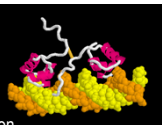
REVIEW: Ch. 16



1. Diagram the structure of an operon, including 3 DNA components and 3 other molecules involved in its regulation.
2. ** Compare the utility and regulation of an inducible system for catabolic genes, and a repressible system for anabolic genes.

Ch. 16b Objectives: Students should be able to....

1. Compare & contrast the 3 different mechanisms of **Horizontal Gene Transfer** in bacteria: ***Transformation***, ***Conjugation***, ***Transduction***. Define and explain the mechanism of *Transposition*.
2. **Ch. 16B:** Describe 4 factors that **differentiate eukaryotic chromosomes/genome** from a **prokaryotic** chromosome/genome…..
3. Compare/contrast **4** differences between **Eukaryotic and Prokaryotic *GENE* structure** (including regulatory sequences)…..
4. Describe and explain the function of **3 modifications that occur to a eukaryotic transcript** before it is translated.
5. Diagram/define and **explain the utility** of ***2*** examples ***EACH*** of eukaryotic Transcriptional, Posttranscriptional, Translational & Posttranslational regulation.
6. **Ch. 11:** Describe and Diagram the **4 phases of the cell cycle**, and how they are regulated by **Cyclin/CDK complexes**.
7. Diagram and compare the 4 main phases of Mitosis, Meiosis I & Meiosis II.
   - Chromosome, Chromatid, Centromere, Centriole, Spindle, Cortical MT's, Spindle fibers/ MT's, Sister Chromatid, Homologous Chromosome, Nuclear Envelope, Cytokinesis (plants/animals).

❖ ***Objectives and Study Guide Questions are your HOMEWORK between classes!!! DUE every Wed/Thur at the end of Lecture!!***

1

B. A Repressible System: *trp* Operon

- The *trp* operon is a **repressible system**:
  - ***the end product of a BIOSYNTHETIC PATHWAY (anabolic) represses the synthesis of enzymes involved in its own synthesis.***
    - Eg: tryptophan is such an end product
- Tryptophan acts as a ***corepressor*** by binding to an inactive repressor protein and making it active (******ALLOSTERIC REGULATION!!******).
  - When the activated repressor binds to the operator, transcription is turned OFF.

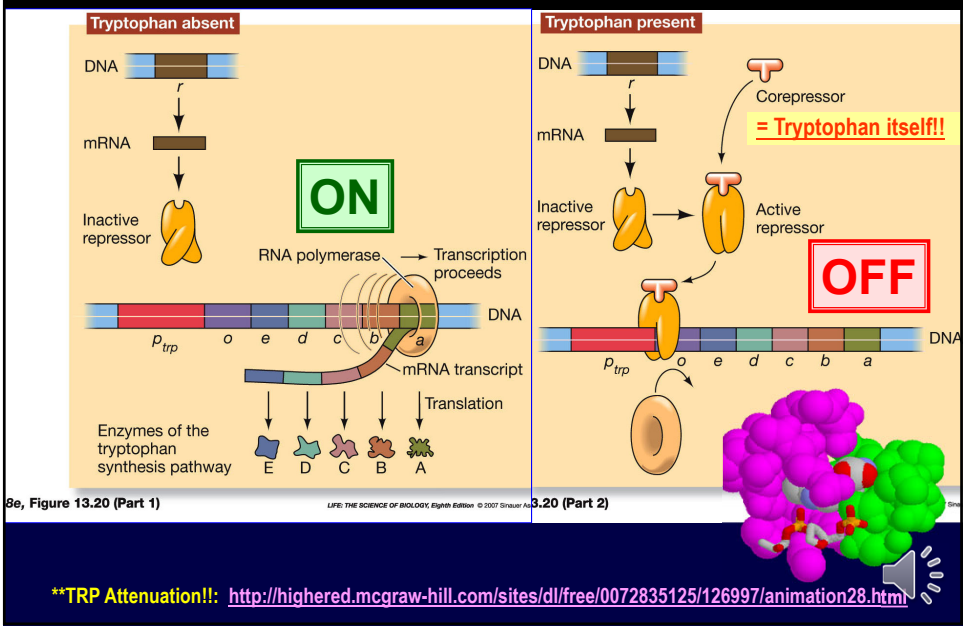
http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120080/bio26.swf::The%20Tryptophan%20Repressor

http://nortonbooks.com/college/biology/animations/ch14a03.htm

http://bcs.whfreeman.com/thelifewire/content/chp13/1302002.html

2

(B.) *TRP* Operon



**TRP Attenuation!!: http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation28.html

3

Operon Regulation: Summary

OPERON TYPE	ACTIVITY of	"Ligand" Molecule Absent	"Ligand" Molecule Present
***Inducible*, ~lac (Catabolic)**	Repressor Protein	**ON**	**OFF** (lactose "inducer")
	CAP Protein	**OFF**	**ON** *(if ↑cAMP; NO glucose!)*
	Operon (transcription)	**OFF**	**ON**
***Repressible*, ~trp (Anabolic)**	Repressor Protein	**OFF**	**ON** (tryptophan "corepressor")
	Operon (transcription)	**ON**	**OFF**

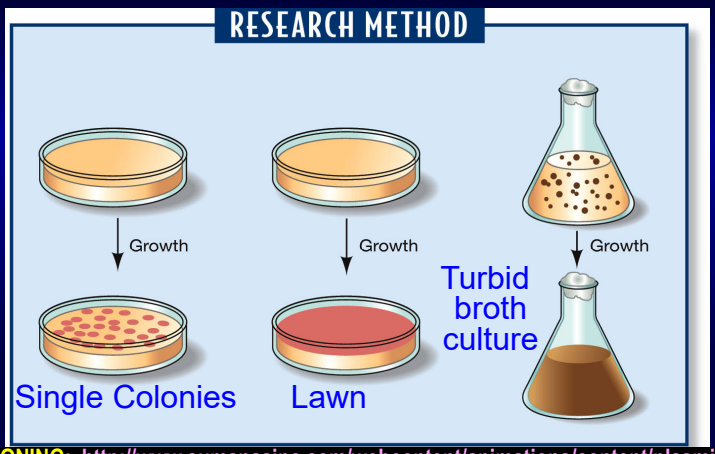
[Reminder: both signal molecules/ Ligands bind to the Repressor protein, **allosterically** changing its activity. Inducer, or Corepressor?]

- **Positive Control**: Regulatory Protein **ACTIVATES** genes.
- **Negative Control**: Regulatory protein **REPRESSES** genes.

4

16.4) Prokaryotes:
Reproduction, Mutation, & Recombination

- When bacteria divide, they form clones of identical cells, which can be observed as colonies when grown on solid media.



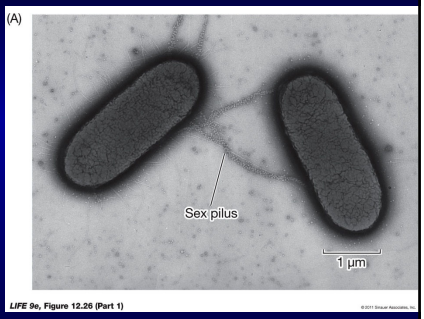
CLONING: http://www.sumanasinc.com/webcontent/animations/content/plasmidcloning.html

LIFE 8e, Figure 13.9

5

Prokaryotes: Recombination
A. Conjugation

- **HORIZONTAL GENE TRANSFER:** A bacterium can transfer its genes to another bacterium by ***conjugation***, ***transformation***, or ***transduction***.



LIFE 9e, Figure 12.26 (Part 1)

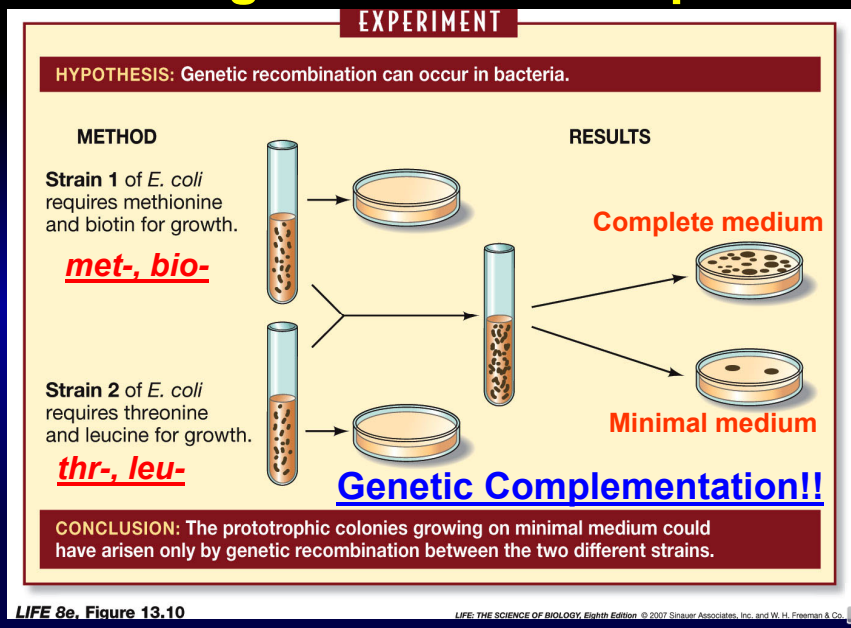
- In ***Conjugation***, a bacterium attaches to another bacterium and passes a partial copy of its DNA to the adjacent cell.

http://www.blackwellpublishing.com/trun/artwork/Animations/conjugation/conjugation.html

E. coli cell strx: http://www.blackwellpublishing.com/trun/artwork/Animations/Overview/overview.html

6

Lederberg and Tatum's Experiment

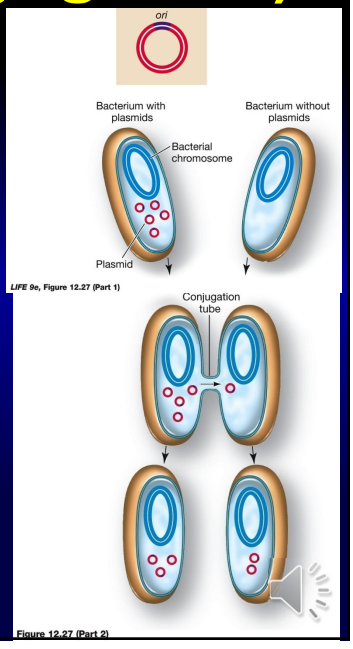


http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter13/animation_quiz_3.html

7

R-Factors (conjugation)

- ***Plasmids*** are small bacterial chromosomes independent of the main chromosome.
- ***R factors***, plasmids that carry genes for ***antibiotic resistance***, are a serious public health threat.



http://www.hhmi.org/biointeractive/animations/conjugation/conj_frames.htm

8

"Gene Replacement"

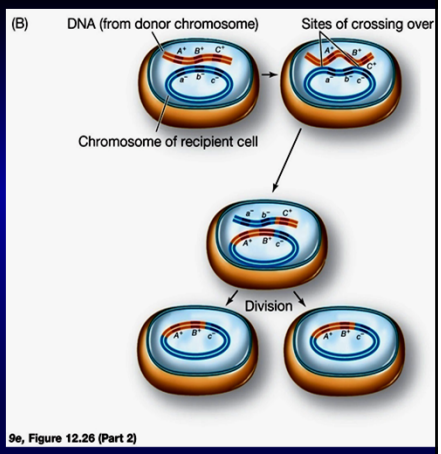
Homologous Recombination:

→ "Gene Replacement"

➢ **RecA Protein, RecBCD.**

➢ DNA taken up into prok. cell:

- must replicate independently, *OR*
- be integrated into the host genome!!



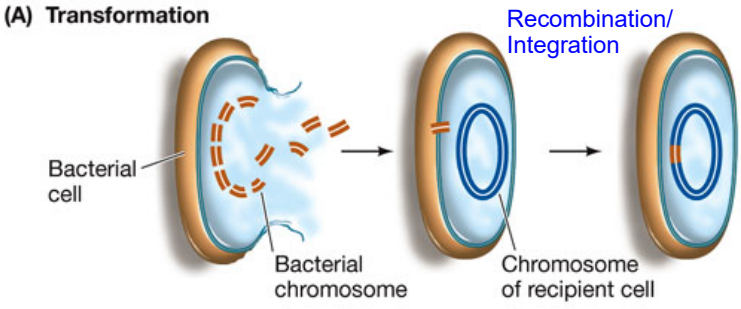
http://www.blackwellpublishing.com/trun/artwork/Animations/Recombination/recombination.html

http://www.1lecture.com/Microbiology/Bacterial%20Transformation-Bacterial%20Transformation.swf

9

Recombination:
B. Transformation

- In ***Transformation***, genes are transferred between cells when fragments of (bacterial) DNA are taken up by a cell from the medium. (Frederick GRIFFITHS!)
  - ***Source of DNA often irrelevant! (RE's, etc.)***
- These fragments may recombine with the host chromosome, permanently adding new genes.



Method:
http://www.dnalc.org/resources/animations/transformation2.html

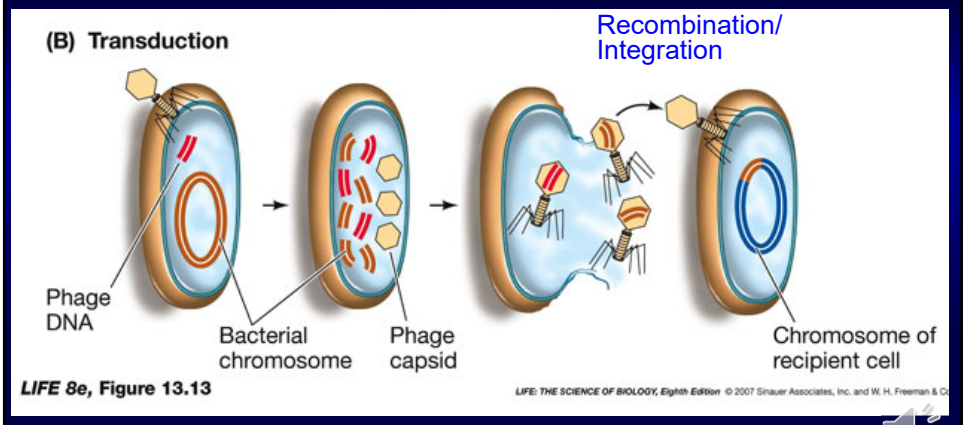
8e, Figure 13.13

http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter13/animation_quiz_1.html

10

Recombination:
C. Transduction

- In ***Transduction***, phage **capsids** carry bacterial **DNA** from one bacterium to another.

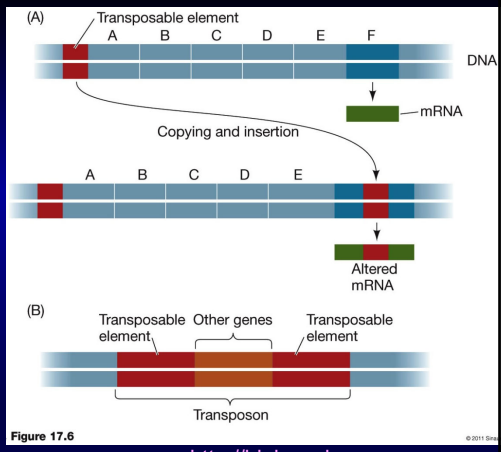


http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter13/animation_quiz_2.html

11

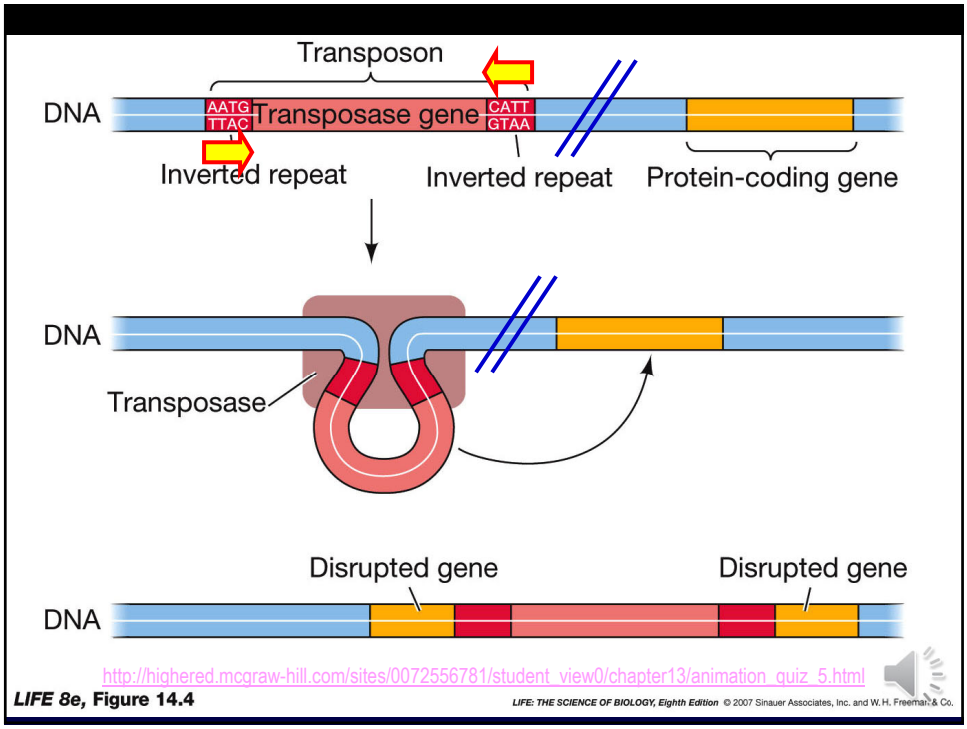
Prokaryotes: Recombination
D. *** Transposition

- ***Transposable Elements:***
  - movable stretches of DNA
  - can jump from place to place on the bacterial chromosome
    - by actually moving (***non-replicative***) or
    - by making a new copy, inserted at a new location (***replicative***).
    - Also called "***Insertion Sequences***" in bacteria.
- ***Can disrupt/ inactivate genes!!***
  - ***"Transposon Mutagenesis"***
- ***Complex Transposon:***
  carries other genes besides *Transposase* (excision & integration)
  - eg: antibiotic resistance



http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter13/animation_quiz_5.html

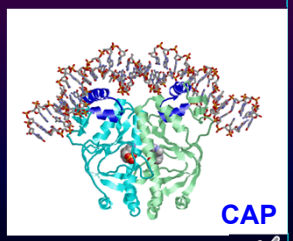
12



13

BIOL 230 – Molecular Biology
Major Concepts (Chs. 13-16a, Prok.)

1. **Central Dogma:** DNA→RNA→Protein; **5'→3' = N→C**.
2. ***One Gene-One Polypeptide*** – ARG$^-$ mutants ***(Beadle/Tatum)***
3. Transcription of a gene begins at a ***promoter***, ends at a ***terminator***
4. Translation starts with **charging a tRNA** → ribosome + AA-tRNA + mRNA → at AUG to STOP (UAA, UAG, UGA)
5. Point mutations– ***Silent, Missense, Nonsense, Frameshift***
6. **Bacteriophages** – lytic or lysogenic cycles; prophage; provirus, **Retrovirus**.
7. **OPERON Regulation in prokaryotes** – many genes/ mRNA transcript ***= polycistronic***; 1 promoter & 1 operator
   a) Positive & Negative Regulation;
   b) Constitutive vs. Inducible genes
   c) Repressible vs. Inducible operons – ***LAC*** & ***TRP***
8. **Horizontal Gene Transfer:**
   a) Conjugation
   b) Transformation
   c) Transduction
   ❖ Other Recombination: Transposition, Transposase



14

16.5) The Eukaryotic Genome

- Eukaryotes have more DNA in their genomes than prokaryotes.
  - But in some cases there is no apparent relationship between genome size and organism complexity.
- Humans: Protein-coding regions make up **~ 1.2 %,** <20,000 genes (18-19K!!)
  - An average gene has 27,000 base pairs.
  - **All** human genes have many **introns**. **TITIN** has 362; 38K aa.
  - Over 50 percent of the genome is **transposons** and other **repetitive sequences.**

A Comparison of Prokaryotic and Eukaryotic Genes and Genomes

CHARACTERISTIC	PROKARYOTES	EUKARYOTES
Genome size (base pairs)	10^4–10^7	10^8–10^{11}
Repeated sequences	Few	Many
Noncoding DNA within coding sequences	Rare	Common
Transcription and translation separated in cell	No	Yes
DNA segregated within a nucleus	No	Yes
DNA bound to proteins	Some	Extensive
Promoters	Yes	Yes
Enhancers/silencers	Rare	Common
Capping and tailing of mRNA	No	Yes
RNA splicing required (spliceosomes)	Rare	Common
Number of chromosomes in genome	One	Many

LIFE 8e, Table 14.1

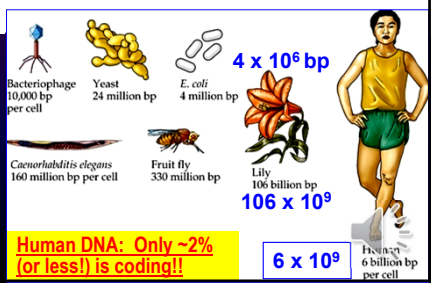


Figure 14.1 (2004)

15

A. The Eukaryotic Genome

- Eukaryotic DNA is separated from the cytoplasm within a **nucleus**. ***Nuclear Envelope***!! → More levels of control!
  - The initial mRNA transcript of the DNA often modified before it is exported to the cytoplasm.

TABLE 17.1

Representative Sequenced Genomes

ORGANISM	HAPLOID GENOME SIZE (Mb)	NUMBER OF GENES	PROTEIN-CODING SEQUENCE
Bacteria			
M. genitalium	0.58	485	88%
H. influenzae	1.8	1,738	89%
E. coli	4.6	4,377	88%
Yeasts			
S. cerevisiae	12.5	5,770	70%
S. pombe	12.5	4,929	60%
Plants			
A. thaliana	115	28,000	25%
Rice	390	37,544	12%
Animals			
C. elegans	100	19,427	25%
D. melanogaster	123	13,379	13%
Pufferfish	342	27,918	10%
Chicken	1,130	25,000	3%
Human	3,300	24,000	1.2%

Mb = millions of base pairs

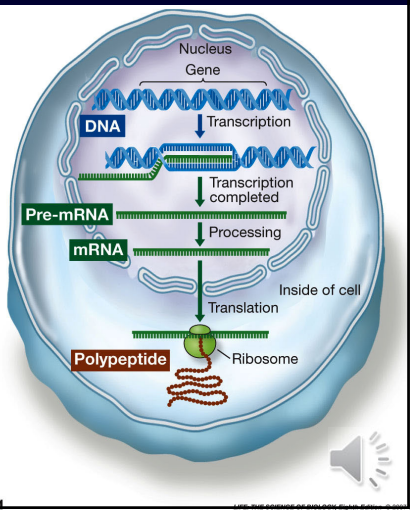


Figure 14.1

16

B. Repetitive Sequences in the Eukaryotic Genome

1. Highly repetitive DNA is present in up to millions of copies of short sequ's.
   a) It is not transcribed. Its role is unknown.
   b) **Satellite DNA** → Forensic DNA Fingerprinting!
   c) *Eg: VNTR's, ~ D1S80!!*

2. **TELOMERES**: Telomeric DNA is found at the ends of chromosomes – *prevent degradation of linear chromomosomes from loss of 5' ends.*
   – ***Telomerase*** → (5'-TTTTGGGGTTTTG-3')***n*** = produced from RNA template for 5' end of chromosome strand…… (GOOD ANIMATION!)
   • http://bcs.whfreeman.com/lodish5e/pages/bcs-main.asp?v=chapter&s=10000&n=00010&i=10010.03&o

3. Some moderately repetitive DNA sequences, such as those coding for **rRNA's**, are transcribed.
   • Mammals: **Four different rRNAs: 16S, 5.8S, 28S** = transcribed as a single transcript. Humans have 280 copies of the sequence on five different chromosomes; and **5S**.

4. **Transposons:** Some moderately repetitive DNA sequences are transposable, or able to move about the genome (***Transposase***). = *40% of Human Genome!!! (<10% in other euk.)*
   – Eg: *Alu* DNA sequences in humans (used for DNA Fingerprinting – Biol 110/225 Lab!)

17

16.6) Transcriptional Control

• Eukaryotic gene expression can be controlled at the several levels of regulation:

1. **Transcriptional** (1-2),
2. **Posttranscriptional** (3-5),
3. **Translational** (6), &
4. **Posttranslational** (7).

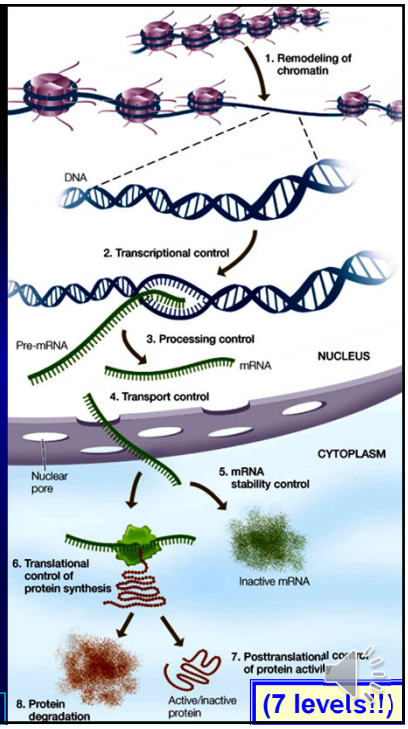


Fig. 16.13

(7 levels!!)

18

Selective Transcription: 3 Rpols

- Major method of control of eukaryotic gene expression is ***Selective Transcription.***
  - by specific proteins binding to regulatory regions on DNA
  - (similar to prokaryotes!! But only ***SOLITARY GENES!)***
- **Conserve energy**: if you don't need it, why even make the mRNA transcript??
  - *Lots of energy to synthesize RNA, process mRNA, export it, translate to protein, and modify protein!!*
  - ***** Transcriptional control saves the most energy!!! *****

****

- **3 Eukaryotic RNA polymerases**; transcribe only:
1. **Rpol-II** → protein-coding genes to mRNA.
2. **Rpol-I** → rRNA coding sequences.
3. **Rpol-III** → tRNA and small nuclear RNAs.

19

A. Transcription Factors

- A series of ***Transcription Factors*** must bind the promoter before Rpol can.
  - ***TFIID*** →
    - *recruits many other factors!!*
- **PROMOTER**: has common sequences (***TATA Box***), and specific sequences.
- Tsc'l initiation by **RNA Polymerase-II** also depends on the binding of **regulatory** proteins: ***Activator*** proteins, and ***Repressor*** proteins.

http://vcell.ndsu.nodak.edu/animations/transcription/movie.htm

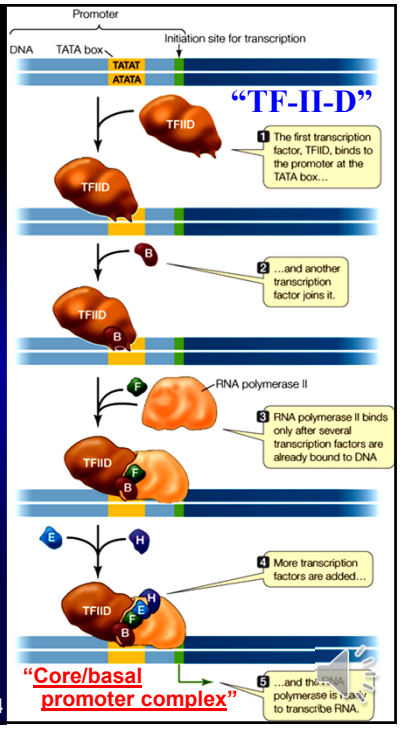
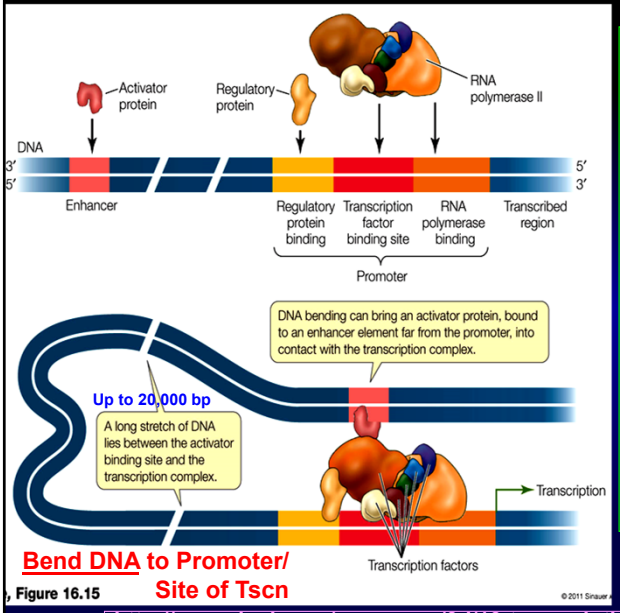


Fig. 16.14

20

Enhancers & Silencers



Enhancers and Silencers

- Far from promoter, in either direction
- Bind a regulatory protein:
  - **Activator** → Enhancer
  - **Repressor** → Silencer

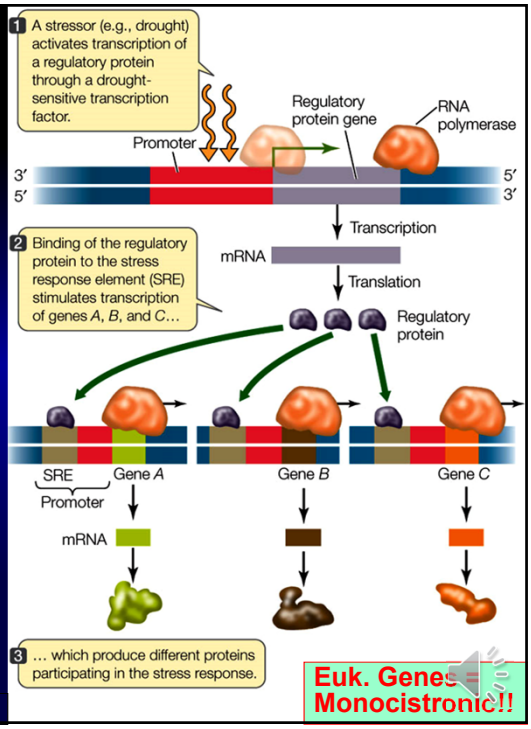
- **Cause DNA to bend** to Promoter/Transcription Complex → **Increase or decrease Tscn.**
  - Various protein interactions determine speed of transcription!

http://www.dnalc.org/resources/3d/13-transcription-advanced.html
http://highered.mcgraw-hill.com/olc/dl/120080/bio28.swf

21

B. Coordinate Regulation

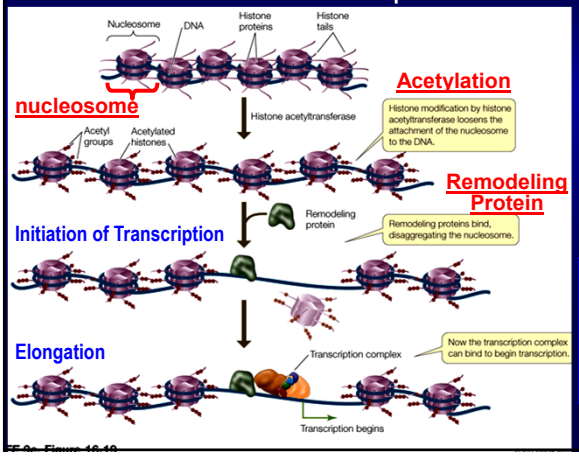
- **Simultaneous control of widely separated genes:**
  - possible by Regulatory Proteins that bind to ***common sequences in their promoters***. =
  - **Coordinate Regulation**
    - Function in same cellular process(es)
    - Eg: Plant drought



22

C. Chromatin Remodeling

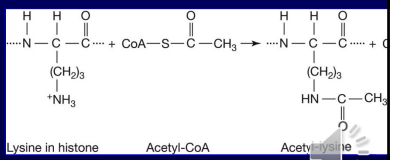
- ***Epigenetics*** → covalent modification of DNA affects transcription: ***Methylation, Acetylation.***
- ***Remodeling of chromatin*** occurs during transcription.
  - ➢ Initiation: Remove ***nucleosome.***
    - Acetylation of histones ***(Acetyl Transferases vs. Deacetylases)***
  - ➢ Then move Tscn complex thru rest of nucleosomes.



ACETYLATION (--$COCH_3$): ***

http://www.chemistry.mtu.edu/~thompson/res/research.html

http://www.youtube.com/watch?v=yQ-GvPJH8T4



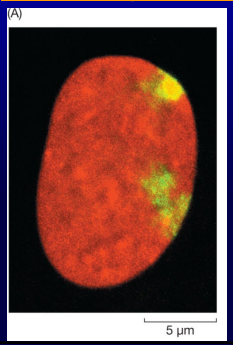
23

Heterochromatin & X-inactivation

- ***Heterochromatin*** is a condensed form of DNA that cannot be transcribed.
  - found in the inactive X chromosome of female mammals.
  - (vs. ***Euchromatin***)

http://highered.mheducation.com/sites/0072995246/student_view0/chapter24/x_inactivation.html

- **C-Methylation** (tsc-inactive genes) **& *XIST* gene**



One active X, all others inactivated

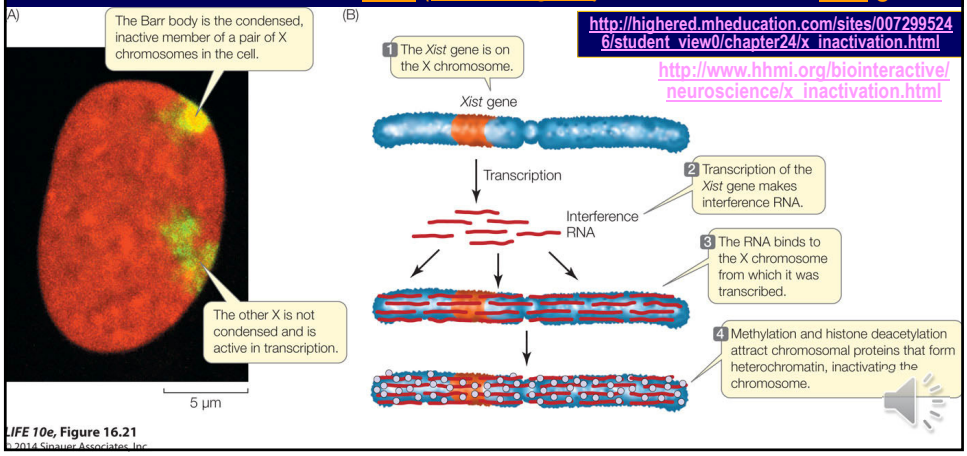
1. **Random (maternal or paternal X is inactivated)**
2. **Regulated by an RNA!**
   - **= Product of the XIST gene**
   - **X-Inactive Specific Transcript**

http://www.hhmi.org/biointeractive/x-inactivation

24

Model for X-Chromosome Inactivation

- The Inactive X has one gene that is only lightly methylated and transcriptionally active, called ***Xist***.
  - The RNA transcribed from *Xist* is not an mRNA; remains in the nucleus.
- This RNA transcript is called **interference RNA (RNAi).**
  - ***Binds the X chromosome that transcribes it; triggers inactivation.***
    - ***Methylation and histone DEacetylation → attract histones → heterochromatin!***
- Active X Chromosome has ***Tsix*** *(anti-Xist gene)* RNA bound to its ***Xist*** gene.



25

16.7) Posttranscriptional Control

- **(A.)** Because eukaryotic genes have several exons, **Alternate Splicing** can ***produce different proteins***
- ***→ from different combinations of Exons!!!!***

ONE GENE:
→ Multiple proteins!!
→ Not in prok.!

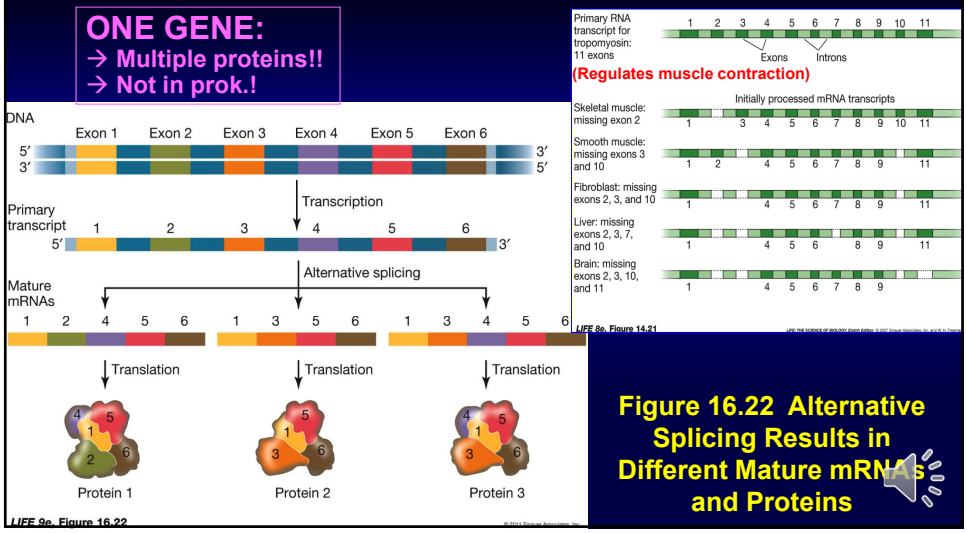


Figure 16.22 Alternative Splicing Results in Different Mature mRNAs and Proteins

26

Posttranscriptional Control

- **(B.)** The **stability of mRNA** in the cytoplasm can be regulated by the binding of proteins.
  - ***Influenced by 5' Cap and 3' Poly A tail***
    - E.G.: tubulin binds its own mRNA when in excess → speeds degradation
  - Specific **AU-rich nucleotide sequences** within some mRNAs mark them for rapid breakdown by a ribonuclease complex called the ***EXOSOME***.
    - Signaling molecules, such as growth factors, are made only when needed and then break down rapidly.
    - Also, **Micro-RNAs** bind mRNA → breakdown, or inhibit Tsln.
- **(C.)** [Includes **RNA Processing** – 4 types!!]
  - 5' G cap, 3' Poly A tail, splicing of introns, (& alternate splicing)

27

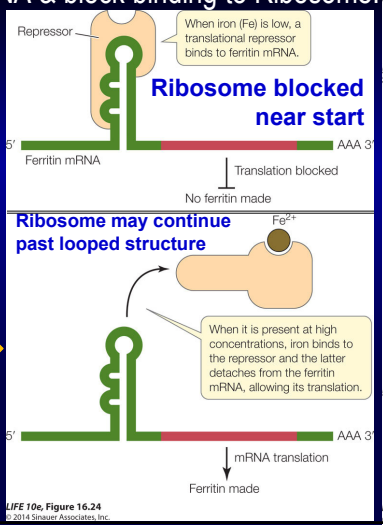
16.8) Translational Regulation

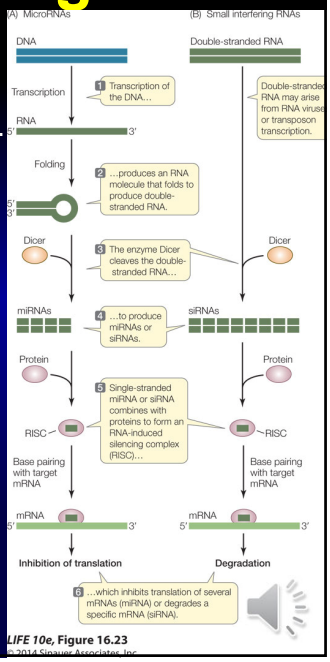
A. Translational repressors &

B. Inhibiting RNAs can inhibit translation of mRNA; Bind mRNA & block binding to Ribosome.

Eg:

- **Ferritin** (binds free iron in cyto) mRNA bound by Tslnl Reprsr when ↓Fe^{++}
  - ↑Fe^{++} → binds Tsln Repr → releases mRNA →
- ***miRNAs***,
- ***siRNAs….***





28

16.9) Posttranslational Control

A. Proteasomes degrade proteins targeted (by ***Ubiquitin***) for breakdown **(signal = N-term. AA).**

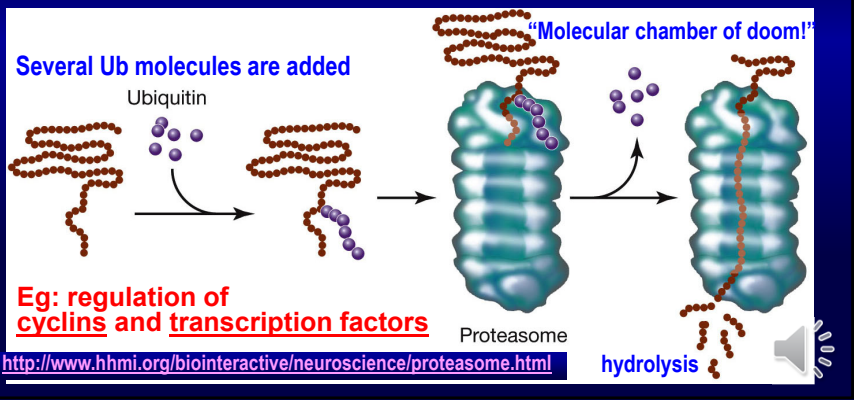
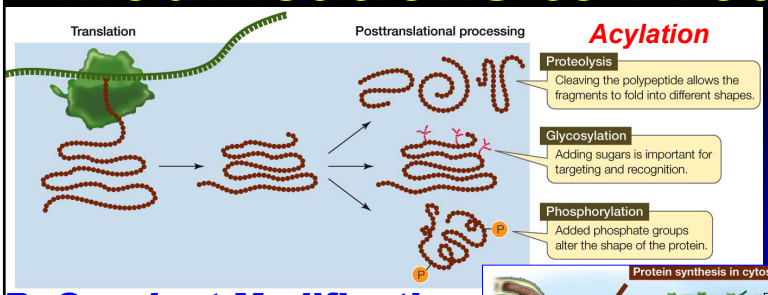


Fig. 16.24

29

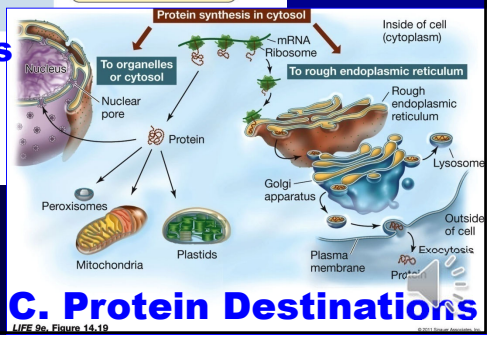
B-C. Post-Translational Modifications to Proteins



B. Covalent Modifications

http://vcell.ndsu.nodak.edu/animations/proteinmodification/movie.htm

LIFE 9e, Figure 14.22



C. Protein Destinations

LIFE 9e, Figure 14.19

30

REVIEW: Eukaryotic Gene Regulation

1. **Transcriptional: (Chs. 14 & 16)**
   - *a) Promoter* (core = TATAA Box); *RNA Pol (3!)*; *Transcription Factors*
   - b) Enhancers (distant DNA sequences) – bound by *Activators* (proteins)
   - c) Silencers (distant DNA) – bound by *Repressors* (proteins)
     - **Bend DNA to promoter & transcription complex!**
   - d) Coordinate Regulation
   - e) Chromatin Remodeling – Heterochromatin, Inactive X
2. **Posttranscriptional: (Chs. 14 & 16)**
   - a) RNA Processing – 5' cap, 3' tail, splicing (exons; alternate splicing)
   - b) Nuclear Export
   - c) mRNA stability
3. **Translational:** – http://vcell.ndsu.nodak.edu/animations/translation/movie.htm
   - http://www.dnai.org/a/index.html --> code --> putting it together
   - Translational Repressors
4. **Posttranslational: (Chs. 14 & 16)**
   - a) Proteolysis – *Ubiquitin, Proteasome.*
   - b) Chemical modification – glycosylation, phosphorylation, acylation (lipid)
   - c) Transport/Delivery – signal peptide cleavage; cellular destination/compartments

31