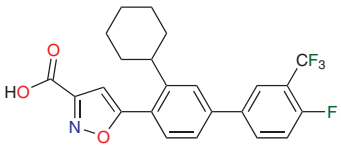
Ligases on display

The evolution of protein enzyme function has traditionally proceeded in a stepwise fashion involving genetic manipulations and selection starting with functional proteins. Now Seelig and Szostak report the *in vitro* evolution of RNA-ligating enzymes starting from a randomized protein library. Szostak and coworkers previously developed 'mRNA display', a protein evolution approach that tethers each protein to the mRNA sequence that encoded it. In the current study, the authors created an RNA-displayed protein library that contained random sequence elements inserted into two noncatalytic zinc finger motifs of a retinoid X receptor domain. The displayed library of ~10^{12} proteins was then screened for its ability to ligate two RNA oligonucleotides, a reaction that enabled the biotin-mediated capture of the active protein sequences. After 17 rounds of the selection-amplification protocol, including an interlude of recombination and mutagenesis, the authors identified several classes of RNA ligase enzymes. The most active enzyme required Zn^{2+} and monovalent cations for activity and enhanced the RNA ligation rate by ~10^6-fold over the background rate. Thermal denaturation and NMR experiments indicate that the enzyme adopts a well-folded three-dimensional structure. Although additional investigations will be required to understand the mechanism and evolutionary history of these ligases, the current study reports the first example of a protein-catalyzed ligation of 3′-hydroxyl and 5′-triphosphate RNA substrates. It also supports the notion that random sequence protein libraries may prove a useful starting point for directed evolution of new enzymes. (*Nature*, published online 16 Aug 2007, doi:10.1038/nature06032) *TLS*

Phosphatase bait and switch



Protein tyrosine phosphatases, which catalyze phosphate hydrolysis, play key roles in regulating signaling pathways. Despite their attractiveness as drug targets, efforts to identify phosphatase inhibitors have met with limited success. Ellman and colleagues have previously developed an approach called substrate activity screening (SAS) to identify protease inhibitors. In a recent paper, Soellner *et al.* adapt their SAS approach to identify inhibitors of PtpB, a protein tyrosine phosphatase that contributes to *Mycobacterium tuberculosis* virulence. Using a library of 140 *O*-aryl phosphates, the authors first identified substrates that were efficiently turned over by PtpB. Focusing on a 'hit' from this initial screen that has drug-like properties, the authors then synthesized and screened a 45-member library of biphenyl phosphates to identify optimal PtpB substrates from this class of compounds. In the final step, these efficient substrates were turned into inhibitors by replacing the substrate phosphate group with a nonhydrolyzable phosphate isostere. One of the resulting compounds, a submicromolar inhibitor of PtpB, is the most potent inhibitor identified to date for this tuberculosis drug target. For the phosphatase inhibitors in this study, the potency of the inhibitor correlated with the K_M of the corresponding substrate. Thus, identifying substrates with low K_Ms may provide a general route for developing inhibitors of other phosphatases. (*J. Am. Chem. Soc.*, published online 18 July 2007, doi:10.1021/ja0727520) *JK*

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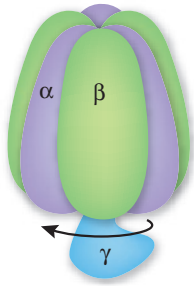
The power of three



The F_1-ATPase reversibly hydrolyzes ATP during a rotation of a γ subunit against the hexagonally arranged α and β subunits of this machine. During these reactions, each of the three ATP molecules is hydrolyzed sequentially by catalytic sites within the three β subunits to power a single 360° rotation of the γ subunit, in three 120° steps. The rotary angle of γ determines whether a single catalytic site is binding ATP, hydrolyzing ATP or releasing ADP and inorganic phosphate (P_i). The single 120° rotations likely involve substeps of 80° and 40°, with the former being driven by ATP binding and the latter by release of ADP or P_i. There is a 2-ms dwell of no γ rotation after the 80° step, the first 1-ms of which is consumed by ATP hydrolysis. Adachi *et al.* sought to determine what chemical reactions happen during that last 1-ms and how it is coupled to the γ rotation. They used a rotation assay to show that this part of the dwell is due to P_i release which then triggers the 40° rotation, which itself accompanies a reduction in affinity of the catalytic site for P_i. The coupling between rotation and affinity change explains how this machine operates in reverse to generate ATP within cells. Kinetic analyses by Scanlon *et al.* suggested that a rate-limiting partial rotation of the γ subunit occurs before P_i release. Adachi *et al.* further monitored the kinetics of ADP and P_i release to show that the ATP that is bound at 0° is released as ADP once it has seen rotation of 240°, simultaneous with binding of an ATP at the same catalytic site. Immediate ATP binding then causes another 80° substep, completing the nucleotide cycle and coupling to γ rotation. (*Cell* **130**, 309–321, 2007; *Biochemistry* **46**, 8785–8797, 2007) *MB*

Forcing the issue

The conformations of proteins involved in cell structure are necessarily different in cells undergoing changes in motility or experiencing shear stress, but the underlying causes for these changes are not known. In particular, whether these changes can be induced by external stress, and whether the underlying energetics are well-represented by artificial techniques such as single-molecule force spectroscopy, have proven unclear at best. Johnson *et al.* now provide evidence for significant force-induced conformational changes using one- and two-dye cysteine labeling in live cells. Introduction of the thiol-reactive dye IAEDANS to red blood cells followed by exposure to shear forces led to the specific and significant labeling of six cysteines within spectrin, a key cytoskeleton protein involved in cell deformability. By fitting the kinetics of force-exposed cysteine labeling by sequential exposure to a second dye, BODIPY-iodoacetamide, the authors further demonstrated that shear stress biases the conformational equilibrium towards the unfolded state with parameters similar to those measured in single-molecule studies. Finally, to investigate the forces induced by other stimuli, Johnson *et al.* then examined the strain involved in the adherence of mesenchymal stem cells to a surface. Cysteine labeling with monobromobimane in the presence and absence of the non-muscle myosin II inhibitor blebbistatin, known to relax the myosin matrix, revealed distinct changes in several proteins including vimentin, another central protein in the cytoskeleton network. These results demonstrate that mechanical force does modulate protein conformation in a cellular setting. (*Science* **317**, 663–666, 2007) *CG*