

T300A — the risk-associated variant of ATG16L1 — carries a single amino-acid change in a region of the protein called the WD repeat. This repeat is a recent evolutionary acquisition, absent from the equivalent autophagy gene of organisms such as yeast, and its function is unknown. As the ATG16L1 T300A variant is also common in the healthy human population, it is likely to have been maintained for a good reason. Understanding the interaction between viruses and the autophagy pathway in cells expressing ATG16L1 T300A should be informative.

Aside from the immunological facets of their model, Cadwell and colleagues' work is noteworthy for providing much-needed evidence for the concerted actions of several environmental and genetic factors in triggering a disease. Before this study, a viral contribution to Crohn's disease would not have featured high in a sweepstake of risk factors, as the incidence of clinical flare-ups of the disease do not correlate with outbreaks of viral infection. But viruses could be operating 'subclinically'.

For example, it is possible that defects in autophagy, or in nucleic-acid sensing mediated by pattern-recognition receptors, lead to abnormal persistence of viruses normally cleared by the immune system. Alternatively, viral infection of the host microbiome could play a part. Yet another possibility is abnormal handling of endogenous retroviruses — those that have integrated into the host genome; this has been implicated in another inflammatory disease, Aicardi–Goutières syndrome¹¹. Mutations in the *TREX1* gene that predispose humans to Aicardi–Goutières syndrome cause an accumulation of nucleic acids derived from endogenous retroelements and an ensuing increase in the interferon response to DNA¹¹.

On the basis of these new results, it seems the Crohn's disease research community has a formidable challenge on its hands. Not only do the remaining genetic risk factors for this disease need to be determined and their functions elucidated, but the contribution of the host's microbiome — and, now, of the host's intestinal viral repertoire — must also be taken

into account in subsets of patients with Crohn's disease who have specific disease traits. That task seems Herculean, but efforts at addressing it will undoubtedly throw up new paradigms that are likely to have ramifications for inflammatory disease in general and for Crohn's disease in particular. ■

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1. Cadwell, K. *et al.* *Cell* **141**, 1135–1145 (2010).
2. Van Limbergen, J., Russell, R. K., Nimmo, E. R. & Satsangi, J. *Am. J. Gastroenterol.* **102**, 2820–2831 (2007).
3. Barrett, J. C. *et al.* *Nature Genet.* **40**, 955–962 (2008).
4. Cadwell, K. *et al.* *Nature* **456**, 259–263 (2008).
5. Saitoh, T. *et al.* *Nature* **456**, 264–268 (2008).
6. Thackray, L. B. *et al.* *J. Virol.* **81**, 10460–10473 (2007).
7. Sabbah, A. *et al.* *Nature Immunol.* **10**, 1073–1080 (2009).
8. Cooney, R. *et al.* *Nature Med.* **16**, 90–97 (2010).
9. Travassos, L. H. *et al.* *Nature Immunol.* **11**, 55–62 (2010).
10. Wobus, C. E. *et al.* *PLoS Biol.* **2**, e432 (2004).
11. Stetson, D. B., Ko, J. S., Heidmann, T. & Medzhitov, R. *Cell* **134**, 587–598 (2008).

SPECTROSCOPY

Attosecond prints of electrons

Olga Smirnova

Attosecond spectroscopy has been used to track the real-time motion of electrons in a krypton ion, and to probe the entanglement between an electron removed from the atom and the ion left behind.

Snapshots of ultrafast dynamics in the micro-world are traditionally made in a 'pump–probe' set-up. A first (pump) pulse of light plays the role of a starter gun, initiating the dynamics. A second, delayed (probe) pulse plays the role of a fast camera, taking snapshots of the moving object at different times. To take snapshots of electrons moving in atoms, the camera shutter must open and close in a fraction of a femtosecond (1 femtosecond = 10^{-15} seconds). On page 739 of this issue, Goulielmakis *et al.*¹ report the first images of electronic motion in atoms taken with an attosecond probe pulse (1 attosecond = 10^{-18} s).

In their experiment, Goulielmakis and colleagues used an intense infrared laser pulse to quickly remove an electron from the outermost (4p) shell of a krypton atom. A krypton ion (Kr^+) was thus created in a superposition of its two lowest-energy states (Fig. 1). These states differ in the way that the spin and the orbital momentum of the created hole (the absence of the electron removed from the atom) add together — the total angular momentum (J) of one state is 1/2, whereas that of the other is 3/2.

As a result of this spin–orbit interaction, the energies of the two states are different. Just as two different notes struck at the same time

produce a beat sound, two simultaneously excited states in Kr^+ that have different energies create a beat in the wavefunction of the hole. The phase of the beat (φ) is proportional to the energy difference (ΔE) between the states, and changes with time (τ). Goulielmakis *et al.* took a 'picture' of the evolving hole using a 150-attosecond extreme-ultraviolet (EUV) probe pulse to excite another electron from the deeper-lying 3d shell of the ion into the hole, while monitoring light absorption.

The way the attosecond 'camera' operates is reminiscent of Young's interferometer, in which light waves travelling along two different pathways add constructively or destructively depending on their relative phase. In Goulielmakis and colleagues' study¹, two excitation pathways running through the two Kr^+ states add together in an excited state of Kr^+ known as the $3d^{-1}$ state (Fig. 1). If the two pathways are in phase, they add constructively to set up a large population of ions in the $3d^{-1}$ state, absorbing a large amount of EUV light. If the pathways are out of phase, they add destructively to give a small $3d^{-1}$ population and weak absorption. In the authors' experiments, the amount of absorbed light varied with the pump–probe delay τ , reflecting the evolving phase ($\varphi = \Delta E\tau/\hbar$, where \hbar is the

reduced Planck constant) and hence the hole dynamics. Because all light frequencies interact with the ion simultaneously, the authors could observe modulation of the absorption signal even when monitoring only one of the absorption lines.

So far, the measurement might look like a typical, albeit technically very challenging, pump–probe experiment. Its other important aspect becomes apparent when we recall that the electron removed by the pump pulse en route to making Kr^+ is lost from the interferometer. Both pathways to the $3d^{-1}$ state share this loss. Because of this, the experiment deals with an open system — one that has not been completely measured. Incomplete measurement is a source of decoherence (the loss of a phase relationship) in the measured part of the system, here the Kr^+ ion. So what does this mean in Goulielmakis and colleagues' study?

Let us recall that, in optics, interferometers are also used to characterize the coherence of optical beams. In the same way, the visibility of the interference fringes in the authors' study measures the coherence of the two interfering pathways in the ion subsystem — that is, the coherence of the spin–orbit dynamics in the Kr^+ ion. These dynamics are coherent if the quantum states of the removed electron are common to both pathways. In contrast, the coherence is lost if these states are orthogonal — not common to both pathways (Fig. 1a). Then, the two subsystems cannot be treated independently: the removed electron and the ion are entangled. Lack of information about the removed electron therefore results in the loss of information about the phase between the two states of the ion. In other words, a high degree of entanglement results in low coherence of the hole motion.

Goulielmakis and colleagues¹ characterized the coherence, and thus the entanglement, of Kr^+ and the lost electron. In their experiments, the intense, ultrashort pump pulse ensures significant overlap of the two quantum states of the removed electron that correlate with two different pathways in the ion's subsystem (Fig. 1b), resulting in a low electron–ion entanglement, a high coherence of the hole's wave packet and high visibility of the interference fringes. The ability to probe decoherence is a very important aspect of the experiment.

The authors' experiment is reminiscent of a two-colour coherent-control scheme². In such schemes, population of a final state is controlled by the relative phase between the two colours of light needed to promote a system from two intermediate states ($J = 1/2, 3/2$) to a final state. One might thus conclude that Goulielmakis *et al.* could have made their measurements without resorting to attosecond pulses — two 'phase-locked' colours, with controlled phase φ between them, would have been enough. From this perspective, the use of a time-delayed attosecond probe can be viewed merely as a convenient way to achieve this goal. Indeed, both colours needed to promote the system to the final $3d^{-1}$ state are naturally present in the ultrashort probe pulse used by Goulielmakis *et al.*, and the relative phase between them changes with the pump–probe delay. Are attosecond probe pulses really needed?

The answer is generally yes, if one deals with open systems. In the authors' study, conducted in the gas phase, decoherence arises only during the preparation of the hole wave packet and does not evolve afterwards. But a notable strength of Goulielmakis and colleagues' technique is that it can also be used in condensed phases (such as liquids and solids). Here, decoherence may quickly evolve during the time delay between the pump and the probe pulses, and so

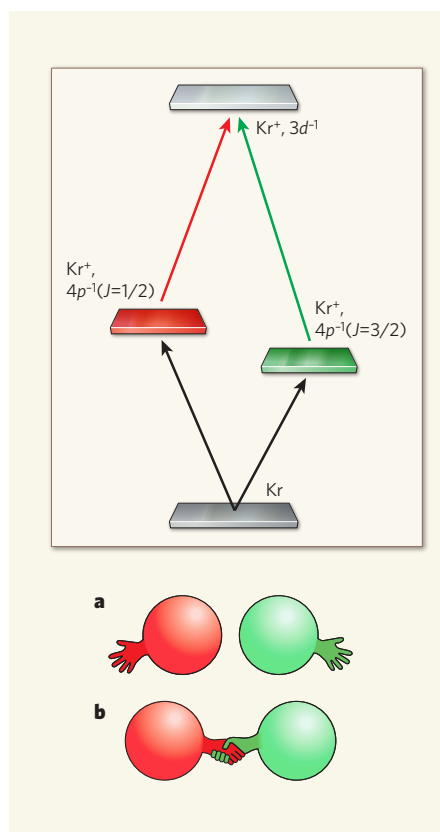


Figure 1 | The first attosecond probe experiments. Goulielmakis *et al.*¹ report a technique for observing electron motion in real time. They irradiated krypton atoms (Kr) with a 'pump' pulse of infrared light lasting a few femtoseconds, liberating electrons to generate Kr^+ ions in a superposition of two states, $4p^{-1}(J = 1/2)$ and $4p^{-1}(J = 3/2)$, where J is total angular momentum. Black arrows indicate the two ionization pathways. The authors then irradiated the ions with attosecond 'probe' pulses of extreme-ultraviolet light, exciting them to a higher-energy $3d^{-1}$ state; red and green arrows indicate the two possible excitation pathways. The complete system constitutes an entangled electron–ion pair. **a**, The different excitation pathways taken by the ion to reach the $3d^{-1}$ state may cause the liberated electrons to adopt orthogonal quantum states. The spheres represent two states of the same electron, in which the red sphere correlates with the $J = 1/2$ state of Kr^+ , and the green sphere correlates with the $J = 3/2$ state. The 'hands' on the spheres don't touch, indicating that the states don't overlap. **b**, In Goulielmakis and colleagues' experiments, strong overlap of the two quantum states (indicated by the held hands of the spheres) lowers entanglement and allows the two possible excitation pathways of the ion to interfere. By measuring the interference, the authors tracked the motion of the hole (the absence of the liberated electron) in Kr^+ in real time, characterizing its coherence and the degree of electron–ion entanglement.

direct time-domain measurements, such as in the experiment¹, become indispensable — a two-colour coherent-control scheme operating with long pulses may not catch ultrafast changes in electron coherence.

Subfemtosecond hole migration across many ångströms has been predicted to occur in large molecules^{3,4}. Such motion may have important implications⁵ for subsequent, femtosecond-scale nuclear dynamics in these molecules. Thus, early-stage hole dynamics may be

connected⁵ to the concept of charge-directed reactivity⁶ — the idea that molecular bonds break in places to which a hole has migrated. This idea assumes coherence of the hole wave packet when it is prepared. The technique of attosecond transient absorption spectroscopy, introduced by Goulielmakis and colleagues¹, is well suited to check this key assumption.

The coupling of hole motion to other electronic and vibrational modes in molecules, which is responsible for charge-directed

NEUROANATOMY

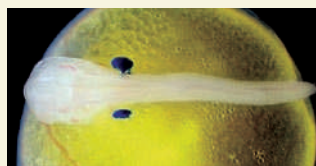
From fin to forelimb

The vertebrate invasion of land was made possible in part by evolution of the tetrapod forelimb from the fish pectoral fin. But what changes occurred in neural control during this transition?

Robert Baker and colleagues have tackled this question using a thorough application of comparative neuroanatomy (L.-H. Ma *et al.* *Nature Commun.* **1**, 49, doi:10.1038/ncmms1045; 2010). Their study centred on the developmental biology of several species of ray-finned fish, which are by far the largest group of extant fish. But it also included lobe-finned fish (a lineage that led to tetrapods) and

cartilaginous fish such as sharks.

Motor-neuron innervation in tetrapods (forelimb) and fish (pectoral fin) arises from the spinal cord. But for ray- and lobe-finned fish, there is evidence that these nerves also originate in the hindbrain. In following up that evidence, the authors looked at the gross anatomy of the developing pectoral fin buds of various ray-finned fish. They found that they all have a similar organization of the buds themselves, of the myotomes that give rise to muscles, and of the neuroepithelium that generates pectoral motor neurons. Using dye-labelled fin buds (pictured



here in a species called the plainfin midshipman fish, attached to its egg yolk), the authors also demonstrated that the motor neurons project from both the hindbrain and the spinal cord.

Studies with transgenic zebrafish, containing a fluorescently tagged enhancer that reports the activity of the developmental gene *hoxb4a* in motor neurons, confirmed the mapping of pectoral-fin neurons. Further work involved injection of the messenger RNA for a photoactive fluorescent protein, kaede, into zebrafish embryos.

The labelled neurons could then be followed during development, crucially showing that they develop *in situ* rather than migrating to their final location.

Baker and colleagues' extension of their study to lobe-finned and cartilaginous fish provided evidence that, in these groups too, pectoral-fin motor-neuron control is exercised from the hindbrain as well as the spinal cord. Overall, the authors conclude that this dual contribution is the ancestral condition in vertebrates. As to the functional context, they speculate that the advent of spinal-only motor innervation of the forelimb allowed another notable characteristic of tetrapods compared with fish — their greater freedom of head movement.

Katie Ridd

reactivity, can be viewed as decoherence evolving over time. This could also be investigated using the authors' approach. Such experiments may address the role of electronic coherence between different potential-energy surfaces at points where such surfaces intersect (known as conical intersections).

Returning to our optical analogy, conical intersections can be thought of as beam splitters. In optics, when a single beam of light strikes a beam splitter, two beams that have a well-defined phase relationship are produced as output. Now imagine reversing the process, so that two rays are sent to a beam splitter. It takes precise control of the rays' relative phases

and amplitudes to obtain a single beam as output — or a well-defined product of a chemical reaction in the molecular context. Similarly, one should expect that the amplitudes and phases of the electronic states that make up a hole's wave packet will affect how this wave packet passes through the conical intersection, and what will appear at the output of such a molecular beam splitter. With charge transfer playing a vital role in many biological and chemical systems, the ability of attosecond transient absorption spectroscopy¹ to characterize attosecond-scale preparation of electronic coherence and its subsequent evolution over tens of femtoseconds opens a

route to discovering and characterizing new mechanisms of chemical reactivity. ■

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1. Goulielmakis, E. *et al.* *Nature* **466**, 739–743 (2010).
2. Brumer, P. & Shapiro, M. *Principles of the Quantum Control of Molecular Processes* (Wiley, 2003).
3. Breidbach, J. & Cederbaum, L. S. *Phys. Rev. Lett.* **94**, 033901 (2005).
4. Hennig, H., Breidbach, J. & Cederbaum, L. S. *J. Phys. Chem. A* **109**, 409–414 (2005).
5. Remacle, F. & Levine, R. D. *J. Phys. Chem.* **221**, 647–661 (2007).
6. Weinkauff, R. *et al.* *J. Phys. Chem. A* **101**, 7702–7710 (1997).

METABOLISM

Malaria parasite stands out

Hagai Ginsburg

One of the hallmarks of cellular biochemistry is the ability to extract energy efficiently from available substrates. The malaria parasite, however, deviates from the norm, and has come up with its own solution.

All living organisms require energy for growth, maintenance and reproduction. At the cellular level, chemical reactions transform energy from one type to another: the energy stored in chemical bonds is turned into ATP — the cell's energy currency — when certain complex molecules are broken down to simpler ones. One such molecule is glucose, which is broken down through a sequence of pathways that lead to the generation of ATP. In this issue (page 774), Olszewski *et al.*¹ show that the malaria-causing parasite *Plasmodium falciparum* does not follow the usual route to generate ATP.

After glucose has been taken up by a cell, it is broken down to two molecules of pyruvate through the cytoplasmic process of glycolysis (Fig. 1). In eukaryotes (organisms whose cells contain membrane-bounded organelles), pyruvate then moves into the mitochondria — the cell's powerhouses — where it is converted to acetyl-CoA and carbon dioxide. There, acetyl-CoA enters the tricarboxylic-acid (TCA) cycle (also called the citric-acid cycle or the Krebs cycle), the central crossroads of intracellular metabolic pathways.

Indeed, the TCA cycle is involved not only in the production of energy, but also in the synthesis and degradation of biomolecules. For its energy-generating activity, acetyl-CoA reacts with oxaloacetate to form citrate. In a series of enzyme-mediated reactions, citrate is then reconverted to oxaloacetate, while two molecules of CO₂ are produced, completing the cycle. Along the way, the movement of protons and electrons (provided by the cofactor NADH⁺ and H⁺) across the inner mitochondrial membrane and the electron-transport

chain, respectively, drives the multiprotein enzyme complex F₀F₁-ATP synthase to produce ATP — a process known as oxidative phosphorylation. In total, for each molecule of glucose that is broken down, 36 molecules of ATP are produced (Fig. 1).

When pyruvate cannot be processed through the TCA cycle, ATP production is much less

efficient. Under certain conditions — such as oxygen shortage, an incomplete ATP-synthase complex or lack of mitochondria — pyruvate is fermented into lactate or ethanol, yielding just two ATP molecules for each glucose molecule.

The biochemical properties of the enzymes that participate in the various stages of ATP production have long been known, and the overall activity of the TCA cycle has been extensively studied. Moreover, recent progress in metabolomics (the study of small-molecule metabolite profiles produced by distinct cellular processes) has enabled specific details of metabolism to be elucidated. And recent advances in liquid chromatography and mass spectrometry allow the detection and quantification of metabolites in ever-smaller amounts of biological material. Furthermore, when

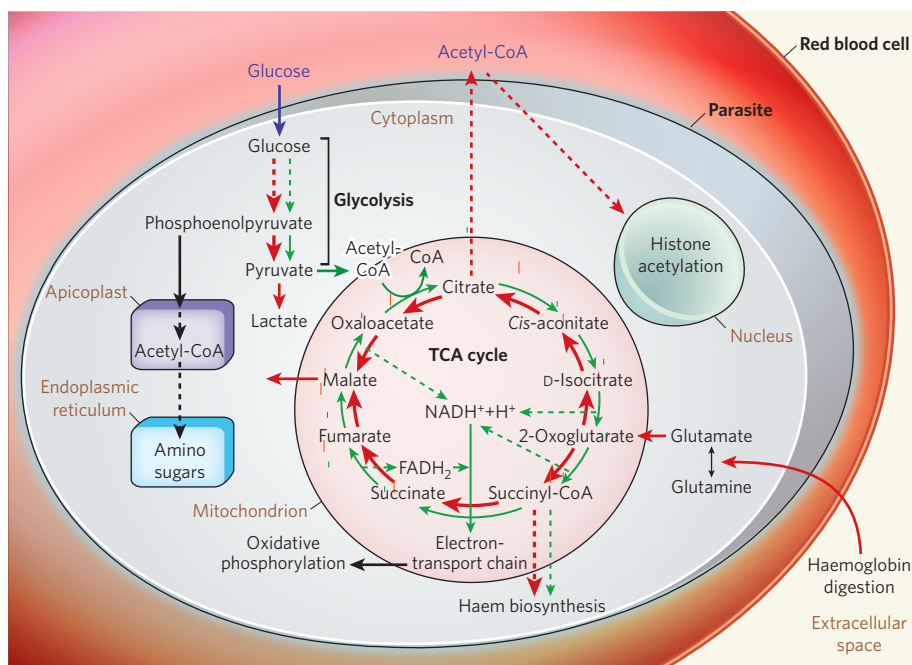


Figure 1 | Canonical intracellular metabolic pathways and those of *Plasmodium falciparum*. A red blood cell infected with a malaria parasite is depicted. The normal tricarboxylic-acid (TCA) cycle is denoted by green arrows, and the branched pathway used by *P. falciparum* is shown in red (with bifurcation starting at 2-oxoglutarate). The main cellular processes that mediate the supply or consumption of metabolites are depicted in black. The host-cell contributions are in blue. Dashed arrows indicate multiple steps.