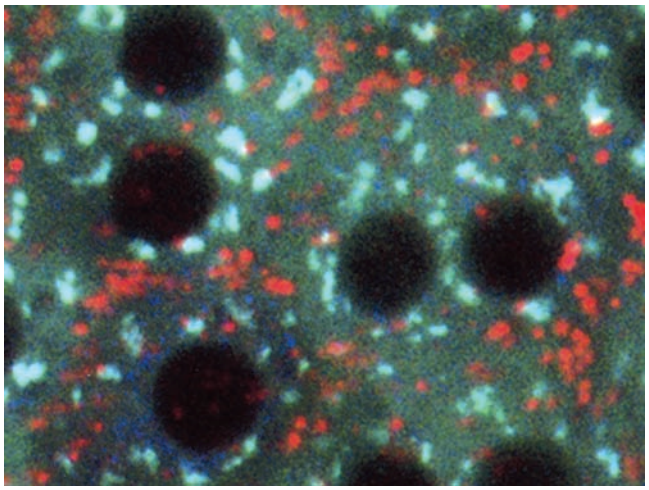
In This Issue

New structure found in plain sight

Like explorers spotting an uncharted island on the horizon, Imanishi et al. (page 373) have identified a previously unknown cellular structure that could be an entirely new organelle. The structure, located in cells of the retinal pigment epithelium, appears to be an essential waypoint in the retinoid cycle—the series of chemical reactions that regenerates 11-*cis*-retinal, the chromophore for rhodopsin, after light converts it to all-*trans*-retinal.

Isolated retinas do not survive long outside of the eye, complicating studies of retinal biology. The authors circumvented this problem by looking directly into the eyes of live mice with two-photon fluorescent microscopy. Retinol and retinyl esters show weak intrinsic fluorescence, producing high-resolution images of intact retinal cells and revealing a fence-like intracellular structure dubbed the retinyl ester storage particle, or retinosome. In wild-type mice exposed to light, retinyl ester levels in retinosomes rise, and then fall, consistent with the recycling of retinoid intermediates produced by light exposure.



Storage sites for retinyl esters called retinosomes (red) do not colocalize with organelles such as the Golgi (blue).

Retinosomes are absent in mice lacking the enzyme LRAT, which produces retinyl esters. Mice lacking RPE65, which is required for processing retinyl esters, accumulate large quantities of the esters in overgrown retinosomes. Biochemical analysis shows that retinosomes also contain adipose differentiation–related protein (ADRP).

The new structure provides a context for understanding the retinoid cycle. By compartmentalizing a portion of the cycle, the retinosome can locally enrich intermediates in the cycle to drive energetically unfavorable reactions. Just as important, sequestering the retinyl esters can prevent toxic reaction intermediates from poisoning the cell.

The retinosome's highly ordered structure suggests that it incorporates other proteins, and the authors are now trying to use ADRP as a hook to isolate pure retinosomes for further analysis. Since defects in the retinoid cycle underlie many forms of congenital blindness, the ability to observe retinosomes directly in intact eyes may also provide a powerful diagnostic tool in the clinic. ■

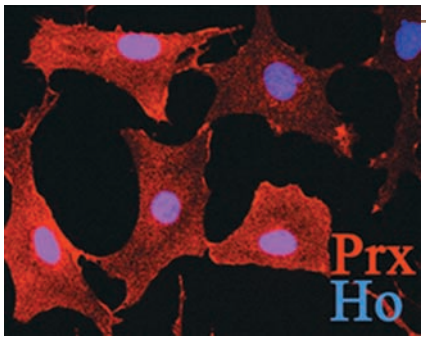
Krox-20 conducts the Schwann song

When Schwann cells begin to myelinate large-diameter axons, they stop dividing, become resistant to apoptosis, and start producing myelin proteins. How are all of these changes coordinated? On page 385, Parkinson et al. identify several new signaling interactions in Schwann cells, and show that the transcription factor Krox-20 is a master regulator of myelination.

The authors found that Krox-20 expression makes Schwann cells resistant to the mitogen NRG-1 and the apoptosis-inducing action of TGF-β. Rather than specifically targeting these signaling molecules, Krox-20 appears to act through a general mechanism, suppressing the activity of the JNK/c-Jun pathway. The data show that JNK/c-Jun signaling is required for both proliferative and apoptotic responses in Schwann cells. Krox-20 expression increases the expression of the scaffold protein JIP-1, a known inhibitor of JNK activity, and also decreases the level of c-Jun protein in the cell, providing two possible ways to inhibit JNK/c-Jun signals.

Surprisingly, expression of Krox-20 in cultured 3T3 fibroblasts, which are not related to Schwann cells, causes the fibroblasts to stop dividing, resist apoptosis, and express the myelin genes periaxin and P_0. The ability to induce so many specialized responses in a different cell type indicates that Krox-20 is a master regulator.

By turning off a single pathway that is activated by both NRG-1 and TGF-β, Krox-20 can coordinate changes in both proliferative and apoptotic activities without affecting other processes activated by growth factors. Its position as a master regulator explains why mutations in Krox-20 often lead to severe hereditary myelination disorders. Parkinson et al. are now trying to determine whether Krox-20 acts directly or indirectly to reduce c-Jun levels and induce myelin gene expression. ■



Krox-20 makes fibroblasts turn on myelin genes.

TEXT BY ALAN W. DOVE
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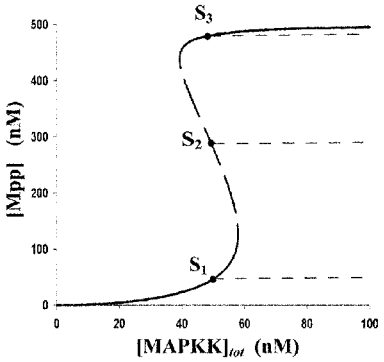
Kinase cascades are binary to the bone

Like electronic relays, many biological signaling pathways are bistable, able to turn "on" or "off" in response to a stimulus, but unstable in intermediate positions. The conventional view is that bistable cellular signaling requires a distinct positive or double-negative feedback signal; but on page 353, Markevich et al. prove that it does not. Instead, signaling pathways like the common MAPK cascade are intrinsically bistable, suggesting that feedback loops function primarily to increase the repertoire and flexibility of cellular responses.



MAPK signaling is all or nothing.

The authors performed a mathematical analysis of a generic MAPK phosphorylation/dephosphorylation cycle, in which MAPK can be phosphorylated or dephosphorylated on two sites. Their assumptions were that the monophosphorylated and diphosphorylated forms of MAPK compete for either kinase or phosphatase, and that the enzymes are nonprocessive. Under these conditions, the equations reveal bistability in the absence of a distinct feedback system. Instead, the competition of substrates for enzymes automatically reinforces a stable "on" or "off" state and makes intermediate states unstable.

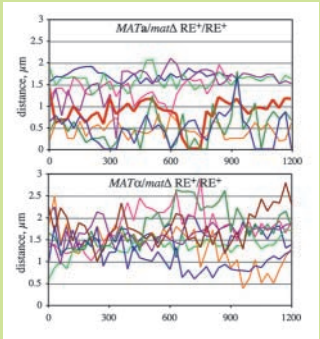
Nonetheless, the ubiquity of biochemical feedback loops implies that they serve some purpose. One possibility is that the combination of intrinsic bistability and a feedback loop could confer multistability, allowing systems like the MAPK cascade to signal varying degrees of "on." ■

Switching mating types with one arm tied

The mating type (MAT) locus on *Saccharomyces cerevisiae* chromosome III can recombine with the HMRa locus near the right telomere or the HMLα locus near the left telomere of the same chromosome. On page 361, Bressan et al. show that the current mating type of a cell determines the spatial configuration of these three loci in the nucleus, suggesting that the outcome of mating type switching is directed by the restraint or release of the left arm of chromosome III.

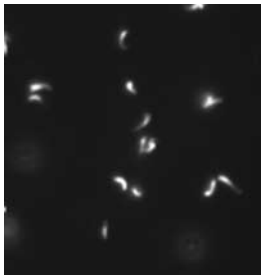
GFP tagging of chromosomes in the nuclei of living cells showed that movement of the left arm of chromosome III is tightly constrained in MATα cells, but relatively free in MATa cells. Deleting the recombination enhancer (RE) sequence on chromosome III keeps the left arm constrained in both types of cells.

RE activity requires the transcriptional activator Fkh1p, and Bressan et al. suggest that Fkh1p competes for DNA binding with tethering factors that restrain the chromosome. In MATa cells, Fkh1p binding prevails, releasing the left arm and allowing HMLα to recombine with the MAT locus, whereas in MATα cells, restraint of the left arm leaves HMRa as the recombination donor. By directing cells to switch periodically to the opposite mating type, the system assures the availability of mating partners in a haploid population. ■



HML is less constrained in MATa cells (top) than in MATα cells (bottom).

Actin organizer takes pathogens for a ride


Beads covered with a Tir peptide drive actin polymerization in an extract.

Enteropathogenic *E. coli* causes a dramatic actin reorganization in intestinal epithelia, erecting intracellular pedestals on the host cells beneath the attached bacteria. On page 407, Campellone et al. reduce this complex phenomenon to twelve amino acids, showing that clustering a small domain of the bacterial Tir protein, which is translocated to the host cell, is sufficient to induce actin rearrangement. The results highlight an interesting evolutionary convergence, and provide a simple model system for studying actin assembly.

After discovering that Tir is the only *E. coli* component required for pedestal formation, the authors further whittled the system down to the C-terminal cytoplasmic domain of Tir. Clustering this domain at the plasma membrane causes its phosphorylation, allowing it to bind to the host protein Nck. Nck binding leads to the recruitment of N-WASP and the Arp2/3 actin nucleating complex, followed by actin pedestal formation. A 12–amino acid piece of the Tir COOH terminus triggers actin assembly in *Xenopus* egg extracts. Interestingly, a similar peptide sequence in vaccinia virus allows the virus to be transported on actin tails, suggesting that two unrelated pathogens have evolved to exploit Nck in similar ways. The authors now hope to determine how Nck binding to the Tir sequence leads to actin rearrangement. ■