Chemically Targeting the Emergent Properties of a Chaperone Complex

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In this issue, Chang et al. (2011) report a small molecule screen against the reconstituted DnaK-DnaJ-GrpE chaperone cycle. Through this approach, they identified myricetin as an inhibitor of DnaJ-stimulated DnaK ATPase activity, indicating the potential for their screening approach to identify modulators of emergent properties of protein complexes.

Intracellular protein homeostasis, or "proteostasis," is maintained by molecular chaperones that bind to unfolded or misfolded proteins, preventing their aggregation in the crowded intracellular environment (Balch et al., 2008). Imbalances in the activity of molecular chaperones are involved in the pathology of numerous human diseases such as diabetes and cancer, and in neurological disorders including Parkinson's disease and Alzheimer's disease. Consequently, chaperones and chaperone complexes represent important targets for the development of small-molecule therapeutics that modulate intracellular proteostasis.

One of the most conserved families of molecular chaperones is the heat-shock 70 kDa proteins (Hsp70s). Members of the Hsp70 chaperone family are found in nearly every organism and intracellular environment and are intimately involved in defining intracellular proteostasis through their involvement in protein synthesis, folding, degradation, and trafficking (Brodsky and Chiosis, 2006). Hsp70 proteins are characterized by a highly conserved two-domain structure comprised of a substrate-binding domain (SBD) and a nucleotide-binding domain (NBD). ATP binding to the NBD induces an open conformation of the SBD, which has low affinity for its misfolded or unfolded protein substrates. Substrate affinity is increased by hydrolysis of ATP to ADP, which allosterically alters the SBD structure, leading to a high-affinity closed conformation. Cycling between the ATP- and ADP-bound states regulates substrate interactions and thus defines the activity of the Hsp70 molecule (Figure 1).

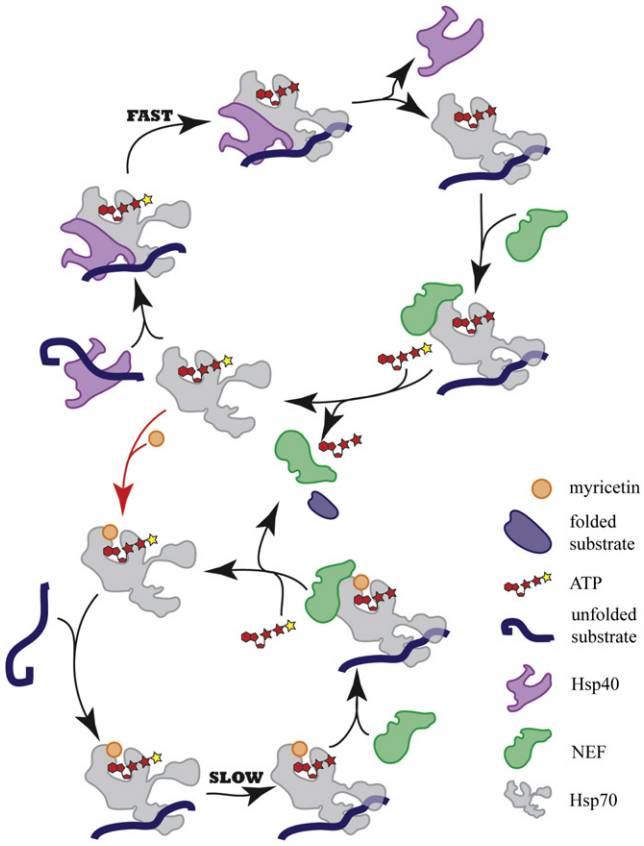

Figure 1. Hsp70 Chaperone Activity Is Assisted by Cochaperone Stimulation of ATP Hydrolysis, by Hsp40, and by Nucleotide Exchange of the NEF
The timing of the Hsp70 chaperone cycle is dictated by the rate of Hsp40-stimulated ATP hydrolysis, which induces substrate binding, and NEF-stimulated nucleotide exchange, which triggers substrate release. Myricetin allosterically blocks the bacterial Hsp40 DnaJ from binding to the Hsp70 DnaK, attenuating ATPase activity to slow, basal levels, and consequently limiting the high-affinity binding of DnaK to its peptide substrate. The activity of the bacterial NEF, GrpE, is unaffected by myricetin, such that substrate release from the Hsp70 cycle is not impeded.

The basic nucleotide-dependent activity of Hsp70s is diversified through interactions with cochaperones such as J-domain containing proteins (including Hsp40s) and nucleotide exchange factors (NEFs) (Szabo et al., 1994; Kampinga and Craig, 2010). J-proteins facilitate Hsp70 activity by both recruiting substrates and stimulating Hsp70 ATPase activity, effectively locking substrates into the SBD. In contrast, NEFs facilitate the ADP-ATP exchange of Hsp70, resetting Hsp70 in the low-affinity closed conformation and promoting substrate release. Hsp70 cochaperones can also define intracellular localization of Hsp70 and substrate specificity, thus increasing the diversity of Hsp70 functions in vivo. The importance of Hsp70 cochaperones in defining Hsp70 activity is emphasized by the nearly 4:1 ratio of J-proteins to Hsp70s in mammalian cells (Kampinga and Craig, 2010).

The diversity of mammalian Hsp70 functions makes this protein family, in isolation, a poor target for therapeutics. The central importance of J-proteins in defining Hsp70 activity offers both the promise of and a significant challenge to developing small molecule strategies to alter Hsp70 function in vivo: identifying molecules that specifically inhibit the Hsp70-J-protein complex. In this issue of *Chemistry and Biology*, Chang et al. (2011) describe a screening approach to identify molecules that specifically inhibit the J-protein-induced stimulation of Hsp70 ATPase activity. Specifically, the authors present what they refer to as "gray box screening," which focuses on identifying small molecules that target emergent properties of a fully reconstituted biological pathway.

The authors screened a reconstituted bacterial Hsp70 pathway including the Hsp70, J-protein, and NEF homologs DnaK, DnaJ, and GrpE, respectively. Specifically, the authors focused on identifying small molecules that selectively inhibited the DnaJ-stimulated ATPase activity of DnaK. Interestingly, when screening against plant extracts, they identified the flavonoid myricetin as a potent inhibitor of DnaJ-dependent DnaK ATPase activity. Importantly, myricetin did not inhibit the basal DnaK ATPase activity nor GrpE-mediated nucleotide exchange, suggesting that it selectively intervenes in DnaJ-dependent steps of the DnaK chaperone cycle (Figure 1). NMR analysis revealed that myricetin bound at a unique site on the NBD of DnaK, distinct from both the nucleotide binding site and the DnaJ binding site. Through biophysical approaches, the authors demonstrated that myricetin binding to the novel site allosterically inhibited DnaJ binding, and selectively disrupted DnaJ-dependent ATPase stimulation. Critically, this is a ligand binding site that could not have been discovered through traditional screening using DnaK alone.

The identification of a small-molecule based screening approach to identify specific inhibitors of the Hsp70-J-protein interactions puts forward the tantalizing possibility of identifying small molecules that can target specific Hsp70-J-protein interactions, allowing selective modulation of Hsp70 activity in vivo. Although the chemical properties of myricetin preclude its use in drug development, the identification of the novel myricetin binding pocket offers a new target for small molecule drug design. A vital next step will be the extension to mammalian J-domain protein-Hsp70 interactions, wherein the critical goal will be to achieve discrimination between J-domain binding partners by small molecule regulators of Hsp70. This is the test that will both determine the therapeutic potential of, as well as pose the greatest challenge to, this screening strategy.

Beyond the details of the Hsp70 system, the authors provide a proof-of-principle, demonstrating the utility of their gray box screening approach to identify small molecules targeting specific emergent properties of a protein complex. While the heterogeneity and sheer size of many biological pathways—particularly those that lack binding-induced enzymatic activity—may preclude the general application of gray box screening, smaller and better characterized pathways such as those between Hsp70 and its cochaperones will benefit from this approach.

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Cystic Fibrosis: CFTR Correctors to the Rescue

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Cystic fibrosis transmembrane conductance regulator (CFTR) correctors are small molecules that target the most common cause of cystic fibrosis: misfolded F508del-CFTR. Using differential scanning fluorimetry, Sampson et al. (2010) identify a CFTR corrector that interacts directly with the CFTR domain affected by the F508del mutation.

Two decades after the identification of the defective gene responsible for cystic fibrosis (CF), symptomatic treatment remains the bedrock of CF patient care. However, there is optimism in the CF community that this might change shortly. By exploiting knowledge and understanding of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel, its physiological role, and dysfunction in CF, the CF community now stands at the cusp of developing therapeutics that target the root cause of the disease. The latest milestone in this quest was the successful completion of