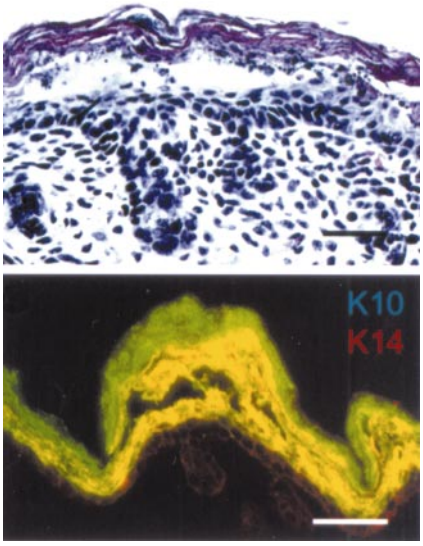
Stem Cells and Mosaic Diseases

Two papers from the same laboratory (Arin et al., page 645 and Cao et al., page 651), describe the use of an inducible gene targeting strategy to develop mouse models for serious human skin diseases. The work has important implications for gene therapy, and also helps to explain why some genetic defects cause a mosaic disease, while others do not. In mosaicism, genetically distinct populations of cells arise from the same lineage in a single tissue; the mechanistic differences between mosaic and nonmosaic genetic diseases are poorly understood.

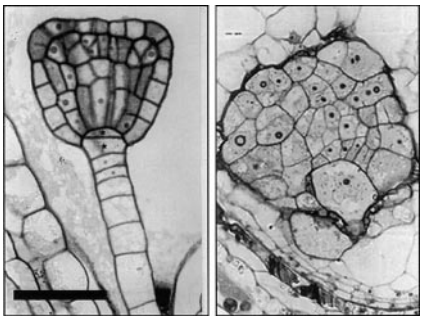


Arin et al. developed an inducible mouse model for epidermolytic hyperkeratosis (EHK), an autosomal-dominant skin blistering disorder caused by mutations in keratin K1 or K10. When a topical inducer is applied to patches of skin on these mice, the activation of a recombinase produces a local population of mutant epidermal stem cells, leading to persistent EHK lesions characteristic of a mosaic form of the disease. In this system, mutant and wild-type stem cells can coexist in the basal layer of the epidermis.

Cao et al. focused on the Dowling-Meara variant of epidermolysis bullosa (EBS-DM), an autosomal-dominant blistering disease that does not have a mosaic form. In Cao et al.'s inducible mouse model for this disease, topical application of an inducer results in EBS phenotypes in the treated areas, but the induced blisters heal rapidly as nonphenotypic stem cells migrate into the wound. Cao et al. speculate that the mutations responsible for EBS place stem cells at a selective disadvantage relative to wild-type stem cells, precluding the development of a mosaic disease and suggesting that substantially different gene therapy strategies will be required to treat EBS and EHK.

A Novel Sec1 Gene in Cytokinesis

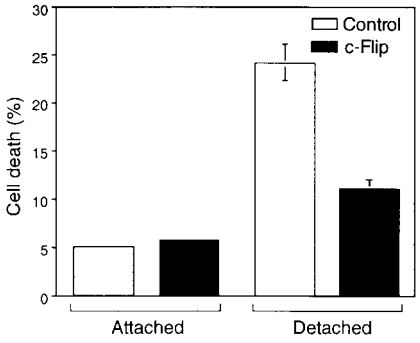
Assaad et al. (page 531) positionally cloned *KEULE*, a gene required for cytokinesis in *Arabidopsis thaliana*, and determined that it encodes a Sec1 protein that interacts with a vesicle-trafficking syntaxin. Sec1 proteins and syntaxins have been shown to interact during vesicle trafficking in a variety of systems, but the new work suggests a novel linkage between this interaction and the cell cycle.



After cloning the gene and identifying it as a putative Sec1 homologue, Assaad et al. determined that KEULE exists in both soluble and membrane-associated forms, and that it binds to the cytokinesis-specific syntaxin KNOLLE in vitro, indicating that it is also functionally homologous to a Sec1 superfamily protein. When wild-type plants are transformed with a *KEULE*–GFP gene fusion, cytokinesis-defective mutant sectors are observed in all somatic tissues, indicating that KEULE is required for cytokinesis throughout the plant life cycle. Database searches of the *Arabidopsis* genome revealed two highly conserved homologues of *KEULE*. Because Sec1 proteins are key regulators of vesicle trafficking, Assaad et al. propose that KEULE may act through KNOLLE to integrate cell cycle signals and transduce them to the cytokinetic vesicle fusion machinery.

How the Extracellular Matrix Regulates Anoikis

Detachment from the extracellular matrix induces apoptosis in endothelial cells, but the molecular mechanisms controlling this biologically important phenomenon, called anoikis, have remained poorly understood. Beginning on page 633, Aoudjit and Vuori report that matrix attachment regulates Fas-mediated apoptosis by modulating the expression levels of the death receptor Fas and the caspase-8 antagonist c-Flip. The findings have important implications both for developing new antiangiogenic tumor therapies and for understanding the pathogenesis of vascular diseases.



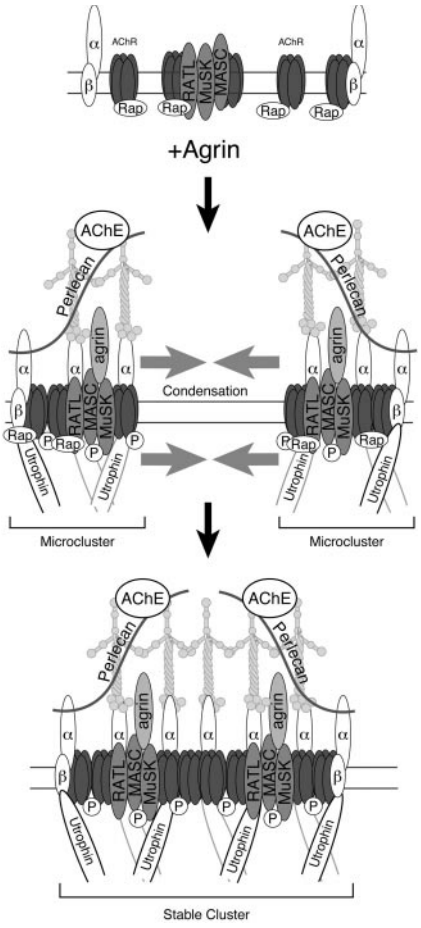
Reasoning that matrix attachment might affect endothelial cell survival by regulating Fas-mediated killing, Aoudjit and Vuori examined whether anoikis is linked to activation of the Fas pathway. They found that even when the Fas receptor is expressed on the cell surface, adherent endothelial cells resist Fas-mediated apoptosis. Matrix attachment modulates apoptosis at two levels, controlling the availability of Fas by regulating the receptor's expression, and controlling receptor-induced activation of caspase-8 by regulating the expression of c-Flip. Detachment from the extracel-

lular matrix enhances Fas expression and inhibits c-Flip expression, activating the Fas/Fas-L death pathway and leading to apoptosis. Because of the importance of caspase-8 in other receptor-induced apoptotic pathways, Aoudjit and Vuori suggest that matrix attachment may regulate apoptosis in a similar fashion in other cell types.

Dystroglycans in Acetylcholine Receptor Clustering

Using both a chimeric mouse model and isolated myotubes generated from embryonic stem cells, Jacobson et al. (page 435) have gained significant new insights into the role of dystroglycans in the organization of the neuromuscular junction (NMJ). The dystroglycans comprise two subunits of a complex of dystrophin associated proteins (DAP), linking the cytoskeleton in a myofiber to its basement membrane in a myofiber. Though the DAP complex was thought to be involved in the formation of nerve–muscle synapses, the mechanisms regulating this process have remained poorly understood. In the new work, Jacobson et al. examined the clustering of acetylcholine receptors (AChR) and basement membrane assembly. Muscle from dystroglycan-deficient mice, and myotubes generated from dystroglycan null ES cells, both develop AChR clusters that are larger, less dense, and less stable than the AChR clusters on their wild-type counterparts. Dystroglycan deficiency also disrupts the association of laminin, perlecan, and acetylcholinesterase (AChE) to the AChR clusters, but does not affect the localization of rapsyn or agrin to the clusters.

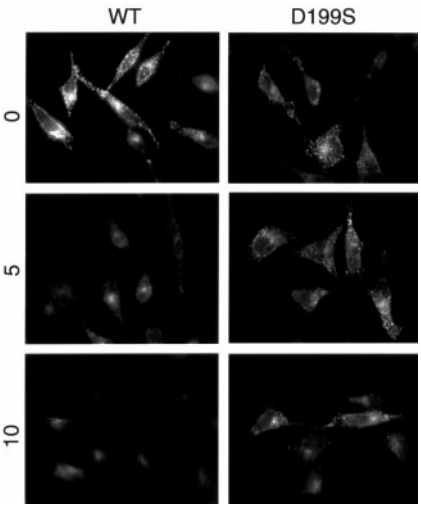
Jacobson et al. previously have shown that dystroglycans cluster under the nerve during synapse formation, and they now propose that this clustering is essential for the proper assembly of the postsynaptic specialization. In this model, dystroglycans



stabilize AChR clusters through their interactions with laminin extracellularly and utrophin intracellularly, while AChE is assembled into the synaptic basement membrane through its binding to perlecan.

Hsc70 Regulates Clathrin-coated Vesicle Disassembly

Newmyer and Schmid (page 607) have demonstrated that the heat shock cognate protein hsc70 not only regulates coat disassembly in the clathrin coated vesicle (CCV) cycle, but also appears to have a broader role in modulating clathrin dynamics throughout the CCV cycle. In addition to confirming another activity of hsc70, the work paves the way for a more complete understanding of the mechanisms controlling cytosolic clathrin maintenance and the formation of coated vesicles.



Previous studies have implicated hsc70 as a CCV cycle component, but in the new work, Newmyer and Schmid directly examined the effect of hsc70 mutations on clathrin dynamics in vivo. When ATPase-deficient forms of hsc70 are overexpressed in cultured cells, CCV uncoating is inhibited and unassembled cytosolic clathrin shifts to an assembled pool, indicating that hsc70 is involved in regulating clathrin disassembly. Since the assembled coat proteins accumulate in the absence of cargo receptors, Newmyer and Schmid suggest that this pool may represent misassembled empty clathrin cages. Overexpressing the mutant forms of hsc70 also blocks the recycling of the transferrin receptor. Based on their results, Newmyer and Schmid propose that hsc70 may act in endosomal trafficking as a chaperone for cytosolic clathrin. In this model, hsc70 might regulate multiple steps in the CCV cycle by preventing clathrin from inappropriately self-assembling.

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