cancer is fundamentally a genetic disease

Dr. Bilal Azab

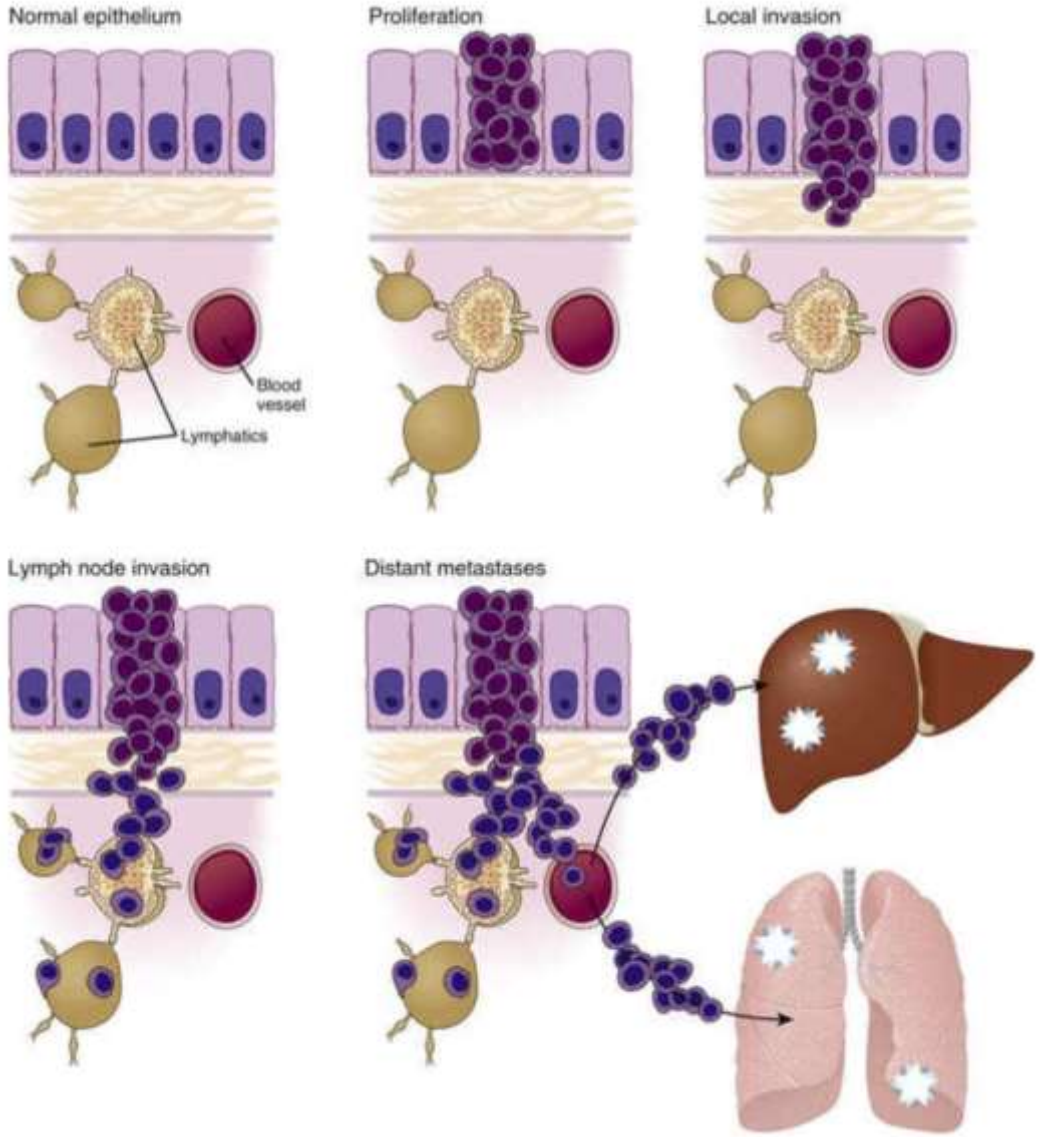
Neoplasia: is a disease process characterized by uncontrolled cellular proliferation leading to a mass or tumor (neoplasm).

Cancer is the name used to describe the more virulent forms of neoplasia

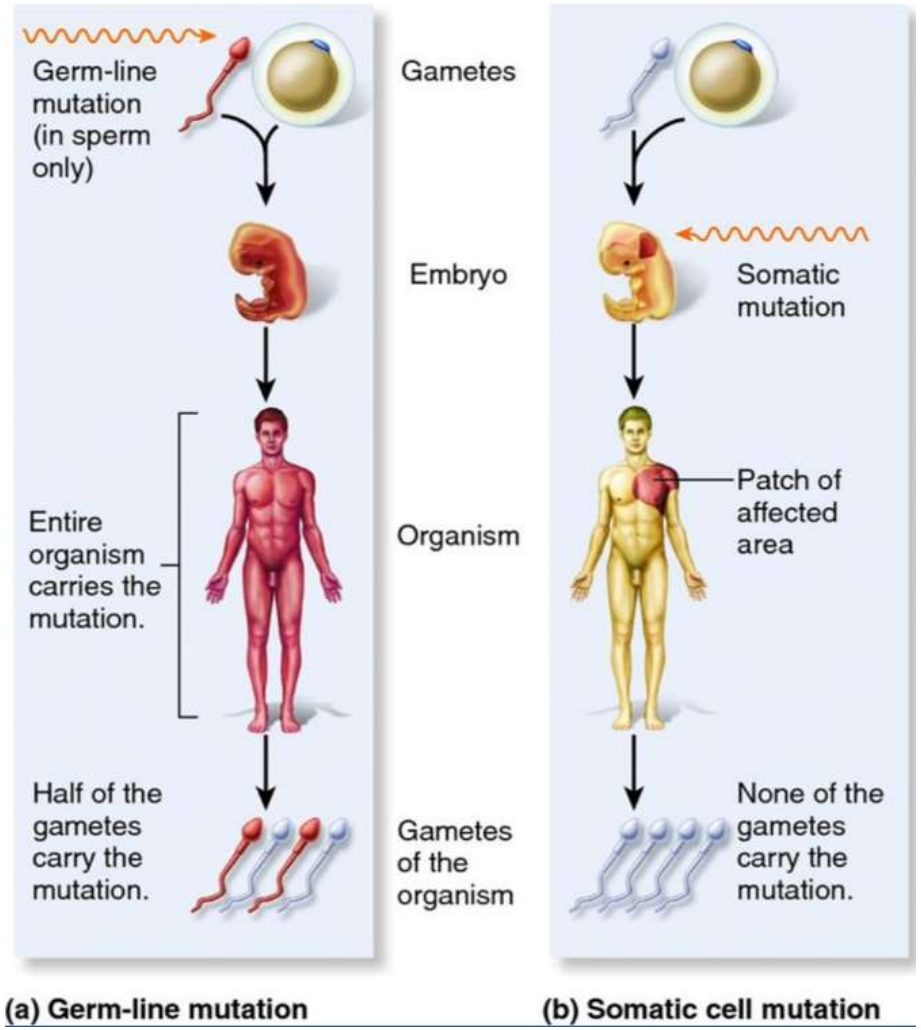
accumulation of cells in a neoplasm occurs because of an imbalance between the normal processes of cellular proliferation and cellular attrition.

For a neoplasm to be a cancer, however, it must also be malignant, which means that not only is its growth uncontrolled, it is also capable of invading neighboring tissues that surround the original site (the primary site) and can spread (metastasize) to more distant sites

Tumors that do not invade or metastasize are not cancerous but are referred to as benign tumors, although their abnormal function, size or location may make them anything but benign to the patient.



General scheme for development of a carcinoma in an epithelial tissue such as colonic epithelium. The diagram shows progression from normal epithelium to local proliferation, invasion across the lamina propria, spread to local lymph nodes, and final distant metastases to liver and lung.



Cancer is not a single disease but rather comes in many forms and degrees of malignancy.

There are three main classes of cancer:

- **Carcinomas**, which originate in epithelial tissue, such as the cells lining the intestine, bronchi, or mammary ducts. Most common

- **Sarcomas**, in which the tumor has arisen in mesenchymal tissue, such as bone, muscle, or connective tissue, or in nervous system tissue

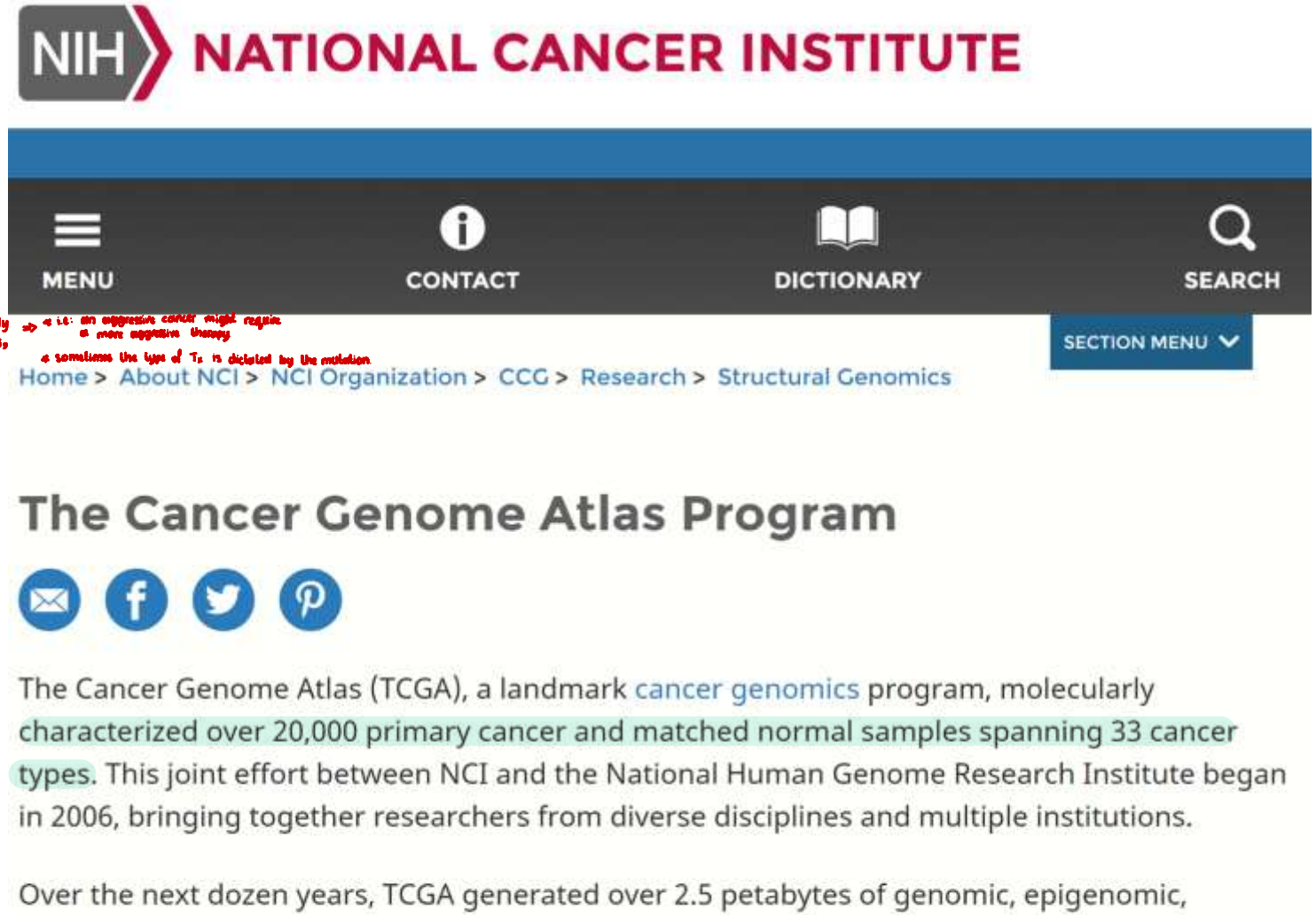
- **Hematopoietic** and **lymphoid** malignant neoplasms, such as leukemia and lymphoma, which spread throughout the bone marrow, lymphatic system, and peripheral blood.

Categories of Cancer

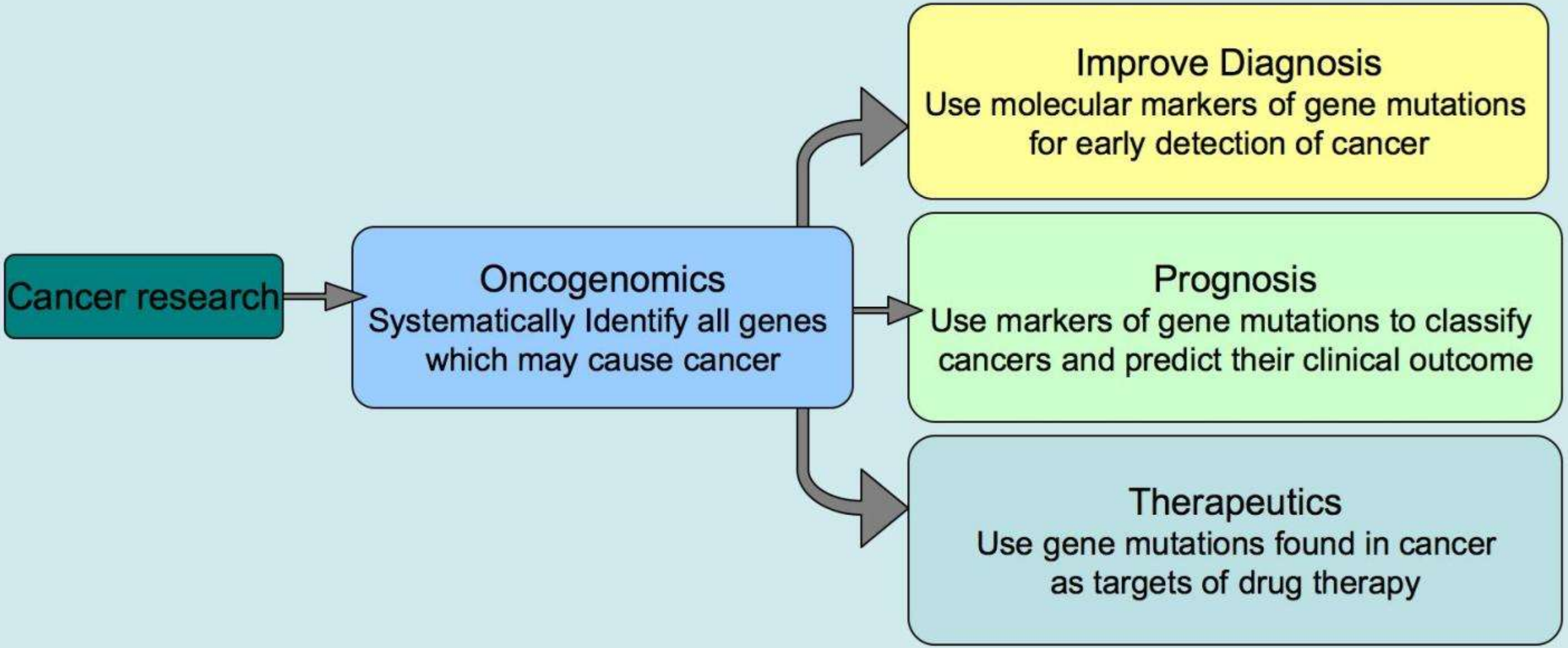
- Carcinoma: Cancer that begins in the skin or in tissues that line or cover internal organs.
- Sarcoma: Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- Leukemia: Cancer that starts in blood-forming tissue such as the bone marrow & causes large numbers of abnormal blood cells to be produced & enter the blood.
- Lymphoma & myeloma: Cancers that begin in the cells of the immune system.

Within each of the major groups, tumors are classified by site, tissue type, histological appearance, degree of malignancy, chromosomal aneuploidy, and, increasingly, by which gene mutations and abnormalities in gene expression are found within the tumor.

Genomics—in particular the identification of mutations, altered epigenomic modifications, and abnormal gene expression in cancer cells—is vastly expanding our knowledge of why cancer develops and is
truly changing cancer diagnosis and treatment.



Overall goals of oncogenomics



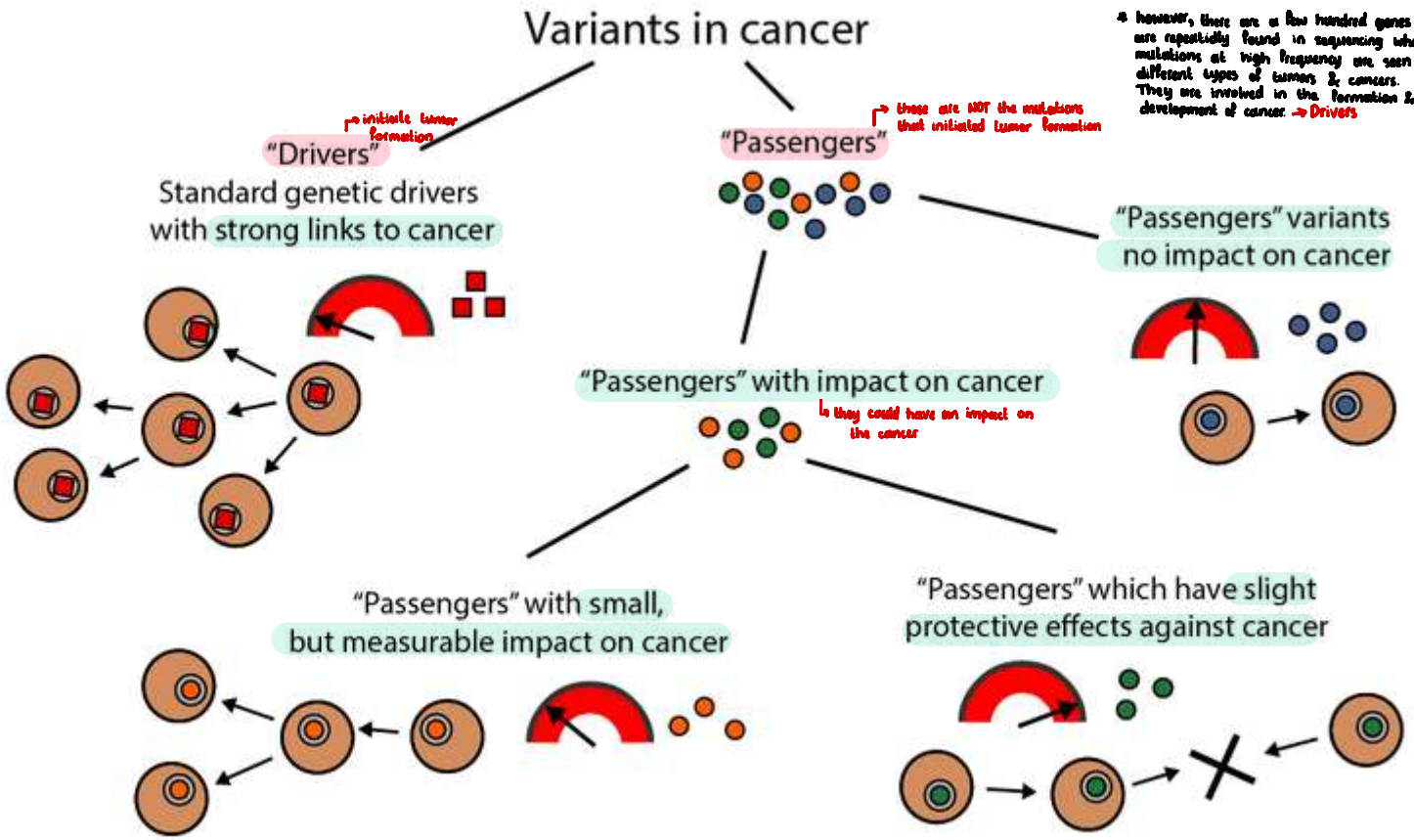
Driver and Passenger Gene Variants

The number of variants present in a tumor can vary from just a few to many tens of thousands.

Most mutations found through sequencing of tumor tissue appear to be random, are not recurrent in particular cancer types, and probably occurred as the cancer developed, rather than directly causing the neoplasia to develop or progress. Such mutations are referred to as "**passenger**" mutations

However, a subset of a few hundred genes has been repeatedly found to be mutated at high frequency in many samples of the same type of cancer or even in multiple different types of cancers, mutated in fact far too frequently to simply be passenger mutations.

These genes are thus presumed to be involved in the development or progression of the cancer itself and are therefore referred to as "**driver**" genes, that is, they harbor mutations (so-called driver gene mutations).



most of the mutations when sequencing is performed appear to be random & not recurrent (other pts with the same cancer don't necessarily have this mutation) They occur as the cancer progresses rather than being directly the underlying cause for the cancer formation → Passengers

however, there are a few hundred genes which are repetitively found in sequencing when mutations at high frequency are seen in different types of tumors & cancers. They are involved in the formation & development of cancer → Drivers

Although many driver genes are specific to particular tumor types, some, such as those in the **TP53** gene encoding the p53 protein, are found in the vast majority of cancers of many different types.

Driver

Although the most common driver genes are now known, it is likely that additional, less abundant driver genes will be identified as The Cancer Genome Atlas continues to grow.

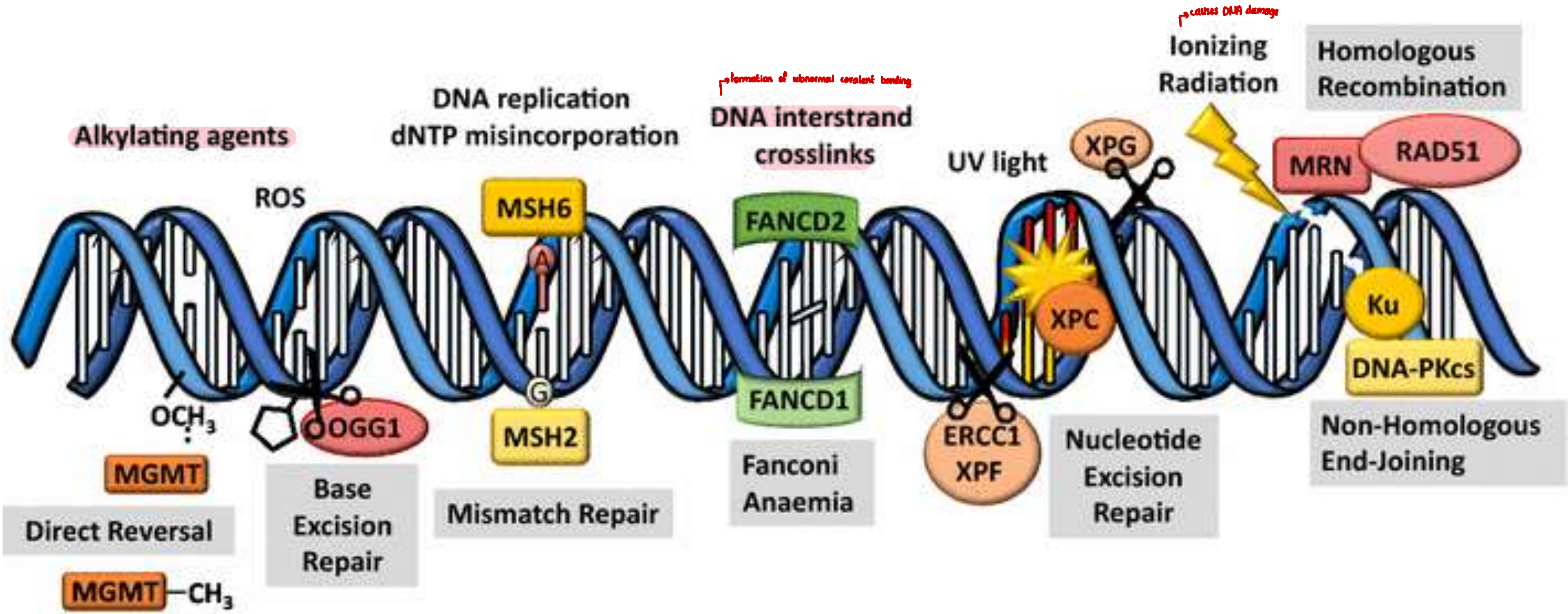
* Where do these driver mutations come from ?

Spectrum of Driver Gene Mutations

↳ error in DNA replication during S phase that is not corrected

↳ external signals, i.e: radiation, UV light ...

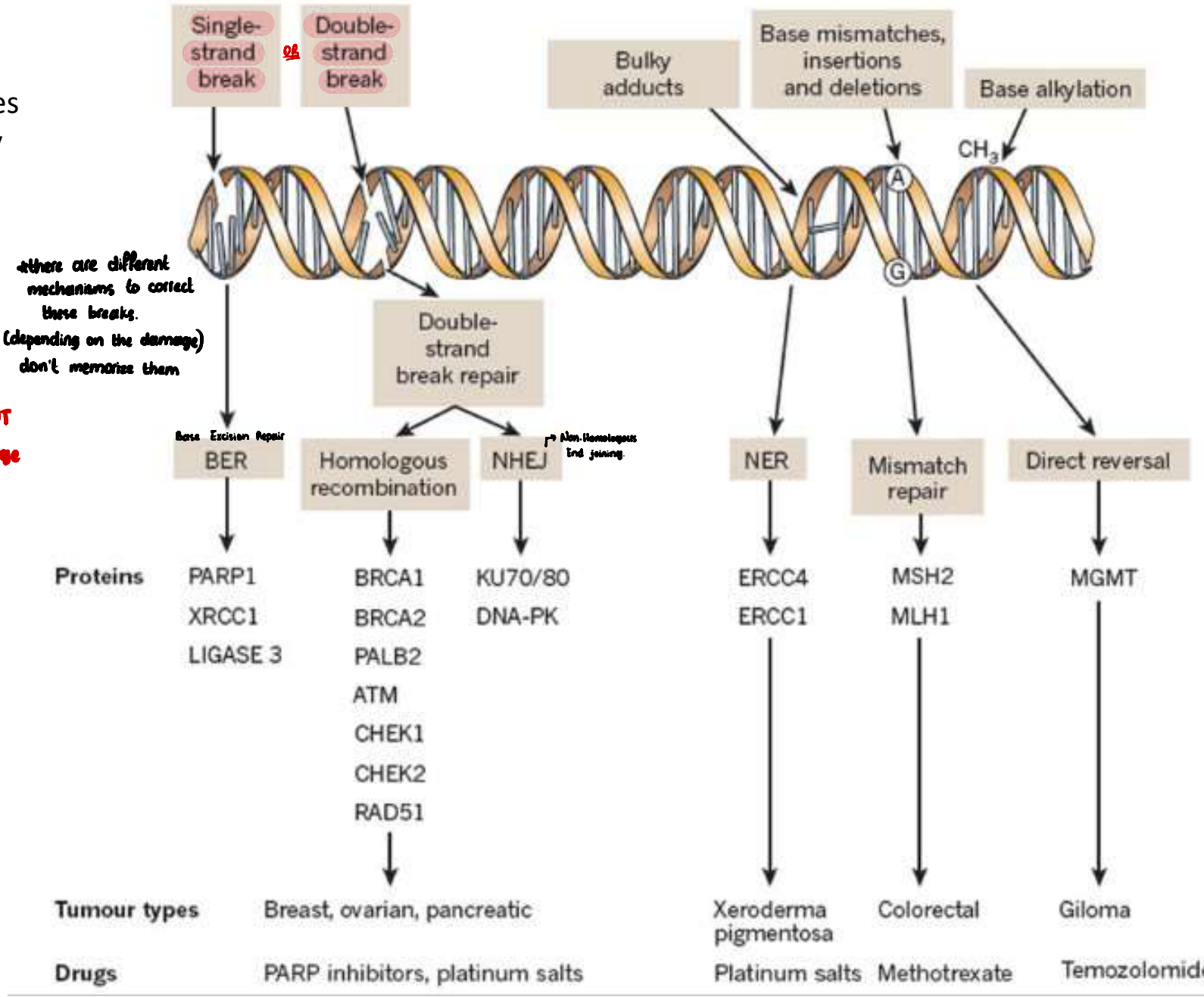
Replication errors, environmental agents and failure of DNA repair could occur to **dividing and arrested** cells will increase the rate of variants around the genome



Spectrum of Driver Gene Mutations

If, by chance, mutations occur in critical driver genes in a particular cell, then the oncogenic process may be initiated.

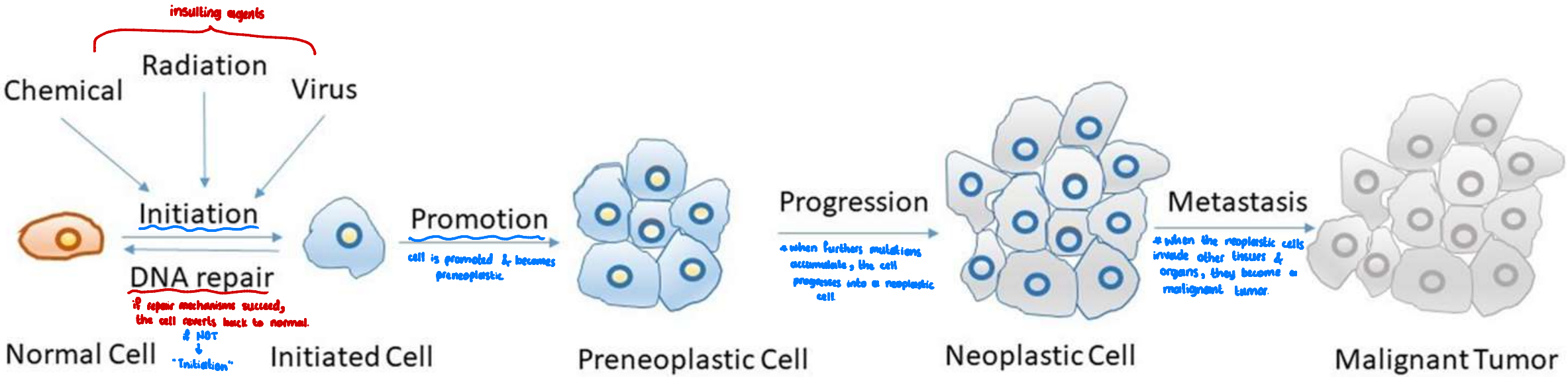
Damage could be:



& Driver & Passenger mutations come around due to internal or external factors, typically they are corrected by repair mechanisms BUT if the repair mechanisms failed to correct this damage & the damage happened to be in a gene involved in cell cycle, division or DNA repair, then the cell could potentially transform into cancer.

Multistep Carcinogenesis

multistep process



if repair mechanisms succeed, the cell reverts back to normal.

if NOT

↓

"Initiation"

* Initiation is NOT enough, more & more mutations start accumulating after.

cell is promoted & becomes preneoplastic

* when further mutations accumulate, the cell progresses into a neoplastic cell

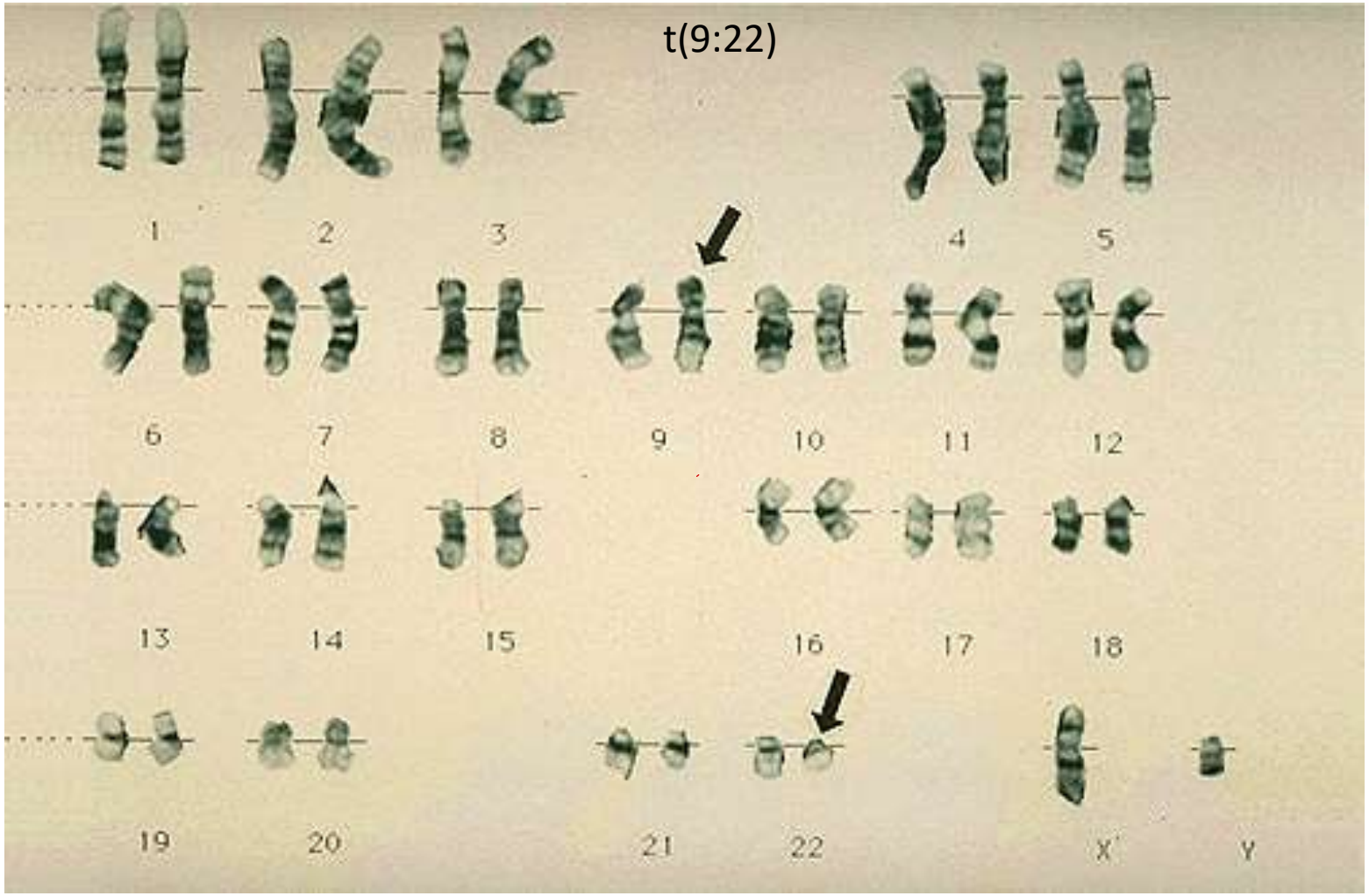
* when the neoplastic cells invade other tissues & organs, they become a malignant tumor

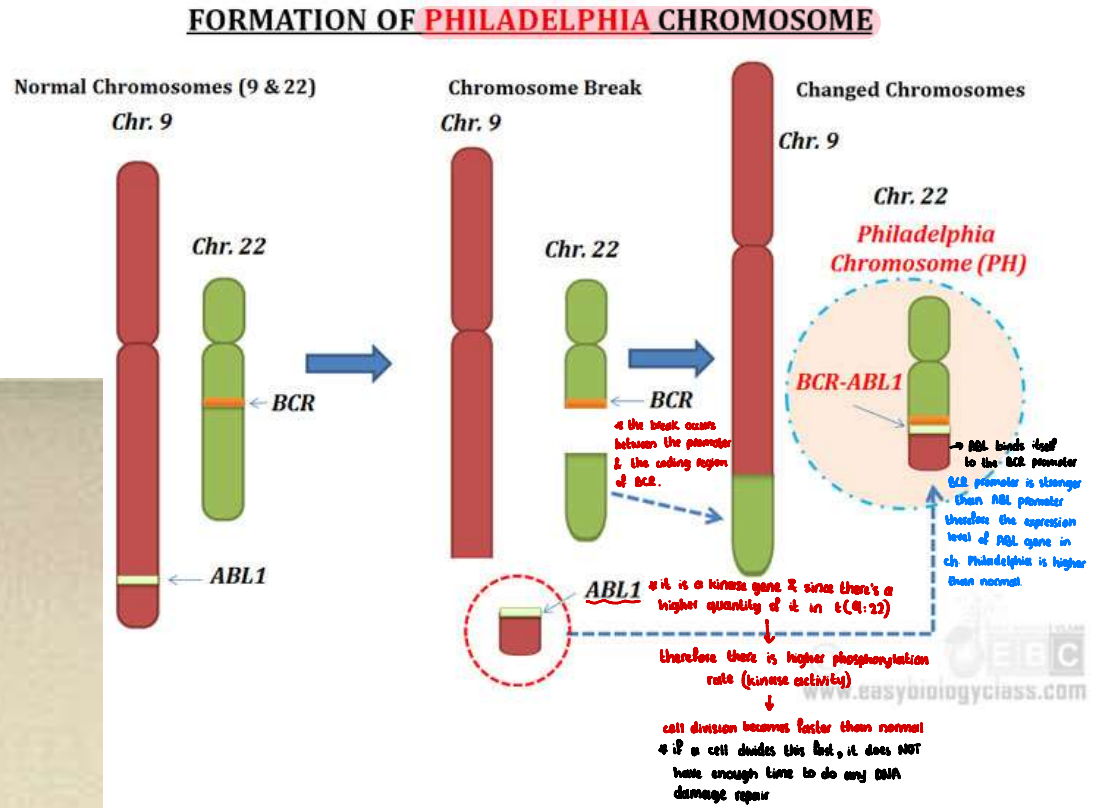
* During this course of "transformation", more driver mutations accumulate along with passenger mutations.

Driver mutations could occur on the chromosomal level

Chromosome and subchromosomal variants can also serve as driver mutations.

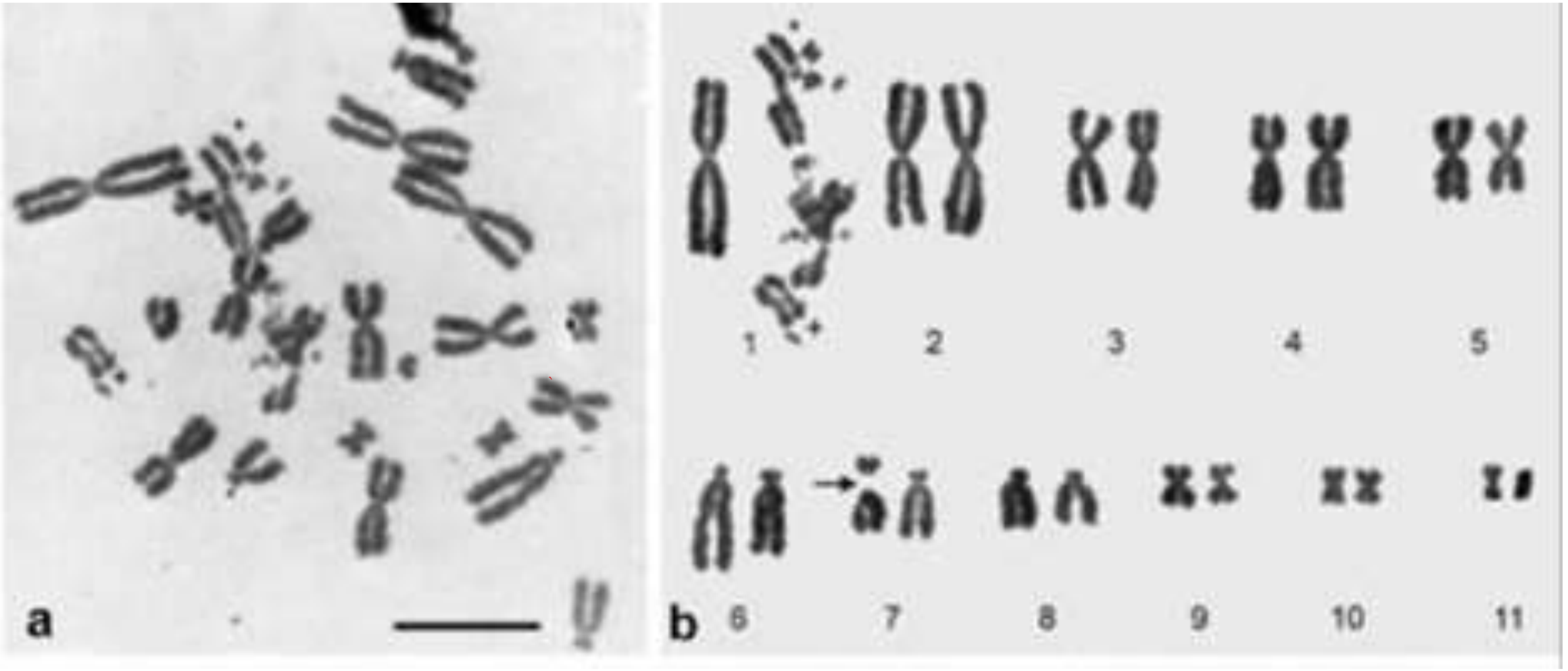
Particular translocations are sometimes highly specific for certain types of cancer ; involve specific genes → Driver mutation for CML
e.g., the BCR - ABL translocation in chronic myelogenous leukemia





Other cancers can show **complex rearrangements** in which chromosomes break into numerous pieces and rejoin (random rejoining), forming novel and complex combinations (a process known as "**chromosome shattering**").

↳ this is an impact of UV light



Metaphase spreads with damaged chromosomes obtained after laser UV microirradiation of nuclei in living Chinese hamster cells.
Nuclei in living Chinese hamster cells were microirradiated (k = 257 nm) at a single nuclear site comprising about 5% of the total nuclear area. Microirradiated cells were followed to the next mitosis (about 3-15 h) in medium with 1 mM caffeine.
a, b Metaphase spread (a) and the corresponding karyogram (b) from a diploid, fibroblastoid Chinese hamster cell reveal a shattered chromosome 1 and a break in a chromosome 7

large genomic alterations involving many kilobases of DNA can form the basis for **loss of function** or **increased function** of one or more driver genes.

i.e: gene amplification.

Large genomic alterations include deletions of a segment of a chromosome or multiplication of a chromosomal segment to produce regions with many copies of the same gene (**gene amplification**).

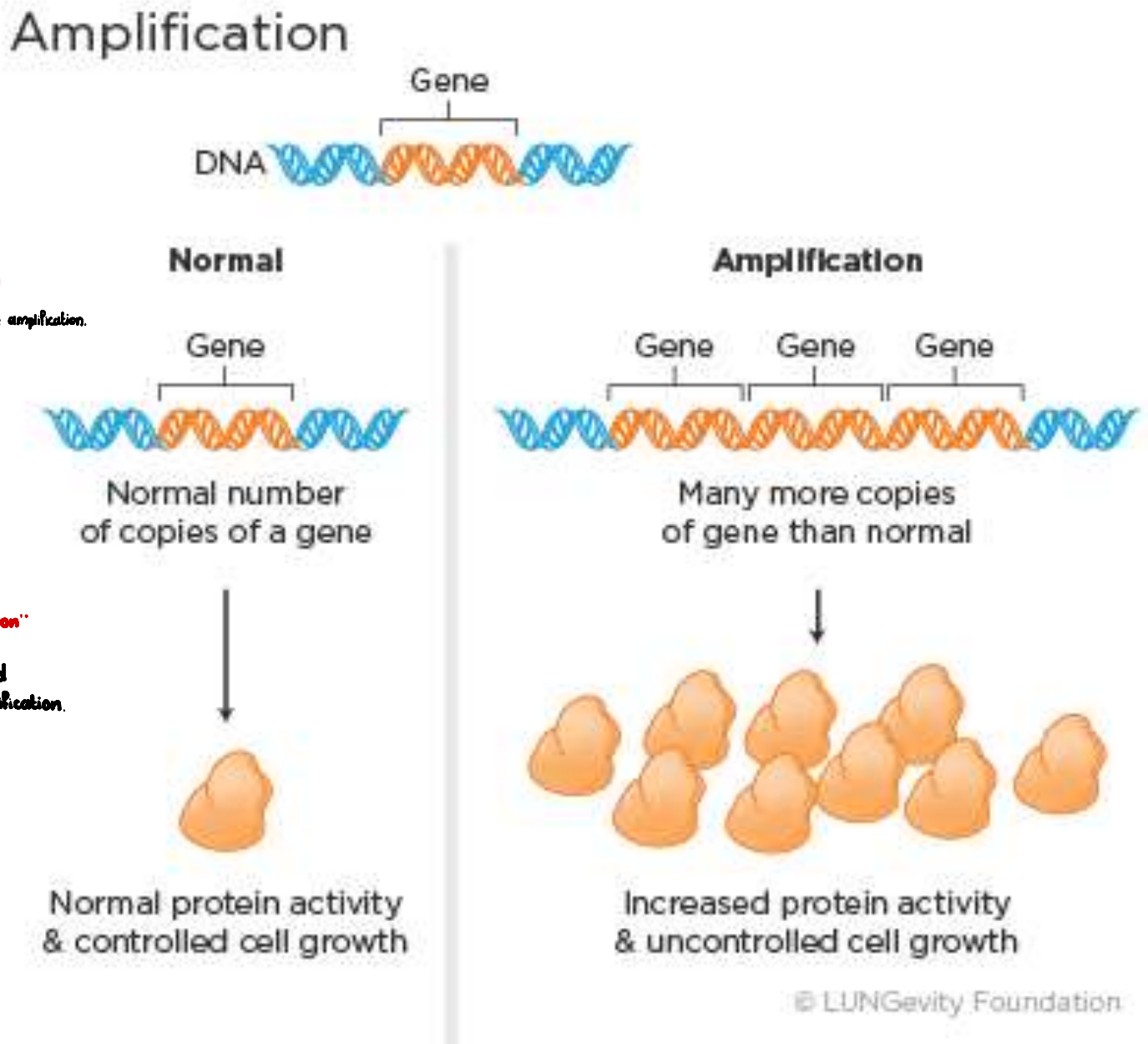
i.e: MYC gene amplification.

* having many copies of the same gene instead of 1

* the gene itself is normal BUT there are much more copies of it which results in ↑↑↑ expression level of the gene.

* if this gene induces cell cycle & division
↓
abnormal cell cycle
↓
cell becomes under potential of "transformation"

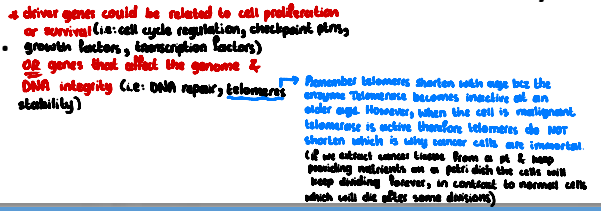
* it's not always a loss of function, it could also be gain of function as in gene amplification.



The Cellular Functions of Driver Genes

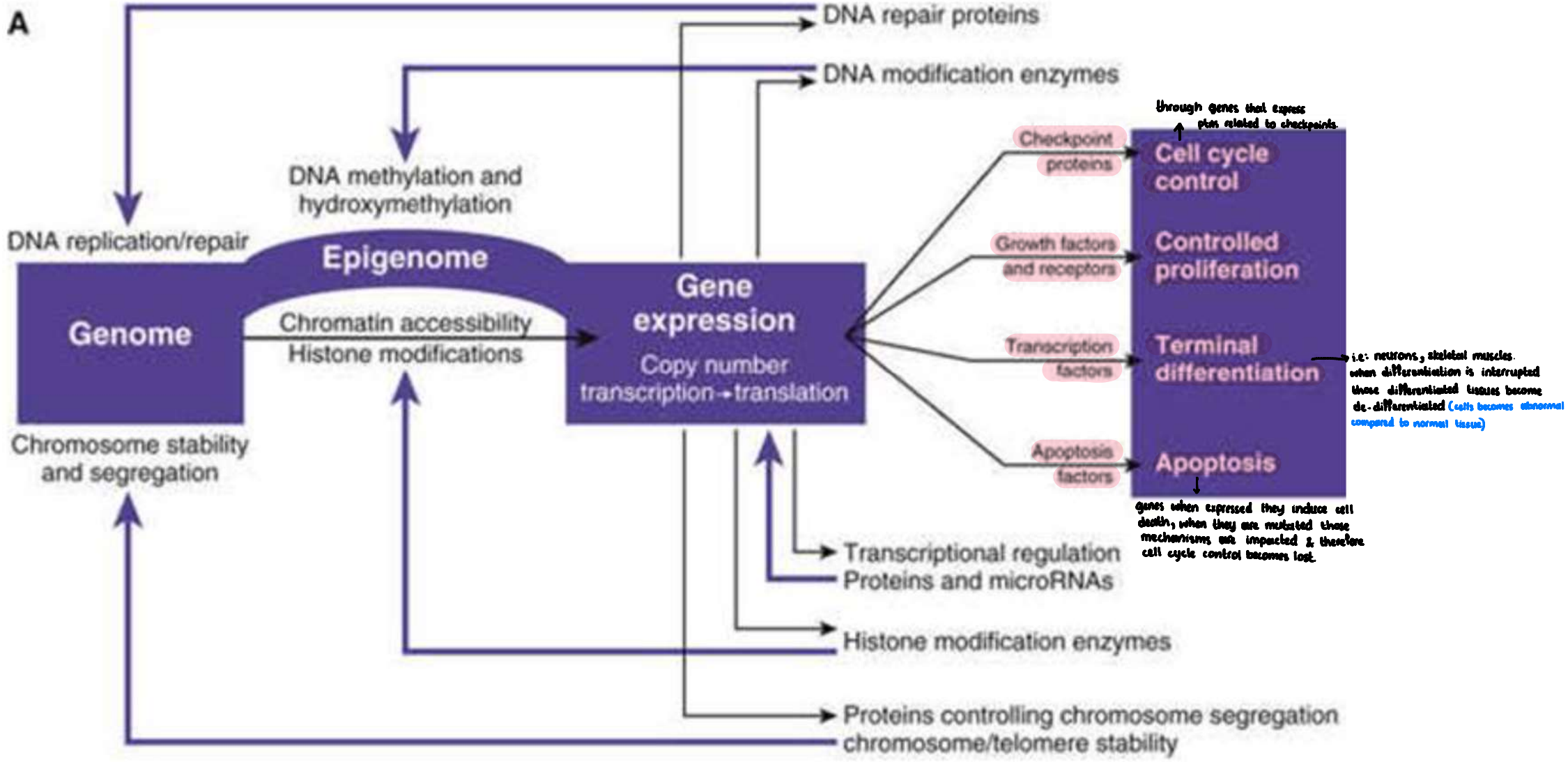
The nature of some driver gene mutations comes as no surprise: the mutations directly affect specific genes that regulate processes that are readily understood to be important in oncogenesis.

These processes include cell-cycle regulation, cellular proliferation, differentiation and exit from the cell cycle, growth inhibition by cell-cell contacts, and programmed cell death (apoptosis).



Classes of driver genes

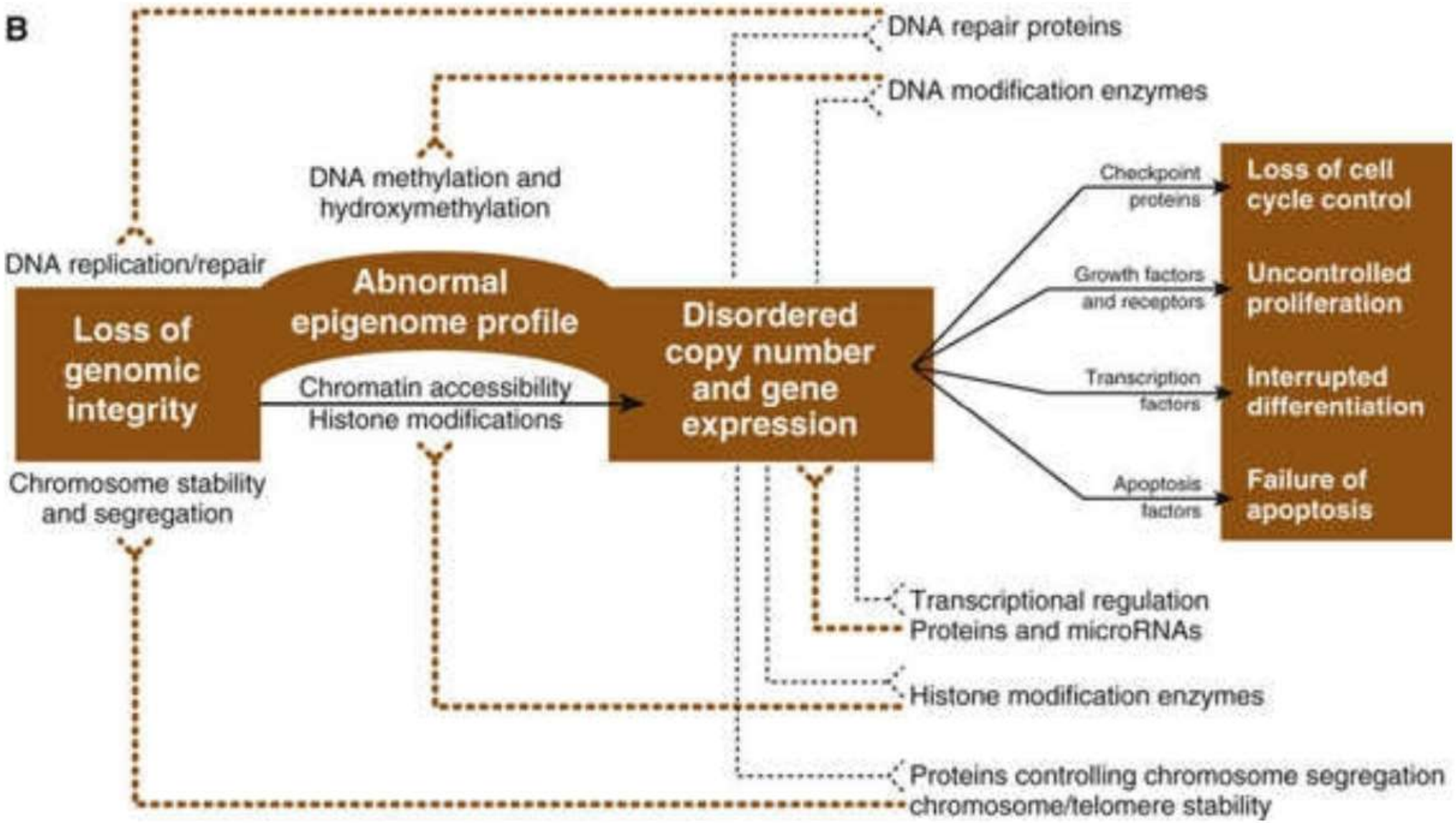
Genes with specific effects on cellular proliferation or survival	Genes with global effects on genome or DNA integrity
Cell-cycle regulation Cell-cycle checkpoint proteins Cellular proliferation signaling • Transcription factors • Receptor and membrane-bound tyrosine kinases • Growth factors • Intracellular serine-threonine kinases • PI3 kinases • G proteins and G protein–coupled receptors • mTOR signaling • Wnt/β-catenin signaling • Transcription factors Differentiation and lineage survival • Transcription factors protecting specific cell lineages • Genes involved in exit from cell cycle into G_0 Apoptosis	Genome integrity • Chromosome segregation • Genome and gene mutation • DNA repair • Telomere stability Gene expression: abnormal metabolites affecting activity of multiple genes/gene products Gene expression: epigenetic modifications of DNA/chromatin • DNA methylation and hydroxymethylation • Chromatin histone methylation, demethylation, and acetylation • Nucleosome remodeling • Chromatin accessibility and compaction (SWI/SNF complexes) Gene expression: post-transcriptional alterations • Aberrant mRNA splicing • MicroRNAs affecting mRNA stability and translation Gene expression: protein stability/turnover



Overview of normal genetic pathways controlling normal tissue homeostasis.

The information encoded in the genome (black arrows) results in normal gene expression, as modulated by the epigenomic state.

Many genes provide negative feedback (purple arrows) to ensure normal homeostasis.



Perturbations in neoplasia.
Abnormalities in gene expression (dotted black arrows) lead to a vicious cycle of positive feedback (brown dotted lines) of progressively more disordered gene expression and genome integrity.

Activated Oncogenes and Tumor Suppressor Genes

"gas pedal" "brake pedal"

Both classes of driver genes—those with specific effects on cellular proliferation or survival and those with global effects on genome or DNA integrity —can be further **subdivided** into one of two functional categories depending on how, if mutated, they drive oncogenesis.

The first category includes **proto-oncogenes**
These are normal genes that, that promotes growth and survival of cells.

when mutated in very particular ways, become driver genes through alterations that lead to ***excessive levels of activity*** ↑↑↑ expression levels

Once mutated in this way, driver genes of this type are referred to as **activated oncogenes** .

not called proto-oncogenes anymore.
a then is gain of function.

Only a **single mutation at one allele** can be sufficient for activation

The mutations that activate a proto-oncogene can **range** from highly specific point mutations causing dysregulation or hyperactivity of a protein, to chromosome translocations that drive overexpression of a gene, to gene amplification events that create an overabundance of the encoded mRNA and protein product

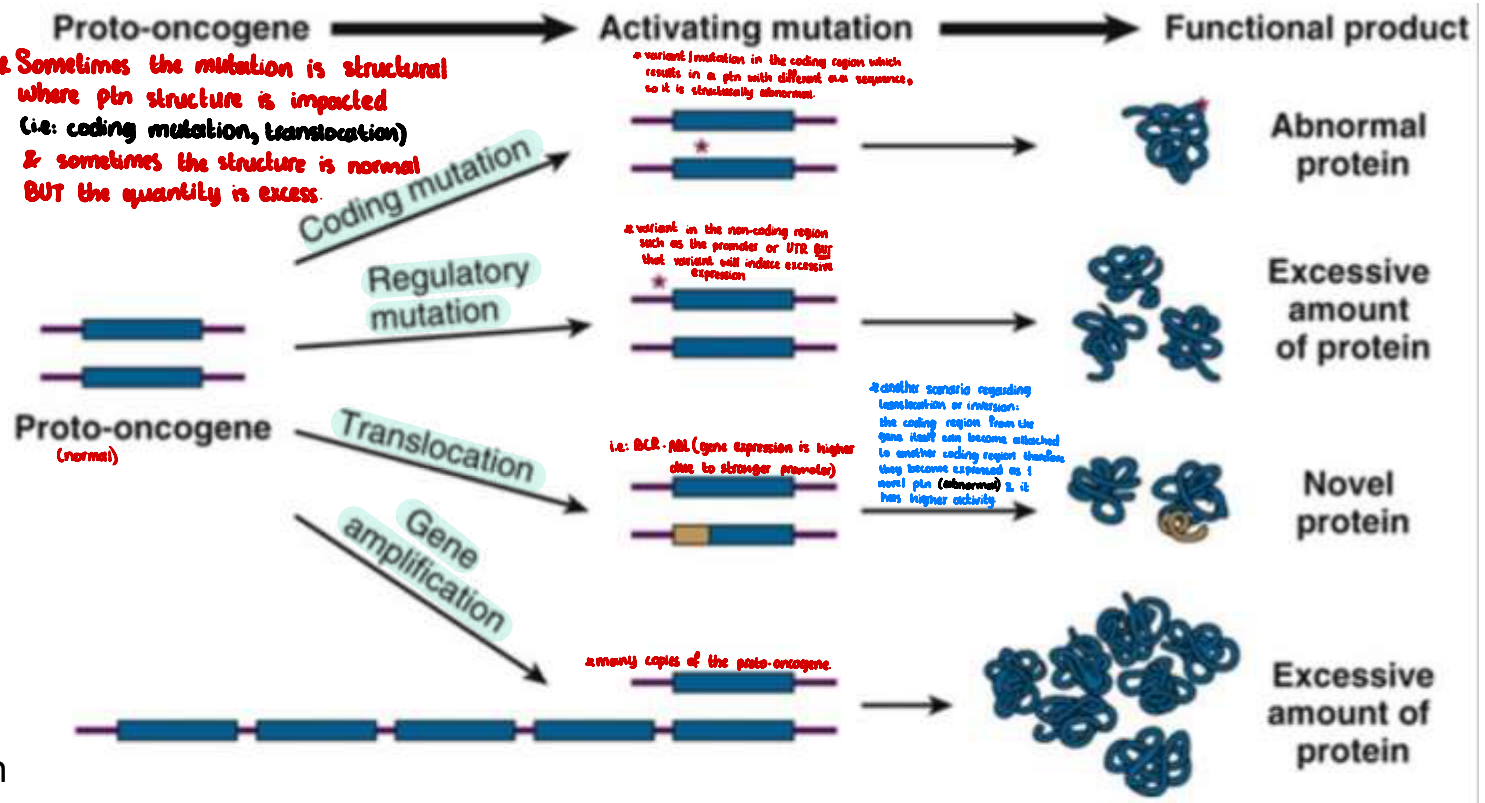


FIGURE 15-3 Different mutational mechanisms leading to proto-oncogene activation. These include a single point mutation leading to an amino acid change that alters protein function, mutations or translocations that increase expression of an oncogene, a chromosome translocation that produces a novel product with oncogenic properties, and gene amplification leading to excessive amounts of the gene product.

4 Example:

RAS is an oncogene

typical function:

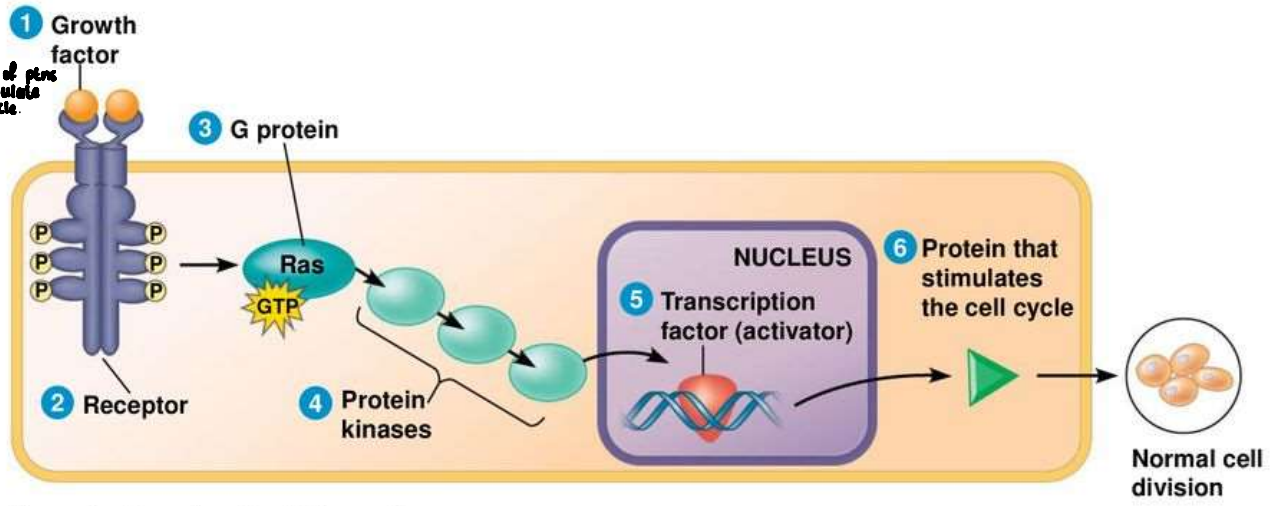
GF binds to receptor on the cell → this receptor activates RAS (becomes phosphorylated) → phosphorylated RAS phosphorylates other pkn → phosphorylated pkn phosphorylates other pkns & so on → these pkns become functional & active → influence TFs to express genes → expression of genes that stimulate cell cycle.

abnormality (RAS mutation):

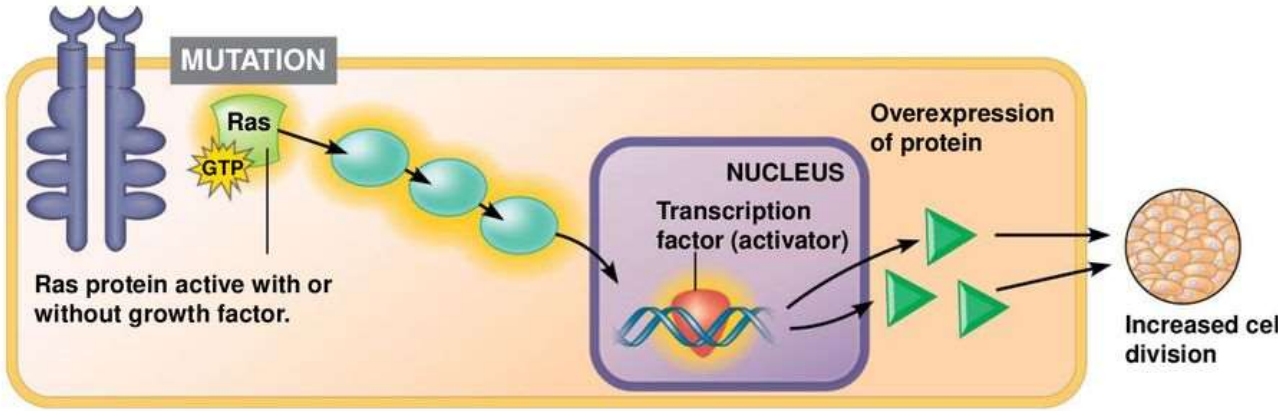
even in the absence of the GF RAS is still functioning → always inducing expression of genes that induce & stimulate cell cycle → abnormal cell cycle results in abnormal division → cell becomes candidate cell to be transform into cancer.

Oncogenes encode proteins such as the following:

- Proteins in signaling pathways for cell proliferation
- Transcription factors that control the expression of growth-promoting genes
- Inhibitors of programmed cell death machinery



Normal cell cycle–stimulating pathway.

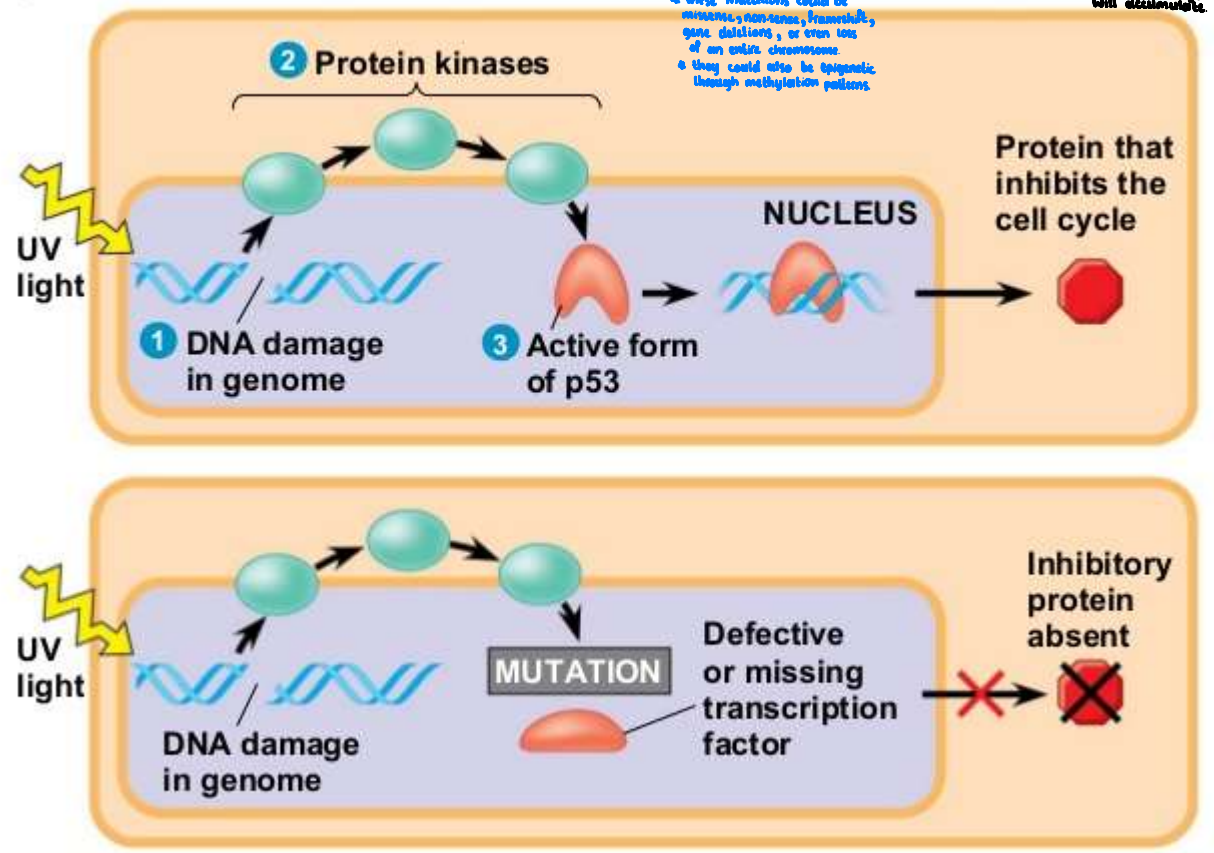


Mutant cell cycle–stimulating pathway.

The second, and more common, category of driver genes includes tumor suppressor genes (**TSGs**), variants in which cause a *loss of expression* of proteins necessary to control the development of cancers.

To drive oncogenesis, loss of function of a TSG typically requires mutations at both alleles.

Figure 16.18



Loss-of-function mechanisms can range from missense, nonsense, or frame-shift mutations to gene deletions or loss of a part or even an entire chromosome.

Loss of function of TSGs can also result from epigenomic transcriptional silencing due to:

- altered chromatin conformation
- promoter methylation
- translational silencing by miRNAs or disturbances in other components of the translational machinery

TSGs encode proteins involved in many aspects of cellular function, including but not limited to:

- maintenance of correct chromosome number and structure
- DNA repair proteins
- proteins involved in regulating the cell cycle, cellular proliferation, or contact inhibition

Cellular Heterogeneity within Individual Tumors

The accumulation of driver gene mutations does not occur synchronously, in lockstep, in every cell of a tumor.

they do NOT occur all at once (stepwise approach)

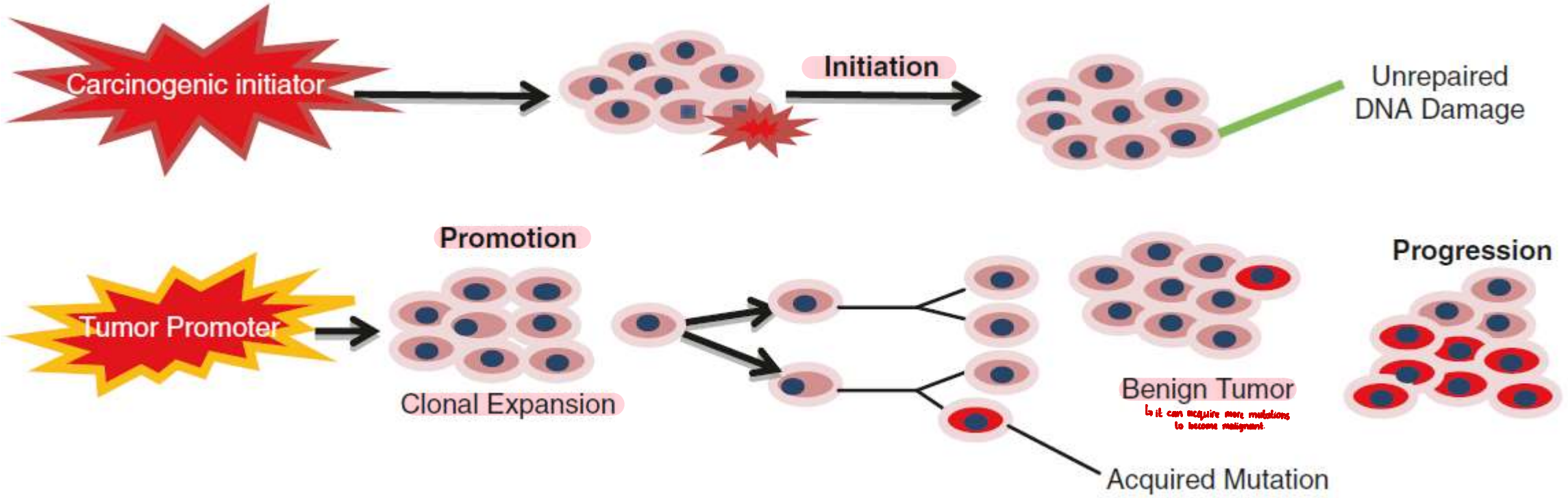
To the contrary, cancer evolves along multiple lineages within a tumor

a if a cell is mutated it is still NOT malignant, it will keep proliferating & gain new mutations every time until driver mutations accumulate enough for the daughter cells to become malignant.

→ that's why there is heterogeneity in cells, some cells have mutations that the other cells have NOT acquired yet.

mutational and epigenetic events in different cells activate proto-oncogenes and cripple the machinery for maintaining genome integrity, leading to more genetic changes in a vicious cycle of more mutations and worsening growth control.

The lineages that experience an enhancement of growth, survival, invasion, and distant spread will come to predominate as the cancer evolves and progresses



↳ it can acquire more mutations to become malignant

A paradigm for the development of cancer

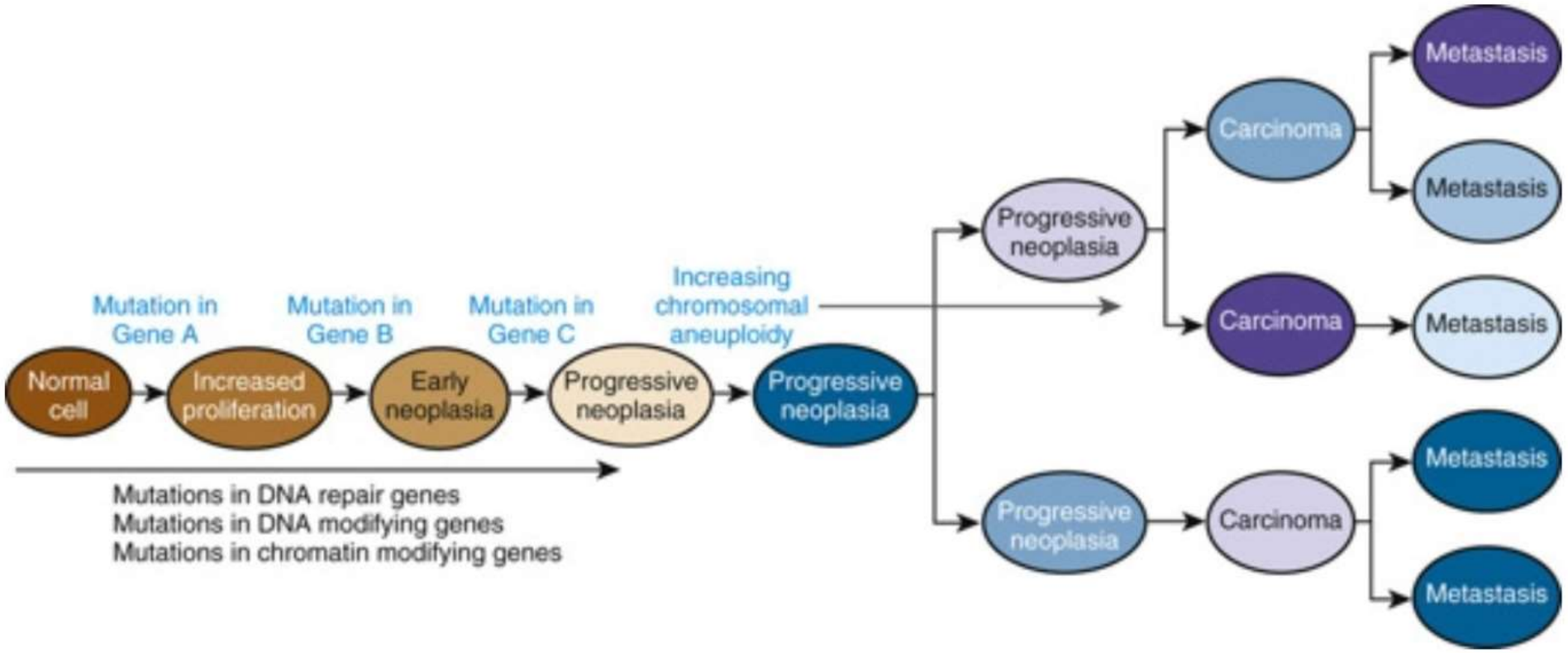


FIGURE 15-4 **Stages in the evolution of cancer. Increasing degrees of abnormality are associated with sequential loss of tumor suppressor genes from several chromosomes and activation of proto-oncogenes, with or without a concomitant defect in DNA repair. Multiple lineages, carrying different mutations and epigenomic profiles, occur within the primary tumor itself, between the primary and metastases and between different metastases.**

the original clone of neoplastic cells evolves and gives rise to multiple sublineages

each carrying a set of mutations and epigenomic alterations that are different from but overlap with what is carried in other sublineages.

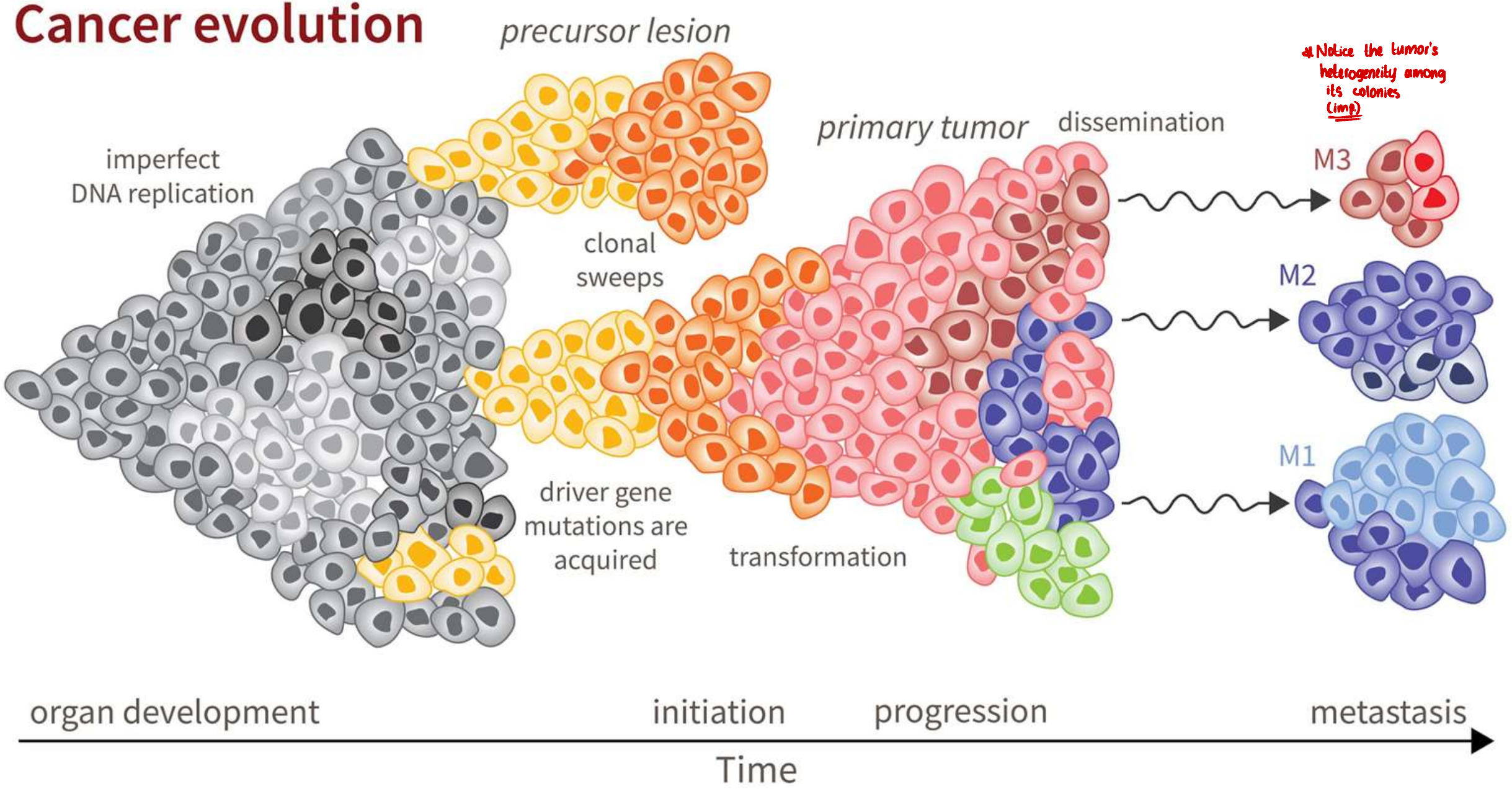
The profile of mutations and epigenomic changes can differ:

Between the primary and its metastases

Between different metastases,

Between the cells of the original tumor or within a single metastasis.

Cancer evolution



* Notice the tumor's heterogeneity among its colonies (imp.)

Cancer in Families

*cancer could be
- *hereditary → all the body tissues have that mutation (including tumor tissue)
- somatic → the mutation is confined to the tumor & absent in the body tissues & may lead to cancer development.

hereditary cancer syndromes follow mendelian patterns of inheritance, where increased incidence is due primarily to inheritance of a single mutant gene with high penetrance.

approximately 100 different genes in which deleterious mutations increase the risk for cancer many-fold higher than in the general population *oncogenic mutations.

https://www.invitae.com/en/physician/tests/01101/

*genetic testing of hereditary cancer from the blood (mutation is found in all body tissues)

There are also many dozens of additional genetic disorders that are not usually considered to be hereditary cancer syndromes and yet include some increased predisposition to cancer (for example, the ten- to twenty-fold increased lifetime risk for leukemia in Down syndrome

Test description

Those genes are known that, when mutated, they increase the risk for cancer

The Invitae Multi-Cancer Panel analyzes genes that are associated primarily with adult-onset, non-syndromic cancer predisposition conditions across major organ systems including, but not limited to, breast, gynecologic (ovarian, uterine/endometrial), gastrointestinal (colorectal, gastric, pancreatic), endocrine (thyroid, parathyroid, pituitary, adrenal glands), genitourinary (renal/urinary tract, prostate), skin (melanoma, basal cell carcinoma), and brain/nervous system. The genetic heterogeneity associated with these cancers can make it difficult to use phenotype as the sole criterion to select a definitive cause. Some genes in this test may also be associated with additional unrelated conditions, which are not included in the list of disorders tested. Genetic testing of these genes may help confirm a clinical diagnosis, help predict disease prognosis and progression, facilitate early detection of symptoms, inform family planning and genetic counseling, or promote enrollment in clinical trials.

This test is specifically designed for heritable germline mutations and is not appropriate for the detection of somatic mutations in tumor tissue.

See all disorders tested

Cancer in Families

Not all families with an apparently increased incidence of cancer can be explained by known mendelian or clearly recognized genetic disorders.* in some of these families, a genetic mutation is NOT necessarily found in all family members → thought to be "multifactorial" where it is affected by environmental factors & genetic predisposition involving a group of genes with low penetrance & small contribution → Collectively the sum of their contributions elevates development to cancer.
These families likely represent the effects of both shared environment and one or more genetic variants that increase susceptibility and are therefore classified as **multifactorial**, with complex inheritance.

Although individuals with a *hereditary cancer syndrome represent ~ 5%* of all patients with cancer, identification of a genetic basis for their disease has great importance both for clinical management of these families and for understanding cancer in general.

*Adenoma is a benign tumor of epithelial tissue of glandular origin or glandular characteristic or both. It can from glands such as the adrenal gland, thyroid gland, prostate...

Activated Oncogenes in Hereditary Cancer Syndromes

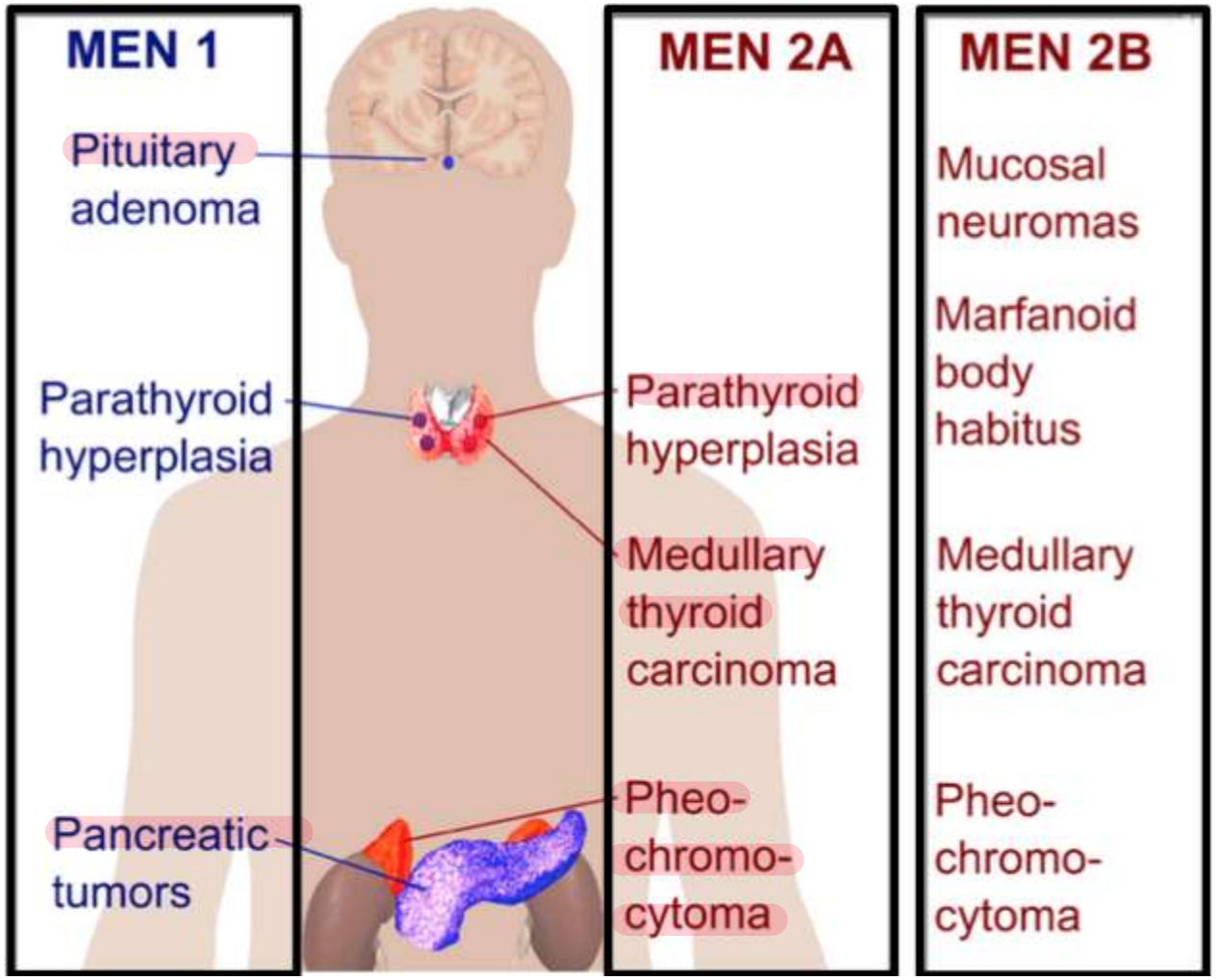
Multiple Endocrine Adenomatosis, Type 2

Adenomatosis: An abnormal overgrowth of, or TUMOUR formation in, two or more of the ENDOCRINE glands *at least 2 glands

*there are other types like MEN2-B & MEN1

MEN2-A is an AD disorder characterized by:
high incidence of medullary carcinoma of the thyroid that is often but not always associated with pheochromocytoma,
benign parathyroid adenomas, or both.

Pheochromocytoma: is a rare, usually noncancerous (benign) tumor that develops in an adrenal gland.



Medullary Carcinoma of the Thyroid

"MENullary Calcinoma of the Thyroid"

- Associated with MEN II (IIa & IIb)
- Tumor is surrounded by Amyloid
- Produces Calcitonin
- Tumor of "C"-cells

Baronerocks.com

Patients with the rarer type B variant, **MEN2B**, have, in addition to the tumors seen in patients with MEN2A,
thickening of nerves and the development of benign neural tumors, known as **neuromas**, on the mucosal surface of the mouth and lips and along the GI tract.

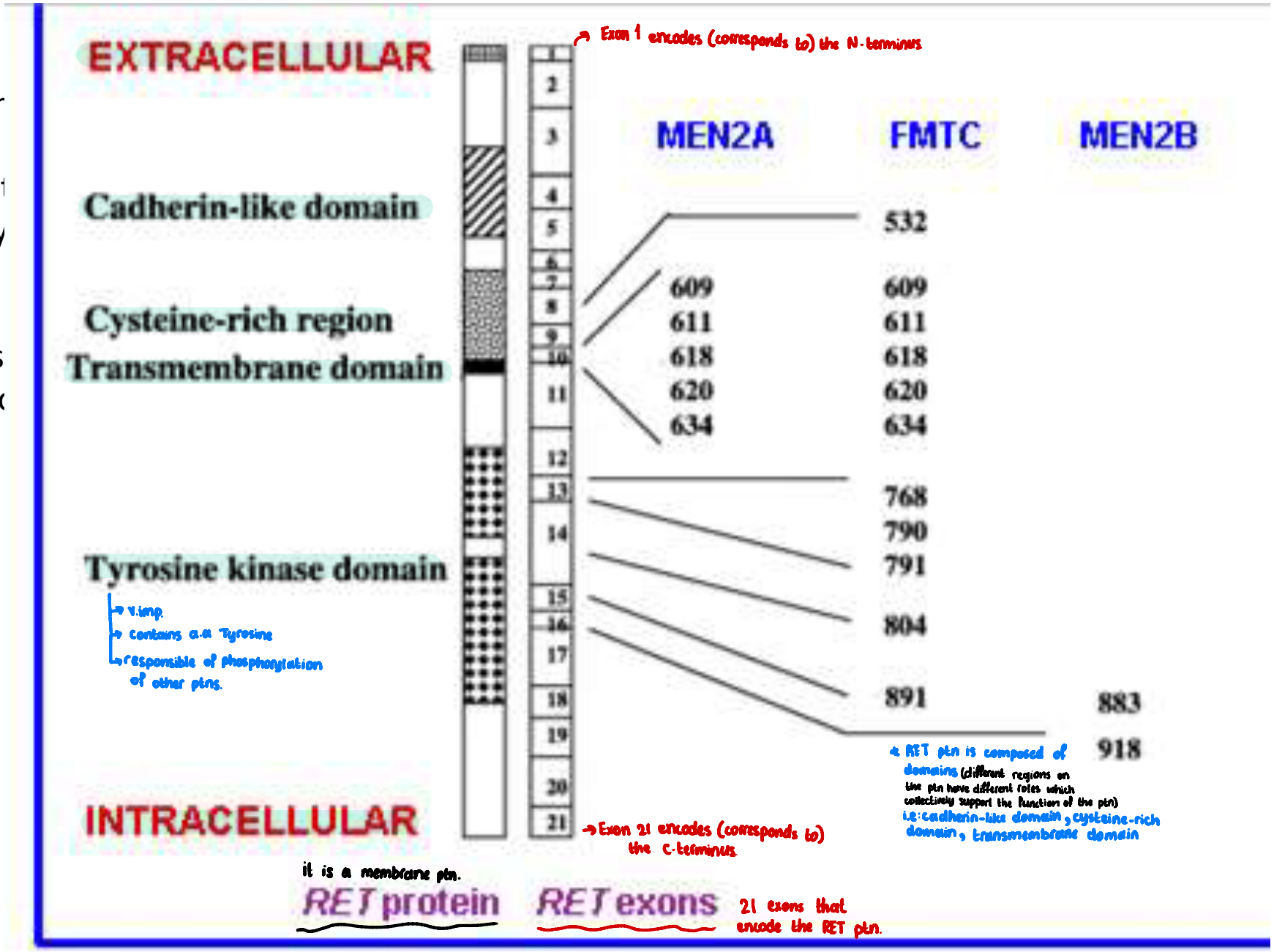
*mucosal neuromas in addition to the tumors seen in MEN2-A

* Variants responsible for MEN2 are located in the RET gene. Individuals who inherit this gene

The variants responsible for MEN2 are ir

Individuals who inherit an activating mut
60% chance of developing a particular ty
(medullary)

More sensitive tests, such as blood tests
catecholamines synthesized by pheochr
above 90% of heterozygotes for MEN2



it is a proto-oncogene

RET encodes a cell-surface protein that contains:

- **extracellular domain** that can bind signaling molecules → i.e: hormone, peptide..
- **cytoplasmic** tyrosine kinase domain

Tyrosine kinases are a class of enzymes that phosphorylate tyrosines in proteins.
Tyrosine phosphorylation initiates a signaling cascade changes in protein-protein and DNA-protein interactions and in the enzymatic activity of many proteins

*RET pin is a type of tyrosine kinase receptors
In order for the kinase to function, a ligand must bind first

Normally, tyrosine kinase receptors must bind specifi signaling molecules in order to undergo the conformational change that makes them enzymatical active and able to phosphorylate other cellular proteins.

The mutations in RET that cause MEN2A increase its kinase activity even in the absence of its ligand (a sta referred to as
constitutive activation → always active regardless of the presence/absence of the ligand.

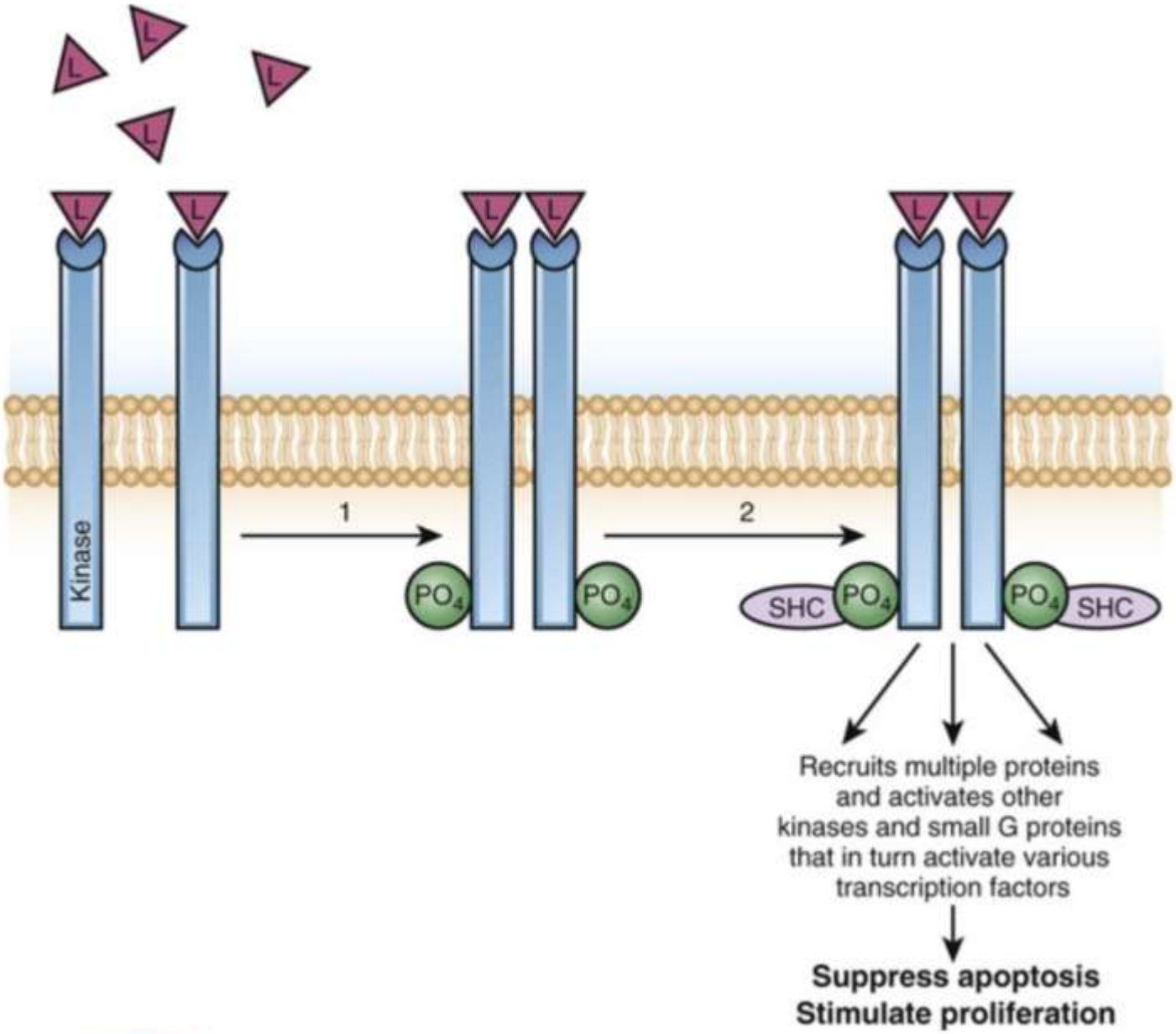
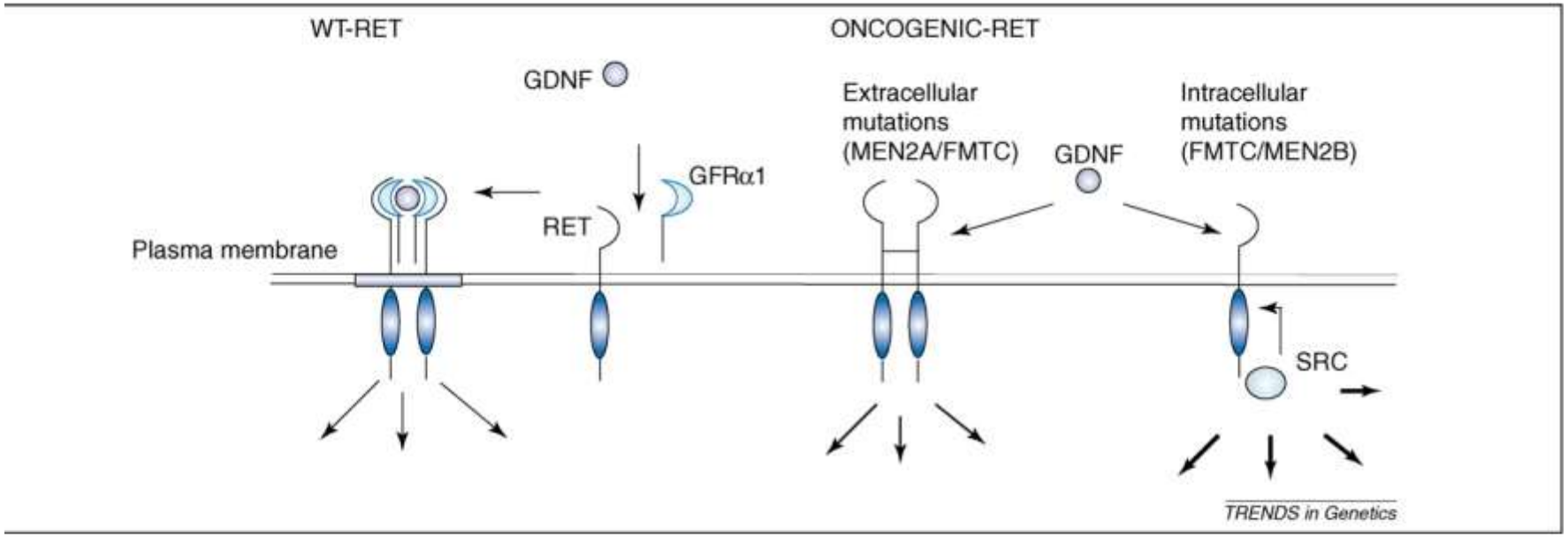


FIGURE 15-5 Schematic diagram of the function of the Ret receptor, the product of the *RET* proto-oncogene. Upon binding of a ligand (L), such as glial-derived growth factor or neurturin, to the extracellular domain, the protein

The RET gene is expressed in many tissues of the body and is required for normal embryonic development of autonomic ganglia and kidney.

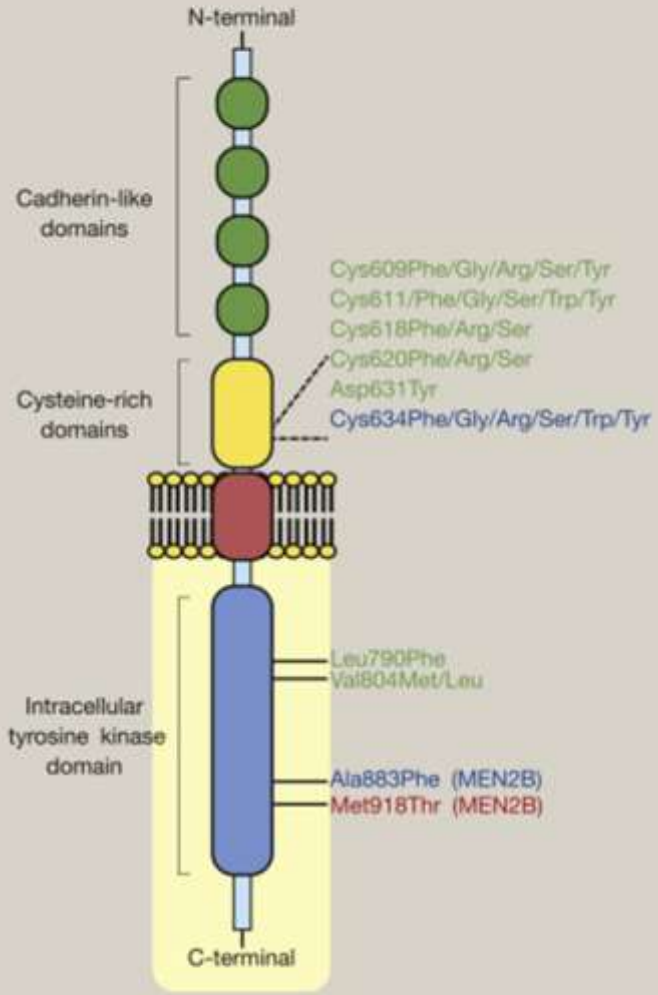
Q: If RET gene is expressed in many tissues, why do RET mutations cause certain cancers in certain tissues & NOT all the tissues in the body where RET expression takes place?

It is unclear why germline activating mutations in this proto-oncogene result in a particular cancer of distinct histological types restricted to specific tissues,
whereas other tissues in which the oncogene is expressed do not develop tumors.



re 5. Possible mechanisms of activation of wild-type RET and MEN2-associated *RET* mutations. **(a)** Activation of wild-type RET: the ligand (GDNF) first binds to the GPI-
or co-receptor 1 (GFRα1); RET is then recruited to form a macromolecular complex receptor. **(b)** Constitutive activation of RET by mutations affecting the cysteine-rich
ain that cause covalent dimerization of the (mutant) receptor. **(c)** Aberrant activation of mutations affecting the tyrosine kinase domain of RET, resulting in monomeric
proteins with altered catalytic activity and altered substrate specificity that preferentially recognize substrates of cytoplasmic tyrosine kinases such as SRC or ABL.

RET receptor structure and location of common MEN2-associated *RET* mutations



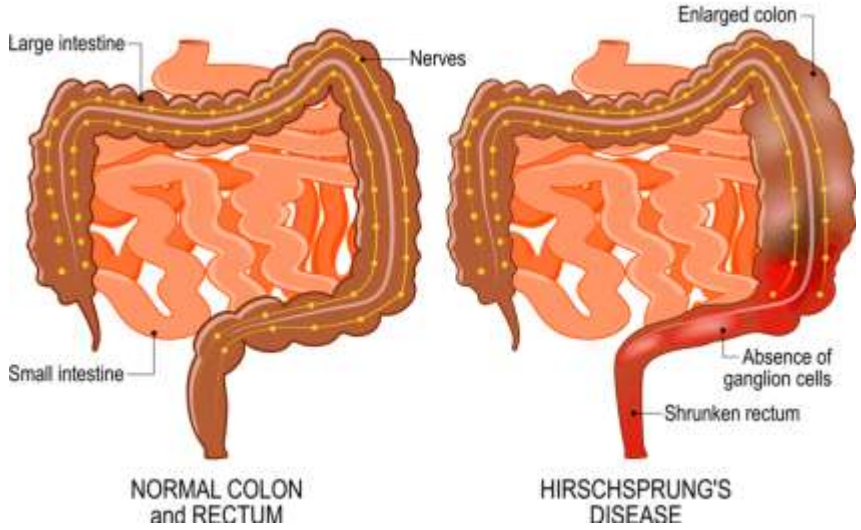
The RET receptor is a membrane-associated tyrosine kinase receptor expressed in cells of neural crest origin. MEN2-associated mutations arise most frequently in the cysteine-rich region of the extracellular domain, or in the intracellular domain associated with intrinsic tyrosine kinase activity, resulting in enhanced receptor signalling. *RET* mutations are described according to the respective missense substitution, with amino acids represented using standard nomencaiture. The American Thyroid Association[3] risk category of each *RET* mutation is represented by colour; red, 'highest' risk; blue, 'high' risk; green, 'moderate 'risk. Mutations associated with MEN2B are noted in parentheses.

Gain of function vs loss of function

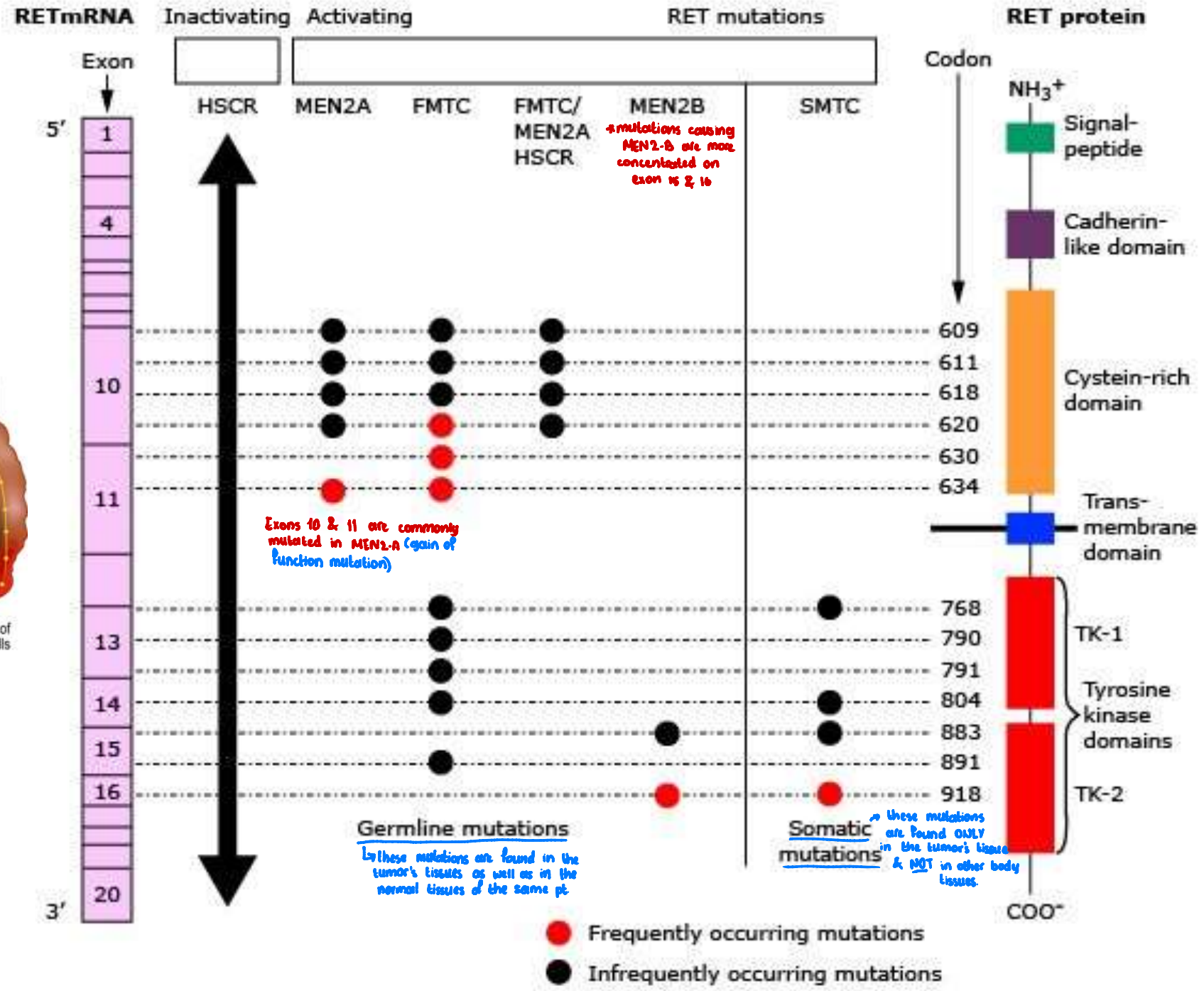
* RET is NOT only implicated in MEN2-A

Interestingly, RET is the same gene implicated in Hirschsprung disease, although those mutations are usually loss-of-function, not activating, mutations. RET becomes always inactive which manifests as Hirschsprung disease.

* there are cases where pts with RET activating mutations (ie: MEN2-A pts) were reported to have Hirschsprung as well which is still unknown why.



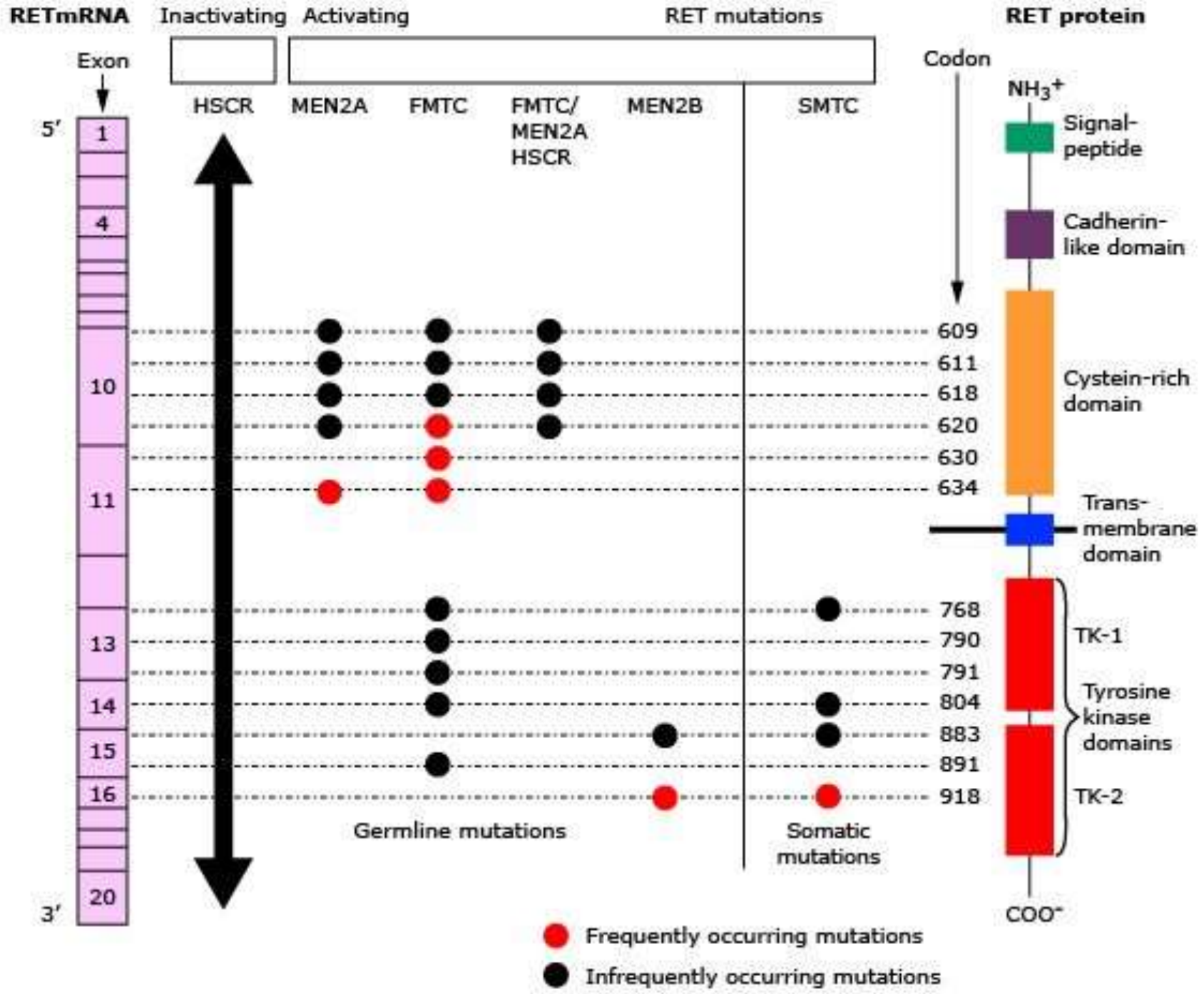
HSCR: Hirschsprung disease → RET loss of function
MEN: Multiple endocrine neoplasia
FMTC: Familial medullary thyroid cancer
SMTC: Sporadic medullary thyroid cancer
} RET gain of function



Gain of function vs loss of function

There are, however, some families in which the same mutation in RET can act as an activated oncogene in some tissues (such as thyroid) and cause MEN2A, while not having sufficient function in other tissues, such as the developing enteric neurons of the gastrointestinal tract, resulting in Hirschsprung disease.

Thus even the identical mutation can have different effects on different tissues.



HSCR: Hirschsprung disease
MEN: Multiple endocrine neoplasia
FMTC: Familial medullary thyroid cancer
SMTC: Sporadic medullary thyroid cancer

The Two-Hit Theory of Tumor Suppressor Gene Inactivation in Cancer

*Remember: oncogenic mutation:
Proto-oncogene — gain of function — 1 mutated allele is enough (dominant-like)
vs
TSGs — loss of function — both alleles must be inactivated (recessive-like)

proteins encoded by proto-oncogenes promote cancer when activated or overexpressed

variants in TSGs contribute to malignancy by a different mechanism,
the loss of function of both alleles of the gene.

The products of many TSGs have now been isolated and characterized

Selected Tumor Suppressor Genes Involved in Human Neoplasms

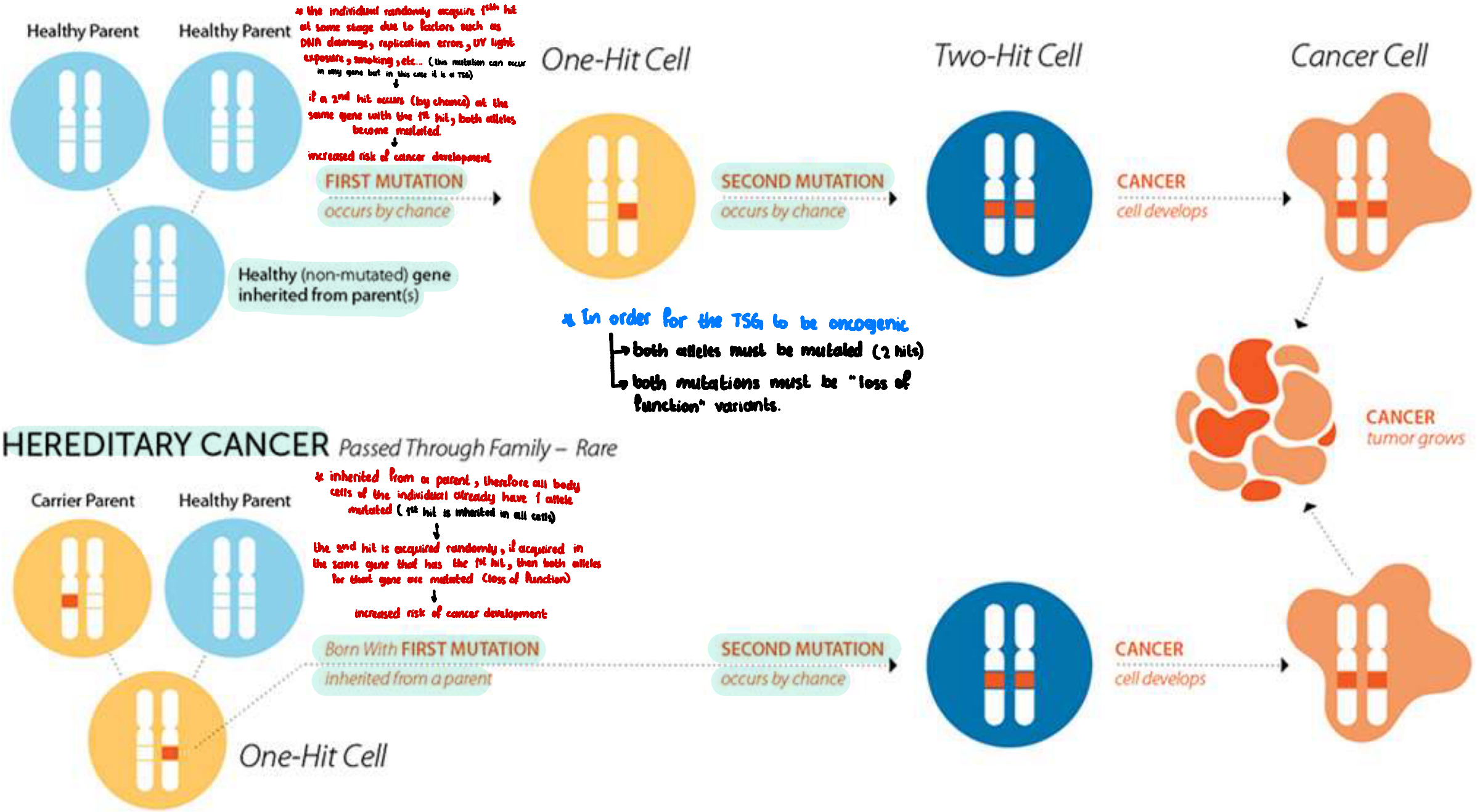
IMP.

TABLE 7-8 -- Selected Tumor Suppressor Genes Involved in Human Neoplasms

Subcellular Locations	Gene	Function	Tumors Associated with Somatic Mutations (mutation presence only in tumor tissue)	Tumors Assocated with Inherited Mutations (mutation in all body tissues)
Cell surface	TGF-β receptor	Growth inhibition	Carcinomas of colon	Unknown
	E-cadherin	Cell adhesion	Carcinoma of stomach	Familial gastric cancer
Inner aspect of plasma membrane	*NF1*	Inhibition of RAS signal transduction and of p21 cell cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	*NF2*	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromastosis type 2, acoustic schwannomas, and meningiomas
Cytosol	*APC*/β-catenin	Inhibition of signal transduction	Carcinomas of stomach, colon, pancreas; melanoma	Familial adenomatous polyposis coli/colon cancer
	PTEN	PI3 kinase signal transduction	Endometrial and prostate cancers	Cowden syndrome
	SMAD2 and *SMAD4*	TGF-β signal transduction	Colon, pancreas tumors	Unknown
Nucleus	*RB1*	Regulation of cell cycle	Retinoblastoma; osteosarcoma carcinomas of breast, colon, lung	Retinoblastomas, osteosarcoma
	p53	Cell cycle arrest and apoptosis in response to DNA damage	Most human cancers	Li-Fraumeni syndrome; multiple carcinomas and sarcomas
	WT1	Nuclear transcription	Wilms' tumor	Wilms' tumor
	P16/INK4a	Regulation of cell cycle by inhibition of cyclindependent kinases	Pancreatic, breast, and esophageal cancers	Malignant melanoma
	BRCA1 and *BRCA2*	DNA repair	Unknown	Carcinomas of female breast and ovary; carcinomas of male breast

PI3 kinase, phosphatidylinositol 3-kinase.

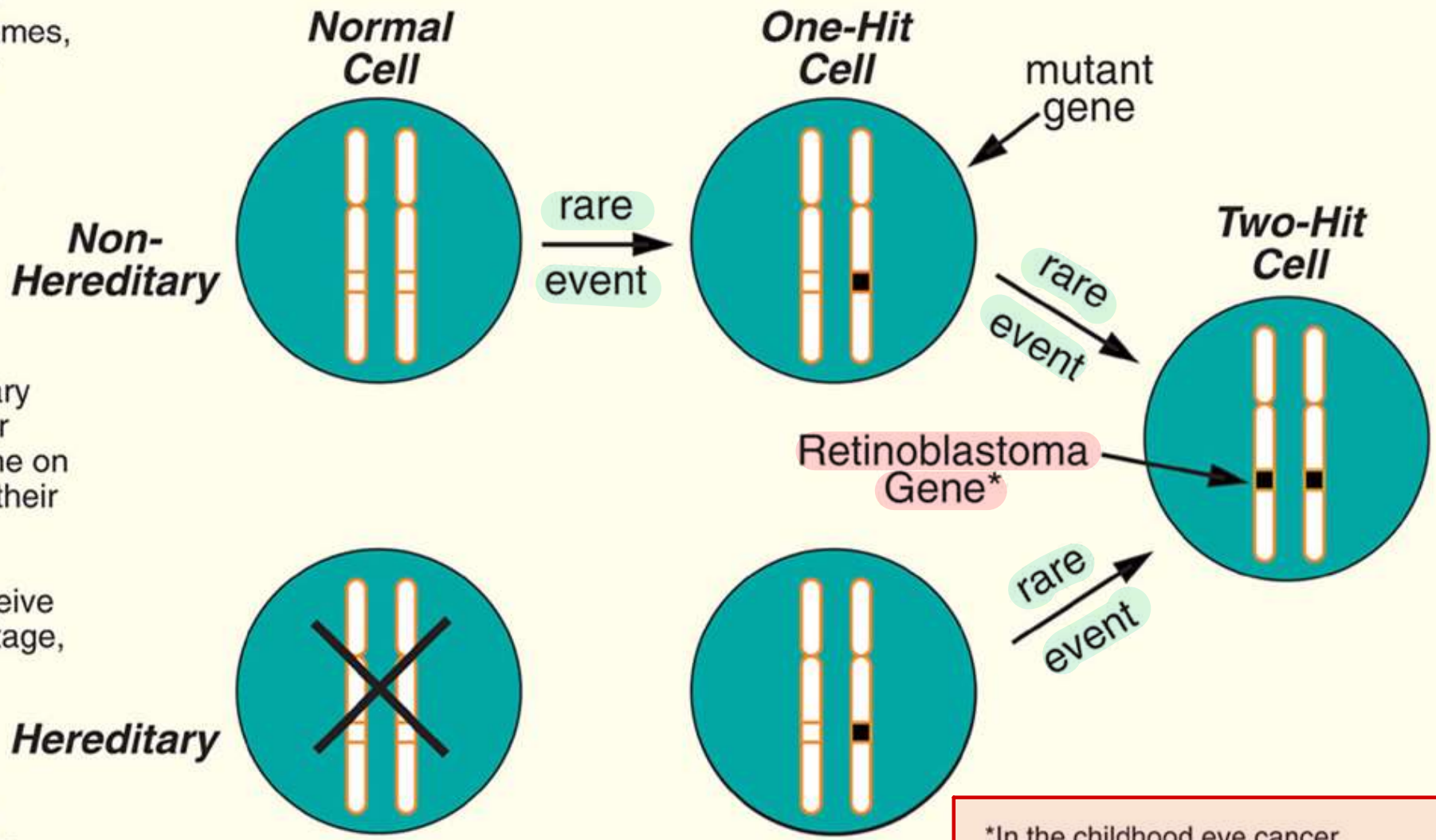
NON-HEREDITARY CANCER *By Chance – Most Common*



HEREDITARY CANCER *Passed Through Family – Rare*

Two-Hit Theory of Cancer Causation

Normal cells have two undamaged chromosomes, one inherited from our mother and one from our father. These chromosomes contain thousands of genes.



People with a hereditary susceptibility to cancer inherit a damaged gene on one chromosome, so their first "hit," or mutation, occurs at conception. Other people may receive the first hit at a later stage, before or after birth.

In either case, if a cell receives damage to the same gene on the second chromosome, that cell can produce a cancer.

*In the childhood eye cancer retinoblastioma, people who inherit the first hit are 100,000 times more likely to develop a second, cancer-causing mutation.

Retinoblastoma familial

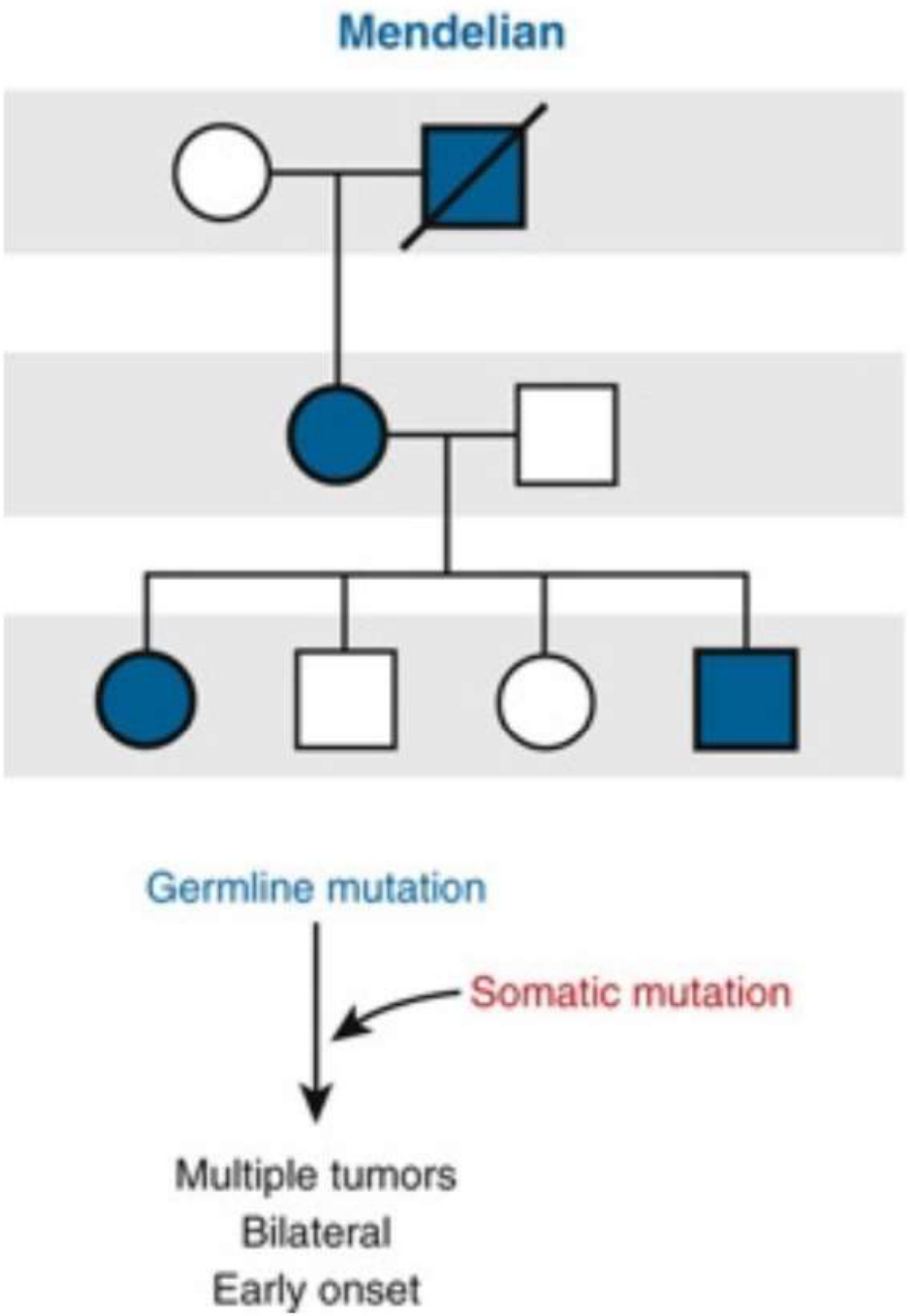
It was suggested that the hereditary form of the childhood cancer **retinoblastoma** might be initiated when a cell in a person heterozygous for a germline mutation in the retinoblastoma TSG

undergoes a second, somatic event that inactivates the other retinoblastoma gene allele.

As a consequence of this second somatic event, the cell loses function of both alleles, giving rise to a tumor.

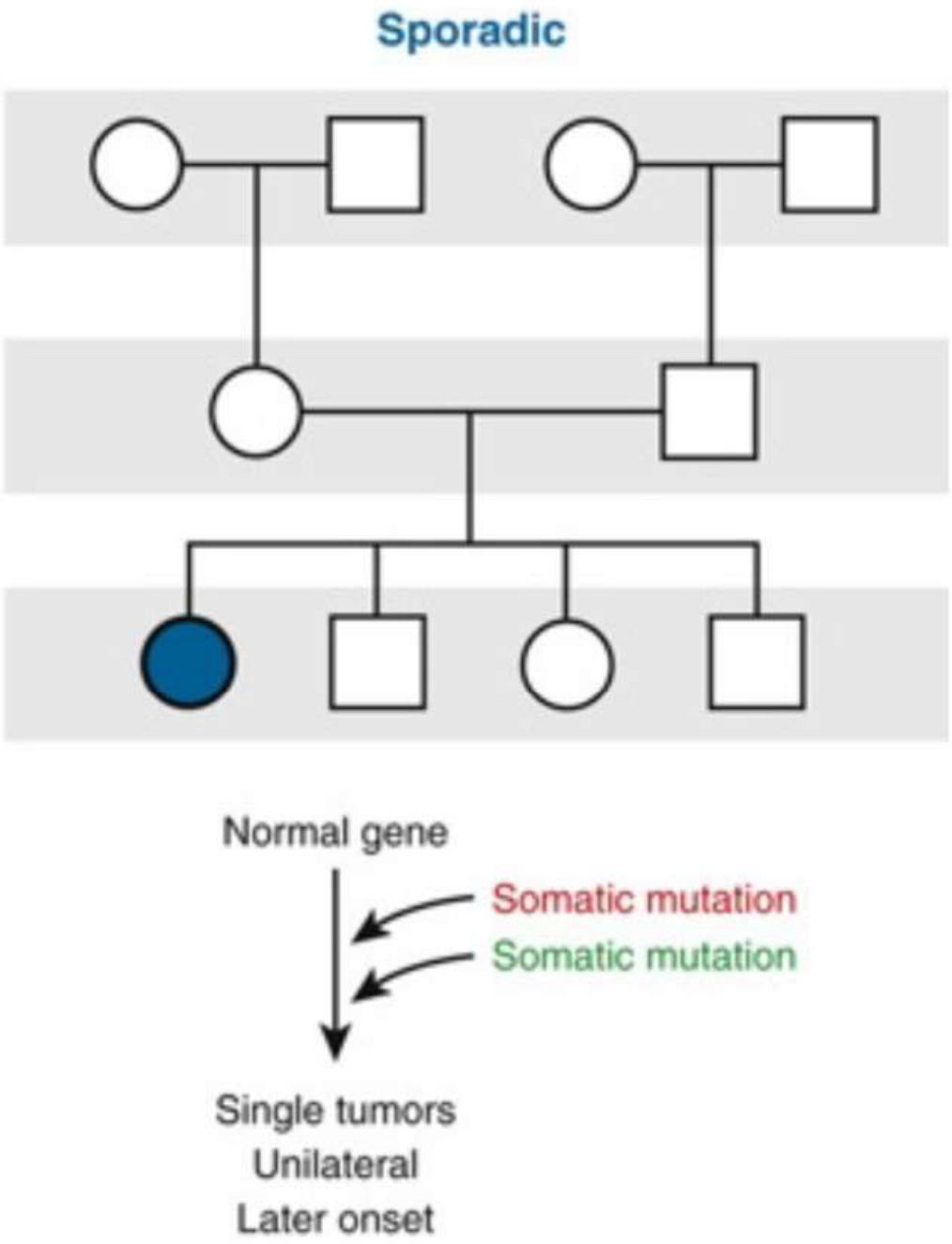
In the sporadic form of retinoblastoma, both alleles are also inactivated, but in this case, the inactivation results from two somatic events occurring in the same cell.

Hereditary RB	vs	Sporadic RB
multiple affected individuals in the family		1 individual only affected (could be hereditary BUT most likely sporadic)
multiple, bilateral tumor		typically single unilateral tumor
early onset (bcz 1st hit is already inherited)		late onset (bcz 1st hit took time followed by 2nd hit which takes more time)



Retinoblastoma sporadic

In the sporadic form of retinoblastoma, both alleles are also inactivated, but in this case, the inactivation results from two somatic events occurring in the same cell.



most tumor suppressor genes require both alleles to be inactivated to cause a phenotypic change

https://www.youtube.com/watch?v=h_sfOYFJTfU&t=51s

Alfred Knudson

In 1971
Knudson performed a statistical analysis on cases of retinoblastoma, a tumor of the retina that occurs both as an inherited disease and sporadically.

He noted that inherited retinoblastoma occurs at a younger age than the sporadic disease.
In addition, the children with inherited retinoblastoma often developed the tumor in both eyes, suggesting an underlying predisposition.

most tumor suppressor genes require both alleles to be inactivated to cause a phenotypic change

https://www.youtube.com/watch?v=h_sfOYFJTfU&t=51s

Alfred Knudson

Knudson suggested that two "hits" to DNA were necessary to cause the cancer.

In the children with inherited retinoblastoma, the first mutation in what later came to be identified as the RB1 gene, was inherited, the second one acquired.

In non-inherited retinoblastoma, instead two mutations, or "hits", had to take place before a tumor could develop, explaining the later onset.

In 1986, RB gene was the first tumor suppressor gene to be identified in medical history

The two-hit model is now widely accepted as the explanation for many hereditary cancers in addition to retinoblastoma, including

I also explain somatic cancers caused by loss of function variants in TSGs.

familial polyposis coli

familial breast cancer

neurofibromatosis type 1 (NF1)

Lynch syndrome

Li-Fraumeni

→ they all follow the 2 hit model

https://www.youtube.com/watch?v=PaEeKZPFuZo

Tumor Suppressor Genes in Autosomal Dominant Cancer Syndromes

4. a heterozygous individual for the RB gene (hereditary RB)
   - on the gene level: AR (requires both alleles to be mutated)
   - on the disease level: AD (v. high risk of disease phenotype following 2nd hit)

i.e.: a woman born heterozygous for the BRCA1 gene is advised to remove her breast tissue even before the 2nd hit takes place & cancer develops bcz she is at a v. high risk

Retinoblastoma

The prototype of diseases caused by mutation in a TSG

Rare malignant tumor of the retina in infants, with an incidence of approximately 1 in 20,000 births

Diagnosis of a retinoblastoma must usually be followed by removal of the affected eye, although smaller tumors, diagnosed at an early stage, can be treated by local therapy so that vision can be preserved

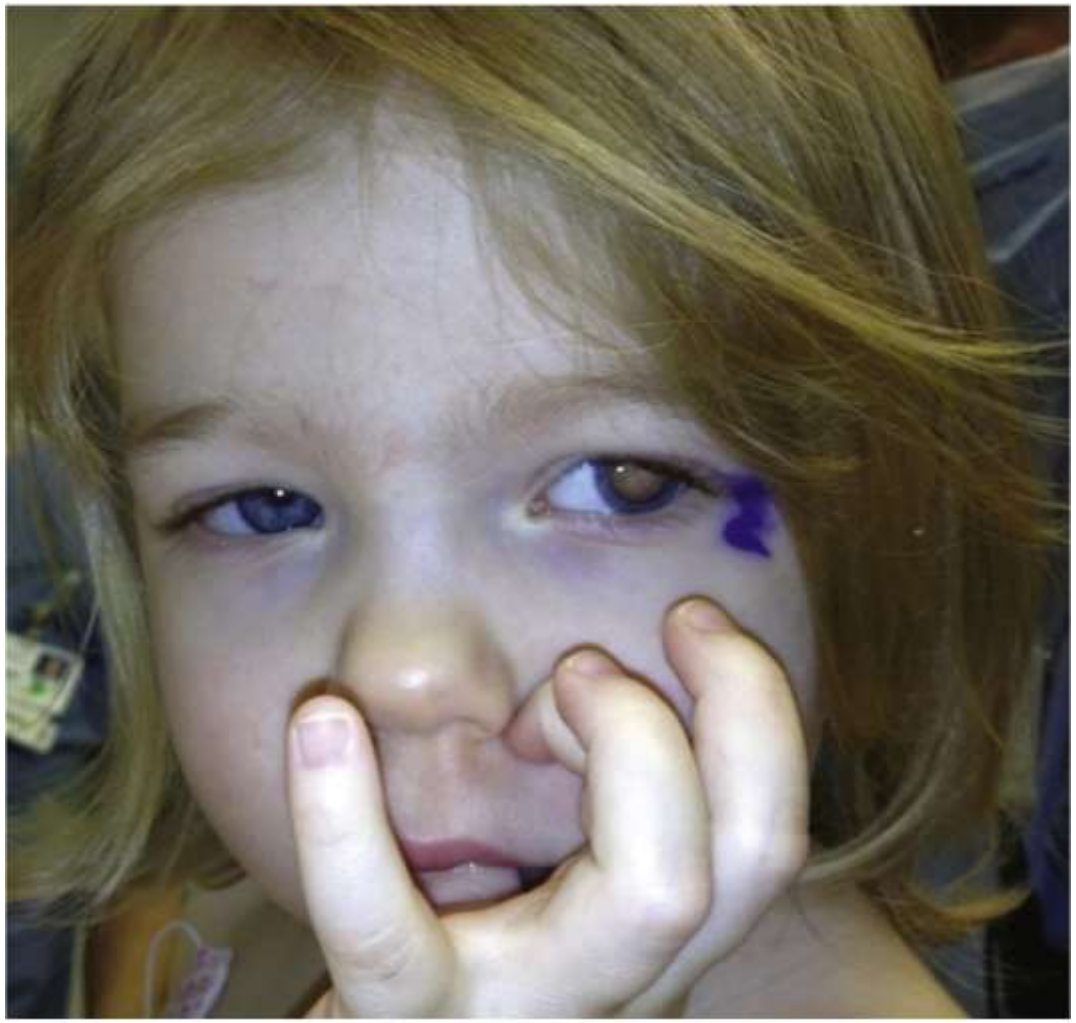


FIGURE 15-7 Retinoblastoma in a young girl, showing as a white reflex in the affected left eye when light reflects directly off the tumor surface. *See Sources & Acknowledgments.*

Approximately 40% of cases of retinoblastoma are of the heritable form, in which the child inherits one mutant allele at the retinoblastoma locus (RB1) through the germline from either a heterozygous parent

Or

more rarely, from a parent with germline mosaicism for an RB1 variant

* gonads (sperms/eggs) contain the RB1 variant BUT not in all other body tissue.

In these children, retinal cells, which like all the other cells of the body are already carrying one inherited defective RB1 allele
suffer a somatic mutation or other alteration in the remaining normal allele, leading to loss of both copies of the RB1 gene and initiating development of a tumor in each of those cells

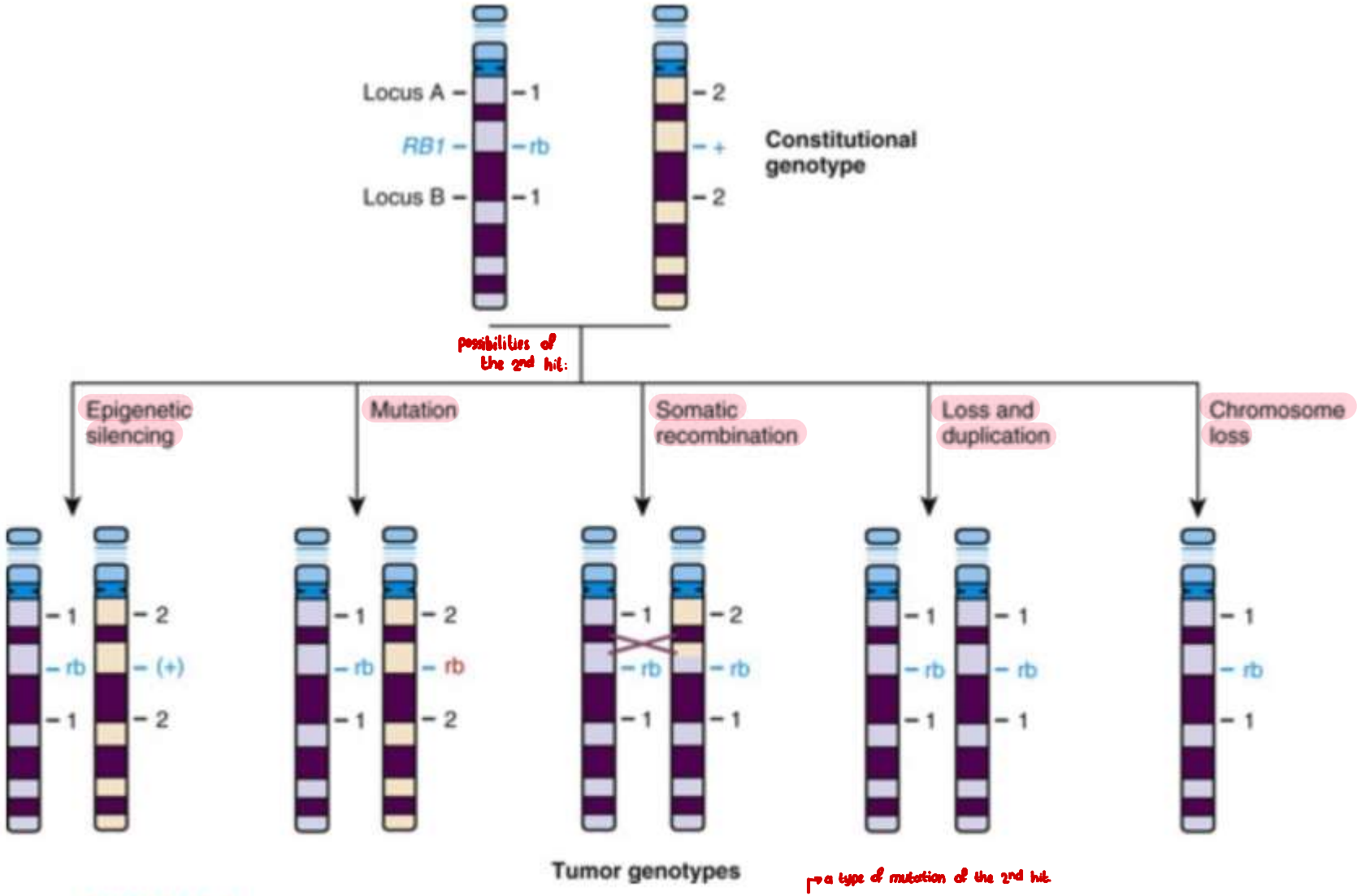
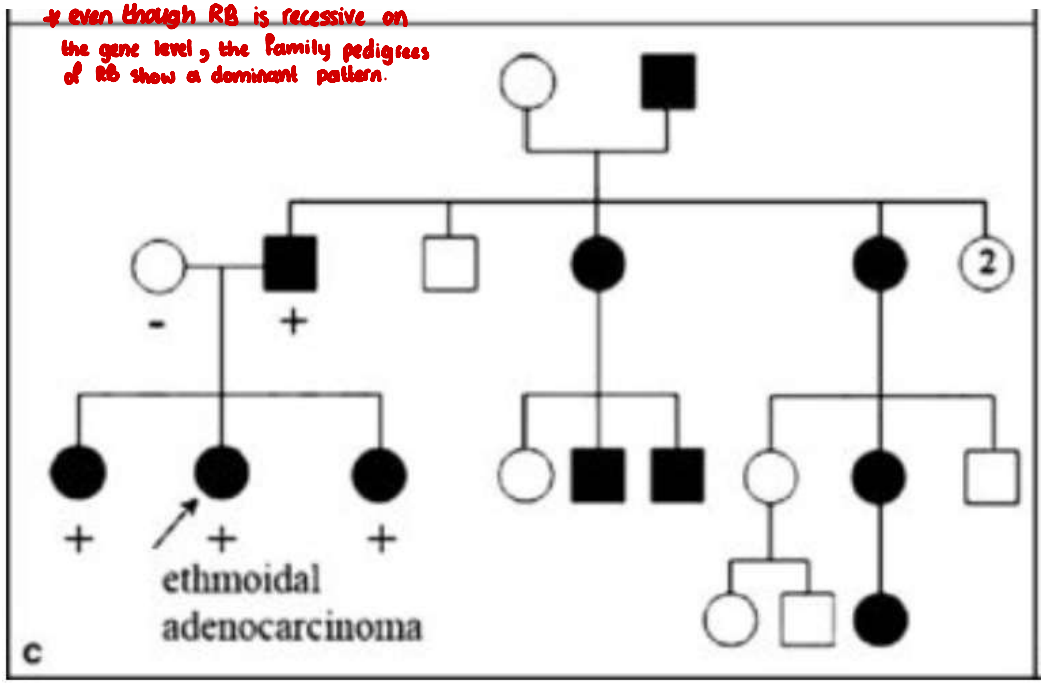


FIGURE 15-8 Chromosomal mechanisms that could lead to loss of heterozygosity for DNA markers at or near a tumor suppressor gene in an individual heterozygous for an inherited germline mutation. The figure depicts the events that constitute the "second hit" that leads to retinoblastoma with loss of heterozygosity (LOH). Local events such as mutation, gene conversion, or transcriptional silencing by promoter methylation, however, could cause loss of function of both *RB1* genes without producing LOH. +, normal allele, rb, the mutant allele.

The disorder appears to be inherited as a dominant trait

because the large number of primordial retinoblasts and their rapid rate of proliferation make it very likely that a somatic mutation will occur as a second hit in one or more cells of the more than10^6 retinoblasts already carrying an inherited RB1 mutation.



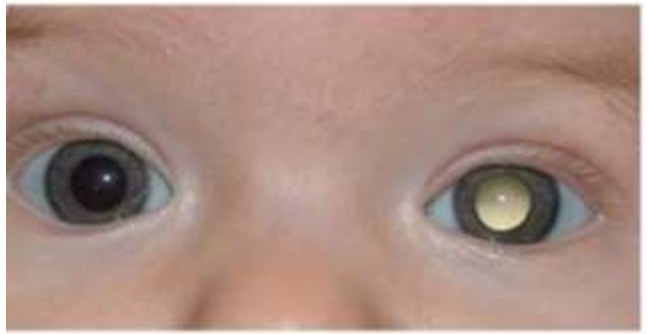


Fig.3 white color in the center circle of the eye

Because the chance of a second hit is so great, it occurs frequently in more than one cell

Thus heterozygotes for the disorder often have tumors arising at multiple sites, such as multifocal tumors in one eye, in both eyes (bilateral retinoblastoma), as well as in the pineal gland (referred to as "trilateral" retinoblastoma).

The occurrence of a second hit is a matter of chance and does not occur 100% of the time; the penetrance of retinoblastoma therefore, although greater than 90%, is not complete.

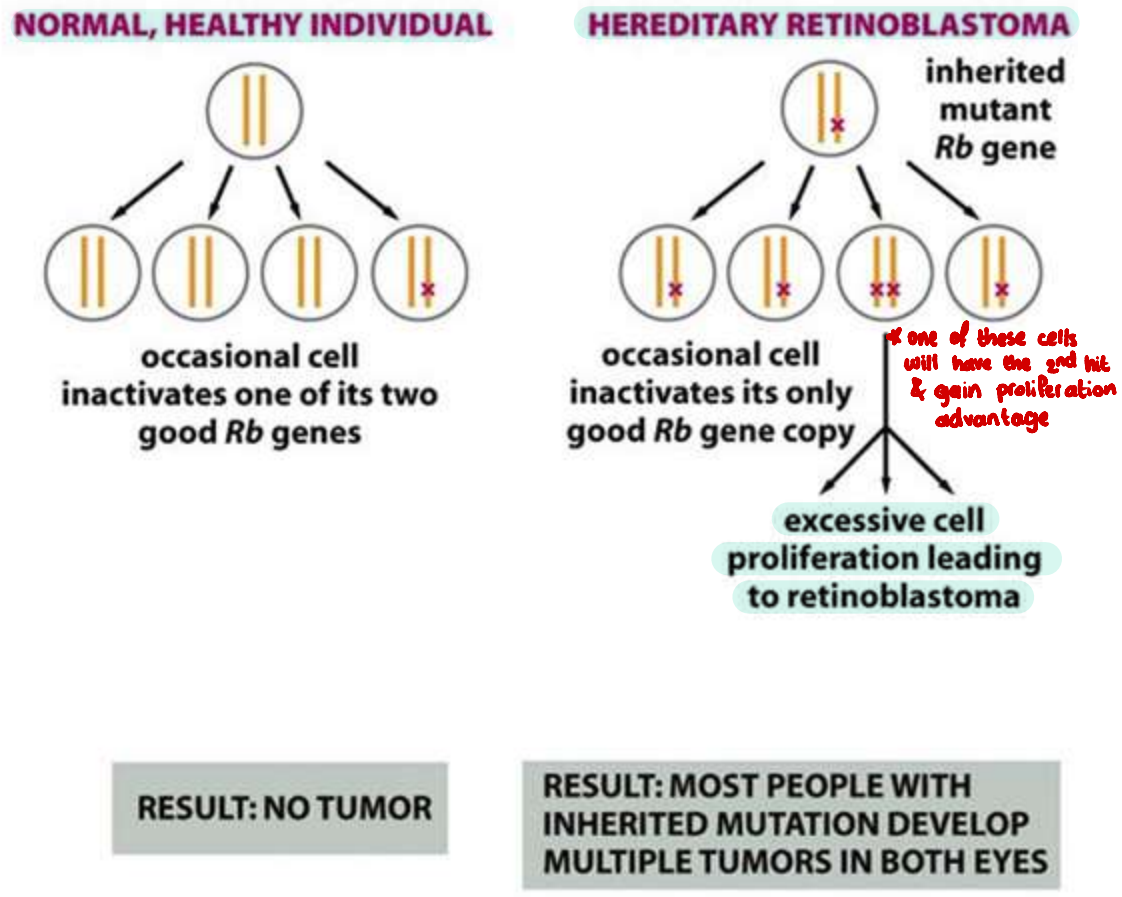


Figure 20-30 *Molecular Biology of the Cell* (© Garland Science 2008)

40% of RB cases are hereditary.

The other 60% of cases of retinoblastoma are nonhereditary

Non-hereditary

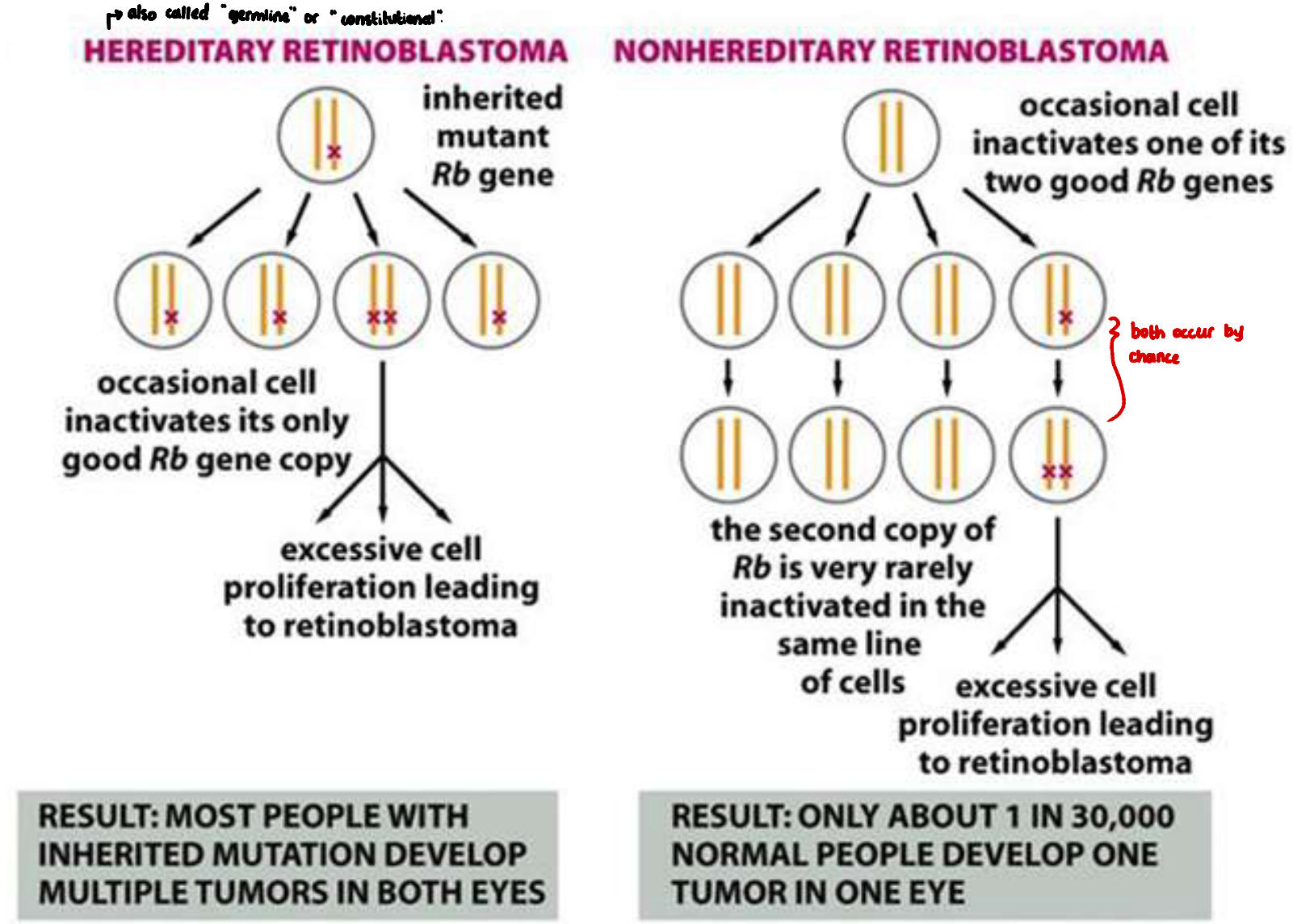
Both RB1 alleles in a single retinal cell have been inactivated independently by chance

Because two hits in the same cell is a statistically rare event, there is usually only a single clonal tumor, and the retinoblastoma is found at one location (unifocal) in one eye only.

Unilateral tumor is no guarantee that the child does not have the heritable form of retinoblastoma, however, because 15% of patients with the heritable type develop a tumor in only one eye.

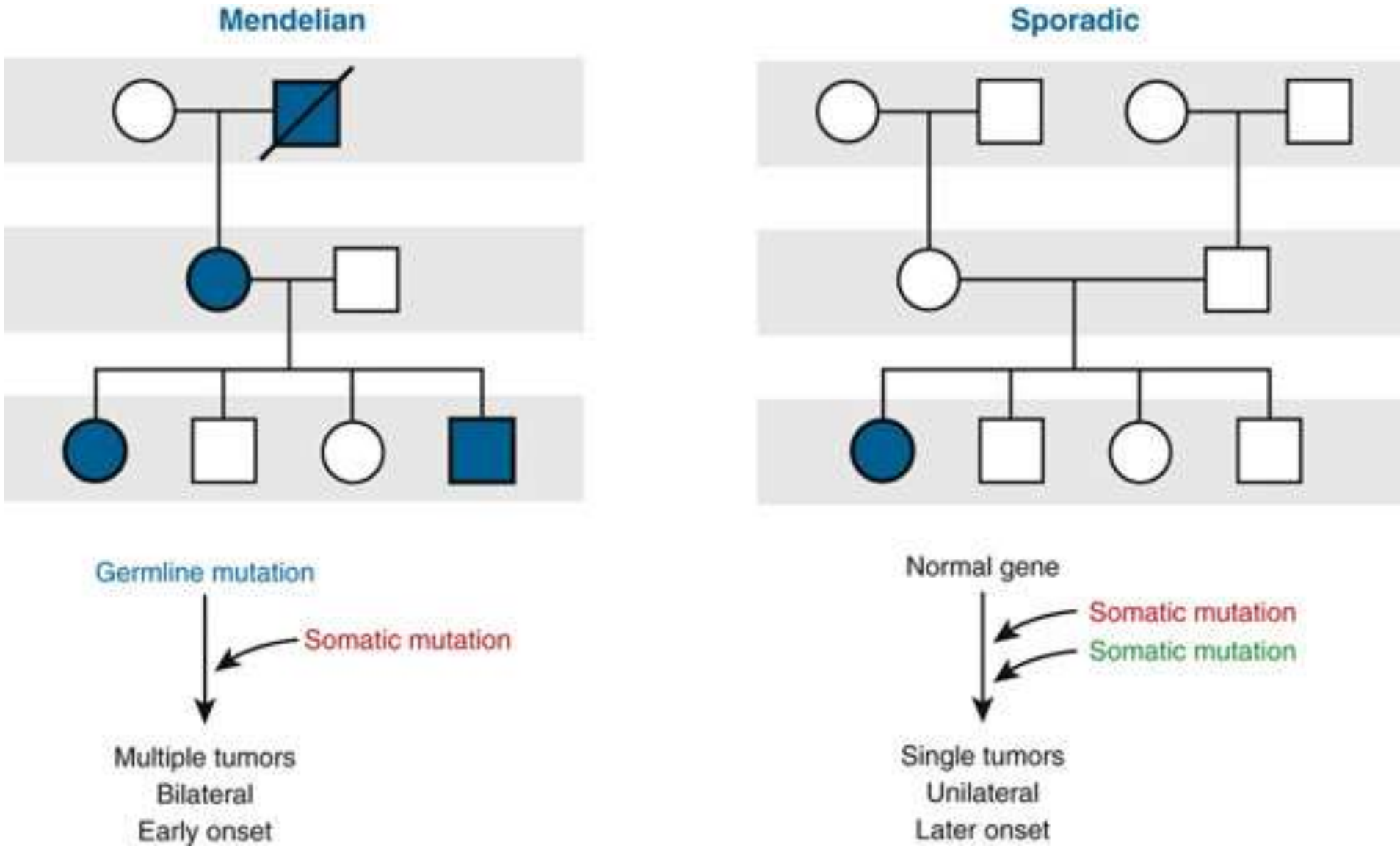
85% of heritable RB develop bilateral tumors. (NOT 100%)

→ also called "germline" or "constitutional".



Another difference between hereditary and sporadic tumors is that the average **age at onset** of the sporadic form is in early childhood, later than in infants with the heritable form

reflecting the longer time needed on average for two mutations, rather than one, to occur.



In a small percentage of patients with retinoblastoma, the variant responsible is a cytogenetically detectable deletion or translocation of the portion of chromosome 13 that contains the RB1 gene.

↳ in this case, damage at the chromosomal level (not a point mutation) in a region that includes RB1 gene & other genes adjacent to RB1. This leads to dysmorphic features.

Such chromosomal changes, if they also disrupt genes adjacent to RB1, may lead to **dysmorphic features in addition** to retinoblastoma.

Nature of the Second Hit

Typically, for retinoblastoma as well as for the other hereditary cancer syndromes, the first hit is an inherited mutation, that is, a change in the DNA sequence.

The second hit, however, can be caused by a variety of genetic, epigenetic, or genomic mechanisms

Although a number of mechanisms have been documented, the common theme is **loss of function** of RB1

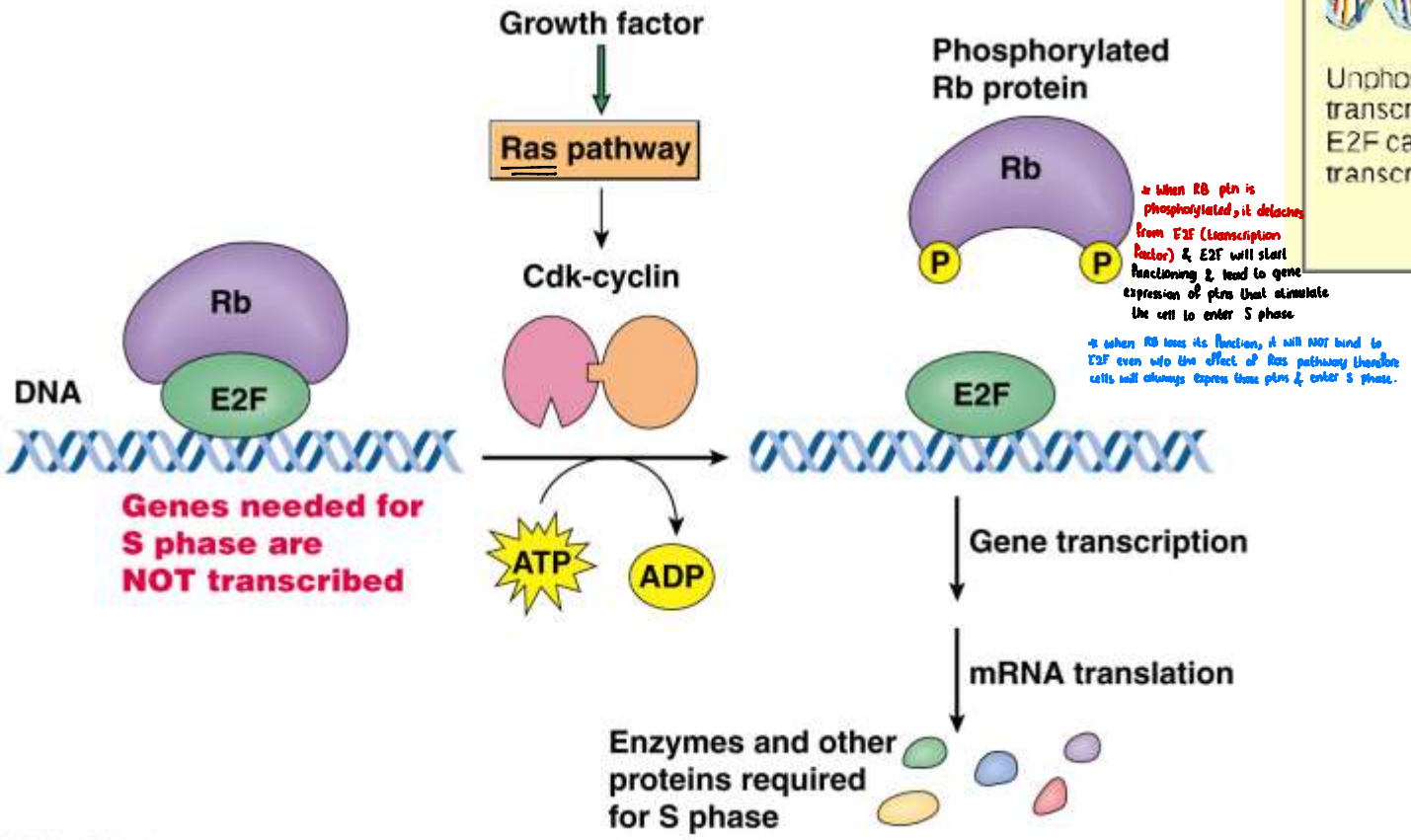
+Regardless of the type of mutation of the hits, the goal is that both hits cause loss of function of the TSG (i.e: RB1 gene).
(if an activating mutation hit the TSG, it would have an opposite effect by decreasing cancer risk)

Oncogenes	Tumor suppressor genes (TSG)
• Activation of the gene product **increases** cancer risk	• Activation of the gene product **decreases** cancer risk
• The mutated form of a proto-oncogene	• A "loss-of-function" mutation can lead to loss of activity, allowing for cancer to occur
• A "gain-of-function" mutation can over-activate a proto-oncogene, turning it into an oncogene	

Mutations in both oncogenes and tumor suppressor genes can have similar effects in enhancing cell proliferation and survival and in promoting tumor development.

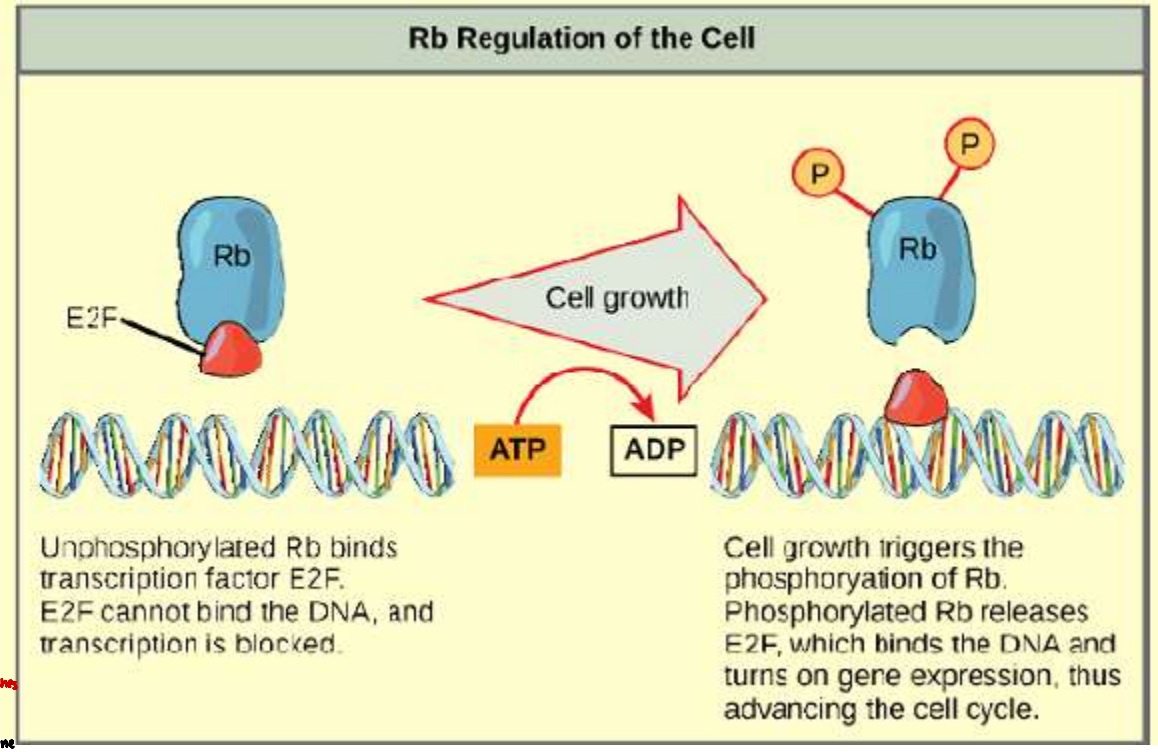
The RB1 gene product, p110 Rb1, is a phosphoprotein that normally regulates entry of the cell into the S phase of the cell cycle. It determines if the cells proceeds to the S phase or remain arrested at G1.

Thus loss of the RB1 gene and/or absence of the normal RB1 gene product deprives cells of an important checkpoint and allows uncontrolled proliferation.



When RB pin is phosphorylated, it detaches from E2F (transcription factor) & E2F will start functioning & lead to gene expression of pins that stimulate the cell to enter S phase.

When RB loses its function, it will NOT bind to E2F even w/o the effect of Ras pathway therefore cells will always express these pins & enter S phase.



Loss of Heterozygosity

* type of mutation especially the 2nd hit

In addition to mutations and epigenetic silencing

a novel genomic mechanism was uncovered when geneticists made an unusual but highly significant discovery when they

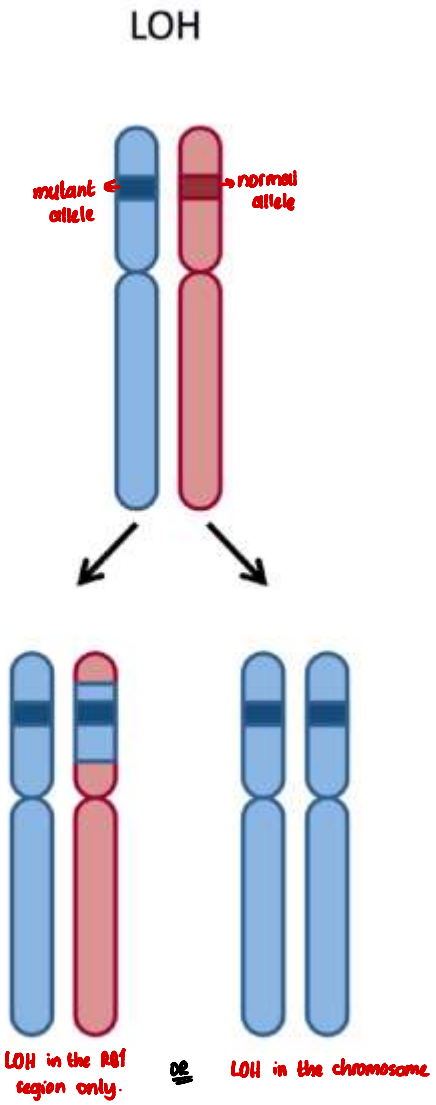
how it was discovered:

compared DNA polymorphisms at the RB1 locus in DNA from normal cells to those in the retinoblastoma tumor from the same patient.

* there are 2 chromosomes (homologues): maternal + paternal. genes are the same but the sequence of DNA is similar but NOT identical. Many regions are heterozygous due to this fact. Therefore, normally speaking, if we sequence RB1 gene & adjacent regions, heterozygosity is found in some regions. BUT if we sequence DNA extracted from a RB tumor, we will find that heterozygosity is lost in the RB1 region even though heterozygosity can still be seen in other regions. (sometimes it can be absent on a chromosomal level)

→ LOH is seen in tumor tissue but NOT in normal tissue.

Individuals with retinoblastoma who were heterozygous at polymorphic loci flanking the RB1 locus in normal tissues had tumors that contained alleles from only one of their two chromosome 13 homologues, revealing a loss of heterozygosity (LOH) in tumor DNA in and around the RB1 locus.

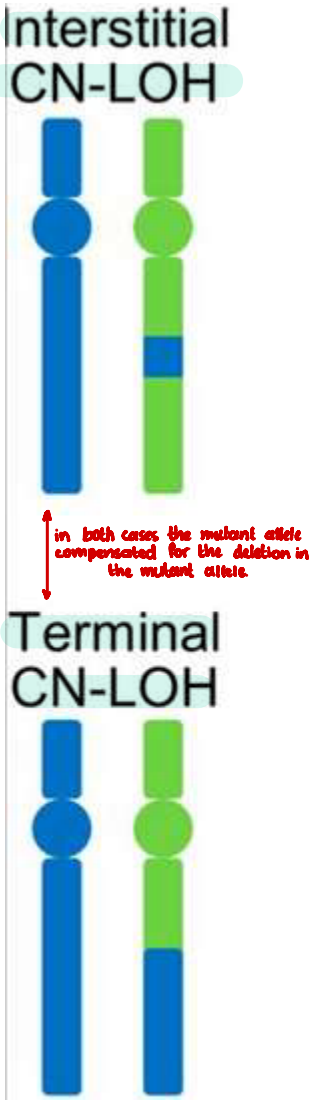


Loss of Heterozygosity

Furthermore, in familial cases, the retained chromosome 13 markers were the ones inherited from the affected parent, that is, the chromosome with the abnormal RB1 allele.

Thus, in these cases, LOH represents the second hit of the remaining allele. LOH is one of the mechanisms that result in the 2nd hit.

LOH may occur by interstitial deletion, but there are other mechanisms as well, such as mitotic recombination or monosomy 13 due to nondisjunction



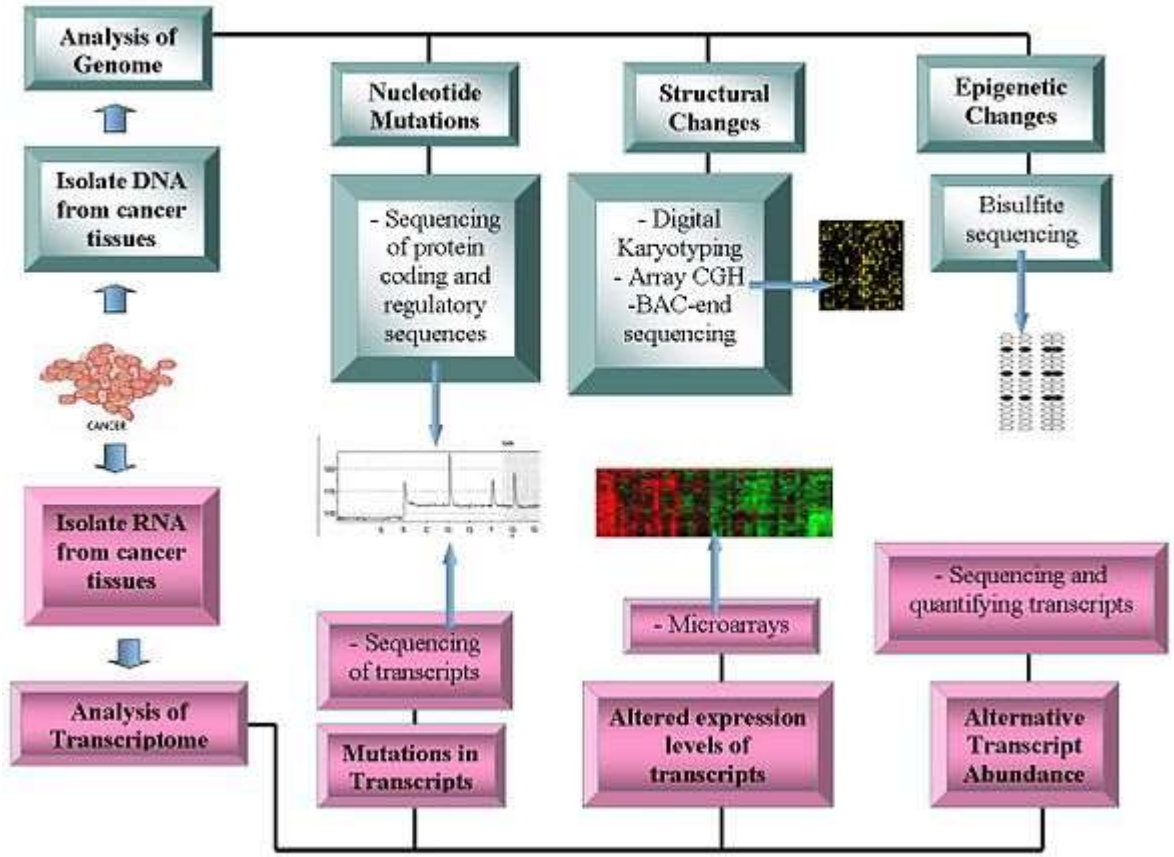
* deletion of the normal allele along with compensation (copy-paste) from the mutant allele. (note that there is NO gain/loss of DNA)

- Copy neutral loss of heterozygosity (CN-LOH) is the most common class of structural mutation.
- Interstitial events are more abundant than Terminal CN-LOH, but affect smaller genomic regions.
- CN-LOH mutation mechanisms are universal to diploid genomes, and play a key role in humans, both in cancer tumor suppressor loss and somatic mosaicism.

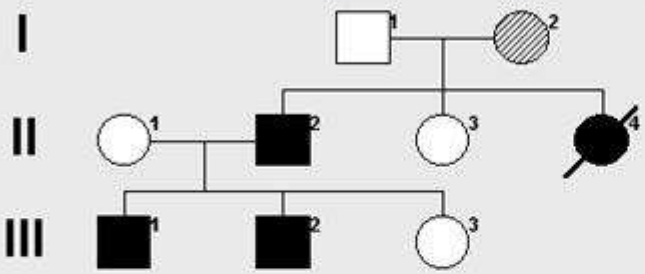
Familial Breast Cancer due to Mutations in BRCA1 and BRCA2

In the general female population. An individual woman has a 1-in-8 (12%) chance of developing breast cancer over an 80-year lifespan

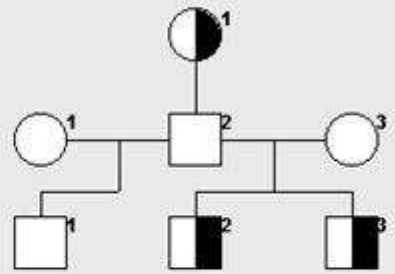
(≈3% to 5%) of breast cancer cases appear to be due to a highly penetrant dominantly inherited mendelian predisposition that increases the risk for female breast cancer fourfold to sevenfold over the 12% lifetime risk observed in the general female population.


Analysis of Genome
Isolate DNA from cancer tissues
CANCER
Isolate RNA from cancer tissues
Analysis of Transcriptome
Nucleotide Mutations
- Sequencing of protein coding and regulatory sequences
- Sequencing of transcripts
Mutations in Transcripts
Structural Changes
- Digital Karyotyping
- Array CGH
-BAC-end sequencing
- Microarrays
Altered expression levels of transcripts
Epigenetic Changes
Bisulfite sequencing
- Sequencing and quantifying transcripts
Alternative Transcript Abundance

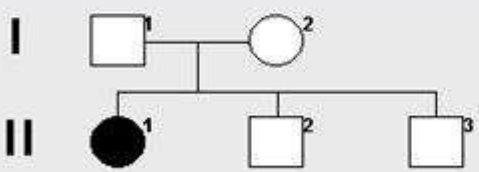
Family history



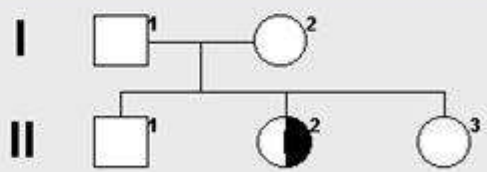
Familial RB



Low penetrance RB



Sporadic bilateral RB



Sporadic unilateral RB