Batchelor's calculations, we can hope for a new theory in 2002. ■

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Functional genomics

# Silent genes given voice

Athel Cornish-Bowden and María Luz Cárdenas

Many genes have little apparent influence on growth rates or metabolic fluxes in an organism. But their roles can be revealed by comparing the effects of mutations on two or more metabolite concentrations.

Most mutations have no noticeable impact on an organism. This implies that changing the activity of some enzyme or other by a substantial factor has little effect; even complete deletion of a gene may not be easily detectable if there are appropriate fail-safe features in the design of the organism. Many genes, up to 85% of those in yeast, do not appear to be required for survival, and a high proportion of these seem to have no detectable effects on metabolic fluxes — the chemical processes that result in energy production or growth. This presents a major barrier to functional studies of a genome. How can we hope to deduce the function of a gene that has no apparent effect?

Writing in *Nature Biotechnology,* Léonie Raamsdonk and colleagues argue that examining metabolite concentrations, which in total are known as the 'metabolome', rather than fluxes, is much more likely to reveal such 'silent' genes (*Nature Biotechnol.* **19,** 45–50; 2001). The authors call their method FANCY, which comes from 'functional analysis by co-responses in yeast' (in this case brewer's yeast, *Saccharomyces cerevisiae*). It uses the fact, long known but often ignored, that typical effects of changes in enzyme activity on metabolite concentrations are much larger than their effects on metabolic fluxes. The authors looked at two mutations affecting 6-phosphofructo-2-kinase, an enzyme whose product fructose 2,6-bisphosphate acts as a signal to regulate energy production; two mutations in the cytochrome oxidase complex, which catalyses a different energy-related process; and a mutation not related to energy metabolism that was used as a control.

Why, though, should changes in enzyme activity have a larger effect on metabolite concentrations than on fluxes? The reason can be seen by looking at what happens when a rock falls into a river. Any transient interruption of the flow of water is rapidly nullified by the increasing level of water just above the rock and the decreasing level just below: as soon as the required pressure is reached, the flow returns to just what it was before. So the rock has no steady-state effect on the flow, although it does have a long-term effect on the water levels, which remain different from their original values as long as the obstacle remains in place. An observer with access only to the steady-state value of the flow can learn nothing about the existence of an obstacle, let alone its location. But an observer with access to the water levels in different places can both detect that an obstacle is present and find out where it is by comparing its effects on the levels above and below it.

As with rivers, so with the genome of an organism such as yeast. Gross properties such as growth rate that depend on fluxes may suggest that most genes are silent. For instance, chemostat culture allows microorganisms to be maintained indefinitely in the phase of exponential growth in a medium of constant composition, the slower-growing strains gradually being eliminated by dilution. Although this approach can in principle reveal very slight differences in growth rates, Raamsdonk *et al.* found that it failed to reveal the deletion of one or other of the two yeast genes that code for 6-phosphofructo-2-kinase. The mutants achieve growth rates equal to those of the wild type by increasing the concentrations of metabolites upstream from the impediment and decreasing those of downstream metabolites (just as for the rock in the river). The effects on a metabolite such as fructose 6-phosphate are not only easily measurable but also detectably different for the two mutants.

Measurements of relatively few metabolite concentrations can thus give voice to apparently silent genes. But individual concentrations are often less informative than one might wish, because quite different mutations may affect the same concentration to a similar extent; in any case, with a completely unknown gene there is no prior knowledge of which concentrations to examine. What is needed is a comprehensive way of studying many metabolites together. The FANCY method provides this, and can reveal quite subtle effects of changes in genotype. The C in FANCY stands for 'co-response analysis' (Hofmeyr, J.-H. S. & Cornish-Bowden, A. *J. Theor. Biol.* **182,** 371–

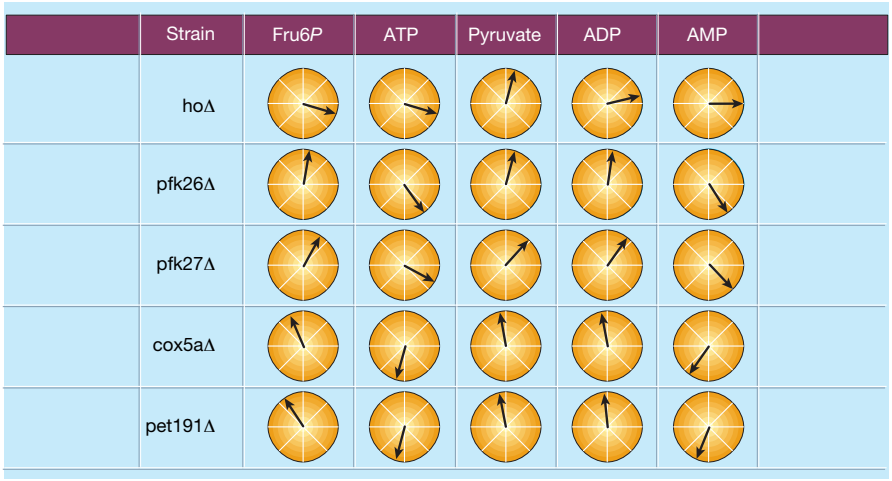


Figure 1 **Study of silent genes using co-response angles, from the experiments described by Raamsdonk *et al.* Five yeast strains are shown (Δ indicates a mutant in which a gene has been deleted). The pfk26Δ and pfk27Δ mutants are defective in 6-phosphofructo-2-kinase activity; cox5aΔ and pet191Δ are mutants of the cytochrome oxidase complex; hoΔ, included as a control, is defective in a part of metabolism not concerned with energy production. Changes in all of the metabolite concentrations shown are relative to changes in the glucose 6-phosphate concentration, which corresponds to the horizontal axis in all cases. The other metabolites are fructose 6-phosphate (Fru6*P*); pyruvate; and the tri-, di- and monophosphate forms of adenosine phosphate. ATP is the primary energy carrier in the cell, and all the metabolites shown are involved in energy production. Note that the different classes of mutants are characterized by different angles. For simplicity, only the angles are illustrated here, but a full analysis would take account of the magnitude as well as the direction of each vector.**

380; 1996). Raamsdonk and colleagues' paper shows how powerful the approach can be.

When two metabolic variables (not necessarily concentrations, but these are often the most revealing) both respond to a change in conditions, their combined response has a direction that can be expressed as an angle. This is better than the corresponding ratio, which suffers from severe problems of statistical instability. The angle is 0° if one does not change at all, 90° if the other does not change at all, 45° if they change by the same amounts in the same direction, and so on. Raamsdonk *et al.* use angles from −90° to +90°, but the signs of the individual concentration changes allow use of the full range from −180° to +180°, with greater sensitivity.

In Fig. 1 the two 6-phosphofructo-2-kinase mutants (pfk26Δ and pfk27Δ) are characterized by similar angles in five different comparisons, easily distinguishable from the angles shown by the other mutants. The two mutants impaired in a different part of energy metabolism (cox5aΔ and pet191Δ) show angles that are similar to one another but different to those of the other mutants. So we can conclude that co-response analysis does provide a sensitive way of grouping mutations into functional classes.

Co-response analysis can classify even completely unknown genes into related groups. Combined with multivariate statistical techniques, such as principal-components and discriminant-function analysis, it allows a large amount of metabolite concentration information, from the nuclear magnetic resonance spectra from different mutants for example, to be put into a map where mutants of related function cluster together. Now that this approach has been shown to perform well on questions where the correct answers are already known, the next step is to begin applying it to the thousands of genes in yeast with no known functions — and that is what Raamsdonk *et al.* are now doing. The problem of how to analyse mountains of data arises in all branches of functional genomics. Two other levels of analysis — the proteome (an organism's protein complement) and transcriptome (its messenger RNAs) — may benefit from similar approaches to those being developed for the metabolome. ■

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