MICROBIAL GENETICS

Embrace your inhibitions!

A thought-provoking new study indicates that inhibiting mutation in the genomes of pathogenic bacteria might be a viable strategy for dealing with the growing problem of antibiotic resistance.

Floyd Romesberg and colleagues used resistance to the synthetic antibiotic ciprofloxacin in *Escherichia coli* as their model system. Ciprofloxacin interferes with topoisomerases such that they introduce double-strand breaks (DSBs) in the DNA, so it is mutations in genes that encode the topoisomerases (for example, *gyrA*) that can confer resistance in bacteria. Previous work indicated that, when challenged with antibiotics, bacteria actively induce proteins that promote such mutations.

The SOS response — a DNA-repair process that is triggered by the autoproteolytic activity of the gene repressor LexA — was thought to be a key component of the mutation-induction process. The authors used a mouse model of thigh infection to show that LexA was involved: a *lexA*-mutant form of *E. coli* (*lexA* (Ser119Ala)) did not develop resistance to ciprofloxacin, whereas 3% of the control strain population did so within 72 hours. Repeating these experiments with an antibiotic from a different class — rifampicin — produced qualitatively similar results.

The authors followed up these studies with *in vitro* experiments that allowed them to compare the number of ciprofloxacin-resistant mutants that arise before and after exposure. From these experiments they estimated that ciprofloxacin induces a 104-fold increase in the rate of evolution of resistance in control strains of *E. coli*. By contrast, the post-exposure mutation rate was 100-fold lower in the *lexA* (Ser119Ala) strain, confirming the *in vivo* finding that LexA derepression was necessary for efficient induction of resistance.

In an attempt to identify the downstream components of the pathway that LexA triggers, Cirz *et al.* then undertook an elegant series of similar studies of pre- and post-exposure mutation rates for different *E. coli* strains with deletions of various genes in candidate pathways. These experiments provided clear-cut evidence that both nucleotide excision repair and recombinational gap-repair pathways are not involved, whereas RecBC-mediated homologous recombination is. Moreover, the authors showed that the effect of deleting any of the three LexA-repressed polymerases (PolII, PolIV and PolV) was equivalent to preventing LexA cleavage: so it seems that LexA functions through derepression of all these enzymes to induce mutations.

The authors suggest that the recombinational DNA-repair pathway might also underlie bacterial responses to other antibiotics, and indeed other cellular challenges. Such a neat system of mutational feedback, which is designed to restore the evolutionary *status quo*, certainly has an intuitive appeal. Moreover, if true, it would also have huge implications for our understanding of, and therapeutic approach to, antibiotic resistance.

Nick Campbell,
NPG Executive Editor, Heredity

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WEB SITE
Floyd Romesberg's laboratory: http://www.scripps.edu/chem/romesberg

ETHICS WATCH

Who regulates genetic tests?

Genetic tests are being sold with the aim of identifying genetic susceptibilities to common conditions, mainly through the internet and alternative healthcare providers. Tests are being marketed alongside 'personalized' skin creams, supplements or advice on medicines or diet. Geneticists[1], including some professional bodies[2], have criticized such sales, although opinions differ on whether tests need better regulation[3], or merely better guidance on their use[4]. Some pharmaceutical and food companies plan to adopt 'genetically tailored' marketing strategies for medicines and functional foods. An important concern is that the information provided on genetic risk, and on recommended interventions, is likely to be incorrect.

So who is responsible for the regulation of commercial genetic tests? Existing controls are limited. In the United States, the Food and Drug Administration has the legal power to assess the clinical validity of genetic tests before marketing. However, it currently assesses only test kits (which are tests marketed by a company to laboratories) and not so-called 'home brew tests' (offered and carried out in-house by a single laboratory). This has allowed the widespread marketing of misleading genetic tests. Some claim to identify susceptibilities to addiction and obesity, although published meta-analyses show no significant associations.

The European Union In-Vitro Diagnostics Directive covers laboratory quality assurance. Its full implementation should ensure that genetic tests correctly identify the DNA sequences they claim to detect. However, although there is some disagreement about interpretation, there is, in practice, no regulatory assessment of the clinical validity of genetic tests (the estimate of risk of having the genetic variant, or its link with a disease). Healthcare services occasionally assess tests on an *ad hoc* basis, relying on the published literature.

Neither the United States nor Europe assess clinical utility. Often, there are no data to determine whether the test is a useful way to decide who should be given which health interventions or advice. Even valid tests might have zero or negative utility (for example, if the people at highest genetic risk have least to gain from the recommended intervention). Nor have policy makers attempted to quantify the health impacts of widespread genetic testing. One concern is medicalization (treating risk factors as if they are diseases). Furthermore, medicines and functional foods marketed to the 'genetically susceptible' could be costly, ineffective and have potential side effects. Another concern is that the tests will undermine public health: for example, they might imply that only a minority of people with 'genetic predispositions' are at risk of diet- or smoking-related diseases.

Who decides what 'knowledge' is in the knowledge-based economy? Who assesses, and takes responsibility for, the impacts of such testing on population health? Governments in both the United States and Europe seem to want to leave the answers to both questions to the market: whatever sells is valid, useful 'genetic information'. Inevitably, different companies will sell different interpretations of genetic risk, and different 'personalized' products. This is bad for public trust, bad for investors and bad for companies who want to market valid, useful tests. Without the regulation of such tests, the human genome will, at best, become a massive marketing scam. At worst, there could be considerable harm to health.

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BEHAVIOURAL GENETICS

Sexual attraction: finding the right switch

Developmental switches — genes that are necessary and sufficient to specify a particular anatomical part, such as the eye — have more or less become household concepts. This logic has now been extended beyond morphological development by the finding that a complicated behaviour is also determined by a switch-like mechanism, one that involves the alternative splicing of a single gene.

In fruit flies, courtship is mostly a male affair: the male's elaborate ritual is rather complex, and therefore would intuitively call for a complex genetic explanation. However, one of the many genes involved in the courtship ritual would indicate otherwise: strong loss-of-function mutations of *fruitless (fru)*, which encodes a zinc-finger protein, specifically abolish male courtship, and weaker ones disrupt individual steps of the process. *fru* is therefore involved exclusively in male courtship and sexual orientation, and does not influence sexual morphology. Could *fru* be the switch gene for this particular set of sexual behaviours? This was first hinted at by the fact that males and females have differently spliced isoforms of the *fru* mRNA, but the idea remained to be tested.

The authors engineered lines of fly that contained male-only (fru^M) or female-only (fru^F) isoforms, and looked at the morphological and behavioural consequences. Consistent with the hypothesis that the male-specific *fru* isoform is required for male courtship, males that contain fru^F do not court. By contrast, females that contain either of the two fru^M isoforms behave like males: they court females (or males that make female pheromones) and do so by carrying out all but the last steps of the male courting ritual. The neat sex role-reversal induced by a single-gene isoform shows that the male-specific splicing of *fru* is necessary and sufficient for sexual orientation and behaviour.

How the sex-specific isoforms induce radically different behaviours is not clear: the authors of a second paper identify a functional circuit of fru^M-expressing neurons; however, the circuits are anatomically similar in males and females, so sex-specific differences must lie in how the circuit functions.

Several complex behaviours have been analysed genetically (see the Review on p521) and are indeed turning out to be quite complicated. However, the courtship example highlights the potential of opening up other innate, sex-specific behaviours to genetic scrutiny.

Tanita Casci

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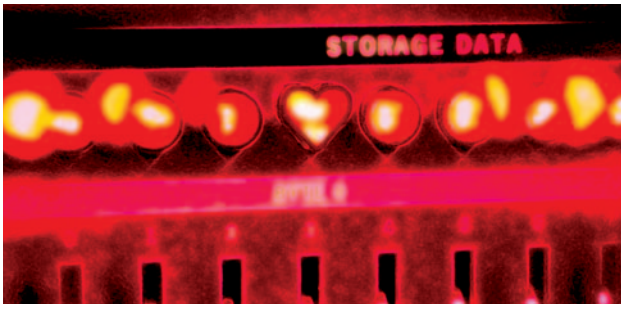

IN THE NEWS

Combing through the genome to find the baldness spot

The mothers are to blame, yet again. "Scientists have discovered a gene that plays a crucial role in male hair loss on the X [chromosome…]. When it comes to going bald, a man is therefore likely to take after his maternal grandfather rather than his father" (*The Independent*, 22 May 2005).

In a collaborative effort, German scientists identified an androgen receptor gene — "long thought to be the root cause of balding" (*Globe and Mail*, 22 May 2005). Professor Nöthen, one of the group leaders, explained that "one variant of this gene was found among men who suffered from premature balding at a very early stage very much more often than among men who still had a full head of hair when over 60" (*Medical News Today*, 20 May 2005).

Balding men probably have too many androgen receptors in the scalp. "Either more androgen receptors are formed among the affected men, or the variant of the receptor which develops as a result of the genetic change is more stable and is not broken down so quickly" (*Medical News Today*).

Of course, male baldness is not a monogenic condition. Other genetic determinants are likely to lie on the autosomes, so we might not be able to blame it all on the mothers, after all. The search is on and scientists are looking for volunteers. "They especially want to hear from men under 40 with severe hair loss who have a brother who is also affected" (*Daily Mail*, 22 May 2005).

In Europe, it is apparently British and German men who are the most susceptible to baldness. Interestingly, "while Germans [go] for treatment, 90% of balding British men [are] too embarrassed to do anything about it" (*The Independent*).

Magdalena Skipper

GENE EXPRESSION

A moving picture of gene expression

Although we know that spatial positioning within the nucleus can reflect the transcriptional activity of a chromosomal region, our picture of this relationship is largely a static one. For example, the localization of certain regions of the yeast genome at the nuclear periphery is known to help keep them transcriptionally silent. However, a recent study indicates that the spatial organization of the genome is a far more dynamic affair: large numbers of genes relocate within the nucleus in response to changing gene expression requirements.

Pamela Silver and colleagues studied how a global change in *Saccharomyces cerevisiae* gene expression affects the association of genes with the nuclear pore complex (NPC), a structure that is located at the nuclear periphery. As well as functioning in mRNA export, the NPC also regulates the transcriptional activity of various genomic regions — both positively and negatively — and some genes relocate to the NPC when they become transcriptionally active. The authors treated yeast cells with the α-factor pheromone to induce the mating response, which triggers large-scale changes in gene expression. They then used chromatin immunoprecipitation to identify genes that bind to NPC components.

Their results showed that 35 out of 49 genes that are highly upregulated during the mating response become associated with NPC proteins, indicating that activation of these genes is correlated to interaction with the mRNA export machinery. These findings applied to genes located on 13 of the 16 yeast chromosomes, showing that this effect is widespread throughout the genome.

But do these changes in binding reflect relocation to the periphery? To confirm this, Silver and colleagues examined the localization of *FIG2* (which encodes a cell-wall adhesin), one of the genes that binds to NPC components after mating response induction. Using fluorescence *in situ* hybridization, they showed that although *FIG2* usually takes up an interior position in the nucleus, it is redistributed towards the NPC when cells are treated with α-factor.

DEVELOPMENT

At the junction between L–R patterning and somitogenesis

Left–right (L–R) asymmetry is required for normal development, but how can symmetry arise in an embryo with L–R polarity? Three groups independently show that symmetry in the somites — which give rise to the skeleton and the muscles — is established by the action of retinoic acid. A fourth group shows that very early in L–R patterning, retinoic acid and Sonic Hedgehog, released in response to fibroblast growth factor (FGF) signalling and encased in membrane-bound vesicles, are transported by nodal flow to establish the initial asymmetry.

Retinoic acid has a newly discovered function — to ensure that somitogenesis occurs synchronously on the left and on the right sides of the embryo. Genetic and embryological experiments in the mouse, chick and zebrafish show that when retinoic acid is absent (either because an antagonist has been administered to the embryo or because the enzyme that synthesizes retinoic acid has been removed genetically), new somites develop asynchronously. Changes in the expression patterns of genes involved in the well-defined process of somitogenesis revealed that *Lunatic fringe* and other so-called cycling genes (such as *deltaC* or *her1*) are asymmetrically deregulated. This asynchrony, which is indicative of a deregulation of the 'clock' — a mechanism that sets the periodicity with which new somites form — is not random: supernumerary somites form on the left. This bias disappears when 'situs' is randomized in the embryo. In the chick, it can be done by inducing Sonic Hedgehog activity on the right side, which does not itself affect somitogenesis, in mouse by using the $iv^{-/-}$ mutants and in zebrafish by inhibiting H^+/K^+ ATPase activity or by preventing *left–right dynein* expression (Kawakami and Raya *et al.* show that both of these function very early in L–R asymmetrical patterning).

Early in embryogenesis, Notch signalling is upregulated on the left, which in turn activates Nodal signalling and the rest of the L–R patterning pathway. Downstream of Notch lies *Lunatic fringe*, a key cycling gene that coordinates somite formation and for which expression becomes asymmetrical in response to the asymmetrical Notch activation. What these new data show is that retinoic acid compensates for this initial asymmetry to allow for symmetrical somitogenesis in the zebrafish, chick and mouse. Interestingly, because *Amphioxus* somitogenesis is asymmetrical, this function of retinoic acid is probably vertebrate-specific.

A related report shifts the focus away from somitogenesis to the earliest events in the establishment of L–R asymmetry. It is well established that cilia-mediated leftward flow around the embryonic node is crucial for the breaking of L–R symmetry in mammals. Kawakami and Raya *et al.* provide the first experimental evidence for the 'nodal flow hypothesis' in zebrafish. Tanaka *et al.* now show that, at least in mouse embryos, this flow transports small (0.3–5 μm) membrane-bound 'nodal vesicular

This study indicates that a large-scale spatial reorganization of the genome takes place when gene-expression patterns are altered. The authors suggest that the relocation of genes to the NPC might increase the efficiency of the production of proteins required for the mating response by speeding up the processing of mRNAs and their export from the nucleus. These findings also provide further evidence against the view that the nuclear periphery is generally associated with inactive regions of the genome, highlighting the complex relationship between nuclear position and gene expression.

Louisa Flintoft

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WEB SITE
Pamela Silver's laboratory: http://research.dfci.harvard.edu/silverlab

parcels' that contain Sonic Hedgehog and retinoic acid. Their pharmacological and molecular genetic experiments indicate that these vesicles are released from the surface of the node and its dynamically protruding microvilli in response to FGF signalling.

With so much new data for the key developmental models we should be able to look forward to some interesting evo-devo studies in the near future.

Magdalena Skipper

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GENOME EVOLUTION

Doubling or splicing: the intimate relationship

Alternative splicing and gene duplication are evolutionary forces that drive new biological functions. But how do these two modes of functional diversification relate to each other?

A study from Kopelman and colleagues now reveals an inverse corellation between the size of a gene's family and its propensity to produce alternative splice variants. Through evaluation of human gene families with reference to species with different times of divergence, the authors show that genes of family size 1 (singletons) use alternative splicing more than genes that have undergone duplications. Furthermore, comparisons between human and mouse orthologues under different duplication models show that the rate of alternative splicing is three times higher in conserved singletons than in genes that have been duplicated in both lineages.

So, do duplicates lose splice variants or do singletons acquire them? The authors found a positive correlation between the age of a duplication event and the fraction of alternative splicing — a model that is further supported by the recent observation that alternative splicing frequently evolves through exon duplication.

These results have implications for understanding the molecular evolutionary mechanisms that lead to genetic innovations, indicating that the pressure for new functions precedes duplication events.

Ekat Kritikou

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WEB SITE
Itai Yanai's web page: http://www.yanaiweb.com/itai.html

GENE REGULATION

miRNAs: tuning Notch responses

Despite rapid progress in discovering miRNA genes in organisms as diverse as *Caenorhabditis elegans* and humans, miRNA target-finding has proved more challenging. Lai *et al.* now show that the Notch signalling pathway is a major target of miRNA-mediated regulation in *Drosophila melanogaster* and provide further insights into miRNA target recognition.

The Notch pathway is a signalling cascade that is is essential for cell specification and development in all metazoan organisms. In *D. melanogaster*, two large families of Notch target genes are clustered at two genomic locations, namely the Enhancer of split-Complex (E(spl)-C) and the Bearded-Complex (Brd-C). Previous genetic and informatics work defined multiple classes of negative regulatory sequence motif that are broadly distributed in the 3′ UTRs of these Notch target genes. These motifs, known as the GY box, the Brd box, and the K box, function to restrict Notch target gene activity during normal development and patterning of the fly nervous system. The authors noticed that the three classes of 'box' motif are perfectly complementary to the 5′ ends of three different families of *D. melanogaster* miRNAs. Taken together, these observations strongly indicate that all of these boxes are in fact binding sites for miRNAs.

Using an *in vivo* assay in *D. melanogaster* imaginal discs to systematically test Notch target 3′ UTRs for regulation by miRNAs, they showed that reporter constructs linked to GY-box-containing 3′ UTRs are inhibited by miR-7, those with Brd boxes by miR-4 and miR-79, and those with K boxes by miR-2 and miR-11. Mutation of the boxes within these 3′ UTRs abolished regulation in this assay, demonstrating that these miRNAs directly target these sites. Other tests using isolated sites further documented the sufficiency and necessity of GY-box, Brd-box and K-box motifs for regulation by complementary miRNAs. This study therefore complements other computational and tissue culture studies with comprehensive *in vivo* proof that Watson–Crick pairing between a 6–7 nucleotide 3′-UTR box and the 5′ end of the miRNA is a characteristic feature of miRNA-mediated regulation.

So what happens when miRNAs are ectopically expressed during *D. melanogaster* development? Although computational studies certainly have predicted these Notch target-regulating miRNAs to also target other transcripts that are unrelated to Notch signalling, their misexpression induced several developmental defects that are characteristic of Notch pathway inhibition. This indicates that these Notch pathway target genes are indeed key endogenous targets of these miRNAs.

As the authors note, future studies will need to address how different miRNA-binding sites collectively contribute to the overall regulation of an individual gene, as most of these Notch target genes contain functional binding sites for two or more different miRNAs. Whether miRNA-mediated regulation is a conserved feature of Notch target genes in other animals remains to be determined.

Ekat Kritikou

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WEB SITE
Gerald Rubin's web page: http://mcb.berkeley.edu/faculty/GEN/rubing.html

BIOINFORMATICS

From genotype to phenotype: a shortcut through the library

A list of genes tells you little about their biological roles: understanding this generally requires time-consuming functional or comparative studies. A new method provides a shortcut on this path from genotype to phenotype — it uses a combination of comparative genomics and literature mining to predict the functions of large sets of sequenced genes.

Peer Bork and colleagues reasoned that if a group of species has a shared phenotypic trait, orthologous genes shared among the species are likely to be involved in the underlying biological process. Such genotype–phenotype correlations have been made in the past, but required initial manual collection of phenotypic information for each species, which is labour-intensive and might lead to biases in the phenotypes examined.

In the new study, this annotation stage was avoided by directly linking species with phenotypic information already available in the published literature. The authors linked 92 completely sequenced prokaryotic species with 172,967 nouns in MEDLINE abstracts (from the database compiled by the US National Library of Medicine). The nouns were grouped according to the species they matched up with, assuming that words that relate more frequently to a particular set of species are likely to be specific to a shared trait. For the same species, 11,026 orthologous gene sets were identified from the STRING (search tool for the retrieval of interacting genes/proteins) database, defining shared sets of genes.

The final stage was to look for correlations between the groupings of MEDLINE nouns and the groupings of shared genes. Bork and colleagues identified 2,700 significant associations between orthologous groups and trait words, which allowed them to relate 28,888 genes to at least one trait. Among these associations, many of the gene–phenotype associations were already known, confirming the validity of the method. However, many new discoveries were also made. For example, previously unknown associations were made between a group of metabolic genes and trait words linked to food poisoning.

Many of the other associations made in this study also linked genes to disease-related phenotypes, which could provide a valuable source of new drug targets. This skew towards clinically relevant phenotypes reflects the fact that pathogens are highly represented among fully sequenced prokaryotes, but as more sequences and more MEDLINE entries become available, this method should provide a way to link genes to a wide range of biological processes.

Louisa Flintoft

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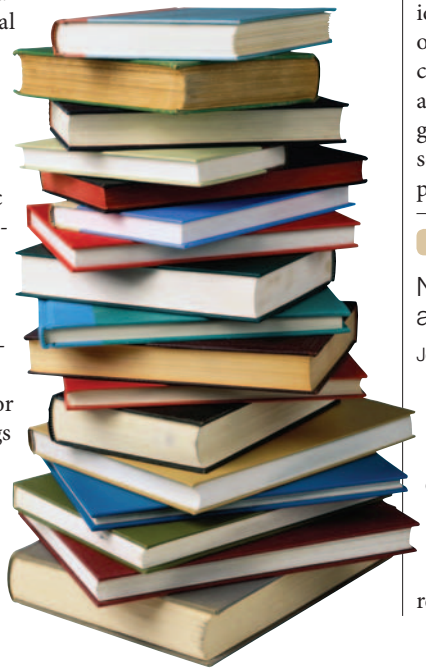
Korbel, J. O. *et al.* Systematic association of genes to phenotypes by genome and literature mining. *PLoS Biol.* **3**, e134 (2005)

WEB SITES

Peer Bork's laboratory: http://www-db.embl.de/jss/EmblGroupsHD/g_27.html

The STRING database: http://string.embl.de



IN BRIEF

FUNCTIONAL GENOMICS

Identification of genes needed for regeneration, stem cell function, and tissue homeostasis by systematic gene perturbation in Planaria.

Reddien, P. W. *et al. Dev. Cell* **8**, 635–649 (2005)

The authors developed an RNAi-based screening strategy to carry out the first large-scale survey of gene function in planarian biology. Among the 1,065 genes that were inactivated, 240 generated phenotypes associated with stem-cell function, regeneration and tissue homeostasis. This study demonstrates the great potential of RNAi for the identification of gene function in under-studied organisms and establishes planarians as a powerful model for studying regeneration.

SYSTEMS BIOLOGY

Cell fates as high-dimensional attractor states of a complex gene regulatory network.

Huang, S. *et al. Phys. Rev. Lett.* **94**, 128701 (2005)

The robustness of development to external perturbations has led to the suggestion that differentiated cell fates represent stable attractor states, which can be reached through more than one pathway. The authors provide the first experimental evidence for this, taking advantage of the fact that the differentiation of human neutrophils can be induced by two different chemicals. Using microarray analysis, they showed that the gene-expression pathways induced by the two chemicals were almost entirely different, but resulted in the same biological endpoint.

METAGENOMICS

Genomic sequencing of Pleistocene cave bears.

Noonan, J. P. *et al. Science* 2 June 2005 (doi:10.1126/science.1113485)

Genome sequencing of extinct species is hampered by the degradation of ancient DNA, but a metagenomics approach could help to address this problem. Noonan *et al.* tested this idea by making libraries from a mixture of DNA fragments obtained from two 40,000-year-old extinct cave bears. They compared these libraries with the annotated dog genome (bears and dogs are closely related) and identified 26,861 bp of cave-bear genome sequence. The phylogeny generated from this and sequences from modern bears is topologically equivalent to bear phylogenies previously obtained using mitochondrial DNA.

GENE THERAPY

Non-viral gene delivery regulated by stiffness of cell adhesion substrates.

Joon Kong, H. *et al. Nature Mater.* **4**, 460–464 (2005)

Considerable efforts are being made to improve the levels of gene transfection and expression achieved using non-viral gene-therapy vectors. Rather than trying to improve the efficiency of this process by manipulating the vector, Joon Kong *et al.* focused on the cellular environment. They show that gene-transfer efficiency and levels of gene expression increase as the rigidity of the cell-adhesion substrate increases. This effect is related to the influence of the substrate on cell proliferation.