Oncogenes and Tumor Suppressor genes

MED 213

The Genetic Basis of Cancer

Oncogenes
Tumor suppressor genes
Repair genes

Environmental mutagens
(biological, chemical, physical agents)

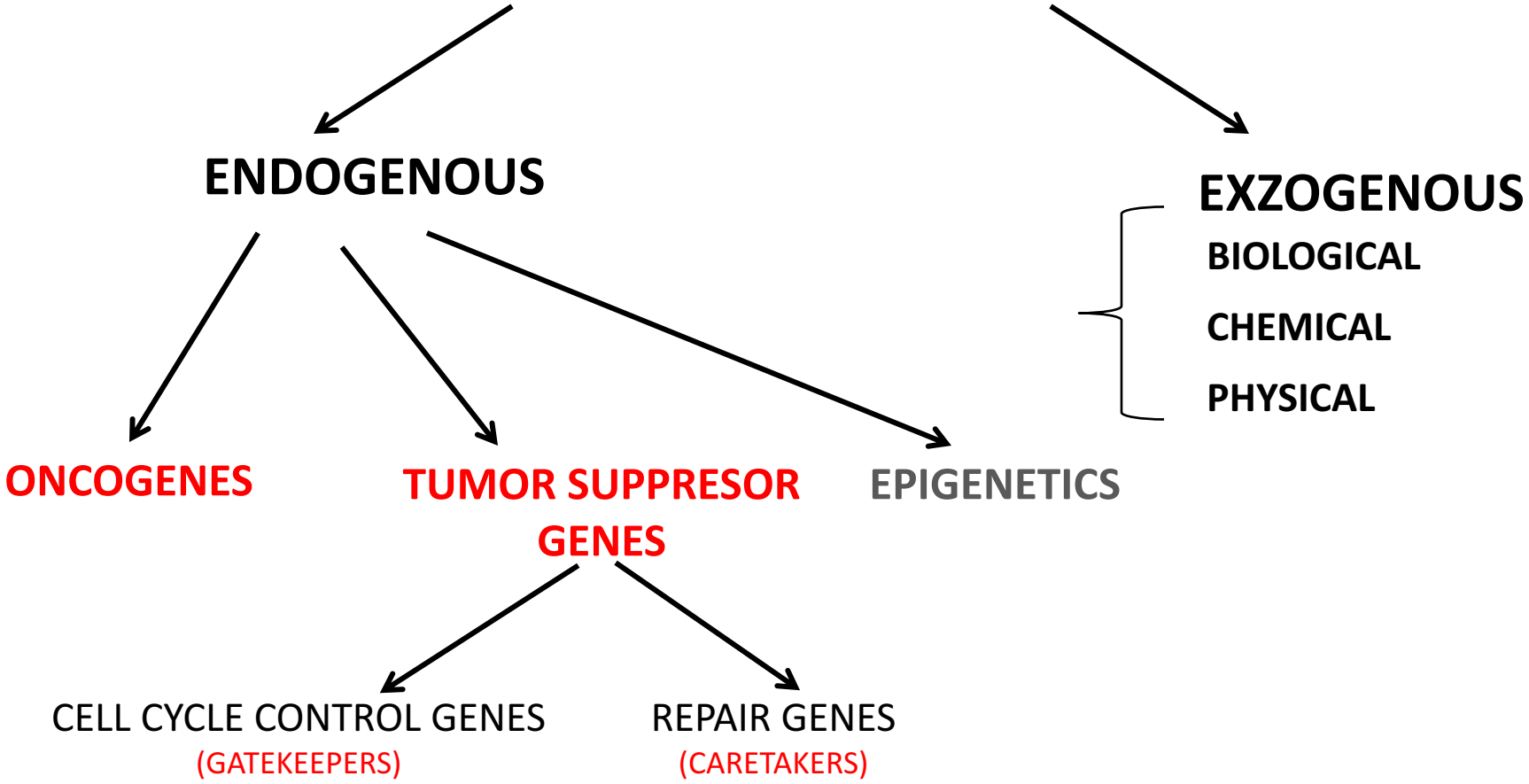
Genetic mechanisms in Familial vs Sporadic Cancers

Pathways in Carcinogenesis

Epigenetics and Cancer

Molecular targets for Cancer Therapy

GENETIC MODIFICATIONS LEADING TO CARCINOGENESIS



What is an oncogene?

- An oncogene is a mutated form of a normal cellular gene – called a proto-oncogene – that contributes to the development of a cancer.

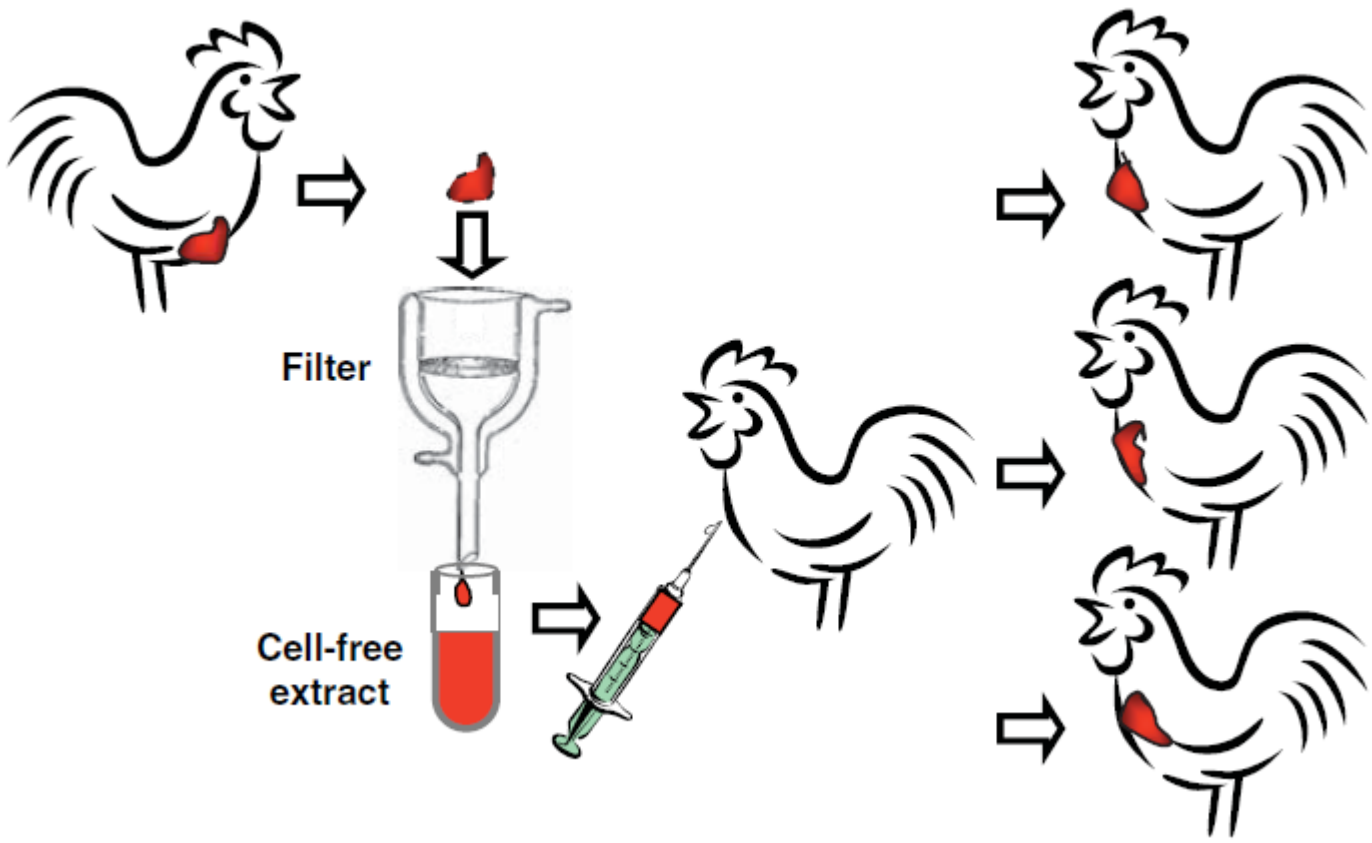
- Oncogenes are caused by mutations that alter, but do not eliminate, the functions of the proteins they encode.

- Most proto-oncogenes encode enzymes. The oncogenic forms of these enzymes have a higher level of activity, either because of an altered affinity for substrate or a loss of regulation.

- It's called gain of function mutations

- Converts proto-oncogenes to oncogenic alleles

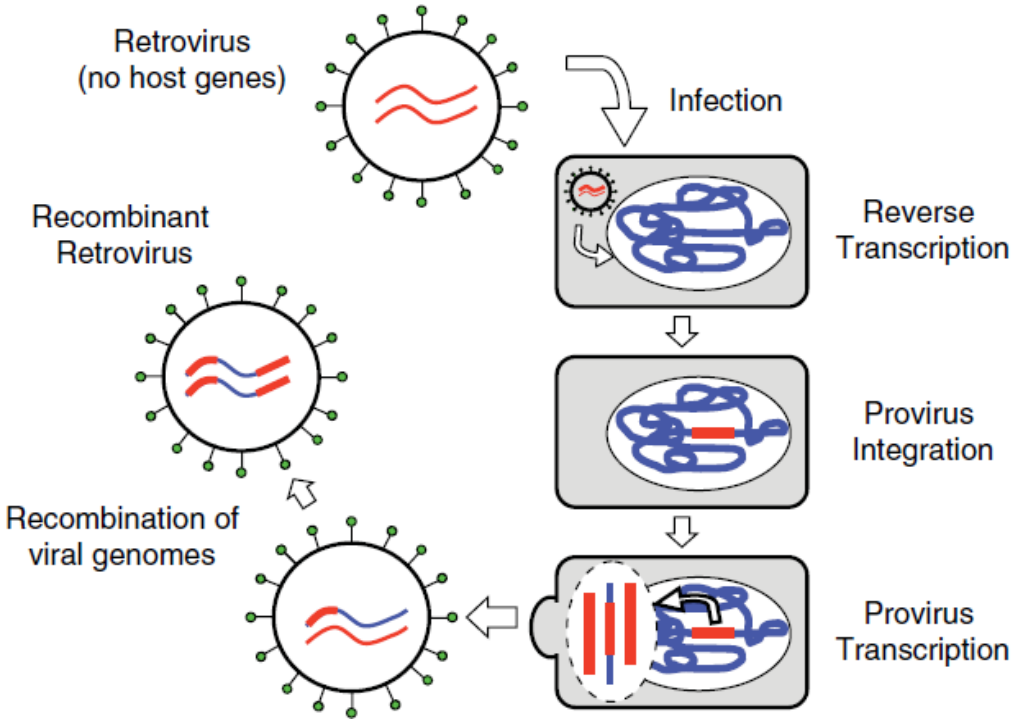
- Known as activating mutations



The Rous experiment:

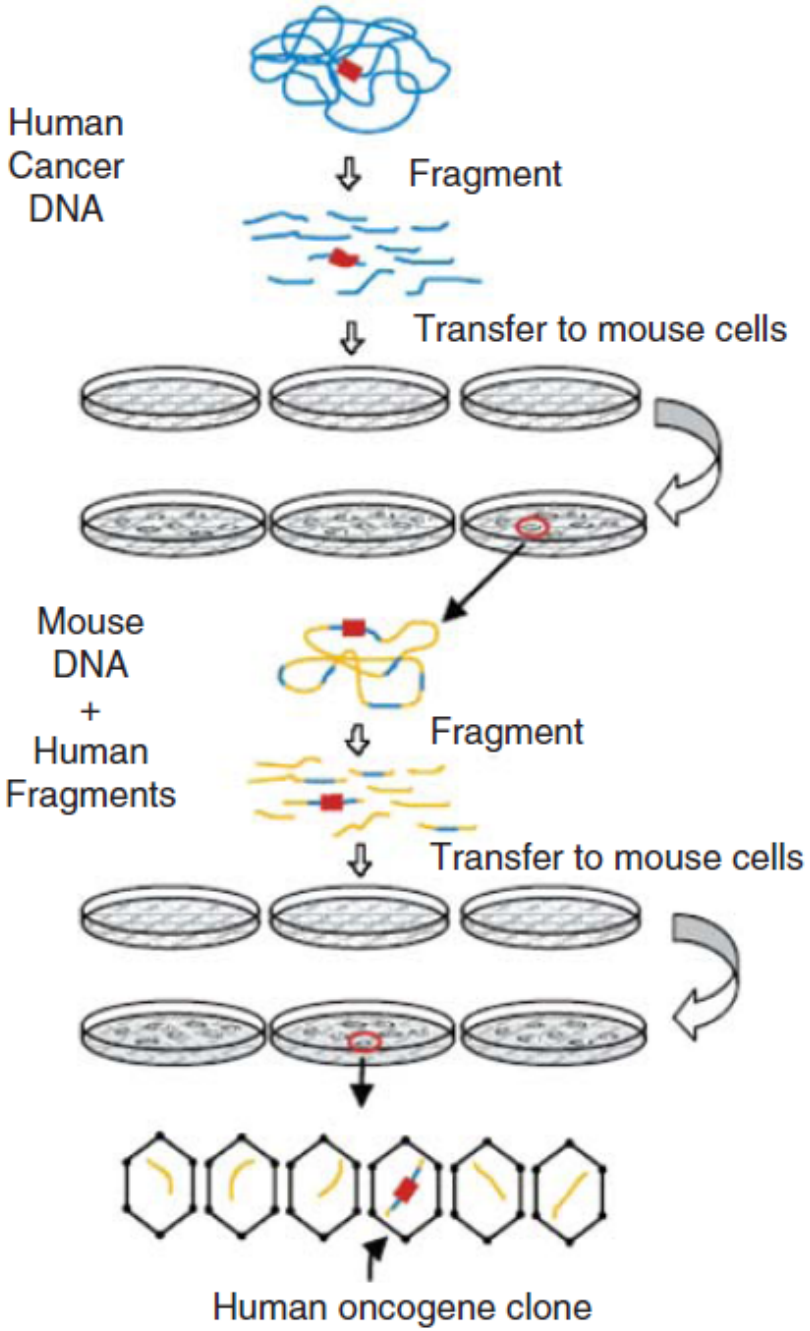
Chicken sarcomas can be horizontally transferred between animals via injection of a cell-free filtrate. This experiment demonstrated the infectious nature of this avian cancer

The acquisition of oncogenes by retroviruses



- The retrovirus capsule contains 2 copies of the viral RNA genome. After infection, the viral genome is copied into DNA by reverse transcriptase and integrates into the cellular genome as a provirus.
- If the provirus is integrated in close proximity to exon sequences, proviral transcripts can be spliced with host cell exons.
- These hybrid transcripts are packaged into a virion, resulting in a heterozygous viral genome.
- The viral genome undergoes recombination during a second round of infection.
- The resulting recombinant virus contains coding genetic elements that originated in the host cell

Oncogene discovery by in vitro transformation



Genes transferred from human genomic DNA (blue) can alter the growth properties of mouse fibroblasts.

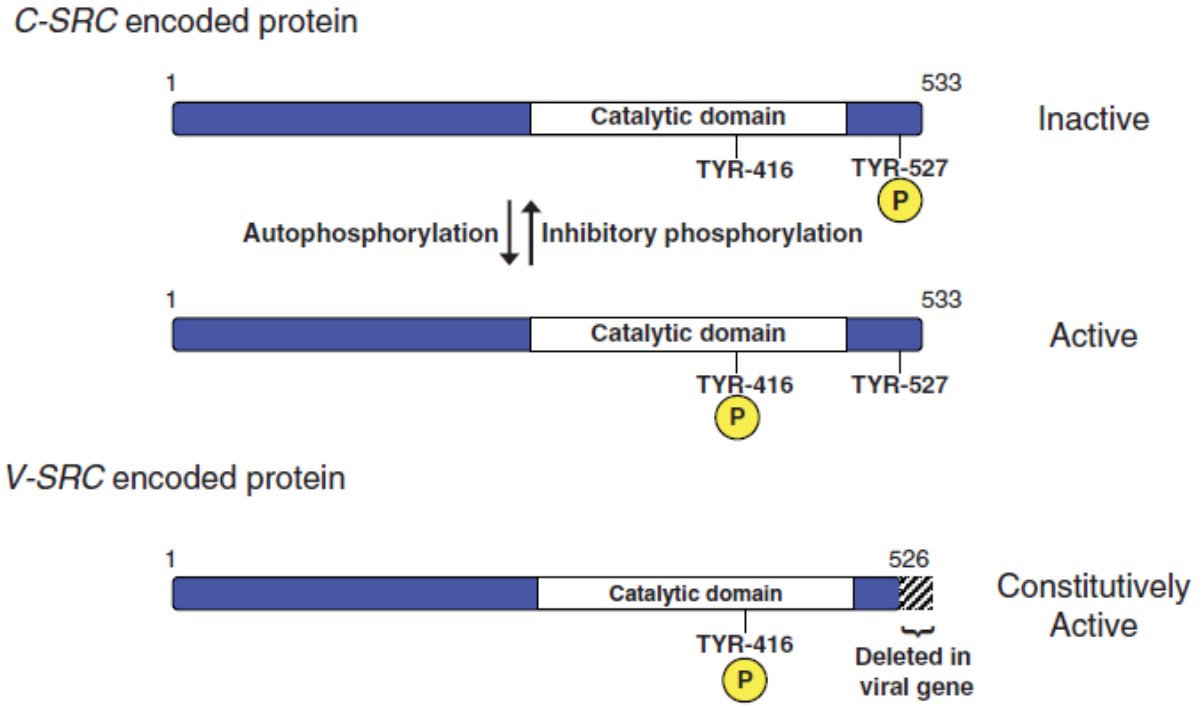
Genomic DNA is sheared into small fragments, which are introduced into mouse cells grown in monolayer cultures.

Appearing after a period of growth, discrete foci represent clones of mouse cells that have altered growth and cell-cell interactions.

Genomic DNA from these clones (yellow) can contain multiple integrated fragments of human DNA. A second round of transfer allows the isolation of individual human fragments.

DNA from the second clone is packaged into a bacteriophage library, which is then screened with a probe corresponding to human genomic DNA-specific repeat elements.

Viral and cellular SRC genes

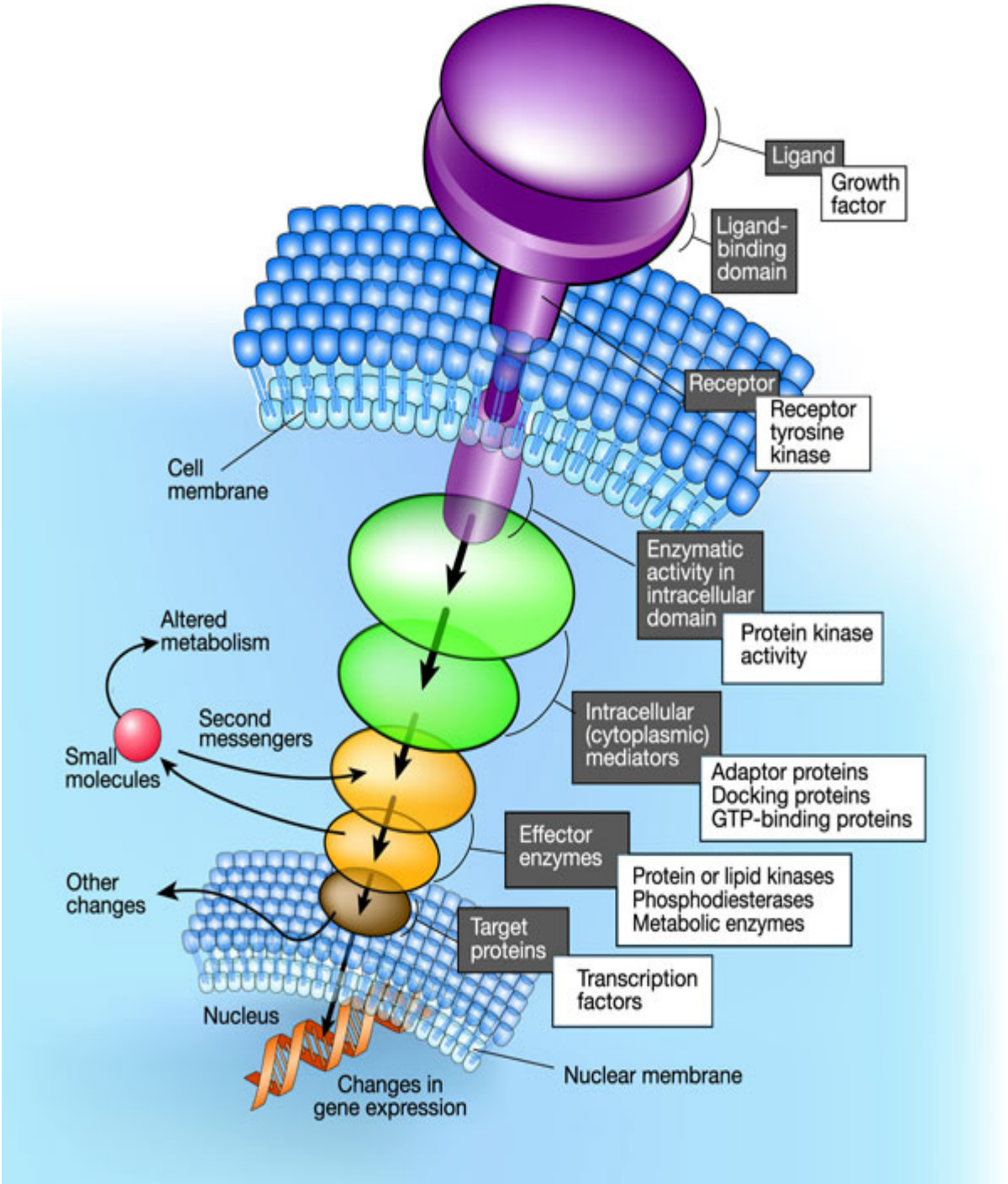


- Viral and cellular SRC genes Cellular SRC is a protein tyrosine kinase that consists of 533 amino acids.
- Tyrosine autophosphorylation at residue 416 within the kinase domain causes a conformational change and results in the activation of kinase activity.
- Phosphorylation at tyrosine 527 by upstream inhibitory kinases prevents SRC – encoded protein activation.
- The viral oncogene V-SRC does not encode the c-terminal 7 amino acids, and therefore does not contain the negative regulatory element

Active Oncogene Effect

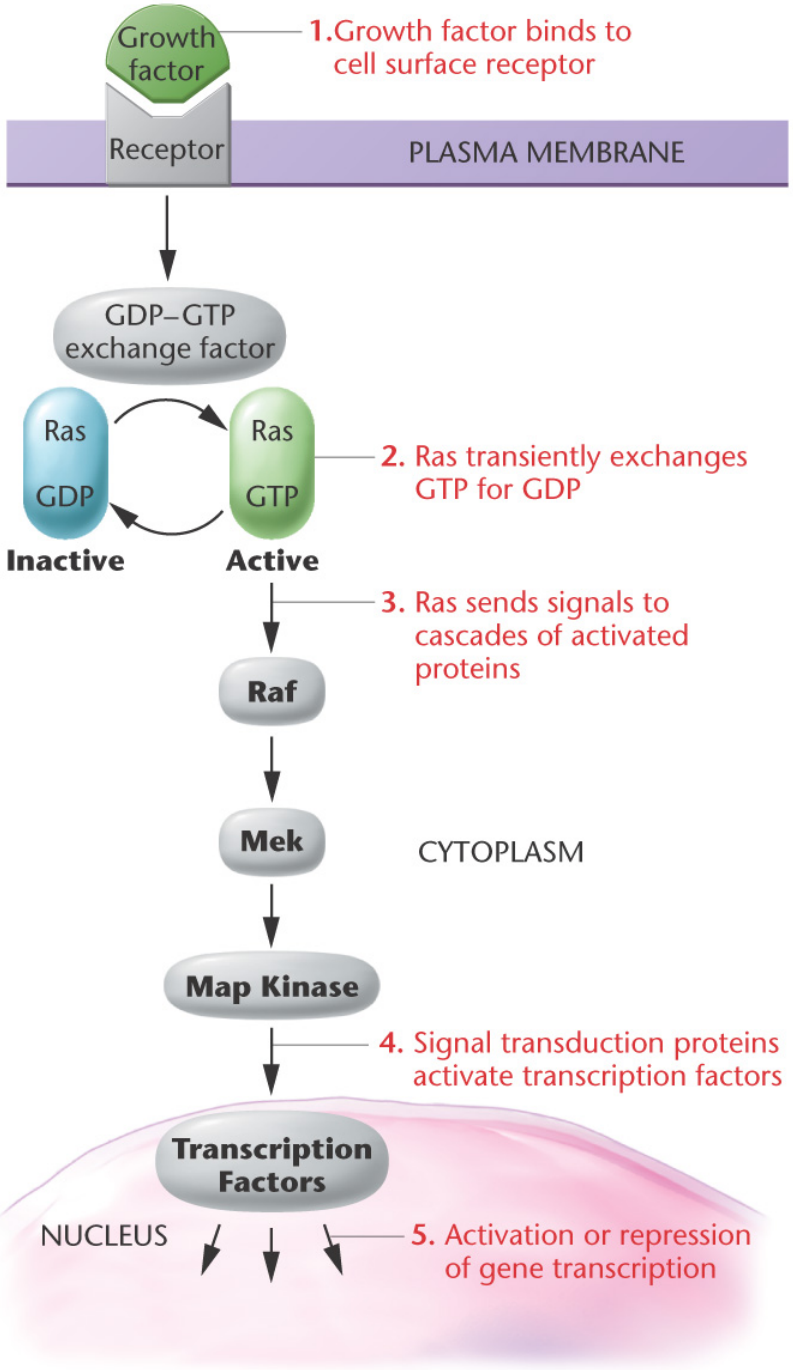
Class		Example	Cancer type
1	GROWTH FACTORS	*SIS*	GLIOMA
2	TYROSINE KINASE RECEPTORS	*RET*	MULTIPLE ENDOCRINE NEOPLASIA 2 (MEN 2)
3	CYTOPLASMIC TYROSINE KINASE	*ABL*	CHRONIC MYELOID LEUKEMIA (CML)
	CYTOPLASMIC SERINE KINASE	*RAF1*	
4	G-PROTEIN SIGNALLING / DOWN STREAM TARGETS	*K-RAS*	PANCREAS CA BREAST, COLORECTAL,
	PHOSPHOINOSITIOL 3-KINASE	*PIK3CA*	GASTRIC, ENDOMETRIAL Ca
5	TRANSCRIPTION FACTORS	*MYC*	BURKITT LYMPHOMA, BREAST, GASTRIC LUNG Ca, SARCOMAS
6	TELOMERASE	TELOMERASE	VARIOUS
7	ANTI APOPITOTIC PROTEINS	*BCL2*	*CML*
8	ONCOMIRS	*mir-21*	BREAST, COLON, LUNG, PANCREAS, LIVER, GASTRIC PROSTATE CANCERS
		mir-17-92	PLUS LYMPHOMAS

General signal transduction scheme



Ras pathway

- Binds to GF receptor
- Ras activity is established with a transfromation from GDP bound form to GTP
- Once active Ras initiates kinase cascade downstream
  - Ras→Raf→Mek→Map
  - Most downstream effectors are transcription factors



RAS Proto-oncogenes

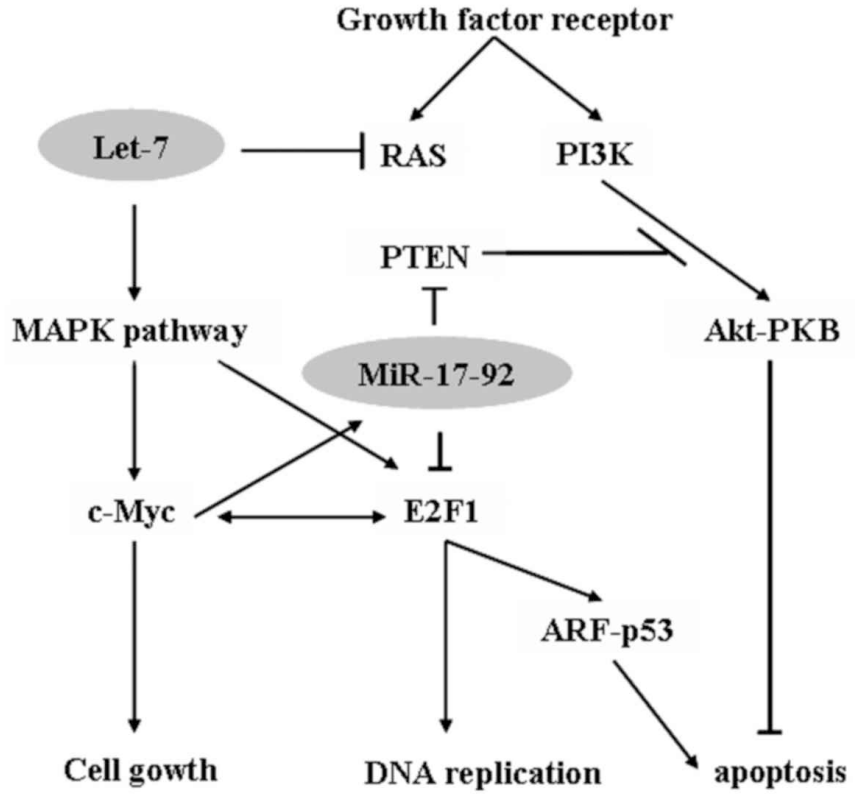
- Signal transduction control from receptors to nucleus

- *RAS* family genes are mutated in 40% of all cancer cases
- >%50 of colorectal cancer cases have *RAS* mutations

Table 2.1 Mutations in the *RAS* gene family

Cancer type	Mutation frequency (%)	RAS family member
Pancreatic carcinoma	95	*K-RAS*
Colorectal carcinoma	50	*K-RAS*
Lung carcinoma	30	*K-RAS*
Acute Myelogenous Leukemia	25	*N-RAS*
Melanoma	10	*N-RAS*

Interaction of miRNAs as oncogenic and tumor suppressor



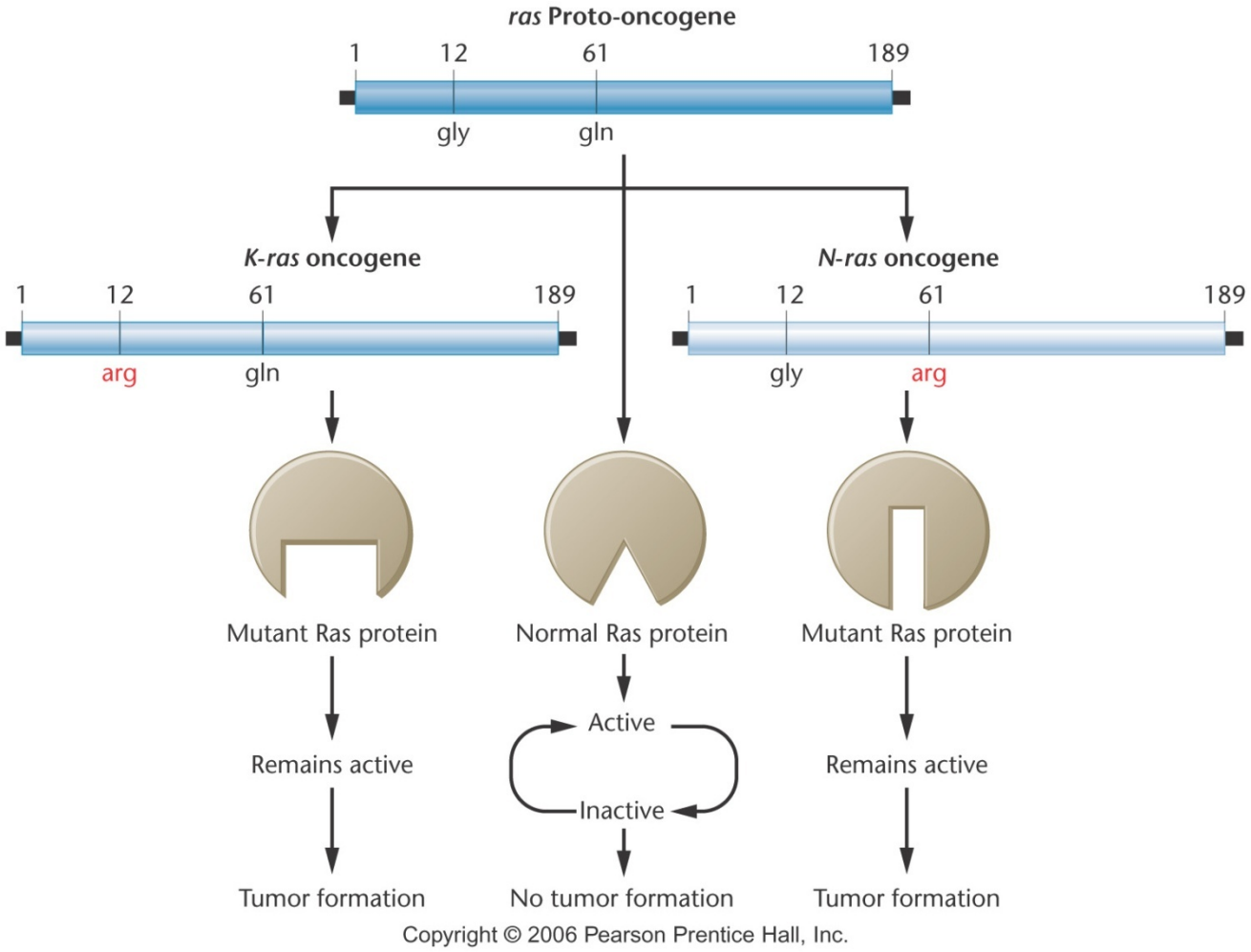
Let-7 suppresses translation of the Ras GTPase genes. The downregulation of let-7 promotes the cell cycle through the Ras-MAPK pathway. miR-17-92 may prohibit oncogene-induced apoptosis.

PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide-3 kinase; PKB, protein kinase B; MAPK, mitogen-activated protein kinase; ARF, alternative reading frame protein of p16INK4a locus. miRNA/miR, microRNA; p53, tumor protein 53; E2F1, transcription factor E2F1; Akt, serine/threonine-protein kinase.

Proto-oncogene activation mechanisms

Mechanism	Activated Gene Type	Effect
Regulator mutations	Growth factor	Increased expression
Structural mutations	Growth factor receptors, signal transduction protein genes	Loss of control in expression
Translocations, retroviral insersions,	Transcription factor genes	Increased expression
Gene amplifications	Transcription factor genes	Increased expression
Regulator mutations, translocations, retroviral insersions,	miRNAs (noncoding regions)	Increased expression, decreased tumor supressor effect
Deletions, inactivating mutations	miRNAs	Loss of expression, increased oncogenic activity

Mutant Ras Proteins



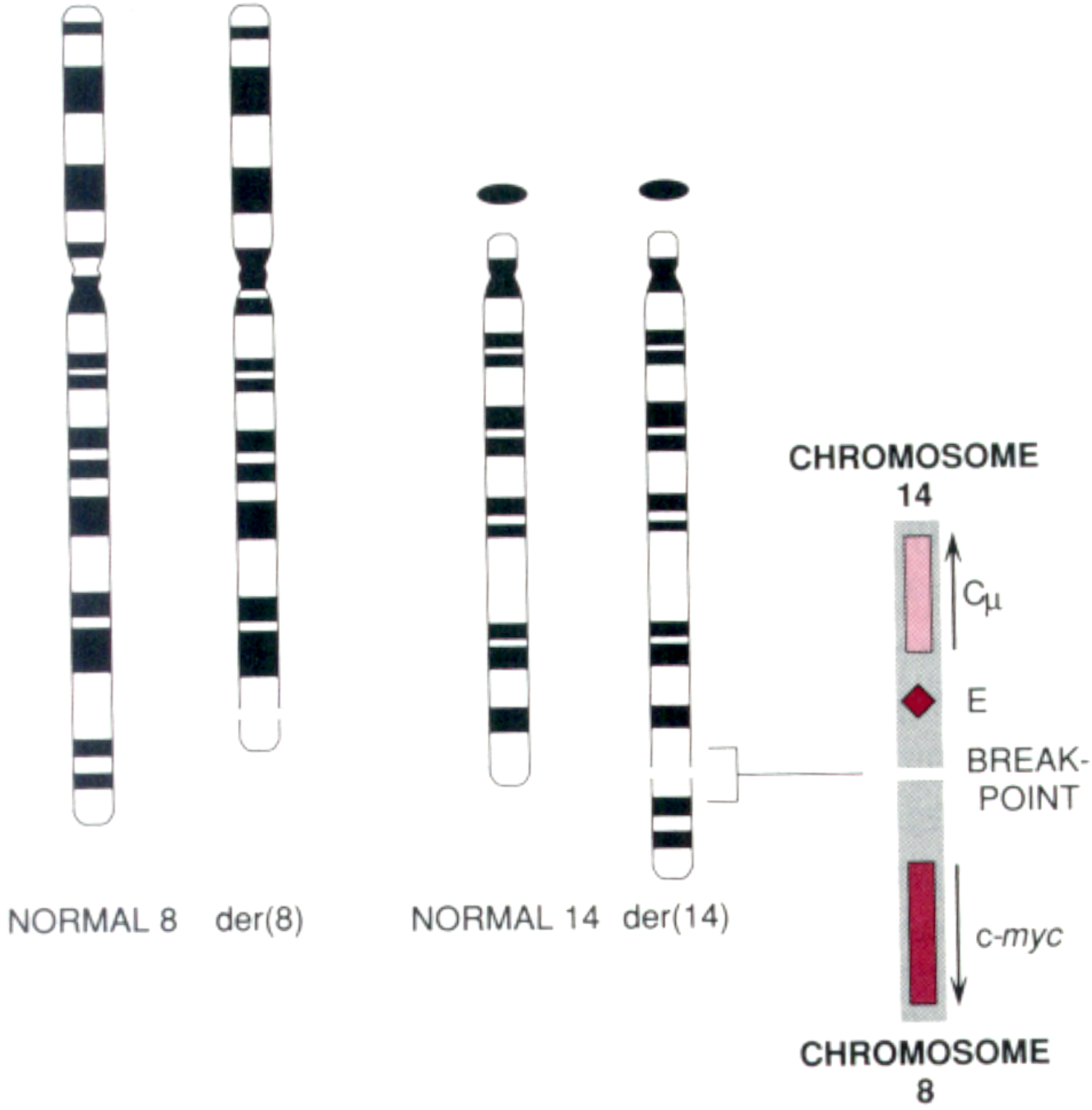
- Single aa change
- mutant ras oncogenes
- N-ras; K-ras

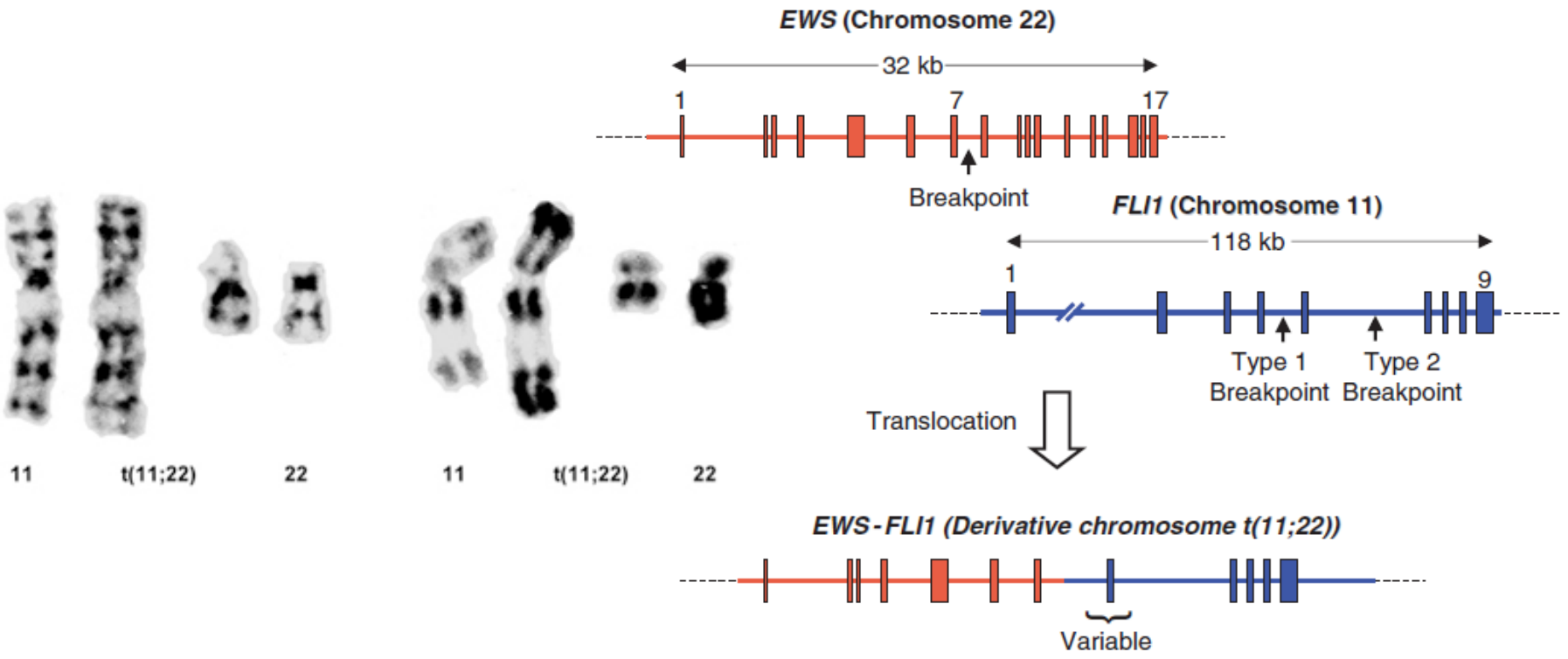
- **Translocations**
  - Proto-oncogenes can be recombined to unusual loci in the genome! If the new locus is under the control of another promoter the transcription control may be lost.
  - This may effect the levels of synthesis

Example:

c-myc: Burkitt lymphoma.

c-myc is translocated to a locus where Ig heavy chain gene promoter is in effect. t(8,14)(q24;q32)

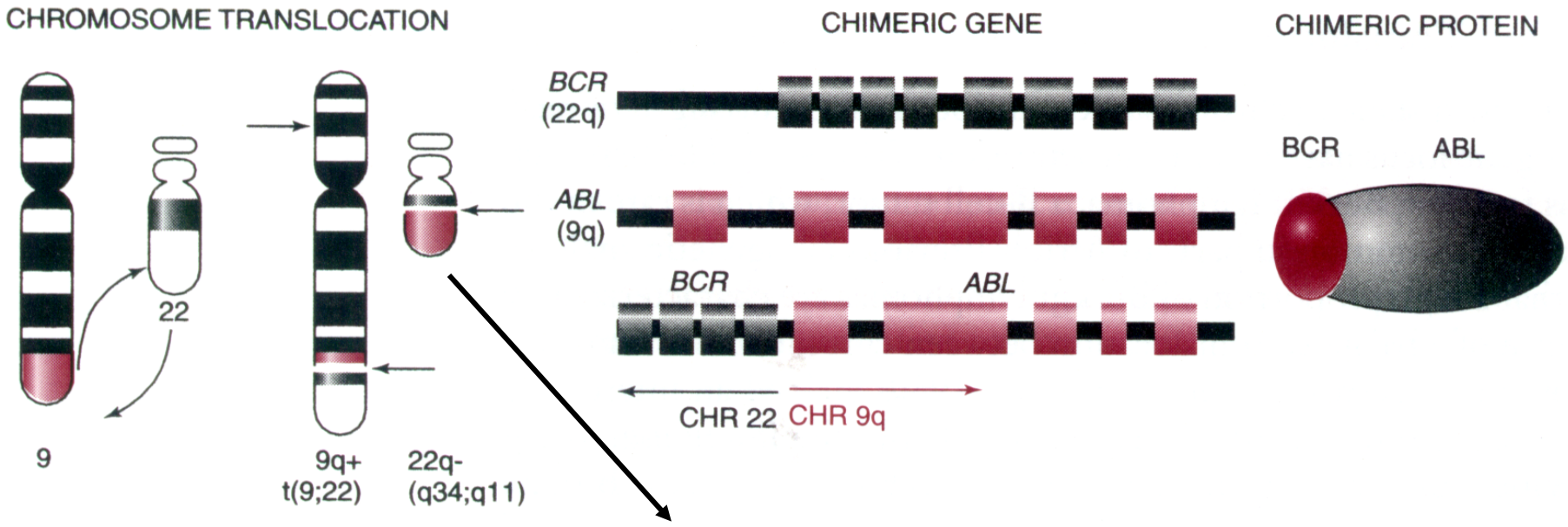

CHROMOSOME
14
Cμ
E
BREAK-
POINT
c-myc
CHROMOSOME
8
NORMAL 8
der(8)
NORMAL 14
der(14)



The creation of *EWS-FLI1 by translocation.*

FLI1 encodes a transcription factor containing a DNA-binding domain. The gene can undergo a t(11;22)(q24;q12) translocation with the Ewing sarcoma gene on chromosome 22, which results in a fusion gene that is present in the majority of Ewing sarcoma cases (90%). An acute lymphoblastic leukemia-associated t(4;11)(q21;q23) translocation involving this gene has also been identified.

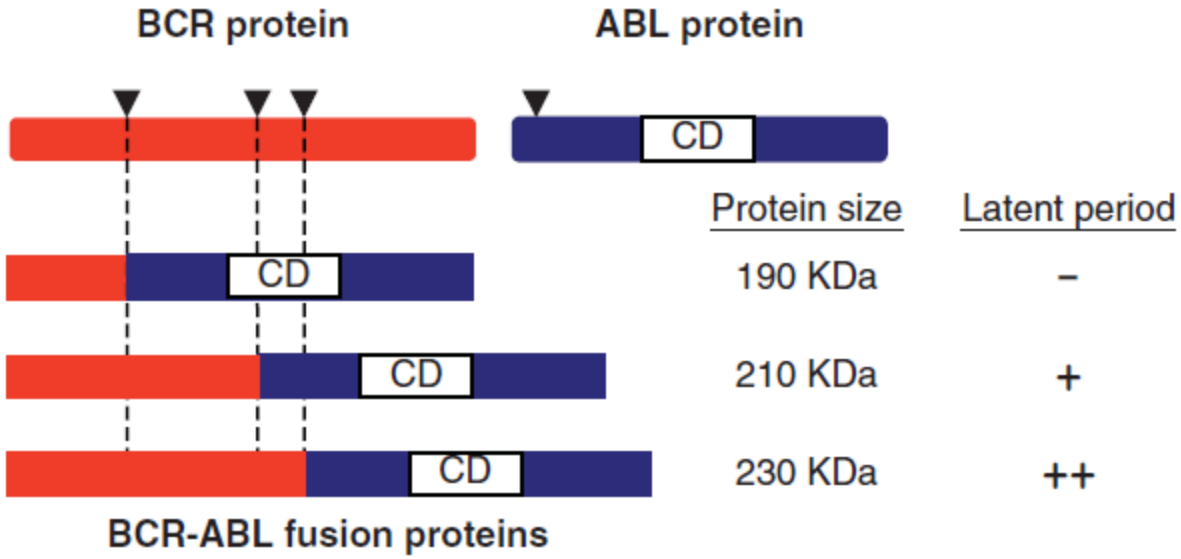
ABL proto-oncogene activation with translocation causing chronic myeloid leukemia (CML)



Philadelphia chromosome (Ph)

ABL encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, division and adhesion

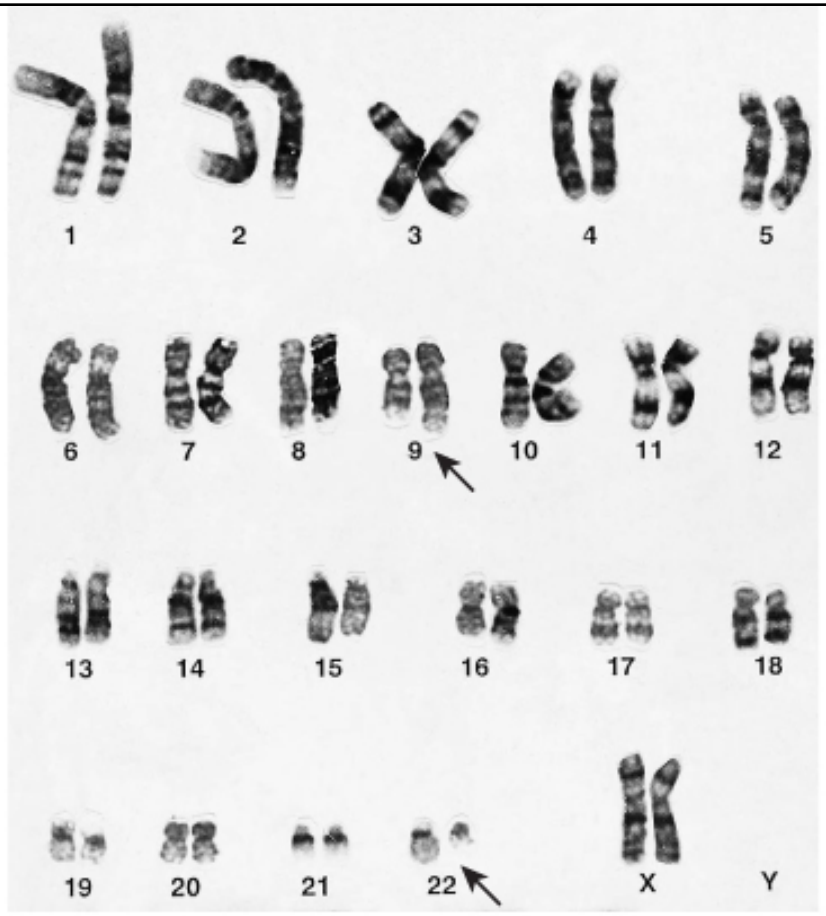
BCR gene product has serine/threonine kinase activity and is a guanine nucleotide exchange factor for GTPases



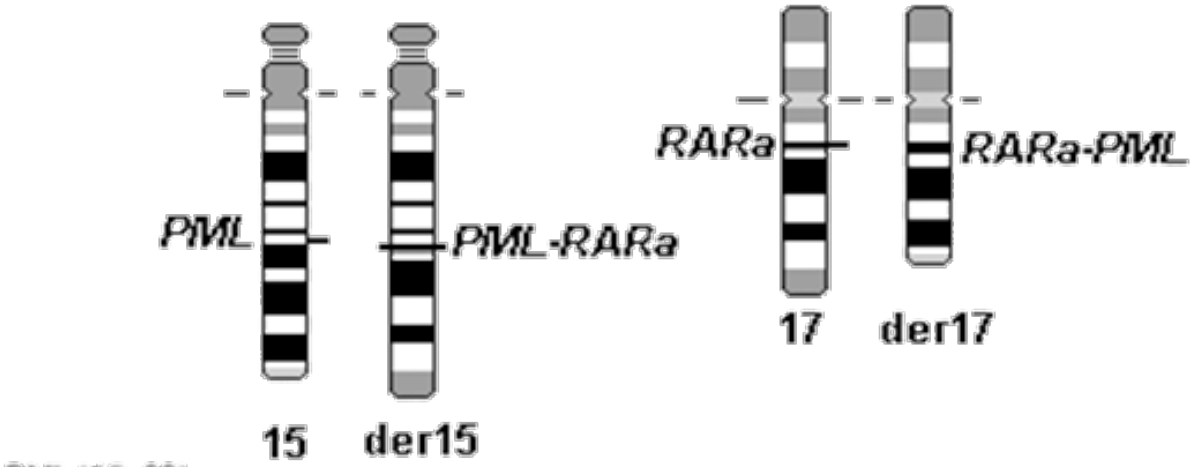
***BCR-ABL* fusion proteins.** Different *BCR* break points lead to distinct fusion proteins (190, 210 and 230 kD)
190 KD ALL
210 KD CML (most frequent)
230 KD CML subtype

Causing increased tyrosine kinase activity

CML patient karyotype



RAR-PML t(15;17)(q24;q21)

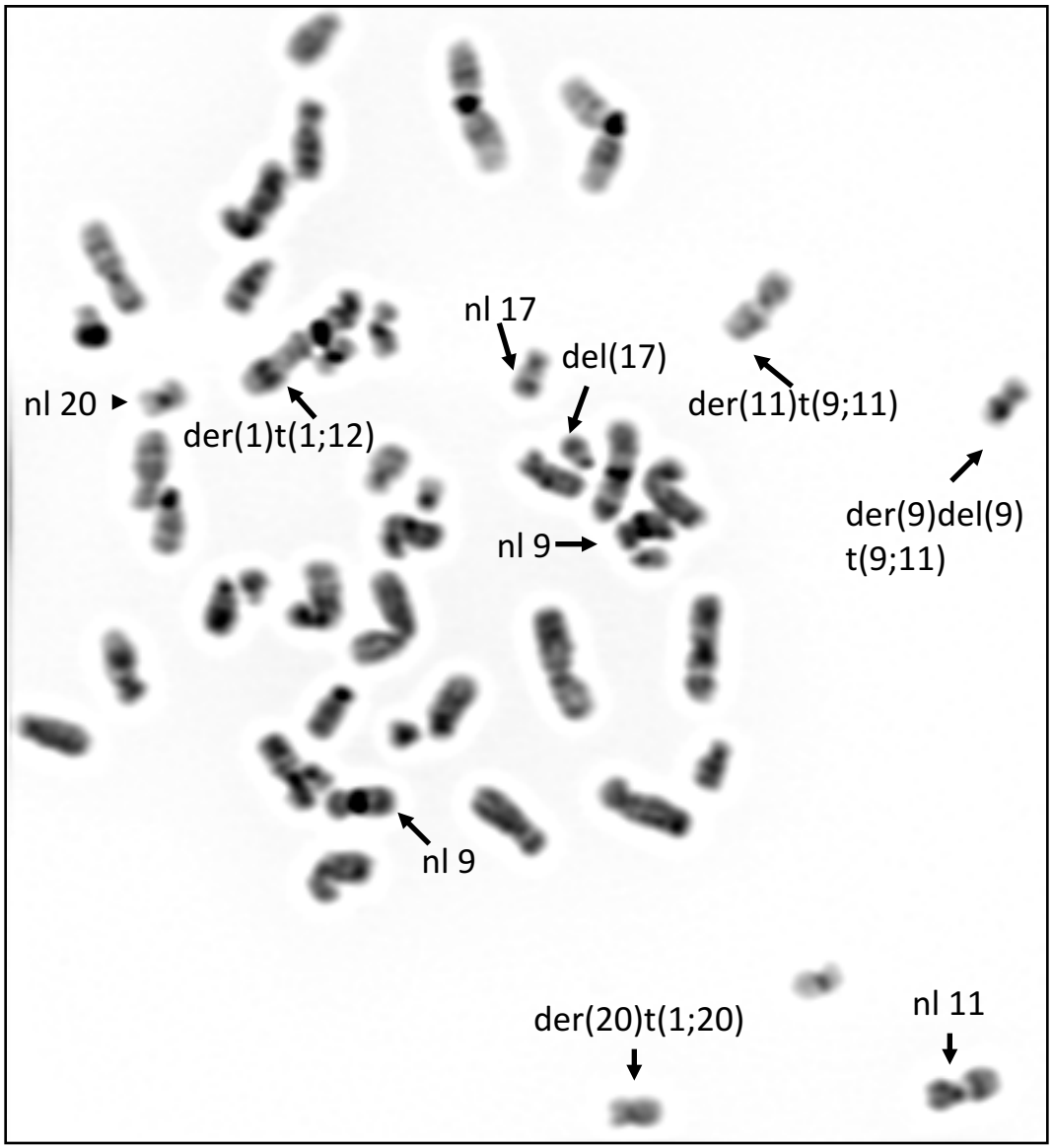


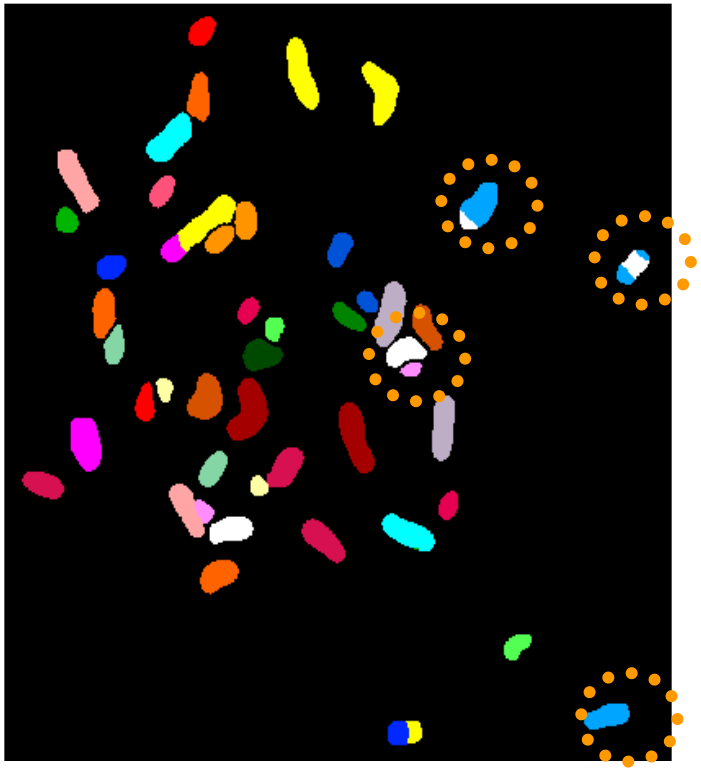
- Causing a fusion protein with retinoic acid alpha receptor gene *RAR* (Chr 17) and promyelocytic leukemia gene *PML* (Chr15)
- This fusion protein binds with enhanced affinity to sites on the cell's DNA, blocking transcription and differentiation of granulocytes. It does so by enhancing interaction of nuclear co-repressor (NCOR) molecule and histone deacetylase (HDAC).
- Leads to APML (APL=acute promyelocytic leukemia)

Frequent Translocations seen in malign neoplasms

Neoplasm	Translocation	Freq.	Effected proto-oncogene
Burkitt lymphome	t(8;14)(q24;q32) t(8;22) (q24;q11) t(2;8) (q11;q24)	80% 15% 5%	*MYC*
Chronic lymphocytic leuk.	t(9;22) (q34;q11)	90-95%	*BCR-ABL*
Acute lymphocytic leuk.	t(9;22) (q34;q11)	10-15%	*BCR-ABL*
Acute lymphoblastic leuk.	t(1;19) (q34;p11)	3-6%	*TCF3-PBX1*
Acute promyelocytic leuk.	t(15;17) (q22;q11)	*95%*	*RARA-PML*
Ewing Sarcoma	t(11;22) ((q24;q12)	90%	*EWS-FLI1*
Chronic lymphoblastic leuk.	t(11;14) (q13;q32)	10-30%	*BCL1*
Follicular Lymphoma	t(14;18) (q32;q21)	100%	*BCL2*

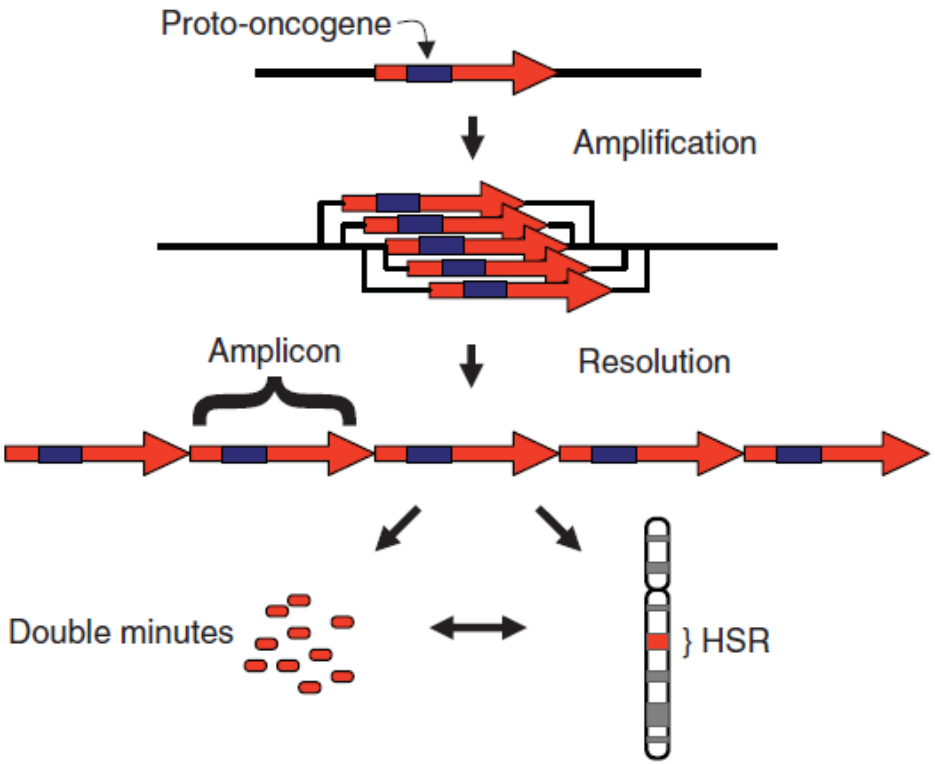
Spektral Karyotyping

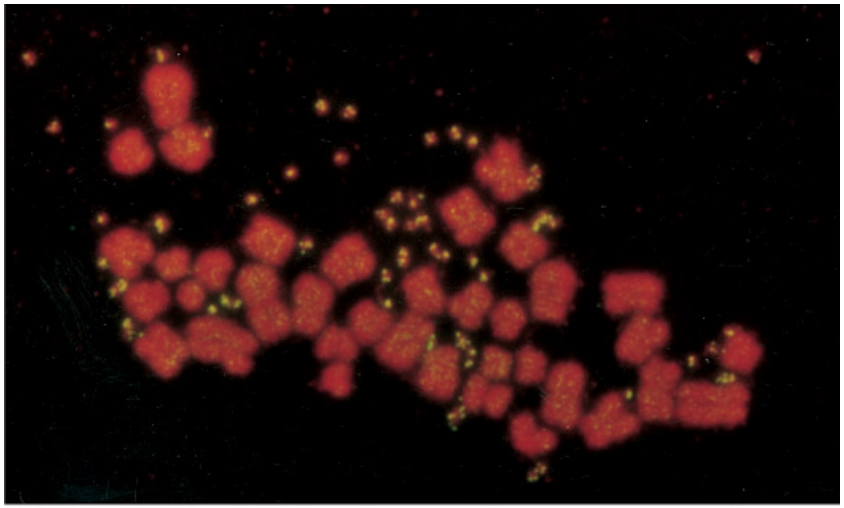




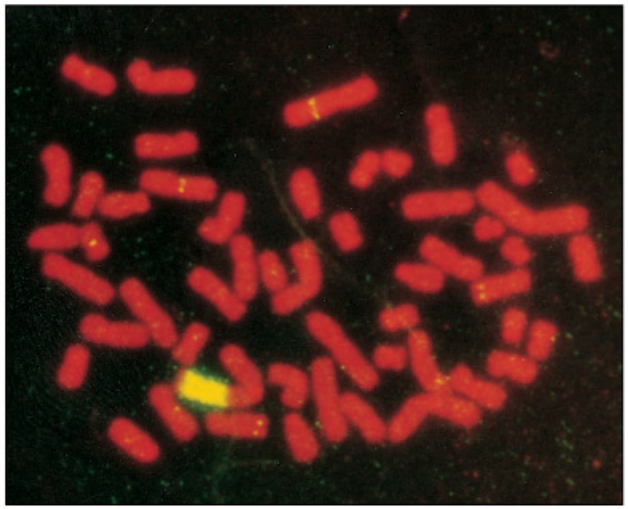
- **Gene amplifications activate proto-oncogenes**
- -increased copy number
  - *HER2/neu; c-erbB2; c-myc* breast cancer

- Amplifications are seen as
  - "Double minutes" (small pieces of chromosomes)
  - Homogenous staining regions (HSR)





(A)



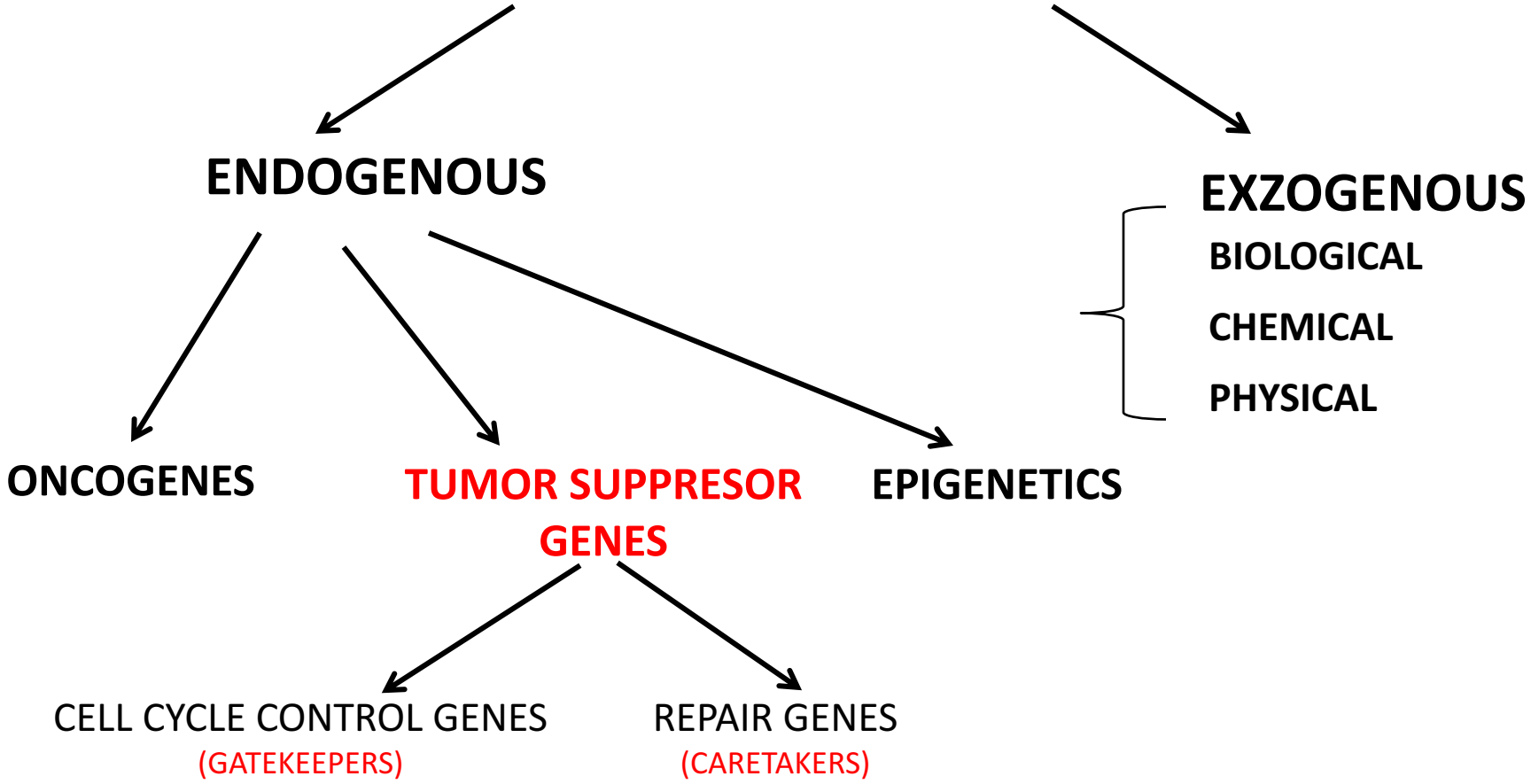
(B)

Figure 23–28. Molecular Biology of the Cell, 4th Edition.

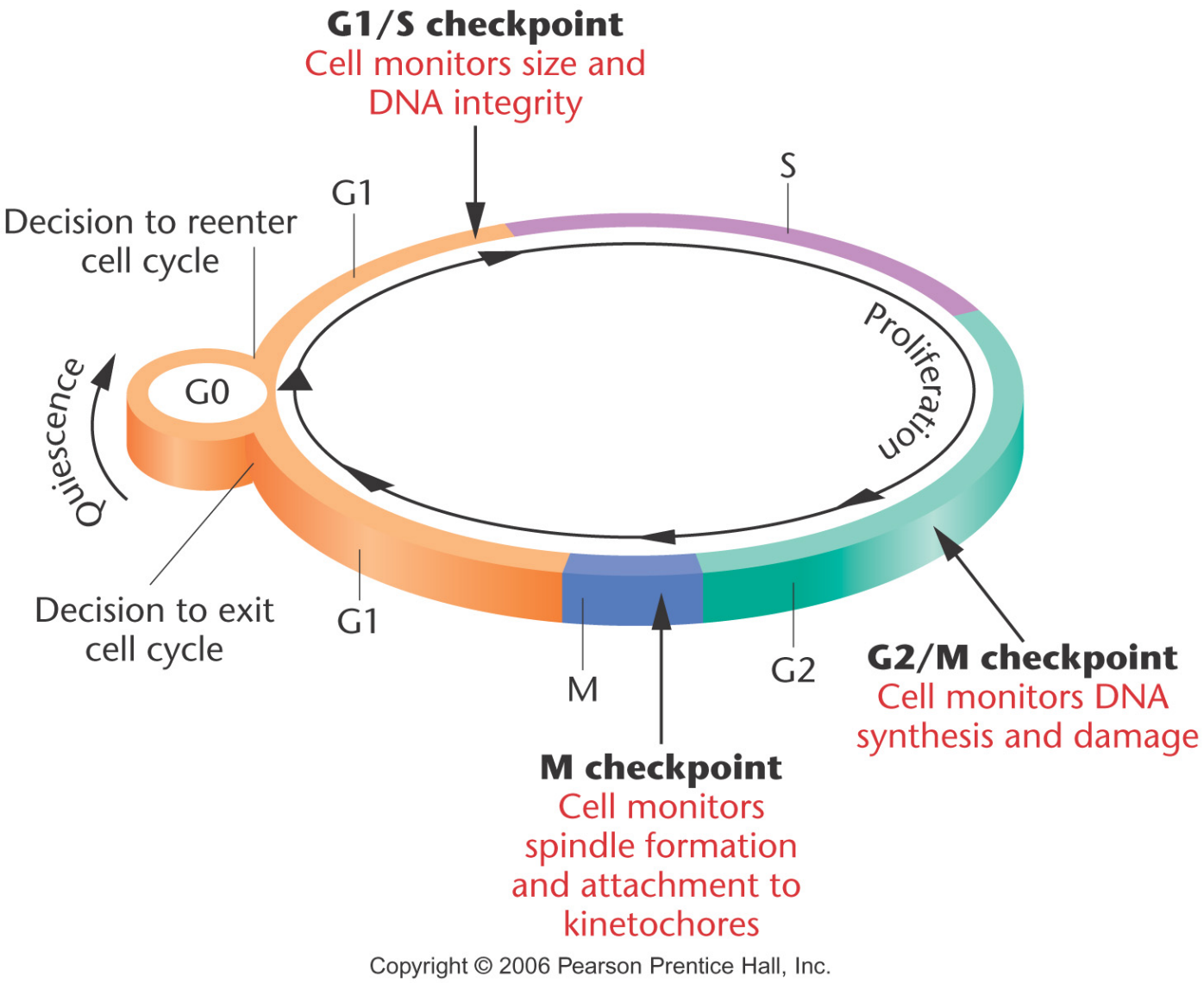
ONCOGENES FREQUENTLY AMPLIFIED IN CANCERS

Oncogene	Cellular function	Type of cancer	%
MYC	Transcription factor	Breast ca	20
		Ovarian ca.	30–40
		Prostate ca.	15
		Pancreatic ca.	15
CCND1	Cell cycle regulator	Esophageal ca.	35
		Head and Neck ca.	25
		Breast ca	15
		Bladder ca.	10–15
CCNE1	Cell cycle regulator	Uterine serous cell ca.	45
		Ovarian ca.	20
CDK4	Cell cycle regulator	Sarcoma	20
		Glioblastoma	20
EGFR	Growth factor receptor	Glioblastoma	30–50
ERBB2	Growth factor receptor	Breast ca	20–35
		Gastric ca	10
MDM2	Regulation of tumor suppressor protein	Sarcoma	20–25
		Glioblastoma	10–15
MET	Protein tyrosine kinase	Breast ca.	20
PIK3CA	Lipid kinase	Lung squamous cell ca.	40
		Ovarian ca	30
		Esophageal ca	20

GENETIC MODIFICATIONS LEADING TO CARCINOGENESIS



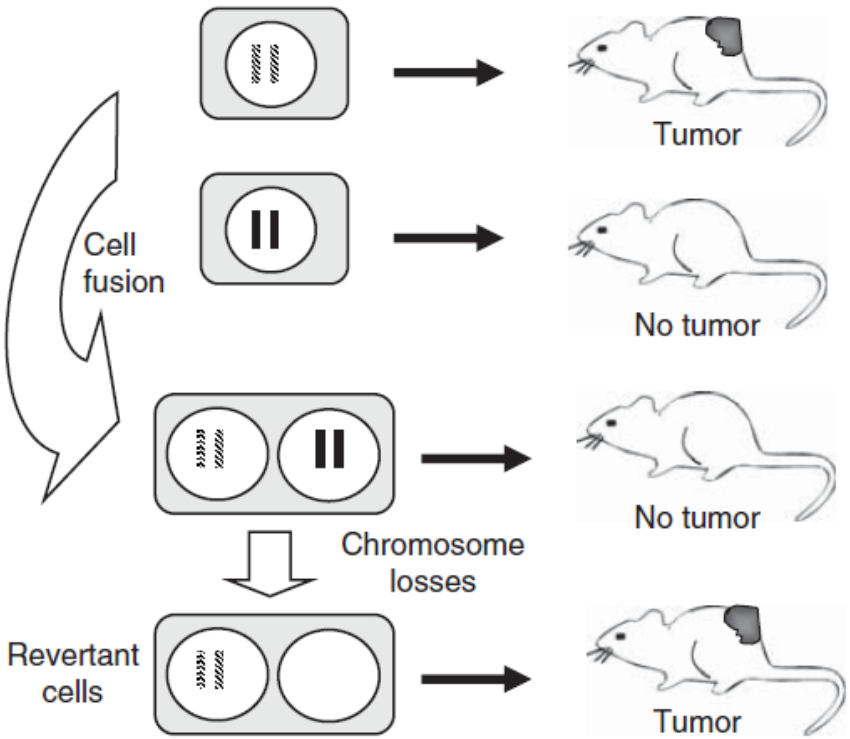
CELL CYCLE



Gene inactivations and Cancer

Tumor supressors (gatekeepers)
Repair Genes(caretakers)

Normal gene >>>>>> allelic loss >>>>>> inactivation



Tumor suppression is a dominant phenotype .

Two distinct types of cultured cells can be distinguished upon their introduction into mice:

Tumorigenic cells (which form tumors when experimentally introduced just below the skin of mice)
and non-tumorigenic cells.

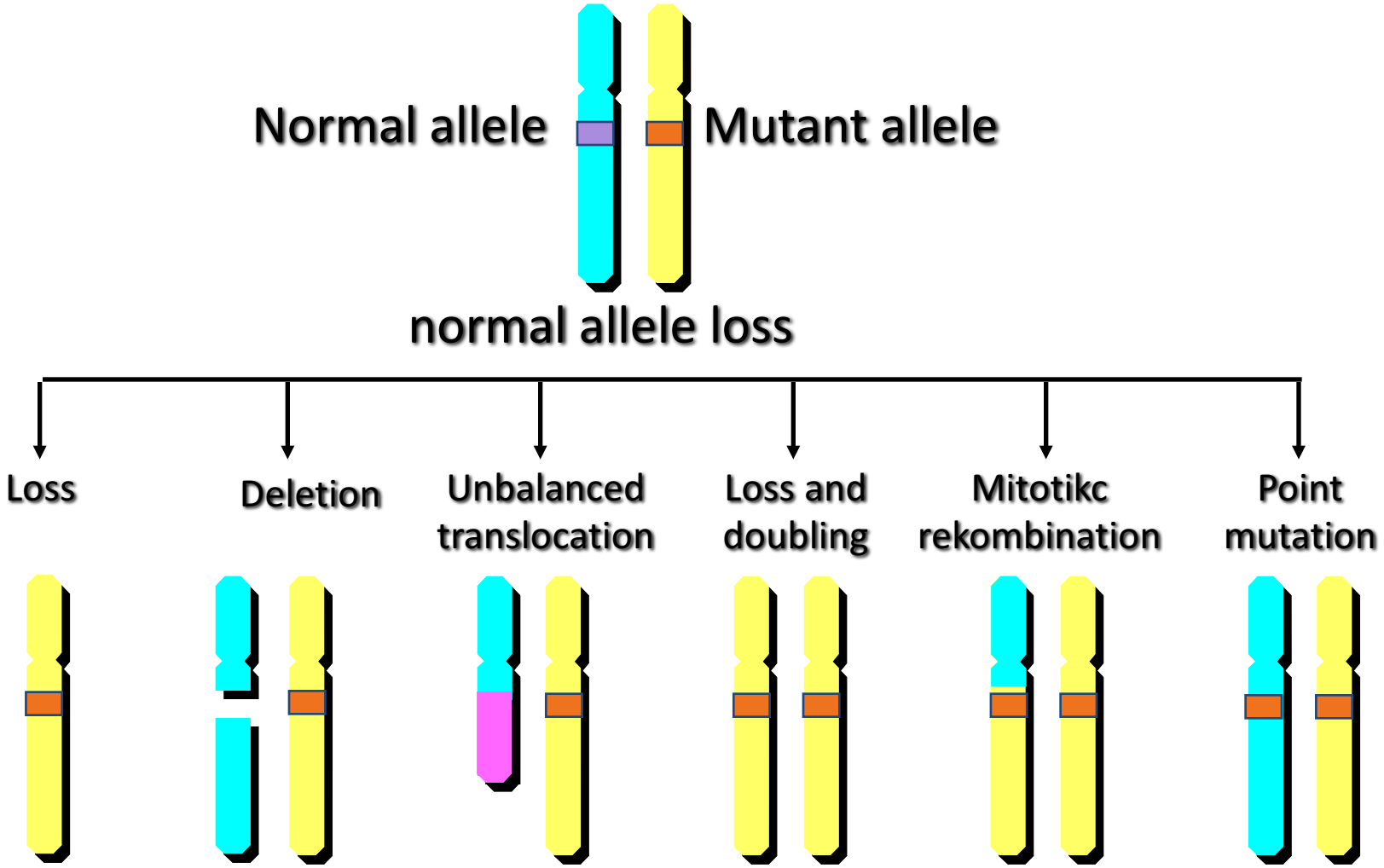
Fusion of these two types of cells allows them to share their genetic material.

Cells containing both sets of chromosomes
are not tumorigenic, demonstrating that the alleles that cause tumor formation (carried on the hatched chromosomes) are recessive.

Because the chromosome complement of the fused cells are unstable, over time cells appear that have lost wild type alleles (carried on the solid chromosomes) contributed by the non-tumorigenic cells.

These rare cells revert to a tumorigenic phenotype. In this simplified illustration, only the relevant pair of homologous chromosomes is shown in each cell

Loss of Heterozygosity



Tumor Supressor Gene Examples

		Disorder	
Gene	**Gen product/function**	**Familial**	**Sporadic**
Gatekeepers			
RB1	p110 / Cell cycle regulator	Retinoblastoma	
TP53	P53 /Cell cycle regulator	Li-Fraumeni syndrome	
APC	APC / Contact inhibition regulator	Familial Adenomatos Poliposis	Colorectal, gastric CA
VHL	Vhl / Controls oxygenation inhibits vascularization	Von-Hippel Lindau sendromu	Renal carcinomes
Caretakers			
BRCA1, BRCA2	Brca1, Brca2 / DNA double strand break repair	Familial breast and ovarian CA	Breast or ovarian CA
MLH1, MSH2	Mlh1, Msh2 / Mismatch repair	Hereditary non polyposis colon cancer	Colorectal CA

TP53 tumor supressor gene

- 50% of cancers possess mutant (inactive) p53.
- Controls >50 genes for transcription.
- DNA damage causes rapid increase in p53 levels.

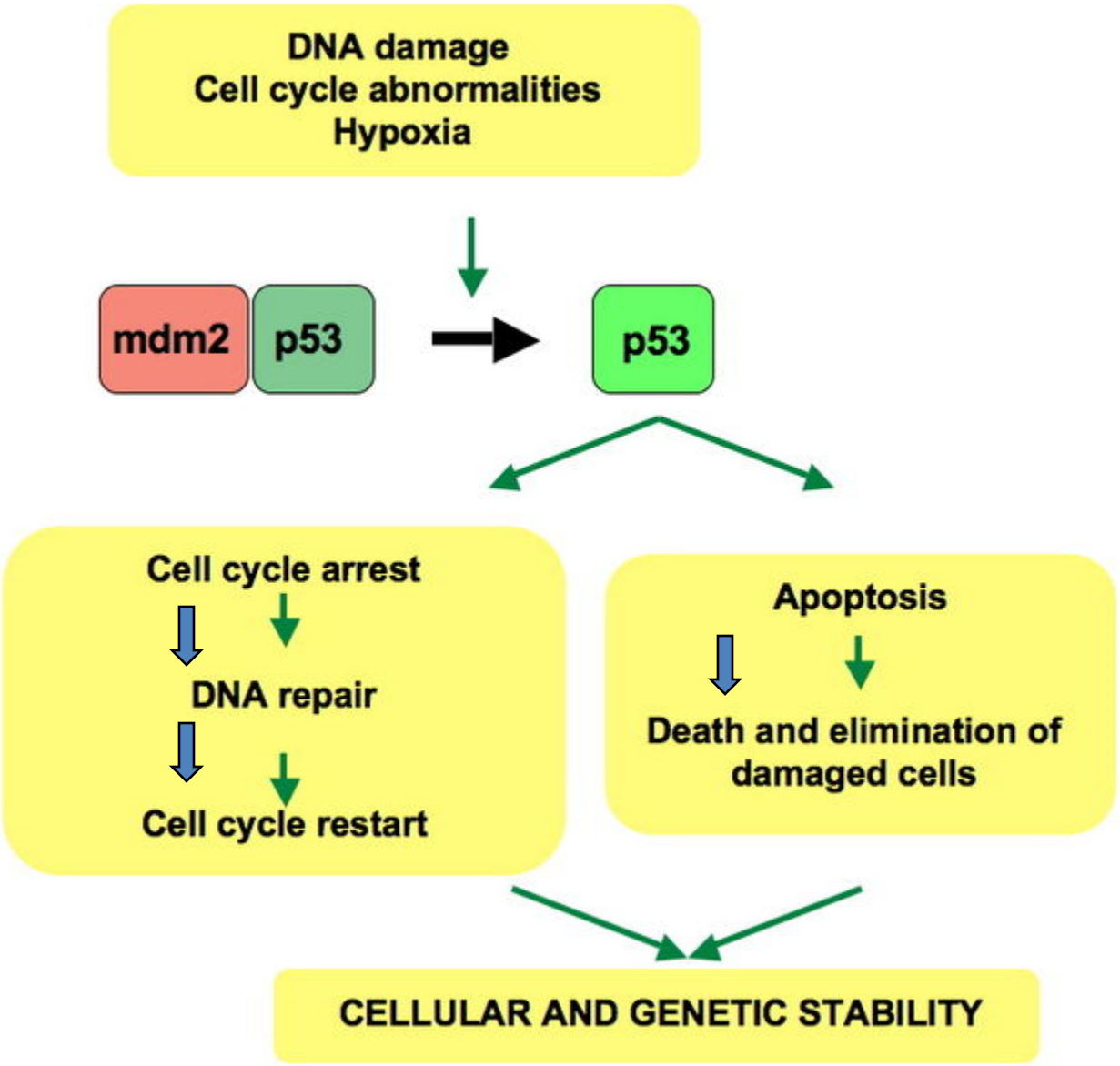
p53 Function

- Active p53 initiates transcription of various genes
  - Suppresses cell cycle; provides time for DNA repair
    - Synthesis of p21 inhibits CDK4/cyclinD1 complex
    - Cell cannot enter the S phase
    - Certain gene products slows down DNA replication
    - Some interrupts at the G2/M stage
  - When DNA repair cannot be done p53 triggers apoptosis
    - *BAX* gene product inhibits *Bcl2* gene
    - Bax homodimers cause cell disruption
    - Mutant p53 cannot be active even in the presence extensive DNA/cell damage

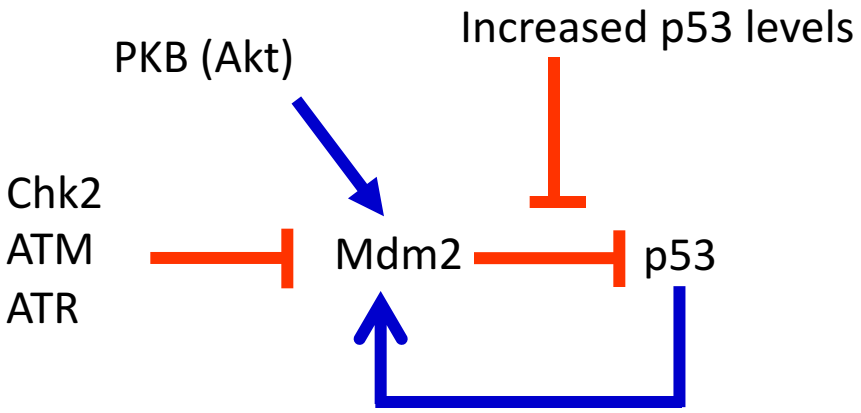
p53 levels is elevated with:

- Ionizing radiation
- UVB
- Hipoxia
- Heat shock
- Oncogene activation
- Cytotoxic chemicals, drugs

- Lack of growth factors


DNA damage
Cell cycle abnormalities
Hypoxia
mdm2
p53
p53
Cell cycle arrest
DNA repair
Cell cycle restart
Apoptosis
Death and elimination of damaged cells
CELLULAR AND GENETIC STABILITY

Post-translational control of p53 after cell damage

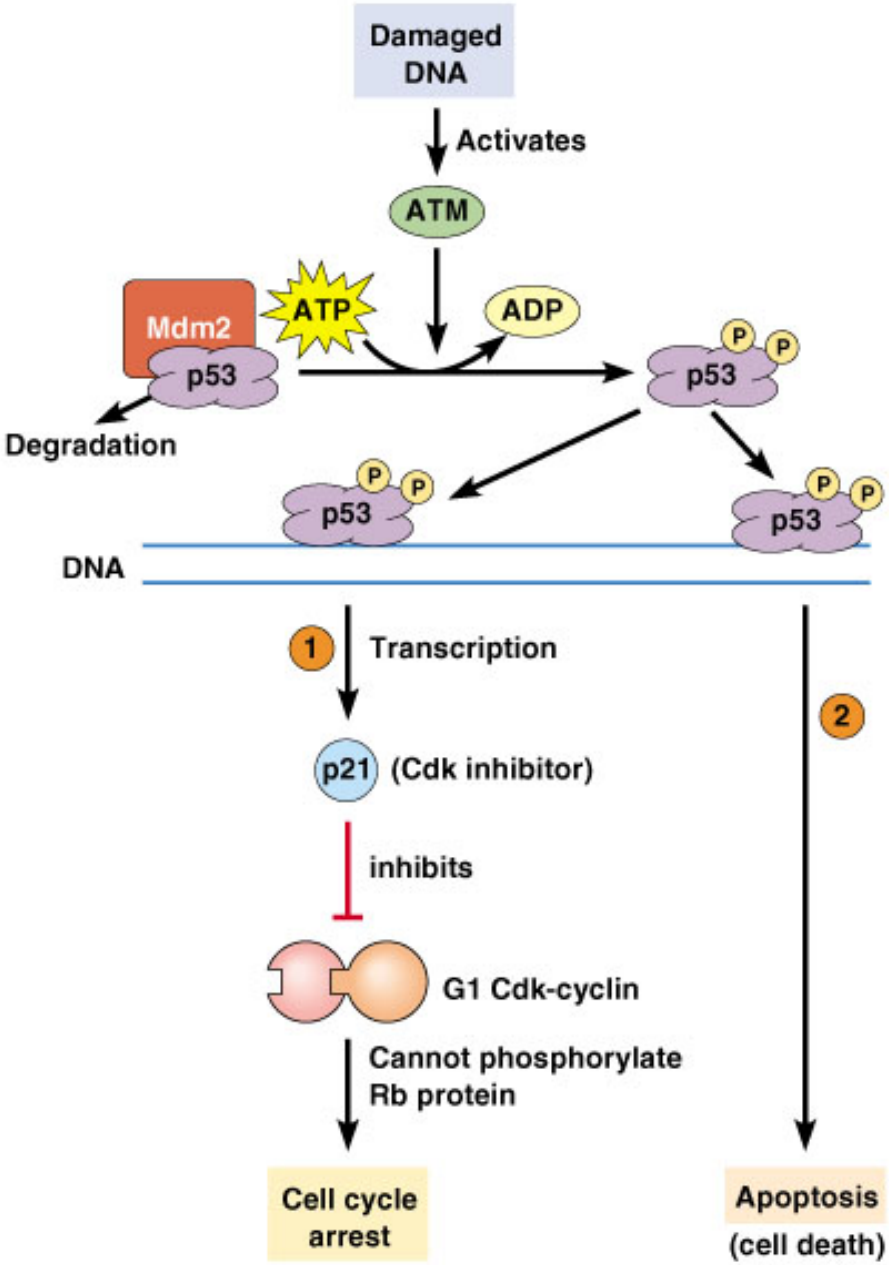


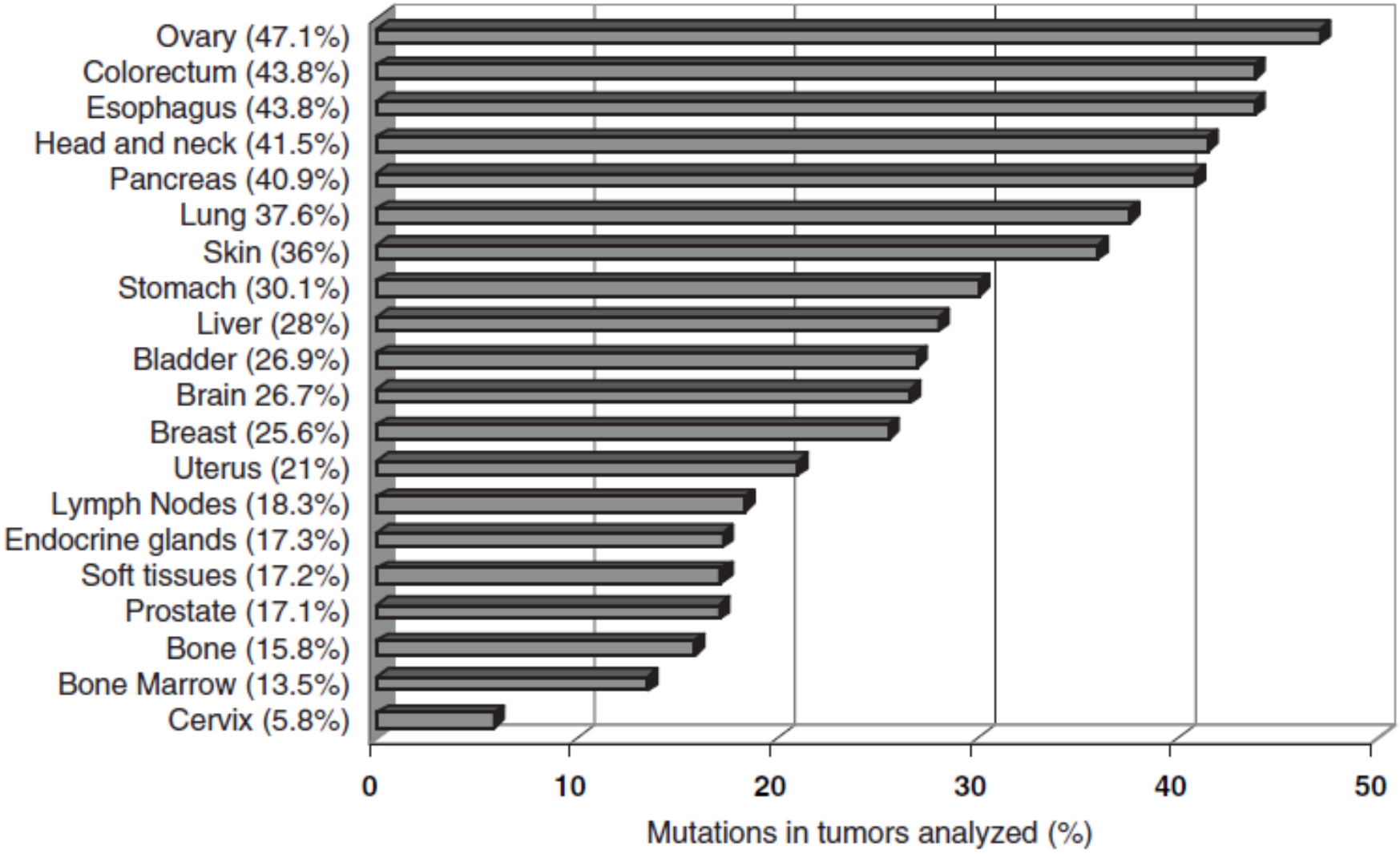
Mdm2: Murine double minute 2

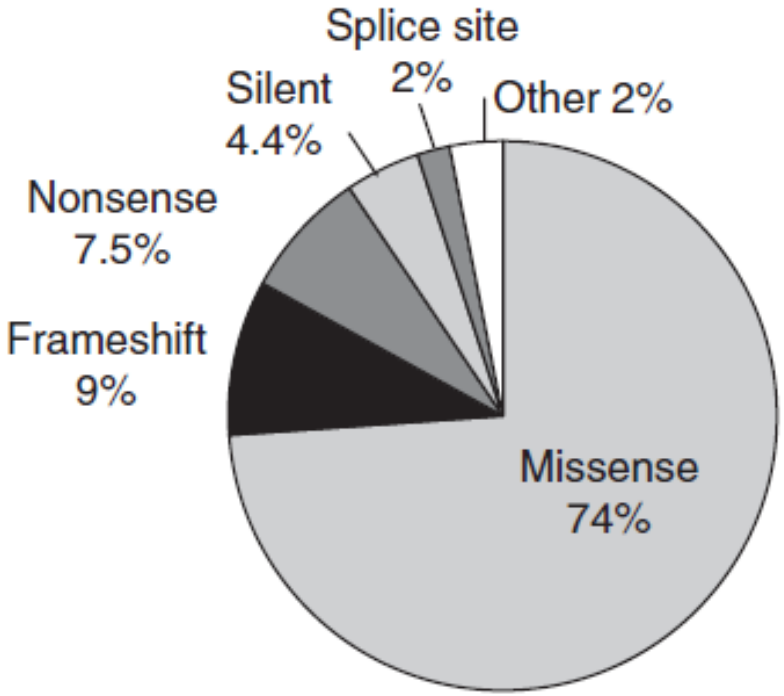
ATM: Ataxia telangiectasia mutated

ATR : Ataxia telangiectasia related

PKB : Protein kinase B




Ovary (47.1%)
Colorectum (43.8%)
Esophagus (43.8%)
Head and neck (41.5%)
Pancreas (40.9%)
Lung 37.6%)
Skin (36%)
Stomach (30.1%)
Liver (28%)
Bladder (26.9%)
Brain 26.7%)
Breast (25.6%)
Uterus (21%)
Lymph Nodes (18.3%)
Endocrine glands (17.3%)
Soft tissues (17.2%)
Prostate (17.1%)
Bone (15.8%)
Bone Marrow (13.5%)
Cervix (5.8%)
0
10
20
30
40
50
Mutations in tumors analyzed (%)

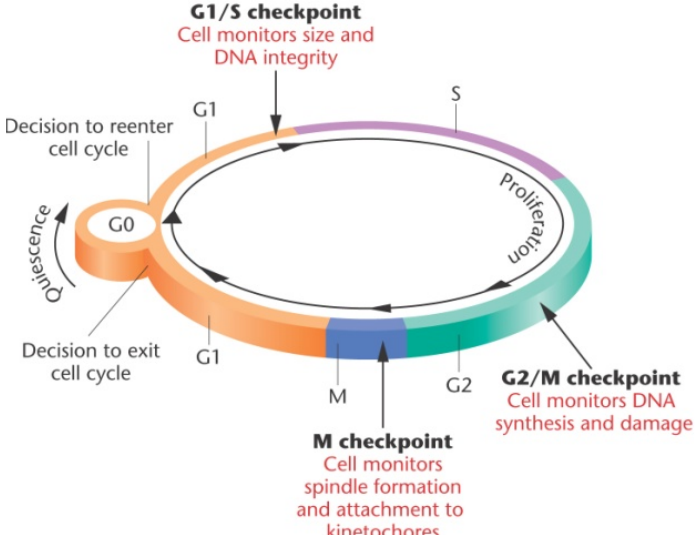


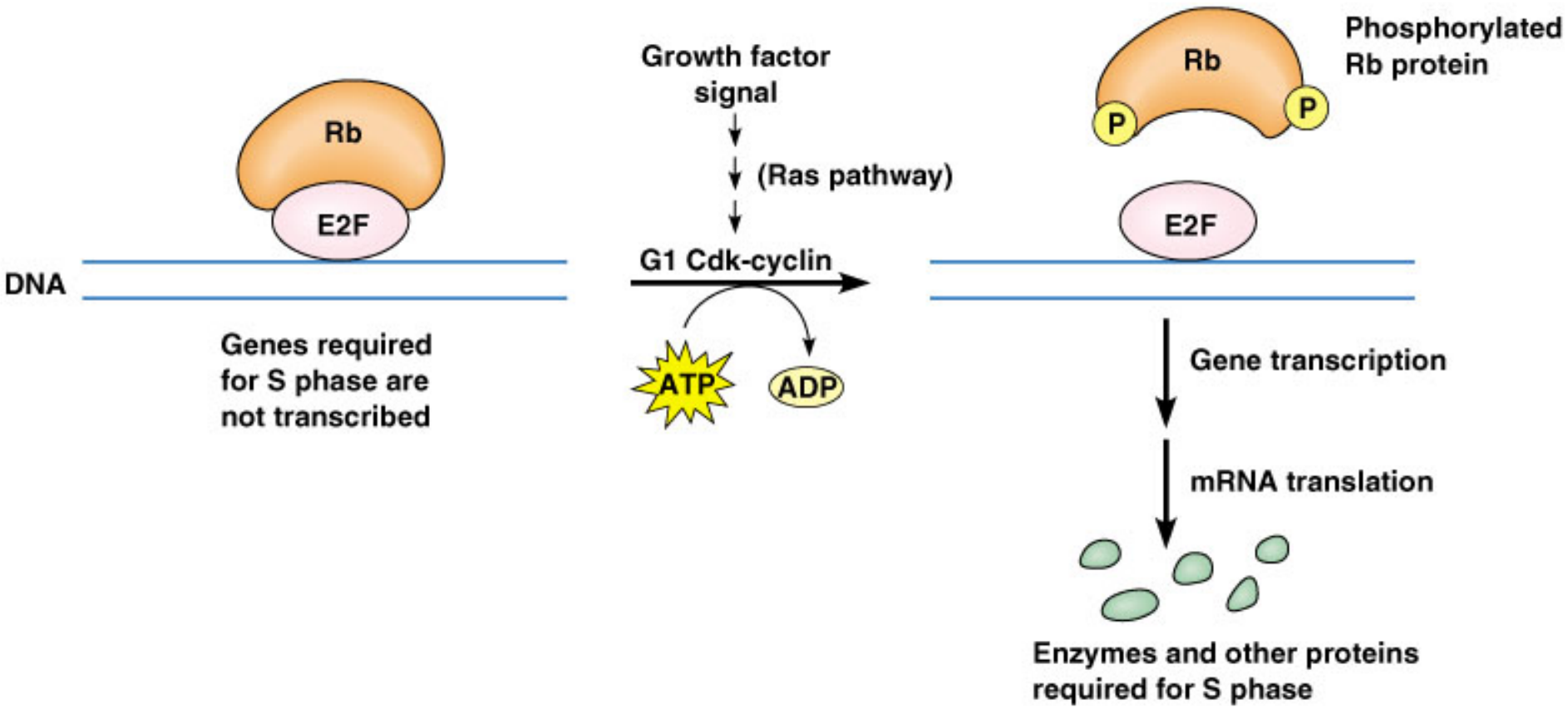
Types of P53 mutations:

Majority of the mutations are point mutations leading to missense variants

pRB (retinoblastoma) Function

- G1/S control point
- Resides in nucleus. Regulated by phoshorylation (Cdk4/cyclinD1 complex)
  - Unphoshorylated RB binds a transcription factor (E2F), and inactivates it
  - Unbound (active) E2F initiates the synthesis of >30 gene products requires to enter S phase





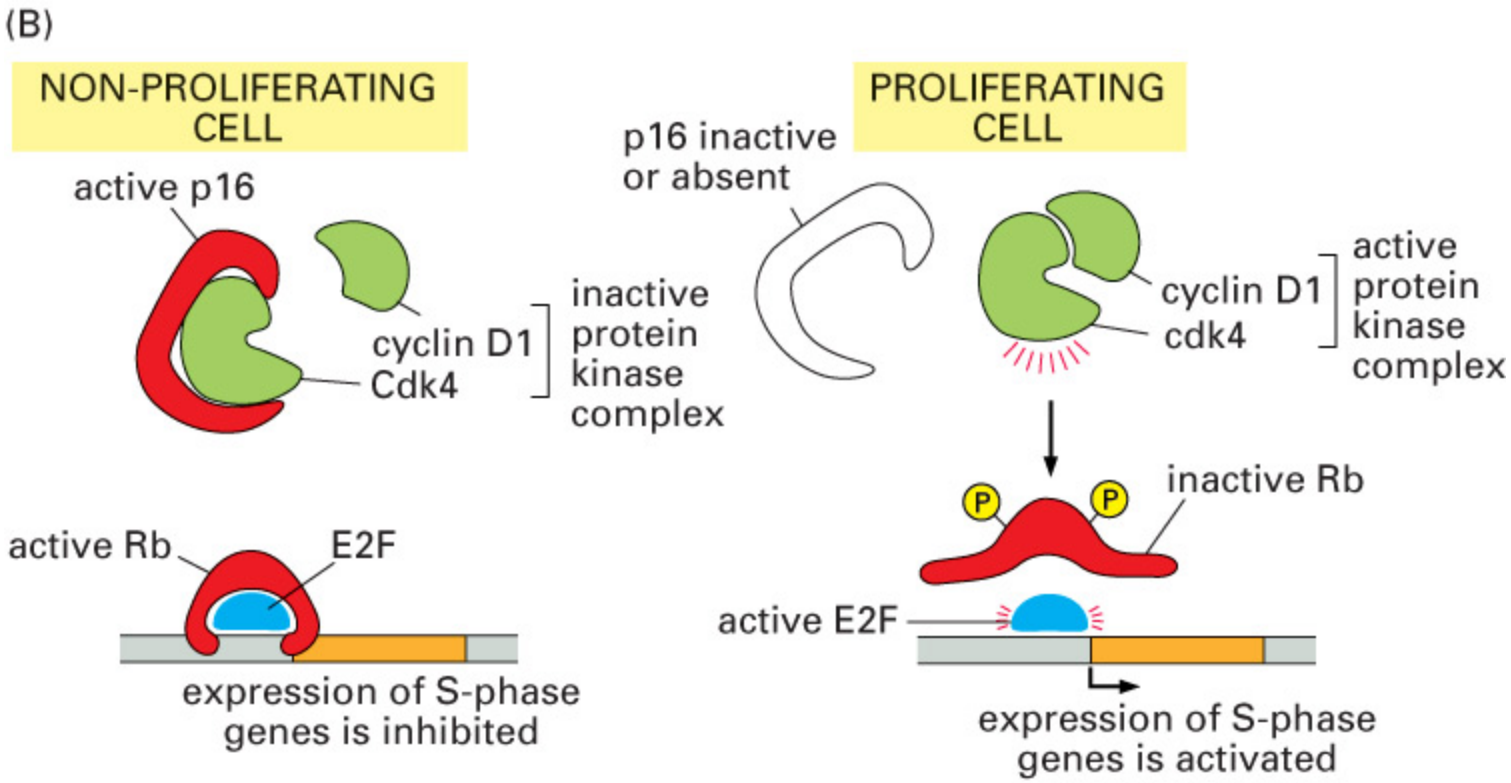


Figure 23–32 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

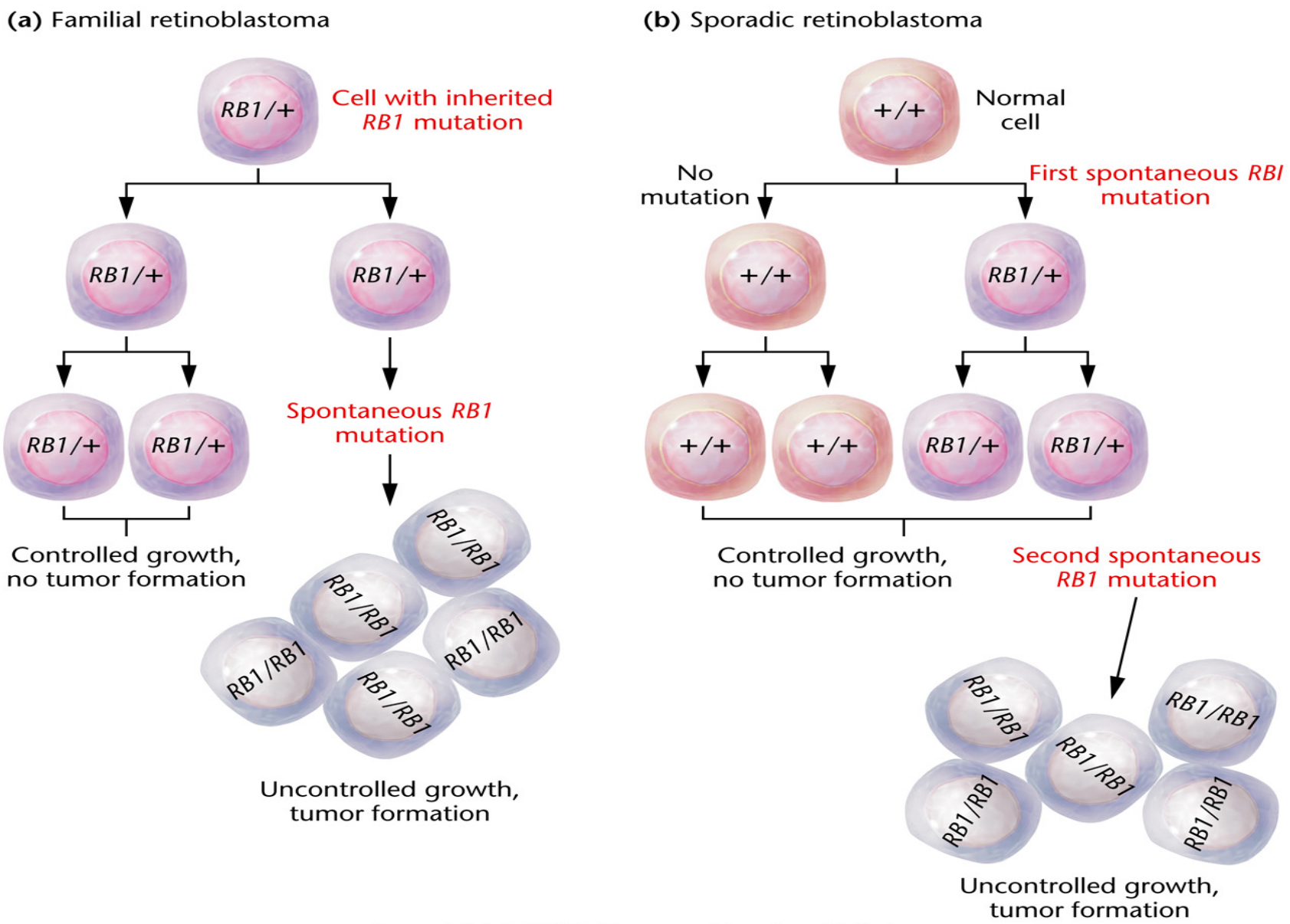
RB1 Tumor Suppressor

- Retinoblastoma 1 gene
- Breast, bone, lung, bladder and retina tumors
- Transmission of one mutant allele can increase the risk of retinoblastoma 85% (compared to 1/14,000-20,000)

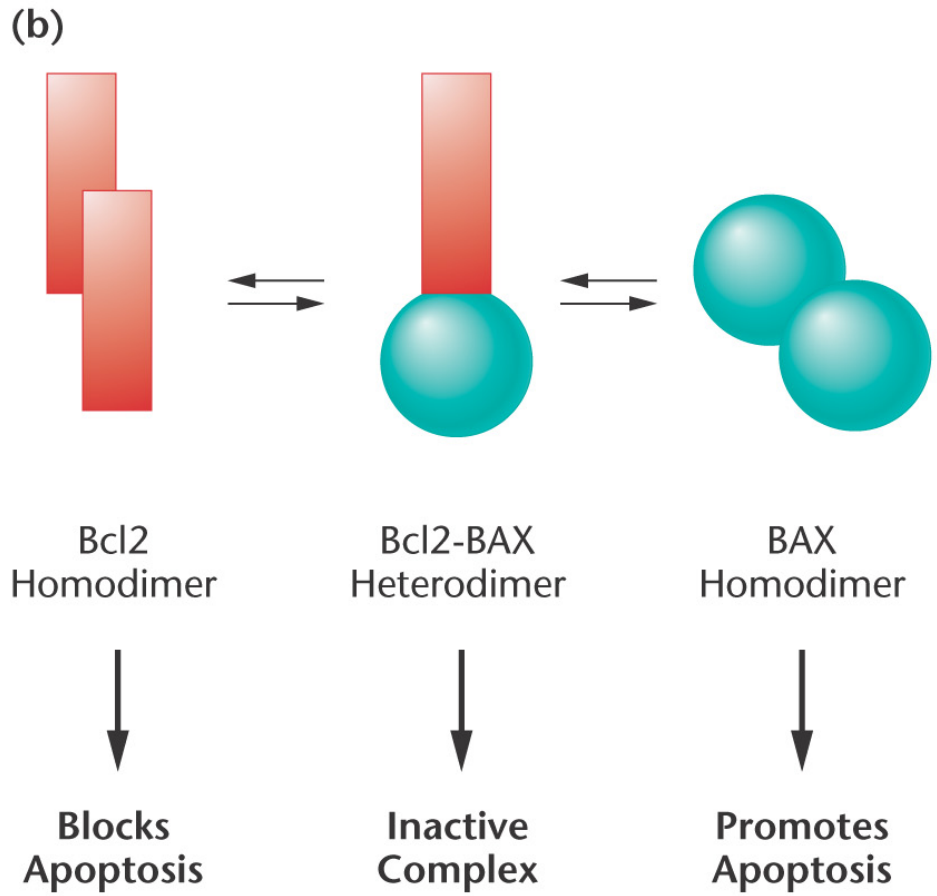
  – Loss of second allele causes the loss of function
  – Loss of two alleles consecutively is a very smaal probability

  – Hence two hit hypothesis works

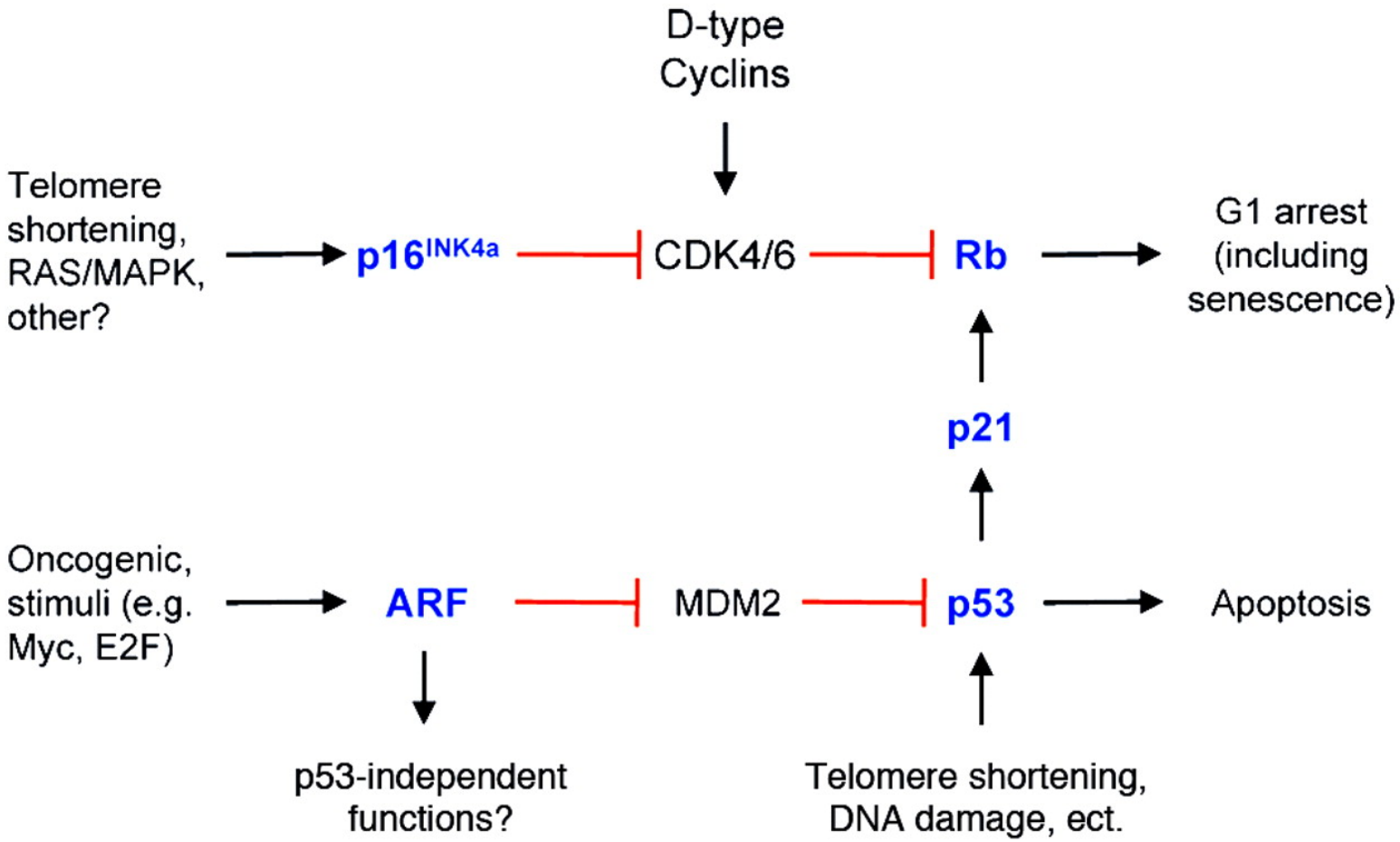
Familyal Retinoblastoma



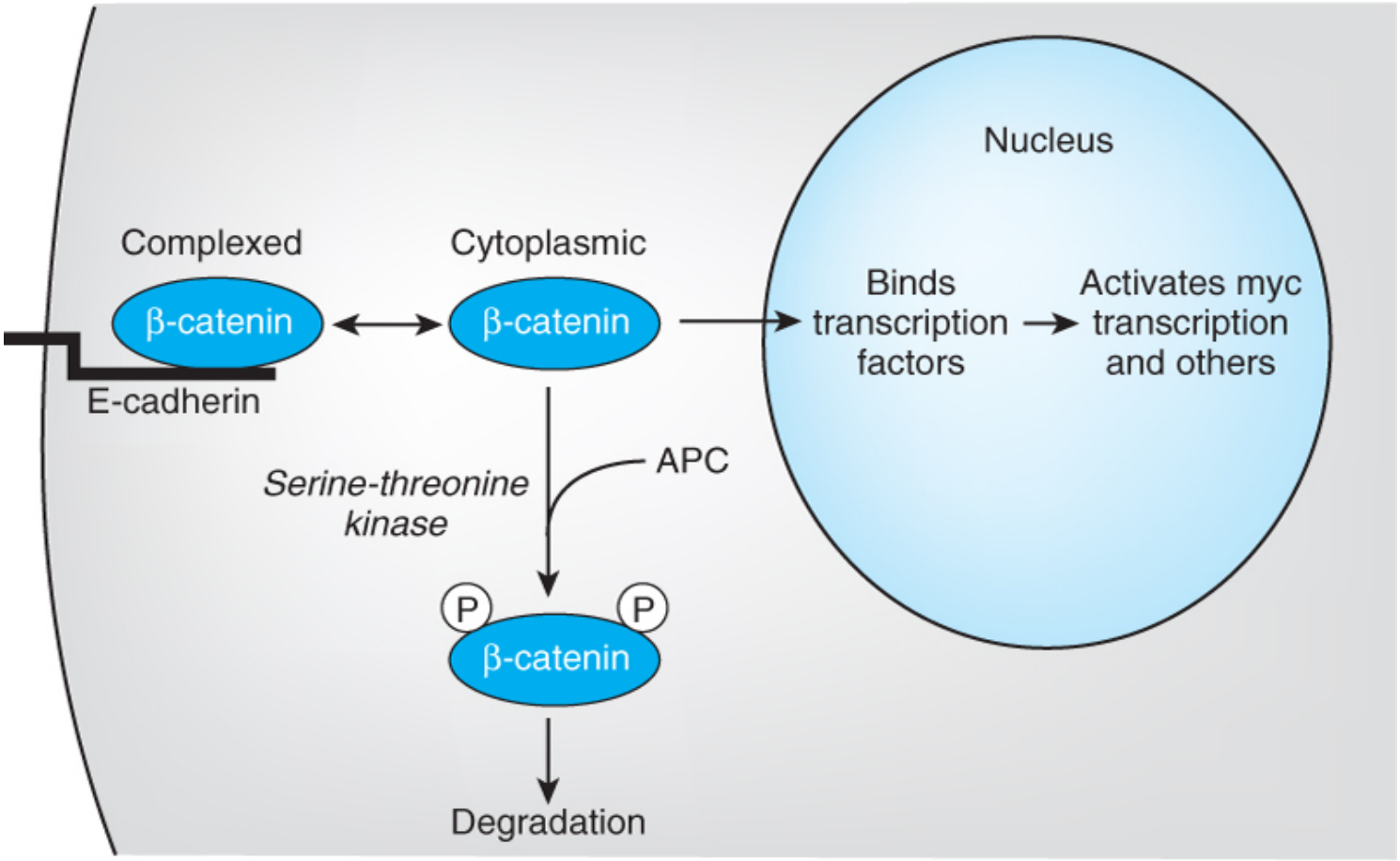
Control of Apoptosis



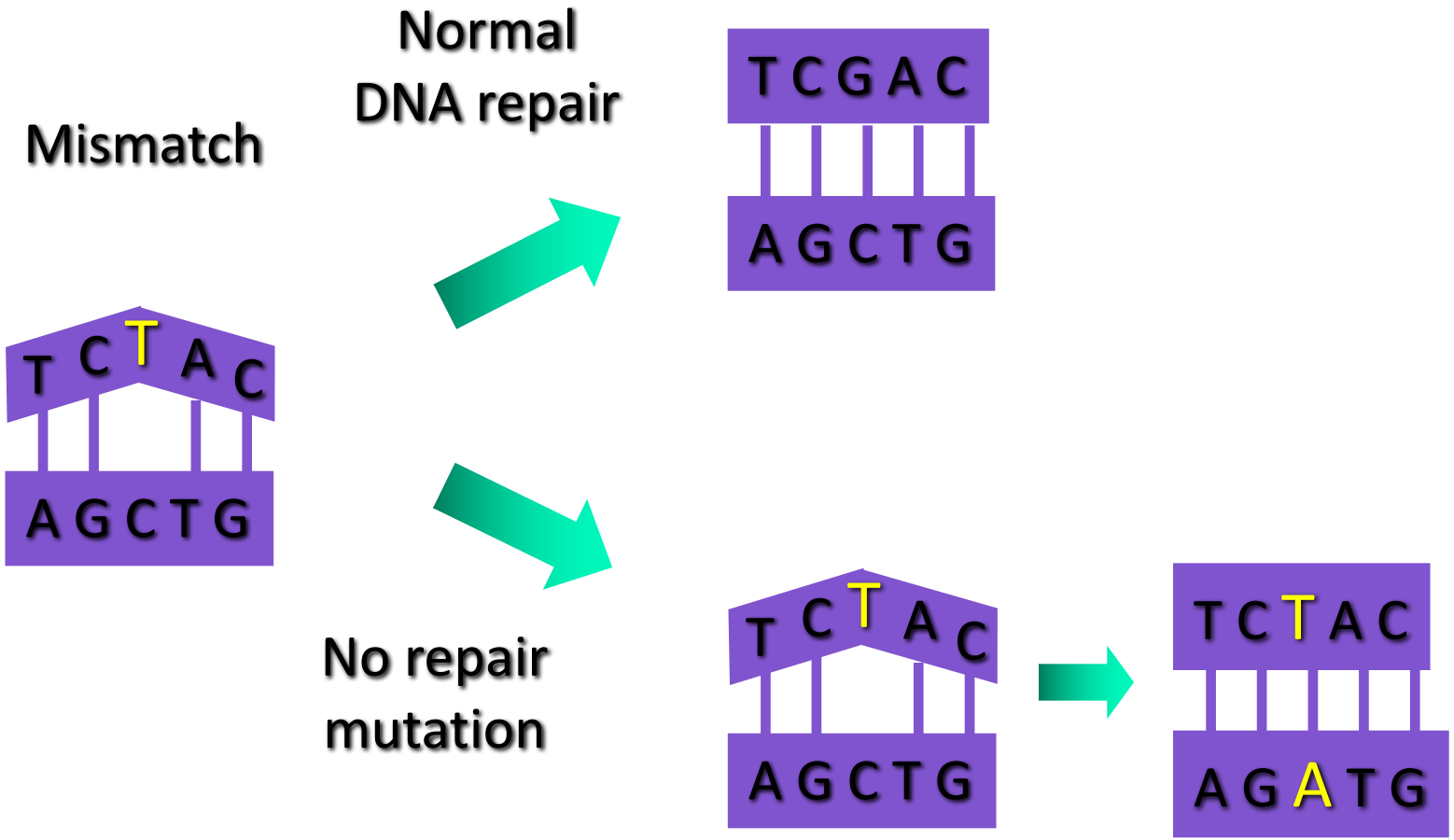
Telomere and p53
At G1 cell cycle control and apoptosis



APC (adenomatous polyposis coli gene) function



DNA Mismatch Repair (MMR genes)

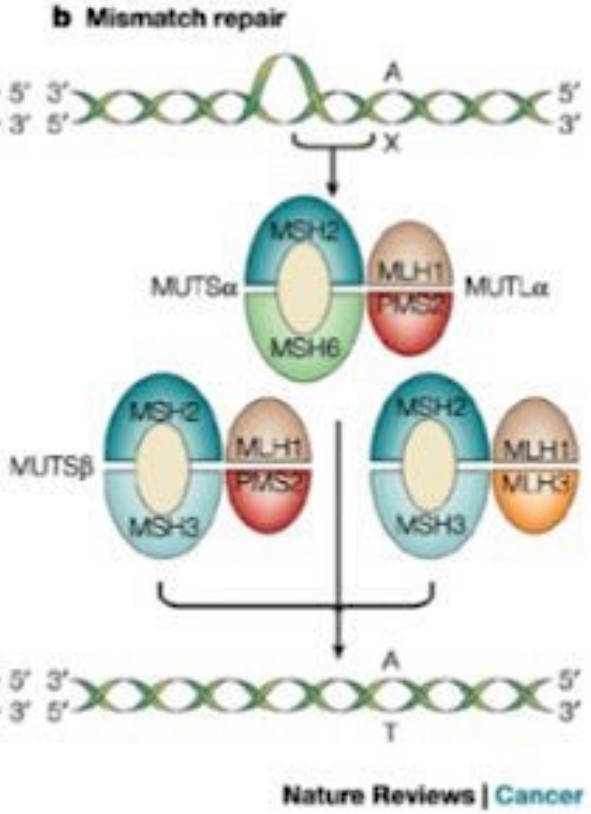


DNA MMR Enzyme Genes

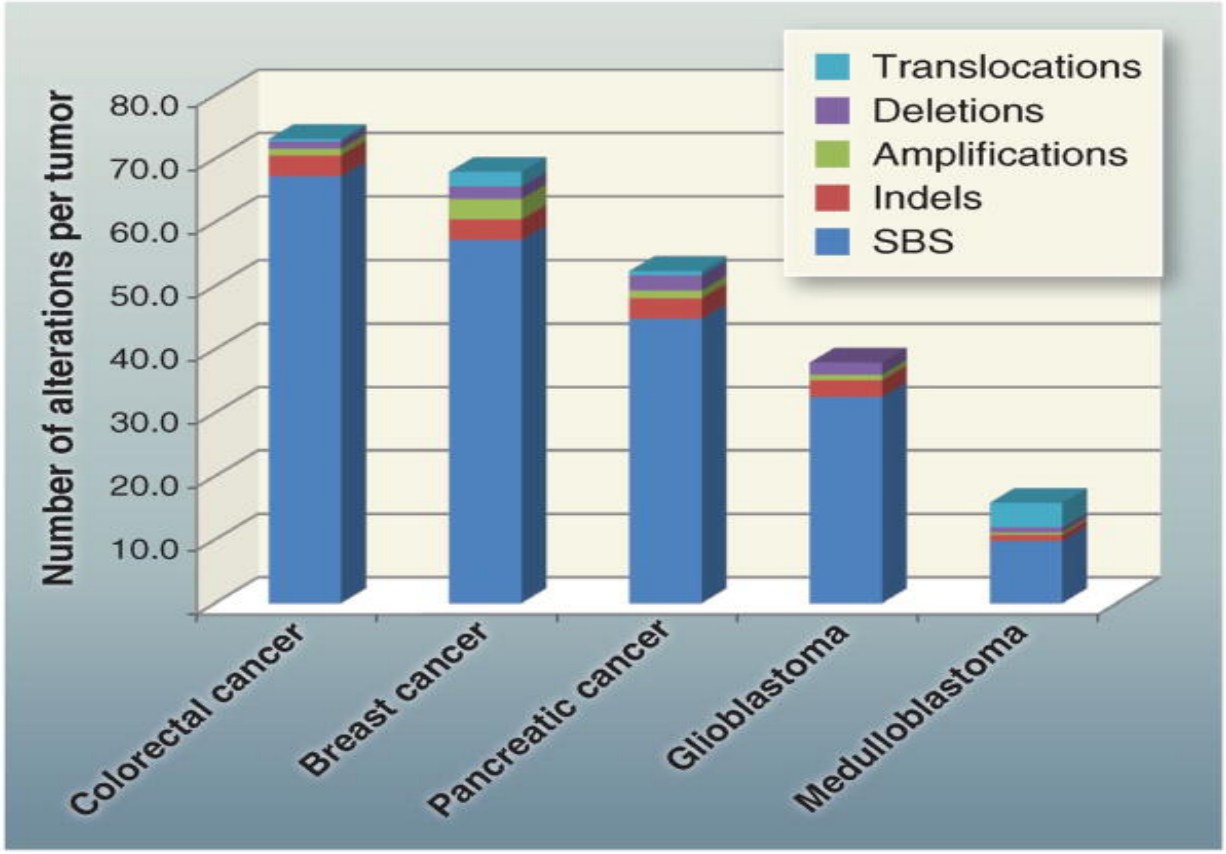
Gene	Location	% Mutations seen
MSH2	2p22	38
MLH1	3p21	32
MSH6	2p16	14
PMS2	7p22	15
PMS1	2q31	-
MLH3	14q24	-
EXO1	1q42	-

Mismatch Repair

- The mismatch repair pathway removes base-base mismatches in homologous recombination intermediates. Mutations in mismatch (*MSH, MLH,* and *PSM)* repair genes lead to **microsatellite instability**
- Precise Mechanism unknown
- **Microsatellite Instability** (small base insertions or deletions) and cancer, especially hereditary nonpolyposis colon cancer (HNPCC).



Total alterations affecting protein-coding genes in selected tumors.



Average number and types of genomic alterations per tumor, including single-base substitutions (SBS), small insertions and deletions (indels), amplifications, and homozygous deletions, as determined by genome-wide sequencing studies.