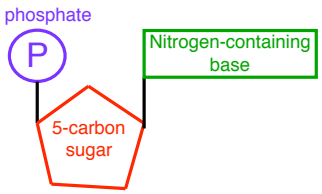
Chapter 7

Microbial Genetics

Chromosomes and genes

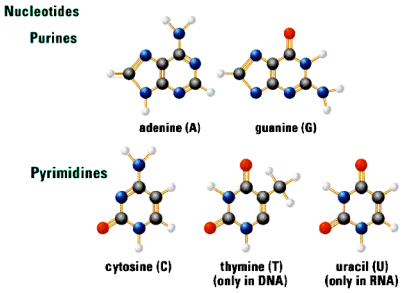
- Chromosome
  - Prokaryotes
    - Circular
      - nucleoid
  - Eukaryotes
    - Linear
      - Nucleus
- Gene - basic unit of heredity
  - Locus - location of a gene on the chromosome
  - Alleles - different versions of a gene at a locus

Basic structure of a nucleotide

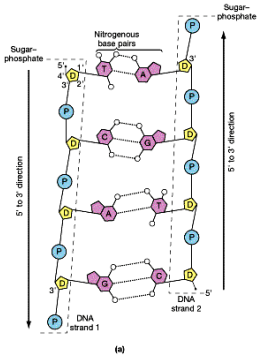


E. coli - 5 million bases

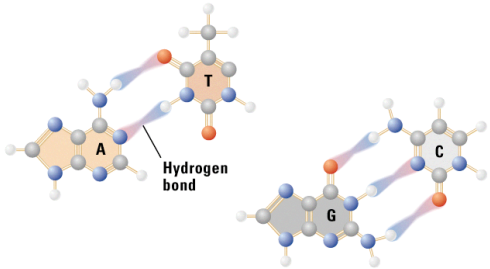
Bases of nucleotides



Structure of DNA



Bases connect or pair in the middle



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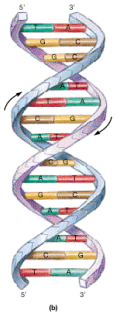
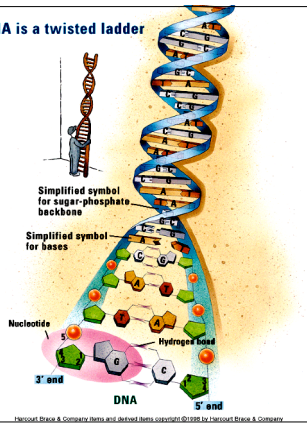
Double helix of DNA



DNA is a twisted ladder

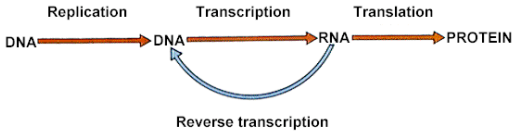


Comparison of nucleic acids

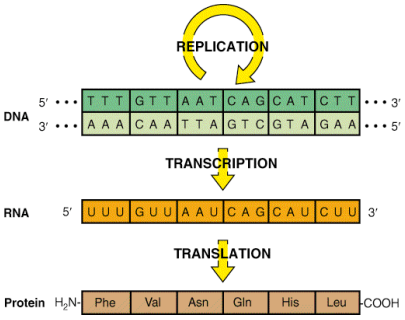
DNA
- Double helix (ds)
- Deoxyribose
- Adenine, guanine, cytosine, thymine

RNA
- Single helix (ss)
- Ribose
- Adenine, guanine, cytosine, uracil

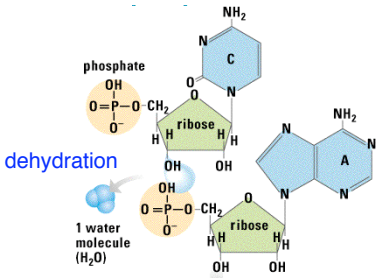
Informational transfer

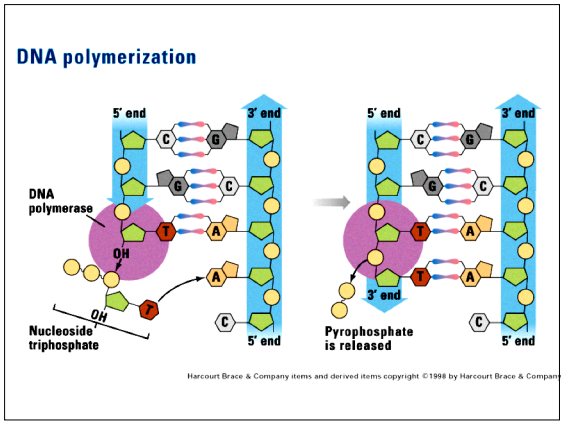


Transfer of information



Nucleotides to Nucleic Acids




DNA polymerization
5′ end
3′ end
DNA polymerase
OH
Nucleoside triphosphate
Pyrophosphate is released
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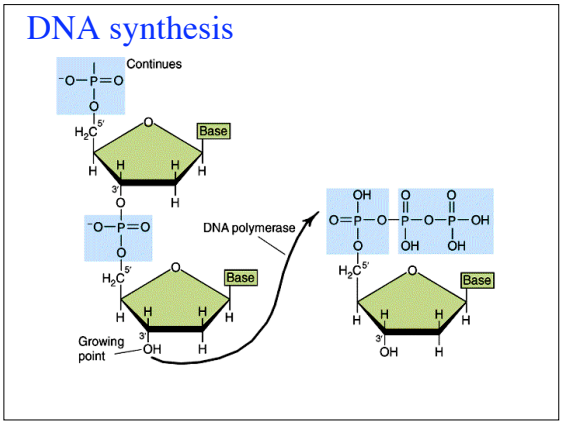
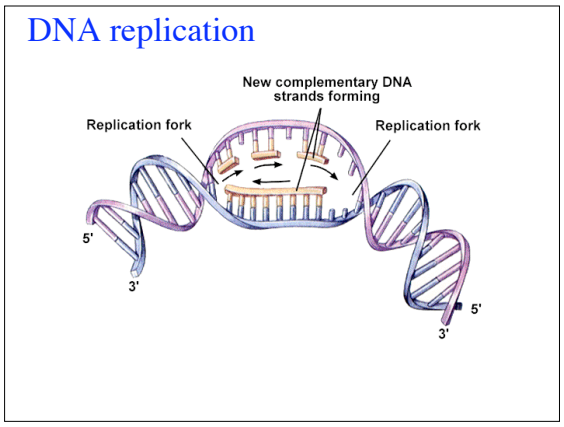
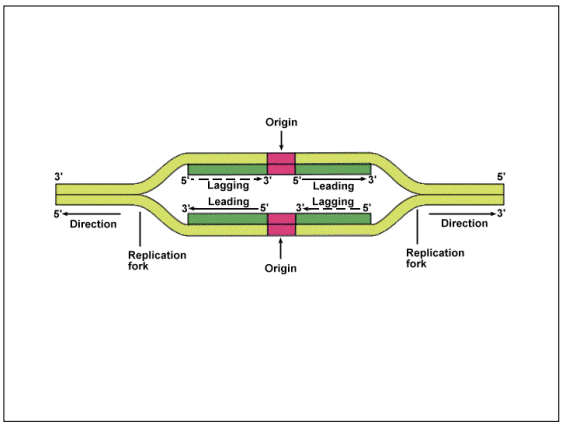
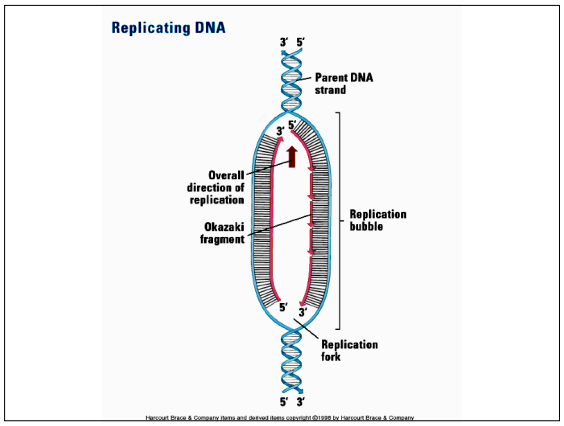

DNA synthesis
Continues
Base
DNA polymerase
Growing point


DNA replication
New complementary DNA strands forming
Replication fork
Replication fork


Origin
Lagging
Leading
Leading
Lagging
Direction
Direction
Replication fork
Replication fork
Origin


Replicating DNA
Parent DNA strand
Overall direction of replication
Okazaki fragment
Replication bubble
Replication fork
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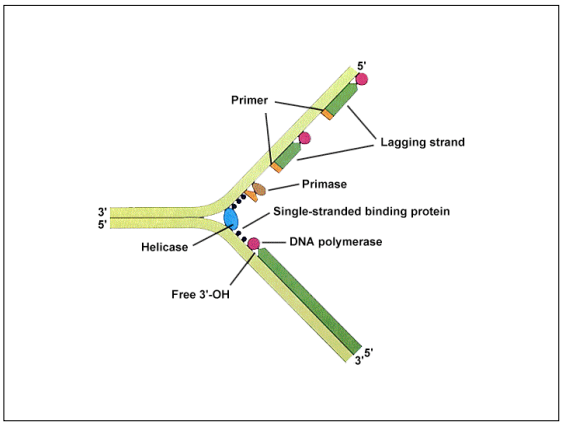
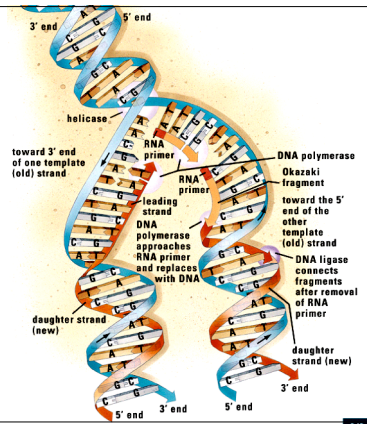
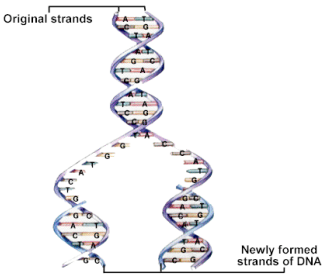

Primer
Lagging strand
Primase
Single-stranded binding protein
Helicase
DNA polymerase
Free 3′-OH

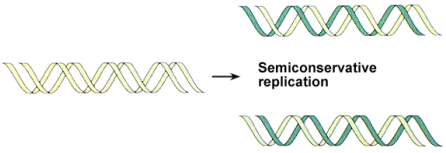
ori site
replication forks
leading strand
lagging strand
helicase
topoisomerase
primase
ss binding protein
RNA primers
Okazaki fragments
DNA polymerase
DNA ligase
3' to 5' directions



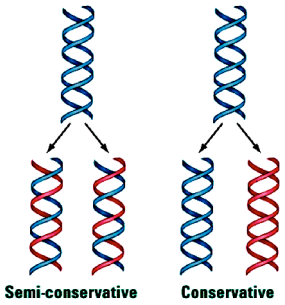
Semi-conservative replication



Semi-conservative replication



Semi-conservative replication



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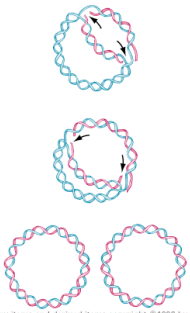
Replication of bacterial DNA



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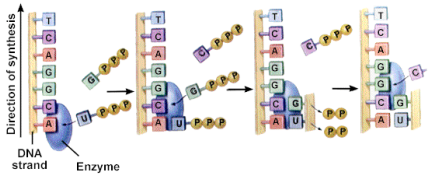
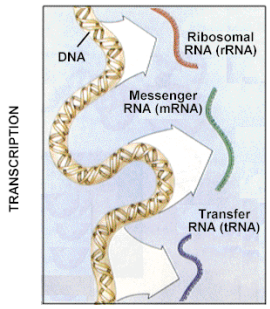
Transcription

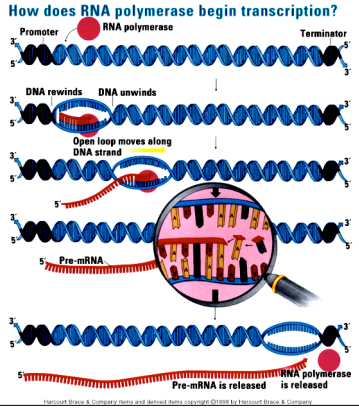


Forms of RNA

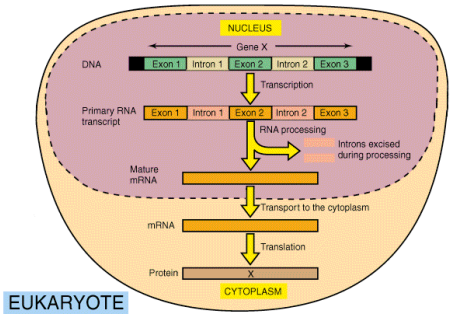
- Ribosomal RNA - rRNA
  - Part of the ribosome subunits
- Messenger RNA - mRNA
  - A template for protein synthesis (polypeptides)
- Transfer RNA - tRNA
  - Transfers amino acids from the cytoplasm to the ribosome

Forms of RNA

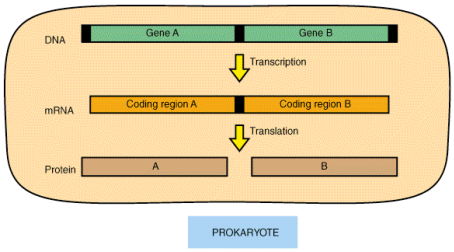




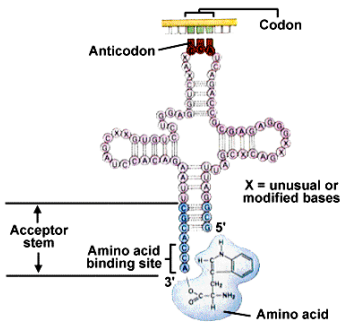
Eukaryote mRNA processing



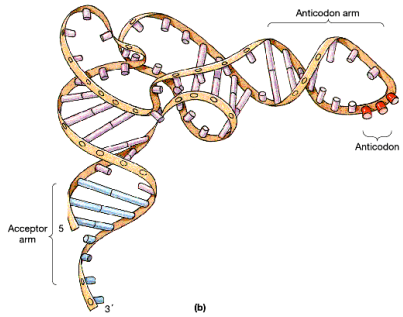
Prokaryote mRNA



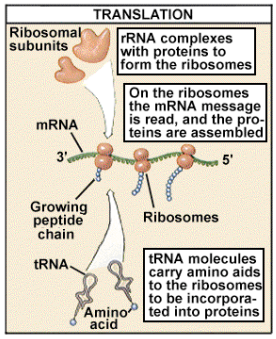
tRNA

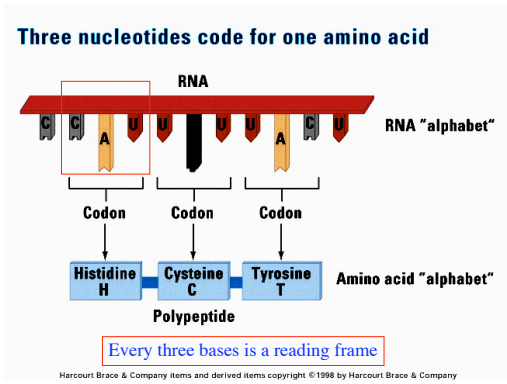


tRNA

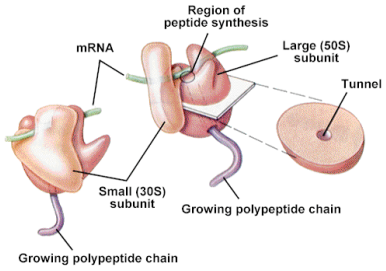


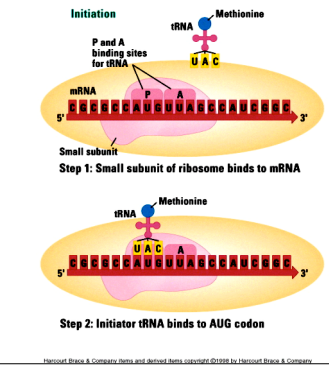
Translation

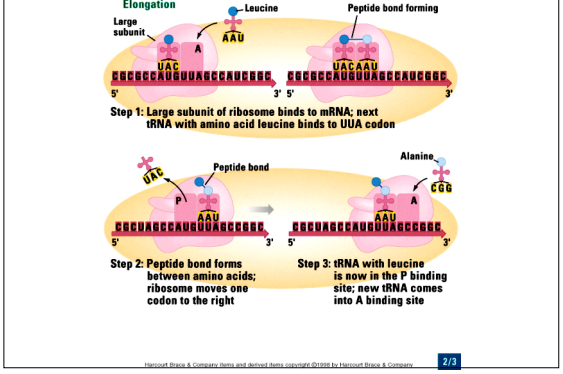


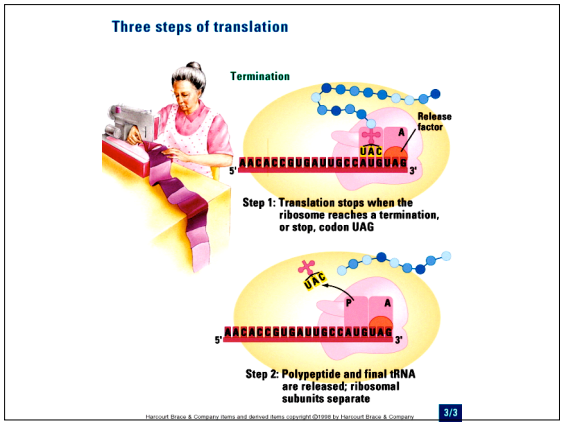


Ribosomes








Three steps of translation
Termination
Release factor
A
UAC
5'
AACACCGUGAUUGCCAUGUAG
3'
Step 1: Translation stops when the ribosome reaches a termination, or stop, codon UAG
UAC
P
A
5'
AACACCGUGAUUGCCAUGUAG
3'
Step 2: Polypeptide and final tRNA are released; ribosomal subunits separate
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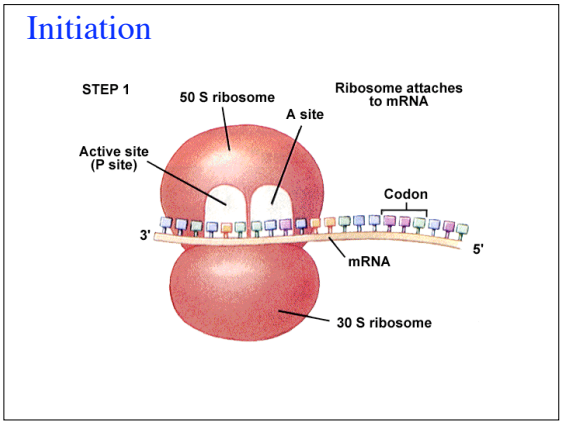
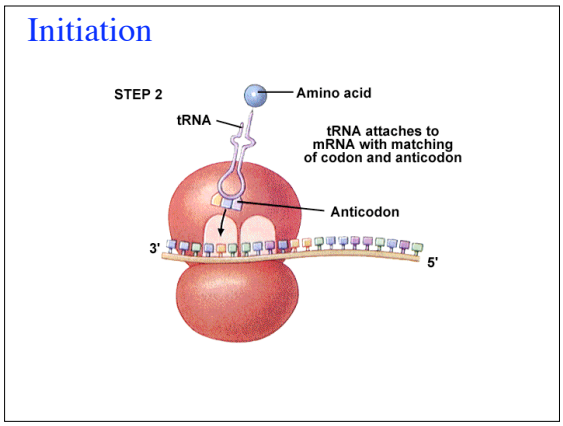
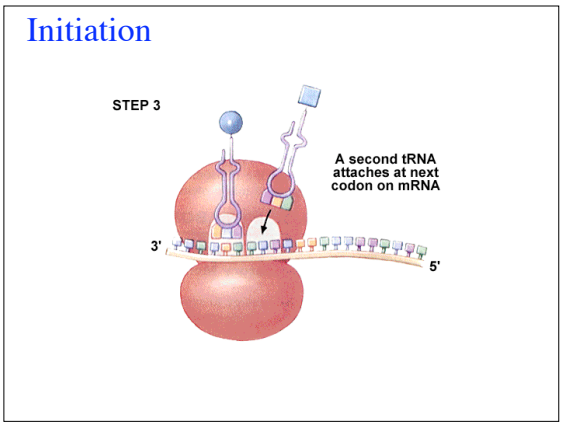
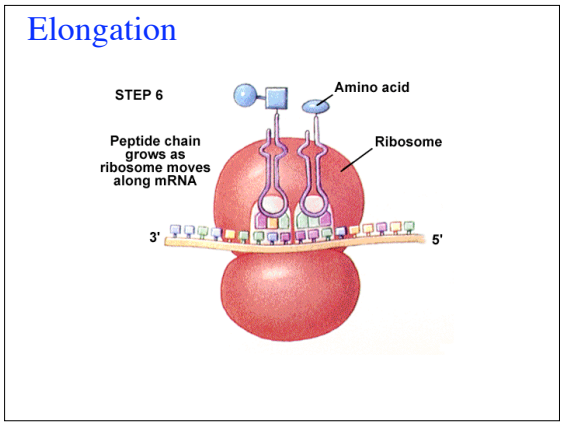
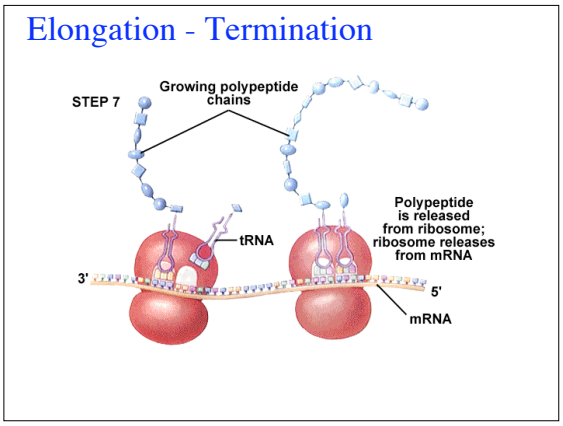
3/3


Initiation
STEP 1
50 S ribosome
Ribosome attaches to mRNA
A site
Active site (P site)
Codon
3'
5'
mRNA
30 S ribosome

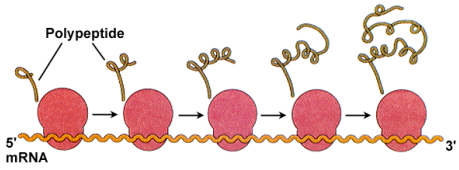

Initiation
STEP 2
Amino acid
tRNA
tRNA attaches to mRNA with matching of codon and anticodon
Anticodon
3'
5'


Initiation
STEP 3
A second tRNA attaches at next codon on mRNA
3'
5'

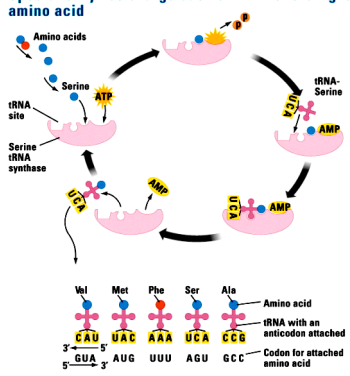

Elongation
STEP 6
Amino acid
Peptide chain grows as ribosome moves along mRNA
Ribosome
3'
5'


Elongation - Termination
STEP 7
Growing polypeptide chains
Polypeptide is released from ribosome; ribosome releases from mRNA
tRNA
3'
5'
mRNA

Polyribosome or polysome



Special enzymes charge each tRNA with the right amino acid



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First position	Second position U	Second position C	Second position A	Second position G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	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

Central dogma

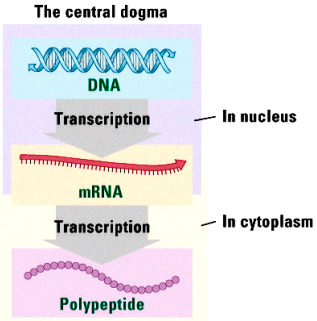

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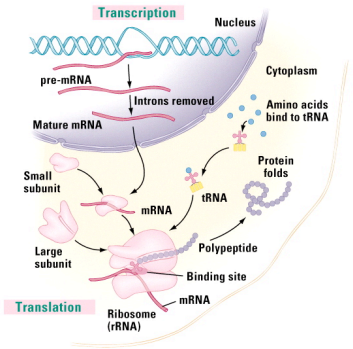
Table 7.4 Major Differences Between Prokaryotic and Eukaryotic Transcription and Translation

Prokaryotes	Eukaryotes
mRNA is not processed.	A cap is added to the 5′ end of mRNA, and a poly A tail is added to the 3′ end.
mRNA does not contain introns.	mRNA contains introns, which are removed by splicing.
Translation of mRNA begins as it is being transcribed.	The mRNA transcript is transported out of the nucleus so that it can be translated in the cytoplasm.
mRNA is often polycistronic; translation usually begins at the first AUG that follows a ribosome binding site.	mRNA is monocistronic; translation begins at the first AUG.

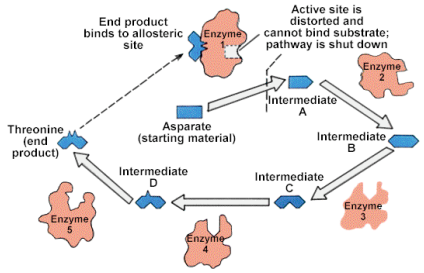
Transcription and translation in a eukaryotic cell



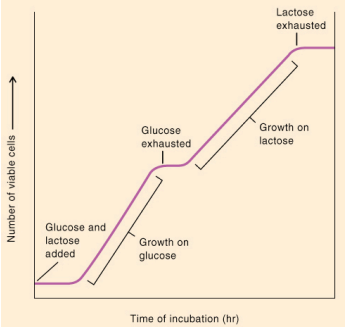
Regulation

- Feedback inhibition
  - – End product inhibition
    - Product inhibits the first enzyme in the pathway
      - – Threonine
- Enzyme induction
  - Constitutive enzymes - glucose metabolism
  - – Inductive enzymes
    - Lactose
    - Lac operon
- Enzyme repression
  - Anabolism
    - – Tryptophan - attenuation

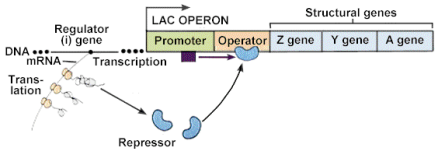
Feedback inhibition



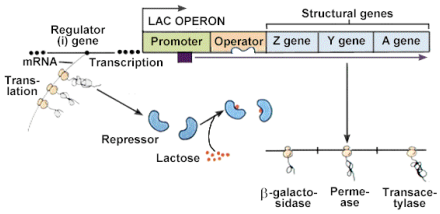
Diauxic growth curve



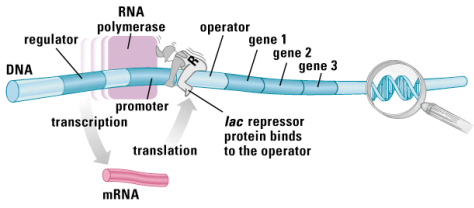
Lac operon (repression)



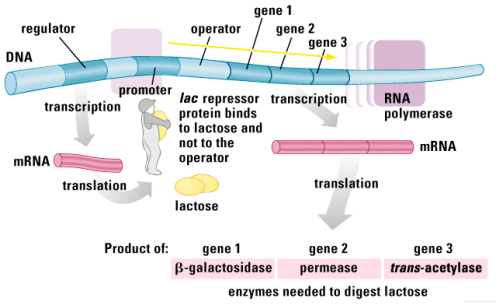
Lac operon (induction)



Lac operon



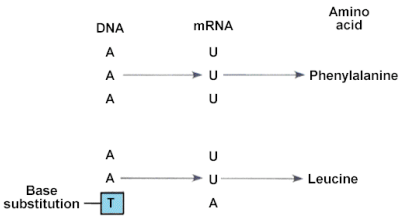
Lac operon



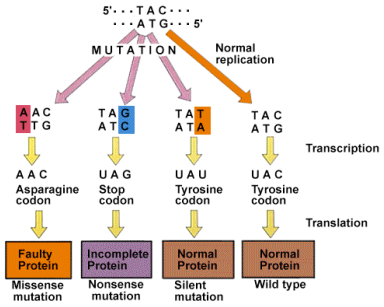
Mutations

- Point mutations
  - A single base substitution
    - Changes the amino acid
- Frameshift mutations
  - deletion or insertion of a base
    - Change the reading frame

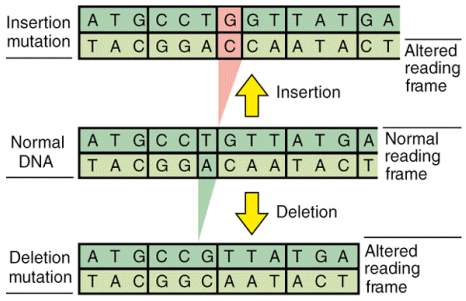
Point mutations



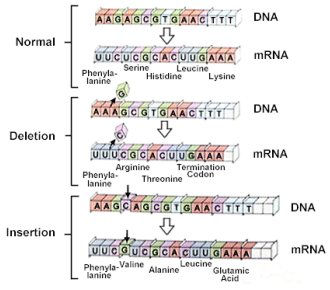
Point mutations



Frameshift mutations



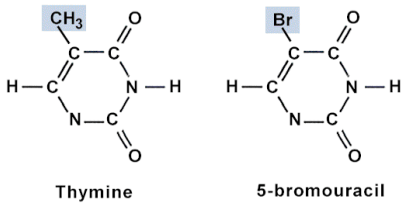
Frameshift mutations



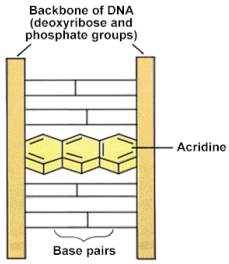
Mutagens

- Base analog
  - 5 bromouracil
    - Replaces thymine - guanine during replication
  - Caffeine
- Alkylating agents
  - Nitrosamines
  - Acridine
    - quinacrine (Atabrine)
- Radiation
  - X-rays
  - UV rays - dimer

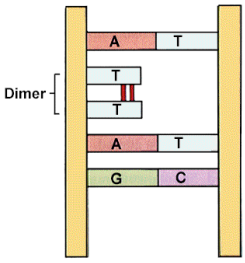
Base analog



Acridine - framshift mutagen



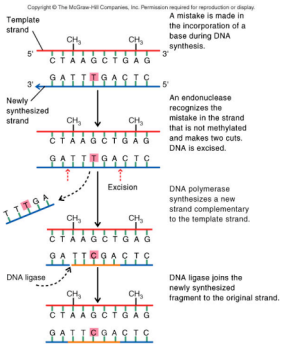
UV - Thymine dimers



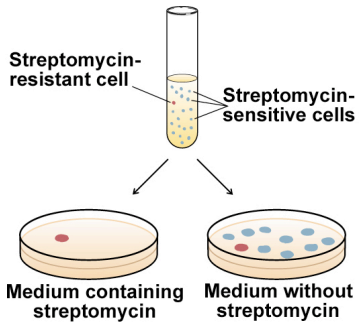
Repair of Thymine dimers

- Light repair
  - Enzyme activated by light removes dimers
- Dark repair
  - Segment of DNA is cut out and replaced

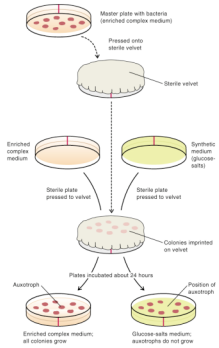
Thymine dimer repair



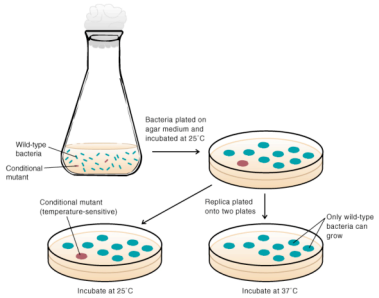
Mutant selection (direct)



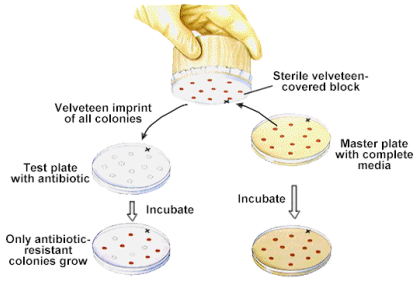
Mutant selection (indirect)



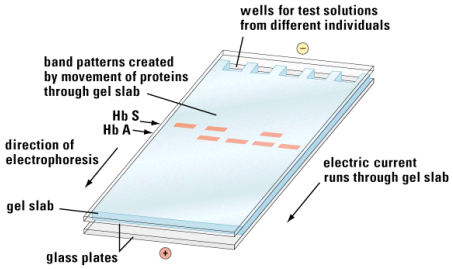
Conditional mutant



Replica plating-testing for resistance



Electrophoresis



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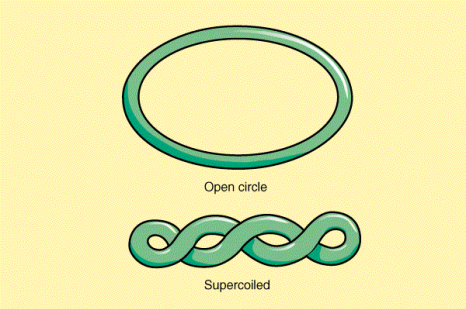
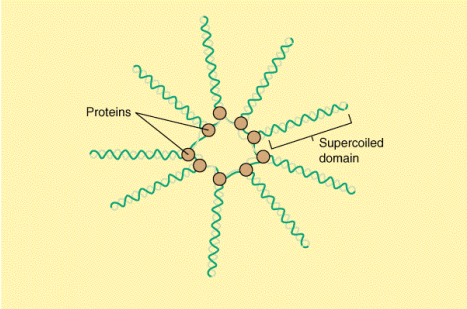
Ames test

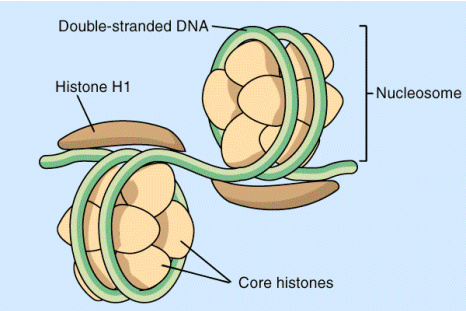
- Bruce Ames
  - Salmonella auxotroph
    - Histidine negative
  - Mutagenic substances cause the Salmonella auxotroph to mutate and grow on His negative medium.

Supercoiled DNA

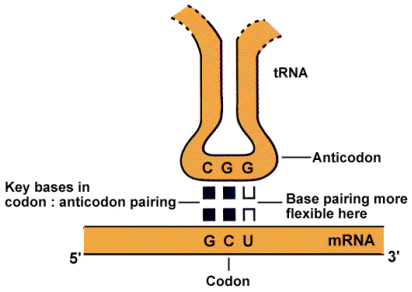


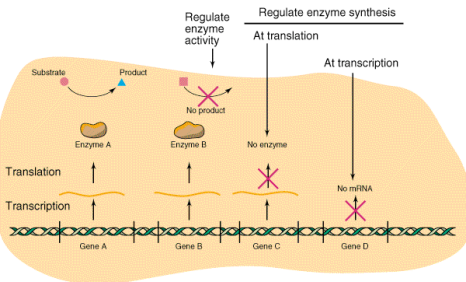


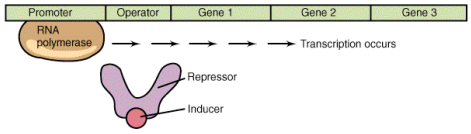
Eukaryotic histones



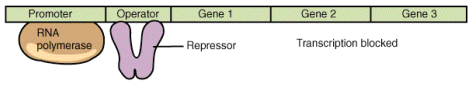
Wobble effect



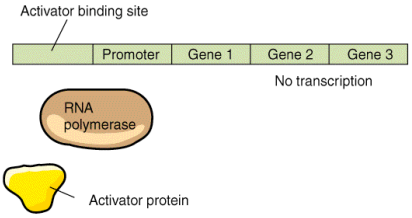




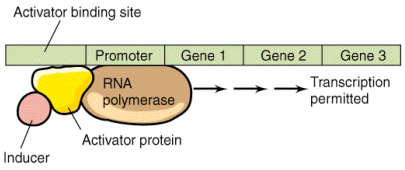
Negative regulation



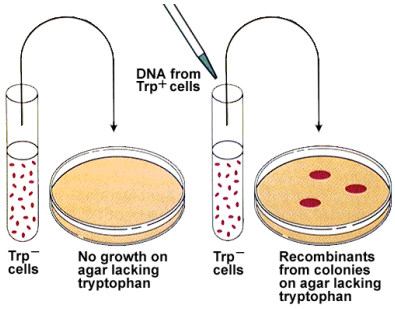
Positive regulation



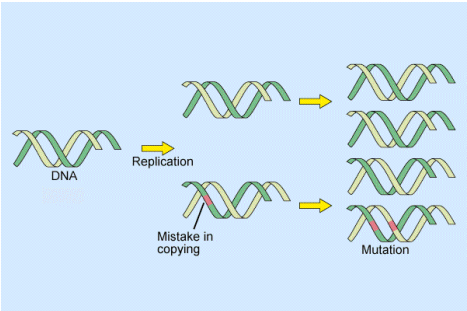
Positive regulation

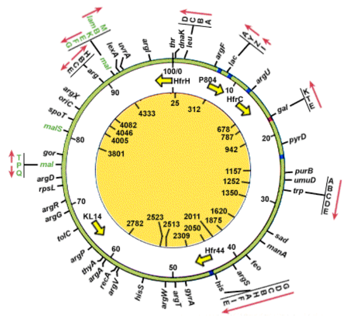


Auxotrophs



Mutations are amplified

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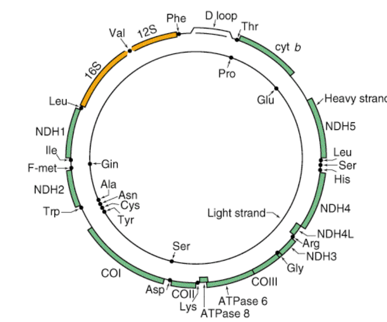
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ATPase 6
ATPase 8
Lys
COII
Asp
COI
Ser
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Cys
Tyr