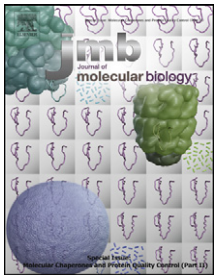
Review



The Mechanism and Function of Group II Chaperonins

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Abstract

Protein folding in the cell requires the assistance of enzymes collectively called chaperones. Among these, the chaperonins are 1-MDa ring-shaped oligomeric complexes that bind unfolded polypeptides and promote their folding within an isolated chamber in an ATP-dependent manner. Group II chaperonins, found in archaea and eukaryotes, contain a built-in lid that opens and closes over the central chamber. In eukaryotes, the chaperonin TRiC/CCT is hetero-oligomeric, consisting of two stacked rings of eight paralogous subunits each. TRiC facilitates folding of approximately 10% of the eukaryotic proteome, including many cytoskeletal components and cell cycle regulators. Folding of many cellular substrates of TRiC cannot be assisted by any other chaperone. A complete structural and mechanistic understanding of this highly conserved and essential chaperonin remains elusive. However, recent work is beginning to shed light on key aspects of chaperonin function and how their unique properties underlie their contribution to maintaining cellular proteostasis.

Chaperonins: The Protein Folding Machines

Among the most striking aspects of protein biology is the manner in which polypeptide chains routinely and rapidly attain an active three-dimensional structure with high fidelity. This property, first presented in historic work by Christian Anfinsen [1], implies that both the native conformation and folding trajectory of a protein are encoded in its primary structure. As Levinthal famously argued, if the sequence of a peptide did not place some restrictions on the conformational landscape accessible at physiological temperatures, an exhaustive search over all conformational degrees of freedom would take an unreasonably long time [2]. These two observations, namely that small globular proteins attain their native conformations autonomously and that they do so on surprisingly short timescales, serve to frame the biophysical problem of protein folding. The combined weight of many folding studies supports the idea that small globular proteins can fold productively in isolation in a two-state fashion [3,4]. Nevertheless, the model of two-state folding does not encompass the breadth of the folding problem under physiological conditions. In particular, the cellular environment places folding polypeptides in an environment that disfavors folding and promotes aggregation and misfolding [5]. The vectorial nature of protein synthesis places a topological constraint upon folding, as N-terminal regions of polypeptides are available for folding before the polypeptide is completed [6,7]. In the cell, proteins also encounter stresses such as temperature, free radicals, and osmolytes that can damage and/or unfold proteins. Unchecked, these perturbations in conjunction with the cytosolic pool of nascent or unfolded polypeptides would lead to protein aggregation en masse in the concentrated cytosol [8]. All of these issues are compounded for the many proteins that cannot fold independently and instead become trapped in intermediate conformations.

To cope with environmental stresses and to facilitate the folding of troublesome or large proteins, cells have evolved a system of molecular chaperones and quality control machinery, often called the

"protein homeostasis" or "proteostasis" network. Chaperones are proteins themselves that bind to unfolded or misfolded polypeptides and induce their folding, sequester them, or facilitate their degradation [8]. Members of this cellular proteostasis network constitute the first interacting partners seen by nascent peptides upon departing the ribosome exit tunnel and can be found in both bacteria and eukaryotes [9,10]. They also commonly represent the final interacting partner of proteins destined for degradation. Among the most important of the molecular chaperones are the chaperonins, large 1-MDa oligomeric complexes comprising two stacked rings, each of which creates a central cavity for polypeptide folding [11,12]. Chaperonins are ATPases that harness the energy of nucleotide binding and hydrolysis in order to encapsulate misfolded proteins in their central cavity such that they may fold in isolation. The chaperonins are present in every kingdom of life and are essential in all sequenced organisms excepting some members of the genus *Mycoplasma* [13]. The chaperonins are subdivided into two families, termed the group I and group II chaperonins.

The group I chaperonins, of which GroE from *Escherichia coli* is the archetype, are present in the bacterial cytosol and in the eukaryotic organelles derived from endosymbiosis. Less frequently, group I chaperonins can be found in archaea [14]. The group I chaperonin system consists of two components, a tetradecameric Hsp60 and a heptameric co-chaperone Hsp10. Hsp60, known as GroEL in *E. coli*, consists of two 7-fold symmetric rings related by a 2-fold inter-ring symmetry axis. Each GroEL ring harbors a central cavity in which client proteins are encapsulated for folding. The co-chaperone Hsp10, called GroES in *E. coli*, binds to GroEL in an ATP-dependent manner acting as a "lid" to prevent substrate egress while greatly expanding the size of the folding chamber [15].

By contrast, group II chaperonins are found in archaea and the eukaryotic cytosol. They also consist of two stacked rings, each composed of eight 50- to 60-kDa subunits, but do not have an obligate co-chaperone in the same manner as the group I chaperonins. Rather, they contain a built-in lid that closes the folding chamber and are thus competent to fold substrates *in vitro* without the assistance of accessory proteins. This should not be taken to mean that the group II chaperonins function in isolation in the cell. On the contrary, the group II chaperonins appear to be at the heart of a complex network of co-chaperones [16–20]. Notable examples include the hexameric prefoldin complex that is often thought to bind to and prevent aggregation of unfolded substrates before handing them off to the chaperonin [21,22] and the phosducin-like proteins that have been shown to enhance TRiC-mediated folding of several substrates [20,23].

The eukaryotic group II chaperonin, which is known as TRiC/CCT (TRiC hereafter), differs from its simpler archaeal homologues in that it is composed of eight paralogous subunits. Most notably, TRiC is absolutely required for folding many essential proteins, including cytoskeletal proteins such as tubulin and actin, as well as cell cycle regulators such as CDC20 and CDH1 [24–26]. It has been estimated that as much as 10% of cytosolic proteins interact with the eukaryotic chaperonin TRiC along their folding trajectory [27].

Architecture of Group II Chaperonins

Like the group I chaperonins, group II chaperonins are composed of two oligomeric rings related by a 2-fold symmetry axis. While group I chaperonins have 7-fold symmetric rings [28,29], the group II chaperonins have 8-fold and occasionally 9-fold [30–34] symmetry within their rings. Unlike GroEL, most group II chaperonins are heteromeric. The extreme case is the eukaryotic chaperonin, TRiC/CCT in which each ring contains eight distinct, paralogous subunits occupying fixed positions in the complex [35,36].

The archetypal group II chaperonin that served as the first structural model for the family is the *Thermoplasma acidophilum* α/β-thermosome. The first atomic-resolution structure of a group II chaperonin was of an isolated apical domain from the thermosome α-subunit [37]. The apical domain, which is the domain that diverges most from the group I in terms of primary sequence, was shown to contain a helical protrusion [37,38] absent from the structures of *E. coli* GroEL. A comparison of the domain structures of group I *versus* group II chaperonins is presented in Fig. 1A highlighting the helical protrusion extending from the apical domain of the group II chaperonin MmCpn [39]. The equatorial domain of the chaperonins forms the ring interface and contains most of the residues involved in nucleotide binding. The intermediate domain forms the apical surface of the nucleotide binding pocket and contains the catalytic aspartate that activates water for nucleotide hydrolysis. The structure of the equatorial and intermediate domains is conserved between the group I and group II chaperonins (Fig. 1A). When the first structure of the full-length thermosome was solved [40], the significance of the apical helix could be appreciated for the first time. The thermosome structure demonstrated that the apical helices form an iris enclosing the folding chamber (Fig. 1B, inset) thereby allowing the group II chaperonins to function without a co-chaperone lid. The structure also revealed how the subunits of one ring are seated directly in register on a subunit in the second ring, in contrast to the staggered inter-ring registry of the group I chaperonins.