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Identification and functional analysis of chaperonin 10, the groES homolog from yeast mitochondria

(protein folding/ribulose-1,5-bisphosphate carboxylase/*Saccharomyces cerevisiae*/molecular chaperones/hsp60)

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ABSTRACT **Chaperonin 60 (cpn60) and chaperonin 10 (cpn10) constitute the chaperonin system in prokaryotes, mitochondria, and chloroplasts. In *Escherichia coli*, these two chaperonins are also termed groEL and groES. We have used a functional assay to identify the groES homolog cpn10 in yeast mitochondria. When dimeric ribulose-1,5-bisphosphate carboxylase (Rubisco) is denatured and allowed to bind to yeast cpn60, subsequent refolding of Rubisco is strictly dependent upon yeast cpn10. The heterologous combination of cpn60 from *E. coli* plus yeast cpn10 is also functional. In contrast, yeast cpn60 plus *E. coli* cpn10 do not support refolding of Rubisco. In the presence of MgATP, yeast cpn60 and yeast cpn10 form a stable complex that can be isolated by gel filtration and that facilitates refolding of denatured Rubisco. Although the potassium-dependent ATPase activity of *E. coli* cpn60 can be inhibited by cpn10 from either *E. coli* or yeast, neither of these cpn10s inhibits the ATPase activity of yeast cpn60. Amino acid sequencing of yeast cpn10 reveals substantial similarity to the corresponding cpn10 proteins from rat mitochondria and prokaryotes.**

The chaperonin system of *Escherichia coli* comprises two proteins, b-cpn60 (groEL) and b-cpn10 (groES) (1). These chaperonins can mediate the refolding of proteins under "nonpermissive" conditions—i.e., conditions that do not allow spontaneous folding. Examples of proteins that undergo chaperone-dependent refolding include ribulose-1,5-bisphosphate carboxylase (Rubisco) (2), citrate synthase (3), and rhodanese (4, 5). In these cases the refolding reaction requires both chaperonin proteins as well as MgATP and K^+ (6). Homologous chaperonin systems have been detected in mammalian mitochondria (7–9) and in chloroplasts (10, 11), and some of these systems have been shown to be functional *in vitro*. The cpn60 from yeast mitochondria, hsp60 (12, 13), has been reported to interact with some proteins newly imported into the matrix compartment (14, 15). However, a yeast mitochondrial homolog of cpn10 (mt-cpn10) has so far escaped detection. We have now identified yeast mt-cpn10, established its structural similarity to cpn10 from other species, and characterized this protein on the basis of its functional properties.

MATERIALS AND METHODS

Materials. All radiochemicals were obtained from DuPont/NEN. Dimeric Rubisco from *Rhodospirillum rubrum* was purified from *E. coli* expressing the plasmid-borne gene (16, 17). A published extinction coefficient was used to determine the concentration of the protein (18). Ribulose 1,5-bisphosphate was synthesized and purified as previously reported (19). b-cpn60 (groEL) and b-cpn10 (groES) were purified from lysates of *E. coli* cells harboring the multicopy plasmid pGroESL (20) as reported elsewhere (21). Beef liver mt-cpn10 was purified by a published protocol (7, 8). Pea chloroplast cpn10 (ch-cpn10) was purified by a method to be published elsewhere (protocol available upon request). The concentration of purified chaperonin proteins was determined by quantitative amino acid analyses and is reported on a protomer basis, unless otherwise specified. Protein concentration was otherwise determined with the Bradford reagent (22).

Enzyme Assays. Rubisco was activated and assayed after folding as described (2, 6). The ATPase activity of cpn60 was determined at 25°C with 100 μM [γ-^{32}P]ATP as substrate in the presence of 50 mM Tris·HCl at pH 7.8, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, and either 1 mM or 100 mM KCl; released $^{32}P_i$ was extracted with molybdate (6, 21). Specific ATPase activity is expressed as mol of P_i released per min per mol of cpn60 protomer.

Chaperonin-Facilitated Refolding of Rubisco. The substrate for folding reactions was dimeric Rubisco, unfolded in 10 mM HCl (23). To quantify refolding, native Rubisco was treated identically, except that KCl was substituted for HCl. The refolding reactions were conducted as before (2, 6) in 0.1 M Hepes–KOH at pH 7.6, 10 mM magnesium acetate, 5 μM bovine serum albumin, 2.5 mM DTT, and 3 mM ATP. When necessary, the reactions were terminated with a glucose/hexokinase trap (2).

Purification of Yeast mt-cpn60. *Saccharomyces cerevisiae* (strain W303; *MATα his3 ura3 trp1 ade2 leu2*) bearing the multicopy plasmid YEpHSP60 (12) was grown and mitochondria were isolated as previously described (24). Mitochondria were suspended at a protein concentration of 5 mg/ml in 20 mM Hepes–KOH at pH 7.4 containing 0.3 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin A, and 1 μM leupeptin and were sonicated on ice. Insoluble material was removed by centrifugation at 150,000 × *g* for 30 min. The supernatant solution derived from 100 mg of yeast mitochondrial protein was applied to a Merck trimethylaminoethyl (TMAE) Superfine anion-exchange column equilibrated with 20 mM Mes–NaOH at pH 6.0. Fractionation was achieved in 20 mM Mes–NaOH at pH 6.0, using the following gradient: 0–10 ml, no NaCl; 10–30 ml, 0.1 M NaCl; 30–50 ml, 0.2 M NaCl; followed by a linear increase to 0.5 M NaCl from 50 to 90 ml. mt-cpn60 was eluted between 0.35 and 0.46 M NaCl. The collected material was concentrated with a Centricon 30

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Abbreviations: cpn60 and cpn10, chaperonins 60 and 10, respectively; the prefixes b-, ch-, and mt- refer to the bacterial, chloroplastic, and mitochondrial chaperonins, respectively; Rubisco, ribulose-1,5-bisphosphate carboxylase; DTT, dithiothreitol; TMAE, trimethylaminoethyl.

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microconcentrator and washed once with 50 mM Tris·HCl, pH 7.2/0.1 mM EDTA/1 mM DTT. For further purification 1 ml of the concentrate (8 mg of protein per ml) was subjected to gel filtration on a Sephacryl S-300 column (2.5 × 40 cm) equilibrated with 50 mM Tris·HCl, pH 7.2/0.1 mM EDTA/1 mM DTT/50 mM NaCl.

Partial Purification of Yeast mt-cpn10. Mitochondria were isolated from lactate-grown *S. cerevisiae* strain D273-10B (24). Preparation of the extract and purification on Merck TMAE was as described for mt-cpn60 (see above). Yeast mt-cpn10 was eluted with the flow-through, which was next applied to a column of FastFlow Sepharose S (Pharmacia; bed volume = 3 ml) equilibrated with 20 mM Mes-NaOH, pH 6.2. The column was developed successively with 6 ml each of 20 mM Mes–NaOH, pH 6.2, containing no NaCl, 0.3 M NaCl, or 0.6 M NaCl. Yeast mt-cpn10 activity, determined by the heterologous b-cpn60/Rubisco folding assay, was confined to the 0.3 M salt eluate.

Amino Acid Sequence Analysis. Yeast mt-cpn10 was electroblotted from an SDS/10–20% polyacrylamide gradient gel (see Fig. 4) onto polyvinylidine difluoride (25) and either cleaved with cyanogen bromide or digested with trypsin (26). The tryptic fragments were separated on a Brownlee Lab Spheri-5RP-18 column (1 × 250 mm) at 40 μl/min with a 60-min linear gradient from 0.05% trifluoroacetic acid in water to 60% acetonitrile/0.04% trifluoroacetic acid. Selected peaks were sequenced by automated Edman degradation using an Applied Biosystems 473A or 477A protein sequenator.

RESULTS

Yeast Mitochondria Contain a cpn10-like Activity. When unfolded Rubisco bound to b-cpn60 was incubated in the presence of MgATP, no active Rubisco was released. However, addition of a yeast mitochondrial extract caused release of a modest quantity of active Rubisco (Fig. 1*A*). This release was proportional to the amount of mitochondrial extract added. This result strongly suggested that yeast mitochondria contain cpn10 and that the b-cpn60–Rubisco complex could be used as a substrate in a functional assay for yeast mt-cpn10 (see also refs. 7, 9, and 11). The use of a functional assay proved to be crucial, as yeast mt-cpn10 was not an abundant protein in the mitochondrial extract used in this study: analysis of the extract by SDS/PAGE and staining with Coomassie brilliant blue revealed only very faint bands in the 10-kDa region, even though the yeast mt-cpn60 band was quite prominent (Fig. 1*C*).

Using the Rubisco refolding assay, we partially purified yeast cpn10 by ion-exchange chromatography (Fig. 1*B*). mt-cpn10 activity was eluted from the Sepharose S column with 0.3 M NaCl, suggesting that yeast mt-cpn10, like other eukaryotic cpn10s examined to date, is a basic protein (P.V.V., unpublished observations). Although the 0.3 M NaCl eluate still contained several proteins, its specific cpn10 activity was about 30-fold higher than that of the unfractionated extract (Figs. 2–4). SDS/PAGE revealed that a 9-kDa protein was enriched in this salt eluate fraction (Fig. 1*C*).

Yeast mt-cpn60 Arrests the Spontaneous Folding of Unfolded Rubisco. The first step in chaperonin-facilitated protein folding is the formation of a stable complex between cpn60 and the unfolded protein (2). For example, the spontaneous folding of denatured Rubisco under permissive conditions was completely arrested by including approximately 0.5 mol equivalent of b-cpn60 oligomer. Native Rubisco was recovered from this binary complex by the addition of MgATP and either b-cpn10 or yeast mt-cpn10 (Fig. 2*A*). Yeast mt-cpn60 also inhibited the spontaneous folding of Rubisco (Fig. 2*B*), although 2–3 times more yeast mt-cpn60 than b-cpn60 was needed for comparable inhibition. When

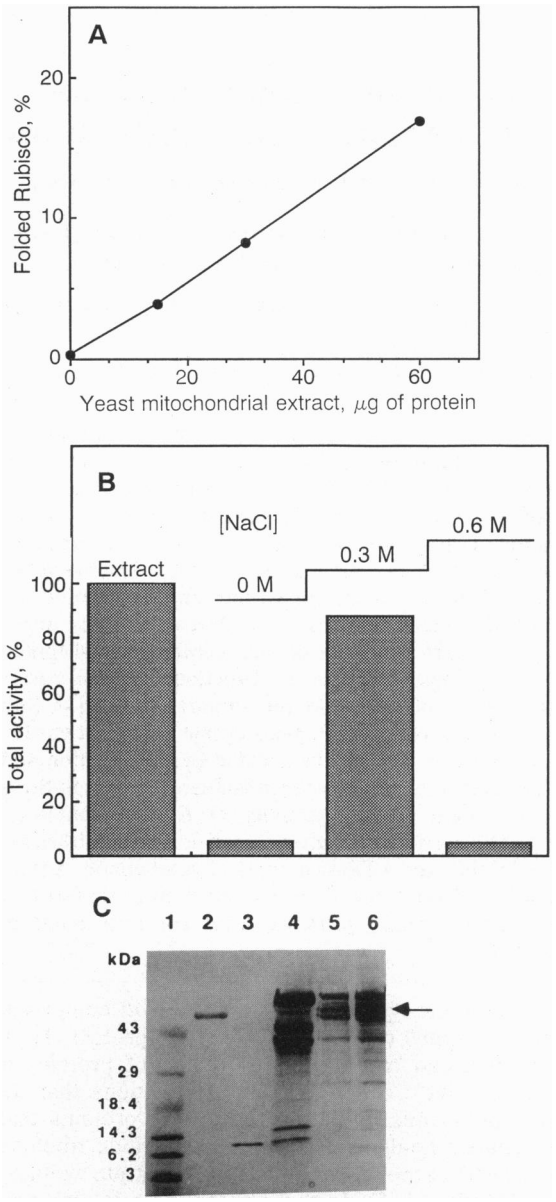

FIG. 1. Identification and partial purification of a Rubisco refolding activity in a yeast mitochondrial extract. (*A*) Folding of Rubisco was tested under conditions nonpermissive for unassisted folding. The mixture contained 2 μM b-cpn60, the indicated amounts of mitochondrial extract, 100 mM Hepes–KOH at pH 7.6, 5 μM bovine serum albumin, 2.5 mM DTT, and 100 nM acid-denatured Rubisco. The final volume was 150 μl. The reactions were started by adding 5 mM magnesium acetate and 2.5 mM MgATP, and folding was allowed to proceed for 1 hr at 24°C. Rubisco activity was assayed as described (2, 6). (*B*) Partial purification of mt-cpn10 on a FastFlow Sepharose S column. mt-cpn10 activity was assayed as in *A* and is expressed as percent of the activity present in the flow-through of the Merck TMAE column. (*C*) Detection of yeast mt-cpn10 after partial purification on a FastFlow Sepharose S column. Samples were subjected to SDS/15% PAGE and stained with Coomassie brilliant blue. Lanes: 1, molecular mass standards; 2, b-cpn60 (2 μg); 3, bovine mt-cpn10 (2 μg); 4, partially purified yeast mt-cpn10 (38 μg); 5, mitochondrial extract (14 μg); and 6, mitochondrial extract (28 μg). The arrow identifies yeast mt-cpn60.

the yeast mt-cpn60–Rubisco complex was supplemented with MgATP and the enriched preparation of yeast mt-cpn10, active Rubisco was recovered (Fig. 2*B*); as with mammalian mt-cpn60 (8), b-cpn10 could not replace yeast mt-cpn10.

Heterologous Chaperonin Systems. The recovery of native Rubisco from complexes of unfolded Rubisco with either

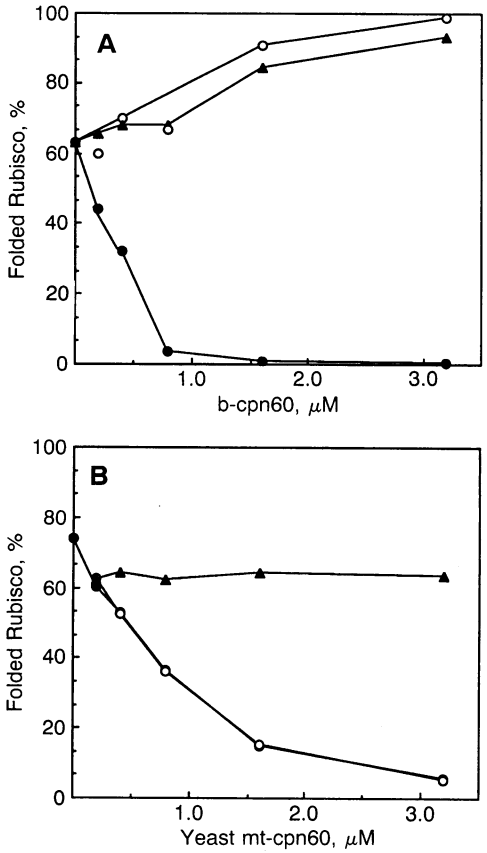

FIG. 2. Inhibition of spontaneous folding of Rubisco by cpn60 and reversal of this inhibition by cpn10. Reaction mixtures contained 100 mM Tris·HCl at pH 7.7, 20 mM $MgCl_2$, 28 mM KCl, 2.5 mM DTT, 5 μM bovine serum albumin, and the indicated concentrations of b-cpn60 (*A*) or yeast mt-cpn60 (*B*). Acid-denatured Rubisco (100 nM) was added to the ice-cold mixtures, and spontaneous folding was allowed to proceed for 5 hr at 15°C. The reaction mixtures were then split into three aliquots. One aliquot was supplemented with 2.5 mM MgATP and 5 mM magnesium acetate (●); the second aliquot was supplemented with 2.5 mM MgATP, 5 mM magnesium acetate, and 10 μM b-cpn10 (○); and the third aliquot was supplemented with 2.5 mM MgATP, 5 mM magnesium acetate, and partially purified yeast mt-cpn10 at 0.2 mg/ml (▲). After 1 hr at 24°C, each aliquot was assayed for Rubisco activity.

b-cpn60 or yeast mt-cpn60 increased with the amount of yeast mt-cpn10 added (Fig. 3). In both cases the recovery of active Rubisco required the presence of yeast mt-cpn10, MgATP, and K^+ (data not shown). The heterologous combination of b-cpn60 and yeast mt-cpn10 was actually more efficient in refolding Rubisco than the homologous yeast chaperonin system. Indeed, b-cpn60 was found to interact functionally with cpn10s from evolutionarily diverse sources (Fig. 3*B*). Yeast mt-cpn60, like bovine mt-cpn60, was more discriminating. b-cpn10 not only was unable to release active Rubisco from the yeast mt-cpn60–Rubisco complex (Fig. 2*B*) but also failed to block the releasing effect of yeast mt-cpn10 (data not shown). Thus yeast mt-cpn60 and b-cpn10 do not form a stable complex. On the other hand, both bovine mt-cpn10 (7, 8) and ch-cpn10 (11) could release some Rubisco from yeast mt-cpn60 in a folded active state (Fig. 3*B*).

ATPase Activity of Yeast mt-cpn60. Like b-cpn60, yeast mt-cpn60 catalyzed the K^+-dependent hydrolysis of ATP. At 25°C the hydrolysis rate per molecule of protomer was about 6 min^{-1} (Table 1), and the K^+ activation constant K_{act} (21), determined with 100 μM ATP, was approximately 1 mM. These kinetic constants are similar to those reported for b-cpn60 (21). Addition of a molar equivalent of oligomeric

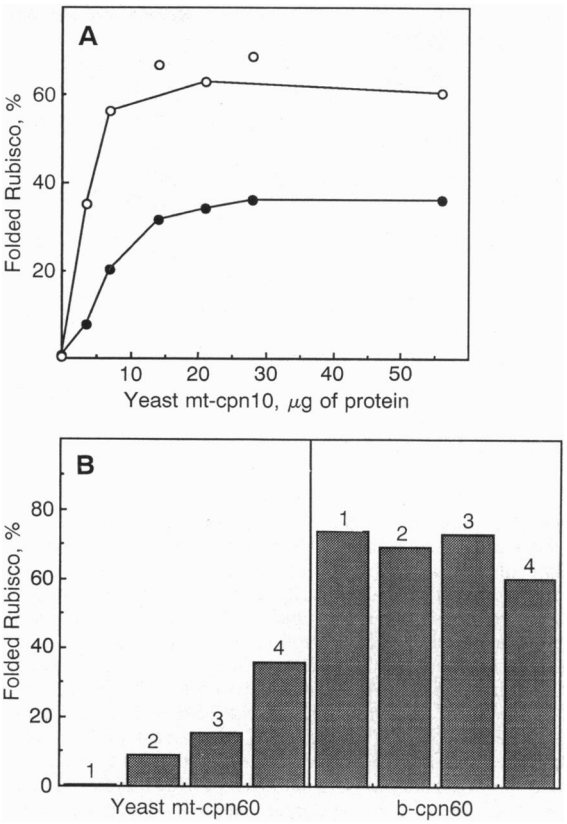

FIG. 3. Comparison of homologous and heterologous cpn60–cpn10 systems. (*A*) Partially purified yeast mt-cpn10 was assayed for its ability to promote refolding of acid-denatured Rubisco that had bound to b-cpn60 (○) or yeast mt-cpn60 (●). (*B*) Effect of different cpn10 preparations on the refolding of acid-denatured Rubisco bound to either yeast mt-cpn60 (*Left*) or b-cpn60 (*Right*). Bars: 1, b-cpn10 (3.75 μM); 2, ch-cpn10 (6.6 μM); 3, bovine mt-cpn10 (4.5 μM); and 4, yeast mt-cpn10 preparation (30 μg). Each assay was saturated for cpn10 protein; doubling the cpn10 concentration in the reaction mixture did not increase the folding activity (not shown). Assay conditions were as described for Fig. 1, with the cpn60 proteins at 2 μM.

b-cpn10 to b-cpn60 inhibited the hydrolysis of ATP; inhibition was complete at 1 mM K^+, and 50% at 100 mM K^+ (Table 1). Yeast mt-cpn10 also inhibited the ATPase activity of b-cpn60, but the inhibition was only partial and was seen only

Table 1. Effect of cochaperonins on the ATPase activity of cpn60 from yeast and *E. coli*

Chaperonins	Specific ATPase activity, mol/min per mol of protomer	
	1 mM KCl	100 mM KCl
b-cpn60	4.6 ± 0.2	5.7 ± 0.2
+ b-cpn10	<0.1	3.0 ± 0.3
+ yeast mt-cpn10	1.9*	5.7*
Yeast mt-cpn60	4.1 ± 0.4	6.8 ± 0.9
+ b-cpn10	4.2 ± 0.4	7.0 ± 0.8
+ yeast mt-cpn10	3.4 ± 0.2	6.6 ± 0.2

Concentrations of pure proteins (as determined by quantitative amino acid analysis; protomer basis) were as follows: b-cpn60, 0.2 μM; yeast mt-cpn60, 1.1 μM; and b-cpn10, 4.2 μM. The approximate concentration of functional yeast mt-cpn10 (0.5 μM in b-cpn60 assay, 1.0 μM in yeast mt-cpn60 assay) was determined by titrating the amount of folded Rubisco discharged from the appropriate cpn60–unfolded Rubisco complex. The measurements with yeast mt-cpn60 were performed in triplicate; results are presented as mean ± SEM.
*Corrected for ATPase activity (0.3 ± 0.1) of the yeast mt-cpn10 preparation alone; these values are from a single experiment.

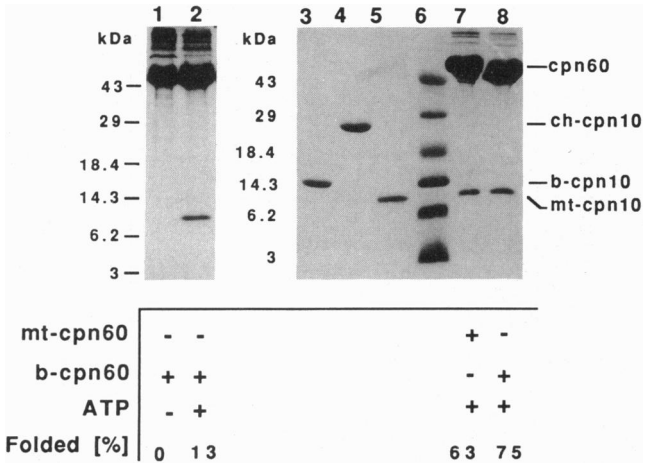

FIG. 4. Yeast mt-cpn10 forms a stable active complex with b-cpn60 or yeast mt-cpn60. Reaction mixtures contained 100 mM Tris·HCl at pH 7.6, 10 mM KCl, 10 mM $MgCl_2$ (buffer A) in a final volume of 200 μl. b-cpn60 and yeast mt-cpn60 were included at concentrations of 11.5 μM and 12 μM, respectively. Partially purified yeast mt-cpn10 (see *Materials and Methods* and Fig. 1 *B* and *C*) was added to a protein concentration of 7 mg/ml (lanes 1 and 2) or 28 mg/ml (lanes 7 and 8). Reaction mixtures 2, 7, and 8 were supplemented with 0.65 mM ATP and incubated for 12 min at 24°C. Each reaction mixture was then applied to a TSK-300 sizing column and fractionated at 24°C in buffer A at a flow rate of 1 ml/min. For samples 2, 7, and 8, 0.25 mM ATP was included in the column buffer. Proteins eluting from the TSK-300 column were monitored at 280 nm. For each sample, the cpn60-containing peak was collected between 9.2 and 10.2 ml. An 800-μl sample of this peak material was precipitated with 80:20 (vol/vol) acetone/water on ice and analyzed by SDS/15% PAGE. The remainder of the peak was assayed as follows for its ability to mediate folding of Rubisco: The material (200 μl) was adjusted to 2.5 mM DTT, and acid-denatured Rubisco was added to 100 nM. The mixture was then divided into two aliquots, which were supplemented with either 2 mM ATP, or 2 mM ATP plus 40 μg of partially purified yeast mt-cpn10. Folding was allowed to proceed for 2 hr at 24°C, and Rubisco activity was then measured. Refolding of Rubisco in the aliquot containing yeast mt-cpn10 is indicated in the table below the photograph. The material shown in lane 2 was not saturated with yeast mt-cpn10. In contrast, the column-purified cpn60–yeast mt-cpn10 complexes shown in lanes 7 and 8 were saturated with mt-cpn10, as addition of yeast mt-cpn10 during the folding assay did not increase the amount of folded Rubisco (not shown). Other lanes: 3, b-cpn10; 4, ch-cpn10; 5, bovine mt-cpn10; 6, molecular mass standards.

at 1 mM K^+. Unexpectedly, the ATPase activity of yeast mt-cpn60 was not inhibited by yeast mt-cpn10 or b-cpn10 (Table 1).

Yeast mt-cpn10 Forms Stable Complexes with Both Yeast mt-cpn60 and b-cpn60. In the presence of MgATP, b-cpn10 and b-cpn60 form a stable asymmetric complex (27), which can be isolated by gel filtration (6, 28) and which contains 0.5 mol of ADP per mol of b-cpn60 protomer (21). The Rubisco refolding results suggested that similar complexes could be formed with the homologous combination of yeast mt-cpn60 plus yeast mt-cpn10 and with the heterologous combination of b-cpn60 plus yeast mt-cpn10. To test for complex formation, b-cpn60 and yeast mt-cpn60 were incubated with partially purified yeast mt-cpn10 in the presence or absence of MgATP. The reaction mixtures were then applied to a gel filtration column (equilibrated with or without MgATP as appropriate), and the eluted cpn60 proteins were analyzed by the Rubisco refolding assay and also by SDS/PAGE. Yeast mt-cpn10 formed active complexes with either b-cpn60 or yeast mt-cpn60, and complex formation required MgATP. The refolding activity of the isolated cpn60–cpn10 complexes shown in Fig. 4 were not increased by adding more yeast mt-cpn10 (lanes 7 and 8); thus the cpn60s recovered in this experiment were saturated with yeast mt-cpn10. SDS/PAGE analyses of these complexes clearly showed the presence of a 9-kDa protein (Fig. 4) that proved to be cpn10 (see below). SDS/PAGE also revealed additional minor proteins in the cpn60-containing fraction; however, these proteins appeared to be nonspecific contaminants, as their cofractionation with cpn60 was unaffected by MgATP.

The complex of b-cpn60 and b-cpn10 as isolated by gel filtration contains, per mol of b-cpn60 protomer, 0.5 mol of ADP that does not readily exchange with free ADP (21). In contrast, when either b-cpn60 or yeast mt-cpn60 was incubated with [α-^{32}P]ATP and yeast mt-cpn10, and the mixture was applied to a gel filtration column equilibrated with unlabeled ADP, the isolated complex retained less than 0.035 mol of ^{32}P per mol of cpn60 protomer, even though the amount of bound nucleotide (as determined by spectral analysis) was not appreciably decreased (not shown). Thus while the homologous bacterial complex of b-cpn60–MgADP–b-cpn10 survives passage through the column, the bacterial/yeast complex of b-cpn60–MgADP–mt-cpn10 and the homologous yeast complex of mt-cpn60–MgADP–mt-cpn10 undergo nucleotide exchange under these conditions.

Amino Acid Sequence Determination of Yeast mt-cpn10. mt-cpn10 purified from rat liver is N-terminally acetylated (29). Initial attempts to perform N-terminal amino acid sequencing of yeast mt-cpn10 were unsuccessful, suggesting that the N terminus was blocked. Cyanogen bromide cleavage of the protein yielded a unique amino acid sequence (peptide 1 in Fig. 5), indicating that yeast mt-cpn10 contains a single internal methionine. Four tryptic peptides (nos. 2–5 in Fig. 5) were also sequenced. Peptides 1 and 2 overlapped and the resultant 15-mer was readily aligned with a conserved region close to the N terminus of other cpn10s (Fig. 5). Peptides 3, 4, and 5 could be aligned with other regions of cpn10s from different species. Peptides 1–5 collectively rep-

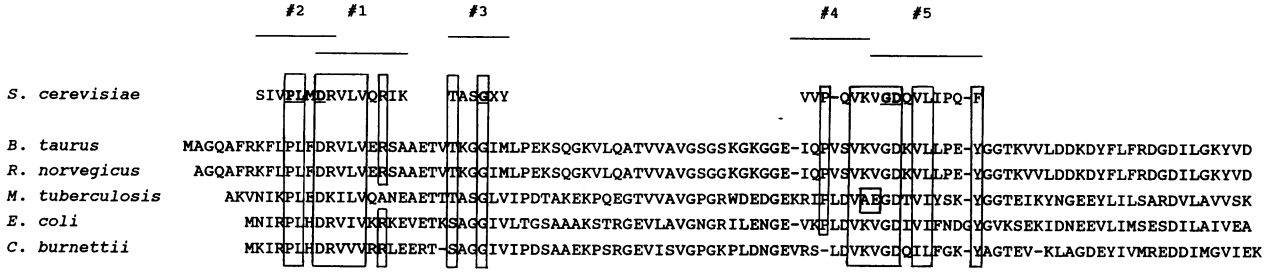

FIG. 5. Sequence comparison of five peptide fragments derived from yeast mt-cpn10 with cpn10 sequences from rat mitochondria (*Rattus norvegicus*, ref. 29) bovine mitochondria [*Bos taurus*; S. J. Pilkington and J. E. Walker (1993) GenBank accession no. X69556], and the prokaryotes *Mycobacterium tuberculosis* (30), *E. coli* (10), and *Coxiella burnettii* (31). Peptide 1 was a CNBr fragment and peptides 2–5 were tryptic fragments. Similar sequences are boxed. Hyphens denote gaps introduced to maximize alignment. Boldfaced and underlined residues in the yeast sequence are conserved in all known cpn10 sequences.

resent approximately one-third of the 9-kDa protein. We conclude that this protein is indeed yeast mt-cpn10.

DISCUSSION

The *in vitro* refolding of Rubisco under nonpermissive conditions requires two proteins, cpn60 and cpn10, in all chaperonin systems studied so far (2, 7–9, 11). Thus the identification of the cpn60 homolog hsp60 in yeast mitochondria (12, 13) implied the existence of a corresponding cpn10. Using a classical biochemical approach, we have now found this protein. Yeast mt-cpn10 can promote the discharge of folded Rubisco from either yeast mt-cpn60 or b-cpn60 in a process that requires both MgATP and K^+; it can form stable complexes with yeast mt-cpn60 and b-cpn60 in a MgATP-dependent manner; it inhibits the K^+-dependent ATPase activity of b-cpn60; its chromatographic properties resemble those of cpn10 from bovine mitochondria; and it exhibits strong sequence similarity to cpn10 proteins from other organisms.

The homologous and heterologous cpn60/cpn10 combinations depicted in Fig. 3 are not equally effective in mediating refolding of Rubisco under nonpermissive conditions. These different efficiencies may reflect the degree to which each cpn10 can couple the hydrolysis of ATP by cpn60 to a conformational change of the cpn60-bound Rubisco molecule.

Although the different cpn10 proteins tested here show considerable evolutionary diversity, they all function best with b-cpn60 in our experimental system. There must be a remarkable conservation of interactive surfaces on b-cpn60 and the diverse cpn10s. Interestingly, b-cpn10 cannot assist either bovine (8) or yeast mt-cpn60 in the refolding of Rubisco, probably because the mt-cpn60s are unable to bind the bacterial cochaperonin: b-cpn10 neither forms a stable complex with nor inhibits the K^+-dependent ATPase activity of yeast mt-cpn60. The inhibition of cpn60-ATPase activity by cpn10 may well be unique to the bacterial system, as it is found with neither the homologous yeast system (this study) nor the mammalian mitochondrial chaperonins that were studied in ref. 8.

Thus, mt-cpn10 can functionally interact with b-cpn60, but the reciprocal combination is not compatible. This result, previously reported for the single toroid mt-cpn60 of mammals (8), has now been extended to the double toroid mt-cpn60 of yeast. Interestingly, the cpn10 of higher plant chloroplasts (ch-cpn10) is partially functional with yeast mt-cpn60, even though ch-cpn10 is a 24-kDa protein composed of two tandemly linked cpn10-like sequences, one or both of which are functional (11).

In summary, the identification of yeast mt-cpn10, the groES homolog from yeast mitochondria, opens the way for purification of this protein, cloning of its gene, and genetic analysis of the mitochondrial chaperonin system in living yeast cells. Up to now, most of our mechanistic knowledge has been derived from studies of the *E. coli* chaperonins, groEL and groES. Comparative studies with the purified yeast homologs will help to reveal which of our present insights are universal and which are merely organism specific.

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