PINChing together integrins and signaling

Integrin-mediated adhesion and intracellular signaling by JNK are both required for proper cell migration, but it isn’t clear what factors integrate the two information systems. Kadrmas et al. show on page 1019 that the integrin effector protein PINCH provides such a link during Drosophila dorsal closure, which involves coordinated migration and adhesion of epithelial sheets.

PINCH interacts with integrin-linked kinase and with Nck2, which is involved in regulation of JNK pathway through MAP4 kinase, making it a likely candidate for bridging the two information sources. Using a series of maternal and zygotic mutants, the team found that PINCH was required for proper dorsal closure and that it acted as a negative regulator of the JNK cascade. Moreover, Kadrmas et al. affinity purified a novel PINCH partner RSU-1, a previously identified suppressor of ras-mediated transformation in mammalian cells. PINCH and RSU-1 stabilized one another and were required for proper JNK activity.

Exactly where in the process of dorsal closure PINCH and RSU-1 are required is the subject of the group’s future work. But the current study does imply that integrin-associated junctional complexes may act as signal coordination points, and that JNK signaling must be finely tuned for proper zipping of the epithelial sheet. JCB

Clustering in integrin binding

The adhesiveness of integrin binding is determined by both the affinity of individual heterodimers for their ligand and the clustering of multiple heterodimers. Kim et al. show, on page 1241, that clustering does not precede binding but rather functions in adhesion strengthening following binding to multivalent ligands.

To probe the question of whether clustering or affinity are primary factors in leukocyte integrin binding, Kim et al. devised a FRET system in which either the α or β subunits of the integrin LFA-1 (αβ2) were labeled with non-dimerizing forms of YFP and CFP. Under basal conditions, no micro-clustering was evident, as cells expressing αL-mCYP, αL-mYFP, and wild-type β2 showed little FRET. Nor did the researchers observe FRET when cells were stimulated to activate LFA-1 adhesiveness. Furthermore, activation of LFA-1 with cytoskeletal-disrupting agents did not induce microclusters or macroclusters, visible by confocal microscopy, in the absence of multivalent ligands.

However, addition of multivalent (but not monovalent) ICAM-1 ligand to stimulated cells induced FRET. Macro- and microclusters did form when cells expressing both the ligand and the integrin heterodimer were cultured together in a manner that stimulated aggregation.

Kim et al. conclude that clustering is involved in strengthening the adhesion force, after initial binding of a multivalent ligand. They hypothesize that conformational changes within the integrin heterodimer—which they were able to confirm with intramolecular FRET—is the key factor in activating integrin adhesiveness. JCB

Measuring insulin

Pancreatic β cells that exocytose insulin must make more insulin. Trajkovski et al., on page 1063, now demonstrate that the signal to induce insulin synthesis after exocytosis comes from the cleavage of the cytoplasmic domain of islet cell autoantigen 512 (ICA512). This protein fragment migrates to the nucleus and drives changes in gene expression.

Previous work showed that ICA512 is a transmembrane protein associated with insulin-containing secretory granules of β cells and that exocytosis of the organelles leads to the insertion of ICA512 into the plasma membrane. Now, using a combination of antibodies and GFP-labeled ICA512 proteins, the team find that once ICA512 is inserted into the plasma membrane, its cytoplasmic tail is cleaved by Ca2+-activated μ-calpain. The liberated cytoplasmic domain moves to the nucleus where it binds PIASy, which is known to regulate various transcription factors.

Transient transfection of the cytoplasmic domain of ICA512 was sufficient to induce expression of insulin mRNA. Furthermore, when Trajkovski et al. blocked calpain activity with either calpeptin or RNAi, insulin expression was not induced, indicating that cleavage of ICA512 was required to induce gene transcription.

Putting these data together, the team proposes that Ca2+ acts a dual trigger, coupling the release of insulin with a feedback signal that modulates gene expression in proportion to the secretory activity of the cell. Because ICA512 is found on secretory granules in virtually all neuroendocrine cells, the researchers think the feedback system may be involved in a wide variety of hormone and neuropeptide biosynthesis and secretion systems. JCB
Targeting the inner nuclear membrane

Proteins destined for the inner nuclear membrane (INM) start out in the peripheral ER. Diffusion from the ER will get them to the contiguous outer nuclear membrane (ONM), but the next step could involve either vesicular transport, short-lived fusions between INM and ONM, or movement along the lipid bilayers surrounding nuclear pores. On page 1051, Ohba et al. suggest that the last explanation applies. Moreover, their data suggest that the process can only occur because the nuclear pore complex, which was thought to be a static structure, is constitutively remodeled.

Previous models suggested that INM proteins move through the nuclear pore membrane, but researchers needed a dynamic assay to test the model. By adapting a protein trapping system (Chen et al. 1995. Proc. Natl. Acad. Sci. USA 92: 4947–4951), Ohba et al. were able to watch fluorescently-labeled FRB reporter proteins move between the peripheral ER and the INM. Under normal conditions, the reporter moved between membranes without restriction. However, when the team added rapamycin to the cells, the FRB reporter accumulated in the INM as the reporter bound to an FK binding protein (FKBP) associated with the nuclear lamina. Thus, the rapamycin-mediated interaction trapped the reporter protein in the INM. The team thinks that native INM proteins become similarly trapped when they associate with nuclear structures.

Only proteins whose luminal and cytosolic domains were under 60 kD gained access to the INM, a limitation noted previously. INM localization was dependent on both energy and temperature, which would be consistent with membrane fusion events. However, because the addition of inhibitors of membrane fusion had no effect on localization in the INM, the team hypothesizes that the energy is required for nuclear pore remodeling. Indeed, addition of antibodies against the nuclear pore protein gp210 blocked localization to the INM.

While the pore structure is busy with the remodeling, small integral membrane proteins may slip by, passing into the INM. The team thinks pore remodeling is constitutive because reporter proteins that lacked any native nuclear protein sequence accumulated in the INM in the presence of rapamycin, suggesting that a signal is not required.

Stick hard, signal fast

Cell type specification by Notch (N) in one cell and Delta (Dl) in another must happen quickly, before morphogenesis further changes the cellular landscape. On page 1217 Ahimou et al. find that the adhesion force between N and Dl peaks and drops in just 10 min, symptomatic of a peak and drop in intercellular signaling. Pulling on Dl can accelerate this process.

To measure the in vivo adhesion force between N and Dl, Ahimou et al. attached a Drosophila cell expressing one or the other protein to a cantilever tip of an atomic force microscope (AFM) and placed cells expressing the other protein in a culture dish. When they brought the AFM tip with the cell near those in the culture dish, they found that, unlike many biologically active molecular pairs, there was no attractive force detected between N and Dl. After pushing the cells together, however, it took up to 14 nN of force to detach them, which is 50 to 250 times the force it takes to dislodge streptavidin from biotin.

Binding of Dl to N is known to cause cleavage of the intracellular, signaling domain of N by presenilin. This proceeded at the highest rate when the adhesion forces between the proteins was high. The force required to separate N and Dl expressing cells dropped off after presenilin cleavage and fell to nearly zero in just 10 min, suggesting that the proteins complete their signaling reaction quickly.

Both the brief and rapid signaling, as well as the increased signaling rate induced by Dl pulling, fits with the role of the N-Dl signaling during development, when there are morphogenetic movements and rapid changes in cell identity. Previous work has identified numerous factors that regulate N-Dl signaling. The team plans to use their new AFM system to decipher the biochemical and biophysical impact of these regulators.