

C8545 Developmental Biology

Lesson 10

Regulation of Gene Expression during Development

Jan Hejátko

Functional Genomics and Proteomics of Plants

CEITEC

and

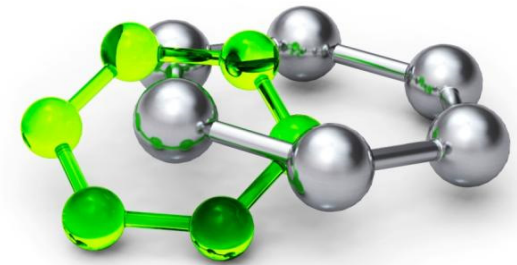
National Centre for the Biomolecular Research,

Faculty of Science

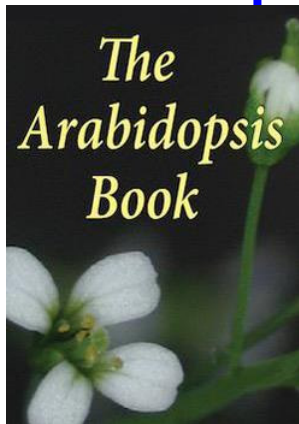
M U N I
S C I

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Literature



- **Fred H. Wilt and Sarah Hake, Principles of Developmental Biology** (W.W. Norton & Company, New York, London, 2004)
- **Capron A, Chatfield S, Provart N, Berleth T 2009.** Embryogenesis: Pattern Formation from a Single Cell. *The Arabidopsis Book*. Rockville, MD: American Society of Plant Biologists, doi: 10.1199/tab.0126, <http://www.aspb.org/publications/arabidopsis/>.
- Selected original papers in scientific journals

Outline of Lesson 10

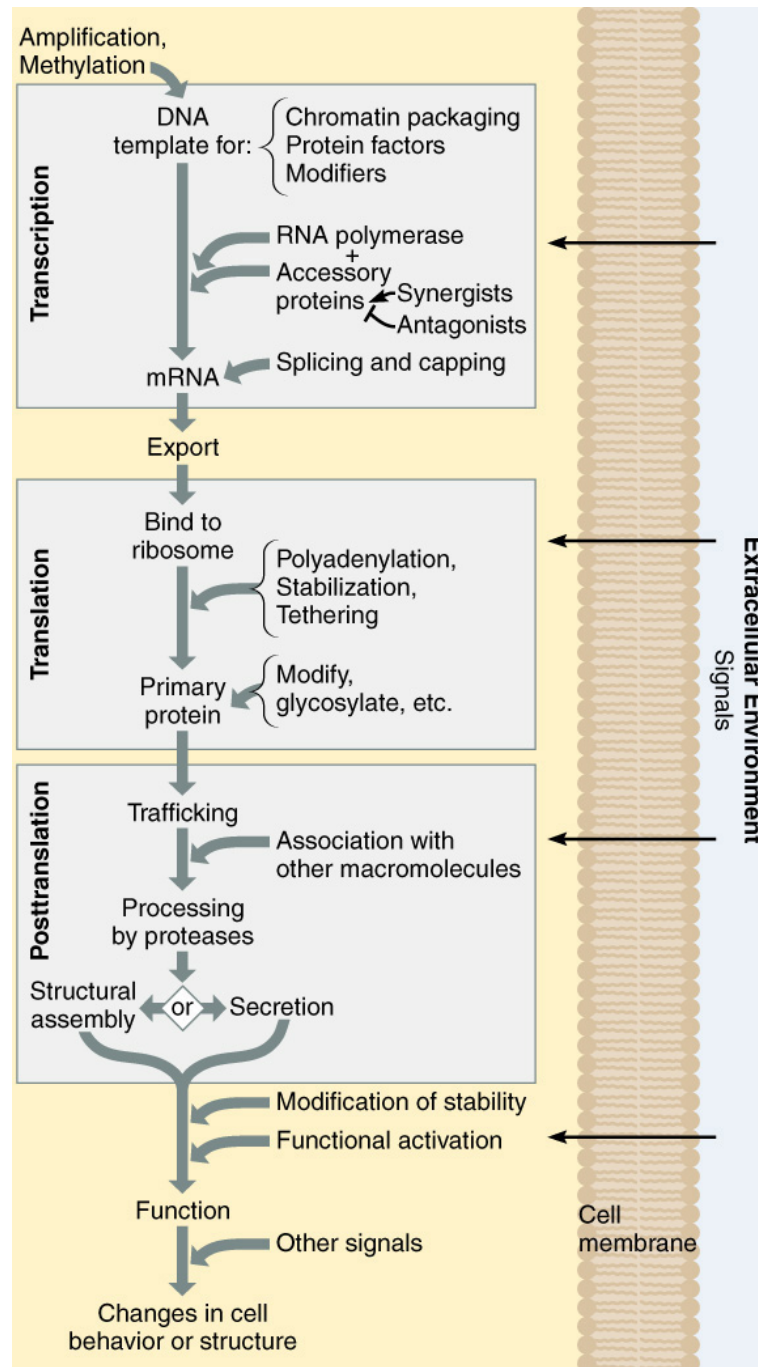
Regulation of Gene Expression during Development

- 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

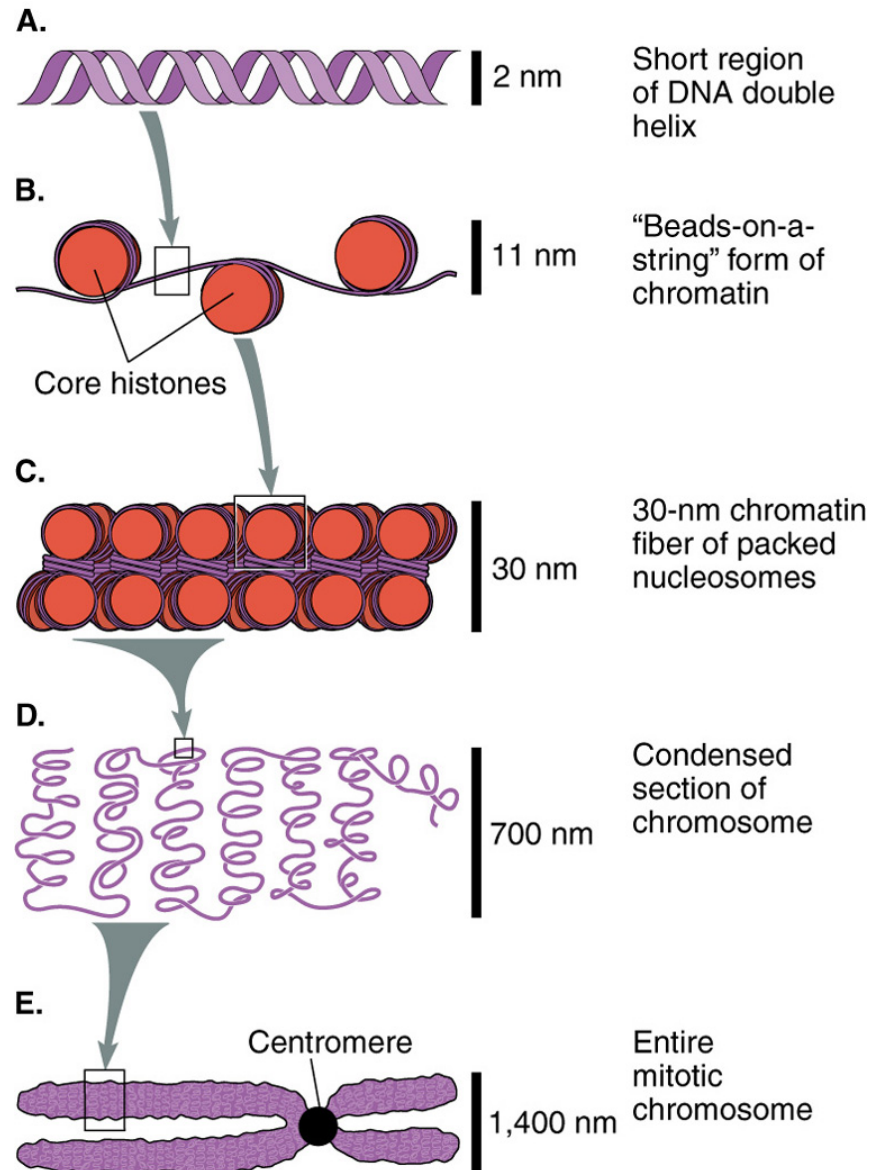
- Overview of levels of gene expression regulation



Outline of Lesson 10

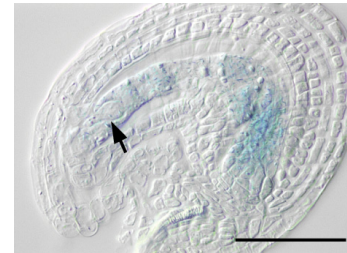
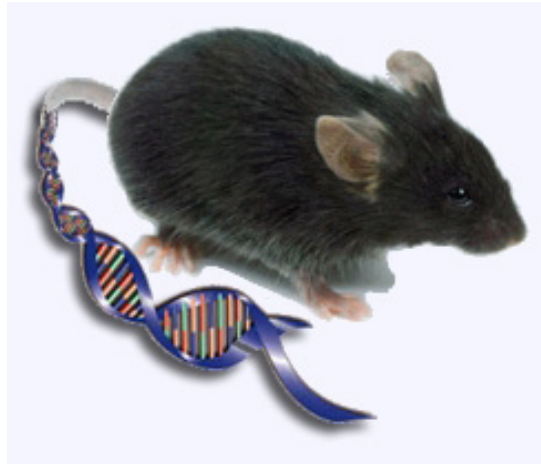
Regulation of Gene Expression during Development

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



Regulation by histone acetyl transferases or histone deacetylases

DNA methylation in animals vs. in plants



CpG

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



Mechanism of transcriptional regulation by DNA methylation mostly unknown



CpG or CpNpG

CpNpNp

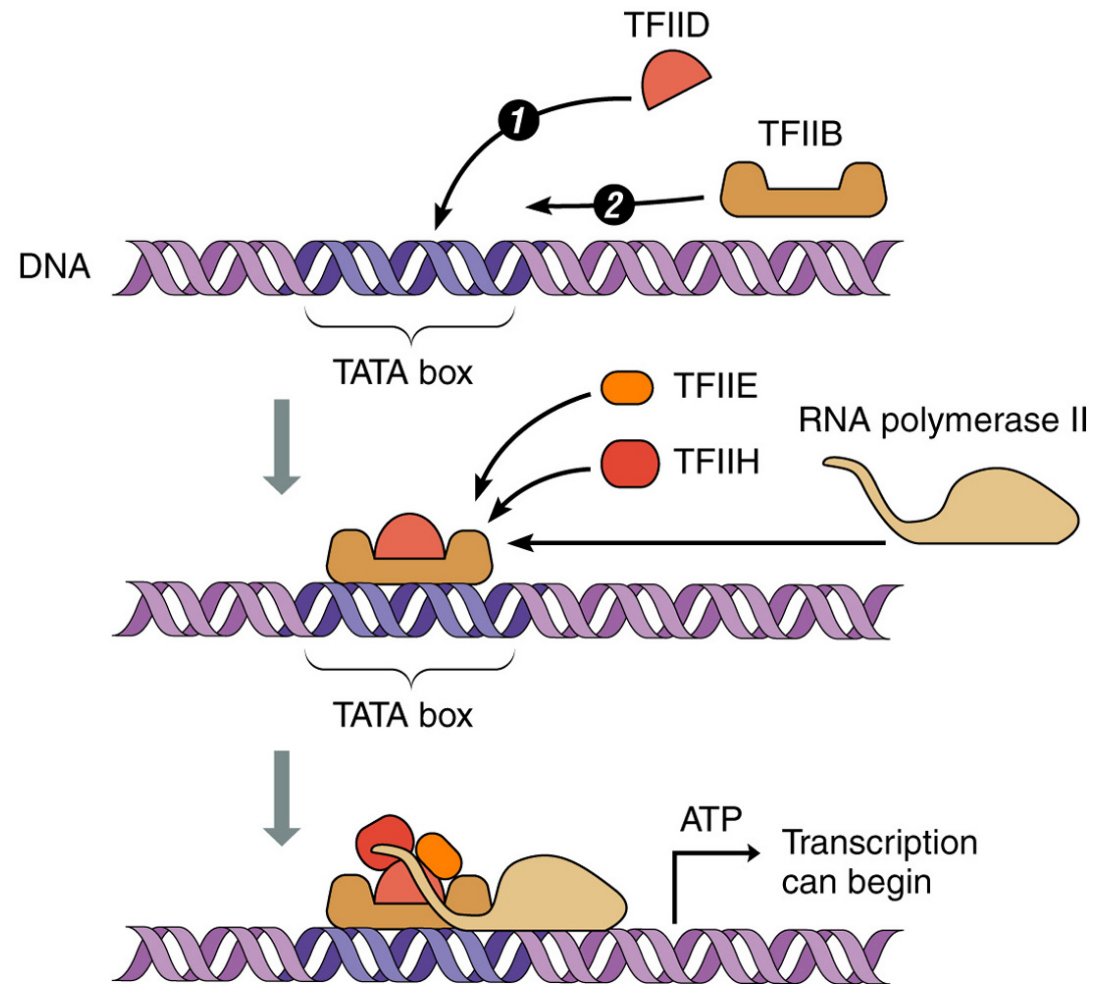
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Regulation of Gene Expression during Development

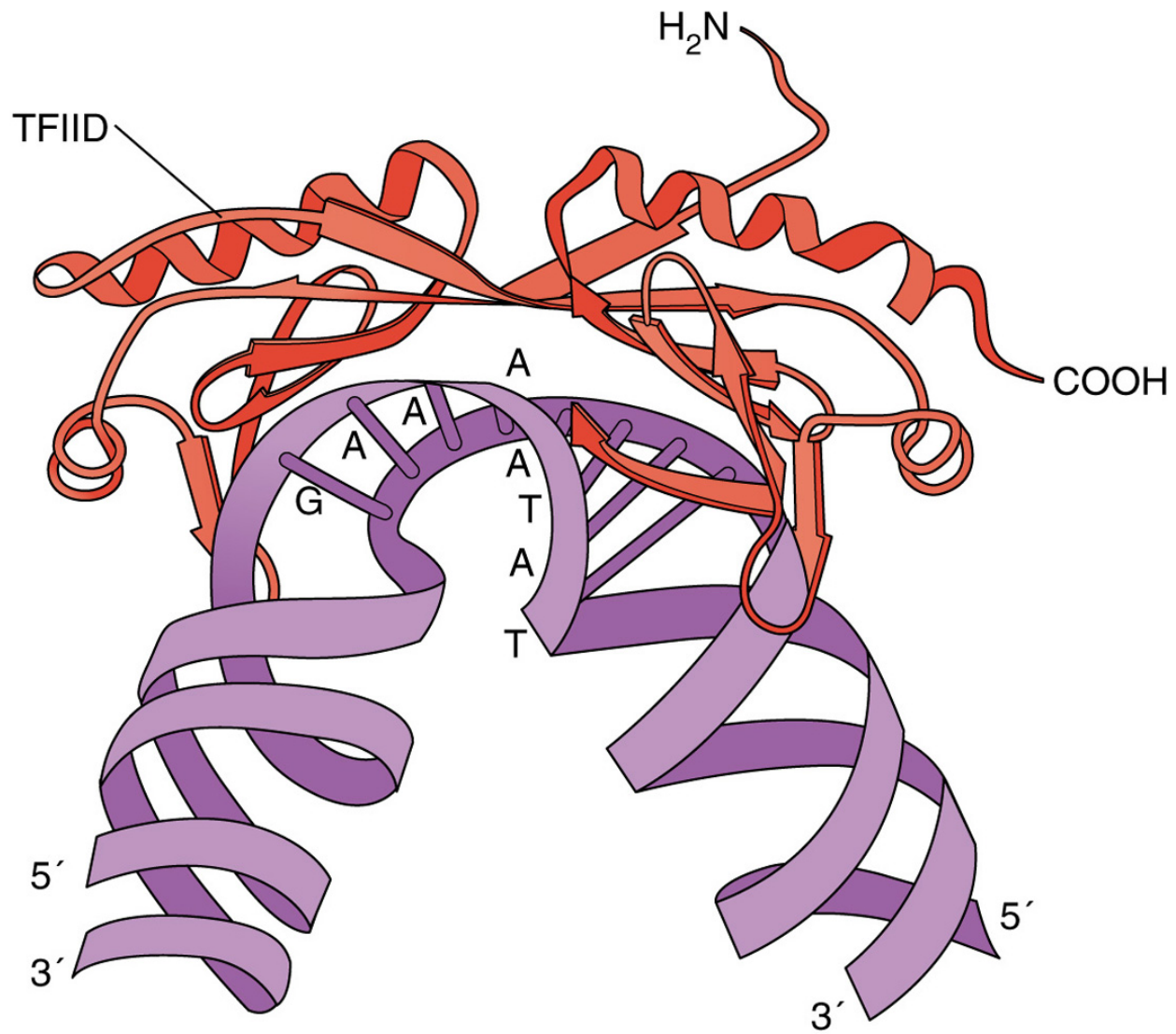
- Overview of levels of gene expression regulation
- Transcriptional gene regulation
 - Modification of the chromatin structure and DNA methylation
 - **Transcriptional activation**

Formation of transcription initiation complex

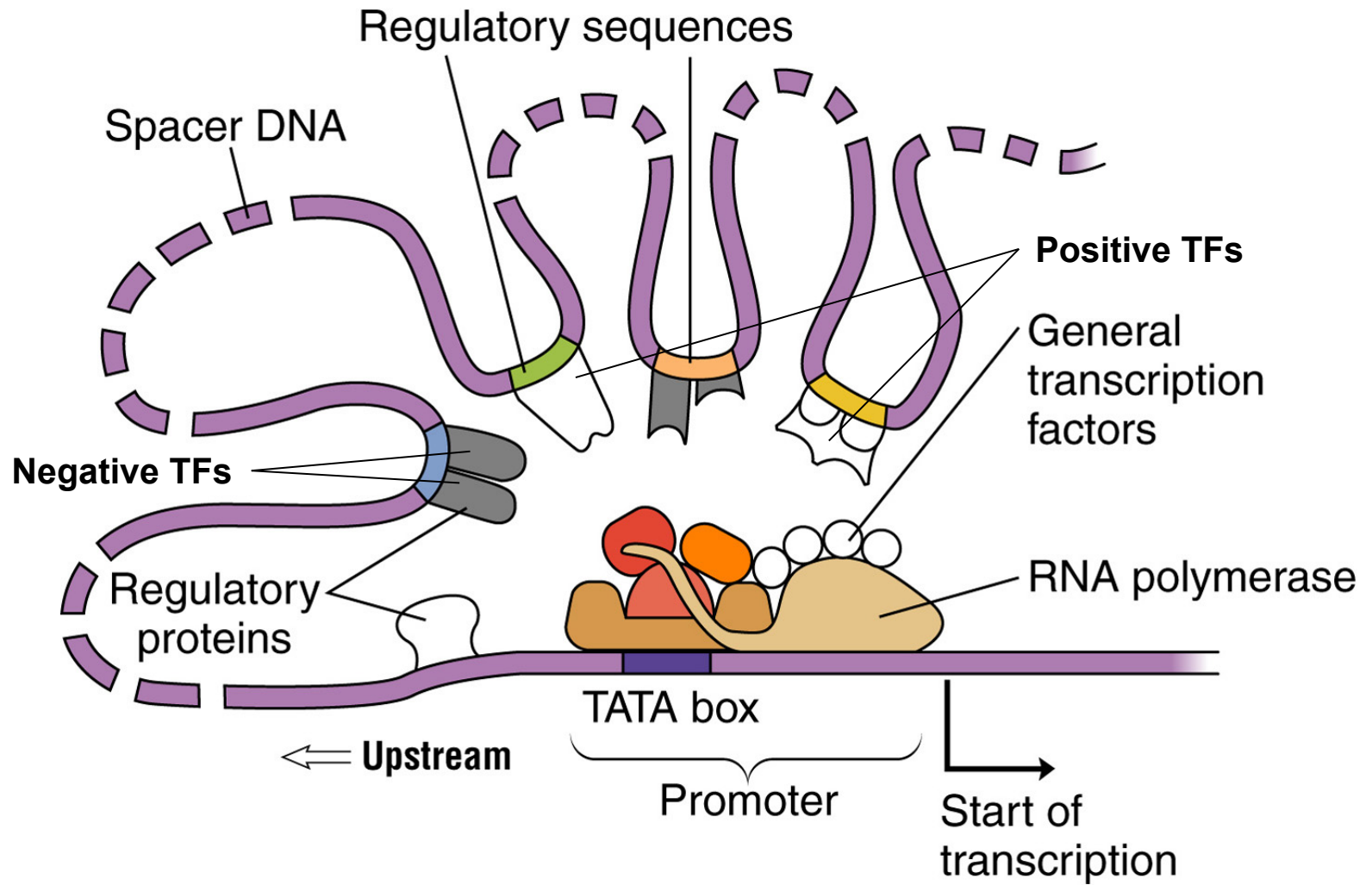
A.



B.



Formation of transcription initiation complex



Mechanism of transcriptional regulation by TAFs

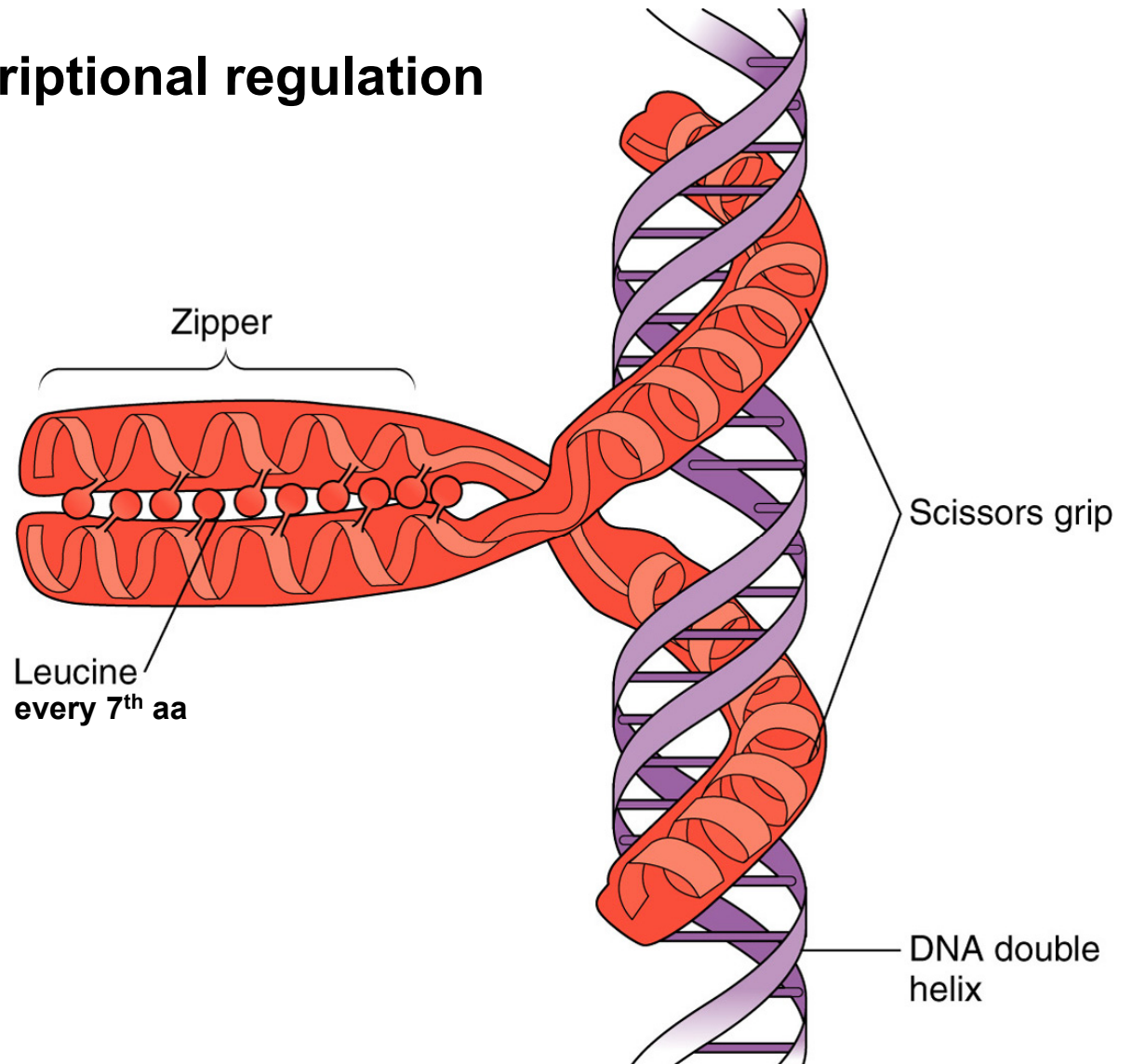
Signal recognition



Dimerization

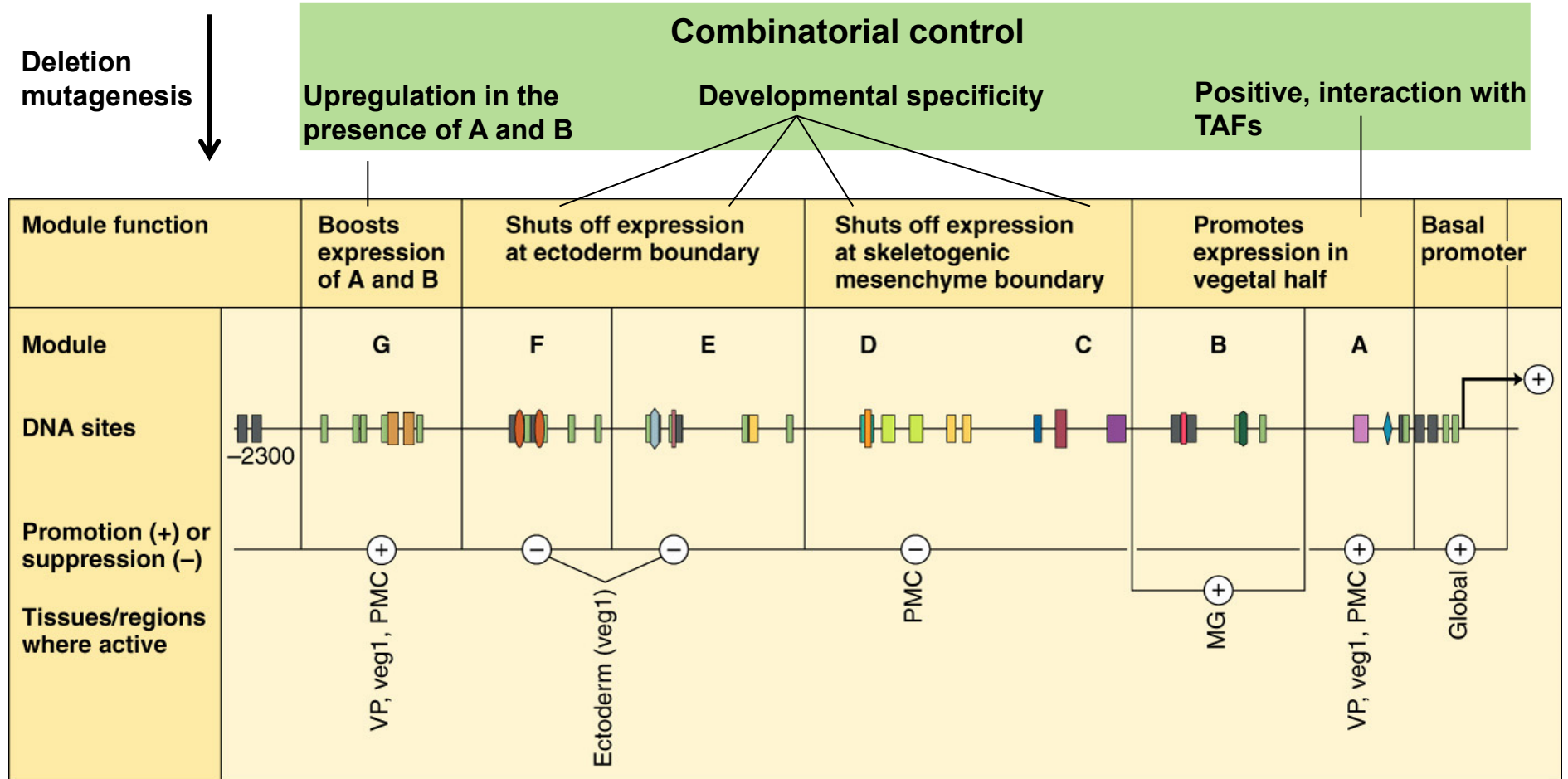


DNA binding and transcription activation



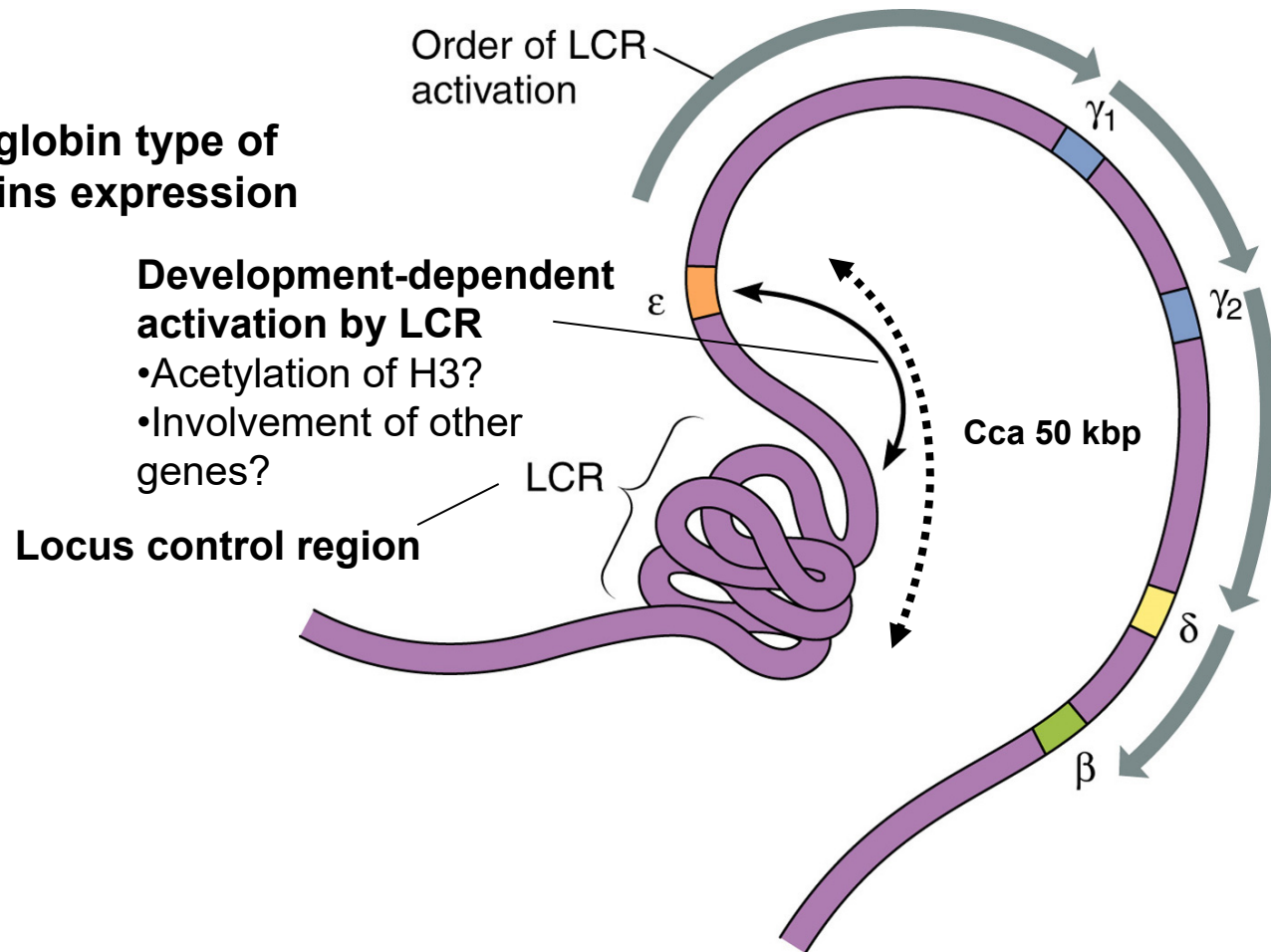
“Microprocessor-like” acting promoters

ProENDO16:REPORTER



“Microprocessor-like” acting promoters

Regulation of β -globin type of hemoglobin chains expression



Outline of Lesson 10

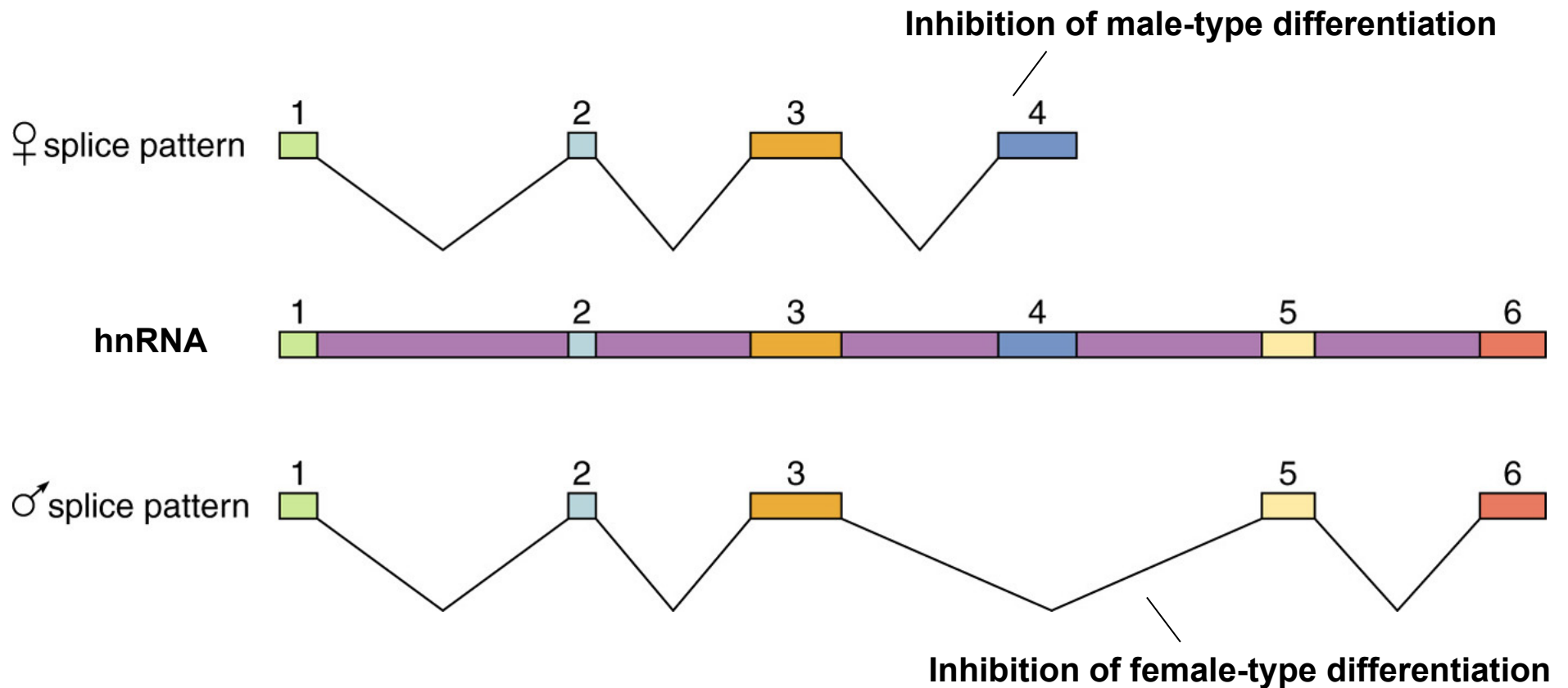
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Splicing of hnRNA



Sex-specific splicing of *DOUBLE SEX (DSX)* hnRNA in *Drosophila*

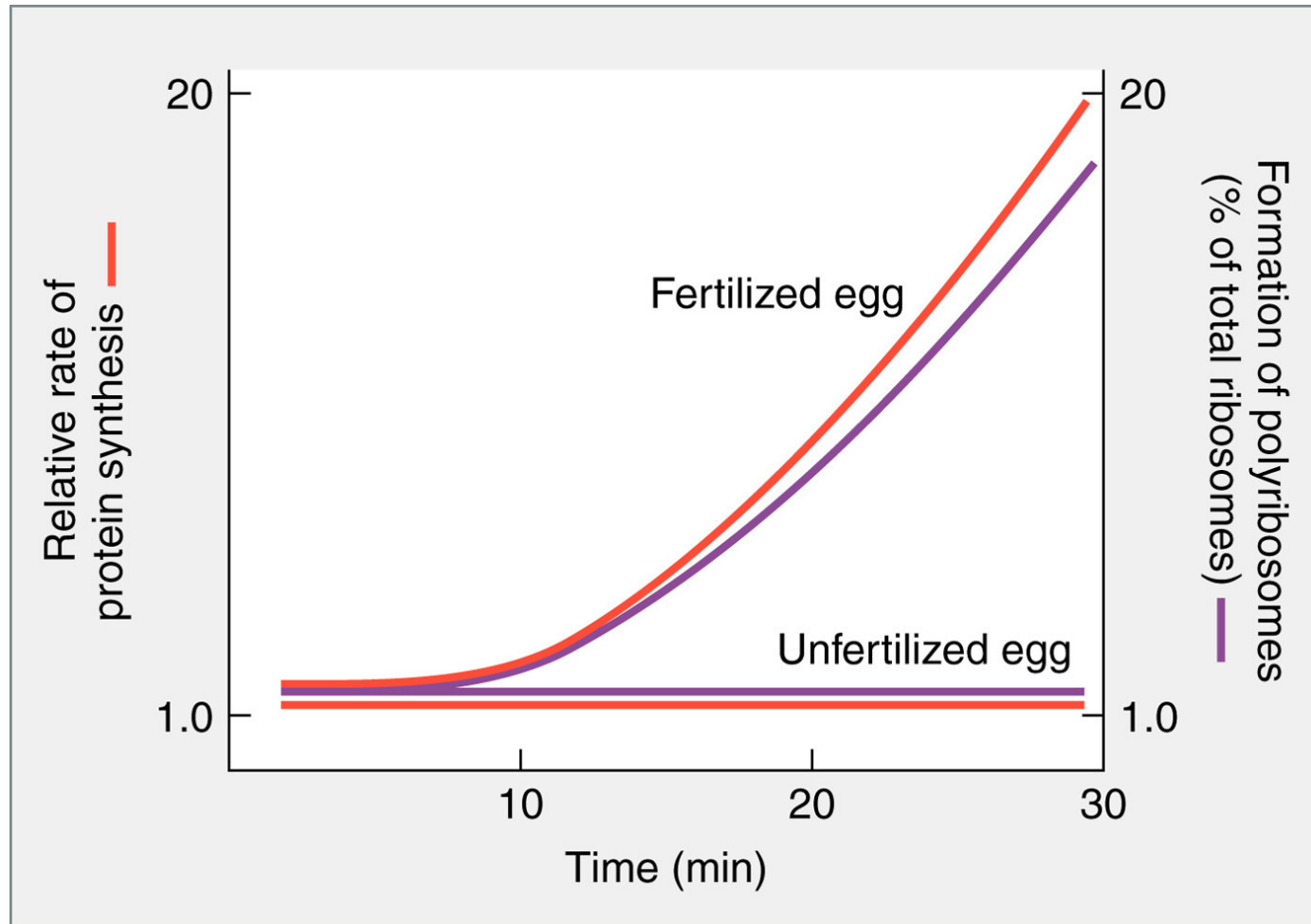


Outline of Lesson 10

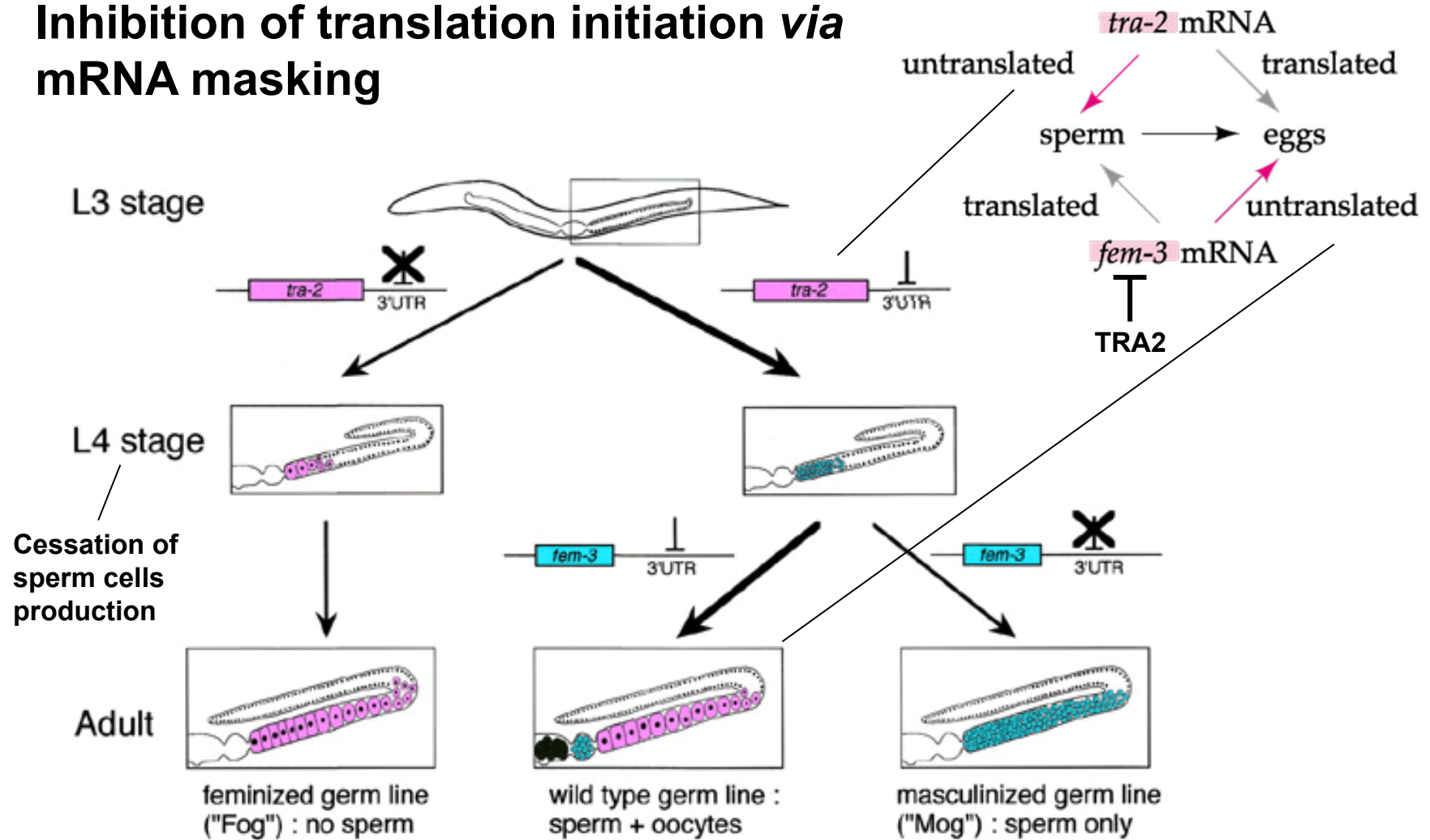
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Translation initiation after egg fertilization



Inhibition of translation initiation *via* mRNA masking



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

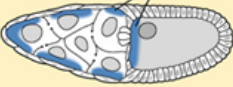
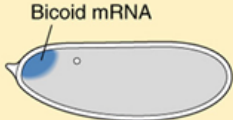
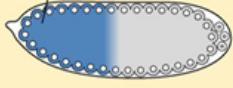
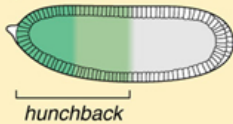
Developmental Biology 8e Online (<http://8e.devbio.com/>)

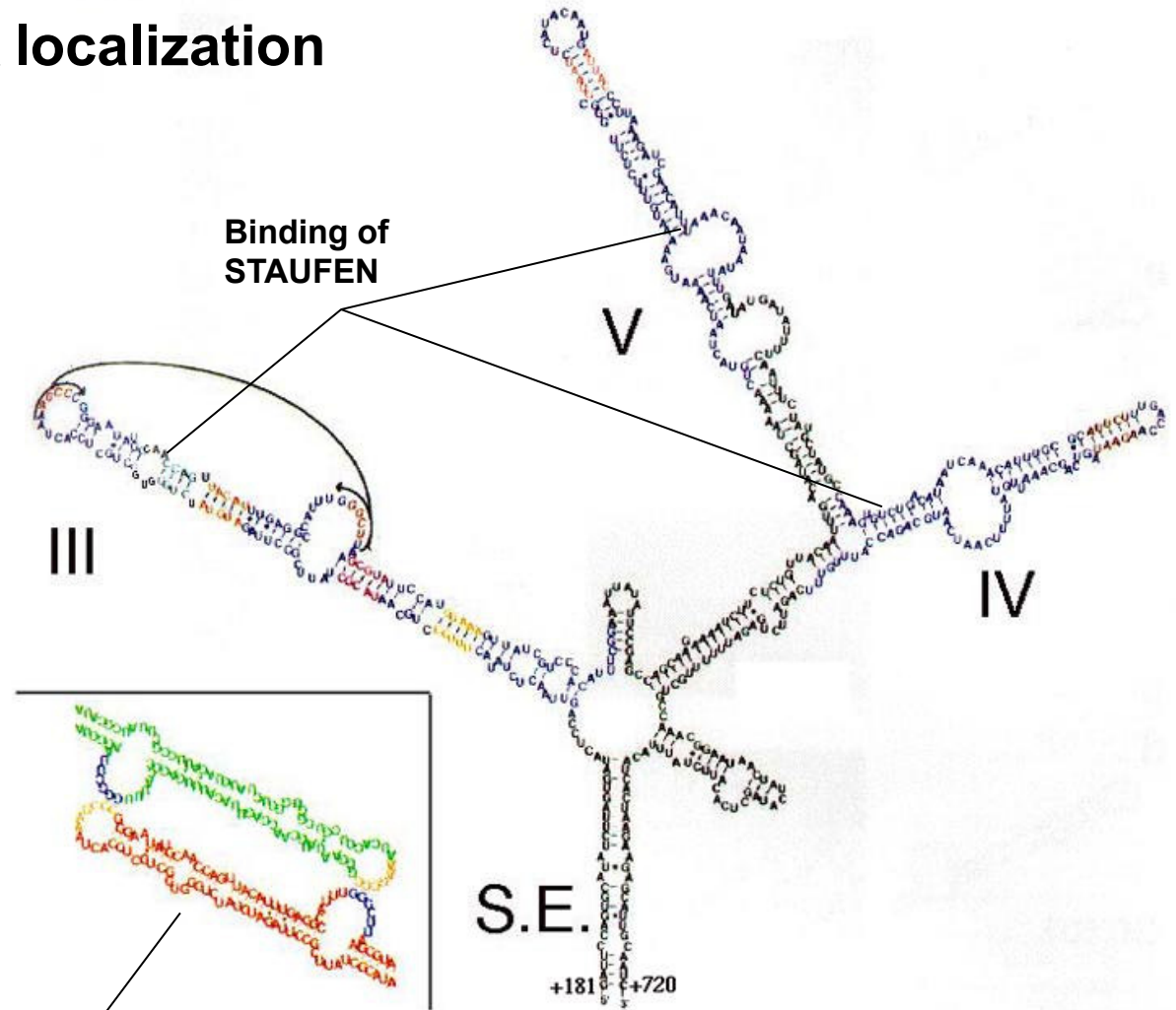
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 - **Localization of mRNA**

Regulation via mRNA localization

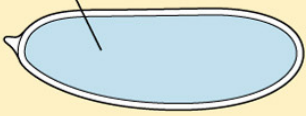
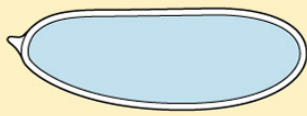
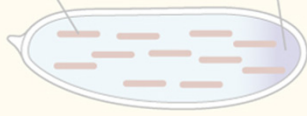
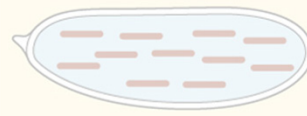
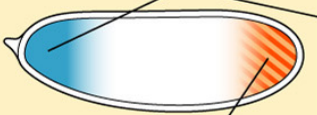
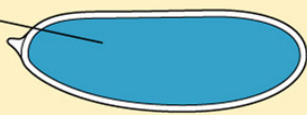
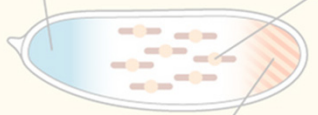
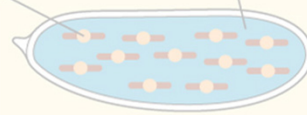




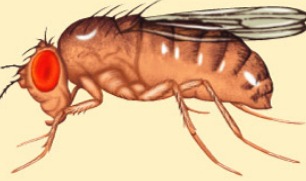

	Anterior:
Oocyte/follicle	Bicoid mRNA 
Fertilized egg	Bicoid mRNA 
512-nuclei stage (cycle 9)	Bicoid protein 
6,000-cell blastoderm zygotic response	Anterior gap genes  hunchback



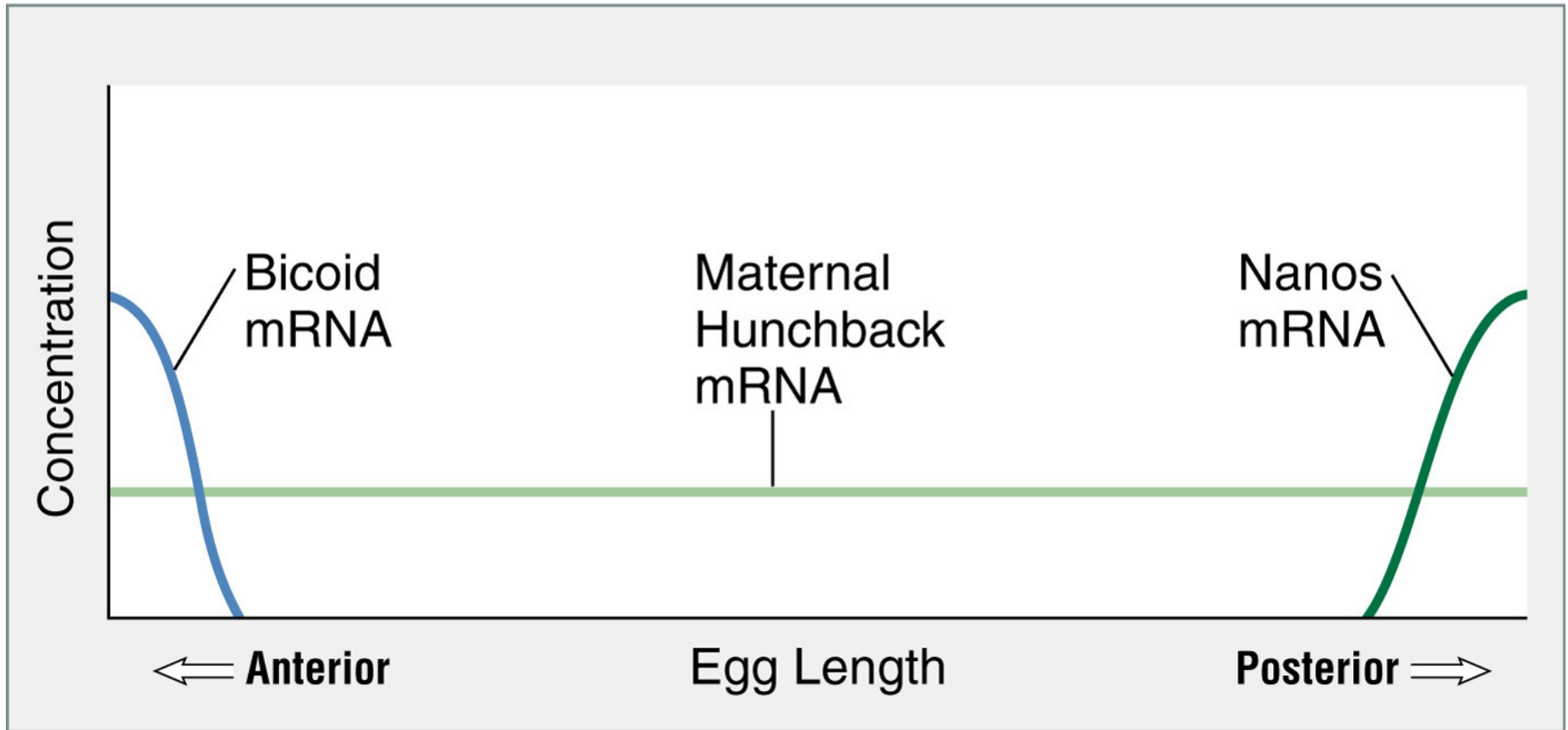
Dimer structure of two *BICOID* mRNAs recognized by STAUFEN

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

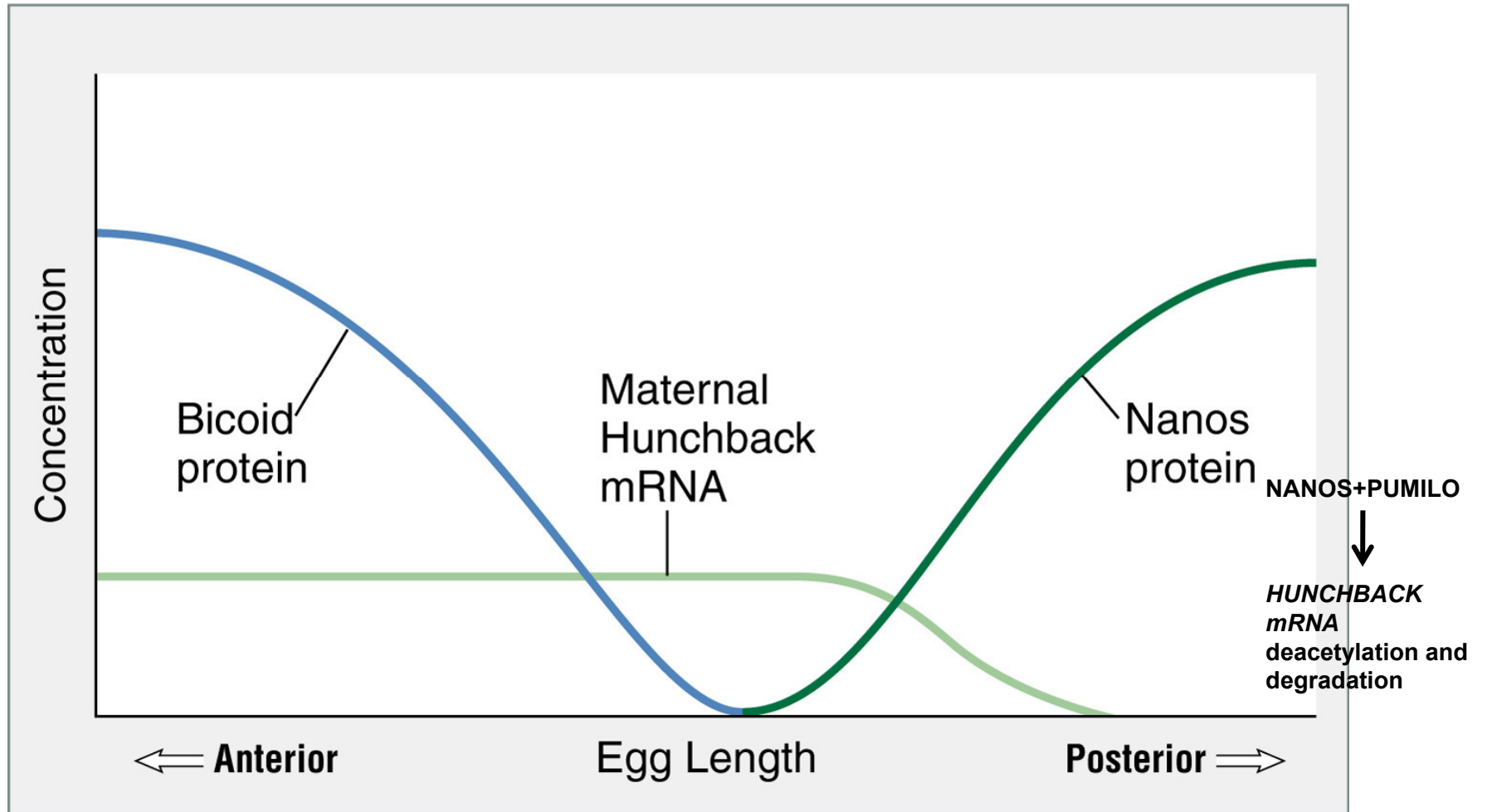
Regulation via mRNA localization

	A. Nanos normal (<i>nanos</i> ^{+/+})	B. Nanos mutant (<i>nanos</i> ^{-/-})	C. Oskar normal (<i>osk</i> ^{+/+})	D. Oskar mutant (<i>osk</i> ^{-/-})
Egg on laying	Hunchback mRNA 		Smaug protein Oskar protein 	
Cleaving egg	Hunchback protein (maternal and zygotic) Localized Nanos mRNA and protein 		Hunchback protein Smaug protein–Nanos mRNA Hunchback protein Localized Nanos mRNA and protein 	
Larva				
Adult		LETHAL		LETHAL

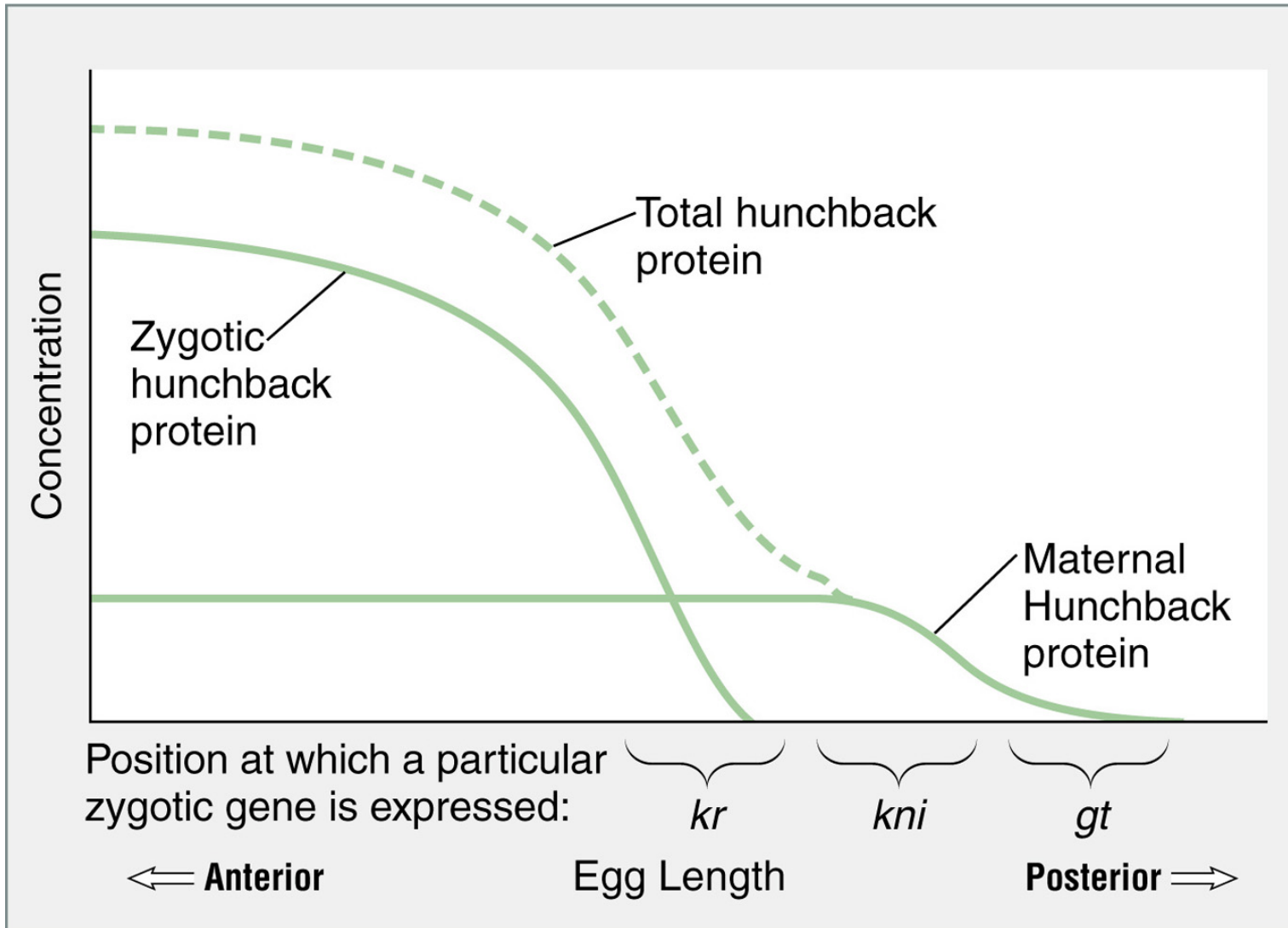
A.



B.



c.



Regulation via mRNA localization

	A. Nanos normal (<i>nanos</i> ^{+/+})	B. Nanos mutant (<i>nanos</i> ^{-/-})	C. Oskar normal (<i>osk</i> ^{+/+})	D. Oskar mutant (<i>osk</i> ^{-/-})
Egg on laying	Hunchback mRNA		Smaug protein Oskar protein	
Cleaving egg	Hunchback protein (maternal and zygotic) Localized Nanos mRNA and protein		Hunchback protein Smaug protein–Nanos mRNA Localized Nanos mRNA and protein	Hunchback protein NANOS+PUMILO
Larva				
Adult		LETHAL		LETHAL

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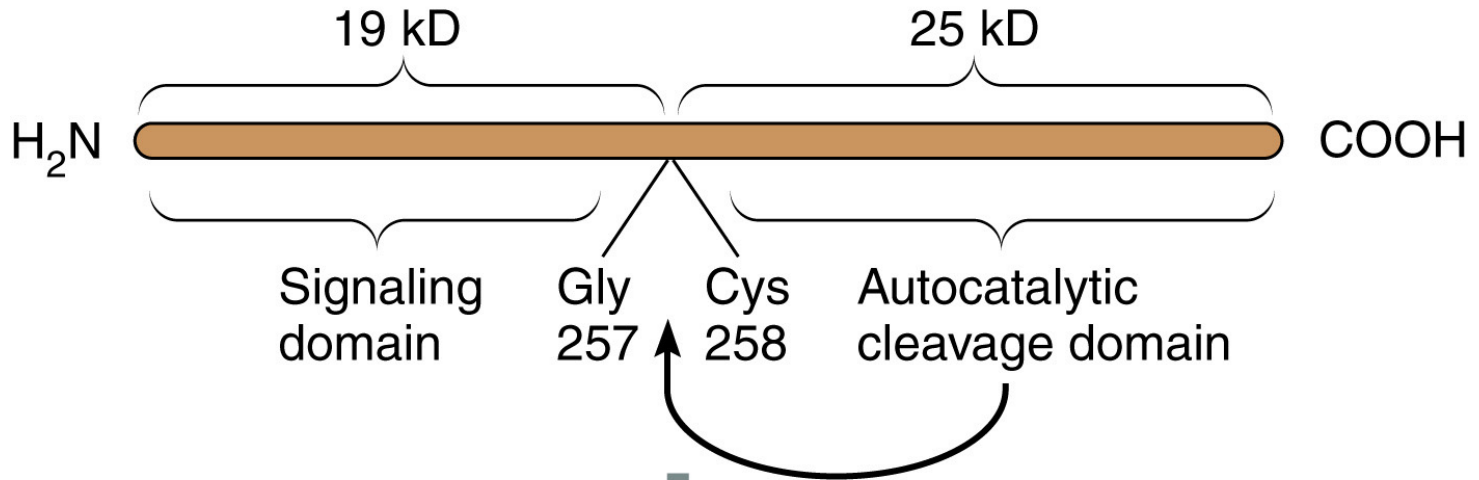
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Regulation via protein localization

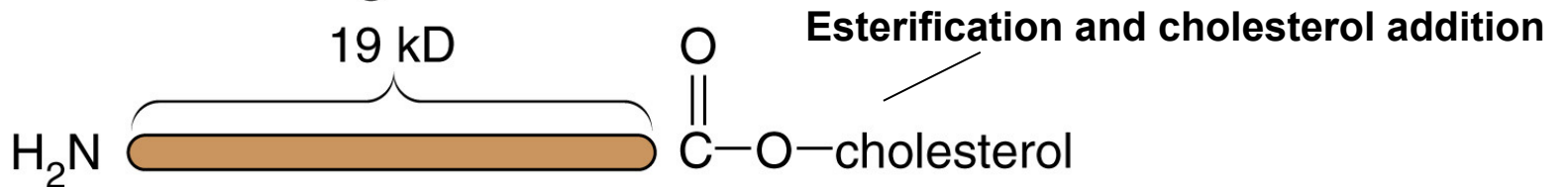
Hedgehog Processing

A. Precursor

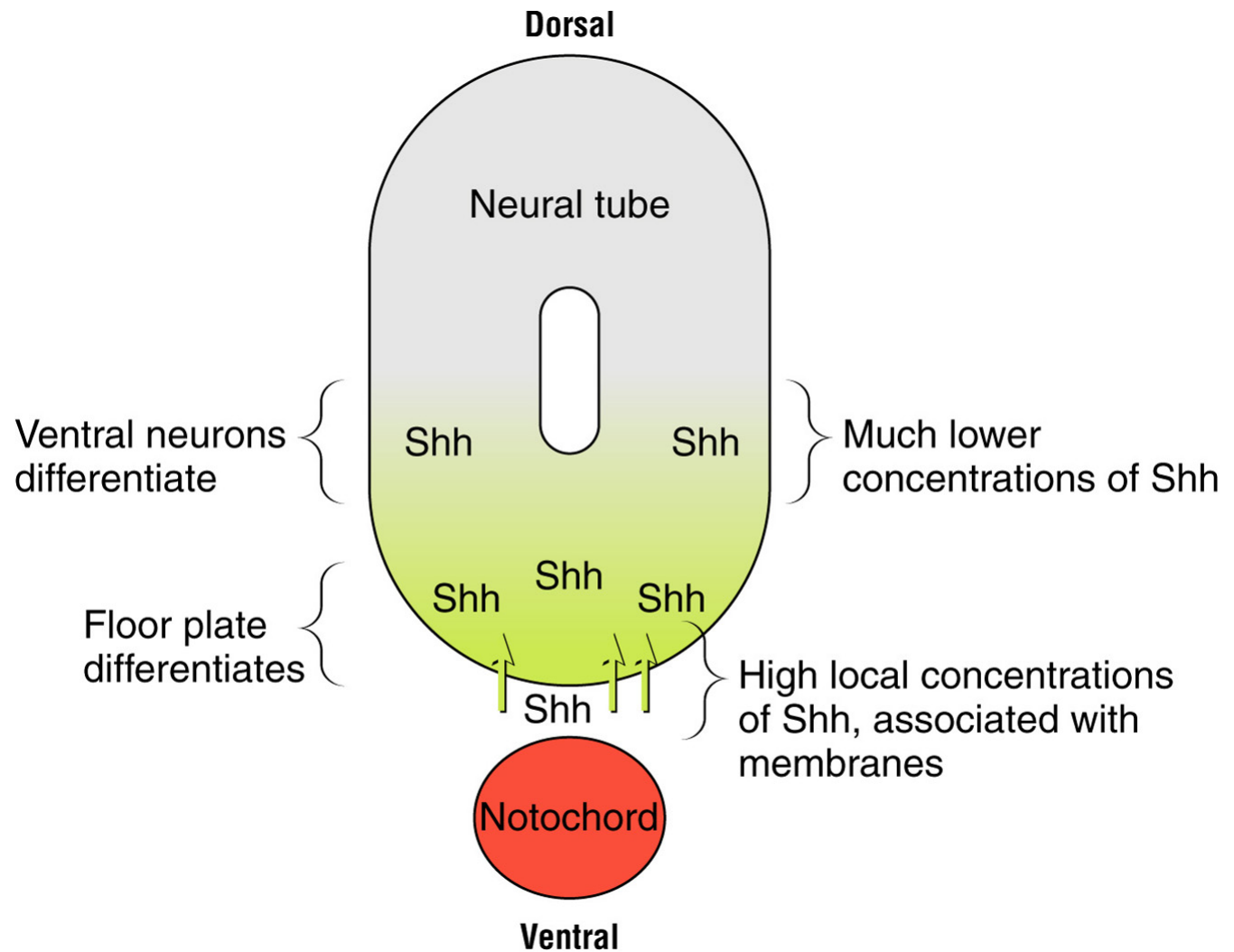


Leaving the Golgi apparatus

B. Active Hh Ligand



Regulation via protein localization



Outline of Lesson 10

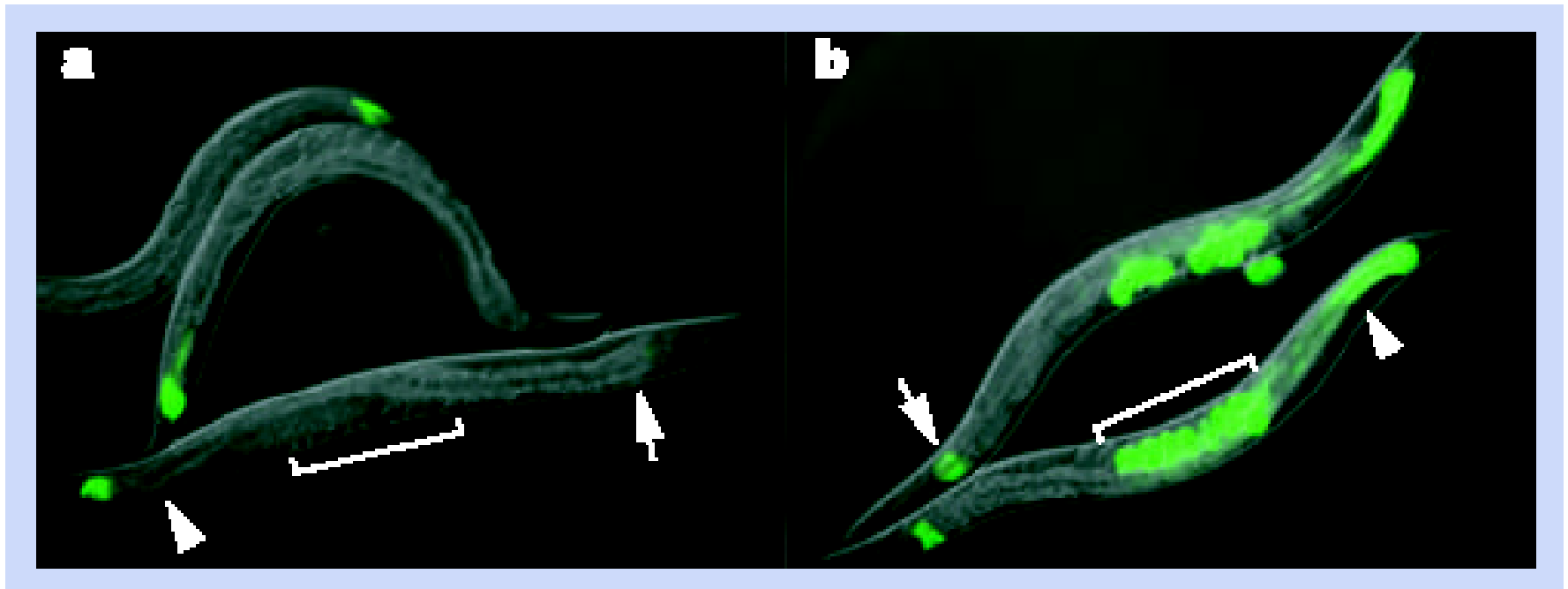
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RNA interference as a natural mechanism of the gene expression

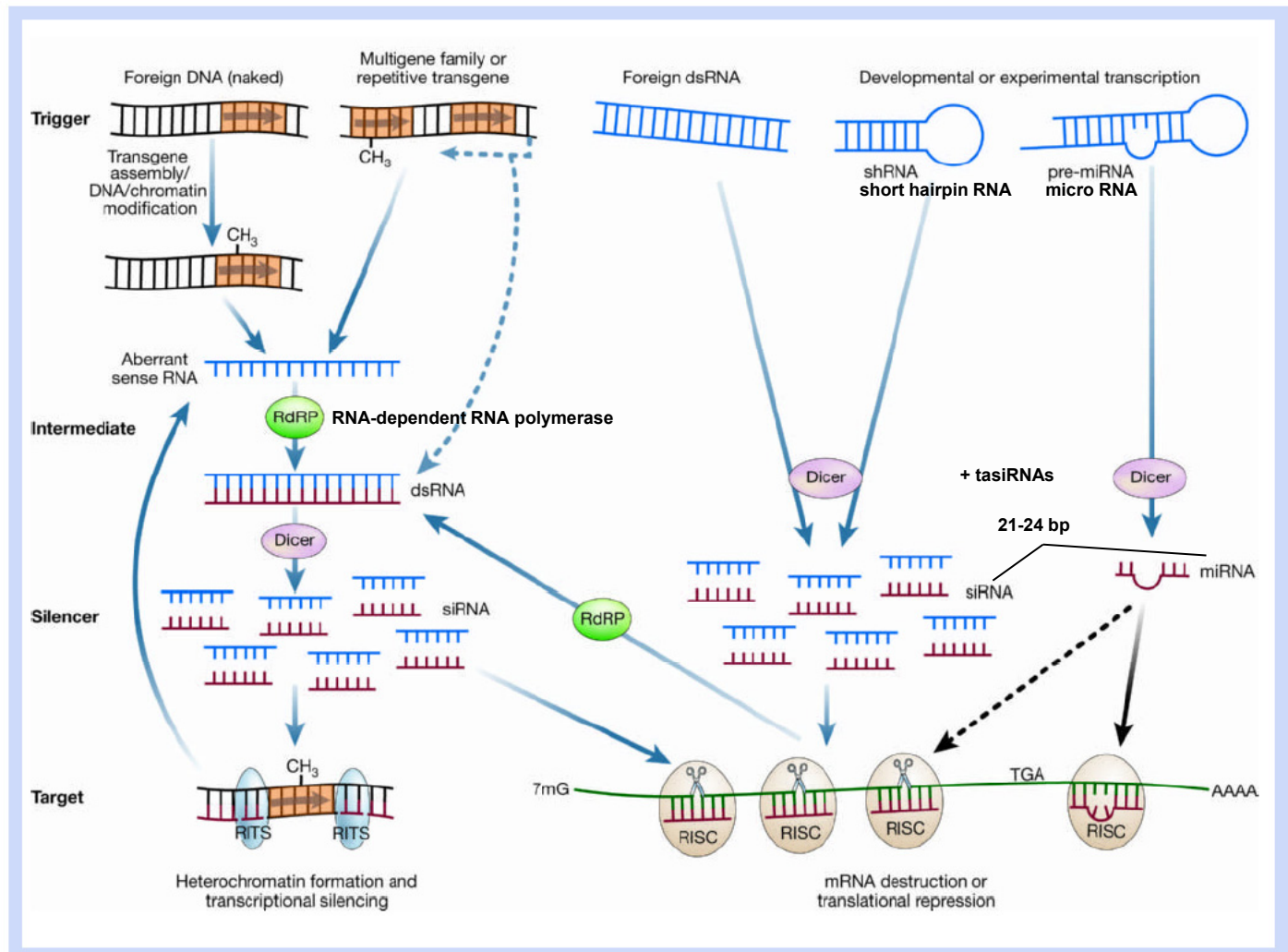
RNAi

rnai



Mello and Conte, *Nature* (2004)

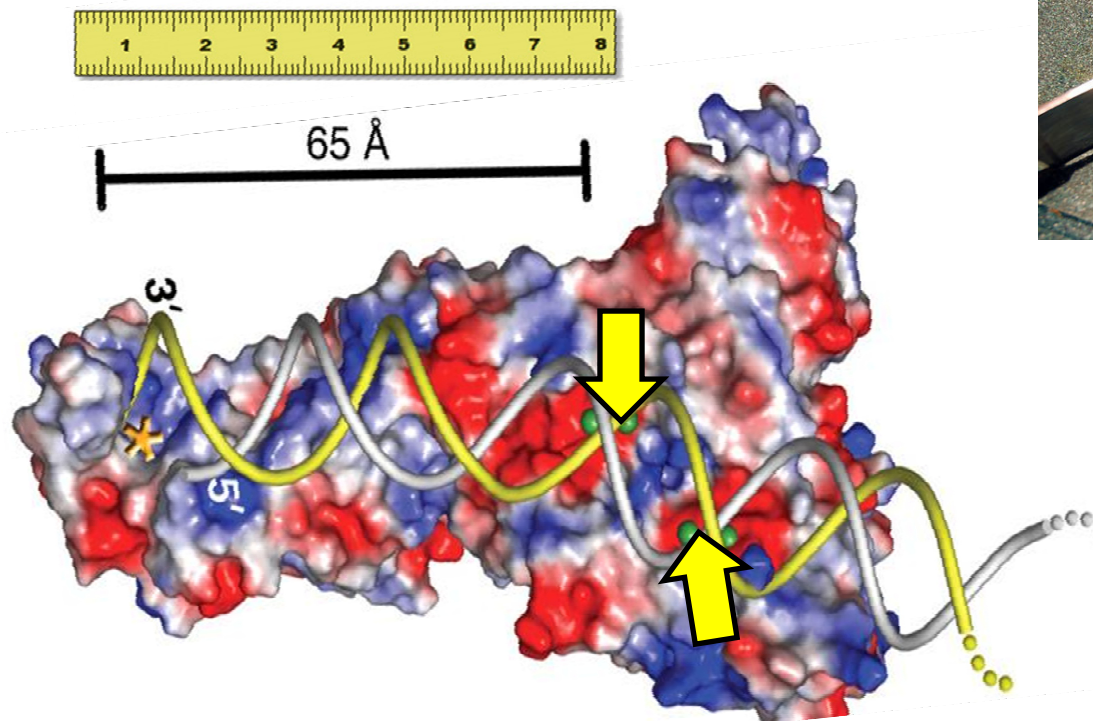
Mechanism of RNA interference



Mello and Conte, *Nature* (2004)

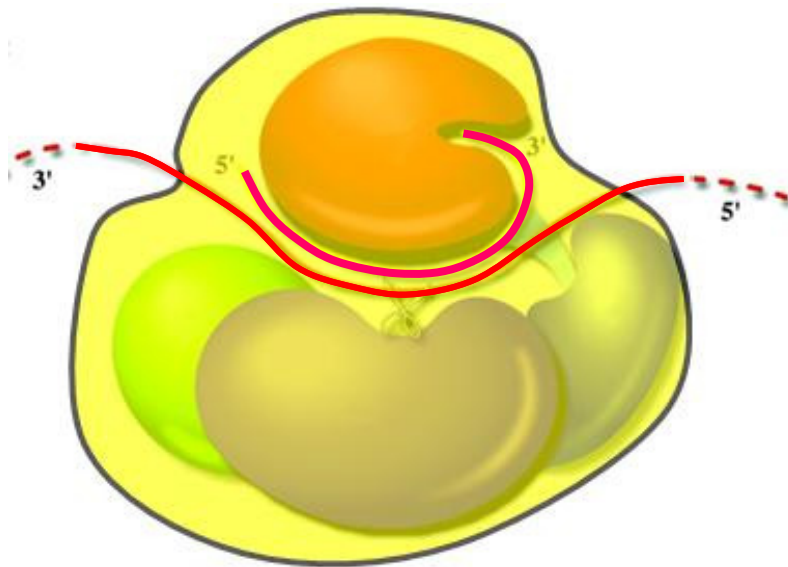
Mello, 2004

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



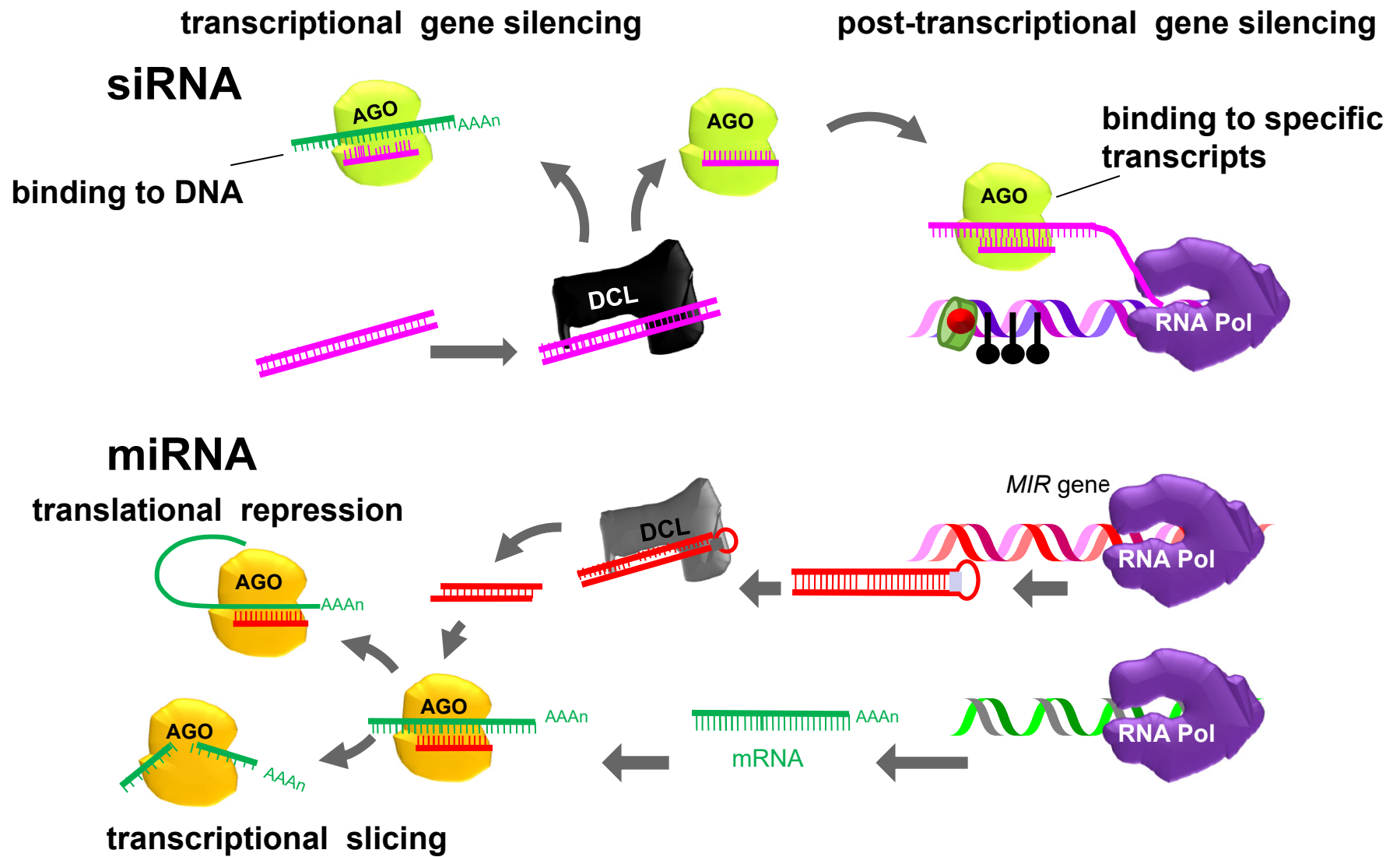
ago1



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.



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

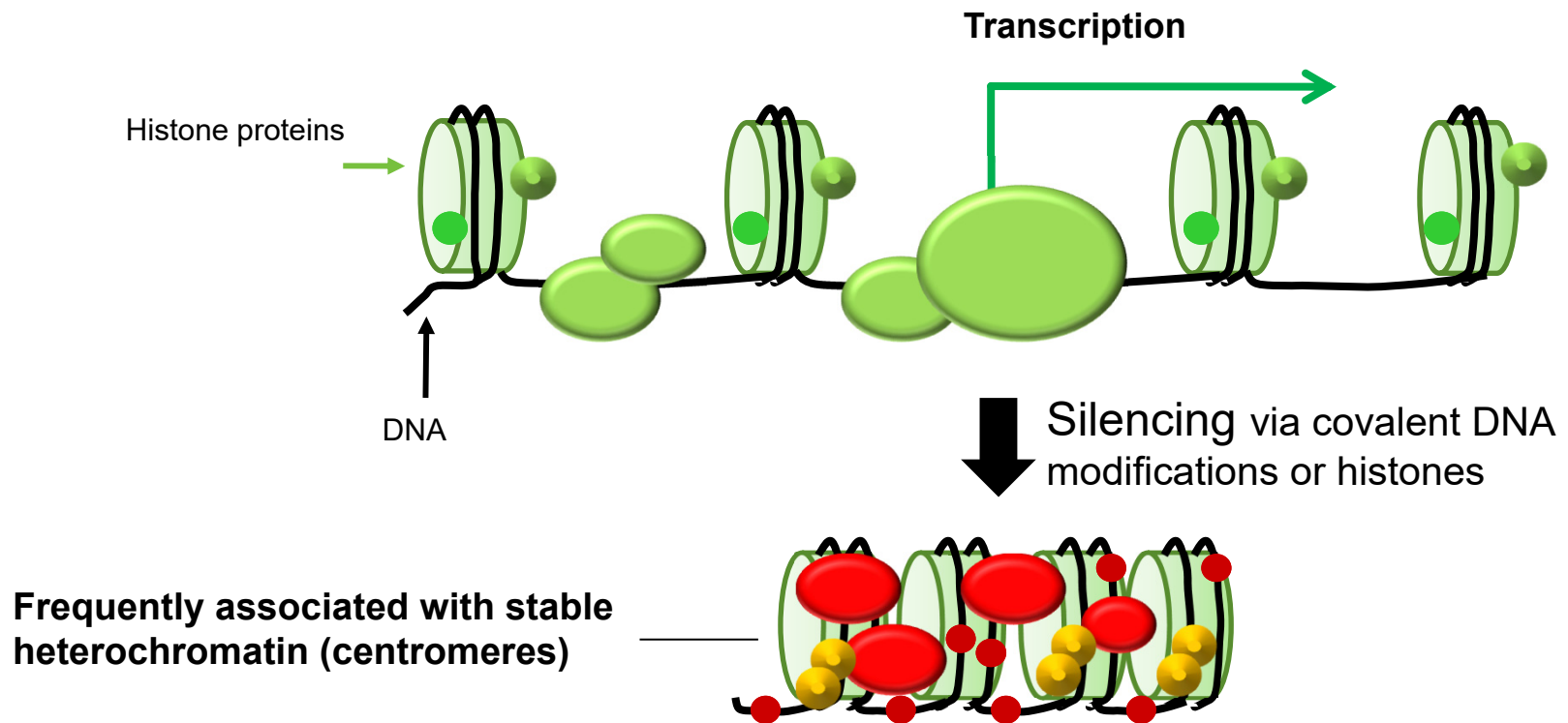
b. 1960

Outline of Lesson 10

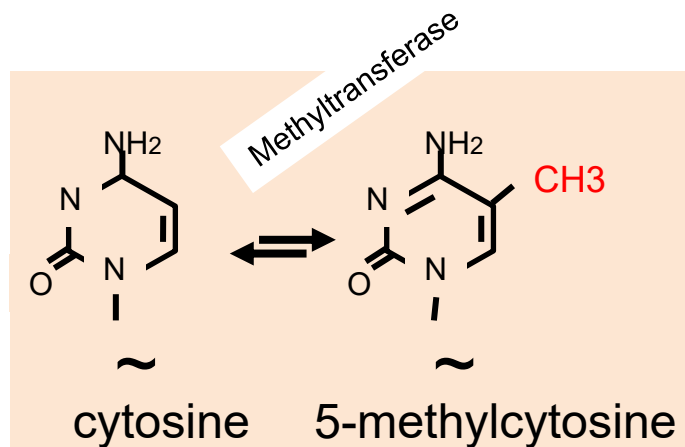
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 - siRNA-mediated silencing

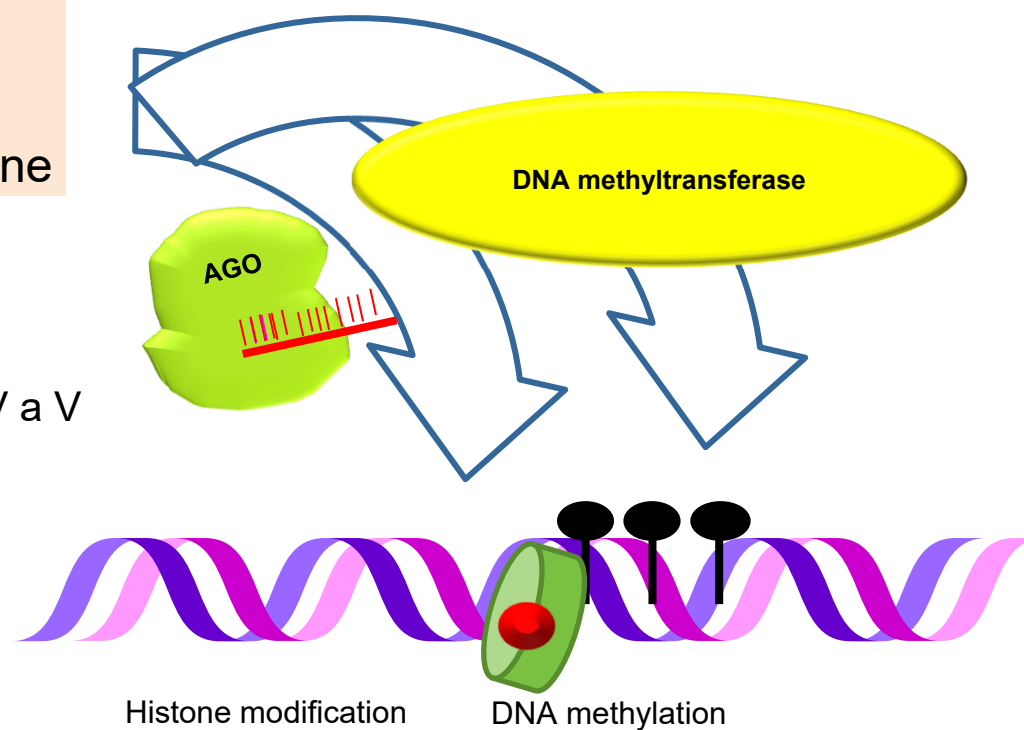
Transcriptional gene silencing via covalent modifications of DNA

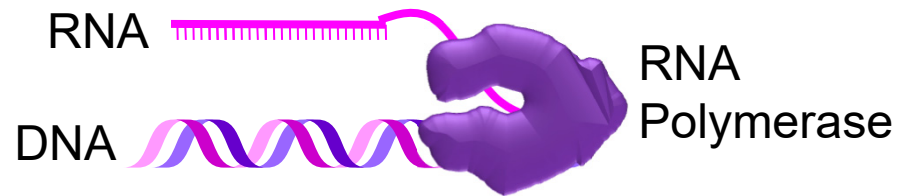


Transcriptional gene silencing via DNA methylation



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

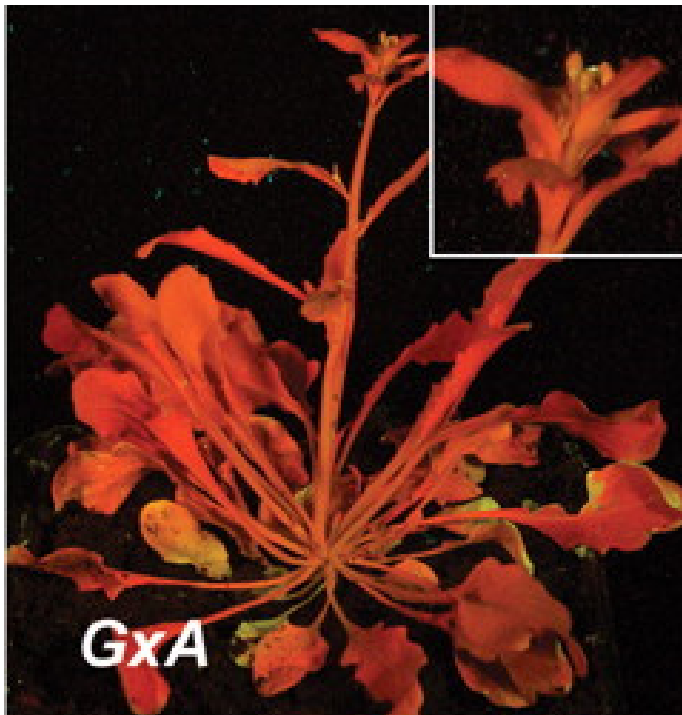




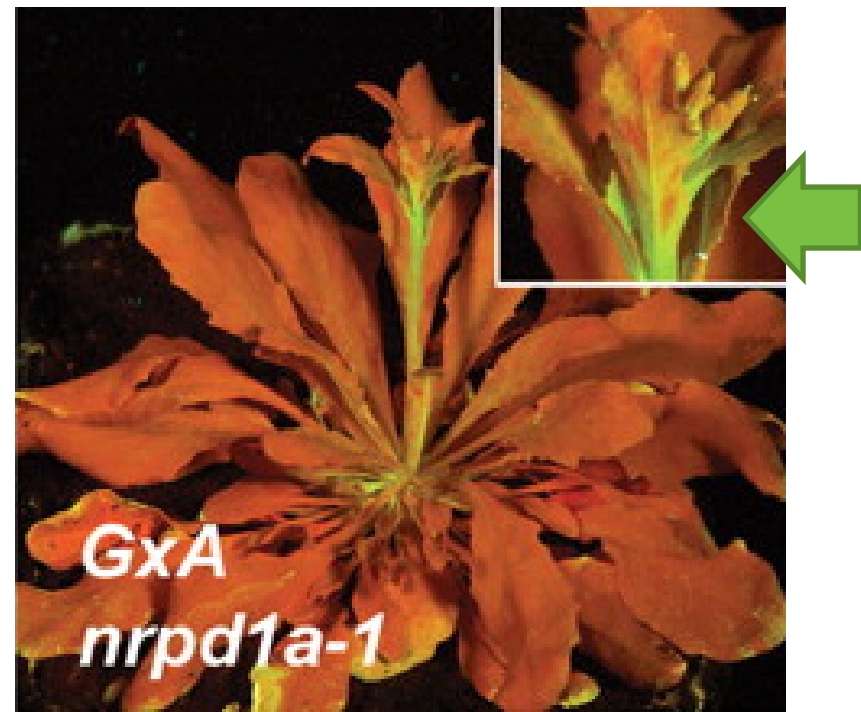
Complex	Distribution	Function
RNA Polymerase I	All eukaryotes	Production of rRNA
RNA Polymerase II	All eukaryotes	Production of mRNA, microRNA
RNA Polymerase III	All eukaryotes	Production of tRNA, 5S rRNA
RNA Polymerase IV	Land plants	Production of siRNA
RNA Polymerase V	Angiosperms	Recruitment of AGO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

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

Arabidopsis with silenced GFP gene

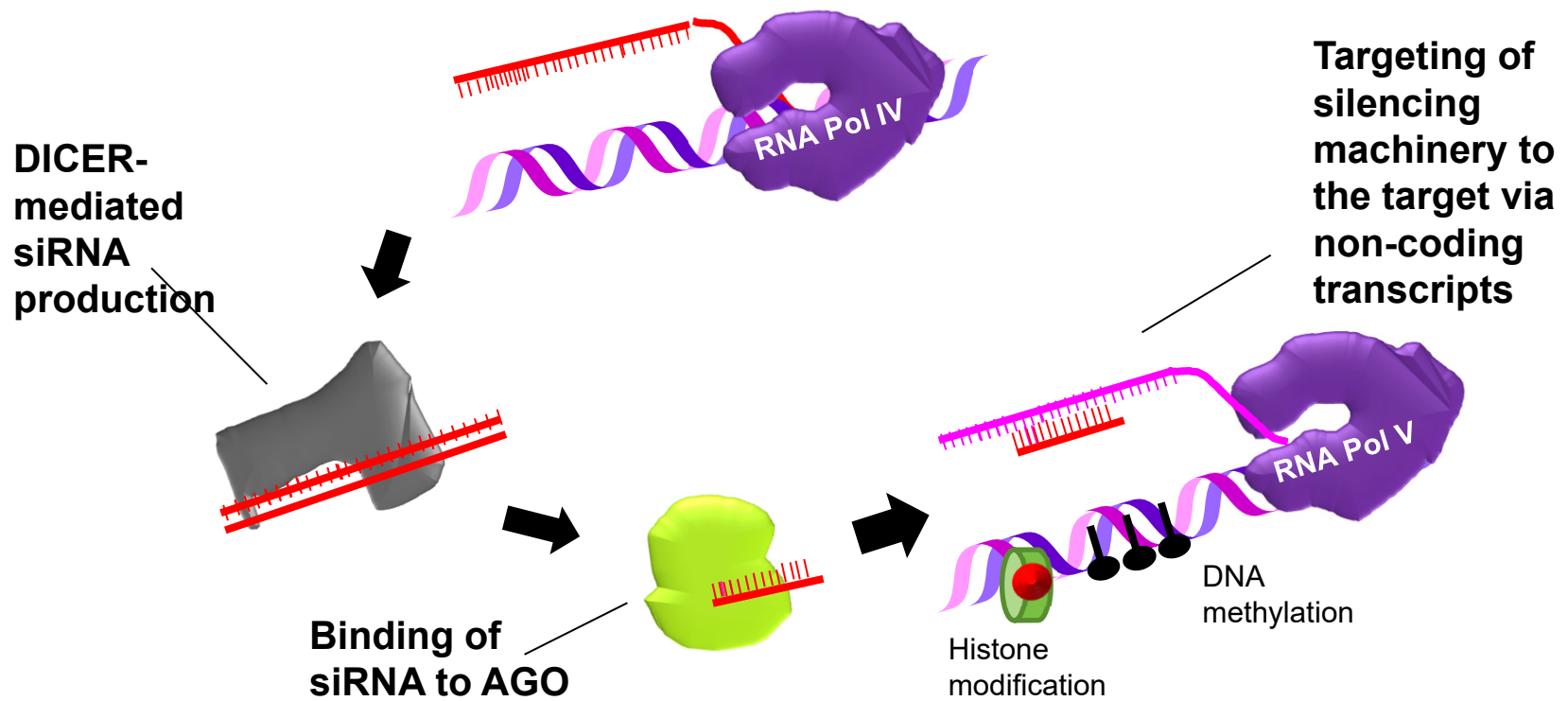


nrpd1a-1

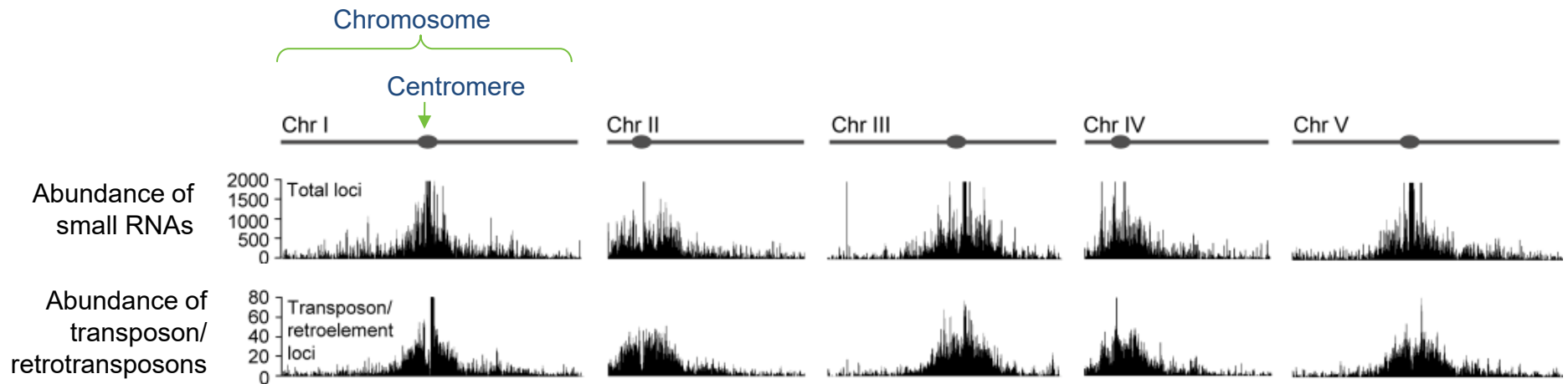


From Herr, A.J., Jensen, M.B., Dalmay, T., and Baulcombe, D.C. (2005) RNA polymerase IV directs silencing of endogenous DNA. *Science* 308: [118–120](#). Reprinted with permission from AAAS.

RNA Pol IV and V are necessary for transcriptional silencing

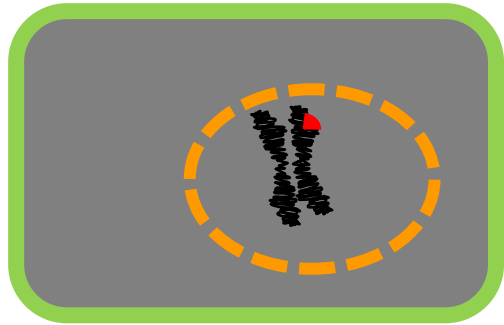


Most siRNAs are produced from transposons and repetitive DNA



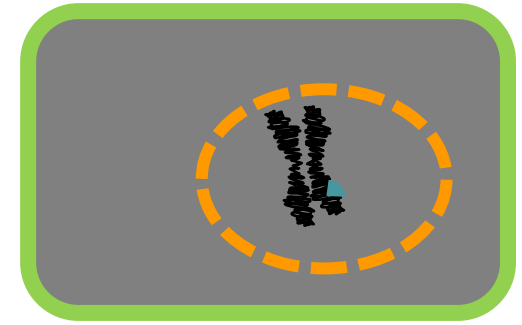
Kasschau, K.D., Fahlgren, N., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., and Carrington, J.C. (2007) Genome-wide profiling and analysis of *Arabidopsis* siRNAs. *PLoS Biol* 5(3): [e57](#).

Transcriptional gene silencing



Pro35S: KAN

X



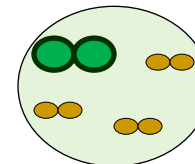
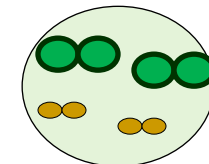
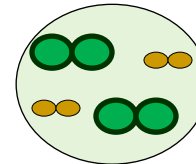
Pro35S: HYG

Expected Results

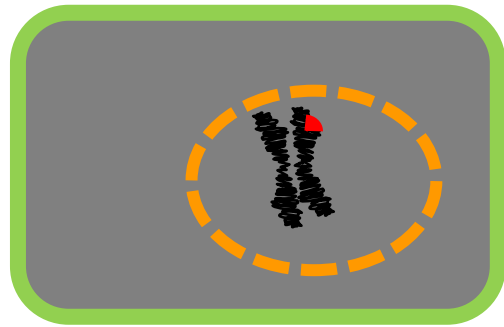
Selection on kanamycin only: 50% KanR

Selection on hygromycin only: 50% HygR

Selection on Kan + Hyg: 25% KanR and HygR

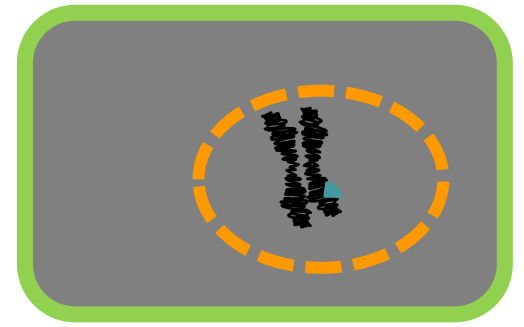


Transcriptional gene silencing



Pro35S : KAN

X



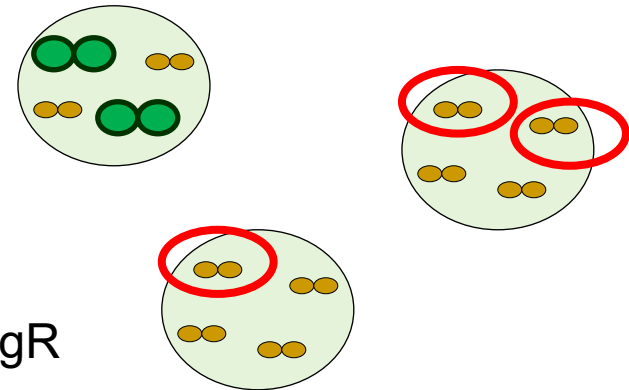
Pro35S : HYG

Observed Results

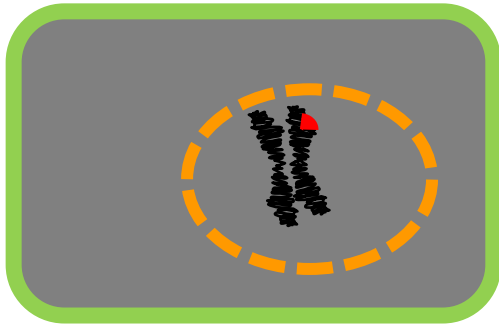
Selection on kanamycin only: 50% KanR

Selection on hygromycin only: 0% HygR

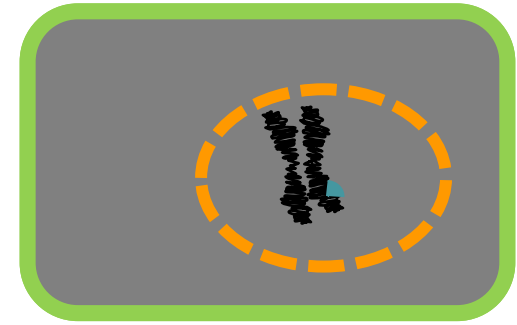
Selection on Kan + Hyg: 0% KanR and HygR



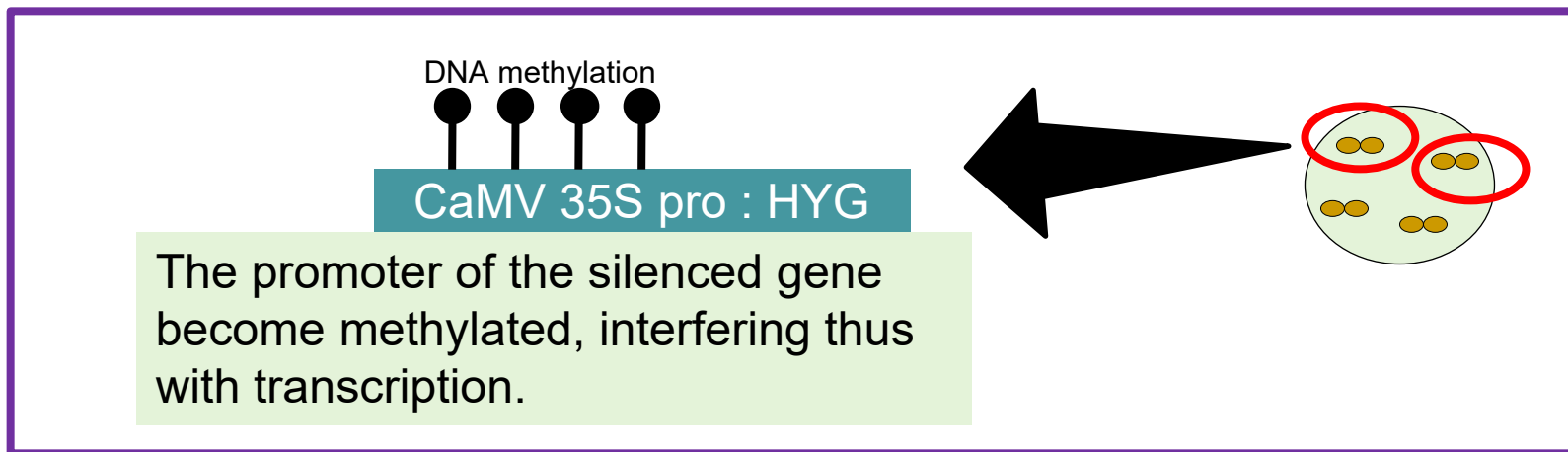
Transcriptional gene silencing



Pro35S : KAN



Pro35S : HYG



siRNAs - summary

- 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

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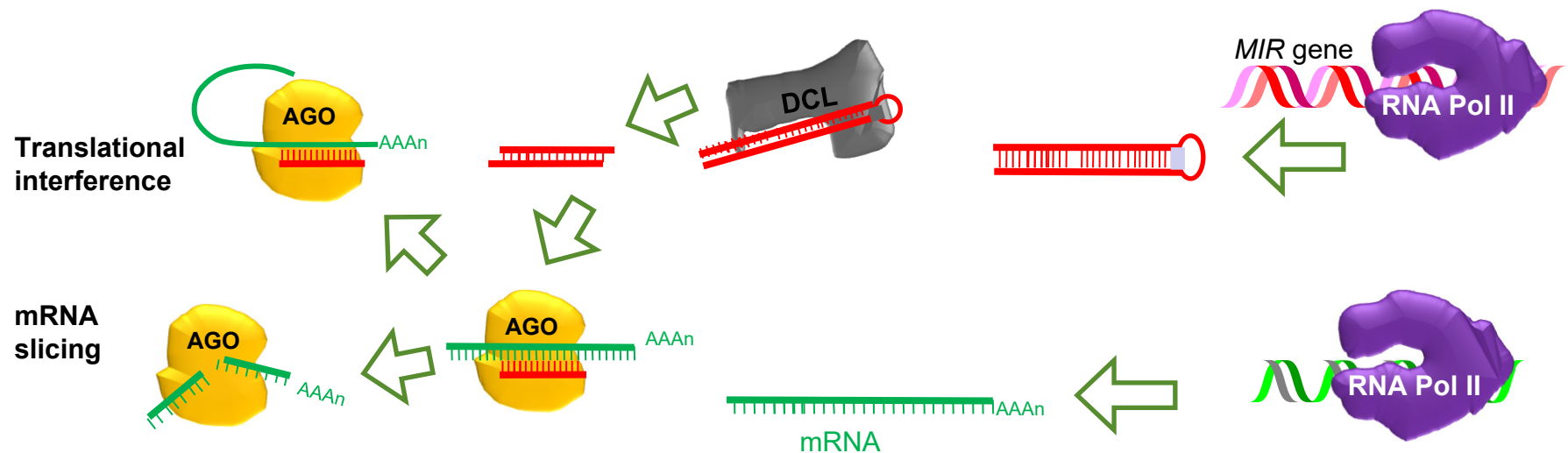
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Mechanisms of miRNAs action

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

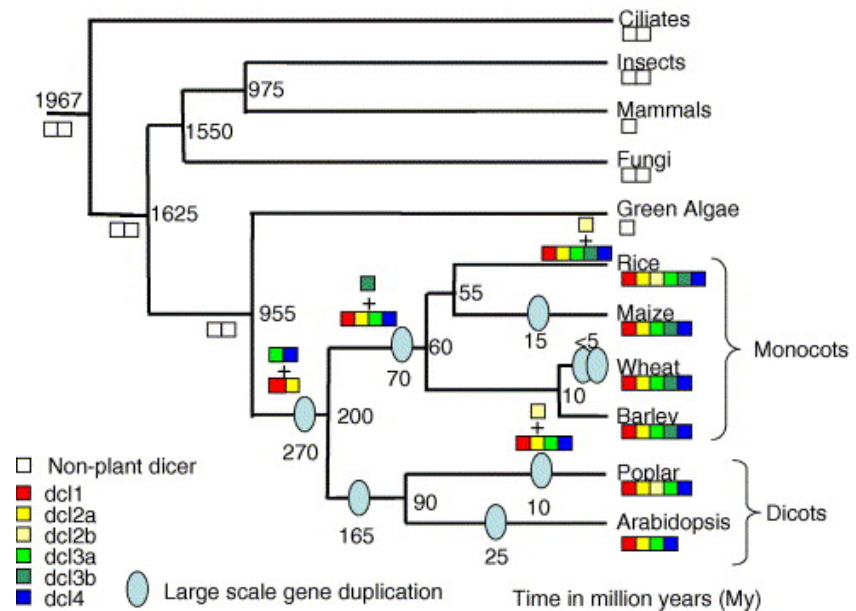


miRNAs and siRNAs are processed by related but different DCL proteins

AtDCL1 produces **miRNA**



AtDCL2 - 4 produce **siRNA**

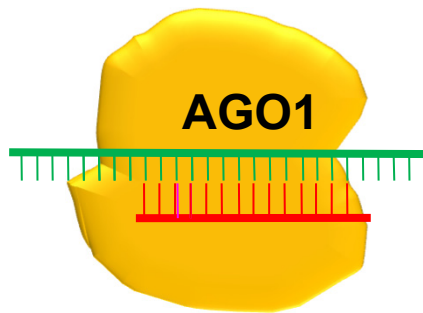


Reprinted from Margis, R., Fusaro, A.F., Smith, N.A., Curtin, S.J., Watson, J.M., Finnegan, E.J., and Waterhouse, P.M. (2006) The evolution and diversification of Dicers in plants FEBS Lett. 580: [2442-2450](#) with permission from Elsevier.

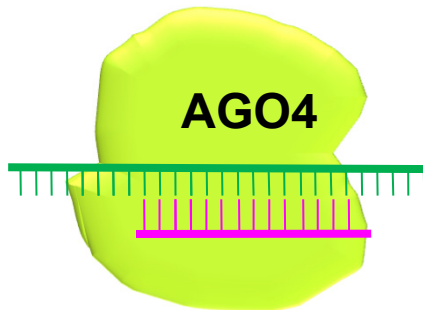
miRNAs and siRNAs associate with several AGO proteins

miRNAs in plants

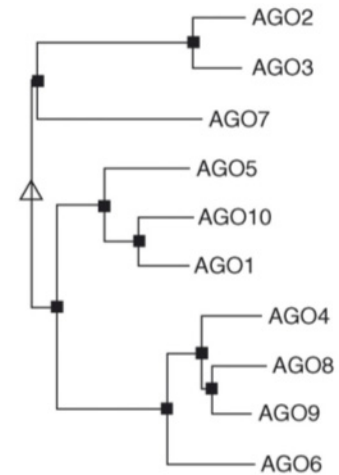
- small # of highly conserved miRNAs
- hing # of non-conserved miRNAs



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

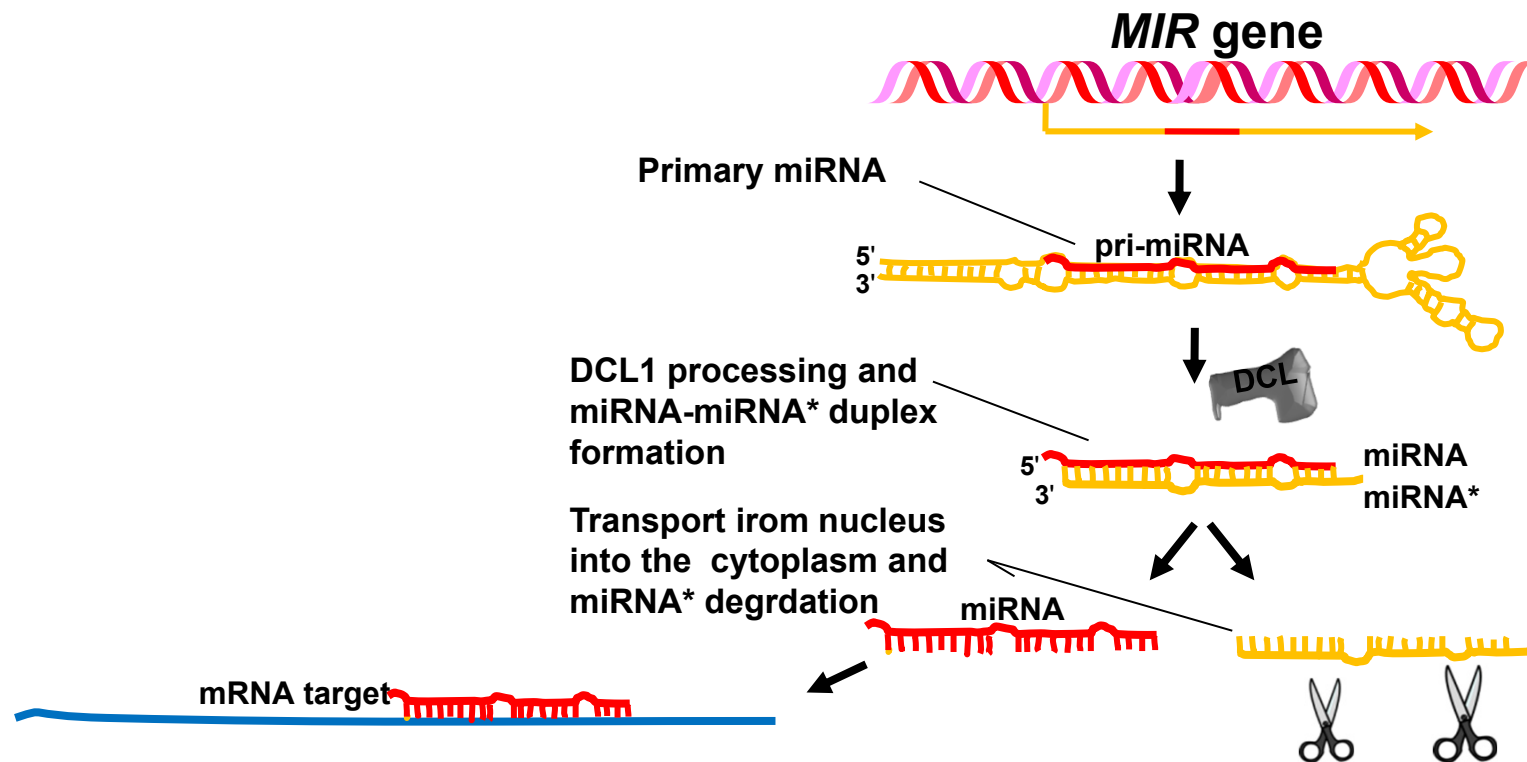


AGO4 preferentially associates with **siRNA** and mediates methylation of source DNA.



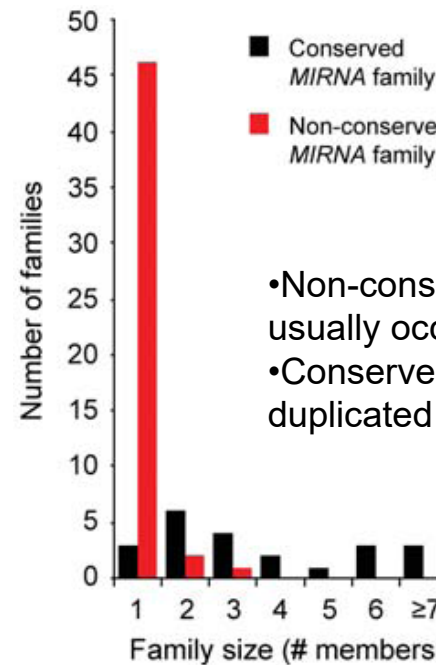
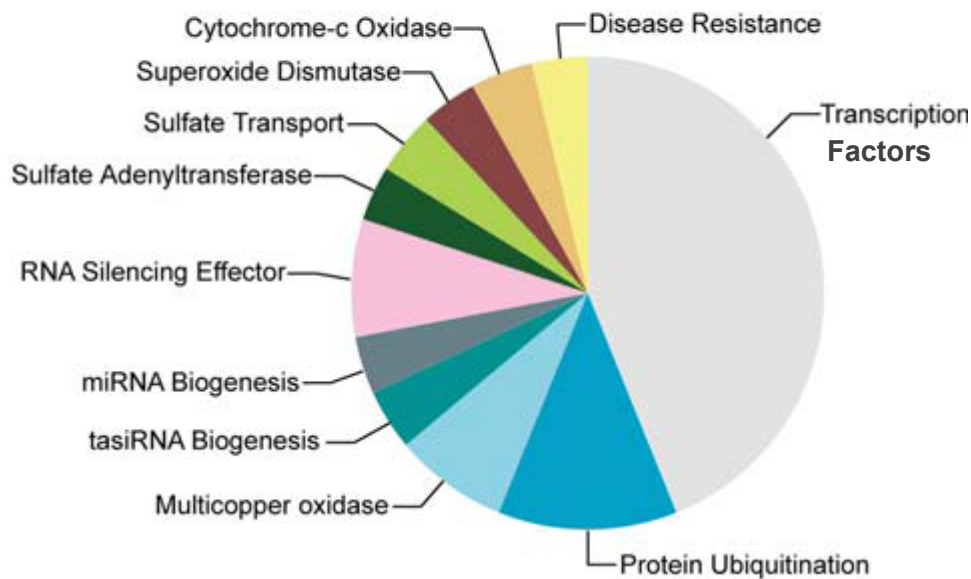
Reprinted from Vaucheret, H. (2008) Plant ARGONAUTES. Trends Plant Sci. 13: [350-358](#) with permission from Elsevier.

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

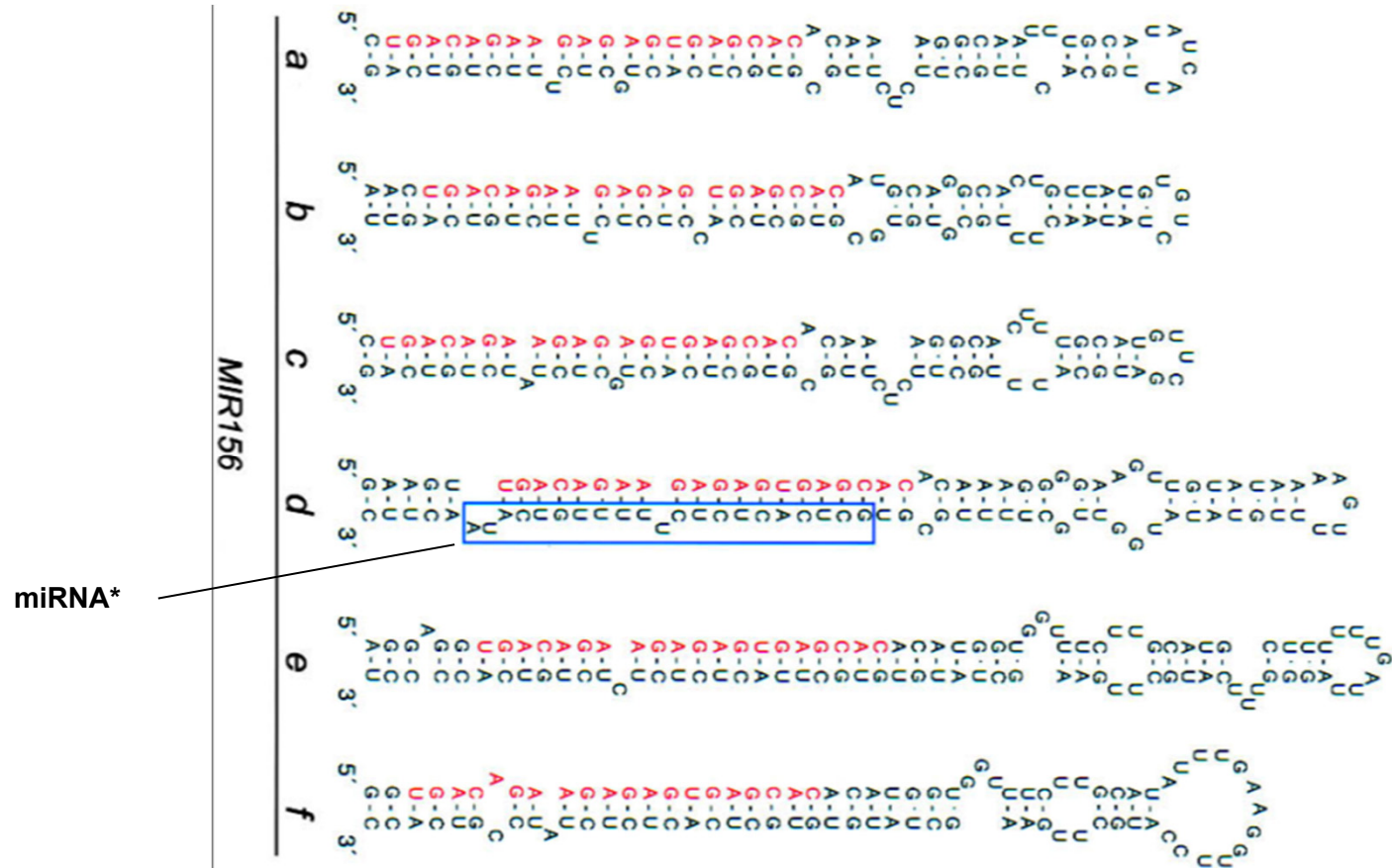


- Non-conserved *MIRNA* families usually occur as single genes
- Conserved ones have often duplicated to larger gene families

Fahlgren, et al., PLoS ONE, 2007

The *MIR156* gene family is highly conserved

Arabidopsis miR156 gene family



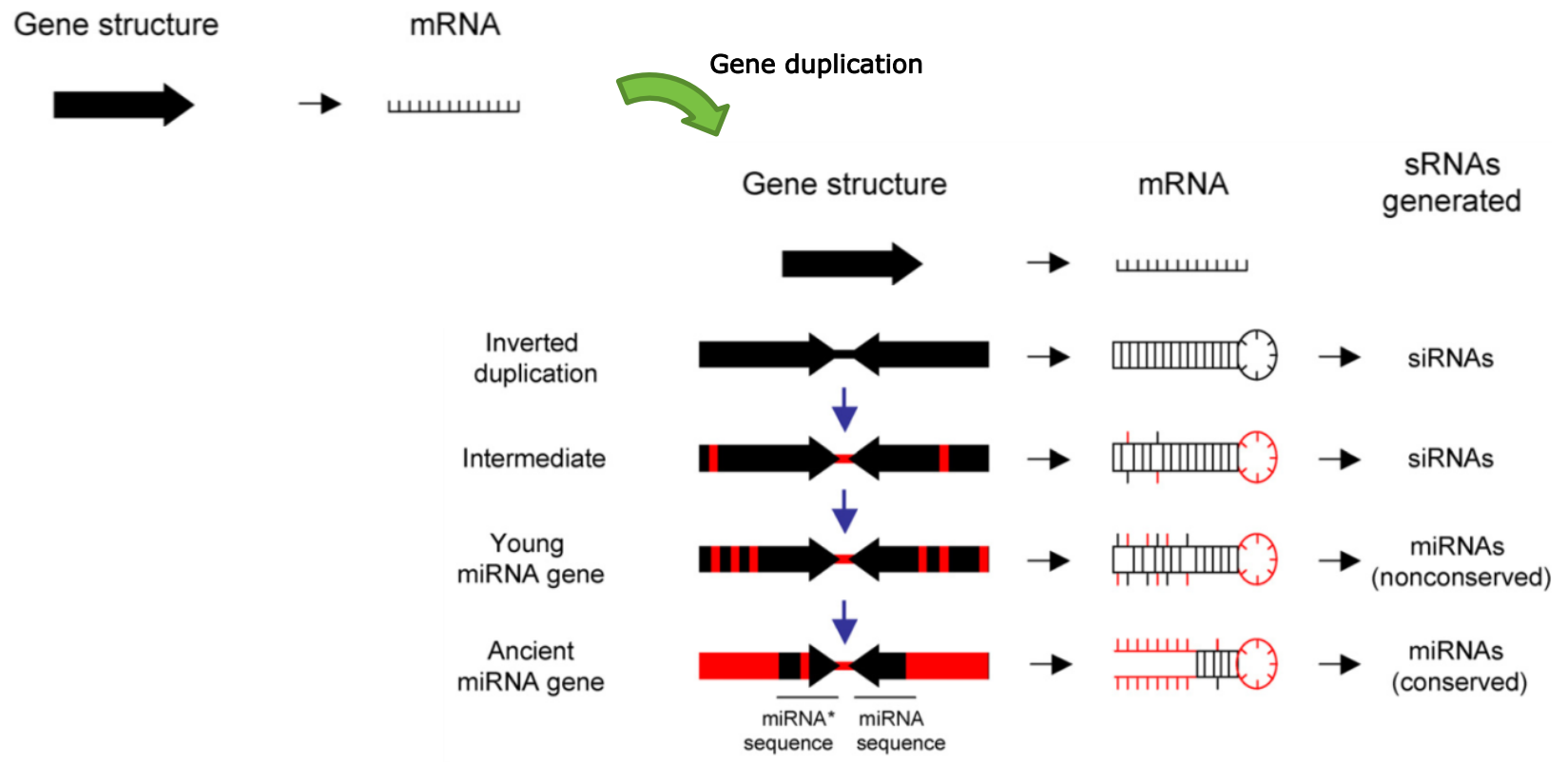
Reprinted from Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., and Bartel, D.P. (2002) MicroRNAs in plants. *Genes Dev.* 16: [1616–1626](#).

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

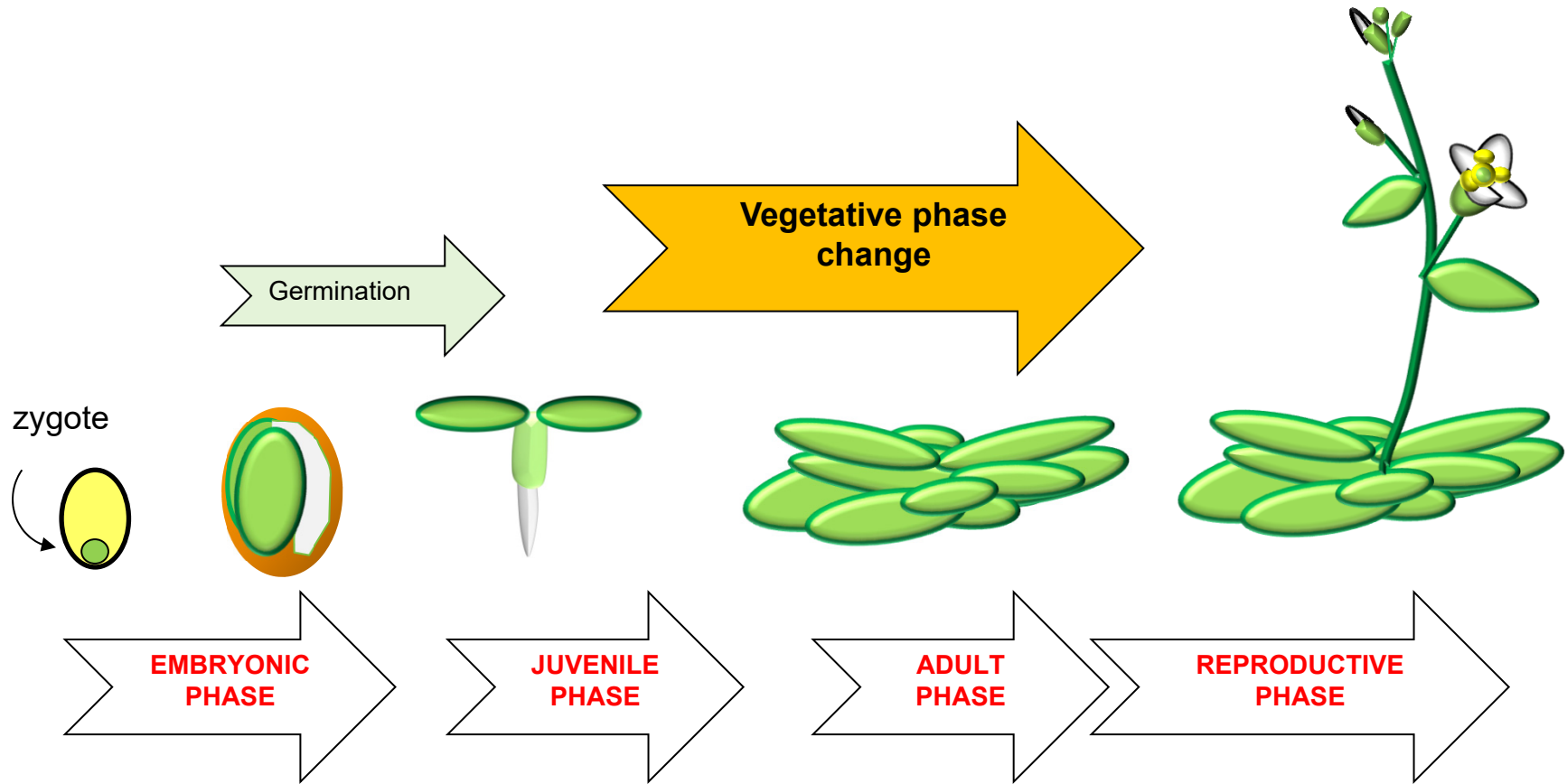
Adapted from Willmann, M.R., and Poethig, R.S. (2007) Conservation and evolution of miRNA regulatory programs in plant development. *Curr. Opin. Plant Biol.* 10: [503–511](#)..

Plant miRNAs are thought to be distantly related to their targets

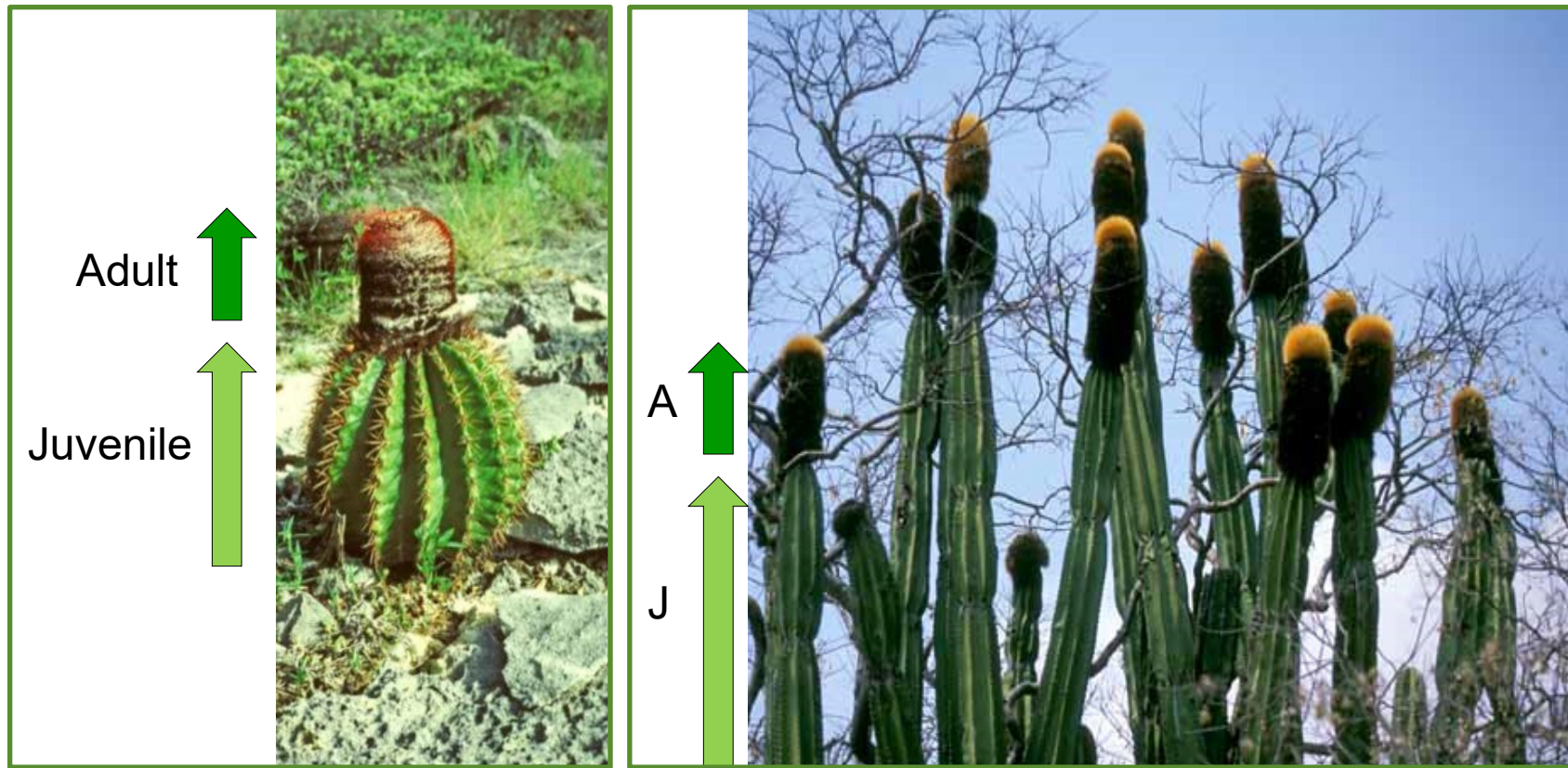


Reprinted from Willmann, M.R., and Poethig, R.S. (2007) Conservation and evolution of miRNA regulatory programs in plant development. *Curr. Opin. Plant Biol.* 10: [503–511](#) with permission from Elsevier.

miRNAs and vegetative phase change

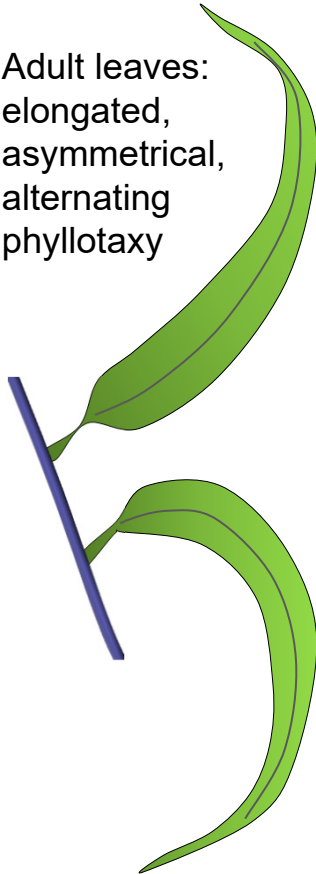


Vegetative phase change affects morphology and reproductive competence

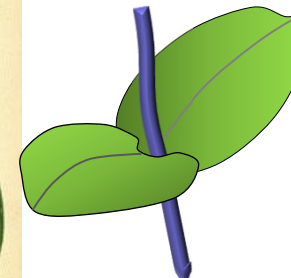


Phase change can affect leaf shape, phyllotaxy, and trichome patterns

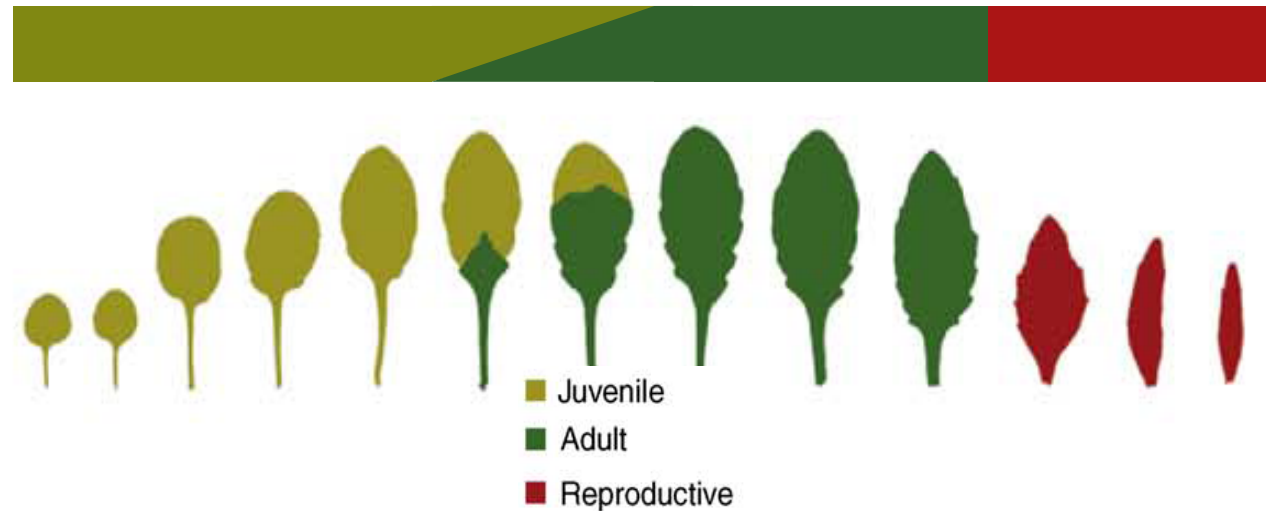
Adult leaves:
elongated,
asymmetrical,
alternating
phyllotaxy



Juvenile leaves:
rounded,
symmetrical,
opposite
phyllotaxy

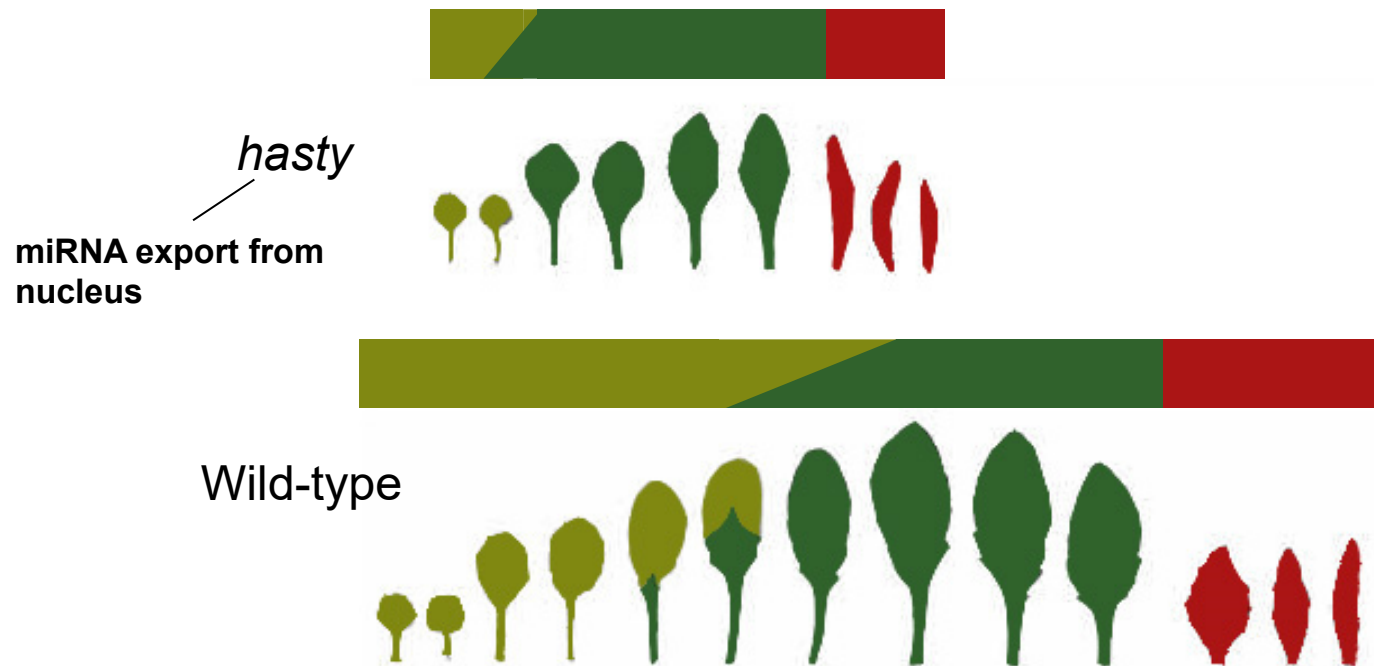


In *Arabidopsis*, phase change affects leaf shape and trichome patterning



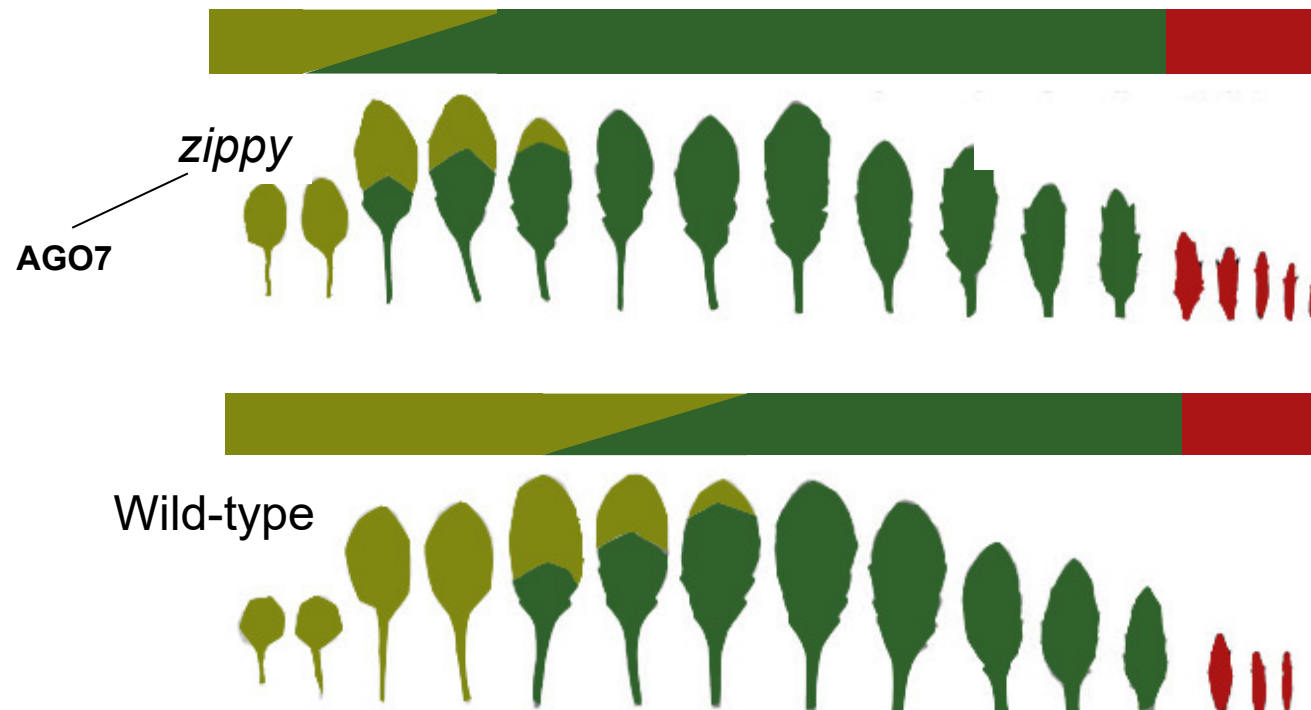
Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. *Curr. Opin. Genet. Devel.* 19: [374-378](#), with permission from Elsevier.

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



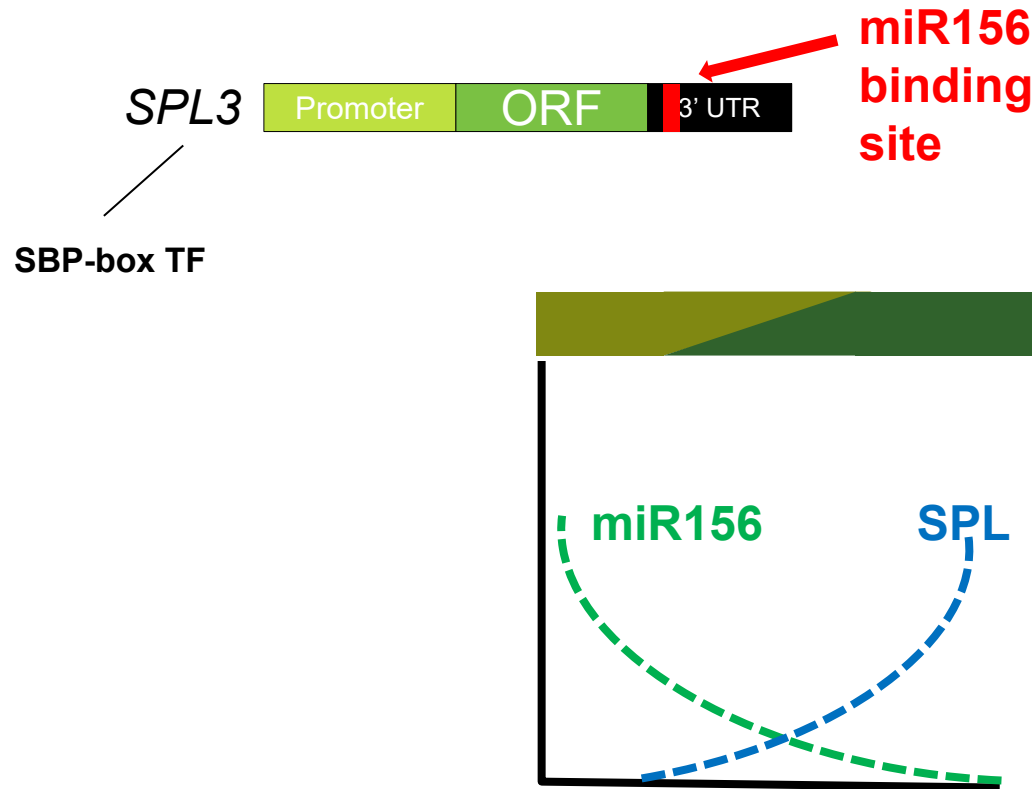
Reprinted from Hunter, C., Sun, H., and Poethig, R.S. (2003) The *Arabidopsis* heterochronic gene *ZIPPY* is an *ARGONAUTE* family member. *Curr. Biol.* 13: [1734–1739](#), with permission from Elsevier.

miR156 overexpression prolongs juvenile phase in *Arabidopsis*

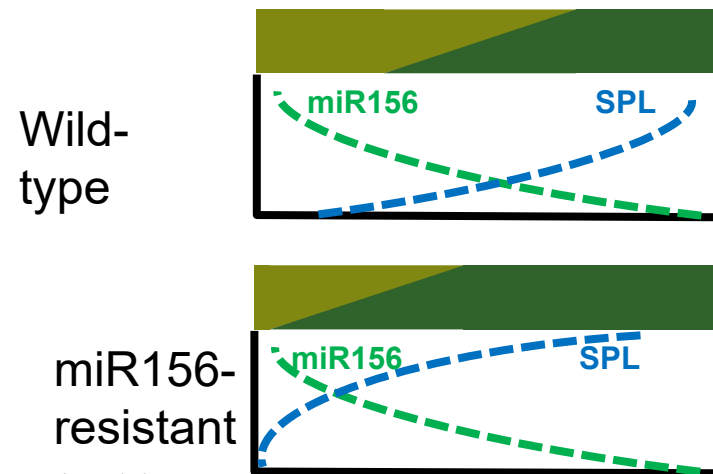
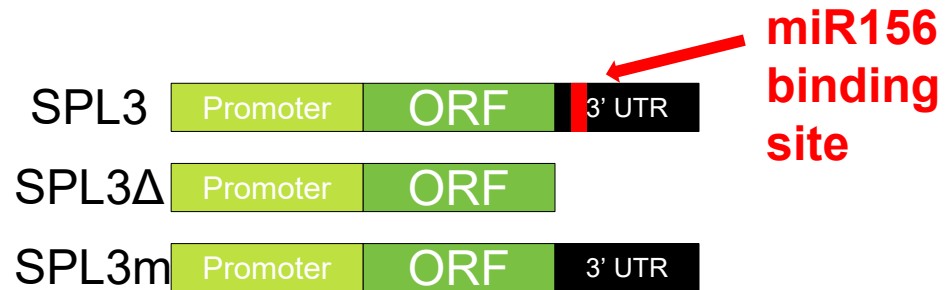
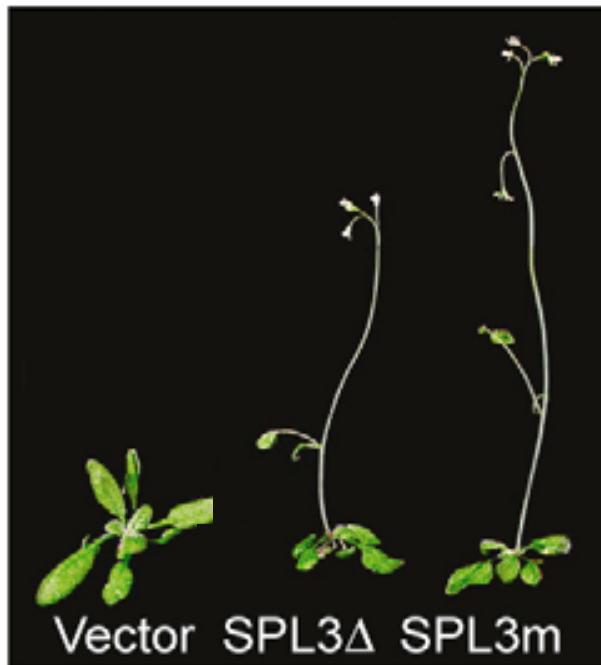


Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. *Curr. Opin. Genet. Devel.* 19: [374-378](#), with permission from Elsevier.

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

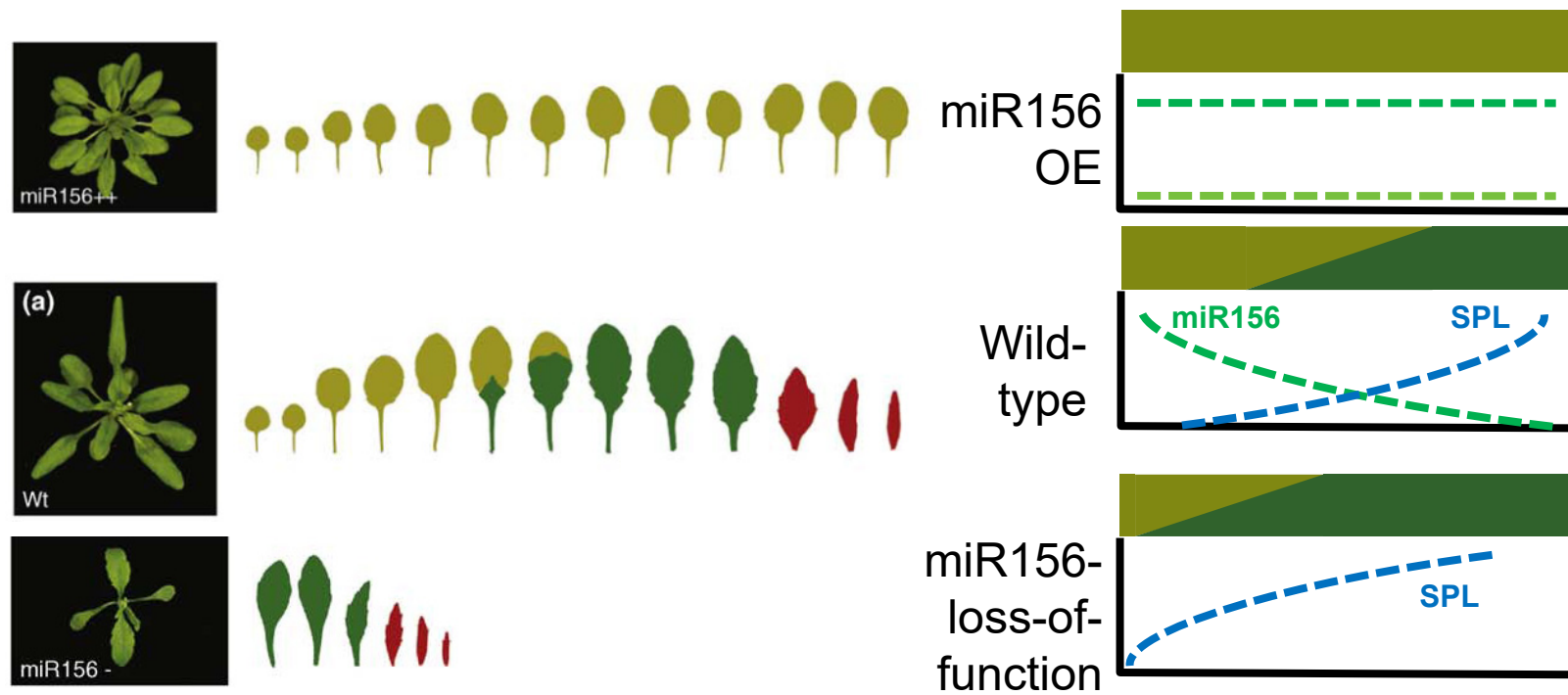


miR156-resistant *SPL* promotes precocious phase change



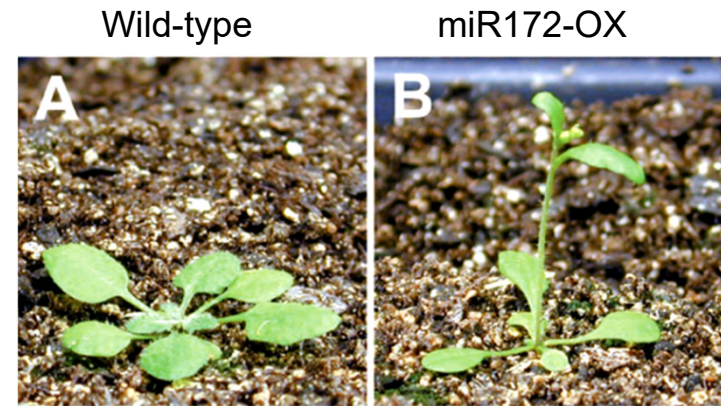
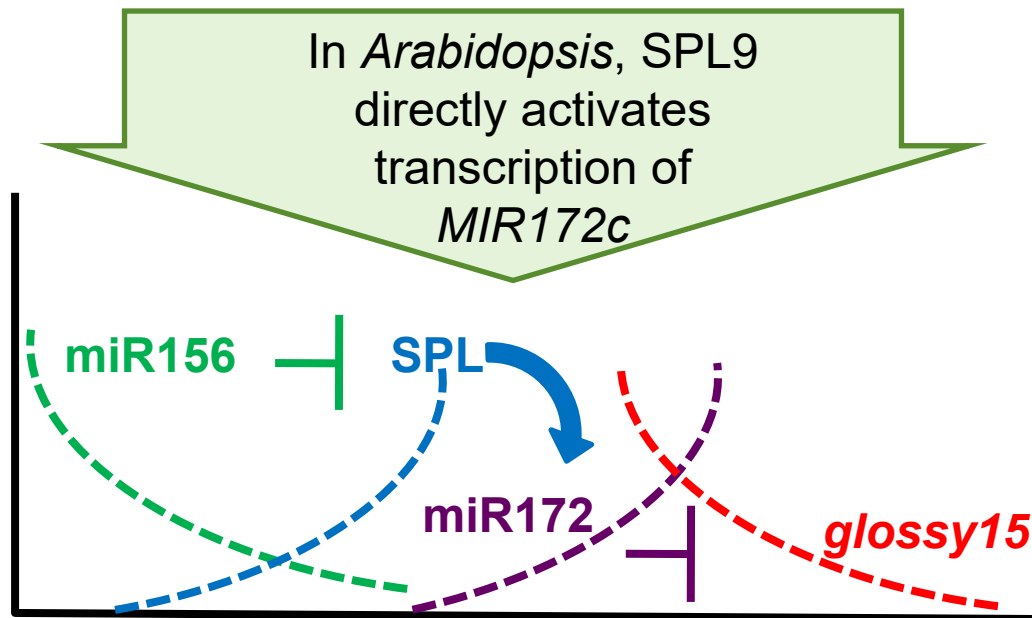
Reproduced with permission from Wu, G., and Poethig, R.S. (2006) Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. *Development* **133**: [3539-3547](#).

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.

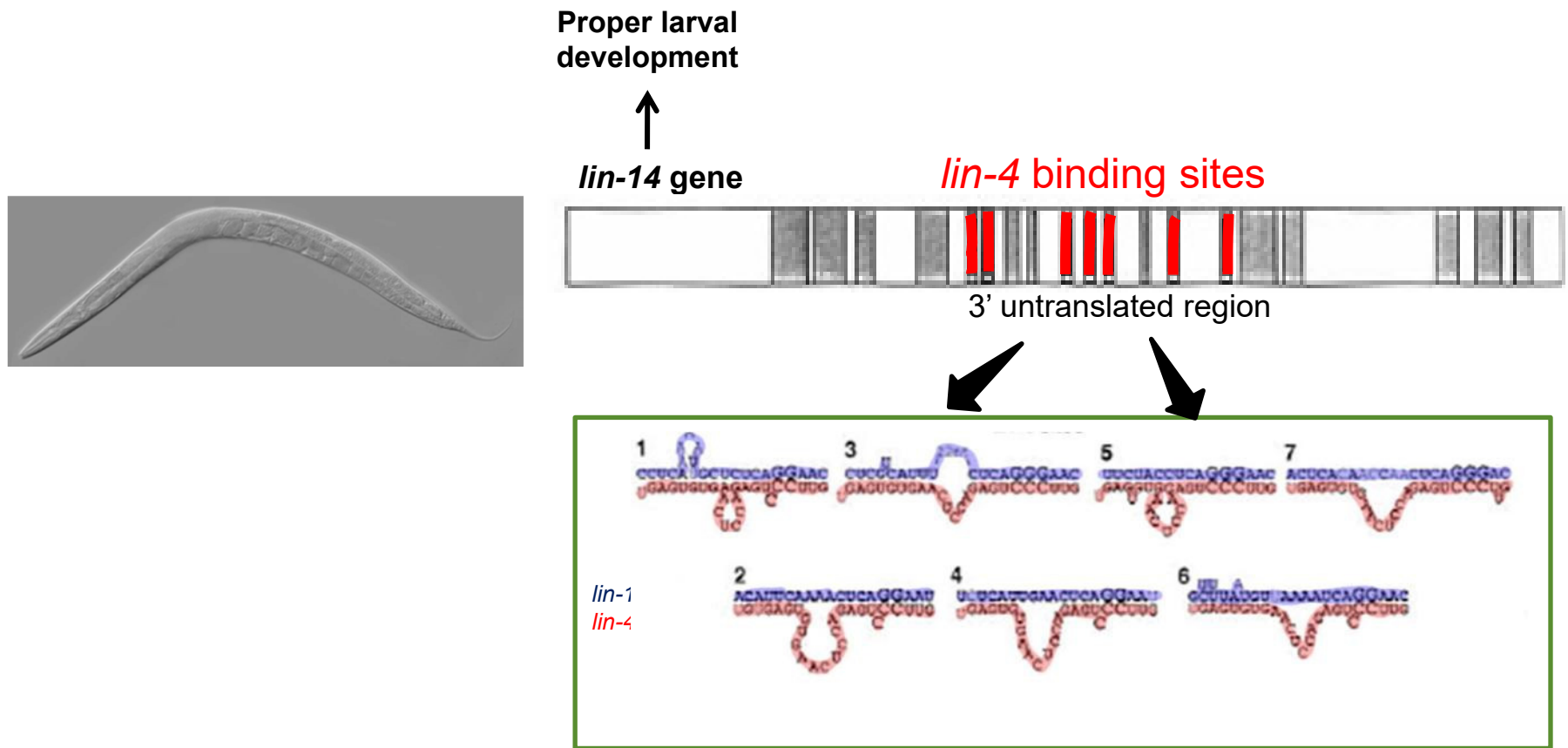
Phase change involves a temporal cascade of miRNAs and transcription factors



Arabidopsis plants overexpressing miR172 flower early.

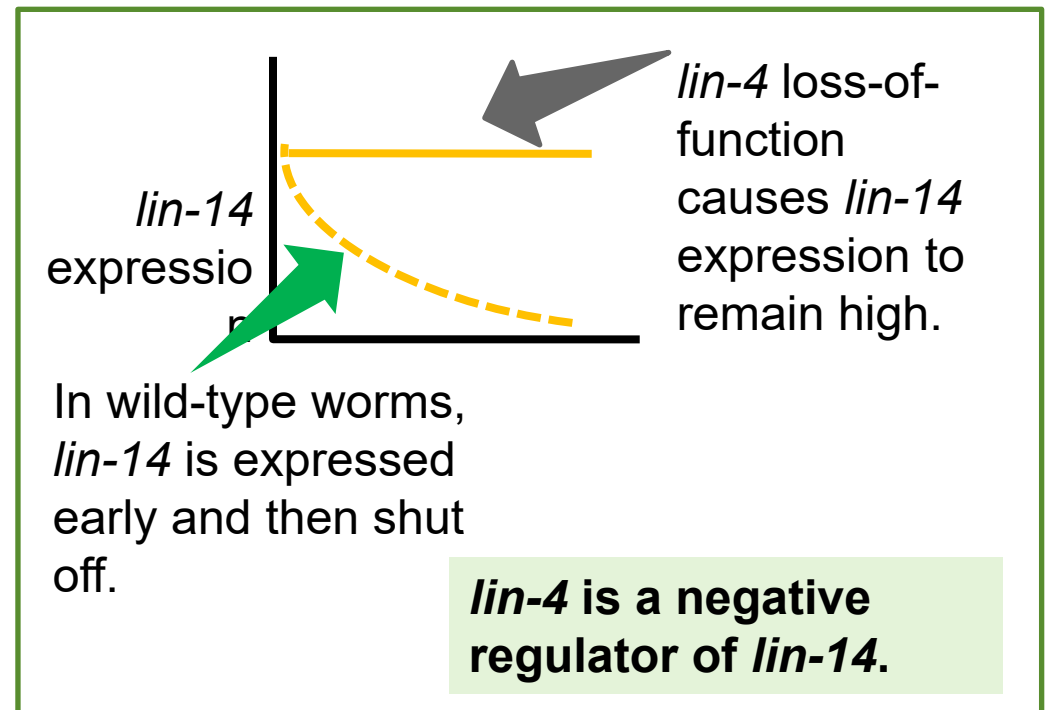
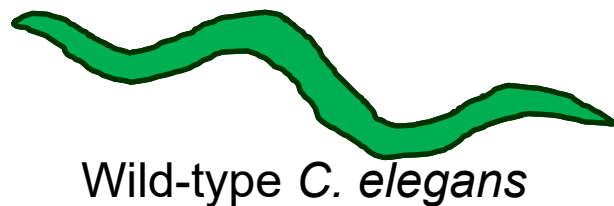
Aukerman, M.J., and Sakai, H. (2003) Regulation of flowering time and floral organ identity by a microRNA and its *APETALA2*-Like target genes *Plant Cell* 15: [2730-2741](#).

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.

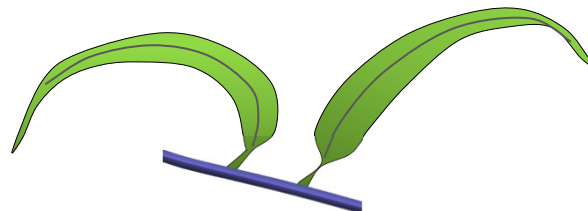
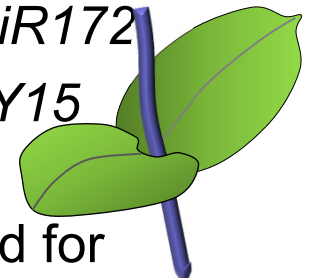
Downregulation of *lin-14* by *lin-4* is necessary for normal development



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

- 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 **posttranscriptional** and **posttranslational**
- **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 posttranscriptional regulations of gene expression
- **RNA interference** is **natural** and powerful **mechanism** allowing regulation of gene expression at both **transcriptional** and **posttranscriptional** 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

Discussion