Actin Polymerization on the Edge

A gradient of 3′ phosphoinositide (PI) lipids, oriented towards a chemoattractant source, is generated by G protein-coupled receptors in both Dictyostelium and neutrophils. On page 1269, Haugh et al. show that a similar polar gradient is generated in fibroblasts in response to platelet-derived growth factor (PDGF) stimulation of a tyrosine kinase receptor.

But Haugh et al. do not stop there. They use sophisticated microscopy to focus only on the base of the cell, where they observe another gradient. This second, radial gradient of PI lipids increases from the center to the periphery of the cell. Haugh et al. propose that cells generate certain PI lipids only on their free, nonadherent surfaces, and that these lipids then diffuse, as they are being degraded, to the adherent surface. The resultant radial gradient may help define a peripheral zone in which actin is preferentially polymerized, explaining how motile cells restrict the majority of actin polymerization to their edges.

Haugh et al. observe this gradient using a fusion protein incorporating green fluorescent protein (GFP) and the PI-binding PH domain of the Akt kinase. When an activated Ras protein is added to the mix, a significant amount of GFP-AktPH localizes to the membrane, even in the absence of PDGF. Addition of PDGF, which is known to result in the creation of PI lipids, initially results in a decrease in GFP-AktPH signal on the attached surface of the cell. Haugh et al. suggest that the fusion protein has been lured away by the PI formed on the nonadherent surfaces. This scenario and the behavior of the radial gradient are consistent with the properties of a mathematical model of the diffusion events.

Steric exclusion of PDGF probably does not explain the difference between adherent and nonadherent surfaces, based on experiments with fluorescent dextrans. An alternative explanation is some form of cross-talk between the PDGF receptor and integrins responsible for cell adhesion. Coordination between these two systems may be particularly important in fibroblasts, which maintain a large, consistently-sized, adhesive surface even as they move.

Profiling Muscular Dystrophy

Transcription profilers have, thus far, concentrated primarily on either perturbing microorganisms or subtyping diseases such as cancer. On page 1321, Chen et al. demonstrate how profiling can be applied to the study of human monogenic disorders, in this case muscular dystrophy (MD). They attempt to minimize the effects of patient-to-patient variability by pooling samples known to have a common genetic basis, and use the results to propose hypotheses for what is occurring as MD progresses.

The essential defect in most cases of MD is a lack of a functional dystrophin complex, which normally imparts structural integrity to the muscle fiber plasma membrane during contraction. For the MD patients with a defect in dystrophin, the primary genetic defect is apparent in the profiles, probably because the nonsense dystrophin mutations lead to mRNA degradation. The same is not true for the patients with missense mutations in α-sarcoglycan, a gene encoding an associated protein. Also not apparent are reductions in mRNAs for many of the other associated proteins that make up the dystrophin complex, even though their protein levels are reduced in MD.

Some transcriptional increases reflect the arrival of new cell types, such as the activated dendritic cells detected here. The most obvious increases attributable to muscle cells are in genes encoding developmental and regeneration proteins. The high levels and widespread distribution of these proteins far exceeds what would be expected based on the relatively modest amount of regeneration. This suggests that MD myofibers are suffering from a chronic state of incomplete differentiation. One explanation may be excessive calcium influx, which results from lack of dystrophin-mediated plasma membrane integrity, and could interfere with calcium-triggered differentiation proteins. Excess calcium influx into mitochondria may also explain the downregulation of many genes involved in mitochondrial biology and energy metabolism. Competing hypotheses of this type should be testable with experiments that perturb one parameter (such as the effects of calcium influx) and subtract the results from the changes presented here.

Asymmetry and Endocytosis

Asymmetric partitioning of Drosophila melanogaster Numb protein controls the determination of certain sensory neurons. Now Santolini et al. present evidence that mammalian Numb is an endocytic protein (page 1345). This raises the intriguing hypothesis that cell fate determination in the nervous
system may rely on the asymmetric partitioning of a component of the endocytic machinery at mitosis.

By immuno-electron microscopy, mammalian Numb localizes to clathrin-coated pits and vesicles, and possibly to the Golgi apparatus and trans-Golgi network (TGN). At the beginning of a temperature upshift, Numb moves from the TGN to coated pits and vesicles, before progressing with internalized receptors to endosomes. Overproduction of a fragment of Numb inhibits receptor endocytosis.

Numb interacts directly with α-adaptin, a clathrin adaptor, and was already known to bind to and antagonize signaling by the transmembrane Notch receptor. The nature of the antagonism was unknown, although Numb was suggested to interfere with translocation of activated Notch into the nucleus, where Notch is proposed to help activate transcription. The results in this issue suggest that Numb may change the rate of endocytosis of Notch, or divert an activated form of Notch so that it no longer reaches the nucleus.

**Making Sure p21 Is On**

Insulin-like growth factors (IGFs) maintain muscle cell viability during differentiation. Lawlor and Rotwein recently showed that p21, an inhibitor of the cyclin-dependent kinases (cdks), is a necessary component of this pathway (Lawlor, M.A., and P. Rotwein. 2000. Mol. Cell. Biol. 20: 8983–8995). Now, on page 1131 they demonstrate that p21 is turned on by two divergent pathways in differentiating muscle cells.

The two pathways share the upstream activation of phosphatidylinositol 3-kinase (PI3-kinase) but then diverge, to either the Akt kinase or the MyoD transcription factor, before converging again on p21. Either pathway alone is sufficient for in vitro cell survival in differentiation media. Thus, even in the absence of MyoD, IGFs still induce p21, presumably through Akt, and the cells still survive. This may mimic the situation early on in the mouse embryo, when somites are exposed to IGFs and express p21 two days before they express MyoD. The Akt pathway may keep these prospective muscle cells alive until MyoD comes along to continue the job and complete the differentiation process.