Understanding substrate binding and delivery through the bi-chaperone Hsp104_Hsp70 supercomplex

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Yeast Hsp104 is a member of the AAA+ family of Hsp100 protein disaggregases and plays critical roles in maintaining proteostatsis by collaborating with the Hsp70 molecular chaperone machinery to bind and solubilize protein amyloids and aggregates. Like other related AAA+ unfoldases, Hsp104 forms a hexameric ring complex comprised of two rings of nucleotide-binding AAA+ domains (NBD1 and NBD2) that bind the substrate polypeptides and power translocation through it's central channel. Previous cryo-EM structural work from our lab revealed that Hsp104 adopts distinct helical spiral conformations bound to substrate. From this work we proposed a model for stepwise translocation in which the protomers sequentially release and re-bind substrate through ATP hydrolysis at the spiral seam, alternating between hexameric states defined by 5 and 6 substrate-bound protomers. Here we sought to expand these structural studies and characterize the Hsp70 collaboration mechanism by determining structures of an Hsp104-Hsp70 supercomplex. Through extensive cryo-EM classification methods and multi-variability analysis, we determined three substrate-bound conformational states that provide additional support of the stepwise translocation mechanism. We identify low-resolution views of the Hsp70 NBD contacting the middle domains (MD) and stabilizing a remarkable bent conformation of the coiled-coil that positions Hsp70 adjacent to the Hsp104 N-terminal domains (NTDs) and channel entrance. Finally, though 3D classification and focus refinement we achieved structures of the NTD ring and channel entrance that reveal a potential rearrangement for substrate transfer to the AAA+ core. Together these structures reveal a network of allosteric interactions and conformational changes that enable substrate hand-off by Hsp70 in coordination with the Hsp104 translocation motor.