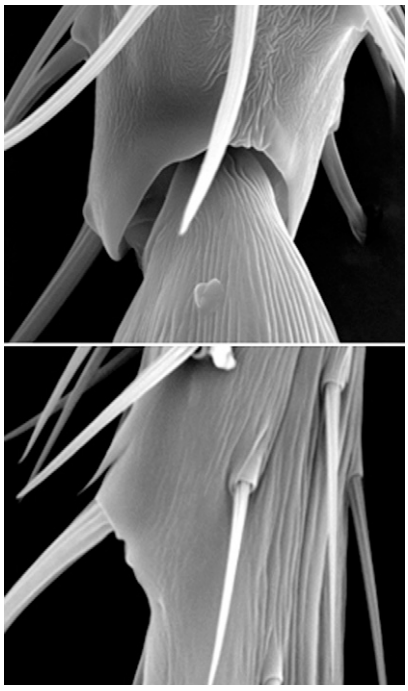
Research Roundup



SUZANNE/MACMILLAN

Apoptosis-defective flies (bottom) lack a well-defined leg joint.

Dying cells make a joint

A leg that doesn't bend is not much use. To make space for joints in the fruit fly limb, a thin band of cells are carved out by apoptosis, according to a new study by Cristina Manjón, Ernesto Sánchez-Herrero, and Magali Suzanne (Autonomous University of Madrid, Spain). The team shows that a sharp switch in morphogen signaling determines which cells get eliminated.

The morphogen in question is decapentaplegic (Dpp). In developing flies, it defines where and in which direction the legs should grow. Manjon et al. now find that, later on, Dpp initiates the formation of joints in the distal segments of the fly leg.

Across the would-be joints, Dpp signaling activity abruptly switches from high to low. At this transition point, a narrow band of cells undergoes apoptosis. Mutants that lack the signaling boundary due to either uniformly high or uniformly low Dpp activity fail to induce apoptosis and do not form bendable joints. Thus it is not the amount of Dpp protein but the switch in Dpp signaling itself which activates cell death.

Although the fly leg has eight joints, the five furthest from the body are the only ones where Dpp gradient-dependent apoptosis seems to be necessary for flexibility. Suzanne speculates that joint formation in the early segments may depend on alternate pathways, just as different gene expression programs drive the formation of different leg sections.

The group is now trying to decipher how the Dpp signal is suddenly shut off and how the transition between high and low Dpp is sensed by the apoptosis pathway. They propose that an interaction between cells on either side of this boundary, perhaps through a transmembrane receptor, might initiate apoptosis. JCB

Reference: Manjon, C., et al. 2006. *Nat. Cell Biol.* doi:10.1038/ncb1518.

Stretchable force sensors

Cells in the body somehow sense that they are continually being pushed and pulled. Yasuhiro Sawada, Michael Sheetz (Columbia University, New York, NY), and colleagues now reveal that cellular tension causes a normally coiled cytoskeletal protein to stretch out and, in so doing, communicate the force downstream. The resulting signals help to direct cells toward areas of lower tension during wound healing and development.

Sheetz's group previously showed that stretching the cell's entire cytoskeleton triggered phosphorylation of a cytoskeletal protein called p130Cas, which is found at cell–matrix contact sites. The inference was that p130Cas itself was stretched and that this stretching was required for its phosphorylation, but formal proof was lacking.

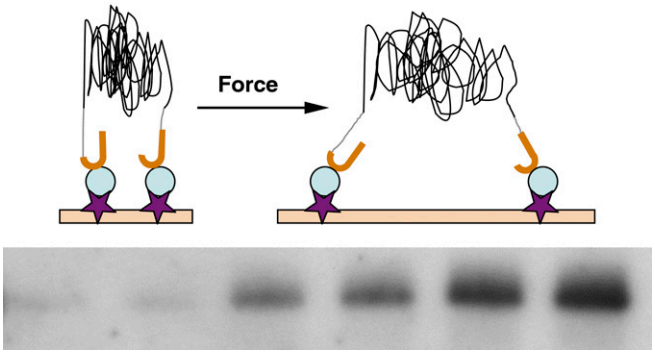
So the group set up a test system with a modified p130Cas. Biotinylation of both ends of p130Cas allowed it to be attached to and stretched by an avidin-coated latex sheet. Stretching was confirmed based on a loss of YFP fluorescence, as the YFP was split between the two ends of p130Cas.

Stretching the latex caused p130Cas to extend and become phosphorylated, presumably because its phosphorylation sites were now exposed. More vigorous stretching of the membrane led to more (and distinct) residues being phosphorylated. These phosphorylated residues allow docking of proteins that turn on the Rap1/MAPK pathway, and having more of them may increase how far the cell spreads in a given direction.

In vivo, the team used antibodies that recognized either the phosphorylated or stretched forms of p130Cas. As they saw in vitro, p130Cas stretching and phosphorylation were correlated. Both antibodies localized specifically to the cell periphery, the region that absorbs most of the pulling in living, moving cells.

The change in p130Cas conformation effectively converts a mechanical stimulus into a biochemical signal. Sawada says this could be a general mechanism for cell signaling and that other cytoskeletal proteins might transduce signals in a similar way. The results suggest that the cytoskeleton is not just a cellular scaffold but also a force sensing radar for the cell. JCB

Reference: Sawada, Y., et al. 2006. *Cell.* 127:1015–1026.



SHEETZ/ELSEVIER

Stretching of p130Cas (top) increases its phosphorylation (bottom).

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Shrinking spines

A pathway from the extracellular matrix to the actin cytoskeleton sucks dendritic spines back into the body of the neuron, say Wing-Yu Fu, Yu Chen, Nancy Ip (Hong Kong University of Science and Technology, Hong Kong), and colleagues.

Spines come and go as synapses remodel during memory formation. Extracellular cues for remodeling eventually cause actin reorganization. Ip's group has now pieced together an entire pathway from cell surface receptor to the actin that causes spine retraction.

The pathway starts with ephrin-A1 whose receptor, EphA4, is a known negative regulator of spine formation. The authors show that ephrin-A1–EphA4 interaction activates Cdk5. This kinase then phosphorylates ephexin1, a guanine nucleotide exchange factor for RhoA GTPase, which probably pulls in the spines by activating actomyosin contraction.

Disruption of this pathway via Cdk5 mutation resulted in abnormal spine morphogenesis and an inability to retract spines in response to ephrin-A1.

Ip speculates that Cdk5 may also be involved in regulating EphB-mediated spine protrusion. As spine dynamics ultimately influences the efficiency of synaptic transmission, it is important to understand the precise roles of Cdk5 in spine growth and retraction. JCB

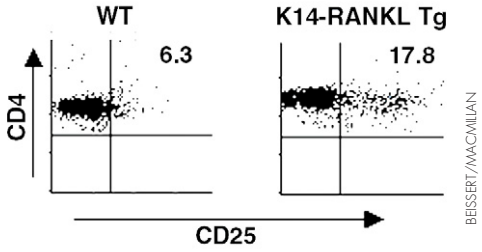
Reference: Fu, W.Y., et al. 2006. *Nat. Neurosci.* doi:10.1038/nn1811.

Skin-based immunity

When skin cells are hit by a blast of UV light, they instruct dendritic cells (DCs) to suppress immunity system-wide, say Karin Loser, Stefan Beissert (University of Münster, Münster, Germany), and colleagues.

UV has the unusual ability to cause immunosuppression by recruiting T regulatory cells (T regs). UV has thus been used to treat autoimmune conditions of the skin, such as psoriasis, but there has been no real understanding of how UV-treated skin manages to attract T regs and eliminate the inflammation.

T reg proliferation and peripheral expansion requires cues from activated mature DCs, which express several receptors including receptor-activated NF-κB (RANK). The authors now show that RANK's ligand, RANKL, is expressed by skin cells (keratinocytes) that have been exposed to UV. The DCs in the UV-treated area are probably activated through their interaction with the keratinocytes, and this helps them recruit T regs.



Immunosuppression results when RANKL (right) triggers production of T regs (right half of panels).

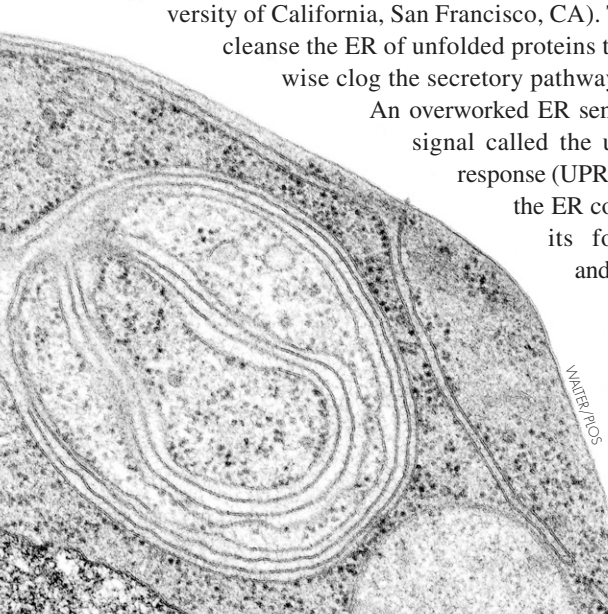
DCs from transgenic mice overexpressing RANKL supported T reg proliferation both in vitro and in vivo. The transgenic mice had 2–3 times as many T regs as normal mice and 6 times as many T regs as mice lacking RANKL. It is not yet clear how RANKL expression specifically attracts benign T regs without also alerting inflammatory $CD8^+$ T cells. Nonetheless, Beissert speculates that a topical RANKL application might provide some relief for patients with inflammatory skin disorders, such as psoriasis or eczema. JCB

Reference: Loser, K., et al. 2006. *Nat. Med.* 12:1372–1379.

Self-eating ERs

When under stress, the ER of yeast cells expands greatly in size but also eats its own membrane stacks to form autophagosome-like structures, say Sebastián Bernales, Kent McDonald, and Peter Walter (University of California, San Francisco, CA). The process may cleanse the ER of unfolded proteins that would otherwise clog the secretory pathway.

An overworked ER sends out a distress signal called the unfolded protein response (UPR). The UPR helps the ER cope by increasing its folding capacity, and jettisoning those proteins that are hopelessly misfolded. The authors found that, under UPR-inducing conditions, most stressed cells had ER networks that are fivefold bigger than normal. But their electron micrographs also revealed many cells containing normal-sized ERs along with autophagosome-like structures packed selectively with ER stacks. The outer membranes of these structures were also derived from the ER, suggesting that the ER could be cannibalizing itself to return to normal size. Thus, says Walter, the ER can not only "counter-balance its expansion but also detoxify to improve the cell's chances of survival".



In stressed cells, expanded ER membranes are cannibalized by ER-derived autophagosomes.

UPR induction of many classical autophagy genes was crucial for cell survival during ER stress. But the sequestration and isolation of unhealthy ER may be more important than its eventual degradation, as cells that lacked a proper degradation system survived under UPR conditions.

It is not yet clear why damaged ER is toxic to cells, or how the ER is labeled for sequestration and packaged so selectively. JCB

Reference: Bernales, S., et al. 2006. *PLoS Biol.* doi:10.1371/journal.pbio.0040423.