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.

REFERENCES

Balch, W.E., Morimoto, R.I., Dillin, A., and Kelly, J.W. (2008). Science *319*, 916–919.

Brodsky, J.L., and Chiosis, G. (2006). Curr. Top. Med. Chem. *6*, 1215–1225.

Chang, L., Miyata, Y., Ung, P.M.U., Bertelsen, E.B., McQuade, T.J., Carlson, H.A., Zuiderweg, E.R.P., and Gestwicki, J.E. (2011). Chem. Biol. *18*, this issue, 210–221.

Kampinga, H.H., and Craig, E.A. (2010). Nat. Rev. Mol. Cell Biol. *11*, 579–592.

Szabo, A., Langer, T., Schröder, H., Flanagan, J., Bukau, B., and Hartl, F.U. (1994). Proc. Natl. Acad. Sci. USA *91*, 10345–10349.

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

the first clinical trial of a CFTR potentiator, a small molecule designed to rescue the defective function of mutant CFTR present at its correct cellular location, the apical membrane of epithelial cells lining ducts and tubes throughout the body (Accurso et al., 2010). But fixing defects in CFTR function is only part of the problem in CF.

The molecular basis of most cases of CF is temperature-sensitive protein misfolding caused by the deletion of the phenylalanine residue at position 508 of the CFTR protein sequence; 90% of CF patients carry at least one copy of the F508del mutation. Because misfolded F508del-CFTR is retained in the endoplasmic reticulum at normal body temperature and degraded by the proteasome, the vast majority of F508del-CFTR fails to traffic to the apical membrane of epithelia (for review, see Riordan, 2008). The small amount of F508del-CFTR that reaches the apical membrane forms unstable Cl^- channels with a pronounced defect in channel gating (the pattern of channel opening and closing). Thus, small molecules with two types of activity are required to restore function to F508del-CFTR: first, CFTR correctors, so called because they overcome the processing defect of F508del-CFTR and deliver the mutant protein to the apical membrane; and second, CFTR potentiators, so called because they enhance ATP-dependent channel gating following CFTR phosphorylation by protein kinase A (PKA) but do not open quiescent CFTR Cl^- channels. To date, very few molecules have been identified with both types of activity, although intensive efforts are being made to develop such molecules, termed CFTR corrector-potentiators or dual-acting molecules.

The strategy of choice to identify CFTR correctors is high-throughput screening (HTS) because there is insufficient information at the current time to design rationally CFTR correctors. HTS exploits a reliable, sensitive, cost-effective assay to screen libraries of drug-like compounds to identify lead compounds for medicinal chemistry optimization. Using heterologous epithelial cells coexpressing F508del-CFTR and a green fluorescent protein with ultra-high halide sensitivity, Pedemonte et al. (2005) identified the bisaminomethylbithiazole corr-4a in a screen of 150,000 drug-like chemicals. Corr-4a has established itself as the benchmark against which all other CFTR correctors are tested. It is equipotent to low temperature at restoring CFTR function to CF epithelia (genotype F508del/F508del), achieving levels of CFTR function about 8% of that of normal airway epithelia (Pedemonte et al., 2005). Restoration of this amount of CFTR function using in vitro assays is considered to have a therapeutic benefit, although we really do not know how much CFTR is enough. Critically, it will be necessary to improve the disease biomarkers and therapeutic endpoints used in clinical trials to address this question.

At the present time, the mechanisms of action of CFTR correctors are not completely understood. These agents might interact with CFTR itself, by acting as either substrate mimics or active site inhibitors. Alternatively, they might target one or more of the bewildering array of CFTR-interacting proteins that orchestrate and control the biosynthesis of CFTR, its delivery to, and its expression at the apical membrane. Targeting regulators of protein synthesis, sorting, and trafficking might unleash all manner of cellular havoc. It therefore seems far better to target CFTR itself to achieve drug specificity. Previous work suggests that some CFTR correctors might indeed interact directly with CFTR. For example, the CFTR potentiator VRT-532 (Van Goor et al., 2006) rescues the misprocessing of F508del-CFTR (indicating that it's a dual-acting molecule), whereas it is without effect on a misprocessing mutant in P-glycoprotein, a closely related ATP-binding cassette (ABC) transporter (Wang et al., 2006). Moreover, the CFTR corrector VRT-325 (Van Goor et al., 2006) inhibited anion transport by low temperature-rescued F508del-CFTR and diminished the apparent ATP affinity of purified reconstituted F508del-CFTR protein (Kim Chiaw et al., 2010). However, both VRT-325 and VRT-532 have toxic effects on cells, making them unsuitable lead compounds for drug development.

In this issue of *Chemistry & Biology*, Sampson et al. (2011) identify the CFTR corrector RDR1 that targets directly the first nucleotide-binding domain (NBD1), the location of the F508del mutation in CFTR. The authors employ an innovative method for drug discovery: differential scanning fluorimetry, which identifies ligands of a target protein by monitoring their effects on the thermal unfolding of the protein. Among 224 hits identified in a previous HTS for CFTR correctors, just one chemical, the substituted phenylhydrazone RDR1, thermally stabilized murine F508del-NBD1 (Sampson et al., 2011). As with previous studies by the Hanrahan and Thomas groups, a battery of biochemical and physiological assays are deployed to investigate F508del-CFTR rescue by RDR1 in heterologous cells, polarized epithelia, and genetically-engineered mice. The authors' data demonstrate that RDR1 thermally stabilizes murine F508del-NBD1, increases the maturation of human F508del CFTR-protein, and augments the function of human F508del-CFTR in vitro and murine F508del-CFTR in vivo. Taken together, these data and the additivity of RDR1 treatment and low-temperature incubation on human F508del-CFTR maturation argue convincingly that RDR1 is a CFTR corrector that targets directly F508del-NBD1 to exert its effects.

Sampson et al. (2011) demonstrate that RDR1 thermally stabilizes murine F508del-NBD1 in a manner similar to ATP, but binds to the protein at a site distinct from that of ATP. Because CFTR potentiators (e.g., genistein) (Moran et al., 2005) bind at a site on NBD1 distinct from ATP and because RDR1 itself potentiates weakly F508del-CFTR Cl^- currents in polarized epithelia (Sampson et al., 2011), the authors investigate whether the CFTR potentiators capsaicin, genistein, and VRT-532 thermally stabilize murine F508del-NBD1. Capsaicin and genistein were without effect, whereas elevated concentrations of VRT-532 induced limited thermal stabilization of murine F508del-NBD1 (Sampson et al., 2011). One interpretation of these data is that the binding site for RDR1 on NBD1 might be distinct from the NBD1:NBD2 dimer interface, the location of the two ATP-binding sites, and one location where CFTR potentiators dock (Figure 1). Indeed, it is tempting to speculate that RDR1 might bind in the vicinity of F508 at the interface between the NBDs and the membrane-spanning domains that form the CFTR pore, where a ligand-binding site has been identified by in silico structure-based screening (Kalid et al., 2010) (Figure 1). But equally, it is important to emphasize that there are

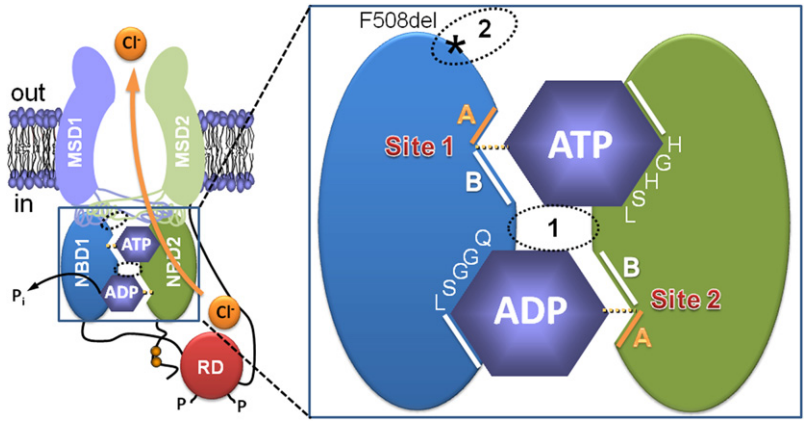

Figure 1. General Location of Some Drug-Binding Sites in CFTR
The simplified model shows the organization of the NBD1:NBD2 dimer in an open CFTR Cl^- channel. Located at the dimer interface, two ATP-binding sites (sites 1 and 2) are formed by the Walker A and B motifs (labeled A and B, respectively) of one NBD and the LSGGQ motifs of the other NBD. Site 2 contains a canonical LSGGQ motif, whereas site 1 contains a noncanonical LSGGQ motif (LSHGH). The position of F508del (surface of NBD1 opposing intracellular loop 4 [ICL4]) is shown by an asterisk, while the general location of two drug-binding sites is indicated by dotted lines. Some CFTR potentiators bind at the dimer interface at a location distinct from the two ATP-binding sites (labeled 1) (Moran et al., 2005). A binding site for CFTR correctors and potentiators has also been identified at the interface between NBD1 and MSD2 in the vicinity of F508 and ICL4 (labeled 2) (Kalid et al., 2010). MSD, membrane-spanning domain; NBD, nucleotide-binding domain; P, phosphorylation of the RD; P_i, inorganic phosphate; RD, regulatory domain. "In" and "Out" denote the intra- and extracellular sides of the membrane, respectively. See the text and Hwang and Sheppard (2009) for further information. Modified with permission from Hwang and Sheppard (2009).

notable differences in the molecular pharmacology of human and murine CFTR (Scott-Ward et al., 2007) that urge caution when interpreting these data. Clearly, identification of the RDR1-binding site on CFTR will be a high priority for future research.

A further important priority for future work is to determine whether RDR1 treatment alleviates the defective channel gating of F508del-CFTR. Once phosphorylated by PKA, wild-type CFTR will chatter open and closed seemingly indefinitely in the presence of intracellular MgATP. By contrast, following low -temperature correction, F508del-CFTR is an indolent channel that soon refuses to open no matter how much it is cajoled. In previous work (e.g., Pissarra et al., 2008), we demonstrated that rescue of F508del-CFTR by second site mutations in *cis* with F508del abrogated, albeit not completely, the gating defect of F508del-CFTR, but had little effect on channel "rundown" (irreversible loss of channel activity). It is therefore critical to learn the effects of RDR1 treatment on the gating behavior and channel stability of F508del-CFTR. If RDR1 not only traffics F508del-CFTR to the cell surface, but restores to it the gating behavior of wild-type CFTR and prevents channel "rundown," we should celebrate! This would be compelling evidence for RDR1 treatment correcting the misfolding defect of F508del-CFTR.

REFERENCES

Accurso, F.J., Rowe, S.M., Clancy, J.P., Boyle, M.P., Dunitz, J.M., Durie, P.R., Sagel, S.D., Hornick, D.B., Konstan, M.W., Donaldson, S.H., et al. (2010). N. Engl. J. Med. *363*, 1991–2003.

Hwang, T.-C., and Sheppard, D.N. (2009). J. Physiol. *587*, 2151–2161.

Kalid, O., Mense, M., Fischman, S., Shitrit, A., Bihler, H., Ben-Zeev, E., Schutz, N., Pedemonte, N., Thomas, P.J., Bridges, R.J., et al. (2010). J. Comput. Aided Mol. Des. *24*, 971–991.

Kim Chiaw, P., Wellhauser, L., Huan, L.J., Ramjeesingh, M., and Bear, C.E. (2010). Mol. Pharmacol. *78*, 411–418.

Moran, O., Galietta, L.J., and Zegarra-Moran, O. (2005). Cell. Mol. Life Sci. *62*, 446–460.

Pedemonte, N., Lukacs, G.L., Du, K., Caci, E., Zegarra-Moran, O., Galietta, L.J., and Verkman, A.S. (2005). J. Clin. Invest. *115*, 2564–2571.

Pissarra, L.S., Farinha, C.M., Xu, Z., Schmidt, A., Thibodeau, P.H., Cai, Z., Thomas, P.J., Sheppard, D.N., and Amaral, M.D. (2008). Chem. Biol. *15*, 62–69.

Riordan, J.R. (2008). Annu. Rev. Biochem. *77*, 701–726.

Sampson, H.M., Robert, R., Liao, J., Matthes, E., Carlile, G.W., Hanrahan, J.W., and Thomas, D.Y. (2011). Chem. Biol. *18*, this issue, 231–242.

Scott-Ward, T.S., Cai, Z., Dawson, E.S., Doherty, A., Da Paula, A.C., Davidson, H., Porteous, D.J., Wainwright, B.J., Amaral, M.D., Sheppard, D.N., et al. (2007). Proc. Natl. Acad. Sci. USA *104*, 16365–16370.

Van Goor, F., Straley, K.S., Cao, D., González, J., Hadida, S., Hazlewood, A., Joubran, J., Knapp, T., Makings, L.R., Miller, M., et al. (2006). Am. J. Physiol. Lung Cell. Mol. Physiol. *290*, L1117–L1130.

Wang, Y., Bartlett, M.C., Loo, T.W., and Clarke, D.M. (2006). Mol. Pharmacol. *70*, 297–302.