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Ecm10, a novel Hsp70 homolog in the mitochondrial matrix of the yeast *Saccharomyces cerevisiae*

Frank Baumann, Irina Milisav, Walter Neupert, Johannes M. Herrmann*

Institut für Physiologische Chemie, Goethestr. 33, 80336 Munich, Germany

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Abstract Members of the heat shock protein 70 (Hsp70) family are found in most of the compartments of eukaryotic cells where they play essential roles in protein metabolism. In yeast mitochondria, two Hsp70 proteins are known: Ssc1 and Ssq1. We identified Ecm10 as a third Hsp70 protein in the mitochondrial matrix. Ecm10 shares 82% amino acid identity with Ssc1 and 54% with Ssq1. Overexpression of Ecm10 mitigates protein import defects in *ssc1* mutants suggesting that Ecm10 can play a role in protein translocation. Like Ssc1, Ecm10 interacts with the nucleotide exchange factor Mge1 in an ATP-dependent manner. Deletion of *ecm10* leads to synthetic growth defects with *ssc1* mutations at low temperature. Our data suggest an overlapping function of Ecm10 and Ssc1.

Key words: Heat shock protein 70; Chaperone; Mitochondrion; Protein import; Ecm10; Ssc1

1. Introduction

Heat shock protein 70 (Hsp70) proteins play essential roles in the biogenesis of both prokaryotic and eukaryotic cells (for review see [1,2]). Members of this family have a characteristic structure: they are comprised of an N-terminal ATPase domain of about 45 kDa, followed by an 18 kDa segment containing the peptide binding site and a variable C-terminal domain of about 10 kDa. The function of this C-terminal tail is not well understood. Hsp70 proteins bind and release hydrophobic stretches of unfolded polypeptides in an ATP-dependent manner. Binding of Hsp70 can thereby stabilize unfolded states of a substrate protein, and controlled release may allow the folding of the substrate into its native conformation. Hsp70 proteins are central components in a large number of cellular reactions, like protein synthesis, folding, assembly, and degradation of polypeptides, protein translocation, and protection against heat and cold stress.

Two Hsp70 proteins were identified in yeast mitochondria so far: Ssc1 is involved in a variety of important processes such as folding of proteins, assembly of oligomers, and protein degradation [3–5]. In addition, it is a central player in the mitochondrial protein import process [6–8]. To facilitate protein translocation across the inner membrane, Ssc1 is recruited to the matrix site of the protein translocase where it binds to incoming polypeptide chains. This binding prevents backsliding of the translocation intermediates and allows a directed movement of the substrates into the mitochondrial matrix. The interaction of Ssc1 with substrates is ATP-dependent and regulated by the nucleotide exchange factor Mge1 [9–11]. The second Hsp70 protein, Ssq1, is less well characterized and appears to play a role in the biogenesis of iron sulfur proteins. Its precise role in this process, however, is unknown [12–14].

We identified Ecm10 as a third Hsp70 protein in the mitochondrial matrix of the yeast *Saccharomyces cerevisiae*. *ECM10* was initially identified in a screen for mutants that show an increased sensitivity to the cell wall perturbing agent calcofluor [15]. Several other genes encoding mitochondrial proteins were also identified in the same screen. The connection between mitochondrial biogenesis and cell wall formation, however, is unclear. We show that Ecm10, like Ssc1, interacts in an ATP-dependent manner with Mge1 and newly imported proteins. In addition, overexpression of Ecm10 suppresses protein import defects into mitochondria of a temperature-sensitive *ssc1* mutant, and therefore both Hsp70 proteins appear to overlap in function and substrate specificity.

2. Materials and methods

2.1. Construction of strains and plasmids

For in vitro transcription and translation of Ecm10, the *ECM10* open reading frame was amplified by PCR using the primers ECM10pGEM-5′ (5′-GGGAAGCTTACCATGTTACCATCATGGAAAGCC) and ECM10pGEM-3′ (5′-GGGGAGCTCTTATTTATTTTCTCTCCCGTTC). The resulting PCR product was digested with *Hin*dIII and *Sac*I and cloned into a pGEM3 vector (Promega, Madison, WI, USA).

Yeast strains are derivatives of the wild type strain YPH500 [16], and of the *ssc1-3* mutant and its corresponding wild type [8]. Manipulations of yeast strains were essentially performed as described in [17]. For disruption of *ECM10*, the *ECM10*-containing pGEM3 plasmid was treated with *Cla*I resulting in the deletion of 1169 bp from the *ECM10* coding sequence which was replaced by a DNA fragment that carried the *TRP1* gene. After linearization with *Eco*RI this plasmid was transformed into yeast cells resulting in wild type and *ssc1-3* mutant strains harboring a disrupted *ECM10* gene. Disruption of the *ECM10* locus by *TRPI* was verified by PCR.

For C-terminal tagging of Ecm10 with the His_6 epitope, the plasmid SSB13 (kindly provided by S. Bednarek, Madison, WI, USA) was used that allowed the amplification of a DNA sequence encoding a His_6 epitope followed by a stop codon, a terminator region and an *URA3* gene. For PCR the primers HH15 (5′-AAGAACAGTGACAATCCTGAAACTAAGAACGGGAGAGAAAATAAAGAAGAACAAAAGCTT) and HH16 (5′-CGTTGAAAGGATATATGGTTTACGCTTACTATTTCGTTTGATGTAAGCGGCCAGTGCCAAGC) were used and the resulting product was inserted directly at the 3′ end of the chromosomal *ECM10* gene by homologous recombina-

*Corresponding author. Fax: (49)-89-5996 270.
E-mail: hannes.herrmann@bio.med.uni-muenchen.de

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tion. Correct insertion was verified by both PCR and Western blotting.

For the expression of N-terminally His_6-tagged versions of Ssc1 and Ecm10 under control of the *GAL1* promoter a derivative of the pYES2.0 plasmid (Invitrogen, Carlsbad, CA, USA) was used that contained a sequence encoding the mitochondrial targeting signal of subunit 9 of the F_0-ATPase of *Neurospora crassa* and an oligohistidine tag (B. Westermann, Munich, Germany, unpublished). The resulting vector was linearized with *Bgl*II/*Sac*I and used to subclone PCR products encoding the mature part of Ssc1 (nucleotides 60–1935) and the homologous region of the Ecm10 sequence (nucleotides 69–1965). For this the primers SSC1pYES-5′ (5′-GGGAGATCTCAGTCAACCAAGGTTCAAGG), SSC1pYES-3′ (5′-CCCCGAGCTCTTTTTACTGCTTAGTTTCACCAG), ECM10pYES-5′ (5′-GGGATCCCAGTCAACCAAAATTCCAGATG), and ECM10pYES-3′ (5′-GGGGAGCTCTTATTTATTTTCTCTCCCGTTC) were used, and the resulting products were treated with *Bgl*II/*Sac*I, and *Bam*HI/*Sac*I, respectively.

Yeast cells were grown either at 24 or 30°C on YP medium (1% yeast extract, 2% peptone) or minimal medium [17] containing 2% galactose and 0.5% KOH-buffered lactate.

2.2. Purification of chemical amounts of Su9(1–69)DHFR

A C-terminal His_6-tagged version of Su9(1–69)DHFR (kindly provided by Thomas Langer, Munich, Germany) cloned into pQE60 (Quiagen, Hilden, Germany) was expressed in *Escherichia coli* BL-21 cells (Novagen, Madison, WI, USA). The Su9(1–69)DHFR protein was isolated by affinity chromatography on a Ni–NTA agarose col-

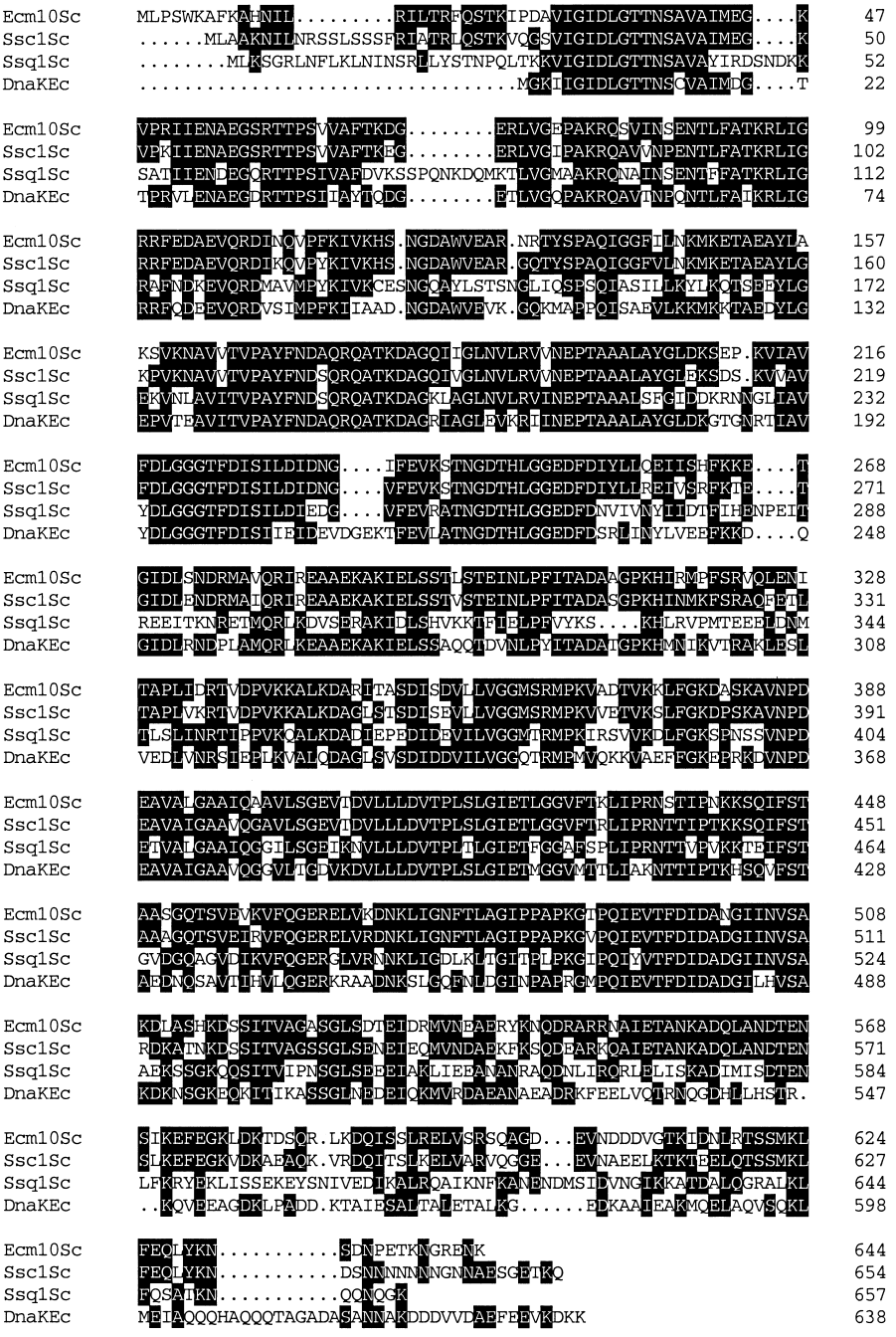

Fig. 1. Sequence comparison of Ecm10, Ssc1, Ssq1 from yeast, and *E. coli* DnaK. Identical residues between at least two of the sequences are indicated by black boxes with white letters. Gaps that were inserted during the alignment are denoted by dots. Alignments were performed by using the DNAMAN software (Lynnon BioSoft, Que., Canada).

umn as described by the manufacturer (Pharmacia, Freiburg, Germany), eluted in 100 mM NaCl, 500 mM imidazole, 50 mM Tris–HCl pH 8, and desalted on a PD10 column (Pharmacia, Freiburg, Germany). Su9(1–69)DHFR containing fractions were pooled and used in a 40-fold dilution for import.

2.3. Subfractionation of yeast cells

Mitochondria were isolated as described [18] and further purified on sucrose step gradients according to published procedures [4]. Fractions enriched in proteins of the cytosol and the endoplasmic reticulum were prepared as described [4]. Carbonate extraction was carried out as outlined in [19].

2.4. Import of precursor proteins into isolated mitochondria

Precursor proteins were synthesized as described in Section 2.2 or by in vitro transcription and translation in the presence of [^{35}S]methionine in reticulocyte lysate as described by the manufacturer (Promega, Madison, WI, USA). Import of precursor proteins in isolated mitochondria and following mitochondrial subfractionation was performed as described [5,19]. For induction of the temperature-sensitive *ssc1-3* mutant phenotype mitochondria were exposed to 37°C for 10 min, and incubated for 5 min at 25°C with an ATP-regenerating system consisting of 2 mM ATP, 2.5 mM succinate, 2.5 mM malate, 10 mM creatine phosphate, and 0.1 mg/ml creatine kinase. Import of chemical amounts of preproteins was carried out at 25°C using 27 μg/ml of purified Su9(1–69)DHFR precursor and 2% reticulocyte lysate containing ^{35}S-labeled Su9(1–69)DHFR precursor. Efficient hypotonic swelling of mitochondria in protease protection experiments was controlled by Western blotting with antisera recognizing marker proteins of the matrix and the intermembrane space.

2.5. Isolation of His$_6$-tagged proteins from mitochondria

Mitochondria (100 μg) were solubilized on ice in lysis buffer (0.2% Triton X-100, 20 mM Tris–HCl pH 8.0, 150 mM Na-acetate, 15 mM imidazole, 1 mM phenylmethylsulfonyl fluoride). After a clarifying spin at 20 000×*g* for 10 min at 4°C the extract was incubated for 2 h with Ni–NTA agarose beads. Then, the resin was washed twice with lysis buffer and once with 20 mM Tris–HCl pH 8.0. Bound material was eluted with sample buffer containing 1% SDS and 500 mM imidazole.

3. Results and discussion

3.1. Ecm10 is a novel mitochondrial Hsp70 protein

The genome of the yeast *S. cerevisiae* encodes 14 genes of members of the Hsp70 family [20]. Nine of these Hsp70 homologs were localized in the cytosol, two in the endoplasmic reticulum (Kar2 and Lhs1), and two in the mitochondrial matrix (Ssc1 and Ssq1). The localization of the remaining Hsp70 protein, Ecm10 (YEL030w), was not assessed experimentally so far.

Ecm10 forms together with Ssc1 and Ssq1 a subbranch within the homology tree of the yeast Hsp70 members [20] that shows close relationship to the bacterial DnaK protein. The amino acid sequence of Ecm10 is highly similar to that of Ssc1 (82% identity), and most differences in the sequence are due to conservative mutations (Fig. 1). Both proteins show striking similarity even in the C-terminal 10 kDa domain which is not well conserved among members of the Hsp70 family. Differences are mainly restricted to the very N-terminus, i.e. the putative mitochondrial presequence (see below), and the very C-terminus. The structure of the short C-terminal tail domain of DnaK has been analyzed by high-resolution NMR, and revealed a highly mobile protein stretch that might serve as a lid on the peptide binding groove [21]. It was suggested that this flexible subdomain makes contacts to the peptide binding site [21–23]. Thereby, it might fine-tune the energetics of peptide binding and release. In the case of cytosolic Hsp70 members, this very C-terminal domain interacts with cofactors that regulate the activity of the chaperone [24,25]. No information exists on the specific role of this domain for mitochondrial Hsp70 proteins. However, removal of the last 21 amino acid residues from Ssc1 did not compromise its function suggesting that this domain is not critical for its activity [26].

The N-terminus of Ecm10 shows the characteristic features of a mitochondrial targeting signal [27], and a putative mitochondrial localization of Ecm10 was therefore proposed [20]. To assess the intracellular distribution of Ecm10 we inserted a DNA sequence into the chromosome downstream of the *ECM10* reading frame allowing the expression of an Ecm10 protein carrying a C-terminal hexahistidine tag (Ecm10-His$_6$). This 'chromosomal tagging' does normally hardly alter protein expression, so that the tagged proteins are present at roughly wild type levels. In this strain Ecm10-His$_6$ could be specifically detected by Western blotting using an antiserum recognizing the hexahistidine epitope (Fig. 2A). Thus, *ECM10*

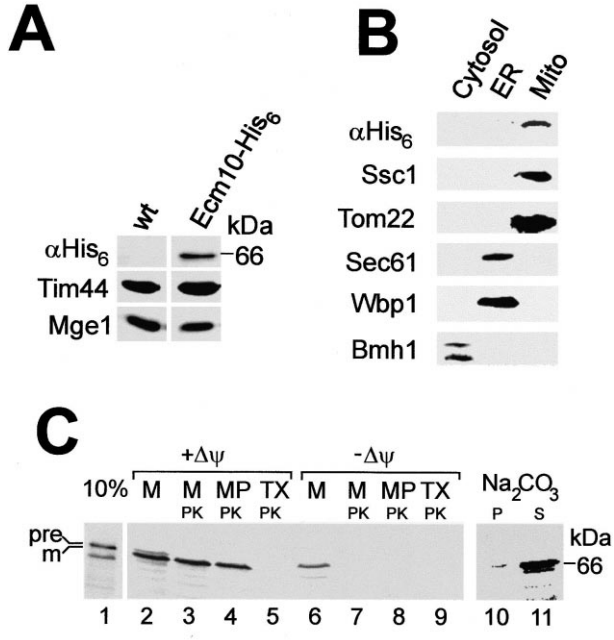

Fig. 2. Ecm10 is located in the mitochondrial matrix. A: Ecm10-His$_6$ can be specifically detected by Western blotting. Mitochondrial protein (50 μg) purified from wild type or Ecm10-His$_6$ cells was resolved by SDS–PAGE, transferred to nitrocellulose and analyzed by Western blotting using a monoclonal antiserum against the His$_6$ epitope (clone BMG-His-1, Roche Diagnostics, Germany), or antisera raised against the mitochondrial proteins Tim44 and Mge1 as loading control. B: Subcellular fractions enriched in proteins of the cytosol, the endoplasmic reticulum (ER) and mitochondria (Mito) were analyzed by Western blotting using antibodies against the His$_6$ epitope, the mitochondrial proteins Ssc1 and Tom22, the ER proteins Sec61 and Wbp1, and the cytosolic protein Bmh1. C: Radiolabeled Ecm10 is imported into isolated mitochondria. Ecm10 precursor (pre) was synthesized in reticulocyte lysate, and was incubated for 25 min at 25°C with wild type mitochondria. In the reactions shown in lanes 6–9 the membrane potential ($\Delta\psi$) was dissipated by addition of 5 μM valinomycin. After import, mitochondria were either mock treated (lanes 2 and 6), treated with proteinase K (PK) (lanes 3 and 7), subjected to osmotic swelling and treatment with proteinase K (MP, lanes 4 and 8), or lysed with Triton X-100 in the presence of proteinase K (TX, lanes 5 and 9). Lanes 10 and 11 show protein recovered after import and carbonate extraction (P, pellet; S, supernatant). For comparison, lane 1 shows 10% of the input of Ecm10 precursor. m, mature Ecm10; M, mitochondria; MP, mitoplasts.

represents an active gene which is transcribed and translated in yeast cells. Ecm10 was present in mitochondria purified by gradient centrifugation (Fig. 2B, lane 3), but was absent in fractions containing proteins of the cytosol and the endoplasmic reticulum (Fig. 2B, lanes 1 and 2).

The mitochondrial localization of Ecm10 was further supported by import experiments in vitro. Ecm10 was synthesized in reticulocyte lysate in the presence of [^{35}S]methionine resulting in a product of an apparent molecular weight of 68 kDa (Fig. 2C, lane 1). Upon incubation with isolated yeast mitochondria (Fig. 2C, lane 2) this preprotein was imported, resulting in a mature form of 65 kDa that remained protease-resistant even upon opening the mitochondrial outer membrane by hypotonic swelling (Fig. 2C, lanes 3 and 4). In the absence of a membrane potential, Ecm10 was not imported into mitochondria and the Ecm10 precursor remained protease-accessible ($-\Delta\psi$, lanes 6–9). Imported Ecm10 was extracted by treatment of the mitochondria with unbuffered carbonate indicating that Ecm10 is not stably associated with mitochondrial membranes (Fig. 2C, lanes 10 and 11). In summary, these subfractionation experiments demonstrate a location of Ecm10 in the mitochondrial matrix. Thus, this cellular subcompartment contains three Hsp70 proteins: Ecm10, Ssc1, and Ssq1.

3.2. Overexpression of Ecm10 can suppress the import defect of an ssc1 mutant

The high degree of similarity of Ssc1 and Ecm10 might indicate a similar function of both proteins. Therefore we tested whether overexpression of Ecm10 can mitigate defects of *ssc1* mutants. For this we chose the temperature-sensitive *ssc1-3* mutant which contains an Ssc1 protein that aggregates upon incubation at 37°C, thereby losing its ability to facilitate protein import [8]. Since Ecm10 and Ssc1 mainly differ in their C-terminal sequences we constructed a plasmid that allows the expression of an N-terminally tagged version of Ecm10 under control of the regulatable *GAL1* promoter. This plasmid was transformed into the *ssc1-3* mutant and mitochondria were isolated after growth of the cultures in the presence of galactose (*ssc1-3* His$_6$-Ecm10↑). We then performed in vitro import experiments employing mitochondria isolated from this mutant. To assess the efficiency of protein import we used a fusion protein consisting of the N-terminal 69 amino acid residues of subunit 9 of the F_1-ATPase of *N. crassa* and mouse dihydrofolate reductase (Su9(1–69)DHFR). This preprotein was expressed in *E. coli*, purified, and mixed with an identical preprotein that was synthesized in reticulocyte lysate in the presence of [^{35}S]methionine, and that served as a radiolabeled tracer in the import reactions. Wild type, *ssc1-3*, or

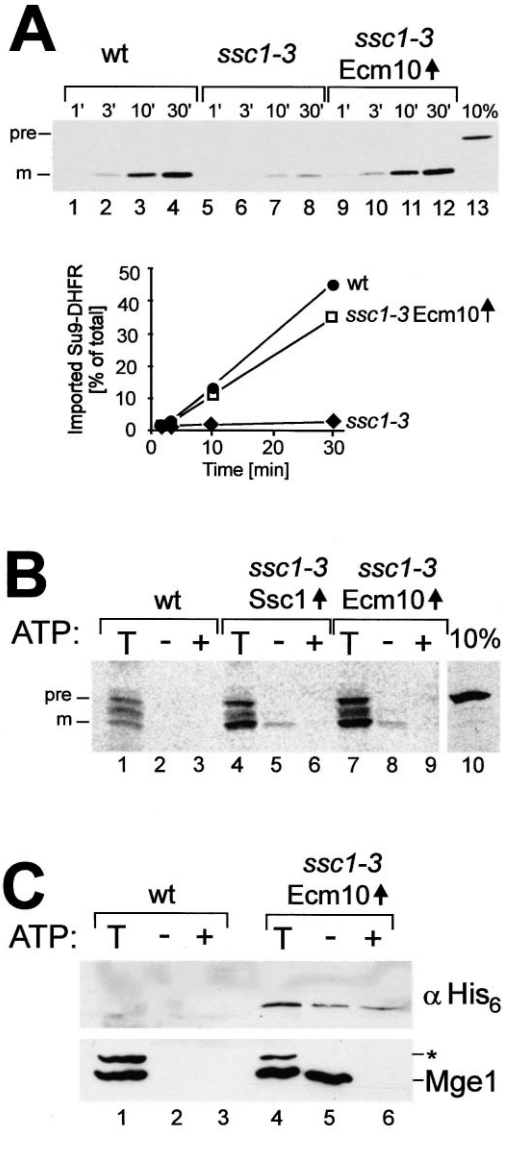

Fig. 3. Overexpressed His$_6$-Ecm10 restores import defect of *ssc1-3* mitochondria. A: Mitochondria were isolated from wild type cells (wt, lanes 1–4), *ssc1-3* cells (lanes 5–8), and *ssc1-3* cells overexpressing N-terminal His$_6$-tagged Ecm10 (*ssc1-3* Ecm10↑, lanes 9–12). Mitochondria were exposed to 37°C for 10 min to induce the temperature-sensitive phenotype of the *ssc1-3* mutants, and incubated for 5 min at 25°C with an ATP-regenerating system. Import was carried out at 25°C using purified Su9(1–69)DHFR precursor (pre). Aliquots were taken at the time points indicated, and treated with proteinase K. For comparison, lane 13 shows 10% of the precursor protein used per import reaction. The signals of the imported mature Su9(1–69)DHFR (m) were quantified on a phosphorimager (Fuji film, Tokyo, Japan) and depicted in the graph as percent of the total Su9(1–69)DHFR precursor used. B: Su9(1–69)DHFR can be copurified with His$_6$-Ecm10 and His$_6$-Ssc1 on Ni–NTA beads. Mitochondria from wild type cells (wt, lanes 1–3), *ssc1-3* cells expressing N-terminal His$_6$-tagged Ssc1 (*ssc1-3* Ssc1↑, lanes 4–6), and *ssc1-3* cells expressing N-terminal His$_6$-tagged Ecm10 (*ssc1-3* Ecm10↑, lanes 7 and 8) were pretreated for 10 min at 37°C, and incubated for 10 min at 25°C with ^{35}S-labeled Su9(1–69)DHFR. 10% of the precursor protein used per import reaction is shown in lane 10. After further incubation in the presence of 50 mU/ml apyrase for 10 min at 25°C the samples were divided. The mitochondria were reisolated and either directly applied to SDS–PAGE (T, total) or lysed either in the absence (−ATP, lanes 2, 5, and 8) or in the presence of 2 mM ATP (+ATP, lanes 3, 6, and 9). After a clarifying spin the extract was incubated with Ni–NTA agarose beads. The resin was washed and bound proteins were eluted and visualized by SDS–PAGE and autoradiography. The total lanes show 10% of the material applied to the beads. C: Wild type (wt, lanes 1–3), and *ssc1-3* His6-Ecm10↑ (*ssc1-3* Ecm10↑, lanes 4–6) mitochondria were pretreated at 37°C, lysed, and the resulting extracts applied to Ni–NTA agarose beads as described in B. The bound material was eluted from the resin, and analyzed by Western blotting using antibodies against the His$_6$ epitope and Mge1. Lanes 1 and 4 show 10% of the material applied to the beads. A cross reaction of the Mge1 serum is indicated by an asterisk. Note that for detection of Mge1 a polyclonal serum was used that produced a much stronger signal than the monoclonal His$_6$ antibody. Therefore the signal intensities do not reflect the relative abundance of both proteins.

ssc1-3 His_6-Ecm10↑ mitochondria were incubated for 10 min at 37°C which caused the aggregation of Ssc1 in both *ssc1-3* mutant samples. Overexpression of His_6-Ecm10 did not protect the mutated Ssc1 protein against aggregation (data not shown). After induction of the *ssc1-3* phenotype, mitochondria were incubated with preproteins for various times, reisolated, and treated with protease to remove non-imported material (Fig. 3A). In wild type mitochondria about half of the preprotein was processed and had reached a protease-protected location within 30 min of incubation (lanes 1–4). In contrast, hardly any preprotein was imported into the mitochondria isolated from the *ssc1-3* mutant (lanes 5–8). The import ability, however, was almost completely restored in the presence of overexpressed His_6-Ecm10 (lanes 9–12). Thus, Ecm10 can functionally replace Ssc1 in the import process of this preprotein. This suggests that, like Ssc1, Ecm10 plays a role in the translocation of preproteins into the mitochondrial matrix.

The binding of Ssc1 to preproteins is essential for their directed translocation across the inner membrane of mitochondria [6,8]. As overexpressed Ecm10 can replace Ssc1 in its role in the import of Su9(1–69)DHFR, Ecm10, like Ssc1, appears to be able to bind to incoming polypeptides. To assess the binding of Ecm10 to imported proteins directly we performed coisolation experiments with His_6-Ecm10 (Fig. 3B). To compare the binding efficiency of Ecm10 to that of Ssc1 an *ssc1-3* strain was constructed that overexpressed His_6-Ssc1 (*ssc1-3* His_6-Ssc1↑). Wild type, *ssc1-3* His_6-Ssc1↑, and *ssc1-3* His_6-Ecm10↑ mitochondria were pretreated at 37°C and incubated with radiolabeled Su9(1–69)DHFR for 10 min. Then, the samples were depleted of ATP by incubation with apyrase to stabilize the Hsp70–substrate interaction. Mitochondria were reisolated, and lysed either in the presence or absence of ATP. His_6-tagged proteins in the resulting extracts were recovered by affinity chromatography on Ni–NTA agarose, and the amounts of coisolated Su9(1–69)DHFR were determined by SDS–PAGE and autoradiography (Fig. 3B). The mature form of Su9(1–69)DHFR was specifically recovered with both His_6-Ssc1 and His_6-Ecm10 in the samples in which ATP was omitted from the lysis buffer (compare lanes 5 and 8 to lanes 6 and 9). Thus, His_6-Ecm10, like His_6-Ssc1, binds to proteins in the mitochondrial matrix during or directly after their translocation across the inner membrane. This suggests that both proteins overlap in their substrate specificity.

3.3. Ecm10 interacts with the nucleotide exchange factor Mge1

The ATP state of Ssc1 is regulated by the nucleotide exchange factor Mge1. Mge1 binds tightly to Hsp70 in the absence of ATP but not if ATP is present [26]. Does Mge1 also interact with Ecm10? In the case of DnaK and Ssc1, the interaction to their nucleotide exchange factors occurs mainly via the N-terminal 44 kDa ATPase domain [26,28]. Since this region is highly conserved between Ssc1 and Ecm10 it seems likely that Mge1 also regulates the ATP state of Ecm10. To assess the interaction of Ecm10 and Mge1 we applied extracts obtained from either wild type or *ssc1-3* His_6-Ecm10↑ mitochondria onto Ni–NTA agarose beads in the presence or absence of ATP. The beads were washed, and the amounts of Mge1 in the bound fractions were assessed by Western blotting (Fig. 3C). No Mge1 was recovered from wild type extracts (lanes 1–3). In contrast, Mge1 was efficiently coisolated with His_6-Ecm10 in an ATP-dependent manner (lanes 4–6).

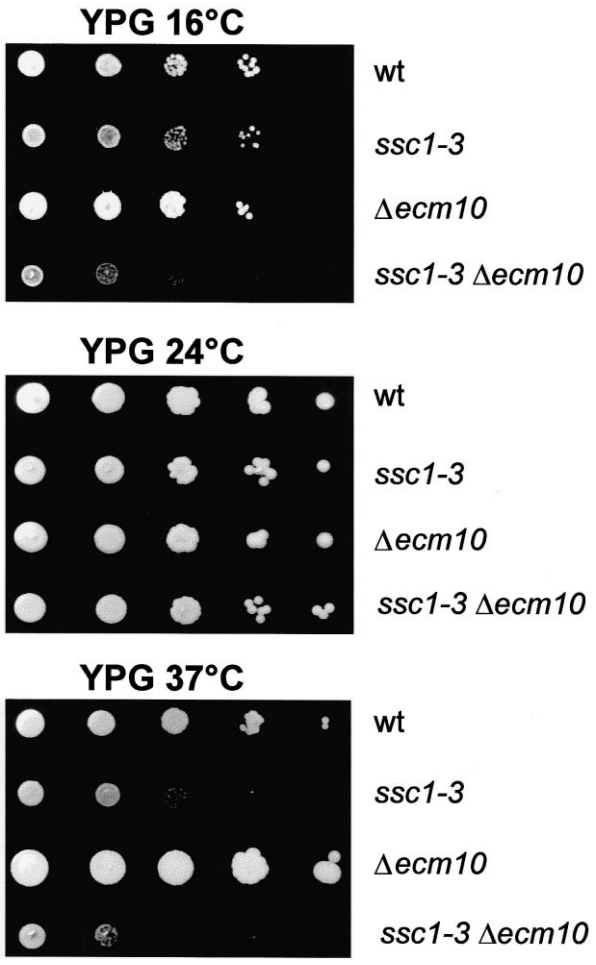

Fig. 4. Growth phenotype of *ecm10* deletion strains. Wild type (wt), *ssc1-3*, *Δecm10*, and *ssc1-3 Δecm10* cells were grown in YP medium containing 2% glycerol (YPG) at 24°C over night. From these cultures 10-fold serial dilutions were spotted onto YPG plates, and incubated at the indicated temperatures for 4 days.

Since Mge1 does not bind to the Ssc1-3 mutant protein [26] and since a large fraction of Mge1 was recovered with the Ni–NTA beads, this cannot be explained by potential traces of Ssc1 in the bound fractions. Thus, Mge1 does interact with Ecm10 and most likely serves as its nucleotide exchange factor in yeast cells.

3.4. Deletion of Ecm10 shows a synthetic growth defect with ssc1 mutations at low temperature

Next we disrupted the *ECM10* gene to study the importance of Ecm10 for mitochondrial biogenesis in vivo. The *Δecm10* single mutant did not show any obvious growth defect (Fig. 4). Since the biochemical experiments revealed an overlap in function of Ssc1 and Ecm10 it seemed possible that Ssc1 might take over the function of Ecm10 in the *Δecm10* mutant to some extent. If so, a synthetic growth defect might be expected if *ECM10* is removed from a strain harboring only a partially functional Ssc1 protein. Therefore we disrupted *ECM10* in the *ssc1-3* background. The loss of Ecm10 did not result in an increased growth defect at 37°C of the *ssc1-3* mutant (Fig. 4, lower panel). However, in contrast to the *ssc1-3* single mutant, the double mutant hardly grew on glycerol at low temperature (Fig. 4, upper panel). Thus, in the

presence of a partially defective Ssc1 protein, Ecm10 is required for growth in the cold. This might indicate a potential role of Ecm10 in the protection against cold stress. A similar cold-sensitive growth phenotype was described for yeast mutants lacking the cytosolic Hsp70 proteins Ssb1 and Ssb2 [29]. These Hsp70 chaperones bind to nascent chains on cytosolic ribosomes and facilitate efficient protein synthesis at low temperature [30]. Ssc1 has a similar chaperoning function on certain mitochondrial translation products [3]. The cold-sensitive growth phenotype of the *ssc1-3 Δecm10* mutant might point to a cooperation of both Hsp70 proteins in this function.

Our data demonstrate significant similarities in both the molecular function and the substrate specificity of Ssc1 and Ecm10. Why does the mitochondrial matrix contain two Hsp70 proteins that appear to overlap significantly in their biological roles? The existence of highly similar isoforms is very common among Hsp70 proteins. For example, the Ssa subfamily in the yeast cytosol comprises four members that strongly overlap in function, and the two Ssb proteins in the same compartment are almost completely identical in sequence. This redundancy might increase the tolerance against mutations in these proteins and thereby might facilitate the (ongoing) evolution of these chaperones. Alternatively, the various isoforms might slightly differ in their properties, and these subtle variations might enable the chaperones to deal with the large variety of different substrates and cellular processes they have to handle. Further studies might reveal these potential functional differences between Ecm10 and Ssc1 in the future.

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