

INDUSTRIAL BIOCHEMISTRY

Industry

- In a general sense, the production of goods and services in an economy
- The term *industry* also refers to a group of enterprises (private businesses or government-operated corporations) that produce a specific type of good or service

Historical perspective

- Winemaking probably began as one of the earliest of human enterprises (8000-3000 B.C.).
- The wine grape was domesticated by at least 4000 B.C. Wine was used for Egyptian worship ceremonies.
- Wine only became a popular beverage about 2000-1000 B.C. in Greece.
- About 600 B.C., wine growing reached France.

Historical perspective

- Most processes do not occur deliberately
- Beer is believed to be over 10,000 years old
- Although no one knows its exact origins
- Agricultural historians believe that the first beer may have been produced accidentally when a stash of grain was soaked by rain and then warmed by the sun, spontaneously fermented by wild, airborne yeast

Historical perspective

- At about 600BC, Samaritans and Babylonians brewed beer
- At about 4000BC Egyptians taking beer
- Around the ancient world several other local alcoholic beverages were produced before industrial application, e.g.
- Palm wine, *BKT*, *pito*, etc
- Others were *dadawa*, *ogiri*, *nono*
- All were done under unsterile condition up to the 16th Century

Sterile condition

- Use Started
 - Between the 18th and 19th Century
 - When the Pasteurization was developed
- Is achieved through working with pure cultures

MICROBIAL GROWTH

MICROBIAL GROWTH

- Nature of microorganisms need to be known since the techniques require them to produce products
- Growth could be in terms of increase in dry wgt or size
- Is the increase in colony size or number of cells or number of colonies
- In liquid or broth, rate is measured at 620 – 650nm wavelength

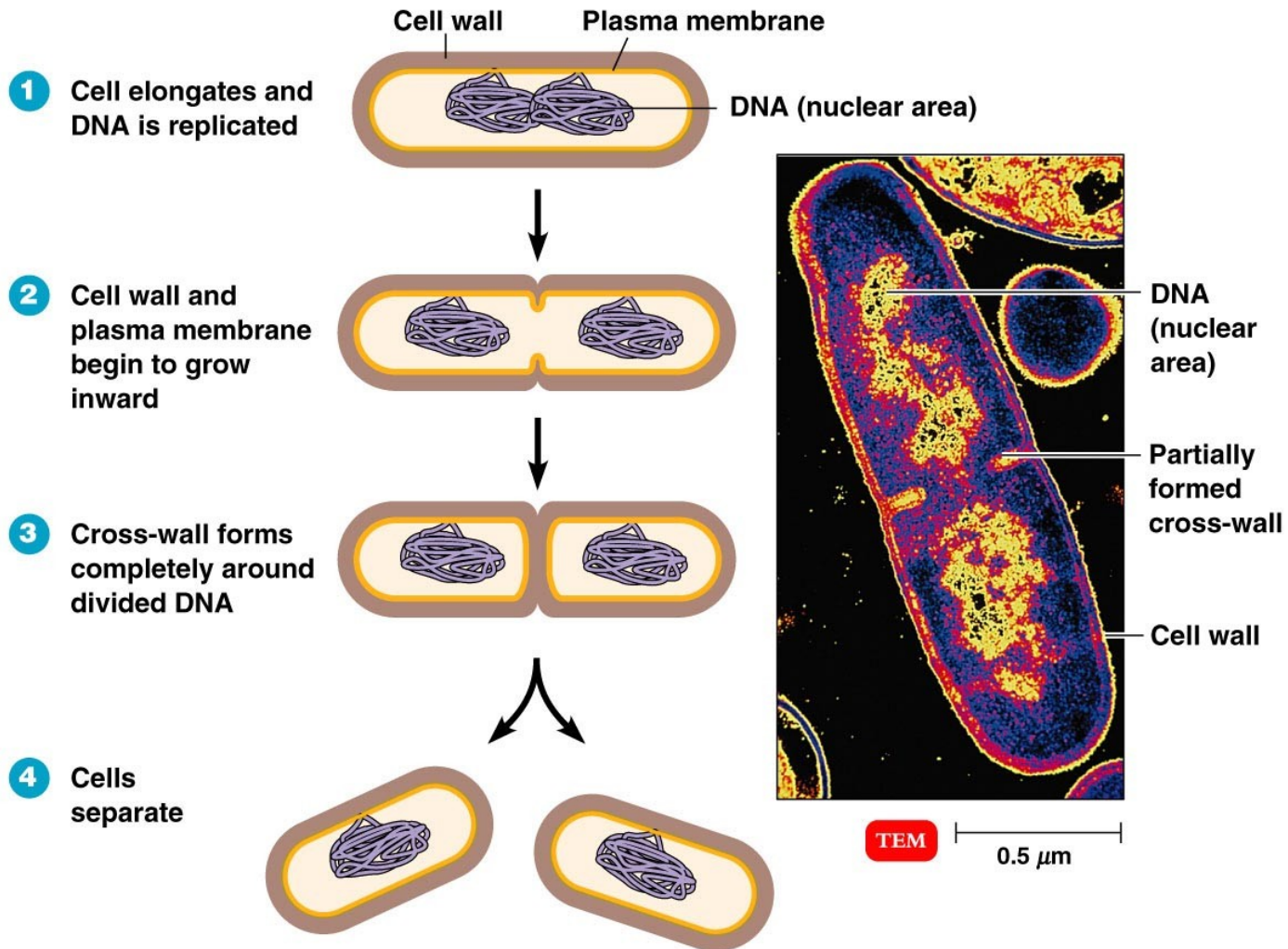
MICROBIAL GROWTH

- There 2 ways to express microbial growth
 - Cell concn – expressed in number of cells per unit vol.
 - Cell density - expressed in dry wgt of cells per unit vol.
- Total or viable count can also be done
 - Total count – counting every cell both living and dead
 - Viable count – counting only living cells

MICROBIAL GROWTH

- Processes of growth include:
 - Binary fission
 - Budding
 - Conidiospores (actinomycetes)
 - Fragmentation of filaments

Binary Fission



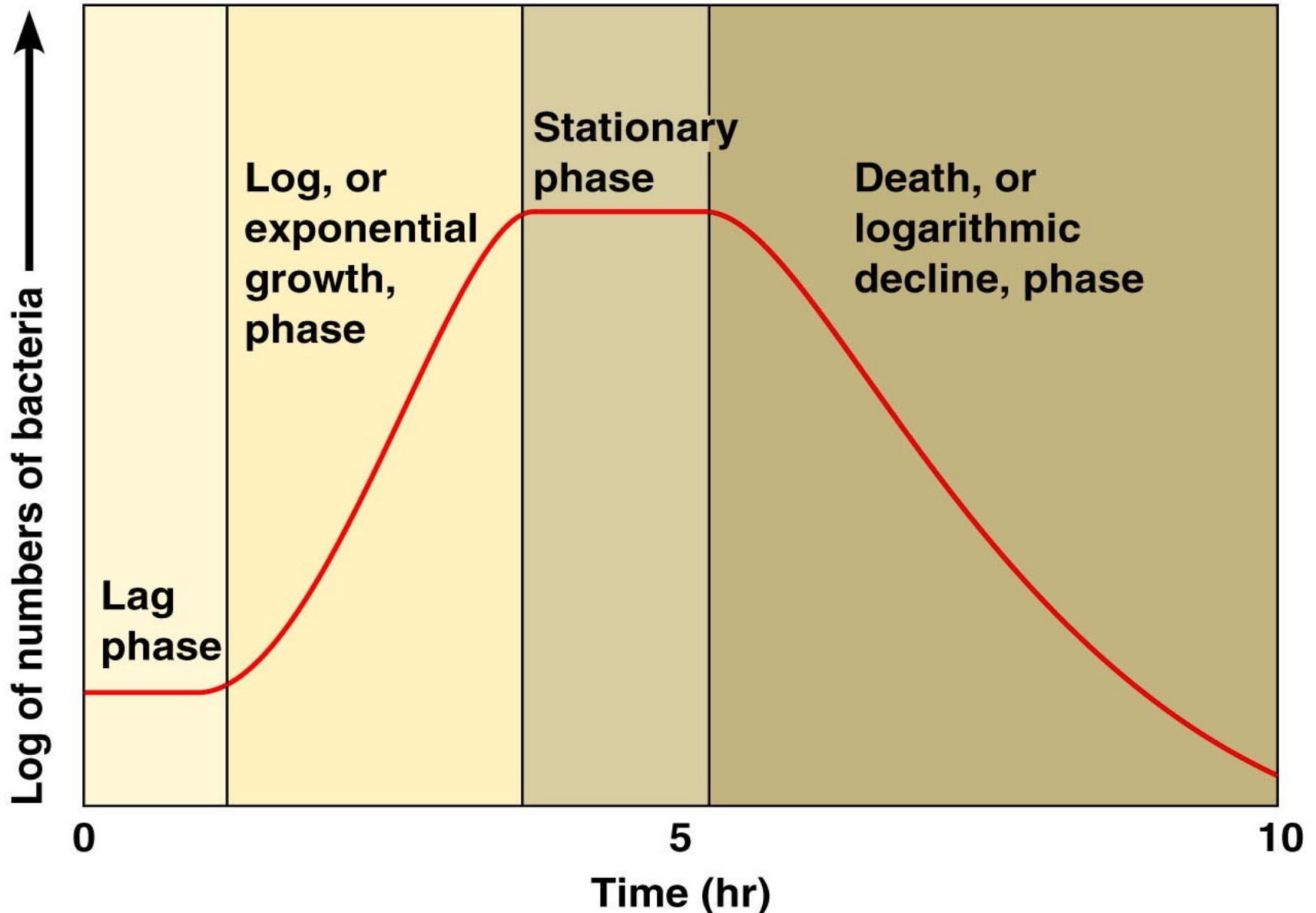
(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Phases of Growth

- 4 main phases:
- Lag phase-
- Acceleration phase
- Log (logarithmic) phase
- Retardation phase
- Stationary phase
- Decline phase or death phase

4 Phases of Microbial Growth



Growth in Batch Culture

- Bacteria growing in **batch culture** produce a **growth curve** with up to four distinct phases.
- Batch cultures are grown in tubes or flasks and are **closed systems** where no fresh nutrients are added or waste products removed.

Growth in Batch Culture

- **Lag phase** occurs when bacteria are adjusting to their medium. For example, with a nutritionally poor medium, several anabolic pathways need to be turned on, resulting in a lag before active growth begins.
- In **log** or **exponential phase**, the cells are growing as fast as they can, limited only by growth conditions and genetic potential. During this phase, almost all cells are alive, they are most nearly identical, and they are most affected by outside influences like disinfectants.

Growth in Batch Culture

- Due to nutrient depletion and/or accumulation of toxic end products, replication stops and cells enter a **stationary phase** where there is no net change in cell number.
- **Death phase** occurs when cells can no longer maintain viability and numbers decrease as a proportion.

Measuring Microbial Growth

Direct methods

- Plate counts
- Filtration
- Direct microscopic count
- Dry weight

Indirect methods

- Turbidity
- Metabolic activity
- Dry weight

Growth of Microbes

- Control of growth is important for
 - infection control
 - growth of industrial and biotech organisms



Factors Regulating Growth

- There are two
 - Internal factors
 - External factors

Factors Regulating Growth

- Internal factors
- These involve the genetic ability of the micro organism such as
- Growth rate e.g. *E.coli* has 20mins; *Mycobacterium tuberculosis* with 18hrs; *S. cerevisiae* with 2hrs, etc
- Lag time – time taken to adjust to new conditions
- Previous history - the phase of growth at which the organism was taken

Factors Regulating Growth

- External factors

- Temperature
- Nutrients
- pH
- Aeration
- Light
- Competition

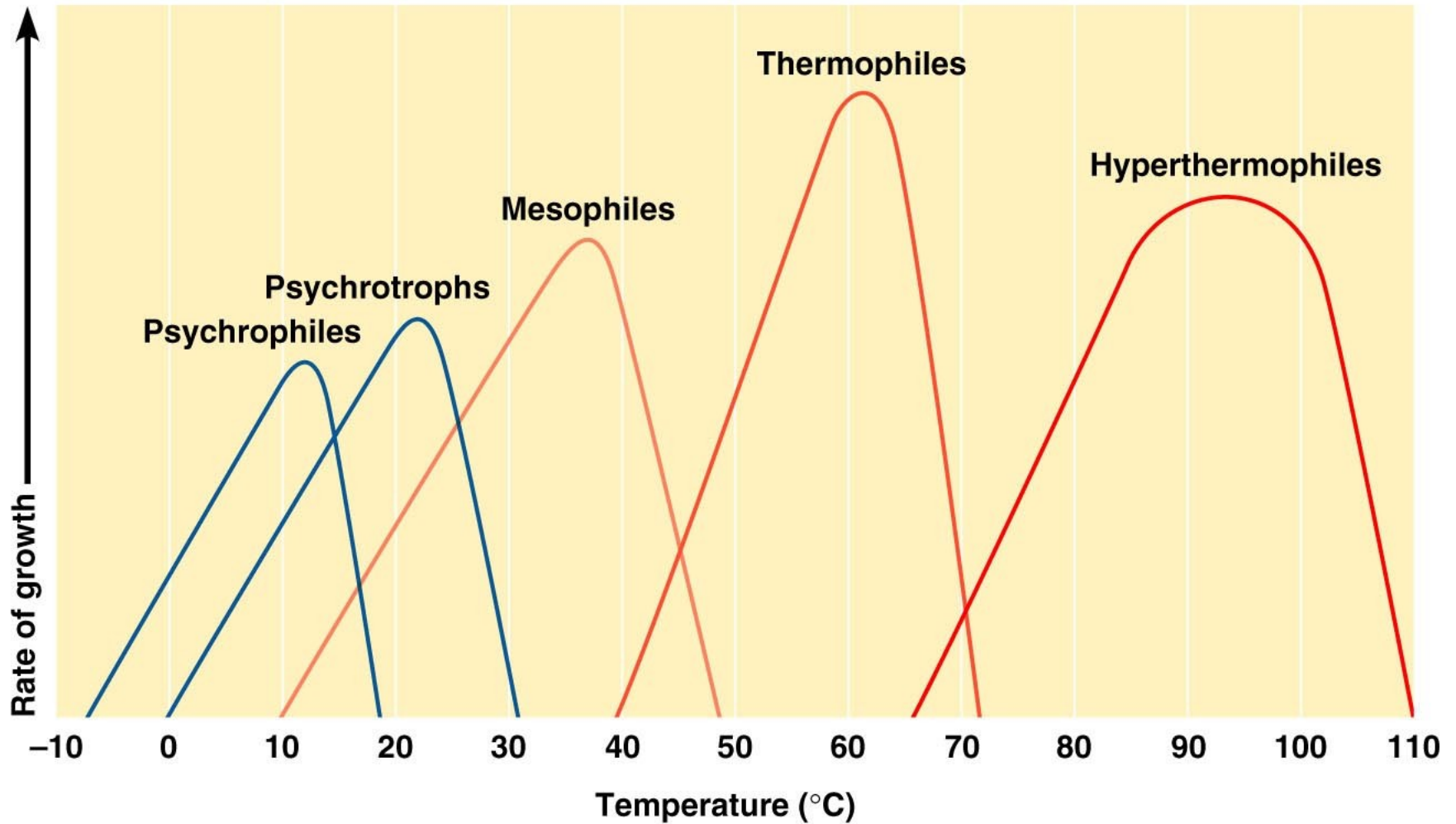
Factors Affecting Bacterial Growth

The Requirements for Growth:

Physical Requirements

- Temperature
 - Minimum growth temperature
 - Optimum growth temperature
 - Maximum growth temperature

Temperature



Psychrotrophs/Psychrophiles

- Grow between 0°C and 20-30°C
- Cause food spoilage

Mesophiles & Thermophiles

- Mesophiles-
- grow best between 25 °C and 40°C
- Thermophiles-
- Heat loving- grow best at 50 - 60 °C
- Obligate thermophiles
- Facultative thermophiles-

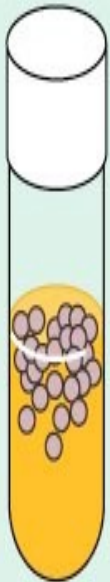
The Requirements for Growth: Physical Requirements

- pH
 - Most bacteria grow between pH 6.5 and 7.5
 - Molds and yeasts grow between pH 5 and 6
 - Acidophiles grow in acidic environments

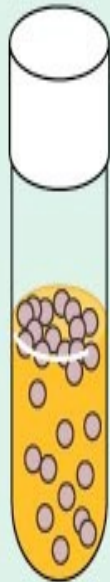
The Requirements for Growth: Physical Factors - Chemical Requirements

Oxygen (O₂)

a. Obligate
Aerobes



b. Facultative
Anaerobes



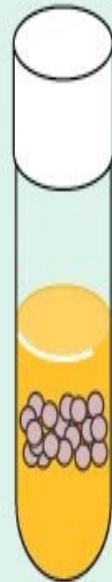
c. Obligate
Anaerobes



d. Aerotolerant
Anaerobes



e. Micro-
aerophiles

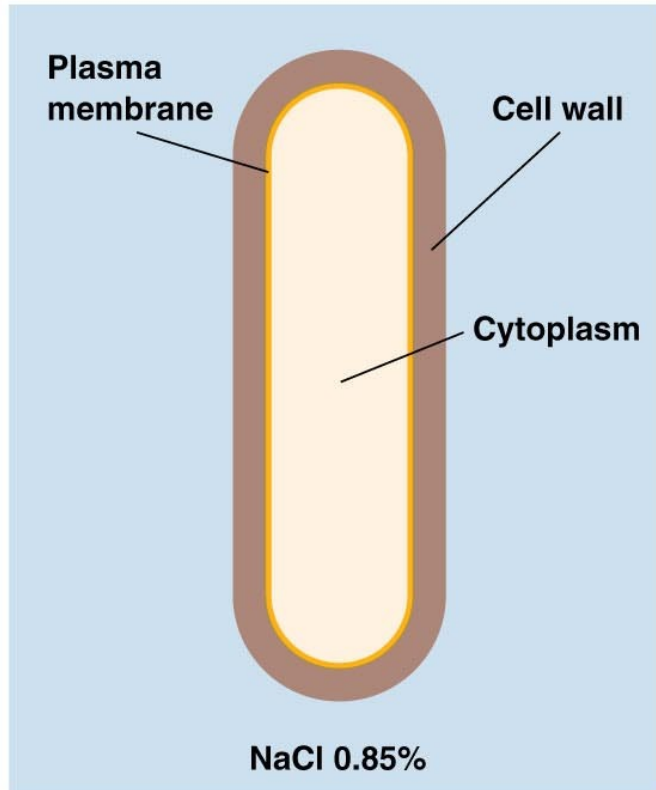


The Requirements for Growth: Physical Factors / Requirements

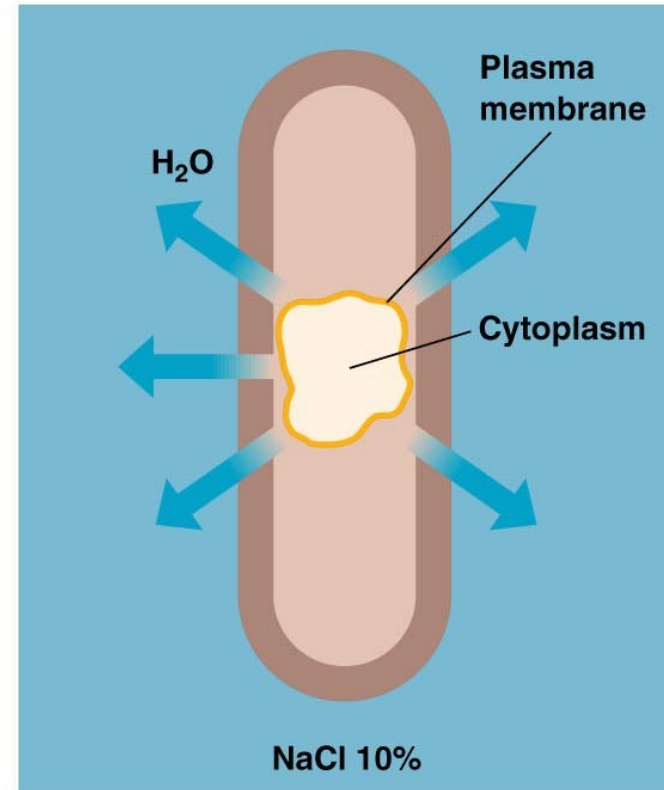
- Osmotic pressure
 - Hypertonic environments, increase salt or sugar, cause plasmolysis
 - Extreme or obligate halophiles require high osmotic pressure
 - Facultative halophiles tolerate high osmotic pressure

The Requirements for Growth:

Physical Requirements



(a) Normal cell in isotonic solution. Under these conditions, the osmotic pressure in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl).



(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

The Requirements for Growth: Nutritional Factors - Chemical Requirements

- Carbon
 - Structural organic molecules, energy source
 - Chemoheterotrophs use organic carbon sources
 - Autotrophs use CO₂

The Requirements for Growth: Nutritional Factors - Chemical Requirements

- Nitrogen
 - In amino acids and proteins
 - Most bacteria decompose proteins
 - Some bacteria use NH_4^+ or NO_3^-
 - A few bacteria use N_2 in nitrogen fixation

The Requirements for Growth: Nutritional Factors - Chemical Requirements

- Sulfur
 - In amino acids, thiamine and biotin
 - Most bacteria decompose proteins
 - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
 - In DNA, RNA, ATP, and membranes
 - PO_4^{3-} is a source of phosphorus

The Requirements for Growth: Nutritional Factors - Chemical Requirements

- **Trace elements**

- Inorganic elements required in small amounts
- Usually as enzyme cofactors
- Vitamins- organic substances and growth factors
- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, and pyrimidines

The Requirements for Growth: Nutritional Factors - Chemical Requirements

- **Trace elements**
 - Nutritional Complexity
 - Locations of Enzymes
 - Adaptations to Limited Nutrients

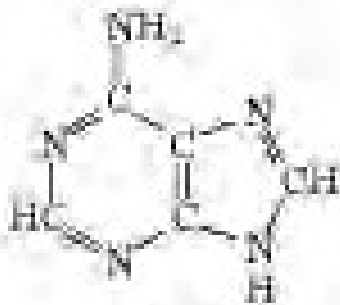
Microbial Genetics

Microbial Genetics

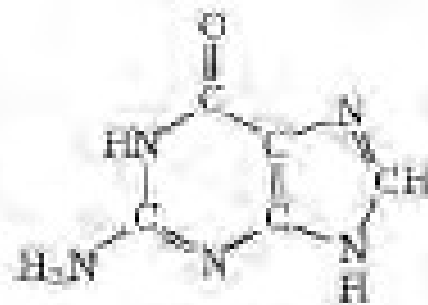
- study of the variations among organisms and how the variations are transferred from generation to generation
- study of the function and behavior of genes
- Understanding this requires the understanding of **genomics**
- genomics is the study of **genomes**

Nucleic Acid Structure

BASES – Purines and Pyrimidines

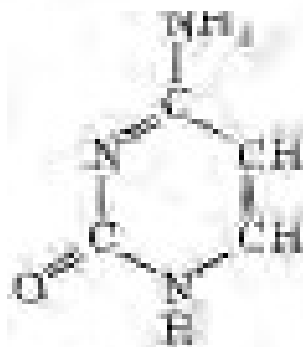


Adenine

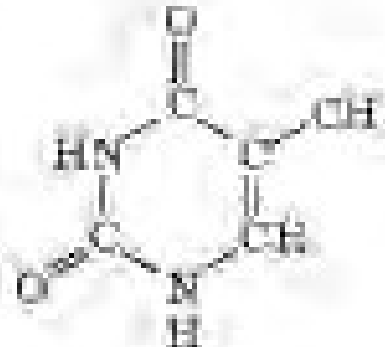


Guanine

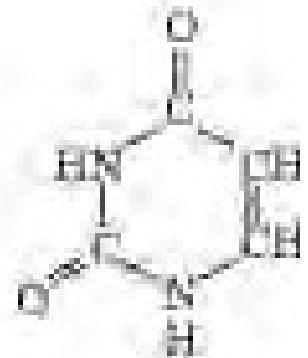
Purines



Cytosine



Thymine
(DNA)



Uracil
(RNA)

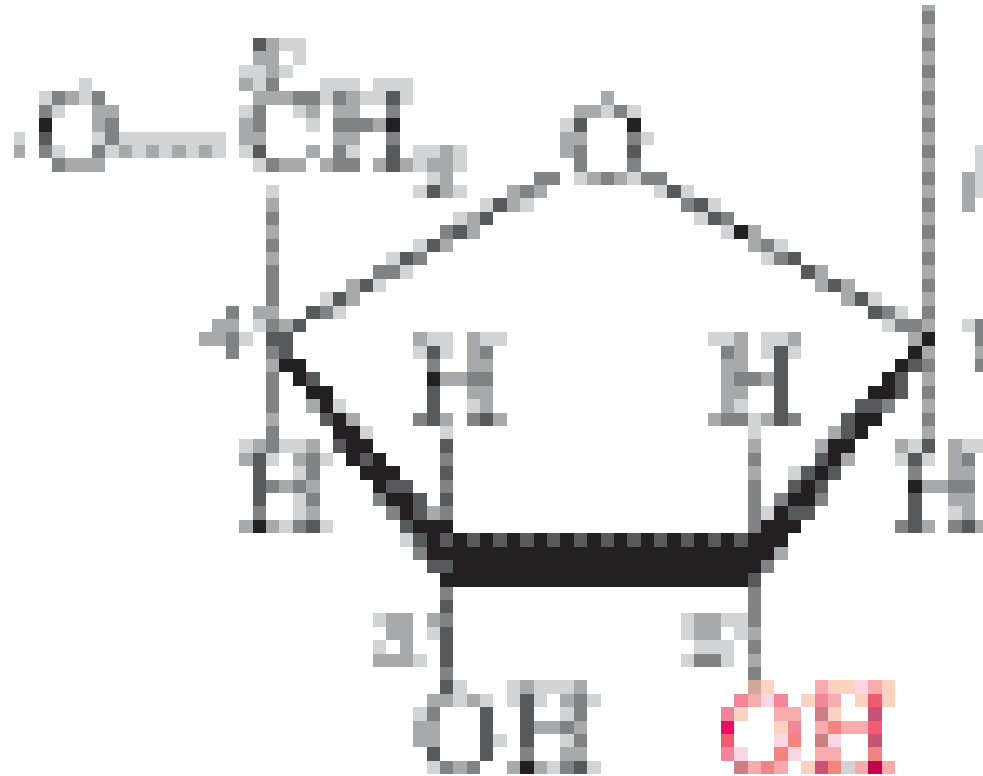
Pyrimidines

Properties of Bases

- Weakly bases hence called bases
- Chemical properties
 - Conjugation
 - Resonance
 - Tautomerism
 - Solubility
 - Stacking
 - Pairing
 - planarity

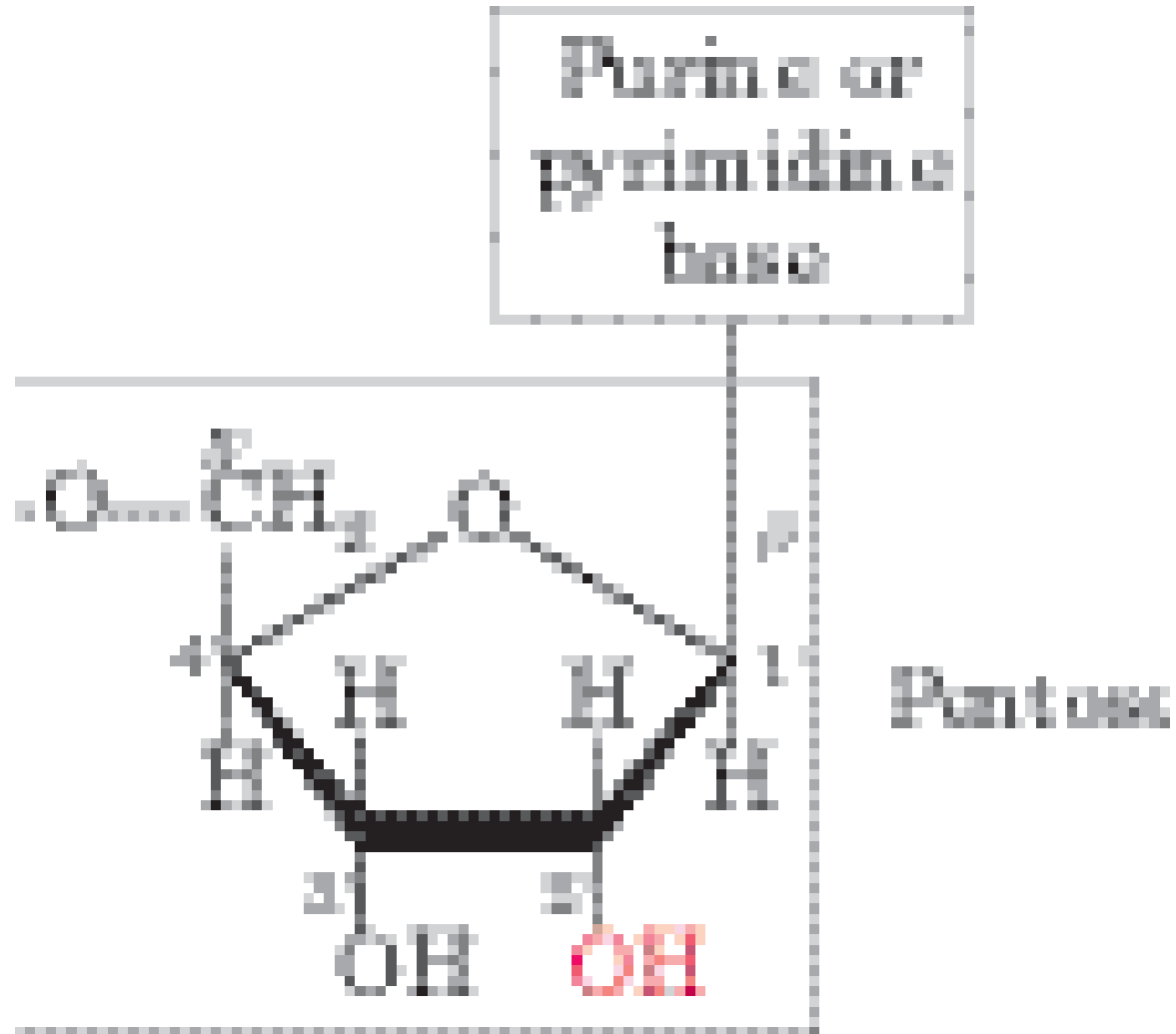
SUARS – Pentoses

- deoxy D ribose and D ribose



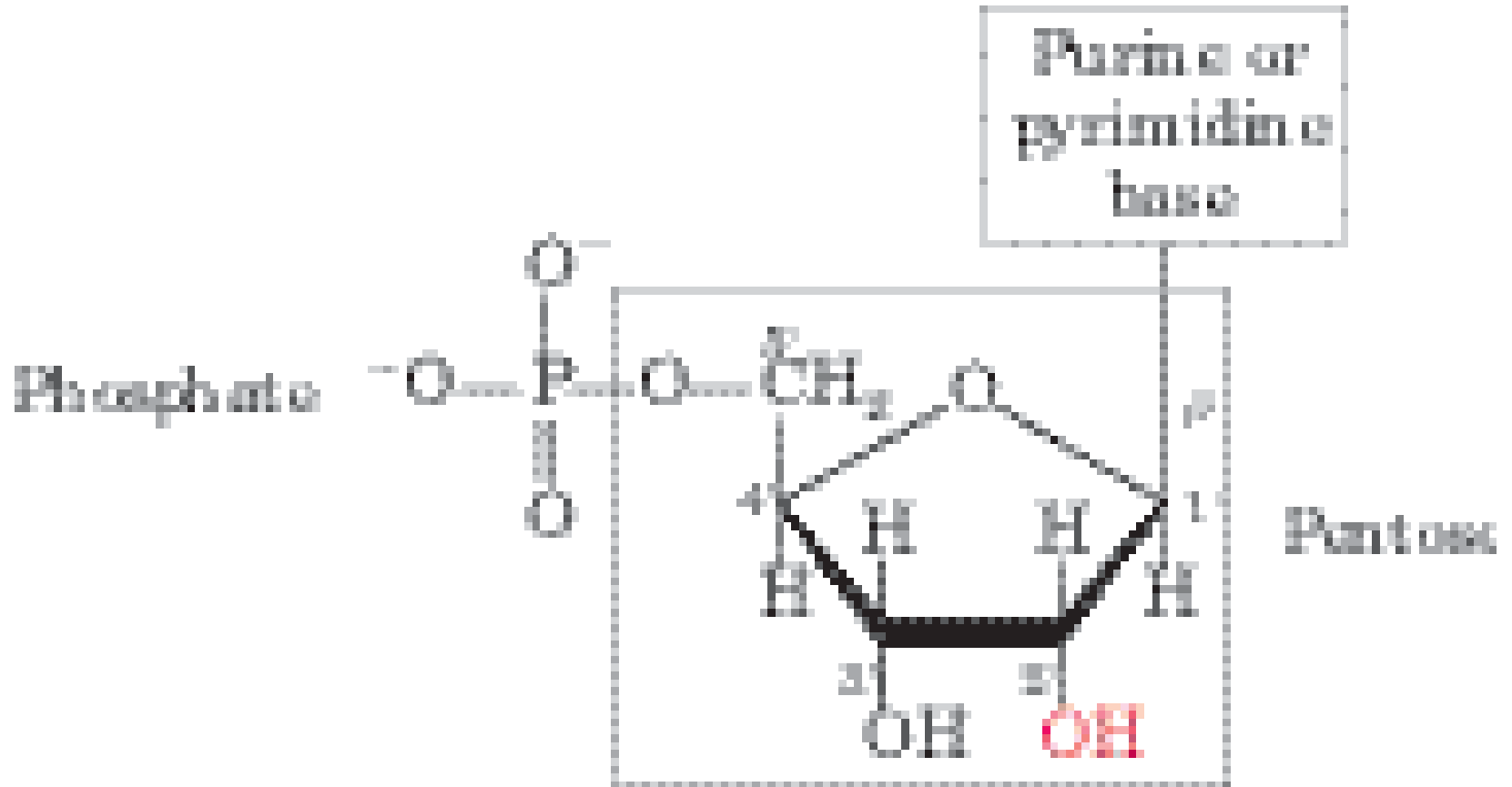
Nucleoside

Base+ Pentose

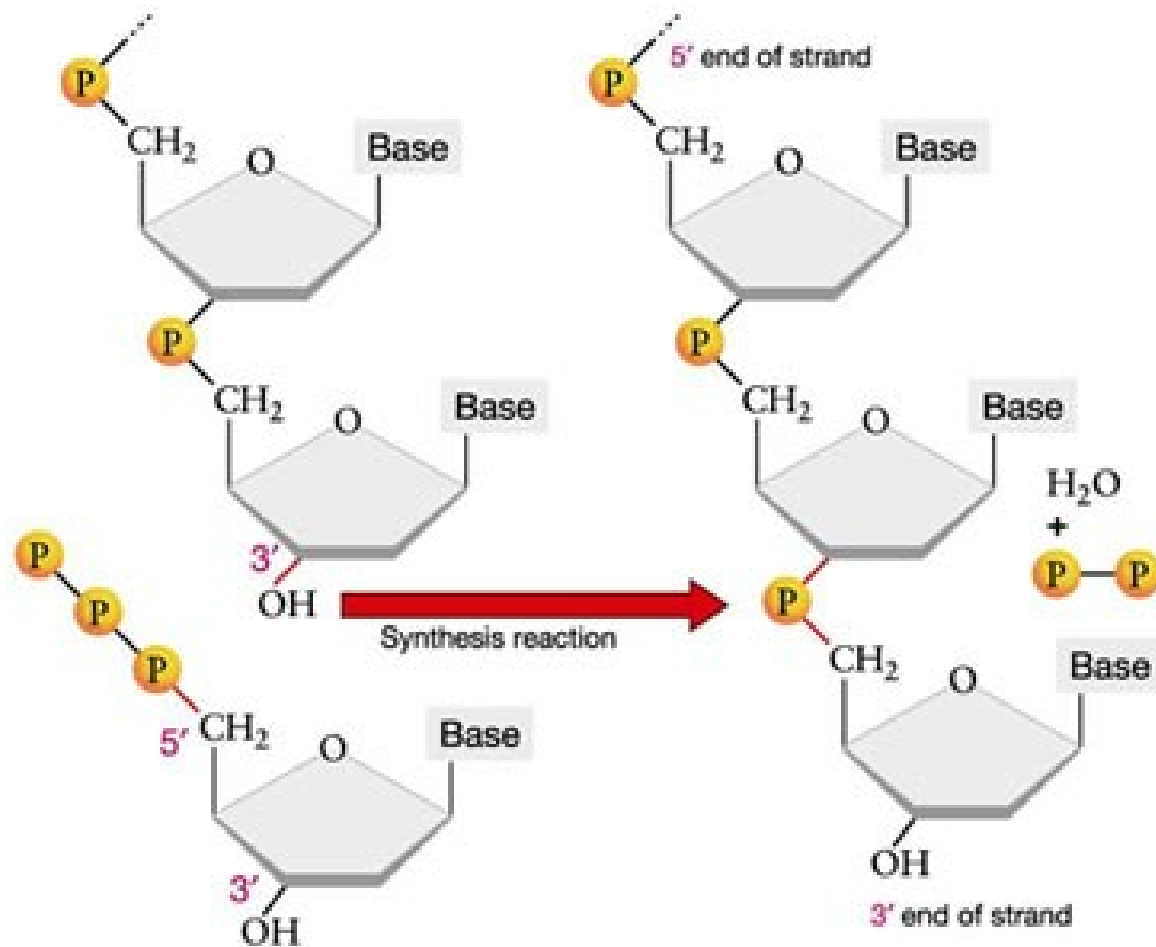


Nucleotide

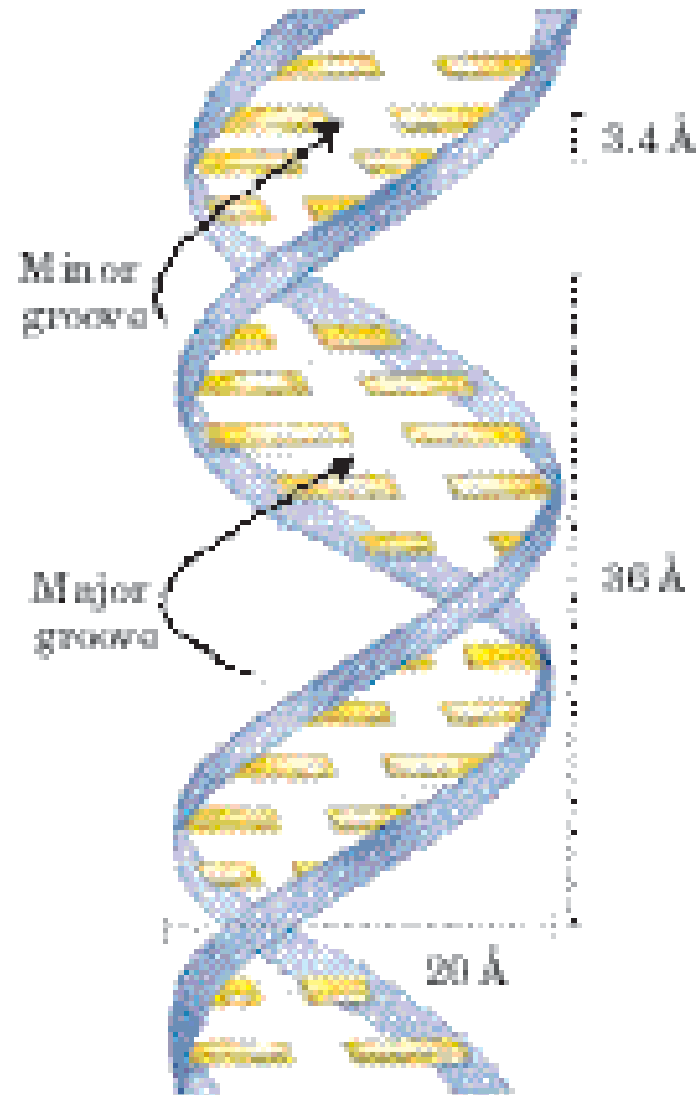
Base+ Pentose+ Phosphate



DNA synthesis



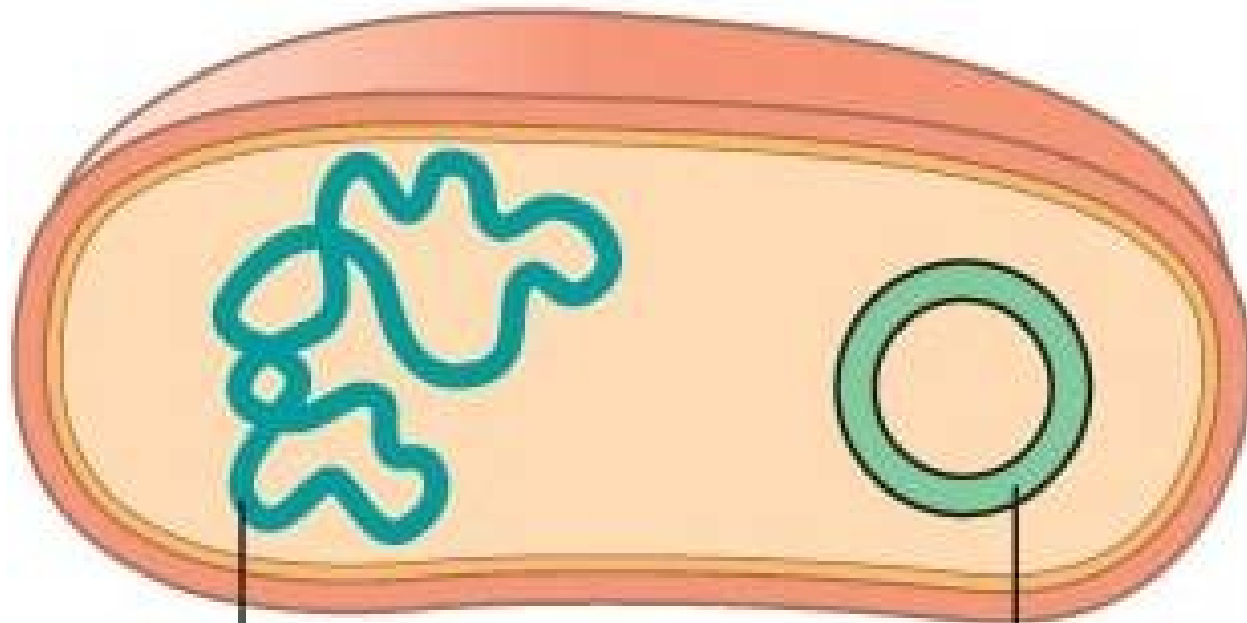
Watson-Crick model for DNA structure



Bacterial Chromosome

- All bacteria were believed to have a single, circular chromosome while the eukaryotes, have linear chromosomes.
- This was due to two reasons:
 - the limited sample of bacterial strains studied
 - the sequencing techniques available to examine the bacterial chromosomes
- This belief has been disproved with the discovery of bacteria with multiple and/or linear chromosomes in 1989.

Bacterium



**Bacterial
chromosome**

Plasmid

Single and Circular Chromosome

- The *E.coli* genome is organized into a single, circular chromosome
- This structure of chromosomal DNA was shown by images obtained from **autoradiography, electron microscopy** and moving pictures of DNA using fluorescence microscopy using **tritium** to label thymidine
- Data of the circular chromosome in *E. coli* was made it became widely used as the primary model to investigate chromosomal replication

Multiple Chromosomes

- The first multiple chromosome in bacteria was found in *Rhodobacter sphaeroides*
- The complete physical map was obtained through restriction digestion with *Asel*, *SpeI*, *DraI*, and *SnaBI*
- *R. sphaeroides* have two circular chromosomes
 - one is 3.1 Mb
 - the other is 0.9 Mb

Linear Chromosomes

- *B. burgdorferi* was the first bacterium to be discovered with linear chromosomes
- The size of the chromosome was found to be around 1.0 Mb
- This chromosome organization compares well to the eukaryotic chromosome organization.

Problems with Linear Chromosomes in prokaryotes

- Two problems arise with linear chromosomes
 - intracellular **nuclease degradation** -the free double-stranded DNA ends need protection
 - Replication of **telomeres**, which are the ends of the linear DNA molecules - will require a different process for DNA replication
- There are two types of telomeres
 - palindromic hairpin loops, in which there are no free double-stranded ends available
 - invertron telomeres with a protein that binds to the 5'-ends

Summary of Bacterial Chromosome Organizations

Bacteria

Agrobacterium tumefaciens

Bacillus subtilis

Bacillus subtilis

Borrelia burgdorferi

Brucella abortus

Brucella melitensis

Brucella ovis

Brucella suis biovar 1

Brucella suis biovar 2

Brucella suis biovar 4

Escherichia coli

Paracoccus denitrificans

Pseudomonas aeruginosa

Rhodobacter sphaeroides

Streptomyces griseus

Vibrio cholerae

Vibrio fluvialis

Vibrio parahaemolyticus

Vibrio vulnificus

Chromosome Organization

One linear and one circular

Single and circular

Single and linear

Two circular

Two circular

Two circular

Two circular

Two circular

Two circular

Two circular

Single and circular

Three circular

Single and circular

Two circular

Linear

Two circular

Two circular

Two circular

Two circular

Bacterial plasmids

- small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently
- They are found in bacteria as small, circular, double-stranded DNA molecules
- plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance
- Plasmids are not generally classified as life

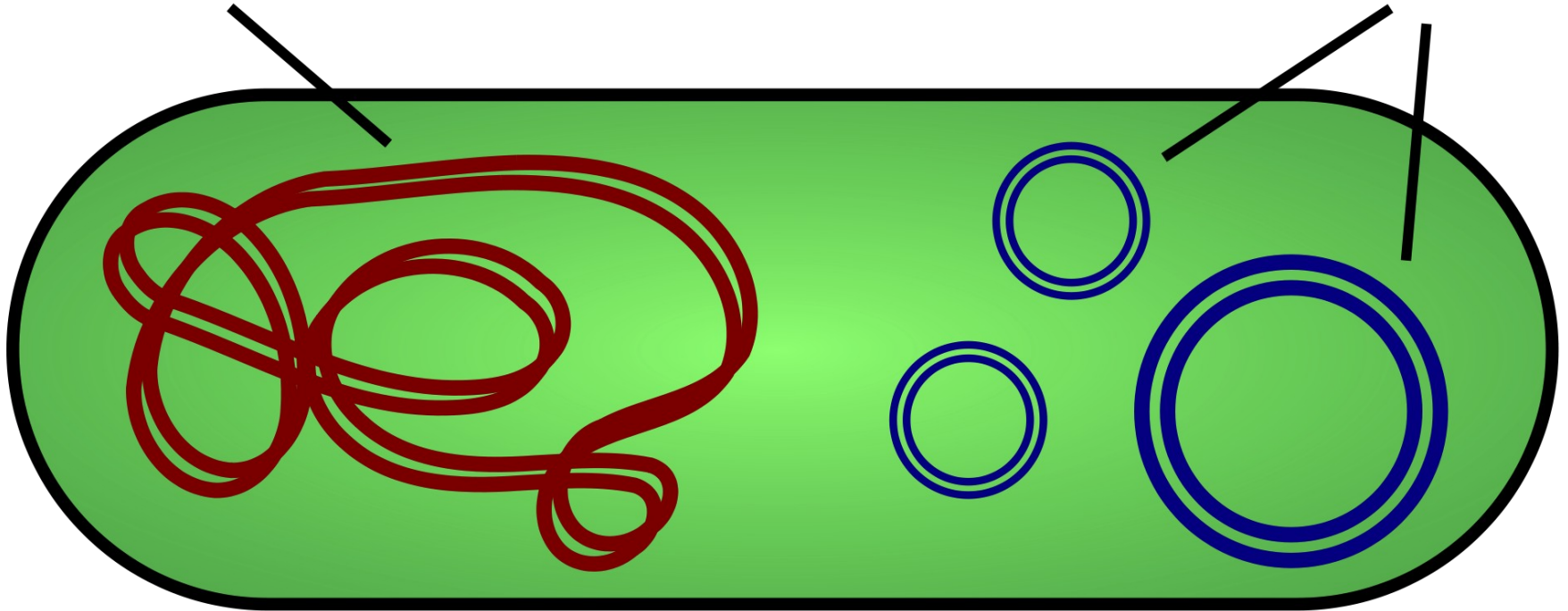
Bacterial plasmids

- Plasmids can be transmitted from one bacterium to another
 - even of another species
- Transmission is through three main mechanisms:
 - transformation,
 - transduction,
 - conjugation

Bacterial plasmids

Bacterial DNA

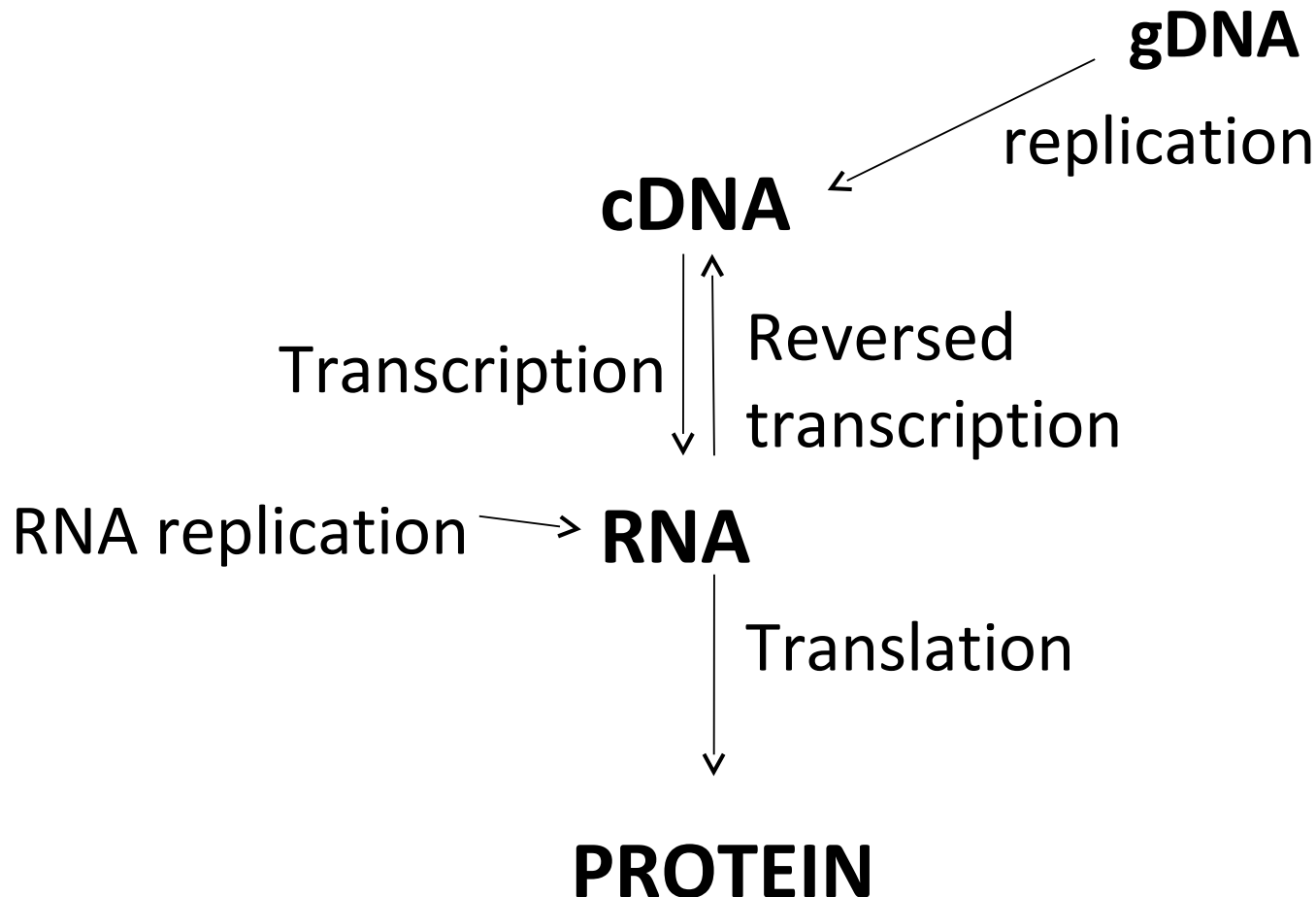
Plasmids



Gene Function

Gene Function in Prokaryotes

- The central dogma of molecular biology, showing the general pathways of information flow via replication, transcription, and translation.



Mutation

Mutation

- A gene mutation is a change in the nucleotide sequence that composes a gene
- Mutations in DNA occur constantly
- these mutations serve the crucial role of providing organisms with previously nonexistent proteins.
- In this way, mutations are a driving force behind genetic diversity, better able to adapt to changes or the emergence of new types of disease

Mutations: Definitions

- Mutations result from alterations in the nucleotide sequence in a gene
- Lethal mutation: results in death of the cell, and therefore cannot be propagated or studied
- Conditional mutation: One that is expressed only under certain environmental conditions; for example, a temperature-sensitive mutation

Mutations: Definitions

- Biochemical mutations: result in change in a biochemical pathway of the cell; for example, an auxotrophic mutation
- Spontaneous mutation: one that arises spontaneously due to error during DNA replication
- Induced mutation: one that has been caused by damage resulting from chemical or radiation treatment (mutagen)

Mutations: Definitions

- Adaptive or directed mutation: The concept that some bacteria have an increased mutational frequency as an adaptive response to certain environmental or nutritional factors (for example, *E. coli* regaining the ability to use lactose, or hypermutation: activation of mutator genes in nutrient-starved cultures).

Levels of occurrence

- Somatic mutations
- Germline mutations

Somatic mutations

- ❖ Arise in the somatic cells
- ❖ Passed on to other cells through the process of mitosis
- ❖ Effect of these mutations depends on the type of the cell in which they occur & the developmental stage of the organism
- ❖ If occurs early in development, larger the clone of the mutated cells

Germ line mutations

- ❖ They occur in the cells that produce gametes
- ❖ Passed on to future generations
- ❖ In multicellular organisms, the term mutation is generally used for germ line mutations

Types of Mutation

- Point mutations
- Insertion or deletion
- Fusion gene
- Dynamic mutation

Mutations: Types of Mutations

- Forward mutation: A mutation in the wild type, causing some notable change in phenotype
- Reversion mutation: A change causing a mutant to appear to revert back to the wild type phenotype
- Back mutation: A reverse mutation in which the mutant nucleotide sequence has truly reverted back to exactly its original wild type nucleotide sequence.
- Suppressor mutation: A reverse mutation in which a mutation in a second gene overcomes the first mutation and restores the wild phenotype
- Intragenic suppression: A second mutation within the same gene, but not restoring the original sequence, restores the phenotype.

Mutations: Types of Mutations

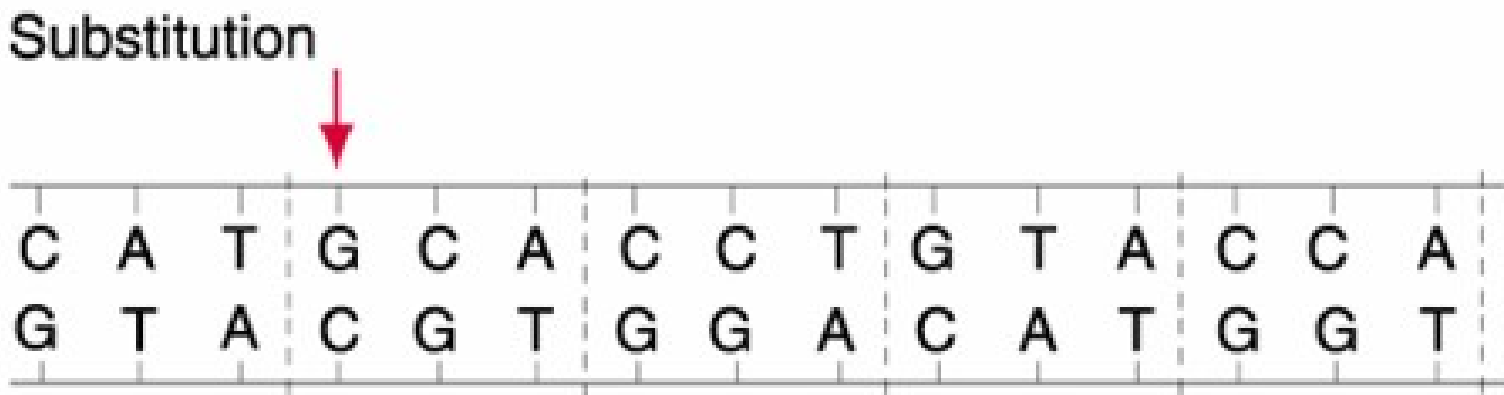
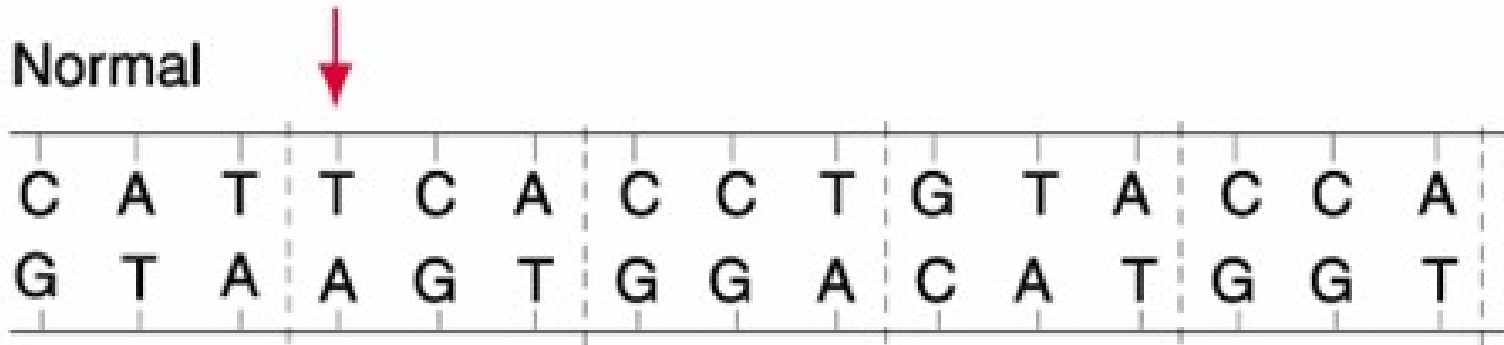
- Point mutation: Substitution of one base for another
- Silent mutation: A point mutation that results in no change in the amino acid sequence of the protein encoded, due to redundancy in the genetic code
- Missense mutation: A point mutation in which there is a change in the codon of one amino acid for the codon for another amino acid, resulting in a protein with a single amino acid substitution. This could lead to anywhere from complete loss of protein activity to no change in the level of activity at all, depending on the amino acid substitution.

Mutations: Types of Mutations

- Nonsense mutation: A point mutation in which a sense codon (encoding for an amino acid) is changed to a nonsense codon (stop codon), resulting in premature chain termination. Usually it results in loss of the protein's function.
- Nonsense suppressor mutation: A mutation in a tRNA gene that makes a mutant tRNA capable of pairing with a stop codon and thereby reversing certain nonsense mutations.
- Frameshift mutation: Insertion or deletion of one or two bases within a gene which cause all of the codons downstream from the insertion or deletion to be misread, so that all of the amino acids past that point are incorrect.

Point mutation

- substitution of one base with another



Point mutation

- Transition
 - purine replaces purine
 - pyrimidine replaces pyrimidine
- Transversion
 - purine replaces pyrimidine
 - pyrimidine replaces purine

Effects

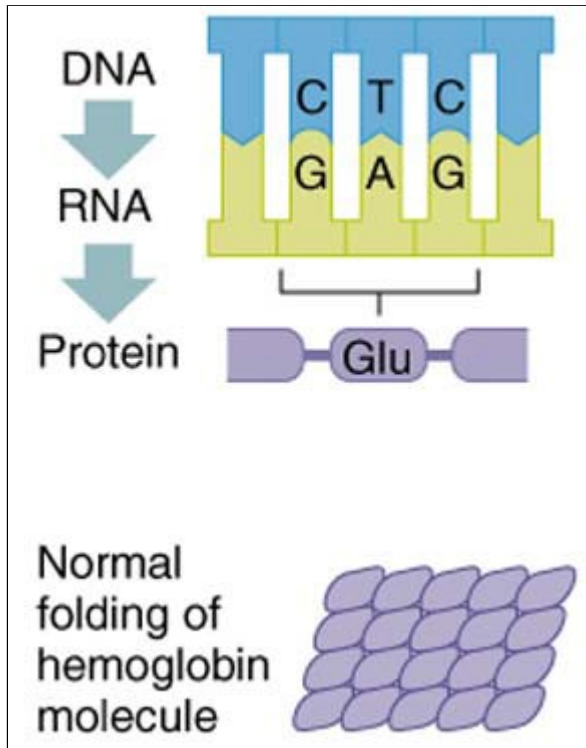
- Silent e.g. GCA to GCC = Ala
- Mis sense eg GGA (Gly) to AGA (Arg)
- None sense eg CAA (GLU) to UAA (stop)

Mis-sense mutation

- A point mutation that exchanges one codon for another causing substitution of an amino acid
- Mis-sense mutations may affect protein function severely, mildly or not at all.

Single base change in hemoglobin gene causes sickle cell anemia

Wild-type Allele

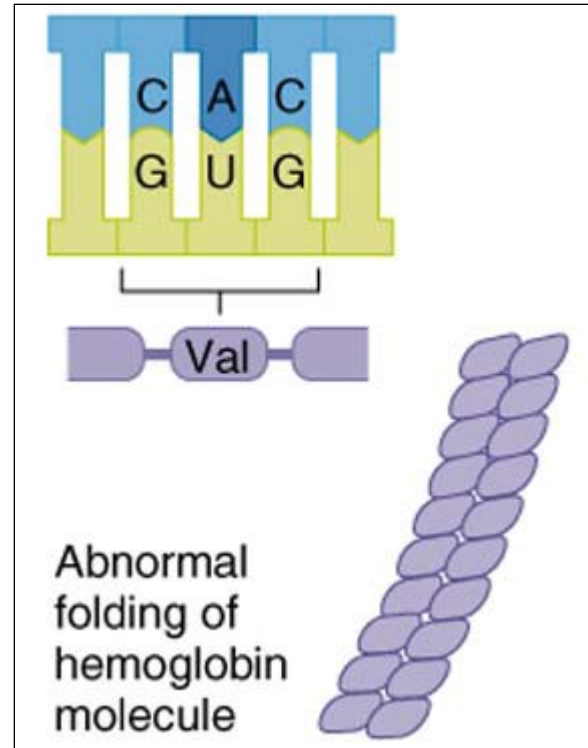


Normal red blood cells

Wild-type phenotype



mutant allele



Sickled red blood cells

mutant Phenotype



Nonsense mutation

- Premature stop codons create truncated proteins.
- Truncated proteins are often nonfunctional.
- Some truncations have dominant effects due to interference with normal functions.

Insertion or deletion mutations

- The genetic code is read in triplet nucleotides during translation.
- Addition or subtraction of nucleotides not in multiples of three lead to a change in the reading frame used for translation. Amino acids after that point are different, a phenomenon called a frameshift.
- Addition or subtraction of nucleotides in multiples of three leads to addition or subtraction of entire amino acids but not a change in the reading frame.

EFFECT: Frameshift Mutation

5' ATG GGA GCT CTA TTA ACC TAA 3'
met gly ala leu leu thr stop



5' ATG GGG AGC TCT ATT A AC CTA A
met gly ser ser ile asn leu

EFFECT: Insertion or deletion of codons

5' ATG GGA GCT CTA TTA ACC TAA 3'
met gly ala leu leu thr stop



5' ATG GGA TTA TTA GCT CTA TTA ACC TAA
met gly leu leu ala leu leu thr stop

EFFECT: Myotonic dystrophy: a triplet repeat disease

- 5 -37 copies of CTG repeat normal phenotype
- 50-1000 repeats myotonic dystrophy
- Genes with 40+ copies are unstable and can gain (or less commonly lose) repeat copies in successive generations.

Myotonic Dystrophy

	<u>Pedigree</u>	<u>Age of onset</u>	<u>Phenotype</u>	<u>Number of copies of GAC mRNA repeat</u>
I	<p>The pedigree chart illustrates the inheritance of Myotonic Dystrophy across three generations. In Generation I, an unaffected female (I-1) and an affected male (I-2) are mated. Their daughter in Generation II (II-1) is affected, while her husband (II-2) is unaffected. They have three children in Generation III: an affected male (III-1), an unaffected female (III-2), and another unaffected female (III-3).</p>	Older adulthood	Mild forearm weakness, cataracts	50–80
II		Mid-adulthood	Moderate limb weakness	80–700
III		Childhood	Severe muscle impairment, respiratory distress, early death	700+

MUTANT GENE

- Gene that has lost, gained or exchanged some material it received from its parent resulting in a permanent transmissible change in its function
- Any gene that has undergone a change, such as the loss, gain or exchange of genetic material, that affects the normal transmission and expression of a trait

Features of mutant products

- Loss of function
 - Null mutant – complete loss of activity
 - Hypomorph – reduced activity
- Gain of function
 - Hypermorph – increased activity
 - Neomorph – new function in a gene
- Suppressors – compensate for other mutants
- Enhancer – enhances phenotype of a mutation

Mutant products

- **If 2 mutations failed to complete**
 - They are alleles of same genes
 - They are allelic to each other
 - They belong to the same complementation group

Mutant products

- **If 2 mutations complement each other**
 - They are alleles of different genes
 - They are not allelic to each other
 - They belong to the different complementation group

DNA Repair

- Proofreading
 - Immediate removal and replacement of a mismatched base during DNA replication; done by DNA polymerase
- Excision repair
 - General system to correct damage that has caused distortions to the double helix
 - Endonucleases remove the wrong bases from one strand, leaving a gap of about 12 bases that is filled in by DNA polymerase I

DNA Repair

- Direct repair of altered bases
 - Photoreactivation: Thymine dimers can be repaired by the enzyme photolyase, which restores the thymines
 - Methyl groups may be removed from alkylated bases by enzymes
- Postreplication repair
 - Mismatch repair system: Correction enzyme scans newly replicated DNA strands; if a mismatched base is found a segment of the strand is excised from around the mismatch and replaced by DNA polymerase

DNA Repair

- Recombination repair
 - Occurs when damage has been so great that there is no template remaining, as when both bases of a pair are missing
 - **RecA protein** cuts a piece of double stranded DNA from another part of the chromosome and splices it into the damaged area
 - **SOS repair**: Occurs when the damage is so great that replication stops completely, leaving many large gaps; recA protein fills in the gaps but the process is highly error prone and results in mutations.

Mutant Detection

- In order to study microbial mutants, one must be able to detect them and isolate them from the population of organisms
- Detection Systems in bacteria are straight forward because there is only 1 copy of the gene therefore any new allele should be seen immediately

Mutant Detection

Screening for autotrophic mutants

- A lysine auxotroph will only grow on media that is supplemented with lysine because it cannot synthesize the amino acid
- Use of replica plating to find mutant

Mutant Detection

Selection for antibiotic resistant mutants

- Technique uses conditions under which only the mutant will grow

Selection of Mutants

- Factors which influence the size of the screening program are:
 - frequency of mutation,
 - extent of yield increases,
 - the amount of time required for a mutation-selection cycle,
 - the available test capacity of the screening program,
 - and the accuracy of the screening test (e.g. antibiotic assay).

Selection of Mutants

- The number of strains, which must be screened to obtain mutants with a yield increase, depends on
 - The strain,
 - The conditions of mutagenesis
 - the biosynthesis pathway
 - the regulation of the product, which is being optimized.
- Normally, several hundred to several thousand isolates per mutation cycle must be tested.

