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 σ32 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
and pMDJ1, using the deletion-insertion of an nonmutated sequences in this region. Plasmid pOD56 was constructed by the pOD50 were exchanged with the wild-type SSC1 cgaagcatt-3 9 5 gene (bp 1 corresponds to A of the ATG, the start codon), using the two MDJ1 I site at bp 69 of the RI/Eco M13mp18 vector (32). These new constructs were used to insert an additional fragment and the gene (36) into the SSC1 site at bp 801 of pOD50. The mutations mdj1-G154D, mdj1-H184R, and mdj1-G207R were introduced into plasmid pOD25 by site-directed mutagenesis, using the primers 5'-cattaaagctgctataga-3', 5'-ggtctgctgctgtttc-3', and 5'-aggctggacagctgcttgc-3', respectively, essentially as described in reference 10. 

**MATERIALS AND METHODS**

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

**TABLE 1. Bacterial strains, bacteriophages, and plasmids used**

<table>
<thead>
<tr>
<th>Strain, phage, or plasmid</th>
<th>Genotype and phenotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli OD38</td>
<td>B178</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>OD25</td>
<td>MC400</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>OD212</td>
<td>AM267 dnak332 ΔgrpE:ΔCam'</td>
<td>This work</td>
</tr>
<tr>
<td>OD220</td>
<td>DA133 ΔgrpE:ΔCam' Kan' (pBR322-gptE')</td>
<td>This work</td>
</tr>
<tr>
<td>OD164</td>
<td>B178 gpt:Cam' Kan' (pOD1)</td>
<td>This work</td>
</tr>
<tr>
<td>OD165</td>
<td>B178 gpt:Cam' Kan' (pOD23)</td>
<td>This work</td>
</tr>
<tr>
<td>OD247</td>
<td>DW608 Δ(pgroEL::lacZ) dnak:ΔTn10</td>
<td>47</td>
</tr>
</tbody>
</table>

**Bacteriophages**

βOD8

**Plasmids**

pBAD22A

pOD1

pWKG90

pWKG100

pOD10

pOD25

pOD26

pOD27

pOD28

pOD40

pOD50

pOD56

pOD51

pOD52

pOD57

pWKG121

pWKG122

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.

**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 Δara714 leu-Tn10 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-, and the Δara714 allele was subsequently screened for the Ara- phenotype on MacConkey arabinose plates, leading to the construction of strain OD273. The Δdnak52 allele (33) was transduced from the OD185 donor strain into the OD258 recipient strain [P1(OD185) × OD258]. The presence of the Δdnak52 allele was selected by Cam', giving rise to strain OD270. The deletion of the chromosomal gptE gene in an otherwise wild-type E. coli background was also performed by P1 transduction as previously described (3). OD260 was used as the donor strain.
and the grpE::Camr allele, linked to a nearby kanamycin marker, was transduced into the OD38 recipient strain carrying plasmid pBAD22A, pOD1, pOD25, pOD26, 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 A₅₉₅ 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 (SSC1Δ23 and MDJ1Δ55) 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 (SSC1Δ23), pOD50 (MDJ1Δ55), and pOD25 (MGE1Δ43) were transformed separately into the wild-type E. coli OD245

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/Pro-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.
Strain OD273 was transformed with plasmid pOD40. The cells were grown at 30°C until log phase and induced with 0.1% L-arabinose for 2 h as indicated. The cell extracts were subjected to SDS-PAGE (15% polyacrylamide gel) and stained with Coomassie brilliant blue R-250. (B) Expression of Ssc1p23 was analyzed by immunoblotting with Ssc1p-specific antibodies. The asterisks and arrows indicate positions of the corresponding induced proteins. M, molecular weight standards.

FIG. 2. Expression of S. cerevisiae Mge1p43, Mdj1p55, and Ssc1p23 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 log phase and induced with 0.1% L-arabinose for 2 h as indicated. The cell extracts were subjected to SDS-PAGE (15% polyacrylamide gel) and stained with Coomassie brilliant blue R-250. (B) Expression of Ssc1p23 was analyzed by immunoblotting with Ssc1p-specific antibodies. The asterisks and arrows indicate positions of the corresponding induced proteins. M, molecular weight standards.

To test for a functional complementation of DnaJ by Mge1p43, pOD25 was transformed into an E. coli strain (OD212) carrying both a deletion of the grpE gene and the dnaK332 compensatory allele and capable of growth at 30°C but not at higher temperatures (29). The expression of Mge1p43 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 grpE520 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 Mge1p43. 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 Mge1p43 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 Mge1p43 protein. When OD38/pOD25 was used as the recipient strain in bacteriophage P1-mediated transduction ex-
experiments, it was shown that the ΔgrpE 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 MGE1Δ43 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 temperature (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 ΔgrpE::Cam' 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 σ32 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 pWK100), 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, termed Mdj1p(55-267), was constructed by inserting a ΔCam' cassette into the unique SalI 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, Mdj1pΔ55, contains the first 267 amino acid residues of Mdj1p, plus an additional 10 residues encoded by the restric-
All mutant proteins were expressed in yeast and shown to result in a total loss of biological activity (Table 3). These mutations were previously characterized and analyzed their effects on cell growth (Fig. 5).

To address the question of whether the simple overexpression of the full-length Mdj1p protein is directly responsible for the observed bacterial toxicity, we used various available DnaJ protein. The results, shown in Table 3, demonstrate that the two Madj1p(55-125)-DnaJ and Madj1p(55-125)-DnaJ12 chimeric proteins indeed suppress the growth defect of E. coli 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 Madj1p 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 Madj1p, 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 Madj1p could function in the context of an otherwise wild-type E. coli DnaJ protein. The results, shown in Table 3, demonstrate that the two Madj1p(55-125)-DnaJ and Madj1p(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 Madj1p(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 Madj1p(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 Madj1p 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 Madj1p protein is directly responsible for the observed bacterial toxicity, we used various available point mutations and small deletions affecting different domains of Madj1p 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 Madj1p Δ55 (data not shown), but only cells harboring the madj1-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 Madj1p Δ55, the presence of an intact Cys-rich motif is clearly needed for its toxicity in E. coli (see Discussion).

The Mge1p Δ43 and Madj1p(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 SecI1p, Madj1p Δ55, and Mge1p Δ43, respectively. As shown in Table 4, Mge1p Δ43 could functionally replace GrpE in this λ plaque-forming assay, whereas SecI1p and Madj1p Δ55 failed to complement for plaque formation, even upon induction under a broad range of L-arabinose concentrations. Furthermore, the fact that the chimera Madj1p Δ(55-125)-DnaJ12 was capable of supporting λ growth, while Madj1p Δ55 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 Madj1p Δ55.

The Madj1p(55-125)-DnaJ and Madj1p(55-125)-DnaJ12 chimeras, but not Madj1p Δ55 and Madj1p Δ55, 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 σ32 heat shock sigma factor (13, 26) but also can activate DnaK to bind σ32 in an ATP-dependent mode, resulting in the effective sequestration of σ32 (13, 14, 26, 28). In this

![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 Mge1p Δ43 as depicted in Fig. 1.](http://jb.asm.org/)

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

<table>
<thead>
<tr>
<th>Recipient</th>
<th>% Cotransduction frequency of the Kan&lt;sup&gt;a&lt;/sup&gt; and Can&lt;sup&gt;b&lt;/sup&gt; markers from OD280 (mini-Kan&lt;sup&gt;a&lt;/sup&gt; near ΔgrpE::Kam&lt;sup&gt;b&lt;/sup&gt;)</th>
<th>Plating efficiency&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>OD38/pBAD22A</td>
<td>0 (0/120)</td>
<td>+</td>
</tr>
<tr>
<td>OD38/pOD1 (grpE)</td>
<td>48 (56/116)</td>
<td>+</td>
</tr>
<tr>
<td>OD38/pOD25 (MGE1 Δ43)</td>
<td>54 (78/144)</td>
<td>+</td>
</tr>
<tr>
<td>OD38/pOD26 (mge1-G154D Δ43)</td>
<td>0 (0/64)</td>
<td>+</td>
</tr>
<tr>
<td>OD38/pOD27 (mge1-H183R Δ43)</td>
<td>0 (0/71)</td>
<td>+</td>
</tr>
<tr>
<td>OD38/pOD28 (mge1-G207S Δ43)</td>
<td>0 (0/62)</td>
<td>-</td>
</tr>
</tbody>
</table>

* Values in parentheses are actual number of Cam<sup>a</sup> transductants/actual number of Kan<sup>b</sup> transductants.

+ , large colonies and efficiency of plating of >1.0; –, no colonies (efficiency of plating, <10<sup>-5</sup>).
way, DnaJ together with DnaK can prevent $\sigma^{32}$ from associating with the RNA polymerase core, thus inhibiting transcription from $\sigma^{32}$-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, $\psi$[groE::lacZ], in an otherwise dnaJ-deleted strain. In a similar fashion, we tested the ability of our different MDJ1 alleles to down-regulate the heat shock response in the same $\psi$[groE::lacZ] reporter strain. Our results showed that the Mdj1p55 and Mdj1p55-267 proteins were unable to down-regulate the heat shock response, whereas the Mdj1p55-125-DnaJ and Mdj1p55-125-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 Mdj1p55 is functional in activating DnaK to bind $\sigma^{32}$, while the rest of the Mdj1p55 protein does not apparently participate in the activation of DnaK to bind $\sigma^{32}$. Further experiments are needed to demonstrate whether Mdj1p55’s failure to down-regulate the heat shock response is due to its inability to directly interact with $\sigma^{32}$ or can bind $\sigma^{32}$ 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

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**TABLE 3. Bacterial plating efficiencies**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>30°C</th>
<th>38.5°C</th>
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</thead>
<tbody>
<tr>
<td>pBAD22A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pWKG90 (dnaJ)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pOD50 (MDJ1::55)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pOD51 (mdj1-255)</td>
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<td>+</td>
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<tr>
<td>pOD52 (mdj1-55)</td>
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<td>pWKG121 (mdj1-dnaJ)</td>
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<td>pWKG100 (dnaJ12)</td>
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</tr>
<tr>
<td>pOD56 (mdj1-Ω)</td>
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<td>+</td>
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<tr>
<td>pWKG122 (mdj1-dnaJ12)</td>
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<td>+</td>
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**TABLE 4. Plating efficiency of bacteriophages**

<table>
<thead>
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<th>Phage</th>
<th>Relative plaque-forming efficiency*</th>
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<tbody>
<tr>
<td>pBAD22A</td>
<td>pOD1 (grpE) (MGE1343)</td>
</tr>
<tr>
<td>pOD25</td>
<td>pWKG90 (dnaJ)</td>
</tr>
<tr>
<td>pBAD22A</td>
<td>pOD50 (MDJ1::55)</td>
</tr>
<tr>
<td>pWKG100 (dnaJ12)</td>
<td>pOD56</td>
</tr>
<tr>
<td>pWKG121 (mdj1-dnaJ)</td>
<td>pWKG122 (mdj1-dnaJ12)</td>
</tr>
<tr>
<td>pBAD22A</td>
<td>pOD10 (dnaK)</td>
</tr>
<tr>
<td>pOD40 (SSC123)</td>
<td></td>
</tr>
</tbody>
</table>

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* Determined by spot testing serial dilutions of the indicated bacteriophages onto cell lawns. +, normal plaque size and efficiency of plating of $\approx 1.0$; −/−, no visible plaques (efficiency of plating, $<10^{-3}$).

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The figure (Fig. 5) represents plasmids containing different MDJ1 or dnaJ alleles under an inducible arabinose promoter (pBAD). Open boxes represent the different conserved domains of Mdj1p55, 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 plating efficiency at low and high temperatures of OD259 (dnaJ::Tn10 ΔcbpA::Kanr) transformed with plasmids containing different MDJ1 or dnaJ alleles (Table 3).
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 MGE1Δ43 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 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 has 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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