BIOL 230: Cell & Molecular Biology

Fall 2019 17-205 MW, Nov. 5-7

http://accounts.smccd.edu/staplesn/biol230/

1. Pre-Lab writeups due each Mon. (for both M&W!!) at the start of lab. (*briefly*, **What**? **Why**? **How**? for each expt.). Question & **Hypothesis**?!
2. **LAB THIS week: Genetic Transformation and Operon Gene Regulation!**
3. **Find Anastasia & Alien GEL DATA under *ADDITIONAL MATERIALS*.**
4. **Extra Credit: STEM SPEAKER SERIES**, Weds. @ 5pm-6pm, Sept. 11- Nov. 6. (NOT Oct. 9) in 6-102. *Write 1 page summary by the following week, and upload to CANVAS*. Extra-Extra credit: Ask the speaker a scientific question, and write abosut the answer.
5. **Exams: Midterm 2 was returned. SEE ME ASAP after reviewing your exam, if you scored <70%!!**
6. **NEXT WED.: QUIZ #5 first attempt due!!!**
7. **RESEARCH Outlines due Wed. night!! See me if you have any questions!!**

1

REVIEW

1. **Ch. 14:** Describe **Beadle and Tatum**'s experiments and their contribution to our understanding of molecular genetics & Central Dogma.
2. Diagram and describe how the **Central Dogma** of Molecular Genetics explains the transition from hereditary ***genotype*** to an organism's expressed ***phenotype***.

TODAY's Objectives: Students should be able to....

1. Compare **replication** & **transcription:** product produced, start & end sites, enzymes, & the direction of movement and synthesis.
2. What is the nature of the **Genetic Code**, and describe the experiments that lead to it's discovery. (What are the "translators"??)
3. Define and diagram the roles of **tRNA, rRNA and mRNA** in translation.
4. Compare sites, directions, and molecules involved in **Initiation, Elongation and Termination** of DNA, RNA and Protein synthesis.
5. **(Ch. 15:)** Define and give examples of the four types of **point mutations**. Which are likely to have the most severe phenotype?
6. **Ch. 16:** Compare and contrast **bacteriophage and animal virus** reproductive cycles, citing specific examples.
7. Compare the Lytic & lysogenic **bacteriophage** reproductive cycles, & describe how they are controlled by the ***late*** and ***early*** genes.
8. Diagram the structure of an **operon**, including 3 DNA components and 3 other molecules involved in its regulation.

❖ ***Objectives and Study Guide Questions are your HOMEWORK between classes!!! DUE WED. at the end of Lecture!!***

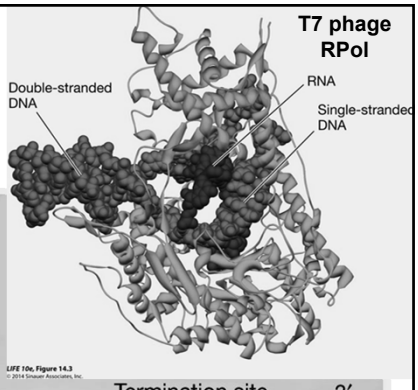
2

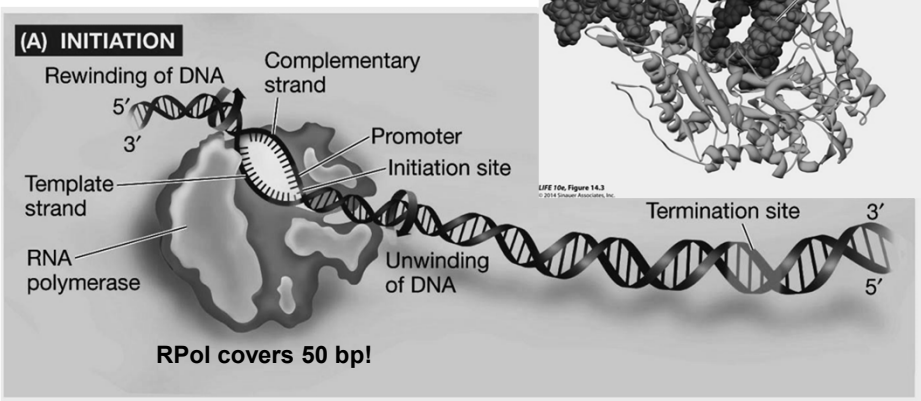
14.3) Transcription:
DNA-Directed RNA Synthesis

1. RNA is transcribed from a DNA template
   - after the bases of DNA are exposed by unwinding of the double helix.
2. In a given region of DNA, only one of the two strands can act as a template for transcription. (***UNIDIRECTIONAL!***)
3. ***RNA polymerase catalyzes transcription from the template strand of DNA.***
4. Transcription starts when RNA polymerase recognizes and binds tightly to a ***PROMOTER Sequence*** on DNA.
5. RNA elongates in a **5'-to-3' direction**, antiparallel to the template DNA. *[Just like DNA synthesis!]*
   - Special sequences and protein helpers terminate transcription.
   - ***EUKARYOTES ONLY: Introns must be removed, and Exons spliced (ligated) together to make Mature mRNA Transcript.***

3

Transcription





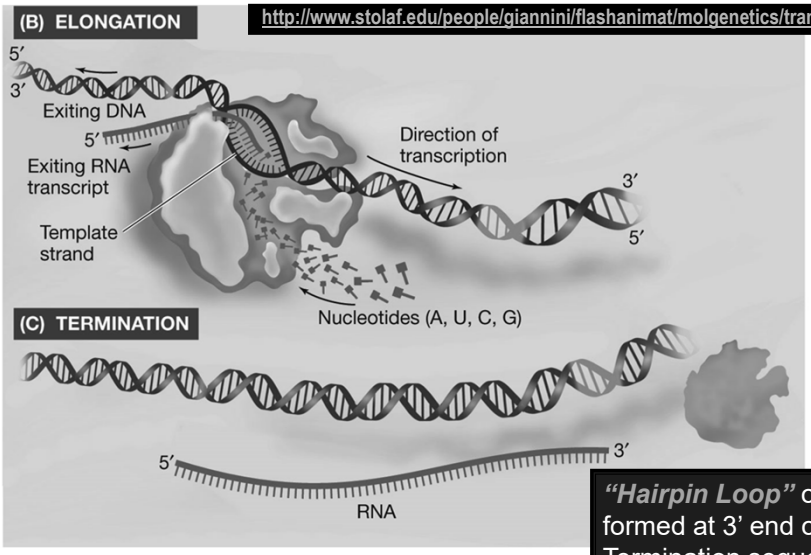
http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/transcription.swf

http://www.hhmi.org/biointeractive/dna/DNAi_transcription_vo2.html

LIFE 9e, Figure 14.4 (Part 2)

4

Transcription



LIFE 9e, Figure 14.4 (Part 3)

"Hairpin Loop" or ***poly-U*** formed at 3' end of RNA = Termination sequence

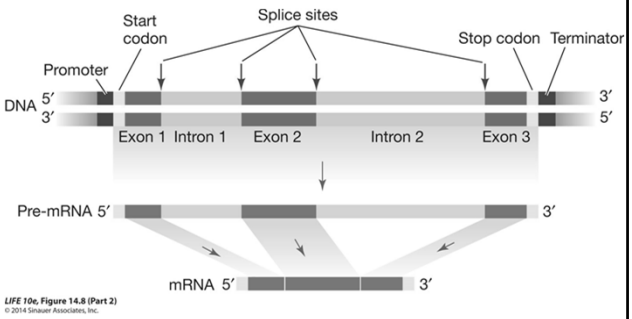
EUK: **http://vcell.ndsu.nodak.edu/animations/transcription/movie-flash.htm**

5

14.4) EUKARYOTIC RNA Processing (post-Tscn):

A. Eukaryotic Protein-Coding Genes ("Structural Genes") → RNA Splicing

1. **Noncoding internal sequences** (***Introns***)
   - Coding sequences = ***Exons***
2. **Flanking sequences** involved in the machinery of tscn/tsln:
   - ***Promoters,***
   - ***Terminators.***
   - ***Enhancers…,***
   - ***Silencers……***



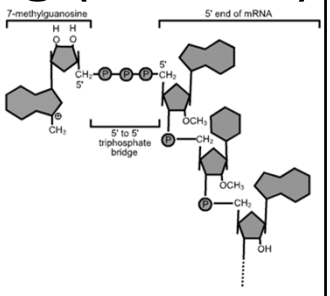
LIFE 10e, Figure 14.8 (Part 2)

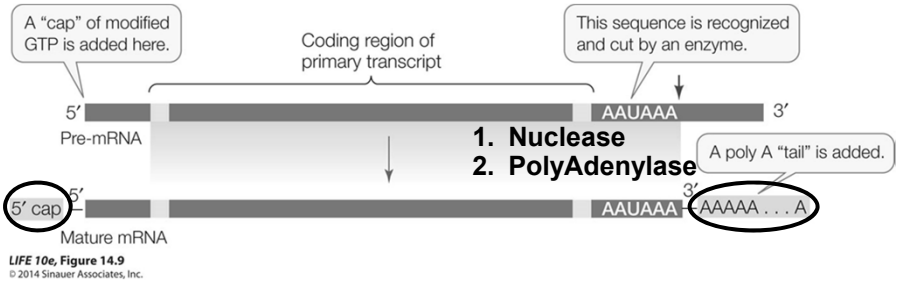
http://vcell.ndsu.nodak.edu/animations/transcription/movie-flash.htm

6

B. Euk. RNA Processing (post-Tscn)

- After transcription, the ends of the ***pre-mRNA*** ***(primary transcript)*** are altered by the addition of:
  1) **5' Methyl-Guanosine (Me-GTP) cap** – ribosome binding/translation!!, mRNA stability.
  2) **3' poly A tail** – mRNA stability, nuclear export!!





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

http://www.sinauer.com/cooper/4e/animations0702.html

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RNA Processing

- (3.) The introns are removed from the ***mRNA precursor*** by the ***Spliceosome***
  - a complex of RNA's & proteins (**snRNPs**).

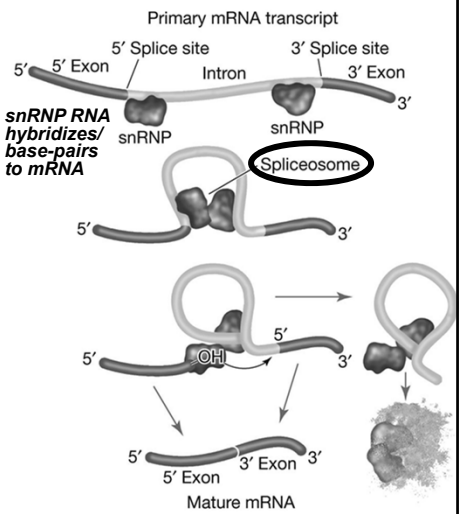

Figure 14.11

Enzymes with both RNA and protein components *(some are **RIBOZYMES**)*:
1) **Ribosomes****
2) **Telomerase**
3) **Spliceosome/snRNPs****

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

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14.5) The Genetic Code

1. The genetic code consists of **triplets** of nucleotides (***codons***).
   a) 4 bases, therefore → **64** codons. *(NON-overlapping!)*
   b) One mRNA codon = start of tsln; codes for **methionine (Start Codon).**
      – Gives DIRECTION and sets READING FRAME for translation!
   c) Three Stop Codons = end of translation.
   d) The other 60 codons code only for particular AAs.

2. 64 codons → only 20 AA's; thus, the ***genetic code is redundant ("degenerate"):***
   a) Some AA's encoded by >1 codon!!
   b) However, a single codon does not specify more than one amino acid. (*codons are not "ambiguous"!!)*

http://www.dnalc.org/view/16494-Animation-22-DNA-words-are-three-letters-long-.html
http://bcs.whfreeman.com/thelifewire/content/chp12/1202002.html

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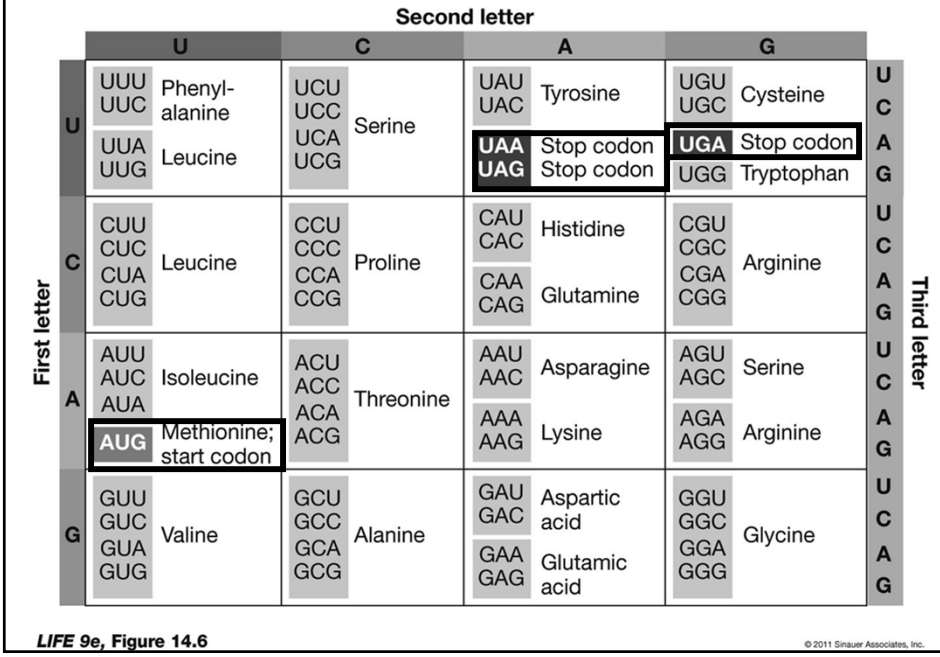
Universal Genetic Code

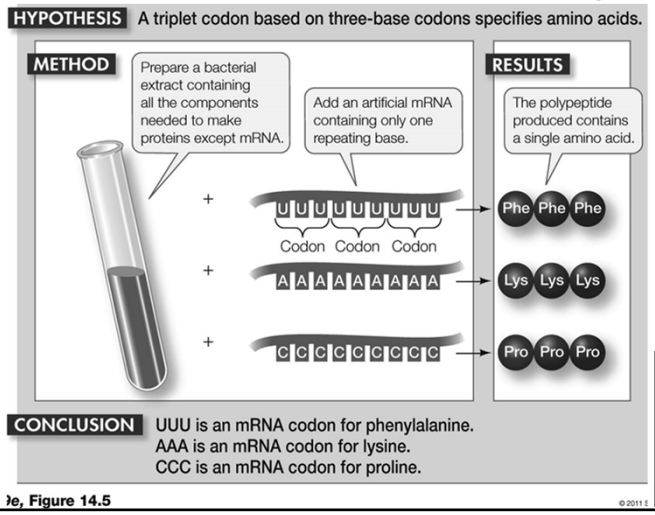


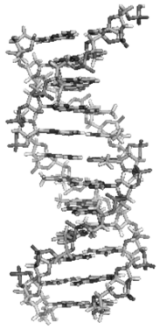
LIFE 9e, Figure 14.6

10

Deciphering the Genetic Code

- Synthetic *polyribonucleotides* – poly***mono***-, poly***di***-, poly***tri***-NTs → directed synthetic Polypeptides
- **Deduction → which RNA triplets gave each AA!!**





1961, by **Marshall W. Nirenberg** and his post doctoral fellow, **J. Heinrich Matthaei.**

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14.6) Preparation for Translation:

Linking RNA's, Amino Acids, and Ribosomes

- In prokaryotes, ***translation begins before the mRNA is completed***.
  - *Tscn & Tsln* = ***simultaneous****!*
- In eukaryotes, transcription occurs in the nucleus and translation occurs in the cytoplasm.
  - *Tscn & Tsln = spatially & temporally* ***separated****!*

Practice Tutorial: http://learn.genetics.utah.edu/units/basics/transcribe/

Complete Gene Expression!!: http://learn.genetics.utah.edu/content/basics/transcribe/

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Preparation for Translation:

Linking RNA's, Amino Acids, and Ribosomes

1. Translation requires three components: tRNA's, activating enzymes, and ribosomes.

2. ***In translation, amino acids are linked in codon-specified order in mRNA.***

3. This is achieved by an adapter, ***transfer RNA (tRNA),*** which binds the correct amino acid and has an ***anticodon*** complementary to the mRNA codon.

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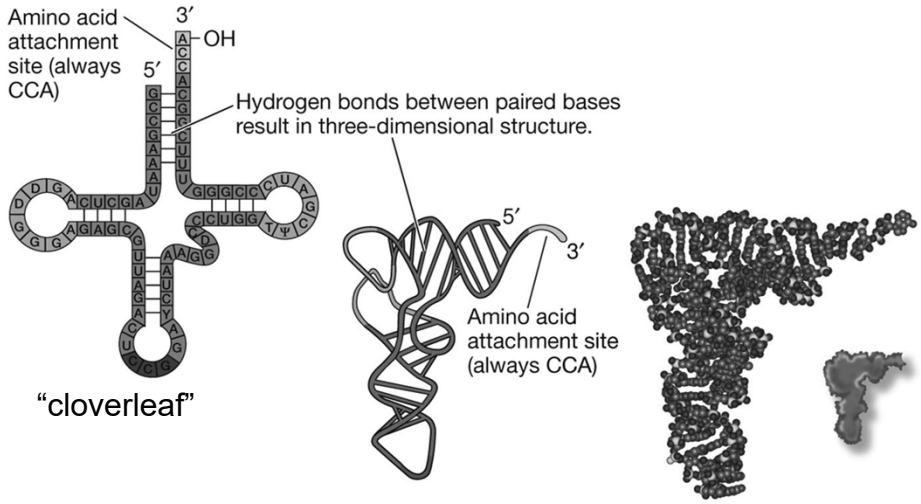
A. tRNA Structure



http://www.hhmi.org/biointeractive/media/DNAi_translation_vo1-lg.mov

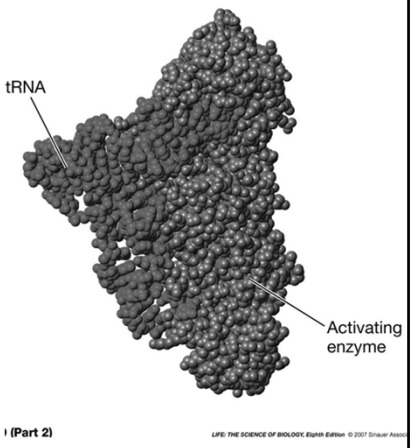
http://www.hhmi.org/biointeractive/animations/index.html

LIFE 9e, Figure 14.12

14

B. Specificity of the Code: Aminoacyl-tRNA Synthetases

- The ***aminoacyl-tRNA synthetases***,
  - a family of activating enzymes,
  - attach specific amino acids to their appropriate tRNA's,
  - forming ***charged tRNA's***.
- ***= the primary determinants of Codon-Amino Acid Specificity!!!***



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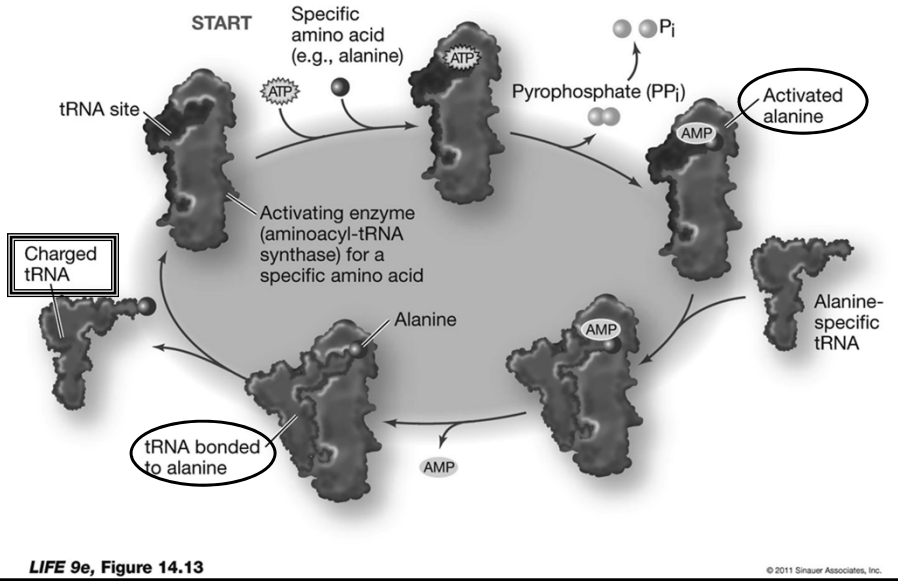
**AminoAcyl tRNA Synthetase: Charging a tRNA molecule **



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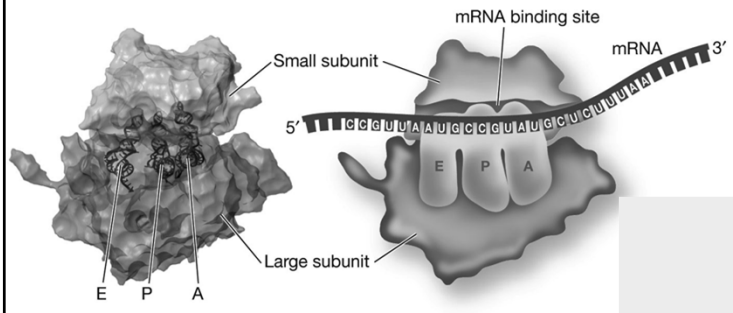
14.7) Translation: RNA-Directed Polypeptide Synthesis

- ***The mRNA meets the charged tRNA's at a ribosome.***



LIFE 9e, Figure 14.14

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

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

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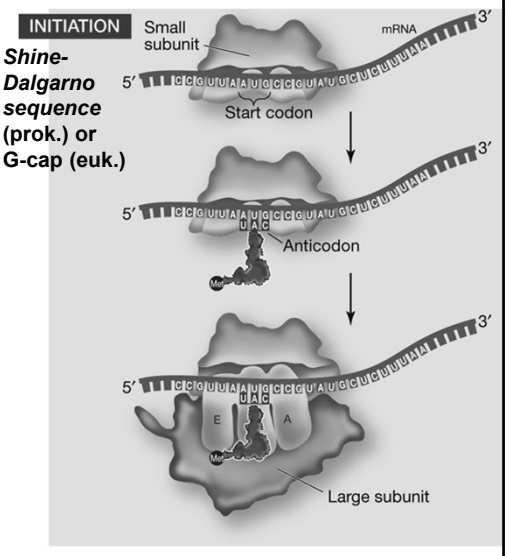
A. Initiation of Translation: mRNA, Charged (aminoacyl) tRNA, Ribosome

A. An ***initiation complex***
  - consisting of an amino acid-charged tRNA
  - & a small ribosomal subunit bound to mRNA
- triggers the beginning of translation.
  - ***READS RNA 5'→3'***
  - ***Synthesizes polypeptide N→ C.***



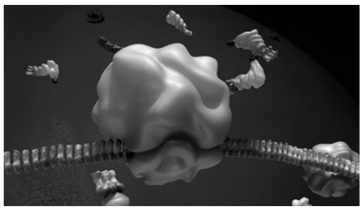
https://youtu.be/oefAI2x2CQM (Amoeba Sisters)

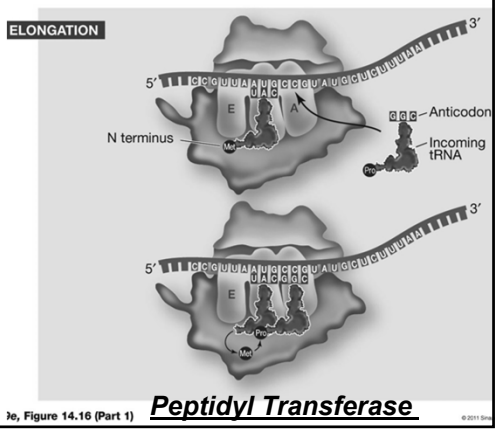
14.15 (Part 2)

18

B. Translation: Elongation

- Polypeptides grow from the **N terminus → C terminus.**
  - *Amino to Carboxyl !!*
  - ***Peptidyl Transferase*** activity is in the **Large Subunit**!! (**rRNAs**)
    - Growing chain in the P-site is cleaved from the last tRNA,
    - then attached by its CARBOXYL group to the AMINO group of the new AA in the A site!!! (condensation rxn!)
- The ribosome moves along the mRNA **one codon at a time.**
  - ***TRANSLOCATION*** *is 3 nucleotides (NT) to the "RIGHT" (3' direction on the mRNA).*
    - *Requires Elongation Factors and GTP!*





Peptidyl Transferase

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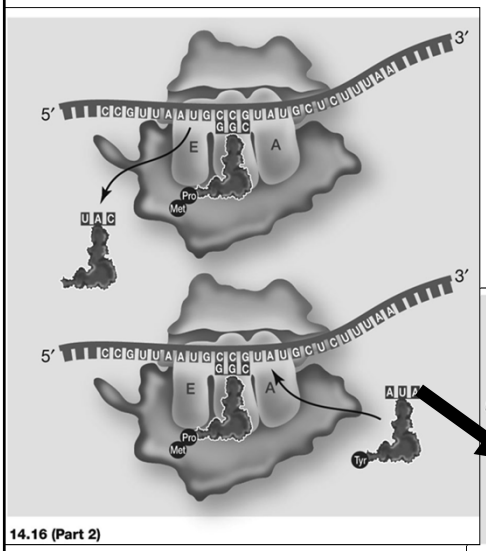
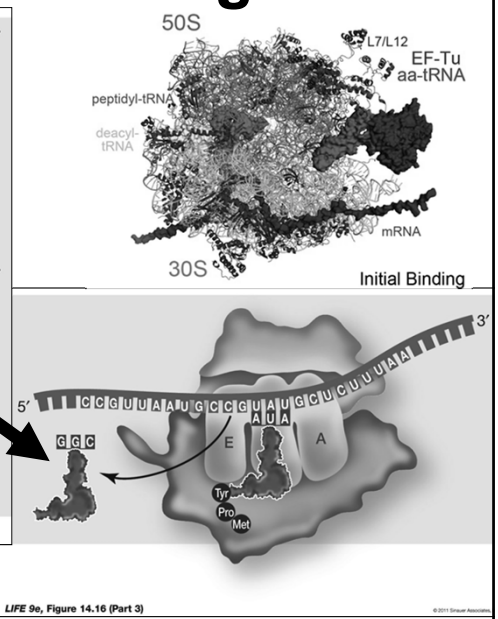
Translation → Elongation



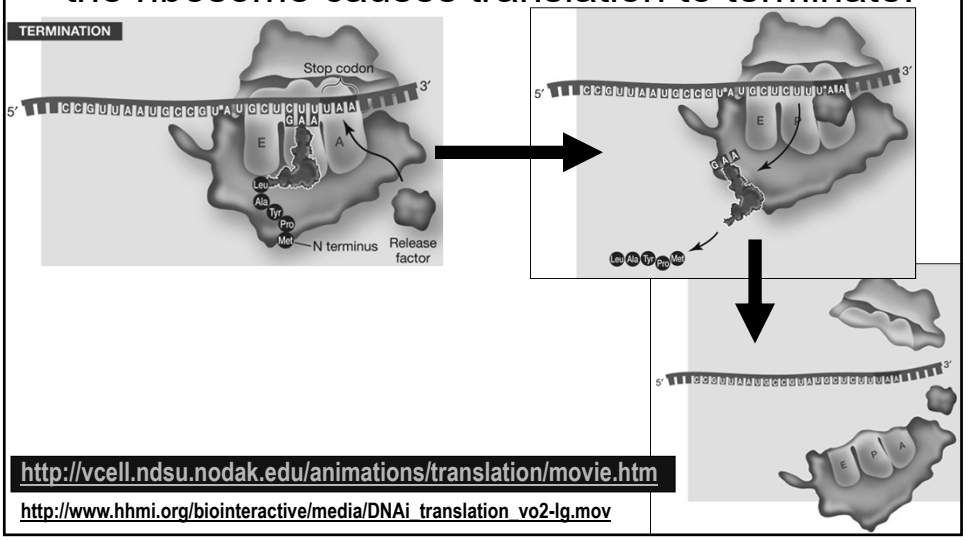


Fun: http://learn.genetics.utah.edu/content/begin/dna/firefly/fireflymon8.swf

20

C. Translation: Termination

- The presence of a stop codon in the A site of the ribosome causes translation to terminate.



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

http://www.hhmi.org/biointeractive/media/DNAi_translation_vo2-lg.mov

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Compare RepIn, Tscn, TsIn:

Process	Initiation	Elongation	Termination
Replication	**At *Origin*: A/T-rich, Helicase, SSB, Primase, DPol3**	**Dpol3, dNTPs, 5'→3'** (leading, lagging) **** *bidirectional***	**Terminator *(ter)* sequences, or end of chromosome** ***(forks meet if circle)*** • ***Euk. Telomeres…***
Transcription	**At *Promoter* – TATAA, A/T-rich, RPol**	**RPol, NTPs, 5'→3'** **** *unidirectional***	**Tsc'I terminator (eg: poly-U, hairpin loop)**
Translation	**At Start Codon (AUG), mRNA, met-tRNA, ribosome (SSU, LSU)**	**Ribosome, AA-tRNA's (anticodons), N→C (follows mRNA 5'→3')**	**Stop codon (nonsense codon): UAA, UAG, UGA** **** Release Factor (protein)**

DNA to Protein: https://youtu.be/gG7uCskUOrA

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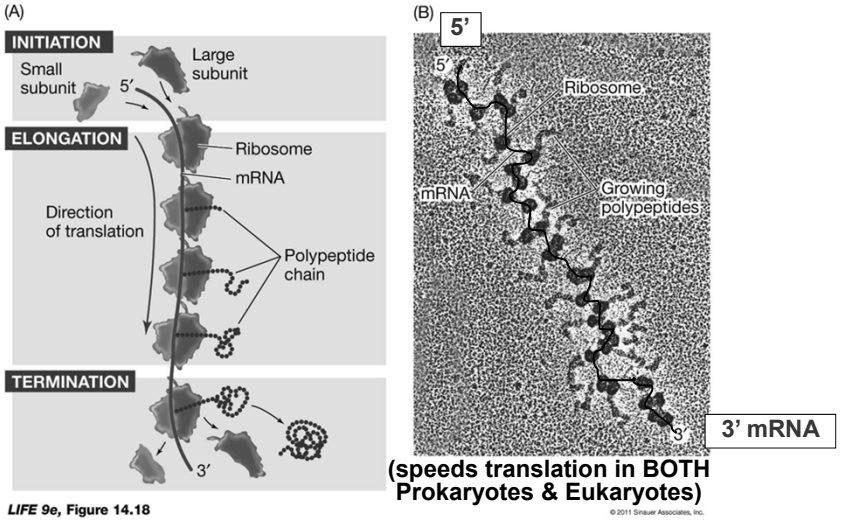
14.8) Regulation of Translation

- In a ***Polysome***, more than one ribosome moves along the mRNA at one time.



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14.9) Posttranslational Events….

1. **Signals** in the AA sequ. of proteins direct them to **cellular destinations**……. (more during Ch. 16)
2. **Protein synthesis begins on free ribosomes** in the cytoplasm.
   a) Proteins destined for nucleus, mitochondria, & plastids
   b) Have signals that allow them to bind to and enter destined organelles.
      - (eg: **pro-pro-lys-lys-lys-arg-lys-val** = nuclear localization signal)
3. Proteins destined for the **ER, Golgi apparatus, lysosomes, and outside the cell**
   a) complete their synthesis on the ER surface.
   b) enter the ER by the interaction of a hydrophobic signal sequence with a channel in the membrane.

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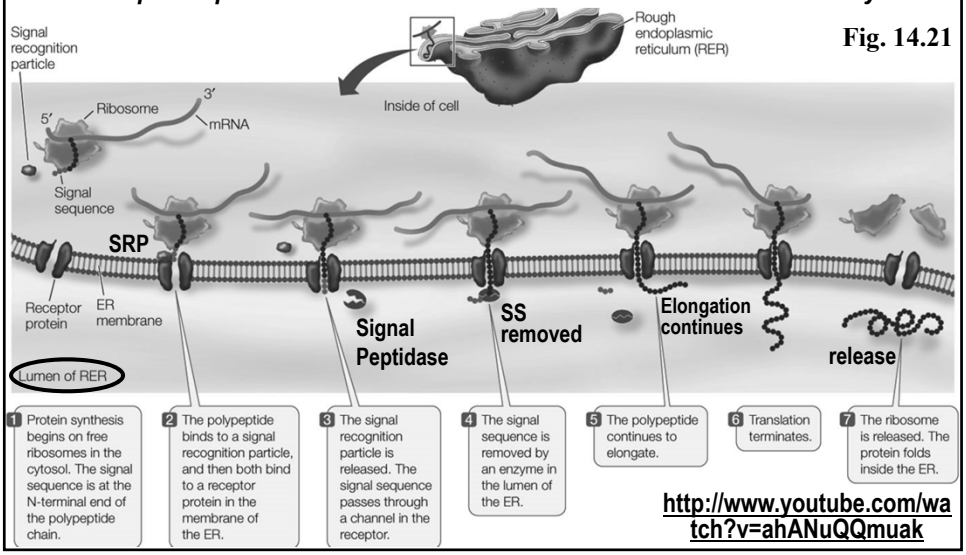
A. Signal Peptides (to RER)

- N-terminal **Signal Sequence** on a newly-translated protein
  - Binds **SRP** in cyto; SRP binds receptor on ER; Signal is cleaved by NZ in **ER lumen;**
  - ***Completed protein released into ER lumen for modification & delivery.***



http://www.youtube.com/watch?v=ahANuQQmuak

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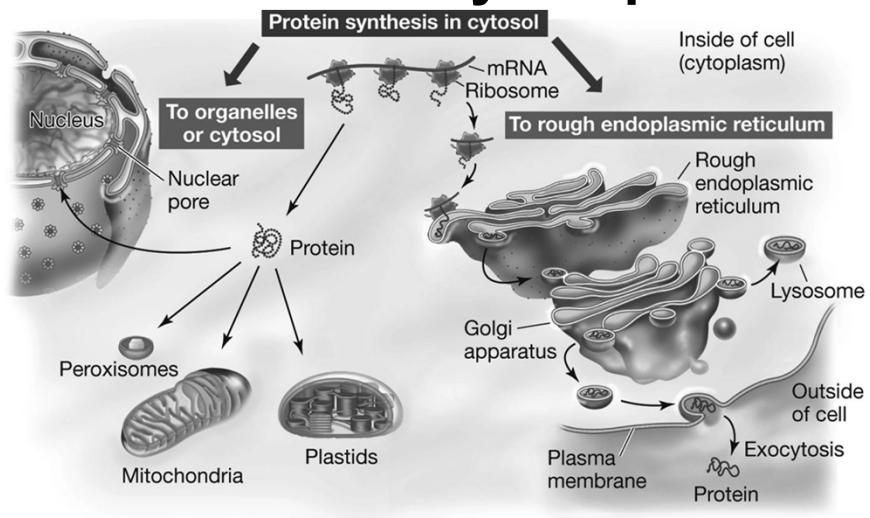
B. Destinations for newly translated eukaryotic proteins



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

http://vcell.ndsu.nodak.edu/animations/mito-pt/movie.htm

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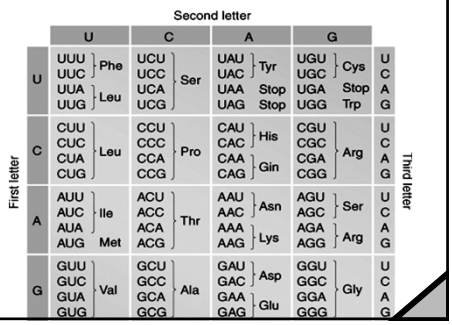
**Apply transcription & translation...



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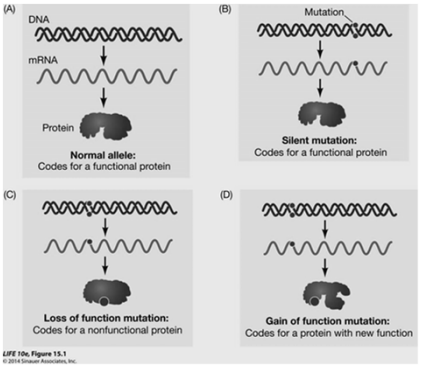
15.1) Mutations:
Heritable Changes in Genes

- Mutations in DNA are often ***expressed*** as abnormal proteins.
- However, the result may not be easily observable ***phenotypic*** changes.



- Some mutations appear only under certain conditions, such as exposure to a certain environmental agent or condition.
  - **= ***CONDITIONAL MUTATIONS***
- Eg: Temperature-sensitive, salt-sensitive, pH-sensitive
- Normal unless moved to ***"restrictive conditions" (vs. permissive)***

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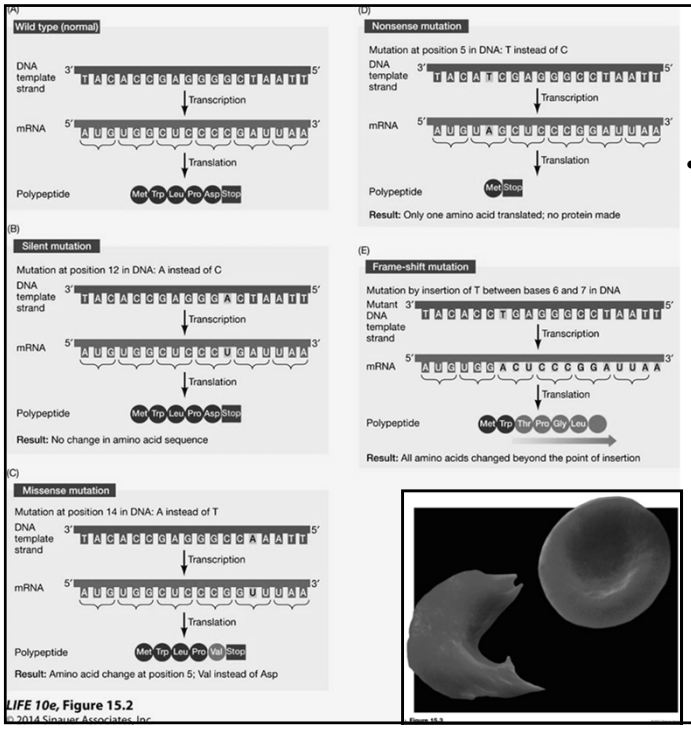

A. Point Mutations

- ***Point mutations*** (***silent, missense, nonsense,*** *or* ***frame-shift***) *result from alterations in single base- pairs of DNA.*
  - *Missense: eg:* ***Glu→Val*** *in HbA→* ***HbS (sickle Cell)***
  - *Only a single bp change!!*

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Mutations in a gene's coding sequence can alter the gene product

(a) Types of mutation in a gene's coding sequence

Wild-type mRNA	5'	GCU	GGA	GCA	CCA	GGA	CAA	GAU	GGA	3'
Wild-type polypeptide	N	Ala	Gly	Ala	Pro	Gly	Gln	Asp	Gly	C
Silent mutation		GCU	GGA	GCC	CCA	GGA	CAA	GAU	GGA	
		Ala	Gly	Ala	Pro	Gly	Gln	Asp	Gly	
Missense mutation		GCU	GGA	GCA	CCA	AGA	CAA	GAU	GGA	
		Ala	Gly	Ala	Pro	Arg	Gln	Asp	Gly	
Nonsense mutation		GCU	GGA	GCA	CCA	GGA	UAA	GAU	GGA	
		Ala	Gly	Ala	Pro	Gly	Stop			
Frameshift mutation		GCU	GGA	GCC	ACC	AGG	ACA	AGA	UGG	A
		Ala	Gly	Ala	Thr	Arg	Thr	Arg	Trp	

Hartwell (2004), "Genetics" Fig. 8.27 a

1. **Silent/ synonymous** mutations do not alter the amino acid specified.
2. **Missense** mutations replace one amino acid with another.
3. **Nonsense** mutations change an amino-acid-specifying codon to a stop codon.
4. **Frameshift** mutations result from the insertion or deletion of nucleotides within the coding sequence.

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Codons & Deletion Mutations

Wild type

One deletion

Two deletions

Three deletions

CAT CAT CAT CAT CAT
• FAT CAT ATE THE RAT
CTC ATC ATC ATC AT
• FTC ATA TET HER AT.
CTA TCA TCA TCA T
• FTA TAT ETH ERA T ...
CTA TAT CAT CAT
• FTA TAE THE RAT …

- DNA
- Words analogy

Junk!

Back into Frame, -1 aa

Deletions in multiples of 3 (Not 1 or 2) = mostly OK → clue to triplet nature of code!!

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

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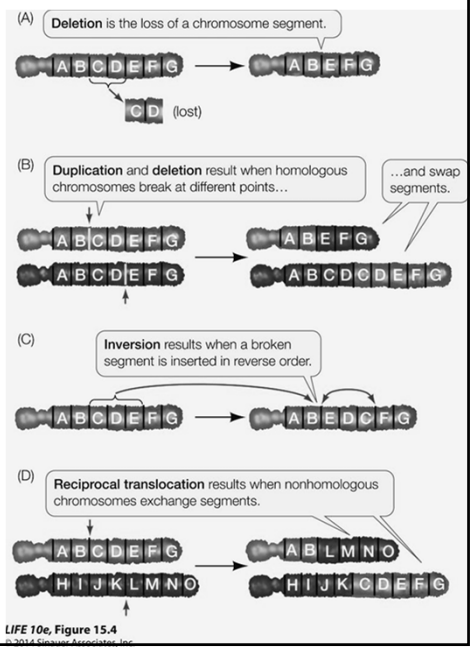
B. Chromosomal Mutations

- **Chromosomal mutations** involve large regions of a chromosome.
  - **a) Deletions**
  - **b) Duplications**
  - **c) Inversions**
  - **d) Translocations**

➢ **Telomeres**:
- *Protect & preserve chromosome ends* during replication
- *Identify ends of chromosomes for breakage repair machinery*



32

C. Mutations = Spontaneous or Induced

1) ***Spontaneous mutations*** occur because of instabilities in DNA or chromosomes. ***Rate: 10^{-9} to 10^{-10}.***
   - Or replication errors.
2) ***Induced mutations*** occur when an outside agent damages DNA.
   - = ***MUTAGEN***
   - **Altered form of C now favorably pairs with A!!**
     - **C*—A pairs!**

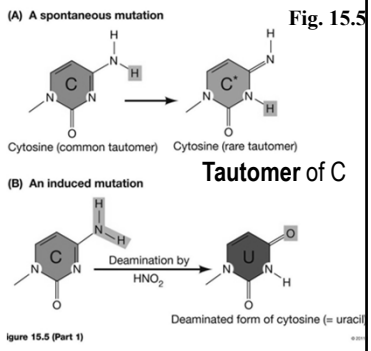
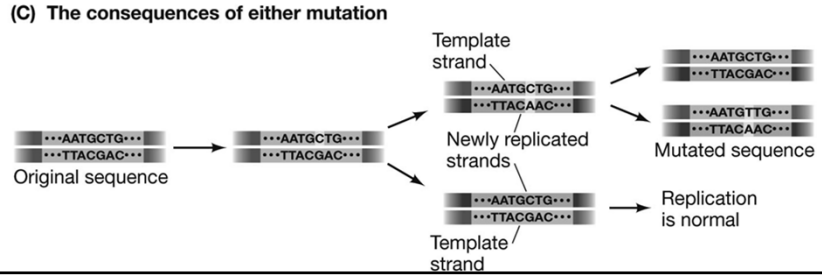


Fig. 15.5



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Summary of the "Universal" Genetic Code & Translation

1. Codon consist of a ***triplet codon***; each specifies an amino acid
   - Code shows a 5' to 3' direction
2. Codons are ***non-overlapping*** – each nt = only in 1 codon!!
3. Code includes ***three stop codons, UAA, UAG, and UGA***
4. Code is ***degenerate*** (>1 codon/aa)
5. ***Fixed starting point*** establishes a ***reading frame at AUG***
6. ***5'- 3'* direction of mRNA → *N- to C-terminus* of polypeptide**
7. Mutations modify message encoded in sequence
   - **a) Frameshift** mutations change reading frame
   - **b) Missense** mutations change codon of amino acid to another amino acid
   - **c) Nonsense** mutations change a codon for an amino acid to a stop codon

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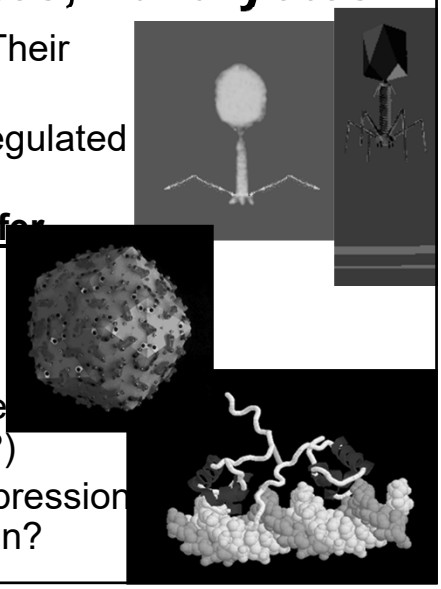
Ch. 16: Regulation of Gene Expression: Viruses, Prokaryotes, Eukaryotes

1. How Do **Viruses** Regulate Their Gene Expression?
2. How Is Gene Expression Regulated in **Prokaryotes**?
3. **How do prokaryotes transfer and recombine genes?**
4. How Is **Eukaryotic** Gene Transcription Regulated?
5. (How Do Epigenetic Changes Regulate Gene Expression?)
6. How Is Eukaryotic Gene Expression Regulated After Transcription?



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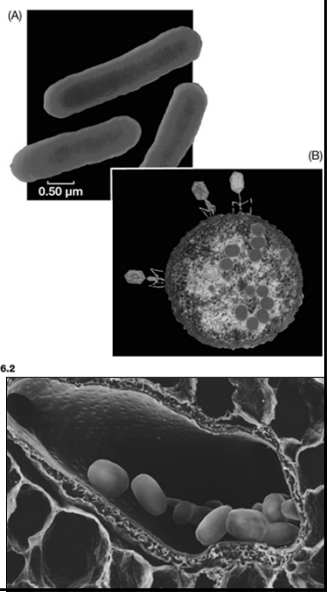
Using Prokaryotes & Viruses to Probe the Nature of Genes

- Prokaryotes and viruses are useful for the study of genetics and molecular biology:
  - they contain ***less DNA*** than eukaryotes,
  - **grow and reproduce rapidly**,
  - and are ***haploid***.
- **= great MODEL SYSTEMS!!!**



Bacillus anthracis

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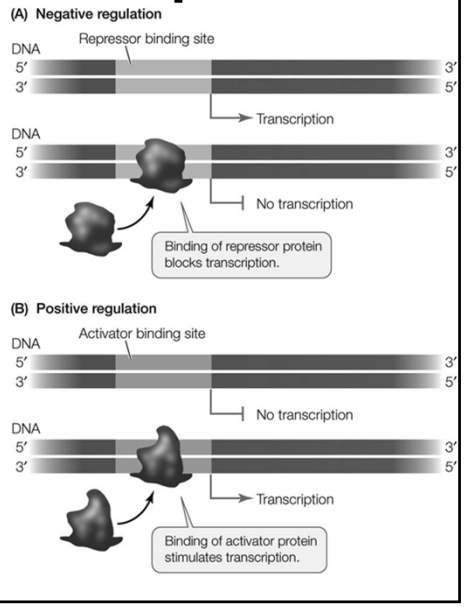
16.1 How Do Viruses & Cells Regulate Their Gene Expression?

One type of regulatory protein binds to/near the promoter:

- **Negative regulation –**
  - Gene may be "normally" transcribed, but binding of a ***Repressor protein*** prevents transcription.
- **Positive regulation –**
  - Gene is not normally transcribed; an ***Activator protein*** binds to stimulate transcription.



16.1

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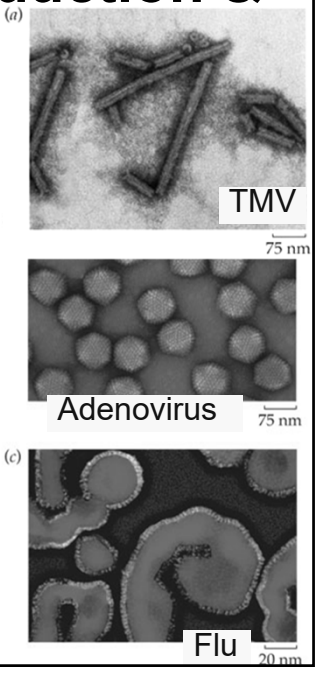
16.2) Viruses: Reproduction & Recombination

- **Viruses:**
  - discovered as disease-causing agents (in plants!)
    - small enough to pass through a filter that retains bacteria!!
  - Viruses are ***obligate intracellular parasites***,
    - need biochemical machinery of living cells to reproduce.
- Consist of:
  - ***nucleic acid genome*** – codes for a few proteins
  - a ***protein capsid***
  - Some have a lipid membrane derived from host membranes ***("enveloped" viruses***).
- Classified by:
  - ***size and shape***, ***genetic material***, ***host organism, & type of damage to host cells***



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A. Viruses: Regulation of Host Genes

1. ***Early genes/stage:*** some viruses have *promoters for host RNA polymerase* – promoter in the viral genome binds host RNA polymerase and adjacent viral genes are transcribed.
   - shut down host transcription, stimulate viral genome replication, stimulate late gene transcription.
   - Viral nucleases digest the host's chromosome for synthesis in new viral particles.

2. ***Late genes/stage:*** encode components of mature viral particle and enzymes to degrade/escape host cell.
   - encode the viral capsid proteins and ***lysozyme*** to release new ***virions.***
   - whole process: from binding & infection to release of new virions takes about 30 min.

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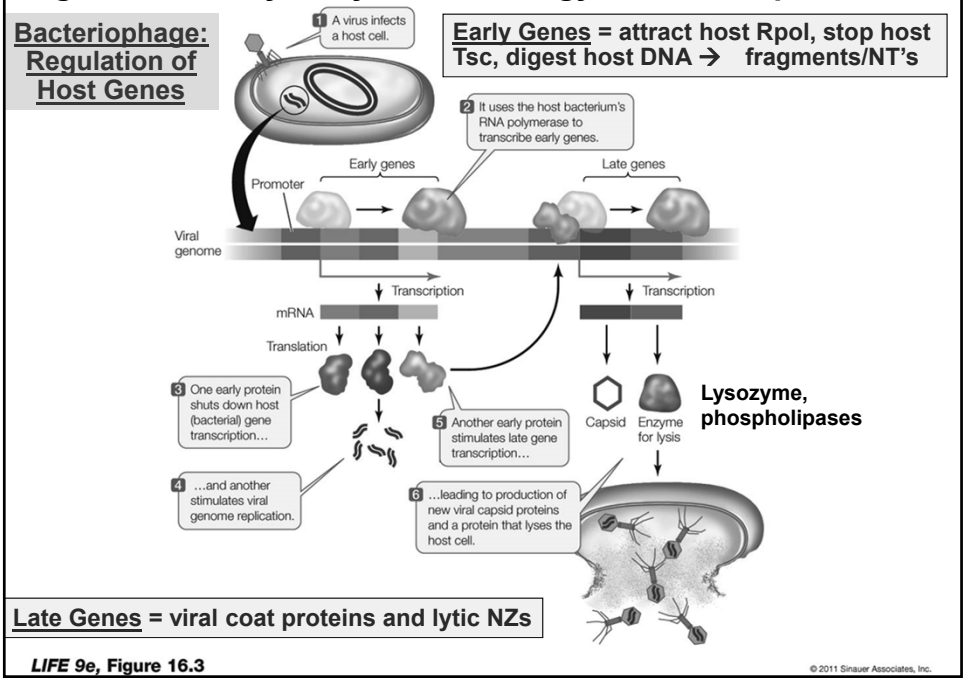
Fig. 16.3 The Lytic Cycle: A Strategy for Viral Reproduction



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B. Viruses:
Bacteriophage Reproductive Cycles

- ***Bacteriophages are viruses that infect bacteria.***

1. ***Lytic Cycle:*** (typical cycle) the host cell breaks open, releasing many new phage particles.
2. Some phages can also undergo a ***Lysogenic Cycle:*** their DNA is inserted into the host chromosome, where it replicates for generations.
   - When conditions are appropriate, the lysogenic DNA exits the host chromosome and enters a lytic cycle.

http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter17/animation_quiz_2.html
http://www.blackwellpublishing.com/trun/artwork/Animations/Lambda/lambda.html
http://www.blackwellpublishing.com/trun/artwork/Animations/t4attacht/t4attacht.html

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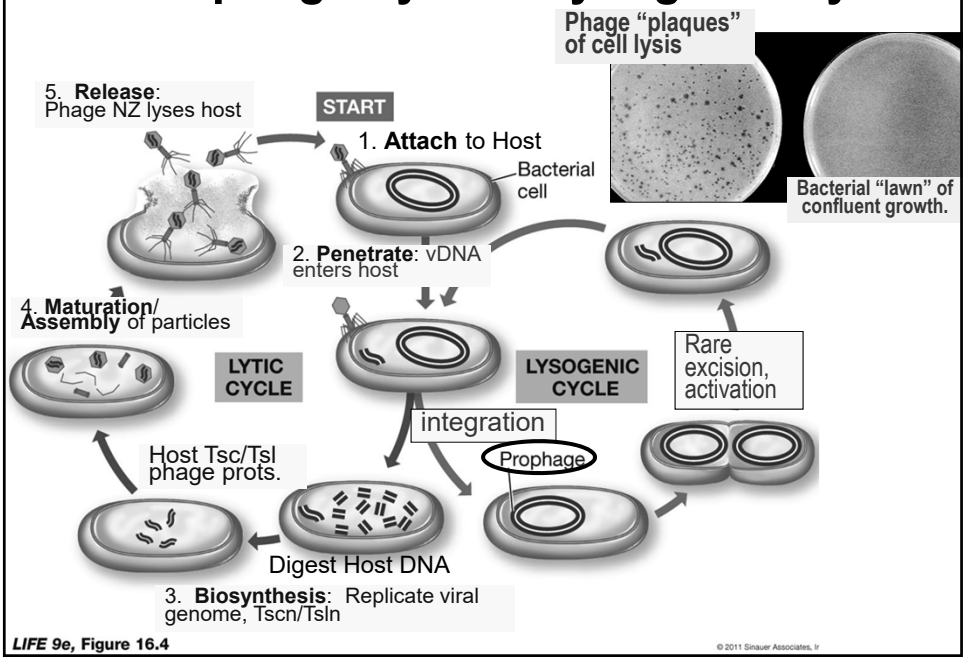
Bacteriophage Lytic & Lysogenic Cycles



LIFE 9e, Figure 16.4

42

C. Regulation of Bacteriophage Gene Expression

1. ***If a host cell is not growing well or is damaged, the virus may switch to the lytic cycle.***
2. A genetic switch senses the host's condition; two regulatory proteins — **cI** and **Cro** — compete for promoters on the phage DNA.
   - The two promoters control viral gene transcription and the regulatory proteins have opposite effects on each promoter.
   - The two regulatory proteins are made early in phage infection and it is a "race" between them.
3. In a rapidly growing host, Cro synthesis is low and **cI is high** — the phage enters a **Lysogenic cycle**.
4. If growth is slow, **Cro is higher** and genes for **Lytic** cycle are activated.

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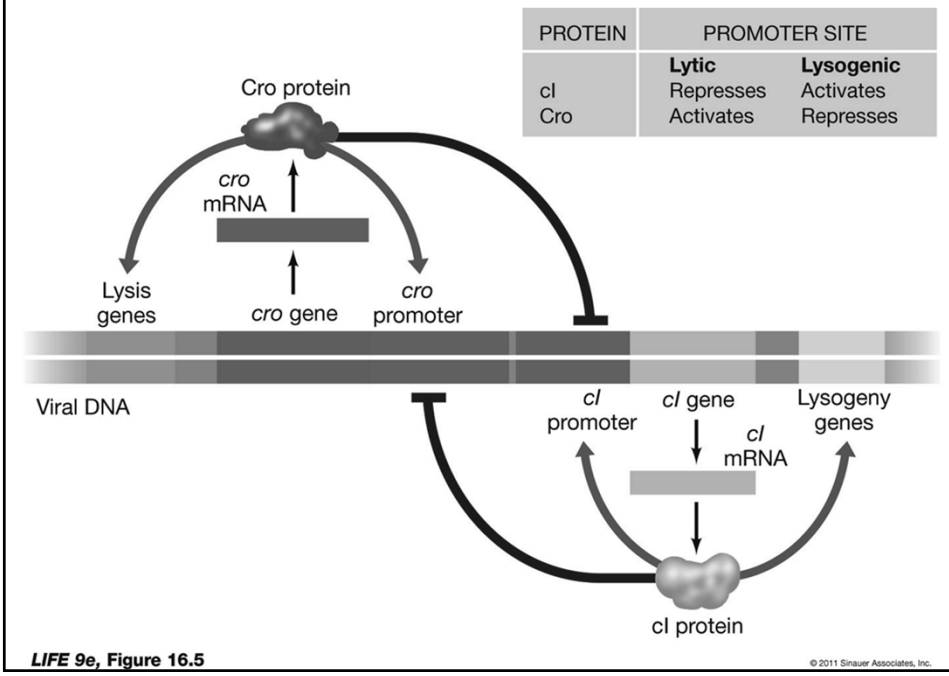
Figure 16.5 Control of Bacteriophage λ Lysis and Lysogeny



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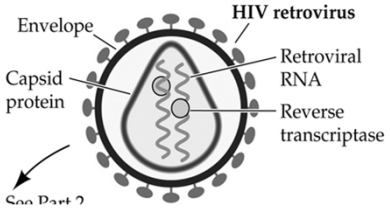
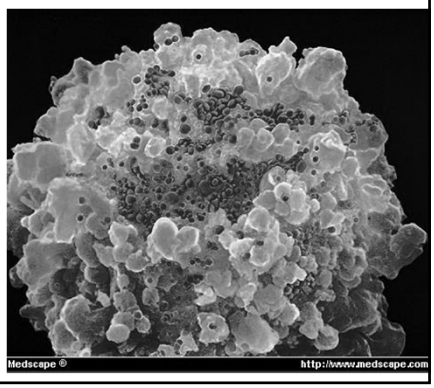
D. Animal Viruses

- ***Most RNA and DNA viruses that infect animals cause diseases.***

1. Some animal viruses are surrounded by membranes derived from ***host plasma membrane***. (**Enveloped Viruses**)
2. **Retroviruses** have RNA genomes that they reproduce through a DNA intermediate.
   - **_____ directed ____ synthesis.**
   - DNA is integrated into host chromosome and is a template for mRNA and new viral genomes.
3. Human immunodeficiency virus (HIV).
   a) An example of viral genome regulation—the reproductive cycle of HIV.
   b) HIV is an **enveloped virus**; it is enclosed in a host cell-derived membrane.
4. Other RNA viruses use their RNA as mRNA:
   a) to code for enzymes and
   b) replicate their genomes without DNA.





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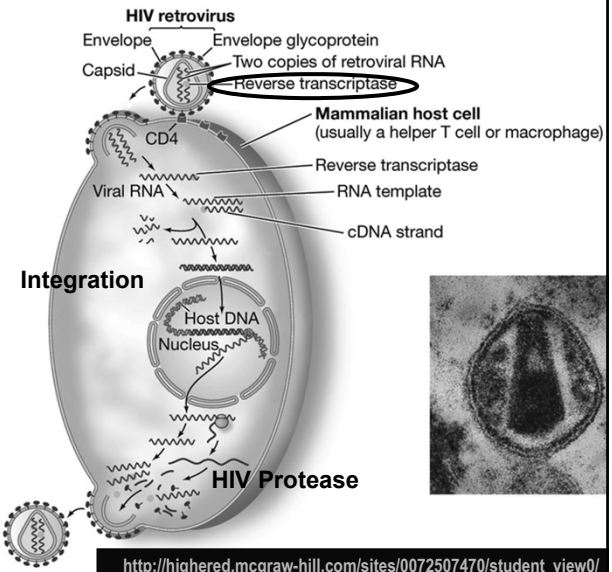
HIV – A Retrovirus (Enveloped)

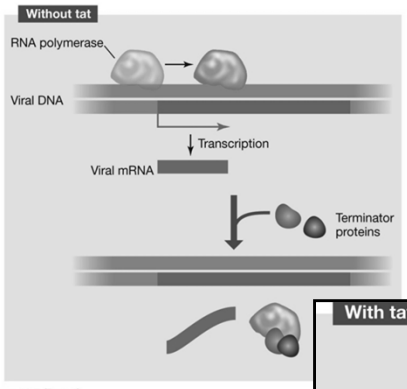
1. Viral glycoproteins bind host receptors.
2. Entry by endocytosis ("**fusion**").
3. Viral envelope fuses with endosome memb., & is released ("**uncoating**")
4. **vRNA → cDNA (RNA-dep't Dpol).**
   - **Reverse Transcriptase**.
   - also makes a copy of the cDNA and double stranded cDNA is integrated into host chromosome.
   - This **provirus** contains promoters recognized by the host's transcription apparatus.
5. Tsln of viral prots; assembly of virion.
6. Release by budding from host PM.



http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter22/animation__how_the_hiv_infection_cycle_works.html

46

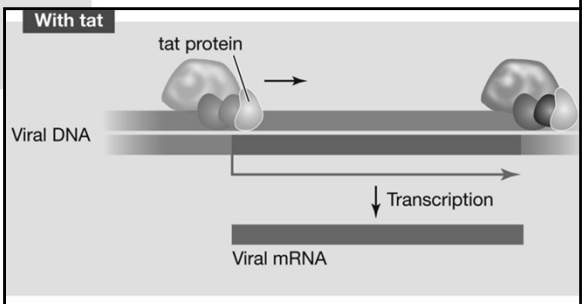
Figure 16.7 Regulation of Transcription by HIV



LIFE 9e, Figure 16.7 (Part 1)

- The host cell has proteins to bind to viral mRNA and prevent transcription,
- but HIV counteracts this with **tat** — a virus encoded protein — in ***antitermination***.

- The ***provirus*** resides permanently in the host chromosome.
  - When activated to make new ***virions***, it is transcribed as mRNA then synthesized by the cell.



Trans-activator of transcription

LIFE 9e, Figure 16.7 (Part 2)

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16.3) Regulation of Gene Expression in Prokaryotes

- In prokaryotes, the expression of some genes is ***REGULATED*** (***Inducible/Repressible genes***)
  - their products are made only as needed.
- Other genes, ***CONSTITUTIVE genes***
  - products are essential at all times
  - are constantly expressed.
  - **Always ON.**
  - **** "Housekeeping Genes" = always ON!**
    - Eg: ribosomes, metabolic enzymes, phospholipid biosynthesis enzymes…..
- A compound that stimulates the synthesis of an enzyme needed to process it is called an ***inducer***.

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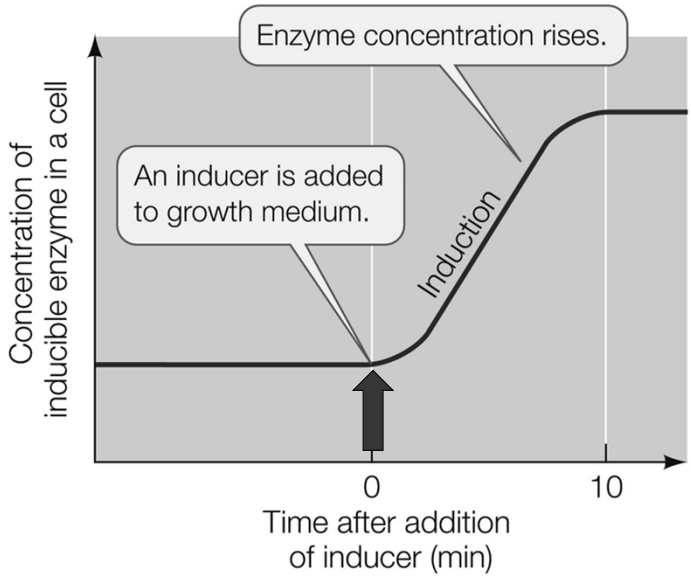
Inducible Enzymes



LIFE 9e, Figure 16.8

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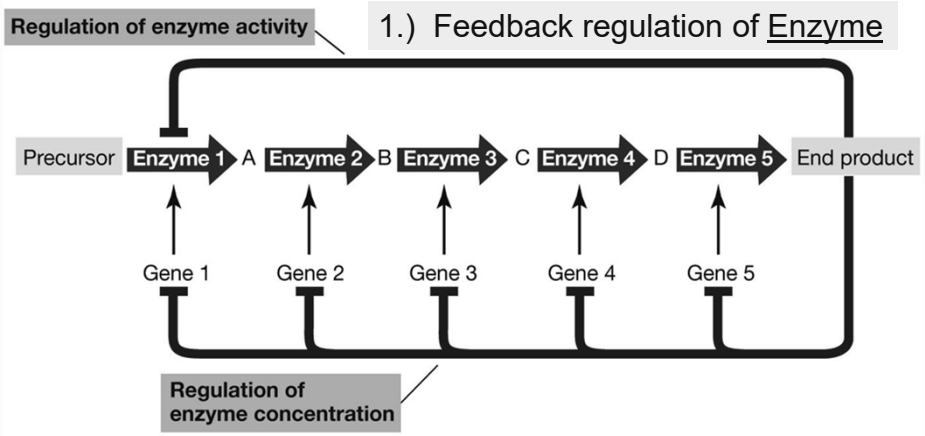
Two Ways to Regulate a Metabolic Pathway



1.) Feedback regulation of Enzyme

2. Feedback regulation of enzyme Gene Expression

LIFE 9e, Figure 16.9

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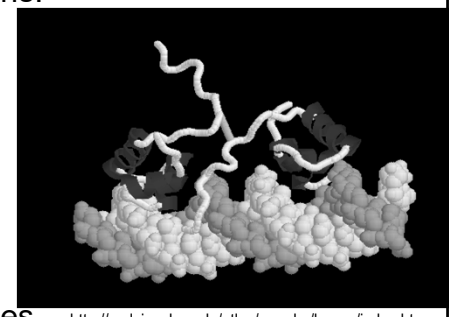
(16.3) OPERONS

- An ***Operon*** consists of a ***Promoter***, an ***Operator***, and ***Structural Genes***.
  - Promoters & operators do not code for proteins,
    - serve as binding sites for regulatory proteins.
  - Structural genes encode proteins.



- When a ***Repressor Protein*** binds to the ***operator***, transcription of the structural genes is inhibited.
  - Repressor proteins are coded by ***constitutive*** regulatory genes.
    - **Always ON.** *("Strong" promoters!! = high affinity for RPol)*

http://molvis.sdsc.edu/atlas/morphs/lacrep/index.htm

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Structure of a Prokaryotic OPERON

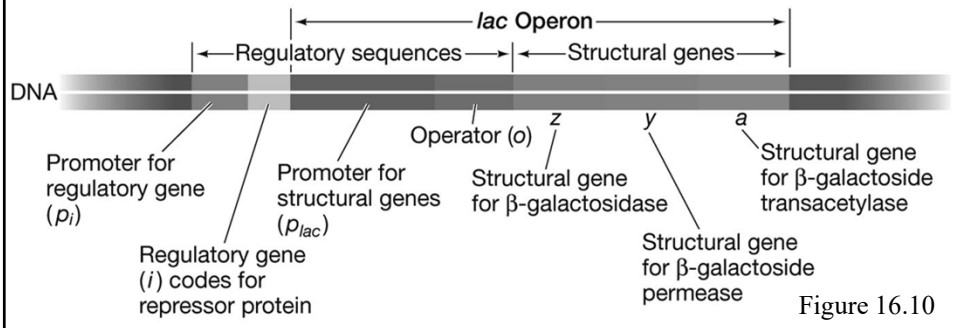

Figure 16.10

1. **Regulatory Gene (*i*)** = encodes repressor or activator protein (***REPRESSOR***)
2. ***Promoter*** = RpoI recognition site for Tscn of all downstream genes (many genes/ 1 promoter/ 1 Transcript!!! = ***PolyCistronic*** genes in prok.)
3. ***Structural genes*** = encode enzymes in a pathway (related prots.)
   - **= coordinated regulation via operon structure!!**
4. **Operator** = regulatory sequence bound by a repressor protein
   - ***(blocks Rpol Tscn)***

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Regulation of Gene Expression in Prokaryotes

❖ **The Expression of Prokaryotic Genes is regulated by:**

1. **Inducible operator–repressor** systems,
   - OFF (repressor bound) unless turned on.
     - ***Catabolic Pathways***
2. systems that **increase the Efficiency of a promoter**, and
3. **Repressible operator–repressor** systems.
   - ON (repressor not bound) unless turned off.
     - ***Anabolic pathways***

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

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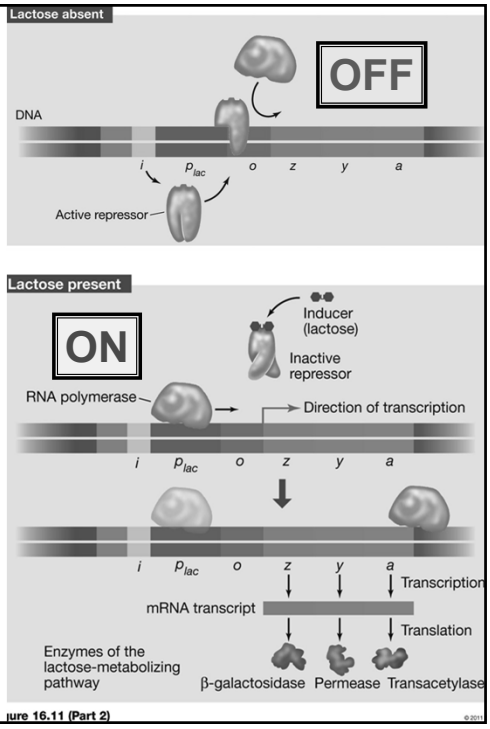
Inducible System: A. *Lac* Operon

- The ***lac operon*** is an example of an ***inducible system*** ***(using NEGATIVE CONTROL)***
  - its proteins allow bacteria to metabolize lactose.

1. **When lactose is absent,**
   - Repressor protein binds tightly to the operator.
   - RNA polymerase from binding to the promoter, turning transcription OFF.
2. **When lactose is present (& glucose is absent),**
   - lactose acts as an inducer by binding to the repressor.
   - changes the repressor's shape so that it no longer recognizes the operator (***allosteric regulation!!***).
   - With operator unbound, Rpol binds to the promoter, and transcription is turned ON.



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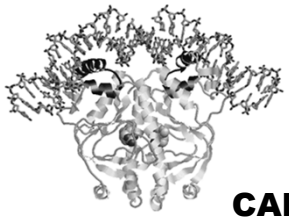
Catabolite Repression Regulates the *lac* Operon:

** cAMP Receptor Protein *(CRP/CAP)*

- The efficiency of Rpol can be increased by regulation of the level of ***cyclic AMP***, which binds to ***CRP***.
  - **= *cAMP Receptor Protein (POSITIVE CONTROL!!!)***
  - Also called **CAP = Catabolite Activator Protein**
  - ***(doesn't activate expression when gluc present/ cAMP low)***



CAP

- ***CRP–cAMP complex***
  - binds to a site near the promoter of a target gene,
  - **enhances the binding of RPol, and hence transcription.**

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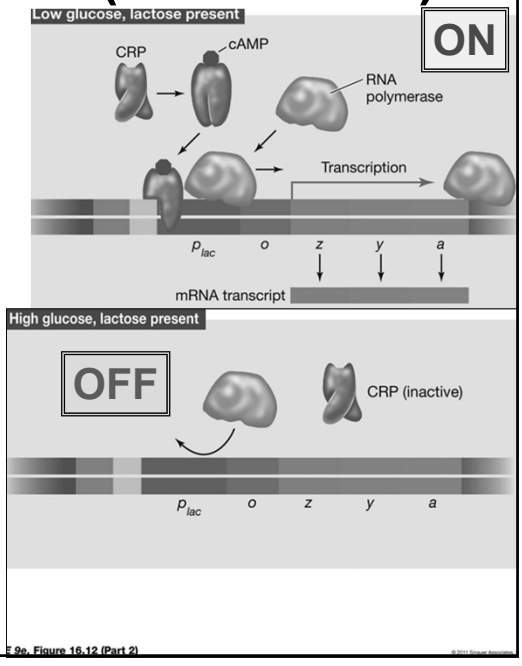
Positive Control (CRP & *Lac*)

- In ***Positive Control***, the regulatory protein is an **ACTIVATOR** (turns genes **ON**).
- In ***Negative Control***, the regulatory protein is a **REPRESSOR** (turns genes **OFF**).

http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter12/animation_quiz_4.html



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Summary of LAC Operon Regulation

TABLE 16.1

Positive and Negative Regulation in the *lac* Operon[a]

GLUCOSE	cAMP LEVELS	RNA POLYMERASE BINDING TO PROMOTER	LACTOSE	LAC REPRESSOR	TRANSCRIPTION OF *lac* GENES?	LACTOSE USED BY CELLS?
Present	Low	Absent	Absent	Active and bound to operator	No	No
Present	Low	Present, not efficient	Present	Inactive and not bound to operator	Low level	No
Absent	High	Present, very efficient	Present	Inactive and not bound to operator	High level	Yes
Absent	High	Absent	Absent	Active and bound to operator	No	No

Negative regulators are in red type.

http://highered.mheducation.com/sites/0072995246/student_view0/chapter7/combination_of_switches__the_lac_operon.html

https://www.sophia.org/tutorials/lac-and-trp-operons-gene-regulation?playlist=biology--11

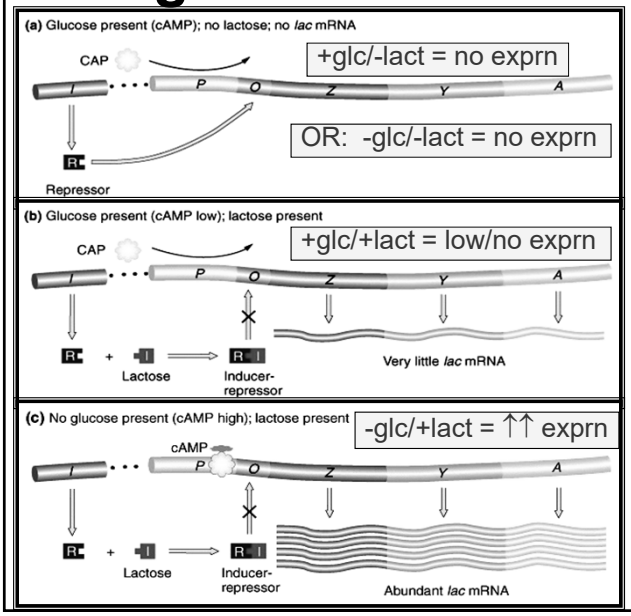
LIFE 9e, Table 16.1

https://highered.mheducation.com/sites/9834092339/student_view0/chapter15/the_lac_operon.html

http://life9e.sinauer.com/life9e/pages/16/162001.html

57

SUMMARY: Transcriptional Regulation of the *lac* Operon



a) **Glucose present (cAMP low): <u>no lactose</u>:**
   - **no lac mRNA**

b) **Glucose present (cAMP low): lactose present:**
   - **very little lac mRNA**

c) **<u>No glucose</u> (cAMP high): lactose present:**
   - **abundant lac mRNA**

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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

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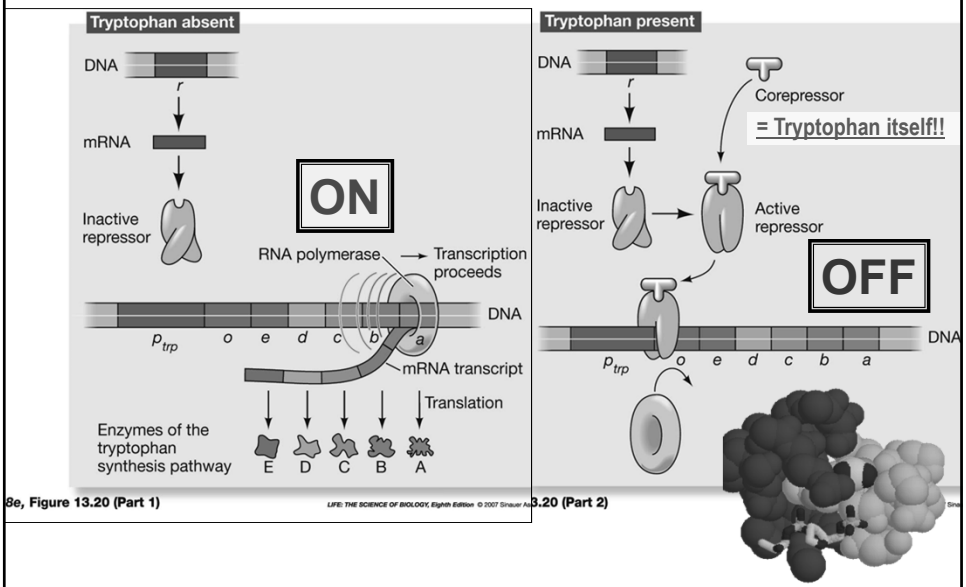
(B.) *TRP* Operon



Se, Figure 13.20 (Part 1) LIFE: THE SCIENCE OF BIOLOGY, Eighth Edition © 2007 Sinauer As 3.20 (Part 2)

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

60