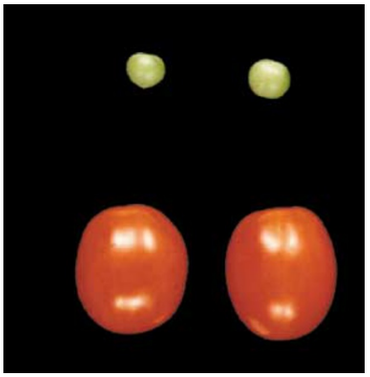
QUANTITATIVE GENETICS

Sizing up developmental timing



Fruits of wild (top) and domesticated (bottom) tomatoes. Picture courtesy of Dani Zamir, and reproduced with permission from Zamir, D. *Nature Rev. Genet.* **2**, 983–989 © (2001) Macmillan Magazines Ltd.

Fruit weight and size are agriculturally important traits, but little is known of their genetic and molecular bases. Many genetic studies on these traits have been done in the tomato, owing to the disparity in size between the fruits of wild and domesticated tomatoes (see picture). Quantitative trait loci (QTL) mapping studies in this plant have identified nearly 30 tomato QTL that affect fruit weight and size.

One such QTL is *fw2.2*, which accounts for ~30% of the difference in fruit weight between wild and domesticated tomatoes. Earlier studies have strongly indicated that altered gene regulation underlies the effects of the large- and small-fruit alleles of *fw2.2* on fruit weight. It has long been believed that such mutations, especially when they affect the timing of development ('heterochronic' mutations), might be a natural force of evolutionary change in plants. In a detailed study of *fw2.2*, Steven Tanksley's group now provide the first experimental evidence to support this theory.

Because previous studies in plants and *Drosophila* have shown that both cell division and expansion are essential factors that determine organ and fruit size, Cong *et al.* analysed cell size and mitotic index (MI) in two nearly isogenic tomato lines in which either a large- or small-fruit *fw2.2* allele was present. Differences in MI were found between the fruits of these two lines, but not in cell size. In the small-fruit *fw2.2* line (TA1144), a rapid but brief rise in MI occurs immediately after fertilization. By contrast, a more gradual and sustained rise in MI occurs in the large-fruit allele line (TA1143), indicating that an extended period of cell division might underlie larger fruit size in this line. Next, the authors found that the *fw2.2* alleles differ in the timing of their peak expression by around one week. This difference in expression timing inversely correlated with changes in mitotic activity during early fruit development, indicating that *fw2.2* might negatively regulate cell division. Moreover, by ~12 days post-fertilization, *fw2.2* levels in TA1144 were more than double those in TA1143. However, only subtle differences in expression patterns were evident between the two lines.

BEHAVIOURAL GENETICS

Worms gang up on bacteria

The nematode worm *Caenorhabditis elegans* can be shy or gregarious when feeding time arrives. New work uncovers some of the neurons and genes that are involved in regulating social feeding in the worm, and points towards multiple systems of antagonistic signalling that control whether, and when, the worms aggregate into feeding groups.

The standard laboratory strain of *C. elegans* is a loner, preferring solitary feeding. But worms with a valine-to-phenylalanine mutation at residue 215 of NPR-1 — a putative G-protein-coupled receptor — form aggregates when they encounter bacteria (their food source). Worms with a deletion at the *npr-1* locus also aggregate, suggesting that a valine-containing receptor (NPR-1 215V) normally suppresses aggregation. In two studies, de Bono and colleagues take advantage of the excellent worm genetics and its small nervous system to delve deeper into the control of social feeding.

The first study investigated how and where NPR-1 acts. The authors constructed a transgene that expressed GFP-tagged NPR-1 215V from the *npr-1* promoter. When expressed in worms with an *npr-1* deletion, this transgene suppressed aggregation. By using different promoters to drive transgene expression in subsets of neurons, the authors showed that expression in just three sensory neurons — AQR, PQR and URX — was sufficient to suppress aggregation.

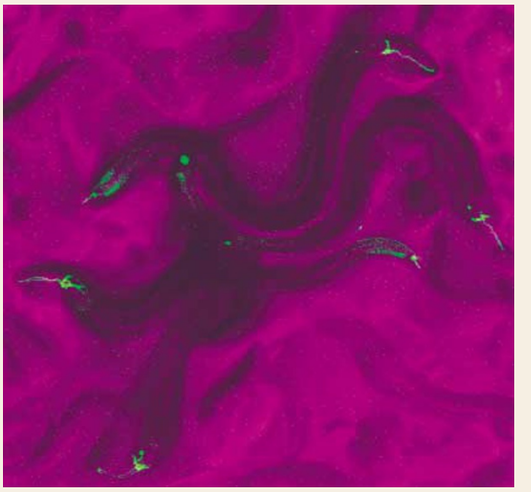
These three neurons are exposed to the fluid in the body cavity. Their ability to mediate social feeding seems to depend on signalling through a cyclic GMP-gated ion channel, as neuron-specific mutations in *tax-2* or *tax-4*, which encode the subunits of the channel, also suppressed aggregation. So, it seems that NPR-1 suppresses aggregation by antagonizing signalling through TAX-2 and TAX-4 in these sensory neurons.

The second study investigated how external stimuli might elicit aggregation. A screen for mutations that suppress aggregation in *npr-1*-deleted animals identified four genes. Two of these, *osm-9* and *ocr-2*, are thought to encode subunits of a TRP-related cation channel in *C. elegans* chemosensory neurons, and are required for avoidance of various noxious stimuli. The other two genes, *odr-4* and *odr-8*, are required to localize a subset of chemosensory receptors to sensory cilia.

Analysis of GFP transgene expression in *ocr-2*; *odr-4* double mutants showed that their expression is required in specific nociceptive neurons — those that respond to noxious stimuli — to rescue social feeding. Laser ablation of these neurons abolished social feeding, confirming the genetic data.

Another piece of the puzzle came from studies of *osm-3* mutants. OSM-3, a kinesin, is required for proper formation of sensory cilia on sensory neurons. Although removing *osm-3* function interferes with the development of the crucial chemosensory neurons, it doesn't suppress social feeding. The authors propose that, as well as blocking the ability of these neurons to promote social

GFP expression in the body cavity neurons of *C. elegans*. Image courtesy of S. Reichett, MRC Laboratory of Molecular Biology, Cambridge, UK.



These results show that the differences in transcript levels between the small- and large-fruit alleles of *fw2.2* are both quantitative (with the small-fruit allele being more abundantly expressed) and qualitative (as evident from the difference in their expression timing). Importantly, these findings provide empirical evidence that heterochronic regulatory changes in gene expression can bring about phenotypic, and probably evolutionary, change in plants. But how *fw2.2* actually modulates cell division remains unknown.

Jane Alfred

References and links

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FURTHER READING Dekkers, J. C. M. & Hospital, F. The use of molecular genetics in the improvement of agricultural populations. *Nature Rev. Genet.* **3**, 22–32 (2002)

WEB SITE

Steven Tanksley's laboratory: http://www.plbr.cornell.edu/PBBweb/Tanksley.html

feeding, lack of OSM-3 blocks antagonistic signals that normally inhibit this behaviour. Indeed, removing *osm-3* function restores social feeding in *odr-4* or *ocr-2* mutants. So, as with the body cavity neurons, nociceptive neurons might be involved in a system of antagonism between signals that promote and suppress aggregation.

As these neurons are required for responses to stressful or aversive stimuli, de Bono *et al.* propose that aggregation is a response to an aversive stimulus that is produced by bacteria. But what the aversive stimulus that promotes aggregation is and how the different control systems interact to regulate when social feeding occurs remains unknown.

Rachel Jones, Senior Editor,
Nature Reviews Neuroscience

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ORIGINAL RESEARCH PAPERS de Bono, M. *et al*. Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. *Nature* **419**, 899–903 (2002) | Coates, J. C. & de Bono, M. Antagonistic pathways in neurons exposed to body fluid regulate social feeding in *Caenorhabditis elegans*. *Nature* **419**, 925–929 (2002)

FURTHER READING Rankin, C. H. From gene to identified neuron to behaviour in *Caenorhabditis elegans*. *Nature Rev. Genet.* **3**, 622–630 (2002)

FUNCTIONAL GENOMICS

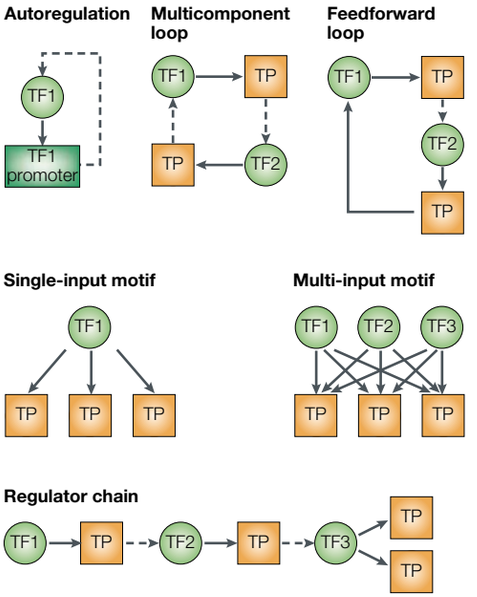
The importance of networking

For many, defining a single pathway was once the ultimate goal. No longer satisfied with understanding individual pathways, researchers now seek to understand how they interact with each other to bring about changes in living organisms. Lee *et al.* now present their *tour de force* approach to mapping transcriptional regulatory networks in the budding yeast. By using genome-wide location analysis (GWLA), they define regulatory motifs, which when combined with global gene expression data allow them to construct a complete regulatory network.

Driven by the desire to know how gene expression is regulated on a global scale, the authors reasoned that they would ultimately need to understand how transcription is regulated. To this end, they used GWLA — a method they previously developed and that allows them to find out which transcription factors (TFs) bind to which promoters. GWLA involves crosslinking TFs that are bound to their target promoters (TPs), recovering the DNA and identifying the TPs by using genomic DNA as a reference. The analysis was done under three growth conditions for 106 out of 141 TFs that could be found in the Yeast Proteome Database.

Lee *et al.* found that many yeast promoters were bound by more than two TFs — a feature that had been thought to be limited to higher eukaryotes. The 4,000 or so interactions fell into six basic regulatory motifs, which the authors consider to be building blocks of larger regulatory networks. They classify these networks as autoregulation, multicomponent loops, feedforward loops, single-input motifs, multi-input motifs and regulator chains (see figure). For example, autoregulation is thought to be important in quick responses to the changing environment, and therefore it is associated with a selective growth advantage. The authors show that 10% of yeast TFs autoregulate; by contrast, in prokaryotes, this figure is thought to be between 52% and 74%. The structure of the feedforward loop suggests that it might be important in response to a sustained rather than a transient signal. It might also provide a way for temporal control.

The authors wondered whether they could use these building blocks to construct a network of interactions. They decided to build a network for regulators involved in the yeast cell cycle because the large amount of information available for this process would make their theoretical model easily testable. To construct their network, the authors used an algorithm that combines the GWLA data with gene expression data. As core regulators that share the same spatial and temporal expression patterns were defined, more regulators with the same expression pattern were added, and so the network grew.



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Astonishingly, the algorithm — which was automated and required no previous knowledge of biology — assigned all the regulators to the correct cell-cycle stages. Moreover, those regulators that had been poorly characterized were now placed in a particular position of the network, which can now be tested experimentally.

All of the interactions are testable, and the approach is applicable to any organism for which good genomic and expression data are available. One important observation that emerges from this work is that the control of cellular processes involves transcriptional regulation of other regulators. This has important implications for mutation analysis — if expression profiling is used to characterize a mutation, it is as likely to reveal direct targets of a mutated regulator as it is to reveal the effects of network disruption.

Magdalena Skipper

References and links

ORIGINAL RESEARCH PAPER Lee, T. I. *et al.* Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* **298**, 799–804 (2002)

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