RNA de-inhibition

Small RNAs are famous for their gene-silencing ability. But new results from Tomoko Kuwabara, Fred H. Gage (Salk Institute, La Jolla, CA), and colleagues show that some tiny RNA species turn genes on, not off.

This new class of RNAs is needed to make neurons. “Small double-stranded RNAs exist in reasonably high concentrations in cells that have just committed to neuronal lineages,” says Gage. These RNAs are homologous to a promoter sequence called NRSE/RE-1, which is found in a wide range of genes that are expressed only in neurons. In other cell types, these genes are known to be shut off by the NRSE/REST repressor.

The group shows that this repressor becomes an activator when NRSE dsRNAs are around. As a result, neuronal genes are turned on, and multipotent adult neural stem cells become neurons.

The NRSE/REST protein binds strongly to the dsRNA, and the two probably sit on promoters as a complex. When both are present, histone acetylases and chromatin-remodeling proteins replace the deacetylases and methyl-DNA binding proteins that are found at neuronal gene promoters when only NRSE is expressed.

The dsRNAs are found in regions of the hippocampus where neurons are differentiating, and their destruction prevents cultured cells from becoming neurons in response to inducing signals. A single molecule RNA switch that creates an entire lineage may not, however, be found outside neurons. “It’s an unusual case,” says Gage, “because of NRSE. It binds to so many promoters that it makes the RNAs generalizable.”

Reference: Kuwabara, T., et al. 2004. Cell. 116:779–793.

Cholesterol hastens Alzheimer’s

Results from Qinghai Zhang, Jeffery Kelly (Scripps Research Institute, La Jolla, CA), and colleagues suggest that evil metabolites may accelerate Alzheimer’s disease (AD) by promoting protein misfolding. This folding effect may explain why inflammation and high cholesterol are risk factors for AD.

AD results from aggregates of a misfolded form of amyloid β peptide (Aβ). Misfolding occasionally results from processing flaws or mutations in the Aβ precursor, but most patients have normal Aβ. Zhang et al. show that even normal Aβ folds abnormally when cholesterol by-products modify it.

Cholesterol itself did not affect Aβ, but cholesterol modified by reactive oxygen species (which are produced during inflammation) to generate an aldehyde group reacted with Aβ and made it more hydrophobic. This altered form aggregates at much lower concentrations than does normal Aβ—concentrations that are found in the brain.

Although brain samples did not show higher levels of the cholesterol aldehydes in AD patients, only small amounts of these metabolites are needed to jump start aggregation. “The creation of compounds that are reactive could be from an event occurring years before an individual presents with AD,” says Kelly. So, as boxers already know, one good knock on the head might do more lasting damage than just a fleeting headache.

Reference: Zhang, Q., et al. 2004. Proc. Natl. Acad. Sci. USA. 10.1073/pnas.0400924101.

The two faces of Mad2

Sequence dictates structure, but for Mad2, one structure is not enough. New results from groups led by Hongtao Yu and Josep Rizo (University of Texas Southwestern, Dallas, TX) show that Mad2 adopts two distinct conformations and that checkpoint activation may consist of switching Mad2 to the right form.

Mad2 holds up anaphase until every chromosome is properly attached to the spindle. Past structural studies of Mad2 showed that Mad1 (its activator), and Cdc20 (its anaphase-halting target) share the same binding site. “It would seem,” says Rizo, “that [Mad1] would be a competitor rather than an activator because they bind in the same place.” But the newly identified second conformation suggests an answer to this puzzle—Mad1 may put Mad2 in an active conformation that is maintained even when the Mads separate.

The two forms can be distinguished based on column chromatography and NMR. The active form has a higher affinity for Cdc20 than does the other form and blocks anaphase in oocyte extracts. In vivo, sequestration of only the active form thwarts the checkpoint.

The forms interchange in vitro very slowly. A fragment of Mad1, however, accelerates transformation of the inactive into the active form. Some Mad2 is always complexed with Mad1 in cells, so the question now is what tells Mad1 to toss off Mad2 (in its active form) so it can bind to Cdc20.

Reference: Luo, X., et al. 2004. Nat. Struct. Mol. Biol. 10.1038/nmb748.
The machine that is a cell is more than the sum of its protein parts. A large step toward understanding how those parts are so effectively put together has been taken by Patrick Aloy, Rob Russell (EMBL, Heidelberg, Germany), and colleagues. Their work has identified ~100 yeast protein complexes and predicted interactions within and among many of them.

“We were working on a large scale to model as many complexes as possible,” says Aloy. Proteins that purified together were assigned to functional groups. The authors built three-dimensional models for as many proteins in these groups as possible, based on known structures and protein homologies. They then predicted which proteins interact directly, and subsequently modeled the structures of complexes containing multiple proteins.

Additional structural data came from EM analyses of the complexes that purified with sufficient quality. “We figured out not just who interacts with whom, but how,” says Aloy. “Understanding function requires structure. At the end of the day it’s what gives you the biochemistry.”

Using known two-hybrid interactions and estimates based on homology, the group also predicted communications between complexes. Some were unexpected connections, such as those between transcription and translation components. Although the accuracy of many of their cross-talk predictions is unknown, the structures suggest suitable sites for mutagenesis by any group interested in a particular interaction pair.

So far, the authors have a good idea of the structure of about a quarter of the estimated total protein complexes in yeast (~400) and has nearly complete structures for 42 complexes. “Our final goal,” says Aloy, “is to model all the associations of all the complexes or organelles at a molecular level.” More structural information should be forthcoming once the group is able to improve their EM using tomographic techniques.

Reference: Aloy, P., et al. 2004. Science. 303:2026–2029.

Mass dedifferentiation

Germline stem cells (GSCs) on their way to differentiation can change their minds and return to pluripotency, according to Toshie Kai and Allan Spradling (Carnegie Institution of Washington, Baltimore, MD). Such a return is a long-sought goal for those hoping to create pluripotent cells for transplantation.

GSCs in the adult fly reside in a niche where they receive Dpp signals telling them to remain undifferentiated. Upon division, one daughter escapes the niche (and the realm of Dpp) and expresses Bam. The freed cell thus differentiates into a cyst—a set of up to 16 cells interconnected by incomplete cytokinesis and a cytoskeletal structure called the fusome.

Kai and Spradling show that these steps toward differentiation can be undone with Dpp. They overexpressed Dpp in flies to form many GSCs, then induced a transient burst of Bam to produce cysts. Hours later, when Bam was gone, the cysts broke down into single cells resembling GSCs.

Using larvae, the group shows that the resulting cells are functional GSCs. As in the adult, transient Bam caused cysts to form, and again these cysts individualized. The resulting single cells developed into normal GSCs as the larvae grew into adults. Reversion of cyst cells may repopulate GSCs depleted by injury or age, for example, although how this choice might be regulated is unknown.

Although other cell types (such as liver) are thought to dedifferentiate on rare occasions, Spradling is excited about the frequency of reversion in cysts. “All the germ cells in the larval ovary become cysts and then, seemingly, all become stem cells again,” he says. So the system should lend itself to finding the factors that direct this backward step. Like cysts, dividing germ cell precursors are transiently linked by a fusome. Spradling wonders whether the severing of this connection is the trigger to stemness.

Reference: Kai, T., et al. 2004. Nature. 10.1038/nature02436.