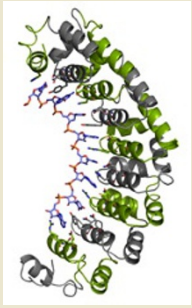
RNA recognition code cracked



By modulating gene expression at the post-transcriptional level, programmable, sequence-specific RNA-binding proteins, such as PUF family members, show promise both as research tools and ultimately as therapies. Until now, however, understanding of the RNA recognition code of PUF family proteins has been incomplete; although the amino-acid determinants for binding to adenine, uracil and guanine have been identified, those for binding cytosine have remained elusive. Filipovska *et al.* elucidate the cytosine-binding amino acids using a yeast three-hybrid system in which reporter gene activation is dependent on the binding of a PUF domain to a cytosine-containing RNA. The highest gene activation and cytosine specificity were observed when PUFs contained an arginine at position 16 and a small or nucleophilic side chain at position 12 of a repeat unit. The authors also engineer PUF proteins that contained 16 instead of the naturally occurring 8 repeats to improve sequence specificity. Future work will show whether the PUF proteins can be fused to suitable effector proteins to manipulate RNA metabolism effectively and with sufficient sequence specificity for practical applications. (*Nat. Chem. Biol.* advance online publication, doi:10.1038/nchembio.577, 15 May 2011) *ME*

Single-molecule immunoprecipitation

Most cellular proteins participate in the dynamic formation of a variety of protein complexes, but current analysis methods are often not able to resolve the complexity, kinetics and functional role of diverse protein assemblies. Jain *et al.* and Yeom *et al.* show that single-molecule immunoprecipitation by antibodies immobilized on a glass surface can be used in combination with fluorescence microscopy to study individual protein complexes. Jain *et al.* used fresh lysates of cultured cells or fresh mouse brain tissue to capture a variety of cellular proteins and their interaction partners. For detection, the proteins were either genetically fused to fluorescent proteins or stained using fluorescently labeled antibodies. The authors show that the stoichiometry of single soluble and membrane-bound complexes can be determined and that, using the PcrA DNA helicase as an example, the surface-bound complexes can be used to measure kinetic parameters of enzyme reactions. Yeom *et al.* concentrate on the terminal uridylyl transferase 4 (TUT4), an enzyme that regulates microRNA biogenesis in embryonic stem cells. The observation of the single complex uridylation reactions revealed that a protein called Lin28 facilitates the processing of the precursor of let-7 microRNA by strengthening its binding to TUT4. Single complex immunoprecipitation experiments will help to elucidate the stoichiometric and functional heterogeneity of protein assemblies. (*Nature* **473**, 484–488, 2011; *EMBO Rep.* published online, doi:10.1038/embor.2011.100, 3 June 2011) *ME*

Traversing the blood-brain barrier

Targeting brain proteins is challenging on two levels: therapeutics must first traverse the blood-brain barrier, and once across may also need to enter brain cells. Researchers at Genentech now describe a bispecific antibody that satisfies both requirements by targeting the transferrin receptor (TfR) as well as a membrane-bound enzyme implicated in the pathology of Alzheimer's, the beta-amyloid (Aβ) precursor protein cleaving enzyme (BACE). An antibody against BACE1 almost halved Aβ production in plasma, but decreased levels by only 20% in the brain. As therapeutic doses in humans would be logistically difficult and prohibitively expensive, the researchers sought to improve uptake into the brain by combining the recognition features of the anti-BACE antibody with those of an antibody against TfR. Antibodies with high specificity for TfR were taken up 11-fold more than control IgG, but remained bound to endothelial cells. Reasoning that antibody with reduced affinity for the target might be released more easily, the authors then reduced affinity for TfR by mutating its cognate complementarity determining regions. Bispecific antibodies with reduced affinities for TfR were visualized in the parenchyma at levels inversely proportional to their affinities for TfR. The most promising candidate reduced brain Aβ by 50% after a single dosing. These findings point to a way of improving uptake of large molecules into brains. However, the consequence of targeting a brain transporter is unknown. (*Sci. Transl. Med.* **3**, 84ra43, 2011; *Sci. Transl. Med.* **3**, 84ra44, 2011) *LD*

Regenerating the heart

Human organs vary widely in their propensity to regenerate after injury. The liver can regrow as much as three-quarters of its mass, but organs such as the heart and brain have little capacity for self-repair. Activating tissue stem cells to promote regeneration could be an appealing strategy for treating various kinds of tissue damage and would have the advantage of avoiding the complexities of cell transplantation. A recent study on cardiac regeneration in the mouse provides preliminary data supporting such an approach. Smart *et al.* found that pre-treatment of mice with the peptide thymosin β4 stimulated epicardial progenitor cells to differentiate into cardiomyocytes after an induced heart attack. The newly generated cardiomyocytes integrated into the heart and led to improvements in ejection fraction, scar volume and left ventricular mass. Because the effects of thymosin β4 were modest, the authors propose to search for small molecules that would be more efficacious in activating resident cardiac progenitor cells. (*Nature* advance online publication, doi:10.1038/nature10188, 8 June 2011) *KA*

Versatile site-specific protein modification

Targeted chemical derivitization of proteins to introduce new functionalities without negatively affecting their activities is of potential value in applications ranging from imaging to enhancing the pharmacokinetic/pharmacodynamic properties of drugs. Incorporation of unnatural amino acids generated by chemical synthesis provides one option for controlling protein functionalization in a site-specific manner. As a more convenient alternative, bacterial and mammalian cells can be engineered to produce the unnatural amino acid pyrroline-carboxy-lysine (Pcl) in the presence of the readily available media supplement D-ornithine. Ou *et al.* show that Pcl can be incorporated into a broad range of proteins and conjugated to >50 reagents that include fluorescent dyes, branched and linear poly(ethylene glycol)s, disaccharides, peptides and phospholipids. The broadly applicable conjugation chemistry they describe is orthogonal to other reactive groups on proteins and enables high-yielding reactions to proceed to near completion in pH and temperature ranges compatible with the stability of most proteins. (*Proc. Natl. Acad. Sci. USA* published online, doi:10.1073/pnas.1105197108, 13 June 2011) *PH*

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