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Hsp104 Gives Clients the Individual Attention They Need

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Yeast heat shock protein 104 (Hsp104), the only known eukaryotic disaggregase, remodels both disordered protein aggregates and cross-β sheet amyloids. To handle this diverse clientele, DeSantis et al. report that Hsp104 hexamers use distinct mechanisms—individual subunits are able to dissolve disordered aggregates, but global subunit cooperativity is required to untangle amyloids.

Proteins are the most conformationally complex and versatile biological macromolecules, and their functions are intimately linked to their three-dimensional structures. Proteins can be intrinsically disordered or can fold into well-defined tertiary or quaternary structures. They can also undergo aberrant concentration-dependent aggregation, affording a spectrum of aggregates ranging from structurally poorly defined oligomers to structurally well-defined cross-β sheet aggregates (amyloid). The protein homeostasis (proteostasis) network keeps the folded proteome and intrinsically disordered proteins within their physiological concentration range, while minimizing misfolding and aggregation (Balch et al., 2008). Central to proteostasis is the competition between chaperone/chaperonin-mediated folding and cellular degradation and, in some species, the ability to disaggregate proteins for refolding or degradation (Figure 1).

The only disaggregase known to remodel both structurally poorly defined aggregates and cross-β sheet amyloid fibrils is heat shock protein 104 (Hsp104), a hexameric ring-like AAA+ ATPase found in yeast (Glover and Lindquist, 1998). ClpB, its *E. coli* homolog, is able to remodel disordered aggregates but lacks the ability to disaggregate amyloid in bacteria (Winkler et al., 2012). How Hsp104 is able to engage and remodel a wide array of structurally distinct client aggregates is the subject of much speculation. Through a series of thoughtful and revealing experiments, DeSantis and colleagues shed light on the distinct mechanisms by which Hsp104 dissolves both amyloid and nonamyloid clients (DeSantis et al., 2012 [this issue of *Cell*]).

How the individual subunits of the Hsp104 hexamer engage clients to facilitate disaggregation can be described by three possible models. In the first, individual subunits bind and disaggregate clients in a probabilistic fashion and function noncooperatively. Here, one subunit out of six can do all the work. In the second and third mechanistic possibilities, subunits cooperate either subglobally or globally, requiring other functional subunits for their own activity. To determine which model is correct for Hsp104-mediated disaggregation as a function of client structure and to understand how this may differ from the mechanism of ClpB, the authors make use of mutations that render individual subunits unable to bind client, unable to bind ATP, and unable to hydrolyze ATP. The authors take advantage of the rapid subunit exchange exhibited by Hsp104 and ClpB, allowing the creation of hexamers containing wild-type and mutant subunits in different proportions. With this clever hetero-hexamer approach (Moreau et al., 2007), the authors ask whether and to what extent cooperativity is necessary in Hsp104 versus ClpB disaggregation of disordered aggregates and amyloid structures of varying stability.

For disordered aggregates, the authors report that Hsp104 couples probabilistic ATPase activity to client disaggregation, requiring only one functional subunit per hexamer. Here, a mutant subunit that cannot hydrolyze ATP or cannot engage client can still stimulate the activity of an adjacent wild-type subunit. Conversely, ClpB couples cooperative ATPase activity to probabilistic client binding for the disaggregation of disordered aggregates, such that mutant subunits that cannot engage client are tolerated, but mutant subunits that cannot hydrolyze ATP inactivate the hexamer.

Whereas ClpB has no amyloid disaggregase activity, even in the presence of chaperones, Hsp104 is able to remodel a wide range of amyloid clients, and this activity is enhanced in the presence of the Hsp70 and Hsp40 chaperone system. Interestingly, Hsp104 requires cooperative ATPase activity and cooperative substrate binding for amyloid clients, and mutant subunits inactivate the entire hexamer, suggesting that intersubunit communication is crucial for the mechanistic switch that allows Hsp104 to disaggregate amyloid. Mutations that still confer thermotolerance in yeast (allowing disordered aggregate disaggregation) but that prevent prion propagation (amyloid disaggregation) disallow this mechanistic switch, preventing intersubunit communication. The authors also show that more subunit collaboration is necessary to disaggregate longer cross-β sheet amyloid cores. Thus, Hsp104 disaggregates amyloid with a mechanism distinct from that with which it disaggregates disordered aggregates, allowing it to remodel structurally diverse client aggregates.

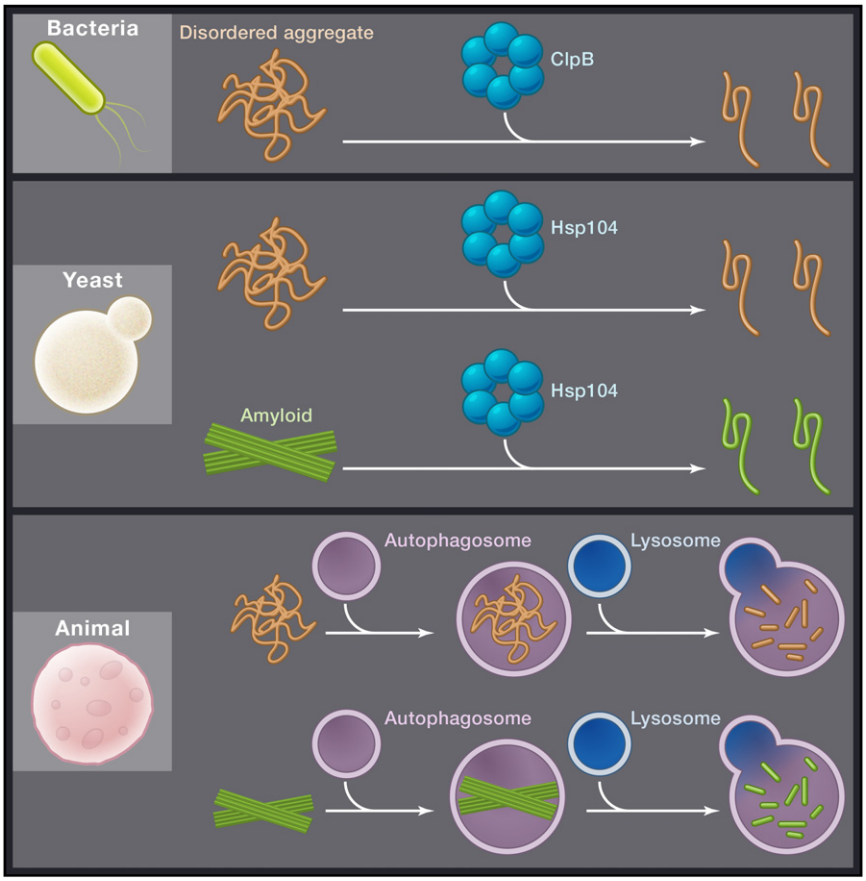

Figure 1. Pathways for Disaggregation

In bacteria, the hexameric AAA+ ATPase ClpB disaggregates disordered aggregates. ClpB requires cooperative ATPase activity between subunits but does not require cooperative client binding. There is no known bacterial amyloid disaggregase. In yeast, the hexameric AAA+ ATPase Hsp104 disaggregates both disordered aggregates and cross-β sheet amyloid. Hsp104 does not require cooperative ATPase activity or cooperative client binding for disordered aggregates. Amyloid clients induce a mechanistic switch in Hsp104, allowing for cooperative ATPase activity and cooperative client handling. In animals, macroautophagy is the only known form of cytosolic aggregate clearance. Here, aggregates (disordered or amyloid) are sequestered in autophagosomes, which then fuse to lysosomes to supply the necessary proteases and denaturing environment. Animals have no known Hsp104 homologs.

The similarities and differences between ClpB and Hsp104 have intriguing evolutionary implications. Even the simplest prokaryotic organisms require a disaggregase activity. Because prokaryotes, unlike eukaryotes, cannot degrade amyloid in the acidic, protease-rich environment of the lysosome (Figure 1), prokaryotes seem to require a disaggregase to combat protein aggregation and to promote proteostasis. The probabilistic translocation of disordered aggregated client is conserved over the 2 billion year gap between bacterial ClpB and yeast Hsp104, yet the yeast disaggregase has lost the requirement for cooperative ATPase activity between subunits for disordered clients and gained the ability to remodel amyloid clients. Interestingly, *E. coli* cytosol has more disaggregase activity for disordered clients than yeast cytosol, suggesting that the bacterial disaggregase ClpB is fine-tuned for disordered aggregates. In contrast, Hsp104 gains an operational plasticity that allows for retuning to a cooperative amyloid disaggregation mechanism, balancing the scales of evolutionary economy. Hsp104's tolerance for subunits that cannot hydrolyze ATP or engage client increases its flexibility as a disaggregase, minimizing the energy required for any given client and providing evolutionary space for further variations. DeSantis and colleagues suggest that Hsp104 gained this operational plasticity to tolerate prions, the remodeling of which can impart selective advantage under certain conditions (DeSantis et al., 2012).

Although bacteria and yeast possess disaggregase activities, metazoan homologs of Hsp104 have not yet been identified, and animal disaggregases remain undiscovered. Numerous amyloidogenic proteins exist in metazoa, and their aging-associated cross-β sheet aggregation leads to the loss of postmitotic tissue (Selkoe, 2003). Although it is possible that disaggregases exist in animals and that we have simply not yet found them, another possibility is that disaggregases like Hsp104 were selected against in the evolution of multicellular organisms. Why might this be the case? We posit that Hsp104 would put multicellular animals at risk by occasionally producing incompletely disaggregated amyloids, perhaps creating toxic and/or seeding-competent oligomers that can spread from cell to cell. This mechanism is gaining favor as the means by which amyloid diseases may spread within and between human tissues (Eisele et al., 2010; Frost and Diamond, 2010; Johan et al., 1998). Because covalent crosslinks within amyloid might stall disaggregases, it is possible that intracellular lysosomal degradation of amyloids in eukaryotes (Martinez-Vicente and Cuervo, 2007) is simply superior to their disaggregation. Animals coat extracellular amyloids with glycosaminoglycans, most likely to render amyloid nontoxic, at least initially. We hypothesize that the intracellular lysosomal degradation of amyloid and oligomers, coupled with the extracellular sequestration of amyloid and oligomers, may simply be superior evolutionary solutions to delaying amyloid disease emergence in animals.

However, it may still be possible to develop Hsp104 variants optimized for the disaggregation of specific amyloid fibrils as biological drugs for symptomatic amyloid diseases. Such a therapeutic disaggregase might facilitate the treatment of particular amyloid-associated diseases, such as Alzheimer's and Parkinson's. Hsp104 variants might also be tailored to enhance disordered aggregate disassembly to maximize the yield of folded, exogenously expressed

proteins in yeast or bacteria, facilitating basic research and enabling the more efficient production of therapeutic proteins.

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TTBK2 Kinase: Linking Primary Cilia and Cerebellar Ataxias

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Mutations disrupting primary cilia cause retinal, renal, and cerebellar defects, and misregulated Sonic hedgehog signaling. A new mouse mutant in the TTBK2 kinase fails to make cilia, and shows neural tube and Sonic hedgehog signaling defects. Ciliary targeting mutations in human TTBK2 are linked to spinocerebellar ataxia, suggesting cilia protect from neurodegeneration.

Primary cilia are highly conserved signaling organelles assembled with a ciliary membrane extended over the centriole-nucleated axoneme with nine microtubule doublets. These tiny hair-like structures are exquisitely positioned within tissues to receive neuroendocrine and sensory signals, notably via G-protein-coupled receptors and olfactory receptors. In vertebrates, cilia are especially important in regenerating tissues, where cell-cycle and specific morphogen pathways, including the Sonic hedgehog pathway (Shh), facilitate developmental patterning and regeneration of ciliated tissue. In Shh responsive cells including neural progenitors, Shh signaling also requires cilia to process pathway components (Goetz and Anderson, 2010). Specific tissues, including the retina and kidney, show distinctive degeneration in genetic diseases that have defects in ciliary function, called ciliopathies. Among neural structures, the neural tube, cerebellum, and corpus callosum appear particularly reliant on coupling cilia to the Sonic hedgehog pathway for neural morphogenesis and maintenance. In this issue of *Cell,* the Anderson lab describes *bartleby*, a new embryonic lethal mouse mutant with strong neural tube and Shh signaling defects. These defects derive from a mutation in TTBK2, a protein kinase important for transitioning centrioles from the cell cycle to postmitotic assembly of axonemes (Goetz et al., 2012). TTBK2 is also an allele of the human SCA11 spinocerebellar ataxia (Houlden et al., 2007), providing a link between cilia and neurodegeneration. Like the obstinate scribe from Melville's classic story, *bartleby* prefers not to make any cilia!

The Anderson lab has been the leader in establishing the cilia-hedgehog link; they used the alkylating agent ENU to induce mutagenesis in the mouse and screen for phenotypes typical of ciliary loss: holoprosencephaly, alterations in body axis and limb formation, randomized heart looping, and mid-gestation lethality (Huangfu et al., 2003). The *bartleby (bby)* mouse showed this characteristic phenotype. Dorsal-ventral patterning markers were defective with a loss of ventral neural tube markers and structures, and Shh markers were lost in the limb bud. Fulfilling their expectations, examination of cilia formation by the marker Arl13b showed no cilia in the neural tube or in *Ttbk2*^{bby} mouse embryo fibroblasts.

Ultrastructure examination showed *bby* embryos presented fully intact mother centrioles, which were appropriately docked to the ciliary membrane, as seen by the CEP164 marker of the membrane proximal distal appendage. The ninein marker of the subdistal appendage, important for microtubule nucleation, was also intact. However, although the basal body was properly assembled, the axoneme, which is normally assembled