Research Roundup

Keeping RNA away from DNA

Newly transcribed RNA represents a threat to genomic stability, say Xialu Li and James Manley (Columbia University, New York, NY). They find that, in metazoan cells, the threat is neutralized by a splicing factor that coats RNA as it it emerges from RNA polymerase.

The danger arises because nascent RNA can anneal to the template DNA strand, thus forming an R loop. The non-transcribed strand is left as a single strand, potentially susceptible to attack by nucleases.

Bacteria combat this tendency by tightly coupling translation to transcription. In eukaryotes, this is not an option as transcription and translation are nuclear and cytoplasmic, respectively.

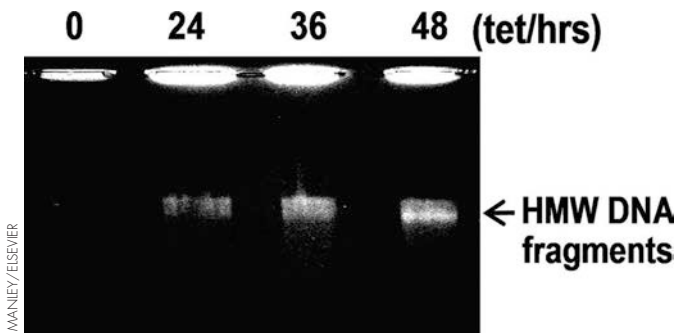**Without an ASF coat, transcribed RNA interferes, resulting in DNA fragmentation.**

Li and Manley did not set out to discover a genome-protective mechanism, but chanced upon it while studying ASF/SF2. The Columbia team put this splicing protein under the control of a tetracycline-responsive promoter. Shutting it off led to cell death, but many revertants became resistant to tetracycline repression.

The ASF transgene in the revertants had, along with many other genes, been shuffled into new areas via DNA rearrangements. Sure enough, markers of DNA double strand breaks appeared within 12 h of turning off ASF, and fragmented DNA appeared within 24 h. These changes were not seen with the tetracycline-mediated shut-off of other essential genes.

R loop structures appeared during the shut-off, and all these shut-off symptoms could be abolished by overexpression of RNase H, which can degrade the RNA in RNA–DNA hybrids. In vitro, ASF suppressed R loop formation during transcription as long as the transcribing RNA polymerase II was phosphorylated on its COOH-terminal domain, as it is in vivo. This phosphorylated domain was already shown to recruit ASF to the transcription reaction.

R loops are not always a bad thing. In B cells they are essential for initiating the DNA rearrangements that mediate class switching of antibody heavy chains. Keeping this machinery away from nascent RNA in other cell types may be one important, presplicing function of ASF and related proteins. JCB

Reference: Li, X., and J.L. Manley. 2005. *Cell.* 122:365–378.

An optical brain

Green algae use a light-activated ion channel to control phototaxis. Now, Edward Boyden, Feng Zhang, Karl Deisseroth (Stanford University, Stanford, CA), and colleagues have used the same channel to control the rapid spiking activity of neurons.

Experimental control of neural activity has become more and more sophisticated, with glutamate uncaging and multineuron patch clamping allowing the targeting of a specific neural area. But there is a catch. "You can't target cell types in that way," says Deisseroth. Usually the individual cell types "are sparsely embedded in the networks."

The obvious solution to this is genetics. Promoters to drive expression in specific cell types are available, as many of the interneuron types express unique neuropeptides or other markers. Initial attempts have met with partial success,

but the complexity of the introduced signal cascades has meant that control has been on the order of seconds and minutes rather than milliseconds.

The Stanford group used rapid optical switches plus the single component channel-rhodopsin-2 from the green alga *Chlamydomonas reinhardtii*. After lentivirus infection of rat neurons with their gene construct, blue light resulted in rapid depolarizing currents. Repeated light pulses could elicit spike trains typical of active neurons, with patterns that were reproducible in either the same or different neurons. In the absence of light, the resting potential, response to injected current, and cell health were unaffected.

Deisseroth is interested in how certain cell types connect with others, and what specific function each one provides. He plans to apply the new technique in the mouse or rat hippocampus

60 mV

1 s
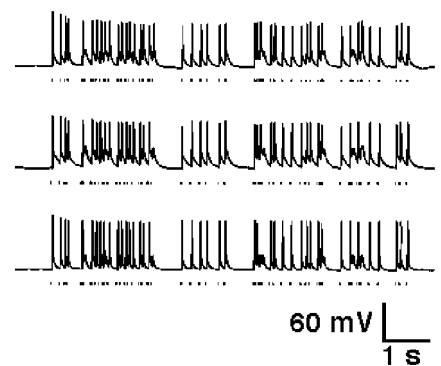
Light pulses give reproducible spikes in different cells.

to get at some of the more enigmatic functions—such as mood—controlled by this brain region. Experiments with brain sections may be followed by experiments in live animals using optical fibers and 2-photon excitation. JCB

Reference: Boyden, E.S., et al. 2005. *Nat. Neurosci.* doi:10.1038/nn1525.

Text by William A. Wells

wellsw@rockefeller.edu

Freedom not aggregation

Glycines are small and thus valued by evolution for their flexibility within crowded and contorted protein environments. Now, Claudia Parrini, Fabrizio Chiti (Università degli Studi di Firenze, Italy), and colleagues find another reason for conserving glycines: they can inhibit protein aggregation.

"We believe that proteins have a general tendency to aggregate into amyloid-like structures," says Chiti. Proteins respond by covering β sheets, or including charges, bulges, or turns.

When Chiti looked at sequences of acylphosphatase (AcP), his folding workhorse, he saw another possibility. The sequences from genome projects had six glycines conserved across the three domains of life. "Glycines are very flexible," says Chiti. "When you put a glycine into an amyloid state it has only one possible conformational shape. So this has a high entropic cost." Theoretically this cost should be a barrier to aggregation.
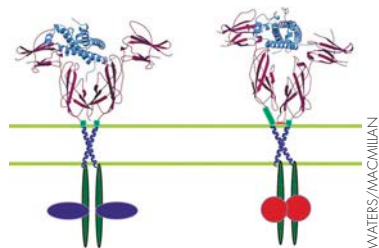
The Italians tested this idea by changing each of the conserved glycines to an alanine. Two were involved in catalysis, and one mutant aggregated in bacteria. The remaining three mutants had no significant defect in either catalysis or protein stability, but they did aggregate significantly faster than the wild-type protein. Enhanced aggregation was seen even when a glycine was replaced by one of several charged residues, which should resist aggregation.

Glycines are well conserved in many proteins. Further study is needed to see whether the anti-aggregation function for glycines can be generalized. JCB

Reference: Parrini, C., et al. 2005. *Structure.* 13:1143–1151.

Turning on by turning

Growth hormone activates its dimeric receptor (the growth hormone receptor [GHR]) by rotating one subunit relative to the other, according to Richard Brown, Michael Waters (University of Queensland, Australia), and colleagues.

"The majority of textbooks today still say that cytokine receptor activation is by dimerization," says Brown.

Receptor rotation aligns activating kinases.

Brown and colleagues add to the accumulating evidence that unbound, inactive receptor is already dimeric by showing that the transmembrane and juxtamembrane domains hold the dimer together in the absence of ligand.

How else might receptor activation work? Another member of the cytokine receptor family, the erythropoietin receptor (EPOR), is thought to be activated by a scissor-like mechanism. The extracellular domains would keep the active faces of the intracellular domains far from each other until ligand binding allows the scissors to close. But when Brown and colleagues crystallized extracellular domains of the dimeric GHR with no ligand bound, they found that it differs little from the published structure that contains ligand.

Studies with EPOR are also consistent with some degree of activation by rotation, but the mechanism is unclear. The Brisbane group find that rotation—achieved by inserting alanines within the transmembrane domain α-helix—can activate GHR. Insertion of four, but not fewer or more, alanines resulted in constitutive activation. Growth hormone apparently achieves the rotation because the two sites for binding GHR are placed asymmetrically. Rotation of transmembrane domains would align cytoplasmic JAK2 kinases so that they can transphosphorylate. JCB

Reference: Brown, R.J., et al. 2005. *Nat. Struct. Mol. Biol.* doi:10.1038/nsmb977.
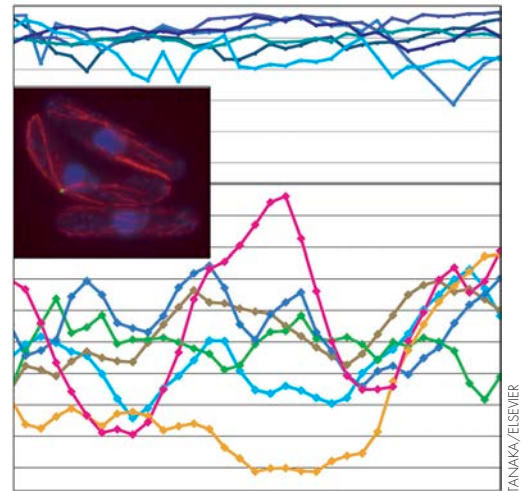
Hrs1p in mitotic cells induces a horsetail astral array (inset) and nuclear oscillation (bottom vs. top).

Getting nuclei moving

Addition of a single fission yeast protein can induce nuclear oscillations in mitotic cells, even though these movements are normally seen only in meiotic cells, according to Kayoko Tanaka, Masayuki Yamamoto, and colleagues (University of Tokyo, Japan).

Fission yeast meiosis includes a thrashing about of the nucleus (also called horsetail nuclear movement [HNM]) that helps align homologous chromosomes. This oscillation is driven by a dynein–dynactin system. Cortex-localized dynein may be switched on at first one end of the cell and then the other. The dynein tugs on an astral array of microtubules called the horsetail astral array (HAA).

Mitotic cells lack both the HAA and any significant amounts of dynein and dynein-anchoring proteins. So it was a surprise when the Japanese group succeeded in inducing the mitotic appearance of the HAA and the HNM by expressing a single meiotic protein. That protein, called Hrs1p or Mcp6p, was discovered by this and another group as a meiosis-specific protein required for HAA formation.

The HNM oscillations were somewhat less organized in the mitotic cells, probably because of the low levels of dynein and associated proteins. But the HAA was quite robust. Hrs1p/Mcp6p appears to interact with both spindle pole body components and microtubule-nucleating components. Tanaka hopes to assemble in vitro complexes with these two activities to see if Hrs1p/Mcp6p can link them together. JCB

Reference: Tanaka, K., et al. 2005. *Curr. Biol.* 15:1479–1486.