BHARATHIDASAN UNIVERSITY

Tiruchirappalli 620024
Tamilnadu, India

Program: M.Sc., Microbiology

Course Title : Microbial Genetics & Molecular Biology

Course Code: 24MICCC3

Unit II: DNA Mutation

Dr. G. Muralitharan
Professor
Dept. of Microbiology

Mutations

- Changes in the nucleotide sequence of the DNA.
- Organisms have special systems of enzymes that can repair certain kinds of alterations in the DNA.
- Once the DNA sequence has been changed, DNA replication copies the altered sequence just as it would copy a normal sequence.

Mutation and Evolution

- Mutation is the source of all genetic variation (e.g.,chromatin remodeling).

- **Natural selection** preserves the combinations best adapted (or NOT) to the existing environment.

Types of Mutations

Somatic mutations

- Occurs in cells not dedicated to sexual reproduction

- The mutant genes disappear when the cell in which it occurred dies and can only be passed on through asexual reproduction.

Germline mutations

- Found in every cell descended from the zygote to which that mutant gamete contributed.

- If an adult is successfully produced, every one of its cells will contain the mutation.

Types of Mutations

Single-base Substitution / point mutation

- Exchanges one base for another.
  - If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a **transition**.
  - If a purine is replaced by a pyrimidine or vice-versa, the substitution is called a **transversion**.

Twelve different base substitutions can occur in DNA.

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Types of Mutations

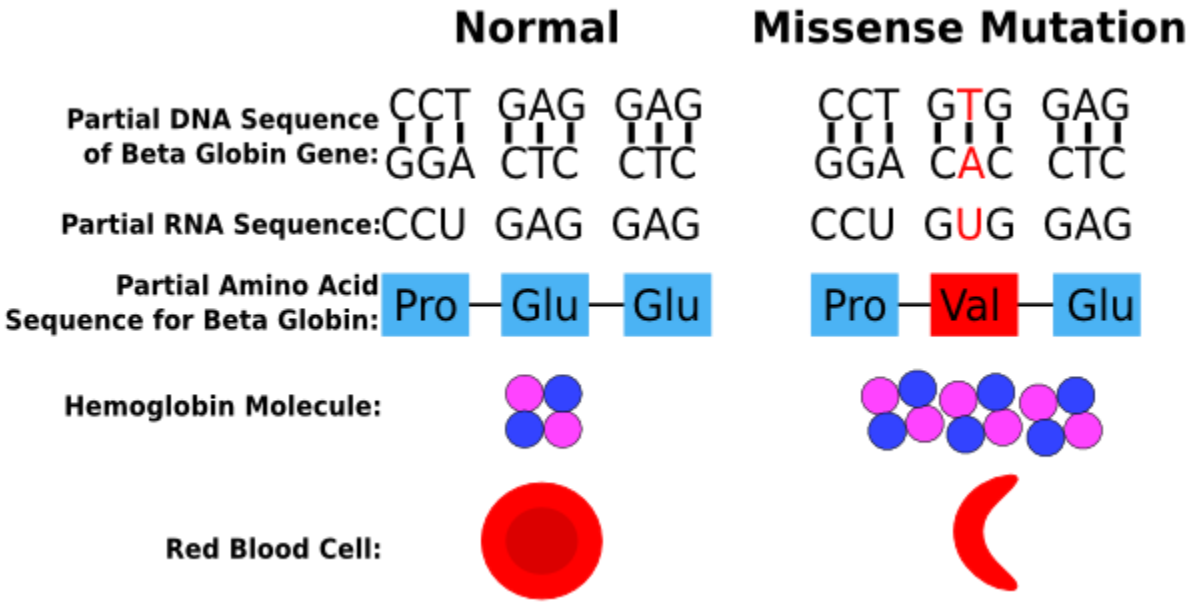
Point mutations continued

A change in a codon to one that encodes a different amino acid and cause a small change in the protein produced = **missense mutation**.

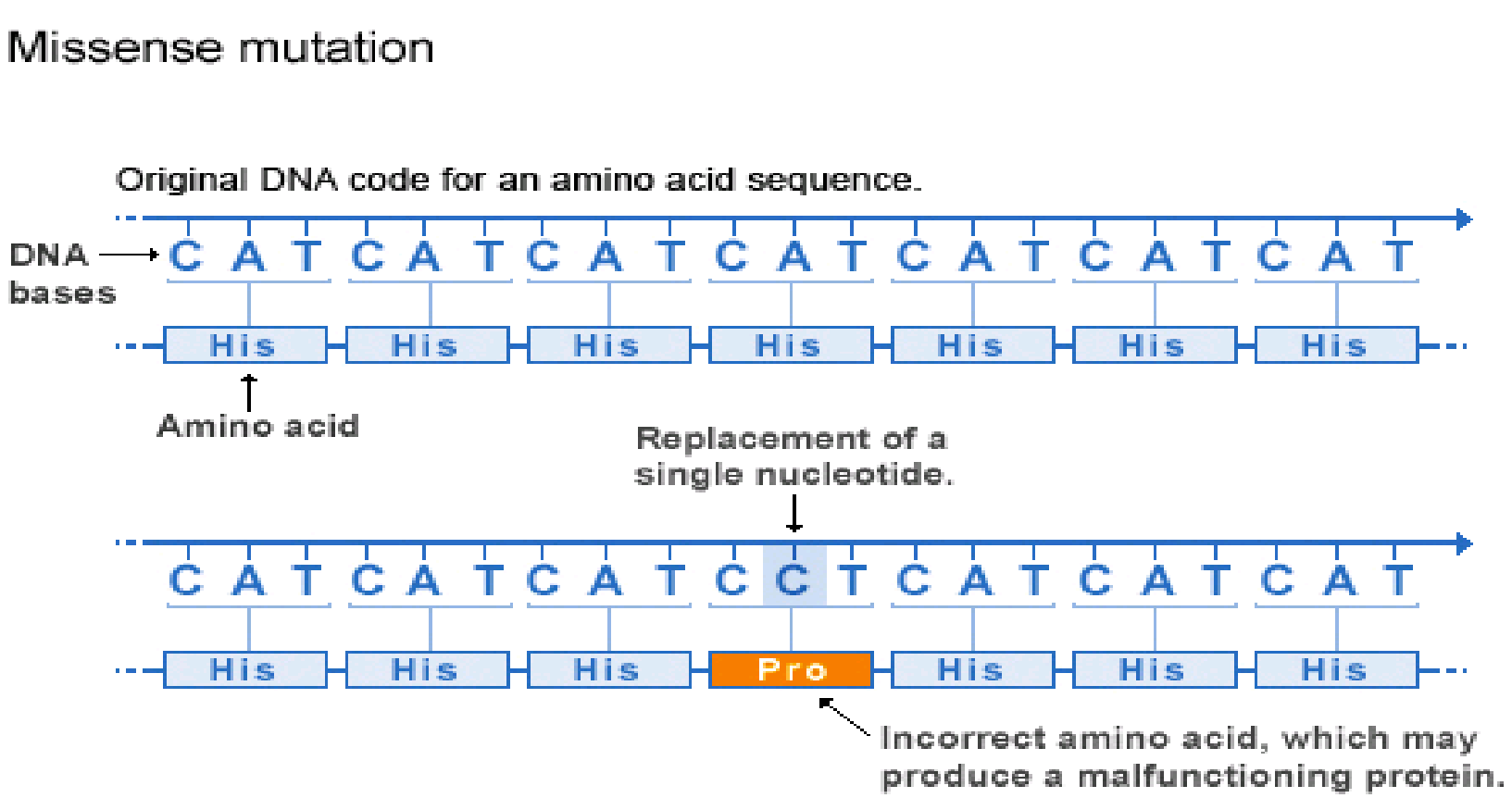
Example **sickle-cell disease**

A → T at the 17th nucleotide of the gene for the beta chain of hemoglobin changes the codon GAG (glutamic acid) to GTG (valine)

Therefore: 6th amino acid glutamic acid → valine



Missense mutation



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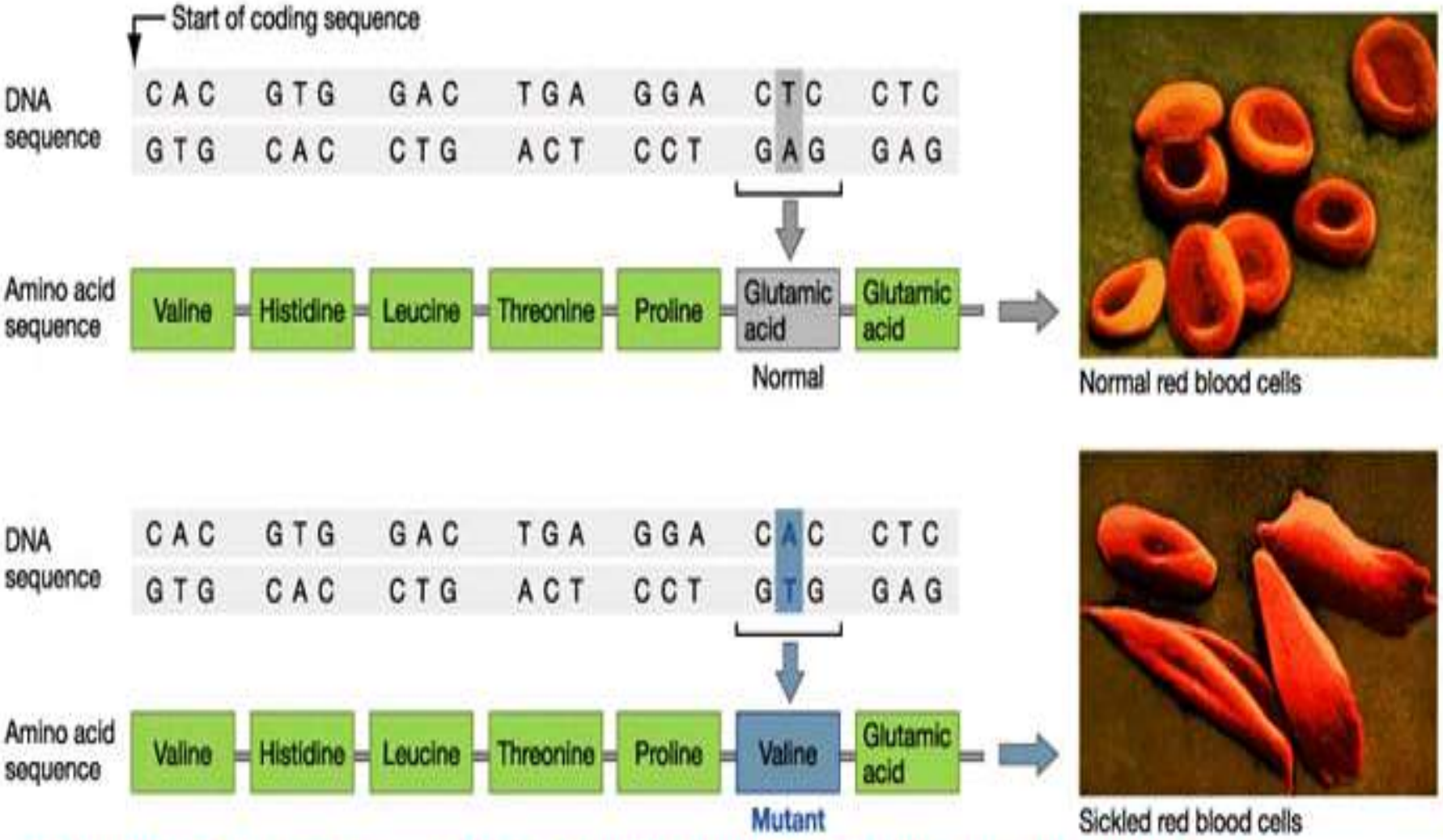


The change in amino acid sequence causes hemoglobin molecules to crystallize when oxygen levels in the blood are low. As a result, red blood cells sickle and get stuck in small blood vessels.

Examples of Diseases caused by point mutations

- Color blindness
- Cystic fibrosis
- Hemophilia
- Phenylketonuria
- Tay Sachs

Types of Mutations

Point mutations continued

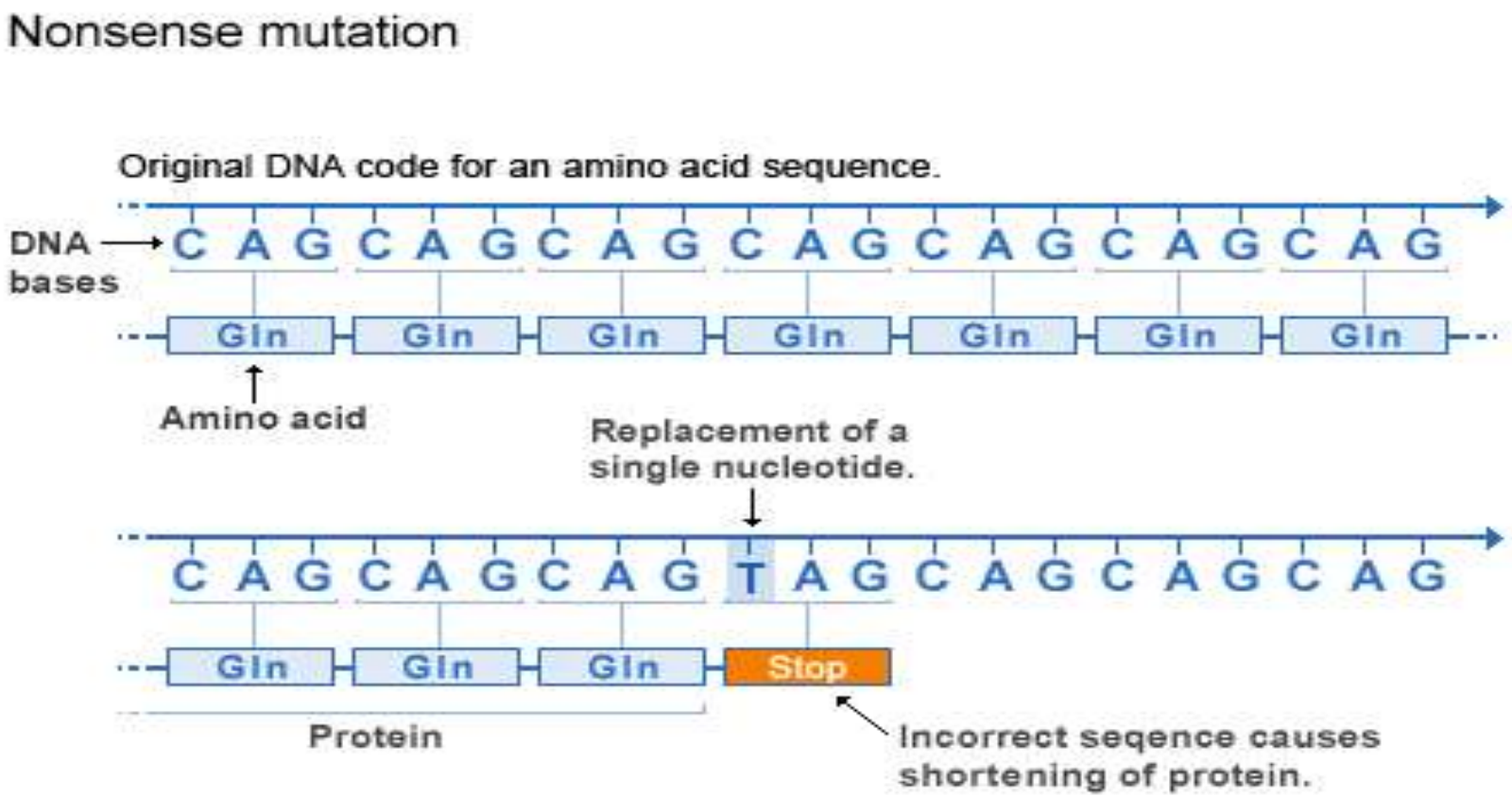
- Change a codon to one that encodes the same amino acid and causes no change in the protein produced = **silent mutations**.

- Change an amino-acid-coding codon to a single "stop" codon → an incomplete protein

  = **a nonsense mutation**

  – can have serious effects since the incomplete protein probably won't function.

Nonsense Mutation



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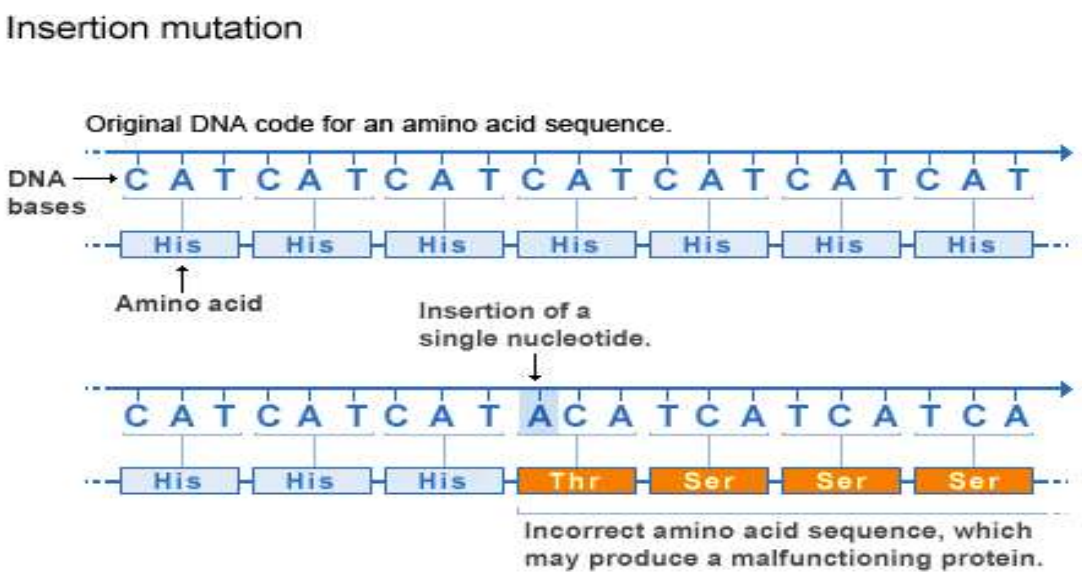
Types of Mutations

Insertion

- Extra base pairs are inserted into a new place in the DNA.

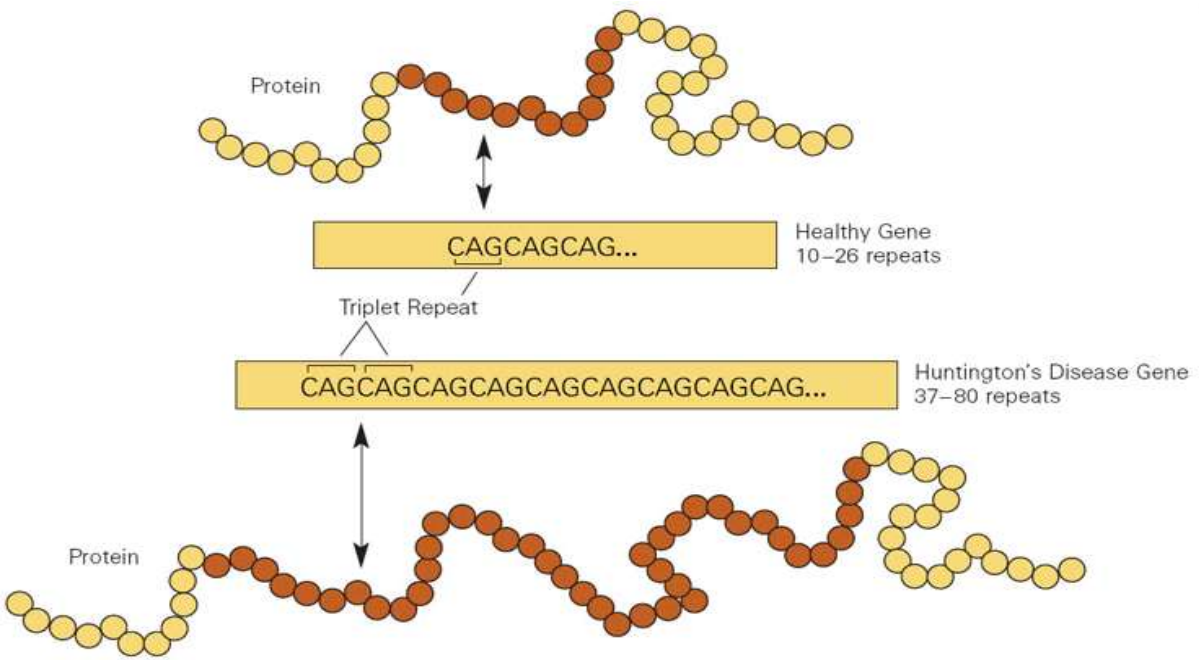
Deletion

- A section of DNA is lost, or deleted.

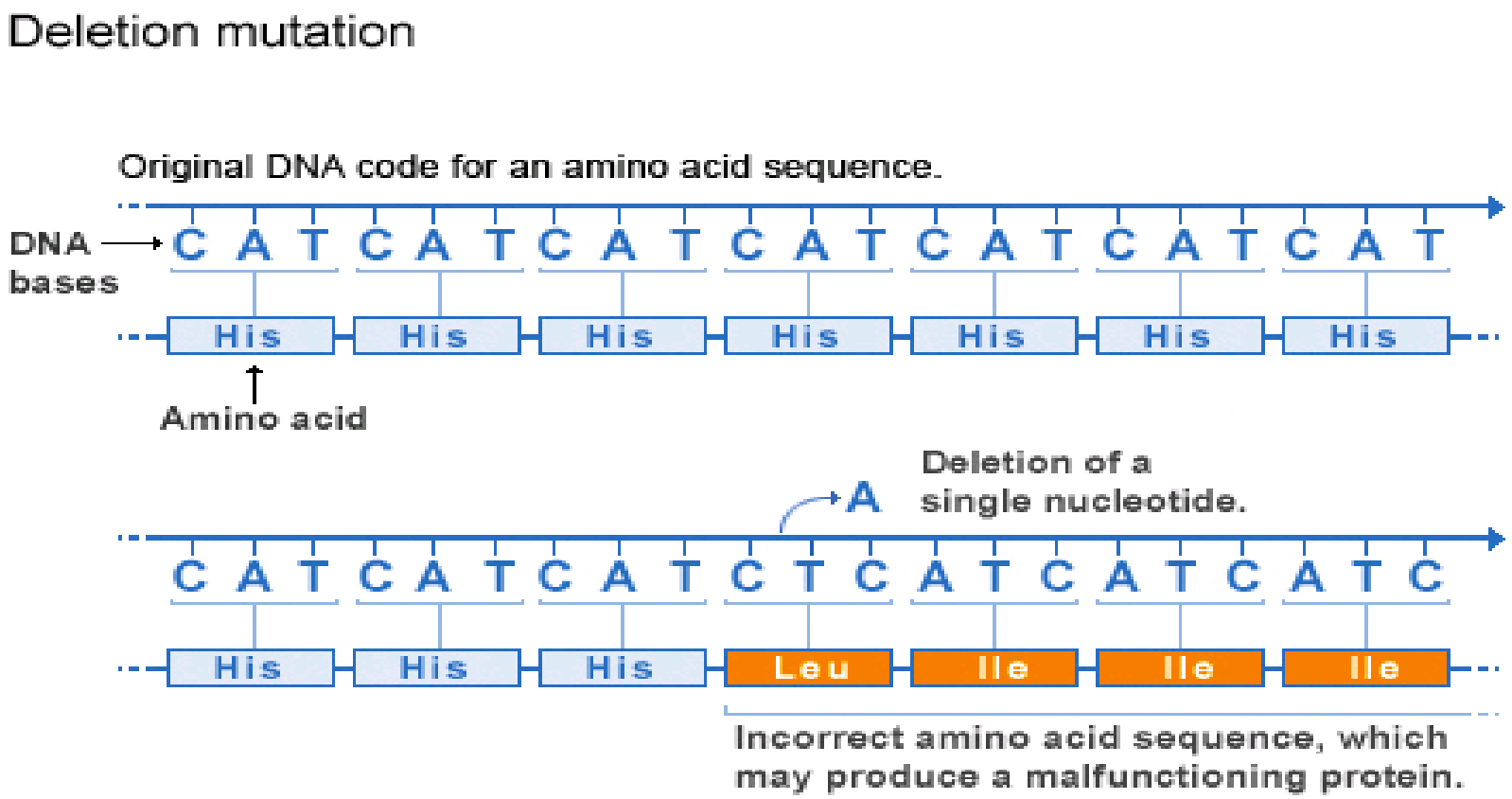


Types of Mutations

- An example of a human disorder caused by insertion is Huntington's disease.
- In this disorder, the repeated trinucleotide is **CAG**, which adds a string of glutamines (Gln) to the encoded protein (called **huntingtin**).
- The abnormal protein increases the level of the p53 protein in brain cells causing their death by apoptosis.



Deletion Mutation



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Examples of Diseases caused by deletions

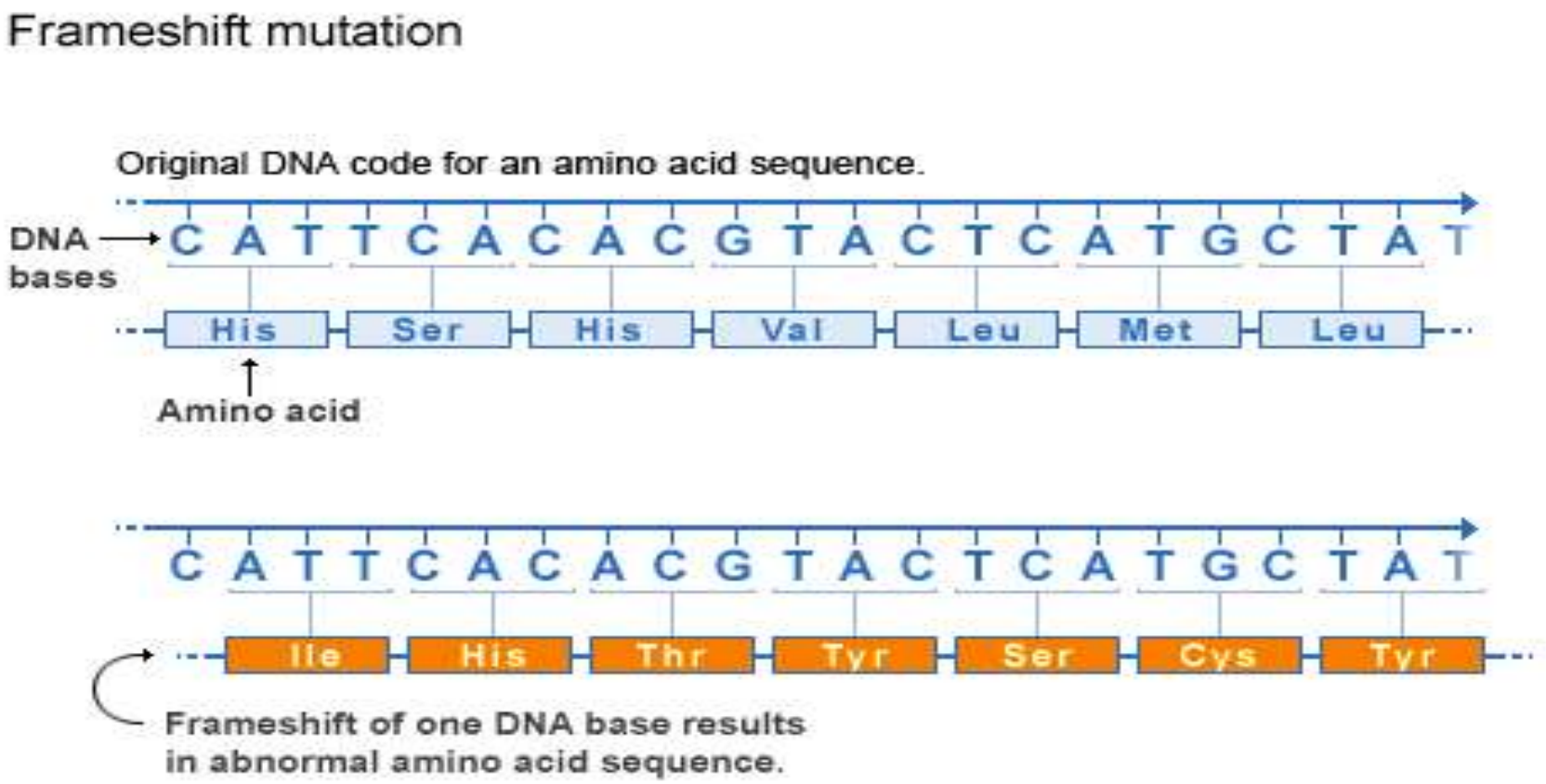
- Cri du chat
- De Grouchy syndrome
- Shprintzen syndrome
- Wolf-Hirschhorn syndrome
- Duchenne muscular dystrophy

Types of Mutations

- Insertion and deletions involving one or two base pairs (or multiples)
  - can have devastating consequences to the gene because translation of the gene is "**frameshifted**"
  - DNA is read in sequences of three bases therefore the addition or removal of one or more bases alters the sequence that follows as the bases all shifted.
  - The entire meaning of the sequence has changed.

- **Frameshifts** often create new **STOP** codons → nonsense mutations

Frame shift mutation



Mutation Frequency

- Frameshift, transition, transversion mutations are **infrequent**
  - Bacteria and phage: 10^{-8} to 10^{-10} per nucleotide pair per generation
  - Eukaryotes: 10^{-7} to 10^{-9} per nucleotide pair per generation

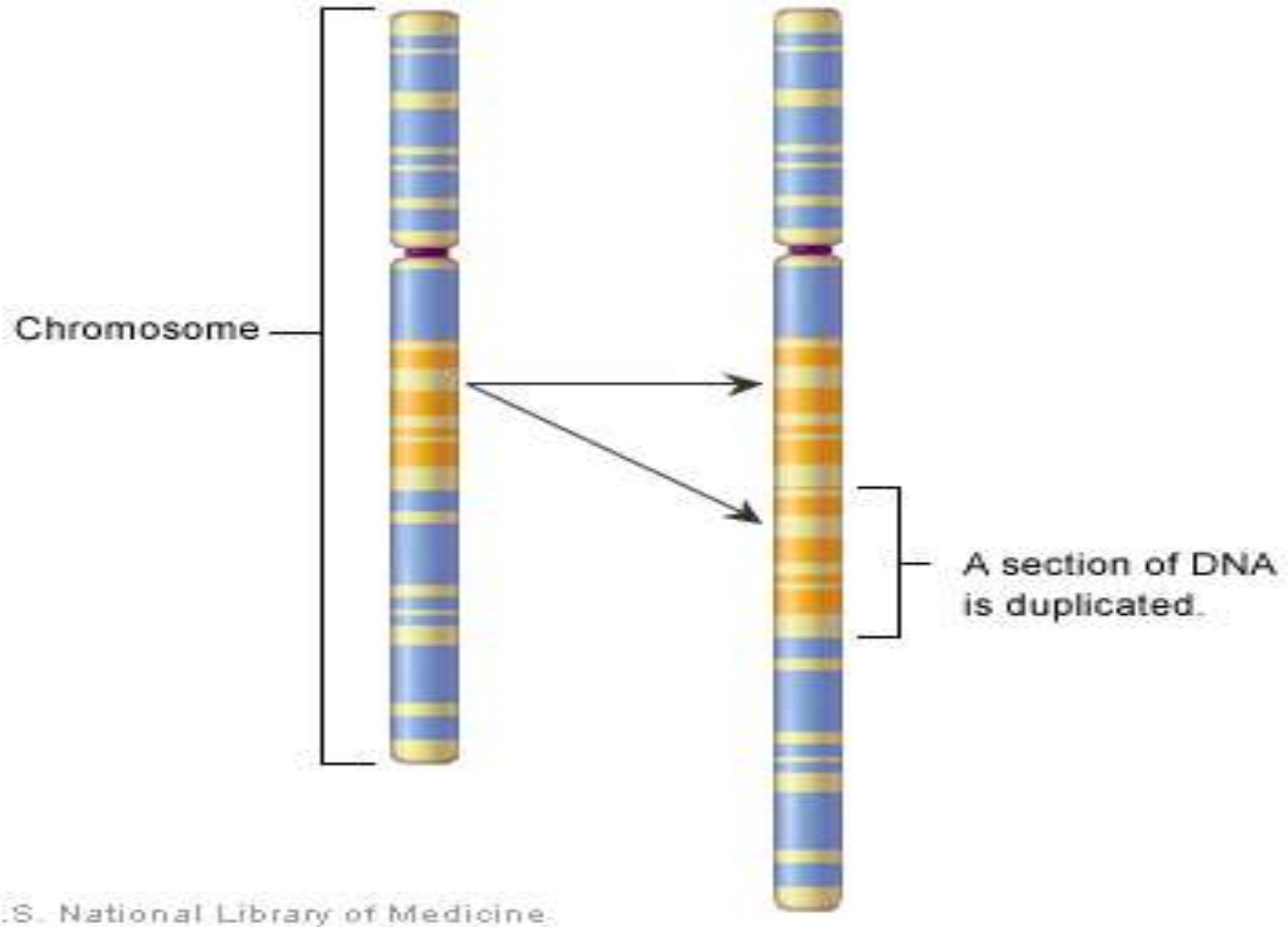
Silent mutation: **UCU=Ser; UCA, UCC, UCG = Ser**

Types of Mutations

Duplications

- Duplications are a doubling of a section of the genome.
- During meiosis, crossing over between sister chromatids that are out of alignment can produce one chromatid with an duplicated gene and the other having two genes with deletions.
  - Example of disease :DM1 (Myotonic dystrophy)

Duplication mutation



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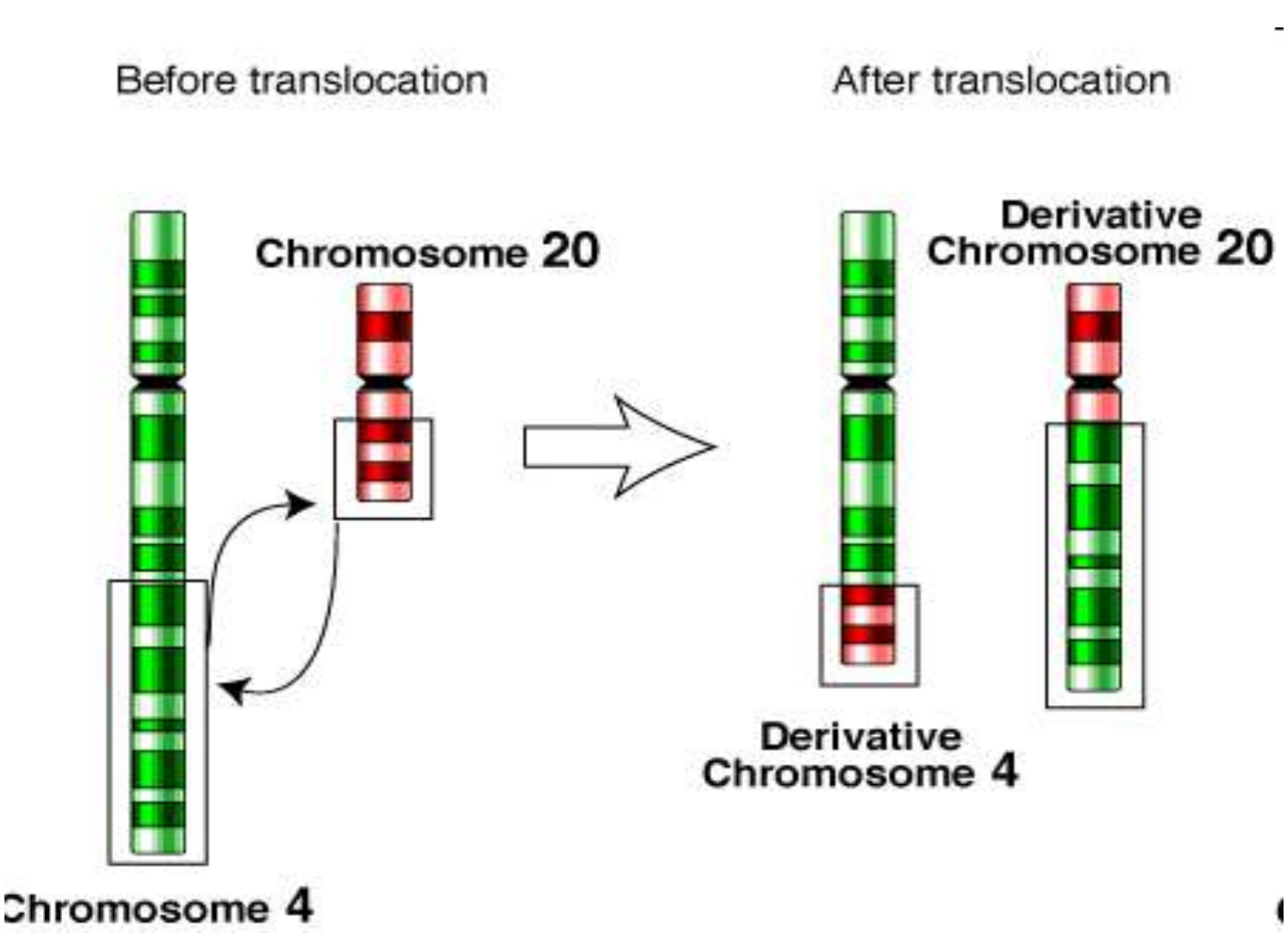
Types of Mutations

Translocations

- Translocations are the transfer of a piece of one chromosome to a **nonhomologous chromosome**.

- Translocations are often reciprocal; that is, the two nonhomologues swap segments.


Before translocation
After translocation
Chromosome 20
Derivative Chromosome 20
Derivative Chromosome 4
Chromosome 4

Types of Mutations

Translocations can alter the phenotype is several ways:

- The break may occur **within** a gene
  - destroying its function
  - creating a hybrid gene.

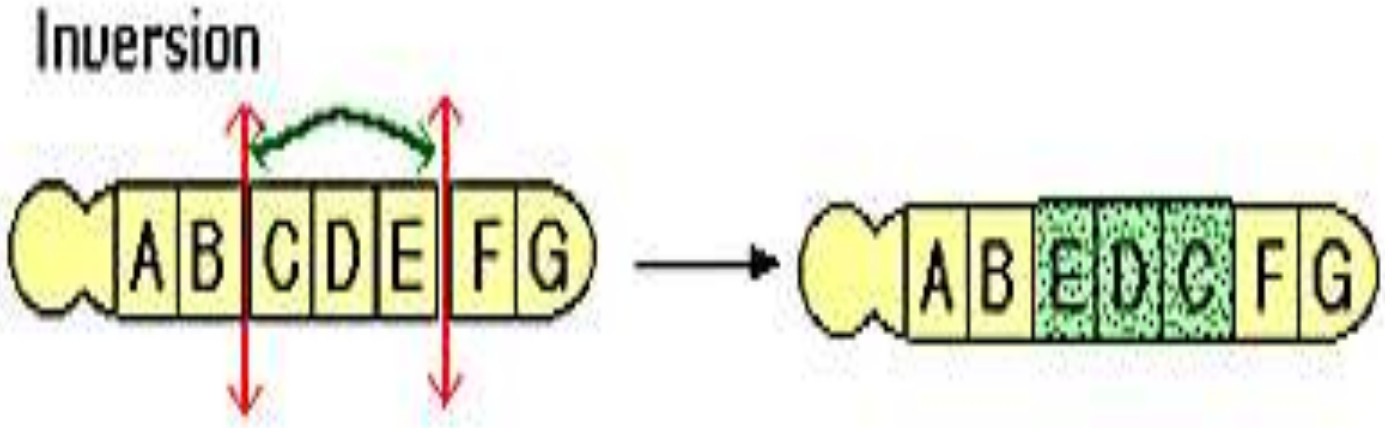
- Translocated genes may come under the influence of different promoters and enhancers so that their expression is altered.

Types of Mutations

Inversion

- An entire section of DNA is reversed.

- A small inversion may involve only a few bases within a gene, while longer inversions involve large regions of a chromosome containing several genes.



Types of Mutations

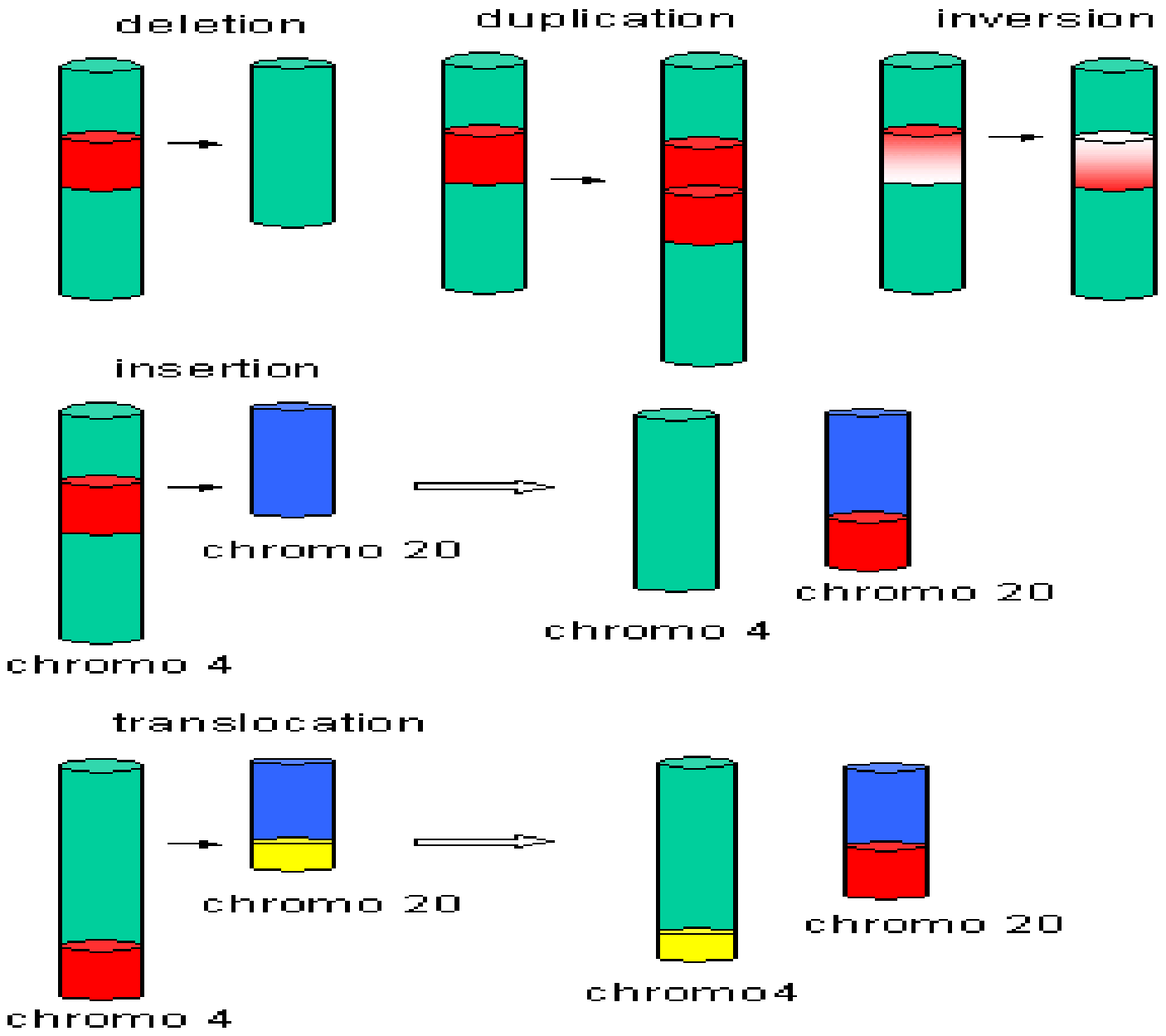
Suppressor mutation

- Partially or completely masks phenotypic expression of a mutation but occurs at a different site from it

  (i.e., causes suppression)
- May be intragenic or intergenic.

- It is used particularly to describe a secondary mutation that suppresses a nonsense codon created by a primary mutation.

Types of Mutations



Mutation: Spontaneous or Induced

- **Spontaneous mutations** occur without a known cause due to unknown agents in the environment.

- **Induced mutations** result from exposure or organisms to **mutagens**, **physica**l and **chemical agents** that cause changes in DNA, such as ionizing irradiation, ultraviolet light, or certain chemicals.

Factors Influencing the Rate of Spontaneous Mutations

- Accuracy of the **DNA replication** machinery
- Efficiency of the mechanisms for the **repair of damaged DNA**
- Degree of exposure to **mutagenic agents** in the environment

Mutation: Usually a Random, Non-adaptive Process

- Is mutation random **(intrinsic)** or directed by the environment?

- **Replica plating** was used to identify the presence of antibiotic (chemical) resistant bacteria prior to treatment with an antibiotic (chemicals).

- **Environmental stress** does not cause mutations but selects for mutants that are best adapted to the environmental stress.

Conditional Lethal Mutations (Experimental)

- **Conditional lethal mutations** are
  - Lethal in the **restrictive condition** but
  - Viable in the **permissive condition**.

- Mutants with conditional lethal alleles can be propagated under the permissive condition, and the phenotype can be studied under restrictive condition.

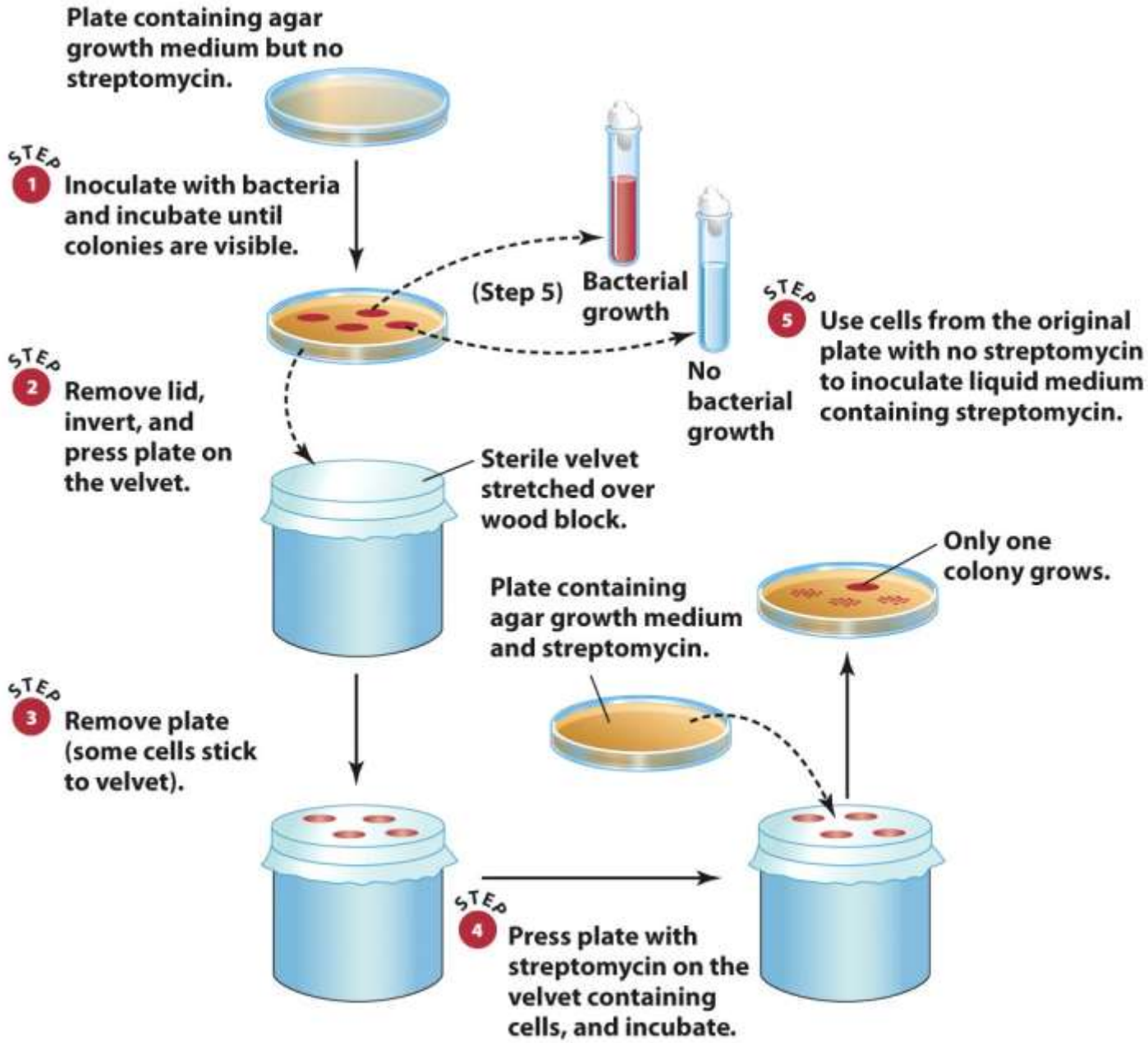
Conditional Lethal Mutants

- **Auxotrophs** are unable to synthesize an essential metabolite that is synthesized by prototrophs. Auxotrophs can grow only when the essential metabolite is supplied in the medium.
- **Temperature-sensitive mutants** will grow at one temperature but not at another.
- **Suppressor-sensitive mutants** are viable only when a second genetic factor, a suppressor, is present.

Isolation and analysis of mutants

- The procedures used to identify and isolate mutants, referred to as *genetic screens*
- The experimental organism
  - Haploid or
  - Diploid
    - Recessive
    - Dominant
- Mutations are induced by treatment with a mutagen
- Mutagenized population subjected to a genetic screen designed to identify and isolate individuals with mutations affecting a particular process of interest

- Characterization of mutants in experimental organisms – fundamental biological process
- Genetic analyses of mutants defective in a particular process can reveal:
  - the number of genes required for the process to occur;
  - the order in which gene products act in the process; and
  - whether the proteins encoded by different genes interact with one another.
- Analysis of mutants help to unravel
  - Metabolic pathways
  - Regulatory mechanisms
  - Developmental process



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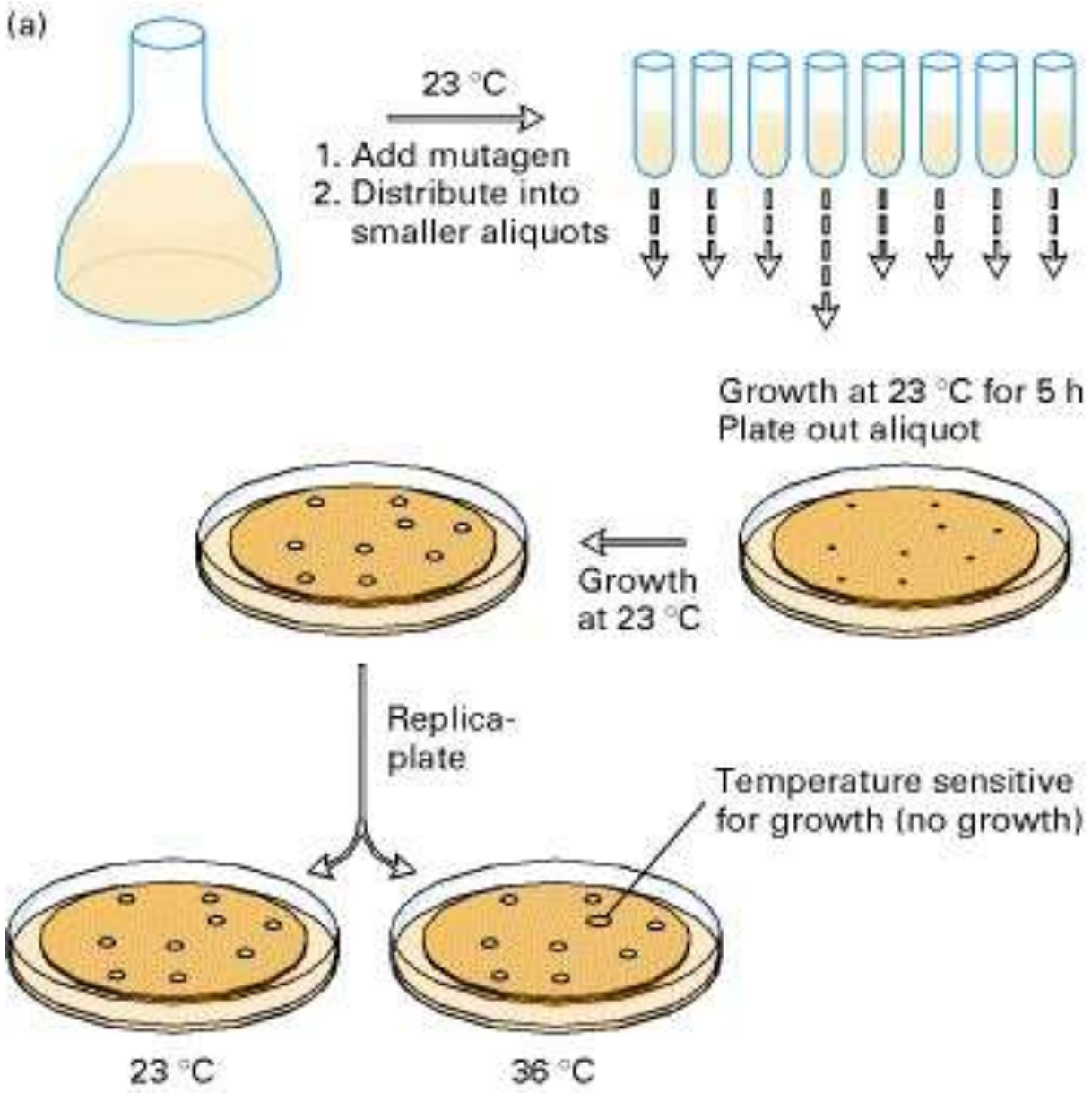
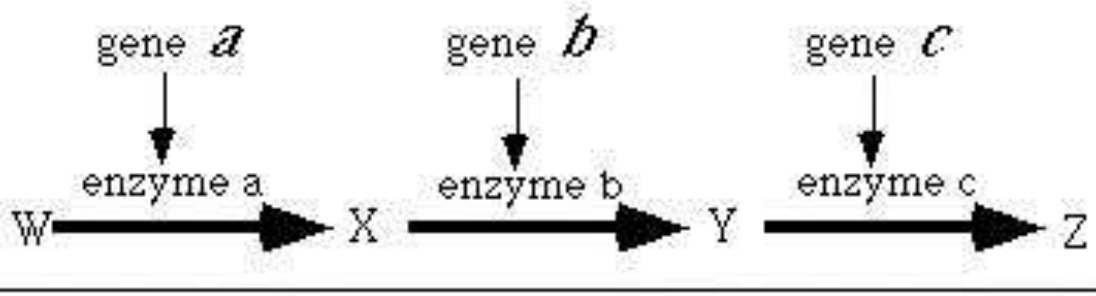
Two-step genetic screen used to identify cell-cycle mutants in yeast



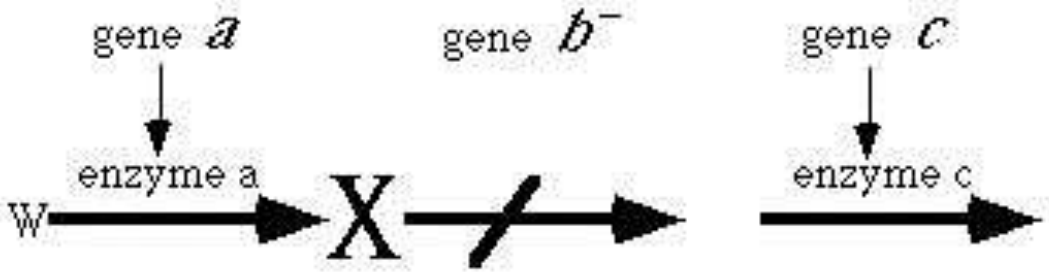
- ✓ Forward genetics – identification of a gene by mutant phenotype
- ✓ Reverse genetics – Using a cloned gene to find a mutant phenotype
- ✓ Genetic analysis of mutants
  - Crossfeeding tests
  - Complementation tests
  - Homologous recombination
    - Epistasis test

Crossfeeding rationale

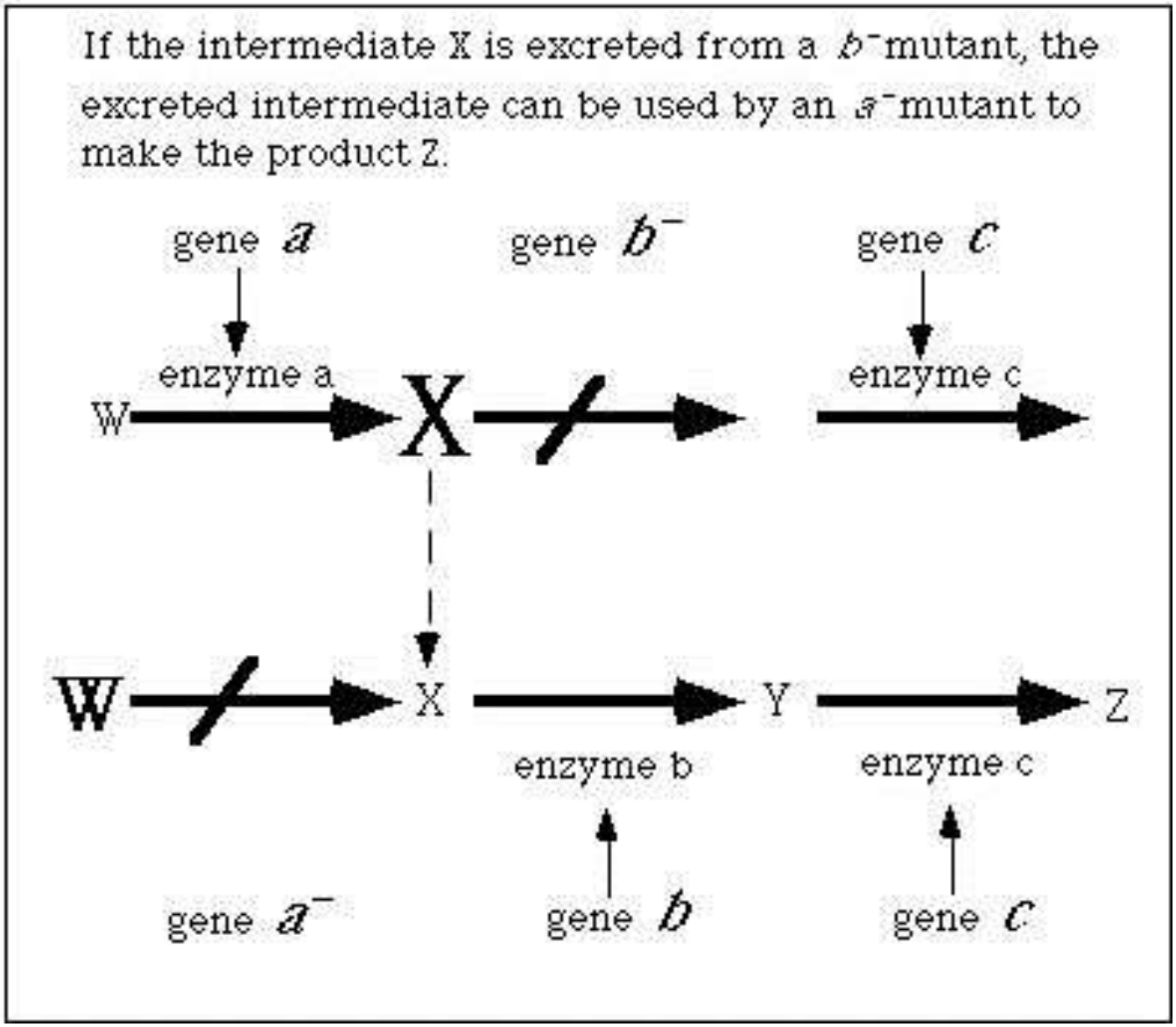
Given the following pathway for the synthesis of Z from A.



A mutant in gene b that does not make enzyme b cannot make Z but instead will accumulate the intermediate X.

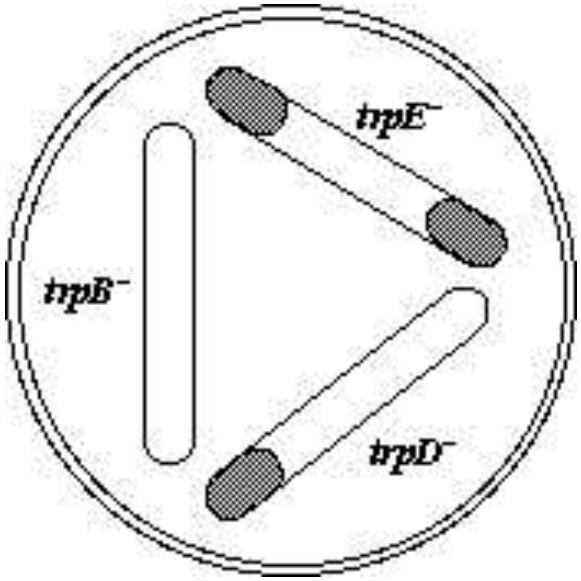


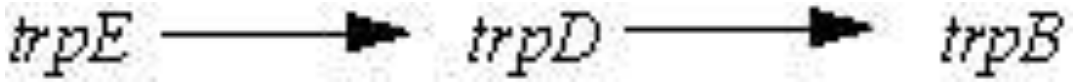
Crossfeeding rationale



Crossfeeding Experiment

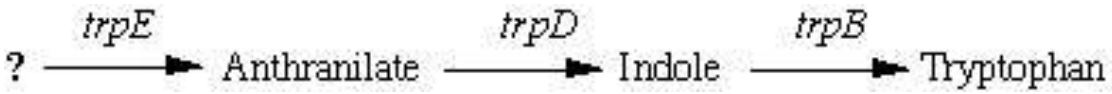
- Crossfeeding – the order of biosynthetic pathway with diffusible intermediates.
- Tryptophan auxotrophs on minimal medium plate supplemented with tiny amount of tryptophan





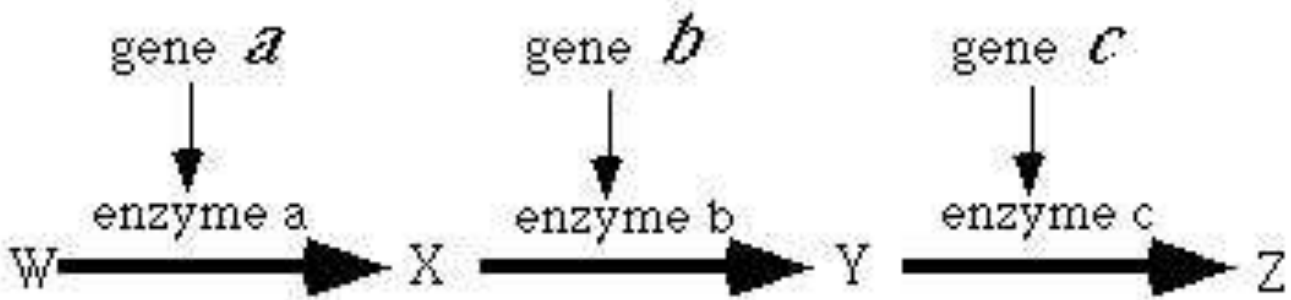
- ✓ It is possible to test which mutant use which intermediate if it is available/known
- ✓ Grow in liquid/agar medium containing the intermediate
- ✓ Simple method to detect – Crystal test
  - Spread the mutant on minimal agar plate lacking the auxotrophic requirement
  - Add few crystals of each intermediate near the edge of the plate
  - Growth near the crystal indicates that the mutant can use that intermediate

Mutation	Growth on minimal medium plus:				Intermediate accumulated
	None	Anthranilate	Indole	Tryptophan	
trp+	+	+	+	+	None
trpE	-	+	+	+	None
trpD	-	-	+	+	Anthranilate
trpB	-	-	-	+	Indole



Complementation Rationale

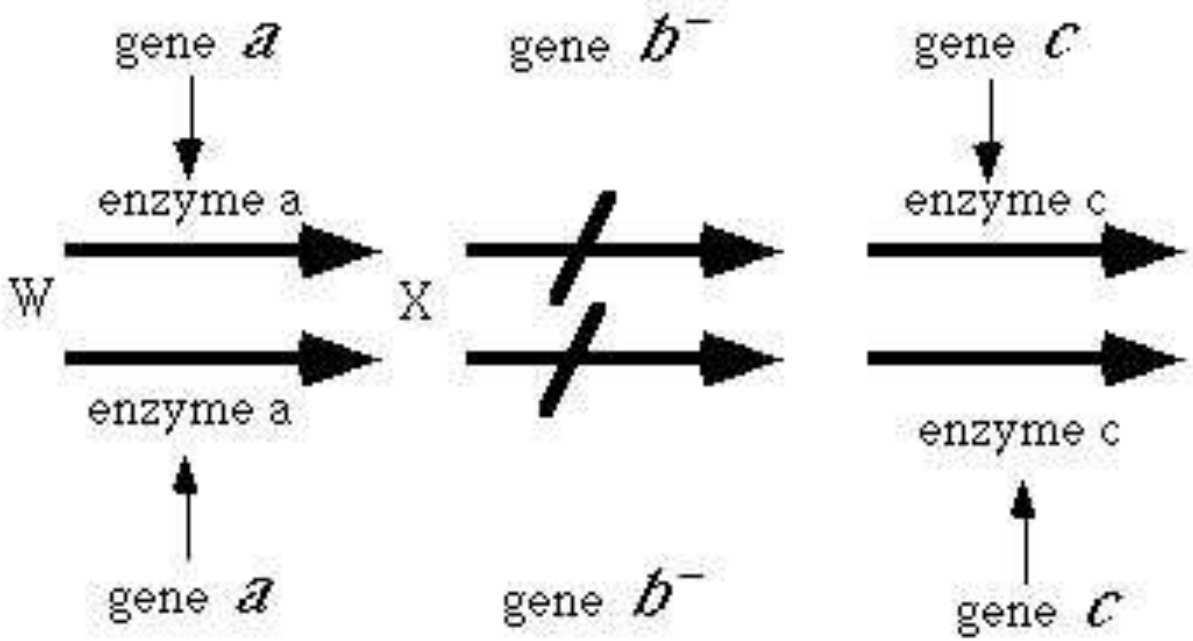
Given the following pathway for the synthesis of Z from A.



A mutant in gene b that does not make enzyme b cannot make Y, therefore will not be able to make the product Z.

Complementation Rationale

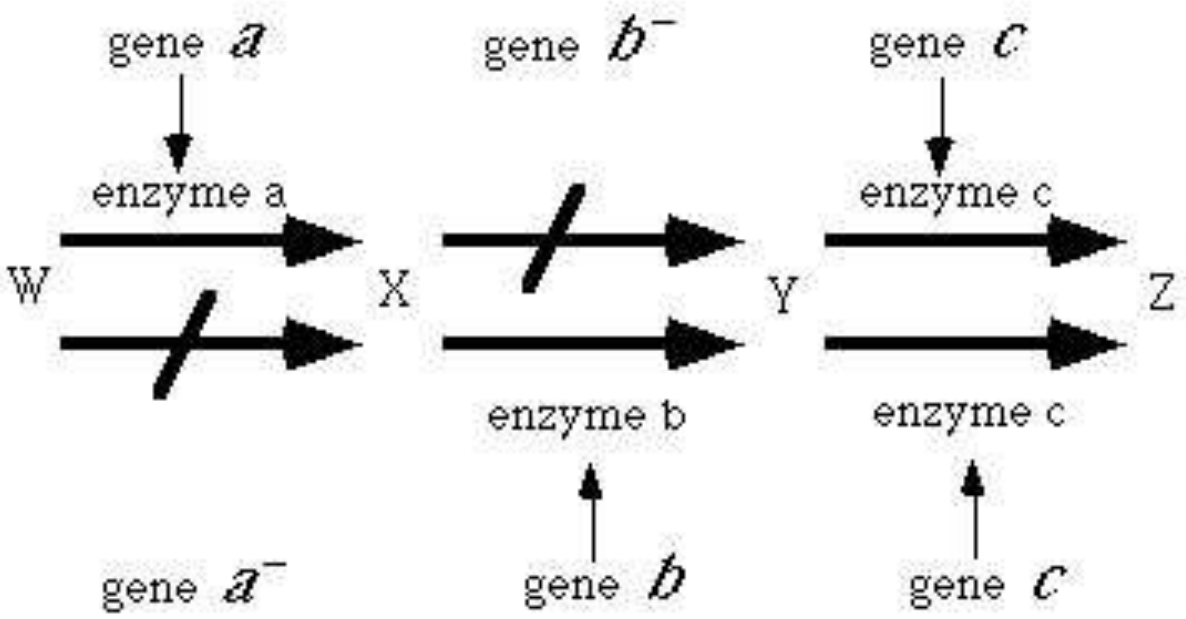
If the cell has two mutant copies of any of these genes, it will be unable to make all the necessary intermediates and, thus, will be unable to make the product Z.



No complementation (because mutations in same gene)

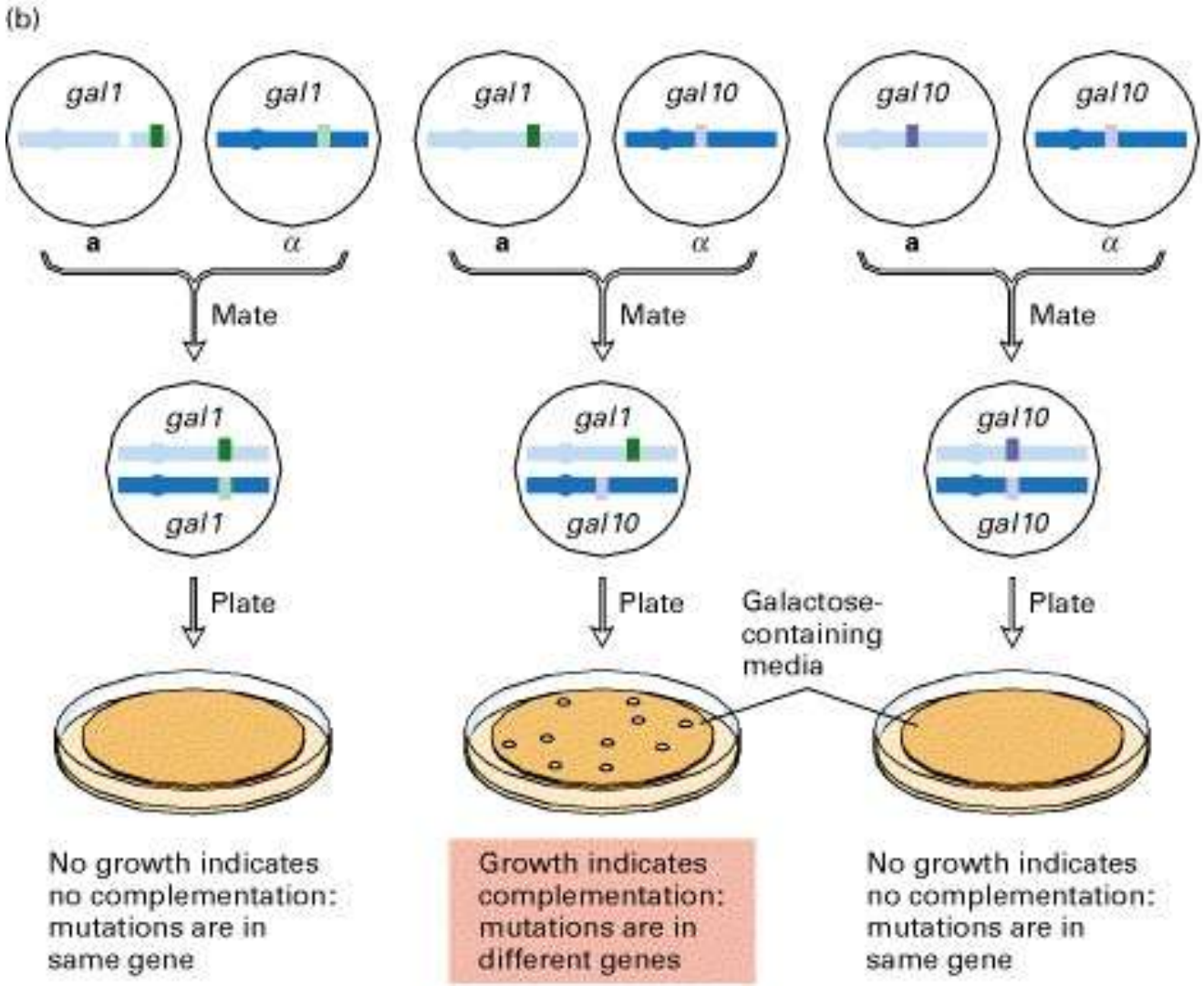
Complementation Rationale

If the cell has at least one good copy of each gene, it will be able to make all the necessary intermediates and, thus, will be able to make the product Z.



Complementation (because mutations in different genes)

Complementation analysis in *S. cerevisiae*



Epistasis

- A double mutant where one mutation masks the phenotype of another mutation
- Different from dominance. Mutation in one gene masks the expression of a different gene
- In dominance, one allele of a gene mask the expression of another allele of the same gene.

Tests of Epistasis

- ✓ Two mutations were isolated with different effects on proline utilization
  - *put-1020* makes cells constitutively express the *putP* gene at high levels,
  - *put-1222* prevents expression of the *putP* gene.
- ✓ To determine if these two mutations affect different "regulatory pathways" or if they both affect the same "regulatory pathway", the double mutant was constructed.

Question

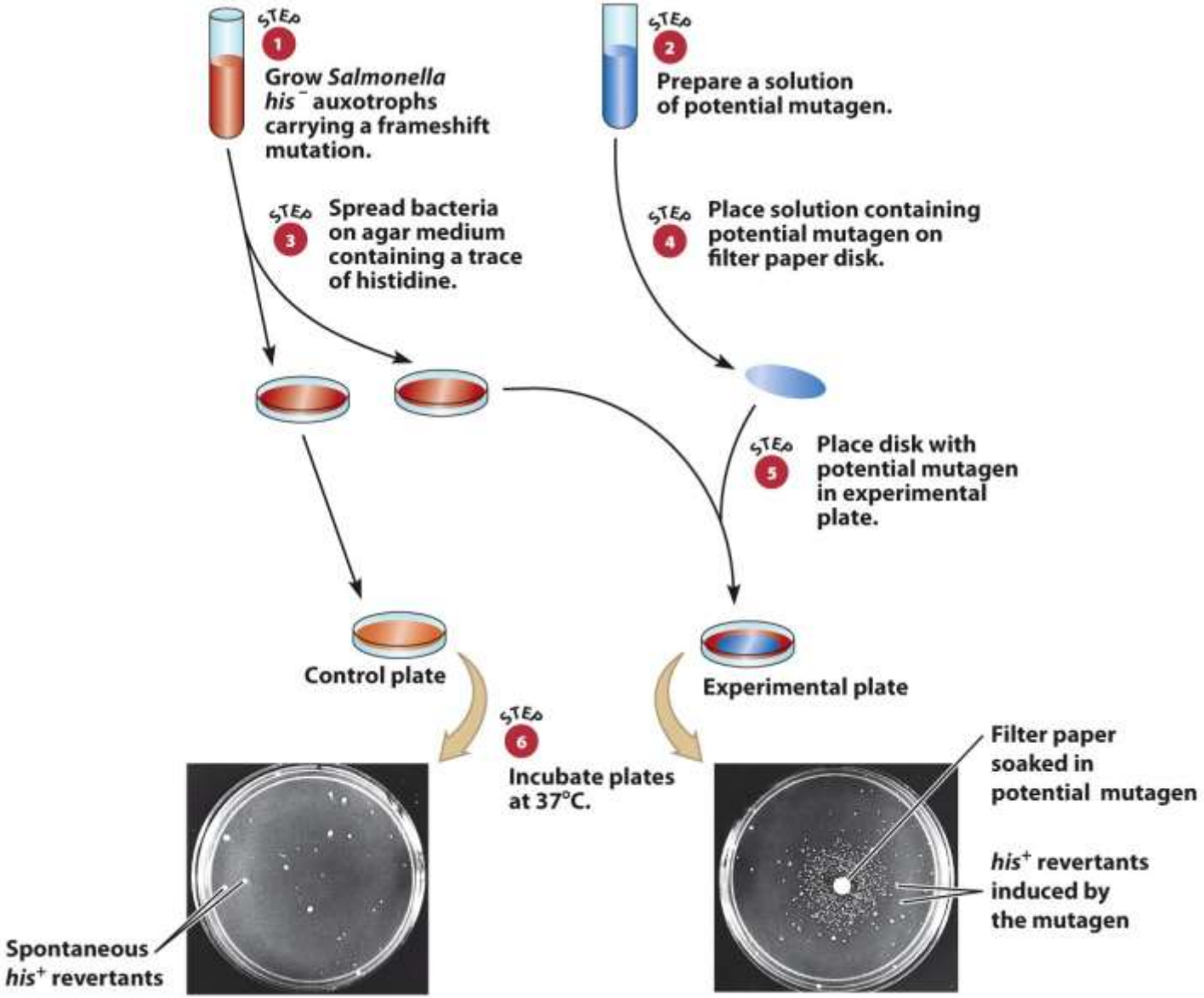
- The phenotype of the *put-1020 put-1222* double mutant is constitutive expression of the *putP* gene – What is your inference?
  - ✓The *put-1020* is epistatic to *put-1222*.
- What results would you expect if the two mutations affected different pathways?
  - ✓ Intermediate between two extremes

Screening Chemicals for mutagenicity: The Ames test

The Ames test provides a simple and inexpensive method for detecting the mutagenicity of chemicals (**Carcinogens)**

- **intracellular**
- **extracellular**
- **enviromental**

Auxotrophic ⟶ prototrophic



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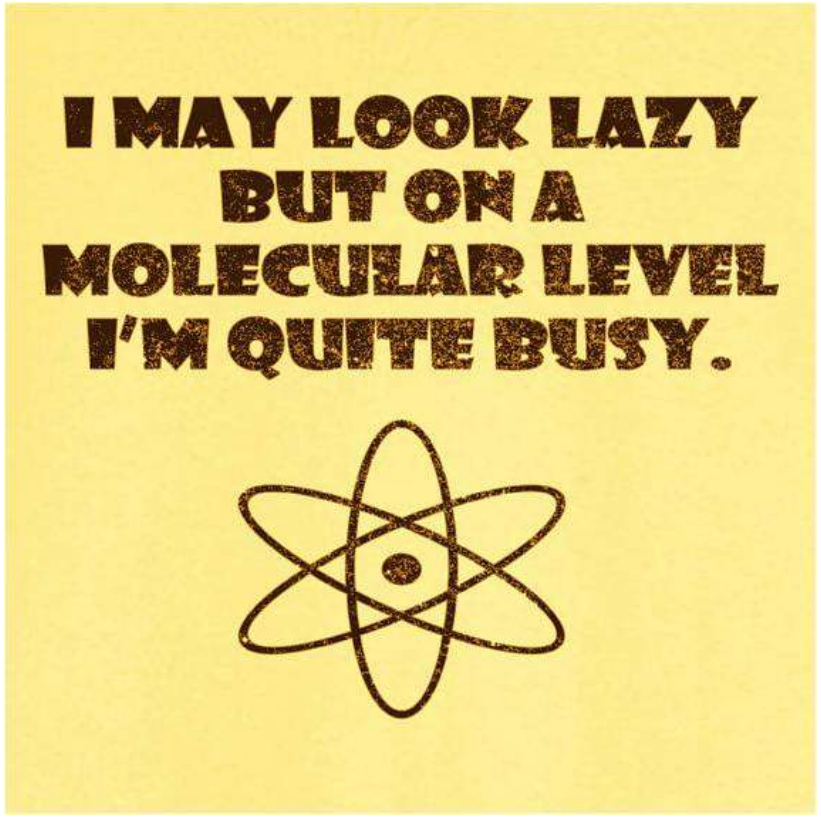
Naming genes

- Given an official name and symbol by a formal committee
- The HUGO Gene Nomenclature Committee (HGNC) – US and UK designates an official name and symbol (an abbreviation of the name) for each known human gene.
- Some official gene names include additional information in parentheses, such as related genetic conditions, subtypes of a condition, or inheritance pattern.
- The Committee has named more than 13,000 of the estimated 20,000 to 25,000 genes in the human genome.
- A unique name and symbol are assigned to each human gene, which allows effective organization of genes in large databanks, aiding the advancement of research.

How are genetic conditions named?

Disorder names are often derived from one or a combination of sources:

- The basic genetic or biochemical defect that causes the condition (alpha-1 antitrypsin deficiency)
- One or more major signs or symptoms of the disorder (sickle cell anemia)
- The parts of the body affected by the condition (retinoblastoma)
- The name of a physician or researcher, often the first person to describe the disorder (Marfan syndrome - Dr. Antoine Marfan)
- A geographic area (familial Mediterranean fever)
- The name of a patient or family with the condition (Lou Gehrig disease)
- Disorders named after a specific person or place are called eponyms.



Thank you