Minireview

Structure and function of a protein folding machine: the eukaryotic cytosolic chaperonin CCT

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Abstract Chaperonins are large oligomers made up of two superimposed rings, each enclosing a cavity used for the folding of other proteins. Among the chaperonins, the eukaryotic cytosolic chaperonin CCT is the most complex, not only with regard to its subunit composition but also with respect to its function, still not well understood. Unlike the more well studied eubacterial chaperonin GroEL, which binds any protein that presents stretches of hydrophobic residues, CCT recognises in its substrates specific binding determinants and interacts with them through particular combinations of CCT subunits. Folding then occurs after the conformational changes induced in the chaperonin upon nucleotide binding have occurred, through a mechanism that, although still poorly defined, clearly differs from the one established for GroEL. Although CCT seems to be mainly involved in the folding of actin and tubulin, other substrates involved in various cellular roles are beginning to be characterised, including many WD40-repeat, 7-blade propeller proteins. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Protein folding; WD40 repeats; Chaperonin; Actin; Tubulin

1. Introduction

Chaperonins are a ubiquitous family of proteins involved in the assistance to the folding of other proteins [1–3]. Their general structure is that of a large cylinder (800–1000 kDa) formed from two rings placed back-to-back, and constituted of ~60 kDa proteins which possess weak ATPase activity. The cavity enclosed by each ring has an important role in protein folding, since it provides the unfolded polypeptide a confined environment, generated after the conformational changes induced upon ATP binding, where folding proceeds. The manner in which the unfolded protein is confined depends on the type of chaperonin.

1.1. Group I chaperonins

These are present in eubacteria and in the eukaryotic organelles of endosymbiotic origin (mitochondria and chloroplast) and are the most well characterised ones, thanks to the structural and functional work carried out with the *Escherichia coli* chaperonin, GroEL [4–7]. This chaperonin, like most members of this group, is composed of two homoheptameric rings. Each monomer is divided into three domains: the equatorial domain, containing the ATP binding site and responsible of most of the inter- and intra-ring interactions; the apical domain, containing the residues responsible for the binding of the unfolded polypeptide; and the intermediate domain, which connects the first two domains. GroEL works in conjunction with a small heptamer termed cochaperonin (GroES in *E. coli*) that caps the chaperonin cavity after the structural rearrangements induced in GroEL upon ATP binding, and forces the release into the cavity of the unfolded polypeptide, which was previously bound to the apical domains.

1.2. Group II chaperonins

This group, found in archaeabacteria and in the cytosol of eukaryotic organisms, although sharing very low sequence homology with its eubacterial counterparts, is more heterogeneous regarding its structure and composition. The archaeal chaperonins, usually named thermosomes, can be octameric or nonameric and can be composed of 1–3 different proteins [8,9]. Little is known about the mechanism and folding of substrates by these chaperonins. However, the atomic structure of the *Thermoplasma acidophilum* chaperonin has been obtained [10,11], and its two component subunits (α and β) reveal the same three-domain arrangement originally described in the GroEL monomer, with a conserved equatorial domain but a less similar apical domain, the substrate binding region. The major difference resides however in an extra region located in the tip of the apical domain, which is not present in the group I chaperonins [10]. This protrusion closes the cavity after the structural rearrangements induced in the chaperonin upon ATP binding, [1,9–15] and probably plays the equivalent role of a cochaperonin, which seems absent in the group II chaperonin system.

2. The eukaryotic cytosolic chaperonin CCT

2.1. Structure and subunit composition

The most different and complex of all group II chaperonins is the eukaryotic cytosolic chaperonin CCT (Chaperonin Con-

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taining TCP-1; also termed TRiC) [1,8,9,16–18]. It is different because, unlike all the other chaperonins characterised so far, it seems to be involved in the assistance of the folding of only a small set of proteins (see below). CCT is also complex because, although is built up like the other chaperonins by two superimposed rings [12,15,19,20], each ring is constituted by eight different, albeit homologous subunits (30% identity) (CCTα, β, γ, δ, ε, ζ, η, θ; CCT1-CCT8 in yeast), whose arrangement within the ring has been determined [21–23]. This unique structural composition seems to be the same in all eukaryotic CCT. In all the organisms and tissues studied each CCT subunit is encoded by an unique gene [18], with the exception of mammalian testis, which contains a tissue-specific CCTζ2 [24]. The overall structure of CCT has been determined at 25–30 Å resolution by cryoelectron microscopy [12,15,19,20] and is very similar to other group II chaperonins [11,13,14,25], a barrel-shaped cylinder with a diameter of ~150 Å and a height of ~160 Å, with every subunit interacting with only one subunit from the opposite ring [11,12], differently from the staggered interaction observed in group I chaperonins [4,5]. Unfortunately, no high-resolution information of the entire CCT assembly has so far been obtained.

The conformational changes induced in CCT upon ATP binding are of different nature to those observed in GroEL (Fig. 1A). In the eubacterial chaperonin nucleotide binding generates small changes that allow binding of the cochaperonin, which in turns induces large rearrangements of the oligomeric structure and the closure of the ring cavity [5,26–28] but ATP binding to CCT (and to group II chaperonins in general) generates large conformational changes in the apical domains that close the ring cavity [11–15,29]. The sealing of the cavity is performed by the extra region present in the tip of the apical domain of group II chaperonins, which acts like an iris [15].

2.2. Folding mechanism of CCT

Like other chaperonins, CCT clearly uses its large conformational rearrangements generated upon ATP binding [14,27] to assist in the folding of other proteins [14]. However, there are some aspects in the mechanism of the cytosolic chaperonin that are different to any other known chaperonin, and which have to do with the interaction between the chaperonin and the substrate, and with the folding mechanism itself. In the case of GroEL, the interaction with the unfolded polypeptide is non-specific and occurs between the hydrophobic surface of the apical domain of any given subunit(s) and the exposed hydrophobic region(s) of the unfolded polypeptide [30–32]. In the case the eukaryotic chaperonin, the interaction with its unfolded substrates, actin and tubulin, occurs between specific CCT subunits [1,20] and the binding determinants of the two cytoskeletal proteins [33–36]. The specific nature of the interaction between CCT and its substrates suggests the presence of charged residues in the regions of the apical domains where the electron microscopy studies of CCT:substrate complexes have located the substrate-binding regions [19,20]. The atomic structure of the CCTγ apical domain [37] shows that this is the case, and the same can be implied of the other CCT subunits, after comparison of their apical domain sequences with that of CCTγ.

Another difference between the two chaperonins is the nature of the folded state of the chaperonin-bound unfolded polypeptide. Numerous studies have shown that GroEL recognises any kind of folding conformation of an unfolded polypeptide as long as the latter exposes a certain set of hydrophobic residues [31]. However, the CCT-interacting conformations of actin and tubulin, although determined at low resolution by cryoelectron microscopy, are compatible with a quasi-folded state which both cytoskeletal proteins may have attained before the interaction with the cytosolic chaperonin [20,39].

Finally, a further important difference between the folding mechanism of GroEL and CCT lies in the interaction between the substrate and the chaperonin after nucleotide binding. In the case of GroEL, the conformational changes brought about by ATP and GroES binding liberate the unfolded polypeptide into the chaperonin cavity (sealed upon GroES binding) where it is given a chance to fold. This is a passive folding mechanism (although see [38]), very different to the active mechanism defined for CCT, where the movements of the apical domains induced upon ATP binding seal the chaperonin cavity and force the change of the bound, open conformations of actin and tubulin towards compact, quasi-native (or native) structures that are not liberated into the CCT cavity but remain bound to the chaperonin [15]. The circular, closed structure of the ring is used in both chaperonins to isolate the unfolded polypeptide from the all the other polypeptides in the cytoplasmic compartment, but the cytosolic chaperonin seems to use the conformational changes that seal the ring to force the folding of its substrates.

2.3. Allostery in CCT

In all chaperonins studied so far, ATP binding and hydrolysis is used for maintaining their functional cycle not only through the conformational changes that induce the closure and opening of the ring cavity, but also through changes in the substrate-binding affinity between the open, substrate-receptive conformation and the closed conformation. These changes are governed by nested cooperativity that involves intra-ring positive cooperativity and inter-ring negative cooperativity in ATP binding [39]. This behaviour is observed in CCT, although kinetic differences with respect to the type I chaperonin GroEL has lead to the suggestion that there may be differences in the intrinsic affinities for ATP among the different subunits in a ring [40]. This is compatible with the fact that the eight different subunits are arranged in a fixed position [21] and with genetic experiments that show that ATP-binding in CCT takes place in a hierarchical manner, different from the concerted manner characterised for GroEL [41].

2.4. The interaction of CCT with actin and tubulin

Actin and tubulin have been characterised as the major folding substrates for folding by CCT. Both cytoskeletal proteins require in vivo and in vitro the interaction with CCT each to fold to their native states [42]. Electron microscopy experiments have shown that actin and tubulin each interact with CCT in a quasi-folded conformation, with the two topological domains of both cytoskeletal proteins separated by a hinge with the nucleotide binding site placed in its vicinity [20].

In the case of actin, the interaction of its two topological domains occurs between two CCT subunits localised in a 1,4 arrangement (CCTδ and CCTβ or CCTδ and CCTε) [19]. The interaction between the two tubulin domains and CCT is

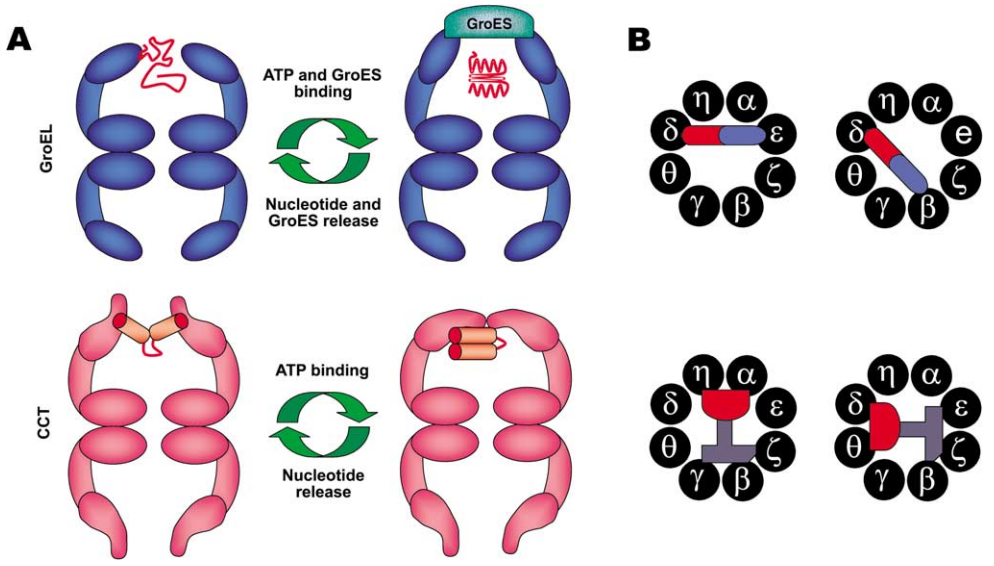

Fig. 1. A: A schematic model of the folding mechanism of GroEL and CCT. Both chaperonins cycle between an open, substrate-receptive conformation and a closed conformation. In GroEL, substrate recognition and binding is performed in the open conformation by a hydrophobic region in the apical domain of any of the GroEL subunits, which recognises any kind of unfolded conformation, provided the denatured polypeptide exposes a set of hydrophobic residues. The closed conformation is generated upon ATP binding in the presence of the cochaperonin GroES, and the unfolded polypeptide is liberated in the GroEL cavity, where folding may take place using the information encoded in its own amino acid sequence. The polypeptide, folded or not, is liberated from the GroEL cavity after GroES release, which is induced upon ATP hydrolysis and nucleotide liberation. In CCT, the apical domains of specific subunits recognise a substrate conformation (at least, in the case of actin and tubulin) that has acquired an important degree of secondary and tertiary structure. The sealing of the CCT, cavity carried out by the movements of the apical domains induced upon ATP binding, is performed by the helical protrusions present at the tip of the apical domains, and the substrate is not liberated in the CCT cavity but remains bound to the apical domains and forced to acquire a more compact, native conformation. B: The actin and tubulin molecules, already in a quasi-native conformation, interact with the nucleotide-free, open conformation of CCT through specific subunits [20]. The N-terminal domains of actin and tubulin are depicted in red, whereas the C-terminal domains are coloured blue.

more complex, with five CCT subunits involved in tubulin binding also in two alternative modes of interaction (see Fig. 1B) [20]. It is possible that CCT may have evolved from a primitive chaperonin for the purpose of, among other things, overcoming specific kinetic barriers in the folding of both cytoskeletal proteins (and perhaps other proteins) through the stabilisation of open conformations. If so, it is possible that the critical steps in actin and tubulin folding, brought about by the conformational changes generated in CCT upon ATP binding, may have to do with the correct folding of their nucleotide binding site. In both cytoskeletal proteins, most of the inter-domain bonds that hold secure their native structures occur through the nucleotide binding site. In the case of actin, unfolding experiments have revealed that its native conformation depends on the degree to which the nucleotide contributes to the connectivity between the two domains of the protein [43]. In the case of tubulin, biochemical experiments show that GTP binding occurs after the closure of the CCT cavity [15,44].

2.5. Other CCT substrates

Although a matter of dispute for some time, it seems clear now that CCT interacts with a large number of proteins (9–15% of newly synthesised proteins [45]; for a comprehensive review of the substrates cited below, see [18,46]). Besides the already cited actin and tubulin, CCT has been found to interact either in vitro or in vivo with other cytoskeletal proteins such as the heavy meromyosin subunit (HMM), actin-related proteins, cofilin and actin-depolymerising factor-1. Additional proteins, involved in various cellular processes have been

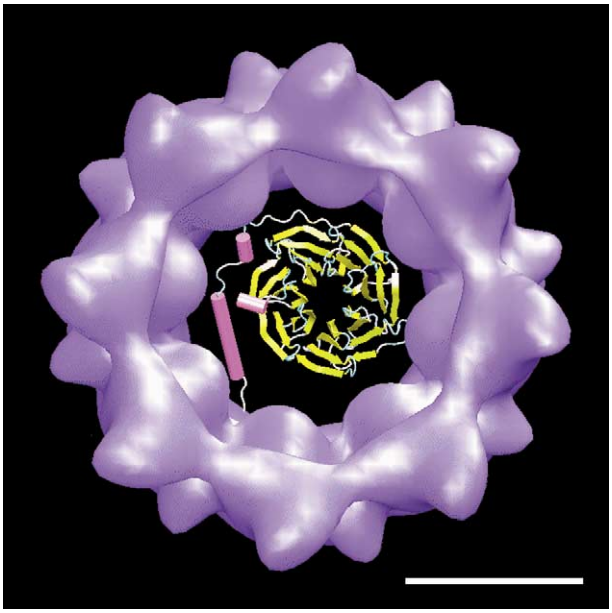

Fig. 2. A model of the interaction of CCT with a WD40 7-blade propeller protein. This model only intends to show that the WD40 protein fits within the chaperonin cavity. The atomic structure of G_{β}-transducin (PDB accession code 2TRC) has been used as a model of a WD40 seven-blade propeller protein, and the eukaryotic chaperonin is represented by a top view of the three-dimensional reconstruction obtained by cryoelectron microscopy of the open, substrate-receptive conformation of CCT [20]. Bar indicates 50 Å.

shown to interact with CCT. Among those, G_{α}-transducin, (involved in retinal phototransduction), cyclin E (implicated in cell cycle control), and luciferase. Other proteins that are folded by CCT are certain viral proteins such as the Epstein–Barr virus-encoded nuclear protein (EBNA-3), the hepatitis B virus capsid and the type D retrovirus Gag polyprotein. It is puzzling that CCT retains the ability to interact so specifically with actin and tubulin and with this host of other non-homologous substrates.

Another CCT-interacting protein is the Von Hippel-Lindau tumour suppressor protein (VHL) [47], which is associated with renal cell cancer. VHL, complexed with elongins B and C, forms part of the VCB–Cul2 complex, involved, like other E3 ubiquitin ligase complexes, in protein degradation processes. CCT has been shown to bind VHL, pending its transfer to the elongin BC complex [47,50]. VHL plays the role in the VBC–Cul2 complex of a protein adapter, linking the complex to its degradation substrate, much like the modular SOCS-box proteins that form part of other E3 ubiquitin ligase complexes [49]. VHL and the SOCS-box proteins contain two domains, an E3 ubiquitin ligase binding site and a target substrate binding site. CCT binds to VHL at this second site, which in many of the SOCS-box proteins contains WD40 repeats [50]. Curiously enough, a proteomic analysis of *Saccharomyces cerevisiae*, using tagged open-reading frames to pull-down multiprotein complexes has revealed a group of at least 21 proteins that interact with the CCT oligomer (Table 1). These proteins are involved in various cellular processes and a large percentage (16 proteins) have in common to posses in their sequence 7 WD40 motifs (Fig. 2) [51]. A hidden Markov model (HMM) search of the WD40 repeat domain, as defined in the Pfam database (http://www.sanger.ac.uk/Software/Pfam), through all *S. cerevisiae* proteins found 97 positive results, containing between three and eight WD40 domains each, thus these CCT-interacting ones represent 17% of the total number of WD40 proteins in yeast. Most of these proteins have molecular masses ranging from 55 to 100 kDa, and some of them are therefore unlikely to be folded within the CCT cavity. It is possible that CCT may a have a different role than folding assistance with regard to these proteins; for example regulating their activity by controlling their liberation into the cytosol or binding to other proteins in assembly as with VHL–celongin C–elongin B complex formation [48].

2.6. The interaction of CCT with other molecular chaperones

It seems now evident that many molecular chaperones work in conjunction to orchestrate the folding process of different proteins. In the case of CCT, prefoldin (PFD, also termed GimC) [52,53] is a chaperone present in archaea and eukarya that can be formally considered as a cochaperonin since it physically interacts with CCT (see [54] and references therein). The heterohexameric structure of PFD resembles that of a jellyfish [55], using its tentacles to protect the nascent chain from unwanted interactions and to transfer the unfolded polypeptide to CCT for its subsequent folding. In the case of the eukaryotic PFD, only a few substrates have been characterised (actin, tubulin and VHL). Other chaperones seem to play a role specifically in the tubulin folding process after its interaction with CCT. α- and β-tubulin, once released from CCT, interact respectively with cofactors B and A and are subsequently transferred with the help of cofactor C, to cofactors D and E, respectively [55,56]. This complex process results in the formation of stable α,β-dimers that can be incorporated into the protofilaments for microtubule formation. Finally, CCT has been shown to interact with Hsp70, either directly [47] or through the interaction with the Hsp70/Hsp90 organising protein (Hop, also termed p60) [57], thus linking CCT with two other major chaperone systems, the Hsp90 system and the one constituted by Hsp70/Hsp40.

2.7. Evolution of CCT

Gene duplication seems to be the driving force behind chap-

Table 1
Other CCT-interacting proteins found in the *S. cerevisiae* genome[a]

Gene name[b]	SwissProt code[c]	Protein name/function[d]	WD40	Mw (kDa)[e]
CAF4/YKR036C	YK16_YEAST	Component of CCR4 transcriptional complex	yes	74.7
CDC20/YGL116W	CC20_YEAST	Cell division control protein 20	yes	67.4
CDC55/YGL190C	2ABA_YEAST	Protein phosphatase PP2A regulatory subunit B	yes	59.7
CDH1/YGL003C	YGA3_YEAST	CDC20 homologue 1	yes	62.8
KSS1/YGR040W	KSS1_YEAST	Mitogen-activated protein kinase KSS1	no	42.7
PEX7/YDR142C	PEX7_YEAST	Peroxisomal targeting signal 2 receptor	yes	42.3
PFS2/YNL317W	YN57_YEAST	Polyadenylation Factor I subunit 2	yes	53.1
PPH3/YDR075W	P2A3_YEAST	Protein phosphatase type 2A	no	35.3
PRP46/YPL151C	PR46_YEAST	Pre-mRNA splicing factor PRP46	yes	50.7
PWP1/YLR196W	PWP1_YEAST	Periodic tryptophan protein 1	yes	63.8
RAD24/YER173W	RA24_YEAST	Checkpoint protein. Cell cycle exonuclease (putative)	no	75.7
RAD28/YDR030C	Q12021	Protein involved in the same pathway as Rad26p	yes	58.2
SEC27/YGL137W	COPP_YEAST	Coatomer beta′ subunit	yes	99.4
SOF1/YLL011W	SOF1_YEAST	U3 snoRNP protein	yes	56.8
STE4/YOR212W	GBB_YEAST	Guanine nucleotide-binding protein beta subunit	yes	46.6
TAF90/YBR198C	T2D4_YEAST	Transcription initiation factor TFIID 90 kDa subunit	yes	89.0
UTP7/KRE31/YER082C	YER2_YEAST	U3 snoRNP protein	yes	62.3
WTM1/YOR230W	WTM1_YEAST	Transcriptional modulator WTM1	yes	48.4
YBL049W	YBE9_YEAST	Unknown	no	16.1
YDL156W	Q12510	Unknown	yes	59.2
YJU2/YKL095W	YKJ5_YEAST	Unknown	no	32.3

[a]The proteins described in this table are those that in [51] have been found to interact with three or more CCT subunits.
[b]According to the '*Saccharomyces* Genome Database (SGD)' ORF names (http://genome-www. stanford.edu/Saccharomyces).
[c]Swiss-Prot protein sequence database entry name (http://www.expasy.ch/sprot).
[d]From the Swiss-Prot 'Protein name' or the SGD 'Gene Product' or 'Description' database entry fields.
[e]Theoretical molecular weight, calculated from the protein sequence composition, in kDa.

eronin evolution [58], both in the simpler archaeal chaperonin systems, composed of one to three different subunits and eukaryotic CCT, made up of its eight different subunits, probably explaining the more complex role of the eukaryotic chaperonin. The evolution of the group II chaperonins towards heterooligomerisation is paralleled by that of their cochaperonin PFD, which in archaea is constituted by two α and four β subunits (α and β being homologous) whereas in eukarya is composed of six different subunits, two α-like and four β-like subunits [54]. The appearance of the eight different subunits in CCT occurred very early in eukaryotic evolution, at the same time that a more complex cytoskeleton evolved to allow the transition from prokaryotes to eukaryotes [18,59]. Actin and tubulin form the core of the eukaryotic cytoskeleton and both precisely require the assistance of CCT for their folding. The domains of actin and tubulin putatively involved in CCT binding are absent or greatly modified in their prokaryote homologues FtsA/MreB and FtsZ, respectively, and most of these domains are implicated in actin and tubulin polymerisation function [60]. It is very likely that CCT evolved in the eukarya kingdom as a direct consequence of the selective pressure imposed by folding problems caused by the emergent cytoskeletal proteins. The functional cycle was then probably parasitised by other proteins to solve their own folding problems, and the cases of the viral proteins assisted in their folding by CCT are paradigmatic. Other CCT-interacting proteins such as the ones containing WD40 motifs are very abundant in eukaryotes and some may have used their interaction with CCT for other purposes in addition to their own protein folding problems [50].

3. Concluding remarks

The biochemical and structural work carried out so far points to CCT as a chaperonin which is very different from the rest of the chaperonin family, not only in functional but also in mechanistic terms. CCT is beginning to be characterised as a unique chaperone placed at the heart of cellular control processes, assisting not only the folding of proteins of critical importance but also controlling cellular pathways such as cycle control of E3 ubiquitin ligases. Many interesting issues remain to be addressed such as the comprehensive characterisation of the conformational changes that govern the CCT functional cycle and its interaction with the new substrates that are beginning to be uncovered.

4. Note

We apologise for not citing many interesting papers found in the literature owing to the limitations of space.

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