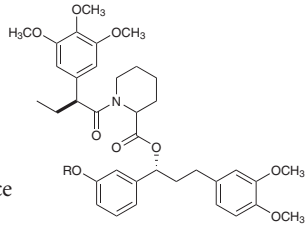
Seeing a ribozyme cofactor

Riboswitches are specialized domains found in the 5′ untranslated region of mRNA. In most riboswitches, binding of a metabolite causes a conformational change that regulates gene expression. The *glmS* riboswitch, which controls expression of glucosamine-6-phosphate (GlcN6P) synthase, is the first example of a ribozyme riboswitch. In *glmS*, GlcN6P binding initiates a GlcN6P-dependent self-cleavage reaction. Biochemical evidence has suggested that GlcN6P may directly participate in catalysis, however the mechanistic details are not known. Klein and Ferré-D'Amaré have now reported the structure of *glmS* bound to the competitive inhibitor glucose-6-phosphate (Glc6P), an isostere of GlcN6P with a 2′ hydroxyl in place of the 2′ amine, as well as the structures of *glmS* in the precleavage and cleaved states. In contrast to the conformational changes typically induced by ligand binding to riboswitches, the structures reveal that the *glmS* ribozyme undergoes very little structural change during catalysis, and the chemical reaction appears to be catalyzed exclusively by the cofactor. Glc6P binds in a conserved active site pocket and makes key contacts with the scissile phosphate. The orientation of Glc6P suggests that GlcN6P could act as a general base to promote nucleophilic attack, electrostatically stabilize the pentacoordinate phosphorous transition state and function as a general acid by donating a proton to the leaving group. Biochemical studies can now be used to probe the mechanistic details of the role of GlcN6P as an essential ribozyme cofactor. (*Science* **313**, 1752–1756, 2006) *JK*

Fine-tuning protein expression

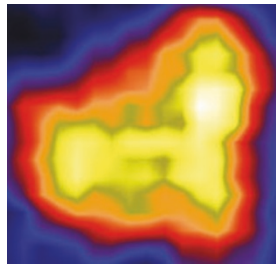


RNA interference (RNAi) has become a broadly used method for examining the biological role of proteins. However, RNAi has important limitations including the difficulty in silencing certain genes and the length of time needed to reduce protein levels. A method that could rapidly and reversibly modulate protein levels in mammalian cells would provide a powerful complement to current approaches for investigating protein function. Banaszynski *et al.* sought to generate a system in which a protein could be conditionally targeted for destruction through the attachment of an unstable domain, which could then be stabilized by small-molecule binding. For the destabilizing domain, the authors chose to make mutants of the FK506- and rapamycin-binding protein FKBP, for which many high-affinity ligands are known. After expressing a library of FKBP mutants fused to yellow fluorescent protein (YFP) in mammalian cells, the authors screened for cells in which fluorescence was initially low, suggesting YFP degradation, but was enhanced by the addition of an FKBP ligand. The mutated domains identified were shown to target YFP to the proteasome in the absence of ligand, but YFP expression was restored upon addition of the FKBP ligand that stabilizes the fusion protein. In contrast to the 48 hours typically required for significant protein knockdown following RNA silencing, removal of the FKBP ligand resulted in substantial protein degradation within 4 hours. The mutated domains were shown to control stability of a variety of proteins including cytoplasmic, nuclear and membrane proteins. In addition, protein expression could be modulated by small-molecule concentration. This impressive tuning of protein expression highlights the advantages of a small molecule–regulated method. (*Cell* **126**, 995–1004, 2006) *JK*

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

A window into membrane composition

The association of membrane proteins into signaling complexes and the observation of 'lipid raft' domains remind us that local organization of membrane components is essential for biological processes. Yet, so far, gaining insight into membrane domain organization on the scale of a few to hundreds of nanometers has remained a major challenge. Kraft *et al.* now report a secondary-ion mass spectrometry (SIMS) method for imaging the composition of membranes on the ~100-nanometer scale. The authors constructed a supported lipid bilayer composed of two phospholipids, 1,2-dilauroylphosphatidylcholine (DLPC) and 1,2-disteroylphosphatidylcholine (DSPC), which were differentially labeled with isotopes (DLPC with ^{15}N and DSPC with ^{13}C). Atomic force microscopy (AFM) of the supported bilayer showed the presence of DSPC domains that had segregated from the DLPC fluid phase. SIMS imaging, or ion-beam bombardment of the surface and mass-spectral analysis of the specifically labeled ion fragments, verified the DSPC phase segregation shown by AFM. Standard calibration allowed the authors to correlate the spectral signal with the fraction of either lipid at a site on the surface. Although most DSPC domains were composed of ~90% DSPC, the authors observed significant heterogeneity in the amount of DLPC present across different DSPC phases. Though AFM techniques allow measurements of membrane topology, the reported SIMS method offers the possibility of *in situ* determination of lipid compositions on a biologically relevant length scale and suggests that it may be possible to image lipid compositions on the surfaces of living cells. (*Science* **313**, 1948–1951, 2006) *TLS*


Mary L. Kraft

Ancient integrase discovered

The insertion of proteins into membranes generally relies on complex machinery, such as the signal-recognition particle (SRP) or the SecYEG translocon. For example, mannitol permease (MtlA) is transported to the membrane via the SRP, and its integration into the membrane is strictly dependent on SecYE. In a recent paper, Nishiyama *et al.* explore membrane insertion and show unanticipated regulation of this process by relatively small compounds. The surprising observation that MtlA spontaneously inserts into simple phospholipid liposomes but not proteoliposomes equipped with much of the integration machinery led the authors to identify diacylglycerol (DAG) as an unexpected inhibitor of spontaneous insertion in inner-membrane vesicles (INVs). Further investigations into insertion using DAG-rich liposomes indicated that the expected protein machinery is not sufficient to cause membrane insertion, thereby demonstrating the existence of a missing integration factor present in the original INVs. Indeed, extraction of INVs returned an active compound that actively facilitates insertion (and in some cases translocation), which led the authors to speculate that this is an ancient integrase. Efforts to identify the factor confirmed that it is a lipopolysaccharide related to lipid A. Further work will be needed to precisely identify this new molecule. (*J. Biol. Chem.*, published online 27 September 2006, doi:10.1071/jbc.M608228200) *CG*