Addressing the biological relevance of a distinct Hsp104 oligomer

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Protein quality control network patrols the proper folding of proteins in the cell where chaperones like Hsp104 are key components in this surveillance mechanism. The yeast Hsp104 disaggregasse is a two-ring ATPase machine that rescues various forms of non-native proteins including the highly resistant amyloid fibers. The structural-mechanistic underpinnings of how Hsp104 promotes the recovery of toxic protein aggregates and how its potent unfolding activity is prevented from doing collateral damage to cellular proteins are not well understood. The current data suggest that Hsp104, as all other AAA unfoldases, is composed of hexameric rings to remodel client proteins in an ATP-dependent manner. As shown by a recent crystal structure, Hsp104 subunits can form a helical filament, in which the basic ATPase building blocks are maintained. Whether such a filamentous organization represents a crystallographic artifact or is of biological relevance remains unclear. To address this point, we aimed to reconstitute the Hsp104 filament in solution and analyze its function by biochemical, structural and *in vivo* approaches. *In vitro*, we managed to reproducibly assemble and stabilize filaments from the *Chaetomium thermophilum* and *Saccharomyces cerevisiae* Hsp104 proteins allowing the characterization of Hsp104 filament by size exclusion chromatography, negative-stain-, and cryo-EM. Reconstruction of the cryo-EM structure of Hsp104-filament is currently ongoing and will reveal the structural mechanism of filament assembly and disassembly. Future experiments aim to correlate the *in vitro* findings with the cryo-EM structure of the filament and the *in vivo* phenotype to unravel the relevance of the Hsp104 filaments in the cell.

References

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[2] - M.Carroni et al., eLife 3:e0248 (2014).