New insights into the regulation of anthocyanin biosynthesis in fruits

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Anthocyanins are important health-promoting pigments that make a major contribution to the quality of fruits. The biosynthetic pathway leading to anthocyanins is well known and the key regulatory genes controlling the pathway have been isolated in many species. Recently, a considerable amount of new information has been gathered on the developmental and environmental regulation of anthocyanin biosynthesis in fruits, specifically the impact of regulation through light. New discoveries have begun to reveal links between the developmental regulatory network and the specific regulators of anthocyanin biosynthesis during fruit ripening. In this opinion article, a simplified model for the different regulatory networks involved with anthocyanin production in fruit is proposed.

Keywords: anthocyanin biosynthesis, fruits, regulation

Anthocyanins in fruits

Anthocyanins are the main pigments in flowers and fruits, generating the characteristic reddish, bluish and purple hues, thereby contributing to the quality of fruits. Anthocyanins are markers of ripening, because most fruits accumulate these compounds only in their ripening phase [1]. Anthocyanins are also recognized as compounds with potential health-benefits [2]. Anthocyanins belong to the flavonoid class of compounds, and structurally, they consist of anthocyanidin aglycon that is bound to one or more sugar moieties. Six anthocyanidins, namely cyanidin (Cy), delphinidin (Dp), pelargonidin (Pg), peonidin (Pn) petunidin (Pt), and malvidin (Mv) occur generally in fruits (Figure 1). Of these cyanidin is most common and can be found in over 82% of examined fruits and berries [3]. Delphinidins and its methylated derivatives, petunidins and malvidins are sources of dark bluish and purple colors, whereas cyanidins and pelargonidins are the main pigments in bright-red colored fruits [3,4]. There are also other factors such as co-pigmentation and pH that affect the perceived hue of a tissue [5]. Regarding anthocyanin production, fruits can be categorized into those which have anthocyanins both in their skin and flesh, those which have anthocyanins only in skin, and those that accumulate anthocyanins in their skin only as response of light stimulus. In some fruits, (e.g. apple [*Malus domestica*] and wine grape [*Vitis vinifera* L.]) all of the three types of varieties can be found. In the first two classes, the developmental regulation of has a major role in anthocyanin biosynthesis, whereas in the third, anthocyanin biosynthesis is more under environmental control. However, in all types of fruits, environmental factors affect the quantitative and qualitative composition of anthocyanins in the ripening fruit. In general, the genetic background of the species/variety determines which anthocyanins occur in fruit, yet environmental factors can affect the concentration of diverse anthocyanins in different ways [6]. Recently, considerable amount of new information has been achieved both on environmental and developmental regulation of anthocyanin biosynthesis in fruits. The transcription factors that directly regulate the expression of the structural genes of the pathway have been identified from many species. The understanding on the light mediated mechanism involved in the regulation of anthocyanin biosynthesis in fruits has increased markedly and links between ripening related anthocyanin accumulation and key regulatory genes controlling fruit development and ripening have been reported. However, many key players of the complicated regulatory network are yet to be identified. This article focuses on known mechanisms involved in the regulation of anthocyanin biosynthesis in fruits and seeks confluences between the regulatory factors in a form of simplified model.

Biosynthesis of anthocyanins

The flavonoid biosynthetic pathway via the phenylpropanoid pathway leading to anthocyanins is well known. The specific flavonoid pathway begins with the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA, which results in naringenin chalcone (Figure 2). This reaction is carried out by chalcone synthase (CHS), before the pathway diverges into side branches leading to different classes of flavonoids, including anthocyanins. Flavanone 3 hydroxylase (F3H), flavonoid 3' hydroxylase (F3'H), and flavonoid 3'5'hydroxylase (F3'5'H) can then direct the route towards cyanidin and delphinidin anthocyanidins. Anthocyanidins are converted from leucoanthocyanins by leucoanthocyanidin dioxygenase/anthocyanidin synthase (LDOX/ANS) and further glycosylated by UDP-glucose:flavonoid-O-glycosyltransferase (UFGT). *O*-methyltransferases (OMTs) catalyze the formation of *O*-methylated anthocyanins such as malvidin, peonidin and petunidin [7,8]. The enzymes involved in the flavonoid biosynthesis pathway are localized in the cytosol. After biosynthesis, flavonoids are transported to vacuoles or cell walls [7]. It has been suggested that the enzymes involved in flavonoid biosynthesis might be acting as a metabolon that influences on the overall efficiency, specificity and regulation of the pathway [9-11]. The individual enzymes also have an impact on the overall stability of the biosynthetic pathway, because the silencing, overexpression or heterologous expression of the single enzyme genes often leads to substantial changes in the flavonoid composition of the target tissue [12-14].

Transcriptional regulation of the anthocyanin biosynthesis

Flavonoid pathway genes are known to be coordinately induced, and transcription factors that directly regulate the expression of the structural genes of the pathway have been identified from a number of species. The regulation of the pathway occurs by the interaction of DNA-binding R2R3 MYB transcription factors and MYC-like basic helix-loop-helix (bHLH) and WD40-repeat proteins [15,16]. The expression pattern and the DNA-binding specificity of MYB proteins and, to some extent, bHLH proteins as well determine the subset of genes that are activated, whereas WD40 proteins seem to have a more general role in the regulatory complex [17]. In Arabidopsis (*Arabidopsis thaliana*) and grapevine, some flavonoid biosynthesis related MYB transcription factors that are activated without a bHLH partner have been identified [18,19].

Plant MYB proteins are involved in the regulation of the pathways of diverse secondary metabolites, signal transduction, developmental changes and disease resistance [15,20]. MYB genes contain structurally conserved, 100- 160 bp DNA-binding domain consisting of single or multiple repeats. The R2R3 MYB genes associated with the flavonoid pathway feature two repeats and represent the most abundant class of MYB genes in plants. In *Arabidopsis,* 126 members of R2R3 subfamily have been characterized, and of these, 13 members are related to the regulation of flavonoid metabolism [16,20]. Most of the MYBs involved in the control of the flavonoid biosynthesis are positive regulators that enhance the expression of the structural flavonoid pathway genes. However, repressors have also been characterized, such as FaMYB1 in strawberry (*Fragaria* x *ananassa* Duch.) and VvMYB4 in the berries of grapevine [16,21]. In fruits, particularly in grapevine, the regulation of flavonoid biosynthesis has been intensively studied and 14 flavonoid biosynthesis related R2R3 MYB family members have been described [16,22]. It is known that different R2R3-MYB family members can control separately the biosynthesis of the end products of different flavonoid pathway branches leading to anthocyanins, flavonols, and proanthocyanins [19,23]. The biosynthesis of anthocyanins in grapevine berries is regulated by VvMYBA1, and VvMYBA2, which are homologs of *Arabidopsis* AtMYB75, AtMYB90, AtMYB113, and AtMYB144 [24]. The homologs of these transcription factor genes have also been shown to be involved in the regulation of anthocyanin biosynthesis in apple (*MdMYBA*/*MdMYB1*/*MdMYB10*) and pear ([*Pyrus communis*], *PcMYB10*), and they have also been isolated from many other members of the rosaceous family [25,26] and some other species [27,28]. Of the allelic homologs, *MdMYB10* has been shown to control apple fruit flesh and foliage anthocyanin accumulation in addition to skin color in certain apple genotypes, whereas *MdMYBA1* and *MdMYBA* have been shown to be expressed in red parts of fruit skin in other genotypes [29,30,31]. A recent study revealed that *MdMYB110a*, a paralog of *MdMYB10*, regulates the anthocyanin accumulation in the red-flesh apple phenotype [32]. Furthermore, the chromosomal location of *MdMYB110a* was found to be consistent with a whole-genome duplication event characterized to occur during the evolution of apple [32]. Polymorphisms of *VvMYBA* family have been reported to be responsible for variation in anthocyanin content in different grapevine varieties [21, 33, 34].

The MYB proteins are believed to be key components in the allocation of specific gene expression patterns, whereas the bHLH proteins, other members of the regulatory complex, may have overlapping regulatory targets [23]. In fruits, bHLH proteins involved in flavonoid biosynthesis have been characterized in grapevine, apple and strawberry [17,35,36,37]. In grapevine, the bHLH transcription factor VvMYC1 was shown to interact with different MYB proteins (Vv MYB5a, VvMYB5b, VvMYBA1/A2, VvMYBPA1) to induce promoters of flavonoid pathway genes involved in biosynthesis of anthocyanins and proanthocyanidins [17]. Involvement of WD40 proteins in the regulatory complex of anthocyanin biosynthesis was first shown for the *Arabidopsis TRANSPARENT TESTA GLABRA*

1 (*TTG1*) locus [38], and since then homologs have been characterized from different fruit species, e.g. apple [39], grapevine [33], pomegranate [40] and strawberry [37].

Developmental regulation of anthocyanin biosynthesis

In many fruits, proanthocyanidins and flavonols are the major phenolic compounds at the beginning of fruit development, with the flavonoid pathway typically switching to the production of anthocyanins at the onset of ripening [4,41]. During this phenomenon, MYB-bHLH-WD40 transcription factor complexes control the transcriptional changes of the flavonoid biosynthesis genes. However, the signaling network behind the ripening-related anthocyanin biosynthesis is still poorly understood.

Plant hormones have a crucial role in the regulation of fruit development and ripening. Climacteric fruits, such as apples, plums (*Prunus domestica*) and pears, produce ethylene linked with respiratory burst at the onset of ripening [42,43]. It is also typical that the content of plant hormone abscisic acid (ABA) increases in fruits just before the ripening stage [44]. In climacteric fruits, maximum ABA contents have been found to precede the ethylene production [45]. In non-climacteric fruits, such as strawberry, grapevine and blueberry (*Vaccinium* spp.), only the peak in ABA production is detected at the onset of ripening [44,46,47]. Recent studies have shown that, in non-climacteric fruits, ABA is involved in the regulation of ripening related anthocyanin biosynthesis [48,49]. Evidence on the central role of ABA in the regulating ripening of non-climacteric fruit as well as accumulation of anthocyanins was demonstrated in strawberry when silencing of an ABA pathway gene, *FaNCED1,* resulted in decreased level of ABA and non-colored strawberry fruits. Furthermore, silencing of a putative ABA receptor also inhibited the ripening and anthocyanin accumulation of strawberries, which could not be rescued by application of exogenous ABA [49]. Jasmonates (JA) have been found to affect the color formation in apples and grapevine possibly via interaction of the ethylene biosynthesis [50,51]. Cytokinins have found to enhance light- and sugar-induced anthocyanin biosynthesis in *Arabidopsis* [52], but reports on the role of cytokinins in fruit ripening related anthocyanin biosynthesis are scarce. Endogenous application of auxin has found to retard anthocyanin accumulation in grapevine [44,53]. Also gibberellins can retard ripening process and ripening related accumulation of anthocyanin in fruits [54]. Plant hormones interact with MYB/bHLH/WD40 complexes either at the transcriptional or posttranscriptional level. In *Arabidopsis*, ethylene inhibits sugar- and photosynthesis induced anthocyanin accumulation by suppressing the expression of transcription factors with positive regulation of the MYB/bHLH/WD40 complex and stimulating the expression of negative R3-MYB regulator MYBL2 [55]. Considering the central role of ethylene in ripening of climacteric fruits, it would be of interest to clarify whether or not a similar mechanism is involved in the crosstalk between developmental and environmental regulation of anthocyanin biosynthesis. In *Arabidopsis*, jasmonates have been found to affect the anthocyanin accumulation via the interaction of negative regulators, jasmonate ZIM-domain (JAZ) proteins, with MYB/bHLH/WD40 complex transcription factors involved in anthocyanin biosynthesis [56].

In fruits, some key genes that are involved in the regulation of fruit ripening have been identified, many of which are *SEPALLATA*- and *SQUAMOSA*-class MADS box or SBP-box transcription factors [43,57,58]. This regulatory network appears to be conserved across fruit bearing species [58,59]. The links between the developmental regulatory factors and the downstream effectors involved in flavonoid biosynthesis have not yet been unraveled.

Although recent studies have revealed the roles of some of these transcription factors in the regulation of flavonoid biosynthesis. A link between ripening-related anthocyanin biosynthesis and SQUAMOSA-class MADS box transcription factor, *VmTDR4*, a homolog of *FRUITFULL* gene (*FUL*) in *Arabidopsis* and *TDR4* in tomato (*Solanum lycopersicum*) and was reported in bilberry (*Vaccinium myrtillus* L.) [60]. The results of the study showed that the expression levels of *VmTDR* were spatially and temporally consistent with anthocyanin accumulation in bilberry fruits and silencing of *VmTDR4* resulted in substantial reduction in anthocyanin biosynthesis. The expression of SEPALLATA-class MADS box transcription factor gene *PyMADS18*, was associated with anthocyanin accumulation in red and green forms of a pear cultivar [61]. In which way the developmental regulatory factors interact with MYB-bHLH-WD40 complexes, is not known.

Environmental regulation of anthocyanin biosynthesis

During the past few years the understanding of the molecular mechanism underlying the environmental regulation of the anthocyanin biosynthesis in fruits has also increased. Numerous studies have shown that light exposure can increase the concentration of anthocyanins especially in fruit skin and that shading of fruit can have the opposite effect [29,62]. In addition to light intensity, light quality also affects the biosynthesis of anthocyanins. Especially UV light but also other specific light qualities, e.g. blue light, has been associated with regulation of anthocyanin biosynthesis in fruits [63,64,65]. High temperatures (30°- 35°C) have been shown to decrease anthocyanin content in the skin of apple and grapevine berries [62,66,67]. Different temperature treatments have also been found to induce quantitative and/or qualitative changes in the anthocyanin profile of grapevine berries, apple and in bilberries [36,62,68]. The combination of a low temperature and light treatment is known to induce anthocyanin biosynthesis in plant leaves [69,70] in addition to stimulating rapid anthocyanin accumulation in the skin of apple and pear [71]. Moreover, excess of nitrogen fertilizer have been found to decrease the anthocyanin contents in fruits along with other phenolic compounds [72]. This phenomenon has been explained e.g. by the carbon/nutrient balance (CNB) hypothesis, which postulates that limited nitrogen availability leads to an increase in the availability of carbon and carbon based secondary metabolites.

Several recent studies have connected the effect of these environmental factors with the changes in the regulatory complex controlling anthocyanin biosynthesis in fruits [36,62,67,73]. It has been discovered that certain R2R3-MYB genes related to flavonoid biosynthesis in fruits react on light or other environmental stimuli in different manner [19,29,62]. The expression of three anthocyanin biosynthesis related MYB genes (*VlMYBA1-3*, *VlMYBA2*, *VlMYBA1-2*) varied greatly in the skin of grapevine berries kept in darkness or light in low (15 °C) or high (35 °C) temperature, whereas some other examined MYB genes (MYB5a, MYB5b) of the pathway did not react to the treatments [62]. Highest anthocyanin levels were measured in grapevine berries kept in 15°C + light and notable qualitative differences were detected between the treatments. Interestingly, highest ABA levels were detected in the same treatment, but the levels were relatively high also in 15°C + dark treatment, which indicates that ABA might have a role in temperature mediated control of flavonoid synthesis. In *Arabidopsis*, it has been reported that higher nitrogen concentration causes a decrease in the expression of *PAP1* and *TT8,* genes from TTG1-GL3/TT8-PAP1 (WD40-bHLH-MYB) anthocyanin biosynthesis regulatory complex and an increase in the expression of three lateral organ boundary domain genes (*LBD37*, *LBD38* and *LBD39*) that act as negative regulators of anthocyanin biosynthesis [74].

Recently, an important piece of the puzzle was reported for the mechanism by which light controls anthocyanin biosynthesis in fruits. The apple MdMYB1/MdMYBA transcription factor has been shown in earlier studies to be a positive regulator for light-controlled anthocyanin biosynthesis [25,29,30]. A new study found that MdMYB1 protein accumulates in light, but is degraded via ubiquitin-depended pathway in the dark [75]. They also demonstrated that MdMYB1 interacts directly with MdCOP1, which acts as a molecular switch of light-induced plant processes [75]. The ubiquititin E3 ligase CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) acts downstream of the light receptors and mediates the degradation of various photomorphogenesis-promoting transcription factors by the Ub-proteasome system. In darkness, COP1 is localized in the nucleus, where it interacts with the target transcription factors, such as ELONGATED HYPOCOTYL5 (HY5) and mediates their ubiquination and degradation via the 26S proteasome pathway [76]. HY5 is a bZIP transcription factor that has been linked to the activation of the *CHS* gene and the accumulation of flavonoids in response to light and UV-B radiation in *Arabidopsis* [77]. A study with apple skin suggested that UV-B irradiation would induce the accumulation of anthocyanins via the MdCOP1-mediated signaling leading to activation and binding of MdHY5 to the promoter regions of *MdMYB* genes [78].

Interaction between the regulatory networks

Detailed studies focusing on comparing the different regulation levels in anthocyanin biosynthesis in fruits have been scarce. One analysis has focused on the influence of genetic, developmental and environmental factors on biosynthesis of flavonoids in strawberry fruits by combining molecular and biochemical information [6]. The results showed that the variation of flavonoid levels, related gene expression, and enzyme activities followed a clear developmental pattern. Anthocyanin and flavan-3-ol levels were affected more by developmental stage and genotype, whereas flavonol levels and proanthocyanidin-related traits showed significant sensitivity to environmental effects. In bilberry, anthocyanin biosynthesis also appears to be under a strong genetic control, because the clones of the northernmost origins were shown to accumulate the highest levels of anthocyanins in comparison with the clones of more southern origin, in different temperature and day length conditions, both in controlled and in common garden experiments [68,79]. Bilberry is one of the best sources of anthocyanins and contains high levels of anthocyanins both in skin and in flesh. It is also a plant that prefers shade and its biosynthesis machinery can lead to high yields of anthocyanins without strong direct light exposure. Consequently, in these type of fruits, developmental regulation based on genotypic information is responsible for the gradually changing composition of phenolic compounds in the course of fruit development and ripening, which can lead - even in limited environmental conditions - to the ripe fruit with species and genotype specific anthocyanin profile. The role of environmental regulation is more to fine-tune the anthocyanin composition within the limits of genotype and developmental stage. In fruits that accumulate anthocyanins in skin only after light stimulus, the role of environmental control is naturally more prominent.

Figure 3 summarizes, in a simplified model, the information acquired from different regulatory networks related to anthocyanin biosynthesis in fruits. The understanding of the signaling mechanism behind the light-related anthocyanin biosynthesis in fruits has markedly increased in recent times. The induction of anthocyanin biosynthesis in low temperatures requires light, and therefore, is presumably regulated via the same mechanism as observed in *Arabidopsis* [66]. However, there is currently no clear evidence whether the observed changes in anthocyanin biosynthesis in fruits affected by other environmental factors are controlled via the COP1 or other ubiquitin-26S proteasome signaling system. The mechanisms behind the developmental regulation of anthocyanin biosynthesis in fruits are still poorly understood. Links between the development related MADS-box transcription factors and anthocyanin biosynthesis regulators have been observed, but the nature of interaction is not clear. Furthermore, the crosstalk between plant hormones, the fruit development related transcription factors and the downstream effectors, such as anthocyanin biosynthesis regulators, require more detailed investigation. The signaling network in crosstalk between environmental and developmental regulation is complex, and there is obviously great variation between different fruit species. The roles of the transcription factors in the flavonoid biosynthesis regulatory complex have been studied in greater depth, but many open questions still exist. The MYB transcription factors have been reported to activate or repress specific parts of the pathway and they also have different roles in development related and environmental factor inducible anthocyanin biosynthesis [15]. It can be presumed that the interplay between branch-specific activating and repressing MYBs has an important role in regulation of the pathway.

Concluding remarks and outlook

Anthocyanins contribute to the quality characteristics of fruits in a significant way and are therefore targets of many breeding programs. The composition of anthocyanins in ripe fruit is formed via the function of complicated metabolic networks regulated by genetic, developmental and environmental factors. Recent studies have markedly expanded the understanding of the mechanism of light-regulated anthocyanin biosynthesis in fruits that occurs via the COP1 signaling center. However, mechanisms related to other environmental factors are still in need of further clarification. Yet new insights have also been gained with regard to the contribution of fruit development-related transcription factors in anthocyanin biosynthesis, but more in-depth studies are required for an understanding of the nature of the interaction as well as crosstalk between other factors that control fruit development and ripening. The characterization and functional analyses of flavonoid biosynthesis-related MYB transcription factors and other members of MYB/bHLH/WD40 regulatory complexes in different fruit species and varieties are currently under active research. These studies will deepen our understanding of the role of interaction between these key players in the regulation of the pathway, which is likely to become one of the most interesting targets for future breeding work. The ongoing release of transcriptome and genome information will enable studies involving a wider range of fruit bearing species, which further widens our understanding on this topic.

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Box 1. Outstanding questions

- R2R3-MYB and bHLH transcription factors regulating the anthocyanin biosynthesis in fruits have been shown to response to environmental stimuli in different manner. How conservative these traits are across the fruit species? What is the regulatory network and what are the mechanisms behind this phenomenon?
- COP1 mediated Ub-proteasome system has recently been shown to have a role in light depended regulation of anthocyanin biosynthesis in apple skin. Are there other mechanisms mediating the light stimulus in fruits? What are the mechanisms mediating the effect of the other environmental stimulus on biosynthesis of anthocyanins in fruits?
- Links between MADS-box transcription factors that regulate fruit development and ripening and anthocyanin accumulation have been shown recently. How do the developmental regulatory factors interact with MYB-bHLH-WD40 complexes?
- What are the mechanisms between crosstalk of plant hormones, the key transcription factors regulating fruit development and ripening and the MYB-bHLH-WD40 complexes regulating the ripening related accumulation of anthocyanins?

Figure legends

Figure 1. Chemical structures of the most common anthocyanidin aglycones in fruits. Cyanidin-glycosides are found in most fruits and they are the major anthocyanidins e.g. in red skinned apples. Dark colored fruits such as blueberries and certain grapevine berries can also contain delphinidin-, malvidin-, peonidin- and petunidin-glycosides. In strawberries, raspberries and cherries pelargonidin-glycosides are found in addition to cyanidin-glycosides.

Figure 2. A schematic presentation of the flavonoid biosynthetic pathway leading to anthocyanins. Enzyme abbreviations: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3' hydroxylase; F3'5'H, flavonoid 3'5'hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase/LDOX leucoanthocyanidin dioxygenase; UFGT, UDP glucose-flavonoid 3-o-glucosyl transferase; OMT, *O*-methyl transferase.

Figure 3. A simplified model of developmental and environmental regulation of anthocyanin biosynthesis in fruits. The transcription of structural anthocyanin biosynthesis genes is regulated by MYB/bHLH/WD40 complex, in which different MYBs can activate specific parts of the pathway and respond differentially on developmental and environmental cues. Plant hormones (auxin, ABA, ethylene, JA, GA) and certain MADS box transcription factors are key factors in development related regulation of anthocyanin biosynthesis. Environmental factors affect fruit ripening and anthocyanin biosynthesis. Light related control of anthocyanin biosynthesis occurs via light receptors that interact with ubiquitin E3 ligase

COP1 that ubiquitinates degradation multiple light-response effectors including HY5, or interact directly with certain anthocyanin biosynthesis related MYB transcription factors to induce transcription of structural pathway genes. Cold temperature can affect together with light response via the same signaling pathway, but it is not understood by which mechanism other observed temperature or environmental effects, such as nitrogen level, regulate the anthocyanin biosynthesis. ABA; abscisic acid; COP1, CONSTITUTIVE PHOTOMORPHOGENIC1; CRY, cryptochrome; GA, gibberellins; HY5, ELONGATED HYPOCOTYL5; JA, jasmonates; PHY, phytochrome (A, B), UVR8; UV RESISTANCE LOCUS; tfs, transcription factors.

Highlights

- Anthocyanin pigments make a major contribution on fruit quality
- R2R3-MYB genes controlling anthocyanin biosynthesis characterized from many fruit species
- Novel interactions between regulators of anthocyanin accumulation shown recently

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- R2R3-MYB and bHLH transcription factors regulating the anthocyanin biosynthesis in fruits have been shown to response to environmental stimuli in different manner. How conservative these traits are across the fruit species? What is the regulatory network and what are the mechanisms behind this phenomenon?
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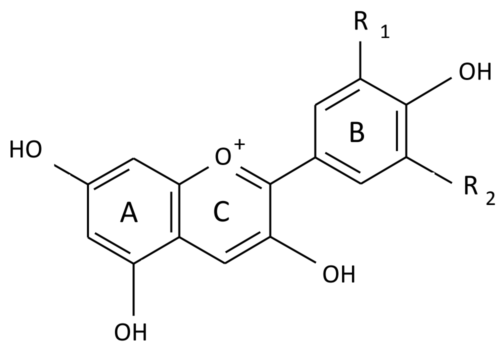


Anthocyanidins:
Pelargonidin $R_1 = R_2 = H$
Cyanidin $R_1 = OH$, $R_2 = H$
Delphinidin $R_1 = R_2 = OH$
Peonidin R_1 $OCH_3 = R_2 = H$
Petudinin R_1 $OCH_3 = R_2 = OH$
Malvidin $R_1 = R_2 = OCH_3$

Fig. 1

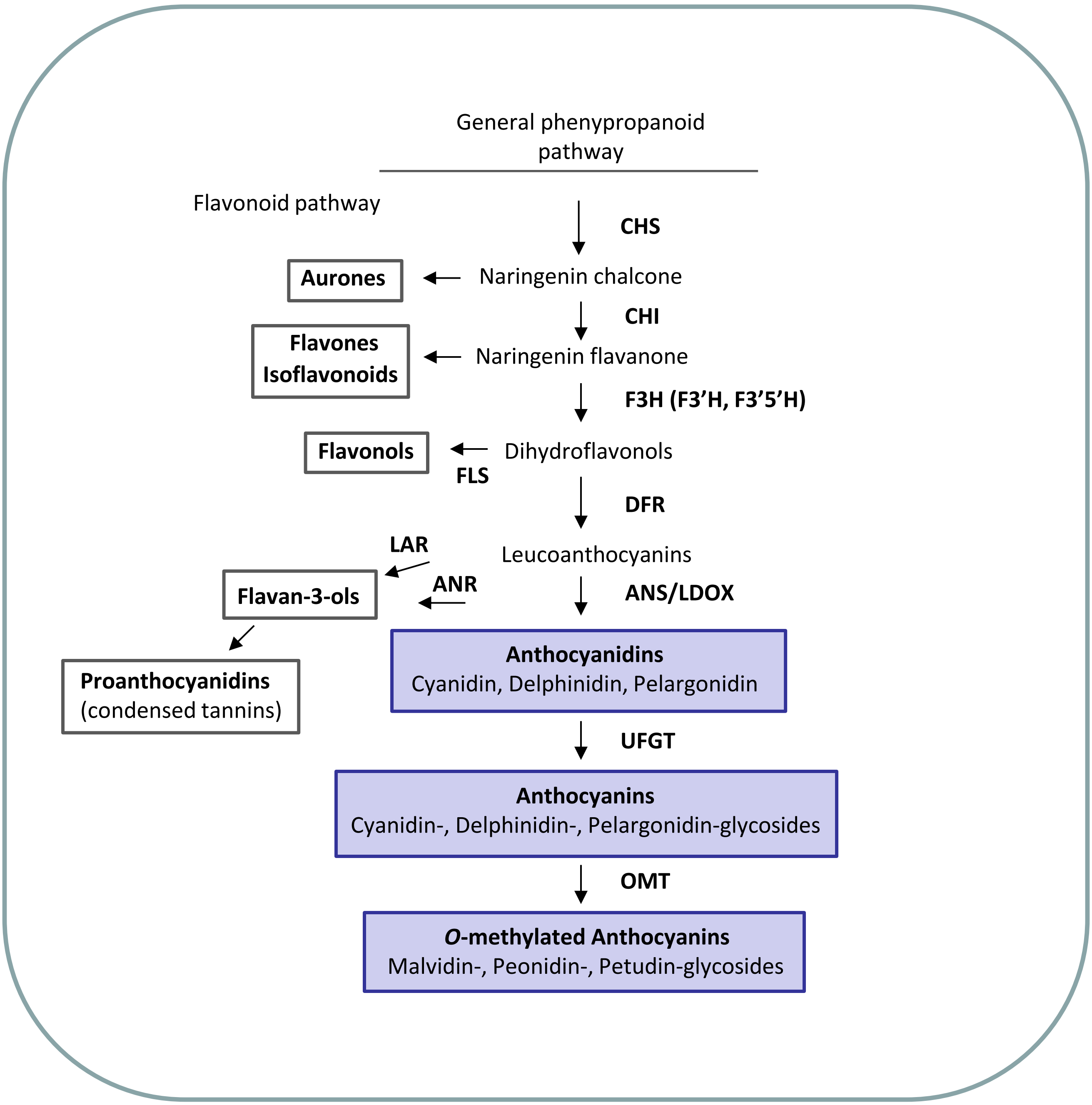
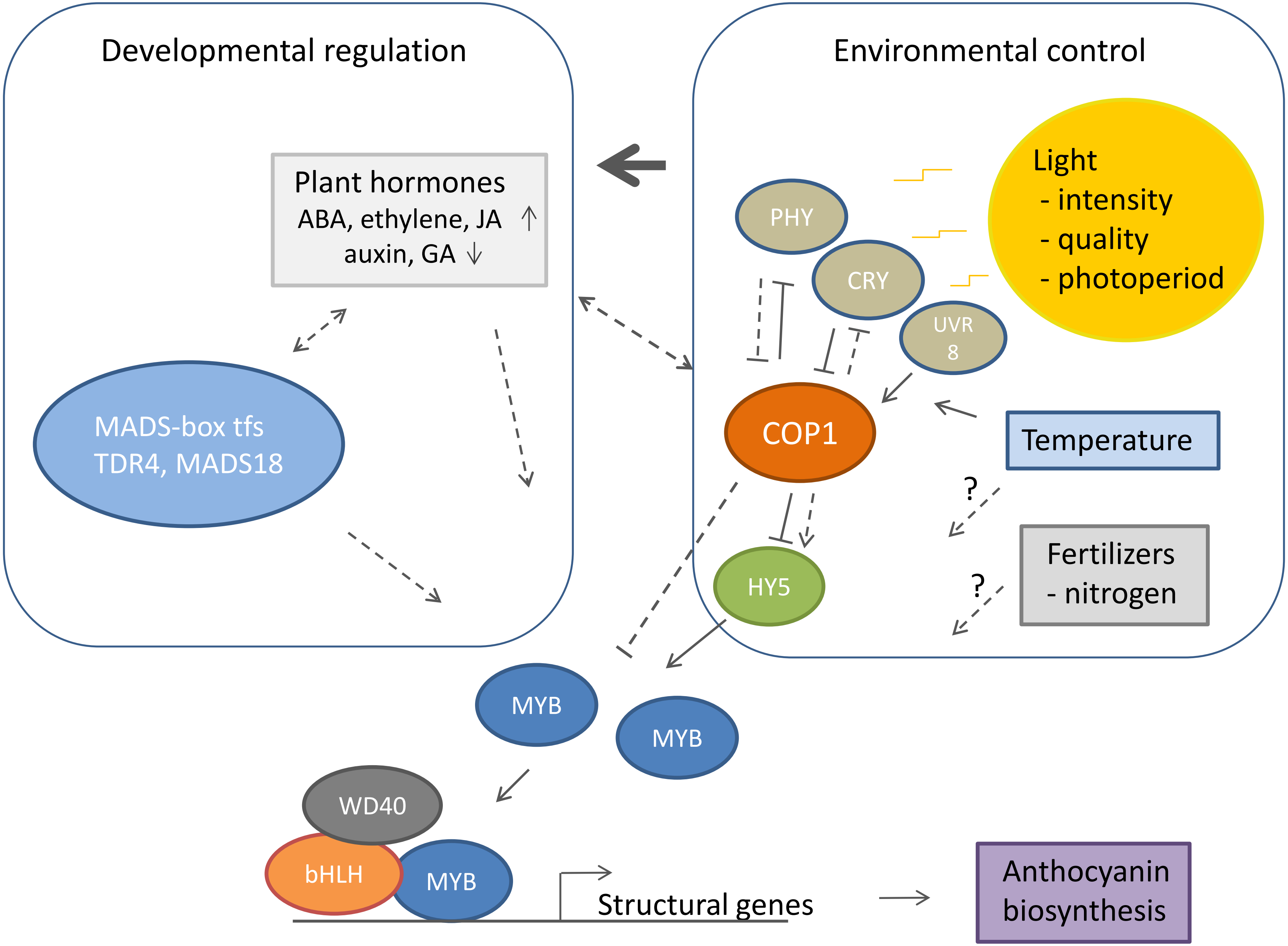

General phenypropanoid pathway
Flavonoid pathway
CHS
Aurones
Naringenin chalcone
CHI
Flavones
Isoflavonoids
Naringenin flavanone
F3H (F3'H, F3'5'H)
Flavonols
Dihydroflavonols
FLS
DFR
LAR
Leucoanthocyanins
Flavan-3-ols
ANR
ANS/LDOX
Proanthocyanidins
(condensed tannins)
Anthocyanidins
Cyanidin, Delphinidin, Pelargonidin
UFGT
Anthocyanins
Cyanidin-, Delphinidin-, Pelargonidin-glycosides
OMT
O-methylated Anthocyanins
Malvidin-, Peonidin-, Petudin-glycosides

Fig. 2



Developmental regulation
Environmental control
Plant hormones
ABA, ethylene, JA ↑
auxin, GA ↓
MADS-box tfs
TDR4, MADS18
PHY
CRY
UVR
8
Light
- intensity
- quality
- photoperiod
COP1
Temperature
?
HY5
Fertilizers
- nitrogen
?
MYB
MYB
WD40
bHLH
MYB
Structural genes
Anthocyanin
biosynthesis

Fig. 3.