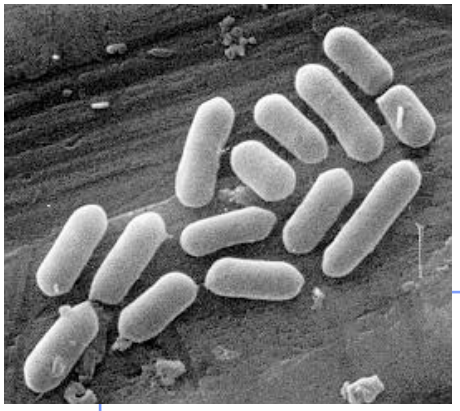
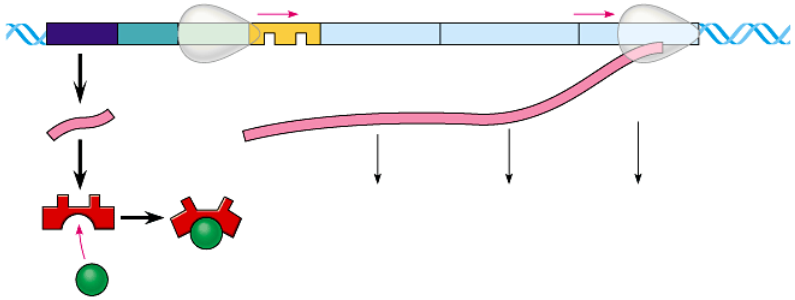
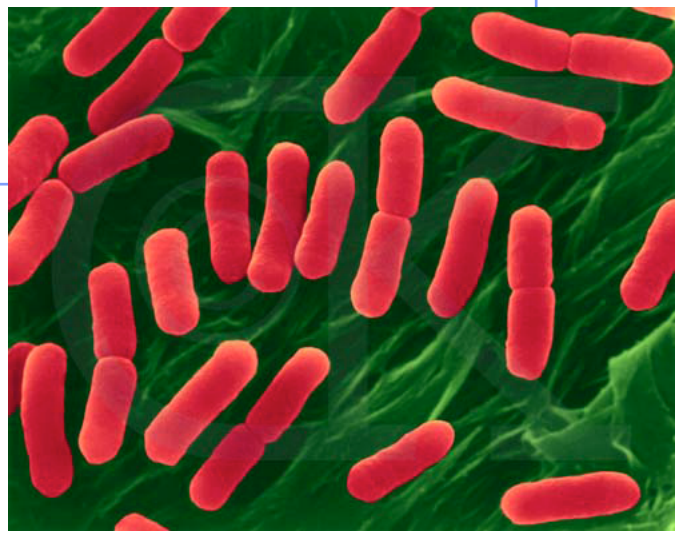
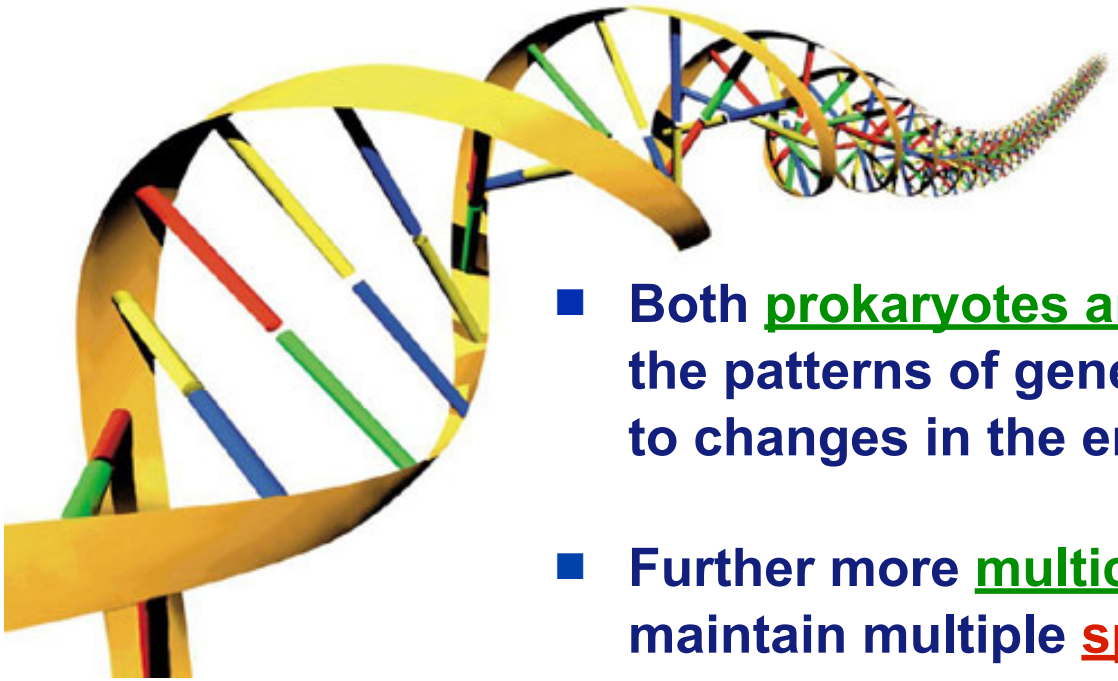




Ch. 18: Control of Prokaryotic (Bacterial) Genes

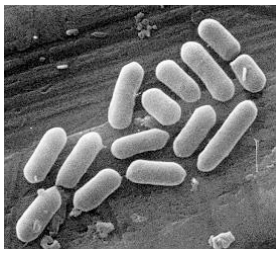


Regulation of Gene Expression

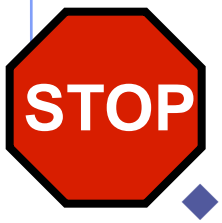


- Both prokaryotes and eukaryotes must alter the patterns of gene expression in response to changes in the environment.
- Further more multicellular eukaryotes must maintain multiple specialized cell types.
  - Recall that all cells in one multicellular organism have identical copies of DNA
    - *BUT* different genes need to be expressed or silenced in different cells to make them produce different proteins so the cells can have different cell functions

Bacterial metabolism



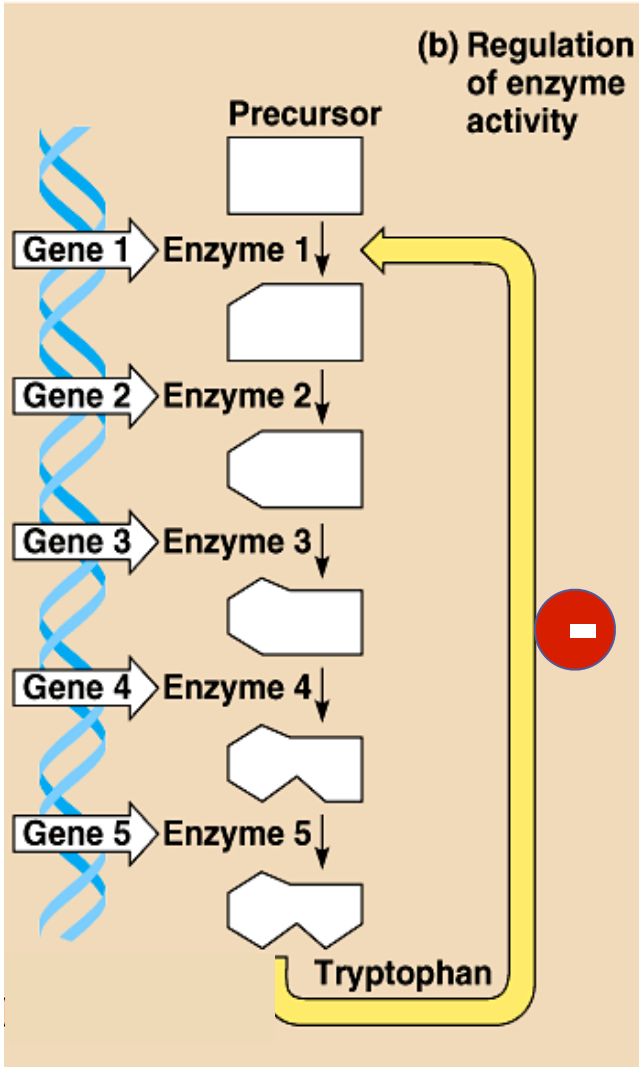
- Bacteria need to respond quickly to changes in their environment
  - if they have enough of a product, they need to stop production of that product
    - why? waste of energy to produce more
    - how? stop production of enzymes for synthesis
  - if they find new food/energy source, need to utilize it quickly
    - why? Need energy for metabolism, growth, reproduction & energy is hard to come by
    - how? start production of enzymes for digestion & proteins for transporting that molecule carrying energy into the cell



1. Controlling Already Made Enzymes

= inhibition

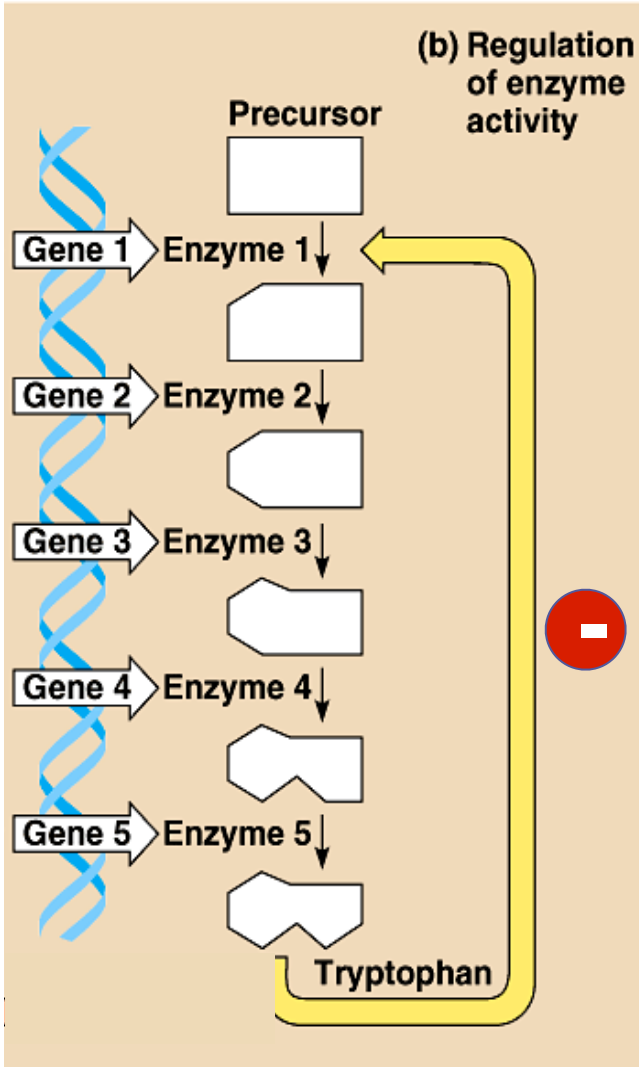
- **Feedback inhibition in amino acid tryptophan biosynthesis:**
  - product of the biochemical pathway (tryptophan) acts as an allosteric inhibitor of 1st enzyme in tryptophan biochemical pathway
    - When the concentration of tryptophan increases (indicating that there is more tryptophan present than needed to support the activities needing tryptophan like protein synthesis), extra tryptophan molecules bump into Enzyme 1s more frequently, inhibiting them.
    - When the concentration of tryptophan decreases (indicating that tryptophan is being used up and more might be needed), fewer tryptophan molecules are available to keep inhibiting repeatedly Enzyme 1s so more an more Enzyme 1s become active again, causing tryptophan to be made again from its precursor.



1. Controlling Already Made Enzymes

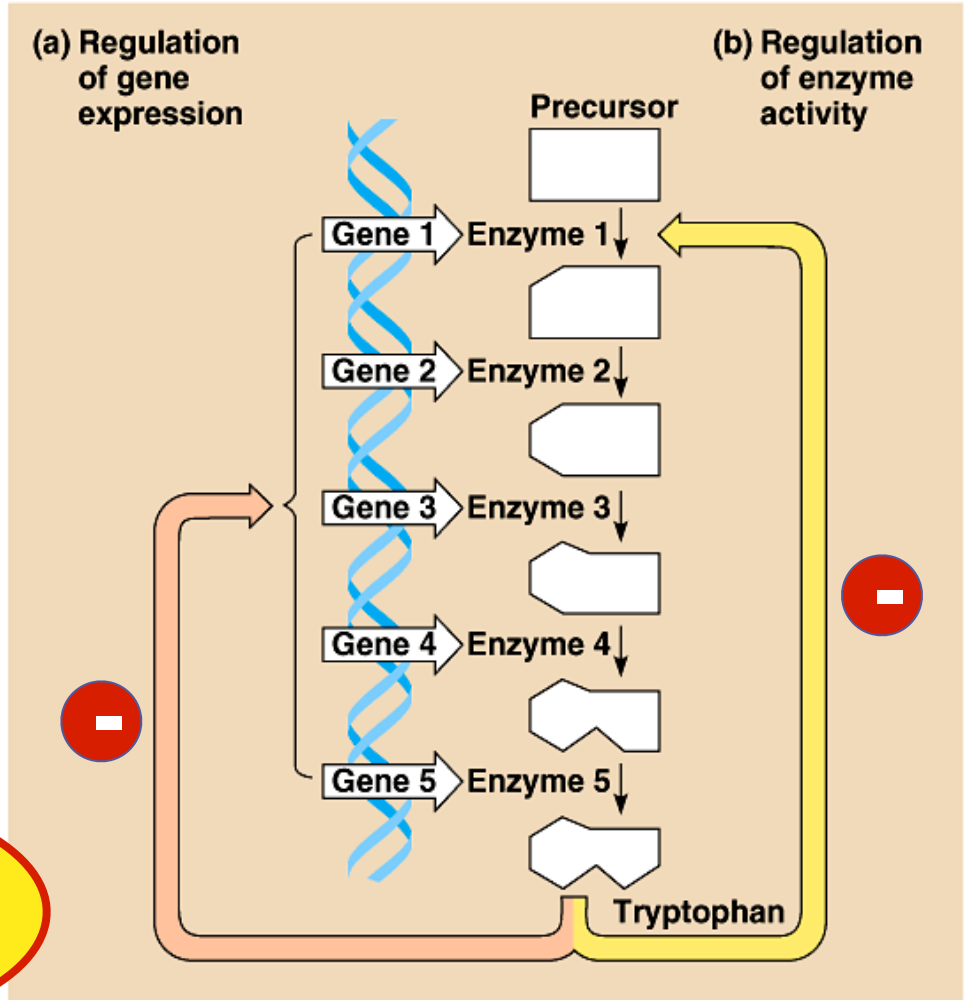
= inhibition

- Feedback inhibition allows for a cell to quickly shut off and turn back on chemistry exactly when it is not longer needed and when it is needed once again...
  - **But** this mechanisms does means that (when the chemistry is not needed) we would be "wasting" energy and amino acids constructing the enzymes (proteins)

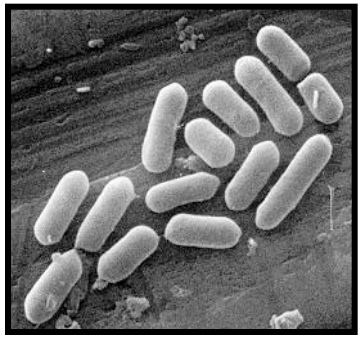


2. Regulating Metabolism by Controlling Transcription of the Gene for an Enzyme

- **Regulating Gene Expression**
  - instead of blocking enzyme function, the cell could block transcription of the genes for ALL the enzymes used in the tryptophan pathway
    - this saves energy by not wasting it on unnecessary protein synthesis



Gene regulation in bacteria



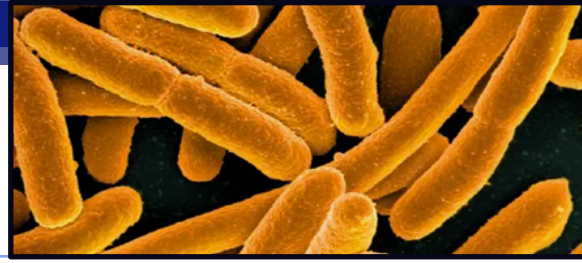
- Cells vary amount of specific enzymes by regulating gene transcription
  - turn genes on or turn genes off
    - turning genes OFF example
      if bacterium has enough tryptophan then it doesn't need to make enzymes used to build tryptophan
      - why use up nucleotides building mRNA, use up amino acids building the tryptophan biochemical pathway enzymes, and use up energy for doing synthesis work, if these enzymes are not needed anyway

- turning genes ON example
  if bacterium encounters new sugar (energy source), like lactose (which it normally doesn't use), then it needs to start making enzymes used to digest lactose

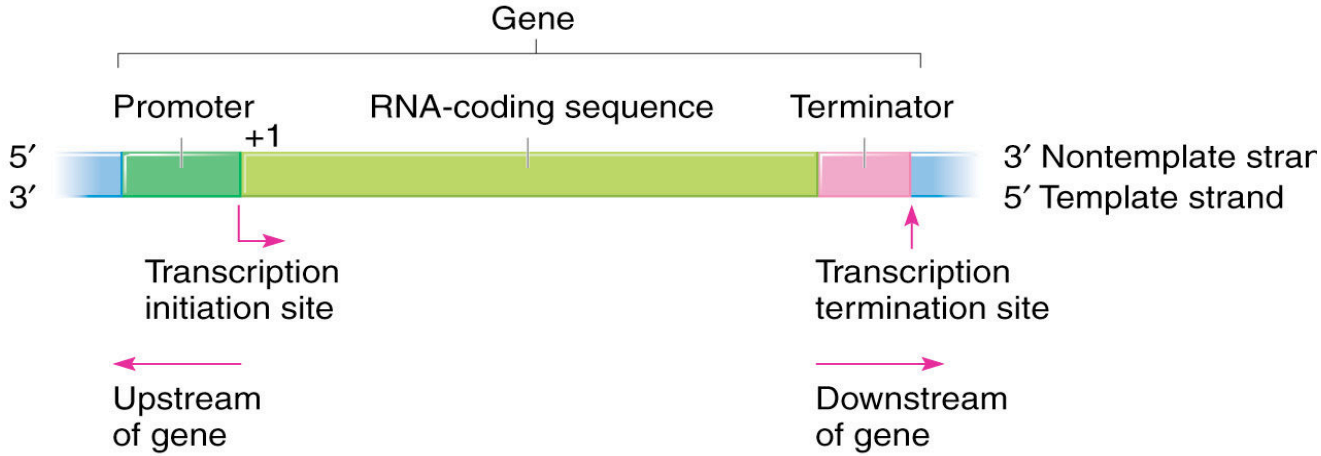
E. coli = rapid growth; new generation every ~20 minutes; one bacteria can make a 10^8 (100 million) sized colony overnight!

Any organism that can put more energy towards growth & reproduction has a (natural) selection advantage or a "selective advantage," we say.

Prokaryotic Transcription

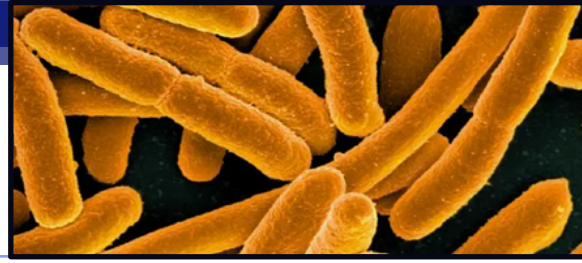


- **The transcription of a basic prokaryotic gene is dependent on:**
  1. **the strength of its DNA promoter (that RNA Polymerase is attracted to) affecting transcription rates of genes**
     - **Many genes in eukaryotes and prokaryotes contain a promoters (where RNA Polymerase binds) and the transcription unit or coding sequence (which encodes the instructions ribosomes use to build polypeptides of proteins from)**

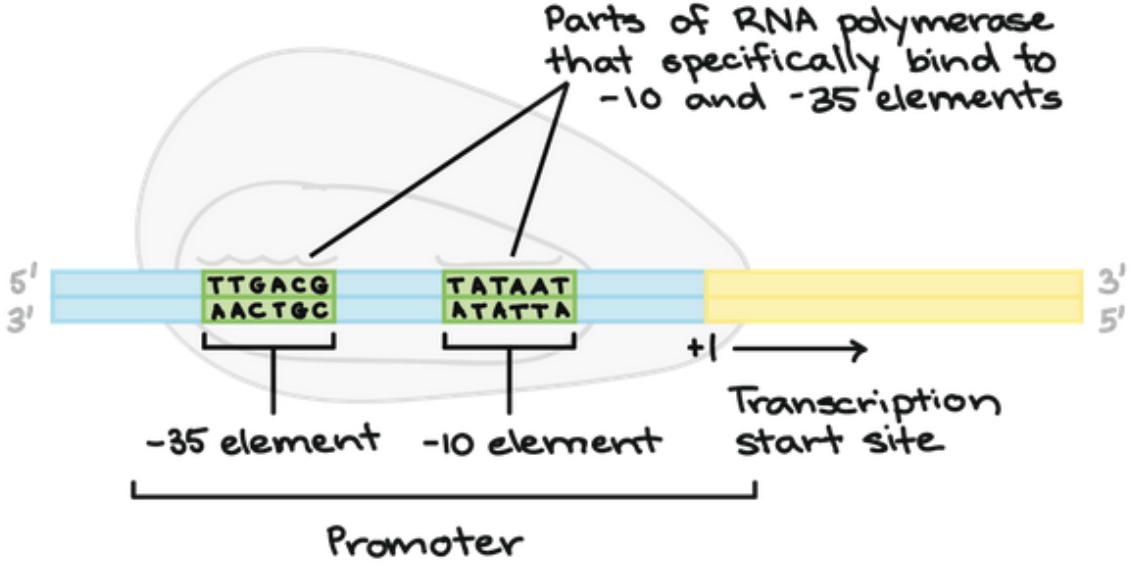


  2. **the presence of activators or repressors which help influence the ability of RNA Polymerase to transcribe certain types of related genes when organized into DNA sections called operons (more about these later**

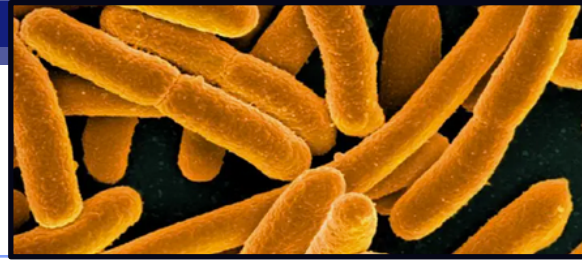
Prokaryotic Promoters



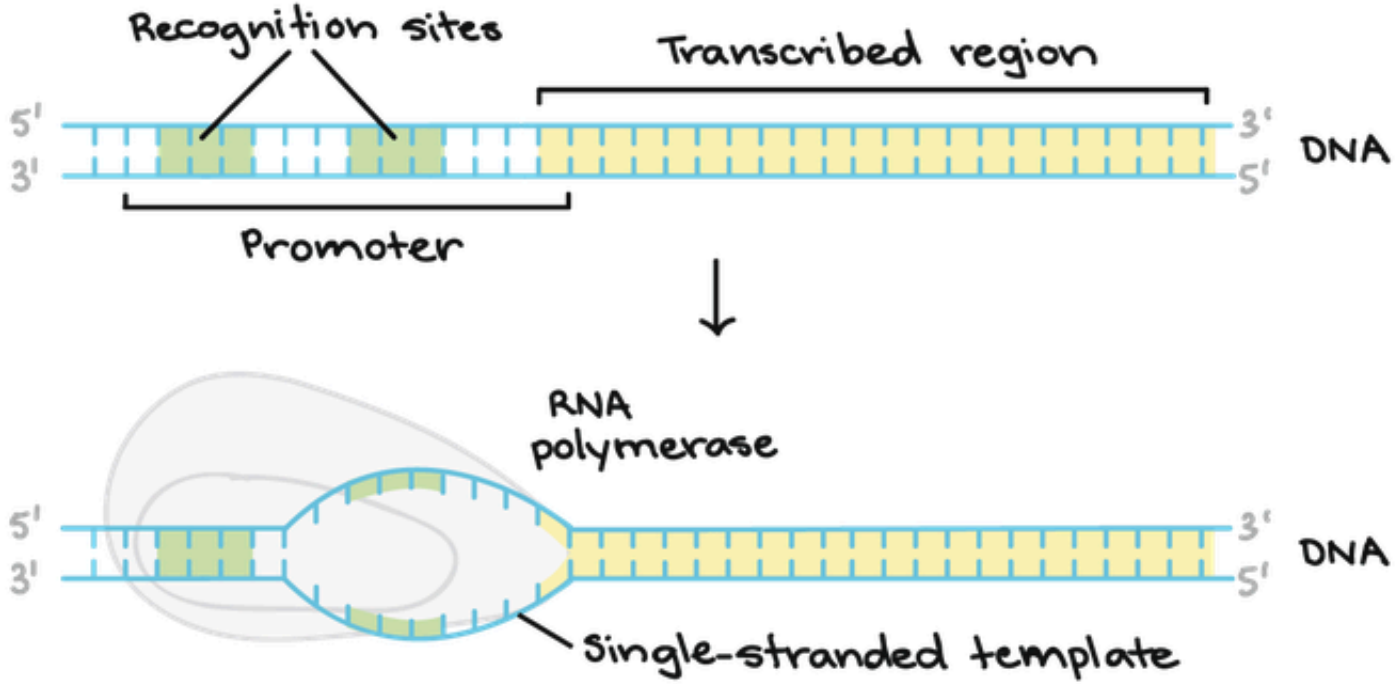
- **Prokaryotic Promoters**
  - **A typical bacterial promoter contains two important DNA sequences, the -10 and -35 elements.**
    - **RNA polymerase recognizes and binds directly to these sequences.**
      - **The sequences position the polymerase in the right spot to start transcribing a target gene, and they also make sure it's pointing in the right direction.**



Prokaryotic Promoters

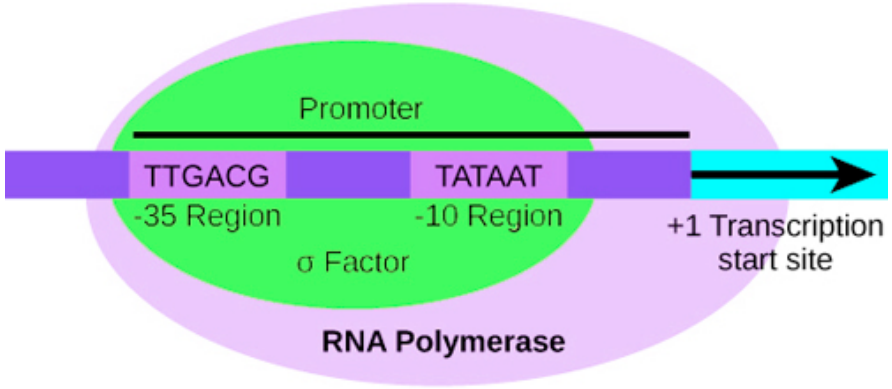


- **Once the RNA polymerase has bound, it can open up the DNA and get to work.**
  - **DNA opening occurs at the -10 element, where the strands are easy to separate due to the many As and Ts (which bind to each other using just two hydrogen bonds, rather than the three hydrogen bonds of Gs and Cs).**

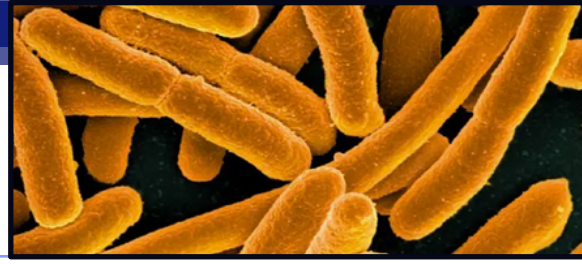


Prokaryotic Promoters

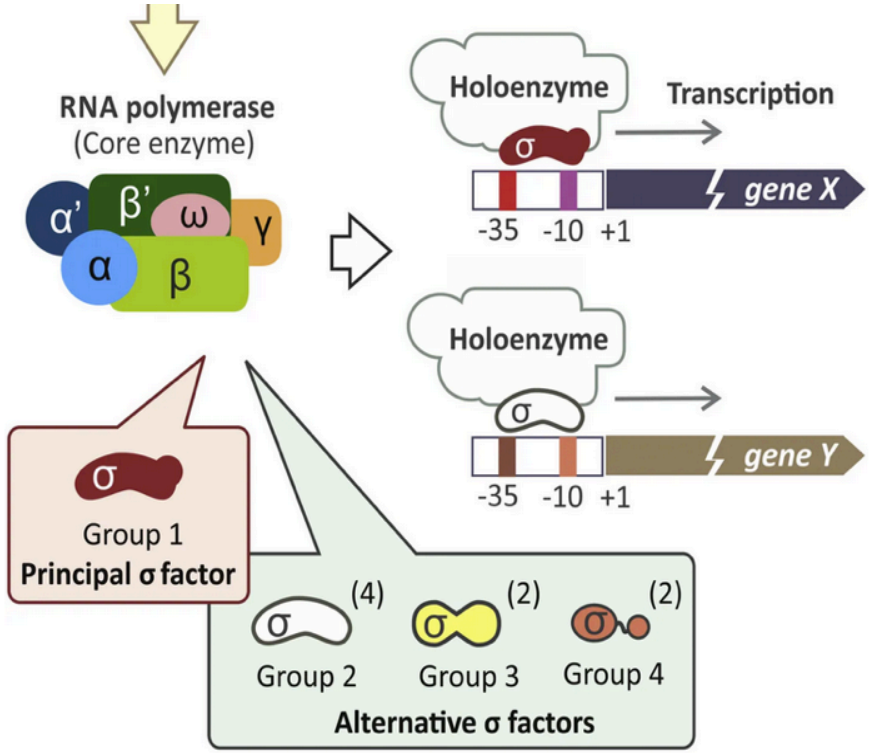
- **In the absence of other regulatory elements, a promoter's sequence-based affinity for RNA polymerases varies, which results in the production of different amounts of RNA transcripts produced by different genes.**
  - **The variable affinity of RNA polymerase for different promoter sequences is related to regions of consensus sequence upstream of the transcription start site within the promoter.**
  - **Sigma factors are specialized bacterial protein subunits that bind to RNA polymerases and allow for the RNA Polymerase to gain a greater affinity for specific gene promoters**
    - **Different sigma factors act as mediators of sequence-specific transcription.**



Prokaryotic Transcription

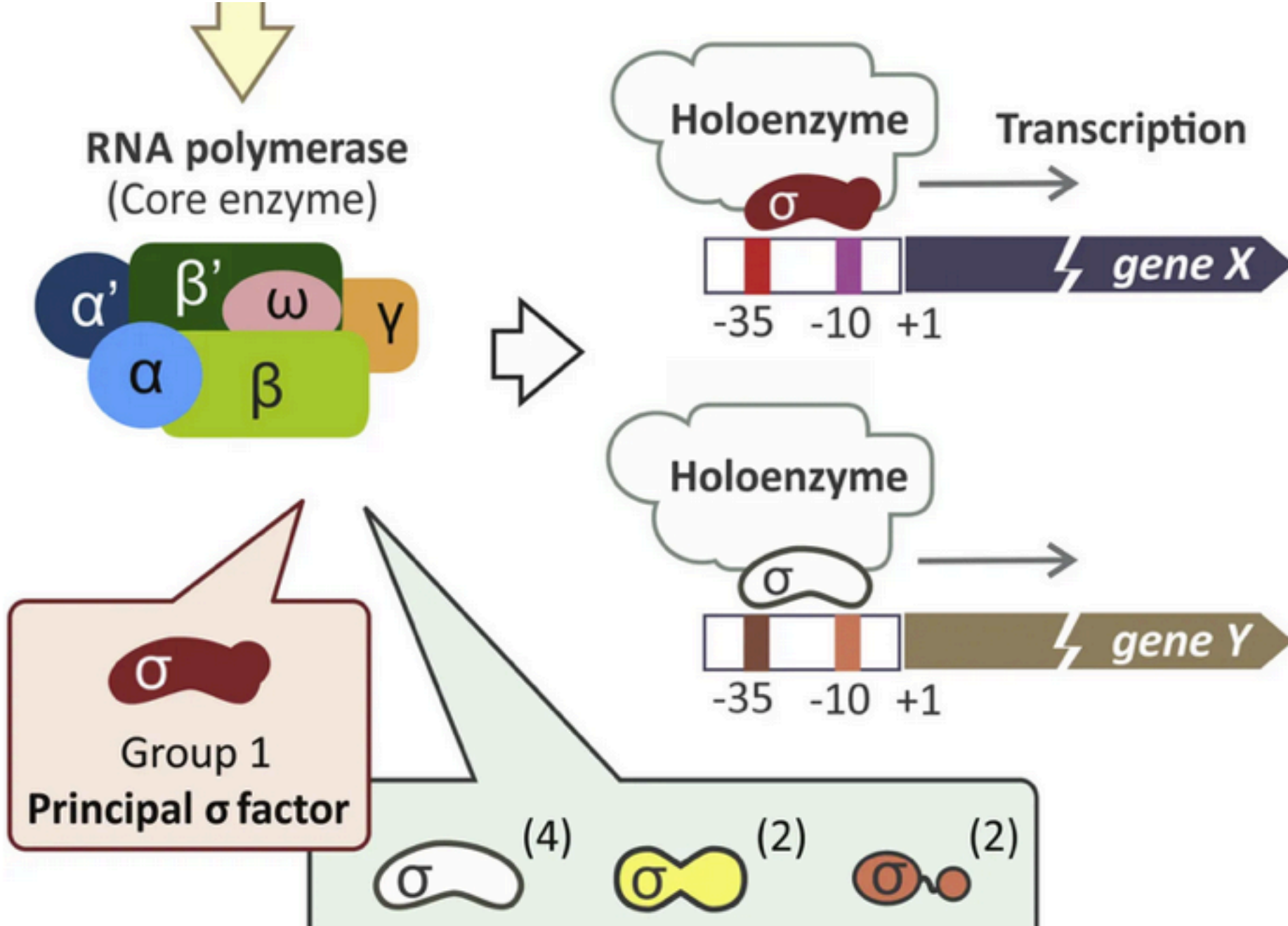


- **Sigma factors bind to RNA polymerase helping the polymerase bind to promoter sequences in order to initiate transcription of DNA into RNA.**
  - **In addition to a primary sigma factor responsible for transcription of most constitutively (always) expressed genes, bacteria typically contain a number of alternative sigma factors, each recognizing a different, specific set of promoters.**
    - **By replacing the primary sigma factor with an alternative sigma factor, the cell can control which sets of genes are expressed at any one time.**

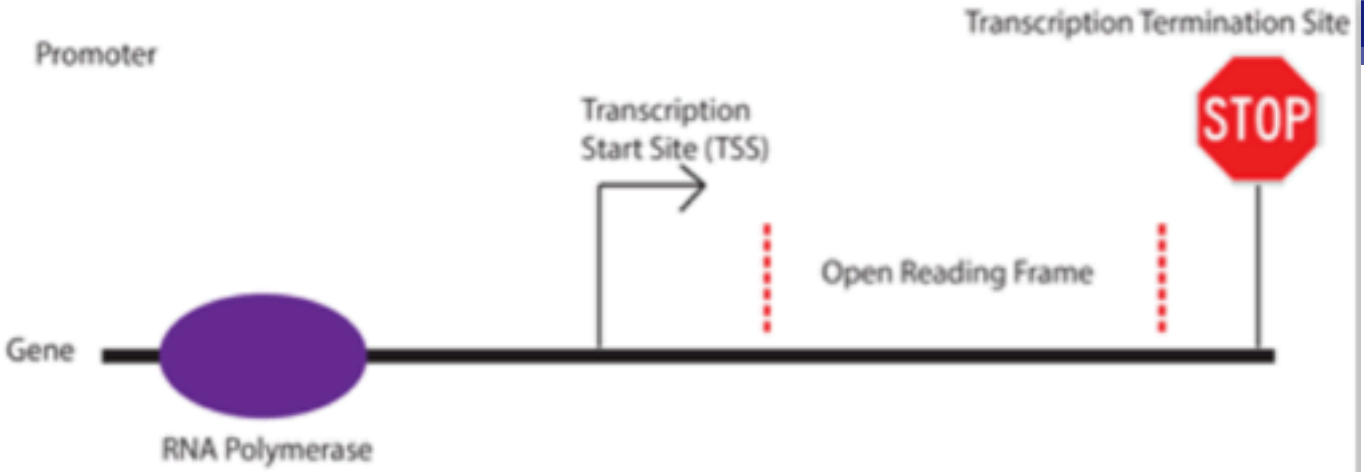


Most Prokaryotic Genes Are "On"

- In the absence of other regulatory elements which our found when genes are organized into operons (as we will see), the default state of a prokaryotic gene is for it to be "on" (getting transcribed), resulting in the production of some amount of transcript all the time.
  - When genes are organized into operons, transcriptional regulation is assisted by additional proteins (as we will soon see), including:
    - Repressors bind to DNA (in/near the Promoter) and inhibit the transcription of the gene by blocking RNA Polymerase, thereby turning the prokaryotic gene OFF.
    - Positive Control Elements (activators) that bind to DNA and incite even higher levels of transcription than normally would occur.
- The prokaryotic strategy for gene regulation is distinct from eukaryotic transcription.
  - In eukaryotes, the basal state is for a gene to be "off," transcription factors being required to attract RNA Polymerase to the promoter in order for transcription to start, the transcription factors being high gene specific.


RNA polymerase
(Core enzyme)
α'
β'
ω
γ
α
β
Holoenzyme
Transcription
σ
gene X
-35
-10
+1
Holoenzyme
σ
gene Y
-35
-10
+1
σ
Group 1
Principal σ factor
σ
(4)
σ
(2)
σ
(2)

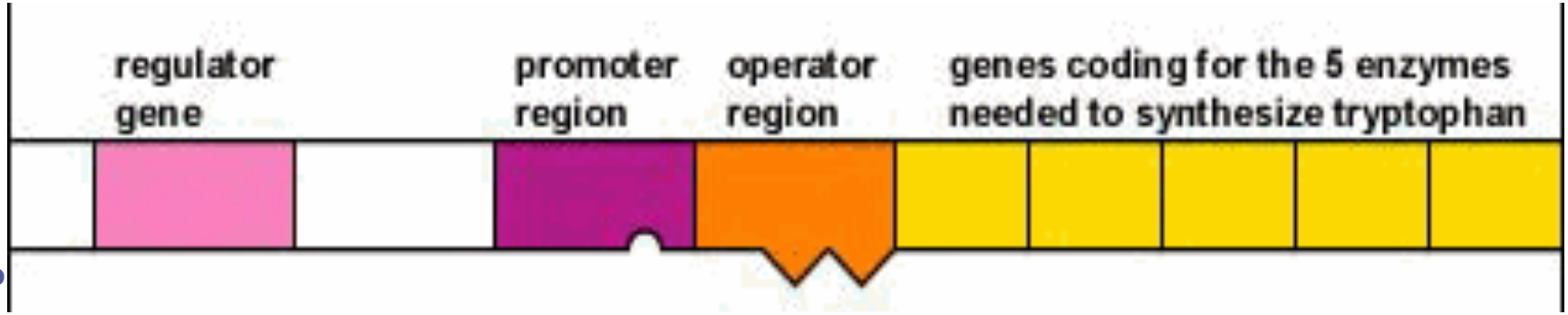
Bacterial Gene Overview



- **In bacterial chromosomes, gene contains a regulatory region and a transcription start site, as well as one or more open reading frames *(transcriptional units/ coding regions)* followed by a transcription termination site.**
  - **Housekeeping Genes or Constitutive Genes: expressed at a more or less constant level because their products are needed constantly - *RNA Polymerase always binding to these promoters and transcribing these genes.***
  - **Regulated Gene Expression: levels of gene product rise and fall in response to molecular signals - *RNA Polymerase not always transcribing these genes.***
    - **Inducible Gene: gene products that increase in concentration due to a particular signal *(process called induction)***
    - **Repressible Genes: gene products that decrease in concentration due to a particular signal *(process called repression)***

Bacteria group some genes together

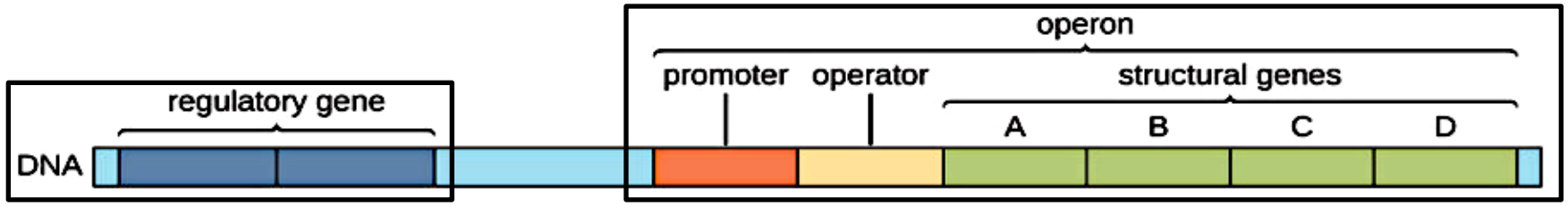
- E. coli synthesized the amino acid, tryptophan, for example, from a precursor molecule in a multistep pathway.
- All five genes' transcription units (open reading frames) that code for these five enzymes are clustered together on the bacterial chromosome with one promoter = a set up called an OPERON
  - ONE PROMOTER serves ALL FIVE GENES
    - All five are transcribed together into one large mRNA transcript that codes for all five polypeptides
  - ADVANTAGE: These genes are under *coordinate control*
    - ONE on-off switch controls the whole group of related genes.



AP

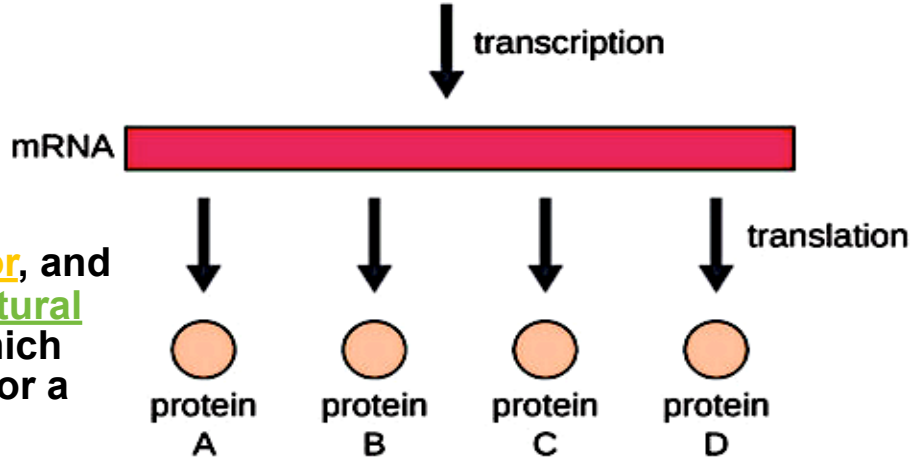
Bacteria group certain genes together in Operons

- **OPERON = Operator, promoter, and genes *(transcriptional units of coding sequences in DNA)***
  - The promoter has simultaneous control over the regulation of the transcription of multiple genes because
    - they will either all be needed at once or none will be needed.
- **The on/off switch is a segment of DNA called an OPERATOR**
  - Located in the promoter or between promoter and the structural genes *(the genes for proteins in the operon)*
    - The operator controls the access of RNA Polymerase to GENES



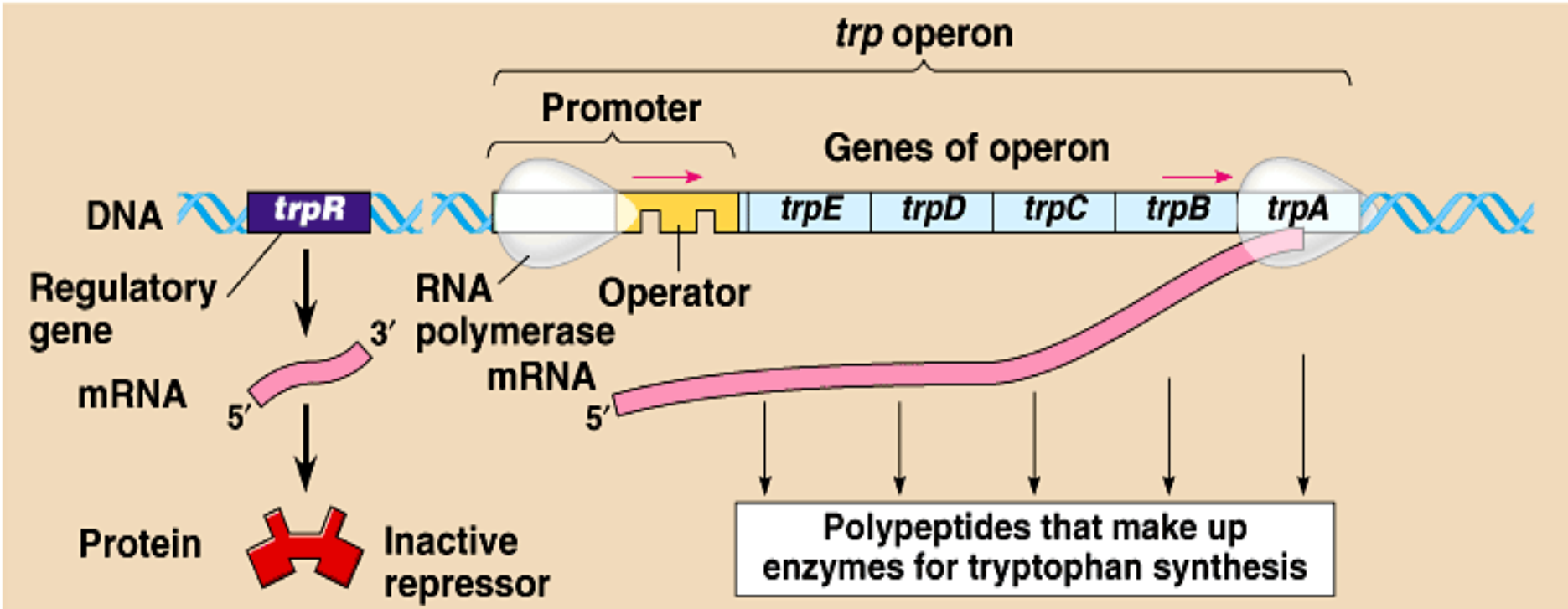
Elsewhere in the prokaryote's chromosome is a regulatory gene (not in an operon) with its own promoter for making the repressor protein that will help turn on or off the genes in the operon

Operon with DNA sequences of the promoter, operator, and the multiple structural protein genes (which encode proteins for a related function)



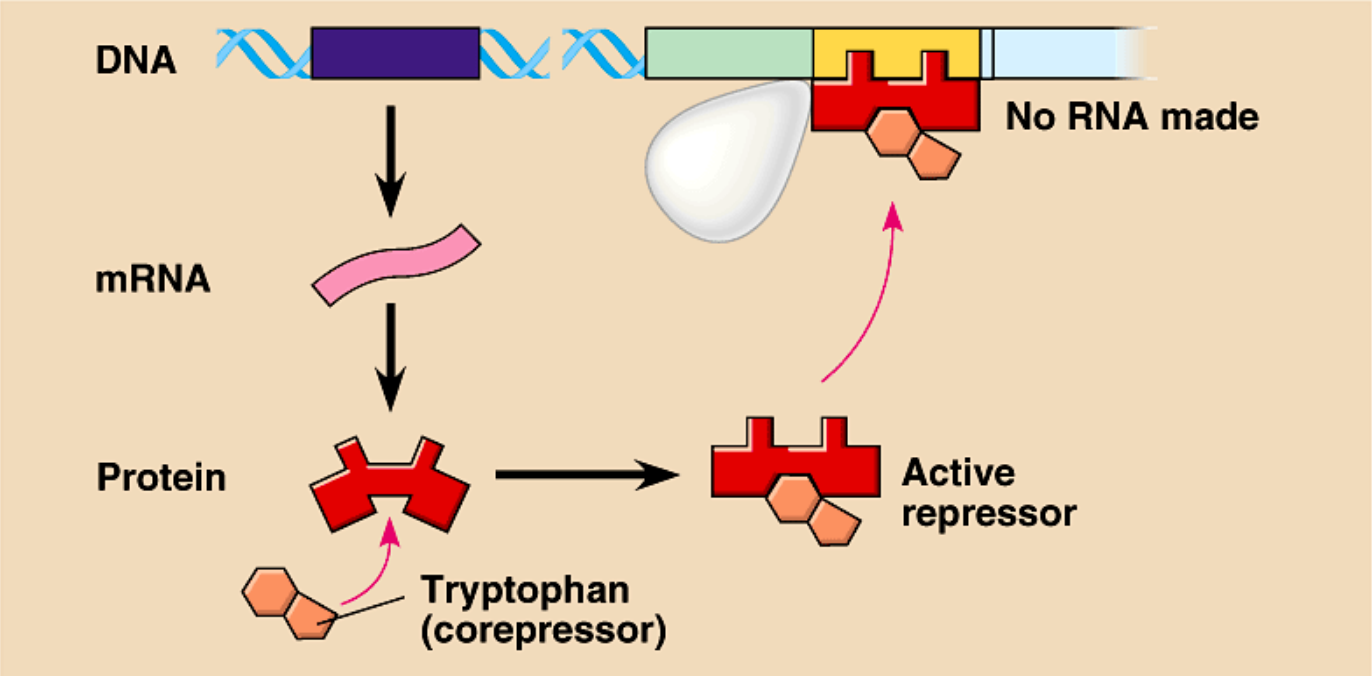
Bacteria group certain genes together

- **Operon**
  - genes with related function grouped together in the DNA
    - promoter = RNA polymerase DNA binding site
    - operator = DNA binding site of repressor protein
- **Repressor** is coded by a gene upstream from the trp operon = **the trpR gene** *(which has its own promotor)*



So how can these genes be turned off?

- **Repressor protein**
  - binds to DNA at operator site
  - blocking RNA polymerase from binding the promoter
    - blocks transcription



Operon model

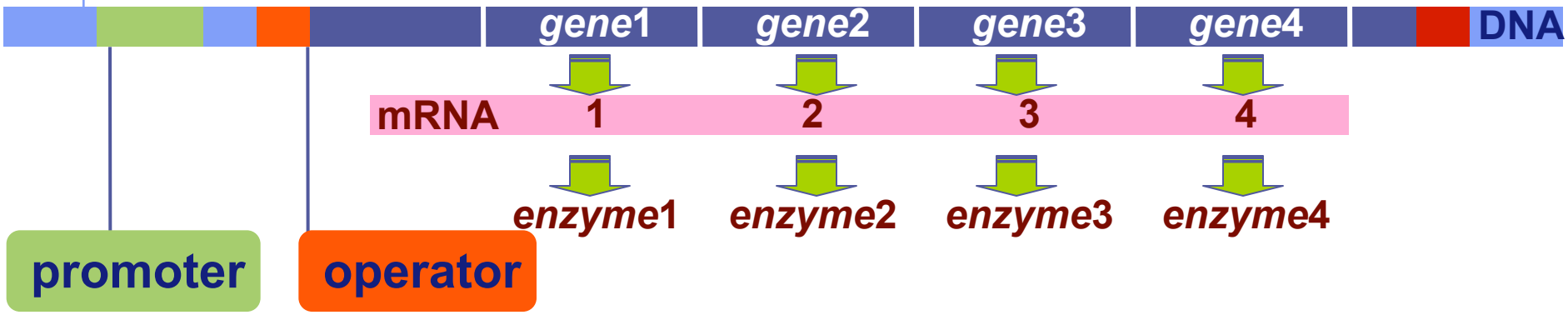
Operon (operator, promoter & genes they control) serve as a model for gene regulation

Repressor protein turns **off** gene expression by blocking RNA polymerase from binding to the promoter, preventing transcription of the genes.

repressor = repressor protein

Operon model

Operon (operator, promoter & genes they control) serve as a model for gene regulation



Repressor protein turns **off** gene expression by blocking RNA polymerase from binding to the promoter, preventing transcription of the genes.

repressor = repressor protein

Operon model

Operon (operator, promoter & genes they control) serve as a model for gene regulation

Repressor protein turns off gene expression by blocking RNA polymerase from binding to the promoter, preventing transcription of the genes.

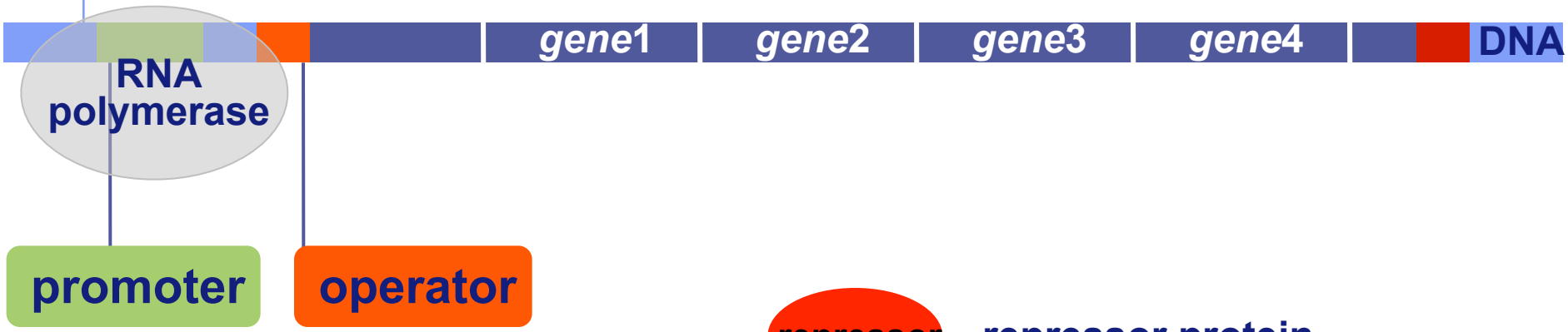
repressor = repressor protein

Repressible operon (operon "on" until repressed)
Ex: Tryptophan Operon (Trp Operon)

Synthesis pathway model

When excess tryptophan is present, it binds to *tryp* repressor protein & triggers repressor to bind to DNA

- **blocks (represses) transcription**



repressor — repressor protein

trp — tryptophan

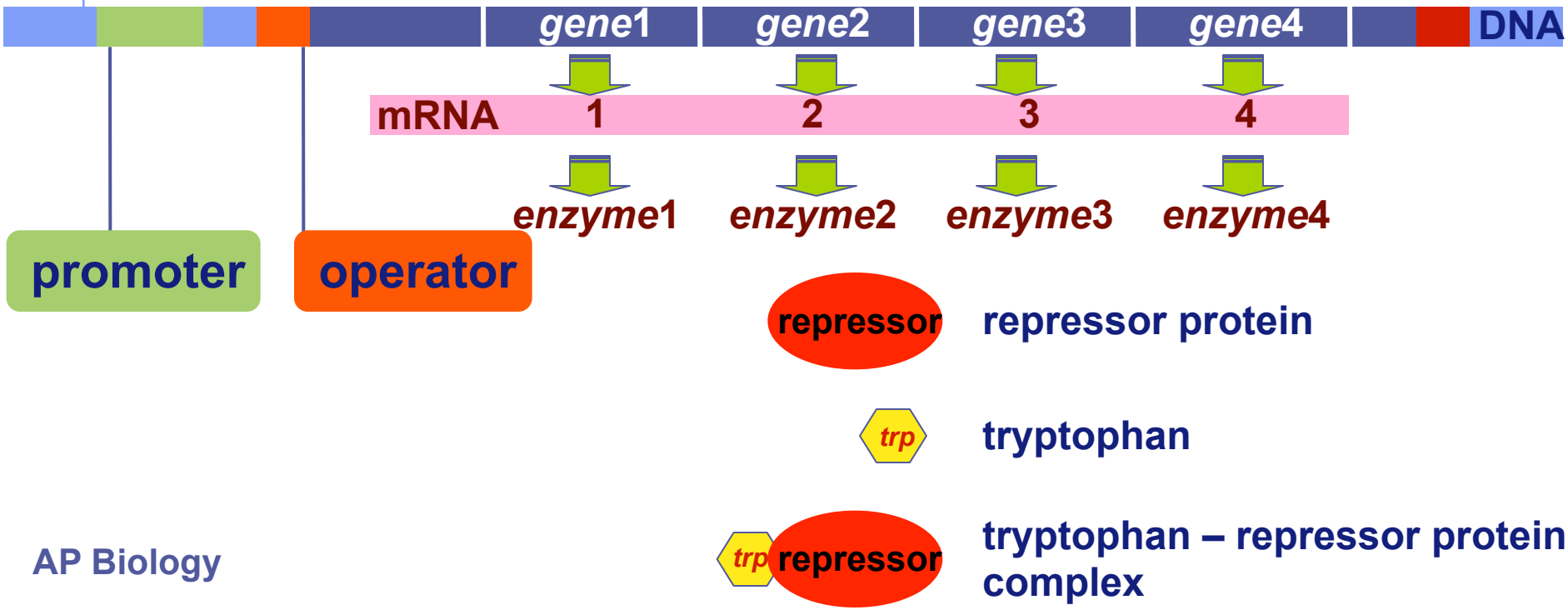
trp repressor — tryptophan – repressor protein complex

Repressible operon (operon "on" until repressed)
Ex: Tryptophan Operon (Trp Operon)

Synthesis pathway model

When excess tryptophan is present, it binds to *tryp* repressor protein & triggers repressor to bind to DNA

- **blocks (represses) transcription**

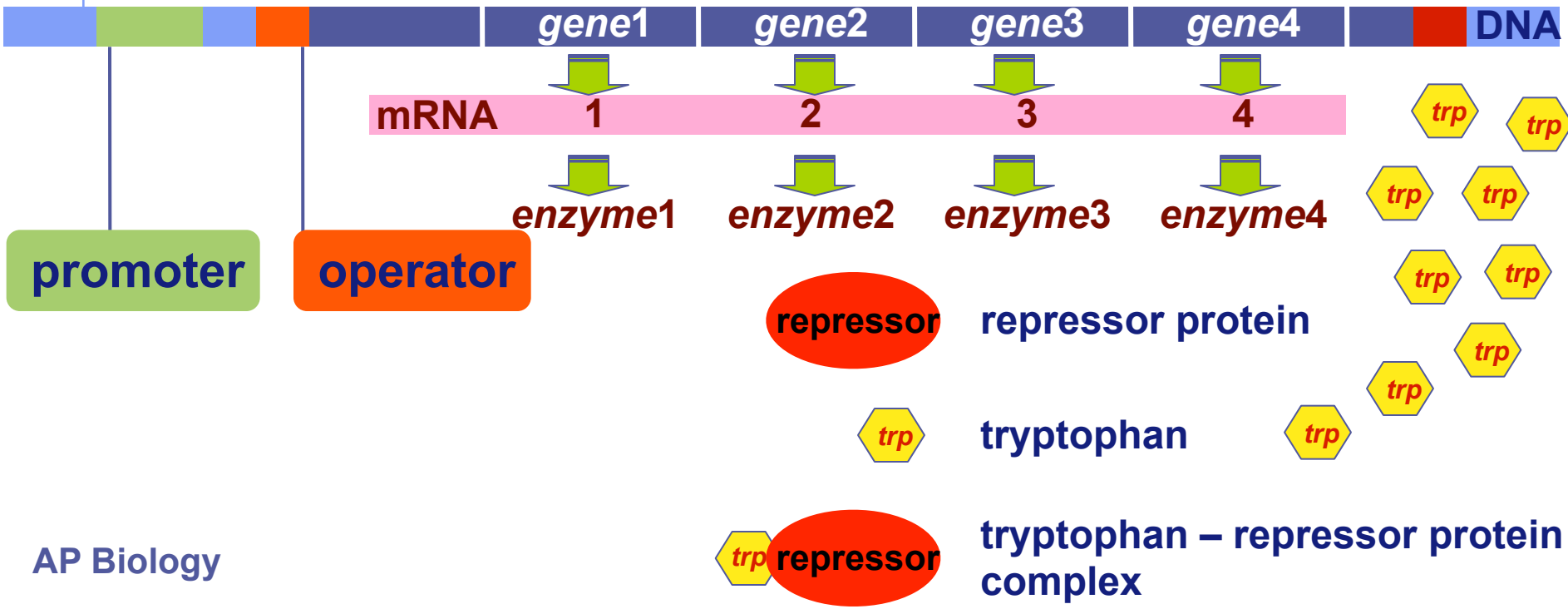


Repressible operon (operon "on" until repressed)
Ex: Tryptophan Operon (Trp Operon)

Synthesis pathway model

When excess tryptophan is present, it binds to *tryp* repressor protein & triggers repressor to bind to DNA

- **blocks (represses) transcription**

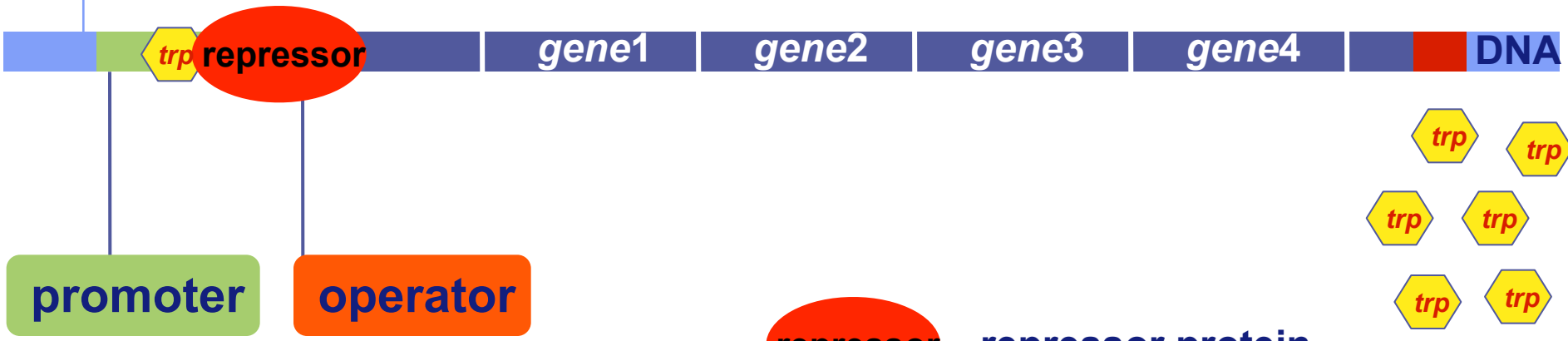


Repressible operon (operon "on" until repressed)
Ex: Tryptophan Operon (Trp Operon)

Synthesis pathway model

When excess tryptophan is present, it binds to *tryp* repressor protein & triggers repressor to bind to DNA

- **blocks (represses) transcription**



repressor — repressor protein

trp — tryptophan

trp repressor — tryptophan – repressor protein complex

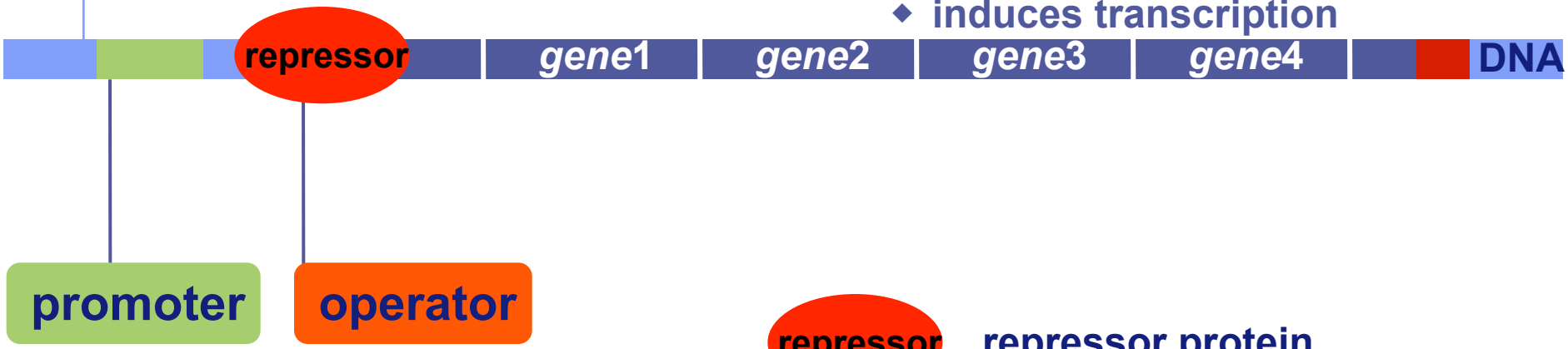
conformational change in repressor protein allows it to bind to the operator!

Inducible operon (operon "off" until activated)
Ex: Lactose Operon (Lac Operon)

Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

- induces transcription



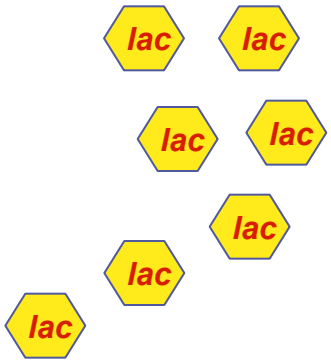
repressor — repressor protein

lac — lactose

lac repressor — lactose – repressor protein complex

Inducible operon (operon "off" until activated)

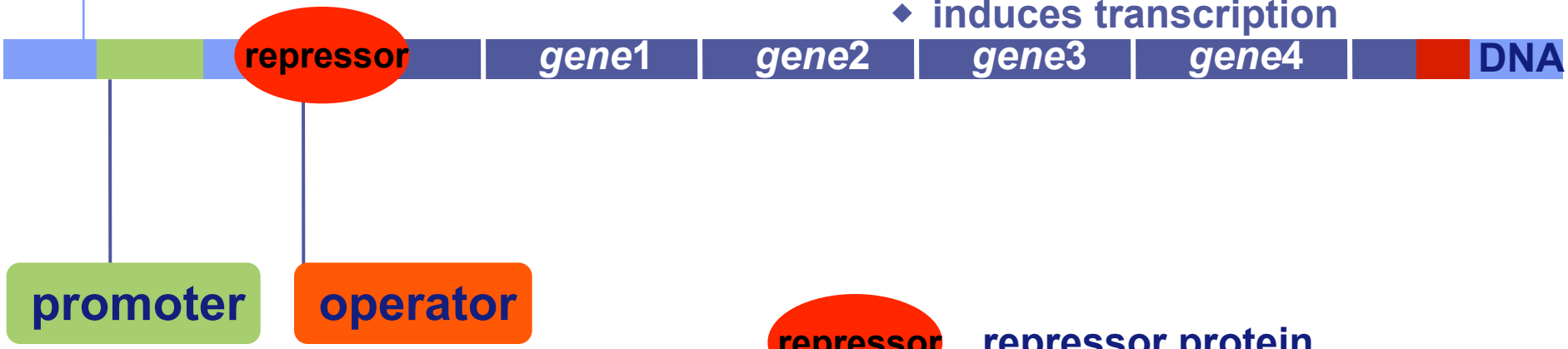
Ex: Lactose Operon (Lac Operon)



Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

- induces transcription

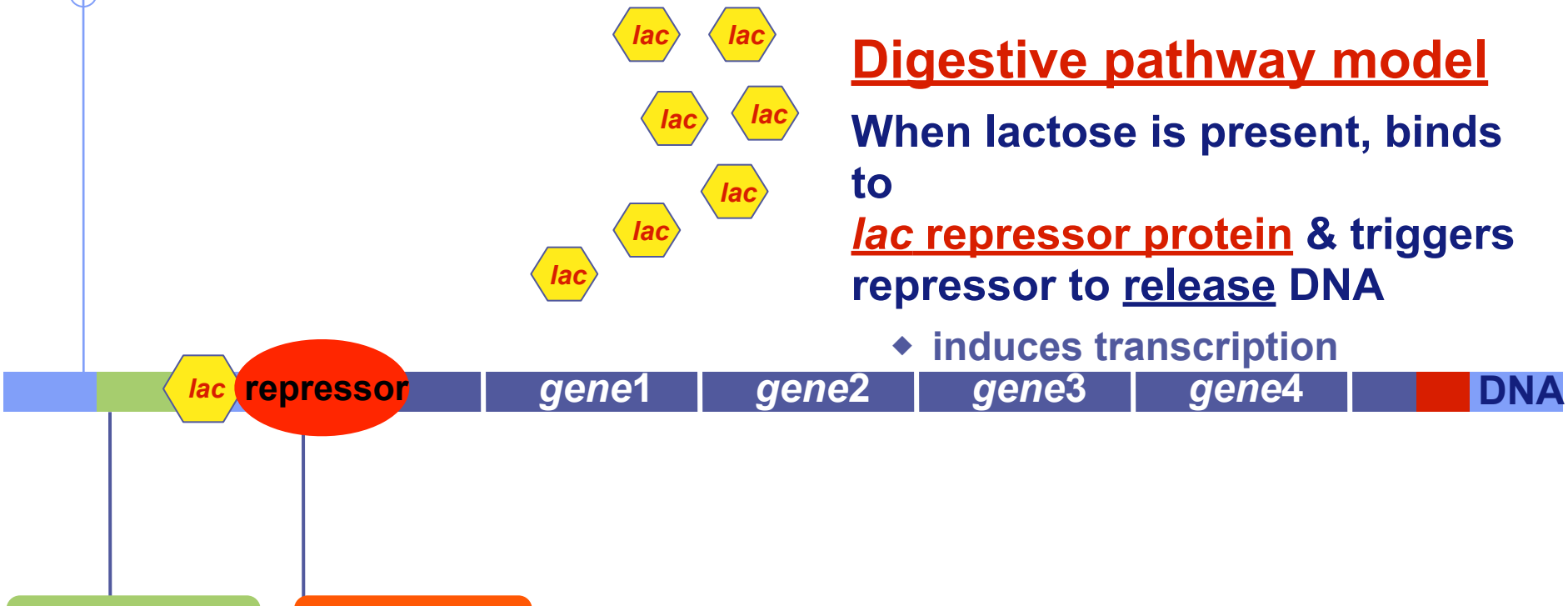


repressor — repressor protein

lac — lactose

lac repressor — lactose – repressor protein complex

Inducible operon (operon "off" until activated)
Ex: Lactose Operon (Lac Operon)



Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

- induces transcription

repressor — repressor protein

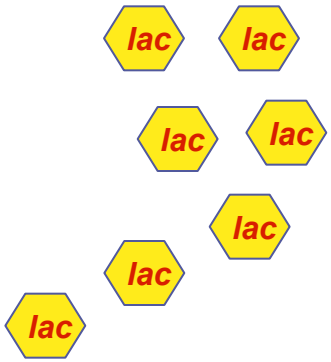
lac — lactose

lac repressor — lactose – repressor protein complex

conformational change in repressor protein!

Inducible operon (operon "off" until activated)

Ex: Lactose Operon (Lac Operon)



Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

- induces transcription

promoter

operator

conformational change in repressor protein!

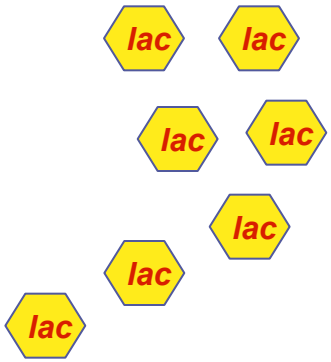
repressor — repressor protein

lac — lactose

lac repressor — lactose – repressor protein complex

Inducible operon (operon "off" until activated)

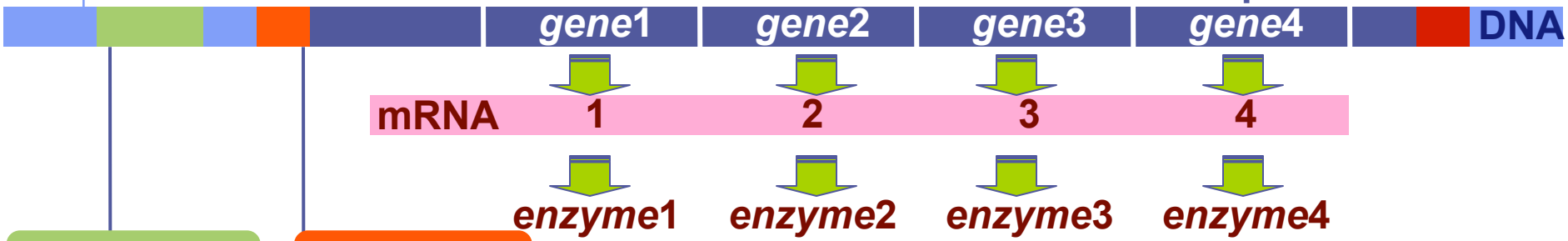
Ex: Lactose Operon (Lac Operon)



Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

- induces transcription



promoter

operator

repressor — repressor protein

lac — lactose

lac repressor — lactose – repressor protein complex

conformational change in repressor protein!

Lactose operon - *Inducible operon*

(normally off unless turned on)

E. coli preferentially use glucose as a source of energy and carbon. But it can use lactose in the absence of glucose.

What happens when lactose is present?

Need to make lactose-digesting enzymes!!!

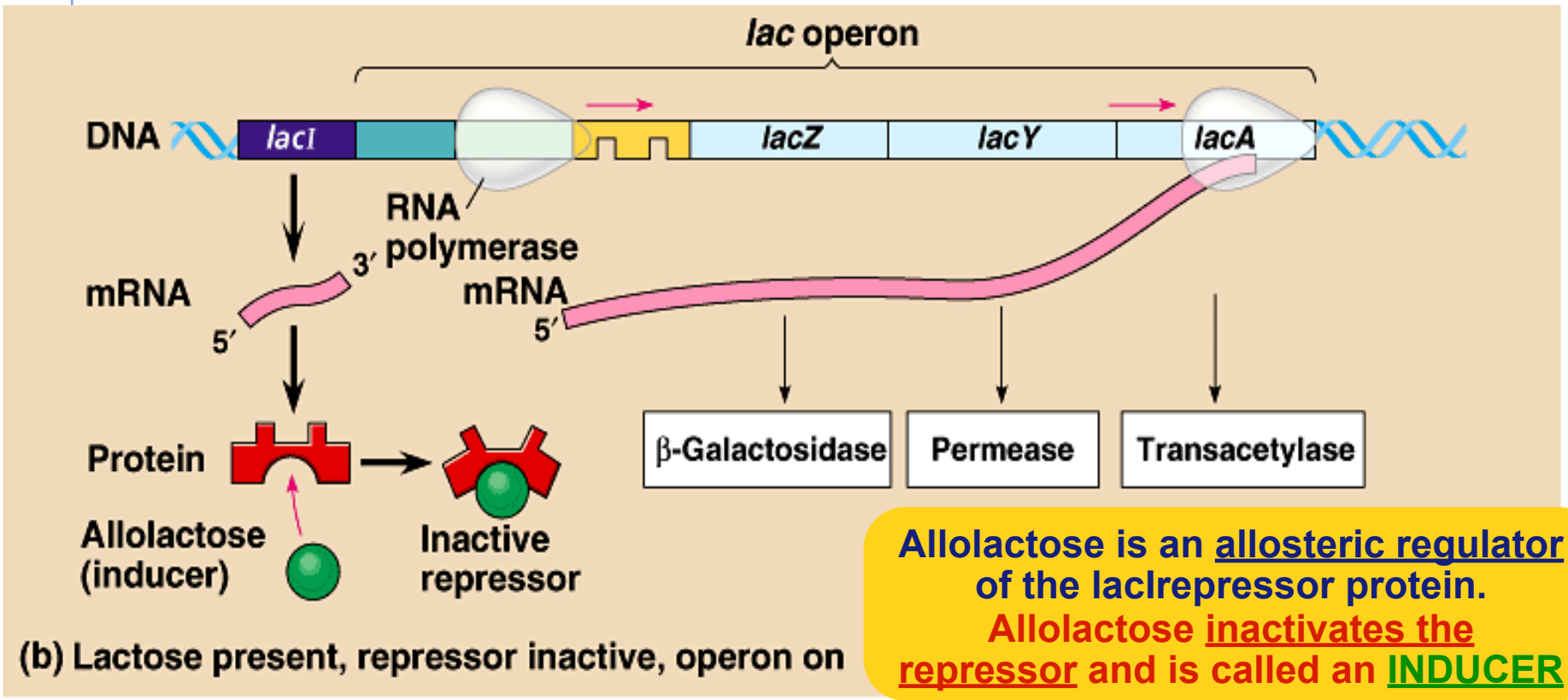
The disaccharide lactose is metabolized by the enzyme β-galactosidase which hydrolyzes it into monosaccharides glucose and galactose.

- **Gene for this enzyme is part of the lac operon.**

A repressor protein is coded by the regulatory gene, *lacI* located outside the operon.

Allolactose is an isomer formed from lactose
(when lactose is present, some of it forms allolactose)

- When lactose is absent, repressor is active & lac operon genes or turned OFF.
- When lactose is present, allolactose binds the lac repressor, altering its conformation, making the repressor inactive & incapable of binding the operator, turning the operon's structural genes ON.

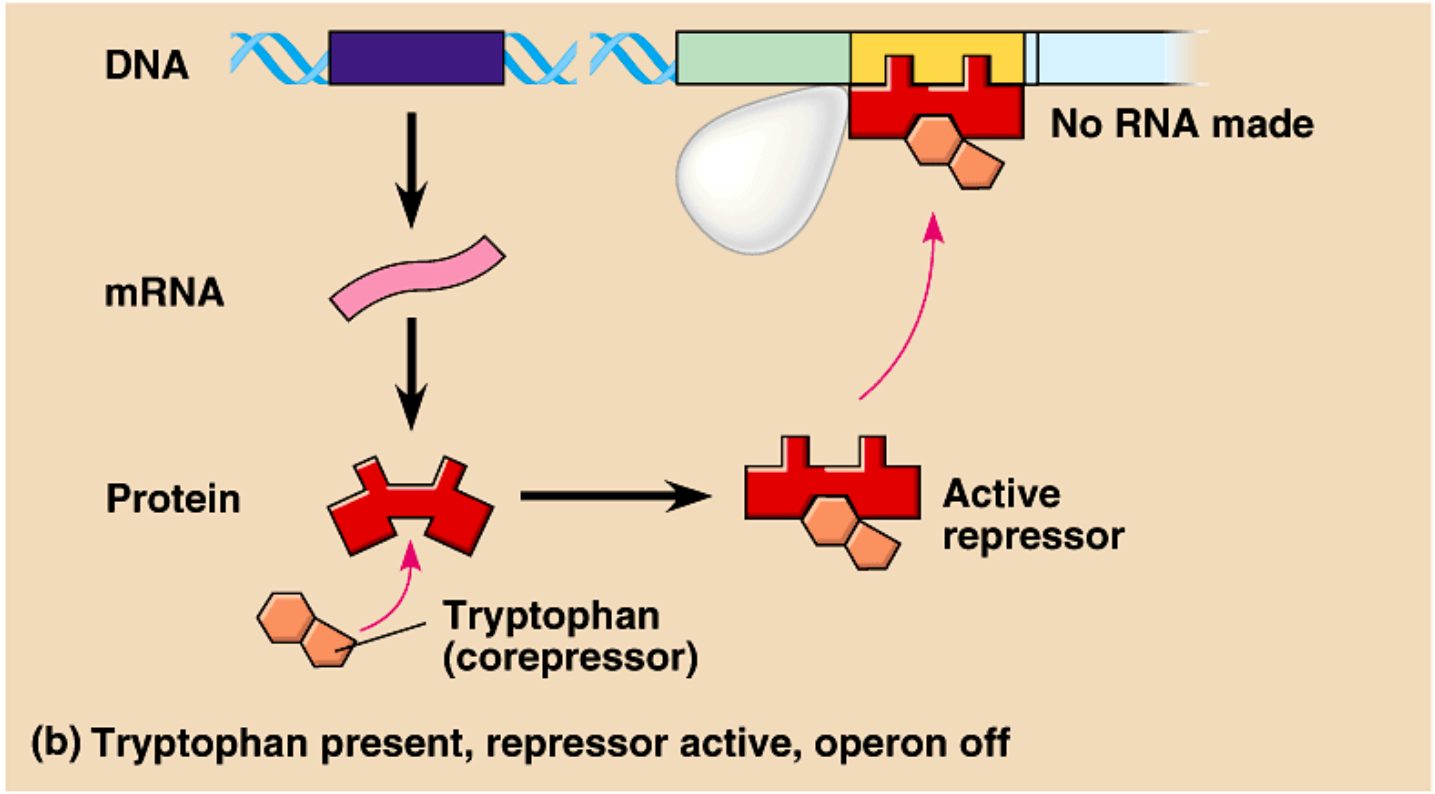


Allolactose is an allosteric regulator of the lacIrepressor protein. Allolactose inactivates the repressor and is called an INDUCER

Tryptophan operon

What happens when tryptophan is present in high concentration?

Don't need to make tryptophan-building enzymes!!!



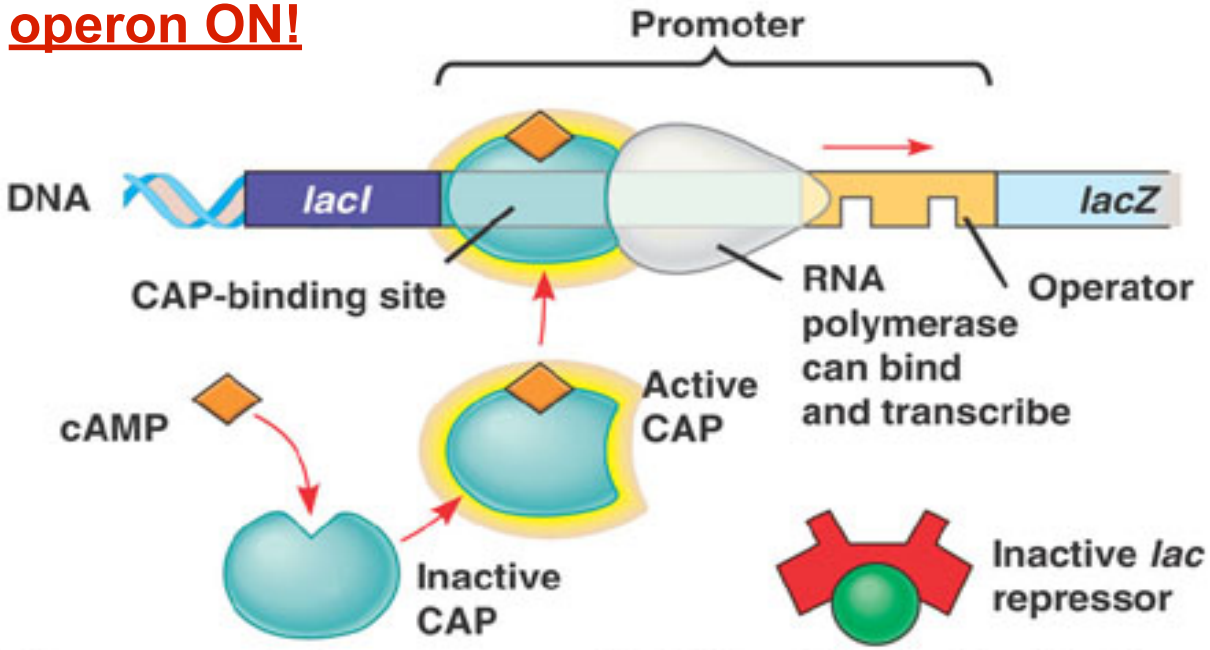
Tryptophan is allosteric regulator (a COREPRESSOR) of the repressor protein

Lac Operons Have a 2nd Level of Control

- In addition to lactose (or allolactose actually) inactivating the repressor there is a second level of regulation.
  - Lactose is not the preferred carb source for *E. coli.*
    - If lactose and glucose are present, the cell will use all of the glucose before the lac operon is turned on even with lactose present!
      - But....*How is the lactose operon not turned on fully when lactose is present if glucose is still present?*
- A second level of control of gene expression exists.
  - The promoter of the lac operon has two binding sites.
    - One for RNA Polymerase & one for a CAP (Catabolite Activator Protein) also known as CRP (cAMP Receptor Protein).
      - CAP/CRP binds to the promoter as a complex between the catabolite activator protein (CAP/CRP) and cyclic AMP (cAMP).

The CAP-cAMP Complex

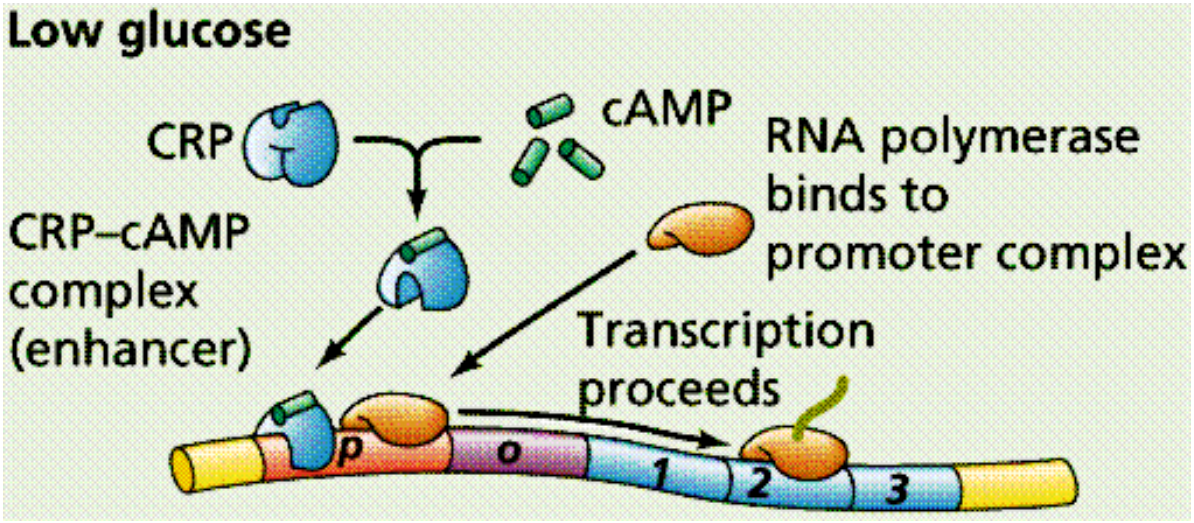
- The binding of the CAP-cAMP complex to the promoter site is REQUIRED for transcription of the lac operon.
  - If glucose is low, then AMP is high and thus cAMP is high
    1. cAMP binds CAP
    2. CAP-cAMP Complex binds promotor
    3. Transcription of lactose operon occurs at a rapid rate = operon ON!



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

The CAP-cAMP Complex

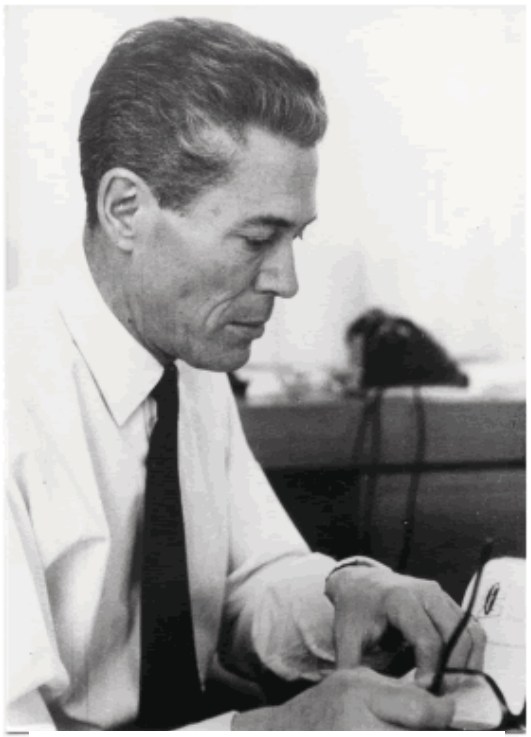
- The binding of the CAP-cAMP complex to the promoter site is REQUIRED for transcription of the lac operon.
  - As the concentration of glucose increases the amount of cAMP decreases!
    - As the cAMP decreases, the amount of complex decreases.
    - This decrease in the complex means RNA Polymerase is less likely to bind to the promoter
      - The lac operon is turned off (even if lactose is present).



Jacob & Monod: *lac* Operon

1961 | 1965

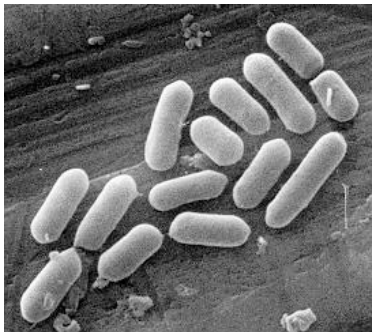
- Francois Jacob & Jacques Monod
  - first to describe operon system
  - coined the phrase "operon"



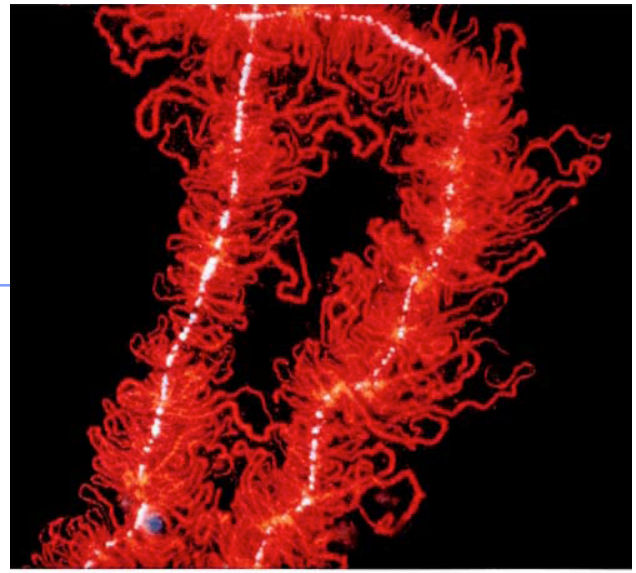
Jacques Monod

Francois Jacob

Operon summary



- Repressible operon
  - usually functions in anabolic pathways
    - synthesizing end products
  - when end product is present in excess, cell allocates resources to other uses
- Inducible operon
  - usually functions in catabolic pathways,
    - digesting nutrients to simpler molecules
  - produce enzymes only when nutrient is available
    - cell avoids making proteins that have nothing to do, cell allocates resources to other uses



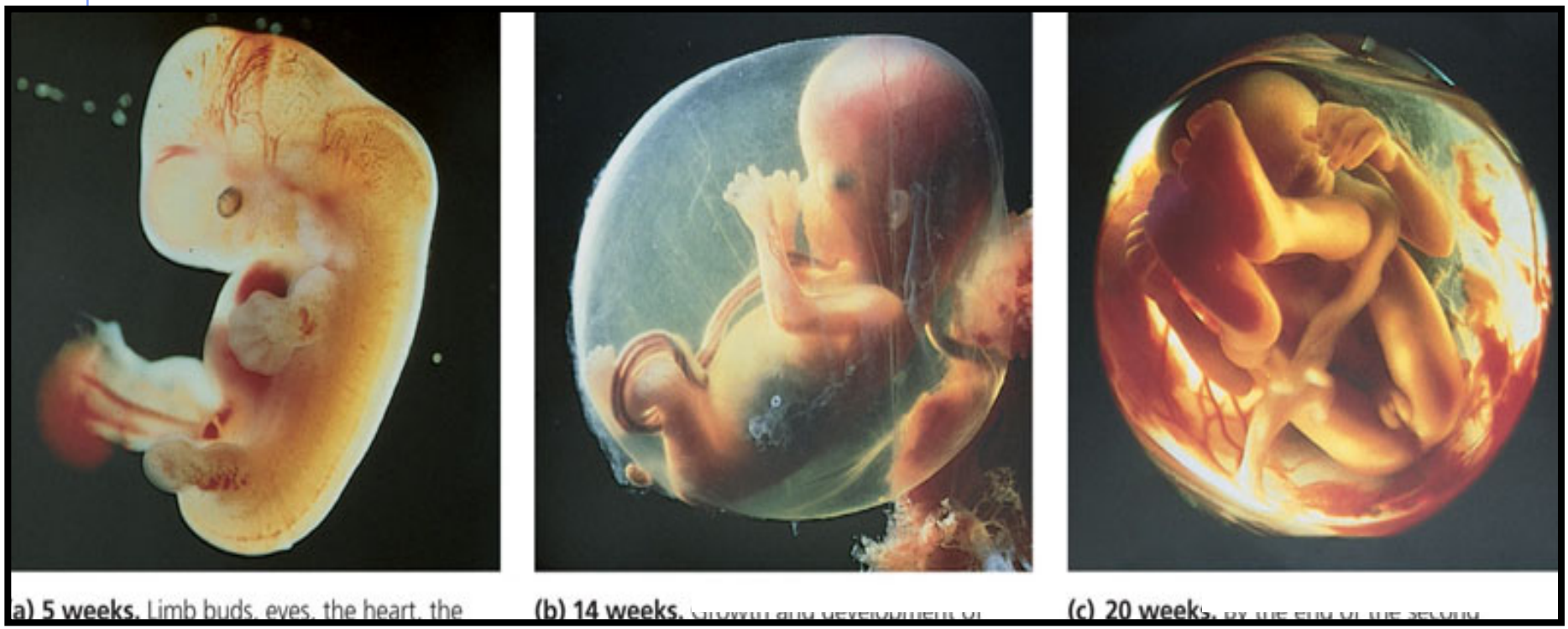
Ch. 18:

Control of the Eukaryotic Genome

2007-2008

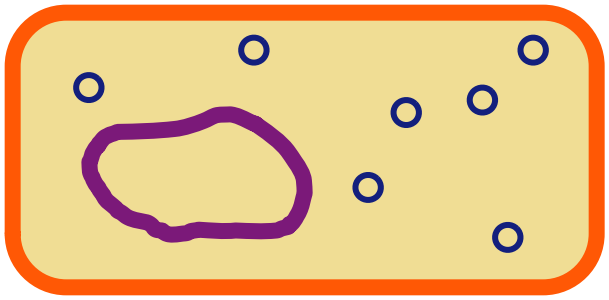
The BIG Questions…

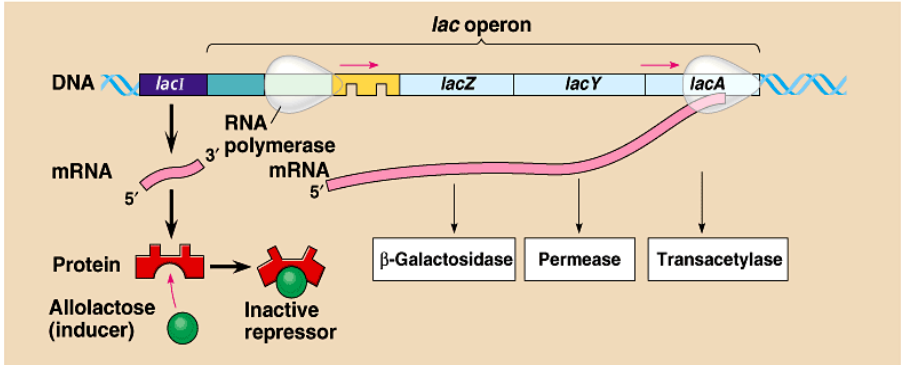
- How are genes turned on & off in eukaryotes?
- How do cells with the same genes "differentiate" to perform completely different, specialized functions?
- Every cell has the same copy of genes so differential gene expression must occur in cells



Prokaryote vs. eukaryote genome

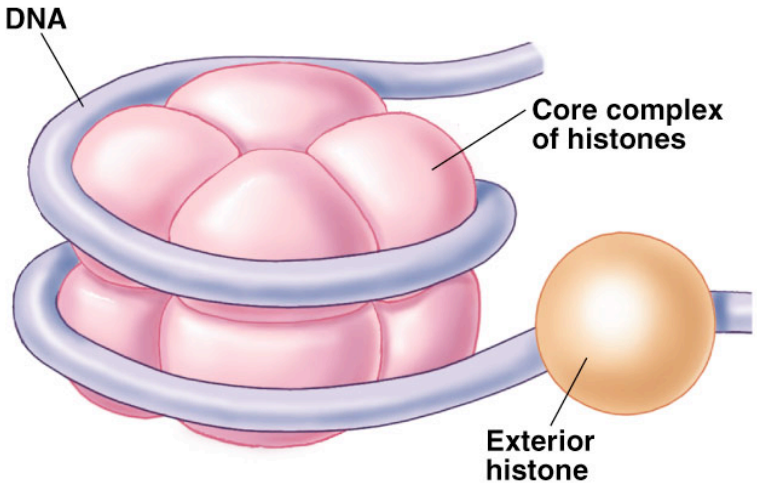
- **Bacterial Prokaryotes**
  - small genome size comparatively
  - circular molecule of naked DNA (no histone proteins)
    - DNA readily available to RNA polymerase so most genes "on"
      - on/off control of transcription happens through regulatory proteins like repressors or CRP/CAP activator proteins for the few genes in operons whose expression can be activated if off and silenced if on
        - i.e. the operon system is NOT USED IN EUKARYOTES
  - most of DNA codes for protein or RNA
    - have no introns in genes, small non-coding DNA sequences within the transcriptional units (open reading frames) of genes
    - do have regulatory sequences: promoters as part of all genes and operators as part of operons





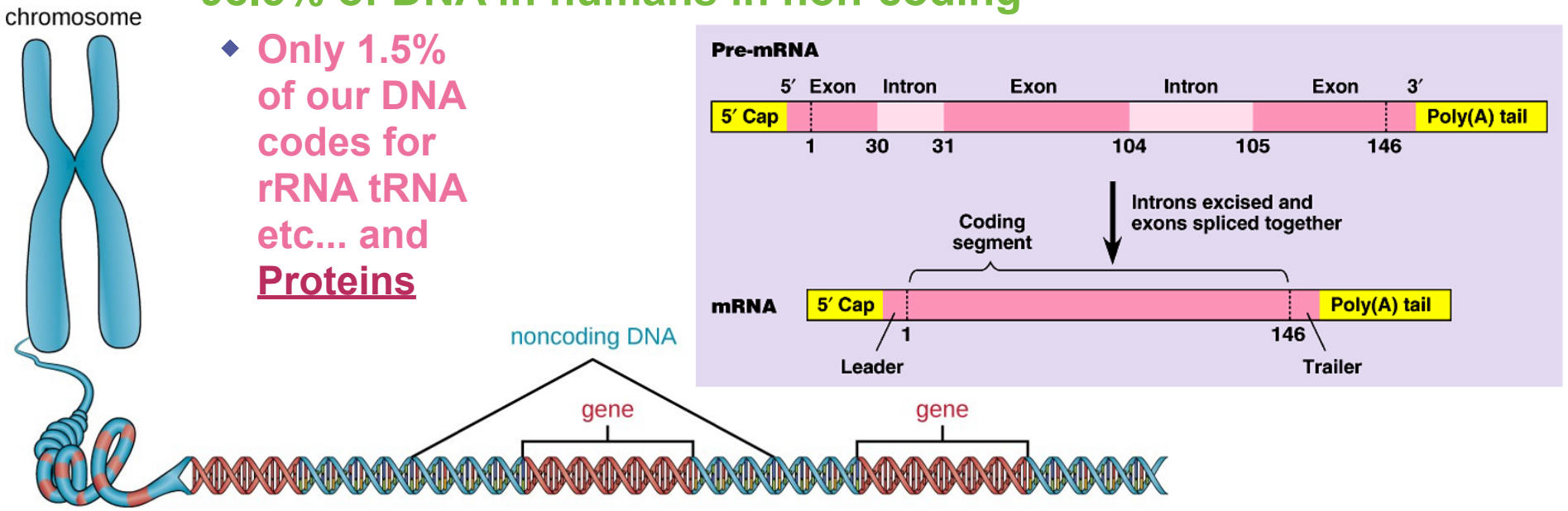
Prokaryote vs. eukaryote genome

- **Eukaryotes**
  - much greater size of genome comparatively
    - how does all that DNA fit nucleus?
      - DNA packaged into chromatin fibers
        - The basic unit of chromatin is the nucleosome = DNA double helix wrapped round complexes of several histone proteins
    - chromatin helps regulate gene expression too
      - The location of nucleosomes along the DNA molecule helps regulate access to DNA (promoters) by RNA polymerase
        - Genes in the regions where the chromatin is more condensed (called heterochromatin) are not expressed ("off")
        - Genes in the regions where the chromatin is less condensed (called euchromatin) may be expressed (can be turned "on")
      - Chemical modifications to chromatin (both to histones and/or the DNA) can influence chromatin structure and thus expression



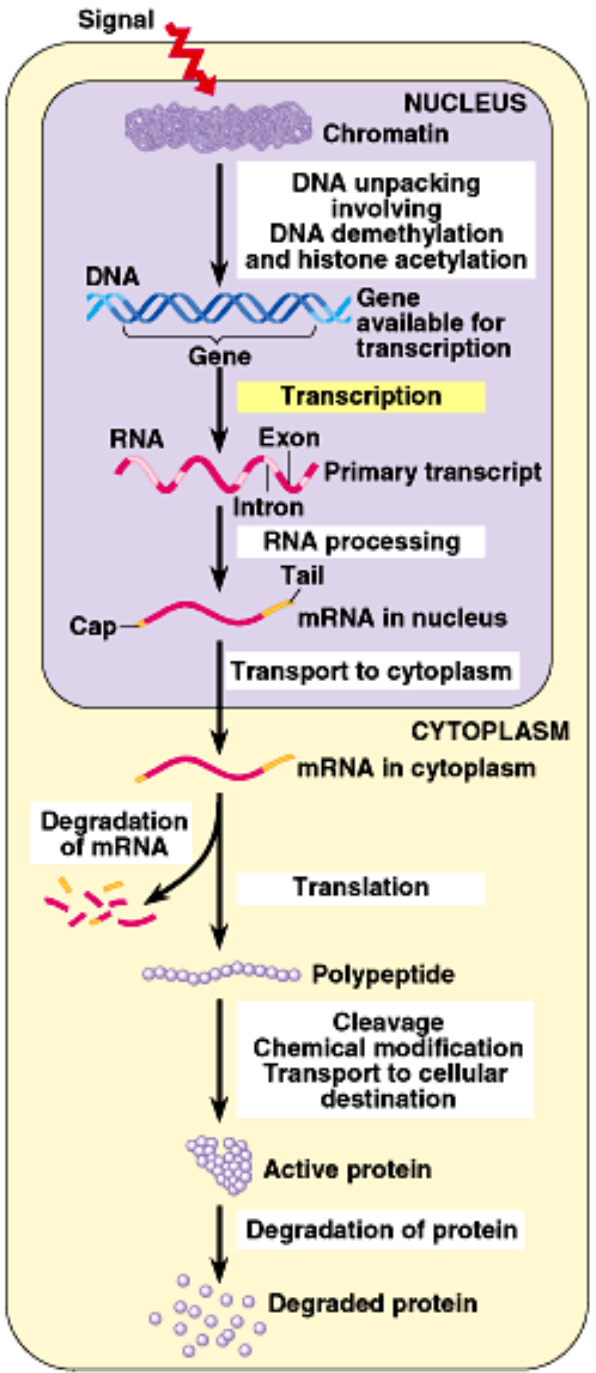
Prokaryote vs. eukaryote genome

- **In Eukaryotes**
  - different cells differentiate (they specialize), gaining distinct features and taking on distinct functions activities
    - specializing requires cells to selectively turn on & off a large numbers of genes at specific times, not all cells activating the same genes.
  - most of DNA does not code for protein (most DNA is not comprised of coding sequences)
    - 98.5% of DNA in humans in non-coding
      - Only 1.5% of our DNA codes for rRNA tRNA etc... and Proteins



Points of Control of Gene Expression in Eukaryotes

- **The control of gene expression can occur at any step in the pathway from gene to functional protein:**
  1. **unpacking DNA - regulating chromatin condensation** *(gene accessibility by RNA Pol)*
  2. **transcription - there can be no, some, or high expression of gene** *(RNA transcript production)*
  3. **mRNA processing - alternative splicing** *(creating variations of a polypeptide)*, **varying length of poly-A tail** *(influencing how many copies of the polypeptide will be created by controlling how fast mRNA is degraded)*, **mRNA cap added** *(to protect mRNA from degradation by nucleases in cytoplasm)*
  4. **mRNA transport - pore complexes control movement of mRNA out of the nucleus**



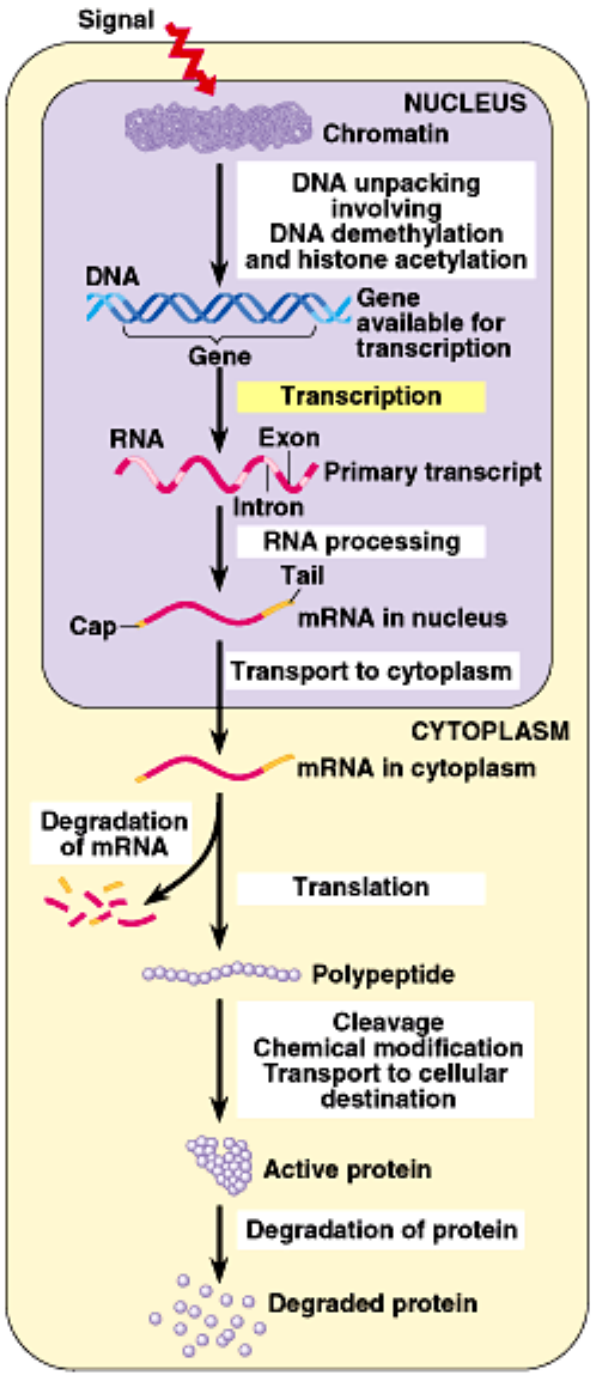
Points of Control of Gene Expression in Eukaryotes

- **The control of gene expression can occur at any step in the pathway from gene to functional protein:**

5. **translation** - mRNA degradation with small RNA binding and dicer protein or blocking ribosomal translation using small RNA to mRNA binding *(prevent polypeptide, protein, formation)*

6. **protein processing** - altering protein structure *(and thus function)* by making changes to the primary structure via chemical modification or cleavage of the polypeptide and altering final protein activity using activators, inhibitors, phosporylation, etc...

7. **protein degradation** - tagging proteins for destruction with ubiquitin *(causing them to then get hydrolyzed into amino acids)*

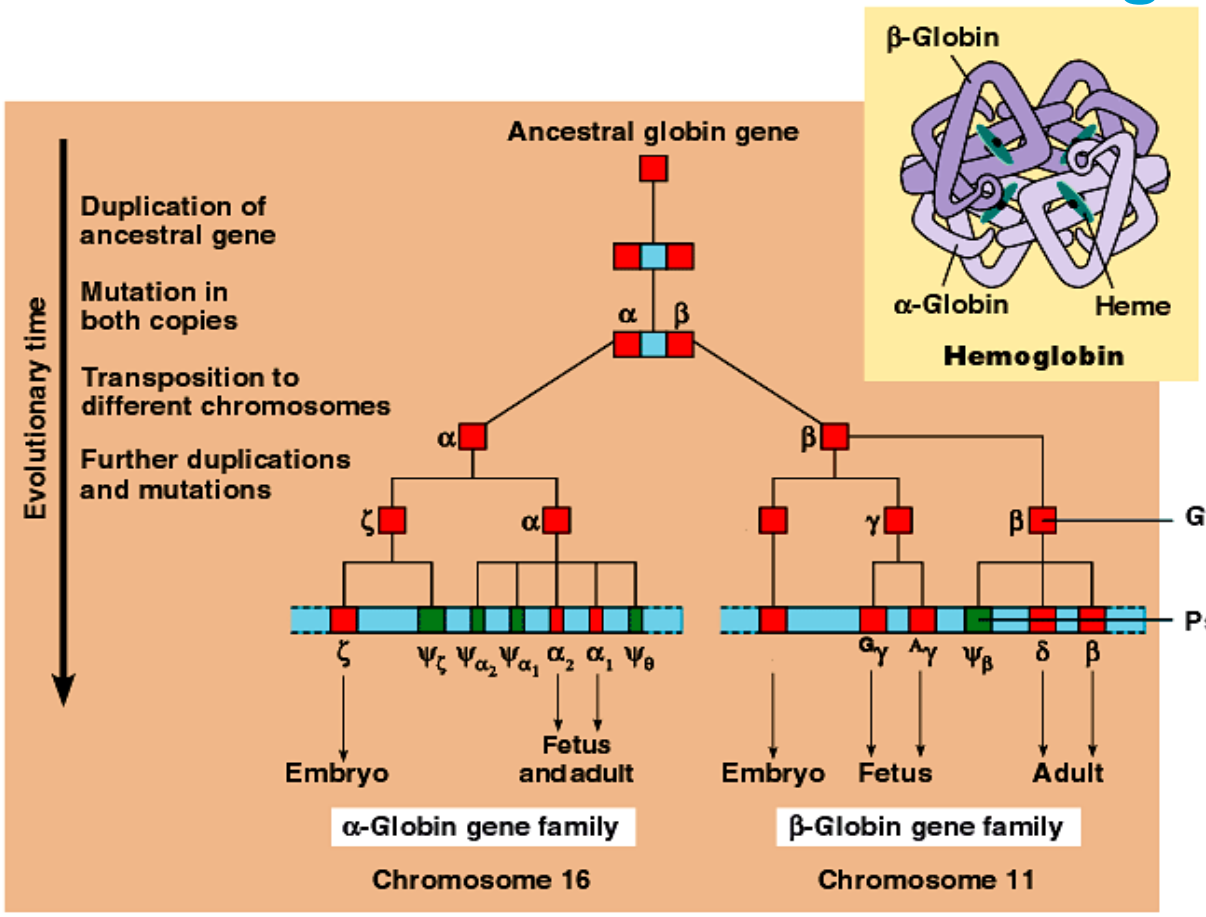


Why turn genes on & off?

- **Cell Specialization *(differentiation)***
  - each cell of a multi-cellular eukaryote expresses only a small fraction of its genes, producing those proteins needed for that cell's specialized functions
- **Development**
  - different genes need to be turned on or off at different points in the life cycle of an organism
    - Remember needing the proteins to do apoptosis to make the individual digits during fetal development?
- **Responding to organism's needs**
  - Living organisms must maintain homeostasis & engage in repair/growth
    - cells of multicellular organisms must continually turn certain genes on & off in response to signals from their external & internal environment

Families of genes exist within the genome

- Human globin gene family
  - evolved from duplication mutations of the common ancestral globin gene



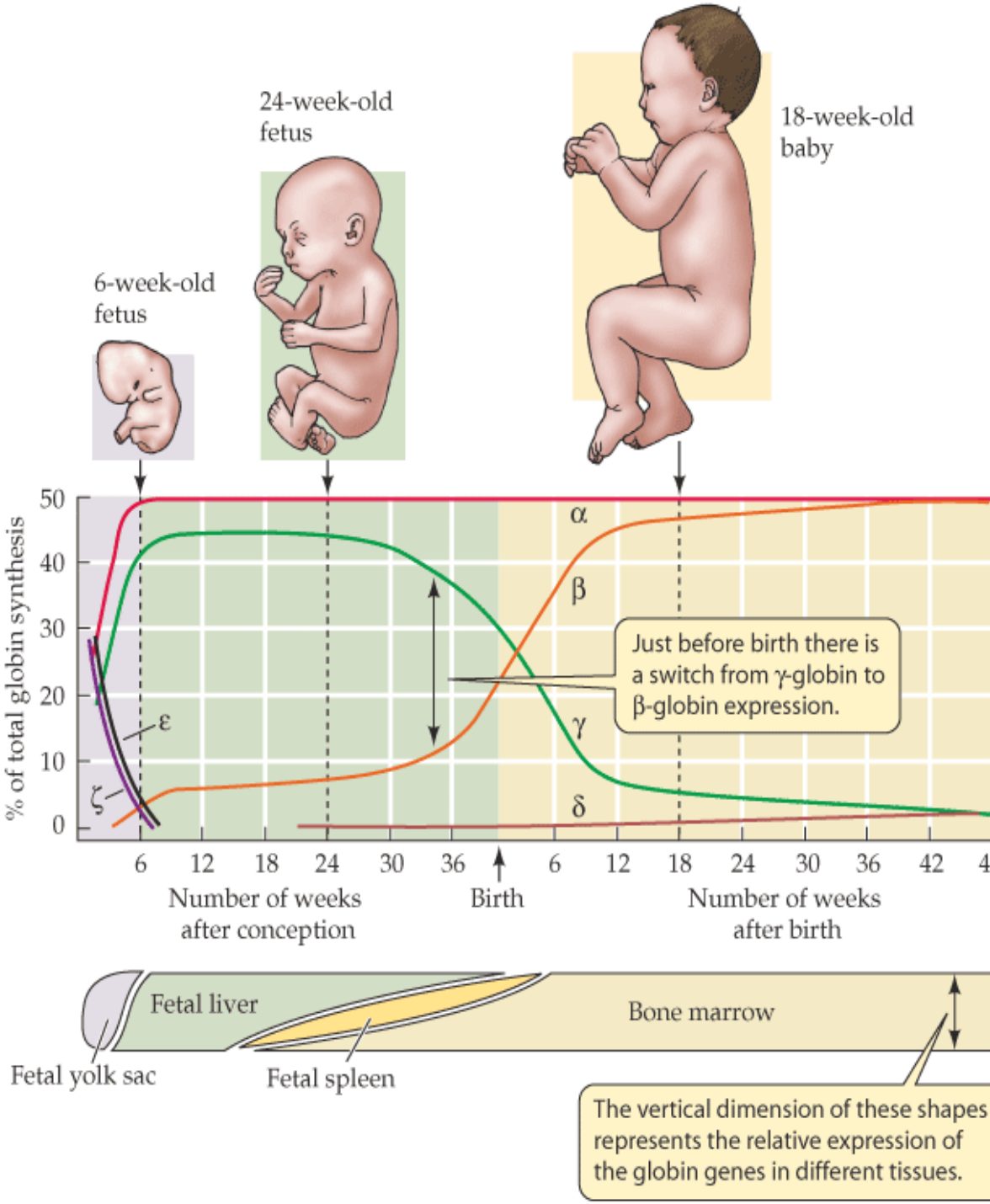
Today, different versions of the globin gene are expressed at different times in development, allowing hemoglobin to function throughout various life stages of a developing animal

- *In the human fetus, the alpha and gamma globin polypeptides make up fetal hemoglobin*
- *After birth, humans hemoglobin is instead composed of alpha and beta globin polypeptides*

Hemoglobin

Differential expression of different beta globin genes ensures important physiological changes during human development

- **fetal hemoglobin is able to bind oxygen with *greater affinity* than the adult form, giving the developing fetus better access to oxygen from the mother's bloodstream.**
  - **fetal hemoglobin is nearly completely replaced by adult hemoglobin by 6 months postnatally.**
  - **In adults, fetal hemoglobin production can be reactivated pharmacologically = useful in the treatment of diseases such as sickle-cell disease**

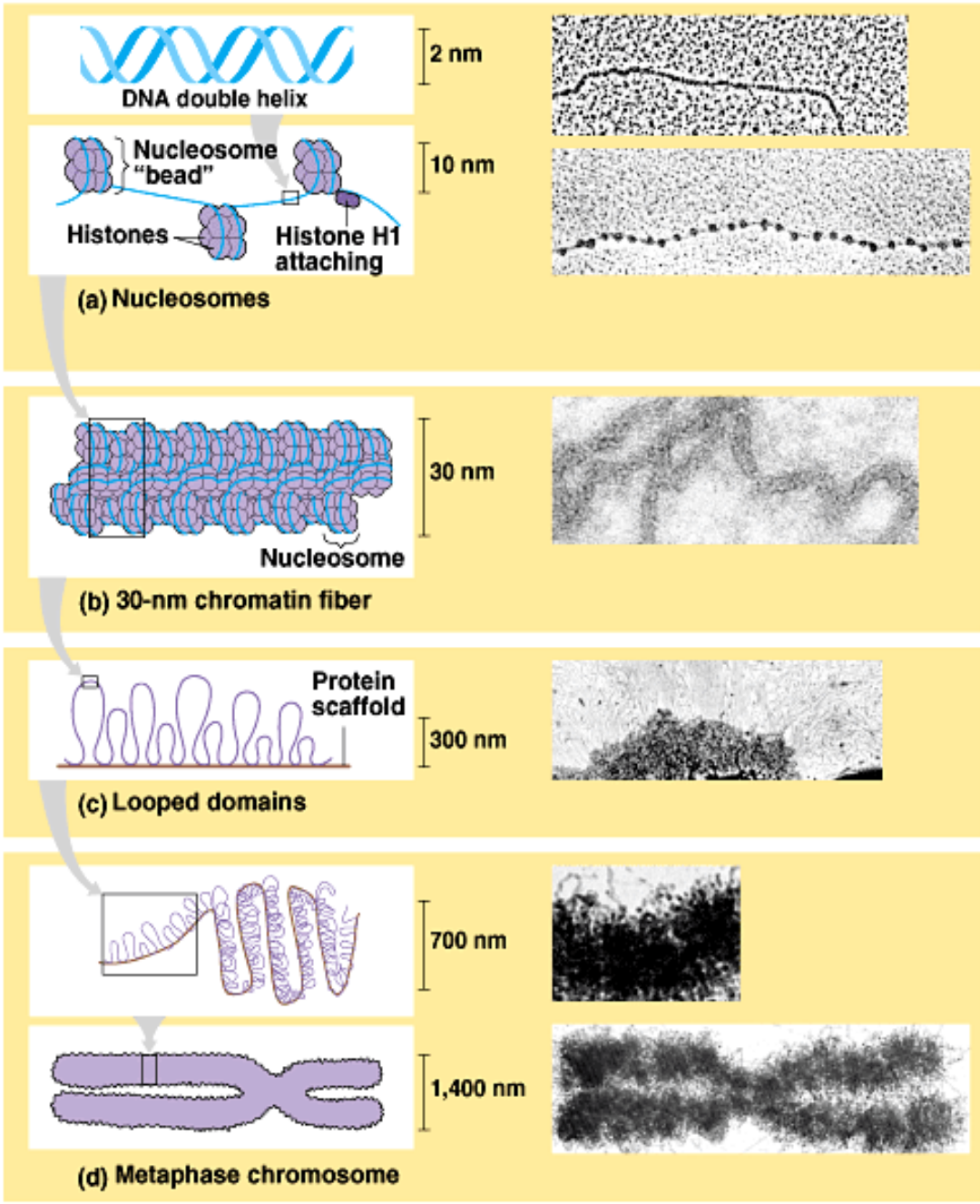


DNA packing

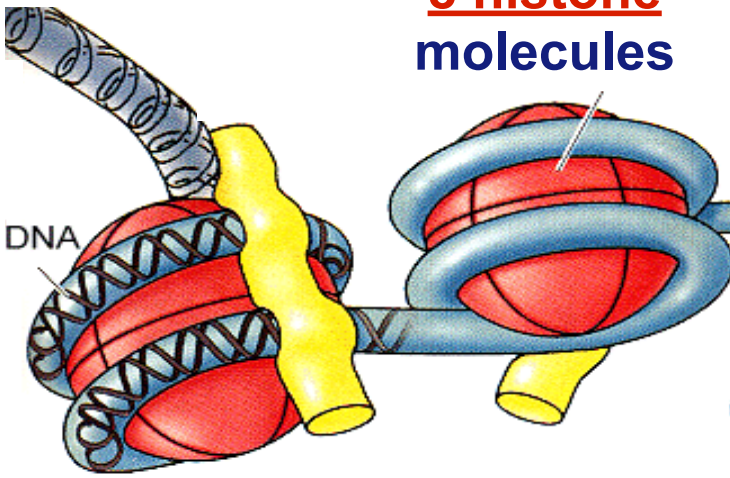
How do you fit all that DNA into nucleus?

- **DNA coiling & folding**
  1. double helix looped around histones into nucleosomes (beads on a string)
     1. First level of DNA packing
  2. chromatin fiber
  3. looped domains creating rosettes
  4. Further condensation into chromosome

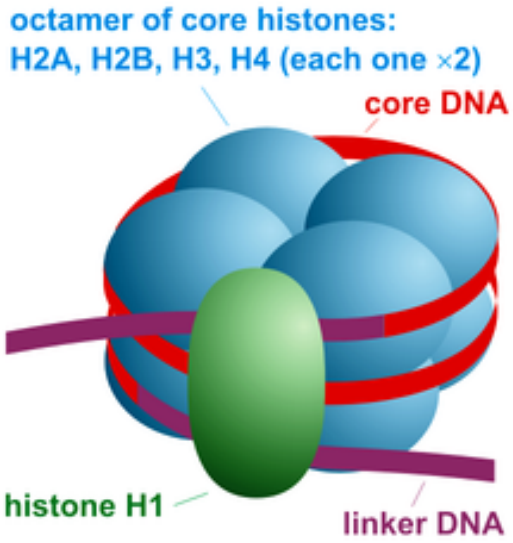
from DNA double helix to condensed chromosome

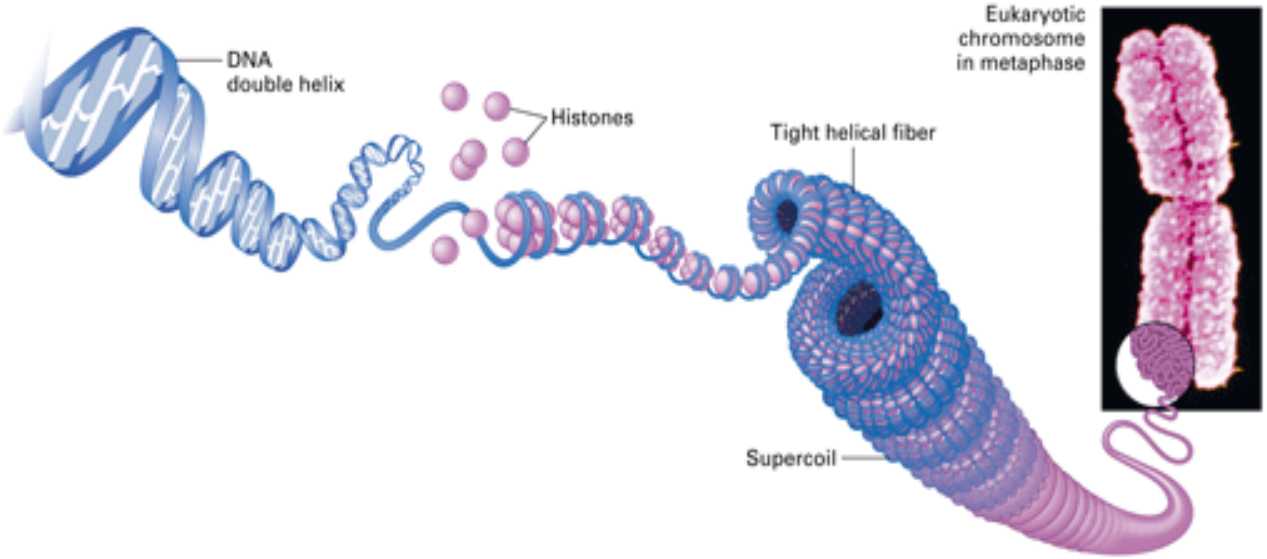


Nucleosomes



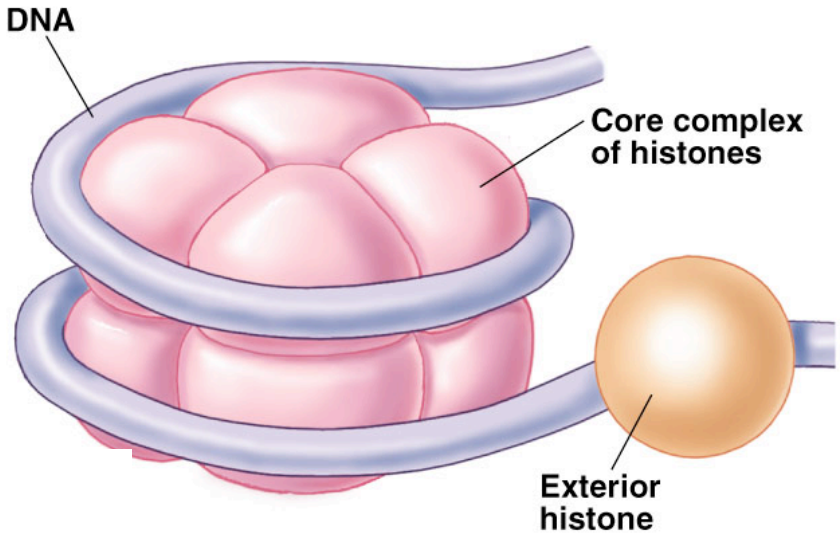
- "Beads on a string"
  - 1st level of DNA packing
    DNA wrapped around 8 proteins
    - histone proteins specifically
      - Tails of histone proteins are made up of positively charged amino acids!
        - arginine & lysine (have positive side groups)
          - bind "tightly" to negatively charged DNA

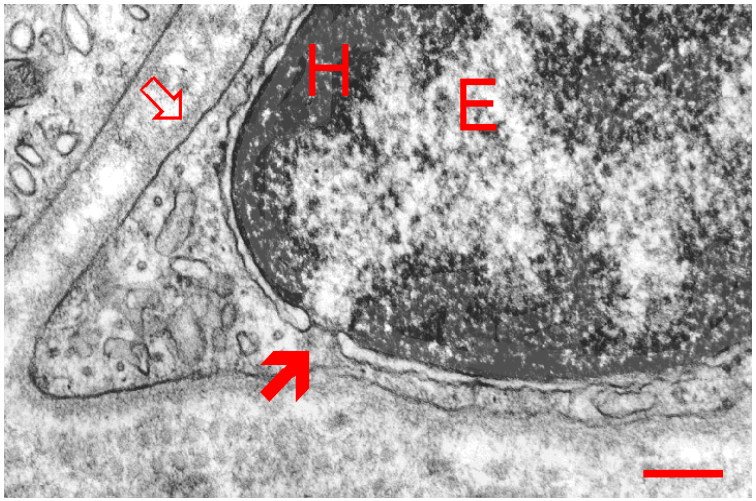




DNA packing

- Degree of packing of DNA regulates transcription
  - tightly packed DNA = no transcription since genes turned off
  - The location of a gene's promoter relative to the nucleosome and the sites where the DNA attaches to the chromosome scaffold or nuclear lamina can affect if a gene is transcribed
    - Heterochromatin: high condensed DNA / Chromatin
      - Genes not expressed
    - Euchromatin: loosely organized DNA / Chromatin
      - Genes accessible by RNA polymerase

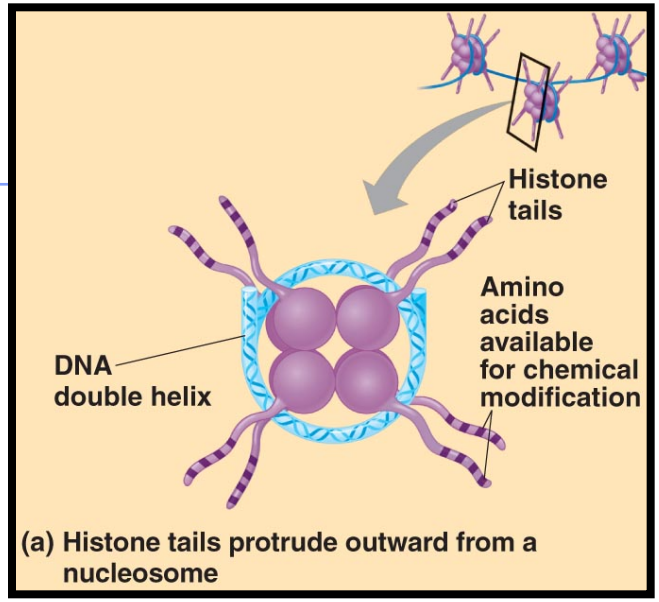


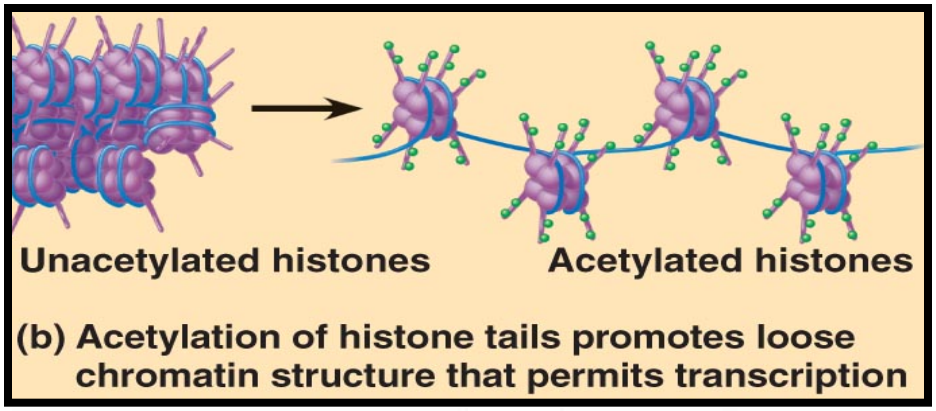


darker DNA (H) = tightly packed
lighter DNA (E) = loosely packed

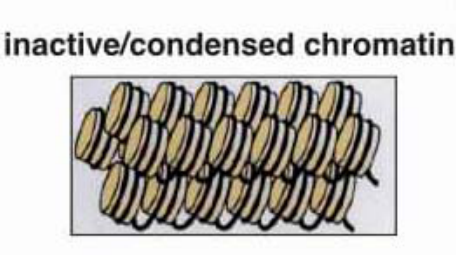
Histone Modification

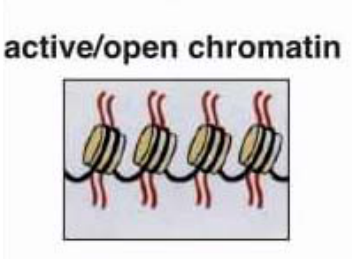
- Histone (polypeptide) "tails" protrude outward from nucleosome.
  - These N-terminal ends can be chemically modified.
    1. Histone Acetylation
    2. Histone Methylation
       - Chromatin condensation
    3. Histone Phosphorylation
       - Chromatin elongation





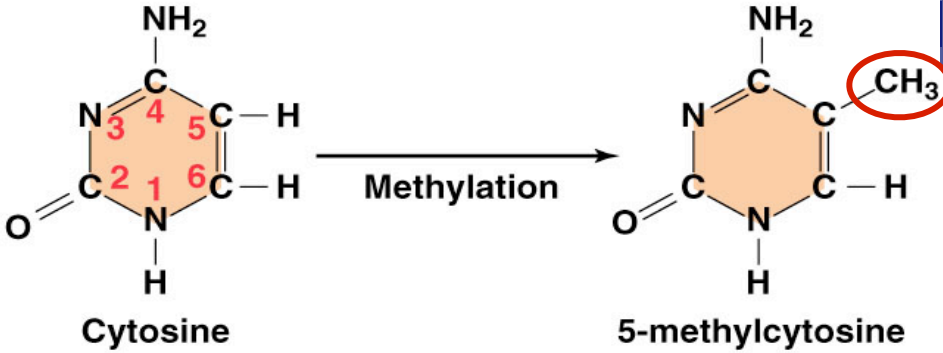
- Acetylation of histones unwinds DNA
  - attachment of acetyl groups ($-COCH_3$) to histones
    - Neutralizes positive charges of Lysines
    - Negative DNA no longer attracted strongly to the histones
    - Conformational change of histone occurs
    - Nucleosomes separate
  - loosely packed chromatin = genes turned on & transcribed

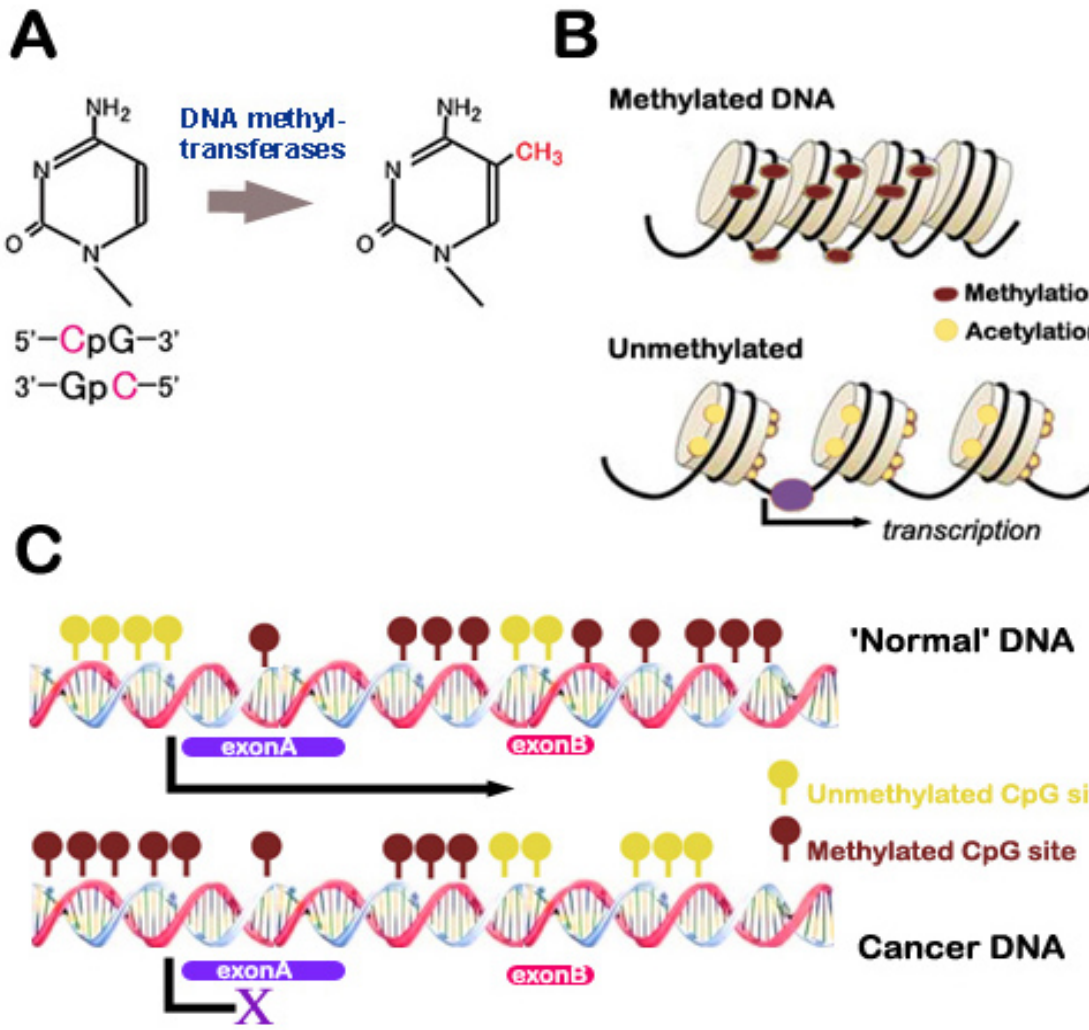




DNA Methylation

- Methylation of DNA blocks transcription factors
  - no transcription = genes turned off
  - involves attaching of methyl groups ($-CH_3$) to cytosine nucleotides in the DNA
    - C = cytosine
  - results in longterm, almost "permanent" inactivation of genes
    - ex. inactivated mammalian X chromosome
      - Once methylated, genes may stay that way even in daughter cells after cellular division





Regulation of Transcription Initiation in EUKARYOTES

- (core) Promoter
  - regulatory sequence in DNA just upstream of transcription unit (coding DNA)
    - Region where RNA Polymerase binds to the DNA
    - Binding of GENERAL (basal) transcription factors (proteins) to regions within the promoter is essential for the transcription of ALL all genes
  - Binding of (same) General Transcription Factors to (any) Promoter attracts RNA Polymerase
  - Establishes a lower "base" rate of transcription for any gene

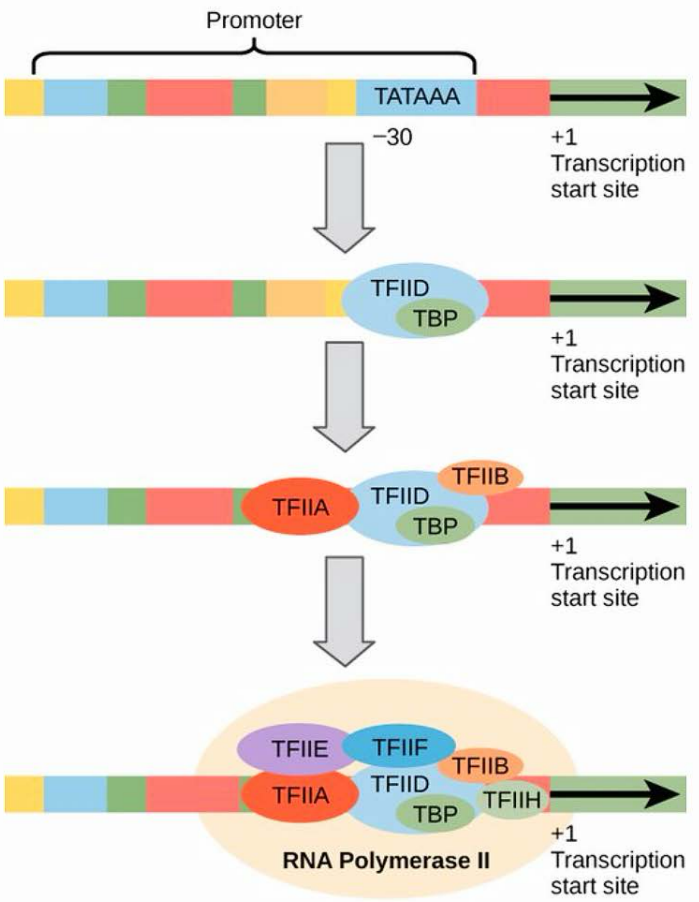


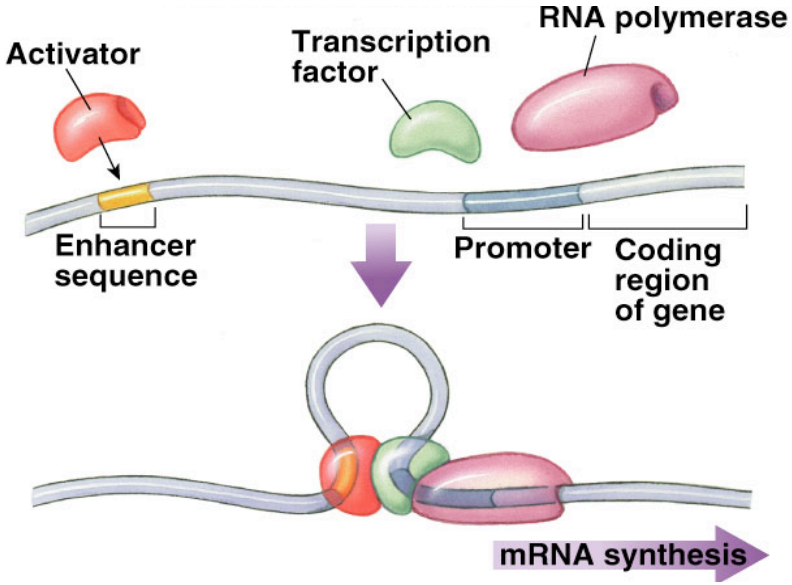
Figure 9. The core transcription machinery assembles on the core promoter, beginning with TBP/TFIID. TFIIB and TFIIA bind next, then recruit the polymerase and additional general transcription factors. Regulatory transcription factors may assemble on other binding sites within the promoter.

Regulation of Transcription Initiation in EUKARYOTES

Control Elements = regulatory sequences outside of the promoter where specific transcription factors (proteins) bind

1. ***Proximal Control Elements***
   - enhancer & silencer DNA sequences
     - sequences in DNA just upstream of (nearby) promoter
       - binding of SPECIFIC transcription factors essential for controlling if JUST that one specific gene is to be transcribed at high rates or not at all.

2. ***Distal Control Elements***
   - enhancer & silencer DNA sequences
     - distant control sequences on DNA
       - 100 or 1000 nucleotides upstream or within introns or following gene
     - binding of SPECIFIC transcription factors essential for controlling if JUST that one specific gene is to be transcribed at high rates or not at all.



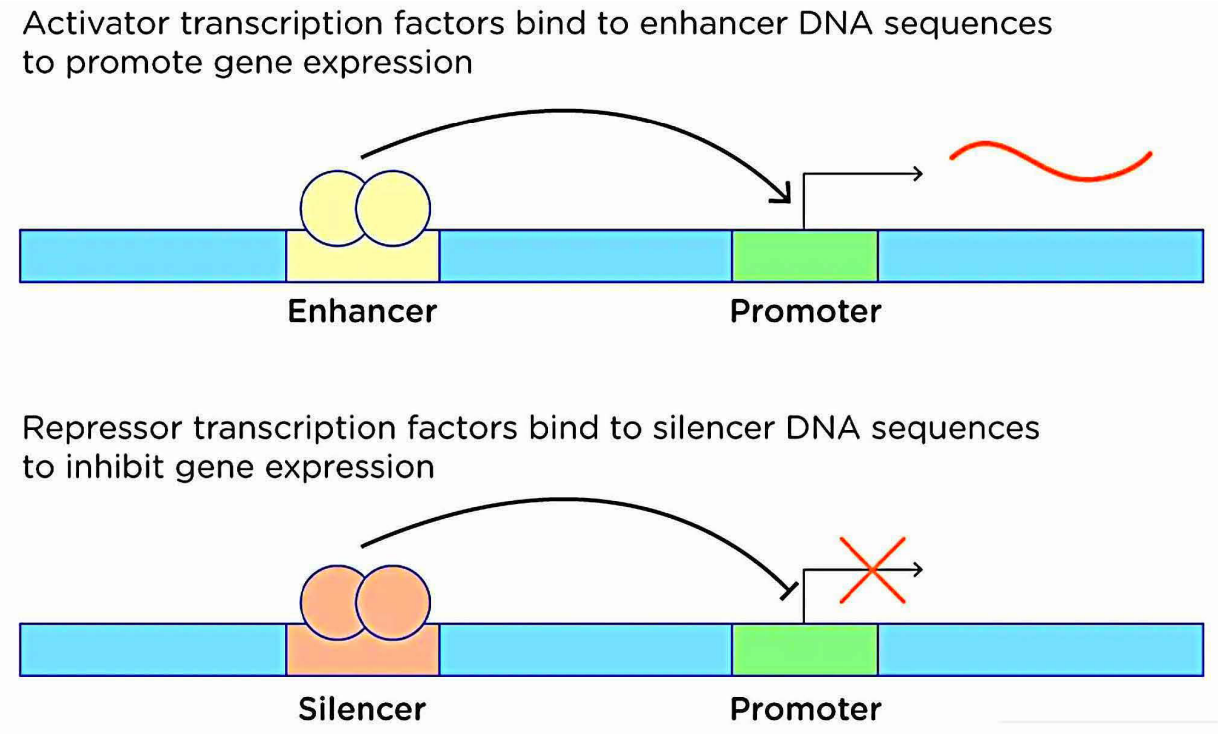
Regulation of Transcription Initiation in EUKARYOTES

Activator proteins are types of Specific Transcription Factors that bind to Proximal & Distal control elements known as **Enhancers**

- activators cause an "enhanced" rate (high level) of gene transcription

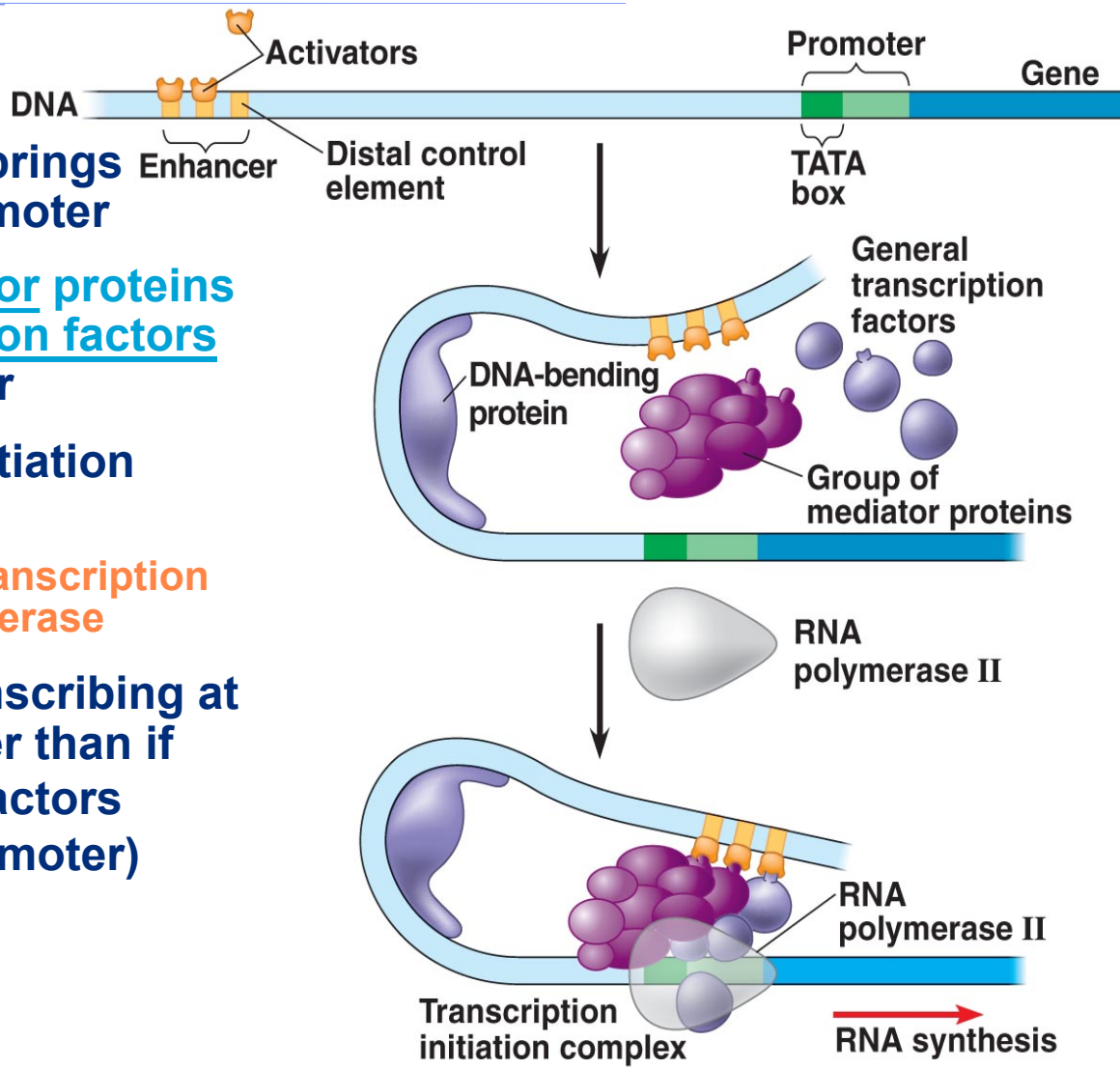
Repressor proteins are types of Specific Transcription Factors that bind to Proximal & Distal control elements known as **Silencers**

- repressors block RNA Polymerase binding, shutting off all transcription (even if general transcription factors for the promoter have been activated)



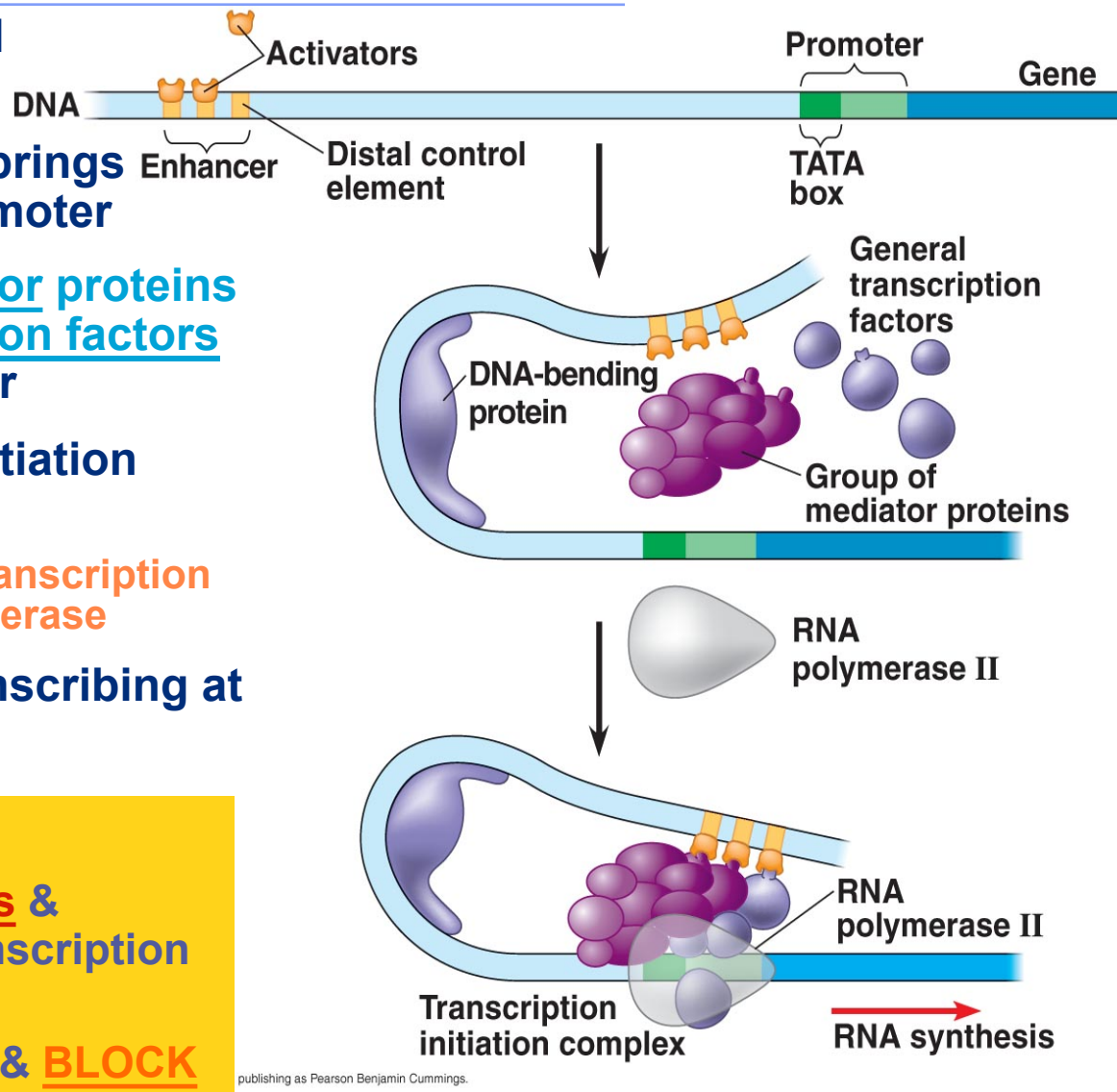
Model for Enhancer action causing High Rates of transcription of a Specific gene

1. Activator proteins bind enhancer sequences
2. DNA bending protein, brings activator closer to promoter
3. Activators bind mediator proteins and general transcription factors binding at the promoter
4. Active transcription initiation complex created
   - ✓ General & specific transcription factors & RNA polymerase
5. RNA pol can begin transcribing at high rates (much higher than if general transcription factors alone bound to the promoter)



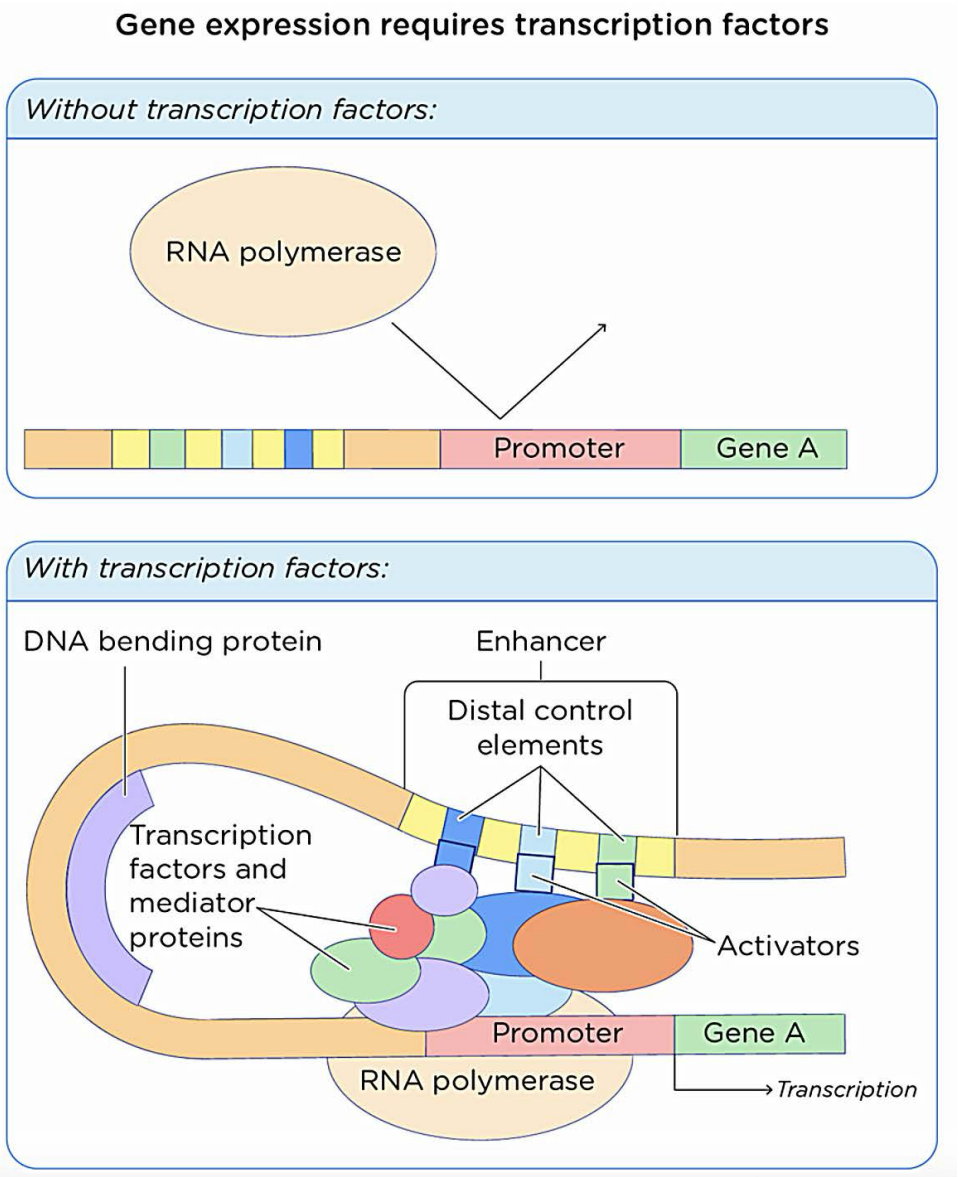
Model for Enhancer action causing High Rates of transcription of a Specific gene

1. Activator proteins bind enhancer sequences
2. DNA bending protein, brings activator closer to promoter
3. Activators bind mediator proteins and general transcription factors binding at the promoter
4. Active transcription initiation complex created
   - ✓ General & specific transcription factors & RNA polymerase
5. RNA pol can begin transcribing at high rates

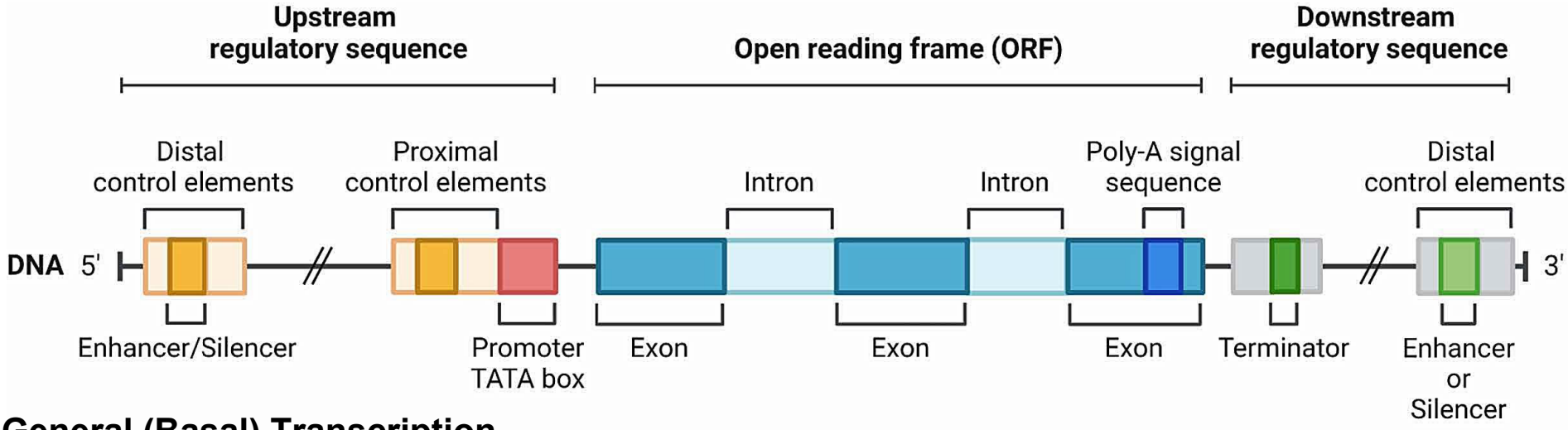


- **Activator proteins**
  - bind to enhancer sequences & stimulates INCREASED transcription
- **Repressor proteins**
  - bind to silencer sequences & BLOCK gene transcription

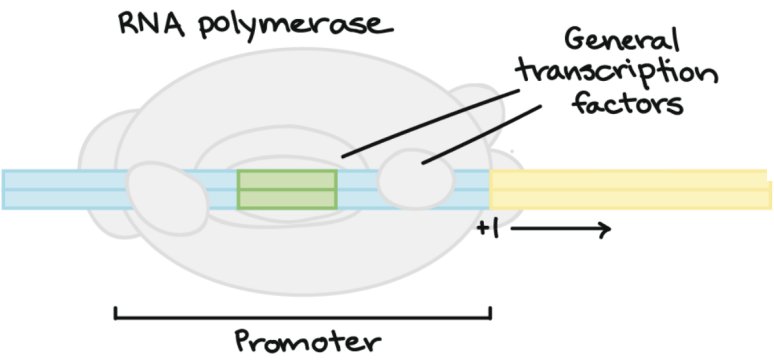
Model for Enhancer action causing High Rates of transcription of a Specific gene

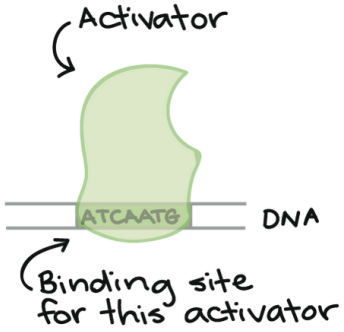


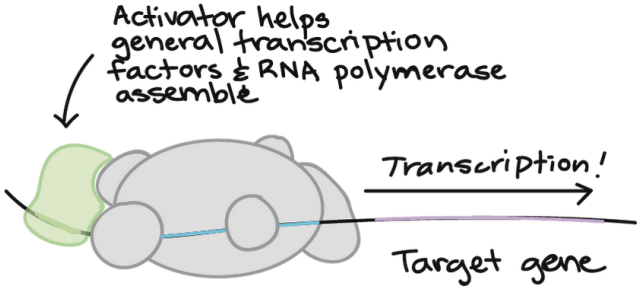
Components of a Eukaryotic Gene



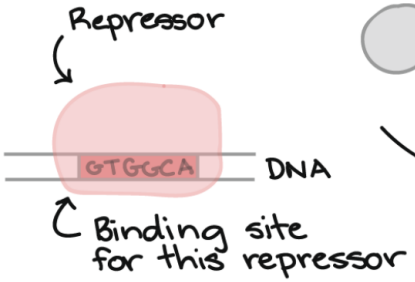
General (Basal) Transcription factors bind to the promoter to help activate low level sof transcription. These proteins are the same for all genes.

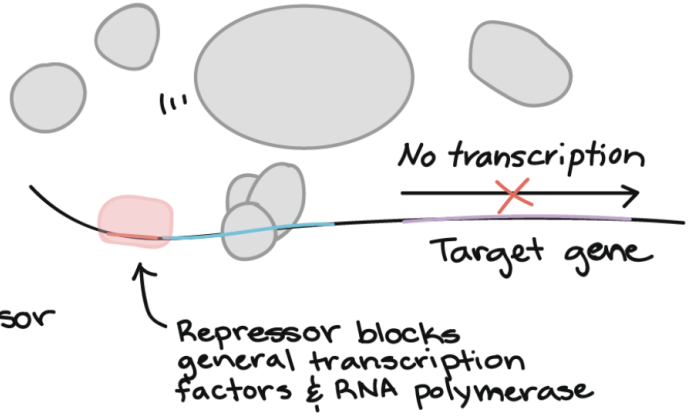




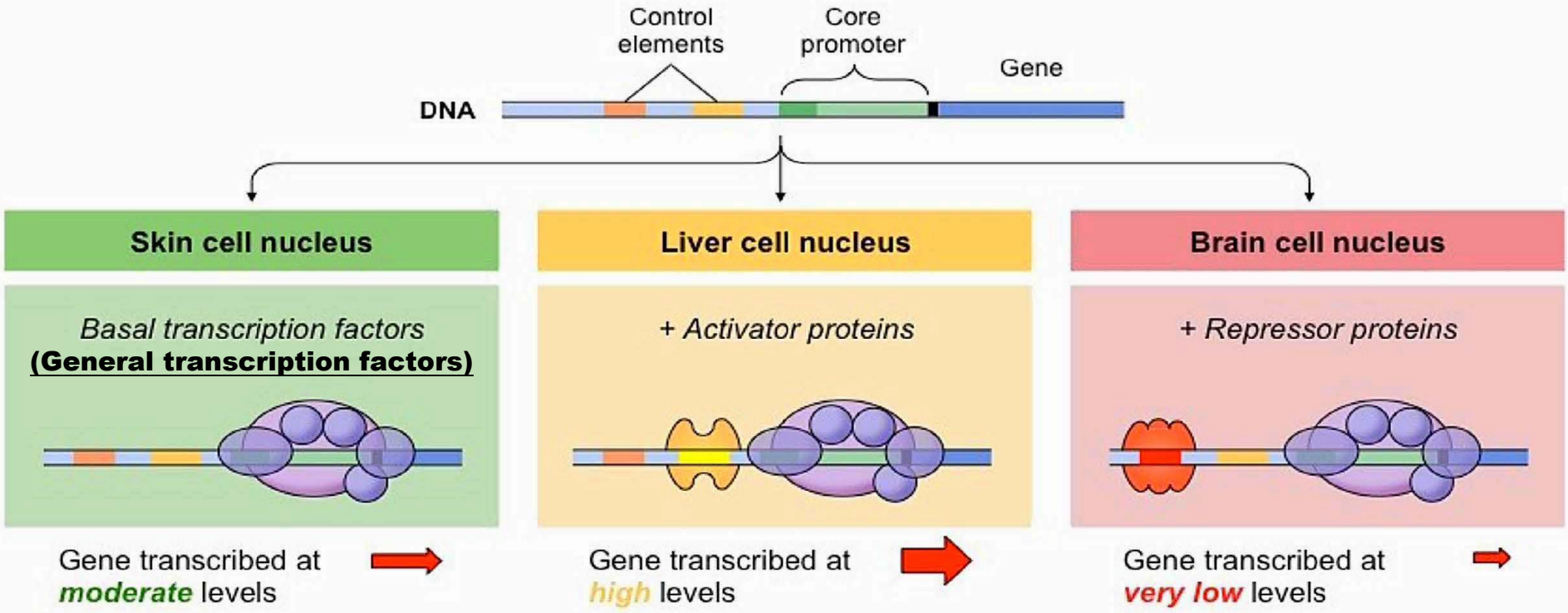


Specific Transcription factors bind to enhancer & silencer sequences, which make up the proximal & distal control elements in DNA, helping increase or prevent transcription, respectively. These proteins & DNA sequences are gene specific.





Differential Gene Expression

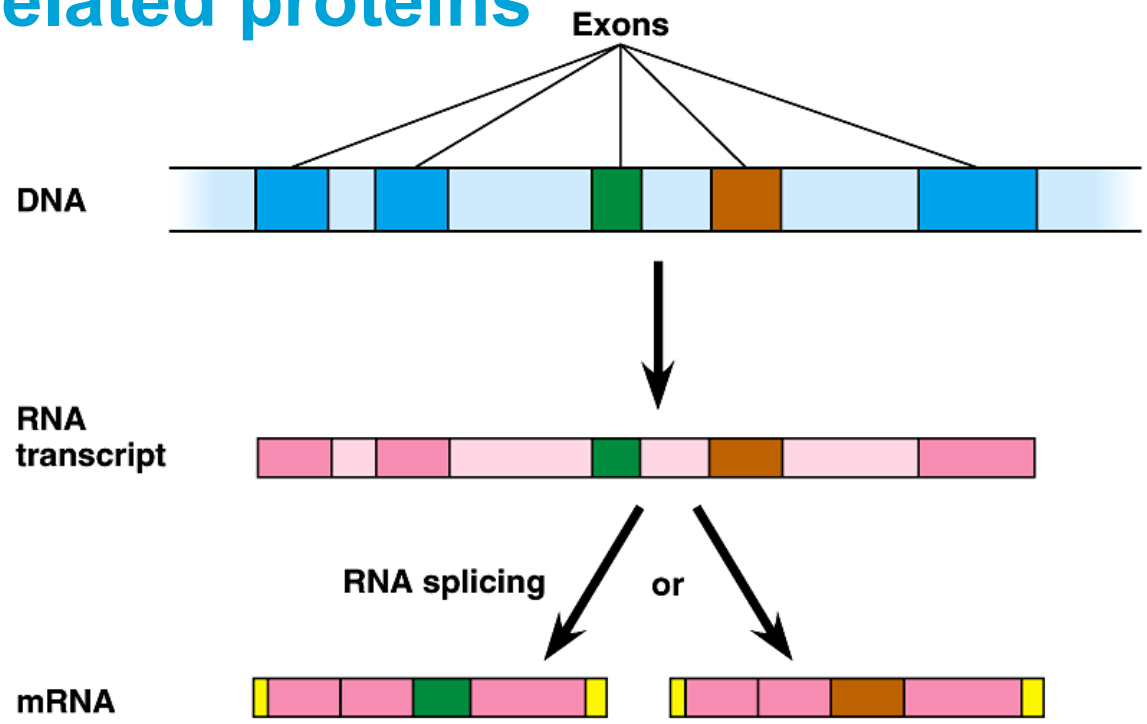


Transcription factors are types of regulatory proteins. They increase or decrease transcription of a gene. Many genes are controlled by both activators and repressors that can bind to many enhancers or silencers in the regulatory regions of a gene. Their combined influence determines the final level of transcription. This allows fine-tuned regulation suited to specific cell types, developmental stages, or environmental conditions, ensuring precision, flexibility, and dynamic control.

Without mechanisms to control gene expression, cells would lack the ability to regulate complex processes such as cell differentiation or cell specialization (= process by which an immature, unspecialized - stem - cell develops into a specialized - tissue- cell type with a distinct structure and function), matinaining homeostasis, or responding to changing conditions.

Post-transcriptional Regulation of Gene Expression

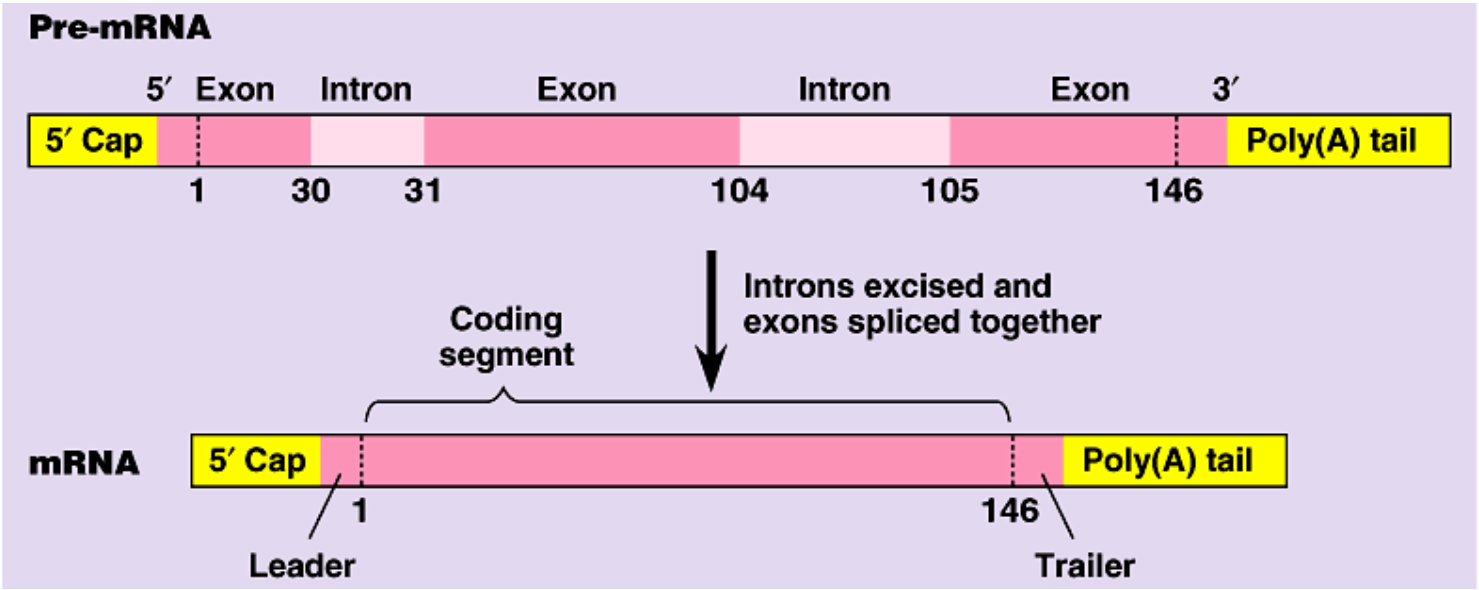
- **Alternative RNA splicing**
  - varying which exons are left in the final mRNA transcript creates a family of related proteins



Post-transcriptional Regulation of Gene Expression

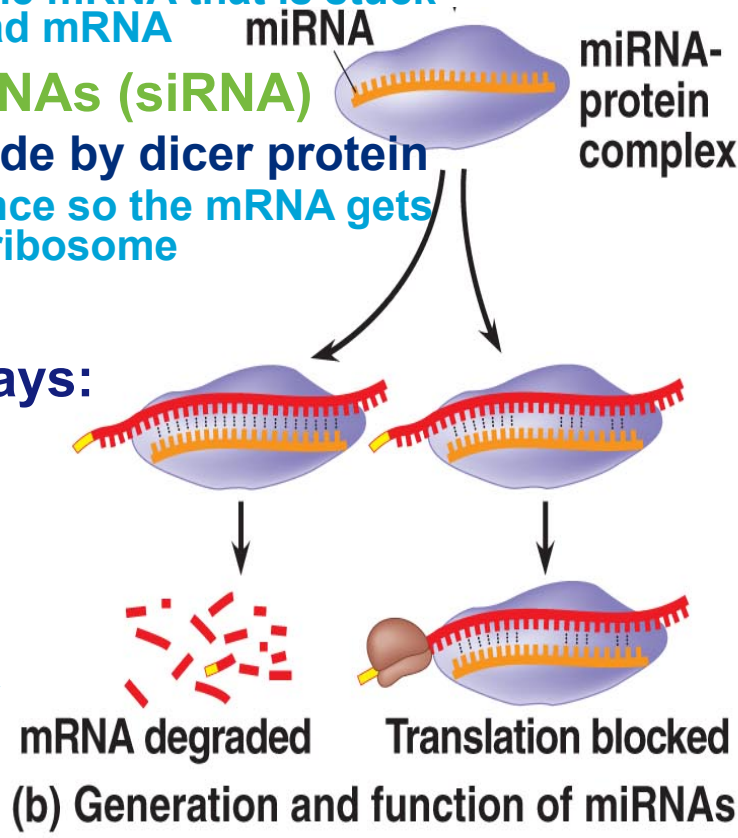
Regulation of mRNA degradation

- Life span of mRNA determines pattern of protein synthesis
  - Longer mRNA lasts, the more times it gets translated
  - Shorter mRNA life span by having enzymes shorten the poly-A tail
  - Removal of cap, causes nucleases to chew up mRNA
    - mRNA can last from hours to weeks



Post-transcriptional Regulation of Gene Expression

- Noncoding (small) RNAs play roles in controlling gene expression
  - MicoRNAs (miRNAs) form complex with proteins
    - Bind, along with proteins, to target mRNA (from specific gene) by base-pairing
      - Repress ribosome's ability to translate the mRNA that is stuck to miRNA strand so ribosome cannot read mRNA
  - RNA interference by Small Interfering RNAs (siRNA)
    - short segments of RNA (21-28 bases) made by dicer protein
      - bind to mRNA with complementary sequence so the mRNA gets destroyed so no translation can occur by ribosome
- BOTH create sections of double-stranded mRNA that stop translation in one of two ways:
  - "death" tag for mRNA that triggers degradation of mRNA
  - mRNA with proteins and RNA cannot bind to ribosome for translation
- cause gene "silencing"
  - *even though this is post-transcriptional control, still turns off a gene*

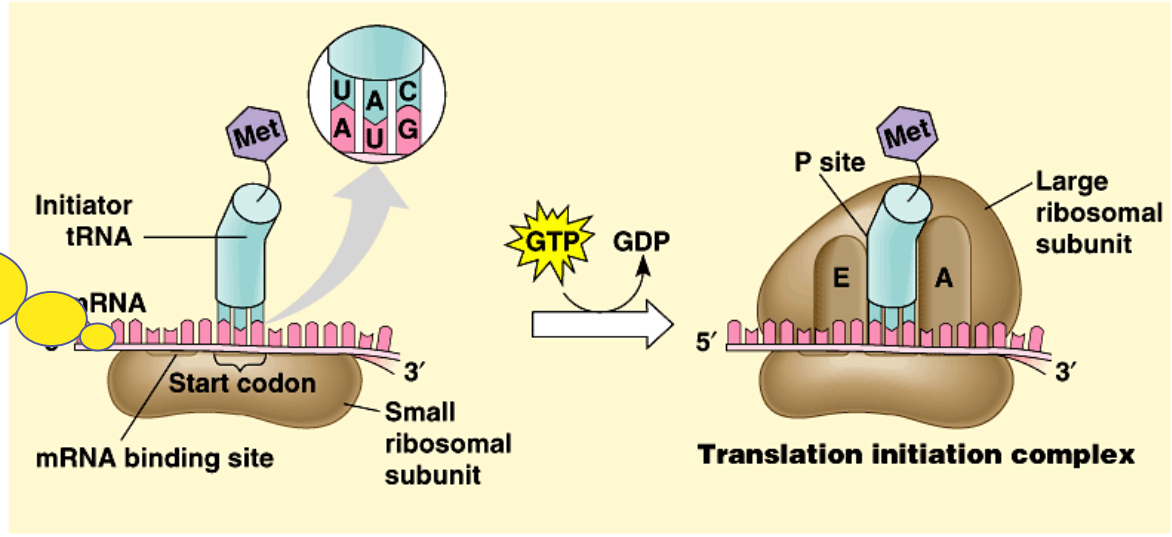


(b) Generation and function of miRNAs

Control of translation by mRNA repressors

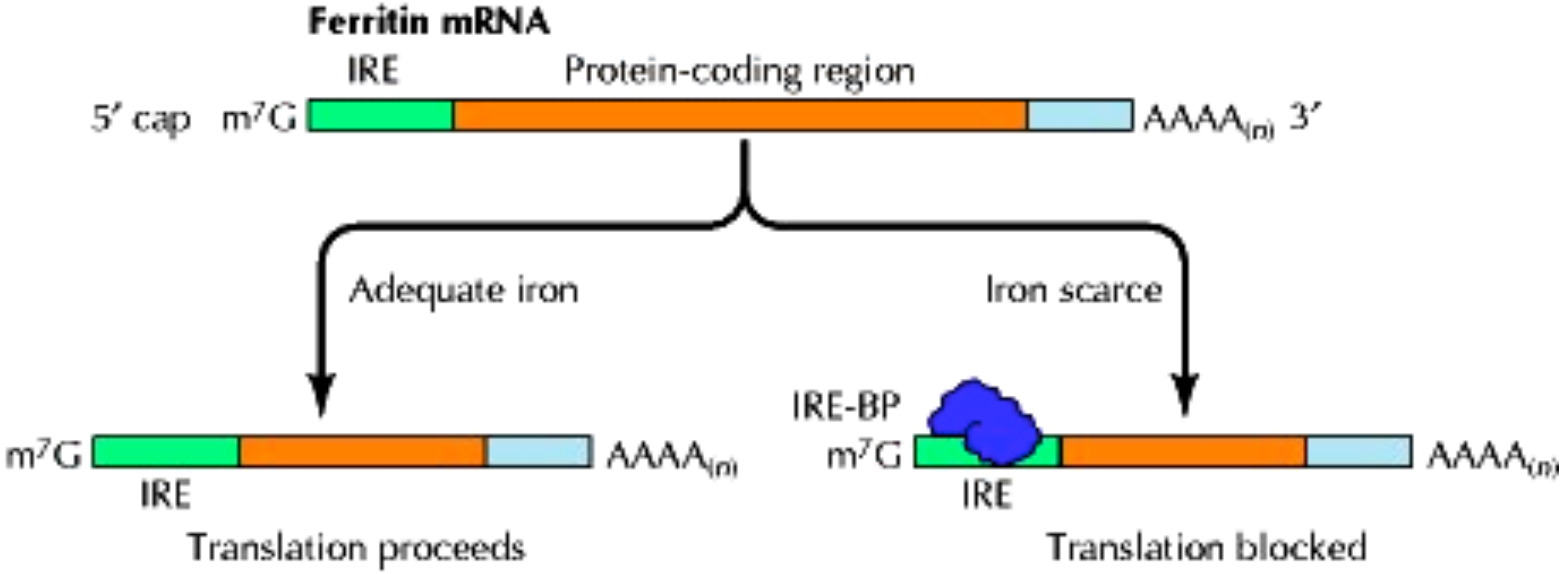
- Binding of repressor proteins to specific mRNA sequences can also block translation of the mRNA by the ribosome.
  - regulatory proteins attach to 5' end of mRNA
    - prevent attachment of ribosomal subunits & initiator tRNA (carrying methionine)
      - ★Effect: block translation of mRNA into protein

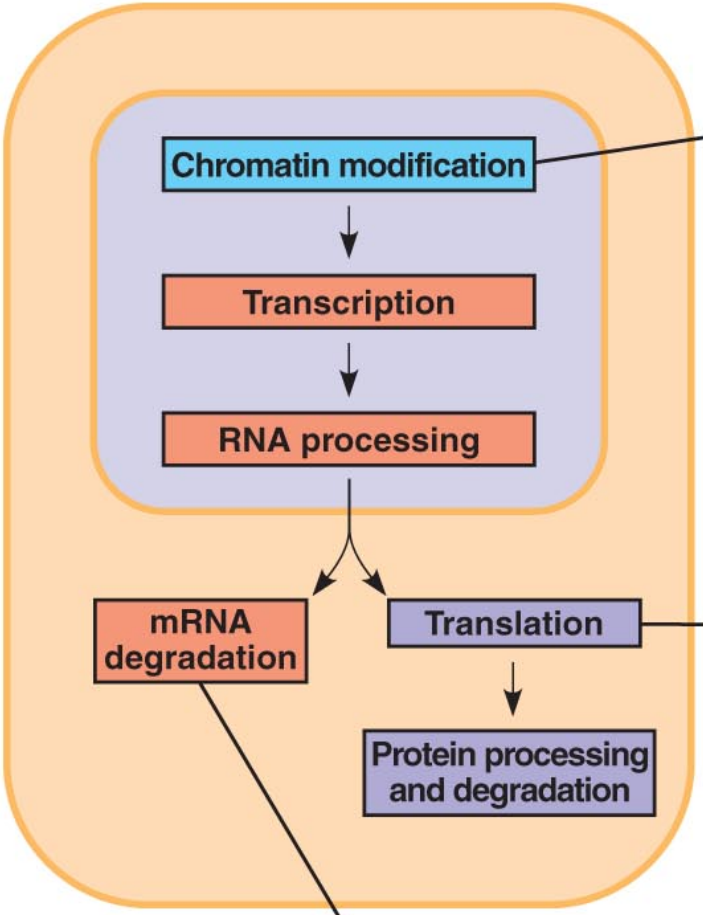
Not gonna happen for us this time buddy :(



Example of repressor binding to mRNA to block translation

- The best understood example of this mechanism in eukaryotic cells is regulation of the synthesis of ferritin
  - Ferritin is a protein that stores iron ions within the cell.
  - Translation of ferritin mRNA is regulated by supply of iron:
    - A protein exists which (in the absence of iron - when no excess iron exists needing to be stored) binds to a sequence (the iron response element, or IRE) in the ferritin mRNA's 5´ untranslated region, blocking its translation.
    - In the presence of iron, the repressor no longer binds to the IRE and ferritin translation is able to proceed.
      - More ferritin protein is synthesized IF iron is abundant



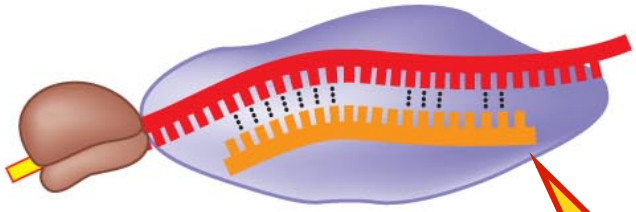


Chromatin modification

- Small RNAs can promote the formation of heterochromatin in certain regions, blocking transcription.

Translation

- miRNA or siRNA can block the translation of specific mRNAs.



Hottest new topic in biology

mRNA degradation

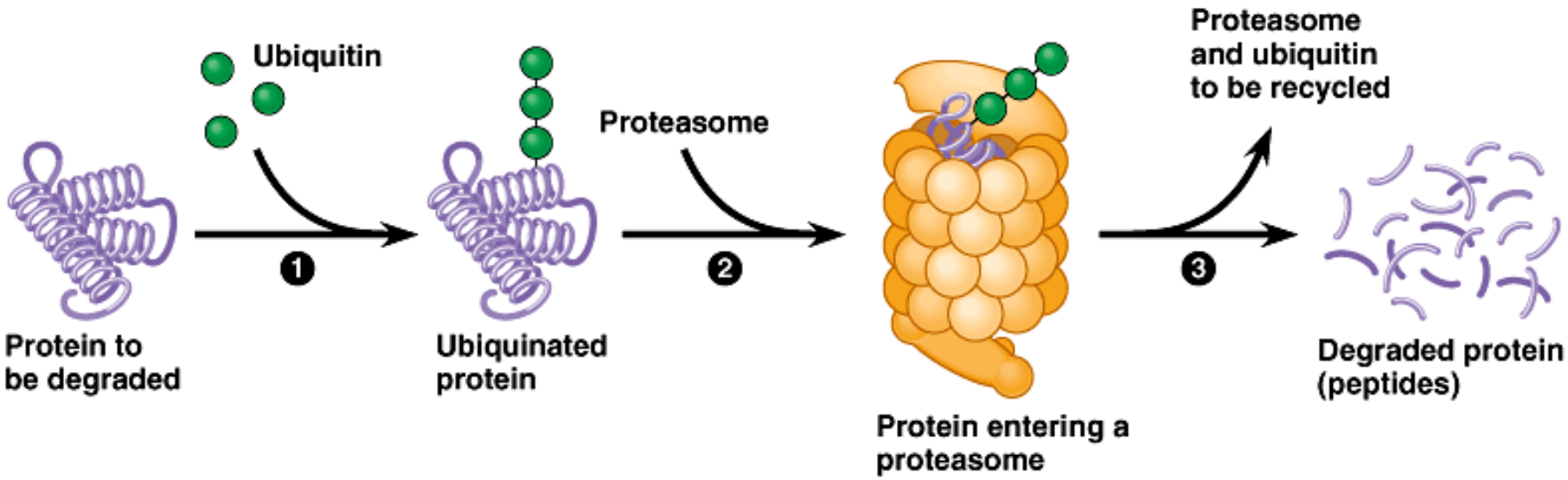
- miRNA or siRNA can target specific mRNAs for destruction.

The Uses of Small RNAs

Post-transcriptional (Post-translational) Regulation of Gene Expression

Protein processing & degradation

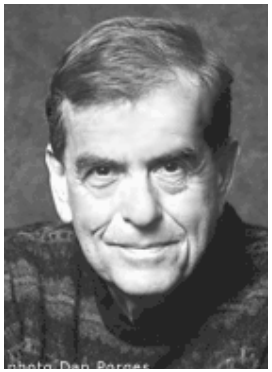
- Protein processing
  - folding, cleaving, adding sugar groups, targeting for transport
- Protein degradation
  - ubiquitin tagging
  - proteosome degradation



Ubiquitin

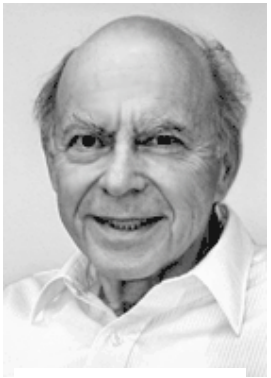
1980s | 2004

- "Death tag"
  - mark unwanted proteins with a label
    - 76 amino acid polypeptide ubiquitin
  - labeled proteins are broken down rapidly in "waste disposer" proteins
    - proteasomes



Aaron Ciechanover
Israel

Avram Hershko
Israel



Irwin Rose
UC Riverside

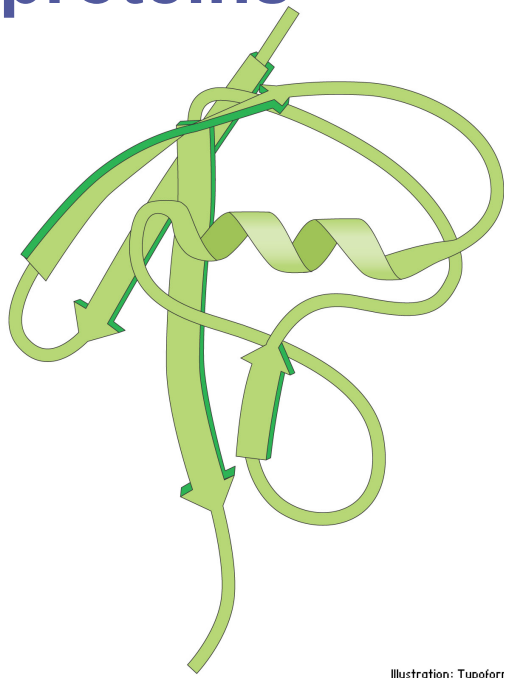
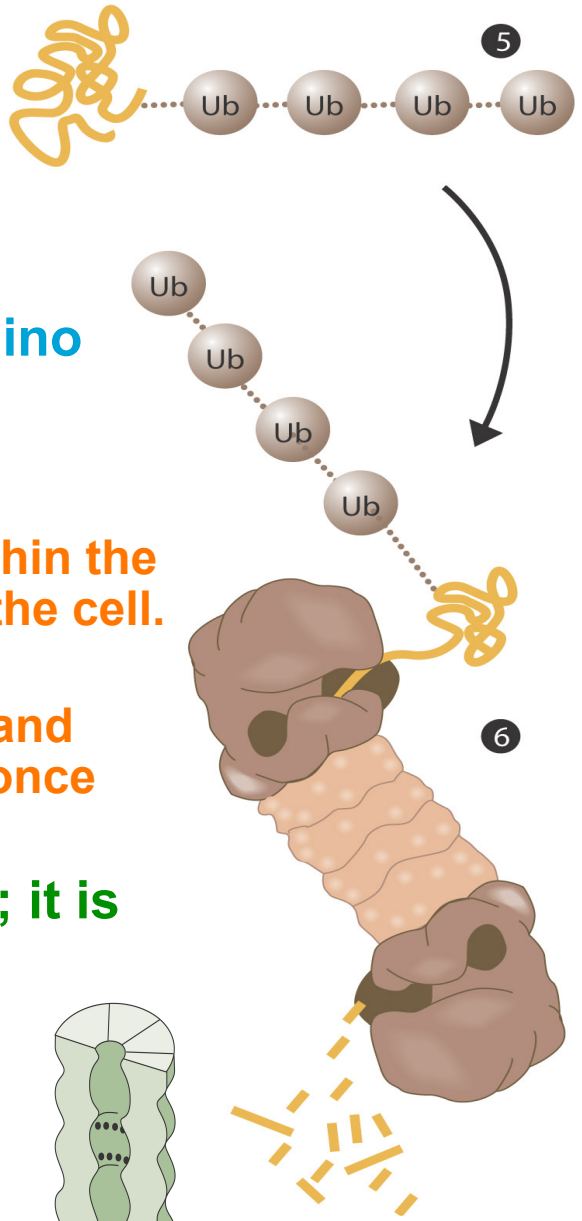
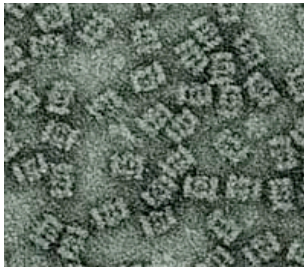


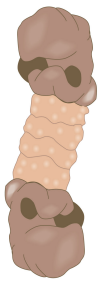
Illustration: Typoform

Proteasome

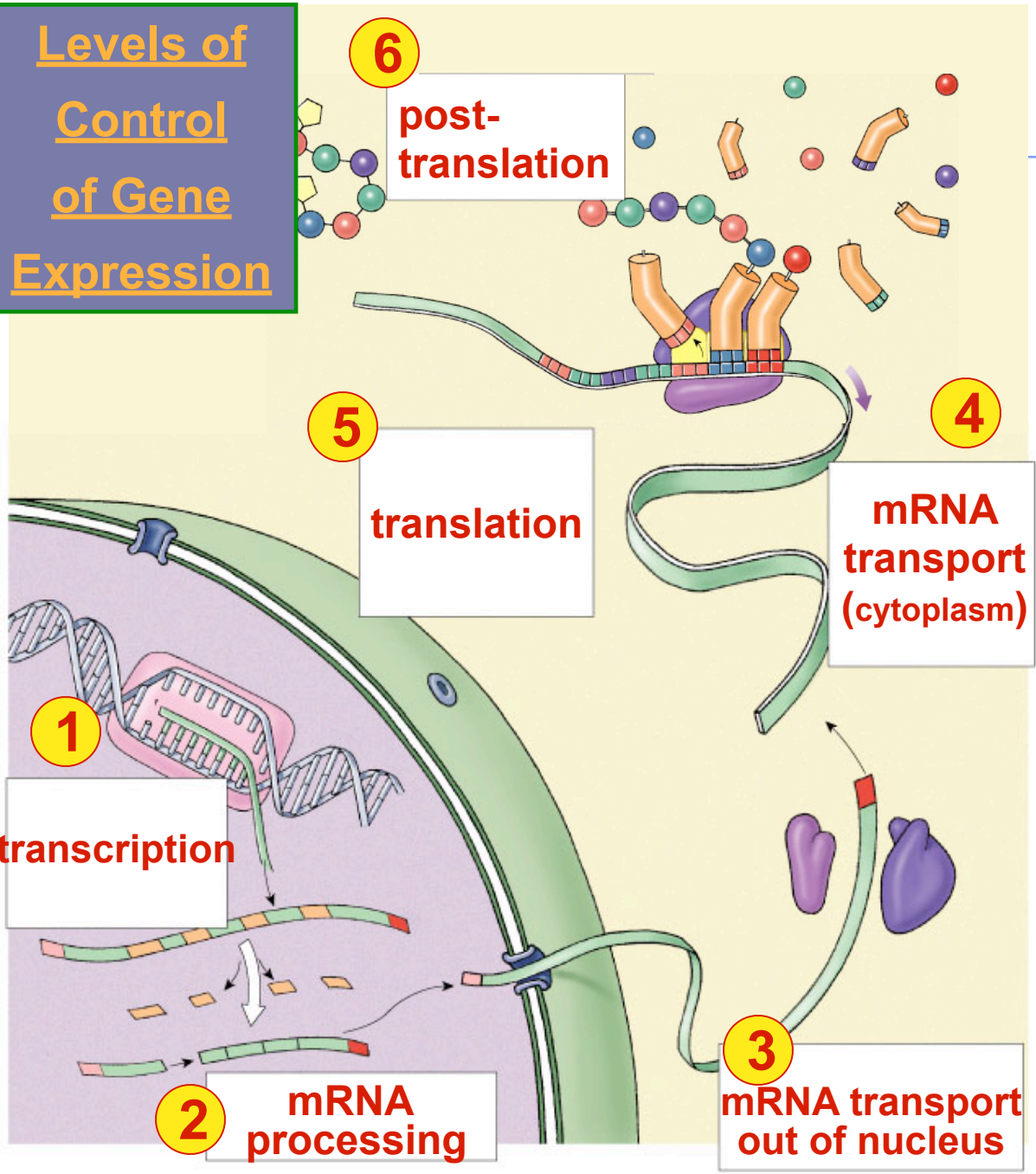
- Protein-degrading "machine"
  - cell's waste disposer
  - can breakdown all proteins into 7-9 amino acid fragments
- A human cell contains about 30,000 proteasomes:
  - The active surface of the proteasome is within the barrel where it is shielded from the rest of the cell.
  - Proteasomes recognize polyubiquitinated proteins, denatures them with ATP energy and admits them to the barrel for disassembly once the ubiquitin label has been removed.
- Proteasome itself cannot choose proteins; it is chiefly the E3 enzyme that does this by ubiquitin-labelling the right protein for breakdown







Levels of Control of Gene Expression



1. Control transcription
- altering DNA packing influences access to a gene
- transcription factor activation

2. mRNA processing
- alternative splicing influences polypeptide made

3. mRNA transport out of nucleus
- breakdown of mRNA by si/miRNA silences gene

4. mRNA transport in cytoplasm
- protection by 3' cap & varying of poly-A tail length influences down degradation of mRNA

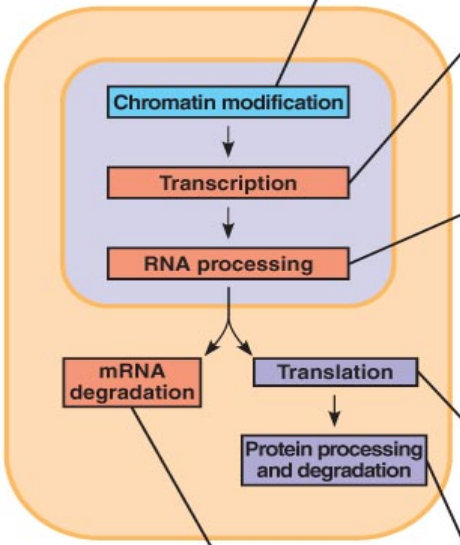
5. translation
- factors which block start of translation
- si/miRNA base paring to mRNA blocks ribosomal translation

6. post-translation modifications
- polypeptides can be chemically modified changing the protein activity
- protein shape, and thus function, can be altered by phosphorylation or by binding of activators and inhibitors
- proteins can be degraded

Chromatin modification

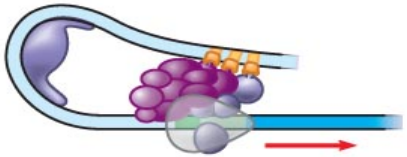
- Genes in highly compacted chromatin are generally not transcribed.
- Histone acetylation seems to loosen chromatin structure, enhancing transcription.

- DNA methylation generally reduces transcription.



Transcription

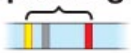
- Regulation of transcription initiation: DNA control elements bind specific transcription factors.



Bending of the DNA enables activators to contact proteins at the promoter, initiating transcription.

- Coordinate regulation:

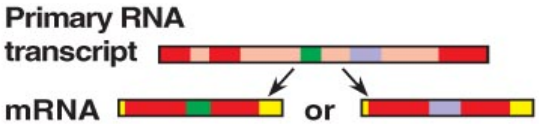
Enhancer for liver-specific genes



Enhancer for lens-specific genes

RNA processing

- Alternative RNA splicing:



Translation

- Initiation of translation can be controlled via regulation of initiation factors.

mRNA degradation

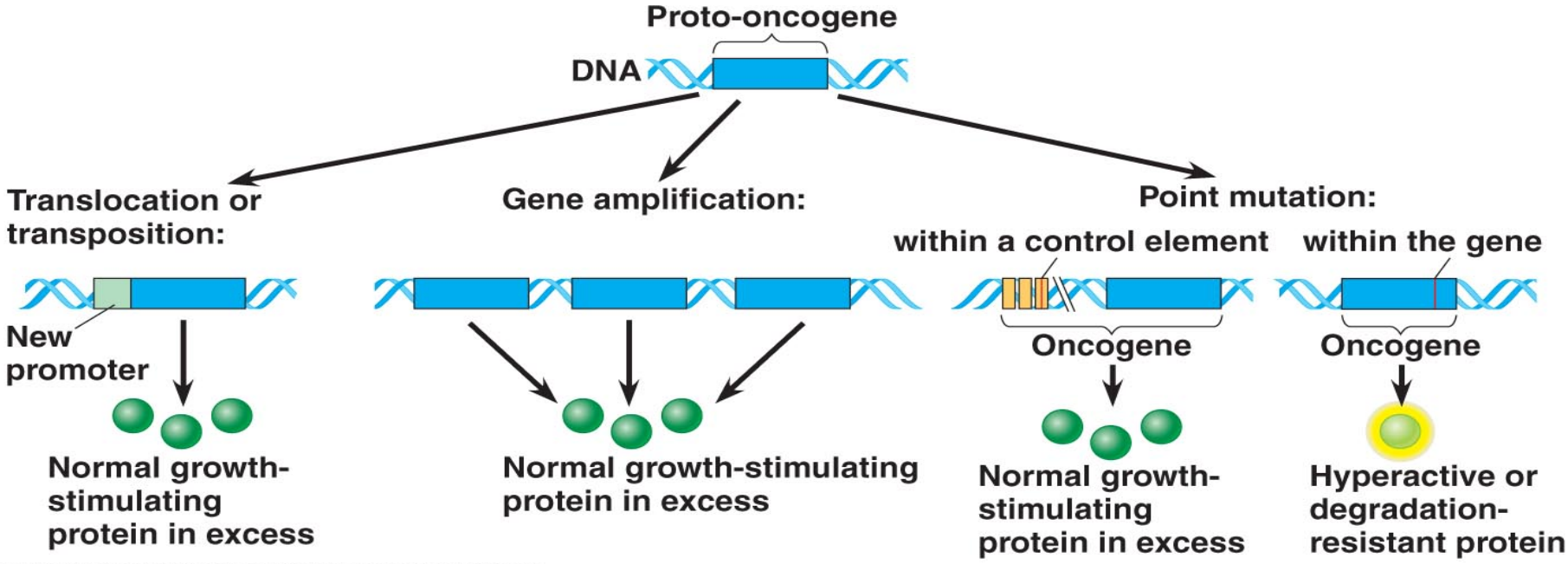
- Each mRNA has a characteristic life span, determined in part by sequences in the 5′ and 3′ UTRs.

Protein processing and degradation

- Protein processing and degradation by proteasomes are subject to regulation.

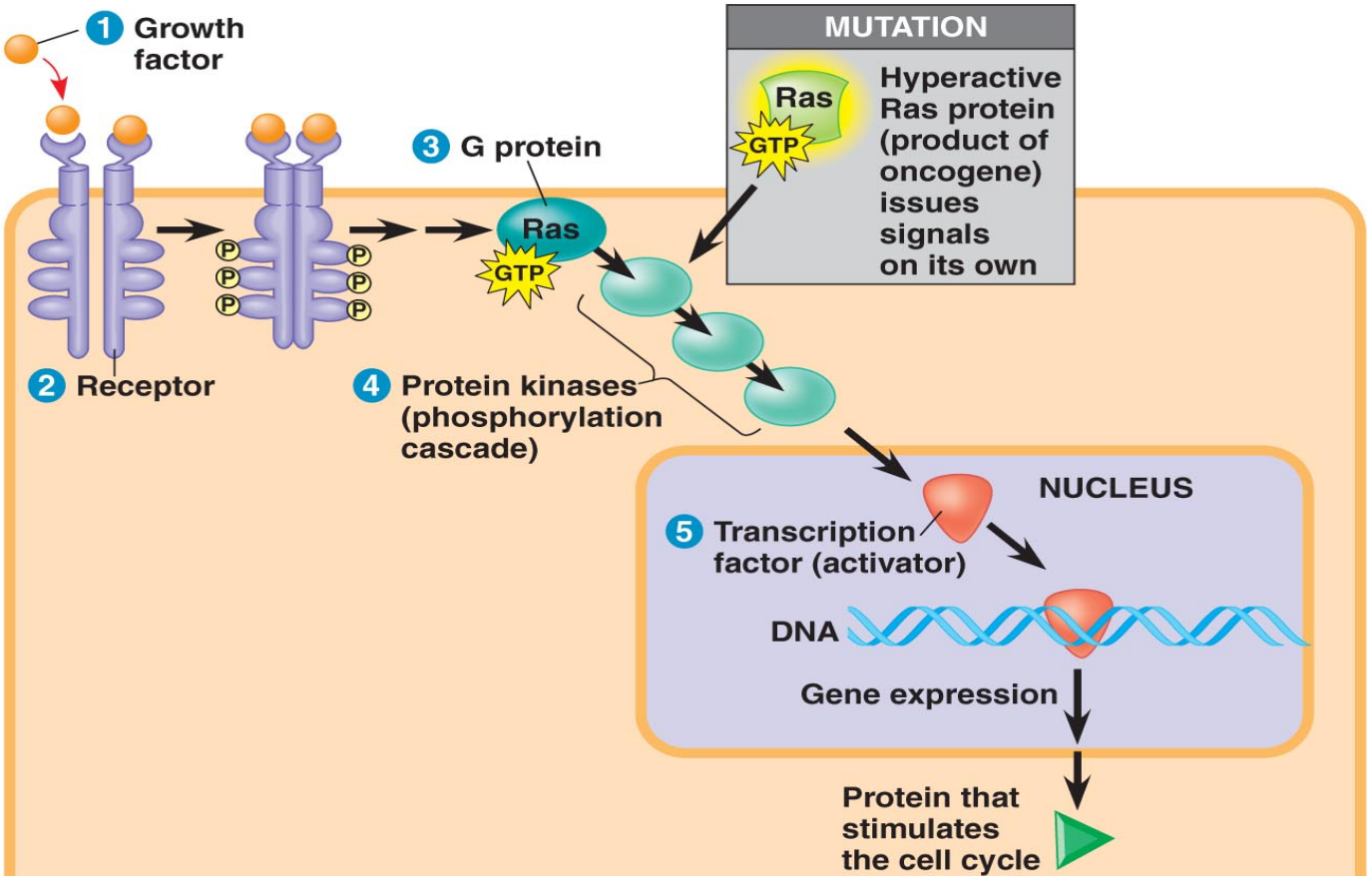
Which types of Gene Mutation Can Lead to Cancer?

- **Proto-Oncogenes**
  - Code for proteins that stimulate normal cell growth & division
    - Ex: ras gene and Ras protein *(a G protein)*
- **Mutated Proto-oncogenes are oncogenes**
  - 'cancer-causing' genes
    - mutations in the gene *(either in controlling elements or transcription unit)* result in either the overproduction of certain proteins that help cells move through the cell cycle or a change in protein structure and thus protein activity in such a way that it continues to aid the cell in moving through the cell cycle even when the cell should not be dividing.



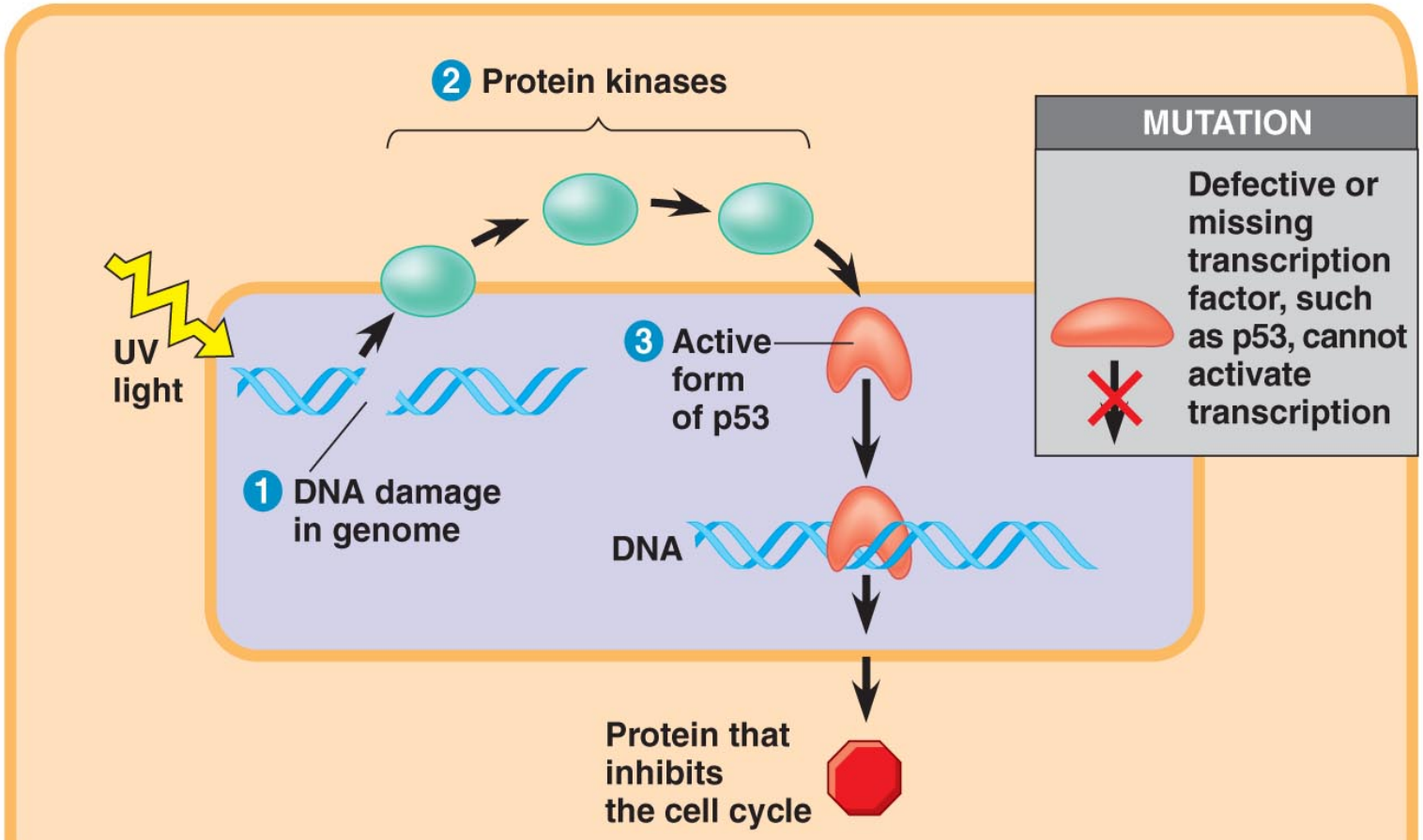
Ras Protein:

- A G-protein coded by the ras gene
  - Normally, it passes the signal from an external growth factor bound to the G-protein-linked receptor on the plasma membrane to protein kinases that pass signal to the nucleus to make proteins needed for dividing through a signal transduction pathway.
    - Mutated ras gene codes for a Ras protein that initiates a signal transduction cascade in the absence of growth factor signal

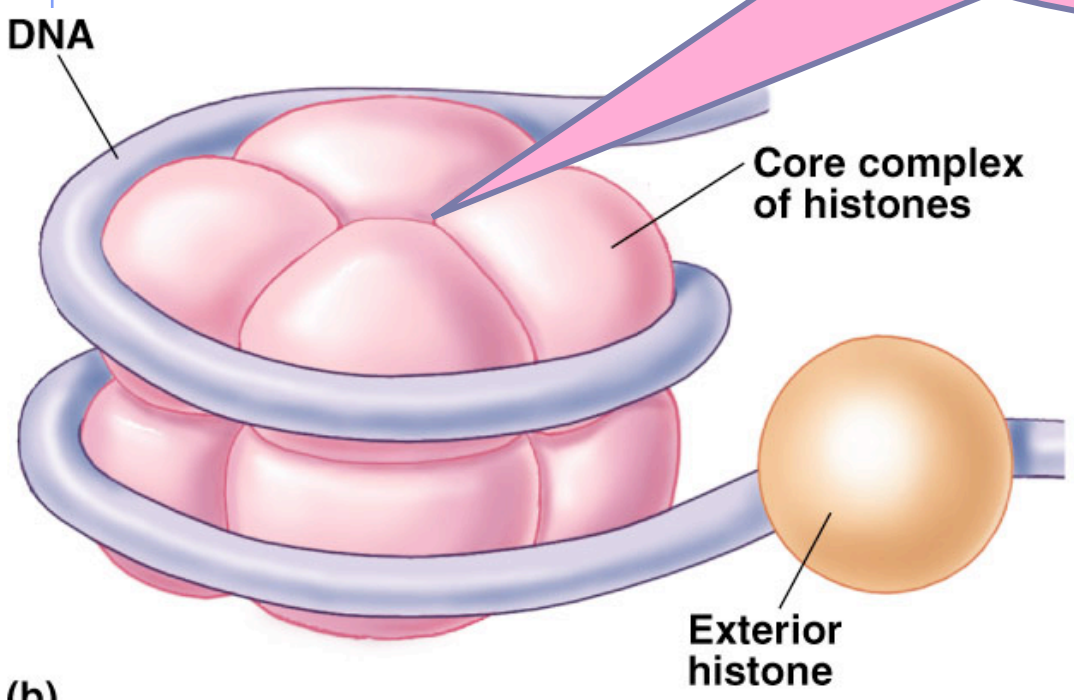


P53 protein/transcription factor

- **Tumor-Suppressor Genes**
  - Code for proteins that help prevent uncontrolled cell growth.
    - Mutations in this type of gene can contribute to cancer by allowing growth in the absence of suppression.
      - Ex: p53 gene codes P53 proteins, transcription factors that promote the synthesis of cell cycle-inhibiting proteins and DNA repair proteins.



Any Questions?
Time to pack it all into your brain!



2007-2008