Guilt-by-association goes global

Stephen Oliver

Clues to the function of a protein can be obtained by seeing whether it interacts with another protein of known function. This principle of guilt-by-association has now been applied to the entire protein complement of yeast.

If two proteins interact with one another, they usually participate in the same, or related, cellular functions. Yeast geneticists have a clever way of seeing whether two proteins can physically associate. They attach each of them to separate fragments of a third protein, called a transcriptional activator, which has the ability to switch on genes. If the two proteins interact, then the two fragments of the activator are reunited and a gene is switched on that produces an easily monitored colour change in the yeast cells. Because it is two hybrid proteins that are actually interacting, this method is called the two-hybrid system.

The availability of the complete genome sequence of the yeast *Saccharomyces cerevisiae*[1] raises the possibility of exploiting the two-hybrid approach to identify all possible pairwise interactions between yeast's 6,000 or so proteins. Although such an exhaustive search has been much talked about[2], so far only individual yeast protein complexes have been analysed in this way[3,4]. Now, a collaborative group from the University of Washington, Seattle, and CuraGen, a biotechnology company, has taken on the daunting task of making a comprehensive two-hybrid analysis of protein interactions in yeast. The group concerned, Uetz *et al.*, report their results on page 623 of this issue[5], and readers may manipulate the data themselves at a public web-site[6].

Uncovering the functions of gene products predicted by the DNA sequences of entire genomes has become a sort of 'range on the 'omes'. Functional genomics involves a number of levels of investigation that have been named the genome, transcriptome, proteome and metabolome[7] (Fig. 1). What distinguishes the last three levels from that of the genome is that they are context-dependent. The entire complement of messenger RNA molecules, proteins or metabolites in a tissue, organ or organism varies with its physiological, pathological or developmental condition. Transcriptome analysis, using DNA microarray techniques to screen large numbers of genes for messenger RNA abundance[8], is yielding huge amounts of data about gene function. But it is an indirect approach — messenger RNAs are transmitters of genetic information, not functional cellular entities.

This is not true of proteins and metabolites, but comprehensive analyses of the proteome and metabolome are more technically demanding. Moreover, 'classical' proteomics, involving the techniques of two-dimensional gel electrophoresis (for protein separation) and peptide mass fingerprinting (for protein identification)[9,10], discards information of immense value in making functional assignments. This is because extracts are immediately placed in denaturing conditions, thereby destroying all protein–protein interactions. Discovering that a protein of unknown function interacts with one of known function provides a valuable clue to the role of the novel gene product, a concept that has been termed guilt-by-association. So a parallel approach to proteome analysis is to examine not the relative abundance of proteins, but their potential interactions with one another.

Two-hybrid analysis (Fig. 2, overleaf) works by separating the coding sequences for the DNA-binding and activation domains of a transcriptional activator and cloning them into separate vector molecules. The coding sequence of a candidate protein whose partners are sought (known as a 'bait') is then fused with the DNA-binding domain. A library of coding sequences for proteins that might interact with the 'bait' (called 'prey') is made in fusion with the activation domain. Yeast (like most sensible organisms) has two sexes, called **a** and α. Therefore, 'baits' and 'prey' can easily be introduced into the same yeast cell by mating. If they physically interact, the DNA-binding and activation domains are closely juxtaposed and the reconstituted transcriptional activator can mediate the switching-on of the gene that effects the colour change.

Uetz *et al.*[5] have used two different strategies in a comprehensive analysis of yeast protein–protein interactions. In array screening, 6,000 yeast colonies, each expressing a different 'prey' molecule, were distributed into microtitre trays. Strains expressing different 'bait' molecules (192 in all) were mated to each member of the array and the positive interactions were identified. One advantage of the array approach is that it rapidly becomes clear which locations produce false-positive interactions, providing reassurance that the system is working

Level of analysis	Definition	Status	Method of analysis
Genome	Complete set of genes of an organism or its organelles	Context-independent (modifications to the yeast genome may be made with exquisite precision)	Systematic DNA sequencing
Transcriptome	Complete set of messenger RNA molecules present in a cell, tissue or organ	Context-dependent (the complement of messenger RNAs varies with changes in physiology, development or pathology)	Hybridization arrays[8] SAGE[13] High-throughput Northern analysis[14]
Proteome	Complete set of protein molecules present in a cell, tissue or organ	Context-dependent	Two-dimensional gel electrophoresis, peptide mass fingerprinting[9,10] Two-hybrid analysis[5]
Metabolome	Complete set of metabolites (low-molecular-weight intermediates) in a cell, tissue or organ	Context-dependent	Infra-red spectroscopy[15] Mass spectrometry[15] Nuclear magnetic resonance spectrometry[16]

Figure 1 **Levels of gene-function analysis. The work of Uetz *et al.*[5], discussed here, applies two-hybrid analysis to the yeast proteome. Details of the methods used are to be found in the references cited; SAGE is serial analysis of gene expression.**

100 YEARS AGO

The proposal to generate electricity on the Canadian side of the Niagara and to sell electric power on the American side, has caused a flutter of excitement among American electricians. The *New York Electrical Review* states that the question has been raised whether foreign-made electricity is not subject to a duty of ten per cent *ad valorum* as an "unenumerated manufactured article." This question has produced a flood of debate, and while it is purely hypothetical as yet, the Merchants' Exchange of Buffalo and the Niagara Falls Power Company, have gone so far as to pass resolutions opposing such taxation. Those who desire discrimination in favour of home-made electricity argue that electric power is a vendable and valuable product of manufacture; that it can be measured easily and accurately, and that foreign-made electricity should pay duty equally with foreign-made cloth or wine. Those who believe in free trade in electricity point out that it is not an article, it is not valuable or sold or saleable, that it has no power to do work, but only serves as a means of transmitting power, and that it is utterly impossible to import it because it instantly returns to its source.

From *Nature* 8 February 1900.

50 YEARS AGO

Throughout Scandanavia most original scientific work is now published in the English language. Some authors write in English and others have their work translated locally; but in many cases it is advisable to obtain the services of an English-speaking man of science to check the manuscript. A most desirable course would be for such material to be checked by an English-speaking specialist who is working on almost identical lines and thereby has a natural interest in the work. The British Council has, therefore, initiated a scheme whereby it will endeavour to place each manuscript which has been submitted through its offices in Scandanavia with a scientific worker in Great Britain who is likely to have an interest in the subject in question and can carry out the checking. In general, the Scandanavians are willing to pay reasonable fees for such services, and the British Council has arranged to undertake the currency conversion for payment in sterling. The Council itself will make no charge for its services.

From *Nature* 11 February 1950.

properly. In contrast, the library screening method does not keep cells expressing the 6,000 different 'prey' molecules separate. Instead, it pools them, and each of the 6,000 strains expressing a different 'bait' is mated with the pool. Hybrid cells are selected and then screened for positive interactions.

The two strategies yield different results. The array method is more efficient, which is only partly attributable to the judicious choice of 'baits'. The library approach, while benefiting from much higher throughput, has the disadvantage that cells in the 'prey' pool compete with one another during growth and mating, so selecting against cells expressing fusion products that retard either process. Thus, of the 12 'baits' that gave positive interactions with both screens, 48 possible partners were identified by the array approach, against only 14 in the library screen.

Uetz and colleagues' analysis[5] has yielded discoveries of three types. First, interactions between proteins of known and unknown function have indeed allowed the role of the latter to be inferred. For instance, two proteins have been linked to the metabolism of arginine (the principal storage amino acid in yeast) because they can interact with ornithine aminotransferase (an enzyme required for arginine biosynthesis).

Second, hitherto unrecognized interactions have been identified between proteins involved in the same biological process. Yeast is an important model system for the study of the cell cycle in eukaryotes (organisms, including ourselves, whose cells have a nucleus; bacterial cells, for instance, don't). Uetz *et al.* have uncovered further interactions between cell-cycle regulators, thus extending our view of the subtlety and complexity of the control of cell growth and division. Specifically, it turns out that the cyclin-dependent kinase Cks1 interacts with each of three different B-type cyclins, implying that Cks1 may allow them to regulate the activity of the Cdc28 kinase that controls the start of yeast's cell cycle.

Finally, and perhaps most notably, the screen has provided clues for seeing how individual biological events are integrated into larger cellular processes. Meiotic recombination is the process that produces new gene combinations during the production of sperm and eggs, and ensures, for instance, that each of us is a unique individual. It involves the exchange of DNA segments between chromosomes inherited from each parent in a process called crossing-over. This takes place in a structure known as the synaptonemal complex and involves the breakage and rejoining of DNA molecules. The subsequent separation of each chromosome pair (segregation) requires the action of the filaments of a structure termed the meiotic spindle.

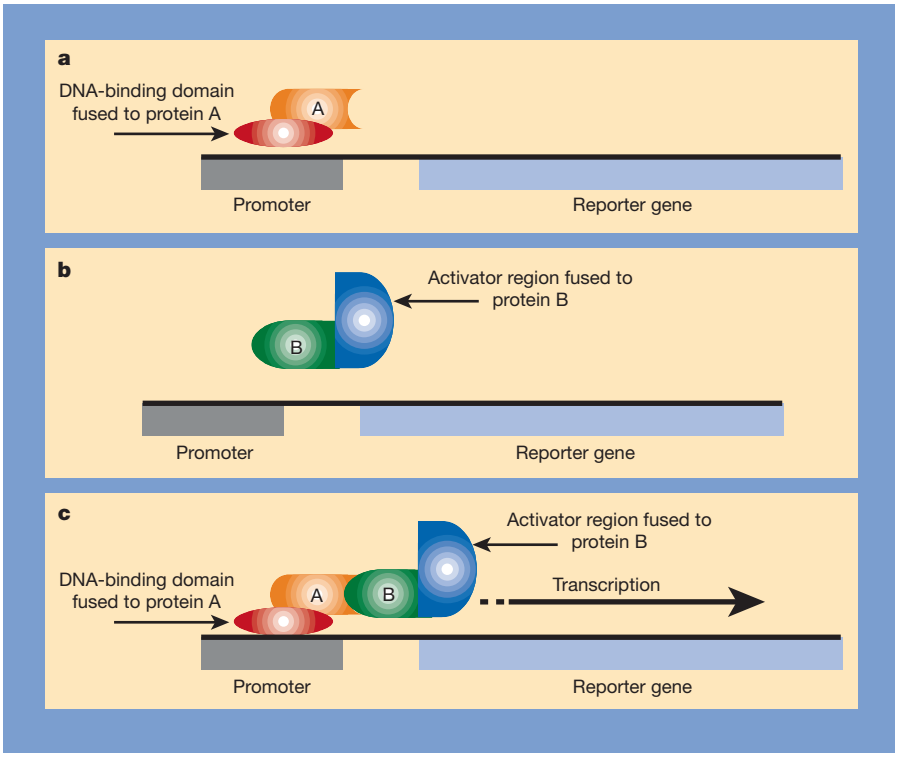


Figure 2 **Detection of protein associations in yeast using the two-hybrid system.** a, **Fusion of the 'bait' protein and the DNA-binding domain of the transcriptional activator cannot turn on the reporter gene.** b, **Likewise, fusion of the 'prey' protein and the activating region of the transcriptional activator is also insufficient to switch on the reporter gene.** c, **When 'bait' and 'prey' associate, however, the DNA-binding domain and activator region are brought close enough together to switch on the reporter gene. The result is gene transcription and a colour change that can be monitored.**

Uetz *et al.* found that Msh5 (a yeast protein required to resolve DNA cross-overs) is able to interact with two proteins implicated, respectively, in the formation of double-strand DNA breaks and the two ends of the meiotic spindle. One of these proteins, in turn, interacts with another that is required for generation of the synaptonemal complex. Thus a network of interactions is revealed that provides an overview of the mechanisms involved in meiotic recombination.

Although comprehensive two-hybrid analysis has the integrative power of all good functional genomics techniques, it lacks the context dependency of classical proteome analysis. It reveals potential protein interactions, but not the biological context in which they happen. Some may occur only when yeast is in a particular physiological state; others may never occur because, in real life, the proteins are located in separate cellular compartments.

New approaches to classical proteomics are required, which exploit separation techniques that do not destroy protein–protein interactions, and which involve methods of mass spectrometry[11,12] and bioinformatics that allow the unambiguous identification of all members of a protein mixture. Such a biochemical approach will complement comprehensive two-hybrid analysis. Together, the two will be a powerful way to discover the functions of newly identified proteins and integrate them into a comprehensive view of the workings of a living cell. ■

Stephen Oliver is in the School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, UK.
e-mail: steve.oliver@man.ac.uk

1. Goffeau, A. *et al. Science* **274,** 546–567 (1996).
2. Fromont-Racine, M. *et al. Nature Genet.* **16,** 277–282 (1997).
3. Mayes, A. E. *et al. EMBO J.* **18,** 4321–4331 (1999).
4. Flores, A. *et al. Proc. Natl Acad. Sci. USA* **96,** 7815–7820 (1999).
5. Uetz, P. *et al. Nature* **403,** 623–627 (2000).
6. http://curatools.curagen.com
7. Oliver, S. G. *et al. Trends Biotechnol.* **16,** 373–378 (1998).
8. Hoheisel, J. D. *Trends Biotechnol.* **15,** 465–469 (1997).
9. Sagliocco, F. *et al. Yeast* **12,** 1519–1534 (1996).
10. Shevchenko, A. *et al. Proc. Natl Acad. Sci. USA* **93,** 14440–14445 (1996).
11. Summerfield, S. G. *et al. Int. J. Mass Spectrosc.* **188,** 1–2 (1999).
12. Link, A. J. *et al. Nature Biotechnol.* **17,** 676–682 (1999).
13. Velculescu, V. E. *et al. Cell* **88,** 243–251 (1997).
14. Planta, R. J. *et al. Yeast* **15,** 329–350 (1999).
15. Teusink, B. *et al. Methods Microbiol.* **26,** 297–336 (1998).
16. Brindle, K. *et al. J. Mol. Recog.* **10,** 182–187 (1997).

Astronomy

Dynamics of Jupiter's atmosphere

Alvin Seiff

Understanding the complex dynamics of Jupiter's atmosphere has been difficult. The planet is mostly fluid, and in this sense is more like a star than the terrestrial planets with their thin atmospheric shells. Observed from a distance, Jupiter's atmosphere below 45° latitude is divided into dark zones and bright bands of cloud in which there are fast-moving jets and long-lived ovals (Fig. 1). The dominant oval is the Great Red Spot, a storm system that has been observed for more than 300 years. Jupiter's weather is altogether remarkably stable: the jets have been flowing east and west at nearly constant speeds for more than 100 years.

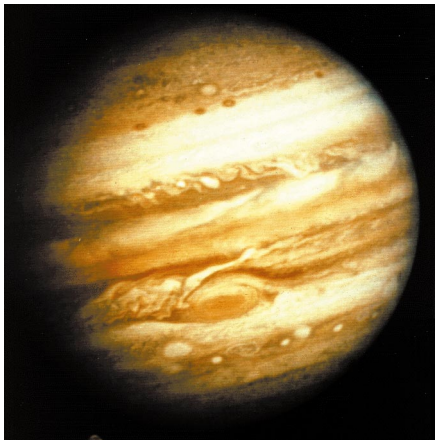
What drives this constant activity — the distant heat of the Sun or the planet's own internal energy? Sunlight, which drives the weather on Earth, is only 4% as strong on Jupiter as on Earth. Jupiter also emits almost twice as much heat as it absorbs from the Sun. The internal heat is not from the nuclear fusion that fuels a star, but is possibly generated by the heavier helium sinking into the deep interior. Solar energy should be important in the surface layers, whereas the transport of internal heat to the surface should drive activity in the deep interior. New observations[1,2] from the Galileo Orbiter, reported on pages 628–632 of this issue, indicate that moist convection — similar to what happens in large thunderstorms on Earth — transports significant energy upwards through Jupiter's clouds.

Moist convection has long been considered a candidate energy source for Jovian dynamics[3], but there has been no firm evidence for its importance on Jupiter until now. Gierasch *et al.*[1] study visible and infrared images taken by Galileo of a region downstream of the Great Red Spot. Just before dawn they observe bright spots due to lightning, and after dawn they find two large storms at the same location. Lightning flashes have recently been seen in Jupiter's clouds[4], but here cloud structure and depth are used to establish that lightning occurs in extremely thick, water-based clouds, up to 50 km below the surface. Such storms are reminiscent of large thunderstorm complexes on Earth, where moist convection occurs. Fluid velocities determined by tracking cloud features reveal an outflow from the storm centre, suggesting, as on Earth, upflow within the storm. Horizontal rotating eddies surround the storm, indicative of large-scale turbulence downstream of the Great Red Spot.

The authors estimate the vertical energy transport due to moist convection in the cloud. They assume that all lightning storms on Jupiter are like this one and multiply the energy transported by the frequency of lightning storms to estimate the global rate of energy outflow. The energy transported by moist convection over the entire planet is comparable to the flux generated by Jupiter's internal heat source. This is still only a rough estimate because of the assump-tions and uncertainties, but it supports the idea that moist convection can carry Jupiter's internal heat up through the cloud levels. The question is how the energy gets transported to and concentrated into these clouds.

In the other paper, Ingersoll *et al.*[2] integrate moist convection into their model of Jupiter's zones and belts. They expand on their earlier idea[5] that jets in Jupiter's cloud layers are driven and maintained by momentum received from small-scale eddies. Jets usually decay into eddies, going from order to disorder in keeping with the second law of thermodynamics, as, for example, in jets emerging from aircraft engines. How Jupiter's eddies then maintain their energy is not clear. Ingersoll *et al.* now argue that moist convection is the energy source that drives turbulent eddies. But how is the vertical kinetic energy of convection converted to angular kinetic energy of the horizontal eddies? The energy is there, but what is the process?

A point not addressed in these papers is the increased wind speed below Jupiter's surface, as observed by the Galileo probe[6]. Wind speed increased from 100 m s^{-1} at the cloud tops to ~180 m s^{-1} at depths greater than 70 km, below which they are constant down to 150 km. This vigorous interior flow would not appear to result from cloud convection,



NASA

Figure 1 **Image of Jupiter taken by Voyager in 1979. The dark and bright bands of clouds on Jupiter and the Great Red Spot — a 300-year-old storm — are clearly visible. What drives Jupiter's turbulent weather has long been a matter of debate. Images taken by the Galileo spacecraft in orbit around Jupiter last year provide new evidence[1,2] that moist convection — similar to what happens in large storms on Earth — is responsible for transporting energy upwards through Jupiter's clouds.**