WEB WATCH

Free associations

- The Genetic Association Database: http://www.grc.nia.nih.gov/branches/rrb/dna/association

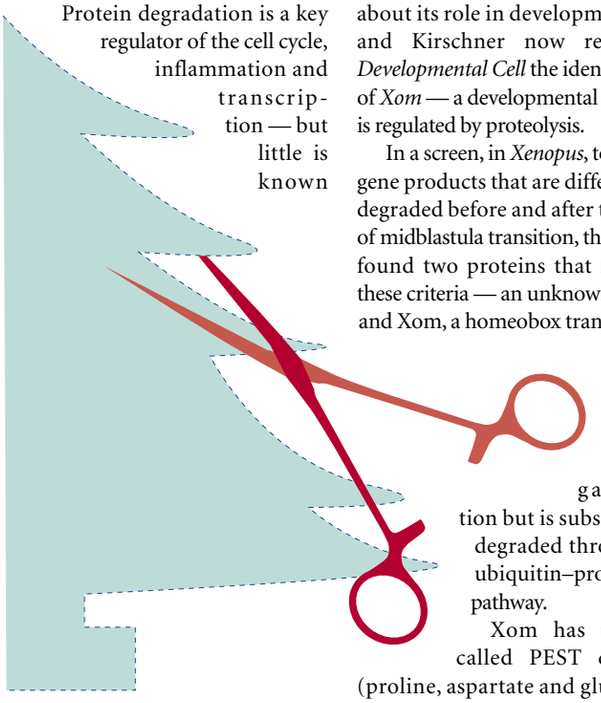
Association studies have finally found a home of their own. The steadily increasing number of these studies — which report a link between one or more genetic polymorphisms and specific human disorders — is filling the literature databases. So, researchers have for some time needed a single, comprehensive repository of all the results that have been published so far. Such a resource has now become available thanks to efforts headed by Kevin Becker at the National Institute on Aging/NIH, who has compiled a freely accessible web site — the Genetic Association Database (GAD) — that catalogues the results of over 700 association studies. The site is still under development and will grow both in content and utility. Nevertheless, its features are clear to see. The studies can be queried according to disease and phenotypic information, as well as according to various aspects of a study's design, such as study sample size or the statistical significance of the association. Links are available, through PubMed, to the original publication, and comments that could be potentially useful to other investigators can also be posted for each study. Importantly, the database also includes studies in which no genetic association was found.

With 75–100 association studies published each week, GAD should attract a lot of traffic. Anyone can submit the results of an association study to the site, provided that permission is obtained from the curator. The future of the database — the only one of its kind — could be made even rosier if Becker realizes his ambition to enrich the site by integrating it with gene-expression and NCBI databases.

Tanita Casci

DEVELOPMENTAL BIOLOGY

Cutting out a pattern



Protein degradation is a key regulator of the cell cycle, inflammation and transcription — but little is known about its role in development. Zhu and Kirschner now report in *Developmental Cell* the identification of *Xom* — a developmental gene that is regulated by proteolysis.

In a screen, in *Xenopus*, to identify gene products that are differentially degraded before and after the onset of midblastula transition, the authors found two proteins that matched these criteria — an unknown protein and Xom, a homeobox transcription factor. Xom is stable during early gastrulation but is subsequently degraded through the ubiquitin–proteasome pathway.

Xom has two so-called PEST domains (proline, aspartate and glutamate, serine, or threonine-rich regions), one of which turned out to be required for Xom degradation. Interestingly, this so-called 'Xom destruction motif' (XDM) resembles the glycogen synthase kinase 3 (GSK3) consensus phosphorylation site, which is conserved in the known substrates of GSK3-dependent proteolysis, such as β-catenin.

In the XDM, the authors found two phosphorylation sites at Ser140 and Ser144. In an *in vitro* assay, an exogenous peptide of the phosphorylated XDM blocked Xom degradation, paradoxically so did the unphosphorylated peptide. However, phosphorylation of XDM seems to be important for Xom degradation because the same peptide, when it contains serine-to-alanine mutations at positions 140 and 144, cannot block Xom degradation *in vitro*, implying that the embryonic extract used in these assays contained a kinase activity, although this turned out not to be GSK3.

Using *in vitro* binding assays, Zhu and Kirschner next found that the E3 ubiquitin ligase Skp1–Cullin–F-box complex (SCF),

HUMAN DISEASE

A clear suspect

Approximately 0.05% of the Western population suffers from systemic lupus erythematosus (SLE) — a complex autoimmune disease. Although several susceptibility loci for SLE have been identified, the nature of the genes and mutations that underlie this disease have remained unknown. Now, Prokunina *et al.* report in *Nature Genetics* the association of the programmed cell death gene 1 (*PDCD1*) with SLE. Importantly, they also propose how a particular sequence variant of *PDCD1* might contribute to the disease's aetiology.

In a previous study of a Nordic population, the authors identified a susceptibility locus for SLE on chromosome 2. One gene in this region stood out as a potential candidate, *PDCD1*. This is because *PDCD1* encodes an immunoreceptor that belongs to the immunoglobulin family and that is known to regulate peripheral self-tolerance in T and B cells. Moreover, *Pdcd1*$^{-/-}$ mice suffer from SLE-like symptoms.

By sequencing *PDCD1* in five healthy unrelated individuals and in five SLE sufferers from the Nordic population, the authors discovered seven SNPs in this gene, three of which constituted a disease-associated haplotype that could account for all of the LOD score seen in the original population sample. These SNPs were then genotyped in five sets of families with different ethnic origins. The results were clear — only one SNP, which lies in an enhancer-like region in intron 4 of *PDCD1*, consistently associated with SLE. This region of intron 4 contains binding sites for transcription factors that are known to be involved in haematopoietic differentiation and in inflammation. In particular, the SNP disrupts a putative binding site for RUNX1, which is inactivated in translocations that lead to acute myeloid leukaemia. Using an electrophoretic mobility shift assay, the authors confirmed that RUNX1 indeed binds to this sequence and that this binding is abolished by the sequence change that is associated with the SNP.

The authors propose that RUNX1 binding to the wild-type

which contains the F-box protein β-TRCP, is responsible for Xom degradation. And they were able to show that a dominant-negative form of β-TRCP could block Xom degradation *in vivo*. Intriguingly, the same E3 ubiquitin ligase complex also mediates the phosphorylation-dependent degradation of β-catenin.

So, what is the role of Xom degradation in early *Xenopus* development? BMP4 is a ventral morphogen that acts together with Xom in an auto-activating feedback loop in which Xom is activated by BMP and vice versa. In addition, Xom inhibits transcriptional activity of dorsal-specific genes that, in turn, inhibit BMP activity. Zhu and Kirschner hypothesize that the proteolysis of Xom might be needed to cease Xom-mediated repression of dorsal-specific genes, such as *goosecoid*, during early gastrulation. If true, the auto-activating circuit would be eliminated, leading to dorsoventral asymmetry in the mesoderm and to the loss of BMP expression on the dorsal side and high expression on the ventral side of the embryo.

Indeed, luciferase reporter assays showed that non-degradable Xom is ~20 times better at inhibiting transcriptional activation of *goosecoid* than wild-type Xom. Consistent with this result, embryos with non-degradable Xom have truncated heads — which is typical of an enhanced ventralized phenotype. This effect is restricted to the dorsal side, not surprisingly, as Xom's effects are restricted to that part of the embryo.

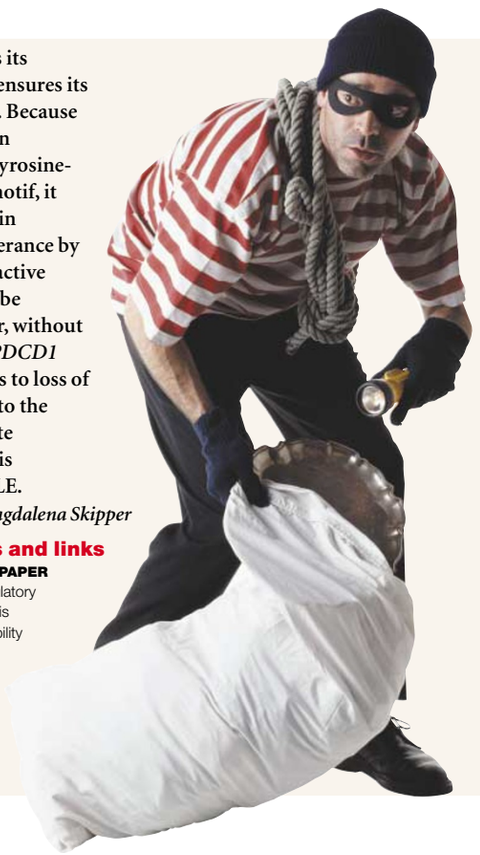
On the basis of these findings, Zhu and Kirschner propose that the correct dorsoventral BMP expression pattern in the mesoderm during gastrulation in the frog depends on the specifically timed proteolysis of Xom. But how Xom is stabilized during early gastrulation and what turns on its subsequent degradation remain unknown.

Arianne Heinrichs, Senior Editor,
Nature Reviews Molecular Cell Biology

References and links

ORIGINAL RESEARCH PAPER Zhu, Z. & Kirschner, M. Regulated proteolysis of Xom mediates dorsoventral pattern formation during early *Xenopus* development. *Dev. Cell* **3**, 557–568 (2002)

WEB SITE
Marc Kirschner's laboratory:
http://cellbio.med.harvard.edu/faculty/kirschner



***PDCD1* modulates its transcription and ensures its correct expression. Because *PDCD1* contains an immunoreceptor tyrosine-based inhibitory motif, it might be involved in preserving self-tolerance by inhibiting auto-reactive cells. It remains to be confirmed whether, without RUNX1 binding, *PDCD1* dysregulation leads to loss of self-tolerance and to the chronic lymphocyte hyperactivity that is characteristic of SLE.**

Magdalena Skipper

References and links

ORIGINAL RESEARCH PAPER
Prokunina, L. *et al.* A regulatory polymorphism in *PDCD1* is associated with susceptibility to systemic lupus erythematosus in humans. *Nature Genet.* 28 October 2002 (10.1038/ng1020)

IN BRIEF

EVOLUTION

Variation in gene expression within and among natural populations.

Oleksiak, M. F. *et al. Nature Genet.* **32**, 261–266 (2002)

By assaying genome-wide gene expression levels in three fish populations, the authors confirm what neutral theory proposes: that much of the significant variation in gene expression levels between populations is probably due to random genetic drift and reflects within-population variation. But, the authors also found differences in gene expression levels that were environmentally, rather than genetically, determined, supporting the theory that important evolutionary adaptations proceed through variation in gene expression rather than coding sequence changes.

IMPRINTING

Regional loss of imprinting and growth deficiency in mice with a targeted deletion of *KvDMR1*.

Fitzpatrick, G. V. *et al. Nature Genet.* **32**, 426–431 (2002)

Beckwith–Wiedemann syndrome (BWS) predisposes to excessive growth, and is characterized by loss of maternal-specific imprinting in a putative imprinting control region, *KvDMR1*. Fitzpatrick *et al.* report that paternal inheritance of a *Kvdmr1* deletion reduces mouse growth and causes the de-repression of six genes in *cis* of *Kvdmr1*, including the cyclin-dependent kinase inhibitor *Cdkn1c*. These findings indicate that methylation loss in BWS patients activates *KvDMR1*-mediated repression on the maternal chromosome to cause abnormal *CDKN1C* silencing.

GENE REGULATION

Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression.

Winkler, W. *et al. Nature* **419**, 952–956 (2002)

Many different post-transcriptional mechanisms for gene regulation exist. Here, Winkler *et al.* show that mRNA can also block its own translation. The authors show that mRNAs that encode *Escherichia coli*'s enzymes of vitamin B_1 biosynthesis can bind vitamin B_1 or its derivatives in the absence of a protein cofactor. This complex binds to other mRNAs of the same species and, by sequestering a ribosome-binding site, prevents their translation.

SEX DETERMINATION

Exploring the envelope: systematic alteration in the sex-determination system of the nematode *Caenorhabditis elegans*.

Hodgkin, J. *Genetics* **162**, 767–780 (2002)

Sex is almost ubiquitous in nature and is determined in various ways — for example, by chromosomal or maternal cues — indicating that its regulation might undergo rapid evolutionary change. Hodgkin has confirmed this hypothesis: by using the detailed knowledge of the sex-determination system in *C. elegans*, he has created a collection of stable worm strains that artificially mimic many of the sex-determination systems found in nature.