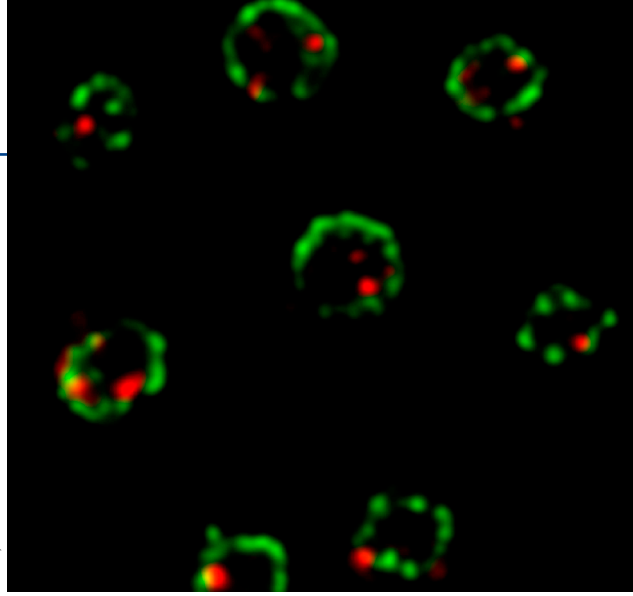
Research Roundup

Anchored telomeres get longer



TADDEI/GASSER

Telomeres (red) stay long if they are near the nuclear envelope (green).

Two groups conclude that factors near telomeres alter the telomeres' nuclear positioning and thus their length.

The lengths of telomeres, which are caps for chromosome ends, are kept consistent by a pathway that "counts" the number of Rap1 molecules bound to telomeres. This pathway signals through Tel1 to recruit the lengthening telomerase enzyme. Cells missing Tel1 have shorter but stable telomeres, which argues for a second pathway regulating length. Searching for clues to the second pathway, Florence Hediger, Susan Gasser (Friedrich Miescher Institute, Basel, Switzerland), and colleagues focused on the proximity of different telomeres to the nuclear envelope (NE)—one place telomerase is thought to concentrate.

Her team shows that the anchoring of individual telomeres near the NE is variable and correlates with the composition of the subtelomeric DNA sequence elements (STEs). The less-anchored telomeres had two STEs with multiple binding sites for Reb1 and Tbf1, factors previously shown to act as insulators against telomere-associated gene silencing. Artificial binding of Reb1 and Tbf1 to normally stably anchored telomeres can also release them from the NE.

Enter Anne-Sophie Berthiau, Eric Gilson (Ecole Normale Supérieure, Lyon, France), and colleagues. They showed that Reb1 and Tbf1 artificially bound to subtelomeric regions causes shortening of telomeres, with more Reb1 and Tbf1 binding sites causing more shortening.

"Our data and Eric's together argue that this pathway, which is not using Rap1-Tel1 signaling, may have more to do with proximity and accessibility of telomeres to telomerase," says Gasser. She proposes that the binding of STE factors changes the folding of telomeres, and subsequently their accessibility or proximity to telomerase.

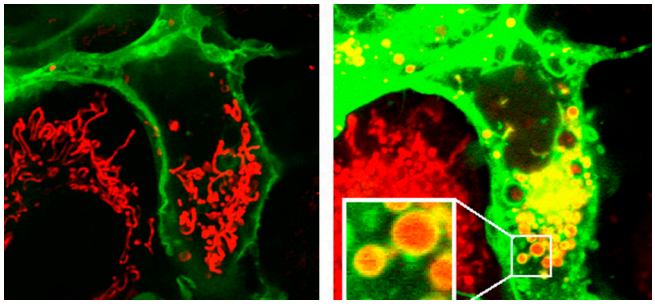
Concentrating telomerase at a membrane might keep it at a low, but effective, amount. Cells need a critical amount of it, but if they get too much then telomeres might get added anywhere a double-stranded DNA break occurs. JCB

References: Hediger, F., et al. 2006. *EMBO J.* doi:10.1038/sj.emboj.7600976.
Berthiau, A.-S., et al. 2006. *EMBO J.* doi:10.1038/sj.emboj.7600975.

Killer K-Ras

K-Ras resides at the plasma membrane (PM) where it promotes cell survival and proliferation. Now, Trever Bivona, Mark Philips (New York University, New York, NY), and colleagues find that fluorescent K-Ras moves to internal cell membranes within a few minutes after activating protein kinase C (PKC), with deadly consequences.

Philips' group happened to stimulate GFP-labeled T cells for an unrelated experiment. "Before our eyes, K-Ras shot off the membrane and into the cell interior," says Philips. K-Ras is attached peripherally to the PM by a farnesyl lipid group and an adjacent polybasic sequence. The team hypothesized that phosphorylation by PKC within the polybasic region acted like a switch, neutralizing the positive charge and releasing K-Ras from the PM. Indeed, blocking this serine-181 phosphorylation by PKC prevented K-Ras internalization.



PHILIPS/ELSEVIER

K-Ras (green) moves to mitochondria (red) when phosphorylated (right).

A phosphate-mimicking Glu-181 mutant gave another shocking result. Cells expressing this K-Ras construct died off rapidly by apoptosis, as internalized K-Ras inhabited ER, Golgi, and—more importantly—mitochondrial membranes.

The outer mitochondrial membrane hosts apoptosis regulators such as the Bcl-2 family members. Philips' group found that the PKC-phosphorylated K-Ras interacted with Bcl-X_L at mitochondrial membranes to promote apoptosis. Human T cells lacking K-Ras were resistant to activation-induced apoptosis.

Although Bcl-X_L is normally antiapoptotic, the group proposes that K-Ras either sequesters Bcl-X_L or converts it to a pro-apoptotic molecule.

Mutated Ras proteins are known to drive uncontrolled cell proliferation in 30 percent of human cancers. PKC agonists may be a possible strategy for treating such cancers, as the group found that oncogenic K-Ras also moved to the mitochondria when phosphorylated by PKC. Furthermore, K-Ras–driven tumors treated with the PKC agonist bryostatin-1 underwent apoptosis. PKC agonists such as bryostatin "cause K-Ras to fall off the plasma membrane," says Philips, "and it goes from being a molecule that drives cell growth to one that kills the cell instead." JCB

Reference: Bivona, T.G., et al. 2006. *Mol. Cell.* 21:481–493.

Text by Kendall Powell
kendall2@nasw.org

Skating on MTs

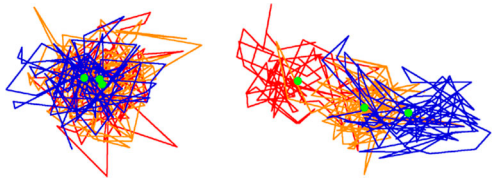
Dynactin can skate along the length of a microtubule (MT), giving a boost to the attached dynein motor's forward progress, according to Tara Culver-Hanlon, Stephen King (University of Missouri, Kansas City, MO), and colleagues.

Dynein is a poorly processive motor compared with others like kinesin. "It doesn't stay on the microtubule worth a hoot," says King. To be efficient, dynein needs dynactin, which has its own MT-binding domains. King's group found that dynactin had not only the well-known CAP-Gly MT-binding domain, but also a neighboring basic domain that bound MTs.

When the group attached the domains to beads for single-particle tracking along MTs, the beads moved in distinctly different ways. CAP-Gly beads missing the basic sequence only swiveled around a relatively fixed point on the MT. Beads with only the basic domain or with both domains could swivel too, but they also skated the length of the MT track while still bound to it.

When the dynein motor was added to the mix, the basic domain alone or full-length dynactin increased the motor's processivity. The CAP-Gly domain alone, however, put the brakes on the motor's speed. The basic domain acts like a space-walking astronaut's tether, says King. "When dynein falls off, [the motor] can rebind and keep moving instead of floating off into the cytoplasm." JCB

Reference: Culver-Hanlon, T.L., et al. 2006. *Nat. Cell Biol.* doi:10.1038/ncb1370.



KING/MACMILLAN

Mutant dynactin circles (left) rather than gliding along a microtubule (right).

Virus diverts checkpoint

Human cytomegalovirus (HCMV) inactivates a DNA damage response by booting two checkpoint proteins out of the nucleus, according to Miguel Gaspar and Thomas Shenk (Princeton University, Princeton, NJ).

When DNA herpesviruses like HCMV replicate, the double-stranded ends of their genomes, which resemble breaks, can trigger a cell's DNA damage checkpoint pathway. Viruses have evolved several mechanisms to inactivate the checkpoint and proceed with replication, such as degrading an essential checkpoint component or using the block in cell cycle progression to their advantage.

Gaspar and Shenk now show that HCMV thwarts the checkpoint in a novel way—by trapping two essential checkpoint proteins, ATM and Chk2, in the cytoplasm. "This is the first time a virus has been found to antagonize a DNA damage checkpoint by mislocalization," notes Shenk.

The checkpoint proteins wind up hanging out with viral proteins in the cytoplasmic "virion assembly zone." Whether ATM and Chk2 get ferried out of the nucleus by the viral proteins or another viral protein blocks the checkpoint proteins' normal nuclear import remains to be answered.

Shenk says the HCMV studies could shed light on the poorly defined nuclear import mechanism—following his motto: "If a virus goes after it, it's probably important." JCB

Reference: Gaspar, M., and T. Shenk. 2006. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.0511148103.

Hungry for better memory

A growling stomach might lend a helping hand to memory, based on new work by Sabrina Diano, Tamas Horvath (Yale University School of Medicine, New Haven, CT), and colleagues.

The growling stomach produces the hormone ghrelin, which stimulates fat storage and appetite by binding neurons in the hypothalamus. But ghrelin also binds neurons in the hippocampus—a center for forming memories.

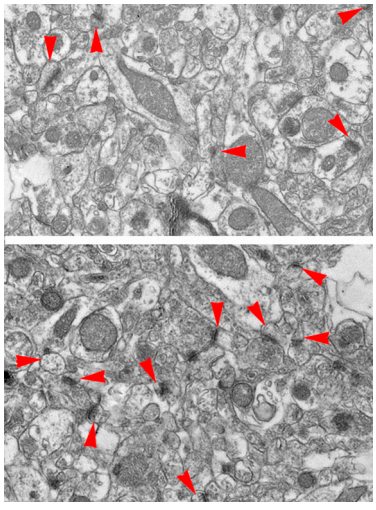
Horvath's team shows that ghrelin's hippocampal action promotes long-term potentiation and a higher synaptic density in the CA1 region of the hippocampus. Both characteristics correlated with improved spatial memory and learning.

The authors injected rodents with ghrelin and used three behavioral tests that rely on hippocampal memory functions—exploring different arms of a plus-shaped maze and two foot-shock avoidance tests. In each, ghrelin improved memory performance in a dose-dependent manner. Performance was improved 20–30% at the highest dose, effectively turning C-grade mice into straight-A students. In a final test, ghrelin knockout mice showed little ability to recognize a novel object unless they got a shot of ghrelin.

Aged SAMP8 mice—a model for Alzheimer's disease—also showed improved memory performance with ghrelin dosing, and the authors propose ghrelin analogues as potential treatments for memory loss. Of course, over-eating and weight gain would be potential side effects.

Horvath says ghrelin represents a primitive system in which a gut hormone acts directly on the higher brain to change synaptic plasticity, and affect cognition. "If you are hungry, you need to be alert and aware of your environment," for example to help in finding the next meal. His group now plans to look for the same mechanism in humans. JCB

Reference: Diano, S., et al. 2006. *Nat. Neurosci.* doi:10.1038/nn1656.



HORVATH/MACMILLAN

Gut hormone ghrelin (bottom) increases synapse density (arrowheads) and improves memory.