Outline

- mutations
  - spontaneous origins
  - types and functional consequences
  - causes
  - repair
- recombination

mutation is a rare process

- 10^{-5} – 10^{-8} mutations per site per generation
- microsatellite loci are often higher: 10^{-3} – 10^{-5}

mutation rates vary

- among species
- among genes within a species

Organism	Character Affected	Gene	Frequency
Bacteria			
Escherichia coli	Lactose fermentation	$lac^+ \rightarrow lac^-$	2×10^{-6}
	Phage T1 resistance	$T1\text{-}s \rightarrow T1\text{-}r$	2×10^{-8}
	Histidine dependence	$his^+ \rightarrow his^-$	2×10^{-6}
Salmonella typhimurium	Tryptophan independence	$trp^- \rightarrow trp^+$	5×10^{-8}
	Histidine dependence	$his^+ \rightarrow his^-$	2×10^{-6}
	Threonine dependence	$thr^+ \rightarrow thr^-$	4×10^{-6}
Algae			
Chlamydomonas reinhardii	Penicillin resistance	$pen\text{-}s \rightarrow pen\text{-}r$	1×10^{-7}
	Streptomycin sensitivity	$str\text{-}r \rightarrow str\text{-}s$	1×10^{-6}
Fungi			
Neurospora crassa	Adenine independence	$ade^- \rightarrow ade^+$	4×10^{-8}
	Inositol independence	$inos^- \rightarrow inos^+$	8×10^{-8}
Plant			
Zea mays (corn)	Kernel color	$C^+ \rightarrow c^-$	2×10^{-6}
	Endosperm composition	$Su^+ \rightarrow su^-$	2×10^{-6}
	Kernel shape	$Sh^+ \rightarrow sh$	1×10^{-6}
Insect			
Drosophila melanogaster (fruit fly)	Eye color	$w^+ \rightarrow w$	4×10^{-5}
	Body color	$y^+ \rightarrow y$	1.2×10^{-6}
	Eye color	$bw^+ \rightarrow bw$	3×10^{-5}
	Body color	$e^+ \rightarrow e$	2×10^{-5}
Mammal			
Mus musculus (mouse)	Coat color	$b^+ \rightarrow b^-$	8.5×10^{-4}
	Pigment pattern	$s^+ \rightarrow s^-$	3×10^{-5}
	Coat color	$a^+ \rightarrow a^-$	4×10^{-5}
	Eye color	$p^+ \rightarrow p^-$	8.5×10^{-4}
Homo sapiens (human)	Stature (achondroplasia)	$A^+ \rightarrow A^-$	5×10^{-5}
	Eye form (aniridia)	$AN^+ \rightarrow AN^-$	5×10^{-6}
	Clotting (hemophilia)	$H^+ \rightarrow H^-$	2×10^{-5}
	Huntington disease	$HUT^+ \rightarrow HUT^-$	2×10^{-6}
	Nail-patella syndrome	$NP^+ \rightarrow NP^-$	5×10^{-6}
	Duchenne muscular dystrophy	$DY^+ \rightarrow DY^-$	1×10^{-4}
	Neurofibromatosis	$NF1^+ \rightarrow NF1^-$	1×10^{-4}

mutation is a rare process

10^{-5} – 10^{-8} mutations per site per generation

microsatellite loci are often higher: 10^{-3} – 10^{-5}

mutation rates vary

- among species
- among genes within a species

transitions: $T \longrightarrow C$, $A \longrightarrow G$

transversions: everything else

Luria & Delbruck (1943) fluctuation test

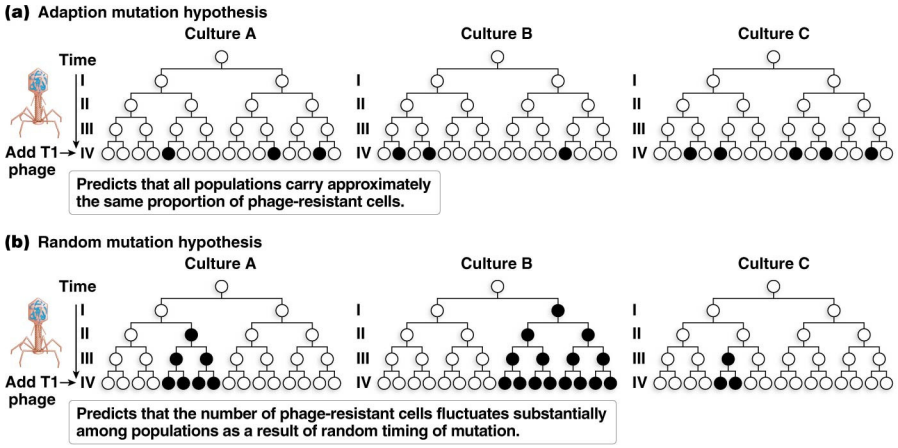
resistance of E. coli to bacteriophage T1

20 small cultures, 1 large culture each grown to 10^8 cells

plate small cultures individually, 20 samples from large culture; growth with/without phage

Hypothesis:

if exposure to phage leads to mutation, all cultures should have similar frequencies of resistance colonies



Luria & Delbruck (1943) fluctuation test

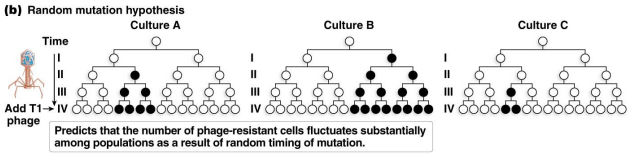
Hypothesis:

if exposure to phage leads to mutation, all cultures should have similar frequencies of resistance colonies

Results:

small: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 3, 5, 5, 6, 35, 64, 107

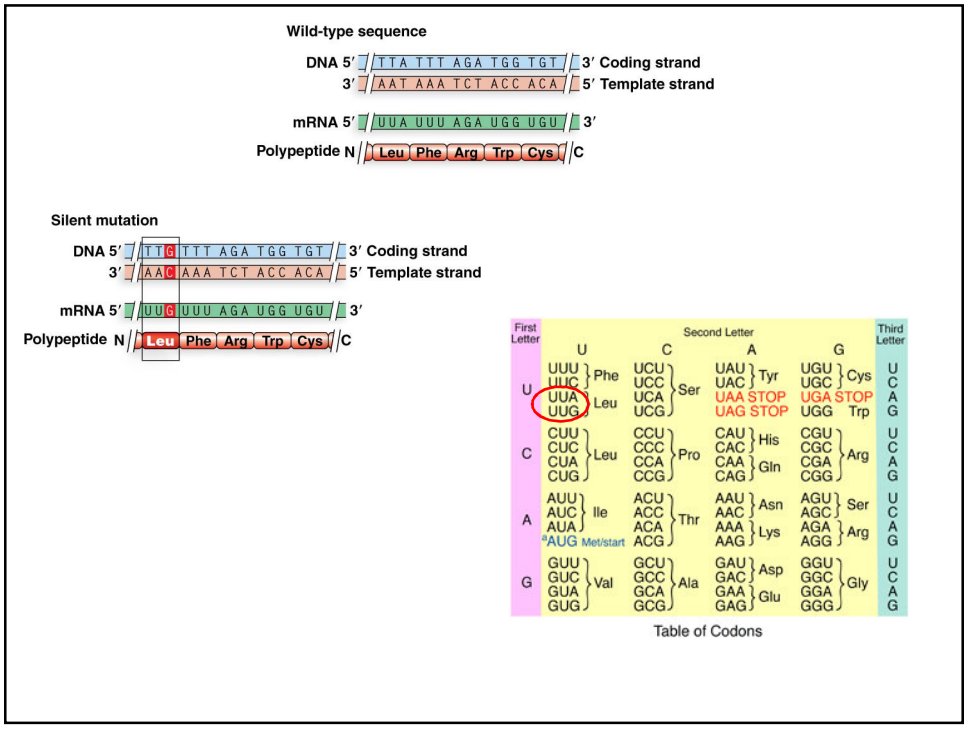
large: 14 - 26

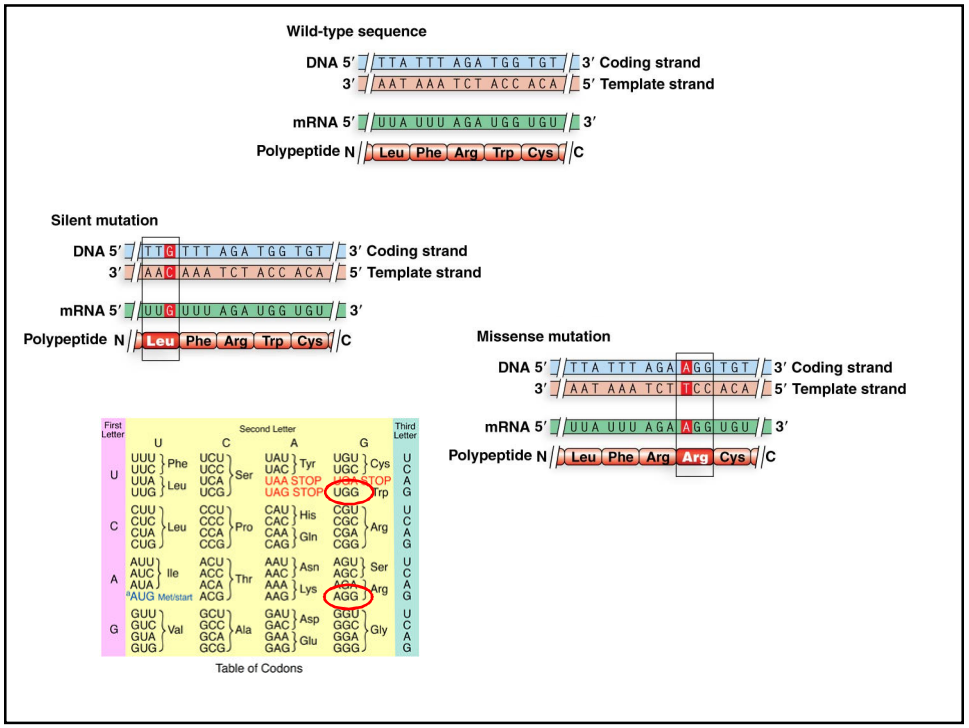


Types of mutations and their consequences

- coding sequence
- promotor sequence
- splicing mutations

- point mutations
- indels


Wild-type sequence
DNA 5′ TTA TTT AGA TGG TGT 3′ Coding strand
3′ AAT AAA TCT ACC ACA 5′ Template strand
mRNA 5′ UUA UUU AGA UGG UGU 3′
Polypeptide N Leu Phe Arg Trp Cys C
Silent mutation
DNA 5′ TTG TTT AGA TGG TGT 3′ Coding strand
3′ AAC AAA TCT ACC ACA 5′ Template strand
mRNA 5′ UUG UUU AGA UGG UGU 3′
Polypeptide N Leu Phe Arg Trp Cys C
First Letter
Second Letter
U C A G
Third Letter
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 STOP UGG Trp G
C CUU CCU CAU His CGU Arg U
CUC Leu CCC Pro CAC CGC C
CUA CCA 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 Lys AGA Arg A
aAUG Met/start ACG AAG AGG G
G GUU Val GCU Ala GAU Asp GGU Gly U
GUC GCC GAC GGC C
GUA GCA Glu GGA A
GUG GCG GAG GGG G
Table of Codons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3′ AAC AAA TCT ACC ACA 5′ Template strand
mRNA 5′ UUG UUU AGA UGG UGU 3′
Polypeptide N Leu Phe Arg Trp Cys C
Missense mutation
DNA 5′ TTA TTT AGA AGG TGT 3′ Coding strand
3′ AAT AAA TCT TCC ACA 5′ Template strand
mRNA 5′ UUA UUU AGA AGG UGU 3′
Polypeptide N Leu Phe Arg Arg Cys C
First Letter
Second Letter
U C A G
Third Letter
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 STOP UGG Trp G
C CUU CCU CAU His CGU Arg U
CUC Leu CCC Pro CAC CGC C
CUA CCA 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 Lys AGA Arg A
aAUG Met/start ACG AAG AGG G
G GUU Val GCU Ala GAU Asp GGU Gly U
GUC GCC GAC GGC C
GUA GCA Glu GGA A
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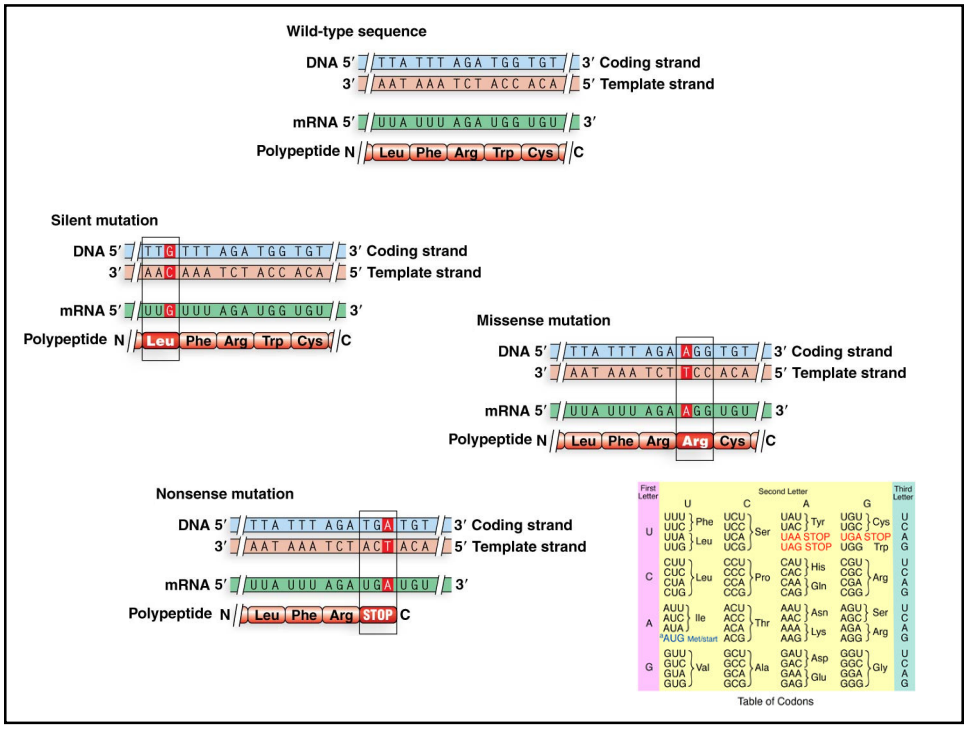
Table of Codons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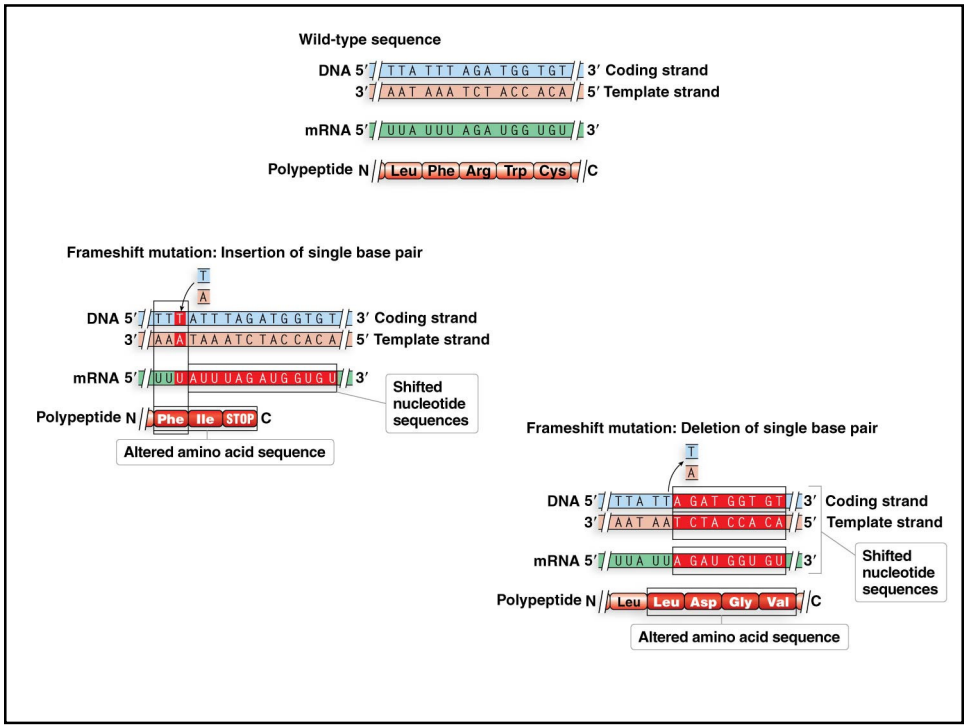
Nonsense mutation
DNA 5′ TTA TTT AGA TGA TGT 3′ Coding strand
3′ AAT AAA TCT ACT ACA 5′ Template strand
mRNA 5′ UUA UUU AGA UGA UGU 3′
Polypeptide N Leu Phe Arg STOP C
First Letter
Second Letter
Third Letter
U C A G
UUU Phe UCU Ser UAU Tyr UGU Cys U
UUC Phe UCC Ser UAC Tyr UGC Cys C
UUA Leu UCA Ser UAA STOP UGA STOP A
UUG Leu UCG Ser UAG STOP UGG Trp G
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
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/start ACG Thr AAG Lys AGG Arg 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
Table of Codons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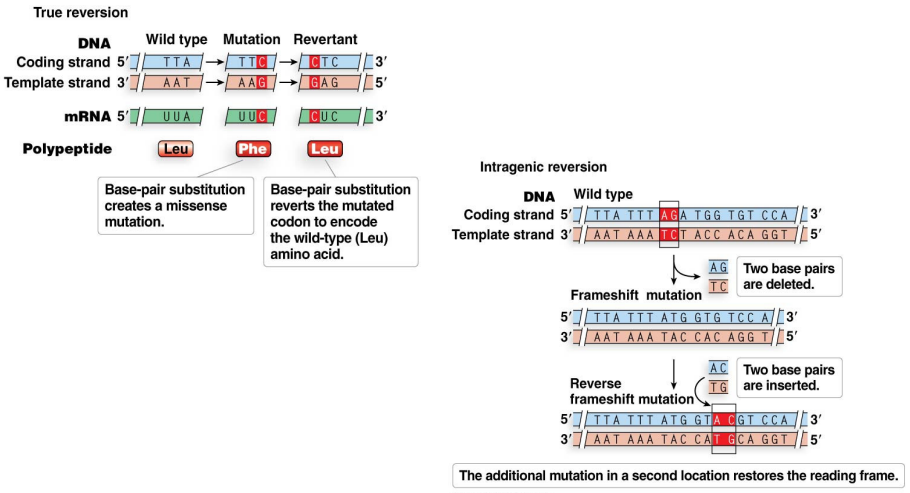
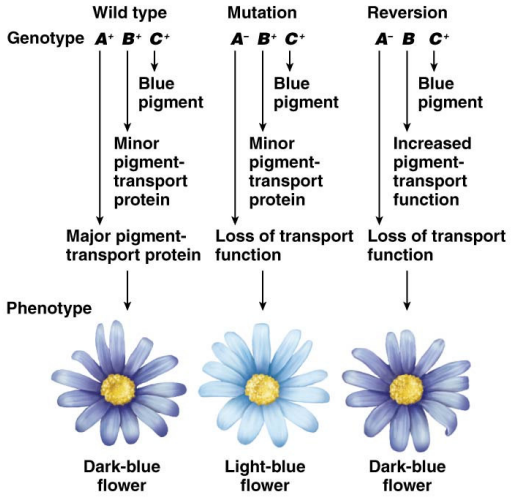
3′ AAT AAA TCT ACC ACA 5′ Template strand
mRNA 5′ UUA UUU AGA UGG UGU 3′
Polypeptide N Leu Phe Arg Trp Cys C
Frameshift mutation: Insertion of single base pair
T
A
DNA 5′ TTT TAT TTA GAT GGT GT 3′ Coding strand
3′ AAA ATA AAT CTA CCA CA 5′ Template strand
mRNA 5′ UUU AUU UAG AUG GUG U 3′
Polypeptide N Phe Ile STOP C
Shifted nucleotide sequences
Altered amino acid sequence
Frameshift mutation: Deletion of single base pair
T
A
DNA 5′ TTA TTA GAT GGT GT 3′ Coding strand
3′ AAT AAT CTA CCA CA 5′ Template strand
mRNA 5′ UUA UUA GAU GGU GU 3′
Polypeptide N Leu Leu Asp Gly Val C
Shifted nucleotide sequences
Altered amino acid sequence

forward and reverse mutation (reversion)



compensatory mutations



What causes mutations?

- DNA replication errors
- spontaneous nucleotide changes (tautomeric shifts)
- spontaneous lesions (depurination)
- mutagens: ultraviolet light, chemicals, free radicals, radiation

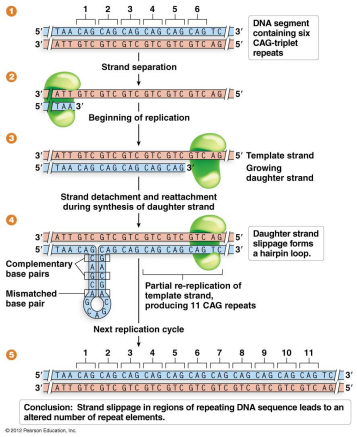
- Ames test

base pair mismatches are rare (10^{-9})

- proofreading by DNA polymerase
- mismatch repair

strand slippage

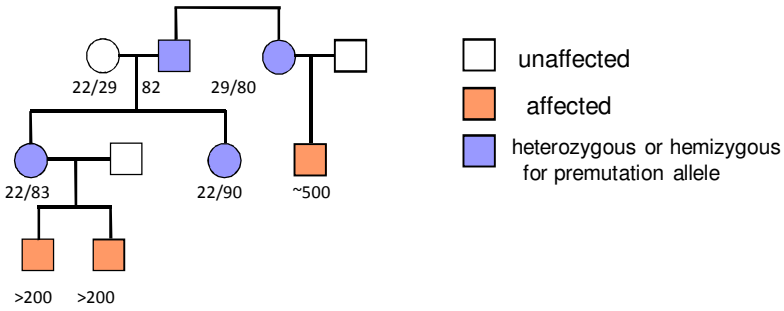
- amplification of repeat motif

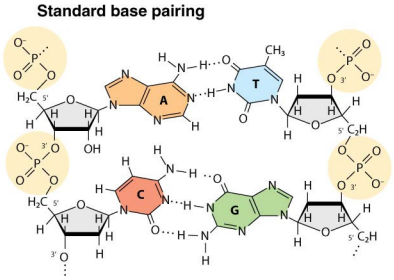


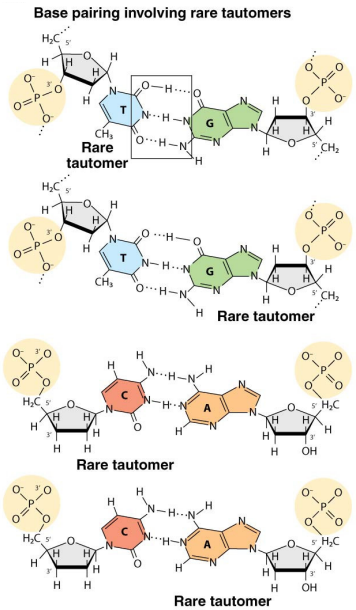
fragile-X syndrome: physical anamolies; mental retardation

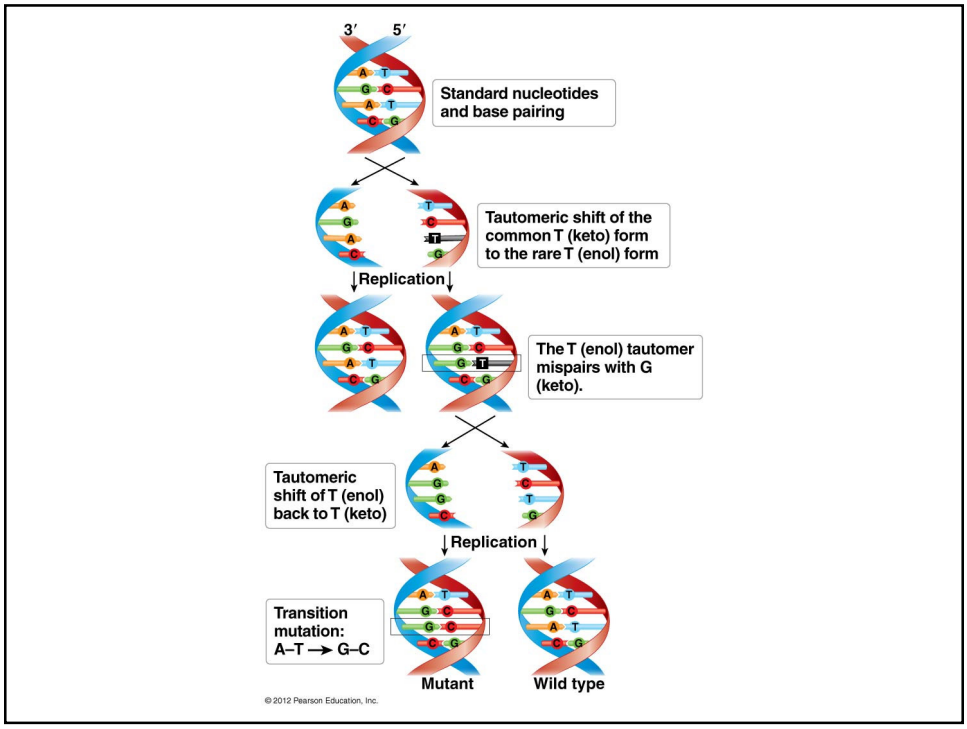
amplification of a *CGG* repeat sequence in the FMR-1 gene

normal (average): 29 *CGG* repeats (<50)
premutation: 55-200 *CGG* repeats
syndrome: 200-1300 *CGG* repeats

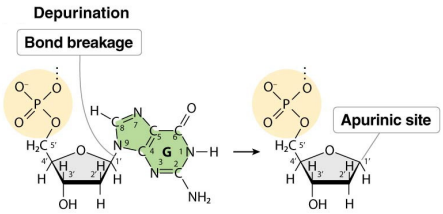


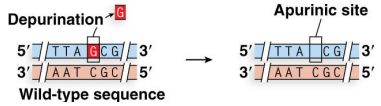


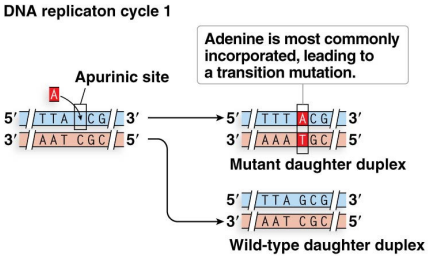




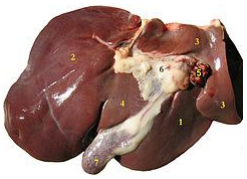
depurination, a common mechanism for transitions







Ames Test -- evaluating mutagenic properties

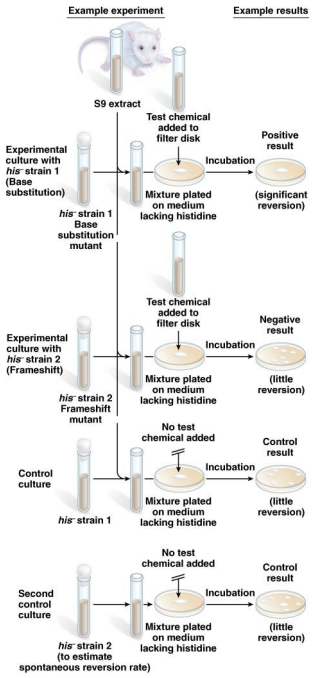


S9 extract - subset of detox enzymes
original compound, byproducts

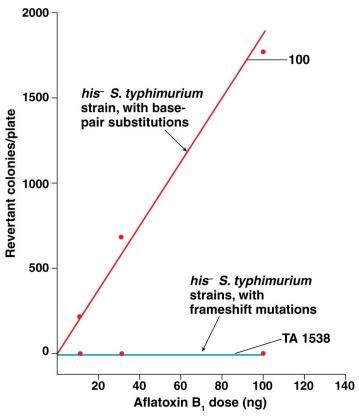
grow his$^-$ strains of *Salmonella typhimurium* with S9 and test compound

- point mutations (transitions and transversions)
- frameshift mutations

vary dosage



Ames test results



[box 12.2]

DNA repair

- homology dependent
- homology independent
- synthesis-dependent strand annealing

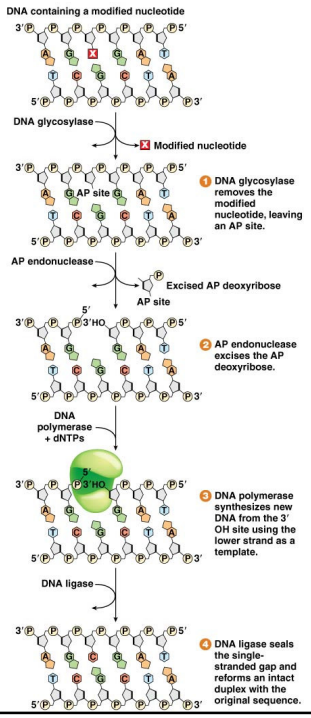
homology dependent:

complementarity to intact strand

nucleotide excision

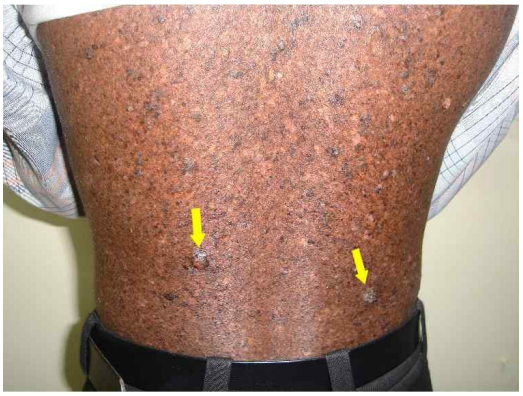
activated by:
- stalled replication forks
- stalled transcription complexes

- recognition of damaged base
- assembly of multiprotein complex
- cutting damaged strand
- undamaged strand as template
- ligation of repaired strand



defects in nucleotide excision repair lead to Xeroderma Pigmentosum

(can recognize stalled transcription complexes, but not repair damage)



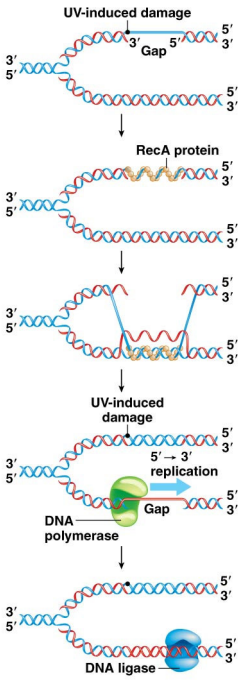
homology dependent:

complementarity to intact strand

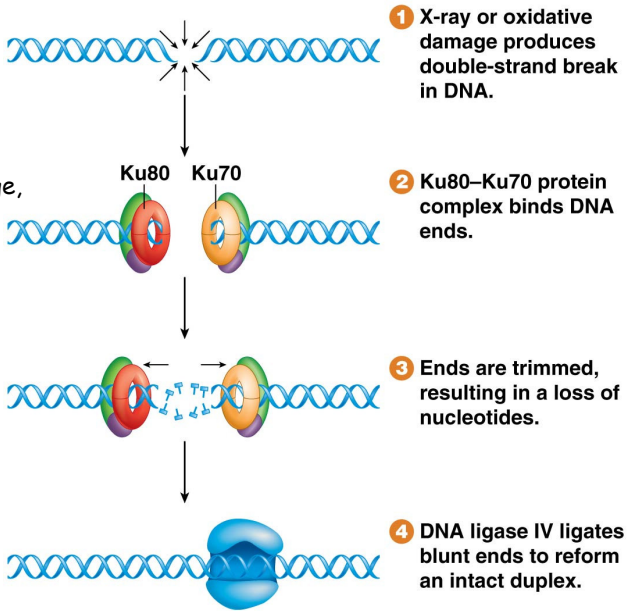
recombination repair

initiated by stalled replication fork

RecA in bacteria
Rad51 in eukaryotes

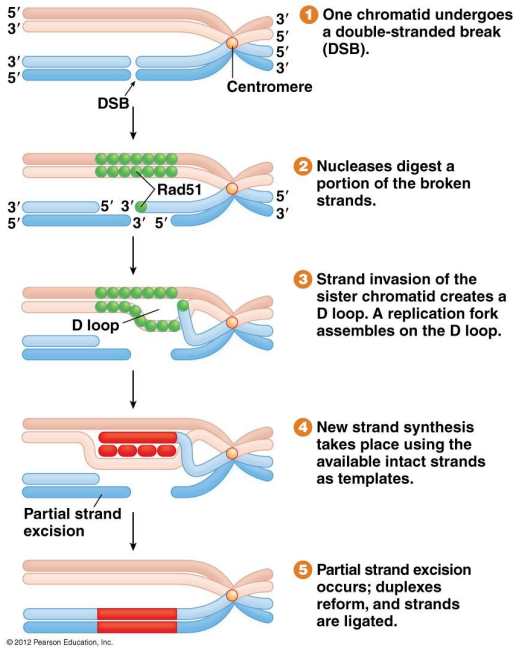


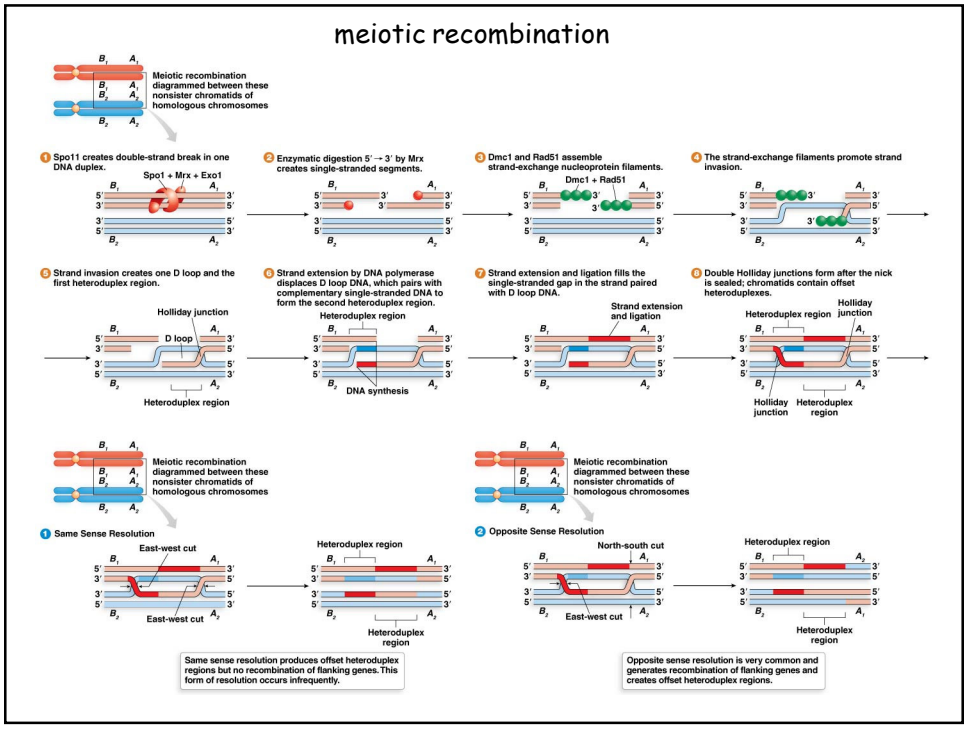
double stranded break repair -- nonhomologous end joining

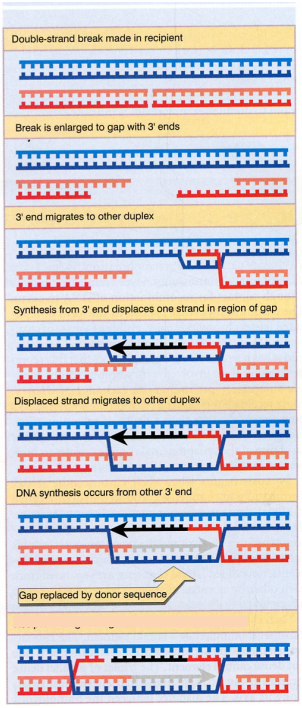


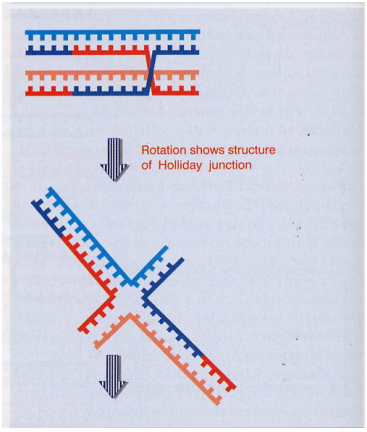
double stranded break repair -- homologous recombination

SDSA: synthesis-dependent strand annealing




meiotic recombination
Meiotic recombination diagrammed between these nonsister chromatids of homologous chromosomes
1 Spo11 creates double-strand break in one DNA duplex.
Spo1 + Mrx + Exo1
2 Enzymatic digestion 5′ → 3′ by Mrx creates single-stranded segments.
3 Dmc1 and Rad51 assemble strand-exchange nucleoprotein filaments.
Dmc1 + Rad51
4 The strand-exchange filaments promote strand invasion.
5 Strand invasion creates one D loop and the first heteroduplex region.
Holliday junction
D loop
Heteroduplex region
6 Strand extension by DNA polymerase displaces D loop DNA, which pairs with complementary single-stranded DNA to form the second heteroduplex region.
Heteroduplex region
DNA synthesis
7 Strand extension and ligation fills the single-stranded gap in the strand paired with D loop DNA.
Strand extension and ligation
8 Double Holliday junctions form after the nick is sealed: chromatids contain offset heteroduplexes.
Heteroduplex region
Holliday junction
Holliday junction
Heteroduplex region
Meiotic recombination diagrammed between these nonsister chromatids of homologous chromosomes
1 Same Sense Resolution
East-west cut
East-west cut
Heteroduplex region
Heteroduplex region
Same sense resolution produces offset heteroduplex regions but no recombination of flanking genes. This form of resolution occurs infrequently.
Meiotic recombination diagrammed between these nonsister chromatids of homologous chromosomes
2 Opposite Sense Resolution
North-south cut
East-west cut
Heteroduplex region
Heteroduplex region
Opposite sense resolution is very common and generates recombination of flanking genes and creates offset heteroduplex regions.


Double-strand break made in recipient
Break is enlarged to gap with 3′ ends
3′ end migrates to other duplex
Synthesis from 3′ end displaces one strand in region of gap
Displaced strand migrates to other duplex
DNA synthesis occurs from other 3′ end
Gap replaced by donor sequence


Rotation shows structure of Holliday junction