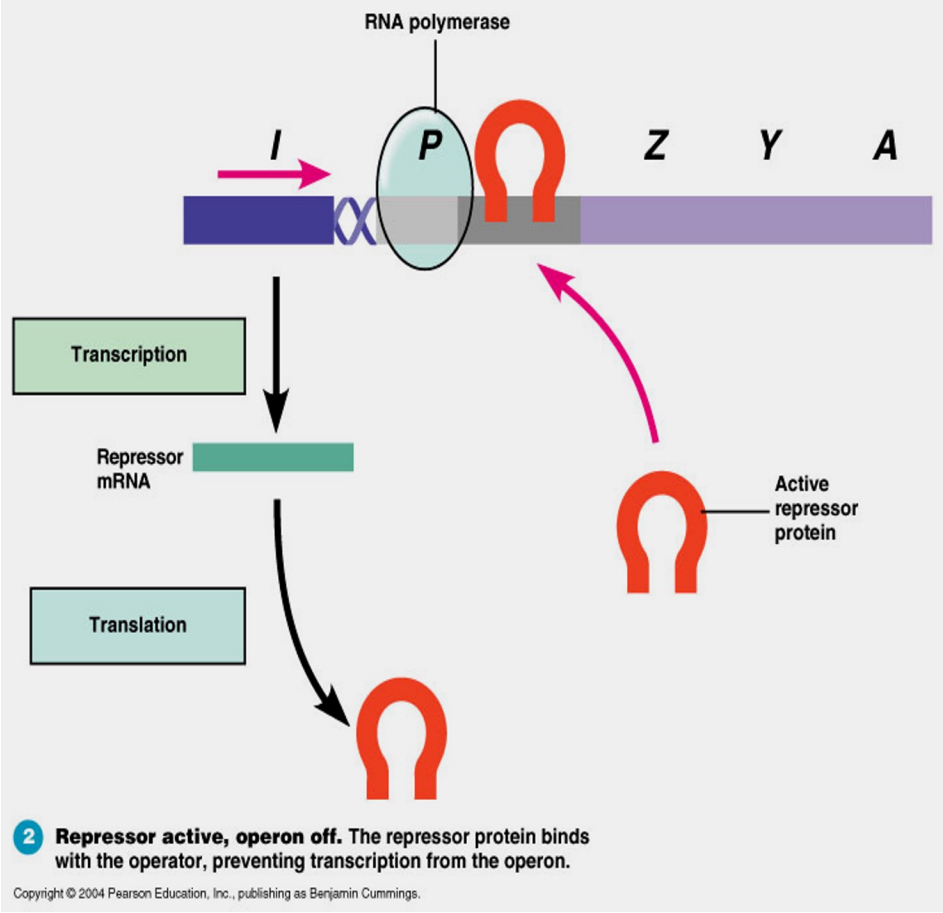
Lecture 29

BT 206

18 April 2023

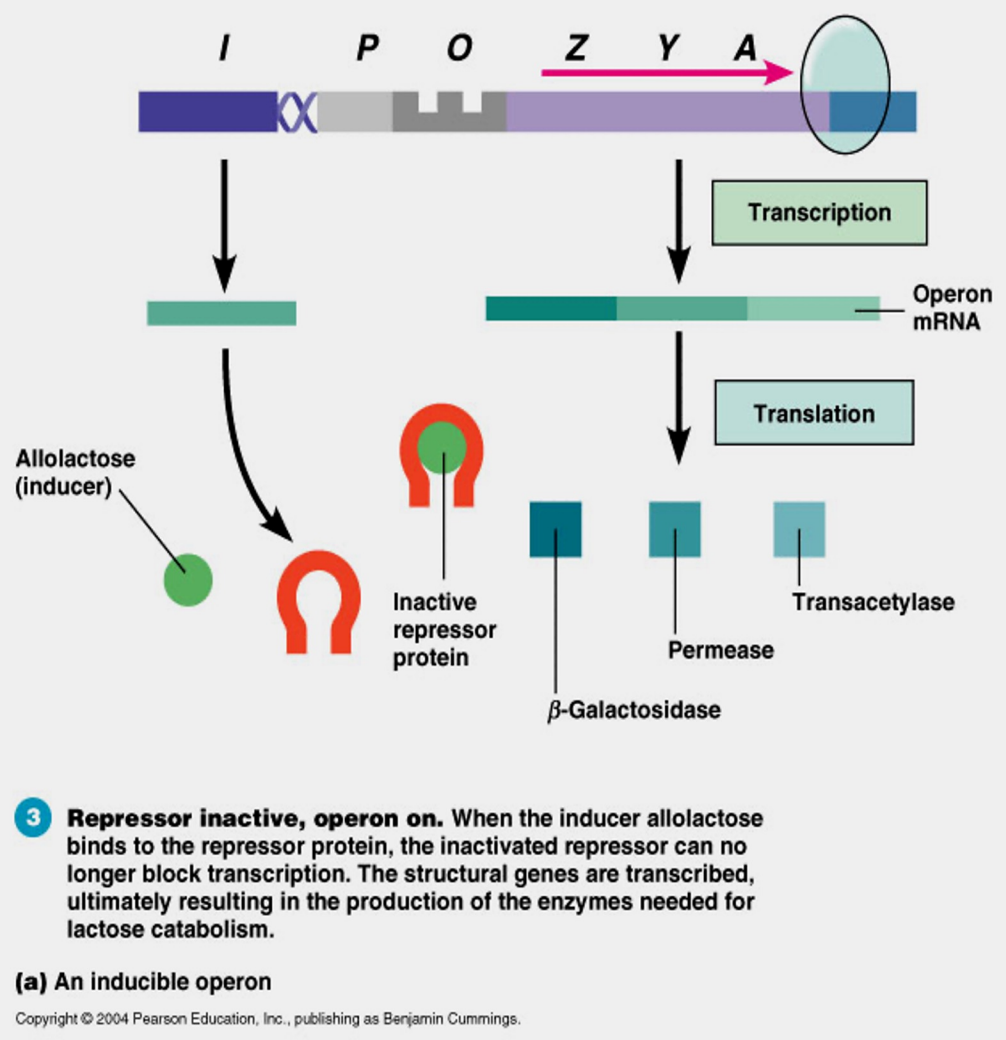
Operon Model

- Lactose is absent
- Repressor binds to operator site
- RNA polymerase is inhibited
- No transcription of structural genes
- No mRNA
- No enzymes are synthesized



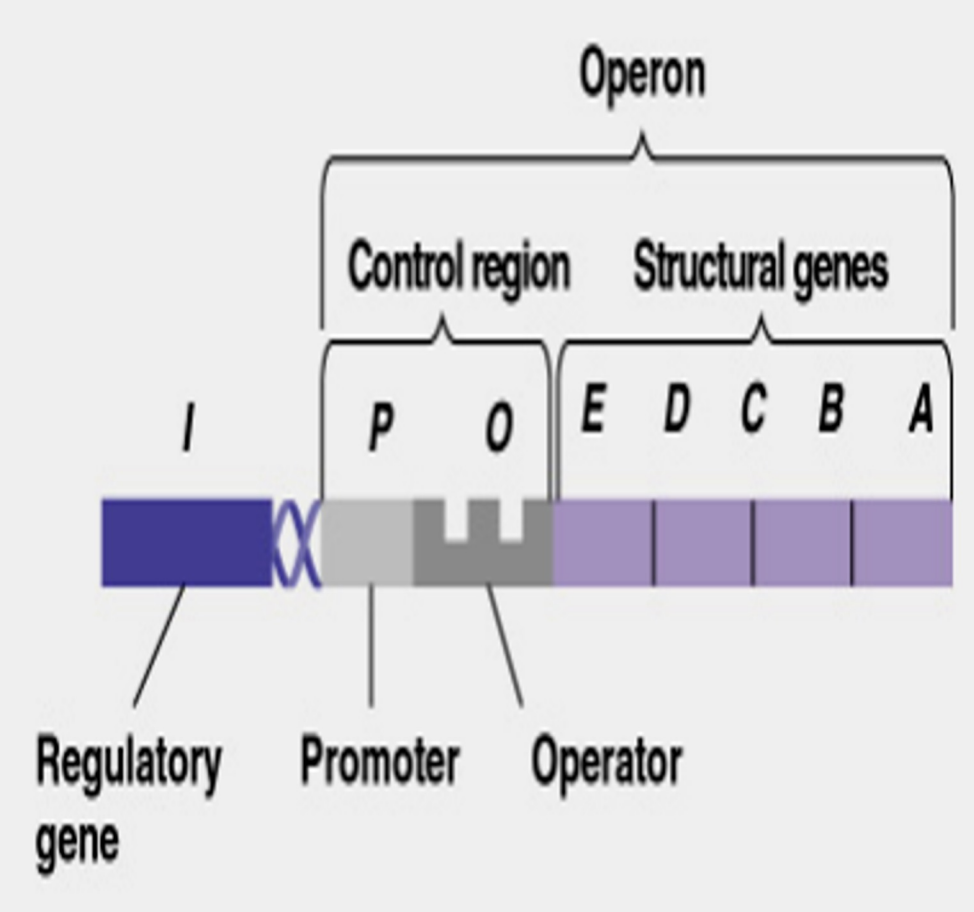
Operon Model

- Lactose is present
- Converted to allolactose
  - Inducer
- Inducer binds to repressor protein
- Repressor protein altered
  - Does not fit into operator site
- RNA polymerase is ***not*** inhibited
- Structural genes are transcribed to mRNA then translated into enzymes
- An inducible operon



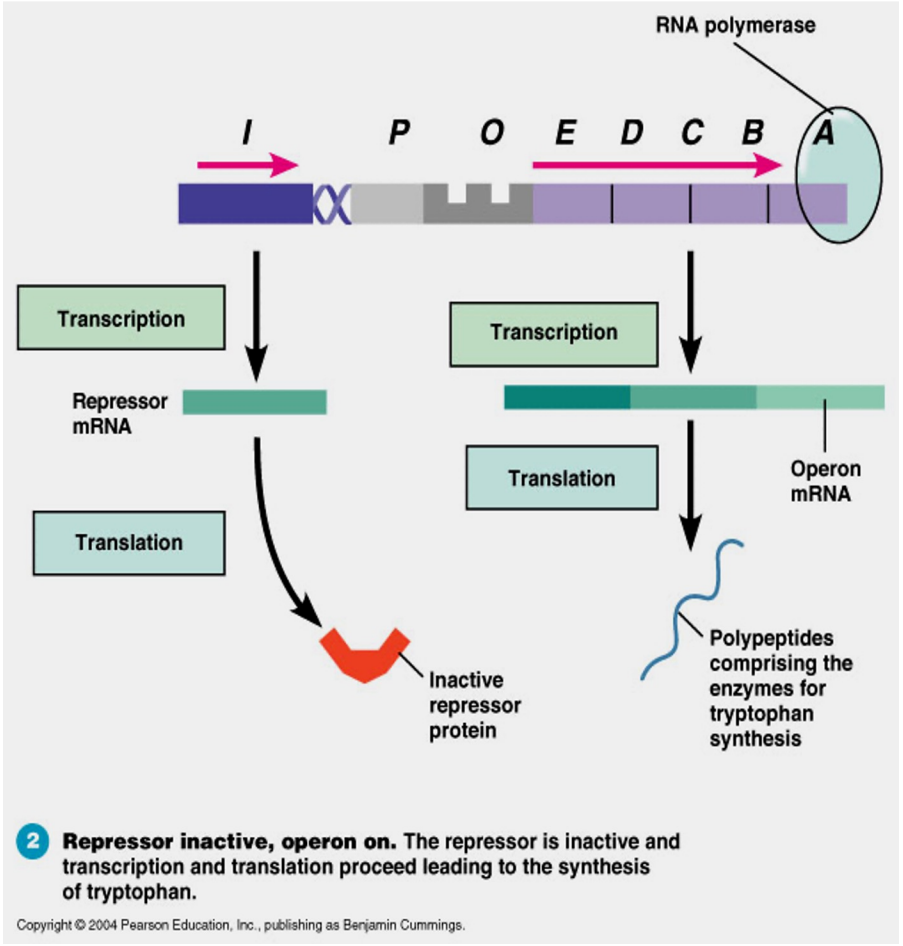
Operon Model

- Repressible operon
  - Tryptophan synthesis
  - EDCBA structural genes
  - Also has promoter and operator region



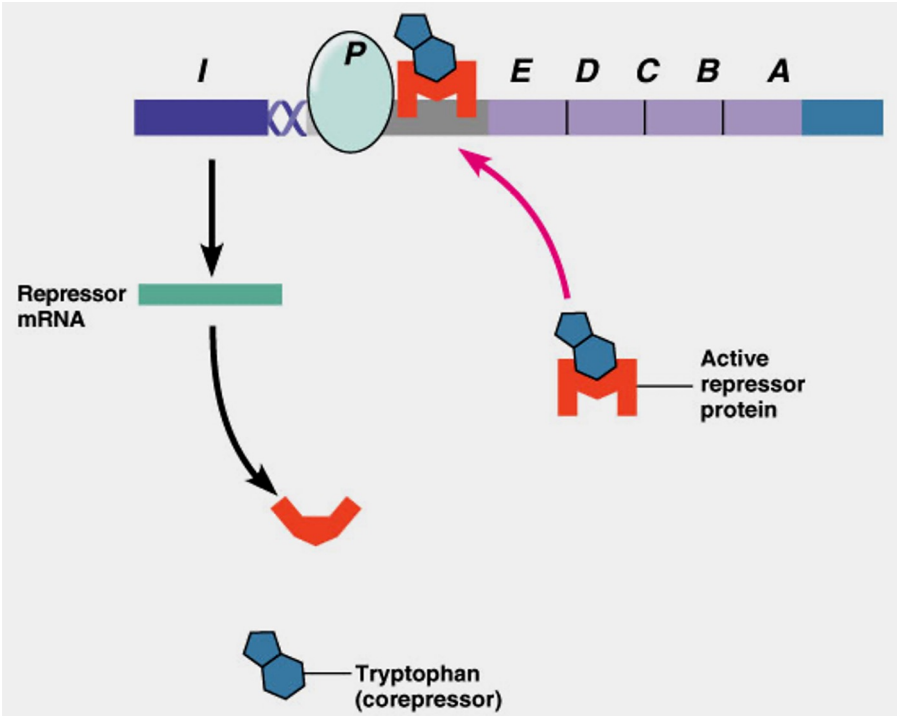
Operon Model

- Repressible operon
  - Structural genes transcribed and translated
  - Tryptophan is synthesized



Operon Model

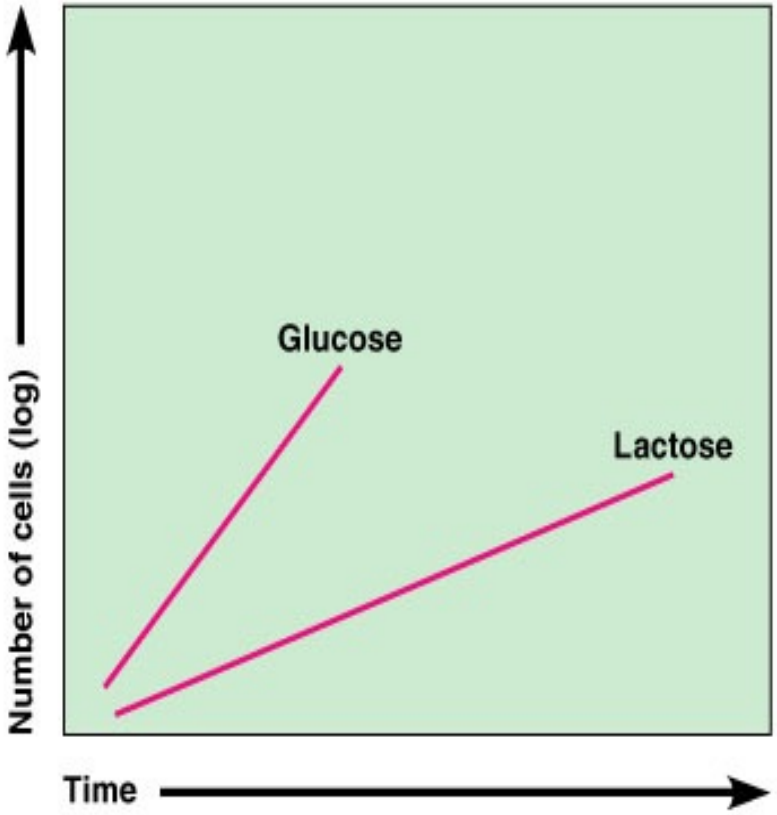
- Repressible operon
  - Excessive tryptophan accumulates
  - Tryptophan acts as corepressor
  - Corepressor binds to repressor protein
  - Repressor protein binds operator and structural genes no longer transcribed



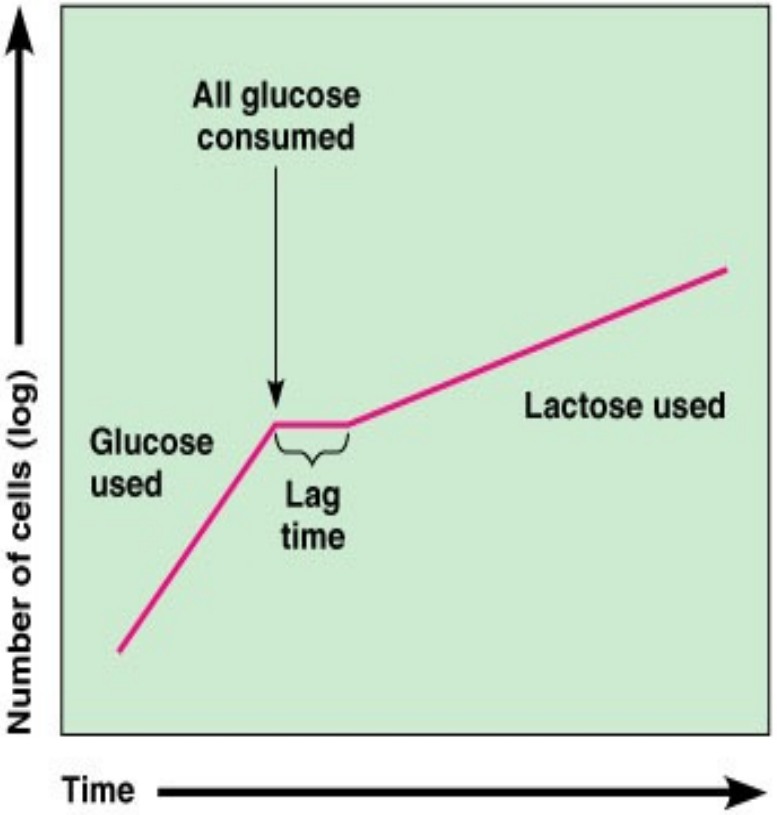
Lactose regulation

- Lactose operon
  - Depends on level of glucose in medium
  - Enzymes for glucose metabolism are constitutive
  - When glucose is absent cAMP (cyclic AMP) accumulates in cell
  - cAMP binds to cAMP receptor protein (CRP)
    - This binds to lac promoter
    - Initiates transcription by allowing mRNA polymerase to bind to the promoter
  - Transcription of lac operon requires
    - Presence of lactose
    - Absence of glucose
  - cAMP is an alarmone
    - Chemical alarm signal the cell uses to respond to environmental or nutritional stress

lac operon



(a) Growth on glucose or lactose alone



(b) Growth on glucose and lactose combined

Lac operon

- Catabolite repression
  - Inhibition of the metabolism of other carbon sources by glucose
    - Glucose effect

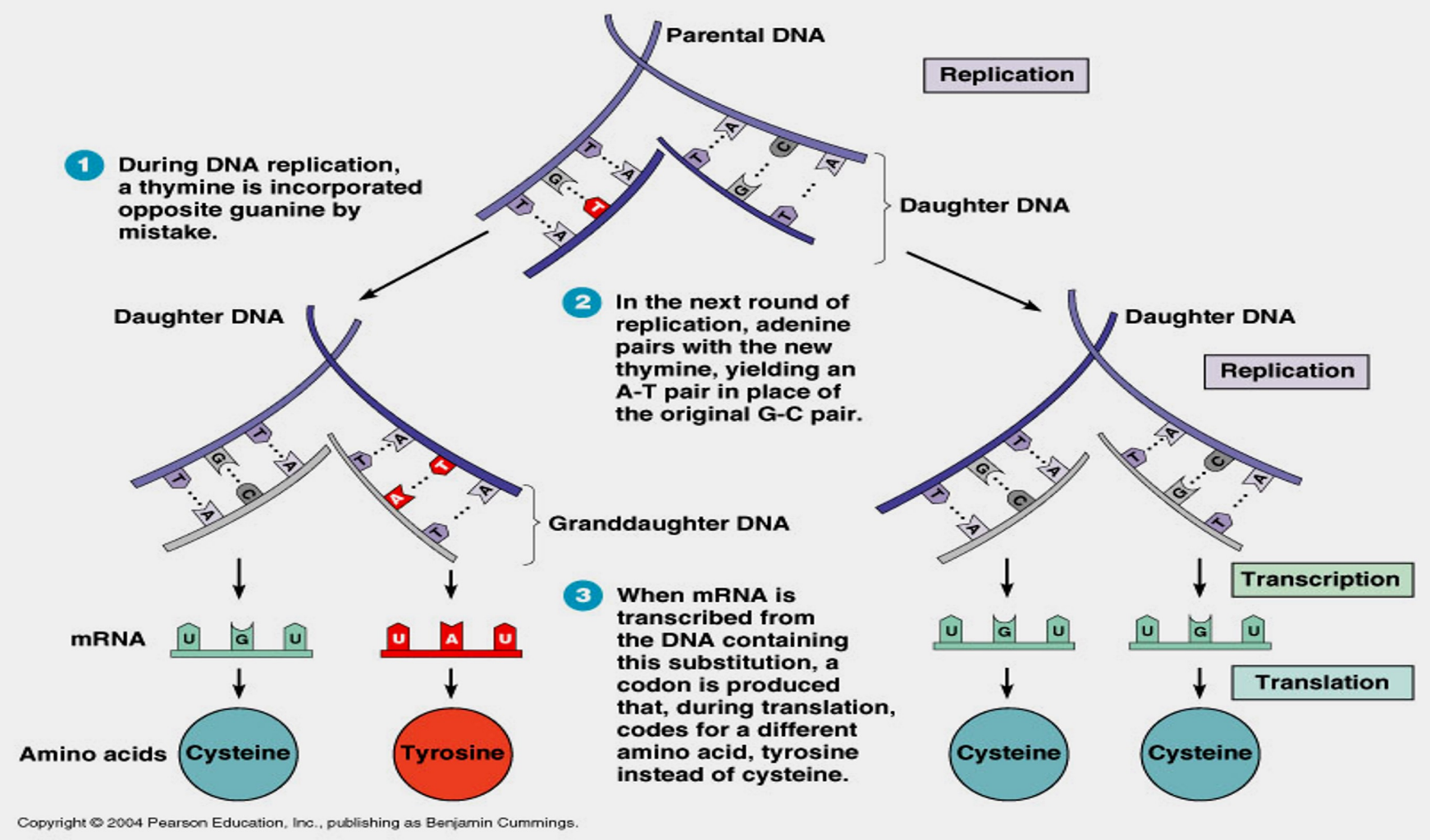
Mutation

- Mutation
  - Change in the base sequence of DNA
    - may cause change in the product coded by the gene
      - Beneficial
      - Lethal
      - Neutral
        - Occur commonly
        - Degeneracy

Mutations

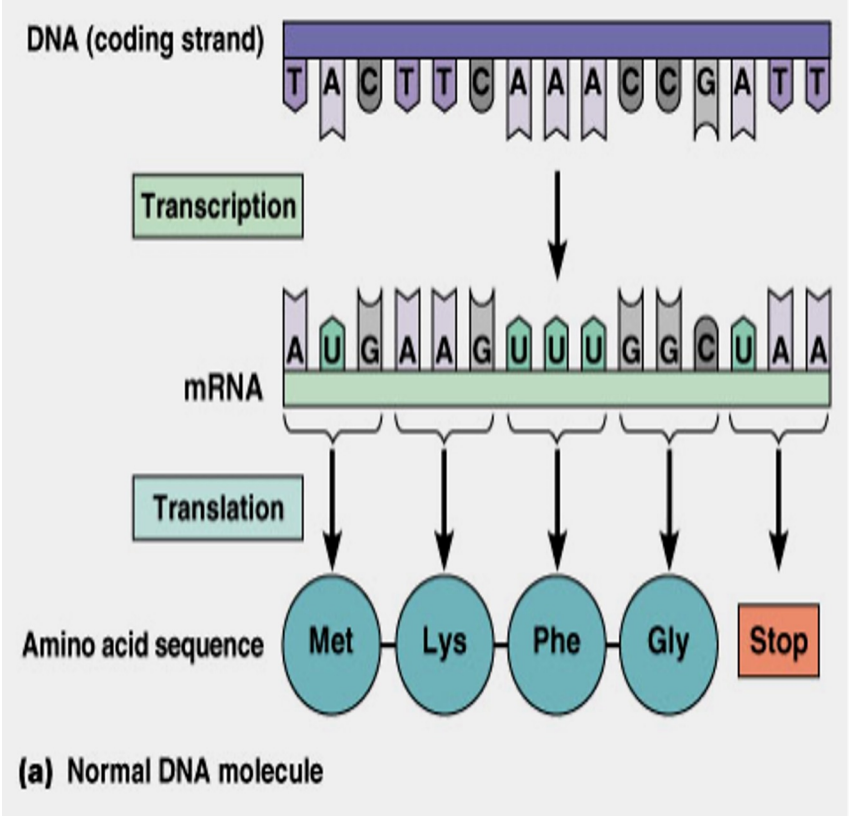
- Types of mutations
  - Base substitution (point mutation)
    - AT substituted for CG
    - mRNA carries incorrect base
    - Translation
      - Insertion of incorrect amino acid into protein
  - Missense mutation, nonsense mutation, frame shift mutation, and spontaneous mutations

Base substitution



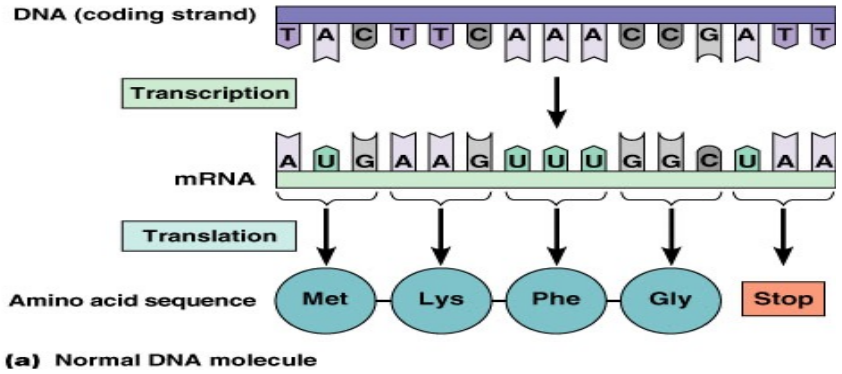
Mutations

- Normal
  - No mutations
  - DNA strand properly transcribed by mRNA
  - Correct sequence of amino acids for protein

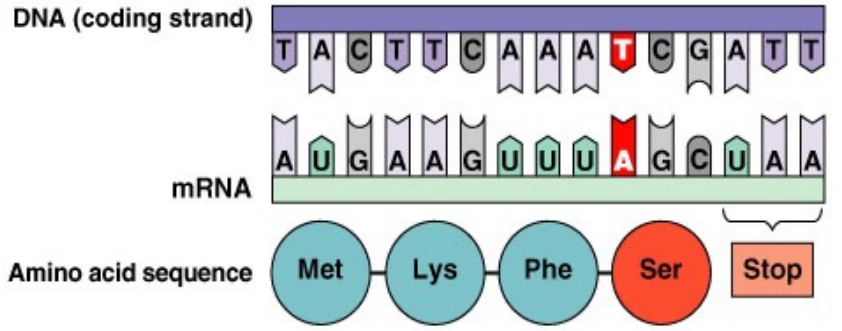


Mutations

- Missense mutation
  - Base substitution results in an amino acid substitution in protein
  - Sickle cell anemia
    - A to T
    - Glutamic acid to valine
    - Hb shape changed during low oxygen



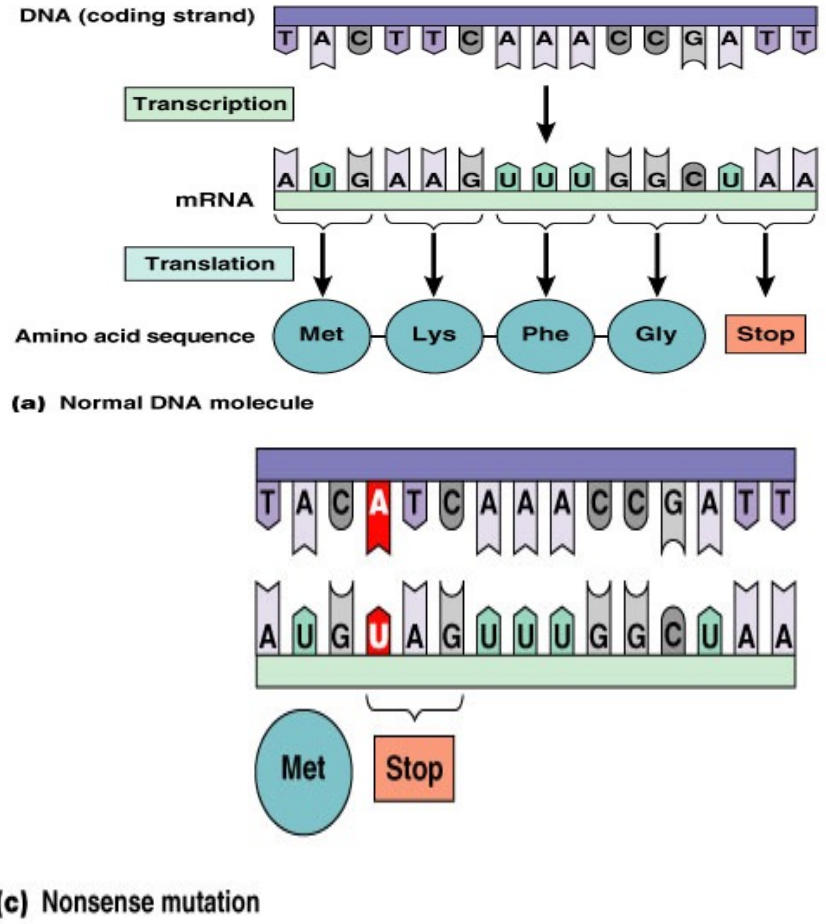
(a) Normal DNA molecule



(b) Missense mutation

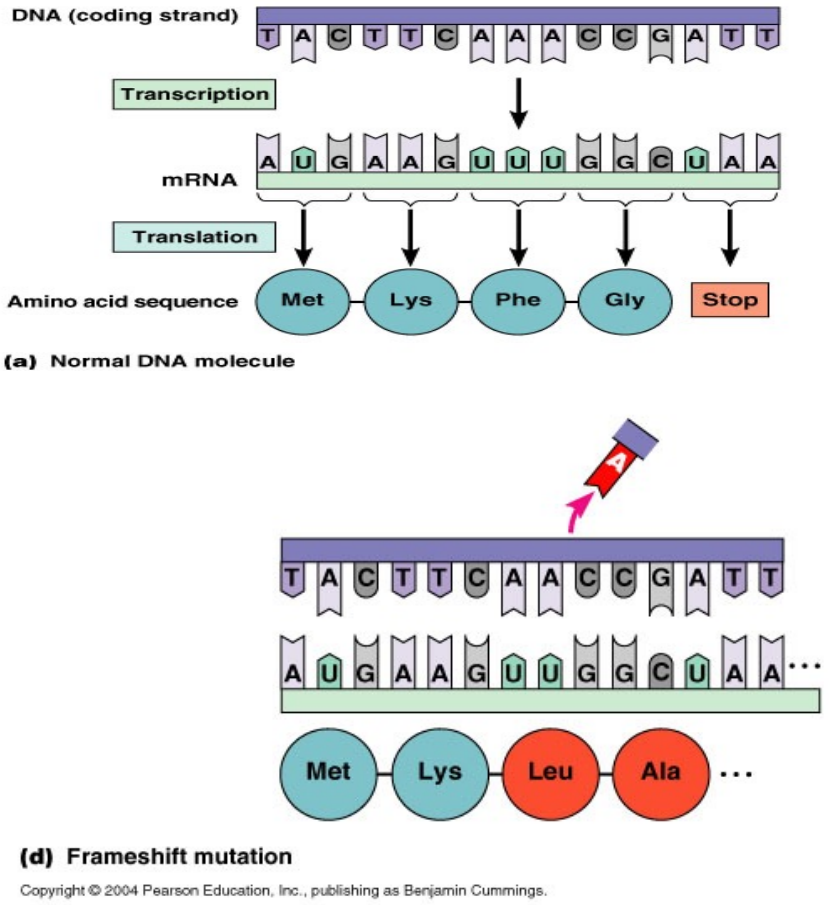
Mutations

- Nonsense mutation
  - Base substitution creates a nonsense or stop codon
  - Functional protein is not produced
  - Only a fragment of protein is produced



Mutations

- Frame shift mutation
  - One or a few nucleotide pairs are deleted or inserted in the DNA
  - Shifts the translation reading frame
  - Almost always result in a long stretch of altered amino acids
  - Inactive protein



Mutations

- Insertion of extra bases into a gene
  - Huntington's disease
- Spontaneous mutations
  - Occur occasionally in DNA replication
- Mutagens
  - Chemical or physical environmental agent that alters DNA or induces genetic mutation.
    - Radiation, ultraviolet light

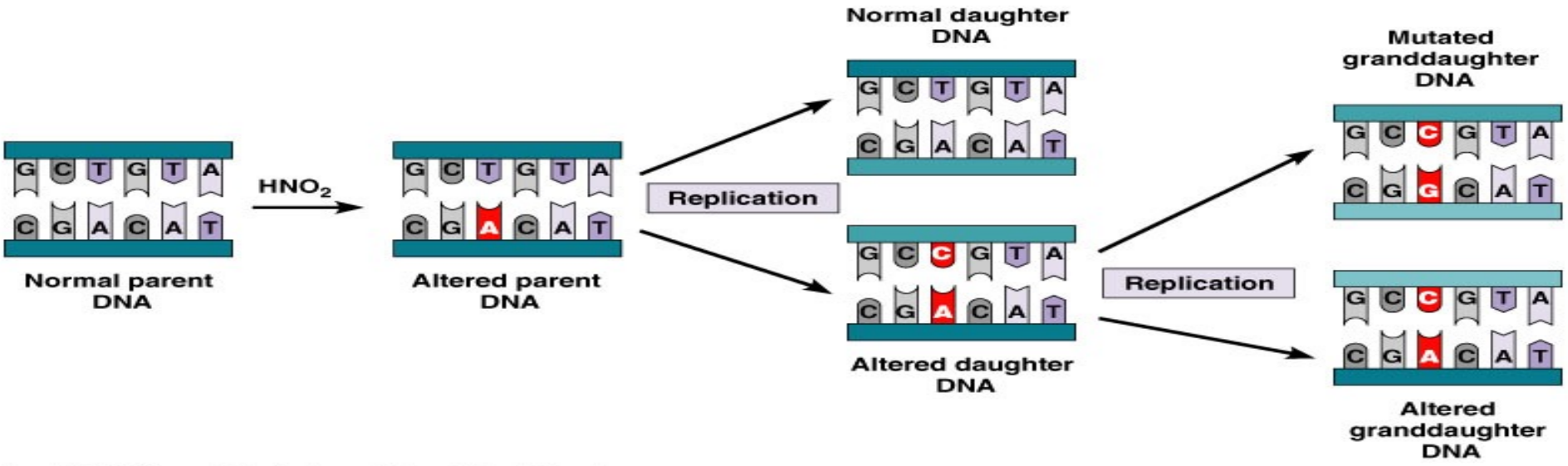
Lecture 30

BT 206

21 April 2023

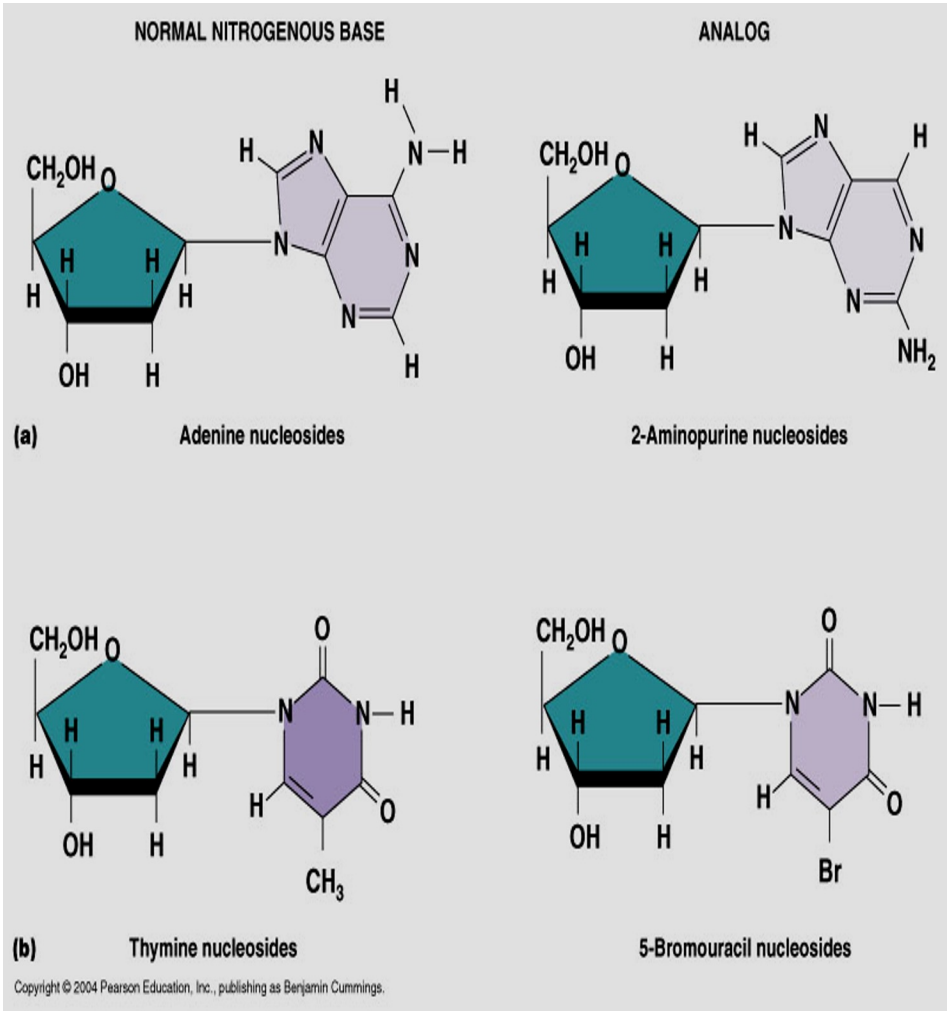
Mutagens

- Chemical Mutagens
  - Nitrous acid
    - Converts adenine (A) to a form that doesn't bind with thymine (T), but instead binds with cytosine (C)
    - Alters base pair on DNA, works on random locations



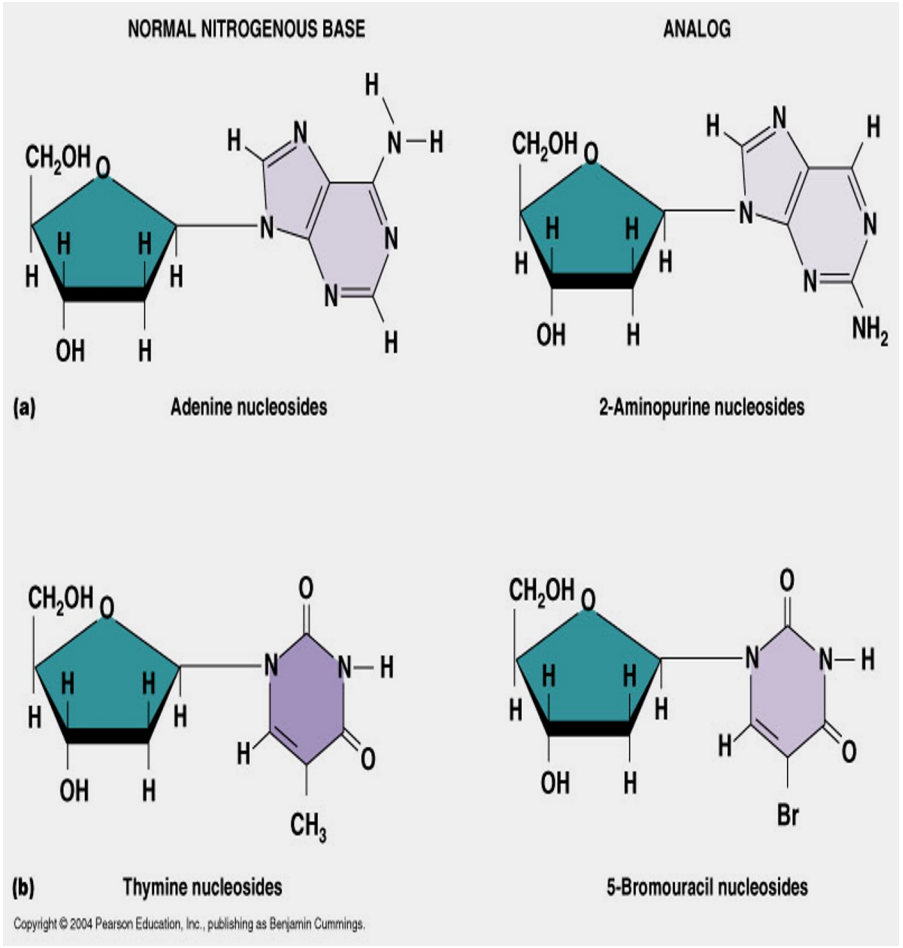
Mutagens

- Chemical mutagens (cont)
  - Nucleoside analogs
    - Structurally similar to normal nitrogenous bases
    - 2 - aminopurine
      - Adenine
    - 5 – bromouracil
      - Thymine analog
      - Will bind with guanine



Mutagens

- Chemical mutagens (cont)
  - During replication analogs cause base pairing mistakes
  - Antiviral and antitumor drugs
    - AZT (azidothymidine)

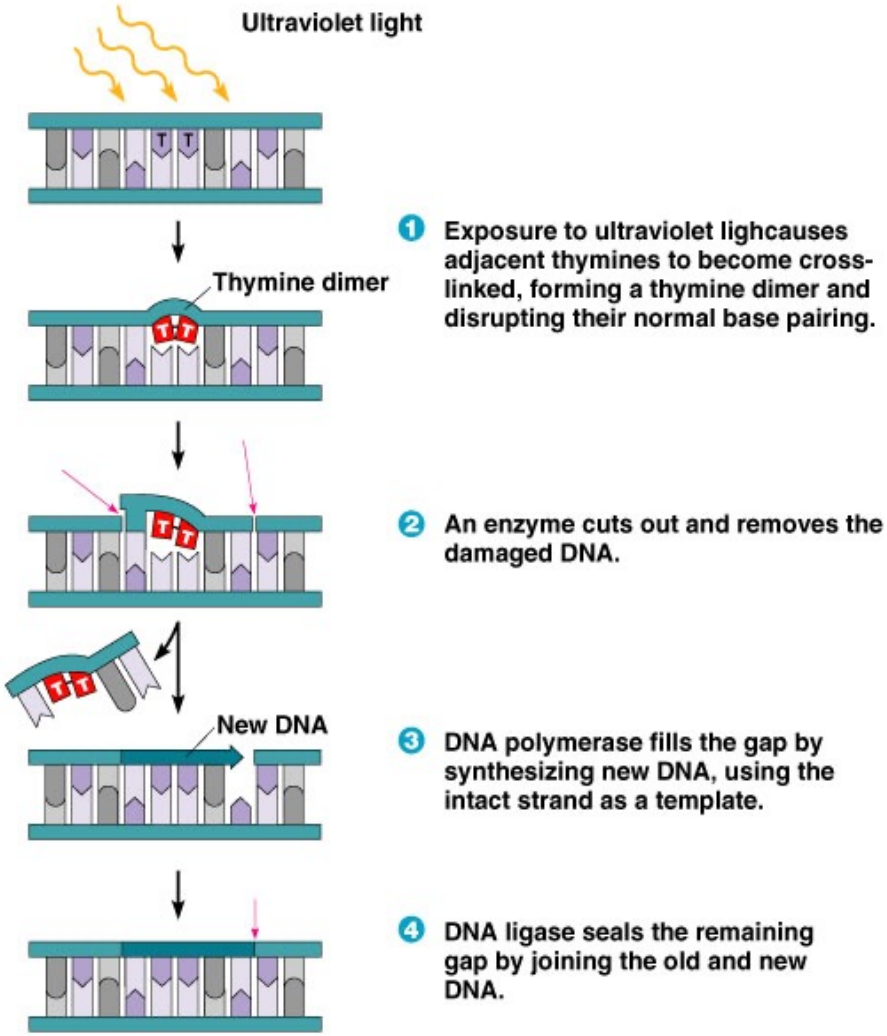


Mutagens

- Chemical mutagens (cont)
  - Other chemicals cause deletions, frameshifts, or insertions
    - Benzoyprene – present in smoke and soot
      - Frameshift
    - Aflatoxin – *Aspergillus flavus*
      - Frameshift

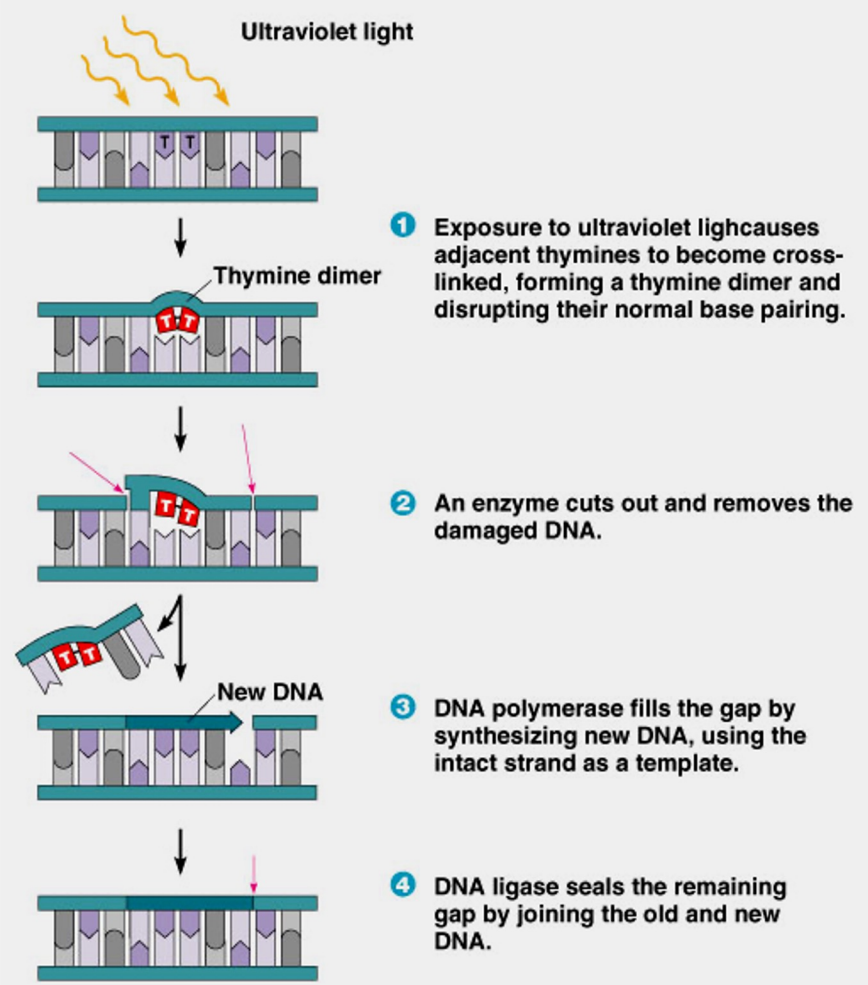
Mutagens

- Radiation mutagens
  - X – rays
  - Gamma rays
  - Ultraviolet
    - Forms covalent bond between certain bases
    - Thymine dimers
      - Death of cell to damage
    - Light repair enzymes
      - Photolyases
        - Use visible light energy to separate dimer



Mutagens

- Ultraviolet damage
  - Nucleotide excision repair
    - Enzymes cut out distorted thymines
    - Creates gap
    - Gap is filled with newly synthesized DNA
    - DNA ligase joins strand to surrounding backbone



Mutation frequency

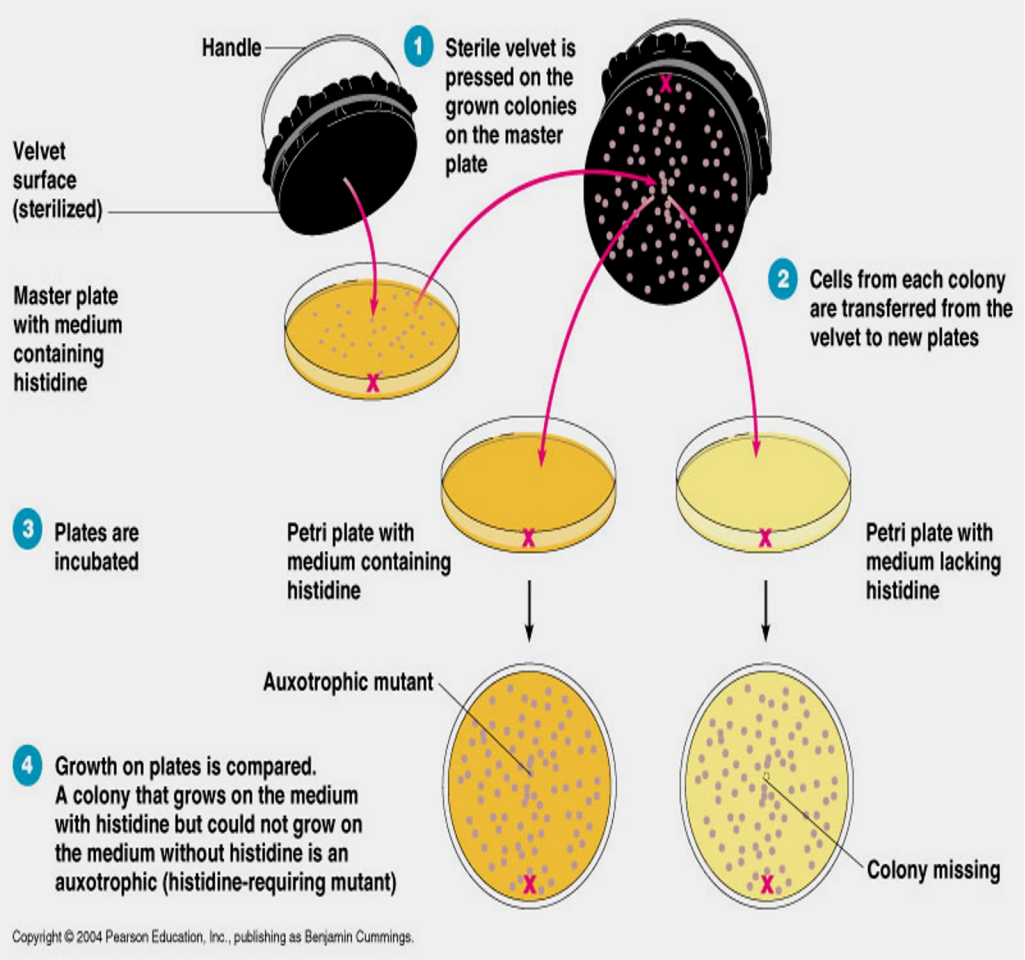
- Mutation rate
  - Probability that a gene will mutate when a cell divides
  - Expressed in power of 10
    - 10^{-4} mutation rate (1 in 10,000 chance of mutation)
    - 10^{-6} (1 in 1,000,000)
  - Mutagens
    - Increase spontaneous mutation by 10 – 10,000 times
    - 10^{-6} becomes 10^{-3} to 10^{-5}

Identifying Mutants

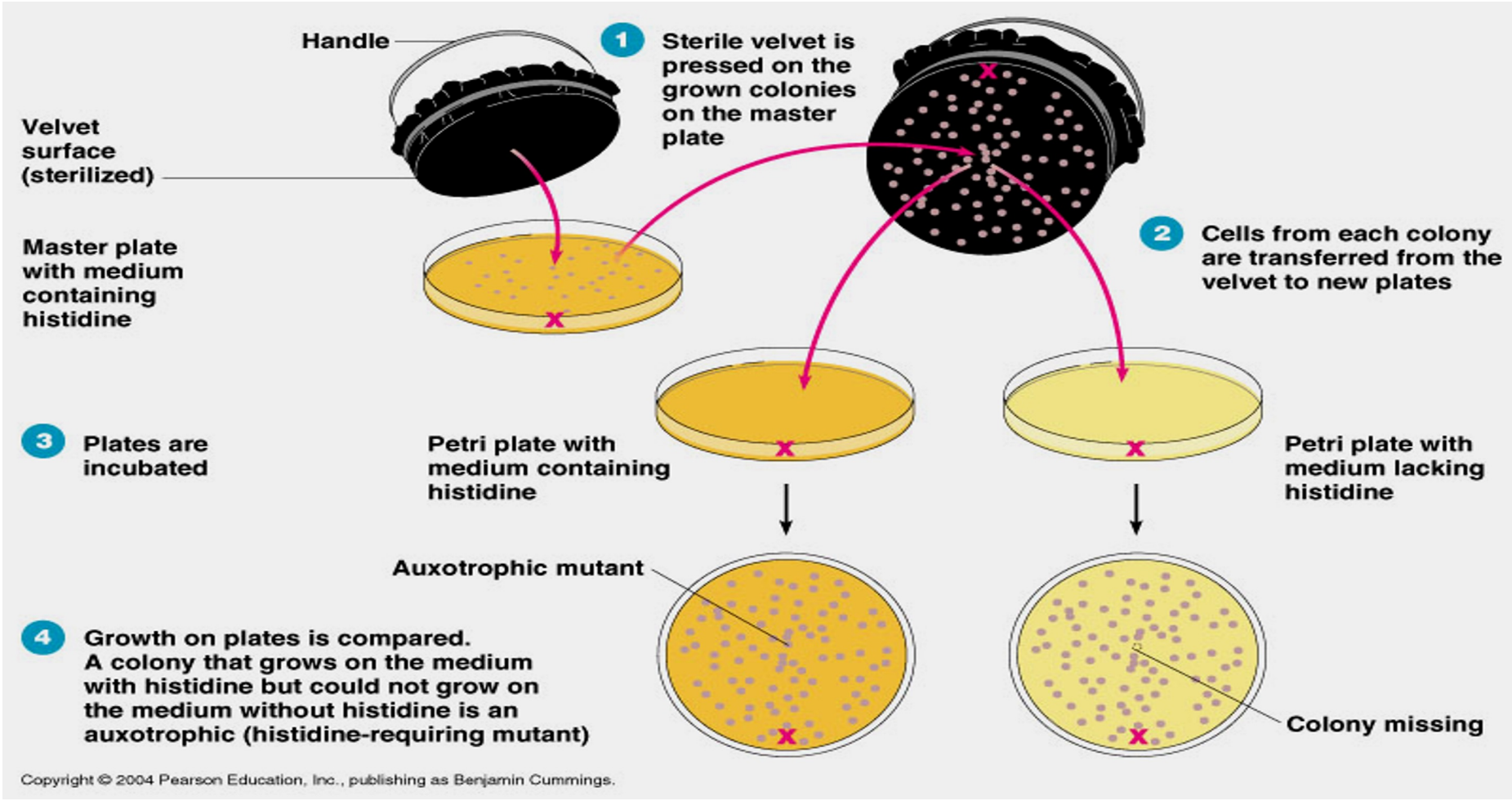
- Positive (direct) selection
  - Detection of mutant cells by rejection of unmutated parent cells
    - Penicillin in agar
    - Unmutated parental cell will not grow
    - Only mutated cells grow

Identifying Mutants

- Negative (indirect) selection
  - Replica plating technique



Replica Plating



Replica plating

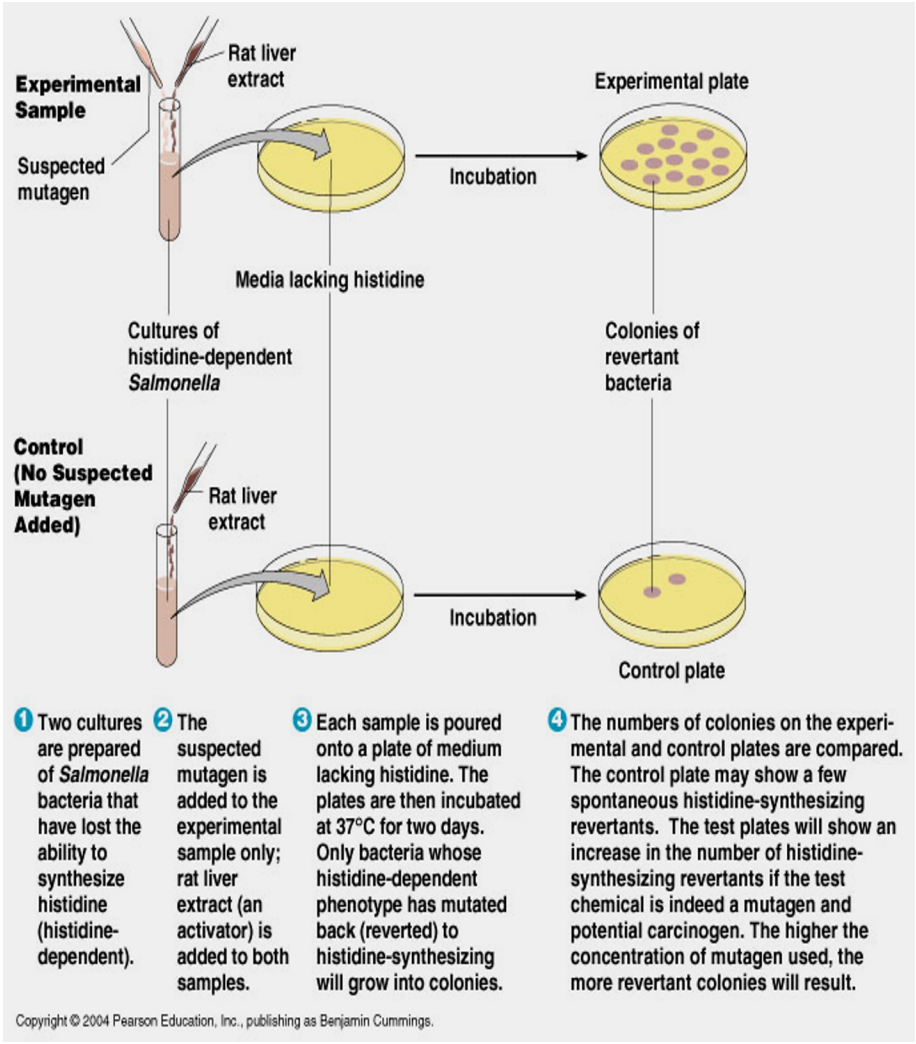
- Auxotroph
  - A mutant microorganism having a nutritional requirement that is absent in the parent.

Identifying Chemical Carcinogens

- Carcinogen
  - A substance found to cause cancer in animals
  - Often mutagens are carcinogens as well
  - Previously used animal testing
    - Time consuming
    - Expensive

Ames test

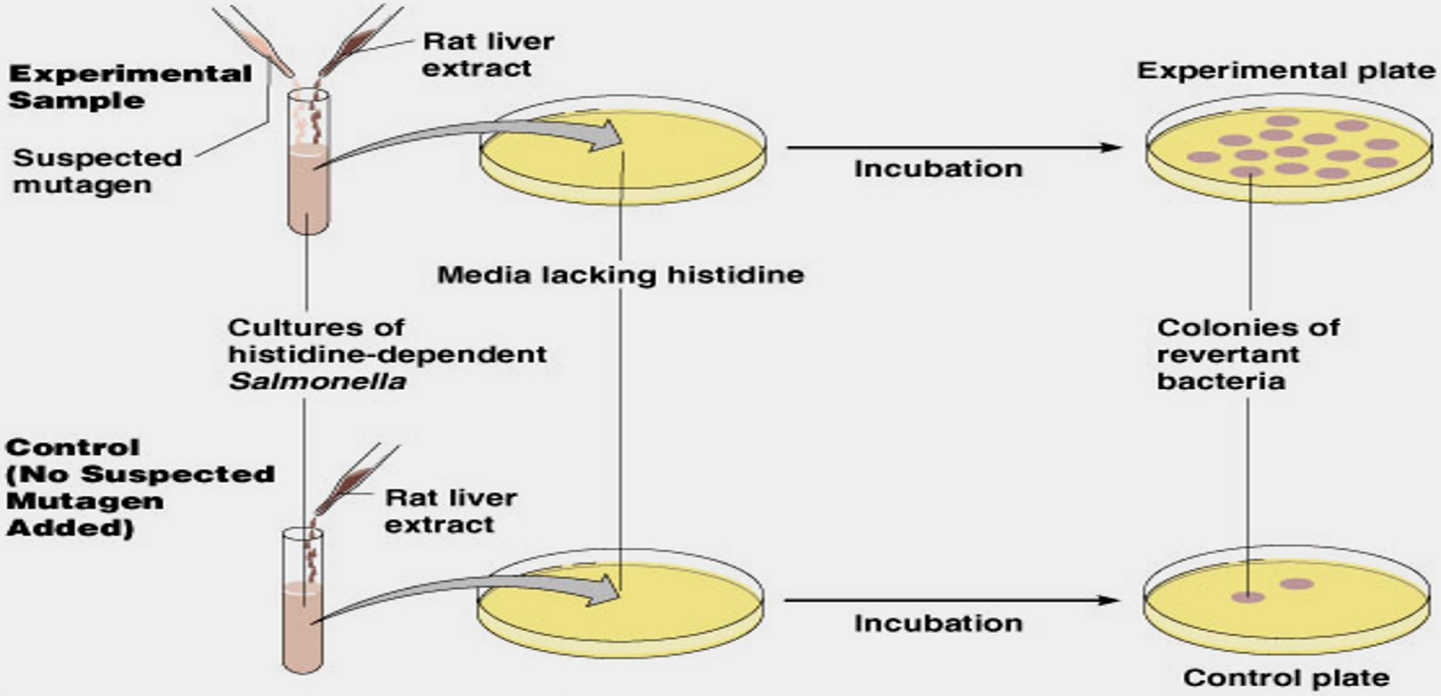
- Ames test utilizes bacteria to act as carcinogen indicator
- Based on observation that exposure of mutant bacteria to mutagenic substance may reverse effect of the original mutation



Ames test

- These are called reversions
  - Back mutations
- Measures the reversion of *Salmonella*
  - Auxotrophs
    - Have lost there ability to synthesize histidine (his^-)
    - (his^+) bacteria have ability to synthesize histidine
- Substances that cause 90% of reversion have been shown to be carcinogens

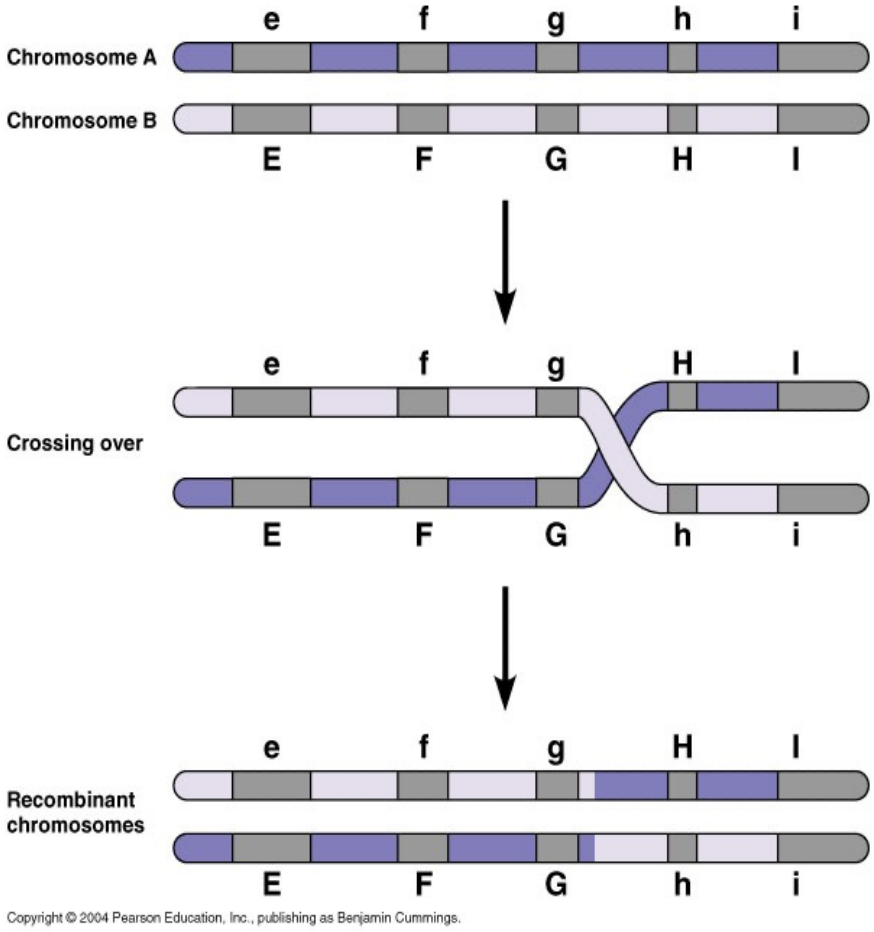
Ames Test



1. Two cultures are prepared of *Salmonella* bacteria that have lost the ability to synthesize histidine (histidine-dependent).
2. The suspected mutagen is added to the experimental sample only; rat liver extract (an activator) is added to both samples.
3. Each sample is poured onto a plate of medium lacking histidine. The plates are then incubated at 37°C for two days. Only bacteria whose histidine-dependent phenotype has mutated back (reverted) to histidine-synthesizing will grow into colonies.
4. The numbers of colonies on the experimental and control plates are compared. The control plate may show a few spontaneous histidine-synthesizing revertants. The test plates will show an increase in the number of histidine-synthesizing revertants if the test chemical is indeed a mutagen and potential carcinogen. The higher the concentration of mutagen used, the more revertant colonies will result.

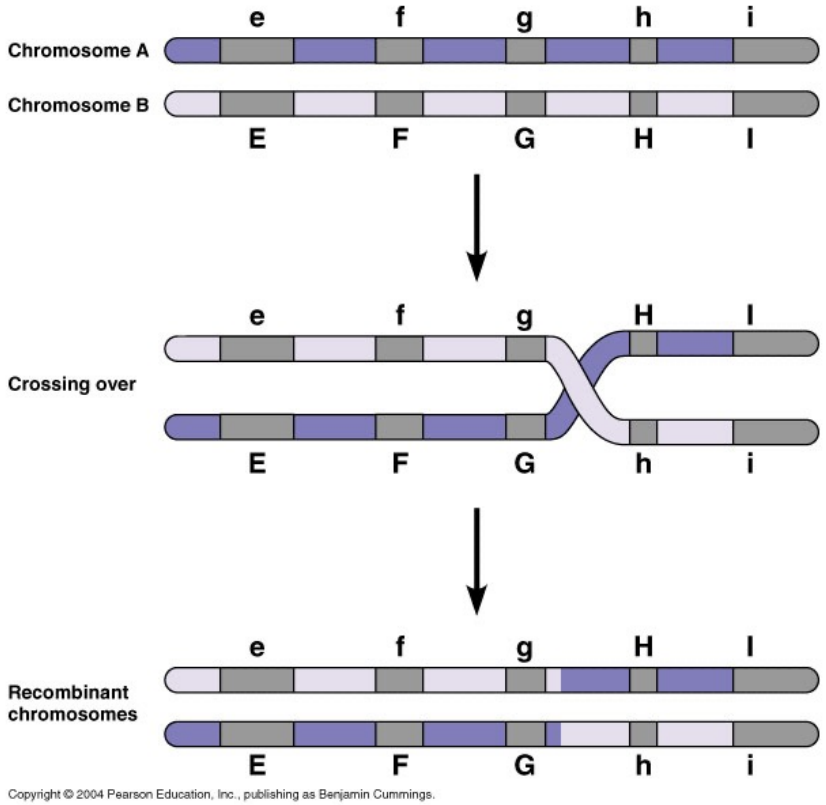
Genetic Transfer and Recombination

- Genetic recombination
  - Exchange of genes between two DNA molecules to form new combinations of genes on a chromosome
  - Crossing over
    - Two chromosomes break and rejoin
  - Adds to genetic diversity



Genetic transfer and recombination

- Eukaryotes
  - Meiosis
    - Prophase I
- Prokaryotes
  - Numerous different ways



Genetic Transfer and Recombination

- Vertical gene transfer
  - Genetic information passed from an organism to its offspring
    - Plants and animals
- Horizontal gene transfer
  - Bacteria transfer genetic information form one organism to another in the same generation
  - Genetic information passed laterally

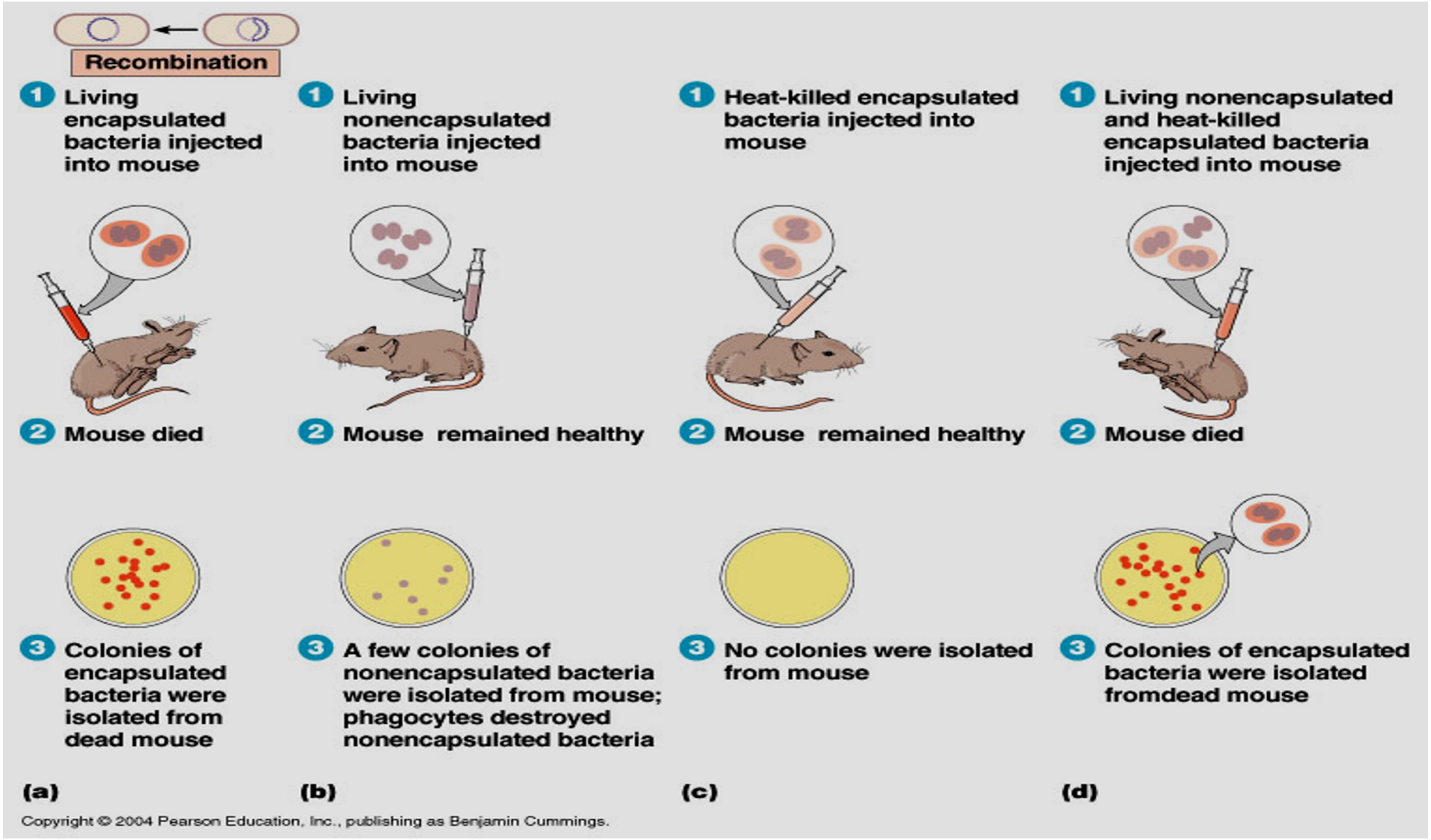
Horizontal Gene Transfer

- Horizontal gene transfer
  - Donor cell
    - Organism gives up its entire DNA
    - Part goes to recipient cell
    - Part is degraded by cellular enzymes
  - Recipient cell
    - Receives portion of donor cells DNA
    - Incorporates donor DNA into its own DNA
      - Recombinant DNA
      - Less than 1 % of population

Transformation

- Genes transferred from one bacterium to another in solution
  - Naked DNA
  - Discovered by Griffith
  - Used *Streptococcus pneumoniae*
    - Two strains
      - Virulent (pathologic) strain
        - Had a polysaccharide capsule resists phagocytosis
      - Avirulent (non- pathogenic) strain
        - Lacked a capsule

Griffith's Experiment



Transformation

- Bacteria after cell death and lysis could release DNA into environment
- Recipient cell can take up DNA fragments and incorporate into their own DNA
  - Resulting in a hybrid (recombinant cell)
  - Recombinant cell must be competent
    - Able to alter cell wall to allow DNA (large molecule) to enter
    - *Bacillus, Haemophilus, Neisseria, Acinetobacter,* and some *Staph and Strep*

Genetic Transformation