FEBS Letters 353 (1994) 197–200

FEBS 14658

Yta10p, a member of a novel ATPase family in yeast, is essential for mitochondrial function

Reimund Tauer[a], Gertrud Mannhaupt[a], Ralf Schnall[a], Alexander Pajic[b], Thomas Langer[b], Horst Feldmann[a,*]

[a]*Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Schillerstraße 44, D-80336 München, Germany*
[b]*Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Goethestraße 33, D-80336 München, Germany*

Received 31 August 1994

Abstract The yeast gene, *YTA10*, encodes a member of a novel family of putative ATPases. Yta10p, as deduced from the nucleotide sequence, is 761 amino acids in length (predicted molecular mass 84.5 kDa). The amino acid sequence of Yta10p exhibits high similarity to two other yeast proteins, Yta11 and Yta12, and to *E. coli* FtsH. Several features of Yta10p are compatible with its localization in mitochondria. We report here that Yta10p is a yeast mitochondrial protein and that import is dependent on a membrane potential and accompanied by processing to a protein of approximately 73 kDa. Disruption of *YTA10* leads to a nuclear *petite* phenotype and to a loss of respiratory competence, as shown by spectrophotometric measurement of the activities of respiratory complexes I–III and IV, respectively. These findings together with the high similarity of Yta10p to several ATP-dependent proteases suggest that Yta10p is a mitochondrial component involved, directly or indirectly, in the correct assembly and/or maintenance of active respiratory complexes.

Key words: Mitochondrion; ATPase; Yta family; *Saccharomyces cerevisiae*

1. Introduction

We recently identified a set of 12 yeast genes, coding for members of a novel ATPase family, which we called the *YTA* family [1]. Our analyses revealed that all of the putative gene products are characterized by the presence of a highly conserved domain of 300 amino acids containing specialized forms of the A and B motifs of ATPases. These features classify the Yta proteins as a distinct subfamily within the still growing AAA-protein family (see [2,3] for reviews). There is accumulating evidence from our work ([1] and unpublished results) and the work of others [4] that particular members of these proteins constitute regulatory components of the yeast 26 S proteasome complex. Another subgroup within the Yta proteins, Yta10p, Yta11p, and Yta12p, exhibit substantial similarity among each other. Yta11p is nearly identical to Yme1p, which was previously shown [5] to be associated with yeast mitochondria and to have high homology to *E. coli* FtsH. FtsH was found to be an essential inner membrane protein with putative ATPase activity [6,7]. Furthermore, FtsH was reported to be identical to HflB, a gene product involved in the stability of the phage lambda cII activator protein and, by several criteria, was proposed to be the ATP-dependent regulatory subunit of an additional proteolytic complex in *E. coli* [8]. We found that Yta10p and Yta12p are more closely related to FtsH/HflB than is Yta11p/Yme1p. The amino acid sequences of Yta10p and Yta12p share several characteristics which are compatible with their mitochondrial location and, together with the above observations, led us to suggest that they might be components of a proteolytic complex in mitochondria [1]. To further address this question, we have chosen to investigate the role Yta10p by genetic and biochemical approaches (see also [9]). Here we report that Yta10p is located in mitochondria and is essential for respiration-dependent growth.

*Corresponding author. Fax: (49) (89) 5996 316.
E-mail: feldmann@dec1.bio.med.uni-muenchen.de

Abbreviations: Abf1, ARS (autonomous replicating sequence) binding factor 1 from yeast; Grf2, general regulatory factor 2 from yeast; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonylfluoride; *YTA*, genes encoding *Y*east (human immunodeficiency virus) *T*at-binding *A*nalogues.

2. Experimental

2.1. Strains, DNA, sequence analysis, Yta10p antiserum, and general procedures

Yeast strain W303D (*MATa ade2–1 his3–11,15 leu2,112 trp1 ura3–52 can1–100*; M.A. Blazquez) was used in genetic experiments and strain D273–10B for biochemical analysis. Cosmid α231 was isolated from a collection of YTA cosmids [1]. Sequence analysis of a set of appropriate subfragments (Fig. 2) cloned into M13 vectors was performed according to the dideoxy chain termination method [10] using [^{35}S]dATP and Sequenase (USB); in some cases, overlaps were determined by using internal oligonucleotide primers. Anti-Yta10p antiserum was obtained from a Chinchilla bastard rabbit after injection of a synthetic oligopeptide containing the 11 carboxy-terminal amino acids of Yta10p, coupled to ovalbumin. Recombinant DNA techniques followed standard protocols [11]. The GCG software package (version 7.2) [12] was used for sequence analysis and searches in public data bases.

2.2. Disruption of YTA10 and complementation

For one-step gene replacement, an intermediate plasmid (pYTA10 :: URA3) was constructed by substituting the 320 bp *Bgl*II fragment in pYTA10 (a plasmid containing the 2.9 kb *Pst*I segment from α231) by the 1170 bp *Bgl*II fragment from pFL39, containing the *URA3* marker. After transformation of W303D cells with the 2.6 kb *Nru*I/*Bam*HI fragment from pYTA10 :: URA3, transformants containing a disruption of one *YTA10* allele were selected on SC medium (minus uracil).The mutant strain, MAY20 (*YTA10/yta10 :: URA3*), was sporulated and the segregants of 10 tetrads were analyzed for growth on different media. Complementation of MAY20 was done by transformation with cosmid α231 to yield MAY20 [α231] (YTA10/*yta10 :: URA3*[*YTA10 HIS3*]), followed by sporulation, tetrade dissection and growth on selective media. All genotypes were confirmed through diagnostic hybridizations. Yeast cell growth conditions, sporulation, and tetrad dissection were according to [13]. Transformation of yeast cells was performed by the lithium acetate procedure [14].

2.3. Analysis of respiratory complexes

Activity of NADH:cytochrome *c* oxidoreductase (complex I–III)

SSDI 0014-5793(94)01045-5

was determined spectrophotometrically in isolated mitochondria according to [15]. Activity of ferrocytochrome *c*:oxygen oxidoreductase (complex IV) in isolated mitochondria was assayed by the procedures as described in [16,17].

2.4. Subfractionation of yeast cells

Yeast mitochondria were prepared according to [18] and further purified by centrifugation on a Nycodenz step gradient [19]. Preparation of microsomal and cytosolic protein fractions followed the procedure in [20,21]. Proteins from the various fractions were separated by SDS-PAGE [22] and identified by immunoblotting [23] using antibodies coupled to horse raddish peroxidase for detection by chemiluminescence (ECL system, Amersham). Markers for the subcellular fractions were AAC (ADP/ATP carrier protein) for mitochondria; Kar2p (BiP chaperone protein) for microsomes; and FBPase (fructose-1,6-bisphosphatase) for the cytosolic fraction. Polyclonal antibodies raised against AAC and FBPase were used. Kar2p was detected by the use of a monoclonal antibody raised against the ER rentention signal HDEL.

2.5. Synthesis of labeled Yta10 protein and import in vitro

5′ GCG GAA TTC ATG ATG ATG TGG CAA C 3′ (*Eco*RI site plus the initial 16 bases of the *YTA10* sequence, 639 through 654 in Fig. 1) and 5′ GCG AAG CTT AAT TTG TTG CTG CAG 3′ (15 terminal bases of the *YTA10* sequence, 2908 through 2922 in Fig. 1, plus a *Hin*dIII site) were used as forward and reverse primers in a PCR reaction. The resulting DNA fragment, EH1, was cloned into pGEM4 (Promega) to yield pTEH1.

Radiolabeled Yta10p precursor protein was synthesized in rabbit reticulocyte lysate (Promega) and incubated with isolated mitochondria for 20 min at 25°C as described in detail elsewhere [9]. After the import reaction, samples were treated with proteinase K (15 min at 0°C). Dissipation of the membrane potential was achieved by the addition of antimycin A, oligomycin, and valinomycin (final concentrations 8, 20, and 0.5 μM, respectively). For control, 40% of the precursor protein added to imports was loaded. To exclude an aggregation artefact, mitochondria were reisolated after import, resuspended in 1% Triton X-100, chilled on ice (10 min) and treated with proteinase K (not shown). Proteinase treatment was stopped by adding PMSF at 1 mM final concentration.

3. Results

3.1. YTA10 *and its putative gene product, Yta10p*

The nucleotide sequence of *YTA10* as determined from cosmid α231 [1] is presented in Fig. 1, together with the deduced amino acid sequence of Yta10p. The translation product of the open reading frame of 761 codons (starting at pos. 639) has a predicted molecular mass of 84.5 kDa. Southern hybridizations indicate that *YTA10* is a singular yeast gene (not shown). The 5′ flanking region of *YTA10* carries a potential TATA box (pos. 464), preceded by consensus sequences for the regulatory DNA-binding proteins Abf1p anf Gfr2p (pos. 319 and 351, respectively). In addition to the features defined for members of the Yta family [1], Yta10p is characterized by the presence of a potential mitochondrial target sequence at the amino terminus [24], two putative transmembrane segments (pos. 116–141 and 222–245 of the amino acid sequence) [25], and a protein signature (pos. 555, VAYHEAGHAV) found in a number of (neutral) zinc metalloproteases [26].

3.2. Disruption of YTA10, phenotype, and complementation

To investigate whether *YTA10* is an essential gene, one of the alleles in the diploid W303D was disrupted by the one step gene replacement procedure (Fig. 2), heterozygous cells [MAY20 (*YTA10/yta10::ura3*)] were sporulated and the segregants analyzed for growth on fermentable and non-fermentable carbon sources. The presence of a disrupted and a wild-type copy of the gene was confirmed by Southern blot analysis (not shown).

```
GCGGCATTTTTAGTCGTGGCCCGCGATATGCTTCTGGCATCTTTATAACGCCTTTATACG  480
CATCTGTTCAGTTTATTCTGGGCCTATGATTTAGATGAGGAATAAATTGGTTCTTACTGT  540
CTATACATAACTTTTACAAGGACCCATTCTATATTAAAGGAGATTAACGCAGTCTTCAAA  600
TAAAGGCATCATCCACTTATTTTATAATATACTAAGTGATGATGATGTGGCAACGATATG  660
                                          M  M  M  W  Q  R  Y  A    8
CAAGGGGTGCGCCACGCTCATTGACATCACTCTCATTTGGTAAAGCTAGCCGCATATCAA  720
  R  G  A  P  R  S  L  T  S  L  S  F  G  K  A  S  R  I  S  T   28
CAGTGAAGCCAGTGCTTCGCTCGGGCATGCCAGTTCACCAGCGTTTGCAGACGTTATCTG  780
  V  K  P  V  L  R  S  R  M  P  V  H  Q  R  L  Q  T  L  S  G   48
GTCTGGCAACCAGAAACACAATACACGTTCTACCCAAATACGTTCCTTCCATATCTCAT  840
  L  A  T  R  N  T  I  H  R  S  T  Q  I  R  S  F  H  I  S  W   68
GGACAAGGTTAAATGAAAATAGGCCAAACAAAGAGGGTGAGGGCAAGAACAATGGTAATA  900
  T  R  L  N  E  N  R  P  N  K  E  G  E  G  K  N  N  G  N  K   88
AAGATAATAATAGCAATAAAGAAGATGGCAAAGACAAAAGAAATGAGTTTGGTTCATTAT  960
  D  N  N  S  N  K  E  D  G  K  D  K  R  N  E  F  G  S  L  S  108
CAGAATACTTCAGATCTAAGGAATTTGCTAATACGATGTTTTTGACCATCGGATTCACAA 1020
  E  Y  F  R  S  K  E  F  A  N  T  M  F  L  T  I  G  F  T  I  128
TTATATTTACTTTGCTCACACCTTCCAGTAACAACTCAGGAGACGACTCTAACCGCGTCT 1080
  I  F  T  L  L  T  P  S  S  N  N  S  G  D  D  S  N  R  V  L  148
TGACTTTCCAGGATTTCAAAACAAAATACCTGGAGAAGGGTCTTGTGTCCAAGATTTACG 1140
  T  F  Q  D  F  K  T  K  Y  L  E  K  G  L  V  S  K  I  Y  V  168
TCGTTAATAAGTTTCTCGTAGAGGCCGAATTAGTCAATACAAAGCAAGTTGTATCGTTCA 1200
  V  N  K  F  L  V  E  A  E  L  V  N  T  K  Q  V  V  S  F  T  188
CCATTGGTTCAGTAGATATTTTTGAGGAACAGATGGACCAGATCCAGGACCTCTTGAACA 1260
  I  G  S  V  D  I  F  E  E  Q  M  D  Q  I  Q  D  L  L  N  I  208
TTCCTCCTCGGGATCGTATCCCCATCAAATACATTGAGAGATCTTCTCCCTTCACATTTT 1320
  P  P  R  D  R  I  P  I  K  Y  I  E  R  S  S  P  F  T  F  L  228
TGTTCCCCTTCCTGCCCACCATCATTCTGCTTGGTGGCCTTTACTTCATAACCAGAAAAA 1380
  F  P  F  L  P  T  I  I  L  L  G  G  L  Y  F  I  T  R  K  I  248
TAAATAGTTCACCACCAAATGCCAATGGTGGTGGTGGAGGAGGCCTCGGCGGCATGTTTA 1440
  N  S  S  P  P  N  A  N  G  G  G  G  G  G  L  G  G  M  F  N  268
ATGTTGGAAAATCCAGAGCAAAGCTCTTCAATAAGGAAACAGACATTAAAATTTCATTTA 1500
  V  G  K  S  R  A  K  L  F  N  K  E  T  D  I  K  I  S  F  K  288
AAAATGTTGCCGGTTGTGATGAAGCTAAACAGGAAATCATGGAATTTGTTCACTTTTTGA 1560
  N  V  A  G  C  D  E  A  K  Q  E  I  M  E  F  V  H  F  L  K  308
AGAACCCAGGTAAGTACACTAAATTGGGTGCCAAGATTCCACGAGGCGCTATTCTTTCTG 1620
  N  P  G  K  Y  T  K  L  G  A  K  I  P  R  G  A  I  L  S  G  328
GACCCCCAGGTACCGGTAAGACTCTCTTGGCCAAGGCCACCGCAGGTGAGGCGAACGTGC 1680
  P  P  G  T  G  K  T  L  L  A  K  A  T  A  G  E  A  N  V  P  348
CCTTCTTGTCAGTATCAGGTTCTGAGTTCGTCGAAATGTTCGTTGGGGTGGGTGCTTCAC 1740
  F  L  S  V  S  G  S  E  F  V  E  M  F  V  G  V  G  A  S  R  368
GTGTAAGAGACTTGTTTACTCAAGCAAGGTCTATGGCCCCCTCGATTATCTTTATAGATG 1800
  V  R  D  L  F  T  Q  A  R  S  M  A  P  S  I  I  F  I  D  E  388
AAATTGACGCTATCGGTAAAGAAAGAGGCAAAGGCGGCGCTCTCGGTGGCGCTAACGATG 1860
  I  D  A  I  G  K  E  R  G  K  G  G  A  L  G  G  A  N  D  E  408
AAAGAGAAGCTACGCTGAATCAATTATTGGTGGAAATGGACGGATTCACTACTTCCGACC 1920
  R  E  A  T  L  N  Q  L  L  V  E  M  D  G  F  T  T  S  D  Q  428
AAGTCGTAGTCCTTGCGGGTACAAATAGGCCCGATGTGTTGGATAATGCTTTGATGAGAC 1980
  V  V  V  L  A  G  T  N  R  P  D  V  L  D  N  A  L  M  R  P  448
CGGGAAGGTTCGATAGACATATCCAAATTGATTCTCCTGATGTCAATGGTAGGCAGCAAA 2040
  G  R  F  D  R  H  I  Q  I  D  S  P  D  V  N  G  R  Q  Q  I  468
TATACCTGGTTCACTTGAAAAGACTGAATCTGGATCCCCTTCTTACAGATGATATGAATA 2100
  Y  L  V  H  L  K  R  L  N  L  D  P  L  L  T  D  D  M  N  N  488
ATCTTTCTGGGAAATTGGCTACGCTTACTCCAGGTTTTACTGGTGCAGATATCGCTAATG 2160
  L  S  G  K  L  A  T  L  T  P  G  F  T  G  A  D  I  A  N  A  508
CTTGTAATGAGGCTGCATTAATCGCTGCCAGACATAATGACCCATATATCACTATCCATC 2220
  C  N  E  A  A  L  I  A  A  R  H  N  D  P  Y  I  T  I  H  H  528
ACTTTGAGCAAGCCATTGAAAGAGTCATTGCGGGACTAGAGAAGAAAACAAGAGTCCTTT 2280
  F  E  Q  A  I  E  R  V  I  A  G  L  E  K  K  T  R  V  L  S  548
CTAAGGAAGAAAAAAGGTCAGTGGCCTATCATGAGGCAGGACATGCCGTTTGTGGTTGGT 2340
  K  E  E  K  R  S  V  A  Y  H  E  A  G  H  A  V  C  G  W  F  568
TTCTAAAATATGCGGATCCACTTCTGAAAGTAAGCATCATCCCGCGGTGGACAAGGTGCTT 2400
  L  K  Y  A  D  P  L  L  K  V  S  I  I  P  R  G  Q  G  A  L  588
TAGGCTATGCCCAGTACCTACCACCGGATCAATATTTGATATCTGAGGAGCAATTCAGAC 2460
  G  Y  A  Q  Y  L  P  P  D  Q  Y  L  I  S  E  E  Q  F  R  H  608
ATAGAATGATCATGGCTCTTGGTGGCCGTGTTTCTGAGGAGCTACATTTTCCATCGGTGA 2520
  R  M  I  M  A  L  G  G  R  V  S  E  E  L  H  F  P  S  V  T  628
CTAGCGGTGCTCATGATGATTTCAAAAAGGTTACACAGATGGCAAATGCCATGGTTACAT 2580
  S  G  A  H  D  D  F  K  K  V  T  Q  M  A  N  A  M  V  T  S  648
CCCTAGGAATGTCACCCAAGATTGGCTACCTGTCGTTTGATCAGAATGATGGAAACTTCA 2640
  L  G  M  S  P  K  I  G  Y  L  S  F  D  Q  N  D  G  N  F  K  668
AAGTTAACAAACCCTTCAGTAATAAAACGGCAAGAACCATTGATTTAGAAGTTAAATCTA 2700
  V  N  K  P  F  S  N  K  T  A  R  T  I  D  L  E  V  K  S  I  688
TAGTAGATGATGCACACCGAGCTTGTACAGAATTGCTAACCAAAAATTTGGACAAAGTCG 2760
  V  D  D  A  H  R  A  C  T  E  L  L  T  K  N  L  D  K  V  D  708
ATCTTGTCGCAAAAGAATTGCTACGTAAGGAAGCAATTACTAGAGAAGACATGATTAGAT 2820
  L  V  A  K  E  L  L  R  K  E  A  I  T  R  E  D  M  I  R  L  728
TATTGGGTCCAAGGCCATTCAAGGAAAGGAACGAGGCTTTTGAAAAATATTTCGGATCCAA 2880
  L  G  P  R  P  F  K  E  R  N  E  A  F  E  K  Y  L  D  P  K  748
AGAGCAATACCGAACCGCCTGAGGCACCTGCAGCAACAAATTAAAGGGGAAATAAAACGG 2940
  S  N  T  E  P  P  E  A  P  A  A  T  N                      761
TGGCTTCTTCATCTCACTTTATATCTTTACCTGATCCCCCTTCCAGGATCCTATCTTATC 3000
TTCTCATTCTTTTAGTAATAATATACATGTATATATATATATTGATAACGAGTAATAAAA 3060
AAAATAAAAAAAAAATAATACATATTCGAAAACAATACTGCTTTTTAGTTCTCAACTTAAA 3120
```

Fig. 1. Nucleotide sequence of *YTA10* and predicted amino acid sequence. Two putative transmembrane spans are underlined. The specialized ATPase A and B boxes are indicated by bold print. The putative Zn-binding sequence is marked by bold italic letters. The complete sequence (3396 bp) can be retrieved from EMBL Data Library (acc. no. X-81066).

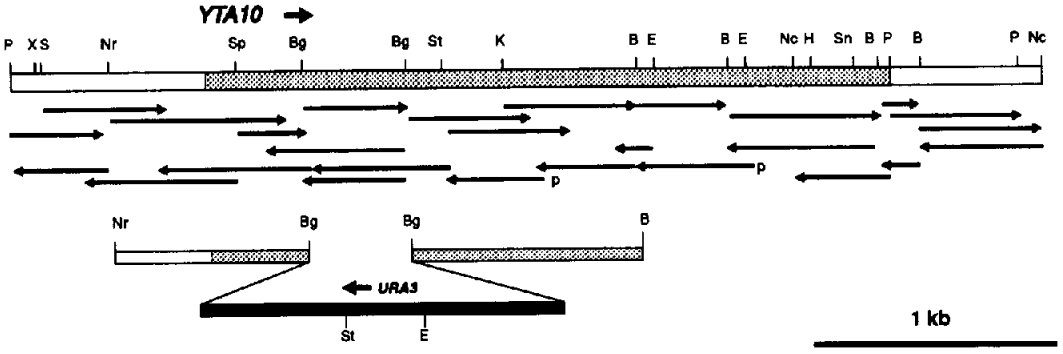

Fig. 2. Sequencing and gene disruption strategy of *YTA10*.

As examplified in Fig. 3A (left panel), the tetrads showed a 2:2 segregation into small and large colonies growing in YPD medium at 30°C. As expected, the slow growth phenotype cosegregated with the *YTA10* disruption and the uracil prototrophy (Fig. 3A, middle panel). On glycerol medium, a non-fermentable carbon source, the *URA*$^+$spores were inviable confirming the petite phenotype (Fig. 3A, right panel).

Transformation of *yta10*$^-$ cells [MAY20 (YTA10/*yta10::URA3*)] with cosmid α231 restored wild-type and respiration competence completely (Fig. 3B). Note that pYc3030 cosmids can be used instead of single- copy *CEN* plasmids in transformation experiments, thus not impairing growth like multi-copy *ARS* plasmids [27].

To examine whether the deletion of *YTA10* might affect components of the respiratory chain, we determined the activities of NADH:cytochrome *c* oxidoreductase (complexes I–III) and ferrocytochrome *c*:oxygen oxidoreductase (complex IV) in isolated mitochondria. Fig. 3C shows that mitochondria from the *yta10* mutant retain only negligible residual activities in these complexes compared to wild-type mitochondria.

3.3. *Subcellular location and import of Yta10p into yeast mitochondria*

Next, we used two approaches to analyze the subcellular location of Yta10p. First, antibodies were raised against the 11 carboxy-terminal amino acids of Yta10p and used for immunoblotting of yeast subcellular fractions. As shown in Fig. 4A, Yta10p was found exclusively in the mitochondrial fraction. In the second approach, radiolabeled Yta10p was synthesized in vitro and was incubated with isolated yeast mitochondria [9,28]. A major translation product with the apparent molecular mass of 84.5 kDa was observed and was imported into a protease-protected location. Import was dependent upon a membrane potential and was accompanied by processing to a protein of approximately 73 kDa (Fig. 4B).

4. Discussion

Among the Yta proteins, a novel family of putative ATPases from the yeast, *S. cerevisiae*, characterized thus far [1], Yta10p exhibits most significant similarity to two other Yta proteins, Yta11p and Yta12p, and *E. coli* FtsH/HflB. Studies with FtsH mutants indicated that this protein is involved in diverse cellular processes, like cell growth and viability [6,29], assembly into and through the bacterial membrane [6,30,31], and, directly or indirectly, in a proteolytic pathway [8]. Following our proposition that Yta10p may be a mitochondrial homologue of FtsH/HflB, we have carried out genetic and biochemical experiments to elucidate the function of Yta10p. In the present study we have shown that Yta10p is indeed a mitochondrial protein, which is essential for respiration-dependent growth of the yeast cells.

When aligned for similarity, the N-terminal part of Yta10p is some 90 amino acids longer than that of FtsH. This extension of Yta10p relative to FtsH shows several features which are characteristic of mitochondrial import signals [24] and which are absent in the prokaryotic protein. First, the N-terminal region of Yta10p reveals a potential amphipathic a-helical structure. Second, up to residue 72, the amino acid sequence of Yta10p is devoid of acidic residues but contains several basic and hydroxy amino acids; a typical mitochondrial matrix processing signal [29] occurs at positions 70 through 72. Our results are in complete accordance with these notions. We found that Yta10p is imported into mitochondria dependent upon a membrane potential accompanied by processing to a protein of approximately 73 kDa. Determination of the exact length of the signal peptide and hence the actual length of the mature protein have to await further experiments. Experimental evidence has been obtained for a membrane topology of FtsH, in which the N-terminal part of the protein spans the membrane twice [7]. Like FtsH, Yta10p reveals two potential membrane spans,

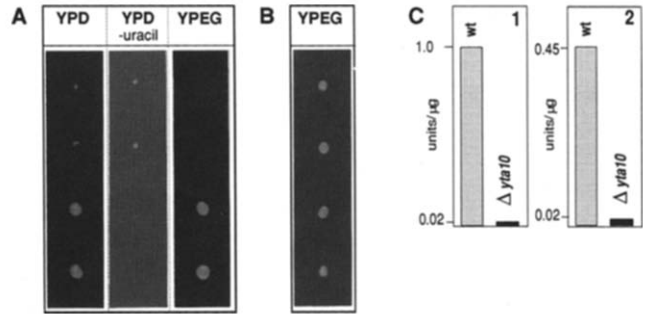

Fig. 3. Disruption of *YTA10*. (A) Replica-plate analysis of a single dissected tetrad from MAY20. Spores from a dissected tetrad were grown on a fresh YPD plate (left panel) and replica-plated onto YPD (minus uracil) (middle panel) and YPEG (right panel), respectively. (B) Complementation. After sporulation of MAY20 [a231], tetrads were dissected, grown on YPD (minus histidine) at 30°C (not shown) and replica-plated onto YPEG. (C) Activity of mitochondrial respiratory complexes from wild-type and mutant cells. Panel 1, activity of NADH:cytochrome *c* oxidoreductase (complex I–III); panel 2, activity of ferrocytochrome *c*:oxygen oxidoreductase (complex IV). p, Yta10p precursor; m, mature protein.

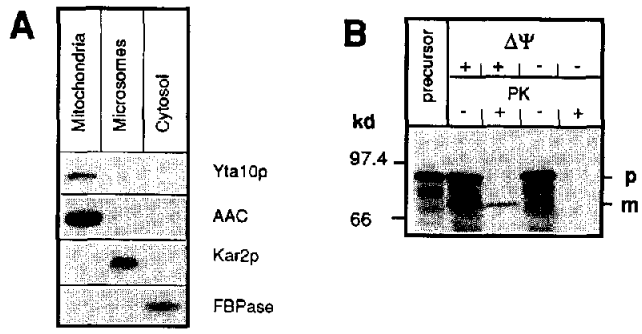

Fig. 4. (A) Subcellular localization of Yta10p. After SDS-PAGE, the markers were detected by immunoblotting. (B) Import of in vitro-synthesized Yta10p precursor protein into isolated yeast mitochondria. Where indicated (PK), samples were treated with proteinase K after the import reaction; DY, intact (+) or dissipated (−) membrane potential. p, precursor Yta10p; m, mature Yta10p.

which have a similar spacing in these two proteins. Experiments complementary to those reported here indicate that Yta10p adopts an analogous topology within the inner mitochondrial membrane [9].

Our experiments have shown that the loss of *YTA10* affects the ability of yeast cells to grow on non-fermentable carbon sources by impairing the activities of the respiratory complexes. As shown for many nuclear *petite* mutants, defects in diverse functions can cause the loss of respiratory capacity [33]. Anticipating that Yta10p fulfills similar functions in mitochondria as does FtsH in the bacterial cell, we favour the possibility that Yta10p is involved, directly or indirectly, in the assembly or maintenance of respiratory complexes. Experiments addressing this issue in more detail are reported in the accompanying paper [9].

Yta10p adds to the group of putative ATPases which are essential for mitochondrial function and have homologues in bacterial systems, such as the yeast Pim1 protein which is a mitochondrial ATP-dependent protease exhibiting over 30% identity with ATP-dependent protease La from *E. coli*, lon from *B. brevis*, and one from *M. xanthus* [34,35]. Additional members of the ATPase family found to be associated with yeast mitochondria but probably different in function, are Yme1p (identical to Yta11p), involved in maintaining the integrity of mitochondria [5] and Msp1p (identical to Yta4p), involved in intramitochondrial protein sorting [36]. It will be interesting to investigate whether all or particular of these mitochondrial proteins may act in concert to guarantee proper mitochondrial function.

Acknowledgements: The expert technical assistance of C. Schwarzlose and I. Vetter is gratefully acknowledged. We would like to thank W. Neupert for discussion and advice. The anti-FBPase antibody was a generous gift from D. Wolf (Stuttgart), the anti-Kar2p antibody was kindly provided by M. Lewis (Cambridge, UK). The Deutsche Forschungsgemeinschaft (SFB190 and SFB184, München) and Fonds der Chemischen Industrie have supported this work.

References

[1] Schnall, R., Mannhaupt, G., Stucka, R., Tauer, R., Ehnle, S., Schwarzlose, C., Vetter, I. and Feldmann, H. (1994) Yeast 10, 1141–1155.
[2] Mian, I.S. (1993) Trends Biochem. Sci. 18, 125–127.
[3] Kunau, W.H., Beyer, A., Franken, T. Gotte, K., Marzioch, M., Saidowsky, J., Skaletz-Rorowski, A. and Wiebel, F.F. (1993) Biochimie 75, 209–224.
[4] Ghislain, M., Udvardy, A. and Mann, C. (1993) Nature 366, 358–361.
[5] Thorsness, P.E., White, K.H. and Fox, T.D. (1993) Mol. Cell. Biol. 13, 5418–5426.
[6] Tomoyasu, T., Yuki, T., Morimura, S., Yamanaka, K., Niki, H., Hiraga, S. and Ogura, T. (1993) J. Bacteriol. 175, 1344–1351.
[7] Tomoyasu, T., Yamanaka, K.,Yuki, T., Murata, K., Suzaki, T., Bouloc,P., Kato, A., Niki, H., Hiraga, S. and Ogura, T. (1993) J. Bacteriol. 175, 1344–1351.
[8] Herman, C., Ogura, T., Tomoyasu, T., Hiraga, S., Akiyama, Y., Ito, K., Thomas, R., D'Ari, R. and Bouloc, P. (1993) Proc. Natl. Acad. Sci. USA 90, 10861–10865.
[9] Pajic, A., Tauer, R., Feldmann, H., Langer, T. and Neupert, W. (1994) FEBS Lett.
[10] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[11] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, a Laboratory Manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, NY.
[12] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.
[13] Sherman, F., Fink, G.R. and Hicks, J.B. (1982) Methods in Yeast Genetics, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
[14] Ito, B., Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol. 153, 163–168.
[15] Hatefi, Y. and Stiggal, D.L. (1978) in: Methods in Enzymology, Vol. 53 (Fleischer, S. and Packer, L., Eds.) pp. 5–10, Academic Press, NY.
[16] Smith, L. (1954) Arch. Biochem. Biophys. 50, 285.
[17] Errede, B., Kamen, D. and Hatefi, Y. (1978) in: Methods in Enzymology, Vol. 53 (Fleischer, S. and Packer, L., Eds.) pp. 40–47, Academic Press, NY.
[18] Daum, G. and Böhni, B.C. (1982) J. Biol. Chem. 257, 13028–13033.
[19] Lewin, A., Hines, V. and Small, G.M. (1990) Mol. Cell. Biol. 10, 1399–1405.
[20] Caplan, A.J. and Douglas, M.G. (1991) J. Cell Biol. 114, 609–621.
[21] Hansen, W., Garcia, P.D. and Walter, P. (1986) Cell 45, 397–406.
[22] Laemmli, U.K. (1970) Nature 227, 680–685.
[23] Schwarz, E., Seytter, T., Guiard, B. and Neupert, W. (1993) EMBO J. 12, 2295–2302.
[24] Hartl, F.U., Pfanner, N., Nicholson, D.W. and Neupert, W. (1989) Biochim. Biophys. Acta 988, 1–45.
[25] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[26] Bairoch, A. (1989) PROSITE: A Dictionary of Protein Sites and Patterns, in EMBL Biocomputing Technical Document 4, EMBL, Heidelberg, Germany.
[27] Stucka, R. and Feldmann, H. (1994) in: Molecular Genetics of Yeast - A Practical Approach (Johnston, J., Ed.) pp. 49–64, Oxford Univ. Press, Oxford.
[28] Glick, B.S. (1991) in: Methods in Cell Biology, Vol. 34 (Tartakoff, A.M., Ed.) pp. 389–399, Academic Press, NY.
[29] Ogura, T., Tomoyasu, T., Yuki,T., Morimura, S., Begg, K.J., Donachie, W.D., Mori, H., Niki, H. and Hiraga, S. (1991) Res. Microbiol. 142, 279–282.
[30] Akiyama, Y., Shirai, Y. and Ito, K. (1994) J. Biol. Chem. 269, 5225–5229.
[31] Akiyama, Y., Ogura, T. and Ito, K. (1994) J. Biol. Chem. 269, 5218–5224.
[32] Arretz, M., Schneider, H., Wienhus, U. and Neupert, W. (1991) Biomed. Biochim. Acta 50, 403–412.
[33] Tzagoloff, A. and Dieckmann, C.L. (1990) Microbiol. Rev. 54, 211–225.
[34] Van Dyck, L., Pearce, D.A. and Sherman, F. (1994) J. Biol. Chem. 269, 238–242.
[35] Suzuki, C., Suda, K., Wang, N. and Schätz, G. (1994) Science 264, 273–276.
[36] Nakai, M., Endo, T., Hase, T. and Matsubara, H. (1993) J. Biol. Chem. 268, 24262–24269.