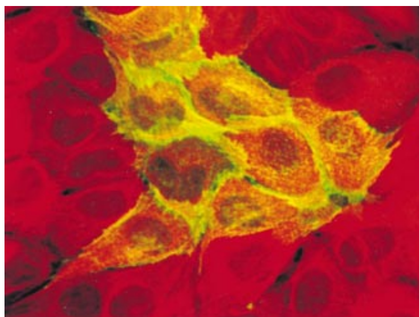
Plakoglobin Helps Cells Come Unglued

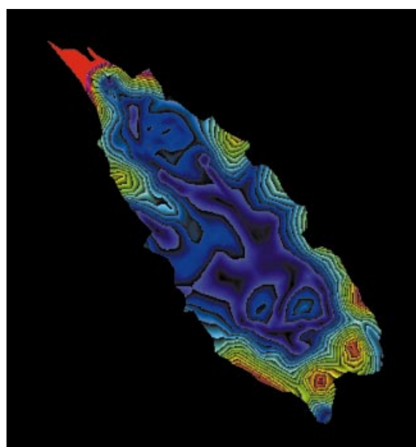
Antibodies against the adhesion molecule desmoglein 3 (Dsg 3) are sufficient to induce the characteristic lesions of pemphigus vulgaris (PV), a severe autoimmune disease that causes skin and mucous membrane blistering. Caldelari et al. (page 823) have now developed an in vitro system to study PV, and have found that plakoglobin is required to mediate the keratin retraction and loss of cellular adhesion caused by anti–Dsg 3 (PV IgG) antibodies. The findings contradict one of the two major models of PV pathogenesis, and also indicate that plakoglobin is required for maintaining normal tissue architecture.



The simplest explanation for the action of PV IgG is that antibody binding sterically hinders Dsg 3–mediated cell adhesion, leading to the formation of blisters. But the antibodies also interfere with intracellular events, so it remained unclear whether PV blister formation was caused solely by steric hindrance, or whether other signaling molecules were required. In the new work, the authors established keratinocyte cultures from the epidermal tissues of plakoglobin knockout (PG −/−) or wild-type (PG +/+) mice. During calcium-induced differentiation, both cell types exhibit desmosomal cadherin-mediated adhesion, but they differ in their responses to PV IgG: PG +/+ cells, but not PG −/− cells, exhibit keratin retraction and loss of adhesion, indicating that plakoglobin is required for PV IgG-mediated adhesion loss. Having determined that PV IgG does not act solely through steric hindrance, the authors plan to use the in vitro system to dissect the plakoglobin-dependent signaling pathway in PV pathogenesis and healthy epidermis.

Younger Focal Adhesions Pull Harder

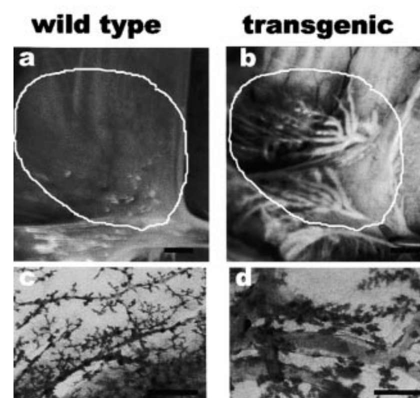
In results that appear to differ from those in another recent report (Balaban et al., *Nat. Cell Biol.* 3:466–472), Beningo et al. (page 881) found an inverse relationship between the size of focal adhesions and the propulsive forces per unit area that they generate during cell migration. The discrepancies between the two reports probably arise from differences in the cells and focal adhesions studied; Balaban and colleagues focused primarily on mature focal adhesions in stationary cells, while Beningo and colleagues examined nascent focal adhesions in migrating cells. The work provides significant new insights into the mechanism of cell migration.



Beningo and colleagues combined GFP imaging with recently developed techniques for analyzing traction forces in order to map both focal adhesions under migrating fibroblasts and traction forces exerted by the cells, at 1-min temporal resolution. In contrast to Balaban and colleagues, who found that focal adhesions of apparently stationary cells exert a constant force per unit area, the new results suggest that small, nascent focal adhesions at the leading edge of a migrating cell provide strong transient forces that move the cell forward. Large, mature focal adhesions, in contrast, exert less force and may serve primarily to anchor the cell and maintain its spread morphology. Beningo and colleagues propose that the recruitment of cytoskeletal components to nascent focal adhesions causes a pulse of propulsive traction force, and that the plaques then either disassemble or mature into larger focal adhesions that act as passive anchors.

Mammary Morphogenesis

In studying the mechanism by which epimorphin regulates morphogenesis in the mammary gland, Hirai et al. (page 785) have also gained significant insight into the activity of CCAAT/enhancer binding protein β (C/EBPβ), a transcription factor implicated in many developmental processes.



C/EBPβ exists in two antagonistic isoforms: a full-length isoform called LAP and a truncated isoform called LIP. In cultured mammary epithelial cells, epimorphin-triggered luminal morphogenesis correlates with an increase in the relative expression of the LIP isoform of C/EBPβ, and overexpression of LIP was sufficient to induce

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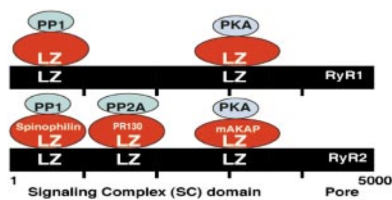
luminal morphogenesis. Overexpression of LAP, in contrast, blocked epimorphin-triggered morphogenesis. Although some previous models of C/EBPβ function have identified LAP as an active isoform and LIP as an inhibitor of LAP, the new data show that the two isoforms have distinct functions and can transduce separate signals.

Epimorphin is encoded by the same gene as the intracellular vesicle targeting protein syntaxin-2. The in vivo localization of epimorphin on the apical surfaces of mammary epithelial cells during the development of the lumina, combined with the presence of truncated but functional epimorphin in mouse milk, demonstrate that the syntaxin gene family products have both intracellular and extracellular functions.

Zipping Up Ion Channels

Providing the first report of what may be a widespread phenomenon, Marx et al. (page 699) demonstrate that leucine/isoleucine zippers (LZ) mediate the targeting of kinases and phosphatases to an ion channel. The work, which focuses on the type-2 ryanodine receptor (RyR2)/Ca^{2+} release channel complex in cardiac muscle



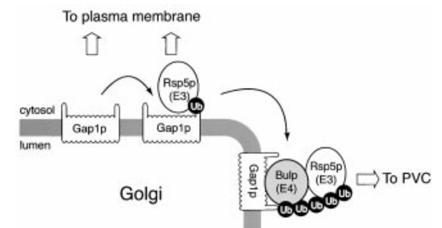
cells, suggests a powerful new approach for the identification of additional ion channel modulators.

Previously, the authors had shown that RyR2 phosphorylation is regulated by kinases and phosphatases that are bound to the channel by targeting proteins, but the identities of the targeting proteins and their mechanism of action remained unknown. Now, Marx and colleagues have identified three LZ motifs on RyR2 that bind to the targeting proteins associated with protein kinase A (PKA) and two phosphatases (PP1 and PP2A). Once bound to the macromolecular complex, the kinase and phosphatases modulate the function of the channel by controlling its phosphorylation state.

Reasoning that the specificity of the LZ motifs would be conserved, the authors then predicted and demonstrated that the RyR1 complex includes PKA and PP1, but not PP2A. Preliminary results suggest that conserved LZ motifs may constitute a general mechanism for targeting kinases and phosphatases to ion channels, which would facilitate the rapid identification and characterization of a wide range of ion channel modulators.

Sorting Out Polyubiquitination

By genetically dissecting the trafficking of the yeast amino acid permease Gap1p, Helliwell et al. (page 649) have determined that polyubiquitination is required as a signal for sorting the protein from the trans-Golgi to the vacuole. Though monoubiquitination was known to act as a signal for endocytosis of membrane proteins, the new work is the first to show a requirement for polyubiquitination in Golgi sorting.



In cells grown on a poor nitrogen source, Gap1p is sorted to the plasma membrane, but when cells are grown on a rich nitrogen source, Gap1p is instead directed to the vacuolar sorting pathway for degradation. In the new work, the authors show that Gap1p must be polyubiquitinated to be sorted to the vacuole, and that the Bul1p and Bul2p proteins, which form a complex with the E3 ubiquitin ligase Rsp5p, are required for this polyubiquitination. Deletion of *bul1* and *bul2* or a mutation in *rsp5* significantly reduces polyubiquitination and causes Gap1p to be redirected to the plasma membrane. Polyubiquitination by an E3 ubiquitin ligase, defined as E4 activity, has previously been described only in vitro, but the new findings suggest that the yeast Bul proteins have E4 activity in vivo that targets Gap1p.

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