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 Antirrhinum10 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

Keywords: Flavonoid, anthocyanin, MYB, bHLH, WDR, transcription factor

*Correspondence to: Nick W Albert; Email: Nick.Albert@agresearch.co.nz

Submitted: 05/29/2014
Accepted: 06/08/2014
Published Online: 06/13/2014
Citation: Albert NW, Davies KM, Schwinn KE. Gene regulation networks generate diverse pigmentation patterns in plants. Plant Signaling & Behavior 2014; 9:e29526; http://dx.doi.org/10.4161/psb.29526

Addendum to: Albert NW, Davies KM, Lewis DH, Zhang H, Montefiori M, Bendolise C, Boase MR, Ngo H, Jameson PE, Schwinn KE. A conserved network of transcriptional activators and repressors regulates anthocyanin pigmentation in eudicots. Plant Cell 2014; 26:962-80; PMID:24642943; http://dx.doi.org/10.1105/tpc.113.122069
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\textsuperscript{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.\textsuperscript{2} These models were 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.\textsuperscript{13-15} We have shown that in petunia, the gene network that regulates anthocyanin synthesis contains the components required to fulfill these models.\textsuperscript{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 fulfill the 2D patterning models proposed by Meinhardt and Gierer,\textsuperscript{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.\textsuperscript{2} Such a mechanism would share significant similarity to that shown to regulate the development and distribution of trichomes in \textit{Arabidopsis} leaves, where an MBW complex with reinforcement mechanisms and mobile WDR and R3-MYB repressors has shown to be involved.\textsuperscript{13,17} However, while this is the case for \textit{Arabidopsis}, trichome regulation and distribution are not regulated by such MBW complexes in many plants.\textsuperscript{18,19} In contrast, the MBW complex has been found to be central to anthocyanin regulation in all higher plants examined to date.\textsuperscript{2} Thus, it appears that the mechanisms that regulate anthocyanin synthesis are highly conserved in plants.

The accumulated studies in \textit{Arabidopsis} (a Rosid) and \textit{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.\textsuperscript{16} We proposed a multi-species model for anthocyanin regulation that integrate hierarchy, reinforcement and feedback activity of the MBW gene regulation network.\textsuperscript{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,\textsuperscript{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 fulfill equivalent roles when these lack flowers? At least some putative MBW components have been found in gymnosperms, specifically MBF1 from \textit{Picea mariana},\textsuperscript{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. 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.

References

1. Noda K, Glover BJ, Linstead P, Martin C. Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. Nature 1994; 369:661-4; PMID:8208293; http://dx.doi.org/10.1038/369661a0
2. Winefield CS, Lewis DH, Swiny EE, Zhang HB, Arathoon HS, Fischer TC, et al. Investigation of the biosynthesis of 3-deoxyanthocyanins in Sinningia candida. Physiol Plant 2005; 124:419-30; http://dx.doi.org/10.1111/j.1365-313X.2005.03498.x
3. Thomas MM, Rudall PJ, Ellis AG, Savolainen V, Glover BJ. Development of a complex floral trait: The pollinator-attracting petal spots of the beetle pollinator Chrysolina americana. Am J Bot 2012; 99:82-91; PMID:22186184; http://dx.doi.org/10.3732/ajb.1100285
4. Ramsay NA, Glover BJ. MYB-bHLH-WD40 protein complexes and the evolution of cellular diversity. Trends Plant Sci 2005; 10:63-70; PMID:15708343; http://dx.doi.org/10.1016/j.tplants.2004.12.011
5. Davies KM, Albert NW, Schwinn KE. From flora to fauna: The molecular regulation of flower colouration and mechanisms for patterning pigmentation. Funct Plant Biol 2012; 39:619-38; http://dx.doi.org/10.1071/FB12195
6. Lowry DB, Sheng CC, Lasky JR, Willis JH. Five anthocyanin polymorphs are associated with an R2R3-MYB cluster in Mimulus guttatus (Phrymaceae). Am J Bot 2012; 99:82-91; PMID:22186184; http://dx.doi.org/10.3732/ajb.1100285
7. Gonzalez A, Zhao M, Leavitt JM, Lloyd AM. Regulation of the anthocyanin biosynthetic pathway by the TTGs/SHH/LHY/MYB transcriptional complex in Arabidopsis seedlings. Plant J 2008; 53:814-27; PMID:18036197; http://dx.doi.org/10.1111/j.1365-313X.2007.03373.x
8. Albert NW, Lewis DH, Zhang H, Schwinn KE, Jameson PE, Davies KM. Members of an R2R3-MYB transcription factor family in Petunia are developmentally and environmentally regulated to control complex floral and vegetative pigmentation patterning. Plant J 2011; 65:771-84; PMID:21235651; http://dx.doi.org/10.1111/j.1365-313X.2010.04465.x
9. Schwinn K, Venail J, Shang Y, Mackay S, Alm V, Buneli E, Oyama R, Bailey P, Davies K, Martin C. A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus Antirrhinum. Plant Cell 2006; 18:831-51; PMID:16531495; http://dx.doi.org/10.1105/tpc.105.039255
10. Schmid M, Venail J, Mackay S, Bailey PC, Lewis DH, Zhang H, Schwinn KE, Jameson PE, Martin CR, Davies KM. The molecular basis for venation patterning of pigment and its effect on pollinator attraction in flowers of Antirrhinum. New Phytol 2011; 189:602-15; PMID:21093563; http://dx.doi.org/10.1111/j.1469-8137.2010.04498.x
11. Glover BJ, Walker RH, Movroud E, Brockington SF. How to spot a flower. New Phytol 2013; 197:687-9; PMID:23293952; http://dx.doi.org/10.1111/nph.12112
12. Martins TR, Berg J, Binka S, Rausher MD, Baum DA. Precise spatio-temporal regulation of the anthocyanin biosynthetic pathway leads to petal spot formation in Clarkia gracilis (Onagraceae). New Phytol 2013; 197:958-69; PMID:23233186; http://dx.doi.org/10.1111/nph.12062
13. Pesch M, Hulskaemp M. Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. Curr Opin Genet Dev 2004; 14:422-7; PMID:15265659; http://dx.doi.org/10.1016/j.cogd.2004.06.007
14. Meinhardt H, Gierer A. Applications of a theory of biological pattern formation based on lateral inhibition. J Cell Sci 1974; 15:321-46; PMID:4858925
15. Turing AM. The chemical basis of morphogenesis. Phil Trans Royal Soc London Ser B 1952; 237:37-72; PMID:18666319
16. Albert NW, Davies KM, Lewis DH, Zhang H, Montefiori M, Brendolise C, Boase MR, Ngo H, Martin C, Glover BJ. Functional aspects of cell differentiation. Mol Biol Evol 2013; 30:526-40; PMID:23188591; http://dx.doi.org/10.1093/molbev/mss260
17. Martin C, Glover BJ. Functional aspects of cell patterning in aerial epidermis. Curr Opin Plant Biol 2007; 10:70-82; PMID:17148837; http://dx.doi.org/10.1016/j.pcbi.2006.11.004
18. Koes R, Verweij W, Quattrochio F. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends Plant Sci 2005; 10:236-42; PMID:15882656; http://dx.doi.org/10.1010/j.splants.2005.03.002
19. Keller A, Machemer K, Braun EL, Grotewold E. Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. Plant J 2011; 66:94-116; PMID:21443626; http://dx.doi.org/10.1111/j.1365-313X.2010.04459.x
20. Nakatsuka A, Yamagishi M, Nakano M, Tazaki K, Kobayashi N. Light-induced expression of basic helix-loop-helix genes involved in anthocyanin biosynthesis in flowers and leaves of Asiatic hybrid lily. Sci Horticult 2009; 121:84-91; http://dx.doi.org/10.1016/j.scienta.2009.01.008
21. Yamagishi M, Shimoyama Y, Nakatsuka T, Masuda K. Two R2R3-MYB genes, homologs of Petunia AN2, regulate anthocyanin biosyntheses in flower tepals, reap spots and leaves of asiatic hybrid lily, Plant Cell Physiol 2010; 51:463-74; PMID:20118109; http://dx.doi.org/10.1093/pcp/pqcp011
22. Albert NW, Arathoon S, Collette VE, Schwinn KE, Jameson PE, Lewis DH, et al. Activation of anthocyanin synthesis in Cymbidium orchids: variability between known regulators. Plant Cell Tiss Org 2010; 100:355-60; http://dx.doi.org/10.1007/s11195-009-9649-0
23. Carey CC, Strahle JT, Selinger DA, Chandler VL. Mutations in the pale aleurone color1 regulatory gene of the Zea mays anthocyanin pathway have distinct phenotypes relative to the functionality of the similar TRANSPARENT TESTA GLABRA1 gene in Arabidopsis thaliana. Plant Cell 2006; 16:450-64; PMID:17442877; http://dx.doi.org/10.1105/tp.1018796
24. Paz-Ares J, Ghosal D, Wienand U, Peterson PA, Saedler H. The regulatory cl locus of Zea mays encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. EMBO J 1987; 6:3553-8; PMID:3428265
25. Ludwig SR, Habera LP, Dellaporta SL, Wessler SR. Lc, a member of the maize B gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the myc-homology region. Proc Natl Acad Sci U S A 1989; 86:7092-6; PMID:2674946; http://dx.doi.org/10.1073/pnas.86.18.7092
26. Bedon F, Grima-Pettenati J, Mackay J, Conifer R2R3-MYB transcription factors: sequence analyses and gene expression in wood-forming tissues of white spruce (Picea glauca). BMC Plant Biol 2007; 7:17; PMID:17397551; http://dx.doi.org/10.1186/1471-2229-7-17