Purification and Biochemical Properties of Saccharomyces cerevisiae Mdj1p, the Mitochondrial DnaJ Homologue*

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The DnaK/DnaJ/GrpE heat shock proteins of Escherichia coli constitute the prototype DnaK chaperone machine. Various studies have shown that these three proteins work synergistically in a diverse array of biological functions, including protein folding and disaggregation, proteolysis, and transport across biological membranes. We have overexpressed and purified the mitochondrial Saccharomyces cerevisiae DnaJ homologue, Mdj1pΔ55, which lacks the mitochondrial presequence, and studied its biochemical properties in well defined in vitro systems. We find that Mdj1pΔ55 interacts with DnaK as judged both by an enzyme-linked immunosorbent assay, as well as by stimulation of DnaK’s weak ATPase activity in the presence of GrpE. In addition, Mdj1pΔ55 not only interacts with denatured firefly luciferase on its own, but also enables DnaK to bind to it in an ATP-independent mode. Using co-immunoprecipitation assays we can demonstrate the presence of a stable Mdj1pΔ55-luciferase-DnaK complex. However, in contrast to DnaJ, Mdj1pΔ55 does not appear to interact well with certain seemingly folded proteins, such as the σ2 heat shock transcription factor or the AP DNA replication protein. Finally, Mdj1pΔ55 can substitute perfectly well for DnaJ in the refolding of denatured firefly luciferase by the DnaK chaperone machine. These studies demonstrate that Mdj1pΔ55 has conserved most of DnaJ’s known biological properties, thus supporting an analogous functional role in yeast mitochondria.

The recent identification of the mitochondrial DnaK (mt-Hsp70/Ssc1p; Ref. 3), GrpE (Mge1p, also termed Yge1p and GrpEp; Refs. 4–7), and DnaJ (Mdj1p; Ref. 8) suggested the existence of a chaperone machine in mitochondria analogous to that of the E. coli DnaK chaperone machine. The Ssc1p and Mge1p proteins have been both characterized as essential components of the translocation of preproteins into the mitochondrial matrix (9, 10). Ssc1p has been shown to bind to and help transport preproteins into the matrix, in a dynamic complex with Tim44p and Mge1p (6, 11–13). Tim44p is a peripheral membrane protein which recruits Ssc1p at the proper position for protein import while Mge1p is a nucleotide release factor required to modulate the nucleotide-dependent stability of Ssc1p-Tim44p complex. Recently, Mge1p was shown to form a stable complex with Ssc1p via a loop structure located on the surface of Ssc1p’s ATPase domain (3). In addition, we previously showed that Mge1p could interact with DnaK and substitute for E. coli GrpE functions both in vivo and in vitro, suggesting a functional conservation of these two proteins (14, 15).

Unlike Ssc1p and Mge1p, Mdj1p is not essential for yeast growth and is particularly dispensable for protein import (8). However, Mdj1p plays an important role for newly translocated and mitochondrially synthesized proteins by preventing their subsequent aggregation (16). In addition, Mdj1p cooperates with Ssc1p and Mge1p to promote protein folding and degradation of misfolded proteins by the Pim1p protease (8). Mdj1p contains three of the characteristic domain structures found in DnaJ. This comprises the extreme N-terminal part, representing the “J-domain,” which makes the primary contact with DnaK (17, 18); the glycine/phenylalanine (G/F)-rich motif which is thought to play the role of a flexible linker between the J-domain and the C-terminal part of the protein (19, 20), and the cysteine (Cys)-rich region which binds zinc and participates in the interaction with the polypeptide substrate (21, 22). Therefore, by analogy to DnaJ, it has been proposed that Mdj1p is required to stimulate both the ATP hydrolysis of Ssc1p, and to guide unfolded substrates to Ssc1p. Recently, we showed that Mdj1pΔ55, a recombinant protein lacking its mitochondrial presequence (1–55 amino acids) could partially suppress the temperature-sensitive phenotype of an E. coli dnaJ mutant (15). In the present study we purified Mdj1pΔ55 and investigated whether it could replace DnaJ in a luciferase refolding assay in vitro. Our results indicate that Mdj1pΔ55 can specifically interact with denatured firefly luciferase, and subsequently stimulate DnaK to ensure efficient luciferase refolding. However, we found that Mdj1pΔ55 has lower affinity for seemingly native proteins, since, in contrast to DnaJ, Mdj1pΔ55 does not interact at all with the AP replication protein and poorly with the σ2 heat shock transcription factor.

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**Materials and Methods**

**Plasmid and Bacterial Strains**—The previously described E. coli OD259 (MC4100 dnlJ::Tn10 σ70 σ28A;kan' SsaI 714 araD139) (15, 23) strain and the pOD50 plasmid (pBAD22AMD/MjΔ55; Ref. 15) were used for the purification of Mdj1pΔ55.

**Purification of Mdj1pΔ55**—A culture of 6 liters of OD259 strain containing plasmid pOD50 was grown with aeration at 30 °C in LB broth supplemented with 100 μg/ml ampicillin to an A600 of 1.0. At that time the inducer arabinoise was added to a final concentration of 0.2% and the culture was grown for an additional 2 h. The cells were then harvested by centrifugation and the pellet (18 g) resuspended in 40 ml of buffer A (50 mM Tris/HCl, pH 8.0, 10% (w/v) sucrose) and lysed by the presence of 10 mM DTT, 10 mM EDTA, 1 mg/ml lysozyme, and 100 μg/ml phenylmethylsulfonyl fluoride for 45 min on ice. During the last 15 min, Brij 58 was added to a final concentration of 0.6%. The cells were subsequently sonicated for 3 min (30-s on/off bursts, power 60%, Sonifier cell disruptor B-30) and cells debris was removed by centrifugation in a Beckman angle 35 rotor at 28,000 rpm for 90 min at 4 °C. The supernatant was directly applied onto a DEAE-Sephacel column (2.5 cm × 12 cm), previously equilibrated in buffer B (50 mM Tris/HCl, pH 7.5, 5 mM DTT, 10% sucrose, 0.1% Triton X-100, 100 μg/ml phenylmethylsulfonyl fluoride). Proteins recovered in the void volume were dialyzed overnight against buffer C (40 mM potassium phosphate, pH 6.8, 0.1 M KCl, 10% (w/v) glycerol, 5 mM DTT, 0.05% Brij 58) at 4 °C. The dialysate was loaded onto a hydroxylapatite column (2.5 cm × 6 cm) previously equilibrated with buffer C. The column was subsequently washed with buffer C containing 140 mM potassium phosphate, and the proteins were eluted with buffer C containing 500 mM potassium phosphate. To avoid precipitation, the eluted proteins were slowly diluted with 3 volumes of 10% glycerol, 5 mM DTT, 0.05% Brij 58, and loaded onto a P11 phosphocellulose column (1.5 cm × 7 cm) previously equilibrated with buffer D (40 mM potassium phosphate, pH 6.8, 0.2 M KCl, 10% glycerol, 5 mM DTT, 0.05% Brij 58). Proteins were eluted with a linear gradient of 0.2–0.8 M KCl in buffer D. The fractions that were enriched in Mdj1p55 protein were pooled and loaded onto a Superox-200 gel filtration column (Pharmacia K16/66) previously equilibrated with buffer E (40 mM potassium phosphate, pH 7.0, 0.4 M KCl, 10% glycerol, 5 mM DTT, 0.05% Brij 58). Fractions containing highly purified Mdj1p55 protein were concentrated onto an hydroxylapatite column (0.5 × 0.5 cm). Proteins were eluted with 0.5 M potassium phosphate and purified proteins were dialyzed against buffer F (25 mM Hepes, pH 7.6, 0.4 M KCl, 15 mM MgCl2, 1 mM DTT, 0.001% Brij 58) and stored at −80 °C.

**DnaJ, DnaK, σ24, and Ap Proteins**—The purification of DnaJ, DnaK, σ24, and Ap proteins was purified according to Zylicz et al. (24, 26, 27) and Liberek et al. (26). Firefly luciferase was purchased from Sigma (L-9009), and anti-luciferase antibody from Promega (E851A).

**ATPase Assay and Kinetic Analyses**—The ATPase activity was performed in 20 μl of buffer G (50 mM Tris/HCl, pH 7.8, 50 mM NaCl, 50 mM KCl, 10 mM MgCl2, 2 mM DTT) and measured as previously described (14, 28). The hydrolyzed ATP was quantified by a PhosphorImager.

**Enzyme-linked Immunosorbent Assay (ELISA)**—The ELISA technique used for detection of protein–protein interactions has been previously described in detail (29). Each given value represents an average of three independent determinations. Briefly, the first protein was incubated at 0.5 μg/well for 1 h in 50 μl of phosphate-buffered saline onto 96-well microtiter plates. The wells were then washed and blocked with phosphate-buffered saline containing 0.2% BSA. The second protein was then added in buffer H (25 mM Hepes/KOH, pH 7.8, 15 mM KCl, 25 mM NaCl, 10 mM MgCl2, 0.1 mM EDTA, 2 mM DTT, 5% glycerol, 0.05% Triton X-100, and 0.2% BSA), incubated for 30 min at room temperature and cross-linked with 0.1% glutaraldehyde. Complex formation was estimated by the anti-second protein antibody, as described previously (29). Prior to use, firefly luciferase was denatured in buffer I (30 mM Tris/HCl, pH 7.6, 6 M guanidinium/HCl, and 5 mM DTT) for 30 min at room temperature.

**Determination of Firefly Luciferase Aggregation and Soluble Firefly Luciferase**—Firefly luciferase at 25 μM was denatured in buffer I for 30 min at room temperature. The denatured firefly luciferase was then diluted 100-fold in buffer J (25 mM Hepes/KOH, pH 7.6, 50 mM KCl, 5 mM β-mercaptoethanol, 5 mM Mg2+-acetate) in the presence of 1 mM ATP and various chaperones, as indicated. Aggregation of firefly luciferase was determined by light scattering at 320 nm using an Uvikon 940 spectrophotometer in a thermostatized cuvette at 30 °C. Subsequently, protein samples (400 μl) were centrifuged in Eppendorf tubes for 20 min at 4 °C and soluble proteins were precipitated with trichloroacetic acid (10% final concentration). Precipitated proteins were separated by means of SDS-PAGE and the amount of firefly luciferase was quantified by densitometry.

**Determination of Firefly Luciferase Refolding**—The renaturation of guanidinium-denatured firefly luciferase (250 nm) was carried out at 25 °C in 100 μl of buffer K (30 mM Hepes/KOH, pH 7.6, 40 mM KCl, 50 mM NaCl, 1 mM DTT, 7 mM Mg2+-acetate) in the presence of 1 mM ATP and various chaperones, as indicated, essentially as previously described (14). The refolding luciferase activity was measured using the Promega Luciferase Assay System (E1500) followed by liquid scintillation counting.

**Co-immunoprecipitation Experiments**—Guanidinium-denatured firefly luciferase was preincubated in 300 μl of buffer K containing 1 mM ATP for 10 min at 25 °C in the presence of various chaperones, as indicated. The reaction mixtures were then centrifuged in Eppendorf tubes for 10 min at 4 °C and soluble proteins were aliquoted into two tubes for co-immunoprecipitation experiments. Two μl of polyclonal DnaK or firefly luciferase antibodies were added to each series of Eppendorf tubes, respectively, and incubated for 1 h on ice. Fifty μl of a 1:1 (v/v) Pansorbin in buffer L (10 mM Tris/HCl, pH 7.3, 140 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS) was added, and following an additional 30-min incubation on ice, Pansorbin was washed three times with buffer L and once with 10 mM Tris/HCl, pH 7.3. Protein complexes associated with Pansorbin were detected following separation by SDS-PAGE and appropriate Western blot analyses.

**Results**

**Purification of the Mdj1pΔ55 Protein, the Saccharomyces cerevisiae DnaJ Homologue**—To purify the mature form of Mdj1p from E. coli, we used a previously described plasmid construct (pOD50; Ref. 15) which carries the Mdj1p gene lacking its mitochondrial leader presequence (1–55 amino acids) and fused to the arabinoise-inducible promoter of a pBAD vector. The pOD50 plasmid was introduced into a dnaJ-deleted strain (OD259) to prevent potential contamination with endogenous DnaJ. Mdj1pΔ55 was induced by the addition of 0.2% t-arabinoise (final concentration) and purified to homogeneity according to the protocol described under “Materials and Methods.” As judged by SDS-PAGE, purified Mdj1pΔ55 protein migrates as a 50-kDa protein, consistent with its predicted molecular mass (Fig. 1). Western blot analysis of the purified Mdj1pΔ55 and DnaJ, using αMdj1p and αDnaJ antibodies revealed the absence of cross-reactivity and confirmed the identity of the recombinantly produced proteins (data not shown).

Previously, DnaJ has been shown to be dimers, either by means of glycerol gradient centrifugation or gel filtration (24). To determine whether Mdj1pΔ55 behaves similarly to DnaJ in solution, we attempted to analyze the quaternary structure of Mdj1pΔ55 by gel filtration. This experiment failed to estimate the accurate molecular weight of Mdj1pΔ55, primarily because

![](image-url)
functions of the wild type DnaJ protein that we tested (15). This includes growth at non-permissive temperatures, suppression of the block on bacteriophage λ growth, and down-regulation of the σ54-dependent heat shock response. DnaJ is known to stimulate up to 50-fold the ATPase activity of DnaK in the presence of GrpE (28). In analogy with this, Mdj1pΔ55 could also stimulate the ATPase activity of DnaK, albeit at a reduced efficiency compared with DnaJ (Fig. 2). This result is in good agreement with that obtained by Horst et al. (30) where a difference of only 2–3-fold was found between mitochondrially-purified Mdj1p and DnaJ.

To further delineate the interaction of Mdj1pΔ55 with DnaK, we tested the formation of this complex by the sensitive ELISA immunodetection technique (Fig. 3). To do this, we first bound DnaJ or Mdj1pΔ55 to the wells of microtiter plates and, following extensive washing and blocking procedures, added increasing amounts of DnaK. It was previously shown that under these conditions ATP hydrolysis is required for the formation of an efficient DnaK-DnaJ complex (29). As expected, Mdj1p and DnaJ were only detected in a complex with DnaK in the presence of ATP, while very little complex was detected in the absence of nucleotide or in the presence of ATPγS, a slowly hydrolyzable ATP analogue. Surprisingly, we found that Mdj1pΔ55 binds more efficiently to DnaK than DnaJ does (see “Discussion”).

**Mdj1pΔ55 Specifically Interacts with Unfolded Firefly Luciferase—**DnaJ has been previously shown to participate with DnaK and GrpE in the refolding of denatured firefly luciferase in vitro (31, 32). During this process, DnaJ can act alone to bind to and prevent the aggregation of unfolded firefly luciferase. In the presence of ATP and DnaJ, denatured firefly luciferase is further stabilized in a complex containing luciferase, DnaK and DnaJ. The addition of GrpE allows the refolding of firefly luciferase by the efficient disruption of the complex and the recycling of the DnaK and DnaJ chaperones. In a recent study, Westermann et al. (16) demonstrated an important role of Mdj1p in the prevention of heat-induced firefly luciferase aggregation, indicating that Mdj1p can interact with unfolded firefly luciferase. To demonstrate a direct interaction between Mdj1pΔ55 and unfolded luciferase in vitro, firefly luciferase was denatured in 6 M guanidinium-HCl and its interaction with Mdj1pΔ55 was tested using the sensitive ELISA technique. As
FIG. 5. Mdj1pΔ55 and DnaK synergistically suppress aggregation of denatured firefly luciferase. A. Guanidinium-denatured luciferase was diluted 100-fold (250 nM), in various reaction mixtures containing 1 mM ATP and the indicated proteins. The aggregation of luciferase was measured at 320 nm. The value of 100% represents that obtained in the absence of additional proteins. ▲, no proteins; ■, DnaK (500 nM); ●, DnaJ (250 nM); ○, DnaK (500 nM) and DnaJ (250 nM); ◆, Mdj1pΔ55 (500 nM); ▼, native luciferase (250 nM); B, following centrifugation, to remove aggregated proteins, the soluble proteins were processed by SDS-PAGE, the gels stained by Coomassie Brilliant Blue, and the amount of luciferase was quantified by densitometry (represented as % of 250 nM native luciferase).

The light scattering experiments, shown in Fig. 5, revealed that Mdj1pΔ55 can replace DnaJ in modulating the interaction between denatured firefly luciferase and DnaK. The light scattering experiments, shown in Fig. 5A, revealed that Mdj1pΔ55 specific complex with DnaK and denatured firefly luciferase. Because the formation of a stable complex between denatured firefly luciferase, DnaJ and DnaK is likely an essential step in the refolding of firefly luciferase, we tested whether Mdj1pΔ55 can create DnaJ in modulating the interaction between denatured firefly luciferase and DnaK. The light scattering experiments, shown in Fig. 5A, revealed that Mdj1pΔ55 specific complex with DnaK and denatured firefly luciferase and works cooperatively with DnaK to prevent firefly luciferase aggregation in the presence of ATP. These results were subsequently confirmed by quantifying the levels of soluble firefly luciferase for each reaction (Fig. 5B). It turned out that in the absence of chaperones approximately 5% of the firefly luciferase was recovered in a soluble form, whereas approximately 80% soluble firefly luciferase was recovered in the presence of either DnaK and DnaJ or DnaK and Mdj1pΔ55. Finally, because we found that the complex containing Mdj1pΔ55 is less stable than that containing DnaJ over a longer period of time (results not shown), we investigated the formation of such complexes by co-immunoprecipitation experiments. As expected, a very weak complex was detected between DnaK and denatured firefly luciferase alone, while the addition of either DnaJ or Mdj1pΔ55 allowed the quantitative co-immunoprecipitation of DnaK with denatured firefly luciferase in the presence of ATP (Fig. 6). Taken together, our results demonstrate the capacity of Mdj1pΔ55 to stabilize the denatured firefly luciferase-DnaK complex, thus preventing denatured firefly luciferase aggregation.

FIG. 6. Co-immunoprecipitation of DnaK and denatured firefly luciferase in the presence of DnaJ or Mdj1pΔ55. Guanidinium-denatured luciferase (250 nM) and DnaK (250 nM) were preincubated in various reaction mixtures containing 1 mM ATP and DnaJ (250 nM) or Mdj1pΔ55 (250 nM) as indicated. A, denatured luciferase that was co-immunoprecipitated by anti-luciferase antibodies was visualized by Western blot analysis using anti-luciferase antibodies. B, DnaK that was co-immunoprecipitated by anti-luciferase antibodies was visualized by Western blot analysis using anti-DnaK antibodies. Denatured luciferase is abbreviated as “D. Lucif.” on figure.
yeast homologues to stimulate the activity of the heterologous DnaK protein in protein folding reactions.

The σ32 Heat Shock Factor Interacts Poorly with Mdj1pΔ55—The E. coli DnaK chaperone machine is involved in the regulation of the E. coli heat shock response and bacteriophage λ DNA replication through an association of the σ32 heat shock factor and the AP replication protein, respectively. Subsequent experiments have shown that the σ32 heat shock factor binds more efficiently to DnaK than it does to DnaJ, whereas AP binds more efficiently to DnaK than DnaJ (29, 33). We tested whether Mdj1pΔ55 could interact with these two seemingly native protein substrates to the same extent as does DnaJ. The interactions of Mdj1pΔ55 or DnaJ with these two substrates were monitored using the ELISA technique described above. It turned out that Mdj1pΔ55 did not interact at all with AP and extremely poorly with σ32 (Fig. 8). These results indicate that, assuming that Mdj1pΔ55 and DnaJ have conserved to a large extent the ability to interact with denatured polypeptide, DnaJ may have evolved further to interact with certain seemingly native proteins, such as the σ32 heat shock factor and the AP replication protein.

**DISCUSSION**

It has been proposed that protein folding in E. coli is likely mediated by the successive actions of the two DnaK and GroEL chaperone machines (1, 34). In this process the DnaK and DnaJ chaperones are the first to bind to newly synthesized proteins. Then, GrpE is required to release the bound polypeptide substrate, thus recycling DnaK. The released polypeptide can either fold on its own or may necessitate interactions with either the GroEL/GroES chaperone machine or potentially another chaperone(s).

The recent identification of all homologues of the DnaK and GroEL chaperone machine members in the mitochondrial matrix of *Saccharomyces cerevisiae* has suggested an analogous protein folding pathway in this eukaryotic organelle as well (35, 36).

The binding and release of denatured polypeptides to DnaK is tightly regulated by the DnaJ and GrpE proteins which jointly stimulate its ATPase activity by at least 50-fold (28). Previously, we showed that the yeast GrpE homologue (Mge1p) could substitute for its bacterial homologue in *in vivo* and *in vitro* and that the J-domain of Mdj1p could replace the J-domain of DnaJ, thus ensuring high levels of DnaK activity (14, 15). In this study, we showed that not only Mdj1pΔ55 can functionally interact with DnaK, but interestingly, DnaK can form a more stable complex with Mdj1pΔ55 than it does with DnaJ. 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 (17, 18). Therefore, we suspect that the J-domain of Mdj1p may possess a higher affinity for DnaK than that of DnaJ. In agreement with this, we have shown by genetic means that a chimeric protein consisting of the J-domain of Mdj1pΔ55 fused to the rest of DnaJ acts as “super-DnaJ” protein, capable of substituting for all of DnaJs functions in an E. coli cell, albeit at much lower concentrations than DnaJ (15).

As with its DnaJ bacterial counterpart, various studies indicate that Mdj1p is a chaperone on its own right. For example, two recent studies reported that Mdj1p can significantly retard the heat-induced aggregation of luciferase and allow the binding of denatured luciferase to Sac1p (16, 36). Here, in agreement with these findings, we showed that Mdj1pΔ55 cooperatively interacts with DnaK to prevent the aggregation of guanidinium-denatured firefly luciferase, as well as its subsequent refolding in the presence of GrpE. Clearly these results indicate that DnaJ and Mdj1pΔ55 have conserved the ability to functionally interact with denatured luciferase and DnaK. Nevertheless, we did find a strong difference in the behavior of DnaJ and Mdj1pΔ55 toward the σ32 heat shock factor and the AP replication protein, notably that Mdj1pΔ55 binds much less efficiently to these substrates. In this context, it is worth noting that denatured firefly luciferase and σ32 most likely bind with differential affinities to various sites on DnaJ, since the deletion of the Cys-rich region of DnaJ drastically reduces its interaction with unfolded luciferase, while its interaction with σ32 is largely unaffected (22). This finding strongly suggests a specificity of DnaJ toward its interactions with certain seemingly native proteins. Finally, the demonstrated poor interaction of Mdj1pΔ55 with σ32 explains our previous finding, namely that Mdj1pΔ55 cannot substitute for DnaJ in the down-regulation the heat shock response in *E. coli* (15).

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**REFERENCES**

1. Hirt, F. U. (1996) *Nature* **381**, 571–580
2. Georgopoulos, C., Liberek, K., Zylicz, M., and Ang, D. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto, R., Tissières, A., and Georgopoulos, C., eds) pp. 209–249, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Miao, B., Davis, J. E., and Craig, E. A. (1997) *J. Mol. Biol.* **265**, 541–552
4. Lalaraya, S., Gambill, B. D., and Craig, E. A. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6481–6485
5. Ikeda, E., Yoshida, S., Mitsuazawa, H., Uno, I., and Toh-e, A. (1994) *FEBS Lett.* **339**, 265–268
6. Basset, J., Maarse, A. C., Krawczynski, K., Kubrich, M., Muller, H., Meijer, M., Craig, K. A., and Pfanner, N. (1994) *J. Cell Biol.* **127**, 1547–1556
7. Bolliger, L., Deloche, O., Glick, B. S., Georgopoulos, C., Jeno, P., Kromodou, N., Horst, M., Morishima, N., and Schatz, G. (1994) *EMBO J.* **13**, 2006–2006
8. Rowley, N., Prip-Buus, C., Westermann, B., Brown, C., Schwarz, E., Barrell, B., and Neupert, W. (1994) *Cell* **77**, 249–259
9. Kang, P. J., and Craig, E. A. (1990) *J. Bacteriol.* **172**, 2055–2064
10. Schneider, H.-C., Westermann, B., Neupert, W., and Brunner, M. (1996) *EMBO J.* **15**, 5786–5803
11. Schneider, H.-C., Berthold, I., Bauer, M. F., Dietmeier, K., Giaud, B., Brunner, M., and Neupert, W. (1994) *Nature* **371**, 708–733
12. Ungermaier, C., Neupert, W., and Cyr, D. M. (1994) *Science* **266**, 1250–1253
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13. Berthold, J., Bauer, M. F., Schneider, H. C., Klaus, C., Neupert, W., and Brunner, M. (1995) Cell 81, 1085–1094
14. Deloche, O., and Georgopoulos, C. (1996) J. Biol. Chem. 271, 23960–23966
15. Deloche, O., Kelley, W. L., and Georgopoulos, C. (1997) J. Bacteriol. 179, 6066–6075
16. Westermann, B., Gaume, B., Herrmann, J. M., Neupert, W., and Schwarz, E. (1996) Mol. Cell. Biol. 16, 7063–7071
17. Wall, D., Zylicz, M., and Georgopoulos, C. (1994) J. Biol. Chem. 269, 5446–5451
18. Karcz, A. W., and McMacken, R. (1996) J. Biol. Chem. 271, 11236–11246
19. Szyperski, T., Pellacchia, M., Wall, D., Georgopoulos, C., and Wuthrich, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11343–11347
20. Pellacchia, M., Szyperski, T., Wall, D., Georgopoulos, C., and Wuthrich, K. (1996) J. Mol. Biol. 260, 236–250
21. Szabo, A., Kurszun, R., Hartl, F. U., and Flanagan, J. (1996) EMBO J. 15, 408–417
22. Banecki, B., Liberek, K., Wall, D., Wawrzynow, A., Georgopoulos, C., Bertoli, E., Tanfani, F., and Zylicz, M. (1996) J. Biol. Chem. 271, 14840–14848
23. Kelley, W. L., and Georgopoulos, C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2679–2684
24. Zylicz, M., Yamamato, T., McKittrick, N., Sell, S., and Georgopoulos, C. (1985) J. Biol. Chem. 260, 7591–7598
25. Zylicz, M., Ang, D., and Georgopoulos, C. (1987) J. Biol. Chem. 262, 17437–17442
26. Liberek, K., Galitski, T. P., Zylicz, M., and Georgopoulos, C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3516–3520
27. Zylicz, M., Ang, D., Liberek, K., and Georgopoulos, C. (1989) EMBO J. 8, 1601–1608
28. Liberek, K., Mrozalek, J., Ang, D., Georgopoulos, C., and Zylicz, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2874–2878
29. Wawrzynow, A., and Zylicz, M. (1995) J. Biol. Chem. 270, 19300–19306
30. Horst, M., Oppiger, W., Raspert, S., Schünfeld, H. J., Schatz, G., and Asem, A. (1997) EMBO J. 16, 1842–1849
31. Schröder, H., Langer, T., Hartl, F.-U., and Bukau, B. (1993) EMBO J. 12, 4337–4344
32. Szabo, A., Langer, T., Schröder, H., Flanagan, J., Bukau, B., and Hartl, F. U. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10345–10349
33. Wawrzynew, A., Banecki, B., Wall, D., Liberek, K., Georgopoulos, C., and Zylicz, M. (1995) J. Biol. Chem. 270, 19307–19311
34. Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer, M. K., and Hartl, F. U. (1992) Nature 356, 683–689
35. Langer, T., and Neupert, W. (1994) in The biology of heat shock Proteins and Molecular Chaperones (Morimoto, R. I., Tissières, A., and Georgopoulos, C., eds) pp. 53–83, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
36. Prip-Buus, C., Westermann, B., Schmitt, M., Langer, T., Neupert, W., and Schwarz, E. (1996) FEBS Lett. 390, 142–146