Regulating Factors in Anthocyanin Biosynthesis

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Introduction

The high level of diversity among anthocyanin biosynthesis genes has allowed biologists to better model degrees of their functional specialization (Ahmed, Maekawa, & Noda, 2009; Khlestkina et al., 2008). These effective, yet slight, variations in pigmentation between species, and even cultivars, suggest that anthocyanin may hold vital roles in survival from abiotic and biotic stress responses during early stages of growth and development. This raises valid questions as to how anthocyanin accumulation and expression are regulated throughout these vulnerable stages of coleoptile development. The presence or absence of anthocyanin in the developing coleoptiles of young seedlings may be important for increasing the plant’s chances of survival when subjected to increasing levels of radiation, temperature fluxes, and herbivory damage before establishment of the cuticle.

The order of expression of various anthocyanin genes has led researchers to classify anthocyanin biosynthesis genes as early biosynthesis genes, EBGs, or late biosynthesis genes, LBGs (Nesi, Jond, Debeaujon, Caboche, & Lepiniec, 2001). When subjected to RT-PCR analysis, transcripts of both EBGs and LBGs throughout the anthocyanin biosynthesis pathway accumulate under light conditions, peaking around the third day following germination (Ahmed, et al., 2009). This is closely correlated with anthocyanin accumulation across multiple species, suggesting that the expression of anthocyanin biosynthesis genes is closely related to the accumulation of anthocyanin pigment compounds in plants. Light, while not necessary for expression, is considered essential for the natural increase and accumulation of anthocyanin flavonoids throughout plant tissues. However, the diversity of anthocyanin’s proposed functions in plants raises further questions about its regulatory pathways.

MYB Transcription Factors

The two classes of transcription factors associated with the anthocyanin biosynthesis pathway are in the R/B and C1/P1 family. MYB proteins differ depending on the number of adjacent repeats in their structure. The three different repeats of the prototypic MYB protein are referred to as R1, R2, and R3, respectively, and can found throughout a wide range of plant species. Of these classes of proteins in plants, R2R3-MYB transcription factors control flavanol accumulation throughout parts of Aribidopsis. MYB11, MYB12, and MYB111 of this subgroup were found to exhibit similar target gene specificity for several flavonoid biosynthesis genes. Triple mutant myb11 myb12 myb111 seedlings specifically lacked flavanol. Mutant myb12 seedlings displayed significantly reduced levels of flavanols in the root and hypocotyl-root transition zones, while myb111 mutants effectively lacked flavanols in the cotyledons, demonstrating roles of these transcription factors in the spatial accumulation of flavanols throughout the
plant. However, these mutant seedlings exhibited no signs of effective reduction in flavonoids such as anthocyanin. In fact, anthocyanin levels of these mutants were observed to be slightly higher in epidermal cells, when compared to that of the wild type (Dubos et al., 2010). This would indicate that precursors of flavanol biosynthesis may also be channeled to help form anthocyanins, and that these flavanol transcription factors may also negatively regulate flavonoid biosynthesis through competition.

R3 type single-MYB proteins such as CPC and TRY had been previously observed as negative regulators over the development of epidermal cells (Tominaga, Iwata, Okada, & Wada, 2007), leading researchers to question similar roles of MYB proteins in regulating anthocyanin biosynthesis. Pale seed color was used as a marker to identify AtMYBL2 as a repressor for effectively suppressing the biosynthesis of anthocyanin. In mutant mybl2 (Loss of function AtMYBL2), enhanced accumulation of anthocyanin was observed through increased expression of DFR and TT8 BHLH proteins. Affected seed color was not observed in trichomes or root hairs, indicating that this biosynthesis does not effectively regulate the biosynthesis of anthocyanin within the seed coat (Matsui, Umemura, & Ohme Takagi, 2008).

Independent of any developmental regulation, high-light has been observed to significantly decrease MYBL2 mRNA levels after only 3 hours. With this inhibitory effect, also came an increase in PAP1, PAP2, and TT8, and as a result, increased accumulation of mRNA encoded by their target genes LDOX and DFR (Dubos et al., 2008). This expression of DFR coincides with the observed protein levels in AtMYBL2 loss of function mutants, suggesting that light may induce anthocyanin production through similar inhibitory function.

The MYB type family of C1/P1 transcriptional factors is rapidly induced by light signal transduction pathways. Inserted viral enhancer sequences adjacent to these MYB transcription factor genes in Arabidopsis was shown by (Borevitz, Xia, Blount, Dixon, & Lamb, 2000) to overcome the natural regulators controlling the accumulation of anthocyanin product in the absence of light. The isolated purple mutant pap1-D exhibited widespread activation of natural phenylpropanoid products throughout all parts of Arabidopsis. From this mutant came the identification of a single PAP1 insertion. The massive overexpression of the PAP1 MYB transcription factor in pap1-D mutants indicates that PAP1 may act as a significant limiting factor in the upregulation and expression necessary to induce anthocyanin biosynthesis.

Conclusions

Given the strong observational similarities between plants under MYBL2 loss of function and PAP1 overexpression, the proposed model of function between them becomes conceivable. The MBW complex, PAP1, may act as a primary activator in the expression of anthocyanin biosynthesis genes, while MYBL2 functions acts as its transcriptional inhibitor. The interactions of these proposed relationships are supported by the ability of these proteins to be influenced by the same DFR and TT8 BHLH proteins. In addition, when exposed to light, the mRNA levels of expression between these complexes was shown by (Dubos, et al., 2010) to be negatively correlated. Moving forward, investigations into the various effects of other possible biotic and...
abiotic effects should be elucidated, and contrasted with further knowledge of internal regulators of MYB and MBW complex expression.

References


