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Review

Mapping the road to recovery: The ClpB/Hsp104 molecular chaperone

Skye Hodson, Jacqueline J.T. Marshall, Steven G. Burston *

School of Biochemistry, University of Bristol, Medical Sciences Building, University Walk, Bristol BS8 1TD, United Kingdom

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ABSTRACT

The AAA$^+$-ATPases are a family of molecular motors which have been seconded into a plethora of cellular tasks. One subset, the Hsp100 molecular chaperones, are general protein remodellers that help to maintain the integrity of the cellular proteome by means of protein destruction or resurrection. In this review we focus on one family of Hsp100s, the homologous ClpB and Hsp104 molecular chaperones that convey thermotolerance by resolubilising and rescuing proteins from aggregates. We explore how the nucleotide binding and hydrolysis properties at the twelve nucleotide-binding domains of these hexameric rings are coupled to protein disaggregation, highlighting similarities and differences between ClpB and Hsp104.

1. Heat-shock protein (Hsp) 100s and protein homeostasis

The process of proteostasis (protein homeostasis) maintains the integrity of the cellular proteome and is essential to cellular survival. Proteostasis includes the biogenesis, folding, trafficking and degradation of proteins in processes that are often modulated via interactions with molecular chaperones, even under optimal growth conditions. One key part of this process is protein folding which is often poised in a delicate thermodynamic balance between folding to a native, functional conformation versus susceptibility to off-pathway misfolding and aggregation events, as well as temporally-controlled degradation (Dobson, 2003). Constitutively-expressed molecular chaperones and proteases act as the protein quality-control system which ensures that the proteome is maintained principally by three mechanisms: (i) Preventing aggregation by shielding exposed hydrophobic regions on unfolded proteins, (ii) degrading off-pathway, partially folded proteins, or (iii) ensuring that misfolded or off-pathway, partially folded proteins are refolded (Walter and Buchner, 2002). However in addition to their constitutive roles, during periods of cellular stress (e.g. heat-shock) the expression of many of the molecular chaperones is significantly enhanced in order to deal with the increase in protein unfolding and misfolding that occurs. If the stress condition is particularly severe then even these chaperone networks can become overwhelmed and the subsequent accumulation of unfolded or partially folded protein species may result in the formation of insoluble aggregates, which, when unchallenged, can build-up and subsequently trigger cell death (Haslberger et al., 2010).

The Hsp100/Caseinolytic protease (Clp) family of molecular chaperones are members of the AAA$^+$ superfamily of ATPases, ATPases involved in various cellular activities (Hanson and Whiteheart, 2005; Neuwald et al., 1999; Ogura and Wilkinson, 2001). Like other AAA$^+$-ATPases, members of this family contain one or two nucleotide binding domains (NBDs) as well as a number of functional motifs including Walker A and B consensus sequences, an arginine finger motif and the Sensor-1 and -2 regions (see Table 1) (Ammelburg et al., 2006; Hanson and Whiteheart, 2005; Tucker and Sallai, 2007). The Hsp100 family can be divided into two sub-classes: the class 1 proteins have two NBDs per protomer (e.g. ClpA, ClpB/Hsp104 and ClpC) while the class 2 proteins have only one NBD per subunit (e.g. ClpX, HslU). Most members of this family function as ATP-dependent unfoldases usually in association with a proteolytic element. For example in bacteria, ClpA, ClpC and ClpX associate with the ClpP protease, while HslU associates with the HslV protease. In each case the Hsp100/Clp protein binds a substrate protein and uses the energy derived from ATP to unfold and translocate the substrate into the associated protease where it is degraded (Martin et al., 2005; Msadek et al., 1994; Schirmer et al., 1996; Schlieker et al., 2005). In contrast, the bacterial ClpB, its yeast cytosolic and mitochondrial homologues Hsp104 and Hsp78 respectively, as well the plant homologue Hsp101, all act as ATP-powered molecular machines but do not interact with a protease, functioning solely in a non-destructive manner to resolubilise protein aggregates (Glover and Lindquist, 1998; Goloubinoff et al., 1999; Mogk et al., 1999; Motohashi et al., 1999;

Abbreviations: AAA, ATPases associated with various cellular activities; Clp, Caseinolytic protease; *E*ClpB, ClpB from *Escherichia coli*; EM, electron microscopy; Hsp, heat-shock protein; NBD, nucleotide binding domain; NEF, nucleotide exchange factor; RCMLA, reduced-carboxylated-α-lactalbumin; *T*ClpB, ClpB from *Thermus thermophilus*.

* Corresponding author. Fax: +44 117 3312168.
E-mail address: s.g.burston@bristol.ac.uk (S.G. Burston).

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Table 1
Common sites of amino acids mutated in order to (partially) inactivate the AAA$^+$ ATPase modules of *Escherichia coli* or *Thermus thermophilis* ClpB (*E*ClpB and *T*ClpB respectively), or *Saccharomyces cerevisiae* Hsp104. Standard single amino acid codes are used but additionally X is any residue and h is any hydrophobic residue. Where no mutation has been published the location of the residue presumed to be equivalent is listed.

Motif	Generalised motif	Key residues				Mutational effects
		NBD	*E*ClpB	*T*ClpB	Hsp104	
Walker A	GXXXXGKT – P-loop interacts with the β and γ phosphates of ATP	1 2	K212 K611	K204 K601	K218 K620	Inhibits nucleotide binding and hence additionally abolishes nucleotide hydrolysis
Walker B	hhhhE – coordinates the Mg^{2+} and activates the water molecules for nucleophilic attack of the β and γ phosphate bond in ATP	1 2	E279 E678	E268 E668	E285 E687	Inhibits nucleotide hydrolysis but still allows nucleotide binding
Sensor-1	Conserved residue, either T or N – coordinates the γ-phosphate of ATP	1 2	T315 N711	T307 N701	T317 N728	Disrupts allosteric communication and inhibits nucleotide hydrolysis but allows nucleotide binding
Arginine finger	Conserved R – contacts neighbouring subunit, forms part of the nucleotide binding pocket and coordinates nucleotide binding	1 2	R332 R756	R323 R747	R334 R765	Inhibits nucleotide hydrolysis and can interfere with oligomerisation
Pore loop	Conserved Y – contacts polypeptide substrates aiding translocation through the axial channel	1 2	Y251 Y653	Y243 Y643	Y265 Y662	Abolishes substrate binding, translocation and disaggregation activity. Oligomerisation and ATPase activity are unaffected

Table 2
Examples of heat-shock protein (Hsp) quality-control systems, which convey thermotolerance to cells. Listed are members of the Hsp100 class I AAA$^+$ ATPases, their associated co-chaperones and nucleotide exchange factors (NEF) identified in eukaryotes and prokaryotes.

Type	Hsp100	Hsp70	Hsp40	NEF
Yeast cytosolic	Hsp104	Ssa1–4	Ydj1 Sis1 Zuo1	Sse1–2 Fes1 Snl1
Bacterial	ClpB	DnaK	DnaJ	GrpE
Mitochondrial	Hsp78	Ssc1	Mdj1	Yge1
Plant	Hsp101	Hsc70	Hsp40	–

Parsell et al., 1994a,b; Zolkiewski, 1999). This disaggregation activity is important for extreme cellular thermotolerance because ClpB and its homologues do not prevent protein aggregation in the same way as other chaperones, instead they mediate the resolubilisation, on return to permissive temperatures, of amorphous protein aggregates formed under extreme heat-shock (Laskowska et al., 1996; Parsell et al., 1994b). To achieve this disaggregation, ClpB and its homologues act in conjunction with the Hsp70 molecular chaperone system; a ubiquitous chaperone system that consists of the monomeric, ATP-dependent Hsp70 chaperone and its co-proteins, Hsp40 and a nucleotide exchange factor (NEF; Table 2).

In addition to its essential role in thermotolerance, yeast Hsp104 also functions to maintain and propagate the yeast prions. These prions are yeast proteins that are able to adopt an altered conformation with a β-rich, amyloid-like fibrillar structure that can then template the conversion of soluble conformers of the protein into the amyloidogenic form, in a manner analogous to the mammalian prions. The best-characterised yeast prion is the translation termination factor Sup35p (Paushkin et al., 1996). Conversion of Sup35p from its soluble form (yeast phenotype [*psi*$^-$]) to its insoluble fibrillar form ([*PSI*$^+$]) is mediated via interactions in its N-terminal, prion-forming domain that is rich in glutamine and asparagine. Maintenance of [*PSI*$^+$] is dependent on Hsp104 as deletion or over-expression of the chaperone results in a loss of the phenotype (Chernoff et al., 1995). This phenomenon can be explained by Hsp104 acting to break up amyloid fibrils and thus seeding further amyloidosis, a mechanism that may be similar to that of amorphous aggregate resolubilisation. Depletion of Hsp104 results in the formation of larger Sup35p fibrils and the production of no new smaller fibril seeds (Wegrzyn et al., 2001), while over-expression results in a transient increase in fibril size, attributed to an imbalance between the functional roles of Hsp104 and the Hsp70 system (Derdowski et al., 2010; Newnam et al., 2011). In addition to Sup35p there are numerous other prion proteins that have been identified, including Ure2p and Rnq1p (Osherovich and Weissman, 2001; Wickner, 1994).

Despite numerous studies on this disaggregase reaction over the past 15 years, especially on the ClpB proteins from *Escherichia coli* (*E*ClpB) and *Thermus thermophilus* (*T*ClpB), as well as the *Saccharomyces cerevisiae* Hsp104, the precise molecular mechanism of how these proteins couple the energy derived from ATP to the disaggregation reaction remains enigmatic. Here we will review what is known about the mechanistic behaviour of ClpB/Hsp104 with particular regard to studies of nucleotide binding and hydrolysis, as well as the allosteric communication between the twelve NBDs, and how this may correlate to successful substrate processing.

2. The AAA$^+$ architecture of ClpB and Hsp104

Each subunit of ClpB and Hsp104 is ~900 amino acids in length (Fig. 1A). Interestingly the prokaryotic ClpB proteins have a secondary (internal) translation initiation site which *in vivo* translates to produce an 80 kDa ΔN-ClpB isoform lacking the amino acids that ordinarily consist of the N-terminal domain. This is produced in addition to the full-length 95 kDa wild-type ClpB, with the two forms present *in vivo* at an approximate concentration ratio of 2:1 for ClpB to ΔN-ClpB (Chow and Baneyx, 2005; Nagy et al., 2010).

The X-ray crystal structure of monomeric *T*ClpB has been solved to 3.1 Å resolution (Fig. 1B) and revealed that following the N-terminal domain, there are two AAA$^+$ nucleotide binding domains, NBD1 and NBD2 (also called D1 and D2), with a coiled-coil middle (M) domain inserted into NBD1 that is uniquely characteristic of just the disaggregases amongst the Hsp100/Clp family (Lee et al., 2003). Since each NBD can bind and hydrolyse ATP then these can be thought of as the core motor units that are common to all AAA$^+$-ATPases. Structurally each NBD can be further defined as consisting of two sub-domains, a more N-terminally located large sub-domain and a C-terminally located small sub-domain; sequentially these are referred to as D1-large, D1-small, D2-large and D2-small. Mutations within conserved regions of these AAA$^+$ modules typically either affect nucleotide binding and/or hydrolysis, or interfere with the coupling of this to the disaggregase function (Table 1). The mobile N-terminal domain of ClpB/Hsp104 plays some role in substrate specificity (Barnett et al., 2005), however its presence is not an absolute requirement for thermotolerance (Beinker et al., 2002; Mogk et al., 2003). *In vivo* studies comparing the individual expression and co-expression of the two ClpB