Advances in the Biosynthesis and Metabolic Regulation of Betalains: Postprint

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Abstract

Betalains are plant-derived, water-soluble natural nitrogen-containing pigments utilized in industries such as food additives and cosmetics. In plants, betalains and anthocyanin pigments are mutually exclusive, and their metabolic pathway serves as an important phytochemical classification indicator. Betalains exhibit pharmacological effects including antioxidant, antitumor, antimalarial, and hepatoprotective activities. Their potential medical and healthcare value, coupled with the uniqueness of their metabolic pathway, has stimulated in-depth research on betalains. This review summarizes domestic and international research progress on key enzymes in the betalain synthesis pathway and synthetic biology strategies for betalain production, providing a reference for establishing synthetic biological methods for betalain production.

Full Text

Preamble

Advances of Betalains Biosynthesis and Metabolic Regulation

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Abstract

Betalains are plant-derived water-soluble natural nitrogen-containing pigments widely used as food additives and cosmetic colorants. In plants, betalains and

anthocyanins are mutually exclusive, making their metabolic pathway an important phytochemical classification index. Betalains exhibit various pharmacological activities including antioxidant, antitumor, antimalarial, and hepatoprotective effects. Their potential healthcare value and unique metabolic pathway have stimulated intensive research. This review summarizes recent advances in key enzymes of the betalain biosynthetic pathway and synthetic biology strategies for betalain production, providing a reference for establishing synthetic biological methods for betalain manufacturing.

Keywords: betalain biosynthesis, synthetic biology, metabolic engineering, betanin, betaxanthin

Betalains are water-soluble nitrogen-containing alkaloid pigments named after their initial discovery in beetroot, comprising two classes: betacyanins and betaxanthins. To date, 75 betalain structures have been identified from 17 plant species [1]. As important natural pigments, betalains are commonly used as food additives and cosmetic colorants, and possess biological activities such as antioxidant, antitumor, and hepatoprotective effects with potential healthcare applications [2]. Additionally, betalains and anthocyanins are mutually exclusive—no plant has been found to contain both pigments simultaneously—making betalains a significant chemotaxonomic marker [3]. Due to their high economic value and scientific importance in plant evolution and molecular classification, research on betalain biosynthetic pathways and production technologies has been actively pursued. With advances in plant genome sequencing and differential transcriptomics, the betalain biosynthetic pathway has been gradually elucidated and key enzymes identified. Betalains are secondary metabolites of L-tyrosine, with betalamic acid as the core structure of all betalains. Condensation of betalamic acid with cyclo-DOPA, followed by glycosylation, yields betacyanins, whereas condensation with amino acids or amines produces betaxanthins. Common betalains and their representative plant sources are shown in Table 1 . This review summarizes research progress on betalain biosynthesis (Fig. 1 [Figure 1: see original paper]) and discusses novel production methods through metabolic engineering and synthetic biology in plants and microorganisms.

Table 1 Common betalains and their sources

Structure	R Group	Betalain Name	Source Plant
Betanidin	H	Betanidin aglycone	Beetroot
Betanidin	Glc	Betanin	Beetroot
Betanidin	H	2-Decarboxybetanidin	*Portulaca grandiflora*
Betanidin	Malonyl	6’ -O-Malonyl-2-decarboxybetanidin	*Portulaca grandiflora*
Betanidin	-Coumaroyl	Lampranthin I	*Lampranthus* spp.
Betanidin	Feruloyl	Lampranthin II	*Lampranthus* spp.
Betanidin	H	Bougainvillein I	*Bougainvillea* spp.

Structure	R Group	Betalain Name	Source Plant
Betanidin	-Coumaroyl	Bougainvillein III	*Bougainvillea* spp.
Betanidin	H	Celosianin I	*Celosia cristata*
Betanidin	-Coumaroyl	Celosianin II	*Celosia cristata*
Betanidin	Feruloyl	Celosianin III	*Celosia cristata*
Betanidin	H	Gomphrenin I	*Gomphrena globosa*
Betanidin	-Coumaroyl	Gomphrenin II	*Gomphrena globosa*
Betanidin	Feruloyl	Gomphrenin III	*Gomphrena globosa*
Betanidin	Sinapoyl	Gomphrenin IV	*Gomphrena globosa*
Betaxanthin	Aspartic acid	Aspartic acid-betaxanthin	*Mirabilis jalapa*
Betaxanthin	Tyramine	Tyramine-betaxanthin	*Mirabilis jalapa*
Betaxanthin	Dopamine	Dopamine-betaxanthin	*Mirabilis jalapa*
Betaxanthin	Histamine	Histamine-betaxanthin	*Mirabilis jalapa*
Betaxanthin	Tyrosine	Tyrosine-betaxanthin	*Portulaca grandiflora*
Betaxanthin	Glycine	Glycine-betaxanthin	*Portulaca grandiflora*
Betaxanthin	Proline	Proline-betaxanthin	*Opuntia ficus-indica*
Betaxanthin	Hydroxyproline	Hydroxyproline-betaxanthin	*Opuntia ficus-indica*

[Figure 1: see original paper]

Fig. 1 Biosynthesis pathway of betalains. 5-GT: Betanidin-5-O-glycosyltransferase, 6-GT: Betanidin-6-O-glycosyltransferase

1.1 Tyrosine Hydroxylase

Betalamic acid synthesis begins with the aromatic amino acid L-tyrosine, which is converted to L-DOPA by tyrosinase. Tyrosinase is the first key enzyme in betalain biosynthesis, a copper-containing protein with two Cu-binding sites and a molecular weight of approximately 40–70 kDa. It has been identified and cloned from various plants including *Portulaca grandiflora* L. [4], *Beta vulgaris* L. [5,6], and the halophyte *Suaeda salsa* L. [7]. The fungus *Amanita muscaria*, which synthesizes betalain pigments, also contains tyrosinase [8].

In higher plants, tyrosinase exhibits both tyrosine hydroxylation activity to produce L-DOPA and oxidation activity to generate dopaquinone. Accumulation of L-DOPA is crucial for betalamic acid production, which requires preventing further oxidation of L-DOPA to dopaquinone. For example, tyrosinase from *Suaeda salsa* shows significantly higher substrate affinity and maximum reaction

rate for L-DOPA oxidation than for tyrosine hydroxylation [9]. Consequently, reductants are indispensable in the betalain metabolic pathway, as they effectively reduce dopaquinone back to L-DOPA for further conversion to betalamic acid. High levels of ascorbic acid have been detected in betalain-rich plants [10]. Additionally, Teng et al. [11] discovered that a catalase-phenol oxidase participates in betalamic acid synthesis. This enzyme from amaranth (*Amaranthus cruentus* L.) exhibits complete tyrosinase activity, including monophenol oxidation of L-tyrosine and diphenol oxidation of L-DOPA, along with catalase activity. The corresponding active sites were identified in its sequence, though no classic copper-binding sites of tyrosinase were found. The expression level of this enzyme positively correlates with betaxanthin production, suggesting its primary involvement in betaxanthin rather than betacyanin synthesis [11].

1.2 DOPA Dioxygenase

L-DOPA is converted to 4,5-seco-DOPA by the second key enzyme, 4,5-DOPA-extradiol-dioxygenase (DODA). The seco-DOPA is unstable and undergoes intramolecular condensation between the amino group and the aldehyde moiety to form the betalain skeleton and chromophore—betalamic acid. DOPA dioxygenase is a non-heme iron protein that catalyzes the ring-opening of catechol derivatives with incorporation of two oxygen atoms [12]. The 4,5-DOPA dioxygenase gene (PgDODA) was cloned from *Portulaca grandiflora* of the Portulacaceae family. Such proteins exist not only in betalain-producing plants but also in other angiosperms and bryophytes, with slight variations in sequences near the catalytic site [13]. Some DODA enzymes from non-betalain-producing plants also exhibit DOPA dioxygenase activity in vitro; these plants fail to produce betalains due to the absence of L-DOPA in vivo [14]. The MjDODA gene from *Mirabilis jalapa* L. was successfully expressed in *E. coli*, confirming DODA activity in vitro and betalamic acid formation [15]. Heterologous expression of beet-derived BvDODA in *E. coli* demonstrated that seco-DOPA spontaneously rearranges to betalamic acid. Both in vivo and in vitro, the S-isomer of betalamic acid predominates (95%), consistent with the S-configuration of most natural betalains [16]. RT-PCR and transcriptomic analysis are currently the primary methods for discovering and identifying functional genes, which led to the identification of PmDODA from *Parakeelya mirabilis* L. as involved in betalain synthesis [17]. In addition to plant-derived genes, the *E. coli* protein YgiD has been reported to possess DOPA dioxygenase activity, synthesizing betalamic acid from L-DOPA in vitro [18].

2.1 Cyclo-DOPA Pathway for Betanidin Formation

Different betacyanins are derived from glycosylation of betanidin (cyclo-DOPA-betalamic acid). Through the oxidative activity of tyrosinase, L-DOPA is further oxidized to dopaquinone, which undergoes spontaneous intramolecular cyclization via nucleophilic attack by the amino group to form cyclo-DOPA [19,20]. Cyclo-DOPA then spontaneously condenses with betalamic acid to generate

betanidin [21]. Silencing the cytochrome P450 oxidase CYP76AD1 in beets abolished betacyanin production while allowing only betaxanthin formation, demonstrating that CYP76AD1 is the cyclo-DOPA synthase [22]. Its homologs CYP76AD5 and CYP76AD6 only exhibit tyrosine hydroxylation activity to produce L-DOPA but cannot catalyze cyclo-DOPA formation [23]. The ability to form cyclo-DOPA is therefore essential for betacyanin production. Among all currently known proteins with tyrosinase activity, only CYP76AD1 can synthesize betacyanins. Due to the instability of cyclo-DOPA, it is readily oxidized by tyrosinase to polymerize into melanin [24]. In betalain-producing plant cells, L-DOPA concentration remains constant, while L-tyrosine accumulates before betalain formation and is gradually consumed during the process [25]. In microbial fermentation systems lacking reductants, melanin rather than cyclo-DOPA accumulates, causing the culture to darken [26].

2.2 Betaxanthin Pathway for Betanidin Formation

An alternative route to betanidin proceeds through betaxanthin conversion. Tyrosine first condenses with betalamic acid to form tyrosine-betaxanthin, which is oxidized by tyrosinase to DOPA-betaxanthin. DOPA-betaxanthin can also form directly from L-DOPA and betalamic acid, and can be further oxidized to dopaquinone-betaxanthin, ultimately generating betanidin through a series of oxidation reactions. Although the reaction conditions remain undefined, this pathway is theoretically more suitable for in vivo implementation. DOPA-betaxanthin accumulation also requires reductants, and tissues with high DOPA-betaxanthin content typically contain elevated ascorbic acid levels [27].

2.3 Glycosylation of Betanidin

Betanin is the 5-O-glycosylated product of betanidin, catalyzed by glucosyltransferase using UDP-glucose. 5-O-glucosyltransferase (5GT) has been identified from beet, amaranth, and the Aizoaceae species *Dorotheanthus bellidiformis* L. Beet-derived Bv5GT shows the highest specificity, while 5GT from amaranth and *D. bellidiformis* can also catalyze flavonoid glycosylation [28]. Additionally, 6-O-glucosylation at the betanidin hydroxyl group produces gomphrenin I. The 6-O-glucosyltransferase from *D. bellidiformis* has been identified, with a sequence distinct from 5-O-glucosyltransferase, and these two enzymes function independently [29]. Another possible betanin biosynthetic route involves glucosylation of cyclo-DOPA by 5-O-cyclo-DOPA glucosyltransferase, followed by condensation with betalamic acid [30]. This enzyme activity has been confirmed in *Mirabilis jalapa* [31].

3 Plant Synthesis of Betaxanthins

Condensation of betalamic acid with various amines or amino acids generates different betaxanthins [32]. This step is considered spontaneous, involving nucleophilic addition of the amine/amino group to the aldehyde group of betalamic acid, followed by dehydration to form an imine [33,34]. The diversity of amines

and amino acids in plants makes it difficult to determine the exact number of natural betaxanthins. Differences in amino acid content among plants determine the predominant betaxanthin types: dopamine-betaxanthin and tyramine-betaxanthin are most abundant in *Mirabilis jalapa*, tyrosine-betaxanthin and glycine-betaxanthin dominate in *Portulaca grandiflora*, and proline-betaxanthin is primary in *Opuntia ficus-indica*. In beets, CYP76AD1 converts L-DOPA to cyclo-DOPA, which more readily condenses with betalamic acid to form betanidin that is subsequently glycosylated to betacyanins. Consequently, beets accumulate primarily betacyanins rather than betaxanthins. Betaxanthin glycosylation is extremely rare in nature; a glycosylated betaxanthin—DOPA-betaxanthin hexoside—was recently discovered in transgenic tobacco [35].

4.1 Betalain Synthesis in Non-Caryophyllales Plants

In nature, betalains are restricted to Caryophyllales plants that do not produce anthocyanins (e.g., beet, amaranth, gomphrena, prickly pear) and some higher fungi (*Amanita muscaria*, *Penicillium novae-zelandiae*) [36]. Biotechnology enables transgenic production of betalains beyond species boundaries, overcoming the barrier between anthocyanin and betalain pathways. Recent achievements in non-Caryophyllales betalain synthesis are summarized in Table 2 .

Expression of the fungal *Amanita muscaria* DOPA dioxygenase gene AmDODA in *Arabidopsis* enabled betalain detection when L-DOPA was supplied exogenously [37]. Expression of the shiitake mushroom tyrosinase gene LeTYR and *Mirabilis jalapa* DOPA dioxygenase gene MjDODA in tobacco and *Arabidopsis* suspension cells produced betaxanthins (primarily proline-betaxanthin) without substrate feeding, confirming that anthocyanin-producing plants outside Caryophyllales can synthesize betaxanthins when equipped with tyrosinase and L-DOPA [38].

Simultaneous expression of BvCYP76AD1, BvDODA, and MjcDOPA5GT in model plant tobacco and economic crops eggplant, tomato, and potato yielded total betacyanin contents of 0.33, 0.25, 0.12, and 0.07 mg/g fresh weight, respectively [35]. Betacyanin production also altered plant coloration: transformation of white petunia with these three genes produced pale purple flowers, demonstrating that this gene combination can modify floral color. Since BvCYP76AD6 possesses only tyrosine hydroxylation activity, its co-expression with the three-gene set in transgenic tobacco generated both betacyanins and betaxanthins, shifting flower color from red to orange-red. In contrast, the BvDODA and CYP76AD6 combination produced only betaxanthins, resulting in yellow flowers. Notably, transgenic tobacco showed significantly enhanced resistance to *Botrytis cinerea* [39]. These modifications not only improve crop nutritional value but also enhance disease resistance, reducing pesticide usage for safer, pollution-free agriculture. Plant synthetic biology for betalain production opens new possibilities for developing ornamental varieties and enhancing crop economic value.

Table 2 Synthesis of betalains from non-Caryophyllales plants

Host Plant & Culture Method	Introduced Genes (Source)	Product & Content (mg/g fresh weight)
Tobacco, cell suspension	TYR (shiitake), DODA (*Mirabilis*), CYP76AD1 (beet)	Betaxanthin 1.02
Tobacco, cell suspension	DODA (beet)	Betanin 0.05
Tobacco, cell suspension	cDOPA5GT (*Mirabilis*), CYP76AD6 (beet), DODA (beet), CYP76AD1 (beet)	Betaxanthin 0.1
Tobacco, callus culture	DODA (beet), cDOPA5GT (*Mirabilis*), CYP76AD1 (beet)	Betanin 0.33
Eggplant, callus culture	DODA (beet), cDOPA5GT (*Mirabilis*), CYP76AD1 (beet)	Betanin 0.25
Tomato, callus culture	DODA (beet), cDOPA5GT (*Mirabilis*), CYP76AD1 (beet)	Betanin 0.12
Potato, callus culture	DODA (beet), cDOPA5GT (*Mirabilis*), CYP76AD1 (beet)	Betanin 0.07

4.2 Microbial Synthesis of Betalains

Yeast is a safe and beneficial microorganism for human applications. Expression of beet-derived BvDODA and BvCYP76AD1 in yeast successfully produced betanidin when L-DOPA was supplied [22]. Since tyrosinase possesses both hydroxylation and oxidation activities, a CYP76AD1 mutant library was screened to identify the double mutant CYP76AD1(W13L,F309L). Expression of this mutant in yeast enhanced tyrosine hydroxylation activity while reducing DOPA oxidation activity, increasing L-DOPA production 2.8-fold to approximately 3.6 mg/L compared to wild-type. Co-expression of the double mutant with BvDODA generated 2.7-fold more betaxanthins than wild-type, demonstrating a strong linear correlation between L-DOPA and betaxanthin accumulation [40]. This directed enzyme modification provides new insights for specialized betalain synthesis.

De novo microbial synthesis of betanin from glucose was achieved in *Saccharomyces cerevisiae*. Co-expression of codon-optimized BvCYP76AD1(W13L) and MjDODA with either MjcDOPA5GT or DbBetanidin5GT produced 16.8 mg/L and 10.4 mg/L betanin, respectively, indicating that cyclo-DOPA glycosylation is more favorable than betanidin glylycosylation for betanin production. Co-expression of MjDODA and BvCYP76AD5 in yeast with various amine substrates generated different colored betalains, including o-dianisidine-betaxanthin that exhibited blue-purple coloration—the most red-shifted non-natural betalain discovered to date. This study not only expanded the betalain color spectrum but also provided a reference for microbial production of diverse betalain pigments [41].

In *E. coli*, recombinant MjDODA protein was induced, purified, and used to establish an in vitro reaction system with various amino acids or amines, synthesizing 24 different betaxanthins. The condensation efficiency of betalamic acid varied among amino acids, with histidine-betaxanthin and arginine-betaxanthin showing the highest yields [42]. In vitro synthesis of various betaxanthins facilitates deeper understanding of betalain formation mechanisms, enables screening of suitable catalytic enzymes, and provides technology for microbial production of diversified betalain pigments.

Currently, most betalain products are extracted from plants, with edible betacyanins limited to beet and amaranth sources. Synthetic biology strategies enable betalain production beyond Caryophyllales, allowing efficient manufacturing of natural and non-natural betalain pigments in model plants. Accumulation of these pigments enhances plant nutritional value and pathogen resistance. Future plant-based betalain synthesis should focus on: (1) Increasing betacyanin content, as the current 0.76 mg/g fresh weight in beets offers substantial improvement potential. Synthetic biology can be used to balance beetroot growth and betalain accumulation for constructing high-yield plant factories specialized in betalain production. (2) The cytochrome P450 genes BvCYP76AD1 and BvCYP76AD6 regulate red/yellow betalain ratios. Expressing these P450 genes with different strengths and combinations in floral or leaf tissues of ornamental plants can generate varying red-yellow pigment ratios to alter color phenotypes. Additionally, engineering endogenous metabolic pathways to produce special amino acids or amines could expand the color palette for horticultural plants. (3) Although anthocyanins and betalains do not naturally coexist, introducing betalain pathways into anthocyanin-producing plants through synthetic biology could enhance stress resistance and nutritional value by producing both pigment types.

Compared with plant systems, microbial engineering using *E. coli* and *S. cerevisiae* offers greater convenience and has become an important host for plant secondary metabolite synthesis [43]. Although betalain synthesis has been reported in yeast, low activity and poor specificity of heterologously expressed enzymes limit yield improvement. Particularly, cytochrome P450 enzymes catalyzing tyrosine hydroxylation and L-DOPA oxidation are difficult to function-

ally express in prokaryotes, explaining the absence of betalain synthesis reports in *E. coli*. Future research should focus on: (1) Mining functional genes suitable for microbial expression. While many Caryophyllales plants and some fungi accumulate betalains, identified biosynthetic genes remain limited. Integrating multi-omics data with enzyme activity assays may identify novel pathway genes, elucidate mechanisms of protein-substrate interactions, and enable rational protein engineering for higher catalytic activity and substrate specificity. (2) For P450 expression in microbes, identifying compatible reductases is essential. These oxidoreductases often form inactive inclusion bodies when membrane-bound. Secondary structure prediction can identify membrane-binding domains for truncation, followed by construction of fusion proteins. This strategy has successfully achieved soluble P450-reductase fusion expression in *E. coli* [44]. P450 engineering to switch from NADPH- to NADH-dependence could enhance utilization of *E. coli* NADH reducing power. (3) Microbial fermentation conditions significantly affect betalain yields. In plants, betalains accumulate stably in vacuoles, whereas microbes lack such compartments, causing excessive L-DOPA oxidation to useless melanin byproducts. Additionally, the condensation mechanism between betalamic acid and cyclo-DOPA or various amines remains unclear, representing another limitation. Combining metabolic engineering to regulate intracellular redox status with optimization of fermentation temperature, pH, and reductant addition can establish rational processes that promote betalamic acid conversion under conditions optimal for both cell growth and product formation, thereby increasing final betalain yields.

References

[1] Khan M I, Giridhar P. Plant betalains: Chemistry and biochemistry [J]. Phytochemistry, 2015, 117(1): 267-295.
[2] Ninfali P, Antonini E, Frati A, et al. C-Glycosyl Flavonoids from Beta vulgaris Cicla and Betalains from Beta vulgaris rubra: Antioxidant, Anticancer and Antiinflammatory Activities-A Review [J]. Phytotherapy Research: PTR, 2017, 31(6): 871-884.
[3] Stafford H A. Anthocyanins and betalains: evolution of the mutually exclusive pathways [J]. Plant Science, 1994, 101(2): 91-98.
[4] Steiner U, Schliemann W, Böhm H, et al. Tyrosinase involved in betalain biosynthesis of higher plants [J]. Planta, 1999, 208(1): 114-124.
[5] Gandia-Herrero F, Garcia-Carmona F, Escribano J. Purification and characterization of a latent polyphenol oxidase from beet root (Beta vulgaris L.) [J]. Journal of Agricultural and Food Chemistry, 2004, 52(3): 609-615.
[6] Gao Z-J, Han X-H, Xiao X-G. Purification and characterisation of polyphenol oxidase from red Swiss chard (Beta vulgaris subspecies cicla) leaves [J]. Food Chemistry, 2009, 117(2): 342-348.
[7] Wang C-Q, Song H, Gong X-Z, et al. Correlation of tyrosinase activity and betacyanin biosynthesis induced by dark in C3 halophyte Suaeda salsa seedlings [J]. Plant Science, 2007, 173(5): 487-494.
[8] Mueller L A, Hinz U, Zrÿd J-P. Characterization of a tyrosinase from

Amanita muscaria involved in betalain biosynthesis [J]. Phytochemistry, 1996, 42(6): 1511-1515.
[9] Chen T S, Xiu Y W, Wang Q, et al. Enzymatic Characteristics of Tyrosinase in Euhalophyte Suaeda salsa [J]. Plant Physiology Journal, 2011, 47(10): 1017-1023.
[10] Jiratanan T, Liu R H. Antioxidant activity of processed table beets (Beta vulgaris var, conditiva) and green beans (Phaseolus vulgaris L.) [J]. Journal of Agricultural and Food Chemistry, 2004, 52(9): 2659-2670.
[11] Teng X L, Chen N, Xiao X G. Identification of a Catalase-Phenol Oxidase in Betalain Biosynthesis in Red Amaranth (Amaranthus cruentus) [J]. Frontiers in Plant Science, 2016, 6(1).
[12] Lipscomb J D. Mechanism of extradiol aromatic ring-cleaving dioxygenases [J]. Current Opinion in Structural Biology, 2008, 18(6): 644-649.
[13] Christinet L. Characterization and Functional Identification of a Novel Plant 4,5-Extradiol Dioxygenase Involved in Betalain Pigment Biosynthesis in Portulaca grandiflora [J]. Plant Physiology, 2004, 134(1): 265-274.
[14] Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids [J]. The Plant Journal: for Cell and Molecular Biology, 2008, 54(4): 733-749.
[15] Sasaki N, Abe Y, Goda Y, et al. Detection of DOPA 4,5-Dioxygenase (DOD) Activity Using Recombinant Protein Prepared from Escherichia coli Cells Harboring cDNA Encoding DOD from Mirabilis jalapa [J]. Plant Cell Physiol, 2009, 50(5): 1012-1016.
[16] Gandia-Herrero F, Garcia-Carmona F. Characterization of recombinant Beta vulgaris 4,5-DOPA-extradiol-dioxygenase active in the biosynthesis of betalains [J]. Planta, 2012, 236(1).
[17] Chung H H, Schwinn K E, Ngo H M, et al. Characterisation of betalain biosynthesis in Parakeelya flowers identifies the key biosynthetic gene DOD as belonging to an expanded LigB gene family that is conserved in betalain-producing species [J]. Frontiers in Plant Science, 2015, 6(1).
[18] Gandia-Herrero F, Garcia-Carmona F. Escherichia coli protein YgiD produces the structural unit of plant pigments betalains: characterization of a prokaryotic enzyme with DOPA-extradiol-dioxygenase activity [J]. Applied Microbiology and Biotechnology, 2014, 98(3).
[19] Li J, Christensen B M. Identification of Products and Intermediates During L-Dopa Oxidation to Dopachrome Using High Pressure Liquid Chromatography with Electrochemical Detection [J]. Journal of Liquid Chromatography, 1993, 16(5): 1117-1133.
[20] Riley P A. Tyrosinase kinetics: a semi-quantitative model of the mechanism of oxidation of monohydric and dihydric phenolic substrates [J]. Journal of Theoretical Biology, 2000, 203(1).
[21] Schliemann W, Steiner U, Strack D. Betanidin formation from dihydroxyphenylalanine in a model assay system [J]. Phytochemistry, 1998, 49(6): 1593-1598.
[22] Hatlestad G J, Sunnadeniya R M, Akhavan N A, et al. The beet R locus encodes a new cytochrome P450 required for red betalain production [J].

Nature Genetics, 2012, 44(7): 816-820.
[23] Sunnadeniya R, Bean A, Brown M, et al. Tyrosine Hydroxylation in Betalain Pigment Biosynthesis Is Performed by Cytochrome P450 Enzymes in Beets (Beta vulgaris) [J]. PloS One, 2016, 11(2): 417-432.
[24] Land E J, Riley P A. Spontaneous Redox Reactions of Dopaquinone and the Balance between the Eumelanic and Phaeomelanic Pathways [J]. Pigment Cell Research, 2000, 13(4): 273-277.
[25] Kishima Y, Suiko M, Adachi T. Betalain Pigmentation in Petal of Portulaca is Preceded by a Dramatic Tyrosine Accumulation [J]. Journal of Plant Physiology, 1991, 137(4): 505-506.
[26] Toivonen P M A, Brummell D A. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables [J]. Postharvest Biol Tec, 2008, 48(1): 1-14.
[27] Gandia-Herrero F, Escribano J, Garcia-Carmona F. Betaxanthins as substrates for tyrosinase. an approach to the role of tyrosinase in the biosynthetic pathway of betalains [J]. Plant Physiology, 2005, 138(1): 421-432.
[28] Das S S, Gauri S S, Misra B B, et al. Purification and characterization of a betanidin glucosyltransferase from Amaranthus tricolor L catalyzing non-specific biotransformation of flavonoids [J]. Plant Science: an International Journal of Experimental Plant Biology, 2013, 211(3): 61-69.
[29] Vogt T. Substrate specificity and sequence analysis define a polyphyletic origin of betanidin 5- and 6-O-glucosyltransferase from Dorotheanthus bellidiformis [J]. Planta, 2002, 214(3): 492-495.
[30] Sasaki N, Adachi T, Koda T, et al. Detection of UDP-glucose:cyclo-DOPA 5-O-glucosyltransferase activity in four o' clocks (Mirabilis jalapa L.) [J]. FEBS Letters, 2004, 568(1-3): 159-162.
[31] Sasaki N, Abe Y, Wada K, et al. Amaranthin in feather cockscombs is synthesized via glucuronylation at the cyclo-DOPA glucoside step in the betacyanin biosynthetic pathway [J]. Journal of Plant Research, 2005, 118(6): 439-442.
[32] Gandia-Herrero F, Escribano J, Garcia-Carmona F. Structural implications on color, fluorescence, and antiradical activity in betalains [J]. Planta, 2010, 232(2): 449-460.
[33] Schliemann W, Kobayashi N, Strack D. The decisive step in betaxanthin biosynthesis is a spontaneous reaction [J]. Plant Physiology, 1999, 119(4): 1217-1232.
[34] Godoy-Alcantar C, Yatsimirsky A K, Lehn J M. Structure-stability correlations for imine formation in aqueous solution [J]. J Phys Org Chem, 2005, 18(10): 979-985.
[35] Polturak G, Breitel D, Grossman N, et al. Elucidation of the first committed step in betalain biosynthesis enables the heterologous engineering of betalain pigments in plants [J]. The New Phytologist, 2016, 210(1): 269-283.
[36] Wang H, Li Y, Zhang K, et al. Feasibility and transcriptomic analysis of betalain production by biomembrane surface fermentation of Penicillium novae-zelandiae [J]. AMB Express, 2018, 8(1).
[37] Harris N N, Javellana J, Davies K M, et al. Betalain production is

possible in anthocyanin-producing plant species given the presence of DOPA-dioxygenase and L-DOPA [J]. BMC Plant Biology, 2012, 12(1): 34-45.
[38] Nakatsuka T, Yamada E, Takahashi H, et al. Genetic engineering of yellow betalain pigments beyond the species barrier [J]. Scientific Reports, 2013, 3(6): 1970-1976.
[39] Polturak G, Grossman N, Vela-Corcia D, et al. Engineered gray mold resistance, antioxidant capacity, and pigmentation in betalain-producing crops and ornamentals [J]. Proceedings of the National Academy of Sciences of the United States of America, 2017, 114(34): 9062-9067.
[40] Deloache W C, Russ Z N, Narcross L, et al. An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose [J]. Nature Chemical Biology, 2015, 11(7): 465-471.
[41] Grewal P S, Modavi C, Russ Z N, et al. Bioproduction of a betalain color palette in Saccharomyces cerevisiae [J]. Metabolic Engineering, 2018, 45(1): 180-188.
[42] Sekiguchi H, Ozeki Y, Sasaki N. In vitro synthesis of betaxanthins using recombinant DOPA 4,5-dioxygenase and evaluation of their radical-scavenging activities [J]. Journal of Agricultural and Food Chemistry, 2010, 58(23): 12504-12509.
[43] Luo Y, Li B Z, Liu D, et al. Engineered biosynthesis of natural products in heterologous hosts [J]. Chemical Society Reviews, 2015, 44(15): 5265-5290.
[44] Leonard E, Yan Y, Koffas M A. Functional expression of a P450 flavonoid hydroxylase for the biosynthesis of plant-specific hydroxylated flavonols in Escherichia coli [J]. Metabolic Engineering, 2006, 8(2): 172-181.

Note: Figure translations are in progress. See original paper for figures.