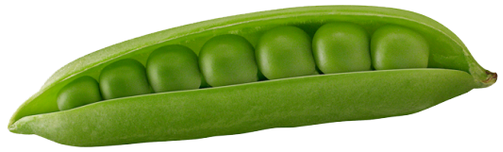
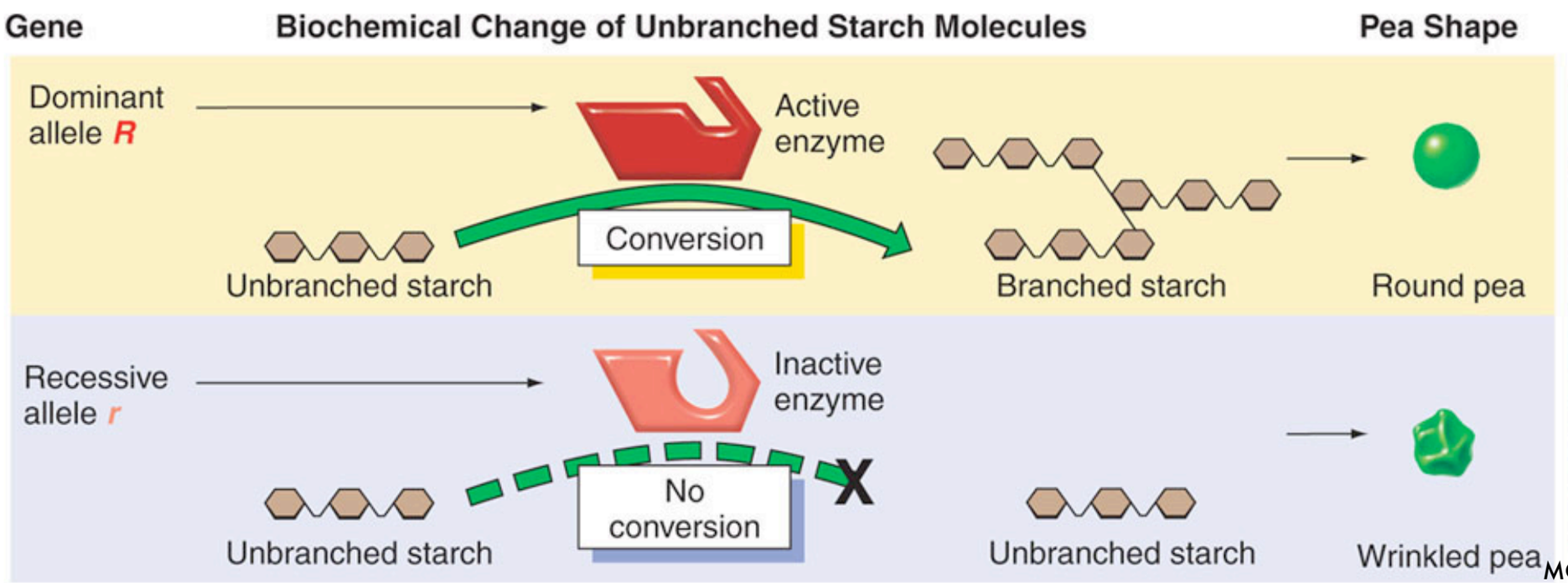
# Genetic analysis is extremely powerful, but also limited in the absence of other types of information

Mendel was interested in variation among peas as a formalism - because he realized that these "phenotypes" could tell him about how traits are transmitted from parents to progeny.



He may have been interested (a bit) in what made one pea wrinkled and one round, but there was no way to figure this out from genetics alone.

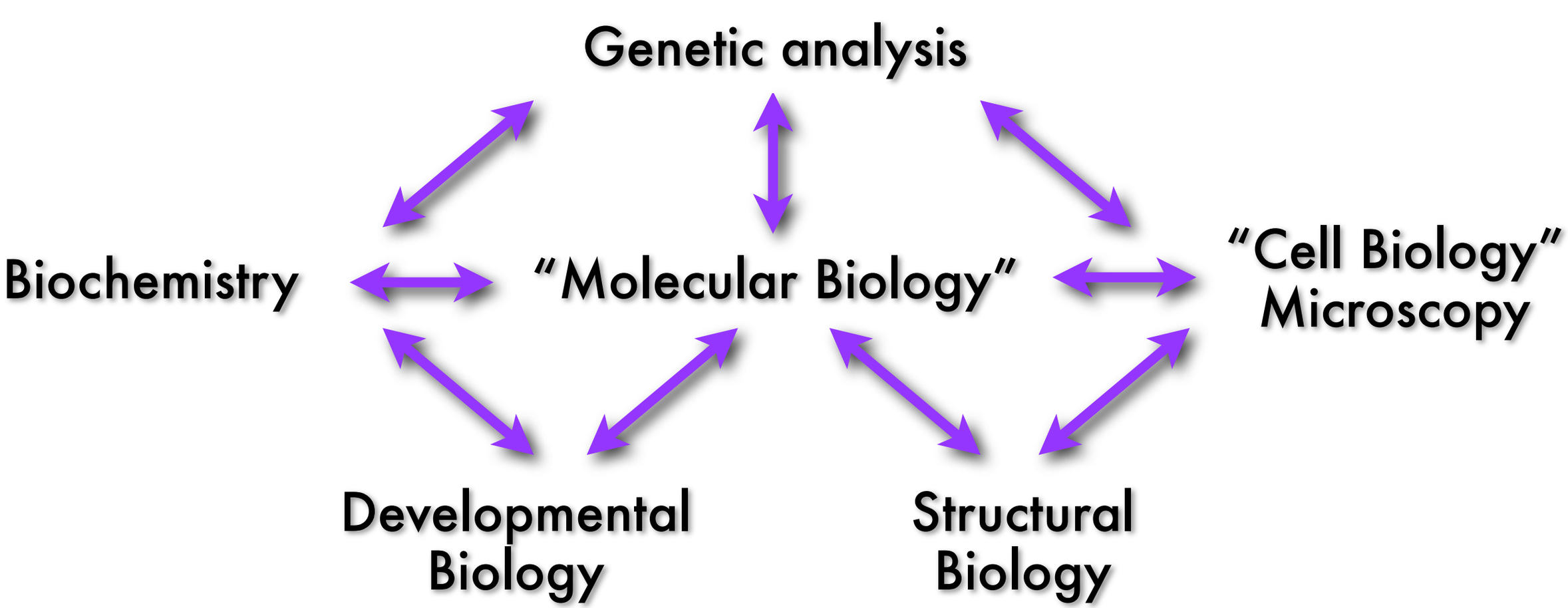

We now know that the "wrinkled" phenotype is due to a defect in an enzyme (a PROTEIN) that converts unbranched starch chains into branched ones.



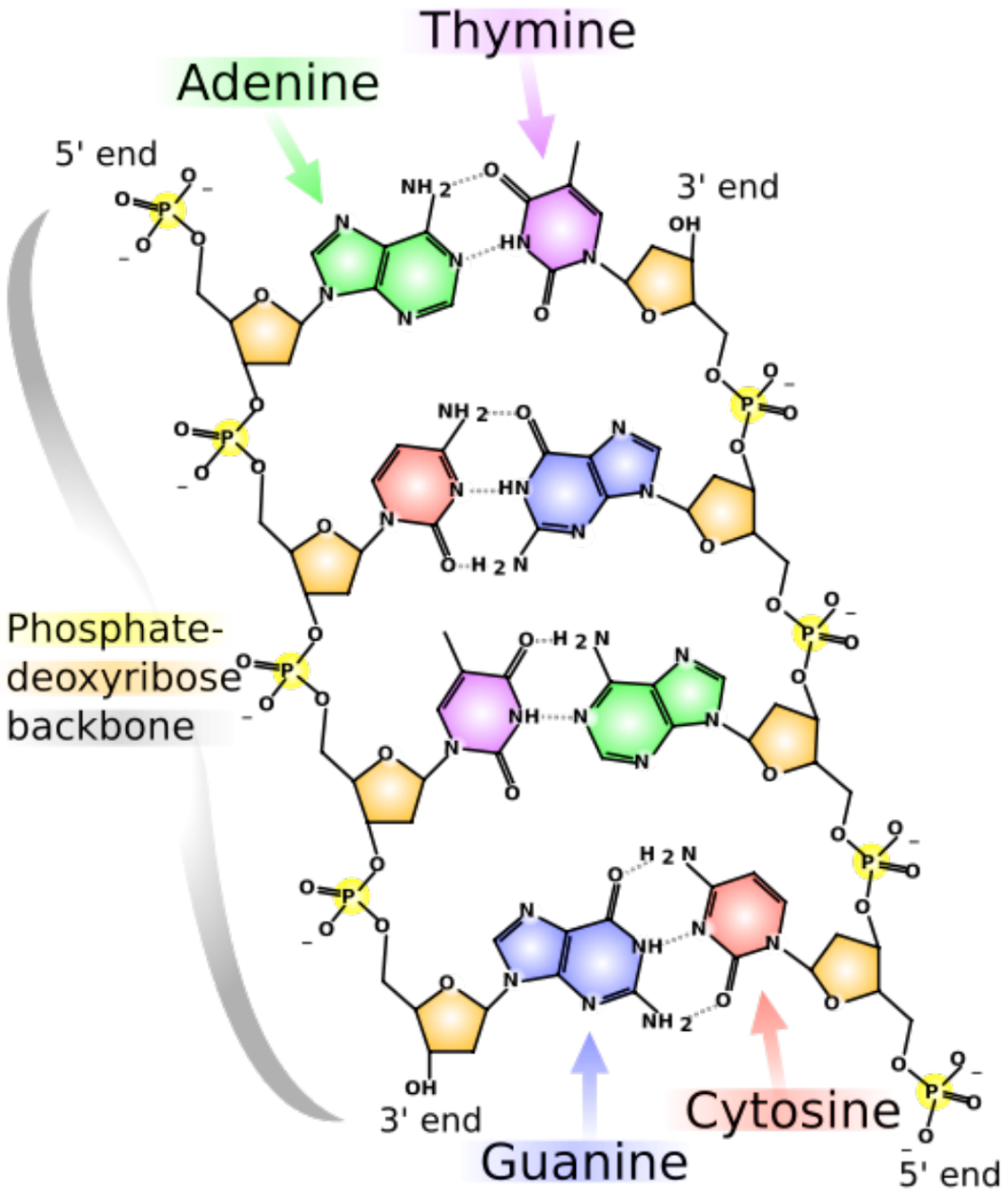

Genetics is both a field in itself (the study of inheritance)
and an experimental tool that has been exploited
in every arena of biological research

Genetic analysis (by Mendel, Sturtevant, Bridges, and many others)
revealed how genetic information is transmitted

To understand how genetic information results in "phenotypes"
required developments in many other areas of biology



# From Lecture 1: Information encoded in DNA generates functional diversity



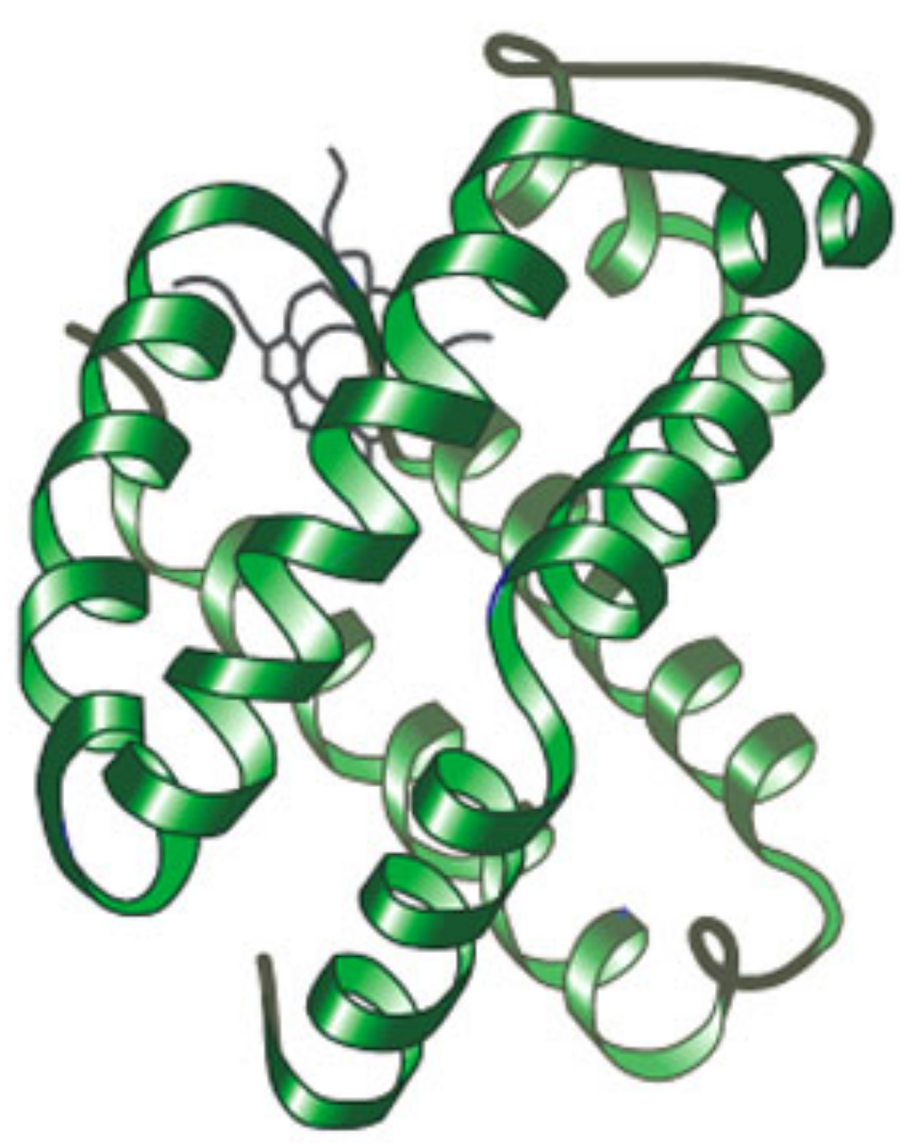

Four bases form the nucleotide building blocks of DNA:

- G (guanine)
- A (adenine)
- T (thymine)
- C (cytosine)

DNA is a double stranded helix composed of A-T and G-C complementary bases.

The DNA sequence "encodes" the amino acid sequence of the proteins that are made. Regulatory information in the DNA specifies when and where the synthesis occurs.

# From Lecture 1:
# Amino acid sequences determine the 3D structures and functions of proteins



Hemoglobin β chain


Lactate dehydrogenase

# Chapter 7
# Anatomy and Function of a Gene: Dissection Through Mutation

To connect genes to phenotype required several major conceptual advances that helped to reveal what genes actually ARE, and how they control function.

Chapter 7 describes some of the key experiments that led to our modern, molecular understanding of gene function.

Some of these are confusing (to me, at least) because they take us away from the diploid genetics we've discussed so far into organisms that transmit genetic information in ways we haven't yet discussed. These include bacteria, like *E. coli*, and viruses that infect bacteria (bacteriophages), like T4.

Also, the chapter alternates between a historical perspective and a more contemporary one. CONFUSING!

So we'll take it slow...

# Mutations:

## Primary tools of genetic analysis

***Mutations*** are *heritable* changes in DNA base sequence that modify the information content of the DNA

***Forward mutation*** = a change from the "wild-type" to a new allele

**Spontaneous forward mutations are rare.**

**How rare, you ask?**

**b**

| Locus[a] | Number of gametes tested | Number of mutations | Mutation rate ($\times 10^{-6}$) |
|---|---|---|---|
| *a⁻ (albino)* | 67,395 | 3 | 44.5 |
| *b⁻ (brown)* | 919,699 | 3 | 3.3 |
| *c⁻ (nonagouti)* | 150,391 | 5 | 33.2 |
| *d⁻ (dilute)* | 839,447 | 10 | 11.9 |
| *ln⁻ (leaden)* | 243,444 | 4 | 16.4 |
| | 2,220,376 | 25 | 11.2 (average) |

[a] Mutation is from wild type to the recessive allele shown.

**Note, this is per gamete, not per cell division.**

***Reverse mutation*** = a change that restores the wild-type allele (a.k.a. ***reversion***)

**Reversion is (usually) much less frequent than forward mutation.**

# Mutations:

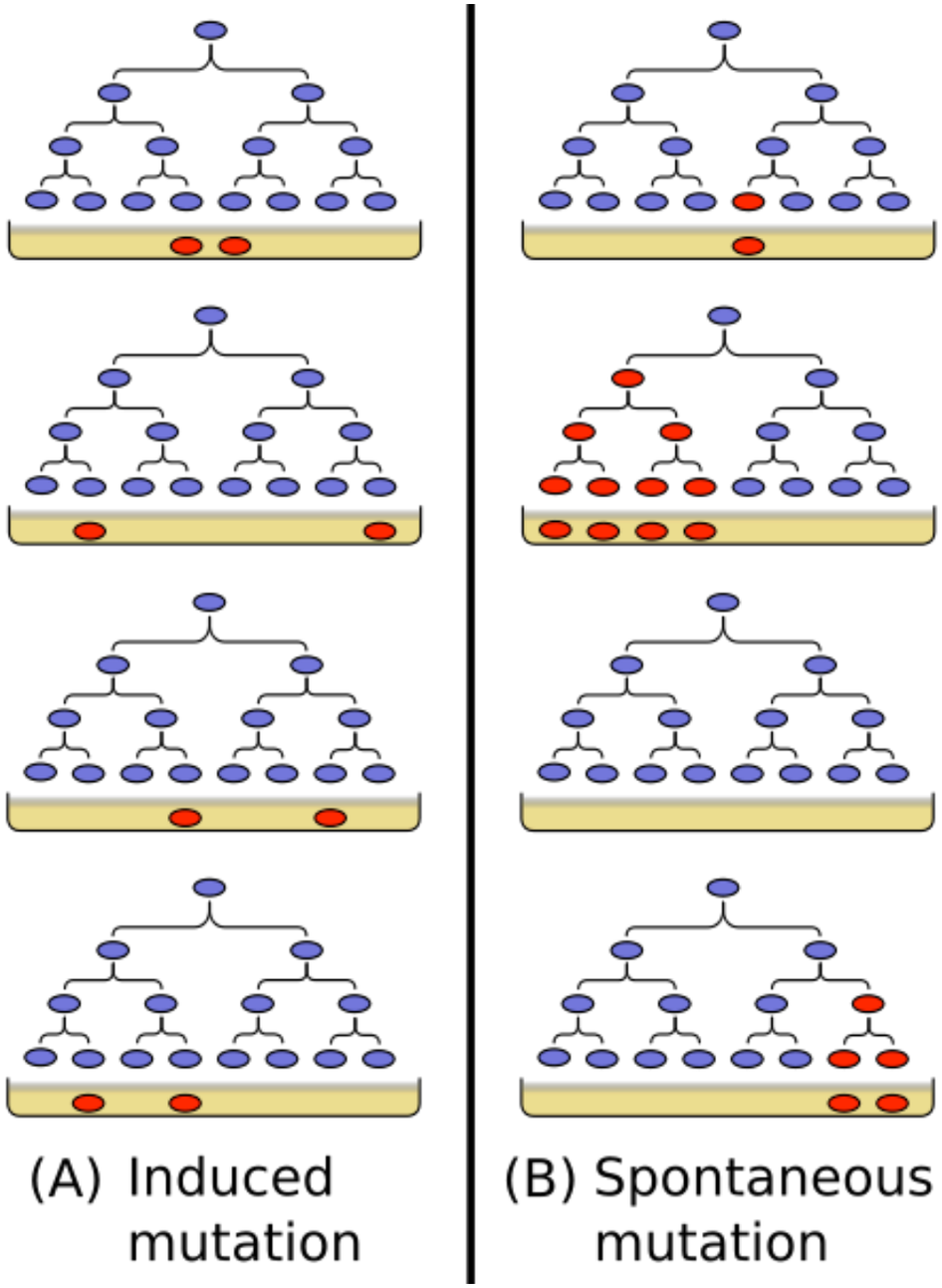
## Primary tools of genetic analysis

***Mutations*** are *heritable* changes in DNA base sequence that modify the information content of the DNA

In sexually-reproducing organisms, a mutation that occurs in the *soma* can only be inherited by daughter cells arising through mitotic cell division. Only *germline* mutations can be transmitted to offsping.

In bacteria, phage, and single-celled eukaryotes (like yeast), there is no distinction between soma and germline. Any mutation can and will be transmitted to the offspring.

# Luria and Delbrück (1943) did experiments to test whether mutations arise at random or in response to "selection"

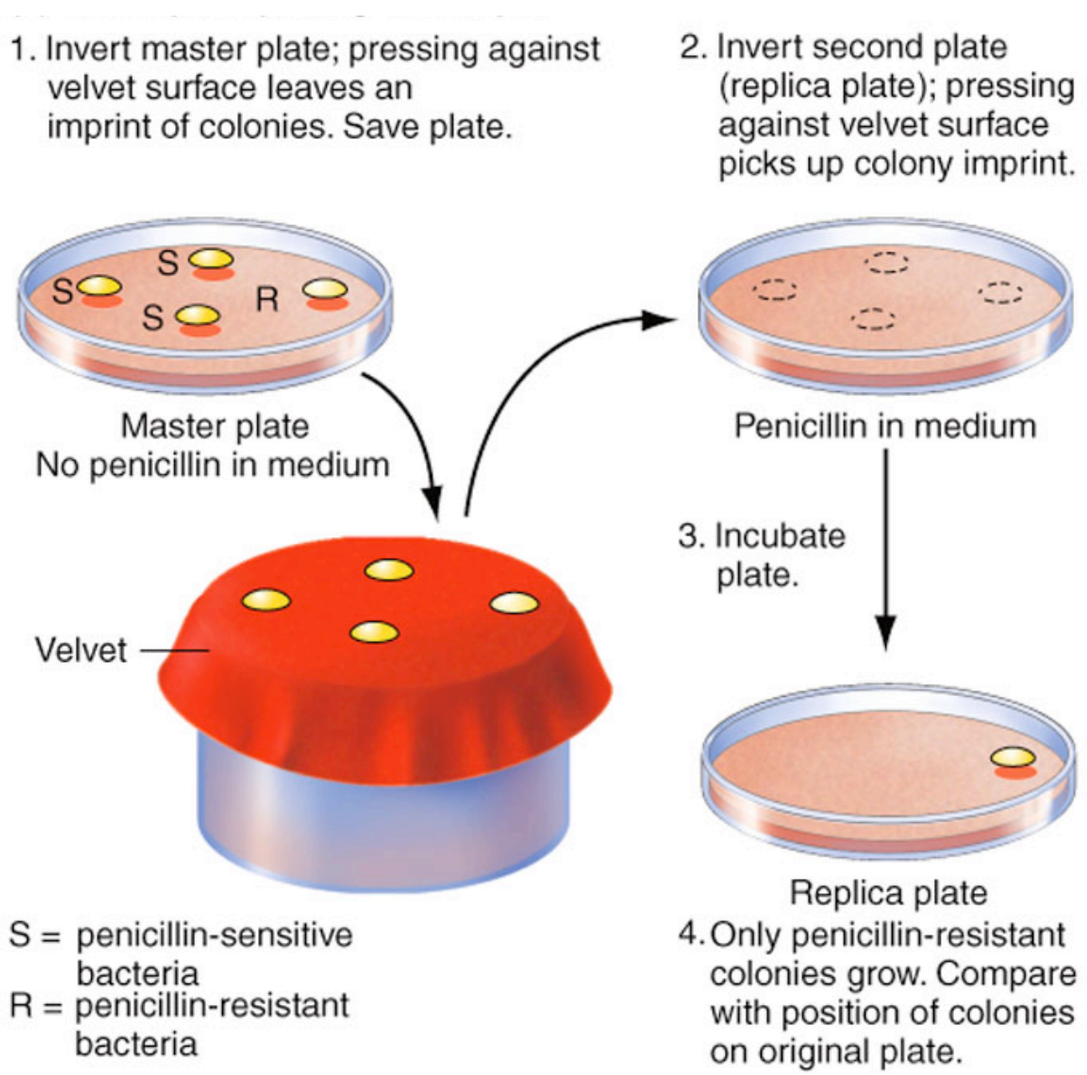


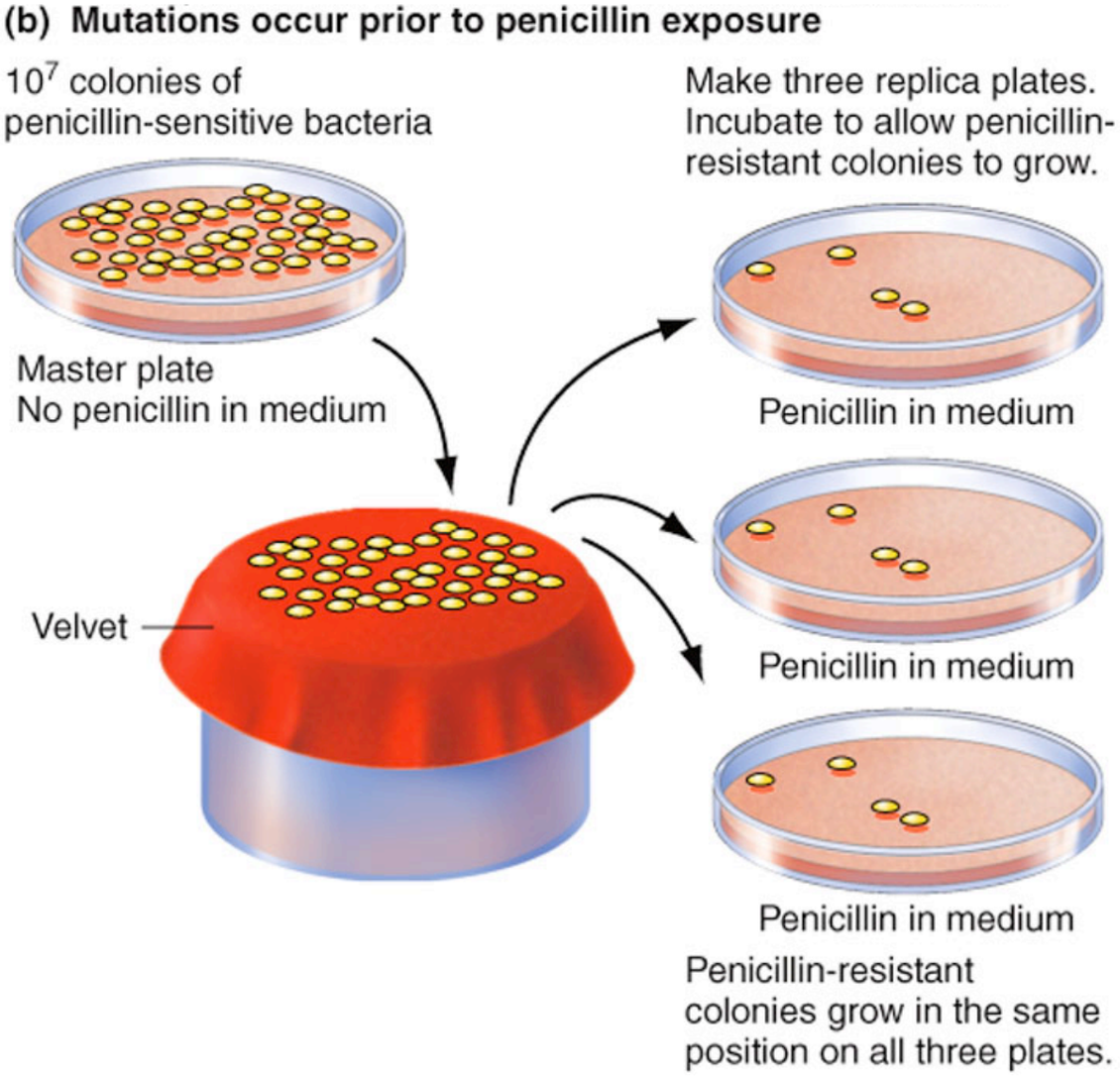

Experiment: split an initially homogeneous population of bacteria into many small subpopulations. Allow these bacteria to grow for many generations, then plate on "selective" media containing an antibiotic.
Some antibiotic-resistant bacteria will arise.
If they arise at random in the culture BEFORE ever seeing the antibiotic, then the total number of resistant bacteria in each subculture should vary greatly (depending on when in the growth of the culture the mutation first arose). On the other hand, if the antibiotic "induces" mutations, the fraction of resistant bacteria in the culture should be more similar (since all cultures should have the same response to this induction).

This "Fluctuation Test" revealed that mutations arise spontaneously in a population, presumably due to some "natural" mutagenic process(es).
This provided strong support for Darwin's ideas about natural selection acting on naturally arising variation.
Salvador Luria and Max Delbrück received the Nobel Prize in 1969 for this important contribution.

# Another way to show the same thing: replica plating

**_E. coli_ "colonies" grown on agar start out as a single bacterium, and typically contain about $10^7$-$10^8$ (10-100 million) bacteria (~25 generations)**



# Where do mutations come from?

1. Spontaneous chemical degradation of DNA

2. Errors in DNA replication that are not corrected

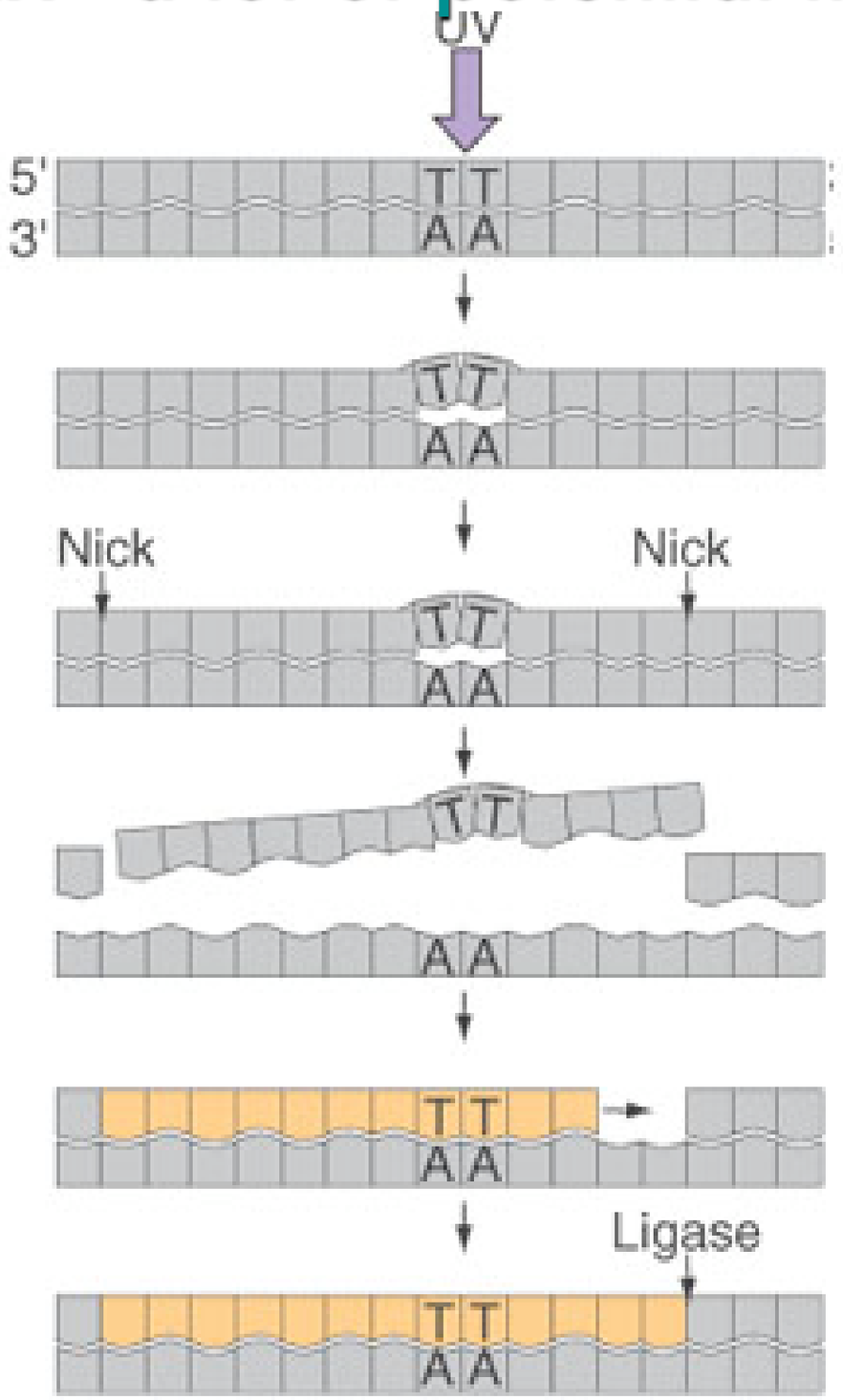
3. Unequal crossing-over or other events during meiosis

4. Transposon ("jumping genes") activity

5. Mutagens - chemicals , radiation, or other agents that damage DNA

# DNA repair mechanisms "fix" a lot of potential mutations

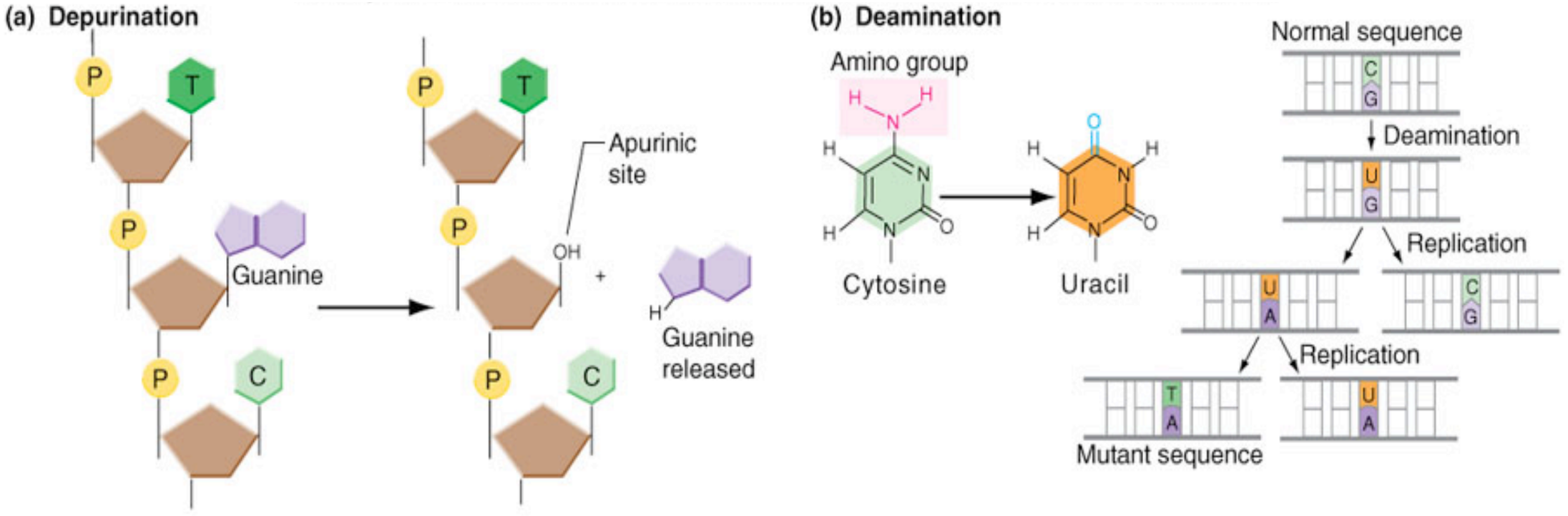
1. Exposure to UV light.
2. Thymine dimer forms.
3. Endonuclease nicks strand containing dimer.
4. Damaged fragment is released from DNA.
5. DNA polymerase fills in the gap with new DNA (yellow).
6. DNA ligase seals the repaired strand.




## Mutations are errors that escape from being repaired

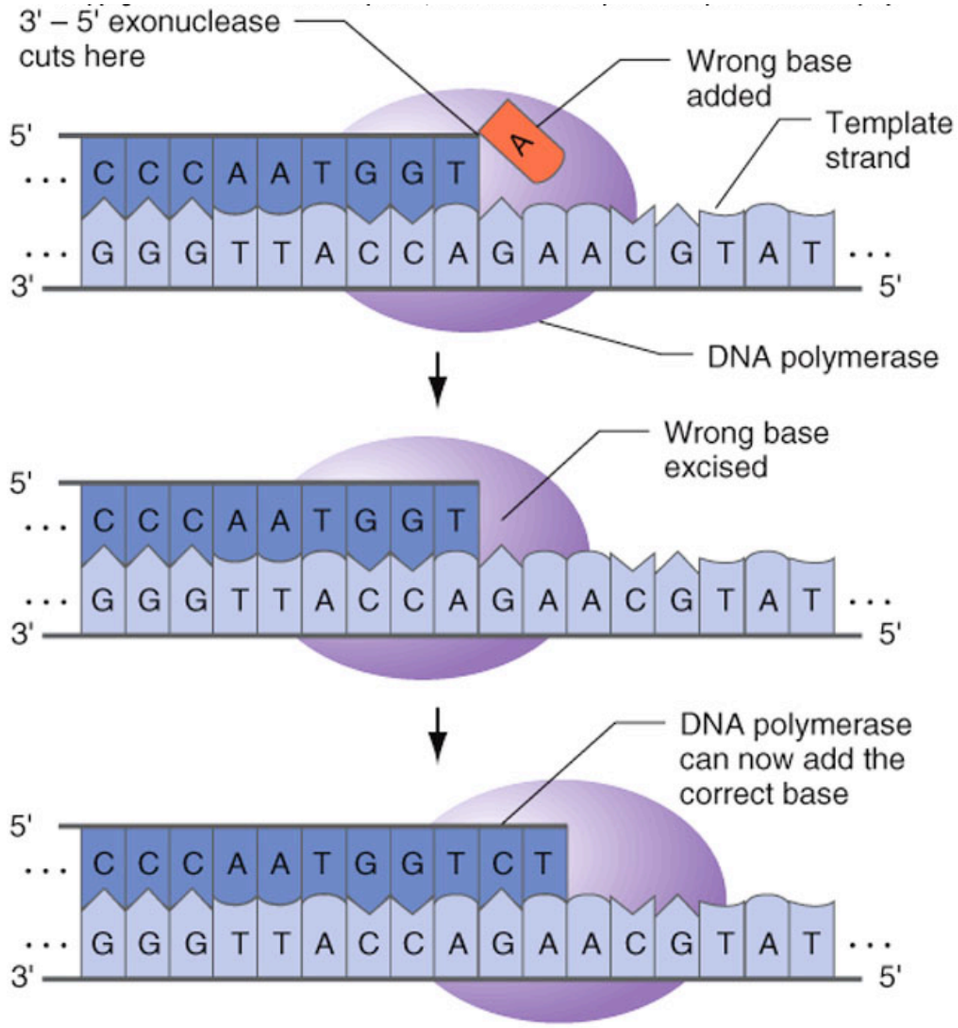
# Where do mutations come from?

## 1. Spontaneous chemical degradation of DNA



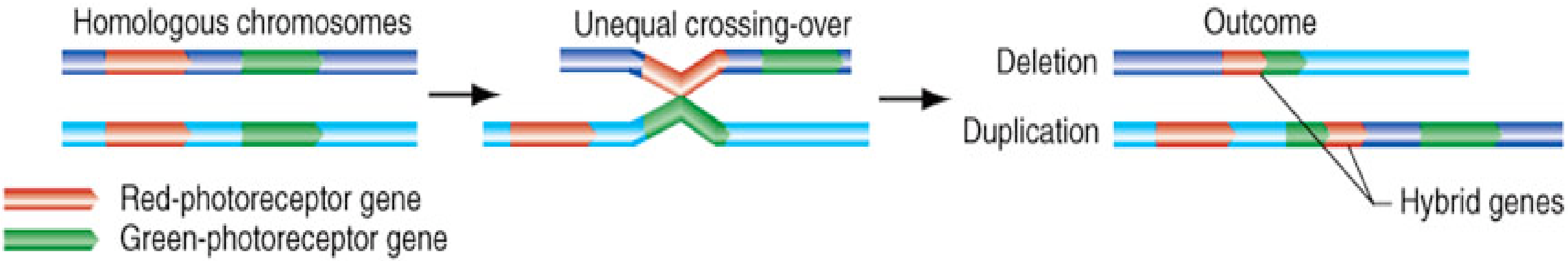
# Where do mutations come from?

## 2. Errors in DNA replication that are *not* corrected



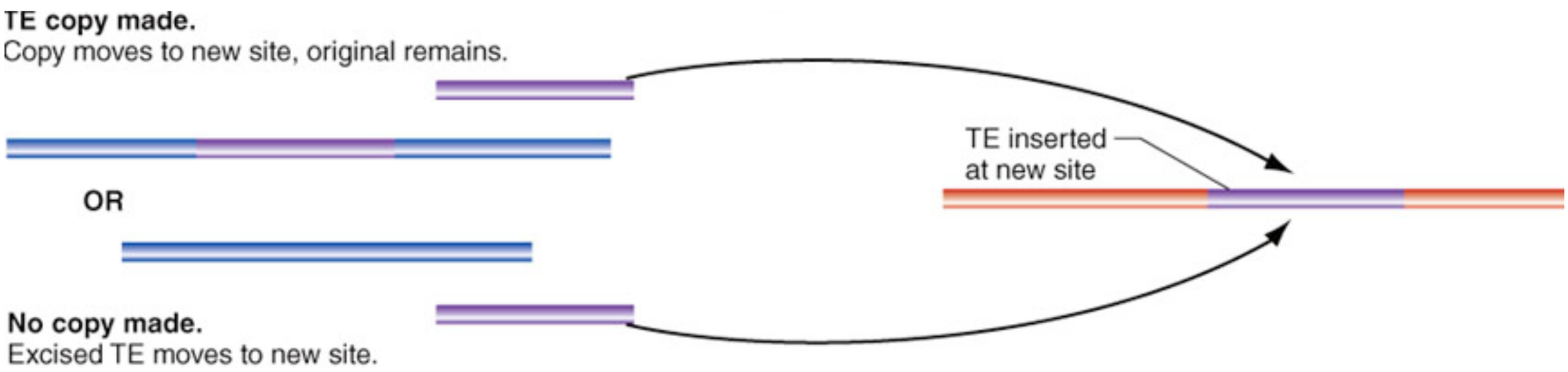
# Where do mutations come from?

## 3. Unequal crossing-over or other events during meiosis



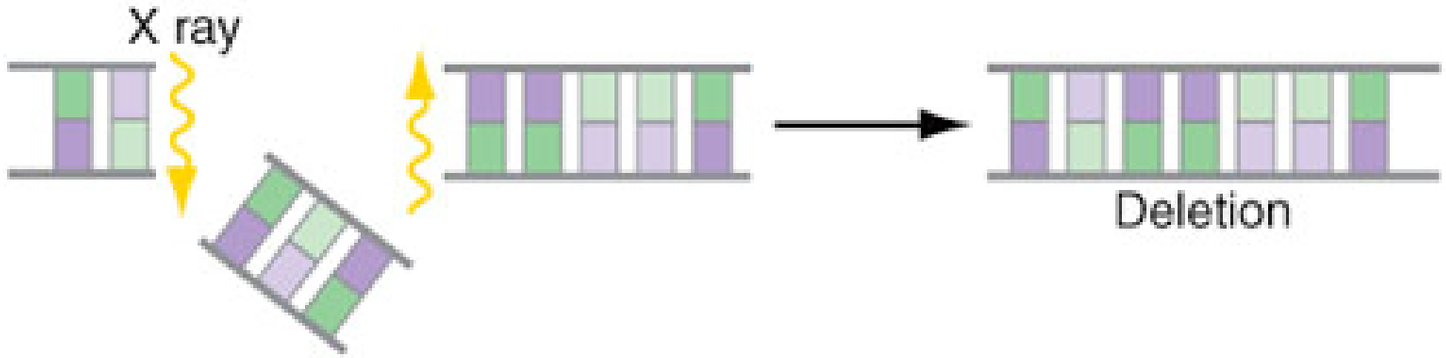
# Where do mutations come from?

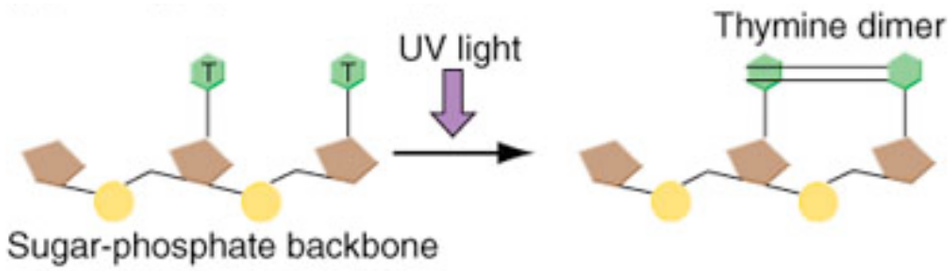
## 4. Transposon ("jumping genes") activity

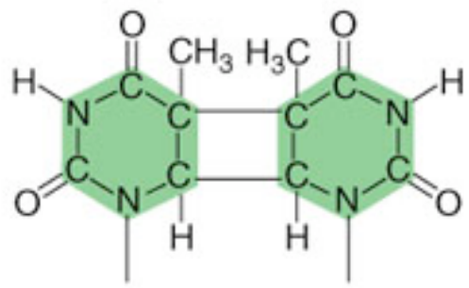


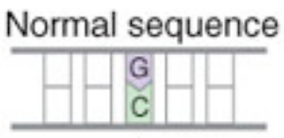
# Where do mutations come from?

5. Mutagens - chemicals , radiation, or other agents that damage DNA



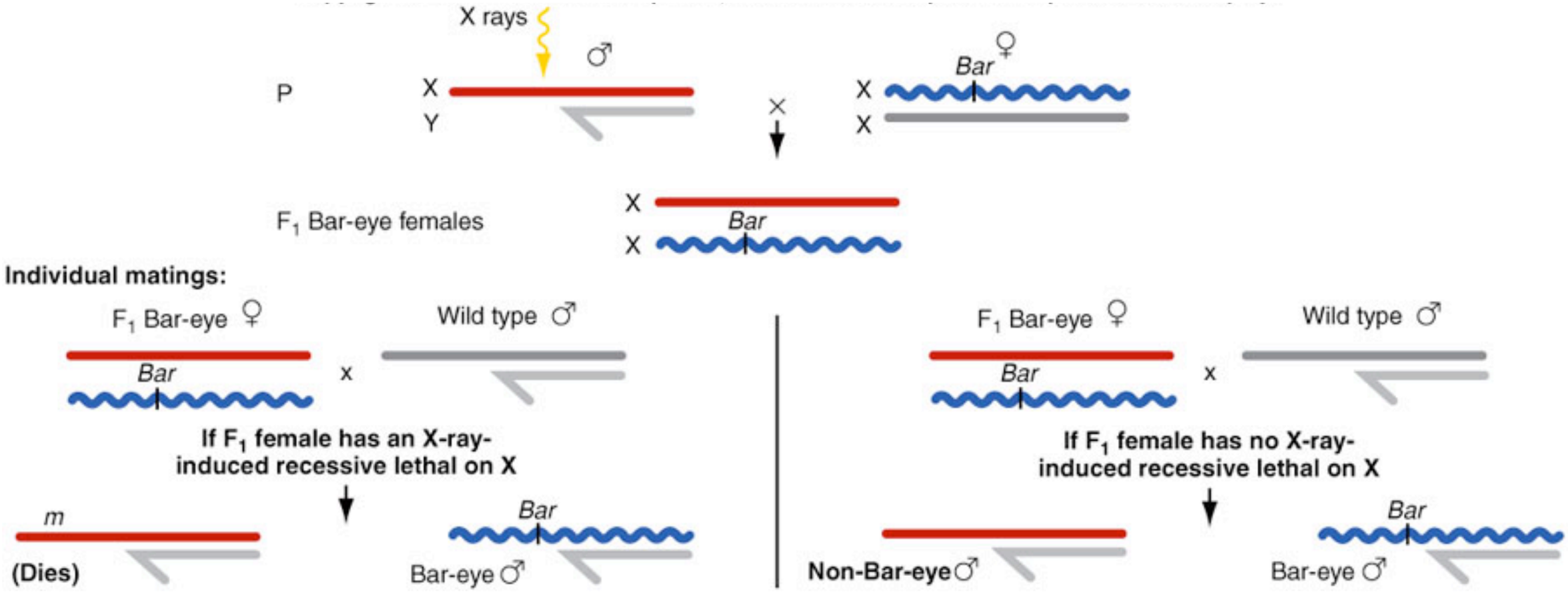



# MUTAGENS: Chemical or physical agents that increase the rate of mutation above the "spontaneous" baseline

Hermann Muller studied mutation in *Drosophila melanogaster* and discovered the first mutagen: X-rays

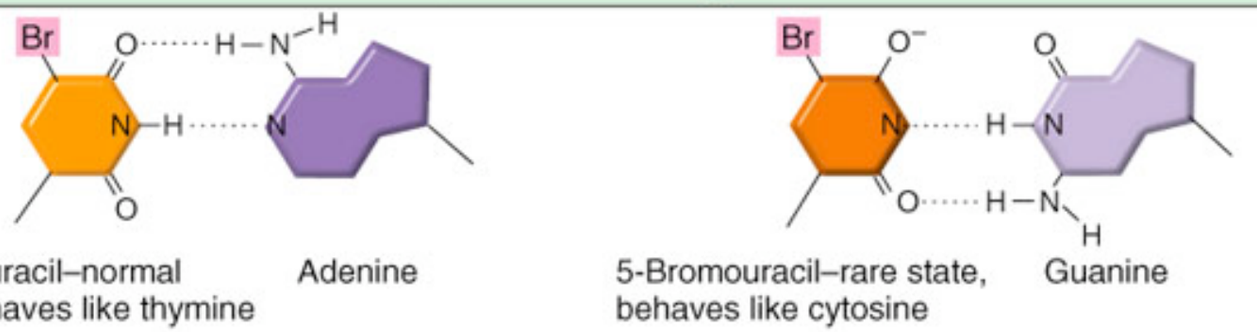
# Quantitative analysis of mutation rates in Drosophila revealed a dependence on radiation dose




In addition to producing genetically-detectable lethal mutations, X-rays produce visible changes in chromosome structure (translocations)

Note: Although it's common to do experiments involving thousands or tens of thousands of fruit flies, it's a lot easier to see spontaneous mutations arise in bacteria, where you can easily sift through BILLIONS of individual organisms

# Many known mutagens are chemicals that interact with DNA and cause damage

| Type of mutagen | Chemical action of mutagen |
|---|---|
| **(a) Replace a base:** Base analogs have a chemical structure almost identical to that of a DNA base. |  |

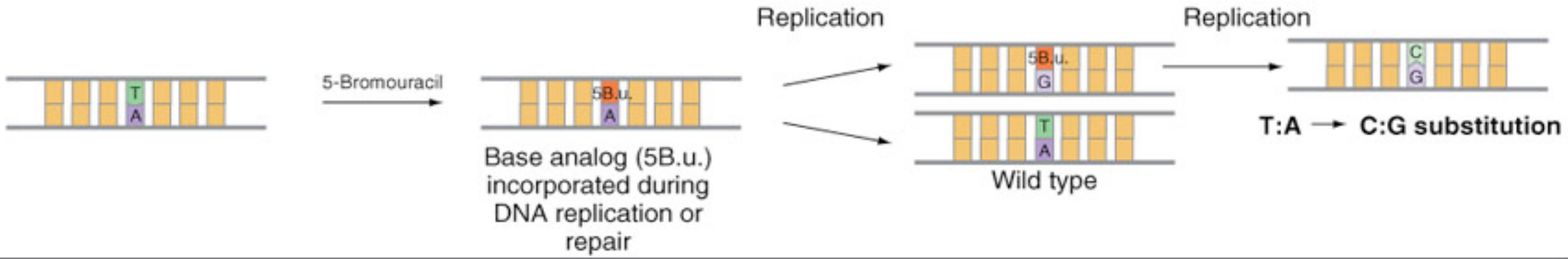


5-Bromouracil–normal state, behaves like thymine — Adenine

5-Bromouracil–rare state, behaves like cytosine — Guanine

5-Bromouracil: almost identical to thymine. Normally pairs with A; in transient state, pairs with G.

**How mutagens induce mutations**





Note: mutagens are often carcinogenic (cancer-causing)

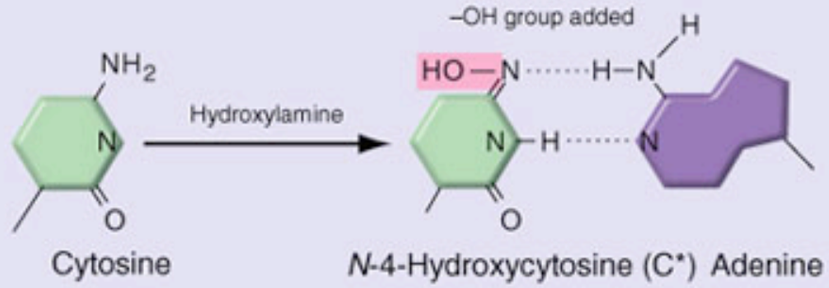
...but they are also used as chemotherapeutic agents!

(e.g. 5-fluoro-2'-deoxyuridine)

# Many known mutagens are chemicals that interact with DNA and cause damage

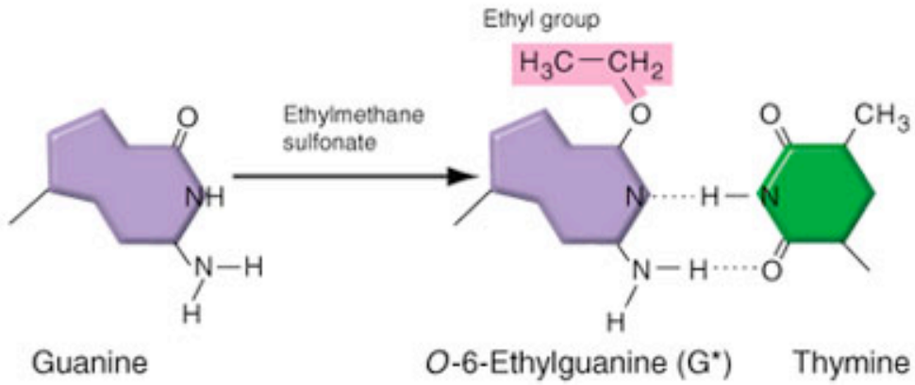
| Type of mutagen | Chemical action of mutagen |
|---|---|

**(b) Alter base structure and properties:**
*Hydroxylating agents*: add a hydroxyl (–OH) group



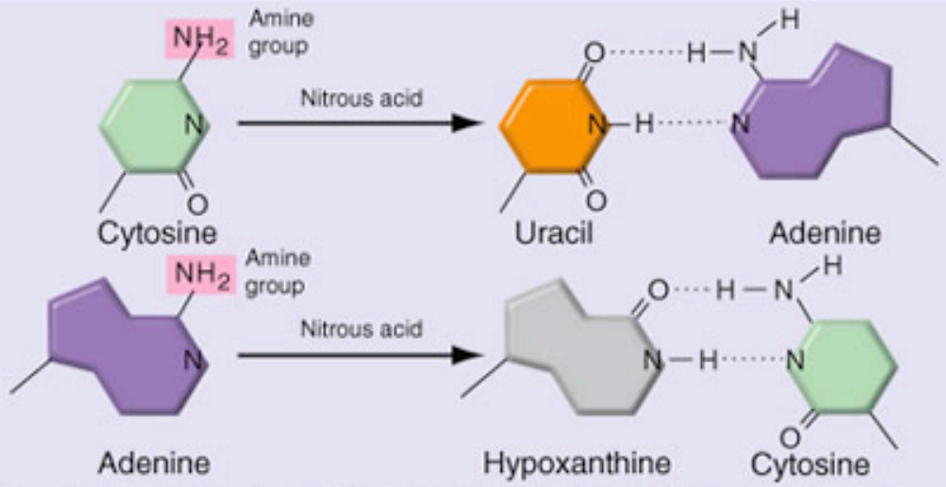

Hydroxylamine adds –OH to cytosine; with the –OH, hydroxylated C now pairs with A instead of G.

*Alkylating agents*: add ethyl ($-CH_2-CH_3$) or methyl ($-CH_3$) groups




Ethylmethane sulfonate adds an ethyl group to guanine or thymine. Modified G pairs with T above, and modified T pairs with G (not shown).

*Deaminating agents*: remove amine ($-NH_2$) groups

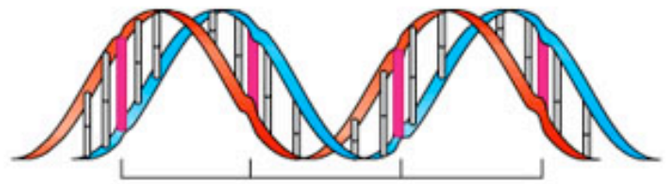



Nitrous acid modifies cytosine to uracil, which pairs with A instead of G; modifies adenine to hypoxanthine, a base that pairs with C instead of T.

# Many known mutagens are chemicals that interact with DNA and cause damage

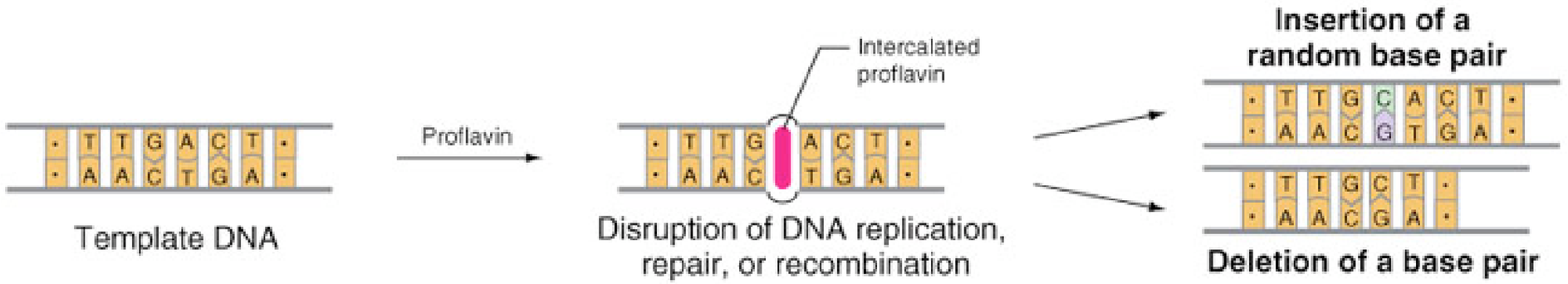
| Type of mutagen | Chemical action of mutagen |
|---|---|
| **(c) Insert between bases:** Intercalating agents | |


Proflavin

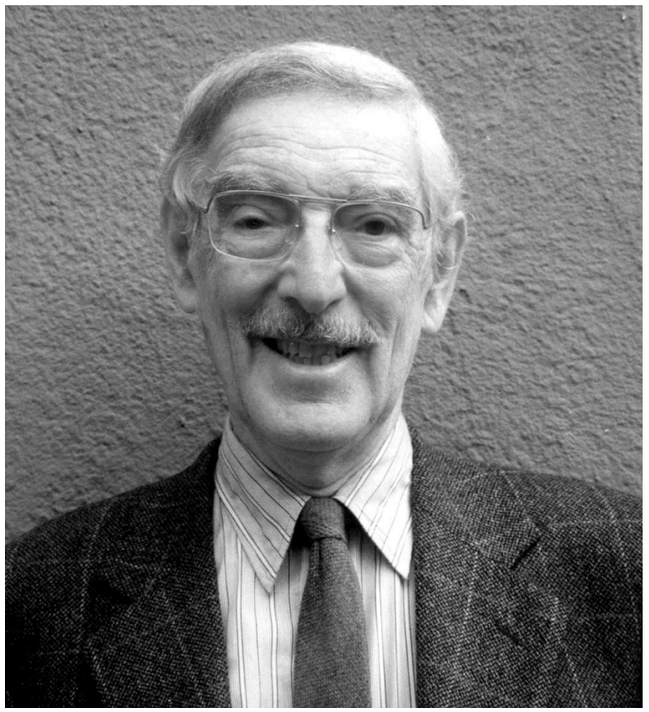


Intercalated proflavin molecules

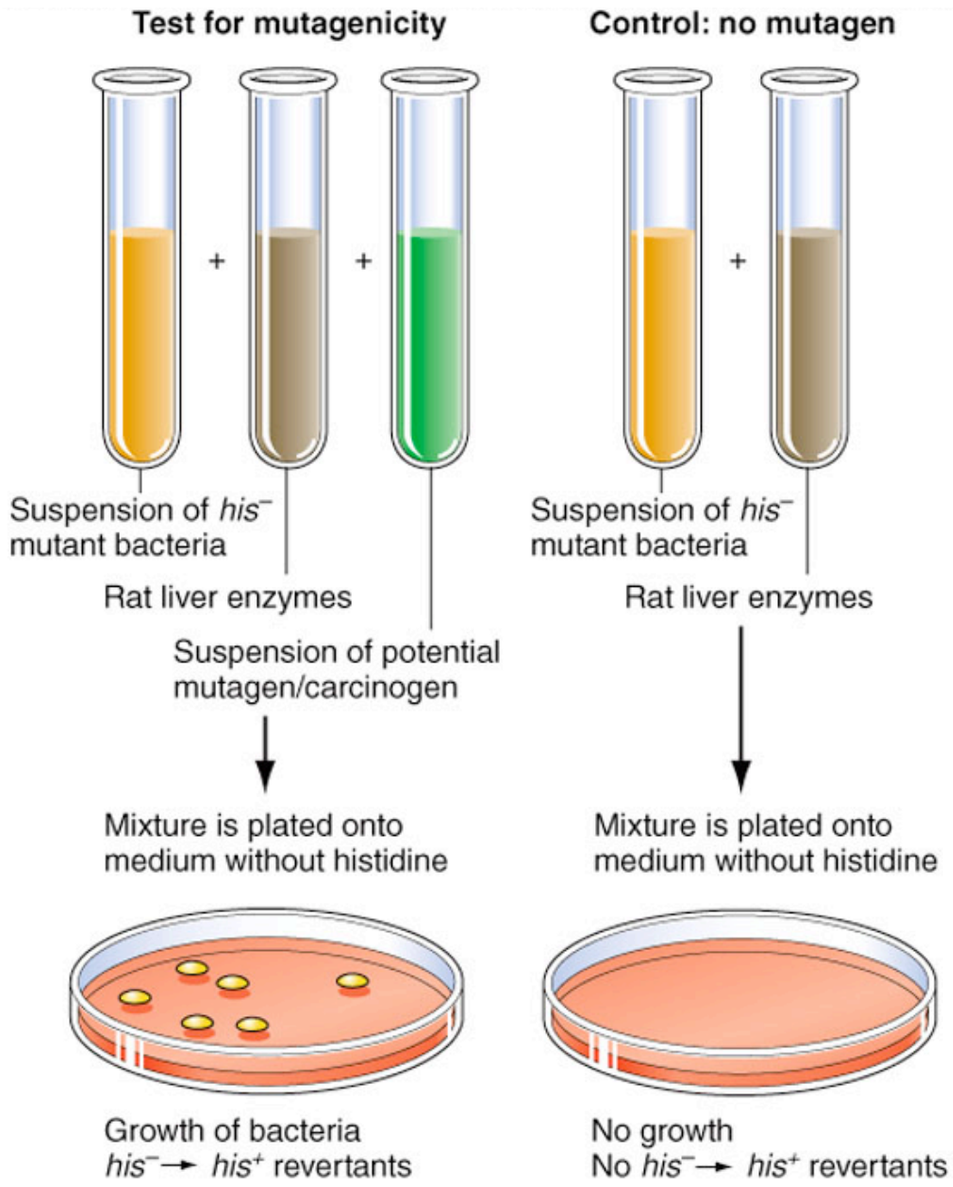
Proflavin intercalates into the double helix. This disrupts DNA metabolism, eventually resulting in deletion or addition of a base pair.



# The Ames test provides a way to quantify the mutagenic activity of chemical compounds



Bruce Ames,
Professor Emeritus UCB




The bacteria used in this test contain *his*⁻ mutations that can be "reverted" by point mutations or frameshifts. They also are deliberately crippled for repair processes that would normally reduce the effects of mutagens.

Rat liver enzymes are added to simulate the metabolic processes of eukaryotic cells - they convert the initial compounds being tested into a range of potential metabolites that would arise in humans.

The Ames test provides valuable information, but the FDA also requires tests in eukaryotic cells and rodents.

# Classification of mutations by their effects on the DNA molecule

- **Substitution**: base is replaced by one of the other three bases
- **Deletion**: block of one or more DNA pairs is lost
- **Insertion**: block of one or more DNA pairs is added
- **Inversion**: 180˚ rotation of piece of DNA
- **Reciprocal translocation**: parts of nonhomologous chromosomes change places
- **Chromosomal rearrangements**: affect many genes at one time

On Friday:

One-gene-one-enzyme (Beadle and Tatum)

Complementation tests