

# Bi8940 Developmental biology

## Lesson 10

### Regulation of Gene Expression during Development

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována  
Evropským sociálním fondem  
a státním rozpočtem České republiky



# Outline of Lesson 10

## Regulation of Gene Expression during Development

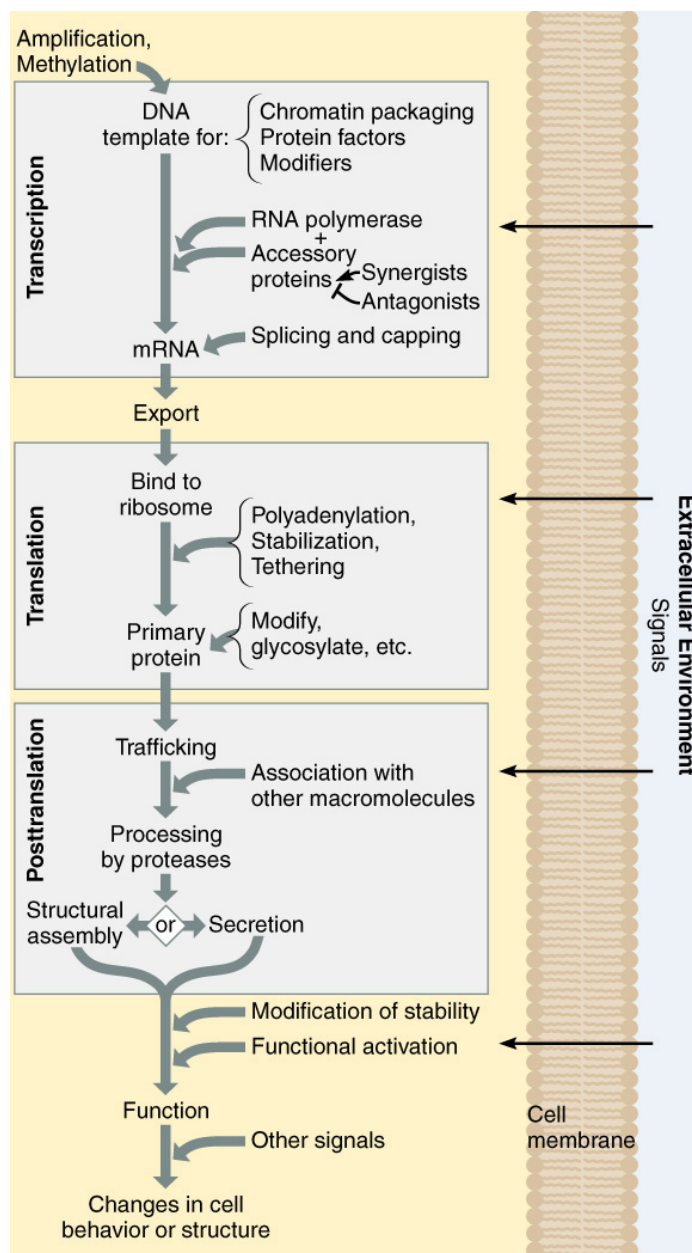
- Overview of levels of gene expression regulation
- Transcriptional gene regulation
  - Modification of the chromatin structure and DNA methylation
  - Transcriptional activation
- Post-transcriptional gene regulation
  - Splicing of hnRNA
  - Translation initiation
  - Localization of mRNA
  - Protein localization
- RNA interference
  - Identification and mechanism of gene expression regulation via RNA interference
  - siRNA-mediated silencing
  - miRNA-mediated silencing



# Outline of Lesson 10

## Regulation of Gene Expression during Development

- Overview of levels of gene expression regulation



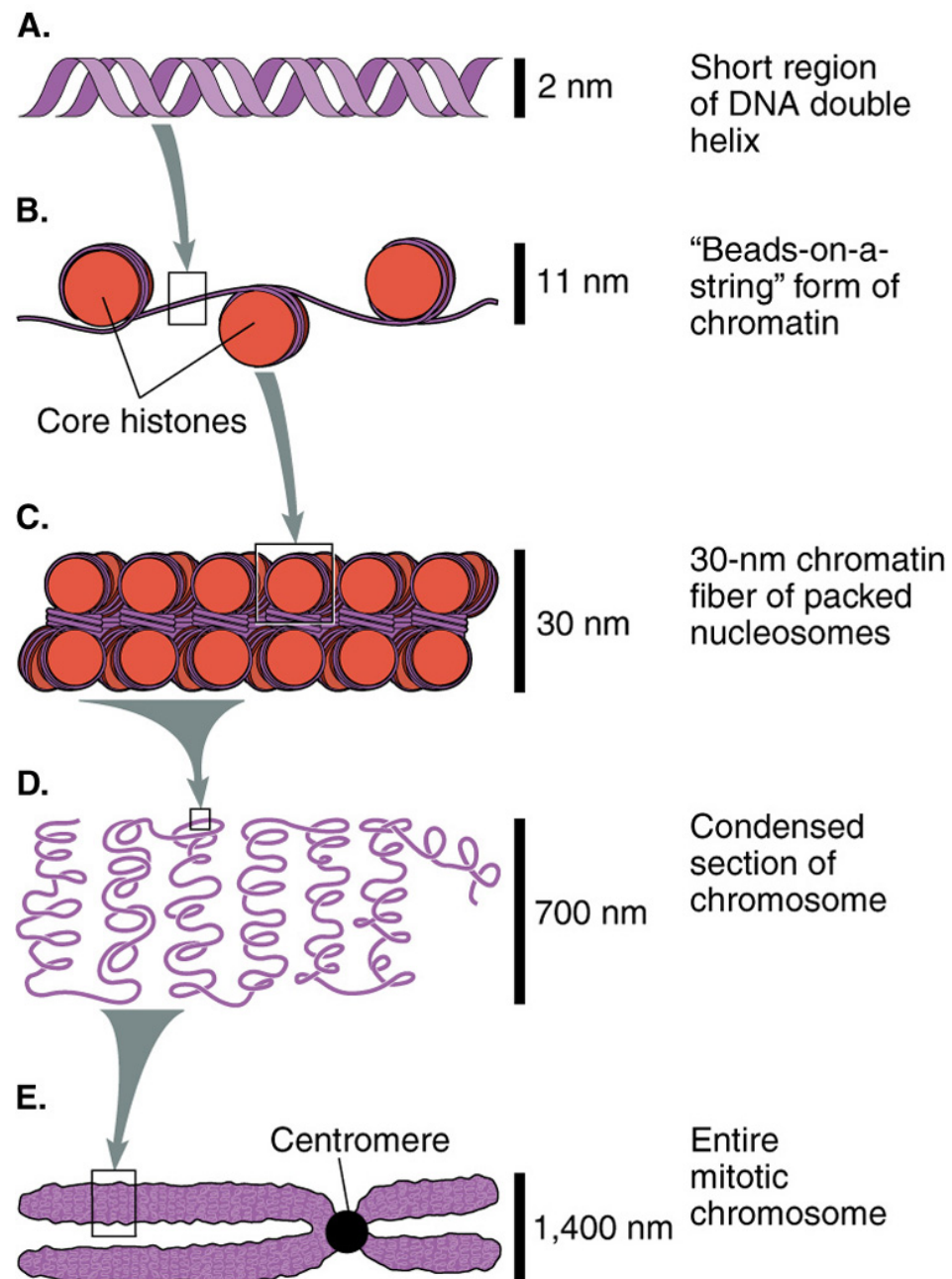




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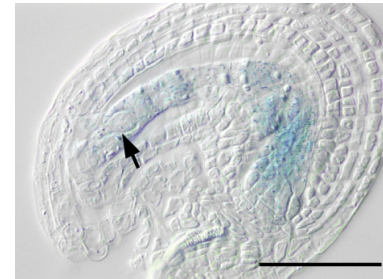
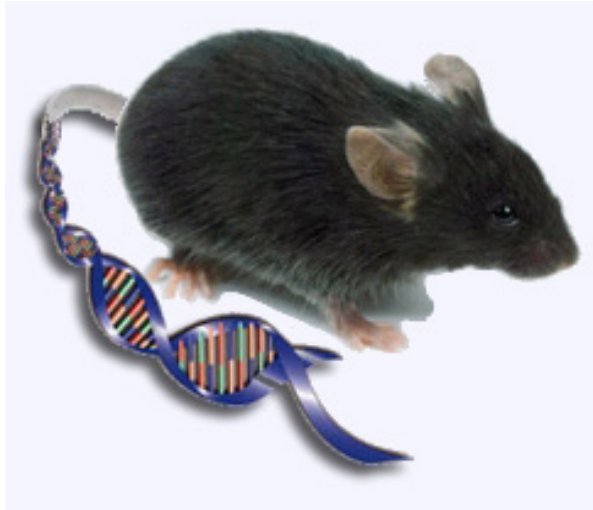
## Regulation of Gene Expression during Development

- Overview of levels of gene expression regulation
- Transcriptional gene regulation
  - Modification of the chromatin structure and DNA methylation



**Regulation by histone acetyltransferases or histone deacetylases**

# DNA methylation in animals vs. in plants



methylation status

**CpG**

Cell-specific methylation allows maintain of tissue-specific gene expression profiles



Mechanism of transcriptional regulation by DNA methylation mostly unknown

methylation status

**CpG or CpNpG**

**CpNpNp**



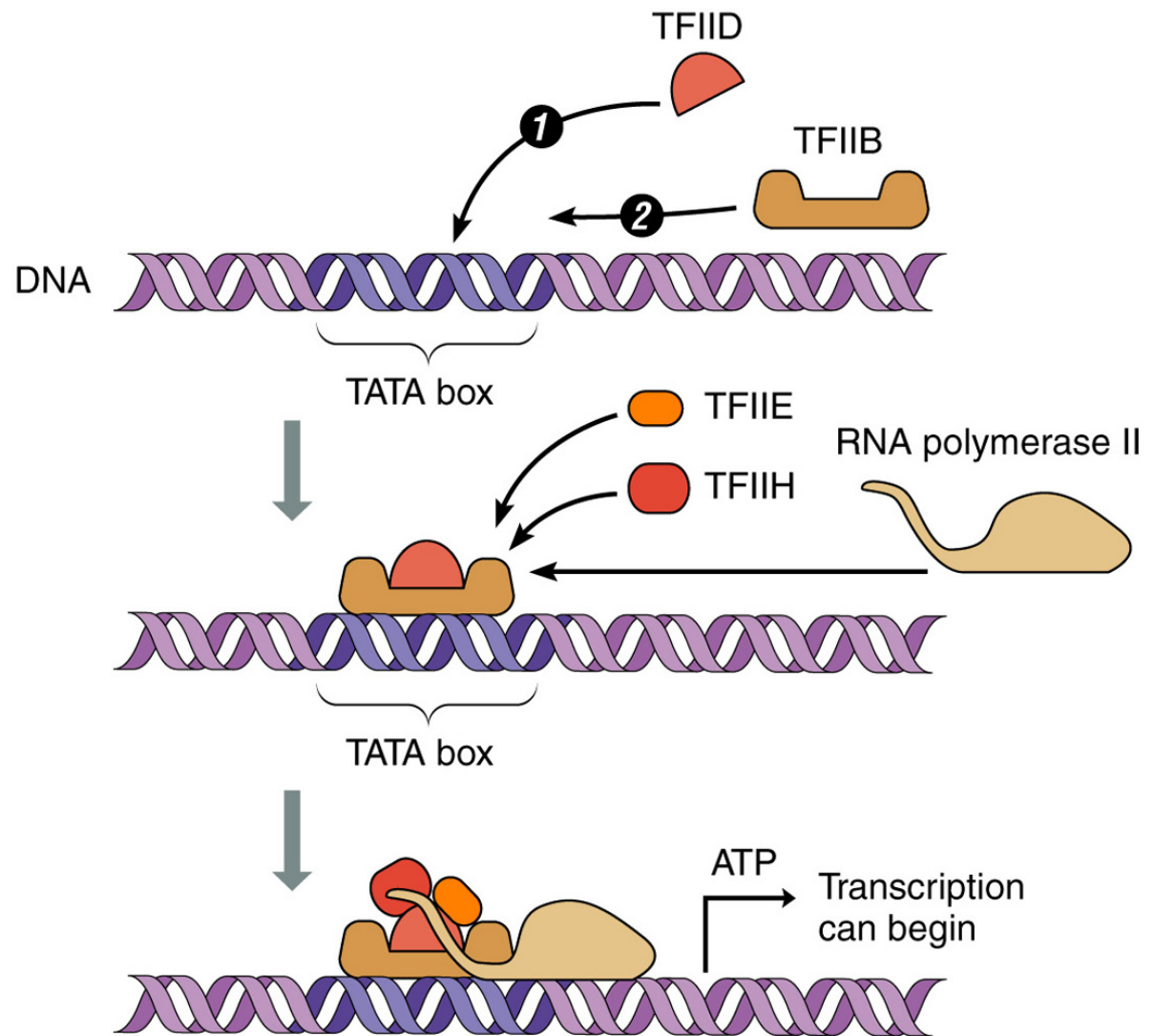
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## Regulation of Gene Expression during Development

- Overview of levels of gene expression regulation
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  - Modification of the chromatin structure and DNA methylation
  - Transcriptional activation

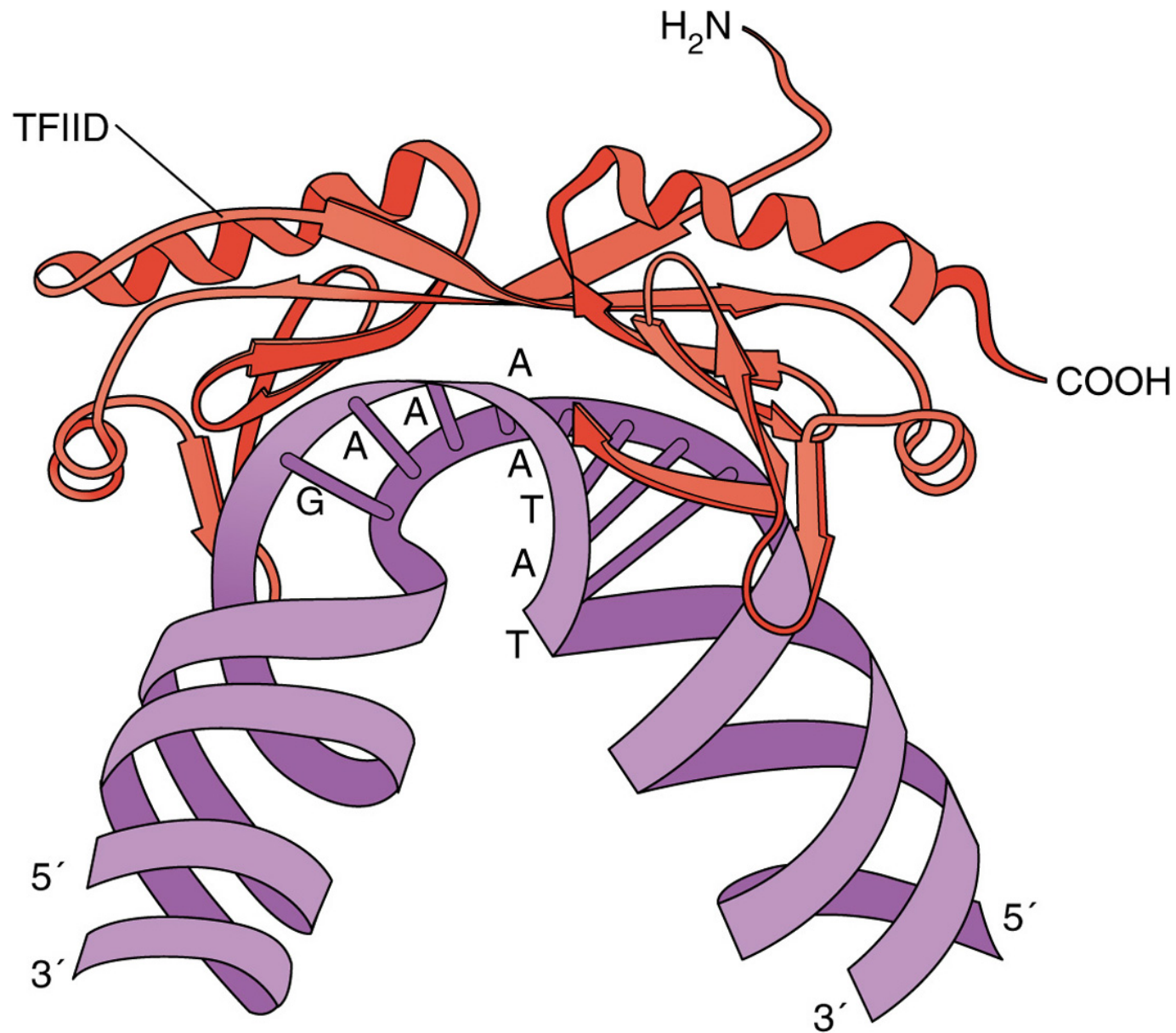
# Formation of transcription initiation complex

A.

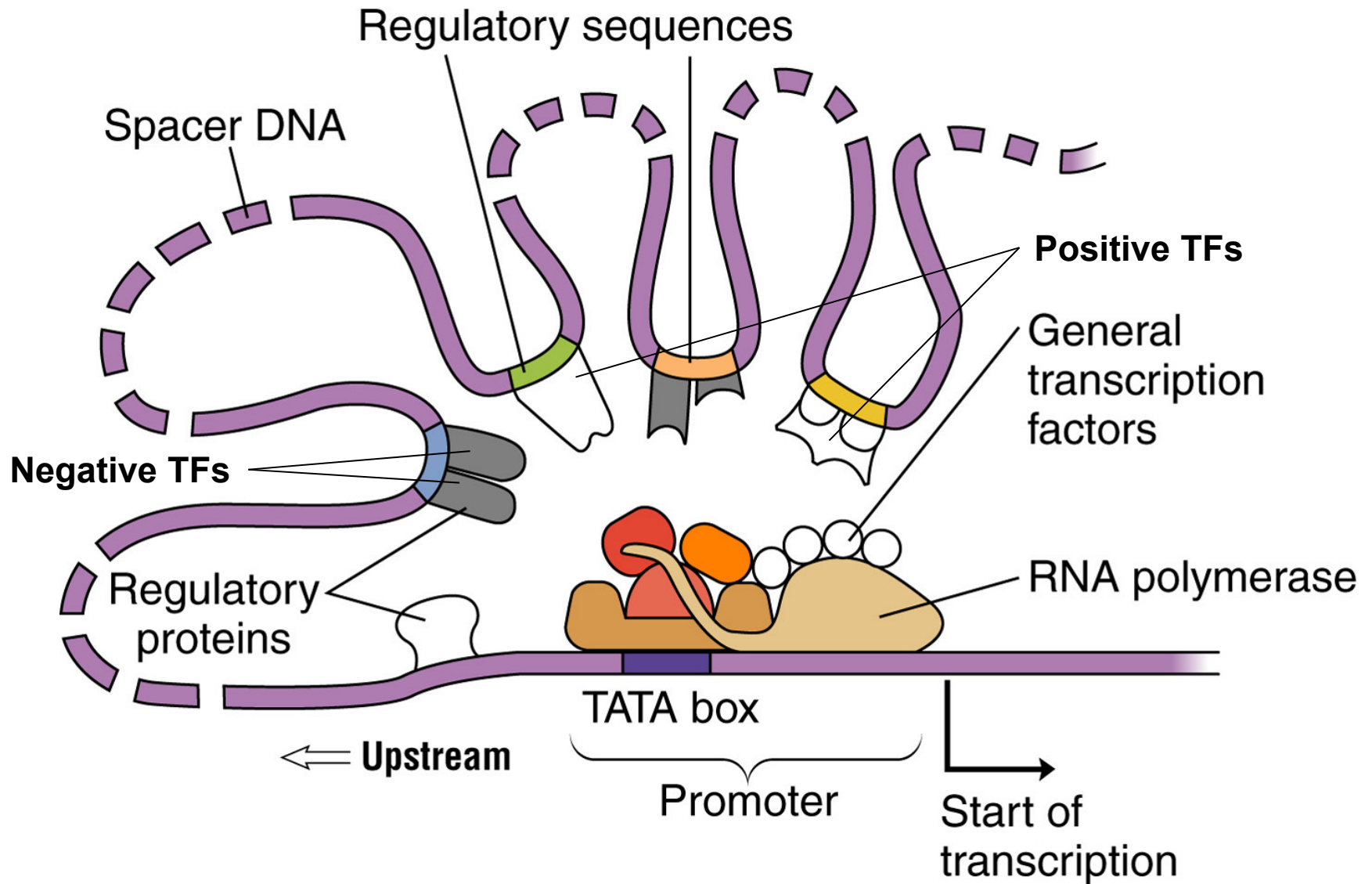




B.



# Formation of transcription initiation complex





# Mechanism of transcriptional regulation by TAFs

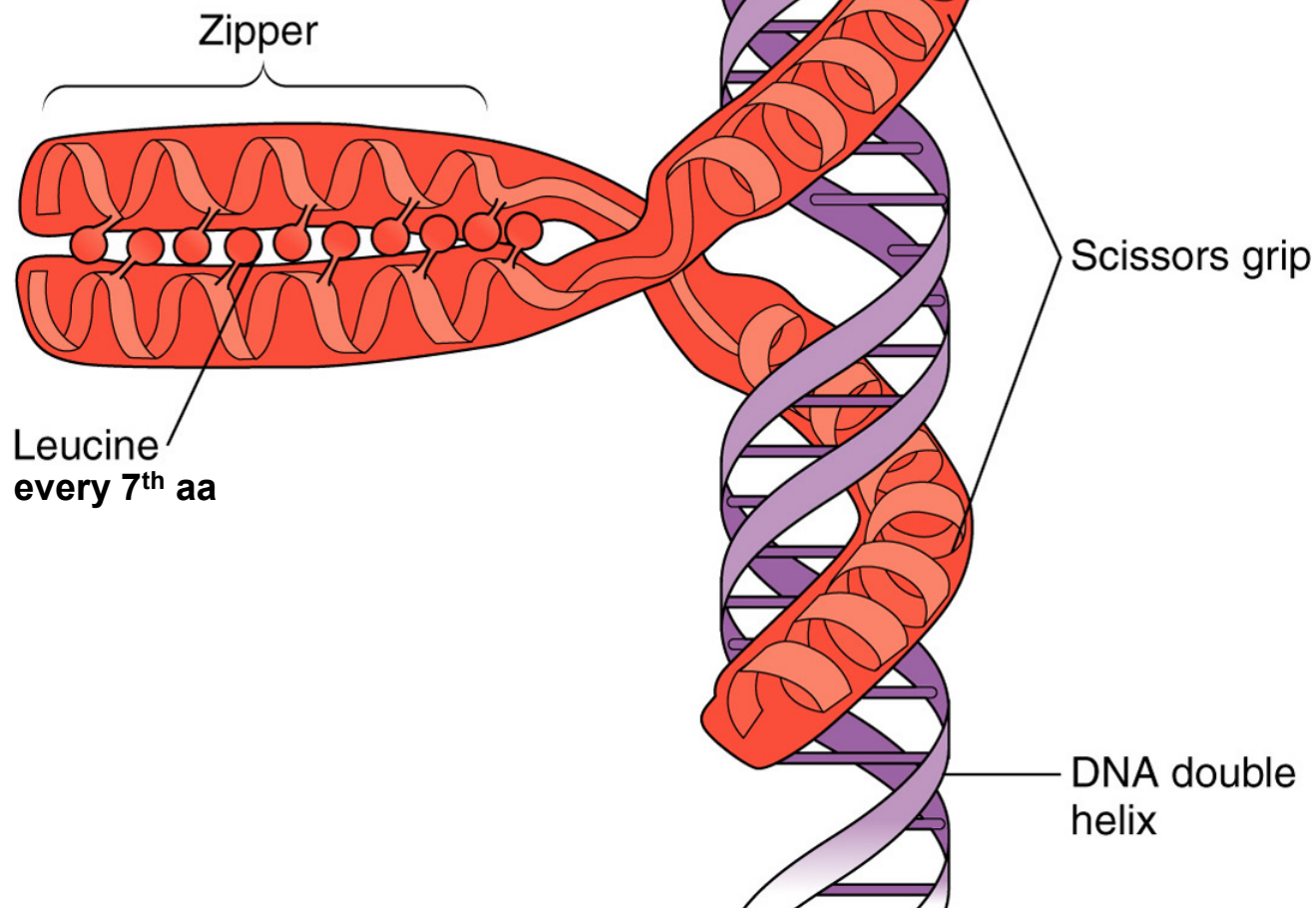
Signal recognition



Dimerization

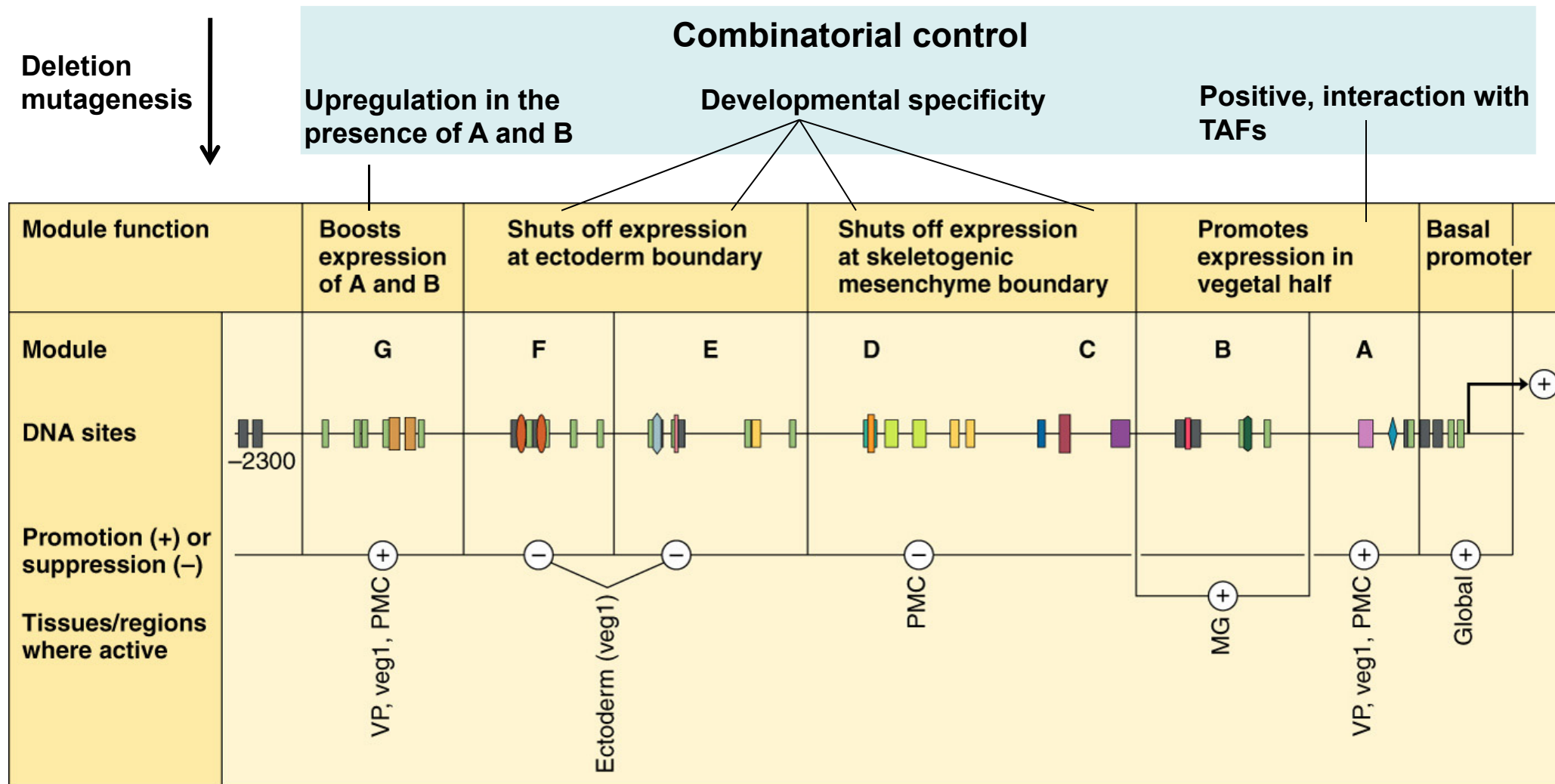


DNA binding  
and  
transcription  
activation



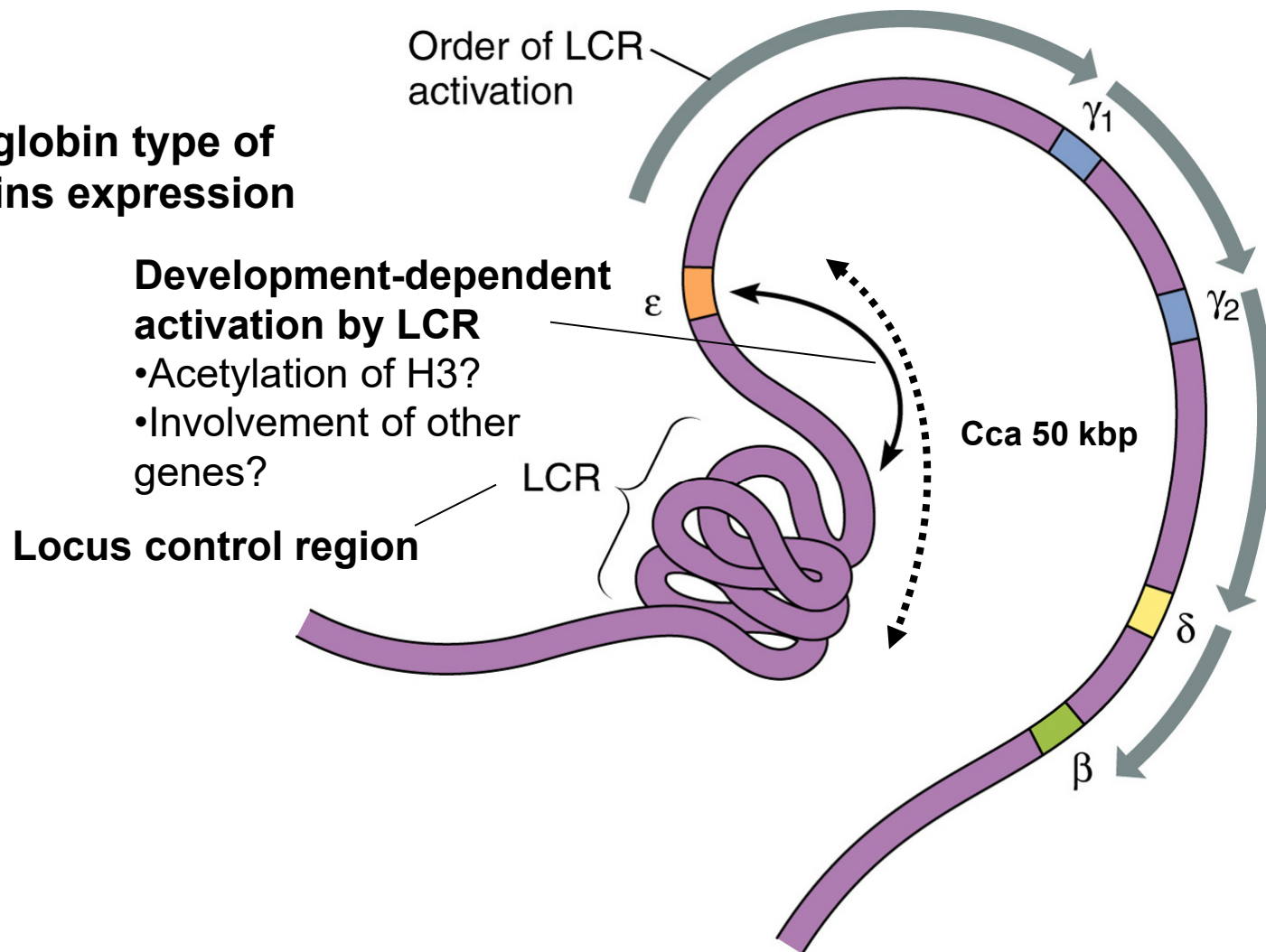
# “Microprocessor-like” acting promoters

## ProENDO16:REPORTER



# “Microprocessor-like” acting promoters

## Regulation of $\beta$ -globin type of hemoglobin chains expression





# Outline of Lesson 10

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  - Transcriptional activation
- Post-transcriptional gene regulation
  - Splicing of hnRNA

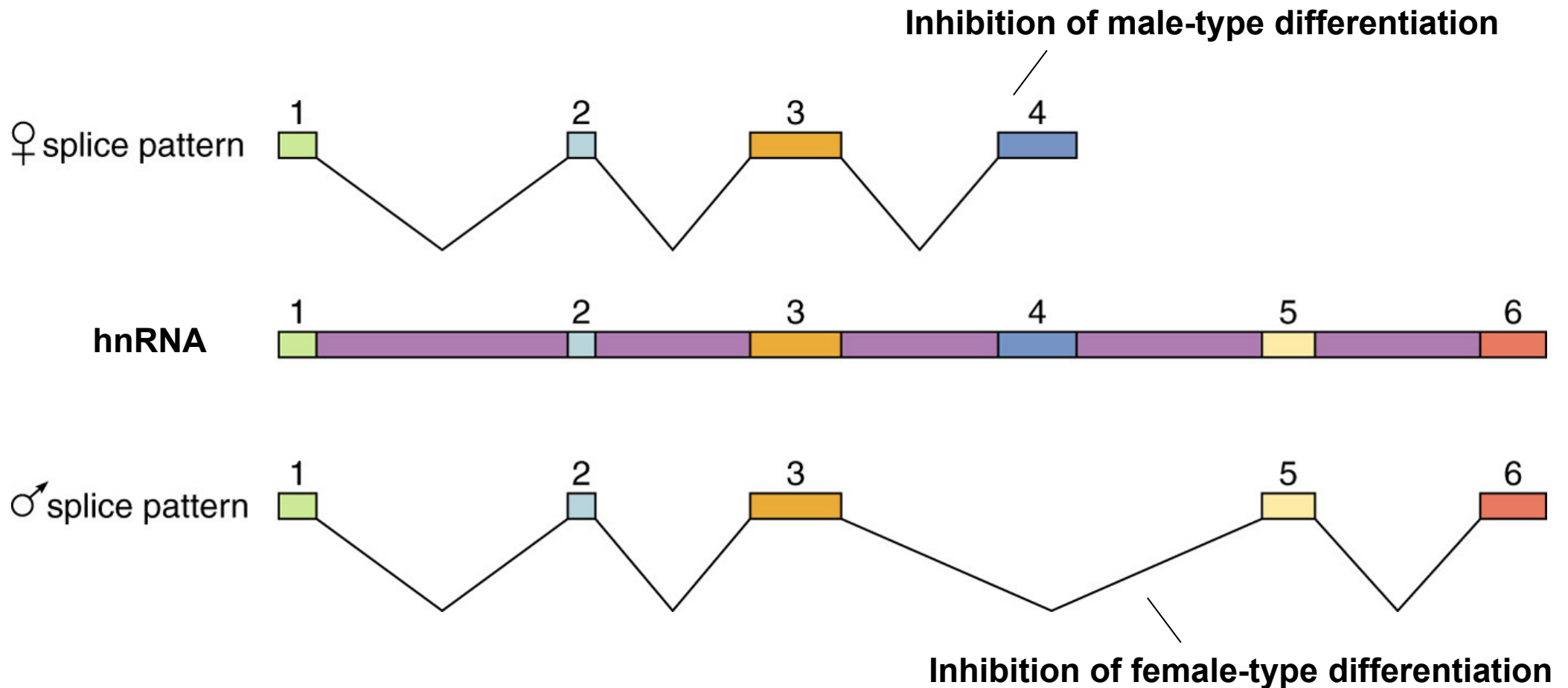
# Splicing of hnRNA



## INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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Evropským sociálním fondem  
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# Sex-specific splicing of *DOUBLE SEX (DSX)* hnRNA in *Drosophila*







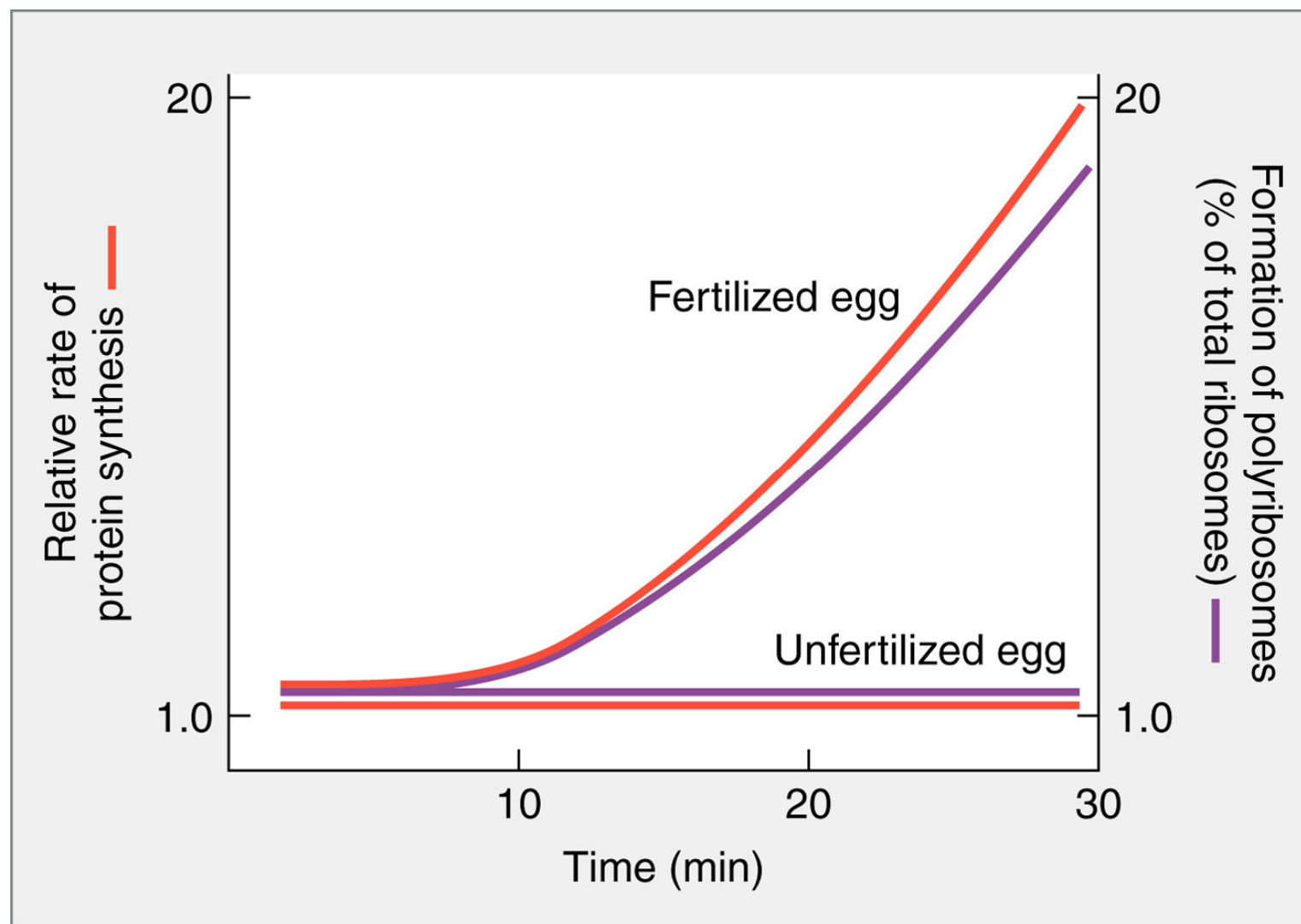
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## Regulation of Gene Expression during Development

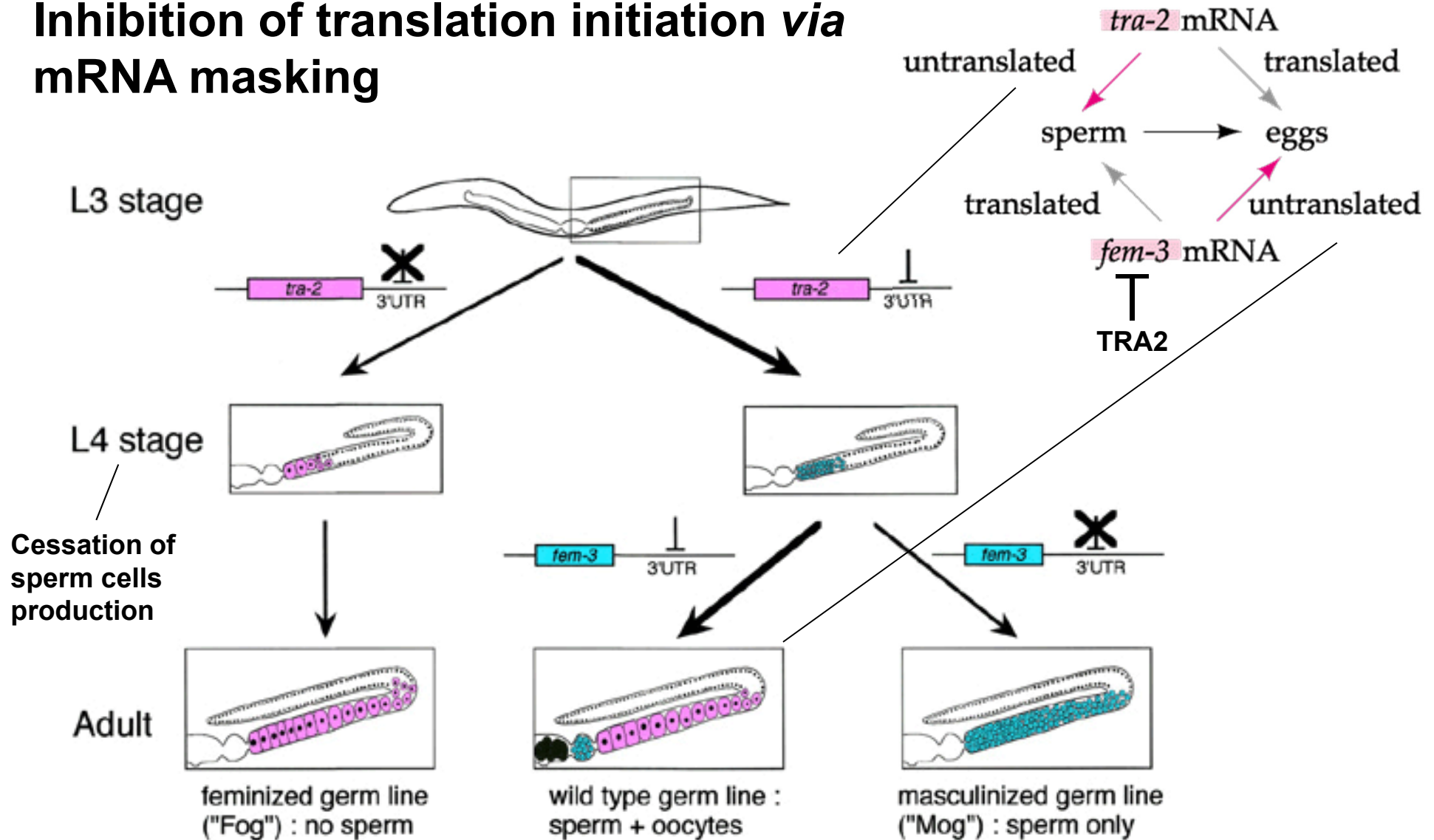
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  - Splicing of hnRNA
  - Translation initiation



# Translation initiation after egg fertilization



# Inhibition of translation initiation *via* mRNA masking



Puoti et al., *EMBO Rep* (2001)

Developmental Biology 8e Online (<http://8e.devbio.com/>)

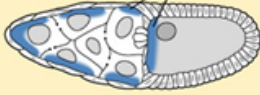


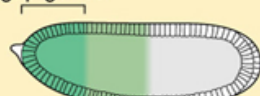


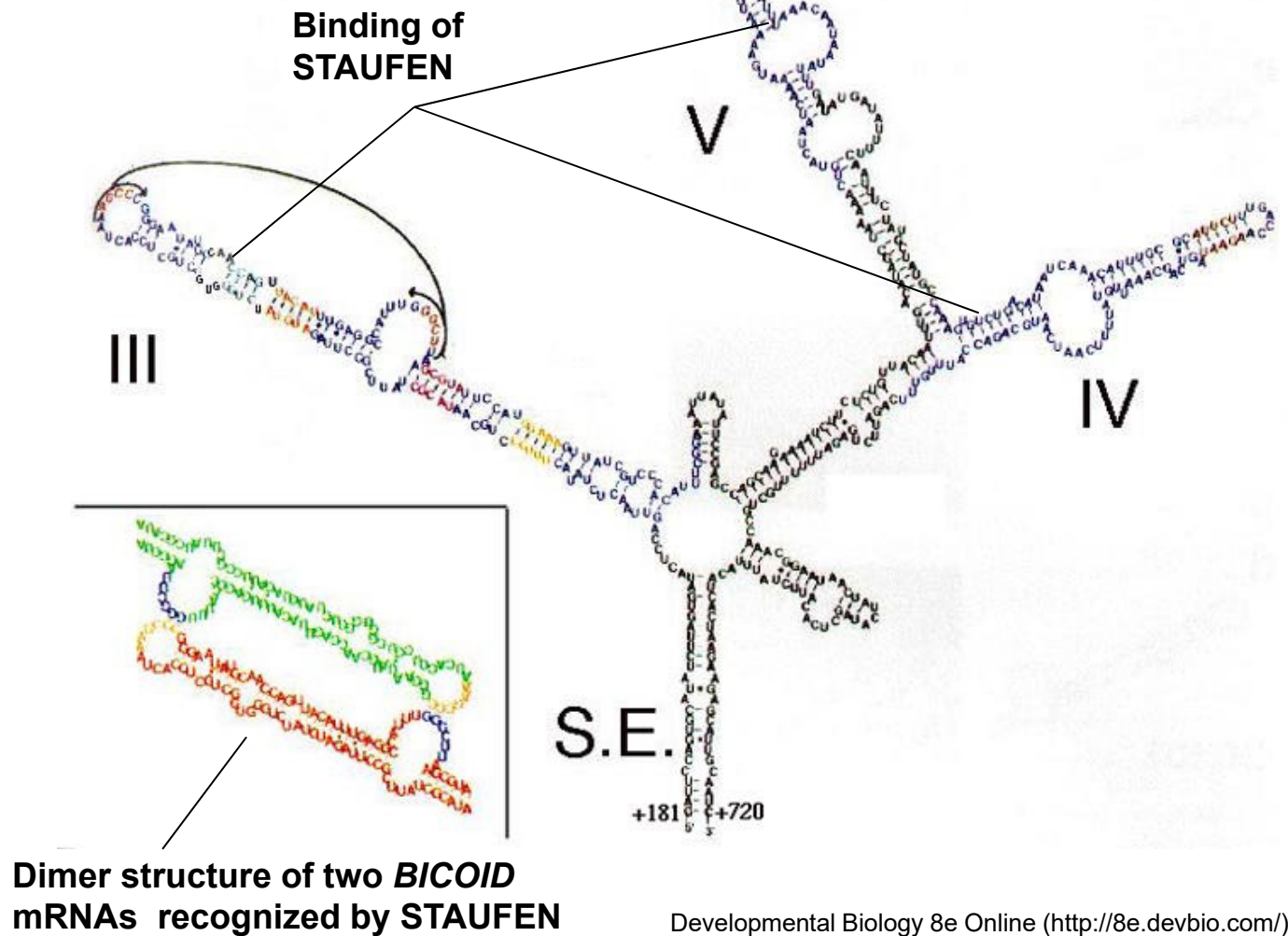
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  - Splicing of hnRNA
  - Translation initiation
  - Localization of mRNA

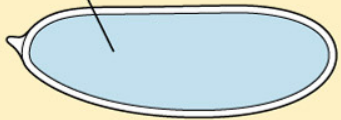
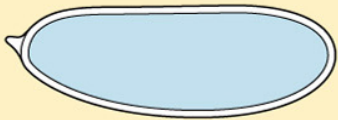
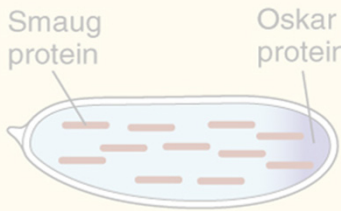
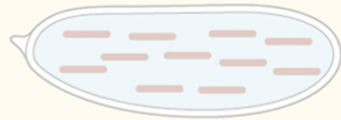
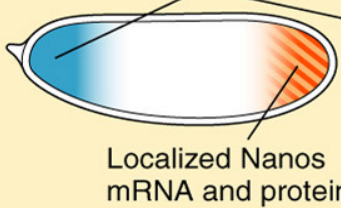
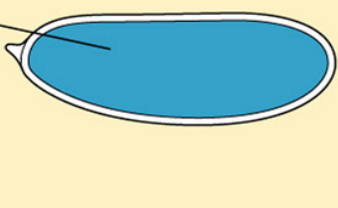
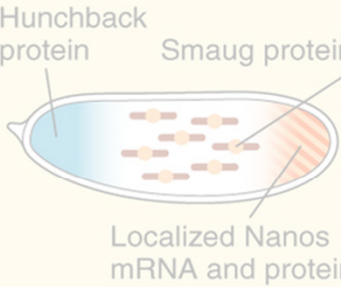
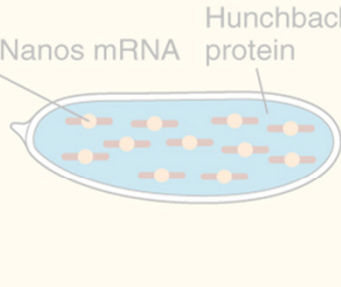




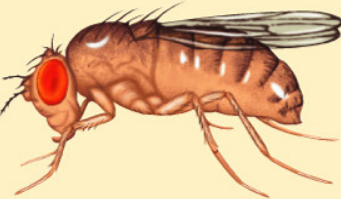

# Regulation via mRNA localization

	Anterior:
Oocyte/follicle	Bicoid mRNA 
Fertilized egg	Bicoid mRNA 
512-nuclei stage (cycle 9)	Bicoid protein 
6,000-cell blastoderm zygotic response	Anterior gap genes  hunchback

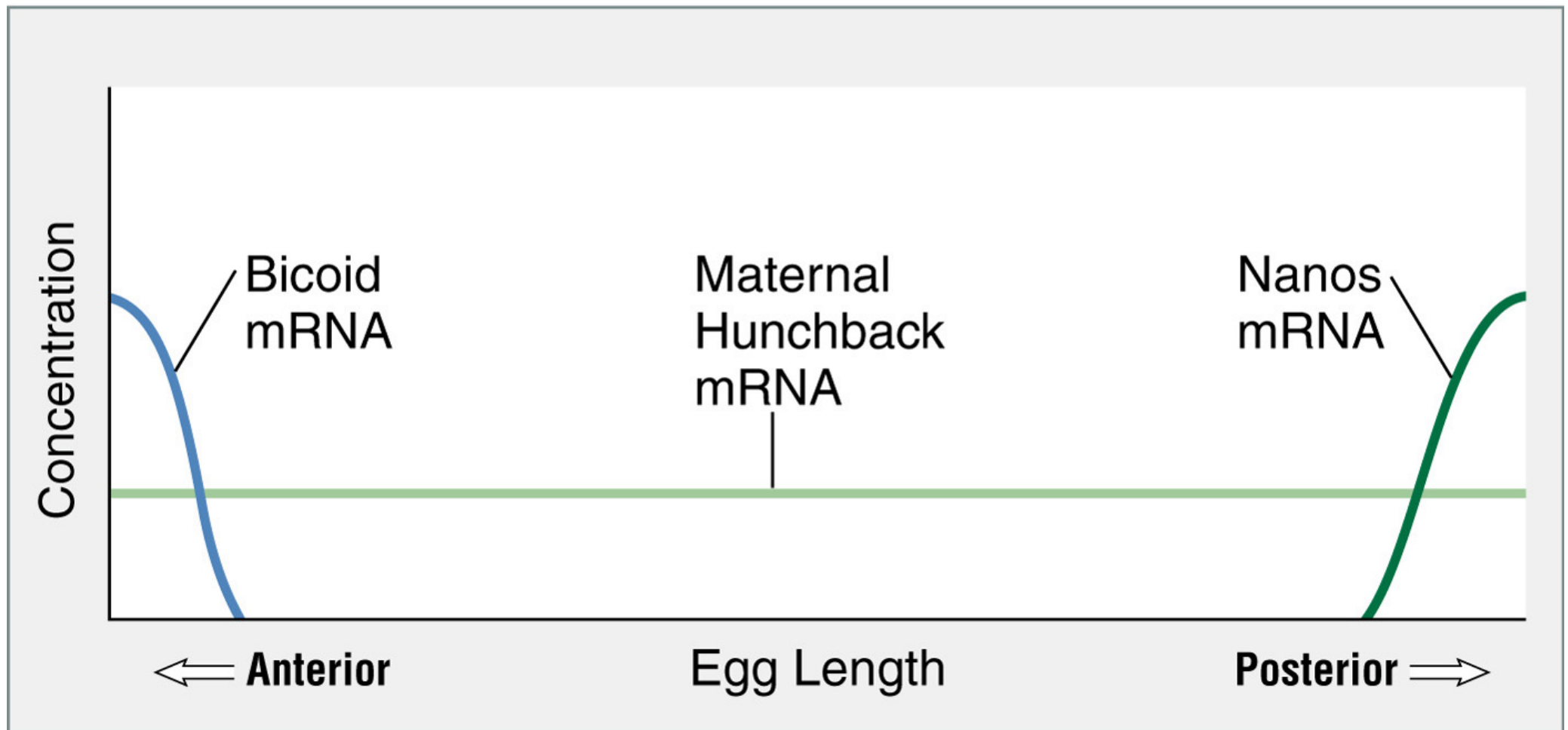




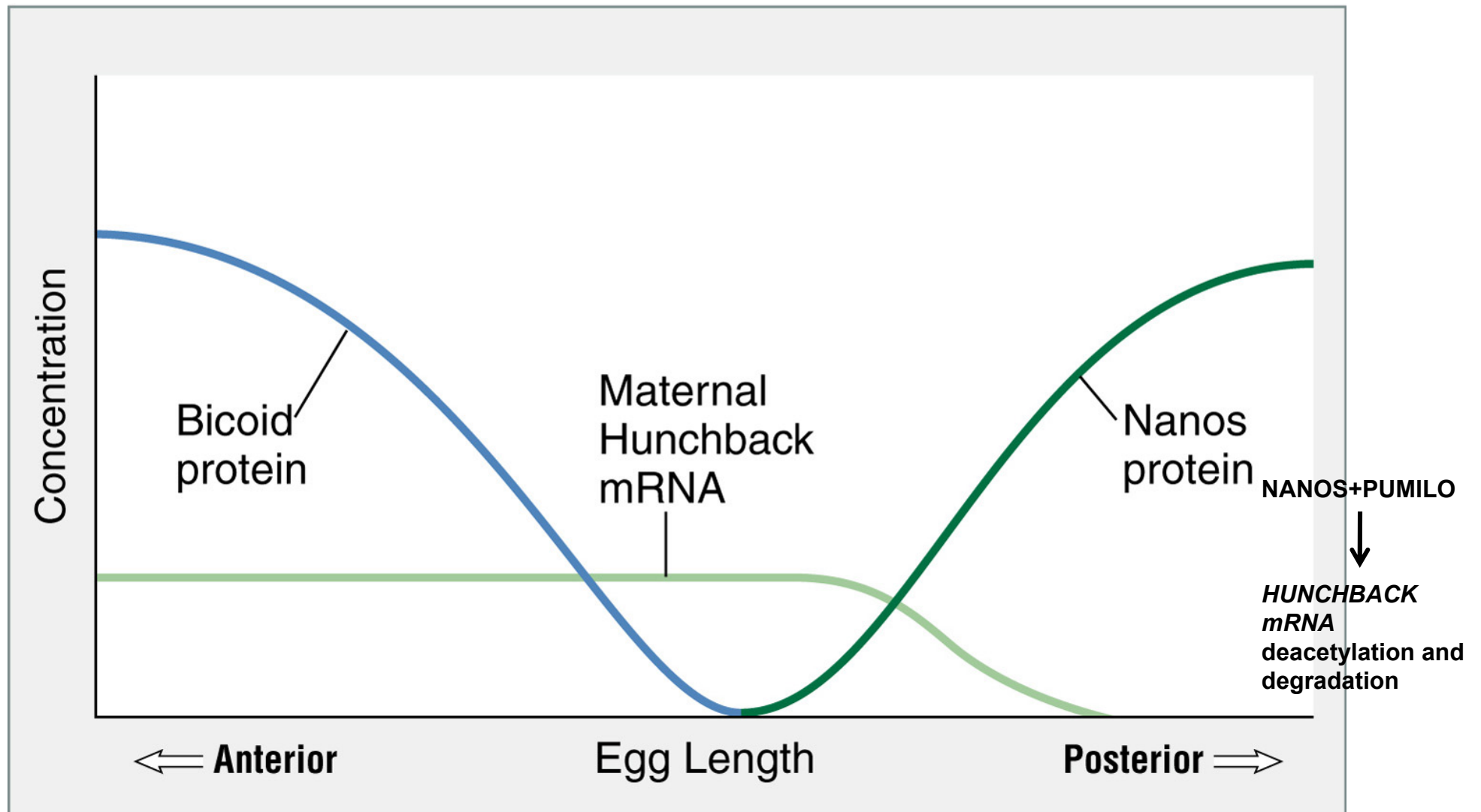
# Regulation via mRNA localization

	A. Nanos normal ( <i>nanos</i> <sup>+/+</sup> )	B. Nanos mutant ( <i>nanos</i> <sup>-/-</sup> )	C. Oskar normal ( <i>osk</i> <sup>+/+</sup> )	D. Oskar mutant ( <i>osk</i> <sup>-/-</sup> )
Egg on laying				
Cleaving egg				
Larva				
Adult		LETHAL		LETHAL

A.

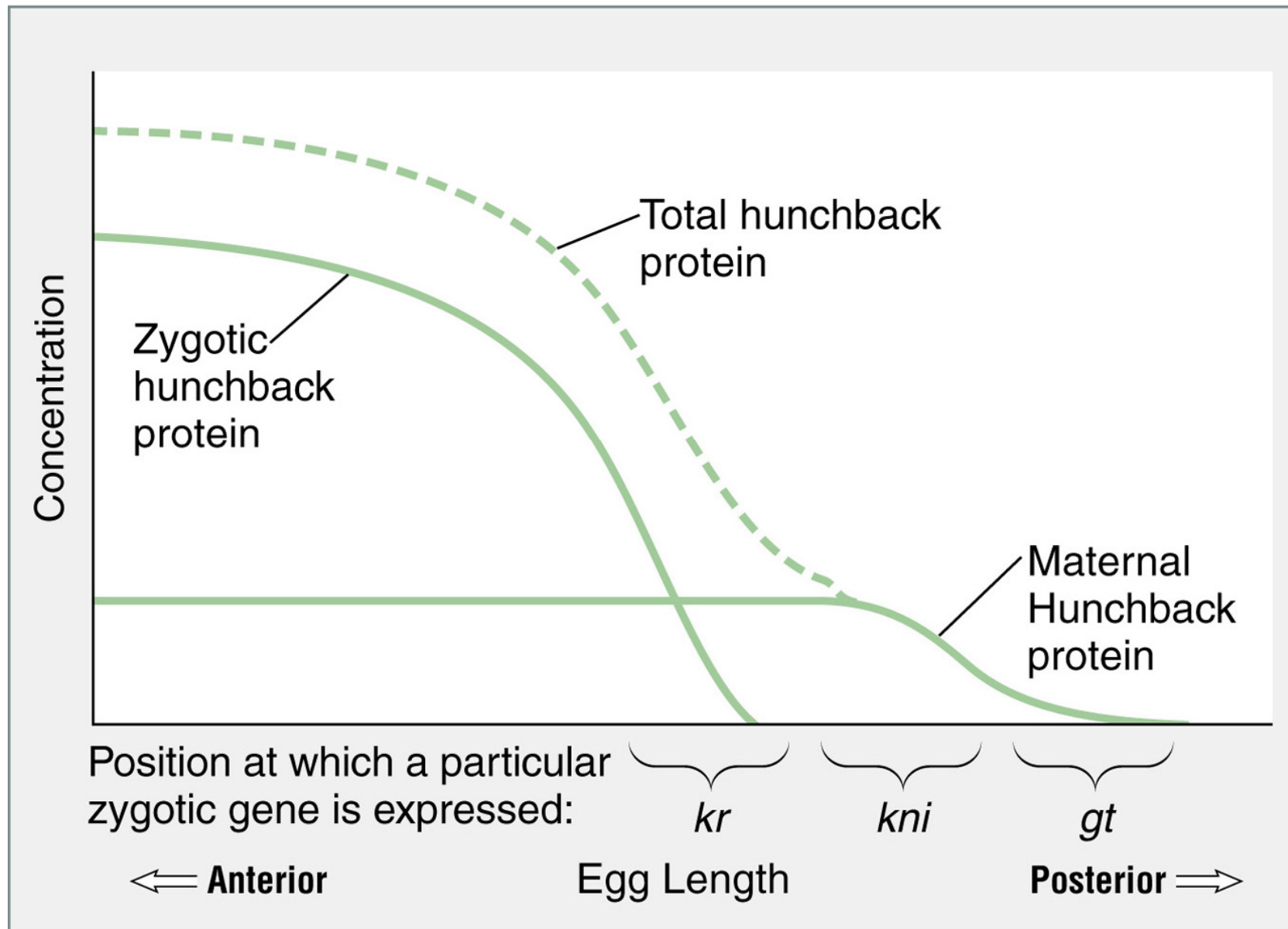


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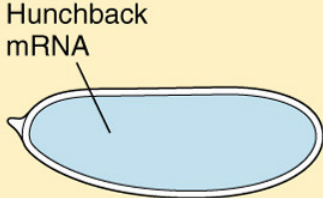

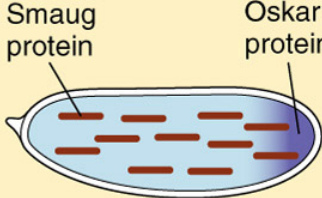
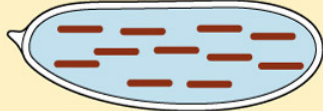
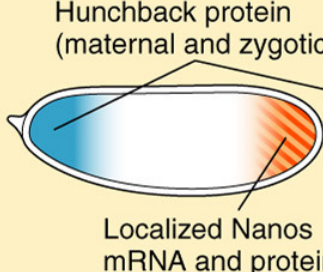

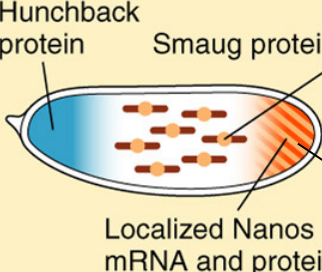
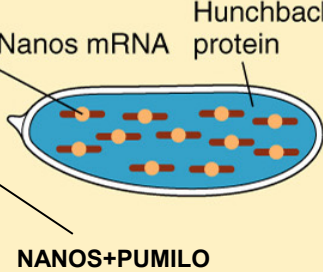










C.



# Regulation via mRNA localization

	A. Nanos normal ( <i>nanos</i> <sup>+/+</sup> )	B. Nanos mutant ( <i>nanos</i> <sup>-/-</sup> )	C. Oskar normal ( <i>osk</i> <sup>+/+</sup> )	D. Oskar mutant ( <i>osk</i> <sup>-/-</sup> )
Egg on laying				
Cleaving egg				
Larva				
Adult		LETHAL		LETHAL



# Outline of Lesson 10

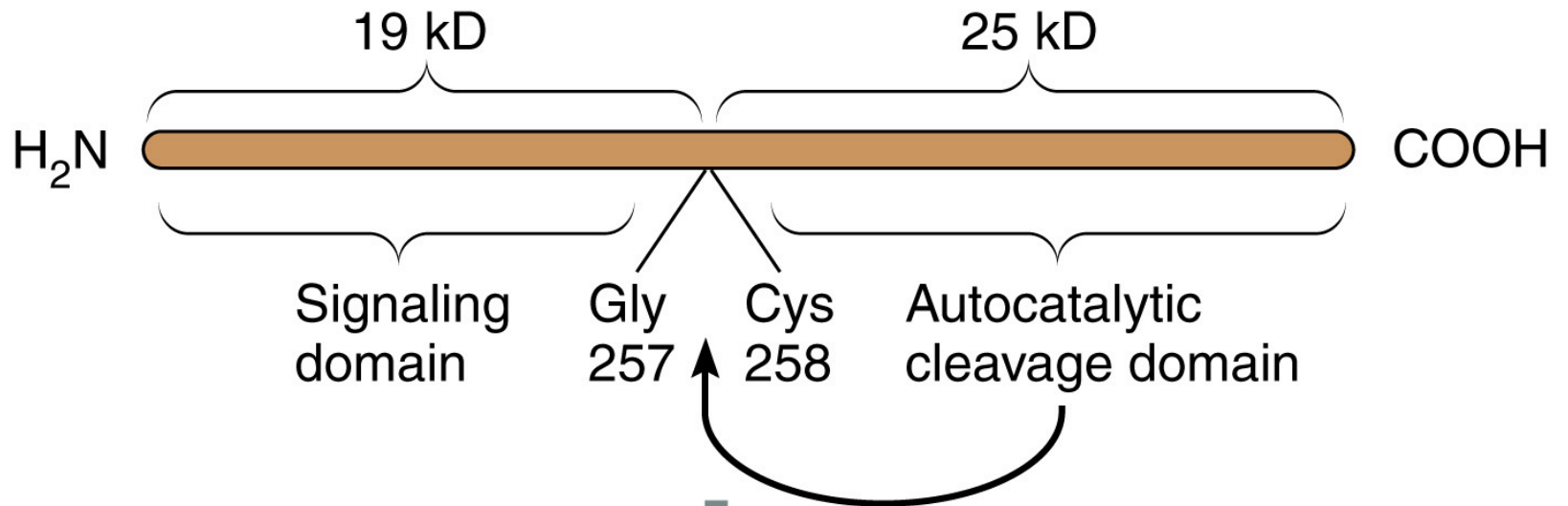
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# Regulation via protein localization

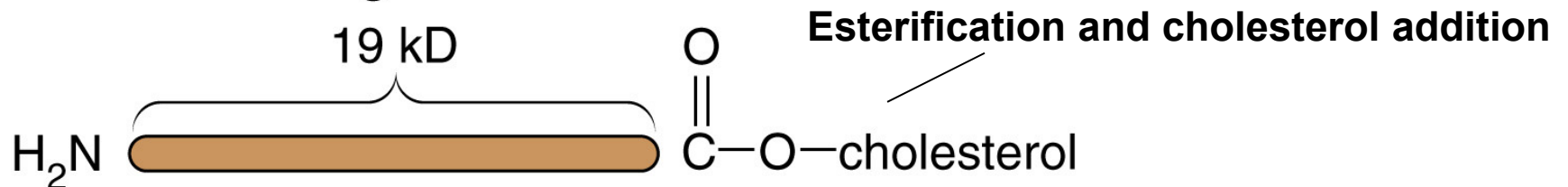
## Hedgehog Processing

### A. Precursor

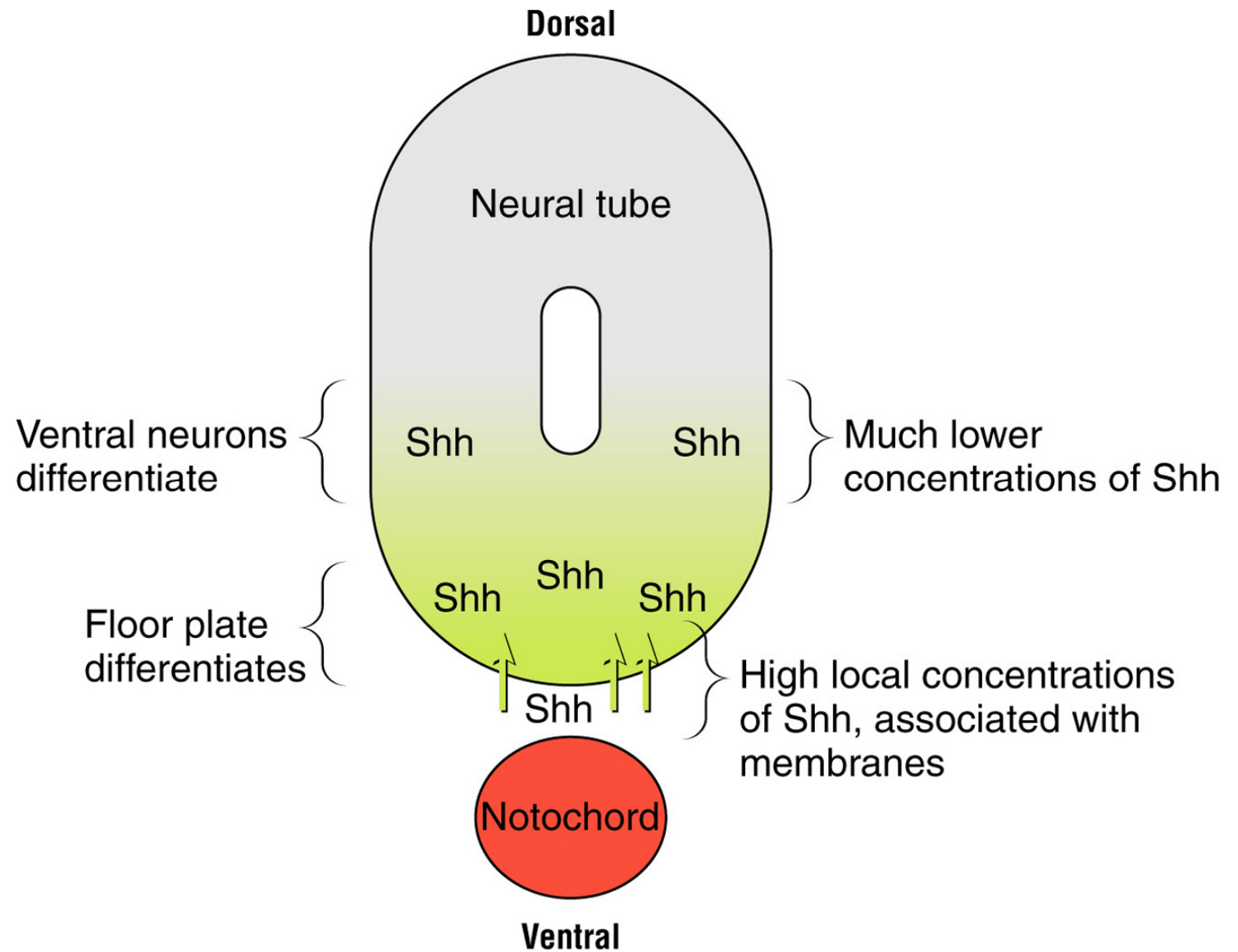


Leaving the Golgi apparatus

### B. Active Hh Ligand



## Regulation via protein localization





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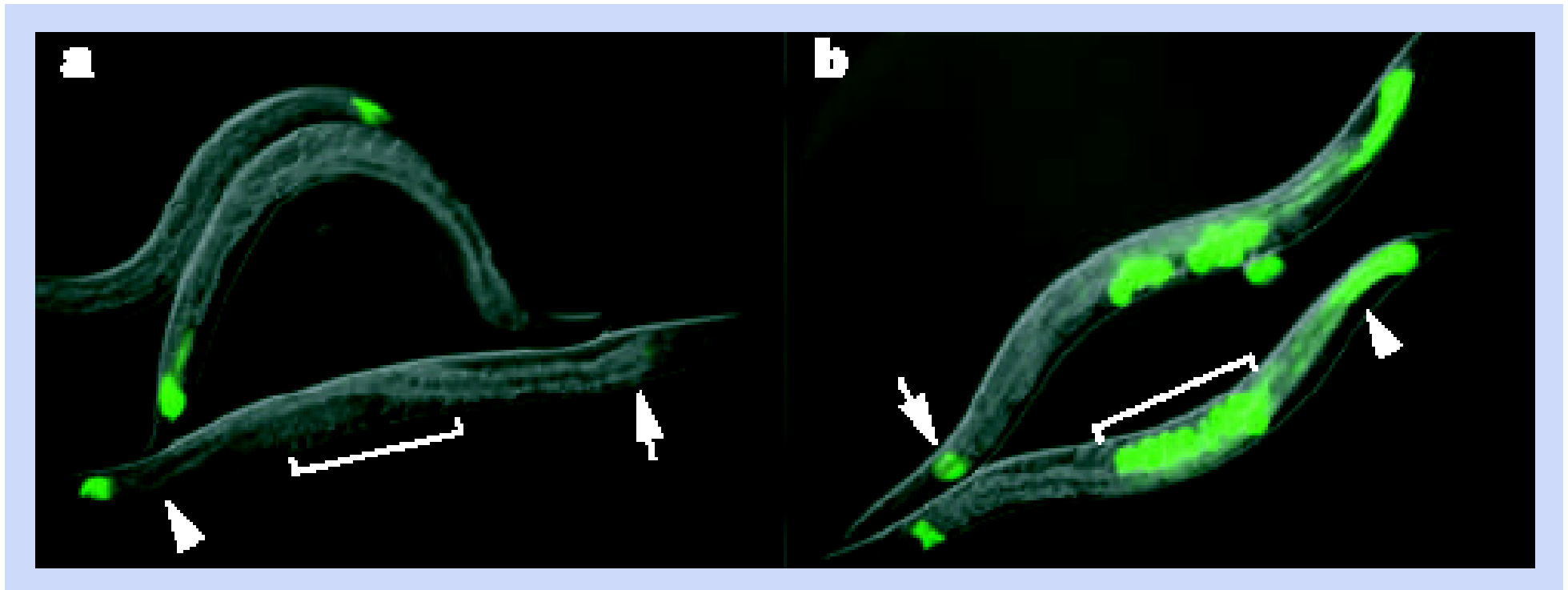
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- RNA interference
  - Identification and mechanism of gene expression regulation via RNA interference



# RNA interference as a natural mechanism of the gene expression

*RNAi*

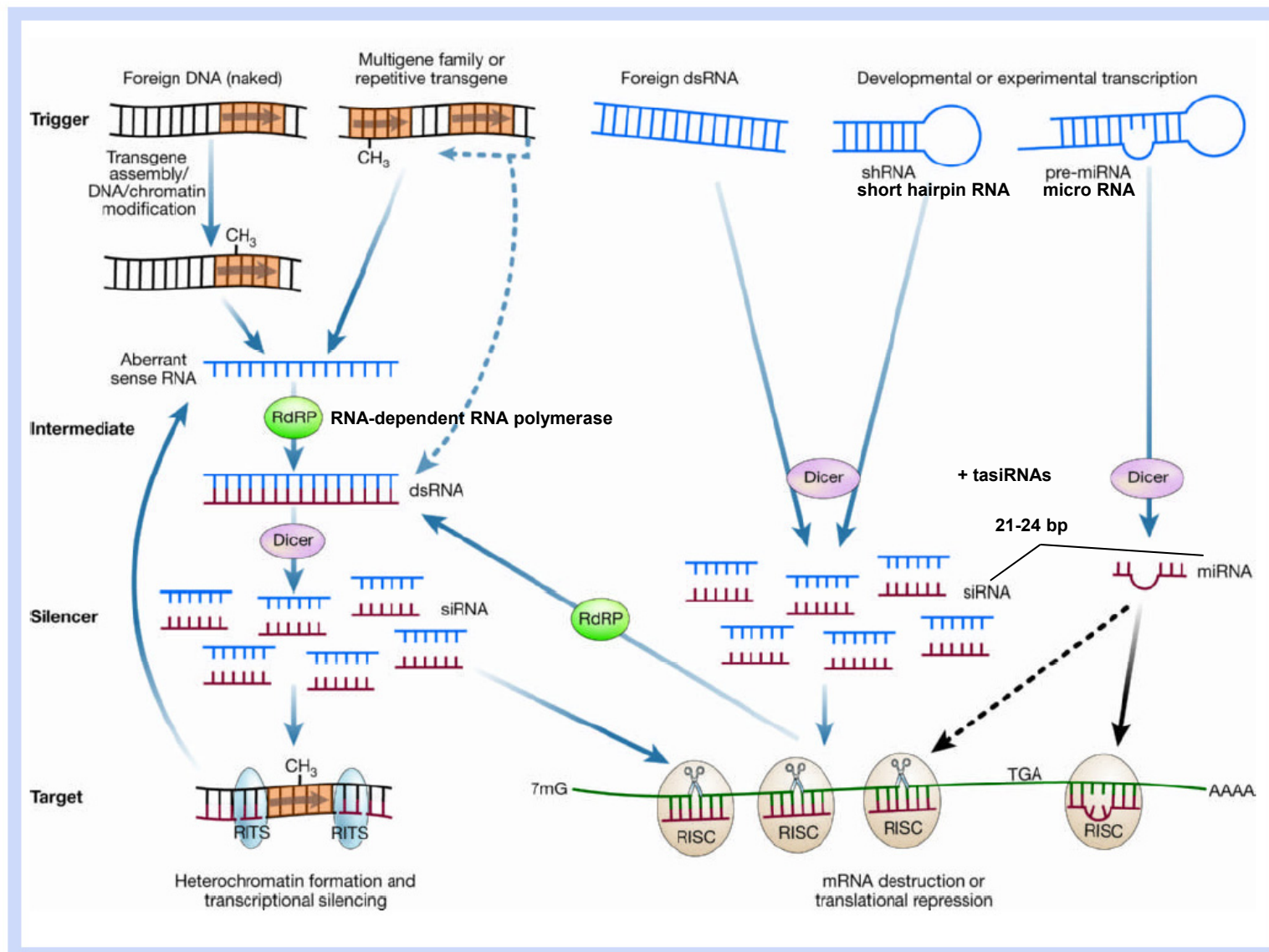
*rnai*



Mello and Conte, *Nature* (2004)

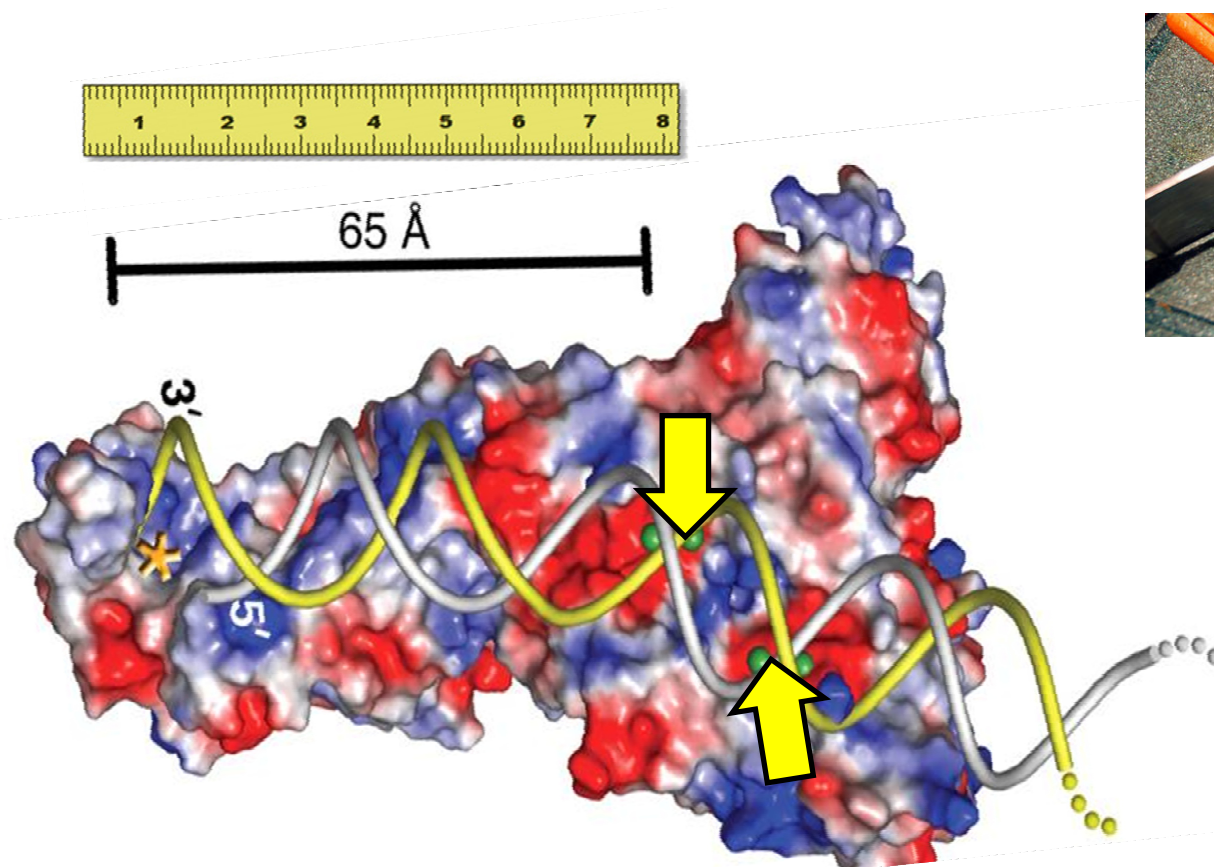


# Mechanism of RNA interference



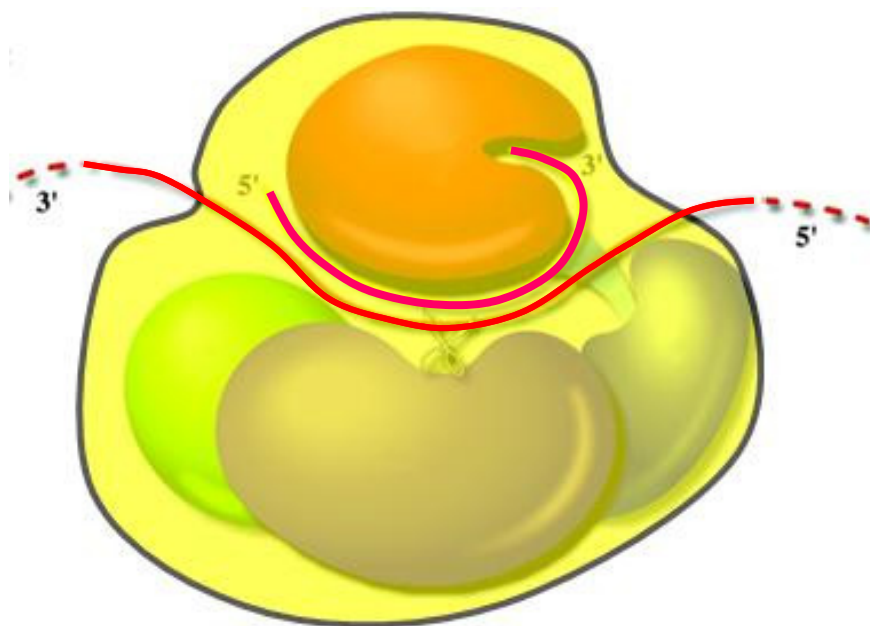
Mello and Conte, *Nature* (2004)

# Dicer and Dicer-like proteins

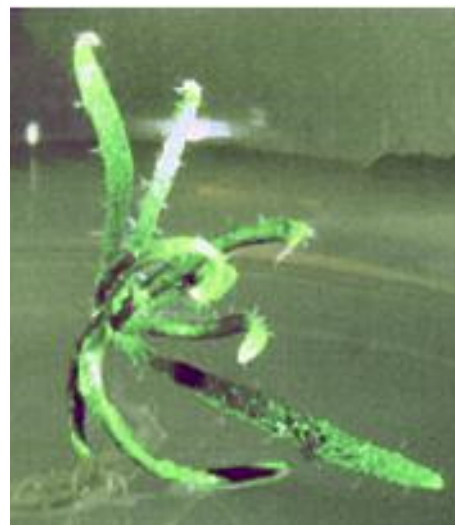


from MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W., Adams, P.D., and Doudna, J.A. (2006) Structural basis for double-stranded RNA processing by Dicer. *Science* 311: [195-198](#). Reprinted with permission from AAAS. Photo credit: [Heidi](#)

# Argonaute proteins



*ago1*



*Argonauta argo*  
**Argonaut pelagický**



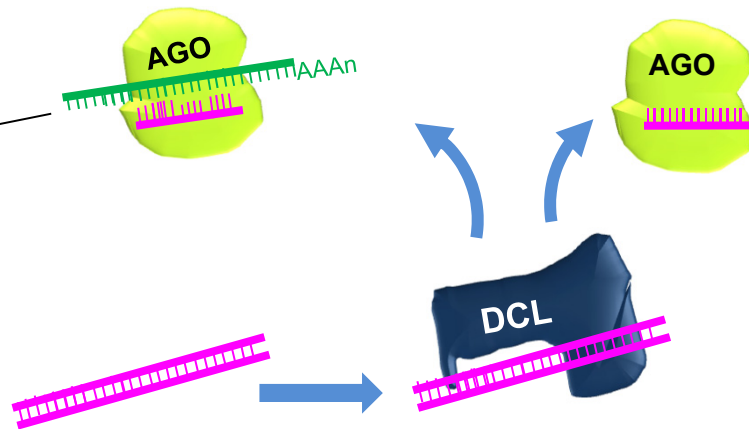
Reprinted by permission from Macmillan Publishers Ltd: EMBO J. Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., and Benning, C. (1998) *AGO1* defines a novel locus of *Arabidopsis* controlling leaf development. EMBO J. 17: [170–180](#). Copyright 1998; Reprinted from Song, J.-J., Smith, S.K., Hannon, G.J., and Joshua-Tor, L. (2004) Crystal structure of Argonaute and its implications for RISC slicer activity. Science 305: [1434–1437](#). with permission of AAAS.



## transcriptional gene silencing

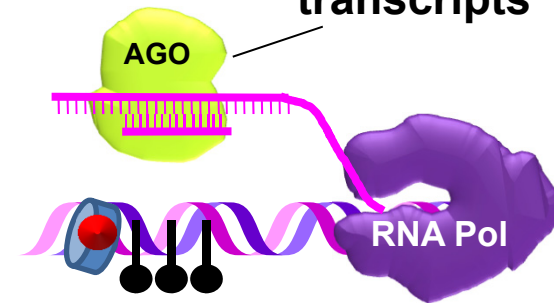
### siRNA

binding to DNA



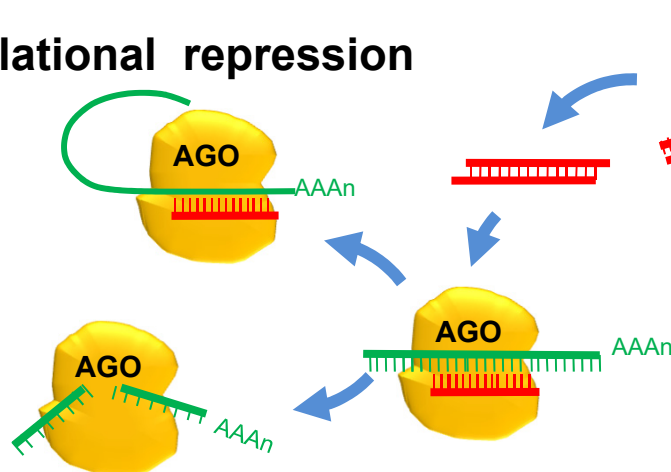
## post-transcriptional gene silencing

binding to specific transcripts

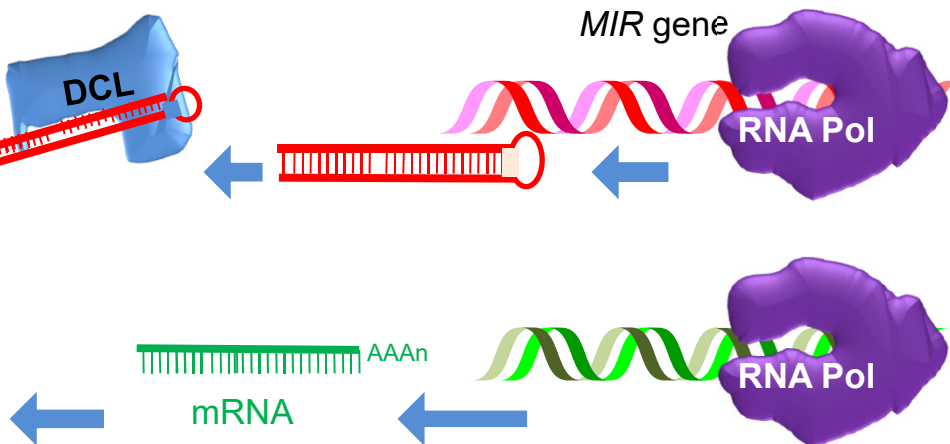


### miRNA

translational repression



transcriptional slicing



# The Nobel Prize in Physiology or Medicine 2006



**Andrew Z. Fire**

USA

Stanford University  
School of Medicine  
Stanford, CA, USA

b. 1959



**Craig C. Mello**

USA

University of  
Massachusetts Medical  
School  
Worcester, MA, USA

b. 1960



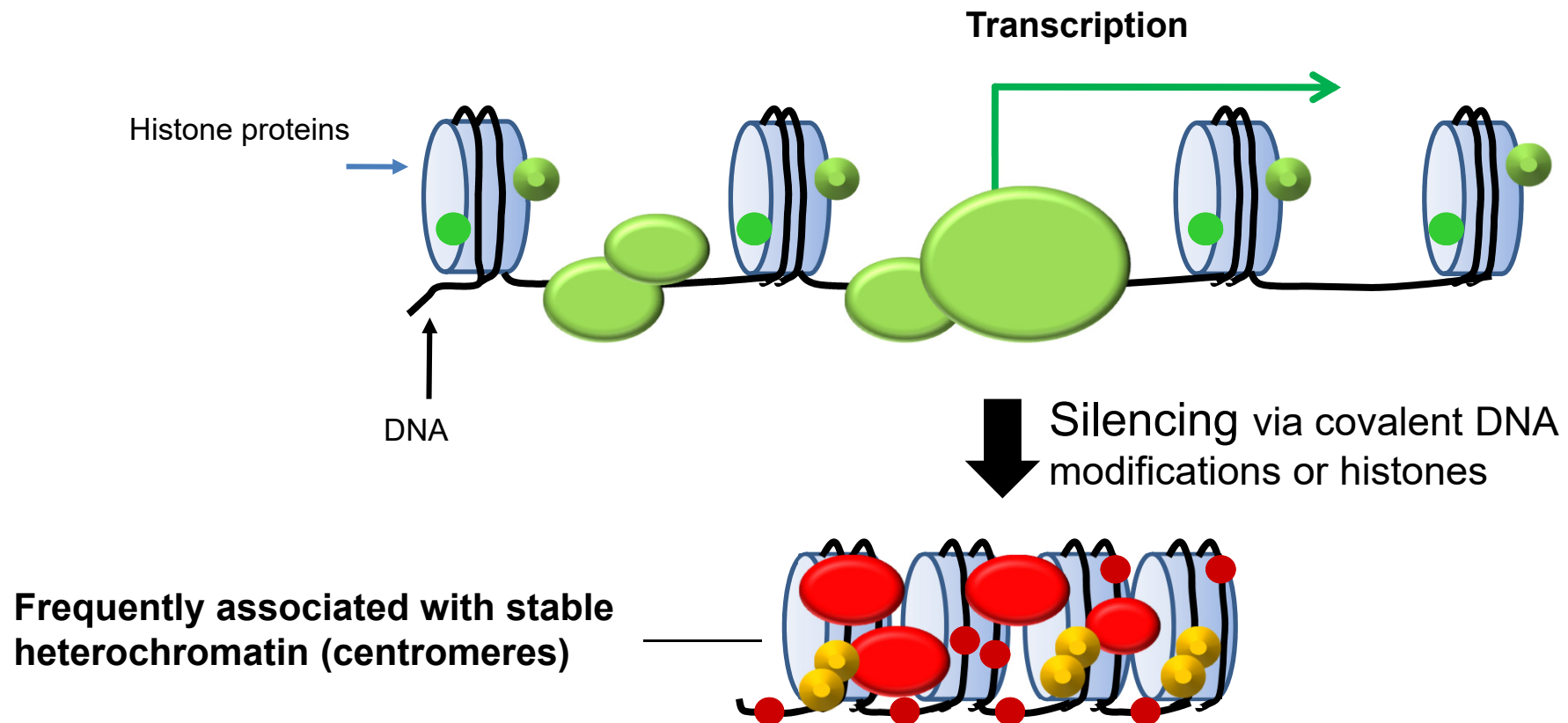
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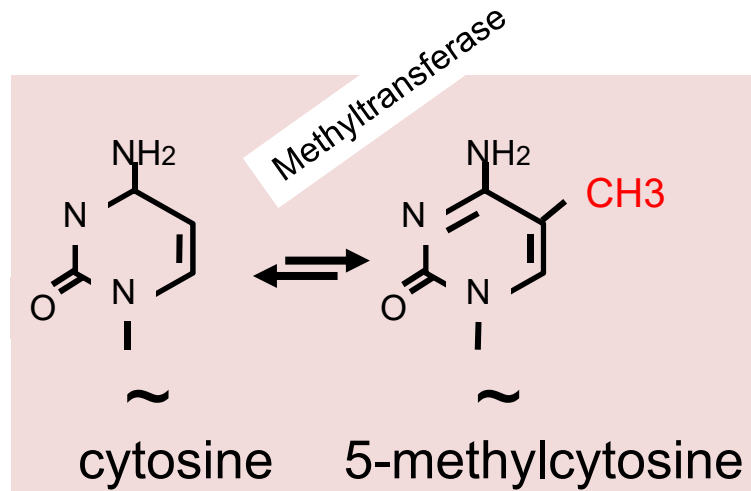
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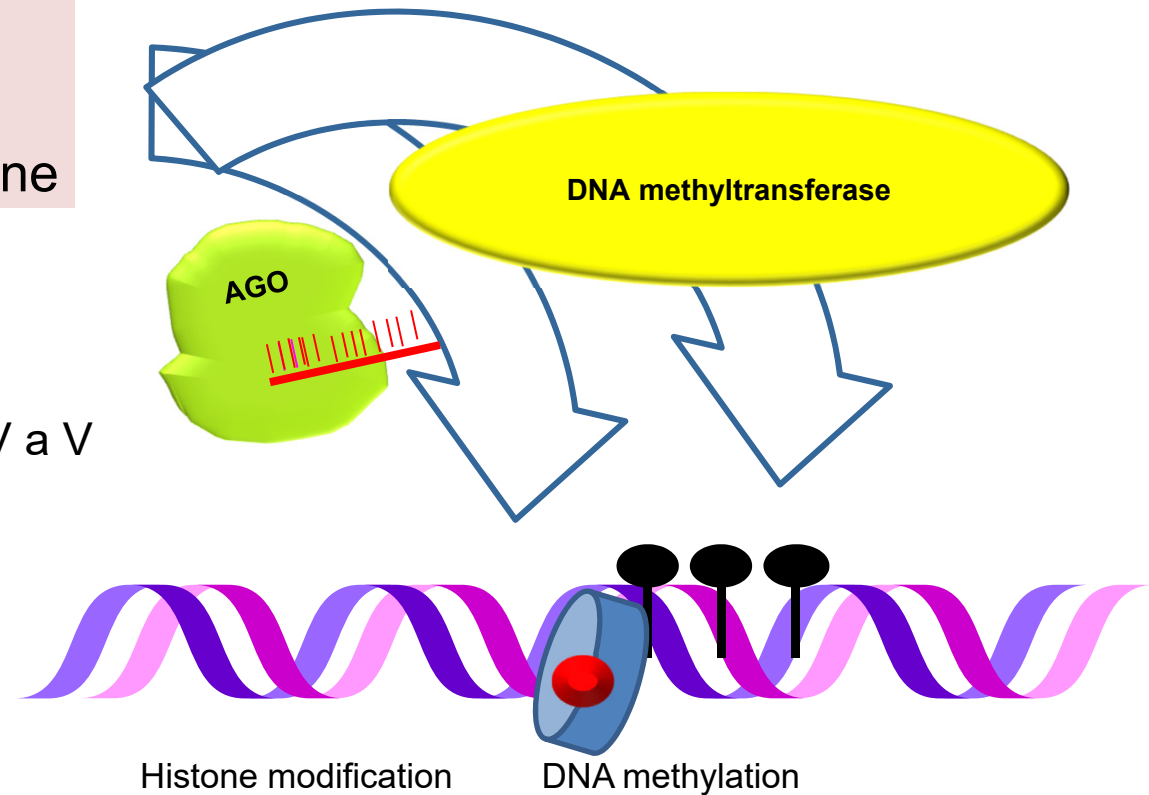
# Transcriptional gene silencing via covalent modifications of DNA

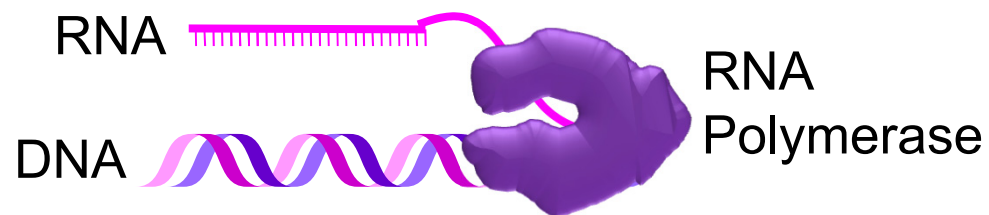


# Transcriptional gene silencing via DNA methylation



- molecular mechanism unknown
- involvement of RNA polymerase IV a V

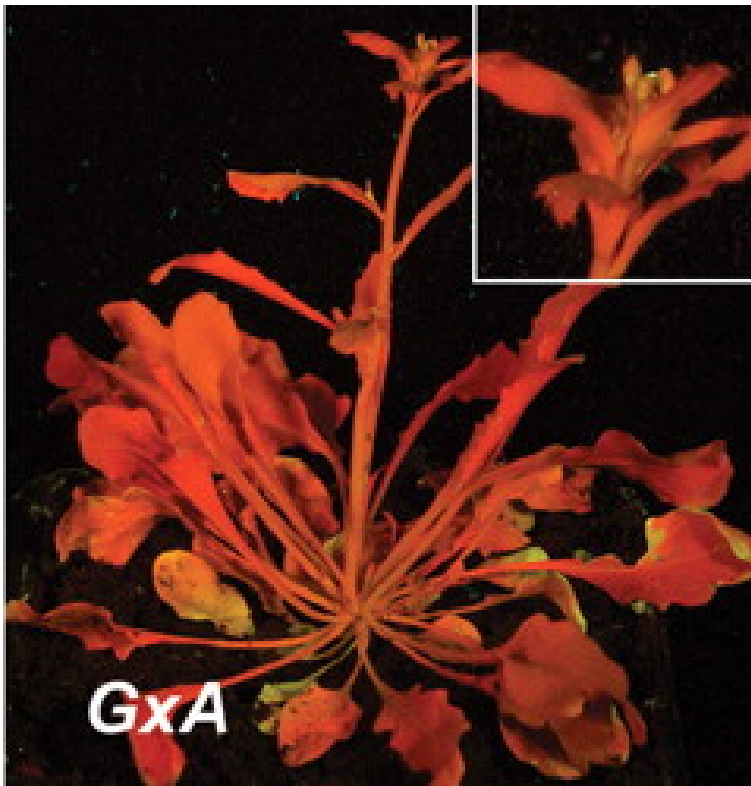




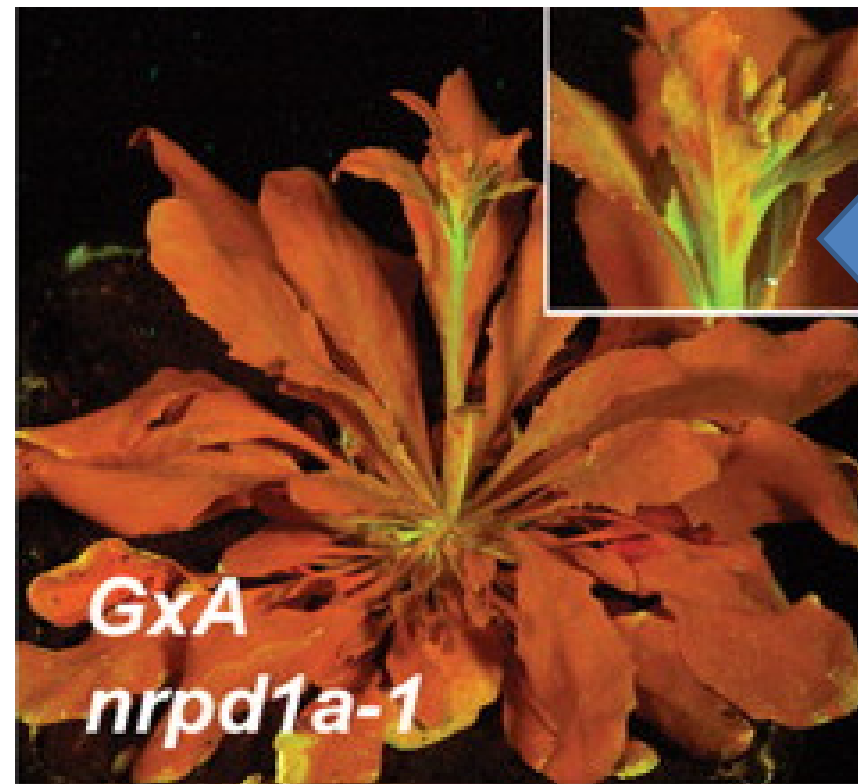
Complex	Distribution	Function
RNA Polymerase I	All eukaryotes	Production of rRNA
RNA Polymerase II	All eukaryotes	Production of mRNA, microRNA
RNA Polymerase III	All eukaryotes	Production of tRNA, 5S rRNA
RNA Polymerase IV	Land plants	Production of siRNA
RNA Polymerase V	Angiosperms	Recruitment of AGO to DNA

# Loss of function of an RNA Pol IV gene interferes with silencing

*Arabidopsis* with silenced  
GFP gene

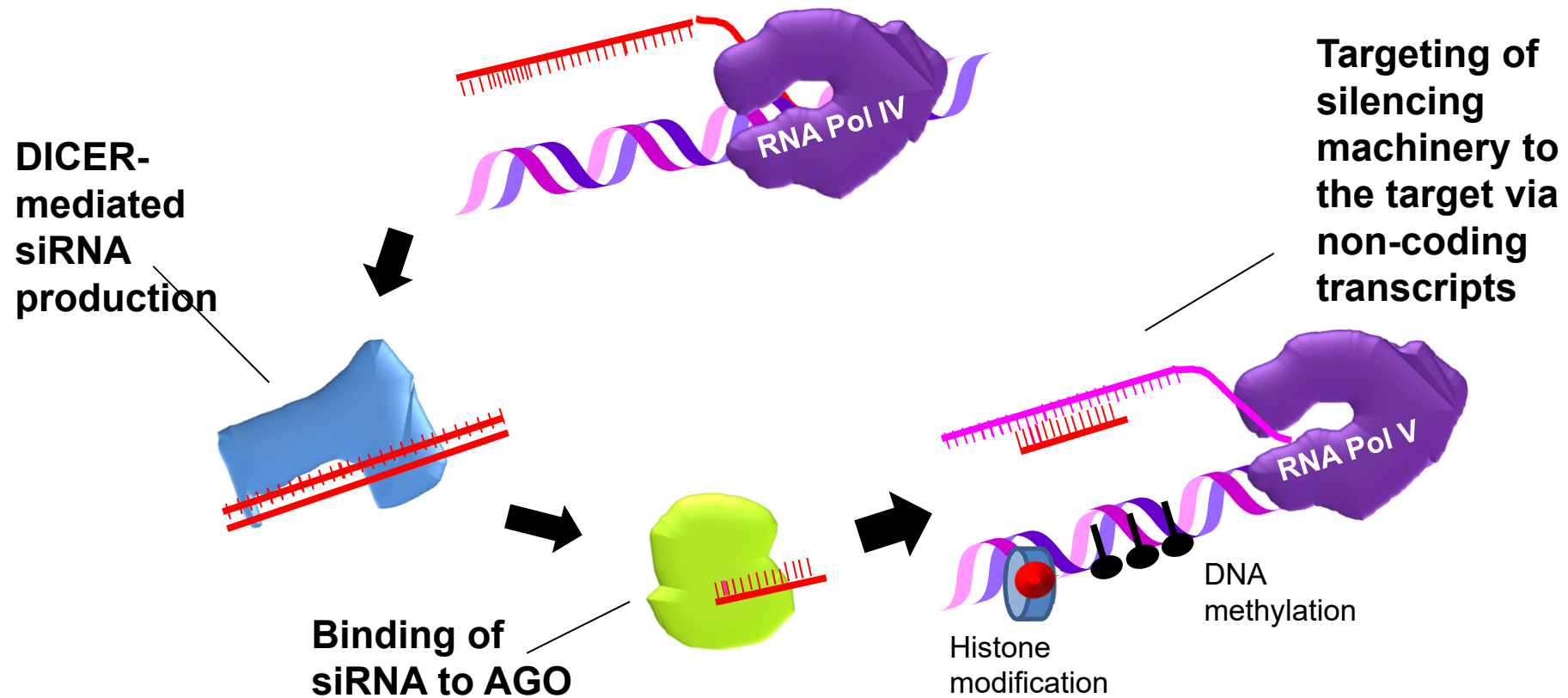


*nrpd1a-1*

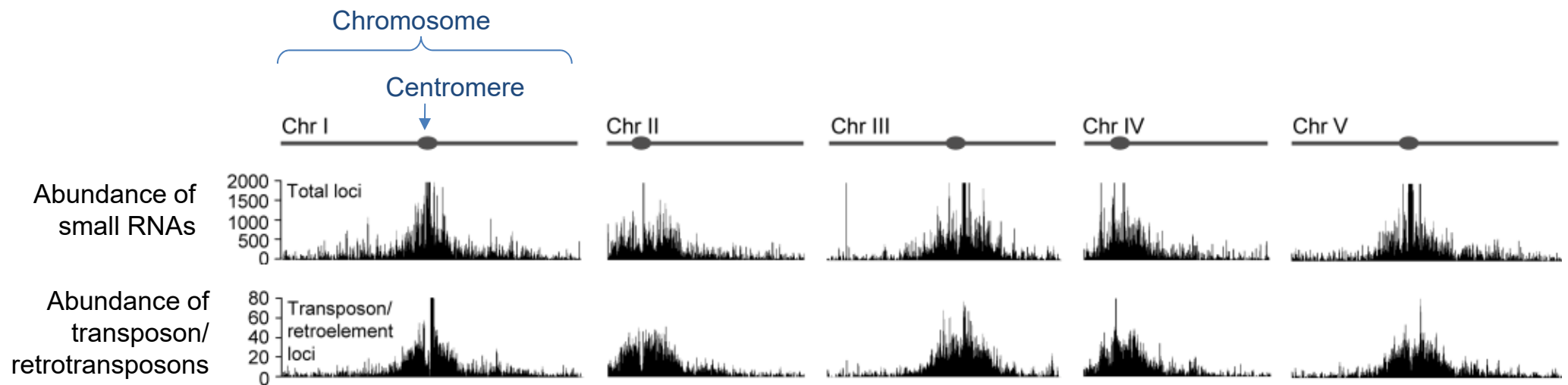


From Herr, A.J., Jensen, M.B., Dalmay, T., and Baulcombe, D.C. (2005) RNA polymerase IV directs silencing of endogenous DNA. *Science* 308: [118-120](#). Reprinted with permission from AAAS.

# RNA Pol IV and V are necessary for transcriptional silencing



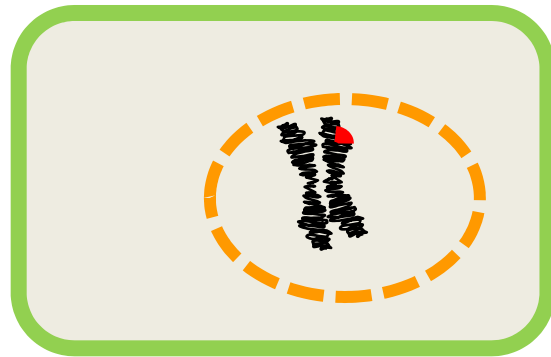
# Most siRNAs are produced from transposons and repetitive DNA



Kasschau, K.D., Fahlgren, N., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., and Carrington, J.C. (2007) Genome-wide profiling and analysis of *Arabidopsis* siRNAs. *PLoS Biol* 5(3): [e57](#).

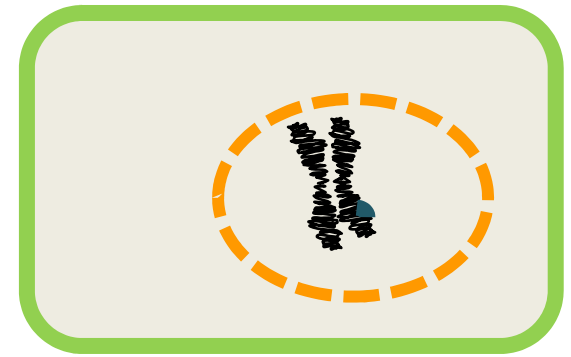


# Transcriptional gene silencing



Pro35S: KAN

X



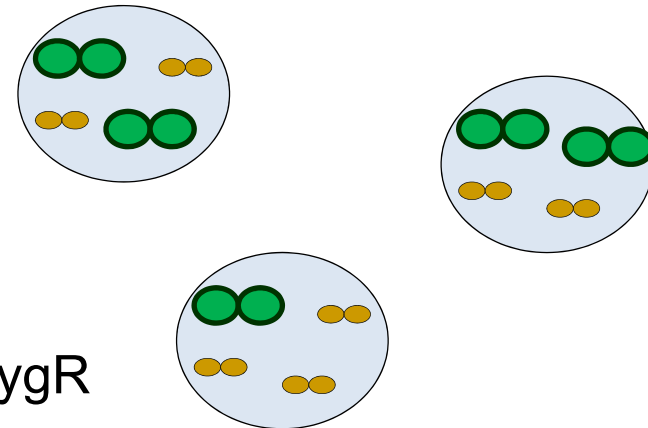
Pro35S: HYG

## Expected Results

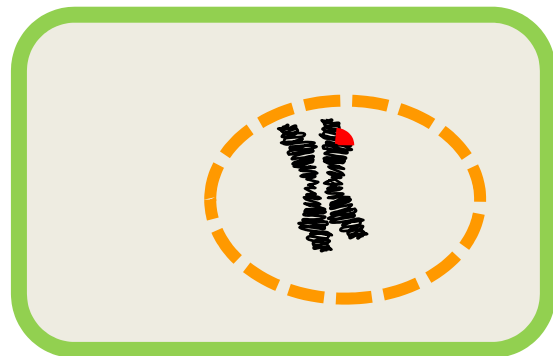
Selection on kanamycin only: 50% KanR

Selection on hygromycin only: 50% HygR

Selection on Kan + Hyg: 25% KanR and HygR

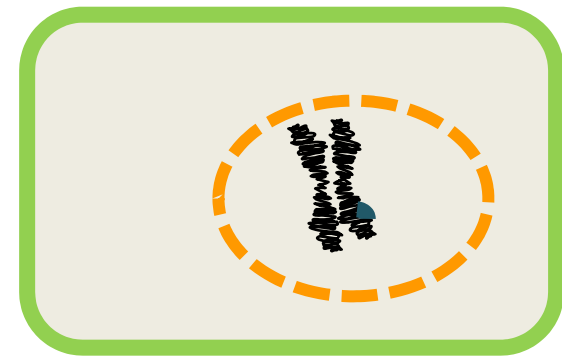


# Transcriptional gene silencing



Pro35S : KAN

X



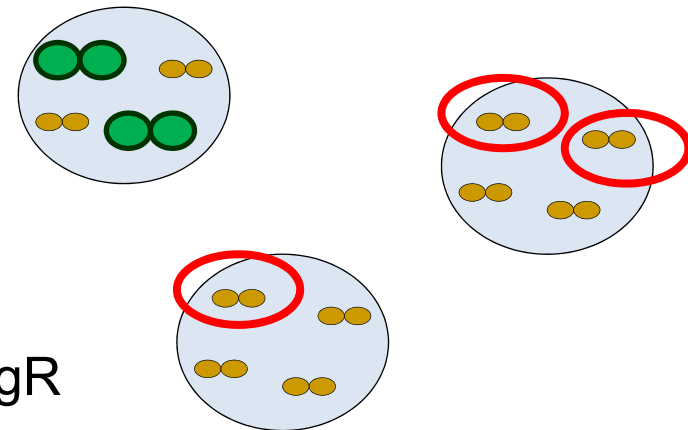
Pro35S : HYG

## Observed Results

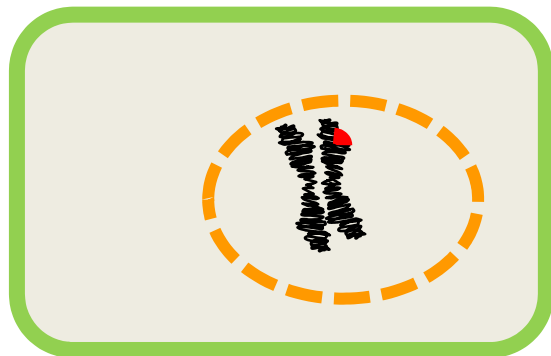
Selection on kanamycin only: 50% KanR

Selection on hygromycin only: 0% HygR

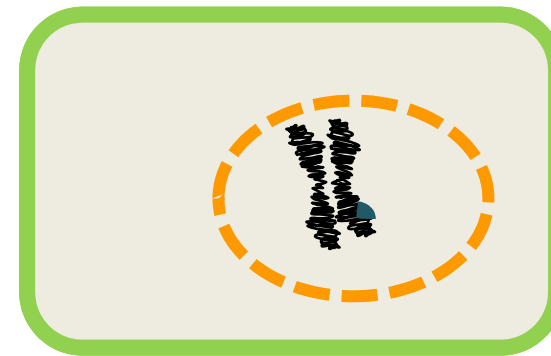
Selection on Kan + Hyg: 0% KanR and HygR



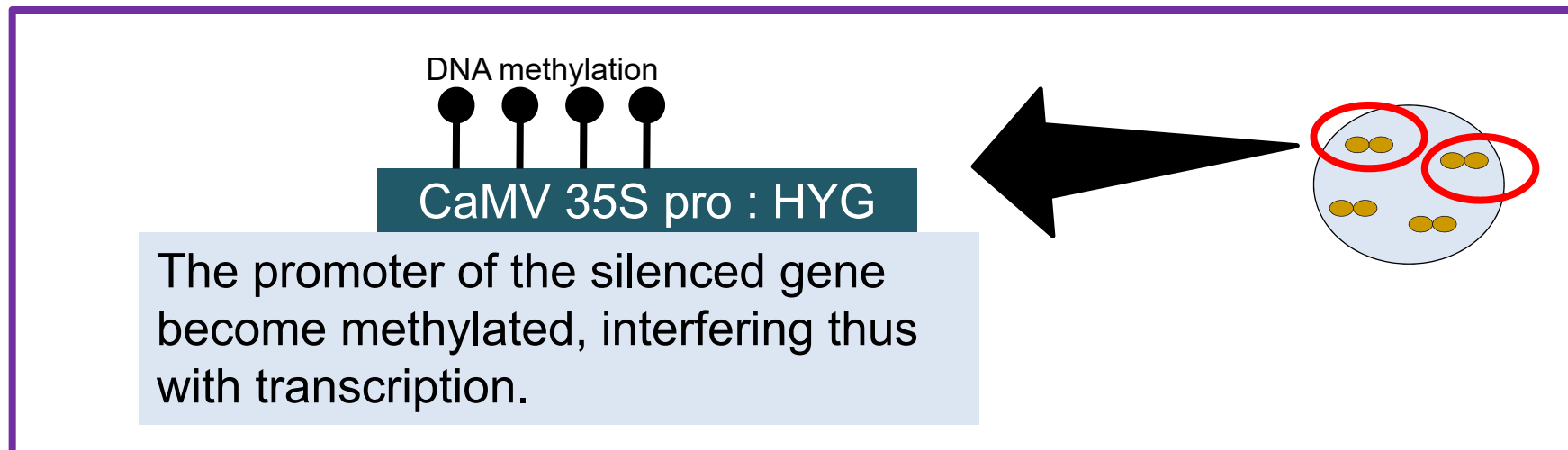
# Transcriptional gene silencing



Pro35S : KAN



Pro35S : HYG



# siRNAs - summary

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- The siRNA pathway silences foreign DNA, transposons and repetitive elements.
- In plants, siRNAs are produced by the action of Dicer-like proteins dicing dsRNA into 24 nt siRNAs
- The siRNAs associate with AGO proteins and form silencing complexes
- The silencing complexes can act post-transcriptionally on RNA targets, cleaving them or interfering with translation
- **The silencing complexes can also act on chromatin, silencing their targets by DNA methylation or histone modification**



# Outline of Lesson 10

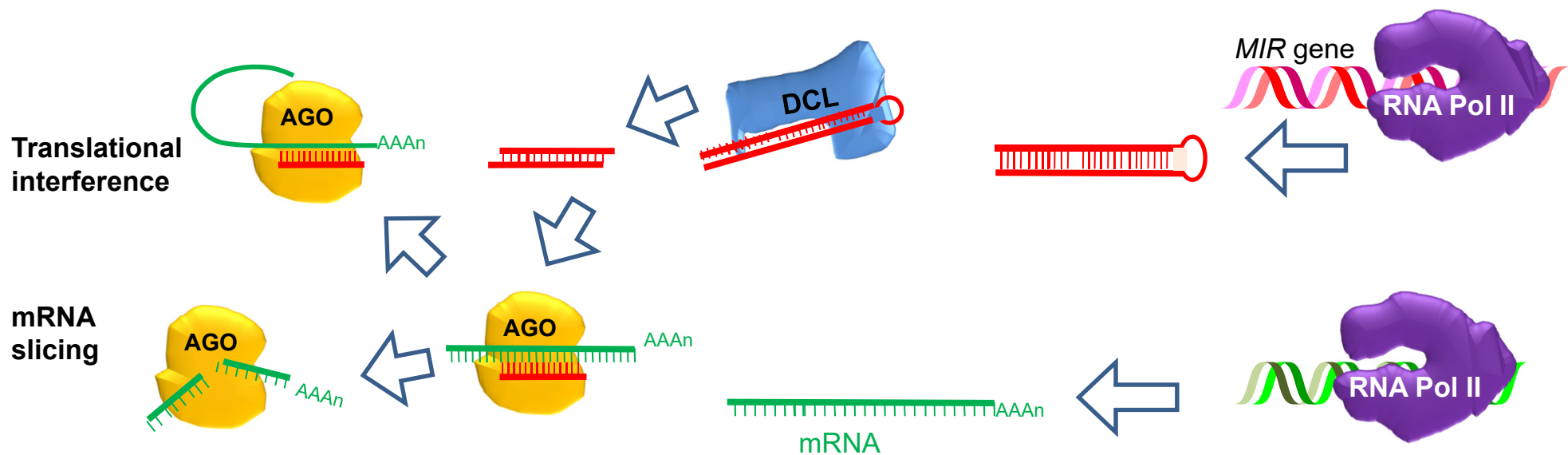
## Regulation of Gene Expression during Development

- Overview of levels of gene expression regulation
- Transcriptional gene regulation
  - Modification of the chromatin structure and DNA methylation
  - Transcriptional activation
- Post-transcriptional gene regulation
  - Splicing of hnRNA
  - Translation initiation
  - Localization of mRNA
  - Protein localization
- RNA interference
  - Identification and mechanism of gene expression regulation via RNA interference
  - siRNA-mediated silencing
  - miRNA-mediated silencing

# Mechanisms of miRNAs action

## miRNAs in plants

- small # of highly conserved miRNAs
- high # of non-conserved miRNAs
- binding to 5'UTR and require almost complete complementarity
- most of the plant miRNA induce slicing of target mRNAs



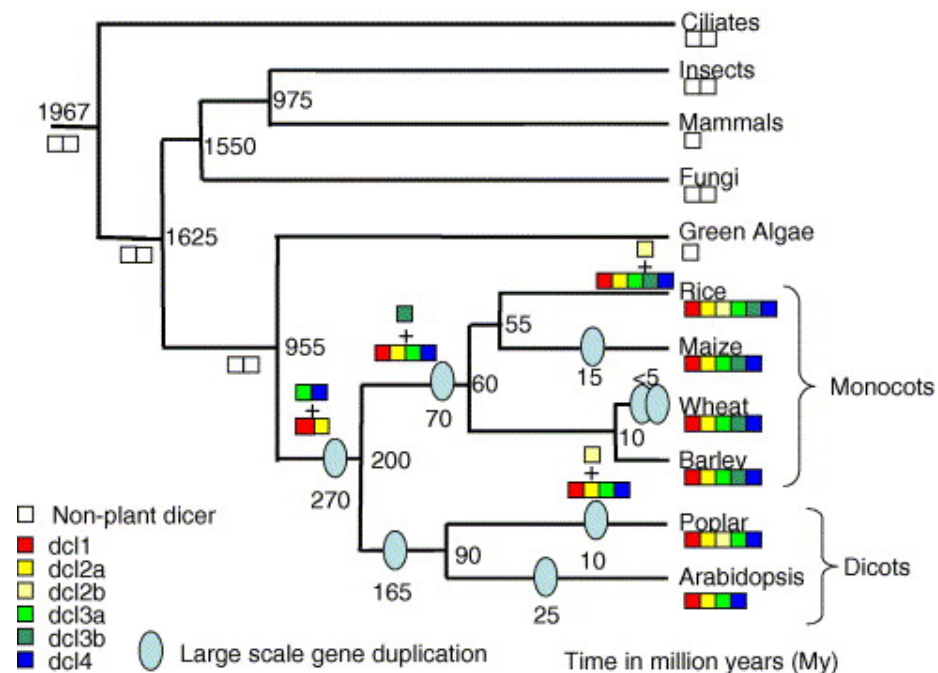


# miRNAs and siRNAs are processed by related but different DCL proteins

AtDCL1 produces **miRNA**



AtDCL2 - 4 produce **siRNA**

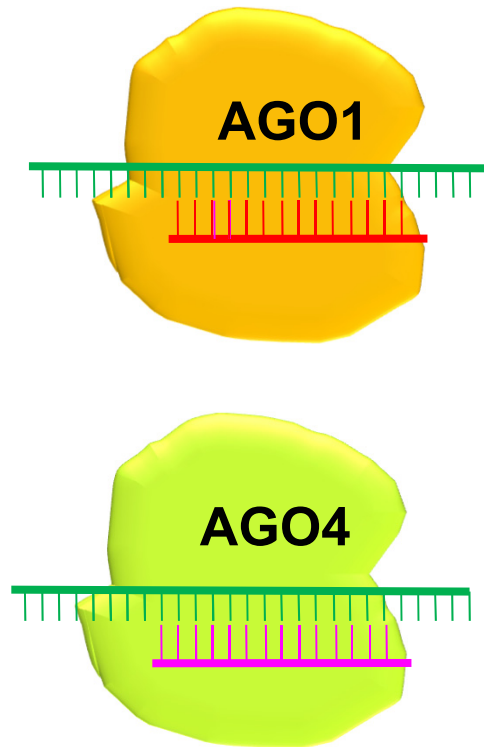


Reprinted from Margis, R., Fusaro, A.F., Smith, N.A., Curtin, S.J., Watson, J.M., Finnegan, E.J., and Waterhouse, P.M. (2006) The evolution and diversification of Dicers in plants FEBS Lett. 580: [2442-2450](#) with permission from Elsevier.

# miRNAs and siRNAs associate with several AGO proteins

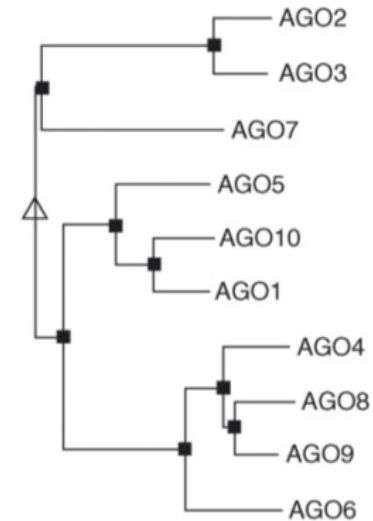
## miRNAs in plants

- small # of highly conserved miRNAs
- high # of non-conserved miRNAs



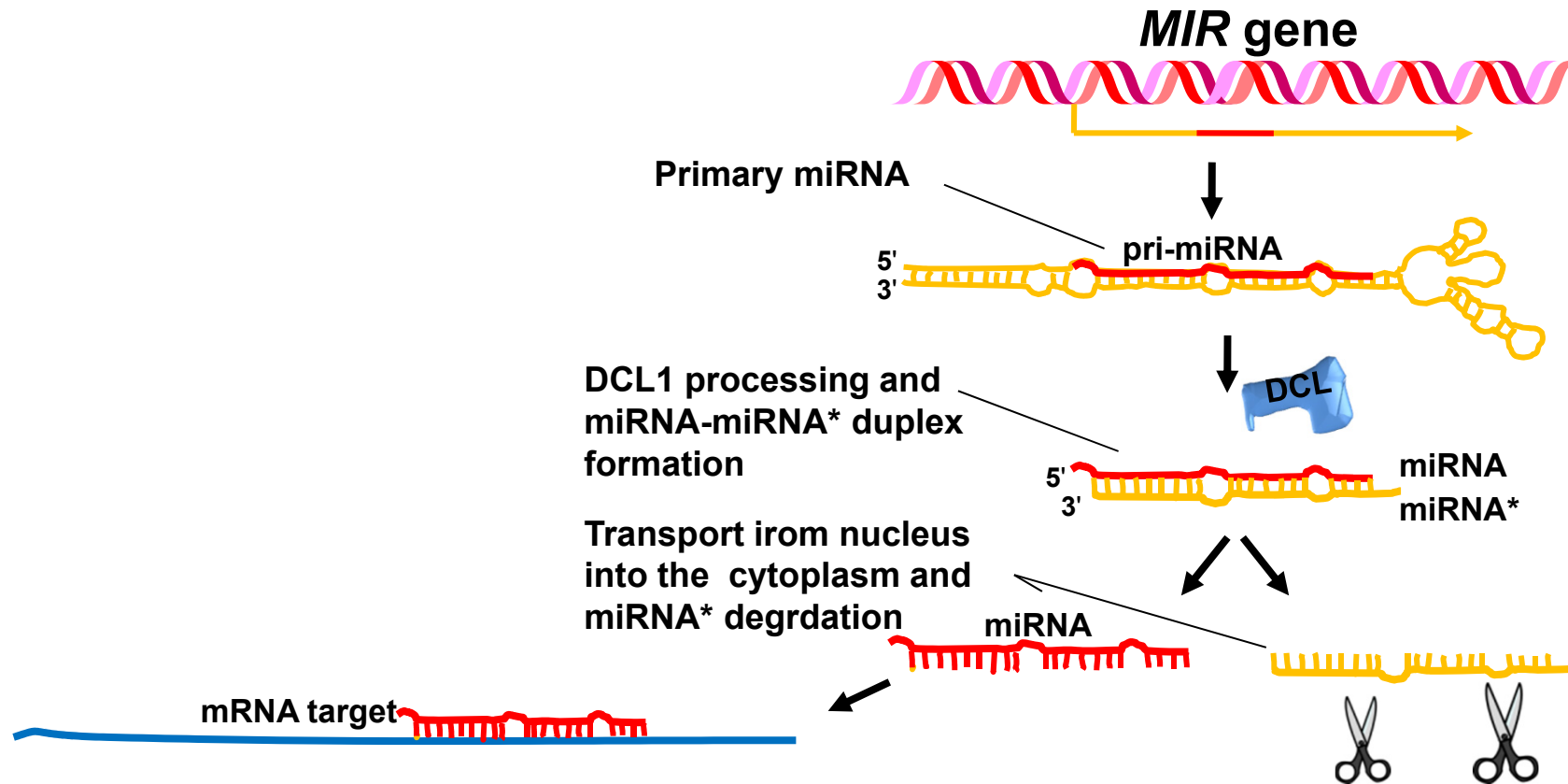
AGO1 preferentially slices its targets and associates with **miRNAs** but also some **siRNAs**

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



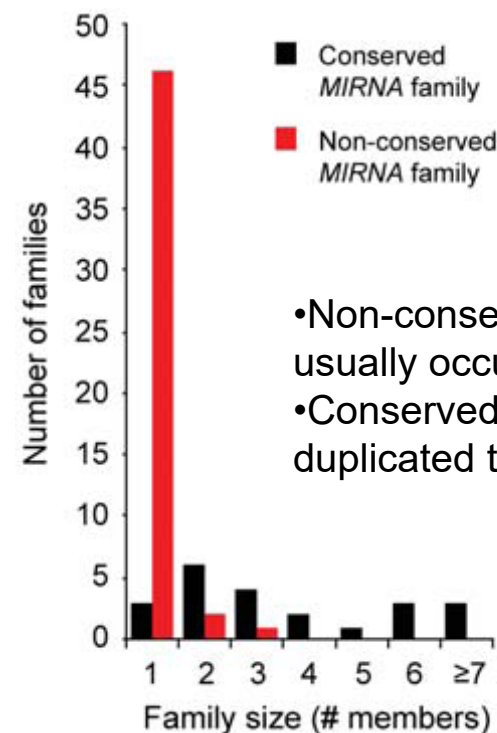
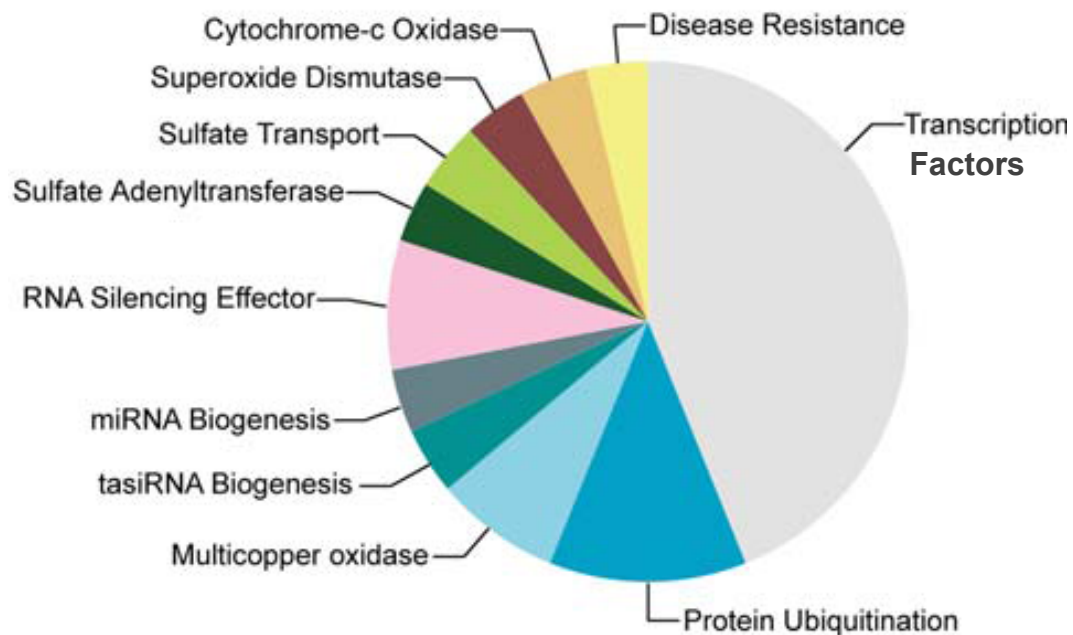
Reprinted from Vaucheret, H. (2008) Plant ARGONAUTES. Trends Plant Sci. 13: [350-358](#) with permission from Elsevier.

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



# Some miRNAs are highly conserved and important gene regulators

Conserved miRNA target functions

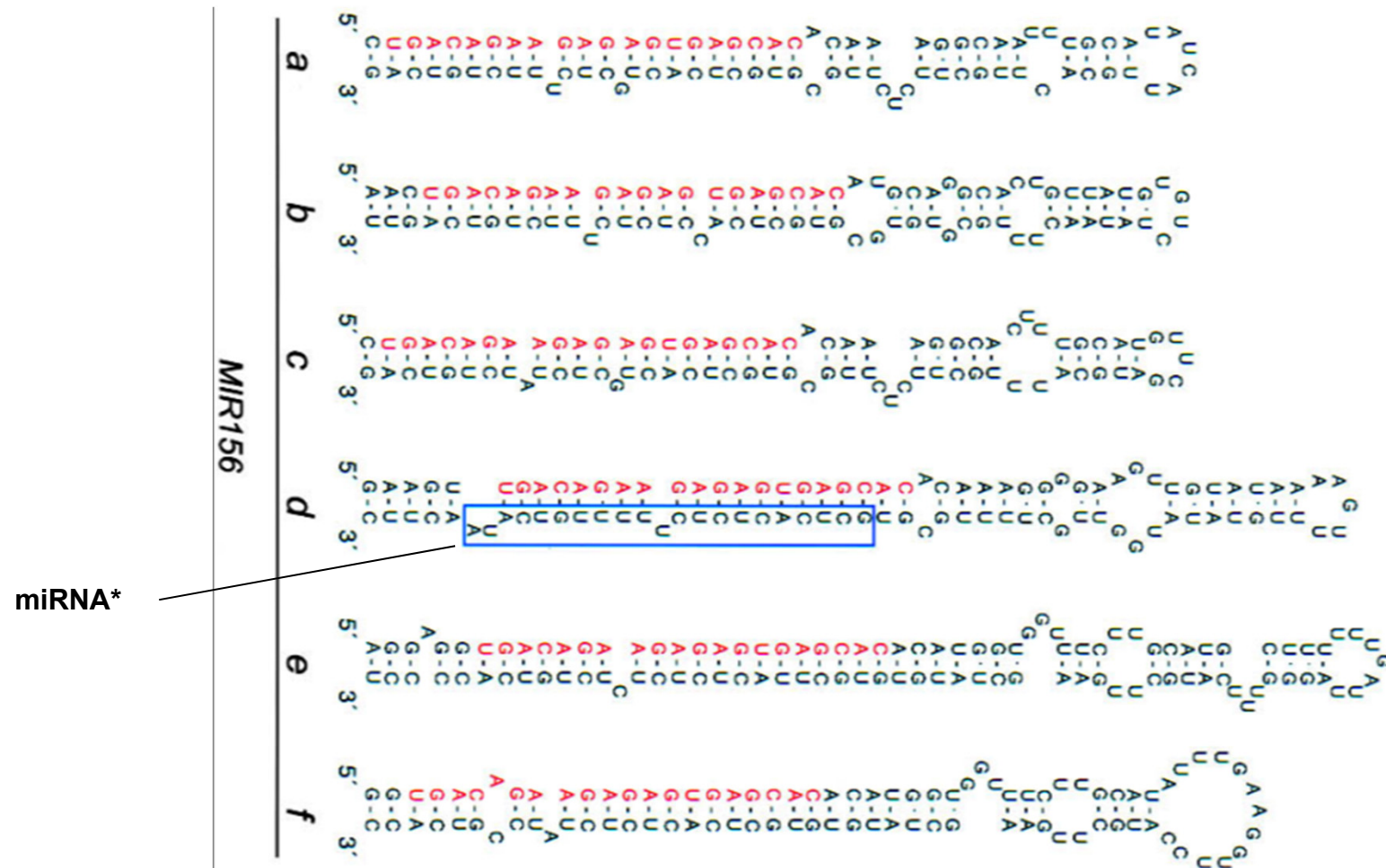


- Non-conserved *MIRNA* families usually occur as single genes
- Conserved ones have often duplicated to larger gene families

Fahlgren, et al., PLoS ONE, 2007

# The *MIR156* gene family is highly conserved

## *Arabidopsis* miR156 gene family



Reprinted from Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., and Bartel, D.P. (2002) MicroRNAs in plants. *Genes Dev.* 16: [1616–1626](#).



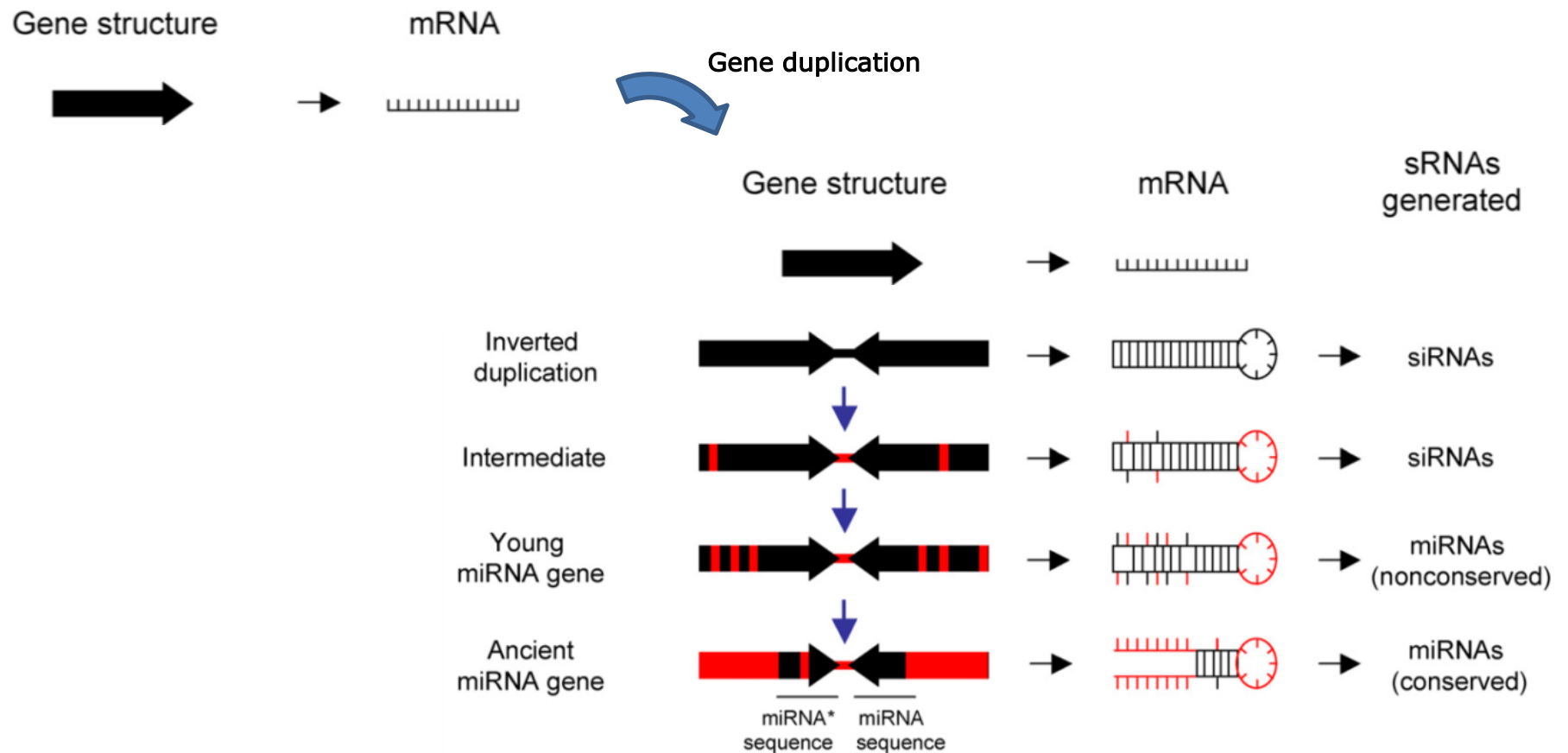
# Targets of some conserved miRNAs

<i>miRNA</i> gene family	Target gene family	Function
156	SPL transcription factors	Developmental timing
160	ARF transcription factors	Auxin response, development
165	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

Adapted from Willmann, M.R., and Poethig, R.S. (2007) Conservation and evolution of miRNA regulatory programs in plant development. Curr. Opin. Plant Biol. 10: [503–511](#).

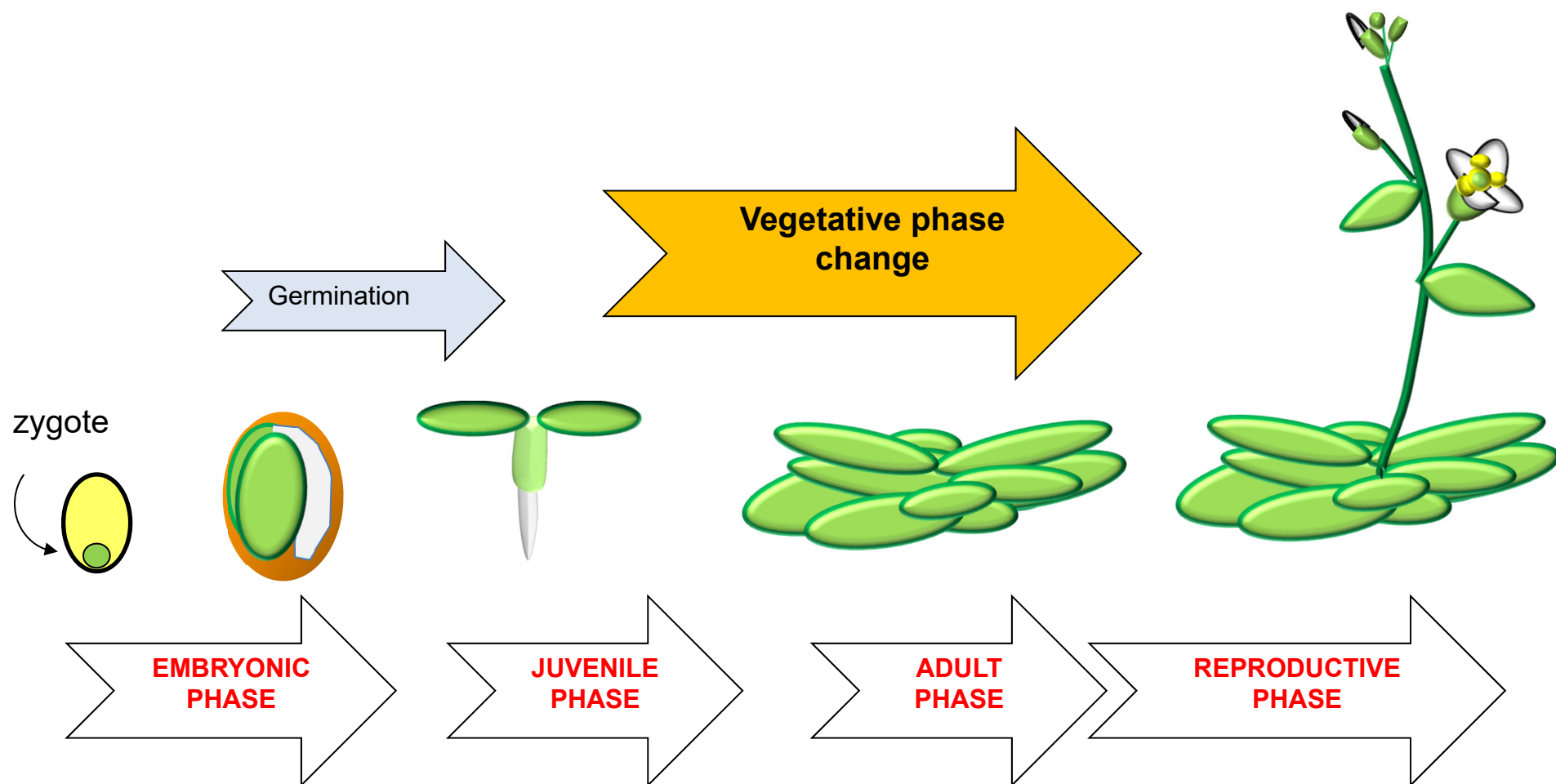


# Plant miRNAs are thought to be distantly related to their targets

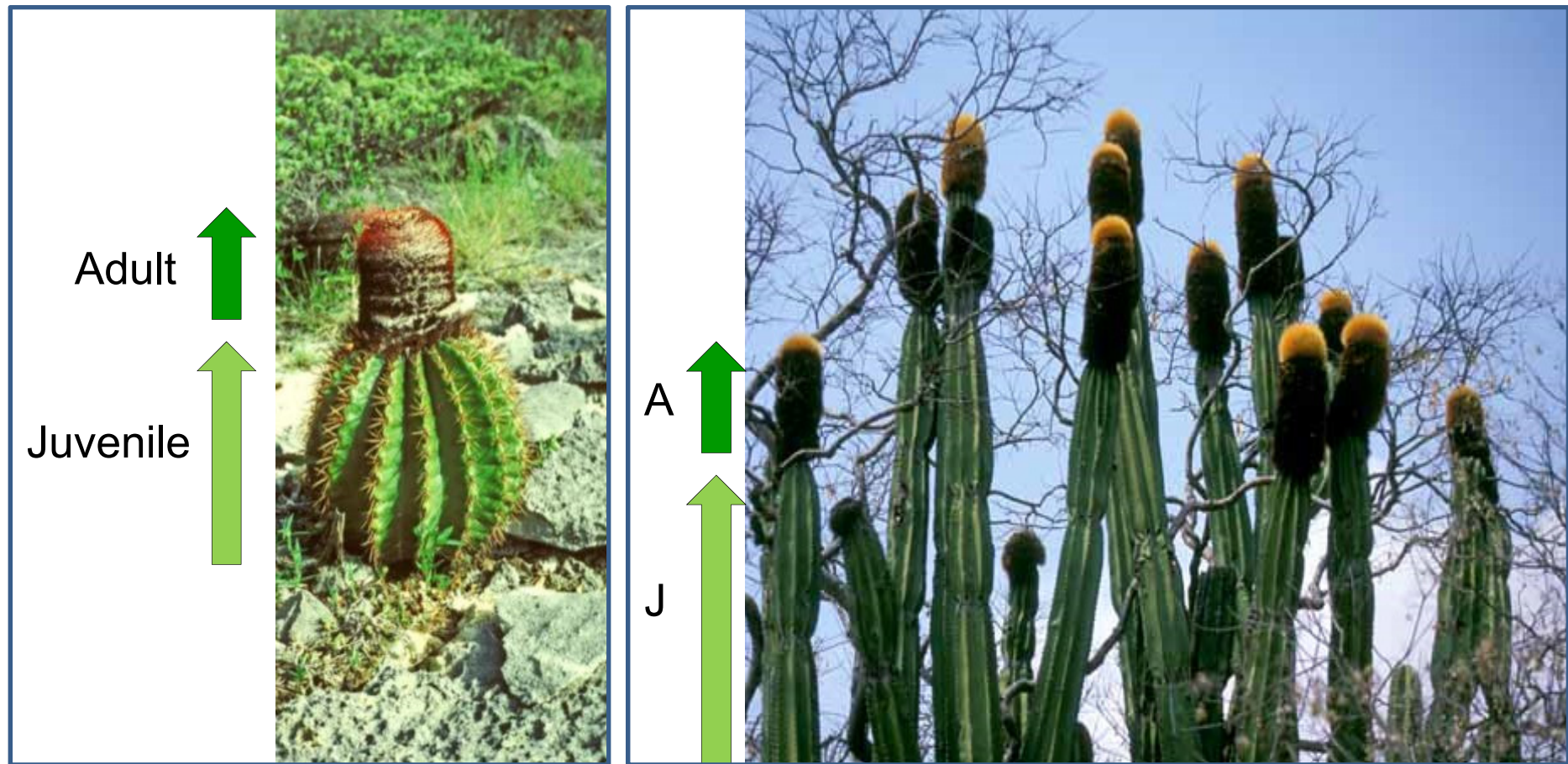


Reprinted from Willmann, M.R., and Poethig, R.S. (2007) Conservation and evolution of miRNA regulatory programs in plant development. *Curr. Opin. Plant Biol.* 10: [503–511](#) with permission from Elsevier.

# miRNAs and vegetative phase change



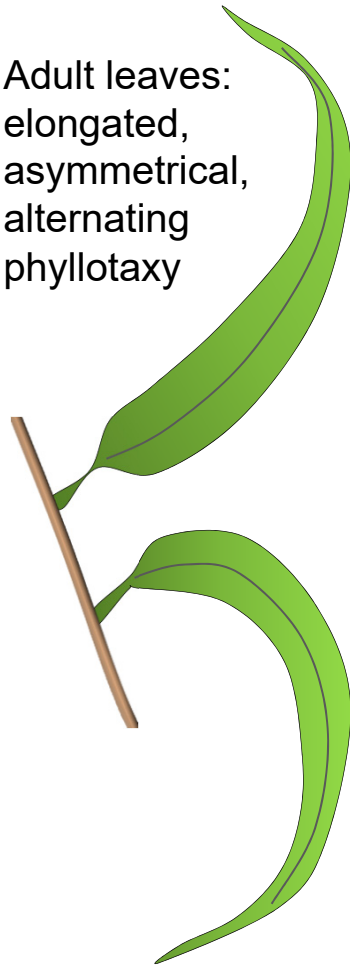
# Vegetative phase change affects morphology and reproductive competence



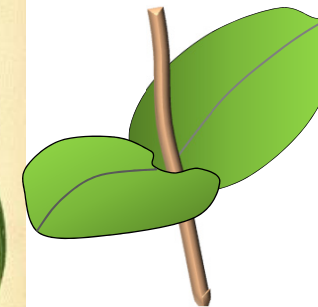


# Phase change can affect leaf shape, phyllotaxy, and trichome patterns

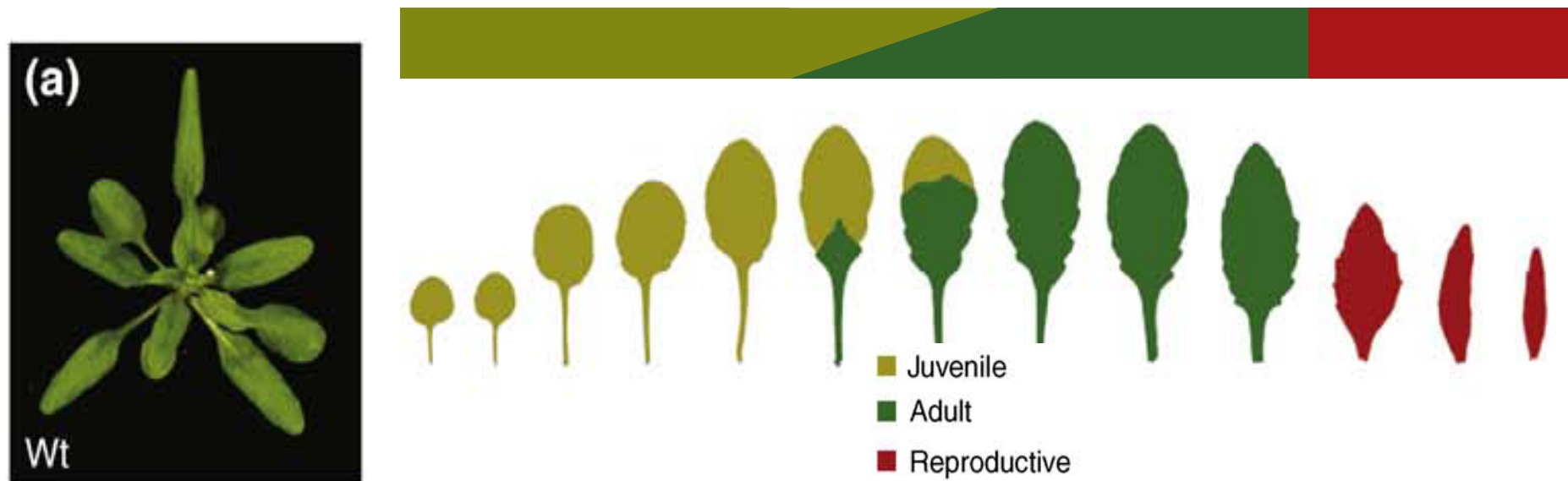
Adult leaves:  
elongated,  
asymmetrical,  
alternating  
phyllotaxy



Juvenile leaves:  
rounded,  
symmetrical,  
opposite  
phyllotaxy



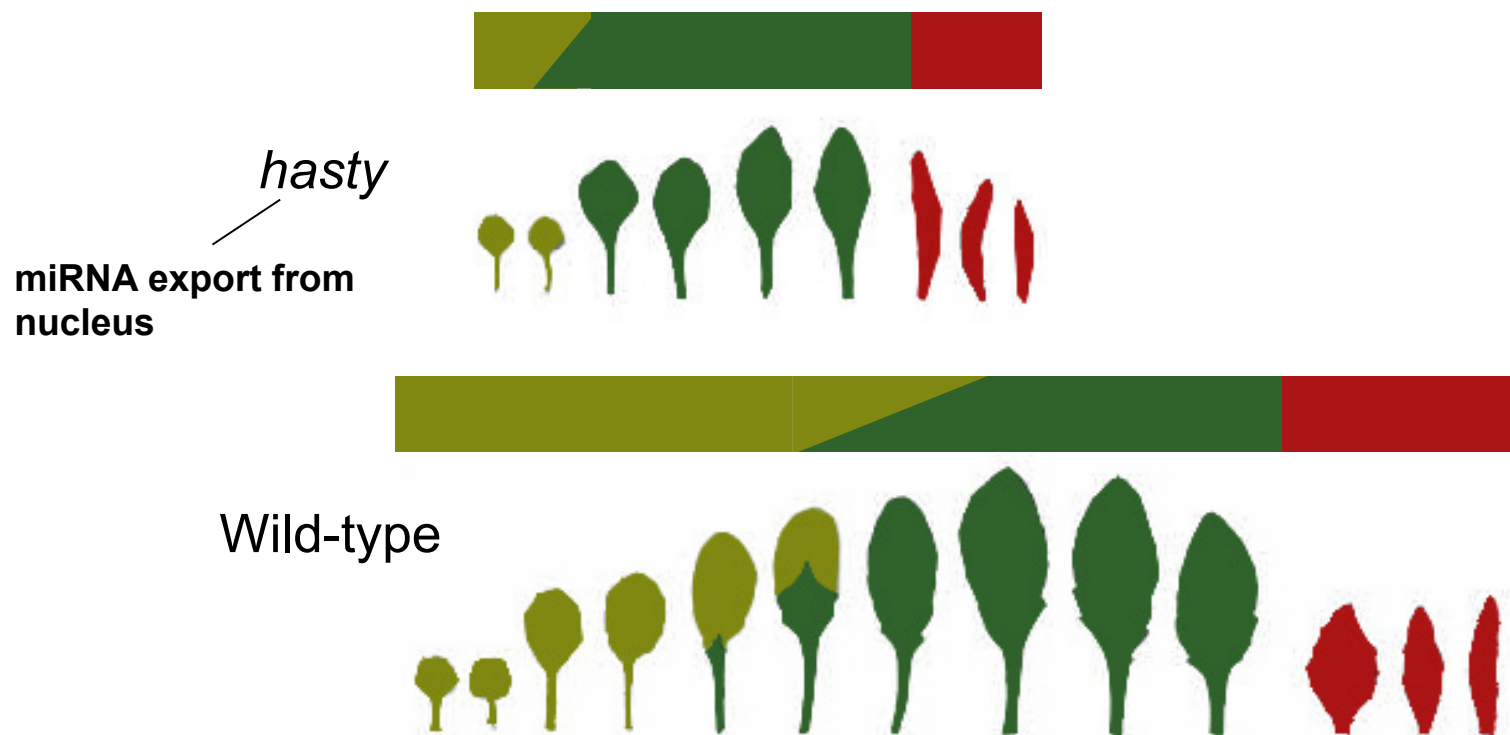
# In *Arabidopsis*, phase change affects leaf shape and trichome patterning



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

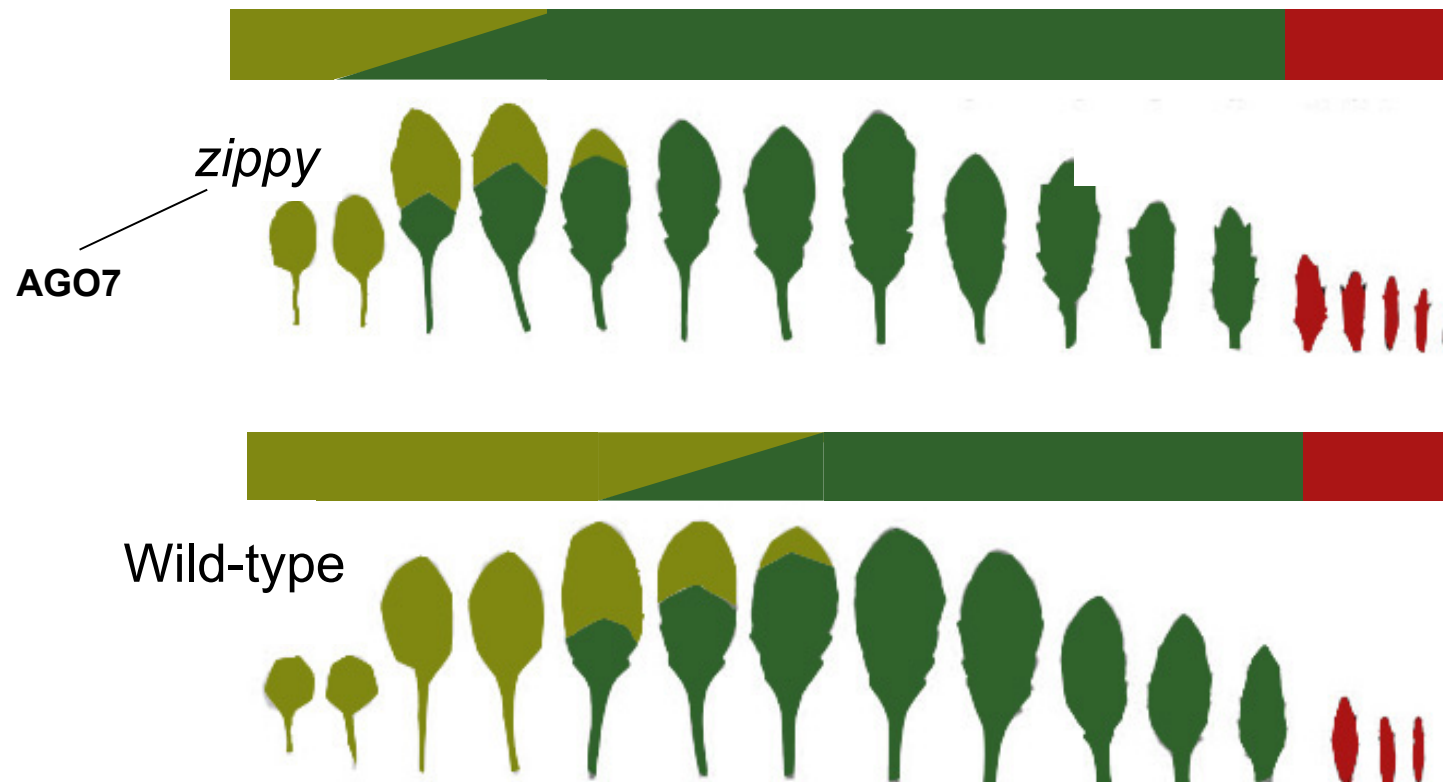


# Phase change is specified by miRNAs



Reprinted with permission from Bollman, K.M. Aukerman, M.J., Park, M.-Y., Hunter, C., Berardini, T.Z., and Poethig, R.S. (2003) HASTY, the *Arabidopsis* ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. Development 130: [1493-1504](#).

# Phase change is specified by miRNAs



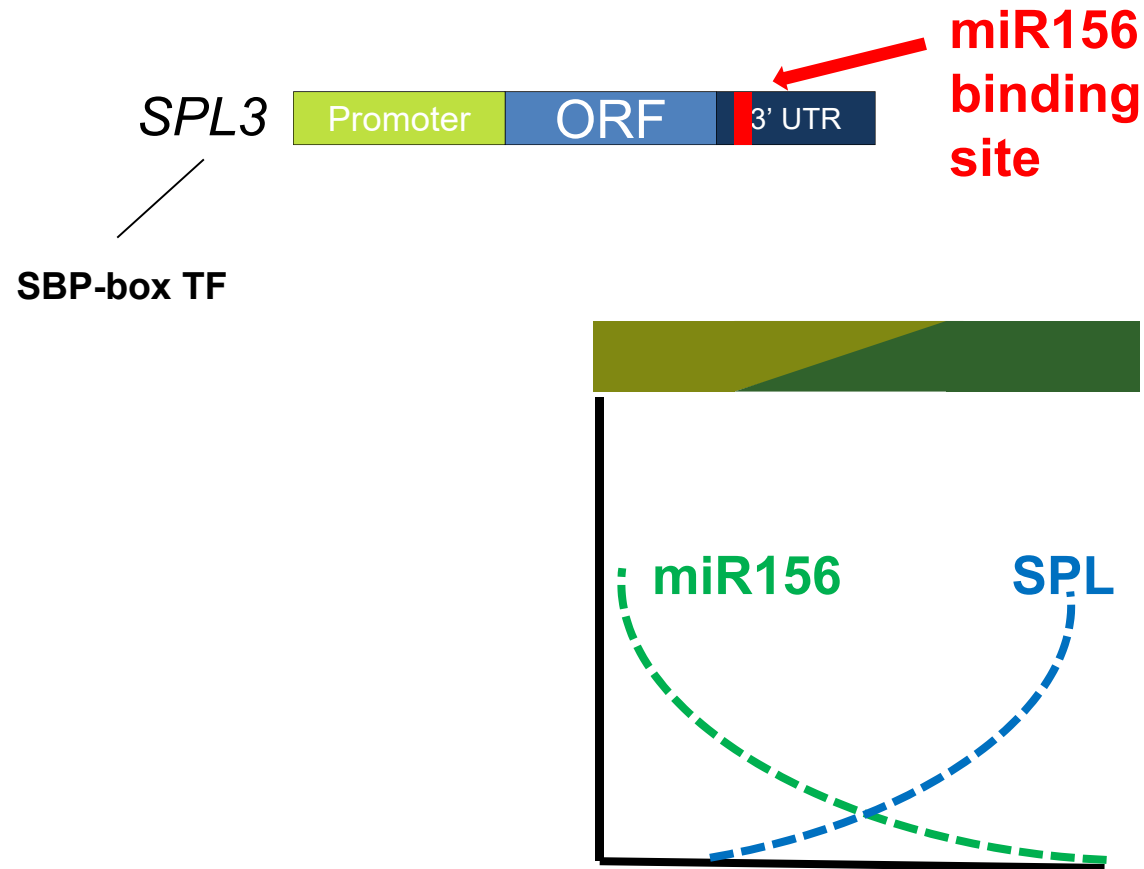
Reprinted from Hunter, C., Sun, H., and Poethig, R.S. (2003) The *Arabidopsis* heterochronic gene *ZIPPY* is an *ARGONAUTE* family member. *Curr. Biol.* 13: [1734–1739](#), with permission from Elsevier.

# *miR156* overexpression prolongs juvenile phase in *Arabidopsis*

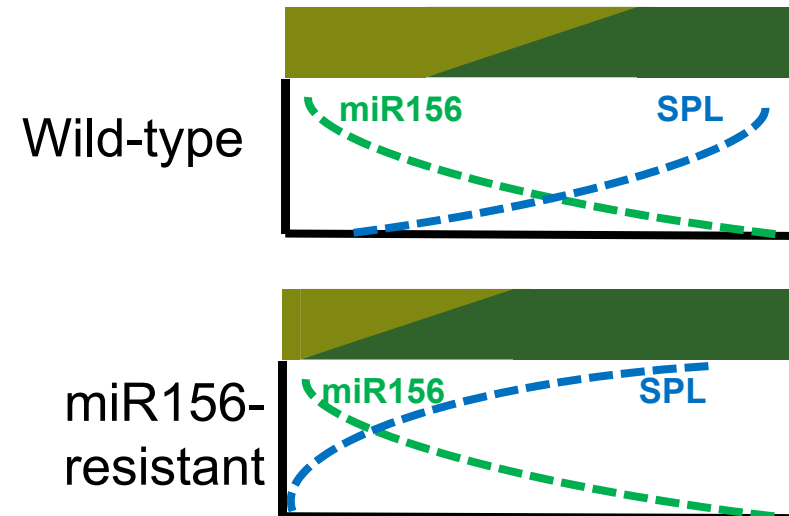
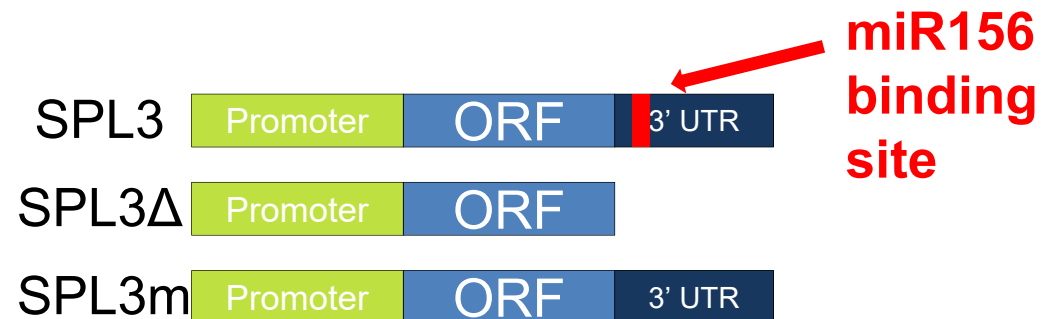
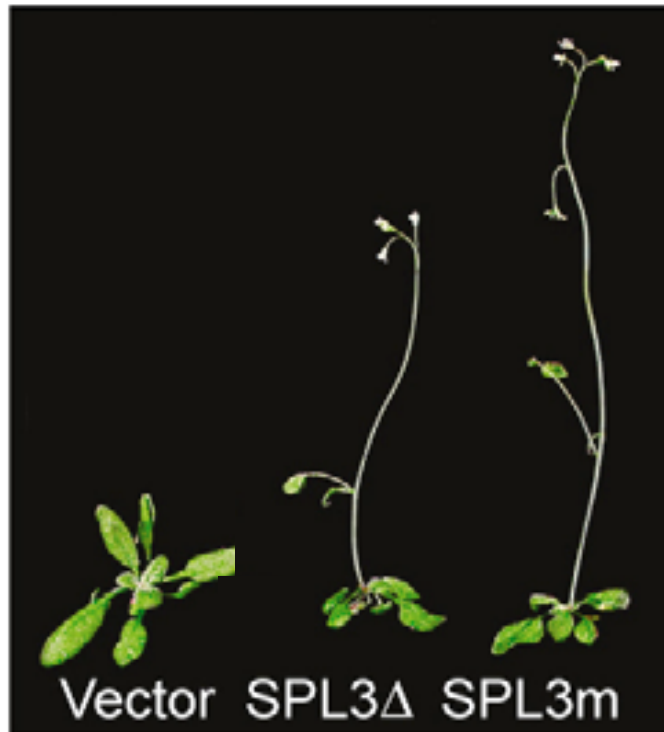


Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. *Curr. Opin. Genet. Devel.* 19: [374-378](#), with permission from Elsevier.

# *miR156 targets SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes, promoters of phase change*



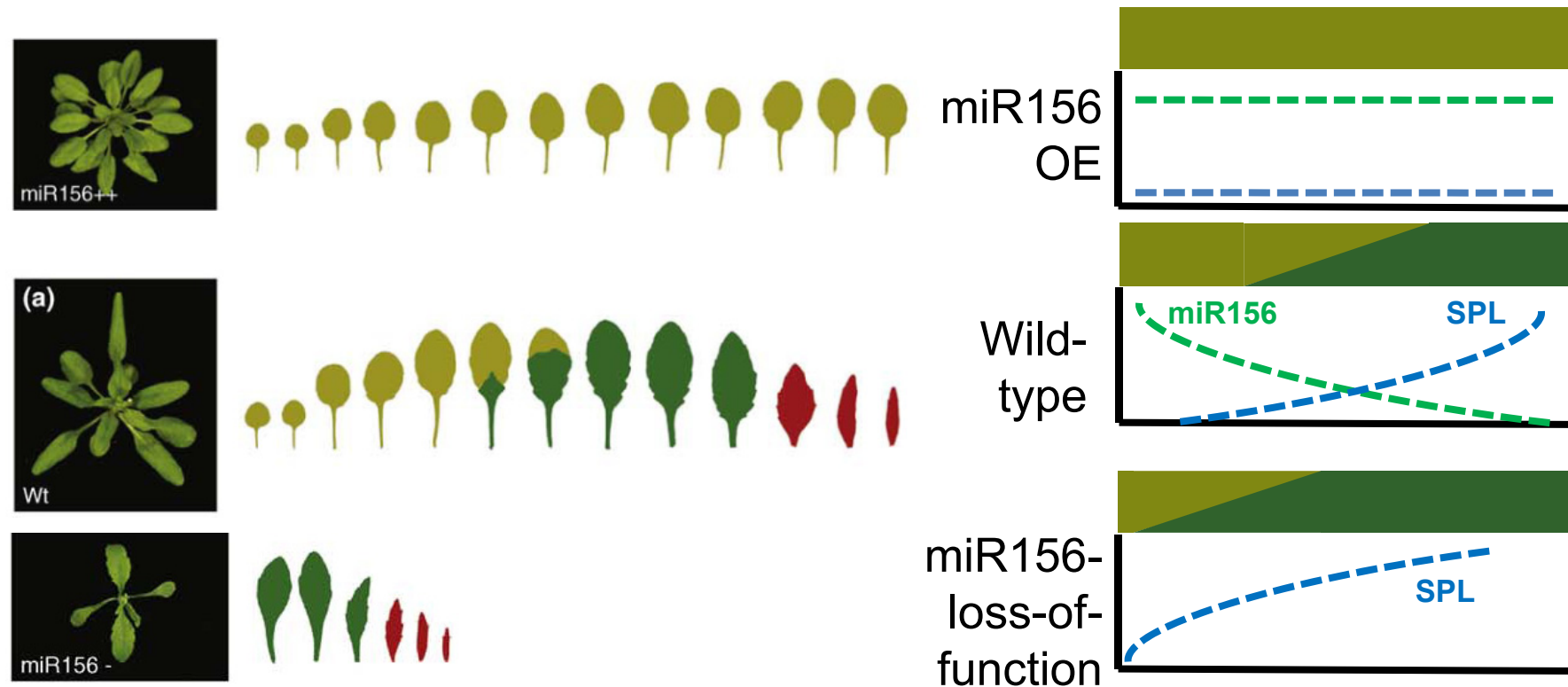
# miR156-resistant *SPL* promotes precocious phase change



Reproduced with permission from Wu, G., and Poethig, R.S. (2006) Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target *SPL3*. *Development* **133**: [3539–3547](#).

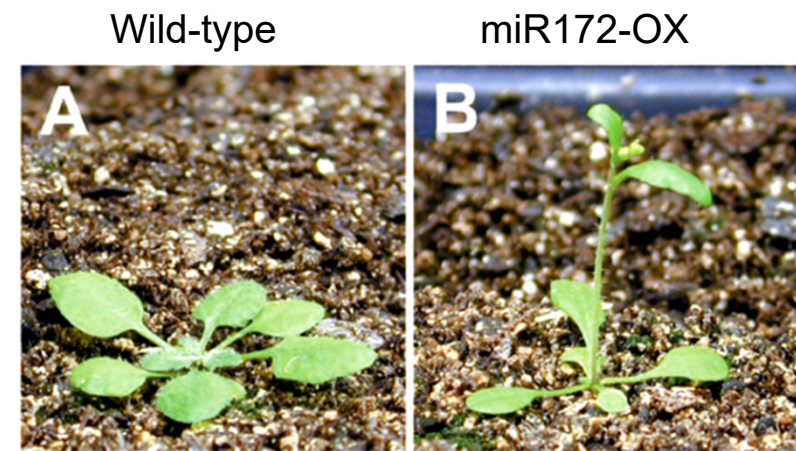
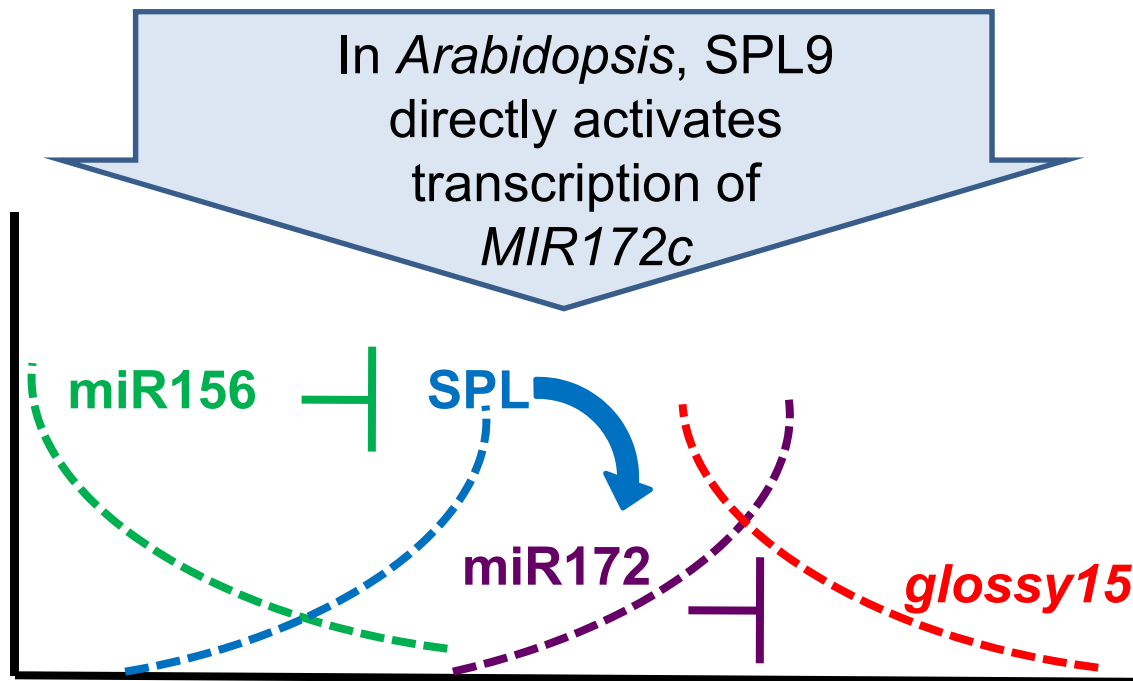


# miR156 loss-of-function promotes precocious phase change



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

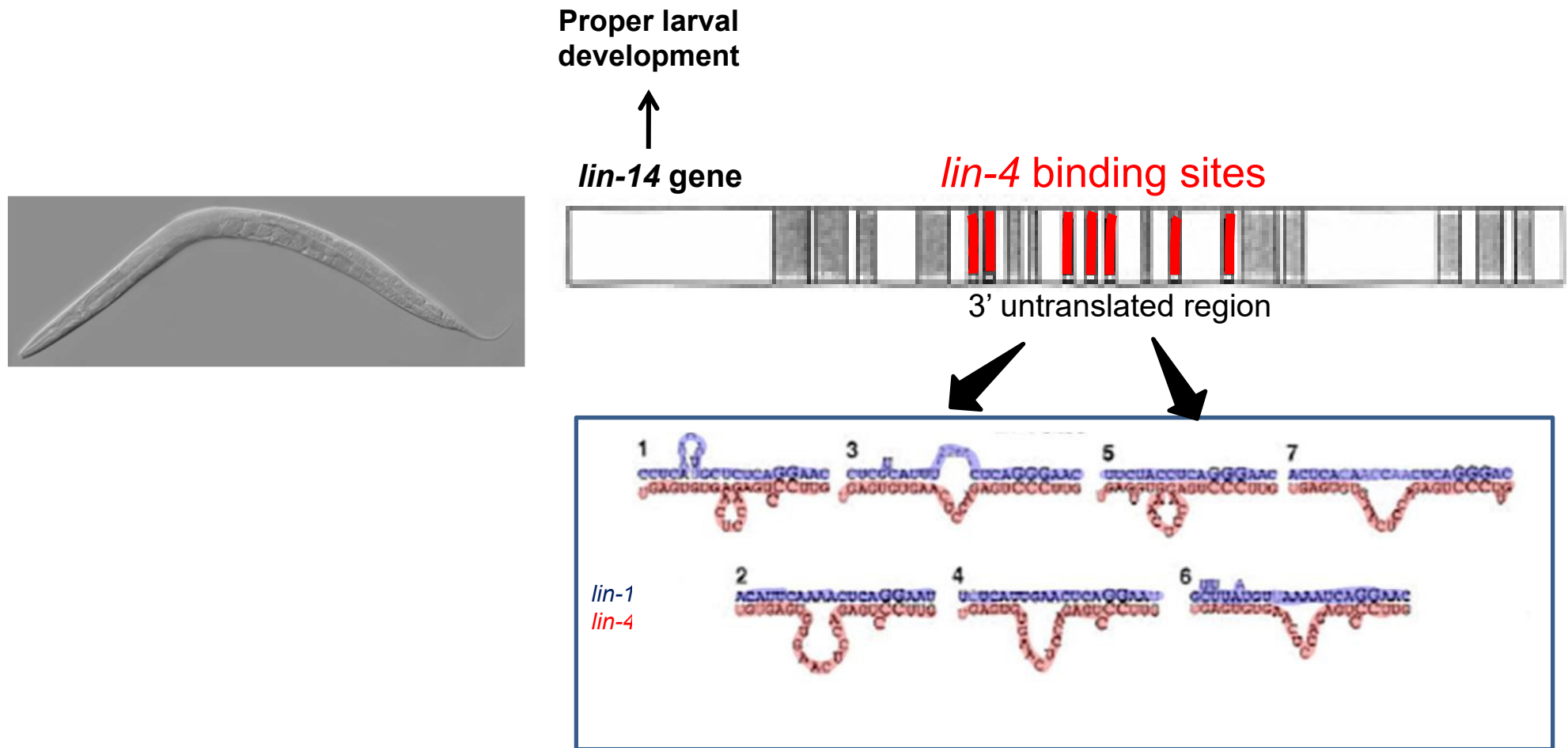
# Phase change involves a temporal cascade of miRNAs and transcription factors



*Arabidopsis* plants overexpressing miR172 flower early.

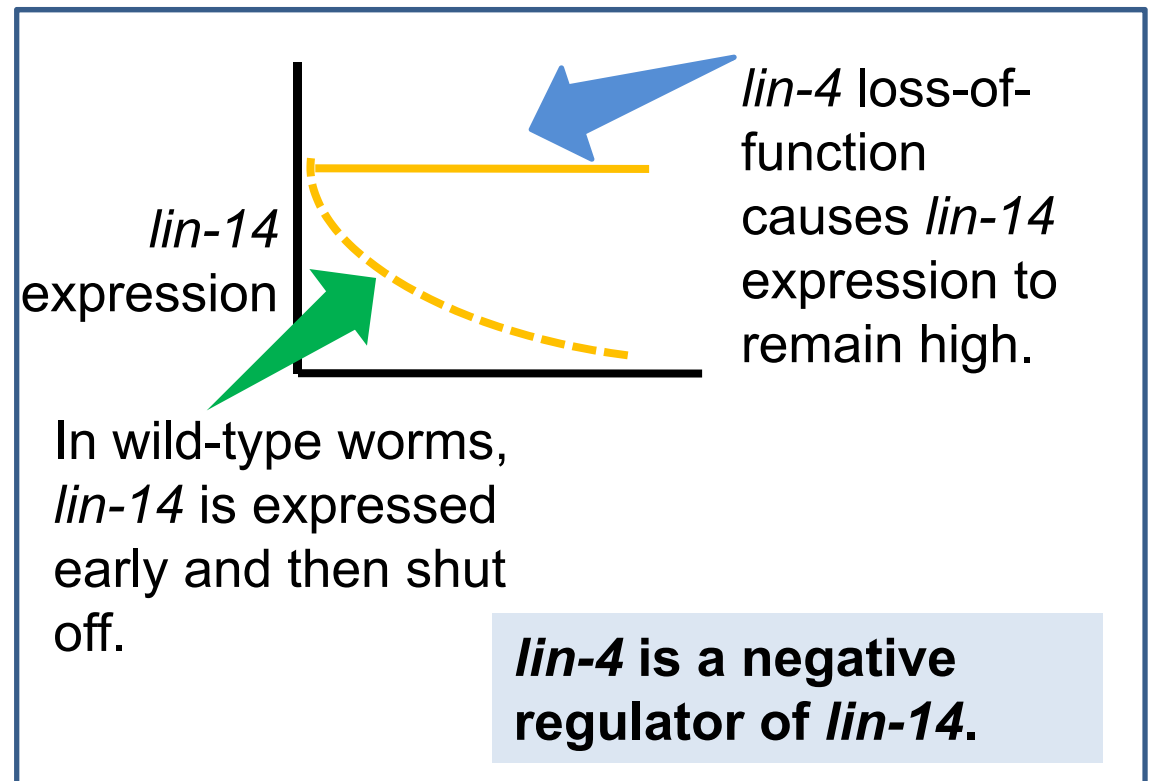
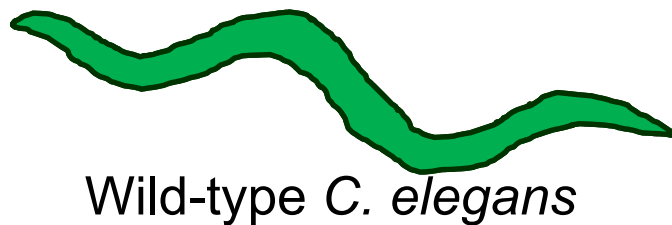
Aukerman, M.J., and Sakai, H. (2003) Regulation of flowering time and floral organ identity by a microRNA and its *APETALA2*-Like target genes *Plant Cell* 15: [2730-2741](#).

# miRNAs regulate developmental timing in other organisms



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

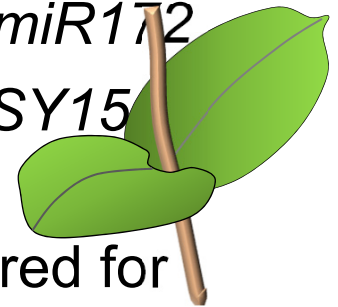


Lee, R.C., Feinbaum, R.L., and Ambrose, V. (1993). The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75: 843–845. Wightman, B., Ha, I., and Ruvkun, G. (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates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

# miRNAs and phase change - summary

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- Vegetative phase change affects morphology and reproductive competence
- miRNAs contribute to the temporal control of gene expression and phase change
  - *miR156* promotes juvenile phase by preventing *SPL* gene accumulation
  - *SPL* genes promote phase change and flowering
  - In *Arabidopsis*, a *SPL* protein promotes transcription of *miR172*
  - *mir172* triggers phase change by interfering with *GLOSSY15* expression
- In the nematode *C. elegans*, *lin-4* silencing of *lin-14* is required for developmental progression







# Key Concepts

## Regulation of Gene Expression during Development

- **Regulation of gene expression** occurs at **different levels**, from **transcriptional** till the **postranscriptional** and **postranslational**
- **Basal promoters** are co-regulated in a **combinatorial way** via spectrum of **positive** and **negative factors**
- **mRNA** and **protein localizations** belong to the most important postranscriptional regulations of gene expression
- **RNA interference** is **natural** and powerful **mechanism** allowing regulation of gene expression at both **transcriptional** and **postranscriptional** levels
- **dsRNA** is either **trigger** or **intermediate** in the **RNAi-mediated regulation**
- **siRNA** and **miRNA** are **two major effector molecules** regulating different and complementary spectrum of target genes