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Biochimica et Biophysica Acta 1757 (2006) 631–638

BIOCHIMICA ET BIOPHYSICA ACTA

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MMI1 (*YKL056c*, *TMA19*), the yeast orthologue of the translationally controlled tumor protein (TCTP) has apoptotic functions and interacts with both microtubules and mitochondria

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Received 17 February 2006; received in revised form 11 April 2006; accepted 13 May 2006
Available online 20 May 2006

Abstract

The yeast orthologue of mammalian TCTP is here proposed to be named Mmi1p (*m*icrotubule and *m*itochondria *i*nteracting protein). This protein displays about 50% amino acid sequence identity with its most distantly related orthologs in higher organisms and therefore probably belongs to a small class of yeast proteins which have housekeeping but so far incompletely known functions needed for every eukaryotic cell. Previous investigations of the protein in both higher cells and yeast revealed that it is highly expressed during active growth, but transcriptionally down-regulated in several kinds of stress situations including starvation stress. In human cells, TCTP presumably has anti-apoptotic functions as it binds to Bcl-X_L *in vivo*. TCTP of higher cells was also shown to interact with the translational machinery. It has acquired an additional function in the mammalian immune system, as it is identical with the histamine releasing factor. Here, we show that in *S. cerevisiae* induction of apoptosis by mild oxidative stress, replicative ageing or mutation of *cdc48* leads to translocation of Mmi1p from the cytoplasm to the mitochondria. Mmi1p is stably but reversibly attached to the outer surface of the mitochondria and can be removed by digestion with proteinase K. Glutathionylation of Mmi1p, which is also induced by oxidants, is not a prerequisite or signal for translocation as shown by replacing the only cysteine of Mmi1p by serine. Mmi1p probably interacts with yeast microtubules as deletion of the gene confers sensitivity to benomyl. Conversely, the deletion mutant displays resistance to hydrogen peroxide stress and shows a small but significant elongation of the mother cell-specific lifespan. Our results so far indicate that Mmi1p is one of the few proteins establishing a functional link between microtubules and mitochondria which may be needed for correct localization of mitochondria during cell division.

Keywords: TCTP; Mitochondria; Microtubules; Apoptosis; Oxidative stress; Ageing, heat shock

1. Introduction

TCTP is a small (19.1 kDa), acidic (pI:4.5) evolutionarily highly conserved protein [1]. It was originally discovered in human tumor cells and therefore called TCTP (translationally controlled tumor protein) [2]. This small protein as well as its transcript is relatively abundant in growing cells [3,4] but is down-regulated in starvation and stress situations [5,6]. Subsequent research in higher cells revealed several interaction partners of TCTP: it binds to microtubuli [2] and is thereby regulated by phosphorylation by a polo-like kinase [7]. Additionally, it binds to the anti-apoptotic proteins Bcl-X_L and mcl1 [8] and is suspected to exert anti-apoptotic function [9].

TCTP specifically antagonizes the eEF1Bβ-mediated GDP/GTP exchange reaction in the elongation reaction of protein synthesis in higher cells. Thus, TCTP functions as an eEF1Bβ-specific guanine nucleotide dissociation inhibitory factor (GDI) during elongation of translation [10]. Co-regulation of TCTP with ribosomal proteins is indicated both by bioinformatic and by experimental data. Finally, the same TCTP protein also has an extracellular function in the mammalian immune system as a

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doi:10.1016/j.bbabio.2006.05.022

cytokine, namely histamine releasing factor [5,11], which is believed to have been acquired recently in evolution by this remarkable molecule.

The structure of the protein from *S. pombe* is known and displays three domains. An α helical domain was shown to interact with microtubules and to bind calcium ions [5], a mainly beta-structured core domain contains the residues interacting with translation elongation factor subunit eEF1Bβ, and an unstructured loop, most interestingly contains the TCTP signature which structurally defines this new protein family [3].

Here we are proposing the name *MMI1* for the yeast gene indicating interaction of the yeast protein with both microtubules and mitochondria.

We are showing that the yeast protein, Mmi1p, after a mild oxidative stress (and various other stresses) is translocated from its normal cytoplasmic localization to the outer surface of the mitochondria. The same translocation occurs also in old yeast mother cells without external stress, but not in young cells. The deletion of *MMI1* is viable but sensitive to benomyl, a microtubule-destabilizing drug, and resistant to mild oxidative stress (hydrogen peroxide) which inhibits growth of the corresponding wild type. Additionally, the deletion mutant displays a small but significant elongation of the mother cell-specific lifespan.

2. Materials and methods

2.1. Yeast strains, media and culture conditions

S. cerevisiae strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), BY4742 (*Matα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*), JC482 (*Matα ura3-52 leu2 his4-539)* and W303-eA (*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 can1-100*) were used. For experiments with deletion strains we used the EUROSCARF deletion mutant collection. Complex medium (YPD) contained 1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) D-glucose; synthetic complete glucose medium (SC-glucose) contained 2% (w/v) D-glucose, 0.17% (w/v) yeast nitrogen base without amino acids, 0.5% ammonium sulphate and 10 ml complete dropout (0.2% Arg, 0.1% His, 0.6% Ile, 0.6% Leu, 0.4% Lys, 0.1% Met, 0.6% Phe, 0.5% Thr, 0.4% Trp, 0.1% Ade, 0.4% Ura, 0.5% Tyr). Agar plates were made by adding 2% (w/v) agar to the media. To select for auxotrophies the respective amino acid was left out from the dropout media [12].

S. cerevisiae apoptotic *cdc48* mutant strain (KFY437) and the corresponding wild-type (KFY417) [13] were used in proteomics experiments. Cells were grown at least for 36 h at 28°C in baffled flasks on YPGal (1% yeast extract, 2% bacto peptone) containing 4% galactose. Pre-cultures were harvested and washed twice in YPGlc (1% yeast extract, 2% bacto peptone, 4% glucose). Following resuspension, YPGlc cultures were inoculated at a ratio of 1:3 wild-type (KFY417, OD_{600}=0.1) to mutant (KFY437, OD_{600}=0.3) cells, in order to ensure comparable cell density after growth. For induction of apoptosis, cells were grown for 16 h at 28 °C in baffled flasks and then stressed for four additional hours with heat shock at 37 °C.

2.2. DNA manipulation and cloning techniques

In this study we used the centromeric vector pUG35 [14] and the low copy plasmid p416GPD [15]. Additionally, we obtained pHISparallel2 plasmid [16] carrying hTPT1 insert from Birgit Simon-Nobbe. The pUG37 was constructed by deletion of the eGFP sequence from pUG35. Further information is found in Table 1.

2.3. Bax-expression

Yeast cells co-transformed with pUG35-*MMI1* and Bax-containing YEp51 were inoculated into synthetic liquid medium and grown to exponential phase. Cells were harvested, washed with sterile water and were transferred to minimal medium containing 3% galactose to induce Bax expression.

2.4. Cell fractionation

Mitochondria for 2-DE analysis were isolated by differential centrifugation as described in Herrmann et al. [17], except that BSA was omitted in the buffers since it may interfere with 2-DE analysis. Mitochondria for localization experiments were isolated according to Rickwood et al. [18]. If

Table 1
Plasmids constructed and used in this study

Name	Primers	Cloning sites	Source	Target
pUG35-*MMI1*	5′-CGCGGATCCATGATTATTTACAAGGATA-3′ 5′-CCGGAATTCGATCTTTTCTTCCACAATA-3′	*Bam*HI *Eco*RI	Genomic yeast DNA	pUG35
pUG35-*hTPT1*	5′-GCTCTAGAATGATTATCTACCGGGA-3′ 5′-CGGGATCCACATTTTTCCATTTCTA-3′	*Xba*I *Bam*HI	Human cDNA	pUG35
pUG35-*SSA2*	5′-GCTCTAGAATACAATGTCTAAAGCTGTCG-3′ 5′-CGGGATCCATCAACTTCTTCGACAGTTG-3′	*Xba*I *Bam*HI	Genomic yeast DNA	pUG35
pUG35-*PRE6*	5′-GCTCTAGAGTTCATCAGGATCAGTTGCA-3′ 5′-CCATCGATATGGTTAGATTTTTTCTTTT-3′	*Xba*I *Cla*I	Genomic yeast DNA	pUG35
pUG35-*HSPA8*	5′-GCTCTAGAATGTCCAAGGGACCTGCAG-3′ 5′-CGGGATCCATCAACCTCTTCAATGG-3′	*Xba*I *Bam*HI	Human cDNA	pUG35
pUG35-*YLR022C*	5′-TAGACTAGTATGCCTATCAATCAACCGT-3′ 5′-TAGGAATTCGTTATGCGTTGTATTATCTAT-3′	*Eco*RI *Spe*I	Genomic yeast DNA	pUG35
pUG35-*ACO1*	5′-CGGGATCCATAGATATACAATGCTGTC-3′ 5′-CGGAATTCTTTCTTCTCATCGGCCTT-3′	*Bam*HI *Eco*RI	Genomic yeast DNA	pUG35
pUG35-*MMI*^{cyc32ser}	5′-TCTACGAAGCCGACTCTGCTATGGTCAATGTCG-3′ 5′-CGACATTGACCATAGCAGAGTCGGCTTCGTAGA-3′	*Dpn*I	pug35-*YKL05Cc*	pUG35
pUG37-*MMI1*(cognate)	5′-GGATCCATGATTATTTACAAGGATAT-3′ 5′-CCATACCATGGTTAGATCTTTTCTTCCACAA-3′	*Bam*HI Van91I	Genomic yeast DNA	pUG37
p416GPD-hTPT1		*Bam*HI *Sal*I	pHISparallel2-hTPT1	P416GPD

In case of PCR-amplified inserts we used Pfu Polymerase (Fermentas, Burlington, Ontario, Canada). For creating the exchange of cysteine to serine we used the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). All restriction enzymes were provided by Promega (Mannheim, Germany). All constructs were sequenced by a commercially available service provider (MWG Biotech AG, Ebersdorf, Germany).

needed, isolated mitochondria were digested with 1 mg/ml Proteinase K (AppliChem, Darmstadt, Germany).

2.5. Preparation of old yeast cells

Preparation of senescent yeast cells, Calcofluor White M2R staining, replicative lifespan determination and lifespan statistics were performed as described in Laun et al. [19].

2.6. Two-dimensional gel electrophoresis (2-DE) and MALDI-TOF mass spectrometry

2-DE was performed according to [20]. Isoelectric focusing (IEF) was done using non-linear immobilized pH gradients (IPG) pH3-10NL (GE Healthcare, Freiburg, Germany) and gradient gels (8–16%T) for SDS-PAGE. 400 μg protein was loaded per gel. Resultant protein patterns were stained with "ruthenium II tris bathophenanthroline disulfonate fluorescent dye" [21]. After staining, gels were equilibrated twice for 10 min in distilled H_2O and digitalized using the Fuji FLA-3000 scanner (Fuji, Düsseldorf, Germany). Prior to mass spectrometry (MS), gels were stained with colloidal Coomassie [22]. Protein spots were subjected to a sequence-dependent protease treatment (100 ng trypsin per gel plug; Promega, Mannheim, Germany), as described by Shevchenko et al. [23]. Resulting peptides were analyzed by peptide mass fingerprinting with the thin layer method [24] using a MALDI-TOF Reflectron (Waters, Eschborn, Germany). Database searches were done in SwissProt using the ProteinLynx Globalserver 1.1 (PLGS, Waters).

2.7. Sensitivity to H_2O_2 and benomyl

Tests for sensitivity to oxidants were performed by spotting cell cultures onto SC-glucose plates containing various concentrations of H_2O_2 (2–4 mM), 50 μM menadione, 1.5 mM diamide, 3.5 mM *tert*-butyl hydrogen peroxide, 30 mM acetic acid and 0.15 mM cumene hydroperoxide and benomyl (10–50 μg/ml). Cells were grown to stationary phase in liquid SC-glucose, serially diluted to OD_{600} values of 3.0; 1.0; 0.3; 0.1 and 10 μl aliquots were spotted onto the appropriate plates. Sensitivity was determined by comparison of growth with that of the wild-type strain after 3 days at 28 °C.

2.8. Structure prediction

The three-dimensional model of Mmi1p was predicted using the online available program 3D-JIGSAW (http://www.bmm.icnet.uk/servers/3djigsaw/). This structure prediction software splits the query sequence into small sequence elements and looks for homologous templates in different sequence databases (Collection of protein multiple sequence alignments and profile hidden Markov models at Sanger Centre/Wash-U/Karolinska Intitutet [PFAM]; Research Collaboratory For Structural Bioinformatics Protein Database [PDB]; Non-redundant database from the National Center for Biotechnology Information [nr]) [25–27].

3. Results and discussion

3.1. Proteomics

During recent years our groups have extensively studied the transcriptome of young and old as well as apoptotic and non-apoptotic yeast cells [28]. In addition, profile gene expression at the proteome level in these cells was started, since for many genes significant differences between the abundance ratio of the messenger RNA transcript and the corresponding protein product were observed [29,30]. Differential 2-DE analysis of mitochondrial fractions of the apoptotic *cdc48*^{S565G} mutant strain compared to the non-apoptotic wild-type strain [13] revealed distinct alterations in the protein pattern (Braun et al., submitted). Differentially expressed proteins were eluted from the gel, digested with trypsin and further characterized by MALDI-TOF (see Materials and methods).

In these experiments the yeast protein Mmi1p, was found to be markedly enriched in the mitochondrial fraction upon induction of apoptosis (Fig. 1) suggesting a potential role of this protein during cell death. Sequence analysis showed that Mmi1p displays high homology to the human *TPT1*/*TCTP* (translationally controlled tumor protein, Fig. 2) which codes for an ubiquitous cell growth-dependent protein of ill-defined primary function [2,3]. The 3D-JIGSAW software was used for *ab initio* modelling of the structure of Mmi1p (see Materials and methods). The resulting predicted structure (Fig. 2C) was strikingly similar to the known structure of *S. pombe* TCTP (Fig. 2B) [3], showing the three domains similar to the ones found in the *S. pombe* structure. The *S. cerevisiae* sequence is 69% identical to the one of *S. pombe* TCTP. In our view this would indicate that not only the amino acid sequence but also the three-dimensional structure of this highly conserved protein has remained relatively constant in evolution.

3.2. Lifespan

The haploid Δ*MMI1* strain was compared to wild-type cells and very little difference in growth on either SC-glucose or SC-glycerol media could be observed (our own unpublished observations). Previously, we could establish a linkage of mother cell-specific ageing and apoptosis in yeast [19]. Therefore we determined the mother cell-specific lifespan of the Δ*MMI1* strain. A small but significant increase of the median lifespan (16% increase of the median; $P<0.02$) was found (our own unpublished observations).

3.3. Oxidative stress resistance

Now, we focused our interest on the analysis of the resistance or sensitivity of the Δ*MMI1* strain to several oxidants. We could

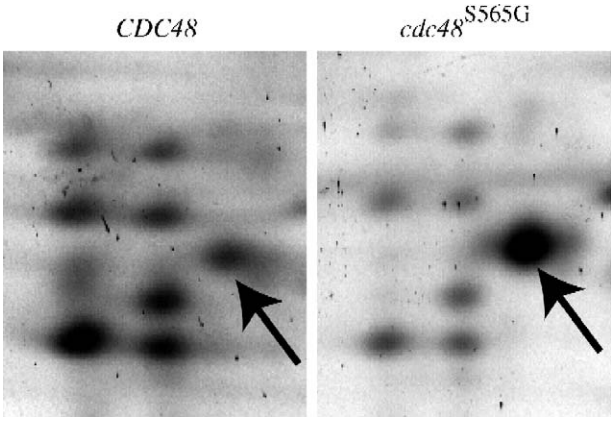

Fig. 1. Mmi1p accumulates in mitochondria isolated from apoptotic yeast cells. Mitochondria from apoptotic *cdc48*^{S565G} mutant (KFY437) and non-apoptotic wild-type strain (KFY417) were isolated by differential centrifugation and analyzed by two-dimensional gel electrophoresis (2-DE analysis). Among the protein spots altered under apoptotic conditions, the protein Mmilp (arrow) was identified by MALDI-TOF mass spectrometry to be markedly enriched under apoptotic conditions.

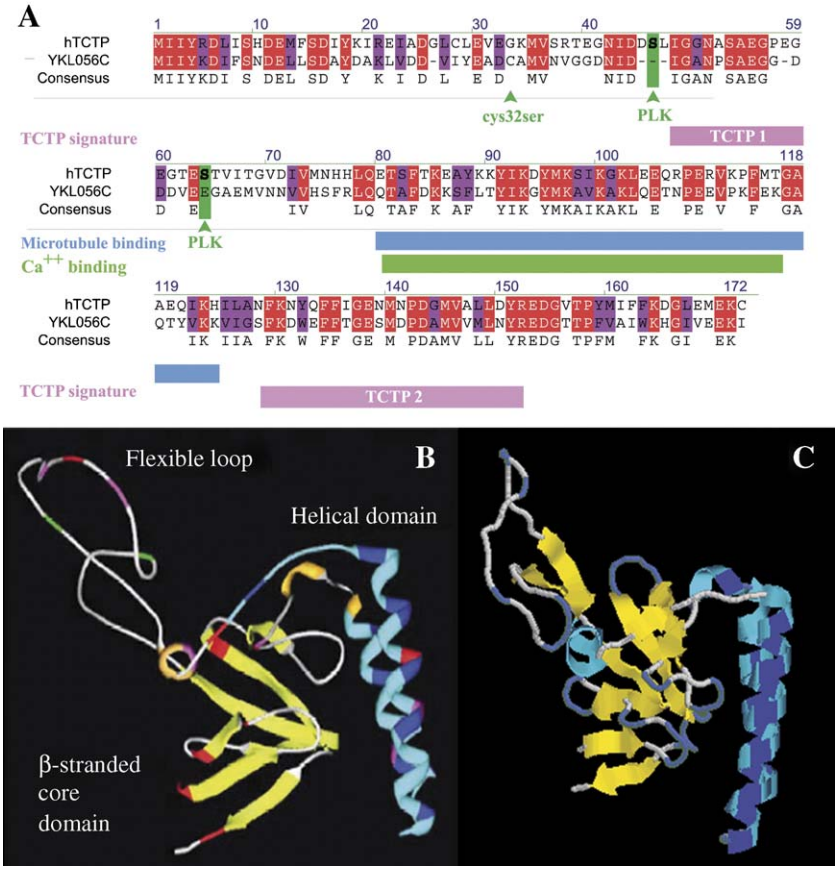

Fig. 2. (A) Amino acid sequence alignment of the *S. cerevisiae* and human TCTP proteins. The two sequences are 41.9% identical. The alpha helical domain (blue) contains the residues shown to interact with microtubules and to bind calcium. The serine residues which are phosphorylated by polo-like kinase are in the flexible loop. The TCTP signature is located in the beta-stranded core domain and in the flexible loop. To investigate the influence of S-thiolation we exchanged the cysteine at position 32 to serine. (B) Structural models for *S. pombe* TCTP (Thaw et al., 2001) and a predicted *S. cerevisiae* Mmi1 structure (C) modeled using 3D-JIGSAW. The color coding is identical to part A.

previously show that mother cell-specific ageing and also apoptosis is strongly influenced by oxidants and other stress stimuli, like heat shock. Spot tests on plates containing different oxidants, showed increased resistance of Δ*MMI1* cells against 3 mM H_2O_2 and increased sensitivity against 50 μM menadione compared to wild-type (Fig. 3). In contrast, the growth is not influenced by 1.5 mM diamide, 3.5 mM *tert*-butyl hydrogen peroxide, 30 mM acetic acid and 0.15 mM cumene hydroperoxide (our own unpublished observations). Additionally, we performed growth curves of wildtype and Δ*MMI1* cells in liquid media containing several different concentrations of H_2O_2. The result confirmed the increased resistance of Δ*MMI1* to H_2O_2 also shown by the spot tests.

3.4. Localization of eGFP-MMI1

We constructed a c-terminal eGFP-tagged fusion protein of Mmi1 to determine its subcellular localization. As expected from the results of Huh et al. [31], fluorescence microscopy of wild-type cells transformed with pUG35-*MMI1*, a plasmid containing the eGFP-tagged Mmi1p showed cytosolic localization. After exposure of the cells to 3 mM H_2O_2, 30 mM acetic acid or heat shock, which are well-known apoptotic stimuli [32,33 and our own unpublished data], the fluorescence signal of the eGFP-fusion protein revealed a mitochondrial pattern (Fig. 4B). No such pattern could be observed when the cells were treated with other chemicals producing oxidative stress like 50 μM menadione, 1.5 mM diamide or 0.15 mM cumene hydroperoxide. After transforming cells containing the eGFP-Mmi1 fusion protein with a Yep51 plasmid expressing murine Bax under control of a *GAL1/10* promotor [34], the localization of the eGFP-tagged Mmi1p was cytosolic in glucose-containing media. After switching to galactose-containing media and thus expression of Bax, the fluorescence signal again showed a mitochondrial pattern (our own unpublished observations). In a control experiment with cells containing a plasmid with only eGFP, as a control, the cells showed cytoplasmatic fluorescence under normal conditions but also under oxidative stress conditions. This shows that the eGFP *per se* is not responsible for the mitochondrial localization of the fluorescence signal. As a second control we randomly chose strains with other eGFP-tagged fusion proteins (*ARA1, ARG1, NIP1,* YLR022C) and treated them like the eGFP-*MMI1*-expressing strain. We could not detect any mitochondrial pattern of the fluorescent signal after stress induction—the localization of the tagged proteins remained cytosolic (Fig. 4A).

In 2003 the group of Chris Grant found that Mmi1 is one out of 15 proteins which are conjugated with glutathione (S-thiolated)

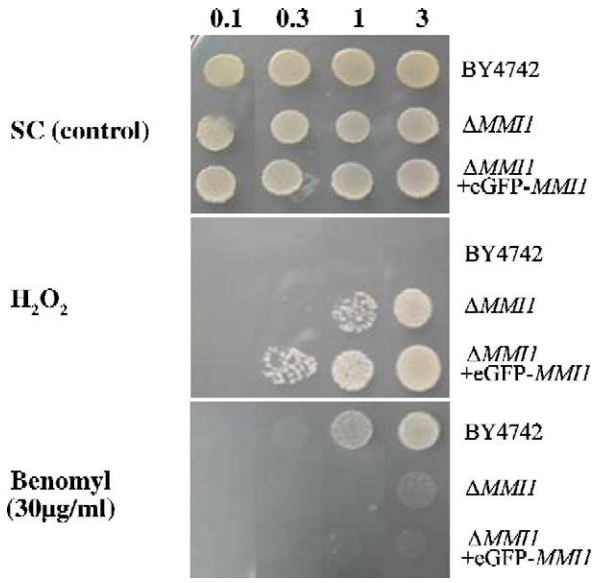

Fig. 3. Wild type cells, cells of the deletion mutant Δ*MMI1*, and cells of the deletion mutant expressing eGFP-Mmi1 were spotted onto plates containing 30 µg/ml benomyl or 3 mM hydrogen peroxide. The deletion cells are resistant to hydrogen peroxide at a concentration at which the wild type cannot grow at all. This effect is not rescued by eGFP-Mmi1p. The Δ*MMI1* cells show a clear benomyl sensitivity which cannot be rescued by the overexpressed eGFP-MMI1 on a multicopy plasmid.

after oxidative stress induction in yeast [35]. The question now was whether this protein modification is the cause for the mitochondrial localization and whether the other S-thiolated proteins, when eGFP-tagged, also show a mitochondrial staining pattern after stress induction. Therefore we tested four other strains expressing eGFP-fusion proteins, whose natively expressed wild-type proteins become S-thiolated (*ALD6, FBA1, PRE6, SSA2*) under stress conditions. All of them behaved like the eGFP-*MMI*-expressing strain suggesting a general connection of the S-thiolation and the mitochondrial localization after stress (Fig. 4C, D). In order to further analyze the mitochondrial pattern we stressed cells expressing eGFP-*MMI1* with H_2O_2 and subsequently isolated mitochondria from these cells. As a control we also isolated mitochondria of unstressed cells. These mitochondria were observed by fluorescence microscopy. Mitochondria of unstressed cells did not show any fluorescent signal whereas mitochondria of stressed cells show an intensive fluorescence staining. After treatment of those fluorescent mitochondria with proteinase K the staining disappeared indicating a localization of the eGFP-Mmi1 fusion protein to the outer membrane of the mitochondria. In contrast to this result the fluorescence emitted by eGFP-*ACO1*, a protein of the mitochondrial matrix, was insensitive to proteinase K digestion. (our own unpublished observations).

In a further experiment we wanted to know whether the human TPT1 (hTPT1) and HspA8 (stress 70 protein family) behave like their yeast orthologues *MMI1* and *SSA2* in oxidatively stressed yeast cells. The eGFP-fusion protein of hTPT1 remained cytosolic after stress with 3 mM hydrogen peroxide, whereas the *SSA2* counterpart HspA8 localized to the mitochondria in yeast. Fratelli et al. [36] showed that stress-70 proteins become S-thiolated after exposure to oxidative stress, in contrast to hTPT1, where no S-thiolation is known at present. In case of hTpt1p, the lack of S-thiolation could be responsible for the missing mitochondrial localization. On the other hand, it is reported, that Bcl-X_L interacts with TCTP, resulting in mitochondrial localization [37]. This interaction does not take place in yeast as no yeast homolog of Bcl-X_L is known and this could be the reason for the lack of translocation of human protein to mitochondria in yeast. In case of HspA8, the mitochondrial localization in yeast could be due to the S-thiolation.

To test whether the translocation to mitochondria is reversible, we added cycloheximide (100 µg/ml) to H_2O_2-stressed eGFP-*MMI1*-expressing cells in order to inhibit new protein synthesis. We observed the fluorescent mitochondrial localization of the fusion protein. After removal of the H_2O_2 stress we further kept track of the fluorescent fusion protein, which now relocated back to the cytosol (our own unpublished observations). Due to inhibition of protein synthesis by cycloheximide, no new eGFP-Mmi1p was produced. Therefore the observed fluorescent signal must be due to previously expressed protein (which was already affected by H_2O_2 stress) and leads us to the conclusion that the process of localization to the mitochondria is fully reversible.

3.5. Role of S-thiolation of Mmi1p

To test if S-thiolation itself is responsible for the mitochondrial localization we constructed an eGFP-Mmi1^{Cys32Ser} fusion protein (Fig. 2), reasoning that yeast Mmi1p contains only one cysteine which is the target of S-thiolation. We still observed a mitochondrial localization of the mutagenized eGFP-Mmi1p after

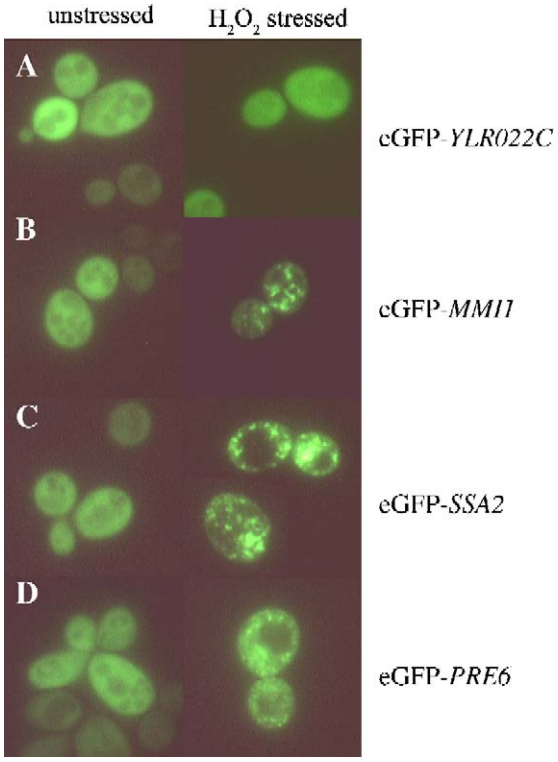

Fig. 4. Subcellular localization of four eGFP-fusion proteins. Late-Log phase cells expressing eGFP-fusion proteins are shown before (left) and after (right) a 30 min H_2O_2 (3 mM) treatment. (A) eGFP-YLR022C stays in the cytoplasm. (B) eGFP-*MMI1*, (C) eGFP-*SSA2*, and (D) eGFP-*PRE6* localize to mitochondria.

Table 2
Genes coding for proteins of the outer membrane of the mitochondria

ARC15, ATP10, CBP3, CBP4, CRD1, DIC1, DNM1, FIS1, FZO1, MDM10, MDM12, MDV1, MMM1, MSP1, OM45, ORT1, PET127, POR1, SCO2, SEN15, SEN2, SEN34, SEN54, TOM20, TOM22, TOM6, TOM7, TOM70, TOM71, UGO1, YHR083W, YPR151C, YPR133W

exposure of the cells to H_2O_2 indicating that S-thiolation is not the major signal leading to the observed mitochondrial localization.

In a 2-hybrid screen it was shown, that Mmi1p interacts with YER140Wp, Tho1p and Nnt1p [38]. Therefore, we transformed eGFP-*MMI1* into ΔYER140W, Δ*Tho1* and Δ*Nnt1* deletion strains to investigate whether the mitochondrial localization still occurs in the absence of the possible interaction partners. In all three deletion strains the eGFP-Mmi1p is still localized to the mitochondria. This leads us to the conclusion that the mitochondrial localization is not caused by a protein–protein interaction between Mmi1p and YER140Wp, Tho1p or Nnt1p. We asked if the localization of eGFP-Mmi1p to the surface of the mitochondria is based on a still unknown protein interaction. To answer this question we transformed the eGFP-*MMI1* into strains deleted for genes coding for proteins of the outer membrane of the mitochondria (Table 2). All of the 33 strains behaved like wildtype concerning mitochondrial localization of eGFP-MMI1. This means that none of the designated yeast mitochondrial outer membrane proteins (according to the MIPS database) is a major or exclusive target for mitochondrial outer membrane binding of Mmi1p. An obvious explanation would be functional redundancy among these proteins for binding of Mmi1p.

3.6. Benomyl sensitivity

The human TCTP was described as a microtubule-associated protein [2]. Therefore we tested the yeast deletion strain Δ*MMI1* for its phenotype in the presence of benomyl. Benomyl and its main metabolite carbendazim bind to and destabilize microtubules and thereby interfere with cell functions such as cell division and intracellular transport [39]. The selective toxicity of benomyl as a fungicide is possibly due to its increased effect on fungal rather than mammalian microtubules. As shown by spot tests the deletion strain of *MMI1* shows increased sensitivity to benomyl (Fig. 5) compared to wild type.

We were interested to know whether the eGFP-fusion protein was able to rescue the benomyl phenotype of a Δ*MMI1* mutant. Interestingly, the benomyl sensitivity is not rescued but increased by expressing the eGFP fusion protein in the deletion mutant (Fig. 3). However, over-expressing the non-fusion protein cloned into pUG37 leads to a partial rescue of the benomyl phenotype and nearly the same partial rescue was also achieved with the human non-fusion TCTP protein (Fig. 5). The latter result points to the fact that considering the interaction with microtubules the human protein can substitute for the yeast protein. One interpretation of these results is that wild-type Mmi1p stabilizes while the lack of Mmi1p destabilizes microtubules which would lead to increased sensitivity to the destabilizing drug, benomyl. The fusion protein eGFP-Mmi1p apparently does not stabilize microtubules presumably due to a structural change that prevents the proper interaction with the highly structured microtubular protein complex. However, the same eGFP-Mmi1p still is transferred to the mitochondrial outer membrane (Fig. 4) like the wild type protein (Fig. 1). This would indicate that the interaction with the mitochondrial surface is not mediated by the same part of the molecule that mediates interaction with microtubules (the alpha-helical domain).

We now investigated the influence of Mmi1p on oxidative stress resistance of yeast cells (Fig. 3). The reasoning was that slightly destabilizing microtubules, which has no major effect on

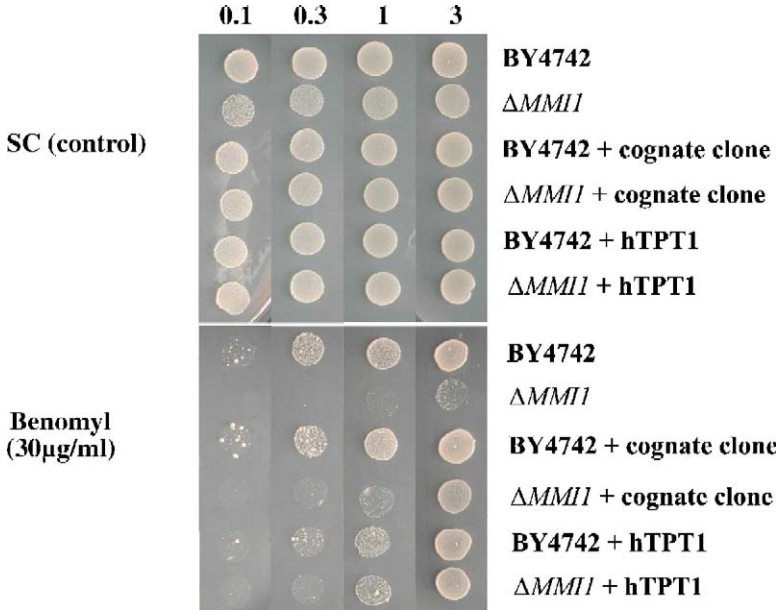

Fig. 5. Wild type cells and cells of the deletion mutant Δ*MMI1*, expressing either cognate *MMI1* or human TCTP, were spotted onto plates containing 30 µg/ml benomyl. Δ*MMI1* cells show a clear benomyl sensitivity which is partially rescued by the overexpressed cognate *MMI1* as well as by human TCTP.

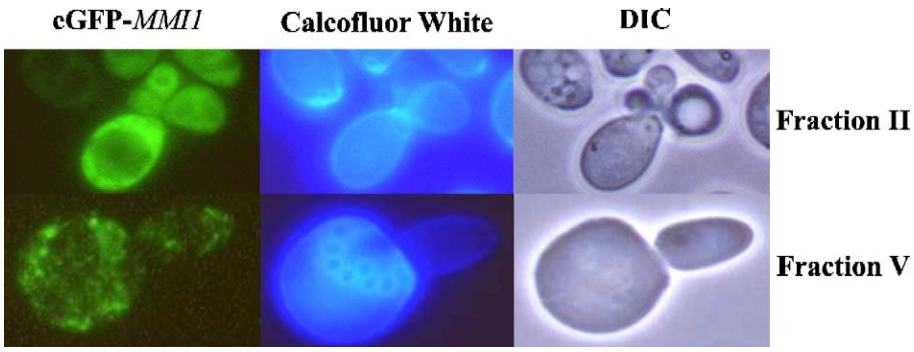

Fig. 6. Expression of eGFP-Mmi1 in old and young wild type yeast cells after elutriation centrifugation. The old mother cells (fraction V) but not young cells (fraction II) show mitochondrial localization of eGFP-Mmi1p. The old and young cell fractions are stained with Calcofluor White M2R to show the large number of bud scars on old mother cells.

cell growth, could make the cells resistant to oxidative stress exerted by hydrogen peroxide. Similar observations had been made concerning the actin cytoskeleton. It was found that point mutations in the *ACT1* gene which destabilize actin fibers as well as a deletion of *SCP1* (homologous to mammalian SM22/transgelin) which has a similar destabilizing effect on this cytoskeletal element, make the cell resistant to oxidative stress, reduce mitochondrial ROS production and lead to an increase in mother cell-specific lifespan [reviewed in 40,41]. This is consistent with the hypothesis that the increase in resistance against hydrogen peroxide is caused by the destabilization of microtubules in a similar way to the effect of actin mutations. As an internal control, we showed that the empty eGFP-vector pUG35 is not responsible for an increased resistance to hydrogen peroxide.

3.7. Localization of Mmi1p in senescent yeast mother cells

We prepared old mother cells (fraction V) and young daughter cells (fraction II) of the wild type strain BY4741 expressing eGFP-Mmi1p by elutriation centrifugation [19]. During preparation both fractions were treated in exactly the same way so that we can exclude any differential stress conditions on the old and young cells. We observed that in old cells the fusion protein is localized to mitochondria while in young cells it is cytoplasmic (Fig. 6). This is in excellent agreement with our previous observations [19] that old cells, but not young cells, contain ROS which show mitochondrial localization and in their final stage of senescence undergo programmed cell death. These observations would further indicate that perhaps the transfer of Mmi1p to mitochondria is an attempt of the cell to react to the apoptotic stimuli created by external (hydrogen peroxide) or internal (ageing) oxidative stress.

4. Conclusions

In the present communication we have for the first time described clearcut phenotypic consequences of the deletion of *MMI1* (YKL056C) in *S. cerevisiae*. These phenotypes are in good agreement with the known physiology of the mammalian TCTP, which has been shown in mammalian cells to bind to microtubules. We are showing here that yeast Mmi1p not only interacts with microtubules but also that it stabilizes them. This function seems to be conserved between the yeast and human molecules since the human TCTP can partially rescue the benomyl sensitivity of the yeast *MMI1* deletion strain.

We have described in detail the transfer of Mmi1p to mitochondria in answer to stresses experienced by the cell. Although at present the functional consequences of this transfer in *S. cerevisiae* are not clear, the comparison with the mammalian system shows that perhaps this transfer serves an anti-apoptotic function [8,9,37] and needs (still unknown) protein partners for Mmi1p.

The molecule, TCTP or Mmi1p, has a surprising number of different functions as described in the Introduction, and we can only speculate at present how these different functions might be interrelated. Some of these functions were only invented in higher organisms, like the ability to be exported from the cell and to serve as a histamine releasing factor. Another proven function of the molecule in higher cells is regulation of the elongation step of translation by acting as a guanine nucleotide dissociation inhibitor (GDI). Comparing the predicted structures and the most conserved amino acids in the sequence of Mmi1p, we think it is possible that the function of Mmi1p in the elongation step of translation is also conserved in yeast.

Acknowledgements

We are grateful to FWF (Vienna, Austria) for project S9302-B05 (to M.B.), to the EC (Brussels, Europe) for project MIMAGE (contract no. 512020; to M.B.), to NGFN2 SMP Proteomics for subproject 9 (FKZ: 01GR0449, to R.J.B.) and to the Austrian Academy of Sciences for a DOC grant (to M. R.).

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