Biochemistry

The chemical know-how of the flame lily

Jing-Ke Weng

The discovery of the biosynthetic pathway for colchicine, a medicine produced by plants, holds promise for the use of metabolic-engineering approaches in producing reliably high yields of this compound. **See p.148**

On page 148, Nett *et al.*[1] shed light on how colchicine, a pharmaceutically and scientifically useful natural product, is biosynthesized in the flame lily *Gloriosa superba*. The authors identify eight *G. superba* genes that encode a series of specialized metabolic enzymes, which collectively convert 1-phenethylisoquinoline, a compound produced early in the biosynthetic pathway, to *N*-formyldemecolcine, the first colchicine precursor that harbours the natural product's signature molecular scaffold. The study not only reveals the ingenuity of plant biosynthesis, but also opens the way to the development of metabolically engineered organisms that make colchicine more efficiently than do its natural producers.

Colchicine is used to treat gout, a particularly painful form of arthritis, and a range of inflammatory diseases[2]. It inhibits the formation of protein filaments called microtubules, thereby arresting the process in which replicated DNA is divided between daughter cells during cell division[2]. This biological activity has also led to colchicine being used to increase the number of sets of chromosomes in plants, which can be beneficial when breeding crop plants[3].

Colchicine is produced by autumn crocuses (plants of the *Colchicum* genus) and flame lilies (the *Gloriosa* genus). Organic chemists have been fascinated by colchicine and its naturally occurring analogues since the 1950s, because the molecules contain an unusual system of rings and a stereocentre (a carbon atom attached to four different atoms or groups), which makes them challenging to synthesize[4] (Fig. 1). Colchicine molecules in nature are produced as just one of the two possible enantiomers (mirror-image isomers) of the compound. An enantioselective synthesis of colchicine was finally achieved[5] in 2017, but is impractical for industrial production. Colchicine is therefore currently sourced from various cultivated autumn crocuses and flame lilies (Fig. 2), but yields are low, and the supply chain can be unreliable.

Nett *et al.* set out to investigate colchicine biosynthesis in *G. superba*. This flame lily is not a model plant like *Arabidopsis thaliana* or rice. Little is known about its genetic make-up, and no tools were available that would enable convenient genetic manipulations and analysis. Finding the gene set for colchicine biosynthesis in the total complement of *G. superba* genes was therefore like finding a needle in a haystack. This is a common problem for plant researchers — efforts to work out the biosynthetic pathways of natural products made by plants have taken decades of extensive work through the painstaking cloning and characterization of putative enzymes in the pathway (see refs 6 and 7, for example).

The starting point for Nett and colleagues' work was a previously proposed biosynthetic pathway for colchicine[8], which had been developed on the basis of experiments conducted in autumn crocus plants and from an analysis of colchicine analogues isolated from related plant species[9]. The early biosynthetic product 1-phenethylisoquinoline is probably derived from the amino acids phenylalanine and tyrosine[10]. This intermediate was proposed to be converted in nine steps to *N*-formyldemecolcine, the first intermediate that contains the molecular scaffold of colchicine (Fig. 1). This putative sequence involves two unusual biochemical reactions: a rare transformation known as a phenol-ring coupling, and an unprecedented reaction that leads to expansion of a molecular ring.

In bacteria, the genes that encode biosynthetic enzymes for a natural product tend to cluster together in the microorganisms' genomes[11], a fact that aids the identification of these genes in bacteria. Plants do not have the same type of biosynthetic gene clusters as bacteria in their genomes, but they do contain a rich variety of tissue types. Because natural products are mostly used to defend against biotic and abiotic stresses under specific circumstances, they tend to accumulate in specific tissues in plants, or be produced in response to certain stimuli. Candidate biosynthetic genes can therefore be identified by correlating the spatial and temporal expression profiles of genes with the formation of the compounds of interest[12].

Colchicine is highly enriched in the rhizome of *G. superba*, but is nearly absent in some other tissues. By comparing tissue-specific transcriptomics data (which characterize gene-expression profiles) with metabolomics data (which characterize all of the small molecules produced in each tissue) for *G. superba*, the authors quickly identified 19 candidate

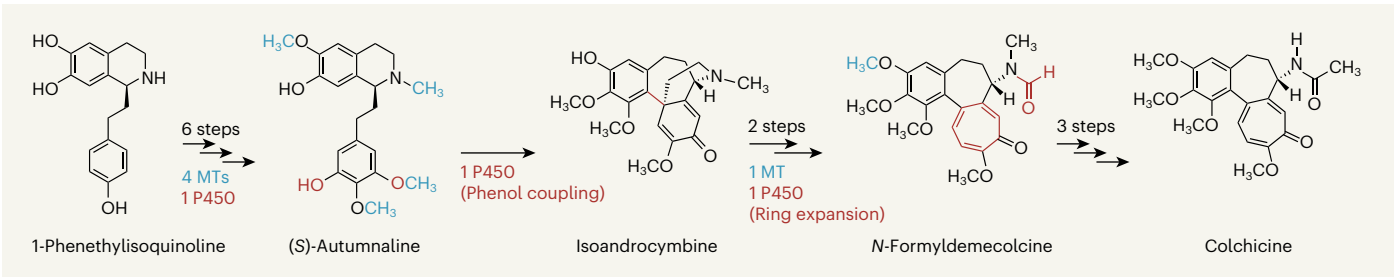

Figure 1 | Part of the biosynthetic pathway of colchicine. Nett *et al.*[1] have identified enzymes that catalyse most of the steps in the biosynthesis of colchicine, a medicinally useful compound produced by flame lilies (*Gloriosa* spp.) and autumn crocuses (*Colchicum* spp.). 1-Phenethylisoquinoline is initially made from the amino acids phenylalanine and tyrosine (steps not shown). It is then converted into (*S*)-autumnaline in six steps by four methyltransferase (MT) enzymes and one cytochrome P450 enzyme (abbreviated as P450); colours indicate parts of the molecule installed by the two types of enzyme. Two other P450s catalyse unusual biochemical reactions: a phenol coupling, which produces isoandrocymbine; and a ring expansion, which produces *N*-formyldemecolcine. A fifth methyltransferase is also needed to convert isoandrocymbine into the substrate for the ring expansion. The enzymes for the final three steps, in which *N*-formyldemecolcine is thought to be converted into colchicine, are still unknown.

ALAMY

Figure 2 | Cultivation of the flame lily. Skilled farm workers hand pollinate the flame lily, *Gloriosa superba*, which is grown as a source of colchicine.

genes that potentially encode biosynthetic enzymes for colchicine: 11 methyltransferases and 8 cytochrome P450 enzymes. Cytochrome P450 enzymes typically catalyse reactions in which a hydroxyl group (OH) is added to a small molecule.

To test the biochemical functions of the enzymes encoded by the candidate genes, Nett *et al.* turned to the plant *Nicotiana benthamiana*, a close relative of tobacco. This plant has become an invaluable tool for plant biotechnology because it can be used in a technique called agro-infiltration[13]. In this technique, bacteria from the genus *Agrobacterium* are used as vectors to transfer genes of interest to the leaves of *N. benthamiana*, which then transiently expresses the genes. The effects of gene expression can thus be observed. Genes can be introduced alone or with others, and can even be transferred together with a solution of a small molecule, to see if the molecule acts as a substrate for any enzymes encoded by the genes.

In this way, Nett and colleagues matched the candidate genes with the enzymatic functions needed to reconstitute various parts of the colchicine biosynthetic pathway in *N. benthamiana* – similar to the way in which a jigsaw is assembled from its pieces. Five of the eleven methyltransferases were indeed found to have roles in the pathway: four of them catalyse the attachment of methyl (CH_3) groups to oxygen atoms, whereas the other catalyses the addition of a methyl group to a nitrogen atom (Fig. 1). The authors also discovered that one of the candidate cytochrome P450 enzymes catalyses two steps in the proposed biosynthetic pathway: the installation of two hydroxyl groups on a benzene ring in an intermediate compound.

Perhaps the most surprising findings were that two cytochrome P450 enzymes catalyse the unusual phenol-ring coupling and ring expansion, respectively – the key reactions that produce the molecular scaffold of colchicine. Finally, Nett *et al.* identified a few more genes, from *G. superba* and from other plants, that encode a set of enzymes that convert phenylalananine and tyrosine to 1-phenethylisoquinoline. When these genes were transiently expressed in *N. benthamiana* with the eight other biosynthetic genes, the total biosynthesis of *N*-formyldemecolcine was successfully reconstituted.

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Nevertheless, the final three steps of the biosynthetic pathway, which convert *N*-formyldemecolcine to the final product, remain unresolved. This is because little is known about the plant enzymes that might catalyse the postulated reactions, precluding a search for candidate genes.

More broadly, the authors have demonstrated that transcriptomics and metabolomics data, together with tools for transiently expressing genes in plants, can unravel biosynthetic pathways from relatively unstudied plant hosts. However, this approach requires that a reliable proposal for the biosynthetic pathway has been developed previously. Moreover, biosynthetic genes that have different expression patterns from those of other genes in the pathway can be easily missed when searching for candidate genes.

Moving forward, activity-guided fractionation – an old technique for identifying enzymes that involves the iterative separation and biological testing of protein mixtures prepared from natural sources – might return. It could now become a routine part of the workflow used to discover the biosynthetic pathways for plant natural products, aided by state-of-the-art tools for characterizing all of the proteins found in a given sample. Alternatively, combinatorial testing of tens to hundreds, or even thousands, of candidate genes in a suitable plant system will greatly facilitate gene discovery, and will require fewer assumptions to be made about which genes to screen than Nett and colleagues had to make.

In the meantime, the reconstitution of the biosynthesis of *N*-formyldemecolcine in *N. benthamiana* using 16 transferred genes (transgenes) is a remarkable feat. However, the quantity produced is still much less than that made by the native plant hosts. Future research should examine the specialized intra- and intercellular mechanisms that support the high accumulation of natural products in their normal hosts. This will be crucial for achieving the ultimate goal of developing genetically engineered plants that efficiently and sustainably biosynthesize pharmaceuticals such as colchicine.

Jing-Ke Weng is at the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA, and at the Massachusetts Institute of Technology, Cambridge, USA.
e-mail: wengj@wi.mit.edu

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