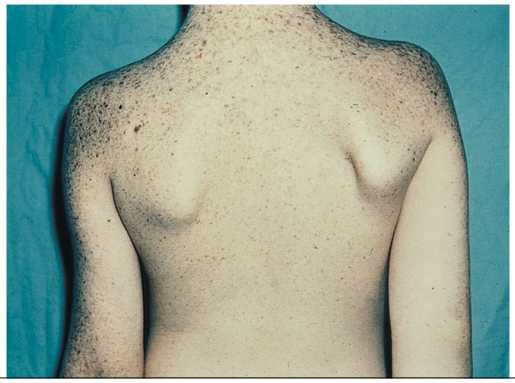
Gene Mutation: Origins and Repair Processes

GAATTC → GTATTC

A → *a*

Mutant colonies of Aspergillus

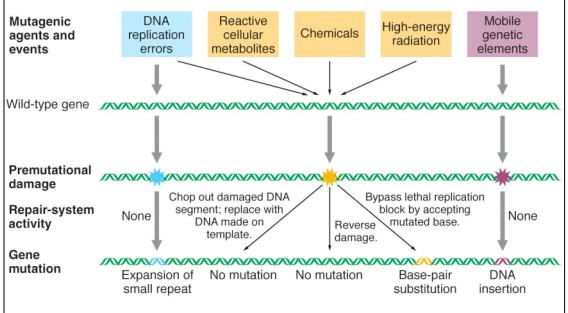
Cancer in xeroderma pigmentosum



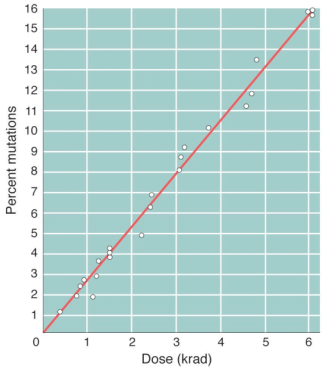
Mutation

- Hereditary change in DNA
- Gene mutations occur within individual genes as a result of change in nucleotide sequence
- Multiple causes
  - integration of transposons
  - mutagens
  - DNA replication errors
- Some types of mutation can be repaired
- Point mutations involve single (or few) base pair changes

Gene mutations



X-ray exposure and mutation (*Drosophila*)

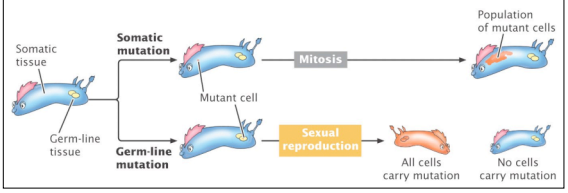


Classifying mutations

- Spontaneous
  - Natural mutations
  - Changes in nucleotides
- Induced
  - Artificial chemical/factor
    - Cosmic
    - UV light
    - Gamma radiation
    - X rays
    - Chemical mutagens

Classifying mutations cont.

- Gametic
- Somatic
- Lethal
  - Loss of function
- Conditional
  - Temperature sensitive mutant



Definitions

- Minimum culture media
  - Allows wild-type to grow, but not mutated cells
- Complete medium
  - Supplemented with vitamins, amino acids, nucleic acids and so forth.
- Prototrophs
  - Wild-type organisms
- Auxotrophs
  - Mutants that require a specific supplement

Mutagens

TABLE 10-1 Mutation Frequencies Obtained with Various Mutagens in *Neurospora*

Mutagenic Treatment	Exposure Time (minutes)	Survival (%)	Number of *ad-3* Mutants per 10^6 Survivors
No treatment (spontaneous rate)	–	100	~0.4
Amino purine (1–5 mg/ml)	During growth	100	3
Ethyl methanesulfonate (1%)	90	56	25
Nitrous acid (0.05 M)	160	23	128
X rays (2000 r/min)	18	16	259
Methyl methanesulfonate (20 mM)	300	26	350
UV rays (600 erg/mm^2/min)	6	18	375
Nitrosoguanidine (25 mM)	240	65	1500
ICR-170 acridine mustard (5 mg/ml)	480	28	2287

Note: The assay measures the frequency of *ad-3* mutants. It so happens that such mutants are red, so they can be detected against a background of white *ad-3*$^+$ colonies.

Point mutation

- Single or few base pair changes
- Origin of point mutation
  - induced by geneticist
    - action of mutagen, an environmental agent that alters nucleotide sequence
    - process of inducing mutations by mutagens is called mutagenesis
  - spontaneous
    - arise in absence of known mutagen
    - may be caused by errors in DNA replication
    - provide "background rate" of mutation
    - critically important to evolution

Types of point mutation

- Base substitution
  - transition
    - A ↔ G (purine ↔ purine) (A·T ↔ G·C)
    - C ↔ T (pyrimidine ↔ pyrimidine) (C·G ↔ T·A)
  - transversion
    - purine ↔ pyrimidine (e.g., A ↔ C) (A·T ↔ C·G)
- Addition or deletion of nucleotide pairs (base-pair addition or deletion)
  - also called *indel* mutations

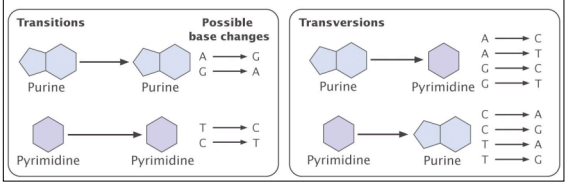
Point mutations

TABLE 10-2 Point Mutations at the Molecular Level

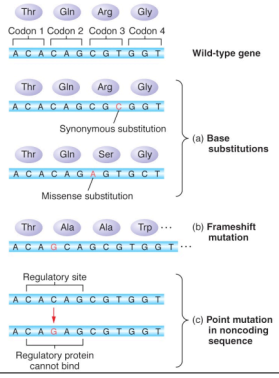
Type of Mutation	Result and Example(s)
At DNA level	
Transition	Purine replaced by a different purine, or pyrimidine replaced by a different pyrimidine: A·T → C·G C·G → A·T C·G → T·A T·A → C·G
Transversion	Purine replaced by a pyrimidine, or pyrimidine replaced by a purine: A·T → C·G A·T → T·A G·C → T·A G·C → C·G T·A → G·C T·A → A·T C·G → A·T C·G → G·C
Indel	Addition or deletion of one or more base pairs of DNA (inserted or deleted bases are underlined): AAGACTCCT → AAGAGCTCCT AAGACTCCT → AAACTCCT
At protein level	
Synonymous mutation	Codons specify the same amino acid: AGG → CGG Both encode Arg
Missense mutation	Codon specifies a different amino acid.
Conservative missense mutation	Codon specifies chemically similar amino acid: AAA → AGA Changes basic Lys to basic Arg; does not alter protein function in many cases.
Nonconservative missense mutation	Codon specifies chemically dissimilar amino acid: UUU → UCU Hydrophobic Phenylalanine Polar Serine
Nonsense mutation	Codon signals chain termination: CAG → UAG Change from a codon for Gln to an amber termination codon AAG ACT CCT → AAG AGC TCC T…
Frameshift mutation	one-base-pair addition (underlined) or AAG ACT CCT → AAA CTC CT… one-base-pair deletion (underlined)

Point mutations (DNA level)

- Transition
  - Py ⇒ Py
  - Pu ⇒ Pu
- Transversion
  - Py ⇒ Pu
  - Pu ⇒ Py
- Indel



Point mutations



Molecular consequences (protein)

- Synonymous mutation
  - changes one codon for an amino acid to another codon for that amino acid
  - no change in amino acid (silent mutation)
- Missense mutation
  - changes codon for one amino acid to codon for another amino acid
  - also called nonsynonymous mutation
- Nonsense mutation
  - change codon for amino acid into translation termination (stop) codon

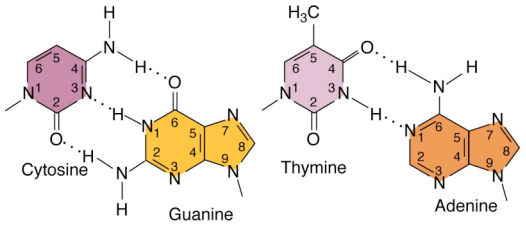
Molecular consequences (2)

- Missense mutations differ in severity
  - conservative amino acid substitution substitutes chemically similar amino acid, less likely to alter function
  - nonconservative amino acid substitution substitutes chemically different amino acid, more likely to alter function
  - consequences for function often context-specific
- Nonsense mutation results in premature termination of translation,
  - truncated polypeptides often are nonfunctional
- Point mutation in non-coding region may or may not have visible effect (e.g., regulatory region)

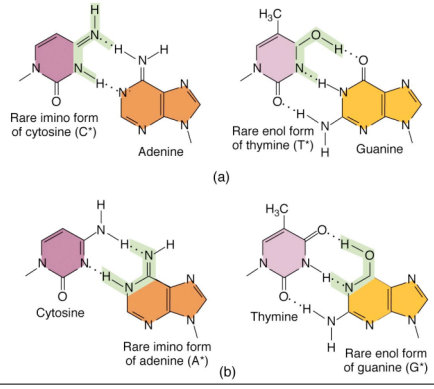
Tautomeric shift

- Natural variation in chemical form of base (isomers)
  - amino (normal) ↔ imino (rare)
  - keto (normal) ↔ enol (rare)
- Results in mutation during DNA replication
  - base in rare tautomeric form pairs with chemically similar base of its normal complement
  - e.g., C·G ↔ C*·G at replication results in C*·A which resolves to C·A upon reverse of shift
  - at next DNA replication, use of A strand results in T·A base pair, a transition
- Contributes to spontaneous mutation rate

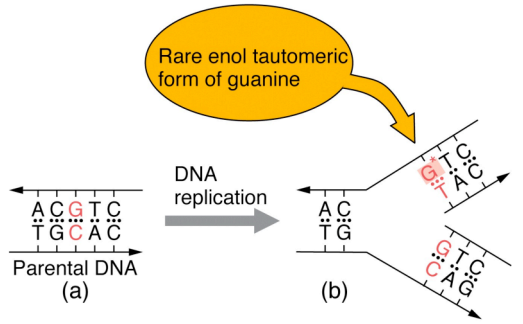
Normal keto pairing



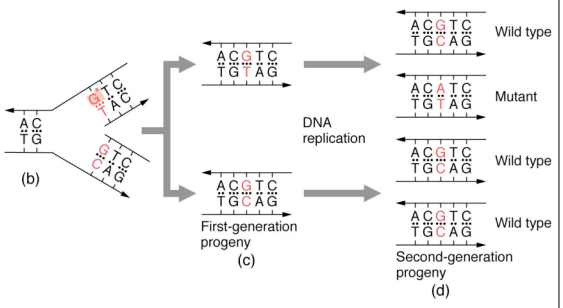
Tautomeric mismatches



Incorporating mutation



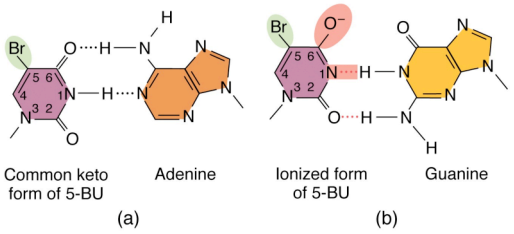
Incorporating mutations



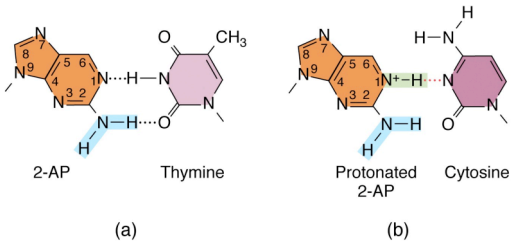
Molecular mechanism (1)

- Mutagens have different mutational specificity
- Base analogs
  - similar to nitrogenous bases of DNA, but have altered pairing properties
  - e.g., 5-bromouracil (5-BU) (base pairs with G) and 2-aminopurine (2-AP) (base pairs with C)
  - result in transitions
- Base alteration
  - alkylating agents modify base structure, resulting in altered pairing
  - e.g., EMS (ethyl methanesulfonate) and NG (nitrosoguanidine)

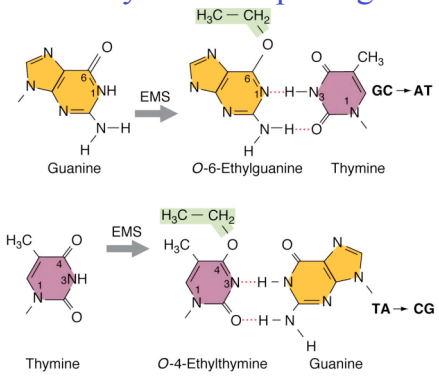
5-bromouracil mispairs



2-aminopurine



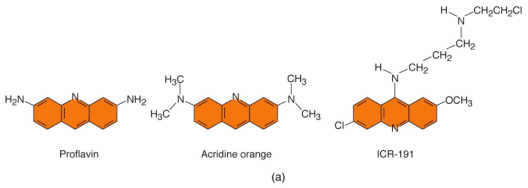
Alkylation mispairing



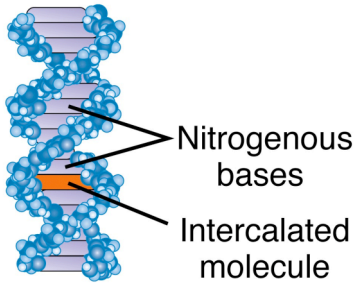
Molecular mechanism (2)

- Intercalating agents
  - flat, planar molecules intercalate between base pairs, disrupt DNA synthesis
  - e.g., proflavin, acridine orange
- Base damage
  - agent alters base so that it has no complement
  - results in replication block and insertion of nonspecific bases by SOS system
- UV light
  - results in pyrimidine-pyrimidine dimers
  - activates SOS system, resulting in insertion of incorrect base

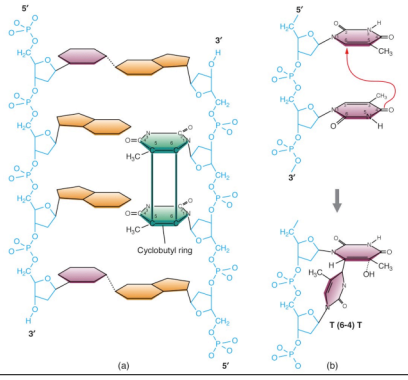
Intercalating agents



Intercalated molecule



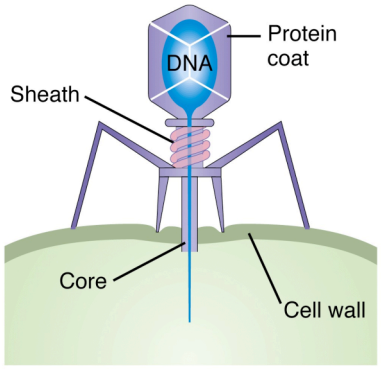
Photodimers



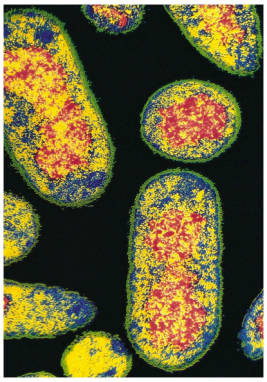
Spontaneous mutation

- Tautomeric shift
- Depurination, spontaneous loss of G or A
- Deamination, converts cytosine to uracil which pairs with adenine at replication
- Oxidative damage to bases
  - caused by superoxide and peroxide radicals
  - chemically alter base pairing properties
- Indel mutations
  - result in translation frameshift
  - often occur in regions of repeated bases

T1 phage (Luria-Delbruck Fluctuation test)

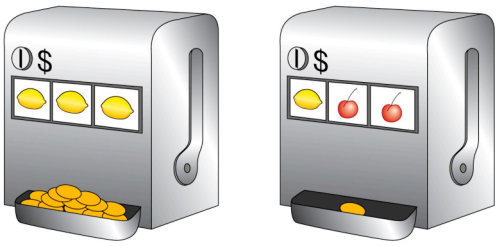


Escherichia coli (Luria-Delbruck Fluctuation test)

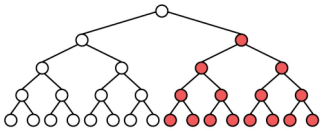


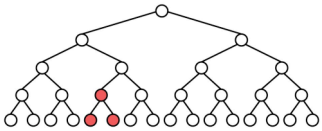
Mutations are random

Adaptation - Increase phage - more mutations
result - same number of cell and phages produced a different number of mutants each time.
Spontaneous- the number of mutants will vary depending on when they arise -early verses late



Early mutation spread more

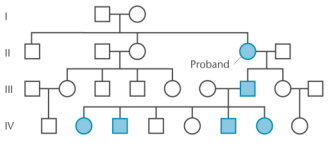


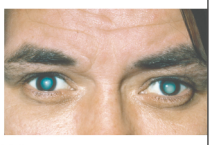


Mutations are selected after they occur

Lac$^-$ mutations

Spontaneous cataract mutation -humans





5-methylcytosine, G*C→A*T transition (*lacI*)

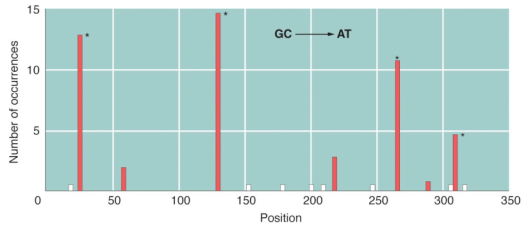
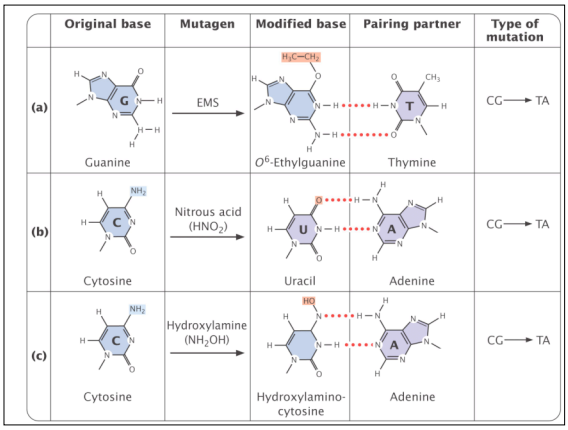
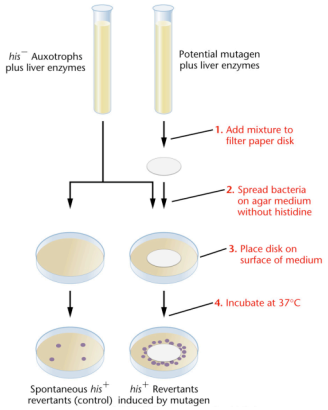


TABLE 15.2 RATES OF SPONTANEOUS MUTATIONS AT VARIOUS LOCI IN DIFFERENT ORGANISMS

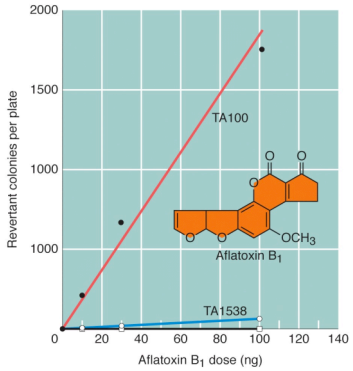
Organism	Character	Gene	Rate	Units
Bacteriophage T2	Lysis inhibition	$r^+ \rightarrow r^-$	1×10^{-8}	Per gene replication
	Host range	$h^+ \rightarrow h$	3×10^{-8}	
	Lactose fermentation	$lac^- \rightarrow lac^+$	2×10^{-7}	
	Lactose fermentation	$lac^+ \rightarrow lac^-$	2×10^{-5}	
	Phage T1 resistance	$T1\text{-}s \rightarrow T1\text{-}r$	2×10^{-8}	
	Histidine requirement	$his^- \rightarrow his^+$	2×10^{-6}	
	Histidine independence	$his^+ \rightarrow his^-$	4×10^{-8}	
E. coli	Streptomycin dependence	$str\text{-}s \rightarrow str\text{-}d$	1×10^{-9}	Per cell division
	Streptomycin sensitivity	$str\text{-}d \rightarrow str\text{-}s$	1×10^{-8}	
	Radiation resistance	$rad\text{-}s \rightarrow rad\text{-}r$	1×10^{-5}	
	Leucine independence	$leu^- \rightarrow leu^+$	7×10^{-10}	
	Arginine independence	$arg^- \rightarrow arg^+$	4×10^{-9}	
	Tryptophan independence	$trp^- \rightarrow trp^+$	6×10^{-8}	
Salmonella typhimurium	Tryptophan independence	$trp^- \rightarrow trp^+$	5×10^{-8}	Per cell division
Diplococcus pneumoniae	Penicillin resistance	$pen^s \rightarrow pen^r$	1×10^{-7}	Per cell division
Chlamydomonas reinhardi	Streptomycin sensitivity	$str^r \rightarrow str^d$	1×10^{-6}	Per cell division
Neurospora crassa	Inositol requirement	$inos^+ \rightarrow inos^-$	8×10^{-8}	Mutant frequency among asexual spores
	Adenine independence	$ade^- \rightarrow ade^+$	2×10^{-8}	
Zea mays	Shrunken seeds	$sh^+ \rightarrow sh^-$	1×10^{-6}	
	Purple	$pr^- \rightarrow pr^+$	1×10^{-5}	Per gamete per generation
	Colorless	$c^+ \rightarrow c^-$	2×10^{-5}	
	Sugary	$su^+ \rightarrow su^-$	2×10^{-6}	
Drosophila melanogaster	Yellow body	$y^+ \rightarrow y$	1.2×10^{-6}	
	White eye	$w^+ \rightarrow w$	4×10^{-5}	
	Brown eye	$bw^+ \rightarrow bw$	3×10^{-5}	Per gamete per generation
	Ebony body	$e^+ \rightarrow e$	2×10^{-5}	
	Eyeless	$ey^+ \rightarrow ey$	6×10^{-5}	
Mus musculus	Piebald coat	$s^+ \rightarrow s$	3×10^{-5}	
	Dilute coat color	$d^+ \rightarrow d$	3×10^{-5}	Per gamete per generation
	Brown coat	$b^+ \rightarrow b$	8.5×10^{-5}	
	Pink eye	$p^+ \rightarrow p$	8.5×10^{-6}	
Homo sapiens	Hemophilia	$h^+ \rightarrow h$	2×10^{-5}	
	Huntington disease	$Hu^+ \rightarrow Hu$	5×10^{-5}	
	Retinoblastoma	$R^+ \rightarrow R$	2×10^{-5}	Per gamete per generation
	Epiloia	$Ep^+ \rightarrow Ep$	1×10^{-5}	
	Aniridia	$An^+ \rightarrow An$	5×10^{-5}	
	Achondroplasia	$A^+ \rightarrow A$	5×10^{-5}	



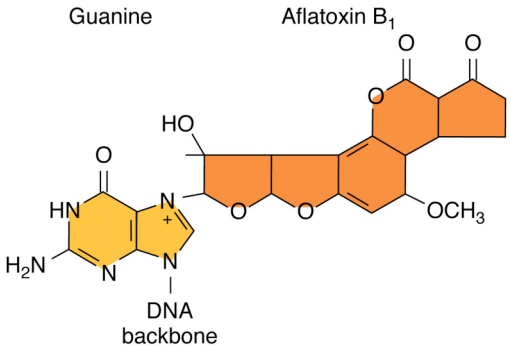
Ames Test



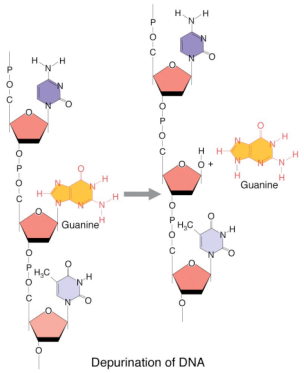
Ames test results of aflatoxin



Aflatoxin activity (apurinic site)

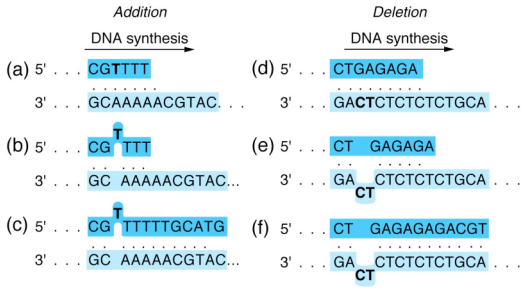


Creating an apurinic site



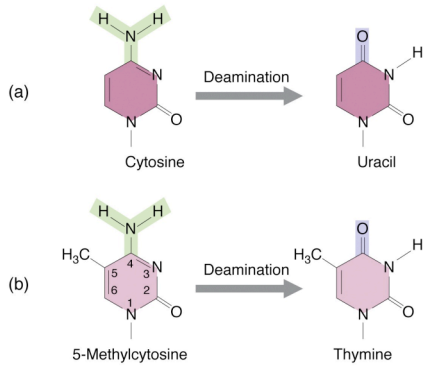
Depurination of DNA

Indel - additions or deletions

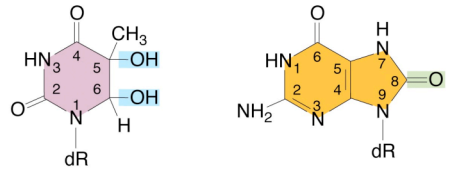


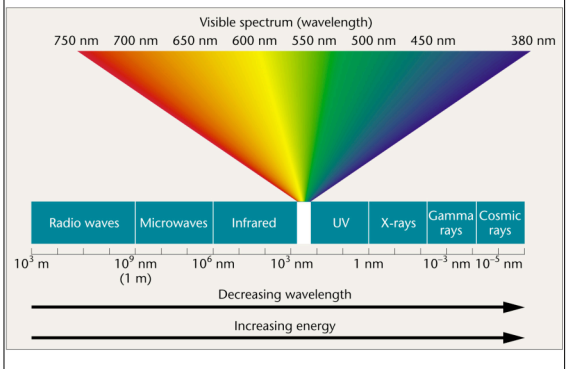
ABO Blood type-type O is a deletion resulting in a stop codon

Spontaneous mutations



Oxidative damaged bases





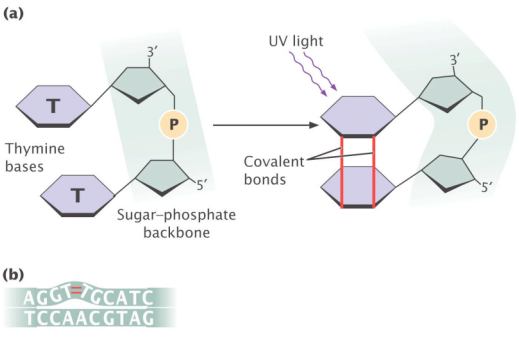


Table 17.4 Theoretical reverse mutations possible by various mutagenic agents

Mutagen	Type of Mutation	Reversal of Mutations by: 5-Bromo-uracil	2-Amino-purine	Ethyl methane sulfonate	Nitrous acid	Hydroxyl-amine	Acridine orange
5-Bromouracil	C-G↔T-A	+	+	+	+	+/−	−
2-Aminopurine	C-G↔T-A	+	+	+	+	+/−	−
Nitrous acid	C-G↔T-A	+	+	+	+	+/−	−
Ethylmethane sulfonate	C-G↔T-A	+	+	+	+	+/−	−
Hydroxylamine	C-G↔T-A	+	+	+	+	−	−
Acridine orange	Frameshift	−	−	−	−	−	+

Note: + indicates that reverse mutations occur, − indicates that reverse mutations do not occur, and +/− indicates that only some mutations are reversed. Not all reverse mutations are equally likely.

Errors in replication (*lacI*)

Red boxes are fast reverting mutations
Gold are deletions

Deletions in *lacI*

S74, S112 75 bases

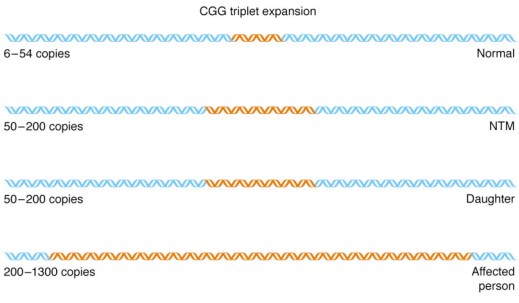
CAATTCAGGGTGGTGAATGTGAAACC------CGCGTGGTGAACCAGG

Site (no. of bp)	Sequence repeat	No. of bases deleted	Occurrences	
20 to 95	GTGGTGAA	75	2	*S74, S112*
146 to 269	GCGGCGAT	123	1	*S23*
331 to 351	AAGCGGCG	20	2	*S10, S136*
316 to 338	GTCGA	22	2	*S32, S65*
694 to 707	CA	13	1	*S24*
694 to 719	CA	25	1	*S56*
943 to 956	G	13	1	*S42*
322 to 393	None	71	1	*S120*
658 to 685	None	27	1	*S86*

Trinucleotide repeats

- Special case of indel mutation
- Characterized by expansion of three-base-pair repeats
  - few repeats to hundreds of repeats
  - expansion may result in abnormal protein, disease
  - number of repeats may expand in subsequent generations
- Thought to arise through slipped mispairing during DNA replication
- E.g., Huntington disease, fragile X syndrome

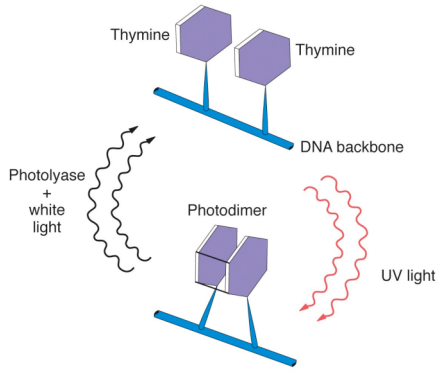
Trinucleotide repeat in humans (fragile X)



DNA repair mechanisms

- Direct reversal of damage
  - photodimer repair by photolyase
    - regenerates pyrimidines in presence of light
  - alkyltransferase
    - remove alkyl groups added by mutagen
  - neither system completely effective
- Homology-dependent repair systems
  - take advantage of complementary nature of DNA molecule
    - excision repair, repairs damage before replication
    - postreplication repair, repairs during or after S phase
  - may have played role in evolution of sex

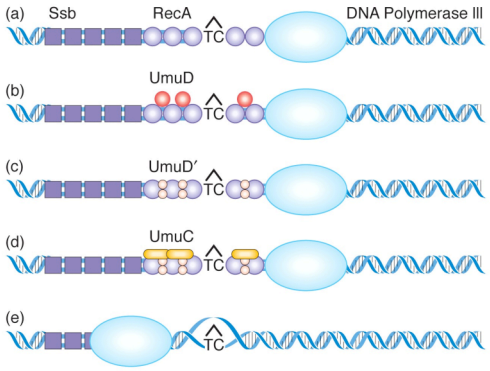
Light repair



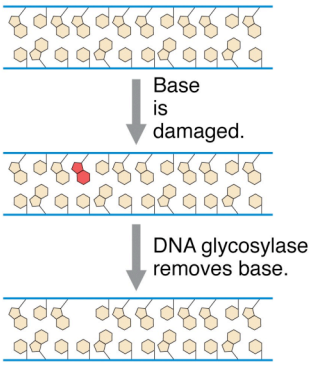
Prereplication repair

- Nucleotide excision-repair system
  - recognizes abnormal base(s) through distortion of helix
  - excises lesion and flanking bases
    - 12-13 nucleotides in prokaryotes
    - 27-29 nucleotides in eukaryotes
  - uses complement to synthesize replacement strand
- Base-excision repair
  - DNA glycosylases remove base
  - repaired by AP site-specific endonuclease pathway which repairs spontaneous loss of purine or pyrimidine

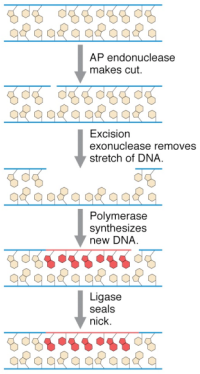
SOS bypass (TC photodimer)



Base excision repair

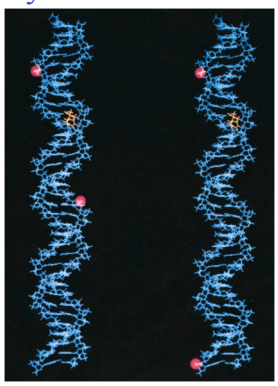


Base excision repair (cont.)



Excising a Thymine dimer

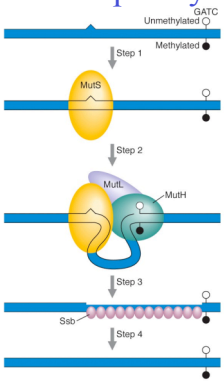
Prokaryotes 12-13 bases
Eukaryotes 27-29 bases



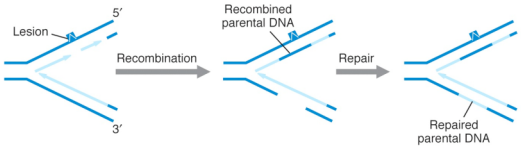
Postreplication repair

- Mismatch repair system
  - recognizes mismatched base pairs
  - determines which base is incorrect one
    - distinguishes old template strand from new strand by delayed methylation that normally occurs
    - methyladenine on old strand in GATC sequence
  - excision of base followed by templated repair
- Recombinational repair
  - recA gene product
  - gap repaired by DNA cut from sister molecule

Mismatch repair system



Postreplication recombinational repair



Double Strand Break

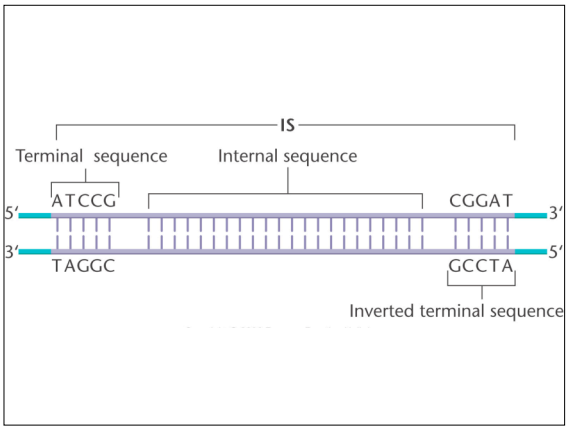
- DNA double-strand break (DSB) repair is activated when both DNA strands are cleaved and is responsible for reannealing the two strands.
  - Homologous recombinational repair fixes a double-strand DNA break by digesting back the 5' ends of the broken helix to leave overhanging 3' ends that interact with a region of an undamaged sister chromatid to allow DNA polymerase to copy the undamaged DNA sequence into the damaged strand.
  - End joining repairs double-stranded breaks but does not require a homologous region of DNA during repair.

TABLE 10-4 Repair Systems in *E. coli*

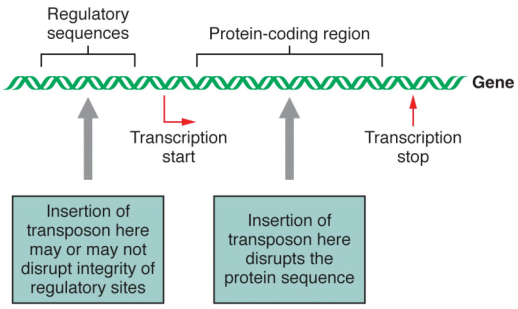
General Mode of Operation	Example	Type of Lesion Repaired	Mechanism
Direct removal of lesions	Alkyltransferases	*O*-6-Alkylguanine	Transfers alkyl group from *O*-6-alkylguanine to cysteine residue on transferase
	Photolyase	6–4 photoproduct	Breaks 6–4 bond and restores bases to normal
	Photolyase	UV photodimers	Splits dimers in the presence of white light
General excision	*uvrABC*-encoded exonuclease system	Lesions causing distortions in double helix, such as UV photoproducts and bulky chemical additions	Makes endonucleolytic cut on either side of lesion; resulting gap is repaired by DNA polymerase I and DNA ligase
Specific excision	AP endonucleases	AP sites	Makes endonucleolytic cut; exonuclease creates gap, which is repaired by DNA polymerase I and DNA ligase
	DNA glycosylases	Deaminated bases (uracil, hypoxanthine), certain methylated bases, ring-opened purines, oxidatively damaged bases, and certain other modified bases	Removes base, creating AP site, which is repaired by AP endonucleases
	GO system	8-oxo dG	A glycosylase removes 8-oxo dG from DNA; another glycosylase converts any remaining 8-oxo dG–A mispairs into 8-oxo dG–C pairs, and the first glycosylase then removes the 8-oxo dG
Postreplication	Mismatch-repair system	Replication errors resulting in base-pair mismatches	Recognizes newly synthesized strand by detecting nonmethylated adenine residues in 5′-GATC-3′ sequences; then excises bases from the new strand when a mismatch is detected
	Recombinational repair	Lesions that block replication and result in single-stranded gaps	Recombinational exchange
	SOS system	Lesions that block replication	Allows replication bypass of blocking lesion, resulting in frequent mutations across from lesion

Mobile elements and mutation

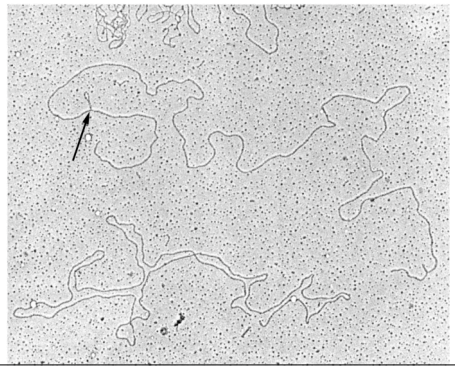
- Also known as transposable elements
- Encode transposase enzyme
- Types of prokaryotic mobile elements
  - insertion sequence (IS)
    - plasmids or chromosome
    - may move from one location to another
    - Transposase & inverted terminal repeats
  - bacterial transposons (TN)
    - include genes conferring drug resistance (R factors)
    - ends consist of identical IS sequences in opposite orientation



Transposable elements



Insertional sequence (IS) in λ*dgal*



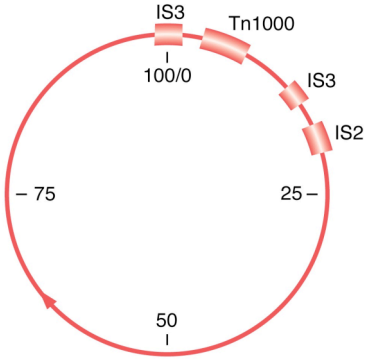
Prokaryotic IS elements

TABLE 10-3 Prokaryotic Insertion Elements

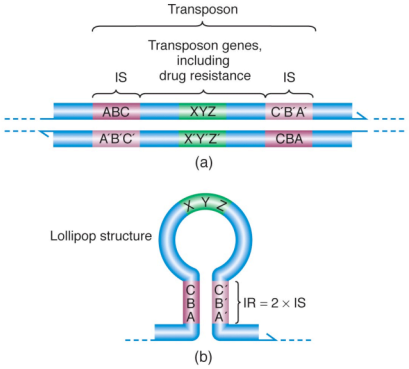
Insertion Sequence	Normal Occurrence in *E. Coli*	Length (bp)	Inverted Repeat (bp)*
IS1	5–8 copies on chromosome	768	18–23
IS2	5 on chromosome; 1 on F	1327	32–41
IS3	5 on chromosome; 2 on F	1400	32–38
IS4	1 or 2 copies on chromosome	1400	16–18
IS5	Unknown	1250	Short

*The numbers represent the lengths of the 5′ and 3′ copies of the imperfect inverted repeats.
SOURCE: M. P. Calos and J. H. Miller, *Cell* 20, 1980, 579–595.

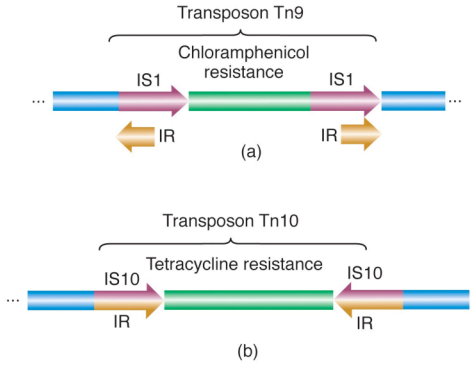
F factor with IS elements



Transposon with inverted repeats



Two different transposons



Transposon

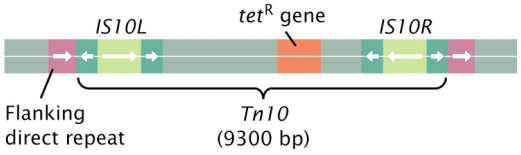


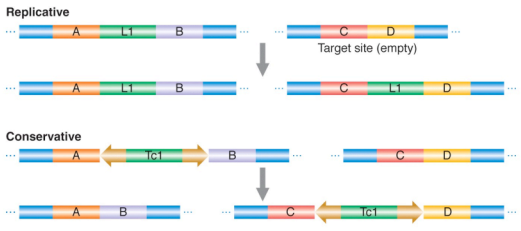
Table 11.5 Characteristics of several composite transposons

Composite Transposon	Total Length (bp)	Associated IS Elements	Other Genes Within the Transposon
Tn9	2500	*IS1*	Chloramphenicol resistance
Tn10	9300	*IS10*	Tetracycline resistance
Tn5	5700	*IS50*	Kanamycin resistance
Tn903	3100	*IS903*	Kanamycin resistance

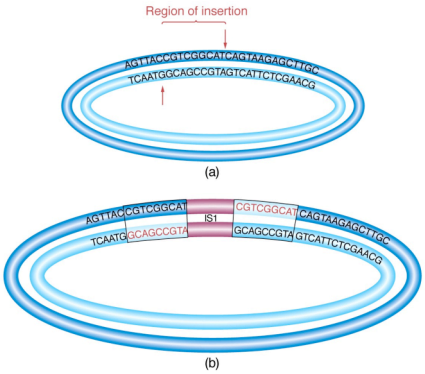
Mechanisms of transposition

- Replicative transposition
  - copy of transposon left behind
  - new copy inserted elsewhere in genome
  - mediated by transposon-specific transposase enzyme
- Conservative transposition
  - excise from location and integrate elsewhere
  - mediate by transposon-specific transposase
  - transposon often flanked by duplicate repeat sequence generated by insertion

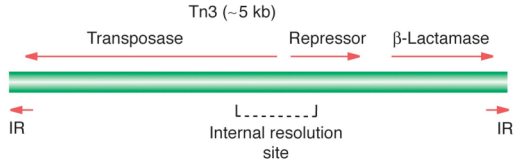
Replicative vs conservative modes



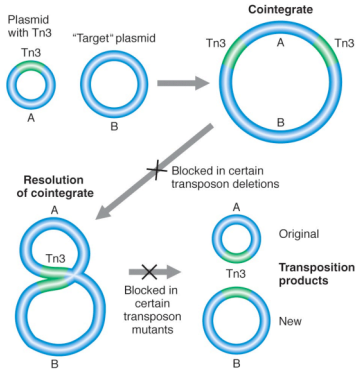
Insertion results in repeats



Tn3 transposon



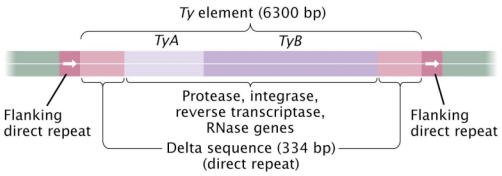
Cointegration



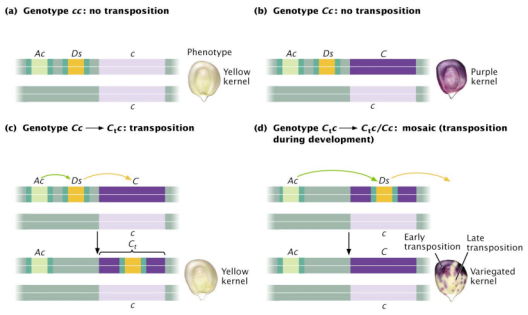
Eukaryotic mobile elements

- Historically, mobile elements were discovered in eukaryotes by genetic analysis
  - Barbara McClintock working with maize
  - Ac (activator) element
  - Unstable mutations
- Mobile elements are common in eukaryotes
  - utilize transposase, as in prokaryotes
  - may cause mutation by insertion into gene
- Examples
  - *Ty* elements in yeast
  - *copia* elements in *Drosophila*
  - *Alu* sequences in humans

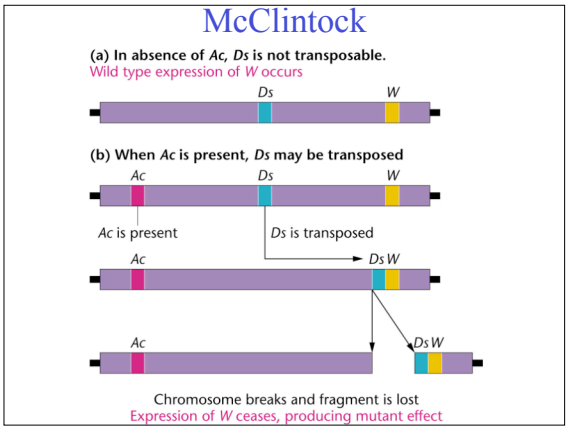
Ty element of yeast



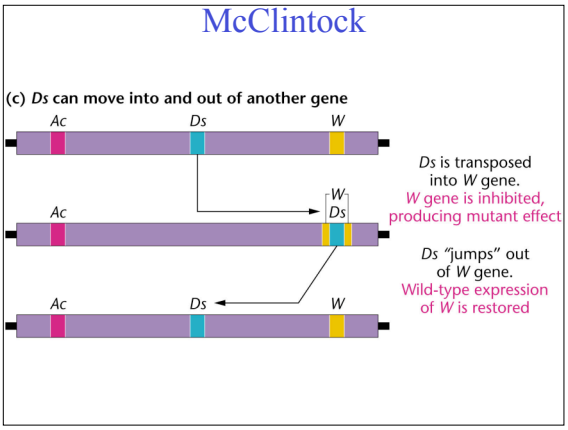
Barbara McClintock



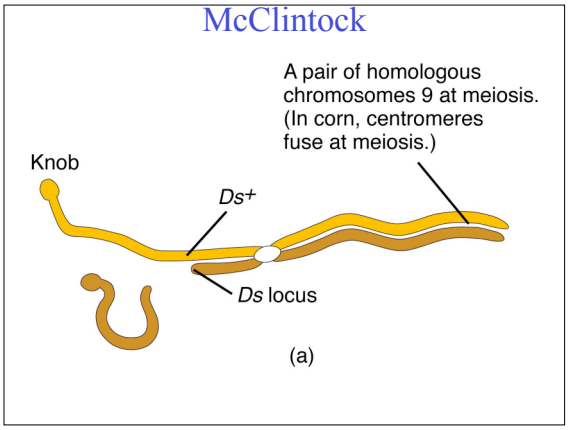
McClintock



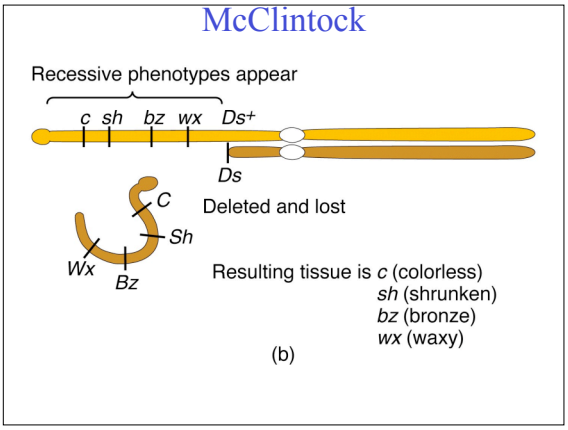
McClintock



McClintock



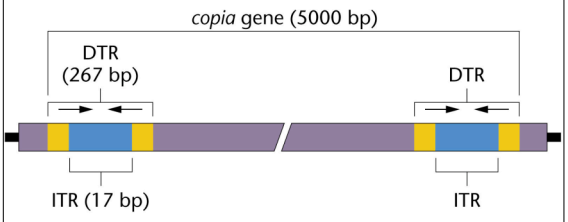
McClintock



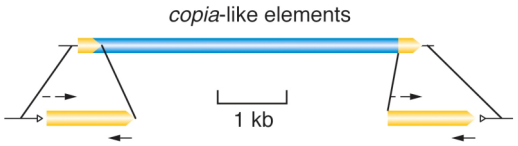
Retroviral-like mobile elements

- Transpose through RNA intermediate
  - e.g., *copia* in *Drosophila*
    - 4-9 kb in length
    - long terminal repeat (LTR)
    - reverse transcriptase copies RNA into DNA
  - may or may not have LTR
- In mammals, includes
  - LINES (long)
    - functional elements
  - SINES (short)
    - nonfunctional elements

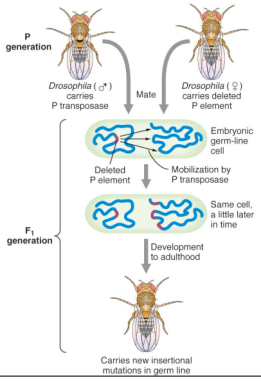
Drosophila



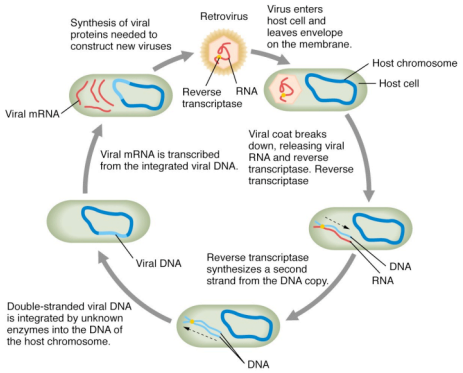
Copia-like elements in *Drosophila*



Crosses



Retroviruses



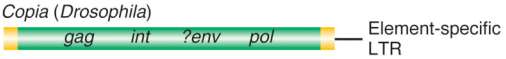
Retrovirus genes

Genes in retroviral DNA and viral retrotransposons

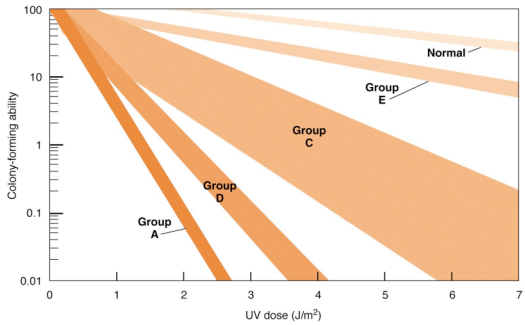
Retroviral DNA

Ty 912 (yeast)

Copia (*Drosophila*)



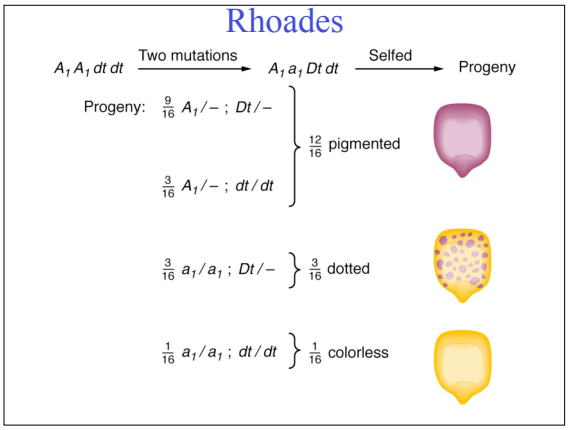
Xeroderma pigmentosum cell sensitivities



Overview

- Mutation changes one allelic form to another and is the ultimate source of genetic variation.
- Mutational variation underlies the study of genetics.
- Mutations are produced by mutagens or occur spontaneously.
- Point mutations include single base-pair substitutions, additions or deletions.
- Some types of mutation can be repaired.
- Specialized forms of mutation include expansion of trinucleotide repeats and insertion of transposable elements.

Rhoades



Rhodes