Methylating mushrooms

The genome of the poisonous mushroom *Omphalotus olearius* provides a potent new biocatalytic strategy for installing backbone *N*-methyl amides on ribosomally synthesized peptides. This discovery could yield new biotechnologies for drug development from peptide macrocycles.

Albert A Bowers

Peptide N-methylation is an important strategy used by medicinal chemists to improve cell permeability, oral bioavailability, and target affinity of peptide-based inhibitors[1]. Correspondingly, *N*-methyl amides appear extensively in bioactive natural products. In the case of the immunosuppressant cyclosporine, for example, specific N-methylation of seven out of ten backbone amide nitrogens in the cyclic decapeptide is thought to allow a conformational 'shapeshifting' that hides polar N–H moieties and facilitates passive diffusion across cell membranes[2]. Until now, N-methylation has primarily been the mark of peptide natural products from complex nonribosomal peptide synthetase (NRPS) assembly lines, and has not previously been found among their cousins, the ribosomally synthesized and post-translationally modified peptide (RiPP) natural products. In this issue, van der Velden *et al.* uncover the biosynthetic origins of the omphalotins, peptide natural products from the bioluminescent fungus *O. olearius* (**Fig. 1a**), and bring peptide backbone N-methylation into the realm of peptide post-translational modifications[3].

Many of the most widely used medicinal natural products, such as vancomycin, penicillin, and cyclosporine, are made by nonribosomal pathways[4]. These typically large, multimodular NRPS assembly lines are capable of extraordinarily diverse chemical transformations, but are notoriously challenging to engineer to make new versions of their cognate compounds. By contrast, RiPP enzymes are usually capable of carrying out chemistry on a wide variety of peptides, as long as they come attached to a leader peptide or short peptide handle that acts as a recognition motif for these enzymes[5]. In many ways, RiPP enzymes are leading the way toward combinatorial biosynthesis, but they appear to be limited in terms of the kinds of chemistry they can do, especially when it comes to some of the most profoundly useful transformations, such as backbone-amide N-methylation.

Enter the omphalotins, a group of heavily methylated, nematocidal cyclic peptides from the bioluminescent mushroom *O. olearius*[6]. Because the methylation pattern seen in omphalotins is similar to that of cyclosporine, the omphalotins were assumed to come from NRPSs. However, van der Velden *et al.* have now identified a gene that encodes the unmodified sequence of the 12-amino-acid omphalotin A, indicating that it is instead a RiPP[3]. A unique feature of

OphA, the protein encoded by the candidate gene, is that the omphalotin peptide sequence is not freestanding, but is actually fused to the C terminus of a much larger, 417-amino-acid enzyme containing a predicted methyltransferase domain (**Fig. 1b**). Consistent with this observation, subsequent experiments demonstrated that OphA, when heterologously expressed in *Escherichia coli*, was capable of iterative autocatalytic N-methylation of the residues at its C terminus that correspond to the sequence of the omphalotin A cyclic peptide (**Fig. 1c**). Additional experiments indicated that OphA acts processively from the N to C terminus to install up to 11 backbone methylations. Remarkably, the authors also found that OphA works *in trans* as a homodimer: each monomer in the complex likely modifies the C terminus of the other.

A hallmark of RiPP enzymes has been their extreme substrate promiscuity. To explore potential applications of OphA, van der Velden *et al.* swapped the C-terminal residues of OphA for sequences closely resembling those of cyclosporin A (CycA) or dictyonamide A (DicA)—a CDK4 inhibitor—both of which are naturally NRPS derived and contain multiple N-methylations. These new fused substrates underwent multiple methylations that were also catalyzed by the methyltransferase domain of OphA: up to five for CycA and up to eight for DicA. Although the final products did not display identical methylation patterns to the natural products, the authors note that the results are proof of principle that OphA or a homolog may one day be engineered for production of custom backbone N-methylated peptides in large quantities. Indeed, the authors were also able to identify and then reconstitute an OphA homolog from basidiomycete *Dendrothele bispora* CBS 962.96, DbOphA.

Biocatalysis has become an increasingly common component of drug discovery, and this influence will presumably grow with advances in whole-genome manipulation and synthetic biology.

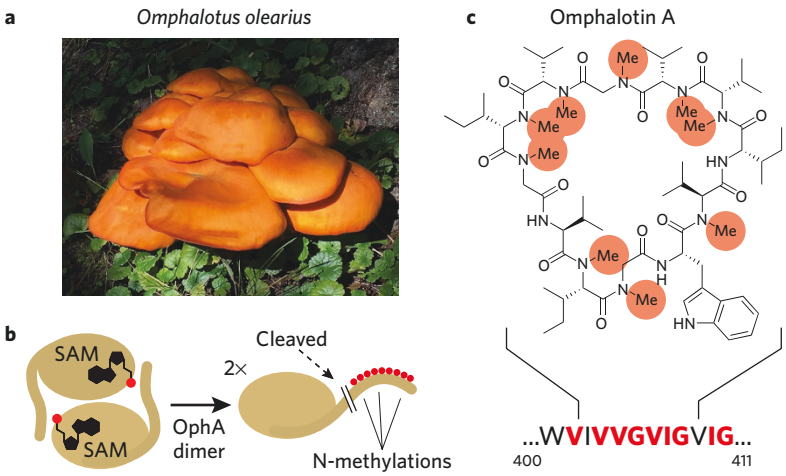

Figure 1 | Structure and origins of omphalotin A. (**a**) The omphalotins are a group of nematocidal peptides isolated from *Omphalotus olearius*, a poisonous, orange-gilled mushroom found primarily in the woods of Eastern Europe. Image reprinted with permission from Claudia Schmidt-Dannert. (**b**) Omphalotin A is derived from the C terminus of OphA, a 417-residue, SAM-dependent methyltransferase. OphA autocatalytically N-methylates nine backbone amides in a section of its own C terminus. (**c**) After modification, this C-terminal region is eventually cleaved from OphA and cyclized to give the structure of the final natural product, omphalotin A.

The discovery of OphA places a very useful chemical transformation into that synthetic biology toolbox by opening the door to whole-cell preparation of backbone N-methylated peptides. Industrial-scale versions of this chemistry would substantially lower the barrier for use of N-methylated peptides as therapeutics by facilitating their large-scale production. One question whose answer will be important for the advancement of OphA in this technology is how the enzyme recognizes and chemically modifies a fragment of its own C terminus. On inspection of the sequence, OphA does not appear to have any of the conserved motifs used by other RiPP enzymes for substrate recognition[7]; understanding the molecular details of how the enzyme recognizes which residues to methylate will be necessary for using OphA or its homologs to generate methylated cyclosporin and/or dictyonamide analogs with high fidelity. Additionally, this work underscores the utility of fungi as important sources of natural products and enzymes for bioengineering. We will likely continue to see examples of useful biological chemistry coming from all kingdoms of life. ■

Albert A. Bowers is in the Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.
e-mail: abower2@email.unc.edu

References

1. Chatterjee, J., Gilon, C., Hoffman, A. & Kessler, H. *Acc. Chem. Res.* **41**, 1331–1342 (2008).
2. Bock, J.E., Gavenonis, J. & Kritzer, J.A. *ACS Chem. Biol.* **8**, 488–499 (2013).
3. van der Velden, N.S. *et al. Nat. Chem. Biol.* **13**, 833–835 (2017).
4. Walsh, C.T., O'Brien, R.V. & Khosla, C. *Angew. Chem. Int. Ed. Engl.* **52**, 7098–7124 (2013).
5. Arnison, P.G. *et al. Nat. Prod. Rep.* **30**, 108–160 (2013).
6. Sterner, O., Etzel, W., Mayer, A. & Anke, H. *Nat. Prod. Lett.* **10**, 33–38 (1997).
7. Burkhart, B.J., Hudson, G.A., Dunbar, K.L. & Mitchell, D.A. *Nat. Chem. Biol.* **11**, 564–570 (2015).

Competing financial interests

The author declares no competing financial interests.