**Tunneling through cells**

S. aureus inactivates RhoA to open tunnels through endothelial cells, report Boyer et al. (page 809). These “macroapertures” may allow the pathogenic bacteria easy access to the endothelial basement membranes for tissue invasion and colonization.

Numerous bacterial virulence factors, including EDIN, target proteins in the Rho GTPase family, deregulating the actin cytoskeleton and changing cell shape and adhesion. EDIN is an ADP–ribosyltransferase that locks RhoA in an inactive state.

Boyer et al. found that when endothelial cells in culture or in rat arteries were exposed to S. aureus expressing EDIN or to recombinant EDIN protein, macroapertures formed in a dose- and time-dependent manner. The timing and number of macroapertures correlated with RhoA–ADP–ribosylation.

Without active RhoA, the actin cytoskeleton was rearranged with a loss of stress fibers. The macroapertures appeared as a result of retraction of the membrane and were not associated with tears or wounds in the membrane.

Once a macroaperture formed, the cell appeared to detect the problem. A dense meshwork of actin encircled the opening and lamellipodium-like structures formed at the edges leading to closure. Therefore, the openings only lasted a few minutes, but that is more than enough time for bacteria to access the basement membrane.

Researchers reported recently that as leukocytes move from the bloodstream to surrounding tissues they can induce similar openings in endothelial cells in a Rho-dependent manner. Pathogenic bacteria may have co-opted this system for more nefarious purposes. JCB

**Rab proteins move integrins**

Proper trafficking of integrins is important for cell adhesion and migration, but the machinery involved has been unknown. On page 767, Pellinen et al. report that small GTPase Rab proteins that are known to be important for endocytosis and exocytosis associate with integrins and facilitate their internalization and recycling.

In epithelial cancer cells, β1-containing integrin heterodimers associated with Rab21. Rab21 expression triggered localization of active β1 integrin and Rab21 to large vesicles, consistent with Rab21 being an early endosomal protein. A large fraction of the integrin rapidly returned to the cell surface.

Cells that overexpressed Rab21 attached to the substrate more efficiently than did wild-type cells, whereas cells treated with Rab21 siRNAs had less affinity for the substrate and migrated less efficiently in a wounding assay.

As Rab 21 did not alter the amount of integrins in the cell, the team hypothesizes that it affects attachment and migration by increasing integrin recycling to newly formed sites of attachment. The researchers hypothesize that the integrins are stripped of their ligands in the vesicle and thus readied to return to the surface to bind new substrate. JCB

**It takes two to regulate**

Two isoforms of the IxB inhibitor of NF-κB are required to turn oscillation into steady regulation during chronic stimulation, according to Kearns et al. (page 659). The use of two out-of-phase regulators may be a common means to control signaling pathways.

NF-κB activation triggers expression of IxBα, which leads to down-regulation of the signaling pathway and a decrease in IxBα transcription. However, under chronic stimulation the NF-κB signaling pathway becomes reactivated as soon as the amount of IxBα drops below a certain level. Thus, in cells engineered so that IxBα is the only IxB isoform present, NF-κB activity oscillates over many cycles. In unmodified cells, however, NF-κB activity is steady, and computational modeling suggested the existence of an active damping mechanism that limits fluctuation.

Kearns et al. found that IxBε expression was also induced by NF-κB. There was, though, a significant delay in its expression relative to IxBα. Mathematical modeling and cell experiments showed that, with the two regulators out of phase due to IxBε’s lag, NF-κB expression was dampened to a steady half-maximal level in chronically stimulated cells after an initial peak.

A recent report showed that two signals that trigger NF-κB activity also induce oscillation individually but lead to an even activity level when combined (Covert et al. 2005 Science. 309:1854-7). Thus, Kearns et al. speculate that this sort of regulatory mechanism may be a way for cells to modulate the level of activity of a signaling pathway, rather than being limited to simple on/off switches. JCB