WEB WATCH

Bugs on the Web

- http://microbiol.org/
  http://www.microbiology-direct.com/

The beauty of the Internet is the ease with which networks can be generated by linking related sites. The Microbiology Network is at the centre of one such web, and aims to serve as a "communication resource for the microbiologist".

The Microbiology Network provides a starting point for various sites of interest to microbiologists, including a 'resource centre' with information on several areas — from contract testing to microbiology software or laboratory equipment and supplies. The site also includes a list of user groups, a page for discussion groups, and 'file libraries', which aim to act as a "repository for computer files of interest to biologists and health care workers in industry, academics, and private practice".

Another web site doing a similar job is Microbiology Direct. Launched in July 2000, this site has a slick design and easily navigable layout. Like The Microbiology Network, its strength lies in the plethora of links to related sites, handily divided into categories (genomics, journals and methods, for instance).

The comprehensive gallery of images includes video clips and links to other photo galleries, and the 'research topics' section contains links to several areas, from phylogenetics and nomenclature to public health microbiology. Many of the 'teaching resources' links are both educational and entertaining, and the news links are regularly added to and updated (an advantage of this site compared with The Microbiology Network). Together, these sites provide a great launch pad for microbiology online.

Alison Mitchell

STEM CELLS

Forever young

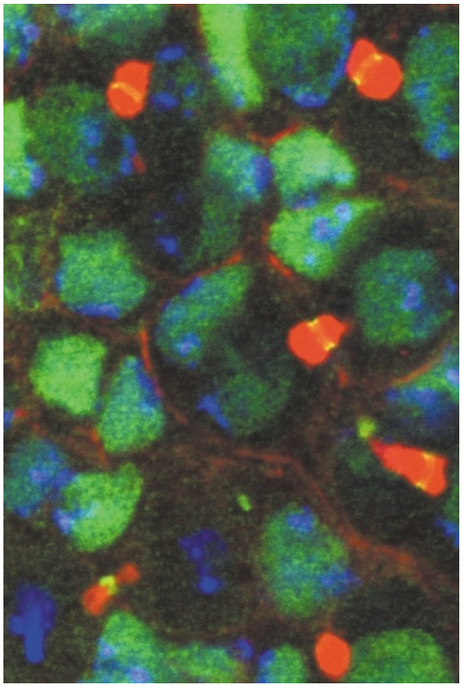
We all know that stem cells can generate any of the body's tissue types, but how do they renew themselves to prevent their own demise? Now, two reports in *Science* reveal some of the biological instructions for self-renewal. They have found that cells in the *Drosophila* testes act as a microenvironment and activate the JAK–STAT (Janus kinase–signal transducer and activator of transcription) signalling pathway, which determines the fate of stem cells.

In male *Drosophila*, the sperm stem cells lie at the tip of the testis, surrounding a cluster of cells called a hub. When the stem cell divides, the daughter cell maintaining contact with the hub remains a stem cell, whereas the cell that is displaced away becomes a blast cell and begins to develop into a male germ cell. What governs the destiny of each cell was unclear.

Kiger and colleagues, and Tulina and Matunis investigated whether cues from within the hub were important. Both groups focused on the JAK–STAT pathway as it is known to activate target genes in both *Drosophila* and mammals. They created mutants for the JAK homologue hopscotch (Hop) or the STAT homologue Stat92E in stem cells, and found that if mutants lacked an active JAK–STAT pathway, the stem cells differentiated but did not self-renew. By contrast, ectopic or constitutive JAK–STAT signalling greatly expanded stem-cell numbers — as shown by the presence of Anillin (red in the picture) or spectrosomes.

But how is the JAK–STAT pathway activated in these cells? Both groups found that cells in the hub act as a microenvironment by expressing a localized source of a signalling molecule called Unpaired, which activates the JAK–STAT pathway in adjacent stem cells. Both groups found that misexpression of Unpaired was sufficient to cause expansion of the germline stem-cell population.

So, it seems that signals within the hub create a stem-cell microenvironment and that any daughter cells that



are displaced will embark on a journey of differentiation into mature germ cells. Signals from surrounding cells that promote differentiation of displaced cells could help prevent excessive stem-cell proliferation, whereas hubs that lack stem cells could instruct both daughters of a neighbouring stem cell to self-renew and repopulate the microenvironment. Although this model will need to be

TRANSCRIPTION

A unique switch

Although histone methylation is known to regulate gene transcription through chromatin modification, no transcription factor has yet been identified as a direct target for methylation. In *Science*, however, Evans and colleagues now describe a unique molecular switch, which enhances the transcription of nuclear receptor (NR)-dependent genes and blocks the transcription of cyclic-AMP-response-element-binding protein (CREB)-dependent genes. This switch is based on the controlled methylation of histones and the transcriptional cofactors CREB-binding protein (CBP)/p300.

CBP/p300, which possess intrinsic histone acetyltransferase (HAT) activity, and coactivator-associated arginine methyltranferase (CARM1), which has intrinsic histone methyltransferase (HMT) activity, are transcriptional coactivators of NR-dependent genes. The cofactor, activator of retinoid and thyroid receptors (ACTR), also possesses HAT activity and interacts with both CBP/p300 and CARM1.

Evans and co-workers used an *in vitro* chromatin-based NR-dependent transcription system to characterize the interplay between the p300-HAT and CARM1-HMT activities. They observed the most striking transcriptional enhancement in the presence of p300, CARM1 and ACTR, indicating that a trimeric coactivator complex might be required for maximal activation of NR-dependent genes.

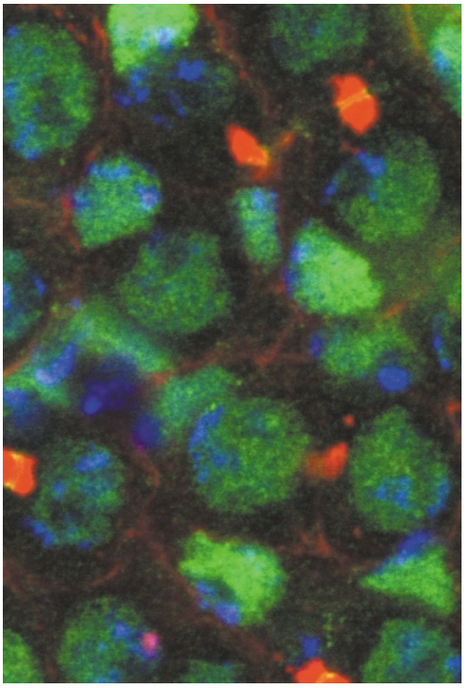
Using a CARM1-HMT-defective mutant, the authors showed that this HMT activity is essential for the enhancement of NR-dependent transcription. They also showed that p300-acetylated histones are more effectively methylated by CARM1 than are nonacetylated histones, indicating that CARM1 might be an NR cofactor through which acetylation and methylation cooperate to modify chromatin and stimulate transcription.

Using a similar system, the authors found that although CBP/p300 enhance this transcription, CARM1 inhibits it, and this inhibition was not observed using a CARM1-HMT-defective mutant. Evans and colleagues found that, in addition to methylating histones, CARM1 also specifically methylates CBP/p300.

As CREB-dependent transcription is determined by the strength of the interaction between the KIX domain of CBP/p300 and the kinase-inducible domain (KID) of CREB, the authors proposed that CARM1-dependent methylation of CBP/p300 inhibits CREB signalling by disrupting this interaction.

Using *in vitro* methylation assays with GST–KIX fusion proteins, the authors found that CARM1 predominantly methylates a single arginine residue in CBP and p300 that is localized to the external surface of the KIX–KID complex. Methylation of this site disrupts the formation of this complex, but does not affect the NR-related functions of CBP/p300.

The authors identified a potential



further defined, it offers an intriguing insight into how stem-cell renewal can be achieved and regulated.

Simon Frantz

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physiological role for CARM1 by showing that CARM1 induces apoptosis in neuronal cells. Nerve growth factor promotes the survival of neurons by inducing CREB-dependent expression of Bcl-2 — an anti-apoptotic factor — in neuronal cells. This CARM1-induced apoptosis was dependent on CARM1-HMT activity and was linked to the inhibition of Bcl-2 induction.

Methylation by CARM1 serves as a unique transcriptional switch, as it can activate the expression of NR-dependent genes, whilst inhibiting the expression of CREB-dependent genes. This study is the first report of direct methylation of a transcriptional cofactor, and, as CREB-dependent signalling pathways are developmentally and physiologically important, methylation by CARM1 could have very broad biological implications.

Rachel Smallridge

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CYTOSKELETON

Comet tales

The GTPase dynamin is crucial for vesicle fission during endocytosis and secretion. But can dynamin also influence actin dynamics? Initial evidence came from reports that dynamin interacts with actin-regulatory proteins and localizes at actin-rich sites. Two papers in the *Proceedings of the National Academy of Sciences* now show that dynamin can control actin nucleation from membranes, thereby regulating comet formation and movement.

To explore a functional link between dynamin and actin, the groups studied the regulation of actin nucleation in actin comets. Comets are induced by infection with *Listeria monocytogenes* or by the accumulation of phosphatidylinositol 4,5-bisphosphate, which induces the activity of actin-regulatory proteins. The groups tagged dynamin 2 with green fluorescent protein (GFP) and stained for filamentous (F-)actin using phalloidin or the actin-binding protein cortactin. The pattern of GFP fluorescence strongly resembled that of F-actin in the comets. Dynamin was further enriched at the tips of vesicles near the membrane.

Live imaging confirmed that dynamin is incorporated into the forming comets. But does it have an active function in these structures? To test this, both groups made use of dynamin mutants to assess any changes in actin tail formation or dynamics. GTPase-deficient dynamin–GFP mutants — which exert a dominant-negative effect on endocytosis — reduced the number and the speed of comets, and caused them to appear short and curled.

The region of dynamin that directly binds to actin-regulatory proteins is its proline-rich domain (PRD). Its involvement in targeting dynamin to actin comets was studied using a PRD-deletion mutant (dynaminΔPRD–GFP), which led to fewer comets. Unlike the GTPase-deficient mutant, however, the comets were longer. Significantly, dynaminΔPRD–GFP couldn't be detected in the comets, indicating that the PRD is required to target dynamin to these — and possibly other — actin structures.

So, what is the functional role of dynamin in actin comets? Given its ability to bind to components of the actin-nucleating machinery, dynamin might regulate actin-nucleation. On the basis that it can also associate with the lipid bilayer, it could direct this nucleation to specific sites, such as the coated pits involved in endocytosis. It is also likely, however, that the interaction between dynamin and actin occurs at non-endocytic sites.

Katrin Bussell

References and links

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Image courtesy of John Laborde, San Diego, California, USA.

APOPTOSIS

Death TRAIL

The road to cell death involves two distinct routes — the 'extrinsic' and 'intrinsic' pathways, which proceed through death receptors or through mitochondrial events, respectively. Although these pathways converge at the level of effector caspases, they are thought to be completely separate before that. However, a report by Xiangwei Wu and colleagues in *Genes and Development* now adds to the growing evidence for crosstalk between these two pathways. They have discovered that death by an extrinsic pathway involving TRAIL relies on intrinsic, mitochondrial events to kill human cancer cells.

Wu and co-workers used cells lacking Bax — a component of the intrinsic pathway — to show that this protein is needed for TRAIL-induced apoptosis. Moreover, whereas Bax was found in the cytosol before treatment with TRAIL, Bax translocated to the mitochondria after treatment. An inhibitor of the TRAIL-mediated extrinsic pathway prevented this translocation, suggesting that this movement depends on the extrinsic pathway.

What's the effect of Bax's translocation to the mitochondria, and how does this link to the intrinsic signalling pathway? Wu and colleagues showed that the loss of Bax blocks the release of intrinsic signalling factors (namely cytochrome *c* and Smac/DIABLO) from the mitochondria. But whereas TRAIL-induced apoptosis could still occur without cytochrome *c*-mediated caspase activation, the liberation of Smac/DIABLO was crucial.

Smac/DIABLO binds to (and removes the inhibitory effect of) a protein called XIAP, which normally inhibits caspase activity and blocks cell death. So Wu and colleagues propose that the TRAIL-mediated translocation of Bax allows the release of Smac/DIABLO from the mitochondria. This lifts the anti-apoptotic effects of XIAP, allowing cell death to proceed.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Deng, Y., Lin, Y. & Wu, X. TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. *Genes Dev.* **16**, 33–45 (2002)