The Past, Present, and Future of Vegetative Phase Change[1]

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Many things change during shoot development. Although the most obvious of these is the production of reproductive structures, the morphology and physiology of leaves and vegetative buds changes as well. Some traits change continuously (either increasing or decreasing), while others are expressed in bimodal (present or absent) or even more complex patterns. Some traits vary in the same way in every species, while others vary in different ways in different species. For example, cell size declines gradually in successively higher leaves, but trichomes may be produced uniformly on all leaves, early in shoot development, late in shoot development, in different patterns on vegetative and reproductive parts of the shoot, or not at all. This phenomenon is termed heteroblasty (Goebel, 1900). Despite the complexity of the changes that occur during vegetative development, it is usually possible to recognize several more-or-less discrete phases based on correlated changes in the expression of multiple traits. These phases are particularly evident in certain woody plants, including *Hedera helix* and some species of *Acacia* and *Eucalyptus*, but are obvious in other species as well (Godley, 1985; Boland et al., 2006). The transition between these phases is referred to as vegetative phase change (Poethig, 1990). Recent studies in Arabidopsis (*Arabidopsis thaliana*) and maize (*Zea mays*) are beginning to provide a clearer picture of the molecular mechanism of vegetative phase change, and may eventually make it possible to manipulate this process in woody species, where it is of major economic and social significance (Brunner and Nilsson, 2004).

THE PHENOMENOLOGY OF VEGETATIVE PHASE CHANGE

Woody plants are favorable systems for studying vegetative phase change because the stability and prolonged duration of various stages in shoot development make these phases easy to observe and characterize. The first experimental study of vegetative phase change was performed by Thomas Andrew Knight with apple (*Malus domestica*) and pear (*Pyrus communis*) trees. In a letter to Sir Joseph Banks, he reported (Knight, 1795): "I took cuttings of some old ungrafted pear-trees, and others from scions which sprang out of the trunks near the ground and inserted some of each on the same stocks. The former grew without thorns, as in the cultivated varieties, and produced blossoms the second year; while the latter assumed the appearance of stocks just raised from seeds, were covered with thorns, and have not yet produced any blossoms" (p. 293). He went on to conclude from this and other experiments that "every cutting, therefore, taken from the apple (and probably from every other) tree, will be affected by the state of the parent stock. If that be too young to produce fruit, it will grow with vigor, but will not blossom; and if it be too old, it will immediately produce fruit, but will never make a healthy tree..." (p. 292). This short report demonstrated that shoots can express stable developmental states, that different parts of the same shoot can simultaneously exist in different developmental states, and that the vegetative character and reproductive potential of the shoot are associated in some way.

Since then, a large number of phase-specific traits have been identified (Brink, 1962; Kerstetter and Poethig, 1998). These include the shape and size of leaves and their pattern of cellular differentiation, branching patterns, disease and pest resistance, the capacity for adventitious root production, and reproductive competence. In some strongly heteroblastic species these traits change in a coordinated fashion over a few nodes to produce two very distinct growth forms, leading to the idea that shoots exist in alternative juvenile and adult phases (Goebel, 1900). However, even in species with well-defined juvenile and adult phases (Fig. 1), the character of the shoot actually varies in a much more complex fashion than suggested by this simple model of shoot development. For example, the first few leaves produced after germination are often distinct from other juvenile leaves (Bongard-Pierce et al., 1996; Telfer et al., 1997; Boland et al., 2006). Although these leaves have many features in common with later-formed juvenile leaves (and hence are usually considered the same leaf type), they are not identical to other juvenile leaves. In addition, reproductive structures are rarely produced immediately after the transition in vegetative morphology; in most cases, the shoot produces adult leaves for an extended period of time before it begins to produce reproductive structures (Brunner and Nilsson, 2004). Finally, reproductively mature shoots continue to undergo morphological and physiological changes (Bond, 2000). This fine-scale variation is described in a nine-phase model of shoot maturation summarized by Gatsuk and col-

Figure 1. Six-month-old *Acacia koa* sapling. During the juvenile phase, this Hawaiian species produces pinnately compound leaves and suppressed axillary buds; in the adult phase, it produces phyllodes and elongated branches.

leagues (Gatsuk et al., 1980). It remains to be determined if these nine phases represent intermediate stages in a single maturation program or are the product of multiple programs regulated in a variety of different ways. In any case, this model makes the point that shoot development is a great deal more complex than is generally recognized.

What is the molecular basis for phase-specific differences in vegetative morphology and physiology? There is surprisingly little information about this question. In maize, the propensity of adult shoots to undergo rejuvenation in culture has facilitated the identification of genes involved in vegetative phase change (Strable et al., 2008). A few phase-specific genes have also been identified in larch (*Larix laricina*; Hutchinson et al., 1990) and English ivy (*Hedera helix*; Woo et al., 1994). However, there is still no comprehensive picture of the changes in gene expression that occur during vegetative growth in any plant species. The existence of phase-specific traits (e.g. the presence versus absence of epicuticular wax in maize or abaxial leaf trichomes in Arabidopsis) suggests that vegetative change is associated with a significant change in gene expression, as is the case for floral induction (Schmid et al., 2003). But, so far, very few differentially expressed genes have been identified in juvenile and adult shoots, and most of the genes that have been identified display relatively small differences in their expression. Whether this reflects reality, or is due to technical issues, remains to be determined.

THE REGULATION OF SHOOT MATURATION BY MIRNAS

The first evidence that vegetative phase change is under genetic control was provided by several gain-of-function mutations in maize that prolong the expression of the juvenile phase (Poethig, 1988). The observation that these mutations do not have a major effect on flowering time or the photoperiodic sensitivity of the shoot (Bassiri et al., 1992) suggested that vegetative phase change is regulated independently of floral induction. Similar results were obtained in a genetic analysis of the timing of vegetative phase change and flowering in two closely related tree species, *Eucalyptus tenuiramis* and *Eucalyptus risdonii* (Wiltshire et al., 1998). These species differ primarily in that *E. risdonii* flowers during the juvenile vegetative phase, whereas *E. tenuiramis* flowers during the adult vegetative phase. Crosses between these species revealed that the duration of the juvenile phase and flowering time are genetically determined and are inherited independently of each other.

Molecular genetic analyses of vegetative phase change in maize and Arabidopsis have since revealed that miR156 plays a particularly important role in this transition (Wu and Poethig, 2006; Chuck et al., 2007; Wu et al., 2009). miR156 is expressed at very high levels during the juvenile phase and declines in abundance during vegetative phase change. Constitutive expression of miR156 prolongs the expression of juvenile traits, whereas loss of miR156 activity eliminates these traits, demonstrating that miR156 is both necessary and sufficient for the juvenile phase.

miR156 targets *SQUAMOSA PROMOTER BINDING PROTEIN* (*SBP*) genes (Rhoades et al., 2002; Schwab et al., 2005). All major plant taxa have multiple members of this plant-specific family of transcription factors, a subset of which is regulated by miR156 (Cardon et al., 1999; Xie et al., 2006; Riese et al., 2007; Guo et al., 2008). The miR156-targeted members of this family have a variety of distinct functions. As a whole, these miR156-targeted genes regulate the same set of traits in different organisms because the phenotypes of plants overexpressing miR156 are quite similar (Wu and Poethig, 2006; Xie et al., 2006; Chuck et al., 2007; Wang et al., 2008). Among other things, these plants display the prolonged expression of juvenile leaf traits, an increase in the rate of leaf initiation, increased branching, an increase in lateral root formation, and floral and inflorescence abnormalities. Determining how these functions are distributed among the

many members of the SBP family is a major challenge, but this picture is beginning to emerge from the phenotypes of mutations in these genes (Wang et al., 2005, 2008; Schwarz et al., 2008; Wu et al., 2009; Chuck et al., 2010; Jiao et al., 2010; Miura et al., 2010).

Several of the SBP genes targeted by miR156 cause early flowering when overexpressed (Cardon et al., 1997; Wu and Poethig, 2006), suggesting that low reproductive competence of juvenile shoots is a consequence of relatively low expression of these genes during the juvenile phase. How do *SBP* genes promote flowering? In at least two cases, the pathway is quite short: SPL3 is a direct transcriptional activator of the floral promoters *FUL*, *AP1*, and *LFY* (Yamaguchi et al., 2009), whereas SPL9 promotes the transcription of the floral promoters *FUL*, *SOC1*, and *AGL42* (Wang et al., 2009). SPL9 also regulates flowering more indirectly, by promoting the transcription of miR172 (Wu et al., 2009), which in turn represses the expression of several AP2-like genes (*TOE1*, *TOE2*, *SMZ*, *SNZ*) that repress the floral inducer *FT* (Aukerman and Sakai, 2003; Jung et al., 2007; Mathieu et al., 2009). An ortholog of these genes in maize, *Gl15*, is also a target of miR172 and represses flowering when overexpressed (Lauter et al., 2005). In both Arabidopsis and maize, these AP2-like transcription factors promote juvenile epidermal identity in addition to repressing flowering, and genetic evidence indicates that they are required for the effect of miR156 on this phase-specific trait (Evans et al., 1994; Moose and Sisco, 1994; Wu et al., 2009).

Most plants normally do not flower during the juvenile vegetative phase, but some species can be induced to flower in this phase, or do so regularly (e.g. *E. risdonii*; Zimmerman et al., 1985; Wiltshire et al., 1998). Although the production of flowers during the juvenile phase might appear anomalous, it is actually consistent with our current understanding of the mechanism of flower induction. As described in more detail elsewhere in this issue (Amasino and Michaels, 2010), flowering is regulated by many pathways, which operate in parallel on the same set of targets. Most of these pathways have little or no effect on vegetative phase change. It is not surprising, therefore, that in some genetic backgrounds these pathways are sufficiently active to overcome the suppressive effect of miR156 on flowering time without simultaneously affecting the vegetative identity of the shoot.

FUTURE DIRECTIONS

Many long-standing questions about vegetative phase remain to be answered: How do plants measure developmental time? Where is the phase-change clock located, and how does it repress the expression of miR156 to bring about vegetative phase change? What role do environmental factors play in vegetative phase change? What is the basis for the stability of juvenile and adult phases in woody plants? What is the functional significance of phase-specific morphological

and physiological traits? These questions have been addressed in a variety of species over the last century, often with conflicting results. Now that some of the major regulators of vegetative phase change have been identified, it is possible to address to these questions with new tools, and with greater insight into the nature of the underlying pathways. Some of these questions can probably best be answered using herbaceous species with well-developed genetic tools, but others will need to be investigated in woody species, where vegetative phase change was first discovered and where it is of the greatest practical significance. The identification of a rapid cycling, strongly heteroblastic tree species amenable to molecular genetic analysis is therefore a high priority.

miR156 not only serves as a master regulator of vegetative phase change, but as a molecular marker for this process: Juvenile shoots have relatively high levels of miR156, and adult shoots have low levels (Wu and Poethig, 2006; Chuck et al., 2007). This makes it possible to investigate the nature of vegetative phase change in species that do not undergo significant morphological changes during vegetative development (homoblastic species; Goebel, 1900), as well as in species where the only evidence for phase change is variation in leaf shape. Although leaf shape is often used as evidence of phase change, this trait is influenced by many different factors and it is still unclear which aspects of leaf morphology are controlled by the phase-change mechanism, and which are under some other form of regulation. miR156 coordinates many agriculturally important traits via its targets, including leaf shape and size, the rate of leaf initiation, the rate of leaf expansion, stem diameter, adventitious root production, branch outgrowth, flowering time, and inflorescence architecture. Defining the specific pathways in which each of these targets operate, and learning how to individually modify the activity of these pathways, are major challenges that must be surmounted to enable precise engineering of shoot development. The temporal decrease in miR156 expression is of crucial importance, and until the mechanism of this event is known our understanding of vegetative phase change will remain juvenile.

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