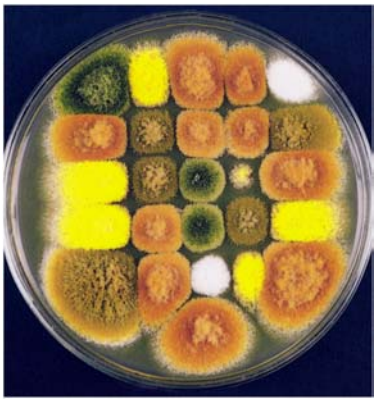
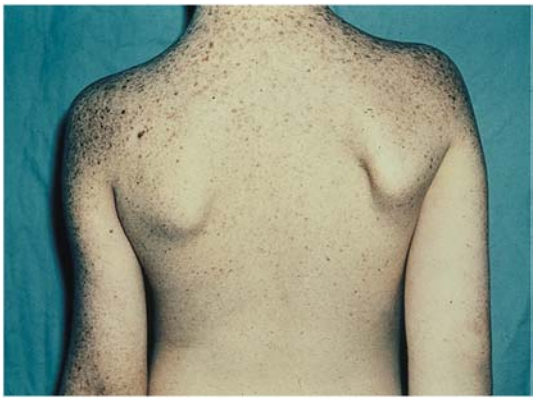
Chapter 10

Gene Mutation: Origins and Repair Processes

GAATTC → GTATTC

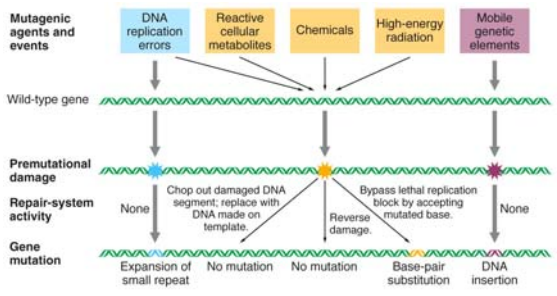
A → *a*





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.



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

Number of Mutations in Flies is Proportional To x-ray dose

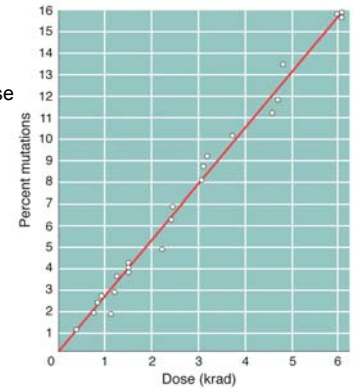


TABLE 10-1 Mutation Frequencies Obtained with Various Mutagens in *Neurospora*

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/mm²/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

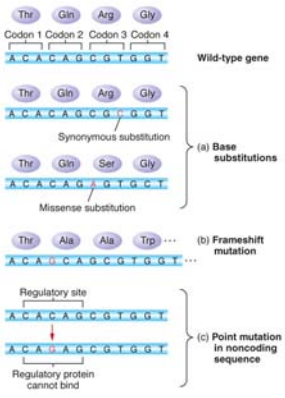
- Single or few base pair changes
- Origin of point mutation
  - induced by geneticist
    - action of mutagen, an environmental agent that alters nucleotide sequence
    - process of inducing mutations by mutagens is called mutagenesis
  - spontaneous
    - arise in absence of known mutagen
    - may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

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)

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



Molecular consequences (1)

- 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)

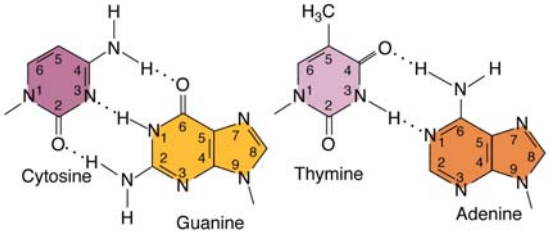
Mechanisms?

- Replacement of a specific base
- Alteration of a specific base
- Damage to a specific base

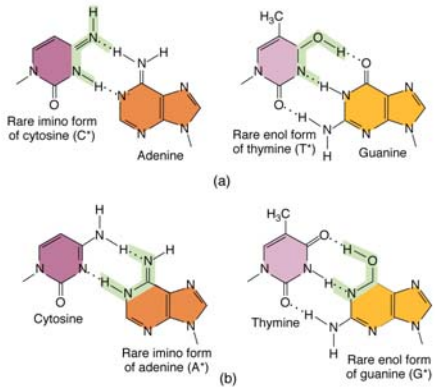
Base analogs are an important cause of spontaneous mutations, since they cause incorrect nucleotides to be inserted, and when that sequence is replicated, the incorrect base sequence becomes part of the genome.

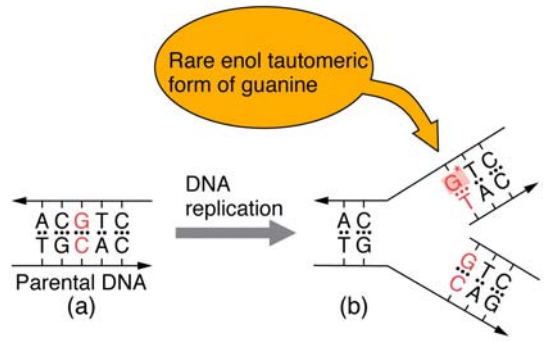
Tautomeric shift

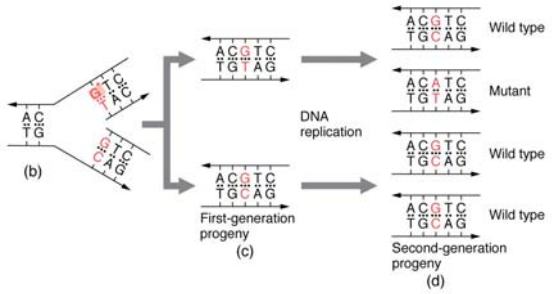
- Natural variation in chemical form of base (isomers)
  - amino (normal) ↔ imino (rare)
  - keto (normal) ↔ enol (rare)
- Results in mutation during DNA replication
  - base in rare tautomeric form pairs with chemically similar base of its normal complement
  - e.g., C·G ↔ C*·G at replication results 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’ base pairing, c:g and t:a



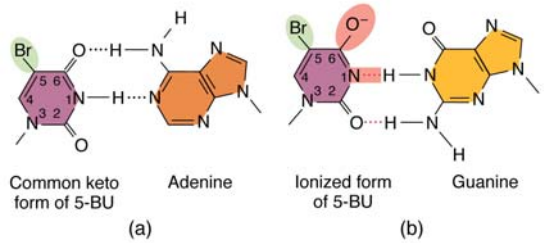




Molecular mechanism (1)

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

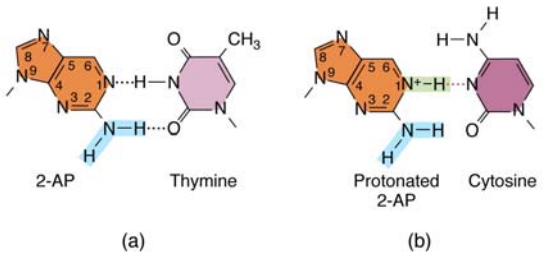
Alternative pairing possibilities for 5-bromouracil (T analog) generating transition mutations at high frequency



here,mimics T, pairs with A

here, mimics C, pairs with G

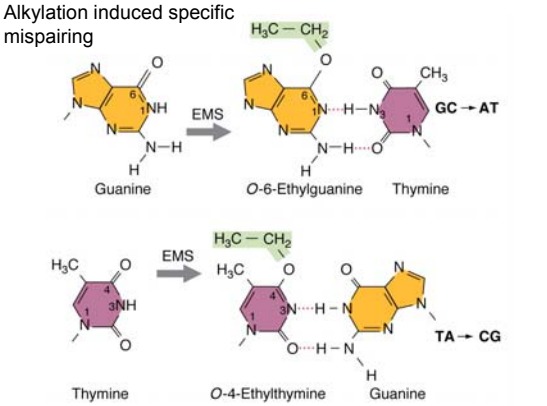
2-aminopurine (A or G analog) specific for transition mutations



Here, it pairs with T

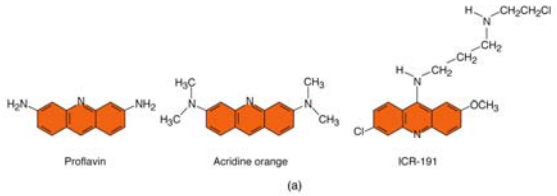
Here, it pairs with C

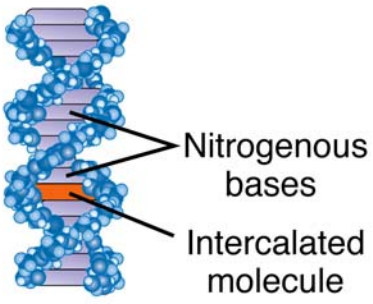
Alkylation induced specific mispairing



Molecular mechanism (2)

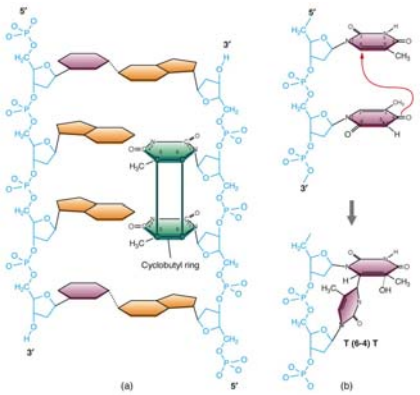
- Intercalating agents
  - flat, planar molecules intercalate between base pairs, disrupt DNA synthesis
  - e.g., proflavin, acridine orange
- Base damage
  - agent alters base so that it has no complement
  - results in replication block and insertion of nonspecific bases by SOS system
- UV light
 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





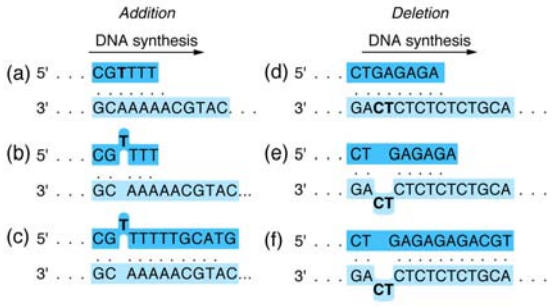
How to bypass a replication block?

- SOS system is a emergency response error prone repair system for allowing cell to survive with significant amounts of DNA damage
- Cells trade off increased level of mutation for ability to replicate DNA, however badly
- Cells survive lethal damage, but are forever changed by mutations, some detrimental



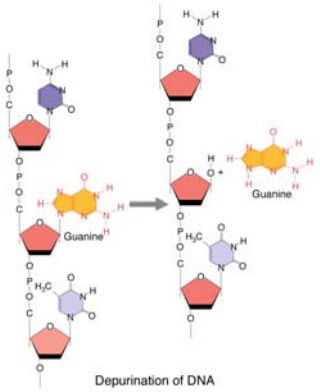
Indel mutations

- Insertion/deletions, usually short repeats of dimer and trimer sequences
- Newly synthesized DNA strands can mispair with the incorrect repeat number on the opposite strand
- Both additions and deletions occur through slip strand mispairing



Spontaneous mutation

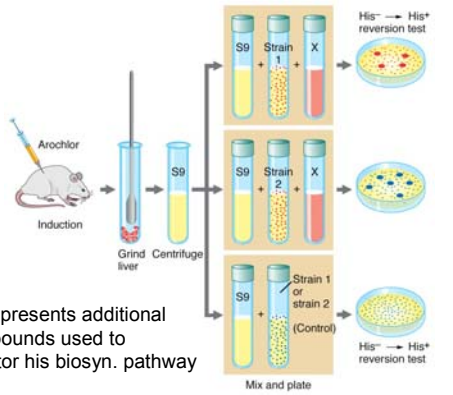
- Tautomeric shift
- Depurination, spontaneous loss of G or A
- Deamination, converts cytosine to uracil which pairs with adenine at replication
- Oxidative damage to bases
  - caused by superoxide and peroxide radicals
  - chemically alter base pairing properties
- Indel mutations
  - result in translation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



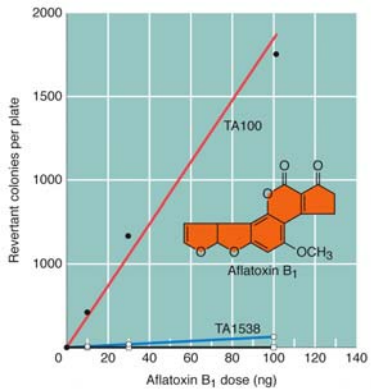
Depurination of DNA

The Ames test for carcinogens

- Bruce Ames recognized that compounds cause cancer because their metabolites mutagenize DNA
- Mutation rates in bacteria can be used to model how dangerous something really is
- Metabolites collected from extracts of rat livers and combined with special strains of Salmonella typhinurium deficient for his biosynthesis
- Bacteria that became his+ (revertants) helped measure mutation rates of compounds tested
- The more carcinogenic a compound is, the higher its rate of his+ colonies scored



'X" represents additional compounds used to monitor his biosyn. pathway



TA 100 is a Base subst. strain

TA 1538 Is an indel (frameshift) strain

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

FMR –1 gene inserts, fragile x syndrome in 5'UTR of mRNA