Research Roundup

Hey, I'm still replicating!

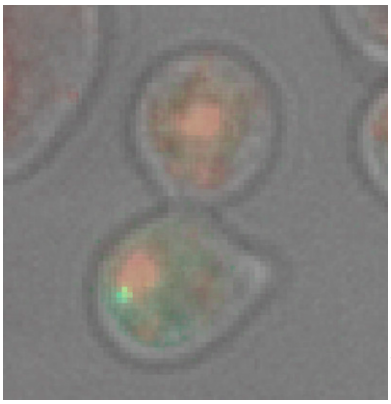
Checkpoints exist to ensure the genome is accurately replicated each cell division. But yeast cells don't bother to check that replication is actually completed, report Jordi Torres-Rosell, Luis Aragón (MRC Clinical Sciences Centre, London, UK), and colleagues.

The team was not initially looking for a replication completion checkpoint. They were studying the function of a yeast complex called Smc5-Smc6 that had been suggested to promote DNA recombination and repair.

Budding yeast that lack Smc5-Smc6 do not survive more than a few cell cycles, so the team synchronized yeast cells in G1, knocked out the complex, and then observed the yeast over one cell cycle. S phase and mitosis appeared to be normal. In the subsequent interphase, however, the Rad53 DNA damage signal was activated. This damage response, the team discovered, was due to a failure of chromosomes to separate correctly at anaphase.

Thinking that this nondisjunction might be caused by a failure to resolve recombination events, the team knocked out critical recombination genes to see whether the problem was bypassed. Loss of recombination only slightly rescued the phenotype, however.

If Smc5-Smc6 was knocked out after S phase, but before metaphase, chromosomes segregated normally, showing that the complex is required during (or before) DNA replication. Loss of Smc5-Smc6 before S phase caused replication forks to persist into metaphase. Labeling of nascent DNA in these cells revealed that large regions of rDNA were still unreplicated. rDNA is a major binding site for Smc5-Smc6 and was also the main site of the nondisjunction.



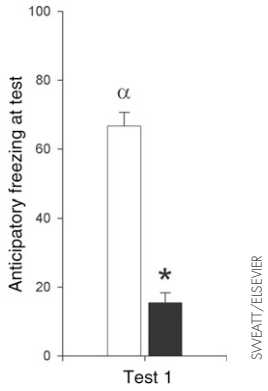
ARAGÓN/AAAS

Yeast without Smc5-Smc6 don't finish replicating rDNA (green) and missegregate this DNA at mitosis.

Aragón suggests that Smc5-Smc6 might act as a chromatin structure modifier that allows the replication machinery to progress unhindered. Despite the slowed replication in the absence of Smc5-Smc6, the cells continued with cell division.

Yeast lack this replication completion checkpoint because they might not need it. There are many unused replication origins that can become active during late S phase, explains Aragón. Thus, it's likely that normal cells can easily replicate their DNA in time for metaphase. JCB

Reference: Torres-Rosell, J., et al. 2007. *Science*. 315:1411–1415.



Rats forget the risk of electric shock when hippocampal DNA methylation is inhibited (black).

Methylate to memorize

A child reaching to touch a hot kettle will be either severely scolded or severely scalded. Learning to avoid hot kettles in the future might be thanks to the state of DNA methylation in the child's hippocampus, according to Courtney Miller and David Sweatt (University of Alabama, Birmingham, AL).

The pair found that the level of DNA-methylating enzymes (DNMTs) increased in the hippocampus when rats learned a conditioned response. For conditioning, rats were placed in an unfamiliar chamber, left to explore for a few minutes, and then given an electric shock and removed from the chamber. When the rats were put back in the chamber a day later, they froze in anticipation of the shock.

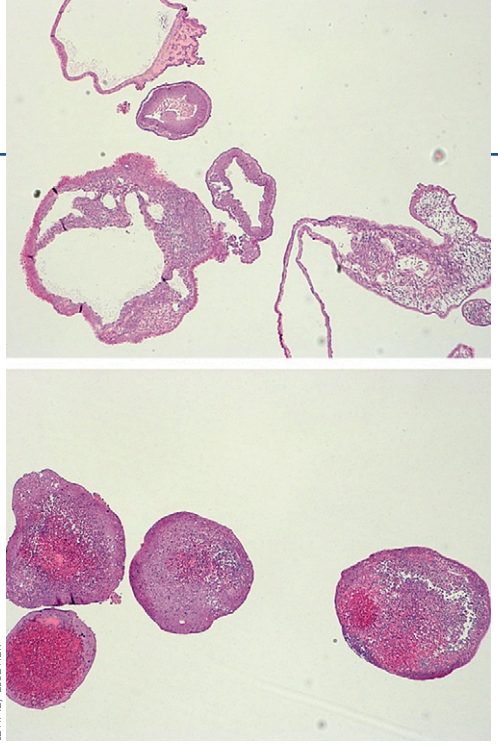
This associative learning was forgotten, however, when the rats were given a DNMT inhibitor immediately after their conditioning. The normal increase in DNMTs upon learning was coupled with an increase in DNA methylation and reduced transcription at a memory suppressor gene. In the presence of the DNMT inhibitor, the suppressor gene remained active.

Despite the increase in DNMT activity during conditioning, a gene that promotes neuronal plasticity was demethylated and thus exhibited increased transcription. This finding came as a bit of a surprise, since DNA methylation was considered to be a stable epigenetic modification in adult tissue.

In the presence of the DNMT inhibitor, the plasticity gene was further demethylated and thus further activated. Since memory was nonetheless impaired, the authors suggest that repressing the memory suppressor by methylation might be the more important cellular event during learning.

Both the methylation of the memory suppressor and the demethylation of the plasticity promoter were apparent just one hour after conditioning and returned to baseline levels by the next day. The lack of permanent change to the hippocampus, explains Sweatt, might be related to the fact that this part of the brain is associated with memory consolidation rather than memory storage. The team now plans to look at the cortex, where memories are stored, to see whether methylation states there become as fixed as our memories. JCB

Reference: Miller, A., and D. Sweatt. 2007. *Neuron*. 53:857–869.



LEVINE/ELSEVIER

Embryoid bodies can't cavitate (top) if their cells don't eat themselves (bottom).

Self-eating embryos

Embryonic cells that cannot eat themselves also can't signal for other cells to eat them, report Xueping Qu, Beth Levine, and colleagues (University of Texas, Dallas, TX).

During embryonic development, the carving out of the body's shape requires a vast number of cells to be eliminated. Coincident with this large-scale programmed cell death, cells also perform autophagy, but whether this self-eating is required for normal embryogenesis was unclear.

To address this question, Qu et al. grew autophagy-deficient embryo-like structures in culture. These embryoid bodies normally develop internal cavities, but, in the absence of autophagy, the bodies remained solid.

The lack of cavitation was not due to a lack of programmed cell death but instead to a failure in clearance of the dead cells. Apoptotic cells normally express signals that tell waste disposal cells to clean-up their dying remains. In the autophagy-lacking embryoid bodies, however, these signals were missing.

The signals could be restored by providing the embryoid bodies with an energy boost. By breaking down and recycling cell components, autophagy provides the cell with energy. The autophagy-deficient embryoid bodies thus had reduced energy production, which seems to prevent their dying cells from calling the clean-up crew. JCB

Reference: Qu, X., et al. 2007. *Cell.* 128:931–946.

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Langerhans cells limit HIV invasion

A suspected entry route for HIV turns out to be a dead-end, report Lot de Witte, Teunis Geijtenbeek (VU University Medical Centre, Amsterdam, Netherlands), and colleagues. Langerhans cells, rather than transmitting the virus to T cells, trap HIV-1 and thus act as a barrier to infection.

The primary targets for HIV-1 invasion are CD4-expressing T cells. HIV-1 uses the CD4 receptor to gain entry. The first immune cells that HIV-1 meets in the body's mucosa, however, are a subset of dendritic cells (DCs) called Langerhans cells (LCs). Most DCs internalize HIV-1 into nonlysosomal compartments and later transmit the virus to CD4-expressing T cells in lymphoid tissues. But in LCs, the team now shows, internalization is the end of the road for HIV-1.

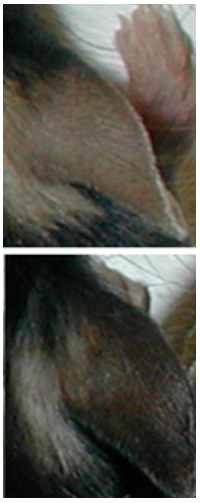
Binding and internalization of HIV-1 to DCs depends on C-type cell surface lectins. The team shows that, on LCs, HIV-1 associates with a C-type lectin called Langerin at the cell surface and in intracellular vesicles. Internalization via Langerin resulted in degradation of the virus and thus prevented transmission.

When the team blocked Langerin, LCs actually increased viral transmission. The LCs probably instead became infected via the small amount of CD4 these cells express. Ordinarily this CD4 route would be out-competed by the abundance of Langerin.

The fate of HIV-1 vesicles in LCs is not yet clear. It is likely that they are targeted to lysosomes for degradation. Because other DCs transmit virus via the lectin pathway, inhibitors of C-type lectins were proposed for use as microbicides. Such an approach, however, would also knock out the ability of LCs to intercept and neutralize invading HIV-1. JCB

Reference: de Witte, L., et al. 2007. *Nat. Med.* doi:10.1038/nm1541.

Tanning with p53



FISHER/ELSEVIER

Mice lacking p53 (top) don't tan.

The guardian of the genome, p53, can induce DNA repair, hold the cell cycle while repair work is done, and induce apoptosis if the damage is too great. Now, Rutao Cui, David Fisher (Harvard Medical School, Boston, MA), and colleagues have discovered a new way in which p53 protects our genomes—it gives us a suntan.

The DNA damage caused by UV exposure has long been known to up-regulate p53. Mice that lack p53 have a propensity to develop tumors upon UV exposure. The team now finds that these mice also fail to tan.

Tanning occurs when keratinocytes make more melanocyte-stimulating hormone (MSH) and thus induce melanocytes to produce large amounts of the pigment melanin. MSH is a cleavage product of the POMC pro-hormone. The team found that p53 directly binds to, and increases transcription from, the *POMC* gene promoter in response to UV treatment.

p53 also promoted POMC and melanin production when induced by factors other than UV, such as the cancer drug etoposide. Melanin provides protection to the skin by mopping up free radicals and by acting as a direct shield from UV radiation. Inducing melanin via the p53 pathway might potentially provide a sunless golden tan. This strategy might be good for reducing cancer risk, although vitamin D levels may need supplementing if the sun were continuously avoided. JCB

Reference: Cui, R., et al. 2007. *Cell.* 128:853–864.