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Review

ATP-dependent molecular chaperones in plastids — More complex than expected☆

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ABSTRACT

Plastids are a class of essential plant cell organelles comprising photosynthetic chloroplasts of green tissues, starch-storing amyloplasts of roots and tubers or the colorful pigment-storing chromoplasts of petals and fruits. They express a few genes encoded on their organellar genome, called plastome, but import most of their proteins from the cytosol. The import into plastids, the folding of freshly-translated or imported proteins, the degradation or renaturation of denatured and entangled proteins, and the quality-control of newly folded proteins all require the action of molecular chaperones. Members of all four major families of ATP-dependent molecular chaperones (chaperonin/Cpn60, Hsp70, Hsp90 and Hsp100 families) have been identified in plastids from unicellular algae to higher plants. This review aims not only at giving an overview of the most current insights on these plastid chaperones, their general and conserved functions but also their specific plastid functions. Given that chloroplasts harbor an extreme environment that cycles between reduced and oxidized states, that has to deal with reactive oxygen species and is highly reactive to environmental and developmental signals, it can be presumed that plastid chaperones have evolved a plethora of specific functions some of which are just about to be discovered. Here, the most urgent questions that remain unsolved are discussed, and guidance for future research on plastid chaperones is given. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. Introduction

Plastid biogenesis and function depend on the orchestrated expression of nuclear and plastid encoded proteins. While the autonomous plastid genome encodes only a small subset of plastid proteins, the majority of the ~3000 plastid proteins are encoded by nuclear DNA and imported into the organelle after their synthesis in the cytosol [1,2]. These precursors are then processed and sorted to their final destination within the organelle. Comparable to other cellular compartments, plastids contain a number of conserved factors dedicated to maintain a healthy proteome. Many of these factors belong to the family of molecular chaperones that transiently bind other proteins (termed substrates or clients) to assist their folding to the native state. These comprise chaperones that only bind to misfolded polypeptides to prevent aggregation (e.g. sHSPs), chaperones that recognize misfolded proteins to locally unfold them under ATP consumption to allow their refolding to the native state (Hsp60s and Hsp70s), or chaperones that disentangle protein aggregates (Hsp100s). In addition, molecular chaperones functionally support protein translocation across membranes, promote complex assembly and disassembly, and participate in many other regulatory processes within the cell [3–7]. Since many chaperones were found to accumulate in cells exposed to heat stress these proteins often are termed heat shock proteins (HSPs).

The functions of molecular chaperones and underlying structural mechanisms are best studied in bacteria and the cytosol/ER of eukaryotic organisms. In contrast, comparably little is known about their function in plastids. This is surprising given the presence of thylakoid membranes as a unique and highly complex compartment in chloroplasts, and the importance of chloroplasts for most life on earth. Despite their homology with eubacterial counterparts, plastid chaperones harbor distinct properties indicating their specific adaptation to the folding requirements of the organelle's unique proteome.

In this review we aim to summarize current knowledge on the composition and function of ATP-dependent molecular chaperone systems in plastids. Thus, we focus on the Cpn60/Cpn10, Hsp70, Hsp90, and Hsp100 classes of molecular chaperones.

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2. Plastid chaperonins

2.1. Architecture and functions of plastid Cpn60s

A general characteristic of chaperonins is their barrel-shaped architecture formed by two stacked oligomeric rings consisting of 60 kDa subunits. Eubacterial (GroEL), plastid (Cpn60) and mitochondrial (Hsp60) representatives are categorized as type I chaperonins. They assemble into heptameric rings and require an oligomeric co-factor forming a lid to encapsulate substrates within a central cavity. This cofactor is GroES in bacteria, Hsp10 in mitochondria, and Cpn10/20 in plastids. The structurally more distinct type II chaperonins are found in archaea (thermosome) and the eukaryotic cytosol (TRiC/CCT) and contain eight subunits per ring with two to eight different isoforms. Here, helical protrusions of each subunit contribute to an in-built lid which substitutes for the function of GroES/Hsp10/Cpn10/20 (reviewed in [8,9]).

Historically, plastid chaperonins were one of the earliest described in literature when they were shown to interact with the newly synthesized large subunit of Rubisco [10]. However, in the following years most knowledge about chaperonin architecture and function was gathered for the bacterial isoform GroEL/ES. In general, all chaperonins have in common that substrate binding and release is fuelled by ATP hydrolysis which drives conformational changes for switching between binding-active and folding-active states of the chaperonin complex. The folding cycle is best understood for the bacterial GroEL/ES system, in which both GroEL heptameric rings act in an asymmetric and anti-cooperative behavior also termed "two-stroke engine" (Fig. 1A). Binding of non-native substrate proteins takes place at the inner wall of the cavity and is mediated through exposed hydrophobic residues at the apical domains (in the *cis* ring). Binding of ATP and GroES to the *cis* ring results in conformational changes of GroEL that enlarge the cavity and change its wall surface from a hydrophobic to a highly hydrophilic, net-negative one. This transition is thought to be an important factor promoting folding. The time required by GroEL to hydrolyse ATP provides about 10 s for substrate folding to take place within the cavity. Binding of ATP and GroES to the opposite *trans* ring results in GroES dissociation from the *cis* ring and subsequent substrate and ADP release (reviewed in [9]). It can be assumed that protein folding by chloroplast chaperonins follows a similar mechanism.

Plastid chaperonins possess an intriguing feature that is not shared by other group I chaperonin family members: different isoforms exist for both Cpn60 and Cpn10 [11,12]. Cpn60s exist as isoforms alpha and beta that share only about 50% amino acid sequence identity. For example, the unicellular green alga *Chlamydomonas reinhardtii* encodes three plastid-targeted Cpn60 members, termed CPN60A, CPN60B1, and CPN60B2 [12] (Table 1). *Arabidopsis thaliana* encodes six plastid members, including three abundant subunits (Cpn60α2, Cpn60β2, and Cpn60β3), and three low abundant ones (Cpn60α1, Cpn60β1, and Cpn60β4). Although the exact subunit composition of formed heptameric rings remains elusive, a number of findings suggest that they are composed of a mixture of alpha and beta subunits (reviewed in [13]) (Fig. 1B). In vitro studies with purified recombinant Cpn60 isoforms from *P. sativum* indicated that Cpn60α needs Cpn60β to assemble into hetero-oligomers. In contrast, purified Cpn60β subunits were able to auto-assemble into functional homo-oligomers. Likewise, *B. napus* Cpn60β expressed in *Escherichia coli* assembled into tetradecameric complexes that were able to fold the large subunit of cyanobacterial Rubisco, while the alpha-isoforms neither assembled into oligomers nor did they show any folding activity [14–16]. Also Cpn60β1, Cpn60β2, and Cpn60β3 from *A. thaliana* were shown to form homo-oligomers. However, despite their close sequence similarity physical properties varied between the individual isoforms regarding their ability to assemble into oligomers, oligomer stability, and preference for co-factors in folding assays. These varying properties were suggested to result from minor amino acid sequence variations within the apical

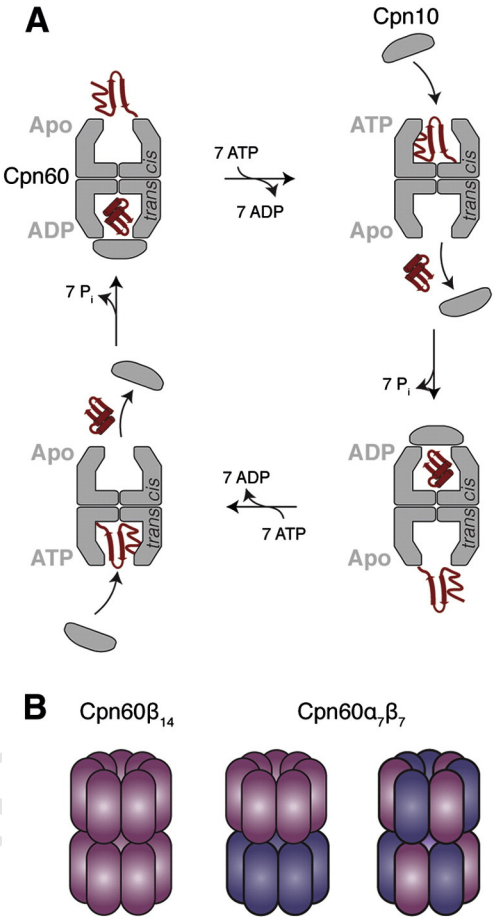

Fig. 1. Schematic folding cycle and composition of plastid chaperonins. A) Folding cycle of plastid chaperonins adopted from that of bacterial GroEL/ES. Unfolded substrates bind to hydrophobic surfaces exposed by the apical domains of Cpn60 subunits in the nucleotide-free *cis*-ring (Apo). Binding of ATP and the Cpn10/Cpn20 oligomer induce conformational changes in the Cpn60 subunits of the *cis*-ring, leading to the exposure of hydrophilic surfaces and to an enlargement of the folding cage. The time needed to hydrolyze the seven ATP molecules in the *cis*-ring provides a period of ~10 s for the substrate to fold in the protected environment of the chaperonin. Binding of ATP and the Cpn10/Cpn20 cofactor to the *trans*-ring causes dissociation of the cofactor from the *cis*-ring and release of substrate and ADP. B) Possible arrangements of Cpn60α (blue) and Cpn60β (pink) within the Cpn60 oligomer. While Cpn60β may assemble into homo-oligomers, Cpn60α requires Cpn60β to form hetero-oligomeric complexes.

domain, which is important for co-chaperonin and substrate binding [17]. In vivo, the composition of Cpn60 oligomers appears to be highly complex. For example, chaperonins were isolated from *A. thaliana* lysates that contained Cpn60α2 together with all four Cpn60β isoforms (50% were Cpn60α2, 15% Cpn60β4, and 35% a mixture of Cpn60β1-3). This particular complex appears specifically dedicated to fold NdhH, a subunit of the chloroplast NADH dehydrogenase-like complex [18].

Studies with *A. thaliana* mutants of the three abundantly expressed isoforms Cpn60α2, Cpn60β2, and Cpn60β3 indicate an essential function for them in chloroplast biogenesis and development: both *A. thaliana* Cpn60α and Cpn60β isoforms were demonstrated to be required for the proper assembly of the FtsZ plastid division ring [19]. *A. thaliana cpn60α2* single and *cpn60β2*/*cpn60β3* double mutants show impaired plastid division, small growth and an albino phenotype. Interestingly, *cpn60β2* or *cpn60β3* single mutants showed less severe phenotypes, hinting to an overlapping function of these isoforms [19,20]. Cpn60s also appear to play a role in protecting plants during heat stress as indicated by their up-regulation during heat shock [21–25]. In this regard, Cpn60β was shown to protect Rubisco activase from

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