WEB WATCH

Computer viruses

We frequently hear about one or another disease in the news, but rarely receive all the information we would like about its biological background. If you wish to find out more about the viruses that cause all this trouble, you could start by looking them up in 'All the virology on the WWW'. The site was put together by David Sander and Robert Garry and it's maintained at Tulane University. Its declared aim is to "be the best single site for Virology information on the Internet", and it certainly succeeds with over 3,000 links to web pages on almost every aspect of virology that you can think of.

This is an index site, and its aim is not to provide new information but to group and organize existing online information. The table of contents is easy to use, linking directly to pages on individual virus families. You can also link to pages containing general information on virology (including some excellent tutorials), virology labs, virology news, related social issues, a collection of images and much more.

One shortcoming is that the search page is not very useful, especially if you don't know the name of the virus. Typing in "foot and mouth", for example, gives an alphabetical listing of viruses (M–P), and you need to read through every entry on the list before finding out that the virus responsible for foot-and-mouth disease is an *Aphtovirus* of the Picornaviridae family. But once you have the name, the information is only a few clicks away, ranging from the basic mechanisms of viral replication to instructions for farmers on how to diagnose the disease in their livestock. You probably won't learn very much about molecular cell biology, but the site is well worth a visit for the sake of improving your general knowledge.

Raluca Gagescu

AGEING

Cheating time

Forget healthy living — the secret to a long life is genetic. And, according to three reports in *Science*, this is the case not only for worms, but also for flies, yeast and maybe even mammals.

Longevity has been best studied in *Caenorhabditis elegans*, where mutations in components of an insulin/insulin-like growth factor (IGF) signalling pathway increase an adult's life span by up to 200%. At the centre of this pathway is the daf-2 receptor which, when stimulated by an insulin-family ligand, modulates the expression of several other genes.

The insulin/IGF pathway is now implicated in controlling how long fruit flies live too. Clancy and colleagues report that mutation of *chico*, which encodes an insulin receptor substrate, extends the median life span of *Drosophila melanogaster* by up to 48%. And Tatar *et al.* show that mutation of the insulin-like receptor (InR) — the fly daf-2 homologue — yields adults with an increased life span of up to 85%.

The results of Fabrizio and colleagues extend conservation of this signalling pathway to the yeast *Saccharomyces cerevisiae*. Their screen for long-lived mutants in non-dividing yeast revealed mutations in, among other genes, *SCH9*. The product of this gene is homologous to Akt/protein kinase B, which is involved in insulin signalling in mammals.

PROTEIN MODIFICATION

Multitasking molecules

The acetyltransferase p300 and its relative CREB-binding protein (CBP), originally discovered as transcriptional co-activators, have more recently been implicated in tumour suppression. Our view of how widespread their functions are during this process is in continual flux as more and more of their targets are identified. The latest update comes from two papers in *EMBO Journal* and *Nature* that now implicate them in regulating p53 stability and aiding DNA repair.

p53 is acetylated in response to several environmental insults, including hypoxia and oxidative stress, emphasizing the importance of this modification in the upregulation of p53. Ito and colleagues show that acetylation of p53 accompanies the kinetics of its stabilization, leading them to ask what factors might mediate this acetylation. Previous studies indicate that p300 and CBP can acetylate p53 *in vitro*, making them prime candidates. To confirm that this is physiologically relevant, the authors overexpressed p300 or CBP *in vivo* and showed that both could induce p53 acetylation.

How does acetylation promote p53 activity? One possibility is that it contributes to protein stability — a possibility that that authors tested and confirmed. In the presence of a deacetylase inhibitor, trichostatin A (TSA), they found that the half-life of p53 increased markedly.

Next, the authors asked whether factors that negatively regulate p53 activity might do so by interfering with this acetylation step. They looked to see whether mouse double minute 2 (MDM2) — an inhibitor of p53 activity — affects the acetylation of p53. Overexpression of MDM2, they showed, reduced p53 acetylation in a dose-dependent manner. As p300 protein levels remained constant throughout this experiment, they concluded that MDM2 has a direct effect on p53 acetylation. This was confirmed by the fact that addition of TSA blocked this inhibition. And importantly, $p19^{Arf}$, an inhibitor of MDM2, restores p53 acetylation.

The conclusion is that p53 stability is regulated by balancing its acetylation, mediated by p300 and CBP, and deacetylation, promoted by MDM2. The authors speculate that reversible acetylation, by inhibiting ubiquitylation, might have a more general role in regulating protein stability — how widespread remains to be seen.

In a second paper, Hasan and colleagues describe a function for p300 in DNA repair. Fibroblasts lacking p300 show a severely reduced ability to synthesize DNA, an observation that led Hasan and colleagues to investigate whether p300 acts as a cofactor for DNA synthesis. Through immunoprecipitation experiments from nuclear HeLa cell extracts, they found that p300 forms a complex with proliferating cell nuclear antigen (PCNA) — an essential processivity factor for DNA synthesis — that does not depend on the S phase of the cell cycle. Next, using an *in vitro* DNA synthesis assay, they showed that this complex could induce DNA synthesis. To confirm that p300 is a cofactor for DNA synthesis *in vivo*, they conducted chromatin immunoprecipitations, and showed that p300 associates with newly

How do mutations in the various insulin signalling pathways contribute to increased longevity? The papers discuss the possible connections between fertility and life span and also between environmental stress and life span. The conservation of these insulin signalling pathways means that these connections can now be investigated further in not one, but at least three, model systems.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPERS Clancy, D. J. *et al*. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* **292**, 104–106 (2001) | Tatar, M. *et al*. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107–109 (2001) | Fabrizio, P. *et al*. Regulation of longevity and stress resistance by Sch9 in yeast. *Science* **292**, 288–290 (2001)

FURTHER READING Strauss, E. Growing old together. *Science* **292**, 41–43 (2001) Guarente, L. & Kenyon, C. Genetic pathways that regulate ageing in model organisms. *Nature* **408**, 255–262 (2001)

synthesized DNA after ultraviolet treatment of the cells. They then narrowed down the reason for this association, and showed that p300 is involved in DNA repair. This role was supported by the observation that p300 also binds XPA, another DNA repair factor.

The authors propose that, as an acetyltransferase, p300 "might change the structure of the chromatin adjacent to DNA lesions, inducing chromatin changes that facilitate PCNA function and DNA repair synthesis, although the p300 complex is unlikely to affect DNA synthesis directly".

Together, these papers add to the transcriptional repertoire of p300 and CBP, indicating that they have more diverse functions in the cell, including DNA repair and protein stability, both of which might contribute to their role as tumour suppressors.

Alison Schuldt

References and links

ORIGINAL RESEARCH PAPERS Ito, A. *et al.* p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. *EMBO J.* **20**, 1331–1340 (2001) | Hasan, S. *et al.* Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. *Nature* **410**, 387–390 (2001)

PROTEOLYSIS

Cleavage mystery comes to N-end

In 1986, Alexander Varshavsky and colleagues proposed the 'N-end rule', according to which a protein can be earmarked for degradation by the nature of the amino-acid residue at its amino-terminal (N) end. Fifteen years on, Varshavsky and collaborators now describe the first physiological substrate of the N-end rule pathway. Reporting in *Nature* they show that cleavage of SCC1 — a subunit of the cohesin complex, which holds sister chromatids together — generates a fragment that is degraded in an N-end-dependent manner.

The defining feature of an N-end rule substrate is a 'destabilizing' residue at its amino terminus. In yeast and other eukaryotes, the substrate is then recognized by a component of the ubiquitin-conjugating machinery, Ubr1, and destroyed by the ubiquitin–proteasome system. Arginine — a strongly destabilizing residue — is found at the amino terminus of the main SCC1 cleavage fragment, leading Varshavsky and colleagues to investigate the fate of this fragment.

Dipeptides bearing a destabilizing amino-terminal residue can block degradation by the N-end rule pathway *in vivo* — this is just what the authors saw for the SCC1 fragment.

They next engineered in a stabilizing residue at the amino terminus of the SCC1 fragment, and found that overexpression of this variant was toxic to the cell. Given that cleavage of SCC1 is normally required for sister-chromatid separation, might the failure to destroy the SCC1 fragment lead to chromosomal instability? To test this the authors studied *ubr1Δ* cells, which lack the N-end rule pathway, and observed a much higher than normal frequency of chromosome loss. And finally, they confirmed that this effect was indeed due to stabilization of the SCC1 cleavage fragment.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Rao, H. *et al*. Degradation of a cohesin subunit by the N-end rule pathway is essential for chromosome stability. *Nature* **410**, 955–959 (2001)

IN BRIEF

OXYGEN SENSING

HIF-α targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing.

Ivan, M. *et al*. *Science* **292**, 464–468 (2001)

Targeting of HIF-α to the von Hippel–Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation.

Jaakkola, P. *et al*. *Science* **292**, 468–472 (2001)

Hypoxia-inducible factor (HIF) is a transcriptional complex that regulates gene activity in response to levels of O_2 — when O_2 is present, HIF is destroyed by a complex involving the VHL protein. These papers show that the HIF–VHL interaction is regulated by hydroxylation of a proline residue on the HIF-1α subunit, suggesting that proline hydroxylation could be key in cellular responses to O_2.

DEVELOPMENT

Drosophila Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton.

Winter, C. G. *et al. Cell* **105**, 81–91 (2001)

The Frizzled (Fz)/Dishevelled (Dsh) pathway mediates planar cell polarity. But how does it regulate cytoskeletal reorganization? Winter and colleagues now report that *Drosophila* Rho-associated kinase acts downstream of Fz/Dsh to phosphorylate nonmuscle myosin II and restrict F-actin bundle formation.

TECHNIQUE

Attomole level protein sequencing by Edman degradation coupled with accelerator mass spectrometry.

Miyashita, M. *et al. Proc. Natl Acad. Sci. USA* **98**, 4403–4408 (2001)

The limit for amino-terminal sequencing has just been pushed down to the low attomole level. The authors have increased the sensitivity of Edman sequencing by introducing accelerator mass spectrometry — a tandem spectrometry tool that counts rare atoms — as a detection system.

EXTRACELLULAR MATRIX

Extracellular matrix composition determines the transcriptional response to epidermal growth factor receptor activation.

Yarwood, S. J. & Woodgett, J. R. *Proc. Natl Acad. Sci. USA* **98**, 4472–4477 (2001)

Integrins transduce signals from the extracellular matrix (ECM) and modulate receptor tyrosine kinases. The authors used DNA arrays to monitor EGF-induced gene expression after attachment of cells to either laminin or fibronectin. Although expression of most genes was not influenced by the type of ECM, certain gene clusters responded differentially. This survey should be valuable for further analysis of the crosstalk between ECM and growth factor signalling.