

Gene regulation networks generate diverse pigmentation patterns in plants

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The diversity of pigmentation patterns observed in plants occurs due to the spatial distribution and accumulation of colored compounds, which may also be associated with structural changes to the tissue. Anthocyanins are flavonoids that provide red/purple/blue coloration to plants, often forming complex patterns such as spots, stripes, and vein-associated pigmentation, particularly in flowers. These patterns are determined by the activity of MYB-bHLH-WDR (MBW) transcription factor complexes, which activate the anthocyanin biosynthesis genes, resulting in anthocyanin pigment accumulation. Recently, we established that the MBW complex controlling anthocyanin synthesis acts within a gene regulation network that is conserved within at least the Eudicots. This network involves hierarchy, reinforcement, and feedback mechanisms that allow for stringent and responsive regulation of the anthocyanin biosynthesis genes. The gene network and mobile nature of the WDR and R3-MYB proteins provide exciting new opportunities to explore the basis of pigmentation patterning, and to investigate the evolutionary history of the MBW components in land plants.

Plants exhibit extraordinary diversity in the coloration and patterning of their flowers, fruits, and vegetative tissues. The red/purple/blue colors in plants are most commonly due to the presence of anthocyanin pigments. These pigments accumulate in response to developmental signals in flowers and fruits, and in vegetative tissues in

response to stress. Floral patterning can be extremely elaborate and can involve structural changes to the tissues, e.g., conical cells,¹ trichomes,² multicellular raised spots,³ as well as multiple pigment classes (e.g., carotenoids, chlorophylls) in addition to anthocyanins. How then, do pigmentation patterns form?

The spatial expression pattern of the biosynthetic genes is responsible for anthocyanin pattern formation, and this is coordinated by a gene regulation network involving a MYB-bHLH-WDR (MBW) transcription factor complex.⁴ Pigmentation patterns can be established by the overlapping expression domains of the MYB, bHLH, and WDR proteins since all 3 components are essential for activating anthocyanin synthesis.⁵ The R2R3-MYB activators in particular are central to pattern formation, as they exist as multi-gene families with diverse spatial expression domains that are associated with distinct pigmentation patterns (e.g., spots, stripes).⁶⁻⁹ Moreover, the MYBs are often more specific in the genes and pathways they target compared with the bHLH and WDR components, which may be shared with MBW complexes regulating other processes (e.g., trichome formation). The mechanism for vein-associated pigmentation (venation) patterning is an excellent example of this, and has been determined in both *Antirrhinum*¹⁰ and *Petunia*.⁸ Anthocyanins accumulate in the epidermal cells overlaying the vasculature because this is where the expression domains of the MYB (transcript gradient from vasculature) and bHLH (epidermal) factors intersect.^{5,10} Questions still remain

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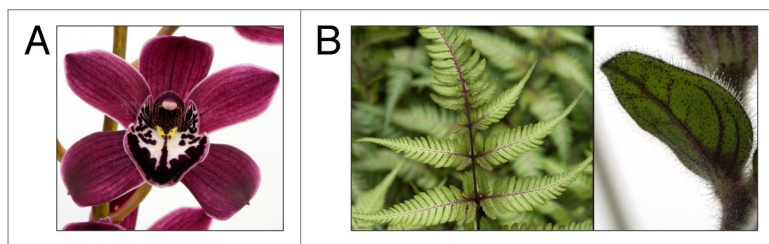


Figure 1. Anthocyanin pigmentation patterns in diverse plant species. **(A)** *Cymbidium hybrida* cv “Ruby Valley Stephen John,” like many orchids, has intricate anthocyanin pigmentation patterns including full tepal pigmentation, venation, spots, and zones that lack pigmentation and co-pigments. **(B)** Vegetative pigmentation is an ancient trait in land plants. Vein and stem-associated anthocyanin pigmentation is present in the leaves of the fern *Athyrium niponicum* (left) and in *Petunia hybrida* (right). The *P. hybrida* plant shown has enhanced vegetative pigmentation due to the silencing of *MYB27*, an R2R3-MYB repressor; venation is activated by the R2R3-MYB gene *DEEP PURPLE* in both flowers and vegetative tissues. The *Athyrium* image was kindly provided courtesy of The New York Botanical Garden.

regarding the signals that regulate the expression of the transcription factors – how is “pigmentation identity” achieved at the cellular level and how does this allow for patterning?

The mechanisms that establish developmentally regulated pigmentation patterns are not well understood (e.g., establishing “petal spot” identity^{11,12}), but recent advances understanding the activity of the MBW complex may provide new insights. Theoretical models have been proposed that would allow the establishment of developmental patterns, which may have relevance for pigmentation patterning.⁵ These models are based upon the diffusion of long-range inhibitors and activators with short-range activity, which act within a regulatory network that exhibits both reinforcement and feedback repression.^{13–15} We have shown that in petunia, the gene network that regulates anthocyanin synthesis contains the components required to fulfil these models.¹⁶ In particular, we demonstrated that the bHLH factor not only activates the expression of the anthocyanin biosynthesis genes (within MBW complex, to establish pigmentation), but also regulates its own expression (reinforcement) and that of genes encoding R3-MYB repressors. To fulfil the 2D patterning models proposed by Meinhardt and Gierer,^{13,14} these inhibitor R3-MYBs should be mobile and limit the activity of the activator (the bHLH, within the MBW activation complex). Our findings demonstrated

that the pigmentation related R3-MYB and WDR proteins are indeed mobile within petal epidermal cells, while the R2R3-MYB activators, R2R3-MYB repressors, and bHLH proteins act cell-autonomously. Thus, the mobility of the R3-MYB and WDR proteins may be important for establishing or maintaining some pigmentation patterns. For example, petal spots are often surrounded by a halo of cells that are less pigmented than the rest of the petal. This suggests that either factors necessary for anthocyanin production are being depleted (WDR) or that inhibitory factors (R3-MYB) are being exported from the spot cells, or that a combination of both is occurring.⁵ Such a mechanism would share significant similarity to that shown to regulate the development and distribution of trichomes in *Arabidopsis* leaves, where an MBW complex with reinforcement mechanisms and mobile WDR and R3-MYB repressors has shown to be involved.^{13,17} However, while this is the case for *Arabidopsis*, trichome regulation and distribution are not regulated by such MBW complexes in many plants.^{18,19} In contrast, the MBW complex has been found to be central to anthocyanin regulation in all higher plants examined to date.^{5,20,21} Thus, it appears that the mechanisms that regulate anthocyanin synthesis are highly conserved in plants.

The accumulated studies in *Arabidopsis* (a Rosid) and *Petunia* (an Asterid) have demonstrated the conservation of the key

components of the MBW complex within Eudicots, including the R3-MYB and R2R3-MYB repressors.¹⁶ We proposed a multi-species model for anthocyanin regulation that integrate hierarchy, reinforcement and feedback activity of the MBW gene regulation network.¹⁶ This model now needs to be tested in diverse plant species, to identify both the elements of the gene regulation network that are functionally conserved, and differences that are specific to particular genera. It does appear that this network holds across the wider Angiosperm group, as similar MBW components have been identified in maize, lilies, and orchids,^{22–27} although the involvement of gene regulation networks and repressor proteins has not yet been demonstrated outside of the Eudicots. The lack of a non-grass monocot model species, with a sequenced genome and genetic resources, has limited the study of anthocyanin regulation within this important and diverse group of Angiosperms. Of particular interest are the Orchidaceae (Asparagales), because of the amazing diversity of floral patterning and specialized pollination strategies present within orchids. Taking into account the hierarchical nature of the MBW gene network, protein mobility and repressive activities may assist in understanding how complex and intricate patterns can form, such as those present in the flowers of orchids (Fig. 1A).

Some of the MBW components in Angiosperms have clearly either evolved for, or have been adapted for, control of pigmentation patterning in petals. So, if similar factors are present outside of Angiosperms do they fulfil equivalent roles when these lack flowers? At least some putative MBW components have been found in gymnosperms, specifically *MBF1* from *Picea mariana*,²⁸ but data from outside of the Angiosperms is limited. Some commonalities in the production of anthocyanin pigments are evident between Eudicots and species as evolutionarily distant as the ferns, mosses, and liverworts. Both the environmental triggers for pigment induction and some of the patterning of pigmentation in vegetative tissues are strikingly similar across the entire range of plant groups.

Such an example for patterning is the association of pigments with the veins of leaves (Fig. 1B). Vegetative pigmentation preceded the evolution of flowers, so it is tempting to speculate that the mechanisms for venation of leaves and petals in the Angiosperms represents this more ancient character.^{5,8} To address this, further data are needed on the regulatory components, whether MBW or other proteins, in non-Angiosperm model species, such as the spikemoss *Selaginella*, the moss *Physcomitrella*, and the liverwort *Marchantia*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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