

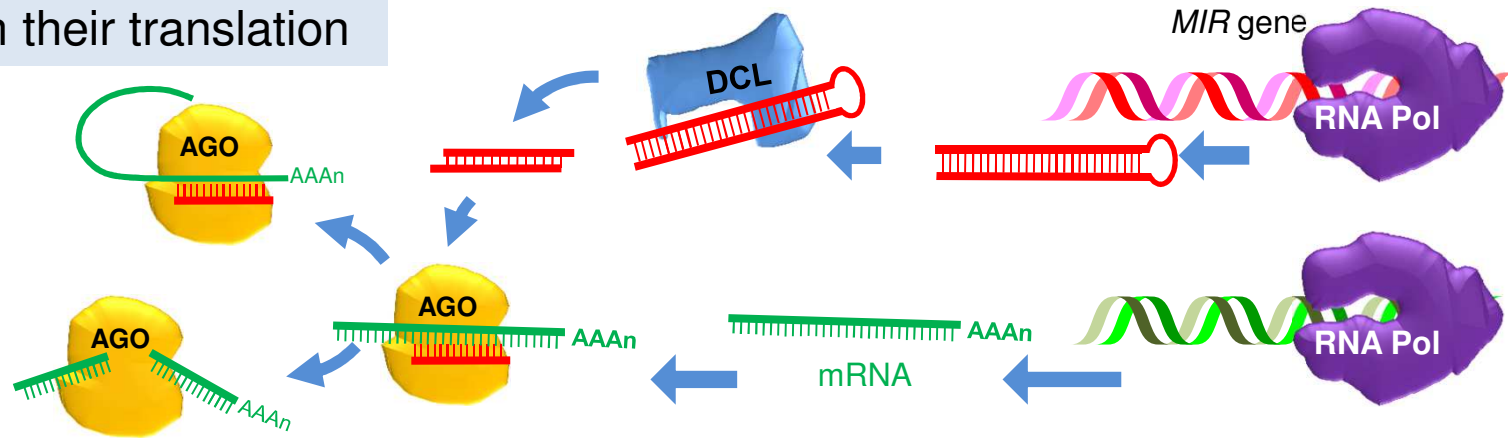
# microRNAs - miRNAs

- miRNAs are thought to have evolved from siRNAs, and are produced and processed somewhat similarly
- Plants have a small number of highly conserved miRNAs, and a large number of non-conserved miRNAs
- miRNAs are encoded by specific *MIR* genes but act on other genes – they are trans-acting regulatory factors
- miRNAs in plants regulate developmental and physiological events



# microRNAs - miRNAs

microRNAs slice mRNAs or interfere with their translation

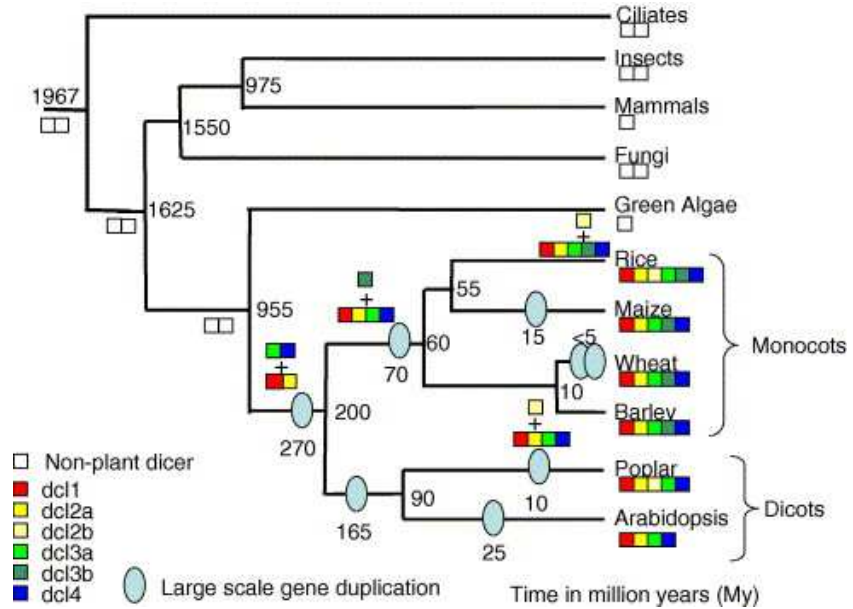


# miRNAs and siRNAs are processed by related but different DCL proteins

AtDCL1 produces **miRNA**

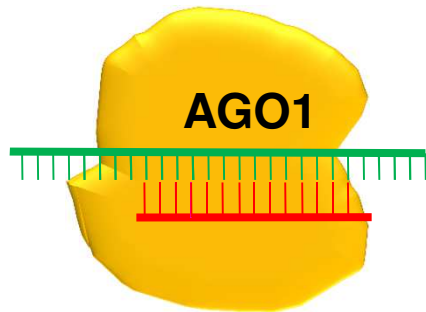


AtDCL2 - 4 produce **siRNA**

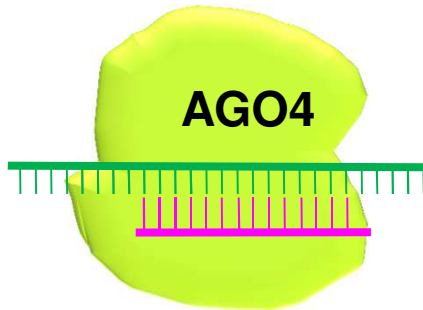


Plants have 4 or more DCL proteins, more than found in other organisms. The amplification of DCL proteins is thought to allow plants great flexibility in pathogen defense responses.

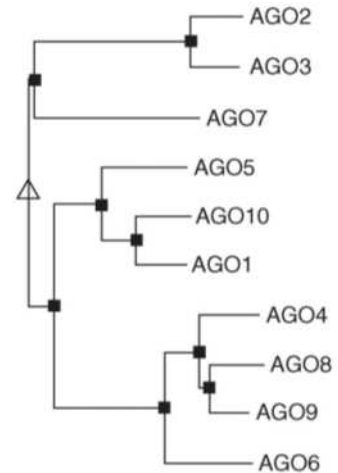
# miRNAs and siRNAs associate with several AGO proteins



AGO1 preferentially slices its targets and associates with **miRNAs** but also some **siRNAs**



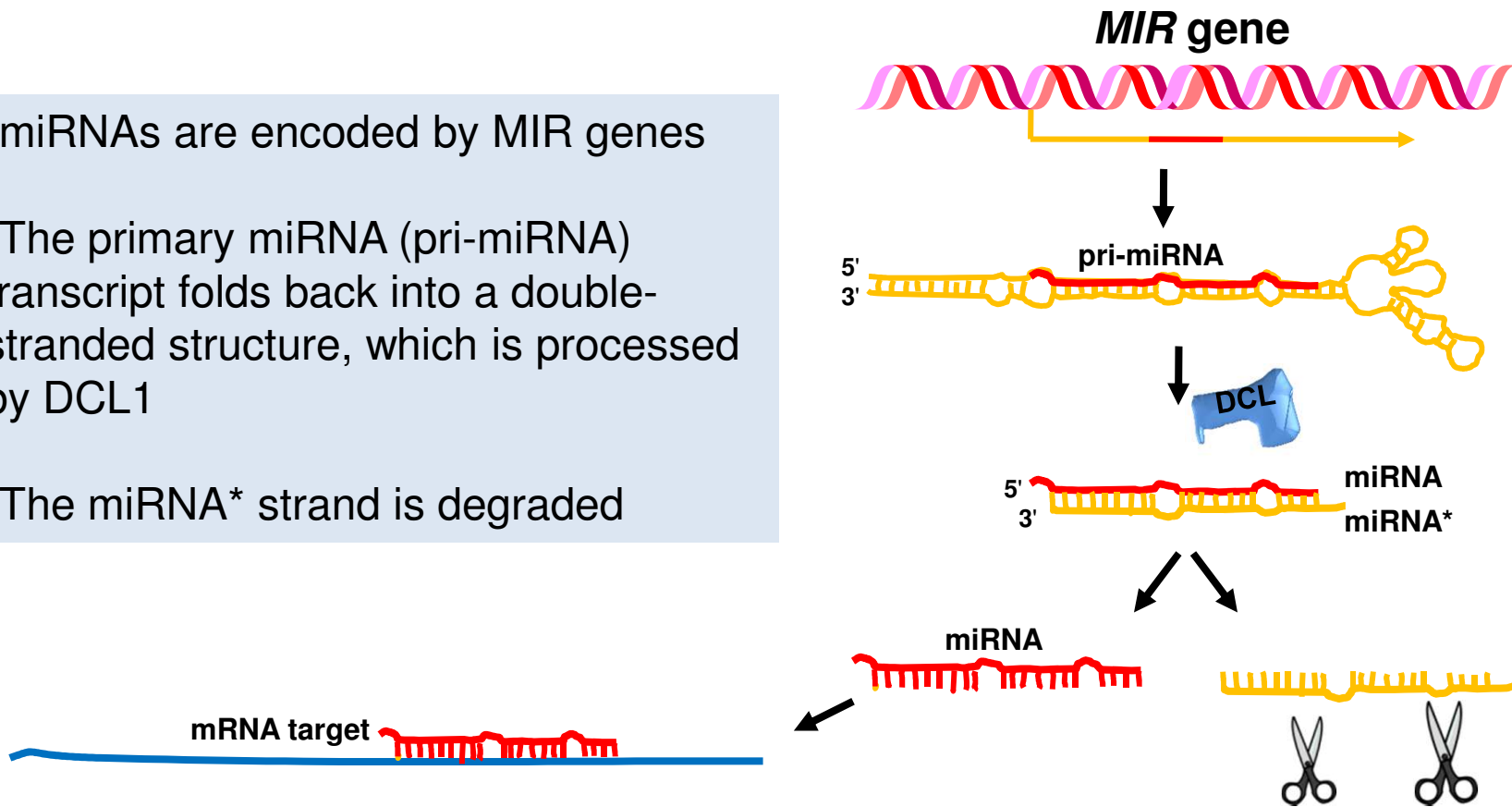
AGO4 preferentially associates with **siRNA** and mediates methylation of source DNA.



Arabidopsis has 10 AGO proteins. They are not all well characterized and there is some functional overlap.

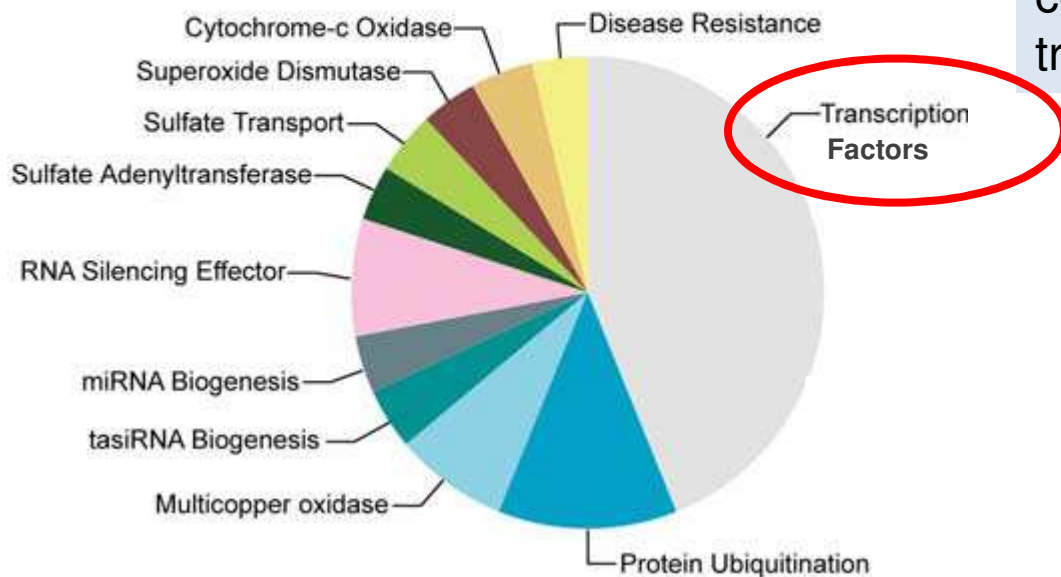
# *MIR* genes are transcribed into long RNAs that are processed to miRNAs

- miRNAs are encoded by *MIR* genes
- The primary miRNA (pri-miRNA) transcript folds back into a double-stranded structure, which is processed by DCL1
- The miRNA\* strand is degraded



# Some miRNAs are highly conserved and important gene regulators

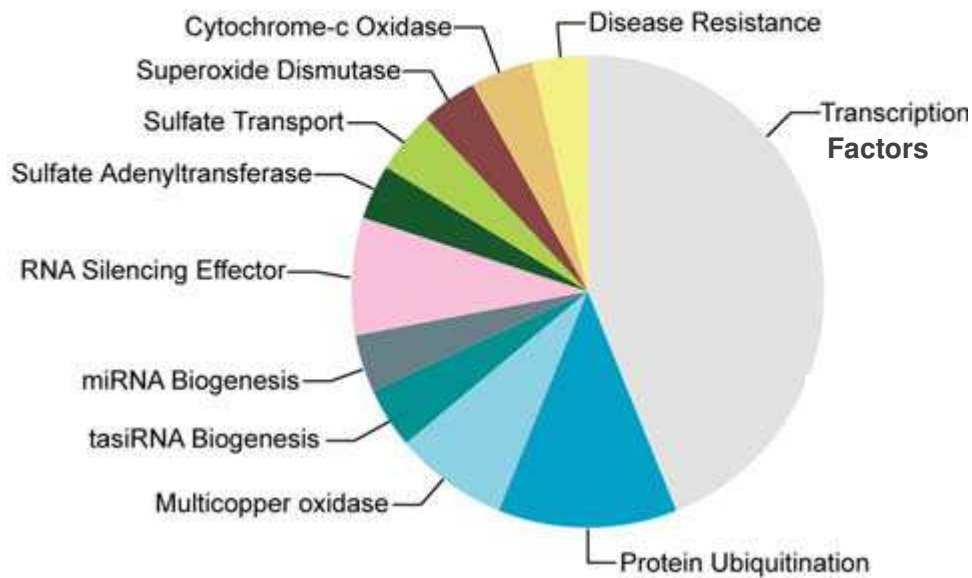
Conserved miRNA target functions



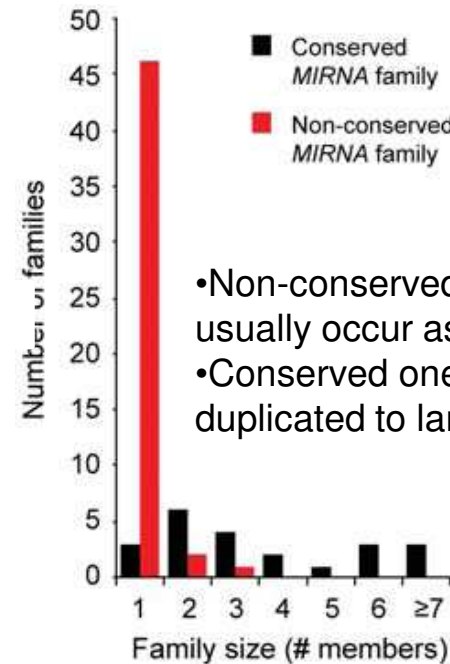
Nearly half of the targets of conserved miRNAs are transcription factors.

# Some miRNAs are highly conserved and important gene regulators

Conserved miRNA target functions



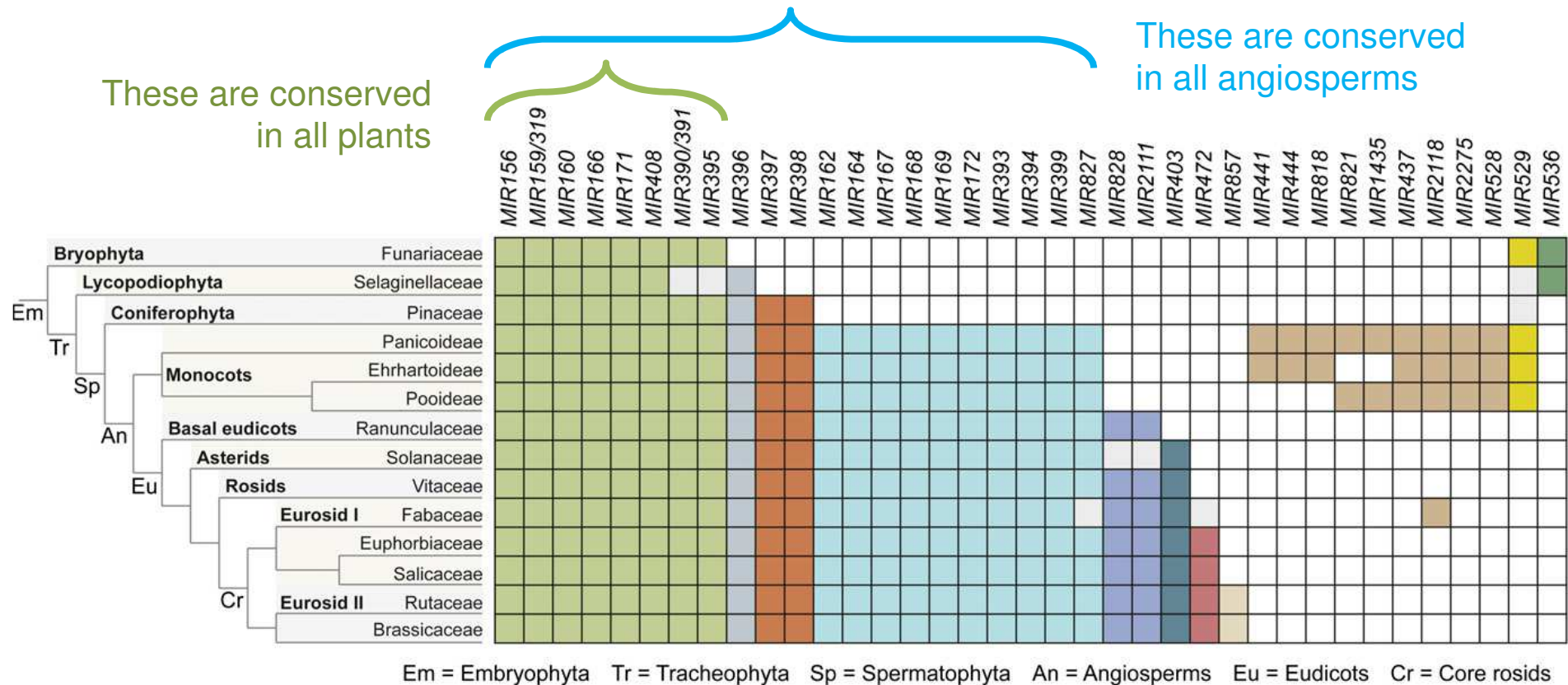
Nearly half of the targets of conserved miRNAs are transcription factors.



- Non-conserved *MIRNA* families usually occur as single genes
- Conserved ones have often duplicated to larger gene families

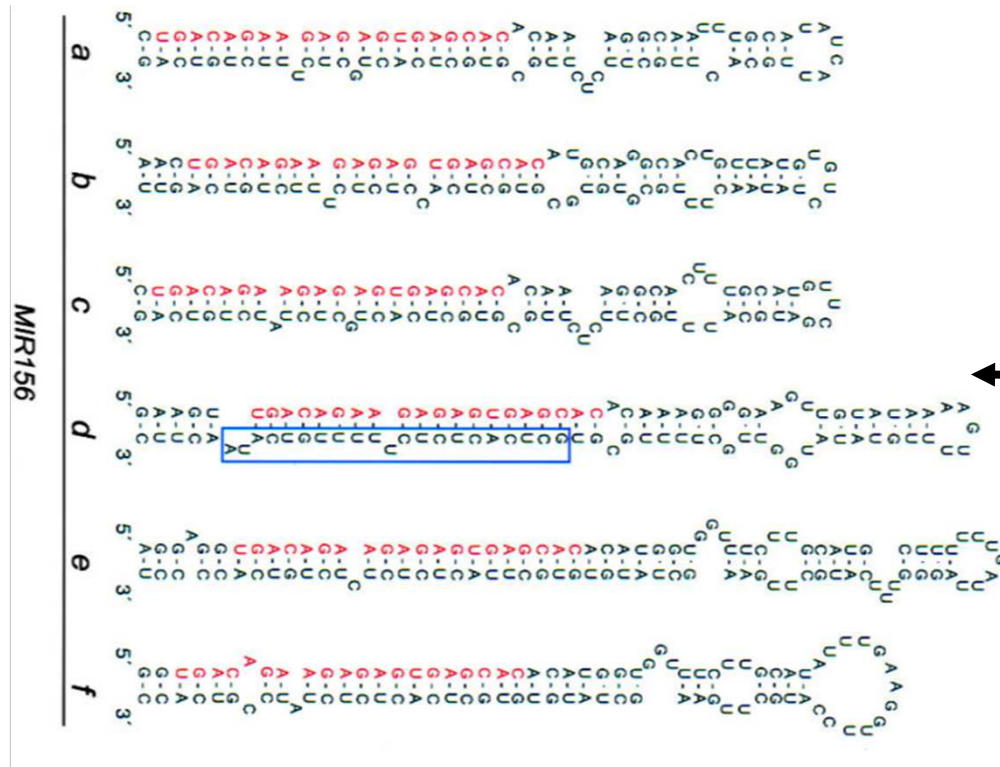
Fahlgren, N., Howell, M.D., Kasschau, K.D., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., Law, T.F., Grant, S.R., Dangi, J.L., and Carrington, J.C. (2007) High-throughput sequencing of *Arabidopsis* microRNAs: Evidence for frequent birth and death of *MIRNA* genes. PLoS ONE. 2007; 2(2): [e219](https://doi.org/10.1371/journal.pone.00219).

# Some *MIR* gene families are present in all plants or all angiosperms





# The *MIR156* gene family is highly conserved



- miR156 is highly conserved within the plant kingdom
- miR156 is found in angiosperms as well as mosses
- miR156 is encoded by six or more genes in Arabidopsis
- miR156 targets transcription factors that control developmental phase changes

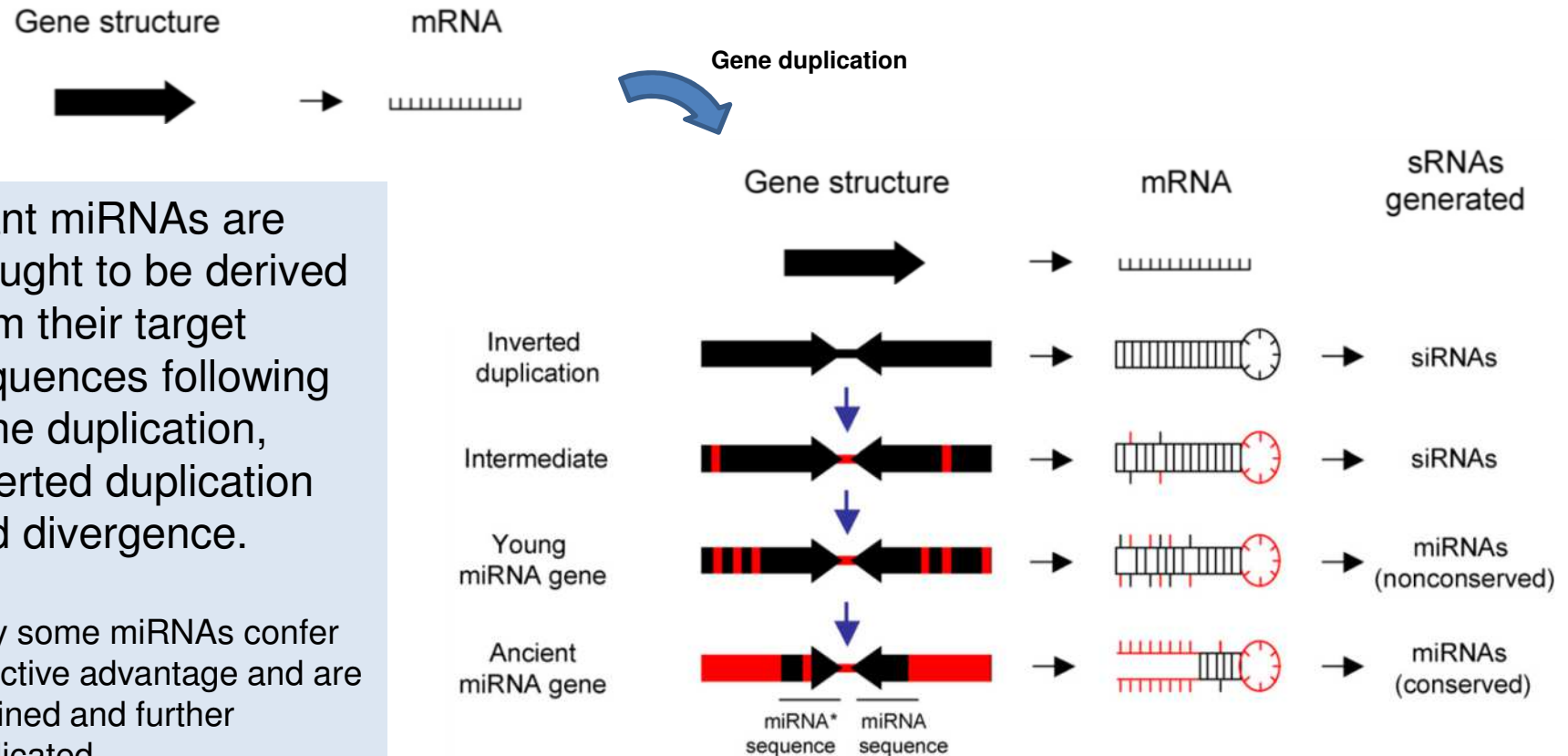
# Targets of some conserved miRNAs

miRNA gene family	Target gene family	Function
156	SPL transcription factors	Developmental timing
160	ARF transcription factors	Auxin response, development
165/6	HD-ZIPIII transcription factors	Development, polarity
172	AP2 transcription factors	Developmental timing, floral organ identity
390	TAS3 (tasiRNA) which acts on ARF transcription factors	Auxin response, development
395	Sulfate transporter	Sulfate uptake
399	Protein ubiquitination	Phosphate uptake

# Plant miRNAs are thought to be distantly related to their targets

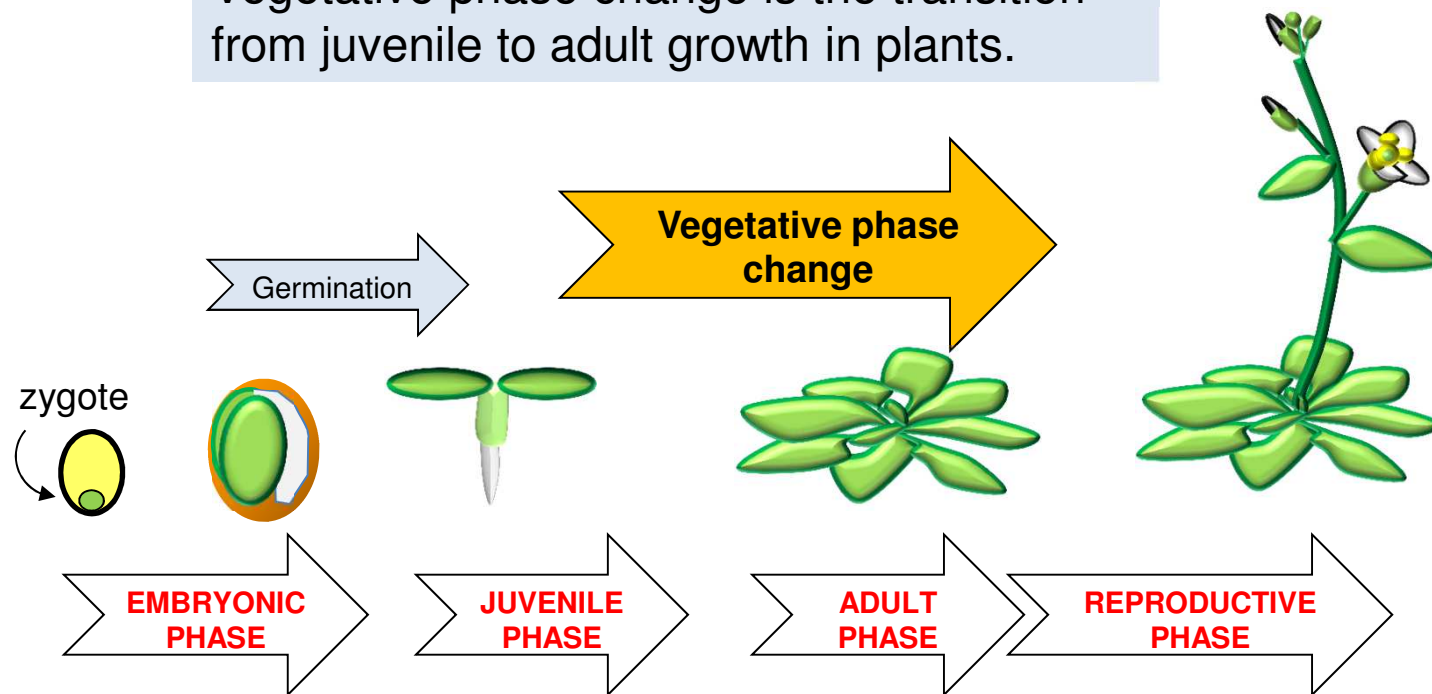
Plant miRNAs are thought to be derived from their target sequences following gene duplication, inverted duplication and divergence.

Only some miRNAs confer selective advantage and are retained and further duplicated.



# miRNAs and vegetative phase change

Vegetative phase change is the transition from juvenile to adult growth in plants.



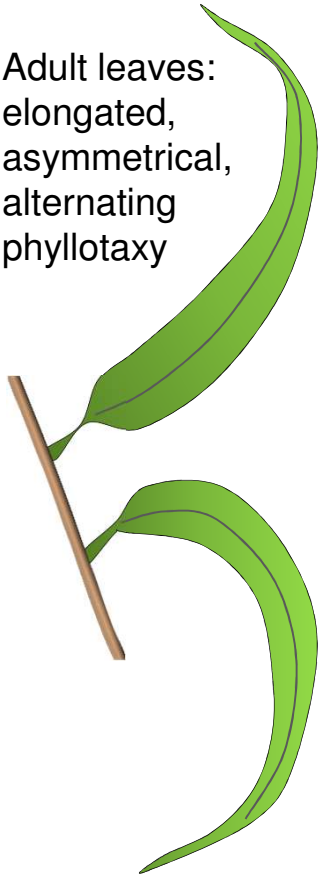
# Vegetative phase change affects morphology and reproductive competence



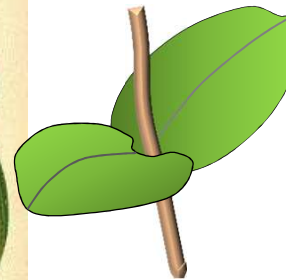
Some cacti have very different juvenile and adult growth patterns.

# Phase change can affect leaf shape, phyllotaxy, and trichome patterns

Adult leaves:  
elongated,  
asymmetrical,  
alternating  
phyllotaxy



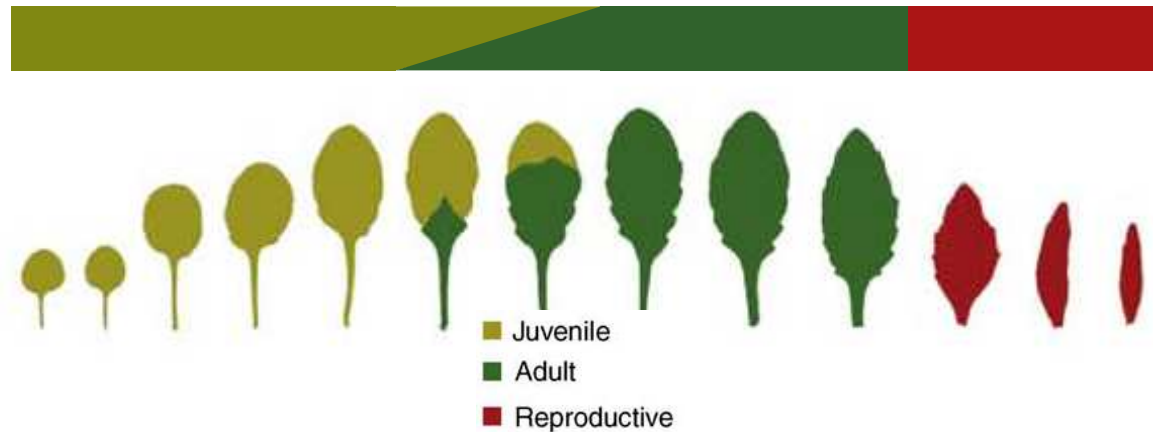
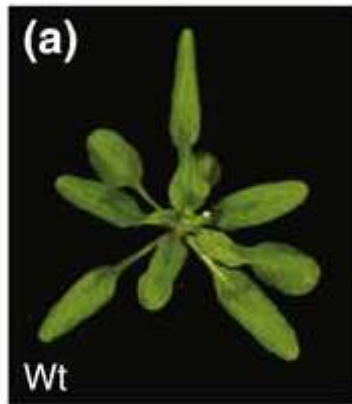
Juvenile leaves:  
rounded,  
symmetrical,  
opposite  
phyllotaxy



Eucalyptus leaves are strongly dimorphic, as are leaves of holly and ivy. In other plants including Arabidopsis and maize the change is more subtle.

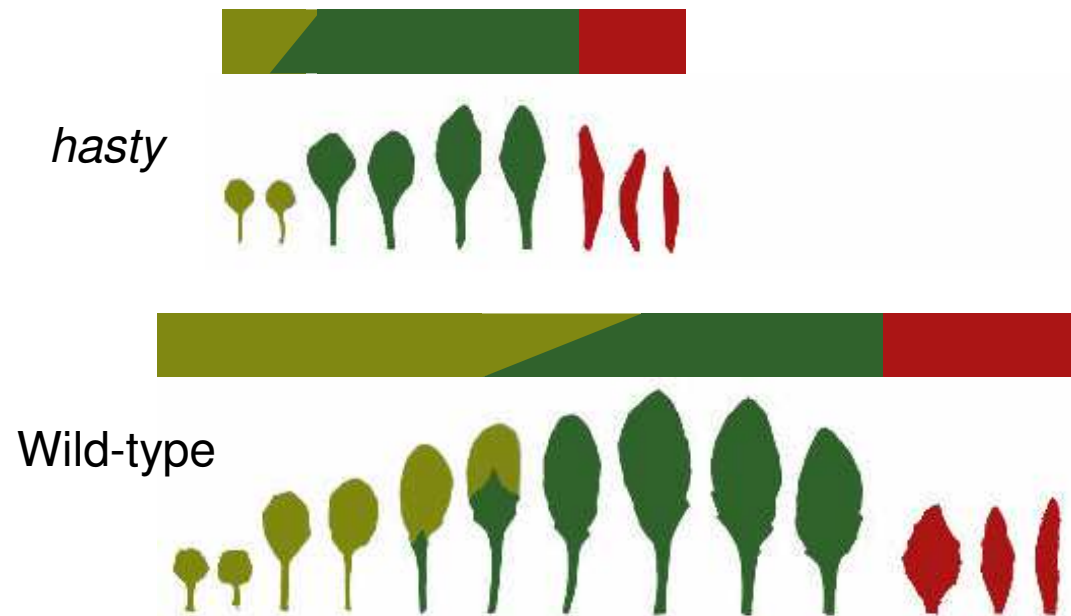
*Eucalyptus globulus*

# In Arabidopsis, phase change affects leaf shape and trichome patterning



Juvenile leaves are rounder, less serrated, and have trichomes only on the upper (adaxial) surface; adult leaves also have trichomes on the lower (abaxial) surface.

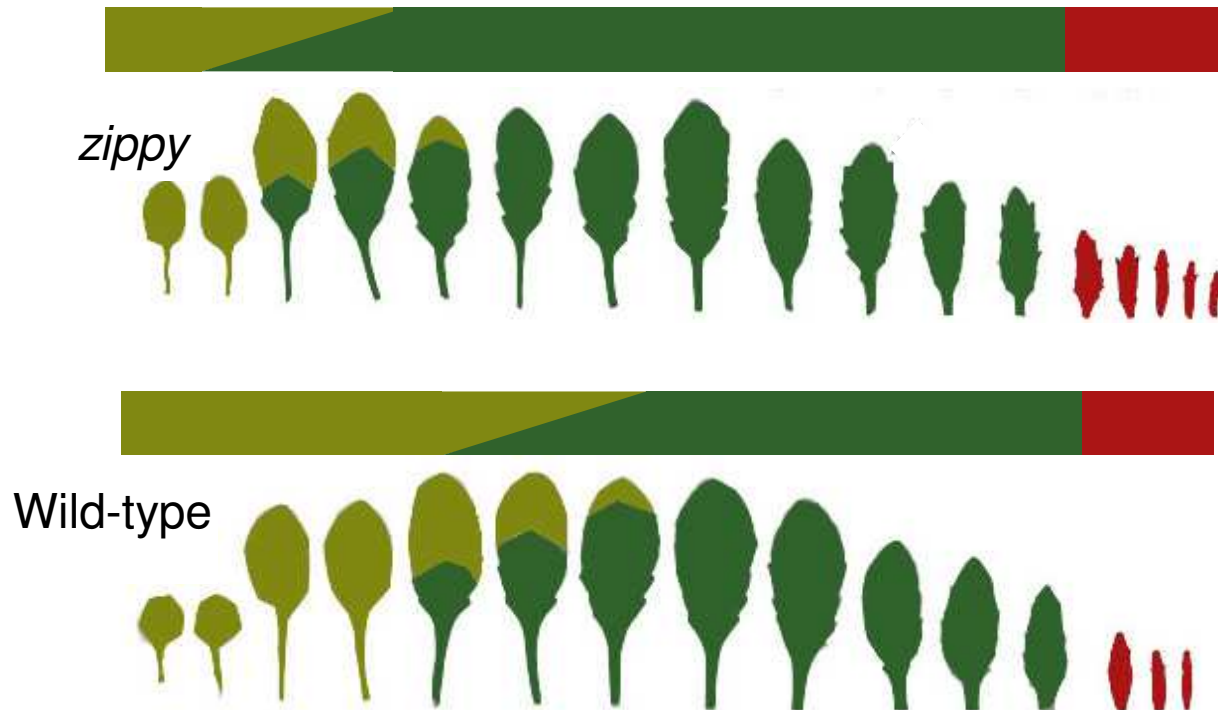
# Phase change is specified by miRNAs



*HASTY*, with a shortened juvenile phase, encodes a protein needed for miRNA export from nucleus to cytoplasm.



# Phase change is specified by miRNAs



Loss-of-function *zippy* mutants prematurely express adult vegetative traits. *ZIPPY* encodes an ARGONAUTE protein, AGO7.

# miR156 overexpression prolongs juvenile phase in Arabidopsis



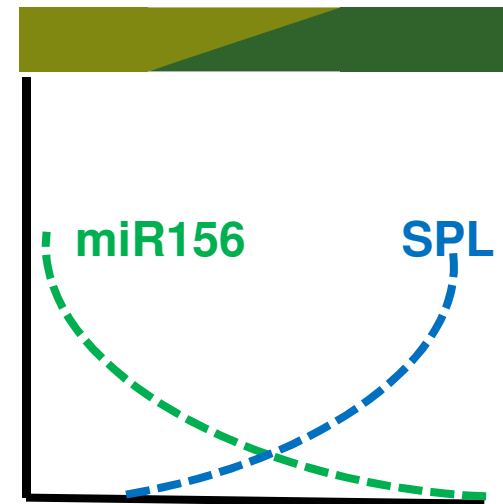
Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. *Curr. Opin. Genet. Dev.* 19: [374-378](#), with permission from Elsevier.

# miR156 targets *SPL* genes, promoters of phase change

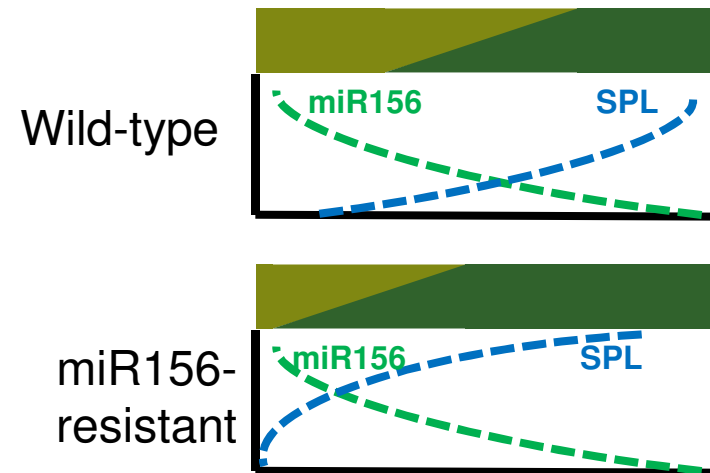
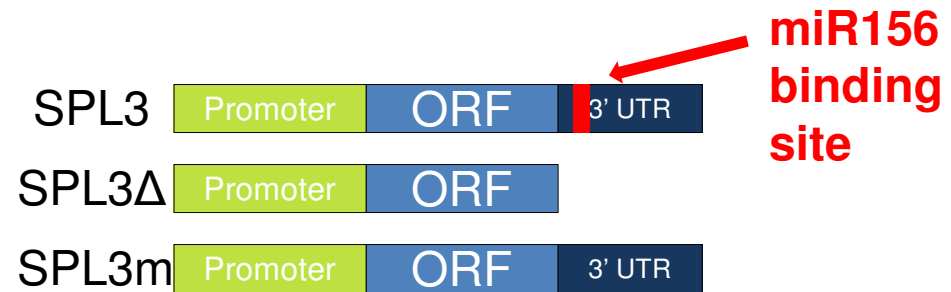
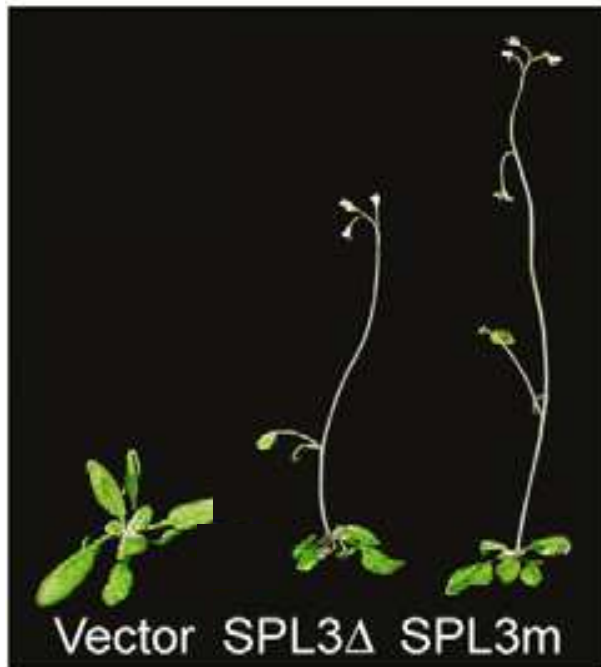
The *SPL* genes are a family of transcription factors that are miR156 targets.



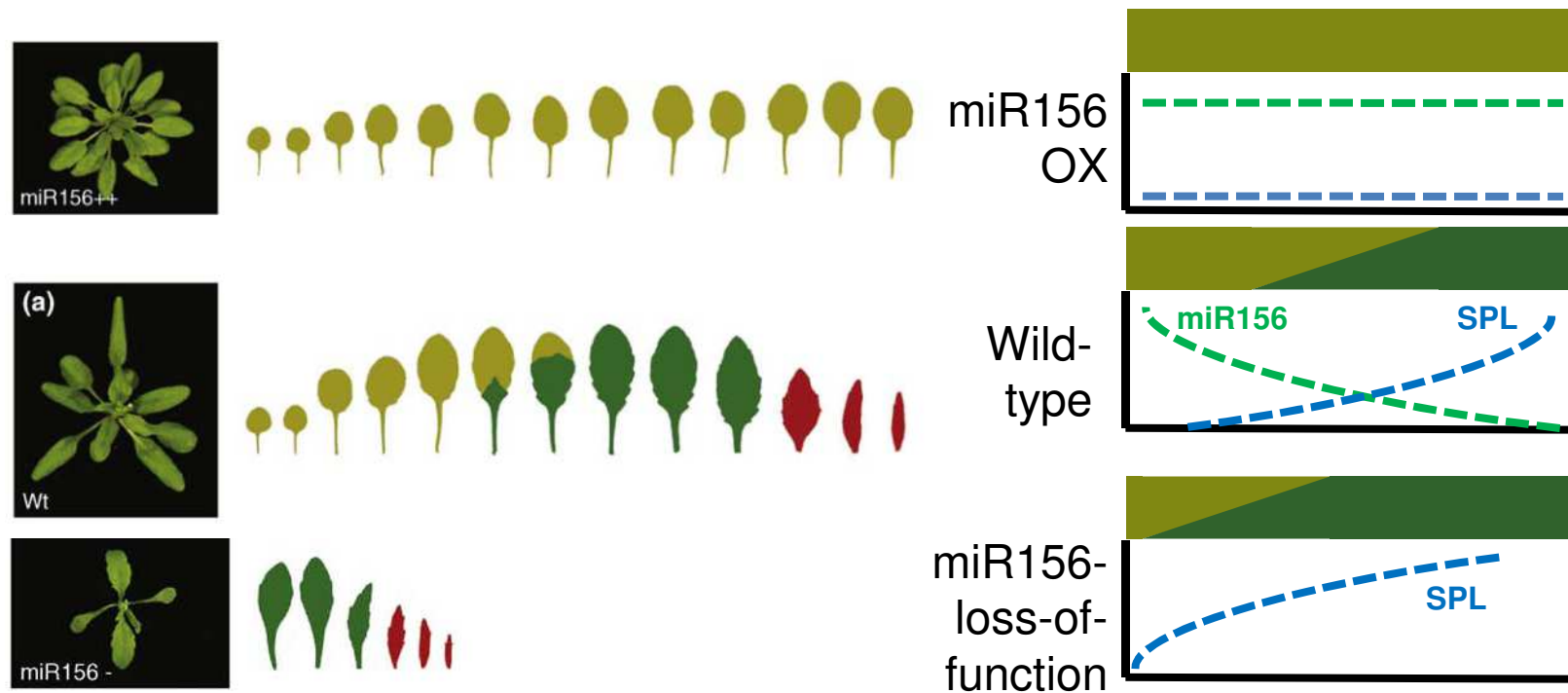
In wild-type plants, miR156 expression decreases with plant age, allowing *SPL* to accumulate and promote phase change.



# miR156-resistant SPL promotes precocious phase change

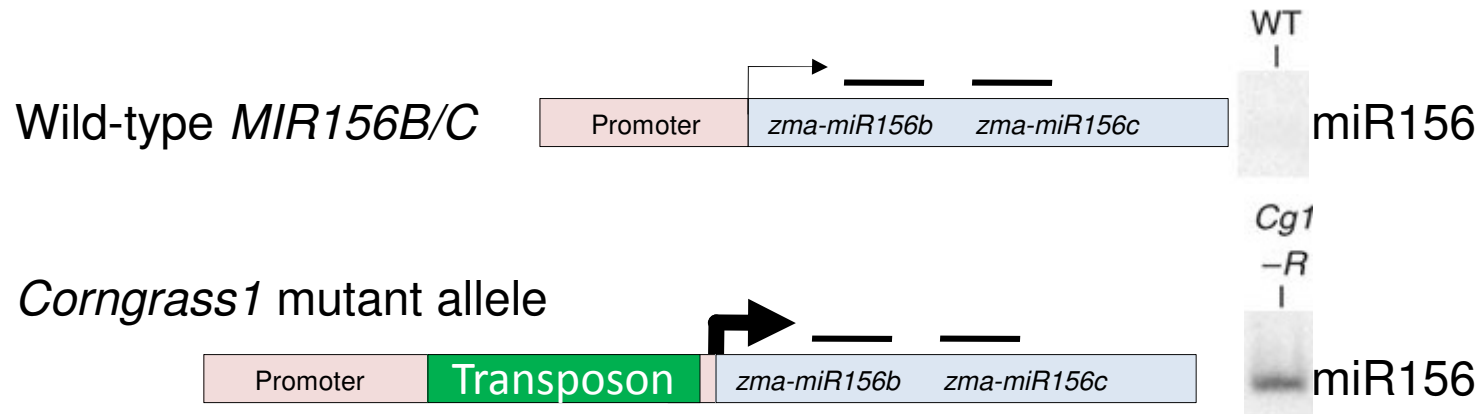


# miR156 loss-of-function promotes precocious phase change



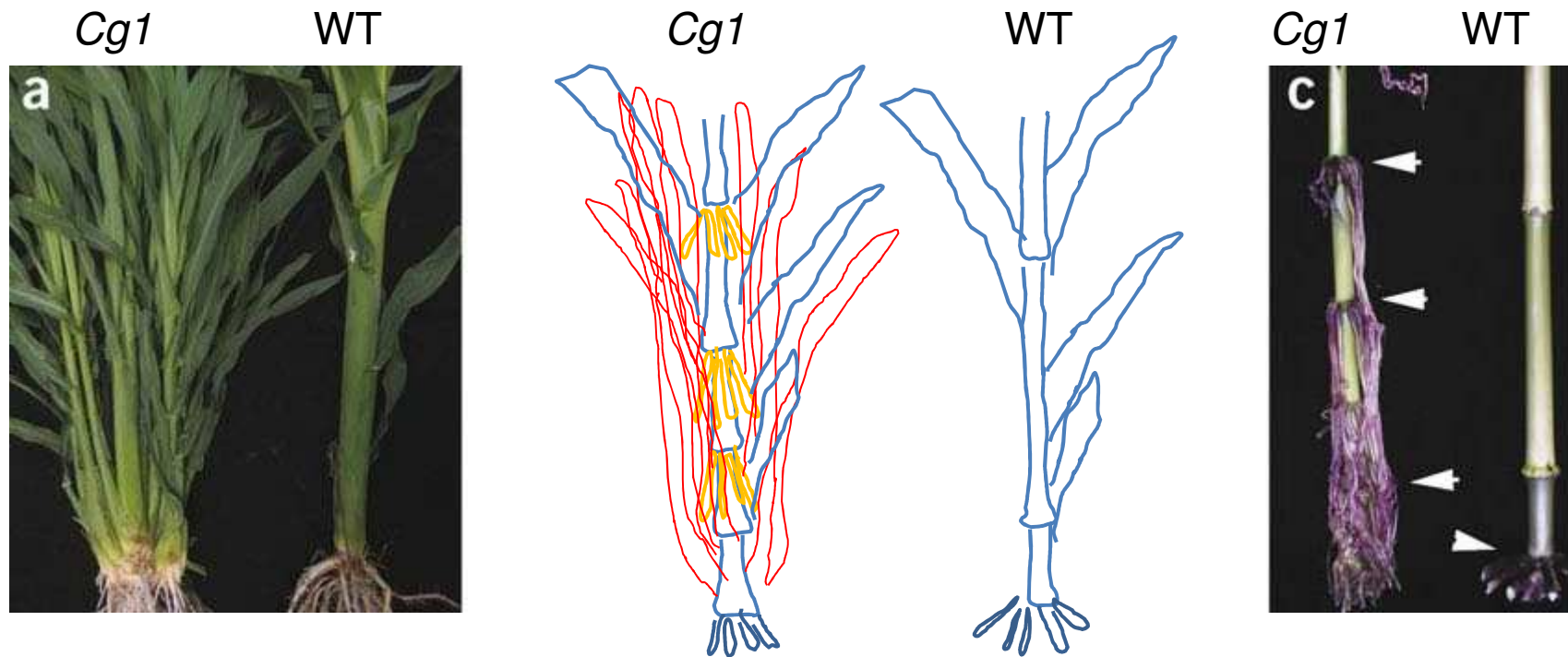
Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. *Curr. Opin. Genet. Devel.* 19: [374-378](#), with permission from Elsevier.

# The function of miR156 in promoting developmental change is conserved



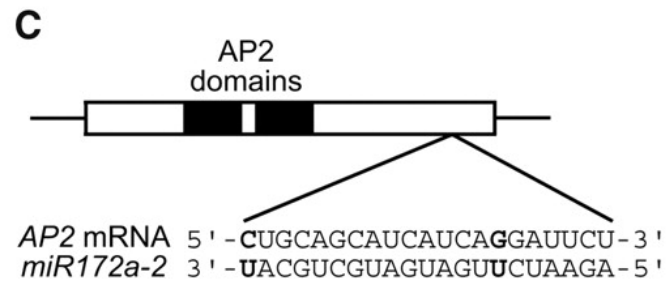
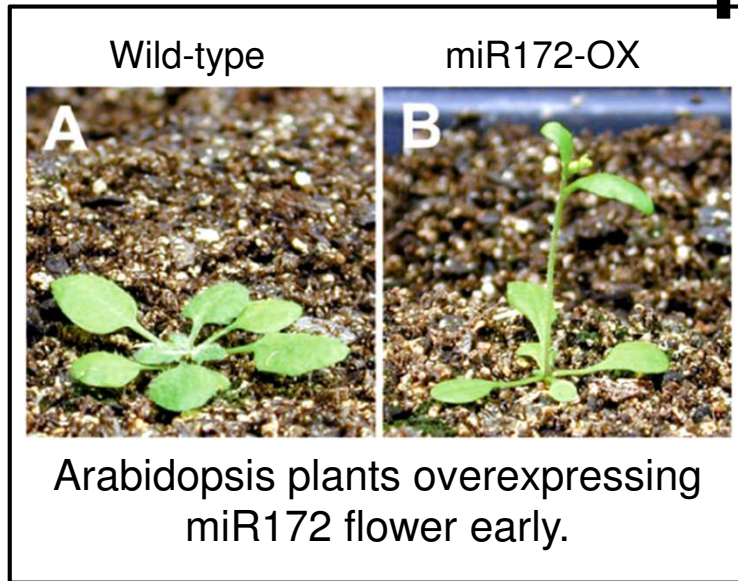
In the *Zea mays* *Corngress1* mutant (*Cg1*), a transposon inserted upstream of the *MIR156B/C* locus increases its transcription level, causing more miR156 to accumulate.

# Elevated expression of miR156 in *Cg1* prolongs the juvenile phase



Adult *Corngrass1* plants express juvenile traits of tillers (red) in leaf axils and roots (yellow) from leaf nodes.

# miR172 promotes flowering in Arabidopsis by targeting AP2-like transcription factors



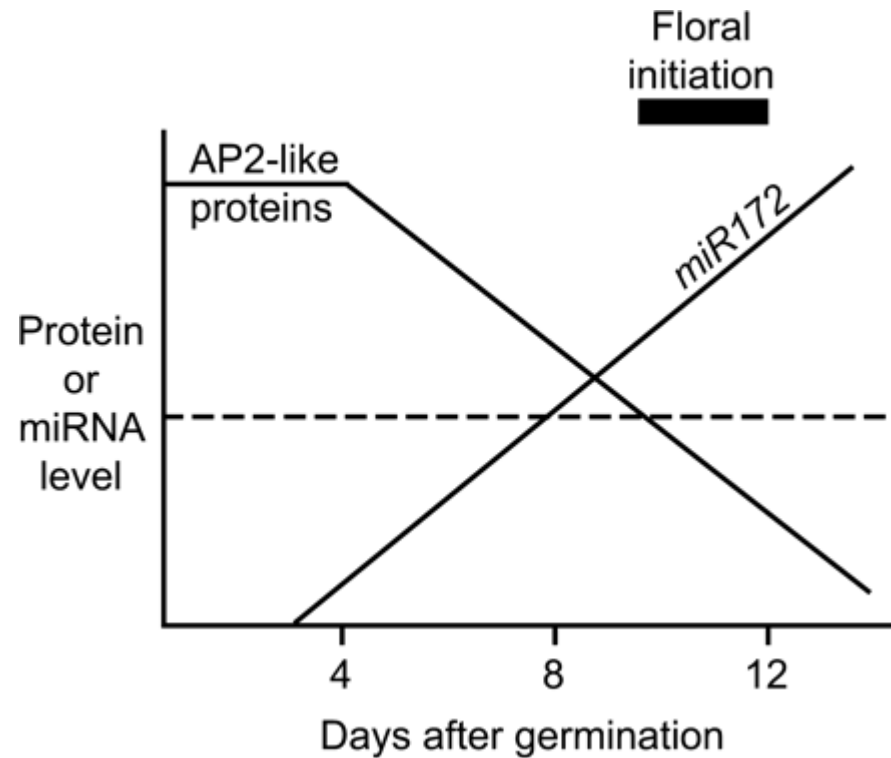
**D**

miR172 homology

AP2	A	C	C	A	A	G	T	G	A	C	A	A	A	T	G	C	T	G	C	A	G	C	A	T	C	A	T	C	A	G	G	A	T	T	C	T	C	C	T	C	A	T	C	A	T	C	A	C	A	A	T	C	A	G	A	1407				
TOE1	C	A	C	C	A	T	T	G	T	T	C	T	C	A	G	T	T	G	C	A	G	C	A	T	C	A	T	C	A	G	G	A	T	T	C	T	C	A	C	A	T	T	T	C	C	G	G	C	C	A	C	A	A	C	C	T	C	1593		
TOE2	C	A	C	C	G	C	C	A	C	T	G	T	T	T	C	A	A	A	T	G	C	A	G	C	A	T	C	A	T	C	A	G	G	A	T	T	C	T	C	A	C	T	C	T	C	A	G	C	T	A	C	A	C	G	C	C	T	C	1320	
TOE3	G	A	A	A	T	C	G	A	G	T	G	G	T	G	G	A	A	T	G	C	A	G	C	A	T	C	A	T	C	A	G	G	A	T	T	C	T	C	T	C	A	A	C	C	T	T	C	C	C	T	T	A	C	C	1106					
IDS1	A	C	G	T	G	C	C	G	T	T	G	C	A	C	C	A	C	T	C	T	G	C	A	G	C	A	T	C	A	T	C	A	G	G	A	T	T	C	T	C	T	A	C	G	C	C	G	C	G	G	G	C	C	A	A	C	G	1567		
GL15	A	C	G	C	C	A	G	C	A	G	C	A	G	C	G	C	C	G	C	T	G	C	A	G	C	A	T	C	A	T	C	A	G	G	A	T	T	C	C	A	C	T	G	T	G	G	C	A	G	C	T	G	G	G	T	G	C	G	G	1692

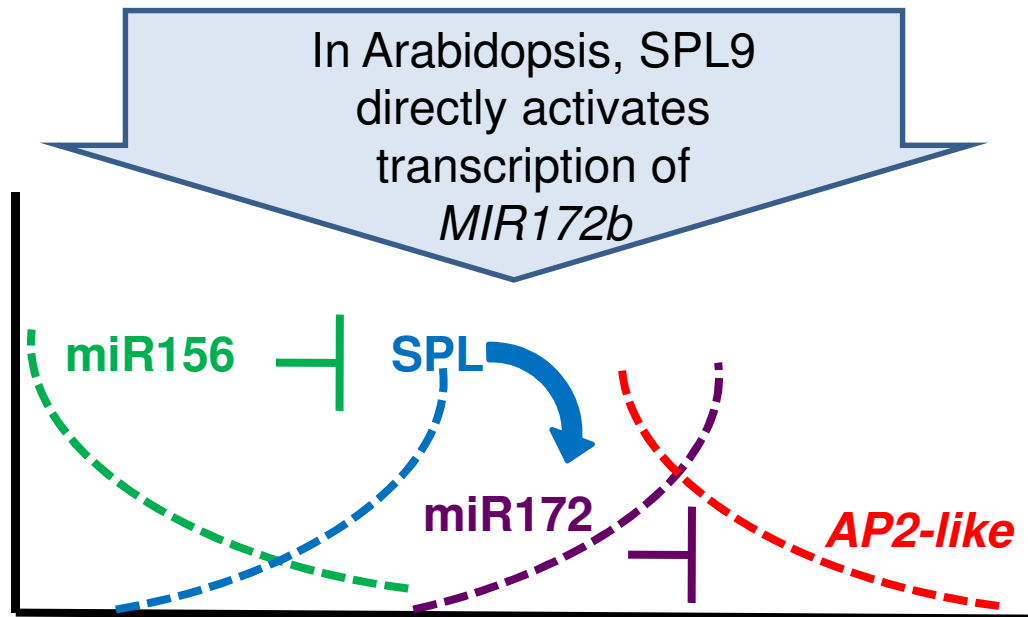


# miR172 expression temporally regulates AP2-like proteins



It is thought that floral initiation can occur when the level of AP2-like floral inhibitors drops below a certain level.

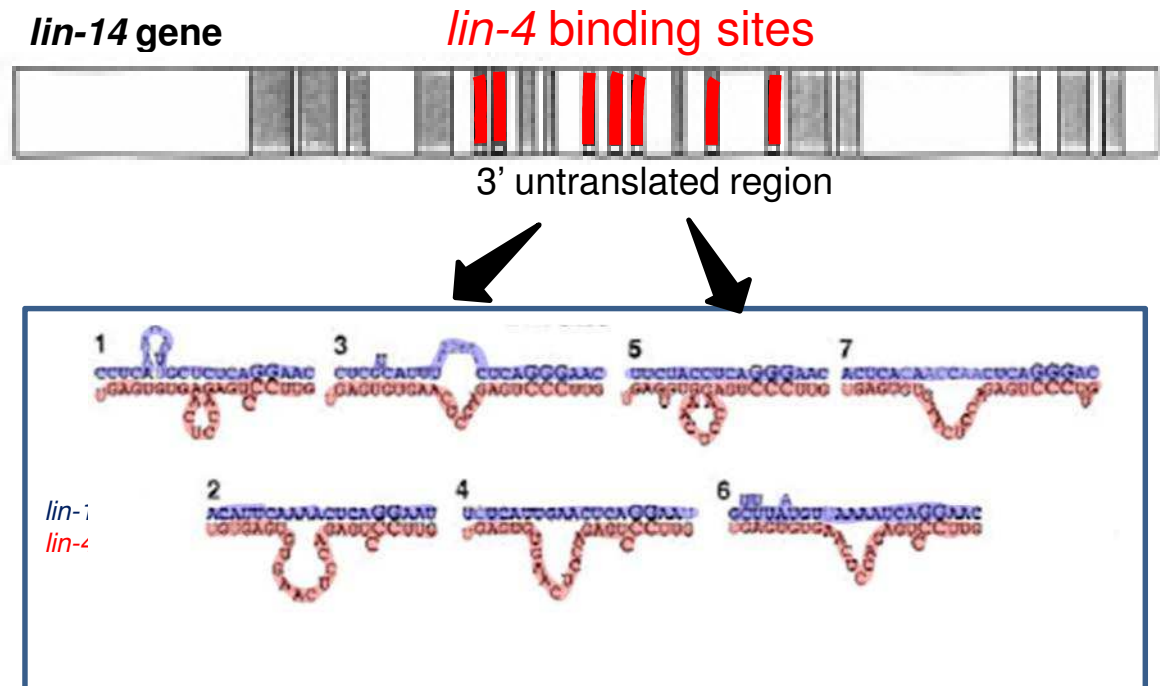
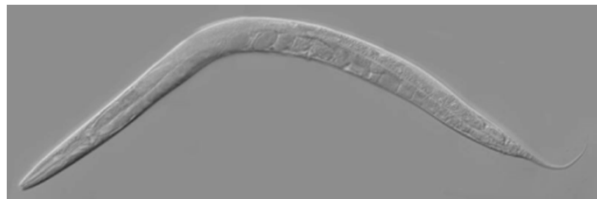
# Phase change may involve a temporal cascade of miRNAs and transcription factors



# miRNAs regulate developmental timing in other organisms

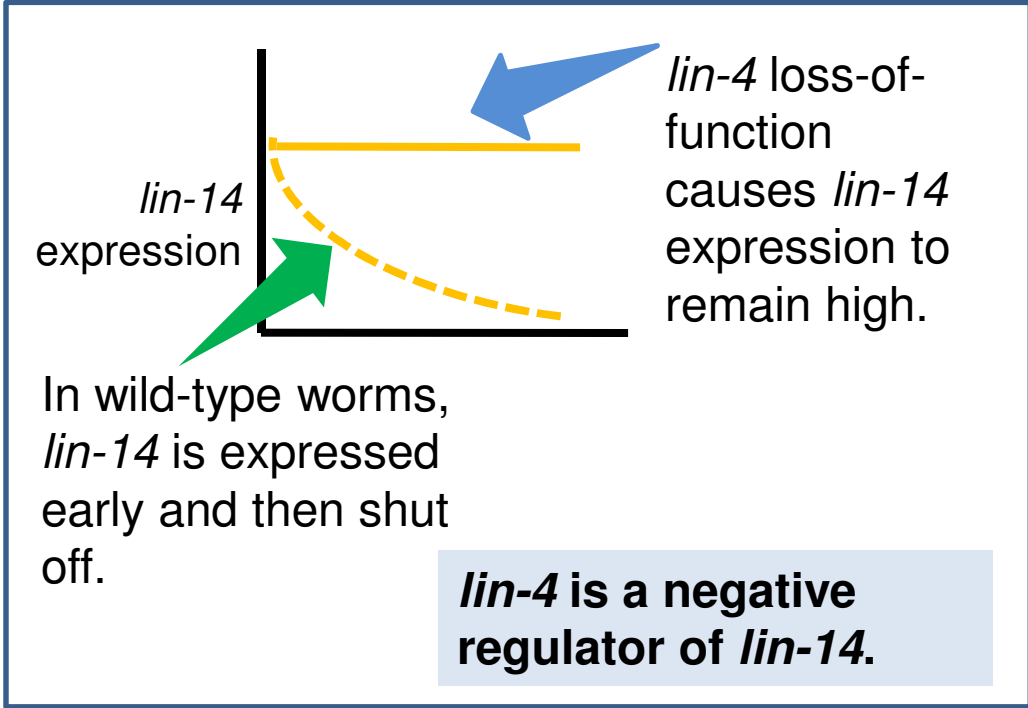
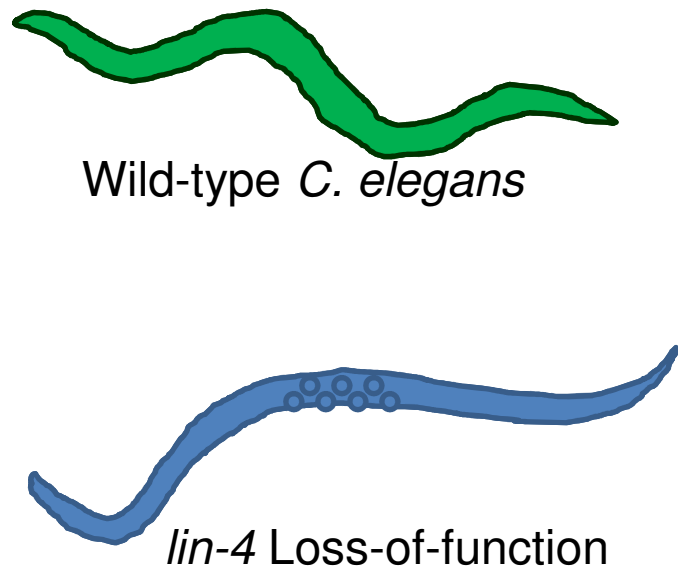
miRNAs were discovered in studies of developmental progressions in the nematode *C. elegans*.

A miRNA encoded by *lin-4* is required for proper larval development.



Lee, R.C., Feinbaum, R.L., and Ambrose, V. (1993). The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75: 843–845. Wightman, B., Ha, I., and Ruvkun, G. (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75: 855–862.

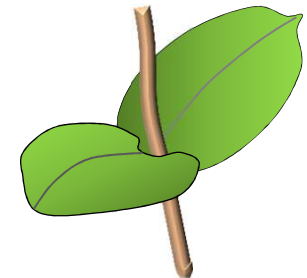
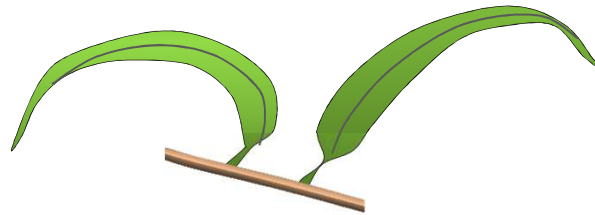
# Downregulation of *lin-14* by *lin-4* is necessary for normal development



Lee, R.C., Feinbaum, R.L., and Ambrose, V. (1993). The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75: 843–845.  
Wightman, B., Ha, I., and Ruvkun, G. (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75: 855–862.

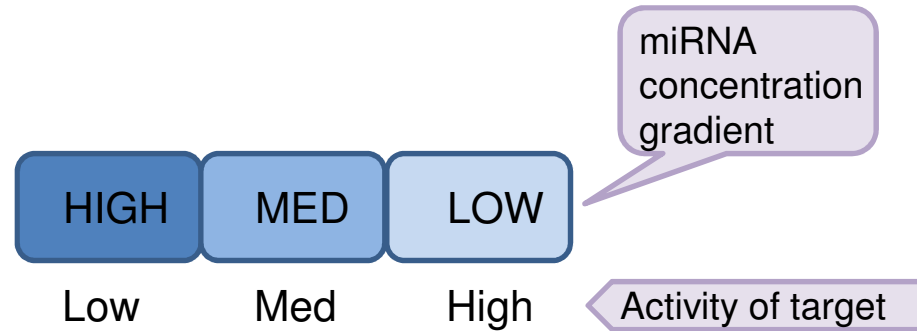
# miRNAs and phase change - summary

- Vegetative phase change affects morphology and reproductive competence
- miRNAs contribute to the temporal control of gene expression and phase change
- In the nematode *C. elegans*, *lin-4* silencing of *lin-14* is required for developmental progression

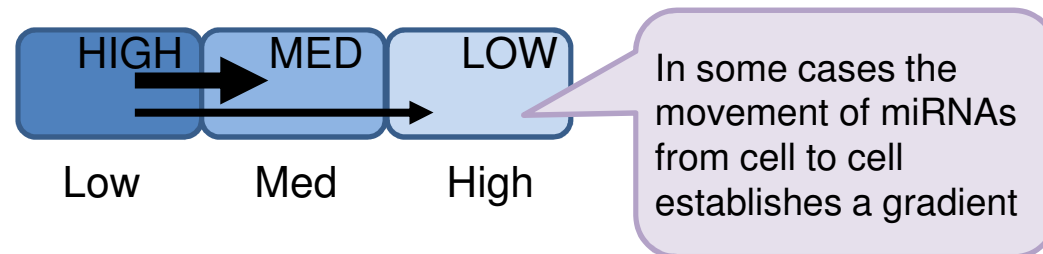


# miRNAs contribute to developmental patterning

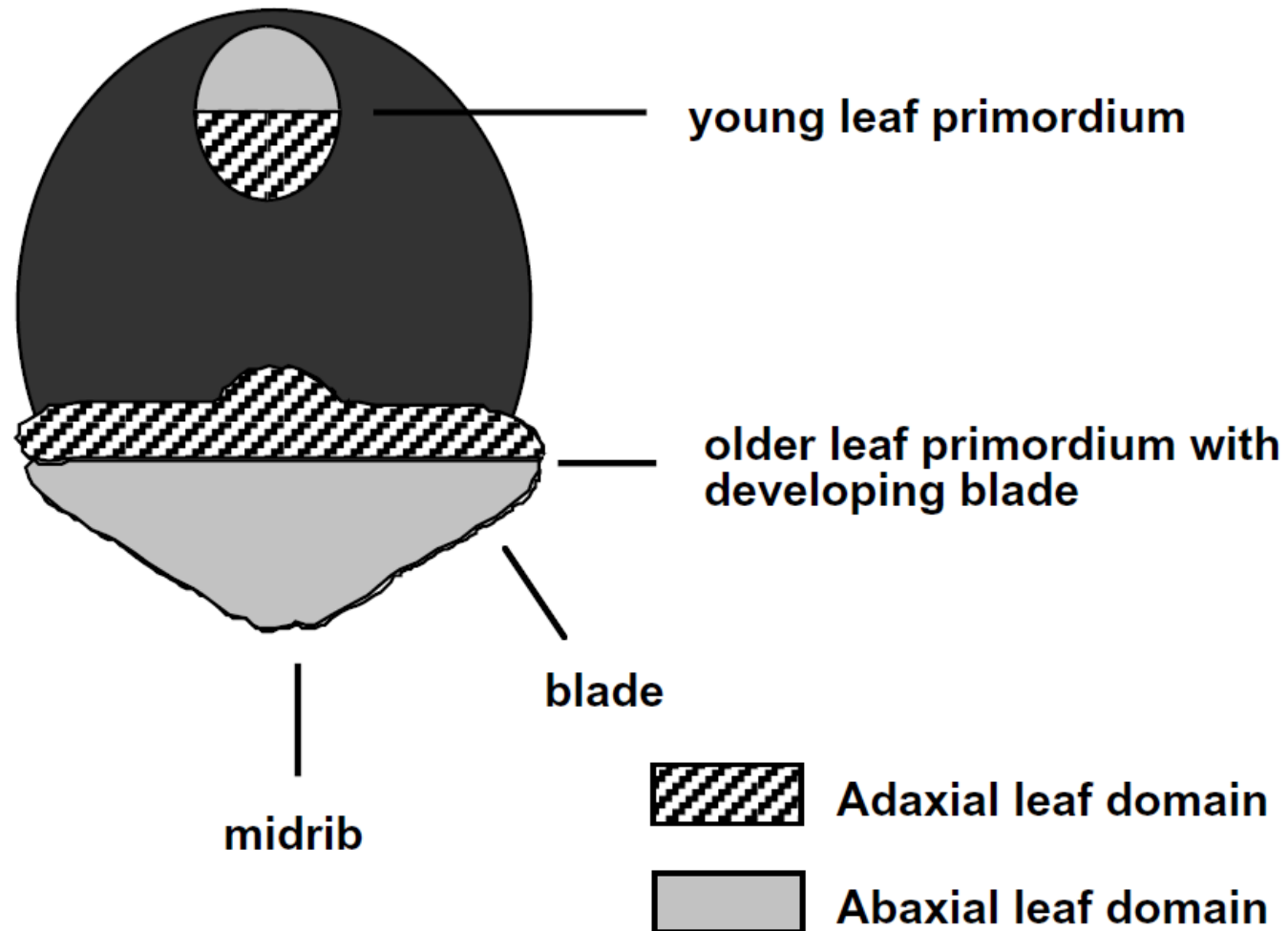
miRNA distribution patterns can spatially restrict activity of their targets



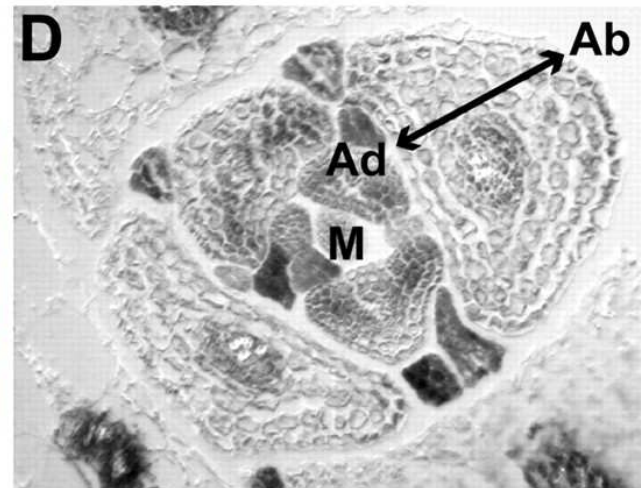
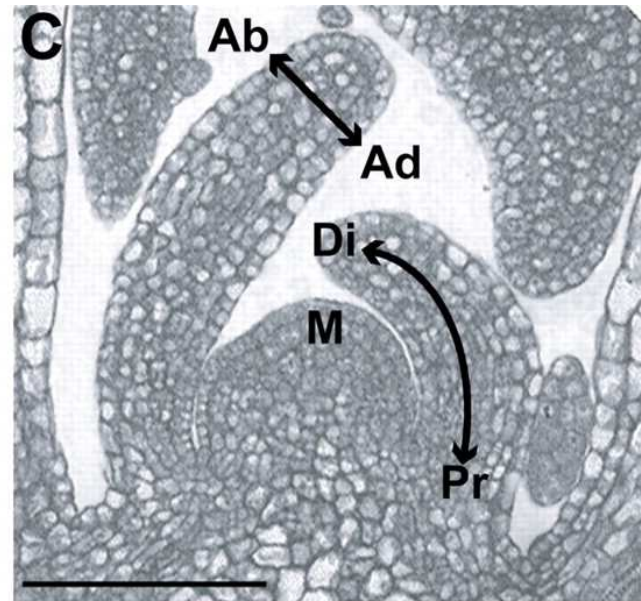
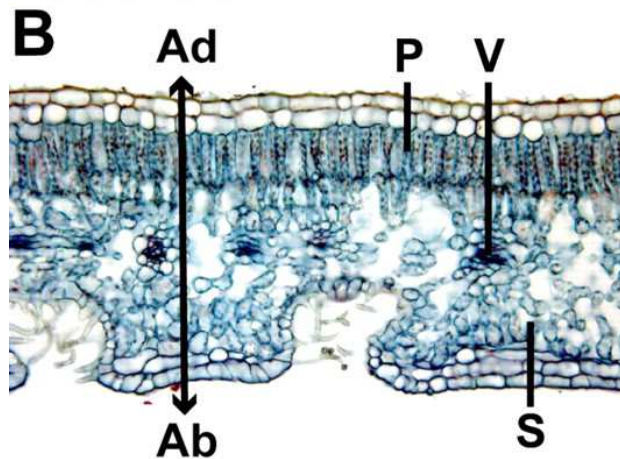
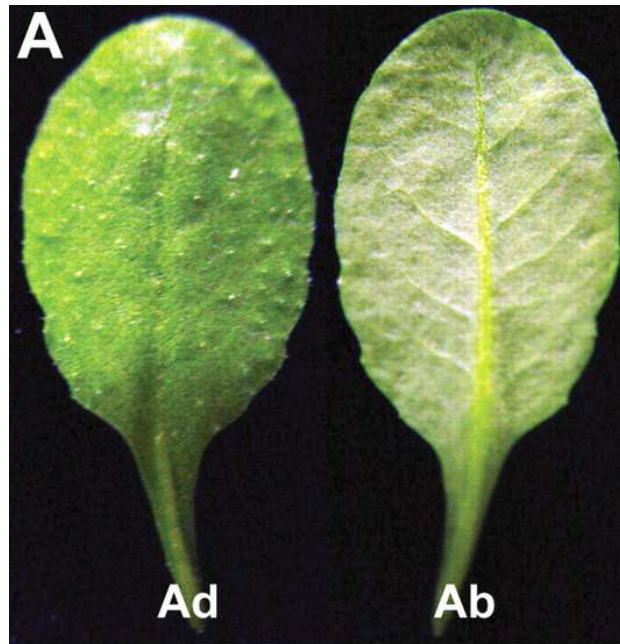
miRNAs can move between cells to spatially restrict activity of their targets



# Asymmetry of leaf primordia reflects adult leaf polarity

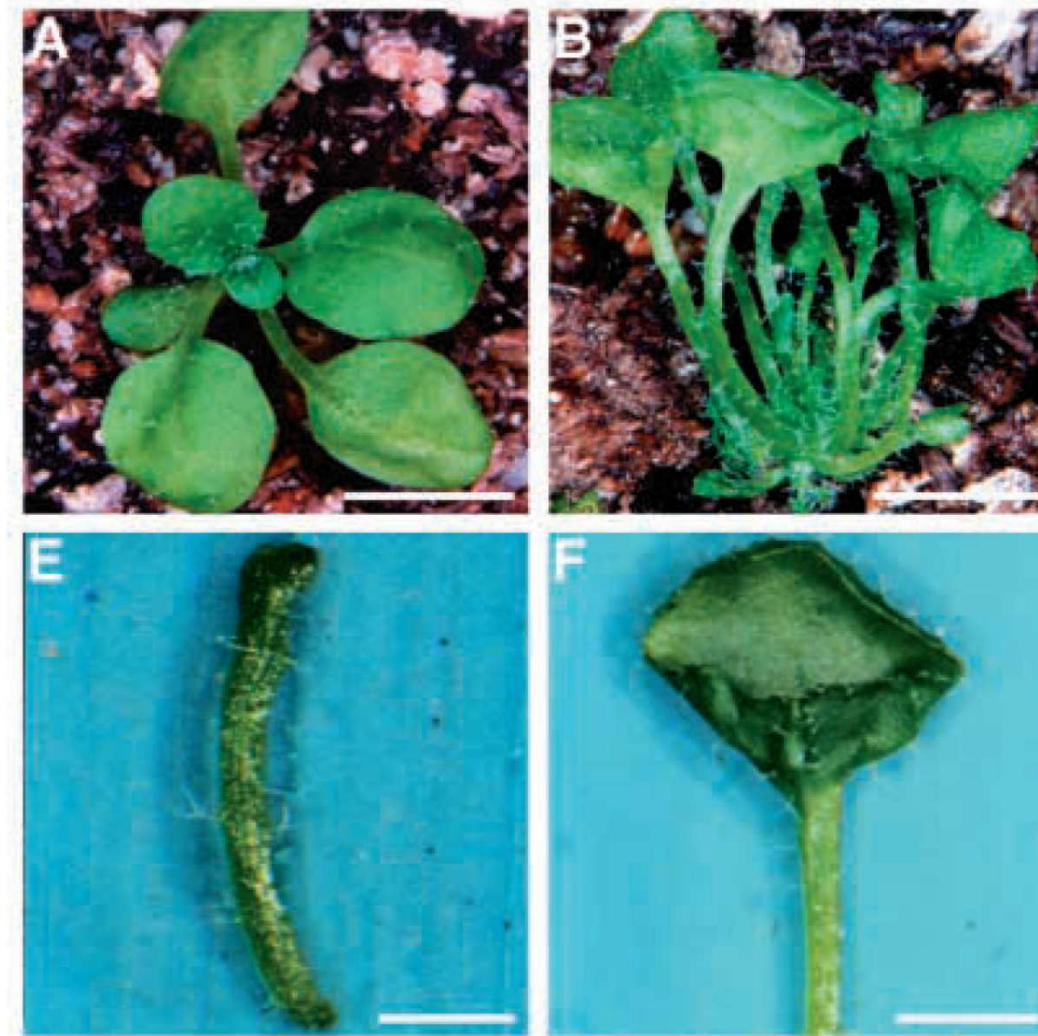


# Asymmetry of leaf primordia reflects adult leaf polarity



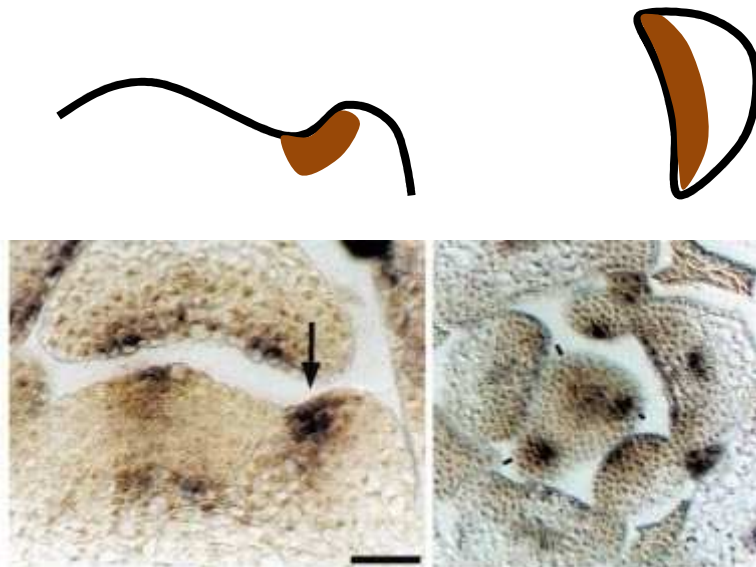


# A dominant mutation in the *PHABULOSA* gene causes leaf radialization



# The *phb-1d* mutation affects *PHB* mRNA distribution

In wild-type plants, *PHB* expression is restricted to the adaxial side of the leaves

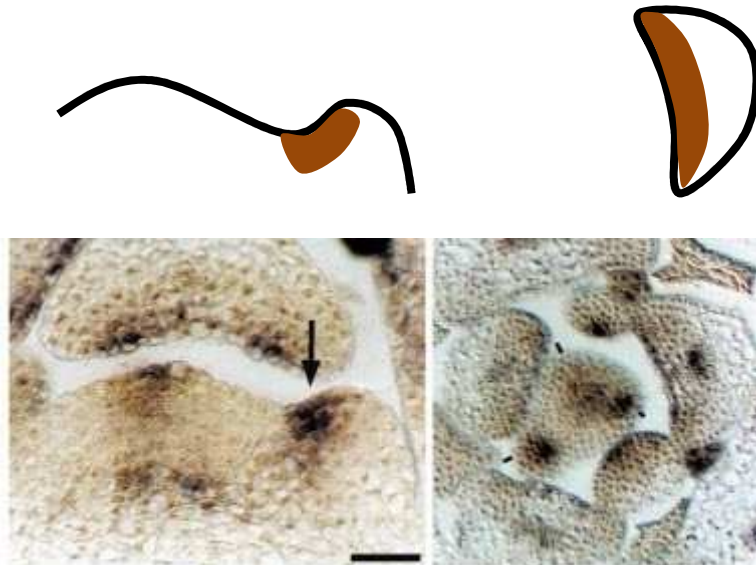


Longitudinal section Cross section

Reprinted by permission from Macmillan Publishers, Ltd: NATURE. McConnell, J.R., Emery, J., Eshed, Y., Bao, N., Bowman, J., and Barton, M.K. (2001) Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots *Nature* 411: [709-713](#), copyright 2001.

# The *phb-1d* mutation affects *PHB* mRNA distribution

In wild-type plants, *PHB* expression is restricted to the adaxial side of the leaves



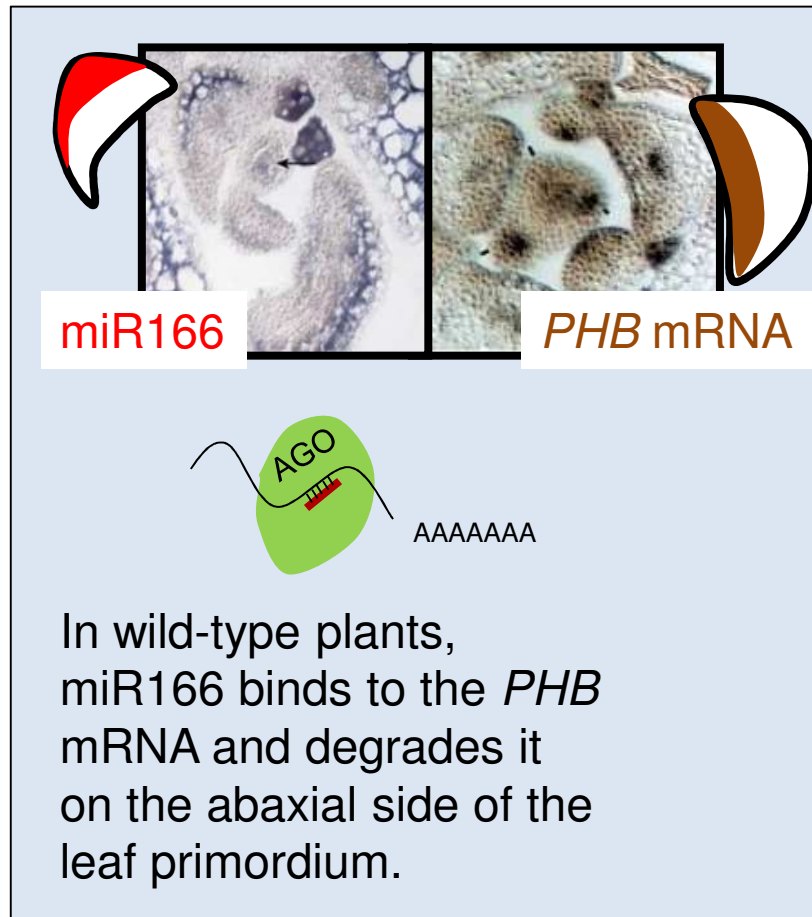
Longitudinal section    Cross section

In gain-of-function *phb-1d* mutants, *PHB* is expressed everywhere, resulting in **adaxialized**, radially symmetric leaves.



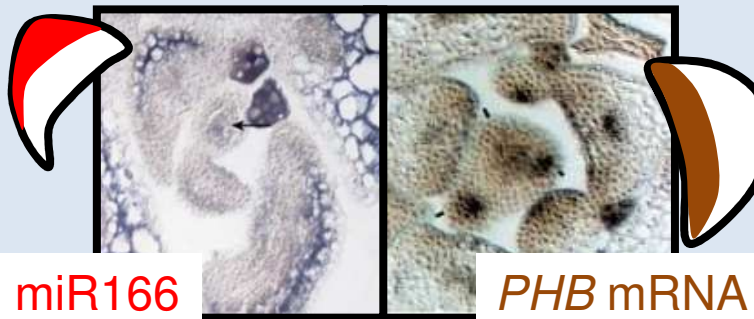
Scanning electron micrograph    Cross section

# *PHB* expression is regulated by a miRNA



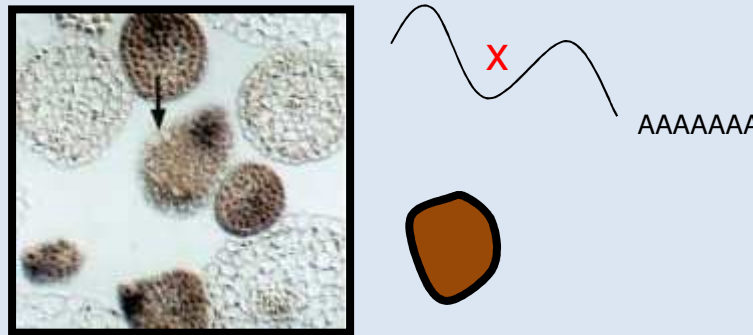
Reprinted by permission from Macmillan Publishers, Ltd: NATURE. Kidner, C.A. and Martienssen, R.A. (2004) Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. Nature 428: [81-84](#), copyright 2004; McConnell, J.R., Emery, J., Eshed, Y., Bao, N., Bowman, J., and Barton, M.K. Nature 411: 709-713, copyright 2001.

# Control of PHB expression by miRNA



The diagram shows two panels of leaf primordia. The left panel is labeled 'miR166' and shows a red and white arrow pointing to a specific region. The right panel is labeled 'PHB mRNA' and shows a brown arrow pointing to a specific region. Below the panels is a green oval labeled 'AGO' with a red bar and a wavy line labeled 'AAAAAAA'.

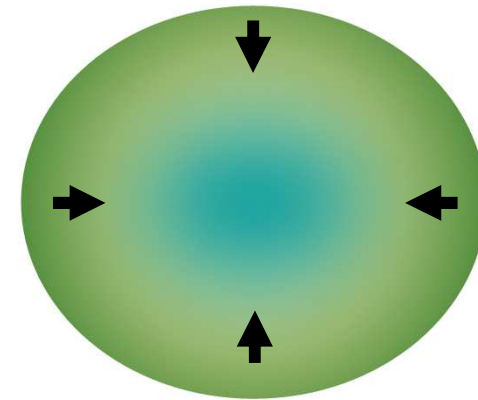
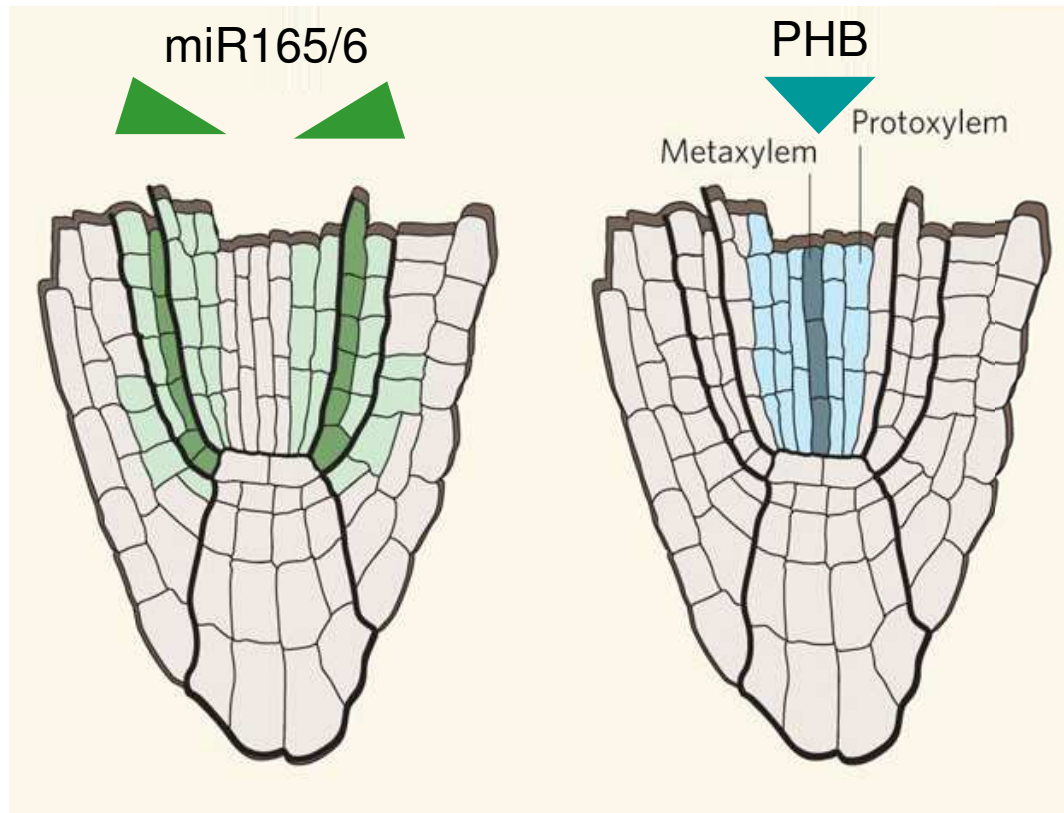
In wild-type plants, miR166 binds to the *PHB* mRNA and degrades it on the abaxial side of the leaf primordium.



The diagram shows a panel of leaf primordia labeled 'PHB-1D mRNA' with a black arrow pointing to a specific region. To the right is a brown oval and a wavy line labeled 'AAAAAAA' with a red 'X' above it.

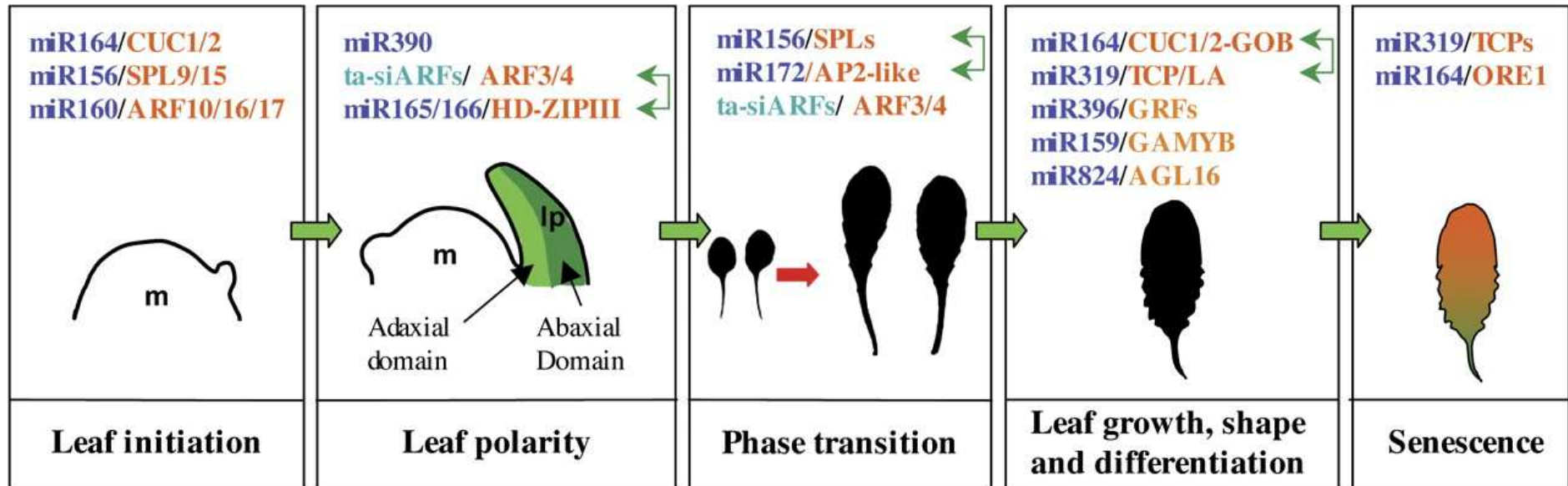
In *phb-1d* plants, base changes in the *PHB* mRNA prevent miR166 from binding to it, allowing it to accumulate throughout the leaf primordium.

# In roots, miR165/6 moves from endodermis into vascular cylinder



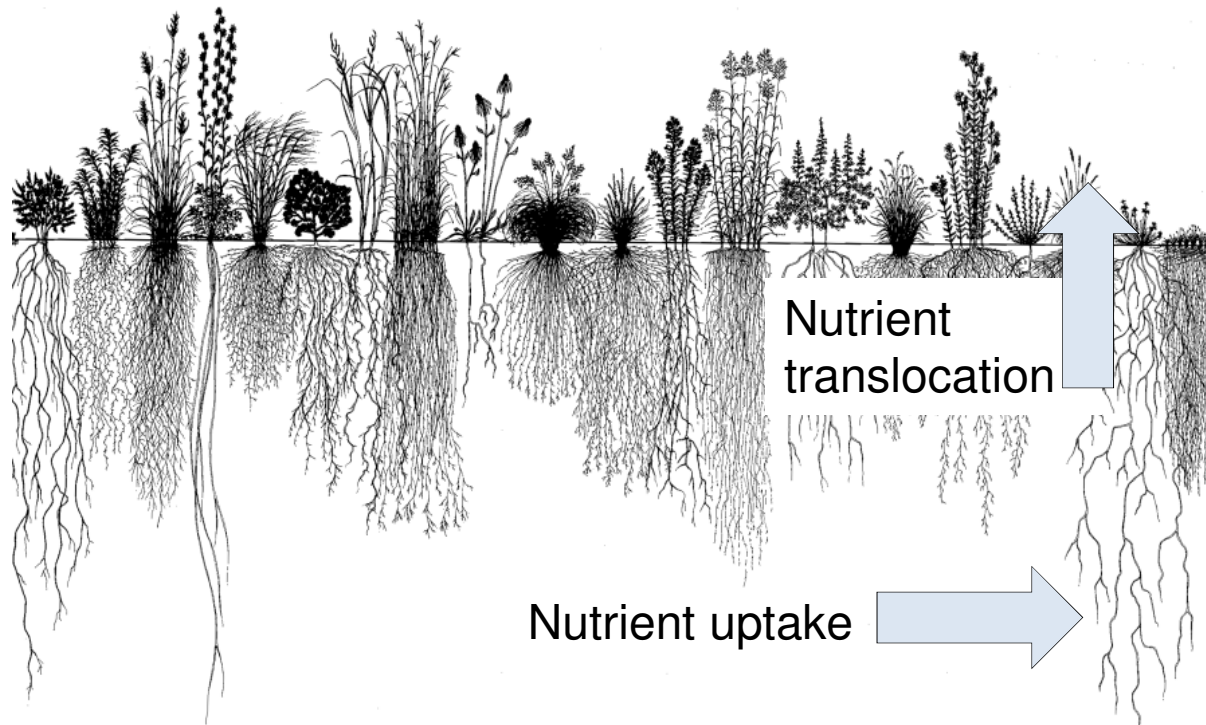
Movement of miR165/6 inwards from the endodermis in which it is produced helps to establish the radial pattern of the root

# Leaves are modulated by miRNA activity throughout development



Pulido, A., and Laufs, P. (2010). Co-ordination of developmental processes by small RNAs during leaf development. *J.Exp.Bot.* 61: [1277-1291](#), by permission from Oxford University Press.

# miRNAs and nutrient signalling

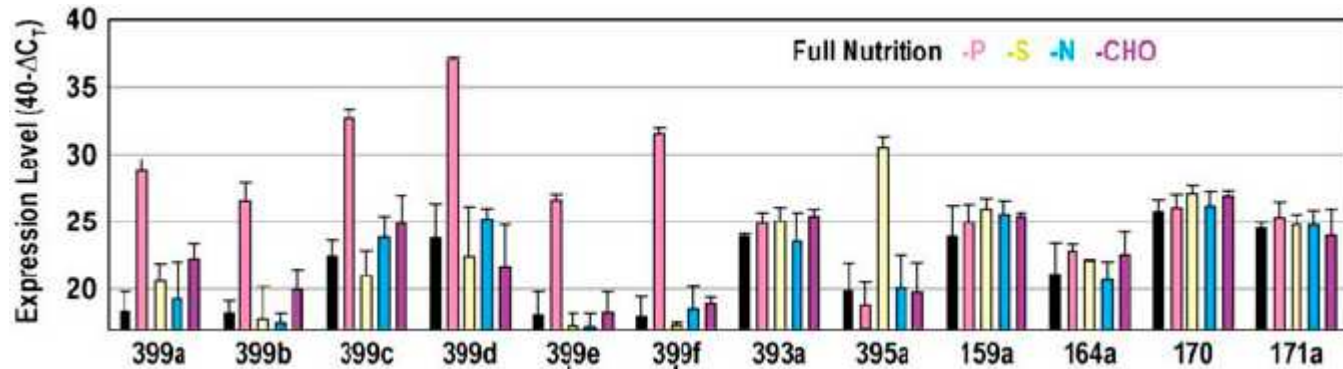


Plants take up nutrients from soil. The shoot sends signals to the root to indicate nutrient demands.

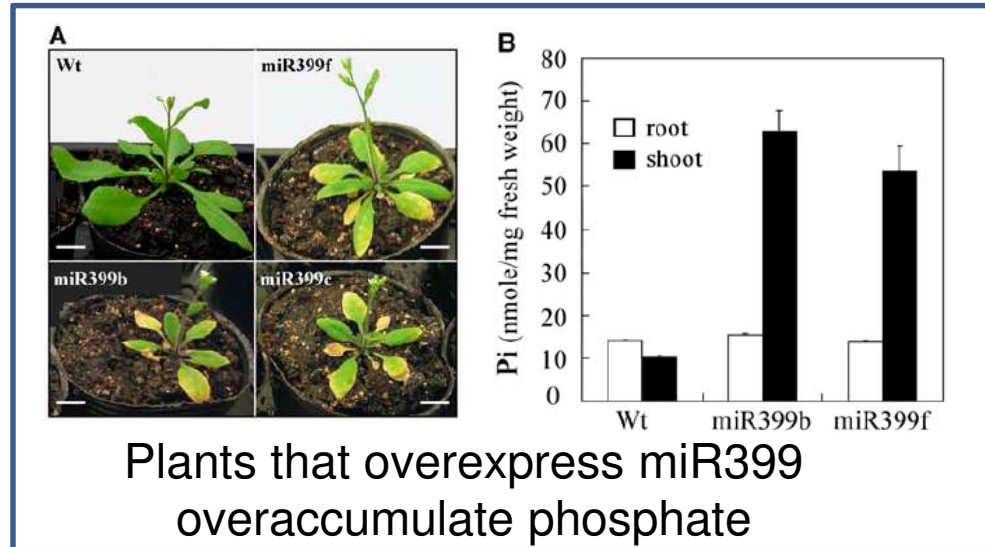
miRNAs move from shoot to root in phloem and regulate nutrient uptake.



# miR399 is induced upon phosphate starvation



Genes encoding miR399 are specifically induced by phosphate starvation.

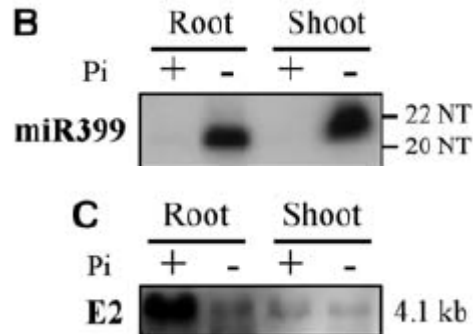


Plants that overexpress miR399 overaccumulate phosphate

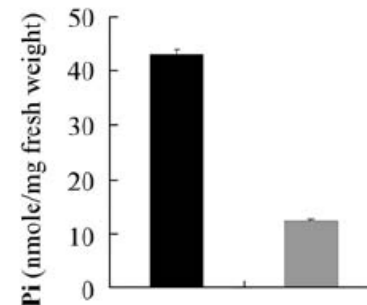
Bari, R., Pant, B.D., Stitt, M. and Scheible, W.-R. (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol.* 141: [988–999](#); Chiou, T.J., Aung, K., Lin, S.I., Wu, C.C., Chiang, S.F. and Su, C.L. (2006) Regulation of phosphate homeostasis by microRNA in *Arabidopsis*. *Plant Cell* 18: [412–421](#).

# A ubiquitin-conjugating E2 is a target for miR399

## E2 gene structure



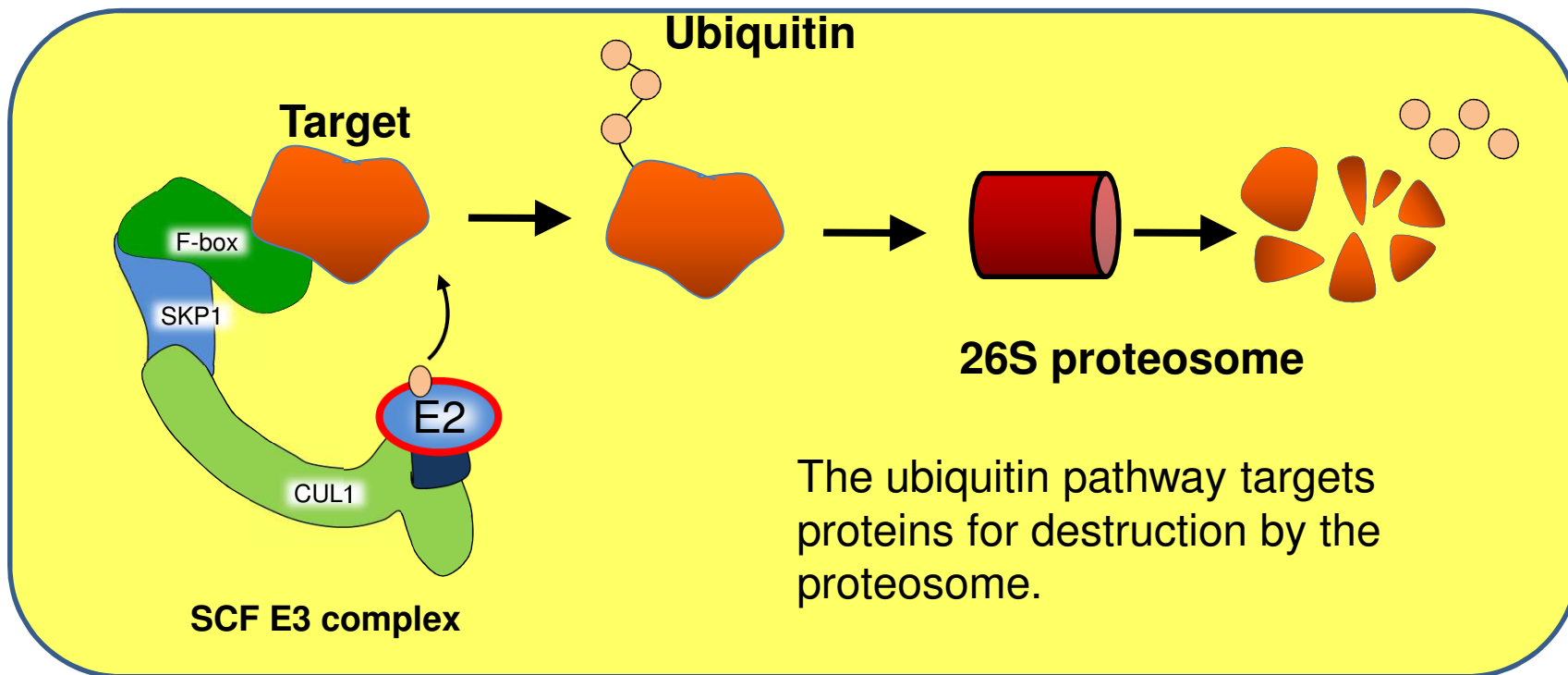
When miR399 increases, E2 expression decreases



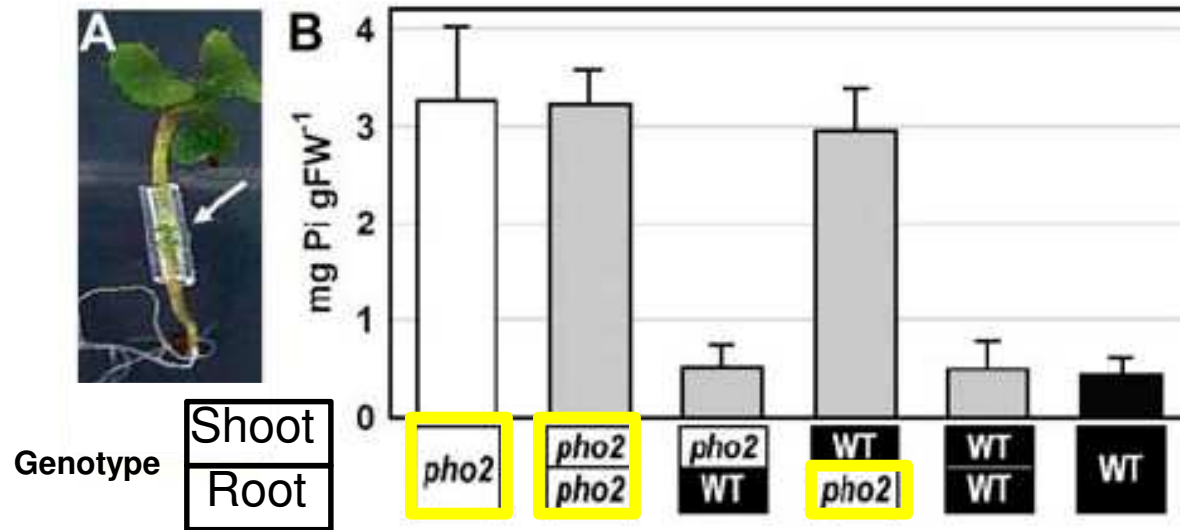
Loss-of-function of E2 (aka *pho2*) (left) causes phosphate overaccumulation

miR399 —| E2 —| Phosphate accumulation

# E2 is a component of the ubiquitin proteolysis pathway



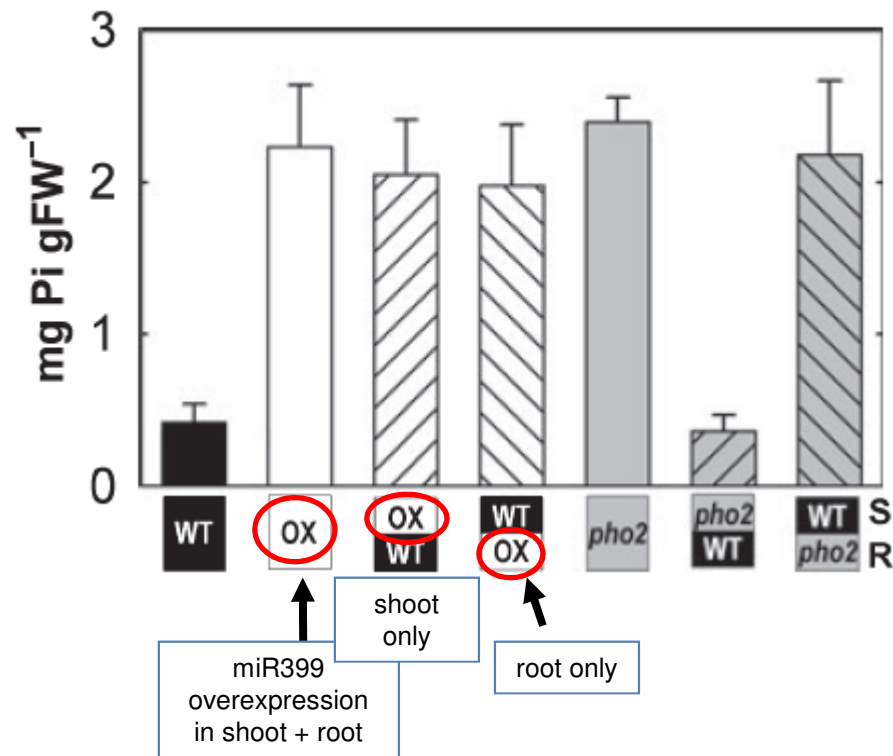
# The E2 conjugase functions in the root, not the shoot



Micrografting of *pho2* and wild-type shoots and roots demonstrates that PHO2 function in the roots is sufficient for normal phosphate uptake.

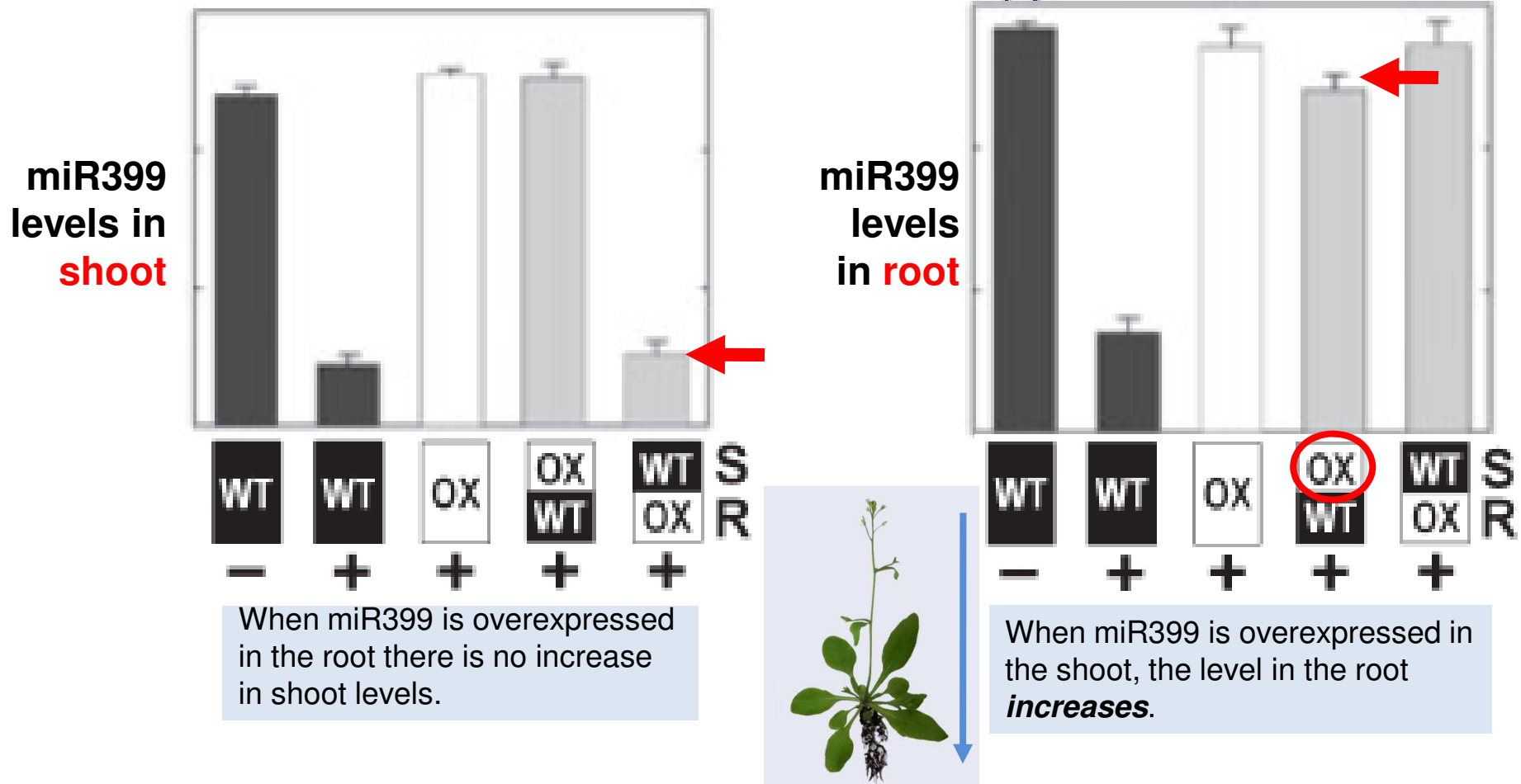
Phosphate overaccumulates only in plants with mutant roots.

# By contrast, overexpression of miR399 in the shoot or root causes increased phosphate accumulation

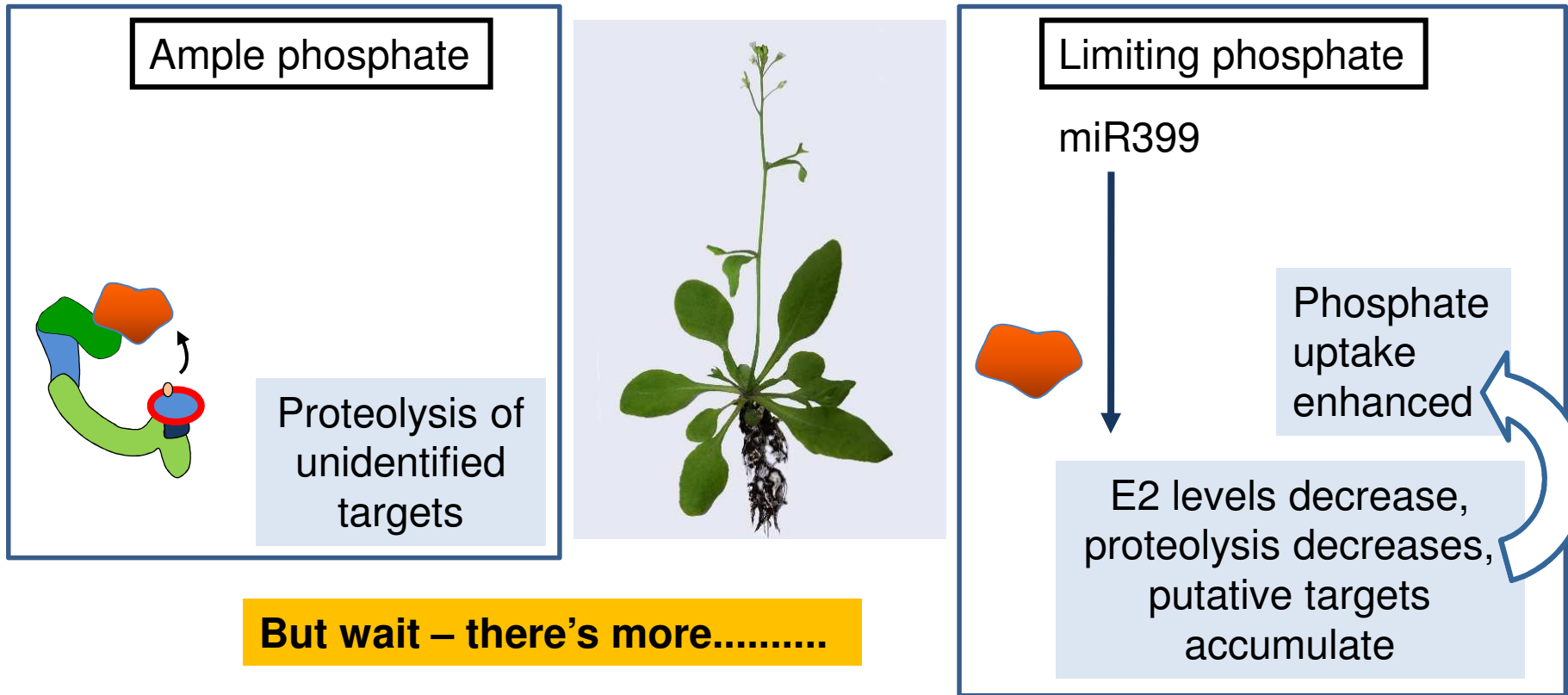


How can overexpression of miR399 in the shoot affect E2 activity in the root?

# miR399 is translocated from shoot to root, but not root to shoot



# miR399 is a phloem-mobile systemic signal



# miR399 activity is regulated by a *target mimic*



miR399 GUCCCGUUUAGAGGAAACCGU  
PHO2 UUGGGCAAUCUCCUUUGGCA

miR399 is highly complementary to its target, *PHO2*



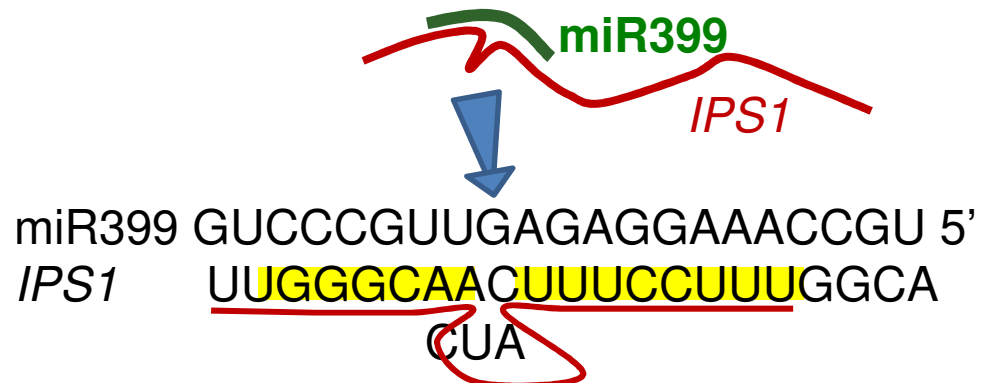
# miR399 activity is regulated by a *target mimic*



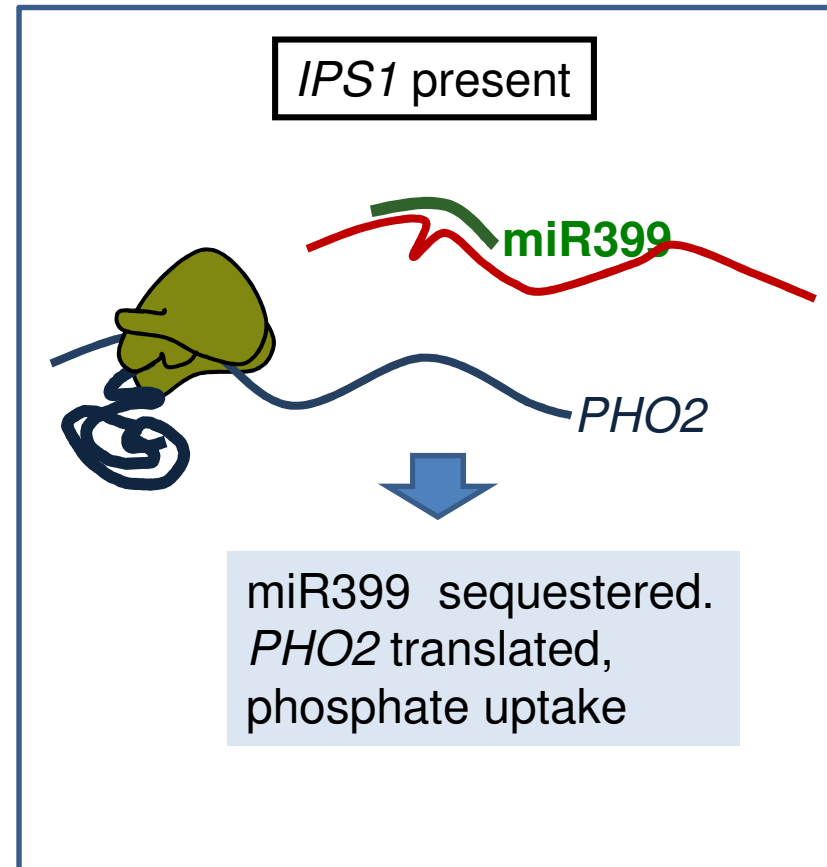
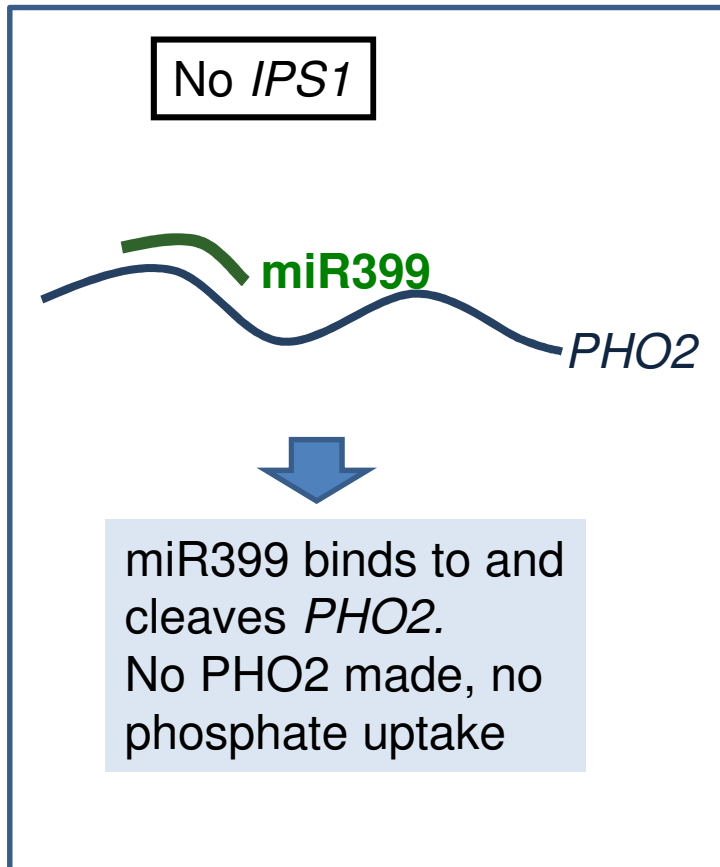
miR399 GUCCCGUUUAGAGGAAACCGU  
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miR399 is highly complementary to its target, *PHO2*

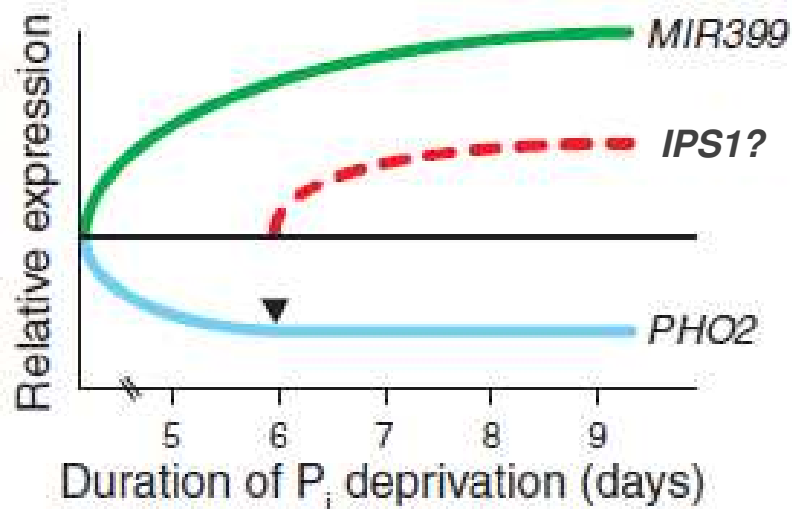
*IPS1* binds miR399 but has additional nucleotides at the cleavage site, so it is not cleaved



# miR399 activity is reduced when *IPS1* is present



# The target mimic *IPS1* may modulate miR399 function



During phosphate deprivation, miR399 is induced, inhibiting production of *PHO2*. After a few days, *ISP1* expression is induced, sequestering miR399 and allowing some *PHO2* expression.

# miRNAs and nutrient uptake- summary

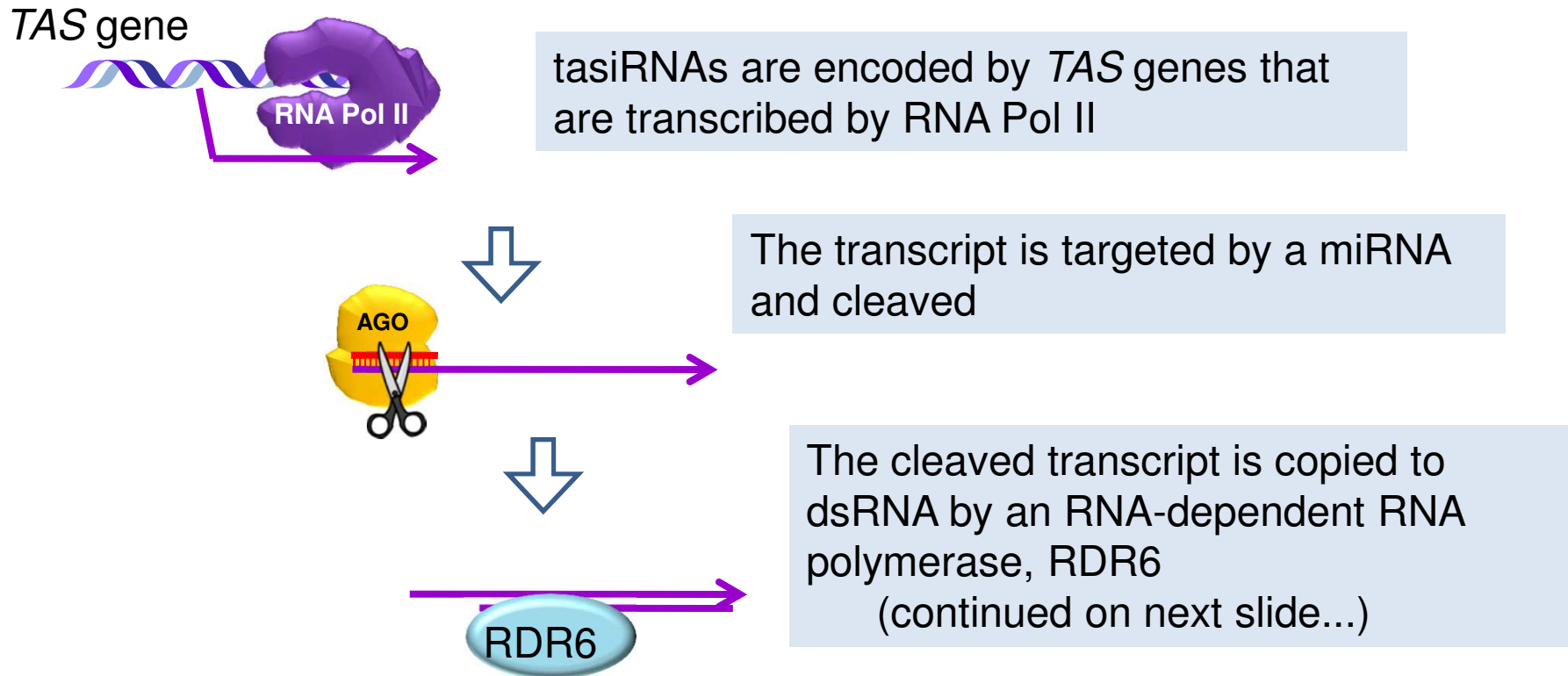
- The rate of nutrient uptake in the roots is regulated by signals from the shoot
- PHO2* encodes a ubiquitin-conjugating E2 that presumably contributes to directed proteolysis
- PHO2* levels are regulated by miR399 which accumulates upon phosphate starvation
- Starvation-induced miR399 moves from shoot to root, regulating phosphate uptake through *PHO2*
- PHO2* expression is also controlled by *IPS1*, a target mimic of miR399

# tasiRNAs

## tasiRNAs – trans-acting siRNAs

Encoded by *TAS* genes

Primary transcript processing initiated by miRNA

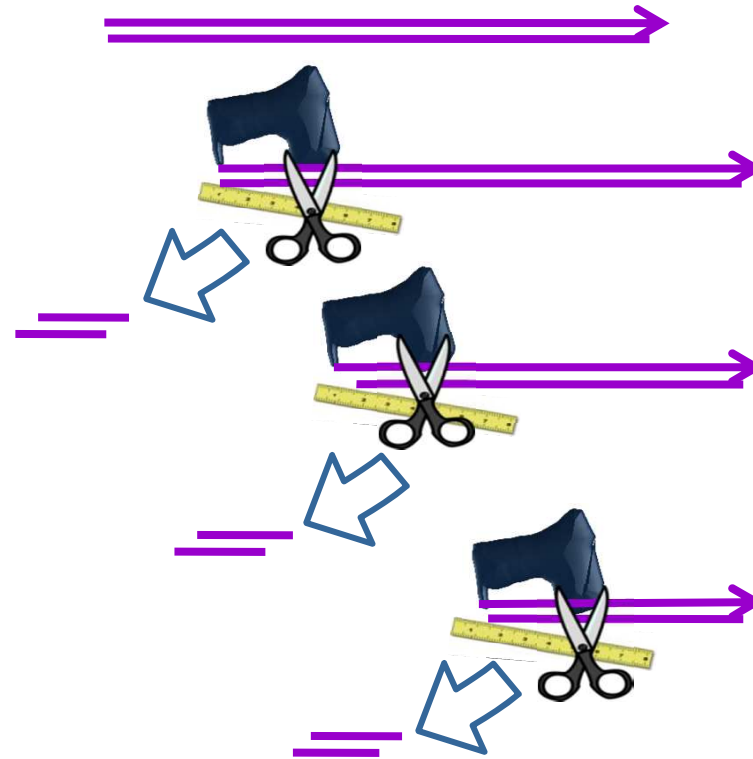


# tasiRNA biogenesis

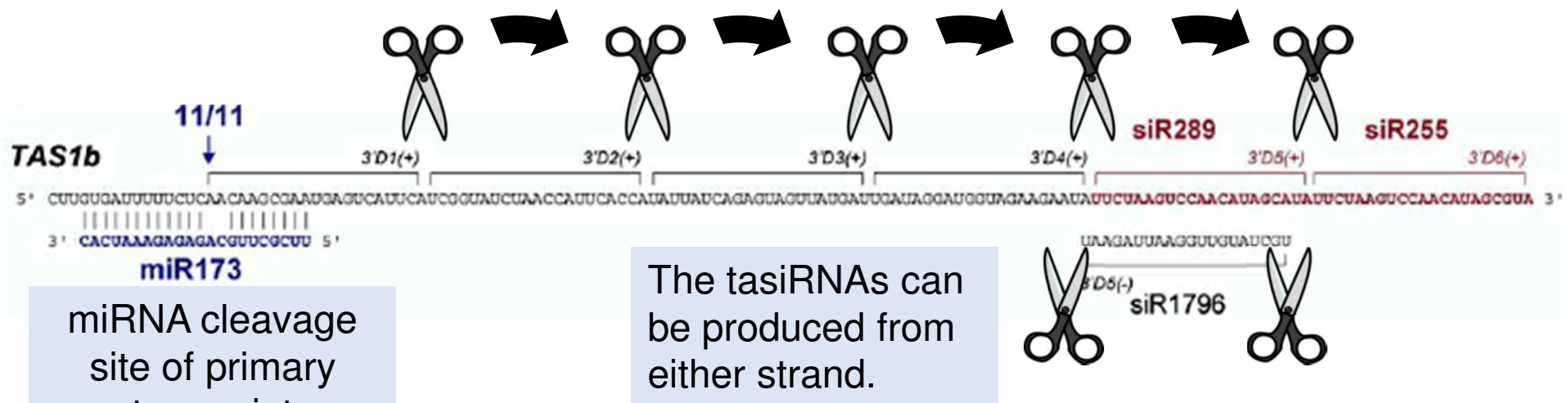
The dsRNA is cleaved by DCL4 into a series of shorter dsRNAs, releasing many tasiRNAs from a single *TAS* gene.

## Arabidopsis has four families of *TAS* genes

- *TAS1* and *TAS2* tasiRNAs target pentatricopeptide repeat genes.
- *TAS3* tasiRNAs target ARF transcription factors.
- *TAS4* tasiRNAs target MYB transcription factors

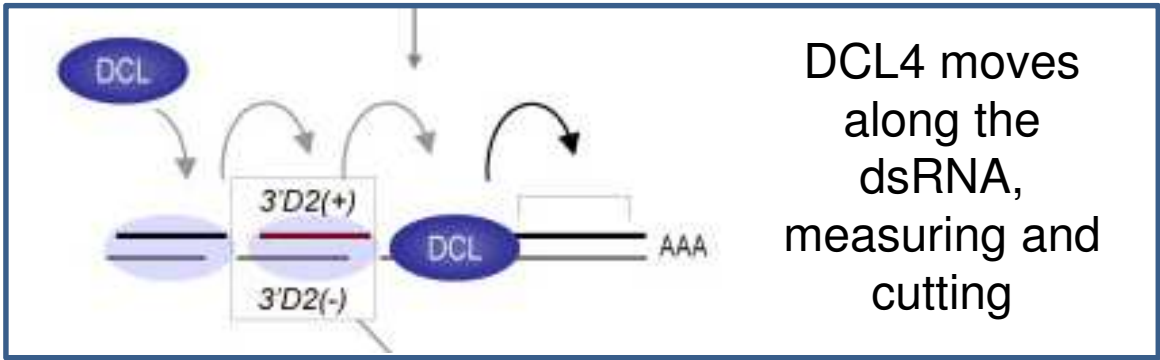


# Several “phased” tasiRNAs are derived from each *TAS* gene



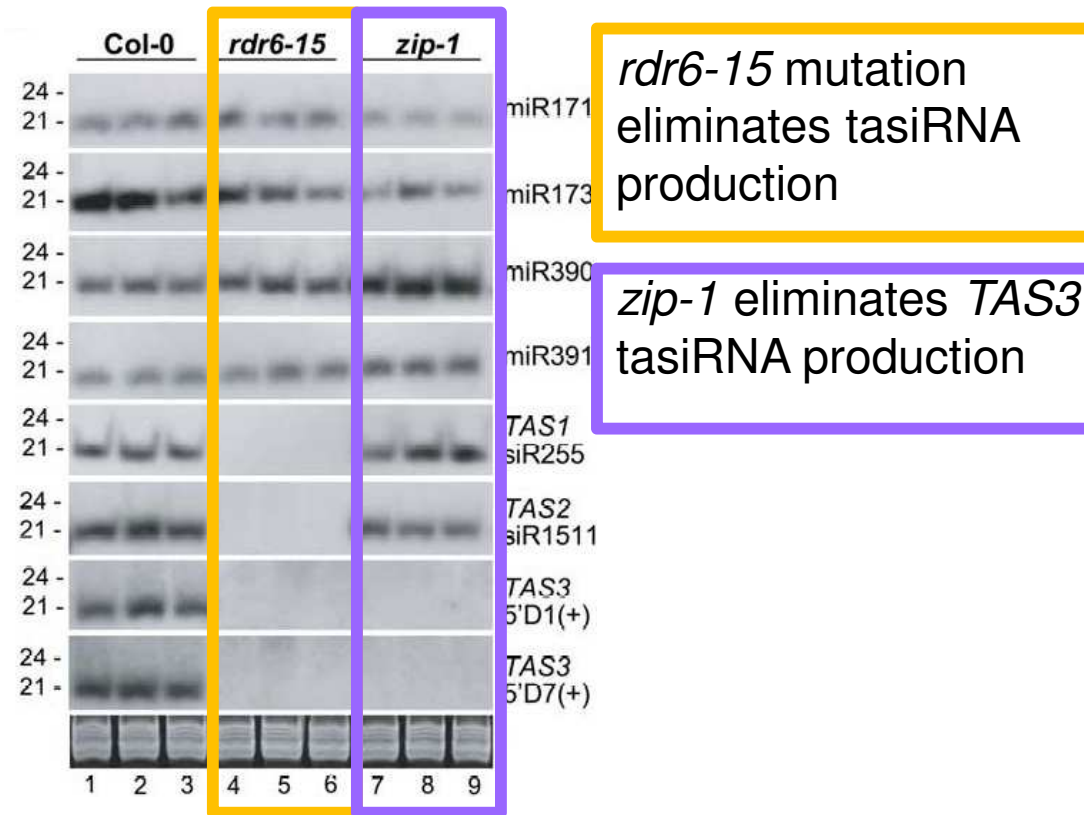
miRNA cleavage site of primary transcript

The tasiRNAs can be produced from either strand.



Reprinted from Allen, E., Xie, Z., Gustafson, A M., and Carrington, J.C. (2005) microRNA-directed phasing during *trans*-acting siRNA biogenesis in plants. Cell 121: 207-221, with permission from Elsevier.

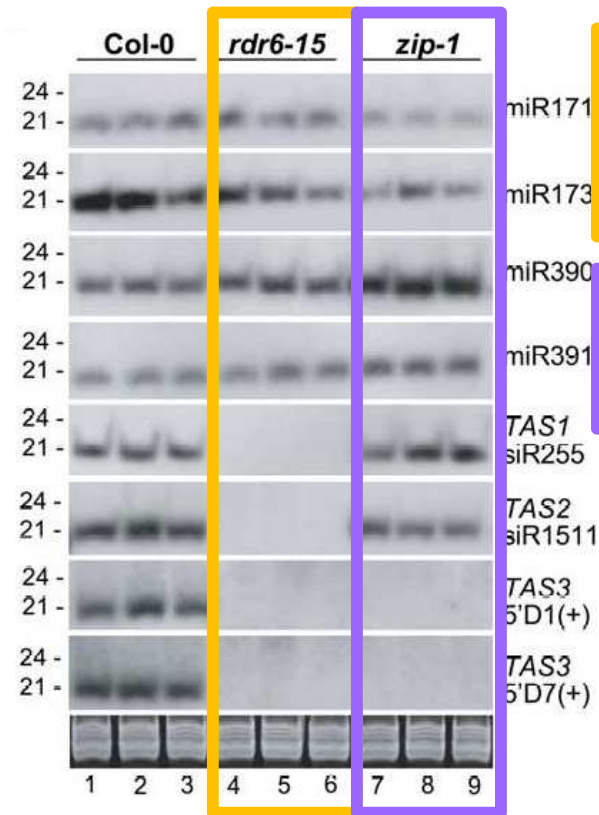
# Mutations that affect tasiRNA production affect phase change



Reprinted from Fahlgren, N., Montgomery, T.A., Howell, M.D., Allen, E., Dvorak, S.K., Alexander, A.L., and Carrington, J.C. (2006) Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. *Curr. Biol.* 16: [939-944](#) with permission from Elsevier.



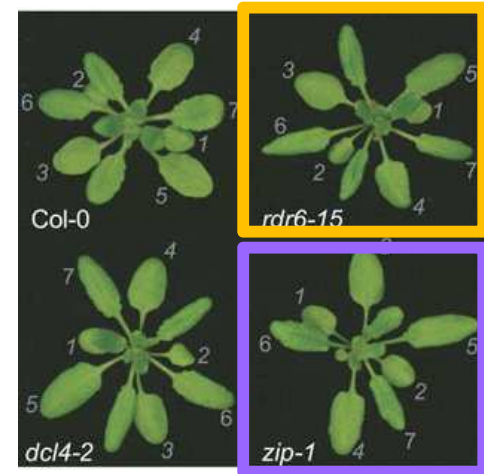
# Mutations that affect tasiRNA production affect phase change



*rdr6-15* mutation eliminates tasiRNA production

*zip-1* eliminates *TAS3* tasiRNA production

Both mutations, as well as *dcl4* and *tas3*, accelerate phase change

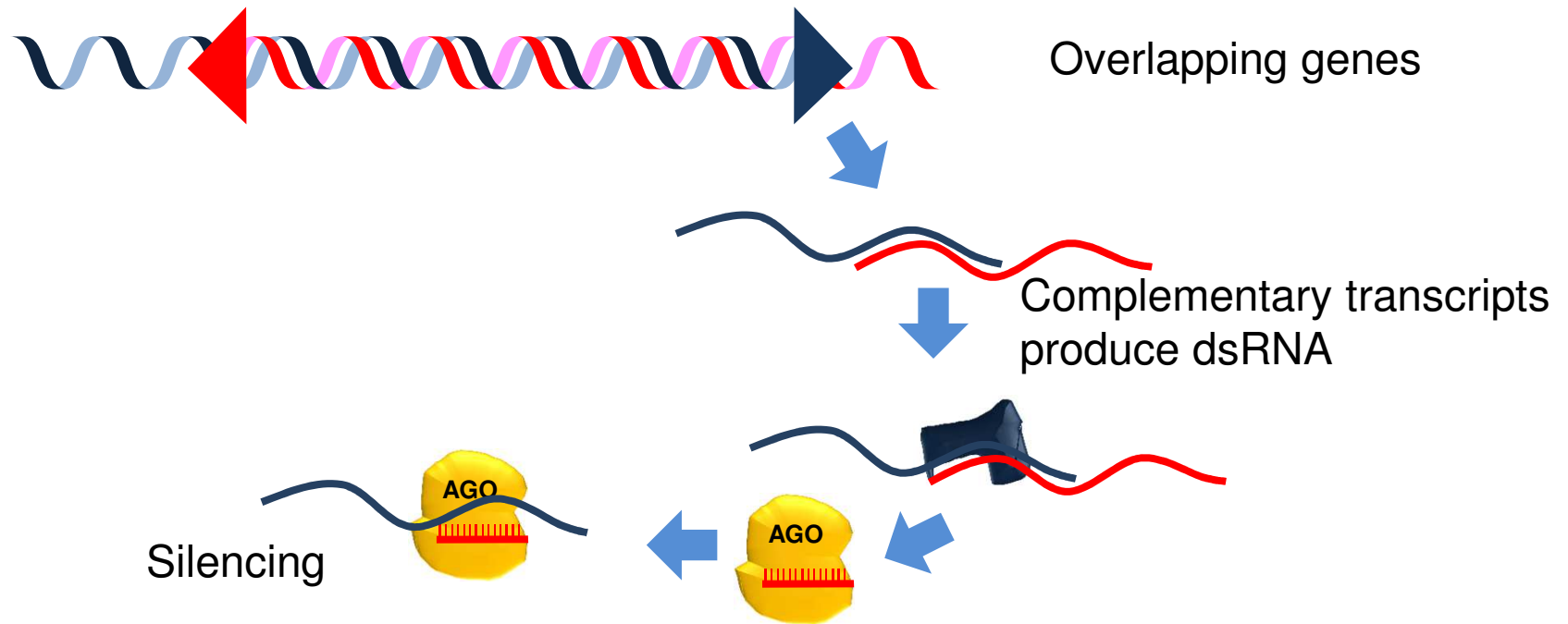


# nat-siRNAs

## Nat-siRNAs – Natural *cis*-acting siRNAs

Derived from overlapping transcripts

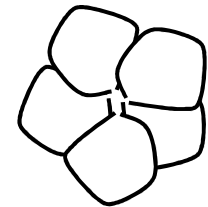
Involved in abiotic and biotic stresses



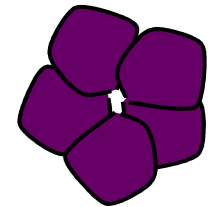
Redrawn from Katiyar-Agarwal, S., Morgan, R., Dahlbeck, D., Borsani, O., Villegas Jr. A., Zhu, J.-K., Staskawicz, B.J., and Jin, H. (2006) A pathogen-inducible endogenous siRNA in plant immunity. *Proc. Natl. Acad. Sci. USA* 103: [18002–18007](#).

# Conclusions

Small RNAs contribute to the regulation and defense of the genome, and confer silencing specificity through base-pairing



siRNA targets include repetitive-rich heterochromatin, transposons, viruses or other pathogens



miRNAs and tasiRNAs targets include regulatory genes affecting developmental timing or patterning, nutrient homeostasis and stress responses

