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Functional analysis of conserved *cis*- and *trans*-elements in the Hsp104 protein disaggregating machine

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ABSTRACT

Hsp104 is a double ring-forming AAA+ ATPase, which harnesses the energy of ATP binding and hydrolysis to rescue proteins from a previously aggregated state. Like other AAA+ machines, Hsp104 features conserved *cis*- and *trans*-acting elements, which are hallmarks of AAA+ members and are essential to Hsp104 function. Despite these similarities, it was recently proposed that Hsp104 is an atypical AAA+ ATPase, which markedly differs in 3D structure from other AAA+ machines. Consequently, it was proposed that arginines found in the non-conserved M-domain, but not the predicted Arg-fingers, serve the role of the critical *trans*-acting element in Hsp104.

While the structural discrepancy has been resolved, the role of the Arg-finger residues in Hsp104 remains controversial. Here, we exploited the ability of Hsp104 variants featuring mutations in one ring to retain ATPase and chaperone activities, to elucidate the functional role of the predicted Arg-finger residues. We found that the evolutionarily conserved Arg-fingers are absolutely essential for ATP hydrolysis but are dispensable for hexamer assembly in Hsp104. On the other hand, M-domain arginines are not strictly required for ATP hydrolysis and affect the ATPase and chaperone activities in a complex manner. Our results confirm that Hsp104 is not an atypical AAA+ ATPase, and uses conserved structural elements common to diverse AAA+ machines to drive the mechanical unfolding of aggregated proteins.

1. Introduction

The ability of bacteria, fungi, and plantae to recover functional protein from aggregates is essential for their cell survival. The Hsp104 AAA+ ATPase is the principal protein disaggregase in *Saccharomyces cerevisiae*. Hsp104 and its bacterial homolog ClpB recognize aggregated proteins and recover functional protein in cooperation with the cognate Hsp70 system (Hsp70/40) (Glover and Lindquist, 1998; Goloubinoff et al., 1999; Motohashi et al., 1999; Zolkiewski, 1999). As a consequence, Hsp104 is essential for thermotolerance development in yeast (Sanchez and Lindquist, 1990), and crucial to the yeast stress response (Parsell et al., 1994; Sanchez et al., 1992).

In addition to its role in protein disaggregation, Hsp104 is also essential for the inheritance and maintenance of yeast prions (Chernoff et al., 1995; Moriyama et al., 2000). It has been proposed that the ability of Hsp104 to remodel amorphous aggregates and prion fibrils relies on a common mechanism (Tessarz et al., 2008; Tipton et al., 2008). Interestingly, despite a similar 3D structure (Lee et al., 2010), the ability to remodel prions is not shared with bacterial ClpB (Inoue et al., 2004; Shorter and Lindquist, 2004), suggesting functional and perhaps mechanistic differences between yeast Hsp104 and bacterial ClpB.

At the molecular level, Hsp104 contains an N-terminal domain followed by two tandem AAA+ domains, and an acidic C-terminal domain unique to eukaryotic Hsp104 members (Fig. S1). The C-terminal tail of Hsp104 features an EIDDDLD motif reminiscent of the EEVD motif present in Hsp70 and Hsp90, and is required for the interaction of Hsp104 with Hsp90 co-chaperones (Abbas-Terki et al., 2001; Moosavi et al., 2010) and with components of the GET pathway that mediate the sorting of tail-anchored proteins to the ER membrane (Wang et al., 2010). The first ATP-binding cassette (AAA-1) features the middle (M)-domain, a hallmark of Hsp104 and ClpB. The X-ray structure of ClpB showed that the M-domain consists of an 85 Å-long coiled-coil inserted within the small α-helical domain of AAA-1, and can be further subdivided into two smaller coiled-coils, termed motif 1 and motif 2 (Lee et al., 2003). While most other Hsp100 proteins lack an M-domain, ClpC features a shorter M-domain (Fig. 1A and Fig. S1). The structure of

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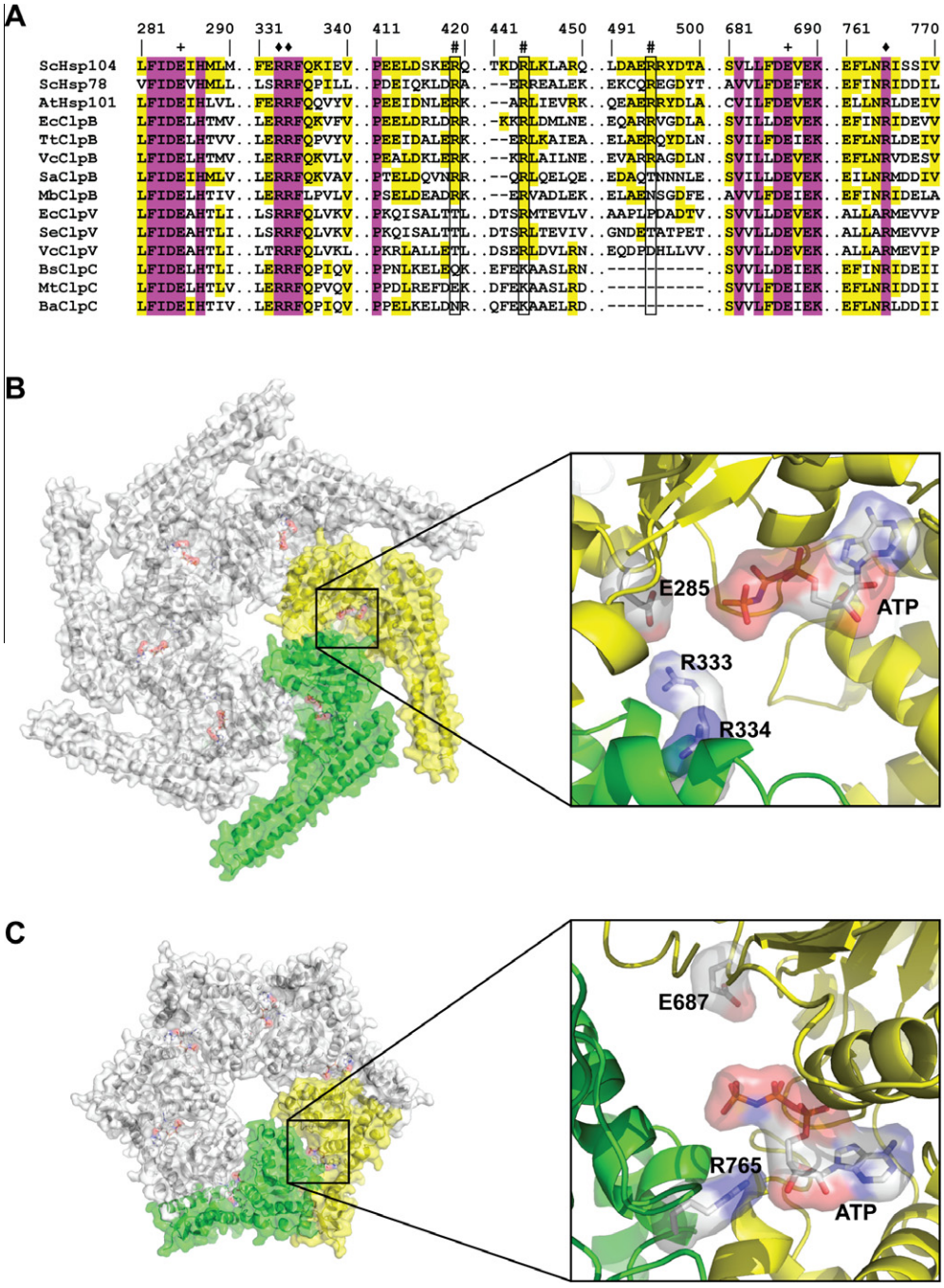

Fig.1. Conserved structural elements in the Hsp104 AAA+ ATPase. (A) Multiple sequence alignment of yeast Hsp104, plant Hsp101, bacterial ClpB, and related Hsp100 proteins. Sc, *Saccharomyces cerevisiae*; At, *Arabidopsis thaliana*; Ec, *Escherichia coli*; Tt, *Thermus thermophilus*; Vc, *Vibrio cholerae*; Sa, *Staphylococcus aureus*; Mb, *Methanococcus burtonii*; Se, *Salmonella enterica*; Bs, *Bacillus subtilis;* Mt, *Mycobacterium tuberculosis*; Ba, *Bifidobacterium animalis*. The numbering refers to the Hsp104 sequence. Strictly conserved residues are highlighted magenta, and residues conserved with yeast Hsp104 are shaded yellow. Arg333/Arg334 and Arg765 are marked with diamonds, and M-domain arginines (Wendler et al., 2007) are marked with "#" and are boxed. The catalytic Walker B glutamates are marked with "+". Top down view of (B) the AAA-1 and (C) the AAA-2 ring of the Hsp104 homo-hexamer based on the atomic structure fit of the single-particle cryoEM reconstruction (Lee et al., 2010). Two adjacent Hsp104 subunits are colored for clarity. The insets depict detailed views of the intersubunit interface in (B) AAA-1 and (C) AAA-2. Glu285 and Glu687 are the Walker B glutamates, and Arg333/Arg334, and Arg765 are the predicted Arg-finger residues in the AAA-1 and AAA-2 subdomains, respectively. The ATP-binding site is located at the intersubunit interface.

the ClpC M-domain is reminiscent of motif 1 of ClpB/Hsp104, and is essential for interaction with the ClpC-specific adaptor protein MecA (Wang et al., 2011). In Hsp104, the M-domain is essential for protein disaggregation (Sielaff and Tsai, 2010), mediates the cooperative interaction with the cognate Hsp70 system (Miot et al., 2011; Sielaff and Tsai, 2010), and is required for inter-domain communication between the AAA-1 and AAA-2 domains (Cashikar et al., 2002). However, the Hsp104 M-domain is not essential for the Hsp104 ATPase activity (Sielaff and Tsai, 2010), consistent with the notion that other Hsp100 proteins which lack an M-domain, such as ClpA, are functional ATPases (Kress et al., 2009).

The fitted cryoEM structures of full-length Hsp104 and an N-terminal domain-truncated Hsp104 variant showed that the 3D structure of yeast Hsp104 and bacterial ClpB is conserved (Lee et al., 2007, 2010, 2003). As seen in other AAA+ machines, the ATP-binding site is formed at the interface between adjacent