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Structure-Function Analyses of the Ssc1p, Mdj1p, and Mge1p *Saccharomyces cerevisiae* Mitochondrial Proteins in *Escherichia coli*

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The DnaK, DnaJ, and GrpE proteins of *Escherichia coli* have been universally conserved across the biological kingdoms and work together to constitute a highly efficient molecular chaperone machine. We have examined the extent of functional conservation of *Saccharomyces cerevisiae* Ssc1p, Mdj1p, and Mge1p by analyzing their ability to substitute for their corresponding *E. coli* homologs in vivo. We found that the expression of yeast Mge1p, the GrpE homolog, allowed for the deletion of the otherwise essential *grpE* gene of *E. coli*, albeit only up to 40°C. The inability of Mge1p to substitute for GrpE at very high temperatures is consistent with our previous finding that it specifically failed to stimulate DnaK's ATPase at such extreme conditions. In contrast to Mge1p, overexpression of Mdj1p, the DnaJ homolog, was lethal in *E. coli*. This toxicity was specifically relieved by mutations which affected the putative zinc binding region of Mdj1p. Overexpression of a truncated version of Mdj1p, containing the J- and Gly/Phe-rich domains, partially substituted for DnaJ function at high temperature. A chimeric protein, consisting of the J domain of Mdj1p coupled to the rest of DnaJ, acted as a super-DnaJ protein, functioning even more efficiently than wild-type DnaJ. In contrast to the results with Mge1p and Mdj1p, both the expression and function of Ssc1p, the DnaK homolog, were severely compromised in *E. coli*. We were unable to demonstrate any functional complementation by Ssc1p, even when coexpressed with its Mdj1p cochaperone in *E. coli*.

The DnaK, DnaJ, and GrpE proteins represent one of the major classes of molecular chaperone machines in *Escherichia coli*. All three proteins have been shown to work synergistically in several biological processes which are crucial for cell survival, namely, to prevent proteins from aggregation during their synthesis or when partially denatured in response to stress, to facilitate protein folding, or to assist targeting of proteins for degradation (11, 16, 18). Chaperones have also the capacity to modulate the structure of seemingly native proteins, for example, by controlling their oligomerization state, their association with other proteins, or their accessibility to proteases. In this way, DnaK, DnaJ, and GrpE have been shown to down-regulate the heat shock response by interacting with and sequestering the σ^{32} heat shock sigma factor (7, 13, 25, 26, 28), initiate the DNA replication of the bacteriophage λ by disassembly of the origin O-some complex and release of DnaB helicase (1, 53), and in the replication of plasmid P1, act by converting inactive RepA dimers to active monomers (50). In all of these reactions, DnaK, DnaJ, and GrpE are thought to bind and release their substrates through a common mechanism (18).

The DnaK (Hsp70), DnaJ (Hsp40), and GrpE (Mge1p) families are highly conserved in nature. Homologs are present in bacteria and in all subcellular eukaryotic compartments, including cytosol, nucleus, endoplasmic reticulum, mitochondria, and chloroplasts (18). DnaK shows 50% amino acid identity to the eukaryotic Hsp70 proteins and can be divided into at least two functional domains, the most highly conserved N-terminal 44-kDa ATPase domain and the C-terminal 24-kDa terminal substrate binding domain (35). The Hsp40 protein family, including DnaJ, is characterized by the combination of four distinct domains. The most highly conserved is the J domain, which is thought to specifically interact with the Hsp70/DnaK ATPase domain (46), the Gly/Phe-rich region, the Cys-rich region, and a less conserved peptide binding domain (40). In contrast, GrpE is the least conserved member of the DnaK chaperone machine. In protein sequence alignment, GrpE-like proteins show five conserved blocks of 10 to 20 amino acids but no apparent conserved structural domains (51).

A model of sequential actions of these three chaperones has been proposed from in vitro protein refolding studies (18, 24, 43). According to this model, cycles of binding and release of substrate by DnaK can be linked to ATP hydrolysis and are finely tuned by the action of DnaJ, which stimulates ATP hydrolysis (27) and presents the protein substrate to DnaK, and GrpE, which promotes nucleotide exchange.

We previously showed that the *Saccharomyces cerevisiae* mitochondrial GrpE (Mge1p, also termed GrpEp and Yge1p) could substitute for *E. coli* GrpE in stimulating DnaK in vitro (10). Within the matrix, Mge1p has been shown to interact with mitochondrial Hsp70 (Ssc1p), and together with Tim44 they promote mitochondrial protein import (19). In addition, Ssc1p and Mge1p are thought to work with Mdj1p (the mitochondrial DnaJ) to prevent aggregation of unfolded proteins (49). In this regard, it appears that these three mitochondrial chaperones cooperate in the mitochondrial matrix in a fashion similar to that of the *E. coli* DnaK chaperone machine. The apparent functional conservation of activities of these chaperone machines in *E. coli* and in the yeast mitochondrial matrix suggests that some, if not all, of its components may be functionally interchangeable.

In this study, we tested to what extent the yeast mitochondrial Ssc1p, Mdj1p, and Mge1p could substitute for their *E. coli* homologs in vivo. Our results show that despite evolutionary divergence, Mge1p and Mdj1p have evidently preserved a

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TABLE 1. Bacterial strains, bacteriophages, and plasmids used

Strain, phage, or plasmid	Genotype and phenotype	Source or reference
E. coli		
OD38	B178	Laboratory collection
OD245	MC4100	9
OD258	MC4100 Δ*ara714 araD139 leu*::Tn*10*	17
OD265	DA281 *dnaK103* Δ*grpE*::ΩCam^r	3
OD273	MC4100 Δ*ara714 araD139 dnaK103* Δ*grpE*::ΩCam^r	This work
OD212	AM267 *dnaK332* Δ*grpE*::ΩCam^r	29
OD280	DA133 Δ*grpE*::ΩCam^r Kan^r (pBR322*grpE*$^+$)	3
OD164	B178 *grpE*::Cam^r Kan^r (pOD1)	This work
OD165	B178 *grpE*::Cam^r Kan^r (pOD25)	This work
OD185	CG2475 Δ*dnaK52*::Cam^r	33
OD270	MC4100 Δ*dnaK52*::Cam^r Δ*ara714 araD139*	This work
OD259	MC4100 *dnaJ*::Tn*10* Δ*cbpA*::Kan^r Δ*ara714 araD139*	22
OD247	DW668 ϕ(p*groEL*::*lacZ*) *dnaJ*::Tn*10*	47
Bacteriophages		
λ*b2c*I$^-$		Laboratory collection
λ*c*I*h*80		Laboratory collection
Plasmids		
pBAD22A	Amp^r *colE1 ori*	17
pOD1	pBAD22A*grpE*$^+$	10
pWKG90	pBAD22A*dnaJ*$^+$	22
pWKG100	pBAD22A*dnaJ12*	22
pOD10	pTTQ19*dnaK*$^+$*dnaJ*$^+$	Dan Wall
pOD25	pBAD22A*MGE1*Δ43	10
pOD26	pBAD22A*mge1*-G154DΔ43	This work
pOD27	pBAD22A*mge1*-H183RΔ43	This work
pOD28	pBAD22A*mge1*-G207SΔ43	This work
pOD40	pBAD22A*SSC1*Δ23	This work
pOD50	pBAD22A*MDJ1*Δ55	This work
pOD56	pBAD22A*mdj1*(55-267)::ΩCam^r	This work
pOD51	pBAD22A*mdj1-2*Δ55	This work
pOD52	pBAD22A*mdj1-5*Δ55	This work
pOD53	pBAD22A*mdj1-6*Δ55	This work
pWKG121	pBAD22A*mdj1*(55-125)-*dnaJ*	This work
pWKG122	pBAD22A*mdj1*(55-125)-*dnaJ12*	This work

functional architecture sufficient to regulate the in vivo activities of DnaK. These results indicate a very high similarity of action between the bacterial and mitochondrial DnaK chaperone machines and underscore the biological significance of the requirement of regulator chaperones to modulate the activity of the Hsp70 class of proteins.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. The various bacterial strains, bacteriophages, and plasmids used during this work are shown in Table 1.

Media. L broth and L agar (2) were used for most of the genetic manipulations. When appropriate, media were supplemented with chloramphenicol (20 μg/ml), kanamycin (50 μg/ml), or ampicillin (100 μg/ml).

Construction of plasmids. The *S. cerevisiae SSC1* and *MDJ1* genes were recloned from pTZ19R*SSC1* (a kind gift of G. Schatz) (8) and p*MDJ1* (a kind gift of E. Schwarz) (36) into the *Eco*RI and *Hin*dIII/*Sac*I sites, respectively, of the M13mp18 vector (32). These new constructs were used to insert an additional *Eco*RI/*Nde*I site at bp 69 of the *SSC1* gene and an *Nde*I site at bp 165 of the *MDJ1* gene (bp 1 corresponds to A of the ATG, the start codon), using the two primers 5′-ccacacgtttggaattccatatgcagtcaaccaag-3′ and 5′-caatcagaaaccatatgaa cgaagcatt-3′, respectively, as previously described (10). The *SSC1*Δ69 *Eco*RI fragment and the *Nde*I/*Sac*1 *MDJ1*Δ165 fragment were then recloned under the inducible arabinose promoter of the pBAD22A vector, to yield pOD40 and pOD50, respectively. The authenticity of the DNA junction regions of pOD40 and pOD50 was verified by DNA sequencing via the Sanger dideoxy sequencing method (37). Finally, the nonsequenced *SSC1* and *MDJ1* genes from pOD40 and pOD50 were exchanged with the wild-type *SSC1* and *MDJ1* from pTZ19R*SSC1* and pMDJ1, using the *Kpn*I and *Bsm*I sites, respectively, to ensure reconstruction of nonmutated sequences in this region. Plasmid pOD56 was constructed by the deletion-insertion of an ΩCam^r cassette into the *Sal*I site (bp 801) of pOD50. The *mdj1-2*, *mdj1-5*, and *mdj1-6* alleles (kind gifts of E. Schwarz) (49) were recloned from plasmids pRS315*mdj1-2*, pRS315*mdj1-5*, and pRS315*mdj1-6* into the *Bsm*I/*Sac*I sites of pOD50. The mutations *mge1*-G154D, *mge1*-H184R, and *mge1*-G207R were introduced into plasmid pOD25 by site-directed mutagenesis, using the primers 5′-cattctaacgtctgtataca-3′, 5′-gttgcttcgcgtttatttg-3′, and 5′-aggtgaaacttaattgttg-3′, respectively, essentially as described in reference 10.

Mdj1p-DnaJ chimeras. A derivative of pOD50 was constructed by oligonucleotide mutagenesis (23) using the primer 5′-cgaaggcagcggtaccaaattgatcg-3′, which introduced a *Kpn*I site at the C-terminal end of the Mdj1p J domain together with the missense mutation P126T. The 219-bp *Eco*RI/*Kpn*I fragment containing the Mdj1p J-domain coding sequence was then excised and cloned into *Eco*RI-*Kpn*I-cut pWKG90-H71T and pWKG100-H71T vectors (22) to create plasmids pWKG121 and pWKG122, respectively. Both plasmids contained the Mdj1p J-domain coding sequence for residues up to and including G125 but lacked sequence corresponding to the first 55 amino acids coding for the mitochondrial targeting presequence. The *Kpn*I site, which defined the J-domain junction sequence, introduced the DnaJ H71T phenotypically silent missense mutation in each case; otherwise, all downstream sequences from and including DnaJ A72 were as in wild-type DnaJ or DnaJ12. The sequences of all constructions were verified.

Construction of strains. Bacterial strains were constructed by P1-mediated transduction carried out by the method of Miller (31) essentially as described in reference 3. The *araD139* Δ*ara714 leu*::Tn*10* locus (22) was transduced from the OD258 donor strain into the OD265 recipient strain [P1(OD258) × OD265]. The presence of the *leu* mutant allele was first selected by Tet^r, and the Δ*ara714* allele was subsequently screened for the Ara$^-$ phenotype on McConkey arabinose plates, leading to the construction of strain OD273. The Δ*dnaK52* allele (33) was transduced from the OD185 donor strain into the OD258 recipient strain [P1(OD185) × OD258]. The presence of the Δ*dnaK52* allele was selected by Cam^r, giving rise to strain OD270. The deletion of the chromosomal *grpE* gene in an otherwise wild-type *E. coli* background was also performed by P1 transduction as previously described (3). OD280 was used as the donor strain,

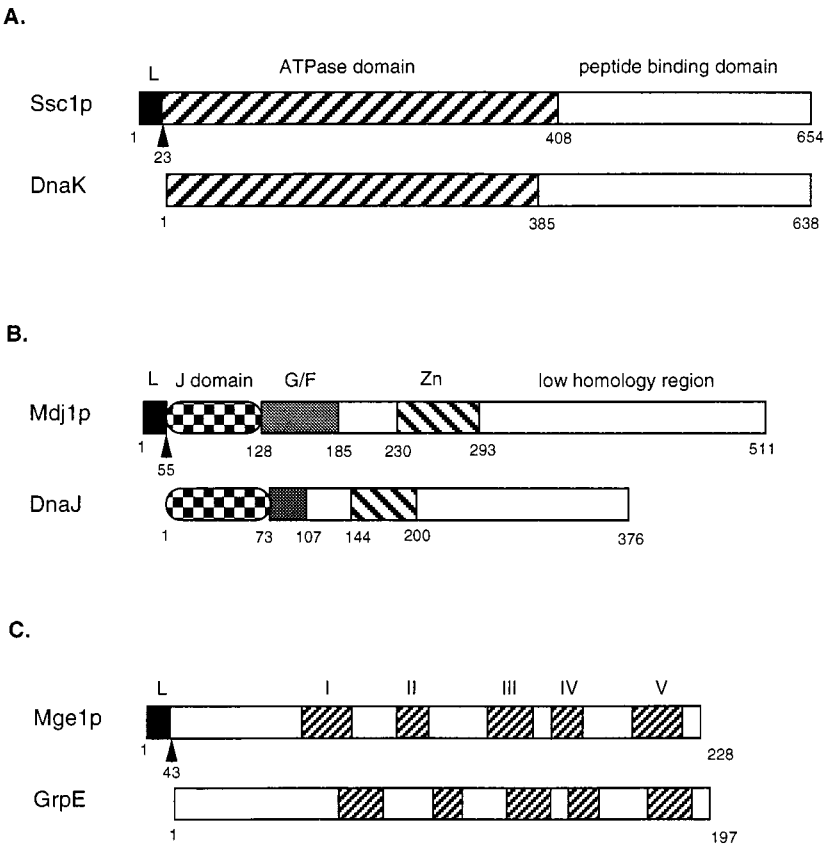

FIG. 1. Representation of the domains conserved between the *S. cerevisiae* and *E. coli* bacterial chaperones. (A) Ssc1p and DnaK are composed of two functional domains which comprise the N-terminal 44-kDa ATPase domain and the C-terminal 24-kDa peptide binding domain. L, leader sequence. (B) Mdj1p and DnaJ contain four distinct domains: the N-terminal J domain, followed by a short Gly/Phe-rich region (G/F), a Cys-rich region of four CxxCxGxG motifs (Zn), and the less conserved C-terminal domain (low homology region). (C) Mge1p and GrpE possess five short highly conserved regions (I through V). *S. cerevisiae* Ssc1p, Mdj1p, and Mge1p are preceded by a leader sequence (L) which is cleaved upon entry into the mitochondrial matrix. The arrows indicate the cleavage sites of these presequences. The major domain boundaries are indicated by residue numbers.

and the Δ*grpE*::Cam^r allele, linked to a nearby kanamycin marker, was transduced into the OD38 recipient strain carrying plasmid pBAD22A, pOD1, pOD25, pOD26, pOD27, or pOD28 [P1(OD280) × (OD38/plasmid)]. To allow transduction of the *grpE* deletion, the plasmid-encoded *grpE* gene was induced for at least 20 min with 0.1% L-arabinose before selection for kanamycin resistance and then on chloramphenicol plates containing ampicillin and 0.1% L-arabinose. The concentration of L-arabinose was reduced to 2×10^{-2}% for the expression the *grpE* gene carried on OD38/pOD1 cells.

Expression of proteins and immunoblot analysis. OD245/pOD25, OD245/pOD50, OD245/pOD40, and OD273/pOD40 were grown in 4 ml of LB medium to an A_{595} of ≈1.0 at 30°C. Each bacterial culture was then divided in two. The synthesis of plasmid-encoded proteins was preferentially induced by adding L-arabinose (0.1% [wt/vol], final concentration) in one tube, while the second tube served as an uninduced control. All cultures were shaken at 30°C for an additional 2 h. An aliquot of each culture was processed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (15% [wt/vol] polyacrylamide gel). Immunoblot experiments were carried out with Ssc1p-specific rabbit antisera (1:10,000 dilution; kindly provided by G. Shatz, Biozentrum, Basel, Switzerland) and visualized with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G as a secondary antibody (Bio-Rad kit).

RESULTS

Expression of functional forms of the mitochondrial Ssc1p, Mdj1p, and Mge1p chaperones in *E. coli*. The members of the DnaK chaperone machine are present in all eukaryotic and prokaryotic organisms and constitute one of the most highly conserved classes of proteins across the biological kingdoms. The recent identification of the Mdj1p and Mge1p proteins in yeast mitochondria as putative regulators of Ssc1p suggested a functional conservation between the protein folding pathway in *E. coli* and that in the mitochondrial compartment. Protein sequence alignments of Ssc1p, Mdj1p, and Mge1p revealed a high structural conservation, with the presence of all distinct domains present in the corresponding *E. coli* DnaK, DnaJ, and GrpE homologs (Fig. 1). We therefore reasoned that some, if not all, of the mitochondrial chaperone homologs might functionally substitute for their *E. coli* counterparts and thus strengthen the notion that a fundamental mechanism has been maintained in the evolution of this particular chaperone machine.

To express only the mature forms of the Ssc1p and Mdj1p proteins found in the mitochondrial matrix as a prelude to our functional studies, the leader mitochondrial targeting presequences were removed, in a manner analogous to that used previously for Mge1p (pOD25) (10). In this regard, the DNA portions coding for the first 23 and 55 amino acids, corresponding to the presequences of Ssc1p and Mdj1p, respectively, were removed by molecular resection (Fig. 1). The corresponding truncated genes (*SSC1*Δ23 and *MDJ1*Δ55) were then fused in frame to an L-arabinose-inducible promoter that supplied the initiating ATG methionine codon, leading to the construction of plasmids pOD40 and pOD50 (see Materials and Methods).

The L-arabinose-inducible expression plasmids pOD40 (*SSC1*Δ23), pOD50 (*MDJ1*Δ55), and pOD25 (*MGE1*Δ43) were transformed separately into the wild-type *E. coli* OD245

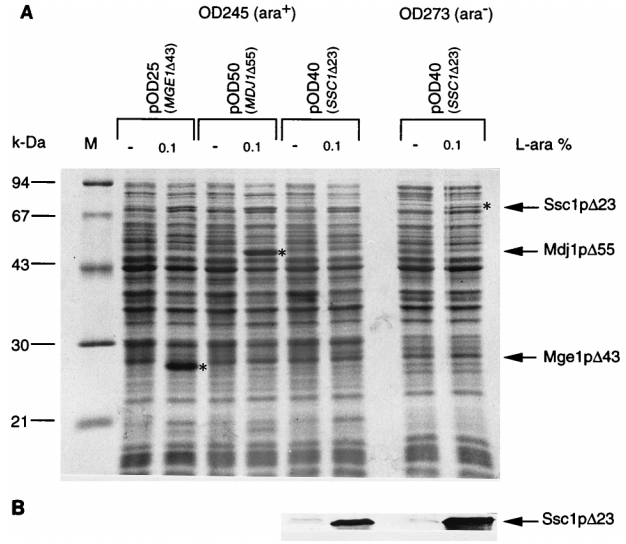

FIG. 2. Expression of *S. cerevisiae* Mge1pΔ43, Mdj1pΔ55, and Ssc1pΔ23 in *E. coli*. (A) Strain OD245 was transformed with plasmid pOD25, pOD50, or pOD40. Strain OD273 was transformed with plasmid pOD40. The cells were grown at 30°C until late log phase and induced with 0.1% L-arabinose for 2 h as indicated. The cell extracts were subjected to SDS-PAGE (15% [wt/vol] polyacrylamide gel) and stained with Coomassie brilliant blue R-250. (B) Expression of Ssc1pΔ23 was analyzed by immunoblotting with Ssc1p-specific antibodies. The asterisks and arrows indicate positions of the corresponding induced proteins. M, molecular weight standards.

genetic background and tested for conditional synthesis of the mitochondrial proteins upon addition of L-arabinose to the medium. Whole-cell extracts of induced and uninduced controls were prepared and visualized following SDS-PAGE (15% polyacrylamide gel) and Coomassie blue staining. The results, depicted in Fig. 2, showed protein bands at molecular masses of 28 and 50 kDa which were clearly visible upon L-arabinose induction of bacteria harboring the *MGE1*Δ43 and *MDJ1*Δ55 plasmid constructs, respectively, which correspond exactly to the expected sizes of the respective mature mitochondrial proteins. Judging by the extent of Coomassie blue staining, we estimate that Mge1pΔ43 and Mdj1pΔ55 were expressed to a level comparable to that of their *E. coli* GrpE and DnaJ homologs when expressed under identical conditions from the same expression vectors (data not shown). In contrast, Ssc1pΔ23 was poorly expressed, even in a strain (OD273) carrying the Δ*ara714* allele, and hence unable to metabolize the L-arabinose inducer. Although a faint protein band of the expected size of 70 kDa was visible, we could only clearly confirm its identity as Ssc1pΔ23, using the immunoblot analysis shown in Fig. 2B. The poor expression of Ssc1pΔ23 in *E. coli* has also been observed by other laboratories and is assumed to be limiting by either a low translation rate or misfolding leading to rapid proteolysis, since the mRNA levels of *SSC1*Δ23 were normally induced (9a).

Ability of Ssc1pΔ23, Mdj1pΔ55, and Mge1pΔ43 to suppress the Ts$^-$ phenotype of various *dnaK*, *dnaJ*, and *grpE* mutant alleles. To test the functional conservation of the yeast proteins, we examined whether Ssc1pΔ23, Mdj1pΔ55, and Mge1pΔ43 could functionally replace their *E. coli* DnaK, DnaJ, and GrpE homologs. Toward this goal, the pOD40 plasmid was transformed into an *E. coli* strain freshly transduced with the Δ*dnaK52*::Cam^r allele (OD270) and unable to grow at 37°C or above. The level of DnaK protein synthesized from the wild-type chromosomal copy of an *E. coli* strain was judged similar to that of Ssc1pΔ23 when induced from our pOD40 plasmid construct in strain OD270/pOD40, as estimated by immunoblot analysis (data not shown). Our results showed that under these conditions, Ssc1pΔ23 failed to compensate for the absence of functional DnaK, as judged by its complete failure to complement for bacterial growth at the restrictive temperature of 37°C (Fig. 3). Furthermore, all of our attempts to increase the Ssc1pΔ23 levels either by using higher concentrations of arabinose or by using minimal M9 medium in order to improve its expression, or to test other *dnaK* mutant alleles, were also unsuccessful in demonstrating any rescue of the bacterial DnaK temperature-sensitive (Ts$^-$) phenotype by its Ssc1pΔ23 yeast counterpart. We also tested the possibility that Ssc1pΔ23 cannot suppress the *E. coli dnaK* defects because it cannot efficiently interact with the *E. coli* DnaJ protein, but instead necessitates the presence of the yeast Mdj1pΔ55. This was done by coexpressing both Ssc1pΔ23 and Mdj1pΔ55 either from the same plasmid or from different, compatible plasmids. Again we found that the coexpression of Ssc1pΔ23 and Mdj1pΔ55 did not suppress either the Ts$^-$ bacterial growth phenotype or the λ resistance phenotype exhibited by various *dnaK* mutant bacteria (see below).

To test for a functional complementation of DnaJ by Mdj1pΔ55, plasmid pOD50 was transformed into the *dnaJ* mutant strain (OD259) unable to grow at high temperatures (22). In contrast to the Ssc1pΔ23 result, a partial suppression of the bacterial growth defect was observed at 38.5°C following expression of Mdj1pΔ55 (Fig. 3). However, very high level expression of Mdj1pΔ55 was clearly toxic for *E. coli* growth at normal and high temperatures, so much so that it completely blocked cell growth when the concentration of the L-arabinose inducer exceeded 0.1% (see Table 3). Although the reason for this toxicity is not clear, it is independent of the presence or absence of the DnaK protein (data not shown).

Finally, to test for a functional complementation of GrpE by Mge1pΔ43, pOD25 was transformed into an *E. coli* strain (OD212) carrying both a deletion of the *grpE* gene and the *dnaK*332 compensatory allele and capable of growth at 30°C but not at higher temperatures (29). The expression of Mge1pΔ43 in such mutant bacteria allowed for the complete restoration of ability to form colonies up to 42°C. This finding confirms and extends our previous published result, namely, that the same pOD25 construct was capable of complementing the Ts$^-$ phenotype of the *grpE280* missense mutant strain at 42°C (10). The overproduction of GrpE under certain conditions can be toxic for *E. coli* growth at high temperatures, as judged by reduced colony formation (Fig. 3 and reference 1a).

Deletion of the chromosomal *E. coli grpE* gene in cells expressing plasmid-encoded Mge1pΔ43. The only known biological function of GrpE is to assist DnaK in carrying out its chaperone activity by promoting the release of all DnaK-bound nucleotides (27). In this respect, *grpE* is an essential gene and cannot be deleted in any *E. coli* wild-type background under all conditions attempted (3). However, several *dnaK* mutant strains were previously shown to tolerate the deletion of the *grpE* gene, and this property was due to the presence of unmapped *dnaK* extragenic suppressors (3) or to certain mutations in the *dnaK* gene, such as *dnaK332* in strain OD212, which allowed *E. coli* to grow in the absence of GrpE or decrease the level of GrpE requirement in the cell (29).

To test to what extent the Mge1pΔ43 protein could substitute for GrpE in an otherwise *E. coli* wild-type genetic background, we tested whether the chromosomally encoded *grpE* gene could be deleted in cells expressing the plasmid-encoded Mge1pΔ43 protein. When OD38/pOD25 was used as the recipient strain in bacteriophage P1-mediated transduction ex-

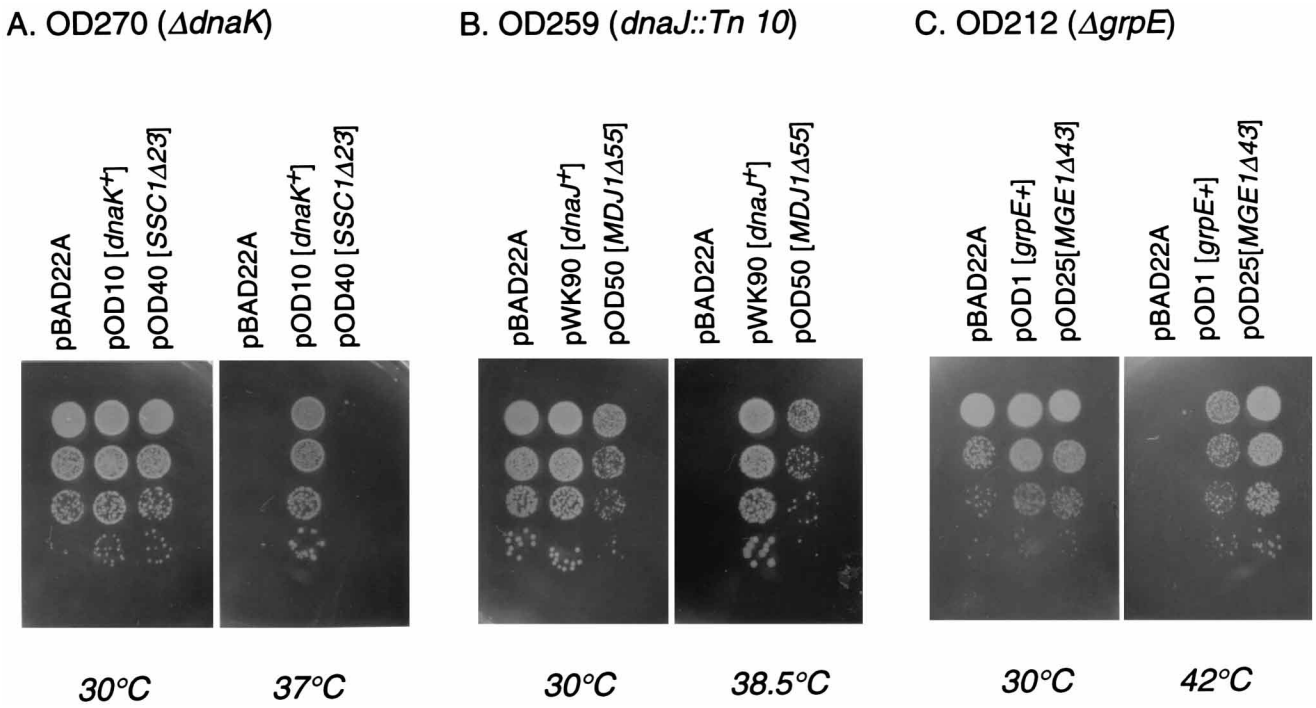

FIG. 3. Suppression of growth defects of *dnaK*, *dnaJ*, and *grpE* mutants by overexpression of the corresponding mitochondrial homologs. (A) OD270, a *dnaK* deletion strain, was transformed with plasmid pBAD22A, or pOD10, or pOD40. (B) OD259, a *dnaJ* mutant strain, was transformed with plasmid pBAD22A, pWKG90, or pOD50. (C) OD212, a *grpE* deletion strain, was transformed with plasmid pBAD22A, pOD1, or pOD25. Cultures of each transformant were diluted 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-7} and spotted on LB agar plates containing ampicillin and 0.1% (wt/vol) L-arabinose. Plates were incubated for between 12 and 18 h at the indicated temperatures.

periments, it was shown that the Δ*grpE* deleted allele could indeed be transferred at the expected cotransduction frequency with an adjacent kanamycin-resistant marker (Fig. 4). As control, a similar cotransduction frequency was obtained with a plasmid-encoded *E. coli grpE* gene (pOD1), while the vector alone (pBAD22A) did not allow the successful transduction of the chromosomal *grpE* deletion (Fig. 4). In contrast to OD212/pOD25 (Fig. 3), it is worth noting that expression of Mge1pΔ43 in our assay can compensate for the total lack of *E. coli* GrpE only at temperatures up to 40°C, presumably because of the absence of other extragenic chromosomal suppressors. This result correlates well with our previous in vitro data showing that Mge1Δ43 specifically failed to stimulate DnaK's ATPase activity at high temperature, as opposed to GrpE (10). Thus, the failure of Mge1pΔ43 to complement for GrpE function above 40°C probably reflects its inability to modulate the ATPase activity of wild-type DnaK at high temperatures. In contrast to this result, as shown in Fig. 3, OD212 cells expressing the mutant DnaK332 protein can grow at 42°C in the presence of Mge1pΔ43. This result may be due to the ability of the DnaK332 mutant protein to partially function without help from GrpE (29) and/or DnaK332's ability to interact with Mge1pΔ43 at 42°C.

To further delineate the functional conservation of Mge1pΔ43, we engineered three conserved point mutations in the *MGE1*Δ43 gene (resulting in the G154D, H183R, and G207S changes [Fig. 4]) which were previously well characterized in the *E. coli grpE* gene and shown to completely block *E. coli* cell growth at high temperatures (51). These three corresponding yeast mutant proteins were synthesized at levels comparable to those of the wild-type Mge1pΔ43, as judged by SDS-PAGE and Coomassie blue staining (data not shown). When tested for the ability to support the introduction of the Δ*grpE*::Cam^r null allele, however, none of the mutant Mge1pΔ43 proteins permitted the deletion of the chromosomal *grpE* gene (Table 2). Taken collectively, these results indicate that Mge1pΔ43 may functionally replace GrpE for cell growth up to 40°C and that the conserved amino acid residues at positions 154, 184, and 207 are crucial for the proper functioning of the GrpE class of proteins in either *S. cerevisiae* or *E. coli.*

Characterization of the Mdj1pΔ55 activity in *E. coli*. Previous studies have established that a truncated DnaJ protein, consisting of only the J domain and the Gly/Phe motif, termed DnaJ(1-108), was sufficient to perform most of the functions of the full-length DnaJ, including (i) stimulation of DnaK's ATPase activity, (ii) regulation of the conformation of DnaK in the presence of ATP, (iii) activation of DnaK to bind σ^{32} in the presence of ATP, and (iv) replication of bacteriophage λ (21, 28, 42, 46).

We have previously shown that the *dnaJ*(1-108) allele, when expressed from the L-arabinose-inducible promoter (resulting in plasmid pWKG100), can complement the Ts$^-$ phenotype exhibited by strain OD259 at 38.5°C (22) (Fig. 5 and Table 3). Based on this result, we tested whether the corresponding truncated version of Mdj1pΔ55, containing only the conserved J domain and Gly/Phe motif, could also act in a manner analogous to that of DnaJ(1-108). For this purpose, pOD56 [*mdj1*(55-267)] was constructed by inserting a ΩCam^r cassette into the unique *Sal*I site (801 bp) in the Cys-rich motif (Fig. 5), resulting in the production of a 26-kDa truncated protein, as judged by SDS-PAGE (data not shown). This protein, Mdj1p(55-267), contains the first 267 amino acid residues of Mdj1p, plus an additional 10 residues encoded by the restric-

FIG. 4. Representation of plasmids containing different *MGE1*Δ43 alleles under the control of an inducible arabinose promoter (pBAD). The hatched boxes represent the five short highly conserved sequences of Mge1pΔ43 as depicted in Fig. 1. The plasmids were used for assays of transduction of the Δ*grpE* deletion allele into a wild-type *E. coli* strain containing plasmid-encoded Mge1pΔ43 and its growth at different temperatures (Table 2).

TABLE 2. Results of transducing the Δ*grpE* allele into various genetic backgrounds

Recipient	% Cotransduction frequency of the Kan^r and Cam^r markers from OD280 (mini-Kan^r near Δ*grpE*::ΩCam^r)[a]	Plating efficiency[b]		
		30°C	40°C	42°C
OD38/pBAD22A	0 (0/120)			
OD38/pOD1 (*grpE*)	48 (56/116)	+	+	+
OD38/pOD25 (*MGE1*Δ43)	54 (78/144)	+	+	−
OD38/pOD26 (*mge1*-G154DΔ43)	0 (0/64)			
OD38/pOD27 (*mge1*-H183RΔ43)	0 (0/71)			
OD38/pOD28 (*mge1*-G207SΔ43)	0 (0/62)			

[a] Values in parentheses are actual number of Cam^r transductants/actual number of Kan^r transductants.
[b] +, large colonies and efficiency of plating of ≈1.0; −, no colonies (efficiency of plating, $<10^{-5}$).

tion sites, and terminates at the first stop codon encoded by the Ω cassette. The overproduction of this truncated protein in strain OD259, following induction by 1% L-arabinose, allowed cell growth at 38.5°C, suggesting that the J domain and Gly/Phe motif of Mdj1p can by themselves modulate DnaK's activity in *E. coli* (Table 3).

In additional tests, chimeric proteins were engineered through the exchange of the DNA regions encoding for the J domain of *E. coli* with that of Mdj1p, in either full-length DnaJ or the truncated DnaJ(1-108) mutant, leading to the construction of plasmids pWKG120 and pWKG121, respectively (Fig. 5). Using these plasmids, we tested whether the J domain of Mdj1p could function in the context of an otherwise wild-type *E. coli* DnaJ protein. The results, shown in Table 3, demonstrate that the two Mdj1p(55-125)-DnaJ and Mdj1p(55-125)-DnaJ12 chimeric proteins indeed suppress the growth defect of OD259 bacteria at 38.5°C. It is also interesting that very low levels of the Mdj1p(55-125)-DnaJ chimera were required for complementation, since even in the absence of L-arabinose, suppression of the bacterial growth defect at the nonpermissive temperature was observed. Control experiments showed that in the absence of L-arabinose, very low levels of the Mdj1p(55-125)-DnaJ chimeric protein were detected by immunoblot analysis, thus eliminating the trivial possibility that construction of this particular chimera caused a deregulation of the pBAD promoter (result not shown). These results suggest that the J domain of Mdj1p is sufficiently conserved, despite only 52% amino acid sequence identity to that of the *E. coli* authentic J domain, and that, if anything, the mitochondrial yeast J domain functions more efficiently than that of *E. coli.*

To address the question of whether the simple overexpression of the full-length Mdj1pΔ55 protein is directly responsible for the observed bacterial toxicity, we used various available point mutations and small deletions affecting different domains of Mdj1pΔ55 and analyzed their effects on cell growth (Fig. 5 and Table 3). These mutations were previously characterized in yeast and shown to result in a total loss of biological activity at high temperature (49). All mutant proteins were expressed in *E. coli* cells at levels comparable to those of wild-type Mdj1pΔ55 (data not shown), but only cells harboring the *mdj1-5*Δ55 mutation (coding for a small deletion within the Cys-rich domain; encoded by pOD52) were viable in the presence of 0.5 or 1% L-arabinose (Fig. 5 and Table 3). Interestingly, our pOD56 construct, whose Cys-rich motif is lacking, was also found to be nontoxic for cell growth when overproduced (Fig. 5 and Table 3). Although we cannot exclude the possibility that deletions in the Cys-rich domain do not affect the overall structure of Mdj1pΔ55, the presence of an intact Cys-rich motif is clearly needed for its toxicity in *E. coli* (see Discussion).

The Mge1pΔ43 and Mdj1p(55-125)-DnaJ/DnaJ12 chimeras can substitute for GrpE and DnaJ, respectively, in λ DNA replication. DnaK, DnaJ, and GrpE were initially identified as host factors required for bacteriophage λ growth, since mutations in any of these genes blocked λ DNA replication (reviewed in reference 15). Therefore, to further evaluate the functional conservation of the mitochondrial chaperone homologs, we tested the ability of bacteriophage λ to form plaques on bacterial lawns of *dnaK*, *dnaJ*, and *grpE* mutants expressing Ssc1pΔ23, Mdj1pΔ55, and Mge1pΔ43, respectively. As shown in Table 4, Mge1pΔ43 could functionally replace GrpE in this λ plaque-forming assay, whereas Ssc1pΔ23 and Mdj1pΔ55 failed to complement for plaque formation, even upon induction under a broad range of L-arabinose concentrations. Furthermore, the fact that the chimera Mdj1p(55-125)-DnaJ12 was capable of supporting λ growth, while Mdj1p(55-267) was not, suggests that the Gly/Phe motif of DnaJ is specifically required for λ DNA replication and cannot be exchanged by the corresponding Gly/Phe motif of Mdj1pΔ55.

The Mdj1p(55-125)-DnaJ and Mdj1p(55-125)-DnaJ12 chimeras, but not Mdj1pΔ55 and Mdj1p55-267, can down-regulate the *E. coli* heat shock response. It was previously demonstrated that mutations in any of the *E. coli dnaK*, *dnaJ*, and *grpE* genes resulted in the overexpression of heat shock genes, even under non-heat shock conditions (39, 41, 44). Subsequent studies established that DnaJ not only possesses a high affinity for the σ^{32} heat shock sigma factor (13, 26) but also can activate DnaK to bind σ^{32} in an ATP-dependent mode, resulting in the effective sequestration of σ^{32} (13, 14, 26, 28). In this

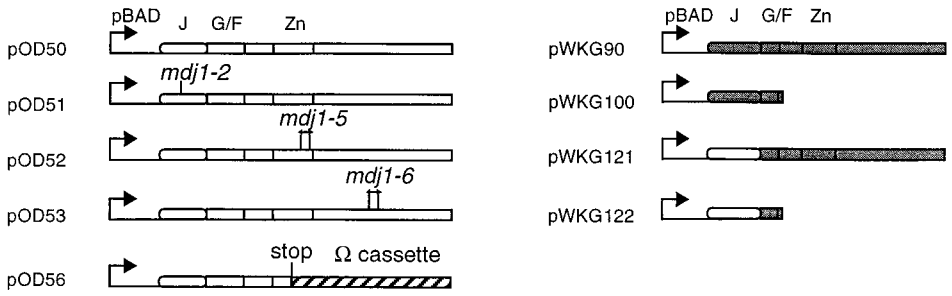

FIG. 5. Representation of plasmids containing different *MDJ1*Δ55 or *dnaJ* alleles under an inducible arabinose promoter (pBAD). Open boxes represent the different conserved domains of Mdj1pΔ55, while solid boxes represent those of *E. coli* DnaJ as depicted in Fig. 1. The hatched box corresponds to the Ω cassette containing a stop codon. These plasmids were used for assays of plating efficiency at low and high temperatures of OD259 (*dnaJ*::Tn*10* Δ*cbpA*::Kan^r) transformed with plasmids containing different *MDJ1*Δ*55* and *dnaJ* alleles (Table 3).

TABLE 3. Bacterial plating efficiencies

Plasmid	Plating efficiency[a]									
	30°C					38.5°C				
	0	0.01	0.1	0.5	1	0	0.01	0.1	0.5	1
pBAD22A	+	+	+	+	+	−	−	−	−	−
pWKG90 (*dnaJ*)	+	+	+	+	+	−	+	+	+	+
pOD50 (*MDJ1*Δ55)	+	+	+/−	−	−	−	−	+/−	−	−
pOD51 (*mdj1-2*Δ55)	+	+	+	+/−	−	−	−	−	−	−
pOD52 (*mdj1-5*Δ55)	+	+	+	+	+	−	−	−	−	−
pOD53 (*mdj1-6*Δ55)	+	+	+	+/−	−	−	−	−	−	−
pWKG121 (*mdj1-dnaJ*)	+	+	+	+	+	+	+	+	+	+
pWKG100 (*dnaJ12*)	+	+	+	+	+	−	−	+/−	+	+
pOD56 (*mdj1*::Ω)	+	+	+	+	+	−	−	−	+/−	+
pWKG122 (*mdj1-dnaJ12*)	+	+	+	+	+	−	−	−	+	+

[a] Determined by spot testing serial dilutions on LB agar plates containing ampicillin and the indicated concentration (percentage) of arabinose. +, large colonies and efficiency of plating of ≈1.0; +/−, small colonies and colony-forming efficiency reduced (0.5 to 10^{-2}) compared to cells containing DnaJ wild type; −, no colonies (efficiency of plating of $<10^{-5}$).

way, DnaJ together with DnaK can prevent σ^{32} from associating with the RNA polymerase core, thus inhibiting transcription from σ^{32}-dependent promoters. Furthermore, Wall et al. (46) showed that the DnaJ12 mutant protein was able to down-regulate transcription from a heat shock reporter gene fusion, ϕ(p*groE*::*lacZ*), in an otherwise *dnaJ*-deleted strain. In a similar fashion, we tested the ability of our different *MDJ1*Δ55 allele constructs to down-regulate the heat shock response in the same ϕ(p*groE*::*lacZ*) reporter strain. Our results showed that the Mdj1pΔ55 and Mdj1p(55-267) proteins were unable to down-regulate the heat shock response, whereas the Mdj1p(55-125)-DnaJ and Mdj1p(55-125)-DnaJ12 chimeric proteins were capable of down-regulating the heat shock response, though to a lesser extent than either DnaJ or DnaJ12 (Fig. 6). These results strongly suggest that the J domain of Mdj1pΔ55 is functional in activating DnaK to bind σ^{32}, while the rest of the Mdj1pΔ55 protein does not apparently participate in the activation of DnaK to bind σ^{32}. Further experiments are needed to demonstrate whether Mdj1pΔ55's failure to down-regulate the heat shock response is due to its inability to directly interact with σ^{32} or can bind σ^{32} but cannot present it properly to DnaK.

DISCUSSION

Over the last few years, various molecular chaperone machines have been discovered in all eukaryotic and prokaryotic organisms thus far tested and have been shown to be conserved throughout evolution (15, 18). By definition, molecular chaperones are proteins capable of interacting with nonnative

TABLE 4. Plating efficiency of bacteriophages

Phage	Relative plaque-forming efficiency[a]												
	OD212 (Δ*grpE*::ΩCam^r)			OD259 (*dnaJ*::Tn*10*)							OD270 (Δ*dnaK52*::Cam^r)		
	pBAD22A	pOD1 (*grpE*)	pOD25 (*MGE1*Δ43)	pBAD22A	pWKG90 (*dnaJ*)	pOD50 (*MDJ1*Δ55)	pWKG100 (*dnaJ12*)	pWKG121 (*mdj1-dnaJ*)	pOD56 (*mdj1*::Ω)	pWKG122 (*mdj1-dnaJ12*)	pBAD22A	pOD10 (*dnaK*)	pOD40 (*SSC1*Δ23)
λ*b2c*I$^-$	−	+	+	−	+	−	+	+	−	+	−	+	−
λ*c*I*h*80	−	+	+	−	+	−	+	+	−	+	−	+	−

[a] Determined by spot testing serial dilutions of the indicated bacteriophages onto cell lawns. +, normal plaque size and efficiency of plating of ≈1.0; −, no visible plaques (efficiency of plating, $<10^{-4}$).

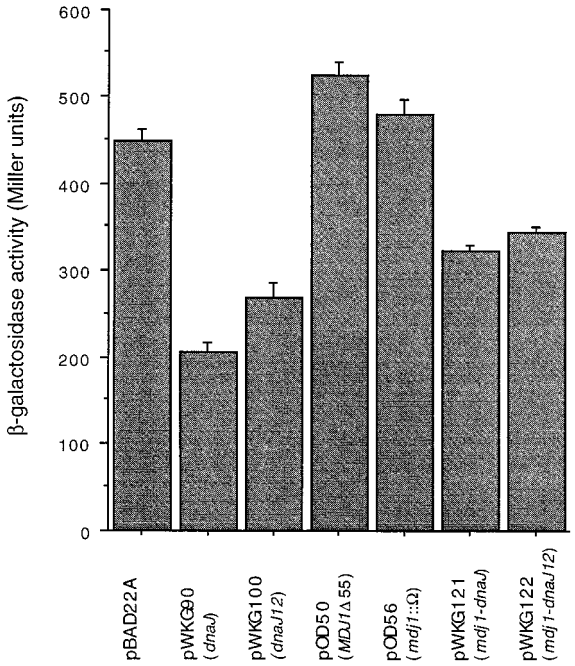

FIG. 6. Abilities of various *MDJ1*Δ55 alleles to down-regulate the *E. coli* heat shock response. Down-regulation of the *E. coli* heat shock response was measured by using a *groEL-lacZ* reporter strain (47). Strain OD247 (*dnaJ*::Tn*10*), which contains the heat shock reporter gene ϕ(p*groE-lacZ*) integrated into the chromosome, was transformed with the indicated plasmids. Fresh overnight cultures of each transformant were diluted 10^{-2} in 3 ml of LB containing ampicillin and grown for 2 h at 30°C. L-Arabinose was then added at 0.1% (final concentration), and the cultures were grown for an additional hour. The β-galactosidase activity (Miller units) was determined at 28°C by the method of Miller (31). The values given are averages of three independent determinations.

polypeptides in order to prevent incorrect folding and aggregation, thus facilitating protein folding (11). The identification of the two major DnaK and GroEL chaperone machines in yeast mitochondria has suggested the possibility of a protein folding pathway in the yeast mitochondrial matrix similar to that in the *E. coli* cytosol (19).

A general feature of the Hsp70 family members, including DnaK, is that the binding and release of their protein substrate is tightly coupled to their ATPase cycle. The two cohort DnaJ and GrpE proteins jointly stimulate the ATPase activity of DnaK by at least 50-fold (27). It is known that in this process, DnaJ increases the hydrolysis of ATP while GrpE accelerates the release of ADP (or ATP) from DnaK. By analogy to its bacterial counterpart, Ssc1p is believed to be similarly regulated by Mdj1p and Mge1p to perform several biological functions in the mitochondrial matrix (19, 30). In a previous study, we showed that Mge1pΔ43 could substitute for GrpE as a nucleotide exchange factor for DnaK in vitro, suggesting a functional conservation between the bacterial and mitochondrial DnaK chaperone systems (10). In this study, we have demonstrated that Mge1pΔ43 can compensate for the total lack of GrpE in *E. coli* cell growth, at temperatures up to 40°C, as well as for λ DNA replication, as judged by the ability to form plaques. In addition, a recent mutational analysis showed that three conserved residues located in the C-terminal domain of GrpE were involved in the modulation of DnaK's function (51, 52). Here, we showed that when the corresponding mutations were introduced in the *MGE1*Δ43 gene, they resulted in the total loss of Mge1pΔ43 activity in an *E. coli* background, indicating that both GrpE and Mge1pΔ43 have similar general structures for interacting with and modulating DnaK's activities.

In contrast to GrpE, DnaJ is a chaperone on its own right and can transiently interact with a large variety of polypeptide substrates (13, 24, 26, 48). DnaJ is also capable of presenting specific protein substrates to DnaK, resulting in a DnaJ-substrate-DnaK complex (14, 18, 24, 28, 35, 48). It is thought that DnaJ can change the conformation of DnaK to a form displaying a higher affinity for the substrate, following ATP hydrolysis (5, 26, 43, 48). It was previously shown that the J domain of DnaJ is absolutely essential for its interaction with DnaK and is specifically required for the stimulation of the ATPase activity of the DnaK chaperone (46). Here, we showed that the J domain of Mdj1p is functionally conserved and can replace the J domain of DnaJ in ensuring DnaK's activity in *E. coli*. Furthermore, recent mutational analysis of DnaJ and its homologs have established that substitutions within the universally conserved HPD tripeptide segment of the J domain of all DnaJ-like proteins (residues 33 to 35 in *E. coli* DnaJ) abolish the interaction with the corresponding Hsp70 member (12, 22, 45, 46, 49). The nuclear magnetic resonance structure of the *E. coli* DnaJ J domain revealed that this tripeptide segment is located in a flexibly disordered loop, representing a good candidate for the initiation of protein-protein interactions between the DnaJ-like protein and its Hsp70 counterpart (34). In this context, the finding that a point mutation localized in this conserved tripeptide loop of the J domain of Mdj1p (*mdj1-2*, H89Q) leads to a total loss of activity at high temperature in a yeast background (49) and in a total loss of activity in an *E. coli* background strongly supports the idea that this region of the J domain makes a crucial initial interaction with DnaK or Ssc1p.

In contrast to the J domain, the remaining domains of Mdj1p (the G/F motif, the Cys-rich motif, and the peptide binding domain) are mostly inefficient in functionally replacing the corresponding domains of DnaJ. The exact molecular functions of these three distinct domains are less understood, but they are thought to play an important role in the interaction with polypeptide substrates and in the stabilization of the DnaK-substrate-DnaJ complex (4, 42, 47). The deletion of the G/F region of the DnaJ protein has been shown to specifically prevent DnaJ from modulating the substrate binding affinity of DnaK (47). Thus, the G/F region is thought to represent a linker between the J domain and the C-terminal substrate binding domain of DnaJ and is needed to orchestrate the appropriate protein-protein interactions leading to the formation of a stable DnaK-substrate-DnaJ complex. In this context, it is worth noting that when the J domain of Mdj1p is linked to the G/F motif of DnaJ, it sufficiently down-regulates the heat shock response and allows λ DNA replication, while the similar construct containing the entire J domain and G/F motif of Mdj1p is totally unproductive in these respects. These results suggest that the G/F motif of Mdj1p, unlike that of the J domain, cannot effectively participate in the modulation of the substrate binding activities of DnaK.

An interesting observation made during the course of this work is that the overproduction of Mdj1pΔ55 is toxic to *E. coli*'s growth. Recent reports characterized the Cys-rich region of DnaJ as a zinc binding finger motif, required to interact with its substrate proteins (4, 42). In this regard, it is worth noting that only those *mdj1* mutations which result in a defective or absent Cys-rich motif could relieve the toxicity resulting from the overproduction of Mdj1pΔ55 in *E. coli* cells.

Although the exact mechanism of toxicity by the overproduced Mdj1pΔ55 is not known, two facts argue in favor of the inactivation, through sequestration, of a key cellular component(s). The first is that the toxicity is not dependent on the

presence or absence of the DnaK protein, suggesting that the cause of the toxicity cannot be the inactivation of the DnaK protein or the sequestration of an important protein(s) by DnaK. The second is that deletion of the corresponding zinc finger region of the *E. coli* DnaJ reduces its activity toward a variety of its polypeptide substrates (4), and in addition, the zinc finger region is important for the binding of a substrate protein in vitro (42). The identity of this putative substrate protein(s) remains unknown.

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