Genetics of Cancer

Introduction

- During development, cell proliferation (genetically programmed) and differentiation occur to form tissues and organs.

a. Skin inner layers, Blood, intestine lining

b. Liver cells

c. Immune cells

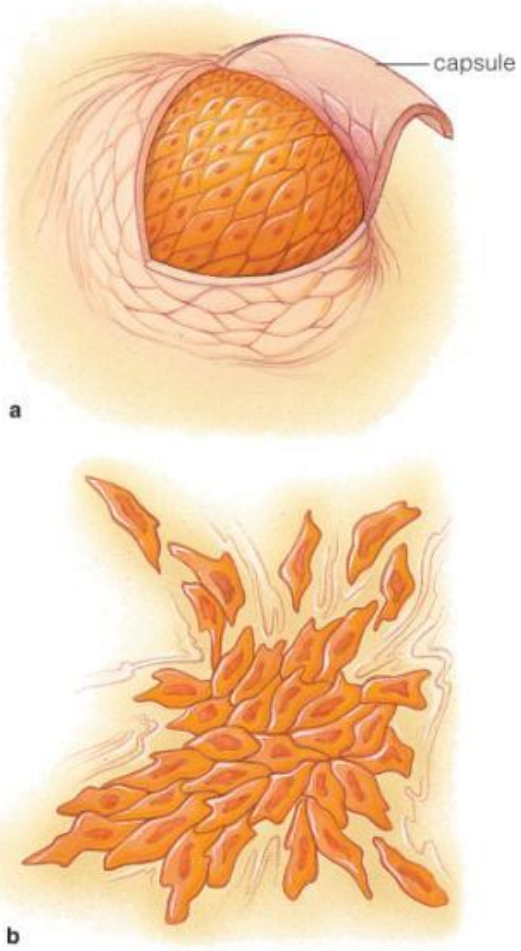
d. Nerve cells and skin surface cells

Cell differentiation correlates with **loss of ability to proliferate**, with the most highly specialized cells terminally differentiated.

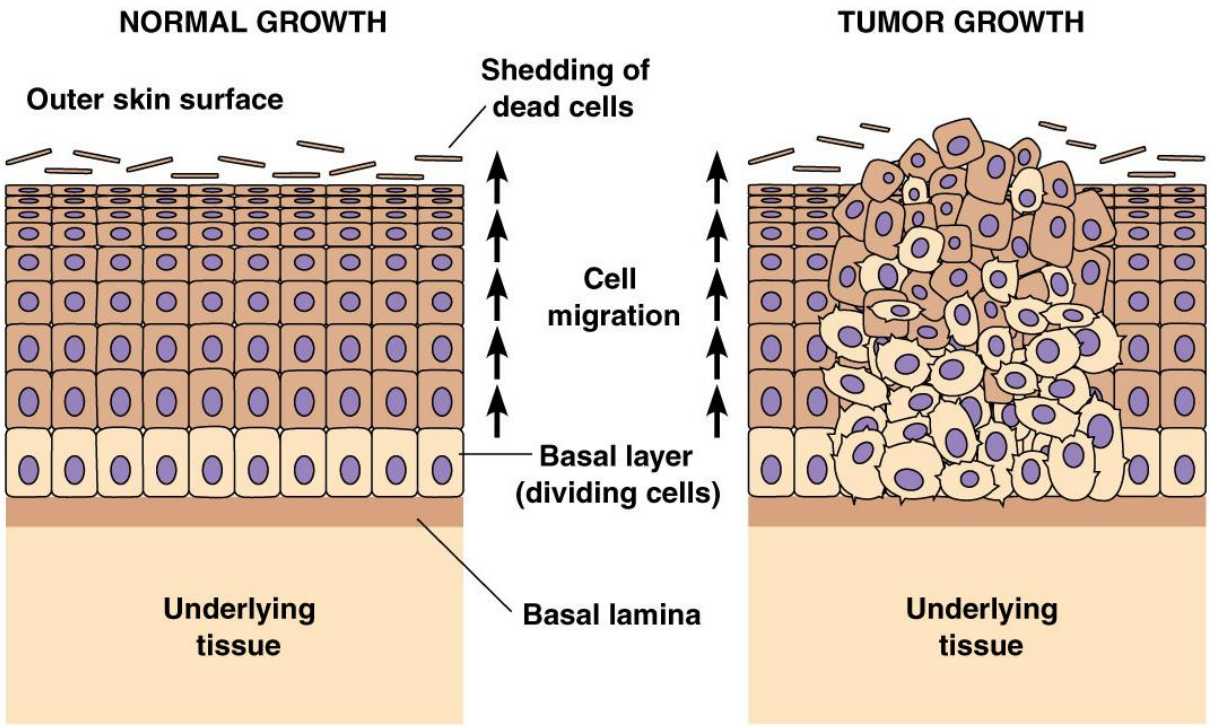
b. Terminally differentiated cells have **a finite life span**, and are replaced with new cells produced from stem cells.

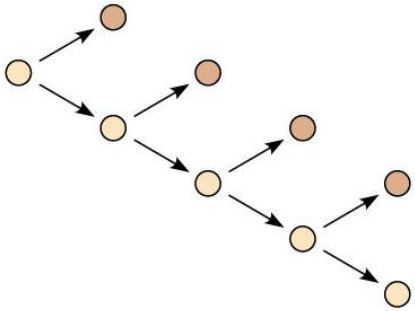
Sometimes, cells deviate from their programmed proliferation and produce a tissue mass called tumor or neoplasm (new growth) (a solid or fluid-filled [cystic] lesion).

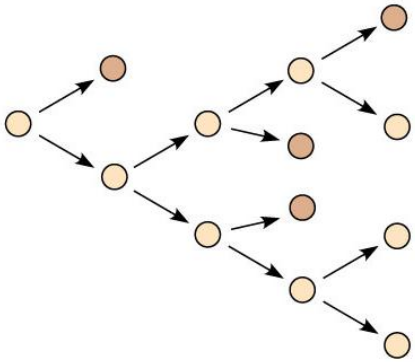
Transformation, Cancer Cells Are Abnormal in Their Growth and Appearance



Comparison of Normal and Tumor Growth in the Epithelium of the Skin



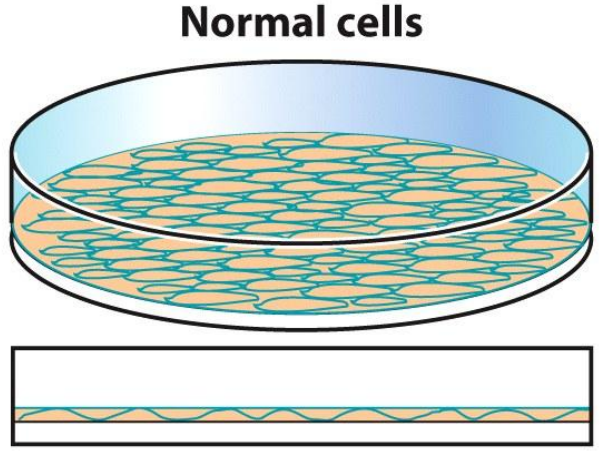




Location/distribution

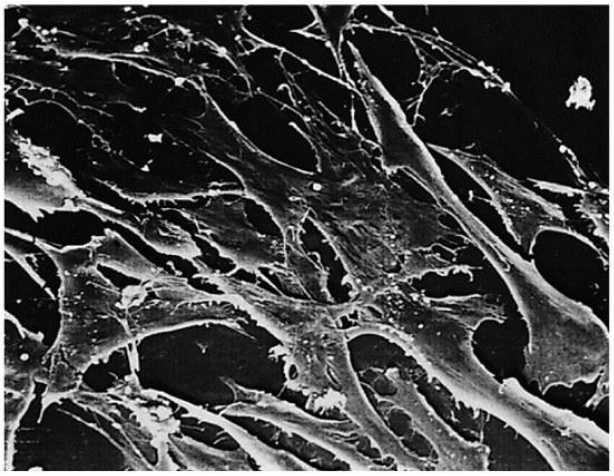
Growth properties of normal and cancerous cells

Contact Inhibition



Normal cells grow in monolayer

(a)



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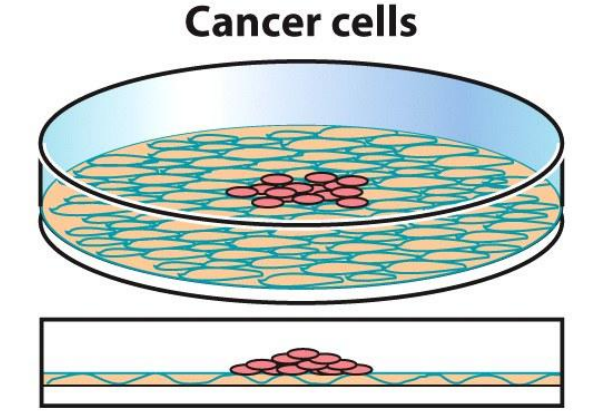
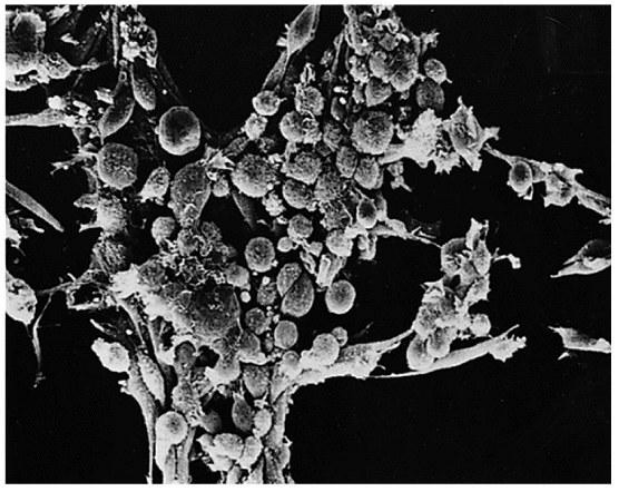
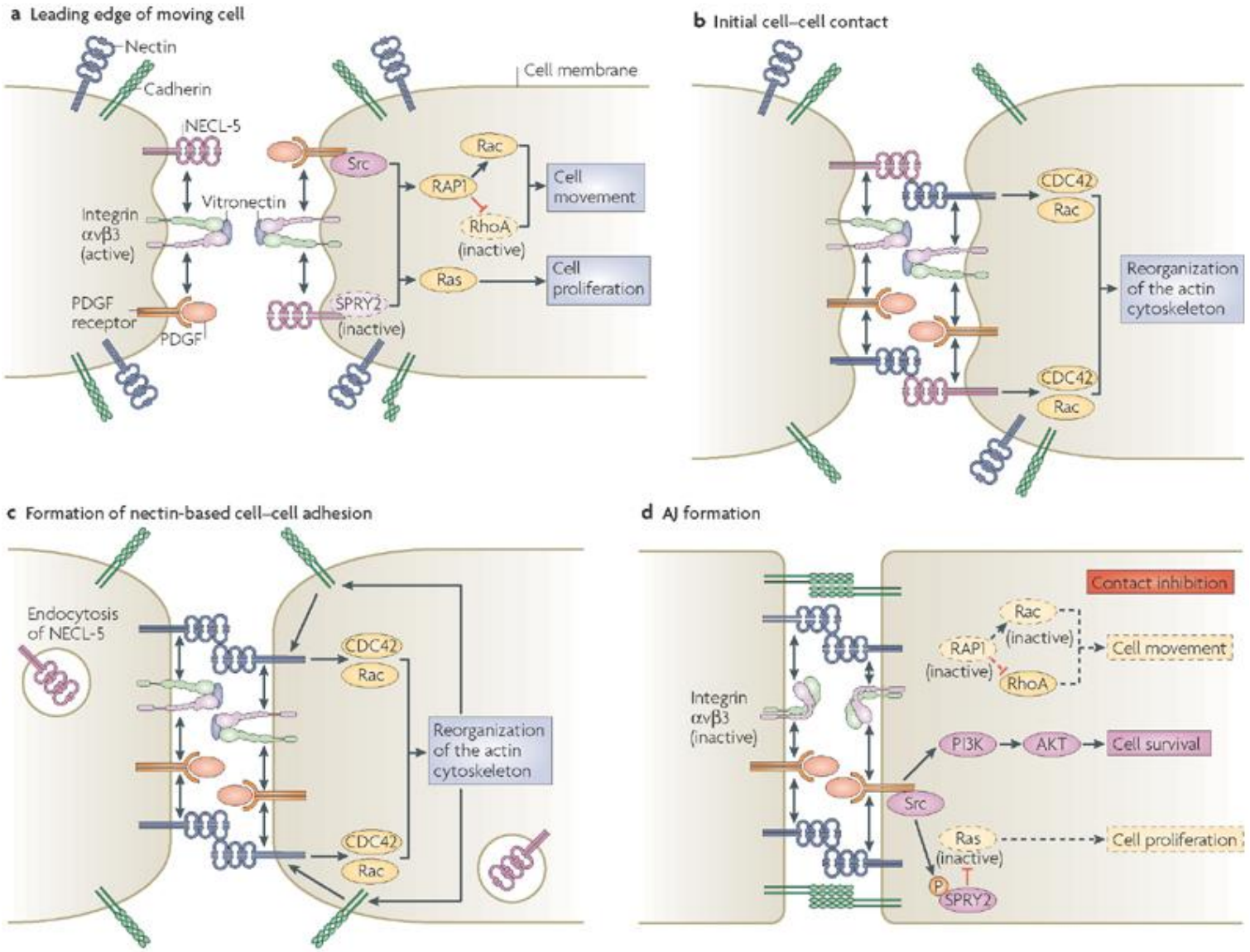


Cancer cells grow in clumps (foci)

(c)



(d)



Nature Reviews | Molecular Cell Biology

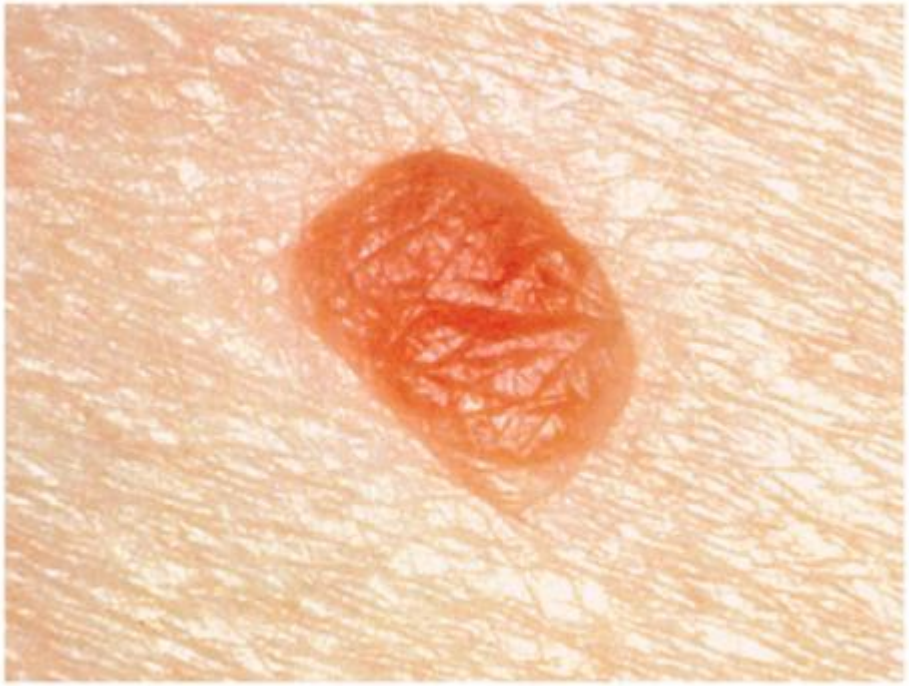
Normal cells vs. Cancer cells

Normal cell proliferation	**Cancer cell proliferation**
Anchorage dependent	Anchorage independent
Density-dependent inhibition	Can grow on top of one another
Limited number of cell divisions	Immortal
Telomere shortening	Telomere maintenance
Proliferation dependent upon extracellular signals	Constant signal to divide
Checkpoints activated at appropriate times	Loss of checkpoint
Apoptosis functional	Apoptosis inhibited

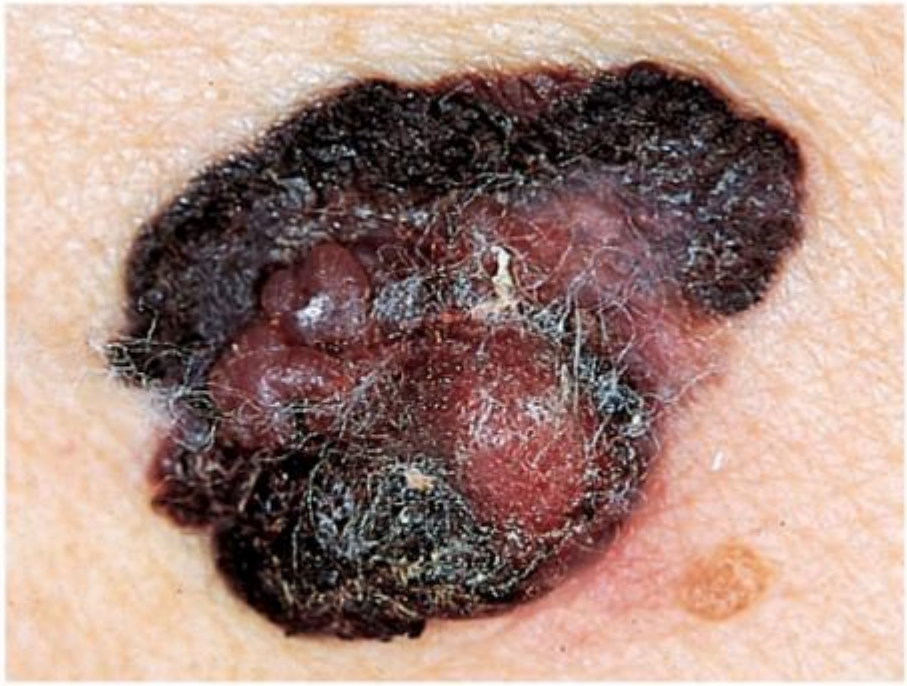
- Oncogenesis (mass birth): Tumor initiation
- Solid tumors: solid mass of cancer cells that grow in organ systems and can occur anywhere in the body.
  - Carcinoma or epithelial tumors which account for 90% of solid tumors, can occur anywhere along that lining …… mouth cancer, throat cancer, esophageal cancer, stomach cancer, bowel cancer, or anal cancer.
  - Sarcoma or connective tissue tumor such as muscles, tendons, fat, nerves and other tissues that connect, support or surround structures and organs in the body: Osteosarcoma .
- Liquid tumors occur in the blood, bone marrow, and lymph nodes. They include leukemia , lymphoma, and myeloma.

- Benign tumor: stay at the same mass, no life threatening, cure by removing the tumor via surgery (exception: brain tumors).
- Malignant tumor: Invading and disturbing surrounding tissues…….. Cancer
- Metastasis (change of state) : malignant tumor spreading to whole body through circulatory system
- Cause of death

Normal Moles Are Common Examples of Benign Growths



a Benign mole



b Melanoma

Main Features of Benign and Malignant Tumors

	Malignant Tumor	Benign Tumor
Rate of growth	Rapid	Slow
Nature of growth	Invades surrounding tissue	Expands in the same tissue
Spread	Metastasizes via the bloodstream and the lymphatic system	Does not spread
Cell differentiation	Usually poor	Nearly normal

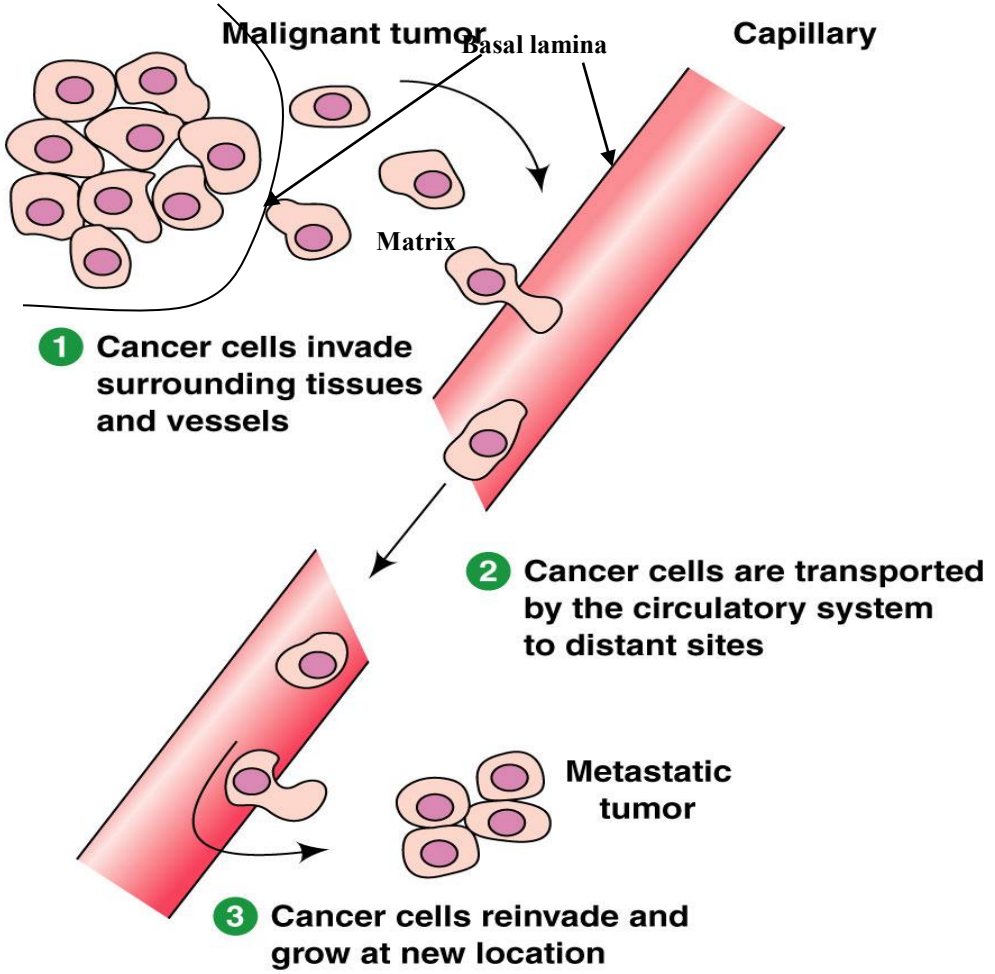
Stages in the Process of Invasion and Metastasis

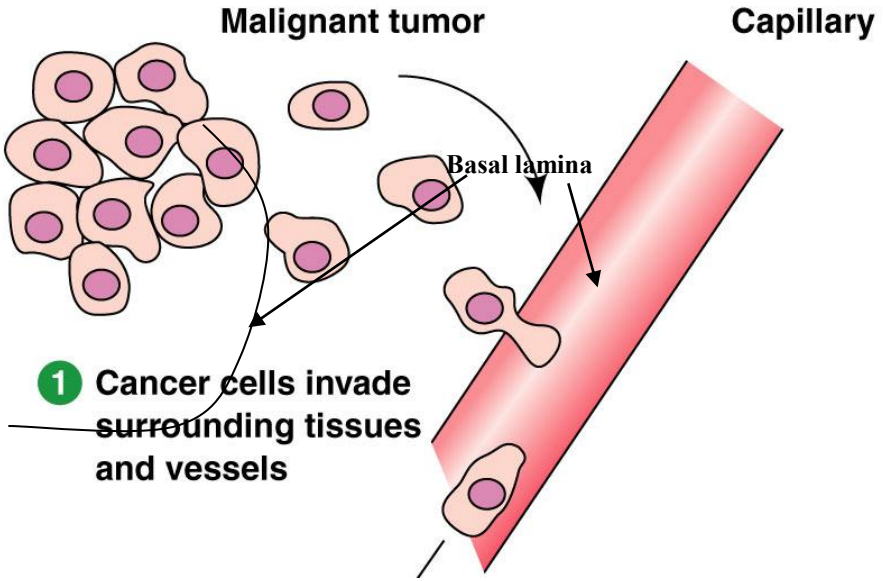
Invasion

Metastasis

Why?

How?





Loss of cell surface proteins involve in cell-cell adhesion

E-cadherin

Increased Motility

Signaling molecules,

Chemoattractants,

Protease activator (Serin protease and MMP)

Relationship of the Cell Cycle to Cancer

Proliferation of eukaryotic cells is described by the cell cycle:

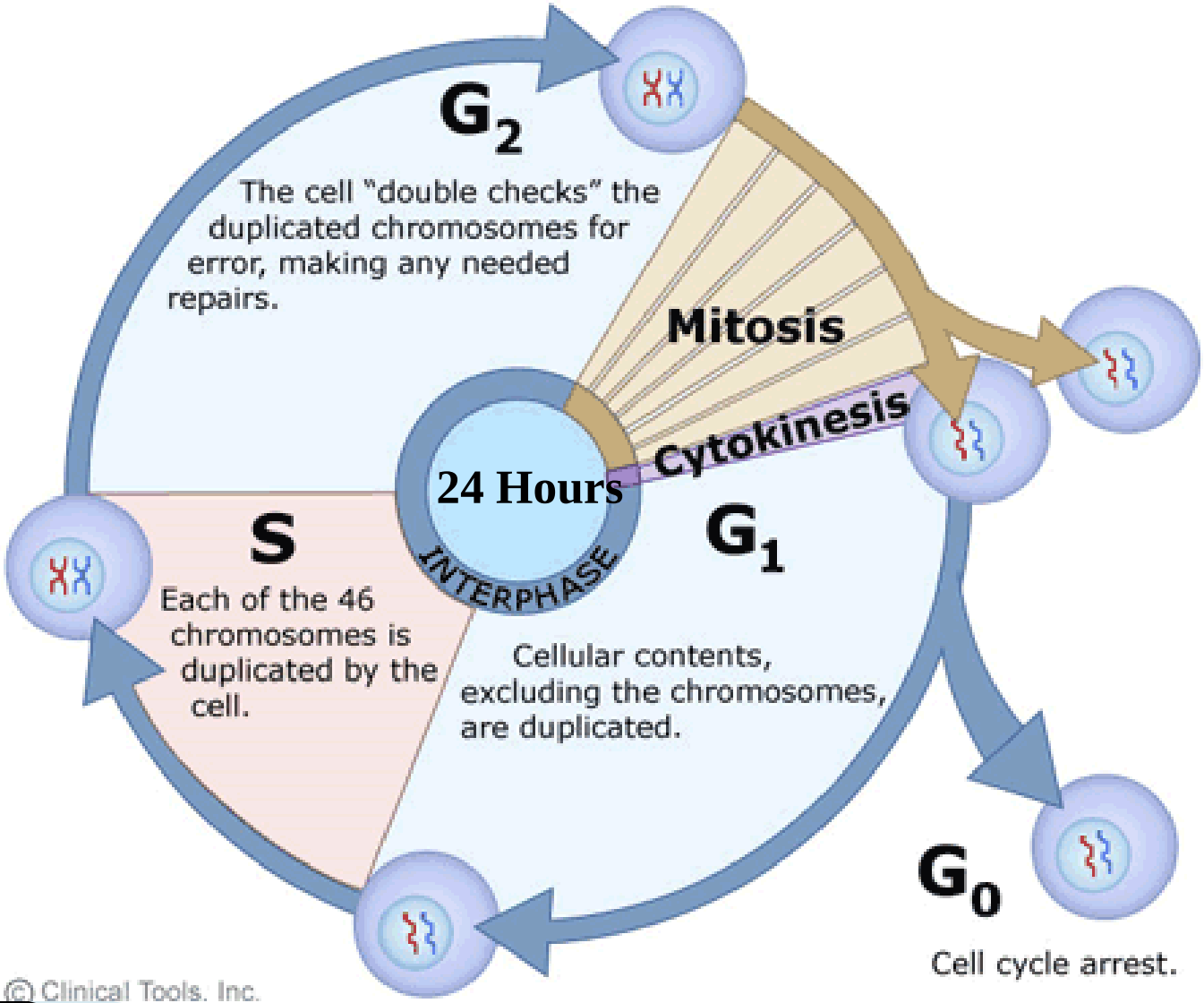
i. M is **mitotic phase**. The rest of the cell cycle is **interphase.**

ii. During G1 (growth phase, high biosynthetic activities) the cell monitors **its size and environment** (under control of p53 gene)

iii If conditions are **appropriate**, it moves into **S phase (DNA synthesis,** During this phase, synthesis is completed **as quickly as possible** due to the **exposed base pairs being sensitive to external factors** such as any drugs taken or any mutagens), and completes the cycle with **G_2 and M.**

Iv A cell that does not commit to DNA replication **may enter G_0 for a long period**, then reenter the cell cycle and proliferate.

Cell Cycle


G2
The cell "double checks" the duplicated chromosomes for error, making any needed repairs.
Mitosis
Cytokinesis
24 Hours
INTERPHASE
G1
Cellular contents, excluding the chromosomes, are duplicated.
S
Each of the 46 chromosomes is duplicated by the cell.
G0
Cell cycle arrest.
© Clinical Tools, Inc.

Animation regarding cell cycle checkpoints

Animation regarding cell cycle in details

Cell cycle regulation:

i. G1 cyclin-Cdk…….TF…….DNA replication proteins, S cyclins, Degrade S phase inhibitors
ii. S cyclin-Cdk …….phosphorylate G1 pre replication protein, prevent making new ones for only one time replication.
iii. Mitotic cyclin-Cdk inactive in S and G2 and active in M, activate proteins involve in chromatin condensation (APC: kinetochore protein degradation, M cycline degradation

- Each phase ends by removing cyclins and many other proteins (tagged with ubiquitin)whose activities must be limited to each phase.

Grwoth factor……cyclin D+Cdk4……Rb phosphorylation……..E2F release from E2F/Dp1/Rb complex…… transcription of cyclin E, A DNAP,…….

Cyclin E + Cdk2……. Transition from G1 to S

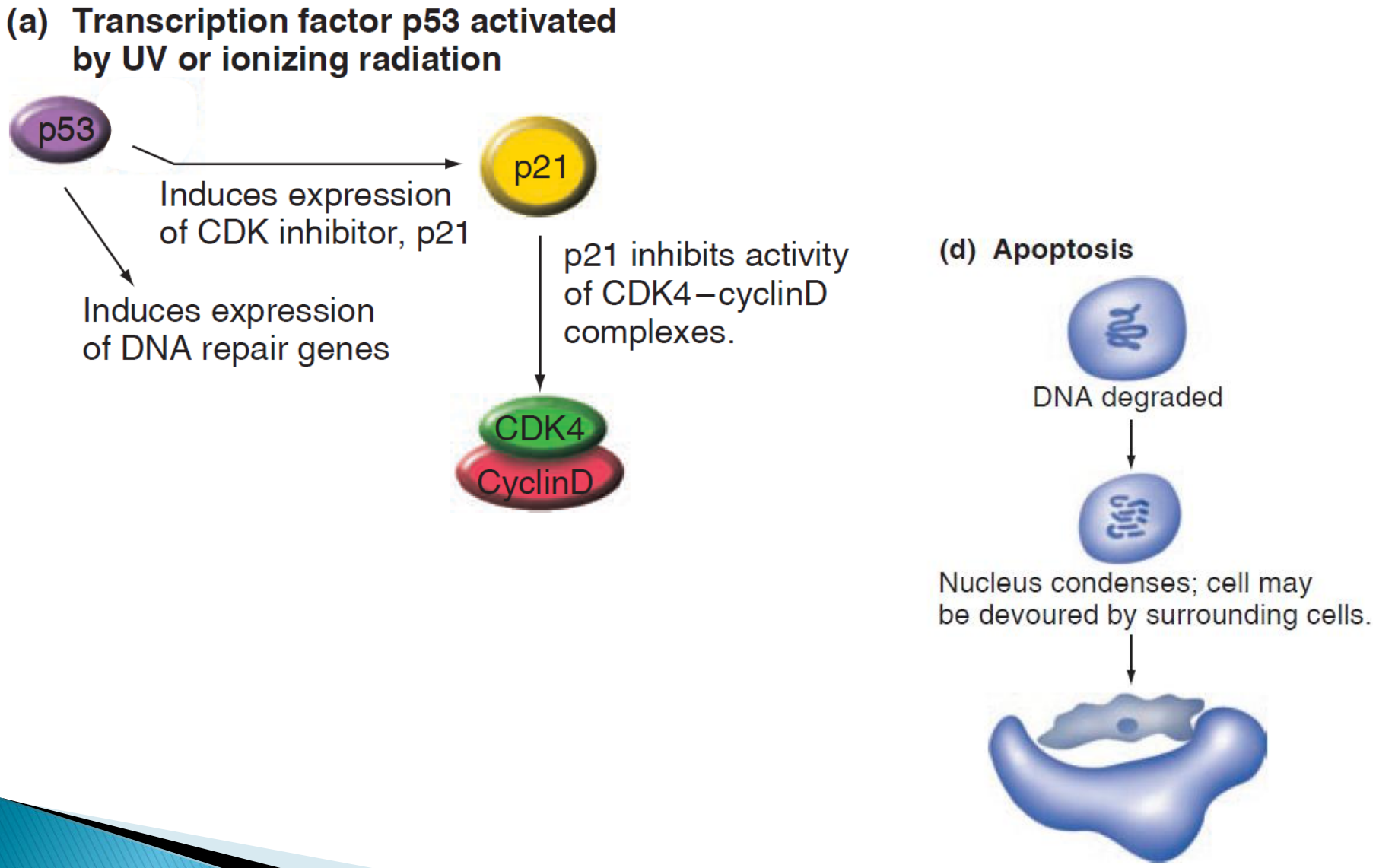
Cyclin B + Cdk1…..Transition from G2 to M ……Prophase and breaking membrane

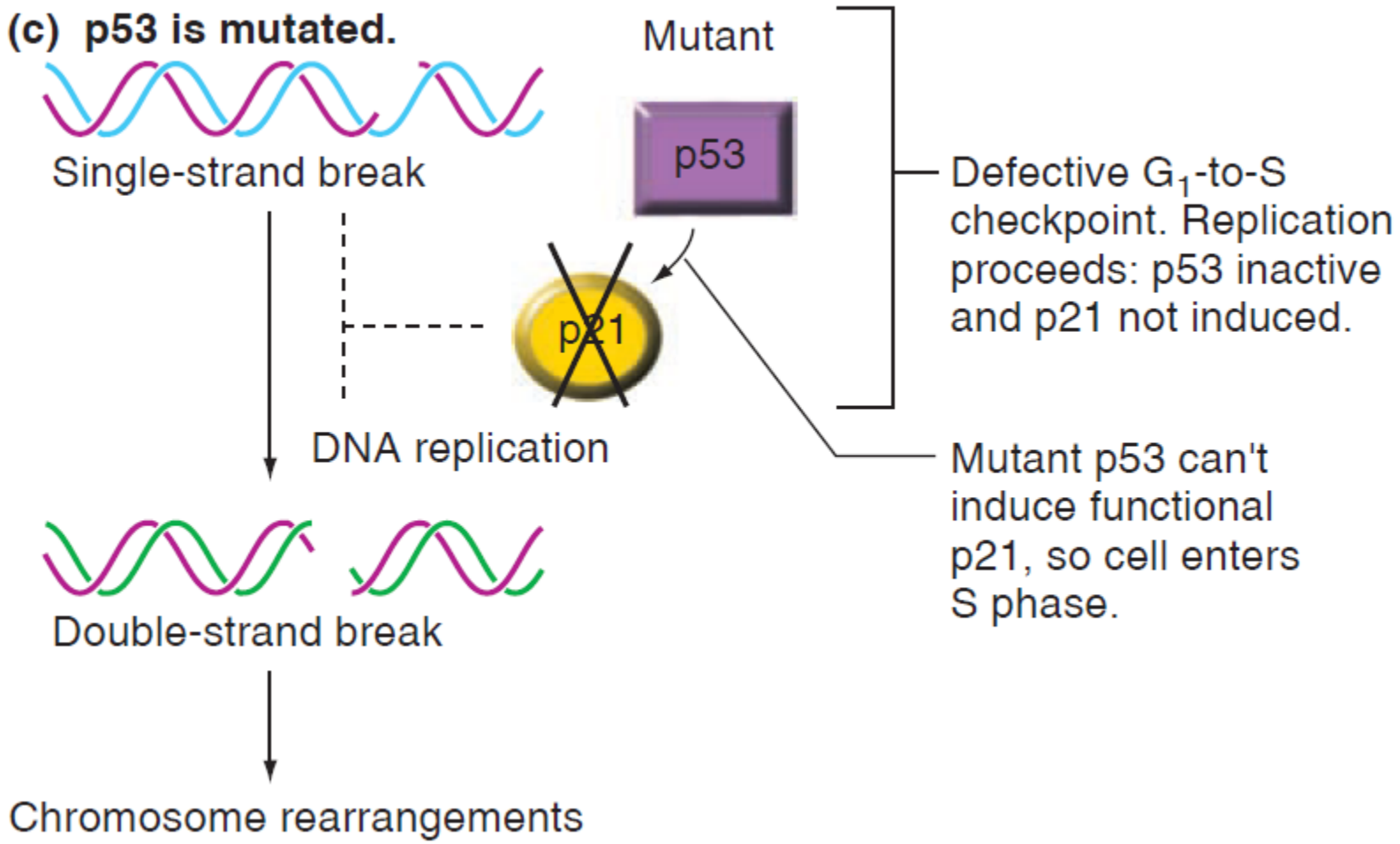
- Damage to a cell's genome (either by environmental agents or random errors of the cellular machinery) can causes serious problem for cells.

- Damage to the cell cycle machinery also causes problems.

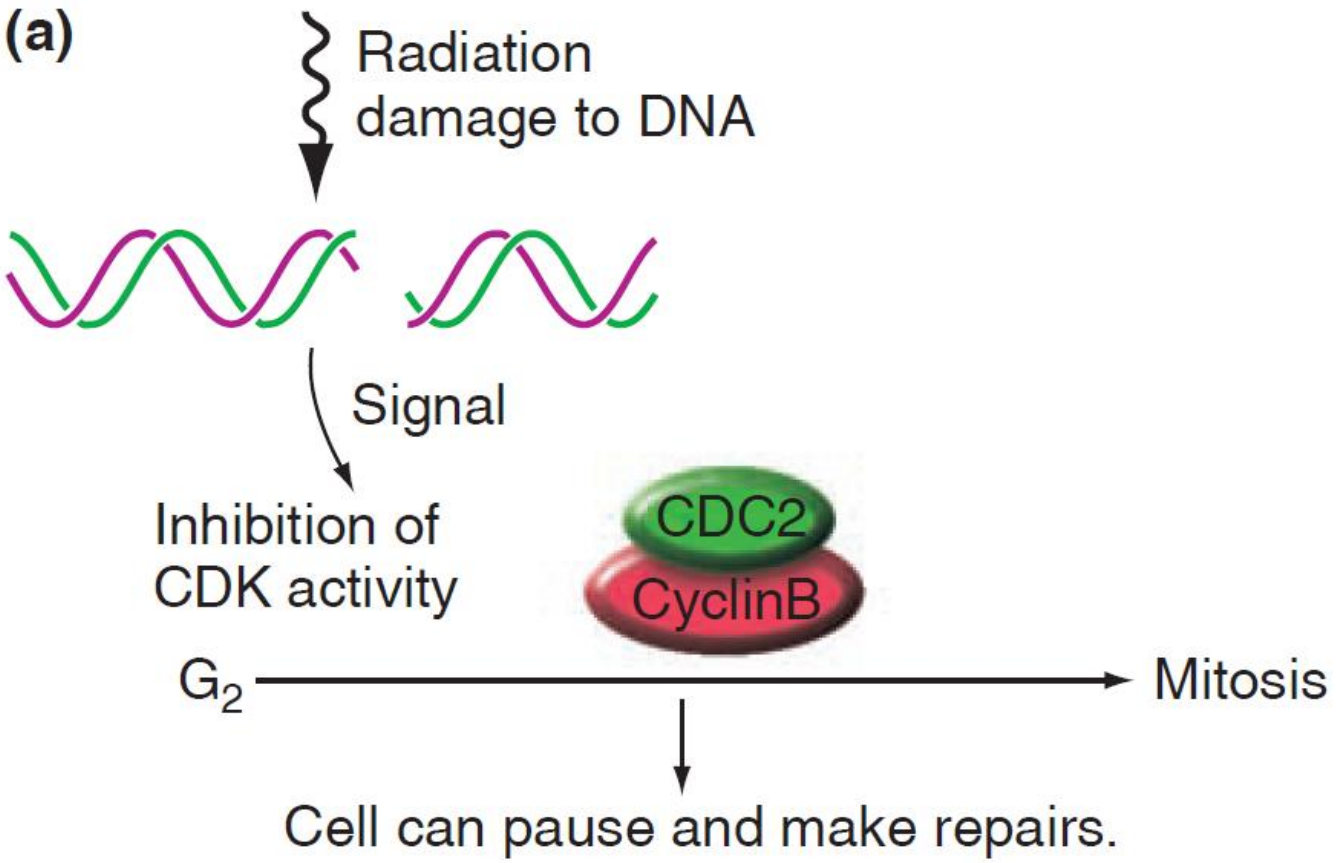
➢ Cell cycle should be arrested while repair take place which controls through checkpoints.

Checkpoints: 1- G1 to S checkpoint, DNA damage in G1:p53

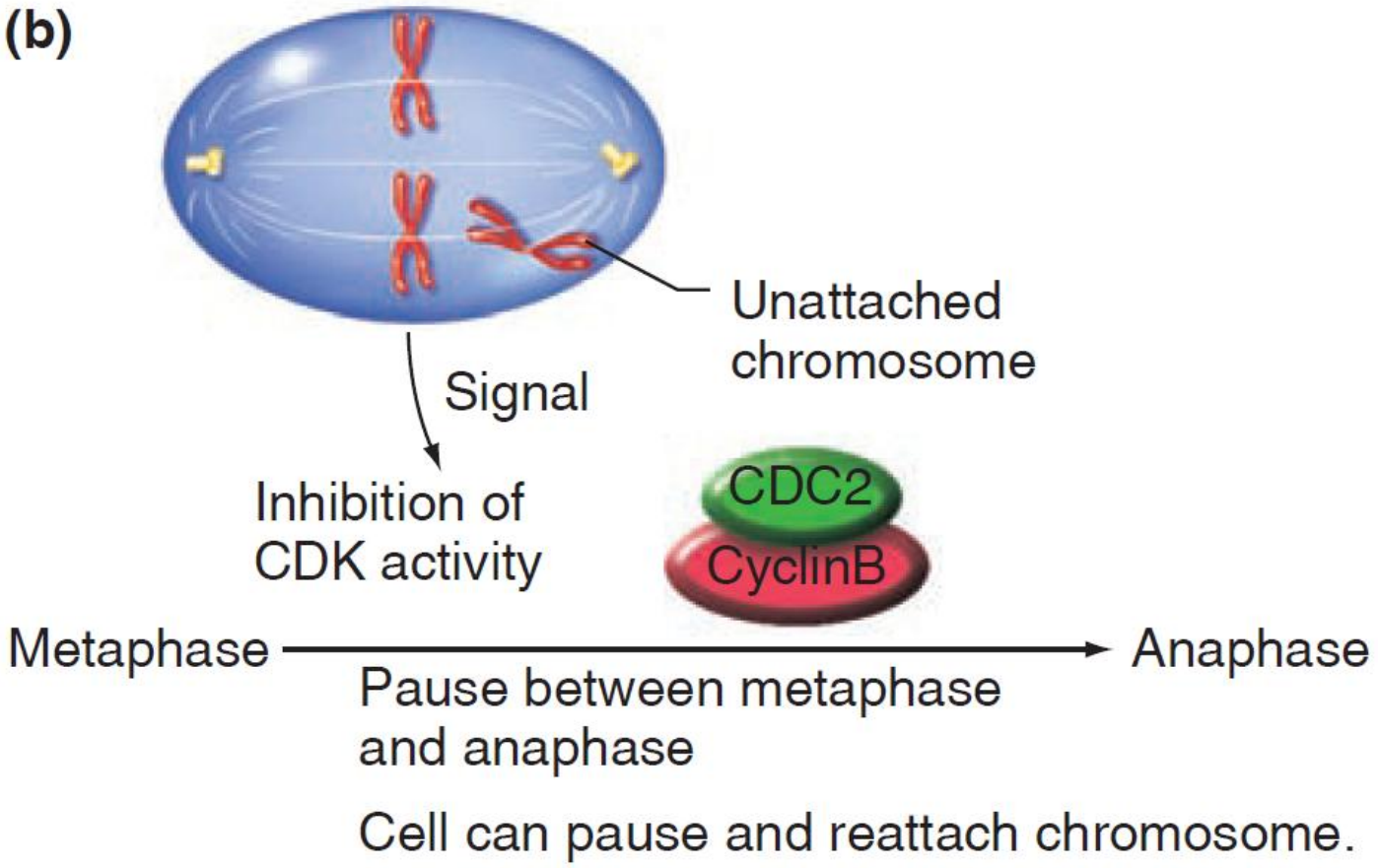



(c) p53 is mutated.
Mutant
p53
Single-strand break
p21
DNA replication
Defective G_1-to-S checkpoint. Replication proceeds: p53 inactive and p21 not induced.
Mutant p53 can't induce functional p21, so cell enters S phase.
Double-strand break
Chromosome rearrangements

2- G2 to M checkpoint, repair before segregation: RAD9 in yeast can pause to repair as many as 200 DSB while mutant RAD9 will die with one unrepaired DSB.



3-Spindle checkpoint



Cell cycle regulation (inhibitors):

- Cip/kip Cdk intracting protein/kinase inhibitory protein

Attach to Cdk4/6 at G1….. Interfere with its connection to cyclin. Block ATP site.

(p21, p27, p57), P21 activated by p53 in response to DNA damage, p27 activated by TGF beta.

Mitogen…… cyclin D synthesis…… inhibitors of Cip/kip + its ubiqutination……promote cdk2-cycE synthesis.

- INK4a/ARF Inhibitor of kinase 4/ alternative reading frame)

p16INK4a attach to Cdk4……G1 arrest

P19ARF prevent p53 degradation

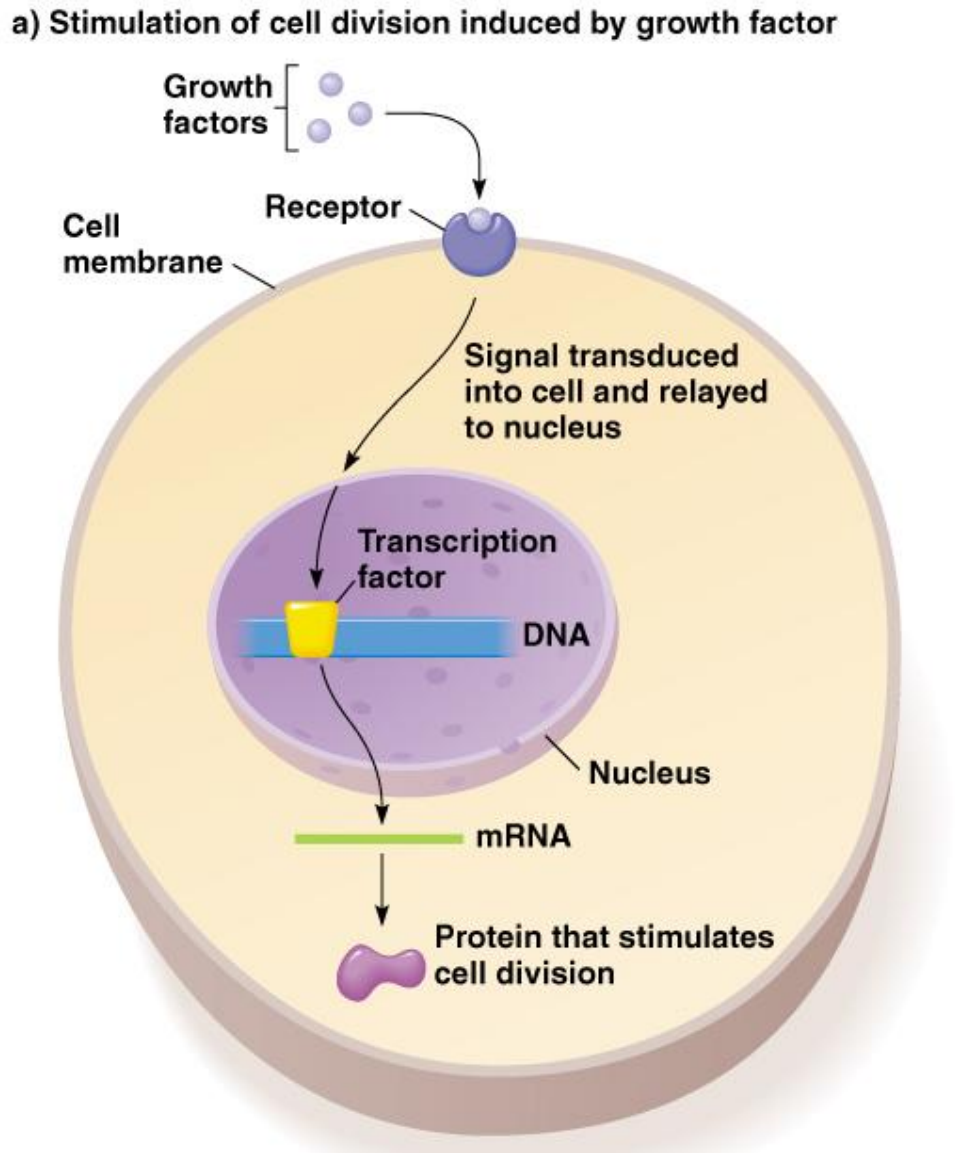
- Normal cell cycle is controlled in several ways. Most important are signal transduction pathways.

Extracellular factors bind to **surface receptors, transmembrane proteins** that relay signals into the cell.

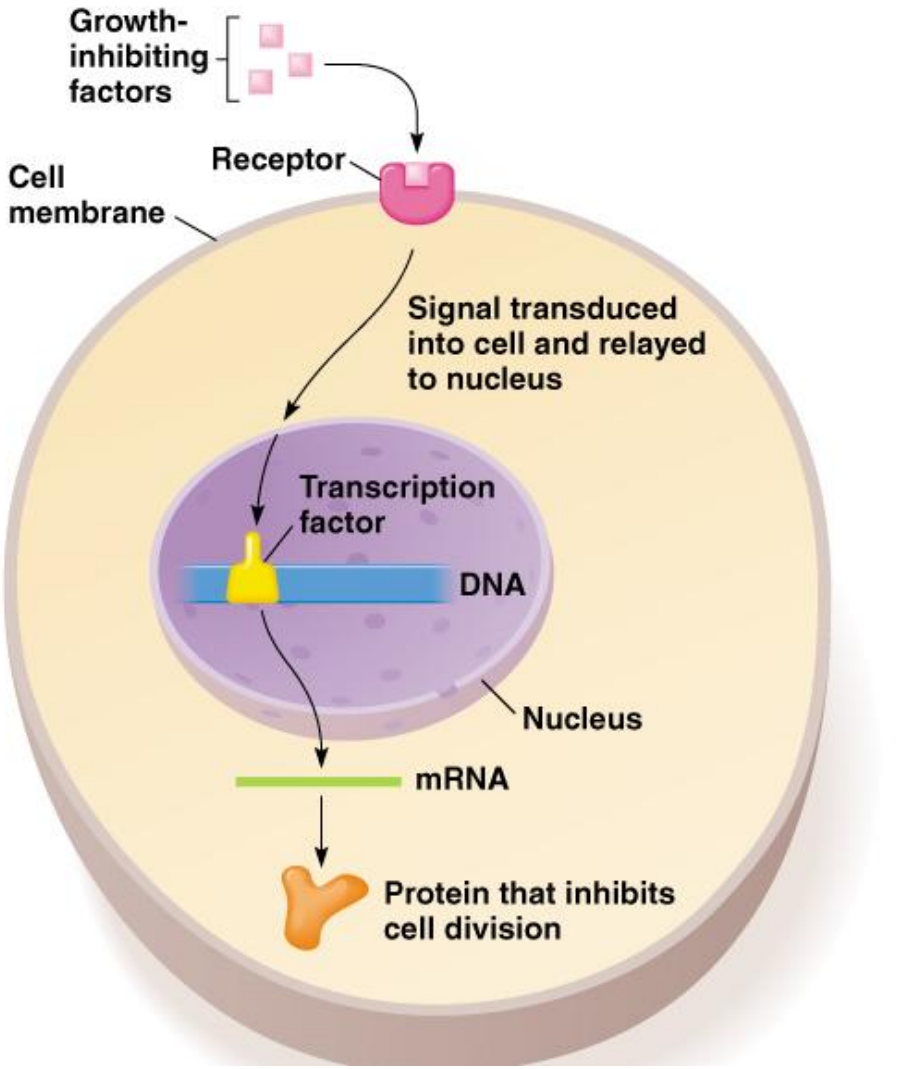
Factors include:

i. **Growth factors** that stimulate cell division.

ii. **Growth-inhibiting factors** that inhibit cell division.


a) Stimulation of cell division induced by growth factor
Growth factors
Receptor
Cell membrane
Signal transduced into cell and relayed to nucleus
Transcription factor
DNA
Nucleus
mRNA
Protein that stimulates cell division

b) Inhibition of cell division induced by growth-inhibiting factor



- Healthy cells produce progeny only when the balance of stimulatory and inhibitory **signals favors cell division.**

- **Neoplastic cells** reproduce without constraint, sometimes because of mutations in inhibitory or stimulatory factor genes or their receptors.

Cancers are genetic diseases

- Familial or sporadic cancers
- Induction of Cancer by viruses
- Clonal descendant of one cancer cells
- Mutagens increase the risk of cancer
- Association of specific chromosomal abnormalities with specific cancers: Burkitt's lymphoma, Chronic Myelogenous leukemia

Cancer

1. Oncogenesis may be due to:
   - Spontaneous genetic changes, such as spontaneous gene or chromosome mutations.
   - Exposure to mutagens or radiation.
   - The action of genes introduced by tumor viruses.

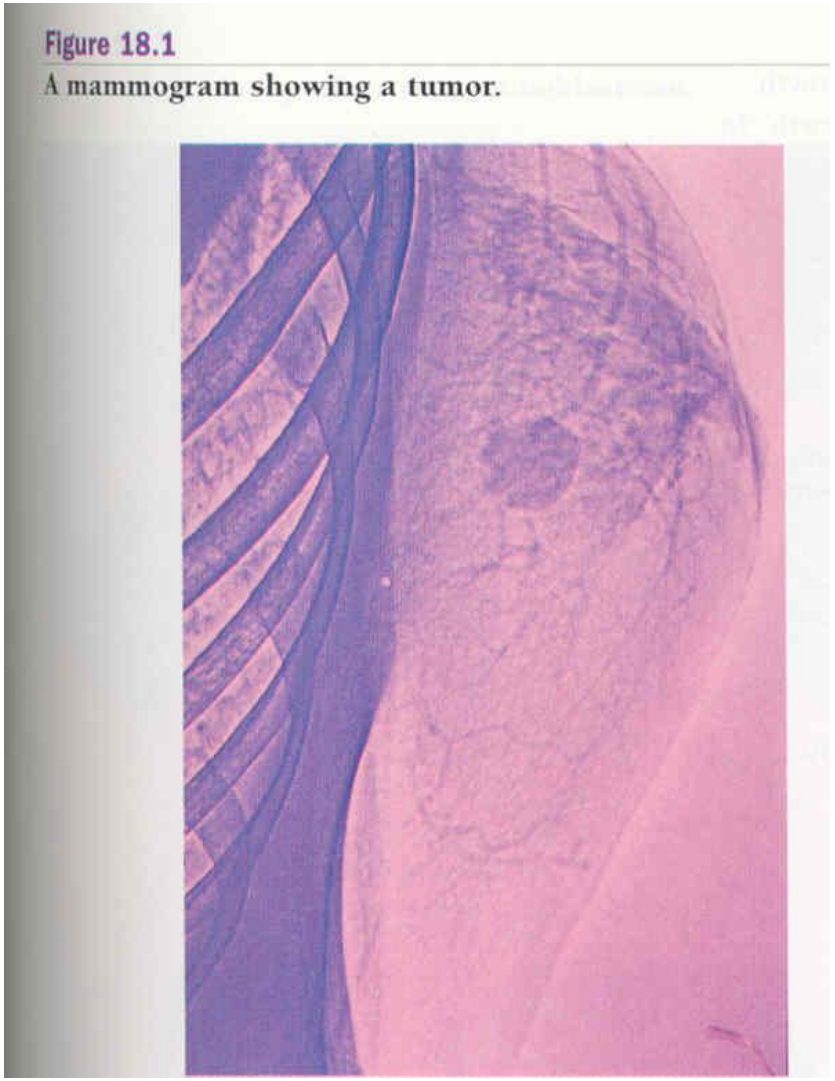


Figure 18.1
A mammogram showing a tumor.

Genes and Cancer

Three classes of genes are mutated frequently in cancer:

a. **Proto-oncogenes**, whose products normally stimulate cell proliferation. After mutation……Oncogenes found in cancer cells which are more active or activate in an inappropriate time.

b. **Tumor suppressor genes**, whose products normally inhibit proliferation.

c. **Mutator genes**, whose products ensure accurate replication and maintenance of the genome……mutation……. Cells prone to mutational error.

http://www.dnatube.com/video/212/How-a-ProtoOncogene-Becomes-an-Oncogene

Oncogenes

- Tumor viruses induce infected cells to proliferate and produce a tumor. There are two types, based on the viral genome:

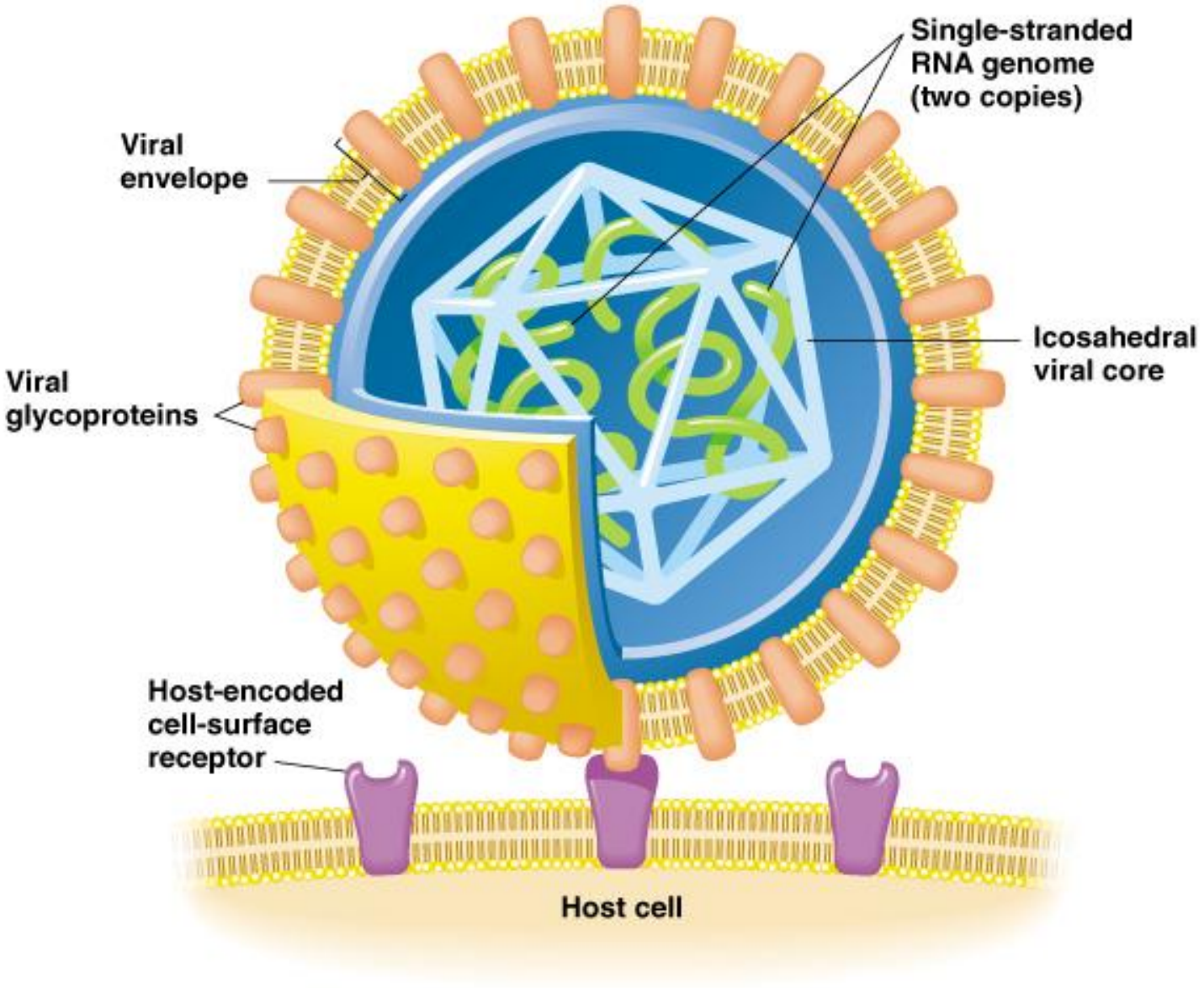
a. RNA tumor viruses transform cells by introducing viral oncogenes. (An oncogene is any gene that stimulates unregulated proliferation.)

b. DNA tumor viruses do not carry oncogenes, and use other mechanisms to transform the cell.

Retroviruses and Oncogenes

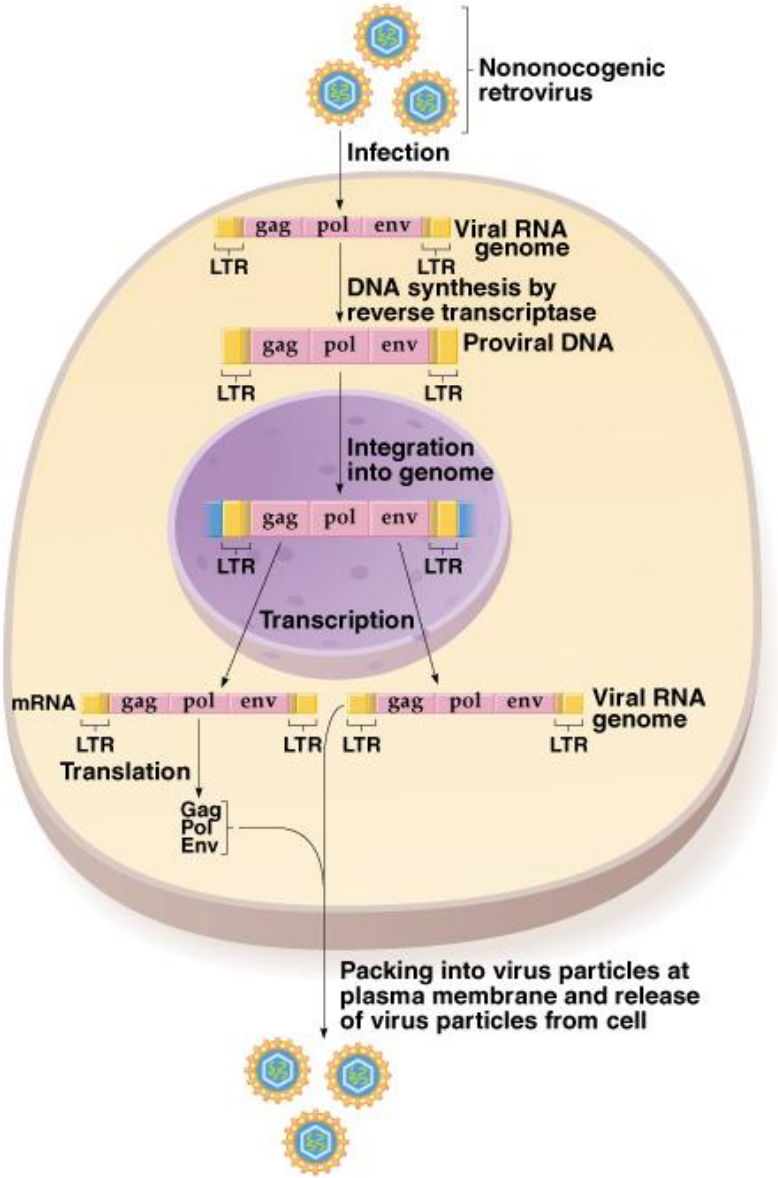
1. RNA tumor viruses are all retroviruses, and their oncogenes are altered forms of normal host genes. Examples of retroviruses include:
   a. Rous sarcoma virus.
   b. Feline leukemia virus.
   c. Mouse mammary tumor virus.
   d. Human immunodeficiency virus (HIV-1, cause of AIDS) (not tumor virus).
2. Structurally, retroviruses have:
   a. Two copies of the 7-10 kb ssRNA genome. + sensed RNA, directely translatable.
   b. A protein core.
   c. An envelope derived from host membrane and bearing viral glycoproteins used to enter a host cell.
3. The retroviral life cycle was first characterized (1910) for a “filterable agent” from a chicken tumor, later named the Rous sarcoma virus (RSV).

Stylized drawing of a retrovirus



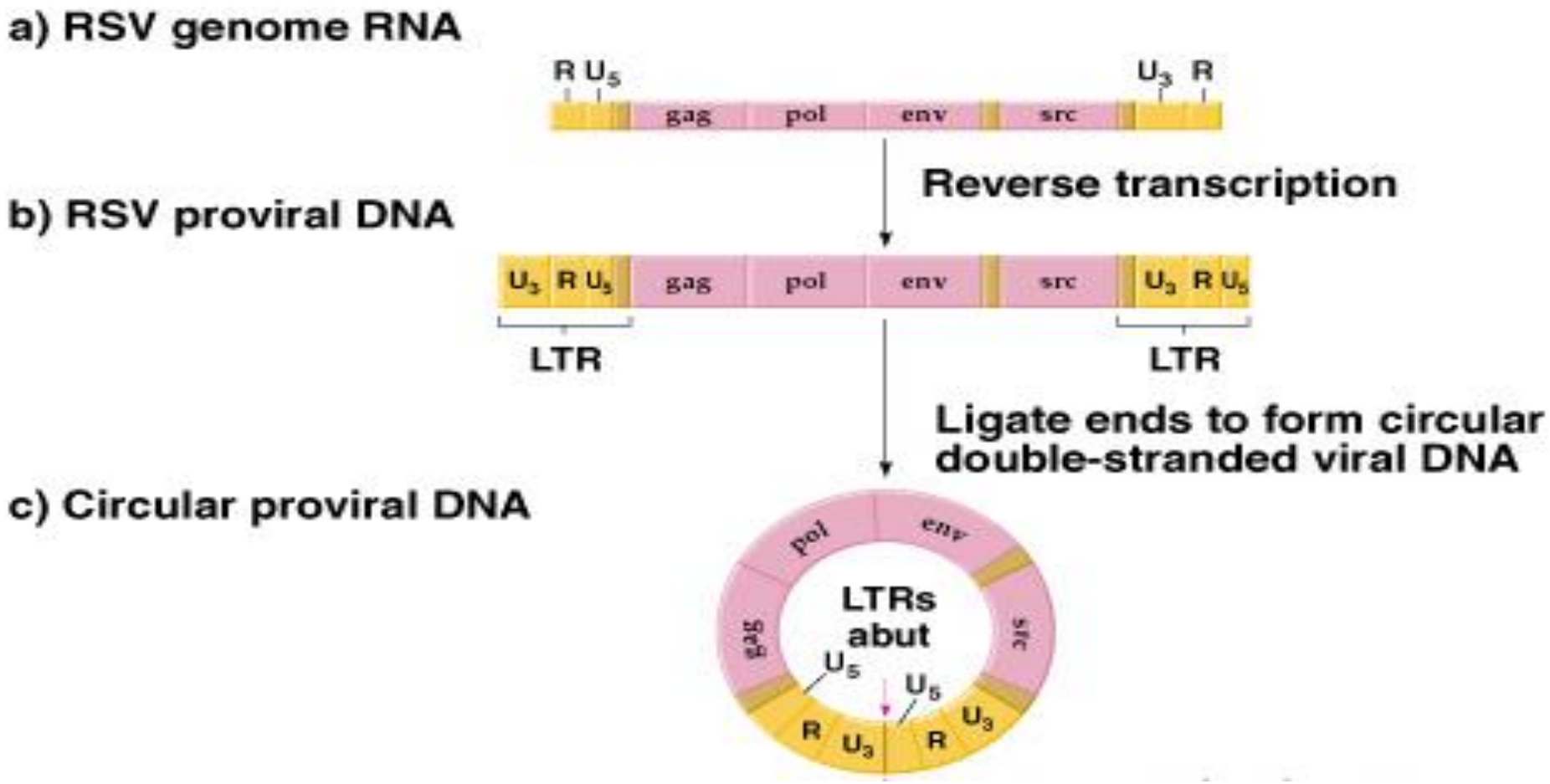
Life cycle of a nononcogenic retrovirus

HIV, a non oncogenic retrovirus, can cause cancer by making immune system less functional.

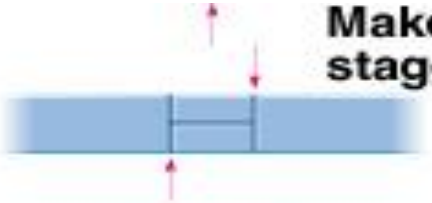


http://www.dnatube.com/video/306/Retroviruses-from-RNA-to-DNA

The Rous sarcoma virus (RSV) RNA genome and the integration of the proviral DNA into the host (chicken) chromosome

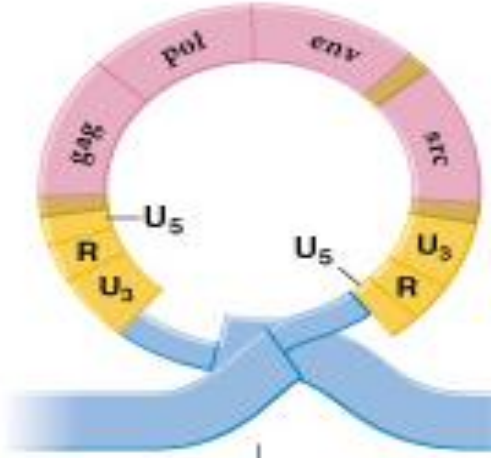


d) Integration of viral DNA begins



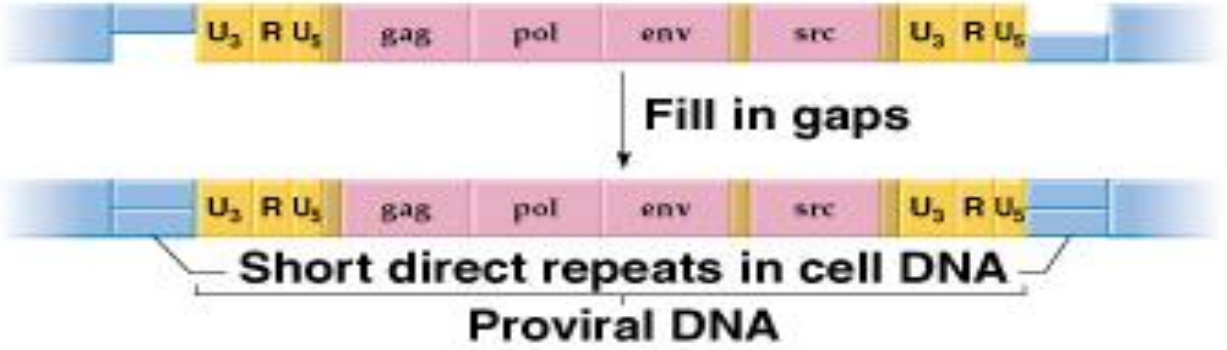
Make cut in viral DNA and staggered nicks in cell DNA

e) Viral ends become joined to the ends of the cell's DNA by recombination



Join ends of viral and cell DNA

f) Integration of viral DNA completed



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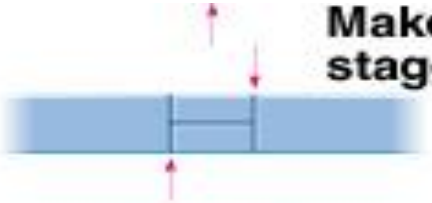

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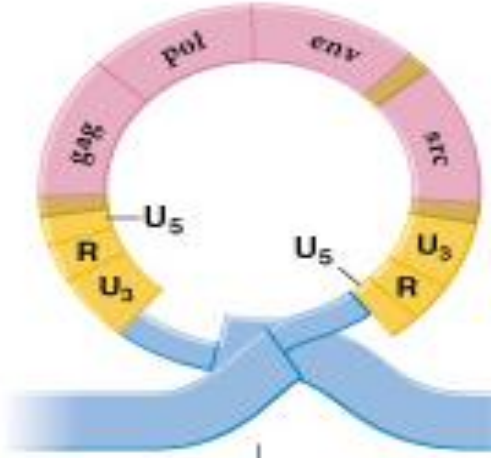

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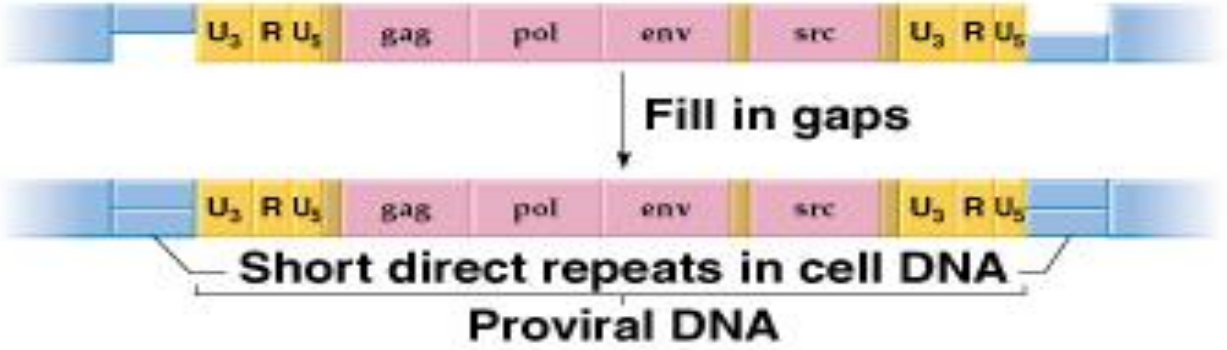


Oncogenic retrovirus (transducing retrovirus)

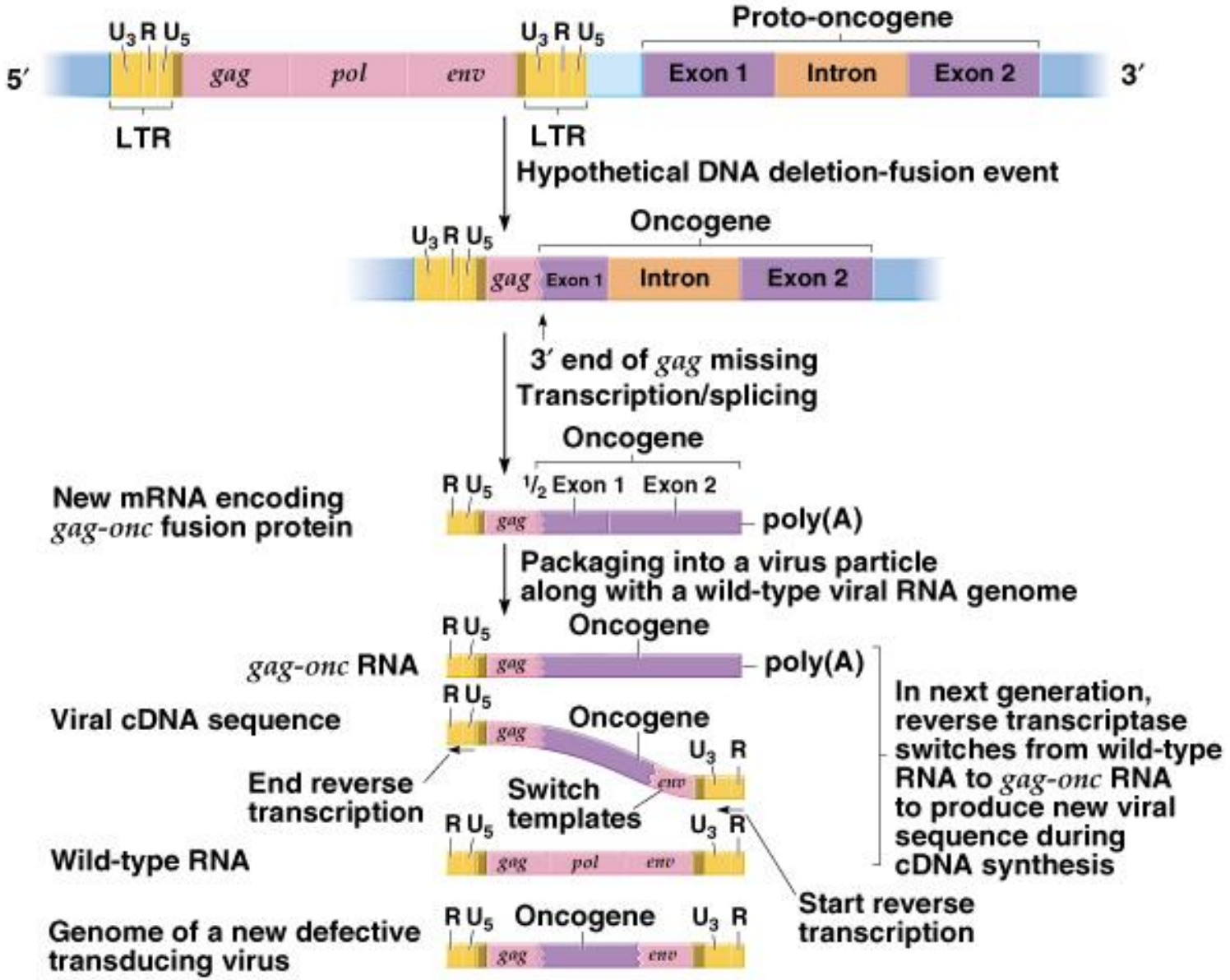
Retroviruses that carry an oncogene **(viral oncogene: v-onc)** are transducing retroviruses.

Different types of cancer are caused by different v-onc genes (e.g., the sarcoma gene, v-src, of RSV).

a. Most transducing retroviruses are defective, lacking one or more genes needed to replicate. If a helper virus supplies the missing gene product(s), progeny can be made.

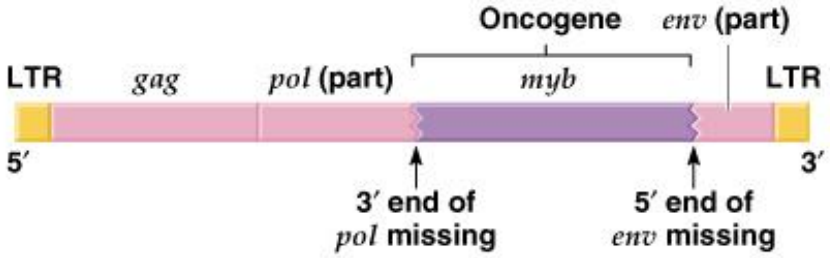
A rearrangment (usually deletion in proviral genes) connect provirus transcriptional unit to cellular genes which go under influence of viral promoter in LTR. If the gene picked up is oncogene or proto oncogene……..a transducing retrovirus has been produced.

a. RSV-infected cells rapidly transform, and also produce progeny RSV particles, because RSV is unusual in having intact gag, pol, and env genes.

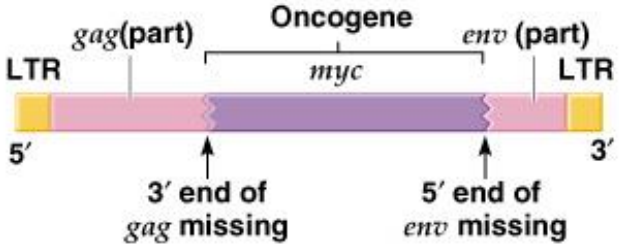


Structures of four defective transducing viruses (not to scale)

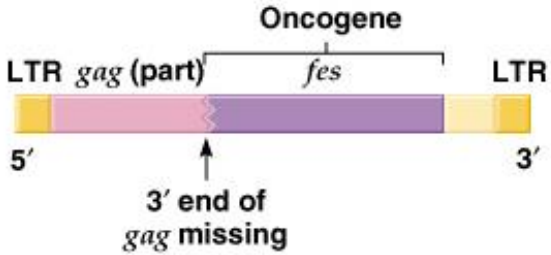
a) Avian myeloblastosis virus (AMV) genomic RNA



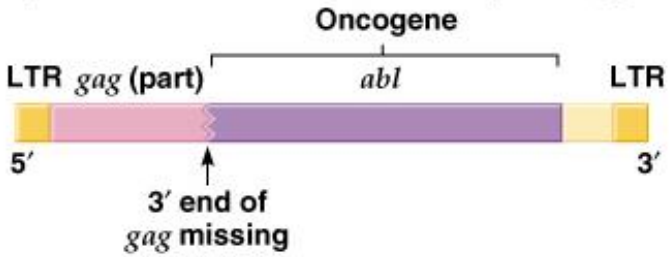
b) Avian defective leukemia virus (DLV) genomic RNA



c) Feline sarcoma virus (FeSV) genomic RNA



d) Abelson murine leukemia virus (AbMLV) genomic RNA



Cellular Oncogene

Genes in normal animal cells with close homology to v-onc genes were described by Bishop and Varmus (1970s) called proto oncogene. Later work by other researchers showed that:

a. Human cells have genes (proto-oncogenes) that are very similar to v-onc genes, but do not induce cancers.

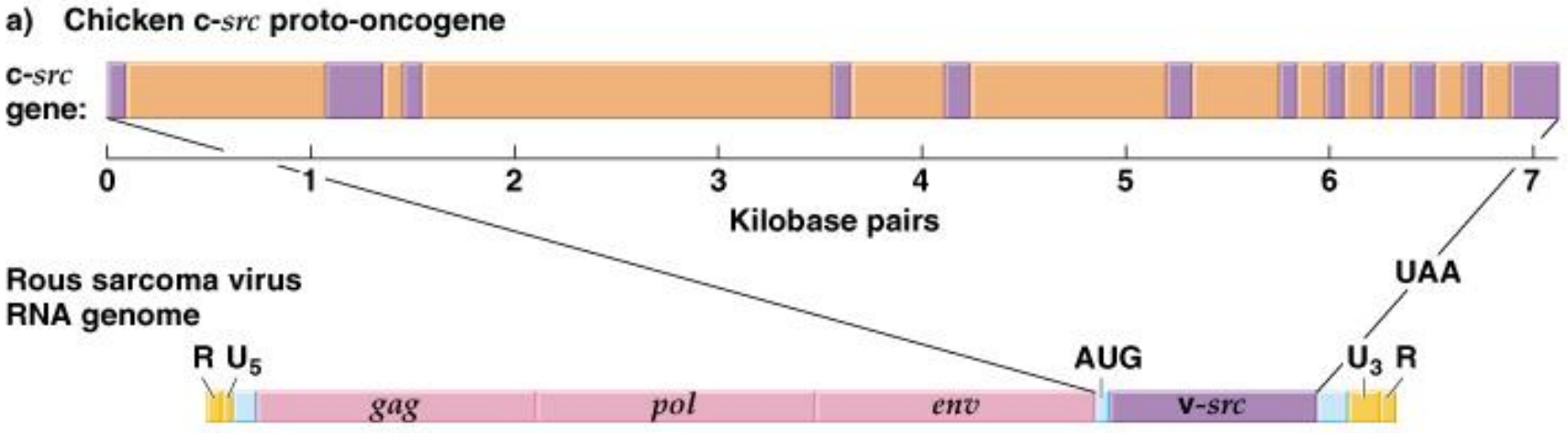
b. Proto-oncogenes are normal cellular genes that regulate cell division and differentiation.

c. Normal cells can become transformed without a tumor virus, if the proto-oncogene mutates to form a cellular oncogene (c-onc). It is a **dominant mutation**.

d. Oncogenes are present in human tumor cells, and cause transformation when introduced into normal cultured cells.

e. Proto-oncogenes contain introns that are missing in the corresponding v-onc.

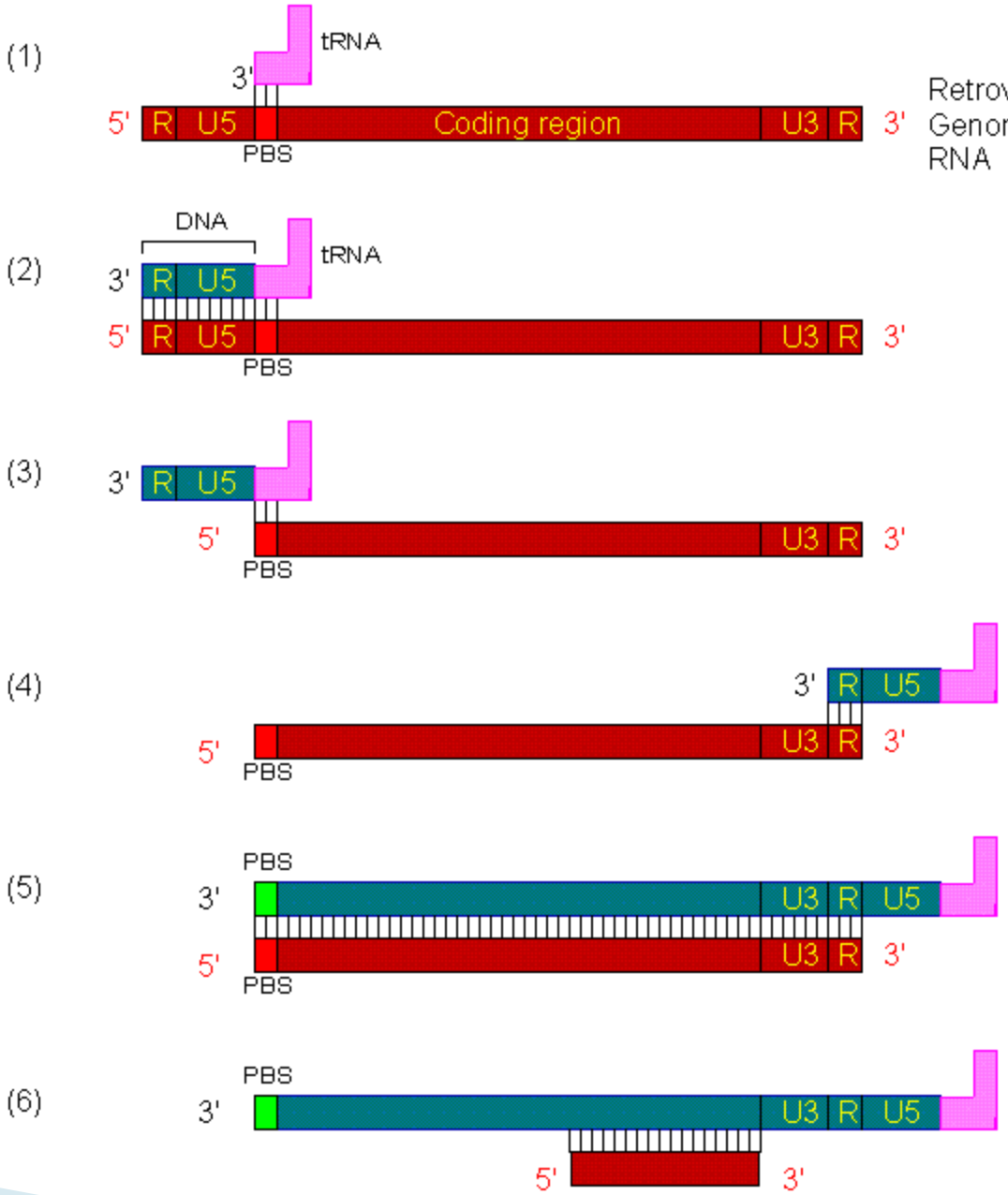
Fig. 18.10 The chicken c-*src* proto-oncogene and its relationship to v-*src* in Rous sarcoma virus

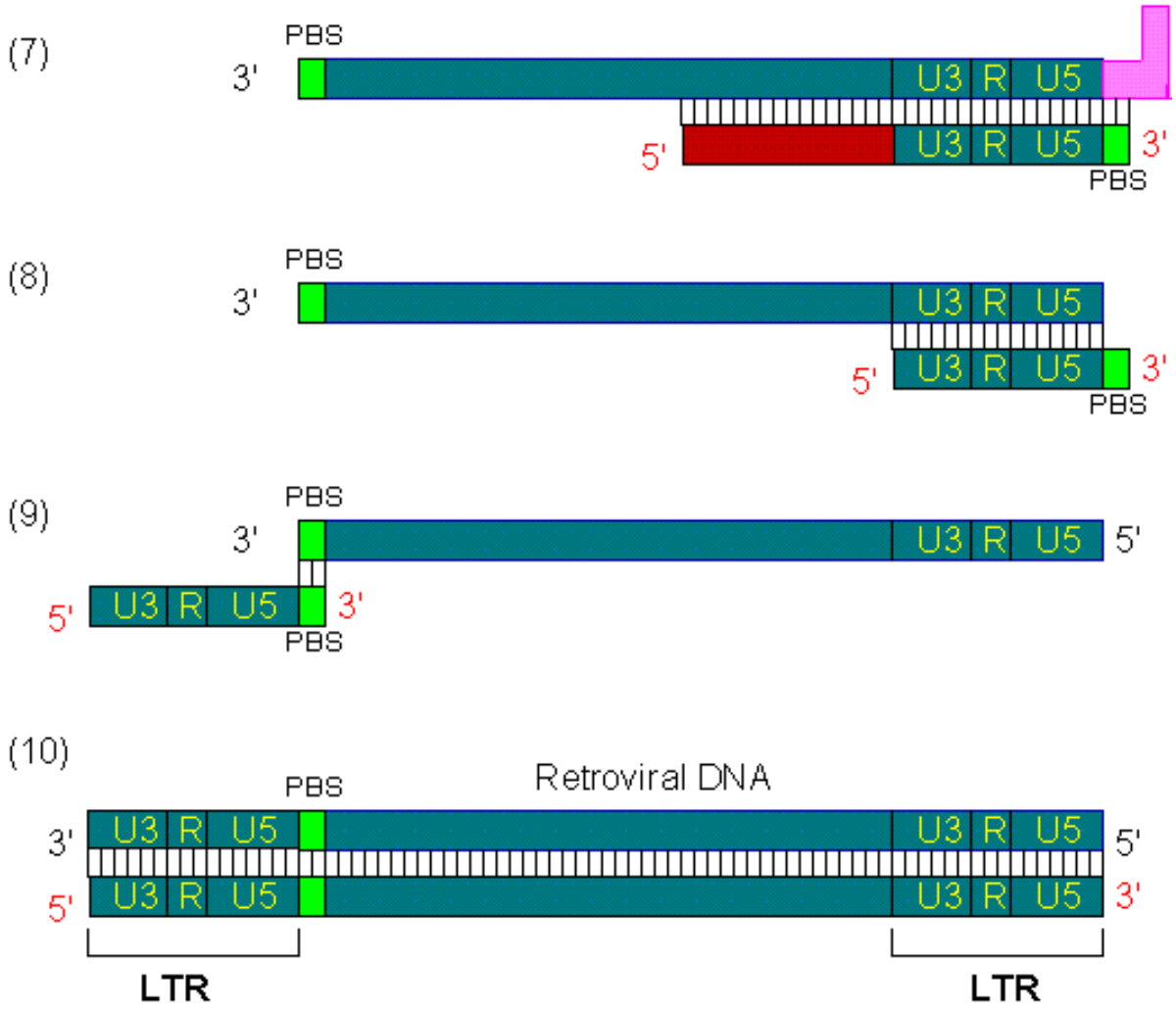


Changing Cellular Proto-Oncogenes into Oncogenes

1. Conversion of proto-oncogenes to oncogenes relaxes cell control, allowing unregulated proliferation. Examples:
   - a. **Point mutations** in the **coding or controlling** sequences can either change the gene product or alter its expression. The **ras** genes are an example:
     i. A point mutation produces a **mutant protein** that can cause cancer in many different types of cells.
     ii. G proteins **lose regulation**, and constitutive growth signals are transmitted to the cell.
   - b. **Deletions** of coding or controlling sequences can change the amount of activity of growth stimulatory proteins, allowing proliferation. The **myc** gene is an example:
     i. The myc gene product is a transcription factor that activates genes involved in cell division.
     ii. Deletions can **remove upstream sequences**, allowing expression from an alternative promoter and changing the amount or activity of the protein product.
   - **c. Gene amplification**, caused by random **overreplication** of regions of genomic DNA, has been found in tumor cells. Multiple copies of ras in mouse adrenocortical tumors are an example.

Reverse transcriptase activity





The entire process is catalyzed by **reverse transcriptase** which has both DNA polymerase and RNase H activities.

Protein Products of Proto-oncogenes (100)

1. Proto-oncogenes fall into classes with characteristic protein products, all of which stimulate cell growth. Some examples are:

   a. **Growth factors:** An example of growth factors is the viral oncogene v-sis, which encodes platelet-derived growth factor (PDGF).

      i. Deriving from mammalian blood platelets, PDGF causes fibroblasts to grow as part of wound-healing.

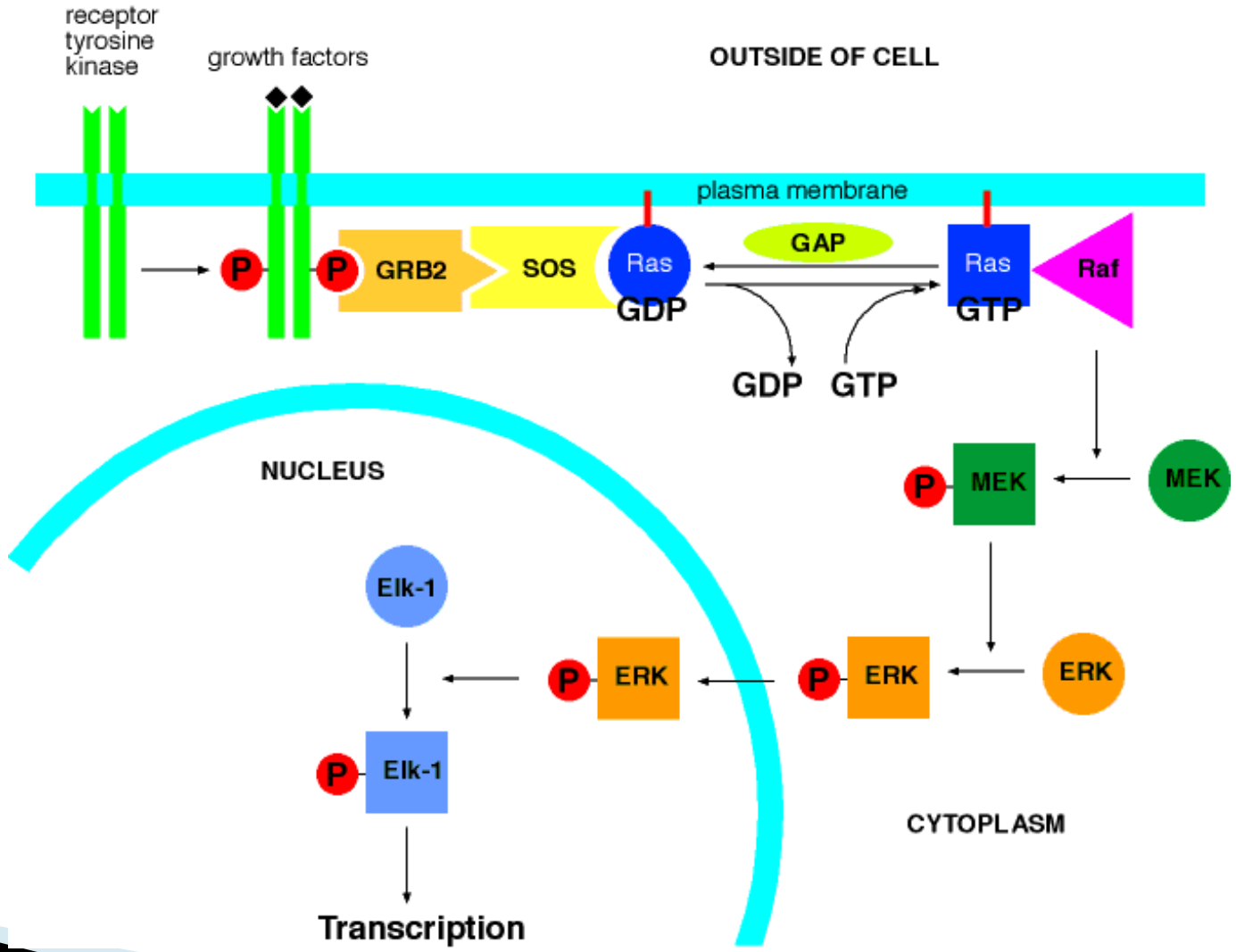
      ii. Introduction of a cloned PDGF gene into cells that normally do not express it (e.g., fibroblasts) transformed the cells.

   b. **Protein kinases:** An example of protein kinases is the src gene product, which encodes pp60src, a nonreceptor protein kinase.

      i. Both cellular and viral versions of the pp60src protein phosphorylate tyrosine (rather than serine or threonine).

      ii. Protein kinases are known to be involved in many aspects of cell signaling and growth regulation.

c. **Membrane associated G proteins activated by surface receptors :** Ras protein



http://www.bio.davidson.edu/courses/immunology/flash/mapk.html

http://www.dnatube.com/video/4100/GProtein-Signaling

Second mechanism of cancer induction by Retroviruses through proto oncogene

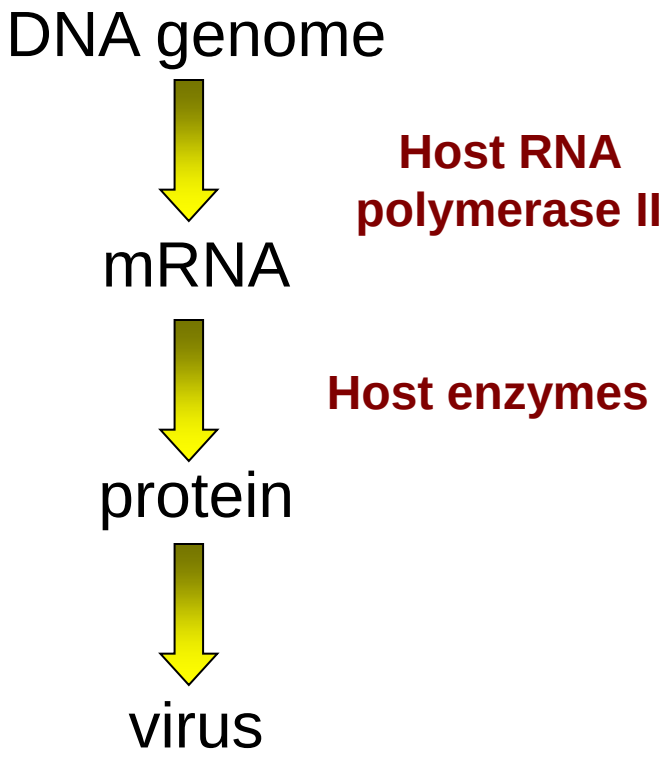
Proviral DNA may integrate near a proto-oncogene, and be transcribed from promoter and enhancer sequences in the viral LTR (insertional mutagenesis, rare in human and animals).

Insertion close to C-myc!

Oncogenesis by promotor insertion

Myc is located on chromosome 8 and is believed to regulate expression of 15% of all genes through binding on Enhancer Box sequences and recruiting histone acetyltransferases (HATs).

DNA Tumor Viruses



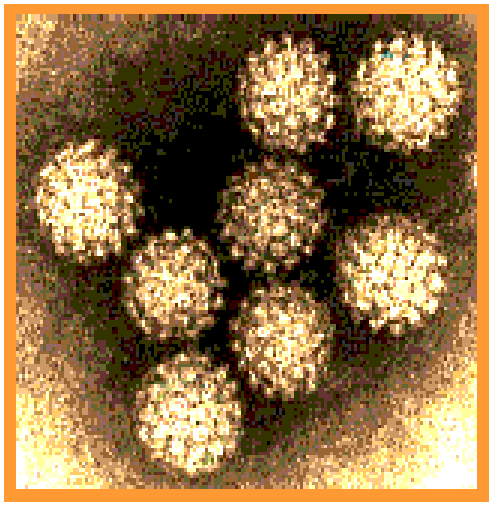
DNA Tumor Viruses

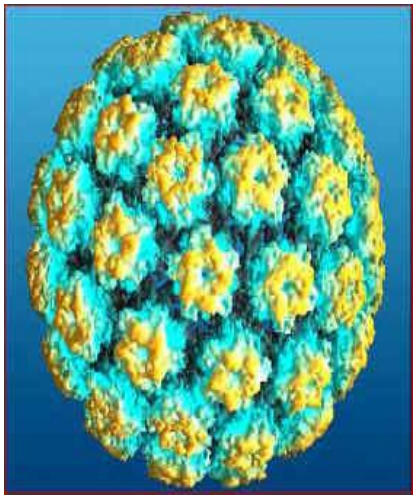
1. Oncogenic DNA viruses do not carry oncogenes, but may transform **cells using viral gene products**. Examples include:
   a. Papovaviruses.
   b. Hepatitis B viruses.
   c. Herpes viruses.
   d. Adenoviruses.
   e. Pox viruses.
2. DNA viruses induce production of cellular DNA replication enzymes, which are **used in viral replication**. Rarely, viral DNA **integrates** into the host genome instead, and may produce protein(s) that stimulate the cell to proliferate. An example:

Papilloma Viruses

The papovavirus group includes many different papillomaviruses, some of which cause:

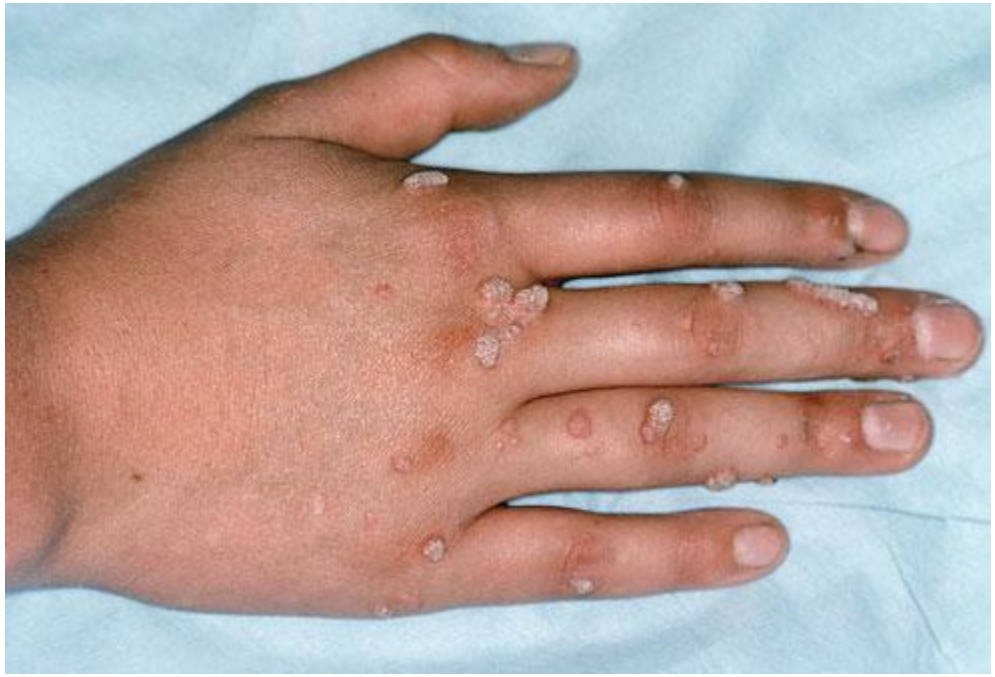
- Natural cancers in animals
- Benign warts
- Cancer in human, mostly malignancies of epithelial cells

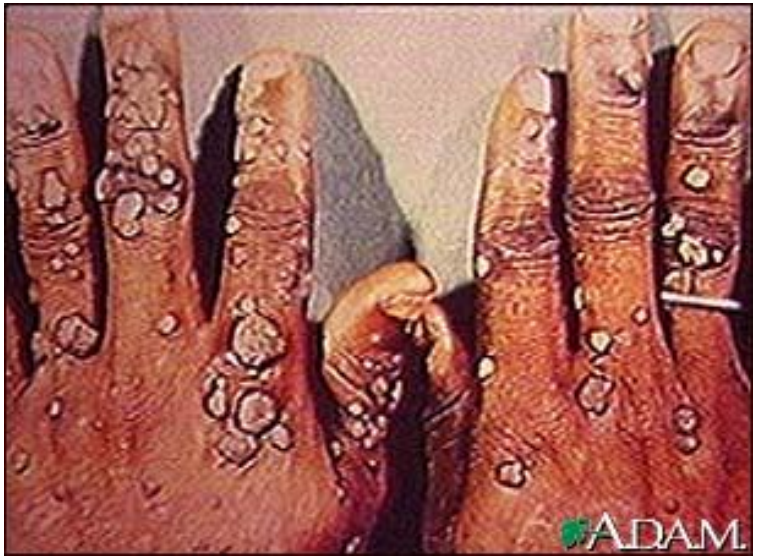




•51 types identified - most common are types 6 and 11

DNA Tumor Viruses: Human Wart
(noncancerous skin growths)





Human Papilloma virus and tree man

http://www.dnatube.com/video/4842/Skin-Warts--3D-Medical-Animation

Papilloma Viruses

ii. Human cervical cancer (HPV-16, HPV-18), due to action of the E6 and E7 genes, which influence cell growth and division.

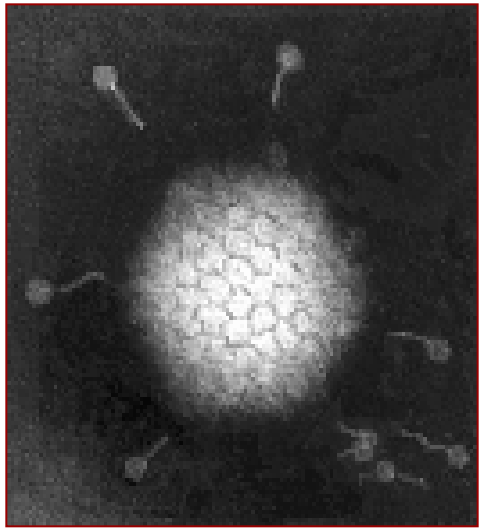
A world-wide study of almost 1,000 cervical cancers indicated that more than 90% contain HPV DNA.

Adenoviruses

Highly oncogenic in animals

Only part of virus genome is integrated

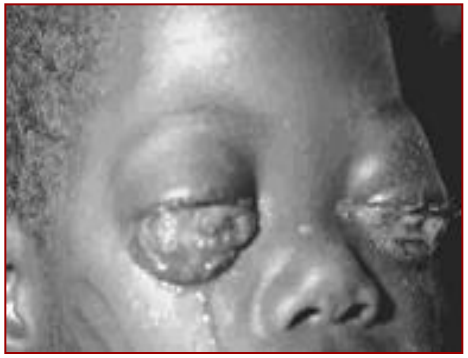
Always the same part



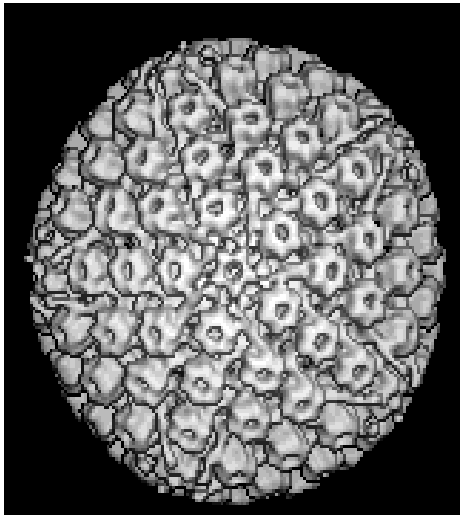
Herpes Viruses

Considerable evidence for role in human cancer

- **Some very tumorigenic in animals**
- **Viral DNA found in small proportion of tumor cells: "hit and run"**



- **Epstein-Barr Virus**
- **Burkitt's Lymphoma**
- **Transforms human B-lymphocytes *in vitro***

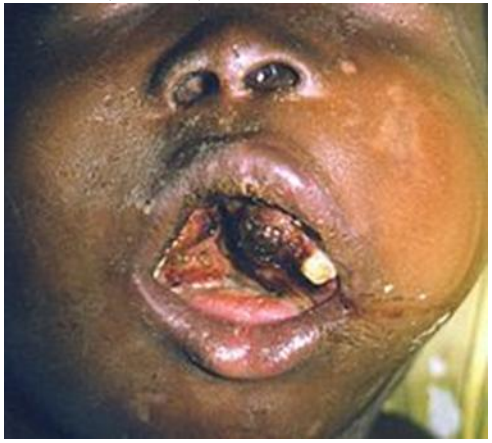


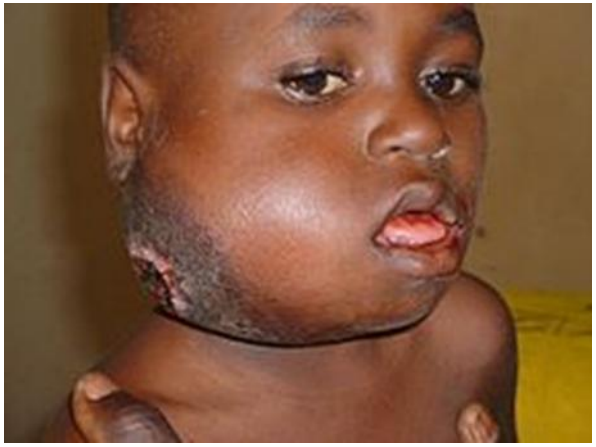
- **<u>Burkitt's lymphoma</u>**, B lymphocyte, disregulation of protooncogene c-myc (8q24), TF for 15% of genes, binding to enhancers and role in chromatin decondensation (recruiting HATs),
- The reciprocal translocation positions the *MYC* proto-oncogene next to an active immunoglobulin gene, resulting in over-expression of *MYC* and development of the lymphoma

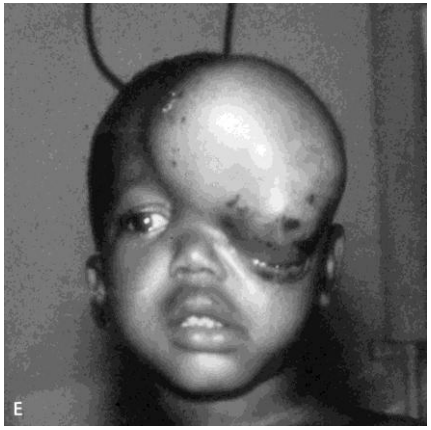
t (8:14) in 85%

t (2:8)

t (8:22)







Chronic myelogenous leukemia (CML; OMIM 151410)

i. Myeloblasts (stem cells of white blood cells) replicate uncontrollably.

ii. 90% of CML patients have the Philadelphia chromosome (*Ph*1) reciprocal translocation.

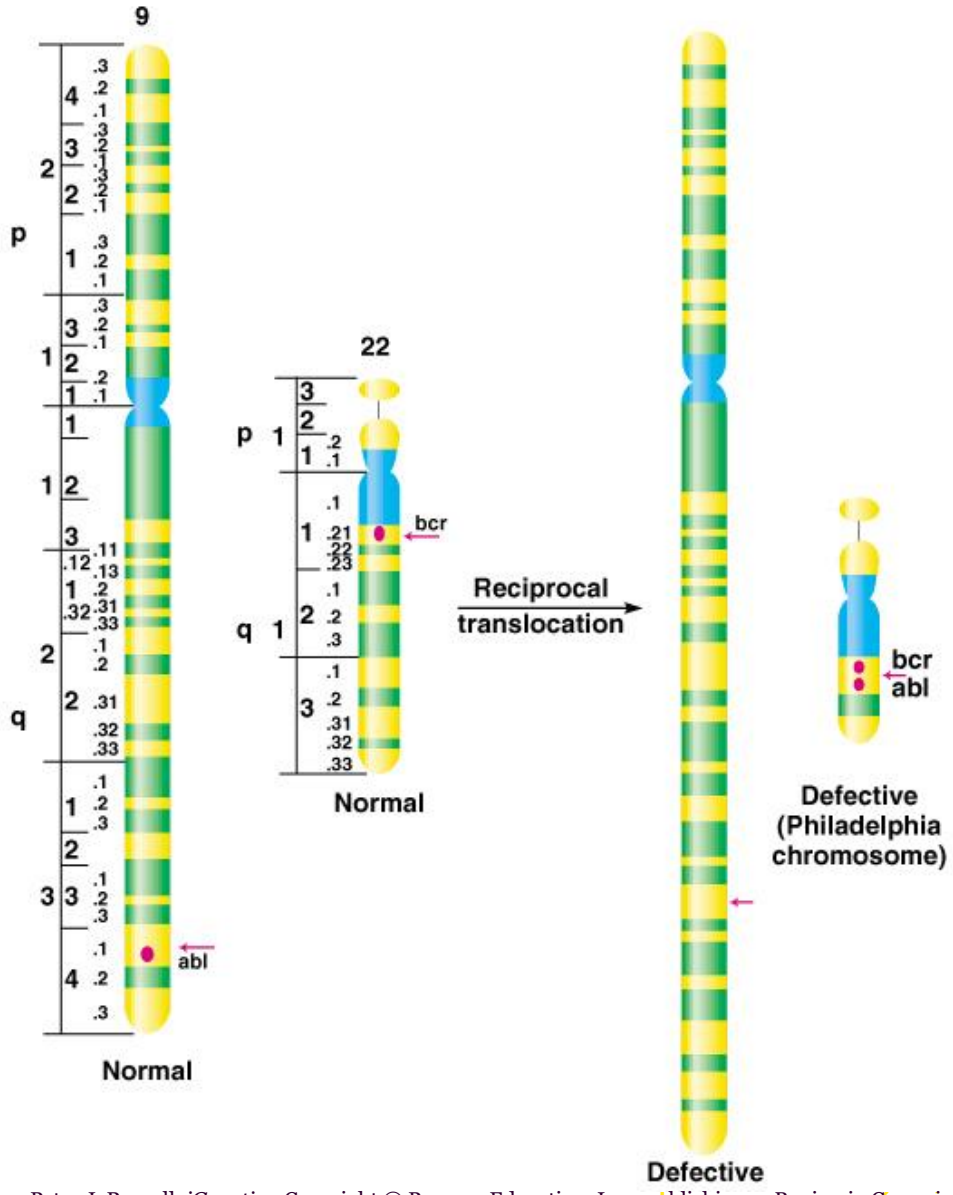
iii. The reciprocal translocation causes transition from a differentiated cell to a tumor cell, by translocating a proto-oncogene from chromosome 9 to chromosome 22, and probably converting it to the *ABL* oncogene.

iv. The hybrid gene arrangement causes expression of a leukemia-producing gene product.

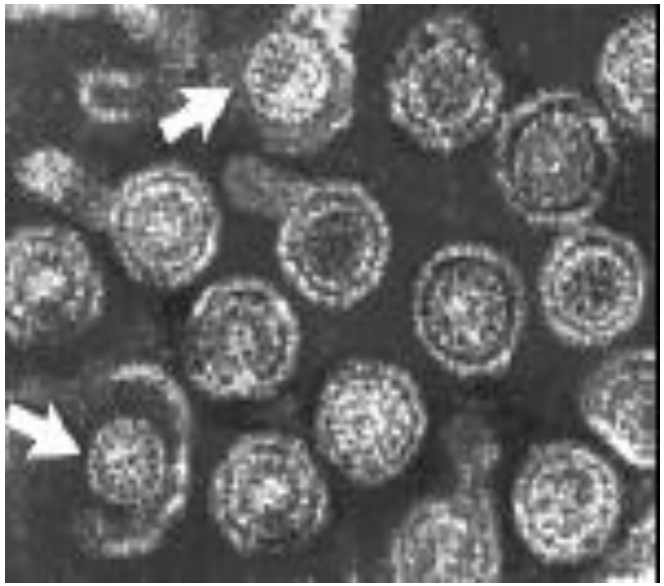
The ABL1 proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response.

BCR: serine/threonin kinase

Fig. 17.13 Origin of the Philadelphia chromosome in chronic myelogenous leukemia (CML) by a reciprocal translocation involving chromosomes 9 and 22



Hepatitis B Virus



DNA genome

↓ **RNA polymerase II**

RNA Provirus

Host enzyme

↓ **Reverse transcriptase**

Viral enzyme

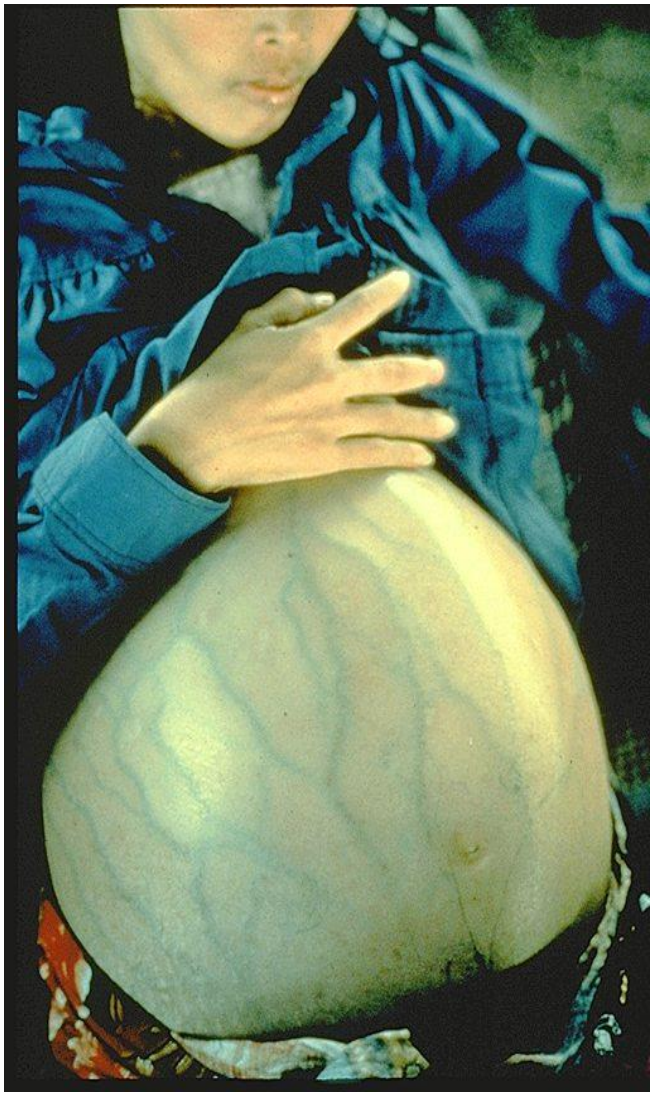
DNA genome

- **Vast public health problem**
- **10% of population in underdeveloped countries are chronic carriers**
- **Long latency**

Epidemiology:

Strong correlation between HBV and hepatocellular carcinoma

China: 500,000 - 1 million new cases of hepatocellular carcinoma per year



This female Cambodian patient presented with a distended abdomen due to a hepatoma resulting from chronic hepatitis B infection. The incidence of hepatocellular carcinoma in patients with a hepatitis B infection is 12 to 300 times greater. The HBV DNA is incorporated into the hepatocytic DNA during the disease pathogenic process.

DNA Tumor Viruses In Human Cancer

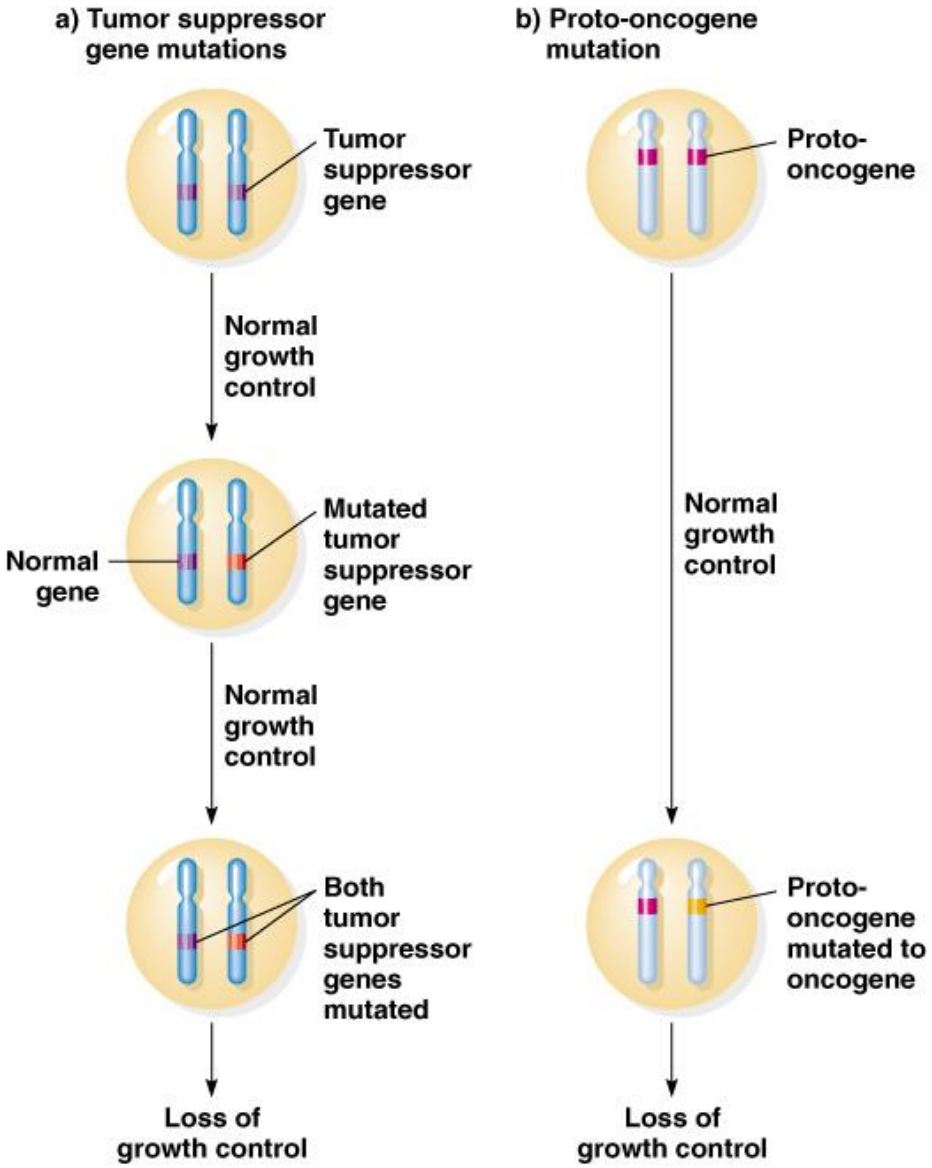
Summary

- **Can transform cells or have lytic life cycle**
- **Often integrate into host genome**
- **In transformation ONLY early genes are transcribed**

Tumor Suppressor Genes (20)

1. Harris (1960s) showed that fusion of cancer cells and normal cells did not always result in a tumor, indicating the existence of tumor suppressor genes.
2. In certain cancers, both homologous chromosomes show deletion of specific regions, the sites of tumor suppressor genes that inhibit cell growth and division. Human examples include:
   a. Breast cancer.
   b. Colon cancer.
   c. Lung cancer.
3. Action of tumor suppressors is the opposite of proto-oncogenes.
4. Both tumor suppressor genes must be lost for unregulated growth to occur (they are recessive), while only one mutation is needed to change a proto-oncogene to an oncogene (it is dominant).

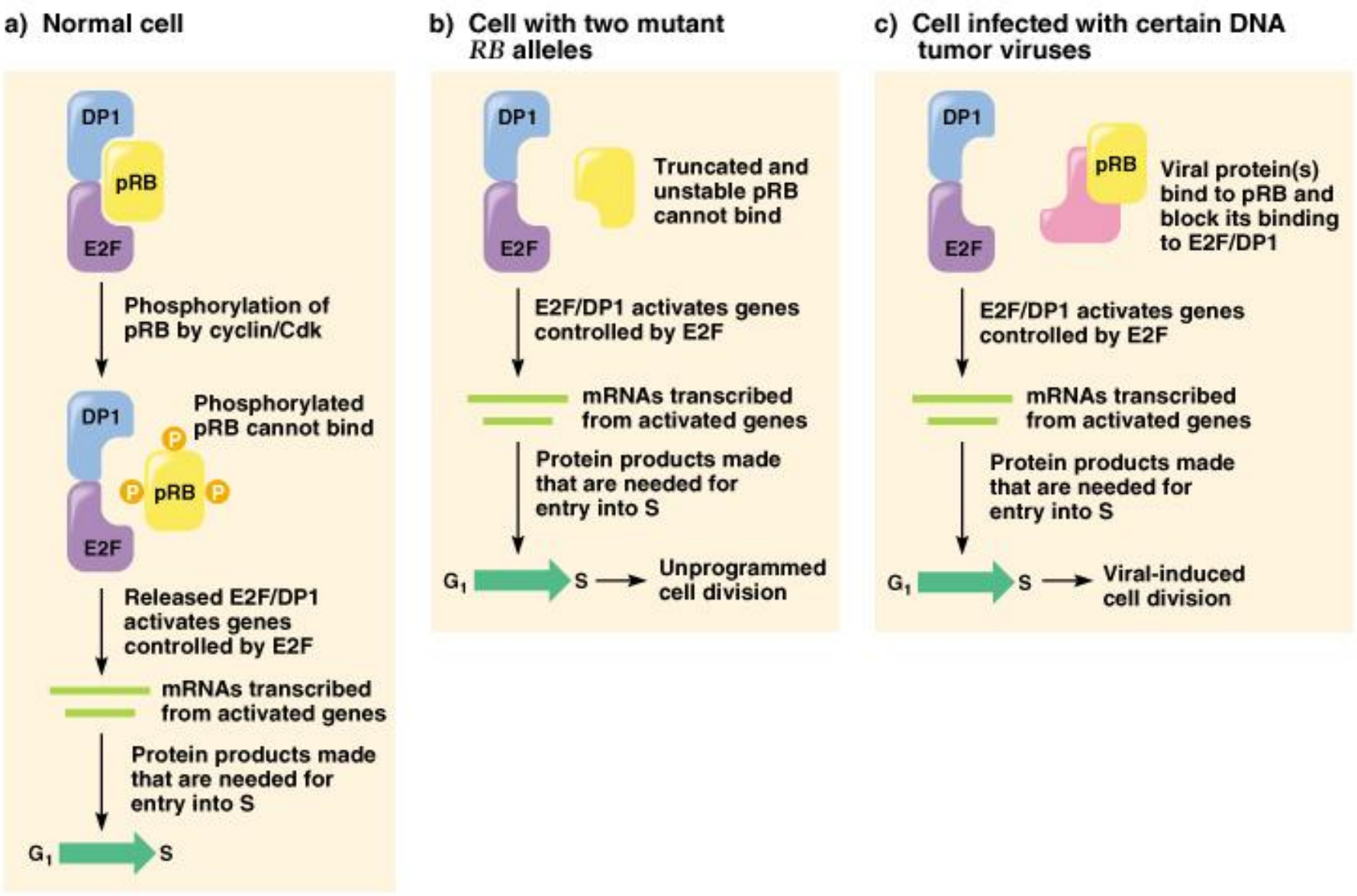
Fig. 18.12 Comparison of the effects of tumor suppressor gene and proto-oncogene mutations



The Retinoblastoma Tumor Suppressor Gene

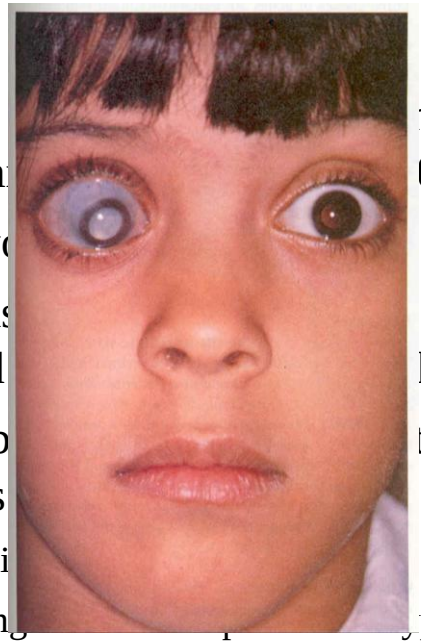
1. The human RB tumor suppressor gene has **been mapped (13q14.1-q14.2) and sequenced**.
   a. Its 180 kb of DNA encodes a 4.7 kb mRNA that produces a 928-amino-acid nuclear phosphoprotein, pRB.
   b. pRB is expressed in **every tissue type examined**, regulating cell cycle and all major cellular processes.
   c. Tumor cells **have point mutations or deletions** in the gene, leading to loss of pRB function.
   d. Karyotype analysis detects about 5% of RB mutants, and the remainder are difficult to detect even with molecular techniques.

2. The cell cycle transition from **G_1 to S** is regulated by pRB, committing the cell to the rest of the cycle.
   a. In a normal G_1 cell, pRB binds two transcription factors, E2F and DP1 (Figure 18.13).
   b. As long as pRB stays bound to the factors, the cell remains in G1 or enters G_0.
   c. At the signal to progress through the cell cycle, cyclin/cyclin-dependent kinase (Cdk) phosphorylates pRB so that it is unable to bind E2F.
   d. Free E2F now binds and activates transcription of genes required for entry into S phase.
   e. After the cell completes mitosis, pRB is dephosphorylated.



Incidence of Cancer

- The **incidence of cancer** falls into two categories:
  a. Sporadic cancers, the more frequent type, do not appear to have an hereditary cause.
  b. Familial (hereditary) cancers run in families.



  - Retinoblastoma is the …nor in children birth to 4 years. Early treatment (usually gam… 0% effective.
  - Retinoblastoma has tw…
    (1) Sporadic retinobla… in children with no family history of retinoblastoma, and …lateral tumor).
    (2) Hereditary retinob…ts typically develop multiple tumors involving both eyes
      (a) Onset is usually earli…
      (b) Siblings and offsprin… pe of tumor.
      (c) Pedigrees of affected families are consistent with a single gene responsible for retinoblastoma.

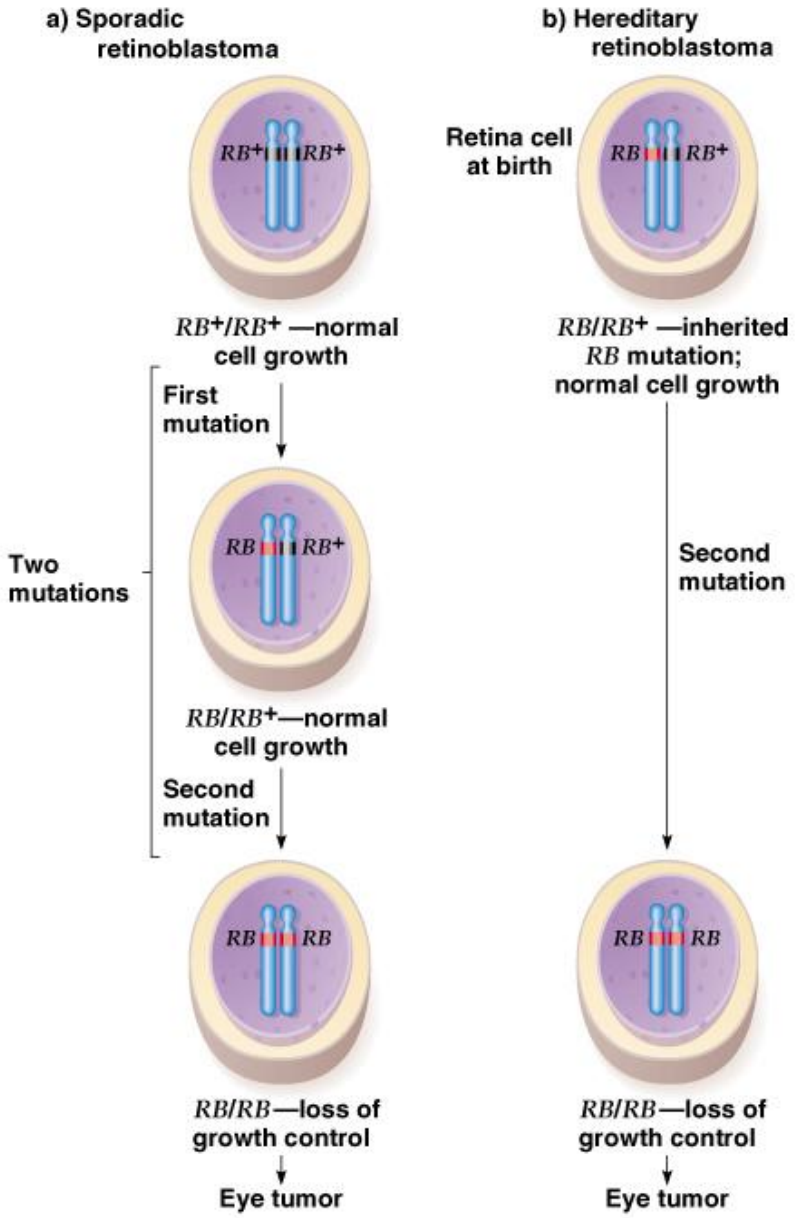
The Two-Hit Mutation Model for Cancer

❑Cancers can be caused by viruses, but most result from mutations in cellular genes. Usually these mutations have accumulated over time, and research has identified the genes involved.

Knudson (1971) proposed the 2-hit mutational model, that two mutations were required for development of retinoblastoma.

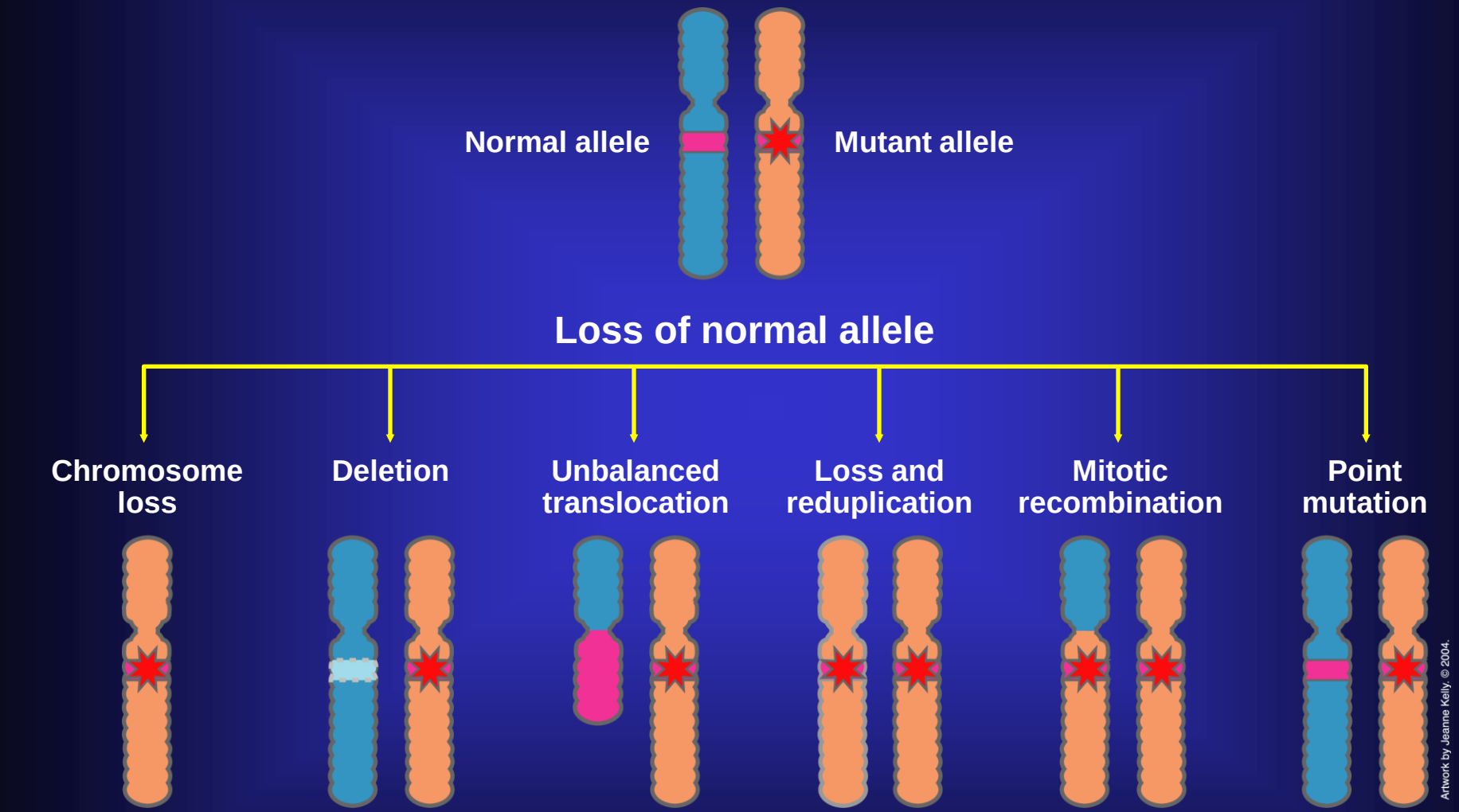
a. In sporadic retinoblastoma, the child starts with two wild-type alleles (RB^+/RB^+).
   i. Both alleles must mutate to produce the disease genotype (RB/RB).
   ii. The probability of both mutations occurring in the same cell is low, so only one tumor forms.
b. In hereditary retinoblastoma, the child starts out heterozygous (RB/RB^+).
   i. Only one mutation is needed for tumor formation (RB/RB).
   ii. Mutations resulting in loss of heterozygosity (LOH) are likely in rapidly dividing cells, and multiple tumors occur.

Knudson's two-hit mutation model



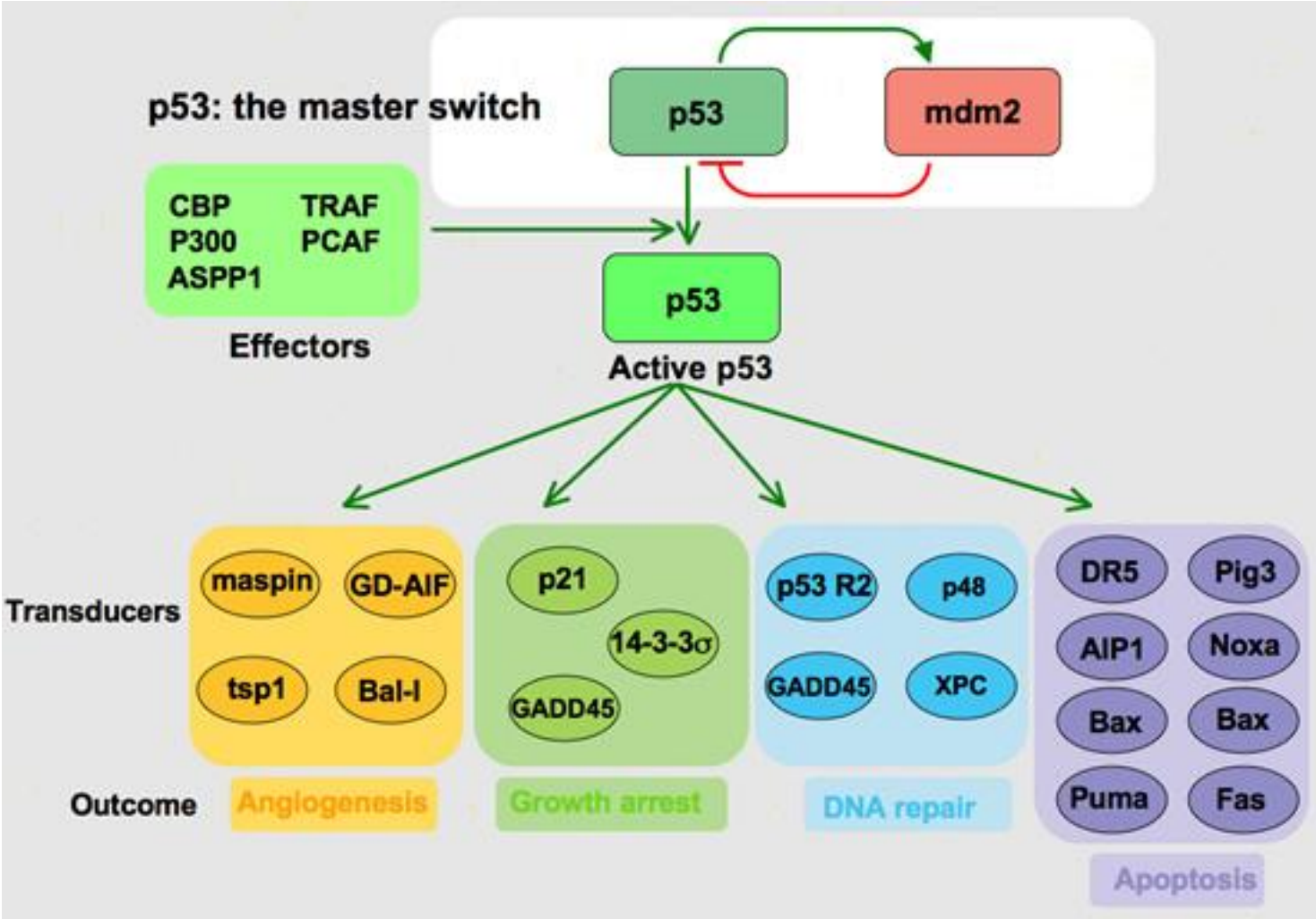
4. In Knudson's model:
   a. Retinoblastoma alleles are recessive, because only homozygotes (RB/RB) develop tumors.
   b. However, in pedigree analysis, the disease appears to be dominant. This is because:
      i. Heterozygous individuals (RB/RB^+) are predisposed to the cancer, since only one mutation is required for the neoplasm. Families with one allele already mutated will have a significant incidence of the disease.
      ii. Homozygous dominant individuals (RB^+/RB^+) develop the cancer only when both alleles in the same cell are mutated. Therefore, most children in the general population do not develop the disease.
5. This hypothesis is supported by later studies of the chromosomes of retinoblastoma patients, which:
   a. Mapped the gene to 13q14.1-q14.2 (long arm of chromosome 13).
   b. Showed that the gene encodes a growth inhibitory factor (tumor suppressor).
6. Retinoblastoma is rare among cancers because a single gene is critical for its development. In most cases, cancers result from a series of mutations in different genes for growth and division.

Heterozygous Condition



The *p53* Tumor Suppressor Gene

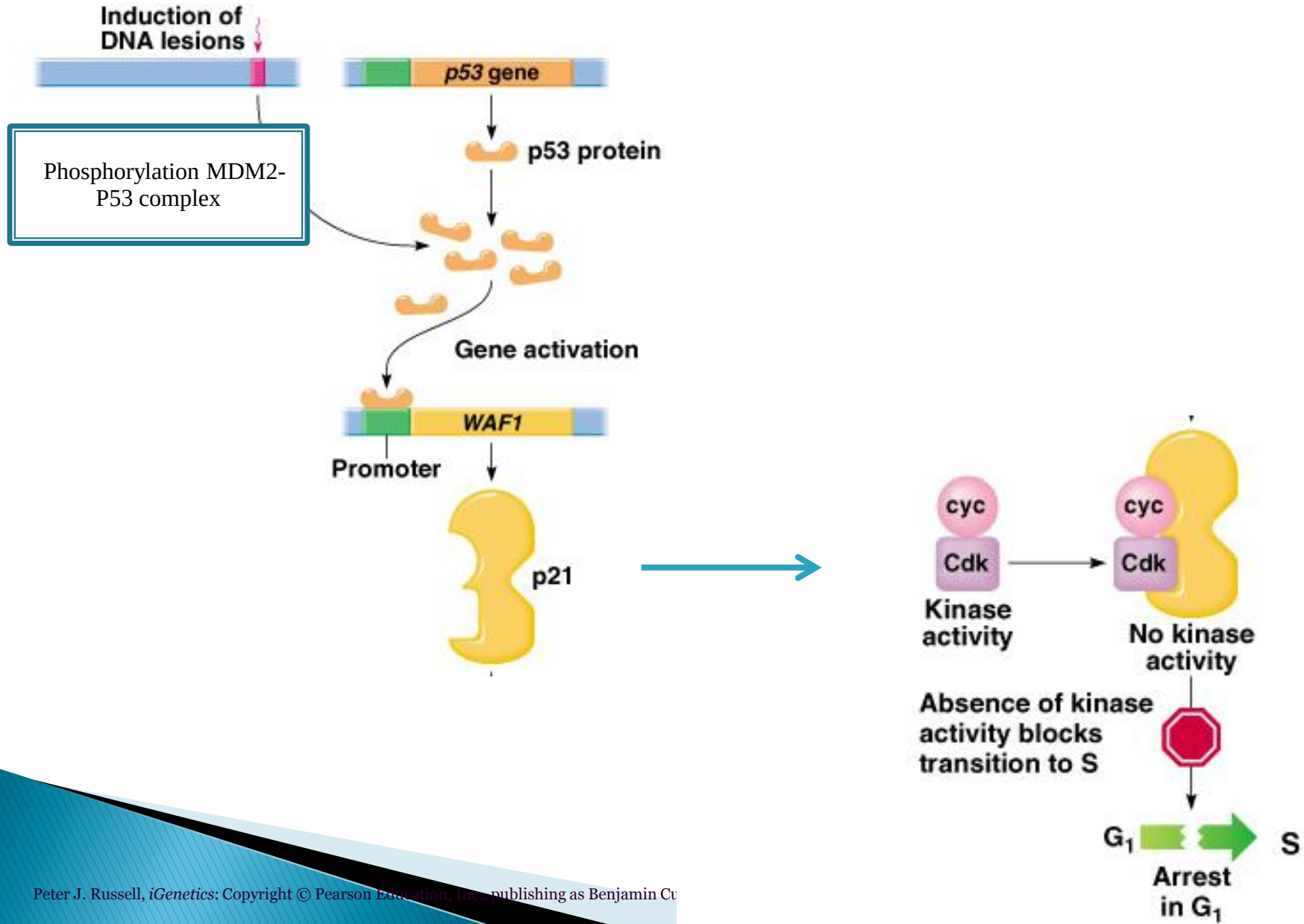
- Most cancers result from mutations in several genes. A gene mutated in about 1⁄2 of human cancers is p53, encoding a 53 kDa tumor suppressor protein (chromosomal location 17p13.1).
  a. Inheritance of 1 mutant p53 allele results in Li-Fraumeni syndrome, with 25 folds risk of developing cancer after age 50.
  b. Tumors arise when the second p53 allele is mutated, so the trait is inherited as an autosomal dominant.
- The p53 tumor suppressor protein (393 amino acids) is involved in many processes, including:
  a. Transcription.
  b. Cell cycle control.
  c. DNA repair.
  d. Apoptosis (programmed cell death).


p53: the master switch
p53
mdm2
CBP
P300
ASPP1
TRAF
PCAF
Effectors
p53
Active p53
Transducers
maspin
GD-AIF
tsp1
Bal-I
p21
14-3-3σ
GADD45
p53 R2
p48
GADD45
XPC
DR5
Pig3
AIP1
Noxa
Bax
Bax
Puma
Fas
Outcome
Angiogenesis
Growth arrest
DNA repair
Apoptosis

- Damage to cellular DNA (e.g., by irradiation) causes *p53* to initiate the cascade of events leading to G_1 arrest.

  The cell has time to repair the DNA damage, before allowing the cell cycle to resume.

Cascade of events by which DNA lesions cause an arrest in G_1



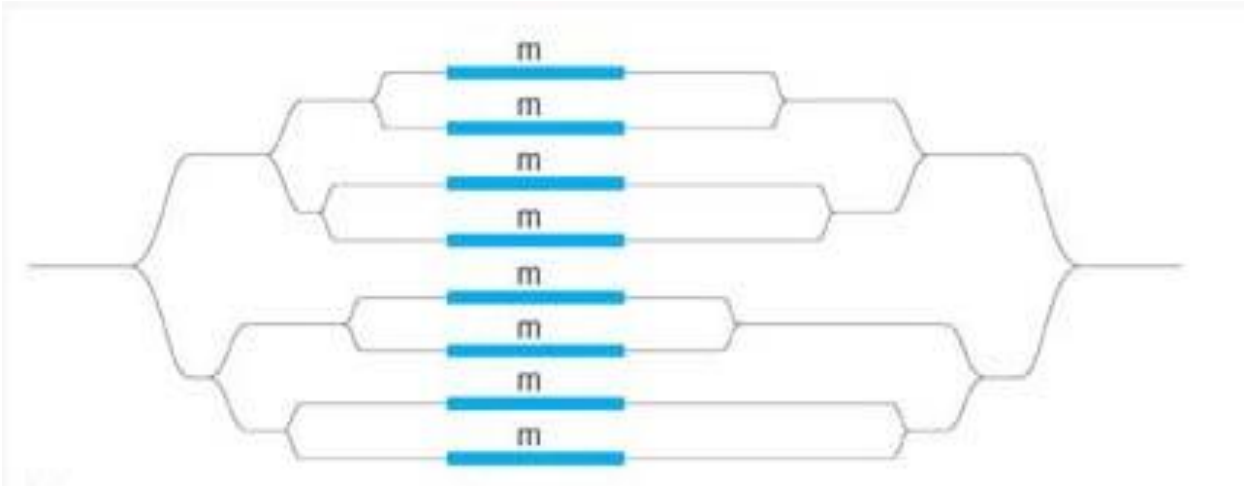
- P53 provide protection against oncogenes. ARF activated by oncogenes (ras), produce p14 which blocks p53 unstability by MDM2.
- If there is too much DNA damage, p53 induces apoptosis instead of DNA repair genes or WAF1. BAX is activated which prevent apoptosis by competing with Bcl2 an anti apoptotic agent.

- Transgenic mice with deletions of both *p53* alleles (knockout mice) are fully viable.
  a. This indicates that *p53* is not essential for growth, cell division or differentiation.
  b. The *p53/p53* knockout mice have one major phenotype, a very high frequency of cancers (100% by the tenth month).

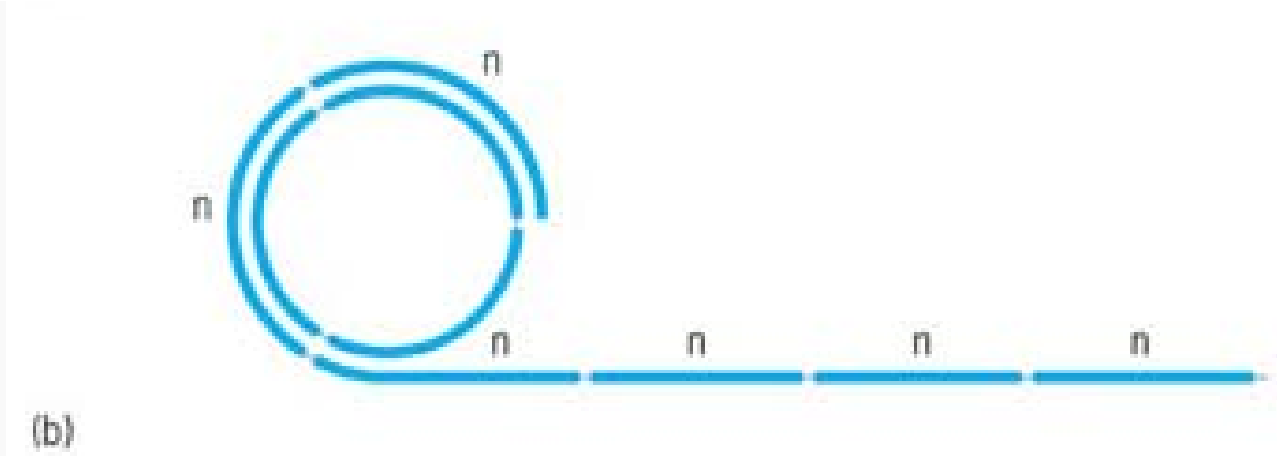
- If both *p53* alleles are inactivated, WAF1 cannot be activated and p21 will not be available to block Cdk activity.
  a. The cell is unable to arrest in G_1, and the cell cycle proceeds to S, regardless of DNA damage.
  b. Apoptosis does not occur without *p53*.
  c. Cell division produces cells with unrepaired genetic damage, allowing mutations to accumulate, and raising the risk of cancer.

Defective G1 to S checkpoint……replication of ss nicks……produce double strand breaks…….chromosome rearrangment…….gene amplification seen in most cancers.

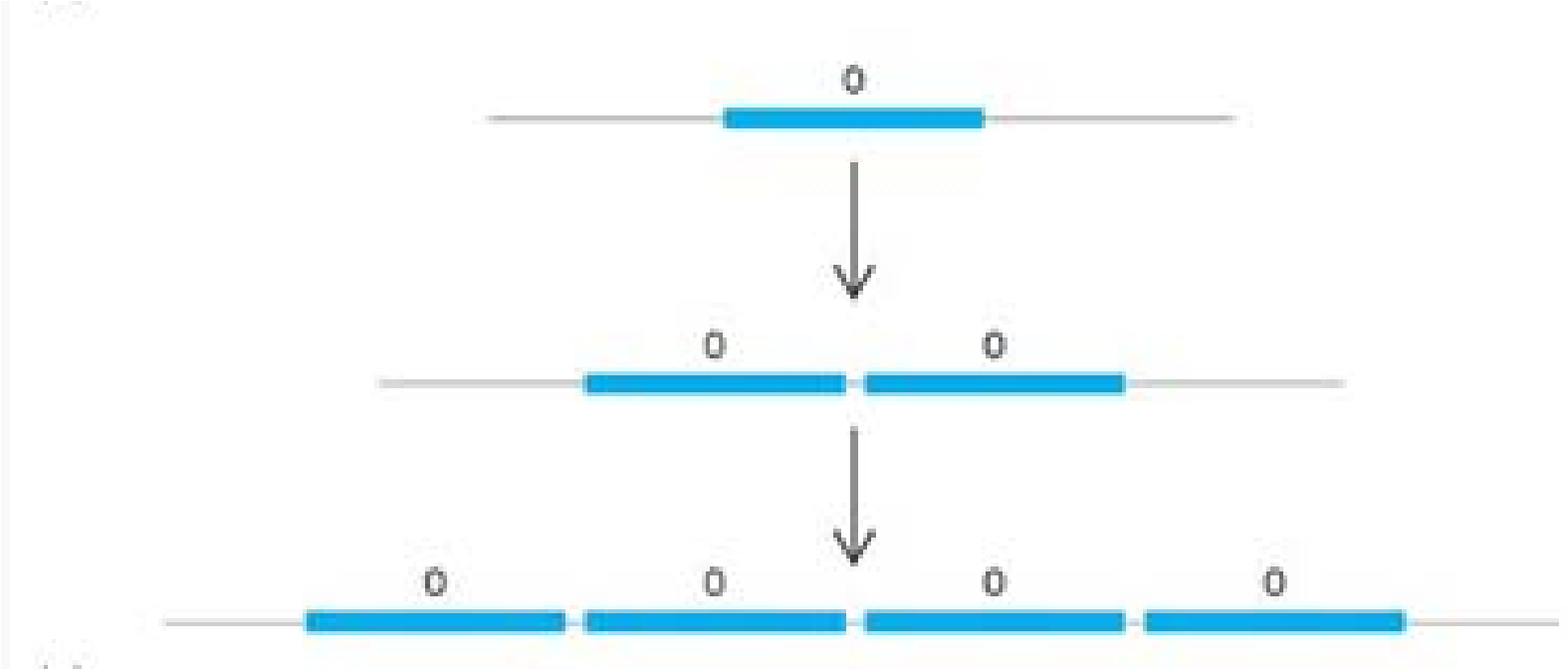
Gene Amplification



Multiple initiation of replication; the replication forks remain unresolved.

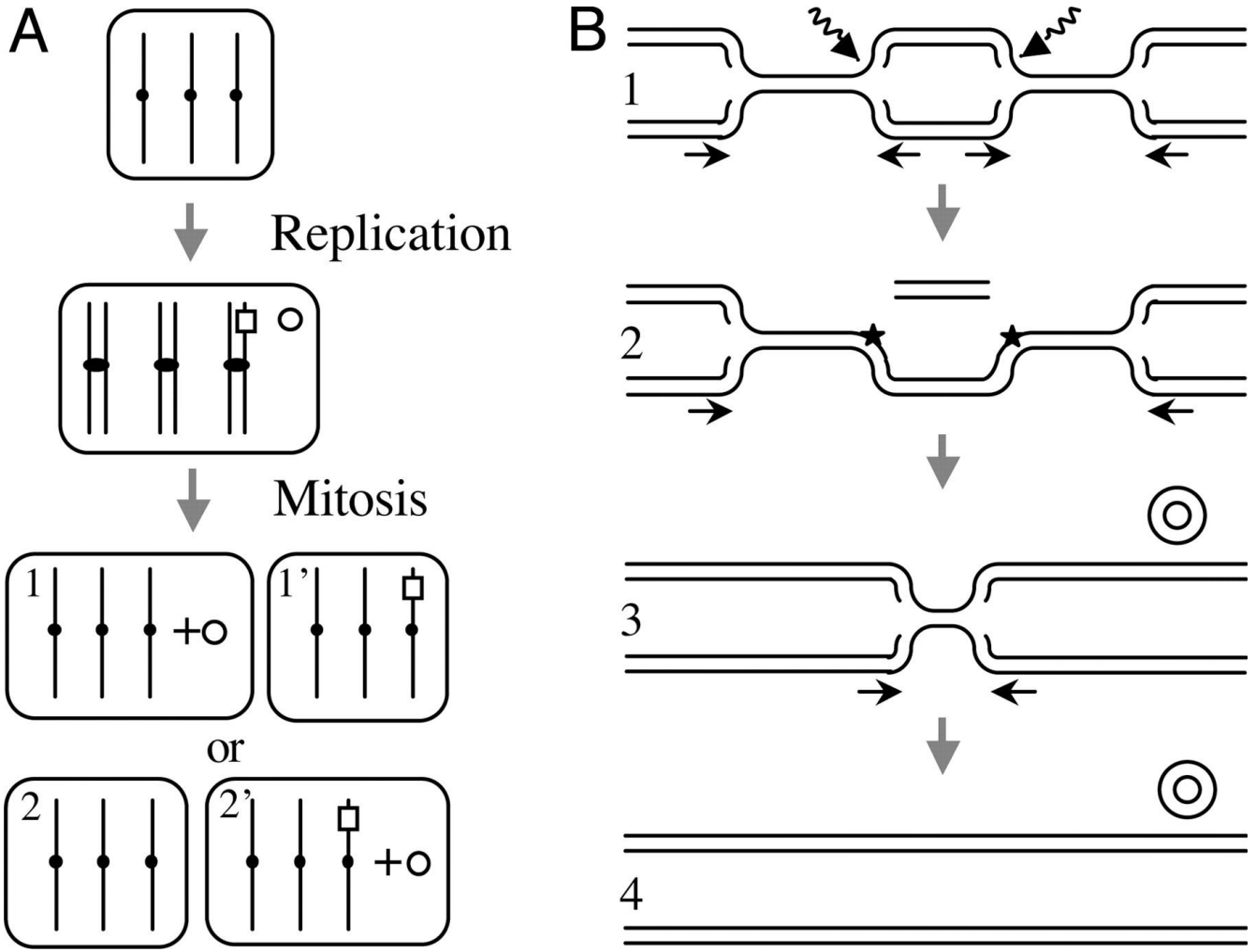


Rolling circle: genes are copied from a single-stranded circle; they become double-stranded subsequently.

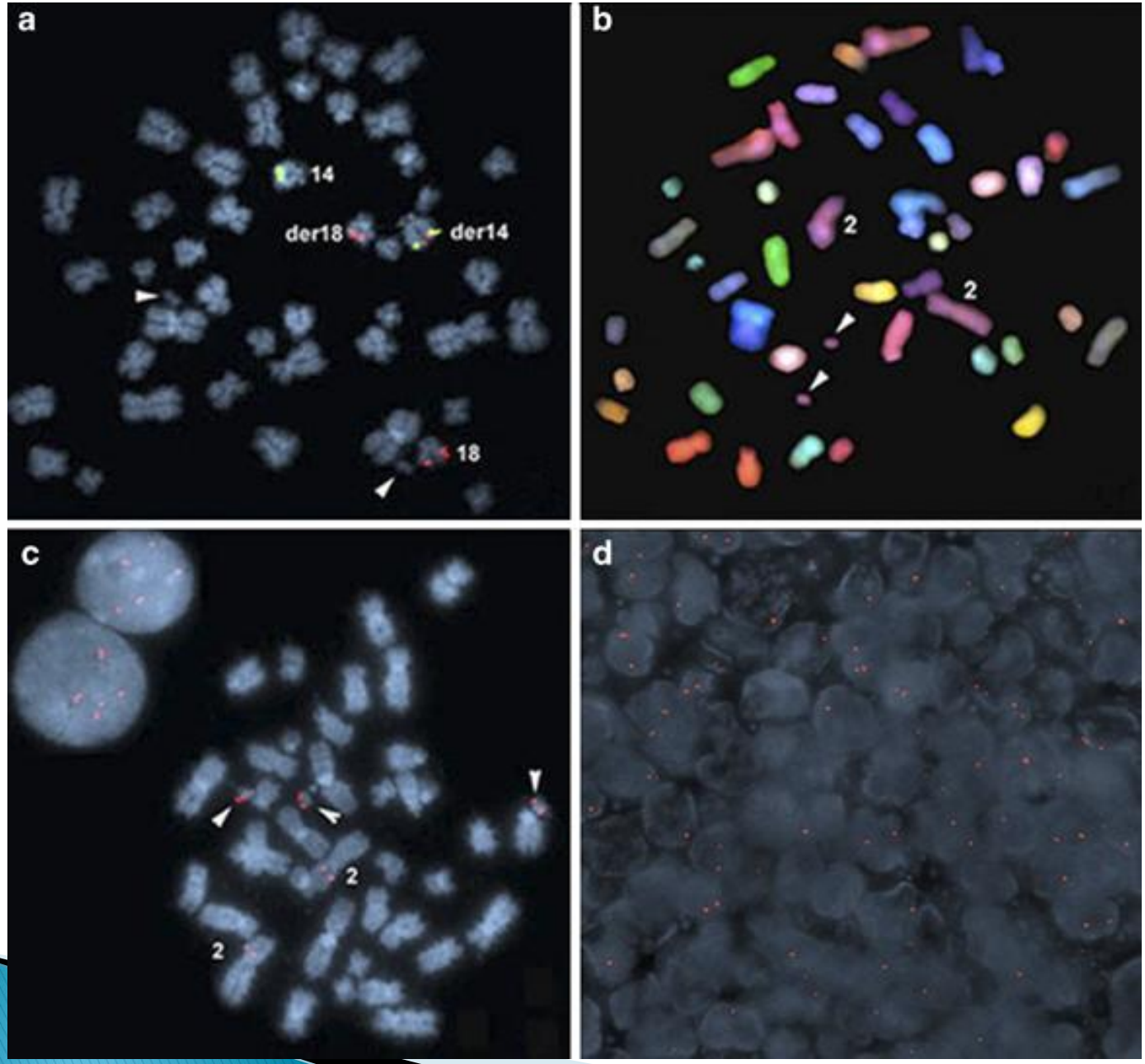


Successive rounds of duplication; integration of duplicates in the DNA strand.

Double minute chromosomes



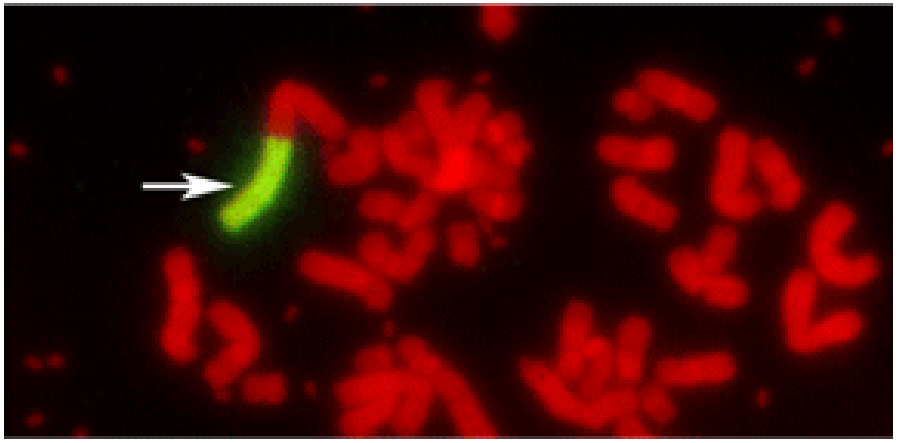
Double minute chromosomes



Homogenously staining region

Homogeneously staining regions (HSRs) are chromosomal segments with various lengths and uniform staining intensity after G banding. This type of aberration is also known as Copy Number Gains or Amplification.

An HSR is one type of change in a chromosome's structure which is frequently observed in the nucleus of human cancer cells. In the region of a chromosome where an HSR occurs, a segment of the chromosome, which presumably contains a gene or genes that give selective advantage to the progression of the cancer, is amplified or duplicated many times. As a result of the duplication this chromosomal segment is greatly lengthened and expanded such that when it is stained with a fluorescent probe specific to the region (Fluorescent in situ hybridization), rather than causing a focal fluorescent signal as in a normal chromosome, the probe "paints" a broad fluorescent signal over the whole of the amplified region. It is because of the appearance of this broadly staining region that this chromosomal abnormality was named a homogeneously staining region.



Target gene amplified by HSRs

CANCER RESEARCH 63, 5281–5290, September 1, 200

Breast Cancer Tumor Suppressor Genes

- Breast cancer is a very common type of cancer. About 5% of breast cancers are hereditary, and like retinoblastoma, the hereditary form often has earlier onset and is bilateral.
- Several genes appear to be involved in familial breast cancer, with two (BRCA1 and BRCA2) thought to be tumor suppressor genes.

a. Mutations in BRCA1 (located at 17q21) are also involved in ovarian cancer. The gene is expressed in many tissues to produce a 190-kDa protein with a role in a number of functions, including:

i. Homologous recombination.

ii. Cellular responses to DNA damage.

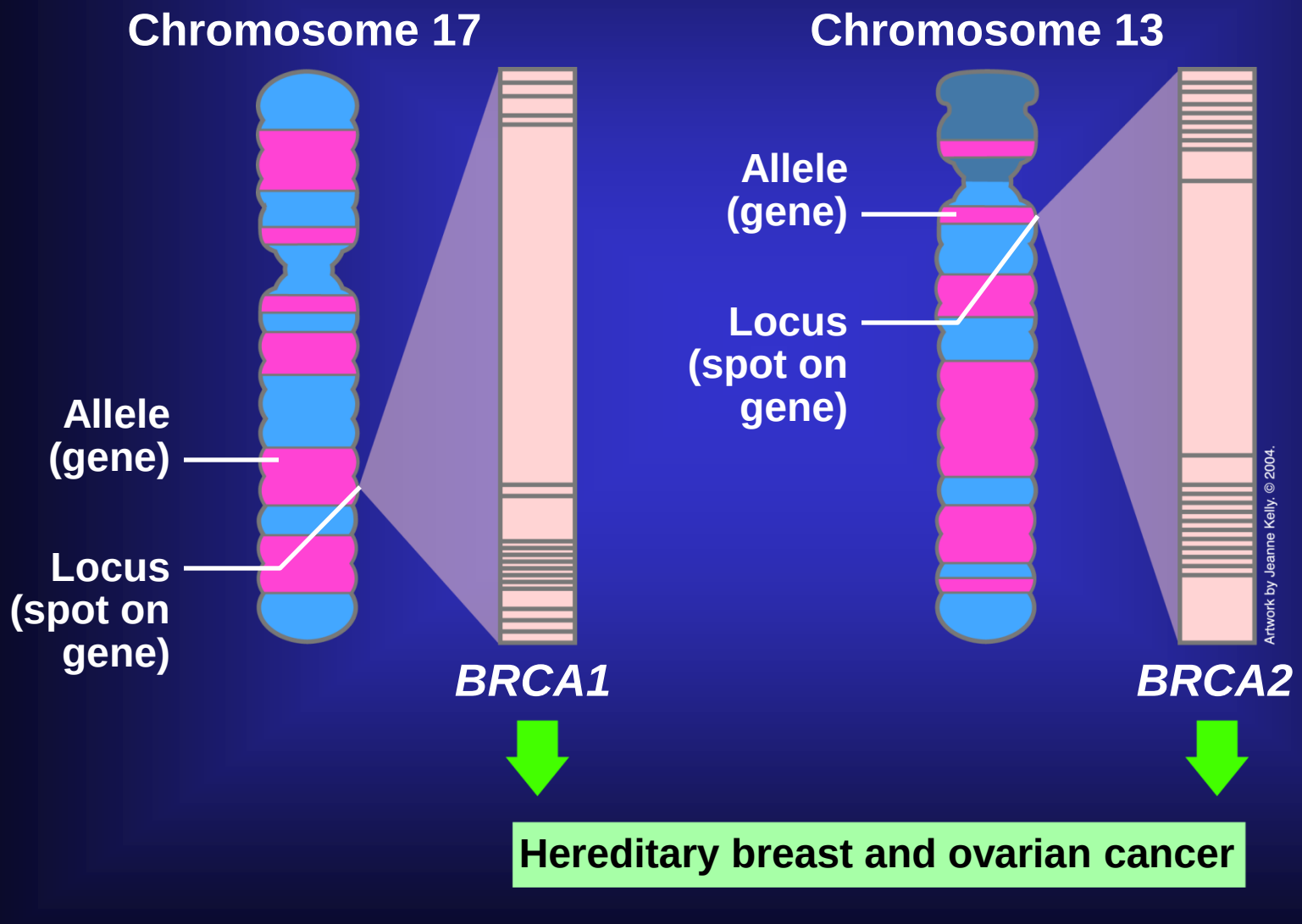
iii. Transcription of mRNA (the BRCA1 protein is part of RNA polymerase II).

b. The BRCA2 gene (13q12-q13) is not involved with ovarian cancer.

i. The large BRCA2 protein has some similarity to BRCA1.

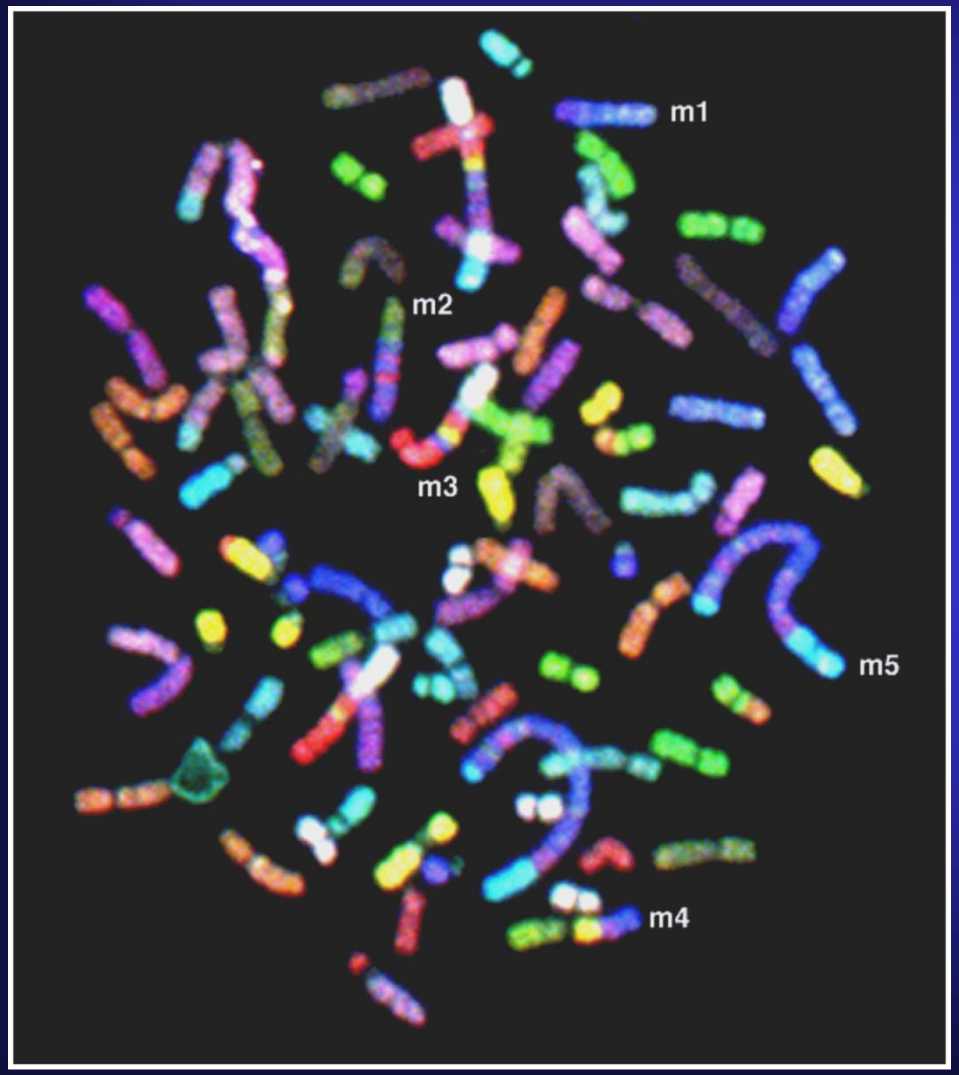
ii. BRCA2 is part of a complex playing a role in timely progression of cells through mitosis.

Different Locus, Different Allele, Same Phenotype



Large Deletions or Insertions

SKY chromosome painting: breast cancer



Artwork by Jeanne Kelly. © 2004.

Normal SKY chromosomes are not multicolored.

Chromosomes in breast cancer appear multicolored because they have exchanged genetic material.

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Mutator Genes

- A gene that increases the spontaneous mutation rate when it is mutated is a mutator gene. Wild-type mutator gene products are involved in DNA replication and repair, so mutations make the cell error-prone.
- An example is hereditary nonpolyposis colon cancer (HNPCC).

a. HNPCC is an autosomal dominant genetic disease that causes early onset of colorectal cancer in which no adenomas (polyps) form.

b. In humans, a mutation in any one of four genes (hMSH2, hMLH1, hPMS1 and hPMS2, mismatch repair) gives hereditary predisposition to HNPCC.

c. A mutation in the single normal allele for any of these genes results in cancer (because it inactivate the remaining normal allel), so its inheritance follows a dominant pattern.

d. All four genes have homologs in yeast and E. coli that are involved in DNA repair, and 1 of the human genes (hMSH2) has been shown to be active in E. coli through complementation.

e. DNA-based blood tests are available for all four genes, allowing carriers

The Multistep Nature of Cancer

Cancer induction may require accumulation of 6–7 independent mutations over several decades, typically involving:

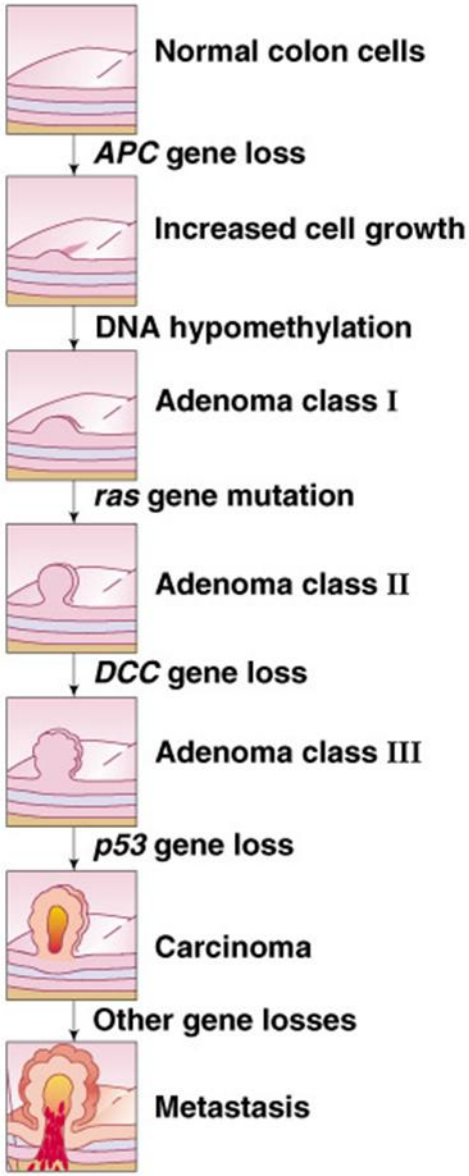
a. Conversion of proto-oncogenes to oncogenes.

b. Inactivation of tumor suppressor genes.

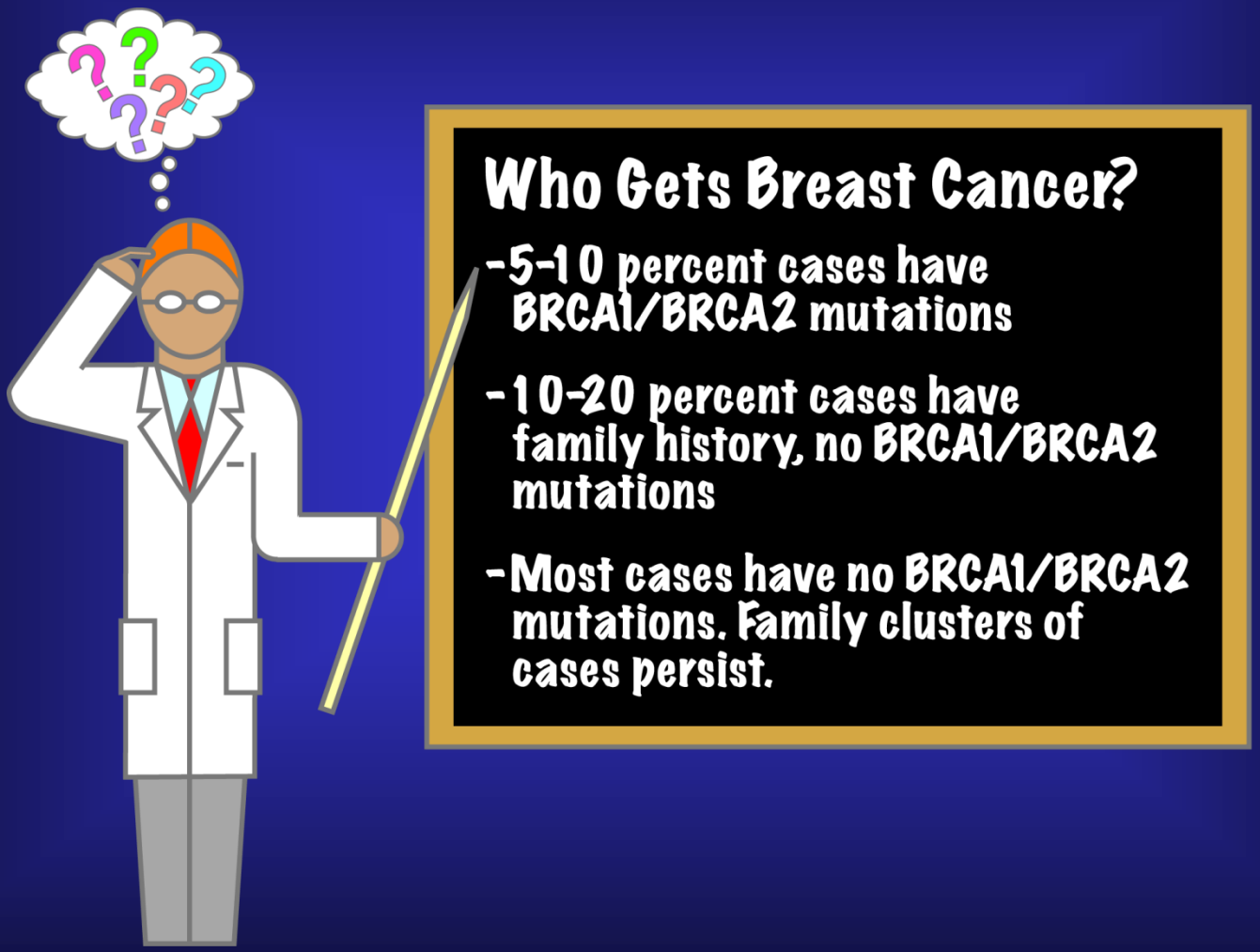
The Multistep Nature of Cancer

An example is Vogelstein's model for a form of colorectal cancer, hereditary FAP .

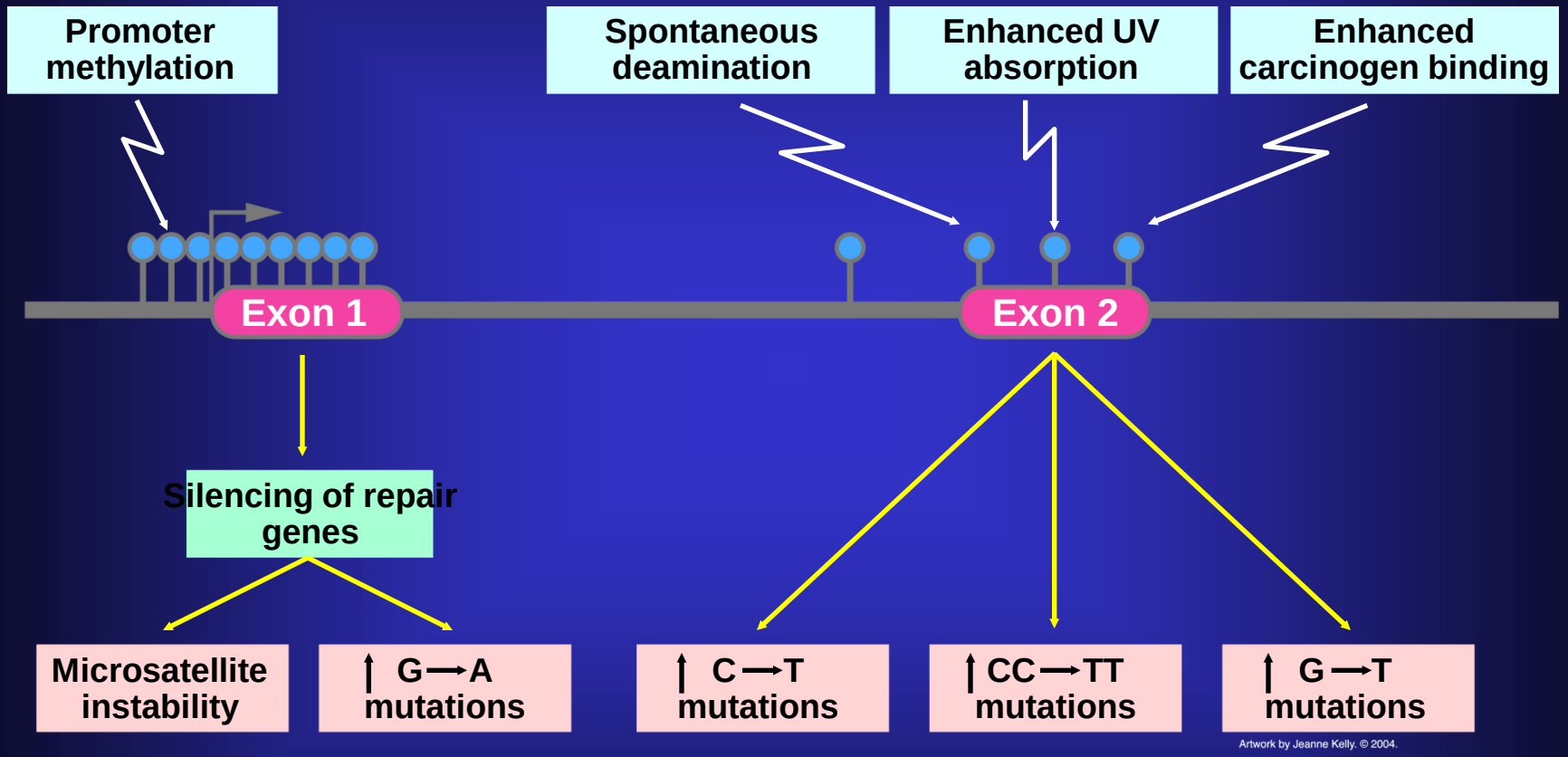
a. Mutation of both alleles of a tumor suppressor gene on chromosome 5, APC (adenomatous polyposis coli), causes increased cell growth.
b. Hypomethylation of the DNA leads to a benign tumor (adenoma class I).
c. Mutation of the chromosome 12 ras proto-oncogene allows cells to form a larger benign tumor (adenoma class II).
d. If both copies of DCC, a tumor suppressor gene on chromosome 18, are lost, an even larger adenoma class III results.
e. Mutation of both *p53* alleles on chromosome 17 results in conversion to a carcinoma.
f. Other gene losses result in the cancer metastasizing.
g. Other paths are possible, but in all cases deletions of APC and mutations of ras occur before deletions of DCC and *p53*.



Cancer Susceptibility: Much Still Unknown



Epigenetic Changes: Much Still Unknown



Source: Jones & Baylin, Nature Reviews|Genetics,3:415–428 (2002)
PMID 12042769

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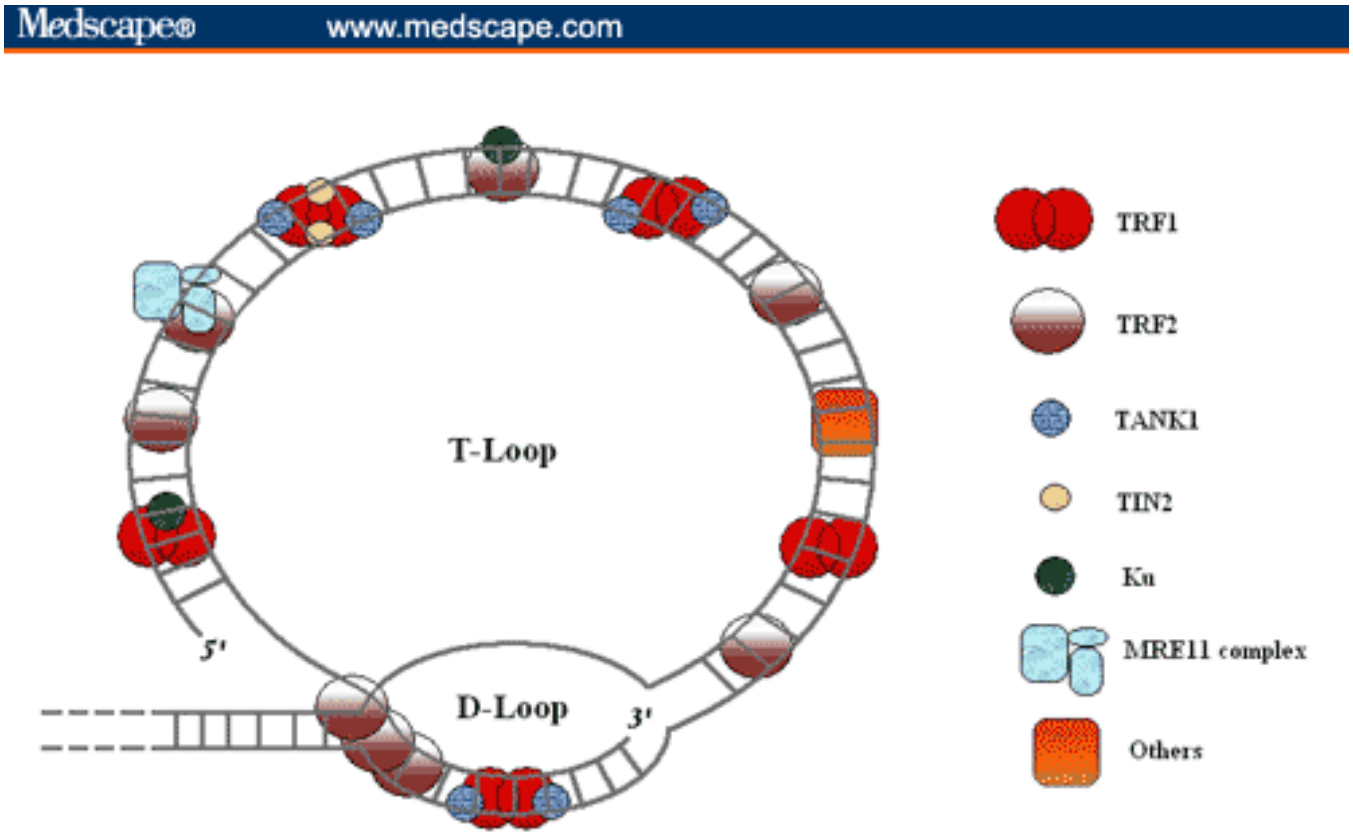
Epigenetic Mechanisms

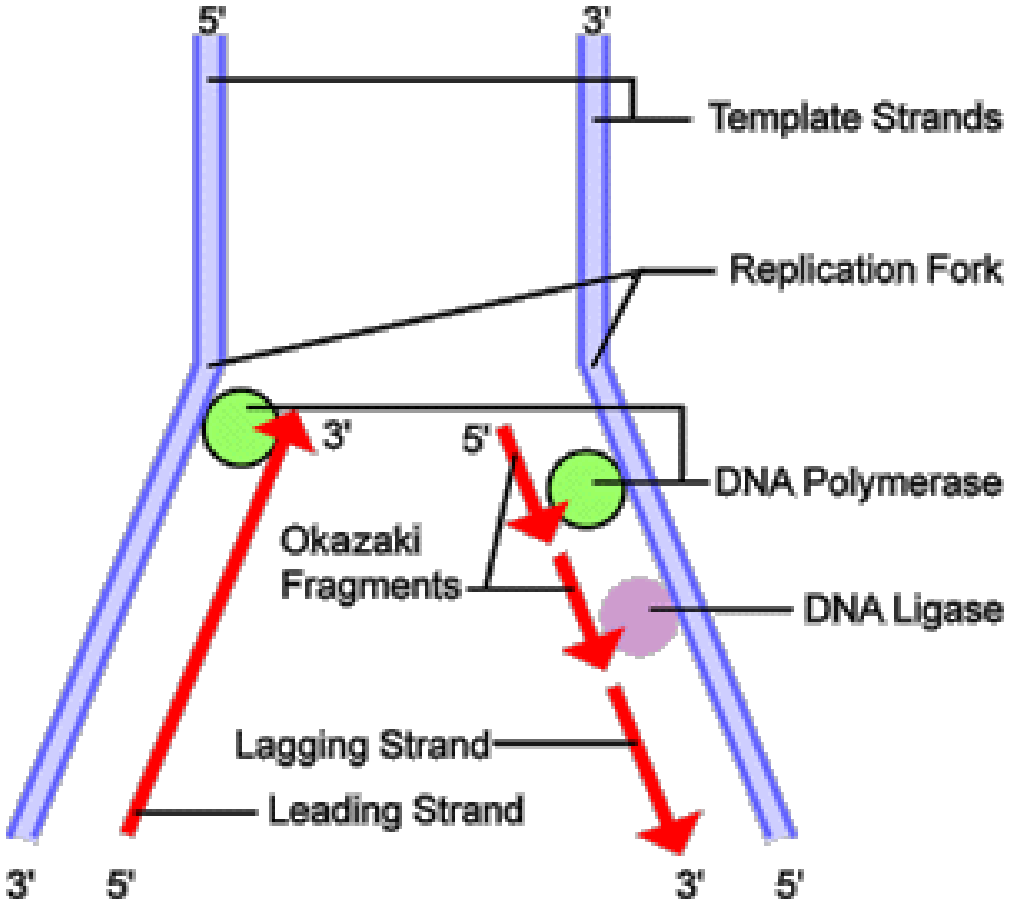
Telomeres compensate for incomplete semi-conservative DNA replication at chromosomal ends.

Telomere structure

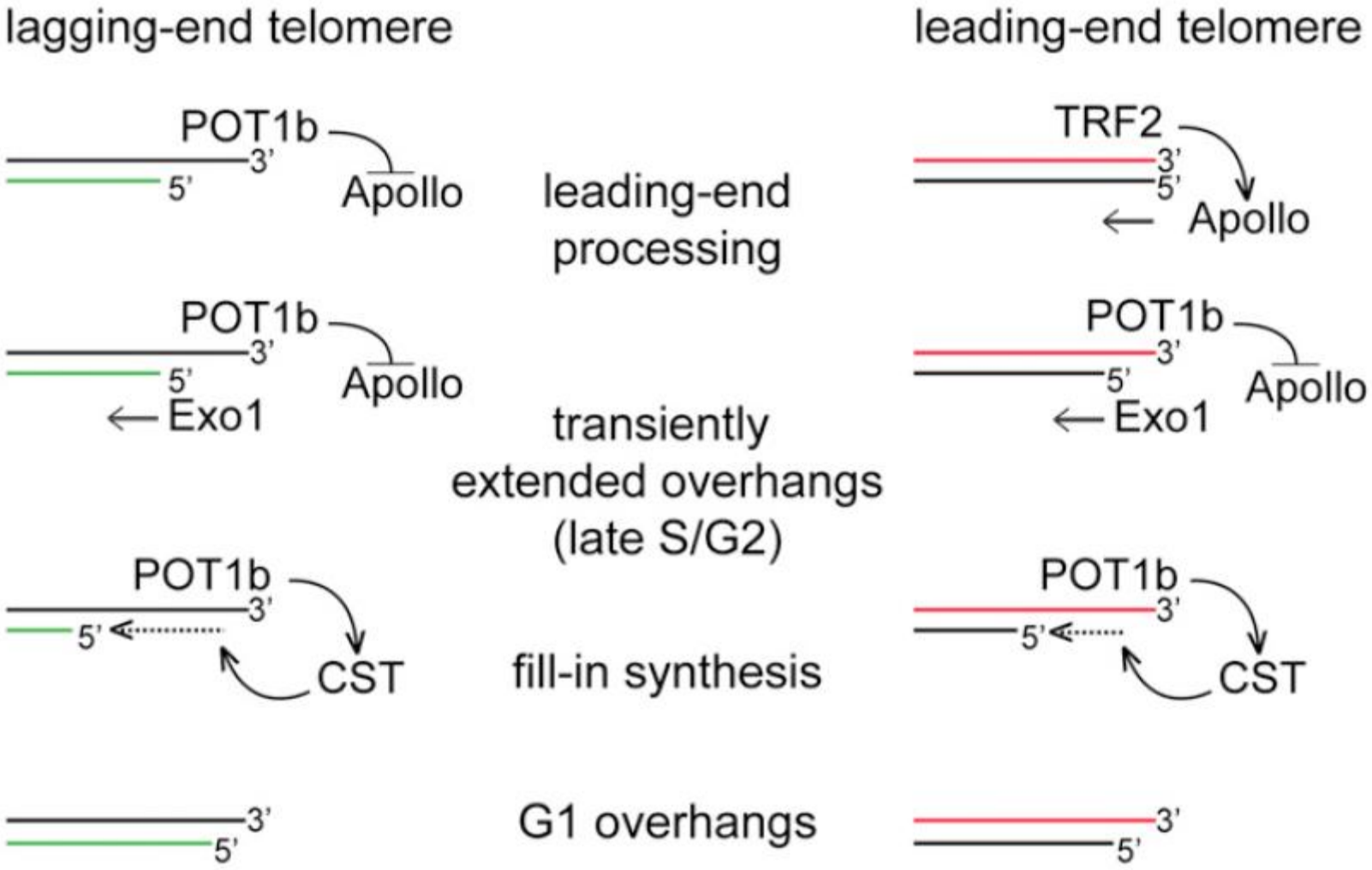


Telomere quadruplexes (

Telomere shortening, telomerase, cancer



Overhang formation

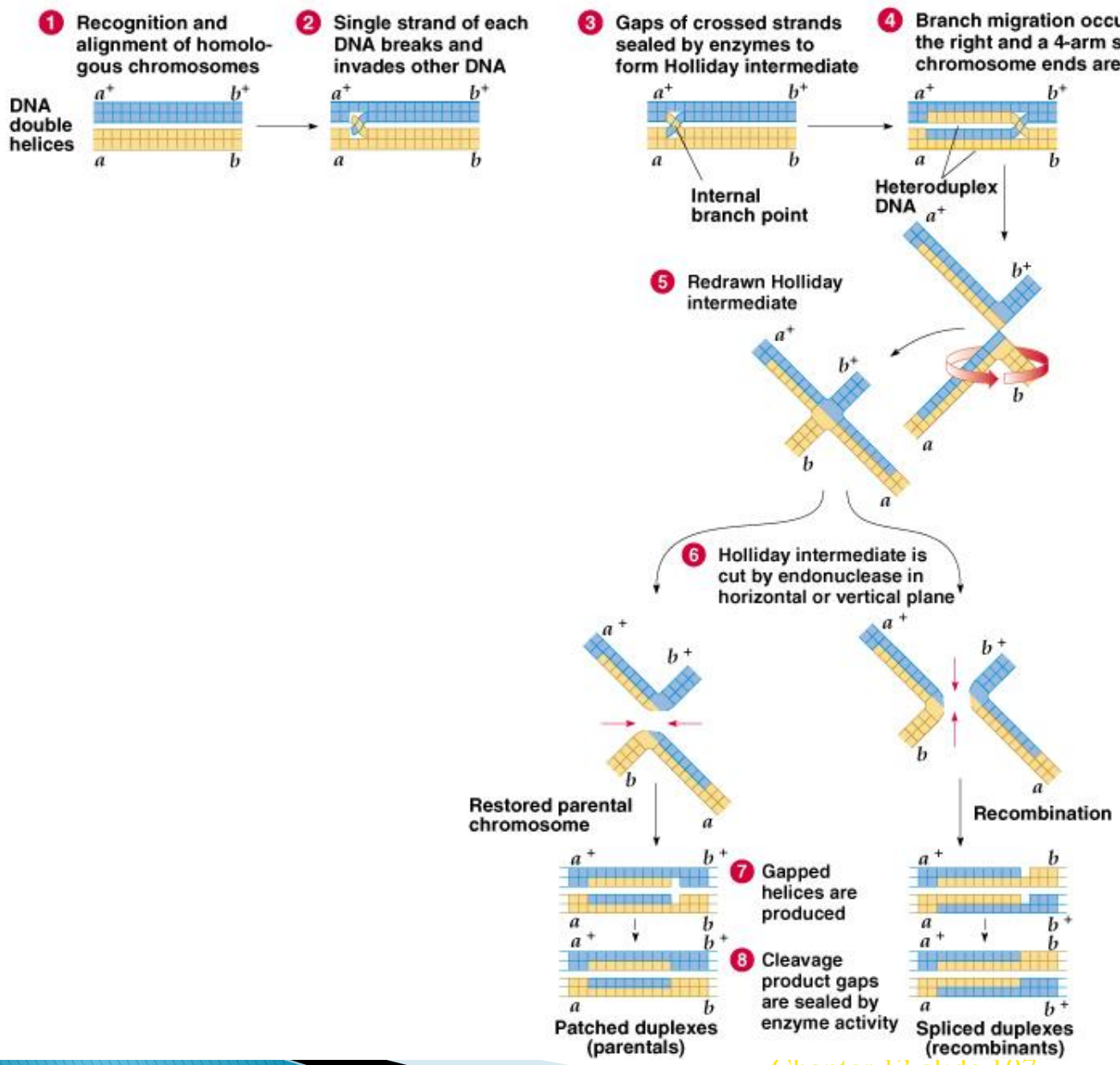



3'
6'

- Transformation:

What is cancer?

Box Fig. 13.1 Holliday model for reciprocal genetic recombination



Gene conversion by mismatch repair at two sites