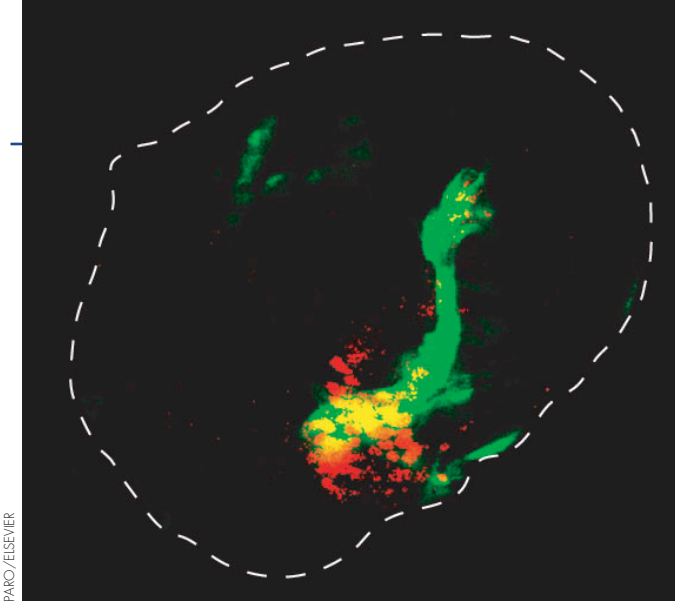
Research Roundup

Wiping the cellular slate clean

A dose of the JNK kinase can reset chromatin states and turn leg into wing, according to Nara Lee, Renato Paro (University of Heidelberg, Germany), and colleagues. The pathway may help cells to become reprogrammable when they find themselves in areas of injury or developmental transition.

JNK has for some time been known to turn on during wound healing, and Polycomb group (PcG) proteins are known to maintain cell fates by clamping down on chromatin. But the two have never been united in one experimental design.

The Heidelberg team took a look at the two processes in fly imaginal discs—the embryonic tissues that will eventually turn into adult structures such as legs. A fraction of the leg imaginal discs are known to transdetermine—or change to a wing cell fate—when they are transplanted and wounded. In the new experiments, transdetermination rates were increased in PcG mutants but decreased in JNK mutants.



PARO/ELSEVIER

JNK signaling (red) and transdetermination (green) overlap.

Transdetermined cells overlapped with cells high in JNK signaling and low in PcG activity. Furthermore, JNK expression down-regulated PcG proteins in both fly cells and mouse fibroblasts.

This experimental system—transdetermination during imaginal disc transplantation—is extremely artificial and not a substrate for natural selection. So where does this fit into normal fly and human biology?

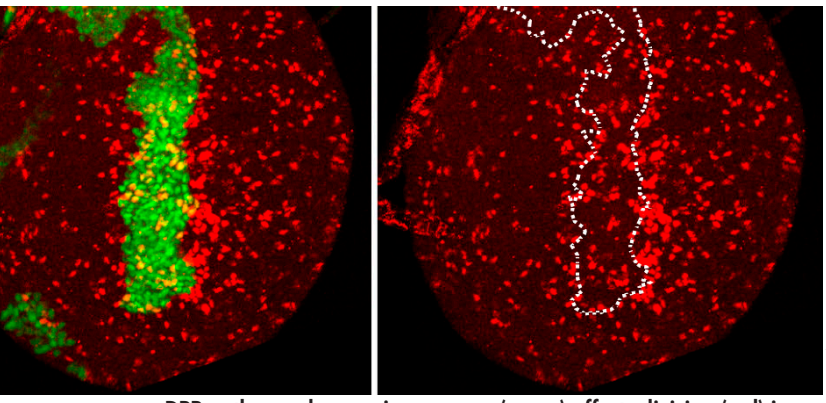
Paro notes that JNK is turned on during injury and major developmental events such as dorsal and thorax closure. These are both times when cells may need to switch allegiances and take up new fates. The JNK pathway and its down-regulation of PcG proteins may open up the cell to new possibilities and allow it to fit into a new environment. Judicious manipulation of this process may, in the future, help researchers to manipulate the differentiation of various adult stem cells. JCB

Reference: Lee, N., et al. 2005. *Nature.* doi:10.1038/nature04120.

Difference drives division

Morphogens that spread from a point or line source are useful for patterning and for defining the outer bounds of a tissue. How to convert such a gradient into uniform cellular growth is, however, far from obvious. Can a pro-growth morphogen avoid encouraging excessive growth near its source and inadequate growth further away?

Dragana Rogulja and Kenneth Irvine (Rutgers, Piscataway, NJ) now provide one possible solution for cells that will become fly wings. The cells, they find, make their division decision in response to a gradient rather than absolute concentrations of the Decapentaplegic (DPP) morphogen.



IRVINE/ELSEVIER

DPP pathway changes in one area (green) affects division (red) in other areas.

The Rutgers group expressed or repressed the DPP pathway in small clones. In both cases they saw new cell division both within the clone and in neighboring areas. This nonautonomous growth had not been evident in previous experiments, probably because the earlier experiments used expression systems with a long lag time. The new experiments used drug-inducible expression to get tighter temporal control.

The researchers believe that cells measure the difference between their own DPP expression level and that of their neighbor, and go through cell division only if there is a difference. This growth effect may be able to spread for several cell diameters both back into the DPP-expressing clone and out into the area expressing lower levels of DPP. Intercell comparisons could be made, for example, by cells expressing receptors that bind homophilically to their partners in a neighboring cell, but signal if there are no more binding partners available.

Further from the DPP source, the gradient effect drops away, probably because in wild-type tissue this area would experience extremely shallow gradients of DPP. The cells in this area seem to depend more on the absolute values of DPP for their instructions. JCB

Reference: Rogulja, D., and K.D. Irvine. 2005. *Cell.* 123:449–461.

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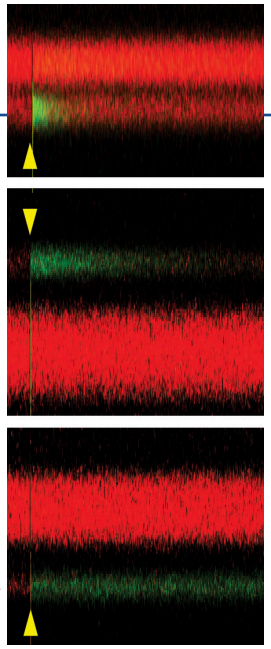


SABATINI/NAS

Over time (left to right), individual dendrites allow the escape of proteins (green) quickly (top), slowly (middle), or hardly at all (bottom).

Squeezing dendritic necks

Active dendritic spines isolate themselves from the outside world, say Brenda Bloodgood and Bernando Sabatini (Harvard Medical School, Boston, MA). The spine necks become a diffusion barrier, which may facilitate the localized build-up of changes needed for synaptic plasticity.

Bloodgood and Sabatini were "playing around" with a sensitive and photoactivatable fluorophore when they noticed that some dendritic spines seemed to be decoupled from the rest of the dendrite. Fluorophore activated inside these spines was slow to leak out, and fluorophore activated outside was slow to diffuse in. Large changes in this diffusion barrier occurred spontaneously in organotypic slice cultures, with more blockage being induced by drugs that favor excitatory transmission.

Sure enough, diffusion restriction was induced in individual dendrites by pairing two excitatory signals from pre-synaptic and post-synaptic sources.

The clampdown at the dendrite neck may involve cross-linking of an actin mesh, or blockage by mitochondria or smooth endoplasmic reticulum. Either way, says Sabatini, "if a spine can hold onto [molecules such as] active kinases, this will have a big impact on signal integration." An isolated spine may be able to retain activated messengers until the next electrical spike arrives to boost the signal further. And truly isolated spines may also be able to act as independent electrical units that store or boost sub-spike electrical inputs. JCB

Reference: Bloodgood, B.L., and B.L. Sabatini. 2005. *Science.* 310:866–869.

Staying smart with age

Some rats, and humans, are better than others at keeping their brains fresh and nimble with age. Hey-Kyoung Lee, Sun Seek Min, Michela Gallagher, and Alfredo Kirkwood (Johns Hopkins University, Baltimore, MD) now find that old rats stay smart by switching from one way of modifying synapses to another.

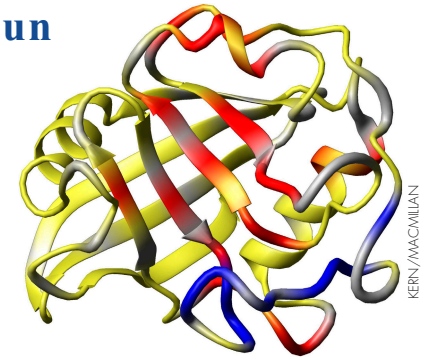
Gallagher has noted before that a subset of older rats maintain learning abilities equivalent to those of young rats. Learning involves the modification of synaptic strength: connections are strengthened via long-term potentiation (LTP) and weakened via long-term depression (LTD). When thinking of learning, says Kirkwood, "most people put the emphasis on LTP, but I have an appreciation for what makes things weaker." When forming a new memory, "making [connections] stronger and making them weaker must be equally balanced."

LTD comes in two flavors, one dependent on and another independent of NMDA receptors (NMDARs). The Baltimore group found that NMDAR-LTD declined with age, but there was no difference between old rats that were either smart or befuddled. But the old and smart rats showed far more non–NMDAR-LTD than either the young rats or the old and befuddled rats.

Thus it appears that some aging rats successfully switch from NMDAR-LTD to non–NMDAR-LTD. This switch is a smart strategy, because NMDAR-LTD can cause excitotoxicity, so decoupling from NMDARs with age "could be a way of managing excitotoxicity," says Kirkwood. Once the non-NMDAR pathway is better characterized, it may make a better target than the NMDAR pathway for enhancing brain functioning in the elderly. JCB

Reference: Lee, H.-K., et al. 2005. *Nat. Neurosci.* doi:10.1038/nn1586.

Catalytic dry run



KERN/MACMILLAN

Moving parts during catalysis (blue) and in the free enzyme (red) are overlapping.

Enzymes are constantly rehearsing, say Elan Eisenmesser, Dorothee Kern (Brandeis University, Waltham, MA), and colleagues. They find that, even before a substrate appears on the scene, an enzyme flexes rapidly through its catalytic motions.

Kern has been looking at enzymes for many years, but in Eastern Europe her work was constrained. "We didn't have big enough NMR machines to look at the protein, so we were looking at the substrate during catalysis," she says. But now money and NMR technologies have caught up with Kern's ambitions. With new NMR methods, different protein conformations can be quantitated, thus yielding the kinetics of protein motion.

The Brandeis group applied these methods to the prolyl cis-trans isomerase cyclophilin A (CypA). They first mapped movements during catalysis, and then found that very similar movements happened with the free enzyme, with frequencies reflecting the turnover number of the enzyme. "All the motions are already there, and constantly going on, even with no substrate around," says Kern. "Nature has selected proteins so they are sampling defined conformations. They are optimized for catalysis."

A single rate constant for these movements was consistent with the existence of an extensive, connected network of moving residues. Chemical shift changes caused by a collection of individual mutations could be cross-correlated, again suggesting the concerted movement of a network of residues. JCB

Reference: Eisenmesser, E.Z., et al. 2005. *Nature.* doi:10.1038/nature04105.