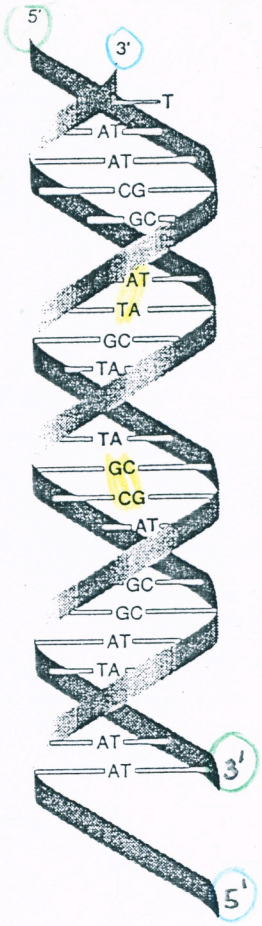
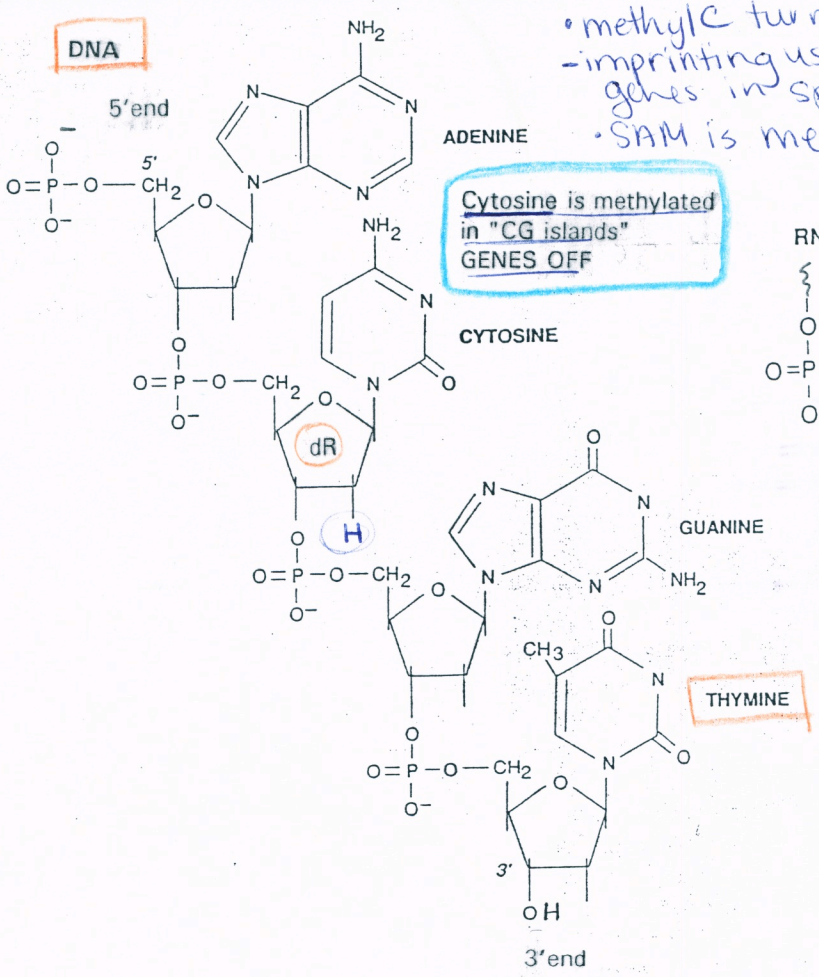
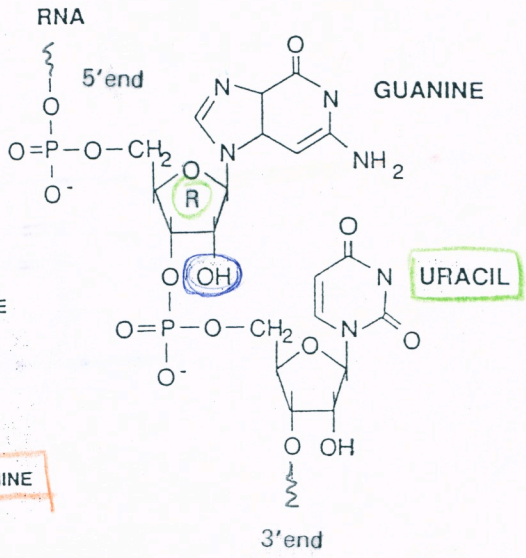




• methylC turns genes off.
- imprinting uses this to turn off genes in sperm/ova (XX?)
• SAM is methylating agent

Cytosine is methylated in "CG islands" GENES OFF



Strand Sequence

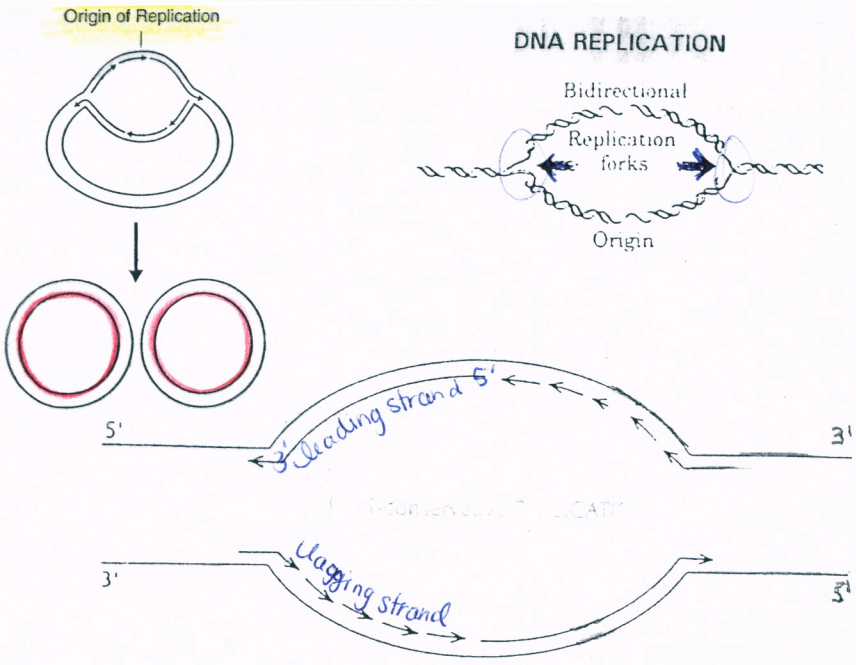
5′ T C G A 3′ or T C G A	Always 5′ → 3′ (L → R)
	must assume 5′ → 3′ (L → R)
3′ A G C T 5′	If written backwards, end must be clearly designated.
5′ p T p C p G p A 3′	Sometimes the PDE bond is indicated.
★ 5′ T C G A 3′ / 3′ A G C T 5′	Strands must be anti-parallel to base pair. If complementary then also anti-parallel.

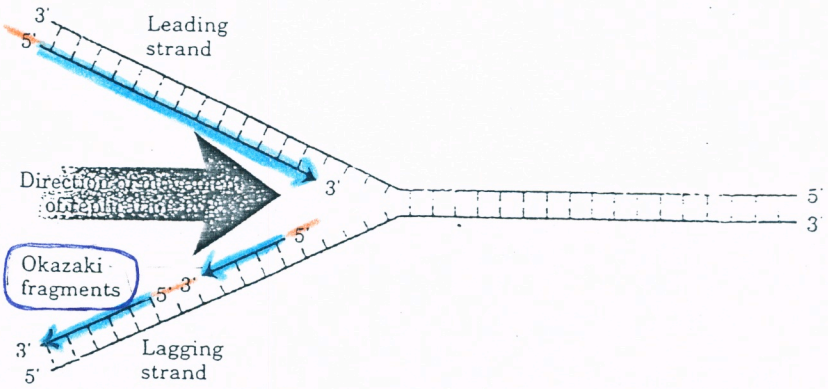
in DNA % T = % A
% C = % G

purines = pyrimidines

* parvovirus is single stranded DNA virus
* held together by H bonds, 3 b/t/n GC means stronger binding

• if As ≠ Ts don't balance, has to be single stranded. ds DNA % should =
• might radiolabel 'T' to see rates of DNA rep vs RNA rep





Comparison of DNA and RNA Polymerases

	DNA Polymerase	RNA Polymerase
Nucleic acid synthesized (5′→3′)	DNA	RNA
Required template (copied 3′→5′)	DNA*	DNA*
Required substrates	dATP, dGTP, dCTP, dTTP	ATP, GTP, CTP, UTP
Required primer (found at 5' end)	RNA (or DNA)	None
Proofreading activity (3′→5′ exonuclease)	Yes	No

*Certain DNA and RNA polymerases require RNA templates. These enzymes are most commonly associated with viruses.

• template is strand that's copied
• helicase unwinds, starting at ORI

• synthesis occurs 5' → 3'

The Steps and Proteins Involved in DNA Replication

	Prokaryotic cells bugs	Eukaryotic cell nuclei us
1. Unwinding of DNA double-helix at replication origin(s)	Helicase (requires ATP)	Helicase (requires ATP)
2. Stabilization of unwound template strands	Single-strand binding protein (SSB)	Single-strand binding protein (SSB)
3. Synthesis of RNA primers	Primase	Primase
4. Synthesis of DNA		
• leading strand	DNA polymerase III	DNA polymerase δ + α
• lagging strand (Okazaki fragments)	DNA polymerase III	DNA polymerase α + δ α β γ δ
5. Removal of RNA primers	DNA polymerase I (5′ - exonuclease)	Unknown
6. Replacement of RNA with DNA	DNA polymerase I	Unknown
7. Joining of Okazaki fragments	DNA ligase (requires NAD)	DNA ligase (requires ATP)
8. Removal of supercoils ahead of advancing replication forks	Topoisomerase II (DNA gyrase) • inhibited by nalidixic acid, norfloxacin (fluoroquinolones)	Topoisomerase II • inhibited by etoposide, teniposide
9. Synthesis of telomeres	Not required	Telomerase RNA

- loss of telomerase is normal aging; increased telomerase is cancer

Other Eukaryotic DNA Polymerases

DNA polymerase γ replicates mitochondrial DNA in eukaryotes.

DNA polymerases β and ε (in eukaryotic cell nuclei) are thought to participate primarily in DNA repair. DNA pol ε may substitute for DNA pol δ in certain cases

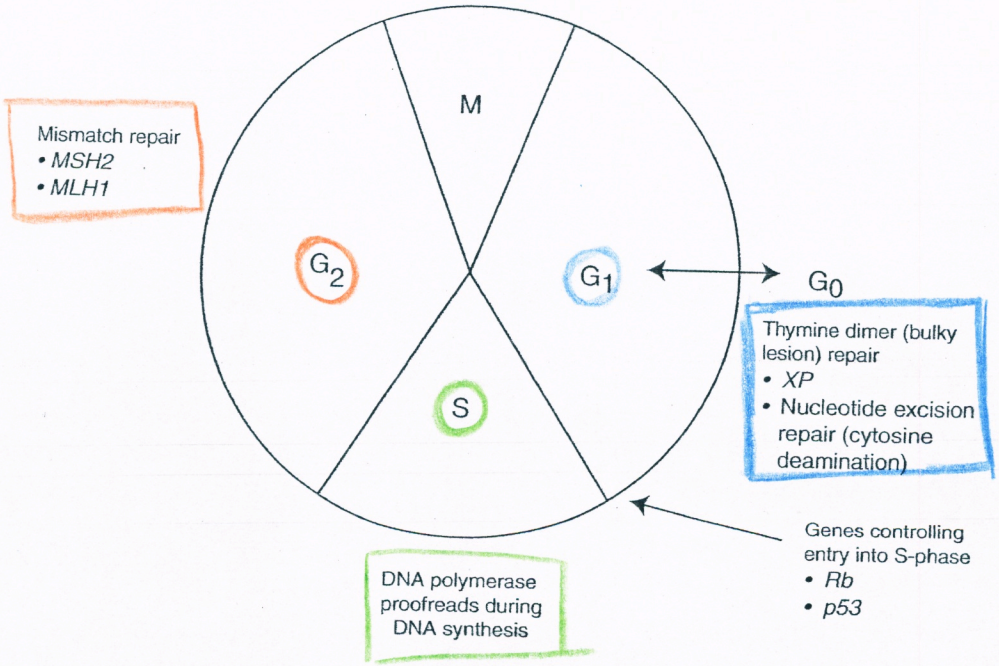
Reverse Transcriptase RNA $\xrightarrow{RT}$ cDNA

Reverse transcriptase, an enzyme found in some viruses, is an RNA-dependent DNA polymerase. This enzyme requires an RNA template to direct the synthesis of new DNA. Retroviruses, most notably HIV, utilize this enzyme to replicate their RNA genomes.

- all DNA polymerases need a primer to get started so RT does too
- RT has no DNA proofreading activity (3′ exonuclease activity absent)
- telomerase is an RT

DNA Repair

Damage	Cause	Recognition/ Excision Enzyme	Repair Enzymes
Thymine dimers (G_1)	UV radiation	Excision endonuclease (deficient in Xeroderma pigmentosum)	DNA polymerase DNA ligase
Cytosine deamination (G_1)	Spontaneous/ chemicals	Uracil glycosylase AP endonuclease	DNA polymerase DNA ligase
Apurination or apyrimidination (G_1)	Spontaneous/ heat	AP endonuclease	DNA polymerase DNA ligase
Mismatched base (G_2)	DNA replication errors	A mutation on one of two genes, hMSH2 or hMLH1, initiates defective repair of DNA mismatches, resulting in a condition known as hereditary nonpolyposis colorectal cancer—HNPCC. Lynch Syndrome	DNA polymerase DNA ligase

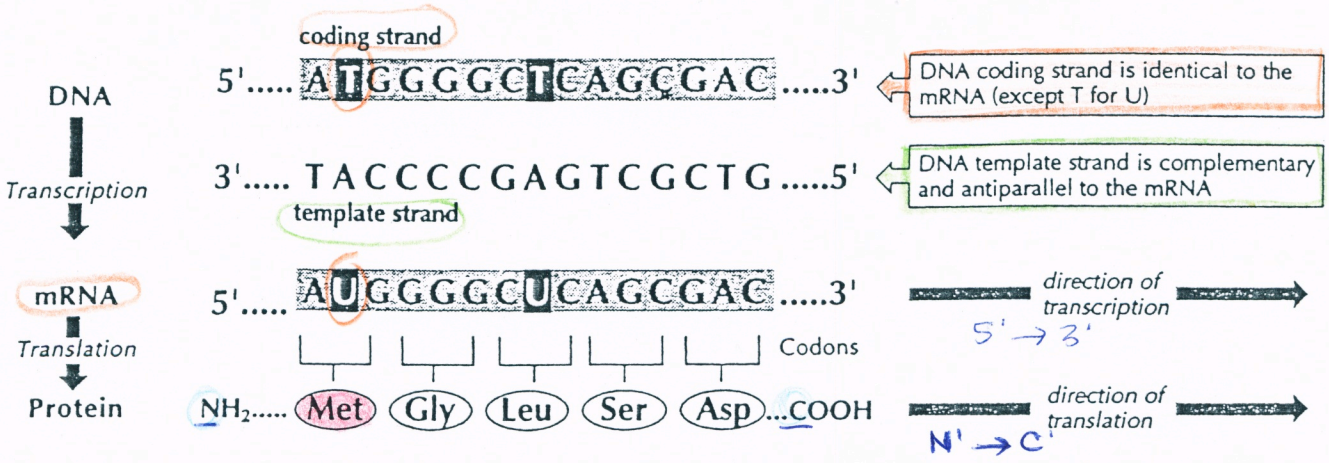


Tumor Suppressor Genes and DNA Repair

DNA repair may not occur properly when certain tumor suppressor genes have been inactivated through mutation or deletion:

- The *p53* gene encodes a protein that prevents a cell with damaged DNA from entering the S phase. Inactivation or deletion associated with Li Fraumeni syndrome and many solid tumors.
- *ATM* gene encodes a kinase essential for p53 activity. *ATM* is inactivated in ataxia telangiectasia, characterized by hypersensitivity to x-rays and predisposition to lymphomas.
- *BRCA-1* (breast, prostate, and ovarian cancer) and *BRCA-2* (breast cancer) required for p53 activity.
- *Rb* The retinoblastoma gene was the first tumor suppressor gene cloned, and is a negative regulator of the cell cycle through its ability to bind the transcription factor E2F and repress transcription of genes required for S phase.

Flow of Genetic Information from DNA to Protein



Second Position

First Position (5' End)	U	C	A	G	Third Position (3' End)
U	UUU Phe, UUC Phe, UUA Leu, UUG Leu	UCU Ser, UCC Ser, UCA Ser, UCG Ser	UAU Tyr, UAC Tyr, UAA Stop, UAG Stop	UGU Cys, UGC Cys, UGA Stop, UGG Trp	U C A G
C	CUU Leu, CUC Leu, CUA Leu, CUG Leu	CCU Pro, CCC Pro, CCA Pro, CCG Pro	CAU His, CAC His, CAA Gln, CAG Gln	CGU Arg, CGC Arg, CGA Arg, CGG Arg	U C A G
A	AUU Ile, AUC Ile, AUA Ile, AUG Met	ACU Thr, ACC Thr, ACA Thr, ACG Thr	AAU Asn, AAC Asn, AAA Lys, AAG Lys	AGU Ser, AGC Ser, AGA Arg, AGG Arg	U C A G
G	GUU Val, GUC Val, GUA Val, GUG Val	GCU Ala, GCC Ala, GCA Ala, GCG Ala	GAU Asp, GAC Asp, GAA Glu, GAG Glu	GGU Gly, GGC Gly, GGA Gly, GGG Gly	U C A G

- correspond to RNA

1 2 3
5'-ACG-3'

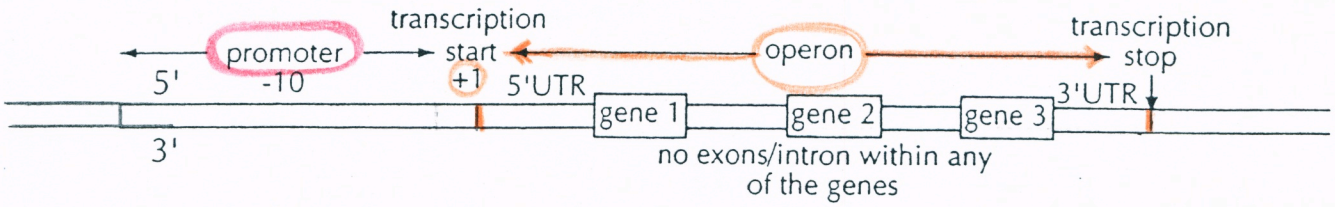
- AUG = start codon for protein synthesis
- UAA, UGA, UAG = stop translation
- 3rd position = wobble position, allows for redundancy "SNPs"

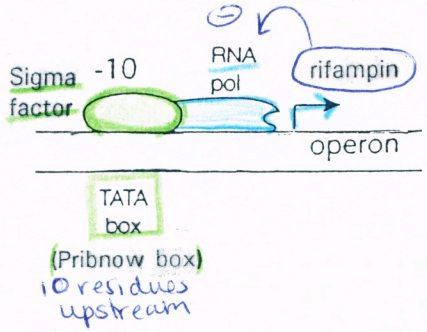
Effect of Some Common Types of Mutations on Protein Structure

POINT

Type of Mutation	Effect on Protein
Silent: new codon specifies same amino acid	None
Missense: new codon specifies different amino acid (HbA vs HbS; glu vs val)	Possible decrease in function; variable effects
Nonsense: new codon is stop codon (TAA, TAG, TGA)	Shorter than normal; usually nonfunctional
Frameshift: deletion or addition of a base	Usually nonfunctional; often shorter than normal (messes up C end)
Large segment deletion (unequal crossover in meiosis)	Loss of function; shorter than normal or entirely missing
Splice donor or acceptor site mutations	Variable effects ranging from addition or deletion of a few amino acids to deletion of an entire exon
Triplet repeat expansion (huntington's & fragile X)	Expansions in coding regions cause protein product to be longer than normal and unstable. Disease often shows anticipation in pedigree.

CYTOPLASMIC EXPRESSION OF A BACTERIAL OPERON

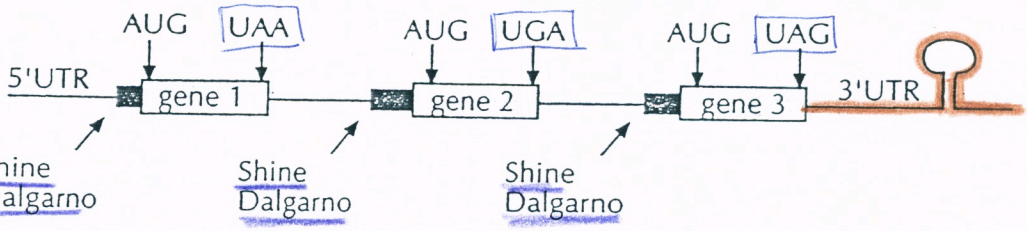




Transcription

stem/loop in mRNA
stops transcription

Polycistronic
mRNA



- Shine dalgarno sequence allows for starting at the right place (bugs)

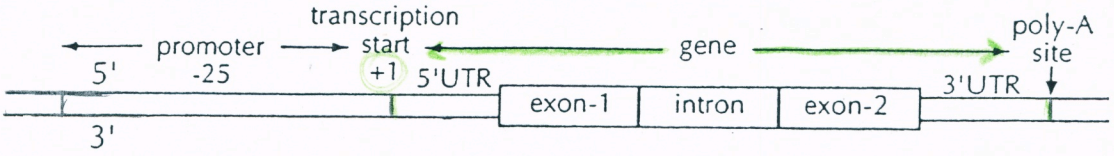
Translation | Translation | Translation

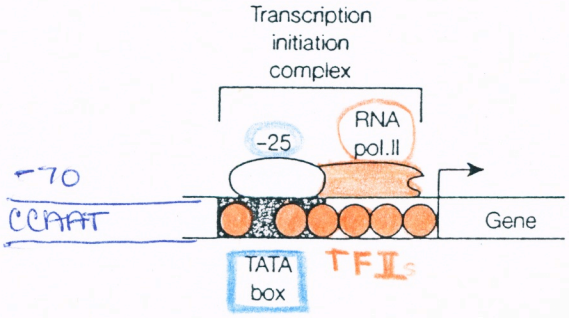
H_2N - protein - COOH (1) | H_2N - protein - COOH (2) | H_2N - protein - COOH (3)

-25 in ppl vs -10 in bug

EXPRESSION OF A NUCLEAR PROTEIN-CODING GENE

class II genes



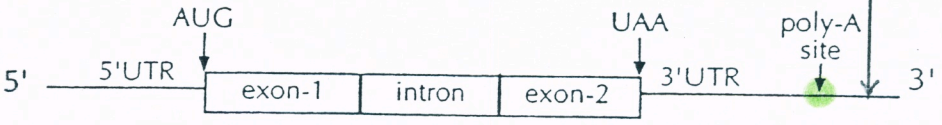


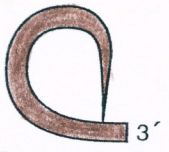
Transcription (nucleus)

- if CCAAT or TATA boxes are damaged, can't start transcription

Nuclease cuts

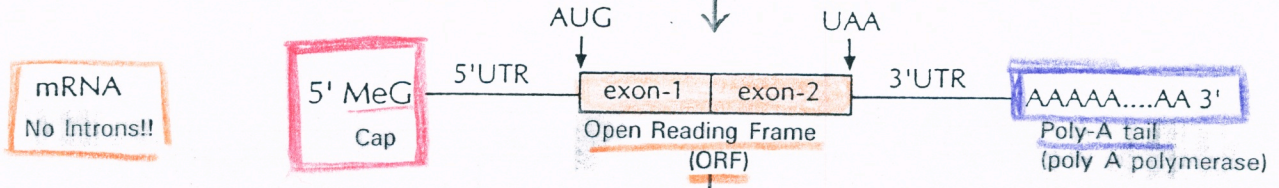
pre-mRNA (hnRNA)





Excised Intron (Lariat) Degraded in Nucleus

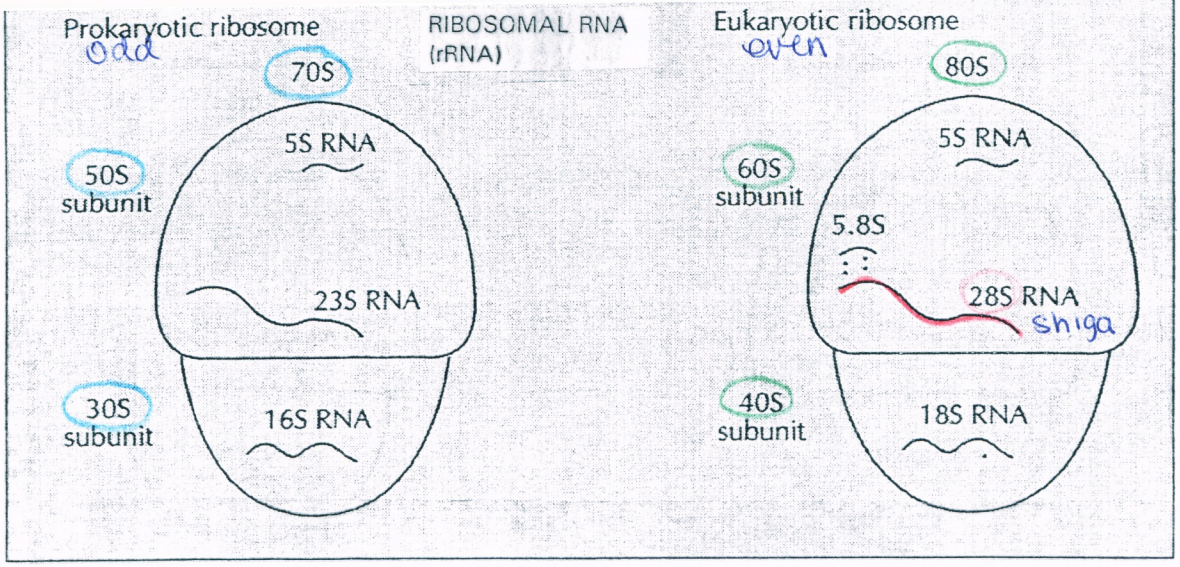
RNA Processing (nucleus)
1. Capping (to initiate translation)
2. Tailing (to prevent RNA degradation)
3. Splicing (to generate an ORF)
   - spliceosome with U-rich snRNA



Translation (cytosol)

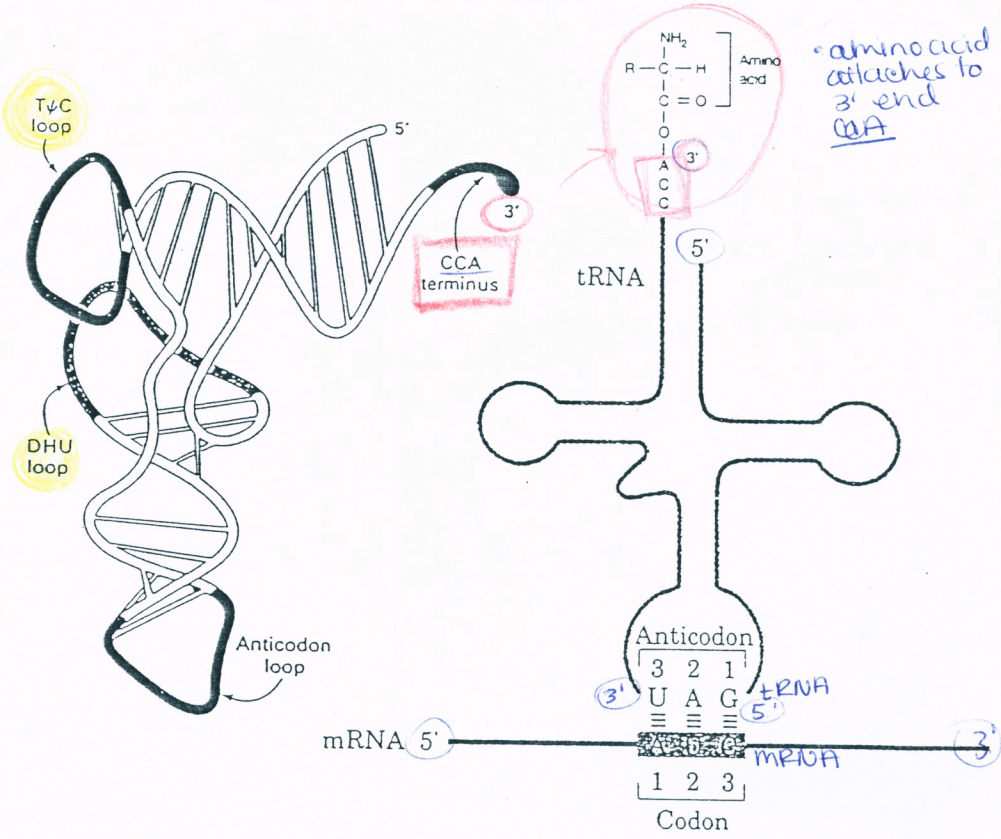
H_2N - protein - COOH

- will generally be one less intron than exons
- ~~TFIIs~~ help RNA pol II identify promoter region
- cap = methyl G



*shiga toxin targets 28S rRNA preventing protein synthesis
- most RNA in any cell is rRNA

TRANSFER RNA (tRNA)



- amino acid attaches to 3' end CCA

- nucleolus makes ribosomes
- tRNA contains unusual bases

Eukaryotic Nuclear RNA Polymerases

Type	α-Amanitin Sensitivity	Subcellular Localization	RNA Product
I	Insensitive	Nucleolus	45S rRNA
II	Very sensitive to low levels	Nucleoplasm	hnRNA (mRNA) and some snRNAs
III	Sensitive to high levels	Nucleoplasm	tRNA, 5S rRNA

hnRNA = heterogeneous nuclear RNA; mRNA = messenger RNA; rRNA = ribosomal RNA; snRNAs = small nuclear RNAs; tRNA = transfer RNA.

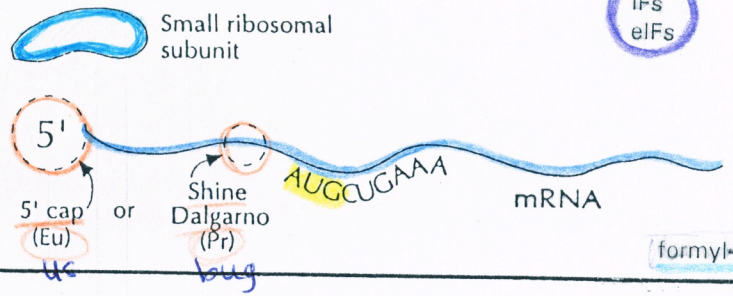
Steps in Translation

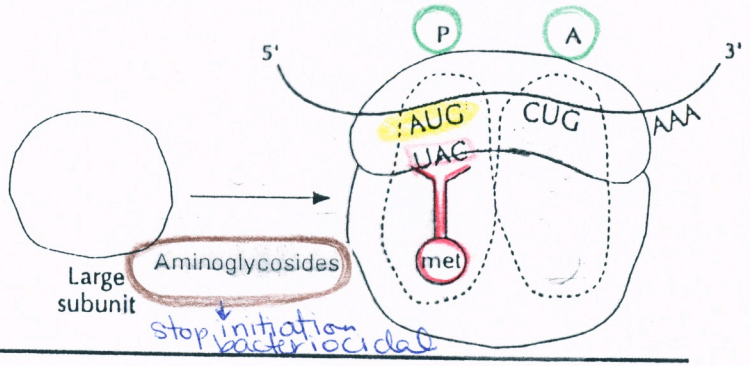
*AUG starts at the P site, A site has next codon

Small ribosomal subunit

IFs
eIFs

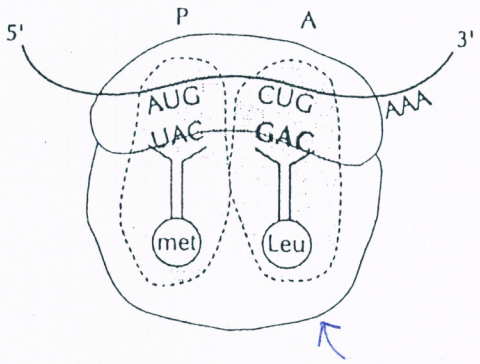
INITIATION





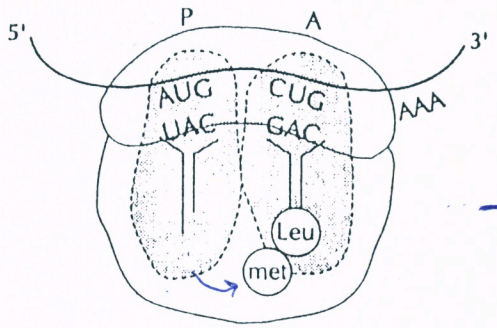
Stop initiation bactericidal

ELONGATION



1. Aminoacyl-tRNA binds to A site

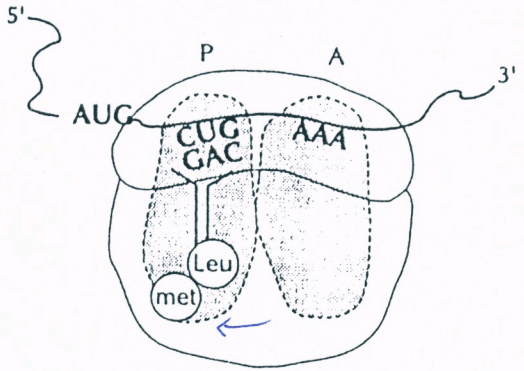
GTP
EFTU and EFTS (Pr)
* eEF-1 (Eu)
Tetracyclines ⊖ 30S
prevents tRNA to A site



2. Peptide bond forms. Peptidyl transferase in large subunit

Chloramphenicol ⊖
inhibits peptidyl transferase (gray baby syndrome)

Elongation cycle repeats for each amino acid added.

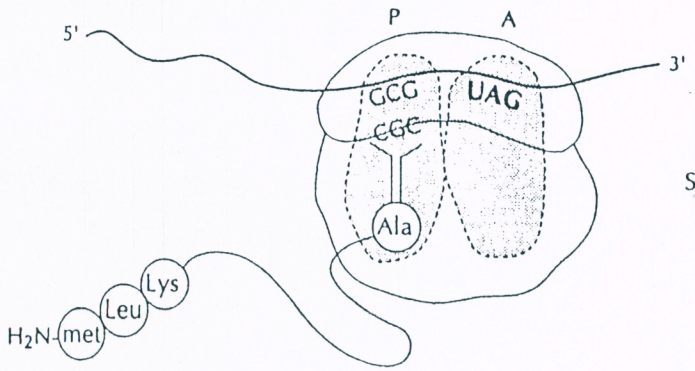


3. Translocation of ribosome 3 nucleotides along mRNA

GTP
EF-G(Pr)
* eEF-2(Eu) ← TOXINS diptheria pseudomonas

Macrolides ⊖ also clinda
Prevent movmt of ribosome

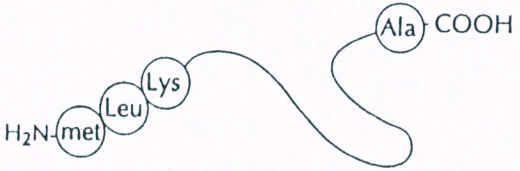
TERMINATION



RFs
eRFs

STOP CODON in A site

Completed protein released from ribosome Ribosomal subunits separate mRNA released



A double-stranded RNA genome isolated from a virus in the stool of a child with gastroenteritis was found to contain 15% uracil. What is the percentage of guanine in this genome?

U = 15 A = 15

G = 35 C = 35

A. 15
B. 25
C. 35
D. 75
E. 85

A medical student working in a molecular biology laboratory is asked by her mentor to determine the base composition of an unlabeled nucleic acid sample left behind by a former research technologist. The results of her analysis show 10% adenine, 40% cytosine, 30% thymine and 20% guanine. What is the most likely source of the nucleic acid in this sample?

A. Bacterial chromosome DS, circ
B. Bacterial plasmid DS, circ
C. Mitochondrial chromosome DS lin.
D. Nuclear chromosome DS lin.
E. Viral genome

%'s don't match so must be ss DNA/RNA, has T so DNA, most likely viral (parvo)

It is now believed that a substantial proportion of the single nucleotide substitutions causing human genetic disease are due to misincorporation of bases during DNA replication. Which proofreading activity is critical in determining the accuracy of nuclear DNA replication and thus the base substitution mutation rate in human chromosomes?

A. 3′ to 5′ polymerase activity of DNA polymerase δ
B. 3′ to 5′ exonuclease activity of DNA polymerase γ
C. Primase activity of DNA polymerase α
D. 5′ to 3′ polymerase activity of DNA polymerase III
E. 3′ to 5′ exonuclease activity of DNA polymerase δ

nuclear DNA replication which needs α + δ

γ is mitochondrial

The proliferation of cytotoxic T-cells is markedly impaired upon infection with a newly discovered human immunodeficiency virus, designated HIV-V. The defect has been traced to the expression of a viral-encoded enzyme that inactivates a host-cell nuclear protein required for DNA replication. Which protein is a potential substrate for the viral enzyme?

SBP holds strands apart following helicase opening

A. TATA-box binding protein (TBP)
B. Cap binding protein (CBP)
C. Catabolite activator protein (CAP)
D. Acyl-carrier protein (ACP)
E. Single-strand binding protein (SBP)

The deficiency of an excision endonuclease may produce an exquisite sensitivity to ultraviolet radiation in Xeroderma pigmentosum. Which of the following functions would be absent in a patient deficient in this endonuclease?

*thymidine dimers

A. Removal of introns
B. Removal of pyrimidine dimers
C. Protection against DNA viruses
D. Repair of mismatched bases during DNA replication
E. Repair of mismatched bases during transcription

The anti-*Pseudomonas* action of norfloxacin is related to its ability to inhibit chromosome duplication in rapidly dividing cells. Which of the following enzymes participates in bacterial DNA replication and is directly inhibited by this antibiotic?

•fluoroquinolones → DNA gyrase

A. DNA polymerase I
B. DNA polymerase II
C. Topoisomerase I
D. Topoisomerase II
E. DNA ligase

Cytosine arabinoside (araC) is used as an effective chemotherapeutic agent for cancer, although resistance to this drug may eventually develop. In certain cases, resistance is related to an increase in the enzyme cytidine deaminase in the tumor cells. This enzyme would inactivate araC to form

C → U w/ deaminase

A. cytosine
B. cytidylic acid
C. thymidine arabinoside
D. uracil arabinoside
E. cytidine

Dyskeratosis congenital (DKC) is a genetically inherited disease in which the proliferative capacity of stem cells is markedly impaired. The defect has been traced to inadequate production of an enzyme needed for chromosome duplication in the nuclei of rapidly dividing cells. Structural analysis has shown that the active site of this protein contains a single-stranded RNA that is required for normal catalytic function. Which step in DNA replication is most likely deficient in DKC patients?

SSRNA indicates reverse transcriptase

RT can be telomerase

A. Synthesis of centromeres
B. Synthesis of Okasaki fragments
C. Synthesis of RNA primers
D. Synthesis of telomeres
E. Removal of RNA primers

During RNA synthesis, the DNA template sequence TAGC would be transcribed to produce which of the following sequences?

5'-TAGC-3'
3'-ATCG-5'

5'-GCUA-3'

A. ATCG
B. GCTA
C. CGTA
D. AUCG
E. GCUA

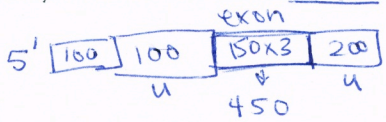
The base sequence of codons 57-58 in the cytochrome β5 reductase gene is CAGCGC. The mRNA produced upon transcription of this gene will contain the sequence:

A. GCGCTG
B. CUGCGC
C. GCGCUG
D. CAGCGC
E. GUCGCG

identical but w/ a U

CAGCGC → don't have to change bc no T

A gene encodes a protein with 150 amino acids. There is one intron of 1,000 bps, a 5′-untranslated region of 100 bp, and a 3′-untranslated region of 200 bp. In the final processed mRNA, how many bases lie between the start AUG codon and the final termination codon?



A. 1,750
B. 750
C. 650
D. 450
E. 150

Stop codon considered part of region for other questions but doesn't create an amino acid, so if asked 150 codons how many aa's, it's 1 less

In the genetic code of human nuclear DNA, one of the codons specifying the amino acid tyrosine is UAC. Another codon specifying this same amino acid is

wobble position = 3rd position

A. AAC
B. UAG → stop codon so NO
C. UCC
D. AUG
E. UAU

Items 2 and 3

A. ATGCAA...→ ATGTAA
B. ATGAAA...→ GTGAAA
C. TATAAG...→ TCTAAG
D. CTTAAG...→ GTTAAG
E. ATGAAT ...→ ATGCAT

The options above represent mutations in the DNA with base changes indicated in boldface type. For each mutation described in the questions below, choose the most closely related sequence change in the options above.

Nonsense mutation A (TAA is stop codon)

Mutation decreasing the initiation of transcription C

mutated TATA box

Accumulation of heme in reticulocytes can regulate globin synthesis by indirectly inactivating eIF-2. Which of the following steps is most directly affected by this mechanism?

A. Attachment of spliceosomes to pre-mRNA
B. Attachment of the ribosome to the endoplasmic reticulum
C. Met-tRNAmet binding to the P-site
D. Translocation of mRNA on the ribosome
E. Attachment of RNA polymerase II to the promoter

A nasopharyngeal swab obtained from a 4-month-old infant with rhinitis and paroxysmal coughing tested positive upon culture for *Bordetella pertussis*. He was admitted to the hospital for therapy with an antibiotic that inhibits the translocation of peptidyl-tRNA on 70S ribosomes. This patient was most likely treated with

A. erythromycin
B. tetracycline
C. chloramphenicol
D. rifamycin
E. levofloxacin