closed because the energy level is too low to be involved in electron transport. Under these conditions, tunnelling requires a single, long — and therefore improbable — jump through the entire 'forbidden' zone (the salt, the molecule and the vacuum gap). Thus, the tunnelling current is small. But application of a voltage large enough to bring the tip's unoccupied levels into alignment with the pentacene's level opens the bridge. This enables a more probable, two-step conduction pathway: electrons can first tunnel out of the pentacene and into the tip, leaving an unoccupied 'hole' on the molecule into which an electron from the gold can tunnel through the salt.

Remarkably, the authors found that a pulse of terahertz laser radiation also opens the pentacene bridge, allowing two-step tunnelling in the absence of the electrical voltage. They propose that the pulse's electric field — which is strongly enhanced at the sharp microscope tip — acts as a transient voltage, switching on the two-step pathway for approximately 100 fs. This represents a completely new mode of single-molecule imaging that enables a snapshot to be captured at a well-defined instant in time.

The characteristics of the terahertz laser pulse are key to the experiment's success. In simplified terms, the pulse can be thought of as half a wave of electromagnetic radiation. The wave therefore biases the system in only one direction, so that current flows in just one direction. By contrast, a full wave would bias the STM system first in one direction, then in the opposite direction, so that the net current would be almost zero — no image would be taken.

Furthermore, the high reflectivity of metals at THz frequencies (typically greater than 99%)[9,10] minimizes light absorption that would result in heating and thermal expansion of the STM tip. Such expansion can easily result in spurious signals[11] because of the tunnelling current's extreme sensitivity to the gap width, and has been a major hindrance to the development of laser-excited STM methods.

Cocker *et al.* applied their imaging method to follow molecular motion on picosecond timescales. To do this, they took advantage of the fact that driving an electron out of the occupied level of pentacene using a pump pulse excites vibration of the molecule up and down with respect to the surface. The authors held the tip at a fixed point above the molecule, and used a probe pulse to excite two-step tunnelling at a well-defined time after the pump. Because the tunnelling current is so sensitive to the distance between the molecule and the tip, they could follow the vibrational oscillations of a single molecule in time (Fig. 1), directly measuring not only its frequency (which has previously been measured for single molecules using another STM method[12]), but also its amplitude and phase.

Several imaging methods that have high spatio-temporal resolution are being developed and show promise (for examples, see refs 3, 13–20), but Cocker and colleagues' approach is unique in its ability to track molecular motion. Nevertheless, further work is needed to develop even more exciting capabilities. For example, a quantitative understanding of the laser-excited tunnelling should be pursued, including detailed computer simulations of the transient electric field generated at the tip by the THz pulse, and of the response of the tip, surface and molecule to that field. It also remains to be seen how this imaging approach may be extended to investigate surface physics and molecular chemistry. Can the damping rates of molecular vibrations be measured? And can molecules be observed as they react on catalytic surfaces?

Single-molecule instant replay will reveal details that will allow dedicated fans — surface physicists and chemists — to appreciate molecular action more deeply. The longer-term challenge is to develop this tool so that referees and coaches — materials scientists and engineers — can use its nanoscale dynamical insights to design, diagnose and improve molecule–surface systems for catalysis, chemical sensors and molecular electronics, all at the single-molecule level. ■

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EVOLUTION

Genomic remodelling in the primate brain

In many mammals, the gene *Ostn* is expressed in muscles and bones. The discovery that the primate *OSTN* gene has been repurposed to also act in neurons provides clues to how humans evolved their cognitive abilities. SEE ARTICLE P.242

JUSTINE KUPFERMAN & FRANCK POLLEUX

The cognitive abilities that separate humans and our primate relatives from other mammals are the product of millions of years of evolution, and stem from differences in how our brains develop and function[1]. Although brain maturation in all mammals relies in part on experience-driven development of neuronal circuits, human cognition depends particularly heavily on the experiential learning that occurs during our prolonged period of growth, which lasts up to two decades after birth[2]. The structural and functional changes that shape neuronal circuits during this developmental period are mediated by genes whose transcription is regulated by neuronal activity[3]. On page 242, Ataman *et al.*[4] describe an unbiased screen to identify genes activated by neuronal excitation in human and mouse neurons. They identify a gene expressed in the bones and muscles of mice and other mammals that, over the course of evolution, was repurposed to act in the neurons of primates.

Because of the importance of experiential learning in humans, identifying gene-expression changes induced by neuronal activity is particularly relevant for understanding the genetic basis of our species' brain evolution. With this in mind, Ataman *et al.* cultured human neurons *in vitro*. These cultures contain a mix of differentiated cell types found in the brain, including glial cells, which

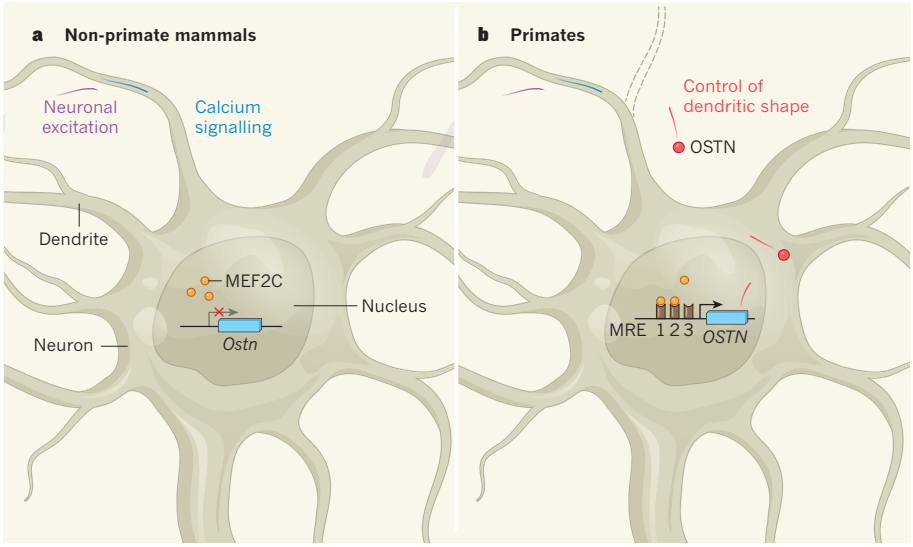

Figure 1 | A gene repurposed. Neurotransmission between neurons leads to intracellular calcium signalling, which in turn activates the transcription factor MEF2C. This protein binds to DNA sequences called MEF2-responsive elements (MREs) to induce transcription of nearby genes. **a**, Ataman *et al.*[4] report that non-primate mammals do not have MEF2C binding sites in the regulatory sequences that lie upstream of the osteocrin (*Ostn*) gene, and their neurons do not express *Ostn*. **b**, By contrast, three MEF2C sites have evolved in this region in primates, and so the equivalent primate gene, *OSTN*, is transcribed in response to neuronal activity (it is not clear whether MRE3 is required for activity-dependent *OSTN* expression). OSTN protein is secreted from neurons and can regulate branching of neuronal projections called dendrites, perhaps regulating the structural changes that neurons undergo during learning in primates.

support neurons and promote formation of the synaptic connections between them, and several subtypes of neuron from the brain's cortex. The authors stimulated these cultures to mimic increased neuronal activity, thereby increasing calcium signalling within neurons and inducing activity-dependent gene transcription.

Next, Ataman and colleagues used RNA sequencing to identify upregulated transcripts and confirmed that several well-characterized[5,6] 'immediate early' genes were rapidly induced within one hour of increased neuronal activity, including the transcription factors *NPAS4* and *FOSB*. They also identified a set of late-response genes induced within six hours, which included previously reported genes[7] such as *BDNF*. At this late time point, the authors found a few transcripts specific to human neurons. The most highly induced of these genes was that for osteocrin (*OSTN*).

In mice, the corresponding gene *Ostn* encodes a secreted protein that is involved in glucose metabolism in muscles and bones[8,9]. *Ostn* is not expressed in the mouse brain, and Ataman *et al.* found that its expression could not be induced by neuronal activity in mouse neuronal cultures. Therefore, *OSTN* expression seems to be regulated by neuronal activity in human but not mouse neurons — a supposition that the authors corroborated through several lines of investigation.

Ataman and colleagues reported that *OSTN* was widely expressed throughout the human neocortex (which is involved in higher cognitive functions, including sensory processing) and was highly enriched in the mature neurons of the developing cortex. They also found that, in the primary visual cortex of macaques, *OSTN* expression was induced by increased sensory-evoked neuronal activity. Together, these lines of evidence point to activity-dependent expression of *OSTN* in primate neurons *in vivo*.

At the genetic level, what underlies this shift from *Ostn* transcription in the bones of mice to activity-dependent regulation of *OSTN* transcription in primate neurons? To address this question, the authors first demonstrated that *OSTN* expression in human neurons is regulated by a 2-kilobase-long promoter region that lies immediately upstream gene. By engineering a series of truncations and point mutations in this region, the researchers identified a minimal 85-base-long region that mediates gene activation in response to neuronal activity. In primates, this region contains three short DNA sequences to which transcription factors of the myocyte enhancer factor 2 (MEF2) family, such as MEF2C, can bind. In mice, however, the *Ostn* promoter does not contain these MEF2-responsive elements (MREs).

In primates but not in other mammals, OSTN might regulate structural changes that neurons undergo during learning.

MEF2 transcription factors are crucial for activity-dependent transcription in neurons and have a role in key aspects of neuronal development[10,11]. The researchers found that two of the three MREs are highly evolutionarily conserved between humans and anthropoid primates, but that there are several differences in these sequences in prosimian primates, rodents, dolphins and several other mammalian species that result in the absence of MREs in the *Ostn* promoter of these species. By replacing the three primate MREs with the equivalent mouse sequences and monitoring gene transcription, the authors convincingly demonstrated that the evolutionary switch to activity-dependent transcription of the *OSTN* gene in primates emerged through a few single-nucleotide mutations that created MREs (Fig. 1).

What is the function of *OSTN* in primate neurons? The authors overexpressed or repressed *OSTN* in human neuronal cultures, and discovered that expression of the gene regulates the shape of dendrites — the branched parts of neurons that receive and integrate synaptic information from other neurons. This result suggests that, in primates but not in other mammals, *OSTN* might regulate structural changes that neurons undergo during learning. Indeed, *OSTN* belongs to a family of genes that encode secreted protein fragments called natriuretic peptides, which have been shown[12] to promote branching of neuronal projections called axons.

Further experiments will be needed to fully determine the impact of *OSTN* expression on primate brain development. Defining the roles of *OSTN* — and of the other activity-dependent genes identified in Ataman and colleagues' study — will improve our understanding of the evolutionary mechanisms that enabled the emergence of primate-specific features of brain development and function. ■

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