Chapter 11

Genes: Structure, Replication, and Mutation

Terms and concepts

- ✓ clone
  - ✒population of cells that are genetically identical
- ✓ genome
  - ✒all genes present in a cell or virus
    - ✑haploid – one set of genes
    - ✑diploid – two sets of genes
- ✓ genotype
  - ✒specific set of genes an organism possesses
- ✓ phenotype
  - ✒set of observable characteristics

DNA as Genetic Material

- ✓ established by several critical experiments
  - ✒Fred Griffith (1928)
  - ✒Oswald T. Avery, C. M. MacLeod, and M. J. McCarty (1944)
    - ✑Transforming principle
  - ✒Alfred D. Hershey and Martha Chase (1952)
    - ✑Genetic material in viruses

Smooth verses rough colonies of *Streptococcus (Diplococcus) pneumoniae*

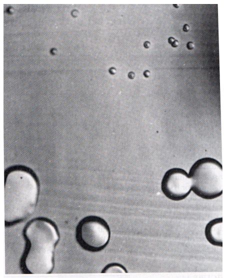
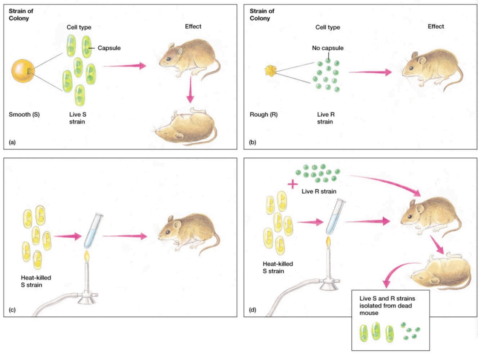


FIGURE 14.5
Transformation of *Streptococcus*. This photo, from the original publication of Avery and coworkers, shows the transformation of nonpathogenic R *Streptococcus* (the small colonies) to pathogenic S *Streptococcus* (the large colonies) in an extract prepared from heat-killed S *Streptococcus*.

Griffith's experiment



Transforming principle

R cells + purified S cell polysaccharide → R colonies

R cells + purified S cell protein → R colonies

R cells + purified S cell RNA → R colonies

R cells + purified S cell DNA → S colonies

S cell extract + protease + R cells → S colonies

S cell extract + RNase + R cells → S colonies

Avery, Macleod, and McCarty’s experiments

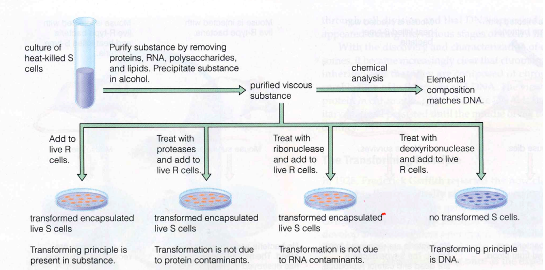
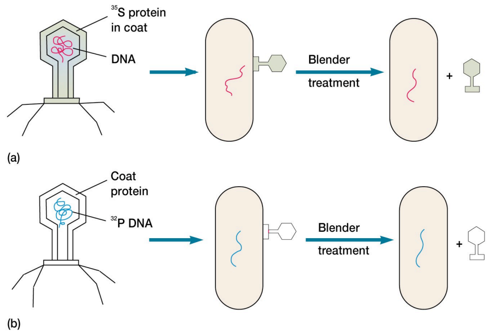


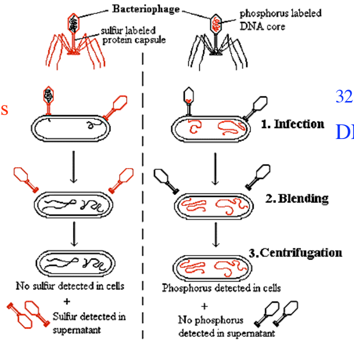
Figure 2.3 Avery, MacLeod, and McCarty’s experiments demonstrating that Griffith’s transforming principle is DNA.

Hersey and Chase



1952

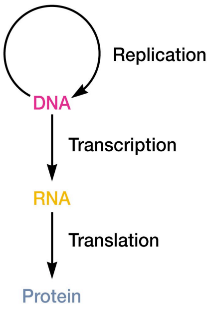
^{35}S labels proteins



^{32}P labels DNA

The Hershey-Chase Experiment

The Central Dogma



Replication

DNA

Transcription

RNA

Translation

Protein

Figure 11.4

Nucleic Acid Structure

Purine and pyrimidine bases

Ribose or deoxyribose

Nucleoside or deoxynucleoside

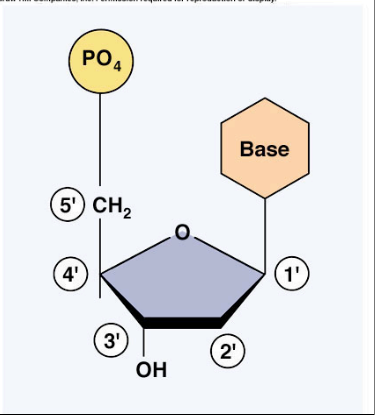
Phosphoric acid

Nucleotide or deoxynucleotide

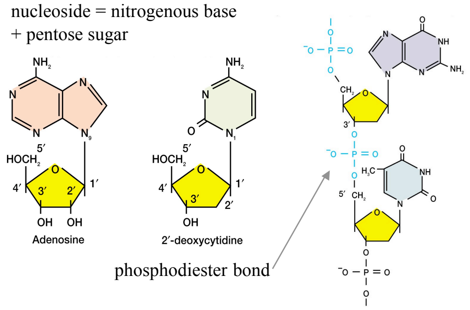
Nucleic acid (RNA, DNA)

Fig. 14.7

Numbering Carbon Atoms in a Nucleotide



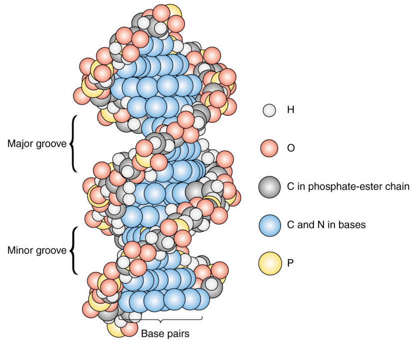
nucleoside = nitrogenous base
+ pentose sugar



phosphodiester bond

DNA Structure

- ✓ nitrogenous bases
  - A, T, G, C
- ✓ pentose sugar
  - deoxyribose
- ✓ usually a double helix, composed of two complementary strands
  - base pairing rules
    - A with T
    - G with C



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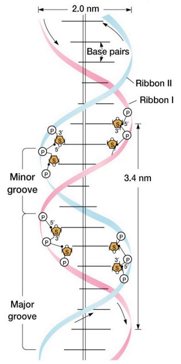
two polynucleotide chains are anti-parallel



RNA Structure

- ✓ nitrogenous bases
  - A, G, C, U (instead of T)
- ✓ pentose sugar
  - ribose
- ✓ usually consists of single strand
  - can coil back on itself
    - forms hair-shaped structures with complementary base pairing and helical organization
    - base pairing rules
      - A with U
      - G with C

Types of RNA

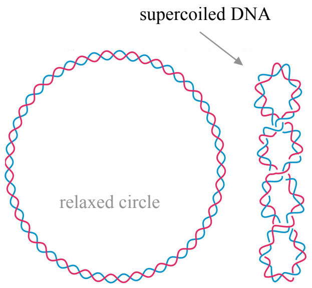
- ✓ three types
  - ribosomal RNA (rRNA)
  - transfer RNA (tRNA)
  - messenger RNA (mRNA)
- ✓ differ from each other in function, site of synthesis in eucaryotic cells, and structure

The Organization of DNA in Cells

- ✓organization differs in two cell types

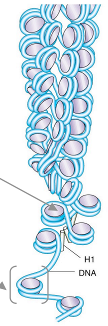
Procaryotic DNA

- ✓ usually exists as closed circular, supercoiled molecule associated with basic proteins



Eucaryotic DNA

- ✓ linear molecules
- ✓ associated with histones
- ✓ coiled into repeating units called nucleosomes
- ✓ 1.75 coils per histone

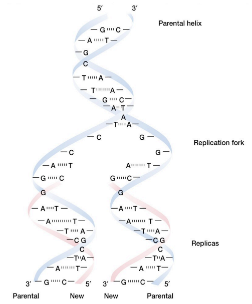


DNA Replication

- ✓ complex process involving numerous enzymes and proteins
- ✓ in general, process is similar in all organisms

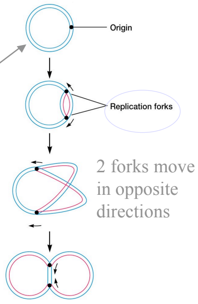
Pattern of DNA Synthesis

- ✓ semiconservative
  - ✑each parental strand is conserved
  - ✑two parental strands separate and serve as templates for synthesis of new strands



Pattern of DNA synthesis…

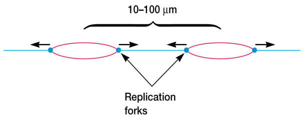
- ✓ in procaryotes
  - ✑bidirectional from a single origin of replication
  - ✑replicon
    - ➯portion of the genome that contains an origin and is replicated as a unit



2 forks move in opposite directions

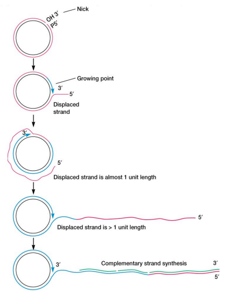
Pattern of DNA synthesis…

- ✓ in eucaryotes
  - ✒bidirectional
  - ✒multiple origins of replication



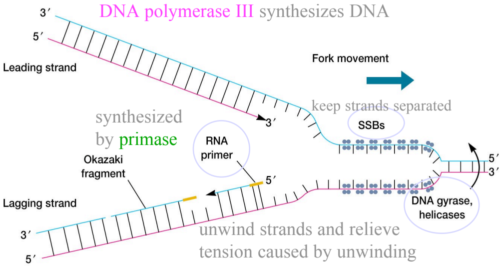
Pattern of DNA synthesis…

- ✓ some small circular genomes (e.g., viruses and plasmids)
  - ✒replicated by **rolling-circle mechanism**



Mechanism of DNA Replication

in bacteria



DNA polymerase III synthesizes DNA

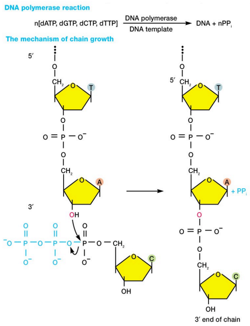
keep strands separated

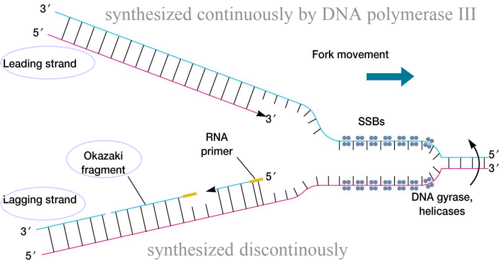
synthesized by primase

unwind strands and relieve tension caused by unwinding

DNA polymerase III

- ✓ uses each strand as template and synthesizes complementary strands



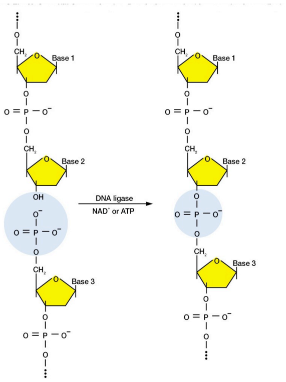


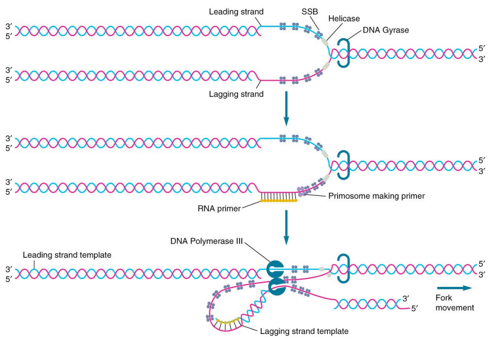
synthesized continuously by DNA polymerase III

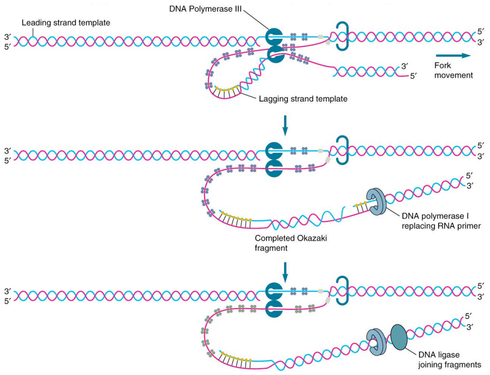
synthesized discontinuously

DNA polymerase I removes primers and fills gaps

DNA ligase joins fragments to form complete strands of DNA







Some amazing facts

- ≥ 30 proteins required to replicate *E. coli* chromosome
- occurs with great fidelity
  - ✑error frequency = 10^{-9} or 10^{-10} per base pair replicated
  - ✑due to proofreading activity of DNA polymerases III and I
- ✓occurs very rapidly
  - ✑750 to 1,000 base pairs/second in procaryotes
  - ✑50-100 base pairs/second in eucaryotes

The Genetic Code

- ✓the manner in which genetic instructions for polypeptide synthesis are stored within genome
- ✓colinearity
  - ✑sequence of base pairs in DNA corresponds to the amino acid sequence of polypeptide encoded

Establishment of Genetic Code

- ✓codon
  - ✑genetic code word
  - ✑specifies an amino acid
- ✓codon meanings deciphered by Marshall Nirenberg, et al. in 1960s

Organization of the Code

- ✓code degeneracy
  - ✑up to six different codons can code for a single amino acid
- ✓sense codons
  - ✑the 61 codons that specify amino acids
- ✓stop (nonsense) codons
  - ✑the three codons used as translation termination signals
  - ✑do not encode amino acids

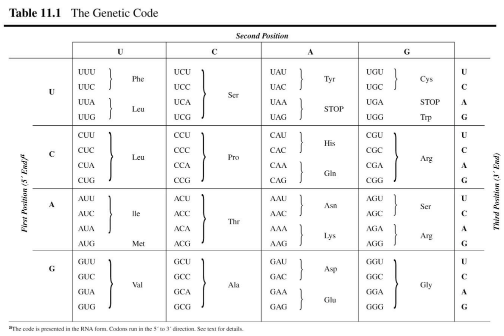


Table 11.1 The Genetic Code

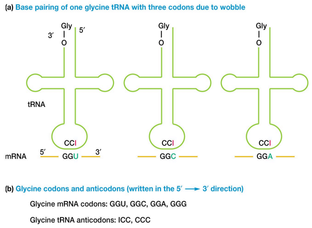
First Position (5′ End)ᵃ	Second Position U		Second Position C		Second Position A		Second Position G		Third Position (3′ End)
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	STOP	UGA	STOP	A
	UUG		UCG		UAG		UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC	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

ᵃThe code is presented in the RNA form. Codons run in the 5′ to 3′ direction. See text for details.

Wobble

✓ loose base pairing
  - ✑3rd position of codon less important than 1st or 2nd

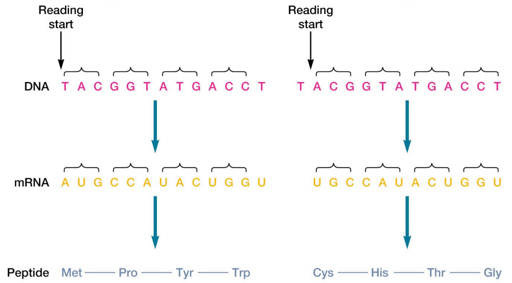
✓ eliminates need for unique tRNA for each codon



Gene Structure

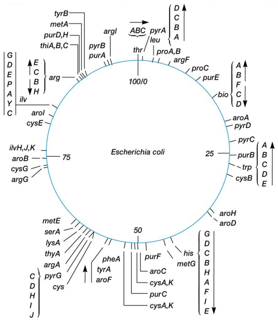
✓ gene
  - ✑linear sequence of nucleotides with a fixed start point and end point
  - ✑encodes a polypeptide, a tRNA, or an rRNA
    - ☞cistron – gene that encodes a polypeptide

✓ reading frame
  - ✑organization of codons such that they can be read to give rise to a gene product

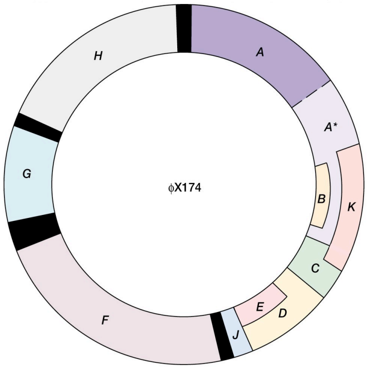


Organization of genes on chromosomes

✓ for most organisms, reading frames do not overlap



• some bacteria and some viruses have overlapping genes

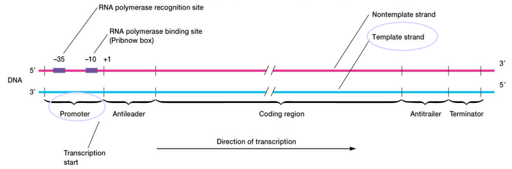


Procaryotic versus eucaryotic genes

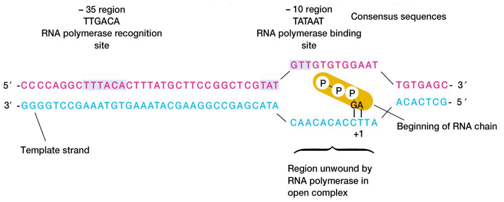
- ✓ procaryotes (and viruses)
  - ✒coding information is usually continuous
- ✓ eucaryotes
  - ✒most genes have coding sequences interrupted by noncoding sequences
    - ☞exons – coding sequences
    - ☞introns – noncoding sequences

Genes That Code for Proteins

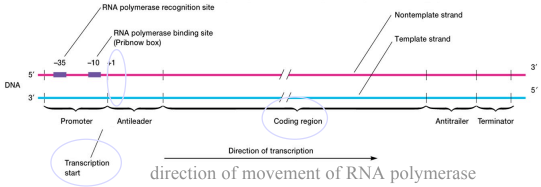
strand that contains coding information
and directs RNA synthesis



serves as recognition
and binding site for
RNA polymerase

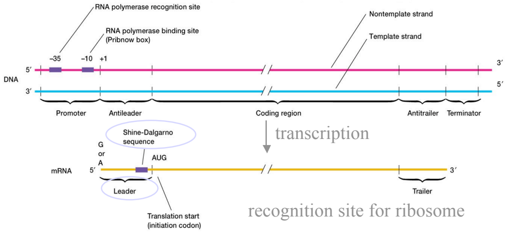


region that specifies sequence of amino
acids in a polypeptide



direction of movement of RNA polymerase

Sequences that are not translated

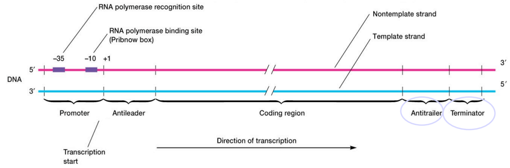


transcription

recognition site for ribosome

Shine-Delgarno 5’AGGA-3’
Translational start 5’-AUG-3’ or 3’-TAC-5’

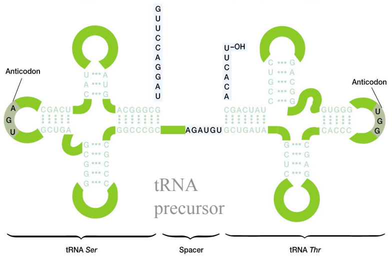
Termination of transcription



transcribed but mRNA
sequence is not translated
Usually a stretch of U’s followed by
a hair-pin loop or rho protein binding

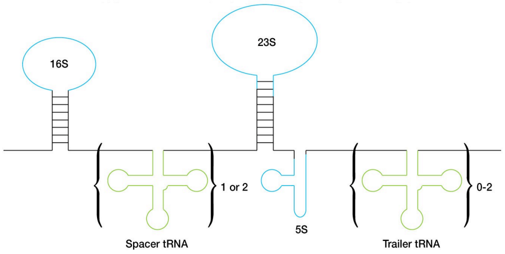
Genes That Code for tRNA and rRNA

tRNA genes have promoter, leader, coding, spacer, and trailer regions



leader, spacer, and trailer removed during maturation process

rRNA genes have promoter, leader, coding, spacer, and trailer regions



spacer and trailer regions may encode tRNA molecules

Mutations and Their Chemical Basis

- ✓ mutations
  - ✑stable, heritable change in nucleotide sequence
  - ✑may or may not have an effect on the phenotype of an organism

Mutations and Mutagenesis

- ✓ mutations can be classified in terms of their effect on phenotype
  - ✑morphological mutations
    - ⊸change colonial or cellular morphology
  - ✑lethal mutations
    - ⊸kill the organism
  - ✑conditional mutations
    - ⊸expressed only under certain conditions (e.g., high temperature)

Other types of mutations

- ✓ biochemical mutations
  - ✑changes in metabolic capabilities
  - ✑auxotrophs
    - ⊸have mutations in biosynthetic pathways
    - ⊸cannot synthesize product of pathway
    - ⊸require product of pathway as nutrient in minimal growth media
  - ✑Prototrophs (wild type)
    - ⊸grow in minimal media without supplements
- ✓ resistance mutations
  - ✑resistance to pathogen, chemical, or antibiotic

How mutations arise

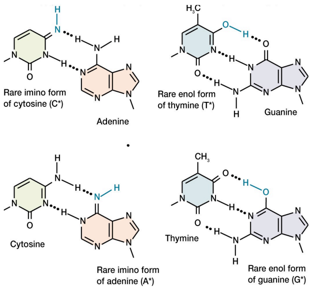
- ✓ spontaneously
  - ✑develop in absence of any added agent
  - ✑usually thought to arise randomly
    - ⊸directed (adaptive) mutation
      - ✐mutations that may result from hypermutation followed by selection
- ✓ induced
  - ✑develop after exposure to a mutagen
- ✓ Transversion
  - ✑Purine to pyrimidine
- ✓ Transition
  - ✑Purine to purine or pyrimidine to pyrimidine

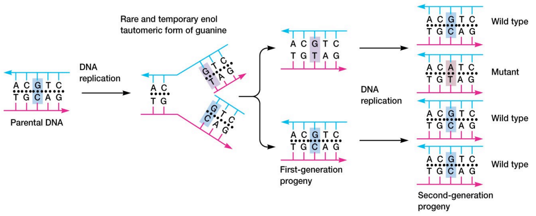
Spontaneous Mutations

✓result of:
- errors in DNA replication
- damage to DNA
- insertion of transposons

Replication errors – tautomeric shifts

- keto form imino or enol form
- alters hydrogen-bonding characteristics of Base
- Produce AC and GT base pairings



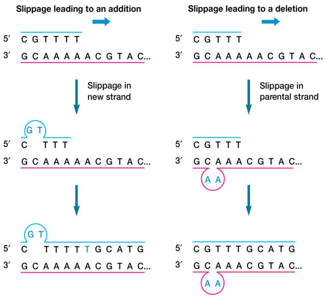


Tautomeric shift from GC to AT pairing

Replication errors - frameshifts

✓deletion or addition of base pairs

✓alters reading frame



Induced Mutations

✓caused by chemical or physical agents that damage or alter the chemistry of DNA, or that interfere with DNA repair mechanisms

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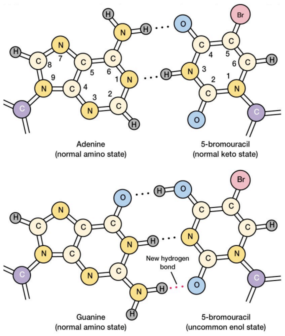
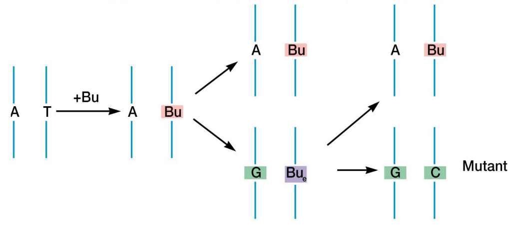
Base analogs

✓similar to nitrogenous bases

✓incorporated into DNA during replication

✓have different base-pairing characteristics

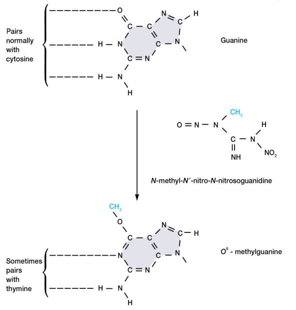




AT to GC transition

Specific mispairings

- ✓ occur when mutagen changes base’s structure and pairing characteristics
  - ✑e.g., alkylating agents

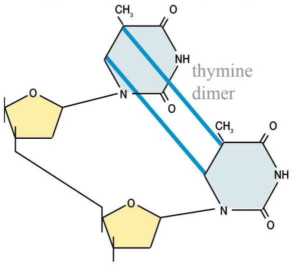


Intercalating agents

- ✓ planar molecules
- ✓ become inserted between stacked bases of helix, distorting DNA
- ✓ cause single base pair additions and deletions
- ✓ e.g., proflavin and acridine orange

DNA-damaging agents

- ✓ severely damage DNA so that it can’t serve as template for replication
- ✓ repair mechanisms allow survival, but also cause mutations



The Expression of Mutations

- ✓ wild type
  - ✑most prevalent form of gene
- ✓ forward mutations
  - ✑wild type mutant form
- ✓ reverse mutations
  - ✑mutant phenotype wild type phenotype

Forward mutations

Table 11.2 Summary of Some Molecular Changes from Gene Mutations

Type of Mutation	Result and Example
Forward Mutations	
Single Nucleotide-Pair (Base-Pair) Substitutions	
At DNA Level	
Transition	Purine replaced by a different purine, or pyrimidine replaced by a different pyrimidine (e.g., AT ⟶ GC).
Transversion	Purine replaced by a pyrimidine, or pyrimidine replaced by a purine (e.g., AT ⟶ CG).
At Protein Level	
Silent mutation	Triplet codes for same amino acid: AGG ⟶ CGG both code for Arg
Neutral mutation	Triplet codes for different but functionally equivalent amino acid: AAA (Lys) ⟶ AGA (Arg)
Missense mutation	Triplet codes for a different amino acid.
Nonsense mutation	Triplet codes for chain termination: CAG (Gln) ⟶ UAG (stop)
Single Nucleotide-Pair Addition or Deletion: Frameshift Mutation	Any addition or deletion of base pairs that is not a multiple of three results in a frameshift in reading the DNA segments that code for proteins.
Intragenic Addition or Deletion of Several to Many Nucleotide Pairs	

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Reverse mutations

Table 11.2 Summary of Some Molecular Changes from Gene Mutations

Type of Mutation	Result and Example
Reverse Mutations	
True Reversion	$\underset{\text{wild type}}{\text{AAA (Lys)}} \xrightarrow{\text{forward}} \underset{\text{mutant}}{\text{GAA (Glu)}} \xrightarrow{\text{reverse}} \underset{\text{wild type}}{\text{AAA (Lys)}}$
Equivalent Reversion	$\underset{\text{wild type}}{\text{UCC (Ser)}} \xrightarrow{\text{forward}} \underset{\text{mutant}}{\text{UGC (Cys)}} \xrightarrow{\text{reverse}} \underset{\text{wild type}}{\text{AGC (Ser)}}$
	$\underset{\text{wild type}}{\text{CGC (Arg, basic)}} \xrightarrow{\text{forward}} \underset{\text{mutant}}{\text{CCC (Pro, not basic)}} \xrightarrow{\text{reverse}} \underset{\text{pseudo-wild type}}{\text{CAC (His, basic)}}$
Suppressor Mutations	
Intragenic Suppressor Mutations Frameshift of opposite sign at site within gene. Addition of X to the base sequence shifts the reading frame from the CAT codon to XCA followed by TCA codons. The subsequent deletion of a C base shifts the reading frame back to CAT.	CATCATCATCATCATCAT (+) (–) $\underbrace{\text{CAT}}_{y}\underbrace{\text{XCA}}_{x}\underbrace{\text{TAT}}_{z}\underbrace{\text{CAT}}_{y}\underbrace{\text{CAT}}_{y}\underbrace{\text{CAT}}_{y}$
Extragenic Suppressor Mutations Nonsense suppressors	Gene (e.g., for tyrosine tRNA) undergoes mutational event in its anticodon region that enables it to recognize and align with a mutant nonsense codon (e.g., UAG) to insert an amino acid (tyrosine) and permit completion of the translation.
Physiological suppressors	A defect in one chemical pathway is circumvented by another mutation—for example, one that opens up another chemical pathway to the same product, or one that permits more efficient uptake of a compound produced in small quantities because of the original mutation.

From *An Introduction to Genetic Analysis*, 3rd edition by Suzuki, Griffiths, Miller and Lewontin. Copyright © 1986 by W. H. Freeman and Company. Used with permission.

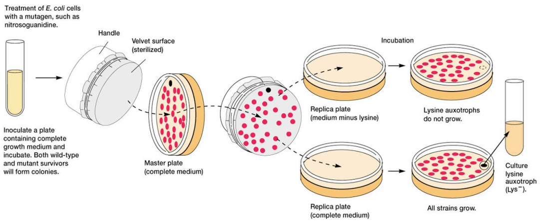
Other mutations

- ✓ regulatory mutations
  - ✑changes in regulatory sequences
  - ✑alter control of gene expression
- ✓ rRNA and tRNA mutations
  - ✑can disrupt protein synthesis
  - ✑some tRNA mutations are suppressor mutations

Detection and Isolation of Mutants

- ✓ mutations are generally rare
  - ✑one per 10^7 to 10^{11} cells
- ✓ finding mutants requires sensitive detection methods and/or methods to increase frequency of mutations
- ✓ observation of changes in phenotype
- ✓ replica plating technique
  - ✑used to detect auxotrophic mutants

Replica plating



Treatment of *E. coli* cells with a mutagen, such as nitrosoguanidine.

Inoculate a plate containing complete growth medium and incubate. Both wild-type and mutant survivors will form colonies.

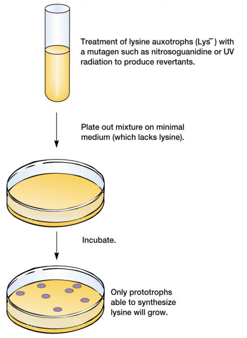
Lysine auxotrophs do not grow.

All strains grow.

Culture lysine auxotroph (Lys^-).

Mutant Selection

- ✓ use of environmental condition in which only desired mutant will grow
  - ✑e.g., selection for revertants from auxotrophy to prototrophy



Treatment of lysine auxotrophs (Lys^-) with a mutagen such as nitrosoguanidine or UV radiation to produce revertants.

Plate out mixture on minimal medium (which lacks lysine).

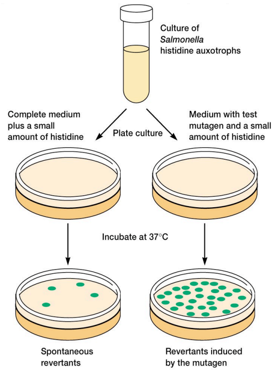
Incubate.

Only prototrophs able to synthesize lysine will grow.

Carcinogenicity Testing

- ✓ based on observation that most carcinogens are also mutagens
- ✓ tests for mutagenicity are used as screen for carcinogenic potential
- ✓ e.g., Ames test

- reversion rate in presence of suspected carcinogen > reversion rate in absence of suspected carcinogen
- then, agent is a mutagen, and may be carcinogen

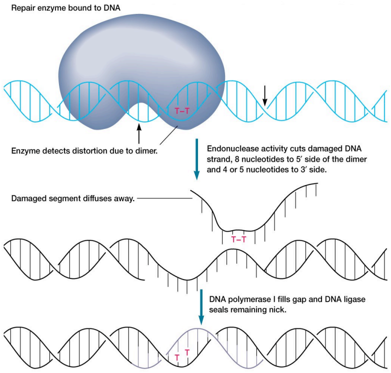


DNA Repair

- ✓ proofreading
  - ✑correction of errors in base pairing made during replication
  - ✑errors corrected by DNA polymerase
- ✓ other repair mechanisms repair incorrect pairings and DNA damage

Excision Repair

- ✓ corrects damage that causes distortions in double helix
  - ✑e.g., thymine dimers
  - ✑e.g., apurinic and apyrimidinic sites
  - ✑e.g., damaged bases



Removal of Lesions

- ✓ photoreactivation
  - ✑used to directly repair thymine dimers
  - ✑thymines separated by photochemical reaction catalyzed by photolyase
- ✓ direct repair of alkylated bases
  - ✑catalyzed by alkyltransferase or methylguanine methyltransferase

Postreplication Repair

- ✓ type of excision repair
- ✓ e.g., mismatch repair system in *E. coli*
  - ✑mismatch correction enzyme scans newly synthesized DNA for mismatched pairs
  - ✑mismatched pairs removed and replaced by DNA polymerase and DNA ligase

DNA methylation

- ✓ used by *E. coli* postreplication repair system to distinguish old DNA strands from new DNA strands
  - ✑ old DNA methylated; new DNA not methylated
- ✓ catalyzed by DNA methyltransferases

Recombination Repair

- ✓ repairs DNA with damage in both strands
- ✓ involves recombination with an undamaged molecule
  - ✑ in rapidly dividing cells, another copy of chromosome is often available
- ✓ recA protein catalyzes recombination events

SOS repair

- ✓ inducible repair system
- ✓ used to repair excessive damage that halts replication, leaving many gaps
  - ✑ recA protein initiates recombination repair
  - ✑ recA protein also acts as protease, destroying a repressor protein and thereby increasing production of excision repair enzymes

Regulation of repair enzymes