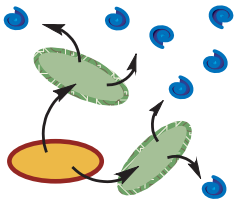
In silico protein enhancements

Attempts to design new or optimized protein function in the laboratory frequently begin with randomization strategies such as error-prone PCR (epPCR). However, it is impossible to physically generate a fully diverse library using these strategies, so complementary strategies for developing protein diversity are needed. Treynor *et al.* used six different computational algorithms to design combinatorial libraries of green fluorescent protein (GFP) variants that first optimize the composition of the sequences by selecting those that are predicted to stabilize the protein. These libraries, which targeted amino acid positions in the core of GFP, were then generated and tested for their emission intensities and for the extremes and ranges of the GFP colors that they produce. Because the computationally-derived libraries are biased against the incorporation of ionizable side chains, each of the libraries fared better functionally than a randomized library that was designed without the structure-based strategies. In contrast, two libraries that were designed with a multiple sequence alignment algorithm were only marginally better than the randomized library by the preservation of function criterion. A library generated exclusively with epPCR performs fairly well by most measures, however, this library mainly produced proteins close to the original green GFP color. These findings clearly demonstrate that protein stability can be used as a surrogate for protein function in library design and that computational methods can be used to complement the limited evolutionary information that might otherwise be used to direct new protein sequences. These computational approaches should prove useful for studies of difficult-to-screen functions such as engineering completely new protein functions. (*Proc. Natl. Acad. Sci. USA*, published online 19 December 2006, doi:10.1073/pnas.0609647103) *MB*

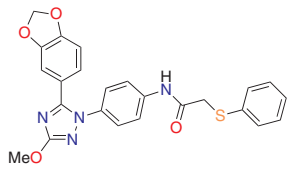
Quorum sensing in tandem



Determining the origin of marine natural products is difficult because of the large number of microorganisms present in oceanic habitats. In addition to this inherent genetic diversity, cross talk between different species can result in the formation of new or altered compounds. Indeed, in a search for new antibiotics, Angell *et al.* discovered a blue compound that is produced in mixed cultures of marine sediments, but is not created by any bacterium cultured alone. A split-and-pool analysis of 60 individual unknown colonies identified two microorganisms that together produce the blue secondary metabolite. Growth experiments indicated that, of the two bacteria, "Pup14A" was the inducer and "Pup14B" was the producer of this compound, which was subsequently determined to be pyocyanin. Pup14A was found to be *Enterobacter* sp.; both this and a second variant of *Enterobacter* ("KM1") were able to induce production of pyocyanin. Pup14B turned out to be *Pseudomonas aeruginosa*, a quorum-sensing bacterium. PCR analysis confirmed that both the pyocyanin synthesis genes and the quorum-sensing inducer region are present in the bacterium, which suggests that induction of pyocyanin production is truly a quorum sensing–like response. Quorum sensing is normally controlled by autoinducers, the concentrations of which rise to the appropriate 'inducing' levels as bacterial cell counts grow. The requirement for a second strain of bacteria in the case of pyocyanin production may thus represent a new mode of bacterial sensing. (*Chem. Biol.* **13**, 1349–1359, 2006) *CG*

Research Highlights written by Mirella Bucci, Catherine Goodman, Joanne Kotz and Terry L. Sheppard

Seizing cytohesins



G proteins are regulated by guanine nucleotide exchange factors (GEFs), which catalyze the exchange of GDP for GTP. Cytohesins are a class of GEFs that are insensitive to brefeldin A, the only known small-molecule GEF inhibitor, which makes it difficult to probe their biological roles. To identify cytohesin inhibitors, Hafner *et al.* screened a small-molecule library for a compound that could displace an inhibiting RNA aptamer from the cytohesin GEF domain. The authors identified a 1,2,4-triazole derivative, SecinH3, that bound to the domain and inhibited nucleotide exchange. In a parallel study, Fuss *et al.* implicated cytohesins in insulin signaling using transgenic flies with a defective cytohesin gene. Insulin signaling is initiated by insulin binding to the insulin receptor, which induces autophosphorylation of cytoplasmic residues that serve as sites for insulin receptor substrate (IRS) protein binding. Using SecinH3 to inhibit cytohesin activity in liver cells, Hafner *et al.* found that cytohesins are required for insulin receptor–IRS complex formation. To probe the involvement of cytohesins in *in vivo* insulin signaling, the authors fed SecinH3 to mice. The *in vivo* inhibition of cytohesins resulted in hepatic insulin resistance, thereby demonstrating that cytohesins are essential for insulin signaling in the liver. This study raises the possibility that cytohesins have a role in the onset of diabetes; it also highlights the use of aptamers to screen for high-affinity small-molecule inhibitors. (*Nature* **444**, 941–944 and 945–948, 2006) *JK*

Informyl histone modifications

Reactive oxygen and nitrogen species induce chemical modifications of DNA and compromise genetic integrity. Recent studies have suggested that reactive electrophilic species, which are common byproducts of oxidative DNA damage, may induce collateral damage by covalently modifying nearby DNA or proteins. A recent study by Jiang *et al.* now provides evidence that oxidative DNA sugar damage results in the N-formylation of lysine residues in histone proteins. The study focused on DNA damage by neocarzinostatin, an ene-diyne antibiotic that oxidizes the 5' position of deoxyribose and produces formylphosphate. Treatment of cells or nuclei containing 5'-tritiated DNA with neocarzinostatin led to an enhancement of tritium labeling of nuclear proteins, particularly in histones H1, H2B and H3. Using an LC-MS/MS method developed to quantify protein modifications, the authors demonstrated that neocarzinostatin treatment induces N^6-formyllysine modifications in a dose-dependent manner in histone proteins. The authors also observed high background concentrations of N^6-formyllysine in histone proteins from untreated cells but not in non-nuclear proteins, which points to N^6-formyllysine as an endogenous histone modification. Additional studies will be required to localize the sites and determine the biological impact of these formylation reactions. However, the current study raises the possibility that modification of lysine residues by secondary damage reactions may block important sites of reversible acetylation or methylation used for epigenetic regulation of gene expression. (*Proc. Natl. Acad. Sci. USA*, published online 26 December 2006, doi:10.1073/pnas.0606775103) *TLS*