Universidade de São Paulo (USP)
Escola de Engenharia de Lorena (EEL)
Engenharia Bioquímica

Aula 5 e 6
Regulação Gênica em Eucariotos

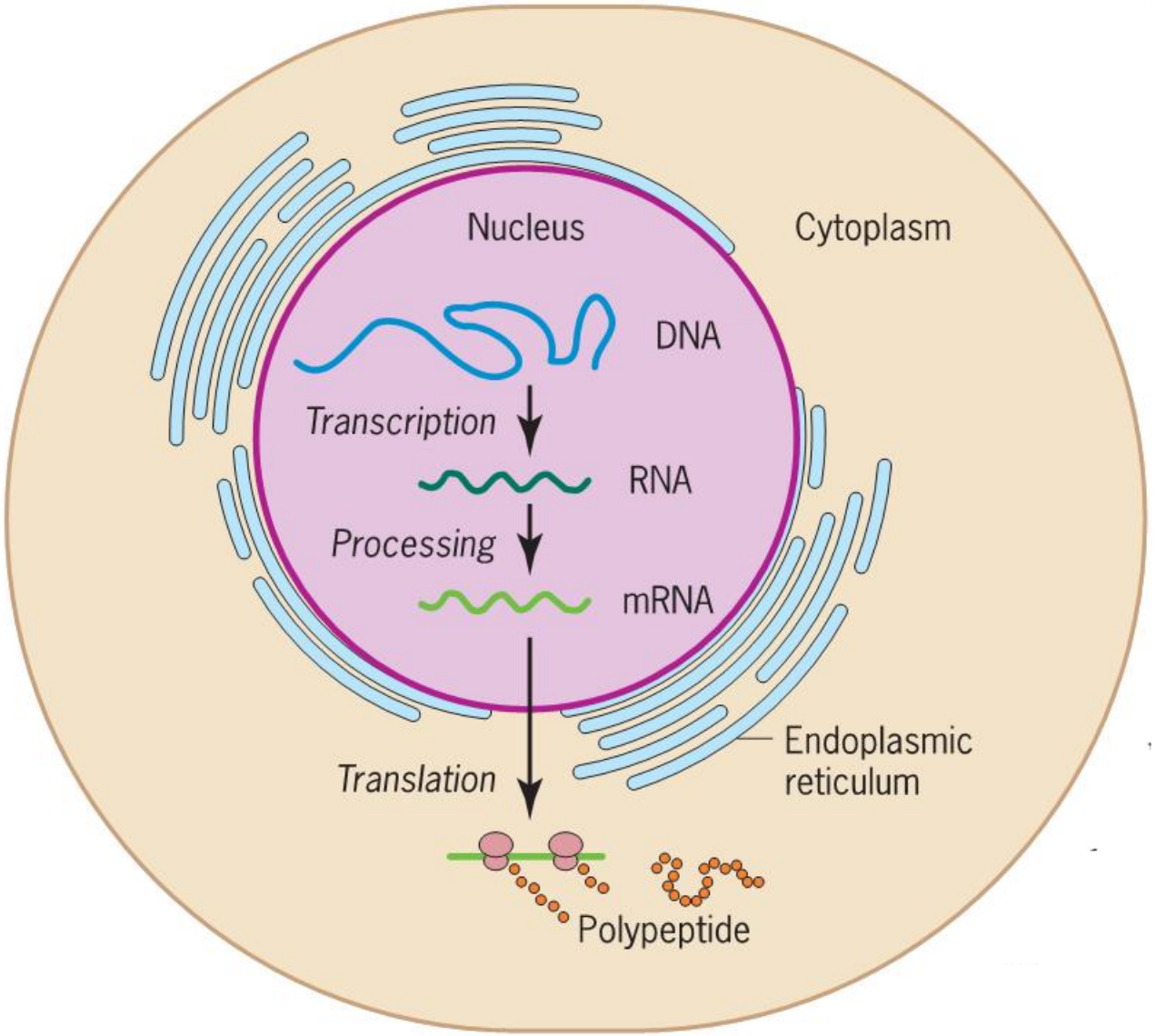
Elisson Romanel

Roteiro da aula

1. Regulação da Expressão Gênica
2. Fatores transcricionais
3. Promotor e elementos regulatórios
4. Regulação Pós-Transcricional
5. Regulação Epigenética

Regulação Gênica Eucariótica

Regulação Gênica Eucariótica



- Anatomia e fisiologia
- Órgãos e tecidos
- Temporal e Espacial
- Sistema elaborado de membranas

Transcrição do DNA é controlada

Recomposição alternativa de RNA

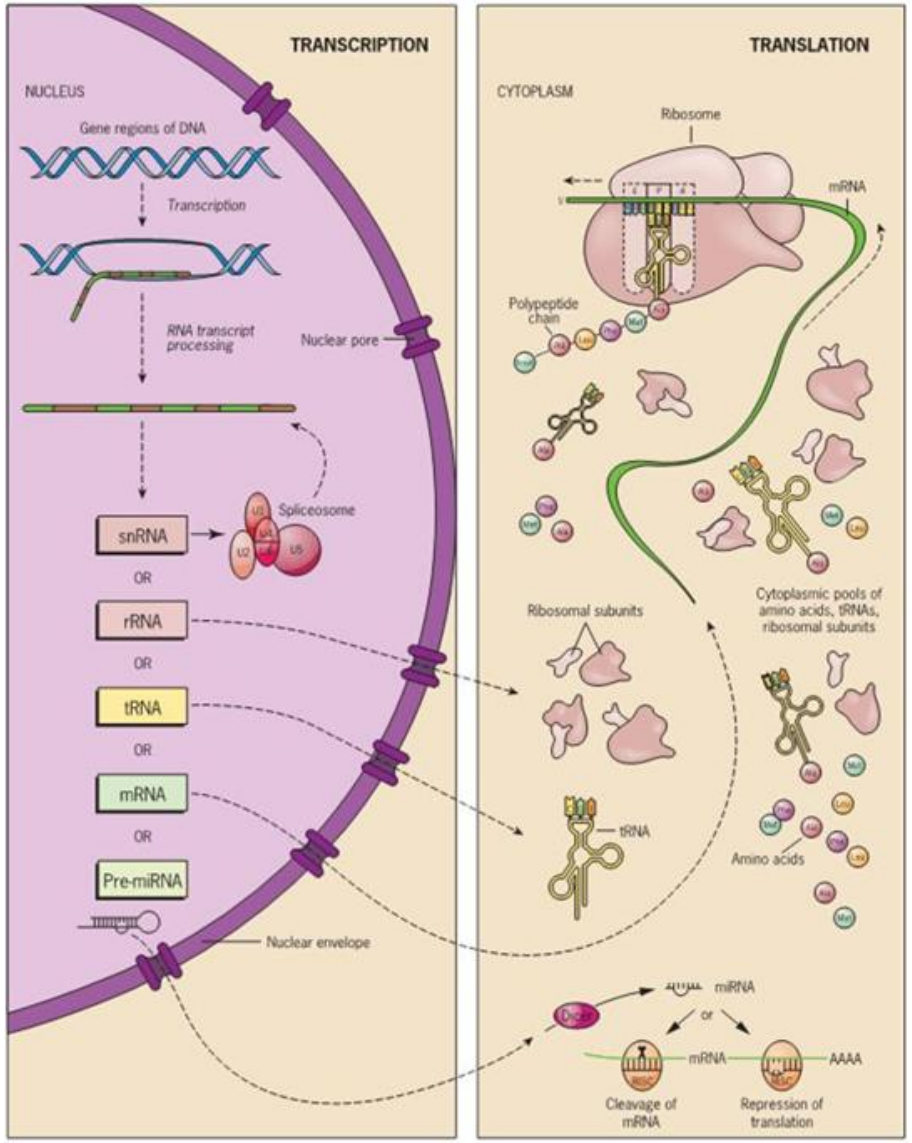
Estabilidade e controle do RNA

Principles of Genetics (Cap. 19)

RNA polimerase

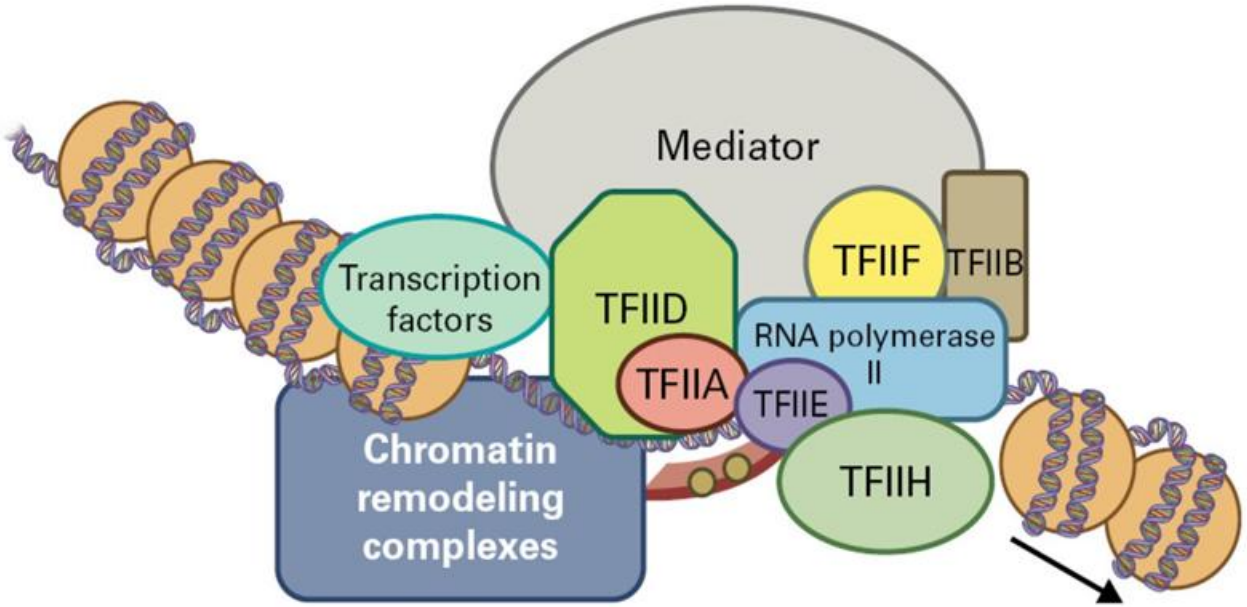
TABLE 9.2 *Plant nuclear RNA polymerases and their products.*

RNAP	RNA synthesized
I	5.8S, 18S and 28S rRNAs
II	mRNAs, miRNAs, snRNAs, ta-siRNAs
III	5S rRNA, tRNAs, snRNAs
IV	siRNAs
V	noncoding RNAs



Principles of Genetics (Cap. 11); Buchanan et al., 2015 (Cap. 9)

Genes x Expressão Gênica



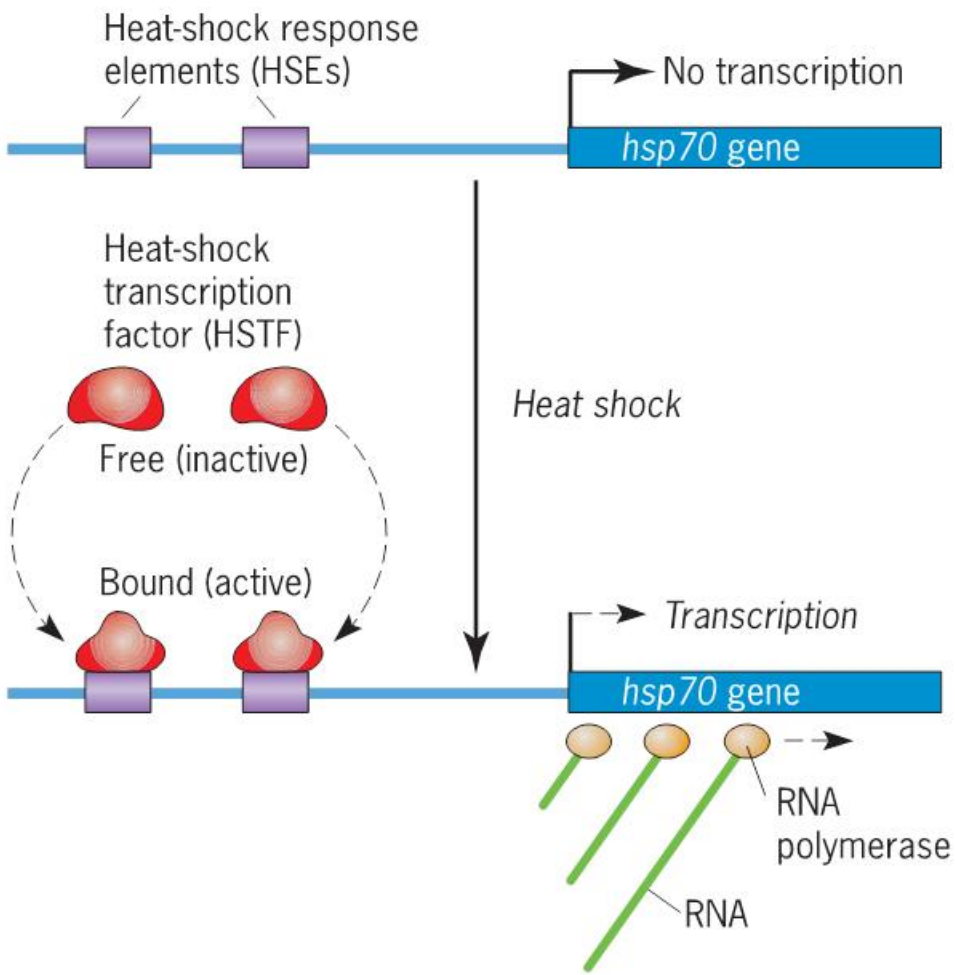
- Genes de expressão constitutiva

- Genes de expressão específica (desenvolvimento, tecido, celular)

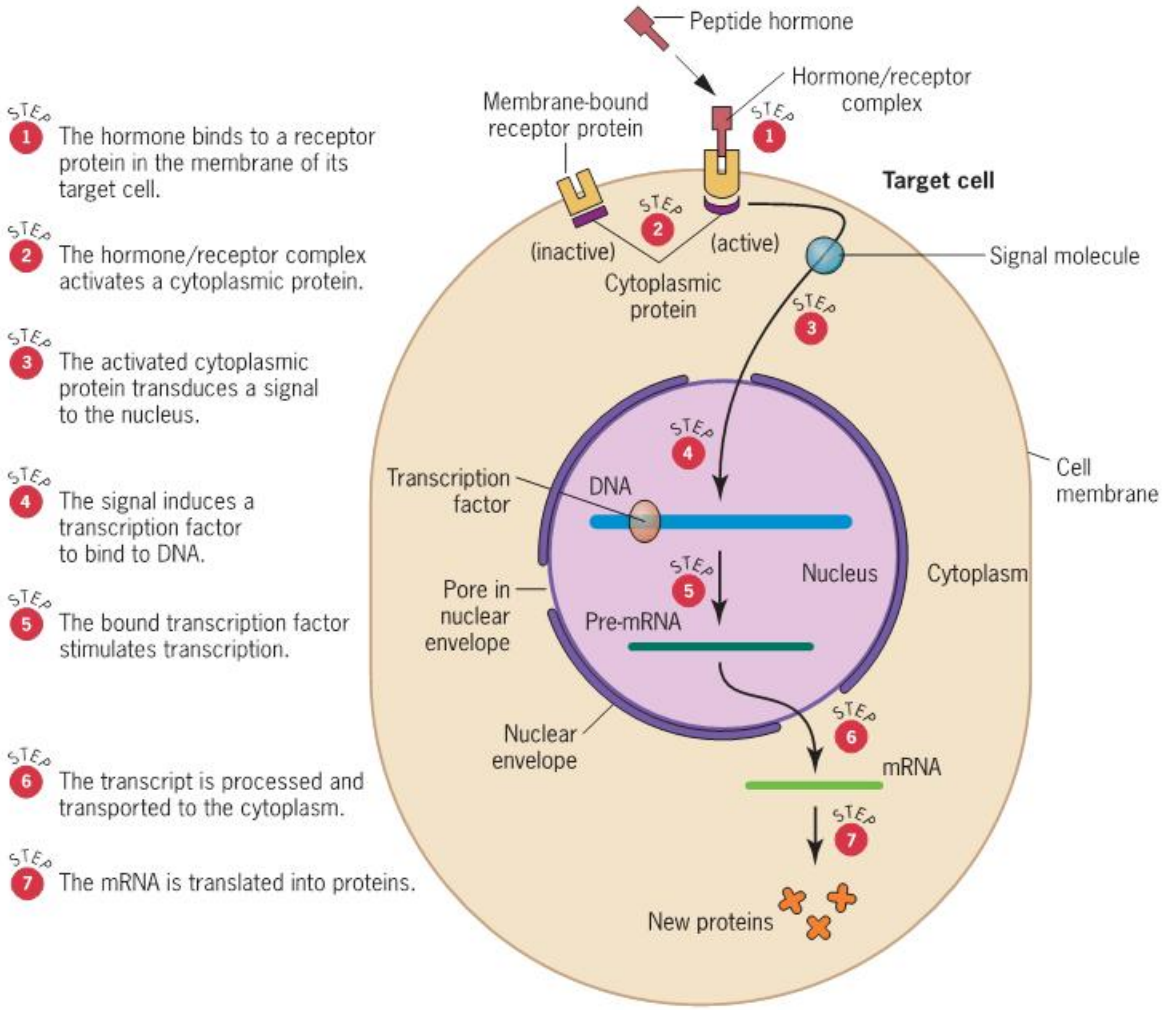
The Cell (Cap. 6); Buchanan et al., 2015 (Cap. 9)

Genes x Expressão Gênica

- Genes induzidos por calor

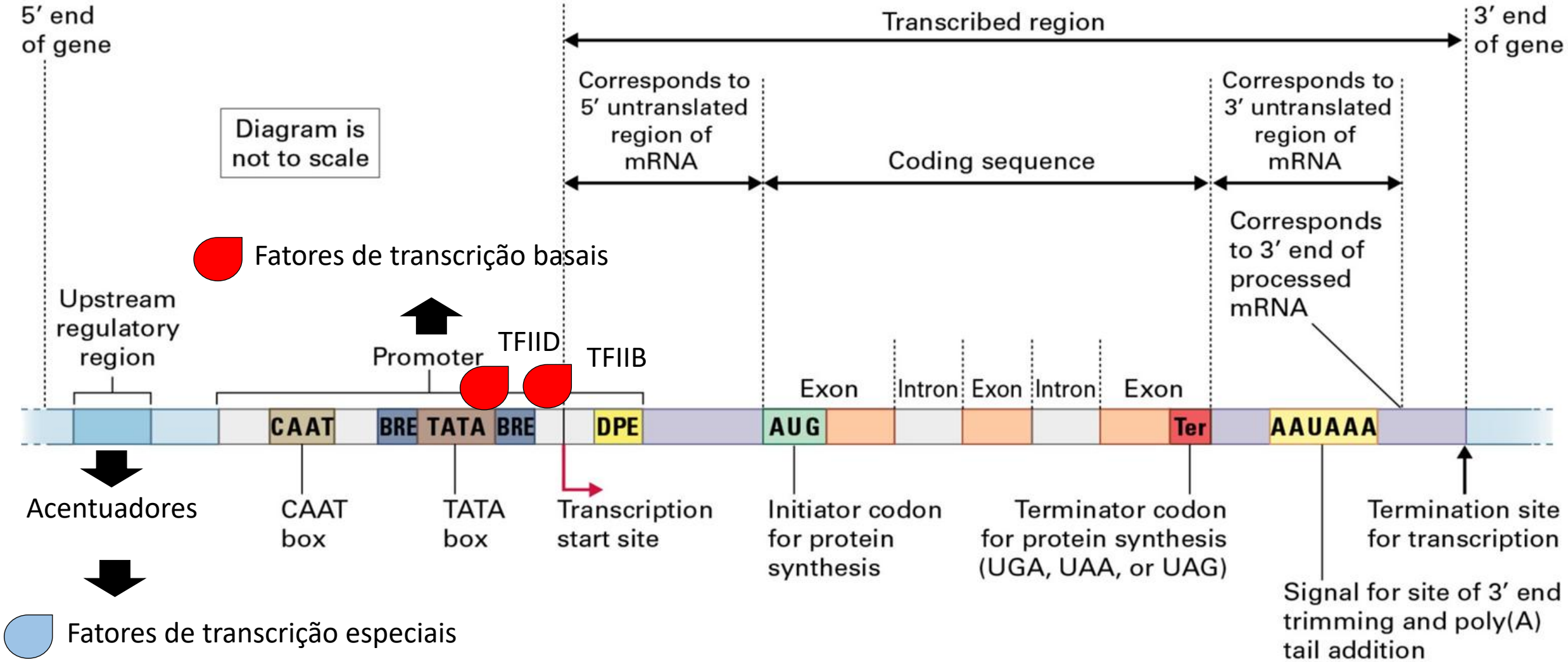


- Genes reguladores por transdução de sinal



Principles of Genetics (Cap.19)

Controle Molecular da Transcrição



Buchanan et al., 2015 (Cap. 9)

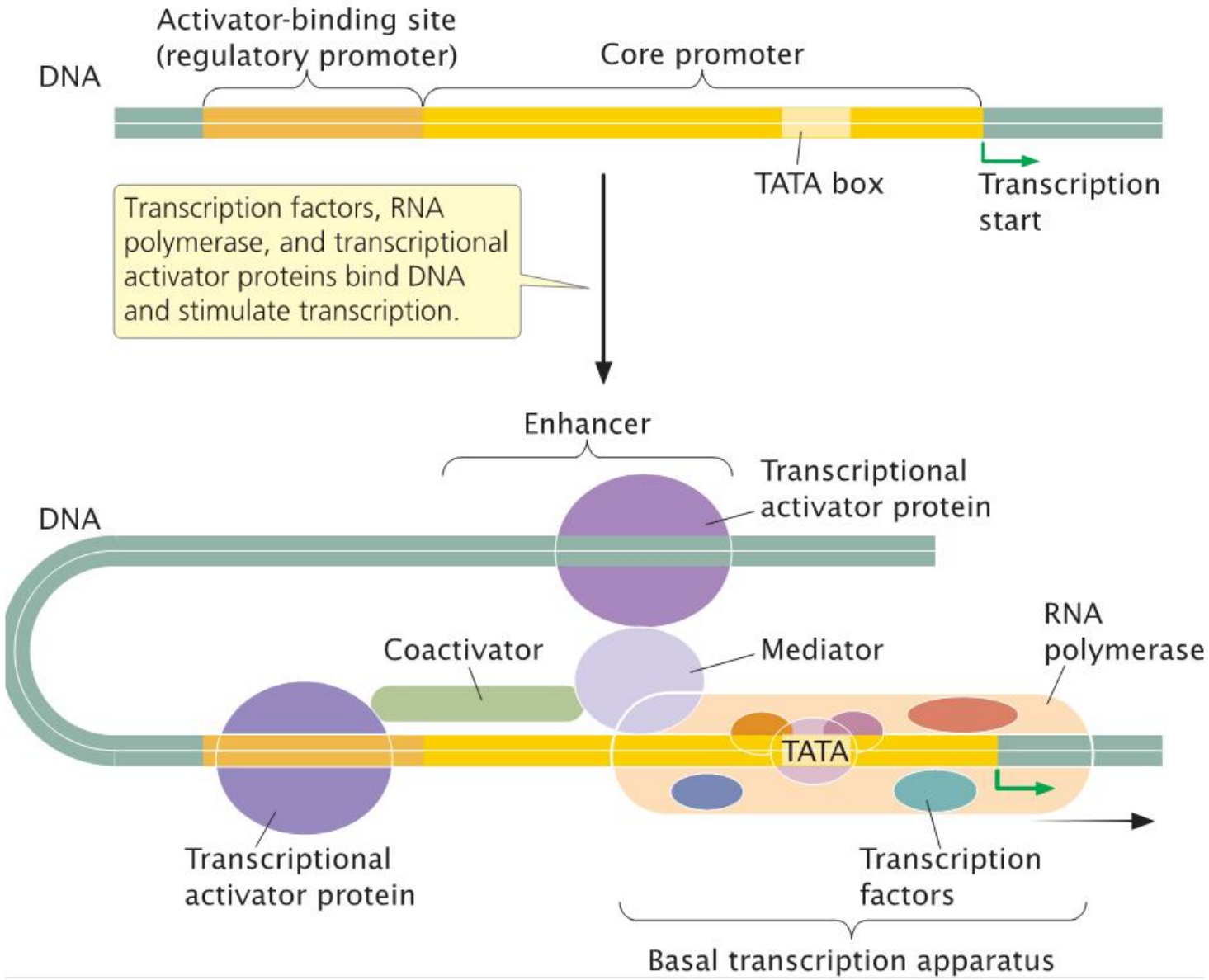
Promotores e elementos regulatórios

TABLE 9.4 *Examples for DNA sequence motifs bound by* Arabidopsis *transcription factors, and their functions in plant growth and development.*

Motif name	Sequence	Bound by	Function
ABA-responsive element (ABRE)	CACGTGGC	ABRE-binding factors (bZIP proteins)	ABA response
Auxin response element (ARE)	TGTCTC	Auxin response factors	Auxin response
CArG box	$CC(A/T)_6GG$	MADS domain proteins	Flower development, etc.
Evening element	AAAATATCT	Certain MYB proteins	Gene regulation by the circadian clock
G box	CACGTG	bZIP and bHLH proteins	Light response, etc.
GATA promoter motif	(A/T)GATA(G/A)	GATA transcription factors	Various

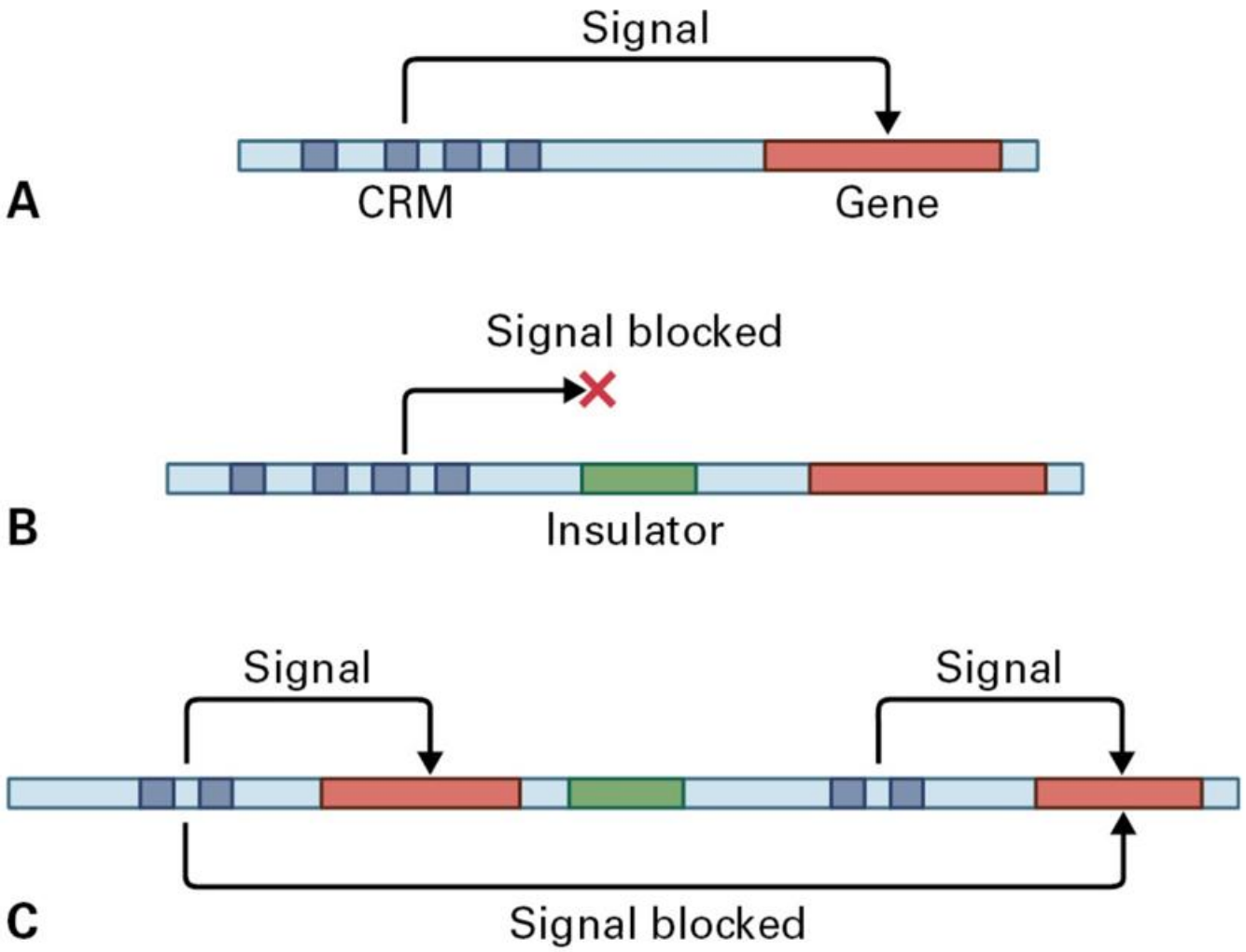
Buchanan et al., 2015 (Cap. 9)

Controle Molecular da Transcrição



Genetics (Cap. 17)

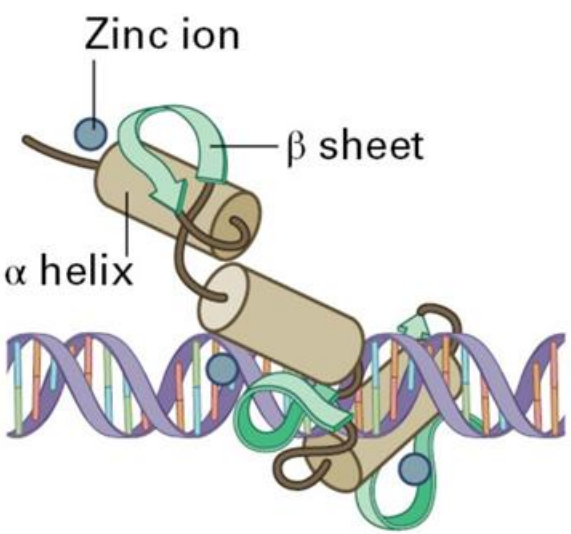
Promotores e elementos regulatórios



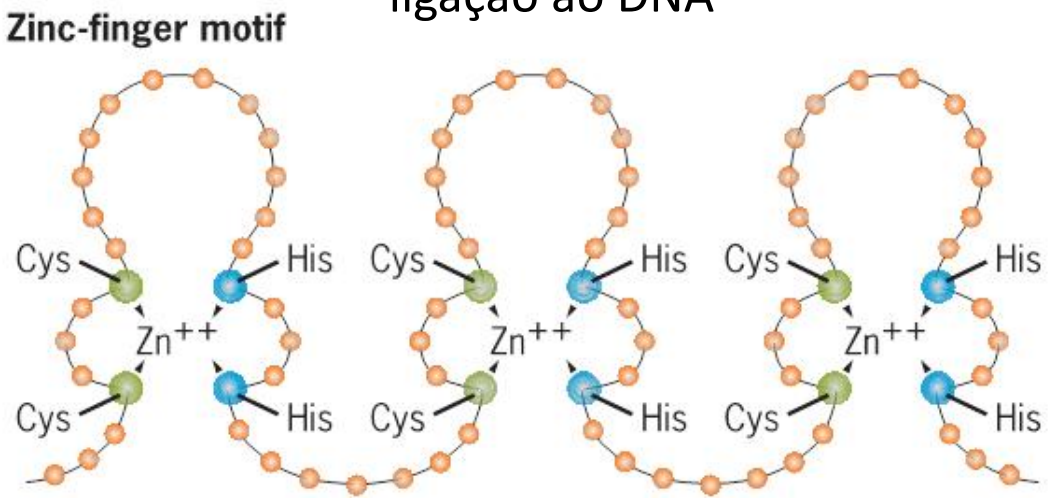
Buchanan et al., 2015 (Cap. 9)

Fator de Transcrição

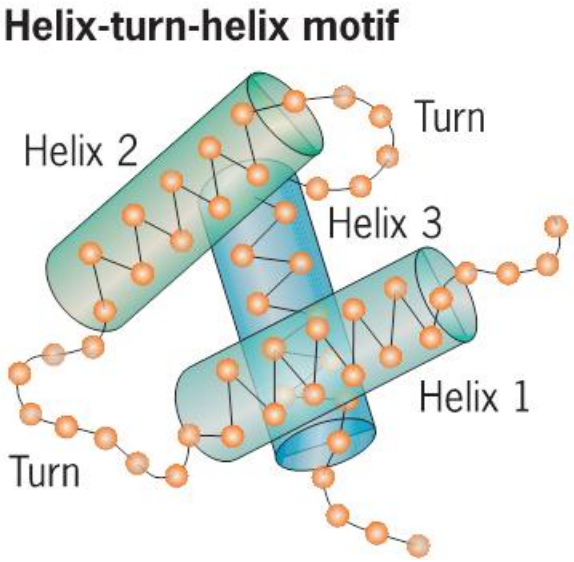
- Domínio de ligação ao DNA (DNA-binding domain)
- Domínio de ativação da transcrição (Transactivating domain)
- Sequencia de localização nuclear (Nuclear localization sequence-NLS)



ligação ao DNA



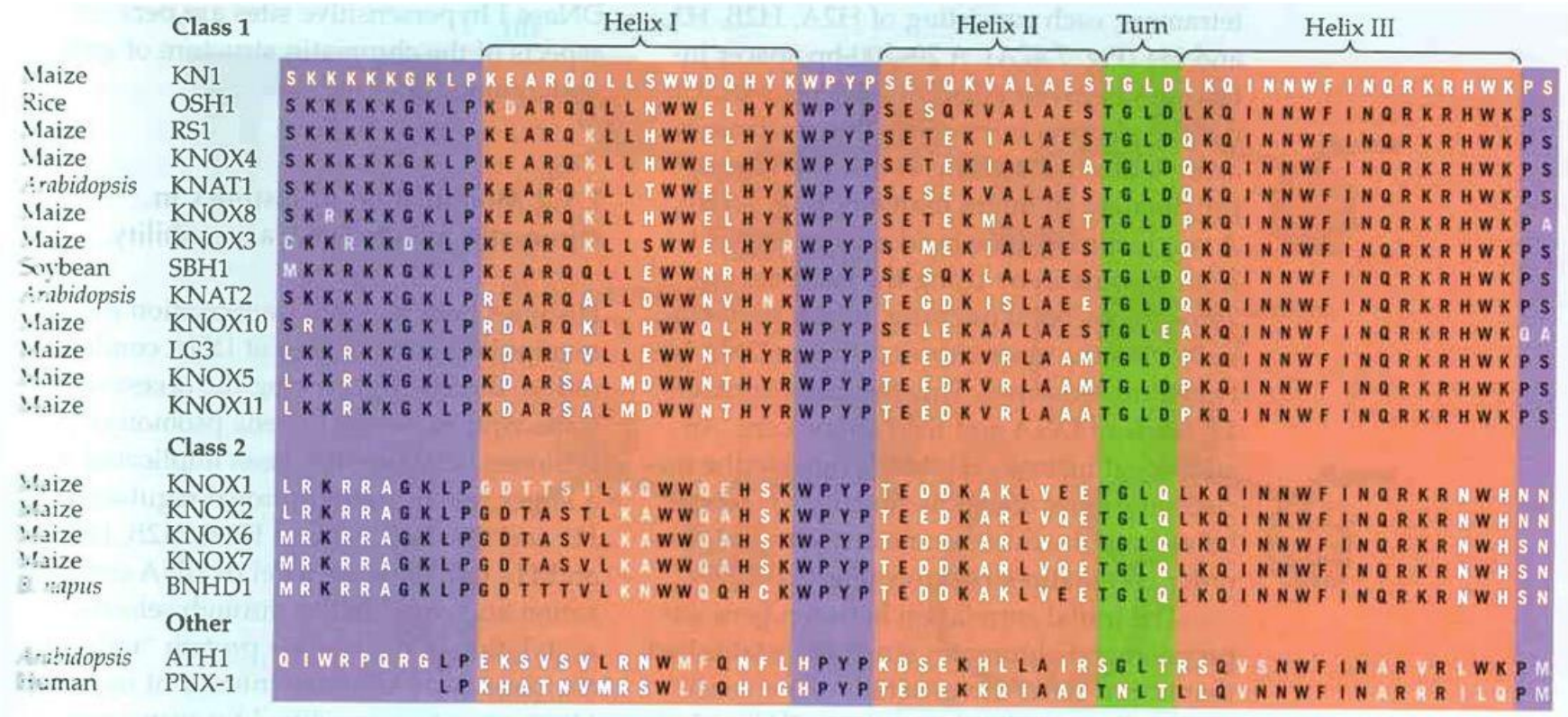
Homeodomínio

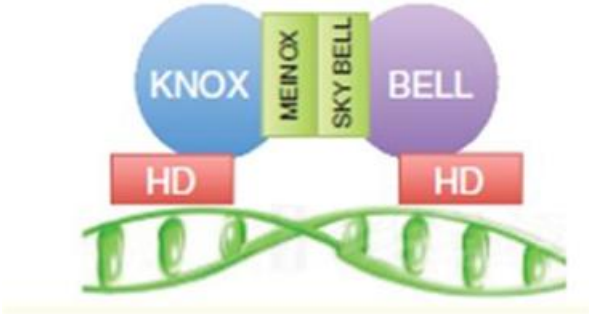


Principles of Genetics (Cap 19); Buchanan et al., 2015 (Cap. 9)

Fator de Transcrição

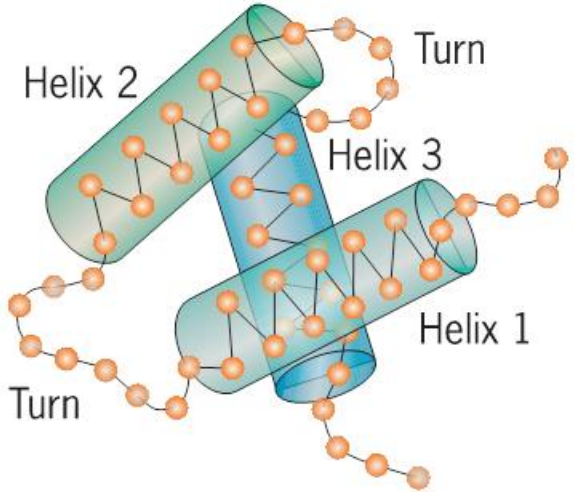
ligação ao DNA





Homeodomínio

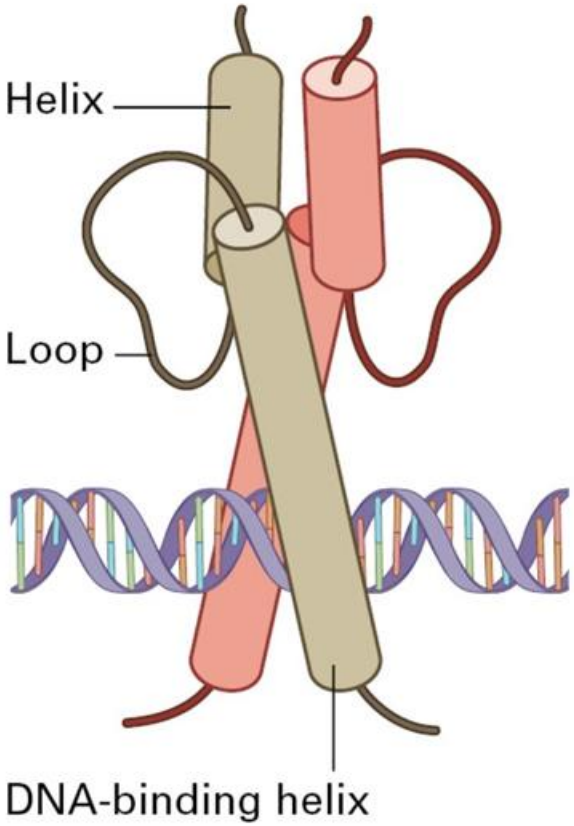
Helix-turn-helix motif

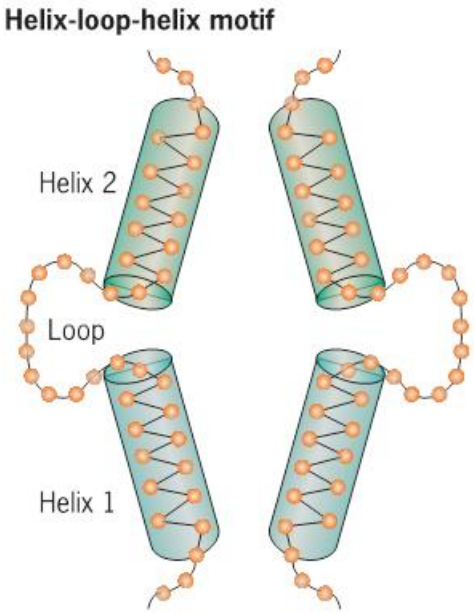


Hay and Tsiantis, 2010 (Development); Principles of Genetics (Cap 19); Buchanan et al., 2002 (Cap. 7)

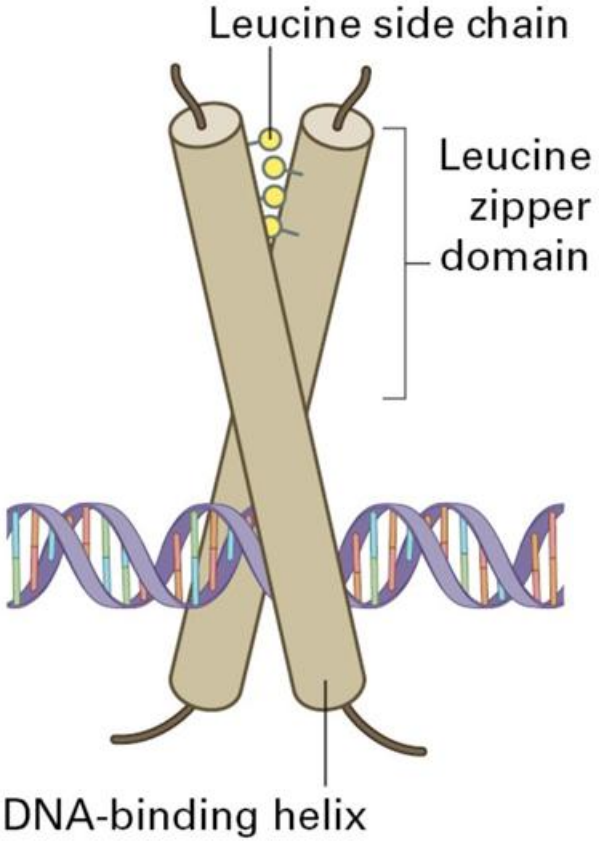
Fator de Transcrição

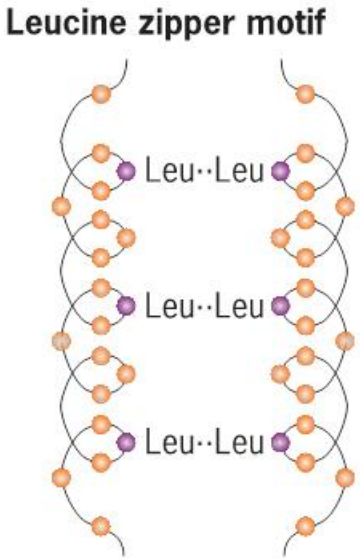
bHLH





bZIP





Homo ou heterodímero (Complexidade x expressão gênica)

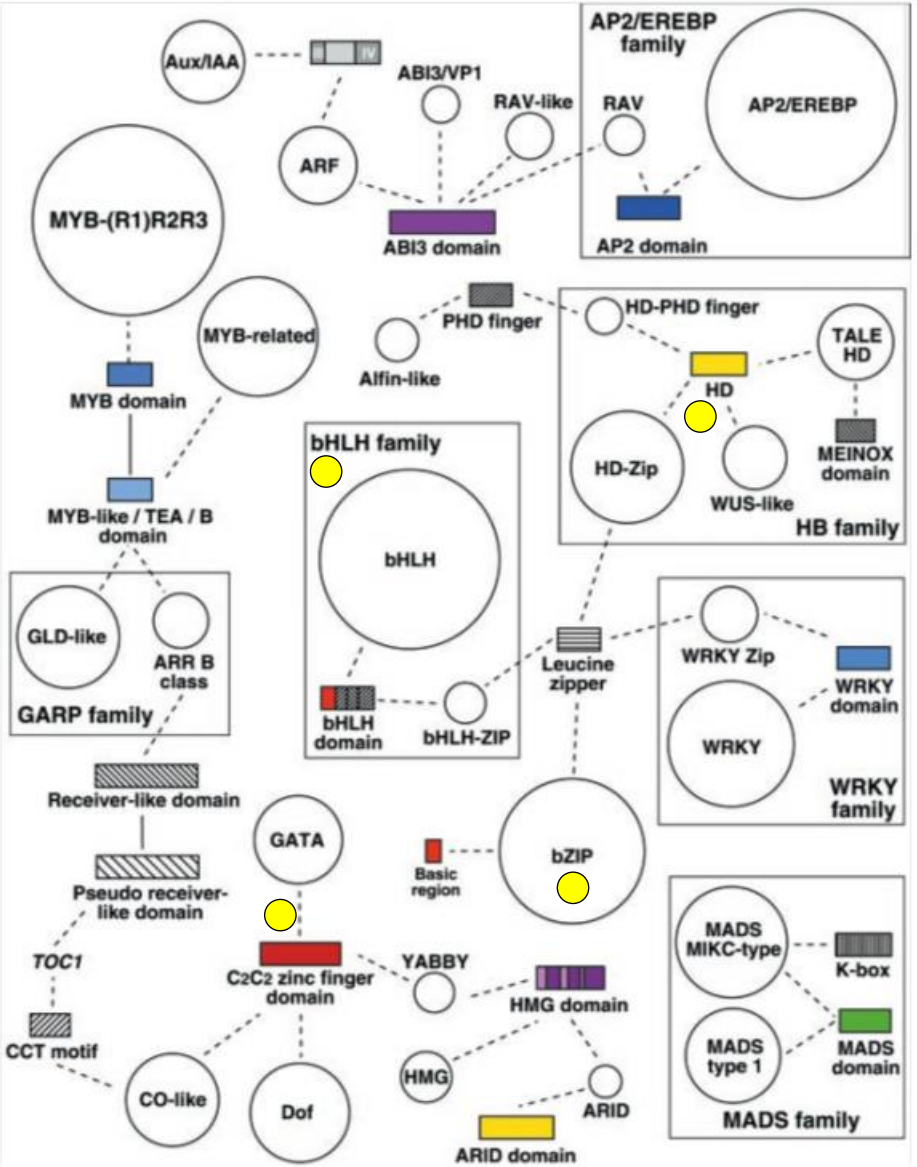
Principles of Genetics (Cap 19); Buchanan et al., 2015 (Cap. 9)

Fator de Transcrição

Organism	Total number of genes	Genes coding for transcriptional regulators	
		Total number	Percentage of total number of genes
A. thaliana	~26,000	1533	5.9
S. cerevisiae	~6,000	209	3.5
C. elegans	~19,000	669	3.5
D. melanogaster	~14,000	635	4.5

Family	*Arabidopsis*	Rice (*Oryza sativa*)	Plant specific
AP2/ERF	147	161	No*
bZIP	74	94	No
GRAS	32	57	Yes
MADS	108	77	No
MYB	198	182	No
NAC	100	149	Yes
WRKY	71	109	No*

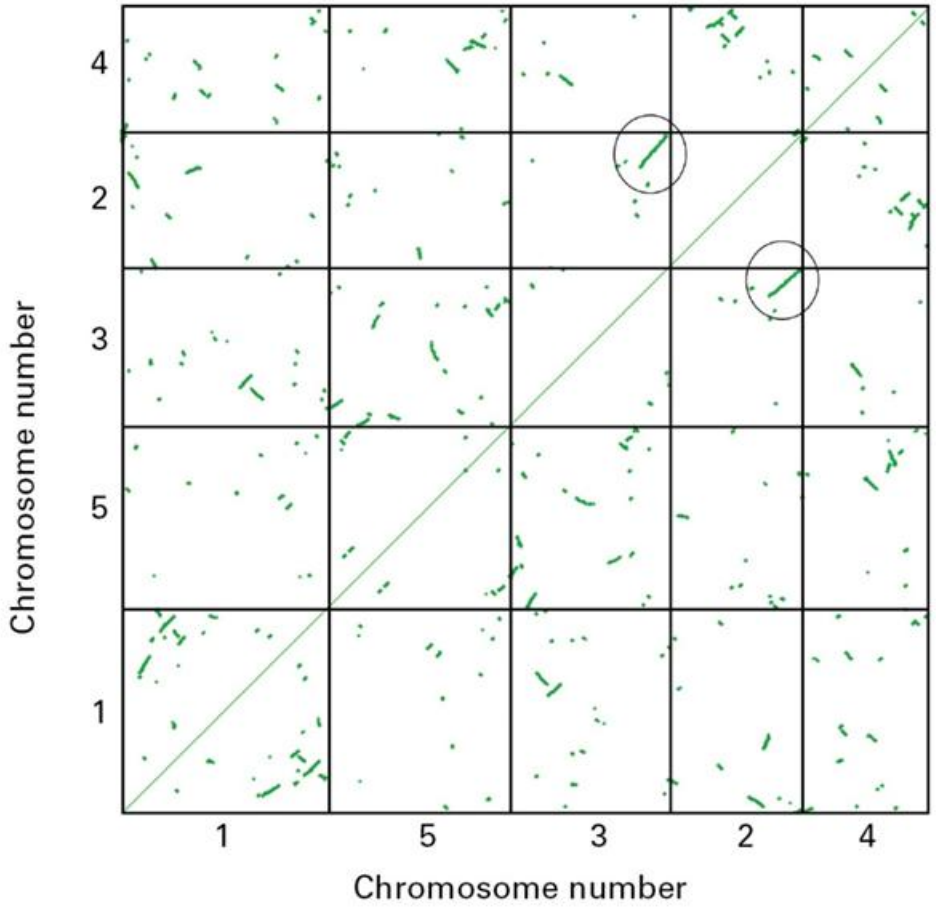
*But predominantly found in plants.

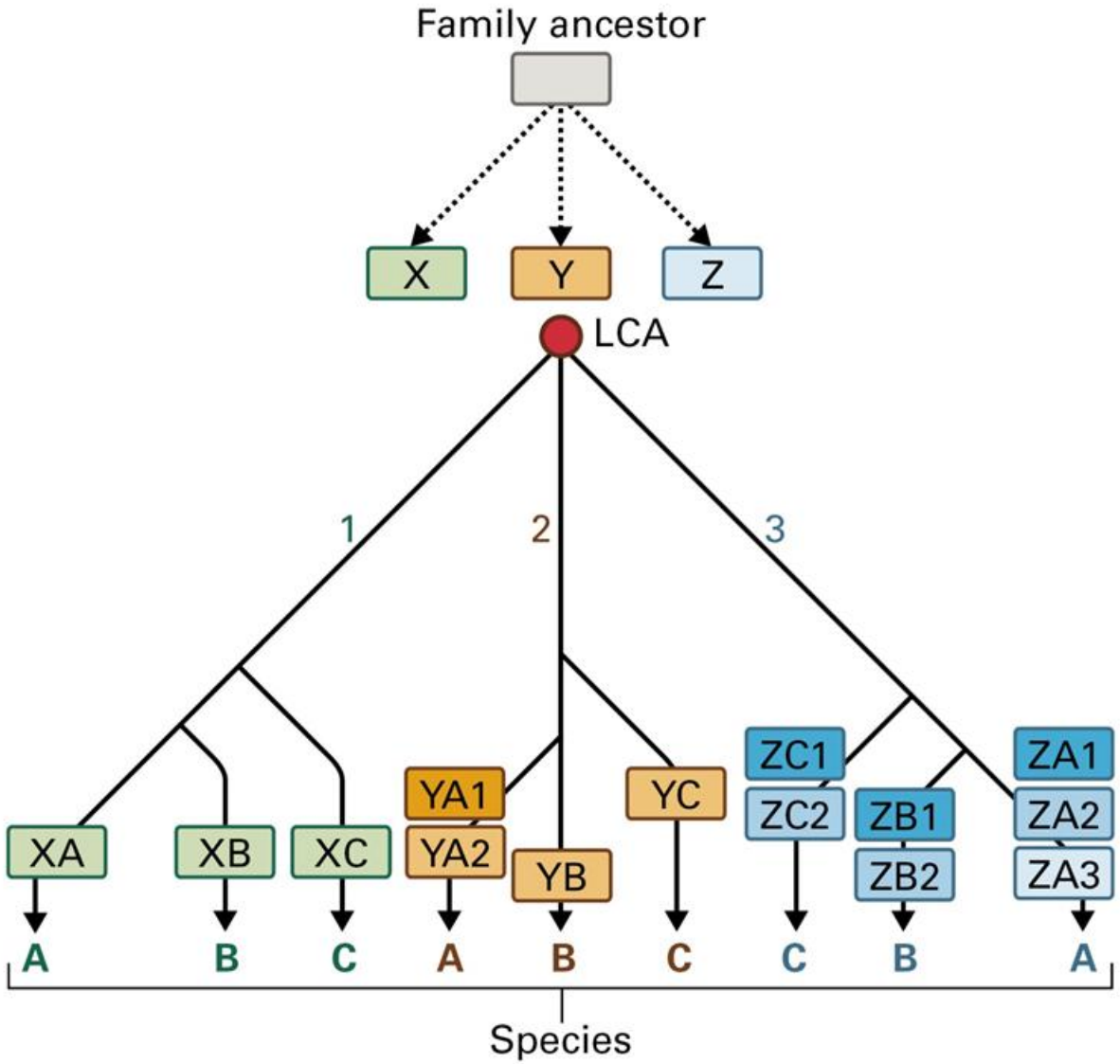


Riechmann et al., 2000 (Science); Buchanan et al., 2015 (Cap. 9)

Duplicação Gênica e Evolução

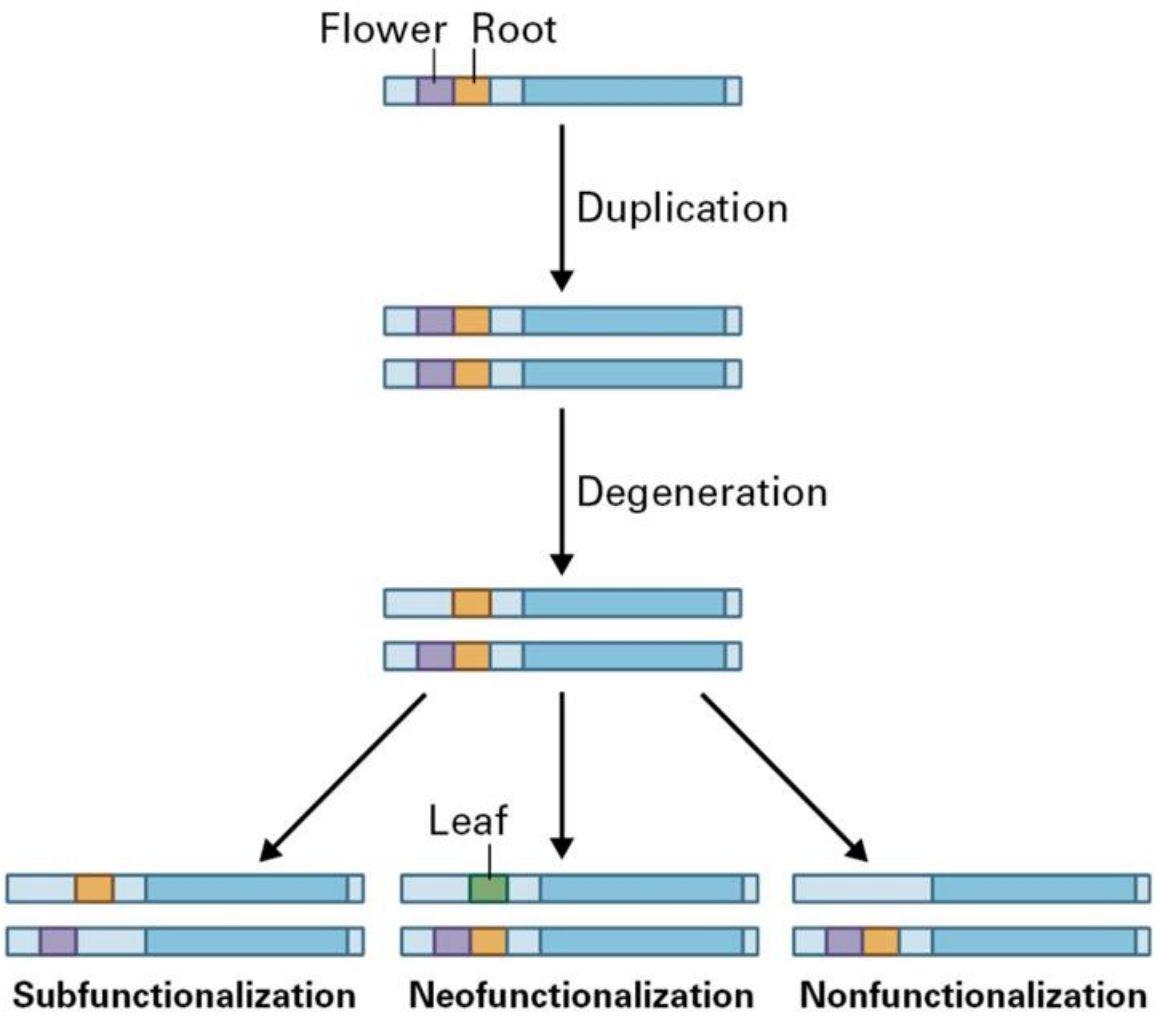
Genoma de *Arabidopsis thaliana*





Buchanan et al., 2015 (Cap. 9)

Duplicação Gênica, Evolução e Expressão



Buchanan et al., 2015 (Cap. 9)

Regulação pós-transcricional

- Interferência de RNA (RNAi)

The Nobel Prize in Physiology or Medicine 2006

Photo: L. Cicero
Andrew Z. Fire
Prize share: 1/2

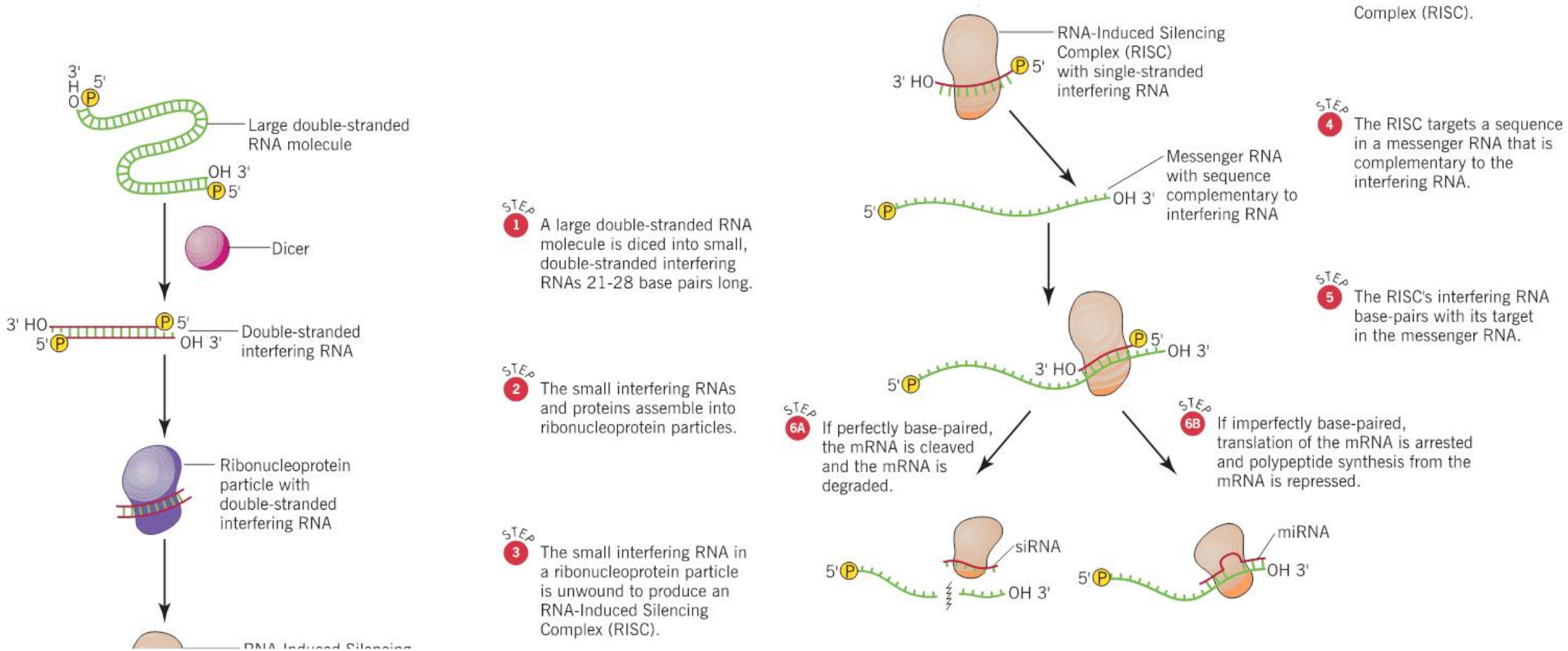
Photo: J. Mottern
Craig C. Mello
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2006 was awarded jointly to Andrew Z. Fire and Craig C. Mello *"for their discovery of RNA interference - gene silencing by double-stranded RNA"*

Principles of Genetics (Cap 19)

Regulação pós-transcricional

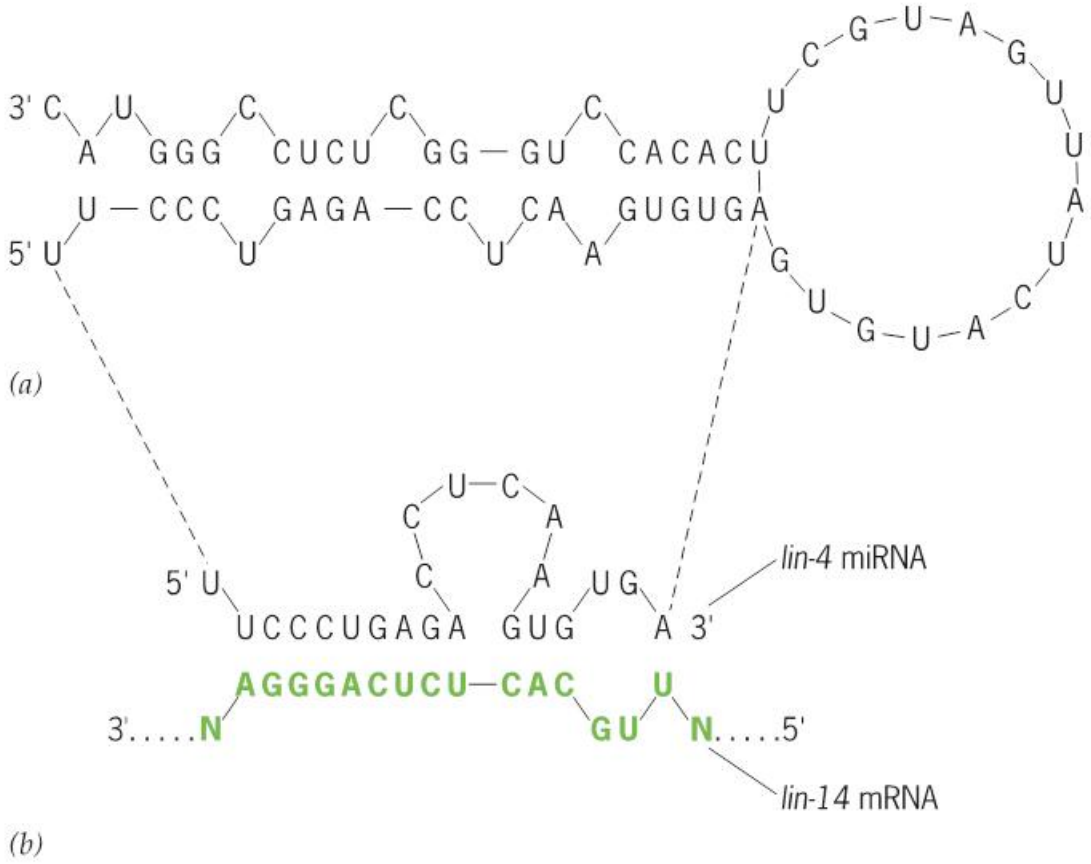
- Interferência de RNA (RNAi)



Principles of Genetics (Cap 19)

Regulação pós-transcricional

- Interferência de RNA (RNAi)



Vídeo

RNA Animation

Principles of Genetics (Cap 19)

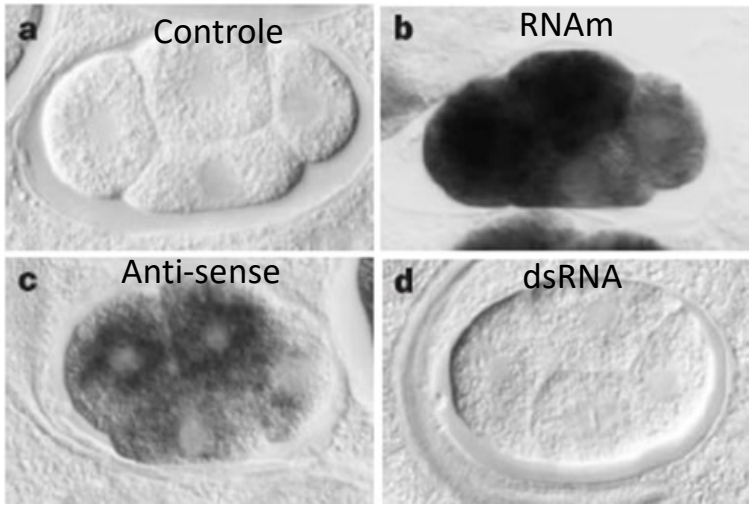
Regulação pós-transcricional

- Interferência de RNA (RNAi)

NATURE | VOL 391 | 19 FEBRUARY 1998

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡



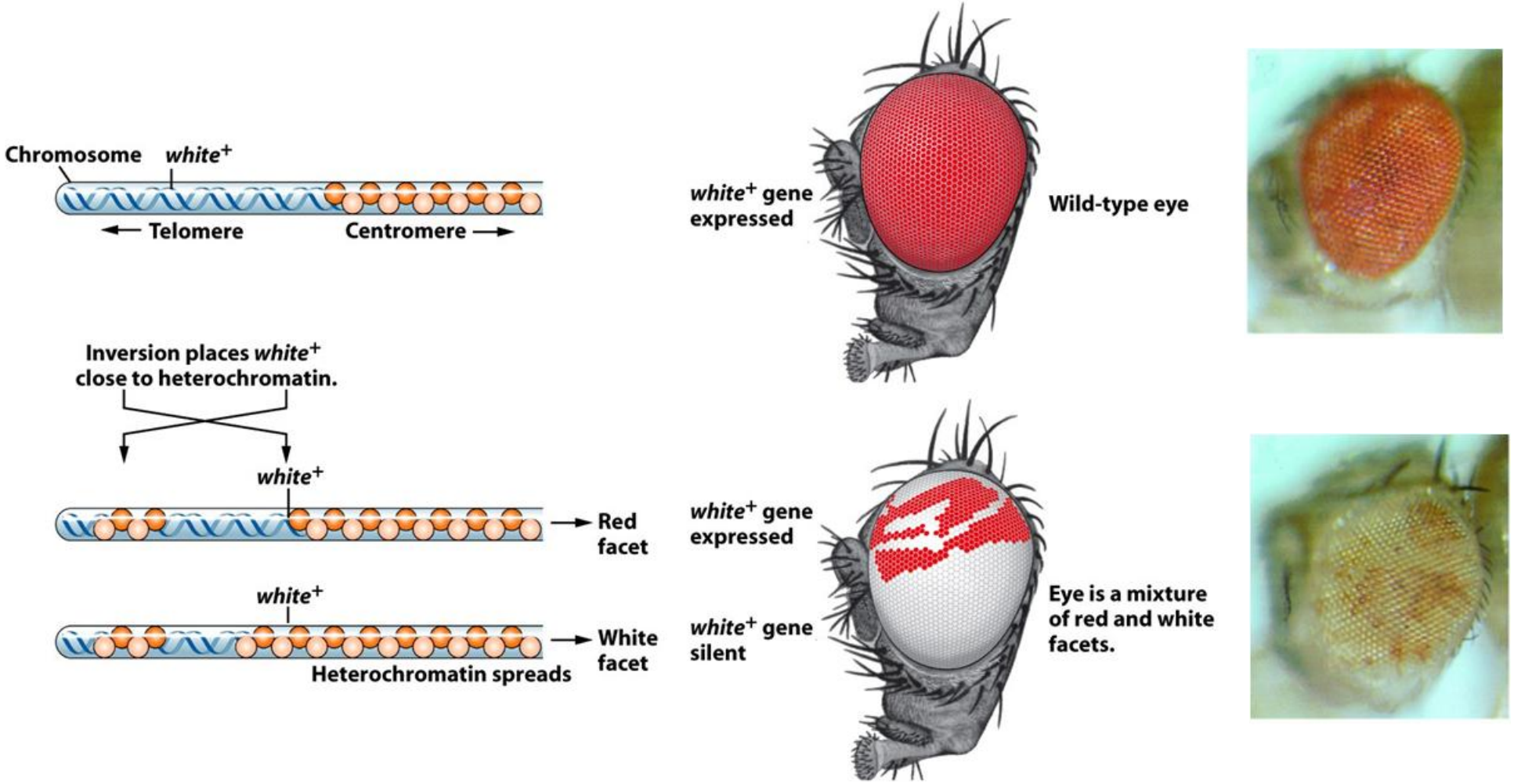
Selvagem

dsRNA

Principles of Genetics (Cap 19)

Organização da cromatina e Expressão gênica - Epigenética

- Variegação por efeito de posição

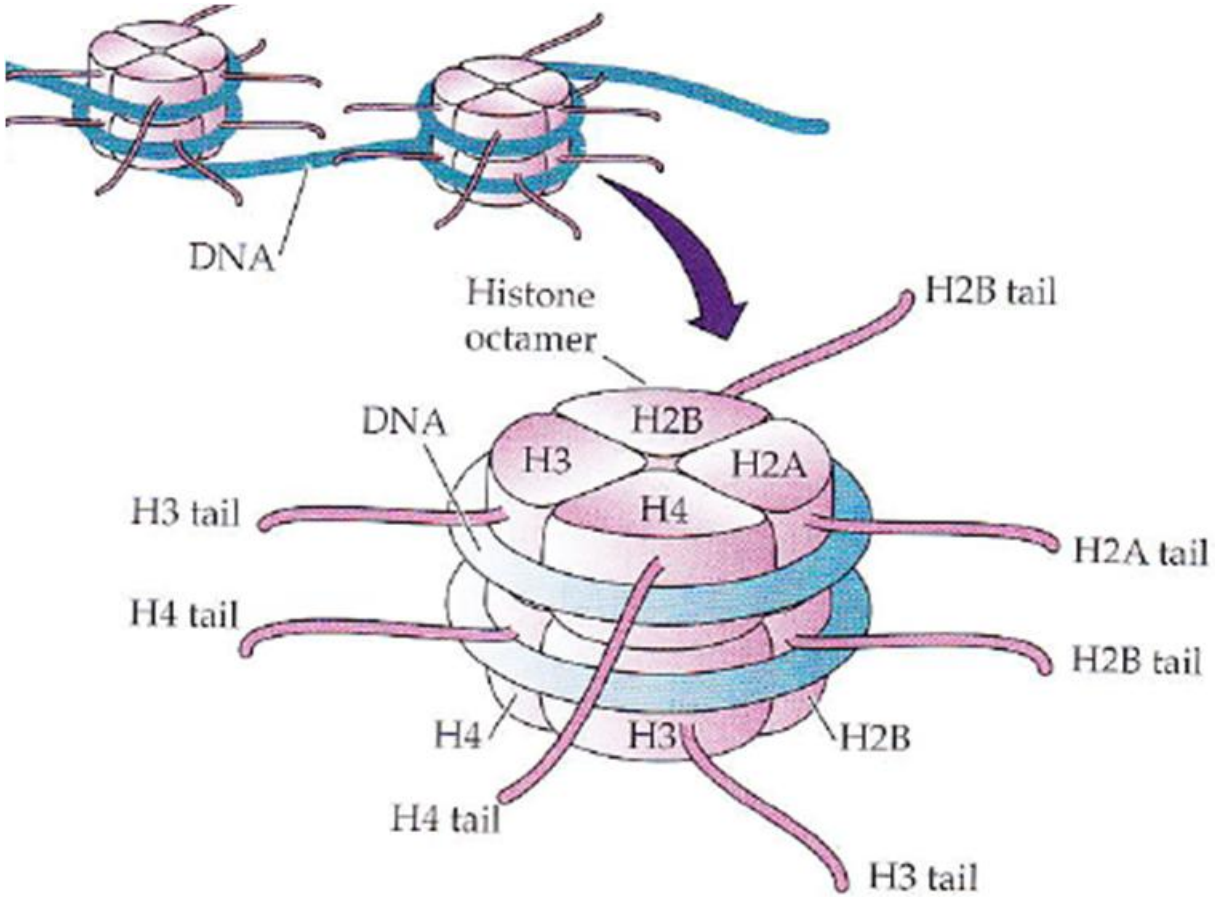


An Introduction to Genetics Analysis (Cap 10)

Organização da cromatina e Expressão gênica - Epigenética

- Remodelagem da Cromatina

- Histona acetiltransferase (Lisina)
- Quinases (serina)
- Histona desacetilases (HDAC)
- Histona metiltransferase (HMT)



Organização da cromatina e Expressão gênica - Epigenética

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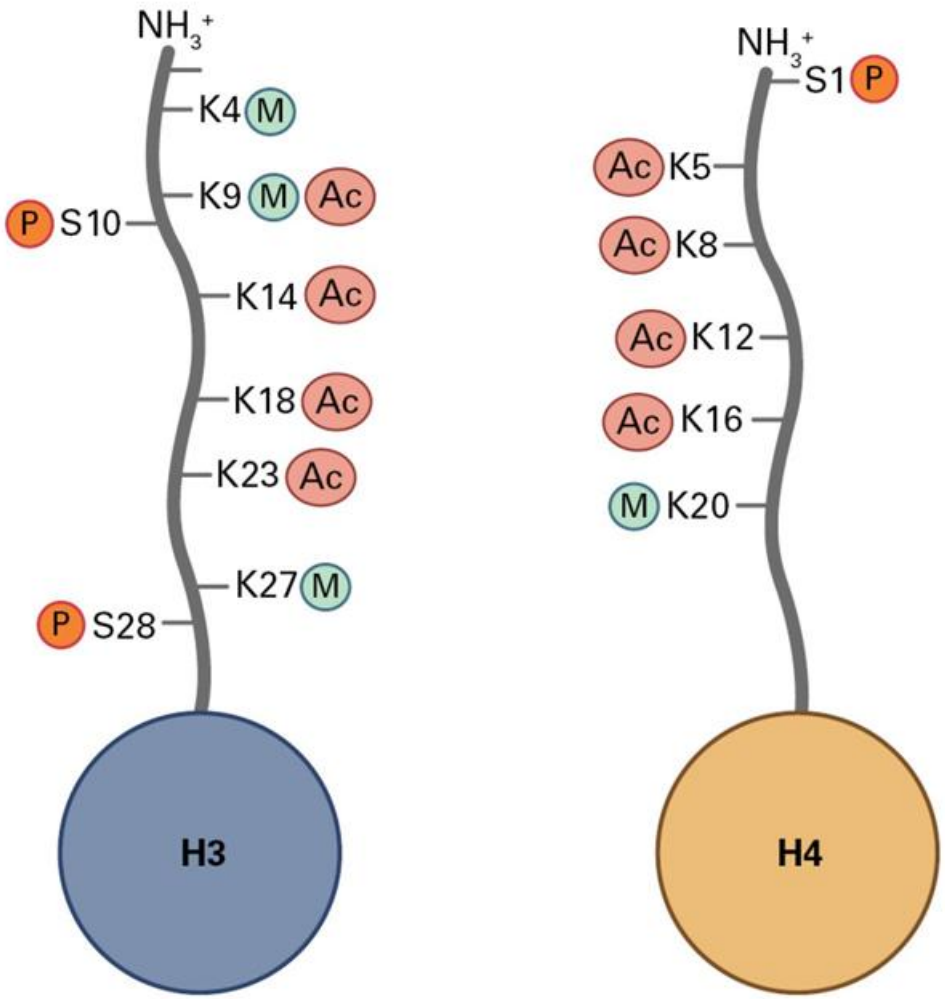

Buchanan et al., 2015 (Cap. 9)

Organização da cromatina e Expressão gênica - Epigenética

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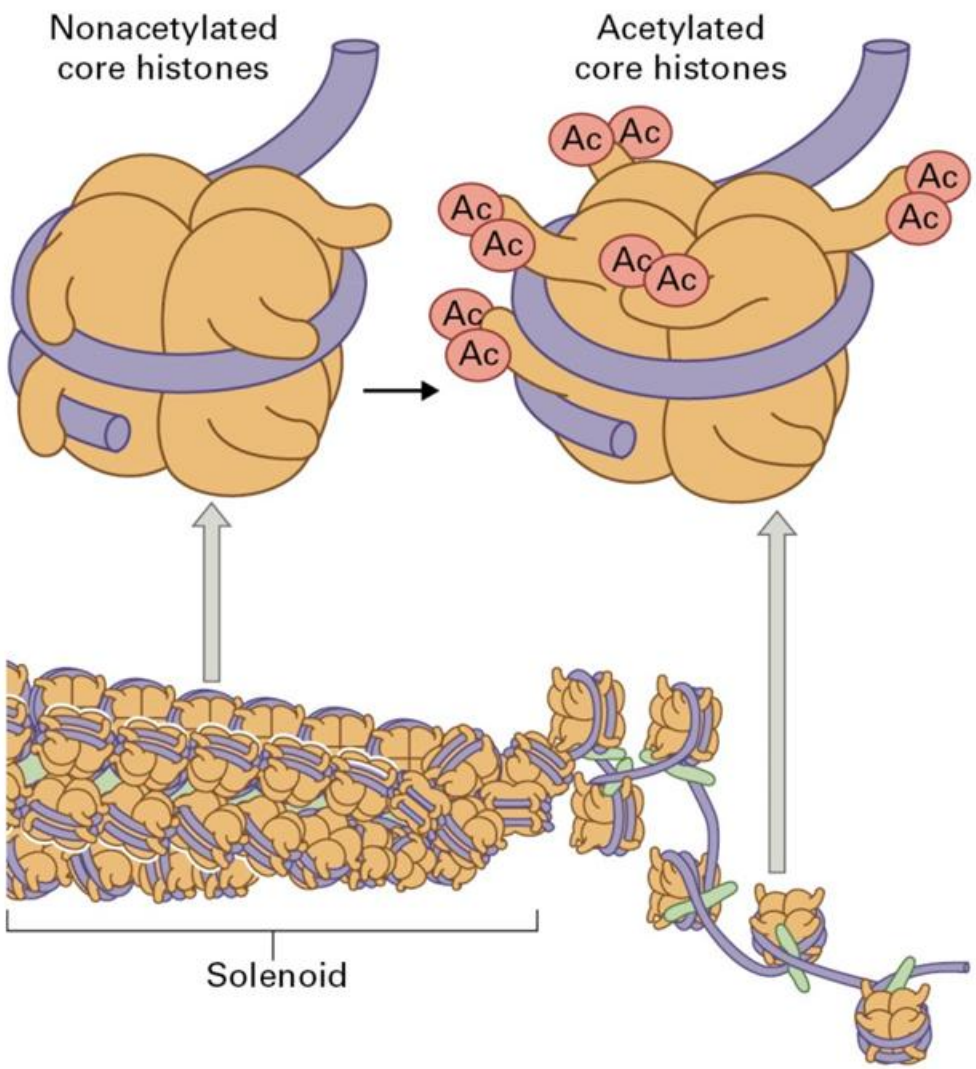

Buchanan et al., 2015 (Cap. 9)

Organização da cromatina e Expressão gênica - Epigenética

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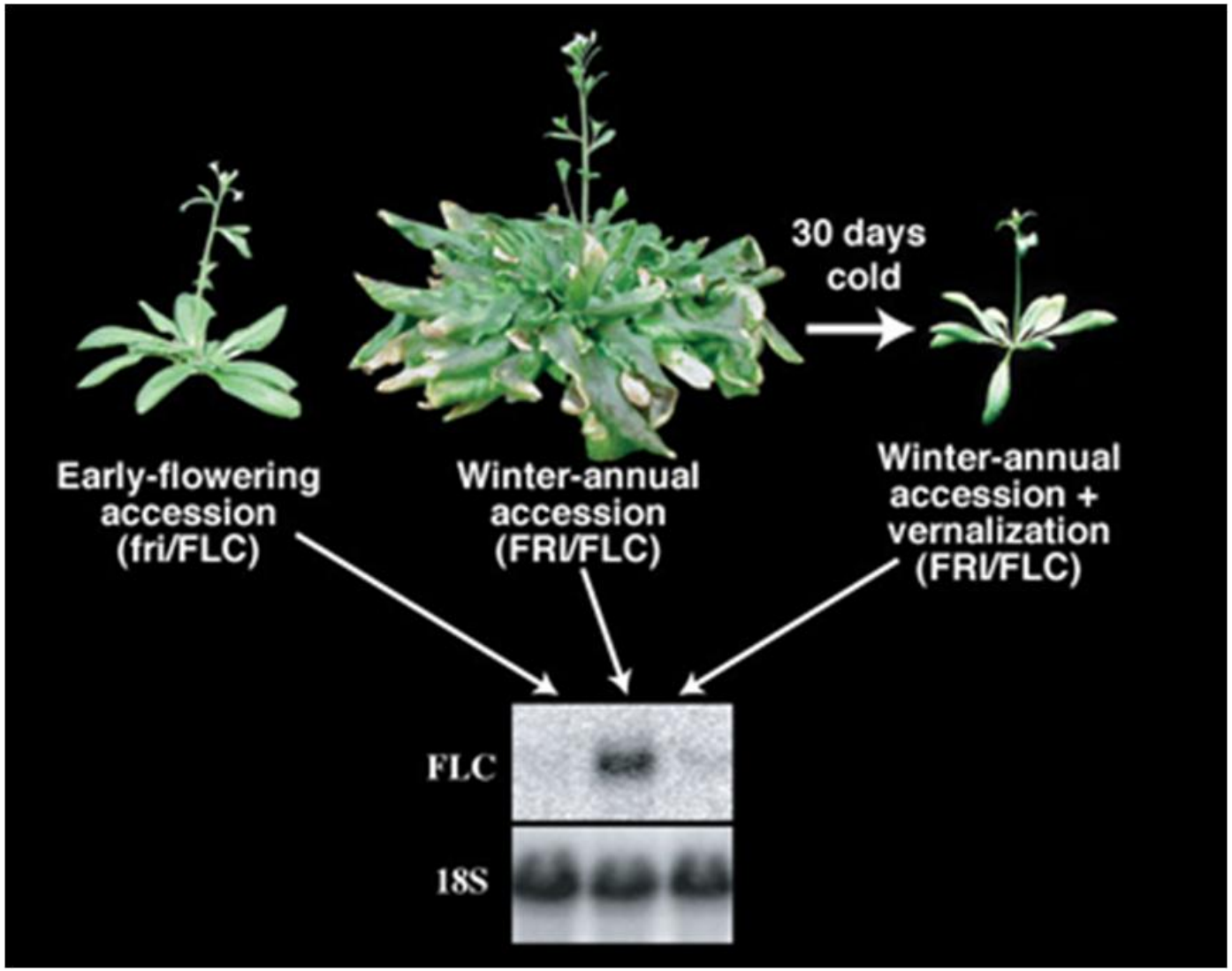
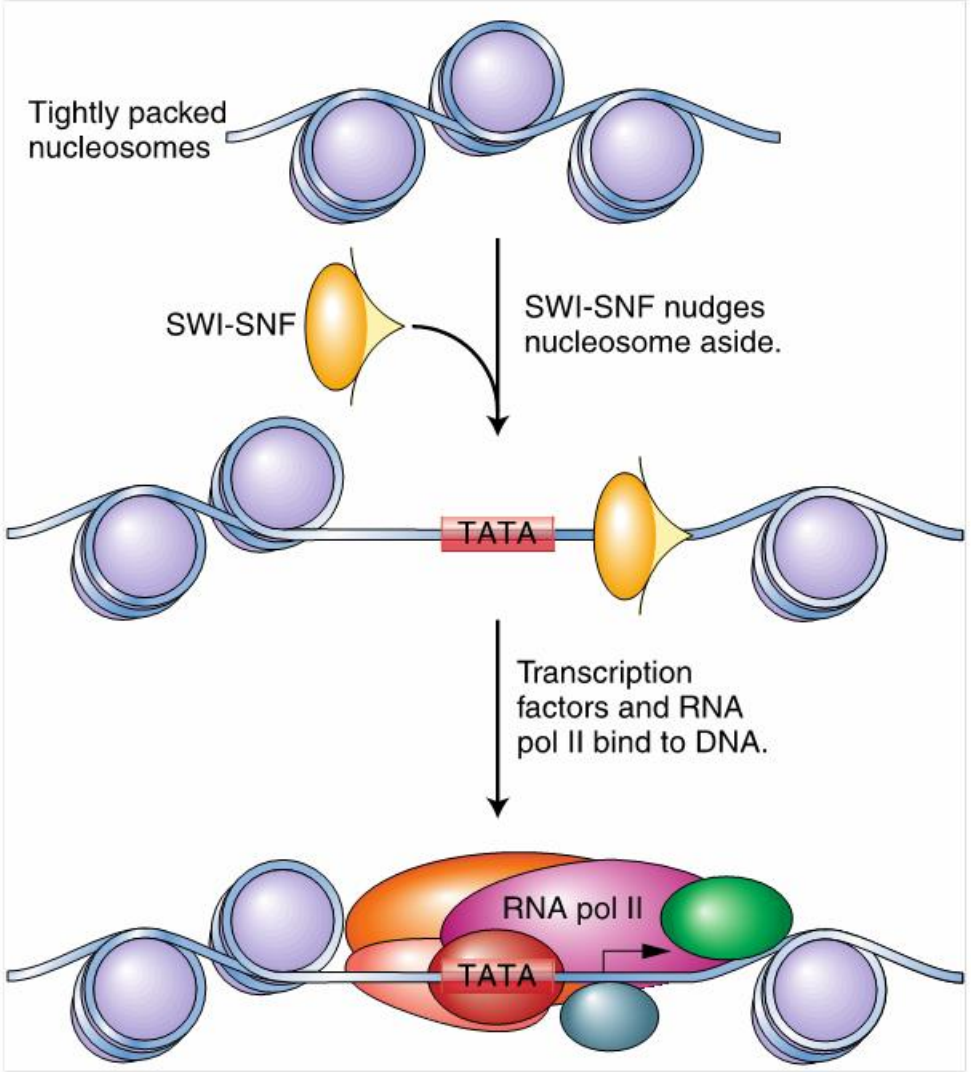
Arabidopsis thaliana



Organização da cromatina e Expressão gênica - Epigenética

- Remodelagem da Cromatina

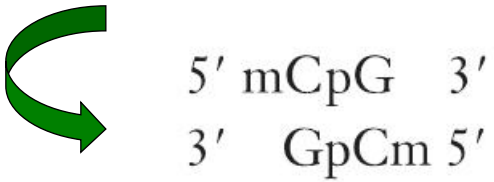
- Histona acetiltransferase (Lisina)
- Quinases (serina)
- Histona desacetilases (HDAC)
- Histona metiltransferase (HMT)
- SWI/SNF (Switching-inhibited/Sucrose nonfermenter)
  - Deslizamento das histonas



An Introduction to Genetic Analysis (Cap. 10)

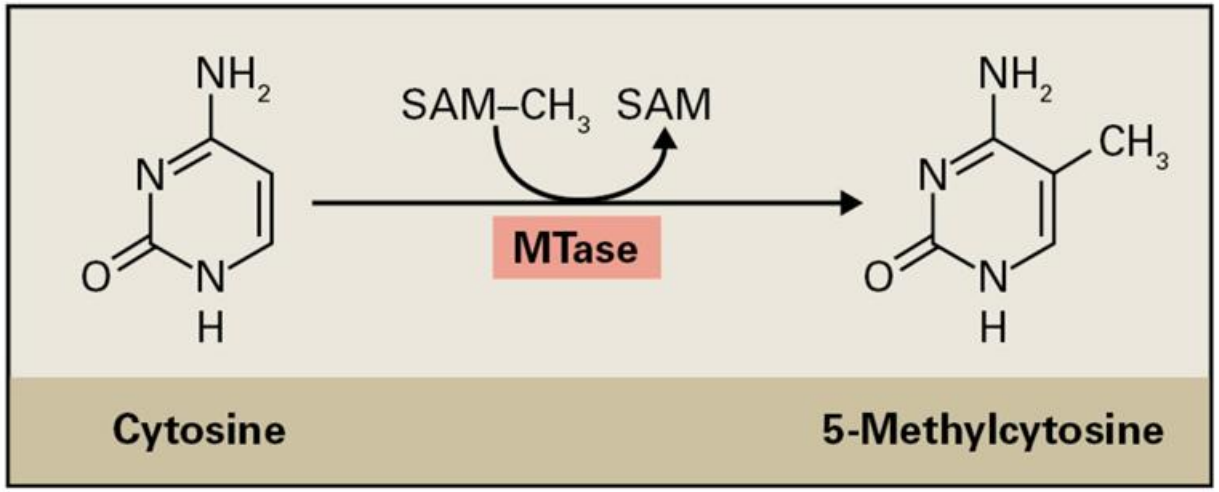
Organização da cromatina e Expressão gênica - Epigenética

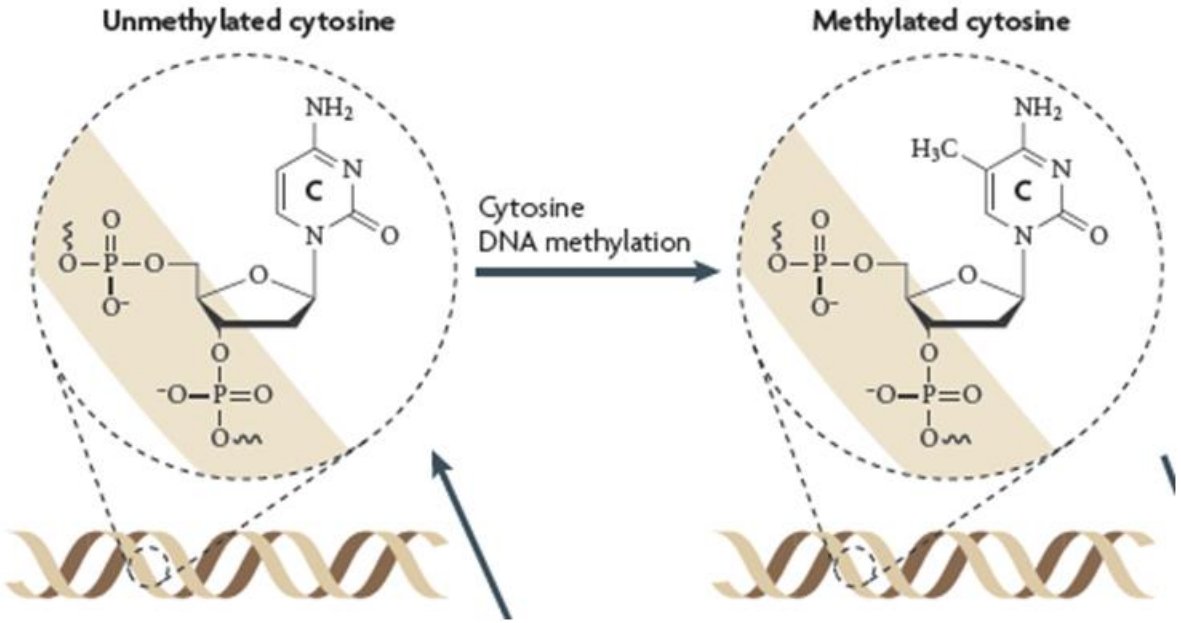
- Metilação do DNA



- Animais 40% CG; 2 a 7% são metilados
- Plantas = CHG e CHH (H=C, T ou A); 20% metilados

- Enzimas de restrição sensível e insensível a metilação (*Hpa*II - CCGG)
- Ilhas CpG, início da transcrição
- DNA metilado, ligação de MeCP2 e modificação da cromatina
- DNA metiltransferase





Buchanan et al., 2015 (Cap. 9)

Organização da cromatina e Expressão gênica - Epigenética

- Metilação do DNA

5′ mCpG 3′
3′ GpCm 5′

- Animais 40% CG; 2 a 7% são metilados
- Plantas = CHG e CHH (H=C, T ou A); 20% metilados

  - Enzimas de restrição sensível e insensível a metilação (*Hpa*II - CCGG)
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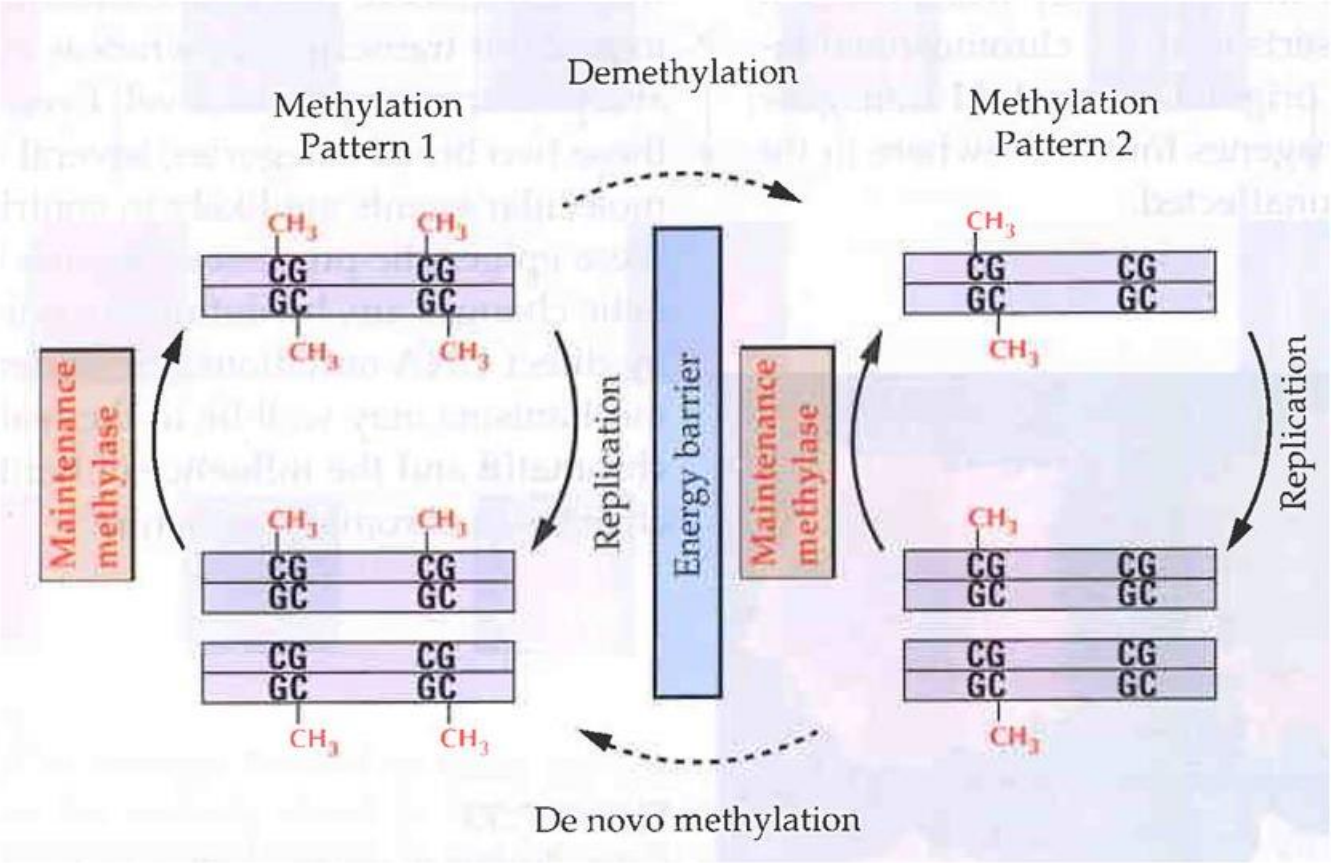


Buchanan et al., 2002 (Cap. 7)

Organização da cromatina e Expressão gênica - Epigenética

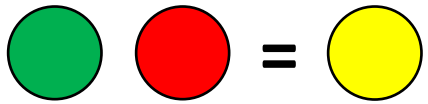
- Metilação do DNA

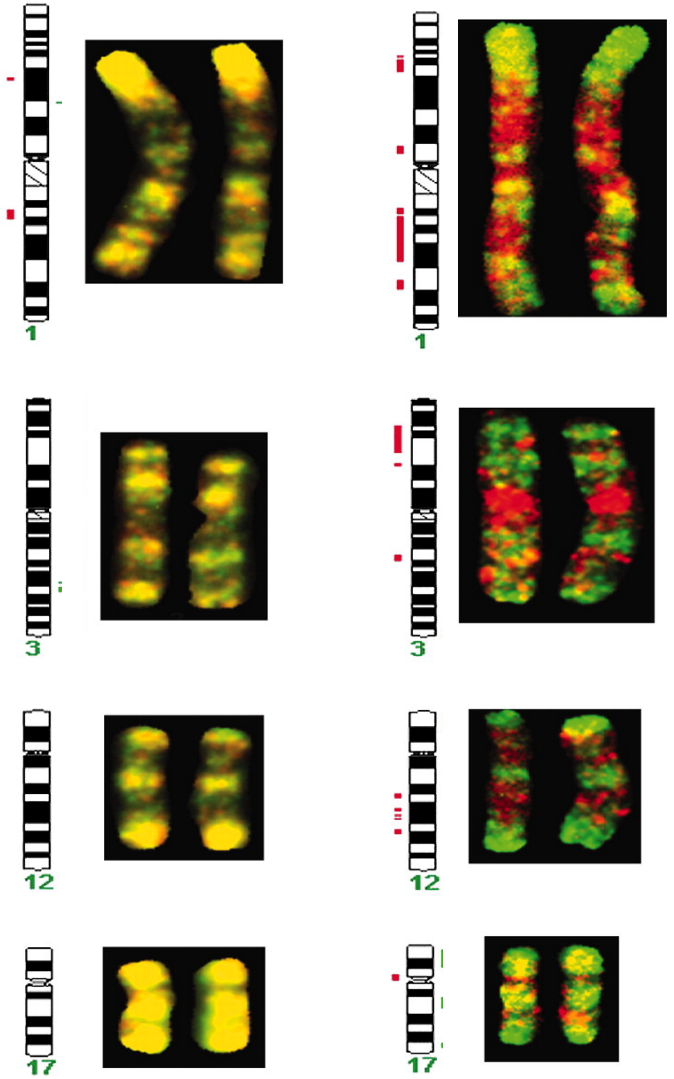
Diana Bozza tranquiliza sua gêmea univitelina, Deborah Faraday, em uma casa de repouso em Front Royal, na Virgínia. Diagnosticada há oito anos com o mal de Alzheimer, hoje Deborah está incapacitada; já Diana não apresenta nenhum sintoma da doença.

National Geografic (Jan/2012)

Organização da cromatina e Expressão gênica - Epigenética

- Metilação do DNA





3 Anos 50 Anos

PNAS

Epigenetic differences arise during the lifetime of monozygotic twins

Mario F. Fraga*, Esteban Ballestar*, Maria F. Paz*, Santiago Ropero*, Fernando Setien*, Maria L. Ballestar†, Damia Heine-Suñer‡, Juan C. Cigudosa§, Miguel Urioste¶, Javier Benitez¶, Manuel Boix-Chornet†, Abel Sanchez-Aguilera†, Charlotte Ling‖, Emma Carlsson‖, Pernille Poulsen**, Allan Vaag**, Zarko Stephan††, Tim D. Spector††, Yue-Zhong Wu‡‡, Christoph Plass‡‡, and Manel Esteller*§§

*Epigenetics, §Cytogenetics, and ¶Genetic Laboratories, Spanish National Cancer Centre (CNIO), Melchor Fernandez Almagro 3, 28029 Madrid, Spain; †Department of Behavioral Science, University of Valencia, 46010 Valencia, Spain; ‡Molecular Genetics Laboratory, Genetics Department, Son Dureta Hospital, 07014 Palma de Mallorca, Spain; ‖Department of Clinical Sciences, University Hospital Malmö, Lund University, S-205 02 Malmö, Sweden; **Steno Diabetes Center, 2820 Gentofte, Denmark; ††Twin Research and Genetic Epidemiology Unit, St. Thomas' Hospital, London SE1 7EH, United Kingdom; and ‡‡Human Cancer Genetics Program, Department of Molecular Virology, Immunology, and Medical Genetics, Ohio State University, Columbus, OH 43210

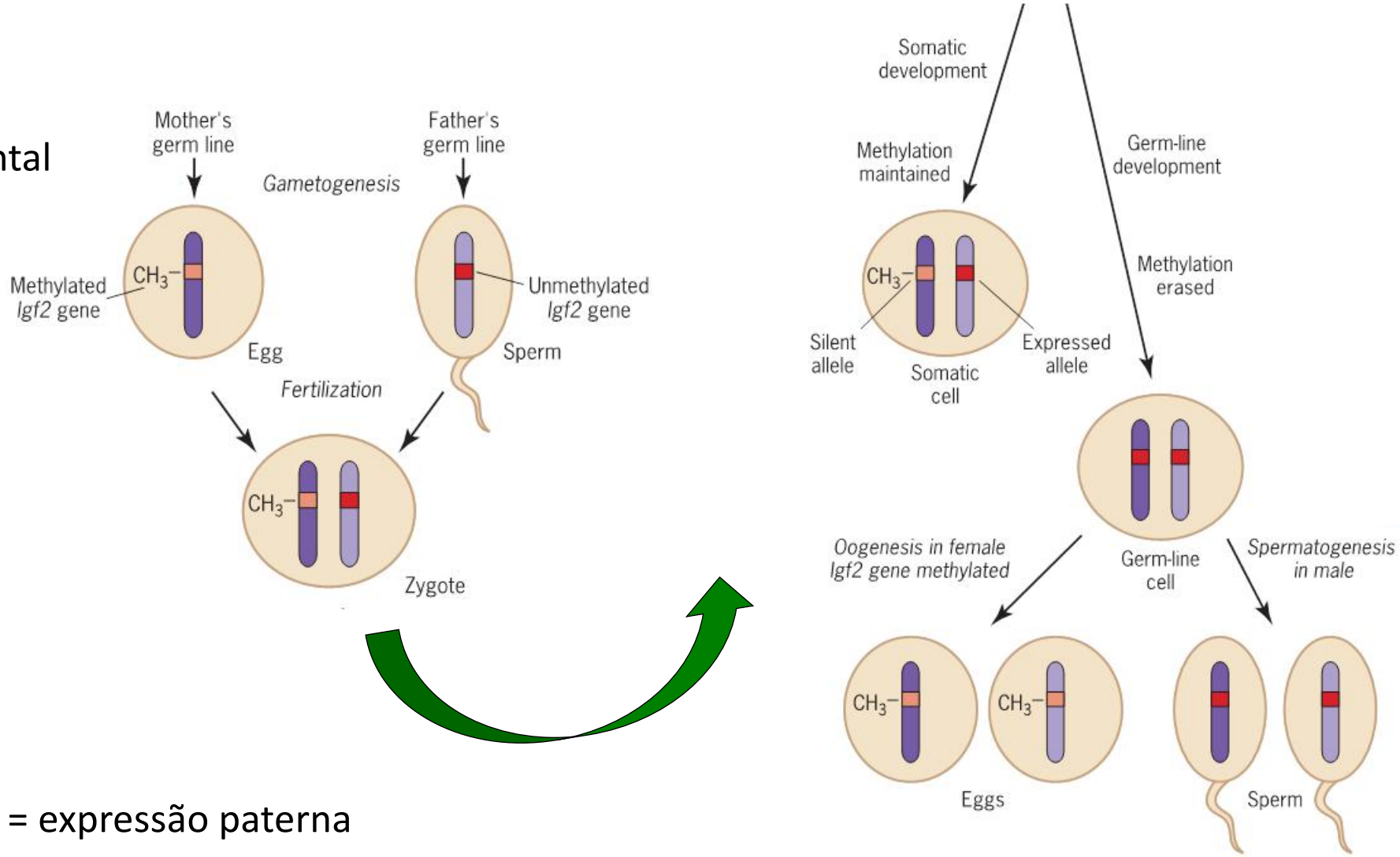
Edited by Stanley M. Gartler, University of Washington, Seattle, WA, and approved May 23, 2005 (received for review January 17, 2005)

Monozygous twins share a common genotype. However, most monozygotic twin pairs are not identical; several types of phenotypic discordance may be observed, such as differences in susceptibilities to disease and a wide range of anthropomorphic features. There are several possible explanations for these observations, but one is the existence of epigenetic differences. To address this issue, we examined the global and locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins. We found that, although twins are epigenetically indistinguishable during the early years of life, older monozygous twins exhibited remarkable differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, affecting their gene-expression portrait. These findings indicate how an appreciation of epigenetics is missing from our understanding of how different phenotypes can be originated from the same genotype.

Organização da cromatina e Expressão gênica - Epigenética

- Imprinting (Estampagem)

  - Depende da origem parental

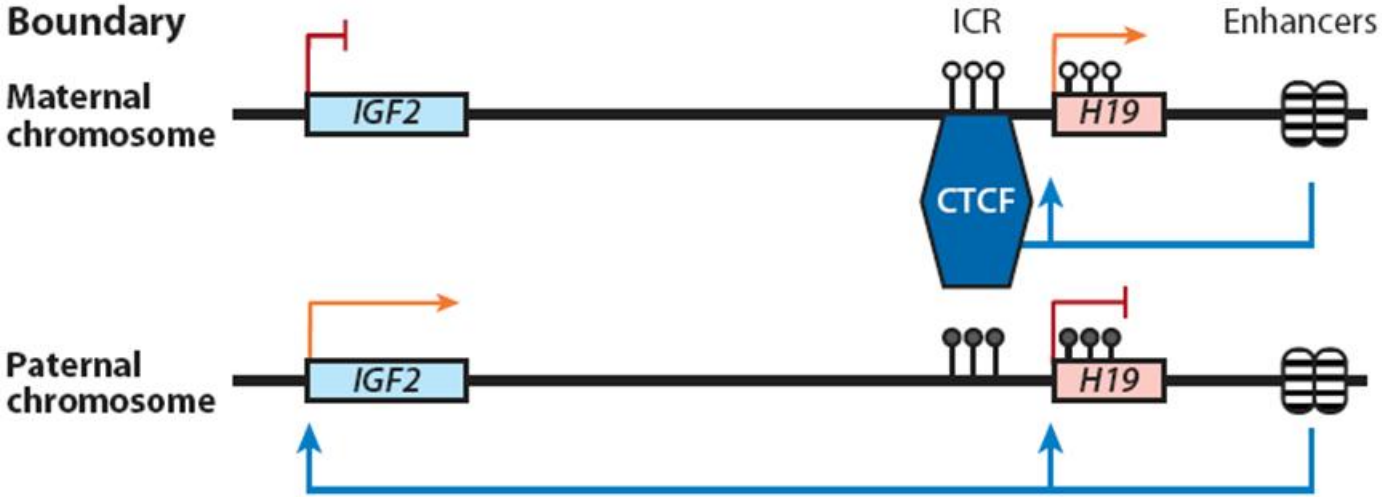


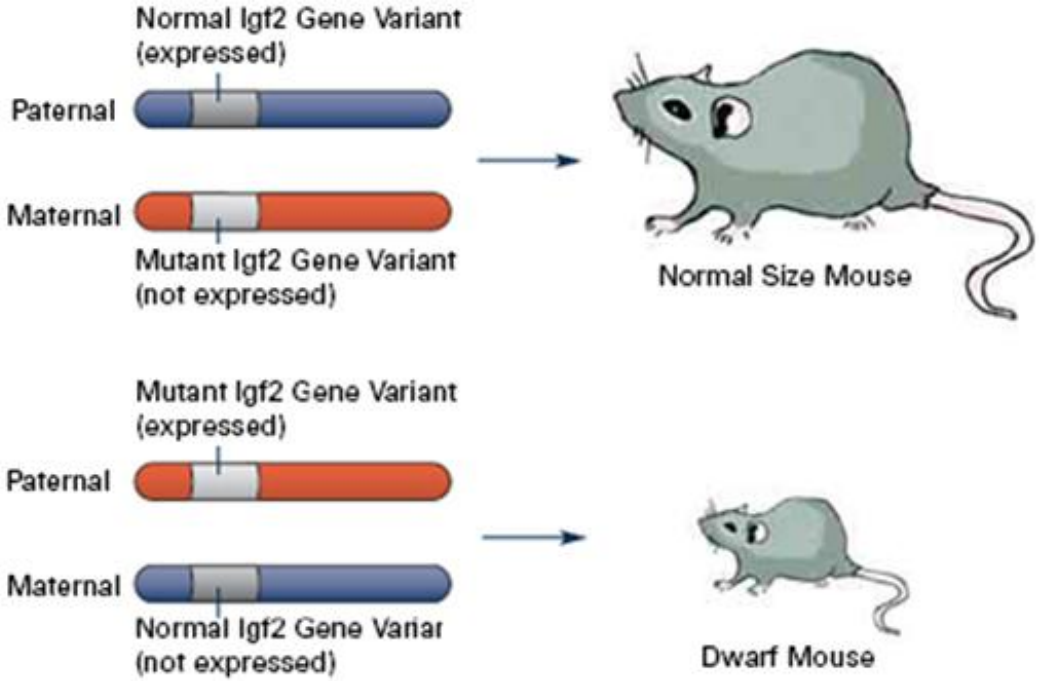
- *Igf2* (Insulin-like growth factor) = expressão paterna
- *H19* = expressão materna

Principles of Genetics (Cap 19)

Organização da cromatina e Expressão gênica - Epigenética

- Imprinting (Estampagem)





Tumor de Wilm

Ocorre expressão de IGF2 materno

Principles of Genetics (Cap 19)

Múltiplos Níveis Regulatórios