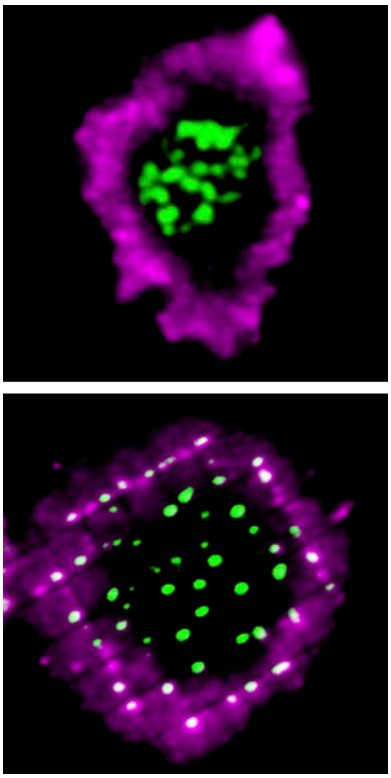
Research Roundup



DUSTIN/AAAS

Phosphotyrosine signaling (purple) remains associated with TCR clusters (green) longer when TCRs are prevented (bottom) from moving inward.

TCRs alive on the periphery

Keeping T cell receptors (TCRs) away from the center of the immunological synapse boosts stimulatory stignals, based on work from Kaspar Mossman, Gabriele Campi, Jay Groves (University of California, Berkeley, CA), and Michael Dustin (New York University, New York, NY).

The immunological synapse—the cell–cell junction between a T cell and antigen-presenting cell (APC)—looks like a bull's eye, with a central cluster of TCRs and their bound antigen–MHC ligands surrounded by a ring of adhesion molecules and their ligands. Active TCR clusters form at the periphery but then move toward the center, where they stop signaling.

To determine whether this change of locale is necessary for TCR shutdown, the group blocked the inward transport. They first replaced the APC with a supported lipid bilayer containing antigen–MHC and an adhesion ligand. They then etched chromium barriers onto the substrate to form variously patterned corrals within which MHC-bound TCRs would be trapped.

TCRs that were stuck in peripheral corrals signaled longer, as measured by their phosphorylation status and ability to elevate cytoplasmic calcium levels. "It's not just a matter of timing," says Dustin. "Location of the TCR clusters is important." It is not clear whether the periphery is a particularly good environment to sustain signaling, the center is a particularly good environment to kill signaling, or both. Positive feedback from dynamic actin in the periphery or negative feedback from centrally located inhibitors, perhaps phosphatases, might be involved.

The group artificially prevented TCR transport, but certain APCs, such as dendritic cells, might have that innate ability. Compared with B cells, dendritic cells are much more potent T cell activators. "So," Dustin wonders, "do they have their own version of these barriers?" Perhaps yes, as at least one report suggested that dendritic cells cause T cells to cluster TCRs in multiple peripheral foci rather than at the typical bull's eye of a B cell. **JCB**

Reference: Mossman, K.D., et al. 2005. *Science.* 310:1191–1193.

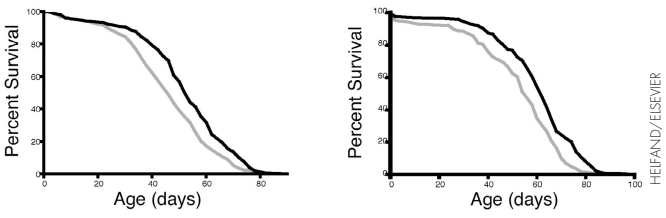
Less p53 for life

New findings from Johannes Bauer, Stephen Helfand (Brown University, Providence, RI), and colleagues show that flies lacking neuronal p53 activity live longer.

Hyperactivation of p53, which kills DNA-damaged cells, reduces tumor incidence in mammals but also shortens their life span. These earlier findings suggested that reducing p53 activity might increase life span. Helfand's group found that this did not pan out for flies lacking all p53—they died earlier than normal, probably because of the requirement for p53 in developmental apoptosis. But if the authors blocked p53 activity only in neurons, the flies lived longer healthy lives and were also more resistant to DNA-damaging agents.

Loss of p53 only in the fat body or muscle tissue did not extend life span. "Maybe," says Helfand, "the nervous system is the weak link. If it goes [via p53-mediated apoptosis, for example], the rest of the body goes." The group does not yet know whether neurons survive longer in the p53 mutants due to less apoptosis, but they provide evidence that traditional caspase-dependent apoptosis is probably not affected. The involvement of caspase-independent apoptosis has not been ruled out.

Another possibility is that the neuronal tinkering causes systemic effects, perhaps via the neuroendocrine system. Indeed, calorie restriction also increases life span in flies, and p53 inhibition seems to lie in this pathway, since both together did not have an additive effect. Calorie restriction has unwanted side effects, however, including reduced fertility and activity levels. Since p53-inhibited flies did not suffer from these problems, the authors have shown that the downstream effects of calorie restriction can be teased apart.



HELFAND/ELSEVIER

Female (left) and male (right) flies lacking (black lines) neuronal p53 live significantly longer.

The p53-inhibited flies might also be expected to have many tumors, but since adult flies are primarily post-mitotic, they are not cancer prone. If only mature human neurons, which are also post-mitotic, were smart enough to rid themselves of p53, perhaps we could all live longer. Says the cautious Helfand, "a judicious decrease in p53 activity might be advantageous." **JCB**

Reference: Bauer, J.H., et al. 2005. *Curr. Biol.* 15:2063–2068.

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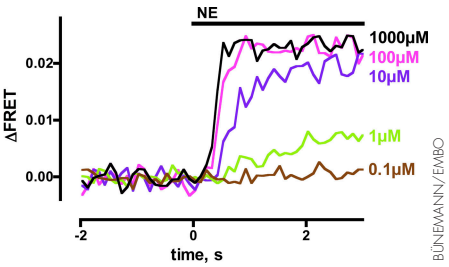
G proteins find partners

In recent years, scientists have identified many complexes containing most or all of the components needed to mount a particular signaling cascade. In contrast, Peter Hein, Moritz Bünemann (University of Würzburg, Germany), and colleagues find that a G protein signaling pathway works just as well, if not better, when receptor and G protein start out apart.

Several groups have hypothesized that speed and specificity of the many different G protein–mediated pathways might be achieved by precoupling the G protein and its receptor, via direct or indirect binding. But the new FRET analyses show that the two are not together until the receptor is activated. Interactions thus depend on random collisions between receptor and G protein. "Lateral diffusion in the membrane is fast enough," says Bünemann.

Kinetic analyses reveal that each interaction between a receptor and G protein lasts only a small fraction of the total time that the receptor and G protein are active. Thus, one receptor can activate many G proteins. This repetition explains why activation of only a very small fraction of the total receptor pool rapidly elicits full activation of its G protein if enough receptors are expressed. Whereas, says Bünemann, "if they were consistently complexed, you'd need a one-to-one ratio of active receptors to G proteins."



BÜNEMANN/EMBO

α_{2A}-adrenergic receptors do not interact with G proteins until the receptor is activated (bar).

The authors are not suggesting that other receptors do not precouple. But at least in this case, any precoupling is more likely to occur between the G protein and its specific effector. JCB

Reference: Hein, P., et al. 2005. *EMBO J.* doi:10.1038/sj.emboj.7600870.

Fragile when condensed

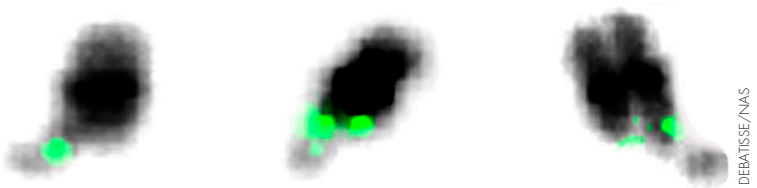
Tumor cells often have chromosomal breaks at conserved sequences known as fragile sites. Now, results from Eliane El Achkar, Michelle Debatisse (Institut Curie, Paris, France), and colleagues suggest that the chromatin can break if it condenses before these fragile sequences are fully replicated.

Fragile sites are especially sensitive to drugs that interfere with S phase. The group found that these same sites are also susceptible to a drug called calyculin A, which induces immediate chromatin condensation. DNA breaks appeared primarily when cells were treated with the drug during S phase or in G2. The further into G2 the drug was added, the fewer breaks were found, suggesting that whatever marks the sites as fragile was progressively corrected before mitosis.

The authors suspect that this "mark" is unreplicated DNA (or replicated DNA that has not yet regained its histone complement). Fragile sites mapped to the transitions between early- and late-replicating sequences, which might might act as barriers to fork progression. As such, fragile sites may be the last sequences replicated (even doing so in G2 rather than S phase) and thus be especially sensitive to premature condensation.

If they are the last to be copied, fragile sites would make ideal spots for G2/M checkpoint proteins to monitor. ATR, for instance, which stabilizes stalled forks, might prevent condensation until fragile sites are replicated. Indeed, fragile sites in cells lacking ATR have been shown to break more often than in normal cells. The authors are now using calyculin A to determine whether ATR sits on unreplicated fragile sites. JCB

Reference: El Achkar, E., et al. 2005. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.0506497102.


DEBATISSE/NAS

Premature condensation causes chromatin to break at fragile sites (green).

History's influence on actin

An imposed load on an actin network leads to exuberant growth when the pressure is released, say Sapun Parekh, Ovijit Chaudhuri, Julie Theriot, and Daniel Fletcher (University of California, Berkeley, CA). Thus, history matters when it comes to actin growth dynamics.

Parekh et al. slowly increased the load pushing against a polymerizing actin network and measured network growth velocity along the way. Velocity remained constant for a while and then slowed as the load approached a stall-inducing maximum.

The authors then returned the system to a lighter load and found that the network grew much faster—even faster than it did previously at this lighter load. A sudden burst of polymerization like that might help a migrating cell push quickly through a weak spot in the surrounding tissue.

To explain how multiple velocities can exist for a given force, Fletcher suggests, "maybe the network is adapting to different loads." Force-mediated activation of Arp2/3, for instance, would increase branching. "With higher load," he explains, "you'd get a net addition in the number of filaments that are pushing the load. When the load is reduced, you now have many more filaments to push the smaller load." Still to be explained, however, is why the higher growth rate under the lightened load was long lasting, i.e., why Arp2/3 activity did not lessen and thereby reduce the number of filaments over time. JCB

Reference: Parekh, S.H., et al. 2005. *Nat. Cell Biol.* 7:1219–1223.