The gene for yeast GTP:AMP phosphotransferase (PAK3) was found to encode a nonfunctional protein in 10 laboratory strains and one brewers’ strain. The protein product showed high similarity to vertebrate AK3 and was located exclusively in the mitochondrial matrix. The deduced amino acid sequence revealed a protein that was shorter at the carboxyl terminus than all other known adenylate kinases. Introduction of a +1 frameshift into the 3’-terminal region of the gene extended homology of the deduced amino acid sequence to other members of the adenylate kinase family including vertebrate AK3. Frameshift mutations obtained after in vitro and in vivo mutagenesis were capable of complementing the adk1 temperature-conditional deficiency in Escherichia coli, indicating that the frameshift led to the expression of a protein that could phosphorylate AMP. Some yeasts, however, including strain D273-10B, two wine yeasts, and two more distantly related yeast genera, harbored an active allele, named AKY3, which contained a +1 frameshift close to the carboxyl terminus as compared with the laboratory strains. The encoded protein exhibited GTP:AMP and ITP:AMP phosphotransferase activities but did not accept ATP as phosphate donor. Although single copy in the haploid genome, disruption of the AKY3 allele displayed no phenotype, excluding the possibility that laboratory and brewers’ strains had collected second-site suppressors. It must be concluded that yeast mitochondria can completely dispense with GTP:AMP phosphotransferase activity.

Adenylate kinases constitute a family of highly conserved soluble proteins that catalyzes the interconversion of nucleoside phosphates (Noda, 1973) and, thus, fulfills an essential function in maintaining the energy charge in cells (Atkinson, 1977). In mammals three types of isozymes exist. They can be divided into short and long isoforms of 21 and 25 kDa molecular mass, respectively. The short form enzyme, AK, resides in the cytoplasm. Based on differences in primary structure, substrate utilization, and subcellular location, two distinct subgroups of the 25-kDa form can be discriminated, called AK2 and AK3 (for a review, see Schulz (1987)). AK2 is located mainly in the mitochondrial intermembrane space and uses ATP-Mg\(^{2+}\) as donor of the high energy phosphate, while AK3, which occurs in the mitochondrial matrix, uses GTP-Mg\(^{2+}\) and ITP-Mg\(^{2+}\) (Tomasselli et al., 1979a, 1986). The latter is thought to play a role in the interconversion of non-ATP nucleoside triphosphates, ITP, and GTP (Tomasselli et al., 1979b), generated by substrate chain phosphorylation through succinic thiokinase in the tricarboxylic acid cycle.

By contrast, procaryotes were shown to contain only a single member of the adenylate kinase family, a long form isozyme (Brune et al., 1985). In yeast, the major isoform of adenylate kinases, Aky2p, is a protein of 24 kDa molecular mass displaying highest homology to mammalian AK2 isozymes. As in bacteria, this protein was considered to be the only adenylate kinase in yeast. However, the finding that haploid disruption mutants of Aky2 are viable although slowly growing on glucose (Bandlow et al., 1988) pointed to the existence of isozymes. Recently we have isolated the genes of two AMP kinase isozymes from yeast, designated URA6 and PAK3 (Liljelund et al., 1989; Schricker et al., 1992a, 1992b). URA6 was isolated as a multi-copy suppressor of Aky2 deficiency, showing that the encoded enzyme has the capacity to phosphorylate AMP (in addition to UMP and CMP). Thus, the yeast enzyme has a substrate specificity that is broader than originally assumed and different from UMP kinase from Dictostylium discoideum (Wiesmüller et al., 1990; Schricker et al., 1992b). PAK3 was isolated by PCR. It is encoded on chromosome V (Schricker et al., 1992a, 1992b) upstream of RAD3 (Reynolds et al., 1985). PAK3 displays closest similarity with mammalian AK3 and is expressed in a carbon source controlled manner. In our studies on Pak3p, we failed to detect enzymic activity with any combination of nucleoside mono- and triphosphate co-substrates tested. In addition, the gene did not complement the temperature-conditional adenylate kinase deficiency of an adk1-1\(^{ts}\) mutant of E. coli or the aky2 null allele mutant of yeast strain DL1-D16. Moreover, a disruption of PAK3, either alone or in conjunction with an Aky2 disruption, did not produce a distinctive phenotype (Schricker et al., 1992a, Konrad, 1993).

Here, we report the conversion of the apparently nonfunctional PAK3 allele into one displaying both enzymatic and biological activity by introducing a +1 frameshift mutation into the 3’-proximal coding region of the gene. We provide evidence that the nonfunctional allele is characteristic of extensively

\(^{1}\) The abbreviations used are: AK, adenylate kinase; Aky2p, yeast major adenylate kinase; Aky2, gene encoding Aky2p; Aky3p, GTP:AMP phosphotransferase in yeast wild strains; AKY3, gene encoding Aky3p; PAK3, expressed, nonfunctional allele of AKY3; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PCR, polymerase chain reaction; Gp5A, guanosine(5’)-pentaphospho(5’)-adenosine; PAGE, polyacrylamide gel electrophoresis.
cultivated strains of Saccharomyces cerevisiae, commonly used for laboratory and brewery purposes. By contrast, functional mannitol. Mitochondria were prepared from the 10,000 cultivated strains of

**EXPERIMENTAL PROCEDURES**

Yeast Strains—AH215 (Hinnen et al., 1978), DL1 (van Loo et al., 1983), DBY593 (Carlson and Botstein, 1982), AB320 (Nasmyth and Reed, 1980), D273-10B (ATCC 24657), TF2 (Fox and Reed, 1979), IL993–3C (Wof et al., 1973), DSY183 (obtained from D. Drubin, Berkeley, CA) YM701 (from M. Johnston, St. Louis, MO), 6657–9B (from J. R. Mattoon, Colorado Springs, CO), SP1 (from J. Thevelein, Heverlee, Belgium), S. cerevisiae-Laureo (brewers’ yeast, from A. Wiest, Weihenstephan, Germany), S. diastaticus, S. capensis (both from The Yeast Genetics Stock Center, Leiden, The Netherlands), wine yeast “Malaga,” wine yeast “Bordeaux” (both from Kitzinger Reinzuchthefen, Kitzingen, Germany) were used for sequence comparison of the ADK1 gene. Yeast strains of all the above-mentioned species and 3500-base pair linear BamHI fragment and transfected into D273-10B. Stable transformants were selected on glycerol/E TOH medium containing chloramphenicol (2 mg/ml) and positive clones checked for correct recombination of the construct at the HIS3 locus by Southern blot analysis. For transformation, a D273-10B his3::CAT strain was transformed by pBR322 carrying YCP-HIS3-HO harboring a 2.5-kilobase EcoRI/Sall fragment encoding the yeast HOG1 gene (Russel et al., 1986). Ygogate were isolated by micromanipulation. The resulting homologous, isogenic 3 diploid strain D273-5d was cured of YCP-HIS3-HO under nonselective conditions and then used to introduce an ayk3 null allele mutation. For this reason, the strain was transformed with a linear 2.5-kilobase EcoRI/EcoRV fragment of the pak3::HIS3 construct described previously (Schiricker et al., 1992a). Proper gene disruption was assayed by Southern blotting. Tetrad analysis of the ayk3-2 strain obtained was performed by micromanipulation using the MSM System (Singer Instrument Co. Ltd., Watchet, Somerset, United Kingdom).

Production of Antiserum—The AKY3 allele of yeast strain D273-10B, comprising the complete coding region but lacking 5' and 3' flanking sequences, was amplified by PCR and thereby equipped with BamHI restriction sites at both ends. This fragment was ligated to the BamHI site of the E. coli expression vector pQE8 (Diagen, Hilden), thus fusing a tag of 6 histidine residues in frame to the amino terminus of Ayk3p. The recombinant protein was expressed under the guidance of the IPTG-inducible lacUV5 promoter and purified from bacterial lysates by two consecutive elutions from Ni2+ chelate columns according to the protocol obtained from the supplier. Two hens were immunized (Gassmann et al., 1990) using about 100 mg of the homogenous protein in complete Freund’s adjuvant per injection. Two boost injections were applied 4 and 8 weeks after the initial injection. The immunoglobulin fraction was prepared by dextran sulfate precipitation (Enzensier et al., 1981) from the yolk of the eggs laid on six consecutive days, which exhibited the highest antibody titers.

**RESULTS**

Gain of Biological Activity by Frameshift Mutations—Comparison of the primary structure deduced from the nucleotide sequence of the PAK3 gene isolated from the S. cerevisiae strain DL1, with the sequences of other adenylate kinase genes, belonging to either short or long isoforms revealed an unusually short carboxyl terminus (Fig. 1). As a consequence, this allele lacks two hydrophobic residues at positions 235 and 239 (family numbering, cf. Magdolen et al., 1992), which are strictly conserved in all other known adenylate kinases. In the cases of Ayk2p from yeast (Magdolen et al., 1992) and AK1 from chicken (Yoneya et al., 1990), it has been shown that deletions of the carboxyl terminus or conversion of amino acid 239 into a hydrophilic residue led to drastically diminished protein stability and/or enzymatic activity. The suspicion that this AK3 null allele, PAK3, might have been shortened by a frameshift mutation that has resulted in an inactive protein was corroborated by the fact that the introduction of a +1 frameshift shortly after

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**Fig. 1. Comparison of the carboxyl-termin al amino acid sequences of adenylate kinases.** Shown are the sequences of Pak3p and Pak3p-ps, presumptive AK3 from yeast and frameshift product; Ayk2p and Ura6p, major AMP kinase and UMP kinase from yeast; BOAK1, -2, and -3, bovine adenylate kinases 1, 2, and 3; ECOAK, adenylate kinase from E. coli. Shading denotes conserved amphiphilic amino acids (positions 235 and 239, family numbering, Magdolen et al. [1992]). Asterisk indicates translational stops.
were obtained. Although all single base pair insertions occurring within seven base pairs of the 3′-end of the coding region of the gene spanning the mutation originally proposed (Fig. 1). At position 234 exclusively, a lysine was tolerated. Interestingly, position 233 showed some variability and displayed either Pro, Arg, or Ala. Since only +1 frameshifts where found, the carboxyl-terminal sequences of all mutants were identical.

We examined whether the introduction of such a frameshift produced an enzymatically active protein. A guanosine residue was inserted into codon 233 by site-directed in vitro mutagenesis, converting the original triplet specifying an arginine, which is not observed in any other nucleoside monophosphate kinase, into an alanine-encoding one. The altered amino acid sequence at the carboxyl terminus of PAK3, deduced from the verified nucleotide sequence, is shown in Fig. 1. To test for biological activity, the gene was inserted into the E. coli expression vector pKK223–3. IPTG-induced expression of the extended yeast gene in the temperature-sensitive adk1 E. coli mutant strain JH17 [pREP4] restored growth under the nonpermissive conditions (39°C), whereas the original PAK3 gene failed to complement (Fig. 2).

We tested whether we could select for biological activity after random mutagenesis of the PAK3 allele from yeast strain DL1. After exposure of JH17 (PAK3), transformant cells to the frameshift-inducing drug, acriflavin, complementing clones were selected by their ability to restore growth of the adk1 mutant strain JH17 at a nonpermissive temperature. By this means, it was possible to isolate 20 complementing clones (derived from nine independent mutageneses) that were subsequently sequenced. The gain of complementing activity was accompanied by a +1 frameshift in each case (Fig. 3, types 2–4) occurring within seven base pairs of the 3′-end of the coding region of the gene spanning the mutation originally proposed (Fig. 3, type 1). At least eight independent isolates of mutants containing mutation type 2, three of type 3, and one of type 4 were obtained. Although all single base pair insertions of position 492 (corresponding to Glu at position 196 in the protein family numbering, Magdon et al. (1992)) potentially lead to proteins that are 9 amino acids longer at the carboxyl terminus than Pak3p, only four nucleotide positions lying within three consecutive triplets were tolerated and led to an active protein product.

Alleles with a +1 frameshift occur in wild yeast strains—The finding that the apparently nonfunctional PAK3 allele from strain DL1 could be converted into a biologically active gene by insertion of a single base pair suggested that in DL1 an originally functional gene had been inactivated by a single base pair deletion. To investigate whether this trait is restricted to strain DL1 or is a general feature of S. cerevisiae and possibly other yeast species, we isolated, by use of PCR, the respective parts of the AKY3 genes from several strains, ranging from industrial and laboratory strains to "wild" yeasts. To have a fair chance to amplify the 3′-ends of the respective AKY3 alleles, the reverse primer was designed complementary to the noncoding strand of the 3′-adjacent RAD3 gene. Among the 18 strains examined this way, two (Saccharomyces uvarum and Saccharomyces carlsbergensis) repeatedly failed to give rise to a PCR product, probably due to either sequence divergence between the primers used and the genomic sequences or to genomic rearrangement in the intergenic region between AKY3 and RAD3. The nucleotide sequences of the remaining alleles fell into two groups (Fig. 4). The majority of them were identical to the one found in DL1. But in the residual five, a frameshift was observed within the Arg codon at position 233 (cf. Fig. 1). A C residue is introduced, changing the triplet for Arg into a proline codon (Fig. 3, AKY3). Most interestingly, all strains harboring the DL1 version of the gene, encoding an inactive protein, were strains used either in research laboratories or in the beer brewing industry. The alleles carrying the insertion and encoding the enzymatically active protein belonged, with the exception of D273-10B, to the more distantly related genera Saccharomyces diastaticus, Saccharomyces capensis, and the wine yeasts Malaga and Bordeaux. D273-10B is a laboratory strain without genetic markers.

The distinction made by the sequence divergence coincided with the results of the functional test in E. coli. Only the insertion-carrying alleles were able to complement the temperature-sensitive phenotype of E. coli JH17 (Fig. 2). We named
the allele encoding the enzymatically active protein version AKY3.

Enzymatic Specificity of Aky3p—The enzymatic specificity of Aky3p was analyzed in extracts of E. coli JH17 transformant strains overproducing the biologically functional isoforms of Aky3p from yeast strain D273-10B and from some mutants obtained from the frameshift mutagenesis experiment or, as a control, overproducing the inactive Pak3p (Fig. 2). As can be seen from Table I, the extracts derived from cells containing a biologically active, i.e. E. coli adk1–1-complementing, AKY3 allele also contained significant GTP:AMP phosphotransferase activity. On the other hand, cells from transformants harboring the PAK3 allele (or URA6 in inverted orientation, negative control, not shown) did not display any activity with these substrates above the level found in the control strain transformed with the vector without AKY3 gene. The background in extracts from the nontransformed JH17 mutant was below 2%. In addition to GTP:AMP kinase activity, a less pronounced ITP:AMP activity was found. With other nucleoside triphosphates including ATP, no significant enzymatic activity was observed. In contrast to any other known adenylate kinase, this isozyme could not be inhibited by the specific adenylate kinase inhibitor, Ap5A (no significant inhibition even at 0.115 mM in the presence of 0.6 mM of each GTP-Mg¹⁺ and AMP), and only very poorly by Gp5A (about 70% inhibition at 0.08 mM Gp5A, 0.03 mM GTP-Mg¹⁺, and 0.03 mM AMP).

To show unambiguously that the observed activity is associated with the enzyme and not caused by nonspecific background, we purified the enzyme. The modification of the amino terminus of the recombinant Aky3p by a histidine tag allowed the one-step enrichment of the product using Ni²⁺ chelate chromatography but did not dramatically impair enzymic activity, since the altered product complemented the deficiency of E. coli adk1–1ts (Fig. 3). Additional purification was accomplished by gel filtration (not shown), but at around 10.5 with the natural Aky3p (Fig. 6). At pH 8.5, the activity was similar with both substrates, but the maximum activity with GTP was about 2-3-fold higher at pH 10.5. Thus, like bovine AK3, also the yeast enzyme displays significantly higher activities with GTP at more alkaline pH values, but still none with ATP as phosphate donor.

Overproduction of Aky3p Fails to Complement AKY2 Deficiency—The complementation of E. coli mutant JH17 adk1–1 by AKY3 demonstrates the capability of the yeast enzyme to substitute for the sole adenylate kinase of E. coli encoded by the adk1 gene. This enzyme is equivalent to Aky2p, the major isoform in yeast. We investigated the capacity of AKY3 also to complement the deficiency of AKY2 in yeast strain DL1-D16.
(aky2). AKY2-deficient yeast cells do not grow on nonfermentable substrates and, thus, display a Pet phenotype, presumably because they are unable to exchange the ATP generated by oxidative phosphorylation efficiently against ADP over the inner mitochondrial membrane due to shortage of the latter. This means that aky2 mutants are likely arrested in state 4 of respiration (Bandlow et al., 1988). Aky3p phosphorylates AMP and produces ADP and potentially can overcome this limitation. To test for complementation, the aky2 mutant strain was transformed with the yeast shuttle vector YEp352 harboring the aky3 allele from strain D273-10B expressing under the adk1 promoter and the level of ADP was adjusted with HCl as indicated.

Subcellular Location of Aky3p—The notion that the yeast AKY3 gene complements the adk1 deficiency of E. coli, which is generally dispensable or functional left us with the question, whether AKY3 is generally dispensable or only in those strains carrying the defective allele, implying that laboratory strains harboring the aky3 allele (PAK3) carry a second site suppressor(s). Such suppressor mutants are frequently observed with aky2 null allele mutants (Bandlow et al., 1988). On the other hand, it was feasible that the loss of a functional AKY3 gene caused a gradual difference of growth rates between these strains.

The active AKY3 allele was found only in wild yeasts lacking genetic markers. Therefore we disrupted HIS3 in strain D273-10B and constructed the diploid homozygous his3 strain, D273-5d (see “Experimental Procedures”). One of the AKY3 alleles then was destroyed in the diploid to allow propagation of the heterozygous AKY3/aky3 alleles under conditions free of constraints that might be selective for second site suppressors. Phenotypic analysis of the meiotic products was subsequently performed with the spore progenies after tetrad dissection. The spores from 12 complete tetrads were assayed for His auxotrophy and prototrophy by replica plating on selective media, and the correct recombinational insertion of the disruption construct was verified by genomic Southern blotting in the progeny of the spores from three tetrads (not shown). In these 12 haploid segregants, the growth behavior in complete medium supplemented with either glucose or glycerol/ethanol was examined. No differences were apparent between haploid AKY3 wild-type and aky3 mutant strains. To make this assay more sensitive, AKY3 and aky3 cells were co-cultivated. Two cultures of cells with the same mating type derived from an AKY3-

![Fig. 6. pH dependence of enzymatic activities of Aky3p. Adenosine monophosphate (0.6 mM substrate) phosphotransferase activity was measured (25 °C) with GTP or ITP (0.6 mM each) as phosphate donors as described (Bandlow et al., 1988). The buffer contained 70 mM triethanolamine, and the pH was adjusted with HCl as indicated.](http://www.jbc.org/Downloaded from)
been a parameter that has played a role in the selection of strains for beer brewing (data not shown). Since AKY3 occurs in single copy in the haploid yeast genome, this gene is apparently dispensable, and its inactivation does not lead to a detectable disadvantage under the growth conditions usually applied in laboratory.

**FIG. 7. Test for complementation by overproduction of Aky3p of the Pet phenotype caused by Aky2p deficiency.** Cells were streaked onto glucose (left plate) or glycerol medium (right plate). The sectors display the following strains: DL1-D16 aky2, transformed with a multicopy plasmid expressing PAK3 under the control of the moderately strong AKY2 promoter; DL1-D16 aky2, transformed with a multicopy plasmid expressing the AKY3 gene from strain D273-10B under the control of the AKY2 promoter; wild-type D273-10B.

**FIG. 8. Western blot of cellular and mitochondrial subfractions (A) and of the pellet and supernatant fractions of mitochondria obtained after extraction with Na2CO3 (B).** mito denotes whole mitochondria; IMS, intermembrane space; IM + OM, inner plus outer mitochondrial membranes. The following antisera (all from rabbit) have been used: Hxk, anti-hexokinase, cytosolic marker; Hsp60, anti-Hsp60 from mitochondrial matrix; Cyt b2, anti-cytochrome b2 from the intermembrane space; Cyt c1, anti-cytochrome c1 from inner mitochondrial membranes. Anti-Aky3 was from chicken eggs (see "Experimental Procedures").

DISCUSSION

Previously, we have isolated the gene, PAK3, a member of the adenylate kinase gene family. In contrast to URA6, which was identified as a multi-copy suppressor of AKY2- and adk1-deficiency by functional complementation (Schricker et al., 1992b), PAK3 was not detected by this procedure. Rather, the gene was amplified by PCR from genomic DNA prepared from strain DL1-D16 (aky2::LEU2) using degenerate primers. Expression of the PAK3 gene was shown to be regulated by the carbon source, but the protein product displayed no activity with cells cultivated under any growth condition and with any combination of nucleoside mono- and triphosphates tested (Schricker et al., 1992a). This suggested that PAK3 is an expressed gene with no enzymic activity of the protein product. We report here that the defect is due to a single base pair deletion and is restricted to a number of laboratory strains, whereas the "wild" yeasts inspected have the intact allele. The frameshift leads to a truncated protein that lacks 9 amino acids from the carboxyl terminus including two conserved hydrophobic residues compared with the "wild" strains. In all other nucleoside monophosphate kinases, the carboxyl-terminal amino acids form part of an amphipathic α-helix, which makes a hydrophobic contact to the purine ring of ATP and stabilizes the protein like a clamp (Diederichs and Schulz, 1991). Their mutation in the adenylate kinase from E. coli (Yoneya et al., 1990) or their partial deletion in Aky2p from yeast (Magdolen et al., 1992) lead to inactive proteins exhibiting increased susceptibility to proteolytic degradation. This explains why Pak3p is inactive also.

PAK3 apparently is an expressed gene occurring in the genomes of culture yeast strains, the protein product of which lacks enzymic activity. Other examples of expressed genes leading to nonfunctional proteins are imp and ho. imp is a mutation found in some strains, causing dependence on the presence of functional mitochondria when grown on galactose (Algeri et al., 1981) and has been identified as a mutated allele of GAL2 encoding the transporter for galactose. The other defective gene, ho, encodes a nonfunctional allele of the mating locus-specific HO endonuclease. The wild-type allele causes
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heterothallism, the molecular basis of mating type switching once per generation and, as a consequence, spontaneous diploidy (Breeden and Nasmyth, 1985). Conceivably, the defective ho allele has been selected in earlier times for reasons of genetic stability to allow genetic analyses. Similarly, the observation that the truncated Pak3p version is restricted to strains used in beer brewing industry and in laboratory research nourished the suspicion that the defective ancestor of the S. cerevisiae strains used today has been selected on purpose also because of some advantageous property. However, the growth and adaptation experiments performed and the determination of the ethanol content in culture media give no indication in this direction. Neither is the adaptation velocity of Pak3-containing strains decreased compared with the wild-type, nor is the growth rate reduced on ethanol-containing media (which might be advantageous, because such strains will use up less of the alcohol they produce by fermentation in a given time), nor can a higher yield in ethanol production be correlated with the possession of the Pak3 allele.

In wild yeasts, the AKY3-encoded protein product displays GTP:AMP and ITP:AMP phosphotransferase activity. No AMP phosphorylation could be detected with ATP as phosphate donor, either in extracts from E. coli cells expressing the yeast gene under the control of an E. coli promoter, in purified preparations of the recombinant enzyme, or in yeast strains overproducing the active protein. AK3 and Aky3p, respectively, are assumed to play a role as scavengers of mitochondrial AMP (or dAMP) at the expense of GTP (or ITP) hydrolysis, which is formed in the Krebs cycle by succinic thiokinase (Heldt and Schwalbach, 1967). Such a scavenger function has to be postulated in mitochondria, because on the one hand AMP is generated in this compartment in a number of hydrolytic reactions (e.g. turnover of DNA or RNA) or in ATP-dependent substrate activations yielding AMP (e.g. tRNA synthetases, PEP carboxykinase, activation of ethanol-derived acetate by acetothio kinase). On the other hand, mitochondrial inner membranes from mammals as well as from yeast lack a transport system for guanosine nucleotides as well as for AMP (Pfaff et al., 1965). Third, physiological observations suggest that a GTP: AMP phosphotransferase activity seems to be required specifically under oxidative metabolic conditions in the presence of ethanol, because expression of the AKY3 gene as well as alcohol oxidation and the generation of AMP (Lindros et al., 1986), e.g. by acetothiokinase, are strongly induced by ethanol. The pronounced activation of the enzyme upon alkalization points in the same direction that the enzyme might contribute to the supply of the ATP synthase with ADP, particularly under conditions of a high ΔpH, i.e. a high membrane potential over the inner mitochondrial membrane as is the case under oxidative metabolic conditions. Studies on AK3 in mammalian adult and fetal tissues show that the enzyme is present at fairly constant levels in all tissues and at all developmental stages tested (Tanabe et al., 1993), suggesting a housekeeping function for the gene at least in vertebrates. This could consist primarily in the regeneration of ADP from AMP, which is to be supplied to the F1F0-ATP synthetase and to a lesser extent in the regeneration of GDP as substrate for the succinic thiokinase, since this task is also fulfilled by other reactions, e.g. by protein biosynthesis.

In yeast, utilization of mitochondrial GTP presumably plays a minor role, particularly during growth on ethanol, where much of the isocitrate formed is channeled into gluconeogenesis via the isocitric lyase shunt, thereby bypassing succinic thiokinase. However, reutilization of mitochondrial AMP remains a problem in the absence of an AMP kinase activity in the matrix. This problem appears even more acute since it has been shown (Lundin et al., 1991, 1992) that yeast mitochondria contain inorganic pyrophosphatase making the theoretically possible pathway of AMP reactivation through acetyl-CoA, pyrophosphate and fatty acid thiokinase unlikely. The fact that wild yeasts, just like laboratory strains lacking Aky3p activity, can dispense with such an AMP scavenger activity proves that this organism must have found an alternative way to tackle the problem of accumulation of AMP in the mitochondrial matrix. The assumption that yeast might have acquired an additional mitochondrial nucleoside monophosphate kinase isoenzyme seems to be highly unlikely, not only because there are no indications, by PCR or enzymatic activity, which would suggest the presence of another such enzyme, but also because of the exceptional metabolic constellation in this organism. Yeast, in contrast to higher eucaryotes, is one of the rare eucaryotes that can live on ethanol as the only carbon source. Under this nutritional condition, however, the flux through the citric acid cycle is greatly funneled into the isocitric lyase shunt as mentioned above, thereby drastically reducing the production of GTP. Under these conditions, the efficient rescue of intramitochondrial AMP by Aky3p would be severely impeded. For this reason, yeast must have evolved an alternative pathway for reutilization of AMP generated in the matrix that simultaneously would render Aky3p dispensable at least in this organism. A plausible model of this alternative rescue pathway is presently not available, but conceivably it could consist in the dephosphorylation of AMP to Ado, which is then reactivated in the cytoplasm.

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