NEWS & VIEWS

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NEUROSCIENCE

Finding the missing fundamental

Robert J. Zatorre

The whole orchestra tunes up to an A note from the oboe — but how do our brains tell that all the different sounds are the same pitch? The discovery of pitch-sensitive neurons provides some clues.

Although Maurice Ravel reportedly came to regret ever having written *Bolero*, it has become a popular staple of the orchestral repertoire. It relies entirely on a single theme, repeated over and over (and over) by different combinations of instruments. Artistic merit aside, the piece raises an interesting question: how do we effortlessly recognize the same melody played by different instruments even though the acoustical structure of the sound reaching our ears varies with the instrument? Bendor and Wang (page 1161 of this issue)[1] have found neurons that figure out what the pitch of a sound is even when they are presented with physically different signals, giving hints to how we come to perceive pitch as a unified entity.

Psychologists are intrigued by the problem of perceptual constancy: essentially, how do we perceive the environment as remaining stable despite huge variability in the inputs reaching our senses? This general question is especially puzzling in the case of pitch, because we have known since the nineteenth century[2] that the pitch of a sound typically corresponds to its fundamental vibrational frequency — even if that frequency is physically absent in the sound reaching our ears. Sounds that have pitch arise from objects that vibrate in a periodic manner, such as columns of air in pipes or the vocal cords (as opposed to aperiodic sounds like wind or rushing water). As Pythagoras knew, if you pluck a string, it will vibrate in its entire extent, as well as in halves, thirds and so on, and each of those vibrational modes will result in a separate harmonic frequency. Yet we usually perceive the pitch as corresponding to the lowest of these, which is the fundamental[3]. For a simple demonstration of the 'missing fundamental' effect, pick up a phone. Most telephone lines cut off the lower frequencies, resulting in a slightly tinny sound, yet the fundamental pitch does not change; a male voice does not sound like Mickey Mouse. The brain seems to figure out the missing pitch.

Bendor and Wang[1] studied the auditory cortex (the region of the brain that enables perception of sound) in the marmoset monkey. They show that there are neurons in this region that respond in essentially the same way to a variety of sounds that all have the same fundamental but do not share any frequencies. For example, a neuron that responds to 200 hertz also responds to the combination of 800, 1,000, and 1,200 hertz because all correspond to the same fundamental. This effect is unusual because neurons usually respond only within their receptive field, which is typically a narrow range of frequencies. The marmoset neurons, however, responded not only to frequencies in their receptive fields, but also when there was no frequency within the receptive field but the other frequencies in the stimulus were harmonically related to the missing one. This property makes psychologists happy, because it provides evidence (if not yet a mechanism) for perceptual constancy. These neurons respond to an abstract property — pitch — derived from, but not identical to, physical sound features. Presumably, therefore, it is thanks to such neurons that we can follow a tune as the instruments change.

One might wonder why marmosets need such a system, given that they don't spend much time listening to iPods. But periodic sounds are important in the natural environment because they are almost exclusively produced by other animals, and so pitch is a good cue to segregate these sounds from background noise[4]. Marmosets are highly vocal creatures, and the development of pitch-sensitive neurons would also be central to communication. From an evolutionary perspective, these abilities could be seen as precursors to human pitch perception, which has led to our unique development of music and is similarly crucial for speech.

The location of the pitch-sensitive cells lateral to the primary auditory cortex, as described by Bendor and Wang, is compatible with studies of the human brain. In human patients, damage to areas analogous to the marmoset pitch-sensitive regions produce specific deficits in perceiving missing fundamental pitch[5]. Moreover, neuroimaging studies in humans demonstrate pitch sensitivity in roughly the same location[6,7]. The human studies typically show specialization for pitch in the right auditory cortex, however. Bendor and Wang do not address this issue, as only a single hemisphere was probed in each of three

monkeys. It will be of interest to determine whether lateralization is present in other species (as others suggest[8]), and is therefore related to basic properties of sound processing, or whether it is uniquely human and thus might be a consequence of the development of language.

Now that we know that there are pitch-sensitive neural units, we have to discover how they work. Sound undergoes many transformations before it gets to the auditory cortex, resulting from the biophysical properties of the cochlea and the many neuronal junctions between cochlea and cortex. We do not yet know precisely how periodic, temporal information available in a stimulus is integrated with the spectral information (or individual harmonics) that is also extracted by the system. We also do not know much about the inputs to the neurons described by Bendor and Wang. Do they come in a hierarchical arrangement from other simpler cells in the auditory cortex? Or do they also receive inputs from subcortical structures such as the thalamus? Perhaps top-down influences from centres associated with complex functions in frontal or parietal lobes are also significant. This last point is relevant, because one technical advantage of this work is that the animals tested were awake rather than anaesthetized, meaning that attentional and other cognitive factors could have a role. The animals were not trained or behaving, however, so it is difficult to know the significance of the stimuli for them. Understanding the interaction between basic perceptual systems and their modulation by higher-order mechanisms will require more attention to these factors. Another interesting question is whether these neuronal properties are somehow hard-wired, or whether they are a consequence of the animals' environmental experience with periodic sounds, which contain harmonically related frequencies.

Ian Whitfield[9] noted that the problem of perception is not to determine that two events are different, which is actually fairly trivial, but rather that events that might seem to be different are actually the same. It is the job of the cortex, he argued, to perform the computations needed to extract invariances despite the different inputs that the environment may provide. The present study[1], and those that will no doubt follow, will lead to a more profound understanding of this fundamental problem. ■

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1. Bendor, D. & Wang, Q. *Nature* **436,** 1161–1165 (2005).
2. von Helmholtz, H. *On the Sensations of Tone as a Physiological Basis of the Theory of Music* (1863) (trans. Ellis, A.; Dover Publications, New York, 1954).
3. Schouten, J. *Koninklijke Nederl. Akad. Wetenschappen Proc.* **41,** 1086–1093 (1938).
4. Bregman, A. *Auditory Scene Analysis* (MIT Press, Cambridge, Mass., 1990).
5. Zatorre, R. J. *J. Acoust. Soc. Am.* **84,** 566–572 (1988).
6. Patterson, R. D., Uppenkamp, S., Johnsrude, I. S. & Griffiths, T. D. *Neuron* **36,** 767–776 (2002).
7. Zatorre, R. J. & Belin, P. *Cerebral Cortex* **11,** 946–953 (2001).
8. Wetzel, W., Ohl, F., Wagner, T. & Scheich, H. *Neurosci. Lett.* **252,** 115–118 (1998).
9. Whitfield, I. in *Cerebral Cortex* (eds Peters, A. & Jones, E.) 329–351 (Plenum, New York, 1985).

BIOLOGICAL CHEMISTRY

Just add chlorine

Nathan A. Schnarr and Chaitan Khosla

Nature provides lessons about developing 'green chemistry' in seemingly out-of-the-way places. One such lesson comes from an enzymatic step in the production of a leaf toxin by a bacterium.

As they describe on page 1191 of this issue[1], a group of researchers led by Christopher Walsh has identified how chlorine is attached enzymatically to an intermediate during the formation of a natural product. This is not surprising in itself — the significance lies in the unreactive nature of the carbon centre concerned.

Many natural products require halogens (chlorine, bromine or iodine) to be strategically placed onto organic molecules at unreactive carbon centres. Halogenation is essential to the biological activity and chemical reactivity of such products (Figs 1a–c), and often serves to generate versatile molecular building blocks for synthetic organic chemists. Ideally, these syntheses would use alkanes — unreactive carbon chains — as their starting materials. These are usually readily available and relatively cheap as they are the main components of oil. Unfortunately, traditional methods for incorporating halogens into alkanes often require environmentally unfriendly reagents and suffer from poor control of specificity (Fig. 2a). In contrast, natural enzymes are benign and do the same job with extra-ordinary specificity, but little is known of the mechanisms of these enzymatic halogenations. Now, Walsh and colleagues[1] have discovered that halogenation of an unreactive carbon centre can be catalysed by a halogenase enzyme, called CmaB, that is α-ketoglutarate dependent and contains non-haem iron.

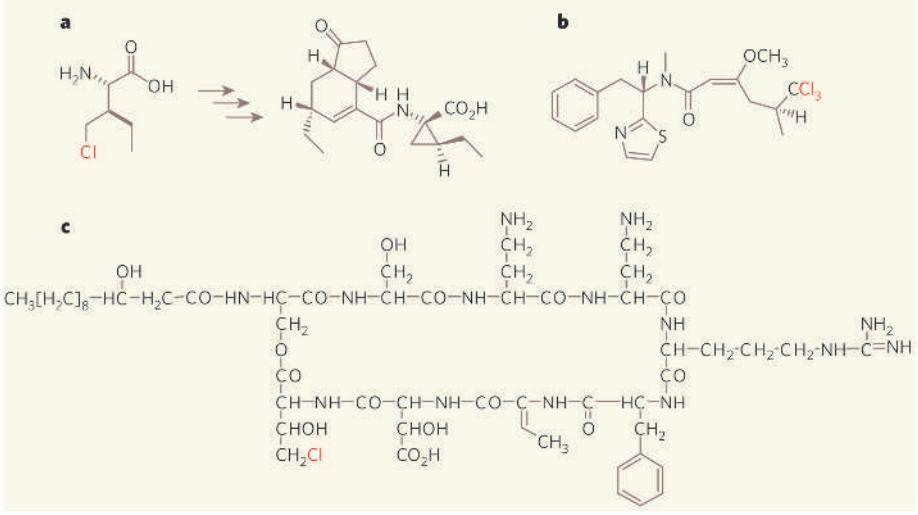

Figure 1 | Chlorine in natural-product synthesis. **a,** Coronatine, a leaf toxin. The chlorinated intermediate (left) goes through several further reactions before coronatine, which does not itself include chlorine, is produced. **b,** Barbamide, a molluscicide. **c,** Syringomycin, an antibiotic. Biosynthesis of all three products involves enzymatic chlorination.

Similar catalysts are known to be involved in oxygenation chemistry carried out by the hydroxylase family of enzymes. Hydroxylases insert oxygen into a carbon–hydrogen bond, an analogous process to halogenation, and have received much attention because of their extraordinary specificity and versatility. In nature, several different types of hydroxylase catalyse such transformations, depending on the substrate. In general, the more reactive substrates require the less reactive enzymes. For example, hydroxylation of highly reactive *p*-hydroxybenzoic acid is readily accomplished by a flavin-dependent hydroxylase. In contrast, hydroxylation of relatively unreactive substrates, such as the amino acids proline or lysine, requires significantly stronger α-ketoglutarate-dependent enzymes containing non-haem iron[2]. In addition to these two extreme cases, a variety of alternative hydroxylases has

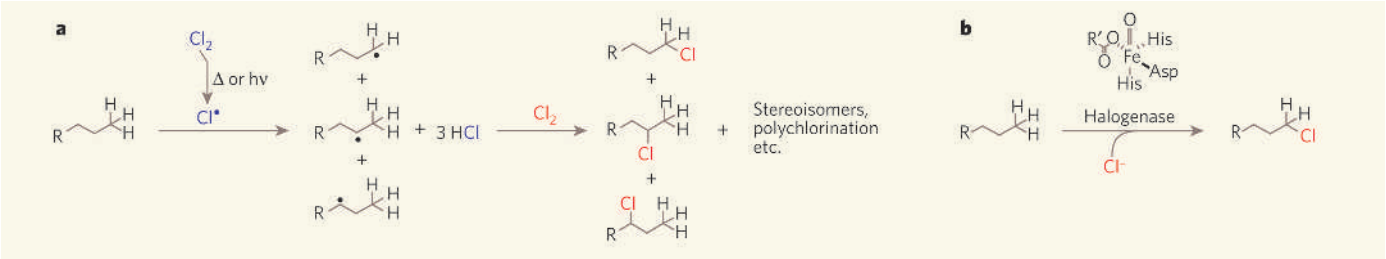

Figure 2 | Chlorinating unreactive organic compounds. **a,** Using purely chemical synthesis without an enzyme, alkanes are chlorinated using highly reactive radical species (black dots). This requires several steps, and it is difficult to control which particular carbon is modified and included in the final stereochemical form of the product. **b,** The reaction is much easier and the products more controllable with a dedicated halogenase, as described by Walsh and colleagues[1]. A chloride anion is oxidized by the oxo-iron species from the halogenase active site for site-specific chlorination of the substrate.

been discovered that act primarily on substrates with intermediate reactivity. By analogy with the hydroxylases, Walsh and colleagues now speculate that natural systems may employ a similar, tunable strategy for halogenation.

In this model, more reactive aromatic substrates rely on gentler, $FADH_2$-derived agents — and this is indeed the case for chlorination of tryptophan during the biosynthesis of the antitumour agent rebeccamycin[3]. Unreactive carbon centres, in contrast, require a more vigorous agent. In the reactions investigated by Walsh and colleagues, the necessary oxidation of a chloride anion to add chlorine to an alkane is evidently carried out by a highly reactive oxo-iron species from the halogenase active site (Fig. 2b). This species presumably breaks an unactivated carbon–hydrogen bond, leading to a high-energy radical species that can be trapped by chlorine.

Analogous enzymes with an apparently similar mode of action are already emerging from other biosynthetic systems. For example, Walsh and colleagues[4] have also identified a putative halogenase in the biosynthetic pathway for the antifungal antibiotic syringomycin (Fig. 1c). This enzyme is presumably responsible for introducing chlorine into the natural product. Similarly, two relatives of CmaB (BarB1 and BarB2) are found in the biosynthetic pathway for barbamide, a potent molluscicide that contains a medicinally interesting trichloromethyl group (Fig. 1b).

Generation of high-energy radical intermediates has emerged as the common thread among many oxo-iron catalysts, whose functions range from natural product biosynthesis to post-translational protein modification and even repair of RNA or DNA. The addition of halogenation to this impressive array of activities illustrates yet another exciting way to generate useful chemical intermediates from comparatively unreactive precursors. Elucidation of the precise mechanism for this transformation will undoubtedly pave the way for novel organometallic halogenation catalysts for chemical synthesis. ■

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1. Vaillancourt, F. H., Yeh, E., Vosburg, D. A., O'Connor, S. E. & Walsh, C. T. *Nature* **436,** 1191–1194 (2005).
2. Hausinger, R. P. *Crit. Rev. Biochem. Mol. Biol.* **39,** 21–68 (2004).
3. Yeh, E., Garneau, S. & Walsh, C. T. *Proc. Natl Acad. Sci. USA* **102,** 3960–3965 (2005).
4. Vaillancourt, F. H., Yin, J. & Walsh, C. T. *Proc. Natl Acad. Sci. USA* **102,** 10111–10116 (2005).

EARTH SCIENCE

Helium not in store

William M. White

The ratio of helium isotopes in some oceanic volcanoes seemed to demand a reservoir of virgin primordial gas in the Earth's mantle. In fact, that might not be necessary — a relief for other geophysical models.

In the 4.5 billion years of its existence, Earth has been continually losing helium through the degassing of rocks from its interior as they melt during volcanic processes. That there is any helium left on Earth at all is largely owing to its replenishment in the interior through radioactive α-decay, principally of the heavy elements thorium and uranium — the α-particle emitted in α-decay is a helium nucleus. But α-decay creates only the heavier helium isotope, ^{4}He. Any trace of the lighter isotope, ^{3}He, on present-day Earth is primordial, dating from the planet's formation.

Admittedly, Earth does not have much ^{3}He: in interior rocks there is only one atom for every 100,000 atoms of ^{4}He. But the ^{3}He/^{4}He ratio is even smaller in the atmosphere — typically eight or nine times lower, but occasionally up to 40 times lower. This imbalance would seem to imply that Earth still retains substantial amounts of primordial helium trapped in its interior. But where? Is this gas largely confined to a single deep reservoir that has remained undisturbed by volcanic activity all this time? Or is it more uniformly dispersed, with degassing just less efficient than we had thought? On page 1107 of this issue, Class and Goldstein[1] argue strongly for the latter.

Volcanoes allow a glimpse into the evolution of processes in Earth's interior. Isotopic analysis of radiogenic elements implies that material from Earth's mantle (the layer between crust and core) that is disgorged as volcanic lava has generally been melted before, at least partially. Helium, a noble gas, is not chemically bound in minerals, so should escape by degassing whenever melting occurs, first to the surface, and from there into space. And although ^{4}He is replaced by radioactive decay, ^{3}He is not; the higher the ^{3}He/^{4}He ratio, therefore, the less melting and degassing has taken place.

The existence of rock with high ^{3}He/^{4}He ratios has led to the notion that there is a reservoir of rock, most reasonably in the deepest mantle, that has escaped melting and degassing and retains much or all of its original helium. This idea is supported by the highest ^{3}He/^{4}He ratios being found in the lavas of oceanic island volcanoes, such as those on Hawaii and in Iceland (Fig. 1). These volcanoes are thought to be produced by convection plumes that carry hot rock from the deep mantle[2,3]. Yet if a deep reservoir of rock exists in its primordial state, it must be isolated from the convection that affects the rest of the mantle and drives plate tectonics. Seismic imaging of Earth's interior has, however, consistently failed to find evidence of any layering in the deep mantle, and implies instead that the whole mantle is involved in convection[4].

The observed ratios of helium isotopes are therefore problematic. They seem to require layered convection that seismologists cannot detect and geodynamicists cannot reproduce in their models. They seem to require a primordial deep mantle, whereas the isotopic ratios of other radiogenic elements indicate that all of Earth's interior has been affected by earlier volcanic activity.

Class and Goldstein[1] attempt to reconcile

these incompatibilities. They investigated the strontium (Sr), neodymium (Nd) and lead (Pb) isotopic ratios of the oceanic island basalts with the highest $^3He/^4He$ ratios and show that these rocks are derived from mantle that is relatively depleted in so-called incompatible elements — elements that are not readily accommodated in mantle minerals and are easily extracted by partial melting.

Against expectation, therefore, it is rocks that have most obviously been melted and undergone degassing that have the highest $^3He/^4He$ ratios. Lower $^3He/^4He$ ratios, indicating a high degree of degassing, are found in those oceanic island basalts whose other isotope ratios are closest to the expected primordial values. The similarity of these rocks to primordial mantle could be coincidence, the result of incompatible-element depletion by melting and subsequent re-enrichment, perhaps by addition of material subducted from Earth's crust.

In the second part of their paper[1], Class and Goldstein report model calculations that show that $^3He/^4He$ ratios as high as those observed in some oceanic basalts could be preserved in the mantle despite extensive melting, volcanism and degassing. The degree to which a model reflects reality always depends on its guiding assumptions: Class and Goldstein assume, for example, that noble gases are not extracted with near-perfect efficiency during melting, but behave like highly incompatible elements, which allows some primordial ^3He to be retained. Furthermore, surface tension dictates that you can no more get all the melt out of a partially molten rock than you can get all the water out of a kitchen sponge. If some of the melt remains in the rock and eventually resolidifies, some of the helium will remain as well. Just how much remains is difficult to judge with our present knowledge; this model may stand or fall with further research on the chemical behaviour of noble gases during melting and on the physics of partially molten rock.

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Figure 1 | Erupting evidence — Kilauea volcano on Hawaii.

The other central assumption of Class and Goldstein's model is Earth's initial helium abundance: the greater this was, the higher the $^3He/^4He$ ratio will be now. The abundance of the noble-gas isotope xenon-129 in the mantle, the decay product of now-extinct iodine-129, indicates that the Earth experienced catastrophic degassing very early in its history[5] — quite possibly as a result of the collision that formed the Moon. This would have released much of the planet's primordial helium. How much was left behind no one knows. This helium abundance, the starting-point of Class and Goldstein's model, is therefore essentially an unconstrained parameter. We shall see how it stands up to scrutiny.

Although some may be reluctant to relegate 'primordial mantle' to the scientific graveyard quite yet, the case made by Class and Goldstein will be hard to rebut. Unsettled controversies remain: for example, there is still a need to maintain separate reservoirs in the Earth's interior to explain variations in other isotope ratios, although for much shorter times than the age of the Earth. This is difficult to reconcile with fairly strong geophysical evidence for convection involving the whole mantle that would destroy that separation. There is much still to learn about the structure and evolution of the Earth's deep interior. ■

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1. Class, C. & Goldstein, S. J. *Nature* **436,** 1107–1112 (2005).
2. Morgan, W. J. *Nature* **230,** 42–43 (1971).
3. Montelli, R. *et al. Science* **303,** 338–343 (2003).
4. Grand, S. P. *Phil. Trans. R. Soc. Lond. A* **360,** 2475–2491 (2002).
5. Staudacher, T. & Allègre, C. J. *Earth Planet. Sci. Lett.* **60,** 389–406 (1982).

BIOLOGICAL CHEMISTRY

Enzymes in focus

Romas Kazlauskas

The technique of directed evolution creates thousands of mutant enzymes from a single original. A new approach helps to search for variants that have an increased range of substrates — and can thus be used for organic synthesis.

Protein catalysts, or enzymes, are useful in organic synthesis largely because they can accept substrates other than their natural ones. Yet they can still distinguish subtle differences in shape between substrates — a characteristic known as stereoselectivity. The substrate range and specificity of an enzyme can be modified by protein engineering. In this case, mutants are created by changing the enzyme's component amino acids. Such mutant enzymes can be used, among other things, to synthesize pharmaceutical building-blocks. Writing in *Angewandte Chemie*, Reetz *et al.*[1] demonstrate a variation on recently developed enzyme-engineering methods[2–6]. They mutate pairs of amino acids in the enzyme's substrate-binding site (active site) to create variants with an increased range of substrates but that retain high stereoselectivity.

Initial efforts at enzyme engineering took a so-called rational-design approach. This involved using knowledge of enzyme structure and active sites, together with computer modelling, to predict precisely the mutations needed. Success was not only measured in terms of increased specificity, but also of stability, the ability to fold, and catalytic activity. Many of the early attempts were disappointing, as these interdependent properties are hard to predict. To increase their success rate, researchers developed techniques such as saturation mutagenesis, in which the effects of each of the 20 normal amino acids are tested at selected positions in the enzyme[7].

The discovery of the polymerase chain reaction (PCR), which copies DNA strands extremely fast, greatly simplified molecular-biological techniques. As a result, the emphasis in enzyme engineering shifted from using rational design to 'directed-evolution' tools that rely on random mutagenesis. Such techniques, for example error-prone PCR and 'gene-shuffling', involve randomly and repeatedly varying amino-acid residues throughout the enzyme. This creates enormous numbers of mutant enzymes. When screened for activity, however, typically only very few of these turn out to be useful.

Surprisingly, many of the mutations identified in directed-evolution experiments were found far from the active site — so far from it, in fact, that the mutated residue did not come directly in contact with the substrate[8]. So was it wrong to focus on the enzyme's substrate-binding site? Are distant mutations instead better at changing enzyme specificity?

The answer turns out to be no. Directed evolution discovers distant mutations not because they are more active, but because they are more common[2,8,9]: there are simply more amino acids far from the active site than close to it. Thorough screening will still find the best mutants; but in practice, it is easier to generate large numbers of mutants than it is to screen them, and incomplete screening favours the more

common, distant mutations. So the emphasis in improving enzyme specificity has returned to the active site, where amino-acid mutations are likely to be more effective[2–6]. This approach is known as focused directed evolution.

Reetz and colleagues' new take on focused directed evolution[1] involves mutating pairs of amino acids in the active site; this process allows more extensive reshaping of the site than would a single mutation. The authors chose amino-acid pairs that pointed towards the substrate, and — simplifying the experiment considerably — chose only pairs that were close together in the linear sequence of amino acids that would form were the folded enzyme unravelled. This meant that for each mutant pair, only one 'mutagenic primer' was required to initiate a PCR process and thereby create a library of mutants. Producing mutations in a pair of amino acids thus becomes no more difficult than in a single amino acid, although, as there are 20^2 different combinations of two amino acids, much more screening is required. Reetz *et al.* elegantly use secondary structure — how the enzyme folds on a local scale — to identify spatially interacting amino acids in the linear sequence (Fig. 1).

The authors tested their paired-mutation approach on a lipase — an enzyme that breaks down fats — from the bacterium *Pseudomonas aeruginosa*. They generated five mutant libraries, picking five different amino-acid pairs from around the active site. Each library contained 400 variants: the original, unmutated enzyme; 38 single mutants, in which one of the 19 'introduced' amino acids occurred at one of the pair of positions; and 361 (19×19) double mutants. The screening process identified eight mutants that typically showed 5–30 times greater activity than the unmutated enzyme. Five mutants were from one library, three from another.

Reetz *et al.* tested the enzyme variants on 11 substrates, and their preliminary results indicate that mutants showing increased activity also show good stereoselectivity. (It should be noted, however, that the mutants of this particular enzyme would not be sufficiently stable for applications in organic synthesis.) A previous directed-evolution study of this lipase[10] identified mainly distant mutations that typically only doubled stereoselectivity. Interestingly, amino acid 162, which was changed in five of the eight best-performing mutants in Reetz and colleagues' study, was also identified as a key position in this previous work.

A surprising finding was that five of the eight best mutants were single mutants. For the 11 target substrates, a single mutant was most active in nine cases, whereas a double mutant (designated M16A, L17F) was most active in the other two. Researchers will need to decide whether the extra chance of success among the double mutants is worth 20 times more screening. One solution might be to use the earlier approach of choosing amino acids that are not adjacent in the linear amino-acid sequence, but are spatially adjacent when the enzyme folds into its natural shape[4–6]. However, this would require the slightly more complex use of two primers for mutagenesis.

It will always be possible to make many more mutants than can be screened, so strategies f or choosing the mutations most likely to improve an enzyme's properties will continue to be important. If the structure (or a homology model) of an enzyme is available, focused directed evolution is currently the fastest approach to altering specificity. For other properties, such as thermal stability, targeting the whole enzyme remains the best approach[9]. ■

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1. Reetz, M. T., Bocola, M., Carballeira, J. D., Zha, D. & Vogel, A. *Angew. Chem. Int. Edn* **44,** 4192–4196 (2005).
2. Park, S. *et al. Chem. Biol.* **12,** 45-54 (2005).
3. Hill, C. M., Li, W.-S., Thoden, J. B., Holden, H. M. & Raushel, F. M. *J. Am. Chem. Soc.* **125,** 8990–8991 (2003).
4. Whittle, E. & Shanklin, J. *J. Biol. Chem.* **276,** 21500–21505 (2001).
5. Koga, Y., Kato, K., Nakano, H. & Yamane, T. *J. Mol. Biol.* **331,** 585–592 (2003).
6. Li, Q.-S. *et al. Biochim. Biophys. Acta* **1545,** 114–121 (2001).
7. Wells, J. A., Vasser, M. & Powers, D. B. *Gene* **34,** 315–323 (1985).
8. Horsman, G. P. *et al. Chem. Eur. J.* **9,** 1933–1939 (2003).
9. Morley, K. L. & Kazlauskas, R. J. *Trends Biotechnol.* **23,** 231–237 (2005).
10. Liebeton, K. *et al. Chem. Biol.* **7,** 709–715 (2000).

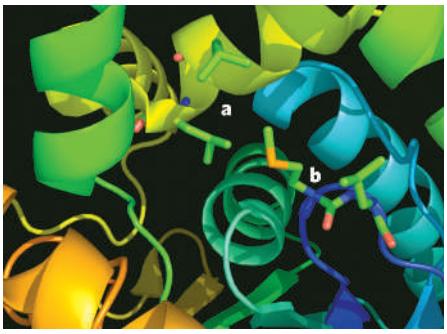

Figure 1 | View of the enzyme active site used by Reetz *et al.*[1] for pair mutation. The local secondary structure of an enzyme determines how far apart in the linear amino-acid sequence two spatially adjacent amino acids are. **a**, In a so-called 3_{10} helix (light green), the two spatially interacting amino acids are three amino acids apart (the green stick-like protrusions are the side chains, while the blue and red tips are the main protein chain, which is shown only as a ribbon for the other amino acids). **b**, By contrast, in a loop (dark blue) the two amino acids are adjacent.

EVOLUTION

A treasure trove of motors

Margaret A. Titus

The myosins are a superfamily of protein motors. Analysis of their sequences in a wide range of organisms reveals an unexpected variety of domains, and provides insights into the nature of the earliest eukaryotes.

Motor proteins use chemical energy, for example from ATP, to generate unidirectional movement along a filamentous track. How a group of proteins acquired and then varied this property to generate a range of movements as evolution proceeded is a fascinating problem in biology. Answers are within reach because of the availability of genome sequences from a diverse cadre of organisms representing various evolutionary groups. This allows in-depth comparative analyses of the sequences of protein families and the incorporation of these data into models of evolution. Richards and Cavalier-Smith (page 1113 of this issue)[1] have performed a comprehensive analysis of the myosin superfamily of motor proteins across a wide sample of eukaryotes (organisms whose cells have nuclei, including plants and animals). The results provide insights into how myosins evolved and into the nature of the earliest common ancestor — the cenancestor — of eukaryotic cells.

The myosins are a diverse group of motor proteins that move along the actin filaments that form a major component of the cell's internal scaffolding. Myosins are best known for powering muscle contraction, cell migration and cytokinesis (the separation of two daughter cells during cell division)[2,3]. These proteins typically consist of an amino-terminal motor domain that binds to the actin track and catalyses nucleotide hydrolysis — the reaction used to harvest energy from ATP — and a carboxy-terminal tail region. This directs the motor domain to targets within the cell, either binding to a cargo that is to be transported around the cell or anchoring the myosin to a particular site[2,3].

Previous comparisons of myosin motor domains identified 18 distinct classes, each associated with specific tail domains, and revealed that the motor and tail appear to have evolved in synchrony[4–6]. Richards and Cavalier-Smith used the highly evolutionarily conserved sequence of the core myosin motor domain to search for myosin genes in genomes from five major taxonomic groups: the amoebae; the opisthokonts (which include fungi and animals); the excavates (which include those protozoans with flagella — whip-like

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50 & 100 YEARS AGO

50 YEARS AGO

There is something very depressing about contemporary biological journals. Paper after paper records observations or experiments, analyses them cautiously, and in a timid and tentative way compares them with previous observations and experiments on the same theme. That is about all: only rarely does the writer disclose how (in his view) his work is related to the broad panorama of biology. There are doubtless sufficient reasons for these omissions: many writers of papers undertake the research they describe for no other reason that that their supervisors 'put them on to it', and many editors of journals consider contemplation out of place in science and do not encourage authors to indulge in it...How refreshing it is, for example, to hear that the choice of a subject for research involves the "art of rejection", and to be told that this art can be compared with the art of the Chinese in designing the empty spaces in their pictures. It is refreshing, too, to be reminded...that the very observations one makes, and *a fortiori*, one's interpretation of them, are limited by the Zeitgeist and by unconscious philosophical assumptions derived from Spinoza.

From *Nature* 27 August 1955.

100 YEARS AGO

A somewhat lamentable aspect of modern science is the vast array of unorganized facts which are awaiting coordination; this is too often because they have been amassed without any definite idea of the purpose which they may serve; consequently it may happen that laborious observations belonging to one science may fail to attract the regard of a neighbouring science merely for want of the mutual acquaintance which would make them serviceable to each other; and in these days of exclusive specialisation the introduction which might lead to a happy union is, perhaps, not brought about for years.

From *Nature* 24 August 1905.

tails that propel them); plants; and chromoalveolates (including dinoflagellates and the apicomplexan parasites such as the malaria-causing *Plasmodium*). The complete sequences of these myosin proteins were then used to find protein domains present in the myosin tails and at the extreme N-termini of some of the molecules.

The analysis uncovered a rich variety of myosins throughout the eukaryotes, extending the catalogue to 37 distinct types of myosin — almost double the number known before. Previously unknown myosin tails containing unique combinations of protein domains were revealed, such as the type 3 myosin from the water mould *Phytophthora ramorum*. This has a series of ankyrin (ANK) protein–protein interaction domains followed by a FYVE domain that binds to the phosphoinositide PI(3)P. Moreover, the analysis confirms that no single myosin is common to all organisms, and that the complement of myosins in any given species ranges from two (in *Entamoeba*) to 13 (in *Phytophthora*). The diversity of myosins is likely to be reflected in the range of actin-based movements that a given cell type or organism can generate, and future functional studies of novel myosins may well reveal a wider range of roles for this group of motor proteins than previously suspected.

Is there a single ancestral myosin? The available data can only narrow down the possibilities to the presence of at least three ancestral myosin subfamilies in the eukaryotic cenancestor (Fig. 1). Previous studies had hinted that two of these, the myosin I and MSD myosins, were present in the earliest eukaryotes, and Richards and Cavalier-Smith's more extensive analysis provides a firm basis for this supposition. It also reveals that a third cenancestral myosin group consists of the MyTH4/FERM myosins, which are present not only in amoebae and multicellular animals (metazoans) but also in chromoalveolates.

So how did the different types of myosin evolve? As one might expect, it seems that following the appearance of the major cenancestral groups, the myosins diversified during eukaryotic evolution by gains and losses of protein domains. Notably, class II myosins, some of the best-studied myosins, are not ancient but arose during the evolution of the unikonts (organisms that have a single flagellum), which include the amoeboid, fungal and metazoan lineages. In addition, certain types of myosins were lost in some groups during evolution. For example, myosin I is missing from the plant lineage and, in an extreme example, no myosins could be found in *Trichomonas* and *Giardia* (both of which are primitive unicellular parasites) or red algae. Myosins in these lineages could have either diverged radically from the rest of the family or been lost altogether: given the existence of myosins in other rapidly evolving groups, it seems most likely that they were lost.

Richards and Cavalier-Smith[1] also address the larger question of the nature of the ancestral eukaryote. Their results are consistent with the emerging hypothesis that a fundamental separation between unikonts and bikonts (cells with two flagella), a group that includes plants, chromoalveolates and the excavates, is the earliest evolutionary divergence. They also infer some of the cellular structures that the common ancestor of these two groups must have possessed: it would have had a single cilium, a centriole and a mitochondrion, and would have had the ability to form a pseudopod. This ancestral cell would have had at least three different types of myosin, with the myosin I perhaps regulating formation of the pseudopod to aid cell movement, the MSD myosin contributing to both cell division and organelle movement, and the MyTH4/FERM myosin having a role in adhesion to substrates and perhaps even contributing to the formation of specialized actin-filled

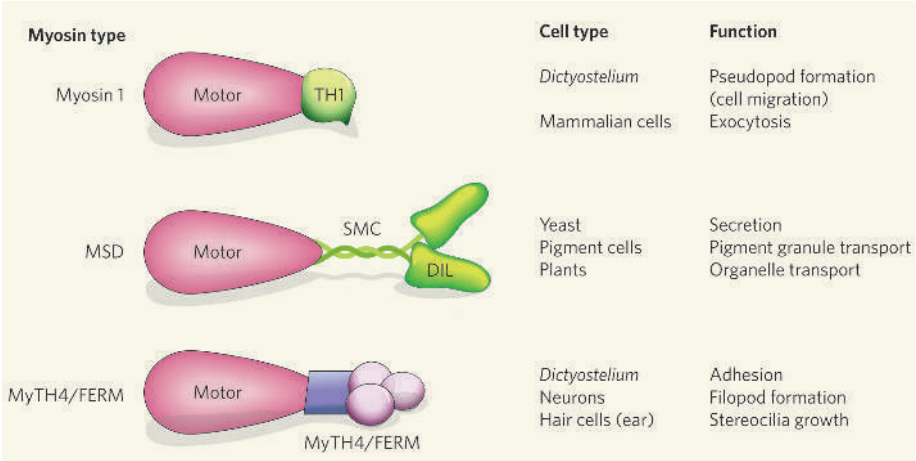

Figure 1 | Three likely ancestral myosins. Richards and Cavalier-Smith[1] propose that there were three ancestral myosins in the earliest eukaryotes, each with distinct tail domain structures. Listed are examples of organisms or cells expressing members of each myosin group and their known functions[2,3]. The TH1 domain would probably bind to charged lipids and target these myosins to membranes. The SMC domain would promote dimer formation and the DIL and MyTH4/FERM domains would target myosins to their cargo or subcellular location.

projections, such as filopodia, which help the cell explore its environment.

The new myosins uncovered by Richards and Cavalier-Smith will keep motor-protein researchers busy for some time. The first task is to characterize them all functionally, including an analysis of their cellular roles and their motor and structural properties. Comparison of these properties will provide more information about the conservation and diversity of motor function in a range of different cellular contexts. It seems likely that other motor-protein families, such as the kinesins, have a similarly large number of different types, and it will be interesting to see if this is indeed the case. Finally, there is a sixth taxonomic group , the rhizarians, for which sequence data are not yet available, so there could well be more myosins to be discovered there. This taxon includes interesting amoeboid organisms such as the foraminiferans, which are distinguished from the amoebae by the reticular structure of their pseudopodia. Including analysis of the rhizarians would not only complete the survey of myosin types, but would help to test the current model of eukaryotic evolution. ■

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1. Richards, T. A. & Cavalier-Smith, T. *Nature* **436,** 1113–1118 (2005).
2. Kieke, M. C. & Titus, M. A. in *Molecular Motors* (ed. Schliwa, M.) 3–44 (Wiley VCH, Weinheim, 2003).
3. Krendel, M. & Mooseker, M. S. *Physiology* **20,** 239–251 (2005).
4. Cheney, R. E. *et al. Cell Motil. Cytoskel.* **24,** 215–223 (1993).
5. Goodson, H. V. & Spudich, J. A. *Proc. Natl Acad. Sci. USA* **90,** 659–663 (1993).
6. Berg, J. C. *et al. Mol. Biol. Cell* **12,** 780–794 (2001).

SYNTHETIC CHEMISTRY

Light on chirality

Yoshihisa Inoue

Reactions that produce only one of two mirror-image forms of a molecule are a hot topic in organic synthesis. A light-driven catalyst provides good results, and the technique could be generally applicable.

Chirality — the non-identity of a molecule with its mirror image — is ubiquitous. It occurs not only in biomolecules (amino acids, sugars, DNA and RNA are examples of chiral molecules), but also in man-made chemicals, materials and drugs. Catalytic asymmetric synthesis — the use of chiral catalysts to transfer and amplify chirality in chemical reactions — has therefore become a central topic in molecular science[1]. Bach and colleagues (this issue, page 1139)[2] now combine two approaches to asymmetric synthesis — the thermal and the photochemical — to control the spatial arrangement of the atoms in a chiral reaction product. The results could be seminal in the field of chiral photochemistry.

In conventional thermal asymmetric synthesis, vibrational energy is supplied to a reaction in the presence of a chiral catalyst or enzyme. This activates ground-state reagent molecules to achieve an asymmetric transformation in which one of two enantiomers — mirror-image forms of a molecule — of a reaction product will be preferentially synthesized. The aim of photochemical asymmetric synthesis is the same, but its tools are different: it uses short-lived, weakly interacting molecular states that have been excited not by heat but by absorbed light. This technique is more difficult to control than its thermal counterpart, and has therefore been less extensively studied, despite its inherent advantages — the low activation energy required for such reactions and the ability to create unstable molecules unique to photochemical reactions, for example.

Nevertheless, chiral photochemistry, or photochirogenesis, has become an area of rapid growth, particularly in the past 10–15 years[3–5]. Like other methods in the realm of asymmetric synthesis, photochirogenesis essentially requires a physical or chemical source of chirality that can be transferred to the reaction products. Such sources come in four main varieties. The first is circularly polarized light, which is used in a technique known as absolute asymmetric synthesis — because a product enriched in one enantiomer is formed from a one-to-one mixture of mirror-image precursor molecules without the intervention of a chiral catalyst. This method is not useful for practical synthetic purposes, but has been discussed in relation to a possible extraterrestrial origin of the chiral homogeneity of biomolecules on Earth[3,6,7].

The second method, known as the chiral auxiliary strategy, uses a molecular group of a particular chirality that binds covalently to an achiral substrate. The irradiation of this augmented substrate creates a new chiral centre in the substrate, often in such a way that the spatial structure of the new chiral centre in the reaction product is determined by that of the chiral auxiliary. Inevitably, however, this technique requires equal molar quantities of the chiral source and the substrate.

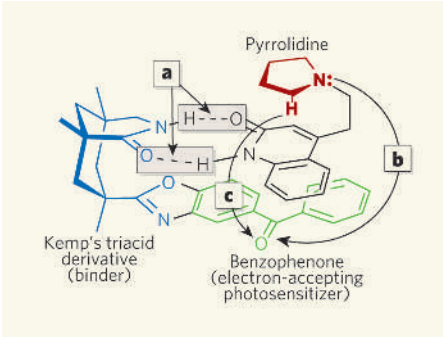

Figure 1 | Choosy catalyst. The photosensitive chiral catalyst molecule developed by Bach *et al.*[2] latches onto a substrate molecule. **a,** A 'Kemp's triacid derivative' group (blue) that forms part of the catalyst attaches to a selected substrate face using two hydrogen bonds (dotted lines). **b,** When activated by light, a photosensitive benzophenone group (green), also part of the catalyst, accepts an electron from a nitrogen atom in a pyrrolidine group (red) in the substrate, creating a radical–ion pair — a process known as photochemical electron transfer (PET). **c,** This is followed by the transfer of a proton adjacent to the nitrogen, setting in a train a sequence of reactions with an enantioselective result — the preferential formation of one mirror-image version of the reaction.

A third and smarter way to transfer chirality is to use a catalytic chiral complexing agent, which is needed in a smaller molar amount than the substrate. Such an agent binds to the substrate in the ground state to provide a chiral environment for a subsequent photochemical process.

Finally, light-absorbing compounds known as chiral photosensitizers can be used to transfer energy or electrons, along with the chiral information, to a substrate. The advantages of this method are, first, that only a small amount of photosensitizer is needed, and, second, that chirality transfer occurs exclusively in the excited state and is thus unaffected by the binding affinity of the photosensitizer for the substrate in the ground state. This does, however, make controlling the structure of the reaction product more difficult, owing to the weak, short-lived interactions in the excited state.

Bach and colleagues' approach[2] combines the advantages of the third and fourth methods. The chiral catalyst that they developed (Fig. 1) contains a photosensitizer component — benzophenone — and a group known as a Kemp's triacid derivative, which uses two hydrogen bonds to attach to a specific substrate like a template, favouring one of the two faces of the substrate's molecular plane. In previous studies[8,9], the authors had exploited the bulky backbone of a simpler template molecule as a 'picket-fence' to prevent a reagent attacking the substrate from the template side.

In their new catalyst[2], the benzophenone group accepts an electron from an atom, in

this case nitrogen, of the bound organic substrate (Fig. 1) when activated by light. This creates a radical–ion pair in a process known as photochemical electron transfer, or PET. (PET processes are well known from, among other things, the conversion of solar to chemical energy in plant photosynthesis.) The benzophenone group then forces a molecular group known as a pyrrolidine ring to remain on one side of the substrate, and thus define which side will participate in the further reaction. This process brings about the desired enantioselection, so that one of the possible two mirror images of the reaction product will form preferentially. Bach and colleagues succeed in obtaining a chiral reaction product consisting of up to 70% excess of one enantiomer, with a yield of 52–64% and turnover numbers (a measure of the amount in mole of a product that is obtained with one mole of catalyst) of between 2.1 and 12.2.

The control of chirality is no trivial task, particularly where PET processes are involved. One factor that can significantly disturb the selective formation of one or other enantiomer is the subsequent dissociation, or separation, of the PET-produced radical–ion pair by solvent molecules. This process spoils the chiral recognition between the sensitizer and the substrate; the reaction will eventually create a racemic product — that is, an equal mixture of two enantiomers. It also means that the polar solvents necessary for PET processes are a mixed blessing: although they accelerate electron transfer and thus increase yield, they simultaneously facilitate dissociation and thus decrease enantioselectivity. A noteworthy previous effort to overcome this trade-off between an excess of one desired enantiomer and high chemical yield was the combined use[10] of a photosensitizer carrying saccharides and a nonpolar solvent. Here, the polar saccharides accelerate PET, whereas the nonpolar solvent prevents the dissociation of the resulting radical–ion pair to secure chiral recognition.

In their current study[2], Bach and colleagues neatly sidestep the acceleration–dissociation dilemma inherent in PET by using neutral radical species produced in the sequence of electron and proton transfers as intermediates, and a hydrogen bond as a tether, to ensure the stability of the chiral environment (Fig. 1). Their photochirogenic process is composed of four steps: the initial PET to produce a radical–ion pair; the transfer of a proton adjacent to the electron-deficient nitrogen to the benzophenone radical anion; the formation of the resulting radical pair into a hydrocarbon ring (cyclization) within the molecule; and finally, the hydrogen initially transferred to benzophenone is returned to the radical centre of the cyclized product. Throughout the whole process, the dual hydrogen bonds tie the substrate to the chiral catalyst in close proximity and in the right orientation. Although all the individual techniques were known previously, they have never before been combined to circumvent the acceleration–dissociation problem.

Bach and colleagues thus provide us with a powerful method applicable to PET photochirogenesis. This is certainly a breakthrough in chiral photochemistry — particularly where synthesis is concerned — that will further stimulate research in this rapidly growing area of science. ■

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1. Rouhi, A. M. *Chem. Eng. News* **82,** 47–62 (2004).
2. Bauer, A., Westkämper, F., Grimme, S. & Bach, T. *Nature* **436,** 1139–1140 (2005).
3. Inoue, Y. & Ramamurthy, V. (eds) *Chiral Photochemistry* (Dekker, New York, 2004).
4. Rau, H. *Chem. Rev.* **83,** 535–547 (1983).
5. Inoue, Y. *Chem. Rev.* **92,** 741–770 (1992).
6. Bonner, W. A. *Origins Life Evol. Biosphere* **21,** 59–111 (1991).
7. Bonner, W. A., Greenberg, J. M. & Rubenstein, E. *Origins Life Evol. Biosphere* **29,** 215–219 (1999).
8. Bach, T., Bergmann, H., Grosch, B. & Harms, K. *J. Am. Chem. Soc.* **124,** 7982–7990 (2002).
9. Aechtner, T., Dressel, M. & Bach, T. *Angew. Chem. Int. Edn* **43,** 5849–5851 (2004).
10. Asaoka, S., Wada, T. & Inoue, Y. *J. Am. Chem. Soc.* **125**, 3008–3027 (2003).