PLANT PIGMENT EVOLUTION

Painting with betalains

Investigations of plant metabolism reveal how an enzyme variant with reduced feedback sensitivity may allow plants to switch their pigment palettes.

Anne Osbourn

Plants make diverse pigments that act as attractants for pollinators and seed dispersal agents, and provide protection against biotic and abiotic stresses. Most plant species produce anthocyanins, flavonoid pigments that appear red to blue, depending on pH. Plants belonging to the order Caryophyllales — which includes cacti, carnations, amaranths, ice plants, beets and many carnivorous species — are the exception to this rule. Most Caryophyllales produce a different class of pigments known as betalains. These red–violet and yellow pH-stable pigments are restricted to Caryophyllales, and never co-occur with anthocyanins[1,2]. The mutually exclusive occurrence of these two pigment types has intrigued evolutionary biologists and biochemists for over half a century. In a recent issue of *New Phytologist* Lopez-Nieves et al. report a breakthrough discovery — the identification of a missing early step in betalain biosynthesis with relaxed feedback sensitivity that probably enables the betalain tap to be turned on[3].

Betalains are synthesized from the amino acid L–tyrosine, while anthocyanins are derived from a different amino acid, L-phenylalanine[1] (Fig. 1). L-tyrosine and L-phenylalanine are amino acids that are required for essential protein biosynthesis in all organisms. The two pigment pathways thus draw these amino acids away from primary metabolism and use them as precursors for the biosynthesis of specialized metabolites. Although primary metabolism is generally highly conserved in plants, specialized metabolism is typified by its tremendous divergence, reflecting the enormous amount of chemical diversity represented in the plant kingdom. The interface between primary metabolism and specialized metabolism is not well understood. Lopez-Nieves et al. provide evidence that betalain-producing plants have developed a strategy for the diversion of enhanced levels of L-tyrosine into specialized metabolism by overcoming a key negative feedback step[3]. They postulate that the relaxation of L-tyrosine feedback inhibition may create a surplus of L-tyrosine at the expense of L-phenylalanine, thereby fuelling the betalain pathway and starving the anthocyanin pathway.

L-Tyrosine is converted to betalains via the hydroxylated intermediate L-dopamine (L-DOPA). The enzymes that catalyse the conversion of L-DOPA to betalamic acid and cyclo-DOPA, which then react to spontaneously form betalains[4], have recently been characterized[5–11]. However, the immediate upstream enzymes that generate the L-tyrosine supply for betalain biosynthesis in Caryophyllales were not known. Lopez-Nieves et al. predicted two possible routes to L-tyrosine from the shikimate pathway precursor prephenate, based on knowledge from other organisms[3]. In microbes, prephenate is normally oxidatively decarboxylated to 4-hydroxyphenyl pyruvate and then transaminated to give L-tyrosine. Conversely, in plants prephenate is usually first transaminated into arogenate and then subsequently decarboxylated to give L-tyrosine (Fig. 1). The enzymes that catalyse the decarboxylation steps are prephenate dehydrogenase (PDH) and arogenate dehydrogenase (ADH), respectively, both of which are present in plants.

Lopez-Nieves et al. showed that protein preparations from red beet (*Beta vulgaris*) could produce L-tyrosine when fed with L-arogenate but not prephenate; that is, red beet has ADH but not PDH activity[3]. Two candidate *ADH* genes were identified, one of which had a similar expression profile to other characterized betalain biosynthesis genes from this species. These two genes, which are in tandem in the *B. vulgaris* genome, seem to have arisen from a recent gene duplication event within Caryophyllales. In plants, L-tyrosine biosynthesis takes place in the plastids and is subject to strong feedback inhibition. Both of the *B. vulgaris ADH* gene products were functional and localized to the plastids. Interestingly, however, the *B. vulgaris* ADH enzyme that was co-expressed with the betalain genes (ADHα) had markedly reduced sensitivity to L-tyrosine inhibition, while its counterpart (ADHβ) did not. This new 'relaxed' enzyme is only found in betalain-producing plants. Intriguingly, two families within Caryophyllales have reverted from betalain to anthocyanin pigmentation, and here the *ADH*α orthologs in these species have undergone putative pseudogenization, and in some cases gene loss. Thus, anthocyanin biosynthesis can be reactivated under such circumstances, perhaps associated with increased availability of arogenate for phenylalanine-based pathways.

Betalains are used as food colourants and dietary supplements[12]. There is also interest in L-DOPA as a therapeutic treatment for Parkinson's disease. Red beet is one of the few edible plants within the Caryophyllales and is the major source of betalains. Heterologous production of betalains in different plant species could enable the generation of genetically modified food crops with enhanced health benefits and improved stress tolerance, as well as the development of new ornamental varieties. It may also open up new opportunities for commercial production of known and new-to-nature betalains for diverse markets. Other researchers have shown that the previously characterized set of core betalain pathway genes is necessary and sufficient to confer betalain production[9,13]. However, yields might be further enhanced by boosting the L-tyrosine precursor supply. Lopez-Nieves et al. demonstrate that transient expression of *ADH*α but not *ADH*β in *Nicotiana benthamiana*, a wild relative of tobacco, increases L-tyrosine levels more than 10 fold[3], suggesting that this strategy may have promise for engineering betalain production in heterologous species. The next step would be to co-express *ADH*α with the core pathway genes to establish the effectiveness of this approach.

It is not known why Caryophyllales have shunned anthocyanins in favour of betalains. Anthocyanins are widespread in plants and so are presumably ancient. Betalains are restricted to Caryophyllales and are likely to have evolved relatively

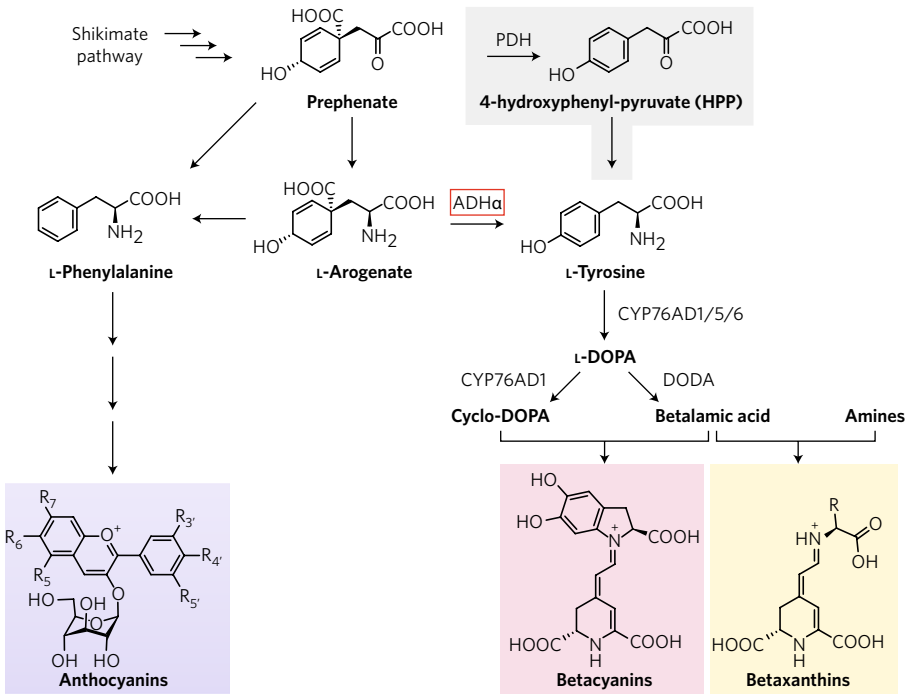

Fig. 1 | Biosynthesis of anthocyanins and betalains. The relaxed enzyme with reduced L-tyrosine feedback inhibition, ADHα, is boxed in red. The core betalain pathway enzymes (the cytochrome P450 enzymes CYP76AD1/5/6 and the DOPA 4,5-dioxygenase DODA) are shown.

recently. The evidence indicates that *ADH*α and the core betalain pathway genes have arisen by gene duplication and neofunctionalization[3,14]. Two functionally related but non-homologous pathway genes are located within 50 kb of each other on chromosome 2 of *B. vulgaris*, along with other genes that are uncharacterized as yet[14]. *ADH*α and *ADH*β are not clustered with these genes and are located on a different chromosome. It remains to be seen whether the other genes form part of a larger biosynthetic gene cluster, as has been shown for a number of specialized metabolic pathways in plants[15]. Examples of clustered pathways for the biosynthesis of L-tyrosine-derived specialized metabolites have been reported from other plants — for the synthesis of cyanogenic glycosides in cassava, *Lotus japonicas* and sorghum. These clusters seem to have arisen independently in these diverse lineages by convergent evolution[16]. Clustering seems to be a feature of specialized metabolic pathways with restricted taxonomic distribution. However, the genes for anthocyanin biosynthesis are dispersed in plant genomes, which may be expected for such a widely distributed and fundamental pathway[17].

The current study opens up many further questions. Which amino acid residue(s) determine the reduced sensitivity of ADHα to L-tyrosine inhibition and can this partial reduction be converted to complete insensitivity through further modification? Why do some plants (such as *Arabidopsis thaliana*) fail to make betalains when provided with the core set of pathway enzymes unless fed with L-tyrosine[11,18]? What are the consequences of manipulating the feedback sensitivity of ADHα for primary metabolism, betalain biosynthesis and anthocyanin production? How are the various pathways co-ordinately regulated at the transcriptional level? Is there a fitness cost of retaining both pathways in the same species? The exciting genetic and biochemical advances in understanding betalain biosynthesis reported by Lopez-Nieves et al. and in other recent publications, in combination with new genome editing technology, now make it possible to address such questions for the first time. ❐

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References

1. Tanaka, Y., Sasaki, N. & Ohmiya, A. *Plant J.* **54**, 733–749 (2008).
2. Brockington, S. F. et al. *New Phytol* **190**, 854–864 (2011).
3. Lopez-Nieves, S. et al. *New Phytol.* http://doi.org/10.1111/nph.14822 (2017).
4. Strack, D., Vogt, T. & Schliemann, W. *Phytochemistry* **62**, 247–269 (2003).
5. Christinet, L. et al. *Plant Physiol.* **134**, 265–274 (2004).
6. Hatlestad, G. J. et al. *Nat. Gen* **44**, 816–820 (2012).
7. Gandía-Herrero, F. & García-Carmona, F. *Trends Plant Sci* **18**, 334–343 (2013).
8. DeLoache, W. C. et al. *Nat. Chem. Biol.* **11**, 465–471 (2015).
9. Polturak, G. et al. *New Phytol.* **210**, 269–283 (2016).
10. Schwinn, K. E. *New Phytol.* **210**, 6–9 (2016).
11. Sunnadeniya, R. et al. *PLoS ONE* **11**, 1–16 (2016).
12. Azedero, H. M. C. *Int. J. Food Sci. Technol.* **44**, 2365–2376 (2009).
13. Polturak, G. et al. *Proc. Natl Acad. Sci. USA* **114**, 9062–9067 (2017).
14. Brockington, S. F. et al. *New Phytol.* **207**, 1170–1180 (2015).
15. Nützmann, H.-W., Huang, A. & Osbourn, A. *New Phytol.* **211**, 771–789 (2016).
16. Takos, A. M. et al. *Plant J* **68**, 273–286 (2011).
17. Medema, M. & Osbourn, A. *Nat. Prod. Rep.* **33**, 951–962 (2017).
18. Harris, N. N. et al. *BMC Plant Biol* **12**, 34 (2012).

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Competing interests

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