Research Roundup

rDNA silencing saves starving cells

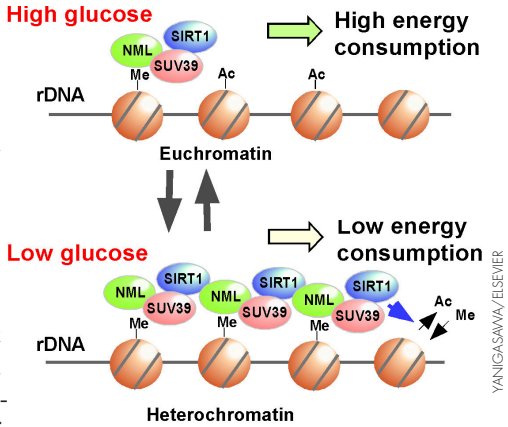
Histone methylation at ribosomal genes saves cells when sugar is in short supply, say Akiko Murayama, Junn Yanagisawa (University of Tsukuba, Japan), and colleagues. The team identifies multiple parts of the machinery that links energy levels to epigenetics.

Ribosome biogenesis demands lots of energy, and rDNA transcription falls during glucose starvation to keep cells from dying. Histone methylation, a modification associated with silencing, has been reported at rDNA. But whether this was the silencing mechanism during glucose starvation—and if so, which factors might be responsible—was unknown.

To find rDNA silencers, the authors purified proteins from nucleoli that bind to histone H3 deacetylated at Lys9—the target substrate for the silencing modification previously reported at rDNA. They identified a known nucleolar peptide and named it nucleomethylin (NML). Transfection with NML increased the number of methylated histones at rDNA and decreased rDNA transcription. Knockdown of NML had the opposite effect.

The histone deacetylase SIRT1 coimmunoprecipitated with NML, and disabling SIRT1's deacetylase activity prevented NML from silencing rDNA. Despite some structural features suggesting that NML might be the methylator, it turned out that that role was played by SUV39H1, a third coprecipitant and known methyltransferase. NML was required for binding, but its other potential functions are unclear.

The authors then showed that calorie restriction increased the binding of the entire complex to rDNA and reduced pre-rRNA transcript production. The authors thus named the complex eNoSC, or energy-dependent nucleolar silencing complex. "We found that without eNoSC, cells cannot adapt to glucose starvation, and die more quickly," Yanagisawa says. SIRT1 is likely the energy-sensing component, since it is known to be regulated by the NAD^+/NADH ratio—a readout of cells' metabolic activity. The authors also suggest that the multiple functions of the complex may explain how silencing spreads along repeated rRNA genes: with NML bound to a deacetylated, dimethylated lysine, SUV39H1 and SIRT1 can modify adjacent lysines, which in turn become binding sites for other eNoSC complexes. JCB



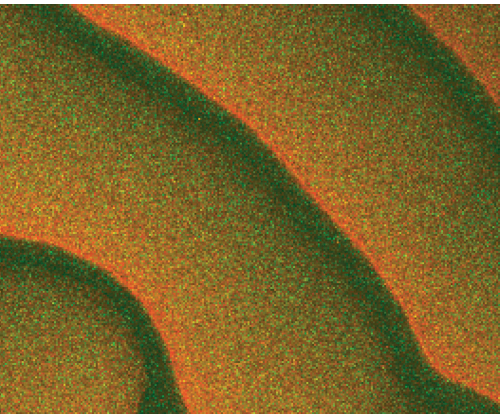
eNoSC complexes silence rDNA to reduce energy use when sugar is low.

Murayama, A., et al. 2008. *Cell.* 133:627–639.

Four proteins, two new cells

Establishing the midpoint as the site of bacterial cell division is the work of three proteins, and constricting the cell is the work of just one, according to two new studies.

In the run-up to bacterial cell division, oscillatory movements of Min proteins occlude both poles of the cell. MinD initially attaches at one end, but MinE binds and drives it off. MinD shuttles to the other end and rebinds, only to be driven off again by MinE, and so the cycle repeats. MinD carries MinC along with it, and MinC prevents membrane binding of a key contractile protein called FtsZ. With MinC guarding both ends of the cell, FtsZ can bind only at the middle.



MinD (green) moves across an artificial membrane and is chased, caught, and detached by MinE (red), leaving protein-free troughs (dark bands) in their wake.

Theoretical models had suggested that MinD and E might drive the oscillations themselves. To test this, Martin Loose, Petra Schwille (Technical University of Dresden, Germany), Karsten Kruse, and colleagues placed MinD on a lipid bilayer and then added MinE. Within an hour, periodic bands of MinD and E appeared, separated by protein-free troughs. The bands marched slowly across the membrane, mimicking the oscillations seen in the bacterial cell. "These two proteins alone are sufficient to create this spatiotemporal structure," says Kruse.

And FtsZ alone is sufficient to constrict the cell. FtsZ links to a membrane tether, and in conjunction with almost a dozen other proteins, forms a contractile ring. Because the other proteins are mainly required for remodeling the cell wall, Masaki Ozawa, David Anderson, and Harold Erickson (Duke University, Durham, NC) wondered whether FtsZ could constrict the cell by itself. The team added FtsZ with a membrane-targeting sequence to purified liposomes. In the presence of GTP, FtsZ formed bands on the inside surfaces and constricted liposomes almost to the point of pinching off. The incomplete division may have been due to the extreme thickness of the liposome walls, Erickson says.

The work by the two teams brings closer a cell-free system for studying the events of cytokinesis. JCB

Loose, M., et al. 2008. *Science.* 320:789–792.
Osawa, M., et al. 2008. *Science.* 320:792–794.

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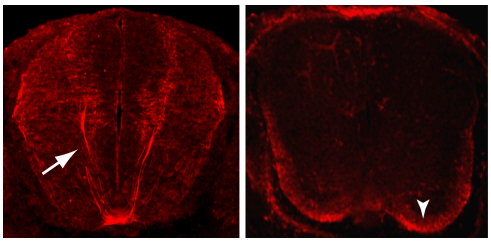
rrobinson@nasw.org



TESSIER-LAVIGNE/ELSEVIER

Precrossing axons express Robo3.1 (arrow). On crossing the midline, an alternative splicing switch turns off Robo3.1 in favor of Robo3.2 (arrow head).

Robo keeps axons moving

For migrating axons, attraction becomes repulsion with the flip of an alternatively spliced switch, according to Zhe Chen, Marc Tessier-Lavigne (Genentech Inc., San Francisco, CA), and colleagues.

A melange of chemoattractants and repellants guides growing axons to their targets. Axons that cross the midline of the spinal cord on their way toward the nascent brain, however, have the complicated task of having to move past local attractants at the midline to complete their journey.

Midline cells also produce repulsive molecules called Slits; but by keeping levels of the Slit receptors Robo1 and 2 low, axons approaching the midline can ignore Slits and instead respond only to attractants. As axons cross the midline, they up-regulate Robo1 and 2, become more sensitive to the repellants, and so are booted out the other side. Precrossing axons also express Robo3, which silences Robo1 and 2. But, curiously, Robo3 is also found on postcrossing axons, when Robo-mediated repulsion is at its strongest. "That was a puzzle," Tessier-Lavigne says. And it's that puzzle they've resolved in the current study.

While sequencing a collection of Robo3 cDNAs, the authors discovered two isoforms, one with and one without the two terminal exons. "We fell off our chairs when we did the immunohistochemistry," Tessier-Lavigne says. Robo3.1 was expressed on the precrossing axonal segment, whereas Robo3.2 was found on the postcrossing segment. Overexpression of 3.1 facilitated crossing but led many axons to recross. Robo3.2 overexpression prevented most axons from crossing all together, but those that did never recrossed.

The results suggest that unlike Robo3.1, Robo3.2 is not a silencer of the Slit receptors, but instead cooperates with them. "Our hypothesis is that Robo3.1 and 3.2 are tied together to give you an all-or-nothing switch," says Tessier-Lavigne. The mechanisms controlling this switch are yet to be determined. JCB

Chen, Z., et al. 2008. *Neuron*. 58:325–332.

Abundance of ITAM prevents autoimmunity

For T cell receptors, more activation motifs means less autoimmunity, say Jeff Holst, Dario Vignali (St. Jude's Children's Research Hospital, Memphis, TN), and colleagues.

The T cell receptor's four CD3 subunits contain a total of 10 tyrosine-based activation motifs (ITAMs). These motifs dock with tyrosine kinases inside the cell to promote signaling, but it's unclear why the receptor needs so many ITAMs.

The authors generated mice that expressed mutant CD3 subunits with different numbers of ITAMs. Mice with seven or more ITAMs on their CD3 subunits developed normally, but with six or fewer, mice were prone to autoimmune disease. "Number was more important than 'flavor,'" Vignali says. Although flavor—the specific ITAM type—also played some part, since two of the four strains with six ITAMs remained healthy.

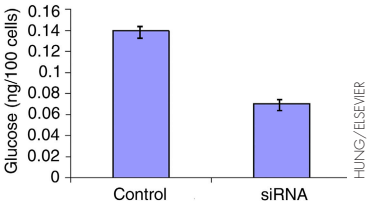
Autoimmunity can be caused by loss of tolerance for self-antigens during development in the thymus. To test whether ITAM number affected self-antigen recognition, the authors created male mice in which either wild-type or mutant CD3 subunits were combined with a T cell receptor specific for a male antigen. Although wild-type mice deleted thymic T cells bearing the receptor, those with few functional ITAMs allowed the T cells to develop and enter the periphery, where they could provoke an autoimmune reaction. "Self-antigens have to be recognized in the thymus even when affinity and density is low," Vignali says. The large number of ITAMs might therefore ensure the message isn't lost. JCB

Holst, J., et al. 2008. *Nat. Immunol.* doi:10.1038/ni.1611.

Sweeter tumors with EGFR

EGFR sweetens cancer cells to keep them alive, say Zhang Weihua, Mien-Chie Hung, Isaiah Fidler, and colleagues (University of Texas, Houston, TX).

Prognosis for many epithelial tumors correlates with the expression level of epidermal growth factor receptor. "It has traditionally been thought that tumor cells with too much receptor have too much tyrosine kinase activity," Hung says. "But therapeutic kinase blockade has been only partially successful."



Knockdown of EGFR reduced cellular glucose by destabilizing SGLT1.

Investigating other mechanisms, the authors showed that knockdown of EGFR reduced intracellular glucose levels, and increased production of energy-scavenging autophagosomes, ultimately leading to cell death. This cell death could be prevented by providing extra glucose.

This effect on cellular glucose is due to an interaction between EGFR and the sodium/glucose cotransporter, SGLT1, the team found. EGFR knockdown reduced expression of SGLT1, and SGLT1 knockdown caused autophagosome-induced death. Transporter loss was not due to lowered transcription, suggesting that the protein became unstable in the absence of EGFR. EGFR's stabilizing effect was dependent on its extracellular domain and remained even in the absence of its kinase activity.

"The excess stabilization of the glucose transporter allows these cells to survive in harsher conditions," Hung says. Targeting this additional function of EGFR might therefore make for a better therapy. JCB

Weihua, Z., et al. 2008. *Cancer Cell*. 13:385–393.

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