Chapter 8
Gene Regulation in Eukaryotes

6学时

Outlines

1. Conserved Mechanisms of Transcriptional Regulation from Yeast to Human.
2. Recruitment of Protein Complexes to Genes by Eukaryotic Activators.
3. Signal Integration and Combinatorial Control.
4. Transcriptional Repressors.
5. Signal Transduction.
6. Gene Silencing by Modification of Histones and DNA.
7. Epigenetic Gene Regulation.
8. RNA Interference.
9. Ubiquitin-Mediated Proteolysis (The Ubiquitin-Proteasome Pathway).
10. Other Eukaryotic Gene Regulations at Steps after Transcription Initiation

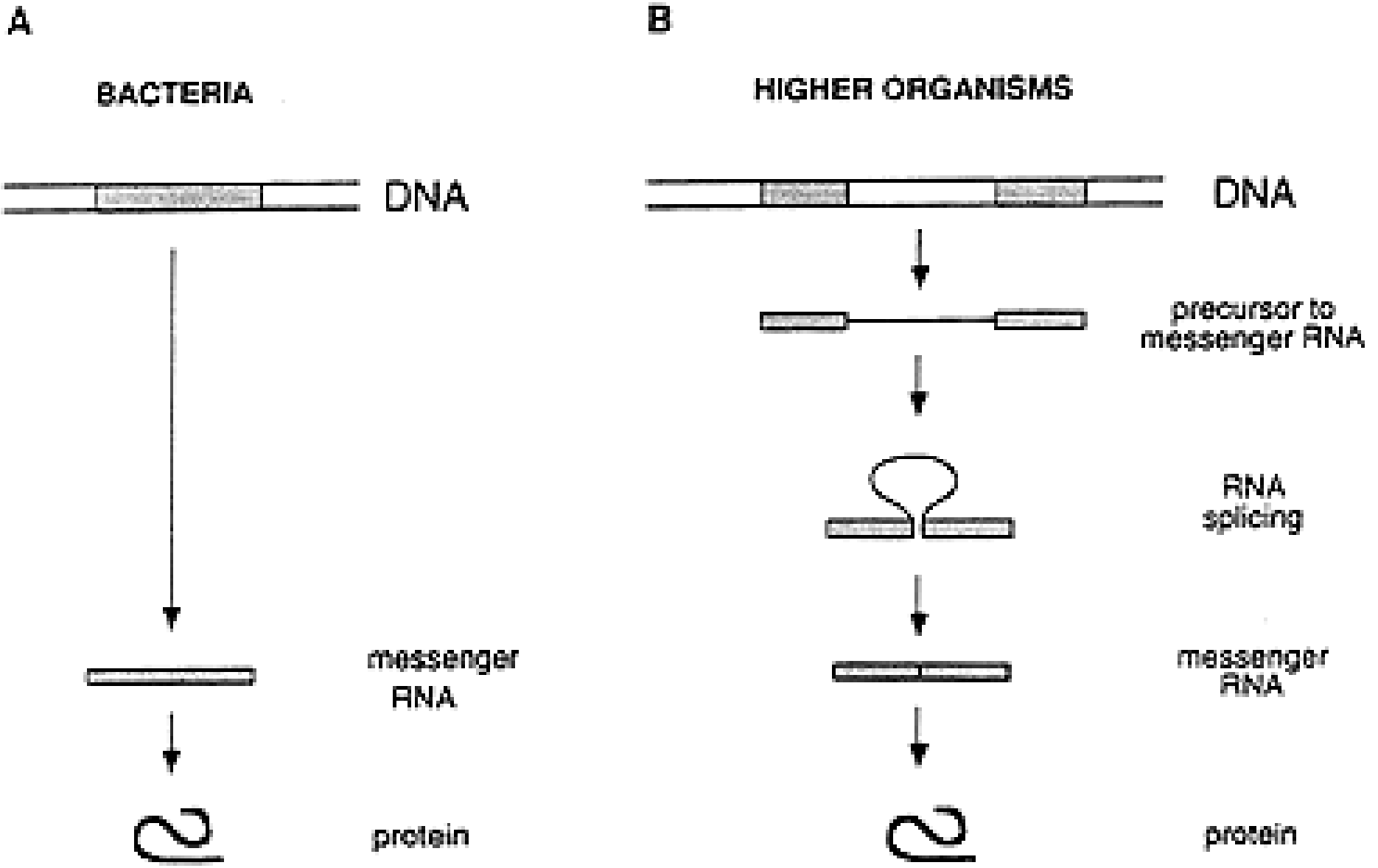
The Nobel Prize in Physiology or Medicine 1993

"for their discoveries of split genes"

Richard J. Roberts

Phillip A. Sharp

RNA splicing in higher organisms



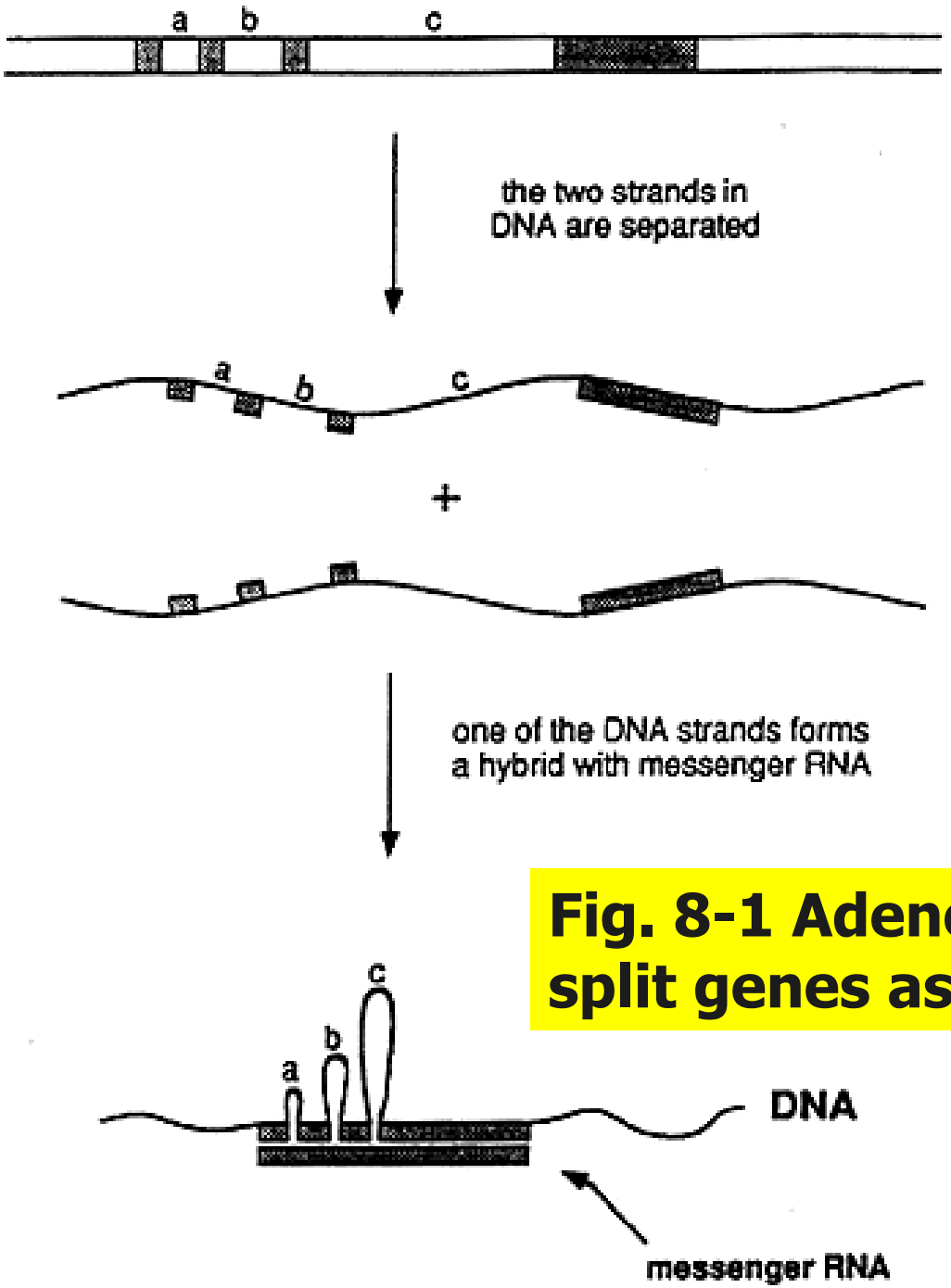


Fig. 8-1 Adenovirus DNA contains split genes as shown by EM in 1977

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

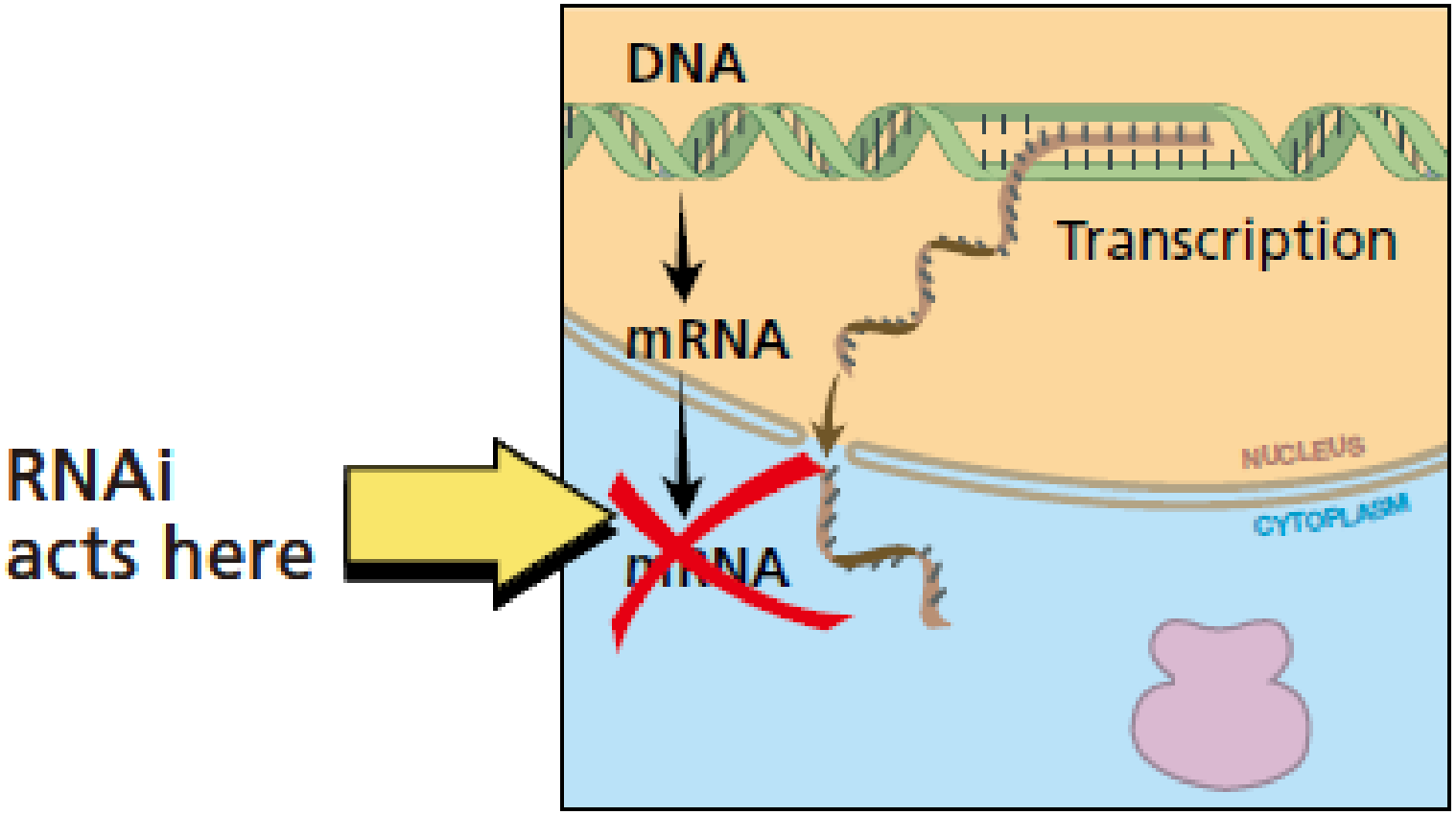
Photo: L. Cicero

Andrew Z. Fire

Photo: J. Mottern

Craig C. Mello

Fig. 8-2 RNA interference (RNAi)



In RNA interference, RNA in double-stranded form breaks down the mRNA for a specific gene, thus stopping production of protein.

Fig. 8-3 Initial C. elegans experiments by Adrew Fire and Craig Mello in 1998

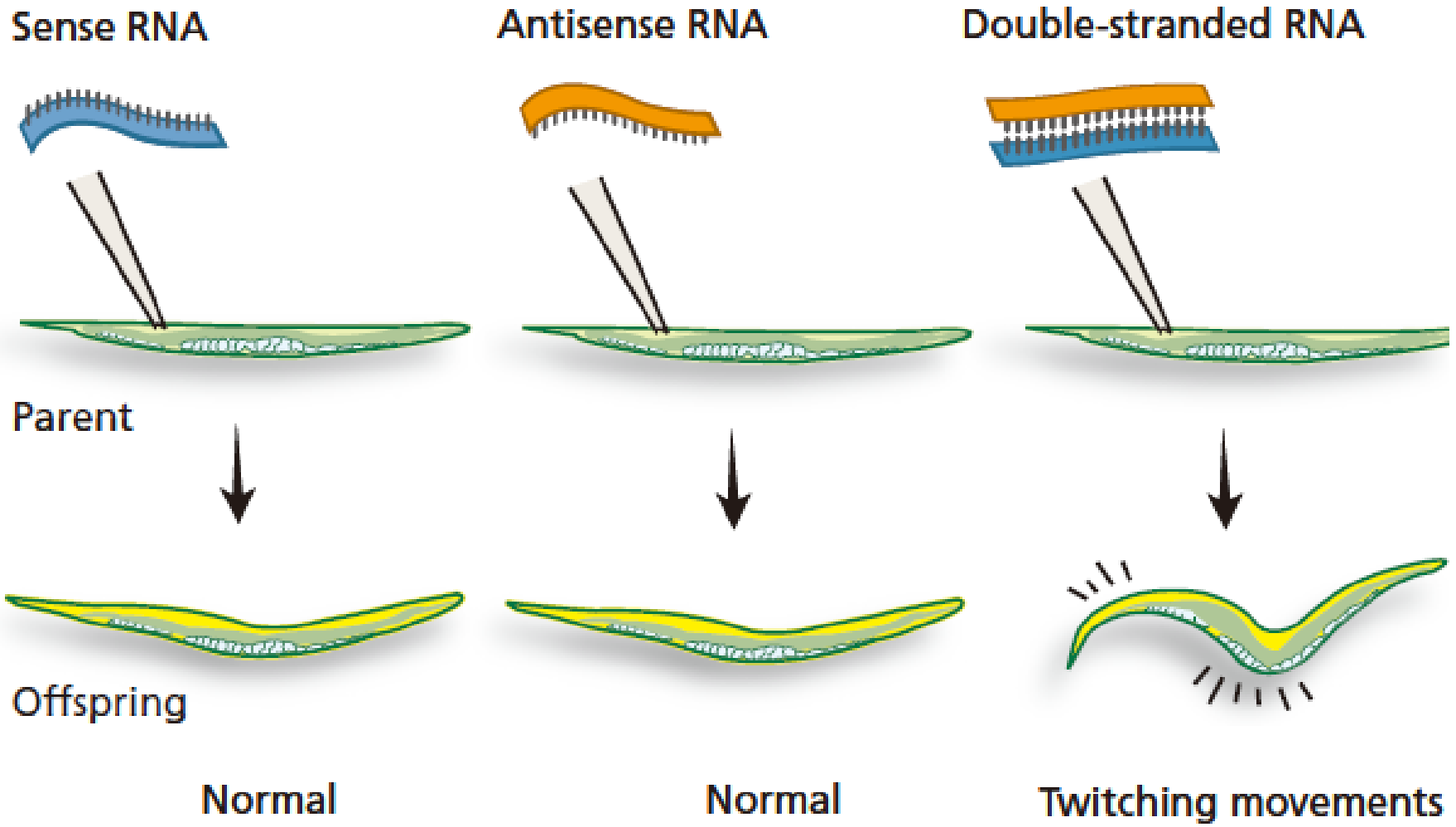
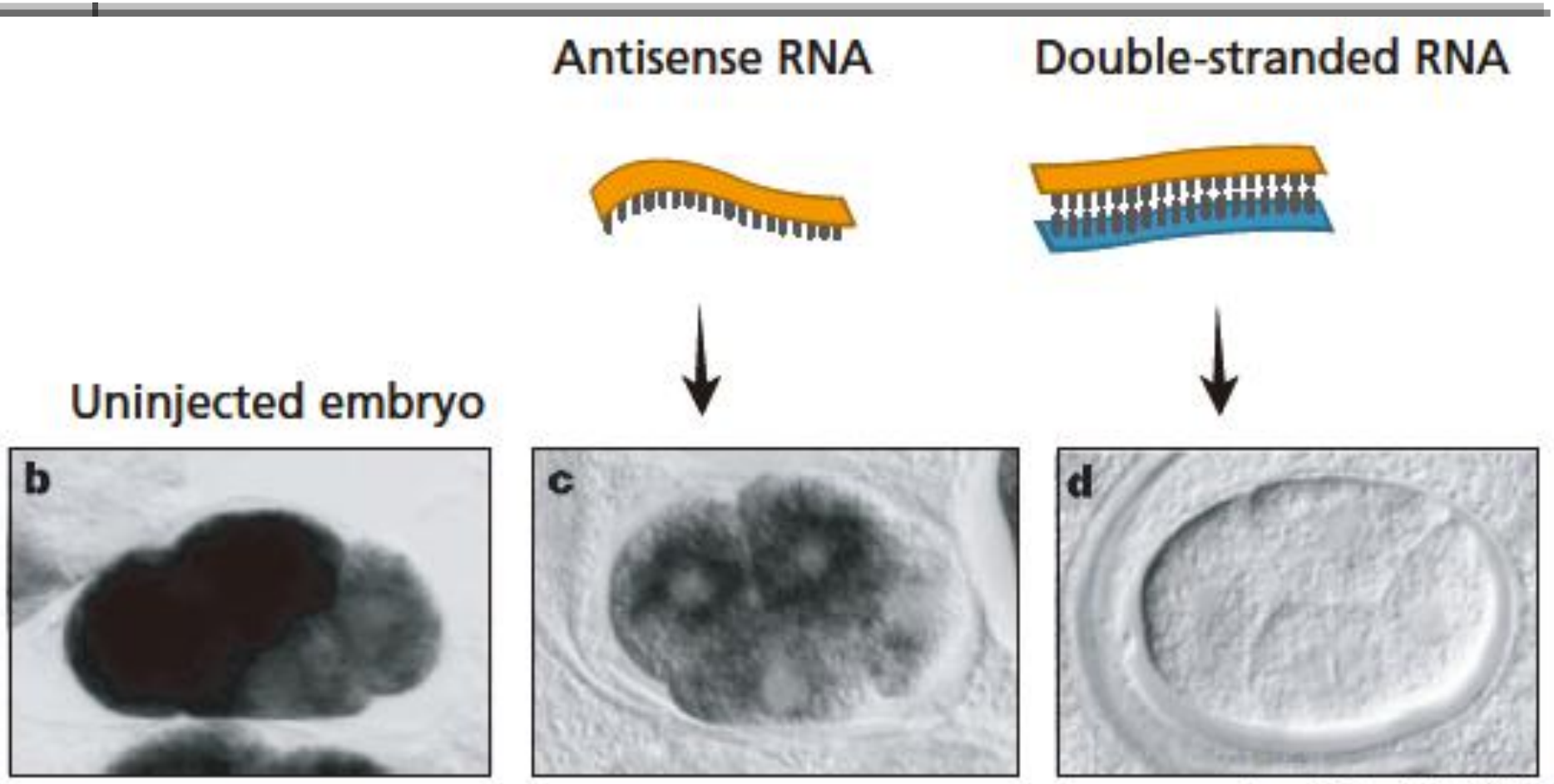


Fig. 8-4 Further experiments in C. elegans by staining targeted mRNA



From *Nature*, Feb. 1998

Albert Lasker Basic Medical Research Award

2008 Winners

Victor Ambros, David Baulcombe, and Gary Ruvkun

For discoveries that revealed an unanticipated world of tiny RNAs that regulate gene function in plants and animals. (More >)

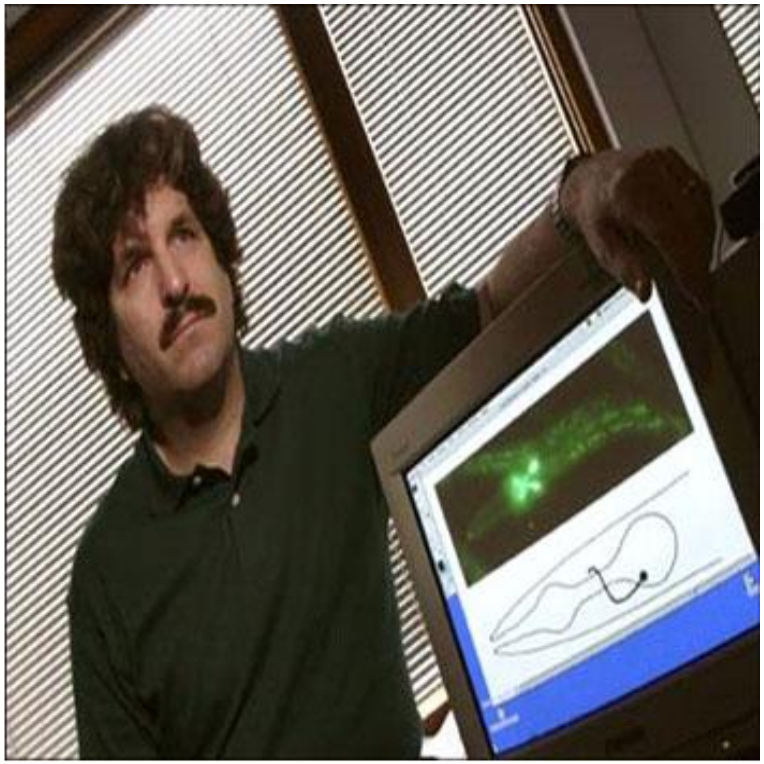
Victor Ambros Ph.D.
University of Massachusetts Medical School

David Baulcombe Ph.D.
University of Cambridge

Gary Ruvkun Ph.D.
Harvard Medical School
Mass. General Hospital

The discovery of miRNAs

Victor Ambros

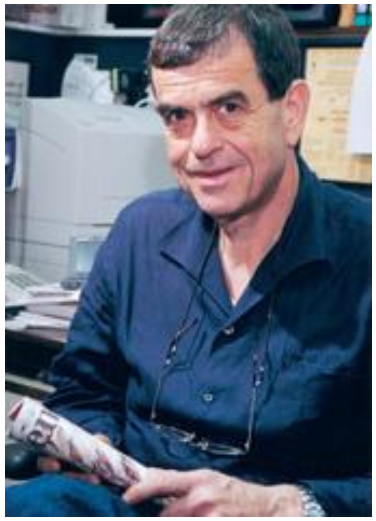


Gary Ruvkun

- **miRNA was first discovered in 1993 by Victor Ambros (*lin-4*)**
- **The second miRNA *Let-7* was discovered in 2000 by Frank Slack as a postdoc at Harvard (Ruvkun lab)**

The Nobel Prize in Chemistry 2004

for the discovery of ubiquitin-mediated proteolysis



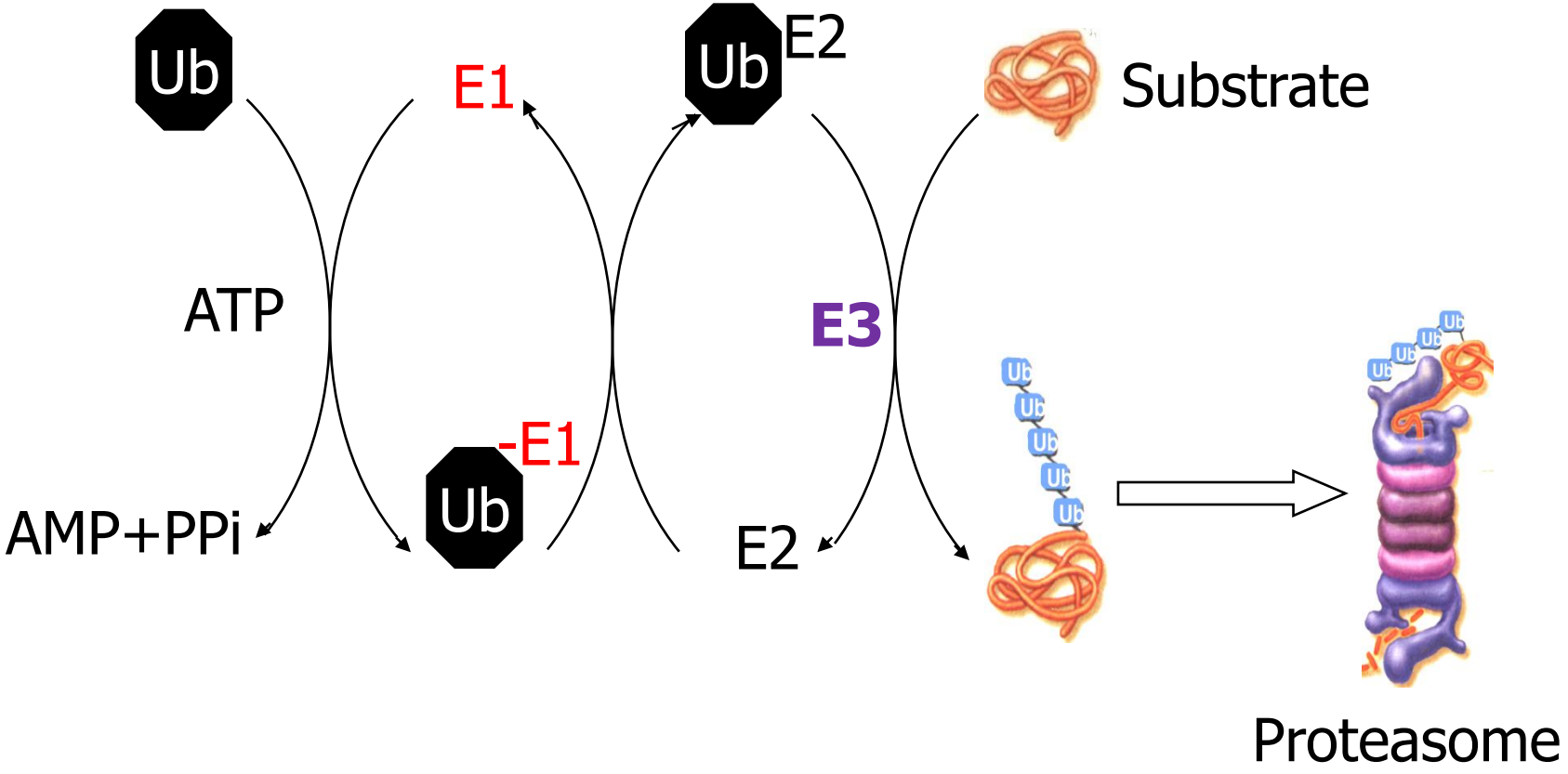
Aaron Ciechanover

Avram Hershko

Irwin Rose

The ubiquitin-proteasome pathway

Responsible for degradation of most cellular proteins



Similarity of regulation between eukaryotes and prokaryotes

1. Principles are the same:
   - signals
   - activators and repressors
   - recruitment and allostery, cooperative binding
2. The gene expression steps subjected to regulation are similar, and the initiation of transcription is the most extensively regulated step.

Difference in regulation between eukaryotes and prokaryote

1. Pre-mRNA splicing adds an important step for regulation.
2. The eukaryotic transcriptional machinery is more elaborate than its bacterial counterpart.
3. Nucleosomes and their modifiers influence access to genes.
4. Many eukaryotic genes have more regulatory binding sites and are controlled by more regulatory proteins than are bacterial genes.

A lot more regulator binding sites in multicellular organisms reflect the more extensive signal integration

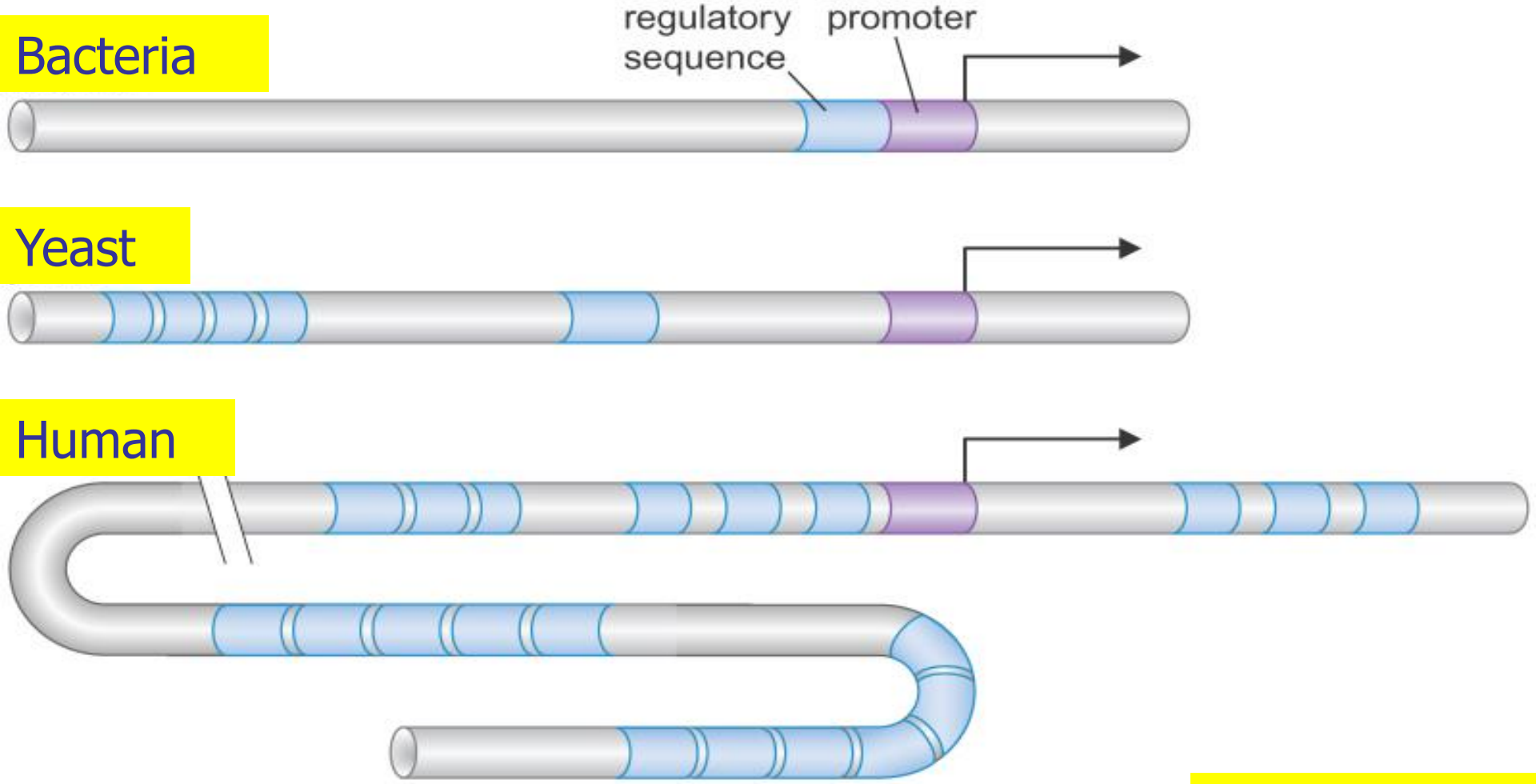


Fig. 8-5

Outlines

1. Conserved Mechanisms of Transcriptional Regulation from Yeast to Human.
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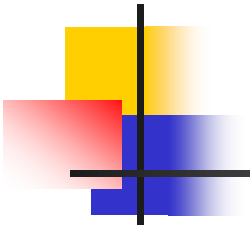


Part 1: Conserved Mechanisms of Transcriptional Regulation from Yeast to Mammals

As in bacteria, eukaryotic activators have separate DNA binding and activating regions

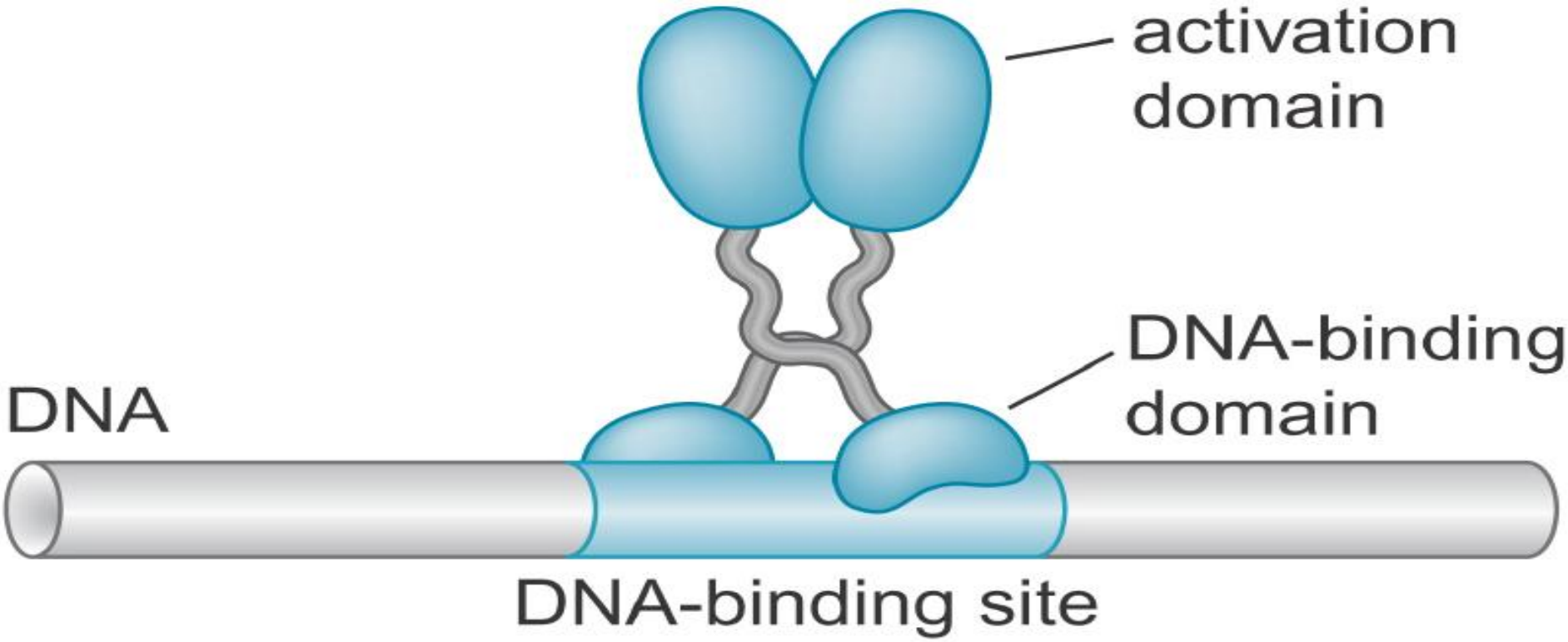


Fig. 8-6 Gal4 bound to its site on DNA

Eukaryotic activators--Gal4

- ✓ **Gal4 activates transcription of the galactose genes in the yeast *S. cerevisae*.**
- ✓ **Gal4 binds to four sites (UAS_G) upstream of *GAL1,* and activates transcription of GAL1 1,000-fold in the presence of galactose**

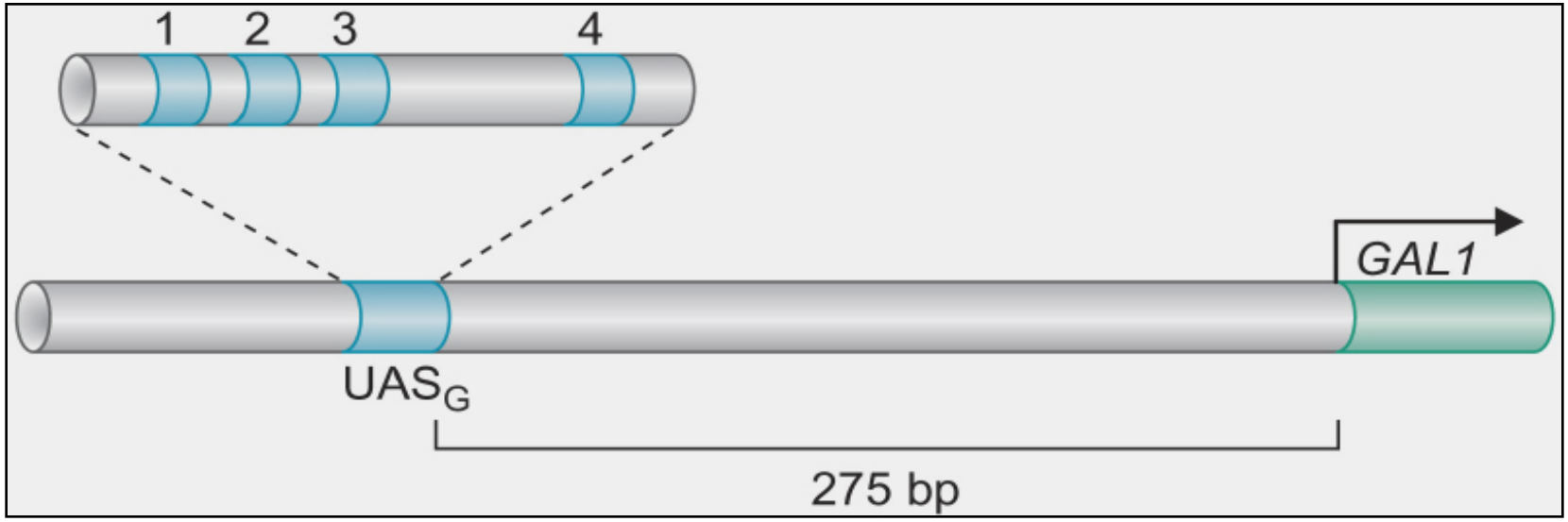
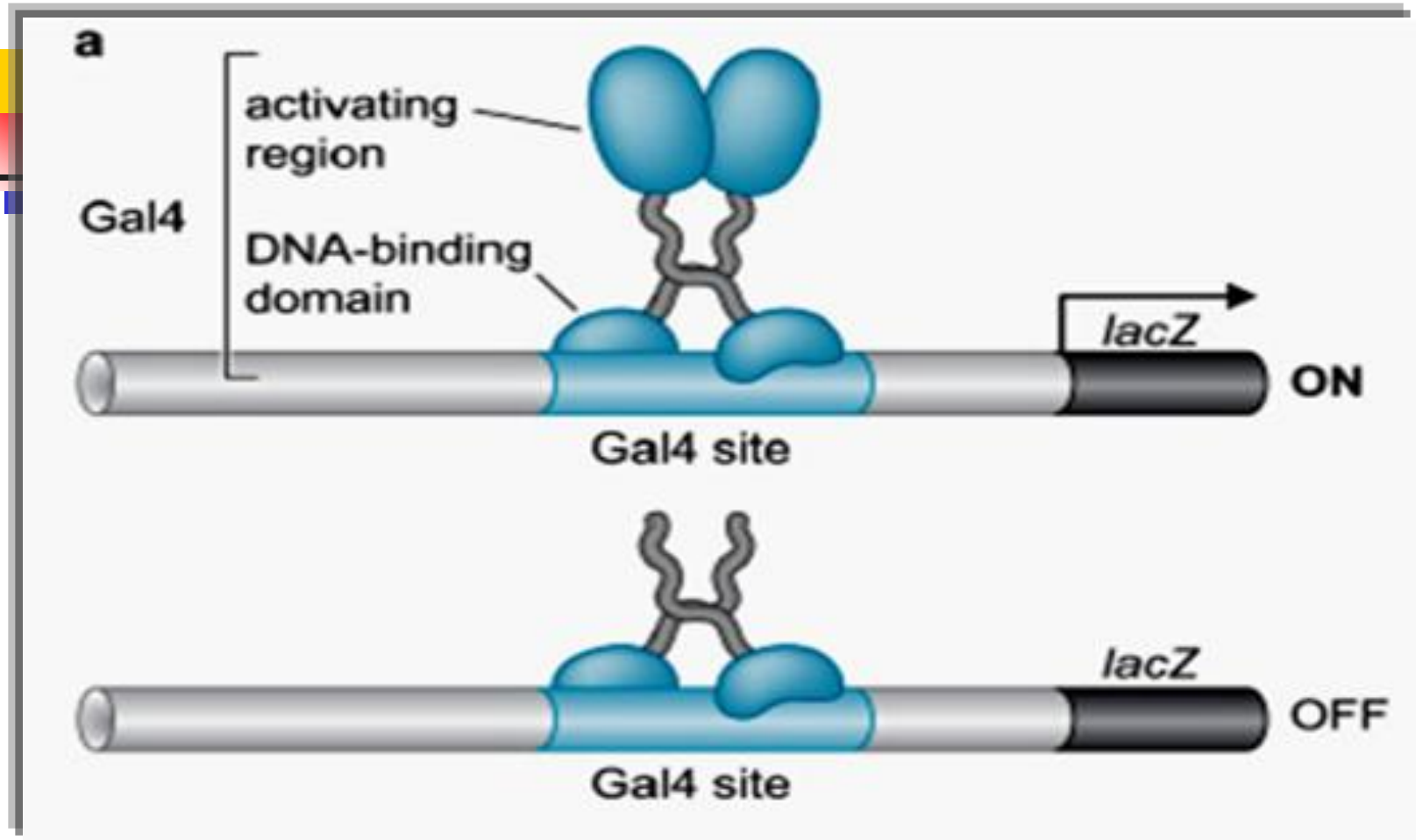


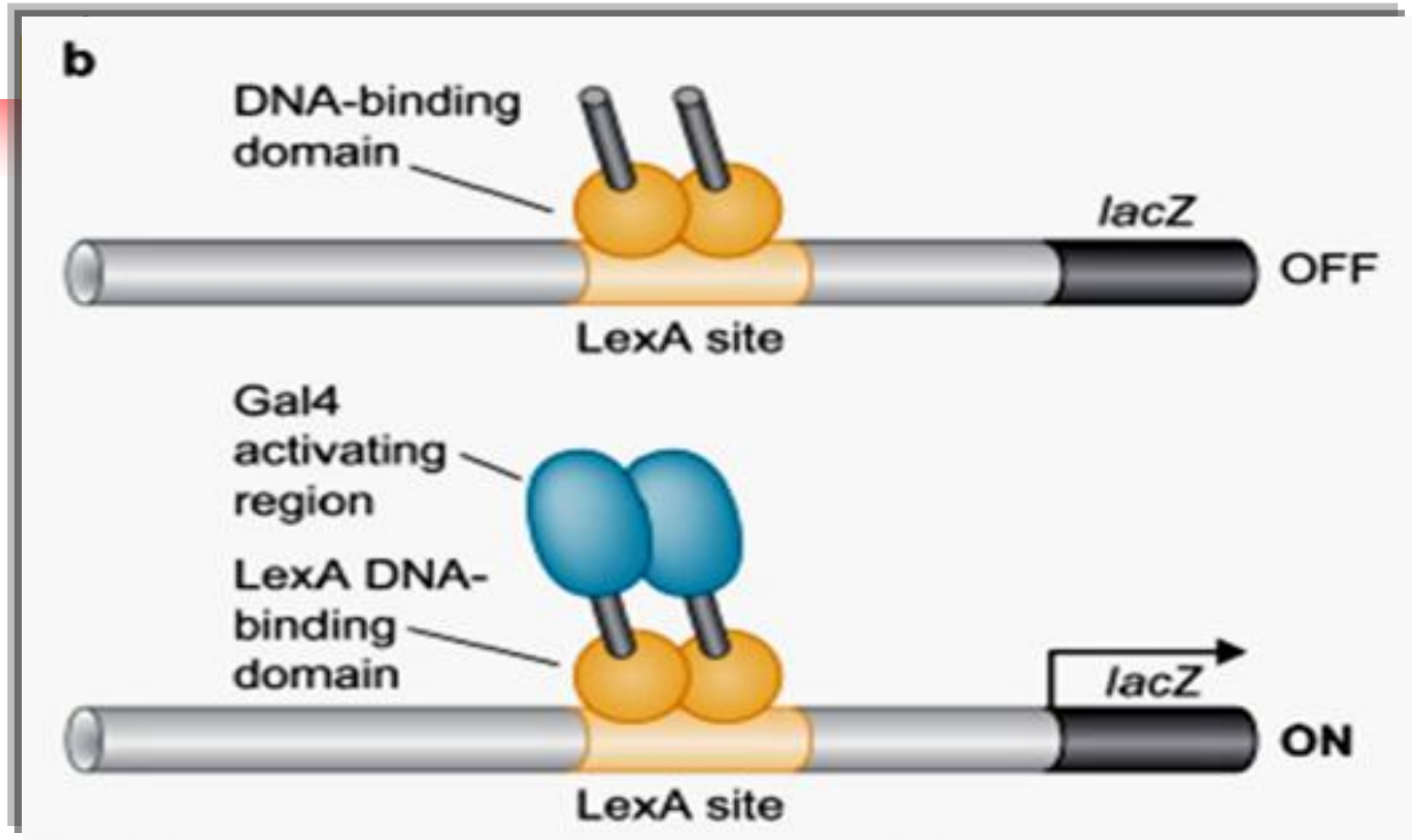
Fig. 8-7 The regulatory sequences of the Yeast *GAL1* gene.

Fig. 8-8 Domain swap experiment



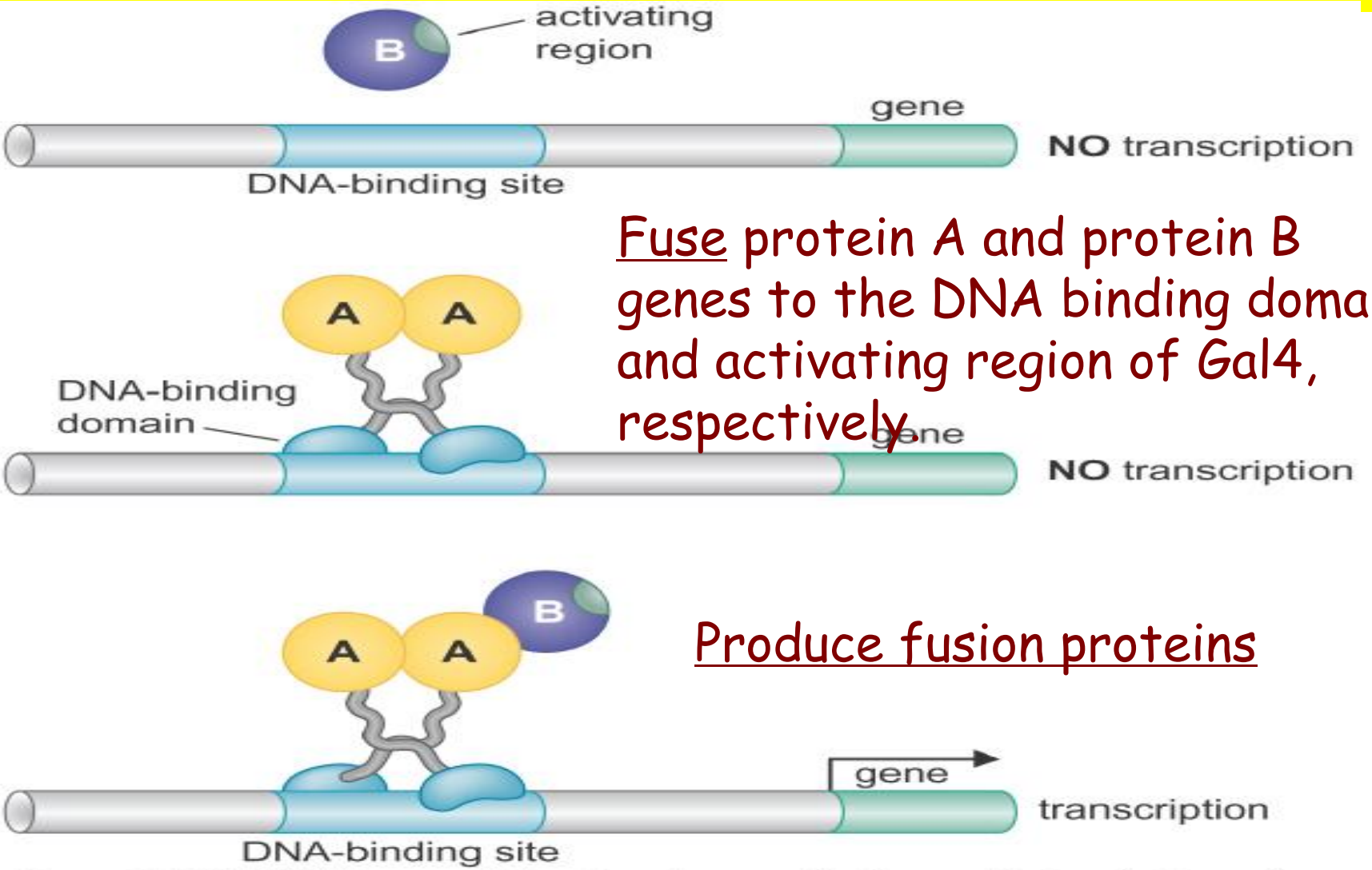
DNA-binding domain of *GAL4* (without activation domain) can still bind DNA, but cannot activate transcription.

Fig. 8-8 Domain swap experiment (continued)



Yeast with a bacterial lacZ reporter plasmid bearing binding sites for bacterial repressor LexA upstream of the GAL1 promoter.

Fig. 8-9 The yeast two hybrid assay for identifying proteins interacting with each other.



Fuse protein A and protein B genes to the DNA binding domain and activating region of Gal4, respectively

Produce fusion proteins

Eukaryotic regulators use a range of DNA binding domains, but DNA recognition involves the same principles as found in bacteria.

- **Homeodomain proteins**
- **Zinc containing DNA-binding domain: zinc finger and zinc cluster**
- **Leucine zipper motif**
- **Helix-Loop-Helix proteins : basic zipper and HLH proteins**

Bacterial regulatory proteins

- Most use the helix-turn-helix motif to bind DNA target
- Most bind as dimers to DNA sequence: each monomer inserts an α helix into the major groove.

Eukaryotic regulatory proteins

1. Recognize the DNA using the similar principles, with some variations in detail.
2. In addition to form homodimers, some form heterodimers to recognize DNA, extending the range of DNA-binding specificity.

The homeodomain has a helix-turn-helix motif (helices 2 and 3) with an arm extending from helix 1 for additional contacts with base pairs in the minor groove.

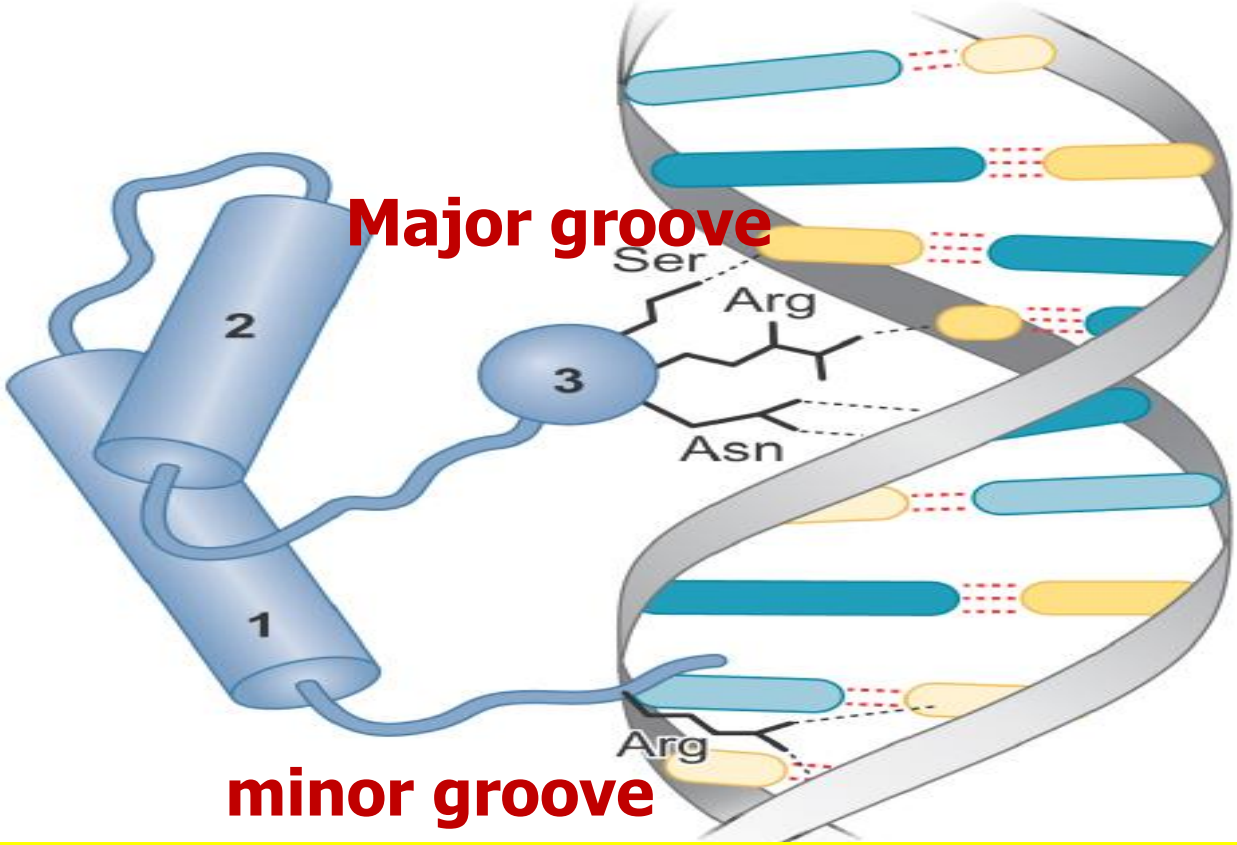


Figure 8-10 DNA recognition by a Homeodomain

Figure 8-11 Zinc finger domain

DNA binding

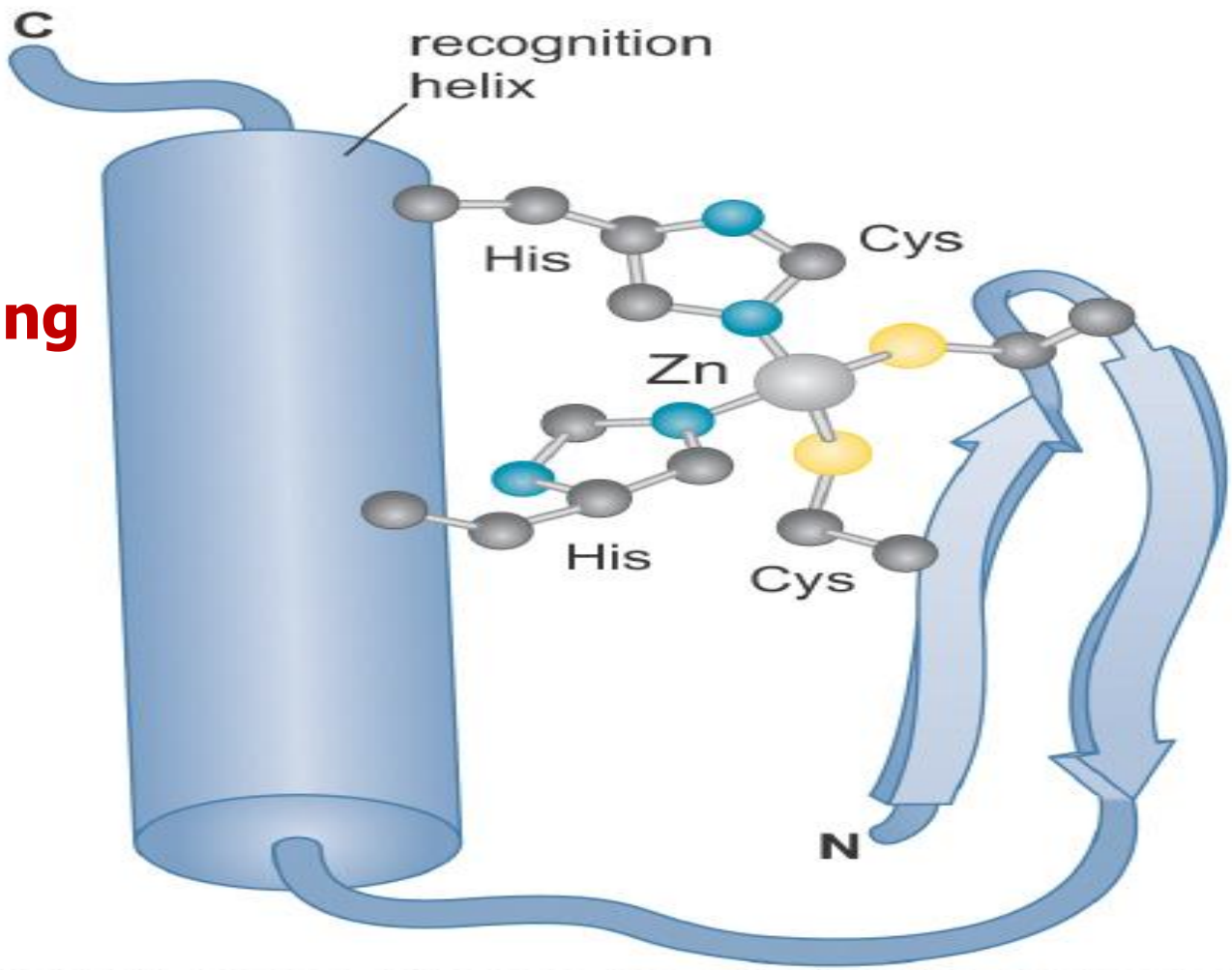
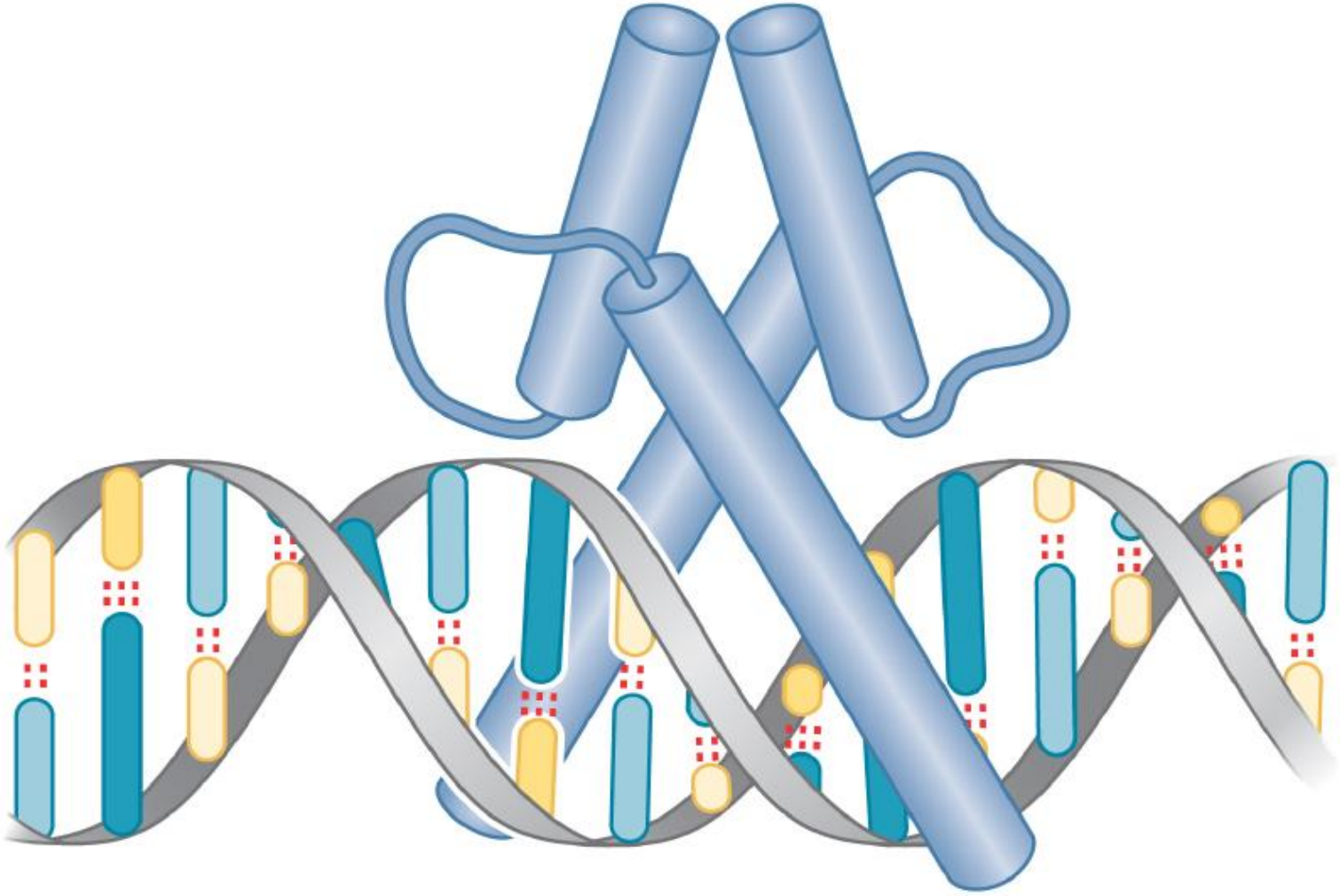


Figure 8-12 **Leucine Zipper Motif combines dimerization and DNA-binding surfaces within a single structural unit.**

Figure 8-13 **Helix-Loop-Helix motif: similar to leucine zipper**



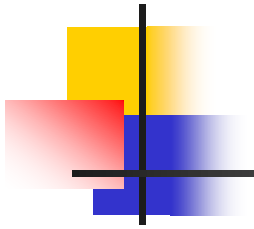
Because the region of the α-helix that binds DNA contains basic amino acids residues, Leucine zipper and HLH proteins are often called **basic zipper** and **basic HLH** proteins.

Both of these proteins use hydrophobic amino acid residues for **dimerization**.

Activating regions are not well-defined structures

The activating regions are grouped on the basis of amino acid content.

- Acidic activation region : contain both critical acidic amino acids and hydrophobic acids. *yeast Gal4*
- Glutamine-rich region : *mammalian activator SP1*
- Proline-rich region : *mammalian activator CTF1*



Part 2: Recruitment of Protein Complexes to Genes by Eukaryotic Activators

Eukaryotic activators also work by recruitment as in bacteria, but recruit polymerase indirectly in two ways:

1. **Interacting with parts of the transcription machinery.**
2. **Recruiting nucleosome modifiers that alter chromatin in the vicinity of a gene.**

Fig. 8-14 Non-polymerase proteins in transcriptional machinery: The Mediator and the TFⅡD complex.

Activators interact with one or more of these non-polymerase proteins and recruit them to the gene.

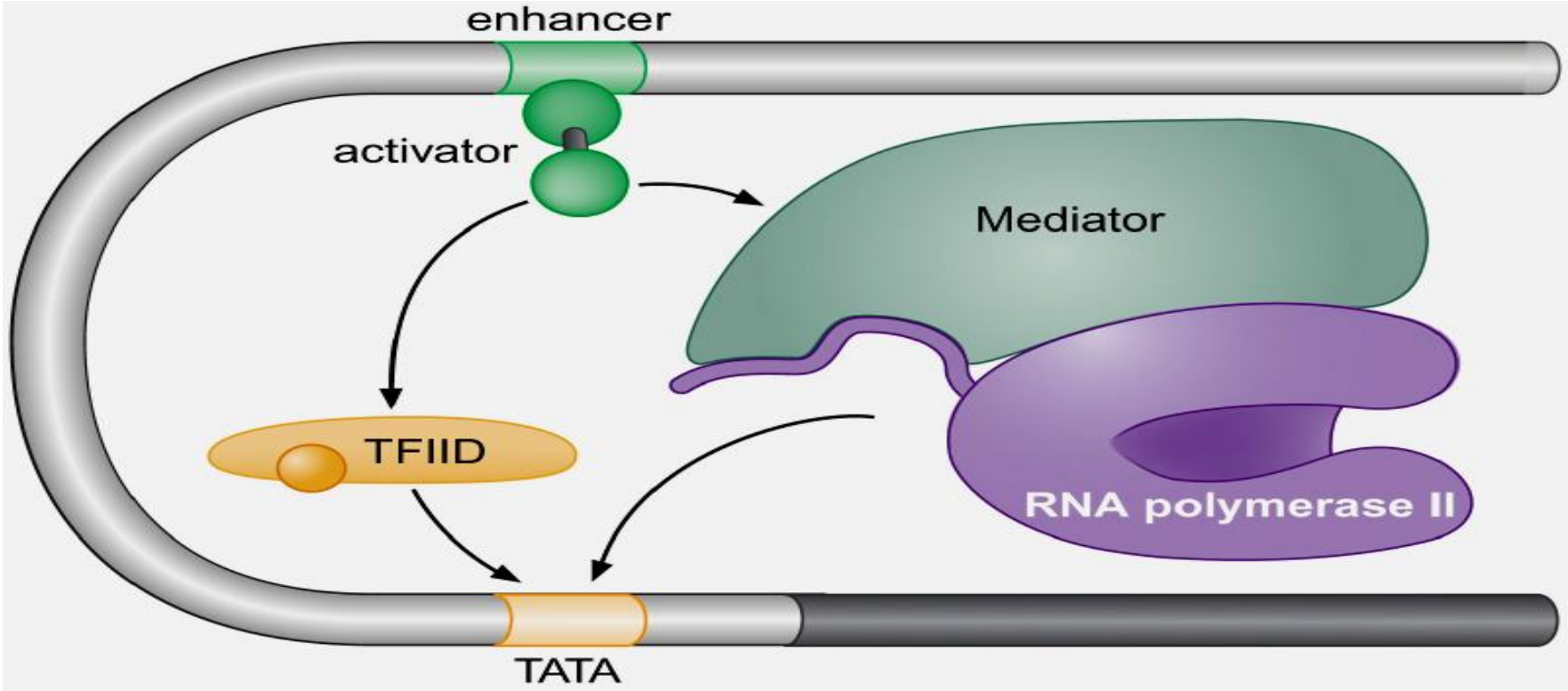
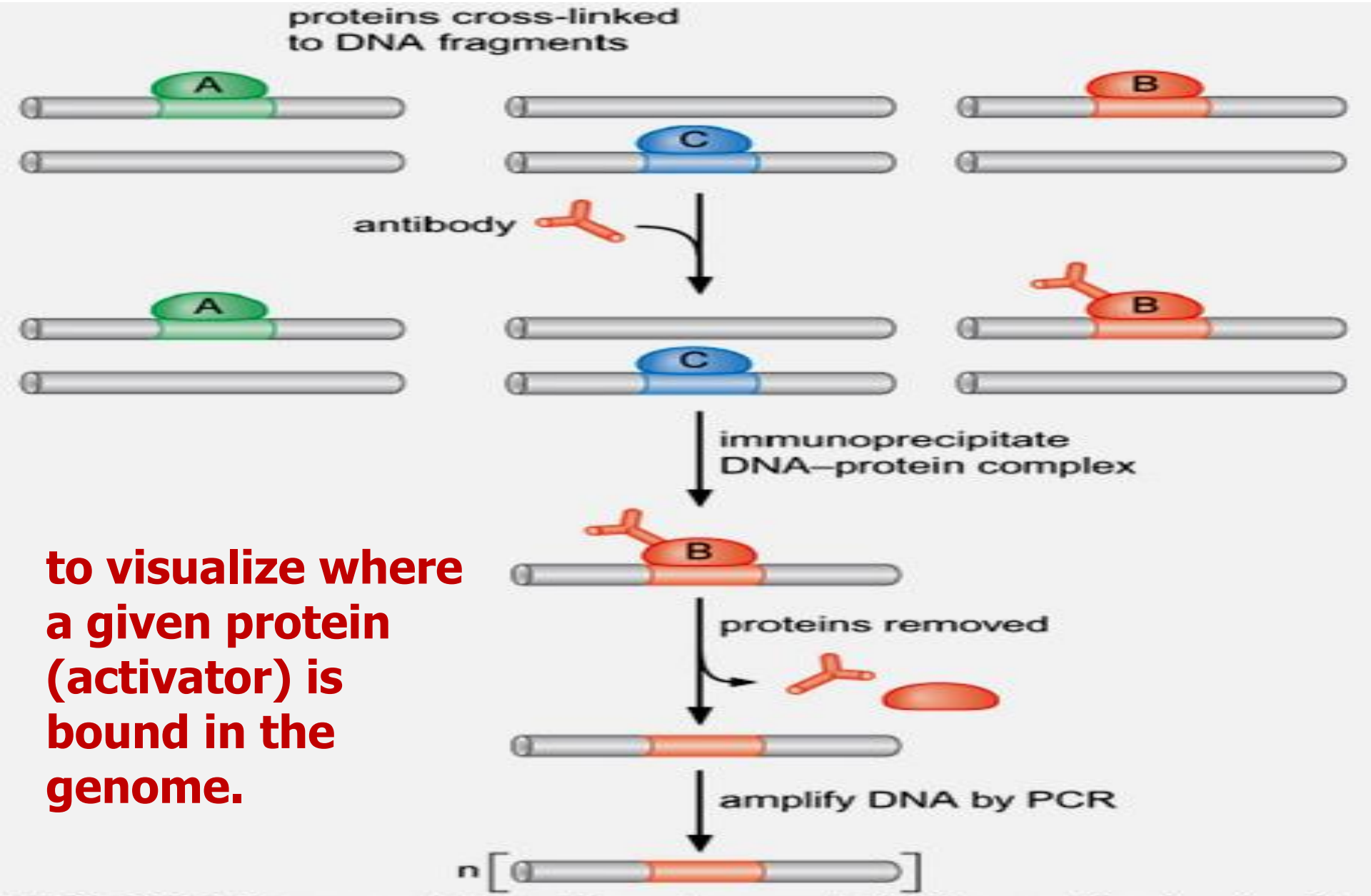
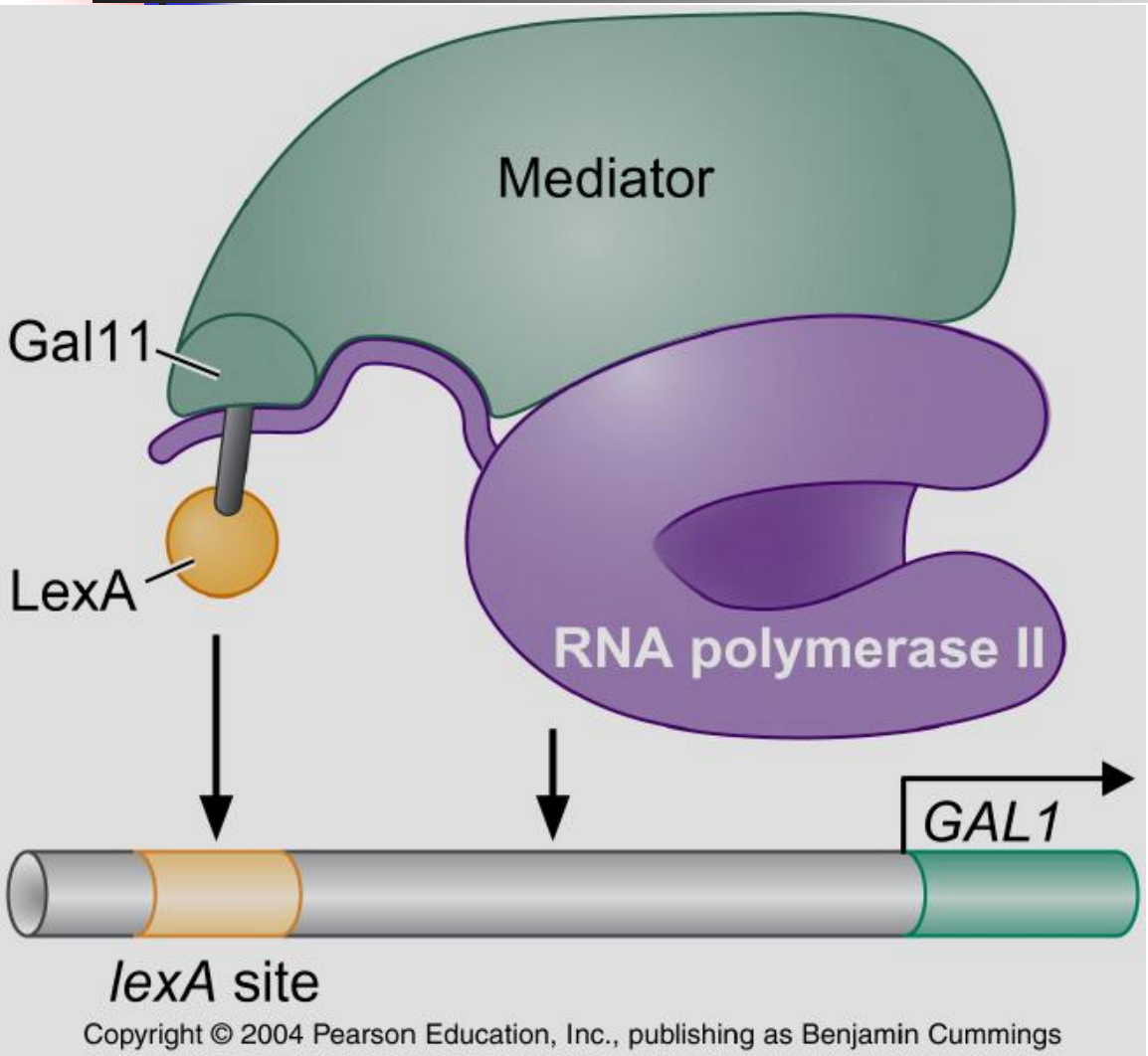


Fig. 8-15 Chromatin Immuno-precipitation (ChIP)



to visualize where a given protein (activator) is bound in the genome.

Figure 8-16 **Activator Bypass Experiment** Activation of transcription through direct tethering of mediator to DNA.



Directly fuse the bacterial DNA-binding protein LexA protein to Gal11, a component of the mediator complex to activate GAL1 expression.

At most genes, the transcription machinery is not prebound, and appear at the promoter only upon activation. Thus, no allosteric activation of the prebound polymerase has been evident in eukaryotic regulation。

Activators also recruit modifiers that help the transcription machinery bind at the promoter

Two types of Nucleosome modifiers :

- **Those add chemical groups to the tails of histones, such as histone acetyl transferases (HATs)**
- **Those remodel the nucleosomes, such as the ATP-dependent activity of SWI/SNF.**

How does the nucleosome modification help activate a gene?

1. "Loosen" the chromatin structure by chromosome remodeling and histone modification such as acetylation, which uncovers DNA-binding sites that would otherwise remain inaccessible within the nucleosome.

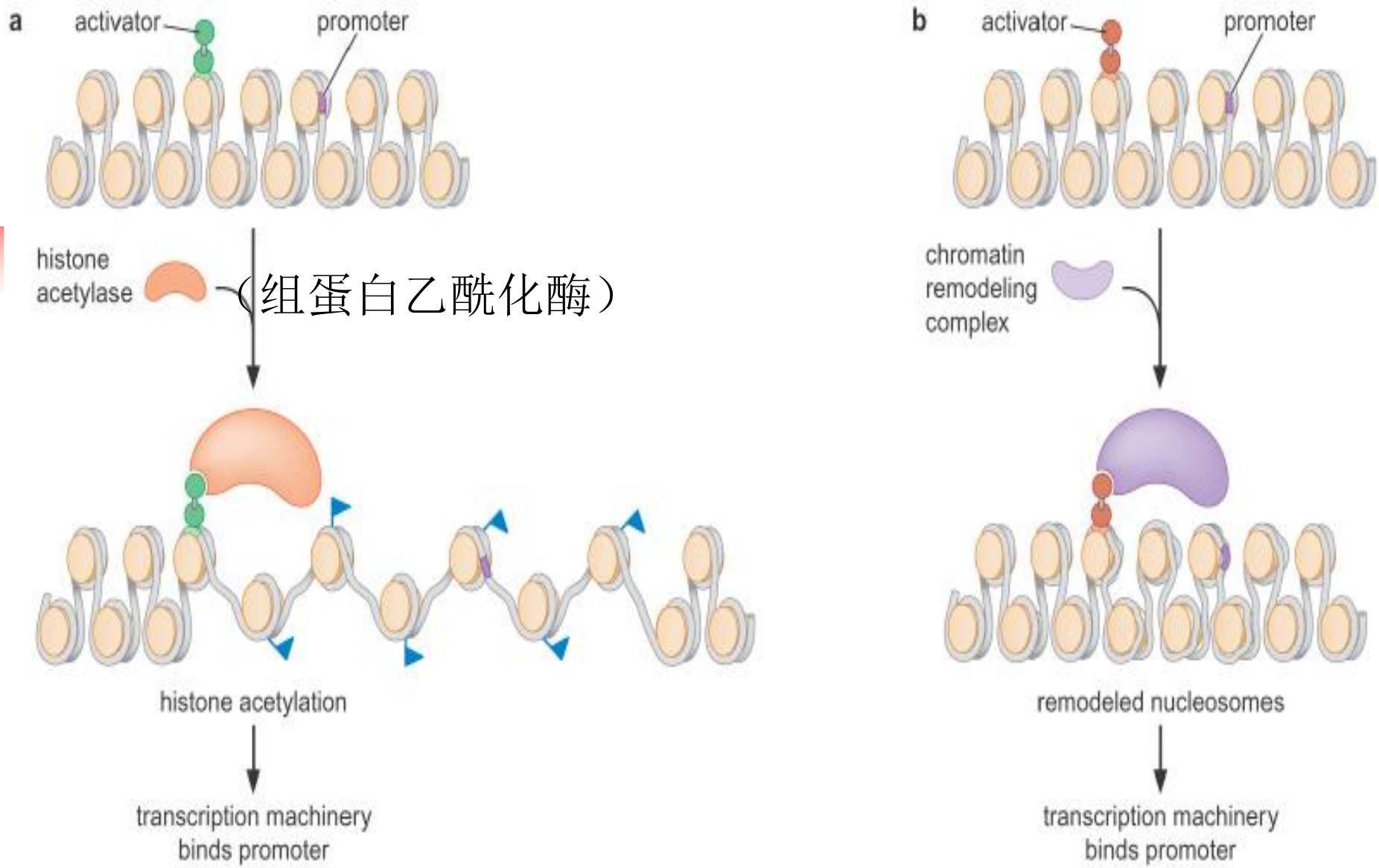
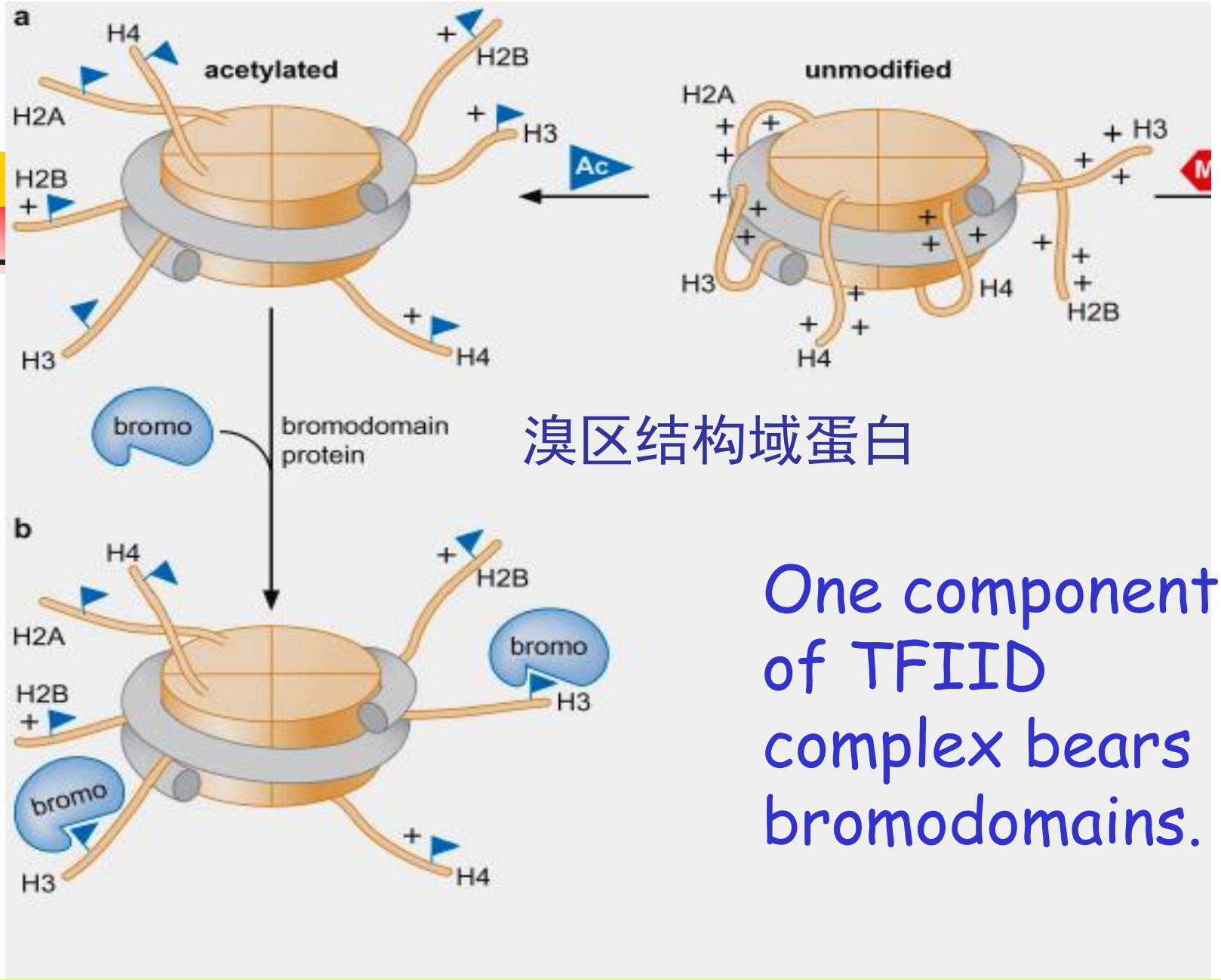


Fig 8-17 Local alterations in chromatin directed by activators

2. Adding acetyl groups to histones helps the binding of the transcriptional machinery.



One component of TFIID complex bears bromodomains.

Figure 8-18 Effect of histone tail modification

- One component of TFIID complex bears bromodomains that specifically bind to the acetyl groups.

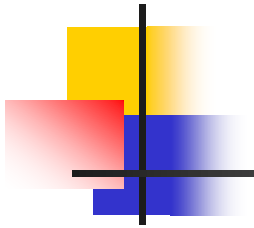
- Therefore, a gene bearing acetylated nucleosomes at its promoter has a higher affinity for the transcriptional machinery than the one with unacetylated nucleosomes.

3. Action at a distance: loops and insulators

Many eukaryotic activators—particularly in higher eukaryotes—work from a distance.

1) Some proteins help. Chip protein in *Drosophila* aids communication between enhancer and gene.

2) The compacted chromosome structure help. DNA is wrapped in nucleosomes in eukaryotes. Chromatin may in some places form special structure that actively bring enhancers and promoters closer.



Specific cis-acting elements called insulators control the actions of activators, preventing activation of the non-specific genes

Enhancer : a given site binds regulator responsible for activating the gene.
Alternative enhancer binds different groups of regulators and controls expression of the same gene at different times and places in responsible to different signals.

Insulators or **boundary elements** are regulatory sequences between enhancers and promoters. They block activation of the promoter by activator bound at the enhancer, and therefore ensure activators to work discriminately.

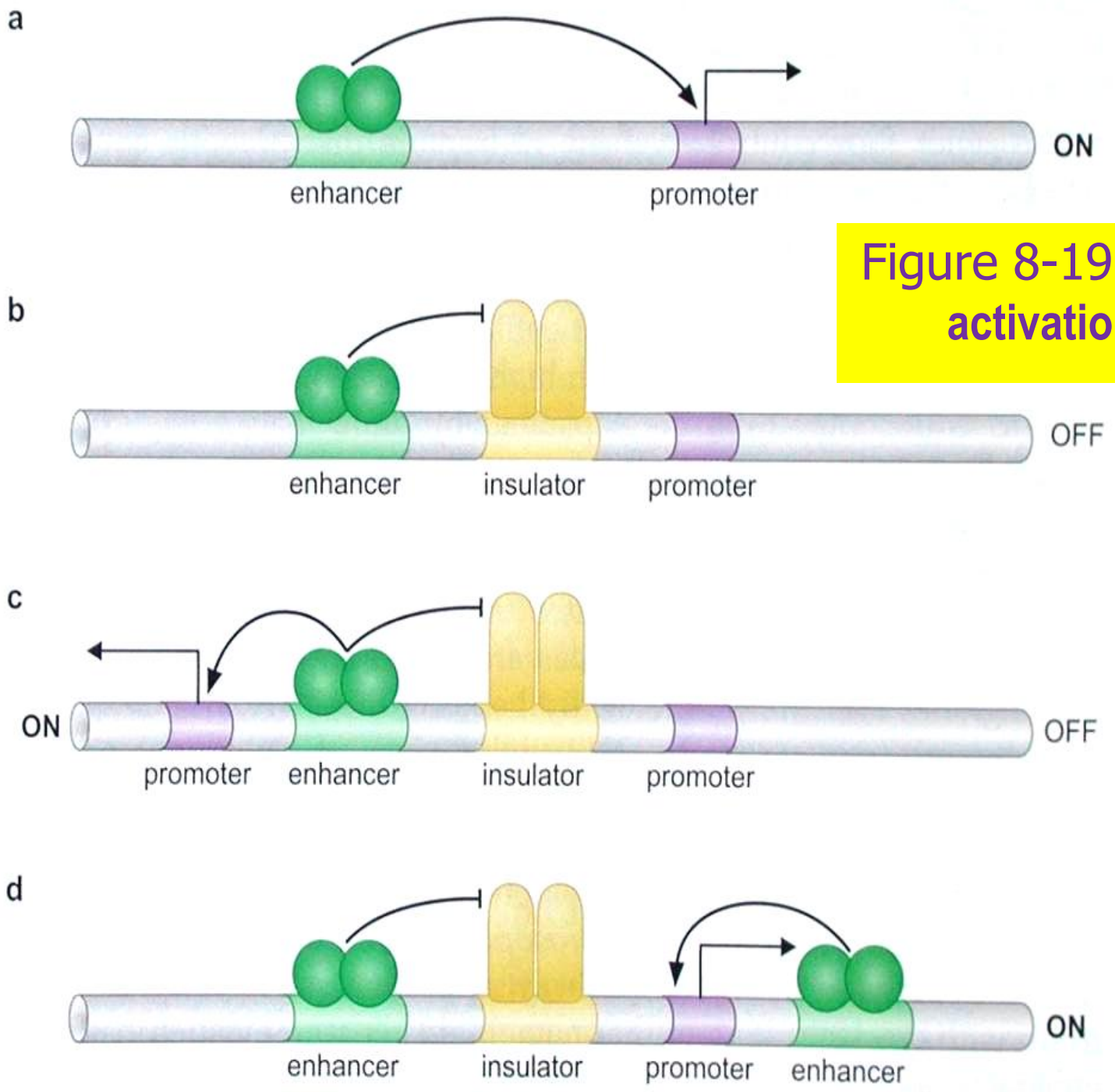


Figure 8-19 **Insulators block activation by enhancers**

Insulators can also protect genes from transcriptional silencing

Transcriptional silencing is a specialized form of repression that can spread along chromatin, switching off multiple genes without the need for each to bear binding sites for specific repressor.

Insulator elements can block this spreading, so insulators protect genes from both indiscriminate activation and repression

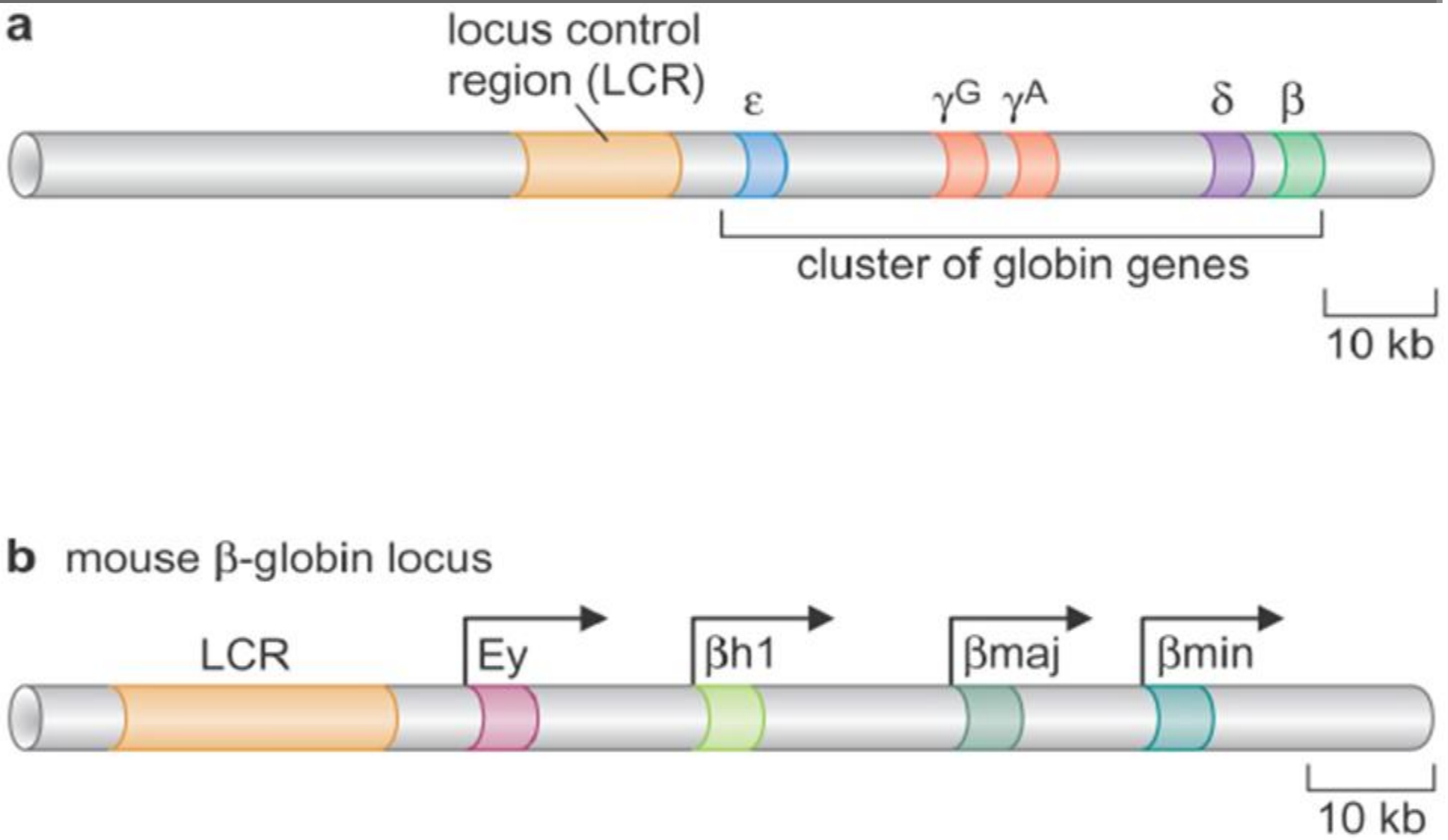
Application of insulators

A gene inserted at random into the mammalian genome is often "silenced" because of formation of heterochromatin, and placing insulators upstream and downstream of that gene can protect the gene from silencing.

Appropriate regulation of some groups of genes requires locus control region (LCR).

1) Human and mouse globin genes are clustered in genome and differently expressed at different stages of development
2) A group of regulatory elements collectively called the locus control region (LCR), is found 30-50 kb upstream of the cluster of globin genes. It binds regulatory proteins that cause the chromatin structure to "open up", allowing access to the array of regulators that control expression of the individual genes in a defined order.

Figure 8-20 Regulation of globin genes by LCR



Another group of mouse genes whose expression is regulated in a temporarily and spatially ordered sequence are called *HoxD* genes. They are controlled by an element called the GCR (global control region) in a manner very like that of LCR.

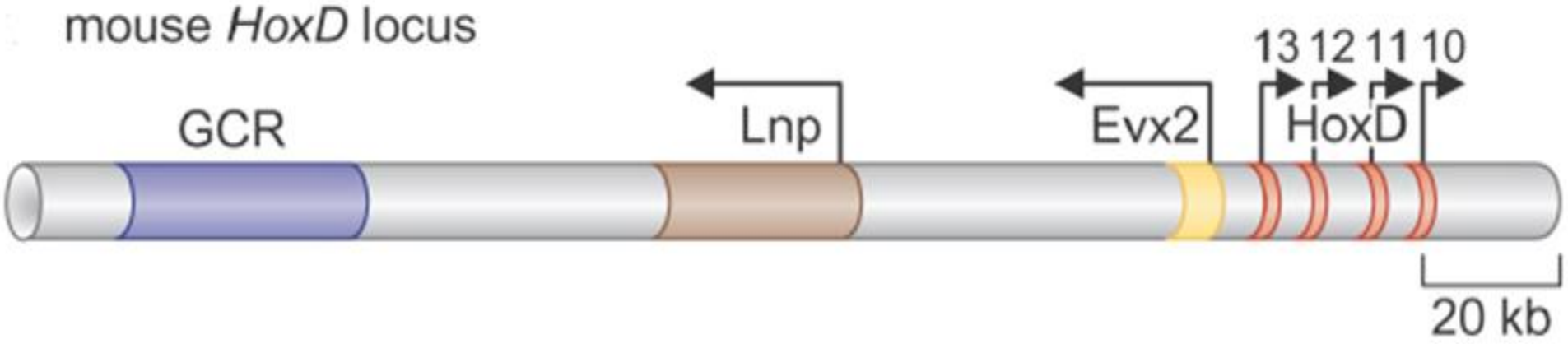


Fig. 8-21 Control of HoxD genes by GCR

Part 3: Signal Integration and Combinatorial Control

In some cases, numerous signals are required to switch a gene on. However, each signal is transmitted to the gene by a separate regulator, and therefore, multiple activators often work together, and they do so synergistically (two activators working together is greater than the sum of each of them working alone.)

Three strategies of the synergy

1. Multiple activators recruit a single component of the transcriptional machinery.

2. Multiple activators each recruit a different component of the transcriptional machinery.

3. Multiple activators help each other bind to their sites upstream of the gene they control.

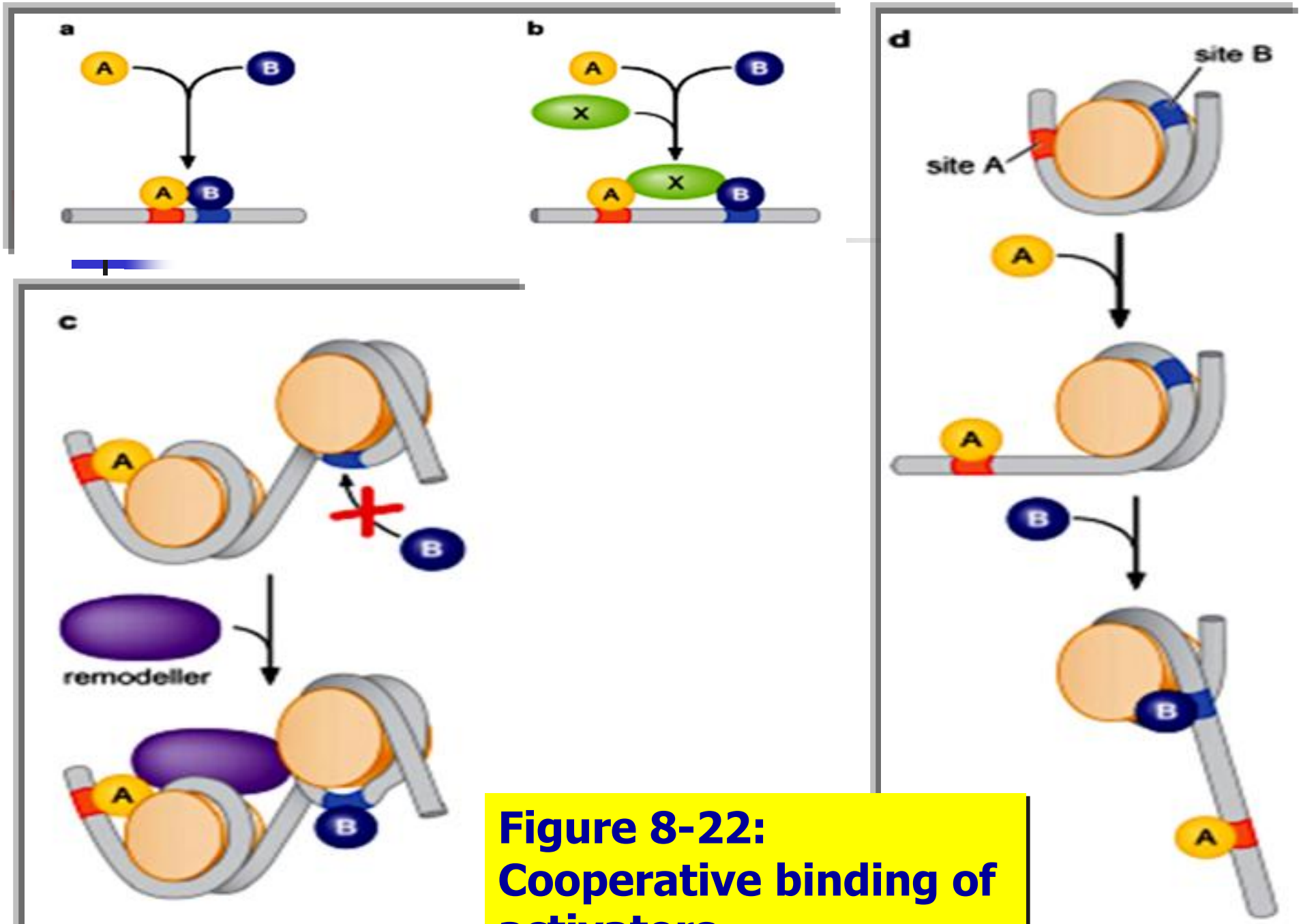


Figure 8-22: Cooperative binding of activators

Homework—select one of the following two items and turn in your answers in either English or Chinese on June 2, Tuesday.

1. What are the essential characteristics of promoters that are activated by recruitment (cooperative binding) and those activated by allostery?

   In an activator bypass experiment, RNA polymerase is brought to a promoter in the absence of a traditional activator. Describe three ways this experiment might be done. How does this experiment distinguish between the two classes of promoter described above?

2. Outline the steps involved in base-excision repair. How does the cell manage to accommodate all of the various types of damaged bases? If the excision repair system fails to remove a damaged base prior to DNA replication, will it inevitably result in a mutation?

Control of the HO gene

The *HO* gene (encoding a sequence-specific endonuclease controlling mating types of budding yeast) is only expressed in mother cells and only at a certain point in the cell cycle.

The **mother cell and cell cycle conditions (signals)** are communicated to **the *HO* gene (target)** by two activators: **SWI5 and SBF (communicators)**.

SWI5: acts only in the **mother cell**, binds to multiple sites some distance from the gene, and recruits enzymes to open the SBF binding sites.

SBF: only active at the **correct stages** of the cell cycle.

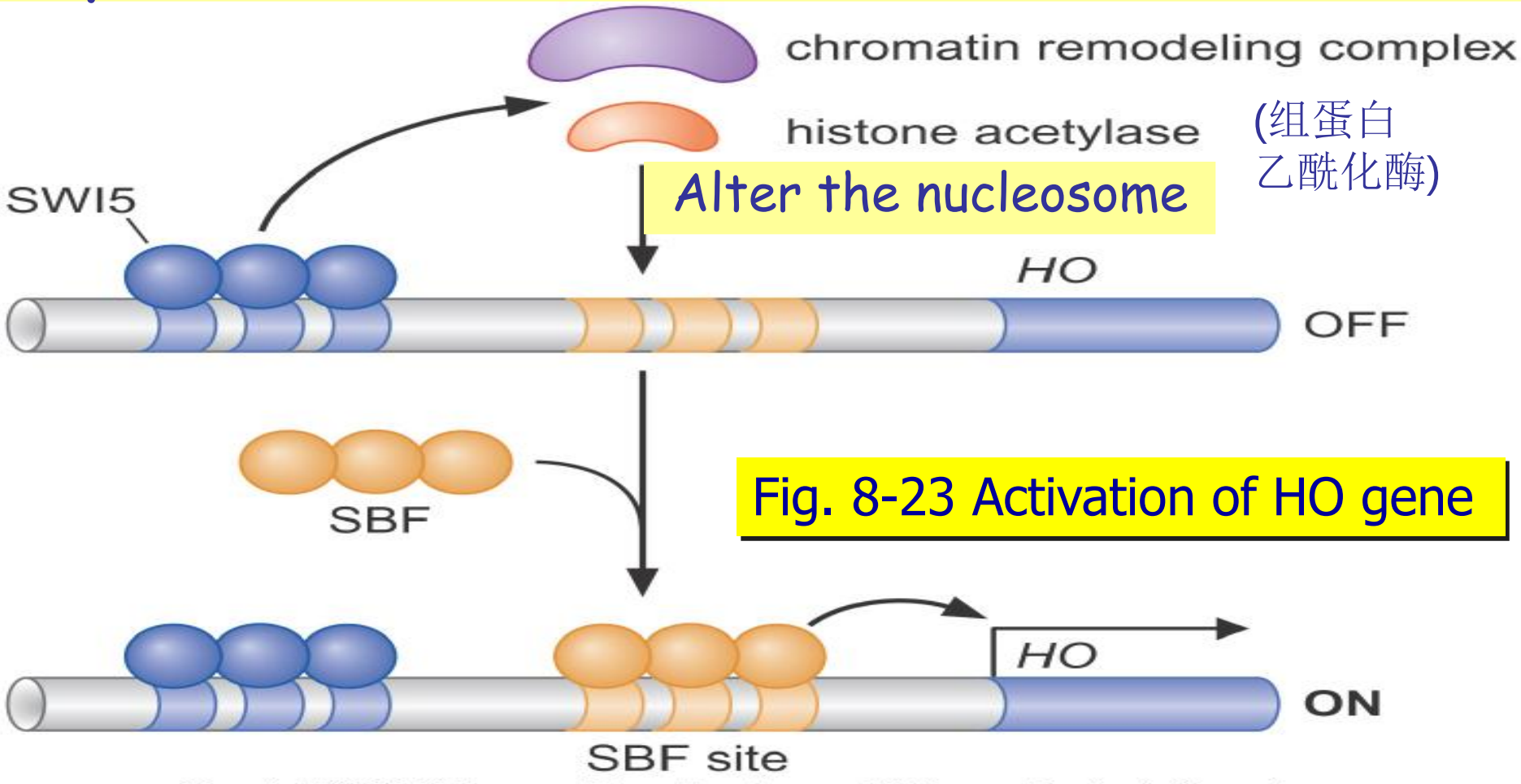
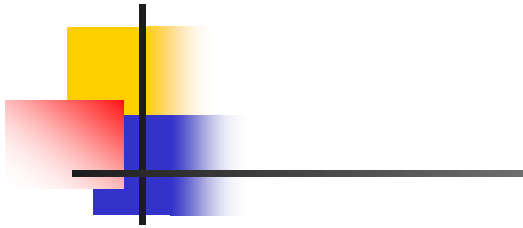


Fig. 8-23 Activation of HO gene



[Watch the animation on “activation of HO gene”]

5月19日 第一节课

Summary for HO gene activation

The *HO* Gene

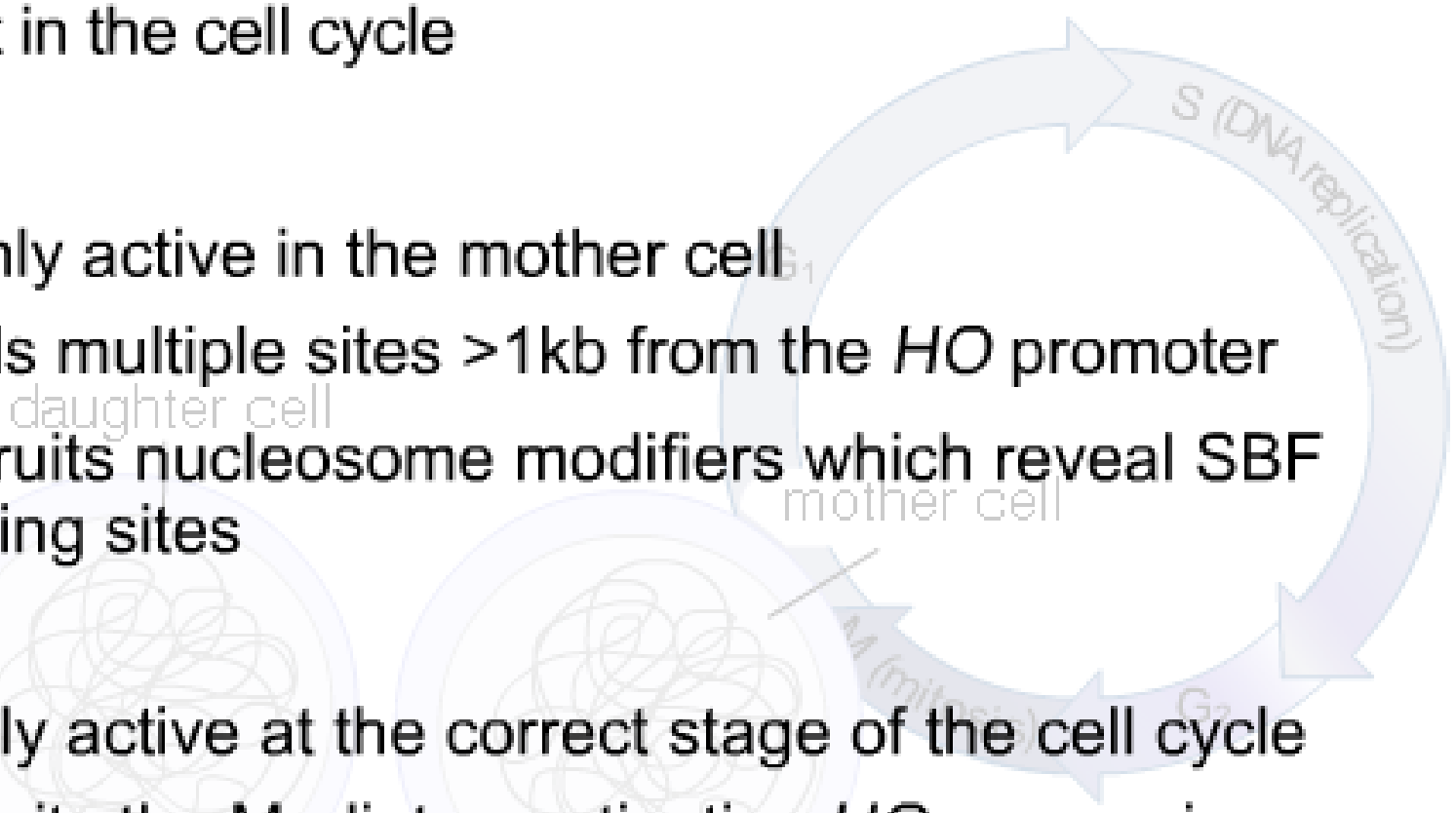
- Codes for HO endonuclease
- Is only expressed in mother cells
- Is only expressed at a certain point in the cell cycle

SWI5

- Is only active in the mother cell
- Binds multiple sites >1kb from the *HO* promoter
- Recruits nucleosome modifiers which reveal SBF binding sites

SBF

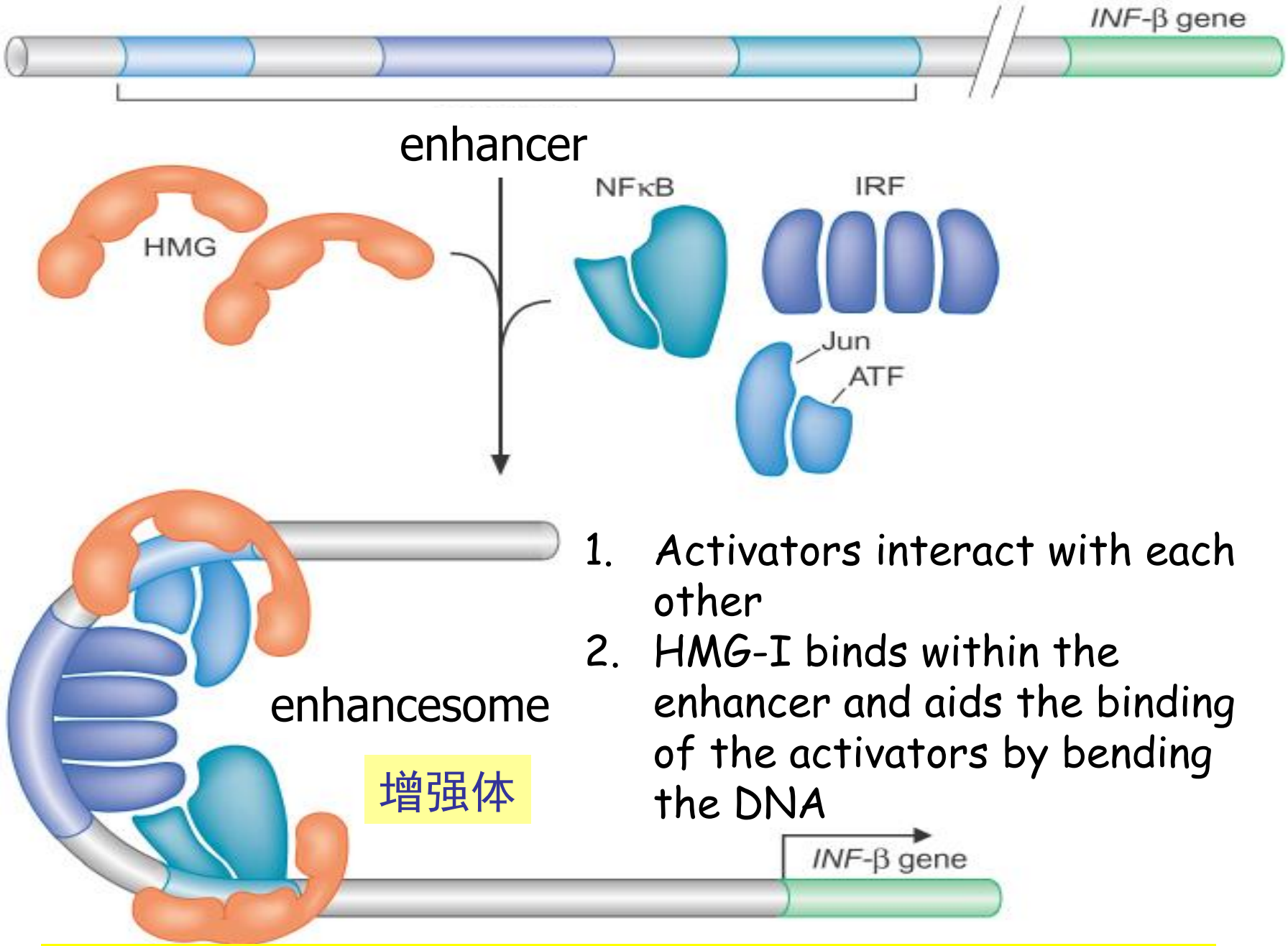
- Is only active at the correct stage of the cell cycle
- Recruits the Mediator, activating *HO* expression



Signal integration: Cooperative binding of activators at the human β-interferon gene.

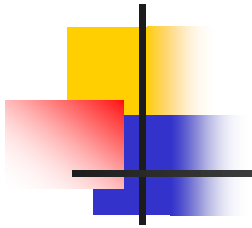
The human β-interferon gene (target gene) is activated in cells upon viral infection (signal). Infection triggers three activators (communicator): NFκB, IRF, and Jun/ATF.

Activators bind cooperatively to sites adjacent to one another within an enhancer located about 1 kb upstream of the promoter, which forms a structure called enhanceosome.



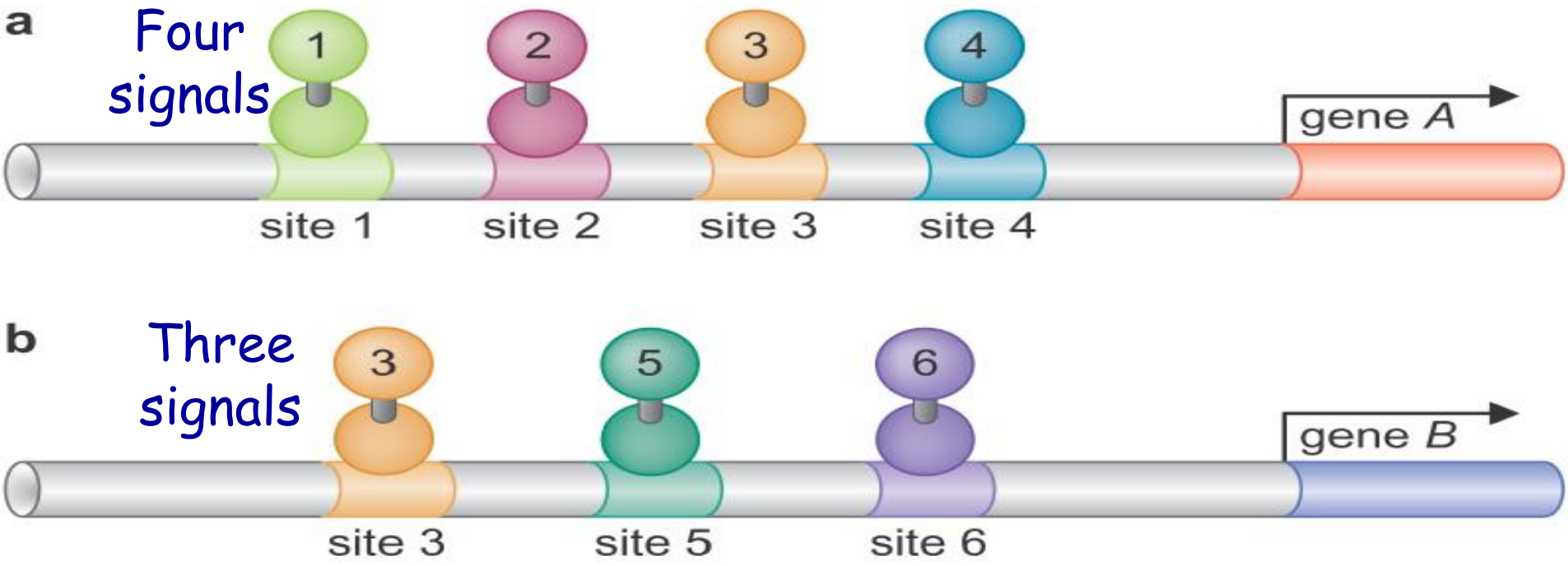
1. Activators interact with each other
2. HMG-I binds within the enhancer and aids the binding of the activators by bending the DNA

Fig. 8-24 The human β-interferon enhanceosome



Combinatory control lies at the heart of the complexity and diversity of eukaryotes

Figure 8-25 Extensive combinatorial controls in eukaryotes.



Combinatory control of the mating-type genes from *S. cerevisiae*

The yeast *S. cerevisiae* exists in three forms:

---two haploid cells of different mating types— **a** and α.

---the diploid cells (**a**/α) formed when an **a** and an α cell mate and fuse.

- **a cells** make the regulatory protein **a1**,
- **α cells** make the protein **α1** and **α2**.
- **Both cell types** express the fourth regulator protein **Mcm1** that is also involved in regulating the mating-type specific genes.

Fig. 8-26: Control of cell-type specific genes in yeast

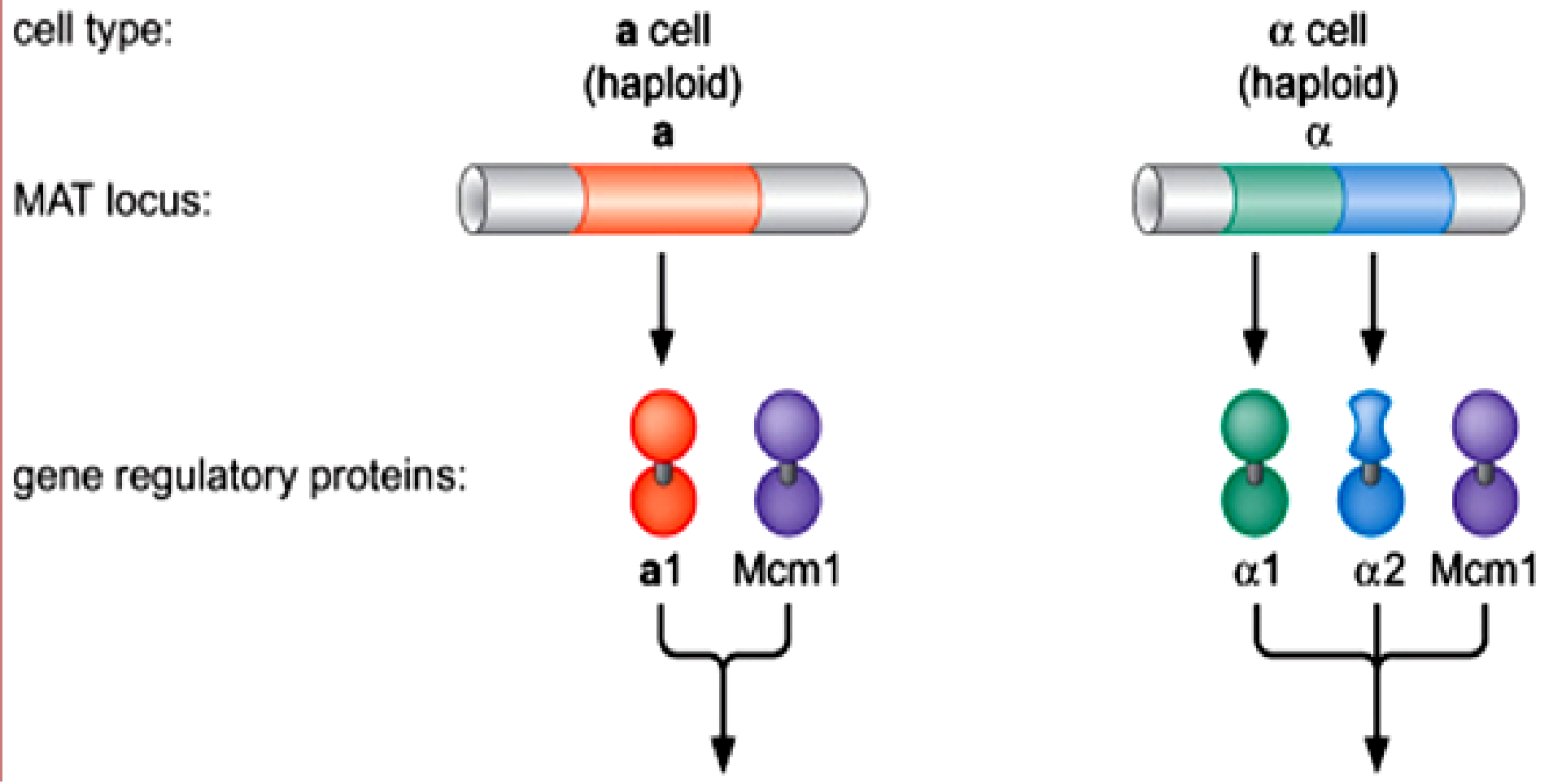
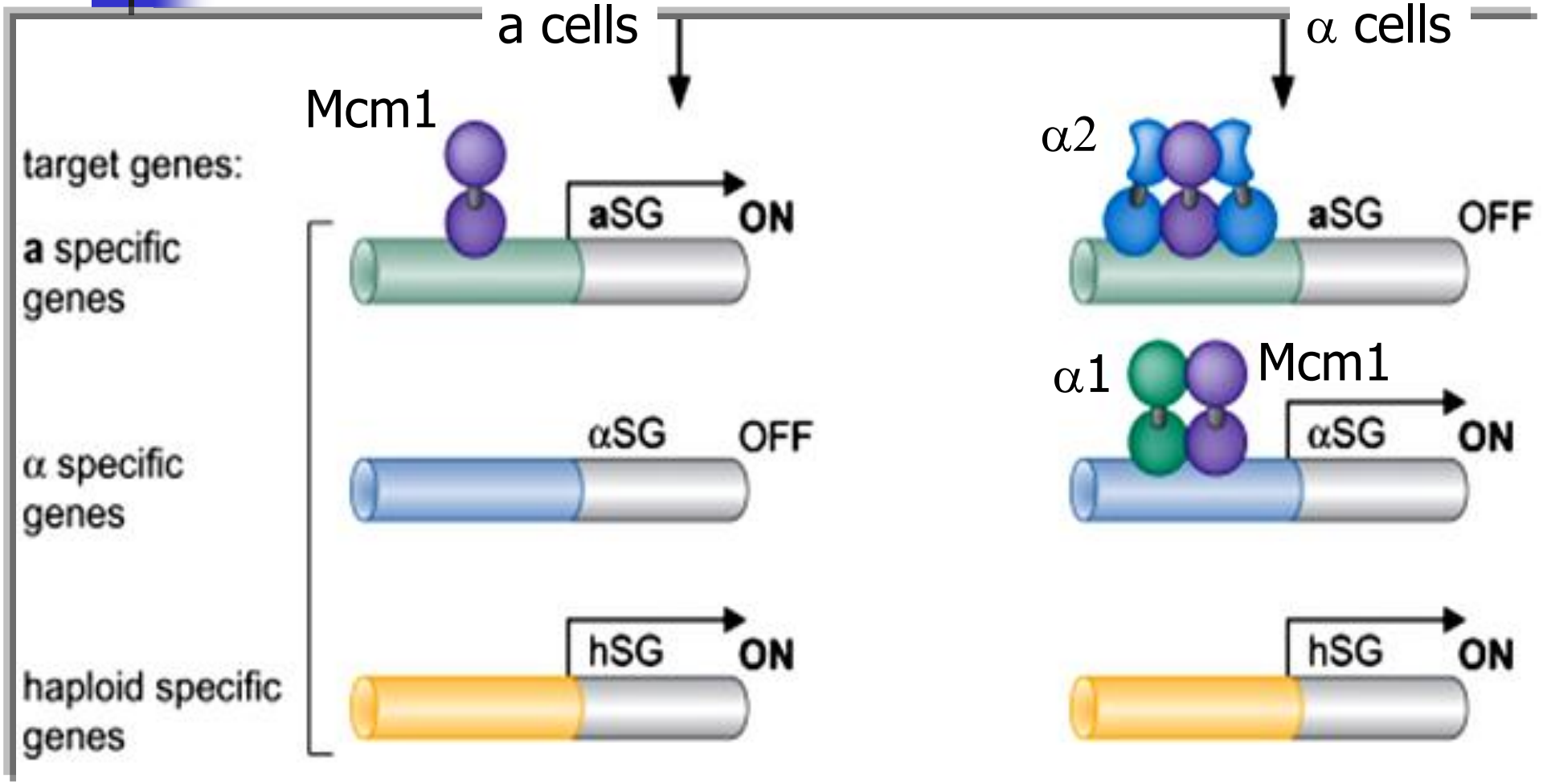


Fig. 8-26: Control of cell-type specific genes in yeast (continued)



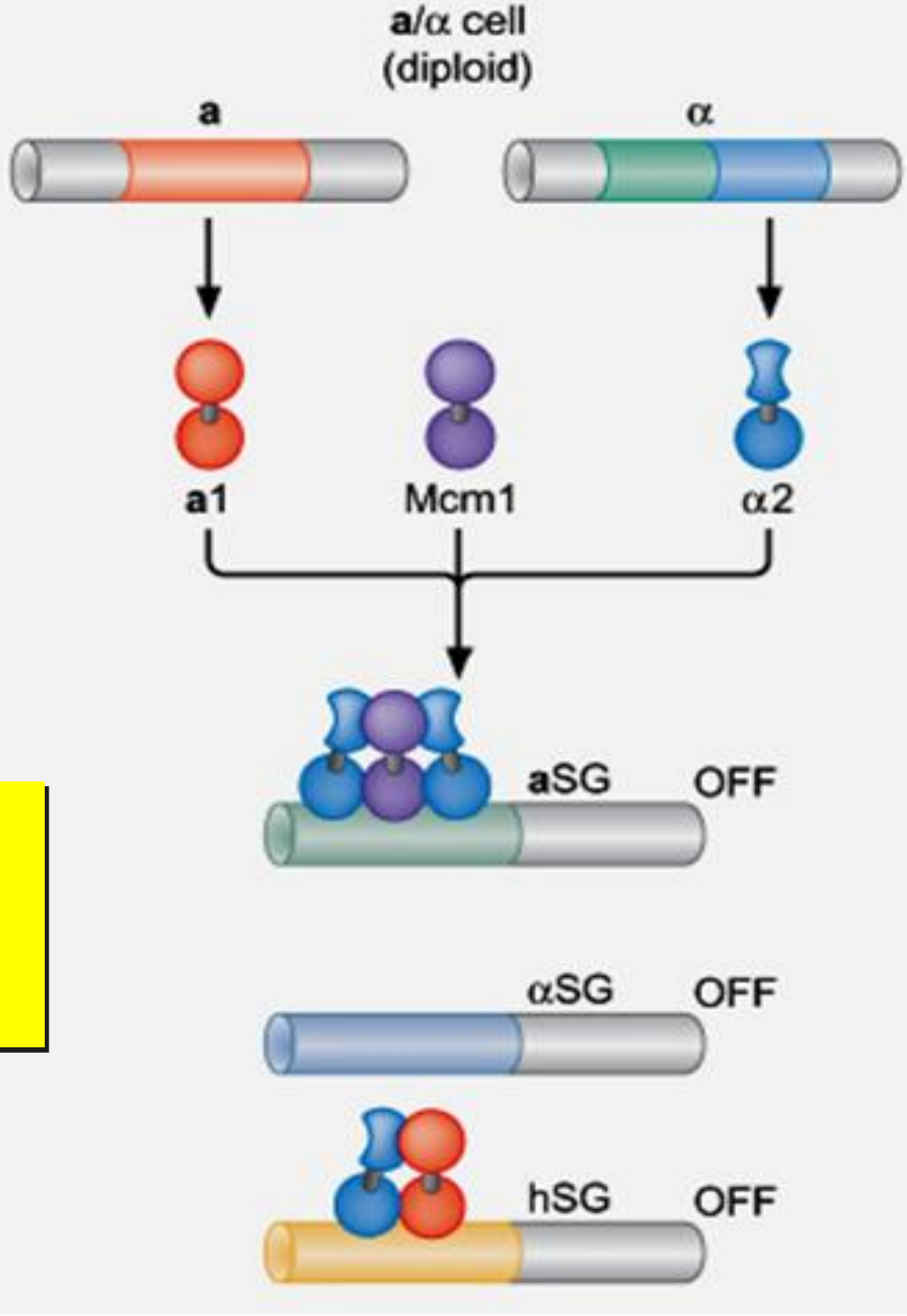
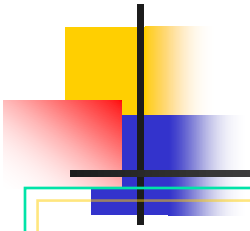


Fig. 8-26: Control of cell-type specific genes in yeast (continued)



Part 4: Transcriptional Repressors

Commonly, eukaryotic repressors **recruit nucleosome modifiers** that compact the nucleosome or remove the groups recognized by the transcriptional machinery.

For example, histone deacetylases repress transcription by removing acetyl groups from the tails of histones.

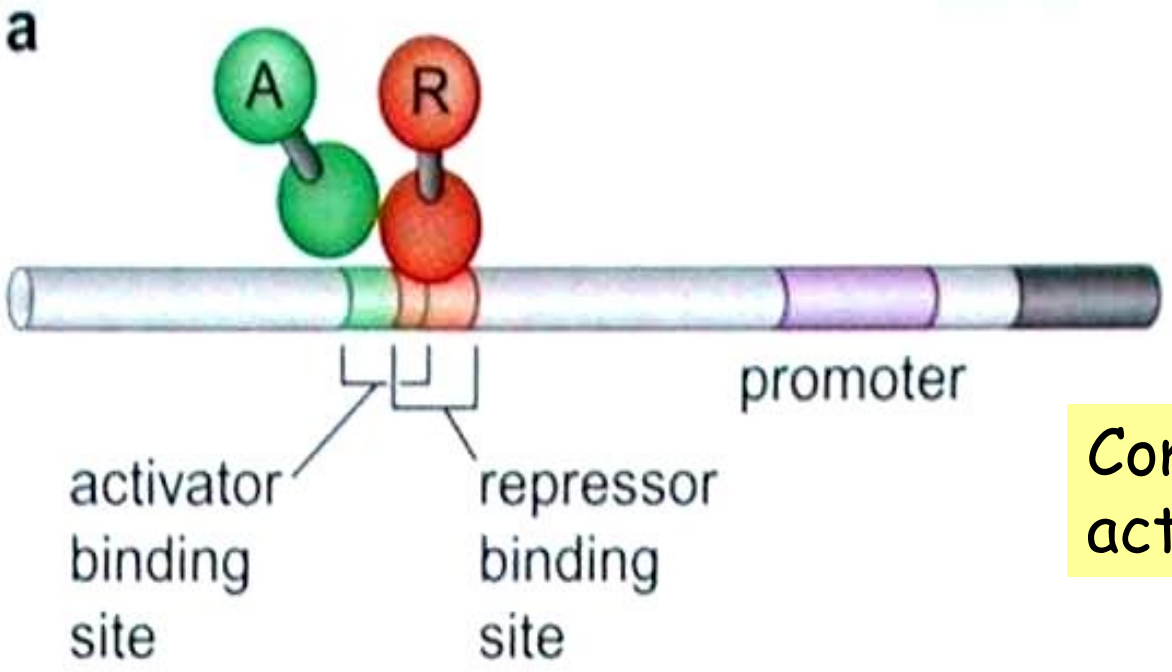
This type of repression is called "**silencing**".

Three other ways in which an eukaryotic repressor works :

(1) **Competes with the activator for an overlapped binding site.**

(2) **Binds to a site different from that of the activator, but physically interacts with an activator and thus block its activating region.**

(3) **Binds to a site upstream of the promoter, physically interacts with the transcription machinery at the promoter to inhibit transcription initiation.**



mechanism:

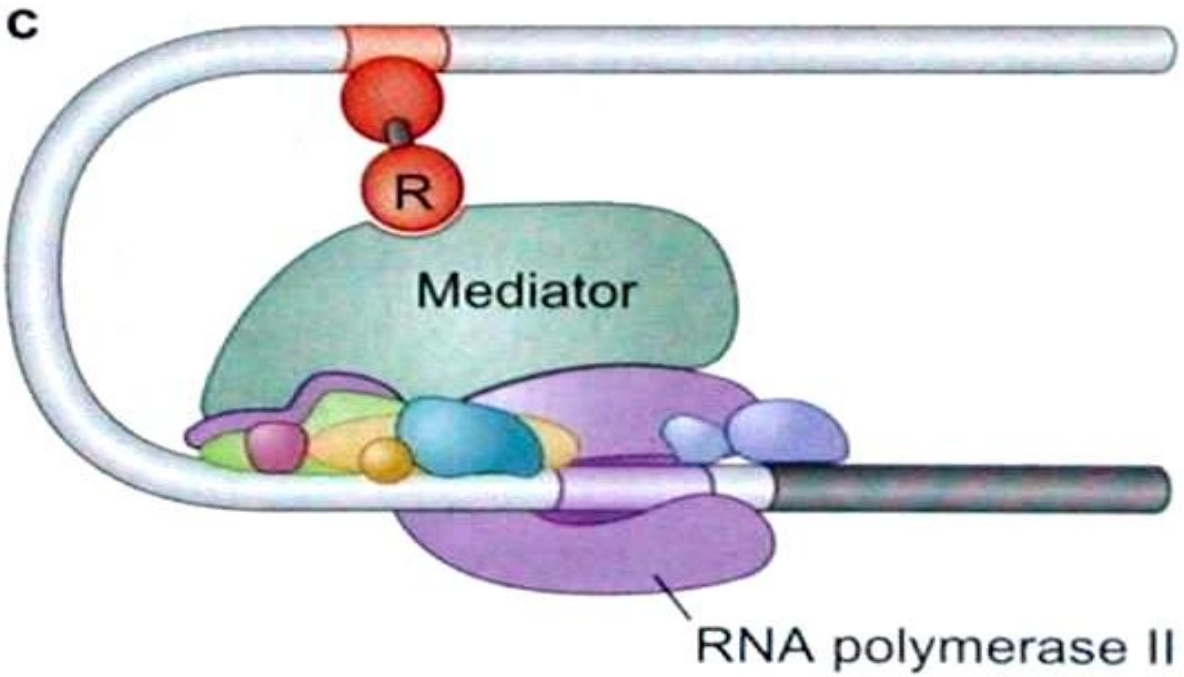
competition

Competes for the activator binding

Inhibits the function of the activator.

inhibition

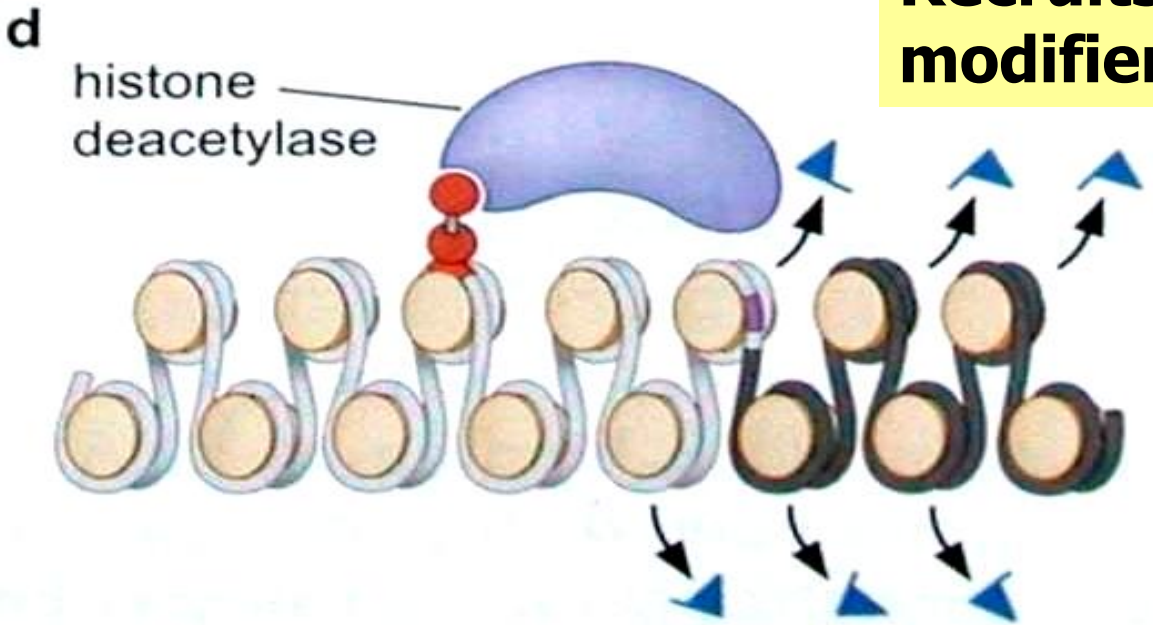
Fig. 8-27： Ways in which eukaryotic repressors work



direct repression

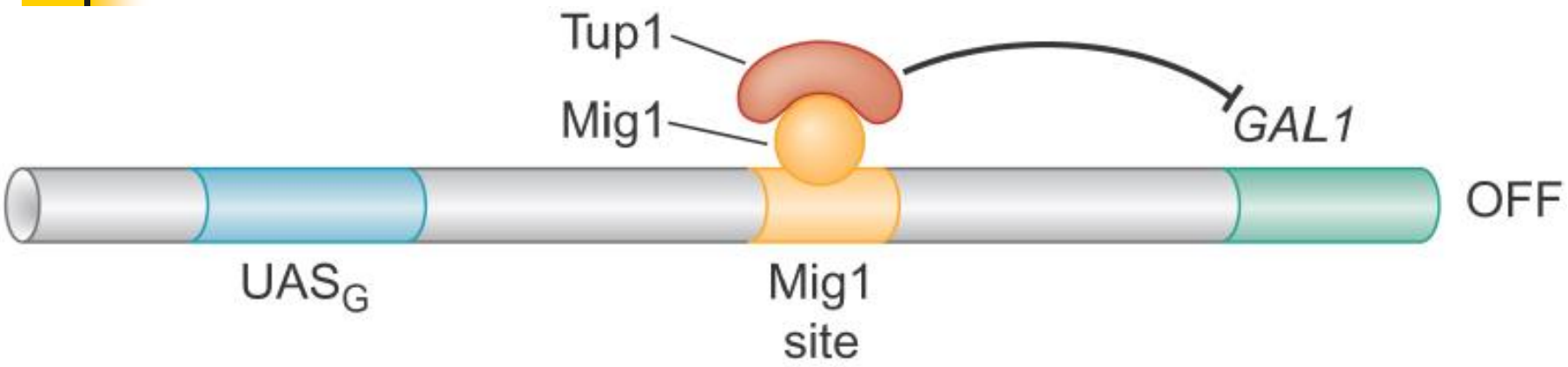
Binds to the transcription machinery

Recruits nucleosome modifiers (most common)

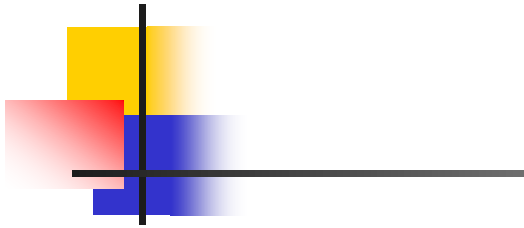


indirect repression

Fig. 8-28 Repression of the GAL1 gene in yeast



In the presence of glucose, Tup1 recruits histone deacetylases, and also directly interacts with the transcription machinery to repress transcription.



Part 5: Signal Transduction and the Control of Transcriptional Regulators

Environmental Signals/Information

1. Small molecules such as sugar, histamine.
2. Proteins released by one cell and received by another.

In eukaryotic cells, most signals are communicated to genes through signal transduction pathway (indirect) in which the initiating ligand is detected by a specific cell surface receptor.

Signal transduction pathway

1. The initial ligand ("signal") binds to an extracellular domain of a specific cell surface receptor

2. The signal is thus communicated to the intracellular domain of receptor *(via an allosteric change or dimerization)*

3. The signal is then relayed to the relevant transcriptional regulator.

4. The transcriptional regulator control the target gene expression .

Figure 8-29：Signal transduction pathway--The STAT pathway

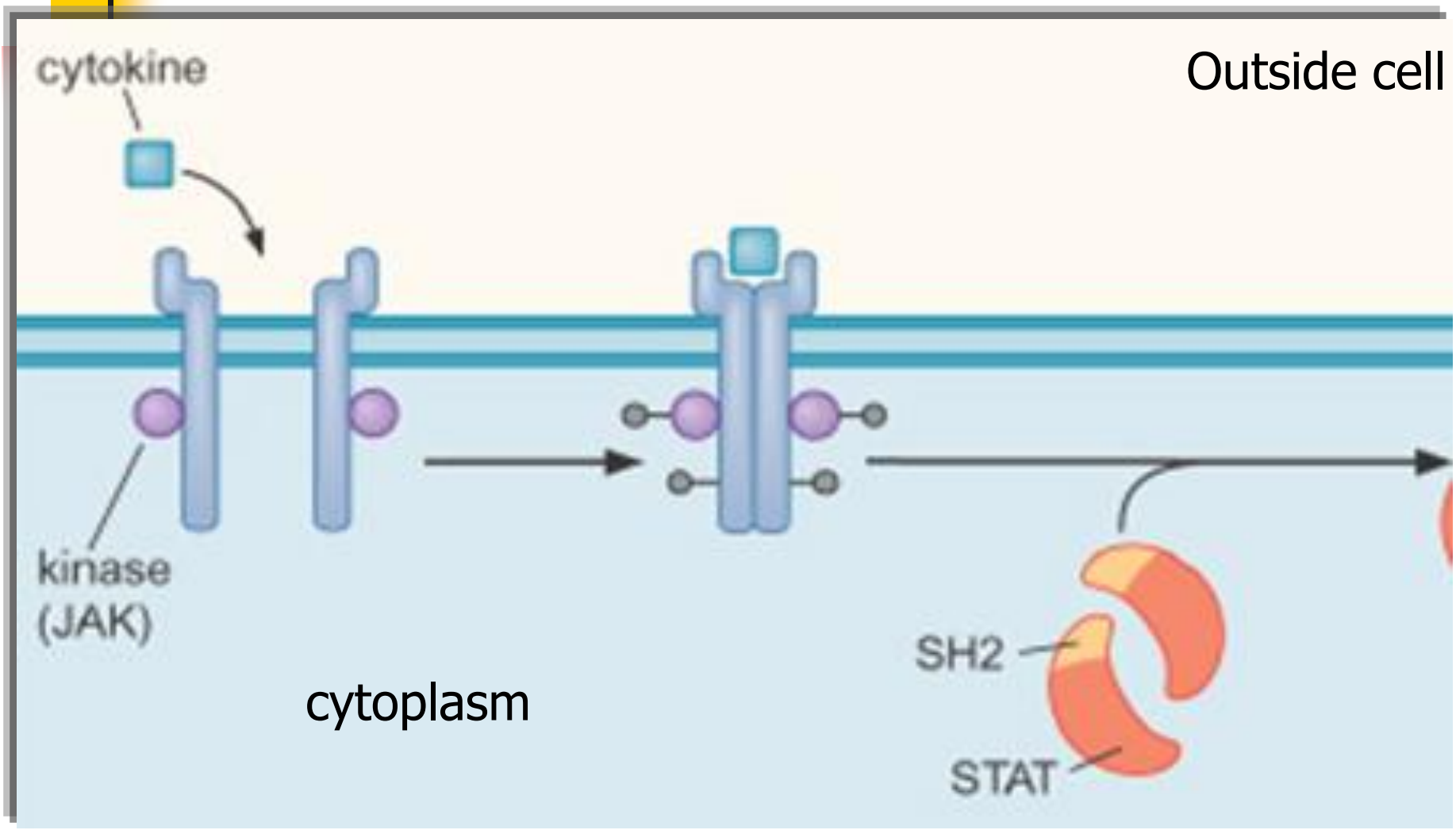


Figure 8-29：Signal transduction pathway--The STAT pathway (continued)

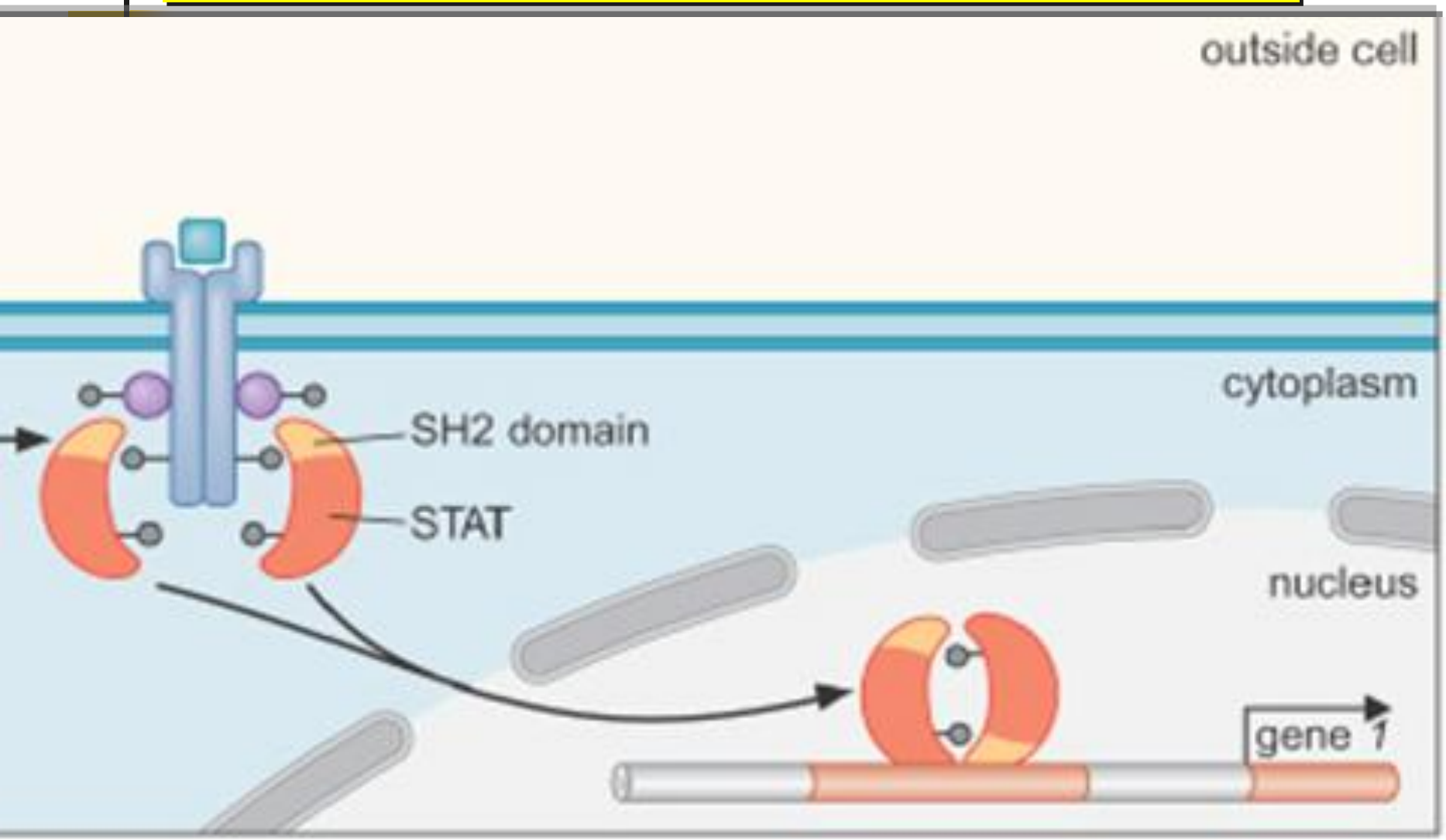


Fig. 8-30 The MAP kinase pathway

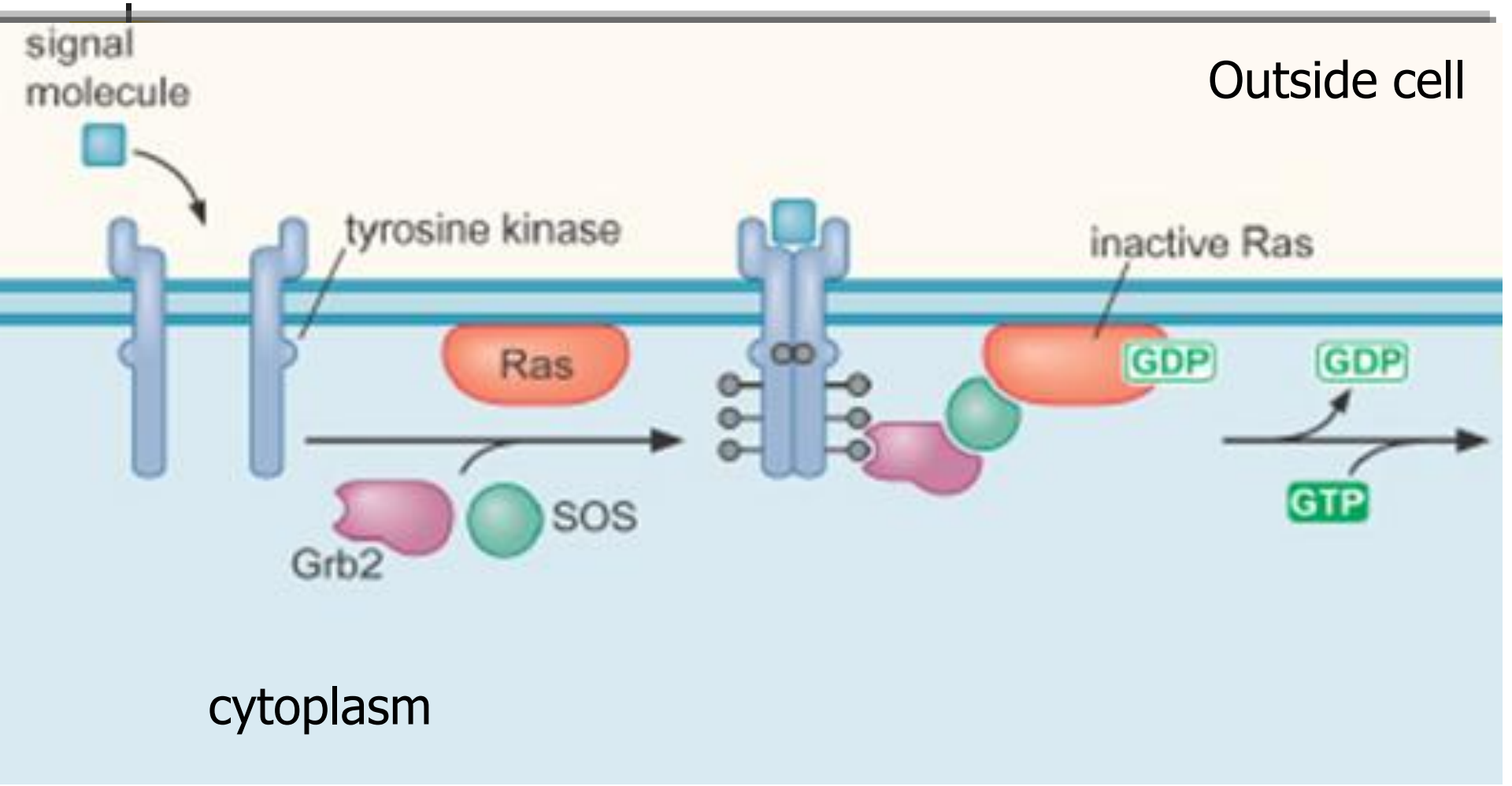
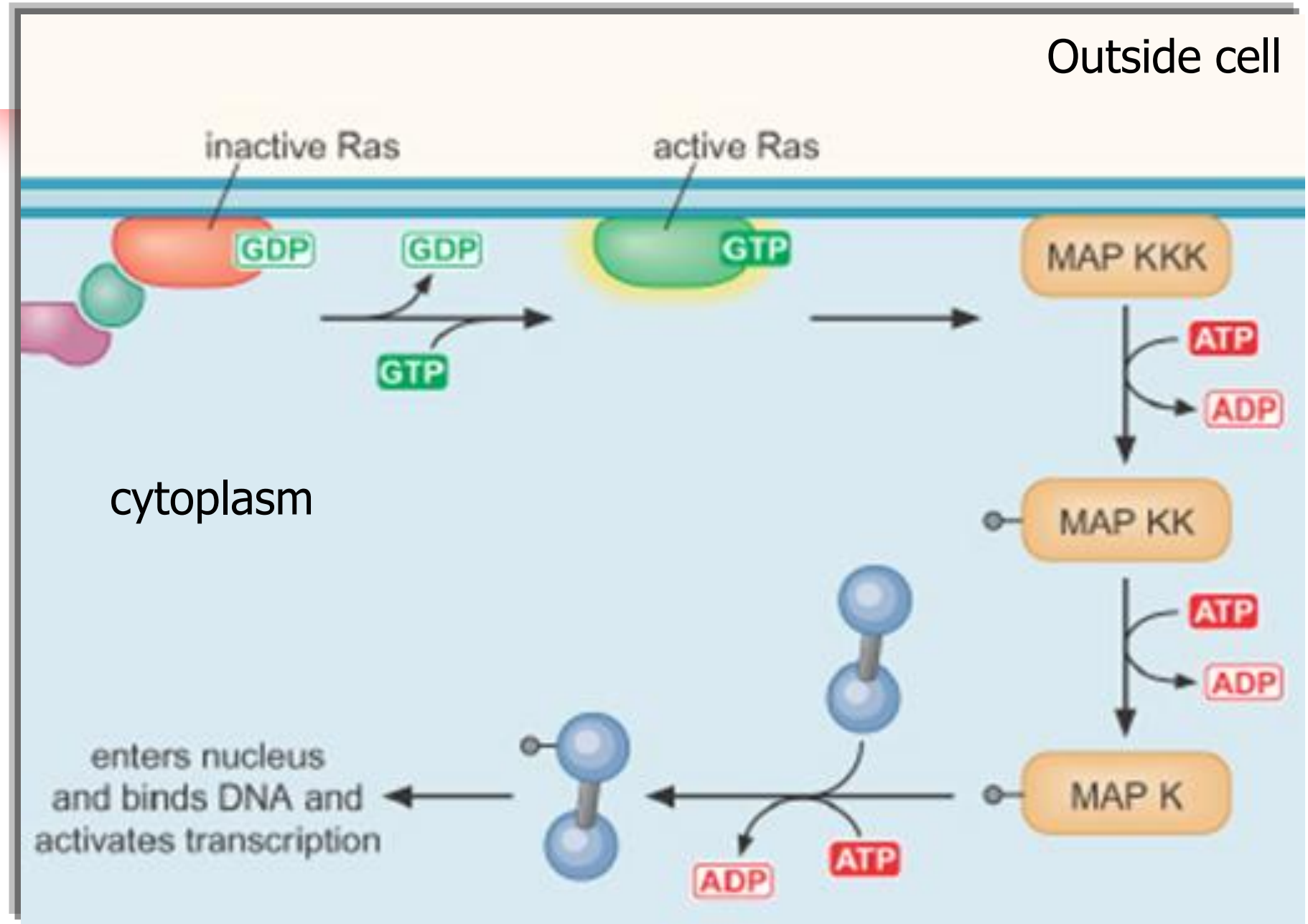
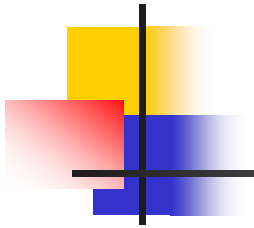


Fig. 8-30 The MAP kinase pathway (continued)





In contrast to those in bacteria, transcriptional regulators in eukaryotes are not typically controlled at the level of DNA binding.

Unmasking an activating region

(1) A conformational change to reveal the previously buried activating region.

(2) Releasing of the previously bound masking protein, leading to an activating region open.

(3) Some masking proteins not only block the activating region of an activator but also recruit (or itself) a deacetylase enzyme to repress the target gene.

Figure 8-31 **Activator Gal4 is regulated by a masking protein Gal80**

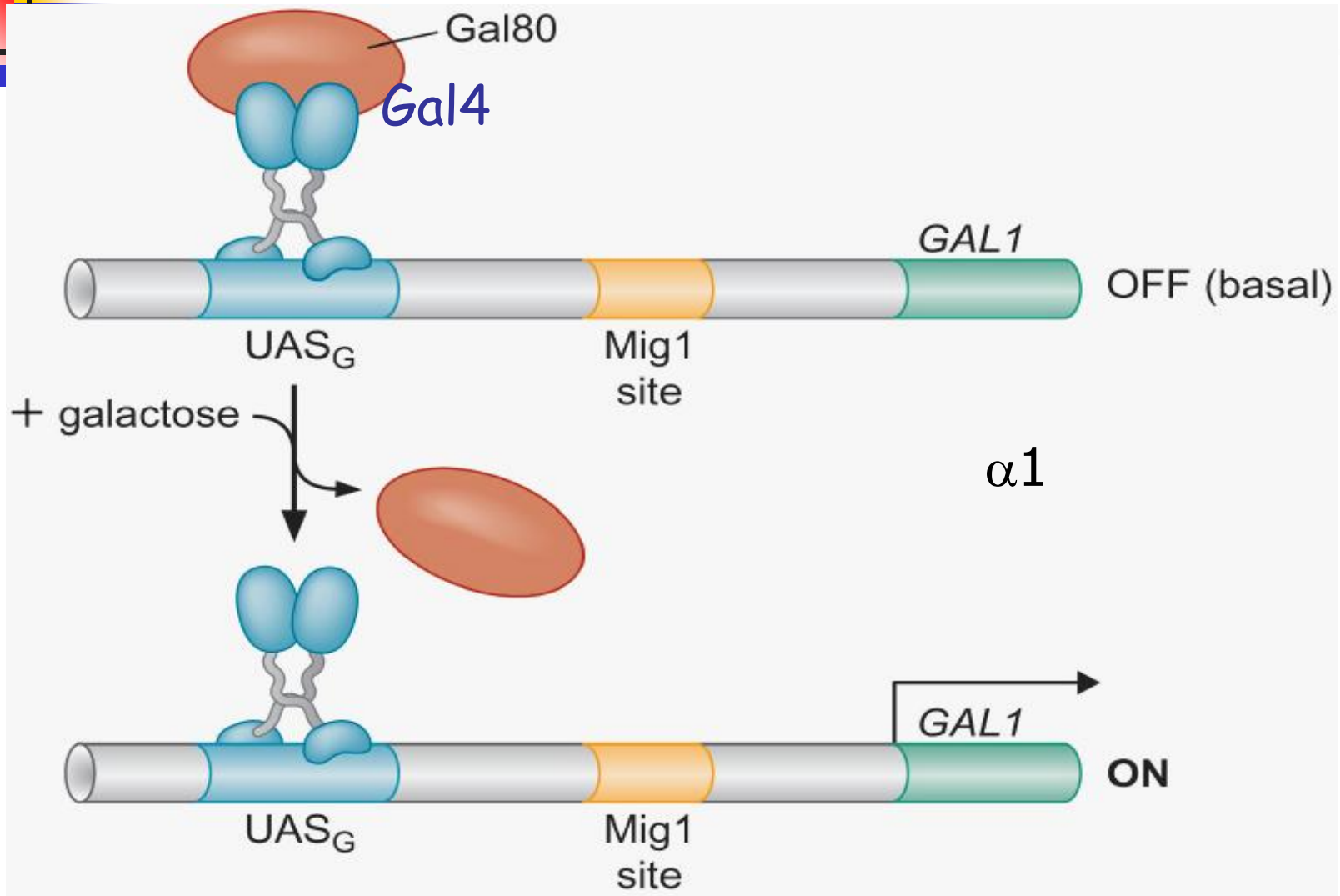
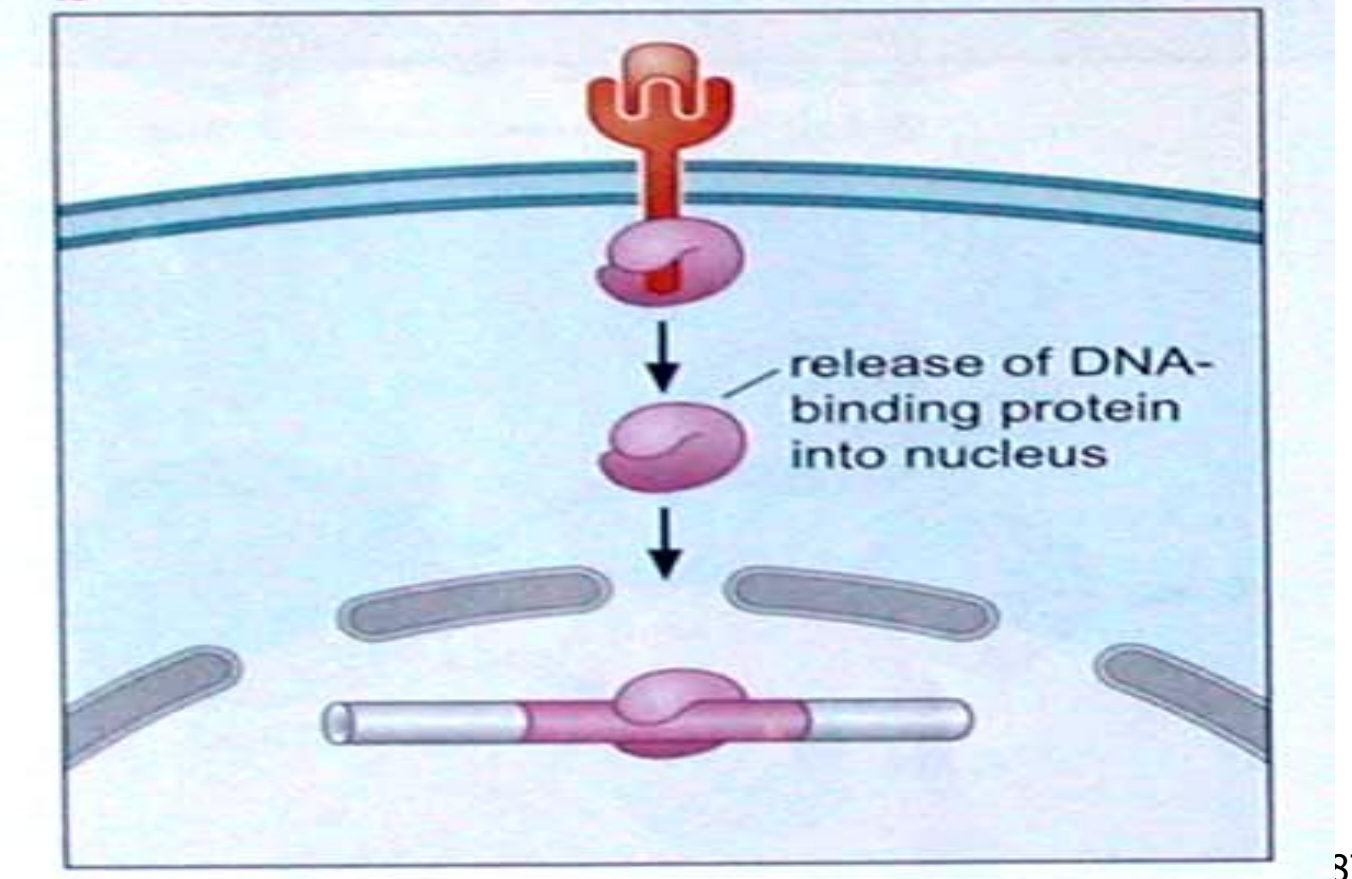


Fig.8-32 **Transport into and out of the nucleus** : When not active, many activators and repressors are held in the cytoplasm. The signaling ligand causes them to move into the nucleus where they activate transcription.

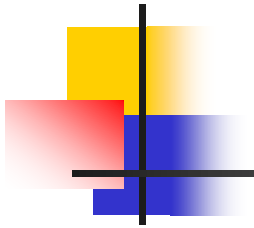


Activators and repressors sometimes come in pieces

The mammalian activator E2F binds sites upstream of its target genes.

A second protein, the repressor Rb, controls the activity of E2F by binding E2F, thus blocking activation and recruiting a deacetylase enzyme that represses the target genes.

Phosphorylation of Rb causes release of Rb from E2F, and thus activation of the genes.



Part 6: Gene "Silencing" by Modification of Histones and DNA

Transcriptional silencing is a position effect.

(1) **A gene is silenced because of where it is located, not in response to a specific environmental signal.**

(2) **(2) Silencing can spread over large stretches of DNA, switching off multiple genes, even those quite distant from the initiating event.**

The most common form of silencing is associated with a dense form of chromatin called "heterochromatin".

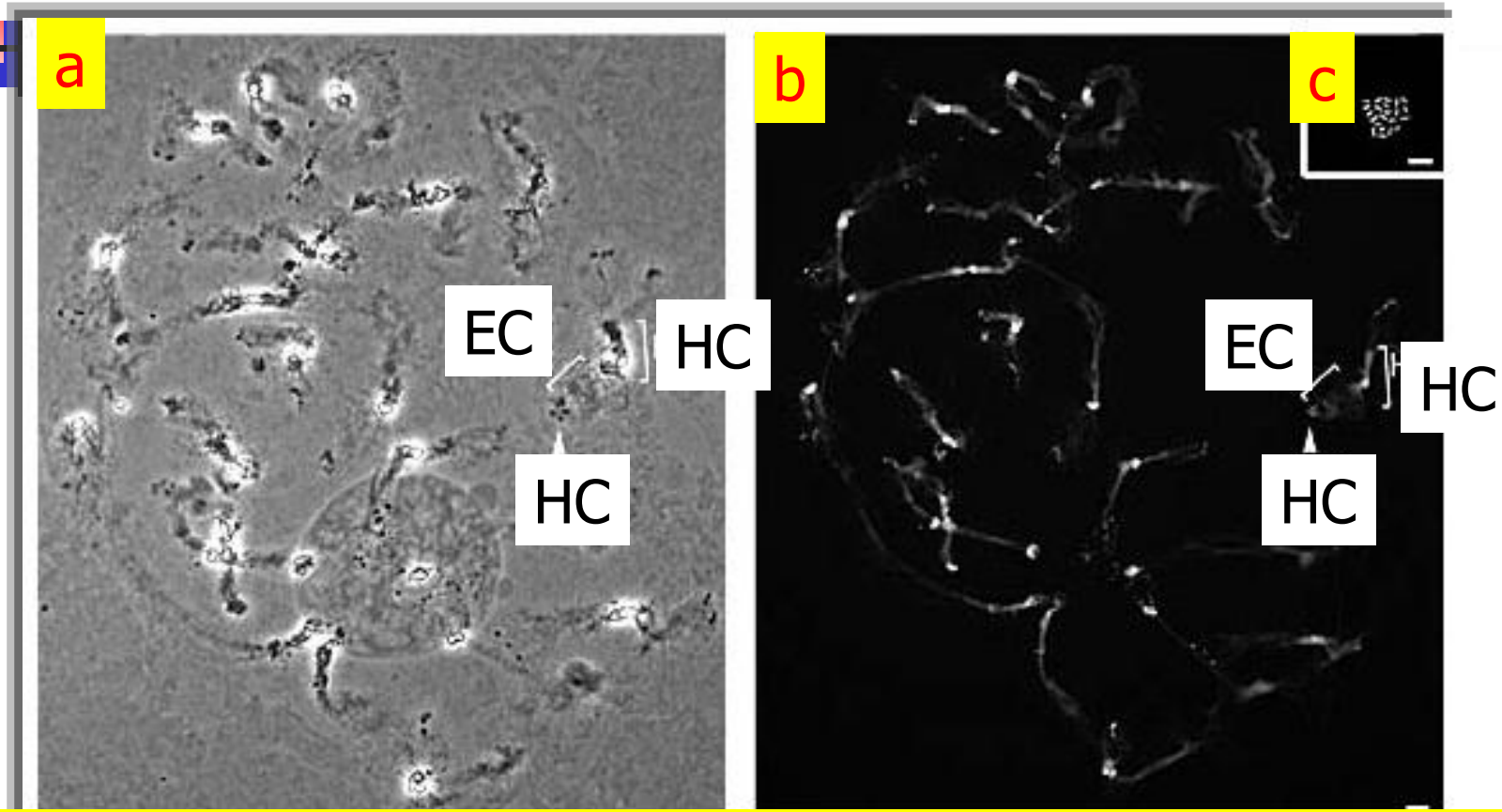
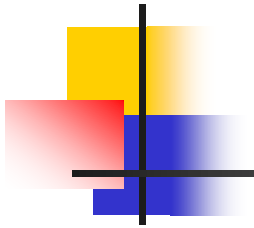


Fig. 8-33 Comparison of euchromatin and heterochromatin of the polytene chromosomes in phase contrast (a) and after DAPI-staining (b and c)



Heterochromatin is frequently associated with particular regions of the chromosome, notably the telomeres, and the centromeres.

In mammalian cells, about 50% of the genome is estimated to be in some form of heterochromatin.

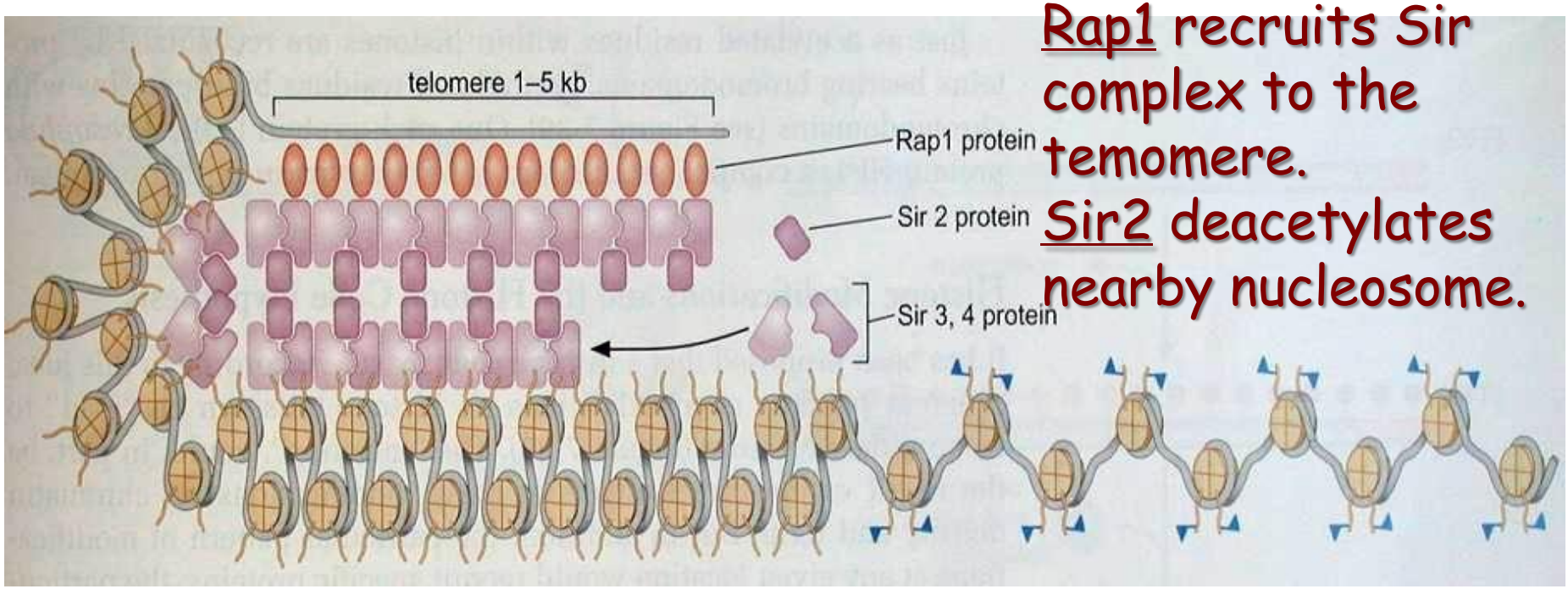
Transcriptional silencing is associated with **modification of nucleosomes that alters the accessibility of a gene** to the transcriptional machinery and other regulatory proteins.

The modification enzymes for silencing include **histone deacetylases, DNA methylases.**

Silencing in yeast is mediated by deacetylation and methylation of the histones

The telomeres, the silent mating-type locus, and the rDNA genes are all "silent" regions in *S. cerevisiae*.

Three genes encoding regulators of silencing, *SIR2*, *3*, and *4* have been found (*SIR* stands for Silent Information Regulator).



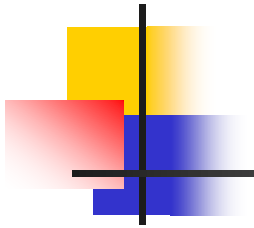
Rap1 recruits Sir complex to the temomere.
Sir2 deacetylates nearby nucleosome.

Fig. 8-34. Silencing at the yeast telomere

Transcription can also be **silenced by methylation of histone** by histone methyltransferase.

This enzyme has recently been found in yeast, but is common in mammalian cells. Its function is better understood in higher eukaryotes.

In higher eukaryotes, silencing is typically associated with chromatin containing histones that both deacetylated and methylated.



In *Drosophila*, HP1 with a chromodomain recognizes methylated histones and condense chromatin.

Proposal for a histone code

According to this proposal, the particular pattern of modifications at any given location would recruit specific proteins, resulting in the direct effects of these modifications on chromatin density and form.

Fig. 8-35 The patterns of histone modifications

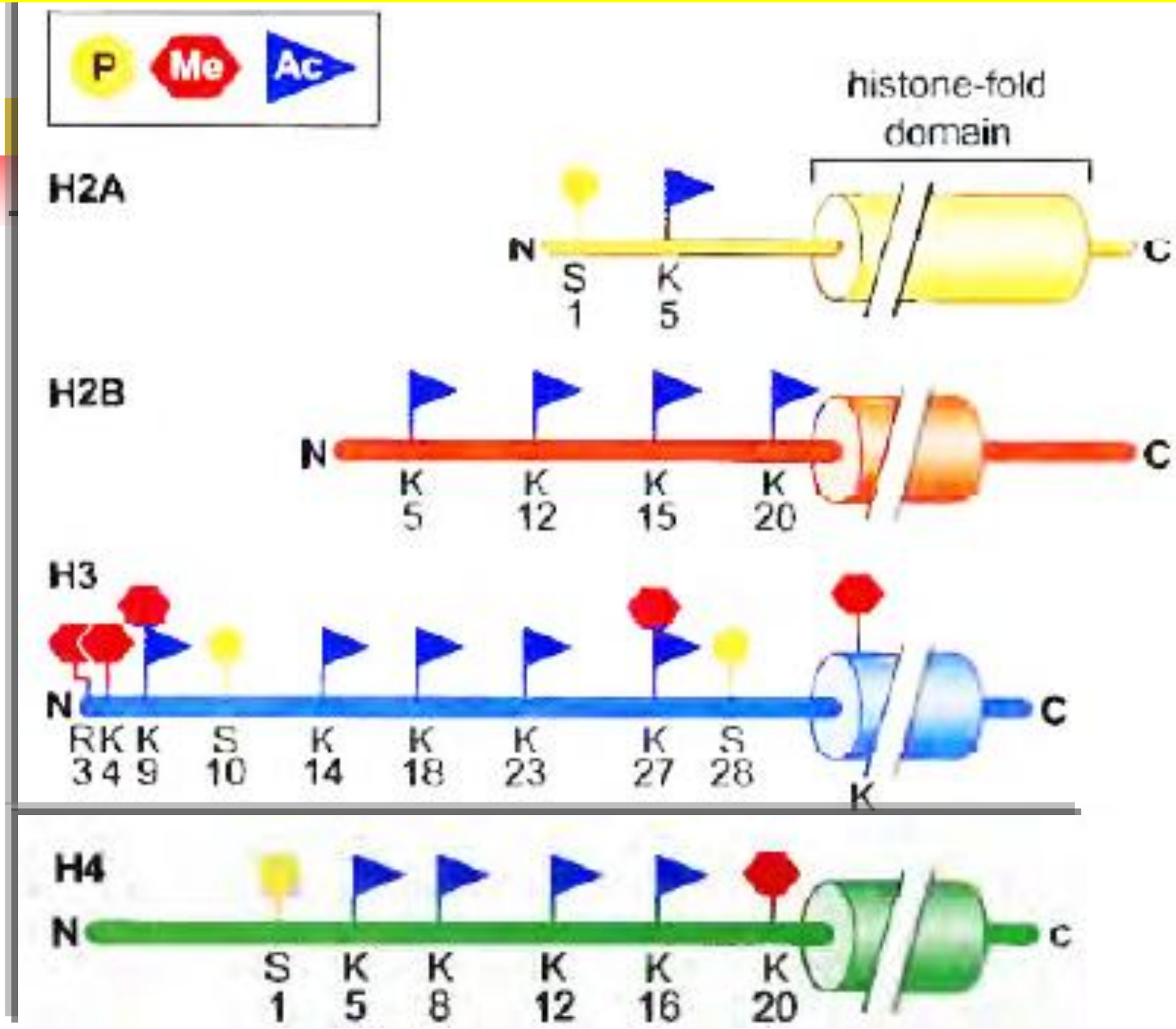
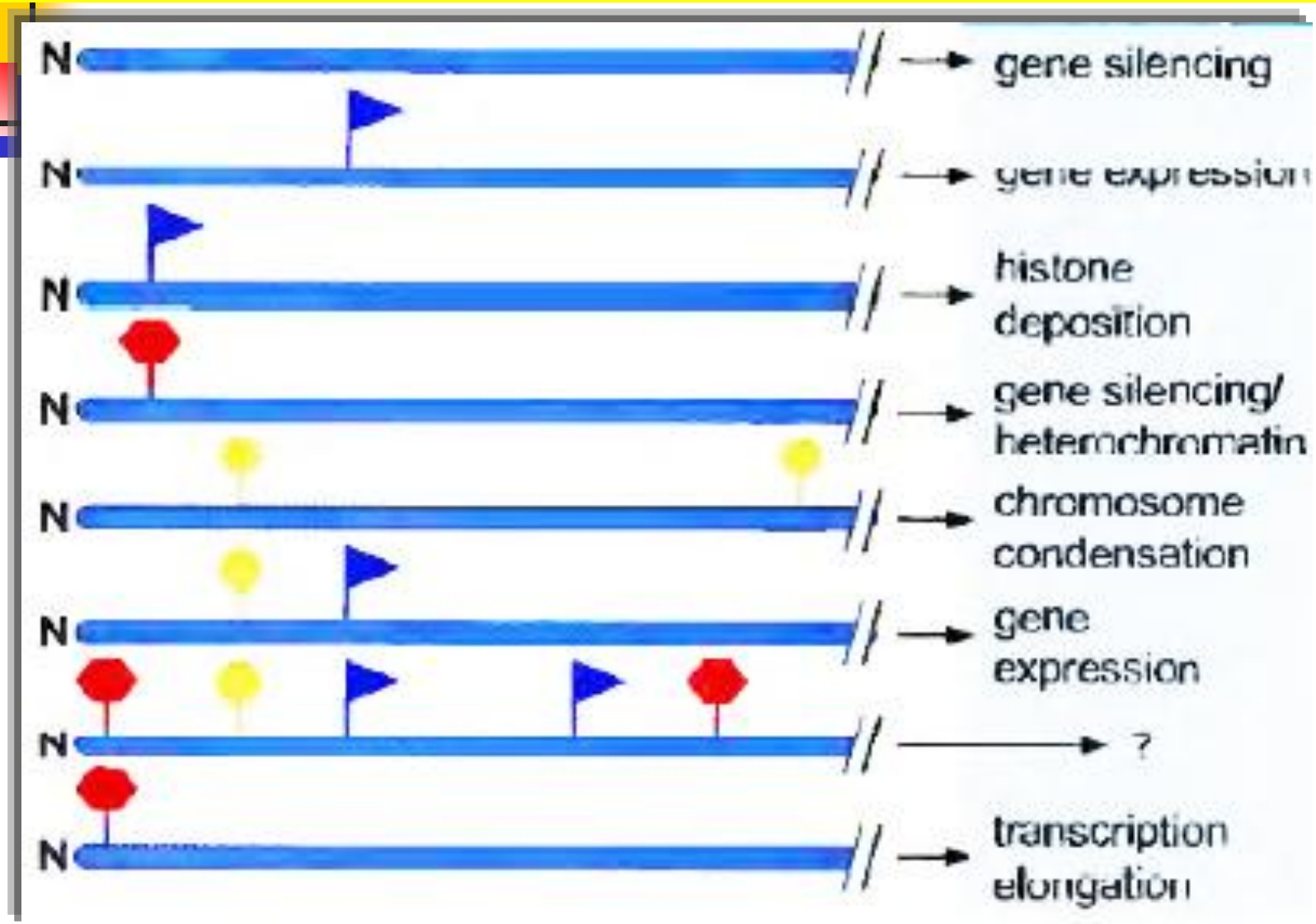


Fig. 8-36 The effects of these modifications on gene expression.



DNA Methylation Is Associated with Silenced Genes in Mammlian cells.

Large regions of mammalian genome are marked by **methylation of DNA sequences**, which is often seen in **heterochromatic** regions.

The methylated DNA sequences are often recognized by DNA-binding proteins (such as MeCP2) that recruit histone decetylases and histone methylases, which then modify nearby chromatin.

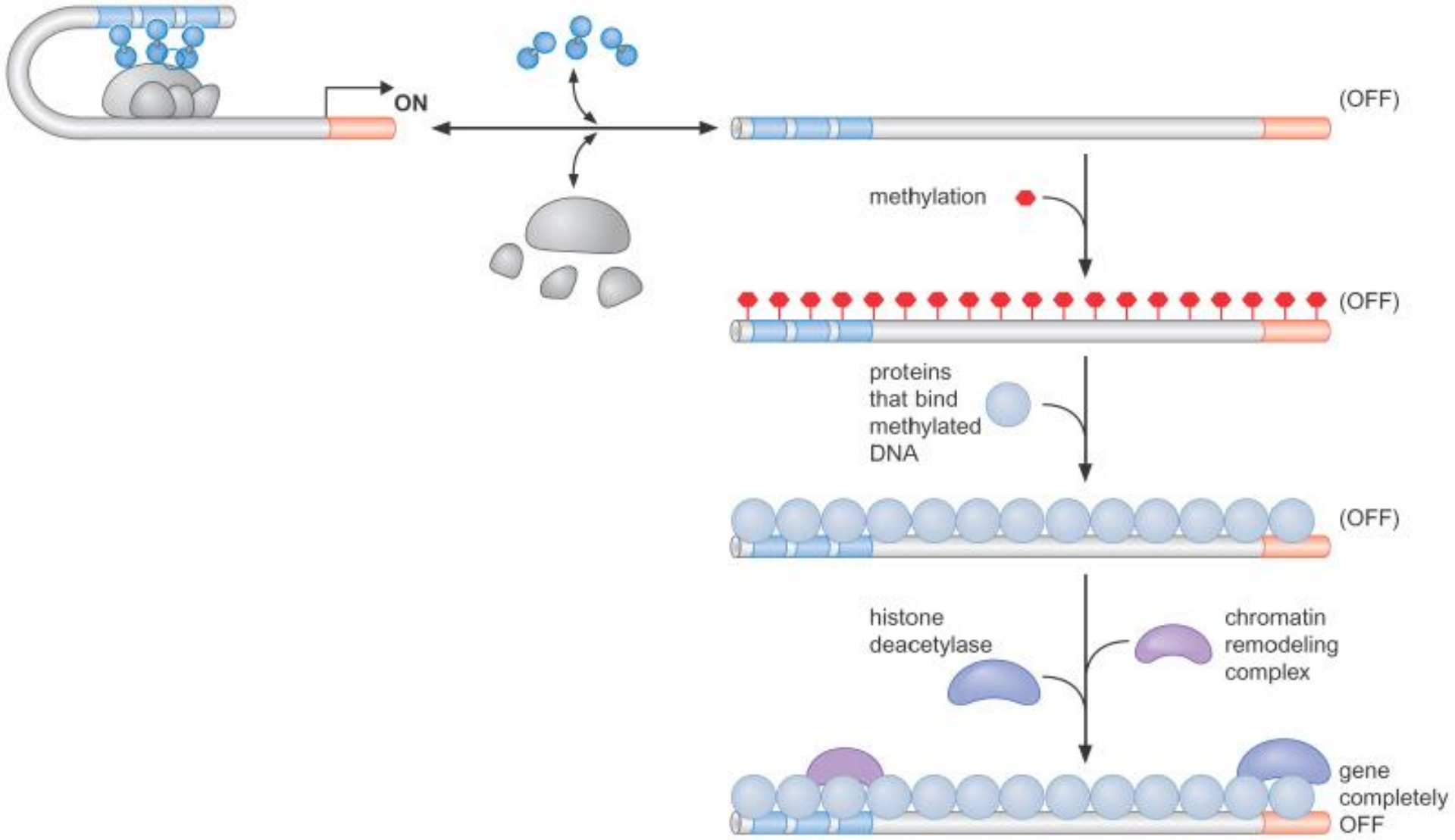


Fig. 8-37 Switching a gene off through DNA methylation and the subsequent histone modification

DNA methylation lies at the heart of Imprinting (胚教)

- Imprinting- in a diploid cell, one copy of a gene from the father or mother is expressed while the other copy is silenced.
- Two well-studied examples: human *H19* and insulin-like growth factor 2 (*Igf2*) genes.

Enhancer: activate both genes' transcription
ICR: an insulator binds CTCF protein and blocks the activity of the enhancer on *Igf2*. Methylation of ICR allows the enhancer to activate *Igf2*.
***H19* repression is mediated by DNA methylation and the subsequent MeCP2 binding to the methylated ICR**

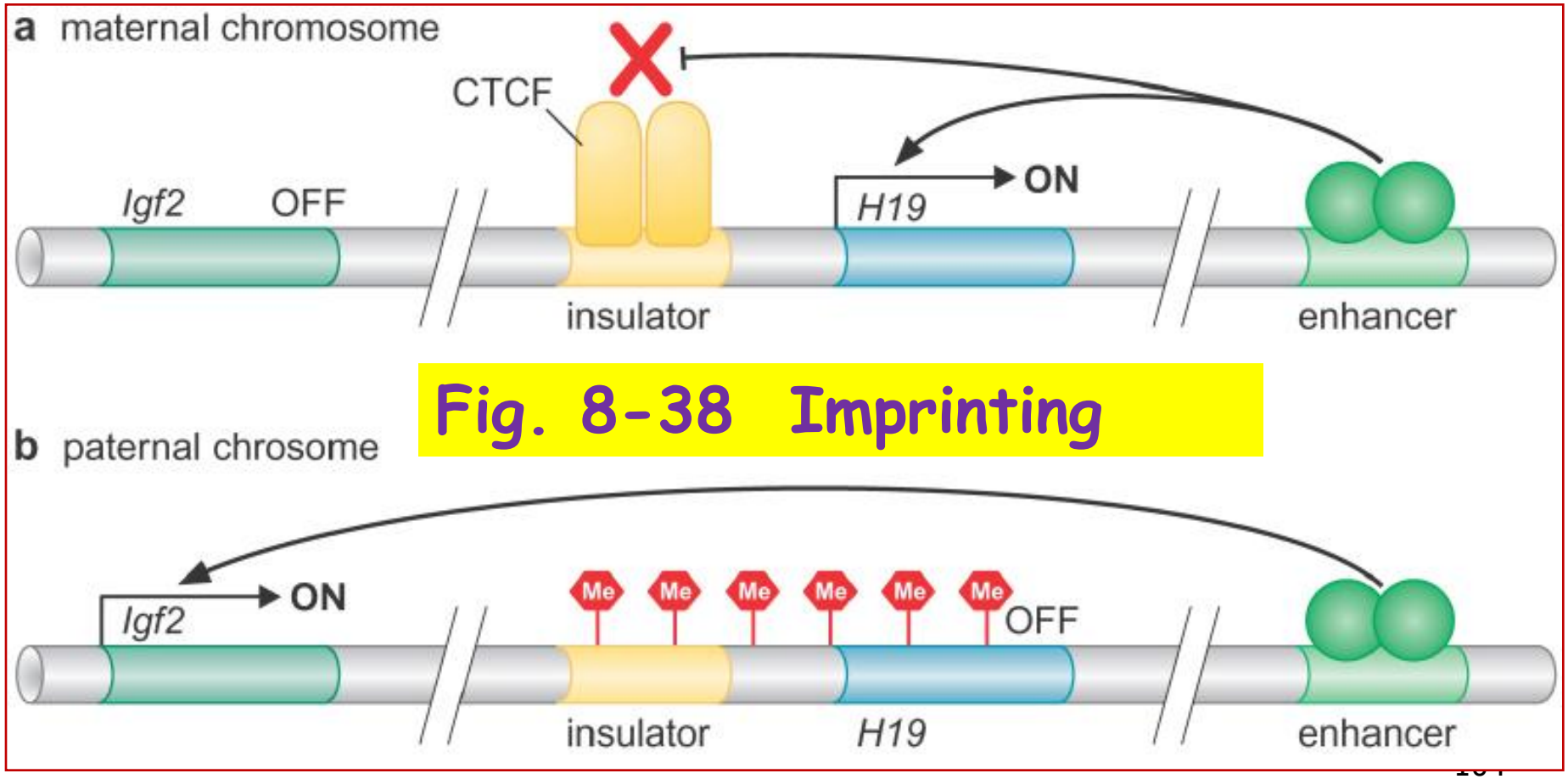


Fig. 8-38 Imprinting

Part 7: Epigenetic Regulation

After the expression of specific genes in a set of cells is switched on by a signal, these genes may have to remain switched on for many cell generations, even if the signal that induced them is no longer present.

The inheritance of gene expression patterns, in the absence of either mutation or the initiating signal, is called epigenetic regulation.

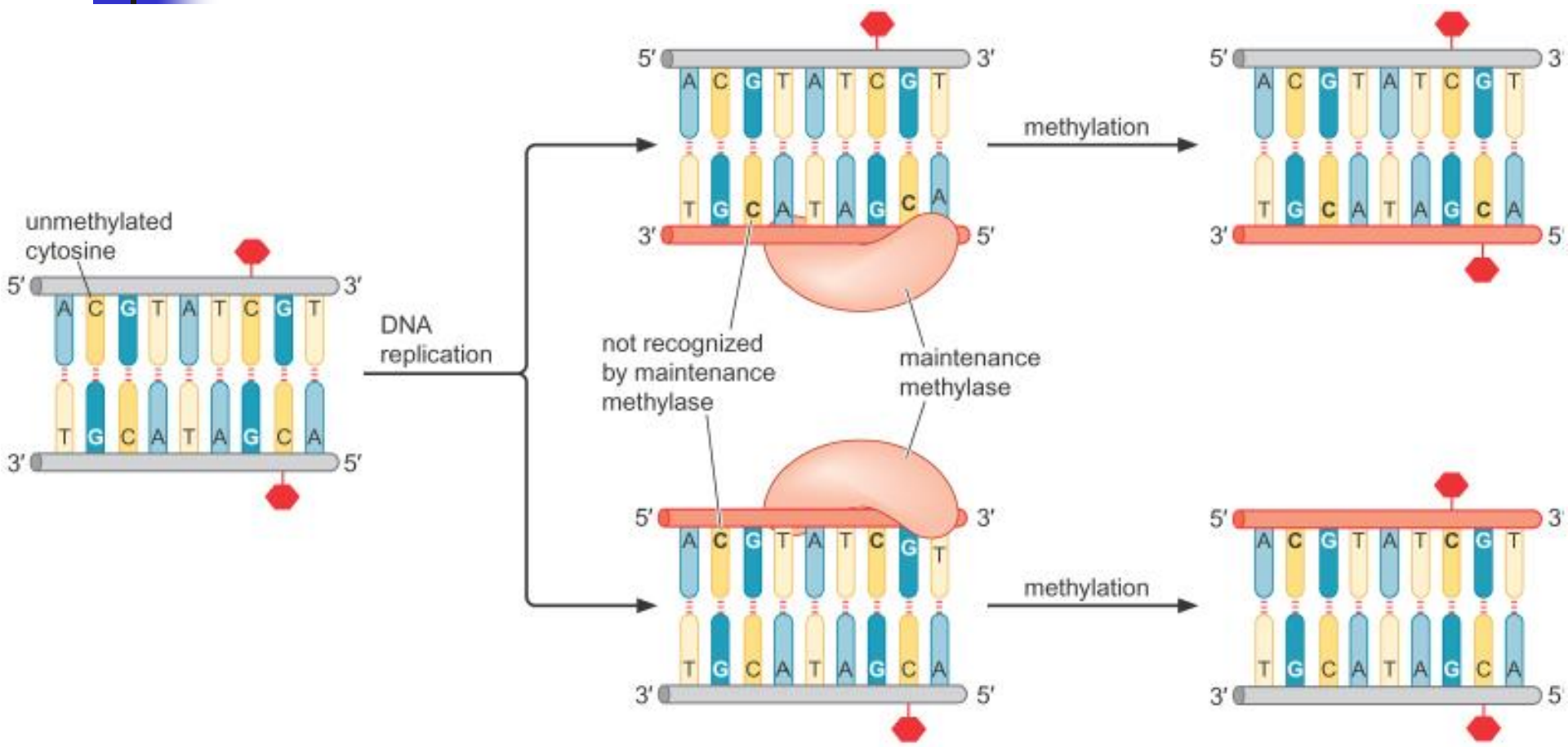
Known examples of epigenetic regulation

1. Imprinting control of Kgf2 and H19 in mammalian cells
2. Maintenance of the lysogenic state of λ lysogens through cell division.

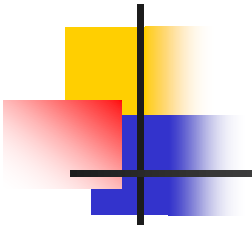
DNA methylation provides a mechanism of epigenetic regulation. DNA methylation is reliably inherited throughout cell division.

Certain DNA methylases can methylate, at low frequency, previously unmodified DNA; but far more efficiently, the so-called maintenance methylases modify hemimethylated DNA—the very substrate provided by replication of fully methylated DNA.

Fig. 8-39 Patterns of DNA methylation can be maintained through cell division



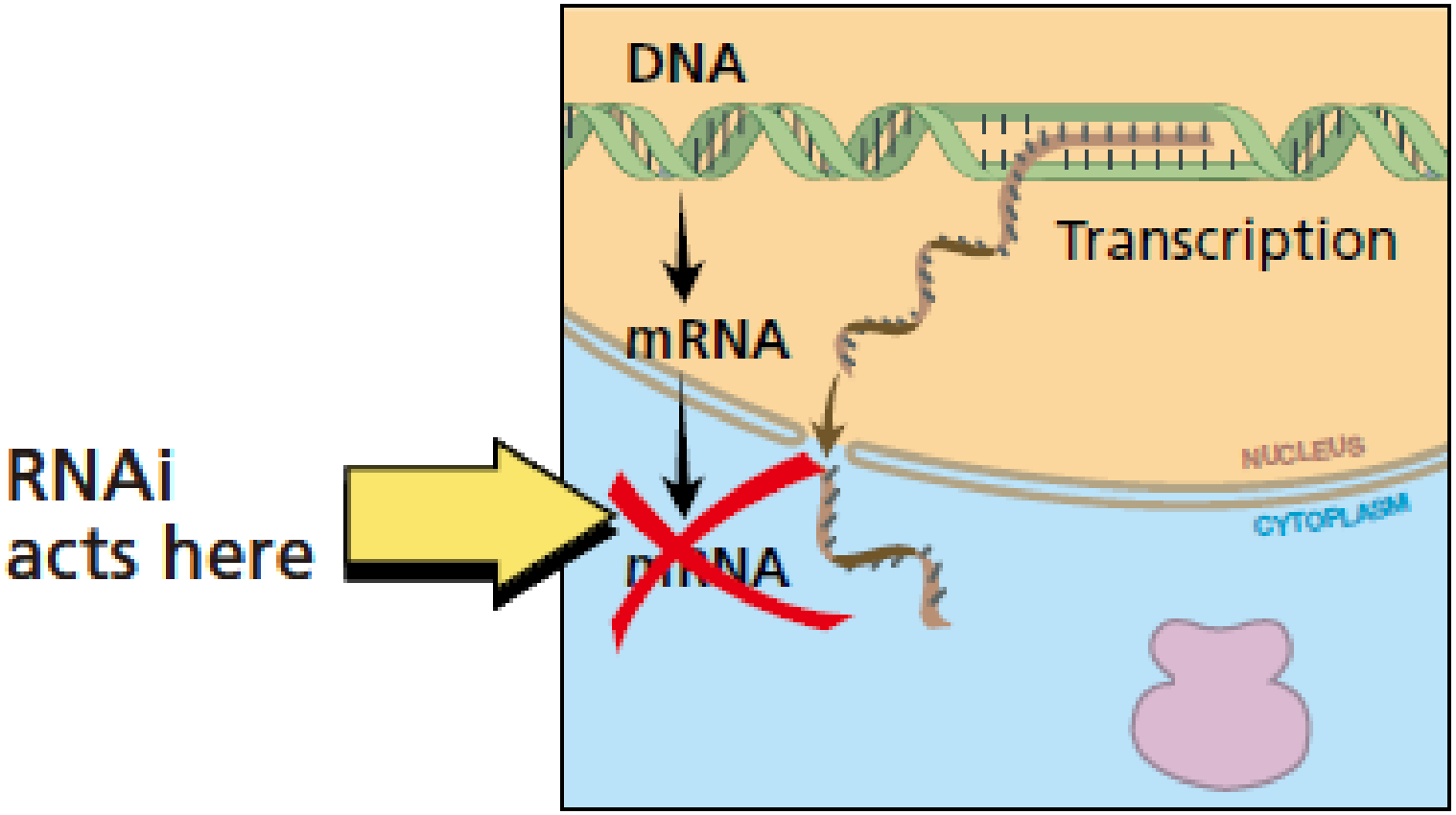
Part 8: RNAs in gene regulation: RNA interference (RNAi)



Double-stranded RNA (siRNA for short interfering RNA) inhibits expression of genes homologous to that RNA. This inhibition is called RNA interference (RNAi).

There are naturally occurring RNAs, called microRNAs (miRNAs), that direct repression of genes in the same way as siRNAs.

Fig. 8-2 RNA interference (RNAi)



In RNA interference, RNA in double-stranded form breaks down the mRNA for a specific gene, thus stopping production of protein.

The impact of RNA interference

As an experimental method, RNAi has had swift and wide impact. It enables to silence any given gene in almost any organism simply by introducing into that organism short dsRNA molecules with sequence complementary to that gene.

The discovery of RNA interference raised hopes for improved treatment of many different disorders.

Chronology of major events in RNA silencing

--Jul. 1969, Britten and Davidson proposed that RNA regulates eukaryotic gene expression

--Oct. 1972, Human cells were shown to contain nuclear double-stranded RNA

--Dec. 1993, The first microRNA, lin-4, discovered

--May 1995, Both sense and antisense RNA found to inhibit gene expression in C. elegans

--Feb. 1998, Double-stranded RNA discovered as the trigger of RNA interference (RNAi)

Chronology of major events in RNA silencing (Continued)

--May 2001, RNAi discovered in human cells
--July 2002, Plant miRNAs discovered
--Nov. 2002, miRNA implicated in cancer
--April 2004, Animal viruses found to encode miRNAs
--Aug. 2004, First "investigational new drug" application filed for a therapeutic siRNA
--July 2005, Primate-specific miRNAs identified
--2006, Fire and Mello won Nobel Prize for discovering RNAi.
--2008, Ambros, Baulcombe and Ruvkun won Lasker Award for discovering miRNA.

Three ways of the RNAi-directed gene silencing

1. Trigger destruction of the target mRNA
2. Inhibit translation of the target mRNA
3. Induce chromatin modification

The RNAi mechanism

1. **Dicer:** an RNaseIII-like multidomain ribonuclease that first processes input dsRNA into small fragments called short interfering RNAs (siRNAs) or microRNAs (miRNA). Dicer then helps load its small RNA products into RISC.
2. **RISC (RNA induced silencing complexes) :** a large multiprotein complex that directs the bound siRNA or miRNA to its target and inhibit the target gene expression.

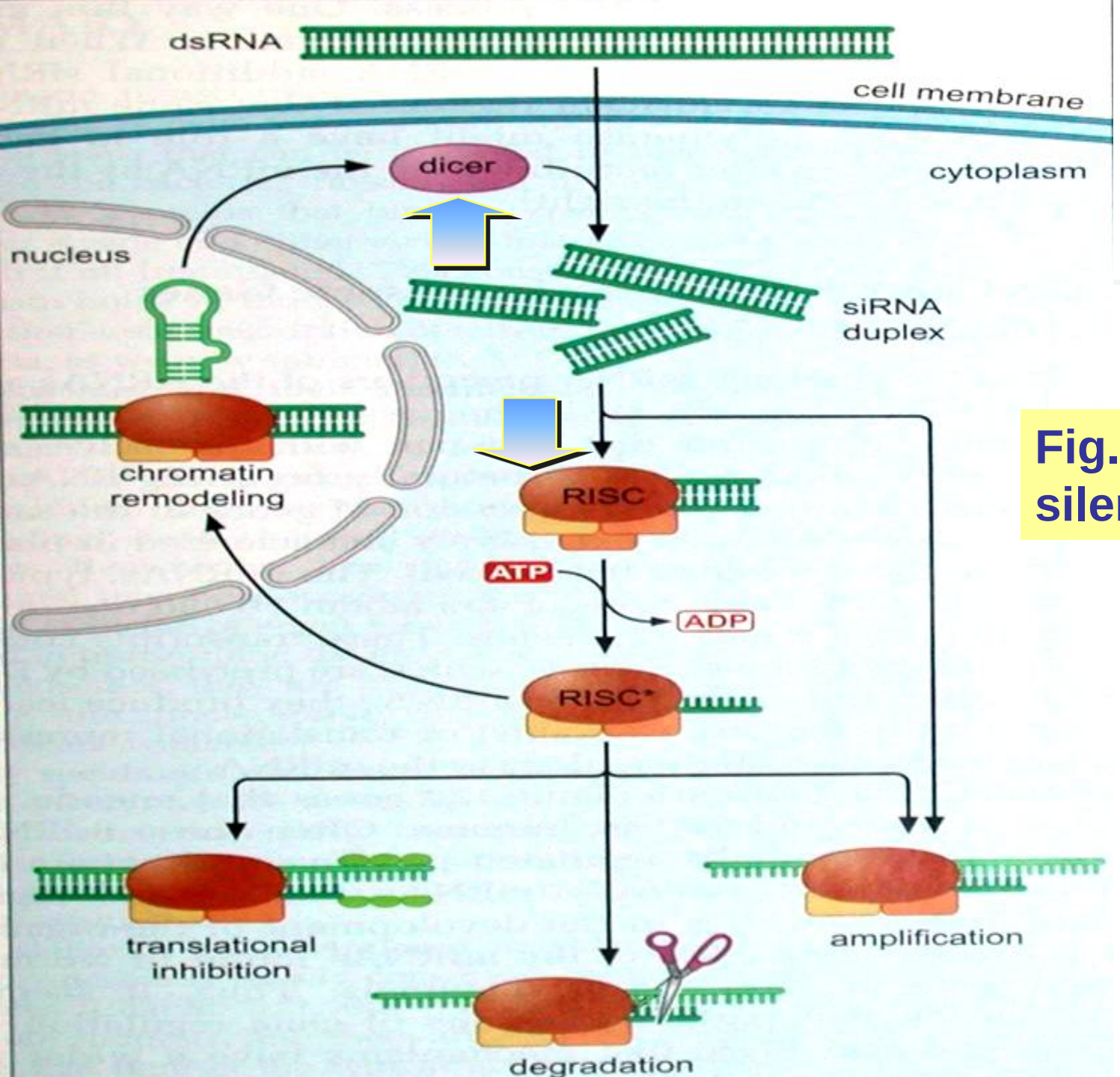


Fig. 8-40 RNAi silencing

MicroRNA (miRNA)

A type of non-coding small RNA (~21–23 nts) produced by Dicer from a stem-loop structured RNA precursor (~70-90 nts). miRNAs are widely expressed in animal and plant cells and functions in the form of RNA–protein complexes, termed miRISCs.

TRENDS in Biochemical Sciences Vol.30 No.2 February 2005

The number of the identified miRNAs is growing rapidly in recent years.

9539 miRNAs have been found by March, 2009 (The miRBase Sequence Database). These miRNAs are from primates, rodents, birds, fish, worms, flies, plants and viruses.

http://microrna.sanger.ac.uk/sequences/

ftp://ftp.sanger.ac.uk/pub/mirbase/sequences/

Han et al., *Cell* 125, 887-901, June 2, 2006

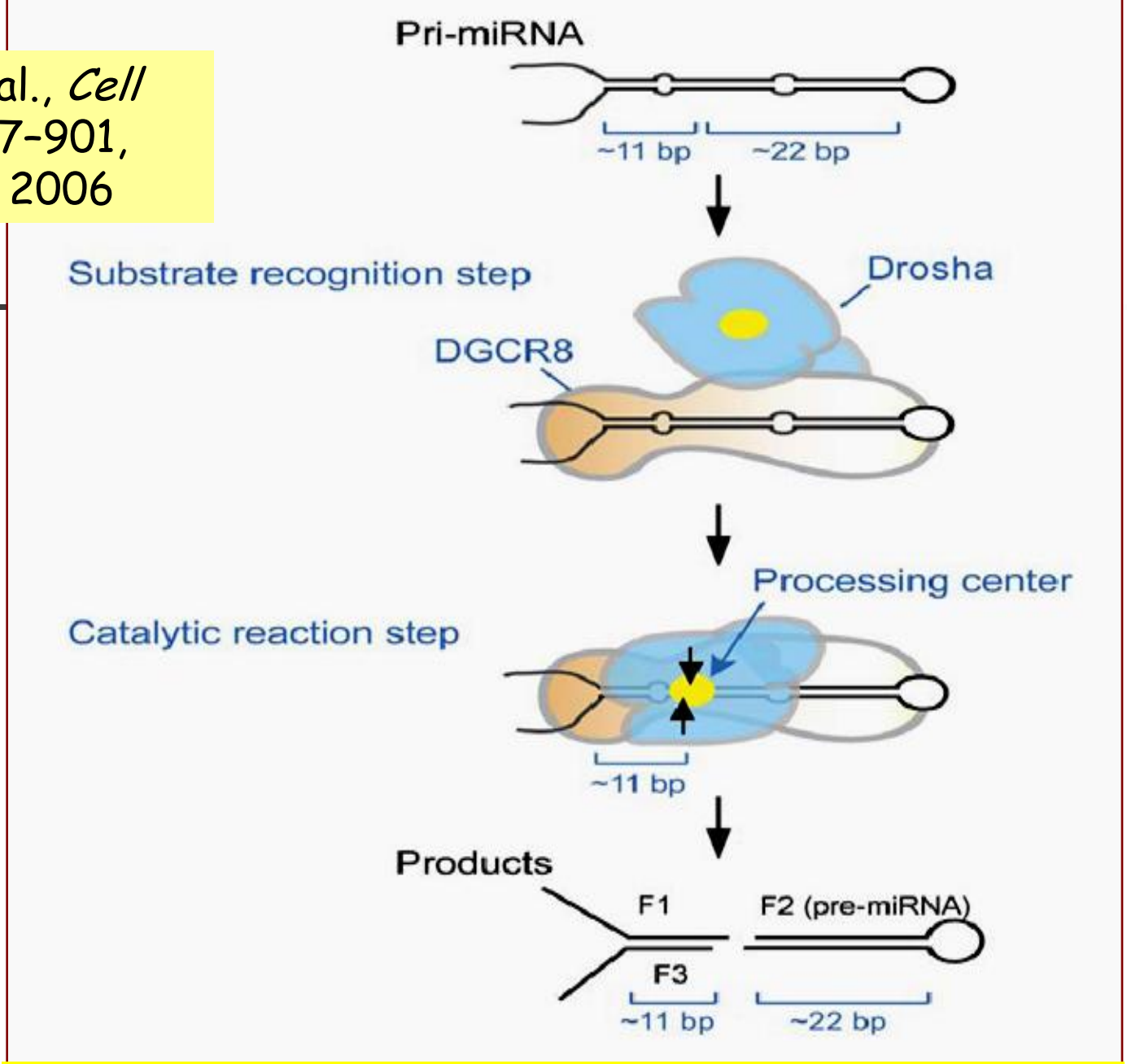


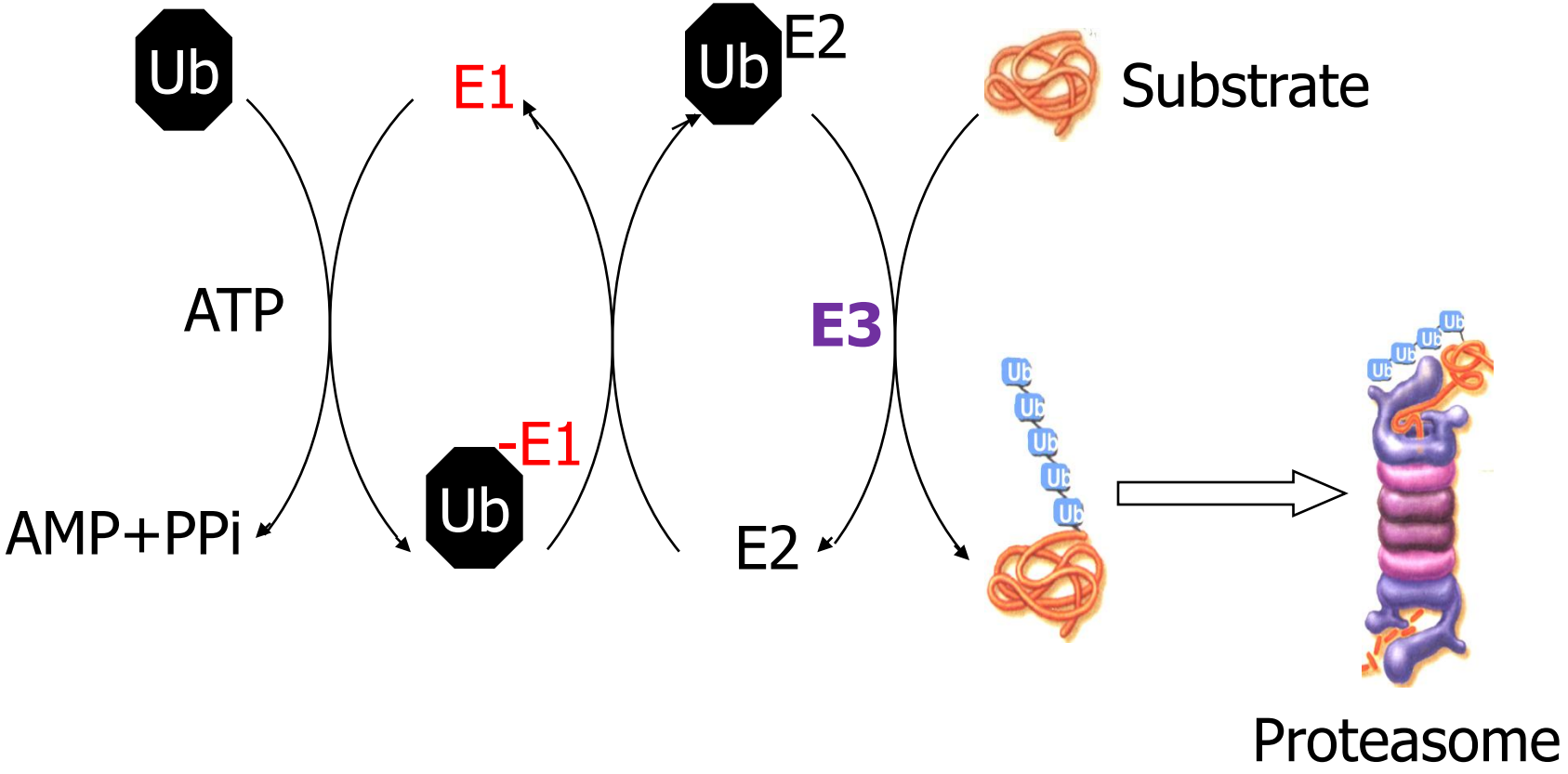
Fig. 8-41 Model for processing pri-miRNA

Part 9: Ubiquitin-Mediated Proteolysis (The Ubiquitin-Proteasome Pathway)

1) Control of transcriptional activators through the ubiquitin-mediated protein degradation by the proteasome.
2) The regulation of protein degradation as a major part of gene expression regulation.

The ubiquitin-proteasome pathway

Responsible for degradation of most cellular proteins



参考书

《泛素介导的蛋白质降解》
Ubiquitin-Mediated Proteolysis

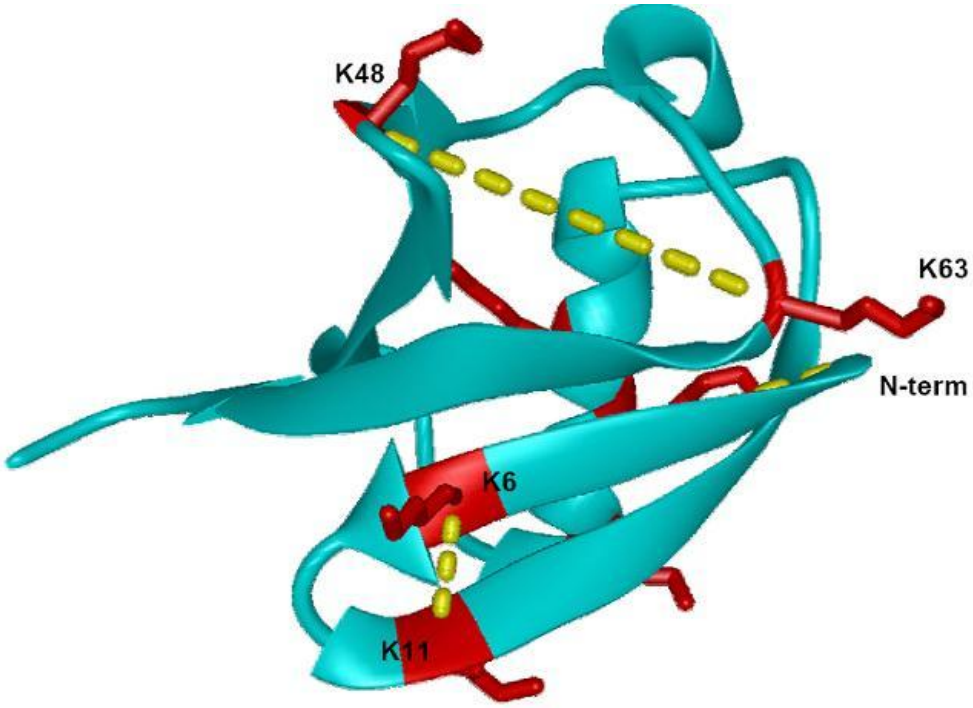
主编：邱小波 王琛 王琳芳

北京协和医科大学出版社， **2008**年

Chronology of major events in Ubiquitin-related researches

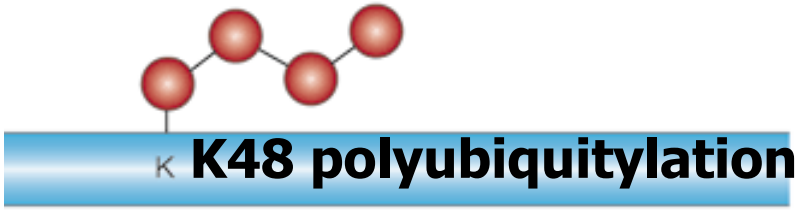
1942年，Schoenheimer发现蛋白质处于不断的产生与分解的动态之中。
1975年，Goldstein发现泛素（Ubiquitin），但以为是胸腺激素。
1977年，Goldknopf和Busch认定组蛋白2A与泛素以异肽键结合。
1977年，Goldberg证明人类细胞中存在一可溶的，直接依赖于能量的非溶酶体类蛋白酶 。
1978年，Ciechanover 和 Hershko发现了APF-1是Goldberg系统中蛋白酶系的必要成分，结果发表在BBRC。
1980年，Ciechanover，Hershko和Rose等人证明了APF-1与当时已被发现的泛素是同一物质 。
1984年，Finley和Varshavsky等发现泛素在细胞周期中的重要性。
1987年，Goldberg和Rechsteiner两个小组几乎同时分离出分子量很大的依赖于ATP并降解泛素化底物的蛋白水解酶。
1988年，Goldberg将这种蛋白水解酶命名为Proteasome－蛋白酶体。
2003年，美国FDA批准了用Velcade来治疗多发性骨髓瘤； Velcade 是在Goldberg发明的蛋白酶体抑制剂MG132的基础上研发而成的。
2004年，Ciechanover，Hershko和Rose因发现泛素介导的蛋白质降解共同获得诺贝尔化学奖。

Figure 8-42 Ubiquitin

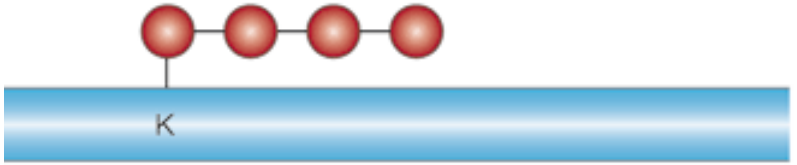


泛素含有**7**个赖氨素残基**(K)**。**K11**、**K29**、**K48**和**K63**均能参与形成泛素与泛素间的异肽键**(Isopeptide bond)**。

Figure 8-43 Ubiquitination Modes



K48 polyubiquitylation



K63 polyubiquitylation

Monoubiquitylation

Multiple monoubiquitylation

靶蛋白必须经多聚泛素(多于4个泛素分子)修饰才能被蛋白酶体识别与降解。

调节蛋白质的活性和定位

调节转录和膜蛋白内吞

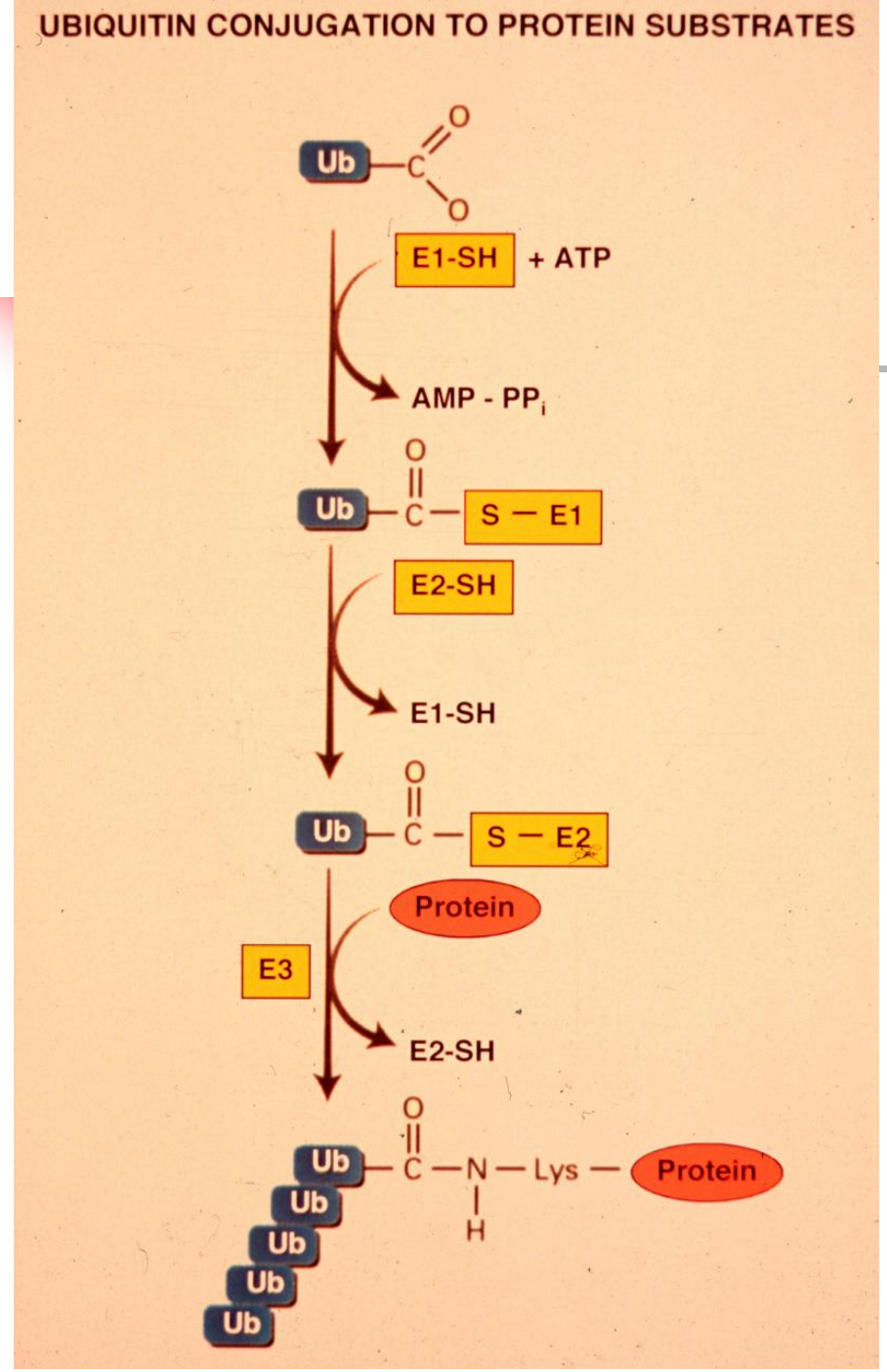


Figure 8-44 Ubiquitination

E1, 泛素激活酶(ubiquitin-activating enzyme)

E2, 泛素载体蛋白 (ubiquitin-carrier protein)

E3, 泛素-蛋白连接酶 （ubiquitin-protein ligase）

E3, 泛素-蛋白连接酶（ubiquitin-protein ligase）

多达几百种，催化被**E2**活化的泛素**C-**端甘氨酸与底物或下一个泛素的赖氨酸间形成泛素**-**异肽键**(Isopeptide bond)**。

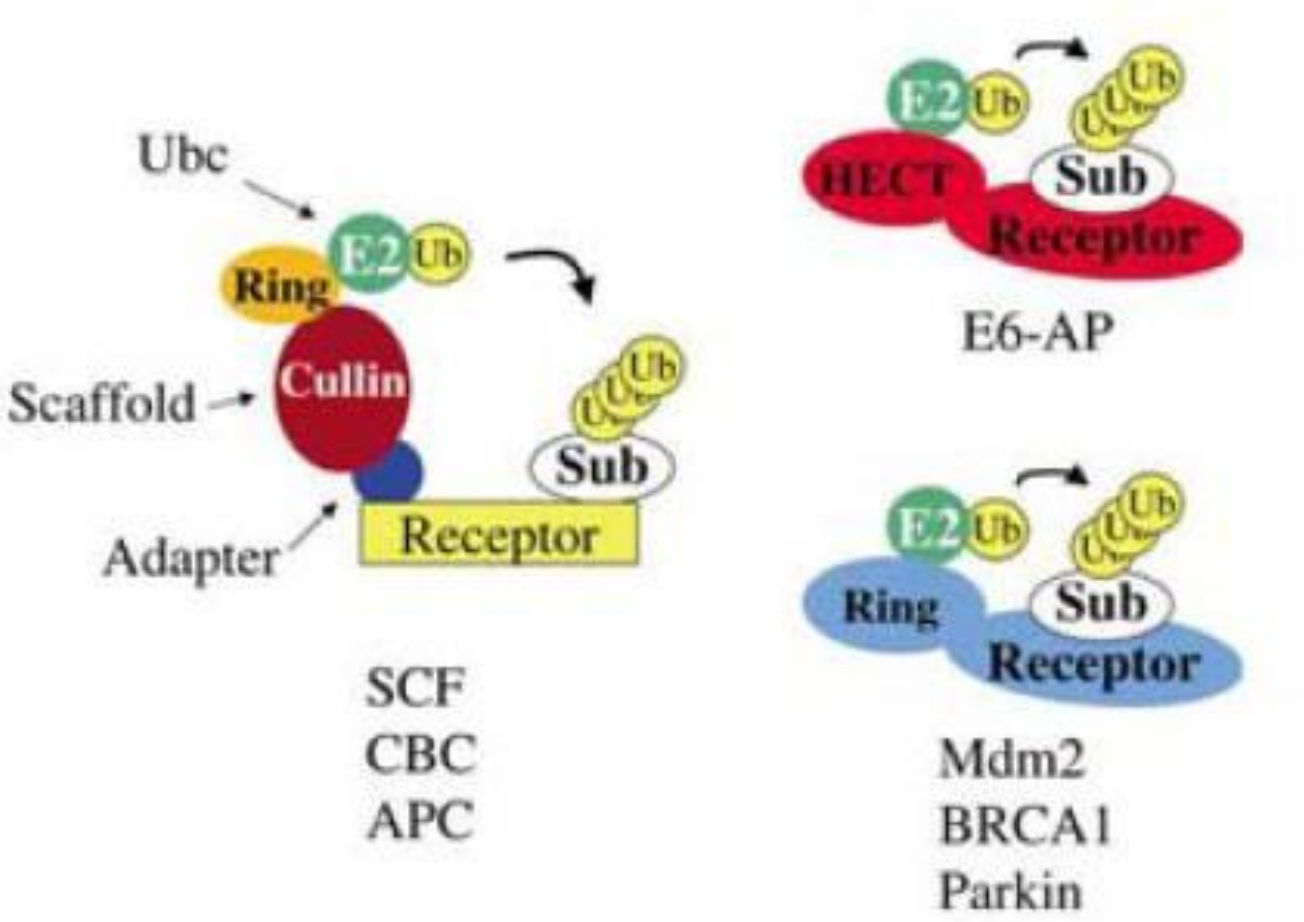
a. RING finger domain（**SCF**复合体，**APC**， **MDM2**，**Parkin, 和c-Cb1**）

b. U-box domain（**CHIP)**

c. HECT domain（能与底物形成硫脂键**)**

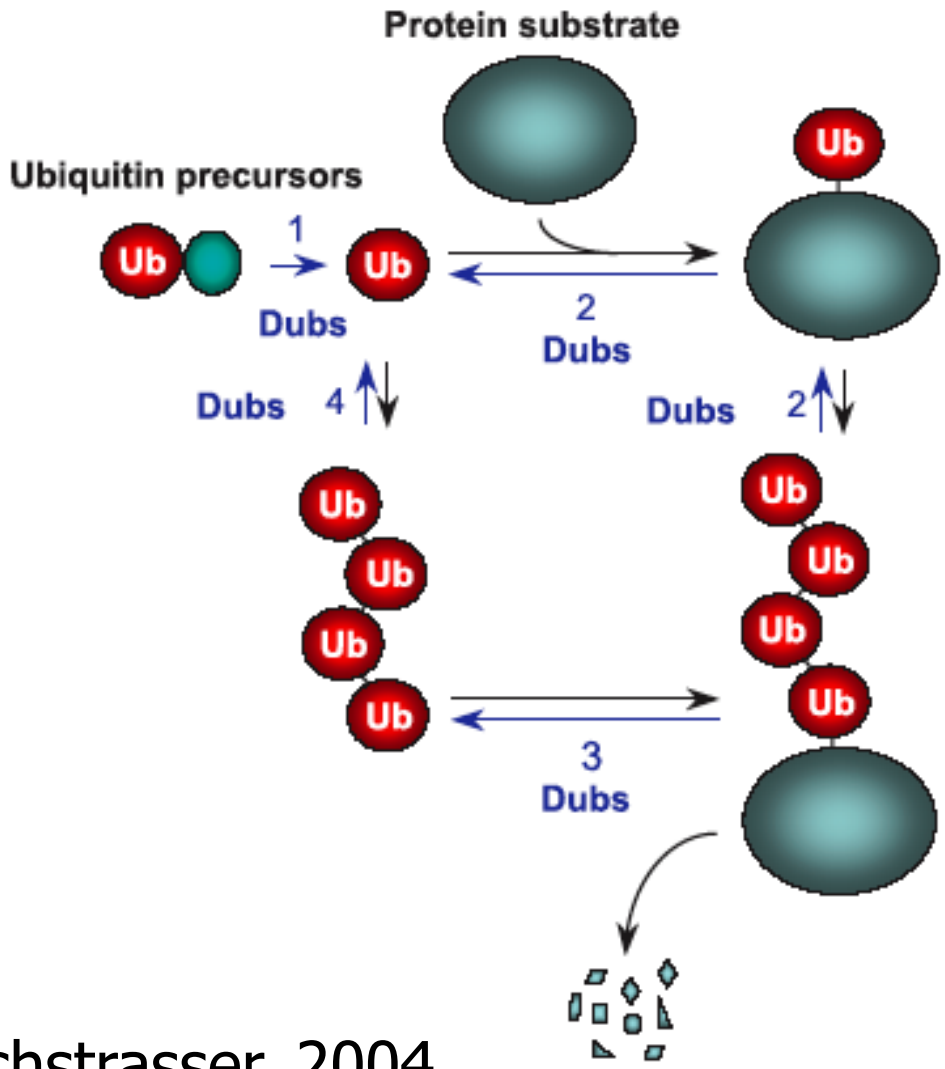
d. N-End Rule

Fig. 8-45 E3泛素连接酶以单体和复合体两种形式存在



Nalepa & Wade Harper 2003 Cancer Treat Rev 29 Suppl 1 49-57

Fig. 8-46 Deubiquitination



Amerik and Hochstrasser, 2004

Deubiquitinating enzymes

去泛素化酶可分为两类：

泛素-C-末端水解酶 ，通常参与蛋白降解后泛素分子的再利用及对多聚泛素链的修饰，也参与由泛素前体产生泛素单体的过程（如UCH37、UCH-L1)。

泛素特异性蛋白酶 ，参与去除蛋白质上的多聚泛素链，也可从短泛素链的末端去掉单个泛素（如USP8)。

去泛素化酶属半胱氨酸蛋白酶，目前已发现90多种。

Ubiquitination plays key roles in almost all the cellular activities, including

- Transcriptional regulation
- DNA repair
- Protein degradation and quality control
- Regulating protein localization & activity
- Cell cycle progression
- Apoptosis
- Immune response

泛素化途径的异常与人类重大疾病

- 神经退行性疾病（如帕金森氏症）：

  Parkin, UCH-L1

- 癌症:

  BRCA1, CYLD, Mdm2, Nrdp1, pVHL

- 传染病病原体的入侵、致病机制:

  E6-AP

Ubiquitination and transcriptional regulation

Ubiquitination occurs on histones H1, H2A, H2B and H3.

This modification on different histones plays distinct roles in regulation of chromatin structures, and hence gene expression and genome stability

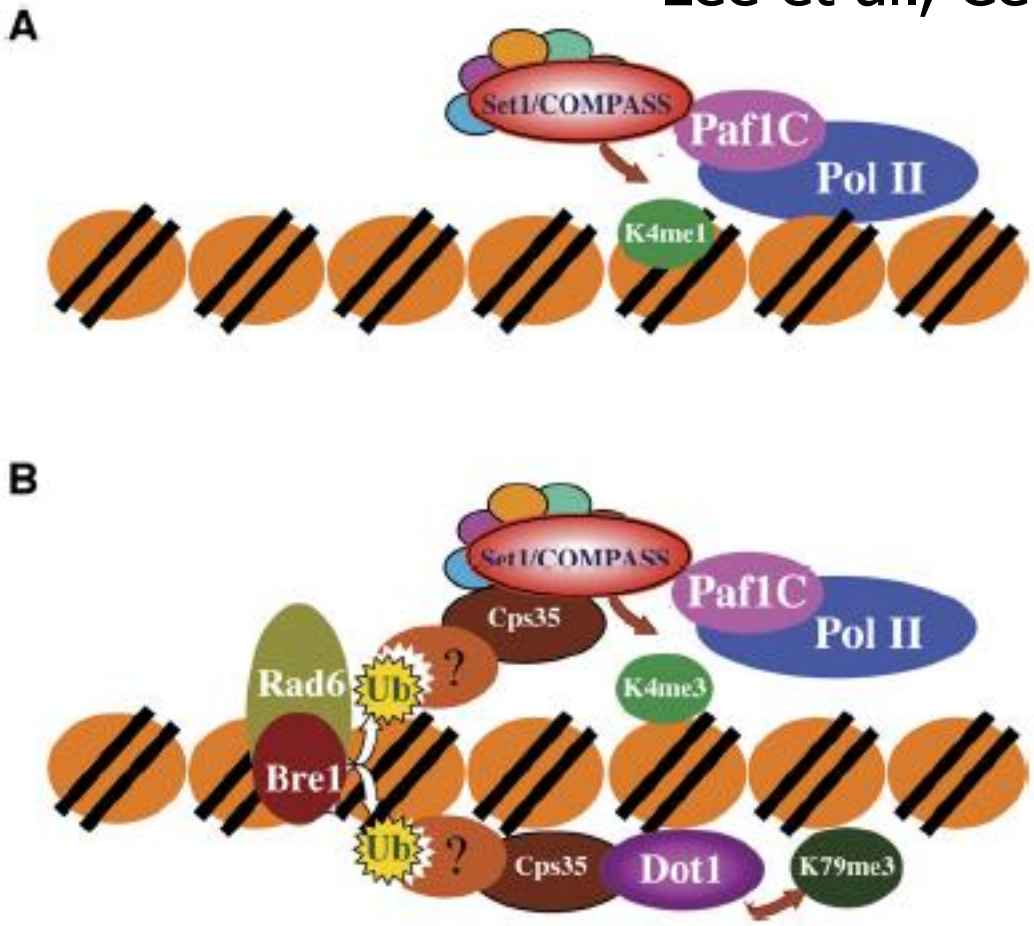
Ubiquitination of histone H2A and H2B has opposite effects on transcription.

Ubiquitination of H2B is associated with gene activation, while H2A ubiquitination contributes to gene silencing

Wang et al., NATURE |VOL 431 | 14 OCTOBER 2004

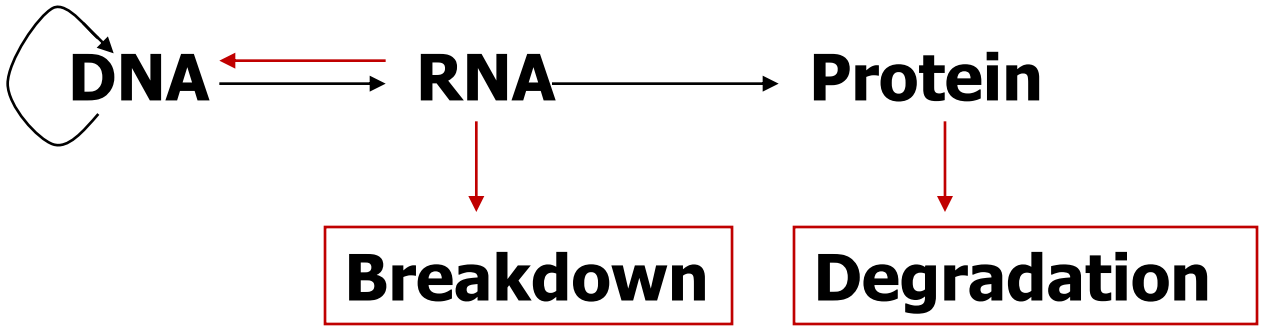
Fig. 8-47 Histone Crosstalk between H2B Mono-ubiquitination and H3 Methylation Mediated by COMPASS

Lee et al., Cell 131, 1084–1096, 2007



Cps35 Is Required for Translating Histone Crosstalk between H2B Monoubiquitination and H3 Methylation by COMPASS

The Central Dogma （Revised)



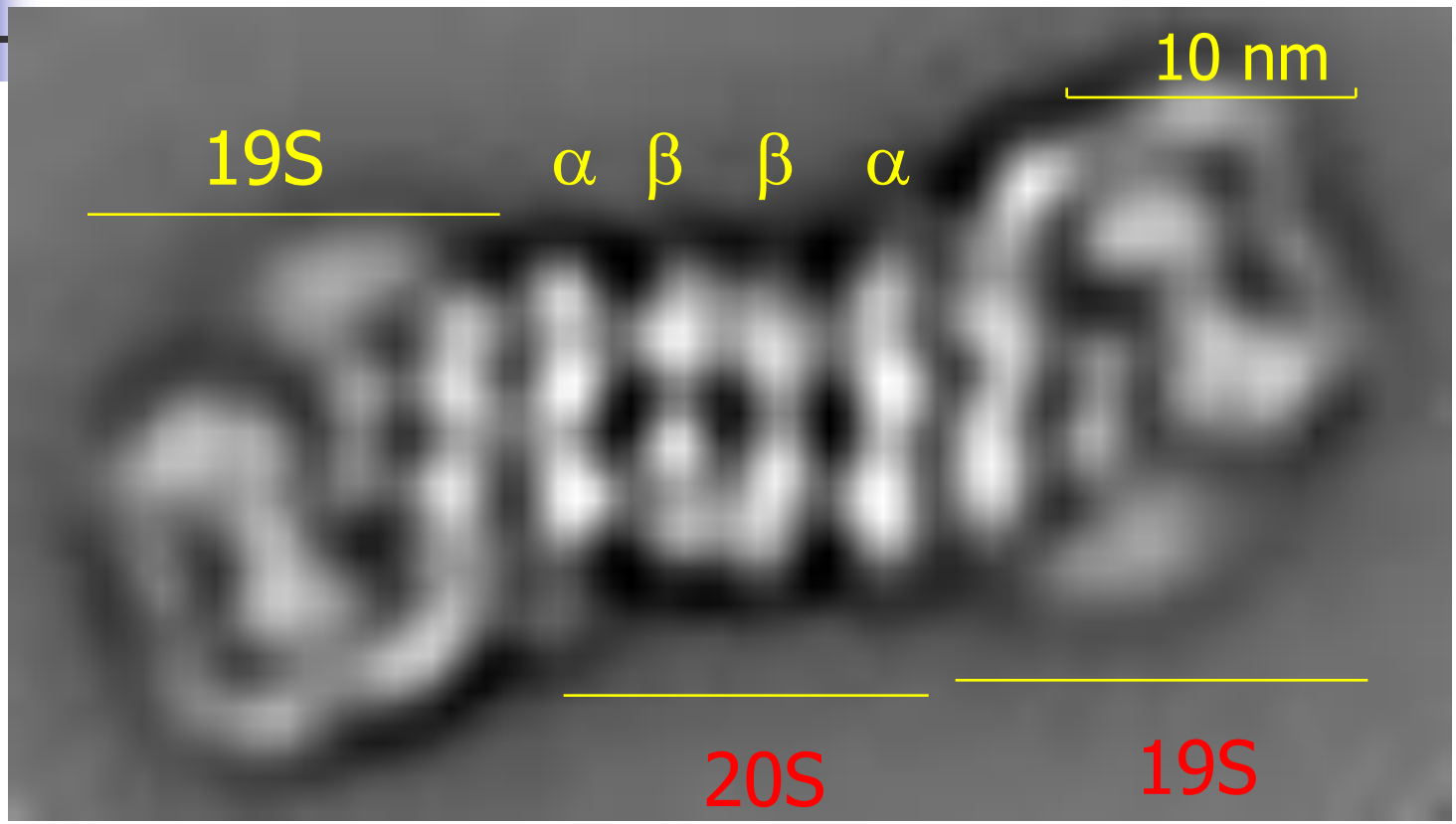
Ubiquitin-mediated proteolysis

泛素-蛋白酶体通路 **(the Ubiquitin-Proteasome Pathway)**

Selective and ATP-Dependent

1) **Regulate biochemical reactions**
2) **Discard unnecessary proteins**
3) **Degrade damaged or misfolded proteins**
4) **Present internalized antigens in immunological responses**

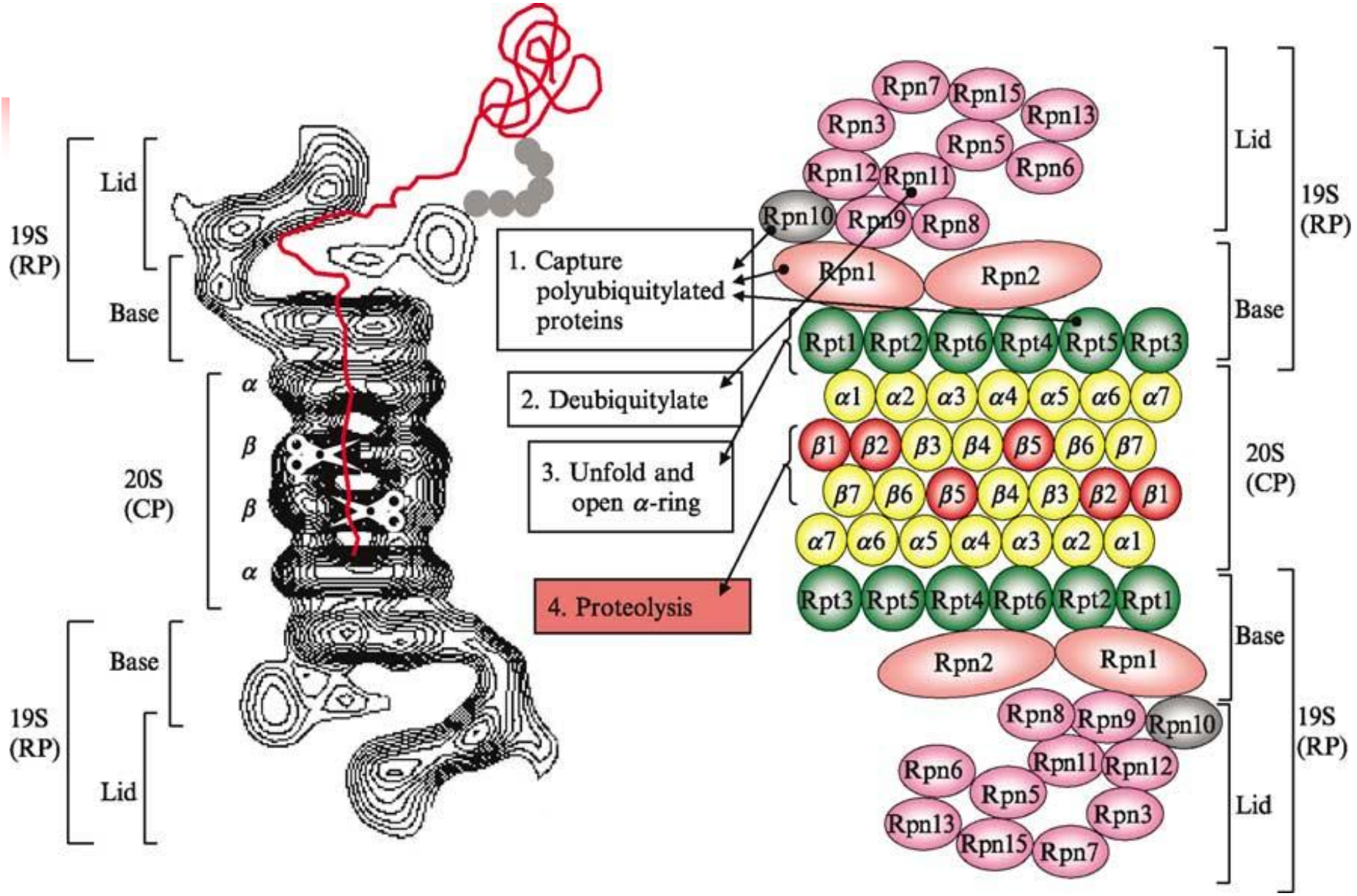
Fig. 8-48 The 26S proteasome under electron microscopy



Catalytic particle
催化颗粒

Regulatory particle
调节颗粒

Fig. 8-49 Subunits of the 26S proteasome



Eukaryotic 20S proteasomes have three types of active sites

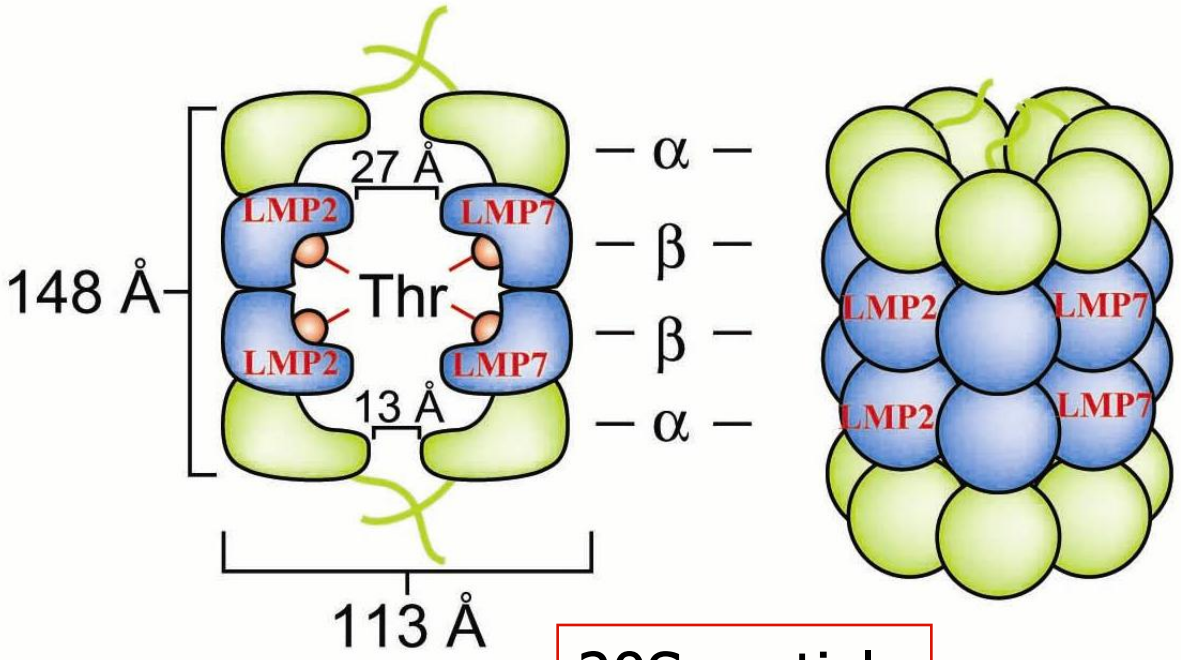
Fig. 8-50

- 2 "chymotrypsin-like"
- 2 "trypsin-like",
- 2 "post-acidic"

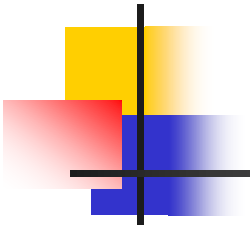
Fluorogenic substrates for:

- "chymotrypsin-like" site:
  - Suc-LLVY-Amc
  - Z-GGL-Amc
  - Suc-FLF-Mna
- "trypsin-like" site:
  - Bz-VGR-Amc
  - Boc-LRR-Amc
  - Z-ARR-Mna
  - Z-GGR-Mna
- "post-acidic" (or "caspase-like") site:
  - Z-LLE-bNa
  - Ac-YVAD-Amc
  - Ac-DEVD-Amc

Fig. 8-51 Immunoproteasome Is Responsible for Antigen Presentation



免疫蛋白酶体的催化颗粒(20S) 有三个特殊亚单位LMP2、LMP7 和MECL-1。其调节颗粒为PA28（11S）。

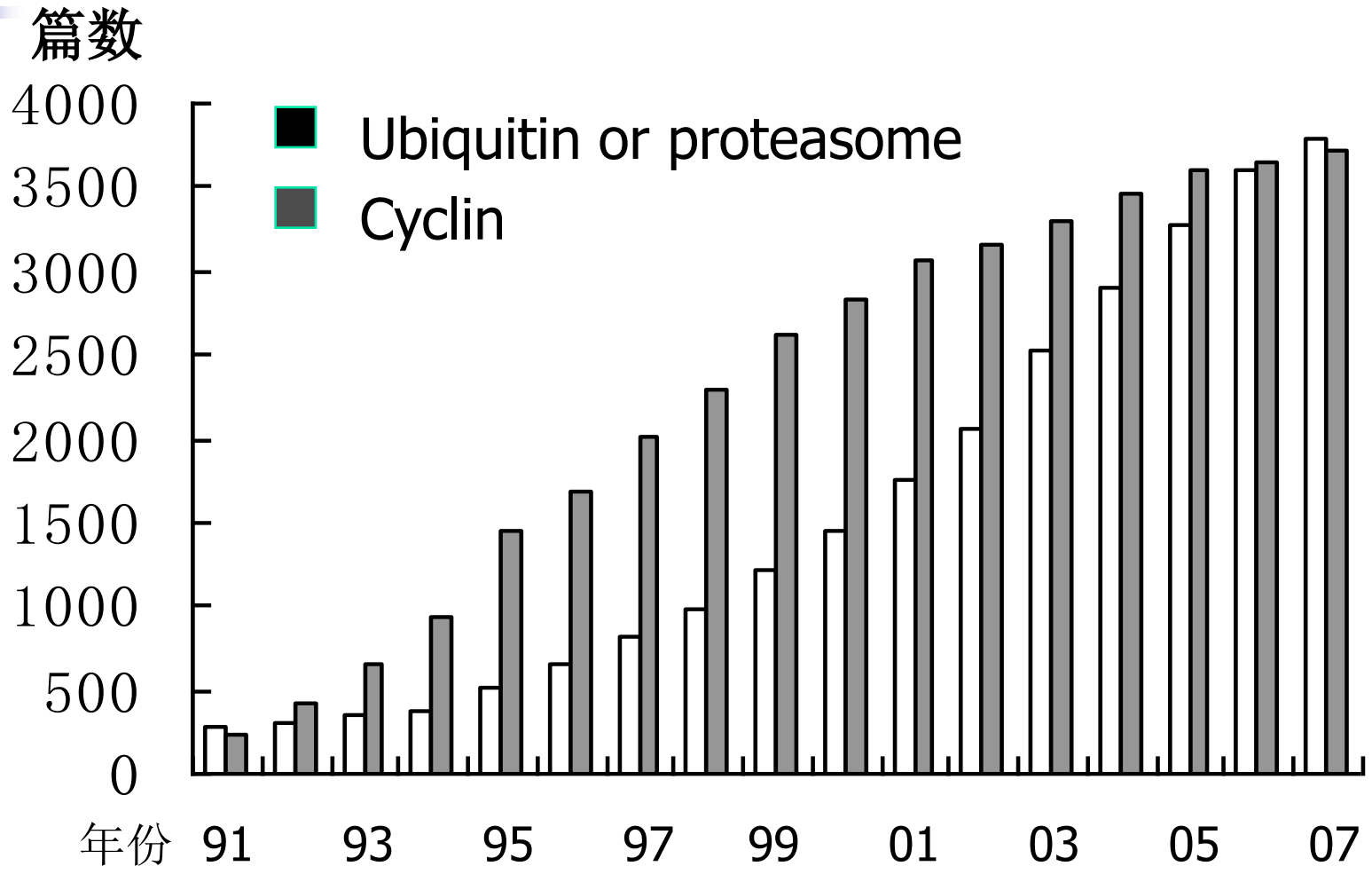


The proteasome inhibitor, Velcade (Botezomib/PS-341), is used to treat multiple myeloma （多发性骨髓瘤）and mantle cell lymphoma, and is promising for treating other diseases.

中药雷公藤中的抗癌活性成分—雷公藤红素**Celastrol**是一种蛋白酶体抑制剂，它能通过控制癌细胞的蛋白酶体活性进而诱发癌细胞凋亡。

Yang, H. et al., Cancer Res. (2006).

Fig. 8-52 近年与泛素—蛋白酶体相关的SCI论文数在快速增长



Part 10: Other eukaryotic gene regulations at steps after transcription initiation

1. **Transcriptional elongation.**
   e.g., HSF recruits a kinase, P-TEF, resulting in the phosphorylation of RNA polymerase tail and keep the transcription of heat shock protein HSP70 gene to proceed.
2. **Control of transcriptional activators at the level of translation.**
   e.g., The levels of amino acids regulate the translation of yeast transcriptional activator Gcn4 that regulates the expression of genes encoding enzymes that direct amino acid biosynthesis.
3. **Regulation of alternative mRNA splicing.**

Fig. 8-53 Early transcriptional regulation of Sxl (sex-lethal) in male and female flies

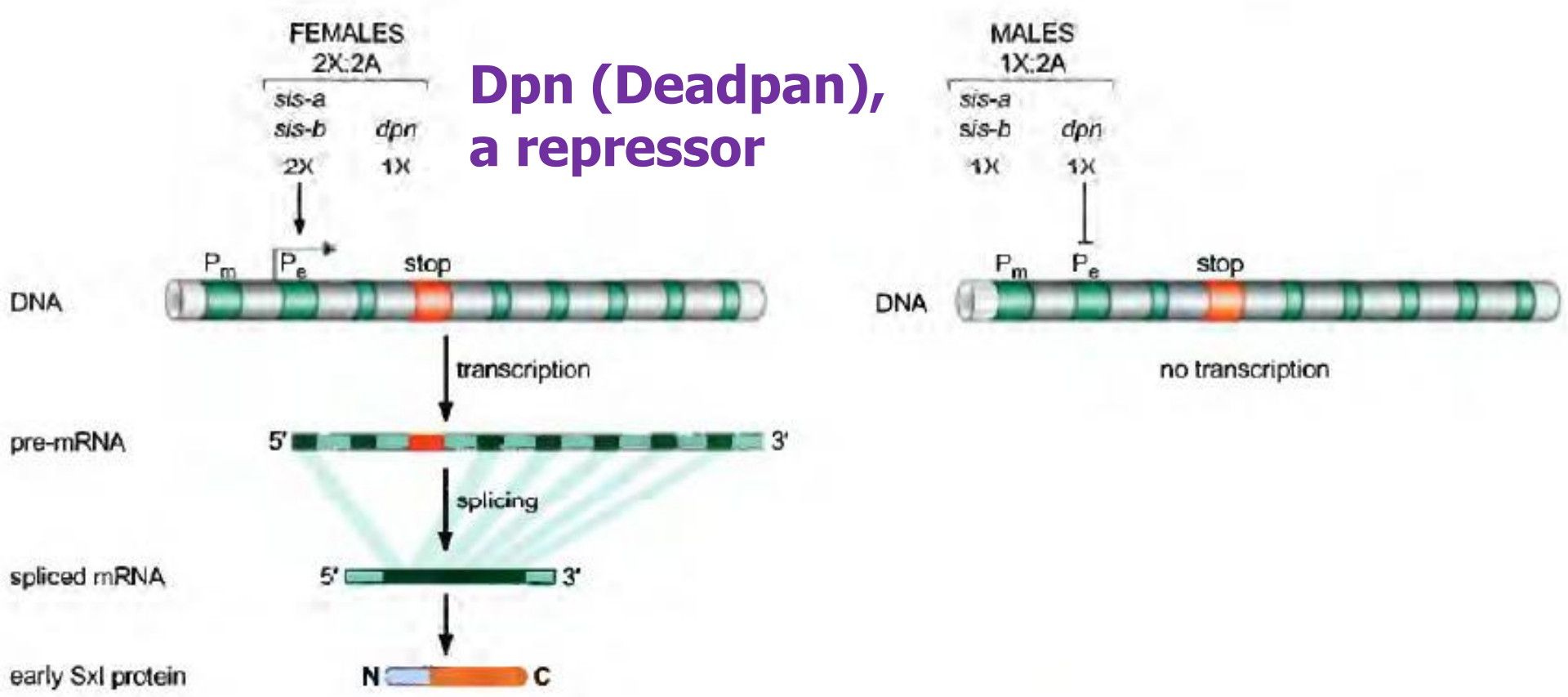


Fig. 8-54 A cascade of alternative splicing events determines the sex of a fly

Sxl, Splicing repressor

Tra, Splicing activator

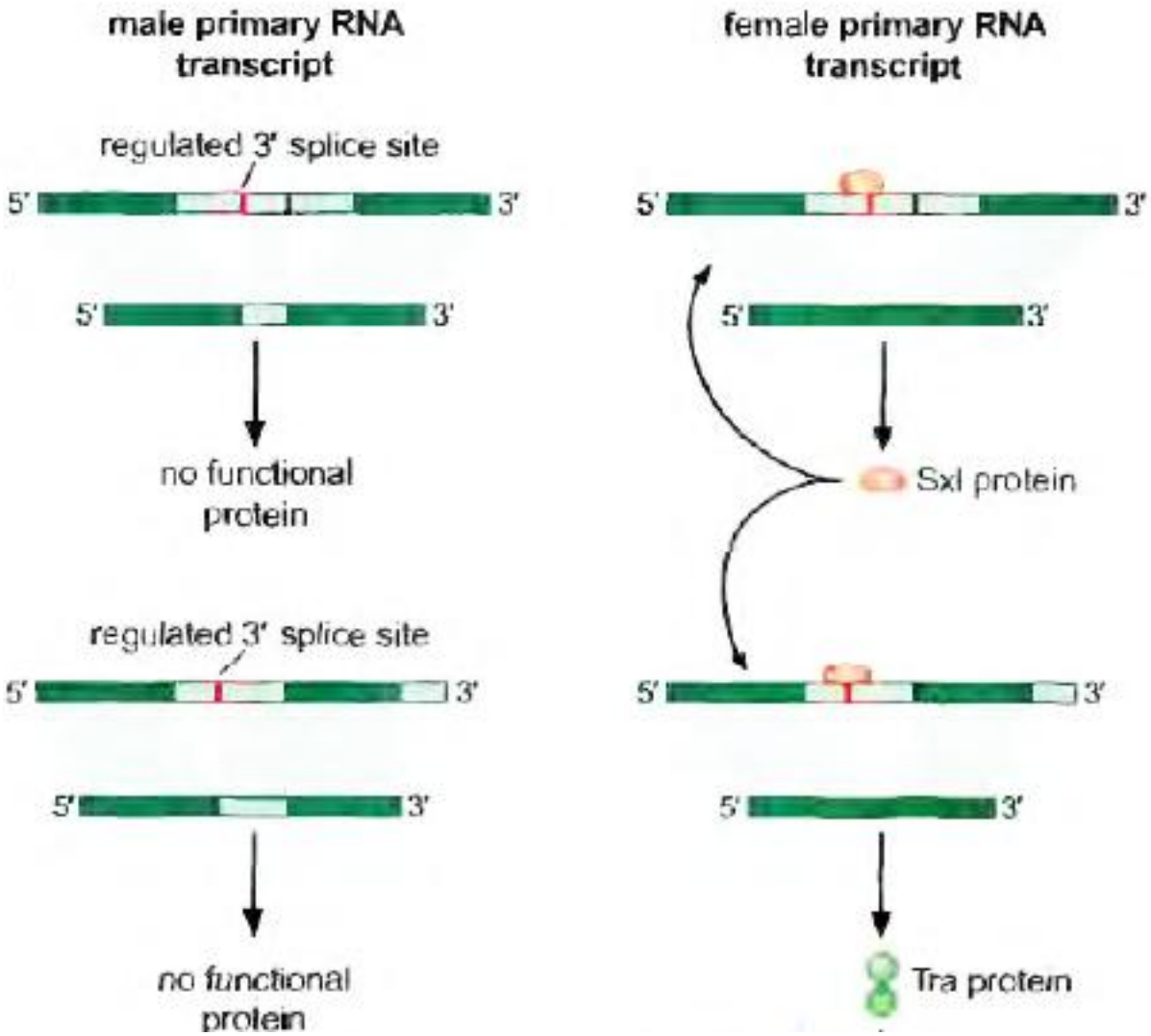
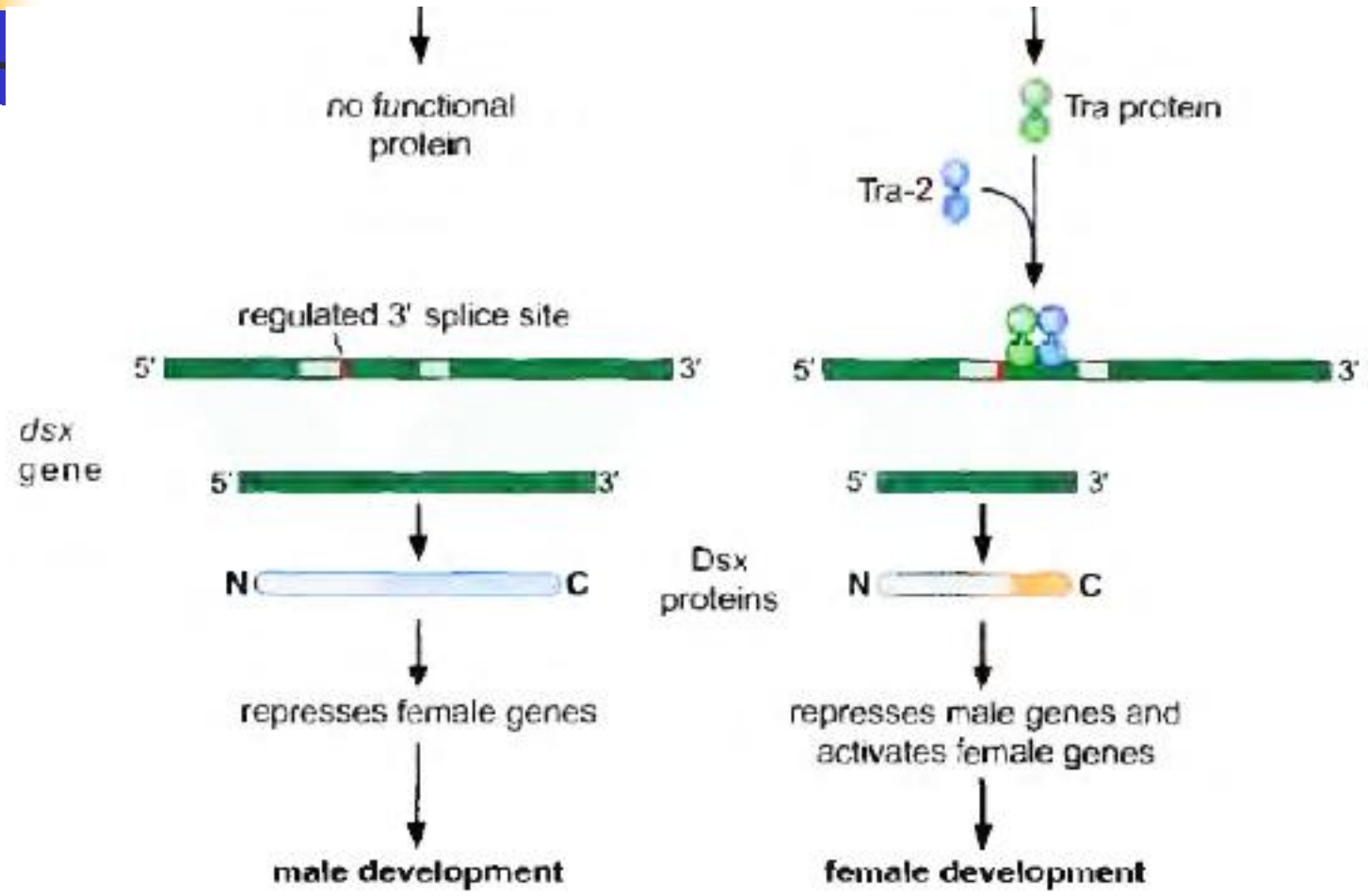


Fig. 8-54 A cascade of alternative splicing events determines the sex of a fly (continued)



Summary of Chapter 8

1. The structure features of the eukaryotic transcription activators.
2. Activation of the eukaryotic transcription by recruitment & activation at a distance.
3. Transcriptional repressor & its regulation
4. Signal integration and combinatorial control
5. Signal transduction: communicating the signals to transcriptional regulators.
6. Gene silencing
7. Epigenetic regulation.
8. RNA interference
9. The ubiquitin-proteasome pathway.
10. Other eukaryotic gene regulations at steps after transcription initiation