TECHNOLOGY

All aboard the synthetic biology train

The vast amount of data that is generated by high-throughput approaches generates hypotheses, all of which need to be tested experimentally. One way to do this is to use experimental synthetic systems — a focus of the growing discipline of synthetic biology. But for the process to be efficient, accurate and cost-effective, gene (and genome) synthesis is required. Tian *et al.* report a new cost-effective and accurate method for multiplex-gene synthesis from DNA microchips and demonstrate the power of their technology by re-creating the 21 protein components of an *Escherichia coli* 30*S* ribosomal subunit *in vitro*.

The motivation for this work was to deal with the high costs and error rate of synthesis. Although synthesizing oligonucleotides from microchips can reduce the costs, currently available chips have a small surface area, so only small amounts of oligonucleotides can be produced. To solve this problem, Tian *et al.* introduced a 10,000-fold amplification step directly after oligonucleotide synthesis.

To solve the second problem — the error rate — the authors used hybridization to 'clean up' the oligonucleotide pools. The newly synthesizd oligonucleotides were hybridized to immobilized complementary oligos and unstable, imperfect pairs were lost during the washes.

Oligonucleotides that are synthesized in this new and improved way are suitable for gene assembly. Tian *et al.* developed a way of synthesizing many genes in parallel in a single step, from a single oligonucleotide pool. The authors call it PAM — (single-step) polymerase assembly mutiplexing reaction. Unlike the traditional assembly methods, which require two or three steps (ligation, assembly and PCR), PAM allows you to combine gene-flanking primer pairs, gene-construction oligos, thermostable polymerase and dNTPs (deoxynucleotide triphosphates) in a single tube.

The initial, challenging target that was chosen for *in vitro* synthesis was the ribosome: one of the largest molecular complexes, and one that is of great interest to

GENE NETWORKS

Epistasis in colour

There has often been a strong temptation to brush epistasis under the carpet, as thrashing out the details of how genes interact with one another was thought to be best left to the few hardy geneticists who had a way with complicated equations and patchy data. Now, however, the pervasiveness and the undeniable importance of genetic interactions in biology, disease suceptibility and evolution has seen epistasis enjoy a welcome comeback. No small part in this revival has been played by the availability of improved statistical tools and global gene-analysis methods. Daniel Segrè, Roy Kishony and collaborators have now applied such a systems-level approach to describe the spectrum of epistasic interactions that occur between metabolic genes in the yeast *Saccharomyces cerevisiae*, and in so doing have transported epistasis beyond the gene level.

For this work — the fruit of a collaboration between the laboratories of Roy Kishony and George Church — the authors took advantage of a computational method known as flux-balance analysis to assess the effects of gene interactions on the growth rate of yeast cells. The fitness of a single mutant is measured as the degree to which the biomass production of mutant yeast differs from that of the wild-type. The presence and type of epistasis could then be evaluated by comparing the fitness value of the double mutants to the product of the fitness values of the single mutants of the same gene pair. From this analysis, the authors concluded that epistasis could be classified as being either aggravating (where one mutant enhances the effect of the other) or buffering (where one mutant mitigates the effect of the other).

This rather neat classification opened the door for a new type of analysis, in which individual gene–gene relationships were used to paint a picture of the overall organization of the metabolic network. The clusters ('modules') of interacting genes that emerged on the basis of annotated gene function were almost always either aggravating or buffering with respect to other modules. This is pretty much what you would expect to find if the genes within a given module were involved in carrying out the same biological function: because they are made up of genes with the same or similar biological function, modules effectively behave as if they are individual genes. These 'monochromatic' relationships between modules largely held true even when the hierarchical clusters were built without previous knowledge of gene function, indicating that the clusters and how they interact are intrinsic properties of this and, by extension, every other gene network. Most of the observed interactions make biological sense; and those that were unexpected can now be used to generate testable hypotheses about the network.

This systematic survey of gene interactions brings epistasis to a new level — one that includes not only interactions between genes, but also between groups of genes. 'Monochromatic modularity', as it is called, is launching epistasis towards a more colourful future.

Tanita Casci

References and links

ORIGINAL RESEARCH PAPER Segrè, D. *et al.* Modular epistasis in yeast metabolism. *Nature Genet.* 12 December 2004 (doi:10.1038/ng1489)

WEB SITE Roy Kishony's laboratory: http://www.cgr.harvard.edu/kishony/prism

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biotechnologists. Importantly, the approach taken by the authors allowed them to circumvent an important problem: *E. coli* codon usage. This is optimized for *in vivo* conditions, but is for some reason inefficient *in vitro*, so they tinkered with the codon usage at the oligo-design stage to maximize translation efficiency.

Tian *et al.* elegantly show how technical obstacles can be overcome on the way to synthetic biology. As more and more hypotheses line up to be tested, the push for advances in this field is likely to increase.

Magdalena Skipper

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WEB SITE

George Church's laboratory: http://arep.med.harvard.edu/

GENE REGULATION

Wading in upstream

Figuring out how gene expression has evolved can take some serious detective work. Because regulatory sequences are short and are surrounded by unconserved, non-coding DNA, spotting similar elements in different species by sequence alignment is often impossible, especially in distantly related organisms. In a recent paper, Audrey Gasch and colleagues describe a new approach to this problem and apply it to the evolution of gene expression in fungi.

The authors predicted that groups of genes that are co-expressed under specific conditions are likely to be controlled by similar regulatory elements and that this could be used as a starting point to identify related elements in different species. On this basis, they first identified groups of *Saccharomyces cerevisiae* genes that were predicted to be co-regulated owing to similar functions or expression patterns. Examining upstream regions revealed 42 different elements that are involved in the co-regulation of different sets of these genes.

The next step was to see whether similar regulatory elements regulate the expression of the corresponding genes in other species. *Saccharomyces cerevisiae* belongs to a group of fungi known as the ascomycetes that are thought to have existed for 500 million to 1 billion years. The authors used a modified BLAST search to identify the orthologues of co-regulated *S. cerevisiae* genes in 13 other ascomycete species and looked at which regulatory elements were present in their upstream regions. In species closely related to *S. cerevisiae*, most of the regulatory elements were conserved in the corresponding groups of orthologous genes. For example, in *Saccharomyces kluyveri* and *Saccharomyces castelli*, this proportion was 50–75%. Even in *Candida albicans* — which is thought to have diverged from *S. cerevisiae* ~200 million years ago — more than a third of the same elements were identified in orthologous gene groups, indicating that *cis*-regulatory elements can be conserved over large evolutionary distances.

Gasch and colleagues also found evidence for evolutionary changes in gene regulation. For example, although there are many cases in which regulatory sequences are conserved, there are also orthologous groups of co-regulated genes that might rely on distinct sets of regulatory elements in different species, with these differences being more pronounced between more distantly related species.

An example of evolutionary change that the authors examined in more detail was the control of gene expression by the transcription factor Rpn4, which regulates proteasomal genes in *S. cerevisiae*. One binding site for this protein was identified as a conserved *cis*-regulatory element in all of the hemi-ascomycetes, a group that includes *S. cerevisiae*. However, in another group — the euascomycetes — a distinct element was identified. Comparison of an Rpn4 protein from three species revealed that the binding specificities are different between fungi, with each binding to elements identified in the corresponding species. So, the *cis*-regulatory elements that are involved seem to have co-evolved with the Rpn4 protein to produce species-specific regulatory interactions.

The evolutionary distances covered in this study are far greater than would have been possible through sequence alignment, providing the opportunity to explore the evolution of gene regulation in unprecedented detail. As more complete genome sequences of related sequences become available, similar studies should become possible across a range of taxonomic groups, leading to a greater understanding of the mechanisms that have shaped organismal diversity.

Louisa Flintoft

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WEB SITES

Audrey Gasch's laboratory: http://www.genetics.wisc.edu/faculty/profile.php?id=159

Michael Eisen's laboratory: http://rana.lbl.gov/

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