Chaperonin-mediated Folding of Vertebrate Actin-Related Protein and γ-Tubulin

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Abstract. The folding of actin and tubulin is mediated via interaction with a heteromeric toroidal complex (cytoplasmic chaperonin) that hydrolyzes ATP as part of the reaction whereby native proteins are ultimately released. Vertebrate actin-related protein (actin-RPV) (also termed centractin) and γ-tubulin are two proteins that are distantly related to actin and tubulin, respectively: γ-tubulin is exclusively located at the centrosome, while actin-RPV is conspicuously abundant at the same site. Here we show that actin-RPV and γ-tubulin are both folded via interaction with the same chaperonin that mediates the folding of β-actin and α- and β-tubulin. In each case, the unfolded polypeptide forms a binary complex with cytoplasmic chaperonin and is released as a soluble, monomeric protein in the presence of Mg-ATP and the presence or absence of Mg-GTP. In contrast to α- and β-tubulin, the folding of γ-tubulin does not require the presence of cofactors in addition to chaperonin itself. Monomeric actin-RPV produced in vitro folding reactions cocycles efficiently with native brain actin, while in vitro folded γ-tubulin binds to polymerized microtubules in a manner consistent with interaction with microtubule ends. Both monomeric actin-RPV and γ-tubulin bind to columns of immobilized nucleotide: monomeric actin-RPV has no marked preference for ATP or GTP, while γ-tubulin shows some preference for GTP binding. We show that actin-RPV and γ-tubulin compete with one another, and with β-actin or α-tubulin, for binary complex formation with cytoplasmic chaperonin.

It is generally accepted that the information contained in the linear sequence of amino acids of a given protein is sufficient to dictate its functional three dimensional structure (Anfinsen, 1973; Jaenicke, 1991). Though in some cases protein folding is a spontaneous process, it is now clear that many proteins assume their correct three dimensional structure via interaction with a class of proteins or protein complexes known as molecular chaperones (for review see Rothman, 1989; Ellis et al., 1991; Gething and Sambrook, 1992). Chaperones use the energy of ATP hydrolysis as part of the mechanism whereby correctly folded proteins are generated, and are thought to function by stabilizing the conformation of intermediates that ultimately lead to the formation of native protein. Two major classes of chaperones have been defined. The first, exemplified by the heat-shock protein hsp70, function as monomers or dimers, and interact with polypeptides so as to maintain them in an unfolded or partially unfolded state (for review see Welch, 1991). The second, exemplified by the heat-shock protein hsp60, function as multisubunit toroidal complexes that provide a sequestered environment in which facilitated protein folding can take place (Bochkareva et al., 1988; Cheng et al., 1989; Martin et al., 1991; Goloubinoff et al., 1989; Ostermann et al., 1989; Viitanen et al., 1992; Phipps et al., 1993). These latter complexes are termed chaperonins.

In the cytoplasm of eukaryotic cells, the folding of actin and tubulin are facilitated via interaction with a multisubunit heteromeric chaperonin (Gao et al., 1992, 1993; Frydman et al., 1992; Yaffe et al., 1992). The folding reaction takes place in two steps: the rapid, ATP-independent formation of a binary complex between the target protein and chaperonin, followed by a slower, ATP-dependent release of the native protein (Gao et al., 1992, 1993; Frydman et al., 1992). To investigate the potential involvement of the same chaperonin in the folding of actin- and tubulin-related polypeptides, we decided to see whether two recently described proteins, actin-RPV (Lees-Miller et al., 1992) (also termed centractin [Clark and Meyer, 1992]) and γ-tubulin (Oakley and Oakley, 1989; Oakley et al., 1990; Zheng et al., 1991; Stearns et al., 1991; Joshi et al., 1992), can be folded via interaction with cytoplasmic chaperonin. These proteins are of particular interest because of their distinctive subcellular localization: γ-tubulin is present exclusively at the centrosome (Zheng et al., 1991; Stearns et al., 1991), while actin-RPV is abundantly present at the same location (Clark and Meyer, 1992) and in association with the dynactin complex, a multisubunit complex involved in microtubule based vesicle motility (Lees-Miller et al., 1992). Microinjection of anti-γ-tubulin antibodies strongly suggests that γ-tubulin is es-
sential for microtubule nucleation at the centrosome (Joshi et al., 1992); however, the solubility and other properties of this low-abundance protein are completely unknown. Here we show that actin-RPV and γ-tubulin both undergo facilitated folding via interaction with cytoplasmic chaperonin, followed by their release as soluble monomeric proteins. We describe the properties of these proteins in terms of their ability to bind nucleotide, to coassemble with their homologs, and to compete with other cytoskeletal proteins for association with cytoplasmic chaperonin.

Materials and Methods

Expression of Labeled Actin-RPV and γ-Tubulin in E. coli

A full length cloned cDNA encoding human γ-tubulin (Zheng et al., 1991) was engineered into the E. coli expression vector pET11a (Studier et al., 1990) in a three-way ligation containing the 4.3-kb Ndel-EcoRI fragment derived from the pET1a vector, a 1.5-kb BglII-EcoRI fragment encoding the downstream sequence from nucleotide 38 of the γ-tubulin cDNA, and the synthetic oligonucleotides 5'TATGCGCAGGAAAAATCATACCTACATACAG-TGGGCAAGTG3' and 5'TGGCCCAACGCAGGTGATGATTTCC-TCGCGCA3', included so as to provide sequences encoding amino acids 1-13. The resulting clone and a cloned cDNA encoding the complete coding region of human actin-RPV in pET42 (Levilliars et al., 1984) were expressed in E. coli BL21DE3 as [35S]-methionine-labeled proteins under conditions where host mRNA synthesis was inhibited by incubation in the presence of rifampicin (Studier et al., 1990; Gao et al., 1992, 1993). The insoluble recombinant proteins were purified from host E. coli cells and denatured by solubilization in guanidine-HCl as described previously (Gao et al., 1992); specific radioactivities were in the range 5 × 106 cpm/μg.

Preparation of Cytoplasmic Chaperonin

Biochemically homogeneous cytoplasmic chaperonin was prepared either from rabbit reticulocyte lysate or from bovine testis by the methods described (Gao et al., 1992; Frydman et al., 1992). The biological activity of each preparation was checked by assaying its ability to facilitate the folding of β-actin in vitro as described previously (Gao et al., 1992).

Purification of Actin and Tubulin

Actin. Actin was extracted from homogenized mouse brains as described (Levilliars et al., 1984). After two cycles of polymerization/denaturation, the product was flash-frozen at 1.25 mg/ml in liquid N2 and stored at −70°C.

Tubulin. Tubulin was prepared from bovine brain by three cycles of assembly and disassembly (Shelanski et al., 1973) followed by chromatography on phosphocellulose (Weinertgen et al., 1975). The preparation was concentrated by ultrafiltration and stored at −70°C in 0.1 M Pipes, pH 6.8, 20 mM MgCl2, 0.5 mM EGTA, 3.4 M glycerol, and 0.2 mM GTP at a concentration of 6 mg/ml.

In Vitro Translation and Folding Reactions

RNA was transcribed from recombinant pET plasmids containing cDNAs encoding complete actin-RPV or γ-tubulin polypeptides (described above) using T7 polymerase (Melton et al., 1984). Aliquots (1 μg) of these RNA were added to micrococal nuclease-treated rabbit reticulocyte lysate (25 μl) (Promega Corp., Madison, WI) supplemented with 10 μCi [35S]-methionine and incubated for 1 h at 30°C. In vitro folding reactions were performed by rapidly diluting labeled, denatured actin-RPV or γ-tubulin (prepared as described above) at a ratio of 1:100 into either reticulocyte lysate that had been cleared of particulate material by centrifugation for 15 min at 200,000 g, or into buffer A (30 mM MES, pH 6.9, 0.1 M KCl, 1.5 mM MgCl2, 0.5 mM EGTA, 1 mM DTT) supplemented with 1 mM ATP and containing purified chaperonin (6 pmol). In some experiments (see text), the in vitro folding reactions also contained 1 mM GTP. Control folding reactions done in buffer A without chaperonin were supplemented with BSA (0.8 mg/ml). Folding reactions were incubated for 2 h at 30°C and applied to a Superose 6 HR 10-30 gel filtration column (Pharmacia LKB Biotech.

ology, Piscataway, NJ) equilibrated and run at 4°C in buffer A without nucleotide and containing 10% glycerol.

Nucleotide-binding Properties of In Vitro Generated Actin-RPV and γ-Tubulin

Material from in vitro translation reactions emerging from the Superose 6 column (see above) with an apparent molecular weight of 40-45 kD was divided into two equal portions; each was applied to a column of either agarose-bound ATP or GTP (Sigma Chem. Co., St. Louis, MO) (0.5 × 4.0 cm) equilibrated in buffer A. The columns were extensively washed with buffer A until no further radioactivity was detectable in the column eluate. Material bound to the columns was eluted with 5 mM Mg-ATP in buffer A (in the case of ATP-agarose) or 5 mM Mg-GTP in buffer A (in the case of GTP-agarose), and quantitated by scintillation counting.

Comassie Blue

Tubulin. Biochemical properties of actin-RPV and γ-tubulin were determined as described above and analyzed by SDS-PAGE. In experiments to determine the binding of γ-tubulin to microtubule ends, phosphocellulose-purified tubulin (60 μM) was polymerized by the addition of 1.5 molar equivalents of taxol. In some experiments, the assembled microtubules were measured by scanning electron microscopy. Microtubules were cut with buffer C containing 40 μM taxol before the addition of in vitro generated γ-tubulin and incubated for 10 min at 37°C. Reaction products were analyzed by sedimentation through sucrose cushions as described above.

Competition Experiments

Unlabeled target proteins for use in competition experiments were expressed in 25-ml cultures of E. coli BL21DE3 at a density of 0.5 (Gao et al., 1993). Labelled, denatured actin-RPV or γ-tubulin was added to increasing amounts of unlabeled, denatured competitor (see text), and the mixtures used as probes in dilution assays containing purified cytoplasmic chaperonin such that each reaction contained 0.4 pmol of labeled target protein. Dilution assays were done essentially as described above, except that the dilution buffer contained 20 mM MES, pH 6.9, 20 mM KCl, 0.5 mM MgCl2, 1 mM EGTA, 1 mM DTT. After incubation for 10 min at 20°C to allow binary complex formation, the reaction products were analyzed on non-denaturing composite agarose/polyacrylamide gels containing 0.5% agarose and 3.0% polyacrylamide, 80 mM MES, pH 6.9, 1 mM EDTA. The gels were run in the same buffer in a Mini-protein gel apparatus (Biorad Labs., Richmond, CA) at 90 V for 1 h, stained with Coomassie blue, de-stained, and fluorographed.
Results

Expression of Actin-RPV and γ-Tubulin in Reticulocyte Lysate

We first characterized the products of in vitro translation reactions containing mRNA encoding human actin-RPV (lane marked A-R) and mRNA encoding human γ-tubulin (lane marked γ). Location of molecular weight markers (97, 66, 44, and 21 kD) are shown at the left. (B and C) Elution profiles of the same in vitro translation reactions shown in A on a Superose 6 gel-filtration column. Arrows show the location of molecular size markers (thyroglobulin, 670 kD; immunoglobulin G, 158 kD; and ovalbumin, 44 kD) run under identical conditions on the same column.

Chaperonin-mediated Folding of Actin-RPV and γ-Tubulin in vitro

The data presented in Fig. 1 suggested that, as in the case of β-actin and α- and β-tubulin, newly synthesized actin-RPV and γ-tubulin polypeptides become associated with cytoplasmic chaperonin as binary complexes which are then discharged as native monomeric proteins. To see whether this was indeed the case, we did folding reactions in which labeled, denatured actin-RPV or γ-tubulin were used as target proteins in folding reactions performed in vitro. Labeled, denatured target proteins for use in these in vitro folding reactions were generated by expressing cDNAs encoding the complete actin-RPV or γ-tubulin polypeptides in E. coli in the presence of [35S]methionine under conditions where host protein synthesis was suppressed (Studier et al., 1990). This resulted in the accumulation of large quantities of recombinant protein (Fig. 2 A). The insoluble nature of the bacterially expressed products enabled us to purify them from the bulk of host proteins, so as to yield material that
Figure 3. Gel-filtration analyses of the products of in vitro folding reactions. Elution profiles of in vitro folding reactions done using labeled, denatured actin-RPV (A, C, and E) or γ-tubulin (B, D, and F) as target protein and applied to a Superose 6 gel-filtration column. Folding reactions were done in which target proteins were diluted into buffer containing BSA (A and B), unfractionated rabbit reticulocyte lysate (C and D), or homogeneous cytoplasmic chaperonin supplemented with Mg-ATP and/or Mg-GTP (E and F). In E, analysis of γ-tubulin in vitro folding reactions done with Mg-ATP and Mg-GTP (△) or with Mg-ATP alone (△) are shown. Arrows indicate the location of the same molecular size markers shown in Fig. 1, B and C.
was at least 80% biochemically pure as judged by Coomassie blue staining of an SDS-polyacrylamide gel (Fig. 2 B), and at least 90% radiochemically pure as judged by autoradiography of the same material (Fig. 2 C). The labeled bacterially expressed insoluble polypeptides were denatured with guanidine-HCl and urea and used as target proteins in folding assays in which they were rapidly diluted from denaturant. After incubation at 30°C, the reaction products were analyzed by gel filtration chromatography.

As a control, we first investigated the effect of diluting the labeled, denatured target proteins into buffer containing BSA as a potential stabilizing protein. These experiments resulted in the emergence of all the radioactivity in the column void volume, presumably reflecting the generation of aggregates of high molecular mass (Fig. 3, A and B, peak 0). In contrast, when the same target proteins were diluted into unfractionated reticulocyte lysate, three peaks emerged. The first (peak 0) appeared in the column void volume, a second (peak I) with an apparent molecular weight of 700 kD, and a third (peak II) with an apparent molecular weight of 40–45 kD; the relative abundance of radioactivity in these peaks was 41, 17, and 42% in the case of actin-RPV (Fig. 3 C) and 43, 23, and 34% in the case of γ-tubulin (Fig. 3 D). Peaks I and II correspond in apparent molecular mass with the actin-RPV reaction products, 66% of the radioactivity was 41, 17, and 42% in the case of actin-RPV (Fig. 3 C) and 43, 23, and 34% in the case of γ-tubulin (Fig. 3 D). Peaks I and II correspond in apparent molecular mass with the actin-RPV reaction products, 66% of the radioactivity was found in the pellet after the first cycle, whereas after the second cycle, ~95% of the labeled actin-RPV was contained in the pellet (Fig. 4 A). In a parallel experiment done with actin-RPV generated in an in vitro chaperonin-mediated folding reaction, ~95% of the labeled actin-RPV was found in the pellet after either the first or second polymerization cycle (Fig. 4 B). The polymerized material obtained in these experiments might represent copolymers with unlabeled brain actin; alternatively, it could represent polymers of actin-RPV on its own. To distinguish between these two possibilities, we did a control experiment in which actin-RPV generated via a chaperonin-mediated in vitro folding reaction was incubated in the absence of carrier actin under conditions in which actin monomers polymerize (Fig. 3 C). No detectable radioactivity was found to sediment in a manner consistent with the behavior of polymerized material. From these data, we conclude that actin-RPV produced either by translation in reticulocyte lysate or by folding in vitro by homogeneous cytoplasmic chaperonin can efficiently copolymerize with native actin.

We investigated the biological activity of γ-tubulin produced in our translation and in vitro folding reactions in parallel experiments by assaying its ability to cocycle with phosphocellulose-purified brain tubulin. After depolymerization of the first cycle pellet, tubulin was assembled into microtubules by the addition of taxol. At the end of each polymerization reaction, polymerized material was sedimented through a sucrose cushion, a procedure that allows only the cosedimentation of proteins that are tightly bound to microtubules (Melki et al., 1991). After the first cycle, ~40% of the labeled γ-tubulin produced by in vitro translation was found in the pellet; upon depolymerization and addition of taxol, virtually all the radioactivity was found to cosediment with microtubules (Fig. 4 A). In the corresponding experiment done with material produced in an in vitro folding reaction containing purified chaperonin, ~80% of the labeled γ-tubulin was found in the pellet after the first cycle, whereas after the second cycle, the pellet contained ~95% of the labeled protein (Fig. 4 E).

Because of its centrosomal location, it has been proposed that the function of γ-tubulin is to nucleate microtubules (Oakley et al., 1990; Stearns et al., 1991; Joshi et al., 1992). This concept implies that γ-tubulin can interact with microtubule minus ends. To test this notion, we did an experiment in which a constant amount of labeled, in vitro generated monomeric γ-tubulin was assayed for its ability to bind to taxol-stabilized microtubules over a range of concentrations. A constant amount of γ-tubulin was incubated with different concentrations of microtubules averaging either 11 ± 3 μm or 1.2 ± 0.4 μm in length (as measured by electron microscopy; the latter polymers were generated by shearing through a narrow gauge needle) and the reaction products were then analyzed by sedimentation through a sucrose cushion. This experiment (Fig. 5) showed that the extent to which γ-tubulin bound to microtubules was highly dependent on the num-
Actin-RPV

Figure 4. Cocycling with brain actin or tubulin of labeled actin-RPV or \( \gamma \)-tubulin translated or folded in vitro. Analysis on 8% SDS-polyacrylamide gels of the supernatants (S) and pellets (P) derived from cocycling experiments in which labeled actin-RPV (A–C) or \( \gamma \)-tubulin (D and E) produced either by translation in vitro (A and D) or by folding in vitro (B, C, and E) were tested for their ability to cocycle through one (1st) and two (2nd) cycles of polymerization and depolymerization with unlabeled mouse brain actin (in the case of actin-RPV) or unlabeled bovine brain tubulin (in the case of \( \gamma \)-tubulin).

ber of available ends. These data, and the copolymerization experiments described above (Fig. 4, D and E), strongly suggest that the products of our \( \gamma \)-tubulin translation and in vitro folding reactions behave as native polypeptides, and are consistent with the interaction of \( \gamma \)-tubulin with microtubules ends.

**Nucleotide-binding Properties of Actin-RPV and \( \gamma \)-Tubulin**

Actin-RPV and \( \gamma \)-tubulin share ~50% and 35% amino acid identity with \( \beta \)-actin and \( \beta \)-tubulin, respectively (Lees-Miller et al., 1992; Clark and Meyer, 1992; Oakley and Oakley, 1989; Zheng et al., 1991). Both might therefore be expected to be nucleotide-binding proteins; in the case of actin-RPV, 14 out of 15 residues that bind Ca-ATP in actin are conserved (Lees-Miller et al., 1992). However, the nucleotide-binding properties of actin-RPV and \( \gamma \)-tubulin are unknown. We therefore measured the binding of in vitro folded actin-RPV and \( \gamma \)-tubulin to agarose-bound ATP and GTP. For comparison, we measured the ability of \( \beta \)-actin to bind to these columns: 52% of in vitro translated \( \beta \)-actin bound to ATP-agarose, while 42% of the same material bound to GTP-agarose (Table I). As a negative control, we examined the binding of in vitro synthesized \( \beta \)-globin to the same immobilized nucleotide columns: no measurable binding was observed. In the case of actin-RPV, 33 and 27%, respectively, of the labeled protein bound to ATP- or GTP-agarose; the corresponding figures for \( \gamma \)-tubulin were 20 and 32%. The relatively low binding efficiencies in all these experiments were not a result of kinetically limited exchange reactions, since repassage of the non-binding fractions over
Table I. Binding of Monomeric β-Globin, β-Actin, Centractin, and γ-Tubulin to ATP- and GTP-Agarose

<table>
<thead>
<tr>
<th>Agarose-bound nucleotide</th>
<th>β-globin</th>
<th>β-actin</th>
<th>Centractin</th>
<th>γ-tubulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>0</td>
<td>52</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>GTP</td>
<td>0</td>
<td>40</td>
<td>27</td>
<td>32</td>
</tr>
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* Measured as the yield of material eluted from the column with Mg-ATP (in the case of ATP-agarose) or Mg-GTP (in the case of GTP-agarose).

fresh nucleotide-agarose columns failed to result in the retention of any further material. It is possible that only a portion of the monomeric products emerging from the gel-filtration columns was native; in addition, it is conceivable that significant denaturation occurred during experimental manipulations after the in vitro folding reactions. Nonetheless, our data suggest that actin-RPV and γ-tubulin are both nucleoside triphosphate-binding proteins, though neither shows an absolute specificity for ATP or GTP.

Interaction between Chaperonin and Actin-RPV or γ-Tubulin

The data described above demonstrate that actin-RPV and γ-tubulin are both folded via interaction with the same chaperonin that folds β-actin and α- and β-tubulin. There is evidence that chaperonin-mediated folding reactions take place within the central cavity that runs along the axis of chaperonin complexes (Creighton, 1991; Langer et al., 1992a,b; Braig et al., 1993), but the nature and specificity of target protein/chaperonin interactions is unknown. We therefore decided to test the ability of actin-RPV and γ-tubulin to compete with one another or with β-actin or α-tubulin for interaction with cytoplasmic chaperonin. In these experiments, increasing quantities of unlabeled denatured competitor protein were added to the labeled, denatured actin-RPV or γ-tubulin probes before dilution into a reaction containing purified chaperonin. The yield of binary complex was then visualized after resolution of the reaction products on a nondenaturing gel (Gao et al., 1993) (Fig. 6). In negative control reactions in which actin-RPV or γ-tubulin was competed with unlabeled α-globin, a polypeptide that does not, on its own, form a binary complex with cytoplasmic chaperonin (Gao et al., 1993), no decrease in the yield of binary complex was detected in the presence of high concentrations of added unlabeled competitor (Fig. 6, A and F). In contrast, a marked decline in the yield of binary complex was detected in competition reactions done with increasing concentrations of unlabeled homolog (Fig. 6, B and G). Competition of the actin-RPV and γ-tubulin probes with one another or with β-actin or α-tubulin all resulted in a similar decline in the yield of labeled binary complex (Fig. 6, C-E, H-J). From these data, we conclude that all these cytoskeletal proteins interact with the same or overlapping sites on the cytoplasmic chaperonin.

Discussion

The chaperonin-mediated folding of β-actin and α- and β-tubulin proceeds via the formation of a binary complex consisting of the target protein bound to chaperonin. These binary complexes have an apparent molecular weight of 700

Figure 5. Binding of γ-tubulin to microtubules as a function of polymerized tubulin concentration and number of polymer ends. Analysis on an 8% SDS-polyacrylamide gel of the supernatants (S) and pellets (P) derived from experiments in which a constant amount of monomeric γ-tubulin generated in vitro was incubated with varying concentrations of taxol-stabilized microtubules (shown in μM in the figure) and the resulting polymers sedimented through sucrose cushions. (A) Unsheared microtubules (average length 11 ± 3 μm). (B) Sheared microtubules (average length 1.2 ± 0.4 μm). (C) Quantitation of the data shown in A (closed circles, solid line) and B (closed triangles, dashed line).
Figure 6. Competition among cytoskeletal proteins for association with cytoplasmic chaperonin. Analysis by native agarose/PAGE of binary complexes formed between cytoplasmic chaperonin and either labeled denatured actin-RPV (A-E) or γ-tubulin (F-J) in the presence of unlabeled competitor protein. Competition reactions shown are with unlabeled denatured α-globin (A and F), actin-RPV (B and I), γ-tubulin (D and G), β-actin (C and H), α-tubulin (E and J). Lanes marked 1-5 represent reactions in which equal amounts of labeled denatured target proteins were competed with a fivefold (lanes 1), tenfold (lanes 2), 20-fold (lanes 3), 30-fold (lanes 4), or 40-fold (lanes 5) molar excess of unlabeled denatured competitor protein.

kD; in the presence of Mg-ATP, Mg-GTP, and (in the case of tubulin) additional protein cofactors, the native proteins are released (Gao et al., 1992, 1993). The appearance of a 700-kD species together with monomeric actin-RPV or γ-tubulin in our in vitro translation reactions was therefore suggestive of the existence of synthetic intermediates consisting of de novo synthesized unfolded polypeptides bound to chaperonin, a conclusion that was confirmed by the generation of identical products in in vitro folding reactions containing homogeneous chaperonin and Mg-ATP (Fig. 3). The soluble nature of monomeric actin-RPV was to be expected, since it is found throughout the cytoplasm, though concentrated at the centrosome (Clark and Meyer, 1992). The generation of soluble monomeric γ-tubulin was more surprising, given the restricted localization of this protein at the centrosome (Oakley et al., 1990; Zheng et al., 1991; Horio et al., 1991; Stearns et al., 1991). Immunofluorescence experiments have shown that the cytoplasmic chaperonin is diffusely distributed throughout the cytosol (Gao et al., 1992; Lewis et al., 1992). Though our data clearly establish that the facilitated folding of actin-RPV and γ-tubulin can be mediated by interaction with cytoplasmic chaperonin, the mechanism whereby the soluble monomeric proteins become incorporated into the centrosome remains unknown.

Previous analyses of actin and α- and β-tubulin folding reactions have shown that while actin is efficiently folded via interaction with chaperonin alone, the proper folding of α- and β-tubulin requires the presence of two additional protein cofactors (Gao et al., 1993). Since γ-tubulin shares ~35% amino acid sequence identity with α- and β-tubulin...
In experiments in which in vitro folded γ-tubulin was tested for its ability to bind to columns of immobilized nucleotide, γ-tubulin showed some preference for GTP binding (Table I). However, the presence of GTP is not strictly required in order to correctly fold γ-tubulin: in vitro folding reactions done in the presence of ATP alone resulted in a product indistinguishable from that of reactions done in the presence of both ATP and GTP, although the yield of monomeric γ-tubulin in reactions done with ATP alone was much diminished (Fig. 3 F). This result contrasts with the requirements of α- and β-tubulin folding reactions, which depend on the presence of both ATP and GTP (Gao, Y., and N. J. Cowan, unpublished observations).

We previously showed that β-actin and α- and β-tubulin compete for interaction with cytoplasmic chaperonin. To see whether actin-RPV and γ-tubulin are recognized by the same sites on cytoplasmic chaperonin (and indeed whether these same sites recognize actin and α- and β-tubulin), we tested the ability of actin-RPV and γ-tubulin to compete with one another and with actin and α-tubulin for the formation of binary complexes. All these cytoskeletal proteins competed efficiently with one another for binary complex formation (Fig. 6). These data strongly suggest the existence of one or more common structural motif(s) that must be recognized by the cytoplasmic chaperonin. Such structural motif(s) may reflect some aspects of secondary or tertiary structure that are common to folding intermediates in actins, tubulins and actin- or tubulin-like proteins.

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