Lecture	e 26 (11/18/20)	Nucleic Acids A. Replication
TODAY		1. Polymerases 2. Fidelity
<ul> <li>Reading:</li> </ul>	Ch27; 1088-1091, 1096-1108	c. Mis-match repair d. Post-replication repair
•Problems:	Ch27 (text); 5,8,10,11,13,16,17 Ch25 (study-guide: applying); 2,3 Ch25 (study-guide: facts); 4,6	i. Direct reversal ii. Base excision iii. Nucleotide excision 3. Sequence determination 4. PCR B. Transcription
END or Exar	n-4 MATERIAL	1. Overview-mRNA     2. Process     3. RNA polymerase <ul> <li>a. Similarities to DNA polymerase</li> <li>b. Differences from DNA polymerase</li> <li>4. Fidelity</li> </ul>
		1. Triplet
NEXT (Lipids):		2. deciphering D. Translation
•Reading:	Ch10; 361-368, 370, 372, 376	<ol> <li>Environmentation (Construction)</li> <li>Protein Biosynthesis</li> <li>Biosome</li> </ol>
•Problems:	Ch10 (text); 1,3,4,8,10,14,16 Ch10 (study-guide: applying); <b>1,3,4</b> Ch10 (study-guide: facts); <i>1-5,6-8</i> Ch11 (study-guide: facts); <i>8</i>	<ul> <li>b. Overview &amp; process</li> <li>c. Process overview; Selection of AUG</li> <li>d. Elongation <ol> <li>b. Decoding</li> <li>Transpeptidation (peptide bond formation)</li> <li>ii. translocation</li> <li>e. Fidelity; two steps</li> </ol> </li> </ul>











Transcription & Translation				
Transcription				
Overview				
Process				
RNA Polymerase				
Fidelity				
Translation				
Genetic Code				
triplet				
decyphering				
tRNA				
Structure				
Anticodon				
Acylation (charging)				
Aminoacyl-tRNA Synthetases				
Mechanism				
Fidelity				
Protein Biosynthesis				
Overview				
Process				
Ribosome review				
Peptidyl Transferase				
Fidelity				





Antibiotic inhibitors of protein synthesis					
Antibiotic	Action				
Streptomycin and other aminoglycosides	Inhibit initiation and cause misreading of mRNA (prokaryotes)				
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)				
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)				
Cycloheximide	Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)				
Erythromycin	Binds to the 50S subunit and inhibits translocation (prokaryotes)				
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (prokaryotes and eukaryotes)				

















## Translation: Protein Biosynthesis

## **Elongation: Fidelity**

How does the EF-Tu•GTP•AA-tRNA•mRNA•Ribosome Complex examine this codon-anticodon interaction via a <u>second</u> method? (much like DNA polymerases and aminoacyl-tRNA synthetases)

It uses a complex of EF-Tu•GDP•AA-tRNA•mRNA•Ribosome to test the codonanticodon interaction via a conformational change that stresses this interaction.

- EF-Tu•GTP•AA-tRNA binds the A-site with a strained anticodon stem-loop • Anticodon-codon interactions in the A-site induce EF-Tu's hydrolysis of GTP
- to GDP. GTP hydrolysis is FASTER for cognate tRNA.
- This results in EF-Tu release from the complex as EF-Tu·GDP
- Once the EF-Tu is gone, the AA-tRNA relaxes, swings its acceptor stem into the A-site on the 50S, pivoting at the codon-anticodon interaction
- Non-cognate tRNAs do not survive this pivot and fall out most of the time

## THEREFORE, GTP HYDROLYSIS IS KEY:

- 1. In its slowness, time is allowed for optimal cognate codon-anticodon interactions
- 2. In its hydrolysis, initiated by these interactions, the process achieves a second test of the fitness by being "hinged" at the codon-anticodon during the pivoting.

Protein Biosynthesis		
P	robability of synthesizing an error-free protein	
Number of amino acid residues		
100	300	1000
0.366	0.049	0.000
0.905	0.741	0.368
0.990	0.970	0.905
0.999	0.997	0.990
р	$=(1-\epsilon)^n$	
p is the probability of an error-free protein $\epsilon$ is the error rate n is the length of the protein		
	P Nun 100 0.366 0.905 0.990 0.999 p is the p ε is the en n is the left	Probability of synthesizing an error-free proteinNumber of amino acid residues1003000.3660.0490.9050.7410.9900.9700.9990.997p = $(1 - \varepsilon)^n$ p is the probability of an error-free $\varepsilon$ is the error rate n is the length of the protein











Translation: Protein Biosynthesis					
ENERGY REQUIREMENTS:					
•2 ATP equivalents for every base in the 3-base codon of the mRNA (mRNA synthesis (transcription))	= 6				
• 2 ATP equivalents for every AA-tRNA (tRNA charging)	= 2				
<ul> <li>1 ATP equivalent for binding with EF-Tu</li> </ul>	= 1				
<ul> <li>1 ATP equivalent for translocation with EF-G</li> </ul>	= 1				
	= 10 total ATPs				
	for every				
	residue				

## END of Material for Exam 4