Gene Regulation in Eukaryotes

- All cells in an organism contain all the DNA:
  - all genetic info
- Must regulate or control which genes are turned on in which cells
- Genes turned on determine cells' function
  - E.g.) liver cells express genes for liver enzymes but not genes for stomach enzymes

Proteins act in *trans*
DNA sites act only in *cis*

- *Trans* acting elements (not DNA) can diffuse through cytoplasm and act at target DNA sites on any DNA molecule in cell (usually proteins)

- *Cis* acting elements (DNA sequences) can only influence expression of adjacent genes on same DNA molecule

Eukaryotic Promoters

trans-acting proteins control transcription from class II (RNA pol II) promoters

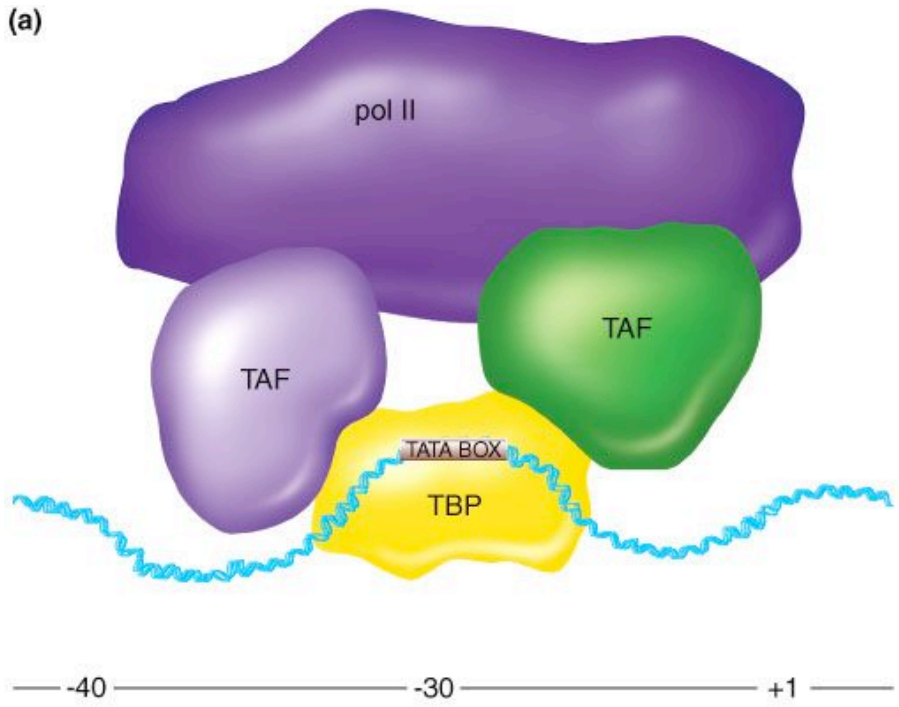


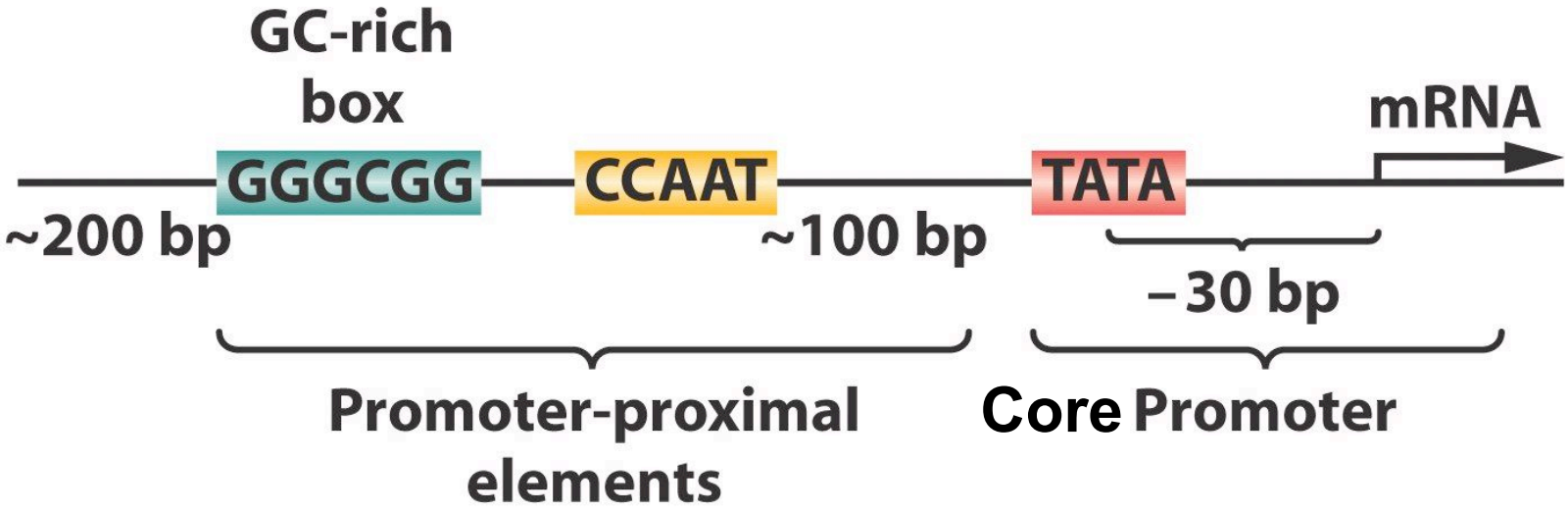
Fig. 17.4 a

- Basal factors bind to the core promoter
  - TBP – TATA box binding protein
  - TAF – TBP associated factors
- RNA polymerase II binds to basal factors

Eukaryotic Promoters

- Promoter proximal elements are required for high levels of transcription.
- They are further upstream from the start site, usually at positions between -50 and -500.
- These elements generally function in either orientation.
- Examples include:
  - The CAAT box consensus sequence CCAAT
  - The GC box consensus sequence GGGCGG
  - Octamer consensus sequence AGCTAAAT

Regulatory elements that map near a gene are *cis*-acting DNA sequences



- *cis*-acting elements
  - Core Promoter – Basal level expression
    - Binding site for TATA-binding protein and associated factors
  - Promoter Proximal Elements - True level of expression
    - Binding sites for transcription factors

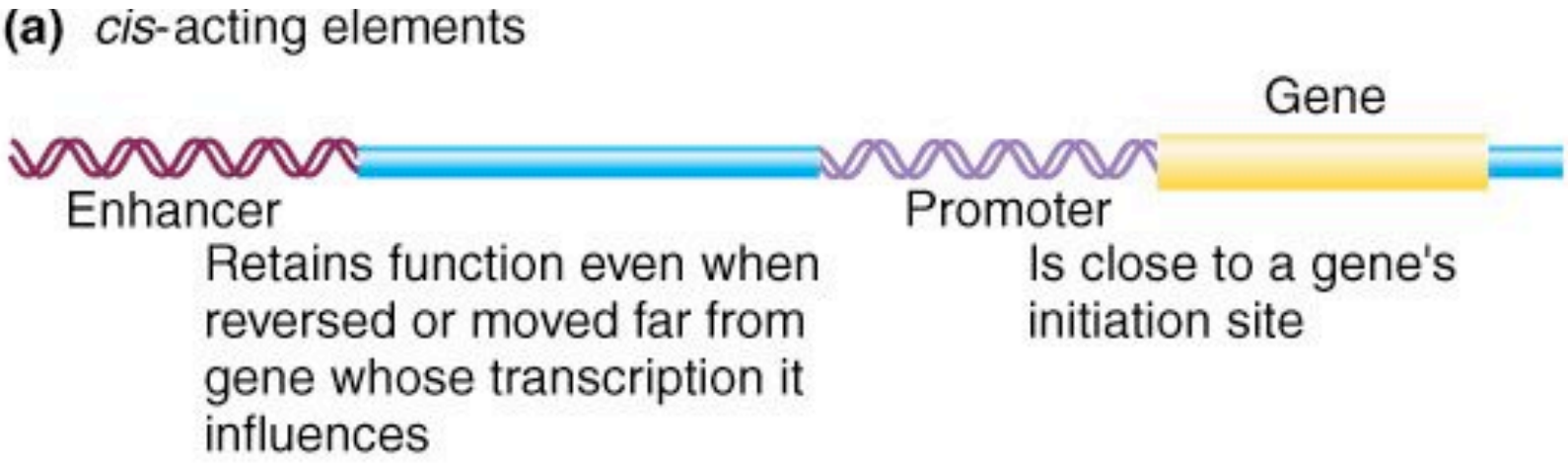
Eukaryotic Promoter Elements

- Various combinations of core and proximal elements are found near different genes.
- Promoter proximal elements are key to gene expression.
  - Activators, proteins important in transcription regulation, are recognized by promoter proximal elements.
  - Housekeeping genes
    - used in all cell types for basic cellular functions
    - have common promoter proximal elements
    - are recognized by activator proteins found in all cells.
  - Genes expressed only in some cell types or at particular times have promoter proximal elements recognized by activator proteins found only in specific cell types or times.

Eukaryotic Enhancer Sequences

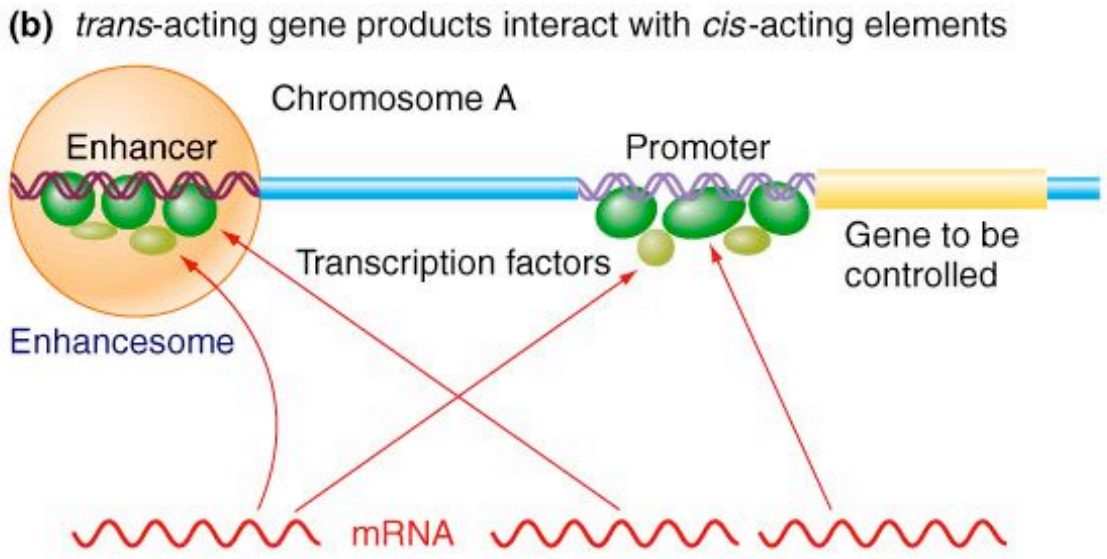
- Enhancers are another *cis*-acting element.
- They are required for maximal transcription of a gene.
  - Enhancers can be upstream or downstream of the transcription initiation site
  - They may modulate from a distance of thousands of base pairs away from the initiation site.
  - Enhancers contain short sequence elements, some similar to promoter sequences.
  - Activators bind these sequences and other protein complexes form, postulated to bring the enhancer complex close to the promoter and increasing transcription.

Regulatory elements that map near a gene are *cis*-acting DNA sequences



- *cis*-acting elements
  - Promoter – very close to gene's initiation site
  - Enhancer
    - can lie far way from gene
    - Can be reversed
    - Augment or repress basal levels of transcription

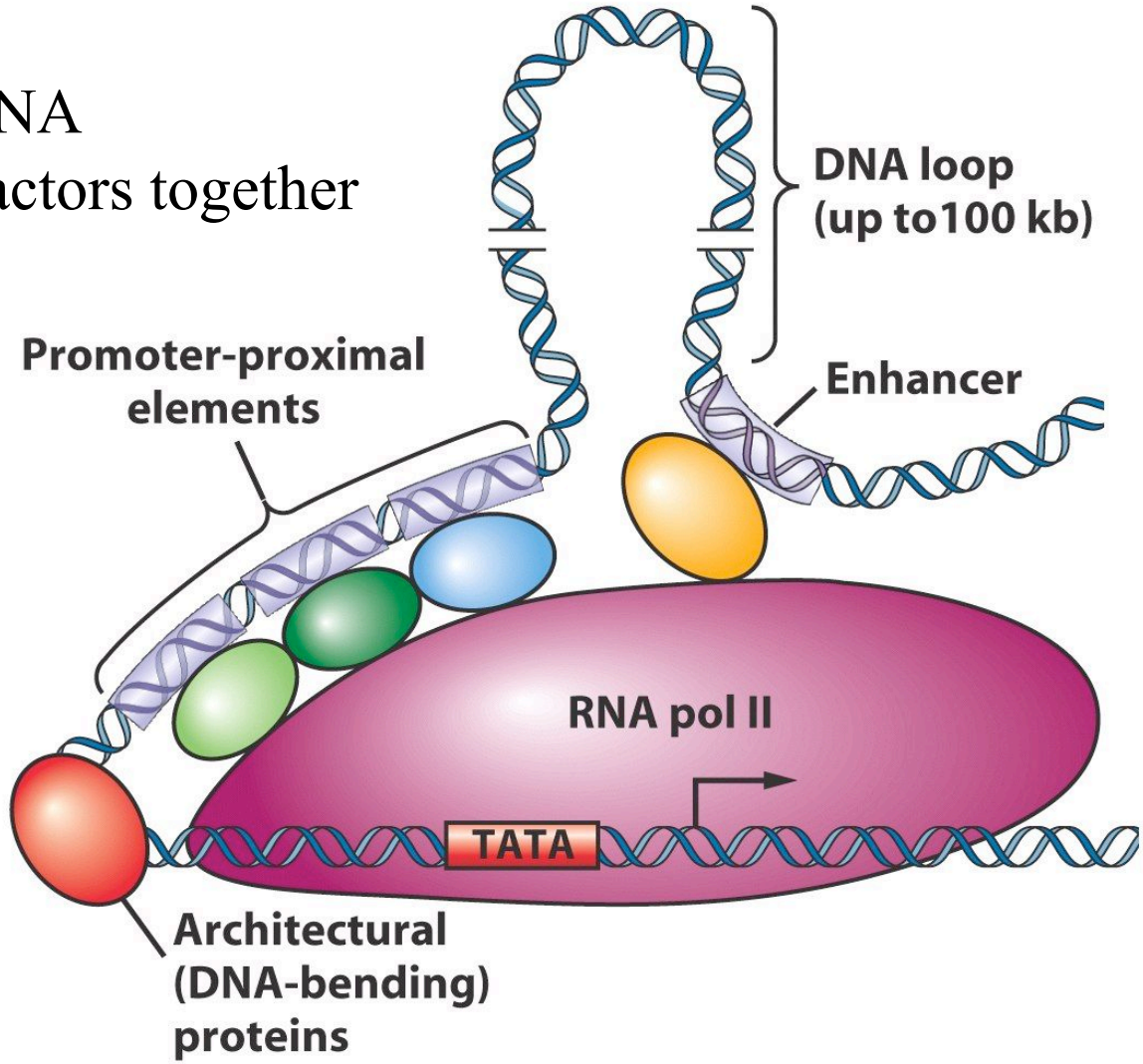
Regulatory elements that act on the promoter or enhancer sequences are *trans*-acting factors



- Genes that encode proteins that interact directly or indirectly with target genes *cis*-acting elements
  - Known genetically as transcription factors
  - Identified by:
    - Mapping
    - Biochemical studies to identify proteins that bind in vitro to *cis*-acting elements

How do Enhancers work if they are so far away from the promoter?

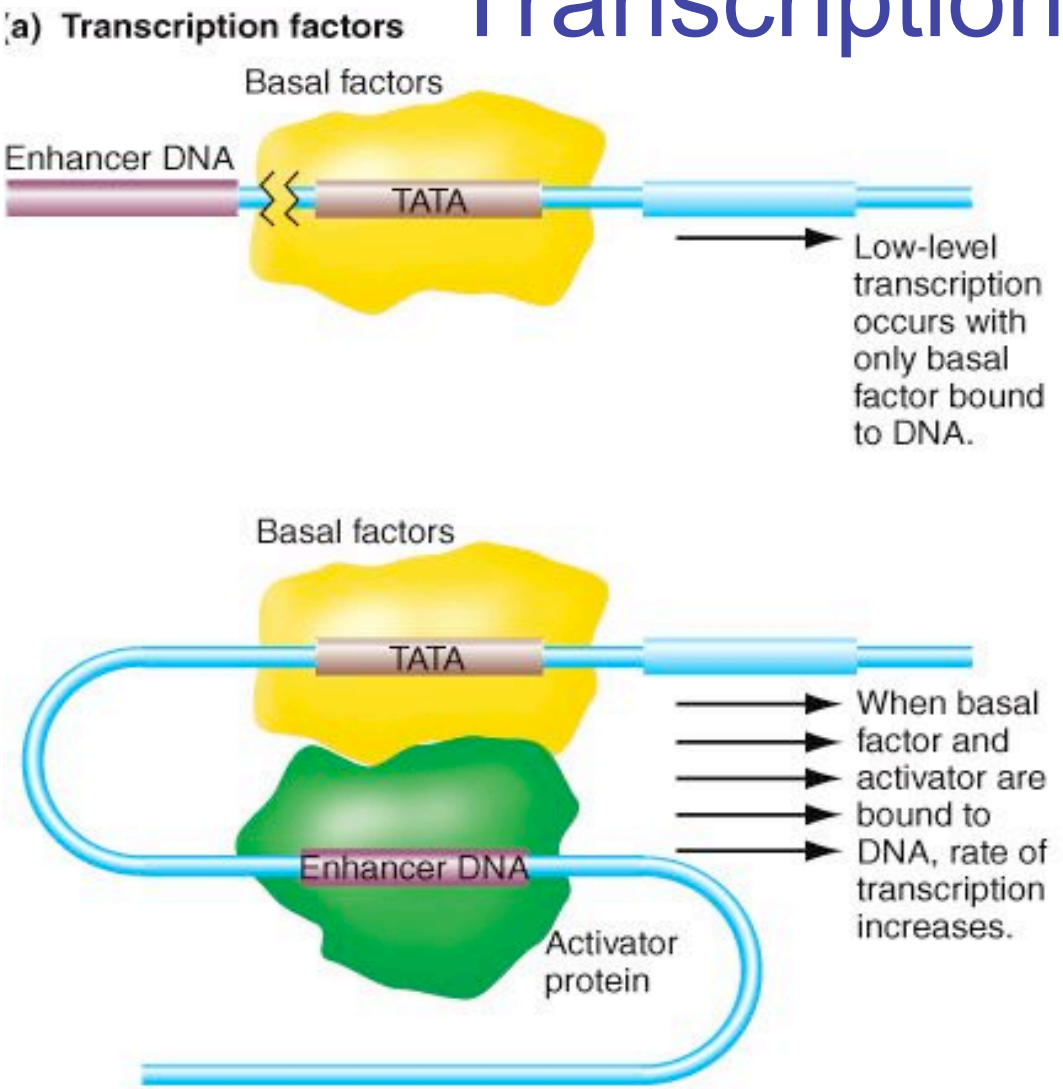
- Possible looping of DNA
- Brings transcription factors together



Transcription Factors

- Also called activator proteins and silencer proteins
- Bind to promoter, enhancer, and silencer DNA in specific ways
- Interact with other proteins to activate and increase transcription as much as 100-fold above basal levels
  - or repress transcription in the case of silencers/repressors
- Two structural domains mediate these functions
  - DNA-binding domain
  - Transcription-activator domain

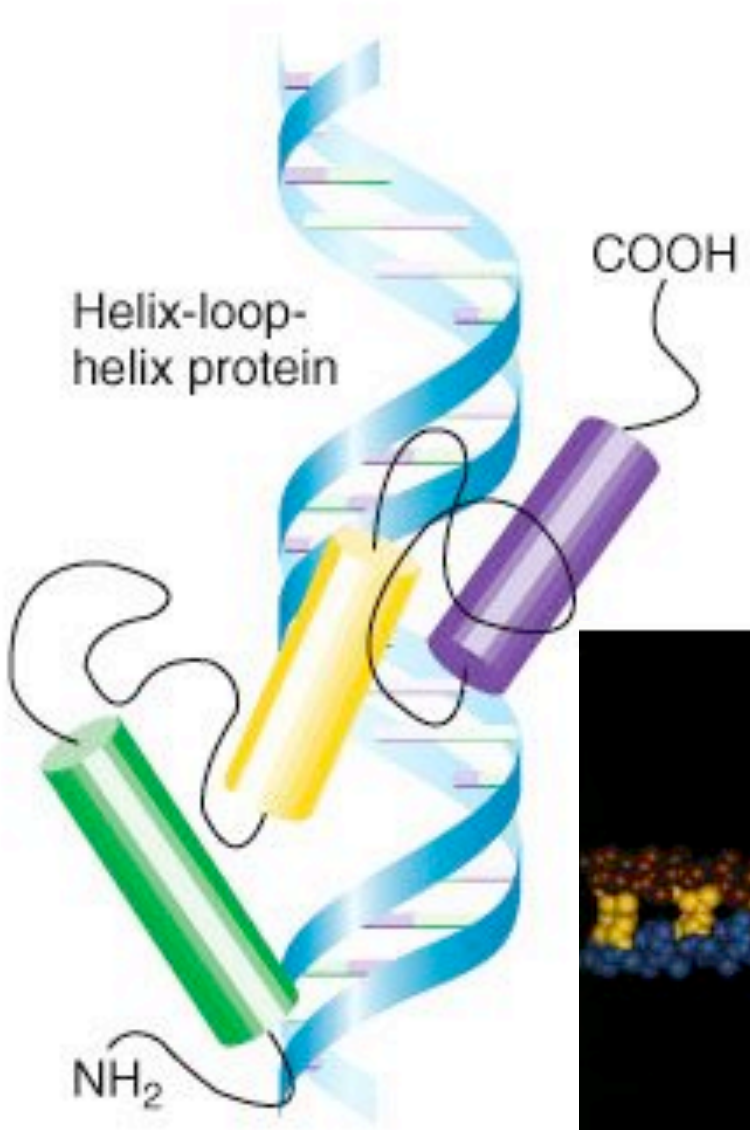
Transcription Factors

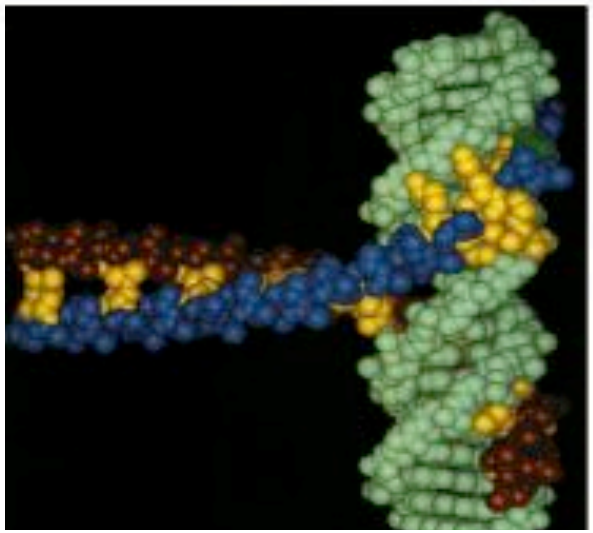


- Transcriptional activators bind to specific promoters and enhancers at specific times to increase transcriptional levels

Fig. 17.5 a

Examples of common transcription factors





- zinc-finger proteins
- helix-loop-helix proteins
- bind to promoter and enhancer DNA
- through their DNA-binding domains

Some proteins affect transcription without binding to DNA

- Coactivator –
  - binds to and affects activator protein which binds to DNA
  - Does not itself bind to DNA

- Corepressors
  - binds to and affects silencer/repressor protein which binds to DNA
  - Does not itself bind to DNA

Localization of activator domains using recombinant DNA constructs

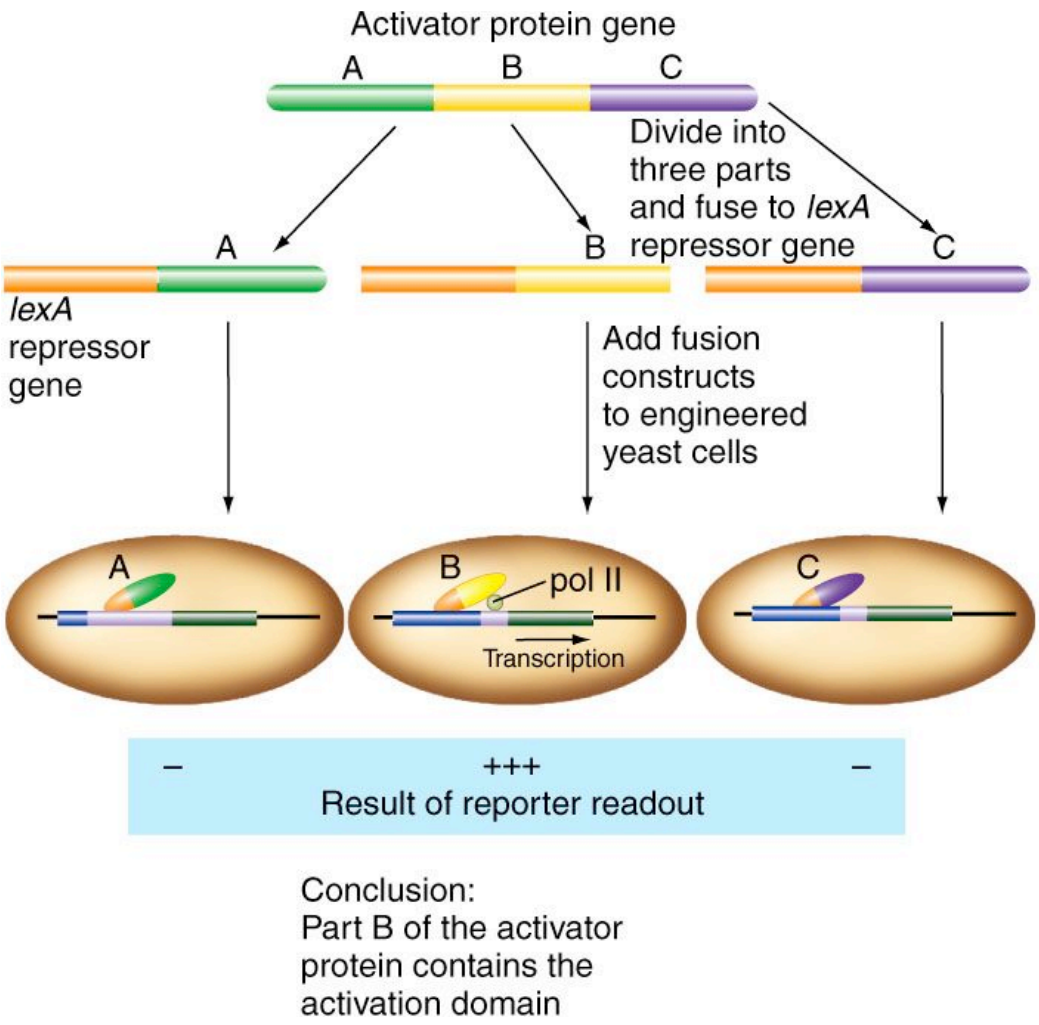


Fig. 17.6

- Fusion constructs from three parts of gene encoding an activator protein
- Reporter gene can only be transcribed if activator domain is present in the fusion construct
- Part B contains activation domain, but not part A or C

Most eukaryotic activators must form dimers to function

- Eukaryotic transcription factor protein structure
  - Homomers – multimeric proteins composed of identical subunits
  - Heteromers – multimeric proteins composed of nonidentical subunits

Fig. 17.7 a

Repressors diminish transcriptional activity

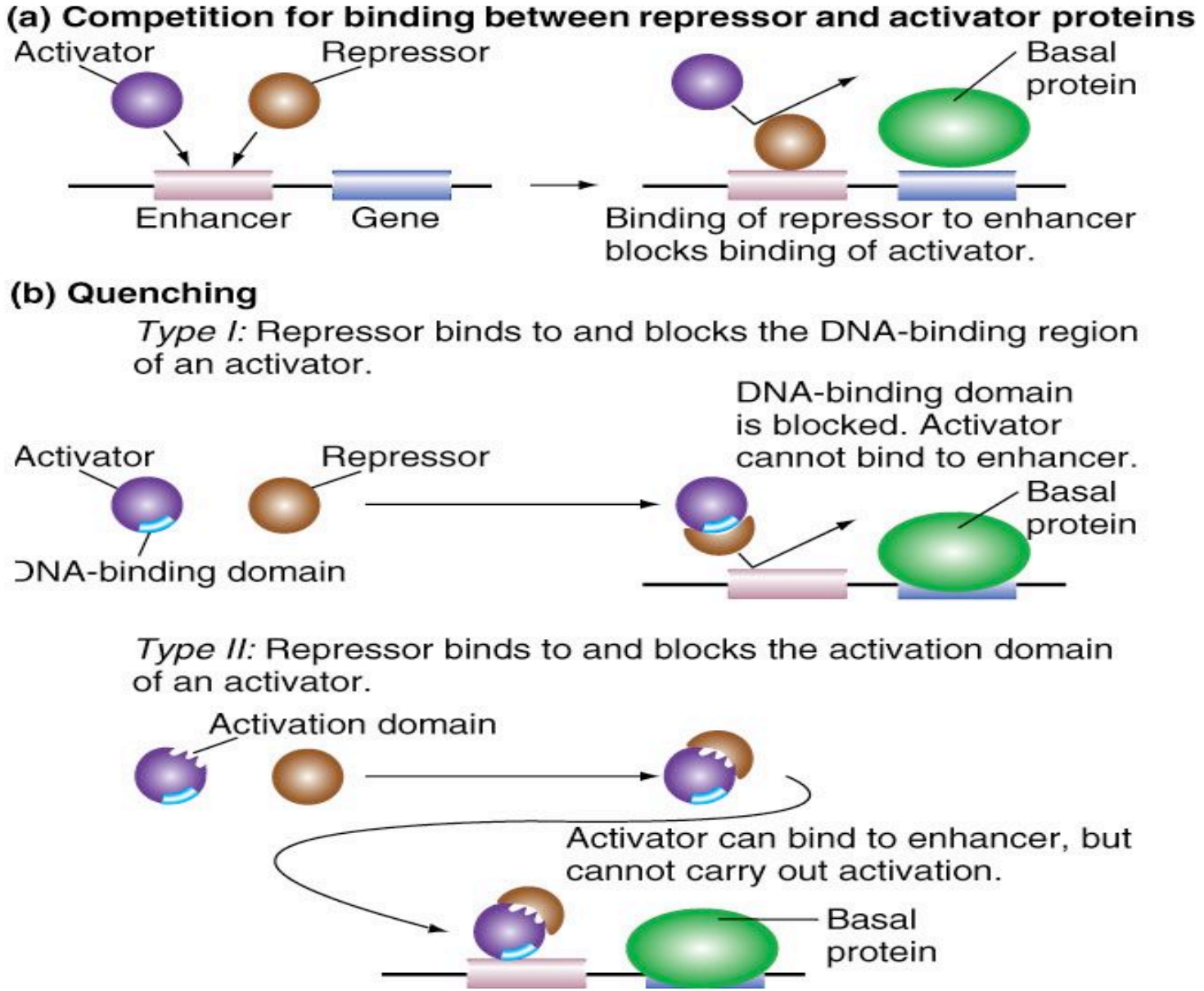
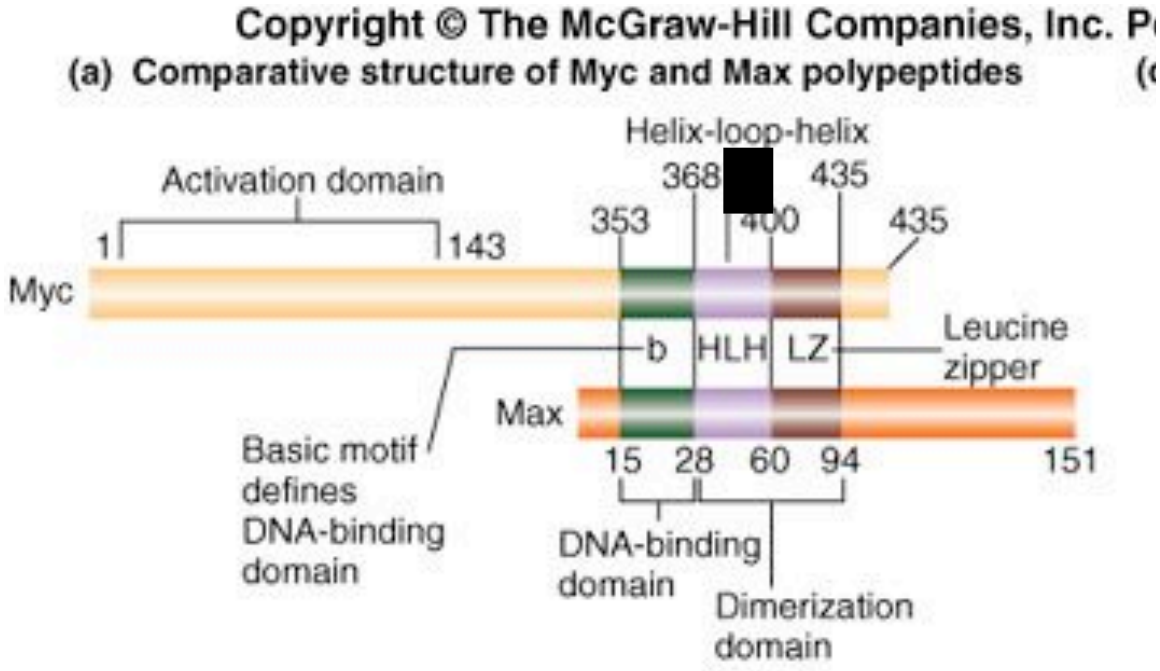


Fig. 17.8

Myc-Max system is a regulatory mechanism for switching between activation and repression



- Myc polypeptide has an activation domain
- Max polypeptide does not have an activation domain

Fig. 17.10

Myc-Max system is a regulatory mechanism for switching between activation and repression

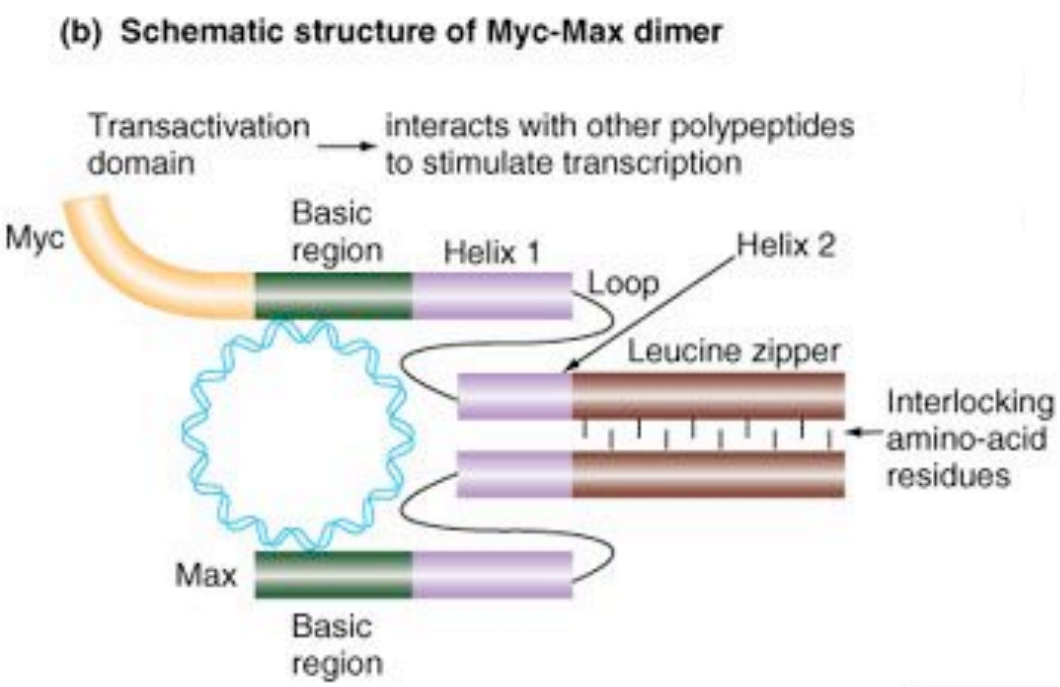


Fig. 17.10

- Myc cannot form homodimers or bind DNA, but has transactivation domain
- Max homodimers can bind DNA, but cannot transactivate (has no transactivation domain)
- Only Myc-Max heterodimer can bind DNA and transactivate

Gene Repression results when only the Max polypeptide is made in the cell

- Gene Activation occurs when both Myc and Max are made in the cell
  - Max prefers Myc as a partner
  - Always heterodimerizes if possible

- Gene Repression results when only the Max polypeptide is made in the cell
  - Only homodimerizes when there is no myc available

Gene Repression results when only the Max polypeptide is made in the cell

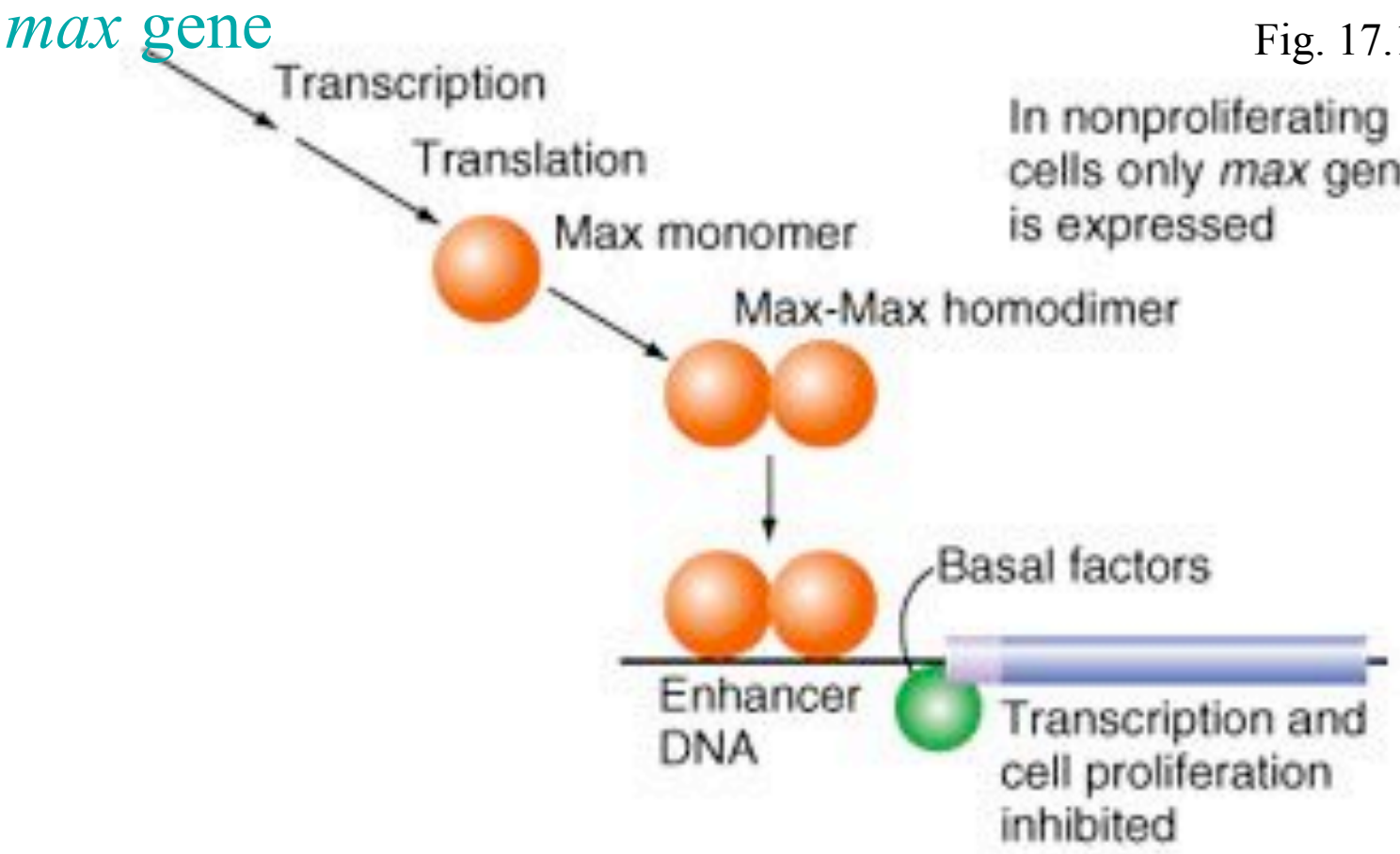


Fig. 17.10 b

Gene activation occurs when both Myc and Max are made in cell

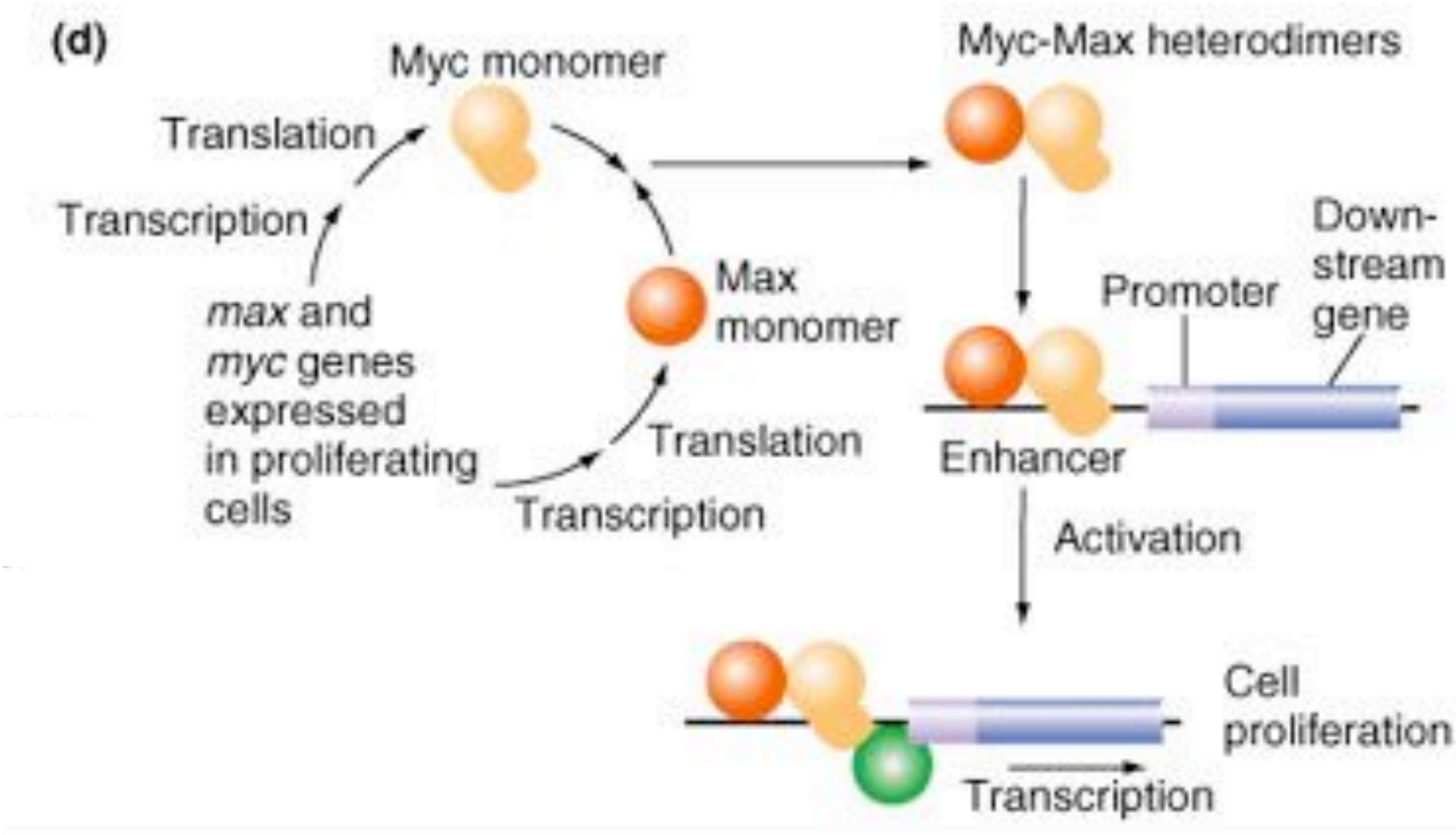


Fig. 17.10

Role of Chromatin in Gene Regulation

- Two broad classes of chromatin:
  - **Euchromatin**: Majority chromatin is in its extended (decondensed) state during interphase, only condenses during mitosis.
  - **Heterochromatin**: Remains highly condensed even in interphase. Accounts for the dark staining regions seen in interphase chromatin. Heterochromatin is further classified as:
    - **Constitutive**: always inactive and condensed: e.g. repetitive DNA, centromeric DNA
    - **Facultative**: can exist in both forms. E.g.: Female X chromosome in mammals.

Epigenetic effects on gene regulation

- Barr bodies:
  - example of heterchromatin decreasing gene activity

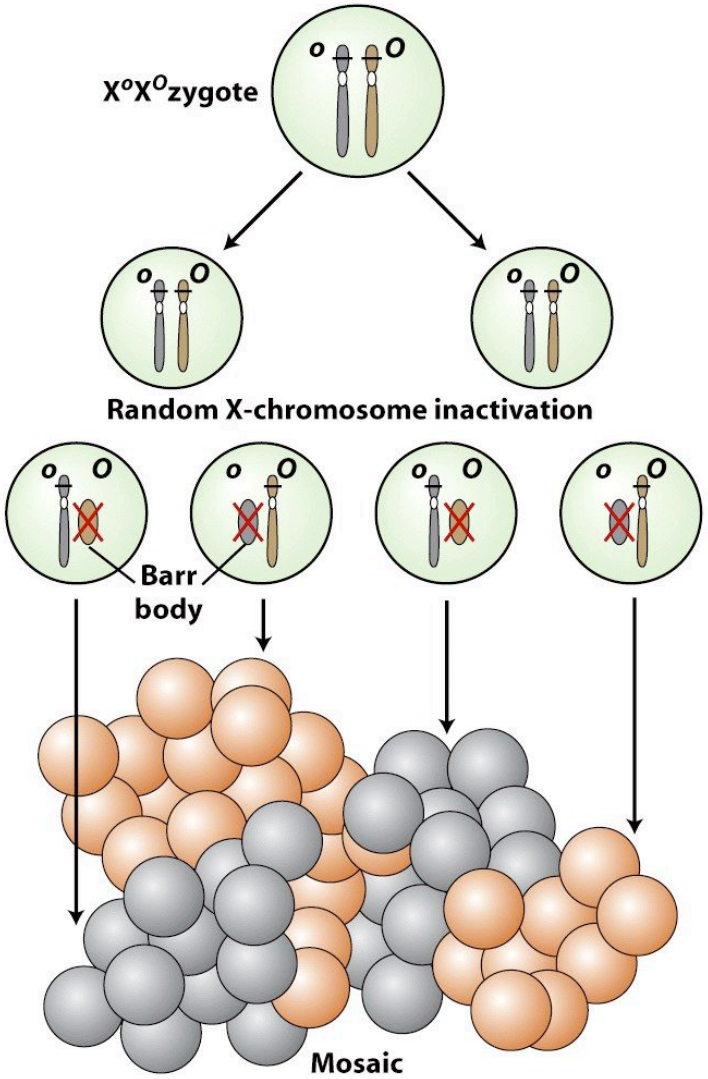
- Barr bodies = X Inactivation

- inactivation of one X chromosome to control for dosage compensation in female mammals
  - One X chromosome appears in interphase cells as a darkly stained heterochromatin mass
  - Most of the genes are turned off on the barr body
  - Random inactivation of one of the X chromosomes early in development.
  - Not the same X in all cells

X Inactivation Example

- Calico cats
- Fur color pattern
- Heterozygous for fur color Oo on X chromosomes
  - O = orange
  - o = black
  - White is caused by another gene present in calicos
- Cells where the O allele chromosome is inactivated produce black pigment
- Cells where the o allele chromosome is inactivated produce orange pigment

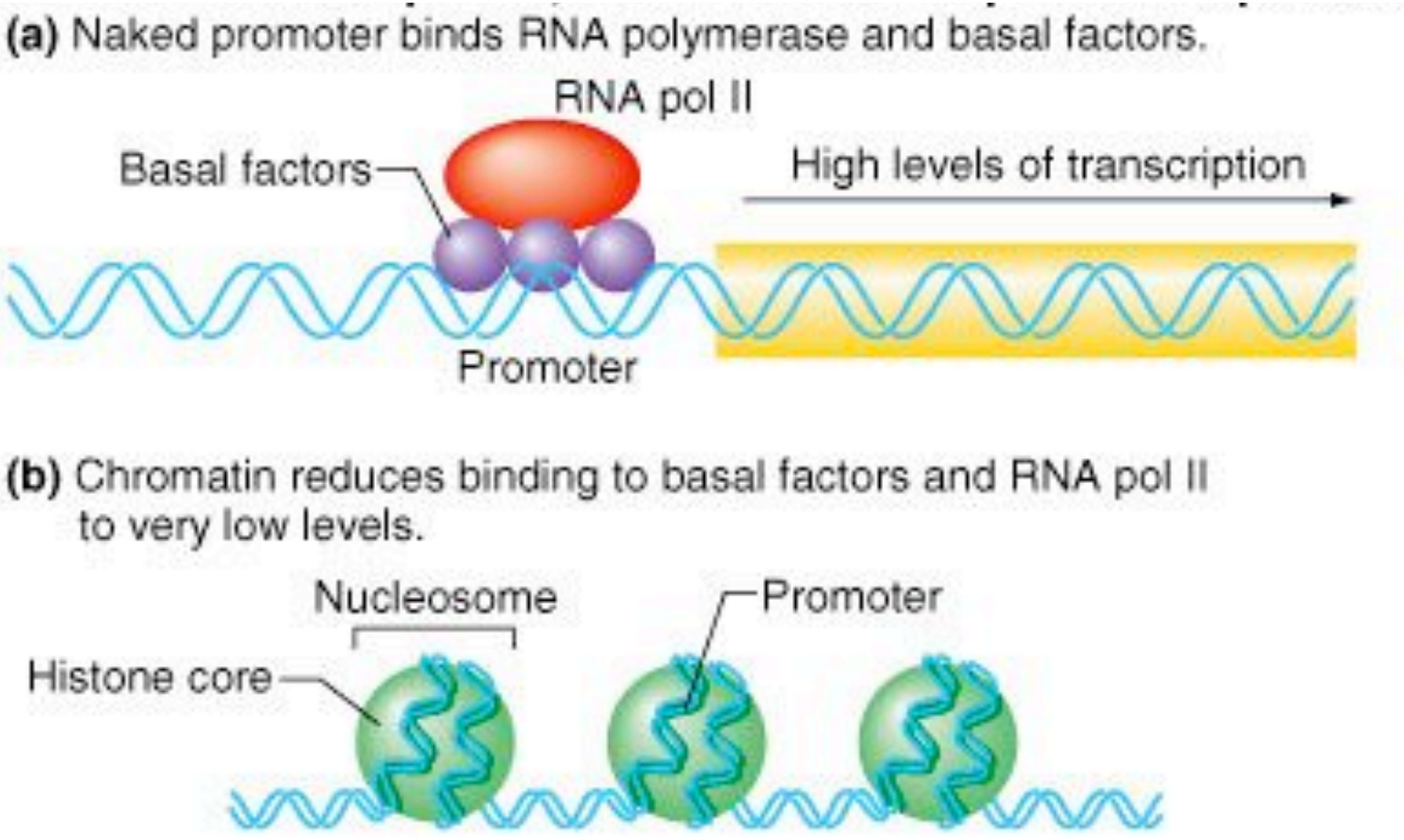
X Inactivation Example



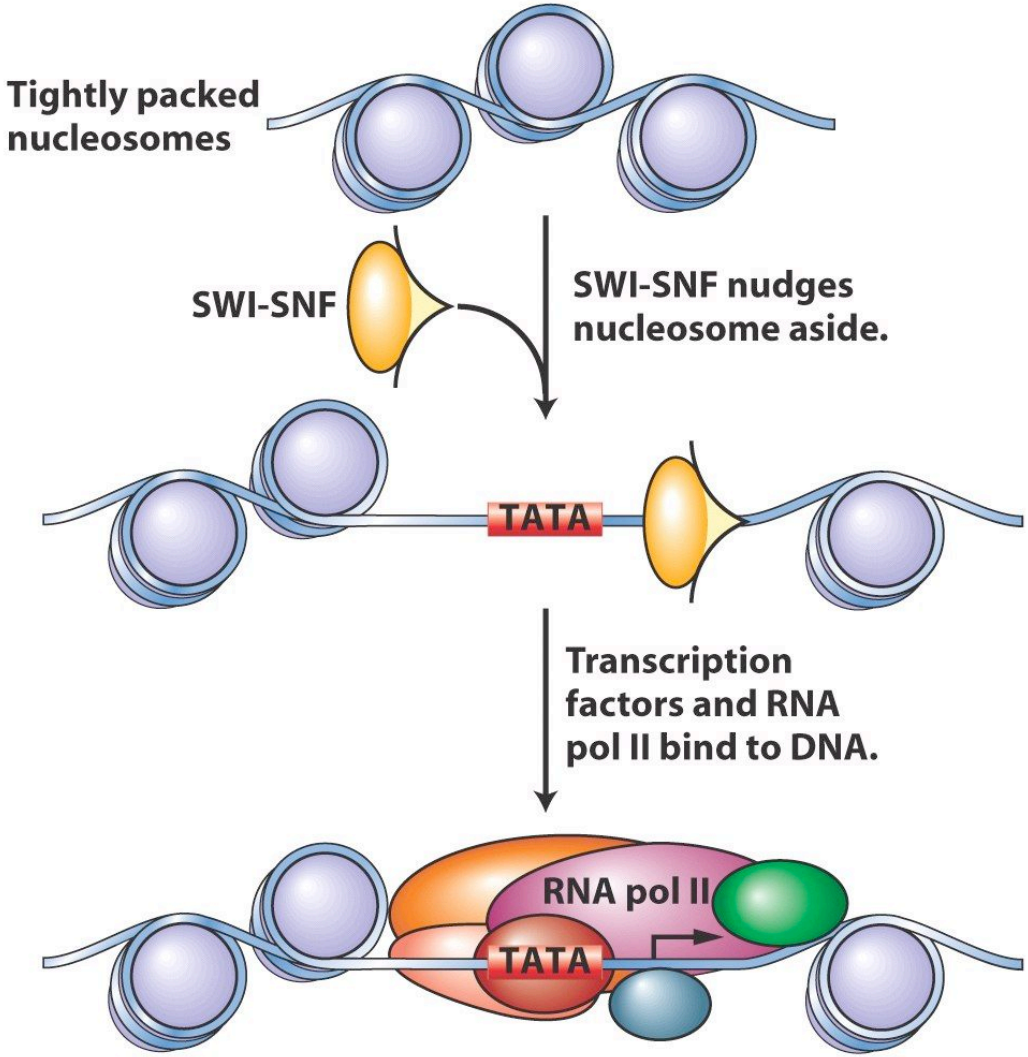
How chromosomal packaging influences gene activity

- Decompaction precedes gene expression
  - Boundary elements delimit areas of decompaction
  - Nucleosomes in the decompacted area unwind to allow initiation of transcription
    - Transcription factors (nonhistone proteins) unwind nucleosomes and dislodge histones at 5’ end of genes
    - Unwound portion is open to interaction with RNA polymerase which can recognize promotor and initiate gene expression

Normal chromatin structure slows transcription



Remodeling of chromatin mediates the activation of transcription



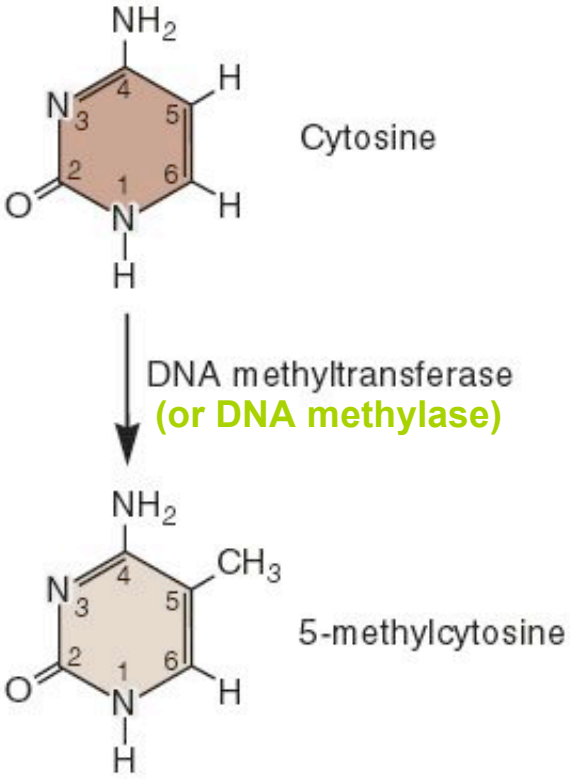
Epigenetic effects on gene regulation

- Chemical modifications of DNA
- Does NOT change base sequence - NOT a mutation
- Usually methylation of Cytosine in CG sequences

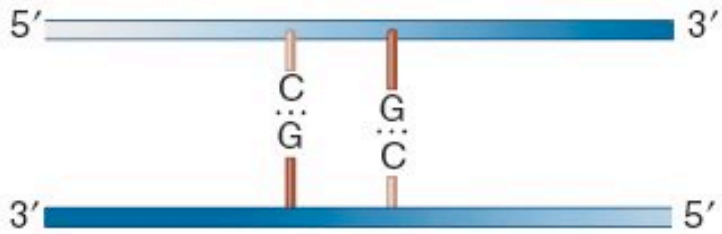
- Example: Extreme condensation silences expression
- Heterochromatin
  - Highly compacted even during interphase
  - Usually found in regions near centromere
  - Constitutive heterochromatin remains condensed most of time in all cells (e.g., Y chromosomes in flies and humans)

- Remember - Euchromatin
  - Contains most genes
  - Active regions

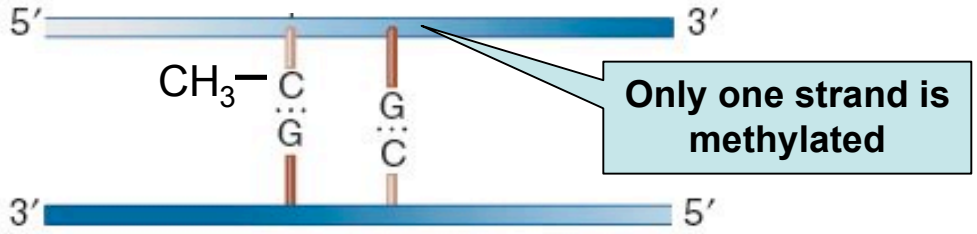
Epigenetic Effect: Methylation



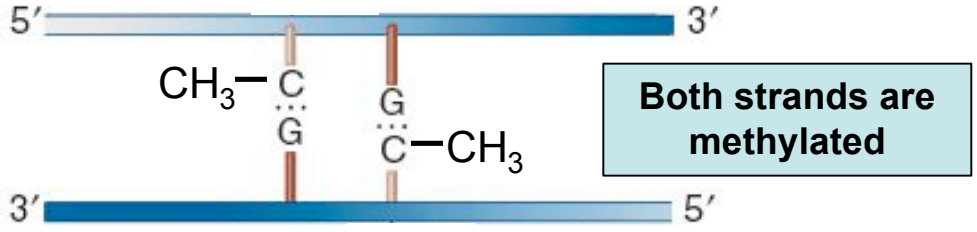
(a) The methylation of cytosine



(b) Unmethylated



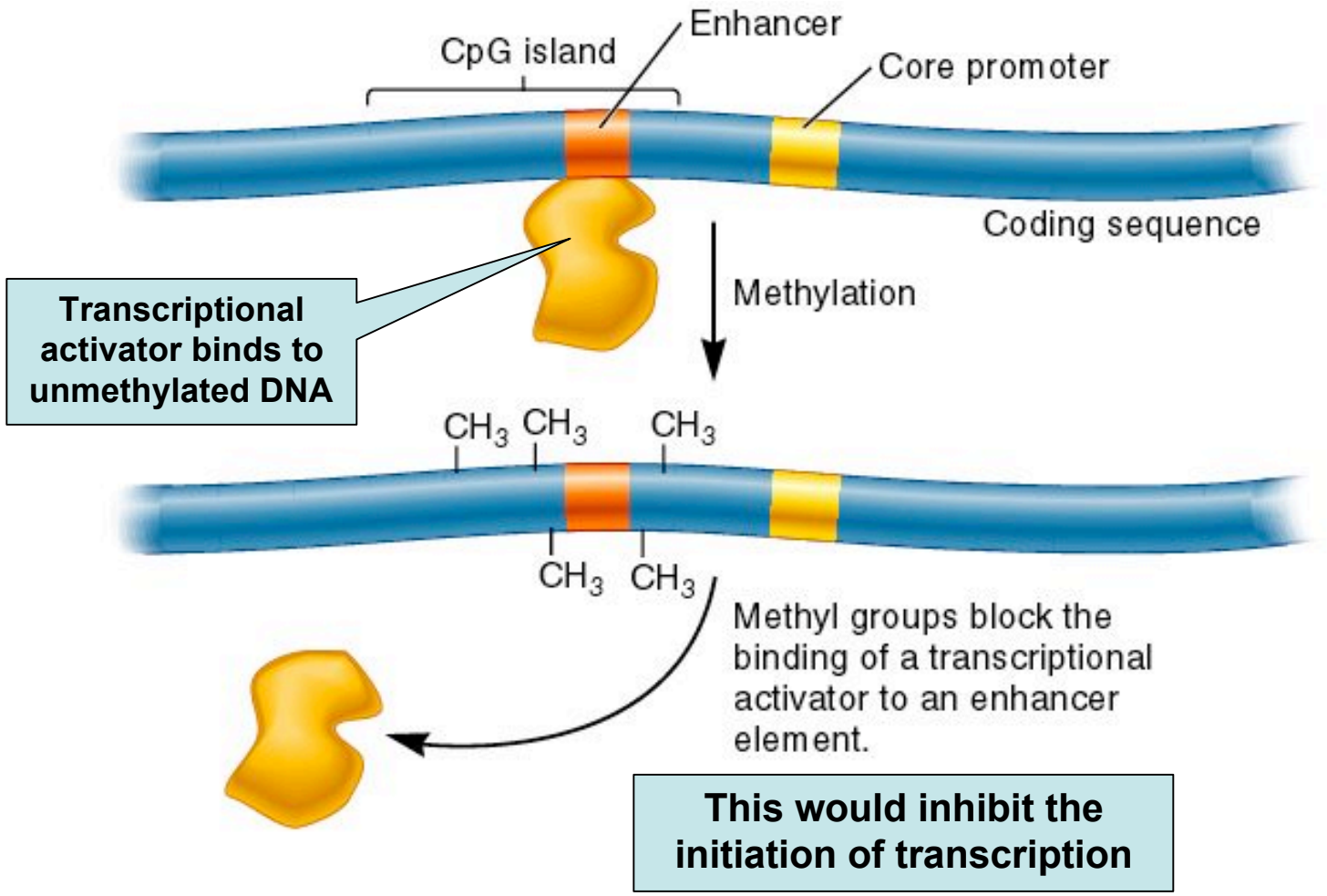
(c) Hemimethylated



(d) Fully methylated

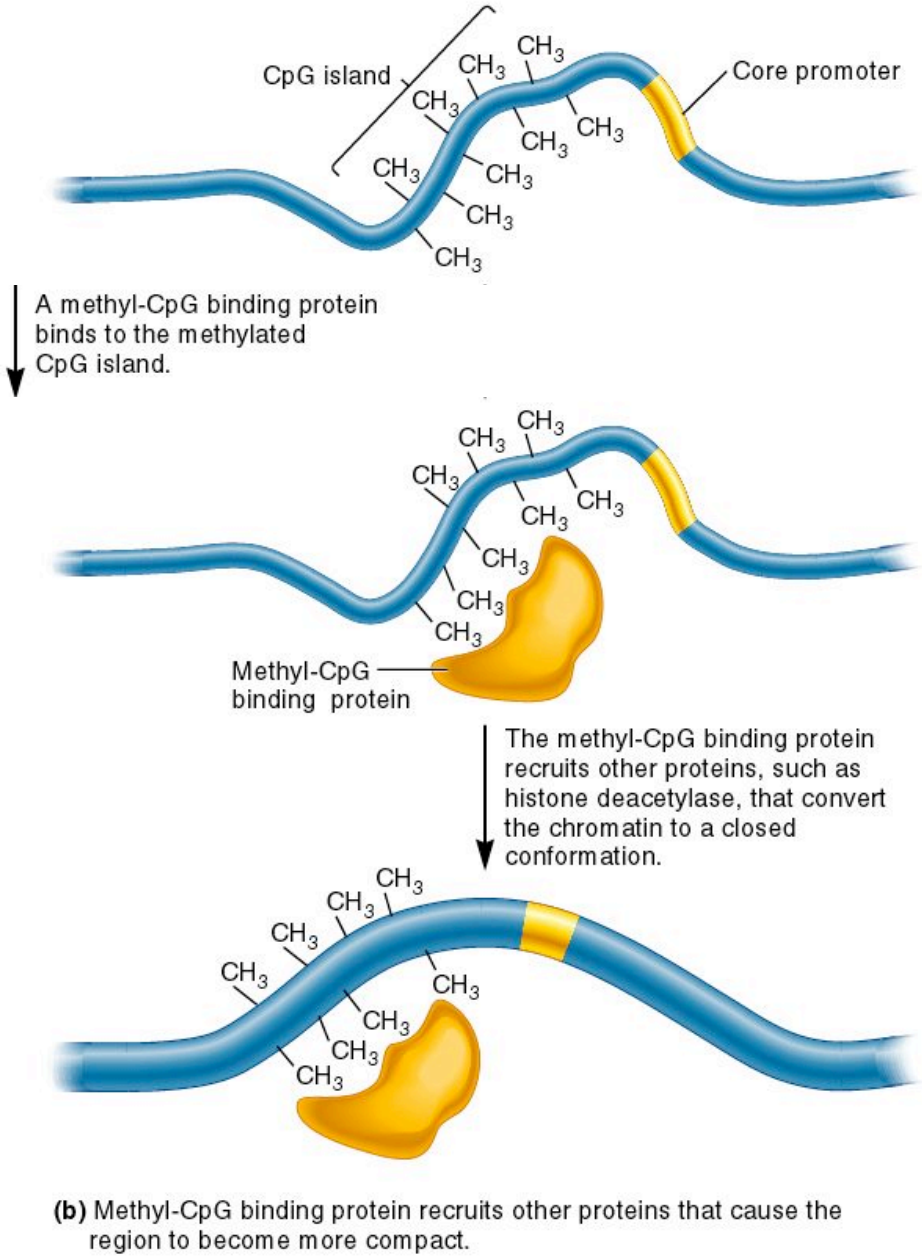
- DNA methylation usually inhibits the transcription of eukaryotic genes
  - Especially when it occurs in the vicinity of the promoter
- In vertebrates and plants, many genes contain CpG islands near their promoters
  - These are area in DNA where there are lots of CG repeats
  - 1,000 to 2,000 nucleotides long
  - In housekeeping genes
    - The CpG islands are unmethylated
    - Genes tend to be expressed in most cell types
  - In tissue-specific genes
    - The expression of these genes may be silenced by the methylation of CpG islands

Transcriptional silencing via methylation: Blocking transcription factor binding



(a) Methylation inhibits the binding of transcriptional activators.

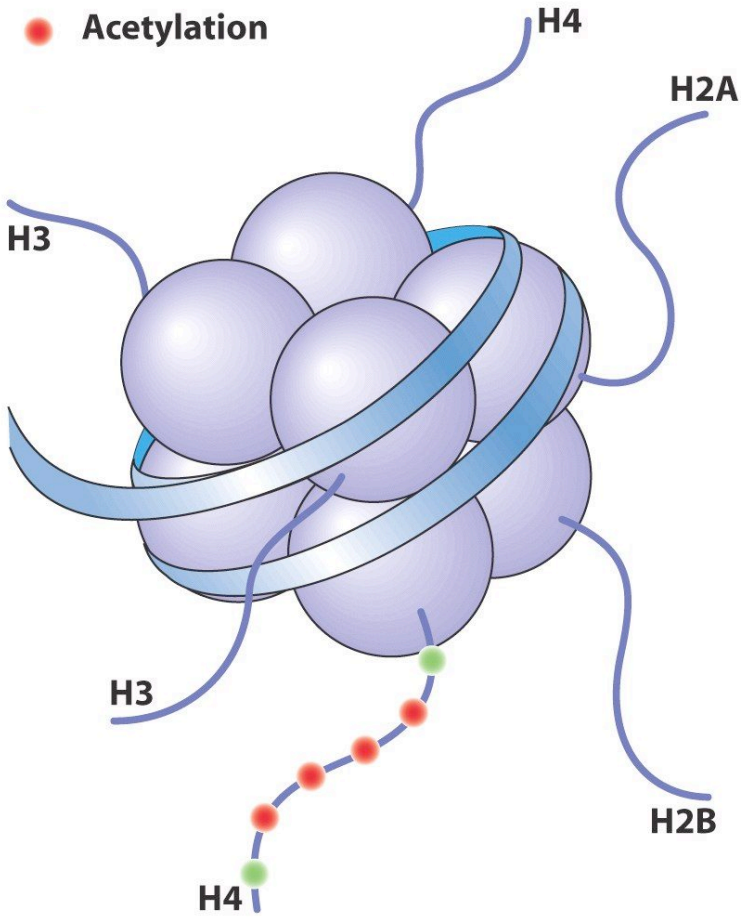
Transcriptional silencing via methylation: Inducing heterochromatin



(b) Methyl-CpG binding protein recruits other proteins that cause the region to become more compact.

Epigenetic effects on gene regulation

- Histone Code is modification of histone tails by acetylation
- Remember:
  - the nucleosome is an octet of histone proteins



Epigenetic effects on gene regulation

- Histone Acetylation = Gene Activation
  - Acetyl groups added to histone tails
- Hyperacetylation = Gene Activation
- Hypoacetylation = Gene Silencing
- Remember:
- DNA methylation = Gene Silencing

Homework Problems

Chapter 20

6, 14,