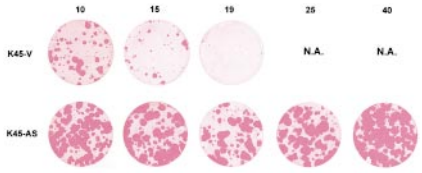
One-Step Immortalization

Human epidermal keratinocytes are born in a basal layer enriched in stem cells but gradually lose their replicative ability as they migrate towards the skin's surface. On page 1117, Dellambra et al. report that the evolution of the keratinocytes from stem to transient amplifying cells can be blocked by downregulating a single gene encoding 14-3-3σ. The new cell line avoids senescence and, thus far, has proven to be immortal. The 14-3-3 family of proteins has been implicated in multiple signal transduction pathways, so the direct biochemical consequence of the intervention is unknown.

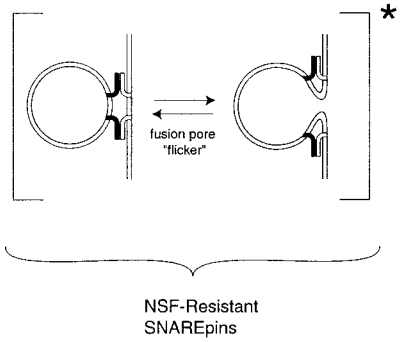


In culture, normal keratinocyte stem cells go through 120–180 cell doublings before they senesce and stop dividing. During this period there is an increase in the number of larger transient amplifying cells, which form paraclones (aborted colonies with only terminally differentiated cells). Addition of an antisense 14-3-3σ construct results, however, in maintenance of small, fast-dividing cells that have thus far undergone ~500 cell doublings with no end in sight. Others have demonstrated similar immortalizations by both turning on telomerase and turning off the cell cycle inhibitor $p16^{INK4a}$, but Dellambra et al. find that the single intervention of antisense 14-3-3σ recapitulates both of these events.

Complete transformation of human cells has been achieved by adding telomerase, SV-40 large T antigen, and oncogenic Ras. The keratinocytes in this study are still anchorage-dependent and require serum for growth, but Dellambra et al. speculate that the addition of a single oncogene such as activated Ras may be sufficient to convert immortalization into transformation. In a recent study, >90% of primary breast carcinomas were shown to have undetectable 14-3-3σ mRNA due to DNA hypermethylation (Ferguson, A.T., E. Evron, C.B. Umbricht, T.K. Pandita, T.A. Chan, H. Hermeking, J.R. Marks, A.R. Lambers, P.A. Futreal, M.R. Stampfer, and S. Sukumar. 2000. *Proc. Natl. Acad. Sci. USA*. 97:6049–6054).

NSF-resistant Fusion

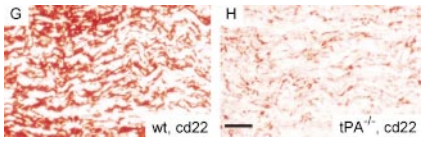
The pairing of a v-SNARE (vesicle SNAP receptor) and a t-SNARE (target membrane SNARE) to form trans-SNARE complexes is necessary for many membrane fusion events. At least for in vitro fusion, the pairing is also sufficient, but whether this sufficiency holds true in vivo is still a matter of contention. Weber et al. (page 1063) remove one objection to the sufficiency argument by explaining how the SNAREs avoid the NSF paradox. NSF recycles SNAREs for additional rounds of fusion by splitting apart SNARE complexes made from proteins in the same membrane (cis-SNARE complexes), but Weber et al. demonstrate that fusion-competent trans-SNARE complexes are somehow resistant to this action of NSF.



NSF can transiently inhibit fusion if it is added at the very beginning of a fusion reaction, probably by dissociating the heterodimeric t-SNARE that is needed to partner with the v-SNARE in the other membrane. If the SNAREs are first allowed to dock at 4°C, however, the subsequent addition of NSF has no effect on fusion at 37°C. The time course for acquiring NSF resistance is similar to the time course for becoming resistant to soluble v-SNARE. Possible explanations for the NSF resistance include steric exclusion of NSF from the closely apposed membranes, or a trans-complex conformation that differs from that of the NSF-sensitive cis-complex. NSF may make fusion vectorial by selectively reacting only with the products of the fusion reaction.

Getting Rid of Fibrin

Too much proteolysis can be a bad thing, but on page 1157, Akassoglou et al. report that at least one proteolytic cascade is beneficial after injury of the peripheral nervous system (PNS).



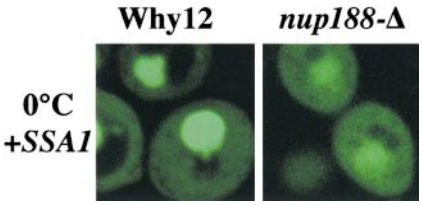
The cascade involves proteolysis of plasminogen by tissue plasminogen activator (tPA) to form plasmin; the plasmin then degrades fibrin, the main protein deposited during the clotting process. Mice that lack tPA or plasminogen show greater axon degeneration after a nerve crush compared to wild-type mice. The increased damage in the mice lacking plasminogen can be rescued by reducing the amount of the fibrin precursor, fibrinogen, either by gene deletion or by administering the pit viper venom Ancrod.

The benefits of tPA in the PNS contrast with its damaging effects during excitotoxic neurodegeneration in the central nervous system (CNS). In the CNS, small amounts of tPA may function to remodel synapses, whereas the larger amounts released during excitotoxicity degrade laminin, thus removing a matrix survival signal for neurons. In the PNS both the substrate and the result are different: tPA is recruited to clear up the mess left when the circulation temporarily encroaches into the nervous system.

Increasing the efficiency of this process with doses of tPA or fibrinogen-reducing medications may help patients suffering from nerve damage or inflammation.

A Channel Opens Wide

On page 1027, Shulga et al. link two budding yeast proteins, called Nup170p and Nup188p, to the elusive nuclear pore complex (NPC) diffusion channel, and suggest that these nucleoporins may also function in NPC gating. The gating mechanism allows facilitated transport of larger proteins through an apparatus that otherwise allows diffusion of only smaller proteins.



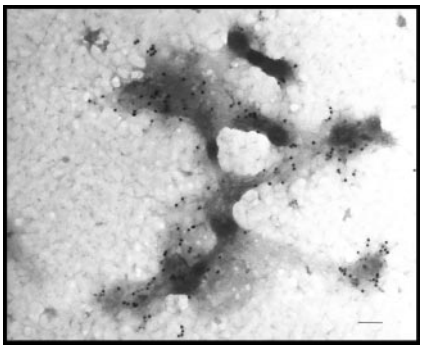
Shulga et al. study passive permeability in strains deleted for 1 of 10 different NPC proteins. At 0°C, only the strains missing Nup170p or Nup188p show passive nucleo-cytoplasmic equilibration of reporter proteins that remain stuck in the nucleus of a wild-type strain. Diffusion into the nucleus is similarly affected. A 36-kD reporter protein shows limited diffusion into the nucleus of a wild-type cell, but larger reporters (of up to 66 kD for nup188-Δ cells and up to 126 kD for nup170-Δ cells) diffuse freely into the nuclei of mutant cells. Signal-directed nuclear transport rates are normal in the mutant cells, suggesting that the NPC is largely intact.

The effect of Nup170p on passive permeability, and the interaction of Nup170p with a facilitated transport docking site, suggest that the diffusion channel and the putative facilitated transporter may be features of the same central structure. If Nup170p and Nup188p can affect the functional diameter of the diffusion channel they may also be involved in gating of the NPC.

Spatial Organization of Signaling

Signaling microdomains in membranes have been studied using detergent extraction, but concerns remain that detergents may be disrupting important interactions or selectively extracting some proteins. Wilson et al. (page 1131) bring the time-tested approach of direct observation to this question. They use immunogold electron microscopy (EM) to delineate a sequence of spatially controlled interactions during mast cell signaling.

Their study subject is the cross-linking of FcεRI, the high-affinity IgE receptor on mast cells. Cross-linking leads to receptor phosphorylation by the Src-related kinase Lyn; Syk kinase then binds to the phosphorylated receptor to continue the signal transduction cascade. Wilson et al. find that both the receptor and Lyn are predominantly in small clusters before activation, with a significant level of interaction between receptor and Lyn clusters. After receptor cross-linking this colocalization of small clusters persists, but most FcεRI is now found in large clusters from which Lyn is excluded. The majority of Syk is intercalated into both the large and small clusters.



Wilson et al. speculate that phosphorylation by Lyn occurs in the small clusters, but then Lyn must be spatially separated from the activated receptor for the signal transduction process to continue. The domains in which subsequent receptor–Syk interactions occur are large enough to have been detected in other studies by light microscopy and detergent extraction, but the spatial organization of the earlier Lyn interaction is revealed here for the first time with the higher resolution of EM.

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