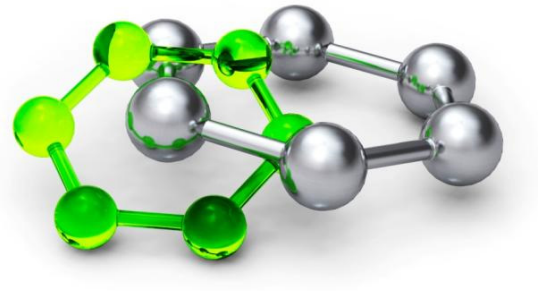
# Bi8940 Developmental Biology
# Lesson 10

## Regulation of Gene Expression during Development

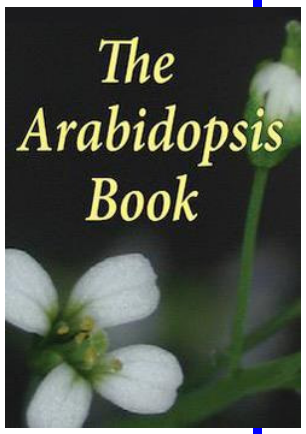
Jan Hejátko

**Laboratory of Molecular Plant Physiology**,
Department of Functional Genomics and Proteomics,
and
**Functional Genomics and Proteomics of Plants**
CEITEC
Masaryk University,
Brno, Czech Republic
hejatko@sci.muni.cz, www.ceitec.eu

MUNI
SCI



# 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

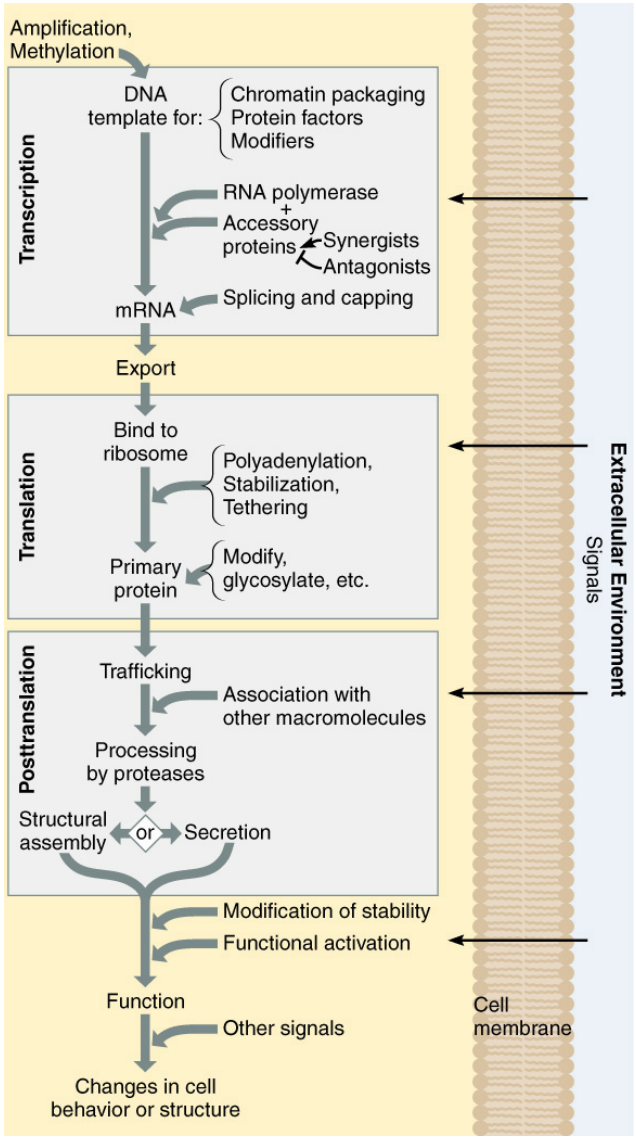
## Regulation of Gene Expression during Development

- 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

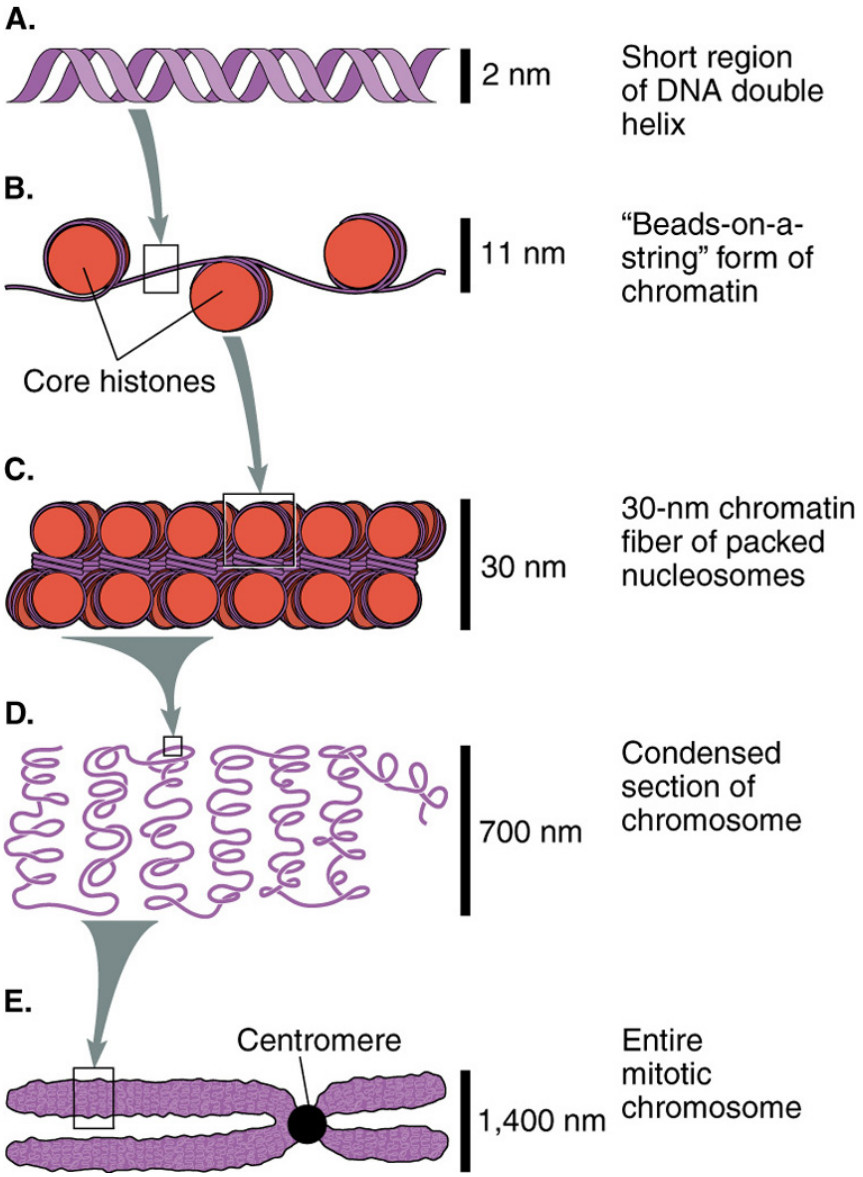
- Overview of levels of gene expression regulation


Amplification,
Methylation
Transcription
DNA
template for:
Chromatin packaging
Protein factors
Modifiers
RNA polymerase
+
Accessory
proteins
Synergists
Antagonists
mRNA
Splicing and capping
Export
Translation
Bind to
ribosome
Polyadenylation,
Stabilization,
Tethering
Primary
protein
Modify,
glycosylate, etc.
Posttranslation
Trafficking
Association with
other macromolecules
Processing
by proteases
Structural
assembly
or
Secretion
Modification of stability
Functional activation
Function
Other signals
Changes in cell
behavior or structure
Extracellular Environment
Signals
Cell
membrane

# 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

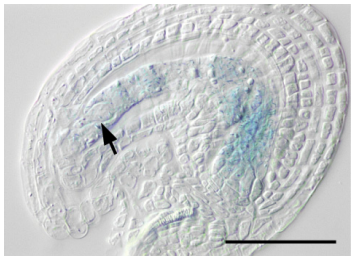



**Regulation by histone acetyl transferases or histone deacteylases**

# DNA methylation in animals vs. in plants

methylation status

**CpG**



methylation status

**CpG or CpNpG**

**CpNpNp**

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

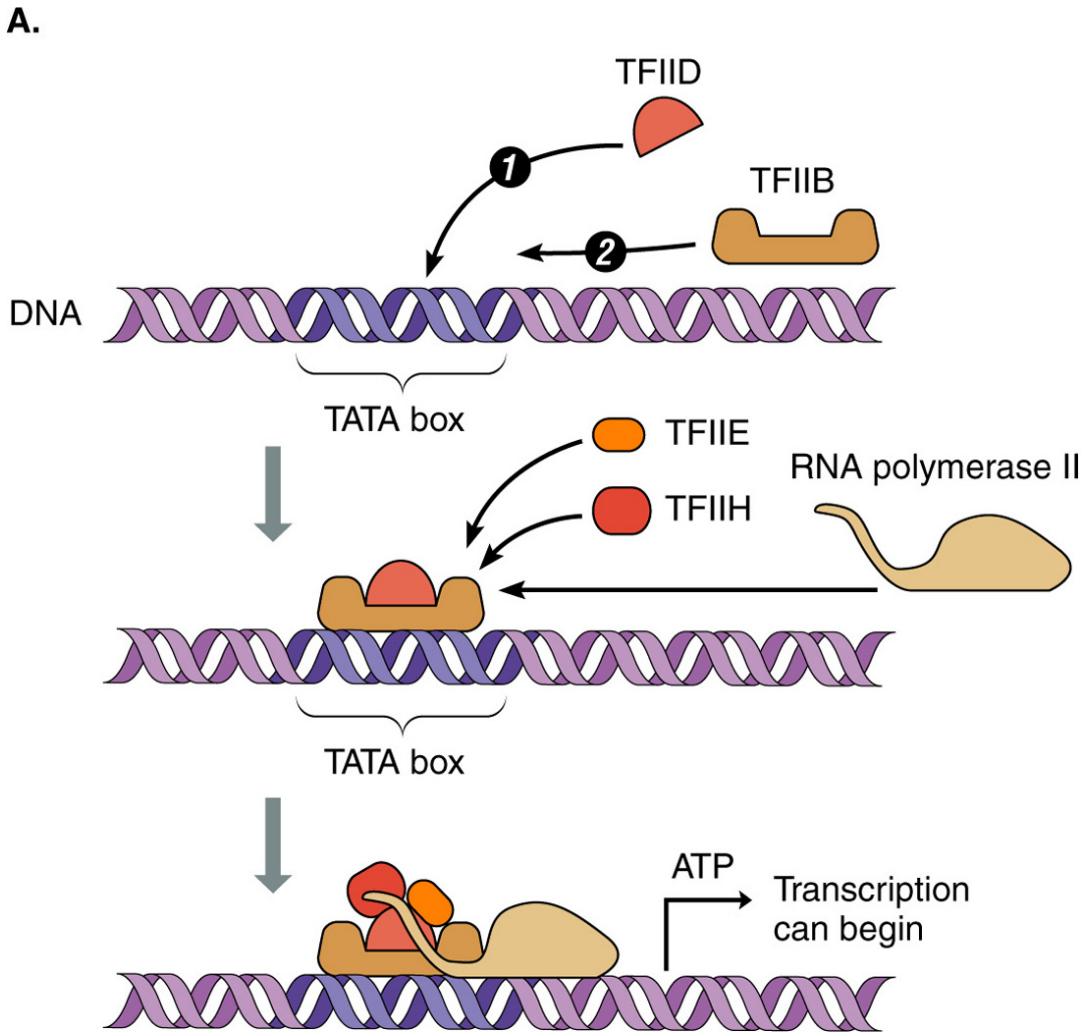
→ **Mechanism of transcriptional regulation by DNA methylation mostly unknown**

# Outline of Lesson 10

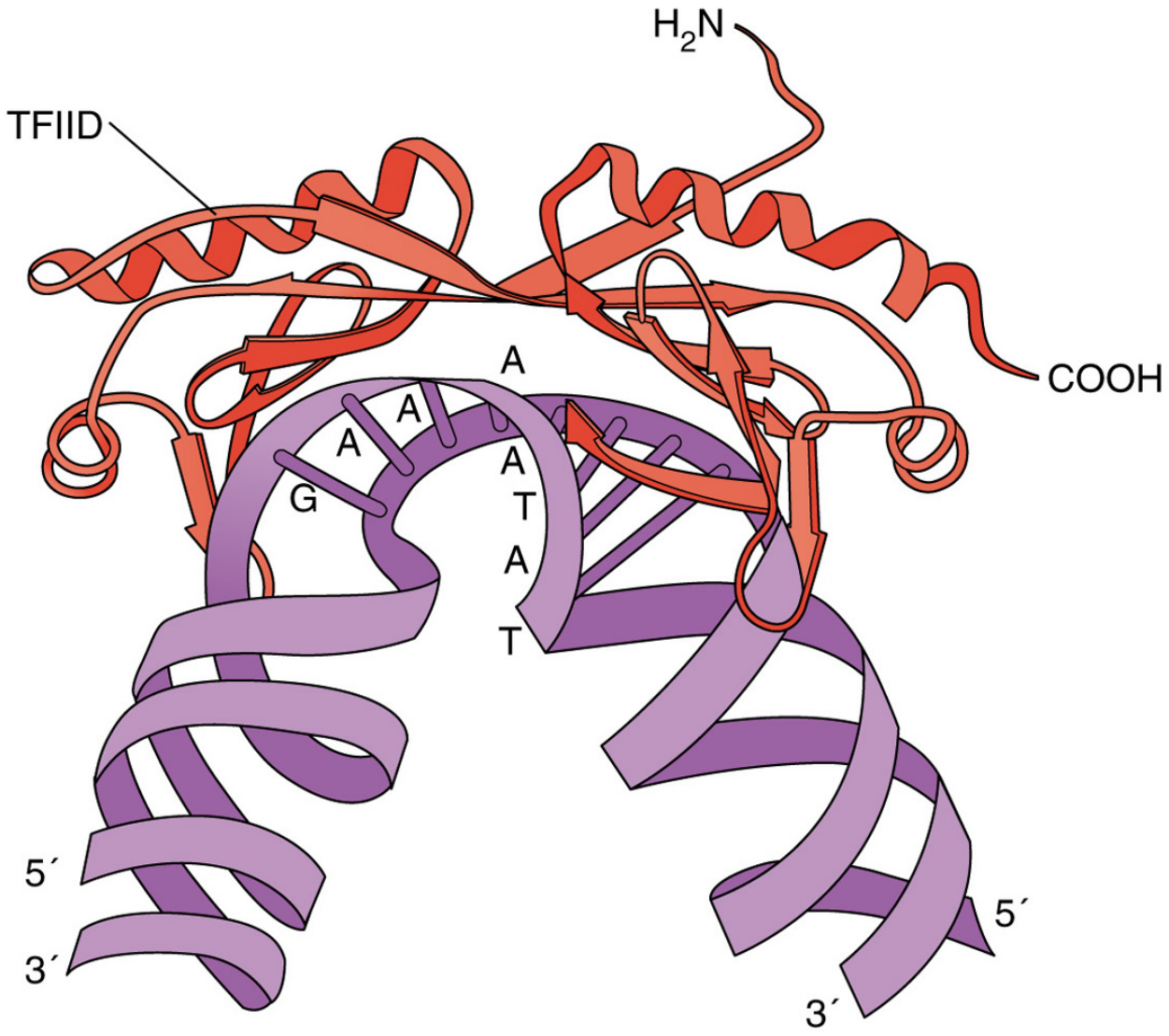
## Regulation of Gene Expression during Development

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

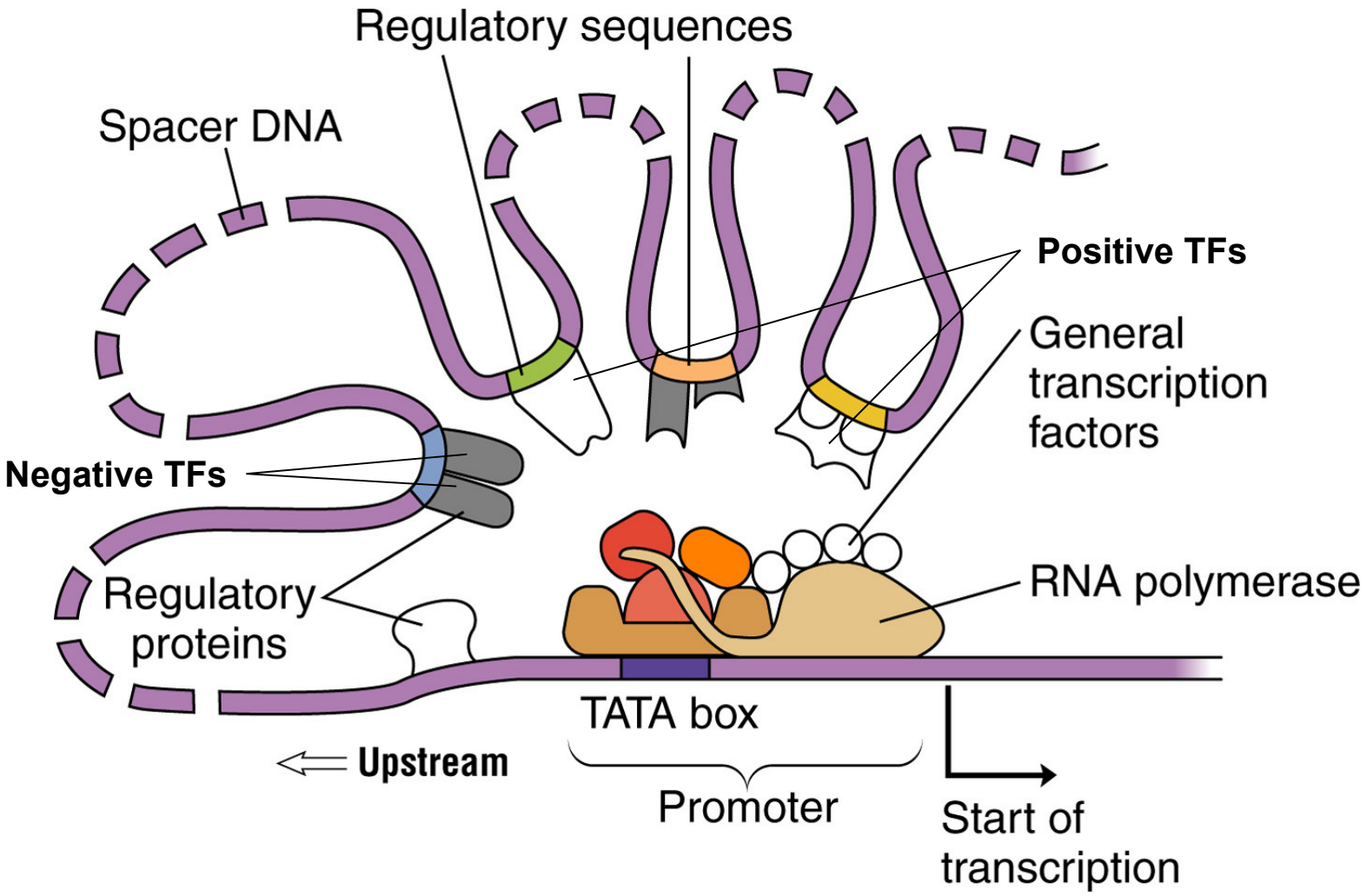
# Formation of transcription initiation complex



**B.**



# Formation of transcription initiation complex



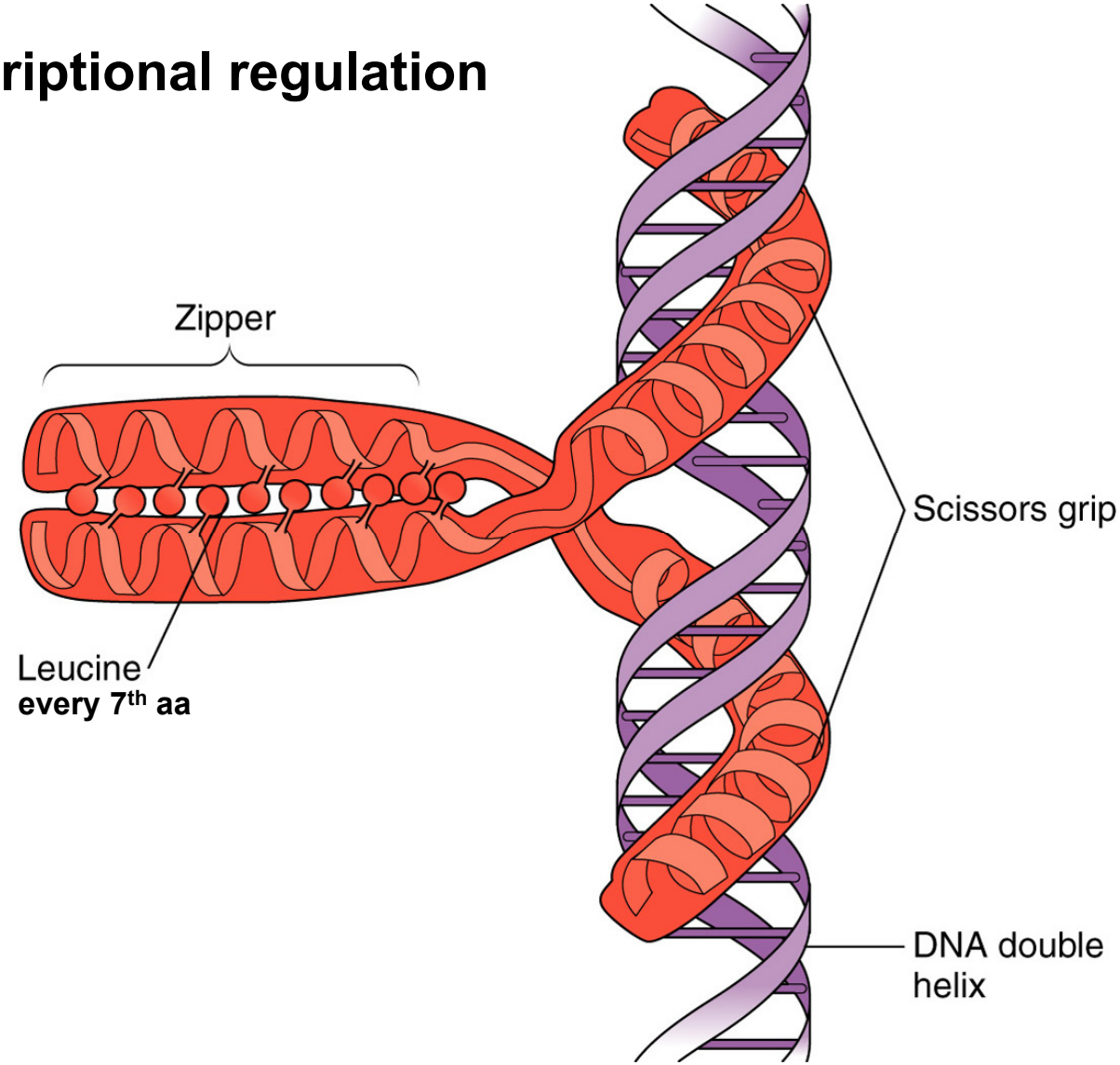
# Mechanism of transcriptional regulation by TAFs

**Signal recognition**

↓

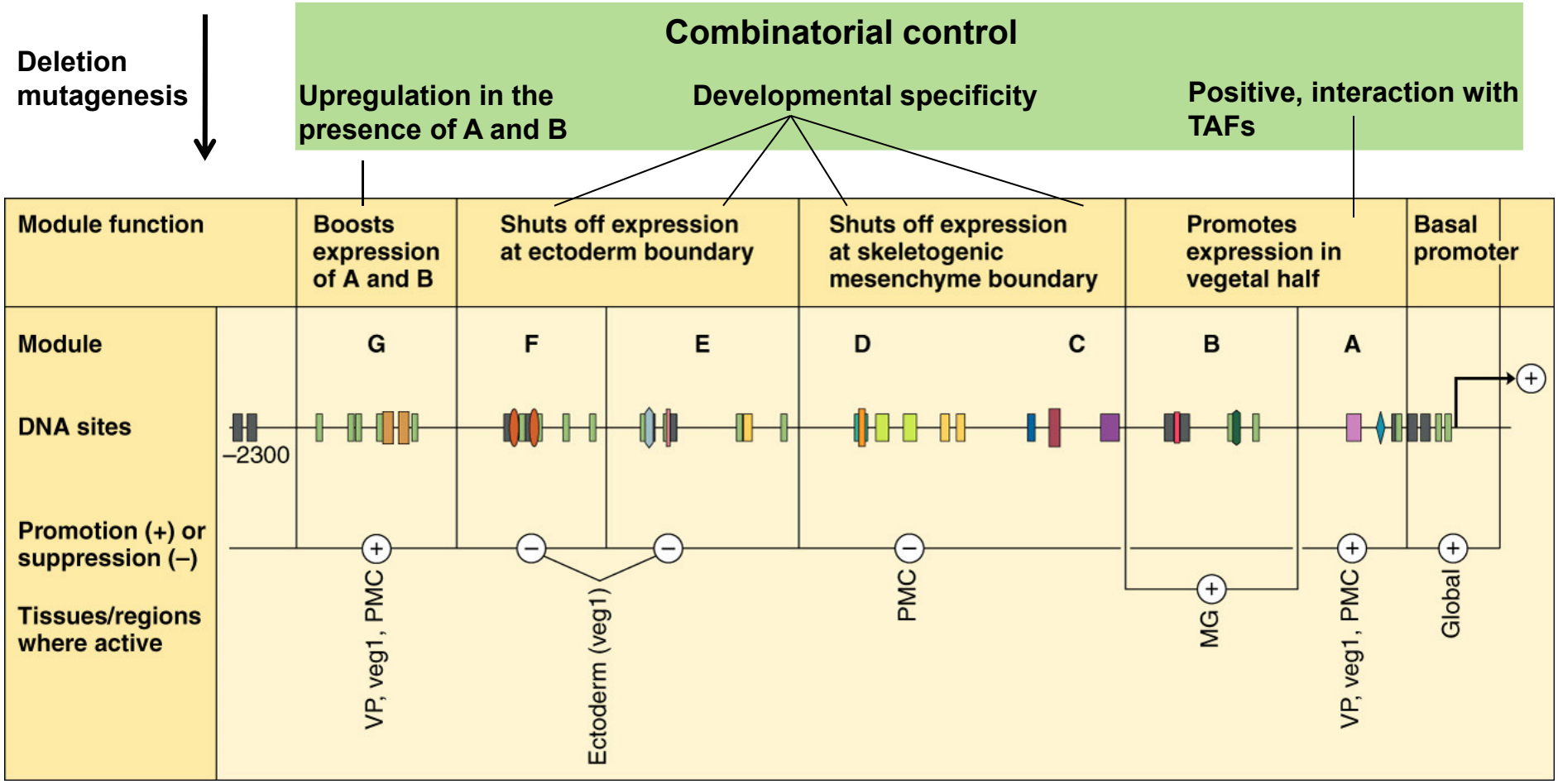
**Dimerization** →

**DNA binding and transcription activation**



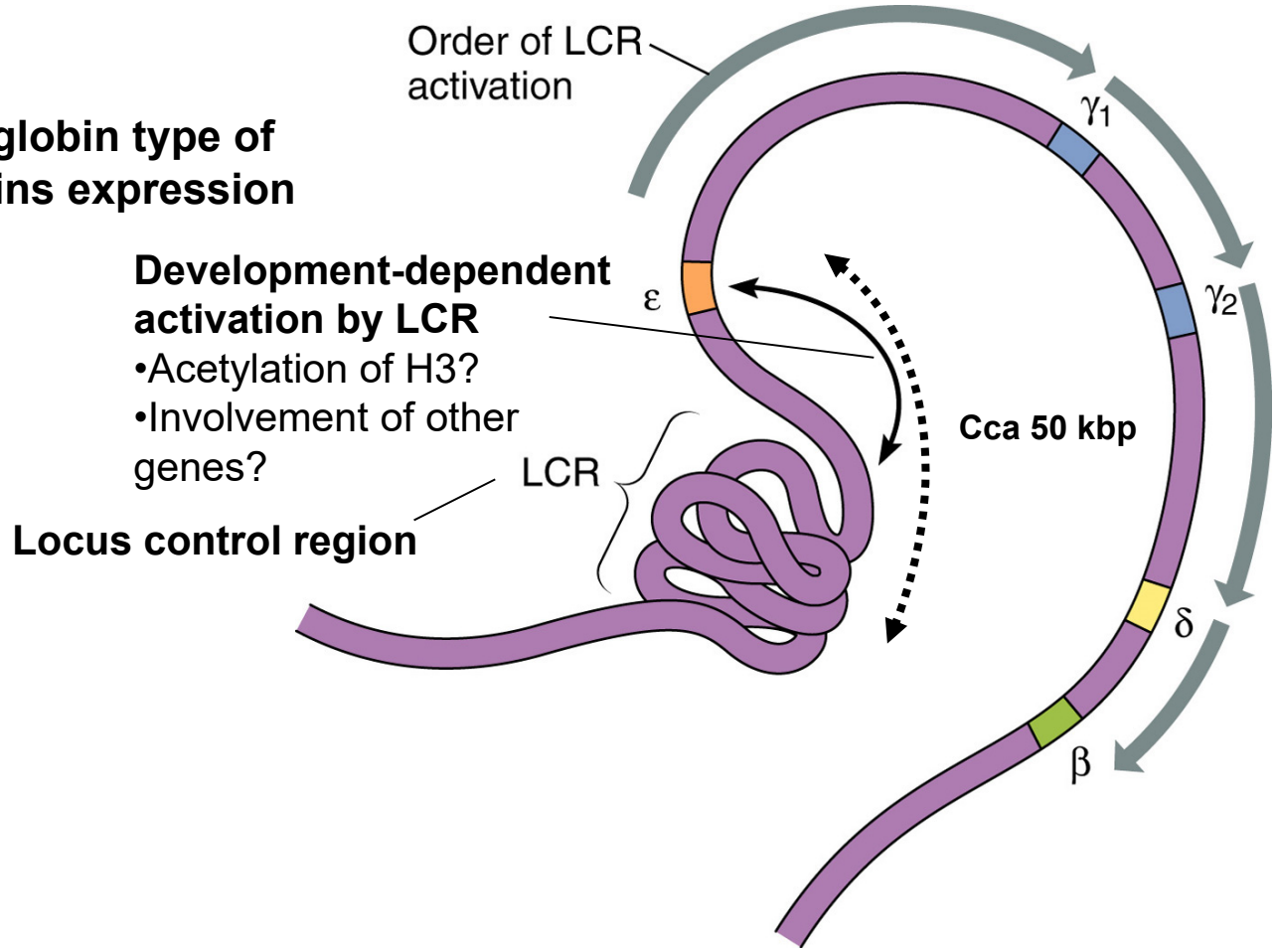
# “Microprocessor-like” acting promoters

***ProENDO16:REPORTER***



# “Microprocessor-like” acting promoters

**Regulation of β-globin type of hemoglobin chains expression**



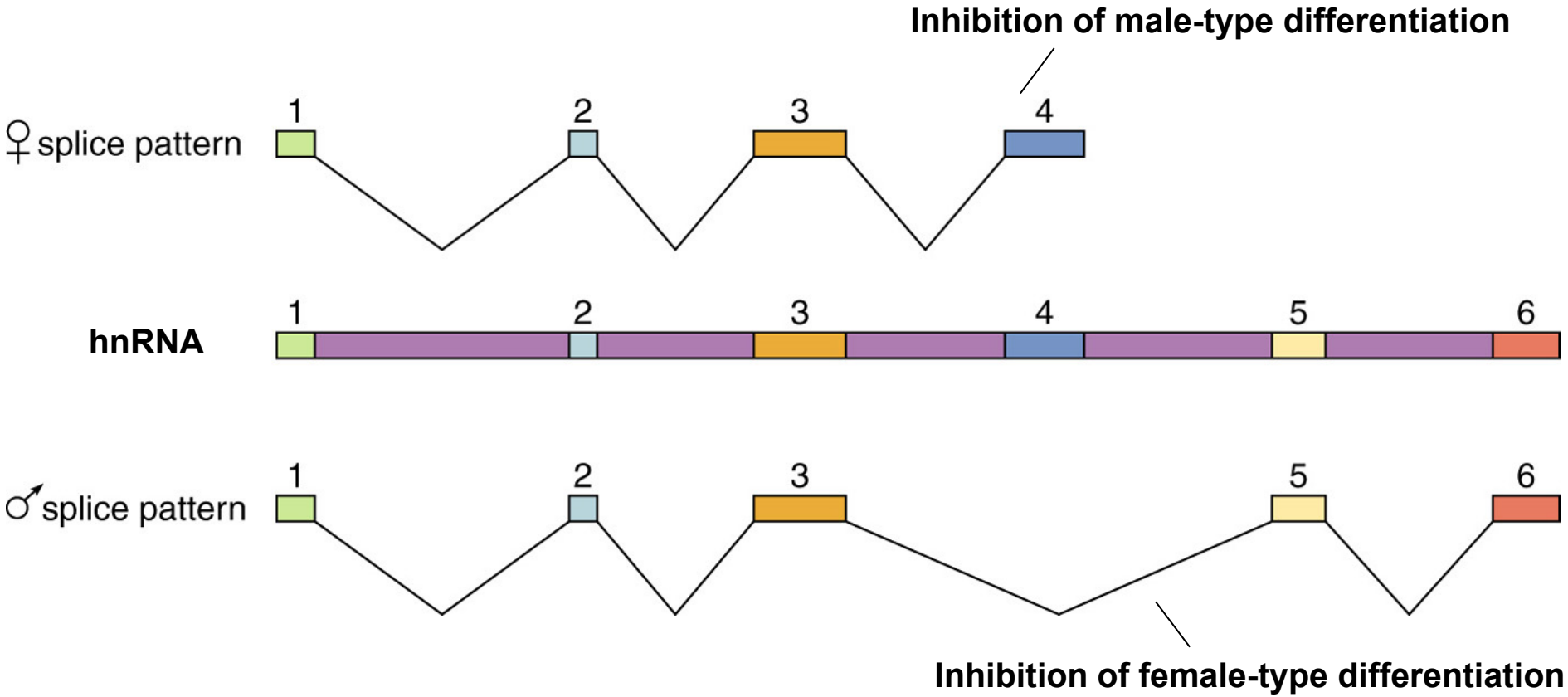
# Outline of Lesson 10

## 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

# Splicing of hnRNA

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

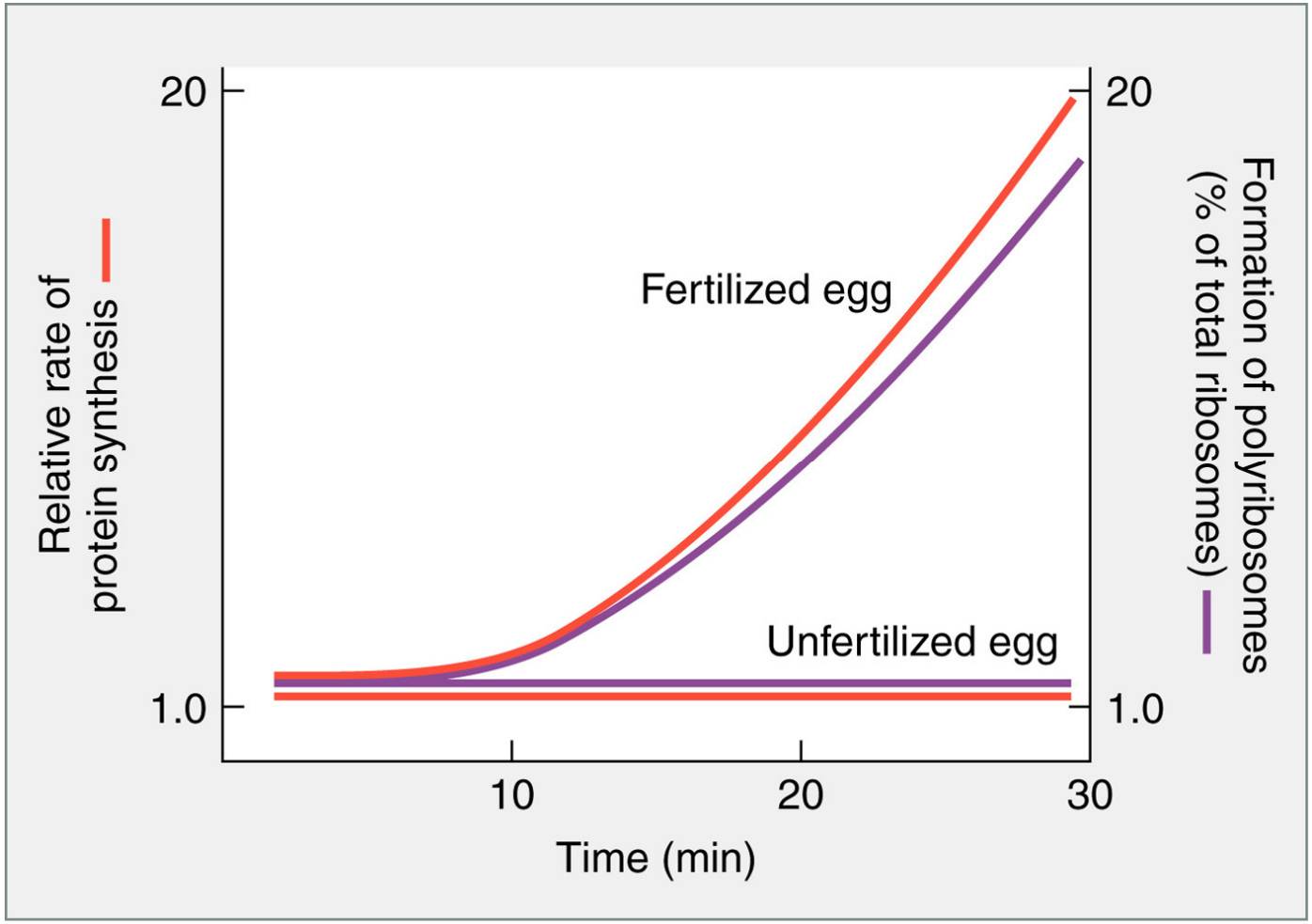


# Outline of Lesson 10

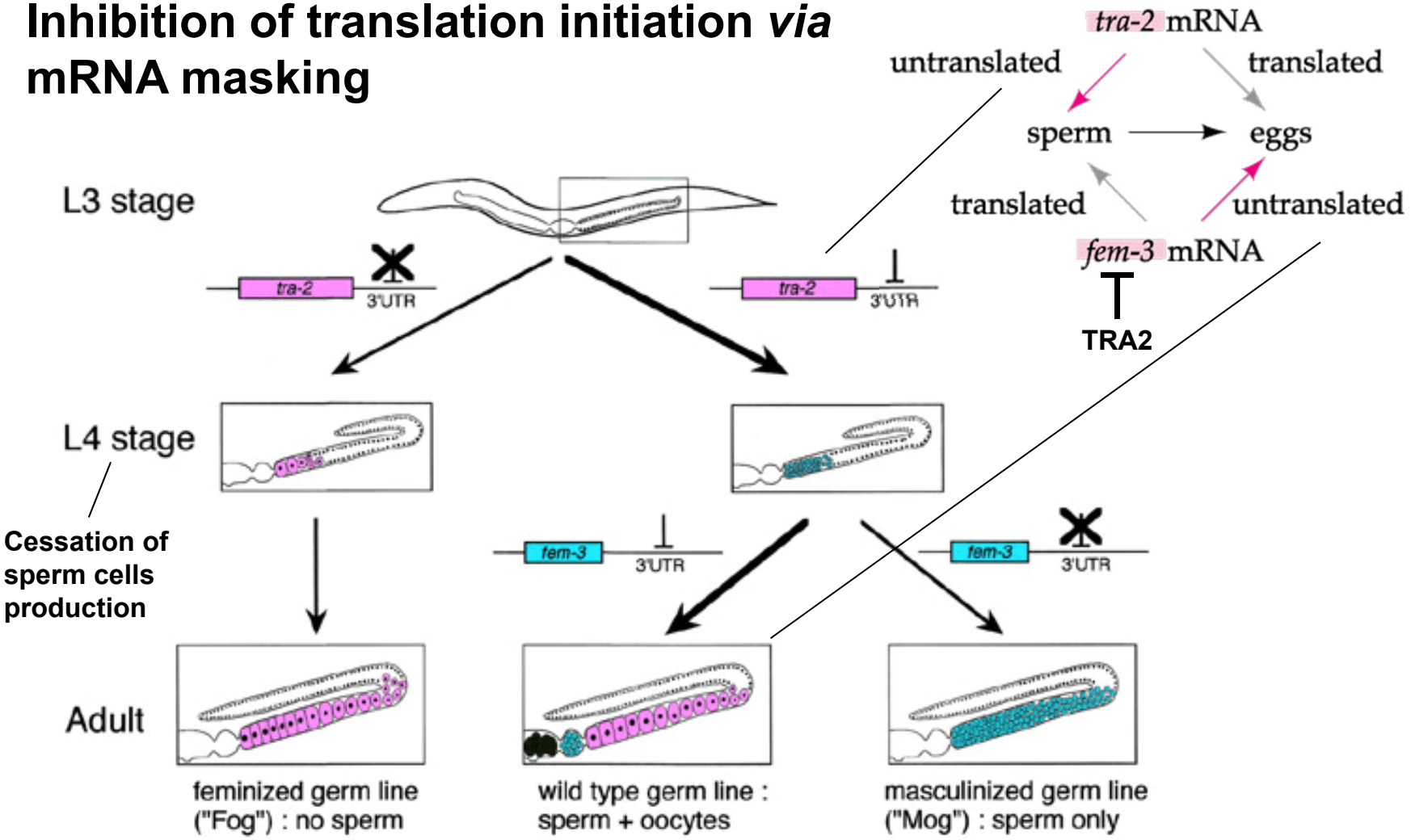
## Regulation of Gene Expression during Development

- 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

# Translation initiation after egg fertilization



# Inhibition of translation initiation *via* mRNA masking




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

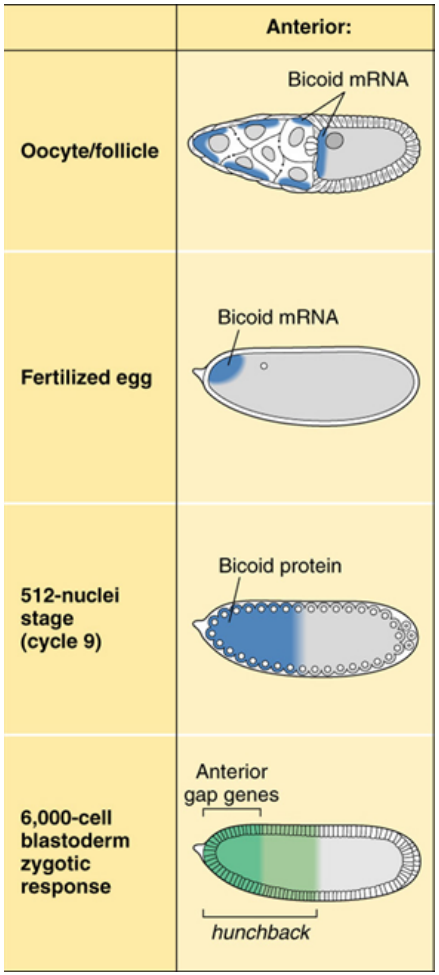
Developmental Biology 8e Online (http://8e.devbio.com/)

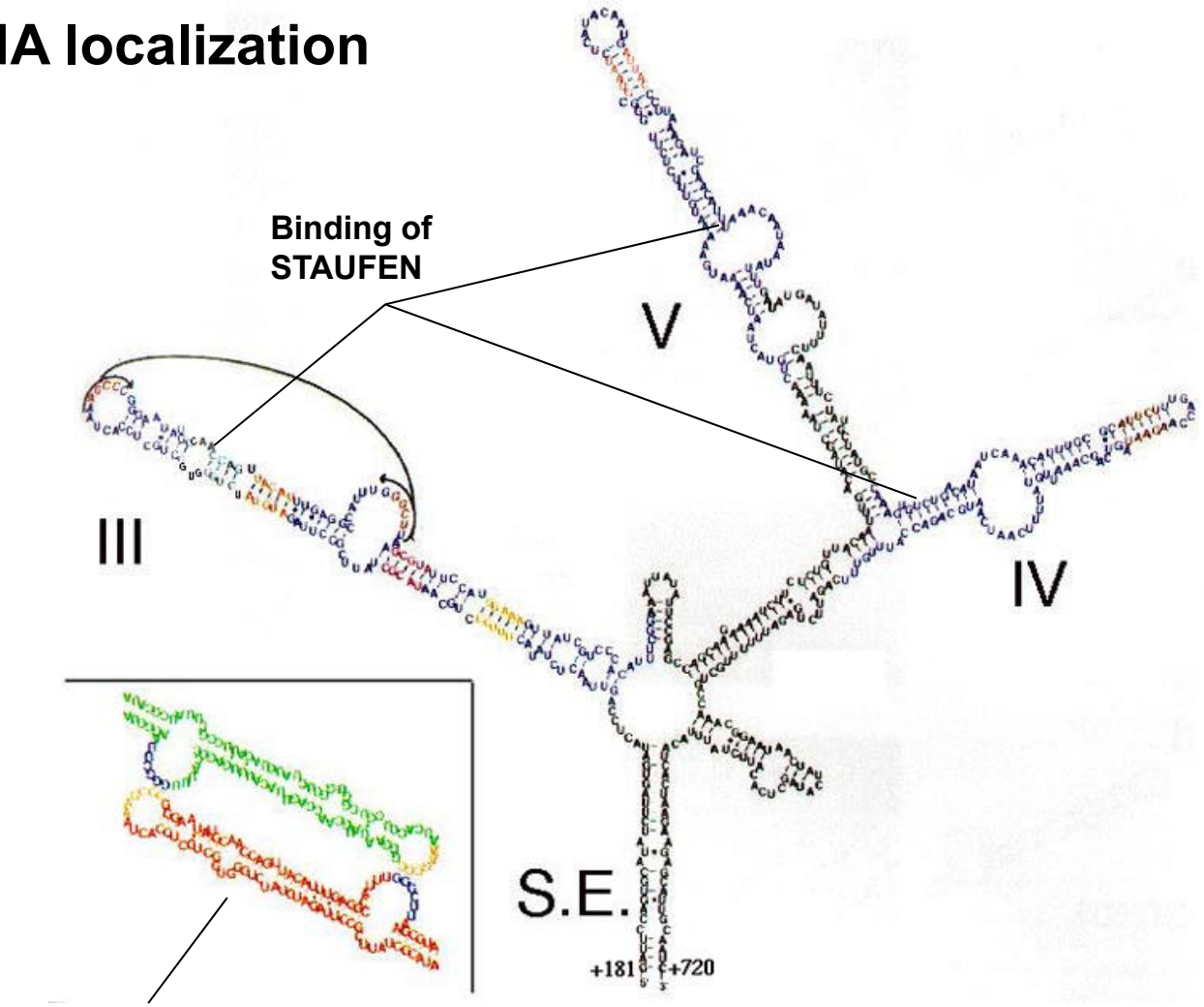
# Outline of Lesson 10

## 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

# Regulation via mRNA localization

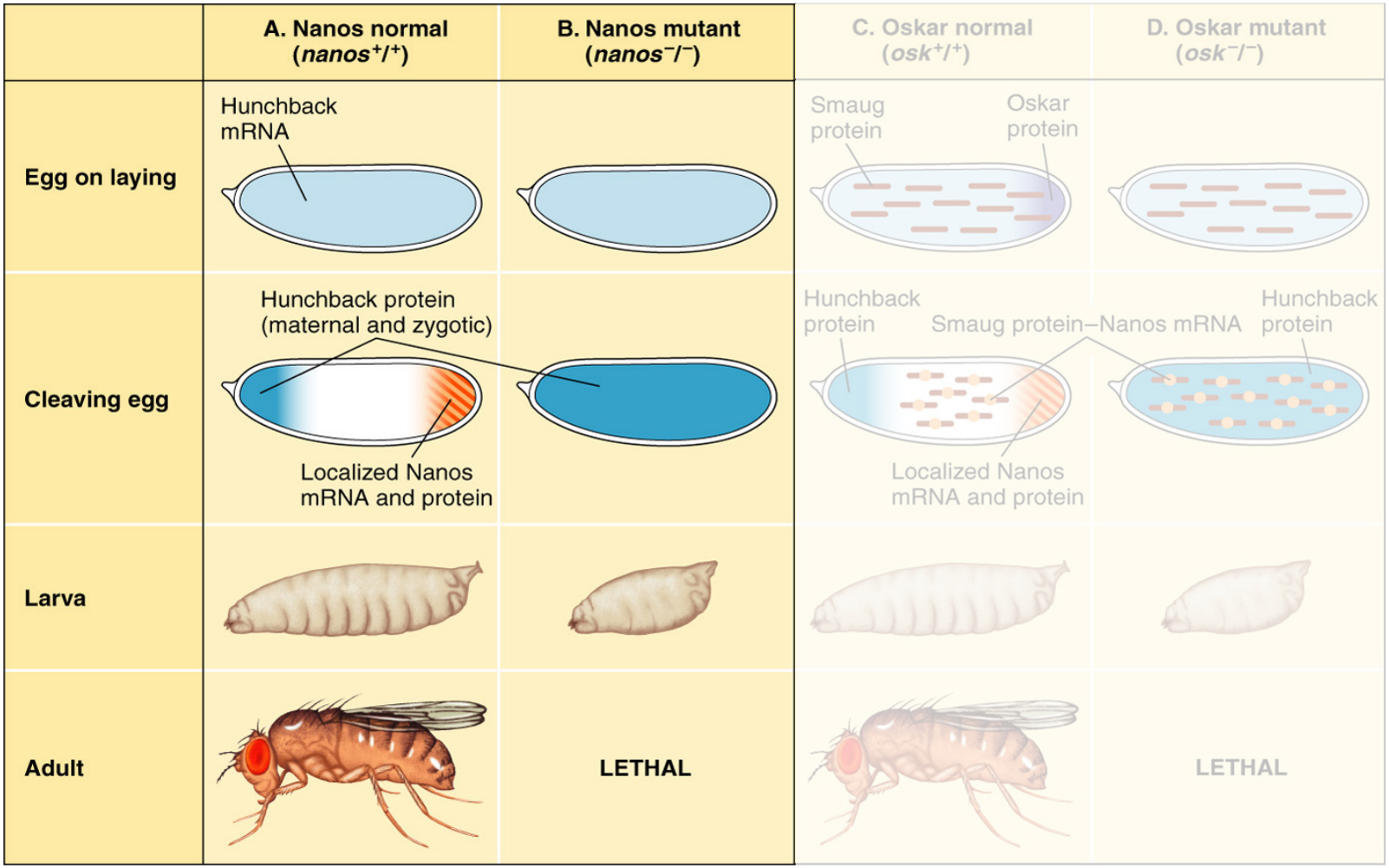


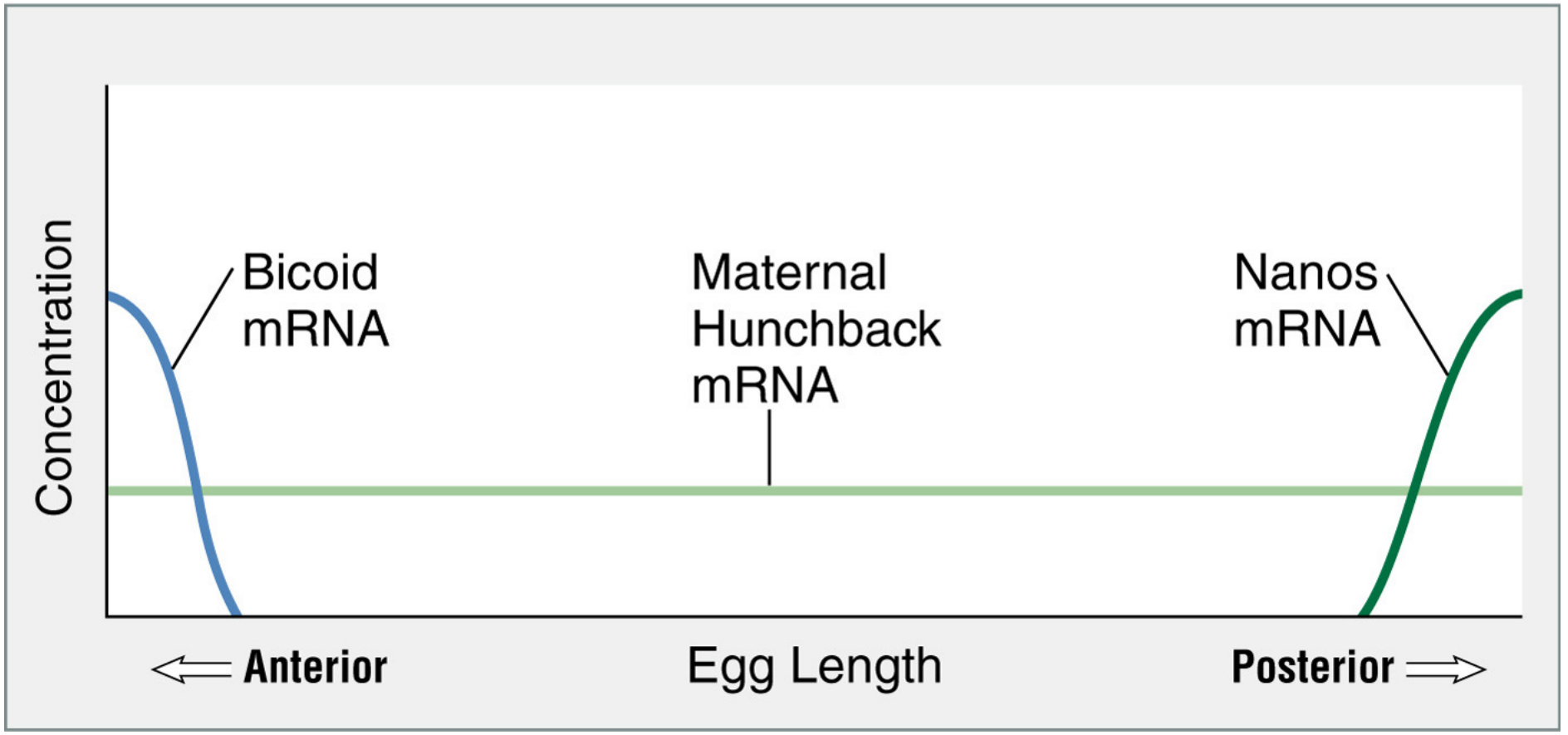

**Dimer structure of two *BICOID* mRNAs recognized by STAUFEN**

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

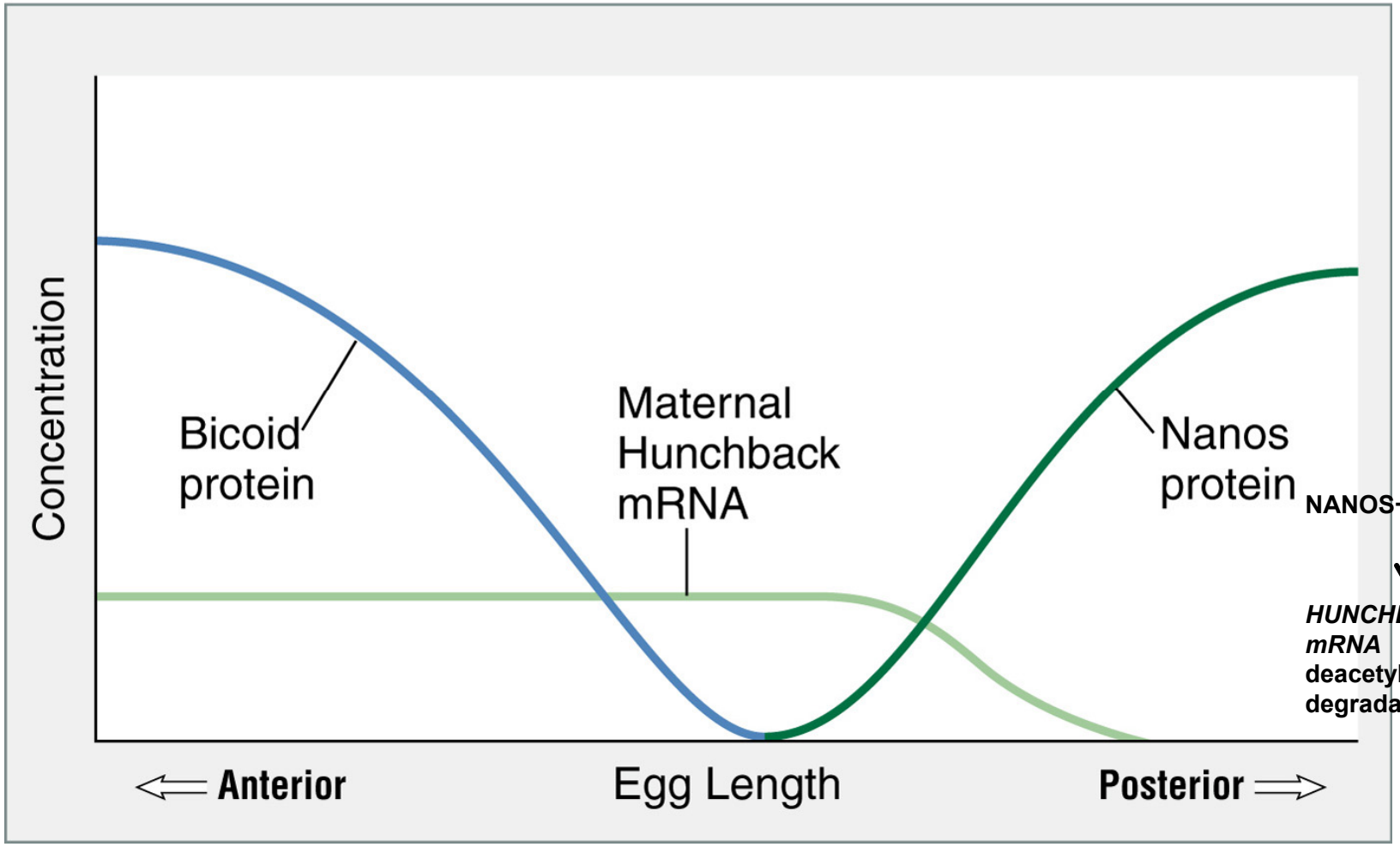
# Regulation via mRNA localization



**A.**


Concentration
Bicoid mRNA
Maternal Hunchback mRNA
Nanos mRNA
Anterior
Egg Length
Posterior

**B.**

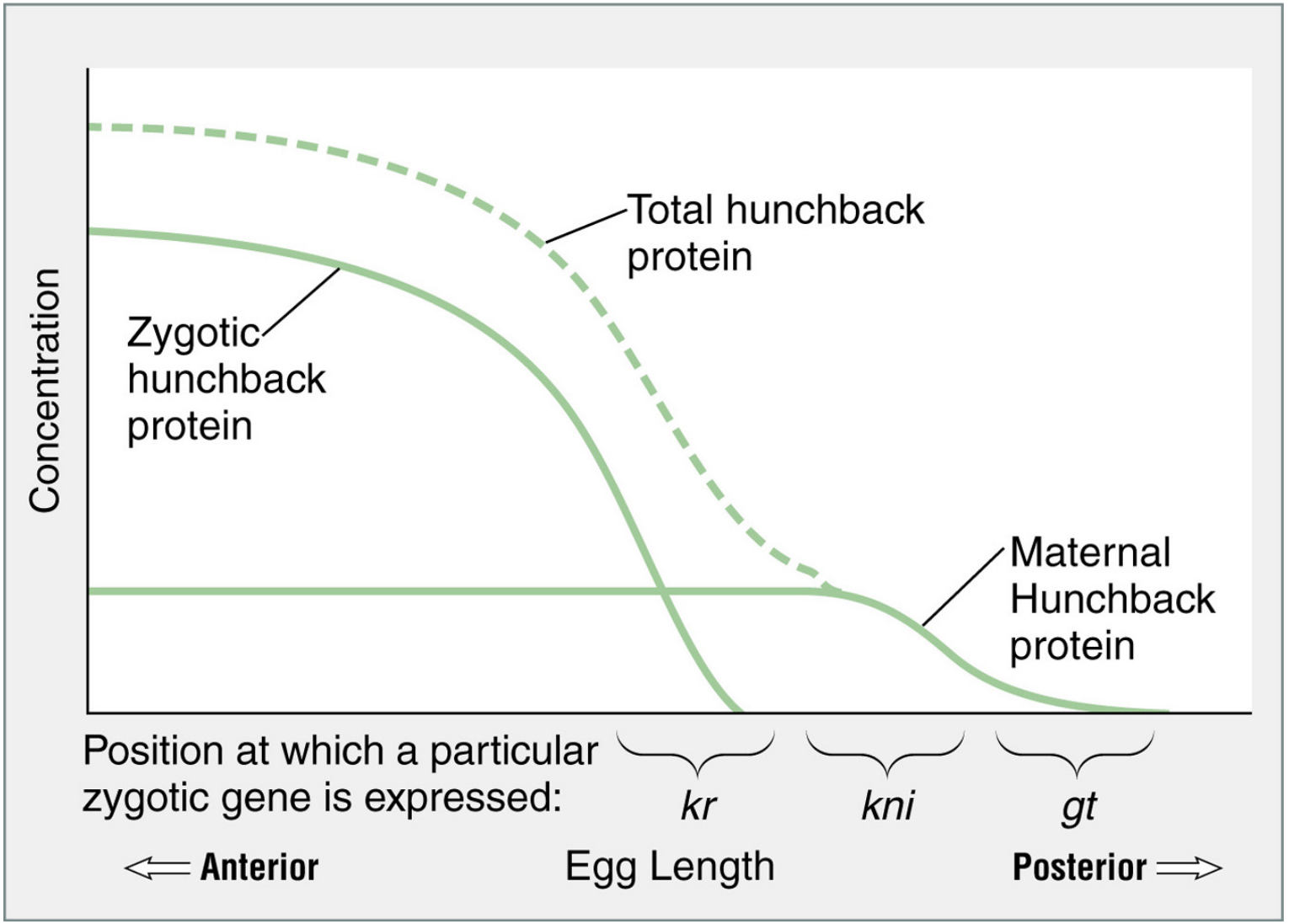



**NANOS+PUMILO**

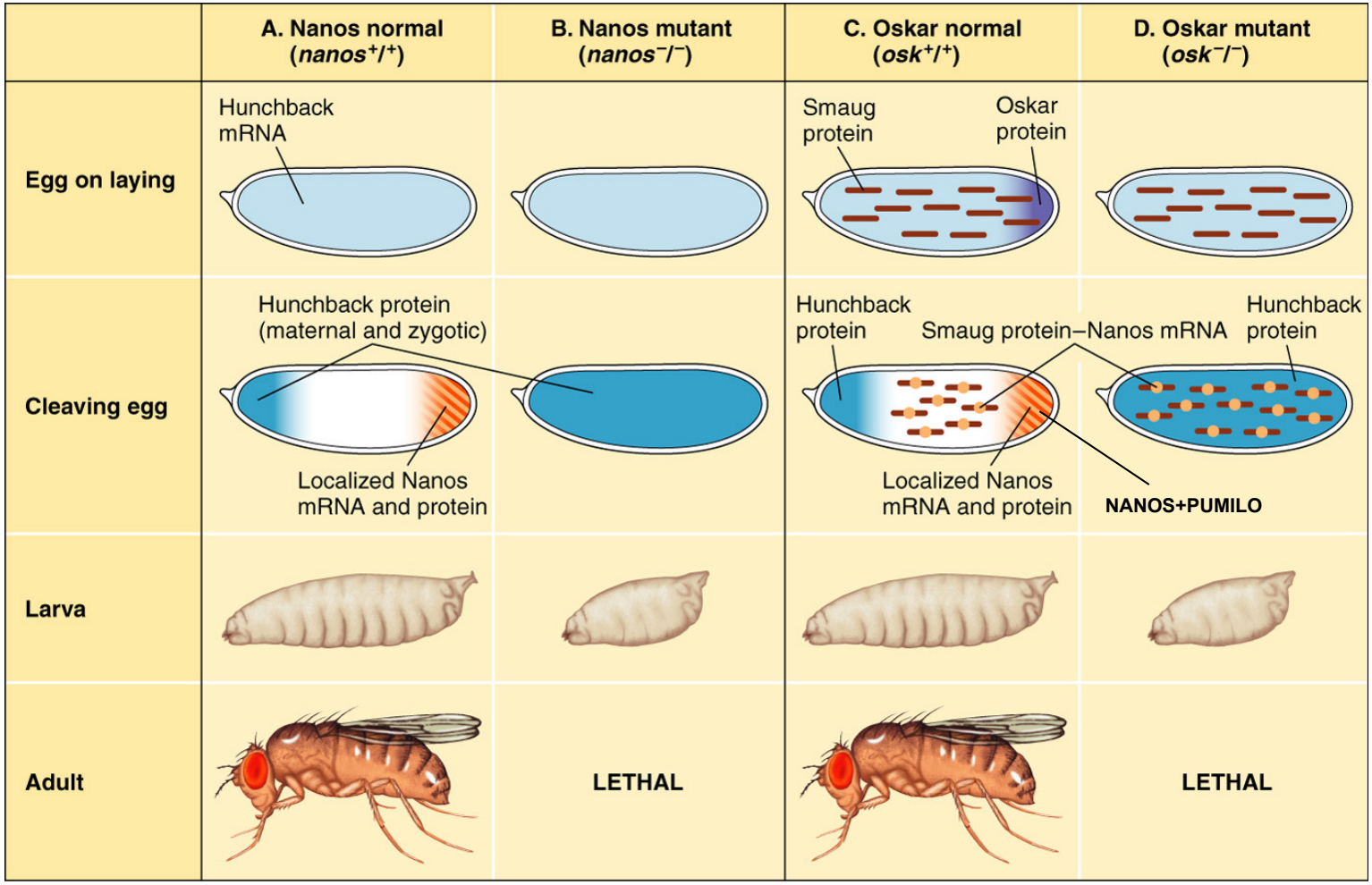
↓

***HUNCHBACK mRNA*** **deacetylation and degradation**

**C.**


Concentration
Total hunchback protein
Zygotic hunchback protein
Maternal Hunchback protein
Position at which a particular zygotic gene is expressed:
kr
kni
gt
Anterior
Egg Length
Posterior

# Regulation via mRNA localization

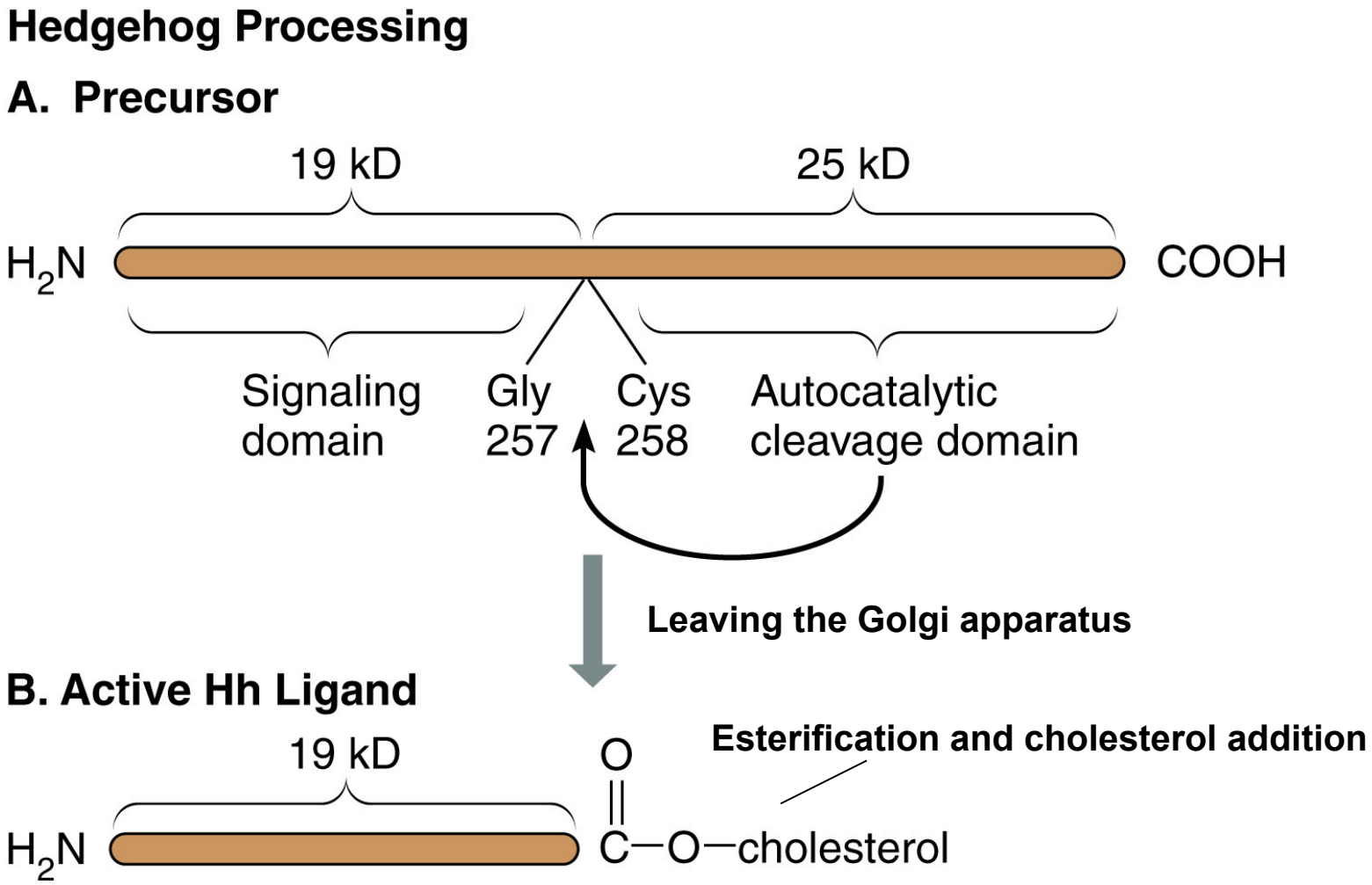


# Outline of Lesson 10

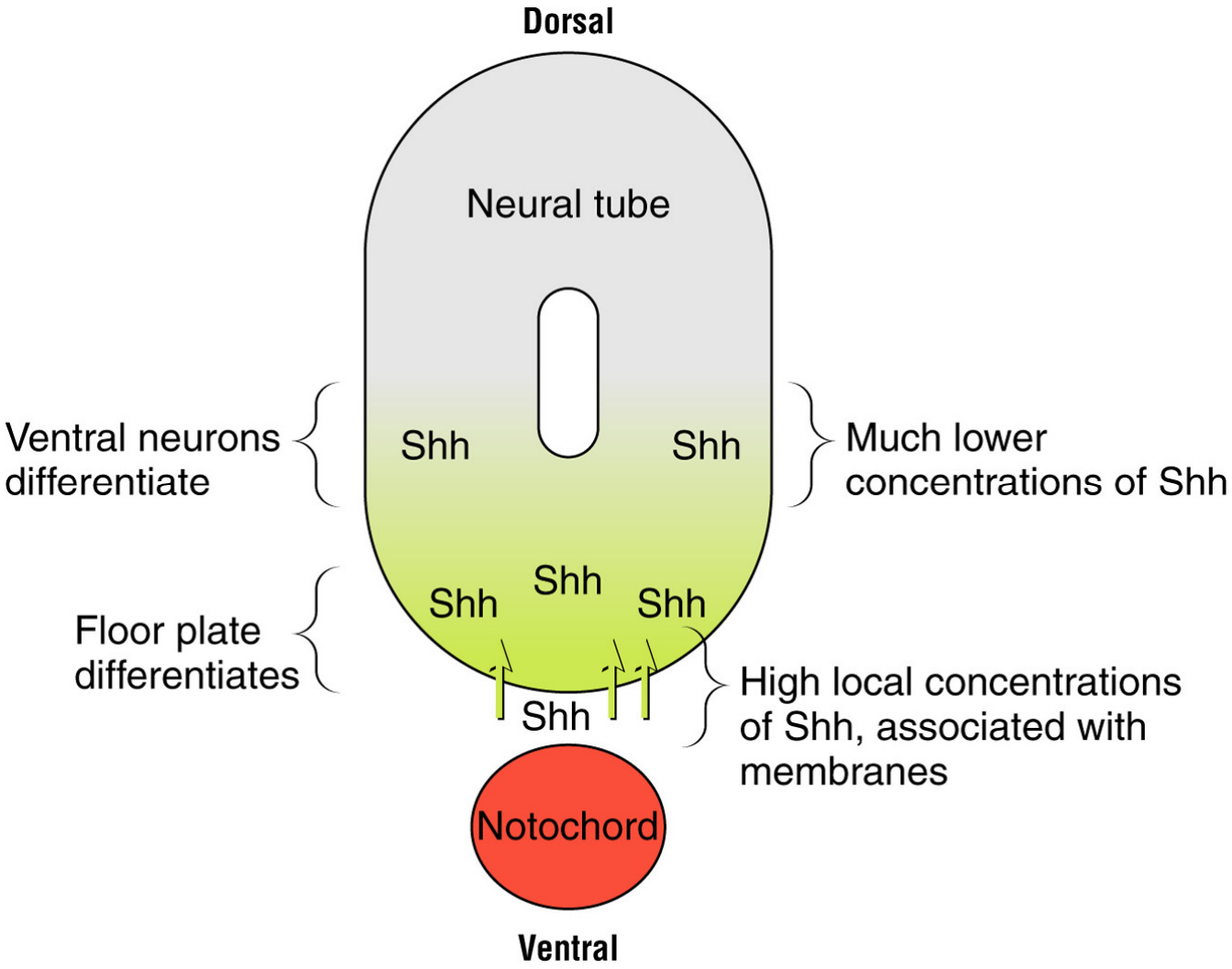
## Regulation of Gene Expression during Development

- 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

# Regulation via protein localization



# Regulation via protein localization



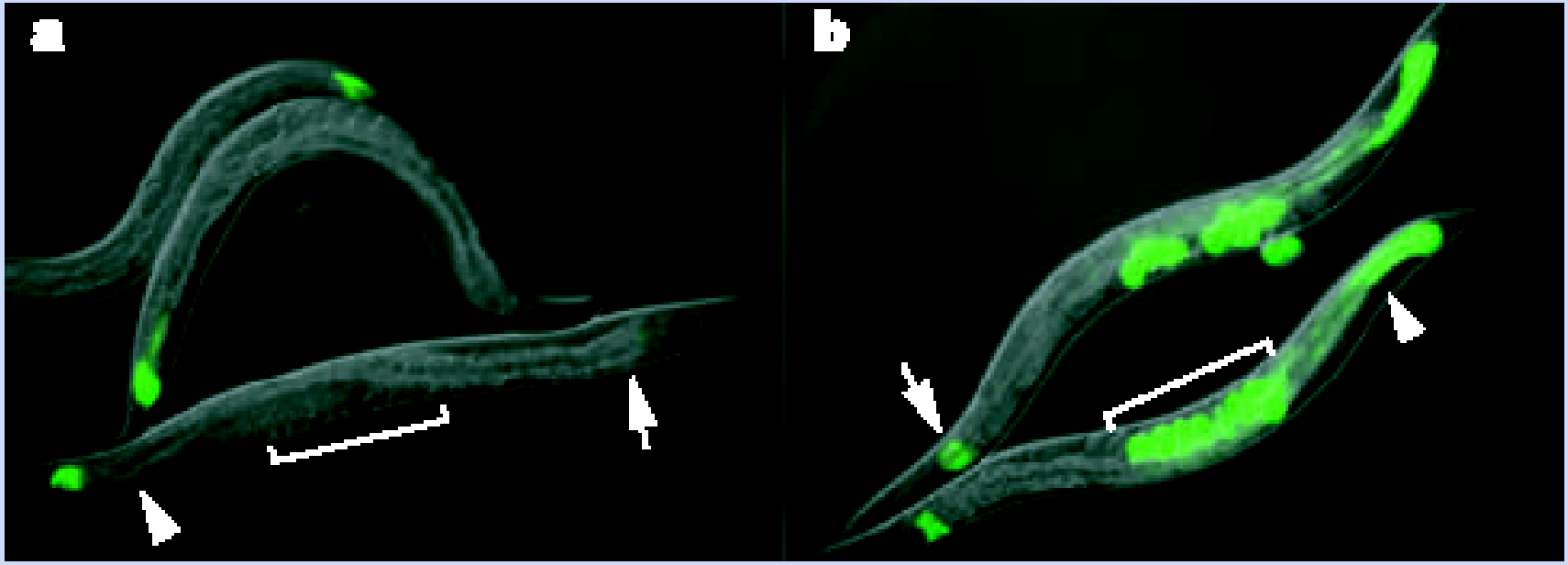
# Outline of Lesson 10

## 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

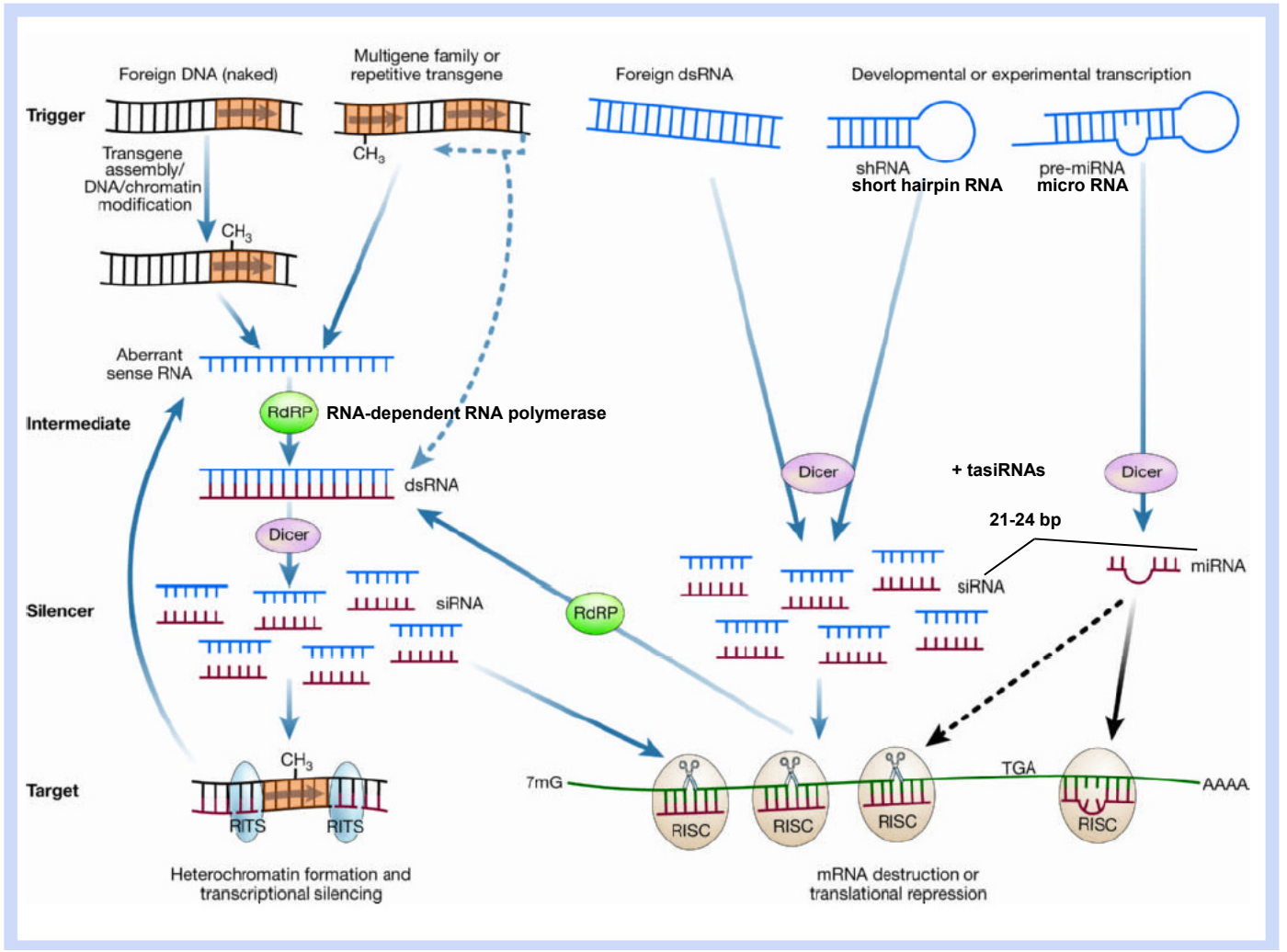
# RNA interference as a natural mechanism of the gene expression

***RNAi*** ***rnai***




Mello and Conte, *Nature* (2004)

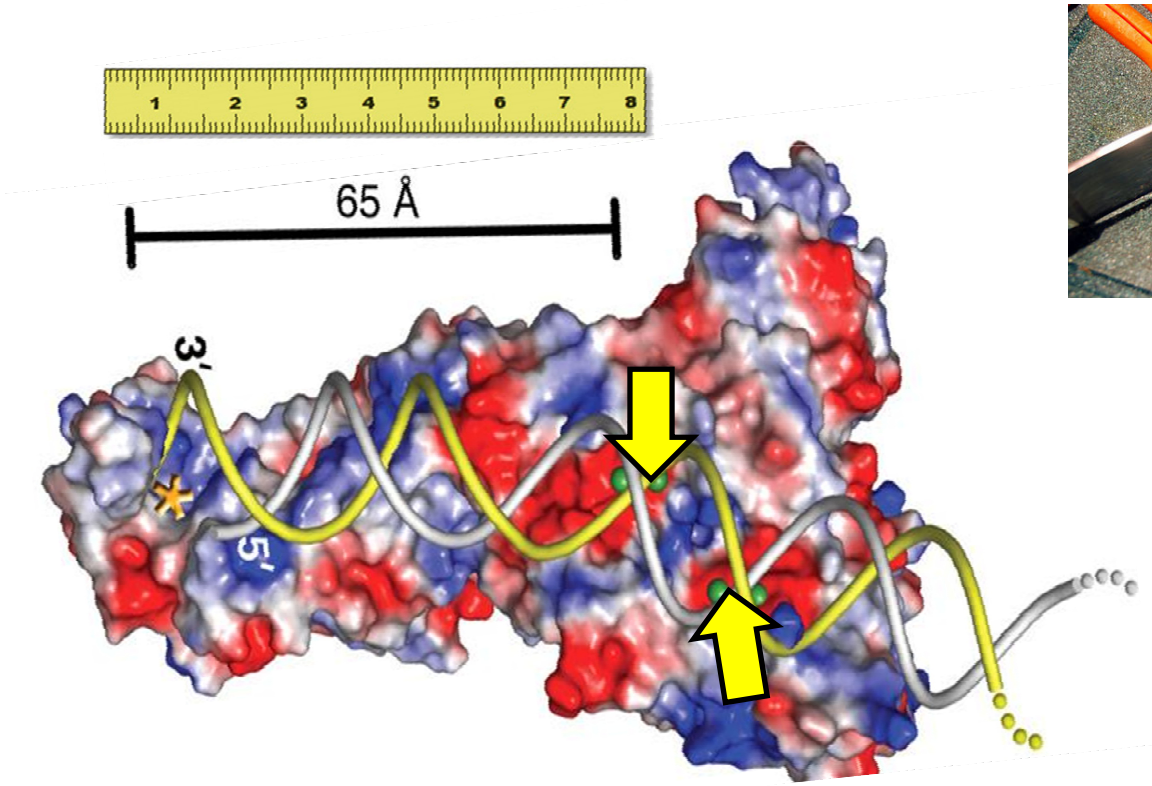
# Mechanism of RNA interference




Mello and Conte, *Nature* (2004)

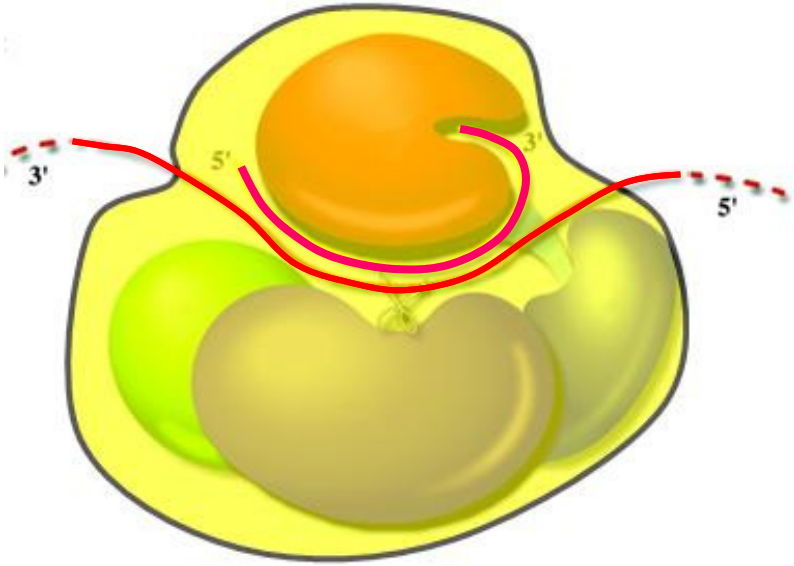
Mello, 2004

# Dicer and Dicer-like proteins

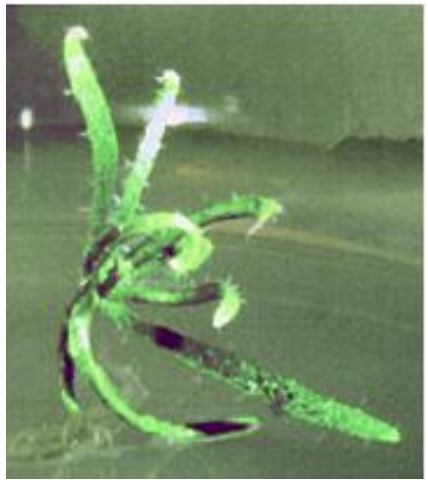



From MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.., Adams, P.D., and Doudna, J.A. (2006) Structural basis for double-stranded RNA processing by Dicer. Science 311: 195 -198. Reprinted with permission from AAAS. Photo credit: Heidi

# Argonaute proteins



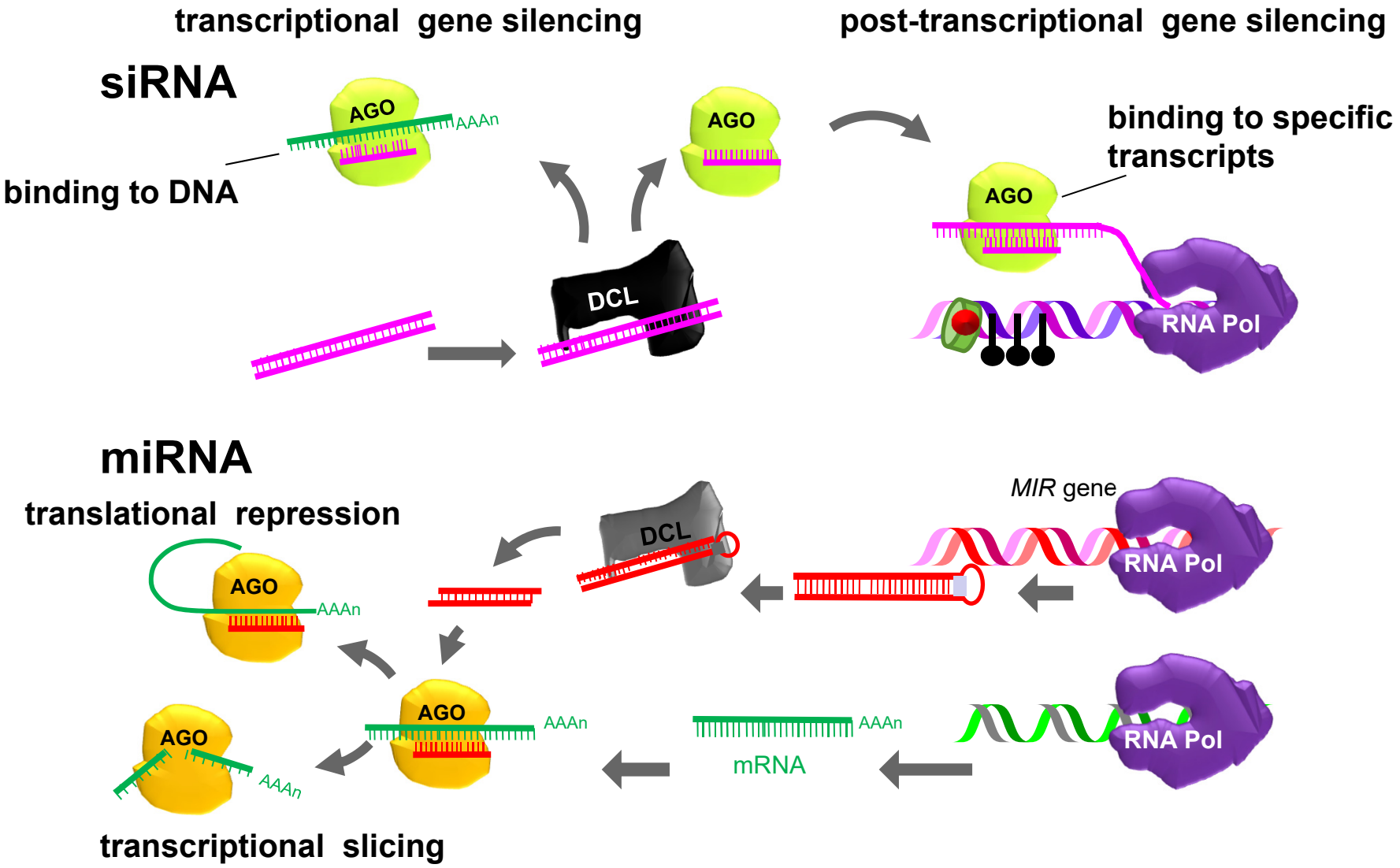

***ago1***



***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.


transcriptional gene silencing
post-transcriptional gene silencing
siRNA
binding to DNA
AGO
AAAn
AGO
DCL
binding to specific transcripts
AGO
RNA Pol
miRNA
translational repression
AGO
AAAn
DCL
MIR gene
RNA Pol
AGO
AAAn
AGO
AAAn
mRNA
AAAn
RNA Pol
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

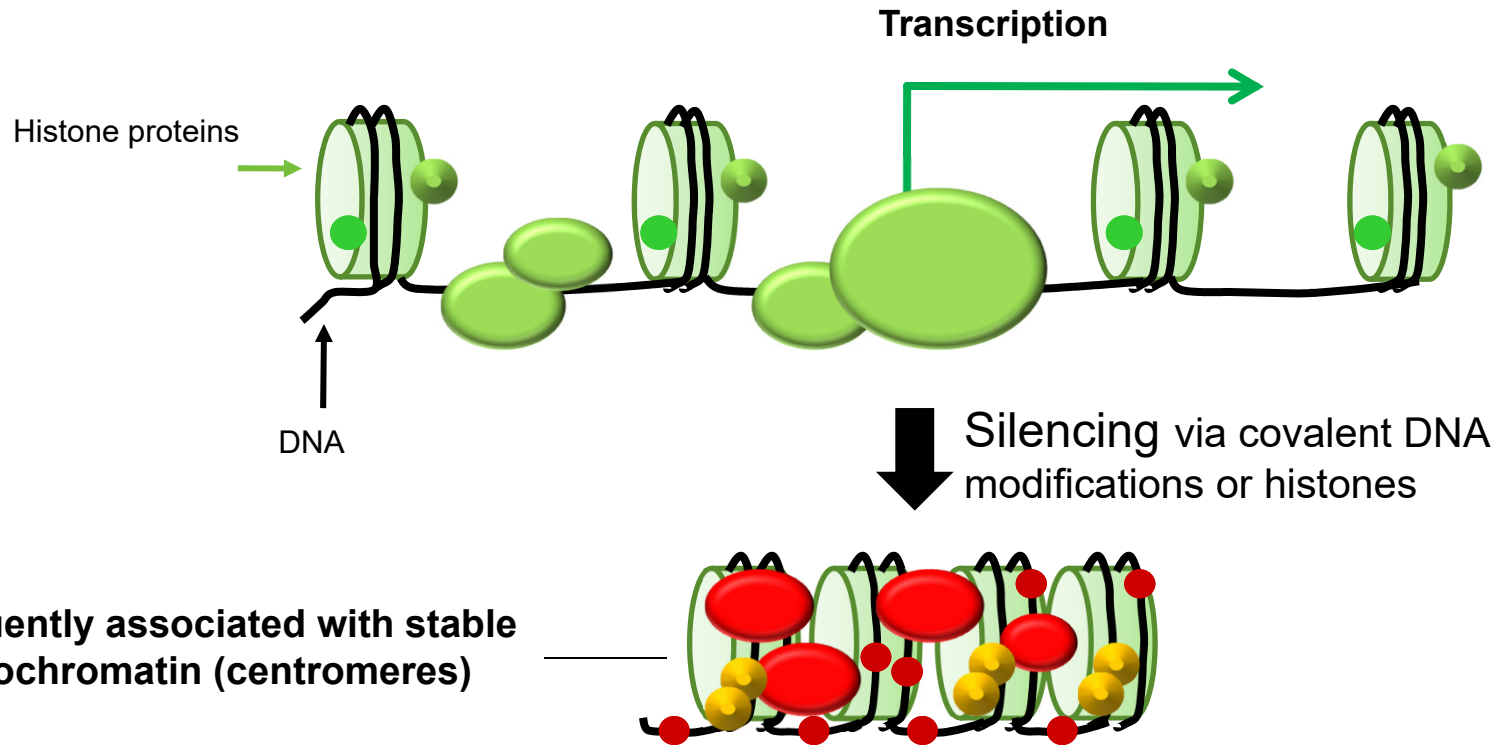
b. 1960

# Outline of Lesson 10

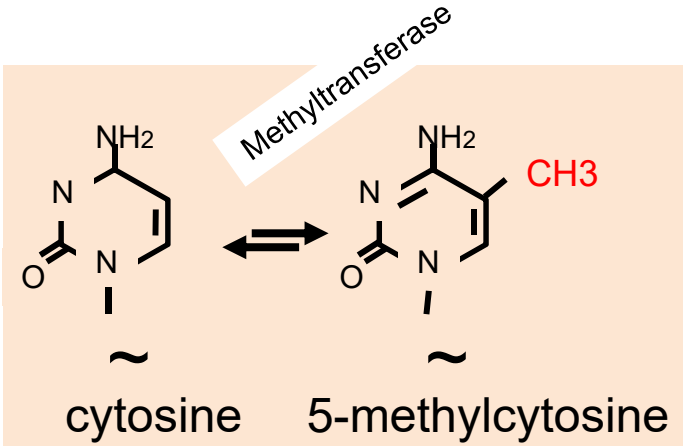
## Regulation of Gene Expression during Development

- 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

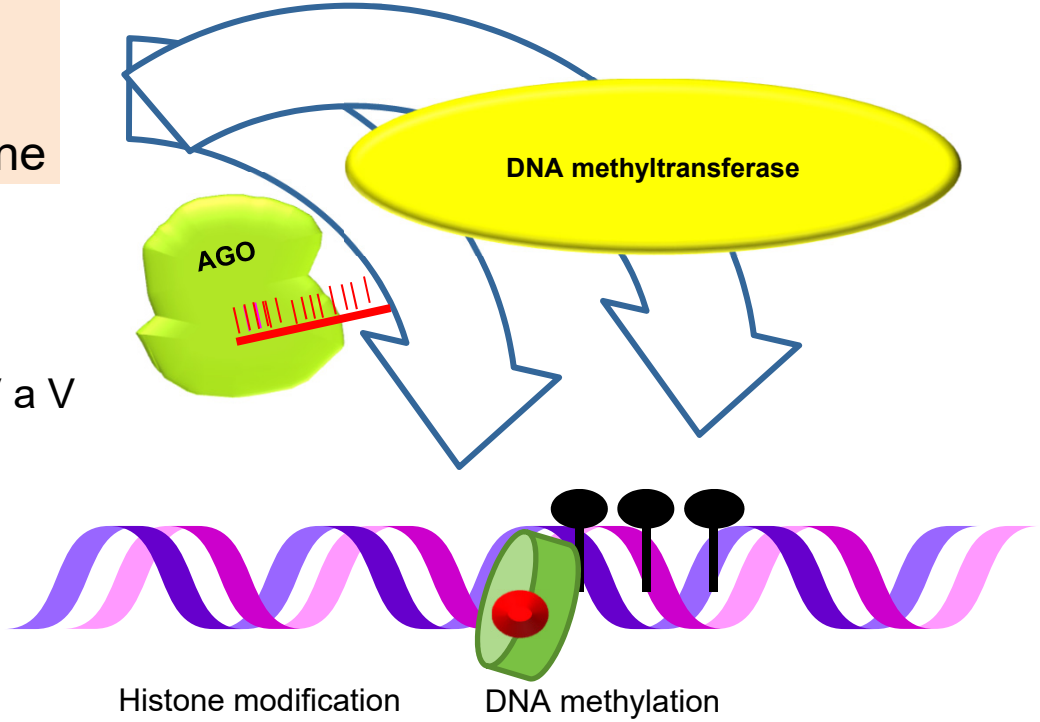
# Transcriptional gene silencing via covalent modifications of DNA

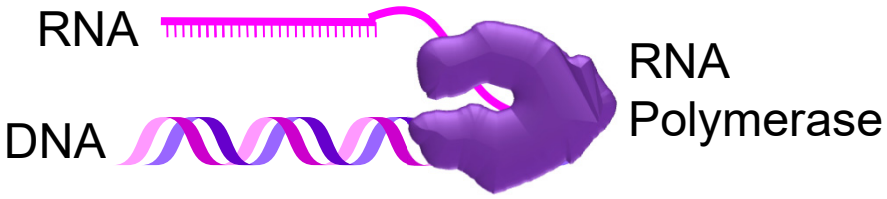


# Transcriptional gene silencing via DNA methylation




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



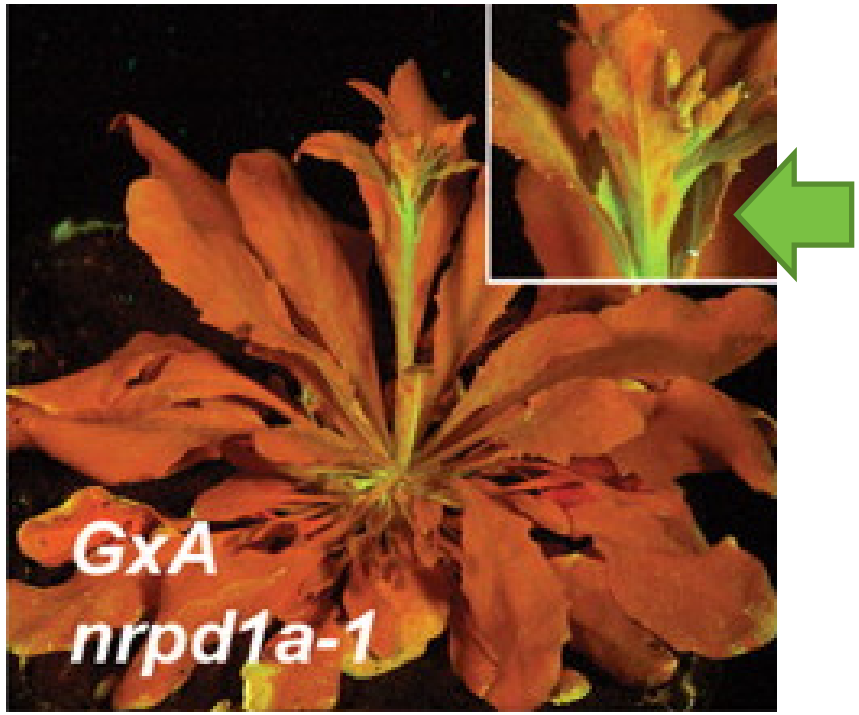



| Complex | Distribution | Function |
|---|---|---|
| RNA Polymerase I | All eukaryotes | Production of rRNA |
| RNA Polymerase II | All eukaryotes | Production of mRNA, microRNA |
| RNA Polymerase III | All eukaryotes | Production of tRNA, 5S rRNA |
| RNA Polymerase IV | Land plants | Production of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

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

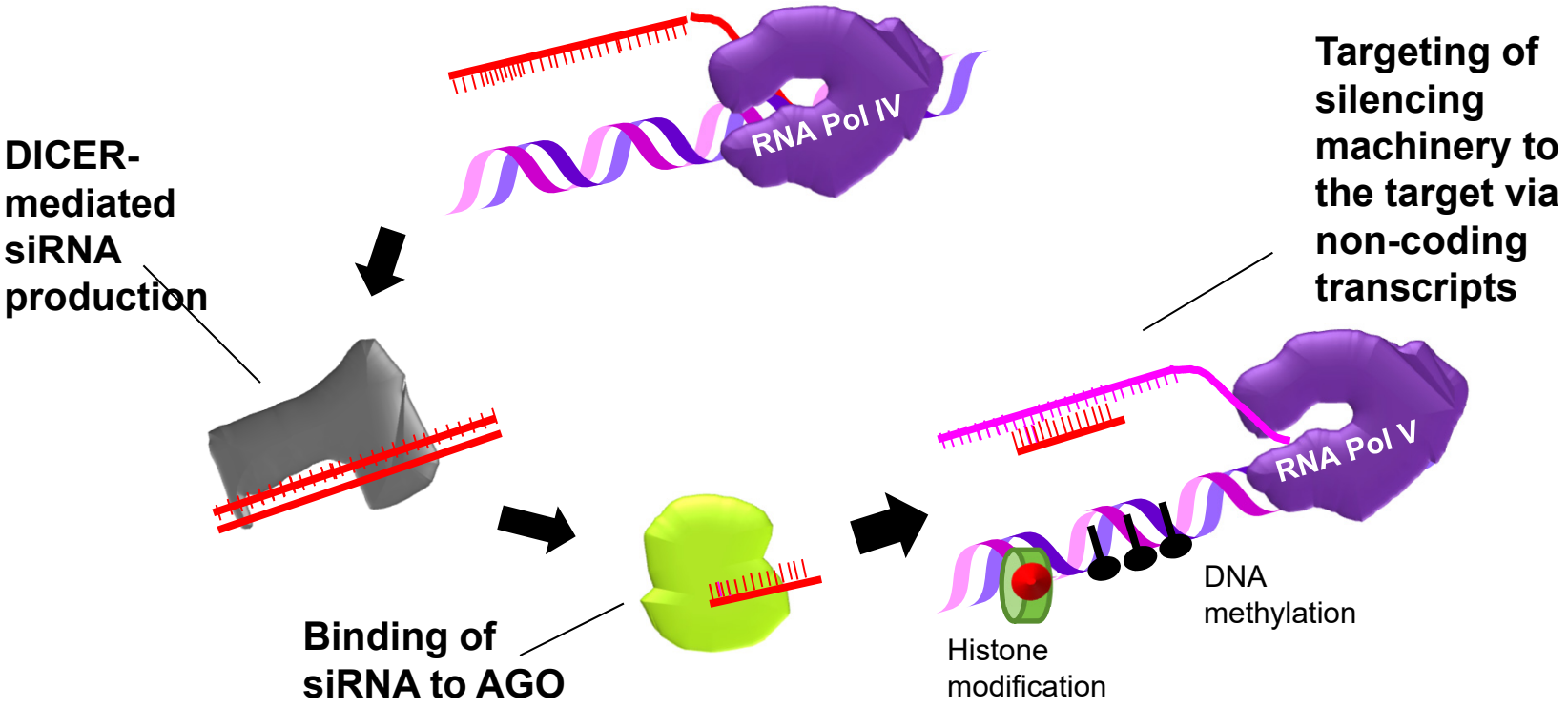
***Arabidopsis*** **with silenced GFP gene**

***nrpd1a-1***

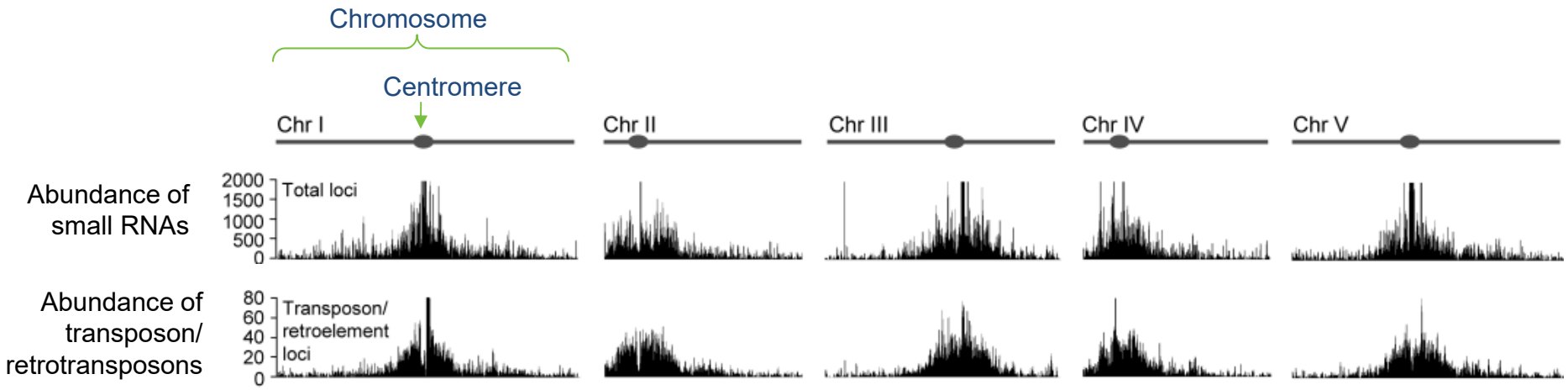




From Herr, A.J., Jensen, M.B., Dalmay, T., and Baulcombe, D.C. (2005) RNA polymerase IV directs silencing of endogenous DNA. Science 308: 118–120. Reprinted with permission from AAAS.

# RNA Pol IV and V are necessary for transcriptional silencing

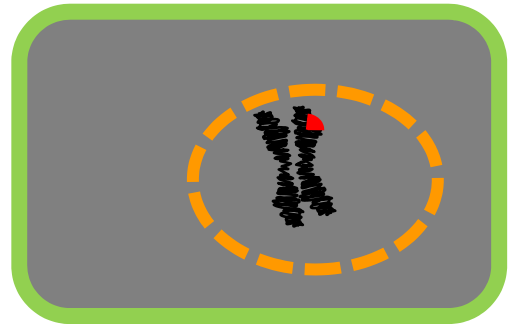


# Most siRNAs are produced from transposons and repetitive DNA



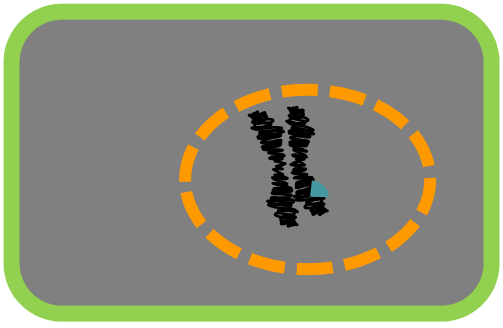

Kasschau, K.D., Fahlgren, N., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., and Carrington, J.C. (2007) Genome-wide profiling and analysis of *Arabidopsis* siRNAs. PLoS Biol 5(3): e57.

# Transcriptional gene silencing



Pro35S: KAN

X



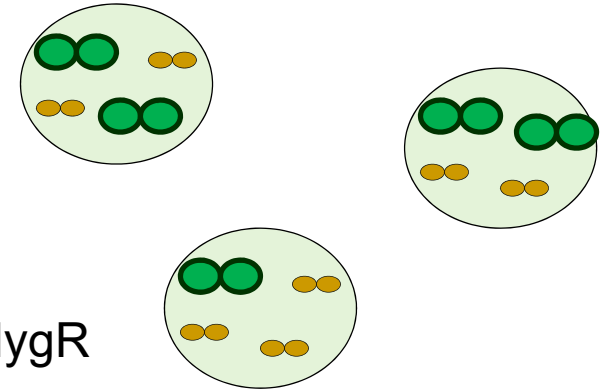
Pro35S: HYG

**Expected Results**

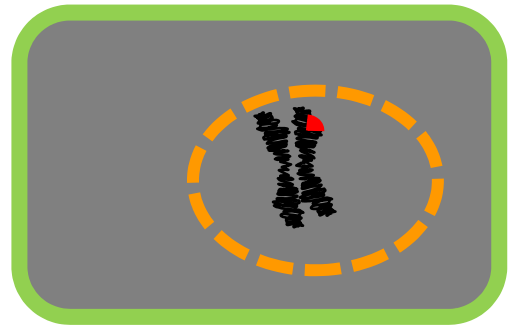
Selection on kanamycin only: 50% KanR

Selection on hygromycin only: 50% HygR

Selection on Kan + Hyg: 25% KanR and HygR

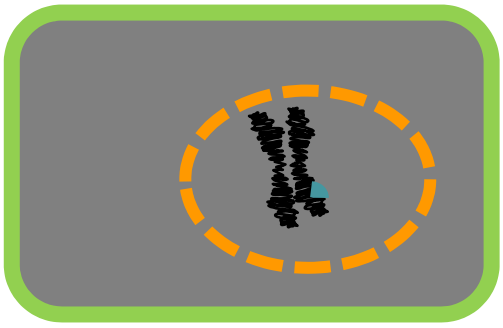


# Transcriptional gene silencing



Pro35S : KAN

X



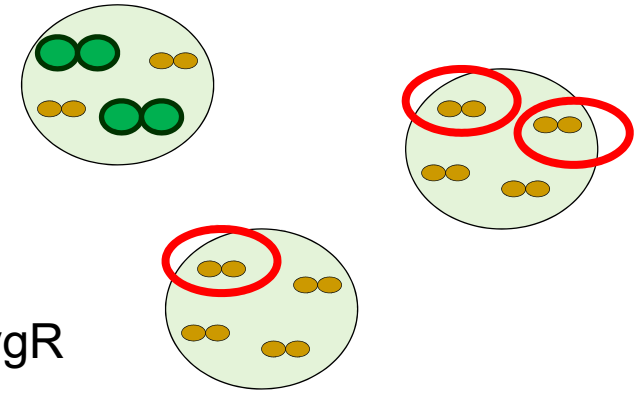
Pro35S : HYG

**<u>Observed</u> Results**

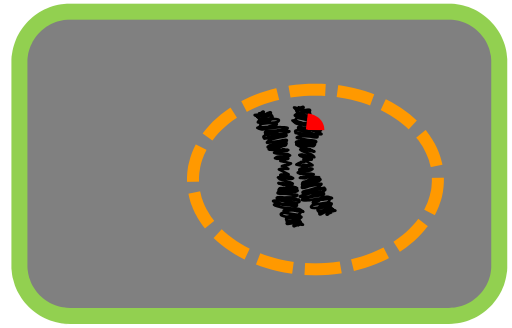
Selection on kanamycin only: 50% KanR

Selection on hygromycin only: 0% HygR

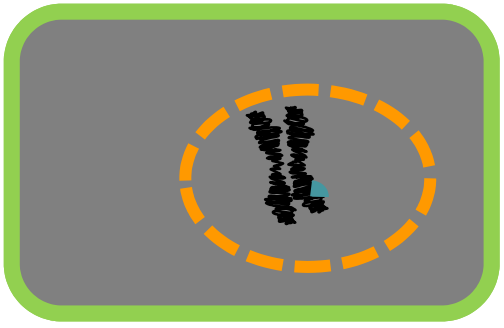
Selection on Kan + Hyg: 0% KanR and HygR



# Transcriptional gene silencing



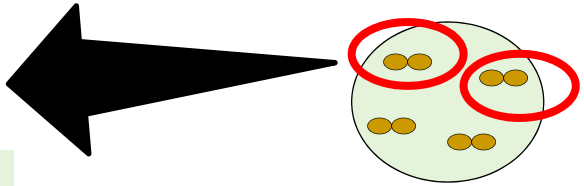
Pro35S : KAN



Pro35S : HYG

DNA methylation

CaMV 35S pro : HYG



The promoter of the silenced gene become methylated, interfering thus with transcription.

# 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

# Outline of Lesson 10

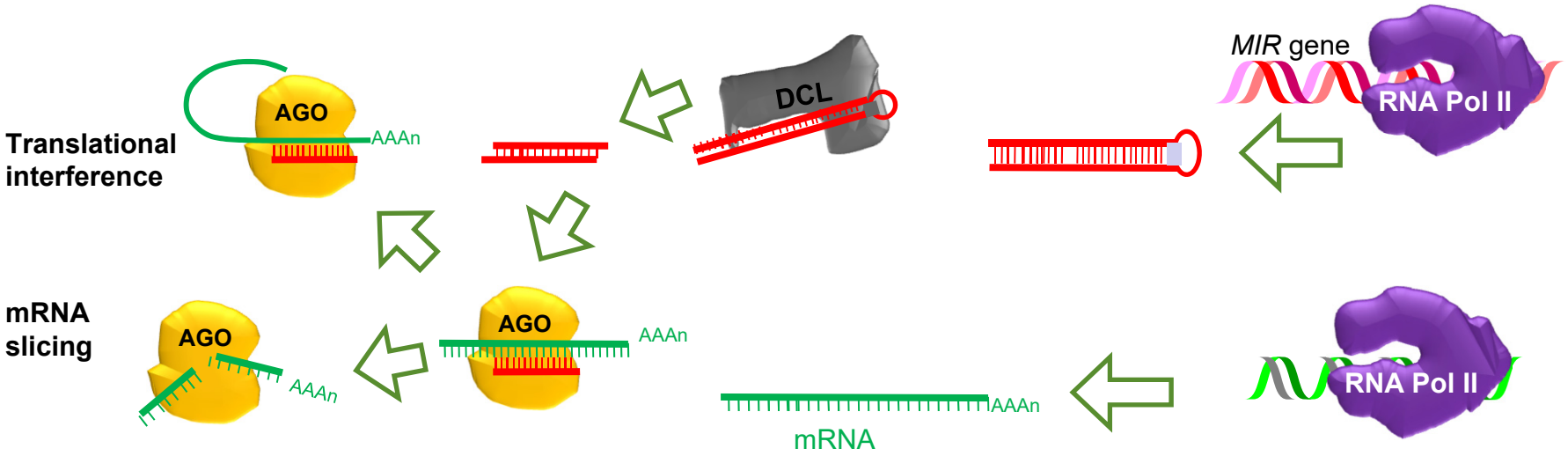
## Regulation of Gene Expression during Development

- 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**

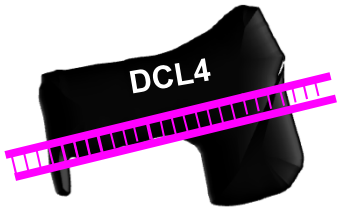
- small # of highly conserved miRNAs
- hing # of non-conserved miRNAs
- binding to 5'UTR and require almost  complete complementarity
- most of the plant miRNA induce slicing of target mRNAs

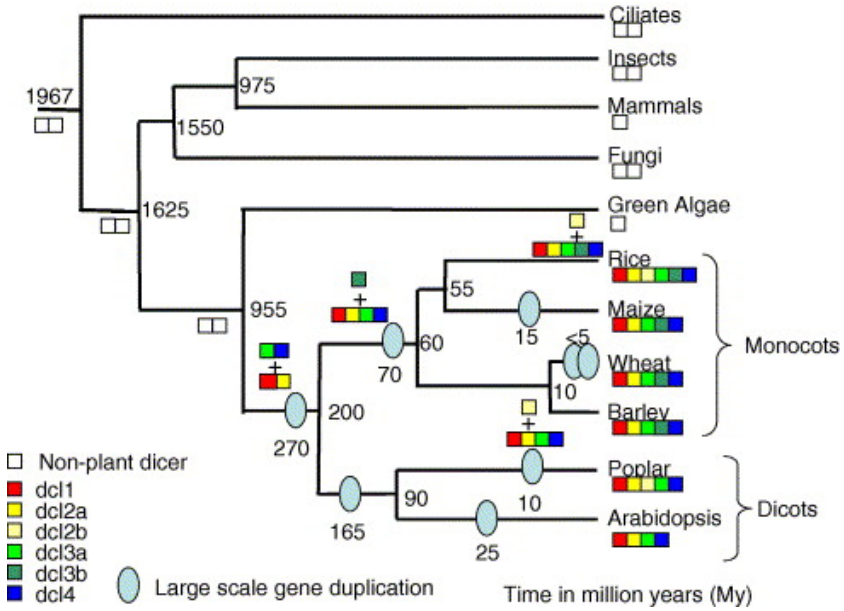


# miRNAs and siRNAs are processed by related but different DCL proteins

AtDCL1 produces **miRNA**


AtDCL2 - 4 produce **siRNA**



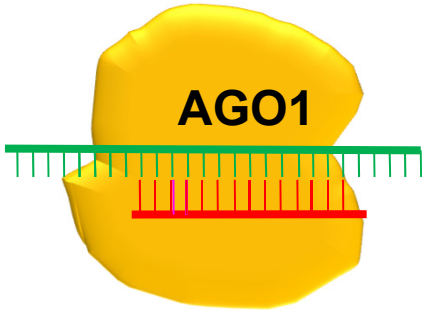




Reprinted from Margis, R., Fusaro, A.F., Smith, N.A., Curtin, S.J., Watson, J.M., Finnegan, E.J., and Waterhouse, P.M. (2006) The evolution and diversification of Dicers in plants FEBS Lett. 580: 2442-2450 with permission from Elsevier.

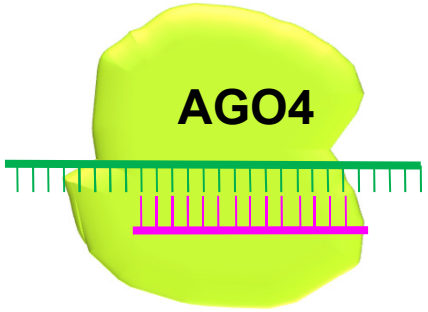
# miRNAs and siRNAs associate with several AGO proteins

**miRNAs in plants**

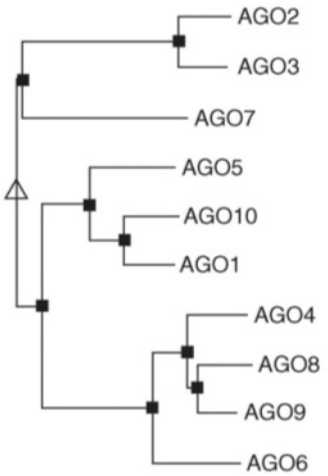
- small # of highly conserved miRNAs
- hing # of non-conserved miRNAs




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

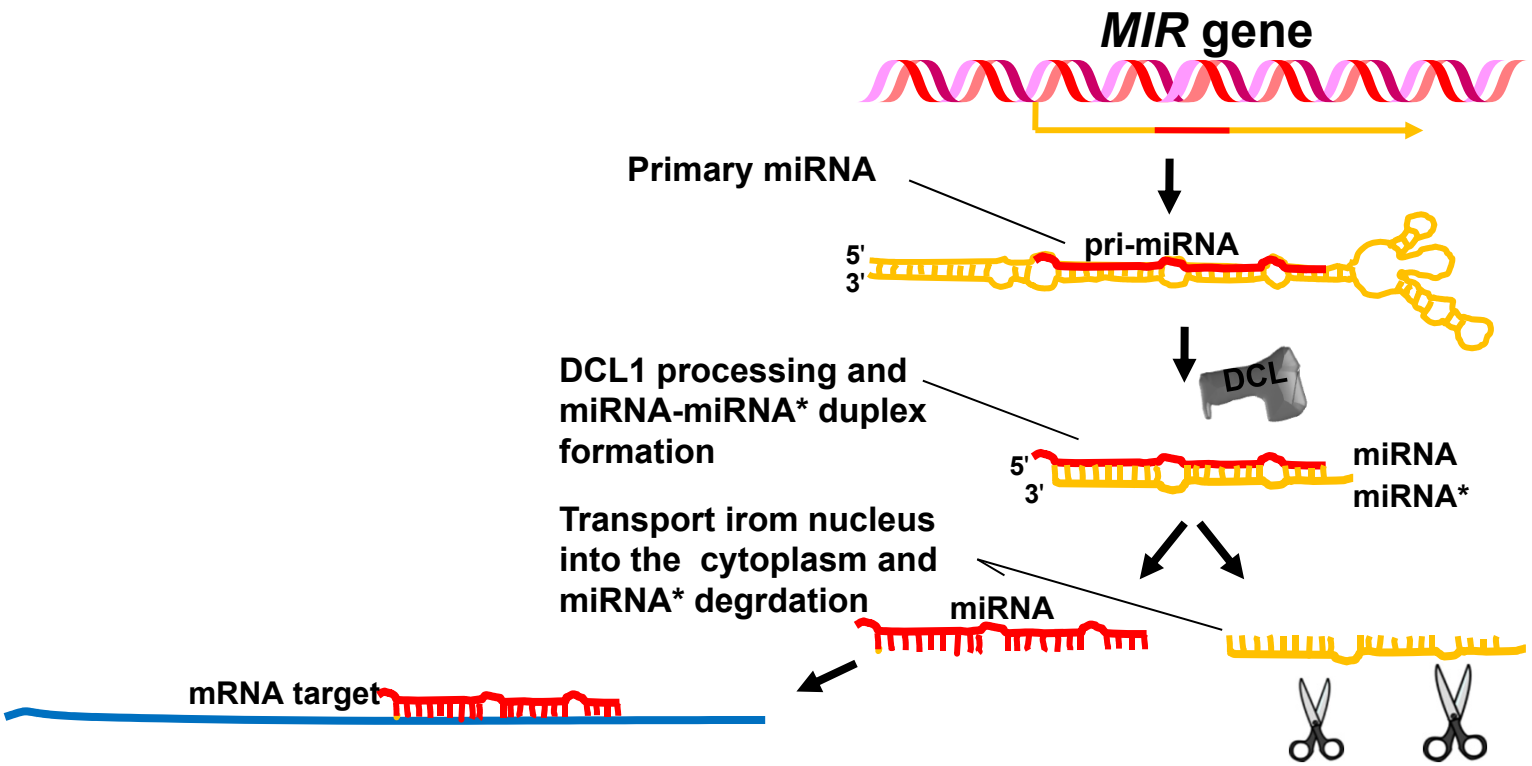



AGO4 preferentially associates with **siRNA** and mediates methylation of source DNA.

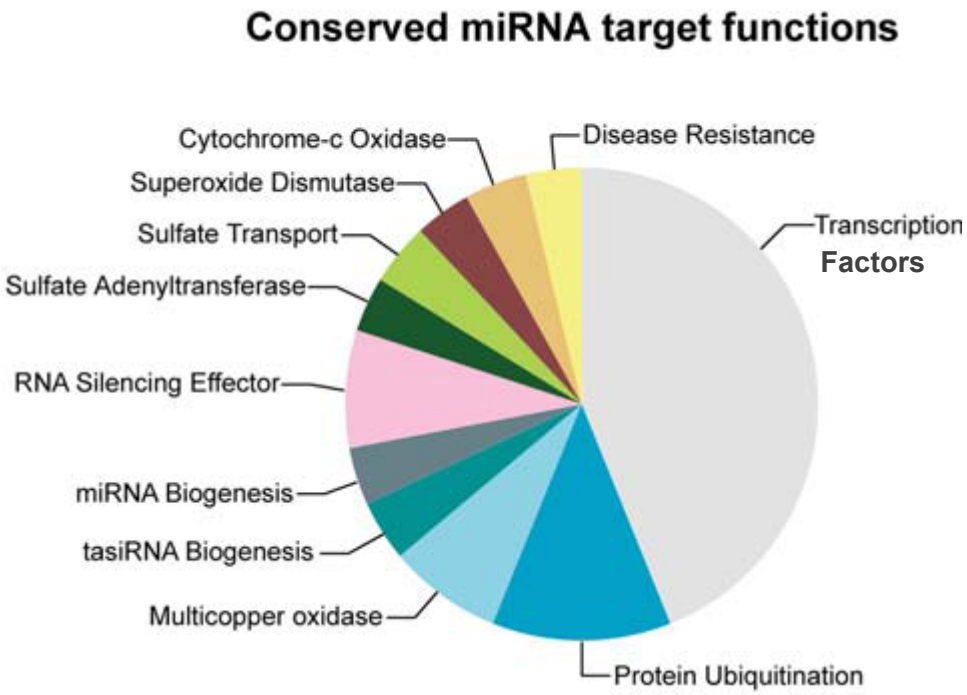


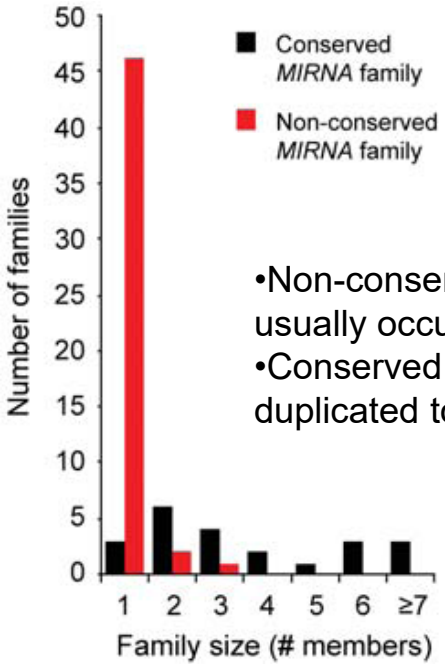

Reprinted from Vaucheret, H. (2008) Plant ARGONAUTES. Trends Plant Sci. 13: 350-358 with permission from Elsevier.

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



# Some miRNAs are highly conserved and important gene regulators



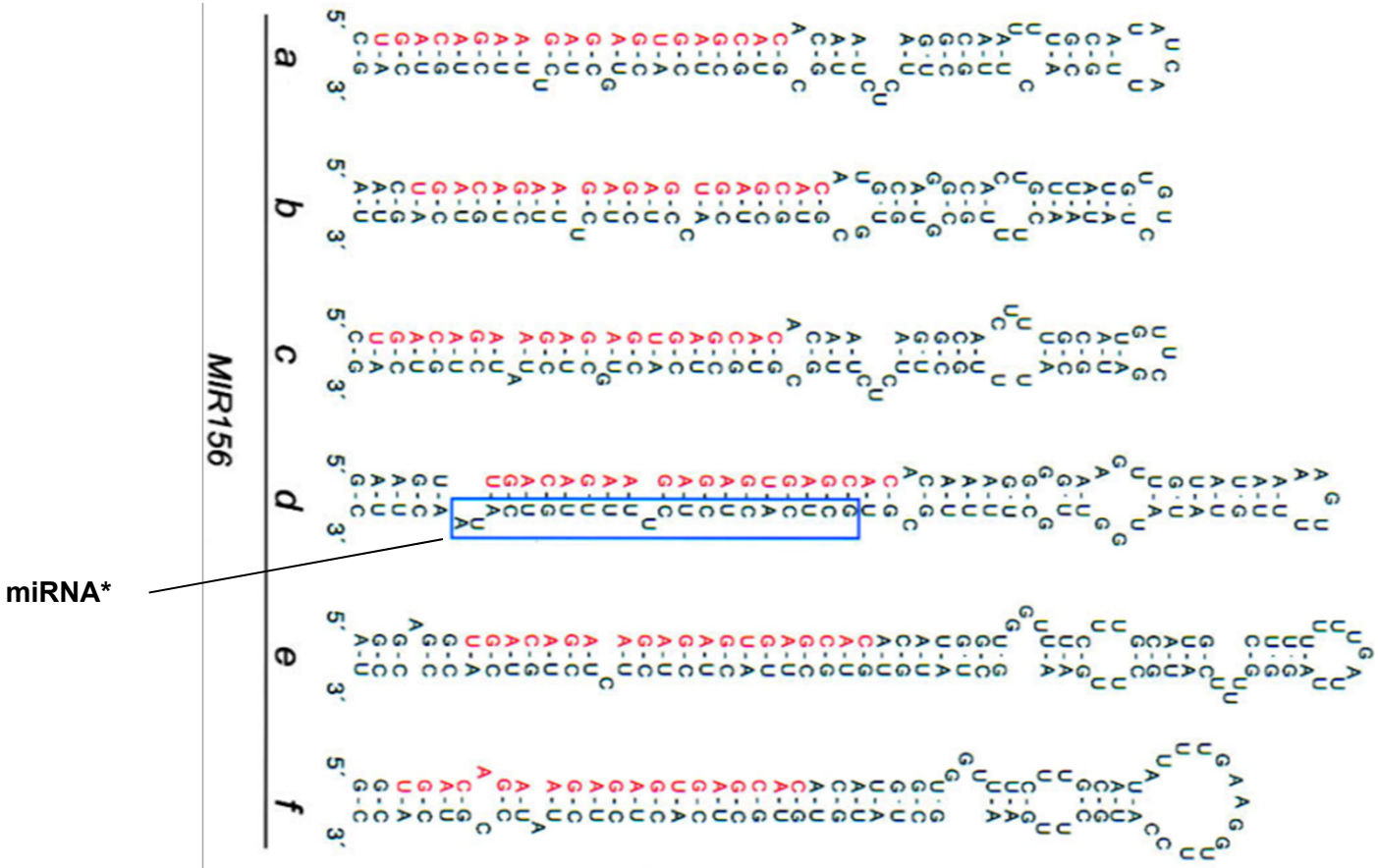




- Non-conserved *MIRNA* families usually occur as single genes
- Conserved ones have often duplicated to larger gene families

Fahlgren, et al., PLoS ONE, 2007

# The *MIR156* gene family is highly conserved

## *Arabidopsis miR156* gene family



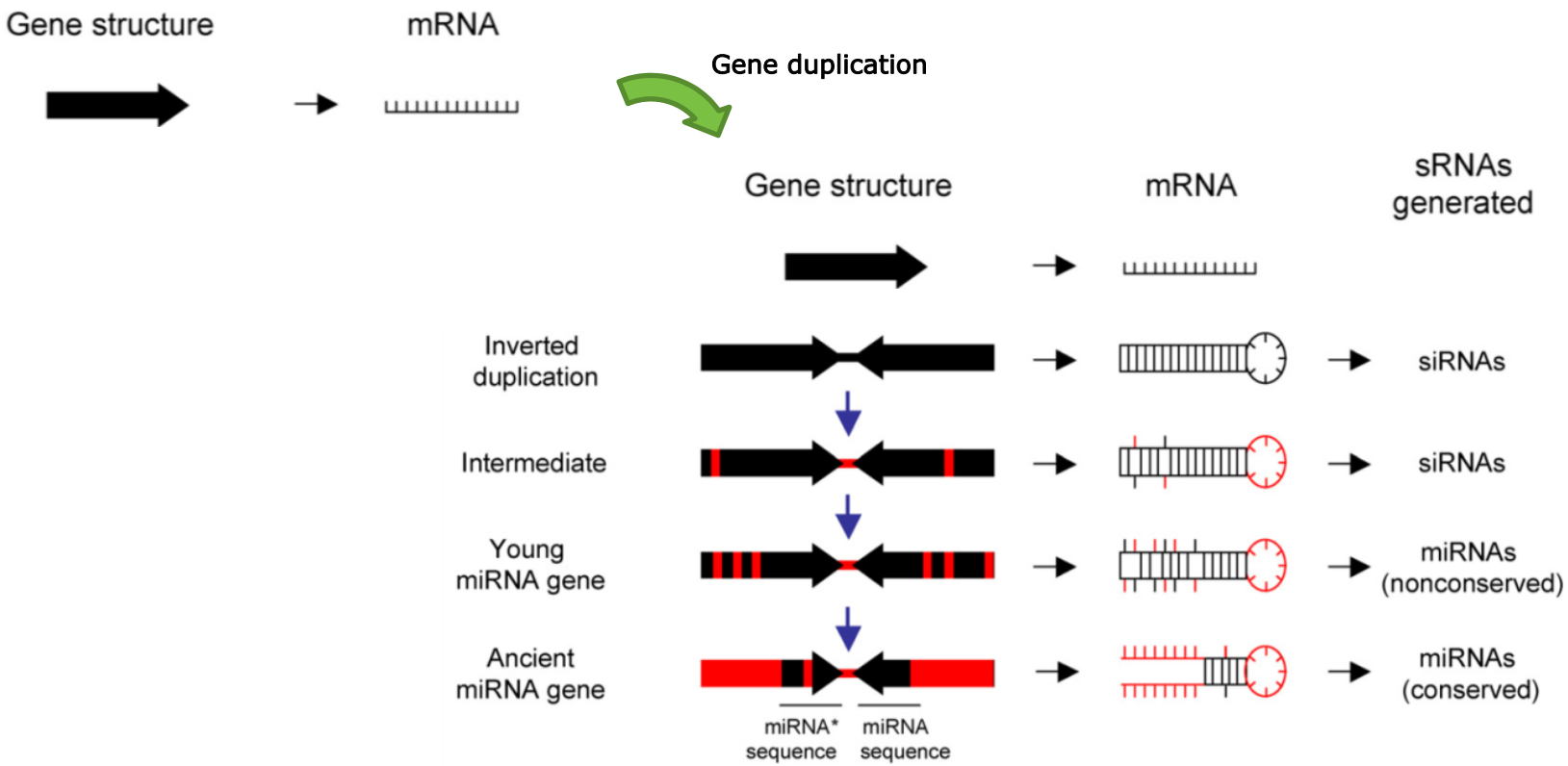

Reprinted from Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., and Bartel, D.P. (2002) MicroRNAs in plants. Genes Dev. 16: 1616–1626.

# Targets of some conserved miRNAs

| *miRNA* 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 |

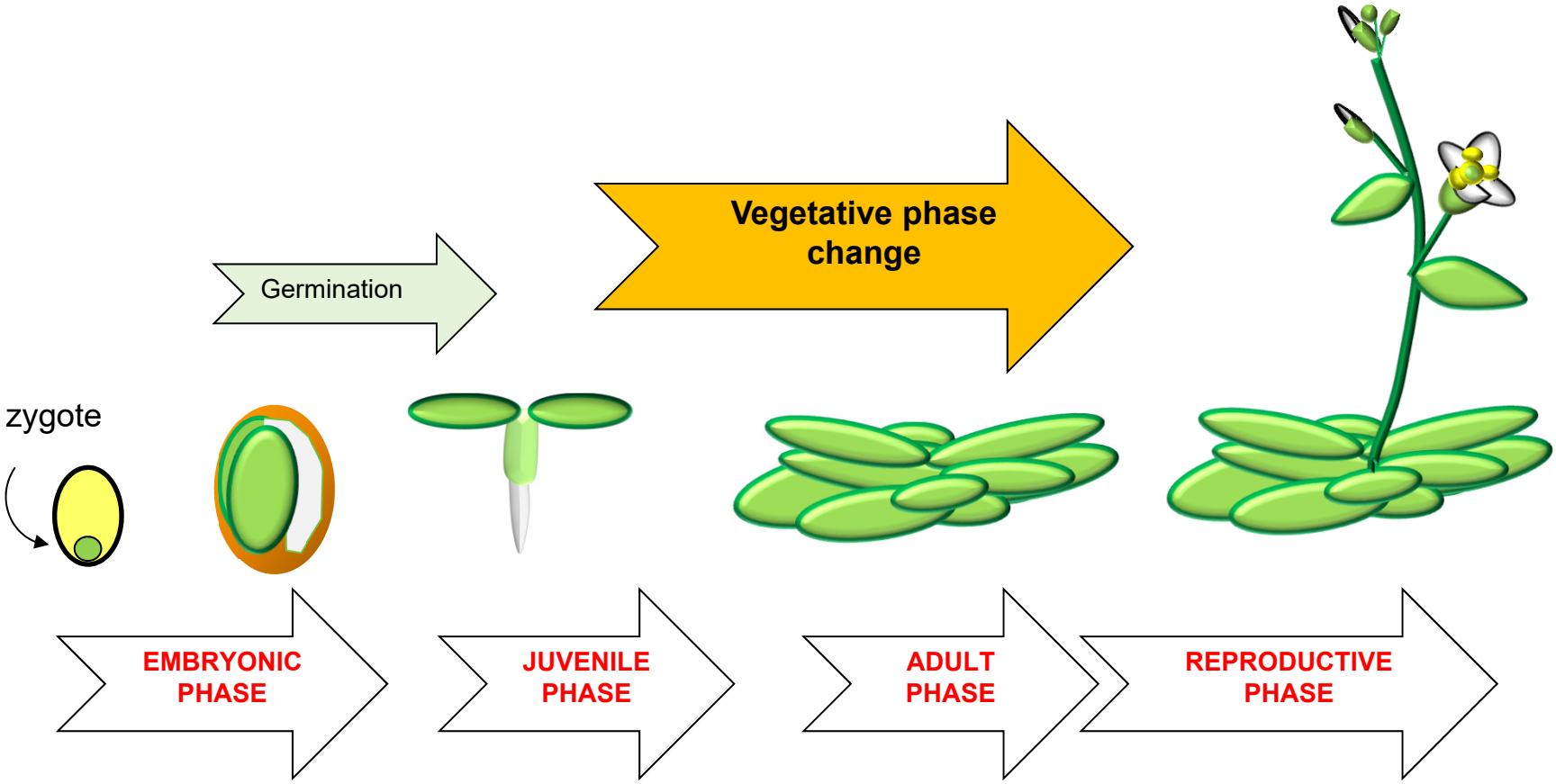
Adapted from Willmann, M.R., and Poethig, R.S. (2007) Conservation and evolution of miRNA regulatory programs in plant development. Curr. Opin. Plant Biol. 10: 503–511..

# 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



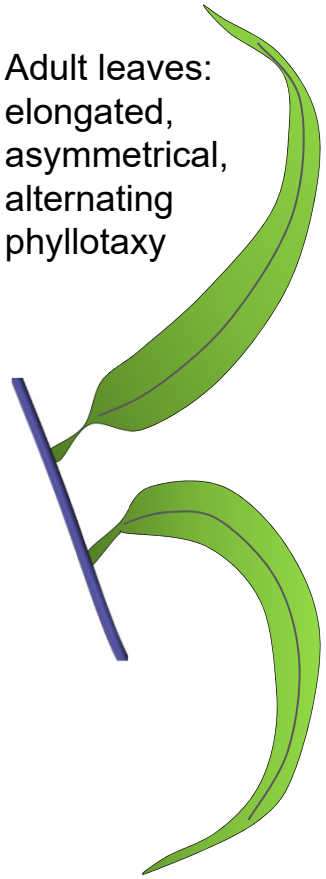
# Vegetative phase change affects morphology and reproductive competence



Photos courtesy of James Mauseth

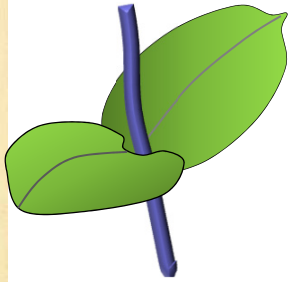
# Phase change can affect leaf shape, phyllotaxy, and trichome patterns

Adult leaves: elongated, asymmetrical, alternating phyllotaxy

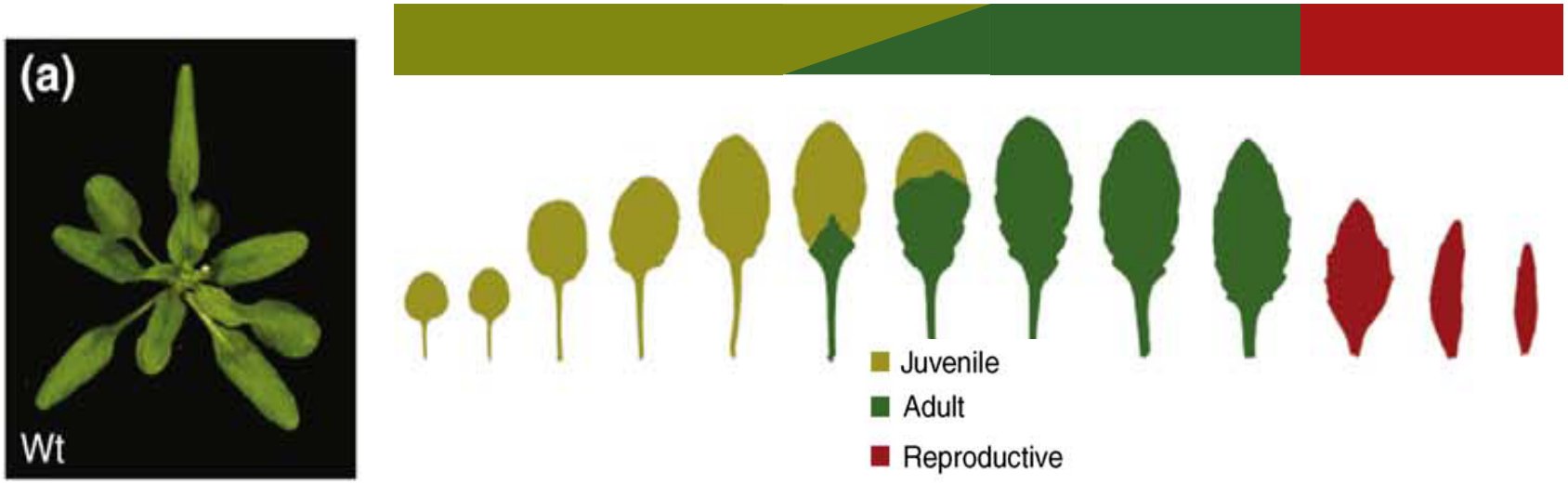



*Eucalyptus globulus*

Juvenile leaves: rounded, symmetrical, opposite phyllotaxy

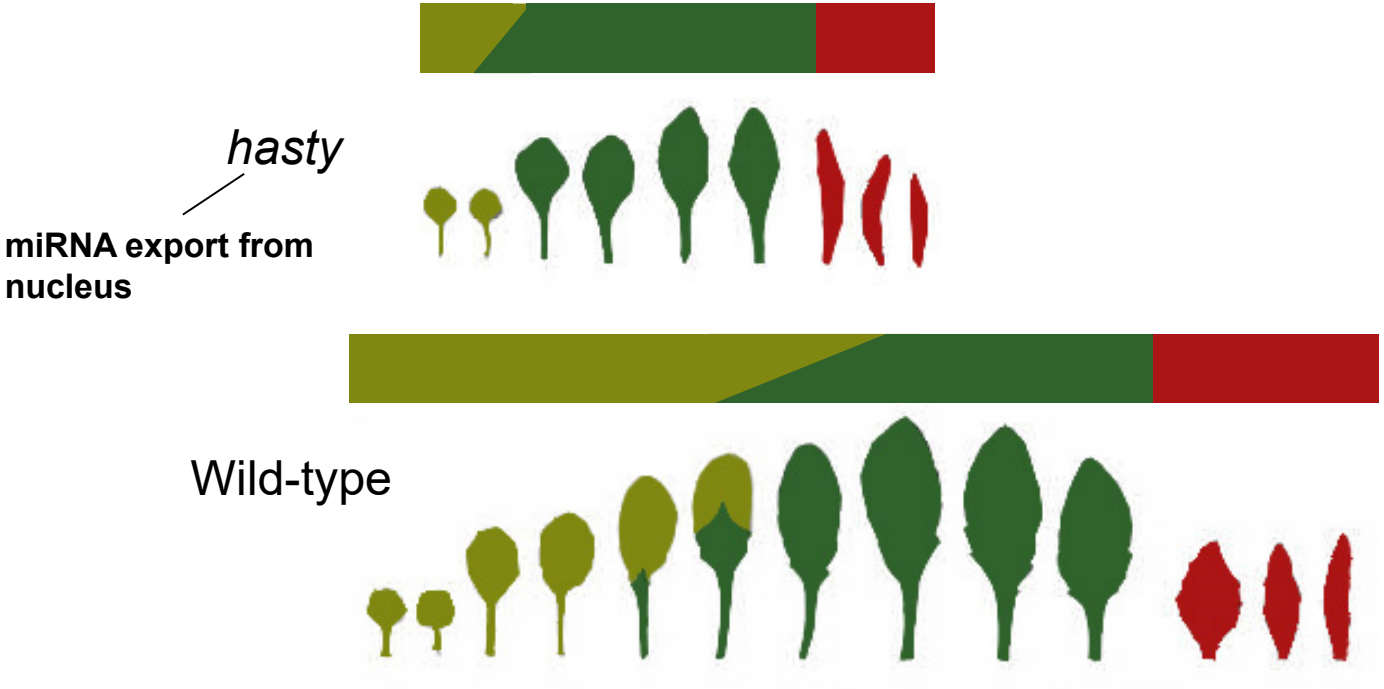


# In *Arabidopsis*, phase change affects leaf shape and trichome patterning



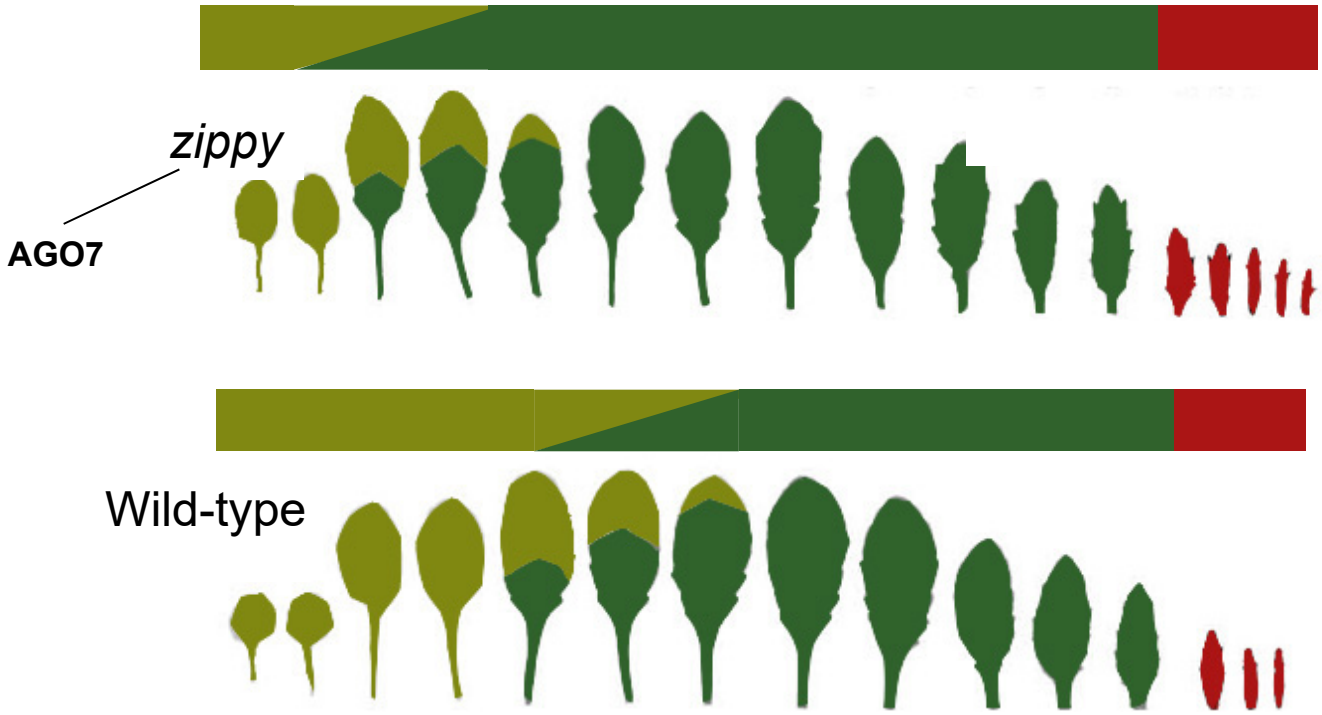

Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. Curr. Opin. Genet. Devel. 19: 374-378, with permission from Elsevier.

# 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

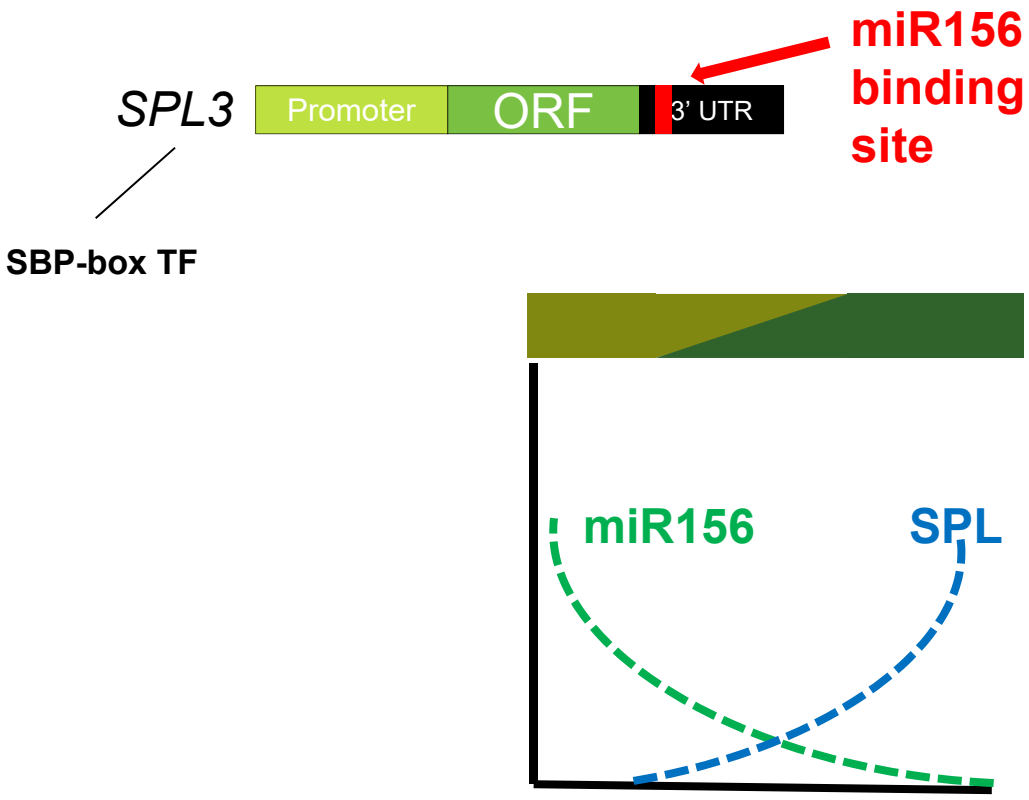



Reprinted from Hunter, C., Sun, H., and Poethig, R.S. (2003) The *Arabidopsis* heterochronic gene *ZIPPY* is an *ARGONAUTE* family member. Curr. Biol. 13: 1734–1739, with permission from Elsevier.

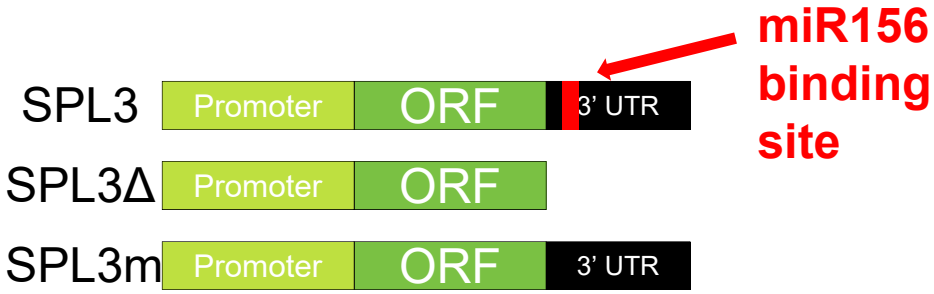
# *miR156* overexpression prolongs juvenile phase in *Arabidopsis*

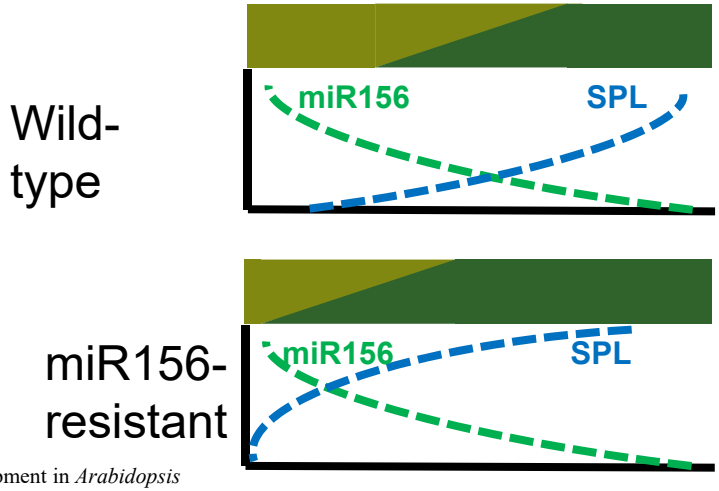

Reprinted from Poethig, R.S. (2009) Small RNAs and developmental timing in plants. Curr. Opin. Genet. Devel. 19: 374-378, with permission from Elsevier.

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



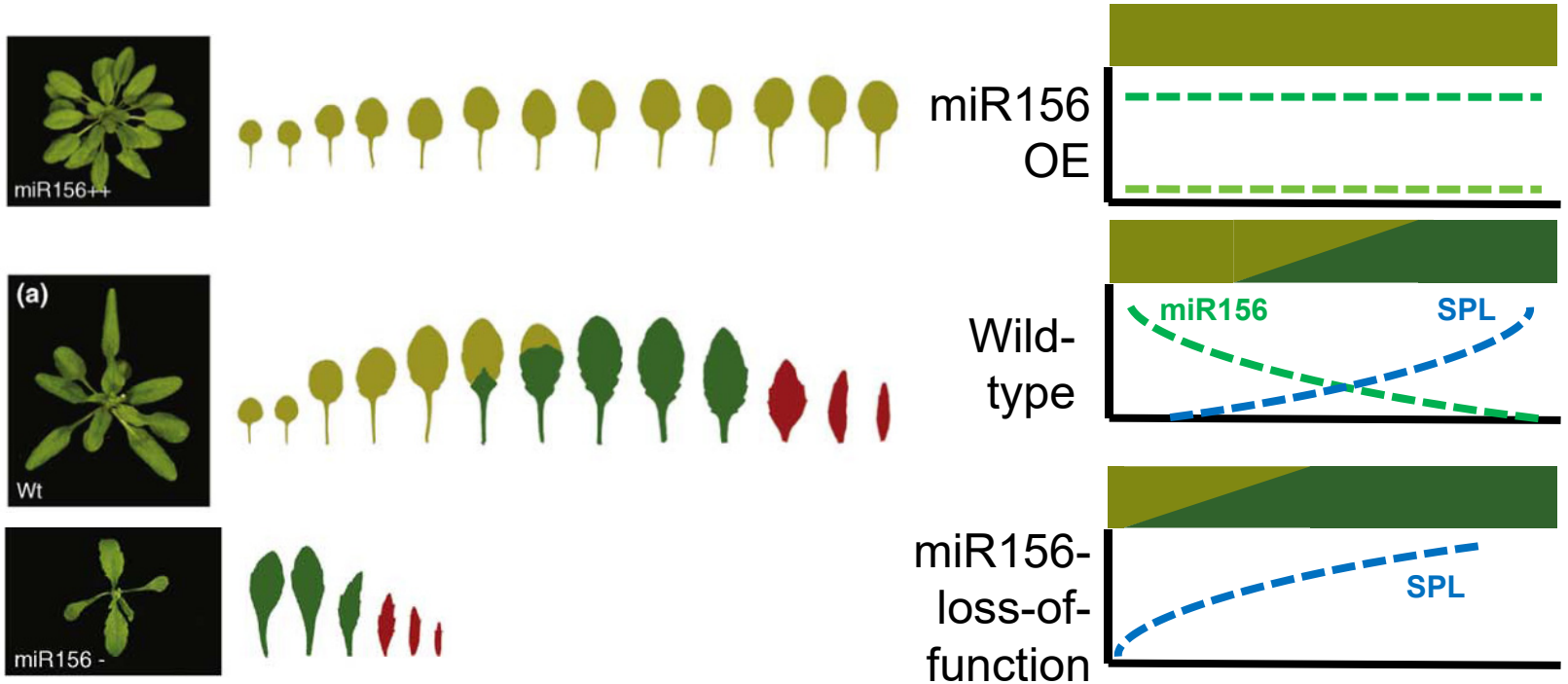
# miR156-resistant *SPL* promotes precocious phase change

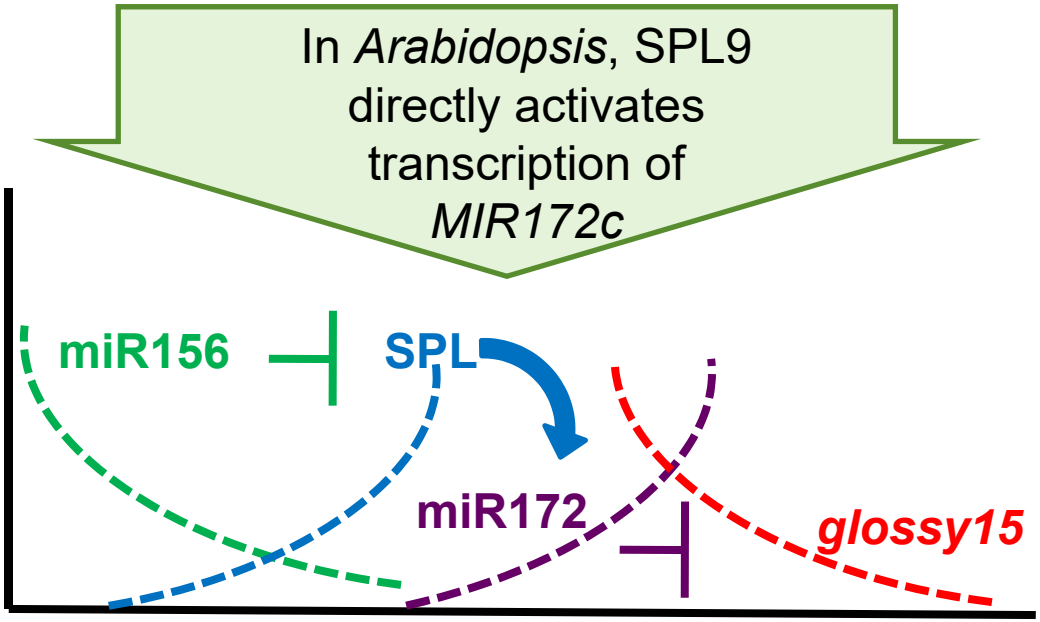

Reproduced with permission from Wu, G., and Poethig, R.S. (2006) Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. Development **133**: 3539–3547.

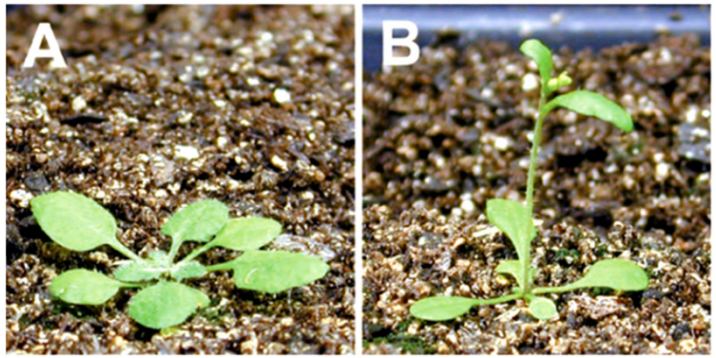
# miR156 loss-of-function promotes precocious phase change




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

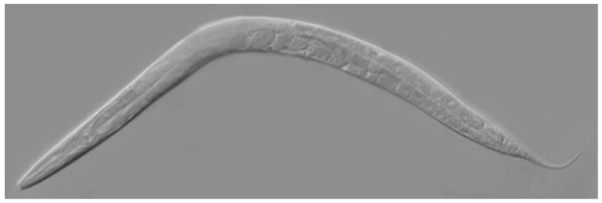


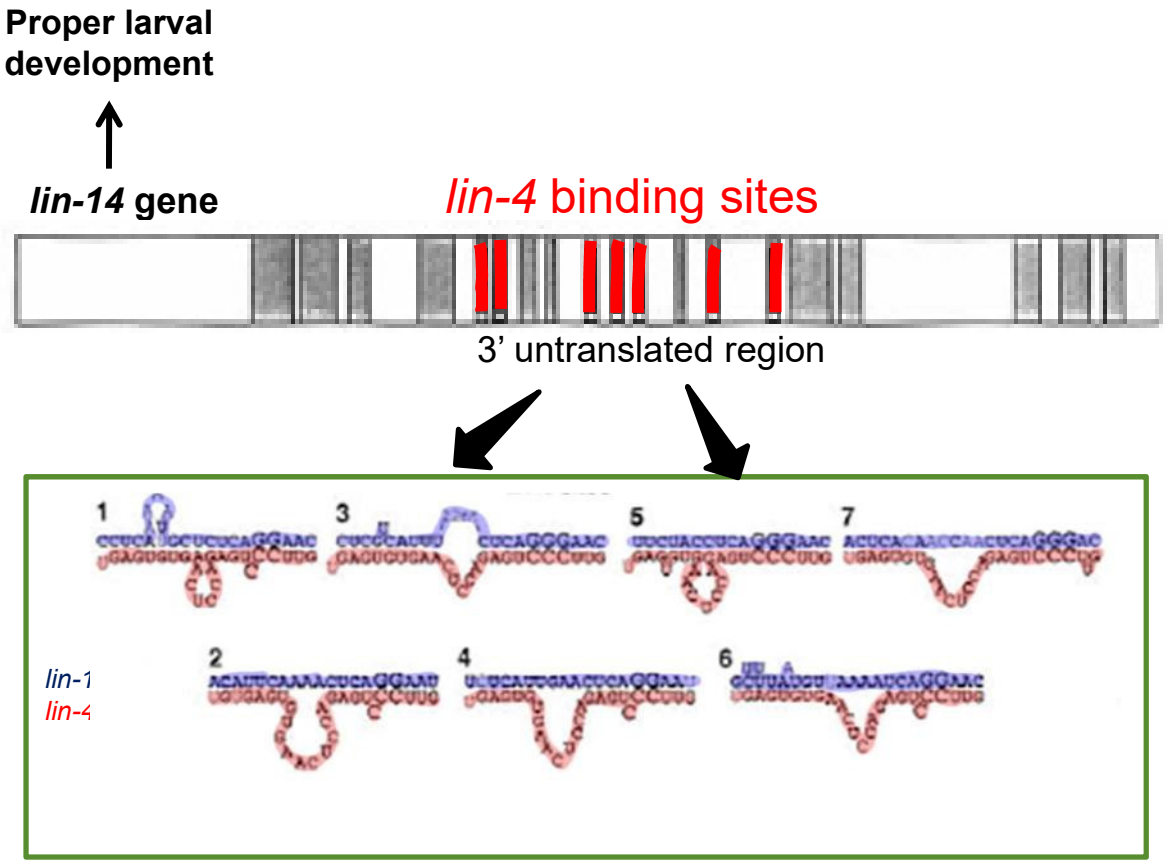




*Arabidopsis* plants overexpressing miR172 flower early.

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.

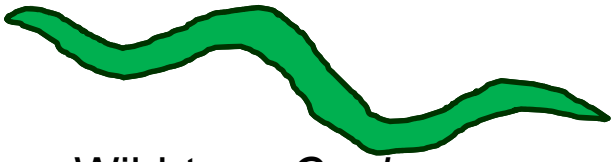
# miRNAs regulate developmental timing in other organisms





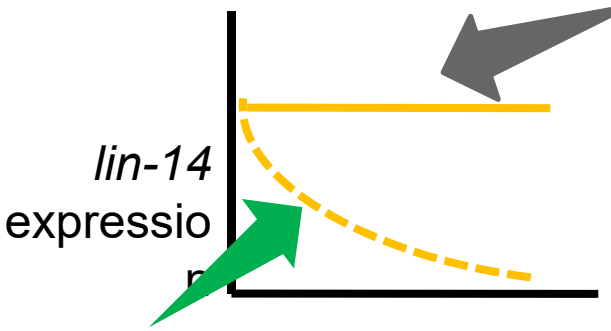

Lee, R.C., Feinbaum, R.L., and Ambrose, V. (1993). The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell 75: 843–845. Wightman, B., Ha, I., and Ruvkun, G. (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates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

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



Wild-type *C. elegans*

*lin-4* Loss-of-function




*lin-4* loss-of-function causes *lin-14* expression to remain high.

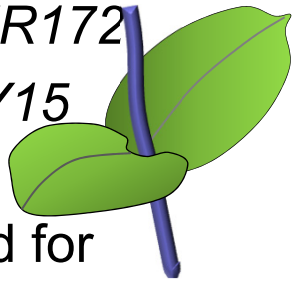
In wild-type worms, *lin-14* is expressed early and then shut off.

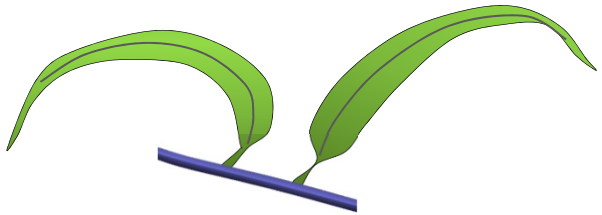
***lin-4* is a negative regulator of *lin-14*.**

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