Lecture 9

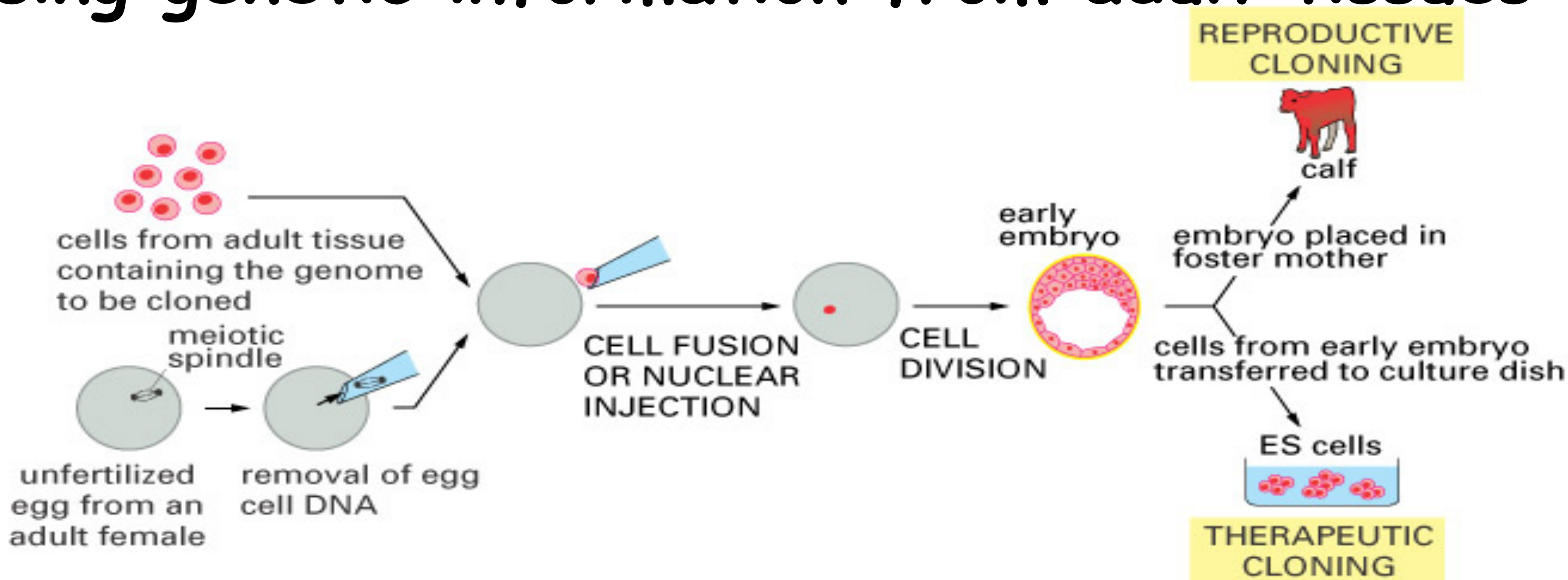
Regulation of Eukaryotic Transcription

April 26, 2016
Pyle

Eukaryotic Gene Regulation

1. Examples of eukaryotic gene regulation
2. Combinatorial and coordinated control
3. Gene regulation and development

Reproductive cloning and therapeutic cloning using genetic information from adult tissues



The cloning of animals demonstrates that nuclei retain a complete complement of genetic information. But how is this regulated to make every cell type in the body?

-Recent work- Human ES cells derived from a cloned embryo (SCNT) containing the DNA from a 32-year-old woman with type 1 diabetes. The researchers also succeeded in differentiating these ES cells into insulin-producing cells..

Why regulate gene expression?

Question:

Almost all cells of an organism are originated from a single cell (zygote -fusion of a sperm and a egg cell), and every cell has the same genome. How do individual cells eventually take unique developmental fates and become different cells, such as blood cells, skin cells, muscle cells, etc?

Short answer:

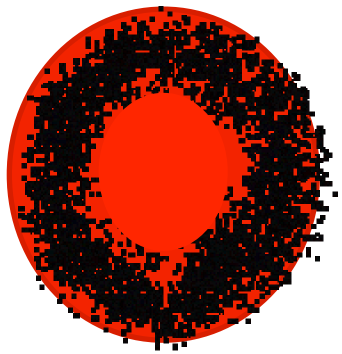
Different cells express different sets of genes, so they have different set of proteins.

New question:

Why are different genes expressed in different ways? What determines when a gene should be expressed?

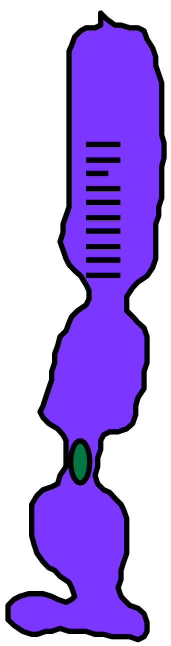
Short Answer: Control of gene expression (combinatorial and coordinated)

Remember...Specialized Cells
Make Specialized Proteins!

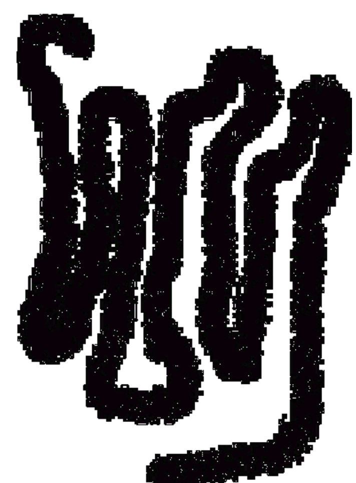


Red Blood Cell

Hemoglobin
Carries Oxygen



Retinal Rod Cell



Rhodopsin
Absorbs Light

Overview of levels of control of gene expression

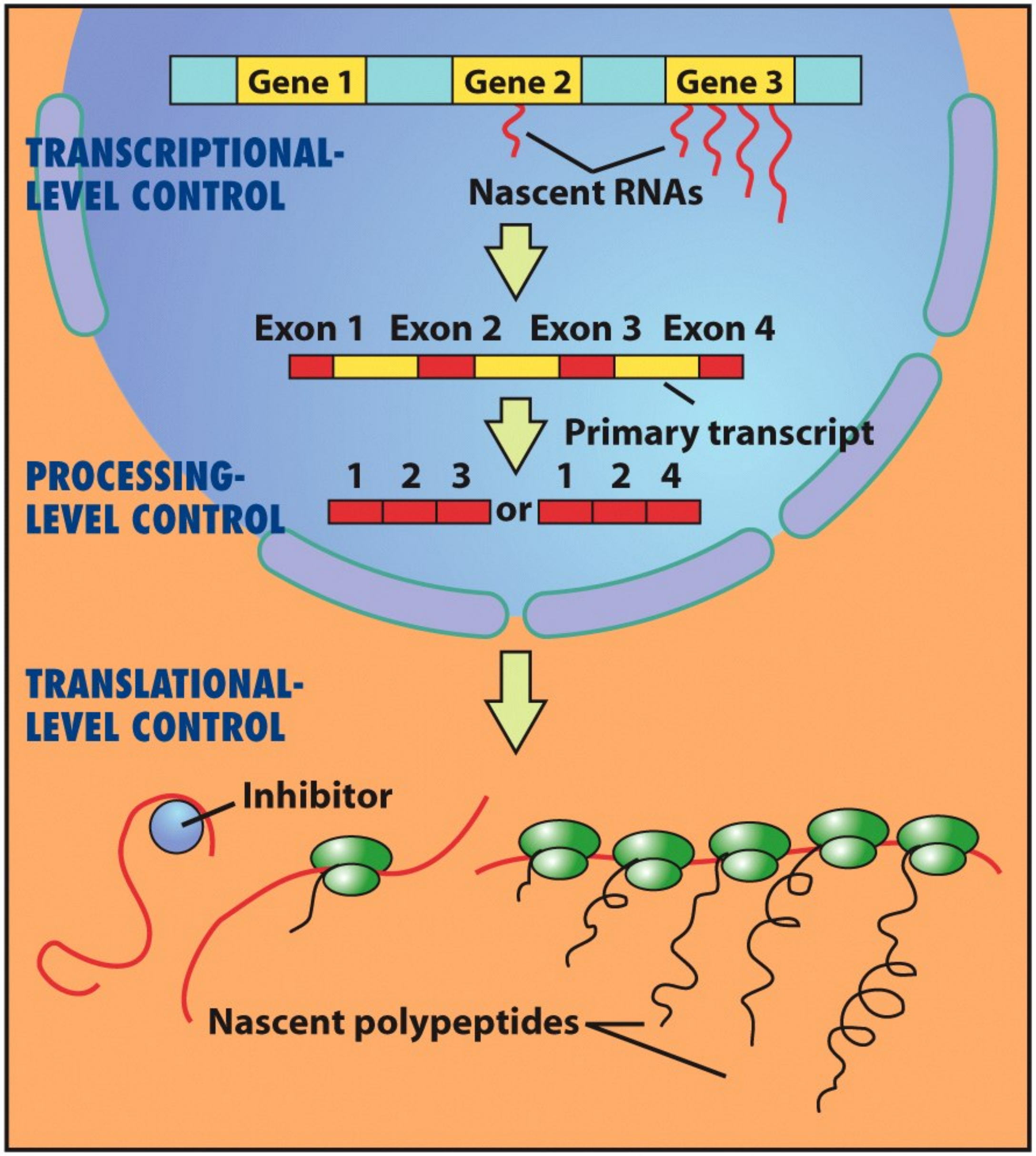
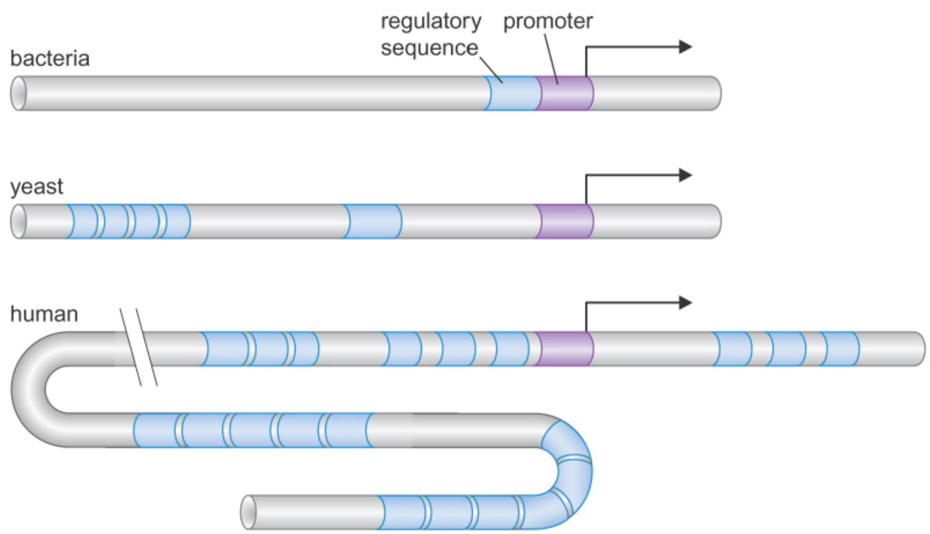
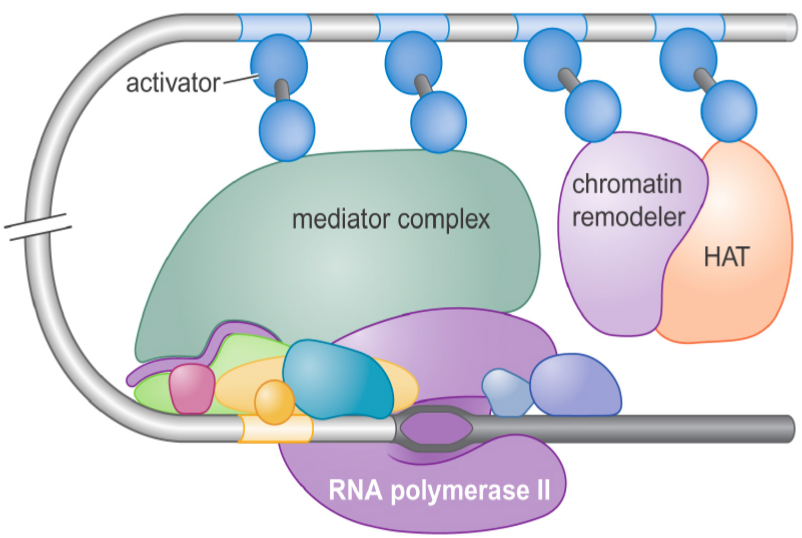
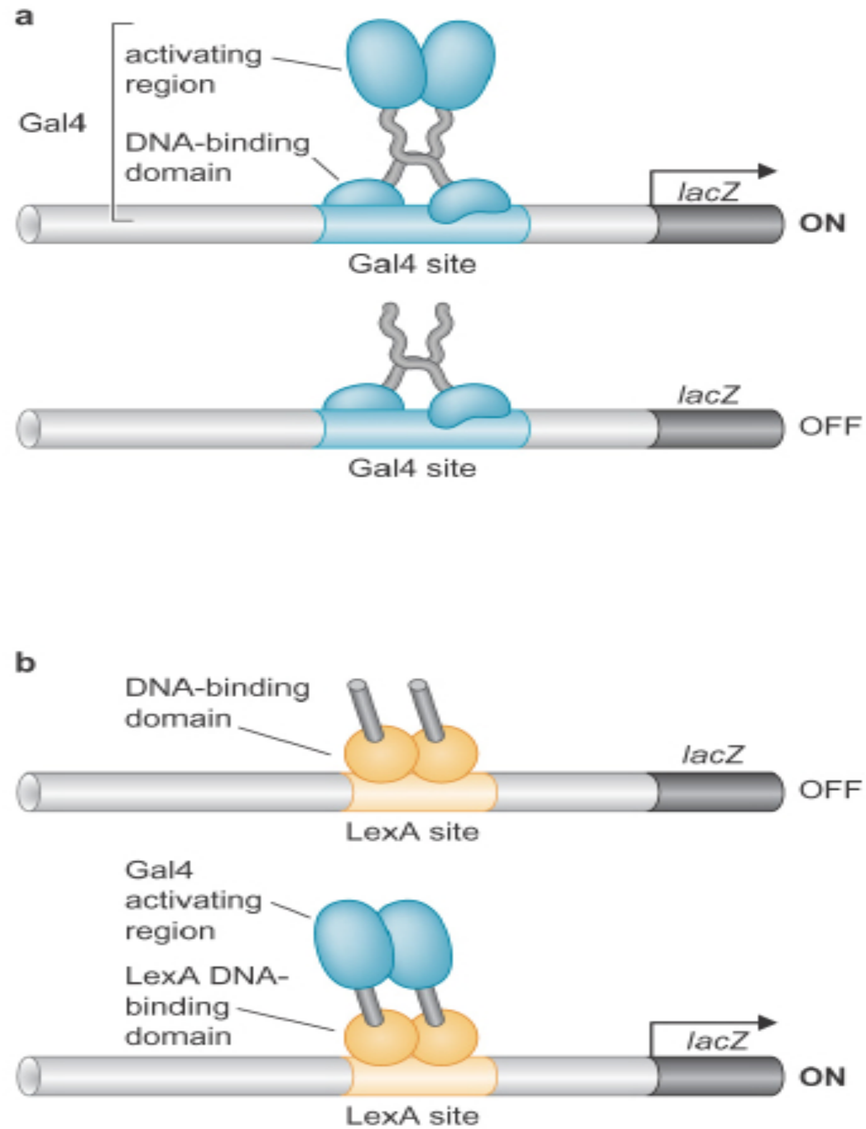


Figure 12-32 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Cis and trans-acting factors involved in the regulation eukaryotic gene transcription







Eukaryotic DNA is packed into chromosomes that consist of both DNA and protein

DNA

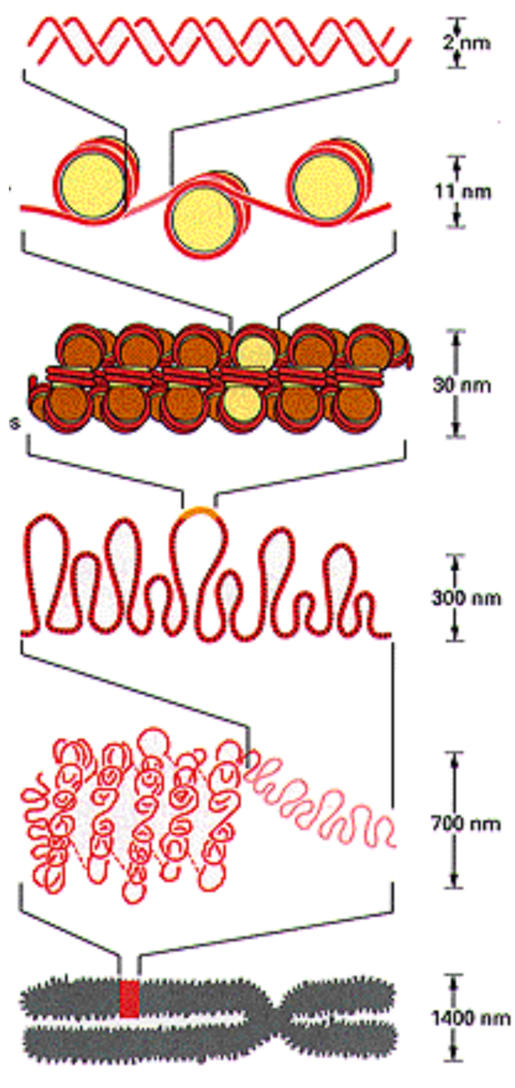
Nucleosomes

30 nm fiber

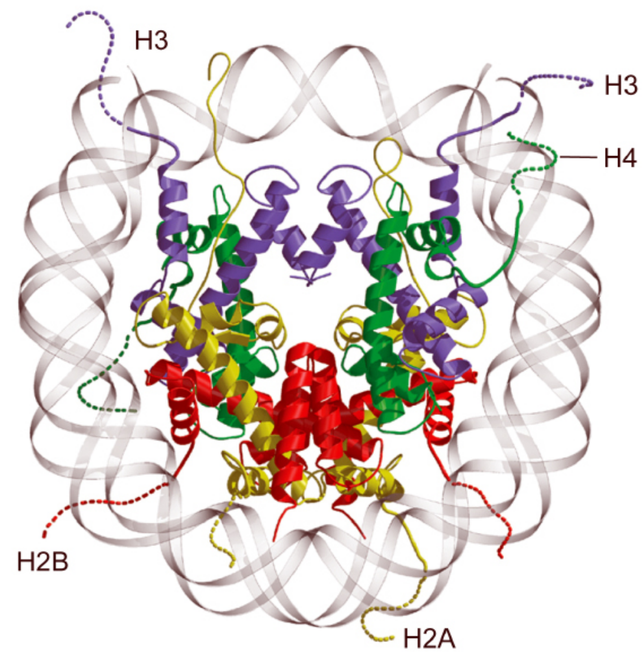
interphase chromosome

metaphase chromosome

entire chromosome

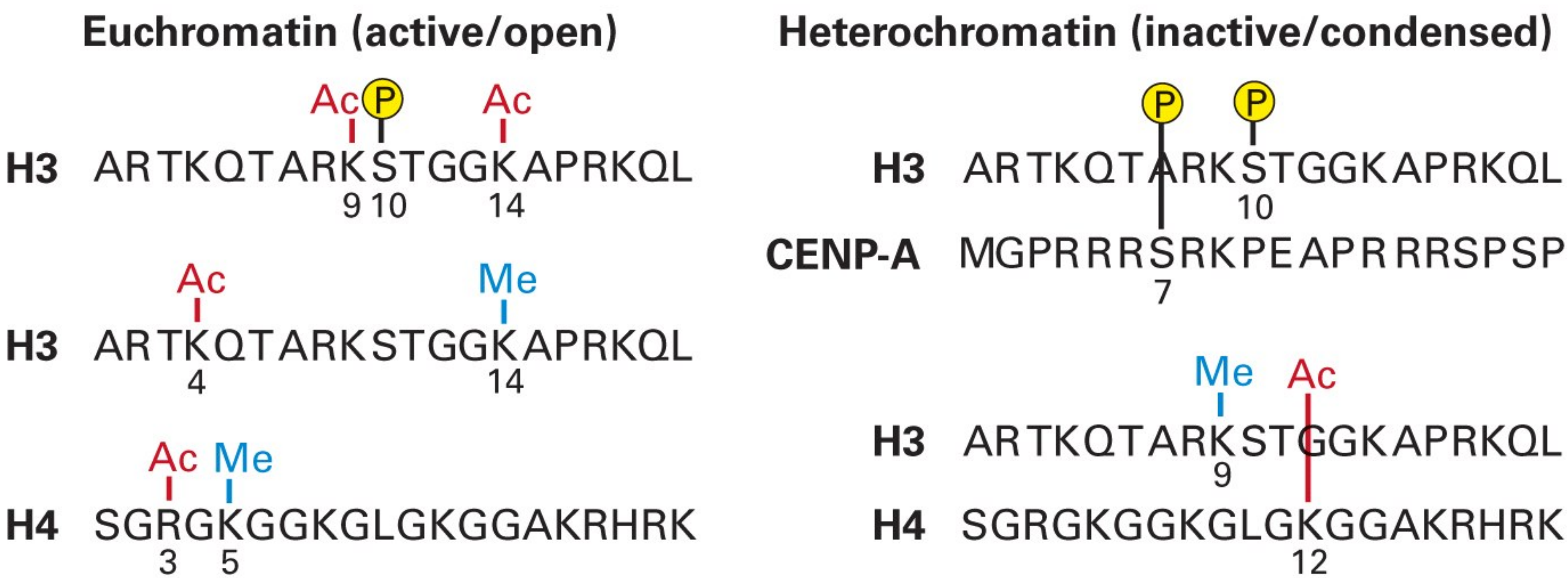


Nucleosome as a structural unit contains core histone complex (2 each of histone H2A, H2B, H3 and H4) and 150-200 bp DNA



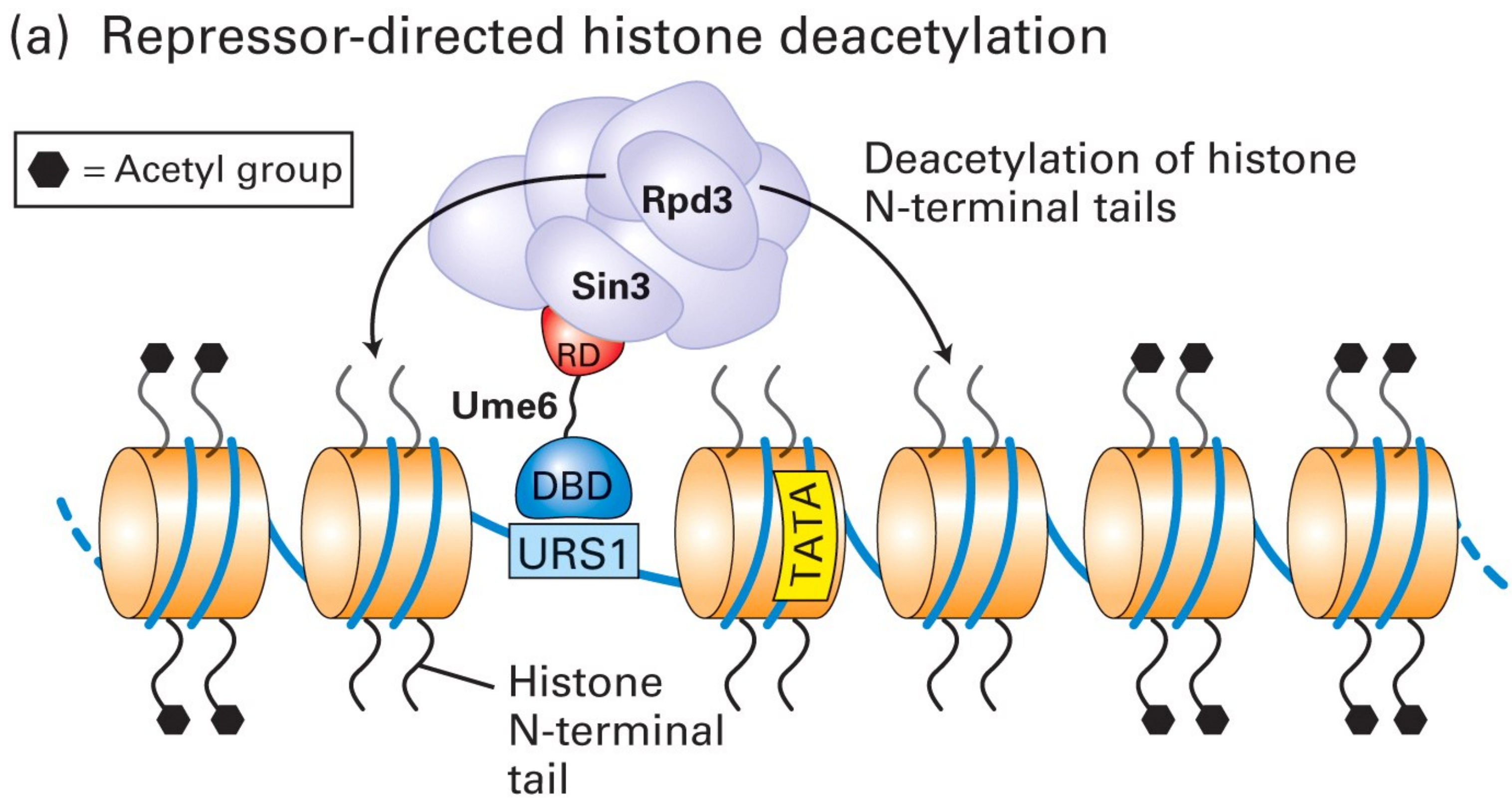
Modifications of histone tails determine chromatin structure and transcription activity

Histones in euchromatin (active) are highly acetylated (hyperacetylation), whereas histones of heterochromatin (inactive) are rarely acetylated (hypoacetylation)



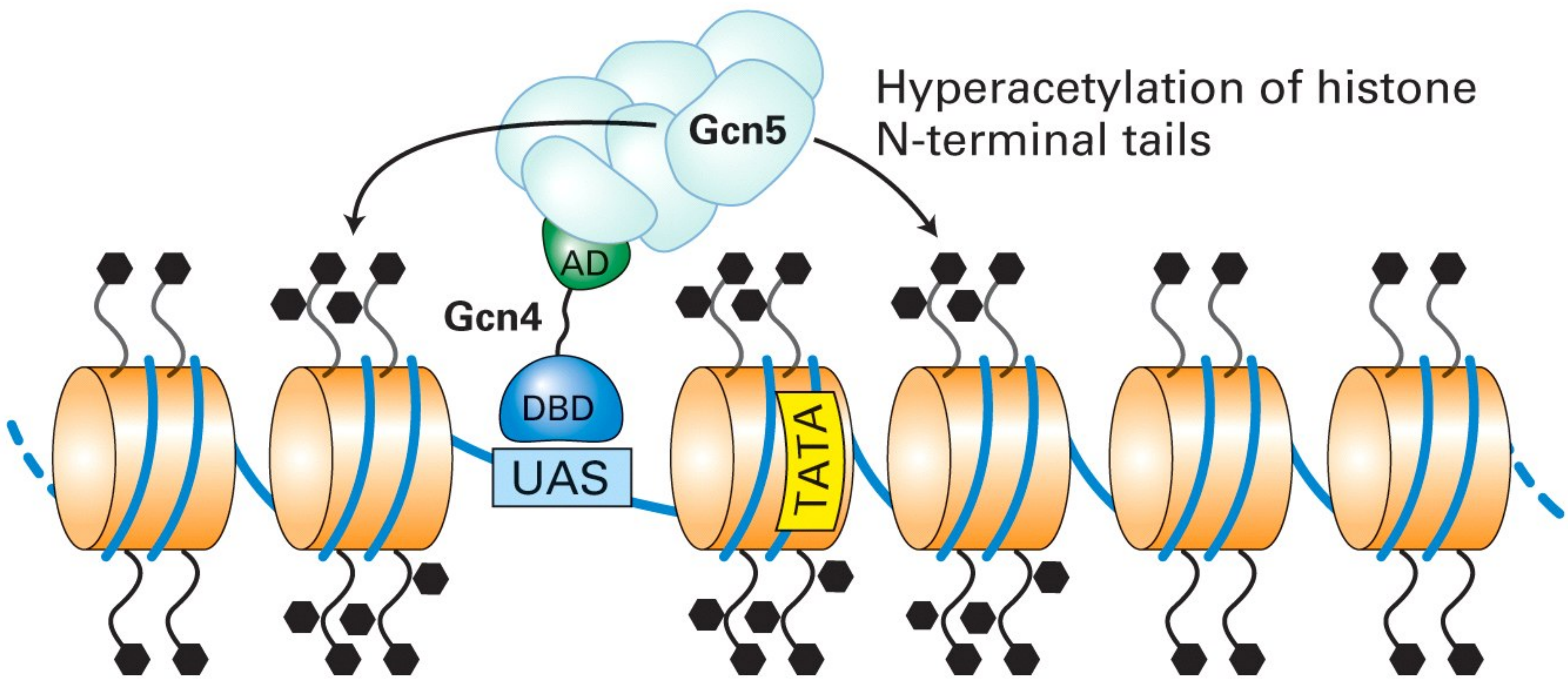
1. How do transcription factors regulate transcription?

(1) Transcription repressors (e.g. Ume6) binds to URS (upstream repression sequence) to recruit histone deacetylase (e.g. Rpd3) to remove acetyl group from histone tail, resulting in inhibition of transcription

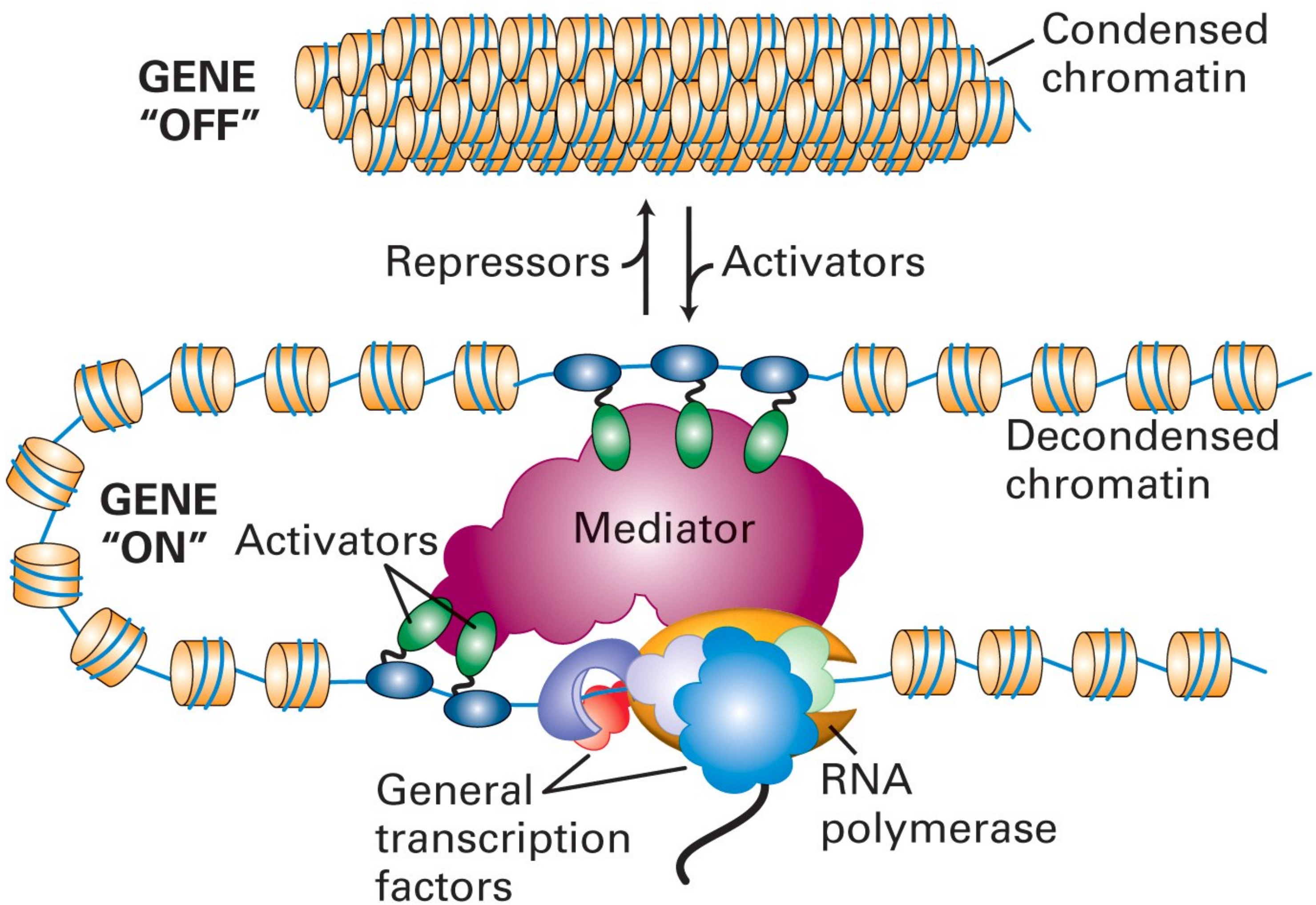


(2) Transcription activator (e.g. Gcn4) binds to UAS (upstream activation sequence) to recruit histone acetyltransferase (e.g. Gcn5). Histone acetyltransferases add acetyl group to histone tail, resulting in activation of transcription

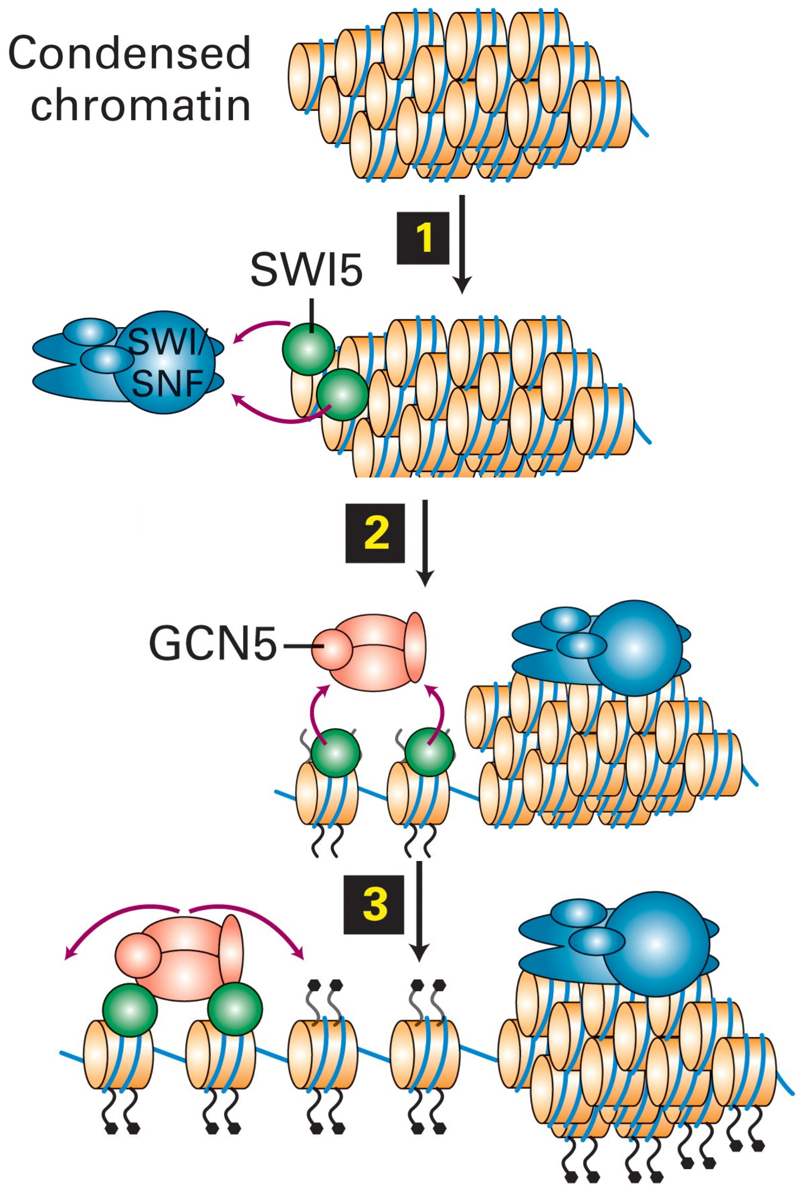
(b) Activator-directed histone hyperacetylation



(3) Transcription factors can recruit co-activator or mediators, which are protein complexes that do not bind DNA but can bind and activate RNA polymerase

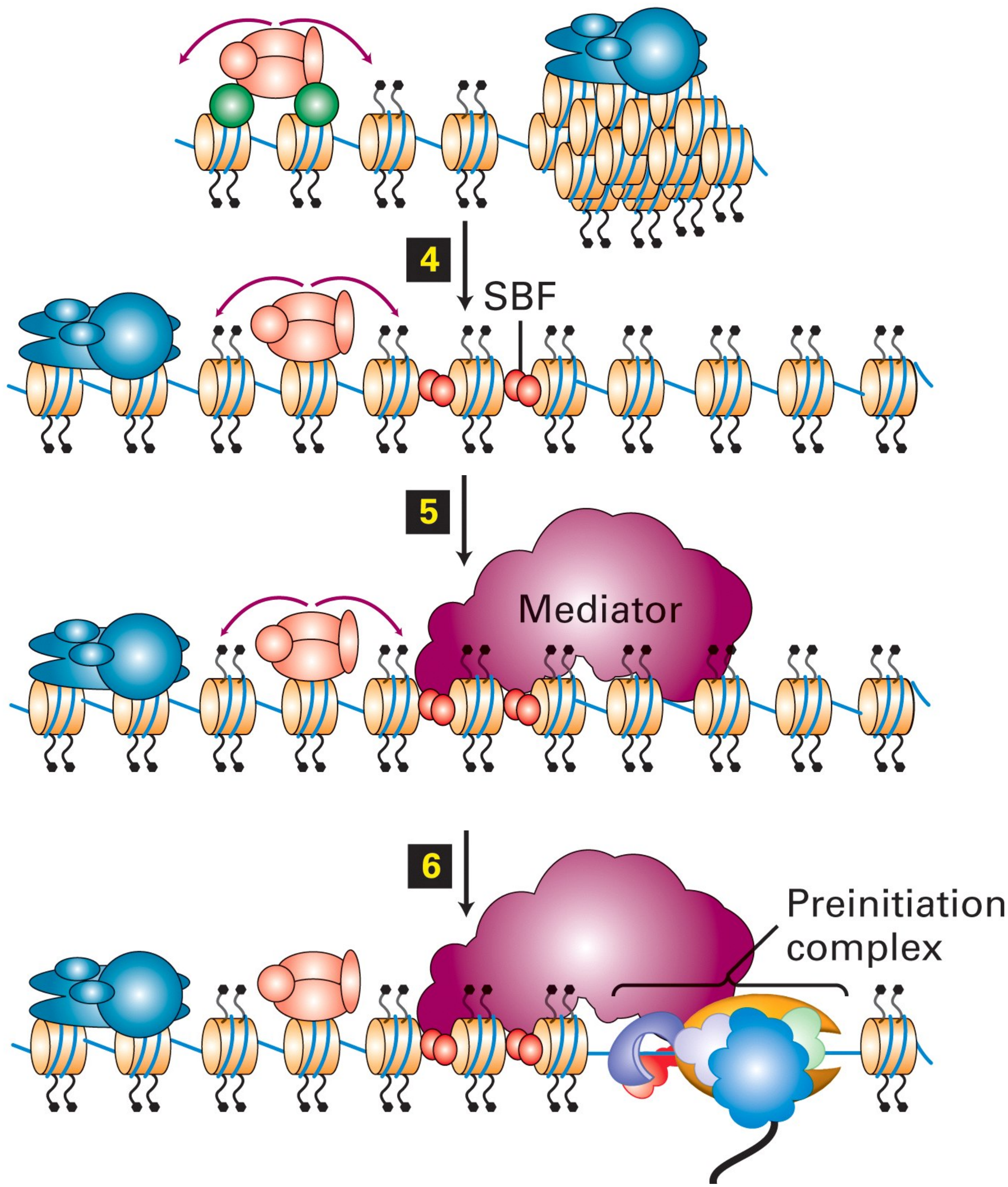


An example of how transcription factors work together to regulate transcription



1-2. The transcription factor SWI5 bind to enhancers. It recruits DNA helicase (e.g. SWI/SNF) to unwind DNA, de-condense nucleosomes, and expose histone tails for modification

3. After nucleosome decondenses, histone acetyltransferase (Gcn5) moves in to acetylate histone tails



4. Histone aceylations further open up nucleosome, allowing binding of another transcription factor, SBF, to the promoter-proximal sequence

5-6. SBF recruits mediator complex, which activate binding of general transcription factors and RNA polymerase II to the promoter

2. Regulation of transcription factor activity

Transcription factors are DNA-binding proteins. Like any other protein, the activity of a transcription factor can be regulated by multiple mechanisms, such as:

- protein modification (phosphorylation, ubiquitination. etc),
- binding to other proteins
- binding to ligands to trigger changes of cellular localization or protein stability

Hormone Control of Gene Expression: An example of induced gene expression

- a steroid hormone receptor

1. Glucocorticoid is a group of steroid hormone synthesized by the adrenal gland. Glucocorticoid is lipid soluble, it can pass through plasma membranes to travel from one cell to another. Steroid hormone regulates immune system, metabolism, energy, etc etc.

2. Glucocorticoid receptor (**GR**) stays in the cytosol in the absence of the hormone. Binding to glucocorticoid results in the change of conformation of GR and entering of the hormone-receptor complex into the nucleus.

3. The hormone-receptor complex binds to a specific DNA sequence called glucocorticoid response element (**GRE**) located in the promoter of a target gene, resulting in a stimulation of transcription.

Activation of a gene by a steroid hormone (cortisol)

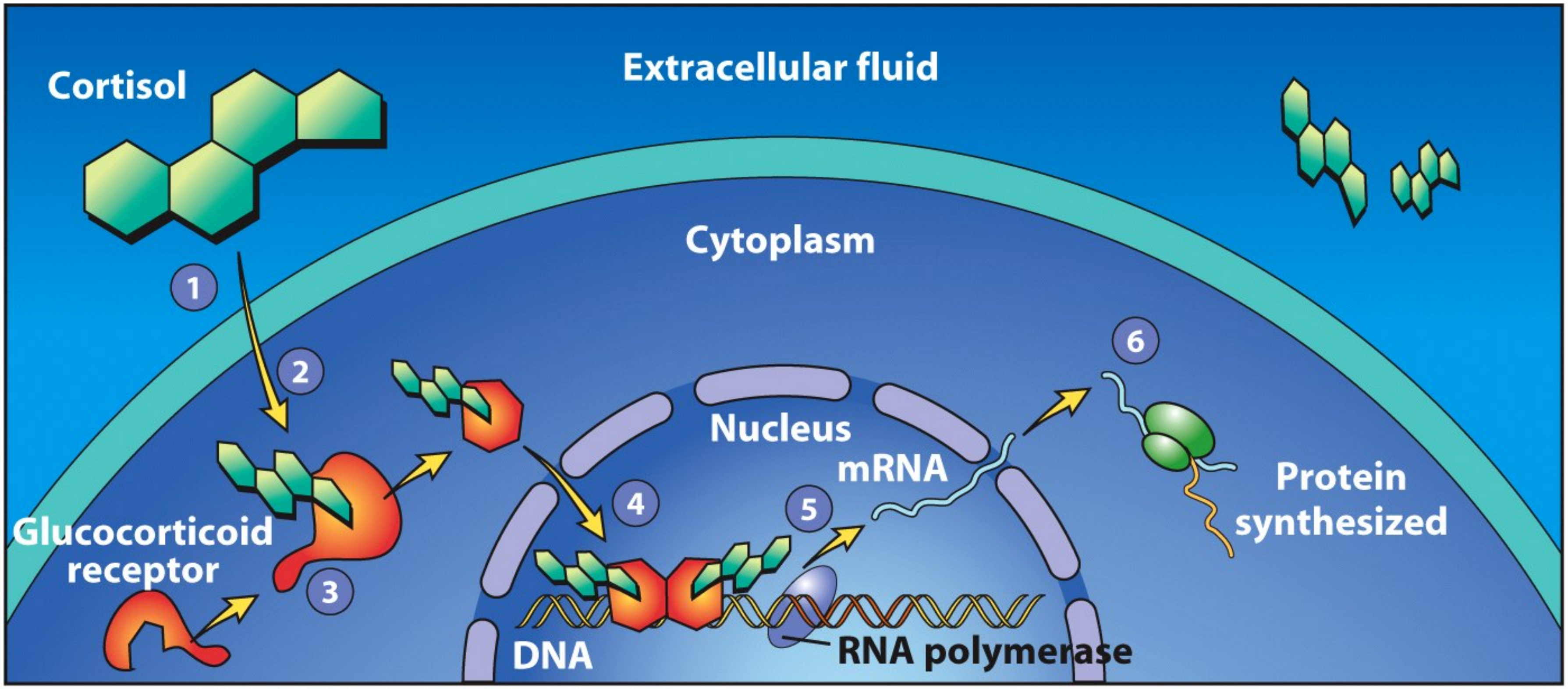
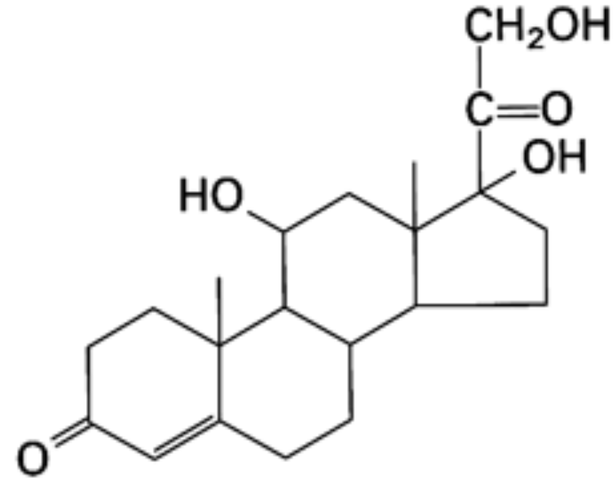


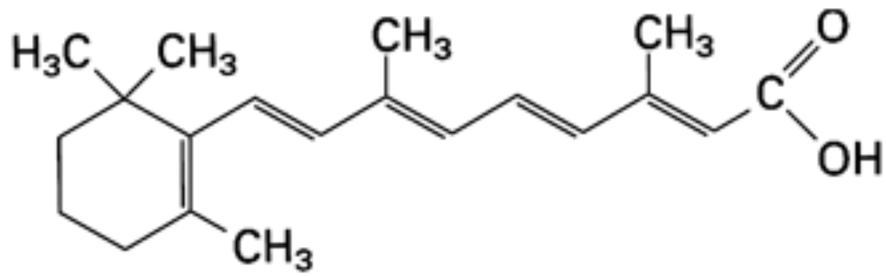
Figure 12-44 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

1. Hormone enters cell, 2. diffuses through the lipid bilayer, 3. binds glucocorticoid receptor, 4. translocates to nucleus and binds to DNA (at response element), 5. transcription is activated, and 6. protein is synthesized in cytoplasm

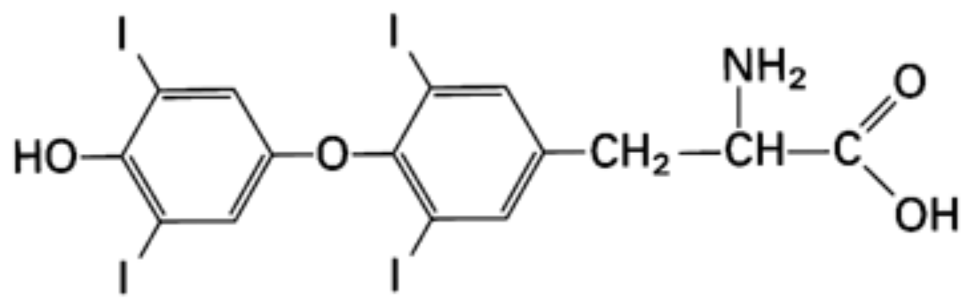
Lipid soluble hormones



Cortisol

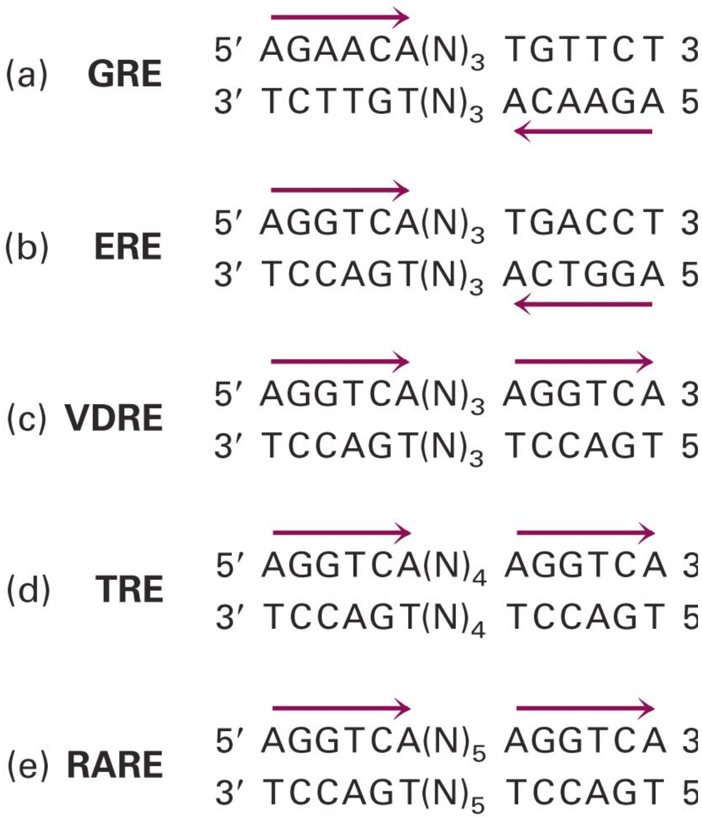


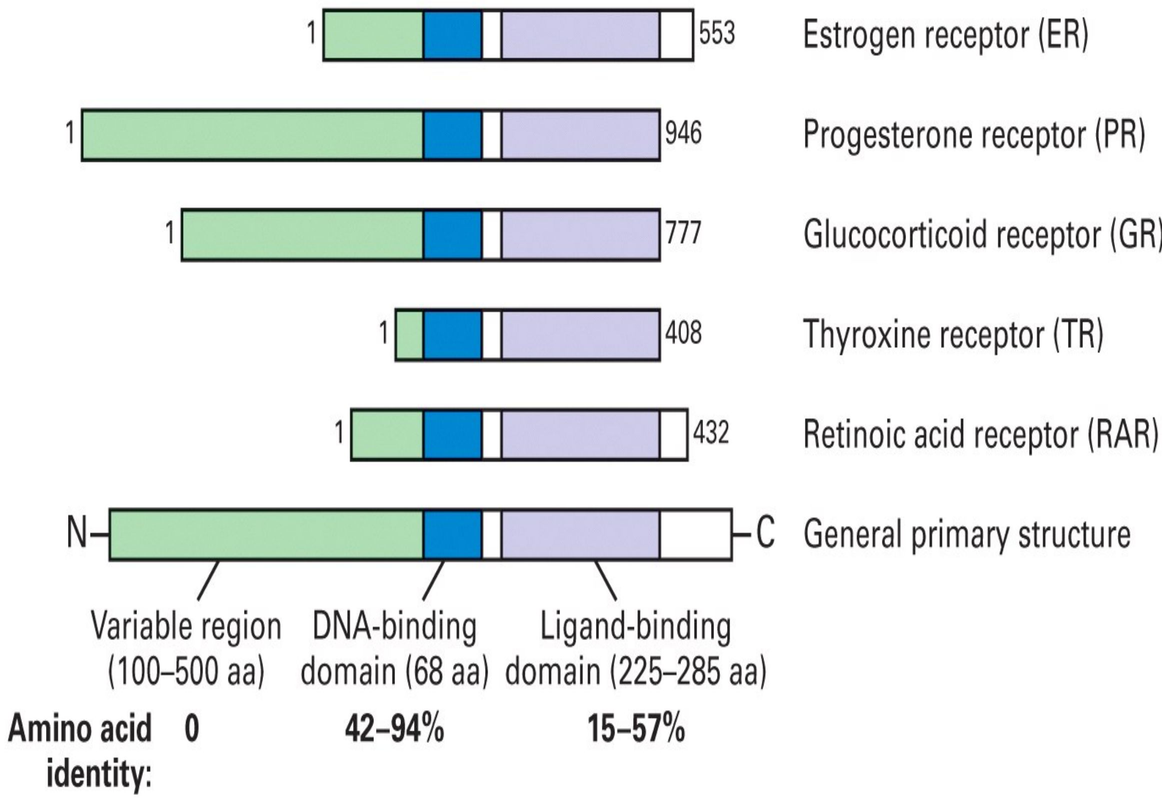
Retinoic acid



Thyroxine

Different hormone binds to different hormone receptor, which binds to different DNA element

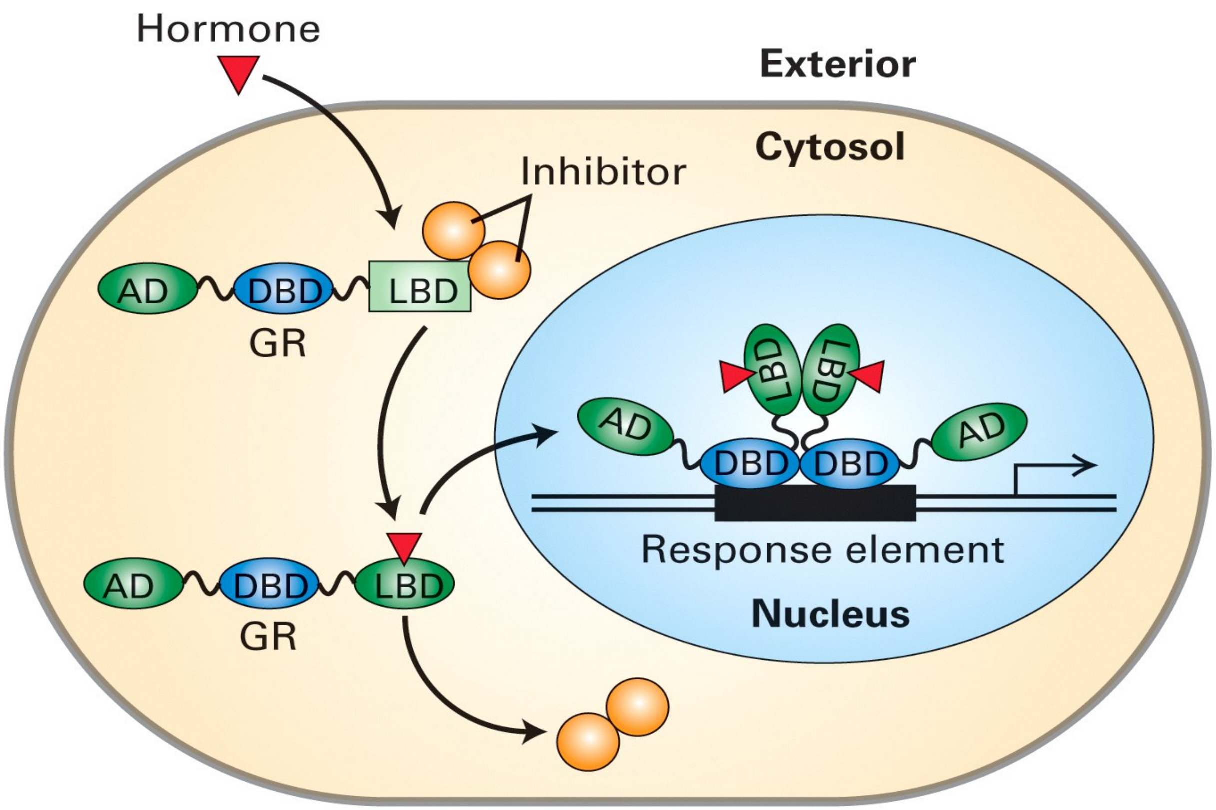




Nuclear receptors bind to different promoter-proximal sequences

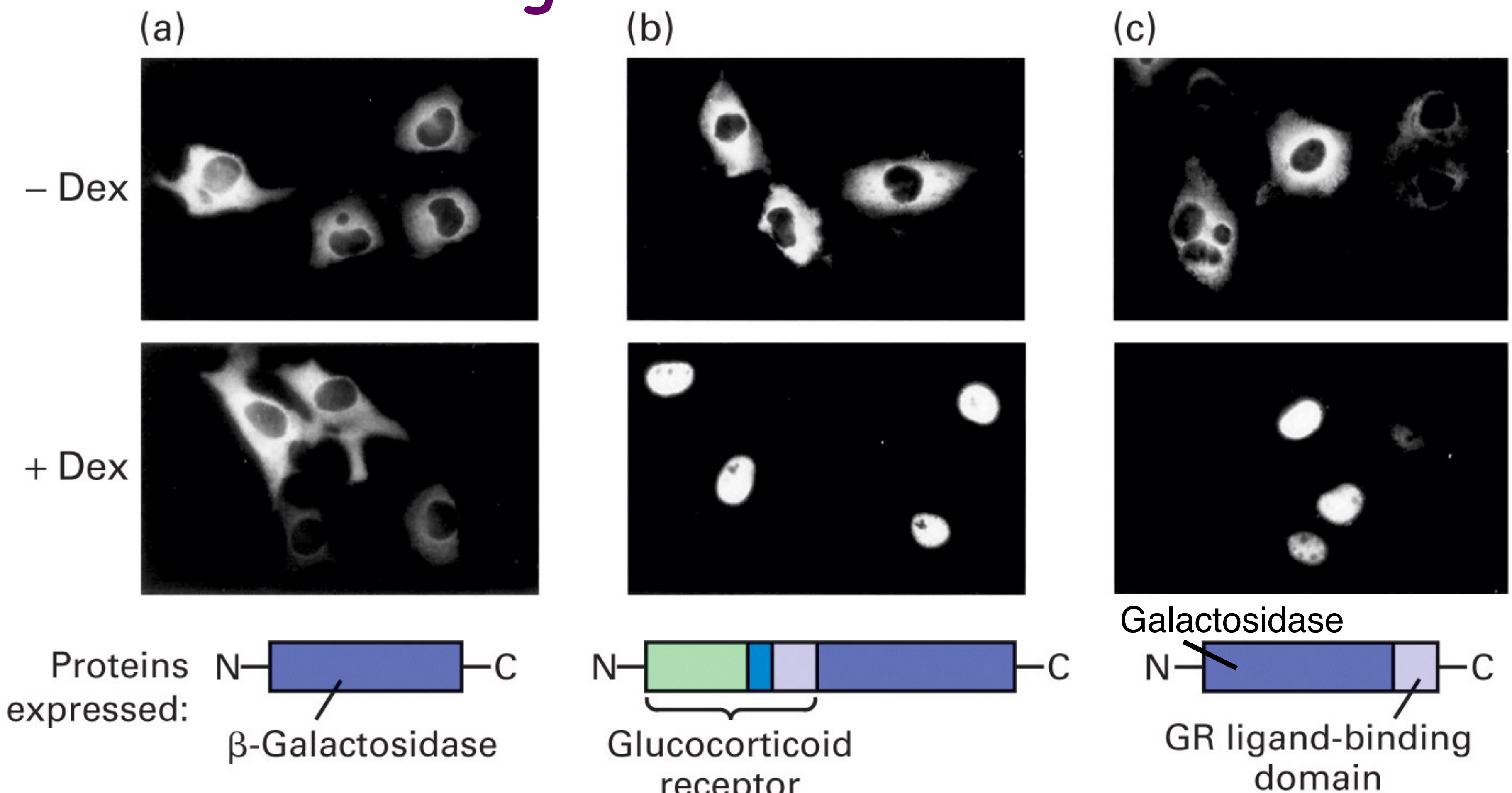
Similar structure of members of the nuclear receptor superfamily

Glucocorticoid receptor (GR) contains different domains and changes its cellular localization after binding to hormone



GR is normally locked in the cytosol by its association to the a cytosolic inhibitor. Binding to hormone removes the inhibitor from GR, allowing GR to transport into the nucleus, bind to DNA, and activate transcription.

An experiment showing the ligand-binding domain of GR is responsible for its ability to change cellular localization



(located in cytosol) (located in nucleus) (located in nucleus)

Dex: Dexamethasone, a synthetic glucocorticoid

Many genes are also regulated by coordinated control

A problem in eukaryotic gene expression:
prokaryotic genes are organized as operons so that proteins needed for the same biochemical pathway can be synthesized simultaneously. Eukaryotic genes are not organized as operons, how could different genes be expressed simultaneously if it is needed?

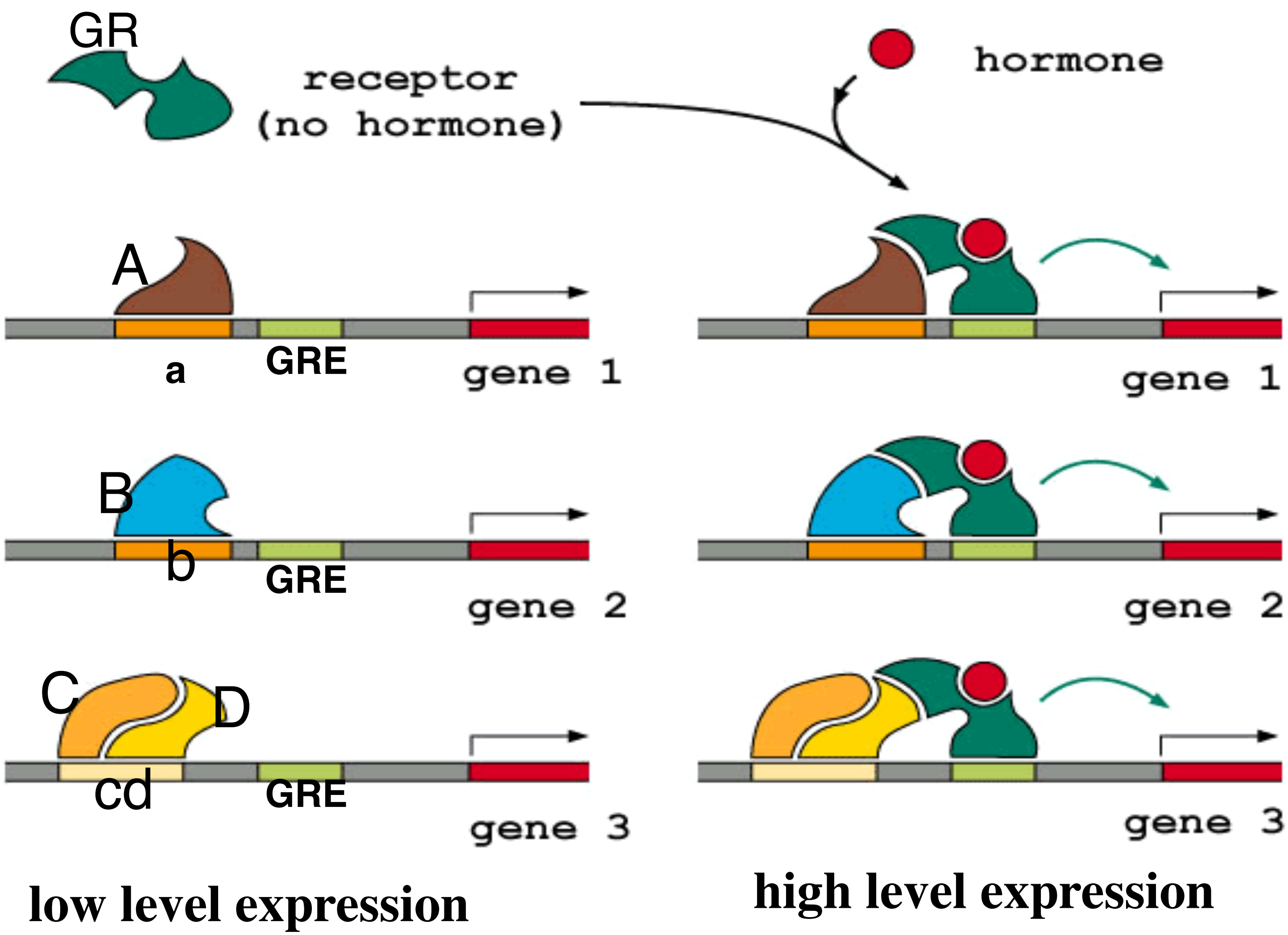
Coordinated control refers to the mechanism that allows different genes to be expressed at the same time. This is often achieved by the presence of an identical DNA regulatory (cis-) element in different genes (either within or outside of the promoter) and binding of the same transcription factor to the cis element.

Global control of gene expression by coordinated control

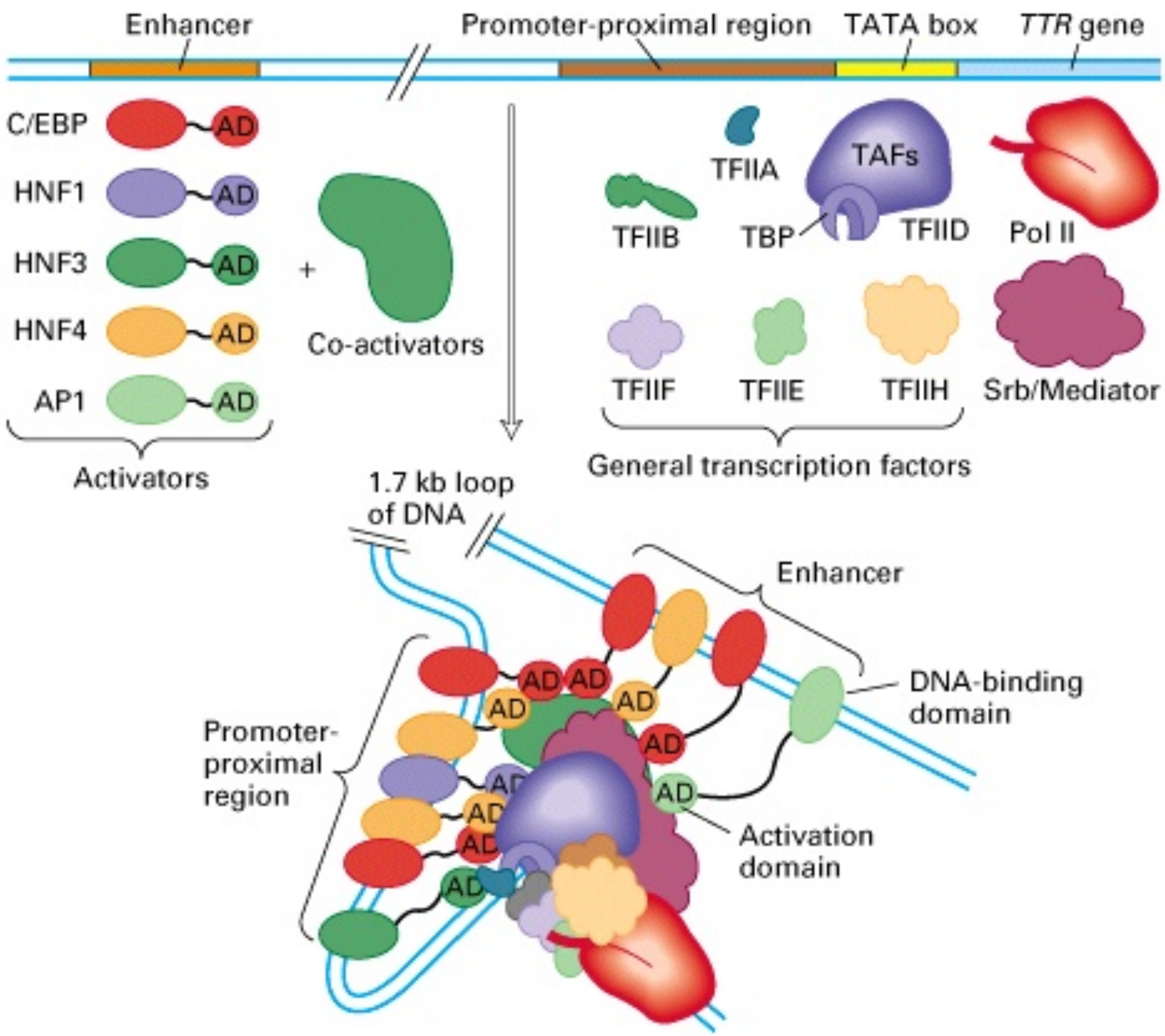
-One transcription factor can coordinately alter the expression of many different genes. Ex. GR-nuclear receptor transcription factor

A, B, C, D are gene-specific factors, they confer low level expression.

A factor such as GR can bind all 3 genes and confer high level expression.



Coordination of multiple transcriptional factors in the regulation of a single eukaryotic gene



Why is transcriptional regulation in mammals so complicated?

1) Most of the 20,000 *genes* in the mammalian *genome* require a unique expression pattern.

2) If the expression of each *gene* were controlled by only one transcription factor (like *CAP* or the lac repressor), 20,000 different transcription factors would be required. 20,000 additional factors would be needed to regulate each transcription factor *gene*, etc, etc....

3) The number of transcription factors required to generate 20,000 unique expression patterns can be dramatically reduced if multiple factors are needed to activate each *gene*. (Combinatorial regulation)

Combinatorial control of gene expression

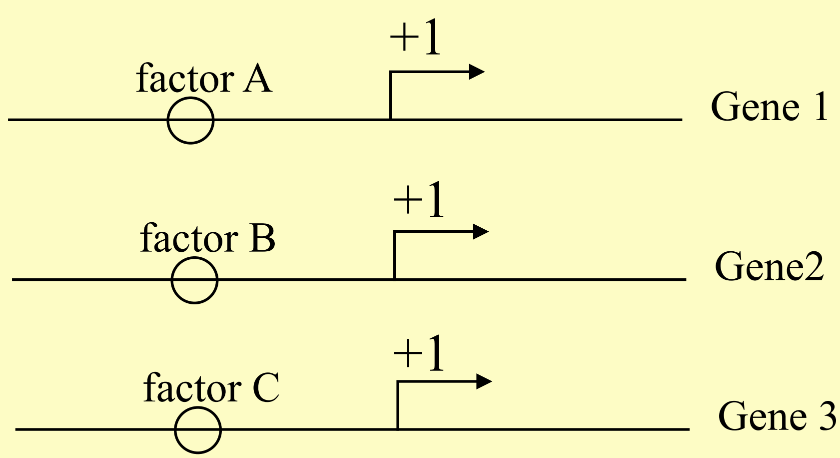
1. Transcription of a gene is often regulated by many transcription factors binding to various DNA sequences. To transcribe or not to transcribe is almost always determined by the combined effect of different transcription factors. The mechanism in which many transcription factors work together to control the expression of a single gene is called combinatorial control.

2. Combinatorial control is like a combination lock: in order to unlock, combination codes need to be entered with all the right numbers in the correct order.

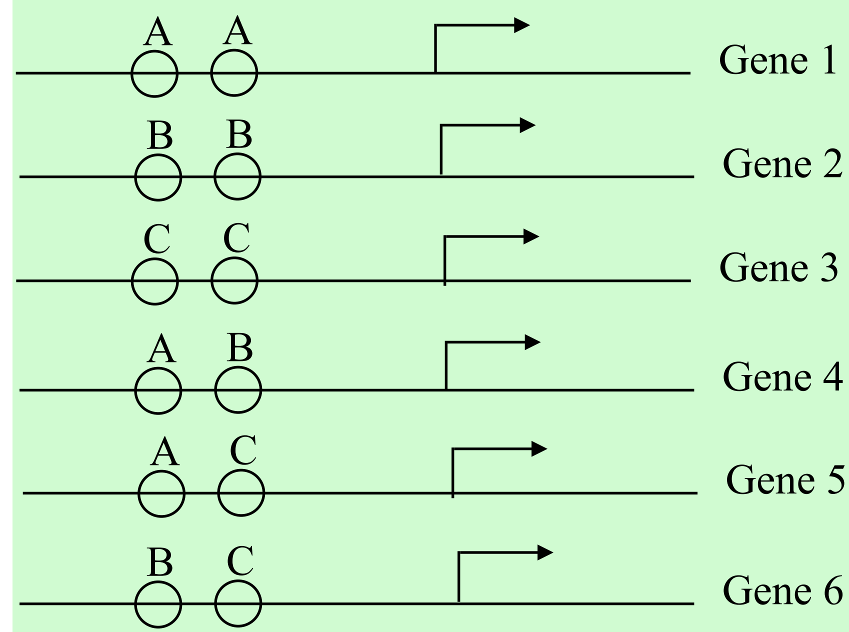
3. A single transcription factor could still have a decisive effect in determining the expression of a gene under the combinatorial control.

Combinatorial Regulation

One factor required to activate each gene:

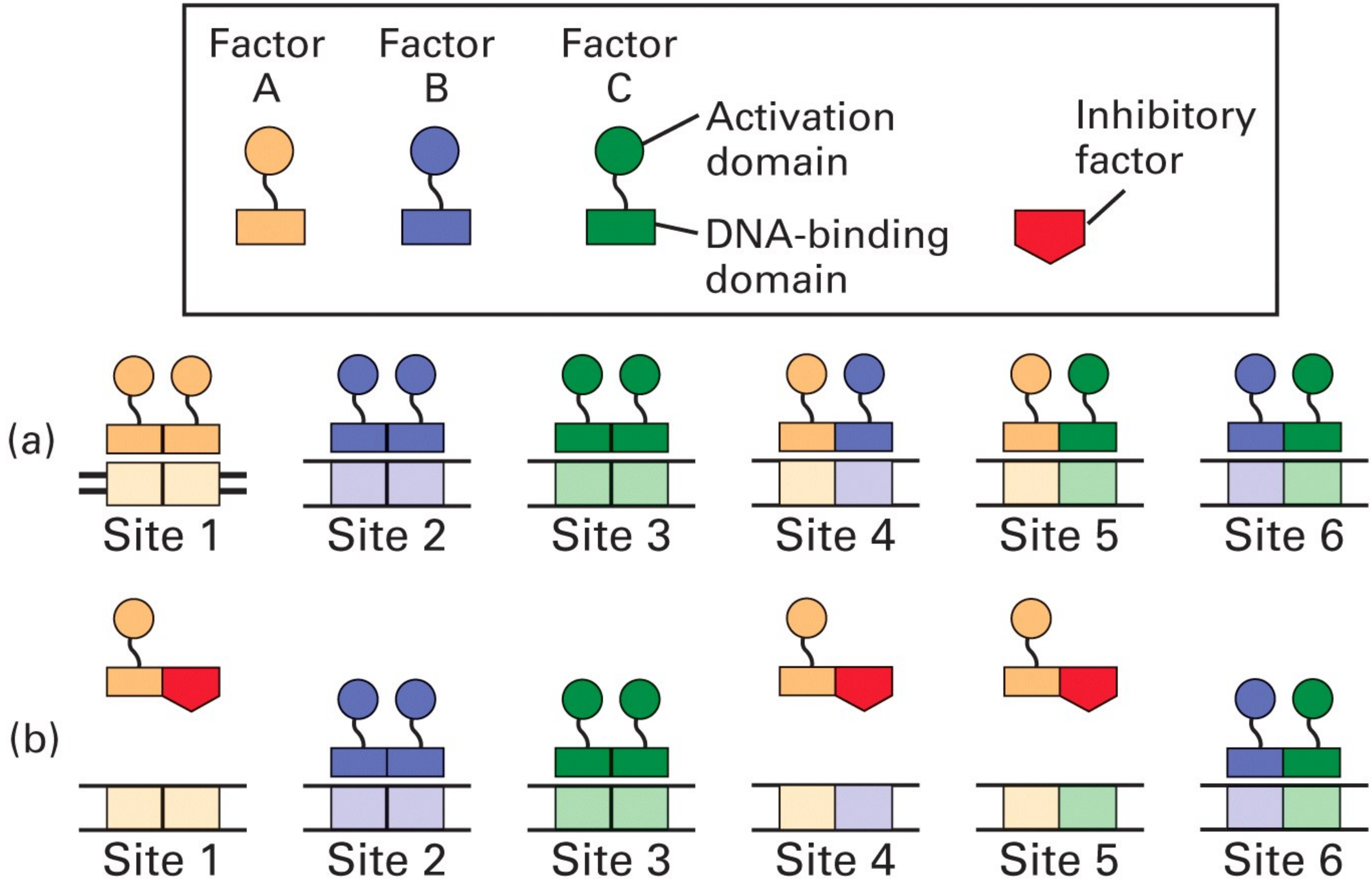


Two factors required to activate each gene:

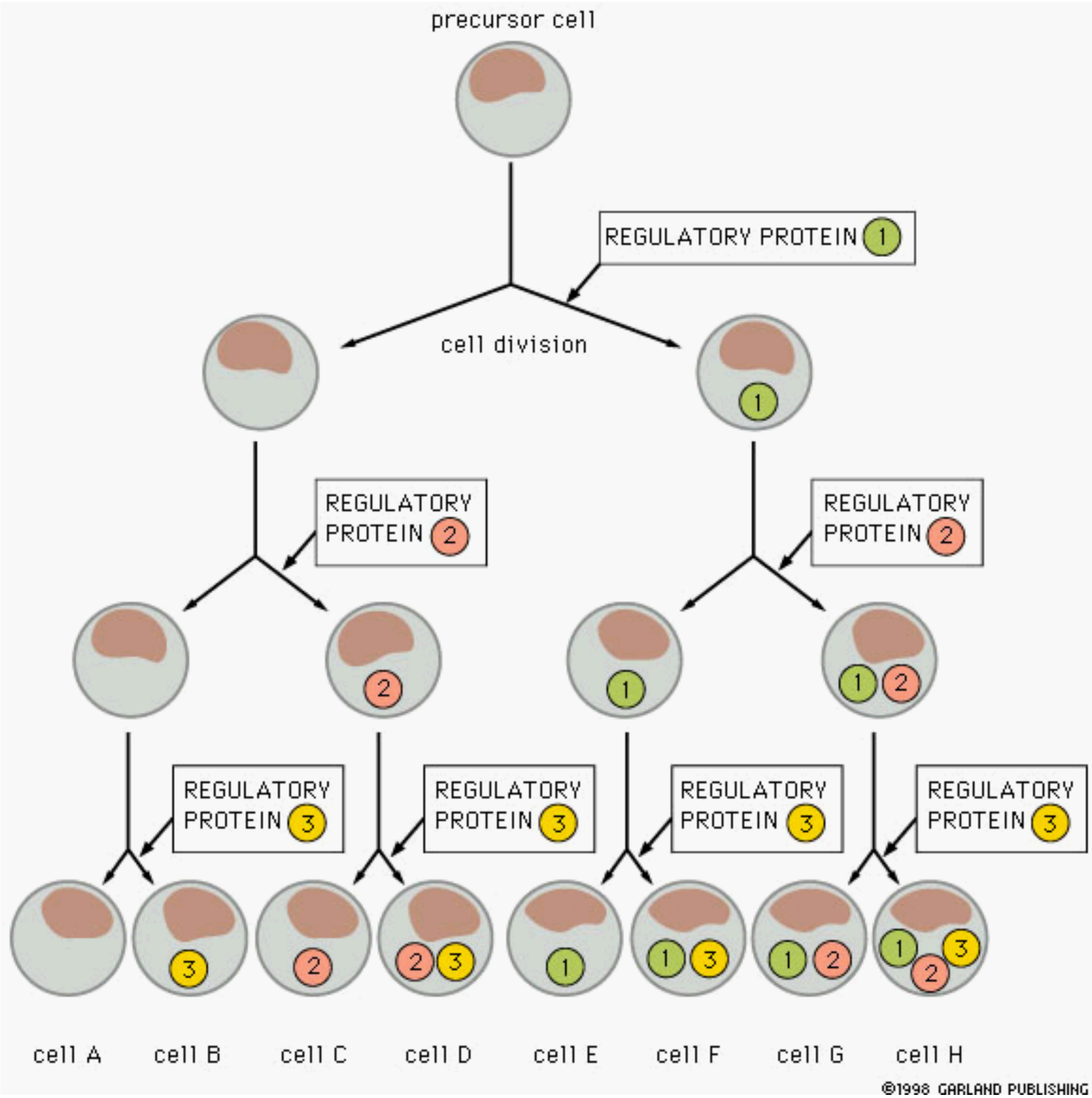


If the expression of each transcription factor is unique, the expression pattern of each of the 6 genes will be unique.

Combinatorial possibilities due to formation of heterodimeric transcription factors



Differential gene expression controls development



Different cell types are generated by both combinatorial and coordinated control of gene expression

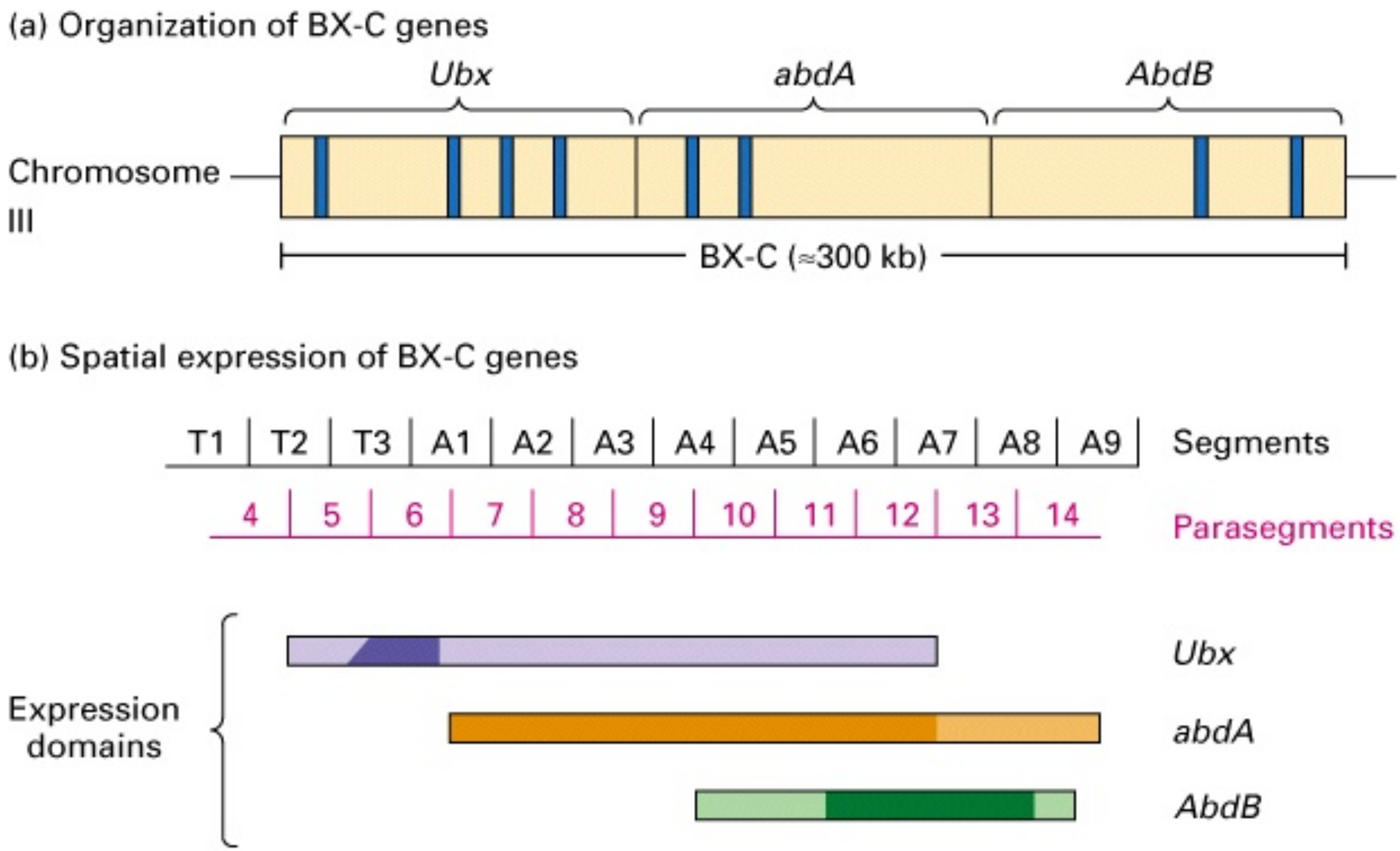
Gene expression and development

Coordinated control predicts that the function of one transcription factor can regulate the expression of multiple genes, that in turn may determine the developmental fate of the cell and the formation of an organ (composed of thousands of cells).

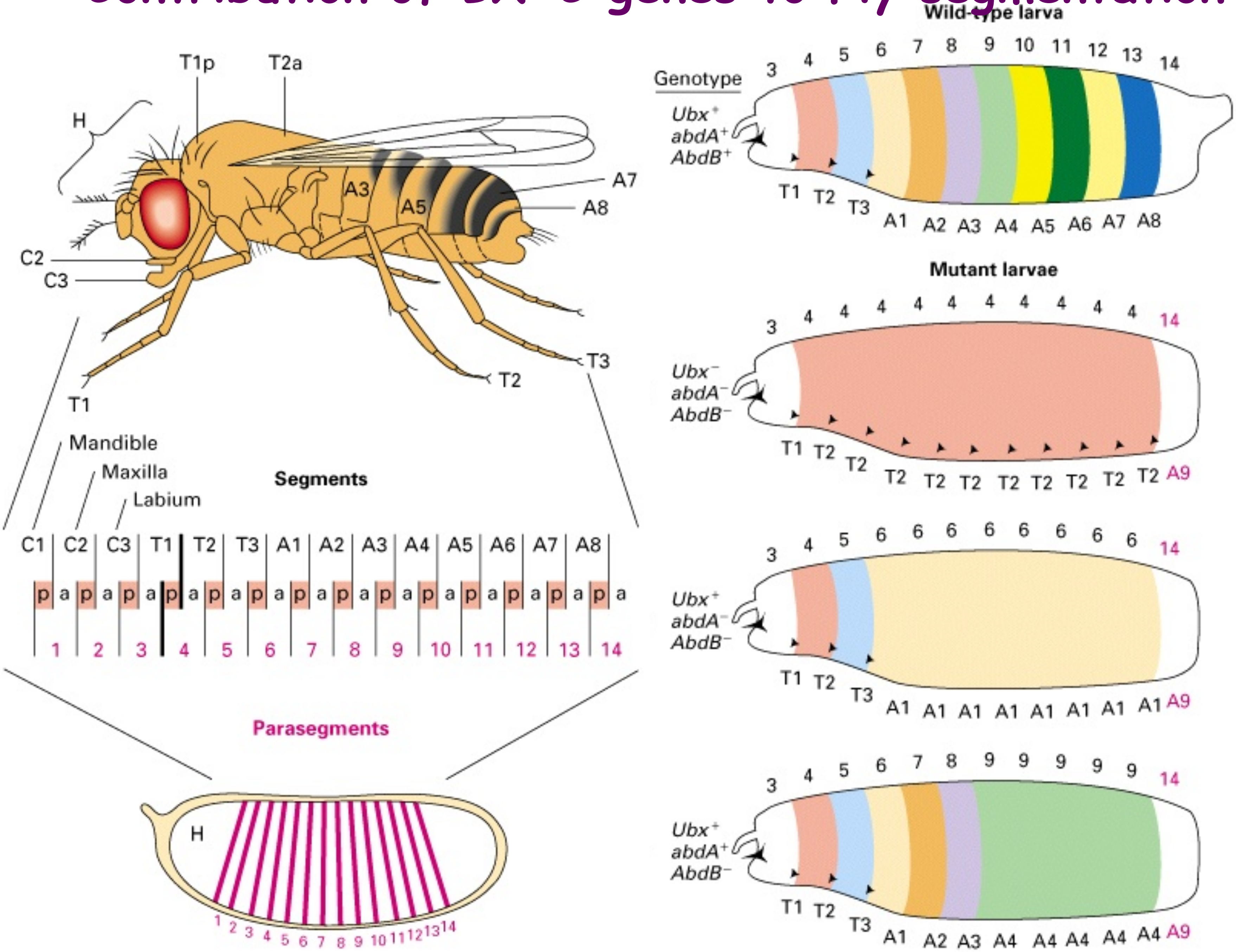
The importance of transcription factors in triggering the formation of a particular organ is evident from studies on regulation of the expression of human globin gene and the function of Drosophila Ey (eyeless) gene.

Many details of how changes in gene expression control development of an organism still remain to be elucidated….

Organization of genes within the bithorax complex (BX-C) on Drosophila chromosome III

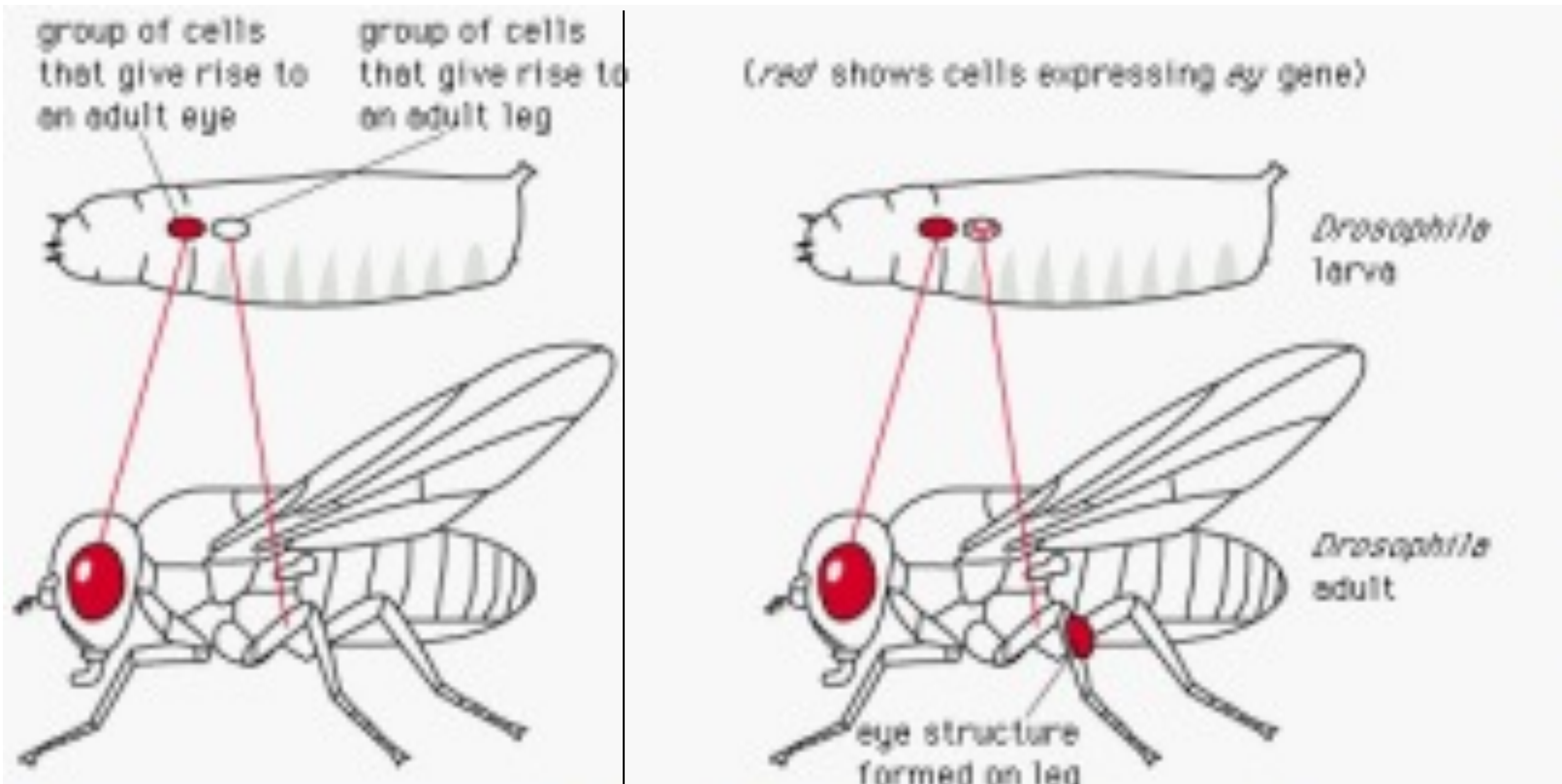


Contribution of BX-C genes to Fly segmentation



Action of one gene can control the formation of an organ

Flys contain a mutation in the Ey gene fail to develop eyes, whereas ectopic (abnormal) expression of Ey in leg or wing precursor cells result in the development of eyes in the leg or wing of the transgenic fly!!

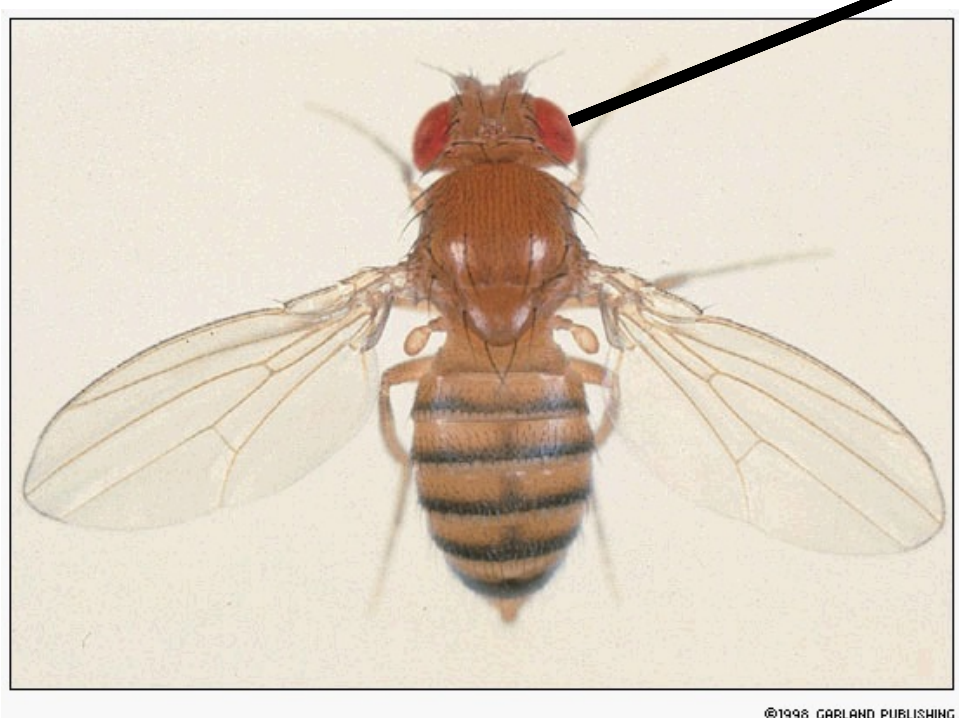


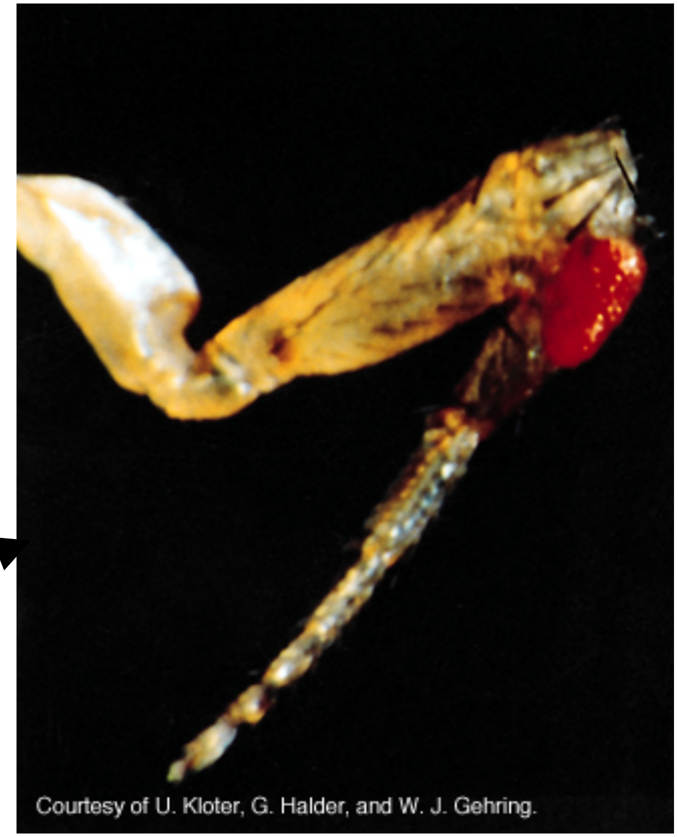
Normal eye development

Ectopic eye development

Development of an insect eye requires the concerted activity of an estimated 2500 gene products.

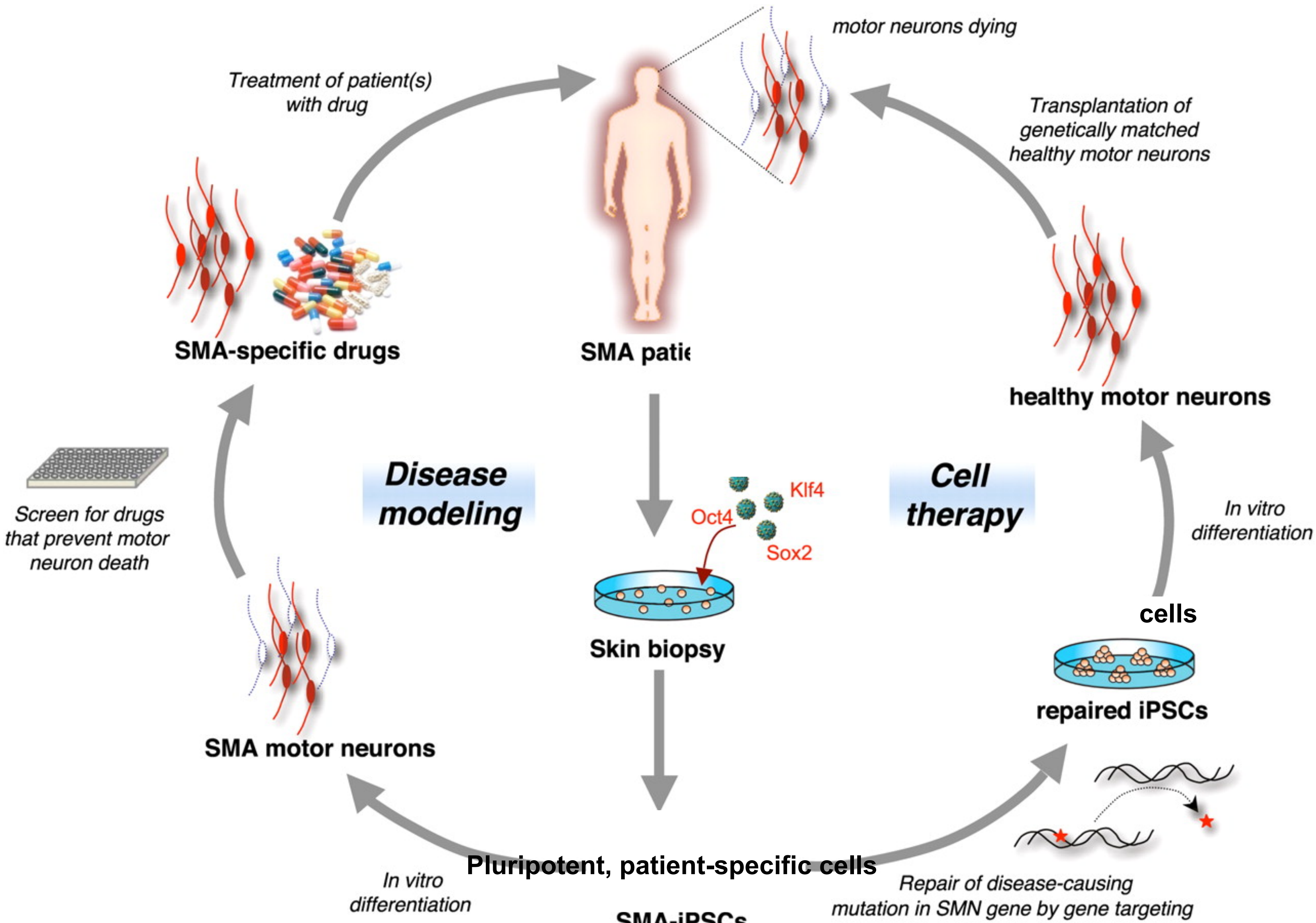
The Ey gene product appears to be the master control the expression of many of those genes.





With ectopic expression of the Ey gene, as many as 14 eyes can be formed in various body parts of some transgenic flies.

Therapeutic applications of reprogrammed cells: Understanding control of mammalian gene expression has enabled development of personalized approaches to genetic medicine



Adopted from Stadtfeld M , Hochedlinger K Genes Dev. 2010