BIOL 240: General Microbiology

Spring 2020 March 23ff

http://accounts.smccd.edu/staplesn/biol240/

1. QUIZ #5 due Friday night (first attempt).

2. *Midterm 2 will be returned THIS WEEK on CANVAS.*

1

Ch. 8: Microbial Genetics

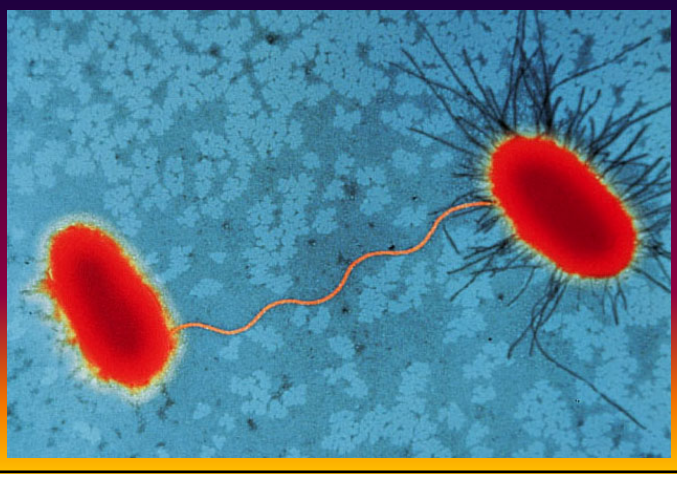
OBJECTIVES: Students should be able to ....

1. **Ch. 8:** Describe the **Central Dogma** of molecular genetics and the three processes that drive the flow of genetic information in an organism.
2. Describe several **properties of DNA** and the process of **DNA replication** that contribute to DNA's central role as the hereditary material.
3. Draw a **replication fork** and label **5 enzymes involved in DNA replication**. Describe the function of each enzyme.
4. Compare and contrast DNA Synthesis (**Replication**) with RNA Synthesis (**Transcription**). Why is it suitable that RNA is a less stable molecule than DNA? ***(THINK: Structure → Function!!! ..... ALWAYS!!)*** *[Make a table!]*
5. ** List the **three types of RNAs** and their functions in protein synthesis. How do they "read" the genetic code? Define a "***codon***", and explain the functional mechanisms of a ***start codon*** and a ***stop codon***.
6. ** Compare the stages of **Initiation, Elongation, and Termination** in **Replication, Transcription and Translation**. Indicate the direction of synthesis in each, and the names of the start and stop sites. *[Make a table!]*
7. ** Diagram an **Operon**, including **6 DNA and protein components** important to its function. *Explain the purpose of this structure/organization.*
8. Explain and give examples of **Inducible & Repressible Operons**. *Describe what types of protein products or pathways each controls, and explain why this is appropriate.*
9. List three types of possible **point mutations**, and explain how some types might be more severe than others. *Explain how can mutations be prevented or repaired.*
10. Outline two methods for **identifying** and **isolating mutant** bacteria.
11. Define, diagram & compare the **3 mechanisms of Horizontal Gene Transfer** **in prokaryotes.**

❖ ***Objectives are your HOMEWORK between classes!!!*** ***Read, Review, Draw!!***
- ***Outline the concepts, define terms, DRAW structures and processes, and PRACTICE!!***

2

Chapter 8
Microbial Genetics



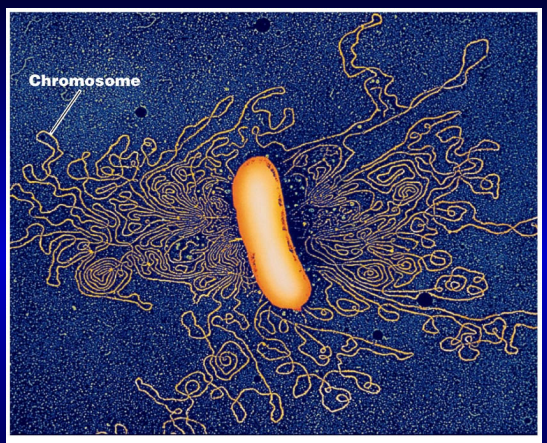
3

Terminology

1. ***Genetics:*** Study of what genes are, how they carry information, how information is expressed, and how genes are replicated; "the science of heredity"
2. ***Gene:*** Segment of DNA that encodes a functional product, usually a protein
3. ***Genome*** = All of the genetic material in a cell
4. ***Genomics*** = Molecular study of genomes
5. ***Genotype*** = Specific forms of genes in an organism
   - Types of alleles present.
6. ***Phenotype*** = *physical characteristics resulting from expression of the genes*

4

E. coli

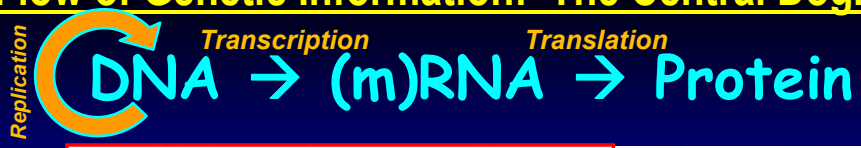


The ***Model Organism*** for molecular biology.

Figure 8.1a

5

Flow of Genetic Information: The Central Dogma



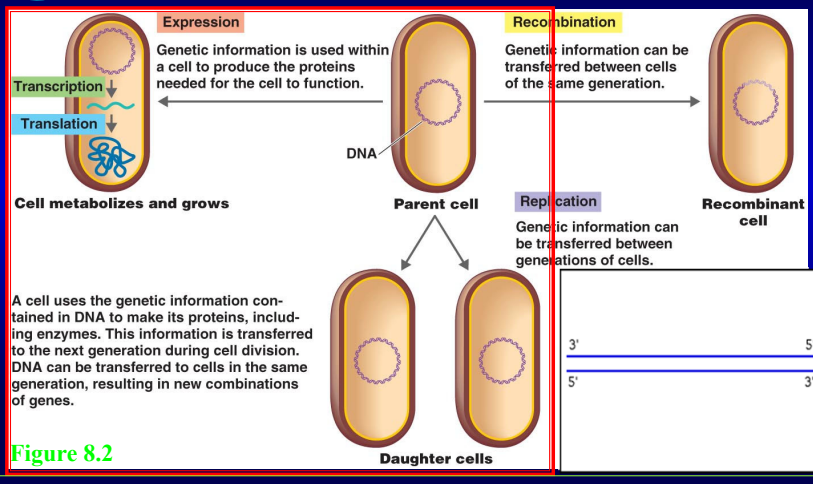


Figure 8.2

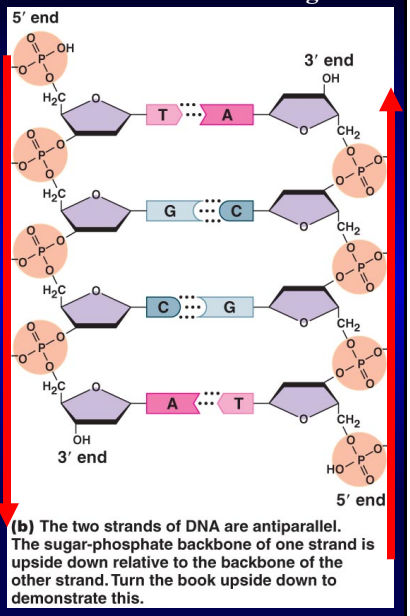
** ***Central Dogma*** of Molecular Genetics **

6

8.1) DNA

1. Polymer of nucleotides: adenine, thymine, cytosine, guanine (**ATGC**)
2. Double helix associated with proteins
3. "Backbone" is deoxyribose-phosphate
4. Strands held together by hydrogen bonds between **A=T** and **G≡C**
5. Strands are ***Antiparallel***
   - ***5' (PO_4) → 3' (OH) polarity***

Figure 8.3



(b) The two strands of DNA are antiparallel. The sugar-phosphate backbone of one strand is upside down relative to the backbone of the other strand. Turn the book upside down to demonstrate this.

7

A. DNA Structure

- ***Semi-Conservative Replication:***
  - Each "parental strand" serves as ***template…***
  - for synthesis of a new "daughter strand"
  - Following ***complementary base-pairing*** rules
    - **A=T**
    - **G≡C**
  - *Rules allow one to predict 2nd strand sequence from 1st!!!*

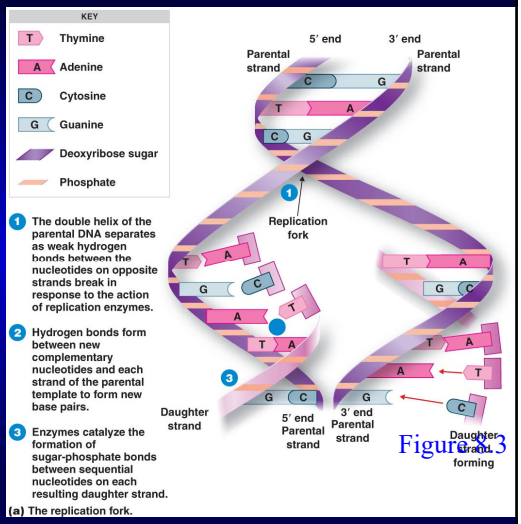
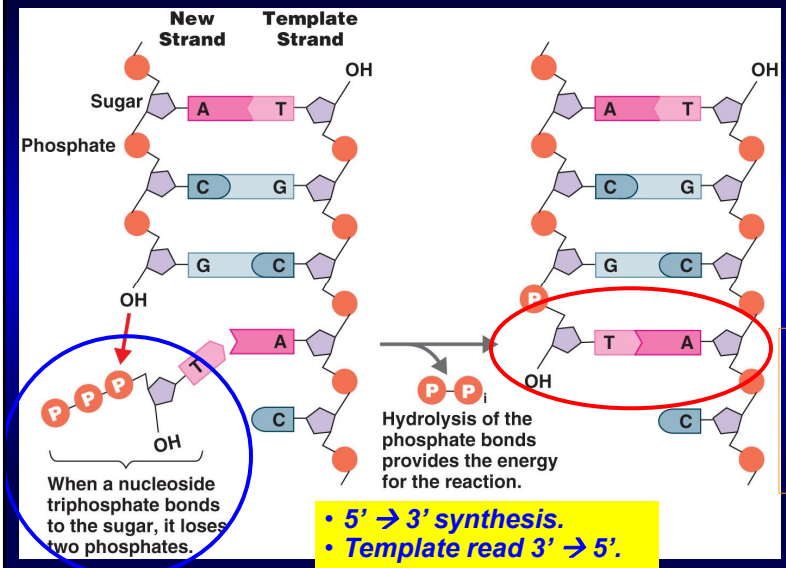


Figure 8.3

GREAT overview: https://youtu.be/TNKWgcFPHgw
http://www.uic.edu/classes/cmeng/cmeng521/DNA_replication.html

8

B. DNA Replication



= DNA-directed DNA synthesis.

http://www.fed.cuhk.edu.hk/~johnson/teaching/genetics/animations/dna_replication.htm

http://www.uic.edu/classes/cmeng/cmeng521/DNA_replication.html

- 5' → 3' synthesis.
- Template read 3' → 5'.

http://www.courses.fas.harvard.edu/~biotext/animations/replication1.swf

Figure 8.5

9

10

DNA Replication Proteins

1. Open helix at ***Origin (Ori)***, lay-down primers:
   - ***a) DNA Helicase*** "melts" strands apart, breaking H-bonds
   - b) Single-Strand Binding Proteins (***SSB***) keep template strands apart.
   - ***c) RNA Primase*** lays down first several nucleotides (RNA!!) – gives "starting block" (free 3'-OH) to begin actual DNA synthesis. [RNA Primers are removed later!]
2. DNA = copied by ***DNA polymerase III*** (Dpol3)
3. In the 5′ → 3′ direction – new nucleotides added to the 3' hydroxyl (-OH) group on deoxyribose in the growing strand
   - Initiated by an ***RNA primer*** (*RNA Primase* enz.)
4. **Leading strand** synthesized continuously
   - follows fork (1/fork; 2/ "bubble")
5. **Lagging strand** synthesized discontinuously
   - Opposite to fork movement → ***Okazaki fragments*** *(unsealed lagging pieces)*
6. RNA primers are removed and Okazaki fragments joined by ***DNA polymerase I*** & ***DNA ligase*** → ***"fill and seal" to finish job!!***

AMOEBA SISTERS!!!!
https://youtu.be/5qSrmeiWsuc

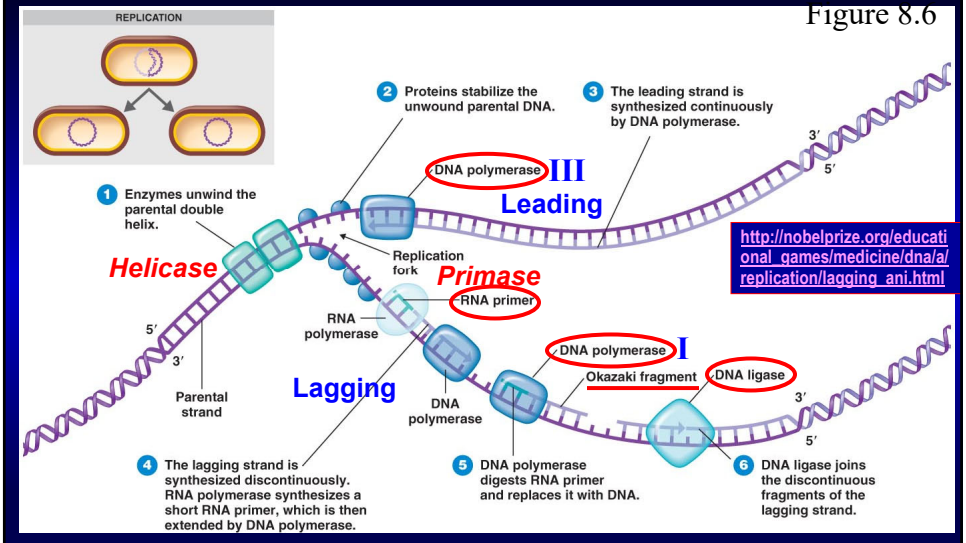
- http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/dna-rna2.swf

Detailed!: http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html

11

C. DNA Replication Fork

Figure 8.6



http://nobelprize.org/educational_games/medicine/dna/a/replication/lagging_ani.html

http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/dna-rna2.swf

http://highered.mcgraw-hill.com/sites/0072943696/student_view0/chapter3/animation__dna_replication__quiz_1_.html

http://www.johnkyrk.com/DNAreplication.html http://www.dnalc.org/resources/3d/DNAReplicationBasic_w_FX.html

12

DNA replication is *Semiconservative* & *Bidirectional*

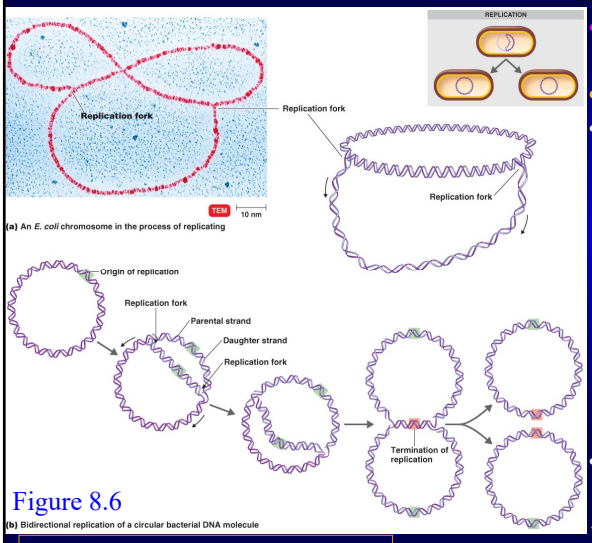


Figure 8.6

- ***= DNA-directed DNA synthesis!!!***
- ***2 forks: opposite directions!***
- Replication results in two daughter DNA duplexes,
  - ***each with one completely new strand, &***
  - ***one old strand (parental strand)***
  - ***= "SEMI-CONSERVATIVE"***
    - ***ONLY synthesize 5'→3'!!!***
- Two replication forks move in opposite directions =
  - ***"Replication Bubble"***

3D REALTIME***: https://youtu.be/6j8CV3droDw

http://www.hhmi.org/biointeractive/dna/animations.html

http://www.wwnorton.com/college/biology/mbio/animations/dna_replication.asp

http://www.sciencemedia.com/website/demos/biochem/ecoliReplication.html

13

8.2) Transcription: RNA Synthesis

1. DNA is transcribed to make RNA (**AUGC**)
   a) **mRNA** = messenger RNA → translated to protein
   b) **tRNA** = transfer RNA → brings amino acid to ribos.
   c) **rRNA** = ribosomal RNA → makes up ribo; catalytic
2. Transcription begins when ***RNA polymerase*** binds to the ***PROMOTER*** sequence
   – *Tells Rpol which strand, and where to start transcribing!!*
3. Transcription proceeds in the ***5′→3′ direction*** (same in ALL nucleic acid synthesis!)
   - new nucleotides added to the 3' hydroxyl group on ***ribose*** in the growing strand
4. Transcription stops when it reaches the ***Terminator Sequence*** *(often many U's or "hairpin loop")*
   - New RNA and Rpol fall off of DNA template.

14

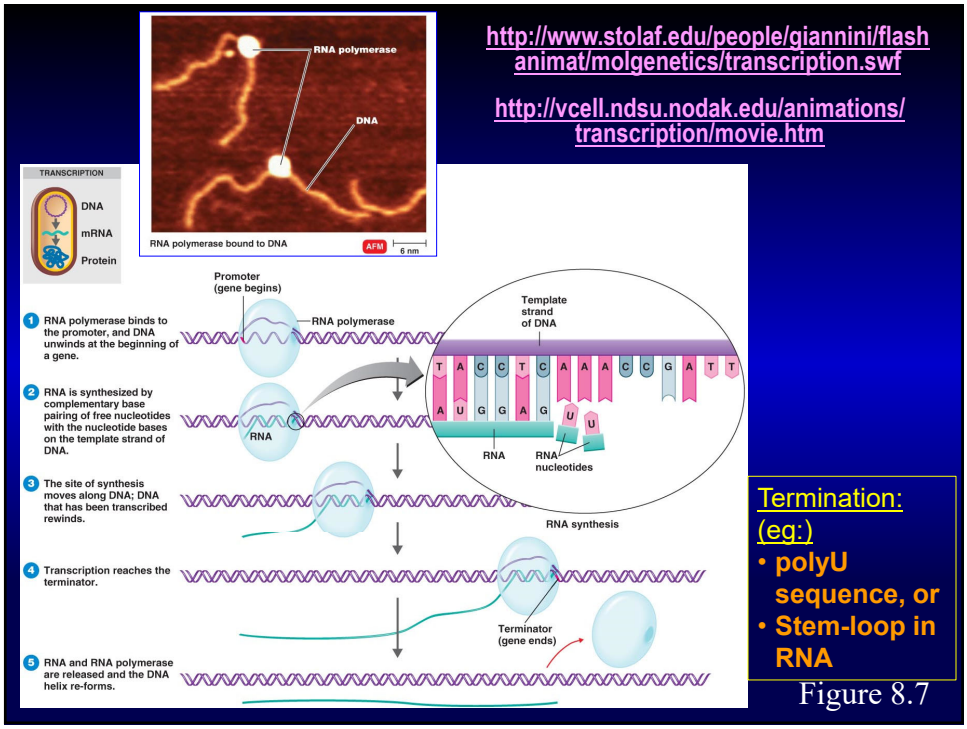


Figure 8.7

15

8.3) Translation

1. ***= RNA-directed Protein synthesis!!!***
2. mRNA is translated in ***Codons.***
   - 3 nucleotide "words"
3. Translation of mRNA begins at the ***start codon:*** ***AUG.***
   - ***Tells ribosome where to start translating,***
   - ***and sets the reading Frame!!!***
4. Translation ends at a ***STOP ("nonsense") codon:*** ***UAA, UAG, UGA.***
   - ***Do NOT encode an amino acid!!!***

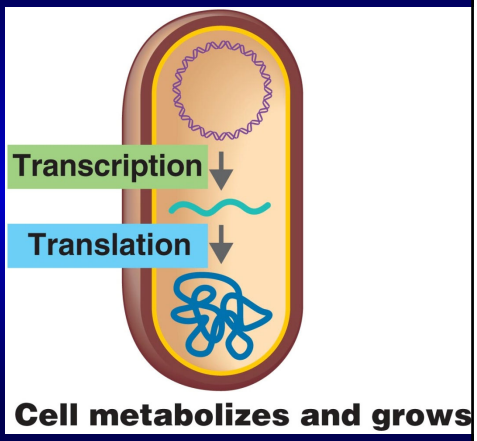


Figure 8.2

16

The "Universal" Genetic Code

Figure 8.8

First position	Second position U	Second position C	Second position A	Second position G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met/start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

- **Codons = non-overlapping**
  - **Start transl'n; set the frame!!**
- **tRNA (transfer RNA)** = adapter molecule that reads "3 letter" codons – via it's complementary "***Anticodon***"!
- Puts in the right amino acid in right order
  - ***Reads mRNA 5'→3',***
  - ***synthesizes N→C polypeptide***
    - **Amino → Carboxy!**
  - ***Anticodon-Codon base-pairing***

https://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter3/animation__how_translation_works.html

http://www.lewport.wnyric.org/jwanamaker/animations/Protein%20Synthesis.html

17

A. Translation – *Initiation*

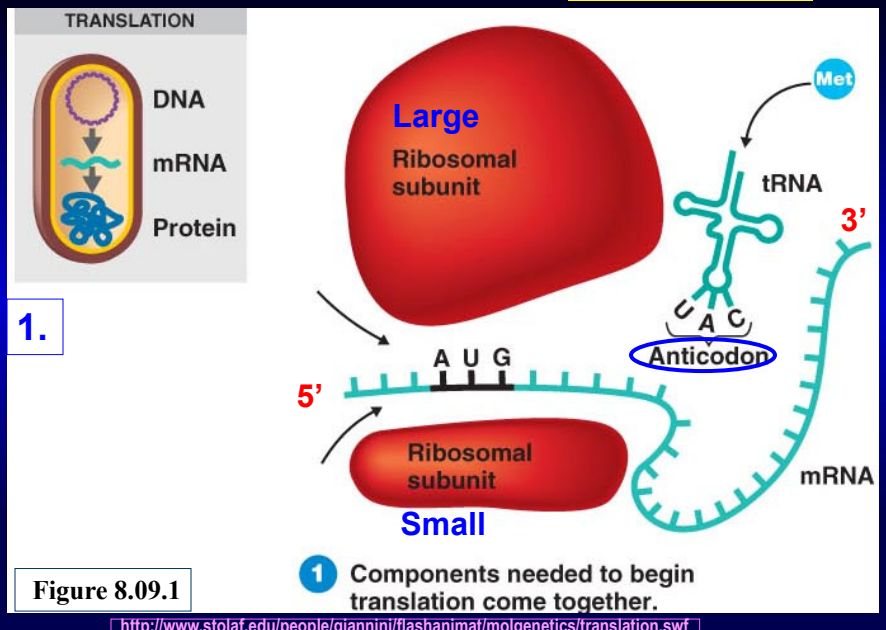


Figure 8.09.1

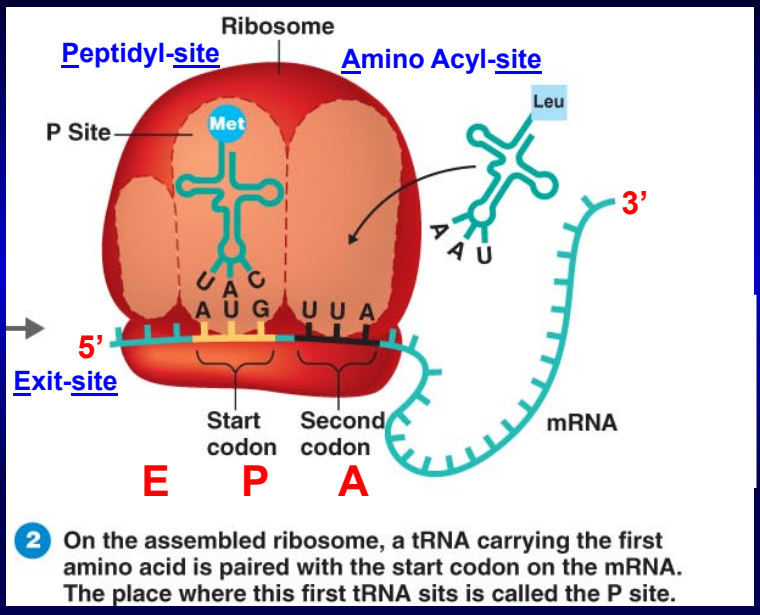
http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/translation.swf

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter15/animations.html#

18

B. Translation - *Elongation*

2.



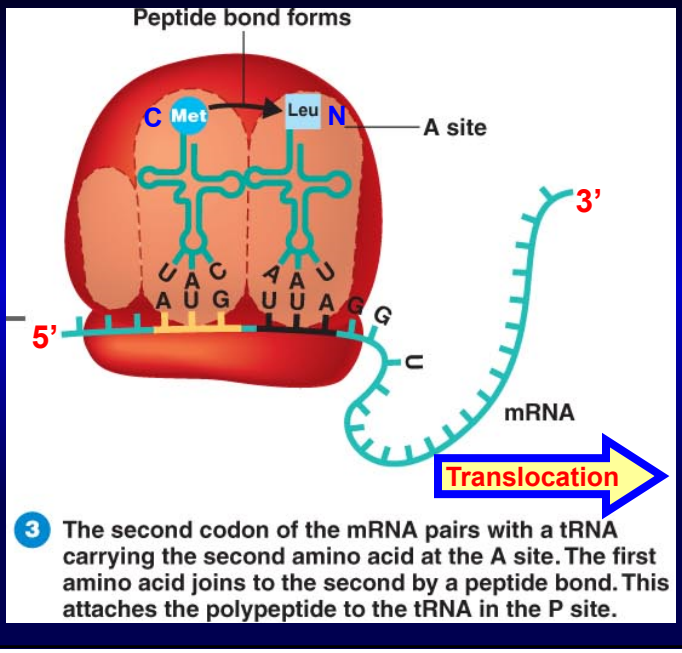
2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. The place where this first tRNA sits is called the P site.

Figure 8.09.2

19

Peptidyl Transferase

3.



3 The second codon of the mRNA pairs with a tRNA carrying the second amino acid at the A site. The first amino acid joins to the second by a peptide bond. This attaches the polypeptide to the tRNA in the P site.

Figure 8.09.3

20

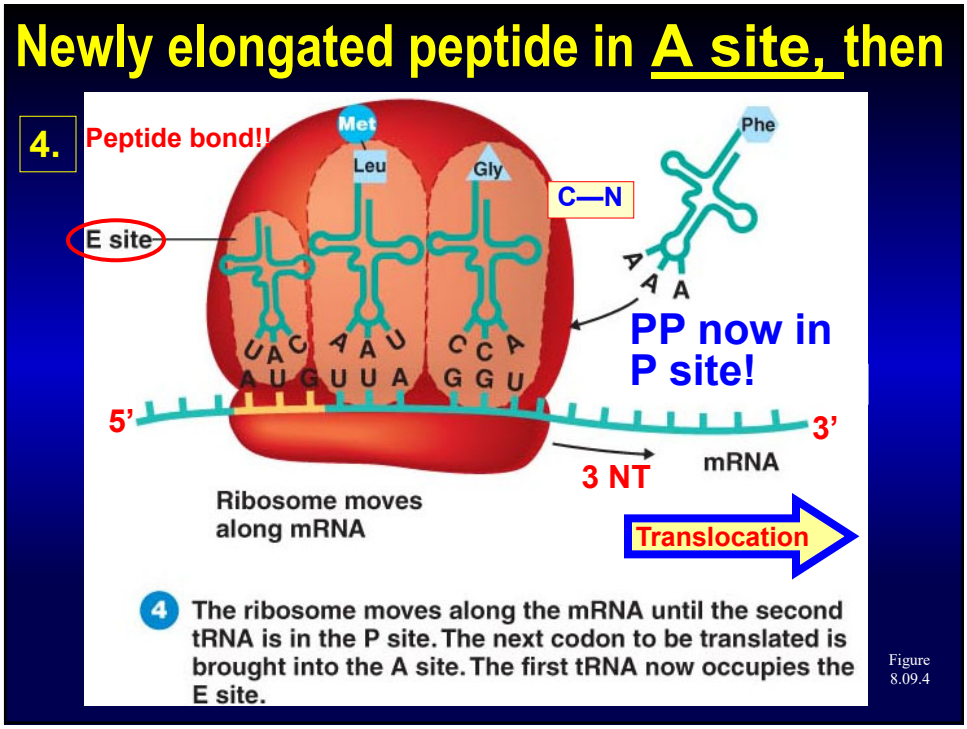


Figure 8.09.4

21

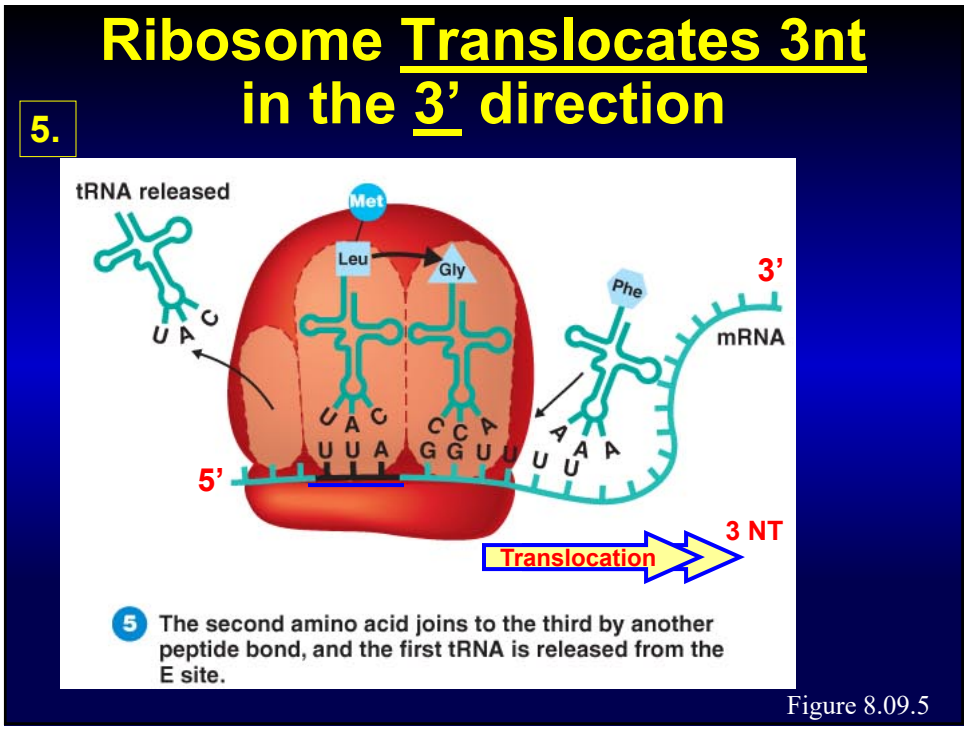


Figure 8.09.5

22

Translation: Peptidyl Transfers & translocations continue

6.

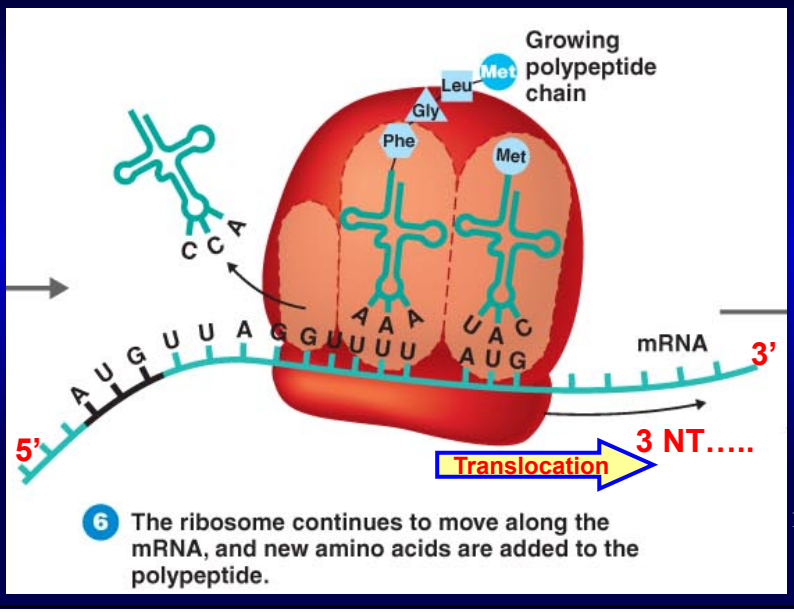
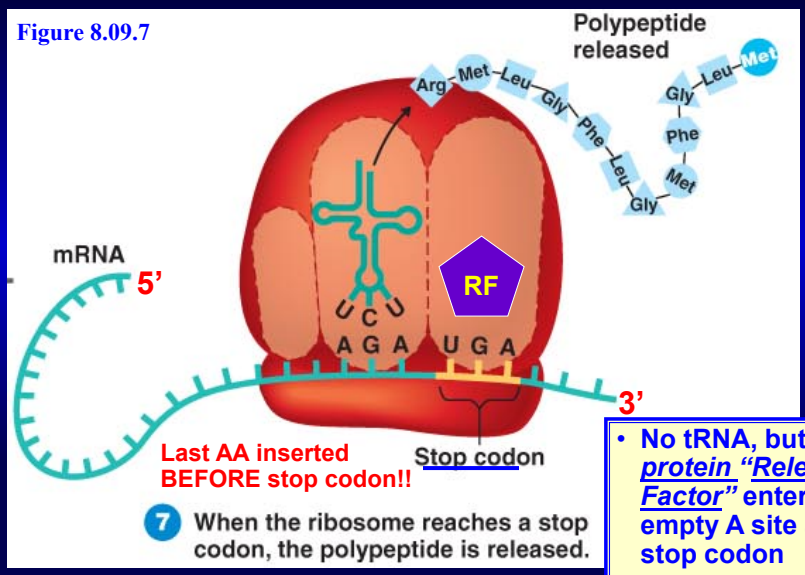


Figure 8.09.6

23

C. Translation - *Termination*

7.



- No tRNA, but a *protein "Release Factor"* enters empty A site at a stop codon

24

TsIn - Termination/ Release

8.

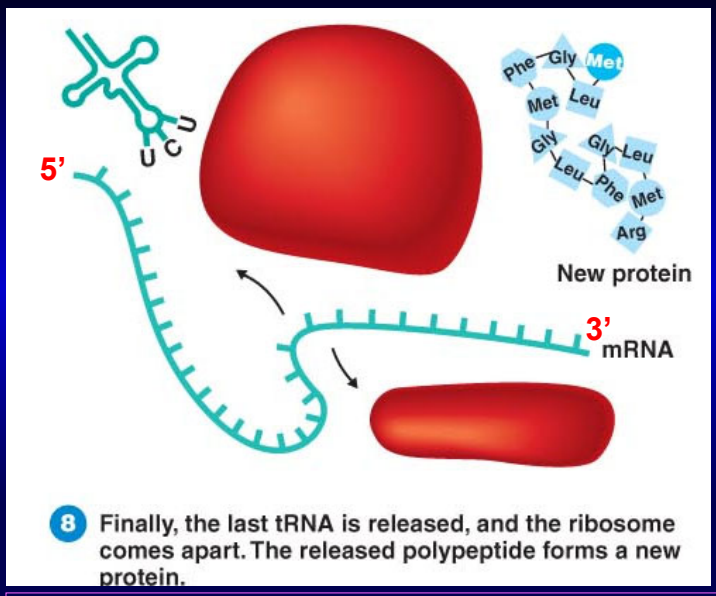
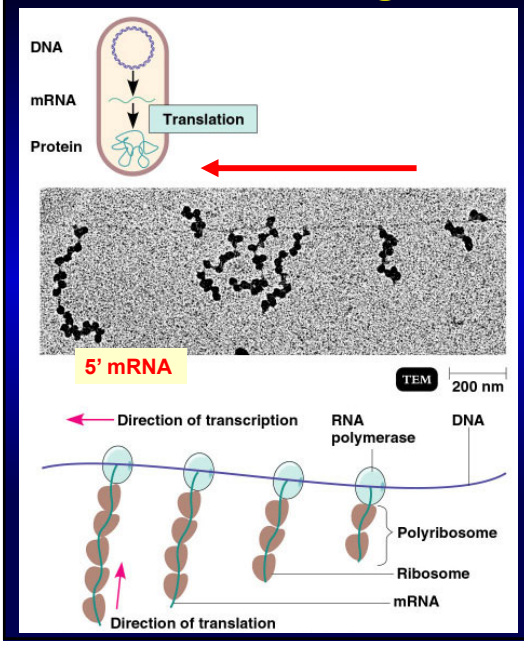


Figure 8.09.8

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

25

** Polyribosomes



- Multiple ribos/ polypeptides translated per mRNA!!!
- = POLYRIBOSOMES (in both Euk. & Prok.!)

Also:

- ***TsIn & Tscn simultaneous in prokaryotes!! (only!)***
  - No nuclear membrane to separate!

Figure 8.10

26

Compare RepIn, Tscn, TsIn:

Process	Initiation	Elongation	Termination
Replication	At Origin: A/T-rich, NZs: Helicase, Primase, DPol3	Dpol3, dNTPs, 5'→3'	Terminator sequences, or end of chromosome (forks meet if circle)
Transcription	At Promoter: AT rich, NZ: RPol	RPol, NTPs, 5'→3'	Tsc'I terminator (poly-U, hairpin loop)
Translation	At Start Codon:(AUG), mRNA, AA-tRNA NZ: ribosome	Ribosome, AA-tRNA's (anticodons), N→C (follows mRNA 5'→3')	Stop codon (nonsense codon): UAA, UAG, UGA •Release Factor (= protein)

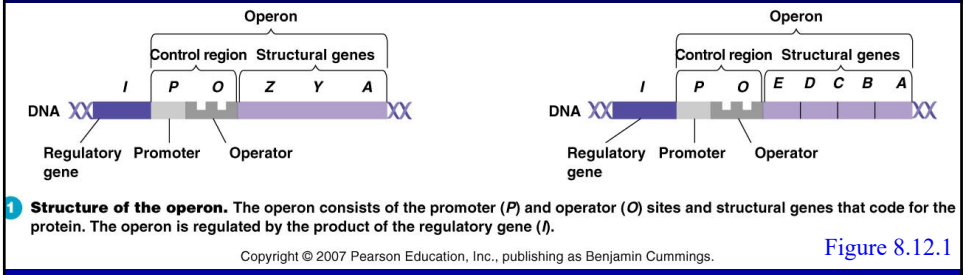
27

8.4) Regulation of Bacterial Gene Expression

- ***Constitutive Genes (enzymes/proteins)*** are expressed at a fixed rate ("always on"!)
  - "housekeeping" functions in cells
- Other enzymes are expressed only as needed ***("regulated enzymes/genes")***
  - ***Repressible Genes/enzymes*** = turned off when not needed; **usually ON.**
  - ***Inducible Genes/enzymes*** = turned on only when needed; **usually OFF.**

28

Operons:
Lactose Catabolism; Trp Synthesis



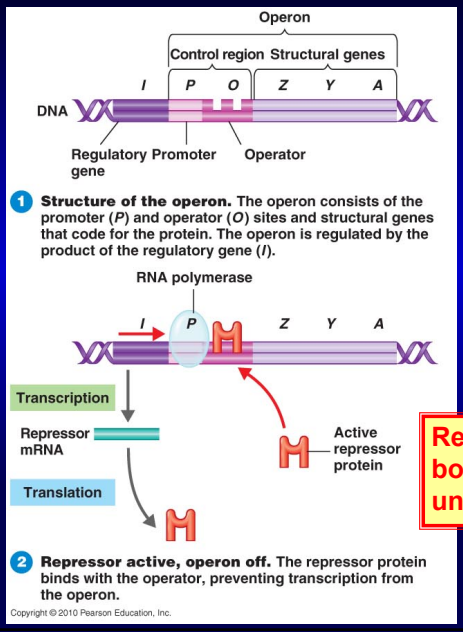
1 **Structure of the operon.** The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein. The operon is regulated by the product of the regulatory gene (*I*).

Figure 8.12.1

- ***OPERATOR*** = target/binding site on DNA.
- Bound by a ***REPRESSOR*** protein, to stop action of RNA Polymerase (→ genes OFF).
  - ***= Negative Regulation!***

29

A. *Lac* Operon: Inducible



Repressor protein usually bound to Operator (OFF!), unless lactose present.

Figure 8.12.2

30

Repression

- Prokaryotes: Related genes organized into ***OPERONS***:
  - Many ***"structural genes"*** (protein-encoding)
  - Under the control of a SINGLE ***PROMOTER*** & ***operator*** (negative control sequence)
  - ***Polycistronic (polygenic) mRNA's!***
    - Single mRNA transcript → encodes several proteins!!
    - NOT in EUKaryotes!!

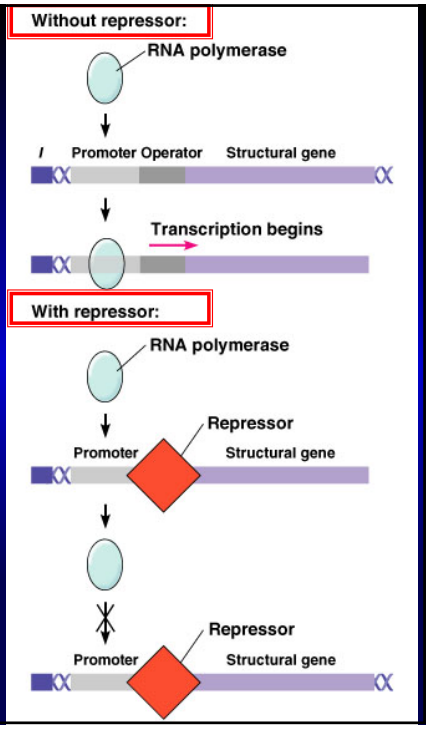
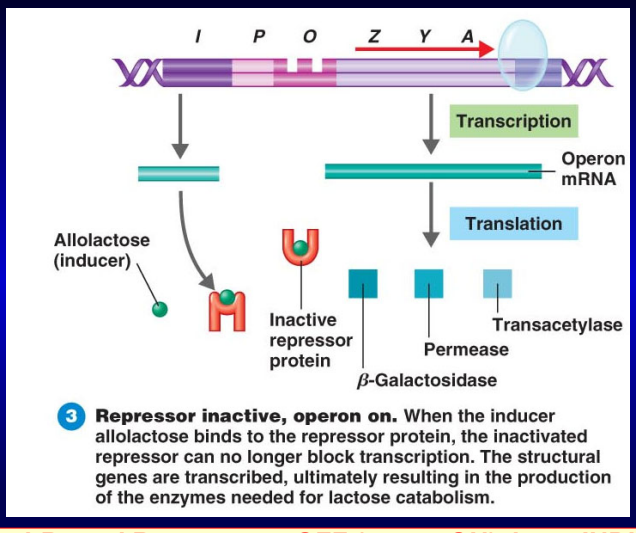


Figure 8.13 (2004)

31

Lac gene induction by Lactose



Signal-Bound Repressor = OFF (genes ON), in an INDUCIBLE system

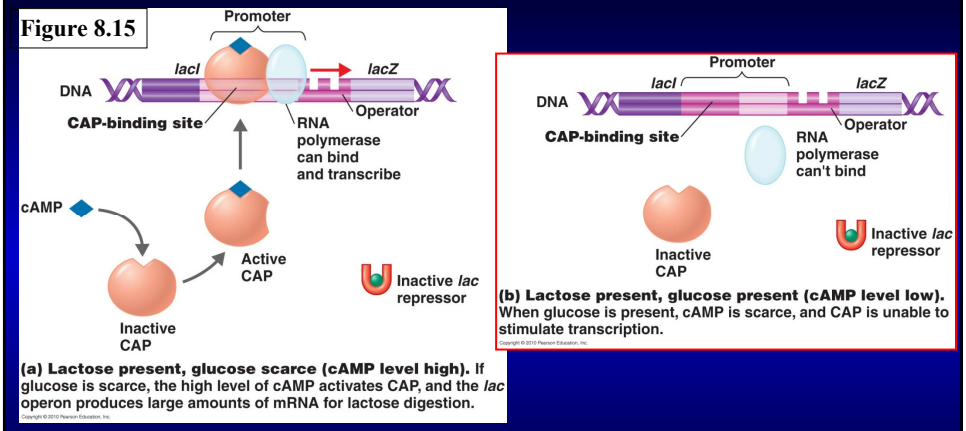
http://vcell.ndsu.nodak.edu/animations/lacOperon/movie.htm

Figure 8.12.4

32

Positive Regulation of the *lac* operon

http://www.biocourse.com/ui/swf/iLabs/lac_operon.swf***

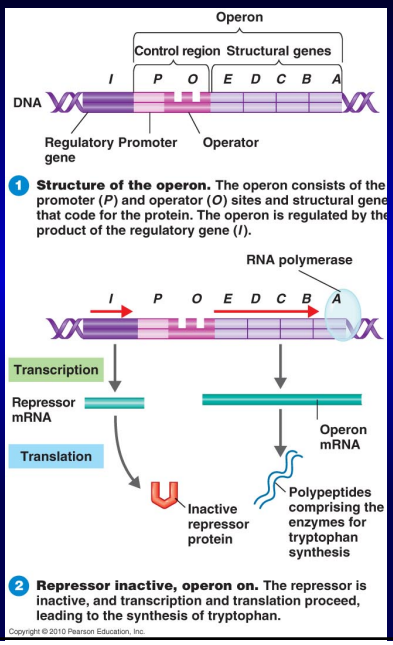


1. Glucose is the preferred energy source in most cells
2. For lactose and other alternative sugars to be consumed, glucose cannot be present
3. With glucose absent, ATP → ADP → AMP → cyclic AMP (starvation signal!!)
4. cAMP binds **CAP (Catabolite Activator Protein)**, which helps RPol bind lac promoter
5. *Full LAC operon activation requires Lactose PRESENT, and Glucose ABSENT!!!*

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120080/bio27.swf::Combination%20of%20Switches%20-%20the%20Lac%20Operon

33

B. *Trp* Operon: Repressible



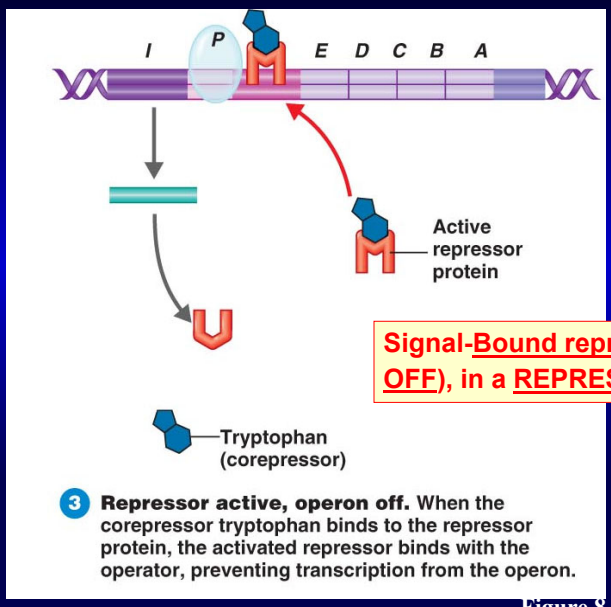
Repressor protein NOT bound to Operator, unless excess TRP present.

- TRP = "corepressor"; activates repressor
  - → genes OFF when excess TRP

Figure 8.13

34

Trp gene repression by excess TRP



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

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

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

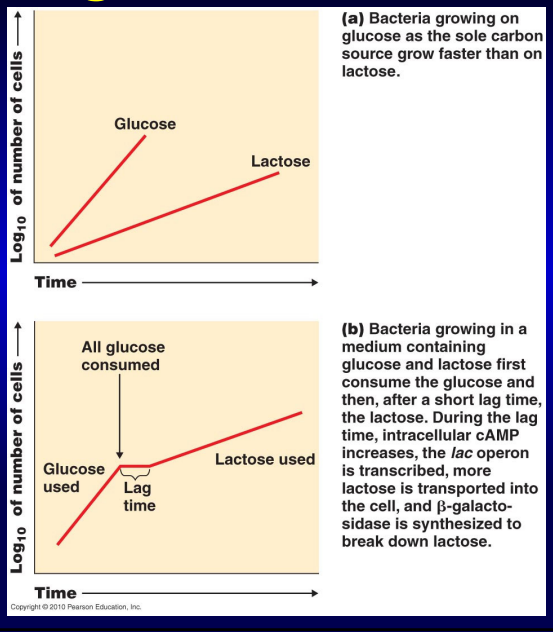
Signal-Bound repressor = ON (genes OFF), in a REPRESSIBLE system

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

Figure 8.13

35

Regulation of Gene Expression



Faster growth on preferred C-source (glc)

Delayed growth (Lag!) on switching to secondary sugar, Lactose

- **Lact catabolic enzymes must be induced & synthesized!!**

36

Operon Regulation: Summary

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

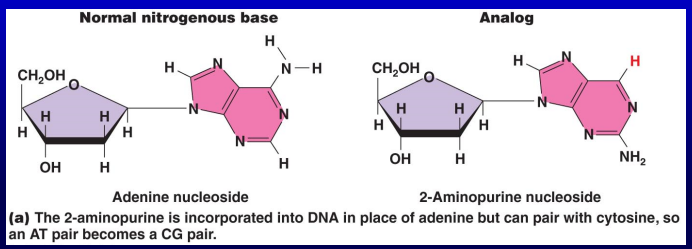
[Reminder: both signal molecules/ Ligands bind to the Repressor protein, **allosterically** changing its activity. Inducer, or Corepressor?]

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

37

8.5) Mutation

- ***= Change in the genetic material***
  - *Source of evolutionary change!! (raw material)*
  - *The most powerful tool in Molecular Biology!!*
- Mutations may be neutral, harmful, or beneficial.
- ***Mutagen***: Agent that causes mutations above spontaneous levels.



(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.

Fig. 8.19a

- ***Spontaneous mutations***: Occur in the absence of a mutagen; due to normal, rare chemistry.

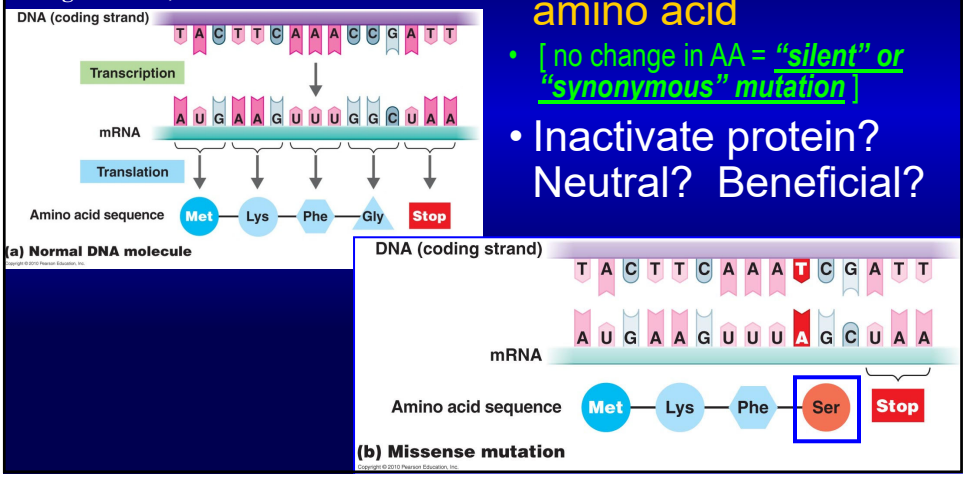
38

A. Point Mutations (base substitution)

1. Missense mutation

- Change in one base
- Result in change in amino acid
- [no change in AA = *"silent" or "synonymous" mutation*]
- Inactivate protein? Neutral? Beneficial?

Figure 8.17a, b



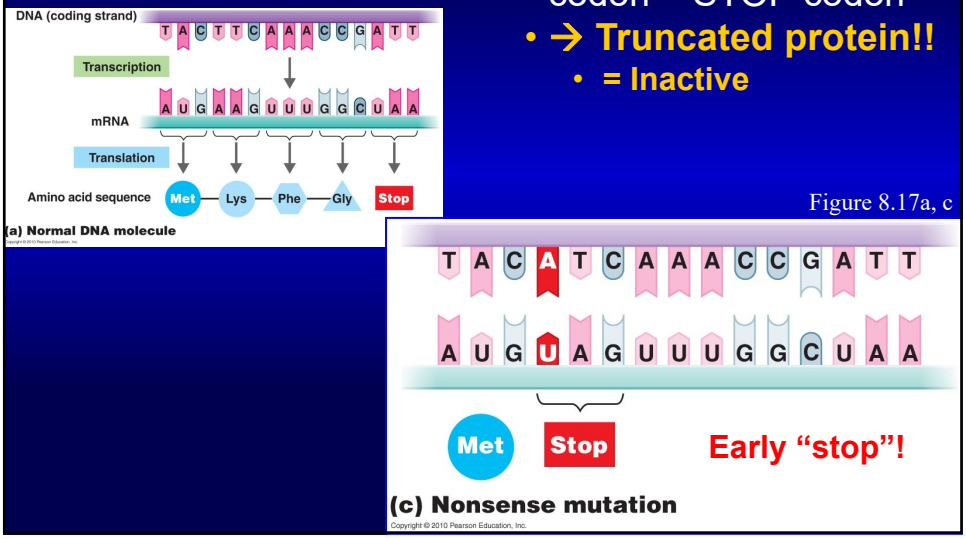
39

Mutation

2. Nonsense mutation:

- Results in a nonsense codon = STOP codon
- → **Truncated protein!!**
  - **= Inactive**

Figure 8.17a, c



40

Mutation

3. *Frameshift mutation:*

- Insertion or deletion of one or more nucleotide pairs *(when not multiple of 3)* – severely change protein sequence from mutation site onward….

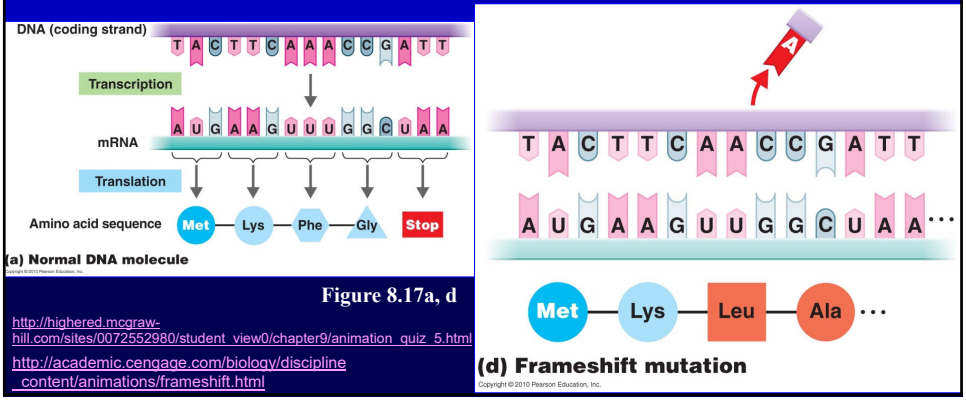


Figure 8.17a, d

http://highered.mcgraw-hill.com/sites/0072552980/student_view0/chapter9/animation_quiz_5.html

http://academic.cengage.com/biology/discipline_content/animations/frameshift.html

41

Codons & Deletion Mutations

Normal

CAT CAT CAT CAT CAT CAT

•FAT CAT ATE THE RAT

One deletion

CTC ATC ATC ATC ATC AT

•FTC ATA TET HER AT.

Two deletions

CTA TCA TCA TCA TCA T

•FTA TAT ETH ERA T..

Three deletions

CTA TAT *CAT CAT CAT*

•FTA TAE *THE RAT* …

• = DNA
• = Words analogy

Junk!

Back into Frame, -1 aa

3 deletions = mostly OK → clue codons = triplets!!

• Insertions or deletions (≠ multiple of 3), cause shift in reading frame
  •= "Frameshift Mutation"→ all codons downstream are JUNK!
    •→ inactive protein!

42

B. Mutagenesis

- Ionizing radiation (X rays and gamma rays) causes the formation of ions
  - can react with nucleotides and the deoxyribose-phosphate backbone → DNA Damage!!
- ***Nucleotide excision repair*** fixes mutations

The Frequency of Mutation

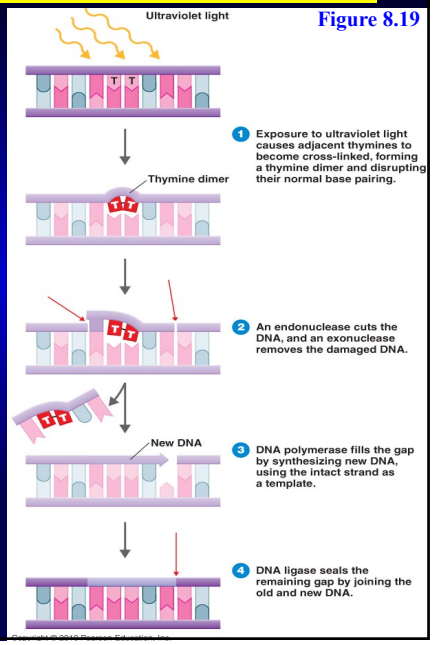
- **Spontaneous mutation rate** =
  - 1 in 10^9 replicated base pairs (10^{-9}) or
  - ~1 in 10^6 replicated genes (10^{-6})
    - (assumes average gene = ~1000 bp)
- ***Mutagens*** increase to 10^{-5} or 10^{-3} per replicated gene (10-1000X more likely!).

43

C.) DNA Excision Repair

- UV radiation causes ***Thymine Dimers***
  - *Pyrimidine dimers!!!*
- ***Light-Activated Repair:*** *Photolyases* separate thymine dimers.

1. **Endonuclease** enzyme clips DNA strand at each side of damage.
   - Recruited to site by ***"Seeker Proteins"***.
2. **Dpol1 & ligase** = fill & seal.



44

D.) Studying Mutations: Selection

1. **Positive (direct) selection** detects mutant cells because they grow or appear different.
   - eg: antibiotic-resistant mutants

2. **Negative (indirect) selection** detects mutant cells because they do not grow.
   - ** ***Replica plating*** – *What does NOT grow on selective media?*
     - eg: ***auxotrophic mutants*** = need "help growing", because lost ability to make a nutrient for themselves

45

Replica Plating – for Neg. selection

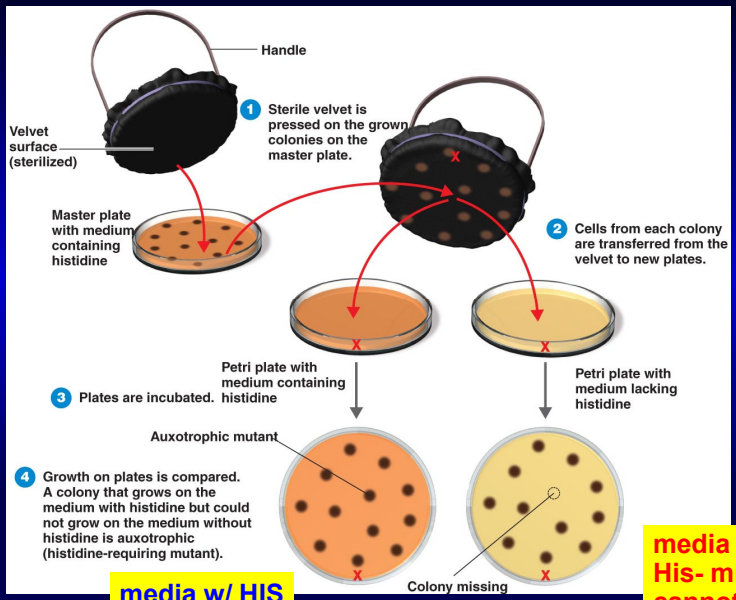


Figure 8.21

46

The Ames Test for Chemical Mutagens/Carcinogens

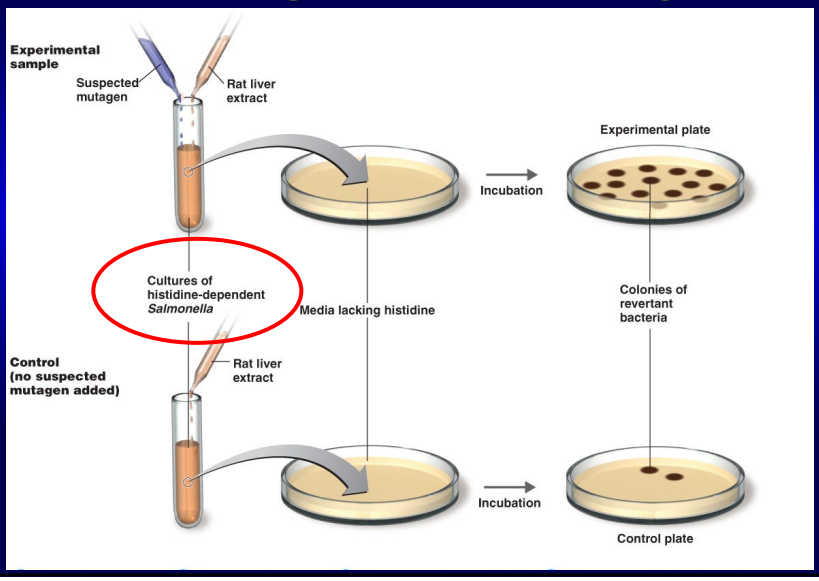
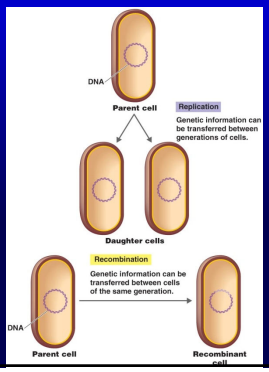


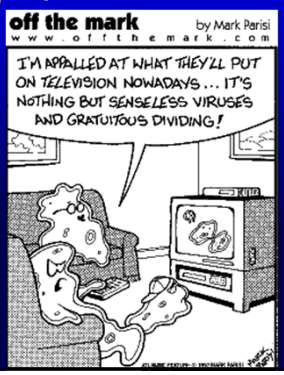
Figure 8.22

47

8.6) Genetic Transfer & Recombination

- ***Vertical gene transfer*** = Occurs during reproduction, between generations of cells.
- ***Horizontal gene transfer*** = Transfer of genes between cells of the same generation.



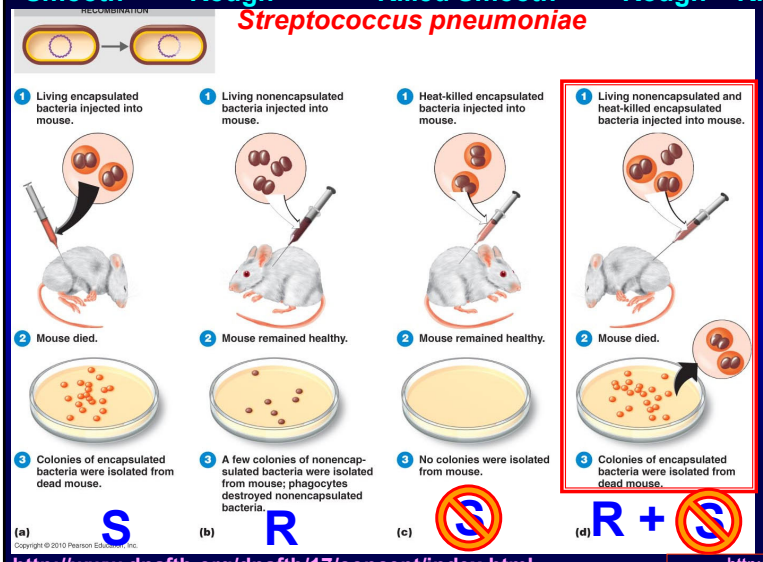


48

A.) Transformation (Frederick Griffith)

Smooth Rough Killed Smooth Rough + Killed smooth

Streptococcus pneumoniae



CONCLUSION:

Some substance from the killed S bacteria had "Transformed" the R → S!!!

The substance later proven to be DNA!

Figure 8.24

http://www.dnaftb.org/dnaftb/17/concept/index.html

http://www.dnalc.org/resources/animations/

http://highered.mcgraw-hill.com/sites/0072552980/student_view0/chapter9/animation_quiz_15.html

49

** Recombination **

Transformation is only stable & hereditary if the DNA is incorporated into the Host / recipient chromosome.

1. **Linear**
2. **No origin of replication**

- *Therefore, must be incorporated into host chromosome to be replicable/ hereditary!!*

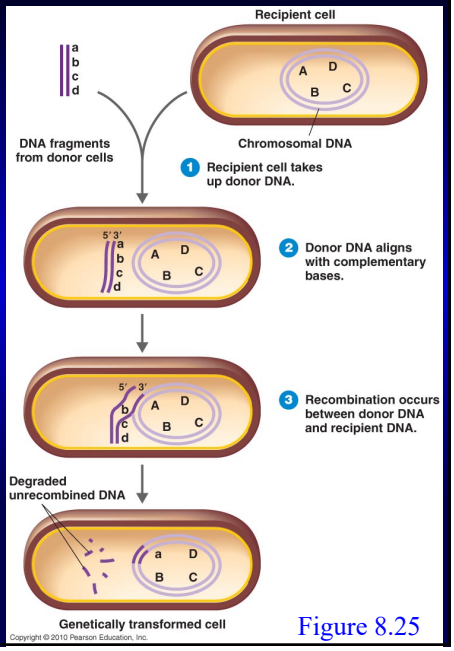


Figure 8.25

50

** Genetic Recombination

- Exchange of genes between two DNA molecules
  - *(Eg: Meiosis-I in Eukaryotes)*
  - Crossing over occurs when two chromosomes break and rejoin
  - *** Bacteria & Archaea are ***Genetically Promiscuous*** cells***!!!***

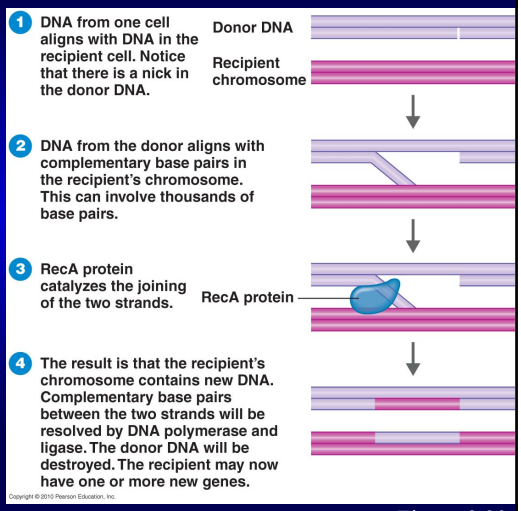
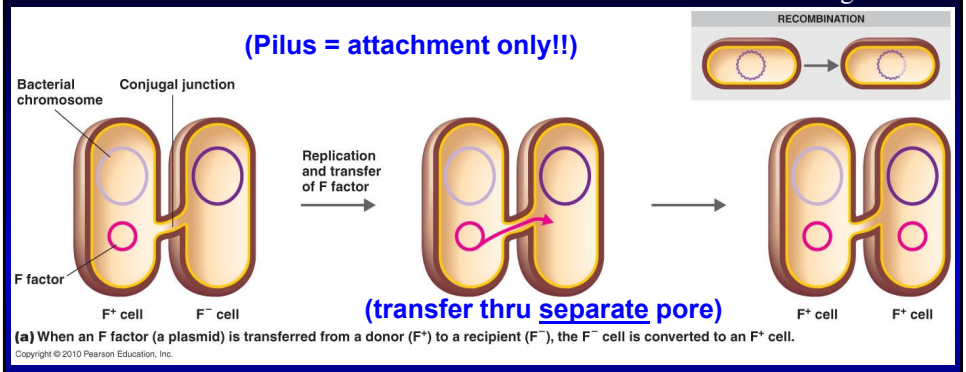


Figure 8.23

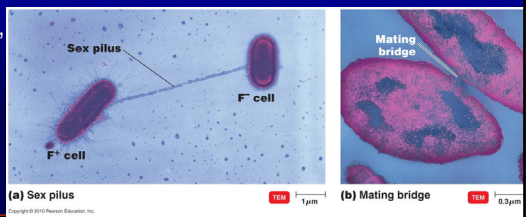
51

B.) Conjugation

Figure 8.27a



- **F factor = Fertility Plasmid** (small, circular, self-replicating chromosome)
- **Can carry genes from donor to recipient strains**
  - **F- recipient becomes F+**



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

Fig 8.26

52

** Plasmids

1. Conjugative plasmid:

- Carries genes for sex pili and transfer of the plasmid

Mating of F+ and F– Bacterial Strains

Animation by Thomas M. Terry

2. Dissimilation plasmids:

- Encode enzymes for catabolism of unusual compounds
  - (*Pseudomonas* = toluene, camphor, petroleum HC's)

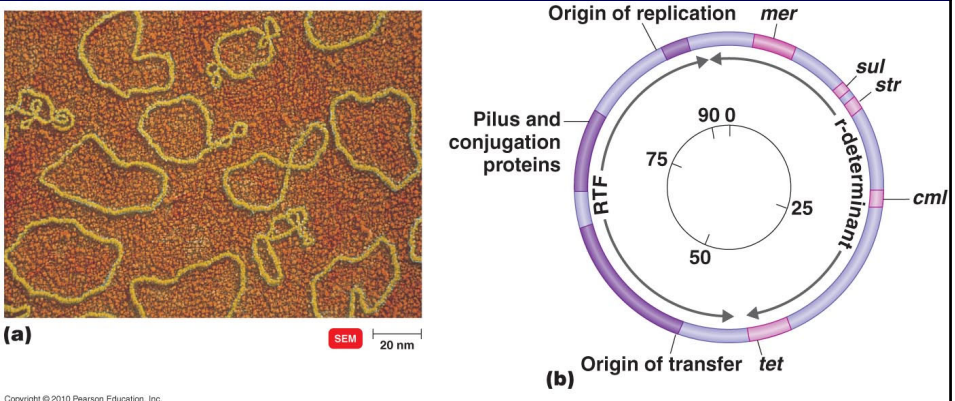
3. R factors:

- Encode antibiotic resistance (eg: MDR)

53

Plasmids

Figure 8.29



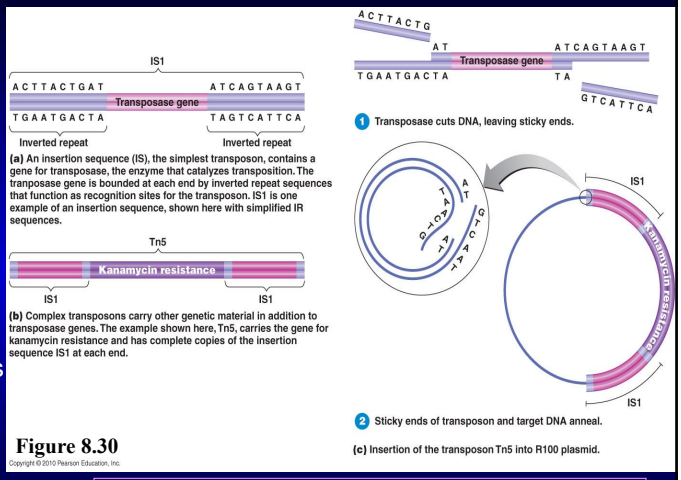
MDR Plasmid

http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation6.html

54

[Transposons]

- Segments of DNA that can move from one region of DNA to another
  - *"Jumping Genes"*
- Contain ***insertion sequences*** for cutting and resealing DNA (***transposase***)
- Complex transposons carry other genes
  - Eg: antibiotic-resistance



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

http://www.dnalc.org/resources/animations/alu.html

http://www.learner.org/courses/biology/archive/animations.html

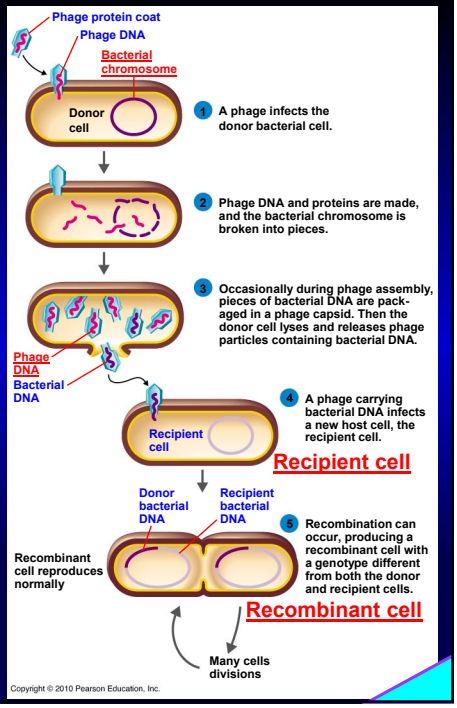
55

C.) Transduction

Figure 8.28:
Transduction by a bacteriophage

http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation7.html

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



56