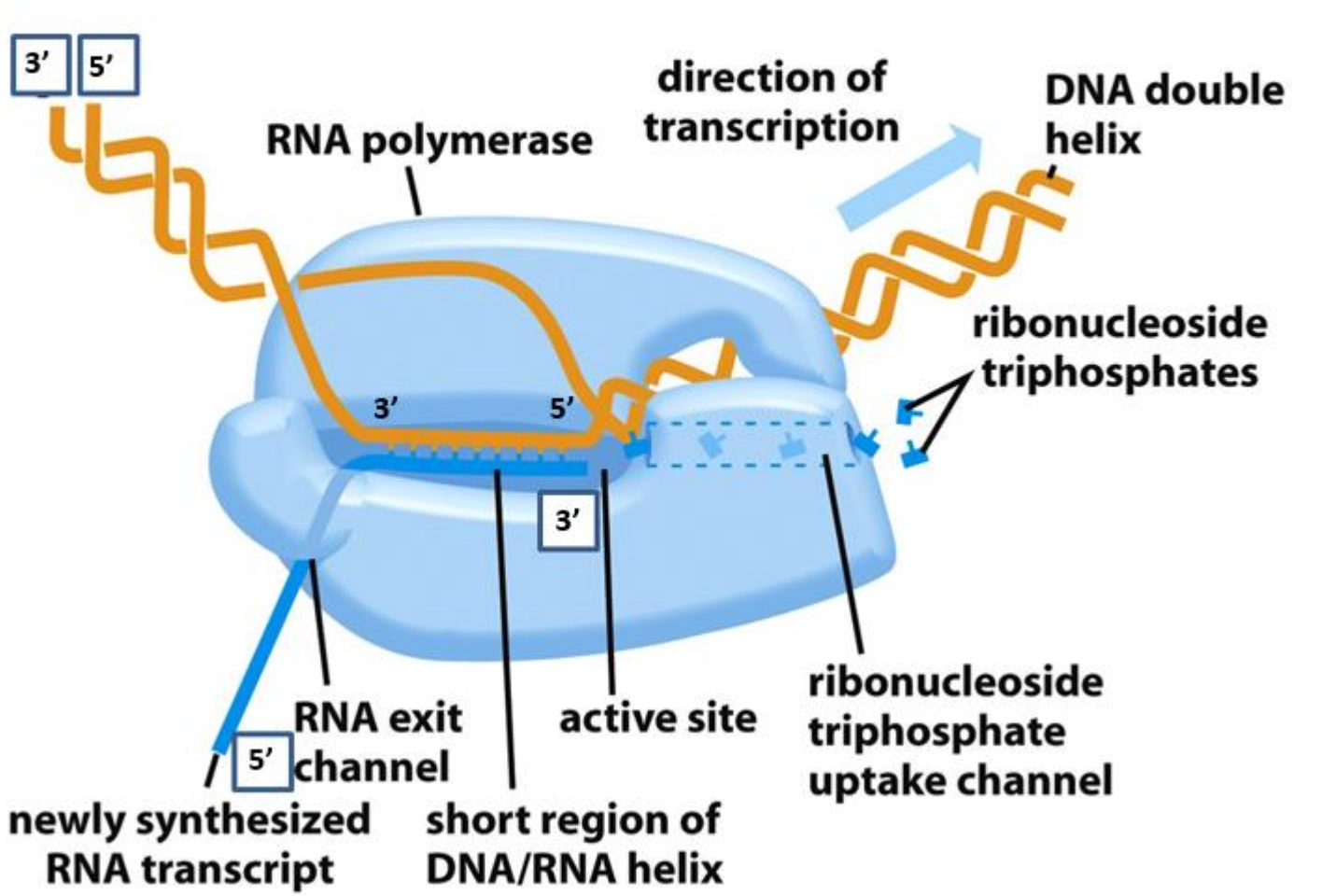
مبانی بیولوژی سلولی و مولکولی، جلسه پنجم

اهمیت تنظیم بیان ژن و مکانیسم های آن


3′
5′
RNA polymerase
direction of transcription
DNA double helix
ribonucleoside triphosphates
3′
5′
3′
RNA exit channel
active site
ribonucleoside triphosphate uptake channel
5′
newly synthesized RNA transcript
short region of DNA/RNA helix

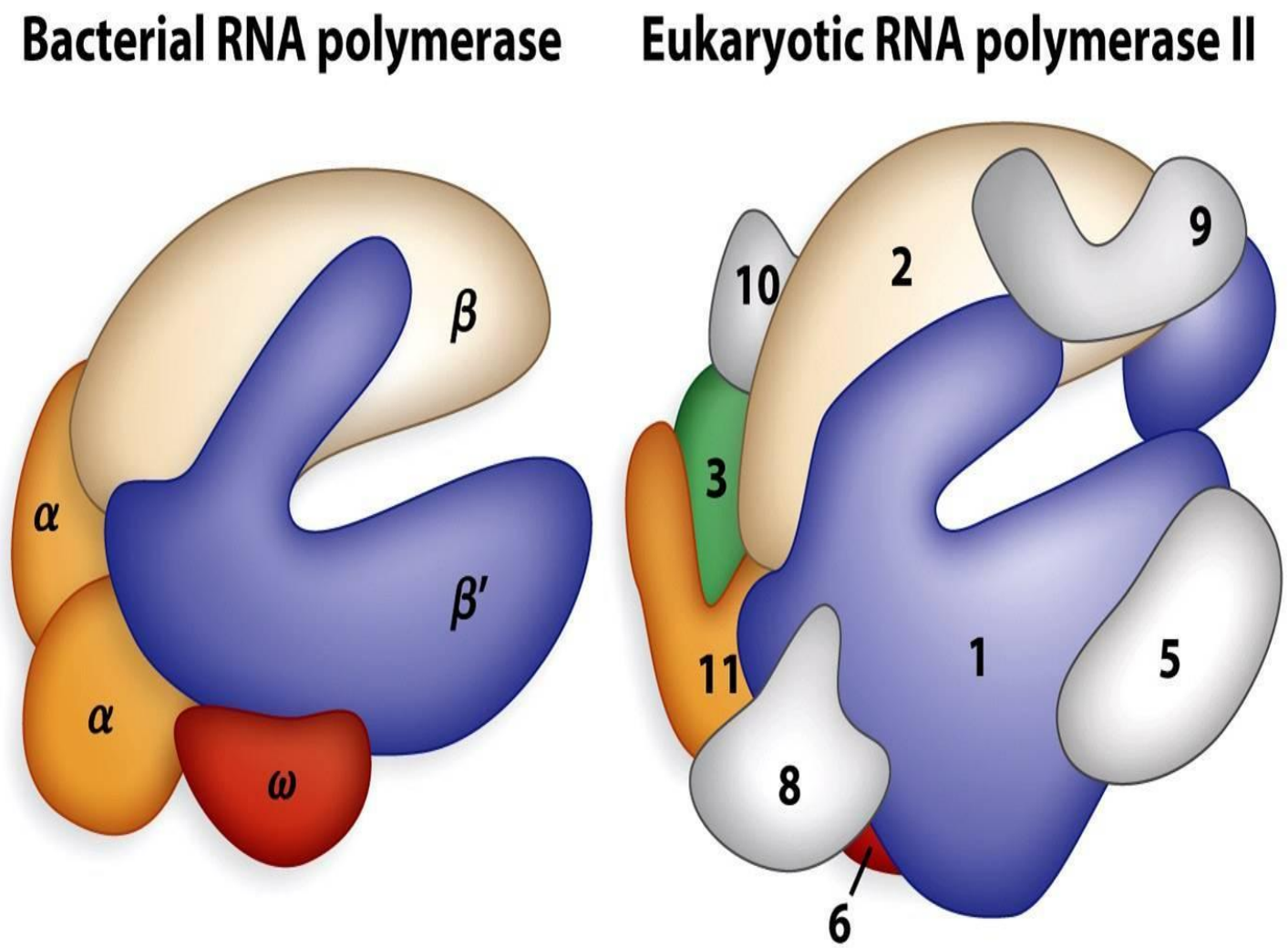


Figure 15-21
Molecular Biology: Principles and Practice

Eukaryotic RNA Polymerases

- Three DNA dependent RNA polymerases:
  RNA Pol I, II, and III
- All 3 are big, multimeric proteins (500-700 kD)
- All have 2 large subunits with sequences similar to β and β' in E. coli RNA polymerase, so catalytic site may be conserved
- All interact with general transcription factors-GTFs
- RNA Pol II is most sensitive to α-amanitin

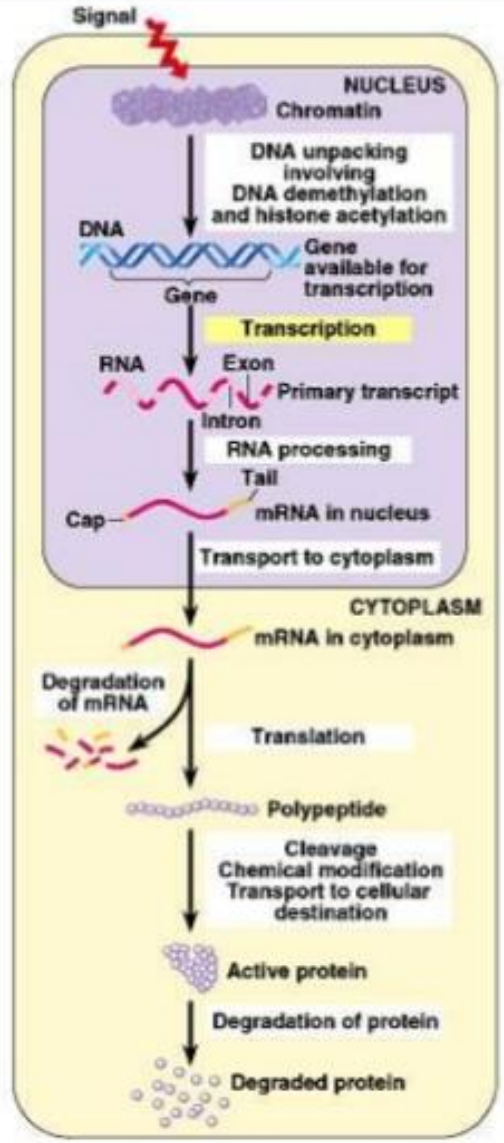
Enzyme	Location	Product
RNA Polymerase I	Nucleolus	rRNA
RNA Polymerase II	Nucleoplasm	mRNA
RNA Polymerase III	Nucleoplasm	tRNA
mt RNA Polymerase	Mitochondria	mtRNA

Mechanism of Gene Regulation in Prokaryotes and Eukaryotes

- In prokaryotes the primary control point is the process of transcription initiation
- In eukaryotes expression of gene into proteins can be controlled at various locations.

Points of control

- The control of gene expression can occur at any step in the pathway from gene to functional protein
  1. packing/unpacking DNA
  2. transcription
  3. mRNA processing
  4. mRNA transport
  5. translation
  6. protein processing
  7. protein degradation



مبانی بیولوژی سلولی و مولکولی، جلسه ششم

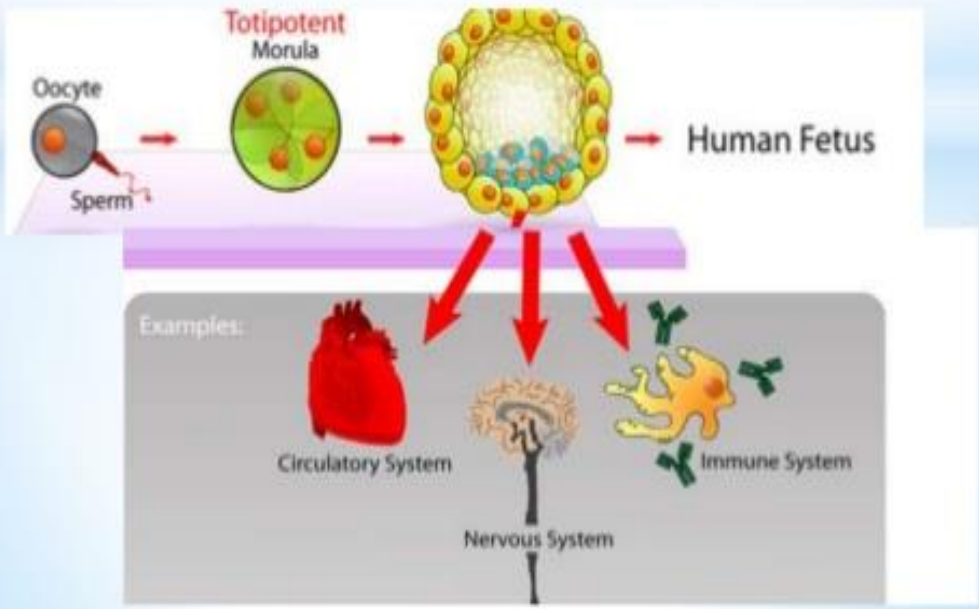
اپی ژنتیک

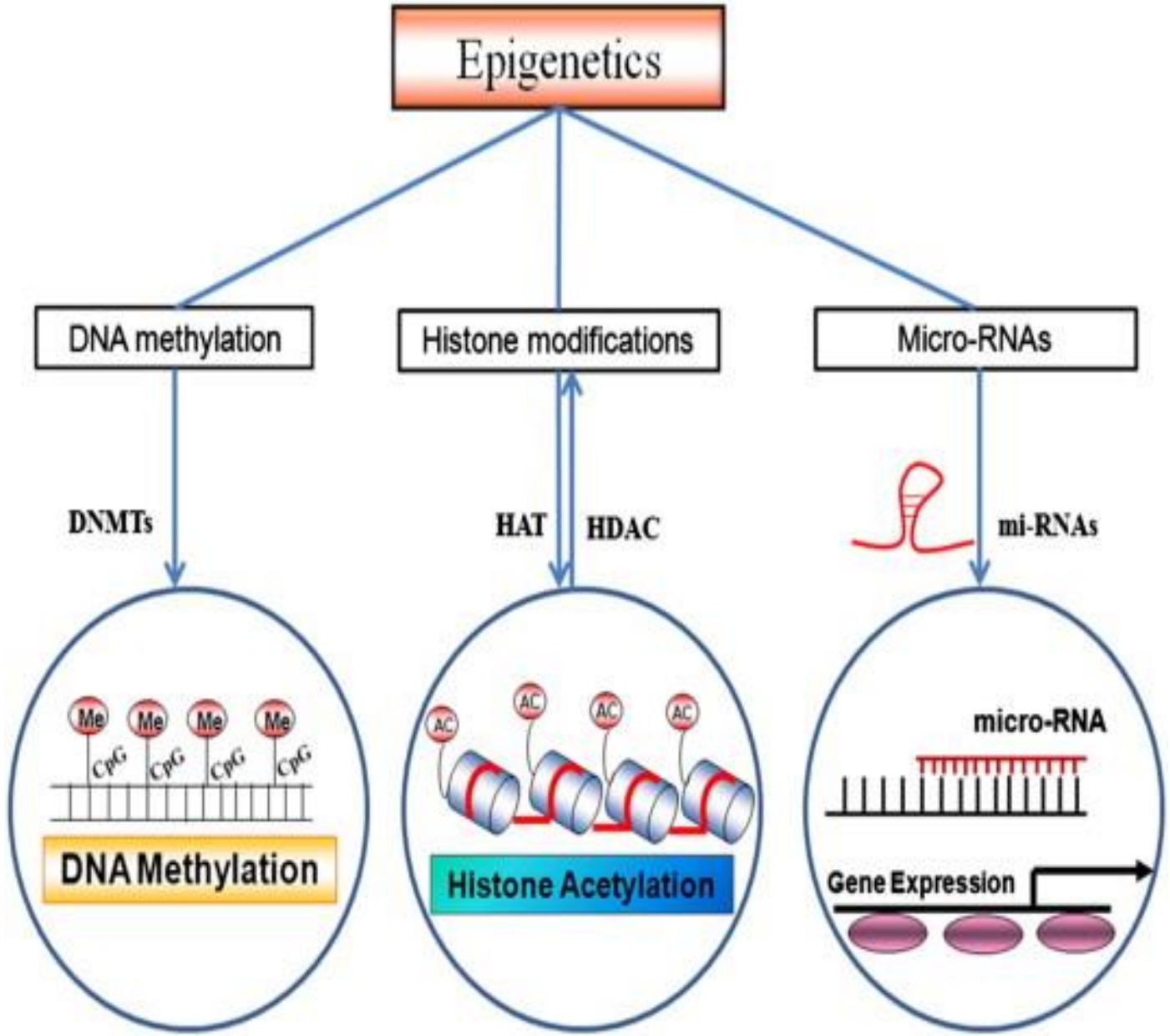
Epigenetics

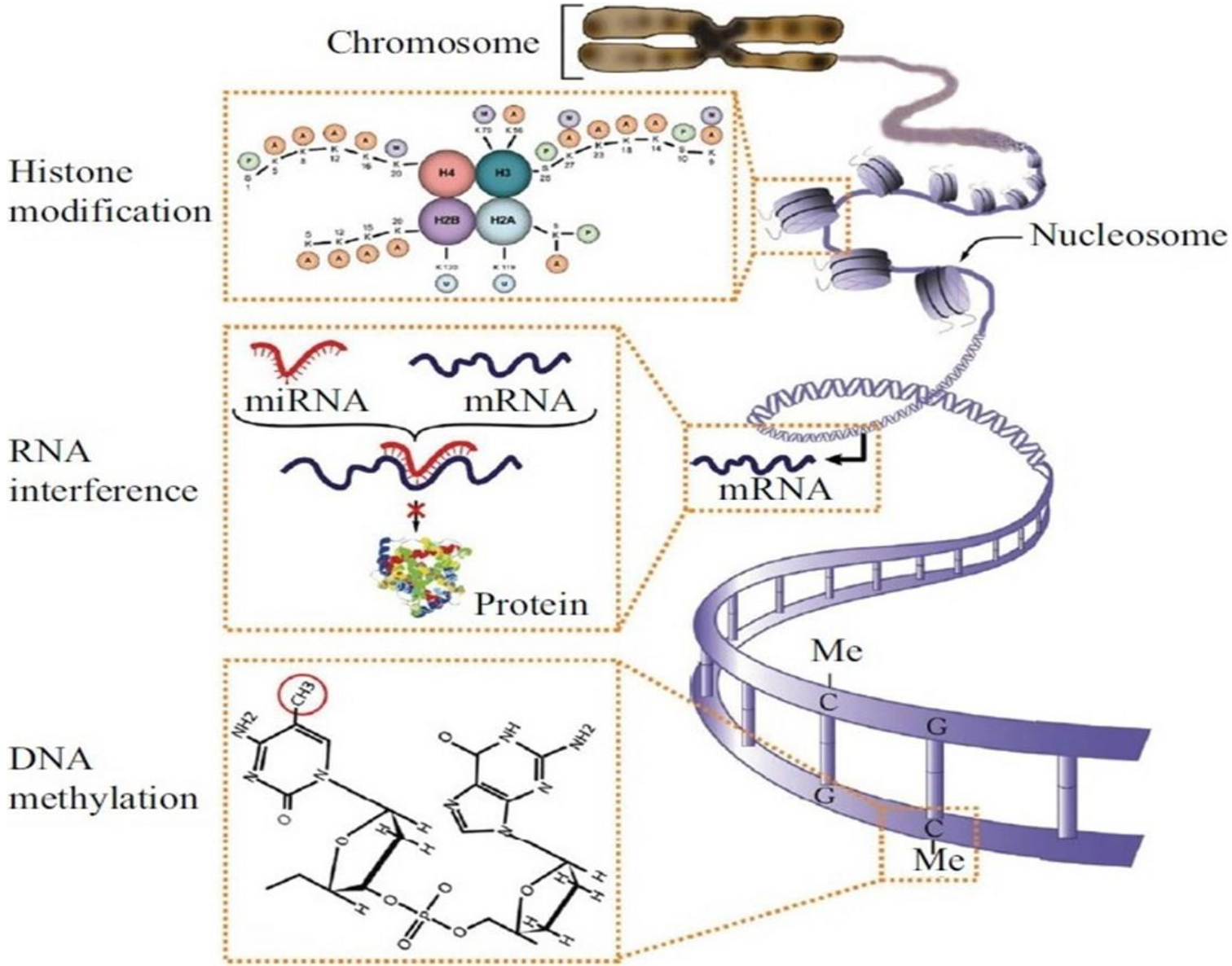
*Changes in gene expression or phenotype that don't involve changes to the DNA sequence[1]

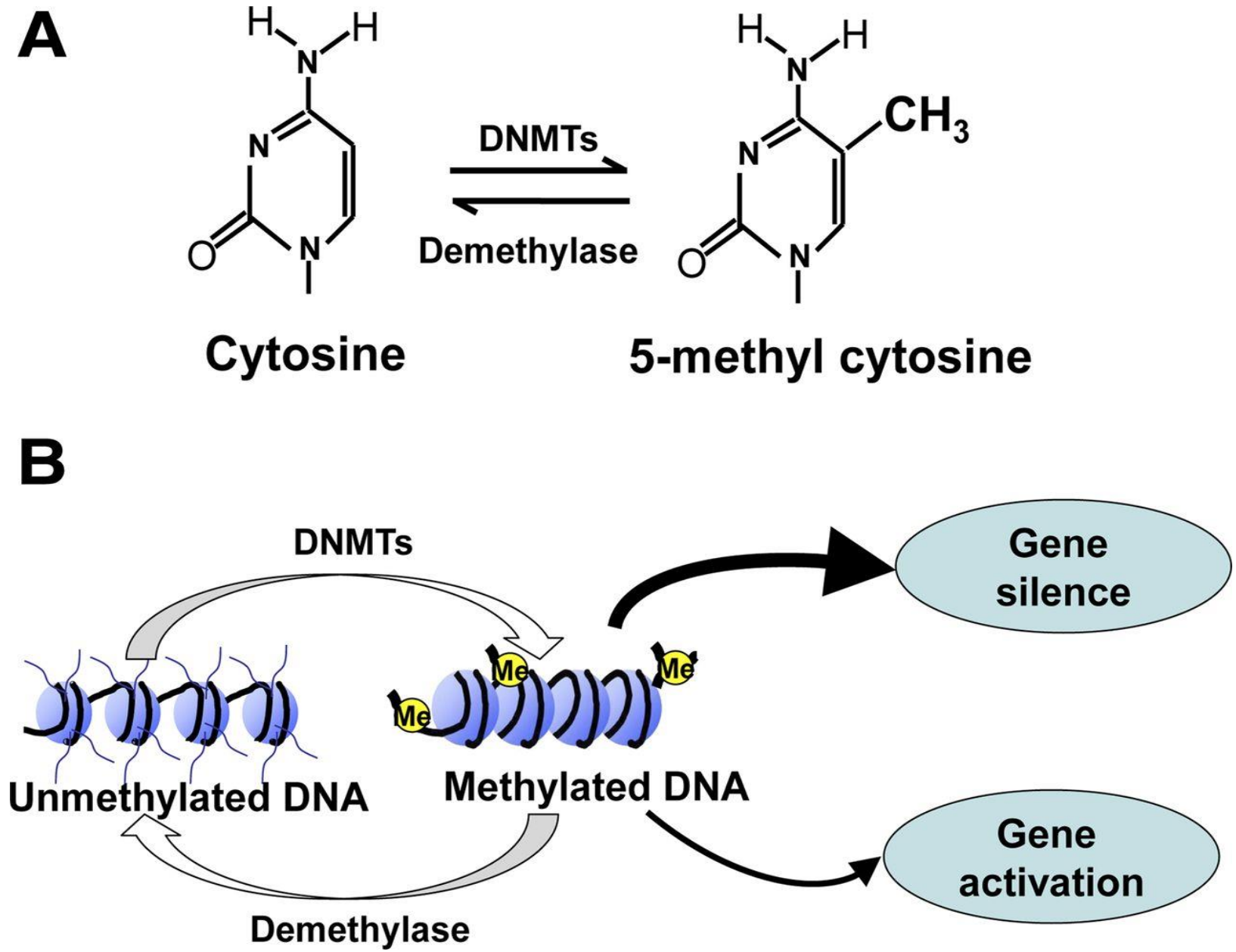
*Its defined as heritable changes in gene activity and expression that occur without alteration in DNA sequence

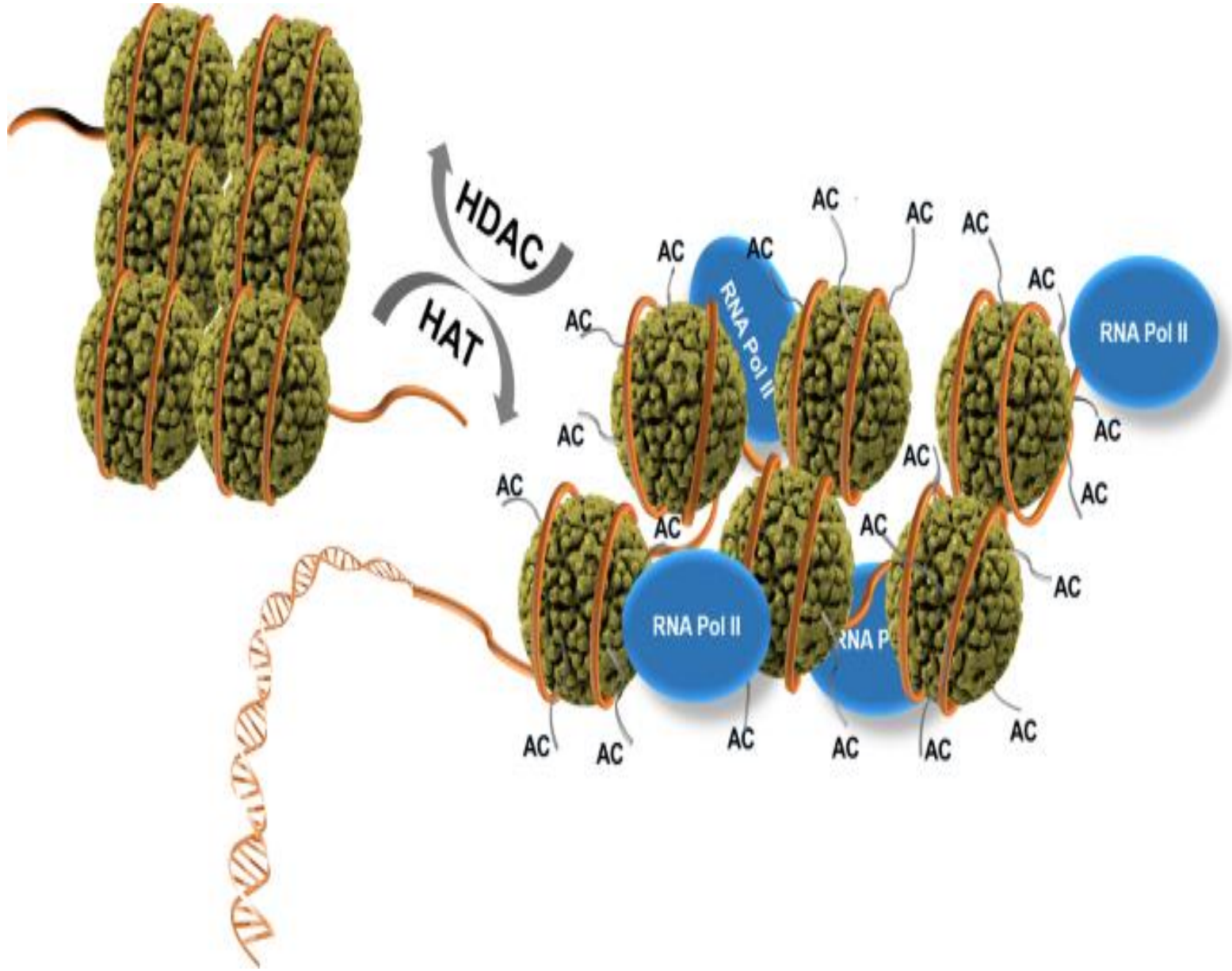
*Modern definition is non-sequence dependent inheritance.

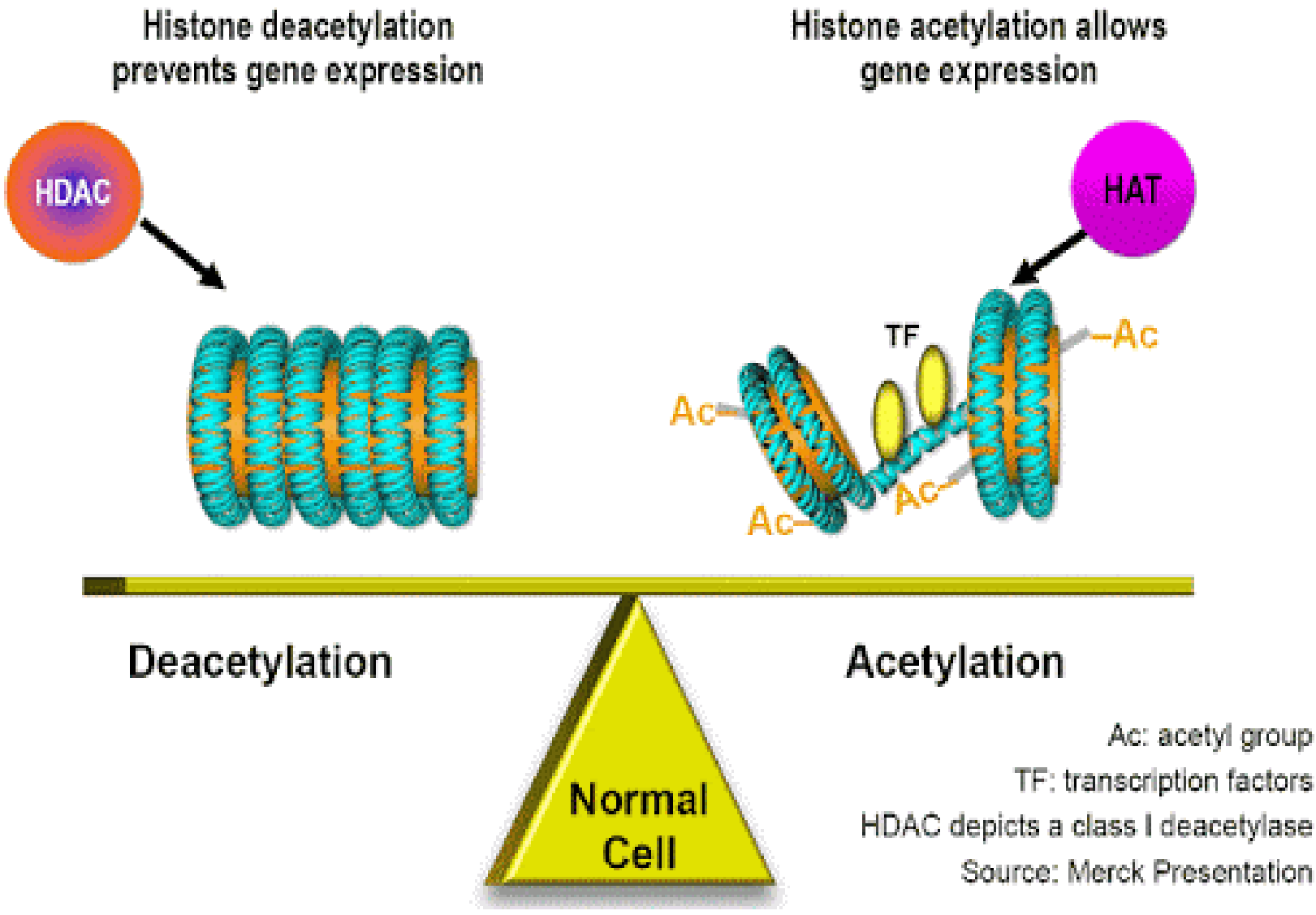



Epigenetics
DNA methylation
Histone modifications
Micro-RNAs
DNMTs
HAT
HDAC
mi-RNAs
Me
CpG
DNA Methylation
AC
Histone Acetylation
micro-RNA
Gene Expression

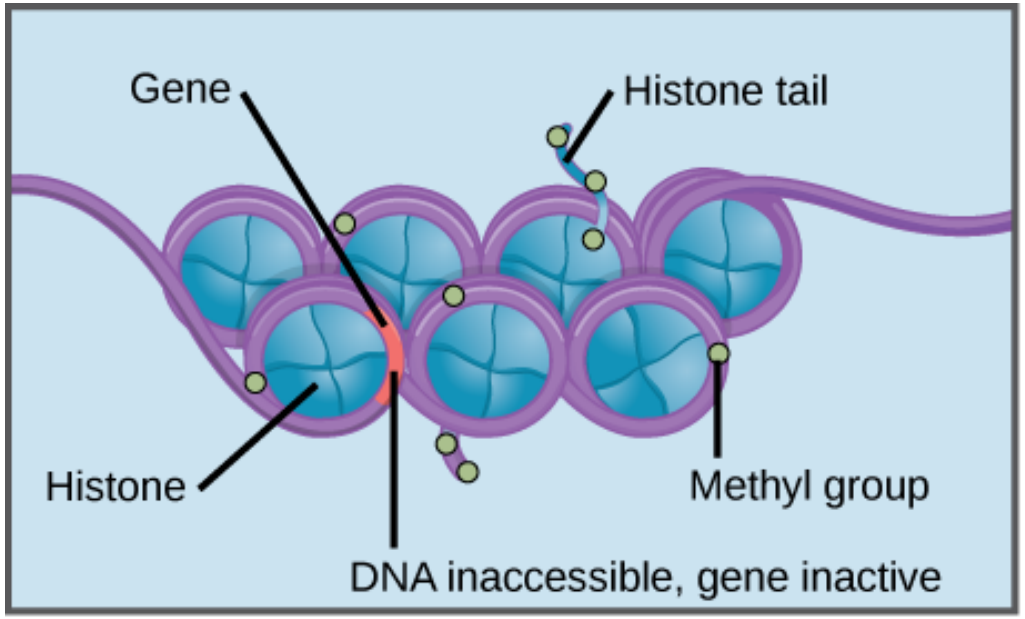

Chromosome
Histone modification
H4
H3
H2B
H2A
Nucleosome
RNA interference
miRNA
mRNA
Protein
mRNA
DNA methylation
CH3
NH2
NH
NH2
Me
C
G
G
C
Me


A
DNMTs
Demethylase
Cytosine
5-methyl cytosine
B
DNMTs
Gene silence
Me
Me
Me
Unmethylated DNA
Methylated DNA
Gene activation
Demethylase

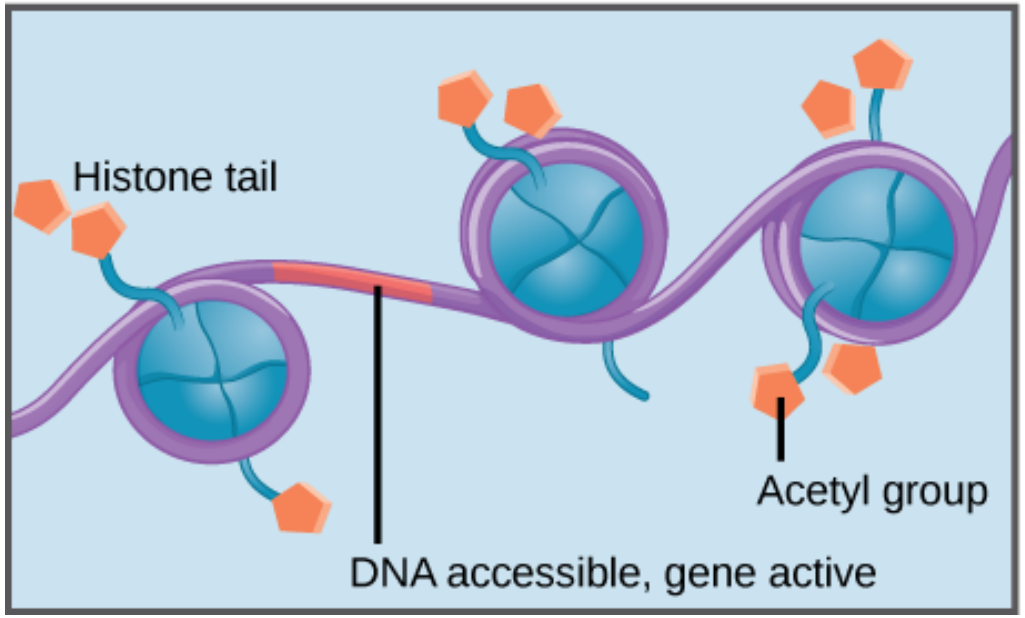

HDAC
HAT
AC
RNA Pol II



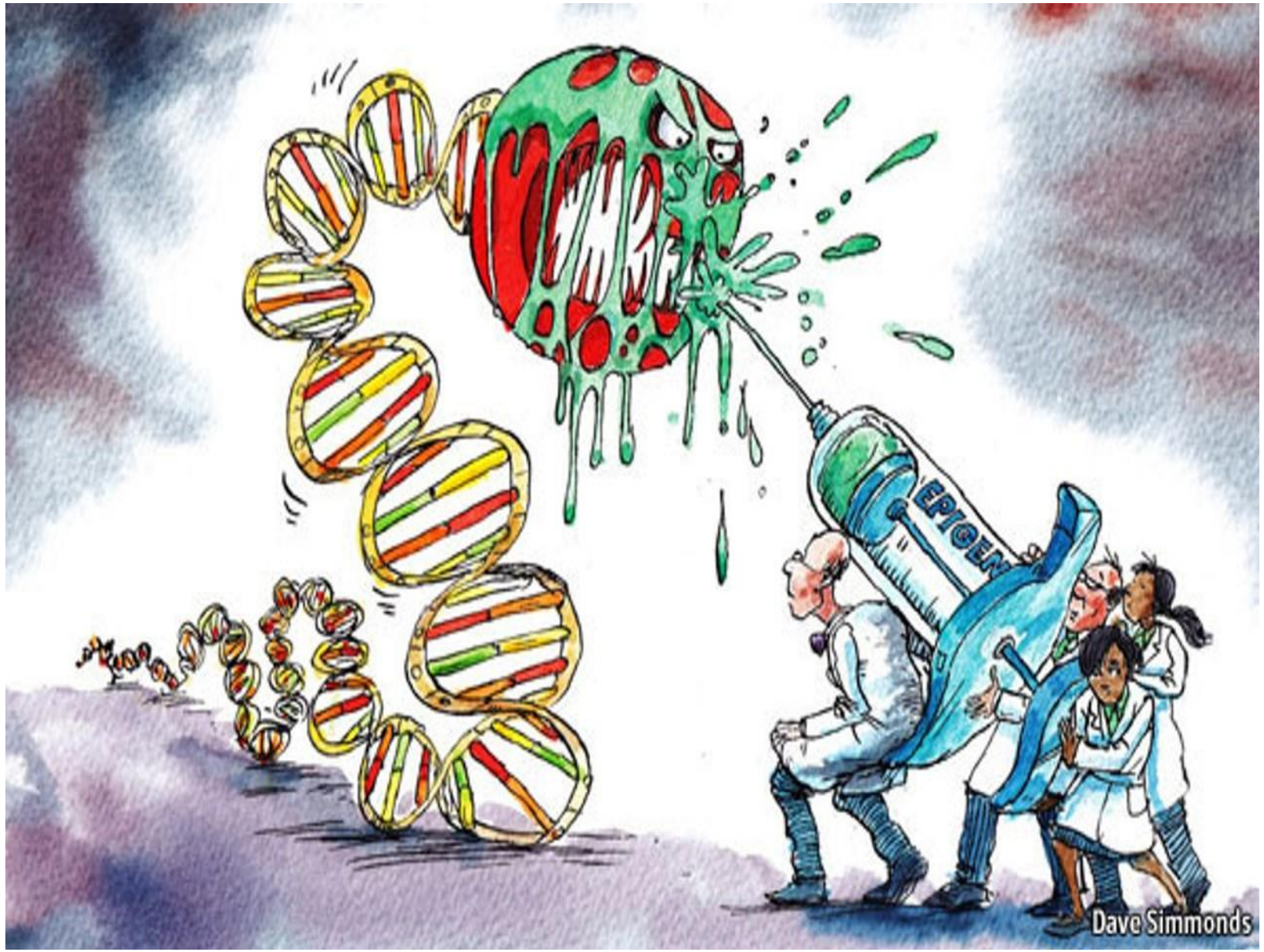
Ac: acetyl group
TF: transcription factors
HDAC depicts a class I deacetylase
Source: Merck Presentation



Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

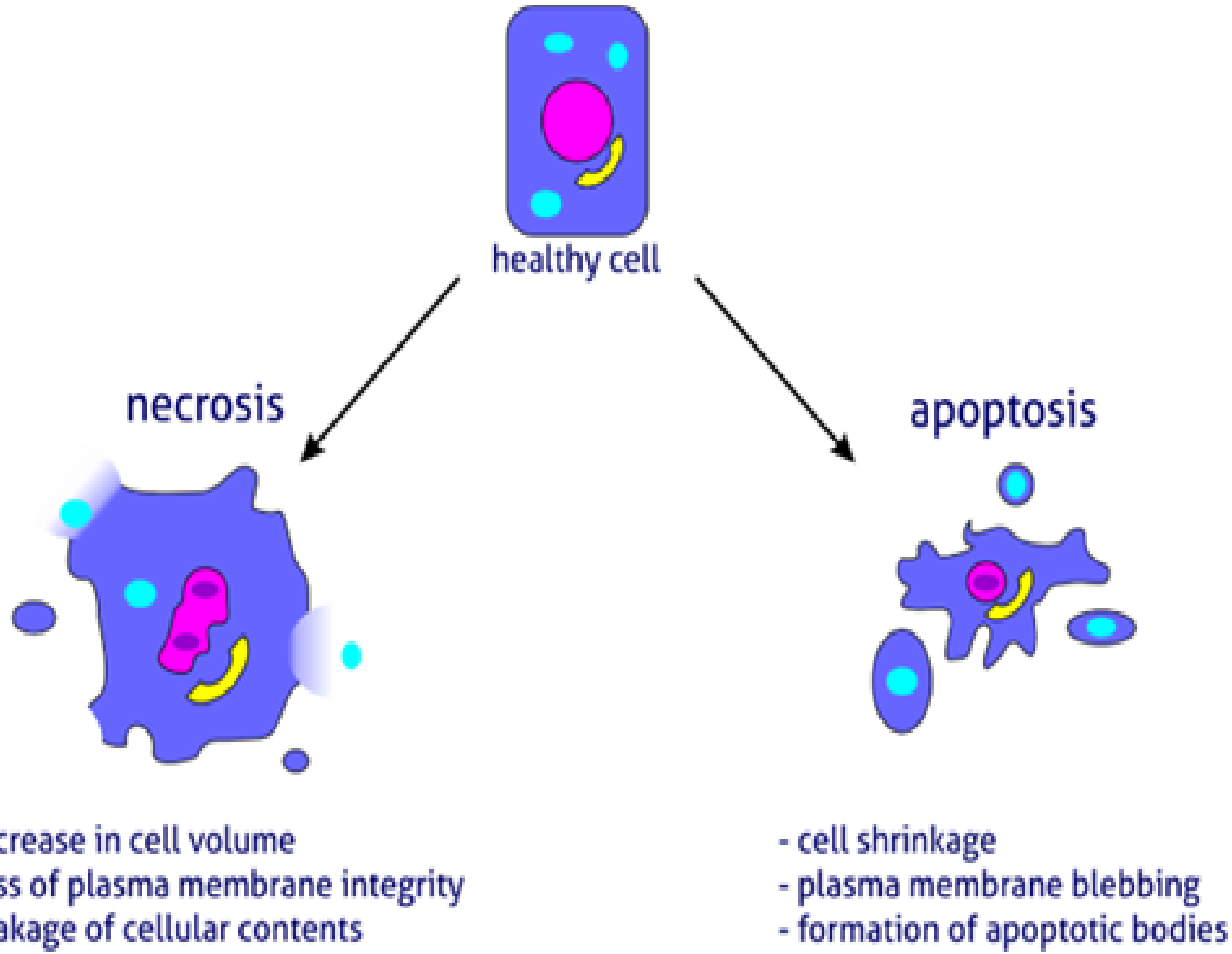


Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

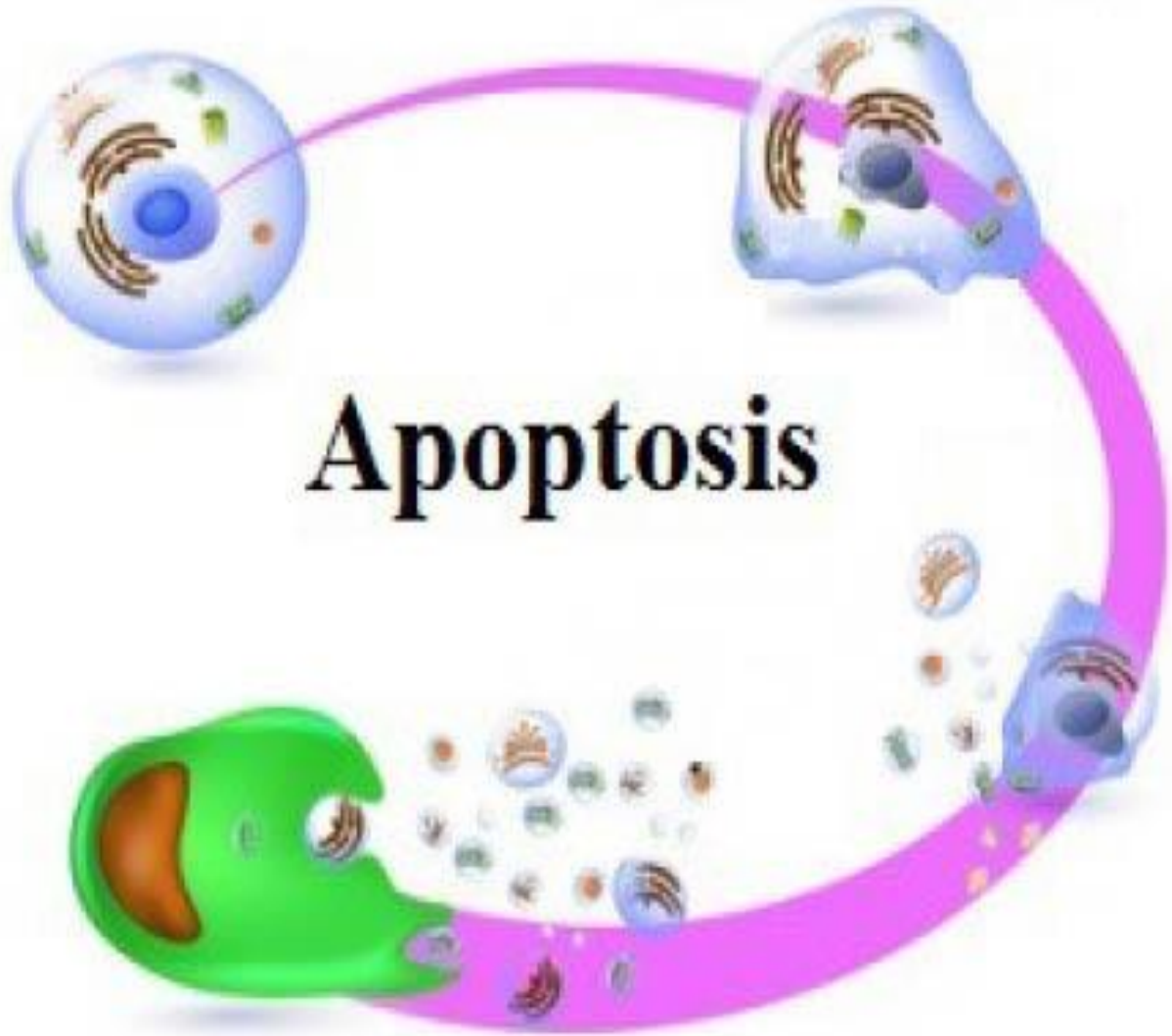

EPIGEN
Dave Simmonds

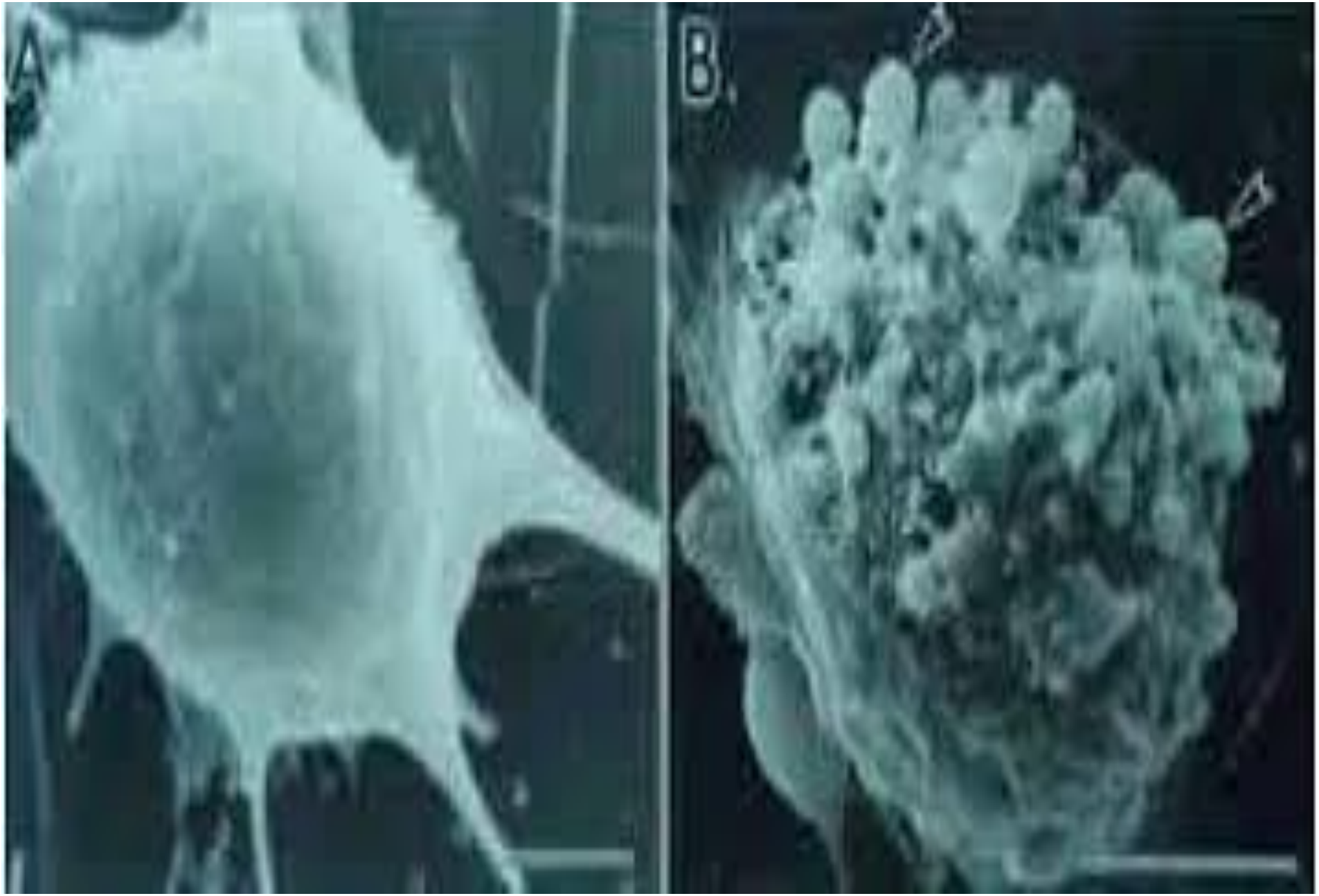
مبانی بیولوژی سلولی و مولکولی، جلسه هفتم

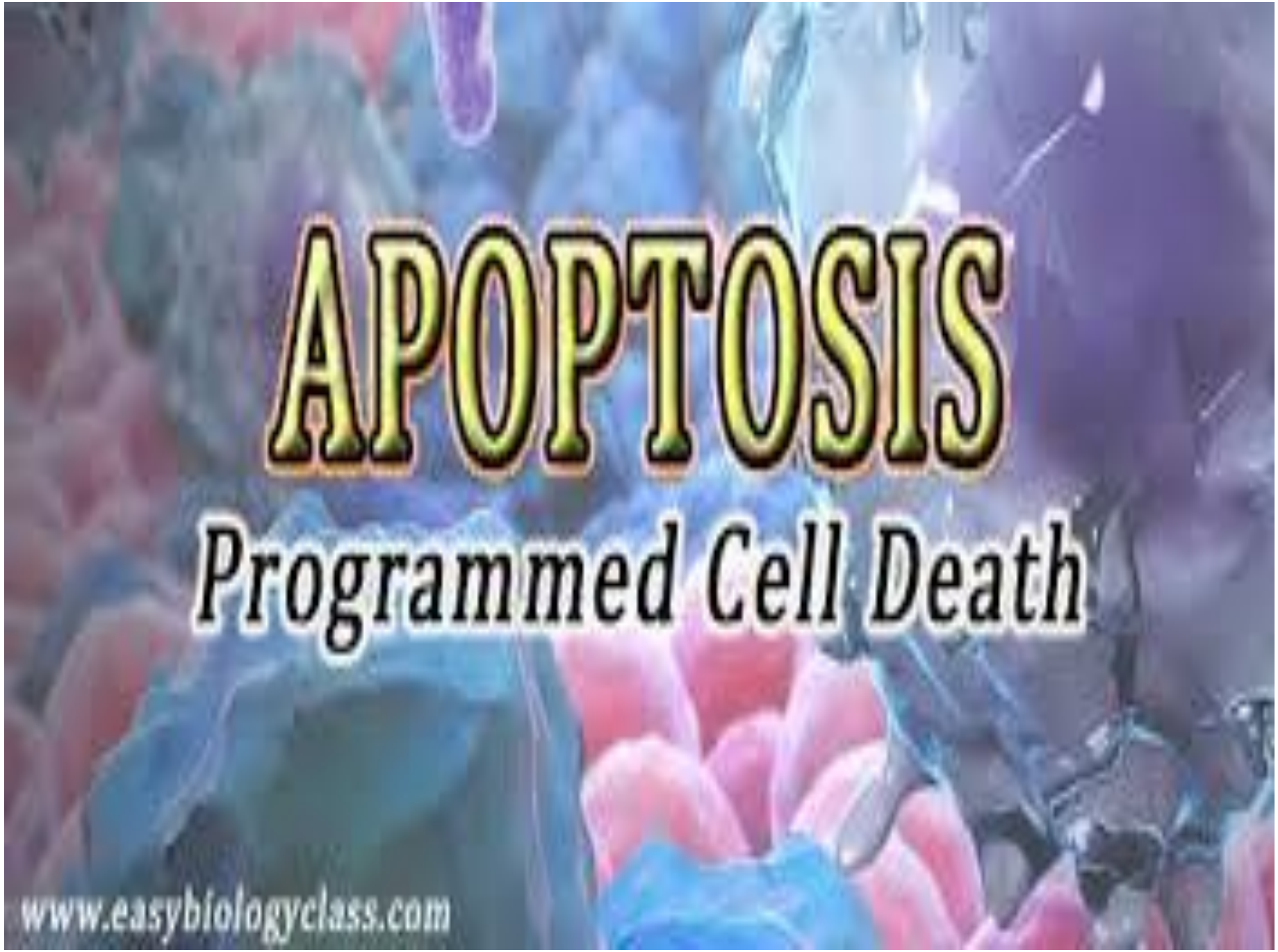
آپوپتوزیس، نقش های زیستی و مکانیسم های مولکولی آن(قسمت اول)


healthy cell
necrosis
apoptosis
- increase in cell volume
- loss of plasma membrane integrity
- leakage of cellular contents
- cell shrinkage
- plasma membrane blebbing
- formation of apoptotic bodies

Apoptosis



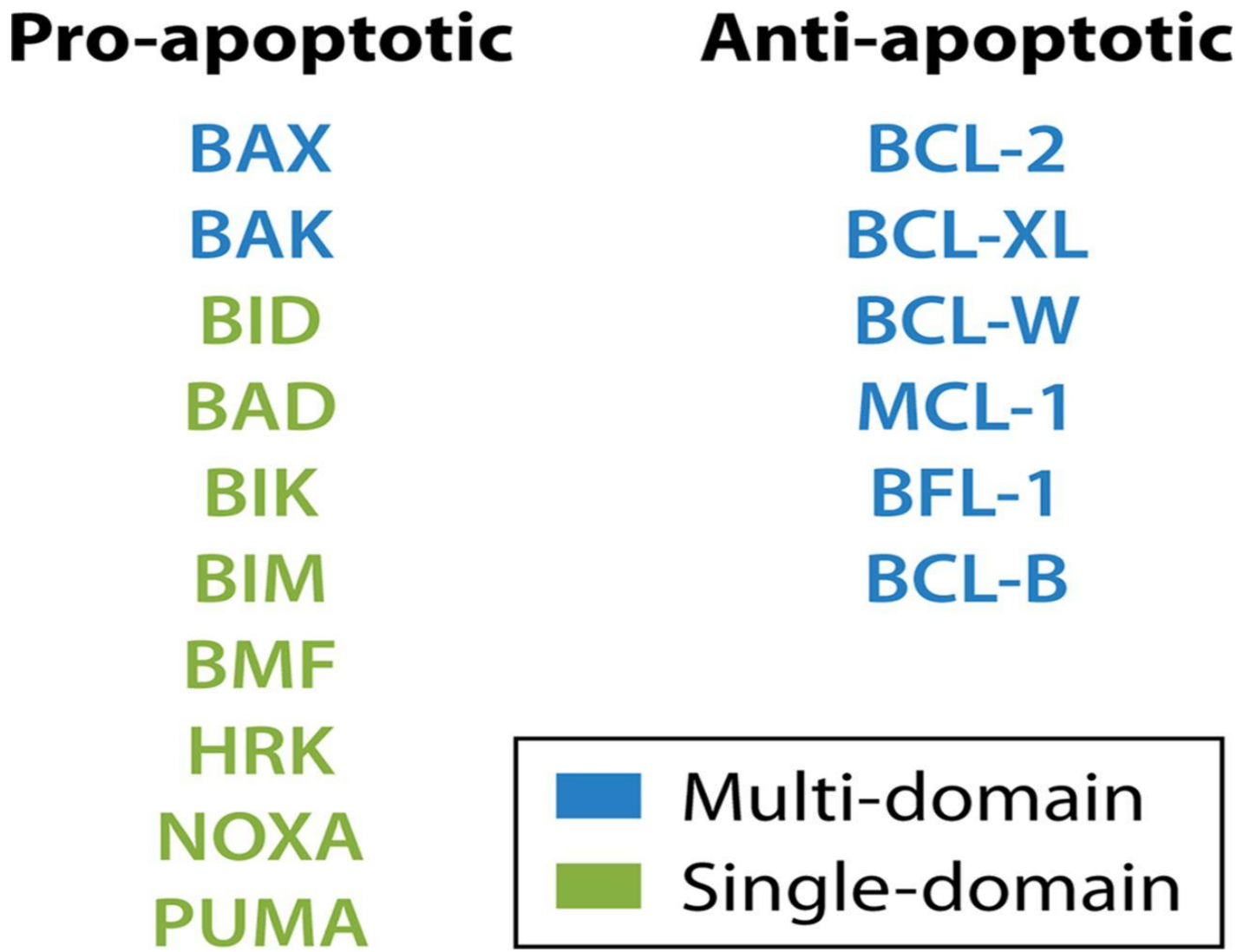

A
B


APOPTOSIS
Programmed Cell Death
www.easybiologyclass.com

Importance of Apoptosis

Pro-survival genes

Pro-death genes


Pro-apoptotic
Anti-apoptotic
BAX
BAK
BID
BAD
BIK
BIM
BMF
HRK
NOXA
PUMA
BCL-2
BCL-XL
BCL-W
MCL-1
BFL-1
BCL-B
Multi-domain
Single-domain

مبانی بیولوژی سلولی و مولکولی، جلسه هشتم

آپوپتوزیس، نقش های زیستی و مکانیسم های مولکولی آن(قسمت دوم)

Medical & Science

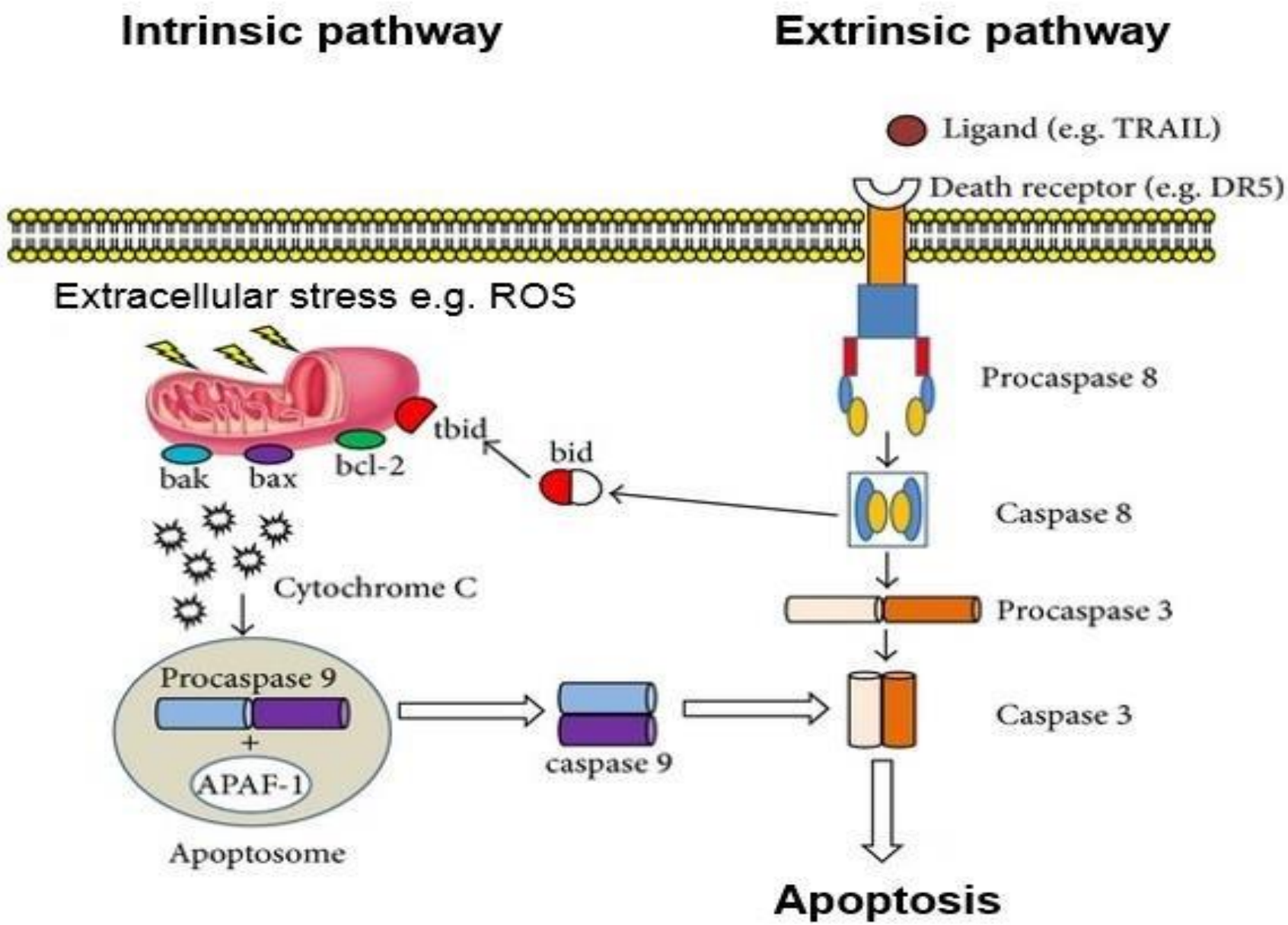
CASPASE

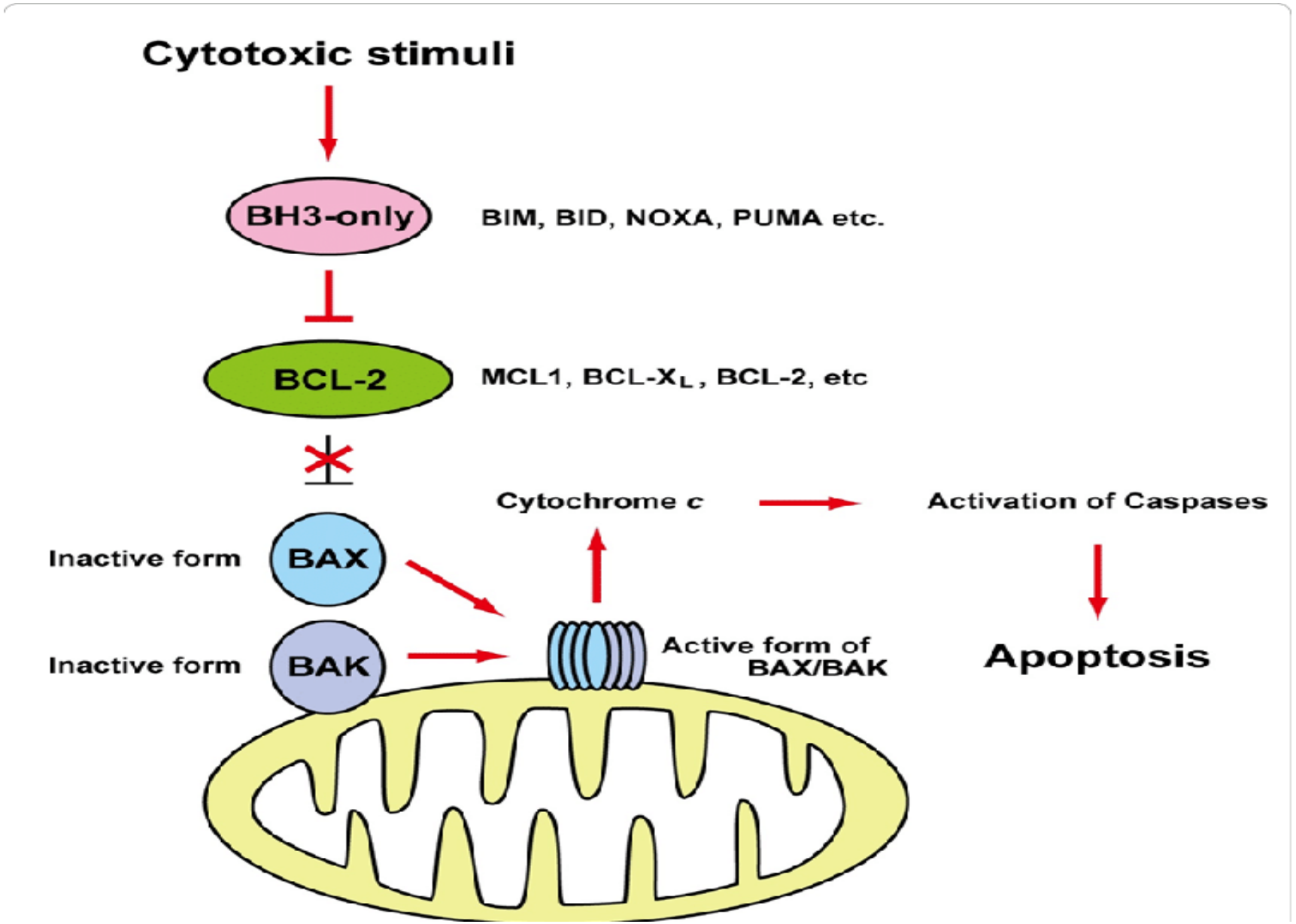
means

Cysteine Aspartic Acid Specific Protease

Caspases

Initiator Caspases	*Executioner Caspases*
Caspase-8	Caspase-3
Caspase-9	Caspase-6
	Caspase-7

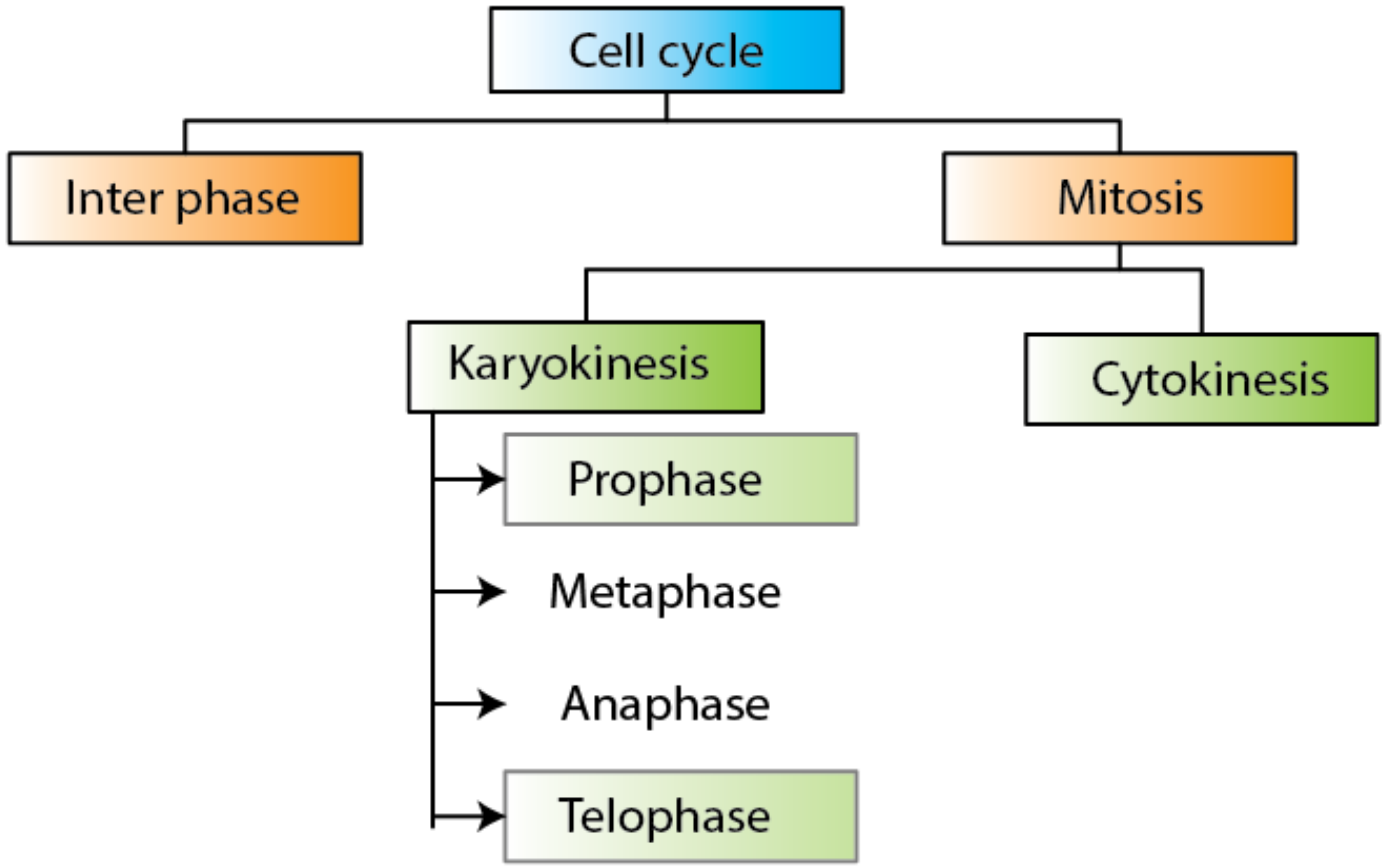

Intrinsic pathway
Extrinsic pathway
Ligand (e.g. TRAIL)
Death receptor (e.g. DR5)
Extracellular stress e.g. ROS
Procaspase 8
tbid
bid
bak
bax
bcl-2
Caspase 8
Cytochrome C
Procaspase 3
Procaspase 9
+
APAF-1
caspase 9
Caspase 3
Apoptosome
Apoptosis

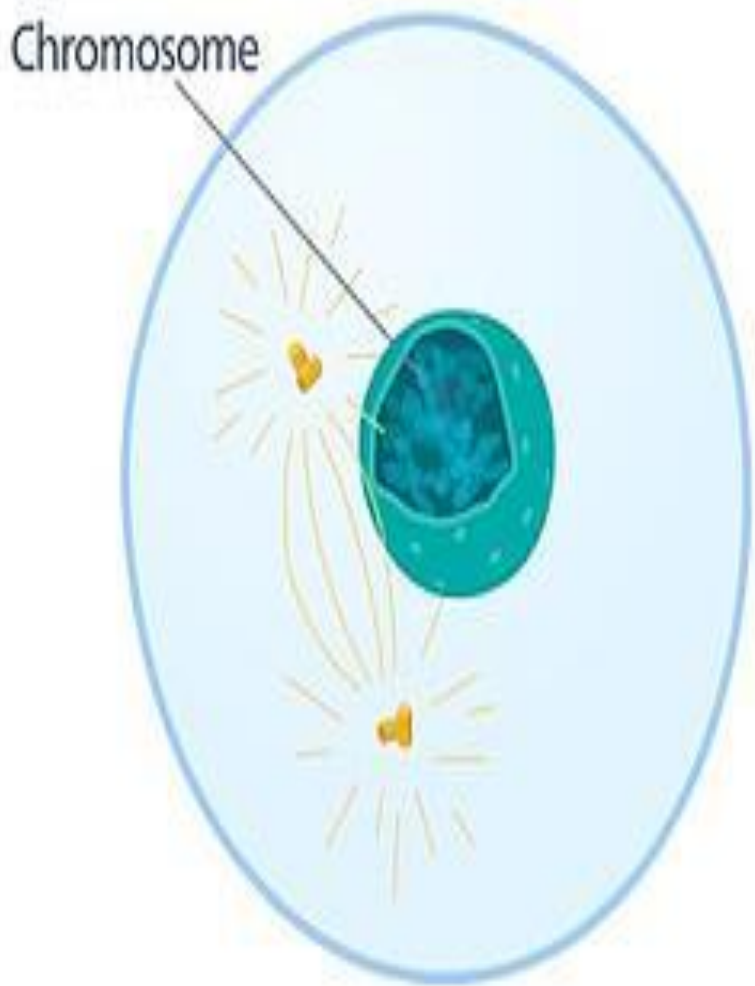

Cytotoxic stimuli
BH3-only
BIM, BID, NOXA, PUMA etc.
BCL-2
MCL1, BCL-X$_L$, BCL-2, etc
Cytochrome c
Activation of Caspases
Inactive form
BAX
Inactive form
BAK
Active form of
BAX/BAK
Apoptosis

مبانی بیولوژی سلولی و مولکولی، جلسه نهم

چرخه سلولی(قسمت اول)

همانند سازی DNA

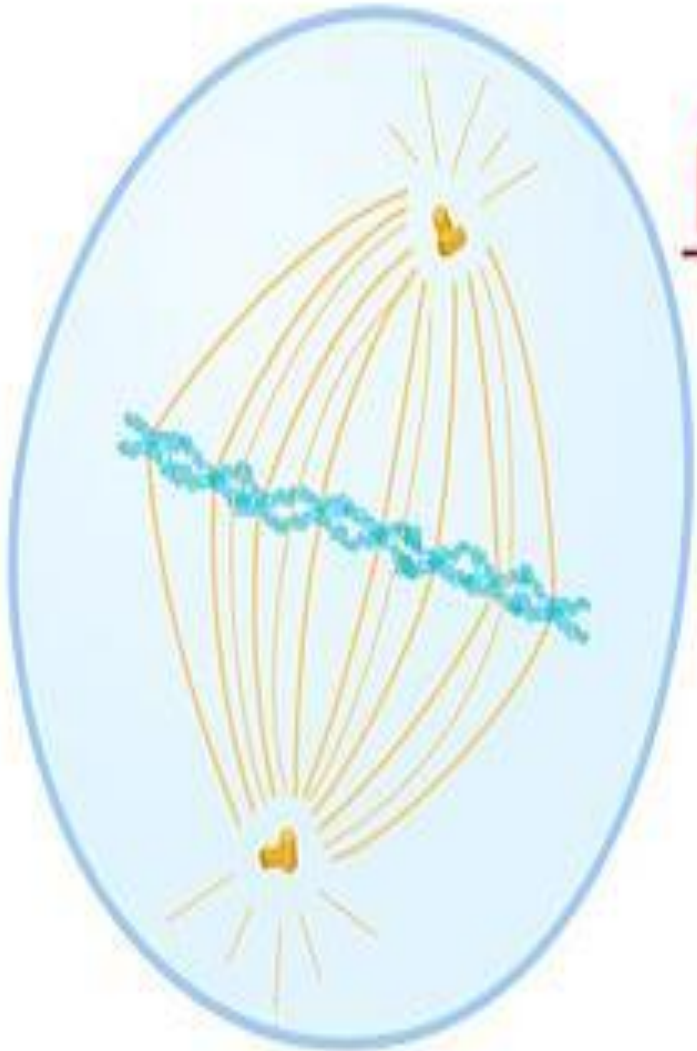

Cell cycle
Inter phase
Mitosis
Karyokinesis
Cytokinesis
Prophase
Metaphase
Anaphase
Telophase



Prophase

Chromatin condenses into **chromosomes**

Nucleolus disappears

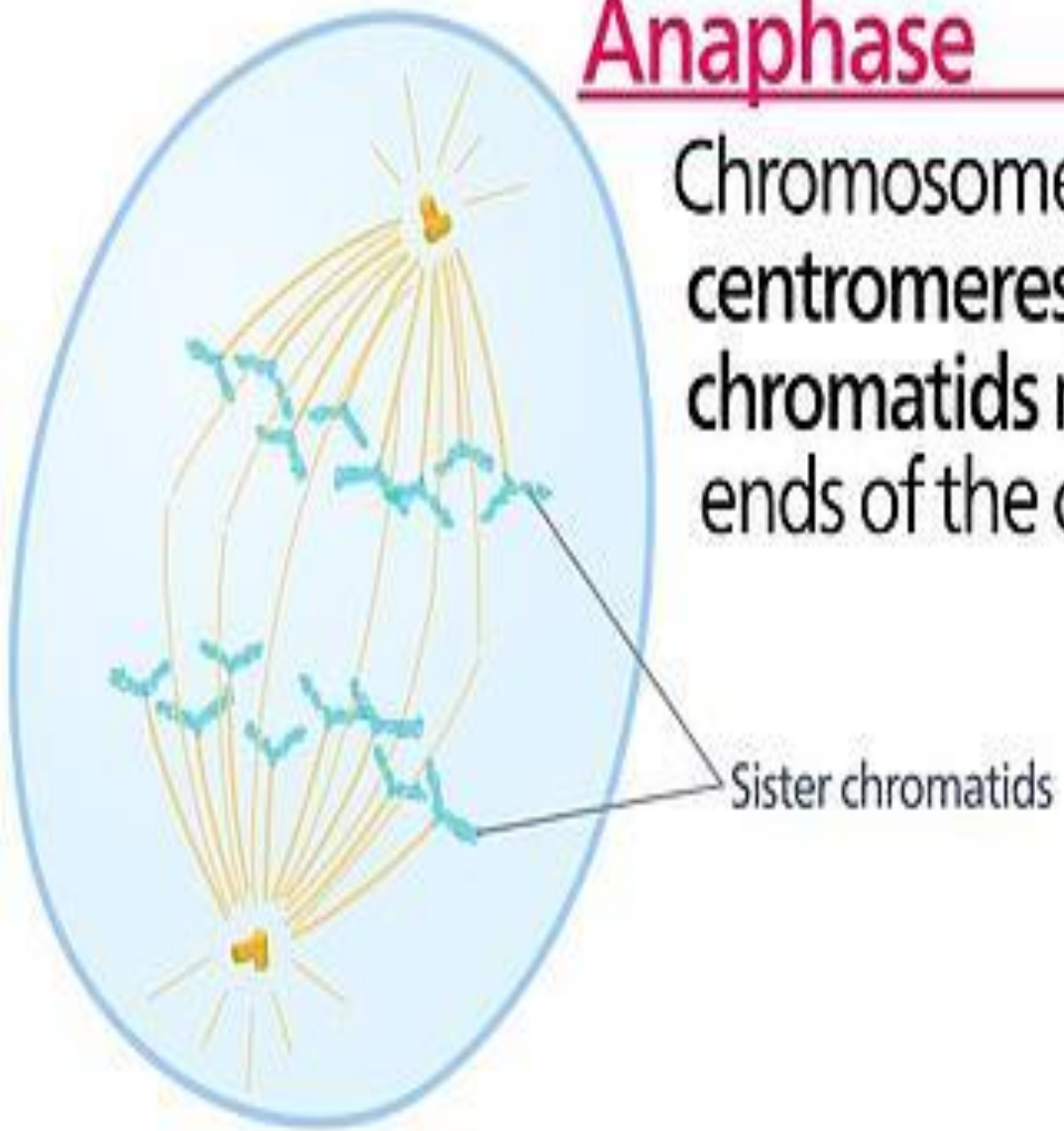


Metaphase

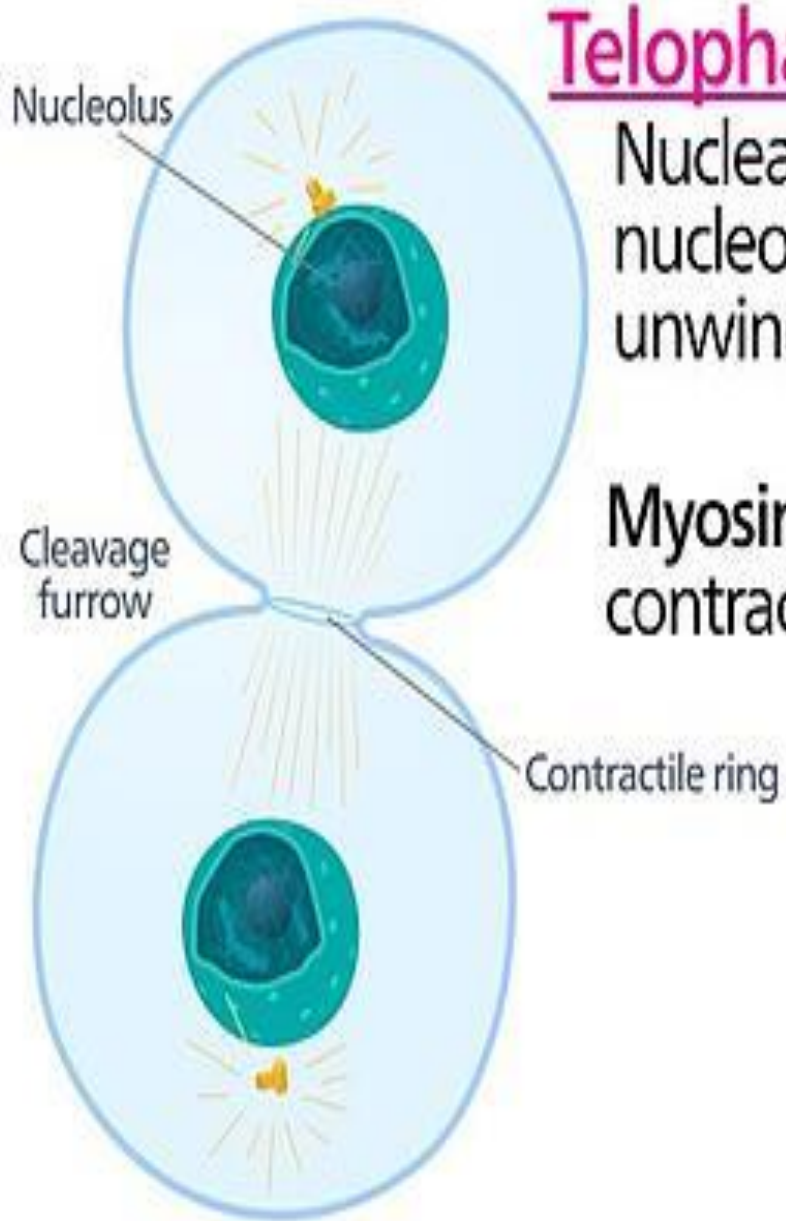
Chromosomes line up along metaphase plate (imaginary plane)

Anaphase

Chromosomes break at **centromeres**, and **sister chromatids** move to opposite ends of the cell

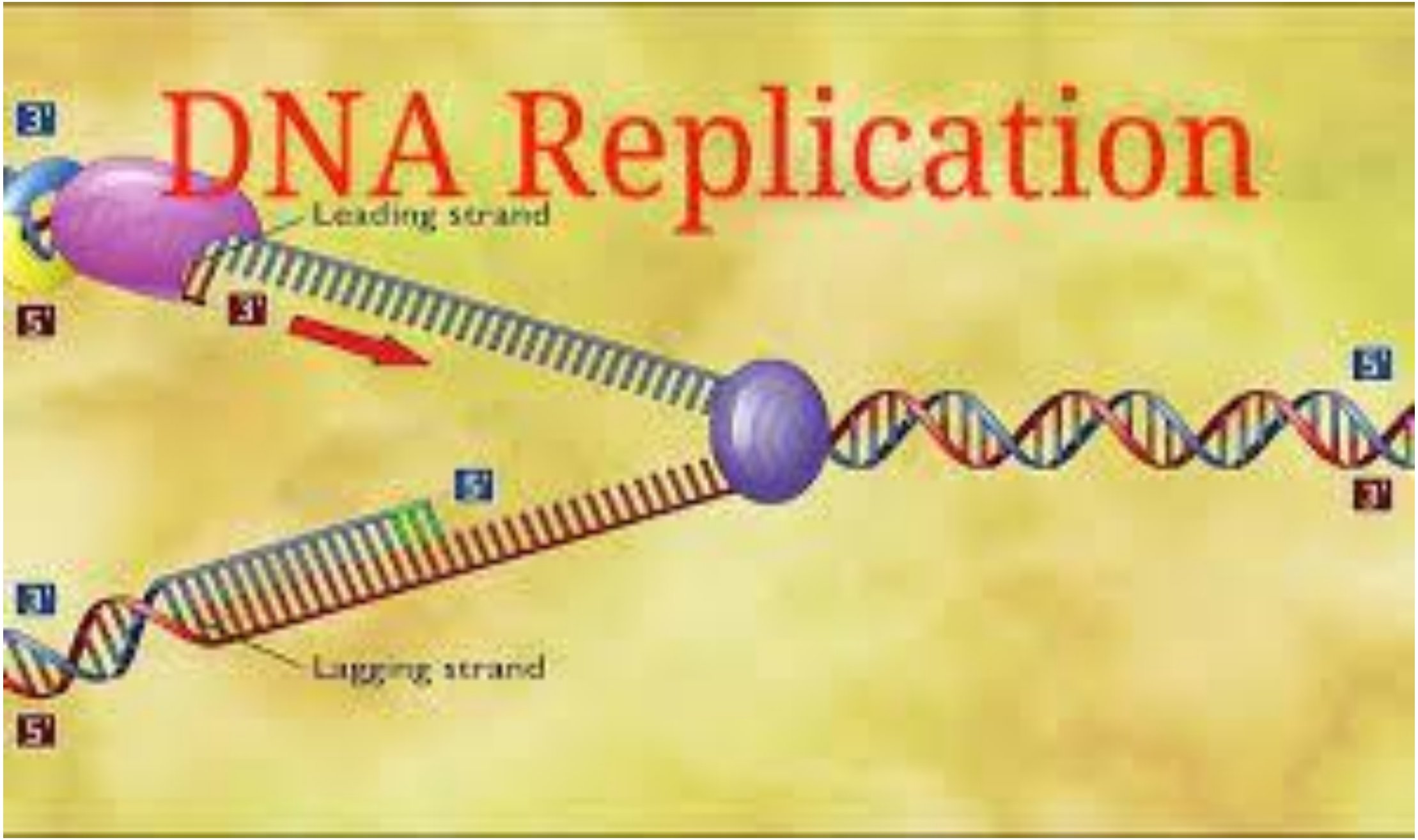


Telophase and Cytokinesis



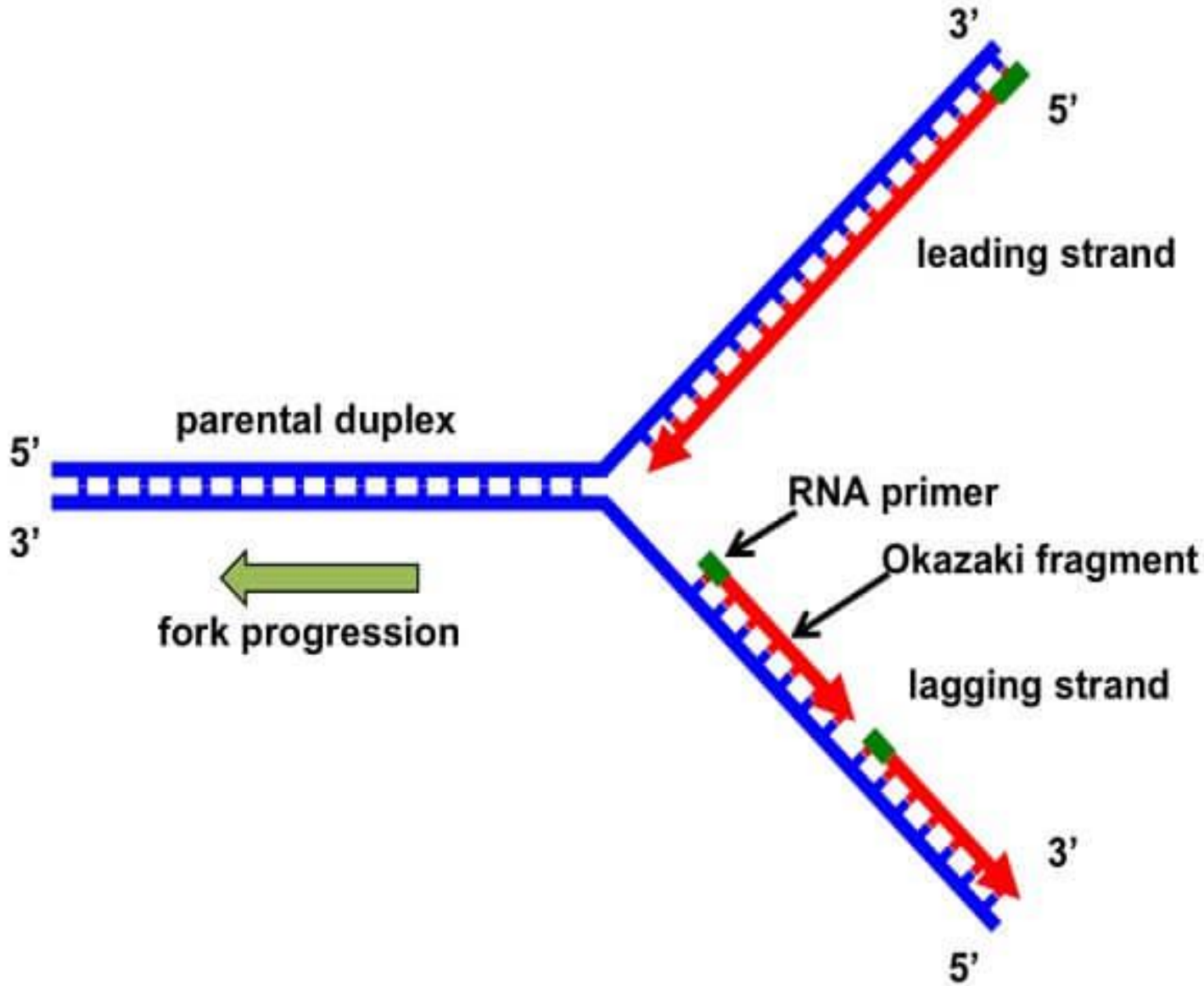
Nuclear membrane reforms, nucleoli reappear, chromosomes unwind into chromatin

Myosin II and **actin** filament ring contract to cleave cell in two


DNA Replication
3'
5'
Leading strand
3'
5'
5'
3'
3'
5'
Lagging strand

Enzymes involved in Replication

- DNA Polymerase I
  - replaces RNA primer with DNA
- DNA Polymerase III
  - adds DNA nucleotides to elongating strand
- DNA Ligase
  - connects DNA backbones
- RNA Primase
  - adds RNA primer
- Helicase
  - unzips double helix
- Single-Strand Binding Proteins
  - keeps helix separated

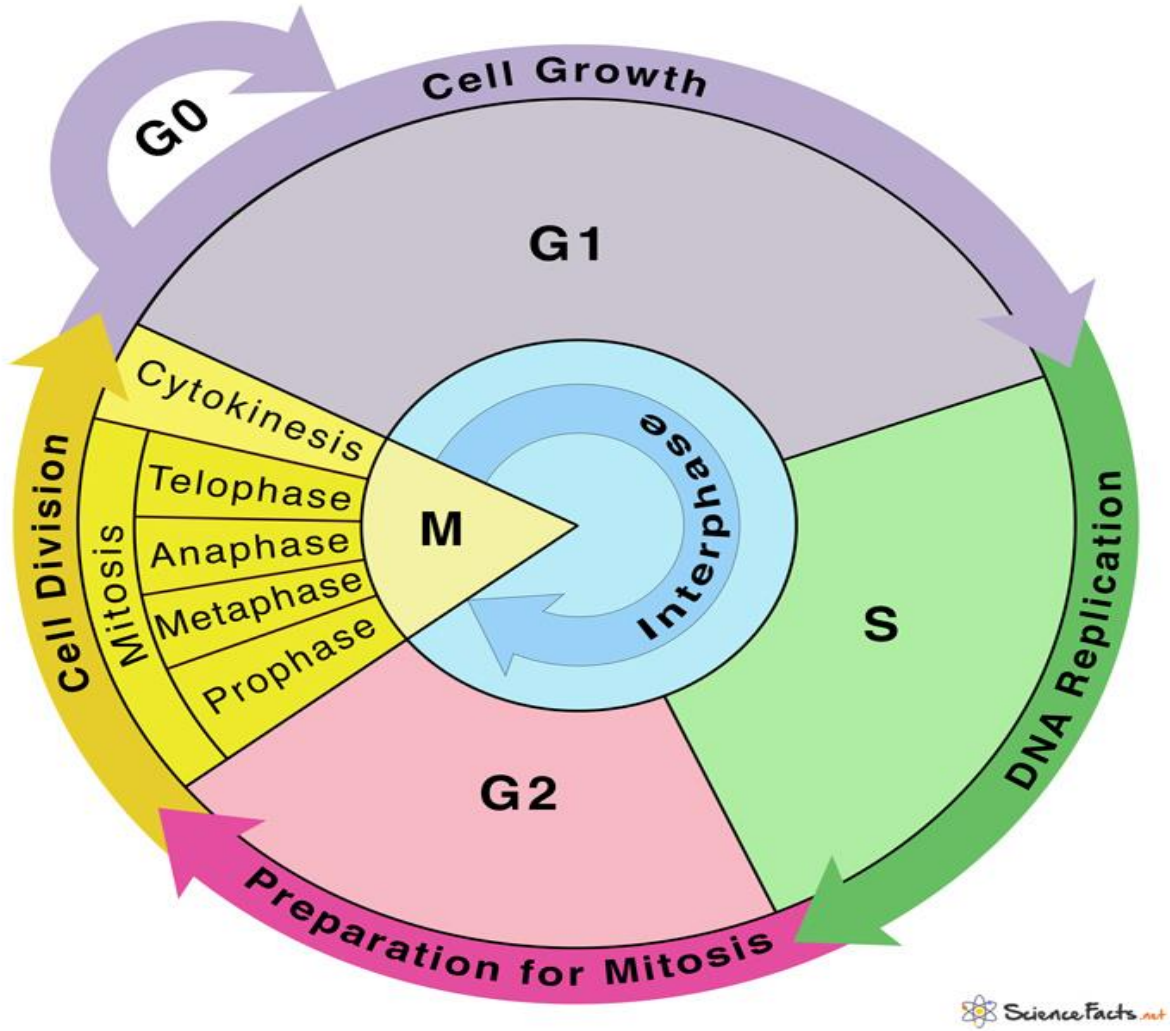

3’
5’
leading strand
parental duplex
5’
3’
RNA primer
Okazaki fragment
fork progression
lagging strand
3’
5’

مبانی بیولوژی سلولی و مولکولی، جلسه دهم

چرخه سلولی(قسمت دوم)

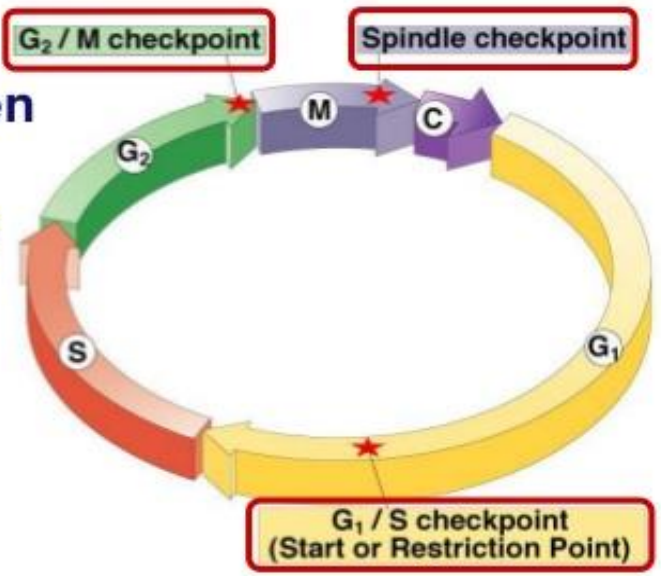
تنظیم چرخه سلولی

Cell Cycle



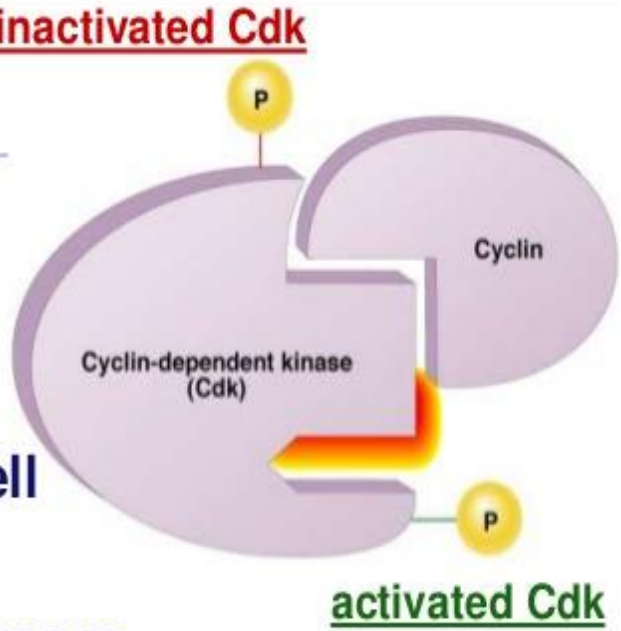
Checkpoint control system

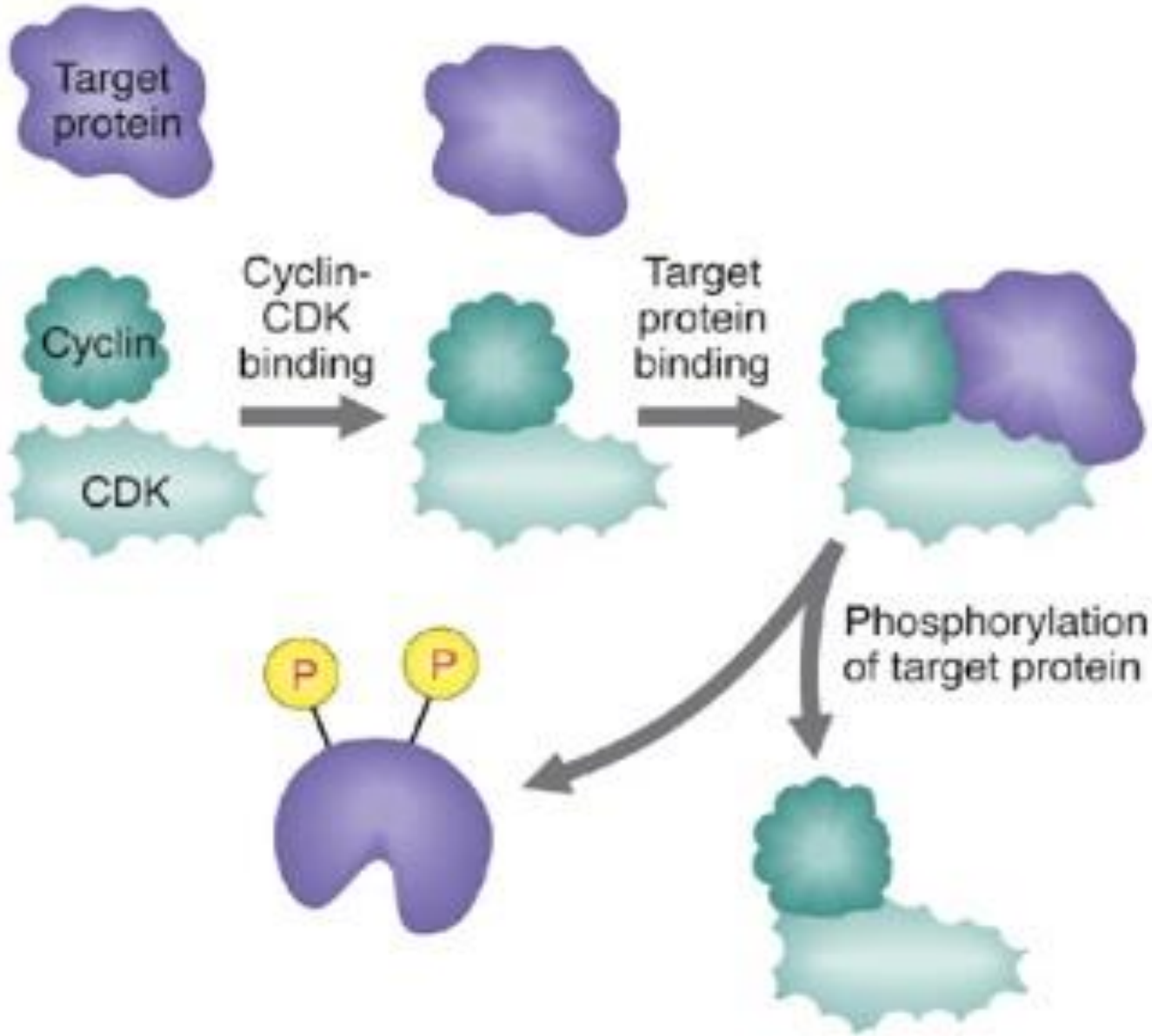
- 3 major checkpoints:
  - G_1/S
    - can DNA synthesis begin?
  - G_2/M
    - has DNA synthesis been completed correctly?
    - commitment to mitosis
  - spindle checkpoint
    - are all chromosomes attached to spindle?
    - can sister chromatids separate correctly?

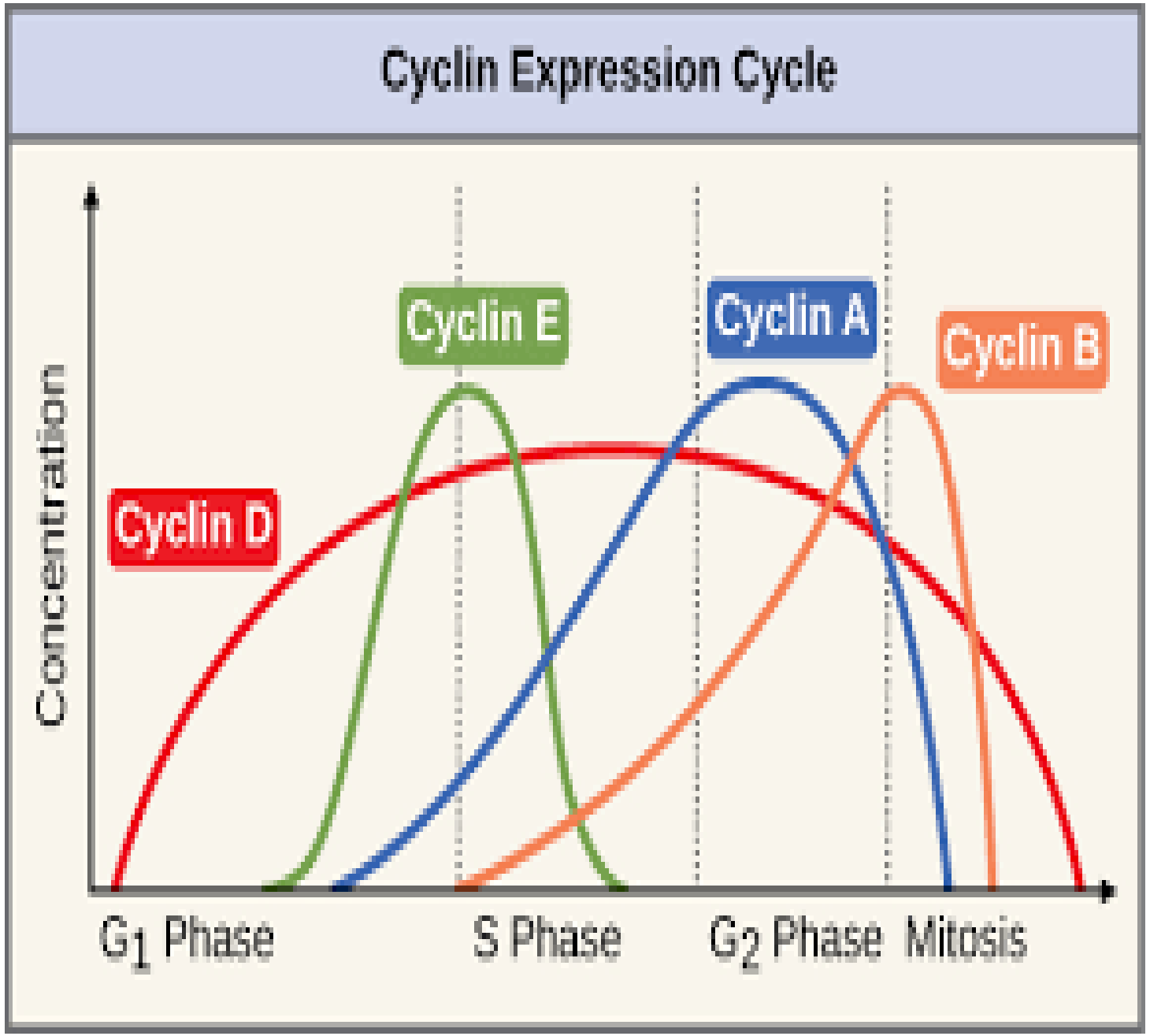


Cell cycle signals

- Cell cycle controls
  - cyclins
    - regulatory proteins
    - levels cycle in the cell
  - Cdks
    - cyclin-dependent kinases
    - phosphorylates cellular proteins
      - activates or inactivates proteins
  - Cdk-cyclin complex
    - triggers passage through different stages of cell cycle




Target protein
Cyclin
CDK
Cyclin-CDK binding
Target protein binding
P
P
Phosphorylation of target protein


Cyclin Expression Cycle
Concentration
Cyclin D
Cyclin E
Cyclin A
Cyclin B
G1 Phase
S Phase
G2 Phase
Mitosis

مبانی بیولوژی سلولی و مولکولی، جلسه یازدهم

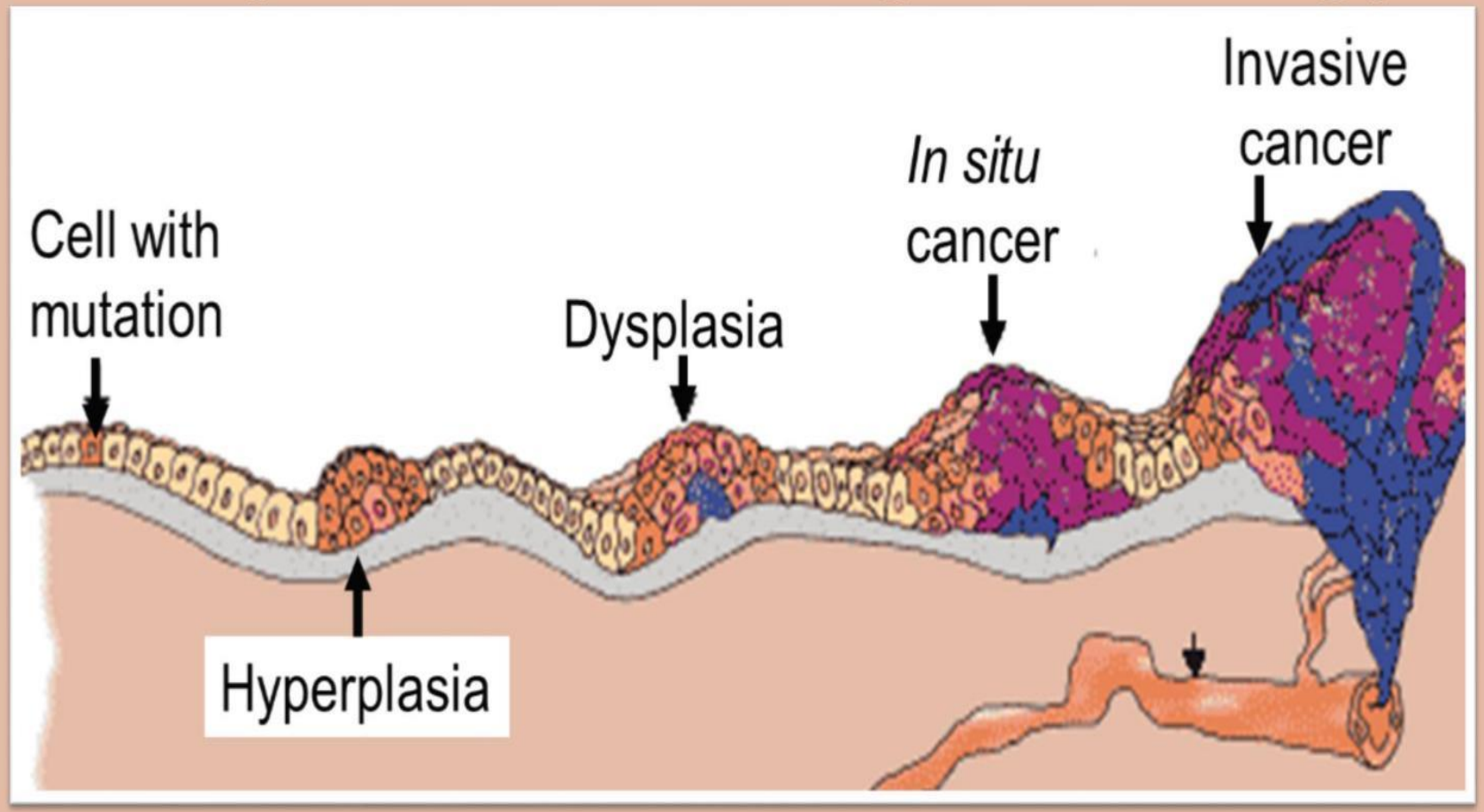
سرطان و مبنای ژنتیکی آن

Definition of Neoplasm

A neoplasm is an abnormal mass of tissue, it's growth exceeds and is uncoordinated with that of the normal tissue and persist in the same excessive manner after cessation of the stimuli which evoke the change.

(Dr. RA Willis)

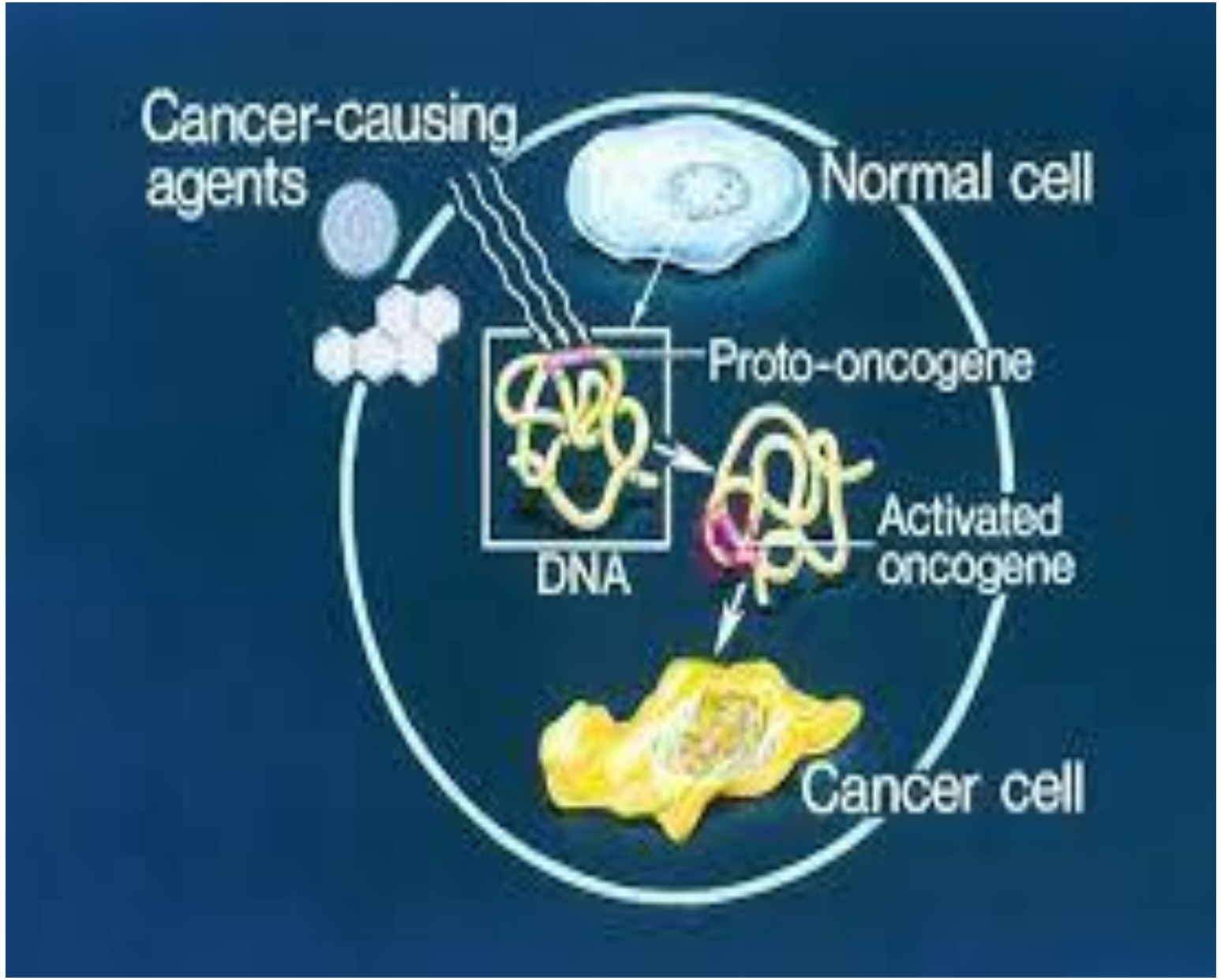
Neoplasia & Oncologic Pathology



Marc Imhotep Cray, M.D.

Definitions

- Oncogene – a gene that when mutated or expressed at abnormally high levels contributes to converting a normal cell into a cancer cell
- Proto-oncogene – the “normal” cellular progenitors of oncogenes that function to promote the normal growth and division of cells


Cancer-causing agents
Normal cell
Proto-oncogene
DNA
Activated oncogene
Cancer cell

مبانی بیولوژی سلولی و مولکولی، جلسه دوازدهم

اهمیت پروتئین ها در بنیان مولکولی بیماری ها

مبانی بیولوژی سلولی و مولکولی، جلسه سیزدهم

روش های متداول در مهندسی ژنتیک(قسمت اول)

Introduction to cloning

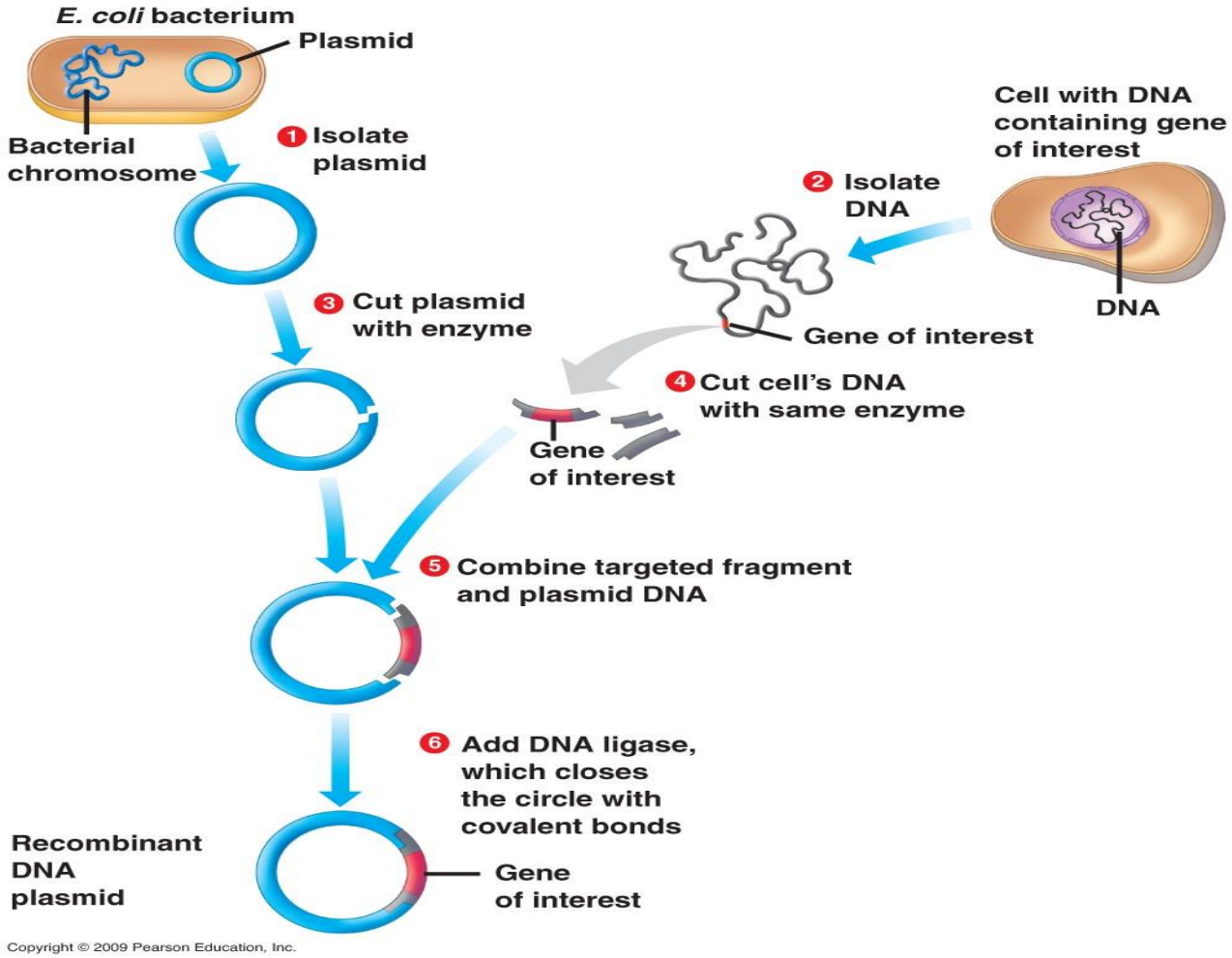
- **There are 3 types of cloning:**
- **Gene cloning** produces copies of genes or segments of DNA.
- **Reproductive cloning** produces copies of whole animals or plants.
- **Therapeutic cloning** produces embryonic stem cells for experiments aimed at creating tissues to replace injured or diseased tissues.

DNA CLONING

- DNA cloning allows a copy of any specific part of a DNA (or RNA) sequence to be selected among many others and produced in an unlimited amount.
- This technique is the first stage of most of the genetic engineering experiments:
  - production of DNA libraries
  - PCR
  - DNA sequencing

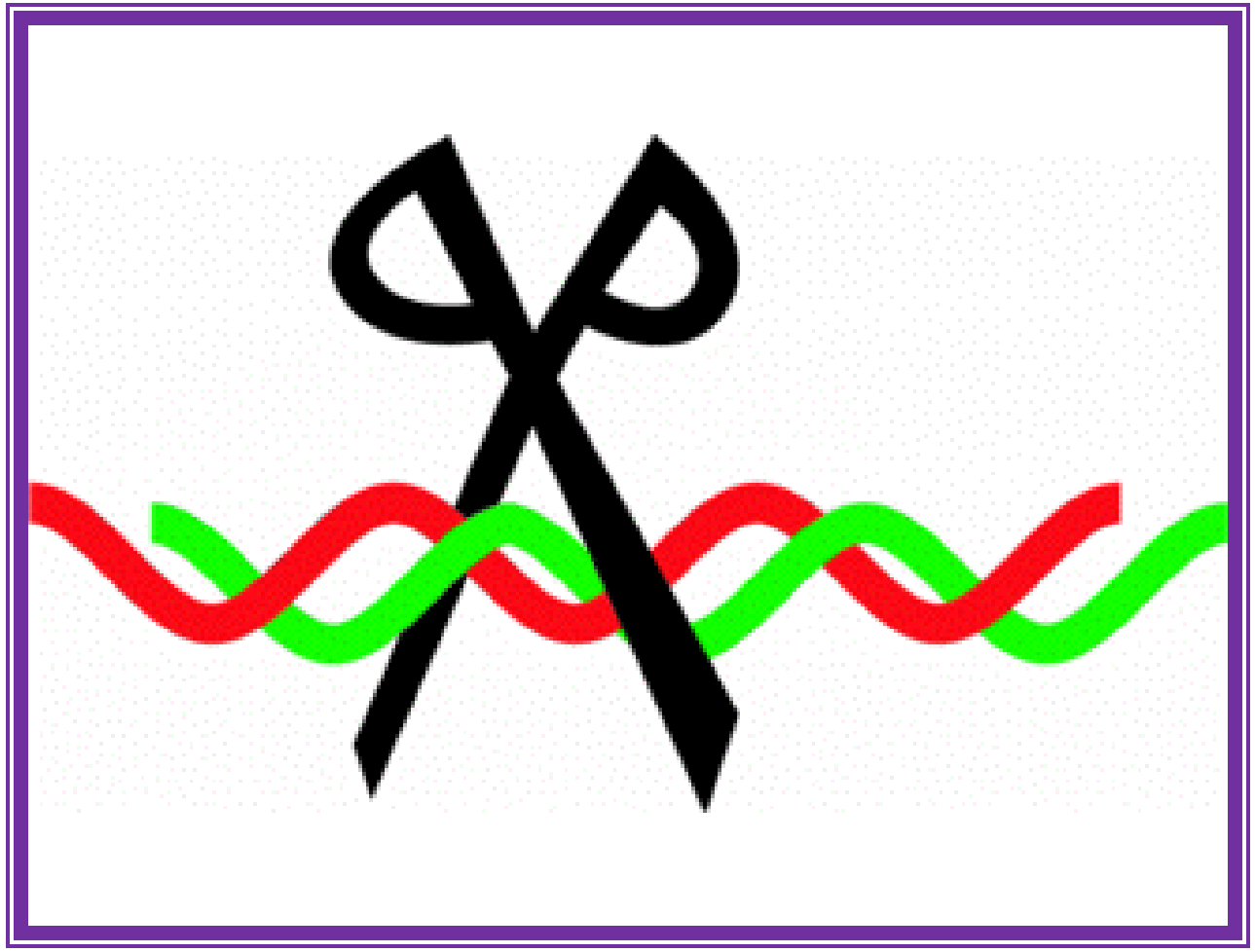
DNA CLONING

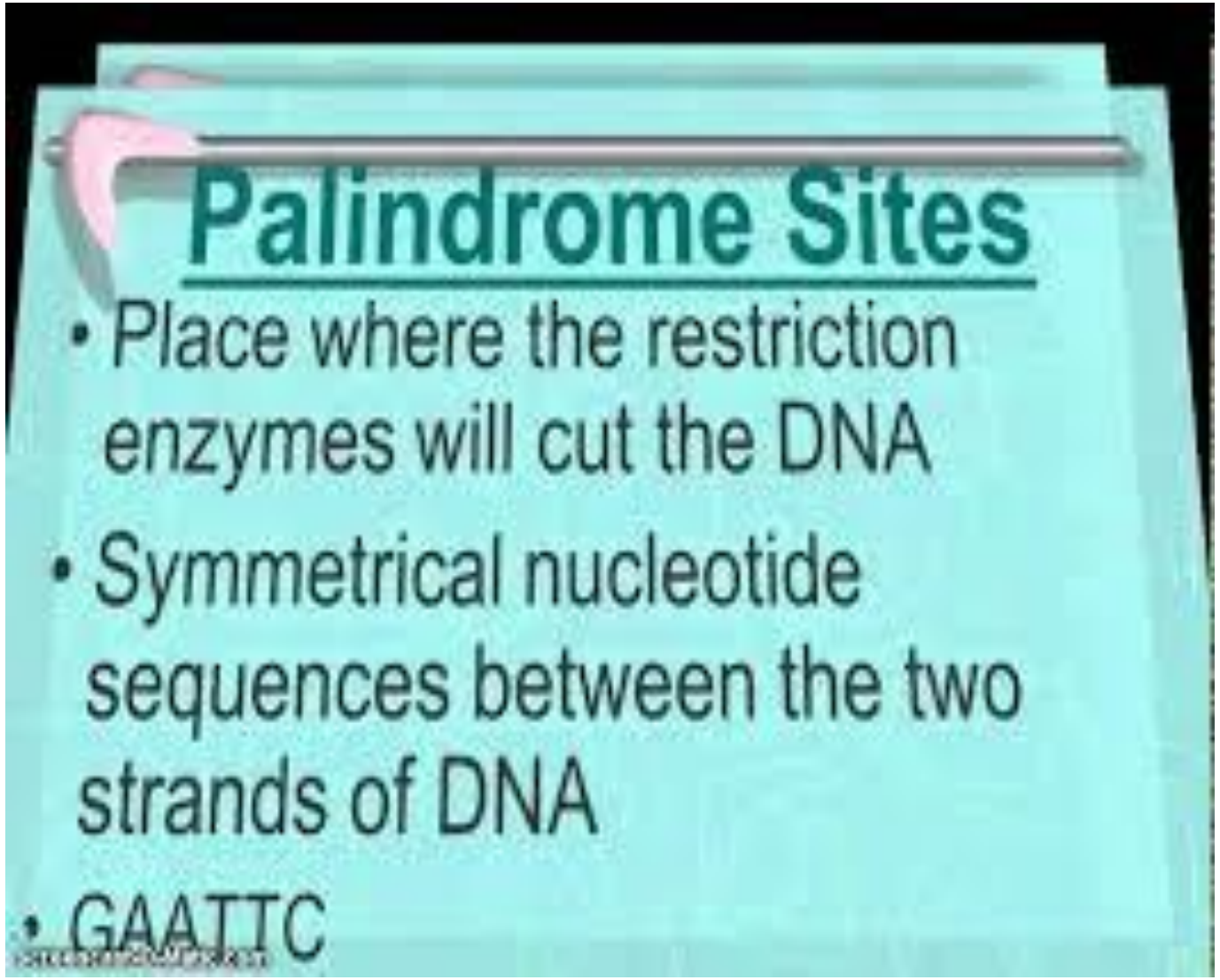
- DNA cloning is a technique for reproducing DNA fragments.
- It can be achieved by two different approaches:
  - cell based
  - using polymerase chain reaction (PCR).
- a vector is required to carry the DNA fragment of interest into the host cell.

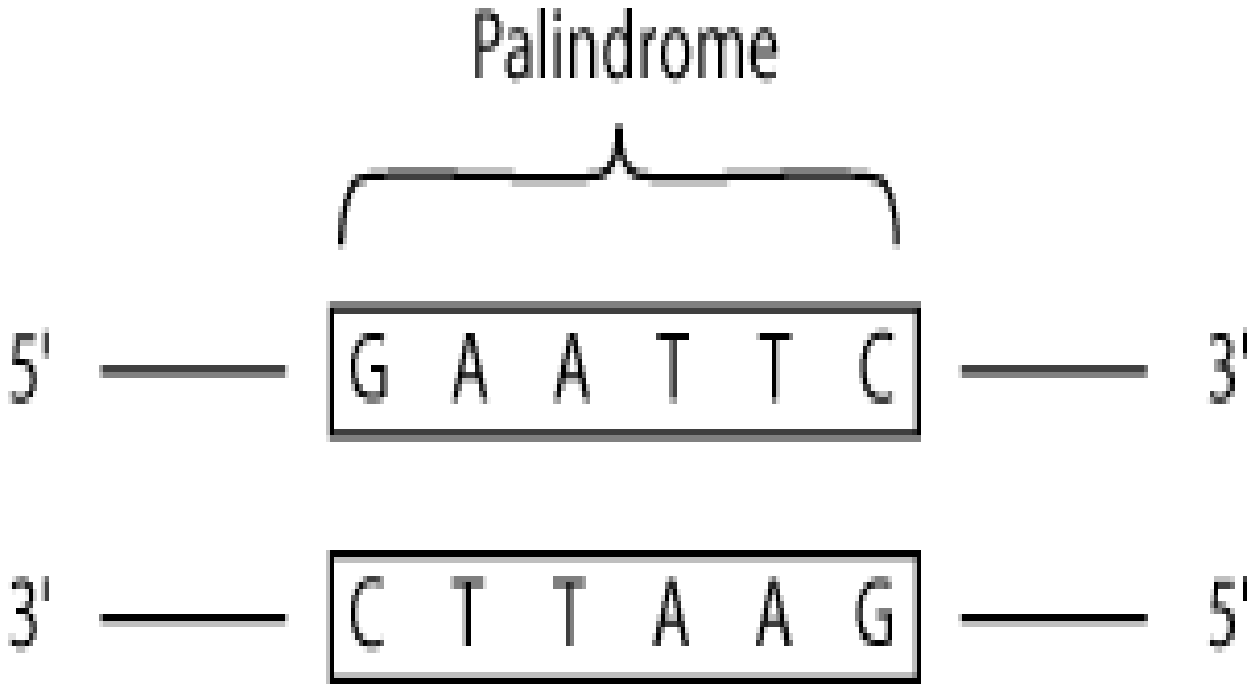


TERMS USED IN CLONING

- **DNA recombination.**
  The DNA fragment to be cloned is inserted into a vector.
- **Transformation.**
  The recombinant DNA enters into the host cell and proliferates.
- **Selective amplification.**
  A specific antibiotic is added to kill *E. coli* without any protection. The transformed *E. coli* is protected by the antibiotic-resistance gene
- **Isolation of desired DNA clones**




Palindrome Sites
• Place where the restriction enzymes will cut the DNA
• Symmetrical nucleotide sequences between the two strands of DNA
• GAATTC

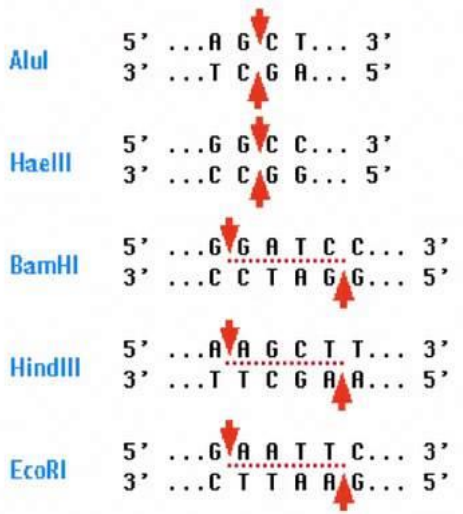


PALINDROME SEQUENCES

- The **mirror like palindrome** in which the same forward and backwards are on a single strand of DNA strand, as in GTAATG
- The **Inverted repeat palindromes** is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (GTATAC being complementary to CATATG)
- **Inverted repeat palindromes** are more common and have greater biological importance than mirror- like palindromes.

Restriction Enzymes

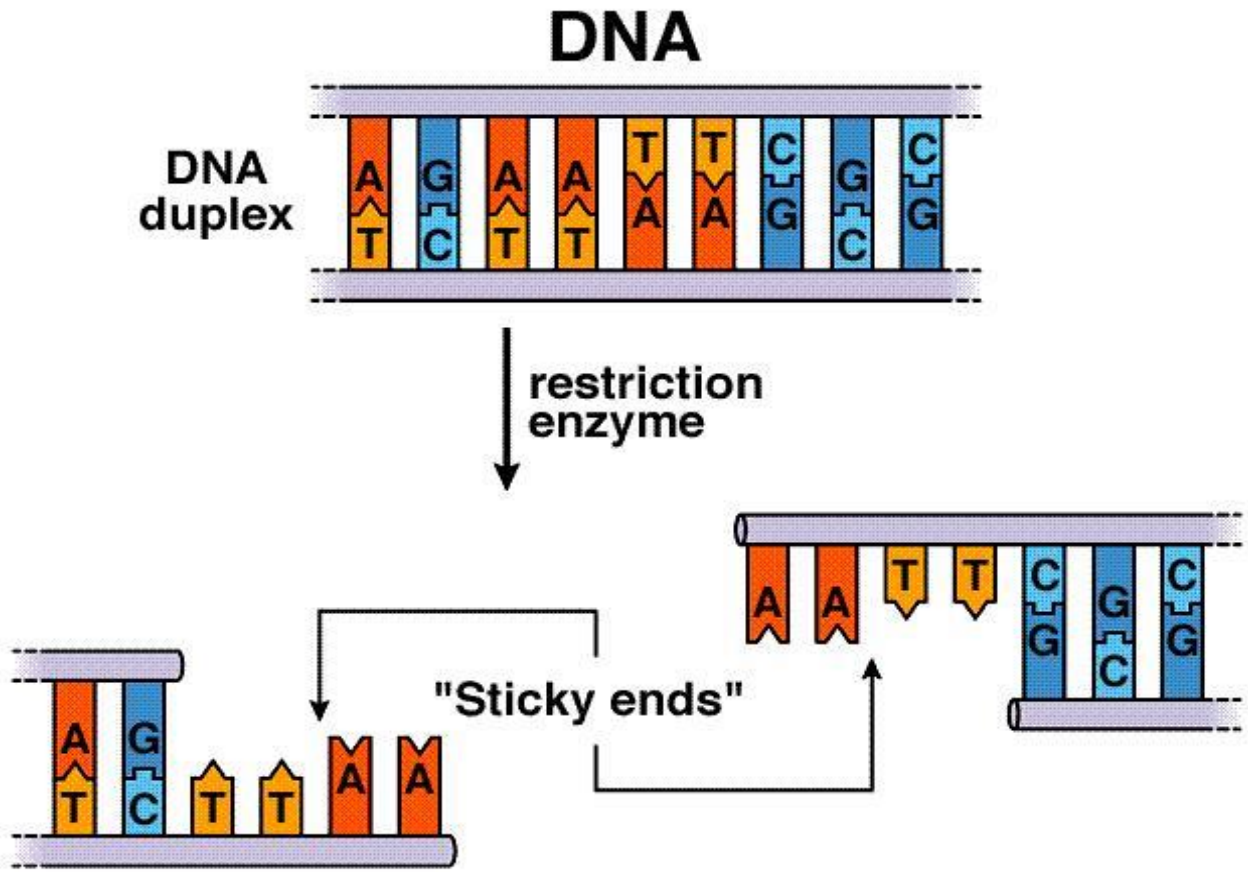
- Restriction endonucleases
  - Digest dsDNA at specific sites
  - Recognition site is palindrome
    - Word example: "Madam I'm Adam"
    - Reads the same on both strands (5' to 3')
  - Recognition sites are different sizes and unique sites
  - Cuts between same nucleotides on each strand



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RestrictionEnzymes.html



Restriction Endonuclease Types

Type I- multi-subunit, both endonuclease and methylase activities, cleave at random up to 1000 bp from recognition sequence

Type II- most single subunit, cleave DNA within recognition sequence

Type III- multi-subunit, endonuclease and methylase about 25 bp from recognition sequence

مبانی بیولوژی سلولی و مولکولی، جلسه چهاردهم

روش های متداول در مهندسی ژنتیک(قسمت دوم)

What is PCR?

PCR is DNA Amplification in vitro

It has the same idea of DNA replication but in a test tube (2 DNA strands seperation, primers anealing and elongation by the DNA polymerase)

9.2 Copying DNA

The **polymerase chain reaction (PCR)** rapidly copies segments of DNA.

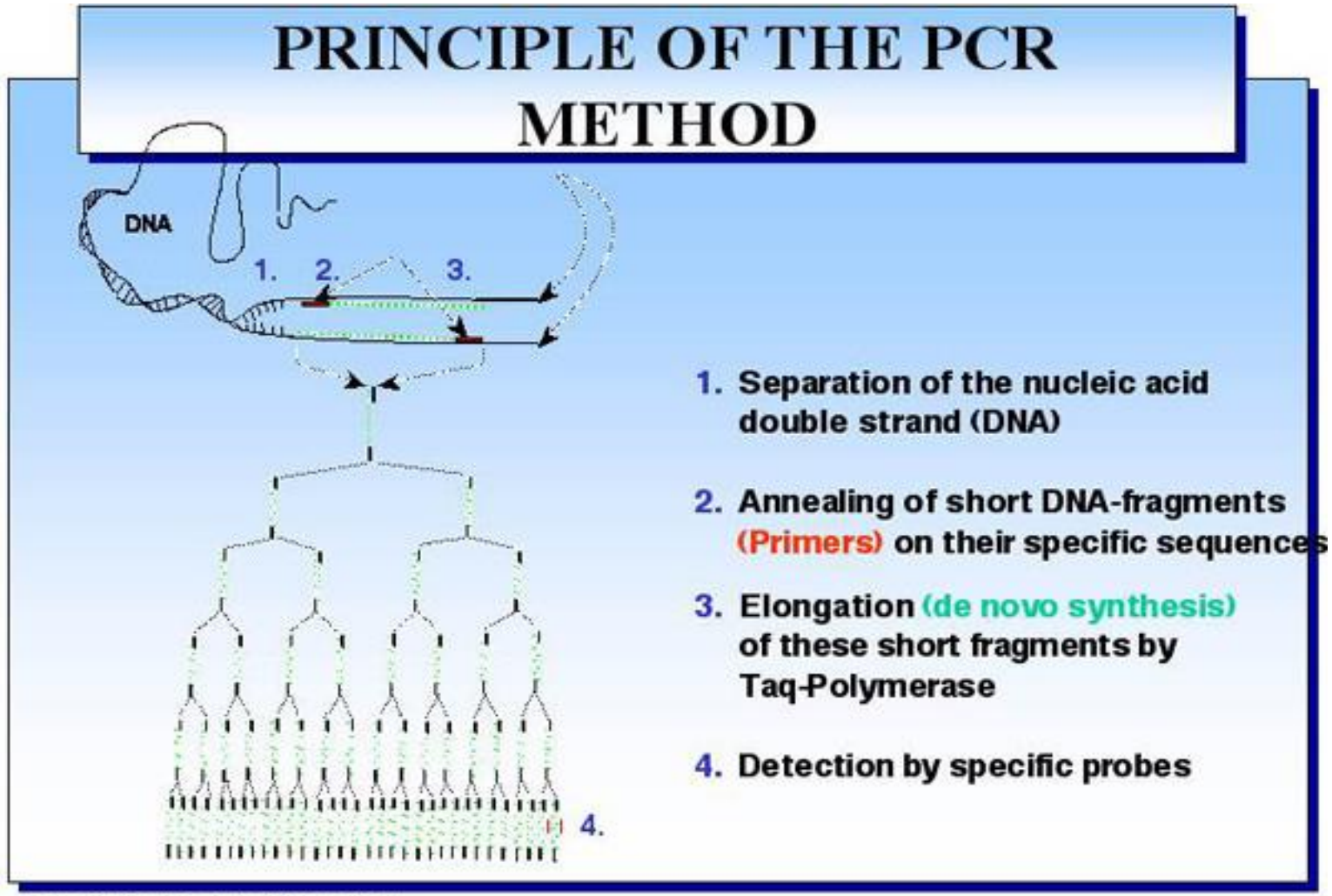
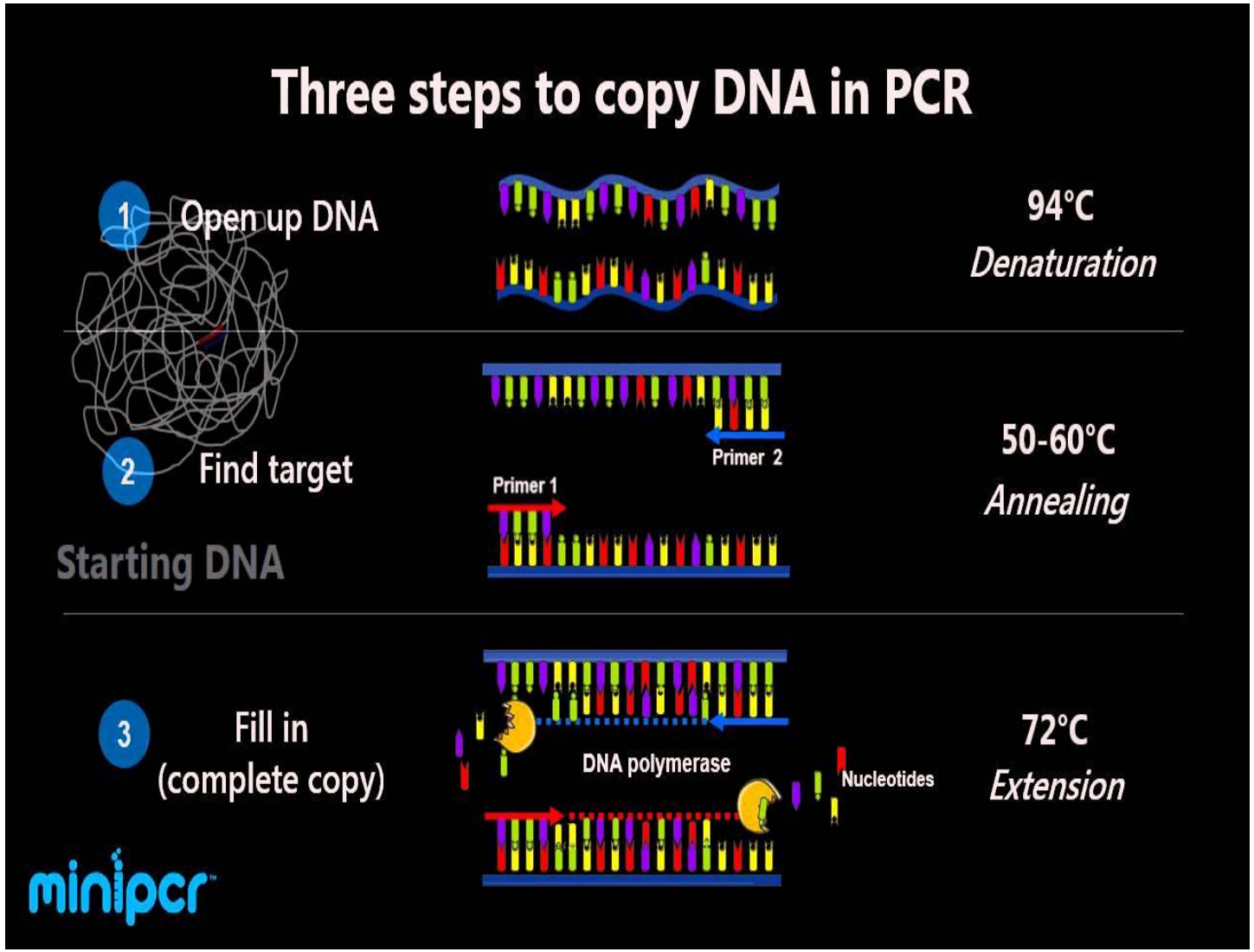
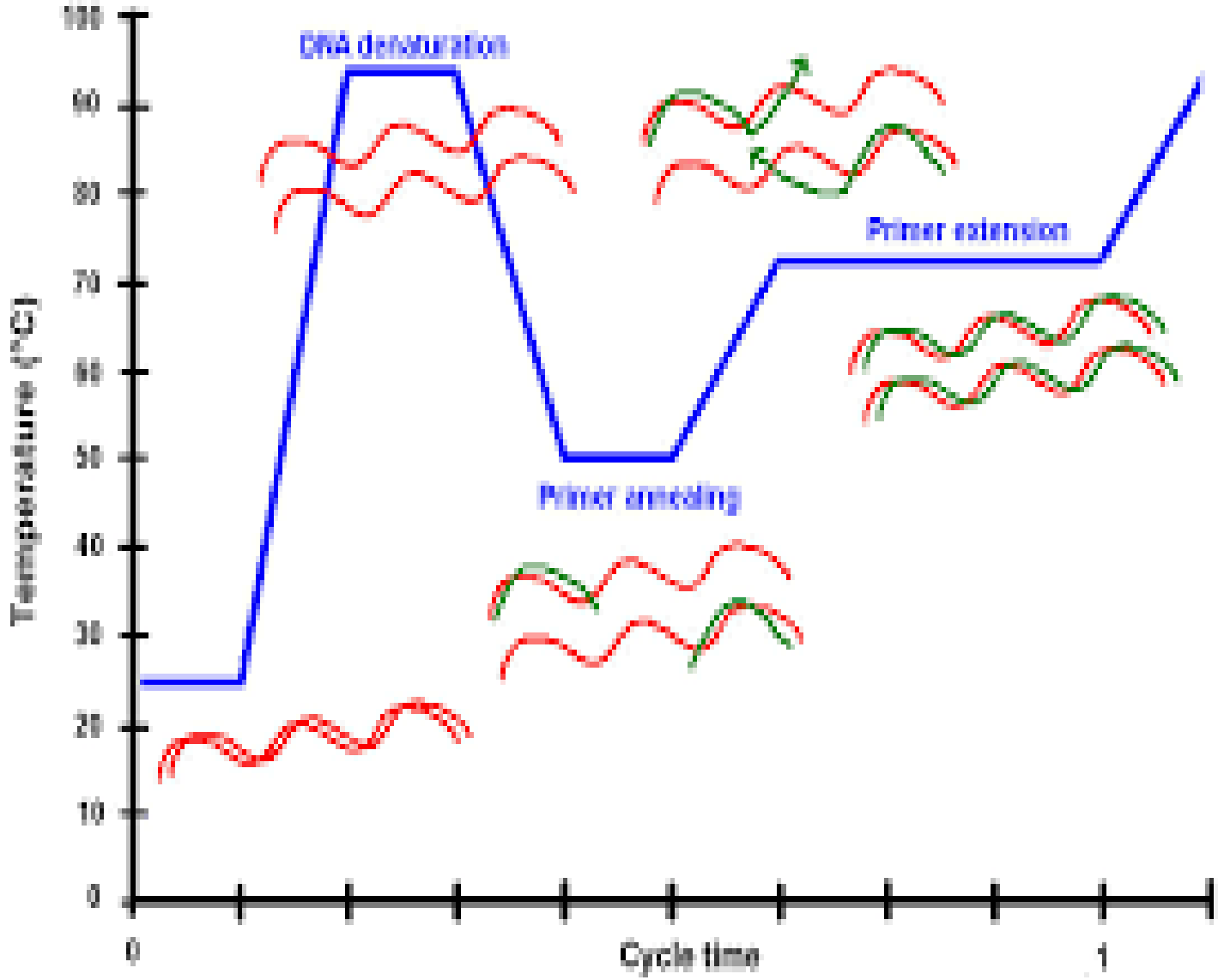


Figure 1. Principle of the PCR method.

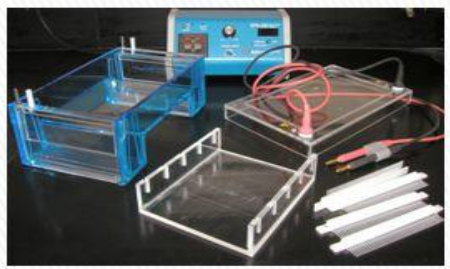

Three steps to copy DNA in PCR
1
Open up DNA
94°C
Denaturation
2
Find target
Starting DNA
Primer 2
Primer 1
50-60°C
Annealing
3
Fill in
(complete copy)
DNA polymerase
Nucleotides
72°C
Extension
minipcr


DNA denaturation
Primer annealing
Primer extension
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
0
Cycle time
1

PCR

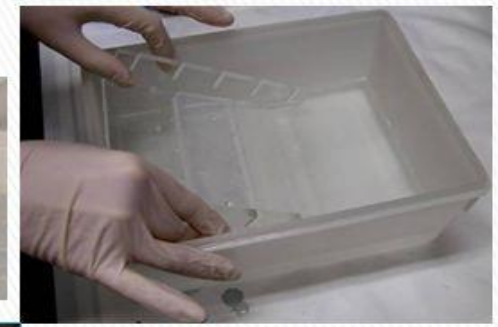
- **PCR instruments includes:**
  - Thermocycler PCR
  - Agarose gel electrophoresis
- **PCR process requires four components:**

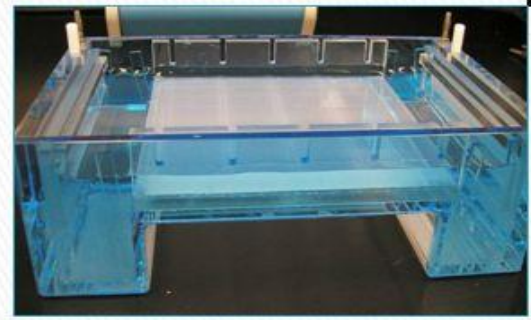
1. **Two primers:** each consisting of 15-20 bases of DNA, containing sequences complementary to the 3' end of target region of DNA that contains the polymorphism or a mutation that causes disease.
2. **Heat- stable DNA polymerase enzyme:** originally isolated from the bacterium *Thermus aquaticus* with a temperature optimum at round 70 C.
3. A large number of **free DNA nucleotides (dNTPs).**
4. Small quantity of **Genomic DNA from an individual** act as a template.

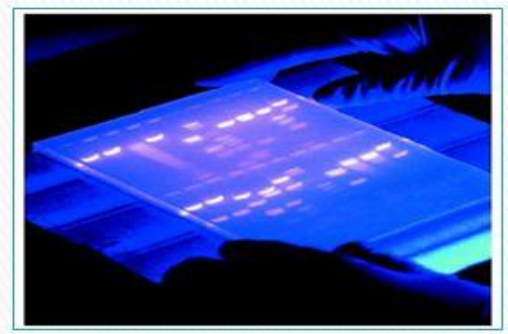


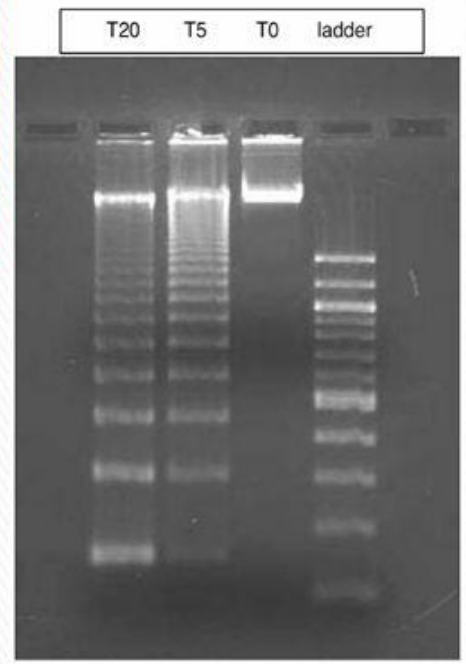
PCR product

- Finally **agarose gel electrophoresis** is employed for size separation of the PCR products. The size(s) of PCR products is determined by comparison with a DNA ladder (a molecular weight marker) which contains fragments of known size, run on a gel alongside the PCR products.

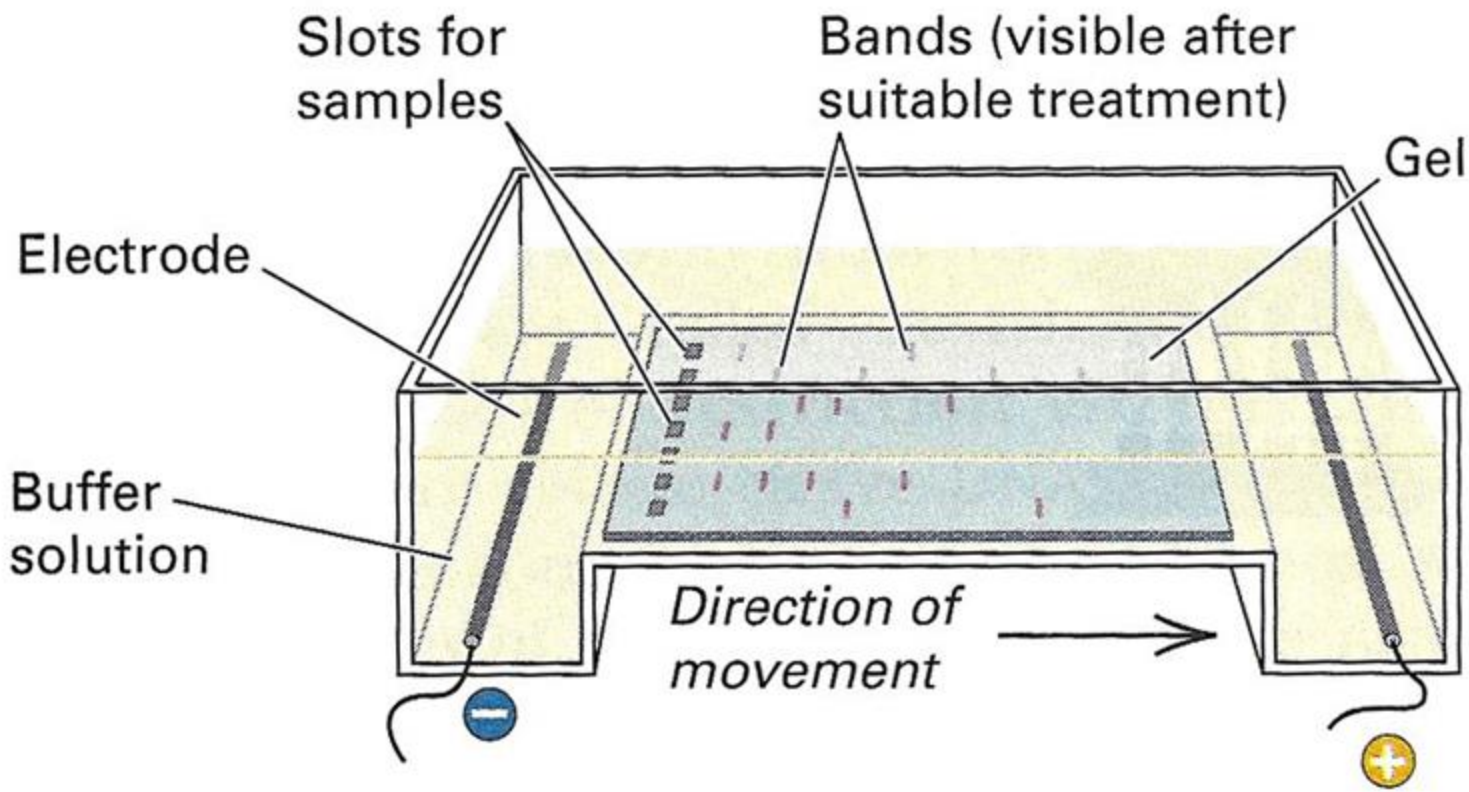




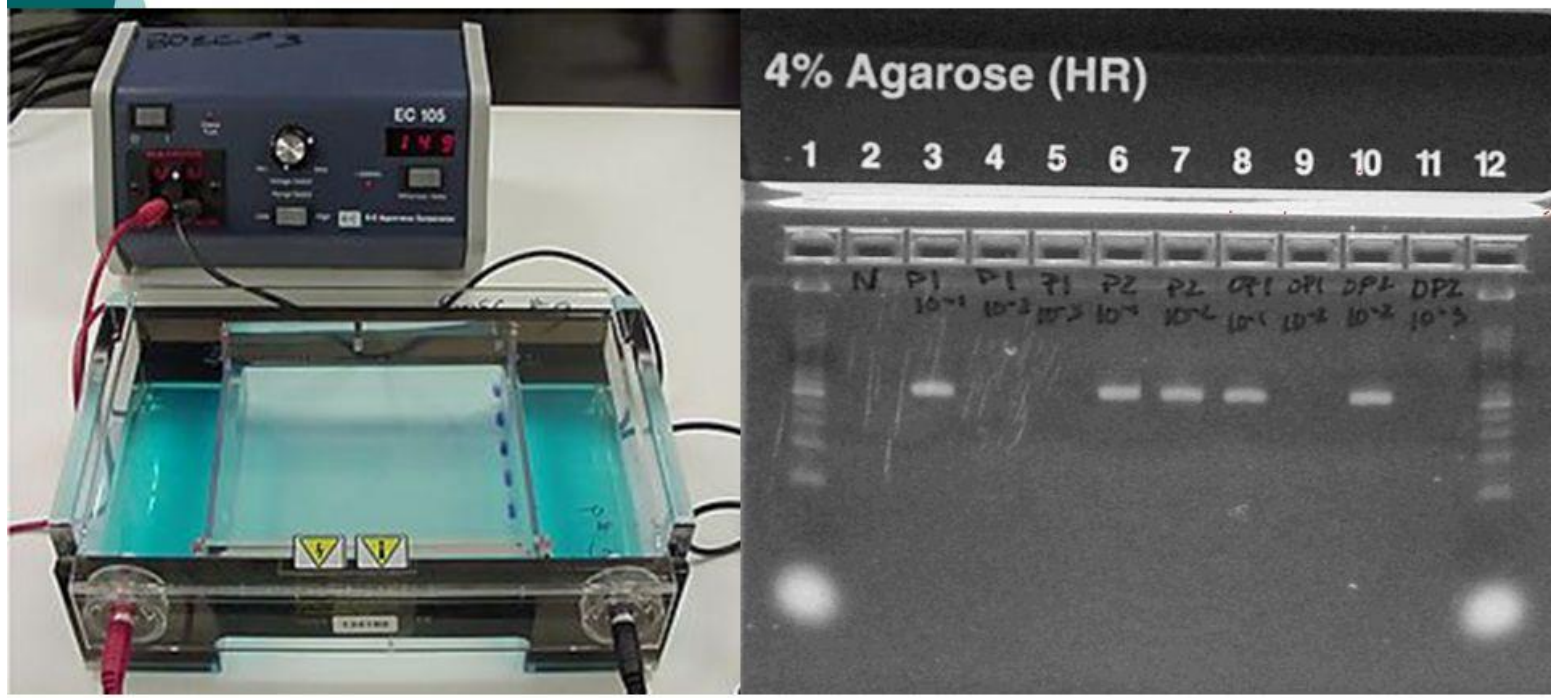




Agarose gel electrophoresis of DNA



Instruments for Viewing PCR Results



•Gel Electrophoresis and Camera image of agarose gel