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Eukaryotic Chaperonins: Lubricating the Folding of WD-repeat Proteins

Dispatch

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Recent work has shown that the eukaryotic chaperonin CCT/TRiC facilitates folding of WD-repeat proteins, vastly enlarging the known clientele for this chaperone beyond actin and tubulin. While the cytoskeletal proteins transit through the cochaperone GimC/prefoldin, an Hsp70 conveys WD-repeat proteins to CCT.

For most proteins of the eukaryotic cytosol, the folding pathways are still largely a mystery, even though many cytosolic chaperones have been identified, including multiple members of the heat shock protein (Hsp) 70 family, Hsp90 and the chaperonin, CCT/TRiC. Particularly puzzling has been the role of CCT, a distant cousin of the prokaryotic chaperonin GroEL, found in eukaryotes and archea. GroEL and CCT both have a heteromeric double-ring structure with a large central cavity at each end in which substrate proteins bind. Two recent papers [1,2] have shed new light, not only on the natural substrates of the CCT chaperonin, but also on the paths taken by different classes of substrate proteins through the cytosolic chaperone network.

About a decade ago, results presented in a flurry of reports made a compelling case that the cytoskeletal proteins actin and tubulin are obligate substrates for CCT *in vivo*. Since that time other proteins, amongst them Gα-transducin, the von Hippel-Lindau tumor suppressor protein (VHL) and histone deacetylase 3 [3], have emerged as possible CCT substrates, as their folding and/or assembly into multiprotein complexes in reticulocyte lysates were found to depend upon CCT. Many other proteins transiently interact with CCT *in vivo* shortly after their synthesis [4]. Particularly intriguing, however, was the identification of twenty-four proteins in a genome-wide screen for interacting proteins in yeast that could be 'pulled-down' with three or more CCT subunits. Sixteen of these proteins contain WD-repeats [5,6].

Such interactions are consistent with the idea that these WD-repeat proteins use CCT to fold into their active conformation, but definitive evidence that they really do need CCT to fold in the cellular environment has proved elusive. Camasses *et al.* [1] have now reported results of an exhaustive set of experiments, which make a compelling case that Cdc20, one of the WD-repeat proteins identified in the genome-wide analysis, requires CCT to reach its functional form. Cdc20 is an integral part of the cellular machinery involved in the regulation of cell division, and is essential for the regulated degradation of regulatory components during anaphase. Specifically, Cdc20 is required for activating the ubiquitin ligase called anaphase promoting complex or cyclosome (APC/C), presumably by recruiting substrates for the ligase [7]. By exploiting the well-established physical interaction of Cdc20 with APC/C and checkpoint proteins such as Mad2 to monitor its functional conformation, Camasses *et al.* [1] were able to show that CCT is required for Cdc20 to fold properly in cells.

The interaction between CCT and Cdc20 occurs within the region of Cdc20 that contains WD repeats. WD repeats are generally found in multiple copies in proteins where they form four β strands and typically have a tryptophan (W)-aspartic acid (D) dipeptide at their carboxyl terminus. The WD-repeats fold into a propeller structure with blades, each consisting of four-stranded β sheets [8]. Experiments with a series of deletion mutants expressed in yeast showed that the region of Cdc20 required for interaction with CCT was limited to two of its seven WD repeats [1]. While the site of interaction may be limited to a subset of the WD repeats of a protein, the central chamber of CCT is large enough to encompass an entire propeller [9].

How general is the requirement for CCT in the folding of WD-repeat proteins? For two other WD-repeat proteins, a case can be made that CCT is needed for proper folding *in vivo*. The Cdc20-related WD-repeat protein Cdh1, a G1-specific activator of APC/C, binds CCT and is functionally compromised in CCT mutant cells [1]. Moreover, Siegers *et al*. [2] report that the WD-repeat protein Cdc55, a regulatory subunit of a tyrosine phosphatase, transiently interacts with CCT immediately after its synthesis, and that CCT mutant cells have lowered Cdc55 activity.

Does CCT play a role in the folding of all WD-repeat proteins? The answer to this question is probably no, though CCT does appear to be required by many WD-repeat proteins. When assayed directly, six of eight WD-repeat proteins were found to interact with CCT *in vivo* [1,2], though two, Bub3 and Cdc4, did not. This failure to detect an interaction might just mean that the interaction is more transient than for other WD-repeat proteins, but Bub3 was also found to be functional in a CCT mutant strain. Bub3 interacted similarly with the kinase Bub1 in wild-type and CCT mutant cells, under conditions where Cdc20's and Cdh1's interactions with their respective partner proteins were defective.

These results paint a picture of CCT acting as a chaperone for many cellular proteins, including the cytoskeletal proteins actin and tubulin and proteins with WD repeats. But another important question is how these substrate proteins arrive at CCT — whether directly from their site of synthesis, the ribosome, or through an intermediary. Earlier work implicated the co-chaperone GimC/prefoldin as an intermediary for newly synthesized actin and tubulin, acting between the ribosome and CCT [10–12]. The data reported by Siegers *et al.* [2] implicate the ribosome-associated

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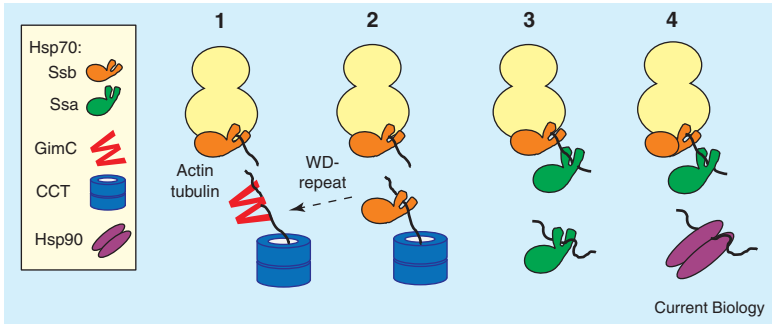

Figure 1. Pathways of protein folding in the eukaryotic cytosol.

Four routes for newly synthesized cytosolic proteins through chaperones is depicted. CCT is involved in folding of the cytoskeletal proteins actin and tubulin, as well as WD-repeat proteins. Normally actin and tubulin are thought to be transferred to CCT by GimC (1) and WD-repeat proteins by the Hsp70 Ssb (2). In the absence of Ssb, WD-repeat proteins may be transferred to CCT by GimC (dashed line). Ssa is thought to be involved in the post-translational folding of many proteins, some unaided by other major chaperones [18] (3), and others in cooperation with Hsp90 (4) [19]. Ssb is shown bound to ribosomes in all panels, as it is thought to be stoichiometrically associated with ribosomes [13], and thus possibly binds to a wide variety of different polypeptides.

yeast Hsp70 Ssb [13] as the intermediary for WD proteins (Figure 1).

Spurred on by the observation that cells lacking both GimC and Ssb Hsp70s are inviable, even though cells lacking either individually are viable (although compromised for growth), Siegers *et al.* [2] looked more thoroughly into the interaction of CCT substrate proteins with other cytosolic chaperones. They found that GimC interacts with actin and tubulin, as expected, but not with any WD-repeat protein. Instead, WD-repeat proteins could be co-immunoprecipitated with Ssb, but not the soluble cytosolic Hsp70 Ssa, suggesting that they might interact transiently and specifically with this ribosome-associated Hsp70 prior to transfer to CCT.

The results in Siegers *et al.* [2] also raise the intriguing possibility that there is a degree of plasticity in chaperone function regarding the transfer of substrate proteins to CCT. While normally Cdc55 binds to Ssb, but not GimC, interaction with GimC was observed in *ssb* mutant cells, raising the possibility that GimC, while not the preferred co-chaperone, can functionally substitute for Ssb in its absence. Such a scenario, particularly if it applied to other Ssb substrates, could well explain the synthetic lethality between *ssb* and *gimC* mutants. Such networking is not a heretical idea. Debate about an overlapping network of chaperones in protein folding has been going on for some time. Indeed some proteins may normally take alternative paths through different chaperones (Figure 1) [14,15]. Certainly in *Escherichia coli* there is functional overlap between the ribosome-bound chaperone trigger factor and the soluble Hsp70 DnaK. Absence of either protein does not have a drastic effect on growth [16,17], but absence of both is extremely deleterious, similar to the situation reported for Ssb Hsp70 and GimC [2].

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