive site centred around −285 bp was identified on the upper strand (Figure 4B, lanes 3–5). These results indicate that the region between −302 and −285 bp is involved in specific DNA–protein interactions in the URS1 region of the AAC3 promoter.

Inspection of the protected DNA region and flanking sequences revealed the presence of cis-acting elements. One of them, complementary to the RAP1-protein-binding site (RMACCCANNCAAY) [21], is located between −300 and −288 bp. Two sequences, one from −301 to −293 bp (AAA-TGCATG) and the other from −324 to −316 bp (AAATGC-GTA), resemble the consensus sequence (AAATGCATACATC)
of the ERA element found in promoters of several genes involved in carbohydrate metabolism [22]. These regions are here referred to as ERA$_1$ and ERA$_2$, respectively. The ERA$_1$ sequence almost completely overlaps the consensus RAP1-binding site.

To confirm that RAP1 binds to the protected DNA region, we used a RAP1-specific antibody in EMSA experiments. As shown in Figure 5, the immune serum abolished the formation of specific protein–DNA complexes, whereas control serum did not, demonstrating the binding of the RAP1 protein to its cognate site in the AAC3 promoter. These results suggest that RAP1 might participate in the transcriptional regulation of AAC3.

Promoter fragment $-378/-296$ bp, containing only the complete ERA$_2$ site from $-324$ to $-316$ bp, did not produce any specific DNA–protein complex with any of the protein extracts examined (results not shown).

**Functionality of RAP1 and ERA sites**

Results of the deletion analysis suggested that the sequences around the RAP1-binding site ($-300/-288$ bp) may be involved in the repression of the AAC3 gene. To assess the functionality of the RAP1 and ERA sites, we introduced mutations in each consensus sequence and studied their effect on the expression of the reporter gene (Figure 6). It should be noted that the mutation of both the ERA$_1$ and the RAP1-binding site eliminated the binding of RAP1 protein in vitro (results not shown).

Data summarized in Figure 6 show that mutation of any of the consensus binding sites affected AAC3–lacZ expression but that each mutation acted differently. In the presence of oxygen, mutation of any of the ERA sites de-repressed the AAC3 gene in wild-type cells regardless of the carbon source. In $\Delta$rox1 cells grown on lactate or raffinose only mutation of the ERA$_2$ site resulted in de-repression of AAC3, whereas mutation of the ERA$_1$ site under these conditions increased AAC3 expression only slightly. In $\Delta$rox1 cells grown on glucose, no significant alteration of AAC3 expression was observed.

Under aerobiosis, RAP1 mutation had no effect in wild-type cells grown on lactate and caused a moderate de-repression of the AAC3 expression in cells grown on glucose. In $\Delta$rox1 cells mutation of the RAP1 site had no effect on expression of the AAC3 gene grown in the presence of oxygen.

In wild-type cells grown anaerobically, results showed that only mutations that abolished binding of the RAP1 protein (RAP1- and ERA$_2$-binding sites) de-repressed the AAC3 expression when cells were grown on glucose or raffinose. The effect was higher in raffinose-grown cells (Figure 6). Mutation of the ERA$_2$ site increased the AAC3 expression in both glucose- and raffinose-grown cells only slightly.

**DISCUSSION**

In the present study we demonstrated that separate negative DNA sequences, different from the ROX1-binding site, in the AAC3 promoter might be linked to the glycolytic metabolism of yeast cells. Deletion analysis identified the presence of repressing sequences (URS$_1$) around $-295$ bp that appeared to be active under both aerobic and anaerobic conditions. Expression of the AAC3 gene in the $\Delta$rox1 strain suggested that these sequences can function independently of the ROX1 repressor and respond to the carbon source.

Results obtained from EMSA using extracts from cells grown on glucose, raffinose or lactate indicated that a common trans-acting factor was bound to URS$_1$. A DNase I footprinting assay mapped the URS$_1$ to a 17 bp region ($-292/-285$) that appeared to be essential for DNA–protein interactions. The sequence of the protected region, the inhibition of DNA mobility shift by anti-RAP1 serum and the mutational analysis demonstrated that RAP1, a ubiquitous transcriptional regulator, binds to URS$_1$ and plays a role in the carbon-source-dependent regulation. Besides the RAP1 consensus sequence the repressing region identified in our study contained two cis-acting elements resembling the ethanol-repression sequence (AAATGCATA, termed ERA) [22] at $-324/-316$ and $-301/-293$ bp. This sequence was first identified as a cis-acting element controlling the expression of pyruvate decarboxylase, an enzyme that acts at the branch-point between fermentation and respiration. The
identity of protein(s) that bind the ERA element has remained unknown. Our results showed that the ERA sequences repressed the expression from the basal AAC3 promoter. Although the DNA–protein binding assays used in this study failed to detect specific protein binding to the putative ERA sequences, the deletions spanning the ERA-containing region resulted in a significant increase in expression, indicating that these elements were essential for physiological expression of AAC3. This was corroborated further by site-directed mutagenesis of the ERA sites. The ratio between the activity of the mutated and the wild-type promoters revealed two important properties of these promoter elements.

First, modification of any of the putative ERA sites in the wild-type strain resulted in a several-fold de-repression of the AAC3 gene in the presence of oxygen regardless of carbon source. In the ROX1-deleted strain, only mutation of the ERA2 site caused de-repression on respiratory carbon sources, whereas the mutation of the ERA3 site had very little effect on expression, indicating a possible co-operation between the two repressors, ERA and ROX1. Under aerobic conditions mutation of the RAP1-binding site had only a marginal effect on AAC3 expression.

Second, the results of mutational analysis indicated that only the RAP1 factor was active under anaerobic conditions since only the mutation of RAP1 and ERA, sites de-repressed expression of the AAC3 gene. We suppose that de-repression of the AAC3 gene by mutated ERA, is due to the simultaneous abrogation of RAP1 binding. Mutation of the ERA3 site had no effect.

Both RAP1- and ERA-binding sites participate in carbon-source-dependent regulation of the AAC3 gene and the involvement of individual factors in this regulation is dependent on oxygen availability. It appears that under anaerobic conditions only RAP1 participates in the regulation of the AAC3 gene, whereas both ERA and RAP1 participate in the repression under oxygen-proficient conditions.

In conclusion, our results demonstrate that the AAC3 gene, encoding the hypoxic isoform of the mitochondrial ADP/ATP carrier, is regulated by two negative cis-acting DNA elements. URS, and URSs. URS, binds ROX1 factor and confers sensitivity to oxygen, whereas URSs responds mainly to the nature of the carbon source used for cell growth. Through URSs, a complex regulation, probably involving more than one regulatory protein (ERA, RAP1) is exerted. Under aerobic conditions, when respiratory carbon sources are present and the mitochondrial ADP/ATP-exchange transport is executed by the major AAC2 isoform, both negative sites completely switch off expression of AAC3. It is possible that the AAC3 isoform is better adjusted for the inverse exchange of cytosolic ATP for mitochondrial ADP than the other isoforms, and this could be necessary for efficient oxidative phosphorylation under the conditions used. The carbon-source-dependent site in the AAC3 promoter, described in this study, is most probably linked to the glycolytic metabolism of yeast cells and it could serve to coordinate the quantity of the carrier that catalyses inverse ADP/ATP exchange with the changing need for ADP/ATP exchange during shifts from oxidative to glycolytic metabolism.

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