ferent phases of vegetative development; the third regulates the differentiation of reproductive structures. The temporal sequence of these differentiation programs is regulated by threshold responses to graded environmental stimuli, such as light quality and endogenous spatial and temporal information. Differentiation programs are synchronized with organ production and growth via the effect of these programs on cell division and cell expansion, and by a thermal clock that is shared by all of these programs.

Much of the research on phase change has focused on a single event—floral induction. How this process is related to earlier developmental transitions remains to be determined, as does the mechanism of these transitions. Model organisms, such as *Arabidopsis* and maize, have produced rapid progress in our understanding of plant development in recent years and will be an important source of information about this central problem in shoot morphogenesis.

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35. I thank C. Hunter and D. Weigel for helpful comments.

REVIEW

Role of MicroRNAs in Plant and Animal Development

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Small RNAs, including microRNAs (miRNAs) and short interfering RNAs (siRNAs), are key components of an evolutionarily conserved system of RNA-based gene regulation in eukaryotes. They are involved in many molecular interactions, including defense against viruses and regulation of gene expression during development. miRNAs interfere with expression of messenger RNAs encoding factors that control developmental timing, stem cell maintenance, and other developmental and physiological processes in plants and animals. miRNAs are negative regulators that function as specificity determinants, or guides, within complexes that inhibit protein synthesis (animals) or promote degradation (plants) of mRNA targets.

The ability of multicellular organisms to produce specific types of cells and organs, in the proper places and at the right times during development, requires control and coordination of large sets of genes. Control of gene expression during development involves perception and integration of cellular and environmental signals. Whereas the roles of proteins as gene regulatory factors are well established, the functions of regulatory RNA molecules in development are just beginning to emerge. miRNAs are a class of small regulatory RNA and have generated considerable excitement recently. These RNA molecules were first discovered in the worm *Caenorhabditis elegans* through genetic screens for mutants that lacked the ability to control the timing of specific cell fate switches during development (*1*, *2*). Several hundred miRNAs from animals and plants have subsequently been identified through computational and cloning approaches (*3*–*13*). Analysis of miRNAs is leading to new paradigms for control of gene expression during development.

miRNAs (~21 to 22 nucleotides in length) arise from larger precursors (Fig. 1A) that are transcribed from non–protein-coding genes. The precursors form self-complementary fold-back structures and are processed by a ribonuclease III–like nuclease termed Dicer (animals) or DICER-LIKE1 [DCL1 (plants)] (*14*). The mature miRNAs function within large complexes to negatively regulate specific target mRNAs. Several miRNAs from animals interact with their targets through imprecise base-pairing, resulting in arrest of translation (*15*, *16*). Plant miRNAs generally interact with their targets through near-perfect complementarity and direct mRNA target degradation (*17*, *18*). Although chemically similar to miRNAs, siRNAs arise by Dicer or DICER-LIKE cleavage of long, double-stranded RNAs rather than specific fold-back structures. siRNAs function during RNA interference (RNAi) within an RNA-induced silencing complex (RISC) to guide sequence-specific cleavage of RNAs (*14*). siRNAs may also guide nuclear events, including histone and DNA methylation, resulting in transcriptional silencing (*19*–*21*).

Despite differences in origin, miRNAs and siRNAs are functionally interchangeable. Most known plant miRNAs guide target RNA degradation in a manner normally attributed to siRNAs (*18*, *22*, *23*). By contrast, most animal miRNAs interact with sufficiently low levels of complementarity to their targets so as not to guide cleavage (*24*). However, if an animal miRNA encounters a target with complete complementarity, it can enter the RNAi pathway and guide target degradation (*25*, *26*). Conversely, an siRNA can repress translation (without cleavage) if the degree of complementarity to its target is reduced (*27*). The activity directed by a miRNA or siRNA, therefore, depends chiefly on how precisely the small RNA anneals to the target.

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miRNAs and Animal Development

Estimates place the total number of distinct *C. elegans* and vertebrate miRNA genes at about 120 and 250 genes, respectively (*11*, *13*, *24*), indicating that miRNAs are a major class of regulatory molecule in animals. About 30% of the worm miRNAs are close in sequence to one or more insect and/or vertebrate miRNA, suggesting that a large fraction of miRNAs could play evolutionarily conserved developmental or physiological roles.

Some miRNAs exhibit temporal or tissue-specific patterns of gene expression (*3*, *4*, *6*, *11*, *24*), consistent with possible regulatory roles for these miRNAs in the control of development, as is the case for the miRNAs *lin-4* and *let-7* (*1*, *2*). For example, up-regulation of *lin-4* RNA in the second larval stage represses the expression of LIN-14 and LIN-28, two key regulators of early larval developmental transitions in *C. elegans* (Fig. 1). Curiously, the mechanism of translational repression by *lin-4* seems to involve intervention at a postinitiation step in protein synthesis (*15*, *16*).

The roles for *lin-4* and *let-7* as temporal regulators of development in other animals are supported by the phylogenetic conservation of their temporal patterns of up-regulation (*28*). In *Drosophila*, *let-7* and the *lin-4* homolog *mir-125* are up-regulated in concert at the onset of metamorphosis (*6*, *29*, *30*). In *Drosophila* and some vertebrates, the *let-7* and *mir-125/lin-4* genes are closely linked and therefore may be coregulated. Perhaps the distinct roles for *lin-4* and *let-7* at different developmental stages in *C. elegans* represent an adaptation of a more widely conserved collaboration between these two miRNAs. Like worm LIN-28 protein, vertebrate LIN-28 homologs are also down-regulated during development. Moreover, the mouse and human *lin-28* 3′-untranslated regions (UTRs) contain predicted *lin-4* complementary sites, suggesting that the *lin-4*–*lin-28* regulatory relationship may also be conserved (*31*).

lin-4 and *let-7* had been the only miRNAs for which regulatory roles were demonstrated by the analysis of mutant phenotypes. However, recent screens for *Drosophila* mutants that exhibit growth defects identified the *bantam* locus, which encodes a miRNA that functions to repress apoptosis and promote cell proliferation in the developing fly (*32*) (Fig. 1). *bantam* miRNA seems to be expressed broadly, and represses the translation of the mRNA for *Hid*, a key activator of programmed cell death. *bantam* is related to *mir-80-82* of *C. elegans* (*24*, *32*), suggesting that the *mir-80* family might control developmental cell death and/or cell proliferation in the worm. In a screen for *Drosophila* genes that oppose the cell death activator Reaper, mutations in the *mir-14* miRNA gene were identified that also affected aspects of fat metabolism (*33*). Given the importance of these cellular processes in animals other than flies and worms, and the conservation of a large proportion of miRNAs across species boundaries, miRNAs are likely to have broad significance in a wide range of developmental processes in animals.

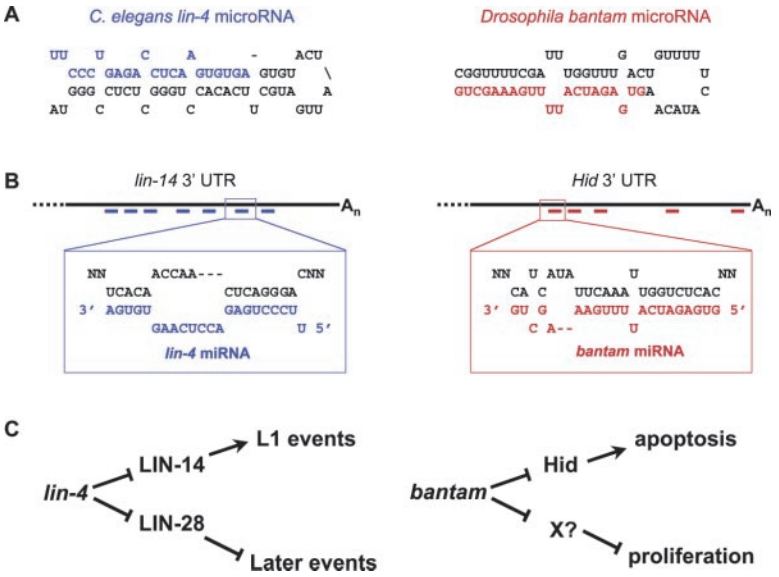

Fig. 1. Structure and function of worm and fly miRNAs. (**A**) The predicted secondary structures for *C. elegans lin-4* (left) and *Drosophila bantam* (right) miRNAs. Positions of mature miRNAs within precursors are highlighted in blue (*lin-4*) and red (*bantam*). (**B**) *C. elegans lin-4* is partially complementary to seven heterogeneous sites (one shown) in the 3′ UTR of *lin-14* mRNA. The 3′ UTR of *Drosophila* Hid mRNA contains five sites that are partially complementary to the *bantam* miRNA. (**C**) Proposed developmental roles for *lin-4* and *bantam* in *C. elegans* and *Drosophila*, respectively. *lin-4* miRNA expression at the end of the worm L1 larval stage results in down-regulation of LIN-14 and LIN-28 protein synthesis, controlling the transition from L1 to later developmental events. *bantam* miRNA in cells of the fly larva acts through the repression of Hid, and probably other targets ("X"), to control the selection of programmed cell death and cell proliferation, respectively.

miRNAs and Plant Development

Realization of the roles of miRNAs in plant development came largely through two routes—analysis of genes required for miRNA formation or activity, and identification of miRNA target genes. Loss-of-function *dcl1* mutants of *Arabidopsis* were recovered in screens for genes involved in embryo, vegetative, and reproductive development (*34*). Defects associated with *dcl1* mutants include overproliferation of meristems (which contain pluripotent stem cells), conversion of normally determinate floral meristems into indeterminate meristems, delayed flower timing, and overproliferation of embryonic suspensor cells (*34*). *dcl1*mutants have reduced levels of miRNAs and ectopically express miRNA target genes (*9*, *10*, *22*). Mutations in other genes that are involved in miRNA formation (*HEN1*), or that are suspected to function in miRNA pathways (*AGO1*), also cause *dcl1*-like phenotypes and leaf polarity defects (*10*, *35*). Further, the RNA-silencing suppressor encoded by *Turnip mosaic virus* interferes with miRNA activity, causes ectopic expression of miRNA target genes, and induces a spectrum of defects that overlap with those of *dcl1*, *ago1*, and *hen1* mutants (*22*). These data suggest that miRNAs function as negative regulators to control meristem cell identity, organ polarity, and other developmental processes. Like animal miRNAs, plant miRNAs exhibit temporal and tissue-specific expression patterns (*8*–*10*).

Numerous *Arabidopsis* mRNA targets were predicted on the basis of near-perfect complementarity with miRNAs, and these predictions were supported by statistical data and phylogenetic conservation in rice (*17*). Fifteen cleavage-type targets were validated recently by in vitro or in vivo miRNA-guided cleavage assays (*18*, *22*, *23*). Strikingly, the vast majority of predicted targets encode members of large families of transcription factors. These include APETELA2 (AP2), CUP-SHAPED COTYLEDONS1 (CUC1) and CUC2, and PHAVOLUTA (PHV) and PHABULOSA (PHB). These and other transcription factors encoded by miRNA target genes are collectively required for meristem identity, cell division, organ separation, and organ polarity. A smaller number of targets correspond to mRNAs encoding factors required for miRNA formation or function. For example, *DCL1* mRNA itself is an miRNA target, indicating that the miRNA apparatus in plants is regulated by a negative-feedback loop (*36*) (Fig. 2A).

Although regulatory roles for target mRNA cleavage during development are predicted, circumstantial evidence supports a role for miR165/166-guided target cleavage in radial patterning of leaves. The HD-Zip transcription factors PHV and PHB control the establishment of adaxial (upper

surface)–abaxial (lower surface) polarity, resulting in structurally and functionally specialized leaf surfaces. The *PHV* and *PHB* genes are normally expressed in leaf primordia cells near the shoot meristem to specify adaxial cell fate, and are turned off in zones that develop into abaxial cells at positions distant from the meristem (*37*). Several dominant *phv* and *phb* alleles contain nucleotide substitutions within the miR165/166 interaction site, resulting in reduced cleavage of *phv* and *phb* mRNAs (*23*, *37*). The mutant alleles are expressed ectopically in meristem-distal zones of primordia, converting cells normally fated for abaxial surfaces to display adaxial-like features. This suggests that miR165/166-mediated regulation of *PHV* and *PHB* controls the transcriptional program that initially distinguishes adaxial and abaxial fated cells (*17*, *23*) (Fig. 2B).

Outlook

We are at the initial stages of understanding the roles of miRNAs during development. Although several animal mRNA targets have been identified, there are likely hundreds more that have yet to be discovered. While target identification can, in principle, reveal the breadth of developmental processes that are under miRNA regulation in animals, the imprecise complementarity of animal miRNA recognition sites makes target validation a major challenge. Do all animal miRNAs act as repressors, and do they all affect translation by similar biochemical mechanisms? What developmental signals control miRNA gene transcription and miRNA processing? Despite the abundance of cleavage-type miRNAs in plants, do plant miRNAs also act by nondegradative modes? We anticipate that the study of these small RNAs will continue to deliver many surprises in the future.

Note added in proof. Several papers describing developmentally relevant miRNA targets were published after this review was completed (*38*–*40*).

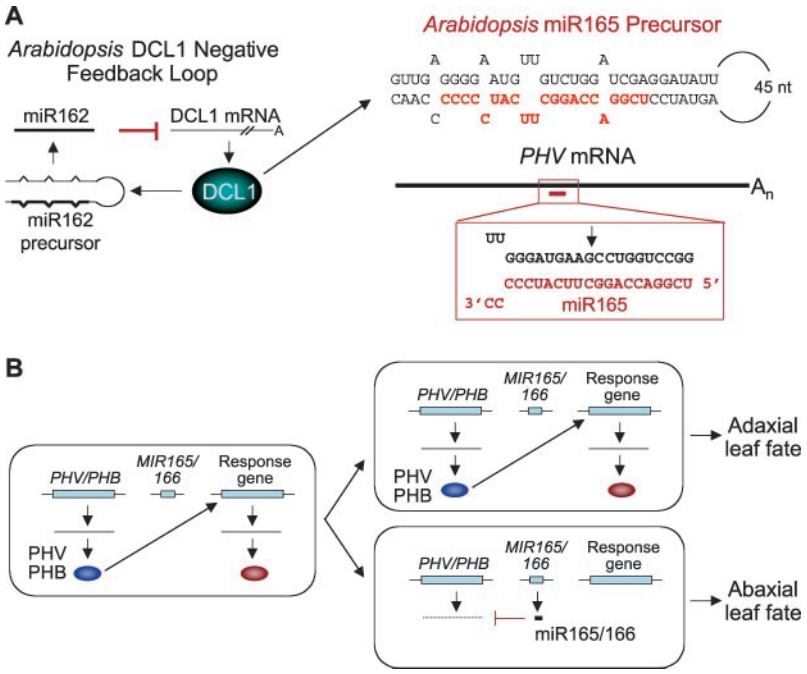

Fig. 2. Structure and function of *Arabidopsis* miRNAs. (**A**) Expression of *DCL1*, which catalyzes miRNA precursor processing, is under negative-feedback regulation by miR162 (left). miR165/166 negatively regulates *PHV* and *PHB* mRNAs by guiding sequence-specific cleavage (right). *PHV* and *PHB* are related genes encoding HD-Zip transcription factors. miR165 and miR166 are related miRNAs that are predicted to interact with *PHV* and *PHB* mRNAs. Only *PHV* mRNA and miR165 are represented. Arrow, miR165-guided cleavage site. (**B**) Model for specification of adaxial/abaxial polarity in *Arabidopsis* leaves. Expression of *PHV* and *PHB* in leaf primordium cells close to the meristem results in a transcription program specifying adaxial fate. Inhibition of *PHV* and *PHB* by miR165/166-guided degradation in cells distant to the meristem specifies abaxial fate.

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41. We acknowledge support from NSF (MCB-0209836 to J.C.C.) and NIH (GM34028 to V.A. and AI43288 to J.C.C.).

Role of MicroRNAs in Plant and Animal Development

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Science **301** (5631), 336-338.
DOI: 10.1126/science.1085242