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Research Article

Recent Advances in the Research on the Regulatory Mechanisms of Anthocyanin Synthesis in Apple Fruits

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Abstract

Anthocyanin accumulation determines peel pigmentation, commodity value and nutritional quality of apple fruit, and its biosynthesis is controlled by an intricate multi-layer regulatory network integrating external environmental cues, transcriptional cascades, epigenetic modification, post-transcriptional modulation and metabolic pathway crosstalk. Unfavorable coloring widely restricts the economic benefits of apple cultivation worldwide, and clarifying the regulatory rules of anthocyanin metabolism is essential for targeted quality improvement. This review systematically sorts out the latest research progress on apple anthocyanin biosynthesis pathways, and elaborates core regulatory mechanisms from four dimensions: structural gene transcriptional regulation centered on the MBW complex, environmental induction (light, temperature, water and nutrients), phytohormone synergistic/antagonistic regulation, and epigenetic modification including DNA methylation, histone modification and non-coding RNA regulation. On this basis, this paper summarizes existing bottlenecks in current research and proposes future research directions relying on multi-omics and gene-editing technology. The conclusion provides theoretical support for regulated coloring cultivation, molecular marker-assisted breeding and precision gene editing breeding of high-color-quality apple varieties.

Keywords

Apple, Anthocyanins, MBW Complex, Transcriptional Regulation

1. Introduction

Anthocyanins, as a crucial class of flavonoid compounds, play multiple biological roles in apple fruits. They form anthocyanidins by binding pigments to sugars via glycosidic bonds, primarily residing in the vacuoles of pericytic cells and imparting apples' vibrant red and purple colors—a characteristic essential for attracting pollinators and seed dispersers [1, 2]. Additionally, anthocyanins exhibit significant antioxidant activity by scavenging free radicals, contributing to the prevention of chronic diseases such as cardiovascular disorders, thus serving as key indicators of fruit quality and consumer preference [2]. Within plants themselves, anthocyanin accumulation enhances resistance to stress conditions like low temperatures and fungal diseases. Studies demonstrate that MdMYB2 and MdSIZ1 in apples promote anthocyanin synthesis while improving cold tolerance [3]; infection by the apple rust pathogen Gymnosporangium yamadae induces anthocyanin accumulation in leaves to combat disease [4]. Consequently, anthocyanins are not only determinants of apple appearance quality but also vital secondary metabolites enabling plant adaptation and health maintenance. Therefore,

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increasing anthocyanin content holds significant importance for improving germplasm quality. At present, inconsistent fruit coloring, poor coloring uniformity and low coloring degree have become prominent restrictive factors limiting the economic benefits of major apple producing areas, and the regulatory network difference among different cultivars has not been fully summarized systematically [5].

With the continuous advancement of modern molecular biology techniques, particularly the widespread application of methods such as genome sequencing, mutagenesis, and mutant analysis, a comprehensive understanding has been achieved of the molecular mechanisms underlying the biosynthetic pathway of anthocyanins in apple fruits. In recent three years, an increasing number of studies have focused on the crosstalk between epigenetic modification and hormone signaling in regulating fruit pigmentation, revealing many new regulatory small-molecule RNAs and transcription factor modules [6]. This paper systematically reviews the regulatory mechanisms governing anthocyanin synthesis in apples, providing significant theoretical insights and practical value for elucidating fruit coloring mechanisms and guiding the breeding of high-quality apple varieties as well as cultivation optimization strategies.

2. Synthesis of Anthocyanins in Apple Fruits

Anthocyanins, as the primary pigments responsible for the coloration of apple fruits, undergo a complex and highly regulated biosynthetic process that begins with the deamination of phenylalanine [8-10]. This core synthetic pathway involves the coordinated action of multiple key enzymes: first, phenylalanine deaminase catalyzes the deamination of phenylalanine to produce cinnamic acid, which is subsequently converted to 4-coumaroyl-CoA by 4-coumaroylase and 4-coumaroyl-CoA ligase [11, 12]. This intermediate condenses with malonyl-CoA under the catalysis of chalcone synthase to form chalcone, which is rapidly isomerized into flavanone by chalcone isomerase. Flavanone is then hydroxylated sequentially by flavanone 3-hydroxylase and flavonoid 3′-hydroxylase to generate dihydroflavanols; these enzymes exhibit substrate specificity, with F3′H preferentially hydroxylating the 3′ position of the B-ring [11-13]. dihydroflavanols are subsequently reduced by dihydromandelan-4-reductase to colorless anthocyanins, which are ultimately oxidized by anthocyanin synthase to form colored anthocyanins [12-14]. In apples, the expression of these structural genes is precisely regulated by various transcription factors, including the MBW complex composed of MYB, bHLH, and WD40, as well as positive regulators such as MdMYB1, MdMYB10, and MdPIF1, which directly bind to the promoter regions of genes like PAL, F3H, and ANS to promote anthocyanin accumulation [7, 16, 17].

After synthesis, anthocyanins require further enzymatic modifications such as glycosylation, methylation, and acylation to achieve stability and exhibit their characteristic coloration [1, 2, 18]. Glycosylation represents the most critical modification step, catalyzed by enzymes like UDP-glucose:anthocyanin 3-O-glucosyltransferase, which attaches sugar molecules to the 3′ or 5′ hydroxyl groups of anthocyanins, forming anthocyanidins. This process significantly enhances their water solubility and stability, while also influencing their subcellular localization within vacuoles [2, 14]. Methylation modifies the chromatic tone of anthocyanins, imparting red or blue hues, whereas acylation improves intramolecular covalent bonding capacity, protecting the pigment from water attack and thereby enhancing its stability in weakly acidic environments [18]. For instance, the glycosyltransferase encoded by the MdUFGT gene in apples serves as a key rate-limiting enzyme in anthocyanidin synthesis, with its expression directly activated by transcription factors such as MdMYB114 [14]. Additionally, the anthocyanin biosynthetic pathway features branching points: dihydropyranoflavones can be directed toward flavonol synthesis (catalyzed by flavonol synthase), while chalcones may branch into dihydrochardihydrochalcones (e.g., raphanin). Competitive regulation of these metabolic pathways also influences the net accumulation of anthocyanins [12, 13, 19]. External environmental factors such as light, temperature, and exogenous hormones (e.g., MeJA) can coordinate the expression of these modification enzymes and related regulatory genes through signaling networks (e.g., the MPK6-HY5 pathway and the ERF109-WER-JAZ2 module), thereby determining the intensity and uniformity of fruit red coloration [20-23]. Additionally, dynamic changes in DNA methylation status (e.g., methylation levels in the promoter region of MdMYB10) can persistently regulate the transcriptional activity of anthocyanin structural genes and modification enzymes, influencing the coloring pattern of the peel or flesh [11, 24, 25]. Thus, a systematic regulatory network spanning core synthetic enzymes to downstream modification enzymes collectively determines the accumulation efficiency, color stability, and ultimate commercial quality of apple fruits.

3. Transcriptional Regulation of Anthocyanins in Apple Fruits

3.1. Transcriptional Regulation of Structural Genes

In the regulatory network of anthocyanin biosynthesis in apple, transcriptional regulation of structural genes constitutes the pivotal factor determining fruit coloration. These structural genes—including MdCHS (encoding chalcone synthase), MdDFR (dihydroflavanol reductase), MdANS (anthocyanin synthase), and MdUFGT (uridine diphosphate-glucosylflavone-3-O-glucosyltransferase)—have promoter regions rich in various cis-regulatory elements that provide precise binding sites for transcription factors [26, 27]. Typical photoreceptor elements (e.g., G-boxes) are highly enriched at binding sites of MYB, bHLH, and WD40 transcription factors. For instance,

the photoperiod-responsive transcription factor MdPIF1 directly binds to G-box elements in the promoters of MdPAL and MdF3H, thereby activating their transcription to promote anthocyanin accumulation [15]. Similarly, MdHY5, as a key integrator of photoperiod signaling, not only initiates cascade reactions by binding to the MdMYB1 promoter but also directly inhibits the transcription of mdm-miR858, thereby relieving its targeted inhibition on MdMYB9 and MdMYBPA1, forming a precise regulatory loop of HY5-miR858-MYB [27]. Furthermore, multiple hormone response elements are also present in the promoters of structural genes: the gibberellin signaling pathway regulates the expression of genes such as MdDFR through the MdRGL2a-MdTCP46-MdMYB1 module [28]; the jasmonic acid signaling pathway influences the transcriptional activity of MdDFR and MdUF3GT via the antagonistic interaction between MdJAZ proteins and MdbHLH162 [29]; while 5-aminoacetopropane (ALA) induces the co-binding of transcription factors MdMADS1 and MdWRKY71 to the promoters of MdCHS, MdUFGT, MdANS, and MdDFR, forming a multiplex activation network [27]. The combination of these elements provides the molecular basis for the multi-layered integration of light, hormonal, and developmental signals, and also explains why the promoters of individual structural genes often simultaneously contain MYB binding sites, bHLH recognition sequences, and photoreceptor signaling elements [8, 30, 31].

The expression of structural genes exhibits strict temporal and spatial specificity, a pattern that determines the differential accumulation of anthocyanins across different developmental stages, tissues, and varieties. During fruit development, anthocyanin synthesis is typically suppressed in the juvenile stage, when chlorophyll and carotenoids dominate, resulting in green fruit coloration. As the fruit enters the color-changing and maturation phases, transcription levels of structural genes such as MdCHS, MdDFR, and MdANS rise sharply, accompanied by chlorophyll degradation, leading to rapid accumulation of red pigments [1, 32]. For example, following bag removal in 'Granny Smith' apples, cis elements in specific regions of the MdMYB1 promoter (-2026 to-1870 bp and-1062 to-964 bp) are activated, driving extensive expression of these structural genes and causing significant reddening of the fruit skin within days [33]. Expression differences among tissues are even more pronounced: MdPRE6-like genes exhibit significantly higher expression levels in flowers and fruit skin compared to roots and leaves; their encoded HLH-domain proteins effectively enhance transcription of MdCHI, MdF3'H, MdDFR, and MdUFGT, thereby promoting specific anthocyanin accumulation in the fruit skin [15, 16]. Among red-fleshed apple varieties, the expression patterns of structural genes exhibit flesh-specific characteristics. For instance, the expression level of mdm-miR858 in red-fleshed apples is significantly lower than that in white-fleshed varieties, leading to derepression of its target genes MdMYB9 and MdMYBPA1, thereby activating downstream structural genes [27]. Additionally, environmental factors significantly regulate spatio-temporal expression patterns: seasonal variations in light intensity and temperature induce circadian rhythmic expression of BBX family members (e.g., BBX1, BBX17), which bind to critical regions of the MdMYB10 promoter to activate structural genes during light periods and suppress them during darkness [34]. Nitrogen and potassium nutrient levels also play a regulatory role: high nitrogen treatment reduces the expression of MdPAL, Md4CL, MdF3H, MdANS, and MdUFGT, whereas increased potassium application partially alleviates this suppression by enhancing sugar transport and hormonal signaling [35]. Comparative studies between varieties reveal significant differences in promoter methylation levels of structural genes between red-skinned and green-skinned varieties. For example, CHH methylation in the MR3 region of the MdMYB1 promoter in 'Fuji' apples exhibits negative correlation with gene expression; this epigenetic marker is maintained via the RNA-directed DNA methylation (RdDM) pathway, resulting in expression disparities across different coloring lines [36]. In conclusion, the transcriptional regulation of structural genes results from the precise integration of light, hormonal, nutritional, and epigenetic signals across temporal and spatial dimensions. This multi-level regulatory mechanism ultimately determines the color differences between the skin and flesh of apple fruits throughout their development from juvenile to mature stages, and provides clear targets for improving fruit appearance quality through molecular breeding [37-39].

3.2. MYB Transcription Factor

The biosynthesis of anthocyanins in apple fruits is governed by a complex transcriptional regulatory network, in which MYB-class transcription factors play a central role. In terms of positive regulation, MdMYB1, MdMYB10, and MdMYB110a have been identified as key genes controlling the red coloration of both the peel and flesh, promoting pigment accumulation by directly activating the expression of structural genes involved in the anthocyanin synthesis pathway [36]. For example, MdMYB114, as an R2R3-MYB transcription factor, directly binds to the promoters of genes such as MdANS, MdUFGT, and MdGST, thereby enhancing anthocyanin biosynthesis and transport; its activity itself is positively regulated by the upstream bZIP transcription factor MdbZIP4-like [12]. Additionally, light-signaling-induced MdWRKY75 can activate the expression of this core MYB family member by binding to the promoter of MdMYB1, further promoting anthocyanin accumulation [14]. Notably, negative regulatory mechanisms also exist for MYB transcription factors: miR172, for instance, indirectly attenuates the positive regulatory effect of MdAP2_1a on MdMYB10 by suppressing the expression of its target gene MdAP2_1a, thereby reducing anthocyanin synthesis [6]; similarly, in other species such as grapes, VdMYB14 acts as a negative regulator that

inhibits anthocyanin synthesis and promotes proanthocyanidin accumulation, suggesting that analogous negative regulatory MYB factors may also exist in apples [9]. Furthermore, the DNA methylation status also influences the activity of MYB genes; for example, low methylation at mCHG in the upstream region of MdMYB10 promotes transcriptional activation, thereby facilitating coloration [9].

3.3. MBW Complex

The bHLH and WD40 proteins collaboratively regulate anthocyanin synthesis by forming an MBW (MYB-bHLH-WD40) complex with the MYB transcription factor. In apples, bHLH transcription factors such as MdbHLH3, MdbHLH33, and MdPIF1 interact with R2R3-MYB proteins and the WD40 repeat protein MdTTG1 to form a ternary complex [7, 17]. This complex efficiently binds to specific cis-element sites (e.g., G-boxes) in the promoter regions of anthocyanin structural genes (e.g., MdPAL, MdF3H, MdDFR), thereby activating their transcription and driving anthocyanin accumulation in vacuoles. For instance, MdPIF1 directly binds to G-box elements in the promoters of MdPAL and MdF3H, promoting their expression [13]. The activity of the MBW complex is subject to multi-layered regulation: on one hand, light signals activate the MdMPK6 kinase, which phosphorylates the bZIP factor MdHY5, enhancing its stability and binding affinity to target genes, thus indirectly strengthening MBW-mediated transcriptional activation [18]; on the other hand, epigenetic modifications such as DNA methylation can influence bHLH gene expression—for example, high methylation of the upstream region of bHLH74 leads to transcriptional suppression, impairing anthocyanin accumulation [9]. Furthermore, the MBW complex itself is subject to feedback regulation by other factors; for instance, the accumulation of flavonoid synthesis intermediates can affect the stability of the complex [17].

3.4. Other Transcription Factors

In addition to the core components MYB, bHLH, and WD40, several other transcription factor families also participate in the precise regulation of anthocyanin synthesis through indirect mechanisms. ERF-class transcription factors play critical roles in responding to light and hormonal signals: MdERF109 not only directly binds to the promoters of anthocyanin structural genes but also forms a protein complex with MdWER (a MYB-related factor), synergistically enhancing anthocyanin accumulation; furthermore, MdWER interacts with the anthocyanin inhibitor MdJAZ2, contributing to the integrated regulation of methyl jasmonate (MeJA) and light signals [19]. Additionally, the expression of MdERF109 is induced by the long non-coding RNA MdLNC499, which is itself regulated by the light-activated transcription factor MdWRKY1, forming a transcriptional cascade of MdWRKY1–MdLNC499–MdERF109 [39]. Members of the bZIP family serve as key nodes at the intersection of light and hormonal signaling: MdHY5 exhibits increased stability after phosphorylation by the light-activated MdMPK6, enabling it to bind to target gene promoters and promote anthocyanin synthesis [18]; MdbZIP43 positively regulates anthocyanin accumulation by upregulating the expression of structural genes such as CHI, F3'H, DFR, and UFGT [13]. The NAC transcription factor is also differentially expressed during fruit maturation and may interact with hormonal signals such as ethylene and abscisic acid to indirectly influence anthocyanin synthesis [40]. WRKY factors such as MdWRKY1 act as upstream activators that not only regulate MdLNC499 but also participate in light response [39]; whereas MdWRKY75 directly activates the expression of core MYB genes [14]. These non-classical regulatory factors form a complex network with the core regulatory module by influencing the assembly, activity, or upstream signaling pathways of the MBW complex, enabling apple fruits to precisely regulate the spatiotemporal accumulation of anthocyanins in response to internal and external signals such as light, temperature, hormones, and developmental stages [2, 24].

4. Regulation of Environmental Factors on Anthocyanin Synthesis in Apple Fruits

The regulation of environmental factors on anthocyanin synthesis in apple fruits is a complex, multi-level process that involves the perception and integration of various external signals such as light, temperature, moisture, and nutrient elements. These signals ultimately influence fruit color quality through sophisticated molecular networks [36, 41].

4.1. Light Exposure

Light is recognized as the primary environmental factor that triggers and promotes anthocyanin accumulation, with its effects depending not only on the presence or absence of light but also on multiple dimensions including light quality, intensity, and photoperiod [18, 24]. At the molecular level, light signals are first detected by various photoreceptors—including photosensitizers, cryptochromes, and the UV-B receptor UVR8—which promptly initiate a cascade of phosphorylation reactions [18]. Studies have demonstrated that the bZIP transcription factor MdHY5 in apples undergoes rapid phosphorylation under light exposure; this phosphorylation depends on direct interaction with the mitogen-activated protein kinase MdMPK6, thereby enhancing the stability of phosphorylated MdHY5 and enabling more efficient binding to and activation of downstream genes involved in anthocyanin synthesis, highlighting the pivotal role of the MdMPK6-MdHY5 phosphorylation pathway in light-induced coloration [18]. Additionally, light signals activate the MdWRKY1 transcription factor, inducing the expression of the long non-coding RNA

MdLNC499, which upregulates the ethylene response factor MdERF109, ultimately promoting the expression of anthocyanin-related structural genes and forming a regulatory cascade spanning from light signals to long non-coding RNAs to transcription factors [39]. Furthermore, light signals can regulate coloration through the miRNA-long non-coding RNA interaction network. For instance, MLNC3.2 and MLNC4.6 act as endogenous target mimics of miRNA156a, protecting the target transcription factor SPL from degradation under light induction, thereby promoting anthocyanin accumulation [42, 43]. Beyond core transcription factors HY5 and ERF109, light signals also synergize with jasmonic acid signaling: MdERF109 forms a protein complex with MdWER, which, as a jasmonic acid-responsive protein, alleviates repression by the inhibitor MdJAZ2, collectively enhancing anthocyanin biosynthesis [19]. These findings demonstrate that light precisely controls the coloration process through multi-layered transcriptional regulation and post-translational modifications.

4.2. Temperature

Temperature is another significant environmental factor influencing anthocyanin accumulation, with high temperatures generally inhibiting and low temperatures promoting red color development in apple fruits [37, 44]. Under high-temperature conditions, the expression of genes involved in anthocyanin synthesis is suppressed, whereas low temperatures effectively induce pigment accumulation in fruits [37]. At the molecular level, cold stress exerts its effects through epigenetic modifications: cold treatment significantly reduces DNA methylation levels in the promoter regions of anthocyanin-synthesis-related genes (e.g., MdCHS, MdCHI, MdF3'H, MdANS, MdUFGT, and the regulatory gene MdMYB10) in both apple leaves and fruits. The DNA demethylase MdROS1 plays a pivotal role in this process by directly binding to promoter regions of certain genes, reducing methylation levels, thereby activating gene expression and enhancing anthocyanin accumulation. Studies have further demonstrated that MdROS1 transcription levels induced by cold are positively correlated with anthocyanin content and the expression of synthesis-related genes, indicating that epigenetic reprogramming is one of the key mechanisms underlying cold-induced coloring [45]. Additionally, under cold stress, plants may enhance their cold resistance by synthesizing anthocyanins as an adaptive response [44]. Notably, different genotypes exhibit varying temperature responses during fruit maturation; breeding varieties adapted to warming climates—such as those selected using marker-assisted selection for specific MdMYB1 genotypes—has become a strategy to mitigate the adverse effects of global warming on apple coloring [37].

4.3. Moisture and Nutritional Status

Environmental factors such as water content and nutritional status also profoundly influence the metabolism of anthocyanins. Drought stress can induce a color change from green to red in the leaves of apple species, which is primarily caused by the accumulation of anthocyanins (particularly astaxanthin-3,5-diglucoside and astaxanthin-3-O-galactoside). Studies have demonstrated that the ethylene-responsive factor MsERF17 plays a positive regulatory role in drought-induced coloring by directly binding to and activating the promoters of MsbHLH3 and MsF3'H, thereby promoting anthocyanin synthesis; overexpression of MsERF17 significantly increases anthocyanin levels, whereas silencing this gene inhibits coloring [46]. Regarding nutrients, low nitrogen conditions typically promote anthocyanin biosynthesis, a process involving the participation of long non-coding RNAs. In apples, the expression of the long non-coding RNA LNC159c is downregulated under low nitrogen conditions, leading to reduced production of miR159c by its host gene, which in turn alleviates inhibition on the transcription factor MsMYB10 and enhances anthocyanin accumulation [47]. Conversely, high nitrogen supply may reduce anthocyanin synthesis by inhibiting key regulatory factors [42]. Sugars, serving as both carbon sources and signaling molecules, are coupled with anthocyanin synthesis: studies have shown a significant positive correlation between soluble sugar content and anthocyanin levels in Xinjiang red apple flesh, indicating that sugar signals may regulate coloring through modulation of sugar metabolism and energy status [13]. Furthermore, plant hormones such as ethylene, abscisic acid, and methyl jasmonate also participate in integrating environmental signals with anthocyanin metabolism. For instance, exogenous methyl jasmonate can synergistically enhance coloration under light exposure, a mechanism partially mediated by the MdERF109-MdWER-MdJAZ2 module [21]. In summary, environmental factors—including light, temperature, water, nutrients, and hormonal signals—collectively form a sophisticated regulatory system for anthocyanin synthesis in apple fruits through complex transcriptional networks, epigenetic modifications, and metabolic interactions.

5. Regulation of Anthocyanin Synthesis by Plant Hormones

5.1. Positive Regulation of Ethylene and Abscisic Acid

Plant hormones play a pivotal regulatory role in the accumulation and coloring of anthocyanins in apple fruits, with ethylene and abscisic acid (ABA) serving as key positive regulators. Ethylene, as a hormone that promotes fruit maturation, significantly induces the biosynthesis of anthocyanins, with critical transcription factors such as EIN3/EILs playing central roles in its signaling pathway. In apples, MdbHLH3, a bHLH transcription factor downstream of the ethylene signal, directly binds to the promoters of ethylene biosynthesis genes

MdACO1, MdACS1, and MdACS5A, activating their transcriptional expression and thereby enhancing ethylene production; this subsequent accumulation of ethylene further feedback-regulates anthocyanin synthesis [48]. Additionally, the ethylene-responsive factor PpERF9 in pears mediates histone deacetylation by binding to the promoters of PpMYB114 and PpRAP2.4 and recruiting the co-inhibitor PpTPL1, thereby suppressing anthocyanin synthesis; however, in apples, ethylene primarily exerts an promoting effect, highlighting the diversity of signaling regulation across species [49]. Abscisic acid also strongly induces anthocyanin accumulation, with its mechanism closely associated with the activation of MYB transcription factors. Studies have demonstrated that ABA treatment significantly elevates the expression level of MdMYB1 in apple skin, subsequently activating the transcription of downstream anthocyanin structural genes [50]. Meanwhile, the ABA-induced bZIP transcription factor MdbZIP44 interacts with MdMYB1, enhancing MdMYB1's binding affinity to target gene promoters and thereby promoting anthocyanin synthesis. In contrast, the BTB protein MdBT2 degrades MdbZIP44 via the ubiquitin-26S proteasome pathway, negatively regulating this process and elucidating the molecular network through which ABA signaling precisely controls anthocyanin accumulation [50]. Synergistic interactions also exist between ethylene and ABA: for instance, the light-induced long non-coding RNA MdLNC610 promotes ethylene production by upregulating MdACO1 expression, thereby enhancing anthocyanin synthesis; conversely, ABA participates in ethylene induction, collectively driving fruit coloring and maturation [51, 52].

5.2. The Typical Negative Regulatory Effects of Auxin and Gibberellin

Unlike the positive regulatory effects of ethylene and abscisic acid (ABA), auxin (IAA) and gibberellin (GA) typically exert negative regulatory effects on anthocyanin synthesis. The expression pattern of the auxin signaling component GH3 gene in red-fleshed apples is negatively correlated with anthocyanin accumulation, suggesting that IAA signaling may reduce anthocyanin production by inhibiting the activity of MYB transcription factors or the MBW complex [53, 54]. The inhibitory effect of GA is mediated through interactions between DELLA proteins and bHLH transcription factors. Studies have demonstrated that the GA signal inhibitor MdRGL2a in apples interacts with the anthocyanin inhibitor MdbHLH162, isolating MdbHLH162 from the MdbHLH162-MdbHLH3/33 complex thereby relieving inhibition on the MdMYB1-MdbHLH3/33 activation complex and promoting anthocyanin synthesis; conversely, GA itself indirectly enhances the inhibitory effect of MdbHLH162 by promoting DELLA protein degradation, exhibiting a negative regulatory characteristic [29]. In certain exogenous GA treatment experiments, GA3 reduced both anthocyanin content and soluble sugar concentrations in apple skin, further confirming its inhibitory effect [55]. In contrast, cytokinin (CTK) exhibits an opposite positive regulatory effect. Transcriptomic analysis revealed that the expression level of the cytokinin signaling component B-ARR gene in red-fleshed apples correlated with anthocyanin accumulation trends, suggesting that CTK may promote anthocyanin synthesis by activating MYB or bHLH factors within the MBW complex [53, 54]. Additionally, nitrogen nutrition status affects hormonal homeostasis: nitrogen application under heavy fruit thinning conditions elevates endogenous concentrations of IAA, GA3, ABA, and ZRs in fruits but delays their peak levels, ultimately reducing skin anthocyanin content, indicating that high nitrogen conditions indirectly inhibit coloring by altering hormonal dynamics [55, 56]. These findings highlight the antagonistic relationship between negative regulation by auxins and gibberellins versus positive regulation by cytokinins, with this balance ultimately determining anthocyanin accumulation levels through modulation of MBW complex activity.

5.3. Stress Regulation of Jasmonic Acid and Salicylic Acid

Jasmonic acid (JA) and salicylic acid (SA), as pivotal stress signaling molecules, induce anthocyanin accumulation in response to environmental stresses to enhance plant resistance. The core component of the jasmonic acid signaling pathway, JAZ proteins, serve as key negative regulators. In apples, MdJAZ1 and MdJAZ2 interact with the GA inhibitor MdRGL2a, disrupting MdRGL2a's isolation effect on MdbHLH162 and thereby releasing its inhibitory activity, ultimately negating anthocyanin synthesis [29]. This mechanism reveals a cross-regulatory dialogue between JA and GA signaling: JA competitively binds bHLH inhibitors through JAZ proteins to DELLAs, thereby modulating MBW complex assembly. Additionally, the MYC2 transcription factor in the JA signaling pathway has been identified as a potential regulator of anthocyanin synthesis, with its expression levels positively correlated with anthocyanin accumulation in red-fleshed apples [53, 54]. Salicylic acid participates in regulation via a distinct pathway; SA treatment increases total phenolic and anthocyanin content in fruits, particularly during early storage, though effects vary with treatment concentration and duration [56]. In apple transcriptome analyses, the SA signaling component NPR1 gene has been annotated as potentially involved in anthocyanin regulation, although its direct mechanism remains unclear [53, 54]. Furthermore, under low nitrogen stress, inhibition of the TOR signaling pathway induces enhanced JA signaling, whereas excessive SA accumulation may trigger programmed cell death, demonstrating that JA and SA balance growth and defense responses in stress adaptation by regulating anthocyanin metabolism [58]. Overall, as stress hormones, JA and SA precisely regulate anthocyanin biosynthesis by modulating the activity of MYB/bHLH transcription factors and their interactions with the MBW complex,

thereby bridging environmental signals and plant secondary metabolism.

6. Epigenetic Regulation

Epigenetic regulation plays a pivotal role in apple anthocyanin synthesis, with mechanisms involving multiple levels such as DNA methylation, histone modifications, and non-coding RNAs, collectively forming a sophisticated gene expression regulatory network [11, 59].

6.1. DNA Methylation

DNA methylation is one of the most extensively studied epigenetic modifications, directly participating in the regulation of fruit color variation by influencing transcription factors and promoter regions of structural genes [11, 59]. For example, in apples, the methylation status of the promoters of MdMYB10 and its homolog MdMYB1 is closely correlated with the intensity of skin redness [11, 24, 36]. Whole-genome re-sulfite sequencing analysis revealed that in apple mutants, the CHH methylation level in specific regions of the MdMYB1 promoter (e.g., the MR3 region) exhibits a significant negative correlation with gene expression; high methylation suppresses transcription, leading to reduced anthocyanin accumulation [23, 36]. Further studies have demonstrated that the RNA-guided DNA methylation (RdDM) pathway is the key mechanism underlying this CHH methylation, wherein the MdAGO4 protein specifically binds to the ATATCAGA motif in the MdMYB1 promoter and forms effect complexes with MdRDM1 and MdDRM2 to mediate the methylation modification [36]. Similarly, investigations of the McCOP1 promoter confirm that the RdDM pathway negatively regulates gene expression through CHH methylation, thereby affecting anthocyanin accumulation; silencing McRDM1 significantly reduces methylation levels and increases anthocyanin content [60]. Furthermore, the activity of DNA demethylases is critically important. During apple fruit development, downregulation of demethylases such as MdDME1 and MdROS1 leads to elevated global DNA methylation levels; particularly, high methylation in promoter regions suppresses the expression of structural genes like MdCHS and MdANS, resulting in paler fruit skin color [61]. Notably, although the correlation between global methylation and gene expression is not absolute, low methylation of the promoters of structural genes ANS and F3H has been consistently observed alongside their upregulated expression across multiple deep red mutants, suggesting that DNA methylation may regulate structural genes through synergistic interactions with transcription factors [62].

6.2. Histone Modifications

Histone modifications also play a profound role in the epigenetic regulation of anthocyanin synthesis. Although direct evidence is not yet widespread in apples, studies have demonstrated that histone acetylation and methylation modifications (e.g., H3K9ac, H3K27me3) can alter chromatin states, thereby influencing the transcriptional activity of anthocyanin-related genes [6, 59]. In apples, histone deacetylases (HDACs) and histone acetyltransferases (HATs) regulate the acetylation levels of core histones in response to developmental signals and environmental stress, thereby modulating the expression of key transcription factors such as MdMYB [64]. For instance, environmental stress can affect the expression of anthocyanin-synthesizing genes by altering the activity of histone modification enzymes, thereby enhancing plant adaptability [6, 63]. Additionally, there is a close interplay between DNA methylation and histone modifications in maintaining the epigenetic state of gene expression; high methylation in promoter regions is often associated with the inhibitory histone mark H3K27me3, which further compresses chromatin structure and silences gene expression [11, 57]. These dynamic changes in modifications enable apple fruits to flexibly adjust their coloring intensity according to developmental stages and environmental conditions [64].

Non-coding RNAs constitute another critical component of the epigenetic regulatory network, exerting precise post-transcriptional regulation over the expression of genes involved in anthocyanin synthesis. MicroRNAs (miRNAs), such as miR156 and miR828, target the mRNA of MYB transcription factors or structural genes, modulating anthocyanin accumulation through degradation or translation inhibition [6]. For instance, miRNAs can bind to transcripts of the MdMYB gene, altering their stability and thereby modifying the activity of the entire anthocyanin biosynthesis pathway [6]. Long non-coding RNAs (lncRNAs) also participate in this regulatory mechanism; they may interact with protein complexes to induce histone modifications or DNA methylation at specific genomic sites, or act as competitive endogenous RNAs (ceRNAs) by binding to miRNAs to suppress the expression of downstream target genes [19, 65]. In studies on apple flesh color fading, it was demonstrated that the expression of MdGSTU22 is associated with a differential methylation region (DMR), suggesting that environmental factors may influence gene expression via non-coding RNA-mediated epigenetic regulation [19]. Additionally, transposable elements (TEs) located within the introns of the MdMYB1 gene exhibit high methylation levels and collaborate with intron-selective polyadenylation (APA) to regulate the conversion of MdMYB1 transcripts from non-functional short transcripts to functional long transcripts, thereby driving fruit coloring processes [65]. These non-coding RNAs interplay with DNA methylation and histone modifications, forming an indispensable epigenetic hierarchy that regulates apple anthocyanin synthesis.

7. Conclusion and Prospects

Current research has systematically demonstrated that apple

anthocyanin synthesis is a complex process precisely regulated by multiple factors, with the core mechanism involving environmental signals (such as light, temperature, and hormones) activating a transcriptional regulatory network centered on the MBW complex through cascade reactions, further refined by epigenetic modifications and post-transcriptional mechanisms. This paper provides a comprehensive review of environmental induction, transcriptional regulation networks, epigenetic modifications, and metabolic interaction mechanisms underlying apple anthocyanin synthesis. However, several key issues remain unresolved: first, the functional redundancy and specificity of critical transcription factors (e.g., specific MYB members) across varieties are not fully elucidated, limiting the generalizability of regulatory network models; second, the molecular mechanisms mediating how environmental signals (e.g., light-temperature interactions) are precisely translated into epigenetic modifications to stabilize gene expression remain incompletely understood; third, the dynamic equilibrium between anthocyanin, proanthocyanidin, and lignin biosynthetic pathways—particularly their spatiotemporal regulation patterns during different fruit developmental stages—remains poorly characterized. Future studies should focus on employing multi-omics approaches to identify variety-specific regulatory modules, investigating noncoding RNA–epigenetic modification interaction networks, and developing gene-editing-based precision control strategies to address fruit coloration heterogeneity and advance targeted breeding for improved apple quality.

Abbreviations

ABA	Abscisic Acid
ANS	Anthocyanidin Synthase
APA	Alternative Polyadenylation
bHLH	Basic Helix-Loop-Helix
bZIP	Basic Region/Leucine Zipper Motif
CHI	Chalcone Isomerase
CHS	Chalcone Synthase
CTK	Cytokinin
DFR	Dihydroflavonol 4-Reductase
DMR	Differentially Methylated Region
ERF	Ethylene Response Factor
F3'H	Flavonoid 3'-Hydroxylase
F3H	Flavanone 3-Hydroxylase
GA	Gibberellin
GA_3	Gibberellin A3
G-box	G-box cis-element, G-box
GST	Glutathione S-Transferase
HDAC	Histone Deacetylase
HAT	Histone Acetyltransferase
IAA	Indole-3-Acetic Acid
JA	Jasmonic Acid
JAZ	Jasmonate ZIM-domain Protein
lncRNA	Long Non-coding RNA
MBW	MYB-bHLH-WD40 Complex
MeJA	Methyl Jasmonate
miRNA	microRNA
MPK	Mitogen-Activated Protein Kinase
NAC	NAM-ATAF-CUC Transcription Factor
NPR1	Nonexpressor of Pathogenesis-Related Genes 1
PAL	Phenylalanine Ammonia-Lyase
RdDM	RNA-directed DNA Methylation
SA	Salicylic Acid
TE	Transposable Element
TOR	Target of Rapamycin
UDP	Uridine Diphosphate
UV-B	Ultraviolet-B Radiation
UVR8	UV Resistance Locus 8
WD40	WD40 Repeat Protein
WRKY	WRKY Transcription Factor
ZRs	Zeatin Riboside

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Conflicts of Interest

The authors declare no conflicts of interest.

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