In This Issue

Hiding proteins in the nucleolus

Cells regulate metabolic pathways by rigidly locking enzymes in the nucleolus, report Mekhail et al. on page 733. Two ubiquitin ligase enzymes bind in the nucleolus in response to signals that block their activity.

Ubiquitin tagging alters protein fates, often marking substrates for degradation. Regulation of the process occurs at the level of the ubiquitin ligases, called E3s, which facilitate the transfer of ubiquitin from a conjugating enzyme to the target protein. Several E3 proteins aggregate in the nucleolus in response to inhibitory signals. The new results show that two different E3 enzymes, MDM2 and von Hippel-Lindau tumor suppressor (VHL), become immobilized in the structure and probably bind nucleolar scaffold proteins.

In the presence of oxygen, VHL tags the hypoxia-inducible factor (HIF), causing its degradation. The authors identified a domain within VHL that detects increased acidity, such as occurs during hypoxia, and somehow induces VHL to move to the nucleolus from the cytoplasm and nucleoplasm, where it normally resides.

Although nucleolar proteins involved in ribosome synthesis move in and out of the structure constantly, the authors saw that VHL was static in the nucleolus, based on FRAP, FLIP, and heterokaryon experiments. Upon neutralization of the culture medium, VHL was released from the nucleolus and resumed its dynamic lifestyle.

MDM2, an E3 that induces the degradation of the p53 tumor suppressor protein, also became fixed in the nucleolus in response to actinomycin-D treatment, which is known to block p53 ubiquitylation.

The authors propose that such nucleolar sequestration, and more generally the concept of switching proteins between a mobile and static state, is likely to be a commonly used mechanism for regulating enzyme reactions. The hypothesis is boosted by preliminary experiments, which indicate that numerous proteins have domains similar to the one that directs VHL’s nucleolar targeting.

Immortal DNA

Karpowicz et al. (page 721) report evidence for asymmetric segregation of the oldest DNA during neural stem cell proliferation.

According to the immortal strand hypothesis, which was first proposed in the mid-1970s, stem cells actively retain the oldest DNA during asymmetric cell divisions. That DNA should, statistically speaking, contain fewer replication-induced errors than DNA resulting from more rounds of replication. While tantalizing for its logic, the hypothesis has been controversial, and numerous studies have failed to find evidence for it.

Working with mouse neural stem cells, the authors labeled cells with BrdU and then looked at how the DNA segregated. When populations of cells were labeled, dispersed into single cells, and allowed to proliferate, they gave rise to neurospheres that frequently contained a few BrdU-labeled cells. The retention of the label after numerous divisions suggested that labeled DNA was asymmetrically segregated to just a limited number of cells, rather than evenly distributed as would be expected if the chromosomes were randomly sorted.

To see whether this interpretation was correct, the team watched clones form under the microscope. A single cell was labeled with BrdU during one division. Subsequent imaging showed evidence of asymmetric segregation of the BrdU-labeled DNA in 6 out of 15 lines.

Similar experiments failed to show evidence for asymmetric retention of older DNA in mouse embryonic stem cells or a fibroblast cell line derived from mouse embryos. Thus, neural stem cells may be particularly fussy about their DNA relative to other stem cell types. If it is true that only some stem cells follow the immortal strand hypothesis, that might explain why previous experiments did not find evidence supporting the hypothesis.

The authors are now turning to other stem cell systems in mice and other metazoans to see whether they can replicate their findings in different contexts—and try to convince still-skeptical colleagues that the immortal strand hypothesis holds value.
A little Rac goes a long way

A slight reduction in the amount of Rac allows cells to move in a more persistent direction, report Pankov et al. on page 793. The small GTPases Rac and Rho modulate migration speed and chemotaxis, with increased expression of Rac in lamellipodia correlating with increased velocity. In the current study, Pankov et al. found that reducing the total amount of Rac1 in a cell by 30% to 50% induced persistent movement and limited random walking. The reduction in Rac reduced the number of lamellae, leaving such structures only at the cell ends. By contrast, knocking down RhoA reduced the rate of migration but did not alter migration style.

Unlike chemotaxis, which also induces persistent movement, the newly identified Rac function did not require phosphatidylinositol 3′ kinase activity, indicating that the two methods of migration control are distinct. Rac activity was affected by the cell environment, however. Cells grown in two-dimensional tissue culture had more Rac and moved in a more random fashion than those grown in a three-dimensional matrix, even when the molecular composition of the two substrates was identical. Rac activity was also dependent on β1 integrin, as expected from previous studies.

Given the strength of Rac’s influence, the authors speculate that such internal control of migration style might allow cells to switch between exploring their environment through random migration to moving as a group in one direction, as they do during wound healing and development. Now the trick will be to find tools that are sensitive enough to see such small changes in Rac levels in vivo, so that the hypothesis can be tested. JCB

Controlling cadherin

When it comes to cadherin regulation, it is not all about α- and β-catenins. Drosophila epithelial cadherin requires a novel regulatory region for cell migration in developing ovaries, according to Pacquelet and Rørth (page 803).

Cadherins are used in cell–cell adhesion and migration in a variety of tissues. The cytoplasmic tail of cadherin contacts the actin cytoskeleton through α- and β-catenin. Studies in tissue culture cells suggested that modulating the interaction between cadherin and the catenins would alter the adhesion strength of cadherin and facilitate migration.

To test whether such mechanisms work in vivo, the authors fused the full-length cadherin or just its transmembrane domain to α- or β-catenin. The full-length cadherin–α-catenin fusion rescued cell–cell interactions and migration in tissues lacking wild-type DE-cadherin or β-catenin. Thus no modulation is required between DE-cadherin and α-catenin for normal migration. If the link between DE-cadherin and the actin cytoskeleton needs to be tempered in vivo, the tempering must happen downstream of α-catenin.

Removing the cytoplasmic tail of cadherin in the α-catenin fusion protein blocked migration in the ovary, though adhesion was normal. Although the specific mechanisms are not yet known, the authors think that this cadherin cytoplasmic domain is required to maximize adhesion force during migration. JCB

Sticky interactions

Upon infection with Neisseria gonorrhoeae, human epithelial cells typically float off the underlying extracellular matrix, reducing the bacterial burden for the host. Now, Muenzner et al. (page 825) show that variants of N. gonorrhoeae and other bacterial pathogens that bind to certain cell–cell adhesion molecules can inhibit this shedding, thus increasing the bacteria’s ability to colonize their hosts.

The adhesion molecules under study are carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), which are thought to be involved in microbial infection. CEACAM engagement by the bacteria triggered expression of CD105, a TGFβ-like receptor that helps organize focal adhesions. In turn, CD105 expression increased host cell adhesion by activating integrins.

No physical association was detected between CEACAMs and CD105 or between CD105 and integrins, so the molecular mechanisms of the pathway are unclear. However, it is apparent that CEACAMs induce CD105 transcription without direct cytoplasmic signaling. CEACAM-6 naturally lacks a cytoplasmic domain and can trigger the response, as can a CEACAM-1 mutant lacking its cytoplasmic tail.

N. gonorrhoeae isolated from patient samples often contain a disproportionate fraction of bacteria that bind CEACAMs, even in controlled experiments where the infection started with bacterial cells that did not bind CEACAMs. The next question is whether bacteria that are beneficial to humans also use CEACAMs. If not, blocking the reaction may be a viable antibacterial strategy. JCB