

Revelations from a bread mould

Jonathan Arnold and Nelson Hilton

The filamentous fungus *Neurospora crassa* is an organism much loved by geneticists. Its genome sequence has now been unveiled, and includes some surprises — such as the relatively high number of genes.

The lowly bread mould *Neurospora crassa* has a venerable history as a subject of scientific research¹. Its fame really began more than 60 years ago, with work by George Wells Beadle and Edward Lawrie Tatum² that would later win them a Nobel prize. Before then, geneticists were studying genes as hypothetical entities that are transmitted from parents to offspring and — in an unknown way — control inherited traits (as illustrated in Fig. 1)³. Meanwhile, biochemists were looking at proteins and their functions in the cell. There seemed at the time to be little connection between the two disciplines. Working with *Neurospora*, however, Beadle and Tatum made the stunning discovery that genes as units of heredity also encode the proteins that carry out much of the work of the cell.

Now, in a paper that promises to be just as revelatory, Galagan *et al.*⁴ present us with the entire list of genes found in the *Neurospora* genome (page 859 of this issue). And on page 893, Selker *et al.*⁵ take a closer look at a particular type of DNA modification — methylation — in *Neurospora*. They propose that this functions largely to defend the genome against mobile genetic elements.

Galagan *et al.*⁴ report the sequence of more than 38.6 million of the 'letters' — base pairs — from the genetic text of this filamentous fungus. They used the 'whole-genome-shotgun' strategy to read the sequence. The approach involved shattering the genome into more than 200,000 fragments, sequencing the pieces, and then looking for overlaps between the text of the fragments so as to put them together in the right order. This strategy is becoming more and more common, although there has yet to be a convincing demonstration that it produces a puzzle that can be put together correctly. A 'physical map' of landmarks in the *Neurospora* genome is in progress, however; if it tallies with the sequence now revealed, it will provide one of the few convincing tests of the approach.

As is usual at this stage of a sequencing project, there are still a few holes in the sequence (*Neurospora* has been estimated to have a genome of 42.9 million base pairs⁶). But at least 90% of it has been completed. Although the authors have only just finished assembling the text, and so have had little time to meditate on its message, they have already revealed a wealth of new information.

One remarkable finding is the estimated



Figure 1 Following genes from generation to generation in *Neurospora crassa*. The segregation of normal and mutant forms of a gene is shown, with black spores bearing the normal gene and grey spores the mutant form. Each line of eight spores is the product of one cross between two parents. The pattern shows the order in which the spores were formed, and hence the pattern of segregation of the gene (that is, its mendelian inheritance). This cannot be observed directly in most other organisms, explaining why *Neurospora* is the geneticists' favourite child. (Reproduced from ref. 3.)

number of 'words' — protein-encoding genes. Identifying genes in amongst other sequence information is never an easy matter, because there are no accurate procedures for doing this⁷; in the case of *Neurospora*, the quest was particularly hard because more than 50% of the genes are made up of non-contiguous sections, making it difficult to determine where gene boundaries lie. So the gene number may need to be revised in the future. But it seems that, although the *Neurospora* genome is respectively about 4 and 100 times shorter than those of fruitflies and humans, the number of genes is not so different. *Neurospora* has around 10,000 genes, compared with some 14,000 in fruitflies⁸ and 21,000–39,000 in humans^{9,10}. In this sense, we are truly not that far in genetic complexity from the common bread mould.

The function of many of *Neurospora*'s genes remains unknown, however. Comparisons with known genes in public databases are one way of determining function, but it seems that only about 1,400 of the *Neurospora* genes have counterparts in fruitflies, worms and other complex eukaryotes (loosely speaking, those organisms, including humans, whose cells have nuclei). Moreover,

more than half of the *Neurospora* genes have no detectable similarity to those of its yeast relatives *Saccharomyces cerevisiae*¹¹ and *Schizosaccharomyces pombe*, the genomes of which have also been sequenced. Much of *Neurospora*'s genetic text awaits exegesis.

What else does the genome sequence tell us? This fungus shares some significant physical characteristics with other complex eukaryotes, notably a biological clock¹². It knows how to tell the time of day, and much of what we understand about this process in other organisms comes from studies of *Neurospora*. The genome sequence should reveal how the biological clock connects with other cellular processes, such as metabolism and light sensing. Furthermore, the sequence provides hints that, like some plants, *Neurospora* can sense both blue and red light. It was already known that it responds to blue light, and Galagan *et al.*⁴ have now found some additional genes that may be involved in this process. But the discovery of genes that may be needed in sensing red light comes as a surprise. Also, the sequence shows that *Neurospora* shares with yeast several of the core signalling pathways for sensing other aspects of the environment (reviewed in ref. 13).

Unlike its yeast relatives, however, *Neurospora* abhors repeated genes and other duplicated sequences in the genome. It has developed a mechanism, known as 'repeat-induced mutation' (RIP), by which such repetitive sequences are destroyed through mutation¹⁴. It may have evolved this mechanism to keep out 'foreign' DNA from viruses and the like, with the result that very little redundancy appears in the text, as Galagan *et al.* show. Nearly every word has a distinct meaning — a feature that may help in decoding how the genome functions. When there is unknown redundancy, it presents a stumbling-block to those who attempt to understand the function of a gene by inactivating it.

So far the focus has largely been the study of one or a few words, but biologists can now begin to see larger syntactic units, such as the coincidence of the 'RIPed' text and its modification with methyl groups. In particular, Selker *et al.*⁵ have found that methylation occurs almost exclusively in the relics of transposons — mobile genetic elements — that have previously been inactivated by RIP. Methylation is generally associated with

gene silencing, and so RIP and methylation together seem to represent a belt-and-braces strategy to ensure that transposons remain inactive. However, Galagan and colleagues' intriguing finding that some regions of the text are methylated even though they are not RIPed raises the possibility that methylation also has another function in *Neurospora*.

Beadle and Tatum² said that, "From the standpoint of physiological genetics the development and functioning of an organism consist essentially of an integrated system of chemical reactions controlled in some manner by genes". With the *Neurospora* genome in hand, researchers can now move towards a specification of the chemical reactions that link genes and proteins in processes such as metabolism, biological clocks and development¹⁵. We may also be able to borrow from computer science to describe the reaction network in terms of a 'biological circuit' and discover more about how this fungus functions. New genomics approaches can measure the responses of the circuit (through RNA and protein profiling) and the links between its components (through protein-protein and protein-DNA

interaction mapping). The genetic text, together with such approaches, should bring a further revelatory consilience of biochemistry and genetics¹⁶.

Jonathan Arnold is in the Department of Genetics and Nelson Hilton is in the Department of English, University of Georgia, Athens, Georgia 30602-7223, USA.

e-mails: arnold@arches.uga.edu
nhilton@arches.uga.edu

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25 years ago in the Nobel-prizewinning work of Hans Dehmelt¹². The magnetic field curls the path of the trapped electron into a spiral-like trajectory. A positively charged torus and two negatively charged caps, aligned on the trap axis, prevent the electron escaping. The upper cap also serves as a sensitive probe for the tiny voltage fluctuations caused by the hovering electron. Through a feedback loop, those voltage changes are amplified and fed back to the lower cap, creating corrective tugs on the electron's motion.

The random voltage that is induced by the thermally fluctuating electron motion along the axis of the trap has been measured in a frequency-selective way. The result is a spectrum of noise power that has a notch at the frequency value that matches the resonant frequency of this axial electronic oscillation — the electron can be represented by a serial circuit that acts, on resonance, as an electric short between the caps. The width of the notch represents how much the fluctuating electron motion is damped, characterizing the dissipation of vibrational electron energy into the electric circuit. Increasing the signal gain in the feedback loop causes this damping to decline, and with it shrinks the width of the spectral notch (Fig. 1).

The temperature of an ensemble of particles quantifies the fluctuations of this system. When this indicator is applied to a single electron, the particle's motional excursions are measured again and again, and the stochastic ensemble of observations can be easily transformed into recorded values of motional energy; these snapshots form, in the equilibrium state, a thermal (or Boltzmann) distribution, with higher energy values being exponentially less likely than lower ones. A tricky way of monitoring such a distribution — called thermometry — has been suggested by Dehmelt^{13,14}. There are, apart from the electron's axial motion, two more, independent modes of vibration: a fast azimuthal motion in which the electron orbits the trap axis, called 'cyclotron motion'; and a slow pulsation of the orbital radius, called 'magnetron motion'. These motions may be excited in differently quantized portions of vibrational energy, and a temperature measurement amounts to finding out how often and for how long the first excited state (and the second, third, and so on) of the quantized motion is randomly occupied by the electron — the mean numbers of occupation forming a Boltzmann distribution when the electron is in thermal equilibrium.

The job of measuring this probability of excitation in a non-invasive and precise way would seem to require a complex strategy — and the patience of indefatigable Sisyphus. The elegant (although not quite straightforward) solution is to make frequency measurements, which are famously precise and convenient, using two coupled oscillators known to be shifting each other's

Quantum electronics

The electron is cool

Peter E. Toschek

Temperature is an awkward concept for a single particle, but the energy of the particle's 'quivering' is a useful substitute. Feedback control of this motion can be used to cool a single electron to very low temperature.

Individual electrons, trapped by electric and magnetic fields, can be used for precise, fundamental measurements. D'Urso, Odom and Gabrielse¹ now report in *Physical Review Letters* that they have succeeded in controlling the residual random motion of a trapped electron using feedback signals. This degree of control could be the basis of experiments to measure elementary constants of nature with even greater precision than before, and to accurately test the quantum theory that governs electrons and radiation — quantum electrodynamics.

The 'calming' of isolated quantum systems, by reducing their kinetic energy, is behind much of the progress in the physics of light and atoms in the past decades. Laser cooling has produced much narrower spectral lines of emitted radiation from free atoms² and bound ions^{3–5}, and hence more precise atomic data⁶. Evaporative cooling of atom clouds in magnetic or optical traps — similar to the way in which coffee cools in a cup — made possible the phase transition to a new state of matter, a Bose-Einstein condensate^{7,8}. Experimenters can force particles such as antiprotons to condense into tight bunches inside the storage ring of

an accelerator by detecting their random excursions and feeding that information back in the form of a changing electrode voltage to coax them back into line. This reduction of random motion is stochastic cooling⁹ — a decisive factor in the discovery of the W and Z bosons, the fundamental particles that mediate nature's weak force.

Feedback, a controlling operation determined by the result of a preceding measurement, may be imposed on any system. In earlier experiments, the residual motion of a single ion inside an electrodynamic trap (consisting of a static and an alternating electric field) has been reduced using a kind of feedback^{10,11}. Here, sequences of almost-resonant light pulses absorbed quanta of vibrational energy from the ion, damping its fluctuations. Now D'Urso *et al.*¹ demonstrate that it is possible to cool an individual quantum particle, an electron, by straightforward, continuous electronic feedback.

In their experiment, an electron is captured and trapped in an electromagnetic cage created from a homogeneous magnetic field and an inhomogeneous electric field that is zero at the centre of the apparatus (Fig. 1). This technique was pioneered some