Malaria

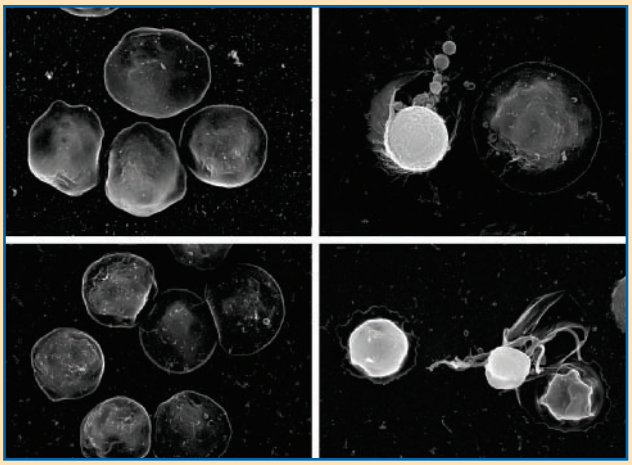
The calcium connection

One of the most visually striking events in the life cycle of the malaria parasite is exflagellation — the process by which the male reproductive cells emerge, complete with propellers, from a precursor cell. As they describe in *Cell* (**117,** 503–514; 2004), Oliver Billker and colleagues have pinned down the initial steps in the underlying cascade of molecular events.

The malaria parasite leads a complex life, relying on two hosts: humans and mosquitoes. It reproduces sexually in the insects. When a mosquito bites an infected human, it takes up red blood cells containing parasite gametocytes — pre-reproductive cells. Within seconds of being ingested, the gametocytes break out of their unwitting host cells, destroying them in the process. The resulting female reproductive cells (gametes) can be fertilized immediately, but the male cells must go through several more stages of development before fully fledged male gametes are formed.

Like all good parasites, the malaria agent takes advantage of its host's own machinery to spark off these events — the mosquito molecule xanthurenic acid acts as the trigger. But what happens next? Billker *et al.* chose to investigate the role of Ca^{2+} ions, common molecular mediators in many organisms, and Ca^{2+}-binding proteins, of which there are many encoded in the parasite genome. To do so, the authors engineered *Plasmodium berghei* — a rodent malaria parasite — to carry a molecule that glows brightly in the presence of Ca^{2+} ions. When they added xanthurenic acid to mouse blood containing these engineered parasites, the gametocytes lit up (the Ca^{2+} detected probably coming from stores within the parasites).

So what occurs downstream of the release of Ca^{2+} ions in the production of gametes? Billker *et al.* wondered if a protein of the calcium-dependent protein kinase (CDPK) family might be implicated.



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Indeed, the authors found that one member of the family, CDPK4, is expressed predominantly in male *P. berghei* gametocytes. They then went on to generate parasites lacking this protein. As the top two images show, mutant male gametocytes were able to emerge successfully from red blood cells (left, red blood cells; right, a rounded male gametocyte emerging from the ghost of its host). But they could not exflagellate: to do so, CDPK4 had to be reinstated (see the lower two images; an exflagellating gametocyte, with protruding propellers, is on the right).

It remains to be seen what comes next in this game of molecular dominoes. Billker *et al.* also want to know where exactly the Ca^{2+} ions are stored inside gametocytes — and is there a counterpart of CDPK4 in the female cells?

Amanda Tromans

mass and the thickness of the light tubes. Perhaps surprisingly, the effective mass of each atom can be easily changed by introducing a standing-wave light field along the length of the tubes, giving rise to a periodic one-dimensional potential. The resulting motion, in which an atom continuously absorbs and emits light as it surfs the standing wave, modifies the effective atomic mass in a way that can be controlled. Using this technique, Paredes *et al.*[1] observed the crossover[7] from the weakly interacting regime, in which the particles can freely pass one another, to a 'fermionized' strongly correlated gas. As proof of the creation of the Tonks–Girardeau gas, Paredes *et al.* measured the momentum distribution of the atoms in the tubes and found that it matched their theoretical prediction for such a state.

It should be emphasized that the Tonks–Girardeau gas only emerges if the gas is sufficiently dilute. At high density, another strongly correlated state with quite different properties may be produced. This is the Mott insulator, which was recently studied in a similar but complementary experiment[8].

One of the most important aspects of Paredes and colleagues' experiment is the striking illustration of quantum control and quantum-state engineering. How this will affect the ongoing pursuit of quantum information and computation, and the investigation of a variety of quantum coherent and strongly correlated phenomena, is not yet clear. But an exciting path has been laid out before us. ■

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Gene regulation

Selfish elements make a mark

Frederic Bushman

Transposons qualify as 'selfish' DNA elements, adding new copies of themselves into our genomes without regard for the consequences. This wilful habit may, however, help in normal gene regulation.

People differ in their genetic make-up, not only through changes in single DNA base pairs but also by the insertion of whole blocks of DNA[1–3]. Human cells contain one type of active mobile element — the L1 retrotransposon — that is responsible for these insertions. Transposons are defined as DNA sequences that can form extra copies of themselves at new locations in an organism's genome; today, L1 and related retrotransposons account for a staggering 17% of the human genome sequence. Mercifully, however, L1 elements 'transpose' only infrequently, minimizing damage to our chromosomes. The forces that keep L1 at bay have long been a mystery, but two papers by Boeke and colleagues in this issue[4,5], together with an earlier study[6], unveil a major control

100 YEARS AGO

A copy of the *Peterborough Advertiser* of May 7 has been sent to us, containing the announcement that radium has been found in beds of Oxford Clay near Fletton, Huntingdonshire. No particulars are given, but a long descriptive article on the discovery suggests that it will make "brickfields better than gold mines." These sanguine anticipations will perhaps be tempered by the following extract from a paper by Prof. J. J. Thomson, read before the Cambridge Philosophical Society on February 15:— "Radium was found in garden soil from the laboratory garden, in the Cambridge gault, in gravel from a pit at Chesterton, in still greater quantities in sand from the sea-shore at Whitby, in the blue lias at Whitby, in powdered glass, in one specimen of flour, and in a specimen of precipitated silica."

ALSO

In the souring of milk the amount of lactic acid developed may reach 0.80 per cent. in three or four days when the milk solidifies. In view of Sir O. Lodge's suggestion (NATURE, October 1, 1903), I have made experiments comparing the rate of acidification, in two to three days, with and without the influence of radium rays… It therefore appears to me that under normal conditions radium rays have little or no effect on the functions of the lactic acid bacillus.

From *Nature* 19 May 1904.

50 YEARS AGO

Mr. A. R. Thomson, R. A., has completed a painting on astronomy to decorate a wall in the entrance hall of the Science Museum, London, near the well-known Foucault pendulum. Mr. Thomson was given *carte blanche* to depict the subject of 'Astronomy', and he has chosen as his main theme the invention and development of the telescope. On the left the small sons of Dutch spectacle-makers are playing with combinations of pairs of lenses… In the centre of the picture Galileo is using his telescope, ministered to by three rather bewildered ladies. On the right the distrust and suspicion of this early period is shown by figures of authority against a background of burning books. The whole picture is dominated by the great two-hundred-inch Hale telescope, and the sky behind is an animated representation of heavenly bodies among the figures of the constellations.

From *Nature* 22 May 1954.

mechanism. In the first paper[4], the authors present this discovery and discuss its implications for understanding genomic evolution. Their second paper[5] describes how they capitalized on these findings to make a modified L1 element that is more than 200 times as active as normal.

L1 and its relatives are termed retrotransposons because their transposition involves reverse transcription — the copying of RNA into DNA. Retrotransposition of L1 begins with transcription of the L1 genomic (DNA) sequence to form messenger RNA (mRNA) copies, a process carried out by a cellular enzyme called RNA polymerase (Fig. 1). The resulting RNAs serve both as templates for the production of L1 proteins (ORF1p and ORF2p) and as templates for retrotransposition. ORF1p binds and organizes the L1 RNAs, whereas ORF2p contains two enzymatic activities — the reverse transcriptase and an endonuclease.

The endonuclease makes a DNA nick in a target chromosome (Fig. 1). The reverse transcriptase then extends one of the freed DNA ends, using the L1 RNA sequences as a template, thereby making a single-stranded DNA copy of the L1 RNAs at the nicked site. Next, the new L1 DNA sequence becomes double-stranded and fully integrated through a series of, as yet ill-defined, further steps. As a result, a copy of the L1 sequence appears at a new location in the genome — often within a gene. The nicking reaction is weakly sequence-specific (it favours DNA that is rich in adenine and thymidine bases), but the target sequence is common enough for L1 elements to be able to hop into much of the human genome.

Nonetheless, the rate of L1 transposition today is very low. This is because the L1 RNAs and proteins accumulate only to very low levels in non-reproductive cells (the proteins are expressed more efficiently in germline cells, explaining why people often differ in the insertions in their genomes). In the first of the new papers, Han, Szak and Boeke[4] describe how they tracked this weak accumulation down to barriers within the L1 DNA sequence that prevent the efficient passage of RNA polymerase. They fused the ORF1 or ORF2 DNA sequence to marker genes, and showed that the hybrid mRNA accumulated much less than did the marker gene mRNA alone.

Curiously, this effect was independent of the orientation of the L1 DNA — but the mechanism varied. When the L1 sequence was in the same orientation as the marker gene, transcription began but elongation of the mRNA was inhibited. In the opposite orientation, the polyadenine — poly(A) — sequence that is characteristic of the tail end of mRNAs was added prematurely (a mechanism that has been suggested previously[6]).

Boeke and colleagues[4] also carried out a bioinformatics study of the human genome,

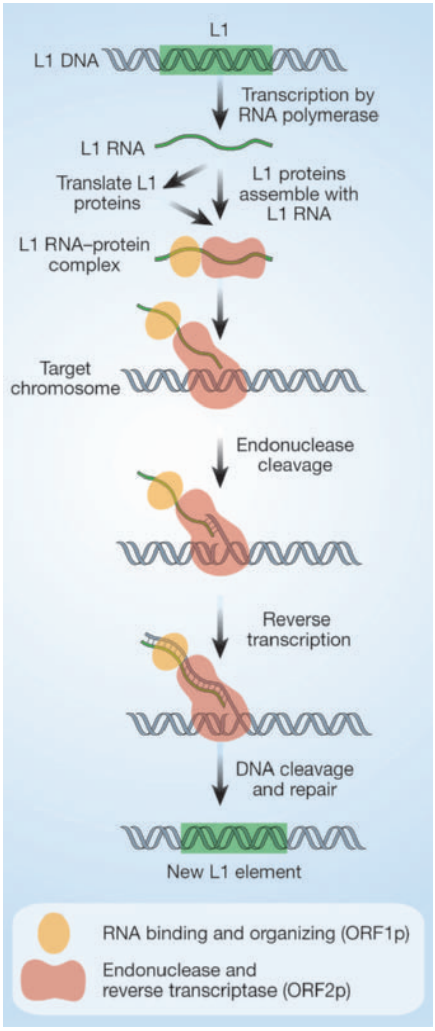

Figure 1 **Retrotransposition of the L1 element. The process begins when the DNA sequence encoding an L1 element is transcribed into RNA by RNA polymerase; the L1 proteins ORF1p and ORF2p are then made, using the RNA as a template, and the RNA and the proteins form a complex. The complex binds to another part of the genome and the endonuclease makes a nick in the DNA, generating a free end. The reverse transcriptase uses this free end as a 'primer', allowing it to start synthesizing a single-stranded DNA copy of the L1 RNA. The RNA is then removed (not shown) and the single-stranded DNA is used as a template to make double-stranded DNA, which is then seamlessly stitched into the nick in the chromosome. Thus, a new L1 element has been made in the genome.**

which revealed that these obstacles can be imposed on a cellular gene through the integration of L1 sequences. Specifically, the authors found that some 79% of human genes have L1 sequences in their non-protein-coding regions (introns) — and that high L1 density correlates with low levels of accumulation of the mRNAs encoded by these genes. These findings support a model in which L1 insertions in introns regulate cellular gene activity by diminishing the efficiency of mRNA elongation or promoting

the premature addition of poly(A) tails, usually without disrupting the integrity of the final protein.

Armed with these findings, Han and Boeke[5] created a mouse hyperactive L1 element (smL1) by resynthesizing the coding sequence of L1. This striking technical feat involved assembling 4,500 base pairs of continuous DNA from short synthetic segments. To make smL1, they replaced a large fraction of the L1 element's protein-coding region with 'synonymous' sequences — sequences that encode the same amino acids but use different triplets of bases to do so. They thereby mutated the sequences that prevent transcriptional elongation or allow premature addition of poly(A) tails. Transposition tests in cultured cells revealed that smL1 transposed more than 200 times more efficiently than wild-type L1.

These findings have several implications. One is technical — if smL1 is as active in intact animals as it is in cultured cells, it might provide a new tool for generating mutations in genetic screens. Human L1 elements have been shown to transpose in mice[7], but use of smL1 instead could greatly increase the rate of mutagenesis. This strategy has the advantage that mutations caused by inserting smL1 would be tagged with smL1 sequences, making it easier to isolate the mutated gene.

Another implication involves the long-term remodelling of the host genome. L1 elements qualify as 'selfish DNA', responding individually to darwinian selection. But the genetic novelty they generate can contribute positively to their host as well, by providing new substrates for natural selection[8]. Previously, researchers have shown that L1 elements can modify the genome by, for example, moving adjacent sequences about with them, hopping into (and so mutating) the coding portions of genes, and contributing portable regions of similarity that allow chromosomes to pair up and swap segments.

The new papers[4,5], together with the earlier study[6], suggest that the insertion of L1 elements into introns can also diminish cellular gene expression in a graded fashion. In the words of Han, Szak and Boeke, such L1 insertions provide a "molecular rheostat" with which to govern gene activity — and their bioinformatics analysis establishes that the mechanism is widely used. Given the similarity of gene complements among species, it seems likely that relatively modest changes in gene activity are a key feature of many speciation events. The new findings may thus have revealed a major mechanism that connects genomic plasticity to the evolution of whole organisms. ■

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Chemistry

Atom tracking

Stuart A. Rice

Diffraction patterns, generated using X-ray pulses of only picosecond duration, reveal the motion of individual atoms as iodine molecules dissociate, then recombine, in solution.

Location, location, location! That well-known saying about real-estate value is an apt paraphrase of what chemists want to know about chemical reactions, as atoms change position to transform one molecule into another. These changes govern the outcome of a reaction, and ultimately determine the biological function of the molecule. As they report in *Physical Review Letters*, Anton Plech *et al.* have extended the technique of X-ray diffraction to track such changes on a shorter timescale than ever before (*Phys. Rev. Lett.* **92,** 125505; 2004). They have monitored the photodissociation of iodine dissolved in carbon tetrachloride at the level of picoseconds — just 10^{-12} seconds.

The general outline of a scheme to track a chemical reaction has been clear for some time: generate a short-lived excitation of the molecule to initiate a reaction, and then, over varying time intervals, follow the changing atomic structure of the reactant. Although suitable excitation sources — such as lasers that generate femtosecond (10^{-15} s) pulses of tunable light — have been available for more than a decade, only recently has it become possible to record the change in structure of a reacting molecule with appropriate time resolution. The development of electron beams composed of picosecond-long pulses, and of techniques to extract picosecond pulses of X-rays from a synchrotron source, have proved crucial. These pulses can be used for electron or X-ray diffraction studies, or in techniques such as 'X-ray absorption near-edge structure' (XANES) and 'extended X-ray absorption fine structure' (EXAFS), to trace the evolution of atomic positions during a chemical reaction.

Electrons interact strongly with matter, so an electron-diffraction signal is usually easy to detect. However, because of that strong interaction, time-dependent electron diffraction can only be used to study the reactions of isolated molecules, or reactions on an exposed surface. The interaction of X-rays with matter is, in general, much weaker than that of electrons. Time-dependent XANES, EXAFS and X-ray diffraction can be carried out in condensed phases. Although XANES and EXAFS measurements provide less precise information on atomic structure than does X-ray diffraction, when applied to solutions they avoid many problems associated with background scattering from the solvent. In X-ray diffraction, the occurrence of scattering from the support medium must be taken into account.

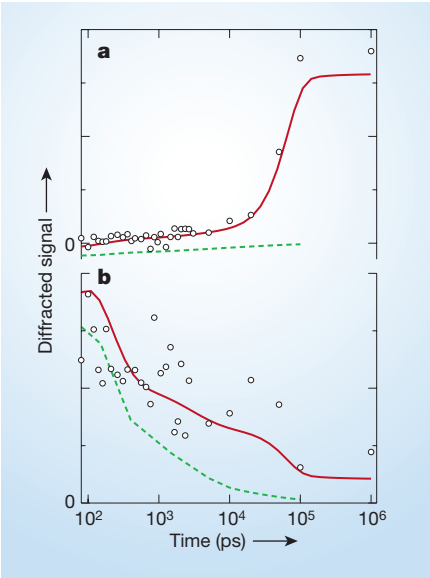

Figure 1 **X-ray diffraction of iodine molecules in carbon tetrachloride solution. Plech *et al.* excited the iodine molecules with a laser pulse, then monitored the molecules' dissociation using X-ray pulses applied at varying time intervals after the excitation. The diffracted signal is shown here for two different spatial scales:** a, **0.6 nm and** b, **0.15 nm. The rise of the data in** a **is attributed to the solvent taking up the energy released by the iodine molecules as they relax to their ground state, and expanding. The behaviour is quite different in** b**: on this scale, both the relaxation of the iodine molecules and the recombination of dissociated atoms contribute to the signal. In both cases, the red curve indicates the predicted signal, taking all the relevant effects into account; the green curve represents the contribution from iodine atoms that do not escape their solvent cage.**