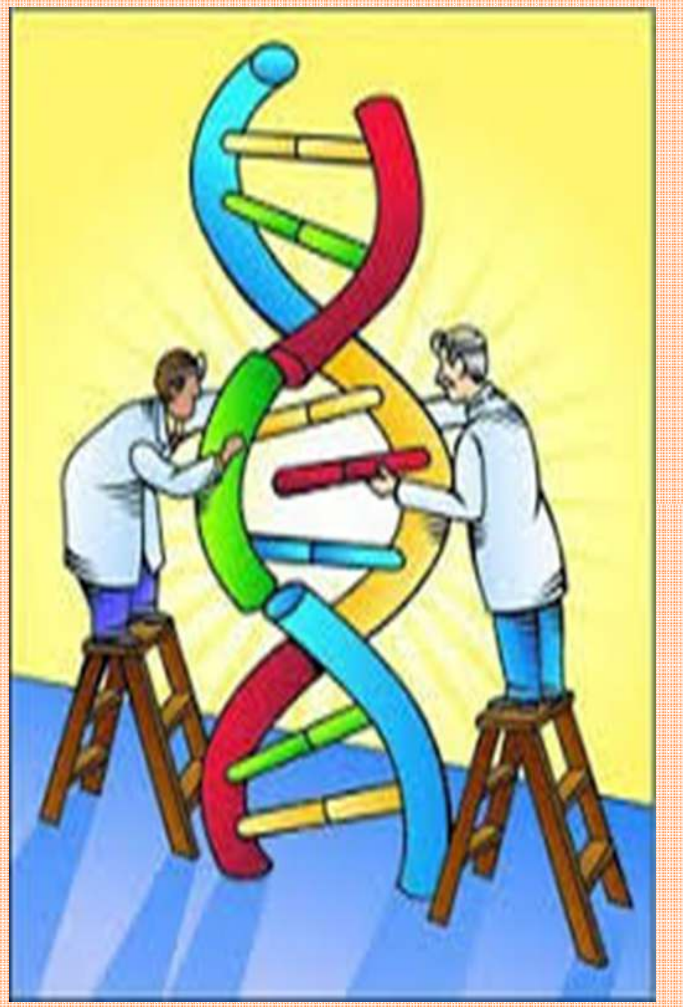
Basics in Plant Genetic Engineering



Dr. Sudin C. Dalave

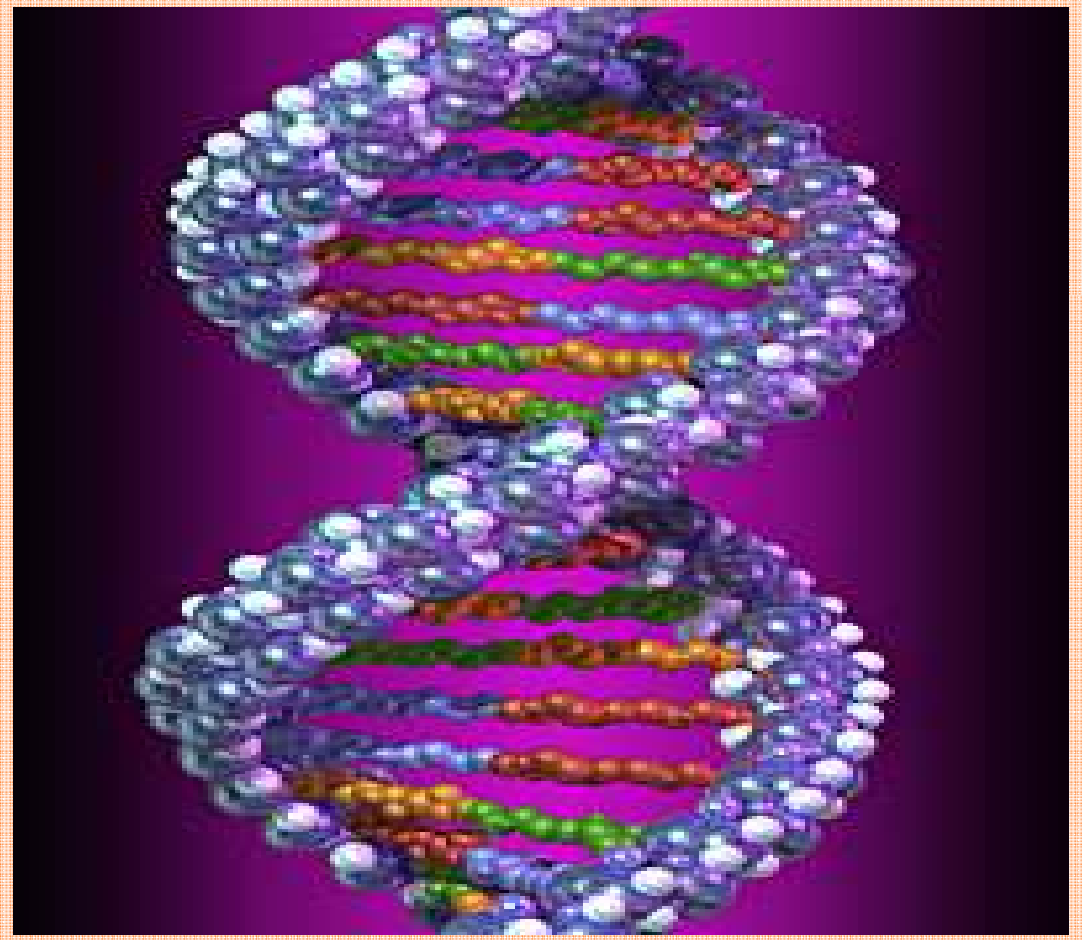
Department of Botany

SNJB's

ARTS, SCIENCE and COMMERCE COLLEGE CHANDWAD

dr.sudin@gmail.com

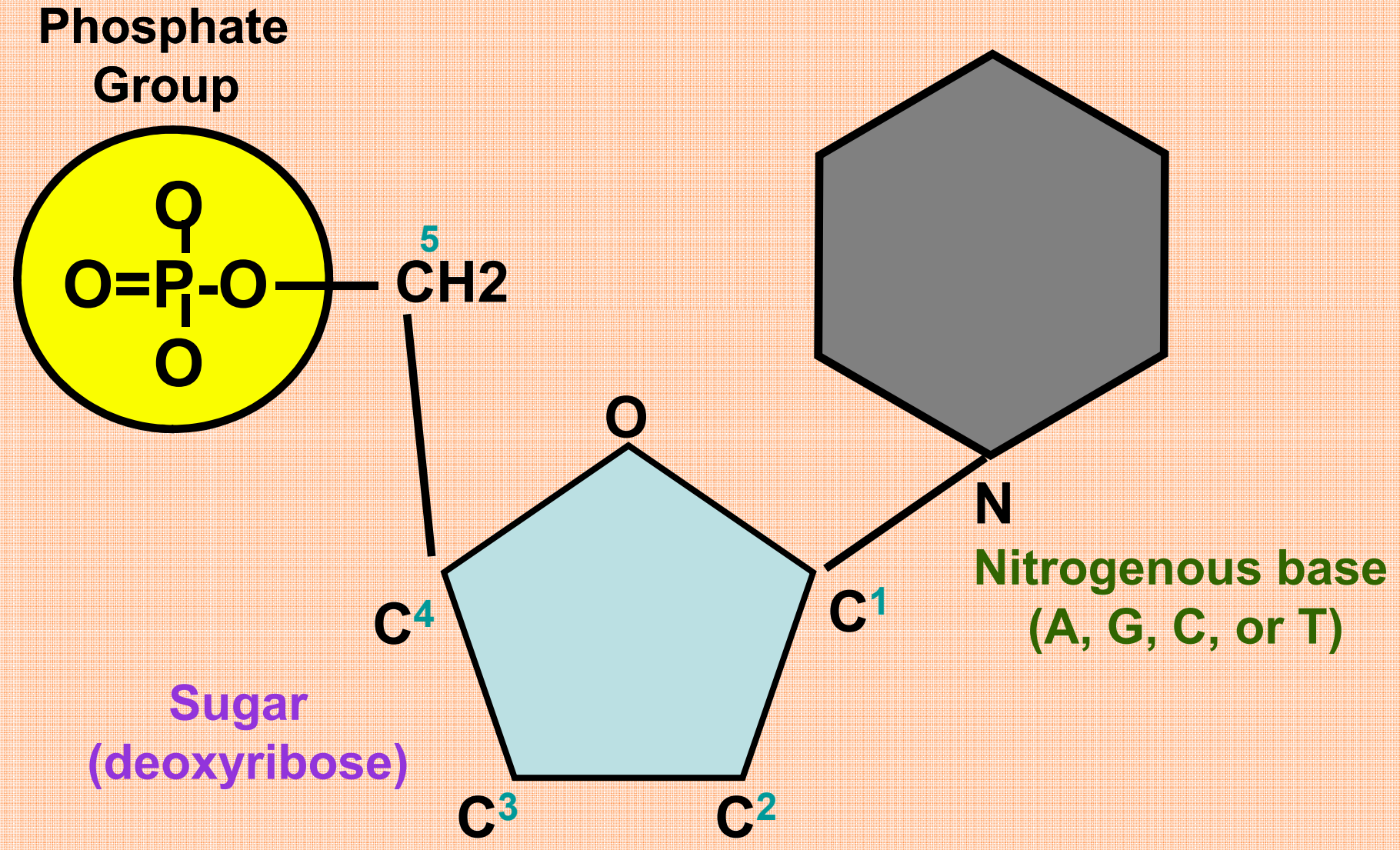
DNA
(Deoxyribonucleic Acid)



Genetic material of cells…

- GENES – units of genetic material that CODES FOR A SPECIFIC TRAIT
- DNA is made up of repeating molecules called NUCLEOTIDES
- It is a composition of Pentose Sugar, Phosphate and Nitrogenous Bases

DNA Nucleotide



A HISTORY OF DNA Discovery

A. **Frederick Griffith** – Discovers that a factor in diseased bacteria can transform harmless bacteria into deadly bacteria (1928)

B. **Rosalind Franklin** - X-ray crystallography of DNA (1952).

C. **Watson and Crick** - described the DNA molecule from Franklin's X-ray (1953).

Watson & Crick proposed…

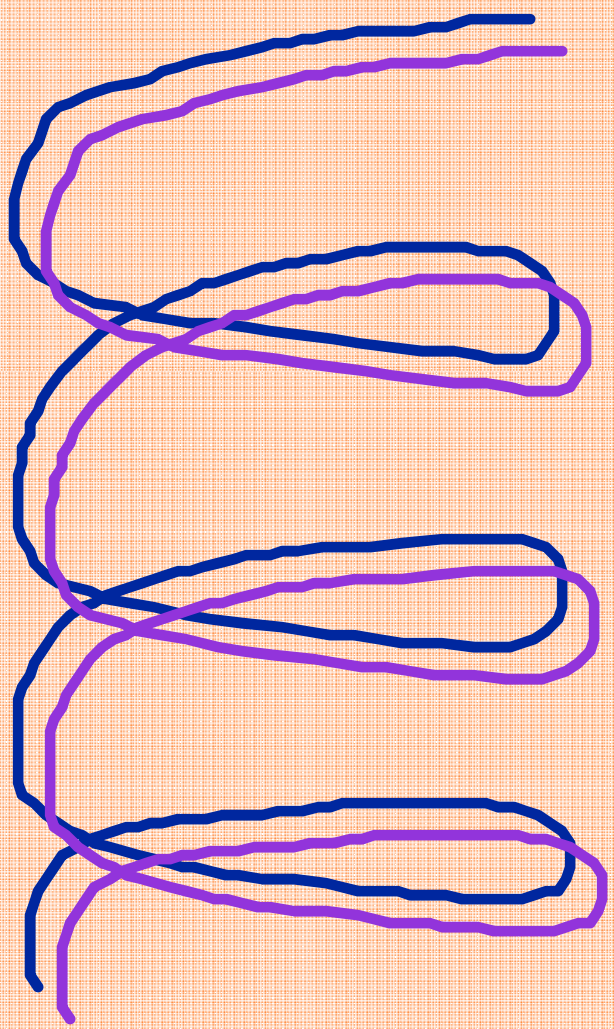
- DNA had specific pairing between the nitrogen bases:

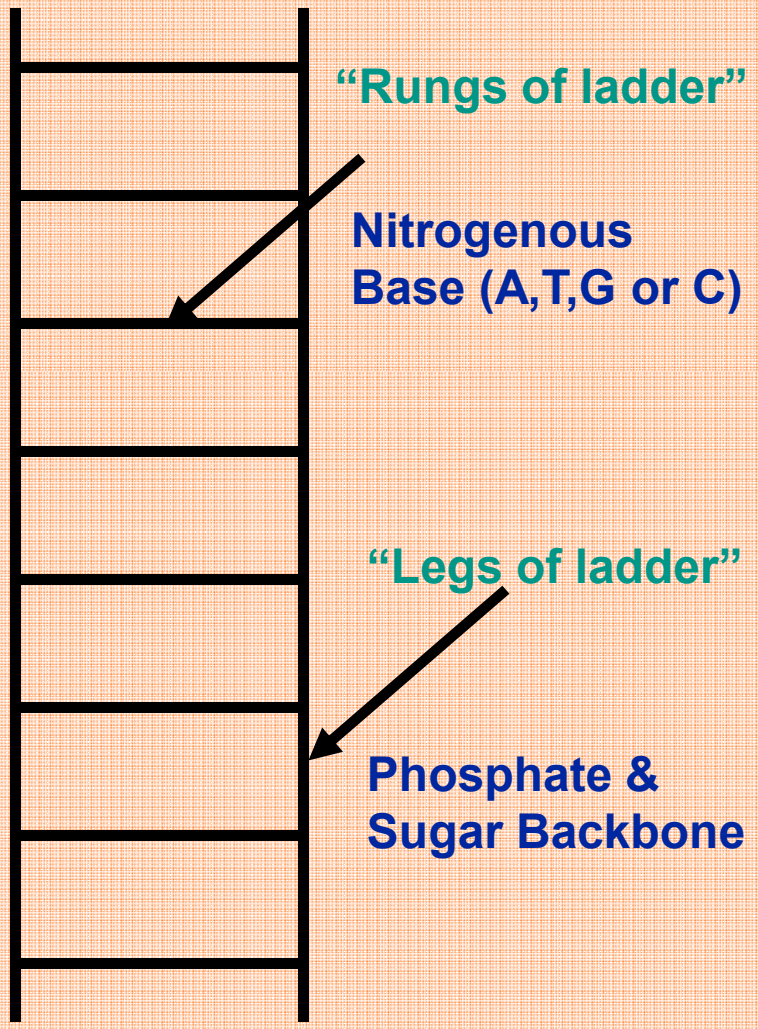
ADENINE - THYMINE

GUANINE - CYTOSINE

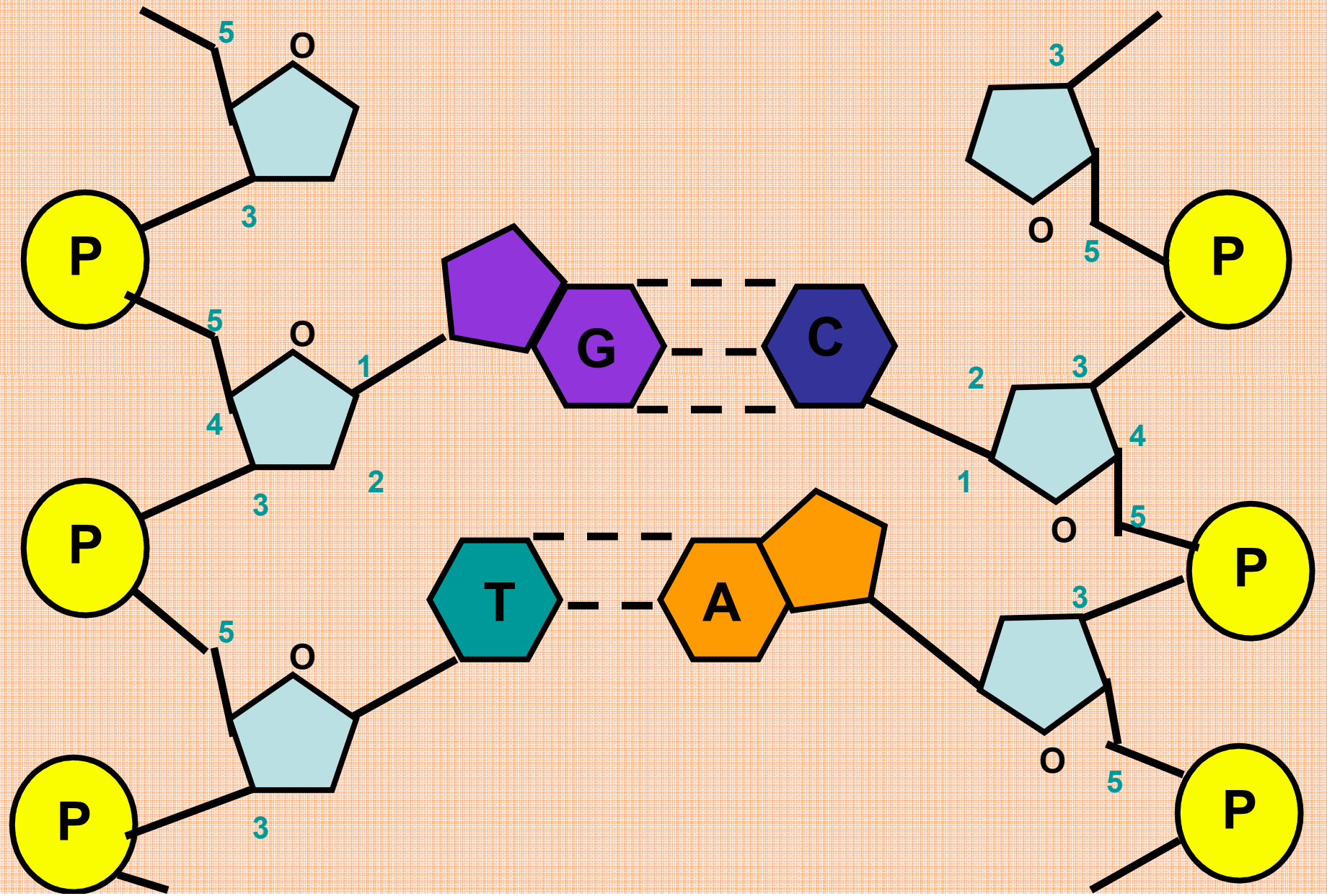
- DNA was made of 2 long stands of nucleotides arranged in a specific way called the "Complementary Rule"

DNA Double Helix



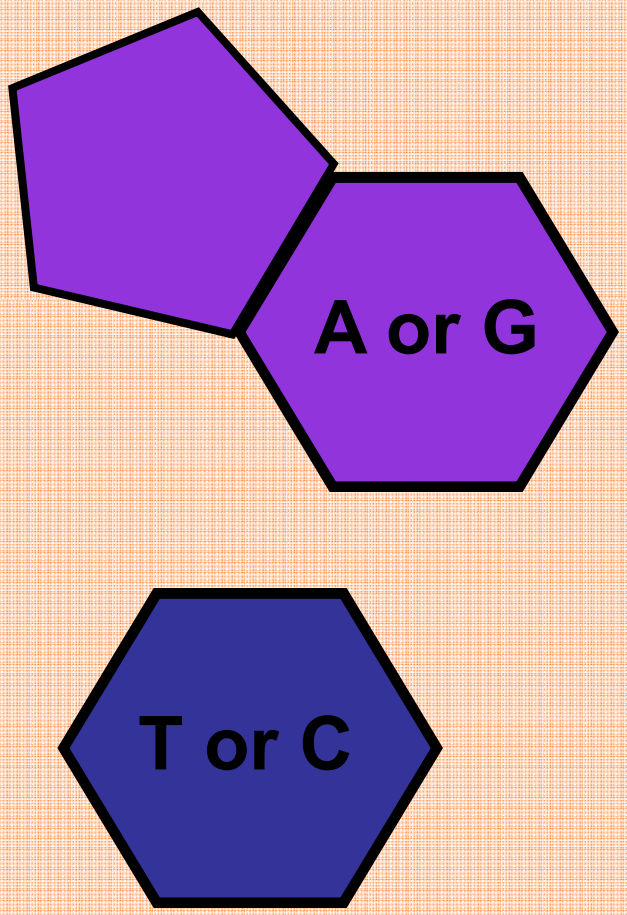


DNA Double Helix



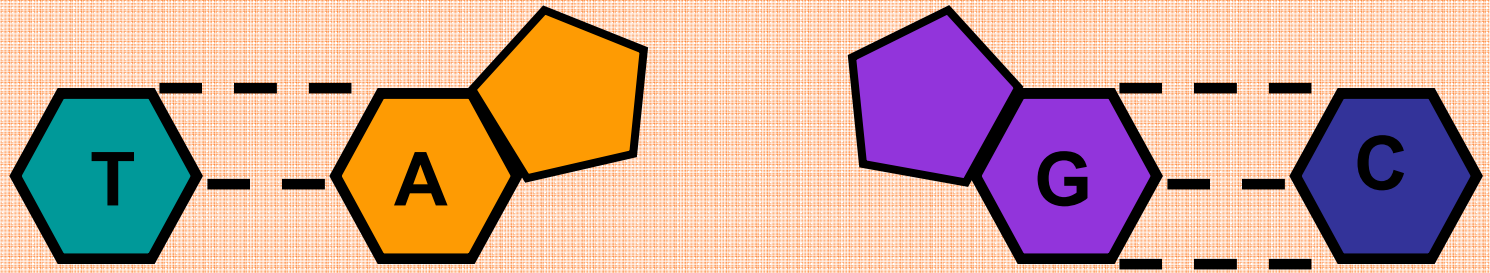
Nitrogenous Bases

- **PURINES**
  1. **Adenine (A)**
  2. **Guanine (G)**
- **PYRIMIDINES**
  3. **Thymine (T)**
  4. **Cytosine (C)**

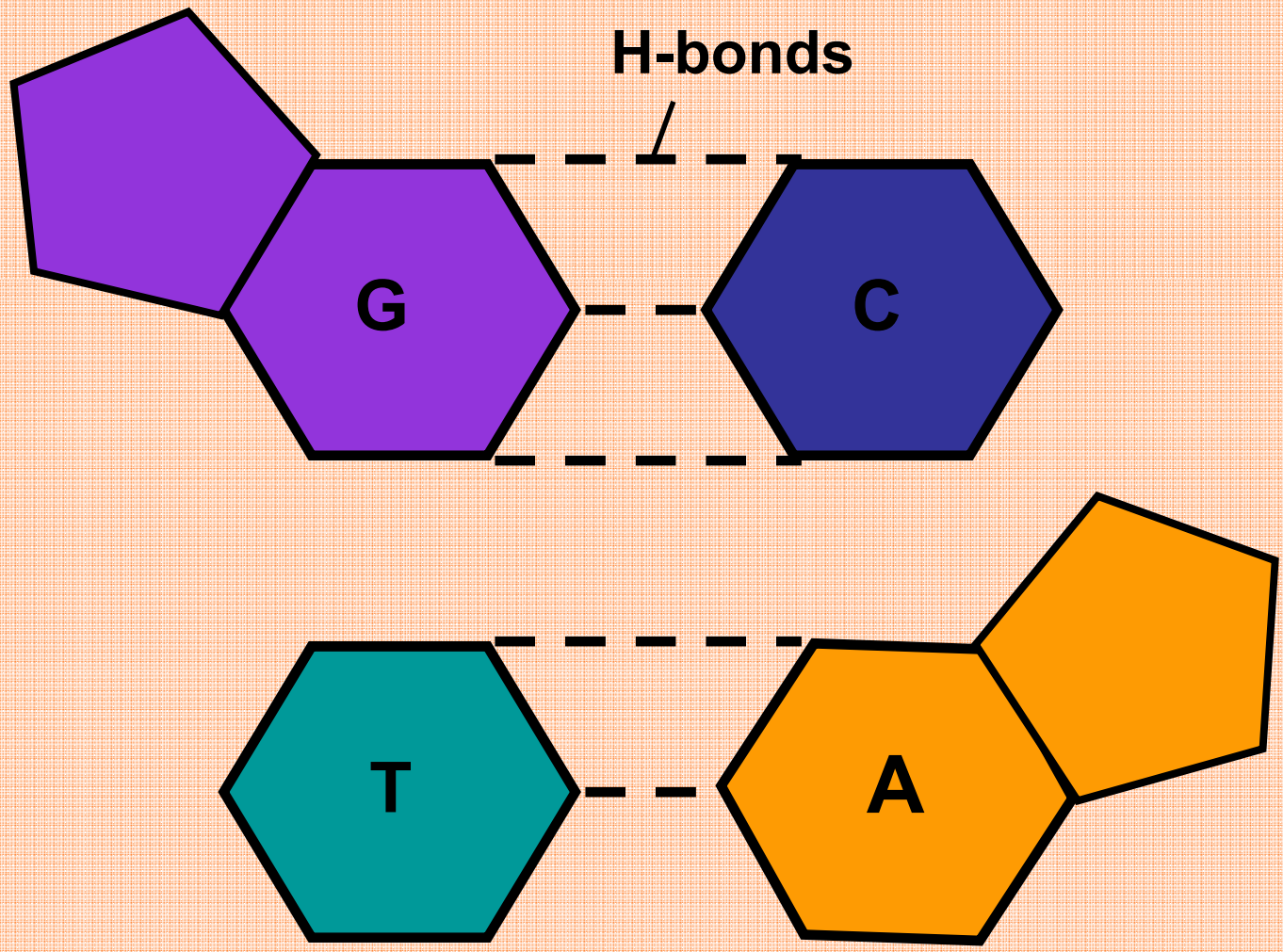


Chargaff's Rule

- **Adenine** must pair with **Thymine**
- **Guanine** must pair with **Cytosine**
- Their amounts in a given DNA molecule will be **about the same**.

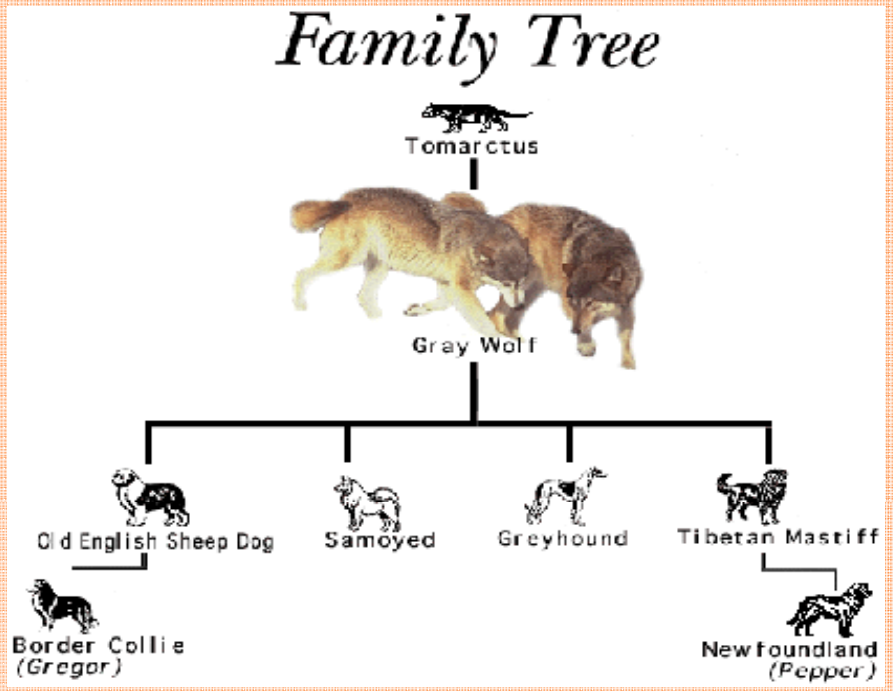


BASE-PAIRINGS



Genetic Diversity…

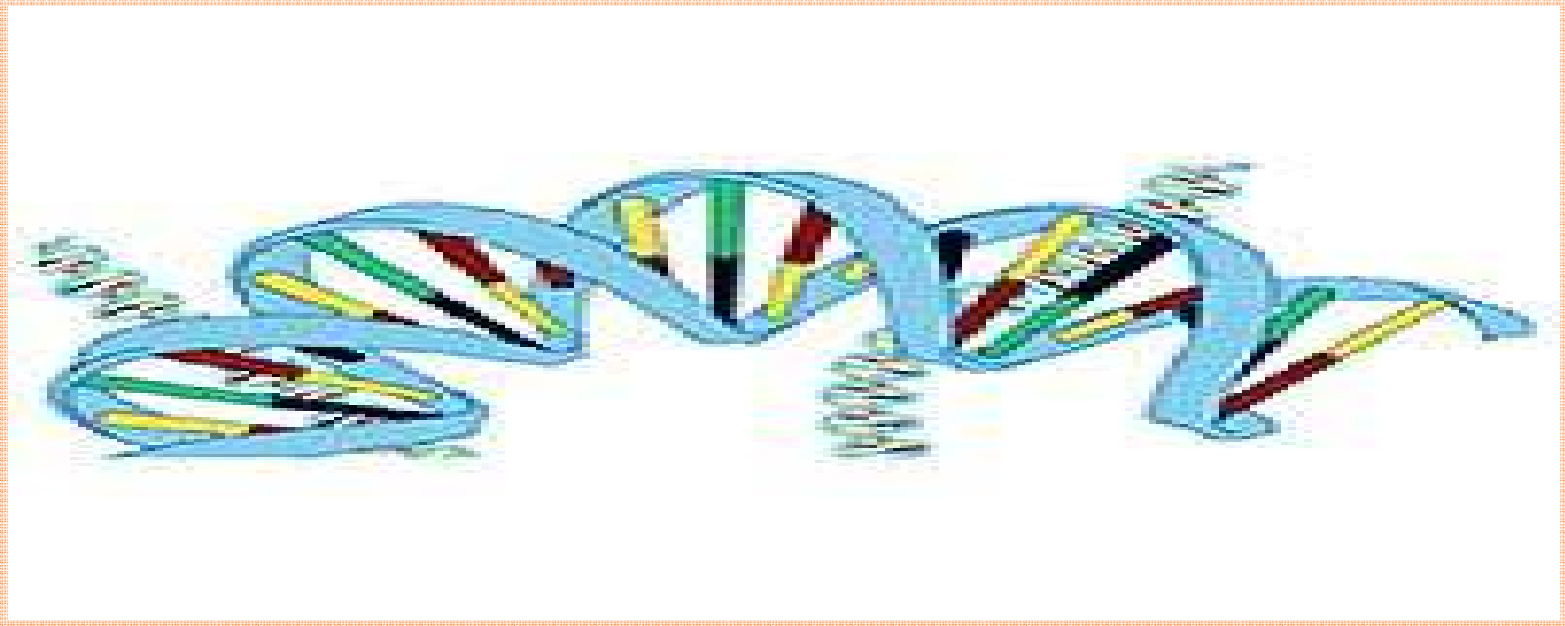
- Different arrangements of **NUCLEOTIDES** in a nucleic acid (DNA) provides the key to **DIVERSITY** among living organisms.

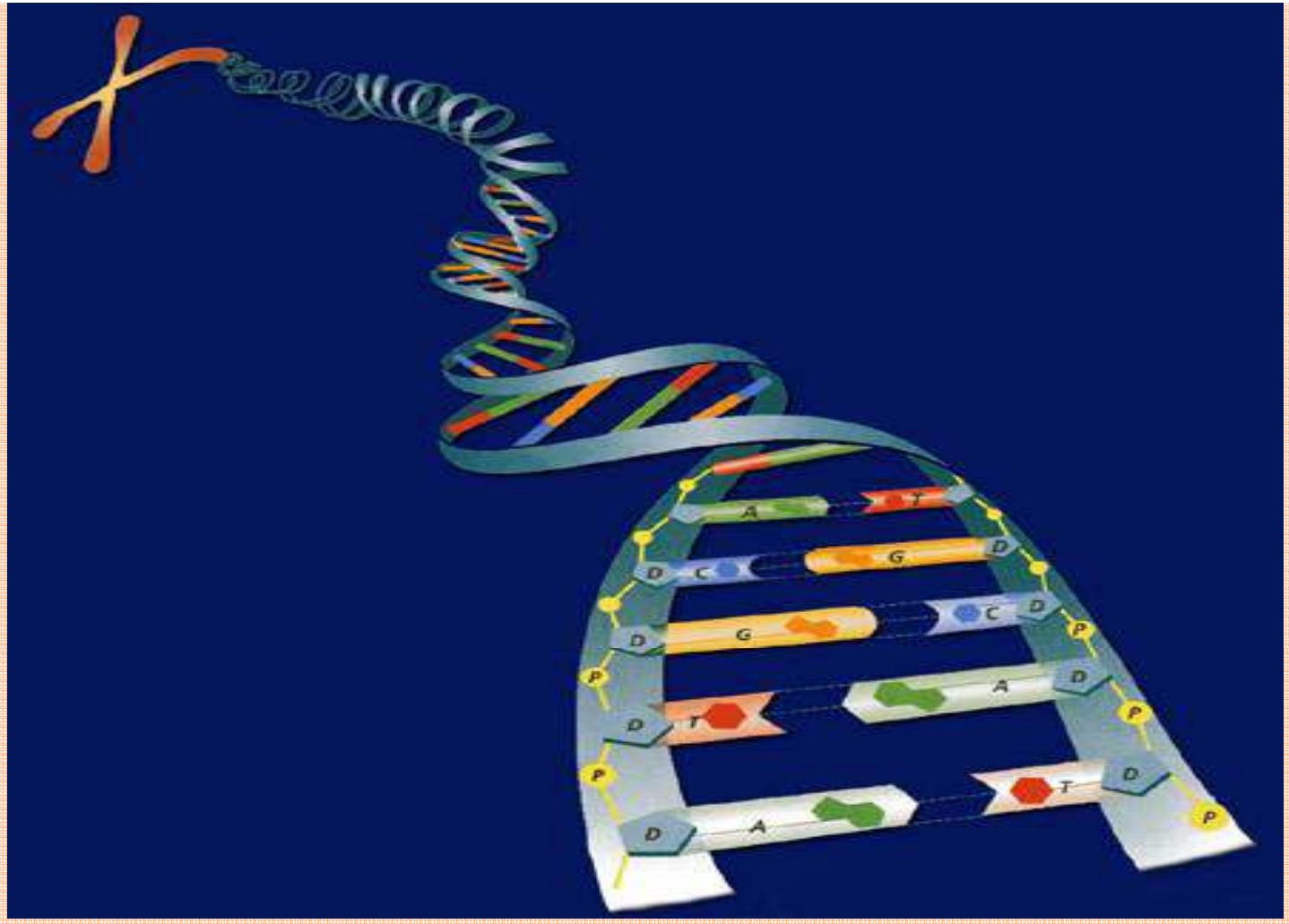


The Code of Life…

- The "code" of the chromosome is the SPECIFIC ORDER that bases occur.

A T C G T A T G C G G…

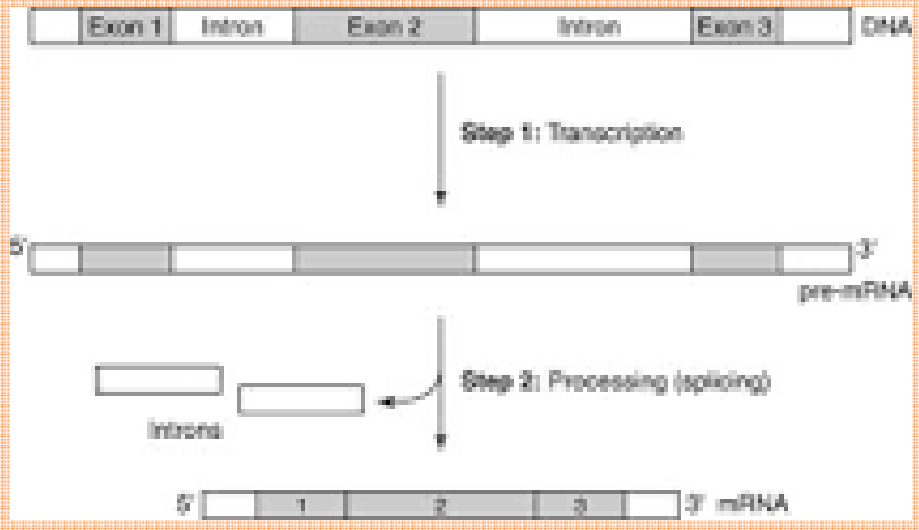




DNA is wrapped tightly around **histones** and coiled tightly to form chromosomes

Structure of Eukaryotic Gene

Genes that are expressed usually have introns that interrupt the coding sequences. A typical eukaryotic gene, therefore, consists of a set of sequences that appear in mature mRNA (called exons) interrupted by introns.



RESTRICTION ENDONUCLEASES

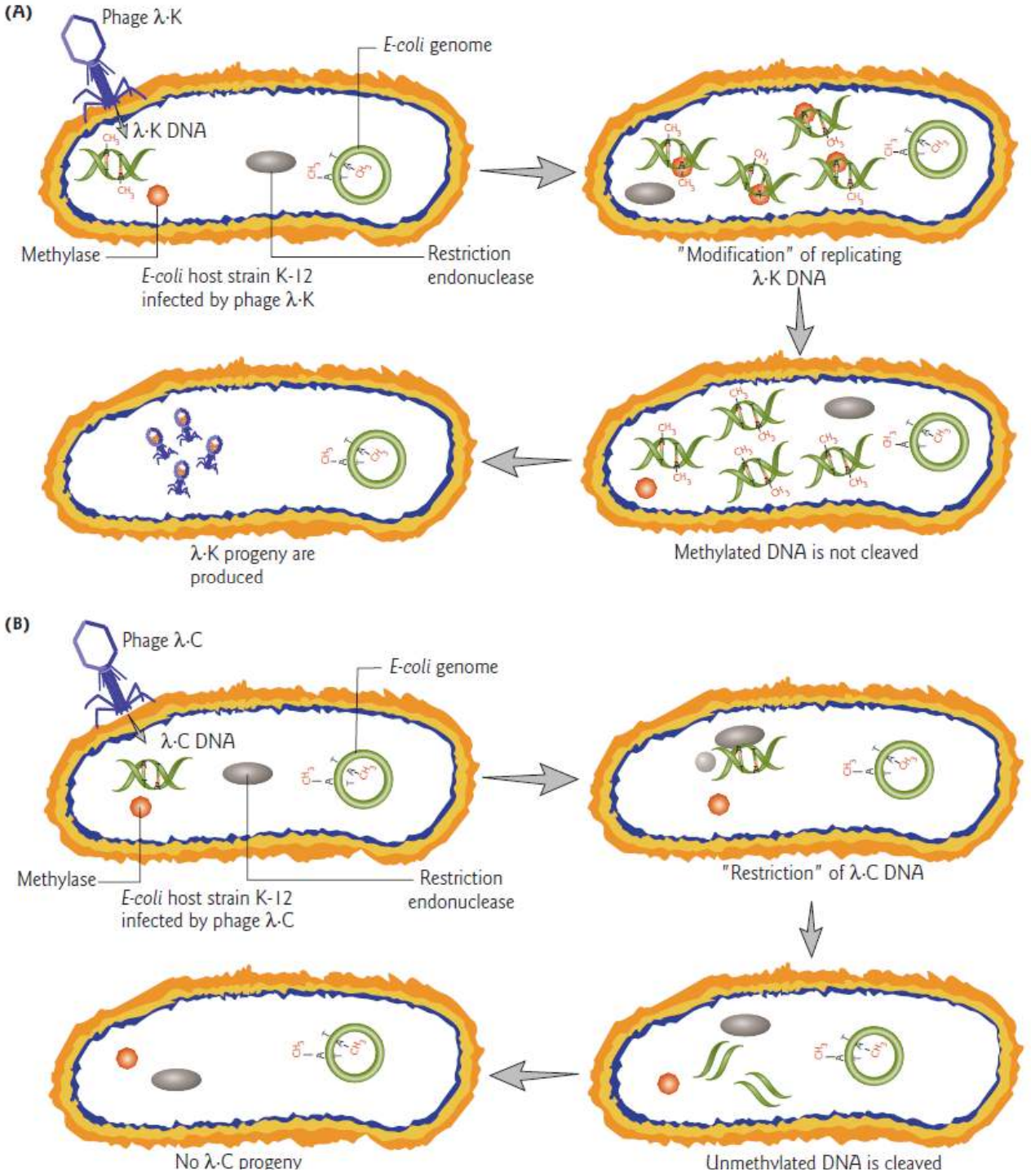
- Restriction endonucleases are naturally occurring enzymes in bacterial cell for disabling bacteriophage DNA.
- These are discovered by Werner Arber of Switzerland a Nobel Prize in 1978.
- The cleavage method makes use of an important class of DNA-cleaving enzymes isolated primarily from bacteria the enzymes are called **restriction endonucleases** or **restriction enzymes** it is also called as **molecular "scissors"**.

- The DNA fragment produced by a pair of adjacent cuts in a DNA molecule is called a **restriction fragment.**
- They are able to cleave DNA molecules at the positions at which particular, short sequences of bases are present.
- *E.g. Bam*HI it recognizes the double stranded sequence and cleaves each strand between the **G** bearing nucleotides

5'-GGATCC-3'

3'-CCTAGG-5'

- **Natural Action of the Restriction Enzymes in Bacterial Cell**



TYPES OF RESTRICTION ENDONUCLEASES

Class	Abundance	Recognition site	Composition	Use in recombinant DNA research
Type I	Less common than type II	Cut both strands at a nonspecific location > 1000 bp away from recognition site	Three-subunit complex: individual recognition, endonuclease, and methylase activities	Not useful
Type II	Most common	Cut both strands at a specific, usually palindromic, recognition site (4–8 bp)	Endonuclease and methylase are separate, single-subunit enzymes	Very useful
Type III	Rare	Cleavage of one strand only, 24–26 bp downstream of the 3′ recognition site	Endonuclease and methylase are separate two-subunit complexes with one subunit in common	Not useful

Nomenclature of the Restriction Enzymes

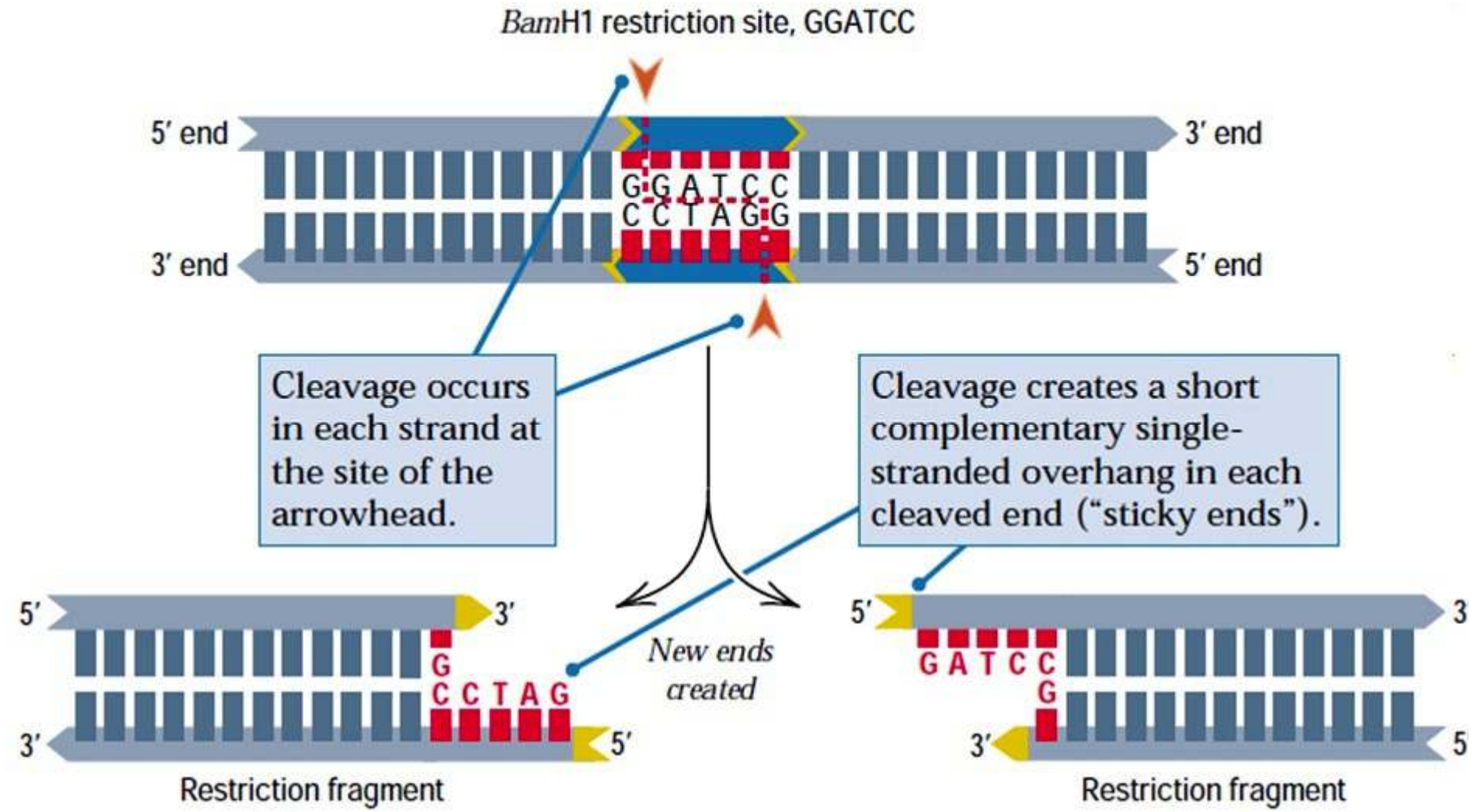
- Most restriction enzymes are named after the species in which they were found.
- *e.g. Bam*HI, was isolated from the bacterium *Bacillus amyloliquefaciens* strain H, and it is the first (I) restriction enzyme isolated from this organism.
- First three letter are belongs to the bacterial name and hence written in italics.

Action of REs

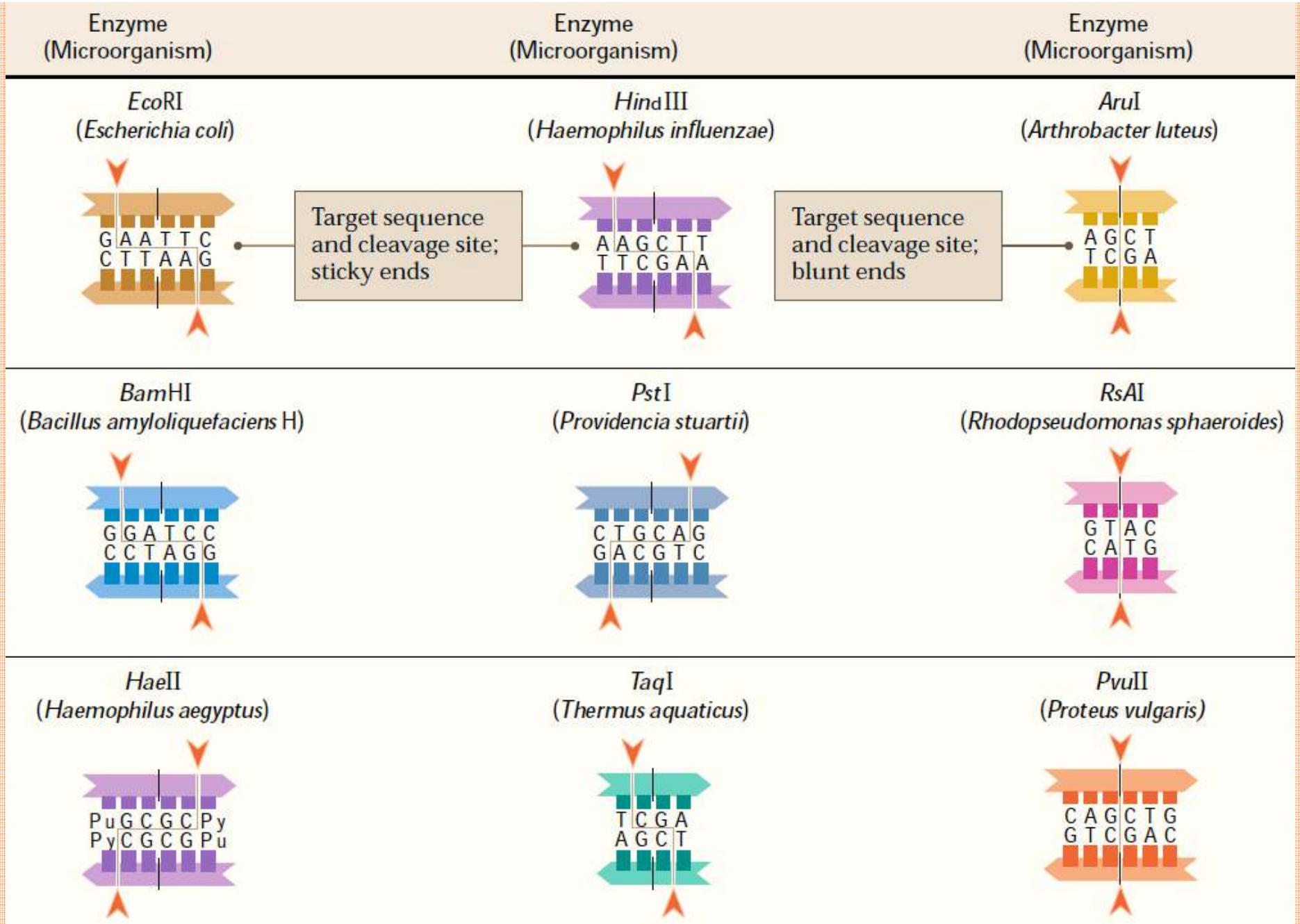
- The enzyme binds with the DNA at these sites and makes a break in each strand of the DNA molecule, producing 3'-OH and 5'-P groups at each position.
- The nucleotide sequence recognized for cleavage by a restriction enzyme is called the **restriction site** of the enzyme.
- Some restriction enzymes cleave their restriction site asymmetrically (at different sites in the two DNA strands), but other restriction enzymes cleave symmetrically (at the same site in both strands).

- Asymmetrical cleavage leave **sticky ends** because each end of the cleaved site has a small, single-stranded overhang that is complementary in base sequence to the other end.
- Enzymes that have symmetrical cleavage sites yield DNA fragments that have **blunt ends.**
- The restriction site of a restriction enzyme reads the same on both strands DNA sequence with this type of symmetry is called a Palindrome.
- e.g. each strand in the restriction site of *Bam*HI reads 5'-GGATCC-3‘
- (in general spelling of MADAM)

•**Each cut creates a new 3' end and a new 5‘ end, separating the duplex into two fragments.**

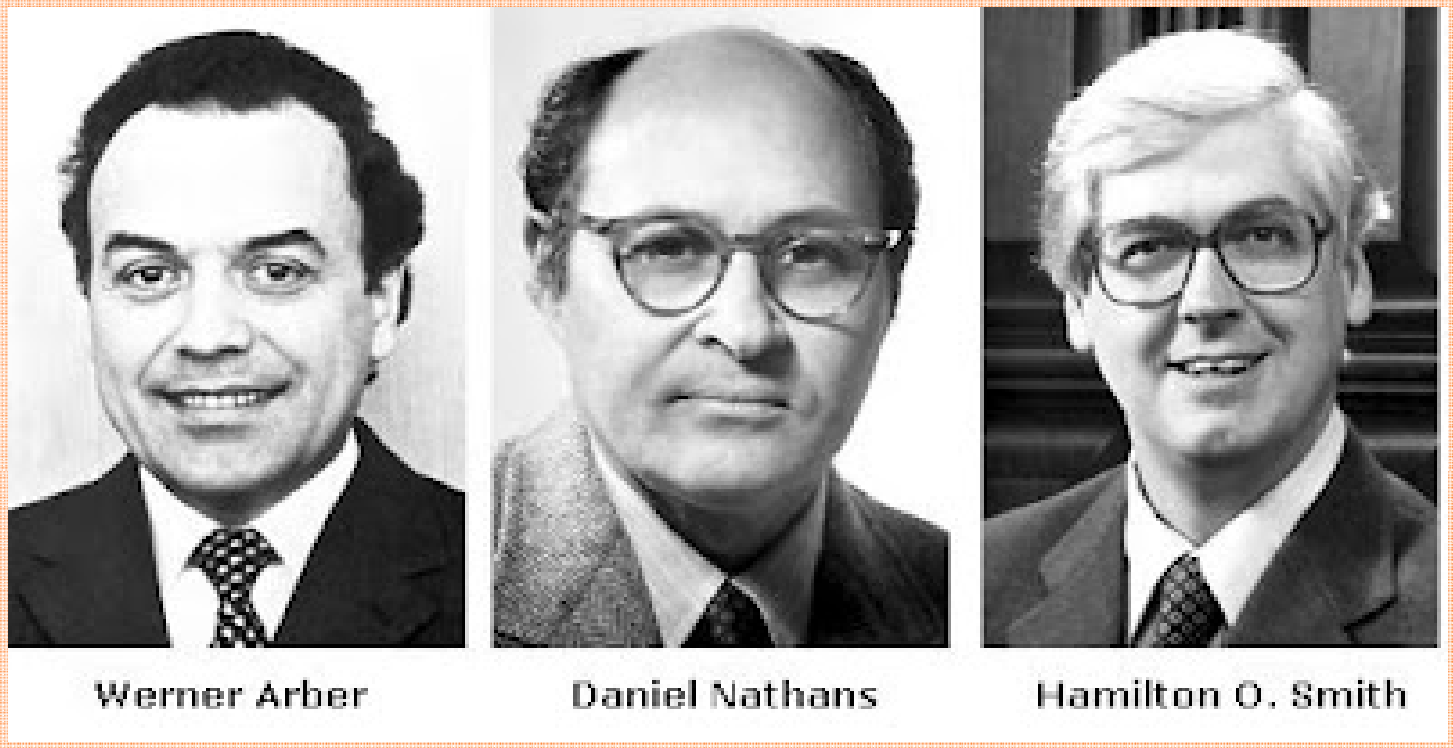


•**Fig: Mechanism of *BamHI***



•**Some restriction endonucleases, their sources, and their cleavage sites**

The Nobel Prize in Medicine 1978



Werner Arber

Biozentrum der Universität, Basel, Switzerland

Daniel Nathans

Johns Hopkins University School of Medicine, Baltimore, MD, USA

Hamilton O. Smith

Johns Hopkins University School of Medicine, Baltimore, MD, USA

Discovery of restriction enzymes and their application to problems of molecular genetics

www.nobelprize.org

Characteristics of Restriction enzymes

- Most restriction enzymes recognize a single restriction site.
- The restriction site is recognized without regard to the source of the DNA.
- Because most restriction enzymes recognize a unique restriction site sequence, the number of cuts in the DNA from a particular organism is determined by the number of restriction sites present.