CUL4B-mediated ERα degradation contributes to dioxin's action as an endocrine disruptor.

Ohtake *et al.* also examined the abundance of ERα and AR in the reproductive tissues of hormone-depleted mice lacking AhR and treated with dioxins and/or steroid hormones. In the absence of AhR, high levels of AR and ERα were insensitive to dioxins, and AhR-negative cells, or normal cells depleted of CUL4B, were unable to suppress hormone-dependent activation of gene expression and cell proliferation.

By showing how dioxins convert AhR into an atypical CUL4B-based E3 ubiquitin ligase that targets ERα and AR for proteasomal degradation, Ohtake and colleagues[5] provide mechanistic insight into the way dioxins disturb endocrine signalling. But several questions remain. Although dioxin promotes the formation of a complex between $CUL4B^{AhR}$ and ERα, a substantial fraction of AhR and ERα exists in CUL4B-independent complexes that are still active in promoting gene transcription[5]. This allows simultaneous hormone-independent activation of steroid-receptor target genes and the blunting of hormone-dependent transcription through steroid-receptor degradation. It is unclear how this partitioning is regulated (Fig. 1) and whether the extent of partitioning is an essential aspect of endocrine disruption.

AhR is best known for its role in detoxification, but it also seems to function in the development of the reproductive organs independently of external ligands such as dioxins[13]. So, another question for the future is whether AhR forms an E3 complex with CUL4B in the context of an as-yet-unidentified natural ligand. There are emerging examples of small molecules that affect the assembly of E3–substrate complexes, such as the interaction of the plant hormone auxin with the TIR1 F-box protein[14]. Therefore, it seems possible that small-molecule-dependent, ubiquitin-mediated protein degradation is more widespread than currently appreciated. Finally, the selectivity of AhR for CUL4B is interesting in light of the finding that CUL4B (but not CUL4A) is mutated in human mental-retardation syndromes linked to the X chromosome[15]. Understanding what makes CUL4B special will be a focus of future research.

The branch of science known as chemical biology involves screening libraries of small molecules to identify compounds that can modulate cellular mechanisms. This pursuit has led to the identification of many molecules, some of which — like dioxins — are deleterious to cells. Ohtake *et al.*[5] have provided an example of how analysis of the interaction between environmental toxins and their targets produces a better understanding of cellular control mechanisms that are crucial to human health. Their results are a poignant reminder that our ecosystem is, in fact, a very large chemical-biology experiment, in which we are both the investigator and the subject. Only time will tell if this experiment is as well controlled as we hope it is. ■

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MICROSCOPY

# Tip-top imaging

Herman Batelaan and Kees Uiterwaal

**Images of nanoscale structures can be constructed using the flow of electrons ejected from a metal probe tip by a fast laser pulse. The technique adds new dimensions to established methods of microscopy.**

How can we see without eyes? One way is with electric fields. Fishes do this all the time: sharks, for instance, use electroreceptors to 'see' distortions of fields caused by nearby objects, and thus locate their prey. The builder's tool known as a stud finder works in a similar way, using electric fields to locate the load-bearing members inside a wall. Writing in *Physical Review Letters*, Ropers *et al.*[1] demonstrate a way to downscale this kind of depth-imaging capability to nanometre sizes.

Technologies for seeing objects too small for the naked eye have a long history. The Dutchman Antonie van Leeuwenhoek reported "with great wonder" the sight of microorganisms through one of his newly developed optical microscopes more than three centuries ago. Optical microscopy has been an important imaging tool ever since, despite an obvious limitation — it cannot be used to see structures smaller than the wavelength of visible light, roughly a micrometre. This shortcoming was bypassed in the 1930s in Germany, when the first microscope was built that exploited the fact that the electron, as a quantum-mechanical particle, is also a wave. An electron's wavelength becomes shorter the faster it moves: modern, fast electron microscopes can therefore spy out objects 10,000 times smaller than the smallest structures visible with an optical microscope.

But, as physicist Richard Feynman remarked in 1959, in a talk[2] often considered to mark the birth of nanotechnology, "there is plenty of room at the bottom". Scanning tunnelling microscopy (STM), invented in the 1980s, was the first of a series of revolutionary imaging techniques that can be used to probe surfaces down to their atomic structure. STM reveals a surface's structure by passing a very sharp needle over the surface, rather as the needle of an old-fashioned record player is dragged over a structured surface to reproduce the sounds imprinted on it. The shape of an STM tip distorts the electric field around it, causing a tunnelling current to flow to a nearby surface. In much the same way, a metal lightning rod on a tower distorts Earth's electric field, allowing a current of electrons stored in the clouds above to flow to Earth (Fig. 1).

D. PLUMMER/ALAMY

**Figure 1 | Tip and flow.** The discharge of current in a lightning strike bears similarities to the electron-imaging technique developed by Ropers and colleagues[1].

The magnitude of the charge flow in STM depends on the distance between the probe and the surface, so an atomic-scale, contoured

APS

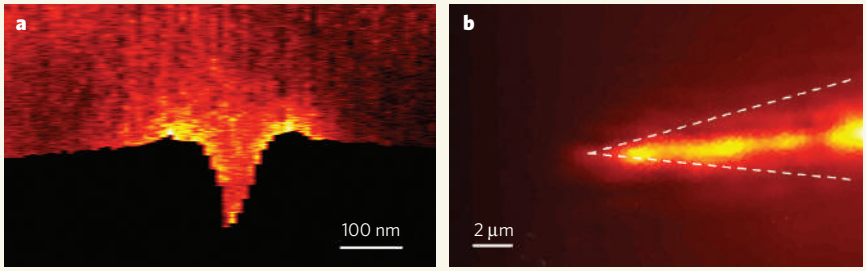


**Figure 2 | Into the groove. a**, Ropers and colleagues' 'tip-enhanced electron emission microscopy'[1] provides a picture of a nanoscale groove in a gold surface. **b**, The laser-illuminated probe tip.

'map' of the surface can be made. What STM cannot easily give us, however, is information on what happens in the third dimension above the surface. Step forward Ropers *et al.*[1], with their 'tip-enhanced electron emission microscopy'.

Like STM, the authors' technique involves measuring the effect of a sample surface on the electrical current flowing through a probe tip. So far, so conventional. But the real beauty of the technique is how that current is generated: it is stimulated by a pulsed laser beam focused on the tip. Because this laser field is affected by any kind of sample that is introduced near to the probe, the device can act in three dimensions. Furthermore, the current flow scales highly nonlinearly with changes in the laser field, making the imaging extremely sensitive to whatever is put near the probe tip.

So what sorts of things could the technique be used to look at? Ropers *et al.* use it to image a nanometre-scale groove on a gold surface (Fig. 2). But anything down to a single metal atom is theoretically possible: the spatial resolution of the authors' technique is given by the size of the metal tip, 20 nanometres, which is certainly scalable to atomic size. Questions such as the distance from which atomic-scale objects would be visible, and whether the technique could also be used for non-conducting nanostructures, will no doubt be addressed soon.

A further dimension is added to Roper and colleagues' technique through its time resolution. The laser pulses that dictate the electron emission are exceedingly short (7 femtoseconds, or $7\times10^{-15}$ s) and have a frequency of 80 megahertz. This admits the exciting prospect of tracking atomic-scale dynamics in real time. For example, the authors suggest[1] that the dynamics of surface polaritons — discrete packets of energy that result from the interaction of an electric field and the vibrations of a material — could be studied using pairs of time-delayed pulses. Surface polaritons have been credited with wide-ranging potential for 'optical' devices that do not suffer the wavelength limitations associated with devices using light propagation.

Another recent study[3] has achieved an electron pulse resolution of below 100 femtoseconds using an identical source, and there is promise for entering the attosecond ($10^{-18}$ s) domain[3]. Ahmed Zewail, who won the 1999 Nobel Prize in Chemistry for his studies of reaction dynamics using femtosecond spectroscopy, recently observed[4] that "Ultrafast electron microscopy should have an impact on all areas of microscopy, including biological imaging". Following Zewail's vision, Ropers *et al.* have made exciting progress in an area that might be called ultrafast near-field microscopy. ■

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EVOLUTIONARY BIOLOGY

# Mass survivals

David Penny and Matthew J. Phillips

**The conclusion that the primary divergences of the modern groups of mammals occurred in the mid-Cretaceous requires fresh thinking about this facet of evolutionary history — especially in ecological terms.**

On page 507 of this issue, Bininda-Emonds and co-authors[1] present an evolutionary tree of more than 4,500 mammals, and conclude that more than 40 lineages of modern mammals have survived from the Cretaceous, some 100 million to 85 million years (Myr) ago, to the present. This is paralleled by Brown and colleagues' analyses for birds, just published in *Biology Letters*[2]: they claim that more than 40 avian lineages have likewise survived from before the extinctions at the Cretaceous/Tertiary (K/T) boundary 65 Myr ago. These numbers of surviving lineages push back the evolutionary history of many mammals and birds much further than earlier estimates based on smaller data sets[3,4]. But strong claims need strong evidence to support them.

However, first things first, concentrating on mammals. Bininda-Emonds *et al.* present an evolutionary tree that includes 99% of living mammal species (4,510 out of 4,554), a major achievement in itself. They used a supertree[5] approach, in which some 2,500 previously inferred subtrees were integrated into a large supertree (see Fig. 1 of the paper[1] on page 508). To date the supertree, they constructed an alignment that included 66 genes consisting of more than 51,000 nucleotides. These, plus 30 fossil calibration points, were used to estimate the times of the divergences and the rates of net speciation against time (Fig. 1, overleaf). Improvements will undoubtedly be made to the tree and its calibration points. However, inferring a good tree of such scale is groundbreaking, and the methods will be used as a model for tree-of-life studies — whether of birds, flowering plants, invertebrate groups or other organisms.

An evolutionary tree is just the initial, descriptive, part of a study; from an evolutionary viewpoint, the real interest is using trees to learn about the processes of evolution. For context, the main divisions of existing mammals are the placental mammals (eutherians), the marsupials and the monotremes (such as the platypus). Each has its further subdivisions into order, then family, and so on.

The authors[1] report a period of radiation of placental mammals around 100–85 Myr ago; all modern orders are inferred to have diverged by 75 Myr ago. In contrast, they do not detect any radiation involving current placental lineages near the end of the Cretaceous. However, they do identify various radiations of modern families from the Eocene through to the Miocene (about 55–10 Myr ago). Modern families seem to radiate at slightly different times for the main lineages (such as for marsupials, Afrotheria, Supraprimates; Fig. 1), making it unlikely that a common physical cause was responsible. But the most challenging aspect of the phylogeny is the inference that more than 40 lineages of living mammals (and of birds, as described by Brown *et al.*[2] ) survived from the Cretaceous to the present.

For mammals, there are three important areas of agreement (or at least non-disagreement) between the fossil record and the supertree results[1]. The first is the initial radiation of modern eutherian lineages (from around