© 2005 Nature Publishing Group http://www.nature.com/naturechemicalbiology

Deoxyribozymes go full circle

Deoxyribozymes, or DNA enzymes, are single-stranded DNA molecules that catalyze chemical reactions. DNA enzymes do not seem to exist in natural systems, but they can be created in the laboratory by means of a combinatorial DNA library technique known as *in vitro* selection. In addition to offering insights into how nucleic acids act as enzymes, deoxyribozymes have shown utility as biochemical tools for applications such as site-specific cleavage of RNA. Wang and Silverman now report a useful small DNA enzyme that synthesizes lariat RNAs, a class of branched RNA molecules. RNA lariats are made during the splicing of pre-mRNA and group II introns by nucleophilic attack of an internal branch-site nucleotide at a 5′ splice site, which cleaves the RNA and produces a cyclic, branched RNA intermediate. Previously, no methods had been available for the efficient synthesis of native and chemically modified RNA lariats for biochemical studies. In earlier work, Silverman and coworkers identified several classes of DNA enzymes that catalyzed the synthesis of branched RNA structures. In the current study, they showed that one of these deoxyribozymes possesses DNA 'arms' that bind RNA substrates and a 39-nucleotide catalytic domain that catalyzes intramolecular lariat formation in one step. The enzyme shows remarkable selectivity by activating a single 2′-hydroxyl nucleophile out of hundreds. The deoxyribozyme tolerates sequence variability in RNA substrates, which allowed the authors to prepare single-nucleotide mutants of a known yeast lariat intermediate. The efficiency and scalability of the approach offers access to biochemically useful quantities of RNA-splicing intermediates, including lariats with modified or mutant nucleotides. (*Angew. Chem. Int. Ed.*, published online 5 August 2005, doi:10.1002/anie.200501643). *TLS*

Sugar signals a starch boost

During the day, sugar utilization in plants is controlled by light-driven photosynthesis of starch. This pathway activates ADP-glucose pyrophosphorylase (AGPase), which catalyzes the formation of ADP-glucose. Subsequently, starch synthases catalyze polysaccharide formation. The dimeric, inactivated form of AGPase is held together by a disulfide bond and is converted to the active, monomeric form upon light-activated reduction. A small sugar, trehalose-6-phosphate (T6P), which is the biosynthetic precursor of trehalose, is known to modulate sugar utilization in plants. However, the biochemistry of this signaling pathway is unknown. In a recent paper, Kolbe *et al.* investigated the possibility that T6P controls sugar levels by initiating starch synthesis. Upon feeding trehalose to *Arabidopsis* leaves, the authors observed an increase in starch synthesis, together with the activation of AGPase. To observe the effects of T6P in living plants, the authors created two transgenic *Arabidopsis* plants, one with increased expression of T6P synthase (causing increased levels of T6P) and another with increased expression of T6P phosphatase (causing decreased T6P). Compared with wild type, levels of AGPase activation and starch synthesis were higher in plants with increased expression of T6P synthase but lower in plants with increased expression of T6P phosphatase. Treatment of isolated chloroplasts with T6P produced activated AGPase, providing direct evidence of the signaling properties of T6P. The authors suggest that T6P promotes thioredoxin-mediated reduction of AGPase, providing an alternative small-molecule-signaled promotion of starch synthesis independent of the light-activated photosynthetic pathway. (*Proc. Natl. Acad. Sci. USA* **102**, 11118–11123, 2005) *GW*

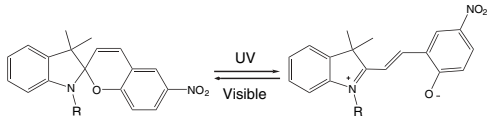
Research Highlights written by Greg Watt, Joanne Kotz and Terry L. Sheppard.

TWO GLYCANS MAKE THE FOLD

In addition to providing stability and structural support to parent proteins, N-glycosylation functions as part of a tagging system during protein folding and quality control. After protein glycosylation in the endoplasmic reticulum, the N-glycan contains a terminal triglucose chain. Glucosidase I (GI) trims the outermost glucose in the chain, and glucosidase II (GII) removes the next one. Monoglucosylated glycoproteins then enter the calnexin/calreticulin (Cnx/Crt) protein-folding cycle, whereupon correctly folded glycoproteins are released when the remaining glucose in the chain is cleaved by GII. In a recent paper in *Molecular Cell*, Deprez *et al.* revealed that more than one glycan is required for entry into the Cnx/Crt cycle. Using SDS-PAGE, the authors found that the outermost glucose was rapidly removed by GI. Unexpectedly, removal of the second glucose by GII was not detected. Furthermore, no immunoprecipitation was observed with antibodies to Cnx, indicating that monoglycosylated peptides do not enter the Cnx/Crt cycle. In contrast, the authors found that peptides containing two N-glycans were trimmed by GII, and the resulting monoglucosylated glycopeptides could bind to Cnx. The authors next explored the effect of multiple glycans on the processing of RNase, a glycoprotein containing three glycosylation sites. In live cells, they found that only RNase with multiple glycans entered the Cnx/Crt cycle, which resulted in slower secretion times than that for monoglycosylated RNase. This work suggests that the level of post-translational processing required by individual glycoproteins is determined by its number of N-glycans. (*Mol. Cell* **19**, 183–195, 2005) *GW*

Channel light switch

Valves that could be quickly and reversibly opened and closed would be useful for generating nanodevices. Light has proven to be one of the best means for rapid and reversible control of material properties and enzyme activities. In a recent paper in *Science*, Feringa and coworkers reported a method for optical control of ion flow in an engineered channel. The authors started with the mechanosensitive channel MscL from *Escherichia coli*, which permits passage of ions and small molecules in response to osmotic stress. Building on previous studies showing that introduction of a polar or charged residue into the pore induces channel opening, the authors generated two different hydrophobic small molecules that became charged upon photoactivation. Irradiation of one of the molecules led to irreversible cleavage of a protecting group to yield an ionic molecule. In the other molecule, UV light induced a ring opening that generated a zwitterion,



which was reversible with exposure to visible light. Upon site-selective attachment of either of these molecules to the pore, ion flow through the channel became photoinducible. Engineered MscL channels introduced into both lipid membranes and liposomes resulted in light-induced pore opening. These studies provide a framework for developing nanovalves for optical control of drug release. (*Science* **309**, 755–758, 2005) *JK*