Mitochondrial and Cytosolic Branched-chain Amino Acid Transaminases from Yeast, Homologs of the myc Oncogene-regulated Eca39 Protein*

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We have isolated a high copy suppressor of a temperature-sensitive mutation in ATM1, which codes for an ABC transporter of Saccharomyces cerevisiae mitochondria. The suppressor, termed BAT1, encodes a protein of 393 amino acid residues with an NH$_2$-terminal extension that directs Bat1p to the mitochondrial matrix. A highly homologous protein, Bat2p, of 376 amino acid residues was found in the cytosol. Both Bat proteins show striking similarity to the mammalian protein Eca39, which is one of the few known targets of the myc oncogene. Deletion of a single BAT gene did not impair growth of yeast cells. In contrast, deletion of both genes resulted in an auxotrophy for branched-chain amino acids (Ire, Leu, and Val) and in a severe growth reduction on glucose-containing media, even after supply of these amino acids. Mitochondria and cytosol isolated from bat1 and bat2 deletion mutants, respectively, contained largely reduced activities for the conversion of branched-chain 2-ketoacids to their corresponding amino acids. Thus, the Bat proteins represent the first known isoforms of branched-chain amino acid transaminases. The severe growth defect of the double deletion mutant observed even in the presence of branched-chain amino acids suggests that the Bat proteins, in addition to the supply of these amino acids, perform another important function in the cell.

ATM1, which codes for an ABC transporter of the mitochondrial inner membrane (1). Yeast cells with inactivated Atm1p display a drastic reduction in growth on fermentable carbon sources such as glucose and do not grow on nonfermentable carbon sources such as glycerol. In the absence of functional Atm1p, yeast cells exhibit a general deficiency in heme-containing proteins, e.g. cytochromes in mitochondria and catalase in peroxisomes. At present, the natural substrate of Atm1p is not known. Heme has been ruled out as a potential substrate, since normal growth of yeast cells lacking Atm1p cannot be restored by the addition of heme (1). A further phenotypical hallmark of atm1 deletion cells is the accumulation of iron in mitochondria. This excludes iron as the compound transported by Atm1p but suggests that the protein plays an indirect role in iron metabolism.

In order to elucidate the functional role of Atm1p, we considered it important to identify proteins interacting with the transporter. Therefore, we screened for high copy suppressors of a temperature-sensitive mutation in ATM1. The mutant cells (strain R64a) contain an Atm1p transporter, which, at the nonpermissive temperature, is degraded. As a result, mutant cells do not grow on nonfermentable carbon sources such as glycerol. Here, we describe the isolation of a suppressor termed BAT1. The encoded protein, Bat1p, is localized in the mitochondrial matrix, and, at increased concentrations, it can prevent the degradation of the mutated Atm1p, thus allowing growth at the nonpermissive temperature.

Based on the immunological cross-reactivity, we isolated a highly homologous protein termed Bat2p, which is localized in the cytosol. Both Bat proteins display a striking sequence similarity to the mammalian protein Eca39. This protein was originally identified by the high overexpression of its mRNA in an undifferentiated mouse teratocarcinoma cell line (3). Subsequently, it was reported that its gene is induced by overexpression of the protooncogene c-myc (4). Homologs of this murine protein are known in humans, rats, and the plant Arabidopsis thaliana. We now identify the two Bat proteins as the yeast branched-chain amino acid transaminases, enzymes that until now have escaped a genetic identification (see Refs. 5 and 6). Several criteria, including the slow growth of the double mutant in the presence of branched-chain amino acids, suggest that the Bat proteins, besides supplying the cell with these amino acids, are required for another important process.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions—The following yeast strains were used: YR6a (Mat a ade2 ura3–52 leu2–3, 112), YPH501 (Mat a ura3–52 lys2–801 ade2–101 trpl–63 his3–200 leu2–1). Cells were grown in 1% yeast extract, 2% Bacto peptone medium (rich medium) supplemented with 2% glucose (YPD) or 3% glycerol (YPG). For selective growth, yeast cells were cultivated in 0.67% yeast nitrogen base (minimal medium) containing 2% glucose (MMG) or 3% glycerol (MMG+Rf; Refs. 7 and 8). The latter media were supplemented with leucine (30 mg/l), adenine, histidine, lysine, tryptophan, and uracil (20 mg/liter each) according to the auxotrophic requirements of the various strains.

Isolation of BAT1—The previously identified cytochrome-deficient pet+ strain R64a was transformed with a yeast genomic DNA library cloned into the Yep13 multicopy yeast plasmid (9) by a published method (10) and incubated on selective medium at 25 °C for 3 days. The resulting 5 × 10$^{4}$ colonies were replica-plated to YP-glycerol medium

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and incubated further for 5 days at 37 °C. Plasmid DNA was isolated from these cells that showed growth at the nonpermissive temperature and used to retransform strain R64a. A plasmid that conferred suppression after the repeated transformation was analyzed in detail. Digestion with HindIII restriction enzyme liberated two DNA fragments besides the plasmid backbone. These DNA fragments were subcloned into YEplac195 episomal plasmid (11) and transformed into R64a. One of these plasmids containing a 1.9-kb nuclear DNA fragment conferred the same suppression as the original YEplc13 clone. This fragment was subcloned into pBluescript II plasmid, and the nucleotide sequence was determined.

Disruption of BAT1 — The pBluescript II plasmid containing the 1.9-kb nuclear DNA insert was used to generate the construct for disruption. The NdeI-BstEII fragment corresponding to amino acids 53–274 of Bat1p was replaced by the yeast URA3 auxotrophic marker. This modified DNA insert was liberated from the plasmid by SalI-EcoRI digestion and used to transform the isogenic diploid strain YPH501. The correct integration of this construct into the resulting diploid strain YPH501Δbat1 was determined by Southern blotting. The haploid disruption strain Δbat1 was derived from the transformed diploid after sporulation and tetrads dissection. For analysis of the Δbat1 phenotype, the disrupted cells were transformed with a centromeric yeast plasmid YCplac111 (11) containing the LEU2 yeast marker and tested at different growth conditions.

Production of Antibodies against Bat1p — The 1.2-kb HindII fragment of BAT1 was cloned into the BamHI site of pQE9 plasmid (Quaigen). This created an amino-terminal fusion of the hexahistidinyl tag harbored in the pQE9 plasmid to amino acid residues 56–393 of Bat1p. The recombinant plasmid was transformed into X.LBue cells. Synthesis of the plasmid-encoded protein was induced by the addition of isopropyl-β-n-thiogalactopyranoside. After 2 h of induction, the expressed fusion protein was found exclusively in inclusion bodies. The inclusion bodies were isolated, washed three times with phosphate-buffered saline containing 1% Triton X-100 detergent, dissolved in SDS-sample buffer, and subjected to SDS-PAGE. After blotting the proteins onto nitrocellulose, bands containing the fusion protein were excised and used for immunization of a rabbit.

Isolation of BAT2 — BAT2 cDNA was isolated by screening the λ ZAPII yeast cDNA library (Stratagene) with the antibody raised against Bat1p. Approximately 2 × 10^7 phage were screened, and 10 of them that gave strong immunoreaction during the repeated screening were selected for further examination. The plasmids from these clones were liberated using a helper phage and analyzed by restriction digestion.

In addition to BAT1-harboring fragments, two other inserts were isolated, and the DNA sequence was determined. One of them showed high homology to BAT1 and was selected for further examination.

For the isolation of the genomic clone for BAT2, we made use of the leucine, isoleucine, and valine auxotrophy of the double-disrupted Δbat1Δbat2 cells. These cells were transformed with the YEplc13 yeast genomic DNA library and plated on minimal medium plates lacking branched-chain amino acids. Ten complemented colonies were selected for further analysis. Plasmid DNA was isolated from them and subjected to restriction analysis. Three of these plasmids showed the same restriction pattern as BAT1, whereas the others proved to be identical to BAT2. Determination of the nucleotide sequence of the 5′-region of BAT2 was completed using one of these plasmids as a template.

Disruption of BAT2 — The plasmid harboring the BAT2 cDNA was digested by HpaI and BstEII restriction enzymes, and the liberated 550-base pair insert (corresponding to amino acid residues 29–221 of Bat2p) was replaced by the TRP1 yeast auxotrophic marker. The modified BAT2 insert was excised from the plasmid by EcoRI digestion and used to transform YPH501Δbat1 diploid cells. DNA integration was confirmed by PCR. One of the transformed clones was sporulated and subjected to tetrad dissection to yield parental type, Δbat1, BAT2-disrupted (Δbat2), and double-disrupted (Δbat1Δbat2) cells.

Miscellaneous Procedures — The following published methods were used: DNA manipulations and the polymerase chain reactions (12), transformation of yeast cells (10), isolation of plasmids from yeast (13), isolation of yeast mitochondria from various strains (14), preparation of whole cell lysates by breaking the cells with glass beads (15), in vitro transcription and translation and protein import into isolated mitochondria (16, 17), fractionation of mitochondria and kits (U.S. Biochemical Corp.).

Amino-acid sequence analysis was performed using the Sequenase sequencing kit (U.S. Biochemical Corp.).

RESULTS

Identification and Sequence of Bat1p — The yeast strain R64a carries a mutation in the ATM1 gene, which encodes a mitochondrial ABC transporter (1). R64a cells are temperature-sensitive for growth on nonfermentable carbon sources, and, at the nonpermissive temperature of 37 °C, the mutant transporter is degraded. We isolated high copy suppressors of this pet phenotype by complementing the R64a cells with genomic yeast DNA. A complementing clone sufficient to restore growth of R64a cells at 37 °C harbored a 1.9-kb DNA fragment that encoded a gene which, for reasons outlined below, was termed BAT1 (Fig. 1A).

The pet phenotype of strain R64a is characterized by largely reduced levels of the holofoms of heme-carrying proteins, e.g. of cytochromes c and c1. We analyzed whether, at the nonpermissive temperature, these effects are reversed in R64a cells that were transformed with high copy numbers of the BAT1 gene. Mitochondria were isolated from these cells as well as from wild-type and R64a cells that had been grown at 25 and...
Bat1p contains a putative mitochondrial targeting signal at its NH₂ terminus. It has the potential to form an amphipathic helical structure with four positive charges on the polar face. A search in protein sequence databases revealed a highly significant homology to a number of proteins from other organisms (Fig. 2). For instance, Bat1p shares about 50% amino acid identity (65% based on chemical similarity) with Eca39 proteins from humans, mice, rats, and the plant A. thaliana. Only a partial sequence derived from the expressed sequence tag (EST) project is available for the plant protein. The murine gene encoding Eca39 protein was identified by virtue of the overexpression of its mRNA in a teratocarcinoma cell line (3). Expression of the gene was later found to be under the control of the mouse oncogene (4). For the rat protein, a function as a cytosolic branched-chain aminotransferase was reported recently (26). Significant sequence homology (44% identity, 64% similarity) was also found for a protein from the Gram-positive bacterium Bacillus subtilis (27). A comparatively weak sequence identity of Bat1p (24% within 237 amino acid residues, 67% similarity) was observed to bacterial branched-chain amino acid transaminases (Ref. 28; Fig. 2). In the enzyme from Escherichia coli, residue Lys™ has been shown to be essential for the formation of the Schiff base intermediate with pyridoxal phosphate (Ref. 29, outlined in Fig. 2). In the Bat1p protein and its homologs, this residue is conserved.

**Bat1p Is Localized in the Mitochondrial Matrix**—To initiate the characterization of Bat1p, its subcellular localization was determined. First, the potential function of the NH₂ terminus as a mitochondrial targeting sequence was tested by performing *in vitro* protein import studies. *B. taurus* was cloned into the pGEM3 transcription/translation vector. Bat1p was synthesized in reticulocyte lysate in the presence of radioactively labeled methionine, and it was incubated with isolated yeast mitochondria in the presence of ATP and NADH (16, 17). Bat1p was efficiently imported into mitochondria and became processed to a smaller species, which was protected against externally added protease (Fig. 3A). Upon lysis of the mitochondria in detergent, this processed form was completely digested. From the size of the cleaved prepiece (about 2 kDa), the processing site in Bat1p can be predicted to be located after amino acid residue 16 (arrow in Fig. 2; see Ref. 30). Import and processing were strictly dependent upon the presence of a membrane potential, Δψ, as is typical for proteins of the inner membrane and the matrix (Fig. 3B; Refs. 31–33).

To analyze the submitochondrial localization of imported Bat1p, mitochondria were subfractionated by swelling in hypotonic buffer. This treatment causes the rupture of the outer membrane but leaves the inner membrane intact (18). As a consequence, proteins outside the inner membrane become accessible to added proteases, whereas proteins localized beyond this membrane remain resistant to digestion. Imported mature Bat1p was not degraded when proteinase K was added after swelling (Fig. 3A) and showed a similar behavior as Tim44, a protein of the mitochondrial matrix (34). An identical observation was made for endogenous Bat1p, which was detected by immunostaining using antibodies raised against Bat1p. In contrast, the intermembrane space marker protein cytochrome b₂ was accessible to digestion by proteinase K. We conclude that Bat1p is exposed to the matrix space.

**Is Bat1p a soluble component of the matrix?** Isolated mitochondria were sonicated in the presence of increasing salt concentrations, and soluble proteins were separated from the mitochondrial membranes by centrifugation. Bat1p was recovered in the supernatant and behaved as other soluble proteins of the mitochondrial matrix such as Hsp70 (Fig. 3C). Membrane-associated proteins like cytochrome c₁, heme lyase were
found in the pellet fraction (cf. Ref. 35), whereas cytochrome c was released from the inner membrane only at higherionic strengths. In conclusion, Bat1p is synthesized with a short mitochondrial targeting sequence, which directs the protein in a ΔΨ-dependentfashion into the mitochondrial matrix space, where it resides asasoluble protein.

Identification and Sequence of the Cytosolic Homolog, Bat2p—During our studies, we detected a protein in postmitochondrial supernatants that was specifically recognized by anti-Bat1p antibodies (Fig. 4). The cross-reacting protein was unlikely to represent a contamination by mitochondrial Bat1p, since it was also present in the postmitochondrial supernatant isolated from Δbat1 cells in which the BAT1 gene had been deleted (Fig. 4). Mitochondria isolated from these cells did not contain any detectable amounts of Bat1p. The cross-reacting protein exhibited a slightly higher mobility on SDS-polyacrylamide gels as compared with Bat1p. The protein was not associated with membranous structures, since it was recovered in a high speed supernatant of the postmitochondrial extract (not shown). These observations suggested the existence of a cytosolic homolog of Bat1p and prompted us to isolate its gene.

Both a cDNA and a genomic DNA fragment were obtained encoding the cross-reacting protein. Sequencing of the DNA revealed an ORF corresponding to YJR148w of yeast chromosome X (36). The encoded protein is 376 amino acid residues long (41,624 Da) and shows extensive homology to mitochondrial Bat1p (77% amino acid identity, 85% similarity). Therefore, the cytosolic protein was termed Bat2p (Fig. 2). The sequence similarity of Bat2p to the other members of the “Bat family” is comparable with that already described for Bat1p.

Analysis of bat Deletion Mutants—Yeast strains were constructed in which one or both BAT genes were deleted. Gene inactivation was confirmed by Southern blotting or polymerase chain reaction (not shown) and by immunostaining analysis of mitochondria and postmitochondrial supernatants isolated from the mutant cells (Fig. 5). The latter analysis also confirmed the exclusive mitochondrial and cytosolic location of Bat1p and Bat2p, respectively. Deletion of a single Bat protein had no significant influence on the cellular level of the homologous partner protein.

The consequences of disruption of the BAT genes for growth of yeast cells under various conditions are summarized in Table I. Deletion of a single BAT gene had no apparent influence on the growth rate at 25 and 37°C on both rich and minimal media containing either glucose (YPD and MMD, respectively) or glycerol (YPG and MMG, respectively) as a carbon source. The only detectable phenotype was a slightly slower growth of Δbat1 cells on YPG at 37°C and on MMD. The latter growth defect could be reversed by the addition of leucine or valine to the medium.

In contrast to the single deletions, the double deletion mutant, Δbat1Δbat2, exhibited a marked reduction in growth on YPD but not on YPG (Table I). We noticed the generation of spontaneous suppressors after several restreaks on this growth medium (not shown; see also below). On minimal media, Δbat1Δbat2 cells required the addition of all three branched-chain amino acids (i.e., leucine, valine, and isoleucine). The leucine auxotrophy is directly linked to the deletion of the two BAT genes and not simply due to the leu2 genotype of the Δbat1Δbat2 cells. This was concluded from the observation that transformation with the LEU2 gene did not yield cells that could grow in the absence of leucine unless the transforming plasmid also contained the BAT1 or BAT2 gene (not shown).
Spontaneous suppression of the branched-chain amino acid auxotrophy was not observed on minimal media, in contrast to what was seen with rich media (see above). Even after supplementing the media with these amino acids, slower growth as compared with wild-type cells was detectable, suggesting that the insufficient supply of branched-chain amino acids is not the sole reason for the observed growth defects of \(\Delta bat1\Delta bat2\) cells.

**The Bat Proteins Function as Branched-Chain Amino Acid Transaminases**—The homology of the Bat proteins to known branched-chain amino acid transaminases and the observed auxotrophy of the \(\Delta bat1\Delta bat2\) double mutant cells for these amino acids prompted us to directly test their potential function as transaminases. A glutamate dehydrogenase-coupled assay was used to measure the generation of leucine, valine, and isoleucine from their corresponding 2-ketoacids, namely 2-ketoisocaproic acid, 2-ketoisovalerate, and 2-ketomethylvalerate. In mitochondria isolated from \(\Delta bat1\) cells, a 10-fold reduction of the leucine- and valine-dependent transaminase activities as compared with wild-type organelles was observed (Fig. 6, upper row). The isoleucine-dependent enzyme activity was reduced 3-fold. In contrast, in \(\Delta bat2\) mitochondria the branched-chain amino acid transaminase activities were hardly affected. The activity of the glutamate-pyruvate transaminase, as a control, was virtually unchanged in all mitochondria, demonstrating that the observed effects on the activity of the branched-chain amino acid transaminase were specific.

In cytosolic fractions prepared from \(\Delta bat2\) or \(\Delta bat1\Delta bat2\) cells, an approximately 2-fold reduction was measured for the leucine- and valine-dependent transaminase activities (Fig. 6, lower row). No significant alteration was observed in the case of the isoleucine-dependent enzyme activity. As with mitochondria, the activity of the glutamate-pyruvate transaminase was virtually unaffected in the various mutants. These data strongly suggest that Bat1p and Bat2p function as branched-chain amino acid transaminases in the mitochondrial matrix and in the cytosol, respectively. Bat1p appears to be the major enzyme for the metabolic generation of leucine and valine in mitochondria, whereas isoleucine may also be synthesized to some extent by other transaminases. The high residual level of branched-chain amino acid transaminase activity in the cytosol after deletion of the \(BAT2\) gene, even in the absence of functional Bat1p, demonstrates the existence of other cytosolic enzymes that can replace the Bat proteins in supplying the cell with branched-chain amino acids. These data suggest that the severe growth defects of \(\Delta bat1\Delta bat2\) cells on glucose-containing media (cf. Table I) are only partially related to an insufficient synthesis of these amino acids.

To support this conclusion, we directly measured the content of free amino acids in wild-type and \(\Delta bat1\Delta bat2\) mutant strains. Cells were cultivated overnight in glucose-containing rich or minimal media and then extracted and deproteinized by lysis with sulfosalicylic acid, and a quantitative analysis of free amino acids was performed. After growth in rich media, most amino acids including leucine and isoleucine were detectable in comparable amounts in wild-type and \(\Delta bat1\Delta bat2\) mutant cells (Fig. 7). As the only exception, valine was reduced 5-fold in \(\Delta bat1\Delta bat2\) cells. However, this reduction cannot account for the severe growth defect of these cells (cf. Table I), since the addition of valine to the medium did not increase the growth rate (not shown). Virtually the same results were observed for cells cultured in minimal media (Fig. 7). Thus, a depletion of the cellular levels of branched-chain amino acids cannot be the explanation for the strong impairment in growth observed after deletion of the two \(BAT\) genes. We therefore conclude that, in addition to their role in the biosynthesis of branched-chain amino acids, the two Bat proteins must fulfill another important cellular function.

**DISCUSSION**

Our study identifies the first two yeast proteins known to function as branched-chain amino acid transaminases. The two enzymes are localized within different subcellular compartments, namely the mitochondria and the cytosol. Thus, the last step in the biosynthesis of branched-chain amino acids (5, 6) can be performed either within mitochondria, where the initial irreversible steps of the synthesis occur, or they may take place after the exit of the 2-ketoacids into the cytosol. Obviously, a carrier in the mitochondrial inner membrane can equilibrate the mitochondrial and cytosolic pools of branched-chain 2-ketoacids and their corresponding amino acids, since the deletion of a single \(BAT\) gene results in no severe phenotype. From our data it appears rather unlikely that the Bat proteins themselves constitute the carrier, as has been suggested for the mitochondrial branched-chain amino acid transferase from rat heart (37). In yeast, both Bat proteins appear to be soluble proteins. Our findings also explain why these enzymes have escaped previous genetic identification. Deletion of only one of the \(RAT\) genes did not result in an auxotrophy for branched-chain amino acids. Although the two Bat proteins perform

### TABLE I
**Growth phenotypes of Bat1p- and Bat2p-deficient yeast cells**

| Strain        | YPD | YPG | MMD | MMG |
|---------------|-----|-----|-----|-----|
| \(\Delta bat1\) | +   | +   | +   | +   |
| \(\Delta bat2\) | +   | +   | +   | +   |
| \(\Delta bat1\Delta bat2\) | +/- | +   | +   | +   |
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The function of the other Bat homologs as transaminases should now be testable by using yeast as a model organism (38). If functional as a transaminase, all of the homologs should be able to complement the growth defects of \( \Delta bat 1 \Delta bat 2 \) cells on glucose-containing media. This analysis will be especially important for the mouse Eca39 protein, which has been reported to be regulated in its expression by the \( \text{Myc} \) oncogene (4). The functional connection of the Eca39 proteins to the \( \text{Myc} \) protein is unclear. Expression of \( \text{Myc} \) is related to the recovery of cells from stationary phase (39, 40). We noticed that yeast cells deficient in a single Bat protein, despite displaying no obvious growth reduction, can only slowly leave stationary phase (data not shown). The Bat proteins may therefore perform a critical function in processes that are necessary for cells to leave the resting phase and to enter the cell cycle. It will be interesting to investigate whether an interaction of the Bat proteins with factors regulating these reactions in yeast can be detected.

Several lines of evidence suggest that the function of the Bat proteins may not be restricted to the supply of branched-chain amino acids by the conversion from their corresponding 2-keto-acids. First of all, the strong growth defect of a \( \Delta bat 1 \Delta bat 2 \) double deletion mutant cannot be explained by the impairment of the cellular branched-chain amino acid transaminase activity. Apparently, the biosynthetic function of the Bat proteins as branched-chain amino acid transaminases can be taken over by other enzymes in the cell, and only a 2-fold reduction in the transamination activity in the cytosol is seen in \( \Delta bat 1 \Delta bat 2 \) cells. This notion is supported by our measurements of the cellular levels of free amino acids. They are close to wild-type levels and should sustain normal cell growth, even in the absence of the two Bat proteins. Furthermore, the strong growth defect observed with \( \Delta bat 1 \Delta bat 2 \) cells on minimal media can only partially be suppressed by the addition of leucine, isoleucine, and valine (see Table I). Thus, in addition to their role in the supply of branched-chain amino acids, the Bat proteins seem to fulfill an additional function that is important to maintain normal growth rates. Strikingly, an additional function has also been found for another enzyme of branched-chain amino acid biosynthesis (41). Deletion of \( \text{Ile}V5 \), encoding the mitochondrial acetyl-CoA reductoisomerase, resulted in unstable mitochondrial DNA producing mostly \( \rho^- \) petite mutants. Two independent functions have also been observed for another pyridoxal phosphate-dependent protein, the bacterial NifS. Initially, NifS was shown to be a cysteine...
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desulfurase required for the formation of the iron-sulfur (Fe/S) cluster in a subunit of nitrogenase (42, 43). More recently, it has been shown that these enzymes may also function as amino acid transaminases (44).

What could be such an additional function of the Bat proteins? Hints to answer this fundamental question may come from the investigation of components interacting with the Bat proteins. We have isolated the BAT1 gene as a high copy suppressor of a temperature-sensitive mutation in ATM1 encoding a mitochondrial ABC transporter.1 High levels of Bat1p, but not of Bat2p, can stabilize the mutant Atm1p against degradation at the nonpermissive temperature. This suggests a direct protein-protein interaction as the molecular basis of the stabilizing effect rather than an indirect effect, e.g. by the interaction of a Bat1p metabolite with Atm1p. This is supported by the observation that overexpression of Bat2p does not suppress the temperature-sensitive mutation in ATM1 (not shown). A further strong indication for a direct functional relationship between Atm1p and Bat1p is the leucine auxotrophy associated with the deletion of the ATM1 gene.1

What may be the functional connection between Bat1p and Atm1p? At present, the physiological substrate of Atm1p is unknown. Atm1p-deficient cells almost completely lack heme-containing proteins such as mitochondrial cytochromes and peroxisomal catalase,1 but heme has been excluded to be the substrate of the transporter (1). The accumulation of iron in atm1 mutant mitochondria suggests that the protein may be indirectly involved in mitochondrial iron metabolism.1 Iron, to be maintained in a soluble state within the cell, has to be complexed by chelating compounds (2, 45). Therefore, one possible scenario is that Atm1p actively transports an iron chelate between the mitochondrial matrix and the cytosol. If transport would occur toward the cytosol, this would result in low concentrations of the free chelator in the matrix. Iron would therefore be more easily available for incorporation into Fe/S proteins and the synthesis of heme. It seems possible, although not very likely, that the chelator is chemically identical to the substrates of the Bat transamination reaction, namely to the branched-chain 2-ketoacids or amino acids. Clearly, also from this point of view it will be important to identify the second cellular function of the Bat proteins.

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