near-edge spectrum; this can be compared with the absorption spectra of standard compounds to deduce the local composition of the material under study. The observed absorption spectrum can thus be thought of as a composite of the individual spectra for components of the catalyst, with the contribution of each component weighted according to its concentration. This means that the spectral map can be used to determine the distribution of pure components throughout the sample.

The STXM technique is ideal for studying heterogeneous catalysts, but has some practical problems associated with it. Soft X-rays are readily absorbed by matter. This means that, for STXM to work, the catalyst particles must be very small, and the distance travelled by the X-rays in the reactor cell — the X-ray gas path length — must be short (less than 100 micrometres). The first STXM study[7] of a catalyst was restricted to experiments using diluted gases at temperatures up to only 533 K. Nevertheless, the technique proved its worth by characterizing the reduction and oxidation of tiny particles of copper oxide (CuO) dispersed on silicon dioxide (silica, a commonly used support for catalysts). The report of de Smit *et al.*[2] describes the design, construction and operation of a much improved STXM cell, which has a gas path length of only 50 micrometres, and which can operate at 1 bar and at temperatures of up to 773 K.

The authors used their reactor cell to examine changes in a catalyst for the Fischer–Tropsch synthesis — a reaction in which 'synthesis gas' (a mixture of carbon monoxide and hydrogen) is converted into hydrocarbons. The Fischer–Tropsch synthesis has been known since the 1920s, but is currently attracting renewed interest as a means of producing transportation fuels from coal, natural gas or biomass. The particular catalyst studied by de Smit *et al.* consists of small iron oxide particles dispersed on silica. However, these are expected to be converted into other compounds after the catalyst has been reduced with hydrogen, and after exposure to synthesis gas.

The authors simulated the reaction conditions of the Fischer–Tropsch synthesis in their cell, and studied the catalyst using X-rays at the absorption edges of carbon, oxygen and iron. In this way, they showed that the iron oxide in the freshly prepared catalyst exists in a single form (known as α-Fe_2O_3), but that, after exposure to hydrogen under the reaction conditions, the particles are reduced to yield metallic iron and a different iron oxide (Fe_3O_4); the authors also observe some formation of an iron silicate (Fe_2SiO_4). After reaction in synthesis gas, the Fe_3O_4 is further converted to iron and Fe_2SiO_4, and the iron in turn reacts to form a compound with carbon (iron carbide). The authors also see evidence for the build-up of hydrocarbon compounds. Observing how the composition of a catalyst alters with changes in reaction conditions and reaction time can reveal insight into the factors controlling catalyst activity and stability.

The current findings[2] demonstrate the potential of STXM for *in situ* chemical imaging of catalysts at the nanometre scale. The spatial resolution of the technique (15 nm) is impressive, but is still not high enough to give a truly atomic-scale view of a catalyst's structure. Improvements in X-ray optics and imaging methods, however, should allow higher spatial resolution, opening the way to a deeper understanding of the structure and composition of multi-component catalysts, and the changes they undergo during a reaction. ■

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BIOCHEMISTRY

Flexible peptide assembly

Jan C. M. van Hest

A jack of all trades is a master of none, as the saying goes. But a protein has been discovered that shuns specialism, and that multitasks to give flexibility to its biosynthetic repertoire.

The biological machinery used by microorganisms to synthesize complex, antibiotic peptides is often compared to Henry Ford's car assembly line: different protein modules line up, each with their own specific synthetic job[1–3], and the molecule under construction is passed from one module to the next, accruing new parts until the desired compound is finally made. This is a sophisticated approach for constructing tailor-made peptides, but building large, multi-protein factories requires quite an investment of resources from the microorganisms involved. This raises the question of whether alternative, more efficient approaches have been adopted by nature — for example, the use of compact protein factories in which one module can execute several operations.

Hamano and colleagues[4] report in *Nature Chemical Biology* the first discovery of a protein that does exactly that. They find that the antibiotic polypeptide poly-ε-lysine (ε-PL) is constructed by a single protein structure that selects the correct building blocks, activates them and then couples them together.

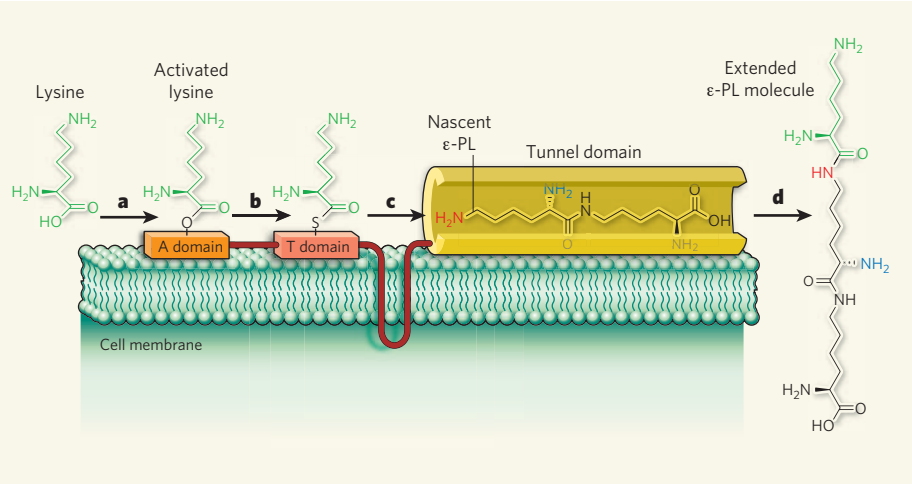

Figure 1 | Antibiotic assembly. Hamano and colleagues[4] have identified the enzyme that synthesizes the polypeptide antibiotic ε-PL, which consists of many lysine amino acids linked together through their side chains. The authors propose the following mechanism for its synthesis. **a**, The first part of the enzyme (the A domain) specifically selects lysine as a building block for ε-PL, and activates the amino acid so that it is ready to react. **b**, The activated lysine is transferred to the next part of the enzyme (the T domain), which holds it in the correct orientation for reaction with the nascent ε-PL. **c**, ε-PL fragments are held in an adjacent tunnel-shaped cavity, in such a way that the side-chain amino group (red), rather than the main-chain amino group (blue), reacts with the activated lysine. **d**, The extended ε-PL molecule may be released from the cavity, or can remain in place, ready to couple with another lysine molecule. For simplicity, the product shown contains three lysine units, but ε-PL actually contains molecules that have 25–35 lysines.

As a potent antibiotic, ε-PL has garnered much attention, and is used in some countries as a food preservative[5]. Constructed from lysine amino acids, its distinctive characteristic is that the lysines are coupled together through their side chains, rather than through groups in their main chains (as occurs between amino acids during normal ribosomal protein synthesis). This mode of coupling suggests the involvement of an enzyme assembly line — a non-ribosomal peptide synthetase (NRPS), to use the technical jargon.

Another quirk of ε-PL is that it is actually a cocktail of peptides, the components of which differ in the number of lysines that make up each molecule. The normal range of chain lengths is 25–35 amino acids. The origin of this chain-length diversity is a topic of debate: it could be the result of degradation of longer ε-PL chains, or the effect of an unusually flexible NRPS synthesis. To solve the mystery, Hamano and colleagues[4] analysed different cell extracts from an ε-PL-producing strain of the bacterium *Streptomyces albulus*. They found one insoluble fraction in which ε-PL was formed, and from this they purified a single active enzyme, which they named ε-PL-synthetase (Pls).

Using an extensive biochemical-characterization procedure, Hamano and colleagues identified within Pls several different domains that are involved in peptide synthesis (Fig. 1). One of these domains is responsible for selecting lysine amino acids (rather than any of the many other naturally occurring amino acids), and for 'activating' the carboxylic acid group in the lysine so that it can form a peptide bond. The authors dubbed this the A domain, after the analogous region in traditional NRPS enzymes. The activated lysine molecule is then connected to another domain, which holds it in the right position for coupling to the nascent ε-PL molecule. Again, by analogy with NRPSs, the authors designated this the T domain.

The recognizable A and T domains comprise the first half of Pls, but the remaining part of the protein acts differently from normal peptide assembly lines. Once activated, lysines cannot be passed on to a neighbouring protein module (as they would be in an NRPS), because there is only one protein involved in the synthesis. Instead, they react with an ε-PL molecule, which is positioned in such a way that the activated lysine can couple only with the side-chain amine of ε-PL. This part of the process is still not completely understood. The most plausible mechanism is that the second part of the Pls protein forms a long, slender tunnel or cavity, which is occupied by the growing ε-PL molecule. Unlike in NRPSs, the nascent peptide is not covalently connected to the protein. After the coupling step with the activated lysine, the extended ε-PL peptide can either be released or remain bound in the cavity. This means that the number of lysines coupled together is not strictly controlled, and would explain the diversity of chain lengths found in ε-PL.

When the working mechanisms of traditional NRPS complexes and the Pls protein are compared, some important differences can be observed. First, each NRPS assembly line allows the synthesis of a complex and perfectly defined peptide, composed of many different amino acids that can form bonds using groups in their main chains or in their side chains. The biological machinery required to create such complex structures is finely tuned, and isn't flexible enough to incorporate amino acids into the product other than those it has evolved to accept. This fine tuning also makes it difficult to modify NRPSs to make analogues of naturally occurring antibiotic peptides (which is desirable for drug discovery).

But the peptide synthesized by Pls lacks the structural complexity of traditional NRPS peptides, because it is composed solely of lysines connected through their side chains. Pls therefore needs only one amino-acid selection domain and one amino-acid activation domain (the A and T domains, respectively). Hamano and colleagues[4] also show that there is some limited flexibility in substrate selection by the enzyme: it can incorporate a few amino acids other than lysine. Furthermore, because the peptide sequence and general domain architecture of Pls are known, it should be possible to change its selectivity even further by protein engineering. Thus, we might one day be able to exploit the biosynthetic flexibility of Pls to make polypeptides with interesting biological activity — not only antibiotics, but potentially also molecules for drug delivery and gene therapy.

So there is more to Pls than meets the eye. At first glance, it might seem less impressive than NRPSs — after all, to stretch the analogy with assembly lines further, if NRPSs produce the peptide equivalent of sophisticated cars, then Pls manufactures relatively simple bicycles. But NRPSs can make only black Model T Fords; Pls meanwhile can produce everything from unicycles to tandems, and, as Hamano and colleagues have shown, might also offer a greater choice of colours. ■

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NEUROSCIENCE

Cool songs

Chris M. Glaze and Todd Troyer

Cooling a specific cluster of neurons in songbirds' brains slows song tempo without changing other acoustic features. This clever technique could be used for understanding neural control of other complex behaviours.

Complex behaviours, ranging from speech and typing to dancing and swimming, all require careful coordination of dozens and often hundreds of different muscles. The resulting behaviour frequently shows hierarchical structure, with sequences composed of primitive movements, each of which is composed of even more basic movements. How is all this coordinated? Researchers have long postulated that the timing of behavioural components is encoded in specialized brain circuits. Without such circuits, movement would quickly become disorganized, like an orchestra performing without a conductor. On page 189 of this issue, Long and Fee[1] provide a remarkable example of such specialization in the brain of the zebra finch by using an equally remarkable experimental approach.

The zebra finch's song consists of a rich set of acoustic features produced with very precise timing. Yet localizing the brain regions that coordinate these features has been tricky. A traditional approach is to measure how neural lesions or electrical stimulation alter song output. But with complex behaviours such as birdsong, such perturbations will often initiate a cascade of disturbance that causes the action to fall apart, making it impossible to isolate the function of the brain area that was initially perturbed.

To circumvent this problem, Long and Fee put a new twist on an old technique. Localized cooling has long been used as a method to selectively and reversibly inactivate particular brain areas[2]. Long and Fee, however, applied only slight cooling, focusing on nucleus HVC — an area of the bird's brain crucial to song production. They find that cooling HVC in this way stretches the temporal fabric of the song, slowing tempo but leaving acoustic features such as pitch largely unchanged. Moreover, the degree of cooling determined the amount of slowing, allowing the authors to effectively 'dial in' a desired tempo.