The Folding of Protein Structures Often Requires the Presence of Molecular Chaperones and/or Chaperonin Complexes. We Here Investigated the Inhibitory Effects of the Chaperone Cofactors Hop/p60 and Hap46. By Immunoprecipitation, We Observed a Direct Interaction of the Eukaryotic Chaperonin-Containing TCP-1 (CCT) Purified from Rabbit Reticulocyte Lysate with Hop/p60. By Contrast, Hap46 Was Not Coimmunoprecipitated. Binding of Hop/p60 to CCT Is Dependent on the Presence of ATP or ADP and Occurs through Carboxyl-Terminal Sequences of Hop/p60. Hop/p60 Significantly Stimulates Nucleotide Exchange on CCT But Not Its ATPase Activity, While Hap46 Has No Effects. We Used Denatured Firefly Luciferase as a Model Protein and Found Decreased Binding to CCT in the Presence of Hop/p60 and ATP. This Coincides with the Inhibitory Effect of Hop/p60 on Luciferase Reactivation in an Assay Using Purified CCT in Combination with Hsc70 and Hsp40. We Also Observed that an Antibody Directed against One of the Subunits of CCT Efficiently Inhibits Refolding in a System Which Depends on Crude Reticulocyte Lysate.

To Be Functionally Active, Proteins Must Attain and Maintain Their Native Conformations. In Many Instances, Such Functionally Correct 3-Dimensional Folding Occurs Spontaneously upon Polypeptide Synthesis or Subsequent to Denaturation of Preexisting Structures. Alternatively, Folding May Be Aided by So-Called Chaperones and Chaperonins Which in Recent Years Have Attracted Growing Research Interests (for Reviews, see Refs. 1–5). One of the Major Functions of Molecular Chaperones, in Particular of Members of Both the 70-kDa and 90-kDa Heat Shock Protein (Hsp)1 Families, Is to Keep Misfolded Proteins from Forming Insoluble Aggregates Through Interactions Between Exposed Hydrophobic Areas. In the Case of the Chaperonin GroEL/GroES of Eubacteria and the Chaperonin-Containing TCP-1 Complex (CCT) of Eukaryotes, Nonnative Proteins Are Individually Encaged Within Barrel-Like Cavities Where They May Attain Their Properly Folded State. In Contrast to the Above-Mentioned Heat Shock Proteins Which Are Mostly Monomers (hsp70) or Homodimers (hsp90), Chaperonins Are Multimeric Complexes of Subunits of ~60 kDa. These Are Arranged in Stacks of Two Rings Each Consisting of 7 or 8 Subunits in Eubacteria and Eukaryotes, Respectively.

The Hsp70 Family of Eukaryotic Chaperones Comprises Stress-Inducible Members as Hsp70 Itself and the Constitutively Expressed Form Hsc70. They Have a Conserved Structure Consisting of the Amino-Terminal ATP-Binding Portion and the Carboxyl-Terminal Domain Which Binds Misfolded Substrate. Recently, Several Structurally Unrelated Proteins Have Been Identified That Associate with Hsp70 and Hsc70 as Cofactors and Affect Chaperoning Activities. Although the “hsc70/hsp70-Associating Protein” Hap46 of Roughly 46 KDa Has Originally Been Detected in Association with Steroid Hormone Receptors (6), It Was Found to Directly Interact with the Amino-Terminal ATP-Binding Domain of Hsp70 and Hsc70 (7–9); It is Also Known as Bag-1 (8, 9). Similarly, the “hsc70-Interacting Protein” Hip/p48 (48 KDa) (10, 11) Associates with the ATP-Binding Domain (10). Hap46 and Hip/p48 Compete for Binding to Hsc70 (9, 12). A Third Factor Is the “hsp70/hsp90-Organizing Protein” Hop/p60 of Molecular Mass 60–66 KDa (13); It Is Unique in That It Binds to Both Hsp70 and Hsp90 Chaperones. Interaction Occurs with the Carboxyl-Terminal Domain of Hsp70/hsc70; However, Binding Is Distinct from That of Misfolded Protein Substrates (12, 14, 15). Even Though Hap46 and Hop/p60 Interact with Different Domains of Hsp70/hsc70, There Appears to Be Some Interference. Hap46 Inhibits the Binding of Hop/p60 to Hsc70, Probably by Steric Hindrance (15).

In Previous Studies, We Used a Standard Protein-Refolding System (16) With Thermally Denatured Firefly Luciferase as a Model for Heat-Damaged Proteins. We Observed That the Hsp70/hsc70 Accessory Proteins Hap46 and Hop/p60 Significantly Inhibited Refolding if Used in Concentrations Roughly Equimolar to Hsc70 (12). This Refolding System Is Rather Complex in That It Not Only Depends on Hsc70 and the Chaperone Hsp40 But Also Requires Small Amounts of Rabbit Reticulocyte Lysate. The Latter Is Known to Contain the Chaperonin CCT (17, 18) and May Contain Additional Factors That Could Possibly Contribute to the Effects Observed with This Crude System. We Now Demonstrate by Use of a Neutralizing Antibody That CCT Is an Essential Component of This Refolding System. In the Present Study, We Also Used Authentic CCT Purified from Reticulocytes and Investigated the Effects of the Above Hsp70/hsc70 Cofactors. In This System of Pure Components, We Again Observed Inhibition of Luciferase Reactivation by Hap46 and Hop/p60. Most Strikingly, We Detected a Direct Molecular Interaction between CCT and Hop/p60.

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‡ The Abbreviations Used Are: Hsp, Heat Shock Protein; Bag-1, Bel-2-Associated Athanogene 1; CCT, Chaperonin-Containing TCP-1; GST, Glutathione S-Transferase; Hap46, Hsp/hsc70-Associating Protein; Hip, Hsc70-Interacting Protein; Hop, Hsp70/hsp90 Organizing Protein; Hsc70, Cognate Form of Hsp70; Hsp40, Heat Shock Protein 40; Hsp70, Heat Shock Protein 70; Hsp90, Heat Shock Protein 90; Tcp, Tailexx Complex Polypeptide.
**Experimental Procedures**

Materials—CCT was purified from rabbit reticulocyte lysate as before (18) and hsc70 from bovine brain (19). Bacterially expressed Histagged human hsp40 and human Hop/p60 as well as GST-Hap46 were as before (7, 12). For obtaining the amino-terminal deletion (residues 1–62) of Hap46 the cDNA (6) was digested with BsrEI and EcoRI. The fragment encoding residues 63–274 was filled in with Klenow fragment to produce blunt ends (20) and ligated in frame into the filled-in EcoRI site of pGEX-2T (Pharmacia) to generate the amino-terminal GST fusion protein. These GST fusion proteins contain thrombin cleavage sites that can be used to produce untagged versions of Hap46 and Hap46(63–274) by proteolysis (20) with bovine thrombin (Boehringer Mannheim). To obtain amino-terminally His-tagged versions of Hop/p60 deletions, we used BamHI pST-28a-Hop (12) that was cut either with NcoI or with BglI and XhoI. Fragments containing codons 1–448 and 116–543 were filled in and cloned into SmaI sites of pQE-30 or pQE-31 (Qiagen), respectively, resulting in pQE-30-Hop(1–448) and pQE-31-Hop(116–543). Expression of proteins was in Escherichia coli strains JM109 and BL21(DE3), and purifications were carried out as before (6, 12). Bovine brain hsp90 was from Sigma and [γ-33P]ATP (10 μCi/μl, 3000 Ci/mmol) from ICN.

**Protein Refolding Assays**—Firefly luciferase (1 μg, Sigma) was thermodenatured for 10 min at 42 °C in the presence of hsc70 and hsp40. Reactivation in the presence of 5% rabbit reticulocyte lysate (Promega) was as before (7, 12) but dithiothreitol was omitted. For refolding in the presence of pure components, luciferase (1 μg) was thermodenatured as above in the presence of hsc70 (2 μg), hsp40 (2 μg), and accessory protein 5 μg each, as indicated, in buffer A (25 mM HEPES buffer (pH 7.2), 75 mM KCl, and 4 mM MgCl2) containing 1 mM ATP and 2.5 mM dithiothreitol; total volume, 20 μl. Purified CCT (25 μg) was then added, and incubation was for 90 min at 30 °C. Enzyme activities were determined with the Promega luciferase assay.

**Immunoprecipitations**—CCT-specific immunoprecipitations were by standard procedures (20) using 5 μg of antibody 91a (Stressgen) in buffer A containing routinely 1 mM ATP. Incubation was at 4 °C for 1 h in a total volume of 150 μl and subsequently overnight with protein G-Sepharose (Sigma). Retained proteins were eluted with SDS-sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting (12). Detection was by antisera C-16 (Santa Cruz) against Hap46 (BAG-1) and E4191 (Promega) against firefly luciferase as well as monoclonal antibodies F5 (Stressgen) against Hop/p60, N27F3-4 (Stressgen) against hsp70/hsc70, MRGSHis (Qiagen) against amino-terminally His-tagged polypeptides, and 91a against the CCT-274). Staining was with peroxidase-conjugated second antibodies and chemiluminescence (Amersham).

**ATP Binding and ATPase Assays**—ATP binding assays were in buffer A containing 2.5 mM dithiothreitol. Following incubations, free nucleotides were removed by centrifugation through MicroSpin G-50 columns (Pharmacia). Protein bound [γ-33P]ATP was analyzed as described (21) by chromatography on polyethyleneimine cellulose (Merck). ATP spots were identified by autoradiography and quantified by liquid scintillation counting.

For ATPase assays, purified CCT (6 μg) was incubated with [γ-33P]ATP (1 μl) and accessory proteins (4 μg each) in a total volume of 20 μl of buffer A containing 2.5 mM dithiothreitol for 20 min at 30 °C. After cooling on ice, 2 μl of each sample were chromatographed on polyethyleneimine cellulose. Released labeled phosphate was quantified as described above. Recombinant Hap46, Hop/p60, and deletion variants thereof were preincubated with ATP-agarose (Sigma) to remove possible contaminating ATPases of bacterial origin.

**Affinity Purification of GroEL—E. coli BL21(DE3) cells expressing Hop/p60 were used. Extracts containing GroEL and Hop/p60 (25–50 μg each) were chromatographed on ATP-agarose as affinity matrix according to a published procedure (22). After extensive washing with saline, eluates with 5 mM ATP were analyzed by SDS-PAGE and immunoblotting with antisemum G-6532 (Sigma) against GroEL and antibody F5 against Hop/p60.

**Results**

**Effects on Refolding of Thermally Denatured Firefly Luciferase**—The protein-folding system that we previously used requires small amounts of rabbit reticulocyte lysate (7, 12). To ascertain that the positive effect of crude lysate on refolding of thermally inactivated luciferase is due to CCT, we used monoclonal antibody 91a directed against α-subunits of CCT (23). Indeed, this antibody was able to completely inhibit enzyme reactivation. As shown in Fig. 1, increasing amounts of antibody added to the folding reaction progressively interfered with reactivation. This result provides clear evidence for the participation of CCT in this refolding system. Binding of antibody 91a to α-subunits may inhibit the function of CCT by sterically fixing these α-subunits within the double-ring structure of CCT.

We then studied protein folding using a combination of pure components, most notably CCT in conjunction with hsc70 and hsp40. This represents the eukaryotic counterpart to the bacterial system of GroEL together with DnaK and DnaJ which has successfully been used in in vitro folding reactions (24) and is known to prevent protein aggregation in vivo (25). In our system, luciferase reactivation was found to depend on CCT and hsc70 (Fig. 2A, Columns 6 and 7 versus Column 1) as well as hsp40 and ATP (not shown). We found that the folding activity steadily increased with the concentration of CCT and reached a level of roughly 30% luciferase reactivation under our experimental conditions. This is almost as efficient as the above refolding system using diluted, crude reticulocyte lysate in which we routinely obtained −40% enzyme reactivation (7, 12). More importantly, the accessory proteins Hap46 and Hop/p60 similarly inhibited reactivation (Fig. 2A, Columns 2 and 4) while GST used as control protein was inert (Column 1). Control experiments showed that inhibition is just as prevalent whether GST-Hap46 or Hap46 itself is used (data not shown).

**Antibody concentration [ng/20 μl assay]]**

![Fig. 1. Refolding of denatured firefly luciferase in a system containing diluted reticulocyte lysate. Denaturation and reactivation in the presence of 5% reticulocyte lysate were carried out as described under “Experimental Procedures.” Increasing concentrations of antibody 91a were added, as indicated. Reconstituted activity in controls without antibody was set as 100%; reactivation levels were roughly 40% of input enzyme activity (7, 12). Average values of 2 independent experiments are given.](image-url)
affects neither its nucleotide- nor substrate-binding activities (15, 26). This is clearly in contrast to Hap46 which, however, binds to a different domain of hsp70 (7, 9, 12, 15). Nevertheless, both Hop/p60 and Hap46 inhibit protein refolding, as pointed out above. We were wondering whether Hop/p60 and/or Hap46 may rather exert some of their actions on the level of the chaperonin complex. We thus checked for interactions with CCT in immunoprecipitation experiments with monoclonal anti-

**FIG. 2.** Refolding of denatured firefly luciferase in a system of pure components. Denaturation and reactivation were carried out as described under “Experimental Procedures.” Reconstituted activity in controls without accessory proteins was set as 100%; reactivation levels were roughly 30% of input enzyme activity. A, refolding in the presence of various proteins, as indicated. Hap46 and Hap46(63–274) were added as GST fusion proteins. Experiments were in triplicate; bars, S.D. B, refolding efficiency of the system containing CCT, hsc70, and hsp40 upon increasing the concentrations of Hop/p60 (■) and Hap46 (●). Average values of 2 independent experiments are given.

**FIG. 3.** CCT-specific coimmunoprecipitation with antibody 91a. Immunoprecipitations were carried out as described under “Experimental Procedures.” A, 120 μl of untreated rabbit reticulocyte lysate (Promega) was used either as such (lanes 1 and 2) or with addition of GST-Hap46 (2 μM) in the experiments of lanes 3 and 4. Lane 1 shows the control without antibody. Detection of coprecipitated endogenous Hop/p60 (lanes 1–3) was by immunoblotting with antibody F5 and of added GST-Hap46 (lanes 3 and 4) with antiserum C-16. B, purified CCT (10 μg) was incubated in 150 μl total volume with hsc70 (10 μg), Hop/p60 (10 μg), and hsp90 (10 μg), as indicated. Staining of CCTα and coprecipitated Hop/p60 was with antibodies as described under “Experimental Procedures.” C, purified CCT (10 μg) was incubated in 150 μl total volume with Hop/p60 (10 μg) in either the absence or the presence of 1 mM ATP or ADP, as indicated. Detection of Hop/p60 was by immunoblotting with antibody F5. D, purified CCT (10 μg) was incubated in 150 μl total volume with deletions of Hop/p60 (10 μg each). Immunoblotting was with antibody 91a detecting His-tagged proteins.
tibody 91a against CCT and protein G-Sepharose. We first asked whether Hop/p60 and Hap46 are coprecipitated with CCT from reticulocyte lysate (Fig. 3A). Indeed, endogenous Hop/p60 was specifically retained and showed up in immunoblots (lane 2). By contrast, endogenous Hap46 was not detectable by this procedure (data not shown). Because the concentration of Hap46 appears to be rather low in reticulocyte lysate, we carried out an experiment in which we added GST-Hap46 to this crude material. Again we did not observe any coprecipitation of Hap46 with CCT (Fig. 3A, lane 4). Moreover, addition of GST-Hap46 did not affect the binding of Hop/p60 to CCT (lane 3). These data clearly demonstrate that CCT interacts with Hop/p60 endogenous to reticulocyte lysate.

Interaction of Purified Hop/p60 and CCT—We extended these immunoprecipitation studies to mixtures of pure proteins. This has the advantage that in particular the effects of heat shock protein chaperones can be tested. Fig. 3B (top) shows that added Hop/p60 is coprecipitated with CCT and the CCT-specific antibody 91a. Interestingly, addition of hsc70 did not affect the interaction between Hop/p60 and CCT (lane 2). We also included hsp90 in CCT-specific immunoprecipitations and found a slight increase (roughly 20%) in the amount of Hop/p60 coprecipitated (Fig. 3B, top, lane 3 versus lane 1). In controls, we checked all immunoprecipitations for CCT and obtained similar signals throughout (Fig. 3B, bottom).

Routinely, these interaction experiments were in the presence of 1 mM ATP. To check the effect of ADP versus ATP on Hop/p60 binding, we carried out the experiment of Fig. 3C. We found that ADP and ATP similarly promoted the interaction with CCT (lanes 2 and 3), but there was minimal binding in the absence of these nucleotides (lane 1).

To investigate the specificity of the above observed interaction between Hop/p60 and the eukaryotic chaperonin, we also tested the bacterial chaperonin complex GroEL/GroES. To this end we used extracts of E. coli containing endogenous GroEL and recombinant Hop/p60. Following affinity chromatography of GroEL on ATP-agarose, eluates with ATP were checked by SDS-PAGE and immunoblotting for GroEL and Hop/p60. We observed no specific copurification of Hop/p60 (data not shown).

The Carboxyl-terminal Portion of Hop/p60 Is Involved in Binding to CCT—Previous studies by Chen et al. (13) have shown that Hop/p60 contains two tetratricopeptide repeat regions which are involved in interactions with hsc70 and hsp90. We constructed two deletion mutants, Hop(116–543) and Hop(1–448), from which either amino- or carboxyl-terminal sequences are missing. Both truncated proteins interacted with hsc70 (15), but Hop(1–448) had a roughly 3-fold higher affinity than Hop(116–543). This agrees with data by others (13, 27) and shows that Hop/p60 preferentially binds hsp70s through its amino-terminal portion. We now tested these Hop/p60 variants for interaction with CCT. Significant amounts of Hop(116–543) coprecipitated with CCT and antibody 91a, but interaction with Hop(1–448) was marginal (Fig. 3D). This unequivocally shows that Hop/p60 interacts with CCT through carboxy-terminal sequences.

Hop/p60 Affects the ADP/ATP Exchange on CCT—Biochemical and electron microscopic analyses have recently pointed to major conformational changes in the complex structure of CCT produced by ATP (18, 28). Moreover, CCT is known to bind both ATP and ADP, but to bind ADP with higher affinity (18). We thus preincubated CCT with unlabeled ADP and measured the ADP/ATP exchange by binding of [$\gamma^3P$]ATP. As shown in Fig. 4, Hop/p60 had no effect (column 1), but addition of Hop/p60 stimulated ATP binding 4–5-fold (column 2). In controls, we found that Hop/p60 by itself does not bind ATP (data not shown). Our experiments also show that Hap46 does not influence the stimulation brought about by Hop/p60 (Fig. 4, column 3 versus Column 2). We thus conclude that the direct interaction of Hop/p60 with CCT (see above) is responsible for the stimulation of the ADP/ATP exchange. Interestingly, both deletants Hop(1–448) and Hop(116–543) exerted no stimulatory effect on the ADP/ATP exchange on CCT (Fig. 4, columns 4 and 5).

We also checked the effect of Hop/p60 on the endogenous ATPase activity of CCT. Addition of full-length Hop/p60 resulted in slight stimulation (15%), while the mutants Hop(1–448) and Hop(116–543) had no effect (not shown). Taken together these data show that only full-length Hop/p60 significantly stimulates the ADP/ATP exchange of CCT but not its ATPase activity.

Hop/p60 Affects Substrate Binding to CCT—Because binding of misfolded substrate proteins to CCT is nucleotide dependent (29), we reasoned that Hop/p60 in the presence of ADP or ATP might influence substrate binding to CCT. Luciferase was again used as the model protein and was denatured by urea pretreatment. It was then diluted into buffer containing hsc70 to prevent aggregation. We checked for interaction with CCT by coprecipitation with the CCT-specific antibody 91a and protein G-Sepharose. In control experiments without antibody, only trace amounts of denatured luciferase were retained (data not shown), but with antibody present we obtained strong signals showing that luciferase is specifically bound to CCT (Fig. 5). We observed some decrease in binding of luciferase protein (roughly 20%) in the presence of ATP rather than ADP (lane 3 versus lane 1). This effect was less pronounced than we had expected, probably due to ongoing ATP hydrolysis and increased substrate affinity in the ADP-bound state (29). Nevertheless, with ATP present, addition of Hop/p60 largely de-
immunoblotting with antiserum E4191. “Experimental Procedures.” Coprecipitated luciferase was detected by antibody 91a and protein G-Sepharose for 3 h at 4 °C, as described under “Experimental Procedures.” Coprecipitated luciferase was detected by immunoblotting with antiserum E4191.

creased the amount of luciferase protein bound to CCT (Fig. 5, lane 4 versus lane 3). By contrast, Hop/p60 had no effect on substrate binding in the presence of ADP (lane 2 versus lane 1).

DISCUSSION

The chaperonin-containing TCP-1 complex CCT of eukaryotic cells, also called TCP-1 ring complex (TRiC) or c-cpn (1, 2), appears to be mostly involved in proper intracellular folding of actins and tubulins. However, this may be a quantitative effect, suggesting that the antibody and Hop/p60 bind to different sites. Further experiments will be required to establish the number of molecules binding per CCT hexamer. All we can tell at present is that binding of antibody 91a to CCT, they provide no stoichiometric information as to how many molecules bind per CCT hexadecamer.

We suggest that the chaperone cofactors Hap46 and Hop/p60 exert their inhibitory effects on protein folding in different ways: Hap46 through hsp70s and Hop mainly through CCT. Our immunoprecipitation experiments did not provide evidence for a direct and specific interaction between Hap46 and CCT. On the other hand, Hap46 readily associates with hsp70s and is known to diminish the binding of misfolded proteins to these chaperones (7, 15). Hop/p60 interacts with both hsp70s (12–15) and CCT, as shown in the present study. It affects substrate binding to CCT (cf. Fig. 5) but not to hsp70s, as has recently been demonstrated (15). We have shown that the eukaryotic chaperonin CCT and the chaperone system hsp70 in conjunction with hsp40 cooperate in luciferase refolding in vitro (cf. Fig. 2A). We propose that this occurs in vivo subsequent to translation or after damage by stress. In this context, it has been reported that newly synthesized actin and luciferase molecules are associated with CCT, hsp70, and hsp40 (33, 34). Folding may then occur in ordered ways similar to the coordinated processes described for the bacterial systems of GroEL/GroES and of DnaK/DnaJ/GrpE in vitro and in vivo (24, 25). In eukaryotic cells, however, the relative expression of the protein factors Hop/p60 and Hap46 may contribute to the regulation of protein-folding reactions involving CCT and hsp70/hsp40. This appears possible in view of the fact that both Hop/p60 and Hap46 inhibit refolding efficiently at concentrations roughly equimolar to those of CCT and hsc70 (cf. Fig. 2B).

Our data show that Hop/p60 does not bind to CCT as substrate. It does not compete with denatured luciferase when CCT is in its ADP-bound form (cf. Fig. 5) which has a high affinity for denatured proteins (29). Furthermore, typical CCT substrates are known to stimulate both nucleotide exchange and ATPase activity of the chaperonin (35), but Hop/p60 affects only the ADP/ATP exchange (cf. Fig. 4) and not the ATPase of CCT.

Stimulation of nucleotide exchange on CCT by Hop/p60 is reminiscent of the Hap46 effect on eukaryotic hsp70s and of GrpE on the prokaryotic DnaK chaperone. However, GrpE in concert with DnaJ leads to strong stimulation of protein-refolding activity of DnaK (36) while Hap46 and Hop/p60 rather inhibit reactivation of misfolded structures by way of interactions with hsp70s and CCT, respectively. When we checked truncations of Hop/p60, we found that interaction with CCT occurs with the carboxyl-terminal portion (cf. Fig. 3D). By contrast, binding to hsc70 involves mainly amino-terminal sequences of Hop/p60 (13, 15, 27), and association with hsp90 occurs through the middle portion of Hop/p60 (13). This is consistent with the observation that hsc70 and hsp90 do not significantly influence the binding of Hop/p60 to CCT (cf. Fig. 3B). While the carboxyl-terminal portion of Hop/p60 interacts with CCT, amino-terminal sequences must be involved in affecting nucleotide exchange, as the respective truncation Hop116–543 did not stimulate ATP binding (cf. Fig. 4).

Previous studies with Hop/p60 purified from rabbit reticulocyte lysate (called recycling factor for hsp70, RF-hsp70) have suggested that it promotes ADP/ATP exchange on hsp70 (37). However, we and others (15, 26) were unable to confirm this observation using recombinant Hop/p60. Presumably, this effect comes about by some contaminant (26). If, for example, a small amount of CCT were copurified with Hop/p60 from reticulocyte lysate, it could well simulate the described ATP-binding effect (37). Rather than affecting nucleotide binding to hsp70, Hop/p60 might have promoted ATP binding to CCT, as is possible according to our data (cf. Fig. 4).

Even though our studies unequivocally demonstrate interaction between Hop/p60 and CCT, they provide no stoichiometric information as to how many molecules bind per CCT hexadecamer. All we can tell at present is that binding of antibody 91a to CCT α-subunits does not interfere with Hop/p60 interaction, suggesting that the antibody and Hop/p60 bind to different sites. Further experiments will be required to establish
which subunit of CCT is involved in the interaction with Hop/p60. Possibly, Hop/p60 can interact with more than one kind of such subunits given that these possess regions of high homology (38). Interestingly, we observed no binding of Hop/p60 to the bacterial chaperonin GroEL even though there are peptide homologies between GroEL and CCT subunits (38).

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