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GENE EXPRESSION

A new take on expression

Variation in gene expression can be studied just like any other quantitative genetic trait. This is the conclusion published in *Nature* by Morley, Molony *et al.*, who used a combination of microarray analysis and genome-wide linkage analysis.

Natural variation in gene expression is widespread. Realizing that herein lies the key to the inter-individual phenotypic variation, including susceptibility to complex diseases, the authors set out to see how amenable this variation would be to genetic study. First, they used microarrays to identify the variable loci. To do this, they measured differences in the expression of ~8,500 human genes from immortalized B cells, and selected the 3,554 most variable genes for subsequent mapping. Second, they used a computer program called SAGE to carry out genome-wide linkage analysis in 14 families from the CEPH (Centre d'Étude du

Polymorphisme Humain) panel, which was also the source of the expression data. Using two stringency criteria (genome-wide $p = 0.001$ and 0.05), they detected linkage for 142 and 984 expression phenotypes, respectively.

The regions that are linked to the expression phenotypes — the regulators — can be in *cis* (according to the authors' criteria, these have to lie within 5 Mb of the target gene) or in *trans*. Although most target genes are associated with regulators in *trans*, few have multiple regulators. The authors point out that rather than reflecting a biological phenomenon, this result might simply be due to the difficulty in detecting multiple regulators, as each of them would have only a weak effect on its target.

The authors found what they term 'master regulators of transcription' — transcriptional regulators that influence many expression phenotypes.

Two hot spots of such regulators were found: one on chromosome 14 and one on chromosome 20. Furthermore, some targets of the master regulators showed coordinated levels of expression.

For the handful of genes, the expression levels of which are regulated by determinants in *cis*, the authors used the results of linear regression to estimate the proportion of the variation that can be attributed to these *cis* factors. It turns out that for some genes, they account for more than half the variation in expression. The authors point out that the remainder of the variation is probably caused by a variety of factors, including the environment.

So, the combination of expression profiling and genome-wide mapping has allowed the authors to identify regulatory elements without any previous knowledge of the regulatory mechanism. It also uncovered a complex network of multiple regulators, including master elements. Morley and Molony *et al.* have shown us that it is possible to move beyond the experimental manipulation of gene expression, towards revealing the genetic interactions that underlie natural variation in expression.

Magdalena Skipper

References and links

ORIGINAL RESEARCH PAPER Morley, M. & Molony, C. M. *et al.* Genetic analysis of genome-wide variation in human gene expression. *Nature* 21 July 2004 (doi:10.1038/nature02797)

WEB SITE

Vivian Cheung's laboratory:
<http://genomics.med.upenn.edu/vcheung>



WEB WATCH

Integr8 genome and proteome browser

• <http://www.ebi.ac.uk/integr8>

With the explosion in genomic and proteomic data stored in a variety of databases, there is an increasing need for this information to be brought together in a way that is easy to access and intuitive to use. In response to this need, the European Bioinformatics Institute has recently launched the Integr8 web site, providing a one-stop shop for researchers exploring gene and protein function.

Integr8 currently houses data on more than 180 species of archaeans, bacteria and eukaryotes, providing information on taxonomy, genome structure and proteomics. For each species, proteins are classified according to sequence similarity, structure and function, giving the user an overview of proteome content. There is also the option of comparing this information across several species. Alternatively, if you have a particular gene or protein in mind, a simple search pulls out a wealth of information, including links to entries in gene and protein databases, structural data, key references from the published literature and a summary of expression patterns and function.

The site also provides several other useful resources. For example, the BioMart tool allows complex searches of several databases using a range of criteria, such as species name, chromosomal region, and protein structure and function. There is also access to FASTA similarity and homology searching, enabling comparisons with sequences from proteome and genome databases.

So, Integr8 truly lives up to its name, and should allow researchers from diverse fields to make the most of the increasing availability of genomic and proteomic information.

Louisa Flintoft

GENOME EVOLUTION

Give and take

With some parasites it's just "take... take... take" and this can include the host's genes! However, other unwelcome guests actually make generous DNA donations to their hosts.

Independent studies of *Trypanosoma cruzi* — the protozoan that causes Chagas disease — and the Rafflesiaceae — a family of plants that rely entirely on their hosts for nutrition — show that there have been horizontal gene transfers between these parasites and their respective vertebrate and plant hosts.

Following up on the hypothesis that frequent integration of *T. cruzi* DNA into the host genome might underlie Chagas disease, Nadjar Nitz and colleagues extracted genomic DNA from 13 patients with Chagas disease. A probe derived from components of the *T. cruzi* mitochondrial DNA (kinetoplast minicircles; kDNA) was then Southern hybridized to these extracts. This hybridization showed

that there were smaller fragments of kDNA than would be expected if it were present in its native form, which indicated that kDNA had integrated into the host genome.

Using 5'-RACE (rapid amplification of cDNA ends), the authors isolated the genomic integration sites in each patient that they studied, identifying integration sites in a total of 5 loci. But could such integration events be observed in an experimental system?

To address this question, the authors examined rabbits that had been experimentally infected with *T. cruzi* for up to 3 years. These rabbits had *de novo* kDNA integrations, which indicated that horizontal transfer of parasite DNA to the host could be a normal part of the infection process. The rabbit and human data also indicated that β -globin loci and long interspersed nuclear elements (LINE-1) are frequent targets for kDNA integrations.

RNA WORLD

RNA stories on a mythical scale

RNA interference (RNAi) hardly disappears off the science headlines. Most recently, the spotlight has been on Argonaute proteins — some of which are involved in the small interfering RNA (siRNA) and microRNA (miRNA) pathways. Okamura and colleagues reveal an interesting division of labour between the Argonautes in *Drosophila melanogaster*: Argonaute1 (AGO1) is required for miRNA maturation and miRNA-directed RNA cleavage, whereas Argonaute2 (AGO2) acts in the siRNA pathway.

siRNA... miRNA... the difference is more than semantic. siRNAs are the key agents for RNAi and mediate RNA destruction in a sequence-specific manner. Although miRNAs

can direct RNA cleavage, they can also block translation of their targets. Both species of small RNAs carry out their functions as part of the RNA-silencing complex (RISC), a multi-protein complex that mediates RNA cleavage. Given that both types of small RNAs associate with the same RISC, do the two pathways converge at this level, or do they differ?

Prompted by previous studies in the worm, Okamura and colleagues decided to resolve the issue by looking at *Ago1* and *Ago2* mutants in *Drosophila*. They mobilized *P*-elements to delete *Ago2* and found that it is required for RNAi *in vivo* and for RISC assembly; in particular, AGO2 is required for the



Interestingly, the authors went on to show that kDNA was also integrated in the genomes of the offspring of chronically infected rabbits and in chickens hatched from *T. cruzi*-inoculated eggs. Importantly, they also found kDNA integrations in the germline of F₂ chickens without persistent infections, conclusively showing vertical transfer to infection-free progeny and so quashing any chance that their results could be artefactual.

unwinding of the siRNA, which is a prerequisite for siRNA-mediated cleavage. Although the RISC is associated with both siRNAs and miRNAs, *Ago2* is required exclusively for the siRNA pathway.

AGO1, on the other hand, seems to be involved only in the miRNA pathway. *Ago1* mutant flies die as larvae with many developmental defects, and miRNA cleavage does not occur in lysates from *Ago1* mutant embryos. *In vivo* analysis indicates that AGO1 directly interacts with Dicer-1, which is required for miRNA production from larger precursors, and that the role of *Ago1* is to stabilize mature miRNAs.

In mammals, siRNA and miRNA pathways seem to converge downstream of Dicer. This is not the case in other organisms, such as worms and flies. Okamura and colleagues have elegantly shown that it is *Ago1* and 2 (as part of RISC) at



that a section of the host mitochondrial genome was transferred to the parasite.

The huge significance of horizontal gene transfer for the evolution of prokaryotes has been known for a long time, as has the large contribution that intracellular endosymbiont ancestors of mitochondria and chloroplasts have made to eukaryotic genomes (see further reading). Studies such as these show that ongoing horizontal gene transfer from a range of parasites and endosymbionts might be more important for eukaryotic evolution than we previously realized — just how important remains to be seen.

Nick Campbell

References and links

ORIGINAL RESEARCH PAPERS Nitz, N. *et al.* Heritable integration of kDNA minicircle sequences from *Trypanosoma cruzi* into the avian genome: insights into human Chagas disease. *Cell* **118**, 175–186 (2004) | Davis, C. C. & Wurdack, K. J. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. *Science* 15 July 2004 (doi:10.1126/science.1100671)

FURTHER READING Brown, J. R. Ancient horizontal gene transfer. *Nature Rev. Genet.* **4**, 121–132 (2003) | Timmis, J. N. *et al.* Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nature Rev. Genet.* **5**, 123–135 (2004)

WEB SITE

Multidisciplinar de Pesquisa em Doença de Chagas: <http://www.unb.br/lb/cel/pg/pt-br/laboratorios/chagas.php>

Charles Davis and Kenneth Wurdack took an entirely different, but no less convincing, approach to their analysis of horizontal gene transfer between plants. Their phylogenetic analyses showed that, although Rafflesiaceae are close relatives of members of the order Malpighiales, the rafflesian mitochondrial *nad1B-C* gene is more closely related to the homologue in its obligate host, *Tetrastigma* (Vitaceae). Clearly, this is strong evidence

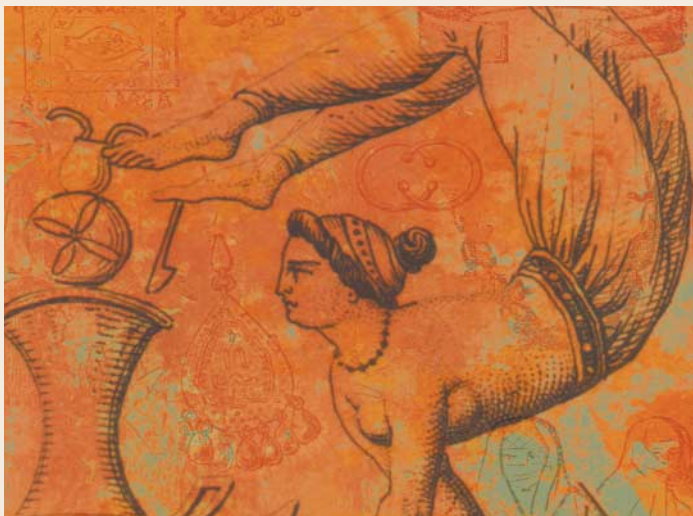
the heart of the difference between siRNA- and a miRNA-mediated RNA cleavage. Undoubtedly, the small RNA world has many more equally elegant stories to tell us.

Magdalena Skipper

References and links

ORIGINAL RESEARCH PAPER Okamura, K. *et al.* Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. *Genes Dev.* **18**, 1655–1666 (2004)

FURTHER READING Liu, J. & Carmell, M. A. *et al.* Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 29 July 2004 (doi:10.1126/science.1102514)



IN BRIEF

PLANT GENETICS

Plant Reproduction

Plant Cell **16** Supplement (2004)

This supplement to the June issue of *Plant Cell* is entirely devoted to plant reproduction, and comprises 19 review articles on flowering, fertilization and on the development of the gametophyte, seed and fruit. The previous supplement was published in 1993, so this is a valuable update on our mechanistic understanding of plant reproduction and its application to agriculture. It will be a great resource for those who teach developmental biology or genetics, for researchers in the plant field and those generally interested in sexual reproduction.

GENE EXPRESSION

Role of transposable elements in heterochromatin and epigenetic control.

Lippman, Z. & Gendrel A.-V. *et al. Nature* **430**, 471–476 (2004)

Making use of microarrays, these authors show that, as predicted from research on other organisms, *Arabidopsis* heterochromatin is composed of transposable elements (TEs) and other related tandem repeats. Their expression is regulated by a chromatin-remodelling protein DDM1 that might be guided by small interfering (si) RNAs that are complementary to the heterochromatic sequences. In addition, TEs can epigenetically regulate individual genes if they lie close to the locus in question, indicating that euchromatic loci such as *FWA* might be imprinted by a siRNA-guided DDM1-dependent mechanism.

PHARMACOGENETICS

Breed distribution and history of canine *mdr1-1Δ*, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage.

Neff, M. W. *et al. Proc. Natl Acad. Sci. USA* **101**, 11725–11730 (2004)

When treated with the anti-parasitic drug ivermectin some dogs, including collies, can die from neurotoxicosis. To investigate the origin of the 4-bp-deletion in the drug transporter gene *mdr1* that underlies this adverse response, Mark Neff and colleagues surveyed dog populations. The causal allele was isolated in seven new dog breeds and found to have originated prior to dog breeds being isolated.

GENETIC DISEASE

Pms2 is a genetic enhancer of trinucleotide CAG·CTG repeat somatic mosaicism: implications for the mechanism of triplet repeat expansion.

Gomes-Pereira, M. *et al. Hum. Mol. Genet.* **13**, 1815–1825 (2004)

The expansion of trinucleotide repeats causes several progressive genetic disorders, but the genetic mechanism underlying repeat instability is unclear. These authors have addressed the issue by studying a transgenic CAG·CTG repeat in somatic tissue in the mouse. The rate of expansion is reduced by 50% in animals that are mutant for the DNA mismatch repair gene *Pms2*, implicating inappropriate mismatch repair in repeat instability.

TECHNOLOGY

No-bake recipe for DNA

Modern genetics relies so heavily on PCR that it is hard to imagine laboratory life without it. However, although PCR was indisputably one of the biggest breakthroughs in genetics — transforming fields from forensic science to disease diagnosis — the thermal cycling at the heart of this technique makes it hungry for energy, time and special equipment. By using a bacterial helicase instead of heat to denature the template, Huimin Kong and colleagues have now overcome the limitations of PCR by devising a means to amplify DNA at a single temperature. This opens the door to the development of quick, hand-held diagnostic devices that could move DNA analysis from the bench into the field.

The new amplification technique, called helicase-dependent amplification (HDA), mimics a process that occurs in nature. A helicase — in this case the *Escherichia coli* UvrD helicase — is first used to separate the dsDNA template molecules. The separated strands are then coated with ssDNA binding proteins to keep them apart, allowing primers

to anneal and be extended by a polymerase. The reaction occurs at 37°C, from start to finish, and can achieve over a million-fold amplification of genomic DNA. It is also sensitive enough to pick up DNA from intact cells and to detect pathogen DNA in blood.

HDA is not the first isothermal reaction to be proposed, but the alternatives involve more complex reactions and rely on an initial denaturing step that requires heat. Essential tweaks are now being carried out to improve the efficiency of HDA. By experimenting with the reaction components — nature has hundreds of helicases to choose from — and their concentrations, Kong and colleagues hope to bring reaction times down to 15 minutes. Machine-free DNA amplification ‘while-you-wait’ might be just around the corner.

Tanita Casci

 **References and links**

ORIGINAL RESEARCH PAPER Vincent, M., Xu, Y. & Kong, H. Helicase-dependent isothermal DNA amplification. *EMBO Reports* 9 July 2004 (doi:10.1038/sj.embor.7400200)

WEB SITE
New England Biolabs: <http://www.neb.com>



GENOME EVOLUTION

Mutations: more common than you thought



Mutation rate, whether per locus or genome-wide, features as a variable in many calculations that underlie evolutionary genetic hypotheses. But a true experimental estimate of mutation rate is difficult to obtain. To this end, several groups have used ‘mutation-accumulation’ lines in both *Drosophila melanogaster* and *Caenorhabditis elegans*: in an attempt to minimize selection pressure, genetically identical flies

or worms are maintained for many generations in a benign environment. Such assays are long and tedious: for one collection, scientists even separated each worm in each generation from everyone else to avoid any selection pressure induced by the stressful dating scene, and instead, self-fertilize.

Previous studies have used fitness-based assays to assess the number of mutations that developed in each line, yielding an indirect estimate of mutation rate. For a more direct estimate, Denver and colleagues have now revisited a collection of these special worm lines and sequenced more than 4 Mb of loci scattered around the genome. In a recent paper in *Nature*, they report the total haploid genomic mutation rate to be approximately 2.1 mutations per genome per generation — an estimate that is an order of magnitude higher than previous best guesses, and 2 orders higher than the indirect estimate from the same collection (although the previous estimates referred to deleterious mutation rate, whereas these authors estimate

total mutation rate). Not only that, but the more frequently observed mutations were insertions, in contrast to reports based on pseudogenes that indicated that most naturally occurring mutations in worms are deletions.

So, with one study, Denver and colleagues prompt the entire field to rethink the process of mutation over time and our measurement of it. It does not take long for this to generate controversy: in a thought-provoking News and Views piece, Rosenberg and Hastings speculate on the mechanisms at work in the Denver study. Either previous estimates were wrong because they only detected mutations that produce phenotypes, as Denver *et al.* would suggest, or the new study uses methods that push the worms to develop more mutations even in the absence of deleterious selection, maybe even through triggering stress-response pathways. Future studies might have to bring the worms out of their posh retirement to settle the question.

Chris Gunter, Senior Editor, Nature

 **References and links**

ORIGINAL RESEARCH PAPER Denver, D. R. *et al.* High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature* **430**, 679–682 (2004)

FURTHER READING Rosenberg, S. M. & Hastings, P. J. Worming into genetic instability. *Nature* **430**, 625–626 (2004)

WEB SITE
NemATOL: <http://nematol.unh.edu/index.php>

IN THE NEWS

Autism Genome Project
The National Alliance for Autism Research has announced the launch of the largest autism study ever conducted.

The study, which is a collaboration between some 170 leading geneticists worldwide, will use Affymetrix DNA microarrays to scan the whole human genome in search of genes that are associated with inherited risk to autism. Approximately 50 academic and research institutes will participate in this public/private research partnership, which will cost in excess of US \$2 million.

In the first phase, approximately 6,000 samples of DNA from 1,500 multiplex families (2 children with autism spectrum disorders and their parents) from all over the world will be analysed. The initial results are expected by early 2005.

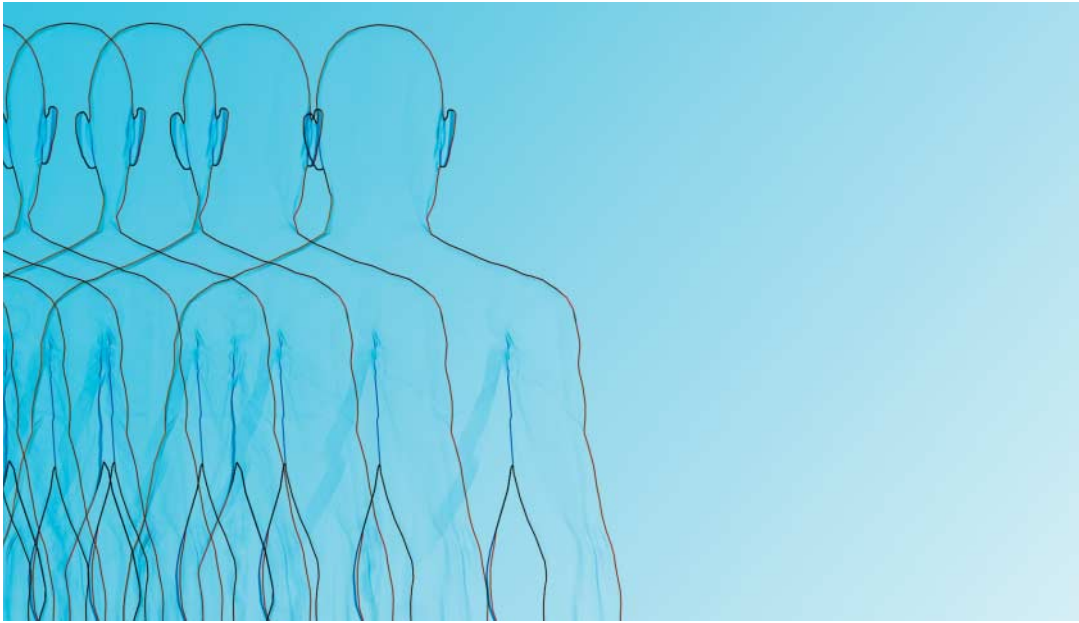
An ancestry test for dessert?
Shish, a London-based restaurant that specializes in Central Asian cuisine, is offering DNA tests to its diners to find out if they are descended from Genghis Khan.

“The unusual promotion is to mark the Mongolian government’s decision to allow citizens to have surnames for the first time since they were banned by the communists in the 1920s” (*The Daily Times*, Pakistan).

Although the offer was available for only a week, the test is offered by Oxford Ancestors, for £195, as part of their Y-clan analysis. As their web site claims — “there are now around 16 million men who have inherited their Y-chromosomes directly from Genghis Khan.”

“The results will take two months and descendants will win a free meal for two” (BBC News) courtesy of Shish!

Magdalena Skipper



HUMAN GENETICS

Variations on a theme

The human genome is littered with small-scale genetic variants, such as SNPs and repeat-length polymorphisms, but little is known about the way in which variants involving larger regions contribute to genetic diversity. Two studies now reveal that large deletions and duplications are more common than was previously thought, prompting a re-evaluation of the way we view human genetic variation.

Large-scale copy-number polymorphisms/variants (CNPs/LCVs) — deletions or duplications of chromosomal segments — have been identified previously from healthy individuals, but technical limitations have prevented an assessment of whether these variants are common on a genome-wide scale. In a collaborative study, Michael Wigler and colleagues developed a method called ROMA (representational oligonucleotide microarray analysis) that enables deletions or duplications to be identified. This involves digesting genomic DNA, amplifying the fragments, attaching a fluorescent label and hybridizing them to an array of complementary probes. The signal strength of each probe indicates the copy-number of the corresponding

genomic region, which can be compared between samples. Using an average of 1 probe every 35 kb, Wigler and colleagues analysed samples from 20 unrelated, healthy individuals from a range of geographical locations. They identified a set of 76 different CNPs, involving regions of 100 kb or more, that varied between individuals, with an average of 11 CNP differences between each pair of subjects. The polymorphisms included both deletions and duplications — most of which have not been identified before — with a mean length of 465 kb. Most regions of the genome had CNPs, although they were noticeably more frequent in some regions, suggesting that there might be CNP ‘hotspots’.

Importantly, many of these CNPs are in regions that contain genes, so this type of variation might influence levels of gene expression and lead to phenotypic differences between individuals. For example, one CNP-variant contained three copies of the gene *PPYR1*, which encodes the appetite-regulating neuropeptide Y4-receptor. CNPs are also present in regions that include genes implicated in nervous-system development, leukaemia and drug resistance. So, it is possible that

large-scale CNPs might underlie variation in a diverse range of phenotypes, from body weight to cancer susceptibility.

In a second study, Charles Lee and colleagues used a similar technique to identify LCVs in samples from 39 healthy individuals. They identified 255 polymorphisms in the human genome: an average of 12 CNPs for each subject.

The authors of both papers point out that their studies are not comprehensive, as the probes that they used represent only a fraction of the genome. Studies using larger sets of probes are planned for the future, which should reveal the full extent to which large-scale polymorphisms contribute to the genetic differences that underlie human individuality.

Louisa Flintoft

References and links

ORIGINAL RESEARCH PAPERS

Sebat, J. *et al.* Large-scale copy-number polymorphism in the human genome. *Science* **305**, 525–528 (2004) | lafrate, A. J. *et al.* Detection of large-scale variation in the human genome. *Nature Genet.* 1 August 2004 (doi:10.1038/ng1416)

WEB SITES

Michael Wigler’s laboratory:
<http://www.cshl.org/public/SCIENCE/wigler.html>
 Charles Lee’s laboratory:
http://labmed.bwh.harvard.edu/pathology/Faculty/Charles_Lee.htm

WEB WATCH

The NHGRI Policy and Legislation Database

• <http://www.genome.gov/LegislativeDatabase>

A new web-based searchable database for US genetics-related policy and legislative documents promises to be a valuable resource for anyone interested in this ever-expanding and important area.

Francis Collins, director of the US National Human Genome Research Institute (NHGRI), said this tool will be useful for everybody "...from academic researchers seeking to patent genetic technologies to average citizens trying to determine what protections exist in their states against genetic discrimination."

Database users can find legislation and laws from specific states through an interactive US map, as well as doing more specific searches for particular combinations of content type (for example, federal legislative materials), topic (such as genetic testing and counselling) and source (for example, the Department of Health and Human Services). Keyword searching is also possible, but only for words in document titles, not those in their content. Perhaps the most useful 'value-added' feature that the database incorporates is a summary, in layman's terms, of each document: this enables quick identification of relevant documents without the need to trawl through pages of legal jargon.

Of course, one of the biggest limitations of this extremely useful tool is that it only encompasses US policy and legislation. However, this focus is understandable given that the US National Institutes of Health fund this resource. Moreover, the addition of further content categories this autumn, such as foreign statutes and laws and policy material from international organizations, will, at least partially, address this limitation.

Nick Campbell

EPIGENETICS

Family feud

Although relatively few in number, imprinted loci have been of intense interest. The *Gnas* locus on distal mouse chromosome 2 is one of the more complex imprinted regions yet discovered, having an antisense transcript and alternatively spliced isoforms that show biallelic as well as maternal- and paternal-specific expression. The parent-of-origin-specific expression of these and other imprinted genes has yielded insights into the mechanisms of gene regulation, whereas the functional role of imprinting has been one of biology's most intriguing mysteries. Two new studies on the *Gnas* cluster published in *Nature Genetics* advance our understanding on both fronts.

The *Gnas* locus encodes alternative transcripts that arise from 4 different promoters, with different first exons spliced to a common exon 2. Among these, *Gnas* encodes G_{α} , the α -subunit of the ubiquitous heterotrimeric G protein G_{γ} , which couples receptors to adenylyl cyclase. Although *Gnas* is biallelically expressed in most tissues, it is preferentially maternally expressed in the proximal tubules of the kidney and in brown and white adipose tissue. Williamson *et al.* have now identified a *cis*-acting element that regulates this tissue-specific aspect of *Gnas* imprinting — the first such control region to be identified.

Deletion of a 2.3-Kb differentially methylated region (DMR) that encompasses exon 1A resulted in increased expression of the paternal allele in the kidney and in adipose tissue. The loss of tissue-specific silencing was confirmed by a clever functional assay, in which mice with parathyroid hormone (PTH) resistance were found to have increased G_{α} -mediated PTH signalling, thanks to increased GNAS production from the paternal allele. The mechanism by which the exon 1A DMR contributes to tissue-specific silencing of the paternal allele remains to be determined.

Plagge *et al.* used a similar gene-targeting approach to examine the function of a paternally expressed *Gnas* transcript, *Gnasxl*, which encodes XL α s. Clues to the function of the various proteins encoded by the *Gnas* locus have come from studies of mice with targeted mutations. Mice that lack G_{α} die shortly after birth, with maternal and paternal transmission showing opposing effects on adipose tissue and metabolic rate. As there is no evidence for exclusive paternal expression of G_{α} in any tissue, attention turned to the paternally expressed GNASXL as having a distinct functional role.

Plagge and colleagues found that mice in which the XL α s-specific XL domain was deleted also died soon after birth. The pups' growth was retarded, they did not suckle and their lipid stores in adipose tissue were depleted. XL α s sites of expression — nuclei that innervate orofacial and tongue muscles, and the pituitary, pancreas and hypothalamus — are consistent with its role in the postnatal adaptation to feeding and in energy homeostasis.

Notably, the XL α s-deficient phenotype provides compelling support for the parental conflict theory of imprinting, which proposes that paternal genes would increase resource uptake from the mother by offspring, and that maternal genes would restrict nutritional demands. Although this theory has received support from mice with mutations in other imprinted genes, the authors note that this is the first example in which an imprinted locus encodes two proteins that act antagonistically in postnatal physiology — regulating cyclic AMP (cAMP) production in this instance. The *Gnas* locus will no doubt continue to provide a unique window into the role of imprinting in negotiating the competing demands of mothers and fathers.

Alan Packer, Senior Editor, Nature Genetics

References and links

ORIGINAL RESEARCH PAPERS Plagge, A. *et al.* The imprinted signaling protein XL α s is required for postnatal adaptation to feeding. *Nature Genet.* **36**, 818–826 (2004) | Williamson, C.M. *et al.* A *cis*-acting control region is required exclusively for the tissue-specific imprinting of *Gnas*. *Nature Genet.* **36**, 894–899 (2004)

FURTHER READING Wilkins, J. F. & Haig, D. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Rev. Genet.* **4**, 359–368 (2003)



EVO-DEVO

Vertebrate face in profile



Faces are one of the most distinctive parts of our anatomy; we use them to tell people apart and to express our opinions and emotions. But how do skeletal, muscle and nerve tissues combine to produce this most engaging part of our body? By analysing a zebrafish mutant, Justin Gage Crump and colleagues show that precise hierarchical interactions between the three tissue types coordinate their assembly in one particular segment of the face.

Vertebrate faces come in many guises but they all develop from seven segmented regions (the pharyngeal arches), among which are intercalated outpockets of endodermal tissue, called 'pouches'. Cranial neural crest cells populate the arches and give rise to cartilage, which the pouches support and pattern. Several mutations disrupt the delicate process that controls the morphogenesis and differentiation of skeletal precursors. For example, mutations in the genes that encode integrins have shown that this conserved family of heterodimeric receptors are involved in signalling and are required for the migration of

neural crest cells to the arches. Now, studies of zebrafish that are mutant for *integrin-α5* have revealed a new link between integrins and face patterning.

The mutant animals, which the authors isolated in a screen for cartilage defects, lack the first pharyngeal pouch and have defects in the cartilage structures derived from the second pharyngeal arch — a phenotype that is linked to the loss of Integrin-α5 function specifically in the first pharyngeal pouch. Embryos with cartilage defects also have muscle and nerve defects within the same face region. The fact that transplanted wild-type pouch endoderm, but not crest, can rescue the cartilage defects of a mutant shows that the gene is required in the pouch rather than in the cartilage. So, it is likely that the cartilage, muscle and nerve defects seen in the mutants are secondary to the pouch defects. Moreover, cell-fate mapping showed that cells closer to the first pouch are the most affected by the mutation and that poor compaction and abnormal death of the neural crest cells that surround the pouch probably cause the cartilage defects.

The most striking feature of the *integrin-α5* mutant phenotype is the hierarchical way in which it triggers face patterning: it seems that the Integrin-α5-dependent endodermal pouch patterns the crest, which in turn might control the development of neighbouring muscles and nerves. Could this interconnected developmental mechanism have shaped the evolution of the head, by allowing the tissues of each head section to vary in size or shape in a coordinated fashion? Perhaps, although it remains to be shown that similar hierarchical relationships hold true for other parts of the vertebrate head.

Tanita Casci

References and links

ORIGINAL RESEARCH PAPER Crump, J. G. *et al.* An integrin-dependent role of pouch endoderm in hyoid cartilage development. *PLoS Biol.* **2**, E244 (2004)

WEB SITE

Charles Kimmel's laboratory: <http://www.neuro.uoregon.edu/ionmain/htdocs/faculty/kimmel.html>

FUNCTIONAL GENOMICS

A better bodyclock

A new approach that uses gene-expression profiles to measure individual body time (BT) promises to make a big difference to the way we will administer drugs in the future.

Having access to an accurate clock or watch is pretty important — as anybody who has been late for an exam or an interview because of an unreliable timepiece can confirm! It can also be important to read the correct time from the body's circadian clock: administering drugs at an inappropriate BT can cause side-effects, whereas at the correct BT a drug's potency might be maximized and its toxicity minimized.

To address the lack of clinically applicable methods of BT detection, Hiroki Ueda and colleagues aimed to create a standard 'molecular timetable' of gene-expression profiles. First, they monitored genome-wide expression profiles from pooled liver samples, taken from BALB/c mice over a 2-day period, under either 12-hour cycles of light and dark, or constant dark conditions. The peak expression times for the 168 genes that had high circadian rhythmicity were distributed over a 24-hour period and did not differ between the two light/dark regimes, which indicated that they could provide a direct measure of the endogenous state of the circadian clock.

Next, the authors used a statistical approach that allowed the BT information to be extracted from the expression profiles of the time-indicating genes. The gradually changing expression profiles of night-indicating and day-indicating genes can be accurately modelled by the best-fitted cosine curve.

Their verification in independent samples indicated that this method could provide accurate estimates of BT. But how clinically useful is such a timetable? In mice, at least, the molecular timetable easily detected rhythm disorders in four *Clock*^{-/-} mutants compared with wild-type mice. Moreover, given that circadian rhythmicity could also be detected in C3H mice, with the timetable that was calibrated in BALB/c mice, it seems this method is robust to changes in genetic background.

So, Ueda and colleagues might have provided a robust means of estimating BT from a single-point measurement: a long-held ambition for many clinicians. Of course, it is a long way from these experiments in inbred mouse strains to a routine clinical tool for humans, but given the number of genes in humans that show circadian expression patterns, there is certainly no obvious reason why the same approach would not be applicable.

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References and links

ORIGINAL RESEARCH PAPER Ueda, H. R. *et al.* Molecular-timetable methods for detection of body time and rhythm disorders from single-time-point genome-wide expression profiles. *Proc. Natl Acad. Sci. USA* **101**, 11227–11232 (2004)

WEB SITE

Hiroki Ueda's laboratory: <http://www.riken.go.jp/engn/r-world/research/lab/cdb/system/Online links>
(Printed image adapted from one provided by Hiroki Ueda.)

