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self-reliant

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In times of perplexity, every now and then it is better to deal with things yourself. It can be a time-saver, and sometimes, too, an energy-saver. If faced, say, by an oncoming downpour, it is wiser to run for shelter rather than wait for someone to bring you an umbrella. Such decisions also exist at the cellular level. Take our immune system for instance. When attacked by a virus, our body begins by rapidly firing off a first round of artillery as it awaits further and more complex lines of defence that involve myriads of other factors. It turns out that the use of fast lanes such as these also occur at a far smaller scale. In this light, scientists recently discovered quite an extraordinary protein, known as ophMA, that belongs to the fungus *Omphalotus orealius*, which methylates its own C-terminal tail instead of depending on another transferase to do the job. The tail is then cleaved and folds into a cyclic peptide, an omphalotin, that has anti-nematode properties. Besides its talent for independence, ophMA also adopts a rare catenane arrangement, very similar to two rings that have been interlocked.

The Wrestlers

by Henri Gaudier Brzeska (1891-1915)

Omphatolins are produced by *Omphalotus olearius* – a mushroom also known as Jack O'Lantern, because of its deep orange colour akin to that of pumpkins and its soft green bioluminescent glow at night. Omphatolins are cyclic highly-methylated peptides and have been known, since the turn of the 21st century, to specifically target the nematode *Meloidogyne incognita*, a common plant pathogen. Since the peptide's structure resembles that of another renowned fungal cyclic peptide, cyclosporine, scientists assumed that omphatolins were synthesized in the same way. That is to say, their synthesis does not rely on the help of ribosomes but rather on huge multi-modular enzymes called non-ribosomal peptide synthetases (NRPs). Such enzymes are able to build peptide sequences – i.e. to create amide bonds – without resorting to the ribosome's complex machinery.

No one, however, was able to pin down what was making omphalotin. Until, much to everyone's surprise, its sequence was spotted on *Omphalotus olearius*' genome. This could imply that omphalotin is synthesized by a ribosome – something no one had expected, let alone considered, given its similarity to cyclosporine. As their name implies, RiPPs (for **Ri**bosomally synthesized and **P**osttranslationally modified **P**eptides) are synthesized via the regal ribosomal machinery with its tango of mRNA, rRNA, amide bonding, post-translational modifications and proteolytic cleavage. This turned out to be partly true for omphalotin: it is a RiPP, but of a different kind. Omphalotin actually forms the C-terminal end of a far longer sequence whose N-terminal end is a methyltransferase – the very methyltransferase that methylates omphalotin! Since this discovery, many other peptides of the same nature continue to surface and are now collectively known as borosins, i.e. macrocyclic peptides whose leader sequence (*gross modo* the methyltransferase) is very long.

OphMA designates both the methyltransferase (M) and omphalotin (A). About 400 amino acids long, ophMA is divided, roughly, into three main

domains: a large N-terminal methyltransferase domain, followed by what has been called a clasp domain and then a C-terminal core peptide that will give rise to the cyclic peptide omphalotin – itself barely twelve amino acids long. When omphalotin is required, the methyltransferase methylates nine out of twelve of the peptide's amide bonds. Methylation is believed to occur, amide bond by amide bond and in the same direction, either as the peptide is being cyclised or once it has been cyclised.

The process sounds straightforward but is actually quite complex as it involves a very rare chemical arrangement known as a catenane. A catenane is a molecular architecture where two – or more – macrocycles are interlocked in the manner of intertwined rings. Remember the Christmas decorations you made at school? Where you took a strip of coloured paper that you glued at the ends to form a circle? Then you took a second strip, inserted one end through the paper circle you had just made, and glued its ends? That is a two-ringed catenane – which can only be disrupted if the covalent bonds holding one ring are broken, or if you tear one of the strips of coloured paper.

OphMA acts as a homodimer where each monomer locks into the other – just like our Christmas decorations – to adopt a catenane arrangement. How does it happen? First, the methyltransferase and the clasp domains of one monomer move towards each other to form a ring-like structure. The clasp domain of one monomer then wraps around the methyltransferase domain of the other, and the core domain (i.e. the future omphalotin) of one monomer inserts itself into the active site of the other monomer. The result is a very rare catenane arrangement of two enzymes – most probably providing stability to ophMA.

The active site becomes an extended hydrophobic tunnel in which sits the peptide substrate, ready to be methylated. Conformational changes abound as methylation occurs – with the help of a cofactor, S-adenosyl methionine or SAM – on the amide bonds, one transfer after another, as the substrate performs 180 degree flips to bring the next residue into the active site. Consequently, each methyltransferase in the ophMA dimer methylates the substrate peptide that belongs to its monomer as opposed to its own.

It is a wonderful strategy. A sort of "you scratch my back, I'll scratch yours". What is more, with the methyltransferase at the substrate's immediate disposal, there is no need to find an external transferase, so to speak, which would only involve additional energy- and time-consuming biological processes. The methylated peptide is cleaved by another enzyme (called ophP) and cyclised to yield the finished cyclic peptide with nine methylated amide bonds conferring both stability and cell permeability. At this point, one would imagine that the catenane arrangement has collapsed – or is on the point of collapsing – as the methyltransferase is discarded and omphalotin is sent to its target.

Despite knowing in great detail how omphalotin is made, how it is toxic to *Meloidogyne incognita* is still not understood. Perhaps, like cyclosporine, it is able to glide easily through the cell membranes and block important enzymes in the cytoplasm. Although no one yet knows which enzymes are blocked… What is particularly interesting for researchers is that omphalotin is only specific to *Meloidogyne incognita*, a known and wide-spread plant pathogen, yet it is not toxic to bacteria or other fungi. So, as a pesticide against a given crop, it should do little harm to the environment. What is more, ophMA does not seem to be very watchful and joyfully methylates residues that have been artificially replaced by others – which could help scientists suggest fine-tuned versions of the peptide for a given therapeutical or agricultural use.

Cross-references to UniProt

Methyltransferase/ribosomally synthesized cyclic peptide omphalotin A precursor ophMA, *Omphalotus olearius* (Jack o'lantern): A0A2R2JFI5

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