Elegant tour de force

When the draft human genome sequence was published it was referred to as the blueprint. But, as with any design plan, the challenge is in establishing how the plan is executed. This task lies at the heart of functional genomics, and now Kamath et al. provide an important contribution to this field with their tour de force systematic RNAi analysis of the *Caenorhabditis elegans* genome. Not only do they uncover the function of scores of worm genes, but they also provide important information on the worm genome structure and its evolution.

To determine RNAi phenotypes for as many *C. elegans* genes as possible, the authors constructed a library of 16,757 bacterial strains (equivalent to 86% of all currently predicted worm open reading frames; ORFs), each of which expressed double-stranded RNA (dsRNA) corresponding to a single worm gene. When worms feed on these bacteria (*Escherichia coli* is their normal diet) the dsRNA is internalized and mediates sequence-specific knock down of the endogenous gene. One by one, the phenotypes were scored, and 10.3% of the analysed ORFs gave consistent phenotypes, which the authors grouped into three classes: nonviable (Nonv), growth defects (Gro) and viable post-embryonic phenotypes (Vpep). The first class contains many universal eukaryotic genes, for example, those that encode essential components of the basal cellular machinery. By contrast, most of the genes in the Vpep class probably represent animal-specific genes, and their products affect processes such as behaviour or body shape.

A closer look at the genomic distribution of genes in each class revealed that genes with similar functions tend to be co-localized in large domains of the genome and are co-transcribed. The size of these clusters, however, suggests that any large-scale transcriptional co-regulation must be mediated by a mechanism other than the previously described open-looped chromatin.

Another interesting finding concerns the X chromosome. The fact that Nonv genes are underrepresented on the X chromosome, whereas those that encode components of signalling pathways and transcription factors are overrepresented, suggests that very different selection pressures operate on genes on the sex chromosomes compared with genes on the autosomes.

Two other studies, in which the same approach was used to address more specific biological questions, have also been recently published. The authors of the second report, published in *Nature Genetics*, sought genes that, when inactivated, increased the *C. elegans* lifespan. Lee et al. followed up their RNAi screen with a classic forward genetics screen and, together, the results showed that worms with impaired mitochondrial and certain metabolic functions tend to be long-lived.
IN THE NEWS

Clone baby?
Doubts have started to surface over the truth of claims that the first human clone has been born, after Clonaid — the company that made the original 27 December announcement — backed away from an independent verification by genetic tests.

The New York Times reported that Clonaid’s self-imposed one-week deadline expired with no evidence forthcoming. Clonaid’s chief executive Brigitte Boisselier said “The parents told me that they needed 48 hours to decide yes or no — if they would do it” (New York Times).

The credibility of the claims took yet another hit when Michael Guillen — the freelance science journalist organizing the genetic tests — pointedly distanced himself from Clonaid after the tests did not go ahead. “It’s entirely possible [that] Clonaid’s announcement is part of an elaborate hoax to bring publicity to the Raelian movement,” he said (The Guardian).

Claude Vorhilon (aka “Rael”), leader of the pseudoscientific sect that funds Clonaid, suggests that a Florida court action aimed at placing the baby under the court’s protection might explain the company’s reticence. “...to take away this poor baby from a mother, I think this is completely crazy, just because she was cloned. So I called Doctor Boisselier, and I said, ‘If I was you, I would not test anything.’” (The Washington Times).

The increasing scepticism of the media has not prevented Clonaid from expanding their claims — according to them a further three human clones will be born in the next month, in addition to the trio already born (The Guardian).

Perhaps the biggest concern for geneticists arising from the whole media furore is the spur it is likely to provide for efforts in the US Congress to ban human cloning (The Guardian).

Nick Campbell

HIGHLIGHTS

Apart from the important biological insights, Kamath et al. have given the community an important resource — the bacterial RNAi library that can be used over and over again. Many more reports of screens such as those by Ashrafi et al. and Lee et al. are bound to follow, the results of which will provide a more complete picture of individual biological processes. The hope is that, as RNAi technology improves, similar systematic screens will also be feasible in mammalian cells.

Magdalena Skipper

EVOLUTIONARY GENOMICS

Compensation or innovation

Gene duplications are often seen as an opportunity to evolve new functions through the accumulation of mutations leading to functional diversification. But, they can also be thought of as a back-up or a buffer against loss-of-function mutations in one of the duplicates. Using yeast as a working example, Gu et al. have shown that gene duplications considerably contribute to genetic robustness against null mutations.

A previous study of genetic robustness — the ability to withstand null mutations — concluded that it was redundant metabolic pathways and networks, rather than duplicate genes, that mainly fulfil this function. However, these conclusions were based only on a few genes, so Gu et al. revisited this problem — this time addressing it on a genomewide scale. They made use of a previous study in which almost all of the genes of Saccharomyces cerevisiae were knocked out and the fitness of the mutants was assessed under five different conditions (see Highlights section in September 2002 issue). When the authors compared the fitness of strains deleted for unduplicated genes with those deleted for duplicates, they found a significant difference — deletions of duplicates were significantly less likely to cause lethality and more likely to have mild effects, or no effects, under each of the five experimental conditions. These observations indicated that duplicated genes compensate for each other, a conclusion that was supported by the fact that deletions of either gene from a duplicate pair showed similar fitness effects. Furthermore, the smaller the divergence between the duplicates, the better they compensate for each other. It also turns out that deleting duplicate genes with higher expression levels has a greater effect on fitness than deleting those that are not as highly expressed.

These data provide strong evidence for the role of gene duplication in genetic robustness against null mutations. Whether this contribution is more or less important than the interactions between unduplicated genes that function in alternative pathways remains to be seen. The role of duplicates in genetic buffering might explain why these genes do not ‘decay’ into pseudogenes as quickly as expected. But, Axel Meyer — the author of the accompanying News and Views — argues for a dual role for duplicate genes, but further whole-genome-sequence comparisons and functional analyses are needed before we can really address this question.

Magdalena Skipper

References and links


Smelling the time

Whether by compelling us to follow the latest fashion or to practise good table manners, the company we keep is known to have a strong influence on our behaviour. But the effect of society, it seems, can reach even deeper. Joel Levine and colleagues have found that internal biological clocks — those that regulate our sleeping rhythm, for example — can be reset by social interactions. Although this work was done in fruit flies, and the external cues were found to be olfactory, it is possible that the clocks of other species respond to the social environment in a similar way.

In all experiments, flies were exposed to a light-dark regime for five days, then placed in constant darkness for two weeks, after which time their clock rhythm — based on locomotor activity — was scored. The clocks of grouped flies were more synchronous after this treatment than were those of flies treated in isolation. If, as this result implies, company keeps flies in time, then genetically asynchronous flies would be expected to disrupt the harmonized clock when added to a wild-type group. This was tested by mixing flies mutant for the period gene (per), which have no sense of time, with wild-type flies, which, indeed, lost their synchronicity. Curiously, per mutants with early activity peaks (‘early birds’) were able to influence the activity of ‘later-rising’ per mutants, but not the other way around.

So, what is it about company that synchronizes the clocks of these flies? Simply exposing individual animals to the air from a chamber in which a group of flies was kept, was enough to synchronize their clocks. As the effect was abolished when the receiving flies had no sense of smell (because of a specific mutation in the paralytic gene), the authors settled on olfactory cues as being a good explanation.

Clocks and social interactions have been linked in many species, from humans to bees, but this is the first study to sniff out — genetically — the underlying sensory cause.

**References and links**


**WEB SITE**

Jeff Hall’s lab: http://www.bio.brandeis.edu/faculty01/hall.html
ETHICS WATCH

The $1,000 genome: ethical and legal hurdles

There is much buzz these days about 24-hour, whole-genome genotyping of individuals for under US $1,000. Craig Venter has announced that he will tackle this $1,000 genome, and some futurists are thinking of how it might be used in medicine. Although the technology for rapid whole-genome genotyping is not yet developed, the tools for doing so might soon be available.

Solving the technical problems fortunately gives us time to resolve the ethical, legal and social issues (ELSIs) that this endeavour presents before it is ready for ‘prime time’. Take the question of ownership of one’s DNA, still an unresolved issue in many jurisdictions. Individuals have the right to decide whether tissue that contains their DNA is removed from their body, but few personal legal rights have been established over the genotyping or testing of DNA that is legally acquired or sloughed off a person.

To gain public acceptance of sequencing an individual’s whole genome, and the testing that this will make possible, legal protections for consent and privacy are essential. However, at present, only 15 US states explicitly protect the rights of consent to testing, and Canada, Australia and most European countries do not explicitly protect an individual’s rights to refuse testing and to have privacy in their test results.

We also need to make clear rules about when genetic information might, or might not, be used in job and insurance decisions. To support a ban on genetic discrimination is easy, but there are some legitimate uses of genetic testing in both settings. A more balanced solution is needed, but we have yet to identify what it would be.

Finally, holders of patents on genes and DNA sequences could block rapid sequencing of the whole genome of an individual by making excessive royalty demands or refusing to license those uses. Changes in the patent system, to allow freer use of gene patents while protecting incentives for innovation, are also needed.

All is not lost, however. Attention to the ethical, legal and social issues raised by the human genome project has identified most of the relevant issues, and this has helped to shape a consensus about how ethically to tackle many issues, including research with stored samples, biobanking, testing minors, setting standards for genetics counselling and the need to rethink patent law.

The prospect of a $1,000 genome is a welcome prod for legal authorities to finish the work that the genomics, medical and bioethics communities have done on ELSI questions. It is time for clear, legal recognition of people’s rights to control the acquisition, testing, use and privacy of their DNA. The $1,000 genome has the potential to increase greatly access to the medical pay-offs of genomics. To get there, we will need to get our ethics and laws straight as well.

John Robertson

REFERENCES

HIGHLIGHTS

GENOMICS

Sea squirt genome released

The sea squirt, Ciona intestinalis is an unusual organism with an unusual history. For more than a century, biologists have pondered over this marine invertebrate due to its peculiar anatomical features and controversial taxonomy. It is now widely accepted that this species is a primitive chordate — that is, it has a cartilaginous column resembling a spine — and so, it is suitably placed to tell us about the evolutionary steps leading from invertebrates to vertebrates such as ourselves. The draft sequence of the C. intestinalis genome — which has been 18 months in the making — has now been published by Dehal and colleagues and represents the first genome of an invertebrate chordate to be published. In their paper, the authors present a preliminary analysis of the gene content of Ciona and how it compares with those of other sequenced animals.

The 160 million bp Ciona genome was sequenced using a whole-genome shotgun approach and is freely available on the web. It was found to contain ~16,000 protein-coding genes. This is similar to the number of genes in Drosophila melanogaster (~14,000) and Caenorhabditis elegans (~19,000), but around half the number estimated to be present in puffer fish (~31,000) and human (~30,000). It has been argued that extensive gene duplication was important in the evolution of the vertebrates. Comparative studies of gene families in Ciona and the vertebrates support this idea, as most of the genes that are present as multiple copies in vertebrates only have a single representative in Ciona.

The two physiological innovations that characterize vertebrates are their complex central nervous system and their adaptive immune system. A major evolutionary question is how and when these features arose. Genes required for these functions are absent from the Ciona genome, suggesting that they originated specifically in the vertebrate lineage. By contrast, there are Ciona homologues of genes required for other aspects of vertebrate development that are absent in the fly and worm genomes, such as thyroid hormone production and detection, and heart development.

As well as shedding light on the origin of the vertebrates, interesting evolutionary insights can be gained from unique features of the Ciona genome. This is the first animal that has been found to have genes involved in the synthesis of cellulose. A putative cellulose synthase has been identified that is likely to be involved in the production of the outer tunic of the adult sea squirt. This gene shares homology with those found in nitrogen-fixing bacteria and so might have been acquired by an ancestor of Ciona through horizontal gene transfer from a bacterial genome. Also of interest is a new family of receptors that contain two domains — a caspase domain and a region with homology to proteins involved in Notch signalling — that have never been observed before in a single protein, thereby providing an example of domain shuffling.

This paper illustrates the power of genome projects to give insight into evolutionary processes. Sea squirts might be only distantly related to humans, but their genome sequence adds another perspective to that given by other sequenced vertebrate and invertebrate genomes. Undoubtedly, the release of the sea urchin genome, expected in the next ten months, will complement this work and will provide more information about metazoan evolution.

Catherine Baxter

References and links


WEB SITE
Ciona genome project: http://genome.jgi-psf.org/ciona4/ciona4.home.html

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The trouble with ART

Assisted reproductive technology (ART), which in its broadest sense encompasses all fertility treatments, has revolutionized reproductive medicine and dramatically changed the lives of many people. Nevertheless, recent reports of an increased incidence of Angelman syndrome following IVF raised the possibility that ART might bring about epigenetic changes in the early embryo and lead to birth defects. Now, in a collaborative study of families with Beckwith-Wiedemann syndrome (BWS), which also involves epigenetic modifications, DeBaun, Feinberg and colleagues show that six out of seven children with BWS were conceived as a result of IVF. The incidence of ART was 4.6% among those who participated in the study, compared with 0.76% in the general US population, leading DeBaun et al. to suggest that the incidence of BWS increases at least sixfold after ART treatment, compared with the general population.

Given the previous reports of Angelman syndrome in association with ART, and the observation that five out of the six BWS cases were associated with classic BWS imprinting alterations, the authors believe that sufficient evidence now exists to suspect that some aspect of ART might interfere with imprinting either in the gametes or in the early embryo. So far, nothing is known about how this might occur, but one thing is certain — ART does not imitate life.

Magdalena Skipper

References and links

ORIGINAL RESEARCH PAPERS

FURTHER READING

IN BRIEF

TECHNOLOGY

Association testing by DNA pooling: an effective initial screen.

High-throughput screening for evidence of association by using mass spectrometry genotyping on DNA pools.

Despite the increasing number of molecular markers that have become available, whole-genome scans for association between such markers and a disease are hindered by the number of genotyping reactions that need to be carried out and by the limiting amount of available DNA. These two studies provide evidence that DNA pooling — in which the case and control samples are genotyped as two large groups — is a valid alternative to sample-by-sample genotyping. Mohlke et al. have shown empirically that pooled SNP genotyping is comparable to individual genotyping in detecting differences in allelic frequency between cases and controls, whereas Bansal et al. have found a significant association between SNPs in the cholesterol ester transferase protein (CETP) gene and levels of serum HDL cholesterol.

POPULATION GENETICS

Genetics affinities of the Andaman Islanders, a vanishing human population.

Disputes over the origin of the now nearly extinct inhabitants of the Andaman Islands have been laid to rest. Although they resemble African pygmies, the islanders have been considered to be more likely descendants of Southeast Asian settlers. The authors’ analysis of mitochondrial DNA, RFLP and microsatellite markers from various local tribes showed that they are the descendants of the early Palaeolithic Southeast Asian colonizers, distinct from their neighbours on a separate, but near-by, group of islands.

ANIMAL MODELS

Zebras as a model organism for the identification and characterization of drugs and genes affecting p53 signaling.

The activity of the p53 gene is crucial in determining tumour progression as it mediates apoptosis and cell-cycle control. By using morpholinos to knock down zebrafish gene activity, Langheinrich et al. show that the key components of p53 signalling are conserved between mammals and zebrafish. As cancer treatment drugs have a similar effect in both zebrafish and mammals, zebrafish could be a good system in which to carry out high-throughput screens for genes and compounds affecting p53 signalling. Results could then inform the development of new cancer therapies.
A double hit for diabetes

Diabetes is one of the fastest growing diseases in the western world, and so finding a cure is a pressing need for the millions of families worldwide that are affected by the various defects in sugar metabolism that this disease encompasses. Individuals affected by lipoatrophic diabetes mellitus, for example, have high levels of sugar in the blood due to insulin resistance. The genetic predisposition to diabetes is probably as varied as the disease itself, therefore, animal models offer an opportunity to understand the molecular and physiological basis of this class of disorder as well as providing a testbed for possible cures. Laustsen, Michael and colleagues have now created a mouse model of lipoatrophic diabetes and have investigated a fruitful route for its cure by gene therapy.

The study stemmed from the authors' interest in four mouse proteins — the insulin receptor substrates (IRS) 1–4 — which relay the insulin signal within cells. Single knockout mice for each IRS gene had shown that IRS1 and IRS2 have important and non-redundant roles in post-natal growth and glucose homeostasis, respectively; by contrast, IRS3 and IRS4 knockout mice had few abnormalities. But the IRS3 and IRS4 null phenotypes could have been compensated for by IRS1 and IRS2 or, conversely, the IRS1 and IRS2 mutant phenotypes could have been ameliorated by IRS3 and IRS4. Double knockout mice showed no redundancy between IRS1 and IRS4; however, the phenotype of IRS1,3 double knockout mice bore a striking resemblance to the features of lipoatrophic diabetes in humans: the mice had high levels of glucose and insulin in the blood, had reduced white adipose tissue (lipoatrophy) and developed non-insulin-dependent diabetes. Furthermore, the diabetes phenotype could be reversed by injecting the IRS1,3–/– mice with an adenoviral vector carrying the leptin gene, which had previously been shown to alleviate the insulin resistance of the other mouse models of lipoatrophic diabetes.

IRS1 and IRS3, therefore, have complementary physiological roles. So, what promise does this hold for human diabetes sufferers? As humans lack the IRS3 protein, they might be more dependent on the function of IRS1, and so sequence variants of this gene should be worth a closer look.

References and links

Original Research Paper

Web Site
Gustav Leinhard's laboratory:
http://www.dartmouth.edu/~biochem/leinhard

Molecular signatures

In order to shed light on the molecular basis of metastasis, Ramaswamy and colleagues used microarrays to compare the gene-expression profiles of metastatic and primary tumours from a range of human tissues, and found that the expression of 17 genes differed between the two classes of tumour and, therefore, provided a molecular signature for tumour-type.

Interestingly, some primary tumours shared the same gene-expression profile as metastatic nodules. Primary lung tumours that had the metastatic molecular signature were associated with worse prognoses compared with tumours that lacked such a signature. Furthermore, primary tumours from breast, prostate and brain with this signature were more likely to develop distant metastases, indicating that the molecular signature is biologically significant and that the basis of metastasis could be shared by different tumour types.

The authors argue that the propensity for a primary tumour to metastasise is linked to its genetic state as indicated by the gene-expression profile. This is in contrast to the current model, according to which the probability of a primary tumour becoming invasive depends mainly on its size — the bigger the tumour the more likely it is that a somatic mutation will occur that confers the metastatic phenotype.

With further refinement and testing, this work could be used to develop diagnostic tools that predict the clinical outcome of primary tumours on the basis of their gene-expression profiles at diagnosis. Microarray-based gene-expression profiling could also be used to provide detailed cancer diagnosis, as illustrated by Dyrskjøt and colleagues in the same issue of Nature Genetics. Their results showed that each class of bladder cancer has a distinct gene-expression profile that could be used in diagnosis or to generate new therapies targeted at specific stages and types of the disease. It is clear that microarray analysis will be invaluable in many aspects of cancer research. We will have to wait and see how long it takes for the practical application of these results to reach the clinic.

References and links

Original Research Papers

Web Site
Whitehead Institute Cancer Genomics:
http://www-genome.wi.mit.edu/cancer
A fertile pursuit of sterility

Whether it is aphids or earwigs, wasps, spiders or cockroaches, most of us have a hate-list of pests we would happily have disappear from the planet. But, facts get in the way of our dreams. Insect pests in particular — which damage crops and transmit deadly diseases to humans and animals — are notoriously difficult to wipe out. The non-specificity of pesticides, as well as the cost of counteracting recurrent drug resistance, has made the chemical line of attack increasingly unpopular. A more economical and ecologically friendly strategy has been to release sterile insects into the wild. Unfortunately, despite its success, this sterile insect technique (SIT) isn’t perfect — the ionizing radiation that sterilizes also reduces competitiveness in the wild. By developing a transgene-based method for sterilizing insects — in this case, for Drosophila — Carsten Horn and Ernst Wimmer have now built on the virtues of SIT while increasing its effectiveness.

Of the various ways of interfering with the reproduction of pests, the authors chose to engineer males whose genetic makeup caused them to produce lethal embryos. The strategy is conceptually simple: the flies to be released would be homozygous for a dominant-lethal gene that is active only in embryos. As the lethal factor, they chose an allele of the pro-apoptotic gene hid, the expression of which was controlled by the regulatory elements of the embryo-specific sry-α gene. The whole expression system was made conditional by using the tetracycline-controlled transactivator system: hid transcription is shut down in the presence of tetracycline, and so the laboratory flies can be reared unharmed by the lethal product of hid through adding the drug to their food. In the wild, where there is no tetracycline, the hid transgene is expressed and can do its deadly deed.

The theory behind the new method translated well into practice: lethality occurred efficiently and was restricted to embryos. When a ninefold excess of sterile males was used, the transgene did not greatly affect the animals’ competitiveness for mates in laboratory experiments, and the progeny from the competitive matings was reduced by nearly 90%.

In addition, although the system was established and tested in Drosophila, all the constructs, selectable markers and genes used in this study should be transferable to most other insect species — such as moths and butterflies — for which germline transformation is possible. As the SIT technique often works more effectively if only males are released, one plan is to aid the sex sorting of the engineered strain by integrating this transgenic method with a similar one that systematically kills adult females.

Transferring the new SIT technique from the bench to the field might take some time, but with luck it will give pests a much tougher bone to chew.

Tanita Casdi

References and links


IN BRIEF

GENOMICS

An active DNA transposon family in rice.

The plant MITE mPing is mobilized in another culture.

Mobilization of a transposon in the rice genome.

These three papers report the discovery of a family of active miniature inverted-repeat transposable elements (MITEs) in rice, which the authors call miniature Ping (mPing). This is an important finding as mPing is the first active MITE to be identified in any organism and the first active DNA transposon to be found in rice. A key feature of this transposon is that it reinserts with high frequency into low-copy coding regions of the rice genome. mPing mobilisation appears to be induced by stress such as gamma-radiation or cell-culture and relies on transpose activity provided in trans, probably to varying degrees by the related DNA transposons Ping and Pong. mPing might be suitable for use in developing gene-tagging programmes in rice. Such a programme could lead to the identification of genes controlling economically important rice traits and, therefore, facilitate the improvement of rice cultivars.

HUMAN GENETICS

Genetic structure of human populations.

The pattern of selection and migration of our ancestors is recorded in our DNA. In the largest survey of its kind, the authors have genotyped 1,056 individuals from 52 populations by using 377 autosomal microsatellite markers. Using a statistical analysis that clusters individuals solely on the basis of their genetic similarity, the authors were able to assign the individuals sampled to the five main geographical regions, and to subclusters that often corresponded to smaller populations. This work also confirms that most genetic variation exists within populations (93–95%) rather than between populations (3–5%).

BIOINFORMATICS

Modeling the percolation of annotation errors in a database of protein sequences.

Functional annotation of protein databases often relies on sequence homology, the functional annotation of which might have also been determined on the same basis. Gilks et al. refer to this possible chain of misannotations as ‘error percolation’ and develop a way to model the annotation quality that clearly shows that this iterative approach quickly leads to decreased database quality. The authors use this as a starting point to build a scoring mechanism to qualitatively evaluate homology-based annotation.