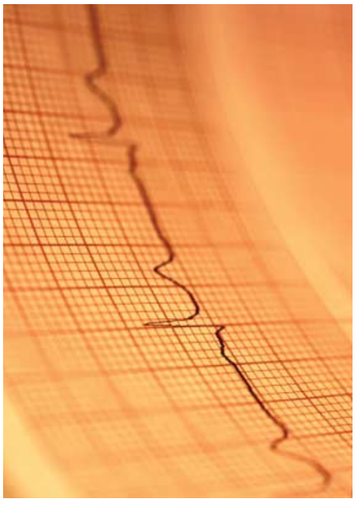
CANCER GENETICS

p53, a protein with a pulse



If there is one protein with little chance of leading a private life, it's p53. This tumour suppressor and its encoding gene have been thoroughly tinkered with over the years, with the result that we have a detailed molecular picture of how this so-called 'guardian of the genome' protects cells from genome damage by either promoting DNA repair or cell death. Galit Lahav and colleagues have now witnessed p53 at work in individual living cells. By fluorescently labelling human p53 and its regulator MDM2, they show that discrete p53 protein levels are activated in discrete quantities when DNA is damaged, and that the number of these 'pulses' depends on the severity of the damage.

The negative-feedback relationship between p53 and its partner MDM2 is well known: DNA damage lowers MDM2 levels, which in turn stabilize the p53 protein so that it can attempt to repair the damage. More p53 also means higher *MDM2* transcription and thereby p53 destabilization. To visualize these dynamics, the authors used time-lapse fluorescent microscopy on living human cells that expressed MDM2–YFP and p53–CFP fusion proteins, which glow yellow and cyan, respectively. The cells were zapped with gamma rays, which breaks DNA, and the levels of each protein were examined every 20 minutes for 16 hours. Fluorescence imaging is carried out routinely, but what is new here is the ability to look at individual cells, as the average signal that is released by a pool of cells would be impossible to resolve.

What the authors expected to see from this experiment was an 'analogue' behaviour, in which the strength of the output of the system matches the input — that is, where the amount of p53 protein increases in a graded manner with the severity of DNA damage. Instead, when the cells were irradiated, the two components of the p53–MDM2 feedback loop were activated in a series of discrete bursts, each containing a fixed amount of protein. The average height and duration of each pulse remained unchanged even when the DNA was more badly damaged; instead, the cells responded by increasing the number of pulses.

COMPARATIVE GENOMICS

Duplicating effort

Arguments have raged about how many times entire-genome duplications have occurred in vertebrate evolution (and indeed whether they have occurred at all!). "At least two" seems to be the answer based on the thorough comparative analysis of the human and *Fugu* genomes by Klaas Vandepoele, Wouter De Vos and their colleagues.

The sequencing of the *Fugu* genome was always going to be a key step in unravelling the twists and turns of vertebrate genome evolution. *Fugu* is a member of the sister group of land vertebrates, the ray-finned fish. In addition to the two genome duplications that are long-suspected to have occurred at the base of the vertebrate tree, some studies indicate that there has been another genome duplication in this group. However, preliminary analyses of the *Fugu* genome did not turn up evidence of an entire-genome duplication.

The authors realized that the most powerful way to address the question of vertebrate genome duplications was to do a comparative analysis. Using BLASTP, they searched for gene families that are shared by *Fugu* and human, and then added in homologous sequences from other fish, the mouse and the outgroups *Ciona* and *Drosophila*. They then built phylogenetic trees for each of the 3,077 gene families identified that had between two and ten *Fugu* genes.

The divergences between duplicated genes were dated relative to the ray-finned fish/land vertebrate divergence event within the 752 gene families in which there was strong statistical support for the relevant branches. The authors then estimated the absolute date of the divergences of gene duplicates in the 488 gene families and found evidence that a molecular clock was operating. A total of 166 (30%) of these divergences seem to have occurred between 225 and 425 million years ago (mya) — that is, after the ray-finned fish/land vertebrate split. Further analyses clearly indicated that a suite of duplicate genes arose approximately 320 mya. The implication is clear: an entire genome duplication event in an ancestor of ray-finned fish probably gave rise to many paralogues in the present-day *Fugu* genome.

However, the distribution of divergence times of *Fugu* gene paralogues is bimodal, with approximately 70% falling in the 500–900 mya window. So, it seems that there was probably at least one, and possibly two (roughly contemporaneous), genome-duplication events much earlier in vertebrate evolution — pre-dating the ray-finned fish/land vertebrate split and even the jawed/jawless vertebrate split (~575 mya). These data indicate that genome duplication has almost certainly had a large role in the evolution of all vertebrates.

These few ancient duplications of vertebrate genomes are fascinating; however, it will take a much more intensive sampling of vertebrate lineages before we will be able to effectively identify and analyse enough similar duplications to start making some generalizations about these events and their consequences.

Nick Campbell

References and links

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FURTHER READING Wolfe, K. H. Yesterday's polyploids and the mystery of diploidization. *Nature Rev. Genet.* **2**, 333–341 (2001) | Aparicio, S. *et al.* Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. *Science* **297**, 1301–1310 (2002)

WEB SITE

Yves Van de Peer's Laboratory: http://www.psb.ugent.be/bioinformatics

This behaviour — which is described as 'digital' because the magnitude of the input is translated into a number of discrete outputs — is important in some biological systems, such as spiking neurons, but defies theoretical expectations of how a negative-feedback relationship should operate. It's a trickly problem to address, but the authors speculate that the gradual increase in p53 protein that is afforded by repeated pulses is a fail-safe mechanism that prevents the downstream repair enzymes from swamping the cell and triggering its death.

Tanita Casci

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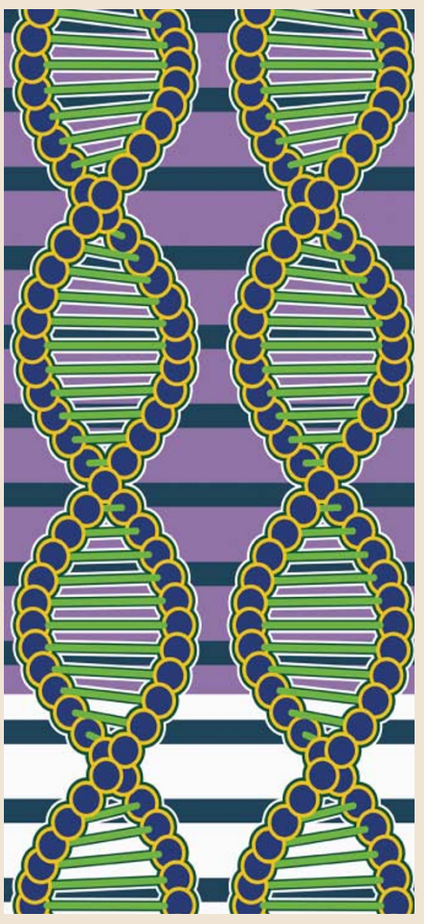
WEB SITE

Uri Alon's laboratory: http://www.weizmann.ac.il/mcb/UriAlon



DEVELOPMENTAL BIOLOGY

Those tricky first steps

Every parent anxiously awaits their little one's first word or wobbly steps, but the most significant developmental milestone happened much earlier, when the oocyte became an embryo and established itself in the lining of the womb. This early stage in a mammal's life is tucked away and so cannot be studied genetically using phenotypic assays. Two groups have now investigated the development of the mouse pre-implantation embryo using microarray-based transcriptional profiling. They show that it is possible to build a temporal profile of gene expression on which to base hypotheses about how genes interact during this early stage of life.

Q. Tian Wang and colleagues examined the expression profile of ~12,000 genes across 12 morphological time points from unfertilized egg to late blastocyst. The expression of a surprising number — more than one-third — of the genes varied by more than fivefold during this period. As well as being sensitive in identifying genes for which transcripts go up or down, the method also faithfully picked up the complexity of a particular stage, such as the increased transcript complexity that occurs following fertilization owing to the transition from maternal gene expression to zygotic genome activation (ZGA). Perhaps the most revealing discovery was that several members of familiar signalling pathways, such as those downstream of Notch, Wnt and BMP, were active at several crucial times — just before implantation, for example — thereby providing candidate genes for further study. The temporal resolution afforded by the array also allows promising candidates to be selected by virtue of their co-expression with known genes.

A similar analysis — this time by monitoring the expression of ~22,000 genes over 7 defined morphological pre-implantation stages — was carried out by Toshio Hamatami and colleagues. A general look at global-transcription trends defines two main developmental transitions — one at the 0–2-cell stage, when ZGA begins, and another, unanticipated one, at the 4–8-cell transition. A thorough study of the behaviour of individual genes that were expressed at each stage revealed some new and useful information: most gene transcription is activated in four transient waves that quickly tail off. This peculiar pattern indicates that many genes are stage-specific (a conclusion also drawn by Wang *et al.*); short bursts of expression presumably ensure that one stage-specific gene product does not spill over into the next stage. But how might these transitions be timed? Experiments *in vitro* that use gene-expression inhibitors support the view that the first wave, which coincides with ZGA, might be activated by maternal factors, with the following waves depending on genes that were expressed in the immediately preceding one, in a stepwise fashion.

The two reports do not always agree: for example, Wang *et al.* found that the main developmental transition occurs at the 2–4-cell stage. Nevertheless, this is the first time that microarrays have been applied to the study of pre-implantation development in the mouse and they have produced a thorough, accurate and quantitative picture of early embryonic development. What's more, they have provided us with a list of genes — thousands of them — to follow up on.

Tanita Casci

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