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Protein Folding in the Cell: Functions of Two Families of Molecular Chaperone, hsp 60 and TF55-TCP1 [and Discussion]

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Protein folding in the cell: functions of two families of molecular chaperone, hsp 60 and TF55-TCP1

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SUMMARY

Two families of molecular chaperone, the hsp 60-GroEL family and the TF55-TCP1 family, have been discovered in evolutionarily related cellular compartments. A member of one of these families, hsp 60, has been shown to play a global role in polypeptide chain folding in mitochondria. We review here studies of both hsp 60 and other family members, discussing their essential physiological roles and mechanism of action.

1. INTRODUCTION

Oligomeric protein complexes comprising ring structures have recently become a focus of interest as mediators of protein folding in the cell. Three such complexes were originally identified, in evolutionarily related cellular compartments: GroEL, in the eubacterial cytoplasm; rubisco subunit binding protein, in the chloroplast stroma; and hsp 60, in the mitochondrial matrix. More recently, complexes have been identified in two distinct evolutionarily related sites, TF55 in thermophilic archaebacteria and t-complex polypeptide-1 (TCP1) in the eukaryotic cytosol. Genetic and biochemical studies have begun to define the physiological roles and mechanism of action of the first, hsp 60 family of components. We discuss these features, then consider the TF55-TCP1 family and how it may resemble or differ from the hsp 60 family.

2. THE HSP 60 FAMILY

GroEL in the *E. coli* cytoplasm, the first member of this family to be identified, was recognized as a chromosomally-encoded product whose deficiency resulted in defective morphogenesis of bacteriophage λ and T4 head structures, and T5 tail structures, suggesting a role in protein assembly (Georgopoulos *et al.* 1973; Sternberg 1973; Zweig *et al.* 1973). Rubisco subunit binding protein was identified several years later by its association in the chloroplast stroma with newly translated large subunits of rubisco (Barraclough & Ellis 1980) and its absence from the mature protein, implying a role in protein assembly. Hsp 60 was identified as a heat-inducible protein in *Tetrahymena thermophila* mitochondria that shared antigenic epitopes with GroEL (McMullen & Hallberg 1987, 1988).

(*a*) *Function in the cell: insights from hsp 60*

Insight into a general function for these components in the acquisition of protein structure was provided by characterization of a mutant strain of yeast defective for assembly of proteins imported into the mitochondrial matrix from the cytosol, and shown to contain a single mutation within hsp 60 (Cheng *et al.* 1989). In this mutant, called mif4 (mitochondrial import function defective), proteins that are normally imported as precursors into the matrix compartment, e.g. the subunits of ornithine transcarbamylase and citrate synthase, were found in the matrix at their mature sizes, but no biological activity was detected. On extraction with nonionic detergent, the proteins were found in insoluble fractions, suggesting that they had aggregated.

In the mif4 mutant, effects were also observed on proteins that are normally imported first into the matrix, then re-exported to the inner membrane or intermembrane space (Cheng *et al.* 1989; Hartl & Neupert 1990; Mahlke *et al.* 1991; Koll *et al.* 1992). These proteins were found as intermediate-sized forms, reflecting that a processing event occurring normally on entry to the matrix had taken place, but that a second processing step that takes place on reexport had not occurred. Because these proteins are apparently normally present in monomeric form during their passage through the matrix compartment prior to reexport, this observation indicated a role for hsp 60 in adjusting polypeptide conformation to enable reexport, suggesting that hsp 60 could act in the biogenesis of oligomeric proteins not only at the level of assembly but at the level of polypeptide chain folding (Cheng *et al.* 1989). A role in folding was firmly established by biochemical studies of the monomeric cytosolic protein, dihydrofolate reductase

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(DHFR), imported into mitochondria via an adjoined leader peptide (Ostermann *et al.* 1989). When imported in the absence of ATP, the DHFR moiety could be isolated in physical association with hsp 60 in a protease-sensitive, apparently unfolded, form; after addition of ATP, but not non-hydrolysable analogues, to the import mixture, DHFR was found to be dissociated from hsp 60 and was relatively protease-resistant, in an apparently native-like form. Simple release from hsp 60 appeared insufficient to produce folding to the native state, because when ATP was added to mitochondria that had been pretreated with the sulfhydryl reagent N-ethyl-maleimide (NEM), DHFR was released from hsp 60 but became aggregated.

(*b*) *Essential nature of hsp 60 and GroEL*

Primary structure analyses of GroEL, rubisco subunit binding protein, and hsp 60 revealed a close resemblance, with nearly 60% amino acid identity (Hemmingsen *et al.* 1988; Reading *et al.* 1989). Presumably, an ancestral version of GroEL in bacteria gave rise to modern-day rubisco subunit binding protein and hsp 60 after endosymbiotic events. Both GroEL and hsp 60 were shown to be essential genes at both normal and heat shock temperature (Fayet *et al.* 1989; Cheng *et al.* 1989; Reading *et al.* 1989). The abundance of these components is heat-inducible, increasing from 1% to 10% of soluble cytoplasmic protein in the case of GroEL (Neidhardt *et al.* 1984), and from 1% to 2% of mitochondrial matrix protein in the case of hsp 60 (McMullen & Hallberg 1987). In addition, heat stress of *E. coli* leads to phosphorylation of a fraction of GroEL molecules, associated with a reduced requirement *in vitro* for a cooperating component to release a bound polypeptide (Sherman & Goldberg 1992). The increased abundance of GroEL and hsp 60 under conditions of stress, where proteins may be subject to loss of native structure, appears to comprise a response to an expanded need for a normal function in protein folding. This interpretation is corroborated both by studies showing that production of misfolded proteins in *E. coli* induces GroEL (Goff & Goldberg 1985; Parsell & Sauer 1989) and by recent studies showing that hsp 60 function contributes to stabilization of pre-existent native DHFR in mitochondria during heat shock (Martin *et al.* 1992).

(*c*) *Role of GroEL: in vivo studies*

The essential nature of GroEL in *E. coli* implies minimally that one essential component of the cell requires the action of GroEL to reach its native form. However, the conditional *E. coli* mutants, isolated by deficiency of λ phage propagation, exhibit a variety of cellular defects after shift to a non-permissive temperature (42°C). They exhibit reduced rates of DNA and RNA synthesis (Wada & Itikawa 1984), reduced UV-mutagenesis (Donnelly & Walker 1992), defective cell division with filamentous morphology (Georgopoulos & Eisen 1974), decreased proteolytic activity (Straus *et al.* 1988), and impaired export of β-lactamase (Kusukawa *et al.* 1989). A recently constructed GroEL carboxy terminal deletion mutant exhibits reduced growth rate throughout the entire range of 20–40°C, and no growth beyond 40°C (A. L. Horwich, unpublished data). Correspondingly, phenotypic defects in this mutant are observed through the entire range of temperature, including filamentous morphology, defective λ phage production, defective DNA repair, and several auxotrophies, further supporting a role for GroEL under non-heat shock conditions. Nevertheless, a global defect of protein biogenesis, like that observed in mif4 mitochondria, has not so far been observed *in vivo* associated with mutations in GroEL. Presumably, only proteins that are preferred substrates for GroEL, i.e. that have the greatest need for its action, are affected in the mutants examined to date. For example, λB protein appears to be a preferred substrate, with critical dependence on GroE function for assembly of λB monomers into a 12-member ring structure that acts as a head-tail connector piece (Kochan & Murialdo 1983; Kochan *et al.* 1984). Perhaps particular structural features of λB and other substrates could provide an indication of the particular requirement for GroEL function.

Perhaps the best evidence to date for a general role of GroEL comes from deletion of the gene for σ32 (rpoH), the transcription factor regulating production of the collective of heat shock proteins of *E. coli* (Kusukawa & Yura 1988; Cowing *et al.* 1985). Incubation of this strain at 37°C leads to global aggregation of newly-translated proteins and formation of inclusion bodies (Gragorev *et al.* 1992). What the contribution of GroE deficiency might be to such a phenotype is unclear, given that many heat shock proteins are simultaneously affected, but, significantly, production of additional amounts of groE in this mutant at least partially alleviates aggregation.

Overexpression of the GroE operon *in vivo* has also, in some cases, been associated with enhanced expression of foreign proteins, consistent with a general function in protein biogenesis (Gouloubinoff *et al.* 1989; Lee & Olins 1992; Dolan & Greenberg 1992; Wynn *et al.* 1992). In some cases, overexpression has permitted rescue of mutationally altered, otherwise inactive, proteins to functional form (Fayet *et al.* 1986; Jenkins *et al.* 1986; Van Dyk *et al.* 1989). As a corollary, overexpression of GroEL could also promote the export of an otherwise poorly secreted lamB-lacZ fusion protein (Phillips & Silhavy 1990).

In vitro binding studies offer additional support for a general role of GroEL: in a bacterial translation lysate, GroEL binds both a newly-translated polypeptide, chloramphenicol acetyl transferase, and the precursor of an exported protein, β-lactamase (Bochkareva *et al.* 1988). Purified GroEL could bind at least 40% of the soluble proteins of *E. coli* after dilution from 5 M guanidine HCL (Viitanen *et al.* 1992a).

In contrast with this, as yet, incomplete case for general involvement of GroEL, *in vivo* experiments with mitochondrial hsp 60 reveal that deficiency in the conditional mutant prevents production of the active conformation of each of ten imported proteins examined to date, including hsp 60 itself (Cheng *et al.* 1989, 1990; Glick *et al.* 1992; S. Caplan and A. L. Horwich, unpublished data; Martin *et al.* 1992). In all

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cases, upon extraction with nonionic detergent, the polypeptide was isolated in an insoluble fraction. The physiological result of the general failure of newly-imported mitochondrial proteins to reach functional form is cell death, predictable from the study of other import mutants, where the failure to produce new mitochondria, supplying essential functions to the cell, results in inviability (Baker & Schatz 1991). In addition to defective biogenesis of imported proteins in hsp 60-deficient mitochondria, the biogenesis of at least one mitochondrially encoded protein, the ribosomal component *var1*, is also affected, with the newly translated protein failing to be incorporated into ribosomes and being recovered in an insoluble fraction (Horwich *et al.* 1992).

(*d*) *Mode of action in polypeptide chain folding*

The observations of aggregation *in vivo* in the setting of deficiency of hsp 60 or GroEL (phage components in the latter case) suggested that the general action of these components might be to prevent aggregation during the folding or assembly of polypeptides. This is essentially a chaperone action, preventing illicit interactions between domains either within non-native polypeptides or between them (Ellis 1987). In addition to this preventive action, an additional role is carried out, describable as 'stepwise release'. Both the experiments with imported DHFR in mitochondria (Ostermann *et al.* 1989) and studies with this same protein and others *in vitro* (described below) suggest that stepwise release of bound polypeptides from hsp 60 and GroEL is critical to reaching the native state. Stepwise release seems likely to favour the formation of productive intermediates, which are critical to reaching an active form as opposed to aggregating, especially in the *in vivo* situation (Rothman 1989). Do these actions constitute catalysis of folding? To date, enhancement in the rate of production of the native state in *in vitro* experiments with GroEL (see below) has not consistently been observed when compared with the rate of non-assisted folding. For example, in the cases of prokaryotic rubisco (Goloubinoff *et al.* 1989) and ornithine transcarbamylase (Zheng *et al.* 1992) the rate has been increased, but in contrast, in the case of DHFR both *in vitro* and in isolated mitochondria (Ostermann *et al.* 1989), the rate appears to be slower than spontaneous refolding *in vitro* (Martin *et al.* 1991; Viitanen *et al.* 1991). While chemical catalysis is not evident, the requirement for protein-mediated folding *in vivo* is absolute; spontaneous folding is not an alternative, at least in the case of mitochondria: in the absence of hsp 60 function, not a single protein examined has reached the native state. Concerning the precise mechanics of chaperone (binding) function and stepwise release (folding) action of hsp 60 family members, only the detailed examination of folding in association with these components, comparing it with folding occurring in their absence, can formally assess the mechanism of action.

(*e*) *Antifolding action in protein export*

In addition to a role mediating folding of polypeptide chains to the native state, GroEL and hsp 60 have been demonstrated to mediate an antifolding role in 'holding' precursor proteins destined for export. GroEL binds preβ-lactamase, proOmpA and prephoE *in vitro* (Laminet *et al.* 1990; Lecker *et al.* 1989), in the first case corroborating observation of defective export in a GroEL mutant. Inside mitochondria, proteins on the 'conservative sorting' pathway, which first enter the matrix and are then reexported to inner membrane or intermembrane space locations, also are bound in intermediate forms to hsp 60 (Koll *et al.* 1992). Such 'holding' of precursor proteins apparently acts to retain the polypeptide chain in a conformation competent for transfer to export components. The 'held' state is probably maintained by the presence of an export signal: the signal domain both contributes to production of a non-native conformation of the precursor protein that is recognizable by GroEL or hsp 60, but its presence also prevents the stepwise release and folding that would occur in the case of the mature-size polypeptide. In the bacterial system, it seems likely that bound proteins are transferred from GroEL to components with a specific role in protein export, e.g. SecB or SecA (Wickner *et al.* 1990). Homologous components may carry out reexport function in mitochondria.

(*f*) *Potential role in oligomeric protein assembly*

Although oligomeric protein assembly has not been observed to be facilitated *in vitro* with either hsp 60 or GroEL, the possibility remains that such an action occurs *in vivo*. To date this possibility has not been distinguishable from effects on polypeptide chain folding. However, the arguments for such an action are strong. Rothman (1989) calculated rates for the assembly of several oligomeric proteins based on bimolecular rate constants for rate-limiting subunit assembly steps, and on intracellular concentrations of the unassembled subunits, and derived estimates of 1 to 100 h, as compared with the observed time in intact cells of usually a few minutes. How could assembly be facilitated? It appears that single GroEL complexes can bind several small polypeptides simultaneously (see below), making it possible that this could facilitate subunit interactions by increasing their local concentration. However, such facilitation should have been detectable in *in vitro* experiments. More likely, facilitation of assembly might occur through the action of favoring the production and stabilization of assembly-competent intermediates, both through chaperone and controlled-release mechanisms, and this favouring could act, particularly under the conditions found in the cell, to facilitate assembly. Finally, it seems also possible that there are as yet unidentified components that might facilitate assembly steps, beyond the steps of folding.

(*g*) *Quaternary structure: rings and a cavity*

All three of the hsp 60 family members were isolated as homo-oligomeric 14mer complexes, composed of 60 kDa monomers (Hohn *et al.* 1979; Hendrix 1979;

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Barraclough & Ellis 1980; Pushkin *et al.* 1982; McMullen & Hallberg 1988). In the electron microscope the complexes appear as cylindrical structures, typically 140 Å† in height and 120 Å in diameter. After negative staining, in top-down views a 40 Å diameter electron-dense 'hole' is observed, surrounded by a ring with seven star-like points of symmetry, corresponding to component monomers. In lateral view four white bars are observed perpendicular to the axis of symmetry, each pair corresponding to a ring (Hutchinson *et al.* 1989). Presumably the white bars correspond to distinct domains within the monomers, which may extend like flower petals from the equatorial region of the cylinder towards its termini (Horwich *et al.* 1992). The most straightforward overall interpretation from the images is that the complexes contain an aperture at either end entering a cavity that may have at its broadest dimension a diameter approximating that of the complex itself.

(*h*) *Co-chaperones: single ring oligomers that associate with GroEL*

Cooperating co-chaperone components have been identified for all three hsp 60 family members. The cooperating component for GroEL, GroES, is present in the same operon with GroEL (Hemmingsen *et al.* 1988). GroES, like GroEL, was shown to be essential (Fayet *et al.* 1989), and mutational defects produced the same phage morphogenetic defects as observed in GroEL mutants (Tilly *et al.* 1981). GroES was identified as a 10 kDa protein (Tilly *et al.* 1983), and it was isolated from the *E. coli* cytoplasm as a seven-member homo-oligomeric ring, 80 Å in diameter with a 20 Å electron dense hole (Chandresekhar *et al.* 1986). Its structure suggested that the GroES ring could directly appose with a ring of GroEL. Adenine-nucleotide-dependent association of the two components was initially demonstrated in biochemical studies (Chandresekhar *et al.* 1986), and, more recently, apposition of the rings has been demonstrated in the electron microscope, both *in vitro*, after addition of purified GroES to GroEL (Saibil *et al.* 1991; Langer *et al.* 1992), and *in vivo*, where a GroEL complex isolated from a strain of thermophilic bacteria, *Thermus thermophilus*, already contained a bound GroES structure (Ishii *et al.* 1992). In both cases, a single GroES ring was bound at one terminus of the GroEL cylinder, producing a 'bullet' appearance.

Both these direct observations, plus biochemical studies examining the relative amounts of GroES needed either to fully suppress the ATPase activity of GroEL (Chandresekhar *et al.* 1986) or to fully reconstitute folding of a polypeptide (Goloubinoff 1989; Martin *et al.* 1991), support a stoichiometry of one GroES ring binding to one GroEL 14mer. This asymmetry of binding could indicate that only one terminus of the GroEL cylinder is capable of binding GroES, suggesting that the GroEL rings are arranged in the long axis (height) dimension, in the form A–B–A–B (where each ring has two nonidentical 'faces', designated A and B). Alternatively, the rings could be arranged in the order A–B–B–A, but upon binding of GroES to one ring, the opposite ring could become altered in conformation to prohibit binding of GroES at that site. Recent electron microscopic studies support the latter possibility (Langer *et al.* 1992), showing distortion of the GroEL rings at the aspect opposite the site of binding of GroES. While the ABBA arrangement is not proven by this observation, it seems more plausible from the standpoint of assembly of the double ring complexes, because an ABAB arrangement seems able to allow progressive attachment of additional rings. As long as the A surface is unable to assemble with another A surface, ABBA comprises a terminally assembled structure.

In the case of hsp 60, the existence of a cooperating component was suggested by the observation that, when DHFR associated with hsp 60 was isolated from mitochondria by digitonin solubilization and gel filtration, addition of ATP produced only a partial degree of protease resistance of DHFR, as compared to that of the native protein, and release failed to occur (Ostermann *et al.* 1989). A mitochondrial homologue of GroES, able to associate with *E. coli* GroEL and cooperate with it to reconstitute activity of a bound prokaryotic rubisco, was subsequently isolated from bovine liver mitochondria (Lubben *et al.* 1990). More recently a rat liver mitochondrial component found to be heat-inducible in cultured hepatoma cells, was purified and found to have similar biochemical features to GroES (Hartman *et al.* 1992). The predicted amino acid sequence of this 10 kDa component is 40% identical to GroES. A putative GroES homologue was identified in chloroplasts by analysis of a cDNA encoding a 30 kDa species (Bertsch *et al.* 1992). The sequence predicts a tandem head-to-tail arrangement of two GroES-like subunits. How and whether such a dimer could form a seven-membered ring structure like that of GroES remains unknown.

(*i*) *Mechanism of action in folding*

The dynamics of action of hsp 60 class complexes have begun to be approached by *in vitro* reconstitution experiments. The mechanism of action can be considered as two sequential steps: binding of an unfolded polypeptide by the 14mer complex; and folding of the polypeptide, initiated upon addition of GroES or MgATP.

(*j*) *Binding: upstream events in the cell*

The molecular nature of binding is not precisely known. In the cell, it seems likely that binding occurs after a protein has emerged completely from ribosomes or from a translocation site, extrapolating from the observation that proteins are fully imported to the mitochondrial matrix before they interact with hsp 60 (Cheng *et al.* 1989, 1990; Ostermann *et al.* 1989). Interaction during translation or translocation appears rather to be a function of hsp 70 class proteins (Beckmann *et al.* 1990; Deshaies *et al.* 1988; Chirico *et al.* 1988; Vogel & Misra 1989; Kang *et al.* 1990;

† 1 Å = 10^{-10} m = 10^{-1} nm.

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Gething & Sambrook 1992; Sanders *et al.* 1992). For example, in mitochondria a matrix-localized version of hsp 70 is required for completion of translocation (Kang *et al.* 1990). Physical association of this component with the entering polypeptide has been demonstrated, and precedes interaction with the hsp 60 oligomer (Kang *et al.* 1990; Scherer *et al.* 1990). Such a progression of interaction can be correlated with a likely progression in development of the structure of the imported protein. For example, *in vitro* studies of an hsp 70 protein, BiP, indicate that it binds to short hydrophobic stretches of amino acids, such as those exposed in newly translated or newly translocated polypeptides (Flynn *et al.* 1991). In contrast, GroEL-bound proteins appear to already contain native-like secondary structure (Martin *et al.* 1991). Indeed transfer has been achieved *in vitro* (Langer *et al.* 1992) between an hsp 70 class protein of the *E. coli* cytoplasm called DnaK, and GroEL, in the presence of components that normally cooperate with DnaK, i.e. the DnaJ and GrpE proteins (S. Wickner *et al.* 1991; Georgopoulos 1992; Hartl *et al.* 1992). In these experiments, when DnaK–DnaJ were first loaded with the nonnative form of the monomeric mitochondrial protein rhodanese, multiple rounds of groE-mediated folding could be observed on addition of GrpE. Whether such a pathway is normally taken *in vivo* remains to be seen, however, because both dnaK and DnaJ, in contrast to GroEL–GroES, are dispensable for *E. coli* at normal growth temperatures (Bukau & Walker 1989; Sell *et al.* 1991).

In the cell, proteins could be delivered to hsp 60 components directly from either hsp 70 homologues or from other components, potentially mediated by binding of the component to hsp 60. Stable interactions between hsp 60 or GroEL and other chaperone components have not been observed to date, however. It seems, rather, that features of a polypeptide itself may be sufficient to direct recognition. For example, after interaction with hsp 70 and ATP-mediated release, a polypeptide could have acquired sufficient structure such that it would no longer be recognizable by hsp 70 but, rather, could be recognized by an hsp 60 family member. This is consistent with *in vitro* observations that α-lactalbumin can be bound by either hsp 70 or hsp 60, depending on whether its conformation is extended or molten globule-like, respectively (Langer *et al.* 1992; Ewbank & Creighton 1991).

(*k*) *Binding in vitro after dilution from denaturant*

It has been possible to study polypeptide binding by GroEL *in vitro* in the absence of other components by first unfolding native proteins in 6 M guanidinium HCl (GuHCl) or 8 M urea and then diluting from denaturant into mixtures containing GroEL (e.g. Goloubinoff *et al.* 1989; Martin *et al.* 1991; Buchner *et al.* 1991; Laminet *et al.* 1990; Zhi *et al.* 1992; Badcoe *et al.* 1991; Höll–Neugebauer *et al.* 1991). The action of binding by GroEL *in vitro* prevented aggregation, corresponding to the observations *in vivo*. Exactly what features of a non-native protein are recognized and whether these are, in fact, the same features that permit stable binding by GroEL, is unknown. However it is clear that native forms of the same proteins are not bound by GroEL. Differential recognition by an accessible binding site in non-native versus native states suggests that hydrophobic surfaces might be the preferred sites of recognition, because they are often accessible in the non-native forms but buried in the native state. Alternatively, particular secondary structures might be recognized, perhaps in defined contexts. This latter possibility is supported by an NMR experiment with a peptide that is found as an α-helix at the amino terminus of rhodanese (Landry & Gierasch 1991). The peptide in solution was present as a random coil but assumed its helical state upon binding by GroEL. Hydrophobic surfaces of amphipathic helices were suggested as potentially preferred sites of recognition. Such peptide binding exhibited a weak association, but the combination of a series of such interactions, perhaps cooperatively (see Bochkareva *et al.* 1992), might result in the stable binding observed for intact polypeptides.

(*l*) *Stoichiometry of binding*

The stoichiometry of binding has been examined by measuring the amount of GroEL needed to prevent aggregation or spontaneous refolding of polypeptide on dilution from denaturant (Goloubinoff *et al.* 1989; Martin *et al.* 1991; Mendoza *et al.* 1991; Bochkareva *et al.* 1992). The collective of studies suggest that one or at most a few molecules could be bound per 14mer complex. Direct examination of gold-labeled DHFR molecules bound to GroEL indicated mostly one molecule bound but occasionally two or three (Braig *et al.* 1993).

(*m*) *Conformation of bound polypeptides*

The conformation stabilized by the binding to GroEL of several different proteins has been examined. In the case of DHFR and rhodanese, the bound proteins were susceptible to proteolysis with low concentrations of protease, suggesting non-organized tertiary structure (Martin *et al.* 1991). Tryptophan fluorescence measurements, feasible because both GroEL and GroES lack tryptophan, indicated that tryptophan residues in the bound proteins were in environments less polar than in the completely unfolded state, but not as apolar as the buried interior of the native state. Similar observations were made with α-glucosidase bound to GroEL (Höll-Neugebauer *et al.* 1991). The ability of the GroEL-polypeptide complexes to bind the hydrophobic dye 1-anilino-8-naphthalene sulfonic acid (ANS) indicated that hydrophobic residues in the bound polypeptide were accessible to solvent, although it could not be excluded that ANS binding occurred at sites formed by interaction of the bound polypeptide with GroEL or at sites produced in GroEL itself upon binding of the polypeptide. The collective findings concerning the environment of tryptophan residues, protease susceptibility, and ANS accessibility suggested that, for DHFR and rhodanese, the conformation stabilized

by GroEL could be that of a 'molten globule', a compact early folding intermediate with native-like secondary structure and non-organized fluctuating tertiary structure brought about by nonspecific hydrophobic interactions (Semisotnov *et al.* 1987; Kuwajima 1989).

An additional approach to studying binding involves testing preformed intermediates for binding by GroEL. Folding intermediates of lactate dehydrogenase formed in various concentrations of GuHCl were tested for their ability to be bound after dilution into mixtures containing GroEL (Badcoe *et al.* 1991). Intermediates likely to have less structure than a molten-globule, including a random coil conformation, appeared to be favoured for binding. Stable folding intermediates of the subunit of a dimeric rubisco have also been formed, either by acid unfolding at high ionic strength (Goto *et al.* 1990), or by a mutation that produces a 'native-like' monomer that fails to dimerize (van der Vies *et al.* 1992). Both intermediates had native-like α-helix content but a non-ordered tertiary structure. Neither, however, was bound by GroEL, while an unstable, aggregation-prone intermediate, produced upon neutralization of the acid-unfolded rubisco, was bound by GroEL. Thus, the general features of early intermediates recognized by GroEL remain to be precisely defined.

The physical site on GroEL where polypeptides are bound has been a subject of speculation, the possibilities including equatorial region between the rings, outer surface of the rings, and cavities inside the rings (Creighton 1991; Horwich *et al.* 1992). An equatorial site of binding seems unlikely given a recent study showing that a single ring version of human hsp 60 overproduced in *E. coli* was able to mediate folding and assembly of a dimeric rubisco *in vitro* (Viitanen *et al.* 1992*b*). Concerning the latter two possible locations, recent studies employing scanning transmission electron microscopic examination of GroEL complexes containing a bound DHFR polypeptide labeled with 1.4 nm diameter gold clusters are consistent with polypeptides binding within the cavity of the rings (Braig *et al.* 1993). This observation indicates that the polypeptide binding sites are likely to be located on the inner walls of the cavity. Bound polypeptides may thus be physically sequestered from the surrounding cellular environment while folding in association with GroEL. Arrangement of binding sites inside the GroEL complex presumably serves also to prevent homotypic interactions between GroEL complexes themselves.

(*n*) *Folding reconstituted in vitro*

Addition of GroES and MgATP to a protein-GroEL complex results in rapid changes of conformation of the bound polypeptide (Martin *et al.* 1991). The complex loses the property of ANS binding within seconds. The tryptophan fluorescence spectrum also changes, with a rapid drop of intensity, followed by a blue shift over several minutes to the wavelength maximum shown by the native form. Protease susceptibility gradually increases, associated with release of the polypeptide and its acquisition of biological activity. At least the initial stage of folding occurs in association with GroEL (Martin *et al.* 1991), but the point along the folding pathway at which release occurs, and whether it lies before or after the most energetic intermediate in the folding pathway, remains undefined.

It seems likely that the conformational changes that occur in association with the GroEL complex are promoted by a cooperative release of bound domains from the complex. The role of GroES in this process could be to couple ATP hydrolysis to such release. For example, in the absence of GroES, DHFR is released and rebound in a cyclical fashion in the presence of MgATP, whereas in the presence of GroES, a single step of release to the native state occurs (Martin *et al.* 1991). For several polypeptides including rhodanese, release in the absence of GroES cannot produce the native state and leads, at least in part, to aggregation. The 'coupling' of folding to GroEL mediated by GroES could involve no more than physical blockage of an exit site from the internal cavity by presence of the GroES heptamer apposed to the GroEL ring (presumably the same ring in which the polypeptide is bound). However, binding of GroES to GroEL has been observed to produce distortion of the normal structure of GroEL (Saibil *et al.* 1991; Langer *et al.* 1992), suggesting that GroES might indirectly regulate chain release by altering the conformation of the GroEL binding sites. Such alteration seems likely to depend on ATP hydrolysis, essential for release, which is itself regulated by GroES. In the absence of polypeptide, GroES suppresses the ATPase activity of GroEL. In the presence of GroES, a burst of ATPase activity is produced by addition of polypeptide, associated with change of polypeptide conformation and release (Martin *et al.* 1991). For example, approximately 100 ATP molecules are hydrolysed in folding a single rhodanese molecule. ATP has been shown to be hydrolysed in a manner that is both potassium-dependent (Viitanen *et al.* 1990) and cooperative (Gray & Fersht 1991; Bochkareva *et al.* 1992). It seems possible that individual GroEL monomers, in particular perhaps those comprising the ring containing bound polypeptide, carry out events of ATP hydrolysis in association with an alteration of their binding sites.

3. THE TF55-TCP1 FAMILY

Thermophilic archaebacteria also contain a major heat shock protein consisting of ~60 kDa subunits (Trent *et al.* 1990). Interest was focused on this protein both because it is essentially the only polypeptide produced by these organisms upon heat shock (Trent *et al.* 1990), and because the organisms themselves are evolutionarily distinct from eubacteria and eukaryotes (Woese & Fox 1977).

The heat shock induction of a single protein in archaebacteria differs strikingly from the heat shock response of other organisms examined to date, where usually a collective of ten or more polypeptides are induced (Lindquist & Craig 1988). In the thermophi-

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lic archaebacterium *Sulfolobus shibatae* a single major translation product was observed, called thermophilic factor 55 (TF55), after transfer from its normal growth temperature, 75°C, to a near-lethal temperature, 88°C (Trent *et al.* 1990). Production of TF55 as the sole translation product correlated with the viability of the strain after subsequent shift to the otherwise lethal temperature of 95°C, indicating a role in the acquisition of thermotolerance. Because components in archaebacteria have been shown to have a closer evolutionary relationship to cognates in the eukaryotic cytosol than to those in eubacteria (Lechner & Bock 1987; Zillig 1987; Iwabe 1989; Auer *et al.* 1989; Rivera *et al.* 1992), it seemed possible that TF55 might not be closely related to GroEL but, rather, might have a relative in the eukaryotic cytosol.

TF55 was isolated from *S. shibatae* grown at 75°C, where the protein is already abundant, and proved to have features both familiar and novel (Trent *et al.* 1991). Most of the protein was found in a 20*S* fraction in sucrose density gradient sedimentation, a behavior resembling that of members of the hsp 60 family. The protein was also virtually the only protein in this fraction, suggesting its occurrence as a homo-oligomeric complex. When the purified protein complex was examined by scanning transmission electron microscopy (STEM), the architecture observed was remarkably familiar: a double-stacked ring appearance. In negative staining, the same lateral view of four light bars was observed as had been seen with the hsp 60 family. In top views, however, usually nine (occasionally eight) discrete monomers were observed per ring instead of the seven members seen with the hsp 60 family. A similar quaternary structure was observed for an abundant heat-inducible component of a second thermophilic archaebacterium, *Pyrodictium occultum*, only in this case eight-member rings were consistently observed, comprised of two polypeptides of 56 and 59 kDa (Phipps *et al.* 1991).

Functional studies of purified TF55 were consistent with a role as a molecular chaperone: the purified complex could bind non-native proteins and exhibited ATPase activity. More particularly, TF55 could bind mesophilic proteins, e.g. bovine serum albumin, as they were thermally unfolded at 56°C or 72°C, but did not bind the thermophilic enzyme alcohol dehydrogenase from *Thermoanaerobium brockii*, which remains in its native state at these temperatures. As a further feature, TF55 exhibited ATPase activity, at a level at 75°C similar to that of GroEL at 37°C. Similarly, the component from *Pyrodictium occultum* exhibited ATPase activity that increased with temperature (Phipps *et al.* 1991).

The nature of its expression suggested that while TF55 is a molecular chaperone under conditions of normal cell growth, during heat-shock it may assume additional functions. Its concentration becomes significantly elevated and it may act to 'protect' other proteins and cellular structures not only via a chaperone function but perhaps by forming higher order matrix or paracrystalline structures.

Although TF55 had features *in vitro* of a molecular chaperone, a demonstration that it mediates folding has so far been lacking. Thermophilic archaebacteria have not, to date, been amenable to targeted genetic manipulation, making *in vivo* analyses difficult. A major issue concerns whether TF55 functions alone as a chaperone in thermophilic archaebacteria. No homologue of hsp 70 has been identified in these organisms. Further, there is no evidence that nascent proteins must interact with such a component or that they must be delivered to TF55. In addition, no small molecular size heat-inducible co-chaperone has been identified during incubation at 88°C, for example, that could be analogous to GroES. It is possible that TF55 could contain as part of its architecture a domain or motif that performs the function of GroES or, alternatively, that such a function might not be required for growth at high temperature.

Analysis of the primary structure of TF55 showed no significant overall relationship to hsp 60 or its relatives (Trent *et al.* 1991). In contrast with this very distant relationship, there was nearly 40% identity between TF55 and a eukaryotic protein, *t*-complex polypeptide-1 (TCP1) (Trent *et al.* 1991). TCP1 was originally discovered as an abundant protein in mouse testis which mapped to the mouse *t*-complex on chromosome 17 (Silver *et al.* 1979). In mice it exists in two polymorphic forms, TCP1A and TCP1B, separable by isoelectric focusing (A = acidic and B = basic), the B form typically being found in common laboratory mouse strains (and designated +). TCP1 has been considered as being involved with transmission ratio distortion (Silver & Remis 1987) whereby sperm containing the normal (+) chromosome 17 are functionally disadvantaged compared with *t*-chromosome sperm, but there is controversy on this point (Lyon 1990). In addition to the presence of TCP1 in spermatocytes and spermatids (Willison *et al.* 1990), to a level of approximately 0.2–0.4% of soluble protein (Lewis *et al.* 1992), TCP1 is found in all mammalian cell types examined so far, except mature sperm, suggesting a general functional role. It is also present in *Arabidopsis thaliana*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and in *Saccharomyces cerevisiae* as an essential gene (Ursic & Culbertson 1991). Among the mammalian sources examined, TCP-1 is highly conserved showing approximately 95% identity in pairwise comparisons; when compared with *S. cerevisiae* and *S. pombe*, there is 70% identity. A faint structural relationship between TCP1 and the hsp 60 family (also called the chaperonins) has led to the suggestion that TCP1 might be a cytosolic equivalent of the chaperonin family (Ahmed & Gupta 1990; Ellis 1990; Hemmingsen 1992; Lewis *et al.* 1992).

(*a*) *TCP1 resides in a hetero-oligomeric complex*

Production of a series of mouse monoclonal antibodies against mouse TCP1 expressed in *E. coli* enabled both cellular localization studies and characterization of the oligomeric state of the protein. Although initial fluorescent studies suggested localization to the trans-Golgi network (Willison *et al.* 1989), it turned out that two of the monoclonal antibodies (23C and 72A) recognized an epitope present in both

TCP1 and a 102 kDa protein at the cytosolic face of Golgi membranes (Harrison–Lavoie *et al.* 1993). In contrast, one of the monoclonal antibodies (91A) recognized only the 58 kDa TCP1 species and produced cytosolic staining. Localization to the cytosol was supported by cell fractionation studies revealing the protein to be in soluble fractions. Interestingly, in staining with 91A, the same diffuse pattern of staining is observed in the nucleus, suggesting possible presence of the TCP1 complex in this compartment as well. This localization seems consistent with an apparent role of the complex in formation of the mitotic spindle (see below), and would indicate, given its size of 900 kDa, that the complex would most likely need to be targeted to the nucleus in less than final form.

In sucrose gradient sedimentation of testicular lysates, TCP1 localized to the 20*S* fraction, suggesting that it resides in a high molecular size complex like that of the hsp 60 family members and TF55 (Lewis *et al.* 1992). In contrast to the homo-oligomeric composition of the TF55 complex, however, TCP1 appears to reside in a hetero-oligomeric complex comprised of at least six to seven polypeptides of apparent size 55–60 kDa (Lewis *et al.* 1992; Frydman *et al.* 1992). This interpretation is supported by the finding that the complex, exclusive of other proteins, maintains this composition through various chromatography steps, and by the observation that immunoprecipitation with anti-TCP1 monoclonal antibodies isolates the same set of polypeptides (Lewis *et al.* 1992). Although these studies provide support for the hypothesis that TCP1 resides in a hetero-oligomeric complex, they do not exclude the possibility that populations of TCP1-containing complexes with various compositions exist, potentially mediating distinct functions. To date, distinct complexes have not been separable. The similarly sized species of heterooligomeric TCP1 complex may be related to one another. In *S. cerevisiae* a second 55 kDa member of the complex has been identified that is 35% identical to TCP1 (Mertens *et al.* 1993). A second TCP1-related protein has also been identified in humans by the ability of its expressed cDNA to rescue an amino acid transport mutant of yeast (Segel *et al.* 1992). Peptide sequence from the fastest-migrating species of bovine testicular complex was related to TCP1 (Frydman *et al.* 1992). Additional TCP1-related proteins have also recently been identified in human, mouse, and *C. elegans* (K. Willison, unpublished data).

The quaternary structure of TCP1 complex from reticulocyte lysate, testis, and *S. cerevisiae*, has been examined by electron microscopy (Gao *et al.* 1992; Lewis *et al.* 1992; D. Miklos, unpublished). A stacked double ring complex like that of TF55 was observed, with mostly eight or nine-membered rings. In at least one study (Lewis *et al.* 1992) the electron dense central 'hole' appeared to be 'obscured', suggesting either a somewhat different architecture to this complex than TF55, for example, or indicating an associated component or substrate protein to be present. In the latter cases candidate proteins include both an hsp 70 class protein, observed to be present in SDS-PAGE analysis of the preparation of the complex (Lewis *et al.* 1992), and tubulin. Overall, the primary and quaternary structures shared with both TF55 and the hsp 60 family suggested that TCP1 complex might also function as a molecular chaperone.

(*b*) *Functional analysis in vivo*

Using the yeast TCP1 gene, it was possible to test the function of TCP1 in intact cells. Yeast cells with a disrupted TCP1 gene were inviable, and a cold-sensitive mutant grew at 14°C at a linear rate instead of the normal logarithmic rate observed for wild-type cells (Ursic & Culbertson 1991). The population of shifted cells contained a high percentage of anucleate and multinucleate cells, suggesting abnormalities of nuclear segregation. Associated with these abnormalities, microtubule staining patterns were abnormal, suggesting a role for TCP1 in microtubule-mediated processes. More recently a stain containing a temperature-sensitive allele of TCP1 has been isolated (Mertens *et al.* 1993). This strain ceases growth after shift to 37°C. The cells fail to produce a normal mitotic spindle, exhibiting at most a short metaphase-like spindle, and are subject to cell cycle arrest as large-budded cells at late G2-M stage. In addition to the major effect on spindle biogenesis, effects have also been observed on several cytosolic enzymes. Two enzymes have been examined, yeast ornithine transcarbamylase (OTC), a cytosolic homotrimer, and cytosolic invertase, an endogenous homodimer. The encoding genes were programmed for inducible expression in cells deficient for the respective endogenous activities by joining their coding sequences to a GAL1 operon promoter (D. Miklos, unpublished). Cells were shifted to non-permissive temperature, then induced by transfer into galactose-containing medium. In each case, induction of enzyme activity to a level approximately 10–20% that in identically programmed wild-type cells was observed. As yet, however, the fate of the newly made polypeptides has not been fully resolved. It is thus so far not clear whether the proteins are rapidly turned-over or whether they are present in insoluble aggregates.

Does the TCP1 complex have specific, or at least preferred, targets for its putative chaperone function? Tubulin in *S. cerevisiae* is not a highly expressed protein, with an estimated abundance of ~0.03% soluble protein. It seems likely then that a putative chaperone function is required not so much by its abundance but by properties of its structure. For example, tubulin subunits are unable to refold to their native state *in vitro* following dilution from denaturant.

The abundance of the TCP1 complex in various cell types fits with the idea that tubulin could be a preferred substrate, as levels of TCP1 complex approximately correlate with the relative abundance of tubulin. For example, testicular tissue has 5–10-fold greater levels of TCP1 complex than other mammalian tissues, corresponding to its higher abundance of tubulin-rich cells. Similarly, *S. pombe* has greater levels of tubulin than *S. cerevisiae*, and a correspondingly greater level of TCP1 complex (K. Willison, unpublished data). Finally, in relation to abundance

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considerations, it should be mentioned that the amount of TCP1 complex is not increased in response to heat shock (Ursic & Culbertson 1992; V. A. Lewis *et al.* unpublished data; D. Miklos *et al.* unpublished data). The significance of this is unclear if one considers that, e.g. hsp 60 which mediates a global function in the mitochondrial matrix, is induced only twofold in response to heat shock.

Could the TCP1 complex have redundancy of function among its distinct subunits such that mutations of one component monomer do not produce a fully defective phenotype? While it seems possible that other subunits of the ring structure could compensate for a mutationally altered member still present in the complex, in the more extreme case, overexpression of TCP1 cannot rescue a lethal deletion of a gene encoding a second member of the complex which is structurally related to TCP1 (Mertens *et al.* 1993). Thus there is apparently no complete overlap of function among at least some component members of the complex. It remains possible, however, that mutational alteration of more than one subunit at a time could produce a more severe phenotype.

(*c*) *Functional analysis in vitro*

The studies *in vivo* of TCP1 in yeast showing defective tubulin-containing structures, found corroboration *in vitro* in the studies of Yaffe *et al.* (1992) who examined newly translated tubulin subunits in rabbit reticulocyte lysate. Newly translated subunits were found to transiently associate with a 900 kDa complex following completion of translation. Several minutes later, the subunits were found either as free monomers or as α-β tubulin heterodimers. Depletion of ATP by treatment of the lysate with apyrase or gel filtration prevented the newly-translated subunits from leaving the complex. Addition of MgATP to the filtered lysate caused release. As a measure of conformation, tubulin subunits were examined for protease susceptibility. The bound subunits were found to be exquisitely sensitive to protease, whereas the released subunits were relatively resistant, resembling native tubulin. When the 900 kDa complex was purified, it was found to contain a collective of 55–60 kDa polypeptides resembling that of TCP1 complex from testis. On immunoblot analysis of this complex with the anti-TCP1 monoclonal antibody, 91A, a 58 kDa species reacted strongly, indicating that the TCP1 complex is responsible for the observed molecular chaperone activity in tubulin biogenesis.

A second study has measured the ability of the TCP1 complex purified from reticulocyte lysate to refold urea-denatured actin. In these studies, actin, metabolically labeled in *E. coli* and purified from inclusion bodies by urea solubilization, was examined for refolding after dilution, using migration in native gel electrophoresis (Gao *et al.* 1992). A portion of the input material was observed to exhibit migration typical of native actin when diluted into reticulocyte lysate fractions containing TCP1 complex. The amount of this form was minor, however, when compared with the quantitative yield in the foregoing tubulin experiments. This low yield raises some questions about the physiological significance of the observations. Indeed, *in vivo* experiments with both the cold and temperature-sensitive alleles of yeast TCP1 fail to reveal any effect on actin function: both alleles could produce budded cells, a function critically dependent on actin. Examination of the temperature-sensitive TCP1 mutant cells by electron microscopy of serial sections failed to reveal any accumulation of transport vesicles, whose transit through ER and Golgi is dependent on actin function (Mertens *et al.* 1993). Staining of the temperature-sensitive mutant with the actin-specific stain rhodamine phalloidin, failed to reveal any difference in pattern from that of an isogenic wild-type strain. Thus a role for TCP1 complex in actin biogenesis seems less certain than its role in tubulin biogenesis.

CONCLUSIONS

The shared features of both primary and quaternary structure and mechanism of function of the hsp 60 and TF55-TCP1 families of chaperone discussed here, suggest that these two groups of chaperone either arose from a common ancestor or evolved convergently, and it has been proposed that both families should be regarded as subgroups of the chaperonins (Ellis 1992). Whatever the case, presumably the present-day components have evolved along with the polypeptides in their respective cellular compartments. Considering the essential nature of protein-mediated folding, it seems likely that this evolution occurred in an interdependent manner: while chaperones became needed to facilitate what would otherwise be inefficient spontaneous folding, proteins themselves may have evolved to successfully utilize these components. Molecular dissections of folding during the next few years, e.g. by further characterizing the intermediates bound and the structures within them that are recognized, and identifying the binding sites within the chaperones themselves, should provide further insight into how the acquisition of protein structure in the cell is mediated by these remarkable components.

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Discussion

N. J. Cowan (*Department of Biochemistry, New York University Medical Center, New York, U.S.A.*). We have recently described the ability of cytosolic TCP1 to fold denatured actin to form the native protein. Have the authors looked at their yeast mutant with respect to actin filament structure?

A. Horwich. We have not seen an actin phenotype. The ability of yeast cells to make buds is critically dependent upon actin so this argues against effects on actin. We have also examined serial sections of the yeast mutant, looking for the accumulation of secretory vesicles whose transport through the secretory pathway is absolutely dependent on actin function, but have not seen accumulation. Perhaps the mutant allele we have described is not critical for actin folding since there are other components of the TCP1 complex; Dr Cowan's *in vitro* experiments use the intact TCP1 complex whereas in our mutant we presume that only one component of the complex, TCP1, is altered. So we cannot exclude from our studies that the TCP1 complex has a role in actin assembly.

P. Viitanen (*Du Pont de Nemours, Wilmington, U.S.A.*). Does the gold-derivatized DHFR attach to chaperonin 60 if chaperonin 10 is bound to the latter?

A. Horwich. We have not tried that or related experiments because of the very low yield of the chaperonin 60 that contains gold-derivatized DHFR. We have found that it is essential to separate the chaperonin 60 from unbound DHFR–gold particles by a filtration step so that no gold particles unbound to the chaperonin 60 appear in the field, but this step lowers the yield greatly.

P. Viitanen. Do gold particles unbound to DHFR appear in the cavities of chaperonin 60?

A. Horwich. No.

P. Viitanen. Have the authors tried binding gold particles to the chaperonin 10 as a means of determining the stoichiometry of binding of chaperonin 10 to chaperonin 60?

A. Horwich. We would like to do this. We are currently trying to bind gold particles to chaperonin 60.

P. Micklethwaite (*Oxford, U.K.*). I am interested in the possible ninefold symmetry of the TCP1 particle in relation to the ninefold symmetry of the centriole which of course contains tubulin. Is there any association between developmental proteins containing epidermal growth factor repeats and the TCP1 chaperonins?

A. Horwich. Not that I am aware of.

M.-J. Gething (*Howard Hughes Medical Institute, University of Texas, Dallas, U.S.A.*). Is it correct that TCP1 alpha and TCP1 beta genes are individually essential for cell viability?

A. Horwich. Both genes are essential; their products either have distinct functions or are both required for making the TCP1 particle.

M.-J. Gething. Are both proteins cytosolic?

A. Horwich. We believe so; neither gene contains a signal sequence.

H. Saibil (*Birkbeck College, University of London, U.K.*). We have evidence from the three-dimensional reconstruction of chaperonin 60 molecules from electron microscope images that there are distinct protein densities inside the cavity at each end of the oligomer; this supports your data suggesting that there are protein binding sites inside each heptameric ring of chaperonin 60.

R. Jaenicke (*Department of Biophysics and Physical Biochemistry, University of Regensburg, F.R.G.*). It has been reported (Phipps *et al.* 1991) that an ATPase with eightfold symmetry accumulates when the thermophilic archaebacterium *Pyrodictium* is heat-shocked by raising the temperature from 102°C to 108°C. We have been trying to determine whether this protein has chaperonin activity, and have found that denatured chains of two heat-resistant enzymes will bind to this ATPase; however, so far we have been unable to release these chains.

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C. Georgopoulos (*Biomedical Institute, University of Geneva, Switzerland*). Am I correct in understanding that there are four or five TCP1 subunits which are about 50% identical to one another, and is it clear that all these subunits occur in the same oligomer or could there be several different oligomers with different combinations of subunits?

F.-U. Hartl (*Memorial Sloan-Kettering Cancer Center, New York, U.S.A.*). We have sequence data for four of the TCP1 proteins and they are related to one another, but the degree of similarity is not yet established. We believe that most of them are in the same complex as they comigrate together on electrophoresis on native gels at different pH values.

A. Horwich. If one overexpresses one TCP1 protein in yeast it forms a homo-oligomer which shows that at least this protein can form oligomers with itself and does not need the other proteins. Whether this homo-oligomer is functional is unknown; it is not toxic to the cell.

P. Mickelthwaite. Is there any significance in the structural similarity at the quaternary level between the TCP1 particle and the proteasome?

A. Horwich. The *in vitro* observations suggest that this particle by itself does not degrade the polypeptides that it binds.

N. J. Cowan. I suggest that the balance of evidence indicates that although the TCP1 particles contain more than one type of protein subunit, they do not themselves form a heterogeneous population. The particles always run as a single band over a range of pH values on native gel electrophoresis; the composition of the particles is also independent of the source of tissue, even when the tissues are making different forms of actin, such as muscle and brain. We have also shown that actin and tubulin compete for binding to the isolated TCP1 particle.

W. J. Welch. This view implies that more than one gene is required to make a functional particle, unlike the case for the bacterial chaperonin 60.

N. J. Cowan. I think that there is no doubt about that.

K. Willison (*Institute of Cancer Research, Chester Beatty Laboratories, London, U.K.*). My experience is that when you purify the TCP1 particle from different tissues the molar ratios of the different subunits are identical. Pulse-chase experiments also give equimolar ratios of labelling of all the subunits at the same time. The subunits, however, do vary in number between testis and cells from tissue culture. We have also shown that many of the subunits are related to TCP1 in sequence. What I should like to emphasize is that there are more TCP1-related genes known than there are polypeptides in the TCP1 particle. My view is that the TCP1 particle is homogeneously composed of four, five or six different proteins, depending on the source. There may be minor variation, but we never see, for example, three times as much of one protein compared to the others. It is still possible that variant particles exist in other types of tissue, but there is no evidence for this. It is also possible that TCP1-related proteins may occur in the enodplasmic reticulum lumen.

N. J. Cowan. I agree that it is possible that there is some tissue-specific variation.

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