Genetics

Subject : Genetics

Lec no : 22

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وقل رب زدني علما

Molecular Genetics of Cancer Cells

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Week 9	• Molecular genetics of cancer cells (part II) • Molecular genetics of cancer cells (part III) • Genetic diseases (part I)
Week 10	• Genetic diseases (part II) • Mitochondrial disorders • Monogenic, multigenic disorders & Genetic disease penetrance
Week 11	• Recombinant DNA Technology • Polymerase chain reaction • Hybridization and blotting techniques
Week 12	• DNA sequencing • Gene therapy

من المحاضرة السابقة:
proteins the regulates the cell cycle ← cyclins
by combination with CDK

Introduction

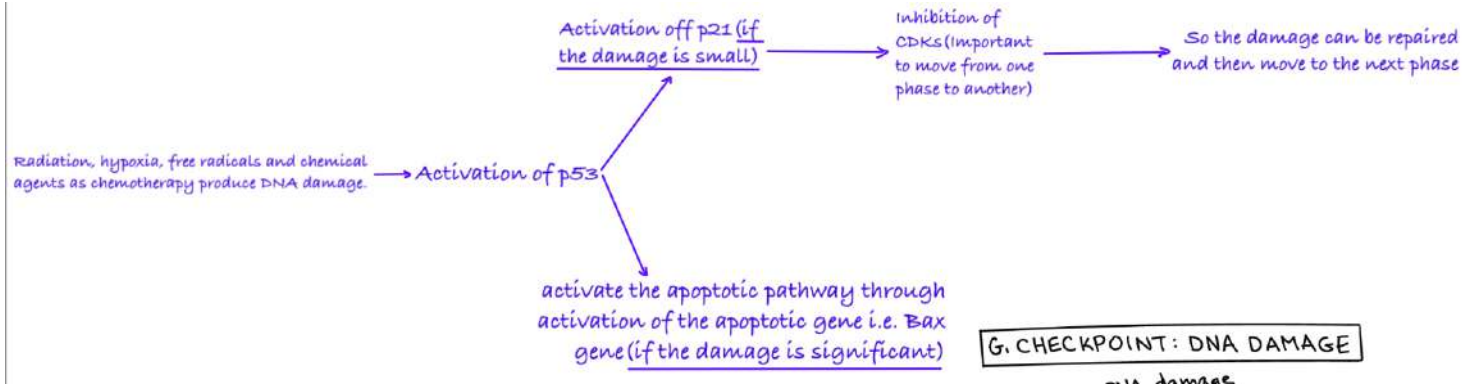
ولكن في cancer cells ← cyclins (regulators) يكون فيهو مشاكل (على الأغلب بسبب أن الجينات التي تشفرها (Alterations) بيصير فيها.
بالتالي cell cycle بتستمر لعدم وجود regulators للتأكد من DNA damage وأصلحه - - - .

Alteration of genes ← بيبدأ من ← cancer ←

- Cancer cells are cells that lose their ability to divide in a controlled way
  - Normal cell division is controlled by genes
  - Cancers are caused by **damage to these genes**
    - Damage usually happens during lifetime
    - Or can occasionally be *inherited*

- Cancer is a disease that starts in the genes.

Introduction



GFs---In normal cells, it is responsible for initiation of cell cycle

- Cancer cells can multiply in ***absence of growth factors*** which are normally needed for normal cells to multiply
  - and are **resistant to signals** that normally *inhibit cell cycle* or *induce apoptosis*

↳ The cell cycle of cancer cells can't be regulated by any signals (لا يمكن إيقافها)

↳ by P21

↳ by 53

- For cancer to grow bigger than pin head, it must grow its own blood vessels (angiogenesis)

- Sometimes, cells move away from original (primary site) and spread to other local/ regional sites due to secretion of proteases → metastasis

Introduction

- In normal cells, both **Bax** and **Bcl-2** are present in balance **(the ratio of apoptosis inhibitors to apoptosis stimulators makes death decision)**.

Induction of apoptosis ⟶ ↑Bax
Inhibition of apoptosis ⟶ ↑Bcl-2

29

Normally
There is a balance between inducers of cell cycle and inhibitors of cell cycle

في cancer cells لا يوجد هذا التوازن
inhibitors أعلى من inducers

Imbalance of cell growth: abnormal cell homeostasis (cell gain vs cell loss via apoptosis)

cell gain أعلى من cell loss

مثلاً ما هكينا الانسان في cancer هو ← Alteration of genes الموروثة من انتج regulators

The genes commonly altered in cancer are either:

1. Proto-oncogenes and oncogenes → In this lecture

2. Tumour suppressor genes (P53) → In next lecture

Proto-oncogenes & Oncogenes

cell division /growth مسؤولين عن

❖**Proto-oncogenes** are genes that are **present in normal cells** and their products play important role in normal cell differentiation (normal cell growth).

- They include *growth factors, growth factor receptors, signal transduction proteins, transcription factors.*

ex: E2F

ex: tyrosin Kinase

activation (phosphorylation) بيعمل of cascades

بيروح على الخلية وبيحفز الجينات المسؤولة عن انتاج cyclins المهمة في بدء cell cycle

- There are normally about 100 types of proto-oncogenes in each human cell which participate in the regulation of normal cell growth.

❖**Oncogenes** are altered proto-oncogenes that encode proteins capable of causing cancer.

هي جينات تنتج بروتينات تسبب Cancer

Extreme cell division, (extreme silly growth)

Mechanisms for conversion of proto-oncogenes to oncogenes

proto-oncogene تحول
إلى oncogene يكون عن
mutation of proto-oncogene طريق

1. Point mutation in proto-oncogene

Normal protein, that is just over express overexpressed
(basically too much of normal protein) → طبيعي cell growth بشكل كبير

2. Gene amplification & overexpression of its' protein products

Alteration (mutation) عبارة عن هو طبيعي غير شيء كلمة هذا

3. Chromosomal translocation

4. Insert mutagenesis (viral carcinogenesis)

I. Point mutation in proto-oncogene

single base تغير في ← يختلف codon ← يعطي مختلف a.a ← يعطي ← Altered protein تتغير

Point mutations

- This produces **changes in the protein product of the gene.**

- The **Ras oncogene** represents the best example of activation by point mutations.

G-proteins هو تابع لعائلة

- Ras protein, the product of the Ras proto-oncogene, is a class of G protein that is found in all nucleated cells.

G protein (trimeric protein) عبارة عن ← Ras protein
يتكون من 3 subunits ← α, β, γ

- It has an **intrinsic GTPase activity.**

hydrolysis of GTP
has GTPase activity
GTP → GDP + P
active form

RAS Proto-oncogene —Mutation→ RAS Oncogene

RAS Proto-oncogene ↓ Normal RAS protein (GTP to GDP+p)

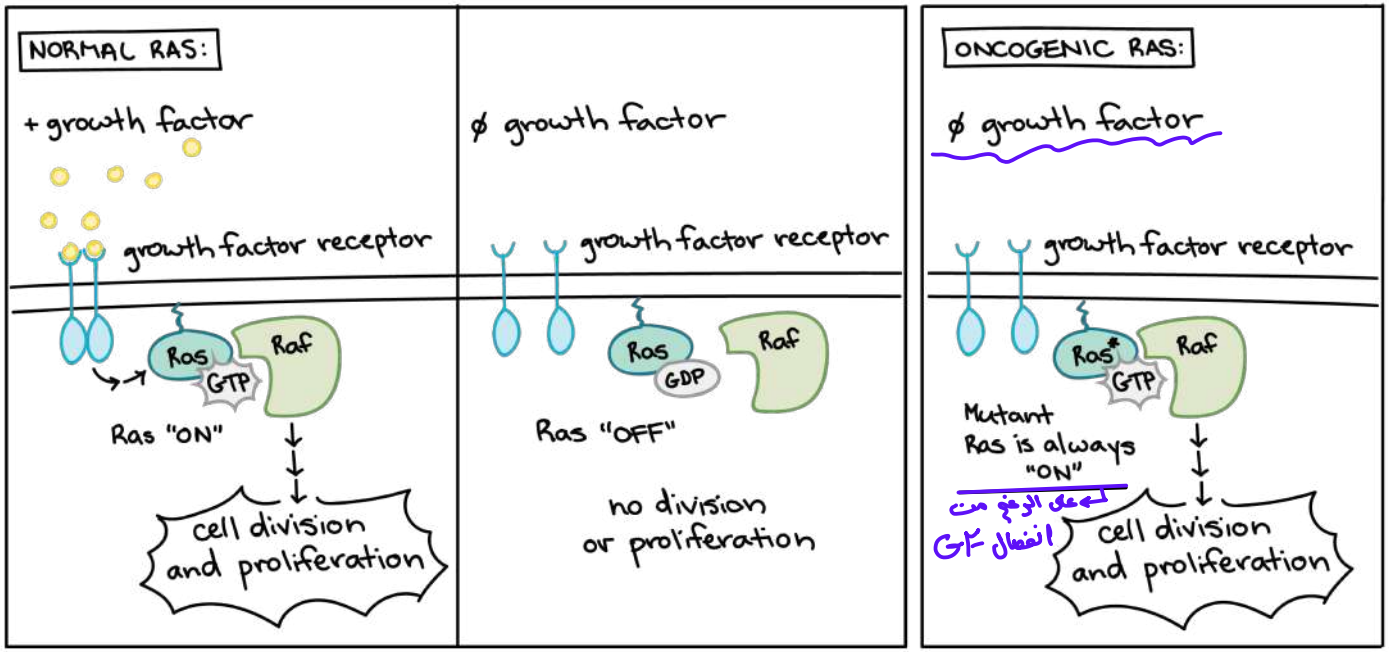
RAS Oncogene ↓ Mutated RAS protein (Decrease GTPase activity) → doesn't have a strong GTPase activity as the normal RAS protein (it has very low efficacy for GTPase) accumulation of GTP → uncontrolled growth

↓

GTP will increase in the cell

RAS protooncogene → RAS protein (مرتبط بـ GTP ⇐ on ، مرتبط بـ GDP ⇐ off) ← يكون مرتبط بشكل طبيعي بـ (guanin GTP) وعندما تكون مرتبطة بـ GTP تكون on (تنقل signal من خارج الخلية إلى داخلها) ولكنها لا تبقى هكذا دائماً بسبب امتلاكها GTPase activity تقوم بتحويل GTP (active form) → GDP (inactive form) (بعد إنهاء عملها مثلاً: عندما ينفصل receptor عن GF) → off رح تصير

RAS protooncogene —mutation→ RAS oncogene (with reduced GTPase activity) → Mutated RAS protein ← ما رح يقدر يحول GTP → GDP عند إنها العمل (عند انفصال GF عن receptor) بالتالي رح يستمر بنقل signal وكأنه GF رابط بـ receptor حتى بعد ما انفصل. → رح يظل on



Ras is a G protein, meaning that it switches back and forth between an inactive form (bound to the small molecule GDP) and an active form (bound to the similar molecule GTP). Cancer-causing mutations often change Ras's structure so that it can no longer switch to its inactive form, or can do so only very slowly, leaving the protein stuck in the "on" state (see cartoon above)

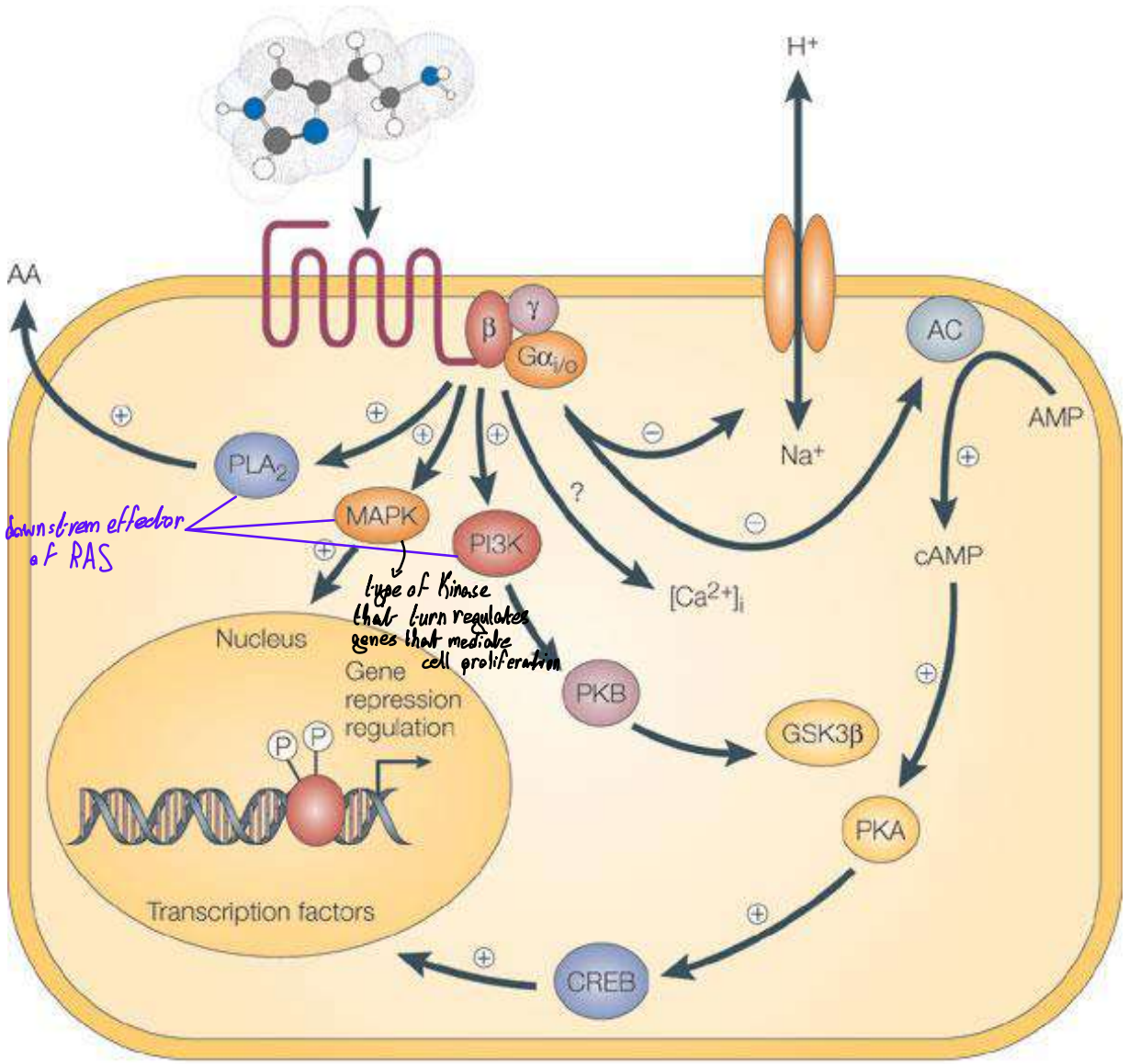
I. Point mutation in proto-oncogene

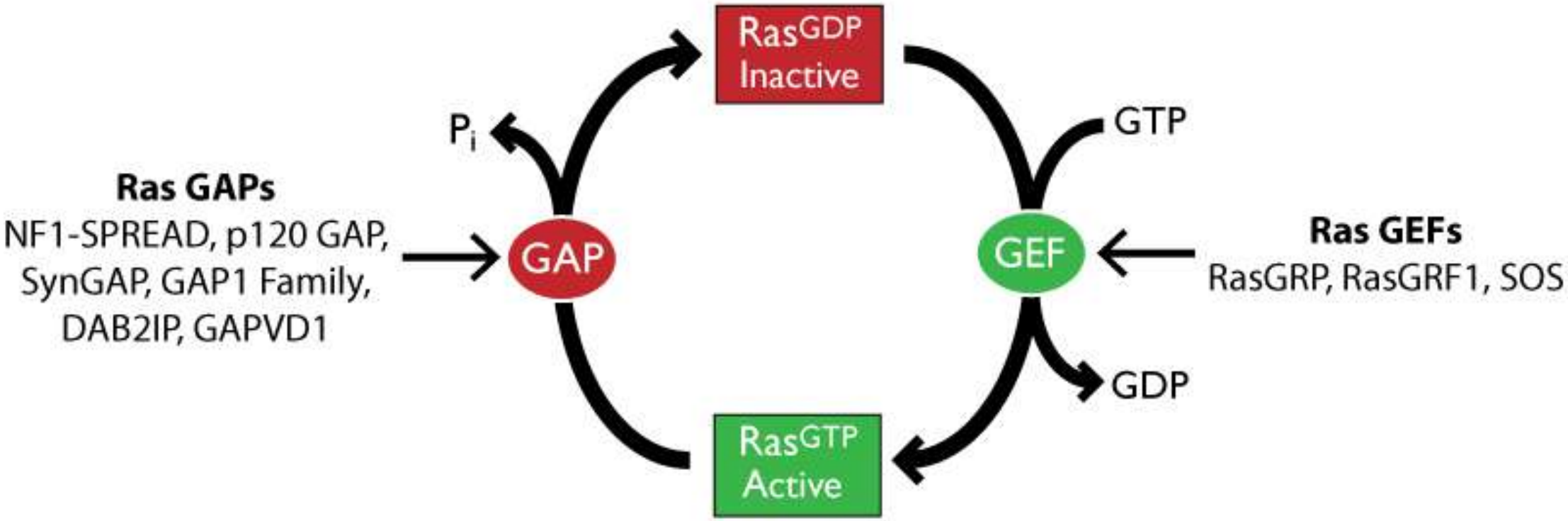
- G proteins also known as ***guanine nucleotide binding proteins***.

  A family of proteins that act as molecular switches inside cells and are involved in transmitting signals from a variety of stimuli outside a cell to its interior.

- When they are bound to GTP, they are 'on' → turn on genes involved in cell growth/ replication, differentiation, survival

- When they are bound to GDP, they are 'off' → inhibit above functions


H+
AA
β
γ
Gαi/o
AC
AMP
PLA2
MAPK
PI3K
?
Na+
[Ca2+]i
cAMP
downstream effector of RAS
type of Kinase that turn regulates genes that mediate cell proliferation
Nucleus
Gene repression regulation
P
P
PKB
GSK3β
PKA
Transcription factors
CREB


Ras GAPs
NF1-SPREAD, p120 GAP,
SynGAP, GAP1 Family,
DAB2IP, GAPVD1
GAP
P_i
RasGDP
Inactive
GTP
GEF
Ras GEFs
RasGRP, RasGRF1, SOS
GDP
RasGTP
Active

I. Point mutation in proto-oncogene

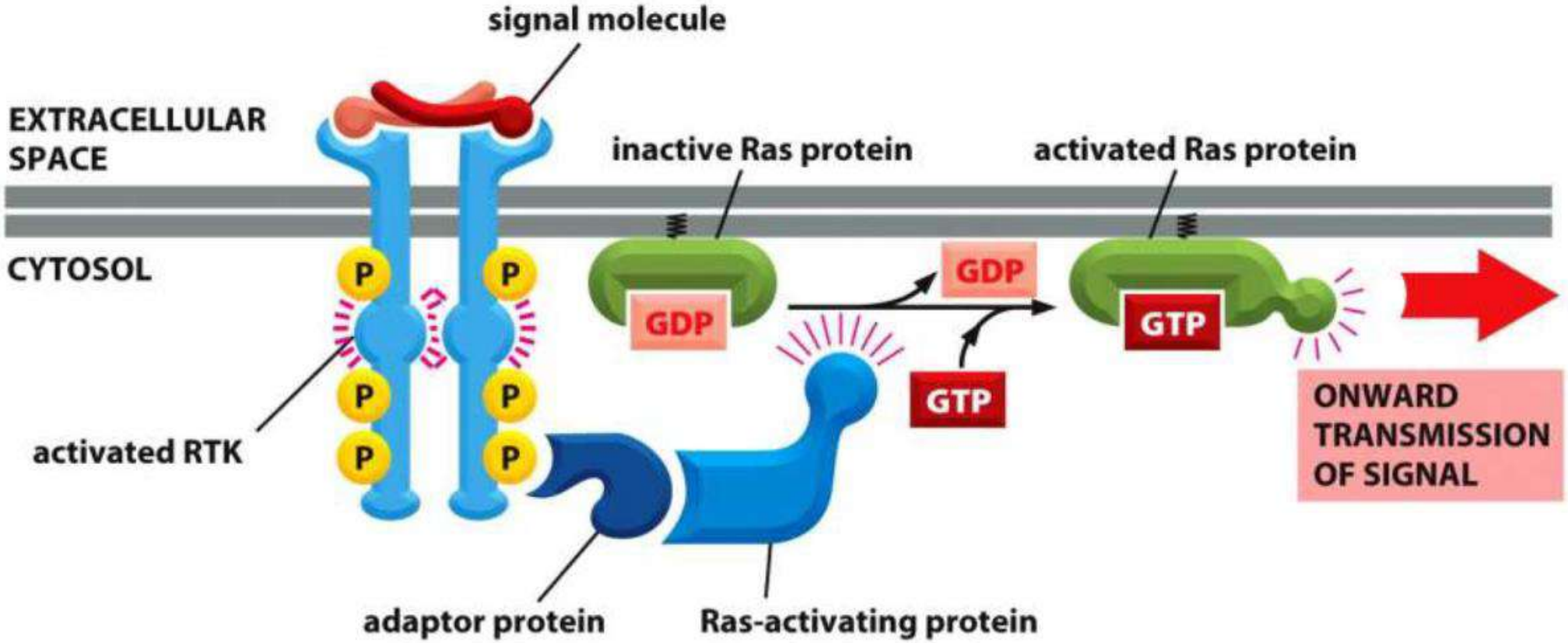
RAS proto-oncogene → mutation

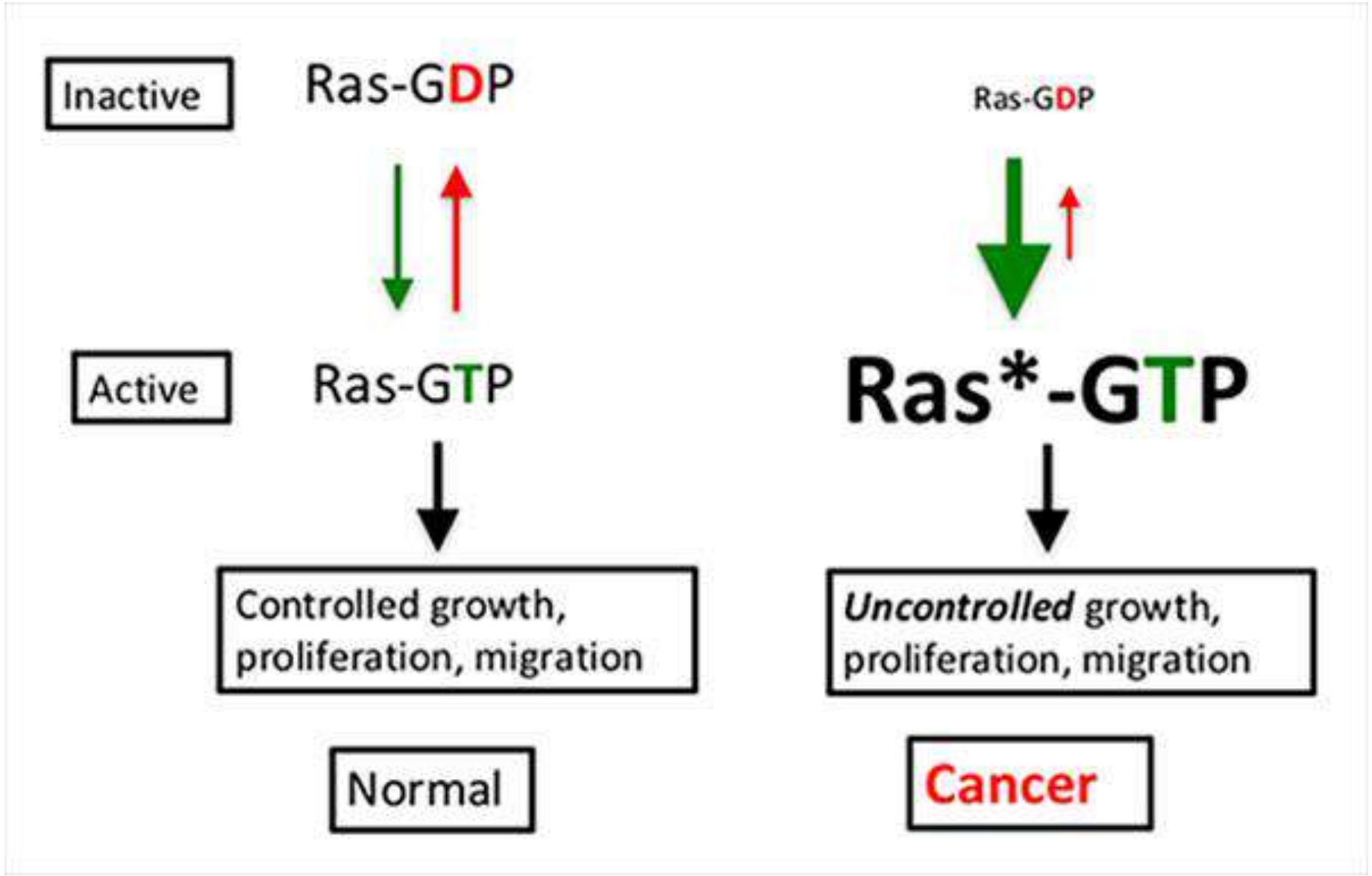
- **Point mutation converts this gene into Ras oncogene** with **reduced GTPase activity.**

- Thus, the effect of **growth factors acting through G protein continues after** the **growth factor dissociates** from the receptor.
  - This mutation is observed in about 15% of cancers.

mutation صايرلها RAS protooncogene بتلاقي colon cancer or bladder cancer * منها نفحص شخص عنده

- In summary: mutated Ras **remains in the active form** (Ras GTP complex) as this point mutation dramatically **reduces the GTPase activity** of the Ras proteins.
  - So mutated Ras acts as a growth promoting signal even in the absence of growth factors.


signal molecule
EXTRACELLULAR SPACE
CYTOSOL
inactive Ras protein
activated Ras protein
GDP
GDP
GTP
GTP
P
activated RTK
adaptor protein
Ras-activating protein
ONWARD TRANSMISSION OF SIGNAL


Inactive
Ras-GDP
Active
Ras-GTP
Controlled growth, proliferation, migration
Normal
Ras-GDP
Ras*-GTP
Uncontrolled growth, proliferation, migration
Cancer

II. Chromosomal Translocations

عملية نقل proto oncogene الى كروموسوم آخر ويصبح تحت رحمة promotor خاص بجين آخر (هذا الجين بطبيعته يحصل transcription بشكل كبير وبالتالي لأنه اصبح تحت سيطرة promotor الخاص بهذا الجين فإنه سيحصل transcription بنفس المعدل).

It can activate proto-oncogenes by:

1. Placing the **normal proto-oncogene near the promotor** (stimulate transcription) of another gene with subsequent overexpression of this proto-oncogene.
   e.g. c-MYC in lymphoid tumors (Burkitt lymphoma).

- MYC (c-MYC) is a regulator gene (proto-oncogene) that codes for a transcription factor. → induces cell proliferation (increase) → وهي يصير انتاج عالي لأنه
  The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that **plays a role in cell cycle progression and apoptosis.**

البروتين الي بينتج عنه nuclear phospho protein

↑ cell cycle ↓ apoptosis

II. Chromosomal Translocations

- In the human genome, MYC is **located on chromosome 8** and is believed to regulate expression of 15% of all genes.

- MYC activation results in numerous biological effects.
  1. The first to be discovered was its capability to **drive cell proliferation** (**upregulates cyclins, downregulates p21**).
  2. It also plays a very important role in **regulating cell growth** (upregulates ribosomal RNA and proteins), **differentiation,** and **stem cell self renewal**.

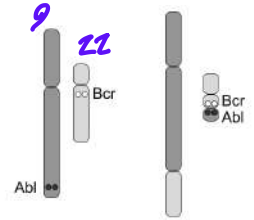
in next lecture

- Myc is a very strong proto-oncogene, and it is very often found to be upregulated in many types of cancers.

II. Chromosomal Translocations

Chimeric protein. Fusion proteins or chimeric proteins are proteins created through the joining of two or more genes

جزء من 9 ينتقل إلى 22
وجزء من 22 ينتقل إلى 9
(recombination)



2. Placing **normal unaltered genes** from **two different chromosomes** to **recombine** and form hybrid genes that **encode growth promoting (chimeric) proteins.**
   - e.g. Philadelphia chromosome.

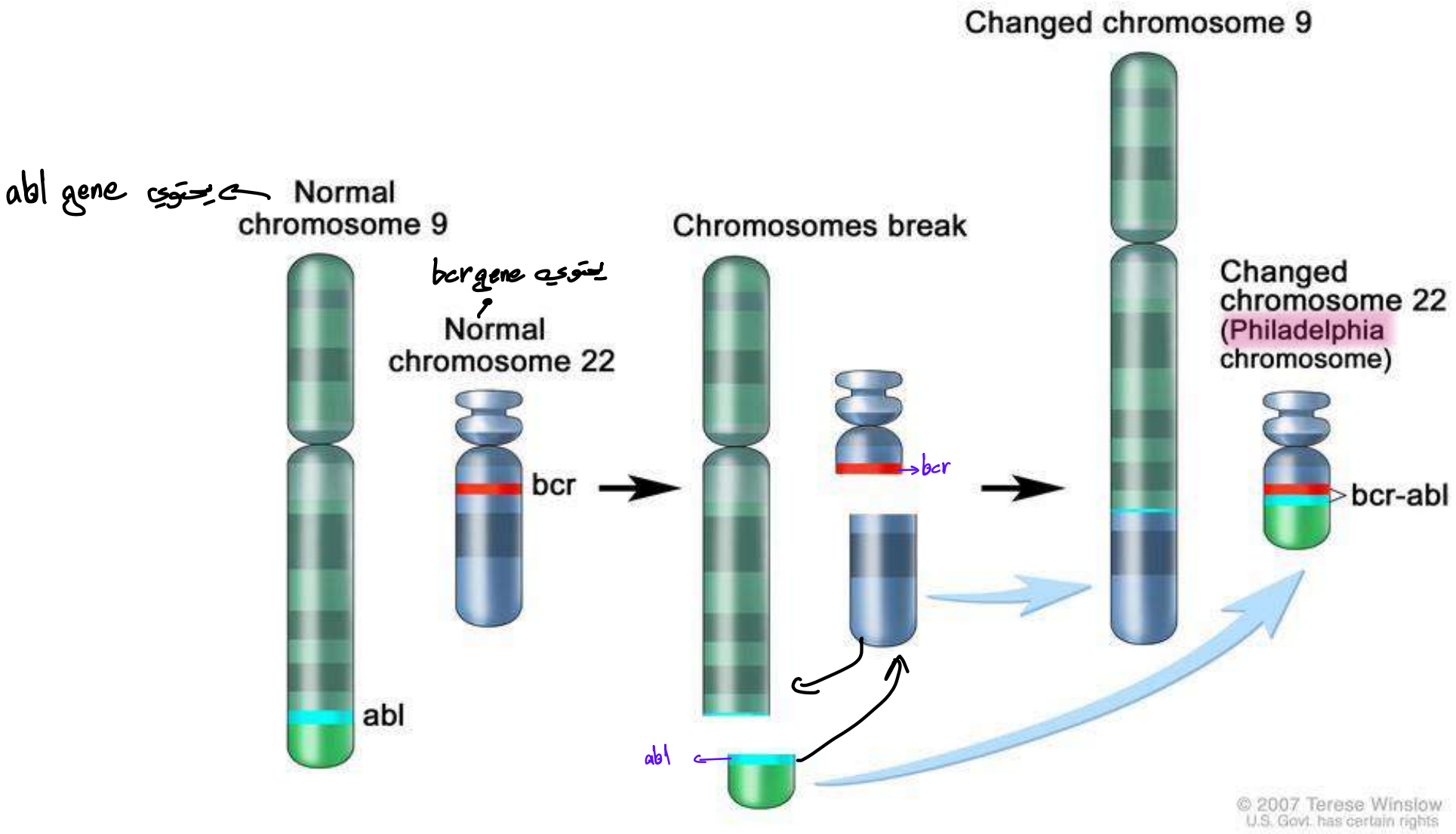
- The Philadelphia chromosome or Philadelphia translocation is a specific **genetic abnormality in chromosome 22 of leukemia cancer cells** (particularly chronic myelogenous leukemia (CML) cells).

II. Chromosomal Translocations

- This chromosome is defective and **unusually short** because of reciprocal translocation of genetic material between chromosome 9 and chromosome 22, and contains a fusion gene called BCR-ABL1 coding for a hybrid protein.

It results in

- A tyrosine kinase signaling protein that is "always on", causing the cell to divide uncontrollably.


abl gene يحتوي
Normal chromosome 9
abl
bcr gene يحتوي
Normal chromosome 22
bcr
Chromosomes break
bcr
abl
Changed chromosome 9
Changed chromosome 22 (Philadelphia chromosome)
bcr-abl
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III.Gene Amplification

growth ← genes مسؤولة عن

proto-oncogene ما بصير amplification بشكل جداً كبير فتتحول على oncogene

كما بيصير لها over expression فبالتالي رح تعطي growth الكثير من products المسؤولة عن

- Such amplification may produce several hundred copies of the proto-oncogene in the tumor cell

  e.g. c-erb B2 in breast cancer; also frequently called HER2 (from human epidermal growth factor receptor 2 and encoded by ERBB2 gene)

in breast cancer → overexpressed

the disease has a poor prognosis ← overexpression of HER2 وبيكون عندها breast cancer معها patients بالعادة لما يكون فيه

Leads to uncontrollable division of cell

بسبب زيادة عدد receptors

- It encodes a transmembrane protein with tyrosine kinase activity

  Tyrosine kinases are important mediators of this signal transduction process, leading to cell proliferation, differentiation, migration, metabolism.

- The over expression of the ERBB2 gene, occurs in approximately 15-30% of breast cancers. It is strongly associated with increased disease recurrence and a poor prognosis.

III.Gene Amplification

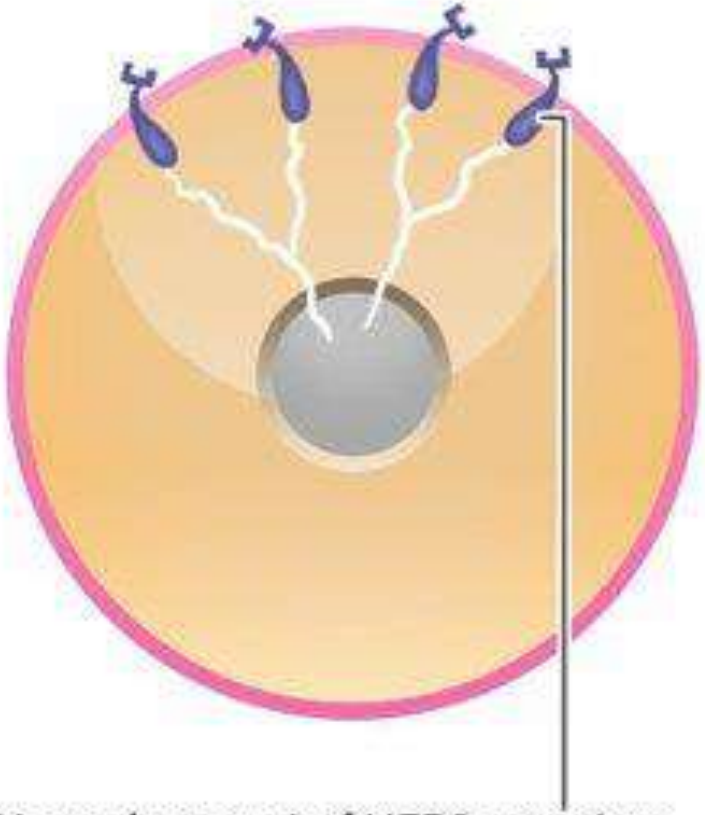
- In summary, signaling through the ErbB family of receptors **promotes cell proliferation** and **opposes apoptosis**, and therefore must be tightly regulated to prevent uncontrolled cell growth from occurring.

dihydrofolate reductase مسؤول عن التلج

dihydrofolate reductase, كثرة فيزيد انتاج amplifications بيصير
لهذا inhibition بيعمل methotrexate فإن
لا يستطيع العمل بسبب عدد الانزيمات الكبير

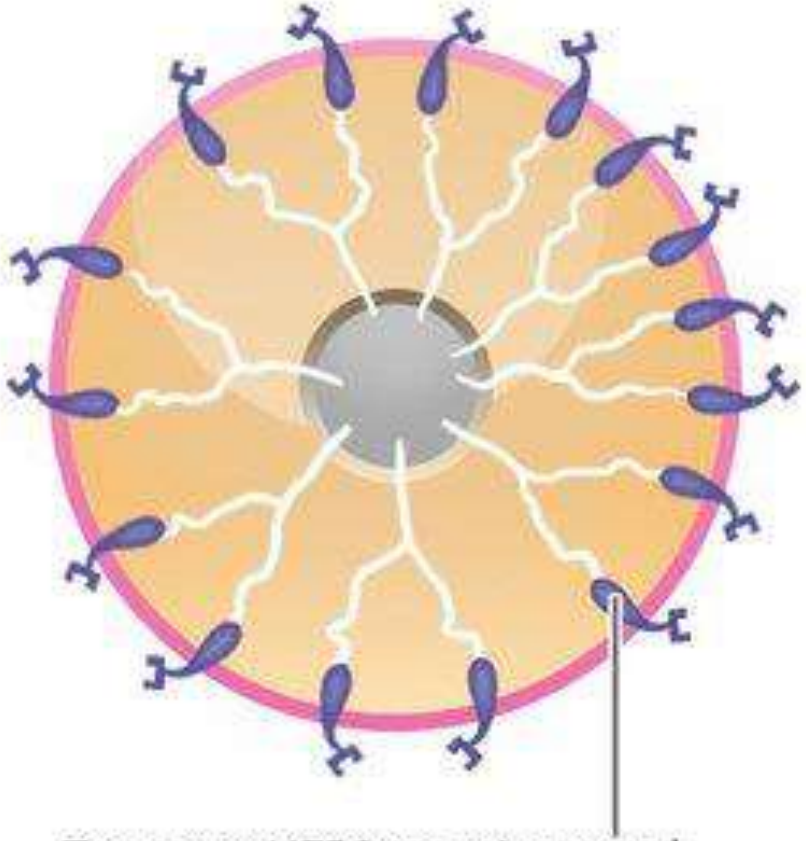
- **Dihydrofolate reductase gene** is amplified in cancer patients (acquired methotrexate resistance) receiving methotrexate, an inhibitor of the dihydrofolate reductase enzyme.

Normal breast cell



Normal amount of HER2 receptors send signals telling cells to grow and divide.

Abnormal HER2+ breast cancer cell



Too many HER2 receptors send more signals, causing cells to grow too quickly.

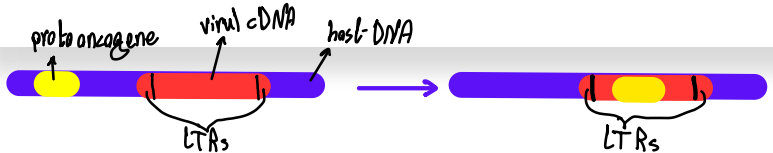
IV. Insertion mutagenesis (viral carcinogenesis)

Retrovirus----RNA virus

1. Retrovirus transduction:

When a retrovirus infects a cell, its RNA genome synthesizes a complementary DNA (cDNA) inside the infected host cell by the viral encoded RNA-dependent DNA polymerase (reverse transcriptase).

The viral cDNA then integrates into the genome of the host cell where it can be copied as the host genome is duplicated during the process of cellular division.



IV. Insertion mutagenesis (viral carcinogenesis)

- At the ends of the retroviral genome there are powerful transcriptional promotor sequences termed **long terminal repeats** (LTRs).
  - The LTRs promote the transcription of the viral DNA leading to the production of new virus particles (viral RNA genome)

يسوّول عن normal cell growth فرع تيج ترجمته بشكل كبير وبالتالي يؤدي الى cancer

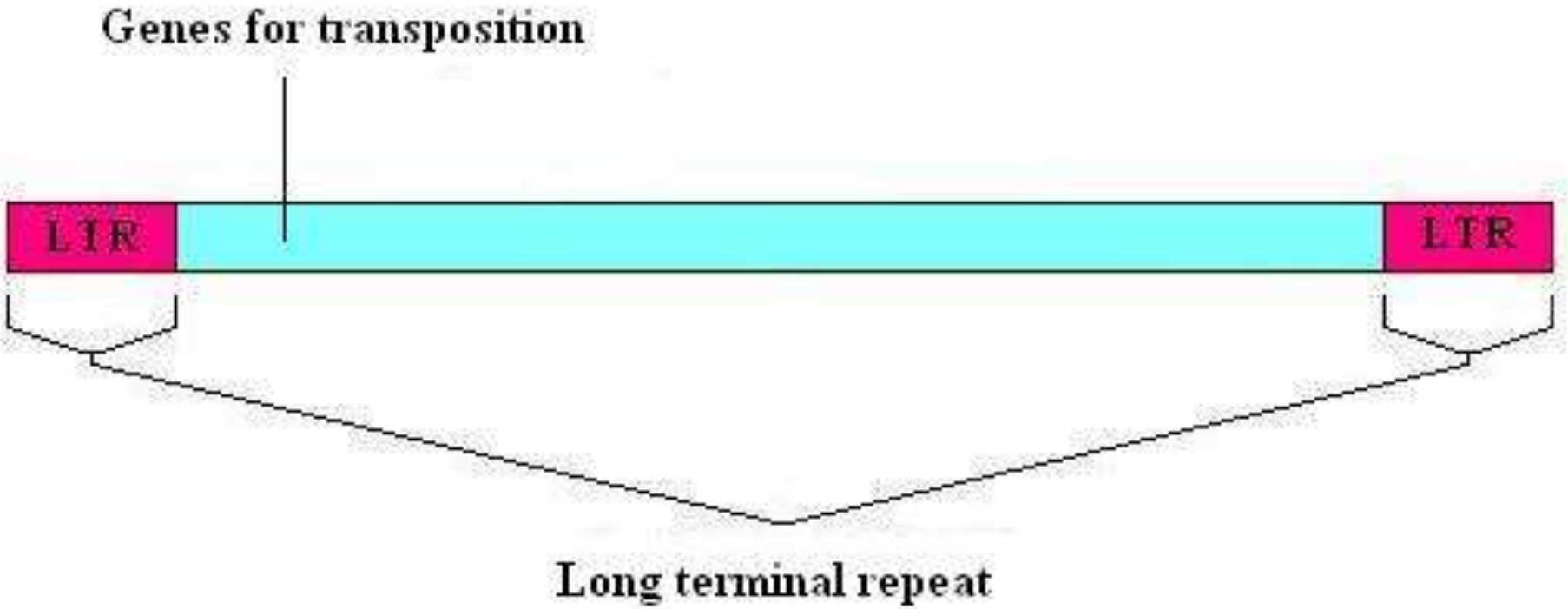
- Occasionally, the virus carries a portion of host DNA. Subsequent infection of a new host by such a virus may introduce new genes to the new host
  - This process is termed **transduction.**

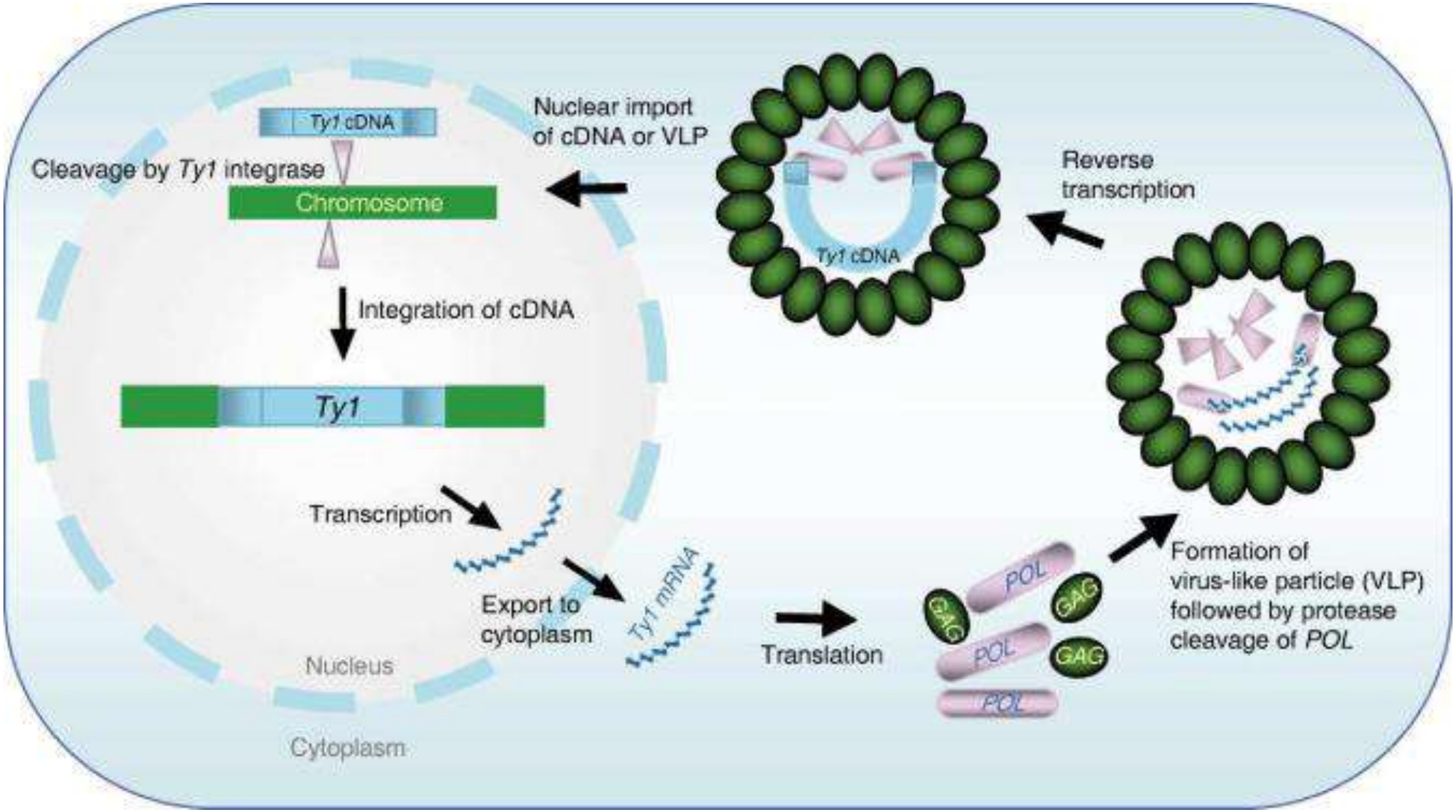
Incorporation of a protein of host genome into the viral genome

IV. Insertion mutagenesis (viral carcinogenesis)

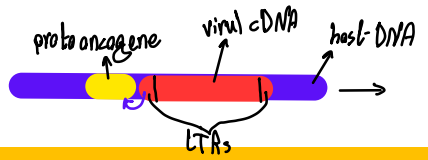
- Occasionally this transduction process leads to the virus acquiring a gene from the host that is normally involved in **cellular growth control.**
- **This gene will be transcribed at a higher rate** due to its association with the retroviral LTRs → the transduced gene stimulates the growth of the infected cell.
- The end result of this process is **unrestricted cellular proliferation** leading to tumorigenesis (cancer formation).

LTR Transposon




Ty1 cDNA
Cleavage by Ty1 integrase
Chromosome
Integration of cDNA
Ty1
Transcription
Export to cytoplasm
Ty1 mRNA
Nucleus
Cytoplasm
Translation
POL
GAG
GAG
POL
GAG
POL
Formation of virus-like particle (VLP) followed by protease cleavage of POL
Reverse transcription
Ty1 cDNA
Nuclear import of cDNA or VLP

IV. Insertion mutagenesis (viral carcinogenesis)



incorporation into the viral genom يصير بدون ما proto oncogene
promotor تأثير تحت فصار promotor of viral genome جنب. كان بالعدوة

2. Retrovirus integration:

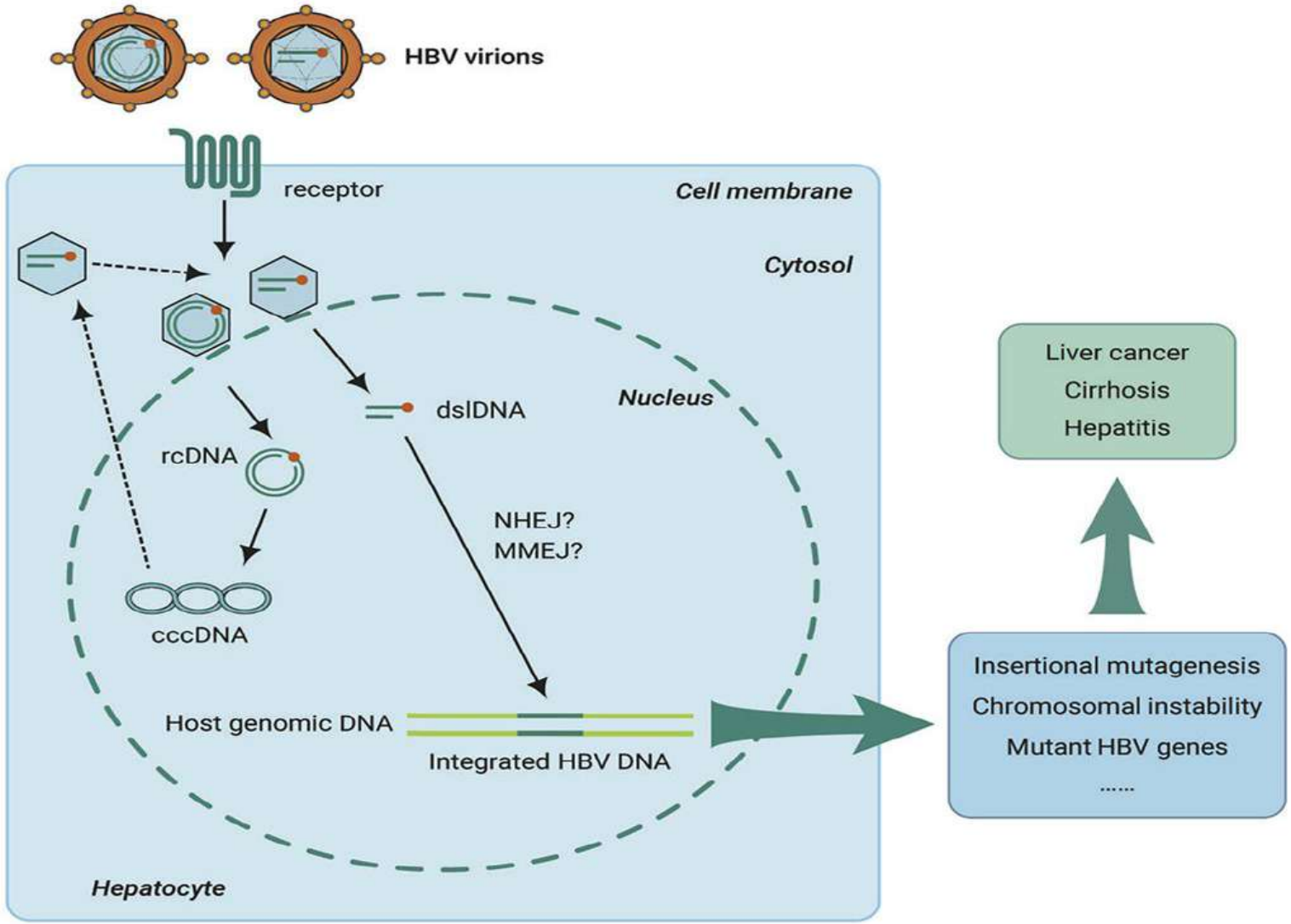
- The second mechanism by which viruses can transform cells relates to the powerful transcription promoting effect of the LTRs.
- When a virus genome integrates into a host genome it does so randomly
  - This is called **virus integration.**
- Sometimes, this integration process leads to the placement of the LTRs close to a gene that encodes a growth regulating protein.

IV. Insertion mutagenesis (viral carcinogenesis)

- If the protein is expressed at an abnormally elevated level it can result in cancer development.
- **A notable example** is the **integration of hepatitis B virus genome** (DNA virus) into the hepatocyte genome leading to induction of liver cancer.
- **HIV (human immunodeficiency virus)** induces certain forms of cancers in infected individuals by this integration induced transformation process.

IV. Insertion mutagenesis (viral carcinogenesis)

- Unlike retroviruses, genomic integration has no role in HBV replication.
- Integrated viral DNA is found in 85%-90% of HBV-related HCCs (hepatocellular carcinoma) and its presence in tumors from non-cirrhotic livers of children or young adults further supports the role of viral DNA integration in hepatocarcinogenesis.
- A significant feature of chronic HBV infection is that HBV DNA fragments are integrated into different locations within the host DNA.


HBV virions
receptor
Cell membrane
Cytosol
Nucleus
dslDNA
rcDNA
NHEJ?
MMEJ?
cccDNA
Host genomic DNA
Integrated HBV DNA
Hepatocyte
Liver cancer
Cirrhosis
Hepatitis
Insertional mutagenesis
Chromosomal instability
Mutant HBV genes
.....

Questions:

- **The Philadelphia chromosome, commonly associated with leukemia, results from a translocation involving which two chromosomes?**

a) 9 and ~~10~~ 22

b) 11 and 22

c) 13 and 14

d) 17 and 18

Answer: A

Questions:

- Which genetic alteration is commonly associated with chronic myeloid leukemia (CML)?

a) BCR-ABL fusion gene

b) BRCA1 mutation

c) HER2 amplification

d) HBV integration

Answer: A

Questions:

- What is the primary role of oncogenes in cancer development?

a) Repair damaged DNA
b) Promote cell division and growth
c) Inhibit cell cycle progression
d) Suppress tumor formation

Answer:B