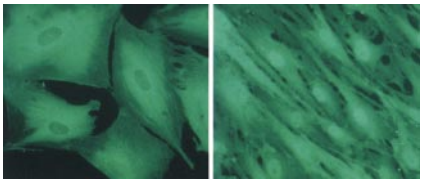
A Contact-Inhibition Signal

On page 1193, Sakaguchi et al. identify S100C as a protein that is downregulated in immortalized cells. But their final conclusion has far greater significance: S100C may be the elusive contact-inhibition signal.



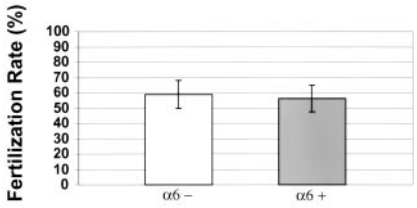
In semiconfluent cells S100C is cytoplasmic, interacts with actin filaments and, when overexpressed, induces the formation of actin-rich membrane protrusions. Expression levels of S100C increase with approaching confluence. In confluent normal cells, but not in confluent immortalized cells, threonine 10 of S100C is phosphorylated, inducing translocation of S100C into the nucleus.

Inhibition of S100C with injected antibodies causes confluent quiescent cells to resume DNA synthesis. The opposite treatment brings the opposite result: forced nuclear entry of S100C in HeLa cells (achieved with a nuclear localization sequence) turns on the cyclin-dependent kinase (cdk) inhibitors p16 and p21 and inhibits DNA synthesis. Thus, S100C is an excellent candidate for the signal that first detects the onset of confluence at adhesion sites before translocating to the nucleus and halting cell division.

An Integrin Not Needed for Fertilization

GoH3, an antibody against the integrin α6β1, can block the binding and fusion of mouse sperm to mouse eggs. But on page 1289, Miller et al. report that α6β1 is not necessary for fertilization, based on the normal sperm binding to and fertilization of eggs lacking the gene for α6.

Egg isolation often starts with removal of surrounding cumulus cells. But this can disrupt the egg's cortical granules, thus modifying the surrounding zona (a web of extracellular matrix) and preventing sperm penetration. For this reason, and to allow a better look at the fusion process, many researchers use chymotrypsin to prepare zona-free eggs for fusion studies. Unfortunately, it seems that chymotrypsin can modify proteins on the egg surface such that GoH3 now inhibits sperm fusion. Other workers recently found that GoH3 penetrates to the surface of zona-intact eggs without blocking sperm fusion, but this could always be dismissed as arising from technical difficulties or a temperamental assay. Miller et al. settle the question by developing a method for culturing eggs from α6 mutant mice (which die soon after birth). If α6β1 does have a role in fertilization, these findings would indicate that its function is redundant with that of other binding molecules.

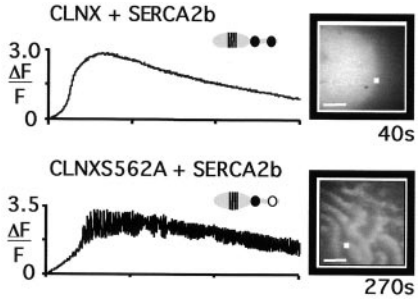


The remaining fusion candidate is the tetraspanin CD9. Its role has been confirmed in a knockout mouse, but it probably works with another protein, possibly an integrin. The lack of α6β1 (which interacts with CD9) in the Miller et al. knockout mouse will make it easier to look for CD9's partner by coimmunoprecipitation.

On page 1171, Snyder et al. call into question a presumed role for another protein. Pex19p's interactions with multiple peroxisomal membrane proteins (PMPs) led to the idea that it was a cytosolic receptor for proteins bound for the peroxisomal membrane. However, Snyder et al. find that the PMPs' motifs for binding to Pex19p and targeting to the peroxisome are often distinct, and that binding to Pex19p takes place in the peroxisomal membrane. Pex19p may regulate the association and dissociation of various Pex protein complexes in the peroxisomal membrane.

Calcium Wave Regulation

When sustained signaling by calcium is needed, but the toxicity of long-term calcium exposure must be avoided, the cell's solution is calcium oscillations. On page 1235, Roderick et al. identify the transmembrane chaperone calnexin as a protein that balances the needs of the cytoplasm with the needs of the endoplasmic reticulum (ER) in regulating these oscillations.

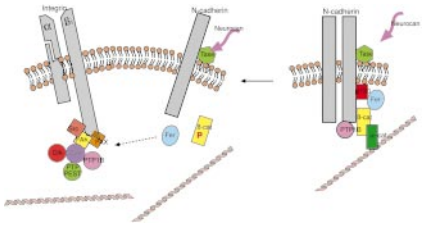


Roderick et al. find that calcium mobilization leads to the dephosphorylation of a serine in calnexin's cytosolic domain. The dephosphorylated calnexin no longer interacts with the calcium-uptake pump SERCA2b, which is therefore free to refill the ER so that protein folding, which requires calcium in the ER lumen, can proceed normally. This cycle explains why overexpressed calnexin inhibits oscillations (by binding SERCA2b) only when the critical serine is not mutated to alanine. The phosphatase acting on calnexin has yet to be identified, although the calcium-sensitive phosphatase calcineurin is a good candidate.

Cadherin–Integrin Coordination

Neurons faced with multiple attractants and adherent substrates need coordinated guidance. On pages 1275 and 1263, Li et al. and Arregui et al. explain one case in which cadherin-

and integrin-mediated adhesion and neurite extension are coordinately downregulated.



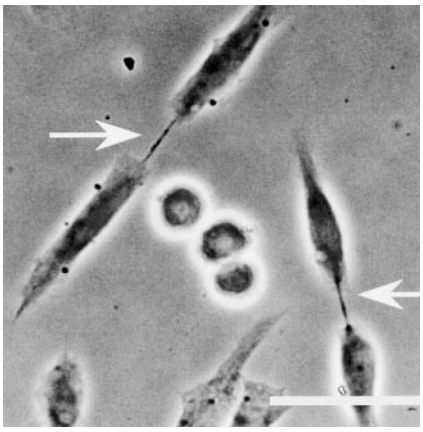
Li et al. identify the extracellular ligand for this downregulation as the proteoglycan neurocan, which binds to the cell surface glycosyl transferase GalNacPTase. Somehow this binding leads to changes in the cadherin complex: loss of the Fer kinase and (possibly as an indirect result) increased phosphorylation of β-catenin. Phosphorylated β-catenin, and thus, cadherin's link to the actin cytoskeleton, is then lost.

Displaced Fer binds to the β1 integrin complex, coincident with the loss of integrin-mediated adhesion. This sequence of Fer displacement and transfer is recreated by Arregui et al. using a peptide that mimics the juxtamembrane region of cadherin. Thus, the loss and then gain of Fer may coordinately turn off two kinds of adhesion to steer neurites away from permissive substrates. This appears to be the case in the developing eye, where Li et al. note that a region with neurocan-positive cells is sandwiched by cells expressing the receptor GalNacPTase.

Lipids in Cytokinesis

Scarce signaling lipids are well known, but on page 1215 Emoto and Umeda gather more evidence that the location of the common lipid phosphatidylethanolamine (PE) is a vital signal in the termination of cytokinesis.

PE is located mainly in the inner leaflet of the membrane bilayer, but towards the end of cytokinesis it concentrates in the outer leaflet near the cleavage furrow. Previously, Umeda's group has shown that a PE-binding peptide can block disassembly of the contractile ring; they now find that this effect is reversible, and can be mimicked by depletion of PE in a cell



line mutant for PE synthesis. The latter result allays concerns that the peptide may have been causing an effect not related to PE. PE may be bending the membrane to form a structure more amenable to fusion, or organizing a protein that interacts with the underlying cytoskeleton.

By William A. Wells, 1095 Market St. #516, San Francisco, CA 94103. E-mail: well@biotext.com