Fusion promoter or fusion inhibitor?

Mononuclear phagocytes can fuse to form osteoclasts or multinuclear giant cells. The latter are hallmarks of Crohn’s disease, granulomas, tumors, and fungal and HIV infections. Though the precise function of these syncytia remains unclear, Takeda et al. (page 945) now provide important new insights into phagocyte fusion, suggesting that the process may differ considerably from other types of cell fusion.

The authors designed a reporter protein that immediately indicates changes in PKC activity. Phosphorylation or dephosphorylation of the reporter causes a change in fluorescence resonance energy transfer. When tethered to the membranes of appropriately stimulated cells, the reporter is phosphorylated and dephosphorylated in sustained oscillations, in step with waves of calcium release. Similar reporter proteins, designed to monitor other signaling parameters, demonstrate that the oscillations can be driven by calcium waves alone, or by concurrent waves of calcium and diacylglycerol.

The rapid oscillations suggest that phosphatases act like brakes that are always on: when PKC activity decreases, dephosphorylation immediately predominates. By directing the reporter to specific intracellular sites, the authors can now ask whether PKC is active when anchored to nonmembrane sites by scaffold proteins, and determine the dynamics of phosphorylation and dephosphorylation at these sites.

Catching a kinase in the act

Using a cleverly designed set of molecular probes, Violin et al., reporting on page 899, provide the first real-time analysis of phosphorylation and dephosphorylation downstream of protein kinase C (PKC). The results provide a striking demonstration of the importance of phosphatases in PKC signaling. When second messenger concentrations decrease, PKC interaction with the membrane loosens, PKC activity is rapidly lost, and the activity probe is quickly dephosphorylated.

The authors designed a reporter protein that immediately indicates changes in PKC activity. Phosphorylation or dephosphorylation of the reporter causes a change in fluorescence resonance energy transfer. When tethered to the membranes of appropriately stimulated cells, the reporter is phosphorylated and dephosphorylated in sustained oscillations, in step with waves of calcium release. Similar reporter proteins, designed to monitor other signaling parameters, demonstrate that the oscillations can be driven by calcium waves alone, or by concurrent waves of calcium and diacylglycerol.

The rapid oscillations suggest that phosphatases act like brakes that are always on: when PKC activity decreases, dephosphorylation immediately predominates. By directing the reporter to specific intracellular sites, the authors can now ask whether PKC is active when anchored to nonmembrane sites by scaffold proteins, and determine the dynamics of phosphorylation and dephosphorylation at these sites.