BIOL 230: Cell & Molecular Biology

Fall 2019 ***17-205*** ***W, Oct. 23***

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 NEXT week: DNA Fingerprinting, #2! ☺ PCR.**
   - **Dr. Paul Welles. (NO lecture meetings next week! Do forensics! ☺)**
3. **Anastasia GEL DATA will be 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. **TODAY!! – Exams returned: Midterm 2. SEE ME after reviewing your exam, if you scored <70%!!**
6. **NEXT WED.: QUIZ #4 first attempt due!!!**
7. **RESEARCH TOPICS Should be finished tonight!! OUTLINE DUE Monday, Nov. 4th online.**

REVIEW

1. Compare the processes of **proofreading** and the types of **post-replicative repair of DNA**. Which enzymes are shared with normal **Replication**?
2. *Draw* and *explain* how Eukaryotic cells **preserve the ends of their chromosomes** after many rounds of replication and reproduction.

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

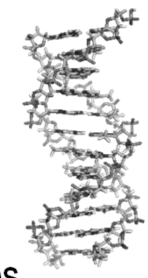
1. **Ch. 14:** Describe **Beadle and Tatum**'s experiments and their contribution to our understanding of molecular genetics & Central Dogma.
2. Diagram and describe how the **Central Dogma** of Molecular Genetics explains the transition from hereditary ***genotype*** to an organism's expressed ***phenotype***.
3. Compare **replication** & **transcription:** product produced, start & end sites, enzymes, & the direction of movement and synthesis.
4. What is the nature of the **Genetic Code**, and describe the experiments that lead to it's discovery. (What are the "translators"??)
5. Define and diagram the roles of **tRNA, rRNA and mRNA** in translation.
6. Compare sites, directions, and molecules involved in **Initiation, Elongation and Termination** of DNA, RNA and Protein synthesis.

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

<u>Chapter 14</u>: From DNA to Protein: Genotype to Phenotype

1. <u>One Gene, One Polypeptide</u>
2. <u>DNA, RNA, and the Flow of Information</u>
3. <u>Transcription: DNA-Directed RNA Synthesis</u>
4. <u>Post-Transcriptional Processing</u>
5. <u>The Genetic Code & Translation</u>
6. <u>Posttranslational Events</u>

(15.) <u>Mutations: Heritable Changes in Genes</u>…..



14.1) One Gene, One Polypeptide

❖ ***<u>Genes:</u>***

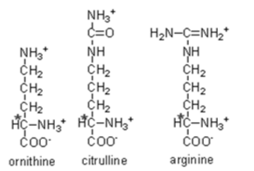
- made up of DNA; = units of hereditary information.
- = *segments of continuous DNA sequence that encode a functional product (usually a protein or an active RNA).*
- ***expressed*** in the ***<u>phenotype</u>*** (physical characteristics) as polypeptides.

- **Beadle and Tatum's experiments:**
  - with the bread mold ***Neurospora crassa* –** haploid!
  - exposure to X-rays resulted in mutant strains lacking a specific enzyme in a biochemical pathway.
  - <u>*Specific* ***Gene*** *expression → Specific* ***protein*** *activity!!*</u>
- These results led to the ***<u>One-Gene, One-Polypeptide Hypothesis</u>***.

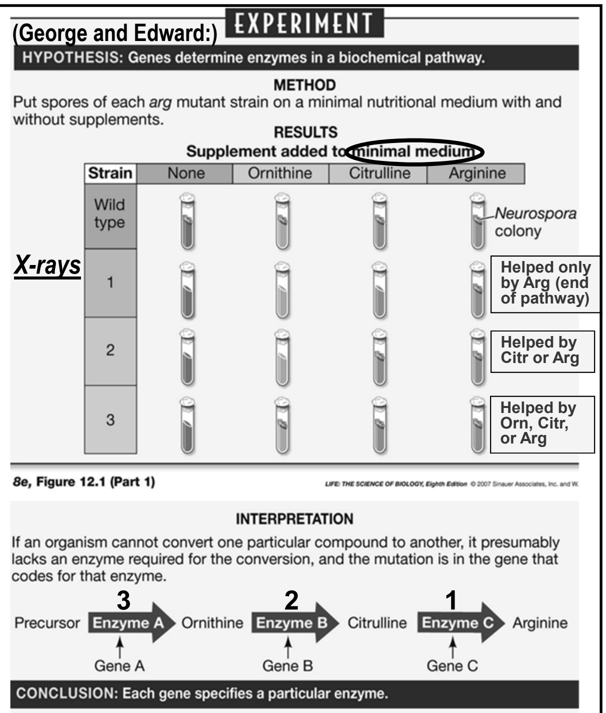
https://www.dnalc.org/resources/nobel/beadle_tatum.html

Beadle & Tatum, 1940s

- Mutants (***auxotrophic***) that cannot synthesize Arginine for themselves
  - *Wild Type* = ***Prototrophs***
- Some *arg-* mutants can use some molecular precursors, but not others, to make Arg.
  - ***The MORE supplements that help the mutant, the EARLIER in the pathway is the mutated gene/enz.***



- ***Each mutated unit of heredity (gene) controlled one enzyme in ARG biosynthesis***



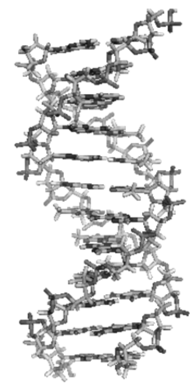
One Gene, One Polypeptide

- The absence of certain enzymes was known to cause by certain hereditary diseases in humans. *("inborn errors of metabolism")*
  - **PKU (**phenyl ketone urea**), AKU (**alkaptonuria**), etc……**
  - **(Dr. Archibald Garrod, ~1900: Gene → enzyme)**
- These observations supported the one-gene, one-polypeptide hypothesis.
  - *[Exceptions in Eukaryotes (Ch. 14)]*
- ***** ESTABLISHED THE CONNECTION BETWEEN GENES & PROTEINS!! *****
  - (Later restated as **DNA → RNA → Proteins**)

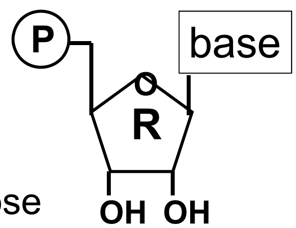
http://www.dnaftb.org/dnaftb/16/concept/index.html

14.2) DNA, RNA, and the Flow of Information

- RNA differs from DNA in three ways:
  1. It is **single-stranded**,
  2. its sugar molecule is **Ribose** rather than deoxyribose,
  3. and its fourth base is **Uracil** rather than thymine.
     - **U-A**, not **T-A** base pairs

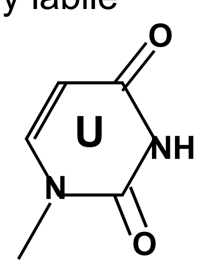


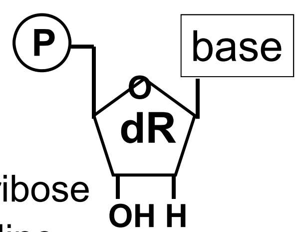
A. RNA vs. DNA



RNA

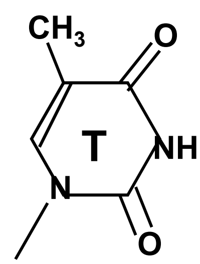
1. Ribose
2. Uracil
3. Single-stranded
4. Chemically labile
   - (2’ OH)





DNA

1. Deoxyribose
2. Thymidine
3. Double-stranded
4. Chemically stable



Three Types of RNA

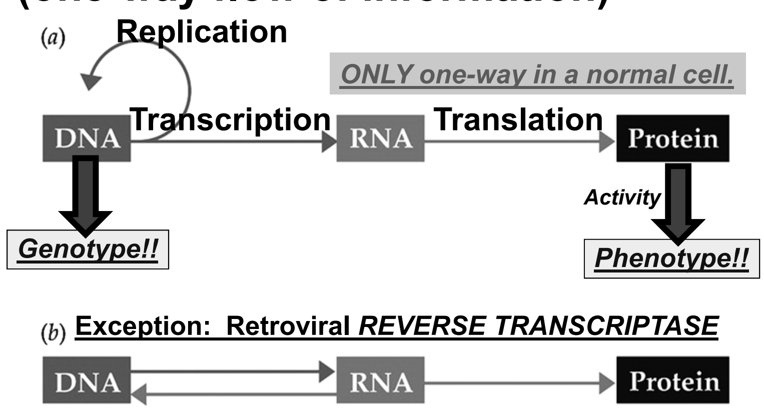
1. **Messenger - mRNA**: serves as an intermediary for the synthesis of proteins (5% of RNA in cell).

2. **Transfer - tRNA**: is the adaptor for converting the nucleic acid code into the amino acid sequence (15%).

3. **Ribosomal - rRNA**: is a central component of the protein synthesizing machinery (80%).

B. The Central Dogma of Molecular Genetics (Crick)

- The central dogma of molecular biology is **DNA → RNA → Protein.**
  - **(one-way flow of information)**



3 Processes Responsible for the Inheritance of Genetic Information

1. Replication: duplicates double-stranded nucleic acids.
- ***DNA-Directed DNA synthesis.***

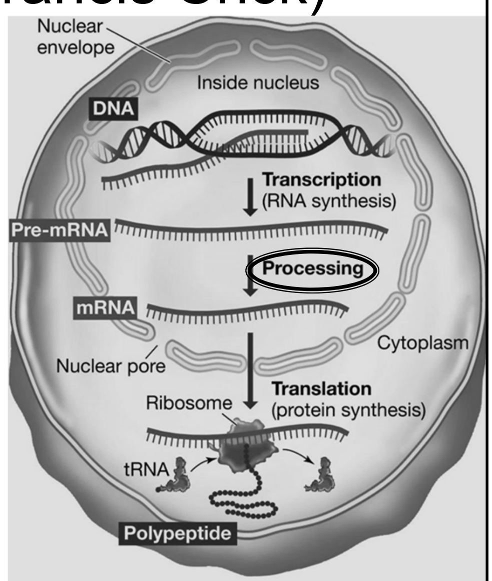
2. Transcription: generates a single-stranded copy of the double-stranded genome.
- ***DNA-Directed RNA synthesis.***
- RNA discovered as the intermediate between DNA in the nucleus, and proteins synthesized in the cytoplasm.

3. Translation: converts the nucleotide sequence into amino acid sequence.
- ***RNA-Directed Protein Synthesis.***

The Central Dogma of Molecular Genetics (Francis Crick)

A gene is *Expressed* in two steps:

1. First, DNA is transcribed to RNA;
2. then (m)RNA is translated into protein.



LIFE 10e, Figure 14.2

An Exception: Reverse Transcription

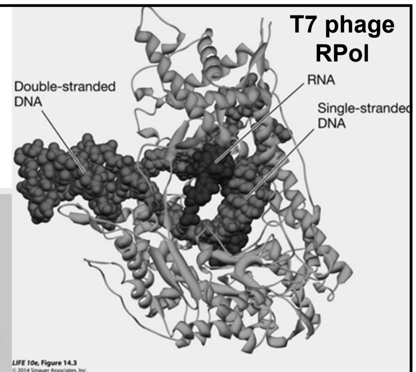
- In ***Retroviruses***, the rule for transcription is reversed: ***RNA → DNA***.
  - Such as HIV, many Tumor Viruses.

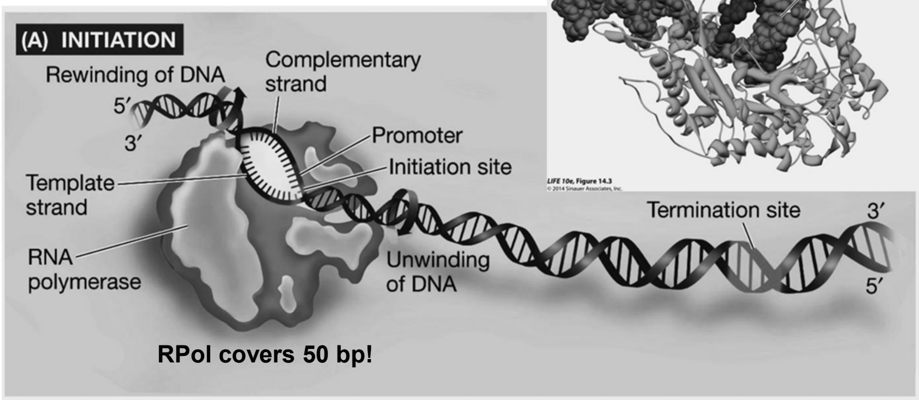
- Other RNA viruses exclude DNA altogether, going directly from RNA to protein.

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.***

Transcription





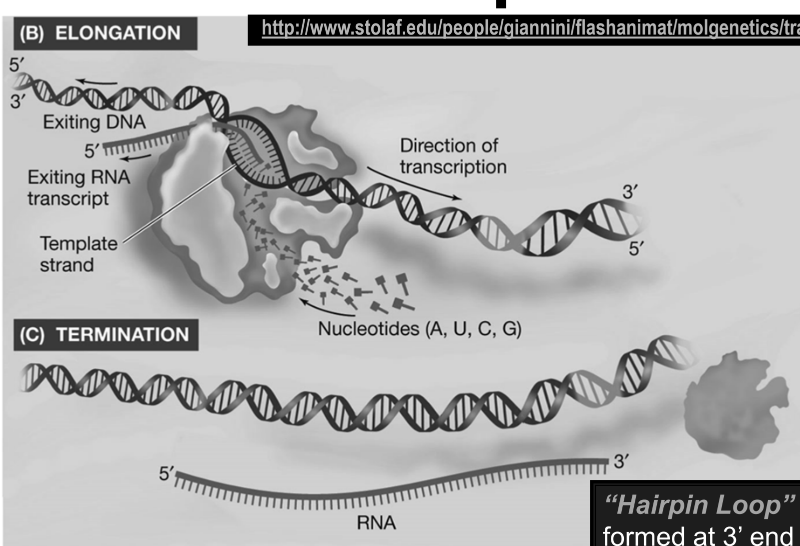
http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/transcription.swf

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

***LIFE 9e*, Figure 14.4 (Part 2)**

Transcription

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



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

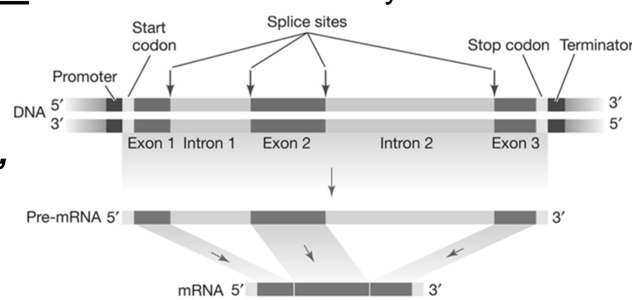
***LIFE 9e*, Figure 14.4 (Part 3)**

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

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…...***

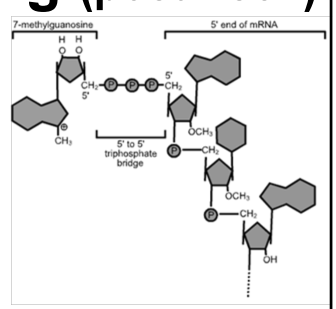


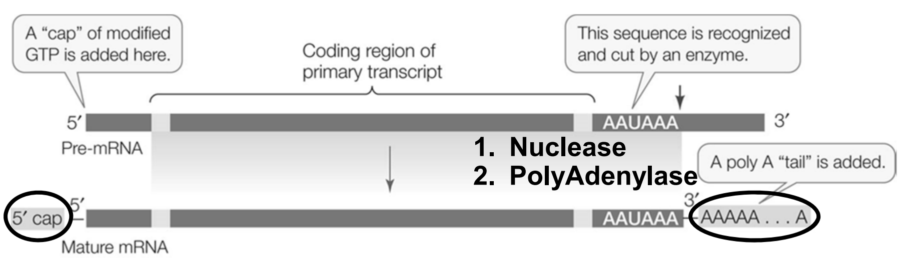
LIFE 10e, Figure 14.8 (Part 2)

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

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!!





LIFE 10e, Figure 14.9

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

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

RNA Processing

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

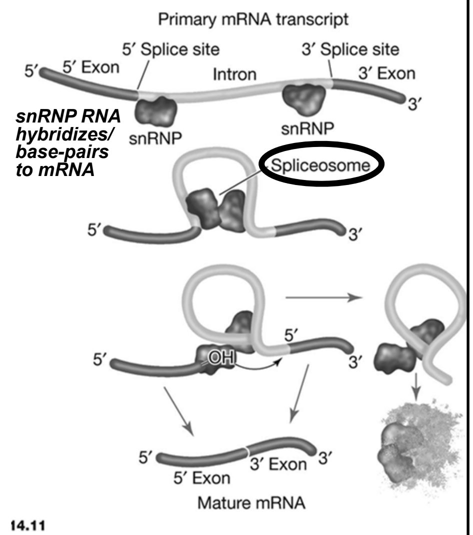


Figure 14.11

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

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

14.5) The Genetic Code

1. The genetic code consists of **<u>triplets</u>** of nucleotides (***<u>codons</u>***).
   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 ***<u>genetic code is redundant ("degenerate"):</u>***
   a) Some AA's encoded by >1 codon!!
   b) However, a single codon does not specify more than one amino acid. (*codons are <u>not "ambiguous"</u>!!)*

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

Universal Genetic Code

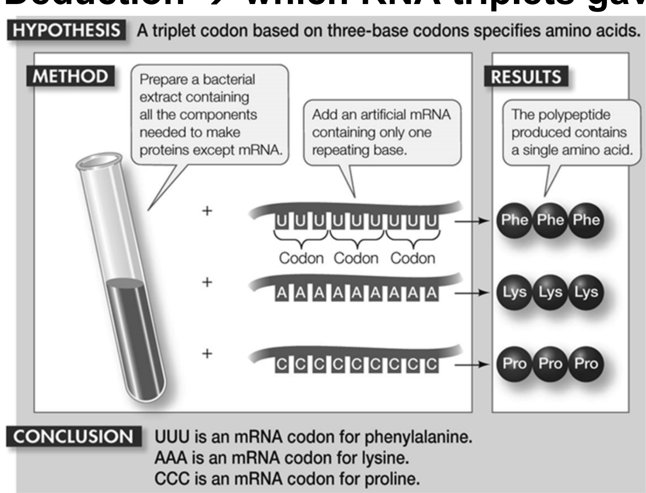
Second letter

First letter	U	C	A	G	Third letter
U	UUU, UUC Phenyl-alanine; UUA, UUG Leucine	UCU, UCC, UCA, UCG Serine	UAU, UAC Tyrosine; UAA Stop codon, UAG Stop codon	UGU, UGC Cysteine; UGA Stop codon; UGG Tryptophan	U C A G
C	CUU, CUC, CUA, CUG Leucine	CCU, CCC, CCA, CCG Proline	CAU, CAC Histidine; CAA, CAG Glutamine	CGU, CGC, CGA, CGG Arginine	U C A G
A	AUU, AUC, AUA Isoleucine; AUG Methionine; start codon	ACU, ACC, ACA, ACG Threonine	AAU, AAC Asparagine; AAA, AAG Lysine	AGU, AGC Serine; AGA, AGG Arginine	U C A G
G	GUU, GUC, GUA, GUG Valine	GCU, GCC, GCA, GCG Alanine	GAU, GAC Aspartic acid; GAA, GAG Glutamic acid	GGU, GGC, GGA, GGG Glycine	U C A G

***LIFE 9e*, Figure 14.6**

Deciphering the Genetic Code

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



9e, Figure 14.5

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

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/

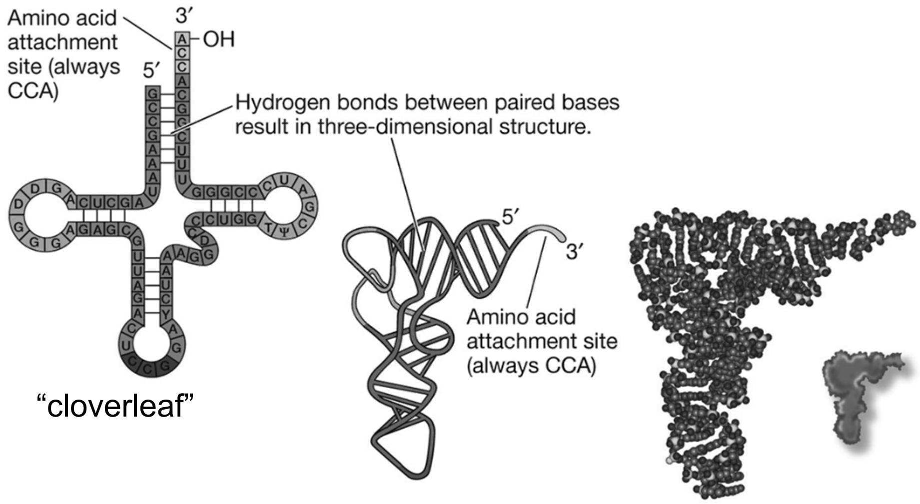
Complete Gene Expression!!: http://learn.genetics.utah.edu/content/basics/transcribe/

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.

A. tRNA Structure



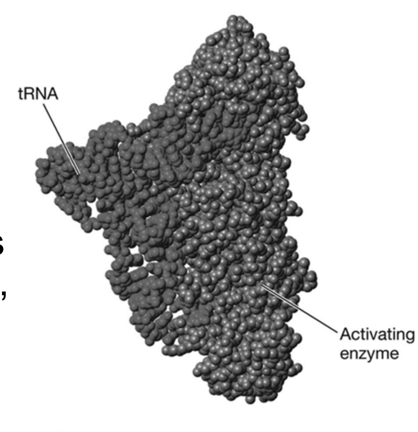
http://www.hhmi.org/biointeractive/media/DNAi_translation_vo1-lg.mov

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

LIFE 9e, Figure 14.12

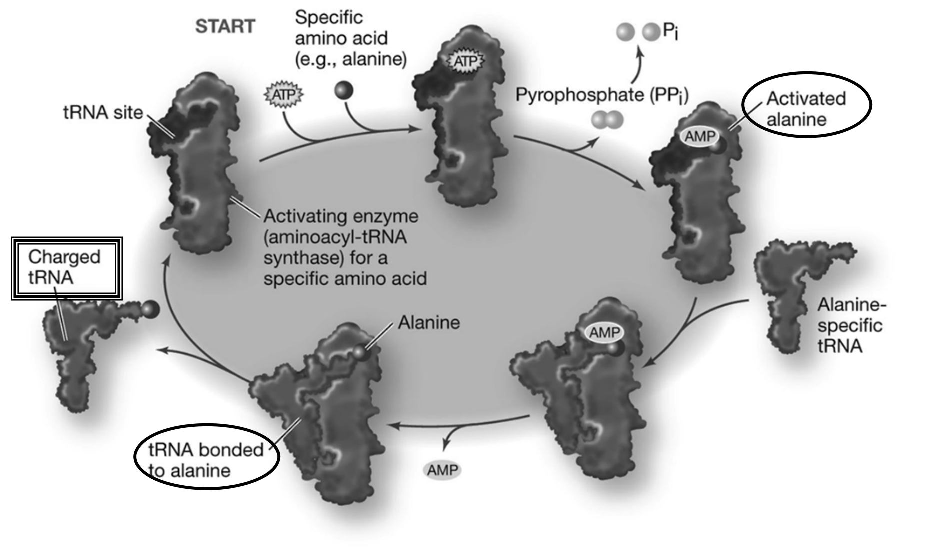
B. Specificity of the Code: Aminoacyl-tRNA Synthetases

- 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!!!***

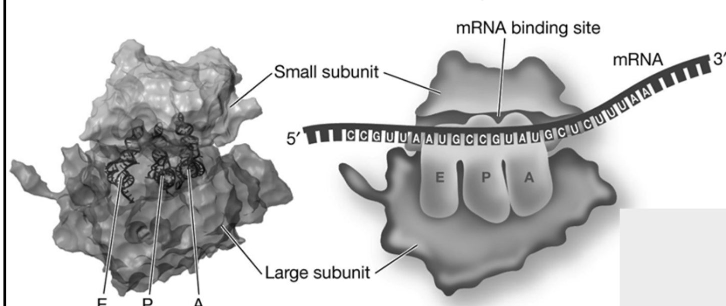
**AminoAcyl tRNA Synthetase: Charging a tRNA molecule **



LIFE 9e, Figure 14.13

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#

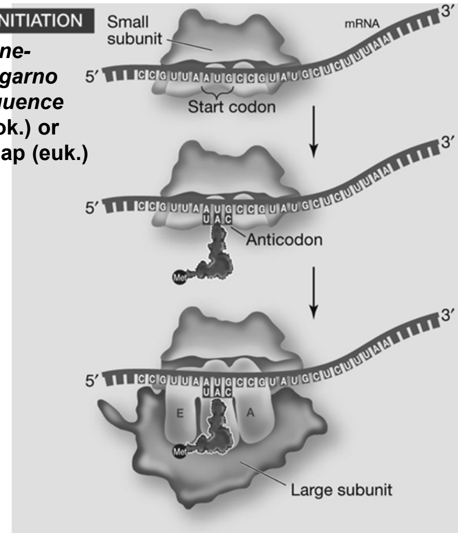
A. Initiation of Translation: mRNA, Charged (aminoacyl) tRNA, Ribosome

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.***

Shine-Dalgarno sequence **(prok.) or G-cap (euk.)**

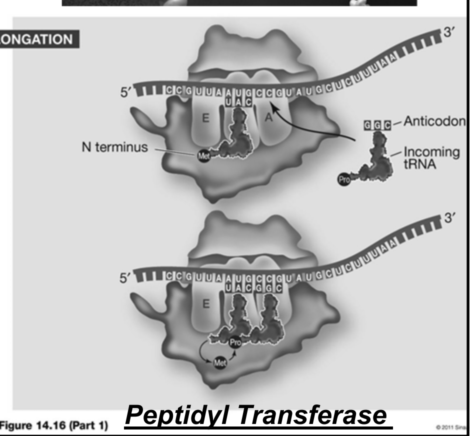


https://youtu.be/oefAI2x2CQM (Amoeba Sisters)

14.15 (Part 2)

B. Translation: Elongation

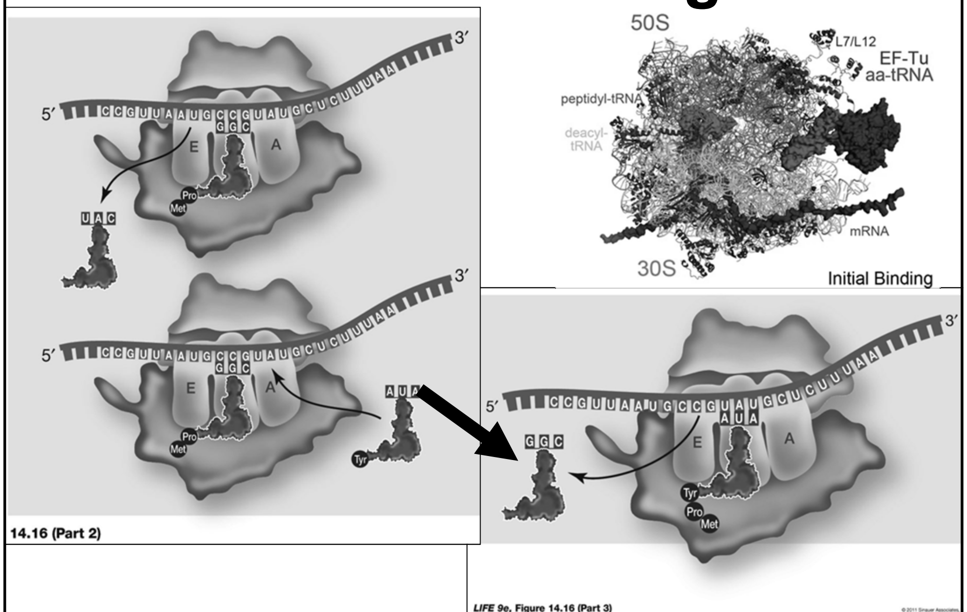
- Polypeptides grow from the **N terminus → C terminus.**
  - *Amino to Carboxyl !!*
  - ***Peptidyl Transferase*** activity is in the **Large Subunit**!! (**rRNAs**)
    - Growing chain in the P-site is cleaved from the last tRNA,
    - then attached by its CARBOXYL group to the AMINO group of the new 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!*

Figure 14.16 (Part 1) ***Peptidyl Transferase***

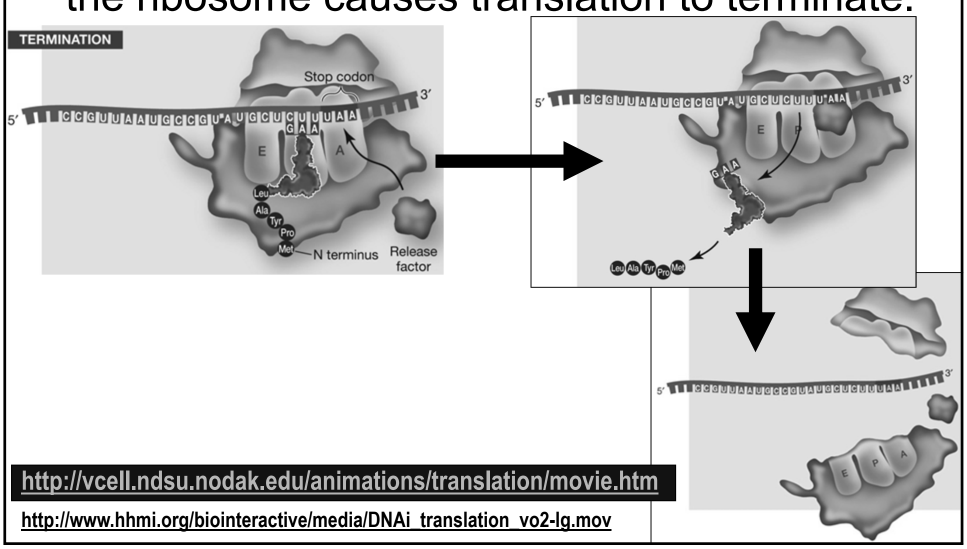
Translation → Elongation



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

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

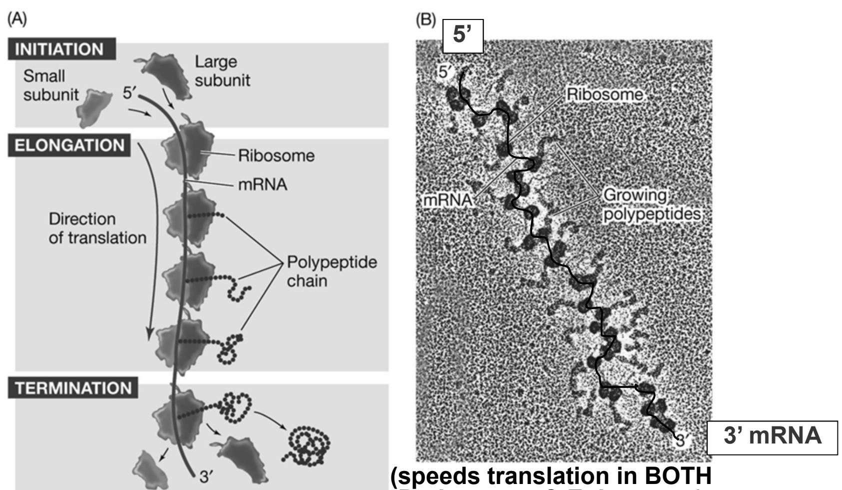
Compare Repln, Tscn, Tsln:

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'l 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

14.8) Regulation of Translation

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



(speeds translation in BOTH Prokaryotes & Eukaryotes)

LIFE 9e, Figure 14.18

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.

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.***

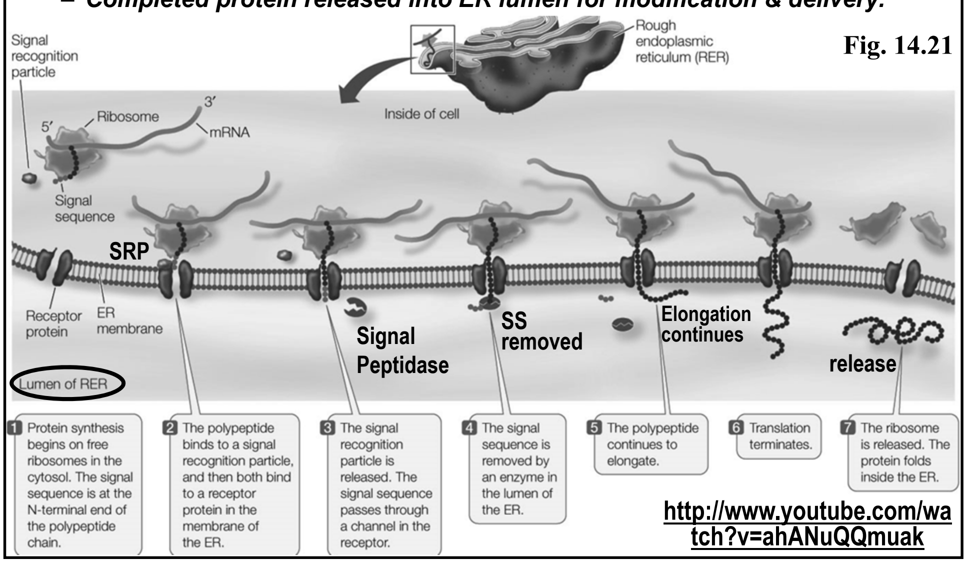
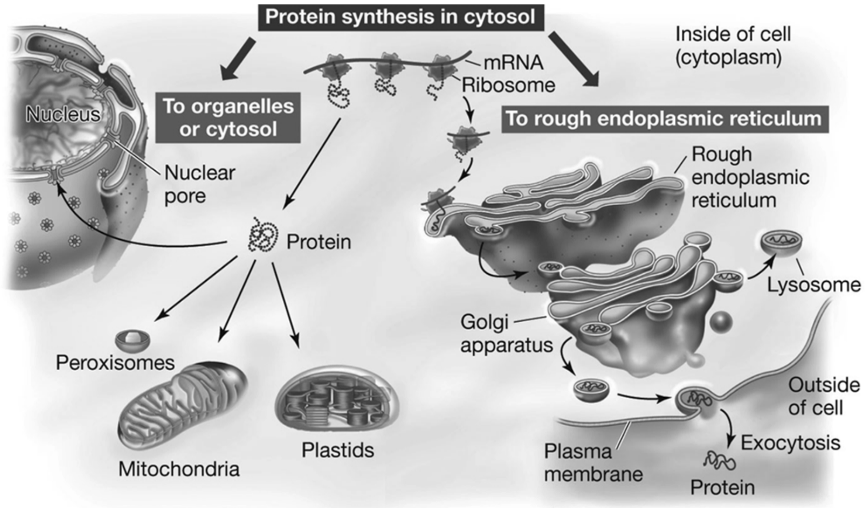


Fig. 14.21

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

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

LIFE 9e, Figure 14.19

**Apply transcription & translation...

- The following stands of DNA encode a 3 AA peptide.
- Label the template and coding (“*mRNA-Like*”) stands, the polarity (5’ and 3’ ends) of all molecules, and write-out the transcript sequence and the amino acid sequence of the peptide.
- 3’ ATACGGTGATATTCGATGTA 5’
- 5’ TATGCCACTATAAGCTACAT 3’

RNA: 5’ U **AUG** CCA CUA.**UAA**. GCUACAU 3’

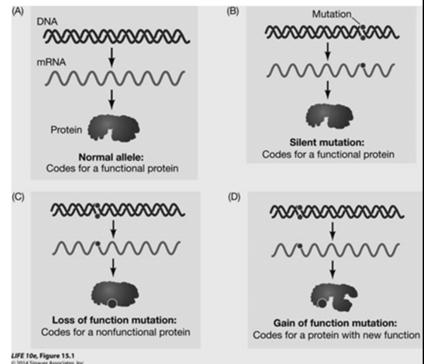
Protein: N- met-pro-leu -C

Second letter

First letter	U	C	A	G	Third letter
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	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

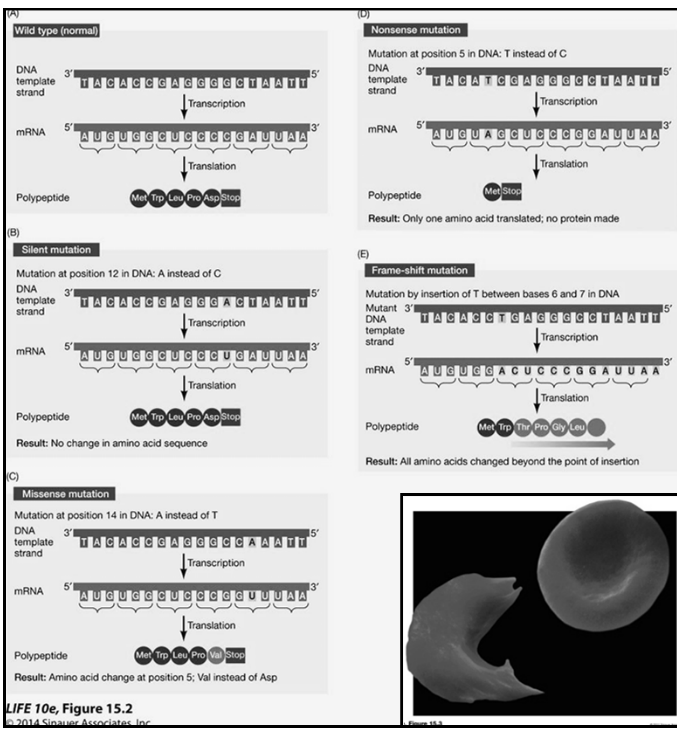
15.1) Mutations:
Heritable Changes in Genes

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



LIFE 10e, Figure 15.1

- 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)***



LIFE 10e, Figure 15.2

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!!*

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.

Codons & Deletion Mutations

Wild type
CAT CAT CAT CAT CAT
- FAT CAT ATE THE RAT

One deletion
CTC ATC ATC ATC AT
- FTC ATA TET HER AT.

Two deletions
CTA TCA TCA TCA T
- FTA TAT ETH ERA T ...

Junk!

Three deletions
CTA TAT CAT CAT
- FTA TAE THE RAT …

Back into Frame, -1 aa

- DNA
- Words analogy

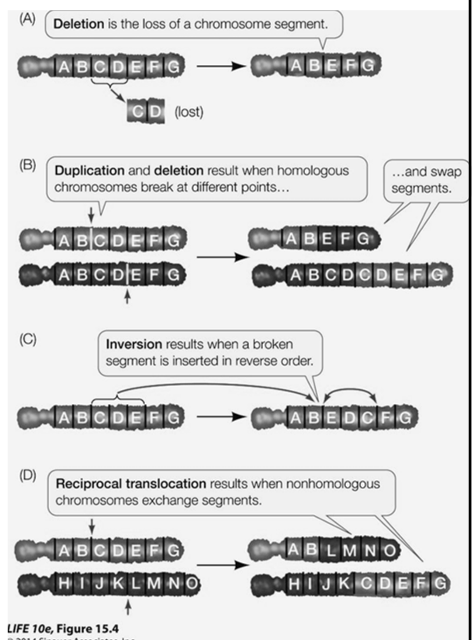
Deletions in multiples of 3 (Not 1 or 2) = mostly OK → clue to triplet nature of code!!

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

B. Chromosomal Mutations

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

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



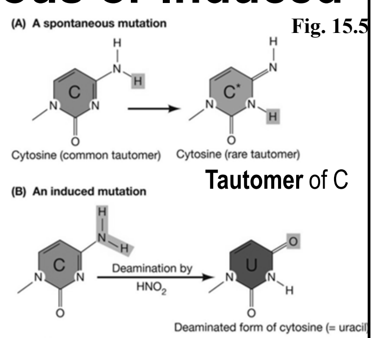
LIFE 10e, Figure 15.4

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.
   - ➢ = ***<u>MUTAGEN</u>***

– **Altered form of C now favorably pairs with A!!**
- **C*—A pairs!**

Fig. 15.5



igure 15.5 (Part 1)