Reminders

Watch the video lectures

- Add to your notes so that you can understand the material
- Replay/re-watch sections as necessary
- Take breaks
- Change the video speed as necessary; use shortcut keys

Read the textbook. Check the textbook for answers to your questions before posting on the Discussion Board.

BIO 230

Lecture 4:

Eukaryotic Gene Regulation

1) Eukaryotic transcriptional activation

2) Eukaryotic transcriptional repression

Readings (Alberts *et al.* custom text)

Pages 310-314, 187-193, 196-197, 198-201

Reminder from a couple of lectures ago…

Transcriptional Regulation

Gene expression in both prokaryotes and eukaryotes is regulated by:

- Gene Regulatory Proteins (transcription factors)

Which bind specifically to:

- Regulatory regions of DNA (cis elements)

Gene regulatory proteins can turn genes:

-ON; Positive regulators; • activators

-OFF; Negative regulators; • repressors

(eg. *Trp* operon)

Transcriptional Regulation

Recall that DNA is transcribed into RNA by the enzyme ● RNA polymerase

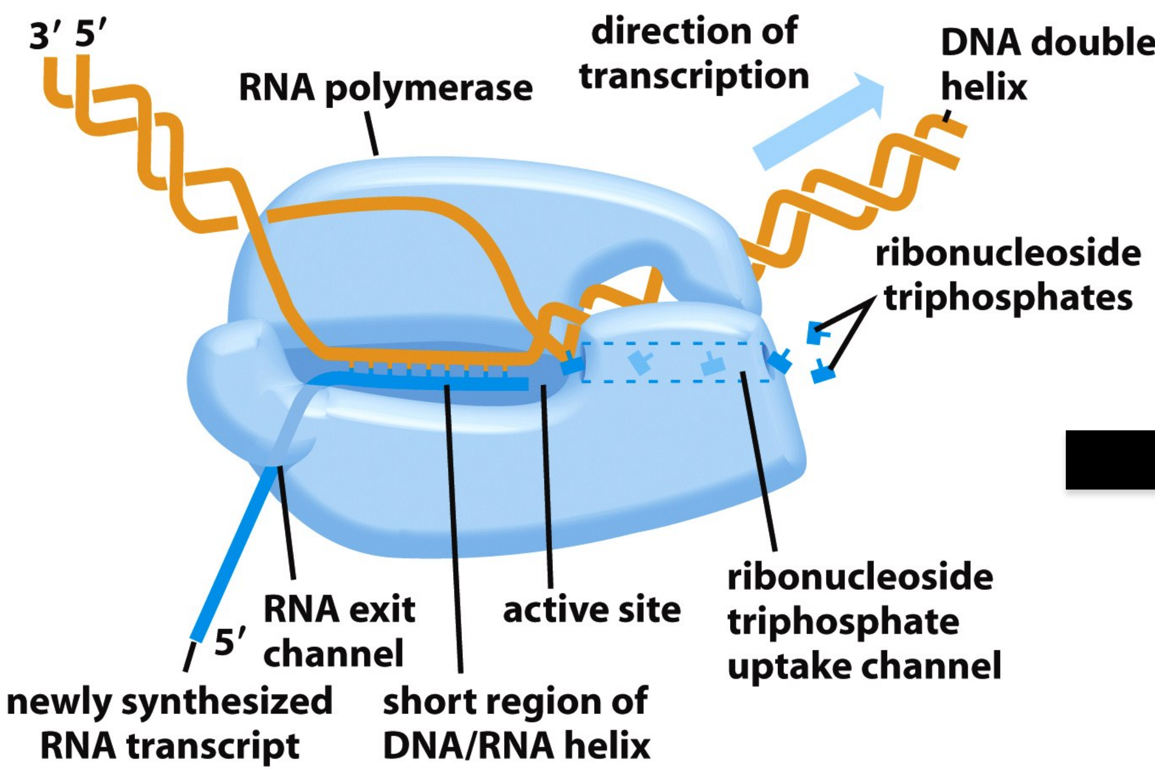


Figure 6-8a Molecular Biology of the Cell 5/e (© Garland Science 2008)

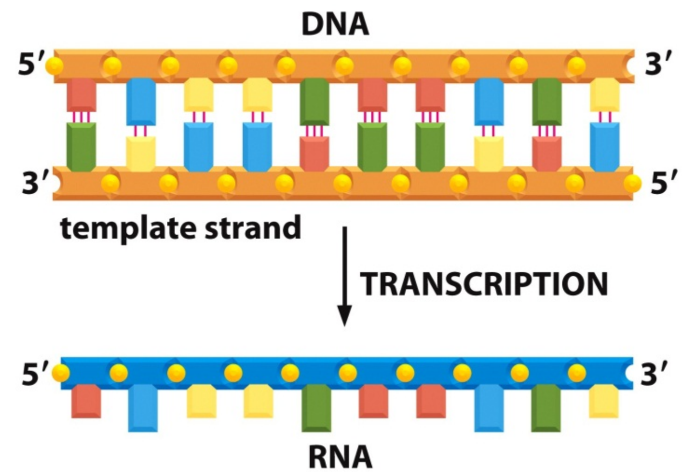


Figure 6-7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcriptional Regulation

Cells produce several types of RNA:

Table 6–1 Principal Types of RNAs Produced in Cells

TYPE OF RNA	FUNCTION
mRNAs	messenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify rRNAs
scaRNAs	small cajal RNAs, used to modify snoRNAs and snRNAs
miRNAs	microRNAs, regulate gene expression typically by blocking translation of selective mRNAs
siRNAs	small interfering RNAs, turn off gene expression by directing degradation of selective mRNAs and the establishment of compact chromatin structures
Other noncoding RNAs	function in diverse cell processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER

Different RNAs transcribed by different RNA polymerases in eukaryotes

Table 6–2 The Three RNA Polymerases in Eucaryotic Cells

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.

Prokaryotes have a single type of RNA polymerase

Transcriptional Regulation

Transcription initiation in eukaryotes requires many proteins:

- general transcription factors

Help position RNA polymerase at eukaryotic promoters

- contain TATA box

Table 6–3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSITION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Table 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Required by nearly all promoters used by

- RNA polymerase II

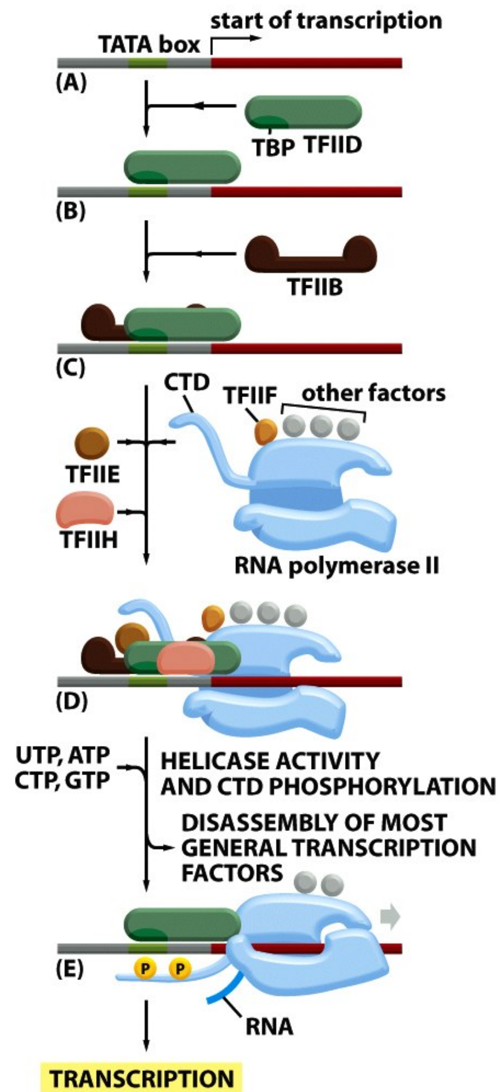


Figure 6-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Eukaryotic transcription

- RNA polymerase II transcribes protein coding genes
- Requires five general transcription factors; TFIID, TFIIB, TFIIF, TFIIE, and TFIIH (prokaryotes only need one; σ factor)
- Eukaryotic genomes lack operons
- Eukaryotic DNA is packaged into chromatin which provides an additional mode of regulation
- Eukaryotic transcriptional activation requires many gene regulatory proteins

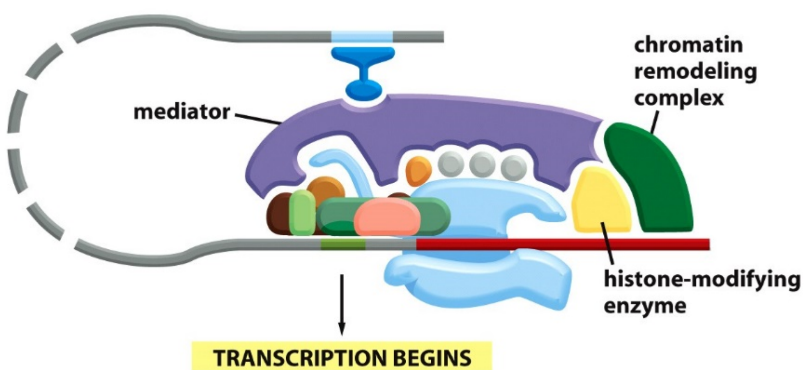


Figure 6-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Eukaryotic transcription

- Mediator acts an intermediate between regulatory proteins and RNA polymerase

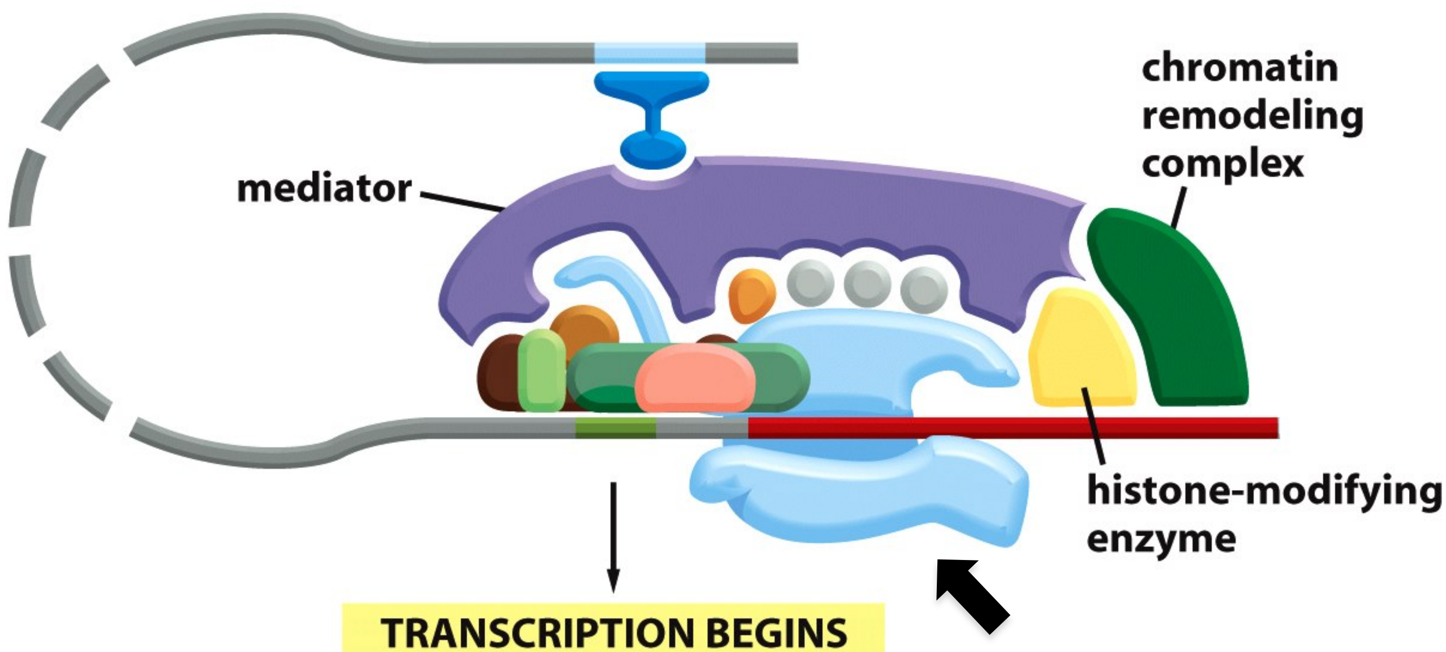


Figure 6-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

RNA Polymerase

Eukaryotic Gene Regulation

-Eukaryotic gene expression controlled by many regulatory proteins (~2000 encoded by the human genome) both activators and repressors
-Gene regulatory proteins can act over very large distances, sometimes >10000 base pairs away
- One mechanism is ● DNA looping

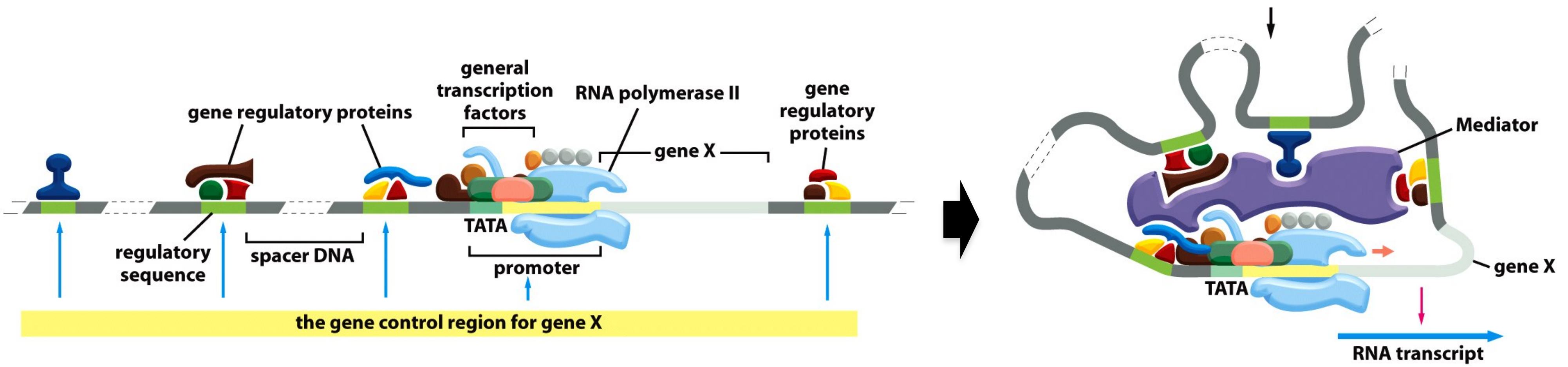


Figure 7-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Eukaryotic gene regulatory proteins often function as ● protein complexes on DNA

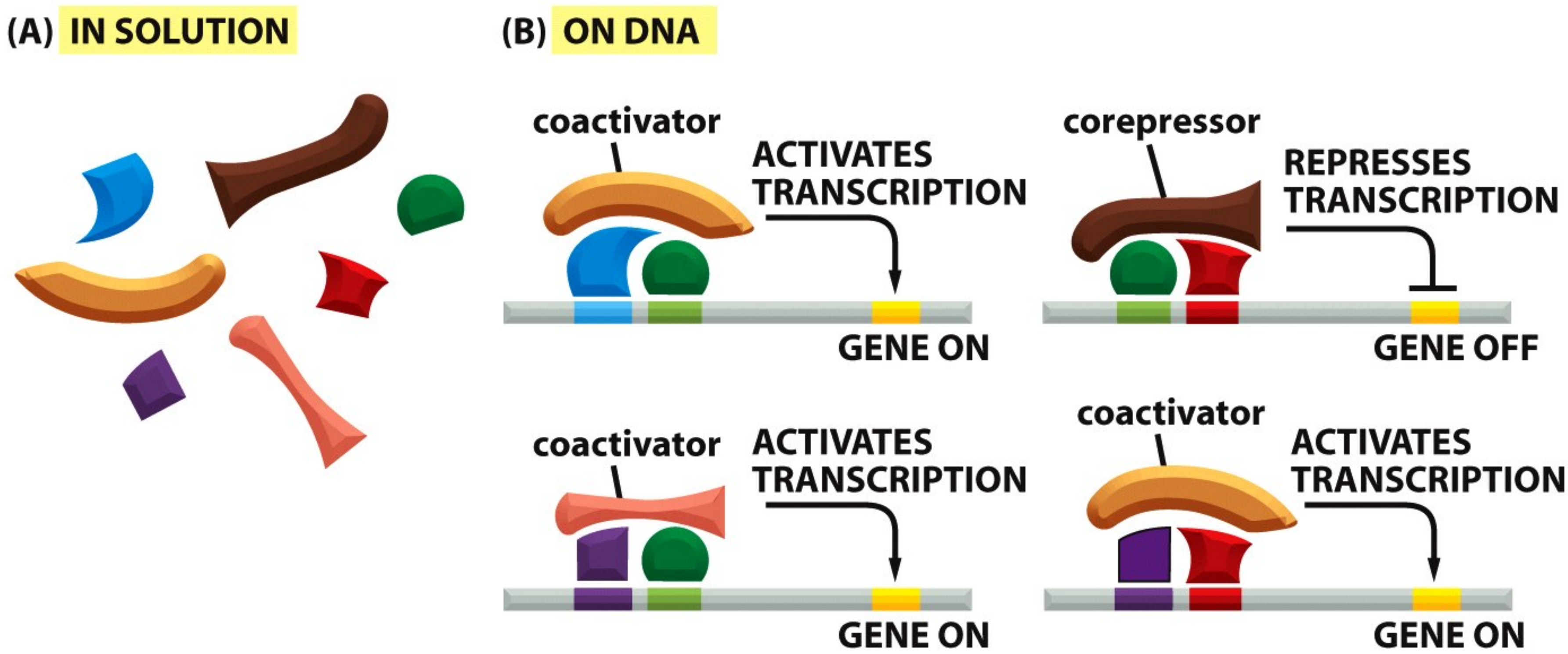


Figure 7-51 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Coactivators and corepressors assemble on DNA-bound gene regulatory proteins ● do not directly bind DNA

Eukaryotic Gene Regulation

Eukaryotic Activator Proteins

Modular design:

1) ● DNA binding domain (DB)
   - recognizes specific DNA sequence

2) ● Activation domain (AD)
   - accelerates rate of transcription

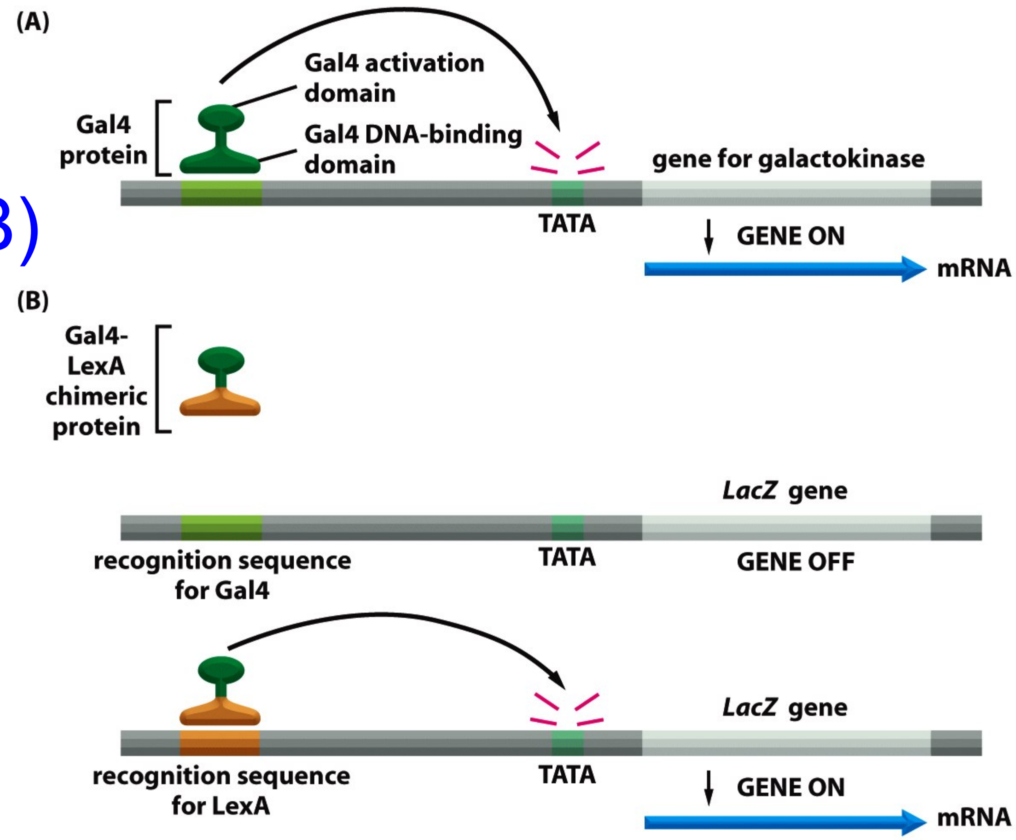


Figure 7-45 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Can mix-and-match DBs and ADs

Eukaryotic Gene Regulation

How do Activator Proteins activate transcription?

Attract, position and modify:

- General transcription factors
- Mediator
- RNA polymerase II

They can do this either:

1) • Directly by acting on these components
2) • Indirectly modifying chromatin structure

Eukaryotic Gene Regulation

1) Activator proteins can • bind directly to transcriptional machinery or mediator and attract them to promoters (like prokaryotic activators)

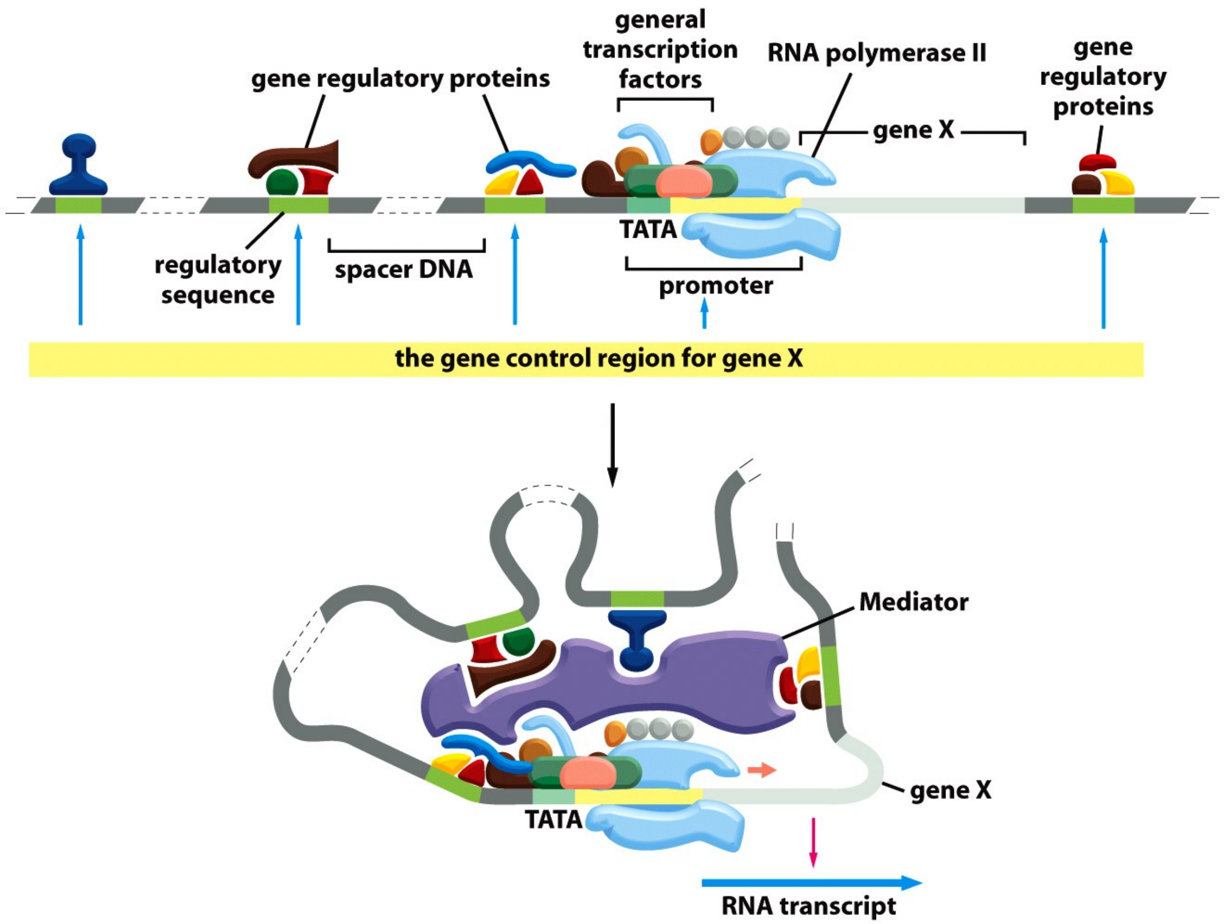


Figure 7-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

2) Activator proteins can alter ● chromatin structure

Nucleosomes are the basic structure of Eukaryotic chromatin
- DNA wound around a ● histone octamer (H2A, H2B, H3, and H4 x 2)

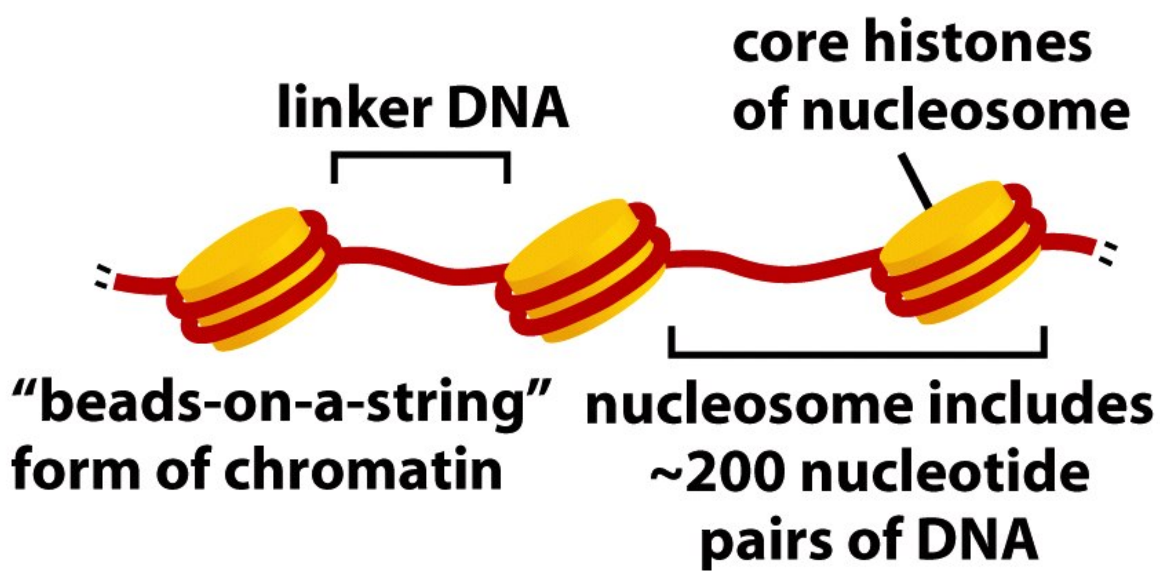


Figure 4-23 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Nucleosomes pack as compact chromatin fibers

Zigzag model

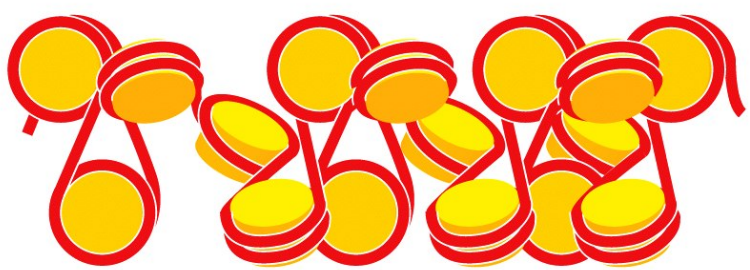


Figure 4-31 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Solenoid Model

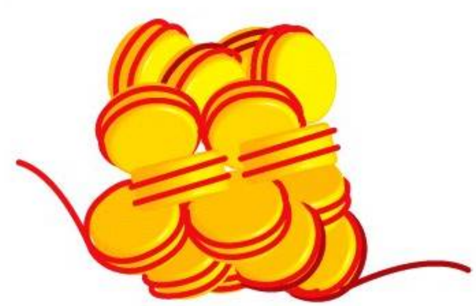


Figure 4-32 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcriptional machinery cannot assemble on promoters tightly packaged in chromatin

Activator proteins can alter chromatin structure and increase • promoter accessibility How?

Eukaryotic Gene Regulation

4 major ways activator proteins can alter chromatin

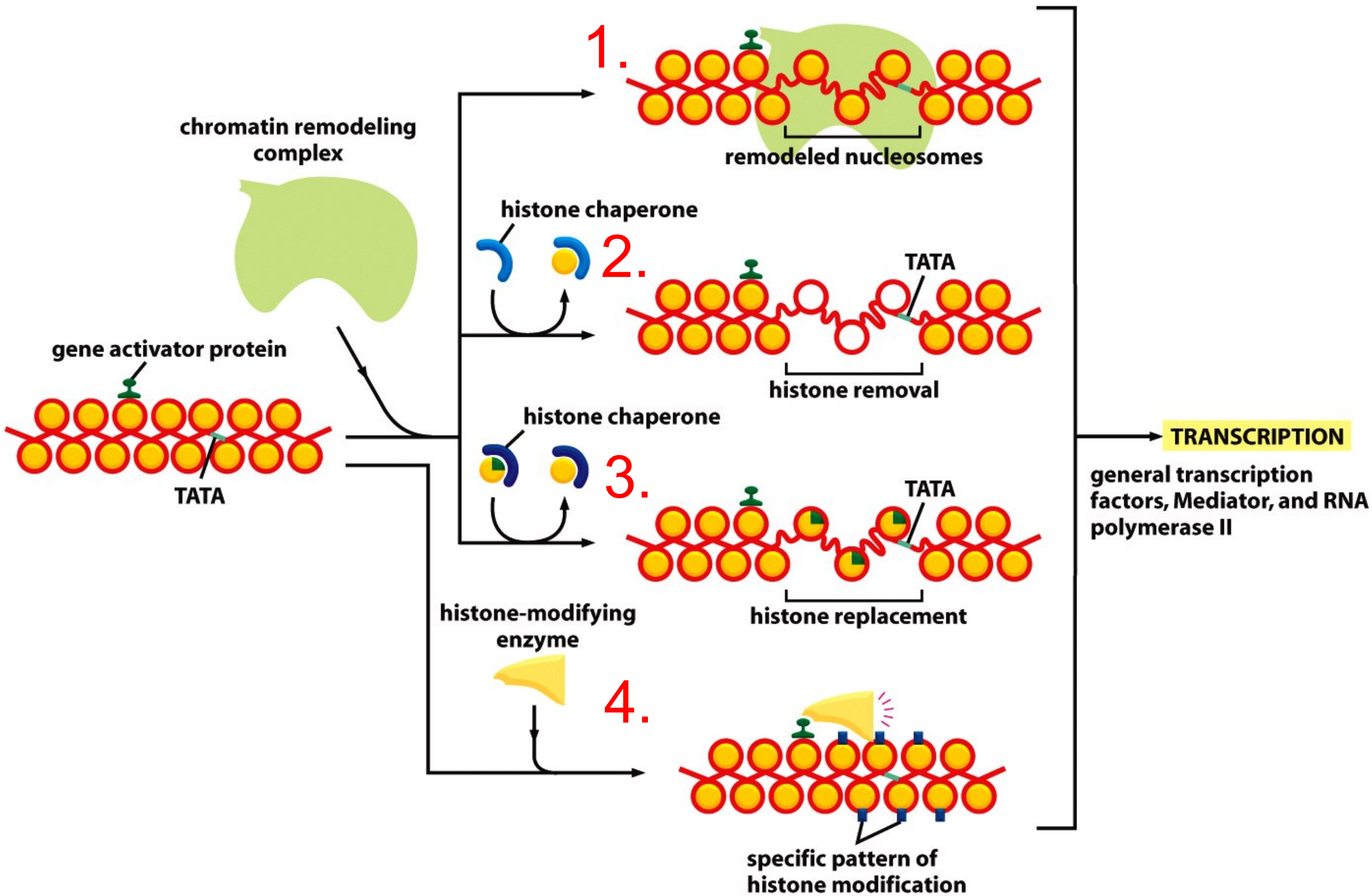


Figure 7-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Nucleosome structure can be altered by
● chromatin remodeling complexes in an ● ATP-dependent manner to increase promoter accessibility

1) Nucleosome sliding

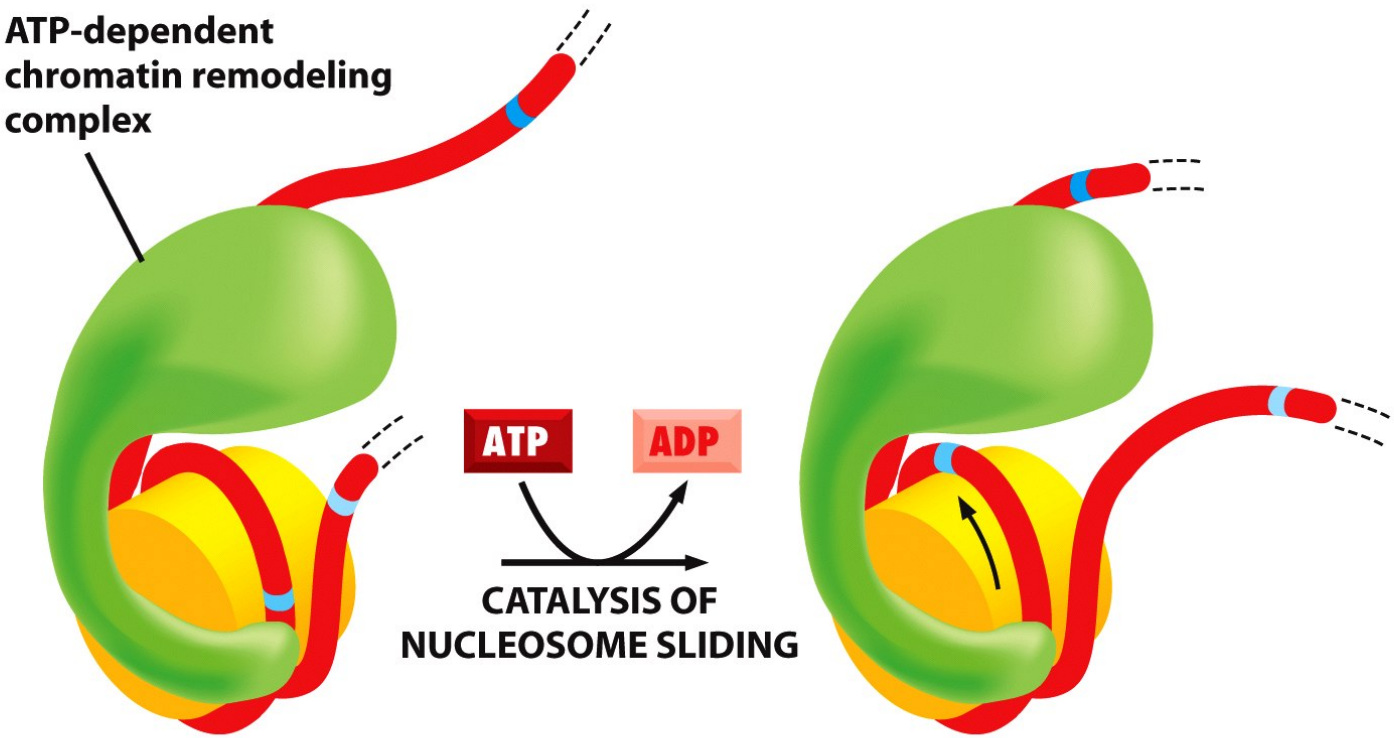


Figure 4-29 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

2, 3) Nucleosome removal and histone exchange

Requires cooperation with
- histone chaperones

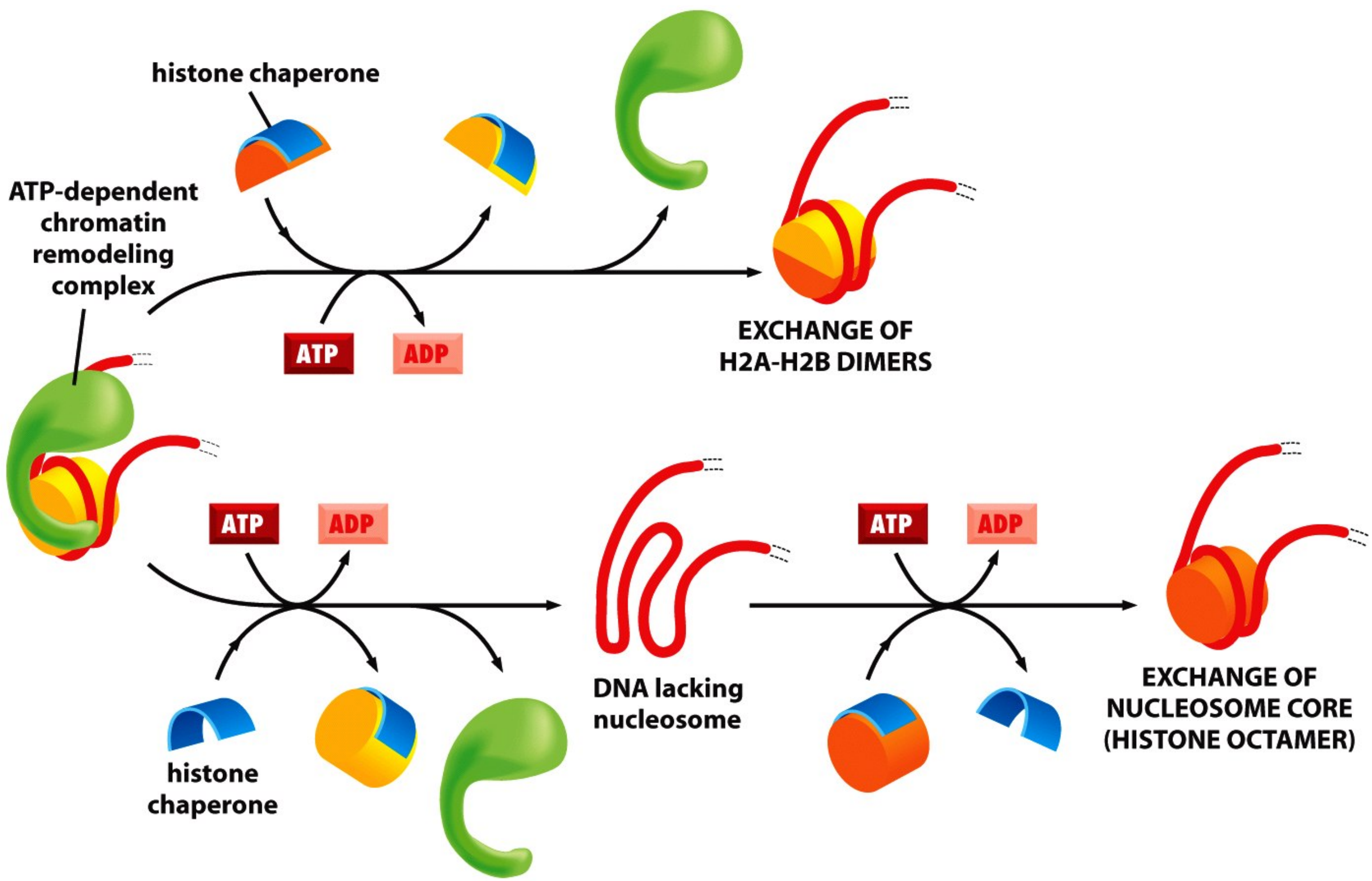
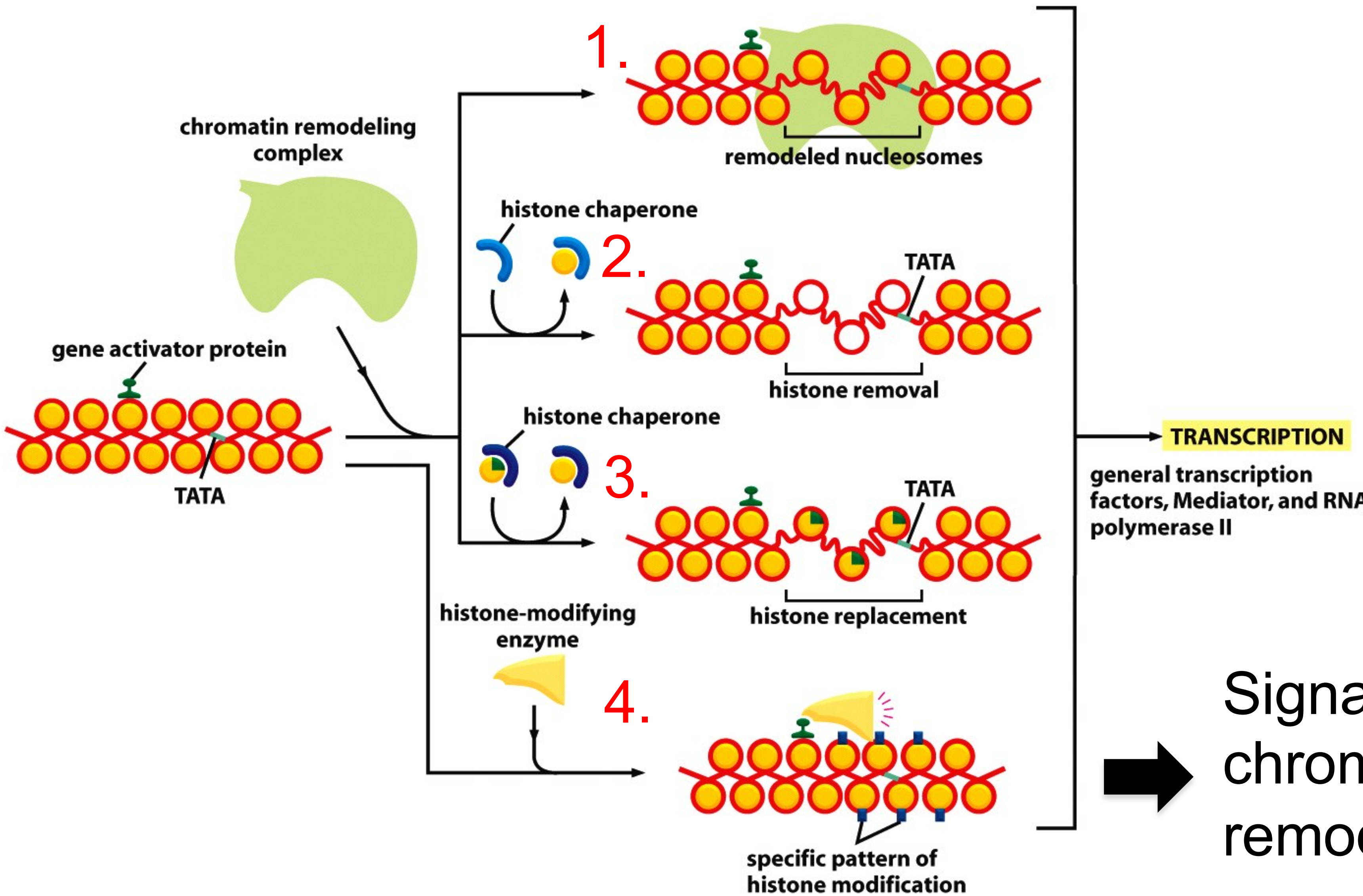


Figure 4-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

4 major ways activator proteins can alter chromatin



Signal for chromatin remodeling

Figure 7-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

4)

Histone modifying enzymes produce specific patterns of histone modifications
- histone code

Addition of phosphate group:
- phosphorylation
- Enzyme: kinase

Addition of acetyl group:
- acetylation
- Enzyme: acetyltransferase

Addition of methyl group:
- methylation
- Enzyme: methyltransferase

Histone modifications occur on specific amino acids of
- histone tails

Eukaryotic Gene Regulation

The histone code:
Specific modifications to histone tails by histone modifying enzymes ● “writers”

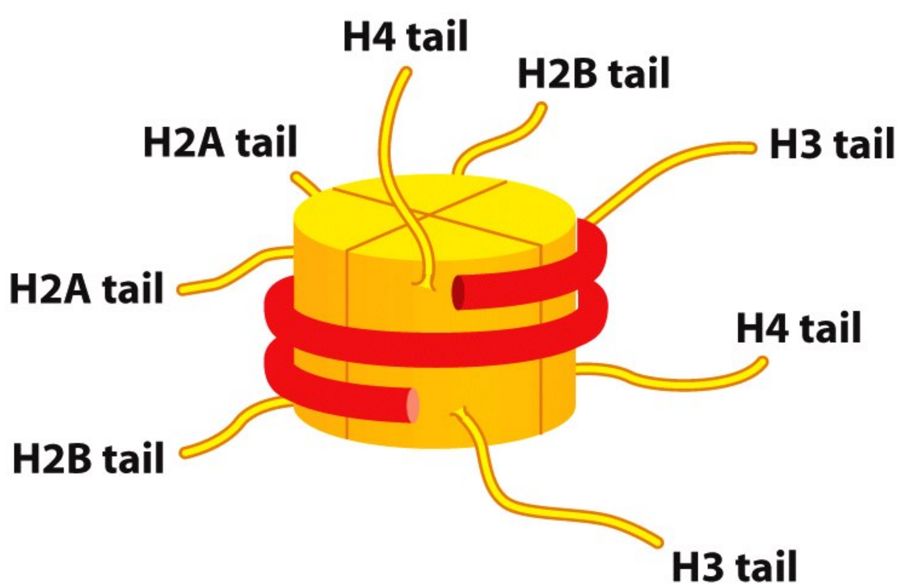


Figure 4-33a Molecular Biology of the Cell 5/e (© Garland Science 2008)

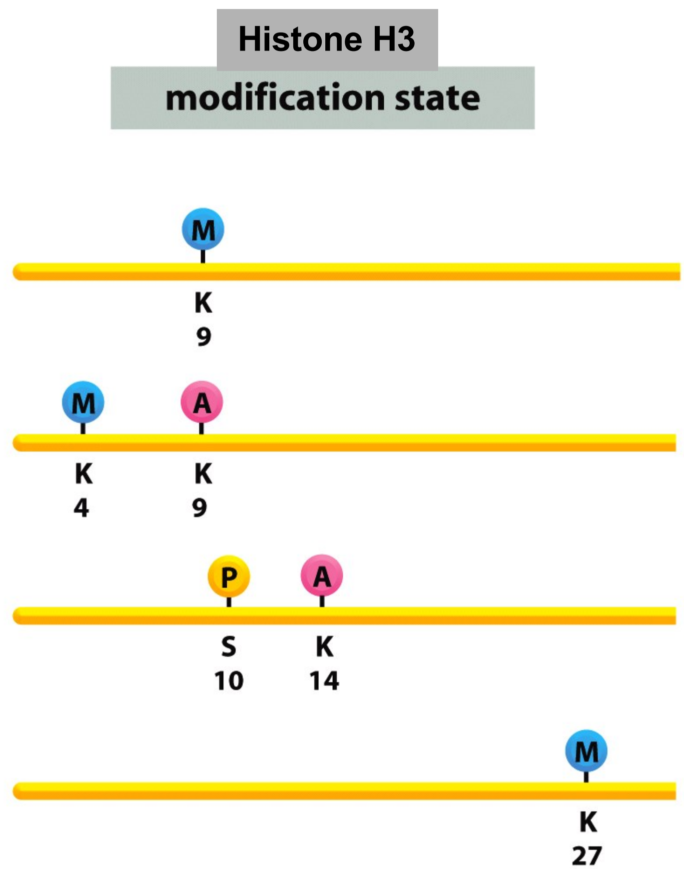
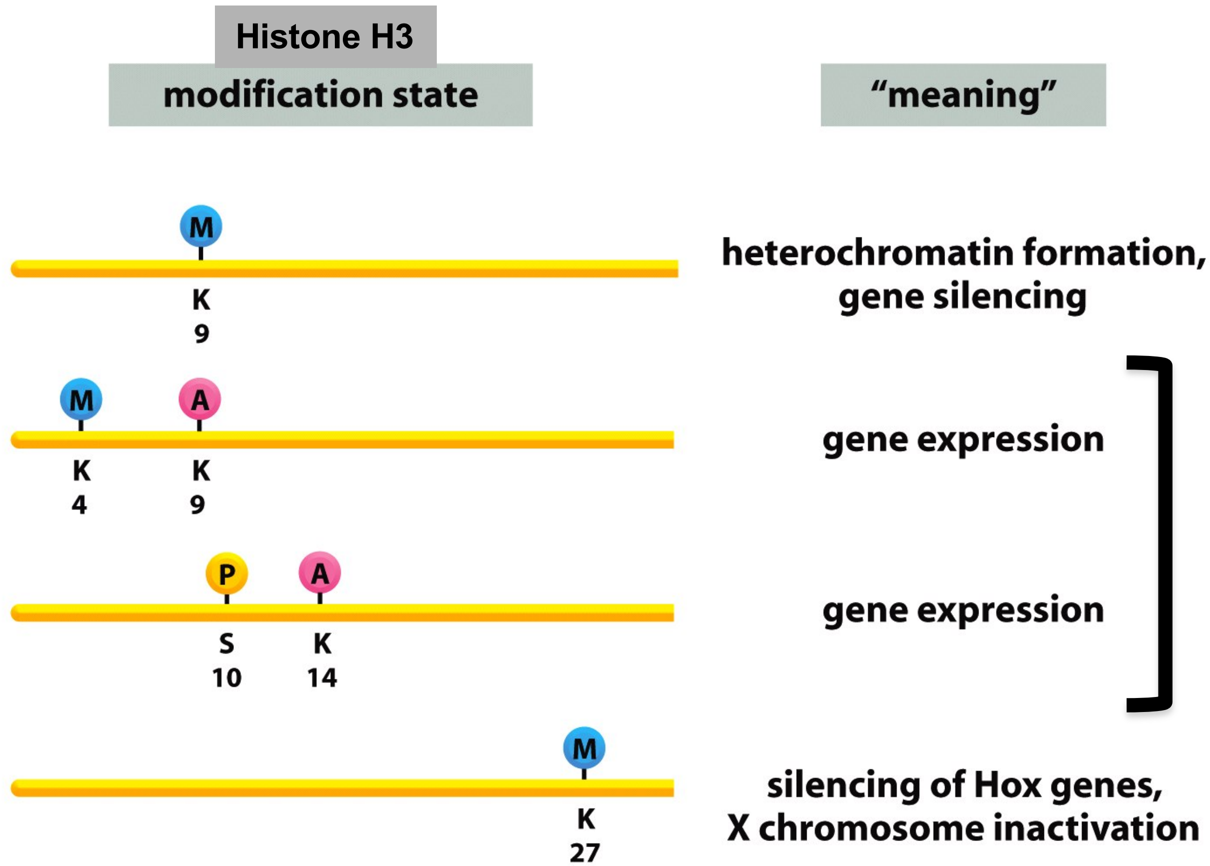


Figure 4-44b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

The histone code:

Code- ● “reader” proteins can recognize specific modifications and provide meaning to the code



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Eukaryotic Gene Regulation

Transcriptional regulation using the histone code

eg. human interferon gene promoter

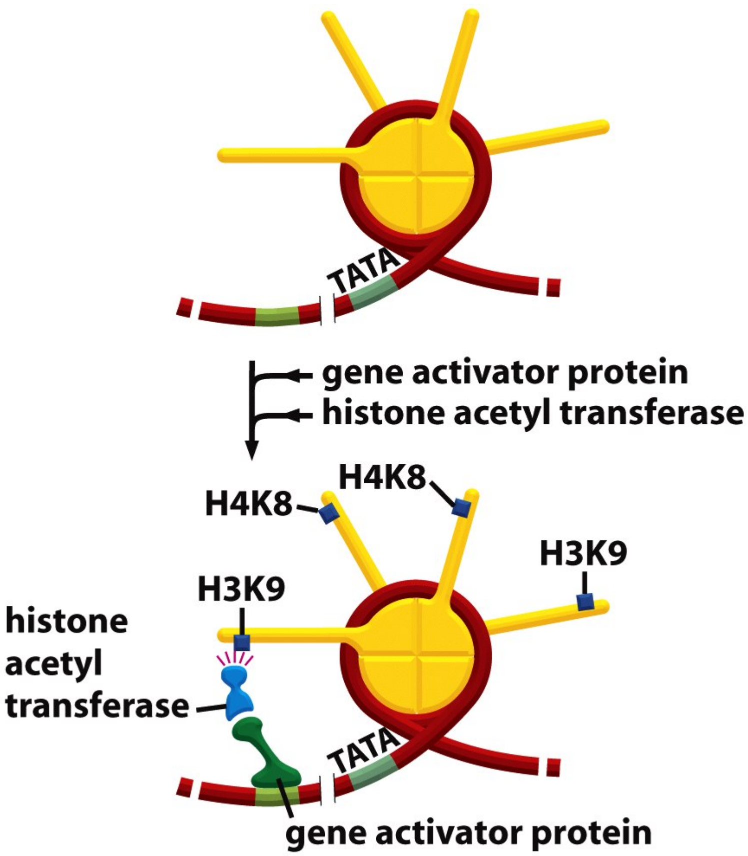

Figure 7-47 part 1 of 4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Step 1: Activator protein binds to chromatin DNA and attracts a

- histone acetyltransferase (HAT)

Step 2: HA acetylates lysine 9 of histone H3 and lysine 8 of histone H4.

Eukaryotic Gene Regulation

Transcriptional regulation using the histone code

eg. human interferon gene promoter

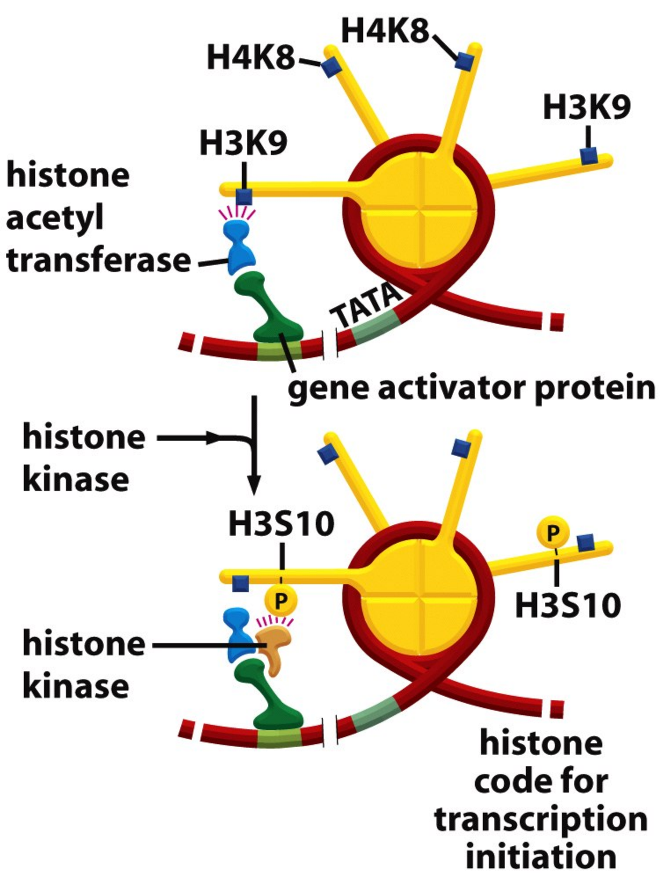

Figure 7-47 part 2 of 4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Step 3: Activator protein attracts a

- histone kinase (HK)

Step 4: HK phosphorylates serine 10 of histone H3. Can only occur after acetylation of lysine 9

Eukaryotic Gene Regulation

Transcriptional regulation using the histone code

eg. human interferon gene promoter

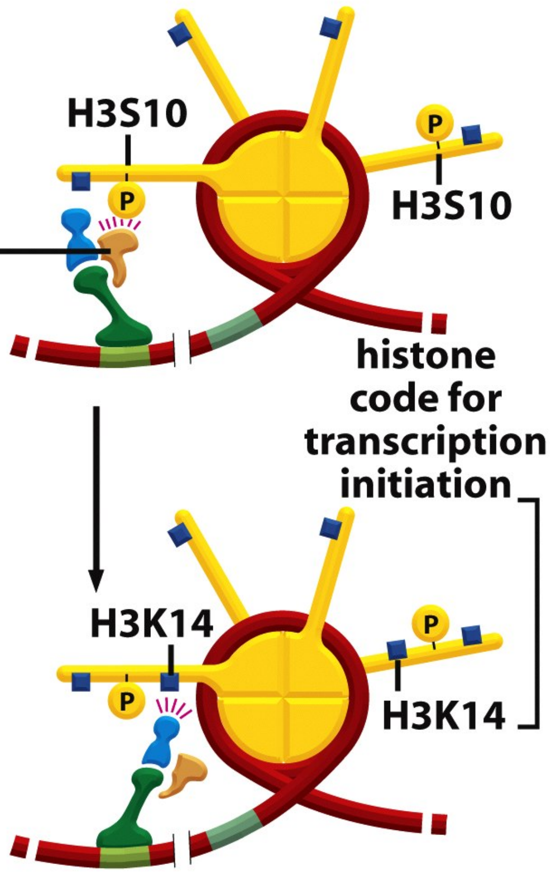

Figure 7-47 part 3 of 4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Step 5: Serine modification signals the acetyltransferase to acetylate lysine 14 of histone H3

Histone code for transcription
Initiation is written

Eukaryotic Gene Regulation

Transcriptional regulation using the histone code

eg. human interferon gene promoter

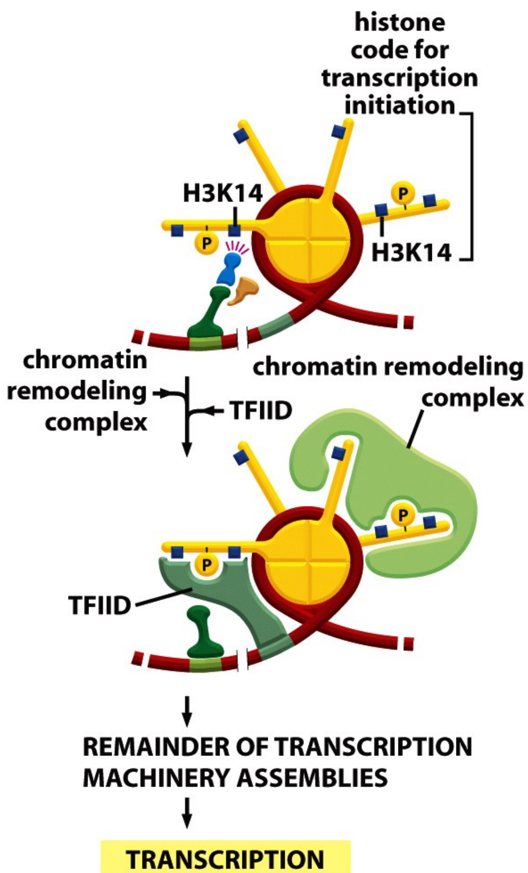

Figure 7-47 part 4 of 4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Step 6:

- TFIID and a chromatin remodelling complex

bind to acetylated histone tails and initiate transcription

Eukaryotic Gene Regulation

Transcriptional Repression

- Unlike prokaryotes, eukaryotic repressor proteins rarely compete with RNA polymerase for access to DNA
- Instead use a variety of mechanisms to inihibit transcription

1) Interfering with activator function

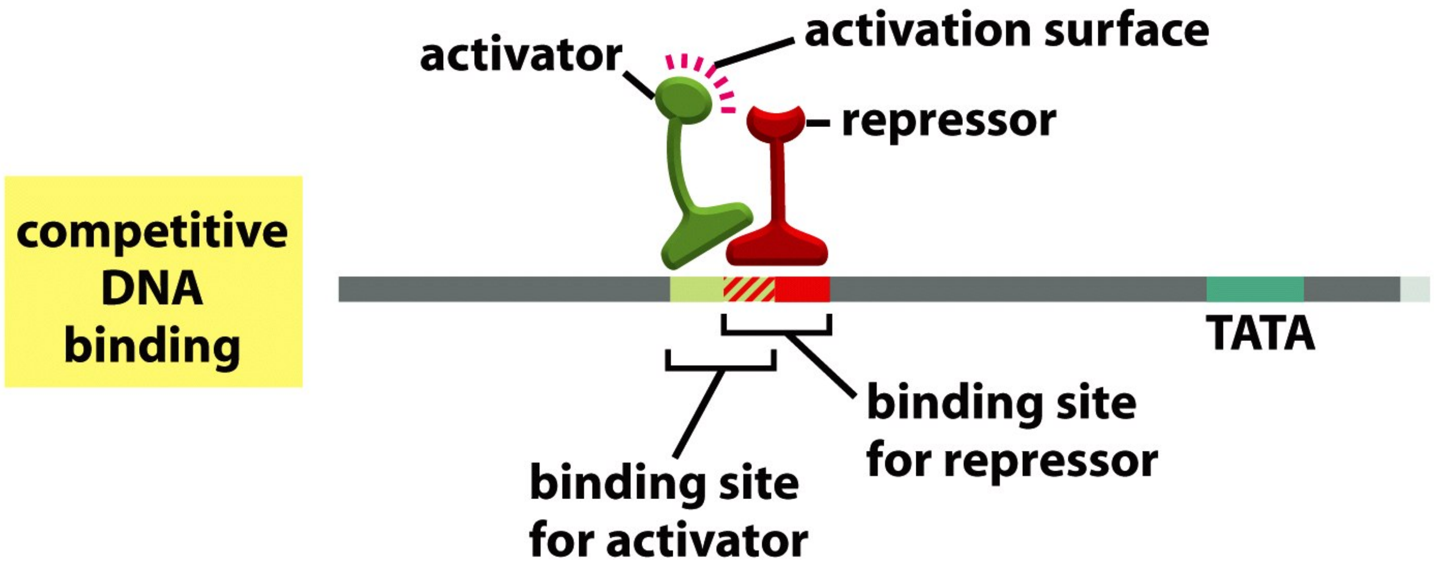

Figure 7-50a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Transcriptional Repression

Interfering with activator function

2)

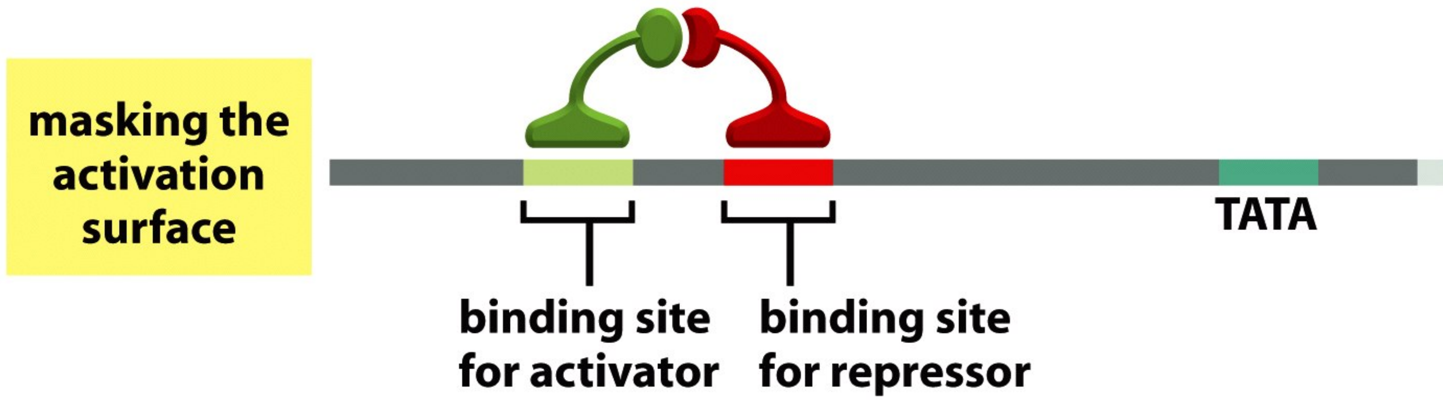

Figure 7-50b Molecular Biology of the Cell 5/e (© Garland Science 2008)

3)

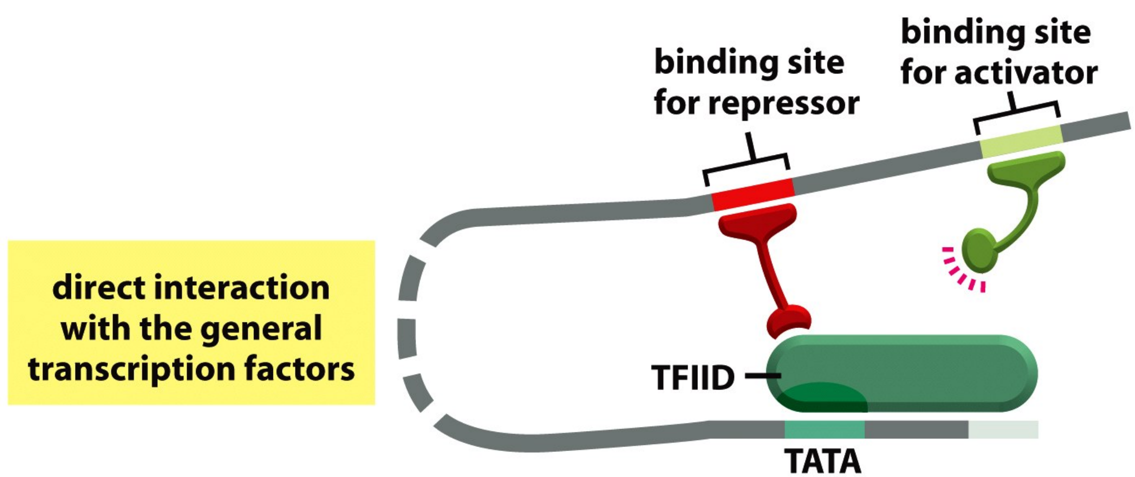

Figure 7-50c Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Transcriptional Repression by altering chromatin structure

4)

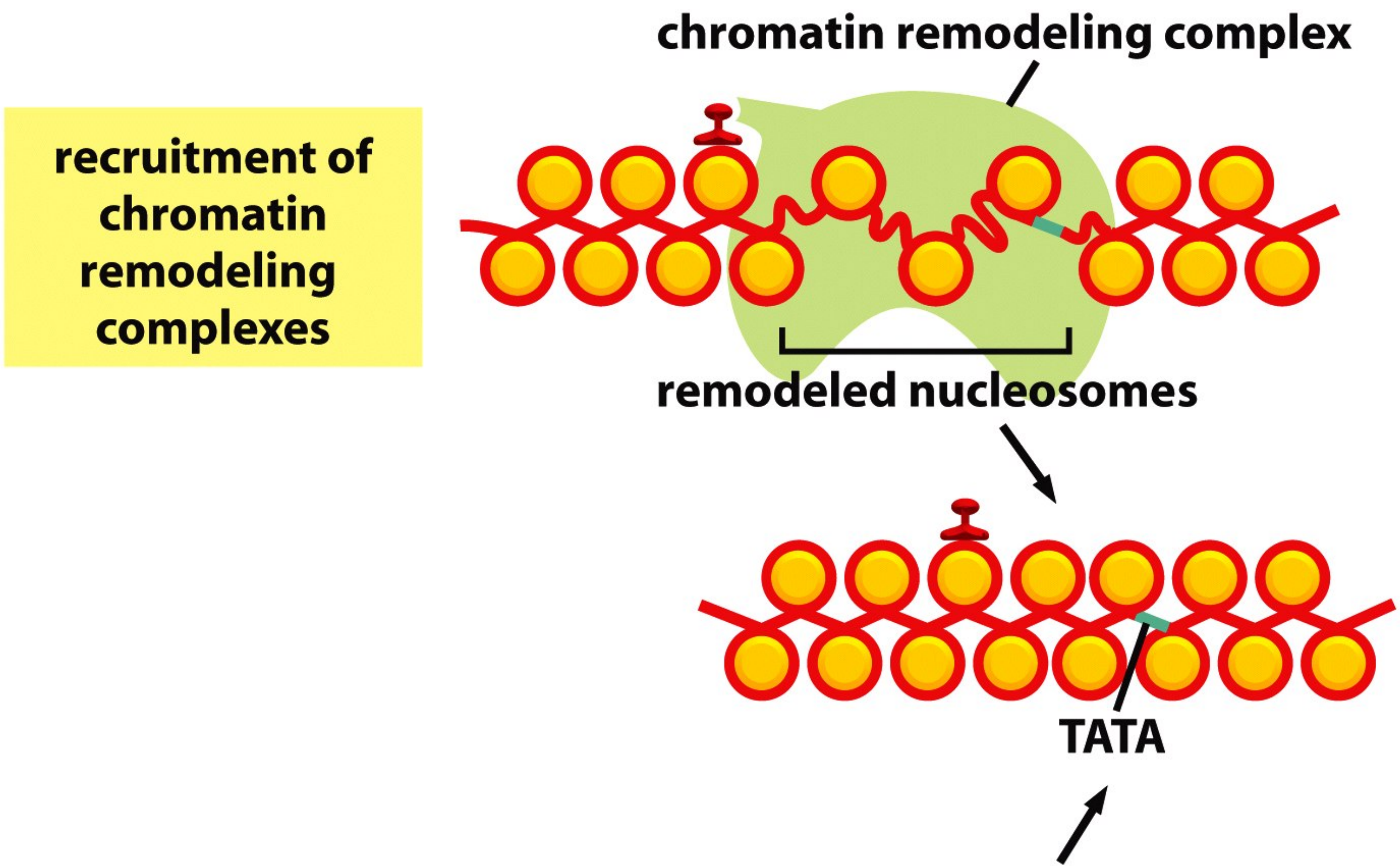

Figure 7-50d Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Transcriptional Repression by altering the histone code

5)

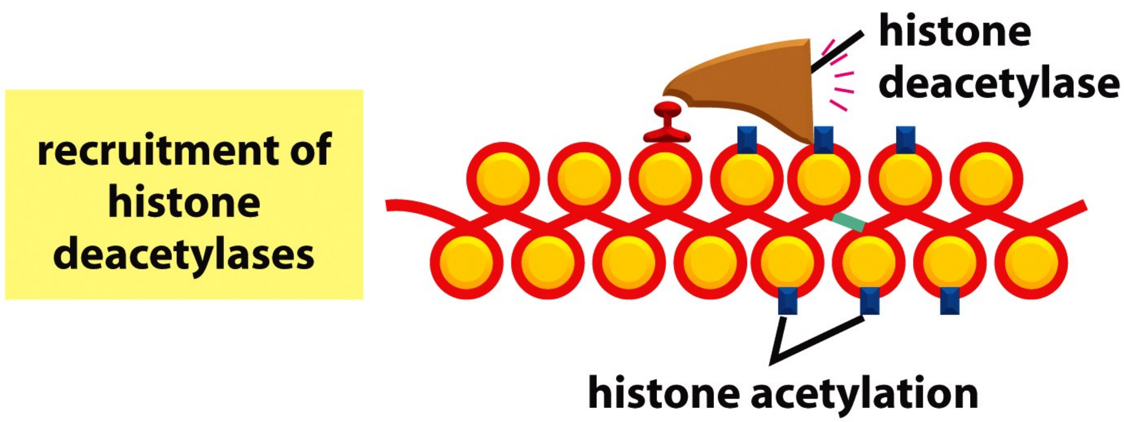

Figure 7-50e Molecular Biology of the Cell 5/e (© Garland Science 2008)

6)

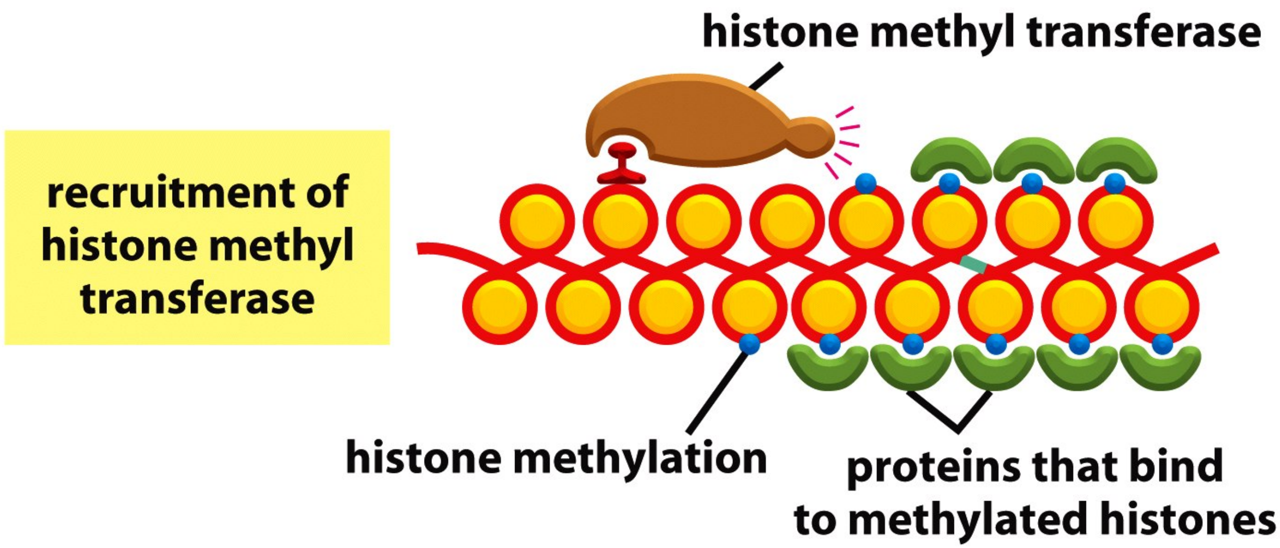

Figure 7-50f Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

Guided by gene regulatory proteins histone "reader" and "writer" proteins can establish a repressive form of chromatin

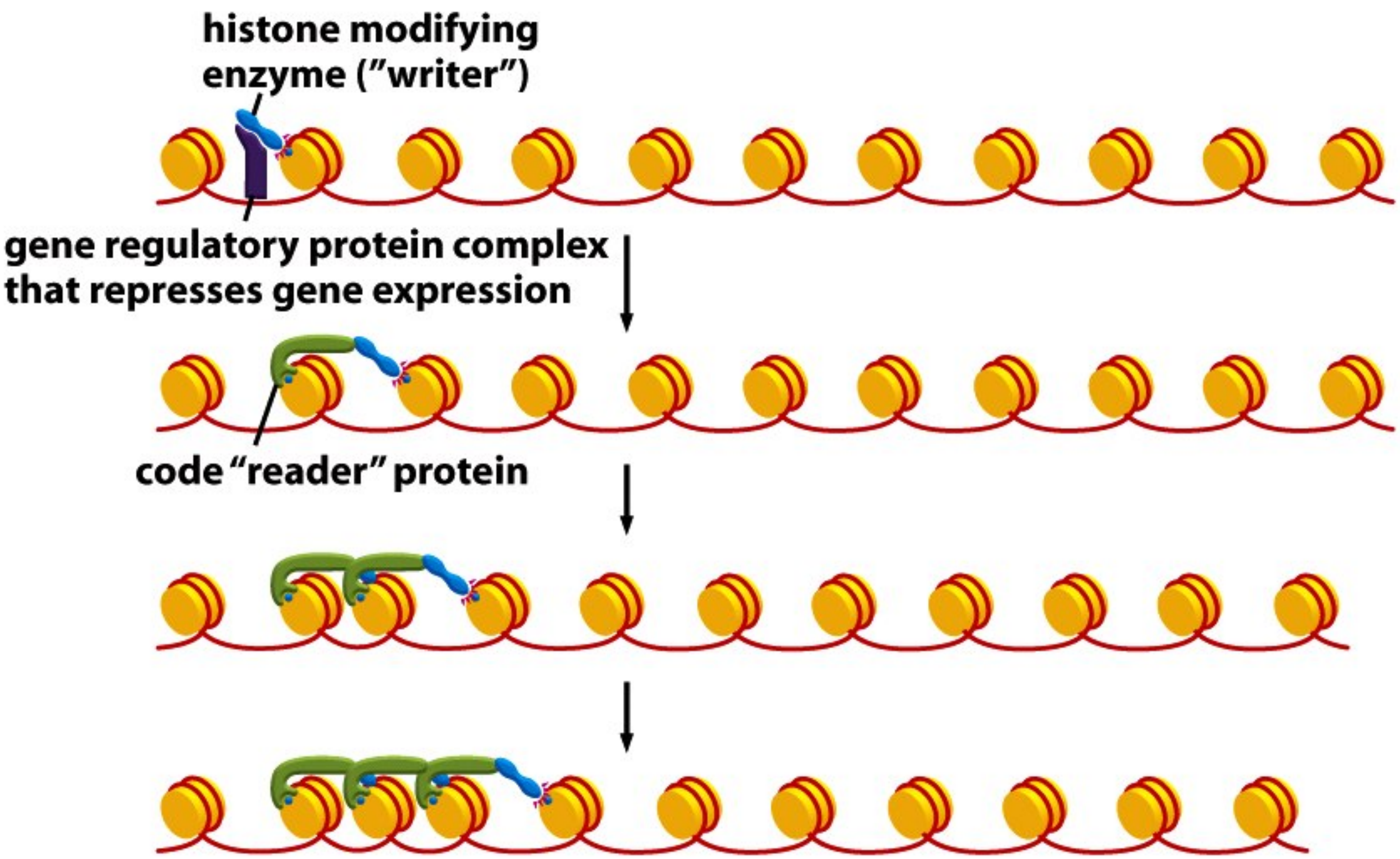

Figure 7-81 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- histone code can spread

This chromatin can be stabilized

Eukaryotic Gene Regulation

Spreading the histone code along chromatin carried out by

- Reader-writer complexes

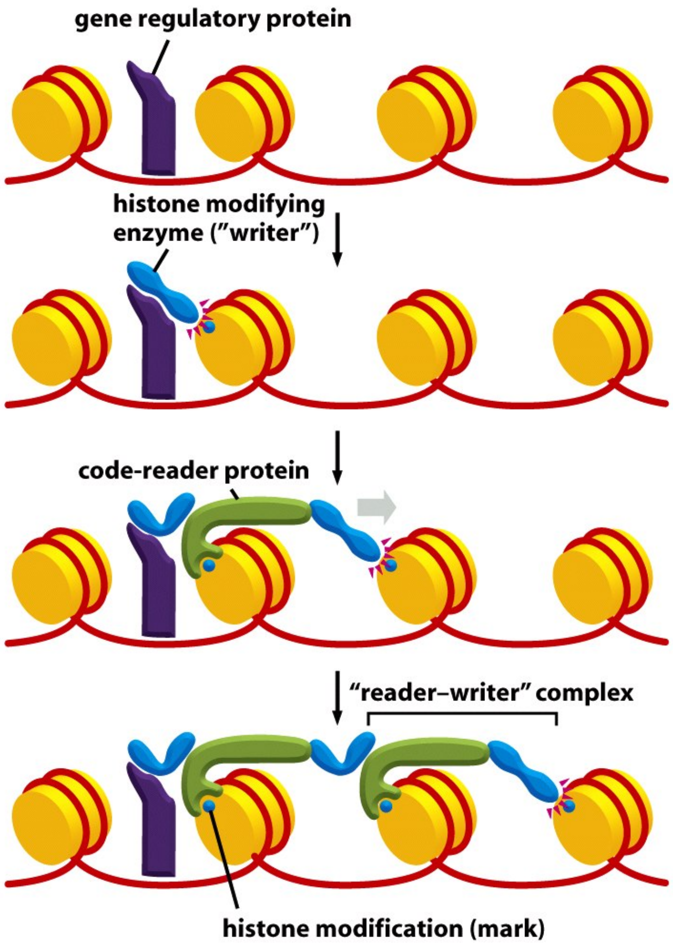

Figure 4-45 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Eukaryotic Gene Regulation

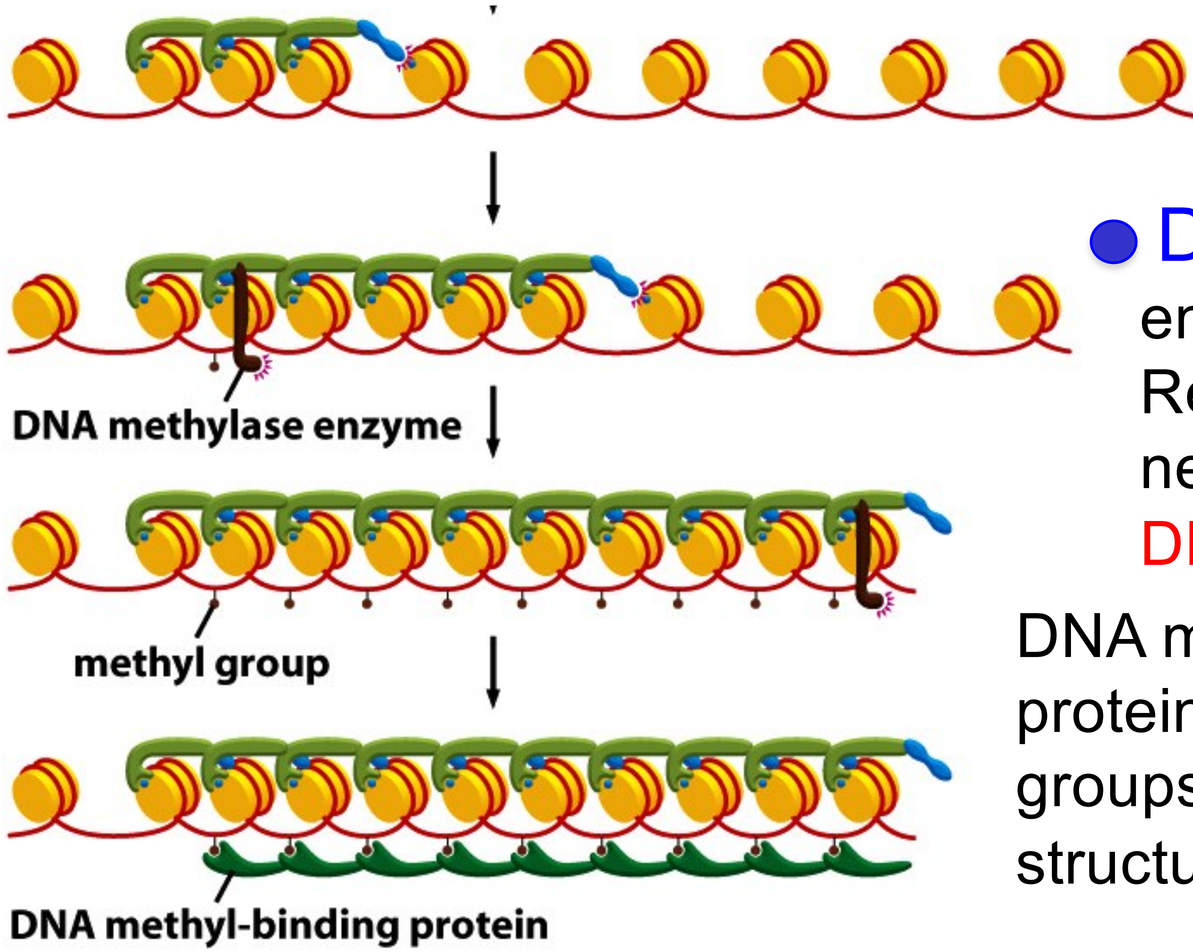

Figure 7-81 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- DNA methylase enzyme is attracted by Reader and methylates nearby cytosines in DNA

DNA methyl-binding proteins bind methyl groups and stabilize structure

-Methylation and therefore gene expression patterns can be inherited
A process called
- epigenetic inheritance

Remember to read the textbook. Check the textbook for answers to your questions.

After reading the textbook, questions are welcome… please ask on the Discussion Board, and/or after classes.

Help one another on the Discussion Board.