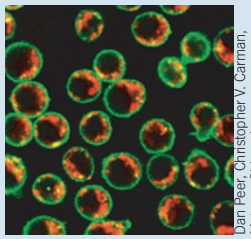
siRNA nanoparticles, fully loaded



Dan Peer, Christopher V. Carman, Motomu Shimaoka

Cyclin proteins drive cells through the different stages of the cell cycle and are therefore therapeutic targets for cancer. Though its function in inflammation and disease is unknown, cyclin D1 (CyD1) is strongly upregulated in both epithelial and immune cells such as leukocytes at inflammation sites in inflammatory bowel diseases (IBDs). To determine the role of leukocyte CyD1 in inflammation, Peer *et al.* developed a strategy that would allow targeting of CyD1 siRNAs to specific leukocyte subsets. Because this would require systemic delivery, a major problem for any siRNA-based therapeutic, their method involved encapsulating siRNA against CyD1 in nanometer-scale liposomes and stabilization of the particles against degradation and the triggering of unwanted interferon responses *in vivo*. To achieve targeted delivery, antibodies against β7 integrins, cell surface adhesion molecules that are highly expressed in gut mononuclear leukocytes, were also attached to the particles. In tissue culture cells, the nanoparticles specifically targeted β7 integrin–expressing cells, and upon intravenous injection into mice they targeted splenic and gut mononuclear leukocytes. CyD1 downregulation caused a decrease in cell proliferation as well as reduced expression of cytokines from proinflammatory T_H1 T helper cells but not anti-inflammatory cytokines from T_H2 cells. Accordingly, in an experimental model of IBD, the targeted nanoparticles could provide protection from intestinal tissue damage, making CyD1 an attractive target for therapy. This siRNA targeting strategy may have broad applications in *in vivo* drug target validation and for potential therapies of various diseases. (*Science* **319**, 627–630, 2008) *MB*

Metals in motion

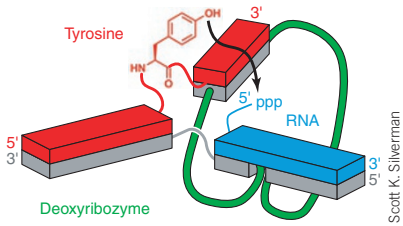
The conversion of water to dioxygen occurs in photosystem II, a multiprotein assembly with an Mn_4Ca cluster at its core. Although this cluster has been studied extensively, the exact mechanism of water oxidation remains unclear. Pushkar *et al.* now provide spectral data that may help to pin down motions occurring during the catalytic cycle. Laser flashes were used to advance the complex through four intermediate states, S_0–S_3, with EPR used to monitor the state of the complex. X-ray absorption spectra focusing on Mn indicated that oxidation of one Mn atom occurs during the S_1-to-S_2 transition in the context of a multinuclear metal complex. By exchanging Ca for Sr, which introduces minimal structural perturbations, the authors gained a second spectroscopic probe; X-ray absorption spectra of this atom confirm that the Sr is not only present but that it is affected by geometric and oxidative changes to the cluster as the reaction proceeds. Fitting of these datasets served to delineate the likely placement of individual metal atoms in each intermediate state and thus the fitting delineates the structural basis of the catalytic cycle. Additionally, the long distances between the metals define the need for several oxo bridges and highlight a central oxygen atom, bound by the Sr and three Mn atoms, as involved in the oxidative pathway toward O-O bond formation. Though much work remains, this study provides a model that can serve as a specific hypothesis for future efforts. (*Proc. Natl. Acad. Sci. USA*, doi:10.1073/pnas.0707092105) *CG*

Written by Mirella Bucci, Catherine Goodman & Terry L. Sheppard

O-glucosylation looking for its enzyme

Signaling through the Notch receptor involves a cell-cell communication between transmembrane ligands Delta and Serrate on the signal-sending cell and Notch on the signal-receiving cell. Subsequent cleavages liberate the intracellular domain of Notch, which is translocated to the nucleus and binds transcription factors involved in processes such as asymmetric cell divisions and maintenance of stem cell populations. Further processing of Notch by O-glucosylation at a serine-containing consensus sequence takes place via an unknown enzyme or enzymes. In a screen to identify *Drosophila melanogaster* genes involved in adult bristle development, Acar *et al.* isolated a strain with a temperature-conditional defect called *rumi*, in which embryos raised at the restrictive temperature of 28 °C have cell fate–specification defects resembling those of *notch* mutant embryos. Genetic interaction and immunostaining experiments with mutant alleles and knockout animals suggested that the Rumi protein is a general regulator of Notch signaling involved in the signal-receiving cell. The presence of an endoplasmic reticulum localization motif in Rumi and a domain implicating it as a polysaccharide modifier led the authors to suspect that Rumi is involved in O-glycosylation of Notch. Indeed, mutational analyses and *in vitro* enzymatic assays showed that Rumi can add a single glucose to consensus sequences within Notch. The absence of some Notch cleavage products in *rumi* mutants at 28 °C and an accumulation of Notch throughout the cell and at the plasma membrane suggest that O-glucosylation of Notch keeps it in a stably folded conformation needed for proper function, particularly at higher temperatures.
(*Cell* **132**, 247–258, 2008) *MB*

Evolving new nucleophiles



Scott K. Silverman

Though cellular DNA adopts a double helix necessary for genetic information storage, in the test tube single-stranded DNA sequences have displayed diverse catalytic abilities. These 'deoxyribozymes' obtained by *in vitro* selection provide insight into nucleic acid catalysis and also may serve as useful reagents for the synthesis of complex biomolecules. Pradeepkumar *et al.* recently demonstrated this concept by identifying a deoxyribozyme that joins RNA to the amino acid tyrosine. The authors presented a random library of DNA sequences with a DNA substrate containing an amino acid that has a nucleophilic side chain (serine, tyrosine or lysine) positioned near an RNA 5'-triphosphate electrophile. After multiple rounds of *in vitro* selection, the authors identified a deoxyribozyme that catalyzes tyrosine-RNA ligation. The DNA catalyst was inactive when nontyrosine amino acids (alanine, serine and lysine) or nucleotides were positioned at the reactive site of the substrate. In the presence of the divalent metal ions Mg^{2+} or Mn^{2+}, this deoxyribozyme catalyzed tyrosine-RNA ligation with a rate enhancement of $>10^5$ times over the uncatalyzed reaction rate, with the tyrosine hydroxyl attacking the RNA 5'-triphosphate to yield a tyrosine-RNA phosphodiester linkage. This first example of DNA-catalyzed bond formation with an amino acid side chain will support further efforts to expand the scope and utility of this ligation chemistry. (*Angew. Chem. Int. Ed.*, published online 23 January 2008, doi:10.1002/anie.200703676) *TLS*