In Brief

Membrane Trafficking by Kinesin II

Kinesin II is a heterotrimer of two motor proteins and a third non-motor subunit. Le Bot et al. (page 1559) find that kinesin II is involved in the movement of membranes between the endoplasmic reticulum (ER) and the Golgi, and Tuma et al. (page 1547) implicate kinesin II in the dispersion of pigment-filled melanosomes, which *Xenopus* uses to change color.

Kinesin II is also needed for the transport of flagellar proteins and is found on mitotic and neuronal vesicles, so the simple idea that a single kinesin performs a single function seems to be inadequate. How the attentions of a single motor might be divided to more than one site is not known.

Both groups study kinesin II function using dominant negative, "headless" versions of the XKlp3 kinesin II subunit that lack the motor domain. The headless protein does not affect lysosome movements or melanosome aggregation, but markedly reduces melanosome dispersion. The residual melanosome dispersion appears to be actin based. Others have suggested that randomly oriented actin ensures an even distribution of melanosomes in the cytoplasm during dispersion. When kinesin II function is compromised, myosin motors may facilitate the spreading of melanosomes in the cytoplasm.

In cultured *Xenopus* cells, Le Bot et al. find that XKlp3 localizes to tubulovesicular structures between the ER and Golgi, and is enriched around the Golgi stacks. Dominant negative XKlp3 halts the transport of newly synthesized proteins from the ER to the Golgi. Directing retrograde, plus end-directed movement from the Golgi to the ER would make more sense for a plus end-directed motor like kinesin II, so either disruption of retrograde movement is rapidly halting anterograde movement, or kinesin II may counteract and regulate dynein's activity in anterograde ER to Golgi protein transport.

Axonin-1 as an Accessory Adhesion Protein

Axonin-1 and NgCAM are both neural cell adhesion molecules that can mediate the fasciculation of neurons, simplifying nerve pathfinding. Between cell and bead, the two proteins can bind to themselves or to each other. But Kunz et al. (page 1673) find that, when axonin-1 and NgCAM are coexpressed in cells, the only contacts between cells are NgCAM–NgCAM links; axonin-1 binds only to NgCAM in the same membrane.

NgCAM accumulates at cell contacts by itself; axonin-1 does so only in the presence of NgCAM. There is no accumulation of either adhesion molecule when a cell with NgCAM contacts a cell with axonin-1.

The failure of axonin-1 to mediate cell–cell adhesion may be a result of its conformation. Kunz et al. map the domains needed for NgCAM and axonin-1 binding. The NgCAM–axonin-1 interaction requires four NH_2-terminal immunoglobulin domains of axonin-1, and four domains of NgCAM: three immunoglobulin domains plus a fibronectin domain closer to the cell membrane. Axonin-1 may loop back to make contact with the NgCAM fibronectin domain, a suggestion that is consistent with a looped structure seen in earlier electron microscopy studies.

This study suggests that soluble axonin-1, which is produced in vivo, may disrupt intercellular links by a novel mechanism. Rather than dissolving axonin–axonin links, the soluble axonin-1 may be displacing the membrane-anchored protein from complexes with NgCAM. The NgCAM that is left should be sufficient to mediate adhesion, but axonin-1 free from NgCAM complexes is known to be associated with high fyn kinase activity, and therefore a more dynamic, less anchored cytoskeleton.

Gap Junction Communication in Migrating Cells

Two reports in this issue suggest that gap junctional intercellular communication (GJIC) helps cells to migrate as an integrated unit. Huang et al. (page 1725) find that the level of GJIC in cardiac neural crest cells is directly correlated with their rate of migration. An increased dosage of a gap junction component, α_1 connexin, led to increased migration, while a knockout of α_1 connexin showed decreased migration. This suggests that the amount of GJIC directly modulates crest cell migration.

Lampe et al. (page 1735) correlate the onset of GJIC with the migration events that occur as keratinocytes spread over a wound, and identify an extracellular ligand that could trigger gap junction assembly.

The first cells to migrate into a wound express integrins that can bind to collagen and fibronectin, the connective tissue components that are exposed by a surface wound. As these leading cells migrate they begin to reestablish the basement membrane (the barrier between the epithelium and the dermis) by secreting laminin 5. The next wave of cells switch to integrin $\alpha3\beta1$, and eventually to $\alpha6\beta4$. Both integrins bind to laminin 5, but $\alpha3\beta1$ promotes motility, whereas $\alpha6\beta4$ mediates anchorage.

Using real wounds and epibole cultures (punches of skin that are an in vitro mimic of a wound), Lampe et al. find that the migrating front of cells makes laminin 5, but forms few or no gap junctions and does not exhibit GJIC. Further back in the migrating wave of cells, however, connexin 43 assembles into apparent gap junctions, and dye transfer shows that there is GJIC.

The signal for gap junction formation may come from the binding of laminin 5 to integrin $\alpha3\beta1$. Anti-$\alpha3$-integrin antibodies, or laminin 5, are sufficient to promote GJIC between cultured keratinocytes. Laminin 5 also induces GJIC between CHO cells transfected with $\alpha3$.

What important messenger is passing through the gap junction? This remains a mystery, but senior author William Carter has some ideas about what GJIC might achieve. "The cells at the leading edge are somewhat autonomous," he says. "But to act as a barrier, the cells that follow must generate a continuous cell sheet. GJIC could facilitate movement across the wound as an integrated group of cells."

Paired Pathways

Death Pathways Converge on p53

Sympathetic neurons that are on target during development are supplied with nerve growth factor (NGF), which binds to the TrkA receptor and keeps the cells alive. Without this signal the cells die in an apoptotic program that is aided by signals from the p75NTR receptor. Although the p75NTR death signal may be generated by an autocrine supply of brain-derived neurotrophic factor (BDNF), it may be increased by any number of neurotrophins that are produced by areas that sympathetic neurons are not supposed to innervate.

Aloyz et al. (page 1691) find that p53 is a common element in the apoptosis pathways downstream of both TrkA and p75NTR. Although p53 has been implicated in neuronal apoptosis triggered by stress, it has not previously been associated with this kind of developmental apoptosis.

Either withdrawal of NGF or addition of BDNF leads to an increase in p53 levels (and, as shown in earlier papers, activation of JNK kinase). Expression of p53 does not change JNK activity, but induces expression of the death mediator Bax, placing p53 downstream of JNK but upstream of Bax. The site at which the TrkA and p75NTR pathways first interact and sum their inputs is not known.

In vivo and in vitro experiments show that p53 is needed for this version of apoptosis: inhibition of p53 with an adenoviral protein allows cells to survive NGF withdrawal, and mice that lack p53 show reduced apoptosis and no reduction in cell number in the superior cervical ganglia. The residual death (and the death seen by other researchers in $p53^{-}$ cells) may be mediated by other p53 family members.

A Phosphodiesterase in Carcinoma Invasion

The integrin α6β4 forms stable adhesive structures by interacting with intermediate filaments (see the gap-junction summary above), but in carcinoma cells α6β4 is also found associated with actin structures at the cell's leading edge, and it has been linked with carcinoma invasion. A pathway involving phosphatidylinositol 3-kinase is essential for this process; on page 1749 O'Connor et al. find that a parallel pathway that leads to phosphodiesterase activation (and thus lowering of cAMP levels) is also required.

Breast carcinoma cells that lack functional β4 will move towards conditioned media or lysophosphatidic acid (LPA), but the rate of chemotaxis is greatly increased if the cells have an intact β4 gene. The α6β4 integrin is being used for signaling not adhesion: of the adhesion-blocking antibodies it is anti-β1 that blocks all cell movement.

Signaling by α6β4 decreases the inhibition of LPA-induced chemotaxis by cAMP. Forskolin treatment (which raises cAMP levels) inhibits LPA-induced chemotaxis, but cells expressing α6β4 are resistant to this effect of forskolin treatment because they induce phosphodiesterase activity. Exogenous phosphodiesterase inhibitors have the opposite effect and can inhibit invasion into Matrigel.

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