PROTEIN BIOCHEMISTRY

Designer modulators

Through convergent engineering researchers modified both the protein surface and a small molecule, creating a specific interaction to manipulate protein activity.

Small molecules can insert themselves into the most awkward places, inhibiting active sites or regulatory regions to change protein function. For years investigators have exploited these small molecules to manipulate proteins, but some proteins, including G proteins, have evaded such control.

Members of the G-protein superfamily bind guanine nucleotides to set the protein conformation for activity: GTP makes G proteins active, whereas GDP turns them 'off'. But the extremely strong affinity of G proteins for GDP and GTP has made isolation of small-molecule modulators difficult. Recently, however, Kavita Shah of Purdue University and her colleagues showed that G proteins can be controlled after modification of both the small molecule and protein through convergent engineering (**Fig. 1**).

As molecular switches, G proteins are important signaling factors, and are often deregulated in cancer and other diseases. Presently available genetic techniques, however, do not allow G proteins to be manipulated during an experiment. In contrast, "small-molecule modulators provide temporal control, which should greatly help in dissecting highly dynamic signaling cascades," says Shah.

To that end, Shah and colleagues designed an H-Ras protein that could be modulated by an orthogonal small molecule. They looked for mutations that would not affect the wild-type function of H-Ras, in particular the nucleotide-directed conformational change. They hoped that substitution with alanines at two positions in the guanine-ring binding site would permit an orthogonal small molecule with bulkier groups to fit into the binding site without disturbing the switch mechanism or substrate specificity.

To isolate small molecules that might regulate the mutant H-Ras, the researchers screened guanine nucleotide analogs. These molecules were a good starting material because, as Shah puts it, "orthogonal small molecules would likely use similar binding modes, and therefore inactivate or activate the mutant enzyme." They identified a doubly phosphorylated guanine nucleotide analog that fit the new binding pocket better than GDP did.

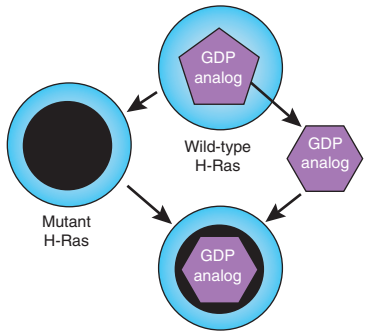

Figure 1 | Convergent engineering. Alteration of the guanine nucleotide binding site on H-Ras by site-directed mutagenesis yielded a mutant H-Ras that binds a nucleotide analog, allowing for specific regulation of the mutant protein.

Using classic H-Ras activity assays, the researchers showed that the new system of mutant protein and guanine nucleotide analog could signal alongside wild-type H-Ras. But the investigators could now specifically activate mutant H-Ras to induce a persistent 'on' conformation. In one experiment, Shah and colleagues isolated complexes of mutant H-Ras and its effectors, and even identified a previously unknown H-Ras effector.

This ability to regulate H-Ras is likely transferable to the other ~200 known G proteins. Shah explains, "the residues mutated in H-Ras are highly conserved across the G- protein superfamily, so three-dimensional structural information is not required, and this approach should be widely applicable." To prove their point, the investigators made the same mutations in another G protein, Rap1B, and found the same responsiveness to the same guanine nucleotide analogs.

It is the strategy, however, that is particularly exciting. By rationally engineering the small molecule-protein interface, Shah and colleagues generated new tools to study the many roles of G proteins. She believes that this technique "can be a valid and valuable approach to specifically modulate individual members of protein superfamilies possessing highly conserved active sites."

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RESEARCH PAPERS
Vincent, F. *et al.* Engineering unnatural nucleotide specificity to probe G protein signaling. *Chem. Biol.* **14**, 1007–1018 (2007).