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Research Roundup

Palindromic protection

Research from Laura Maringele and David Lydall (University of Newcastle, UK) shows that yeast find a way to survive in the absence of telomeres. By forming large palindromes at chromosome ends, yeast mutants proliferate for many generations despite genomic instability, possibly becoming the yeast equivalent of cancer cells.

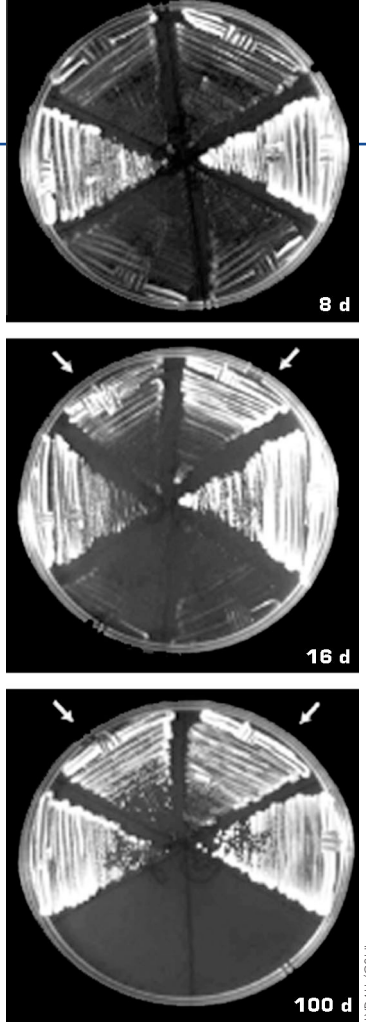
Chromosomes are progressively eroded with each cell cycle if they are not protected by telomeres, in part because the DNA replication machinery cannot copy the ends of linear DNA. Telomeres are maintained by telomerase or, when telomerase is compromised, by recombination. Maringele and Lydall now uncover a pathway to prevent chromosome shortening that is independent of both telomerase and recombination.

This pathway was seen in yeast mutants lacking the Exo1 nuclease. These mutants escape the cell cycle checkpoints that are normally activated by the loss of telomeres, probably because the mutants generate less checkpoint-activating ssDNA at unprotected ends. Further replication of these mutants was expected to cause death from loss of essential telomere-proximal genes, but many survivors were viable for thousands of generations.

Chromosomes in the surviving lines were shortened, but essential genes were protected by large mirror image duplications called palindromes. Palindromes were consistently found to originate at short inverted repeats (IRs). Short IRs have been suggested by others to initiate palindrome formation through a DNA repair mechanism. The extra hundreds of kilobases of DNA that the authors found at chromosome ends should protect chromosomes for thousands of generations. "You can keep chewing up the ends of palindromes until you get close to essential genes, and then repeat," says Lydall.

Mammalian somatic cells, which are low in both telomerase and recombination, might also escape checkpoint controls at the end of their replicative life span due to low levels of Exo1 (or equivalent nucleases) and gain immortality via palindromes. Whether palindromes are formed in precancerous cells and aid in malignancy remains to be seen. JCB

Reference: Maringele, L., and D. Lydall. 2004. *Genes Dev.* 18:2663–2675.



LYDALL/CSHL

Palindromes allow yeast cells lacking telomeres and Exo1 (arrows) to replicate.

SOS needs help getting active

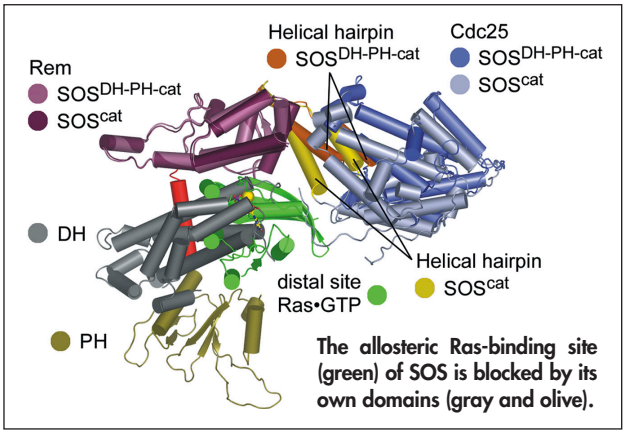
A Ras activator takes care with its own inhibitory domain to prevent an accidental Ras outbreak, according to structural studies from Holger Sondermann, John Kuriyan (University of California, Berkeley, CA), and colleagues.

Ras turns on MAPK pathways that trigger big changes in cell physiology that lead to cell growth, survival, or differentiation. "Its pathways control so many [extensive] outputs, you really want to avoid false activation," says Sondermann. His work now shows that the necessary care is taken by Ras's nucleotide exchange factor, SOS.

SOS helps Ras expel GDP to make room for GTP, which activates Ras. RasGTP then begins a positive feedback loop—previous studies show that RasGTP binds to an allosteric site on SOS and stimulates its exchange activity. But the new structures, which include a larger piece of SOS than previously crystallized in complex with Ras, show that SOS is normally autoinhibited. Two of SOS's own domains block the allosteric binding site for Ras.

When the two intruding domains are removed, the allosteric SOS site can bind to RasGTP or, with a 10-fold lower affinity, RasGDP. This binding activates exchange activity by increasing the affinity of the SOS catalytic site for Ras. SOS mutants that were unable to accommodate Ras in the allosteric site were also unable to stimulate MAPK pathways downstream of Ras.

What pushes aside the blocking domains in vivo is not known, but it probably ensures that a small blip in RasGTP levels does not turn into full-blown MAPK activation unless conditions warrant it. Growth factor stimulation is known to result in SOS membrane localization and thus activation, possibly by making the allosteric site more accessible to Ras. Phosphorylation by receptor tyrosine kinases might also alter SOS's structure and thus move the blocking domains out of the way. JCB



KURIYAN/ELSEVIER

The allosteric Ras-binding site (green) of SOS is blocked by its own domains (gray and olive).

Reference: Sondermann, H., et al. 2004. *Cell.* 119:393–405.

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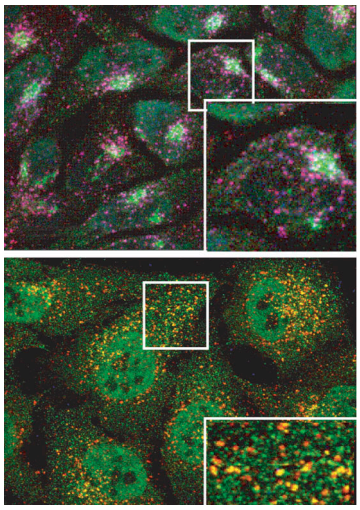
SNX1 hugs the curves

A sorting protein uses the coincidence of a lipid signal and membrane curving to direct its tubulation activity to the correct compartment, as shown by Jez Carlton, Peter Cullen (University of Bristol, UK), and colleagues. Relatives of this sorting nexin, SNX1, may control trafficking to and from a number of intracellular compartments.



CULLEN/ELSEVIER

TGN localization (top) of a receptor (green) is lost when SNX1 (blue) is missing (bottom).

SNX1 chooses its home via two membrane-binding domains. One targeting domain is the PX domain, which is known to bind to the endosomal phosphoinositide, PI3P. The second is a BAR domain, which was shown to target a fly protein to highly curved membranes and tubulate them.

Cullen's group shows that these domains combine to put mammalian SNX1 on the tubular portion of early endosomes (which also have less curved vesicular domains). This placement was needed to recycle a mannose-6-phosphate receptor from endosomes to the TGN. The cargo is probably selected by the retromer complex, which associates with SNX1.

The pinching off of endosomal tubules may be SNX1-driven, but Cullen is not yet convinced, as he needed a lot of SNX1 to get tubulation in vitro. Nine SNX1 relatives have both BAR-like and PX domains. As mammalian PX domains have different PIP binding partners, the nine might direct various trafficking pathways. JCB

Reference: Carlton, J., et al. 2004. *Curr. Biol.* 14:1791–1800.

GTPase modes modeled

A new computational model from Scott Bornheimer, Shankar Subramaniam, and colleagues (University of California, San Diego, CA) predicts how the GTPase cycle operates in one of several modes.

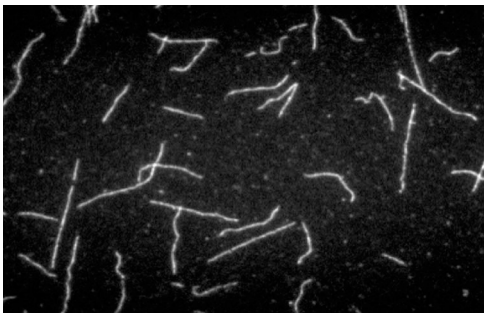
Several GTPase cycle models exist, but many include only the G protein, its activator (often a receptor), GTP, GDP, and phosphate. Subramaniam's group added a GAP, the G protein deactivator, to the equations, and used experimental data from the GTPase cycle of a mouse G protein stimulated by a acetylcholine receptor (largely from Mukhopadhyay and Ross. *PNAS*. 96:9539–9544) to build their model.

"Variability in the concentration of the players in vivo is common," says Subramaniam. "How will the cell achieve maximum or moderate turnover? What happens when it's starved of GAP? How can the cell compensate to accomplish the same end point?" The model can now predict answers to these questions.

Four modes were found in which G protein activity is unaltered by changes in receptor or GAP concentrations. Between these extremes are infinite variations. In some modes, particularly when G protein levels are low, the cycle operates while the G protein and receptor are not physically clustered. In this mode, GAPs are able to shut down G protein signaling entirely.

In other situations, clustering is required for G protein activity, and GAPs can change the signaling amplitude but cannot eliminate it. The authors are now using FRET to determine how local clustering changes with altered component concentrations. JCB

Reference: Bornheimer, S.J., et al. 2004. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.040700910.



CARLIER/ELSEVIER

Profilin helps formin to steer actin monomers into the barbed ends of actin filaments.

Profilin for processivity

Profilin helps formin to speed actin assembly and enhance ATP hydrolysis, according to Stéphane Romero, Marie-France Carlier (CNRS, Gif-sur-Yvette, France), and colleagues.

Formins initiate actin filaments at various places such as filopodia, focal adhesions, and the cytokinetic ring. The authors show that when formin is alone on barbed ends, it slows filament dynamics by binding to and falling off the barbed ends. But when another actin-binding protein, profilin, is added, formin becomes a processive motor for rapid actin elongation.

The profilin/formin combination speeds polymerization two ways. First, it increases by 15-fold the on-rate of actin to barbed ends. "Electrostatic or hydrodynamic properties of formin," suggests Carlier, "may allow profilin–actin to associate much faster at short distances than is [possible when] limited by diffusion." This is a much faster rate than that of Arp2/3. The disparate on-rate constants thus allow for different actin velocities at the same actin monomer concentration.

Formin's second ability is to hasten ATP hydrolysis by actin, which is the rate-limiting step of filament growth at high actin–profilin levels. Enhanced hydrolysis may result from structural changes to the ATP-binding site, which should be revealed by structural studies of actin–formin–profilin. JCB

Reference: Romero, S., et al. 2004. *Cell.* 119:419–429.