

cancer is fundamentally a
genetic disease

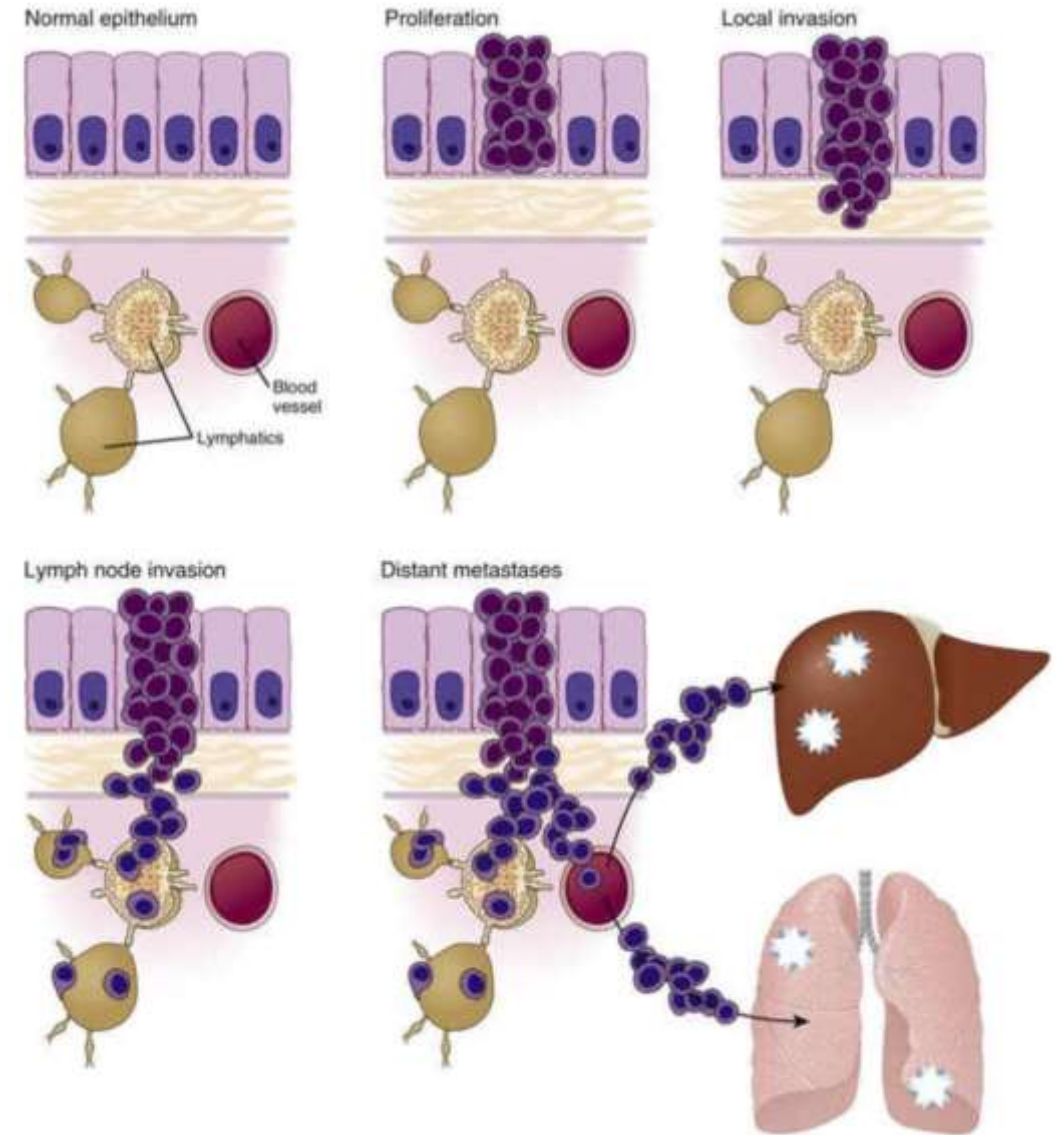
Dr. Bilal Azab

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

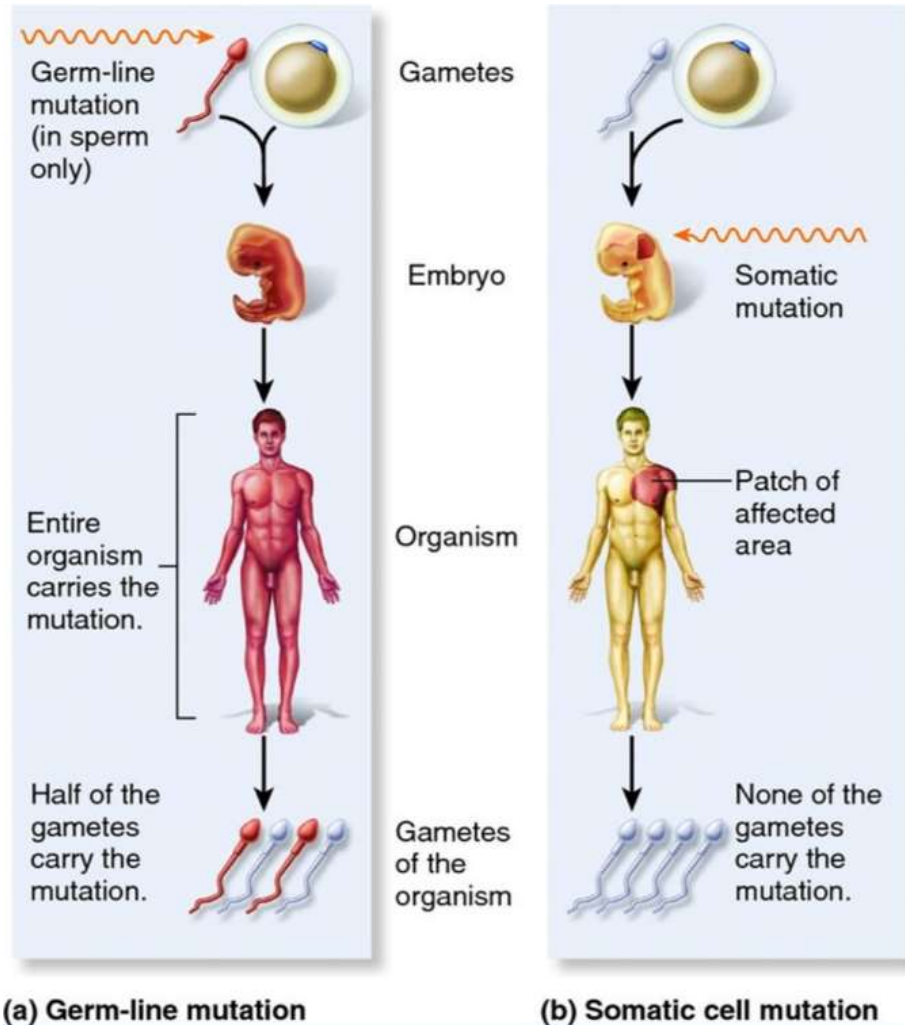
Cancer is the name used to describe the more virulent forms of neoplasia. accumulation of cells in a neoplasm occurs because of an imbalance between the normal processes of cellular proliferation and cellular attrition.

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.

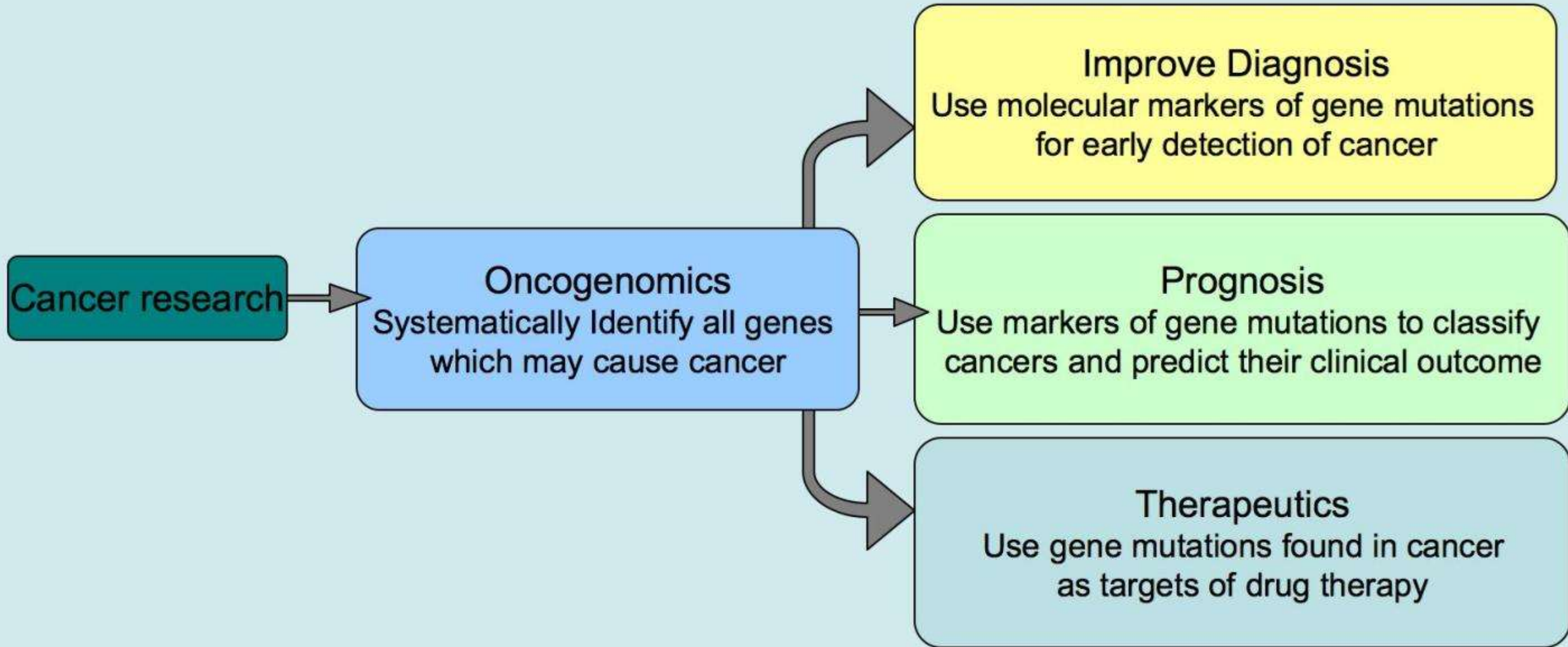
The Cancer Genome Atlas Program



The Cancer Genome Atlas (TCGA), a landmark [cancer genomics](#) program, molecularly characterized over 20,000 primary cancer and matched normal samples spanning 33 cancer types. This joint effort between NCI and the National Human Genome Research Institute began in 2006, bringing together researchers from diverse disciplines and multiple institutions.

Over the next dozen years, TCGA generated over 2.5 petabytes of genomic, epigenomic, transcriptomic, and proteomic data. The data, which has already led to improvements in our ability to diagnose, treat, and prevent cancer, will remain [publicly available](#) for anyone in the research community to use.

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.

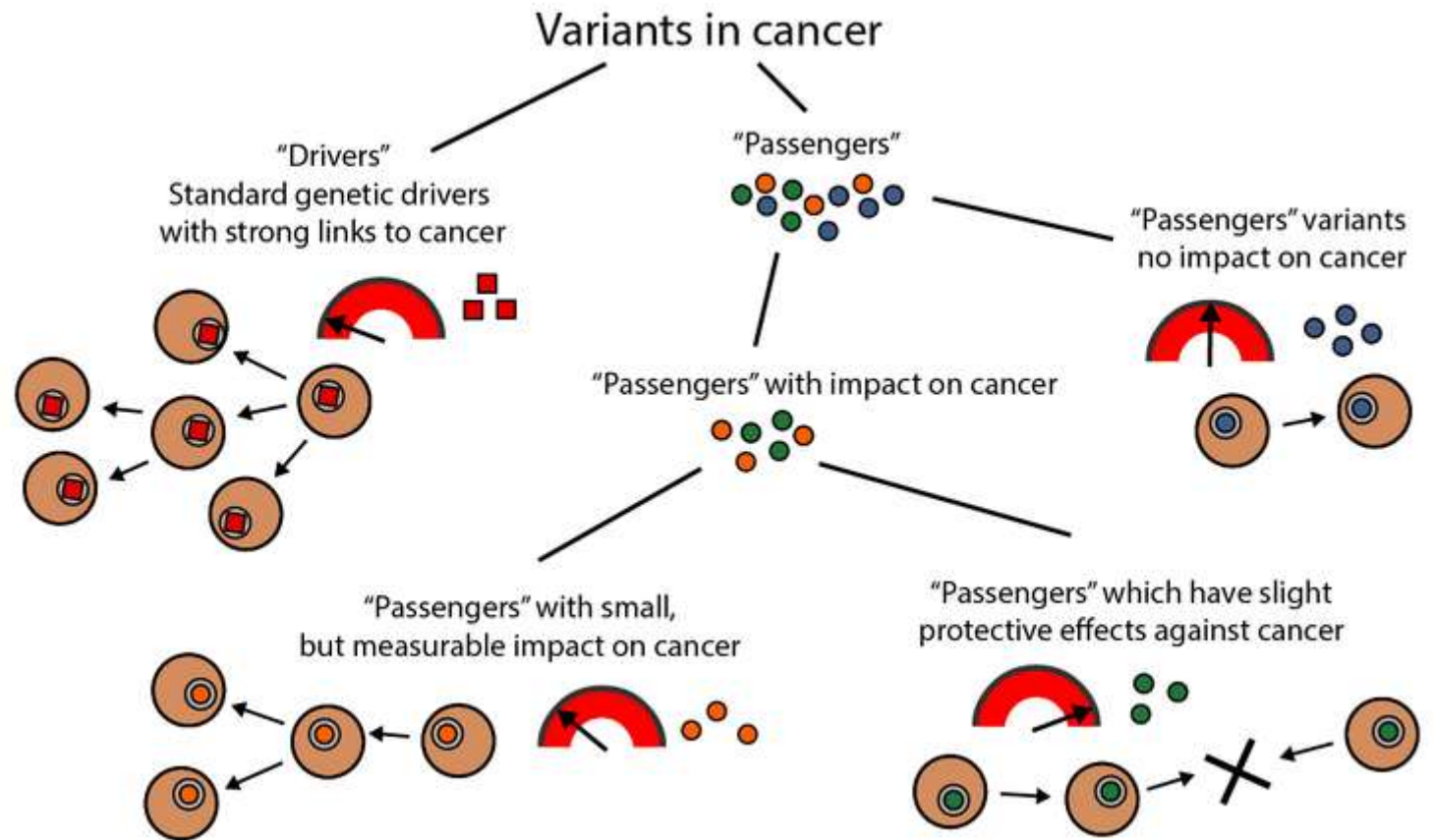
Most mutations found through sequencing of tumor tissue appear to be random, are not recurrent in particular cancer types, and probably occurred as the cancer developed, rather than directly causing the neoplasia to develop or progress. Such mutations are referred to as “**passenger**” mutations

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

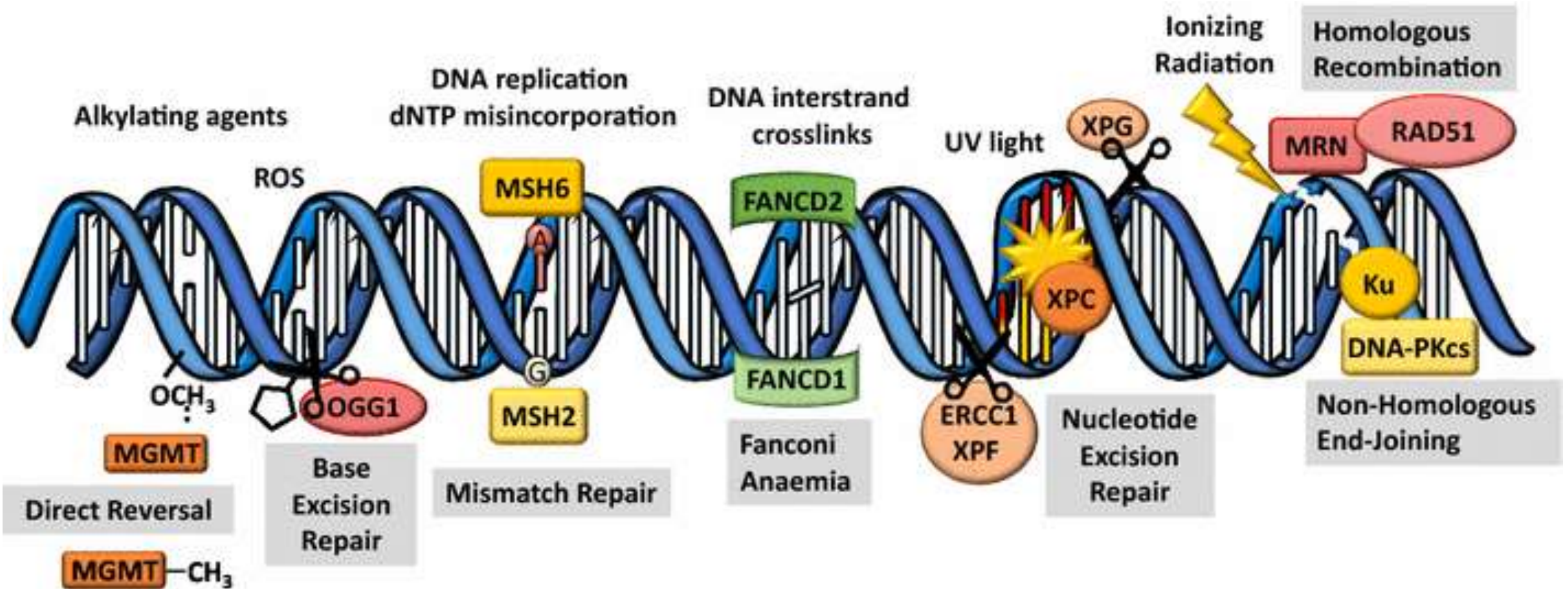
mutations). Although many driver genes are specific to particular tumor types, some, such as those in the **TP53** gene encoding the p53 protein, are found in the vast majority of cancers of many different types.

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.



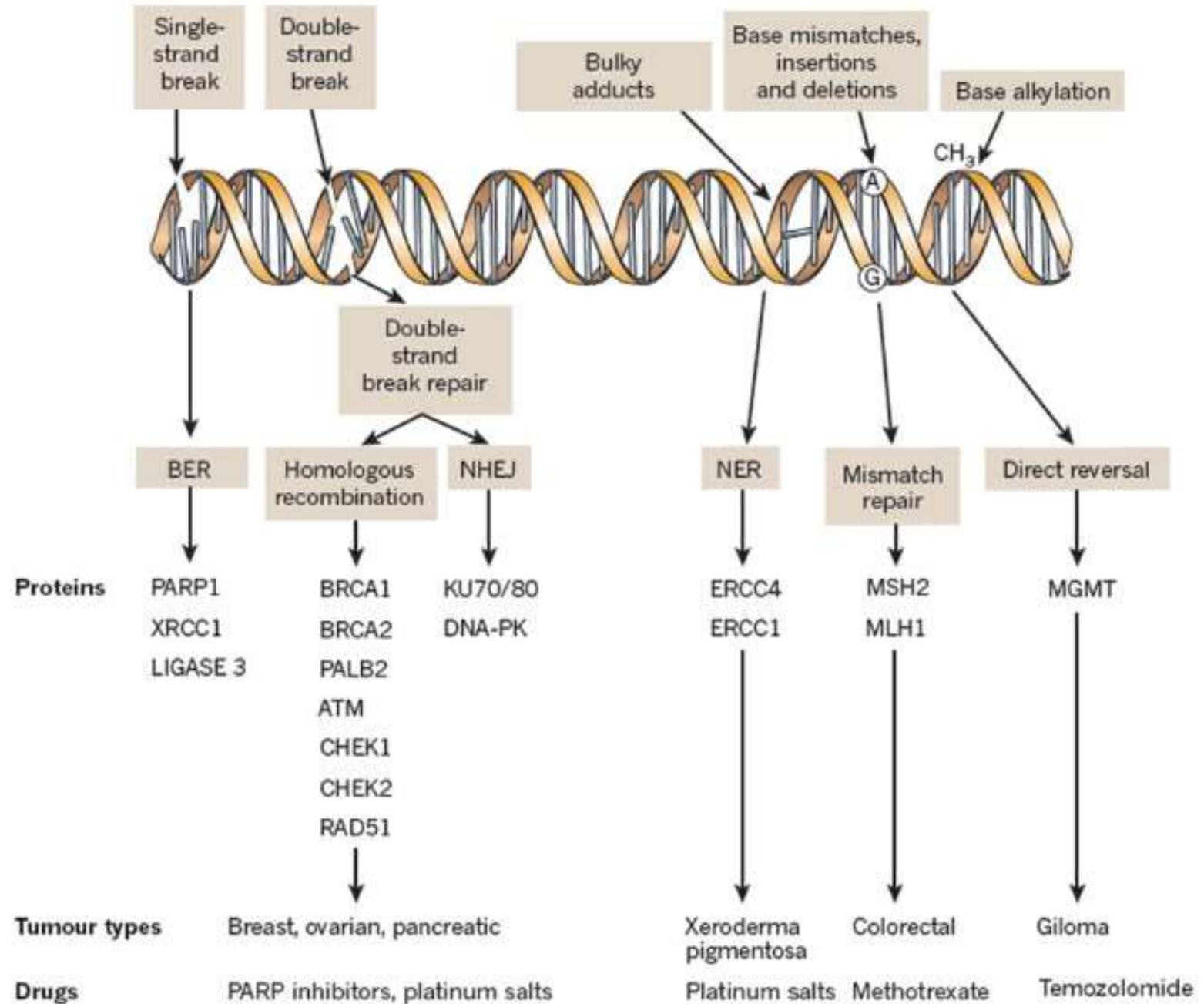
Spectrum of Driver Gene Mutations

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

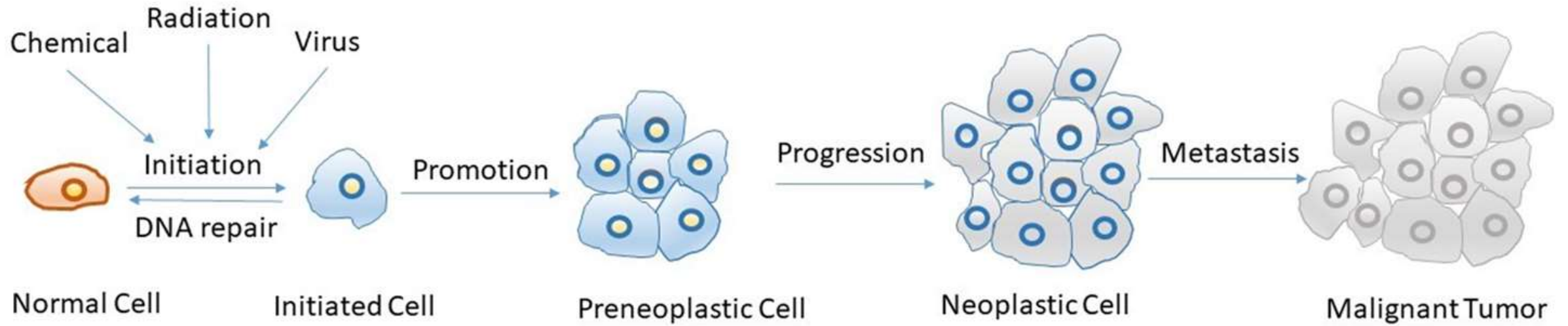


Spectrum of Driver Gene Mutations

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



Multistep Carcinogenesis

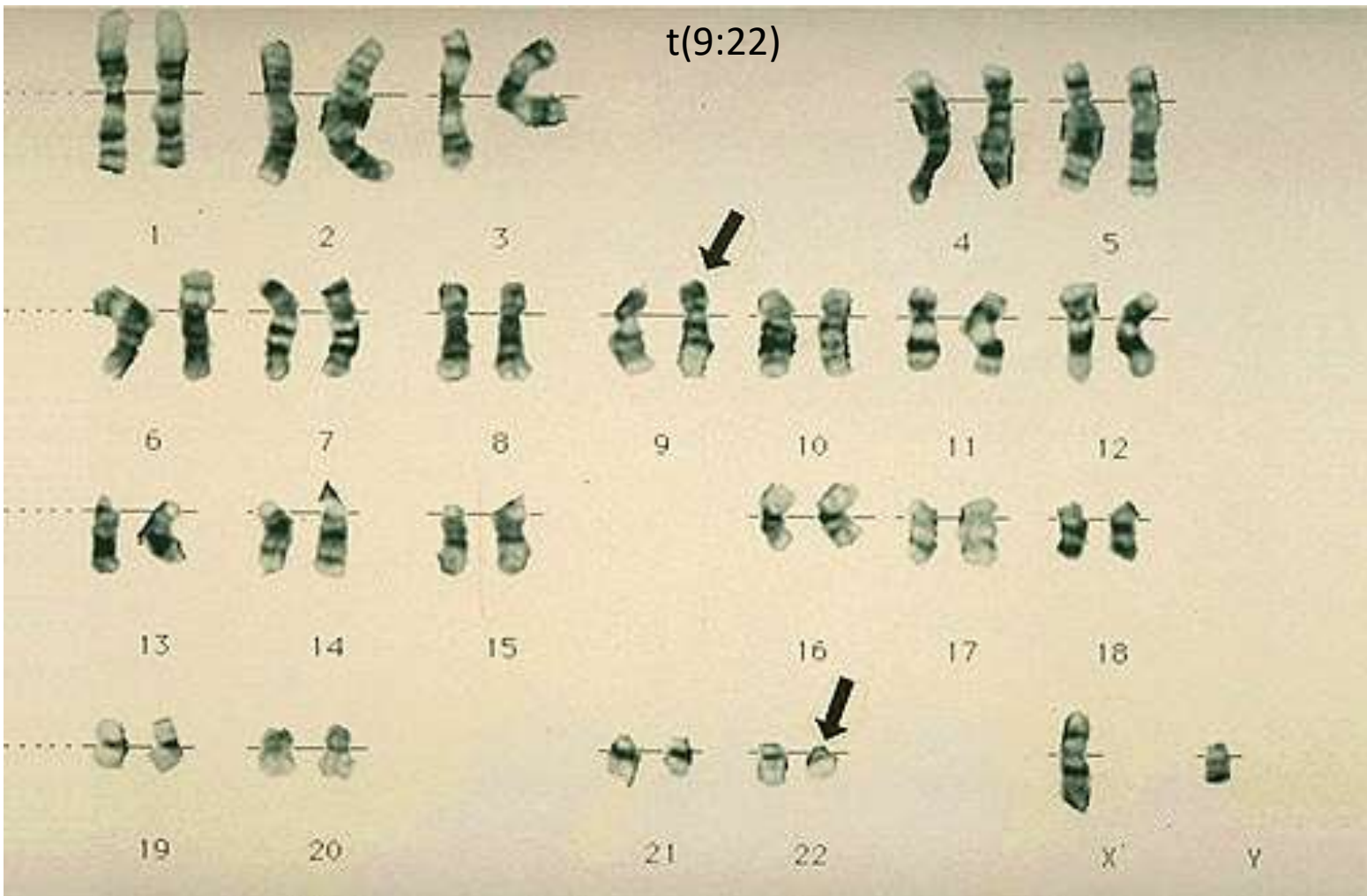
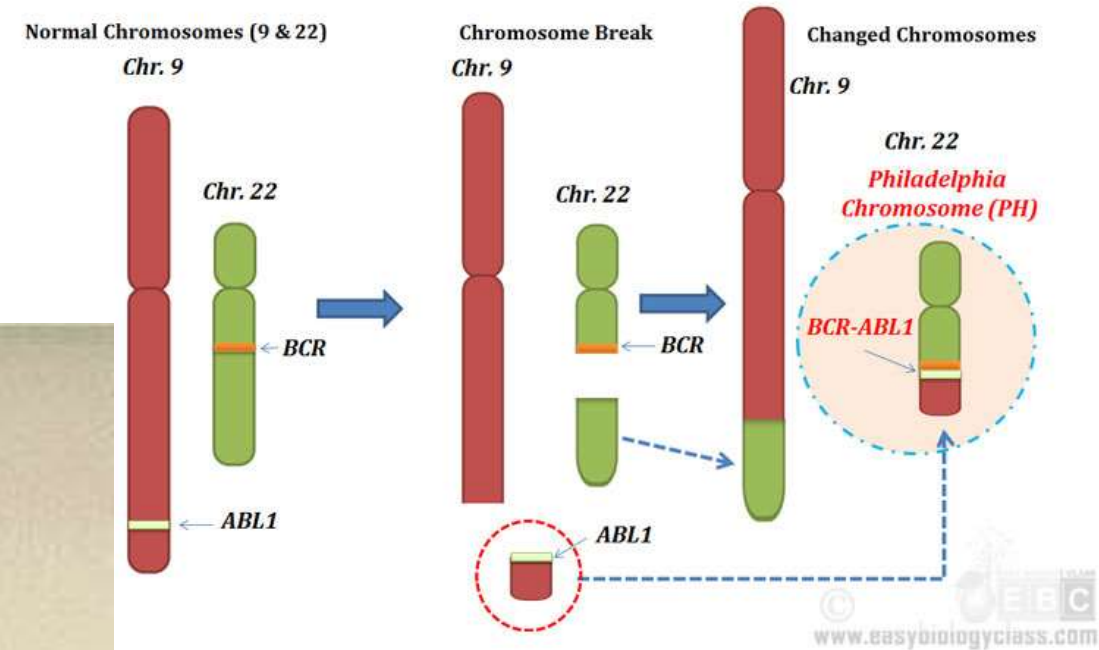


Driver mutations could occur on the chromosomal level

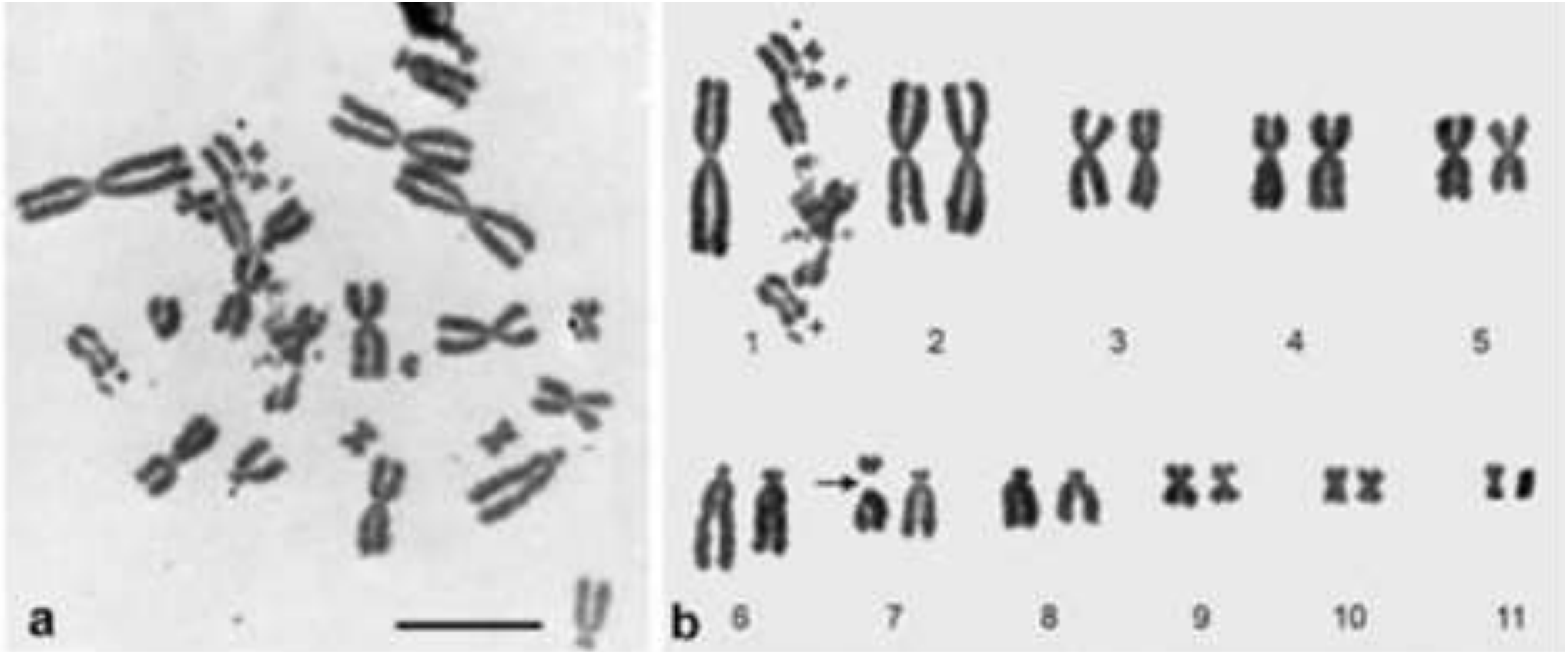
Chromosome and subchromosomal variants can also serve as driver mutations.

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

FORMATION OF PHILADELPHIA CHROMOSOME



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



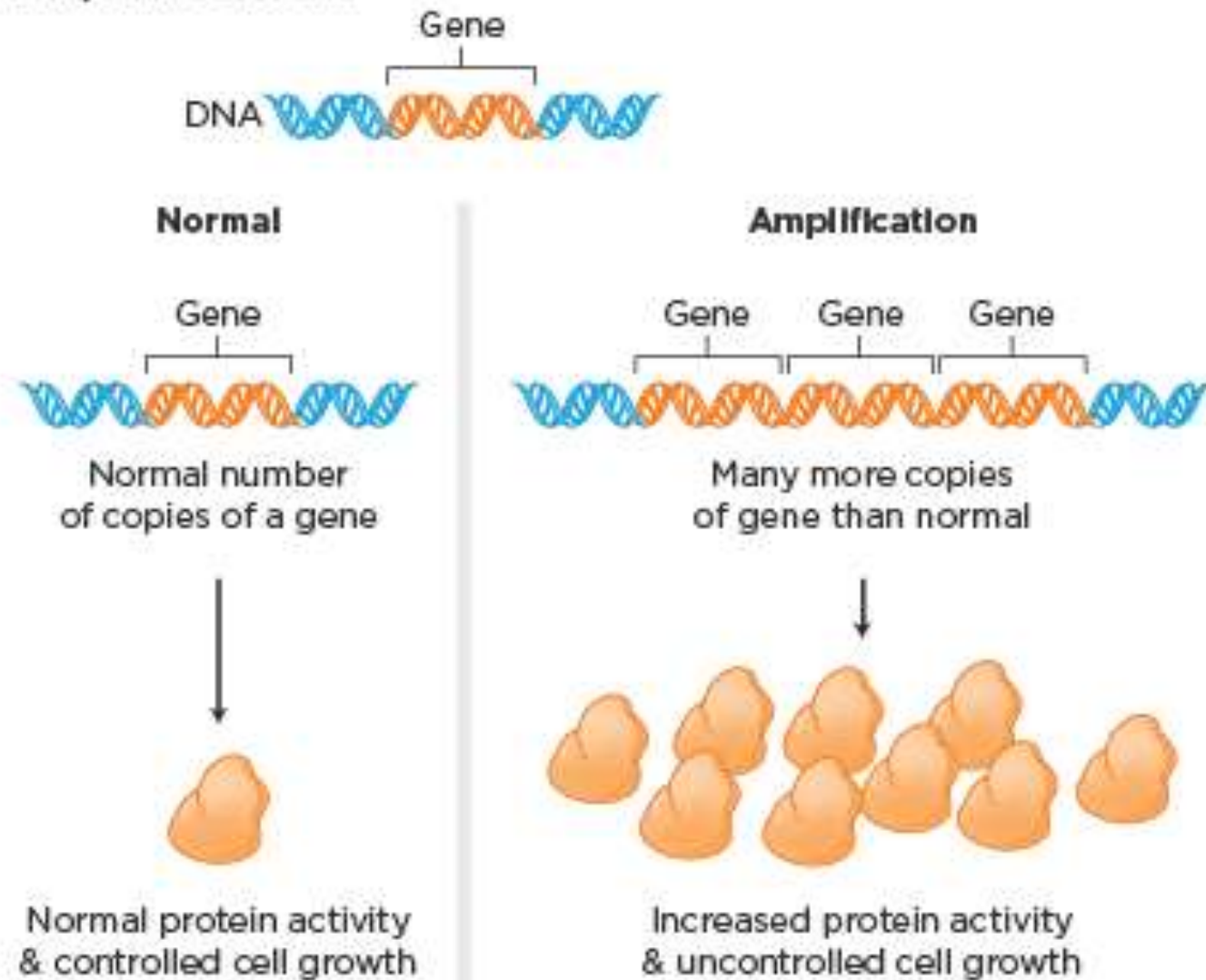
Metaphase spreads with damaged chromosomes obtained after laser UV microirradiation of nuclei in living Chinese hamster cells. Nuclei in living Chinese hamster cells were microirradiated ($\lambda = 257 \text{ nm}$) at a single nuclear site comprising about 5% of the total nuclear area. Microirradiated 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.

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**).

Amplification



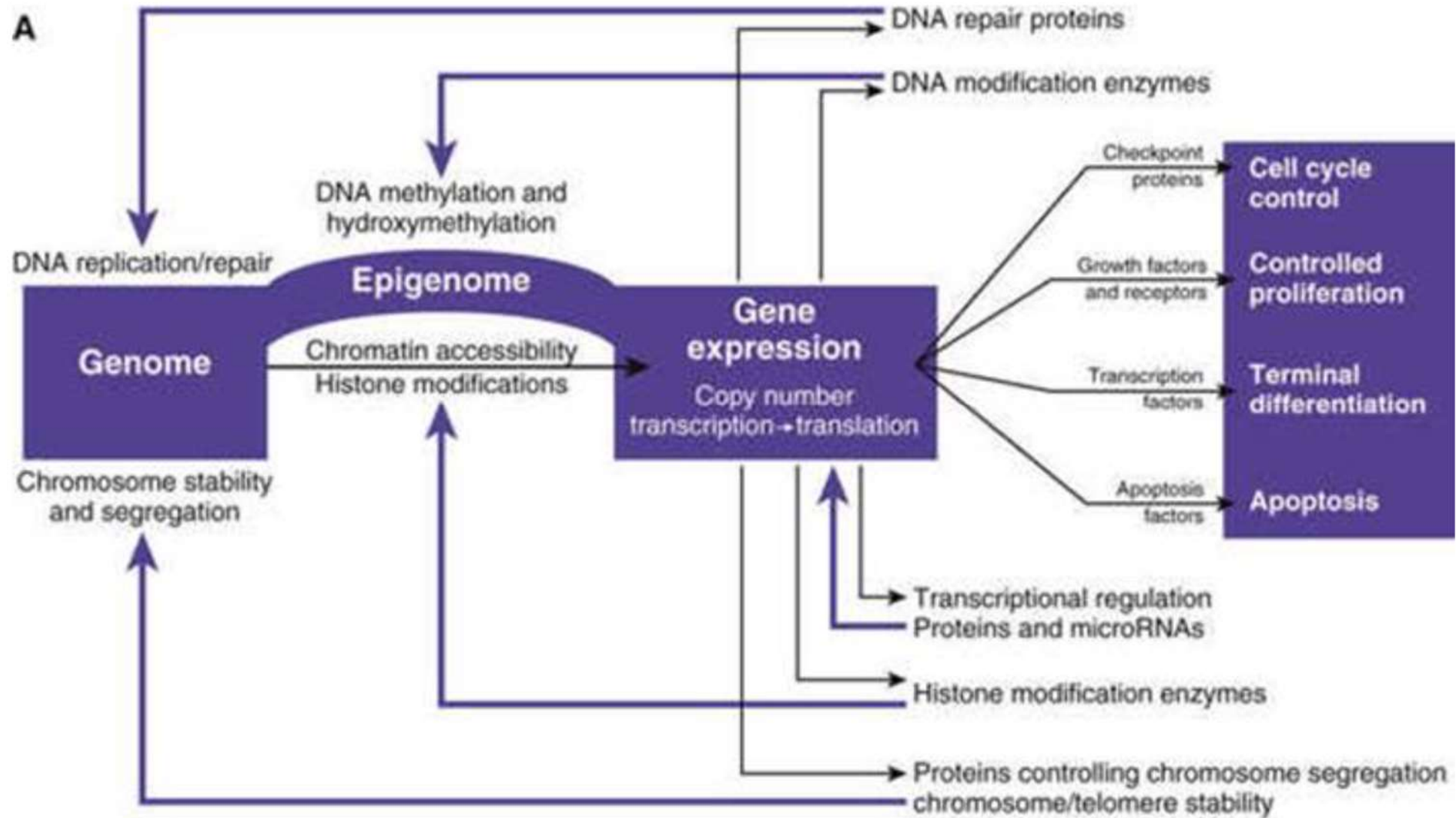
The Cellular Functions of Driver Genes

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

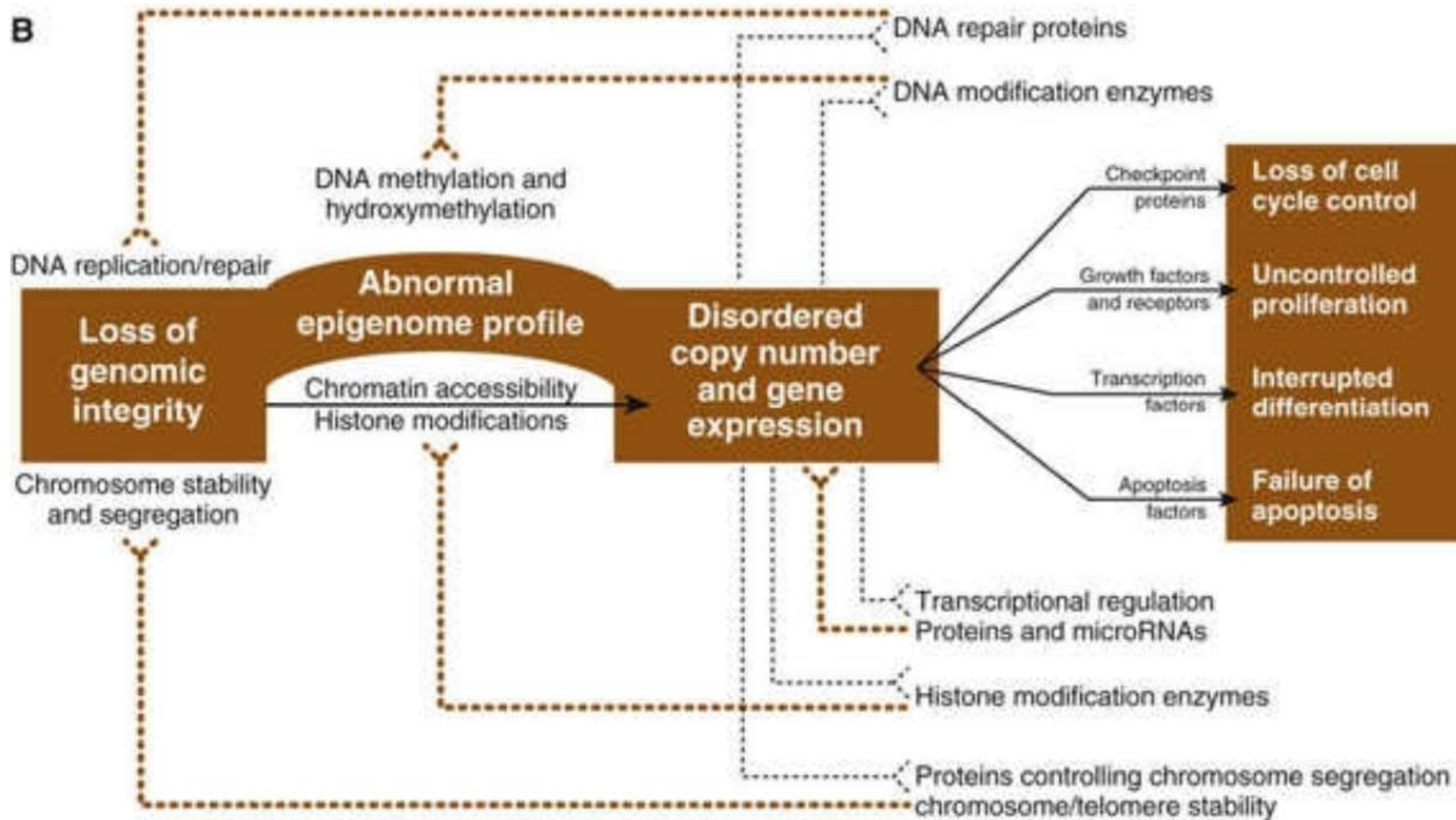
Genes with specific effects on cellular proliferation or survival	Genes with global effects on genome or DNA integrity
<p>Cell cycle regulation</p> <p>Cell cycle checkpoint proteins</p> <p>Cellular proliferation signaling</p> <ul style="list-style-type: none"> • Transcription factors • Receptor and membrane-bound tyrosine kinases • Growth factors • Intracellular serine-threonine kinases • PD kinases • G proteins and G protein-coupled receptors • mTOR signaling • Wnt/β-catenin signaling • Transcription factors <p>Differentiation and lineage survival</p> <ul style="list-style-type: none"> • Transcription factors protecting specific cell lineages • Genes involved in exit from cell cycle into G_0 <p>Apoptosis</p>	<p>Genome integrity</p> <ul style="list-style-type: none"> • Chromosome segregation • Genome and gene mutation • DNA repair • Telomere stability <p>Gene expression: abnormal metabolites affecting activity of multiple genes/gene products</p> <p>Gene expression: epigenetic modifications of DNA/chromatin</p> <ul style="list-style-type: none"> • DNA methylation and hydroxymethylation • Chromatin histone methylation, demethylation, and acetylation • Nucleosome remodeling • Chromatin accessibility and compaction (SWI/SNF complexes) <p>Gene expression: post-transcriptional alterations</p> <ul style="list-style-type: none"> • Aberrant mRNA splicing • MicroRNAs affecting mRNA stability and translation <p>Gene expression: protein stability/turnover</p>



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

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**

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

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

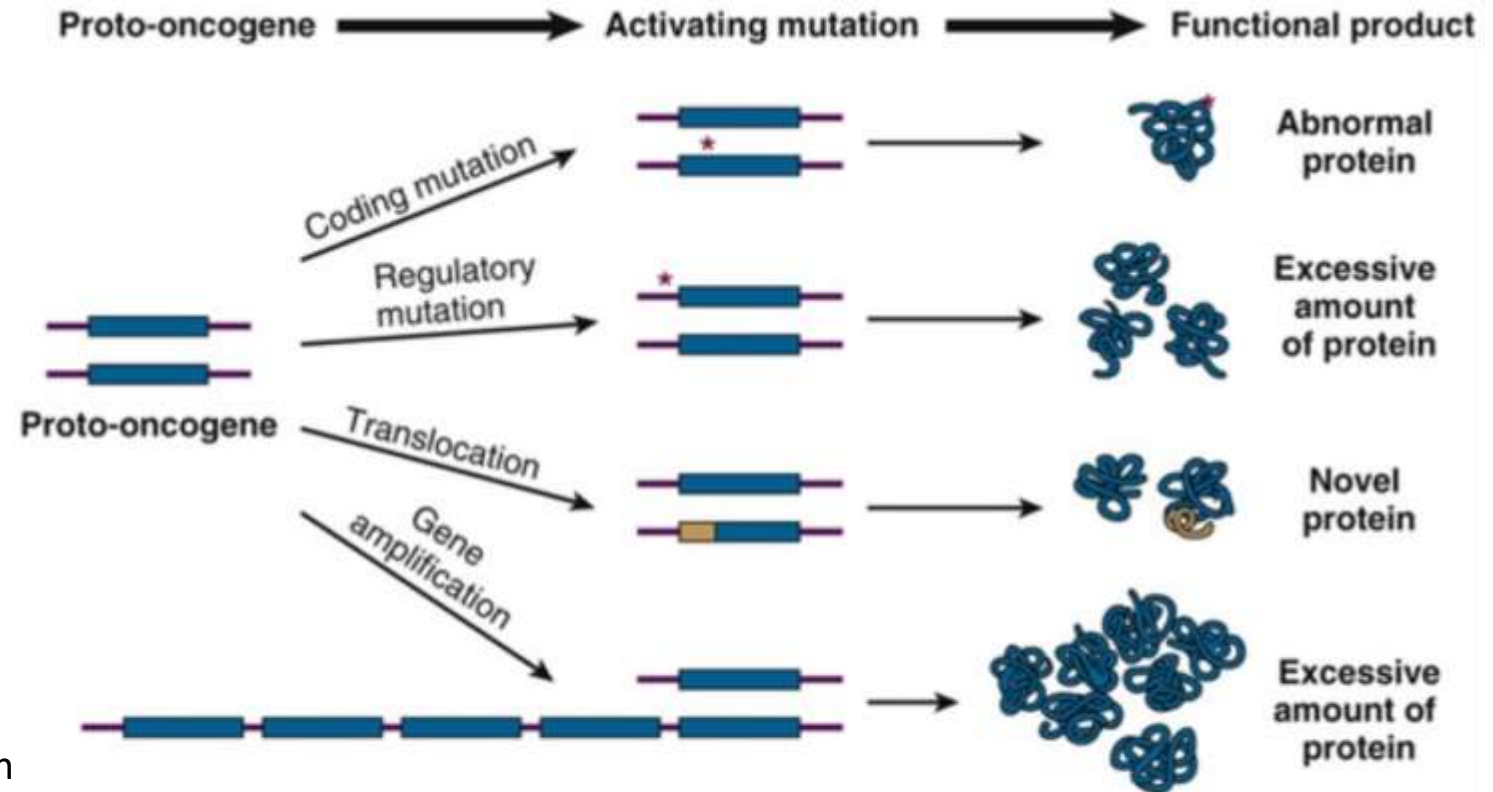
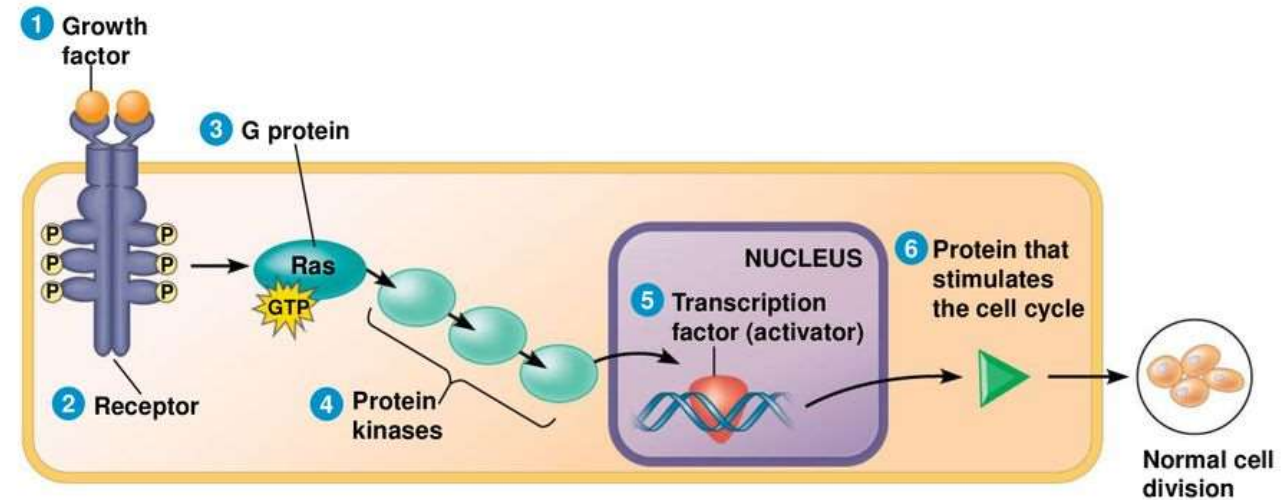


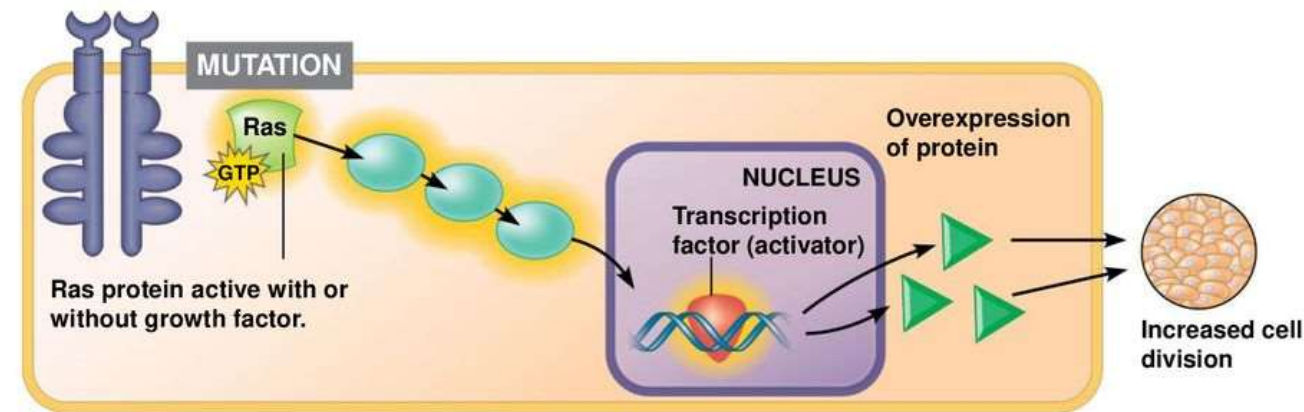
FIGURE 15-3 Different mutational mechanisms leading to proto-oncogene activation. These include a single point mutation leading to an amino acid change that alters protein function, mutations or translocations that increase expression of an oncogene, a chromosome translocation that produces a novel product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

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.

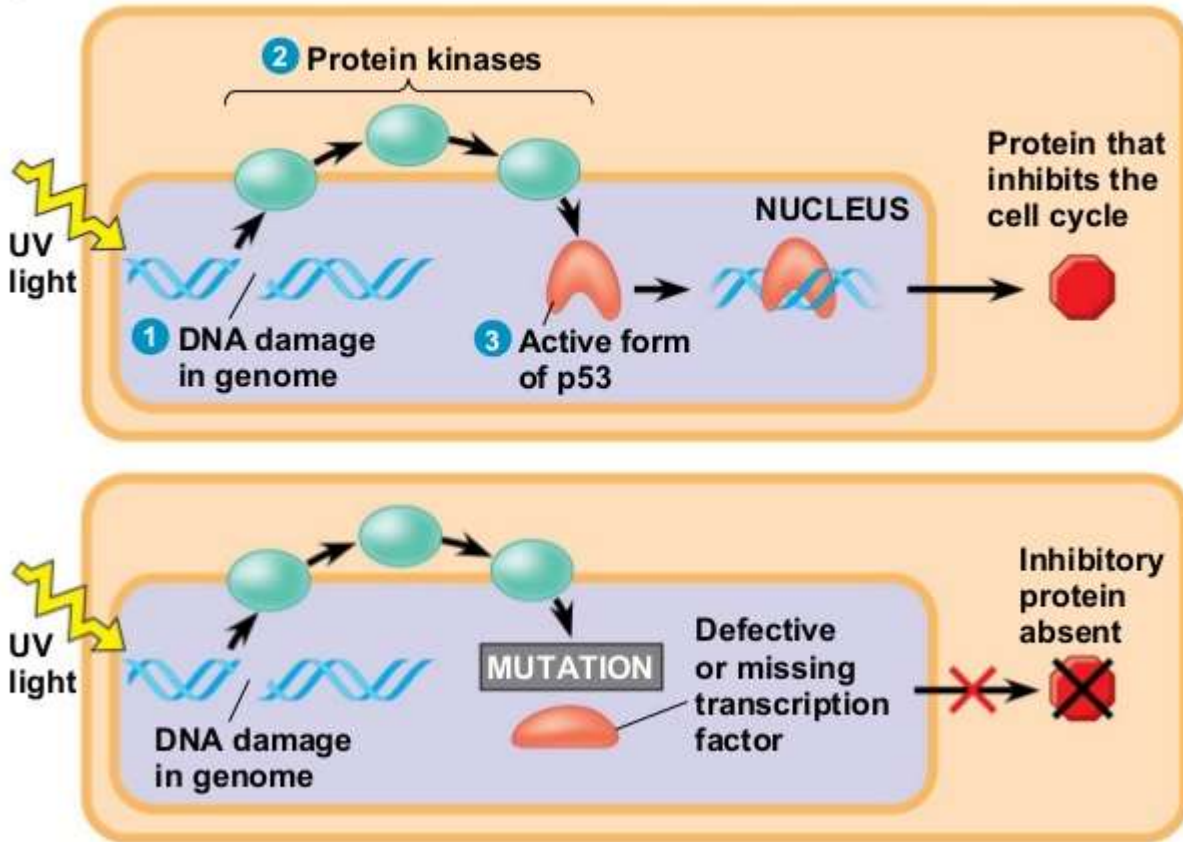


Mutant cell cycle–stimulating pathway.

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

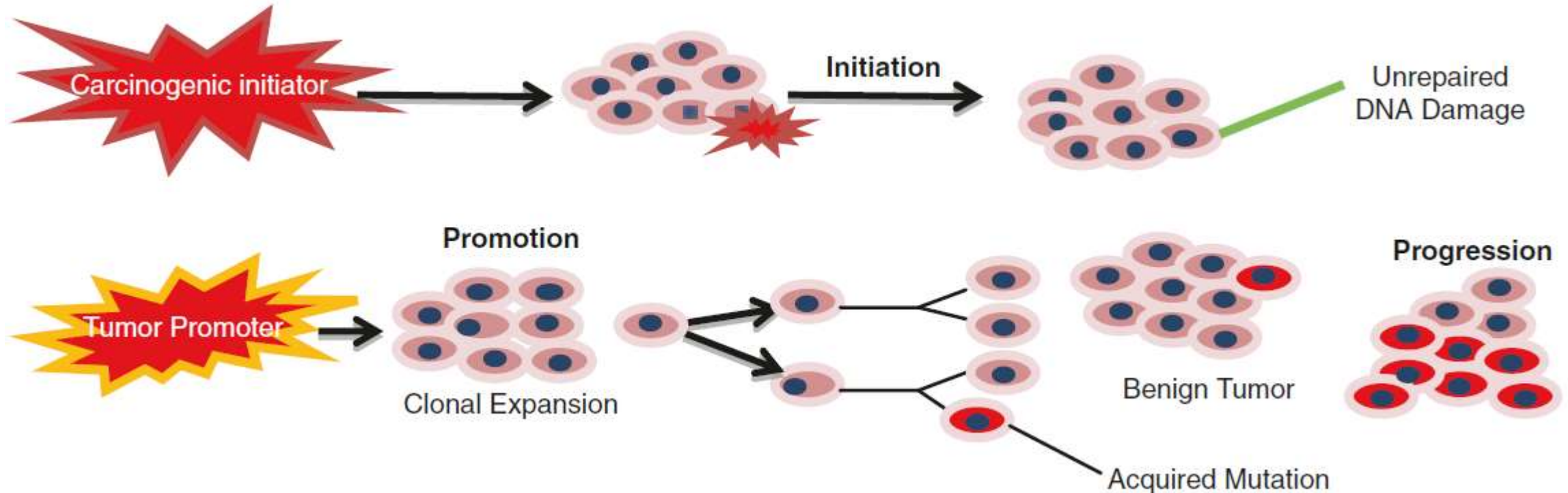
Cellular Heterogeneity within Individual Tumors

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

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

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



A paradigm for the development of cancer

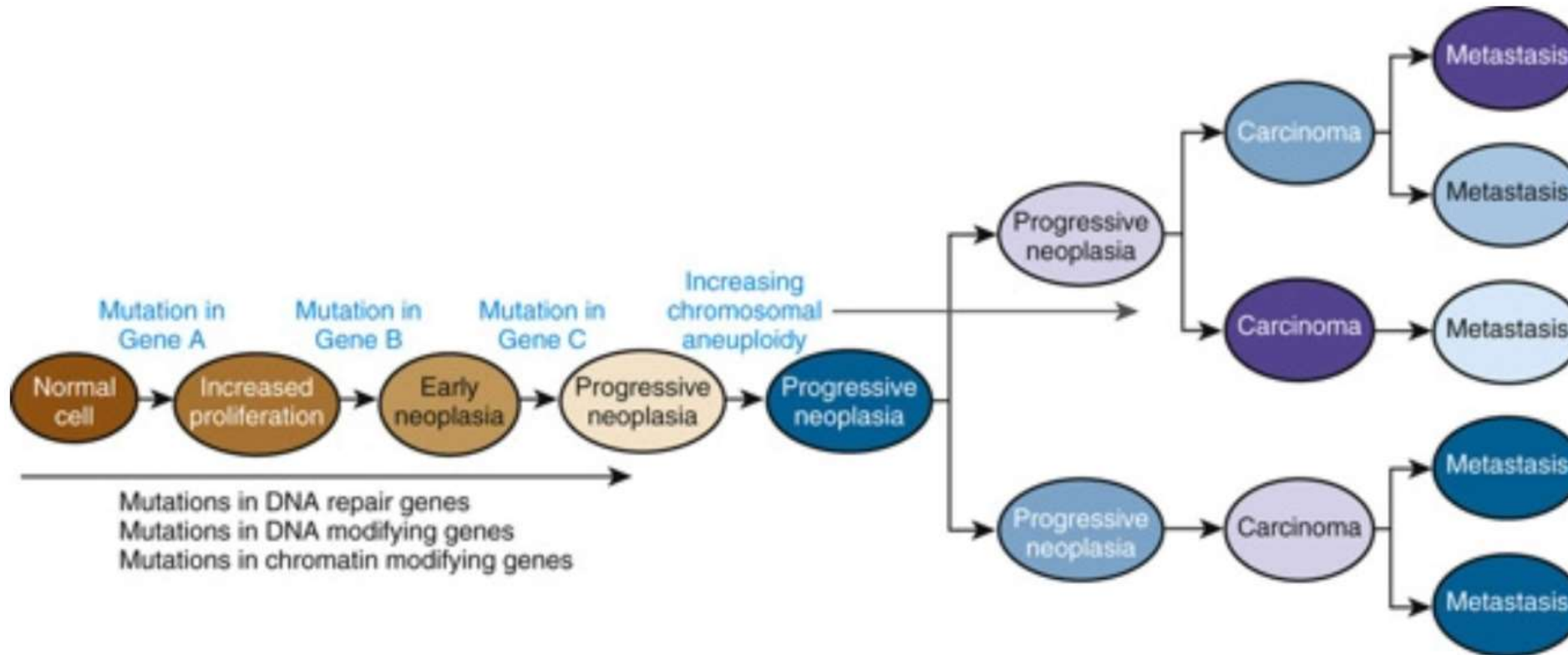


FIGURE 15-4 Stages in the evolution of cancer. Increasing degrees of abnormality are associated with sequential loss of tumor suppressor genes from several chromosomes and activation of proto-oncogenes, with or without a concomitant defect in DNA repair. Multiple lineages, carrying different mutations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

The profile of mutations and epigenomic changes can differ:

Between the primary and its metastases

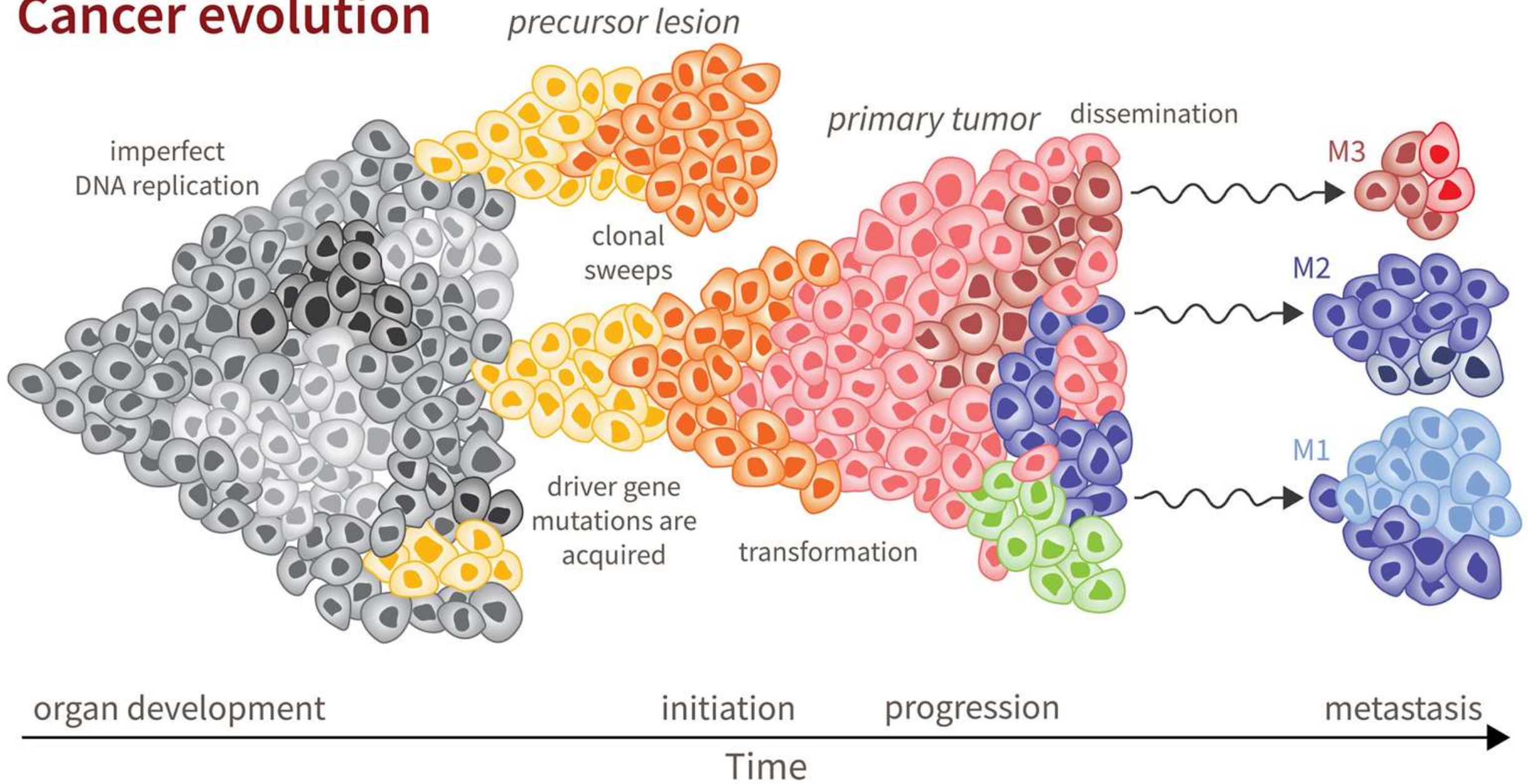
Between different metastases,

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

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.

Cancer evolution



Cancer in Families

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

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

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)

Cancer in Families

Not all families with an apparently increased incidence of cancer can be explained by known mendelian or clearly recognized genetic disorders.

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.

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

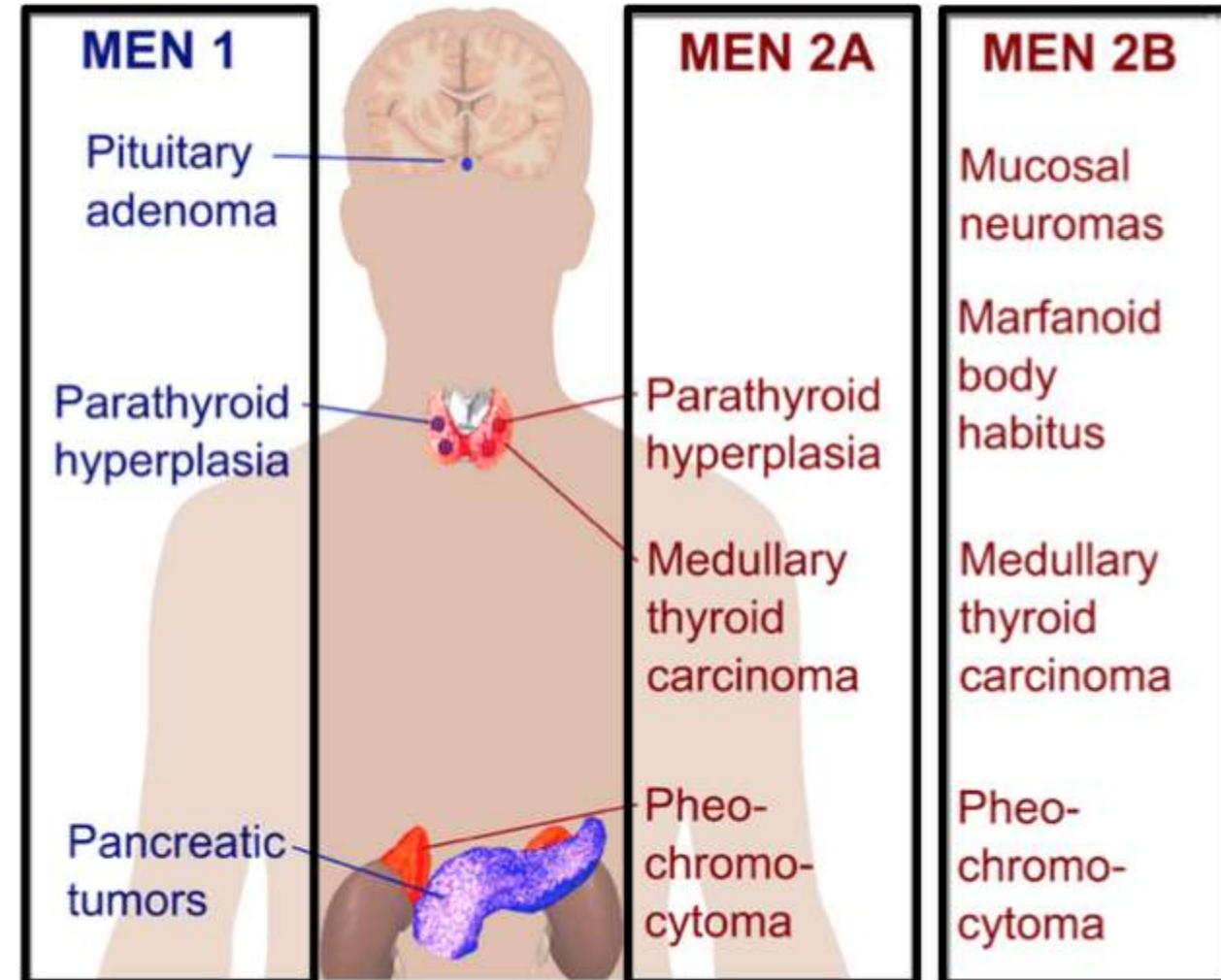
MEN2-A is an AD disorder characterized by: high incidence of medullary carcinoma of the thyroid that is often but not always associated with pheochromocytoma, benign parathyroid adenomas, or both.

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 **A**myloid
- Produces **C**alcitonin
- Tumor of “**C**”-cells

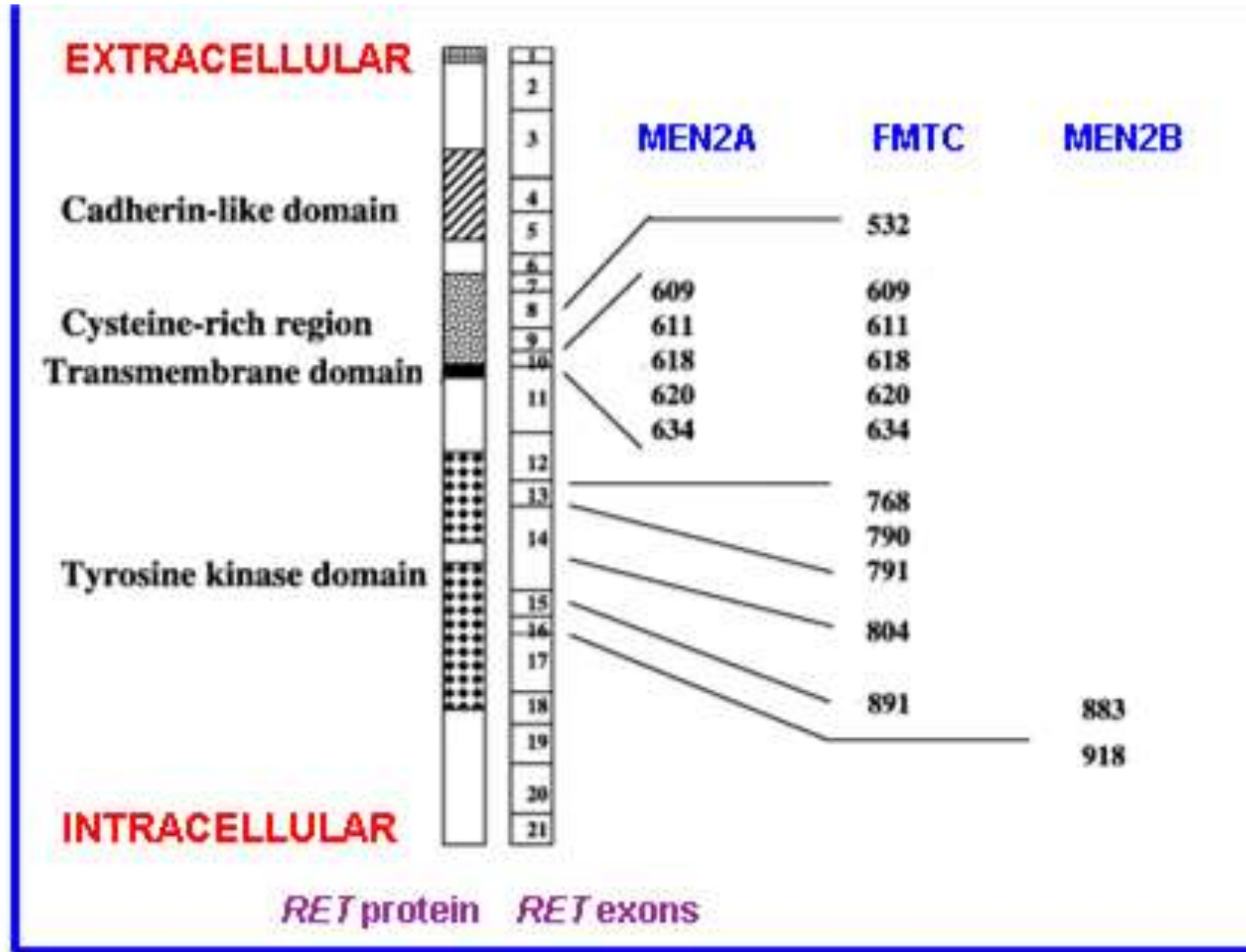


Patients with the rarer type B variant, **MEN2B**, have, in addition to the tumors seen in patients with MEN2A, thickening of nerves and the development of benign neural tumors, known as **neuromas**, on the mucosal surface of the mouth and lips and along the GI tract.

The variants responsible for MEN2 are in

Individuals who inherit an activating mutation have a 60% chance of developing a particular type of cancer (medullary)

More sensitive tests, such as blood tests for catecholamines synthesized by pheochromocytoma, are positive in above 90% of heterozygotes for MEN2



RET encodes a cell-surface protein that contains:

- **extracellular domain** that can bind signaling molecules
- **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

Normally, tyrosine kinase receptors must bind specific signaling molecules in order to undergo the conformational change that makes them enzymatically 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 state referred to as

constitutive activation

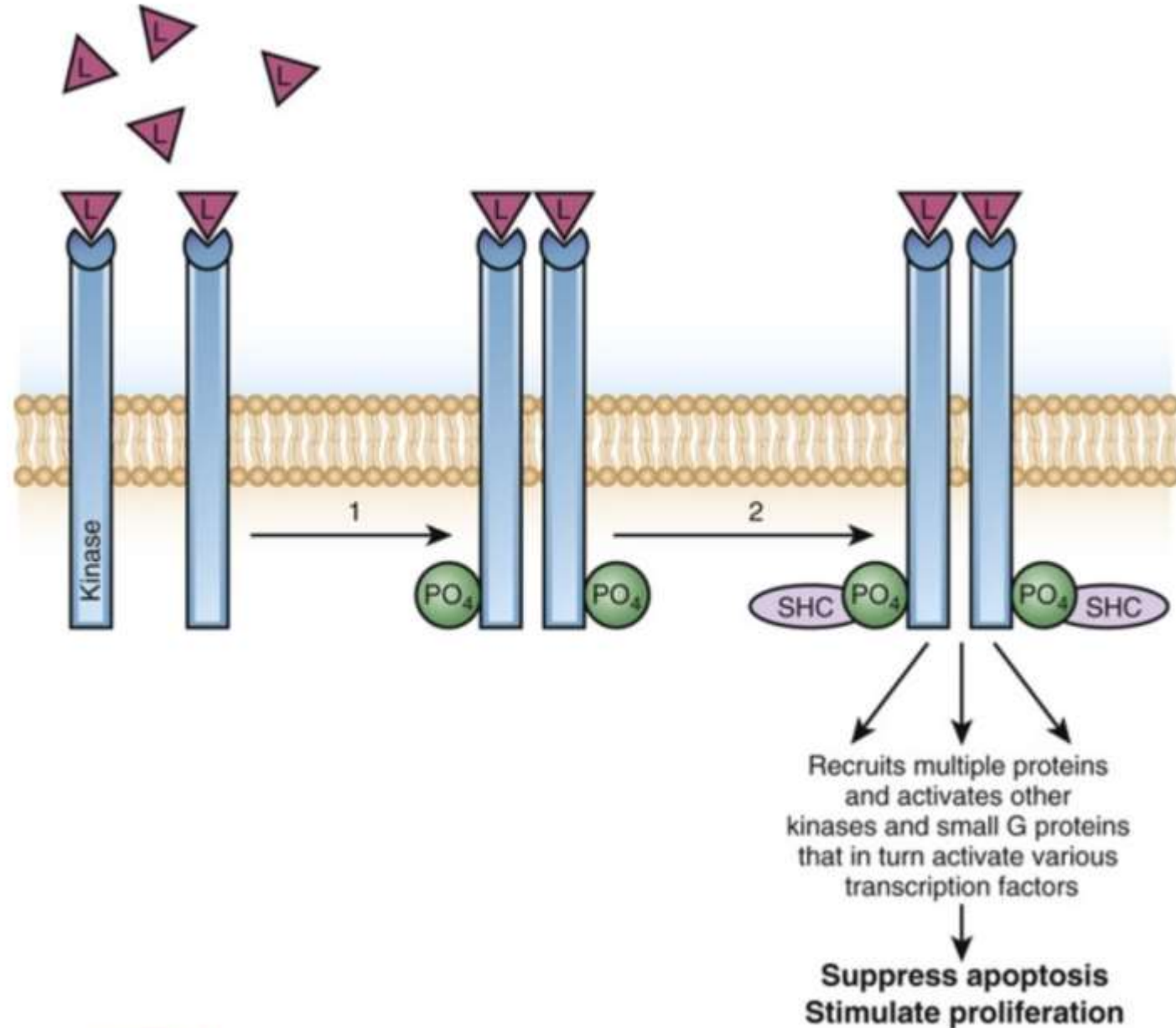
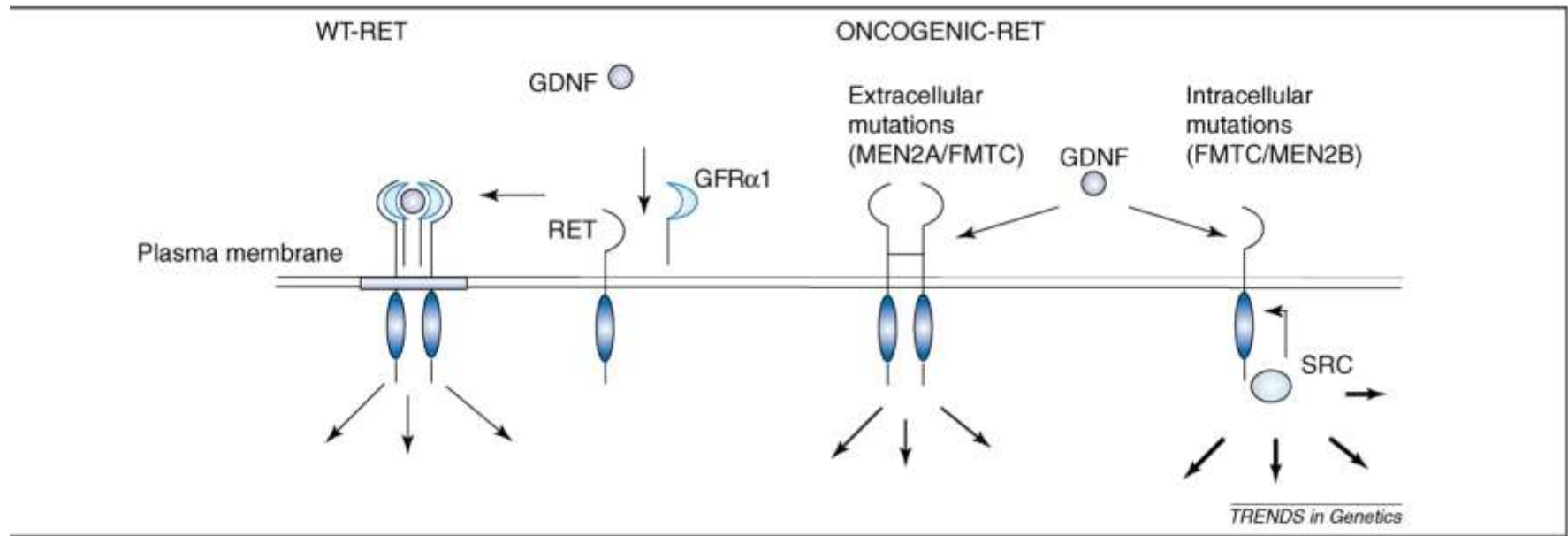


FIGURE 15-5 Schematic diagram of the function of the Ret receptor, the product of the *RET* proto-oncogene. Upon binding of a ligand (L), such as glial-derived growth factor or neurturin, to the extracellular domain, the protein

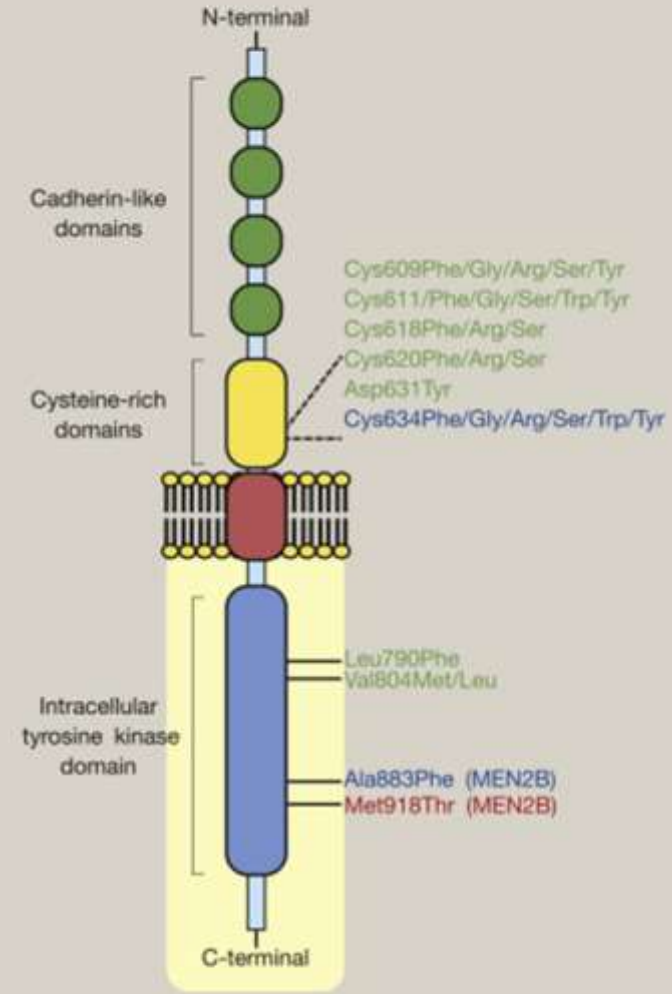
The RET gene is expressed in many tissues of the body and is required for normal embryonic development of autonomic ganglia and kidney.

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 GPIIb/IIIa co-receptor 1 (GFR α 1); RET is then recruited to form a macromolecular complex receptor. **(b)** Constitutive activation of RET by mutations affecting the cysteine-rich domain 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.

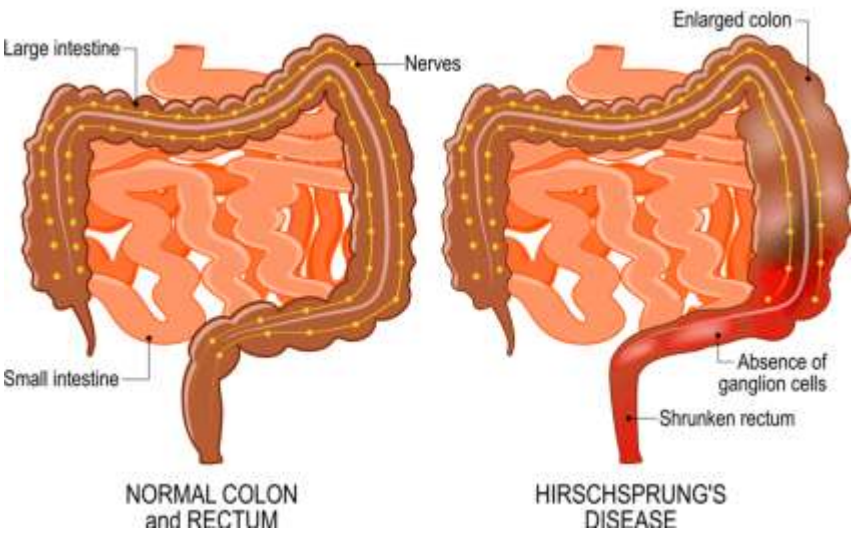
RET receptor structure and location of common MEN2-associated RET mutations



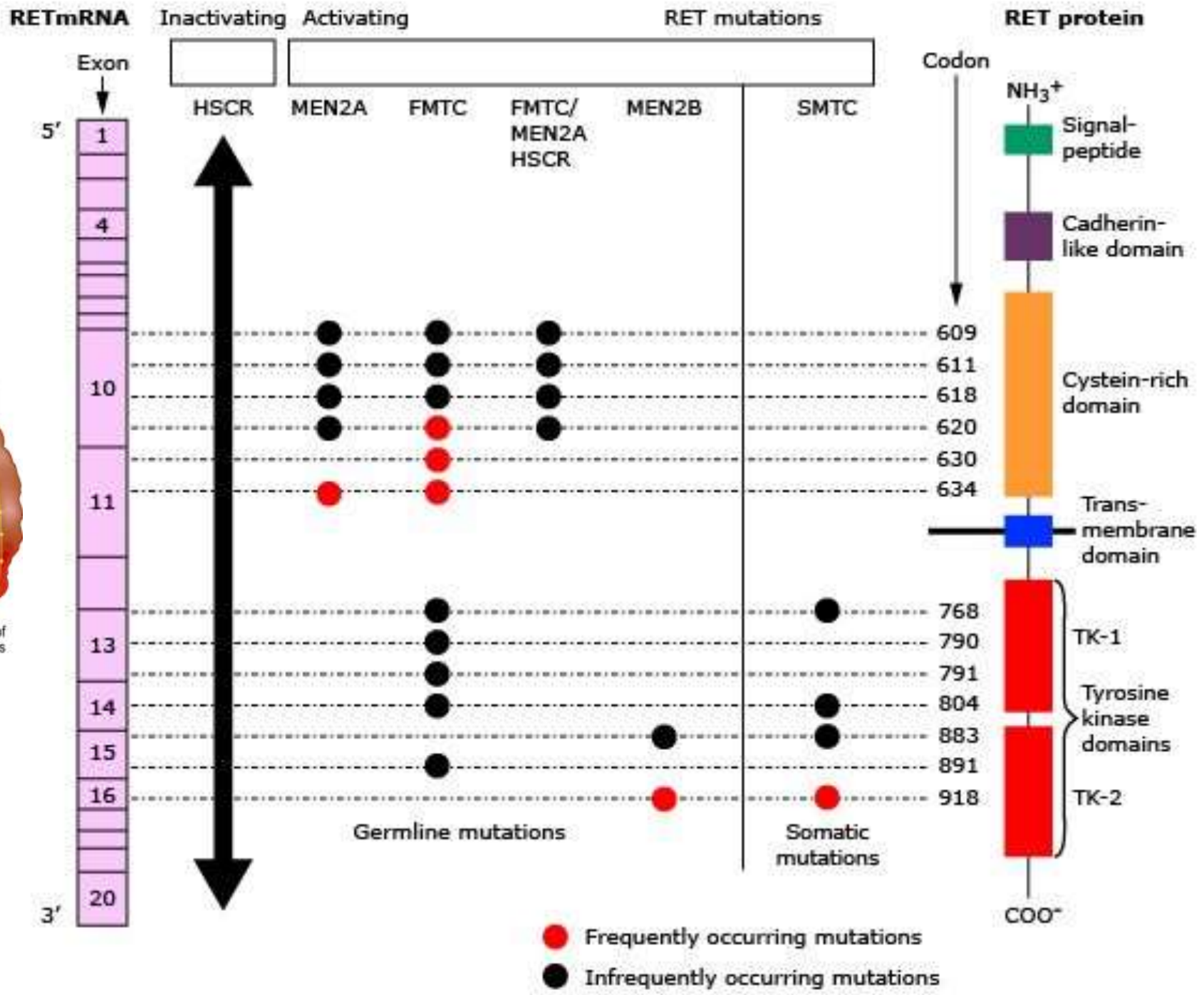
The RET receptor is a membrane-associated tyrosine kinase receptor expressed in cells of neural crest origin. MEN2-associated mutations arise most frequently in the cysteine-rich region of the extracellular domain, or in the intracellular domain associated with intrinsic tyrosine kinase activity, resulting in enhanced receptor signalling. *RET* mutations are described according to the respective missense substitution, with amino acids represented using standard nomenclature. The American Thyroid Association's 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

Interestingly, RET is the same gene implicated in Hirschsprung disease, although those mutations are usually loss-of-function, not activating, mutations.



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

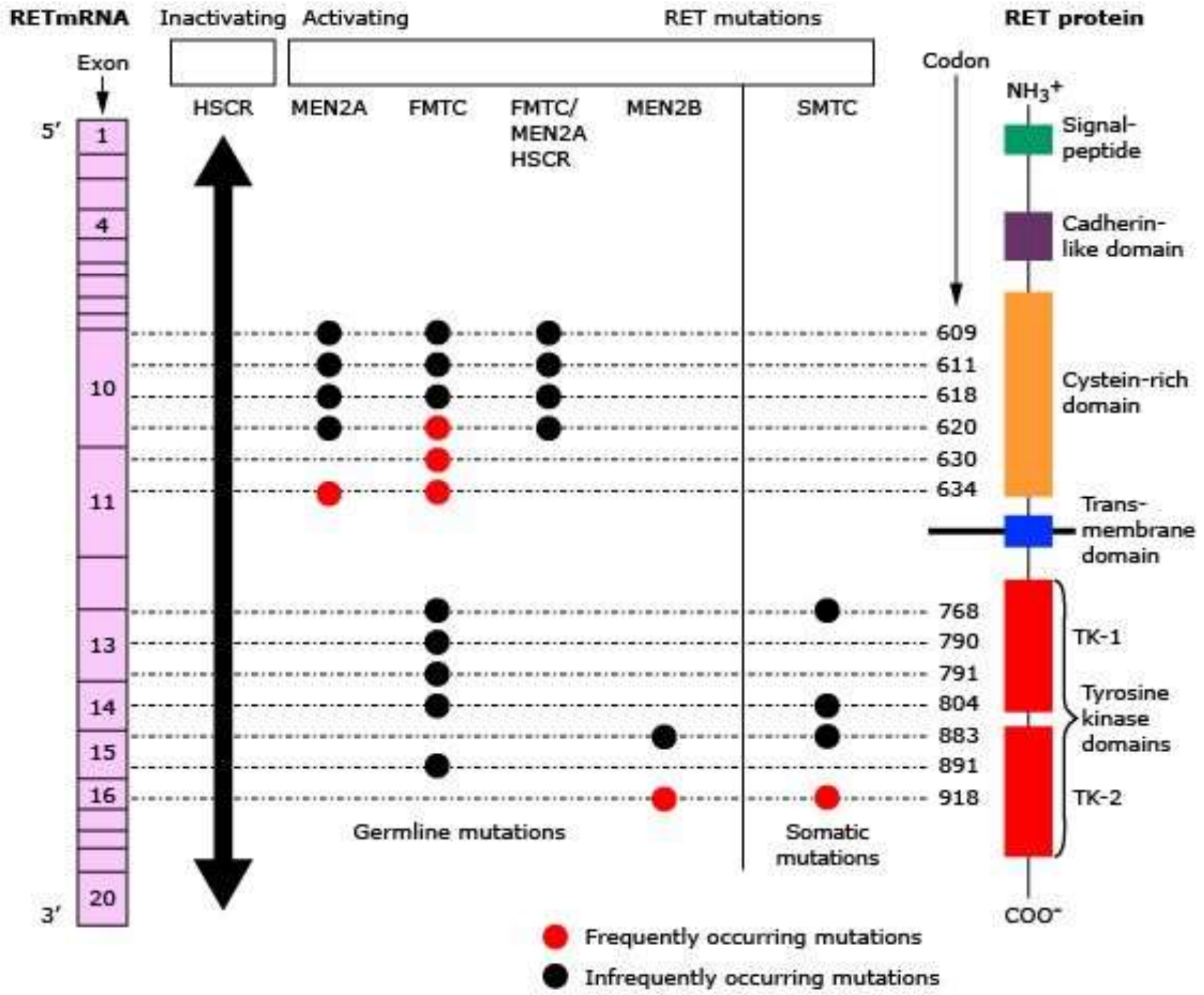


Gain of function vs loss of function

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

Selected Tumor Suppressor Genes Involved in Human Neoplasms

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.

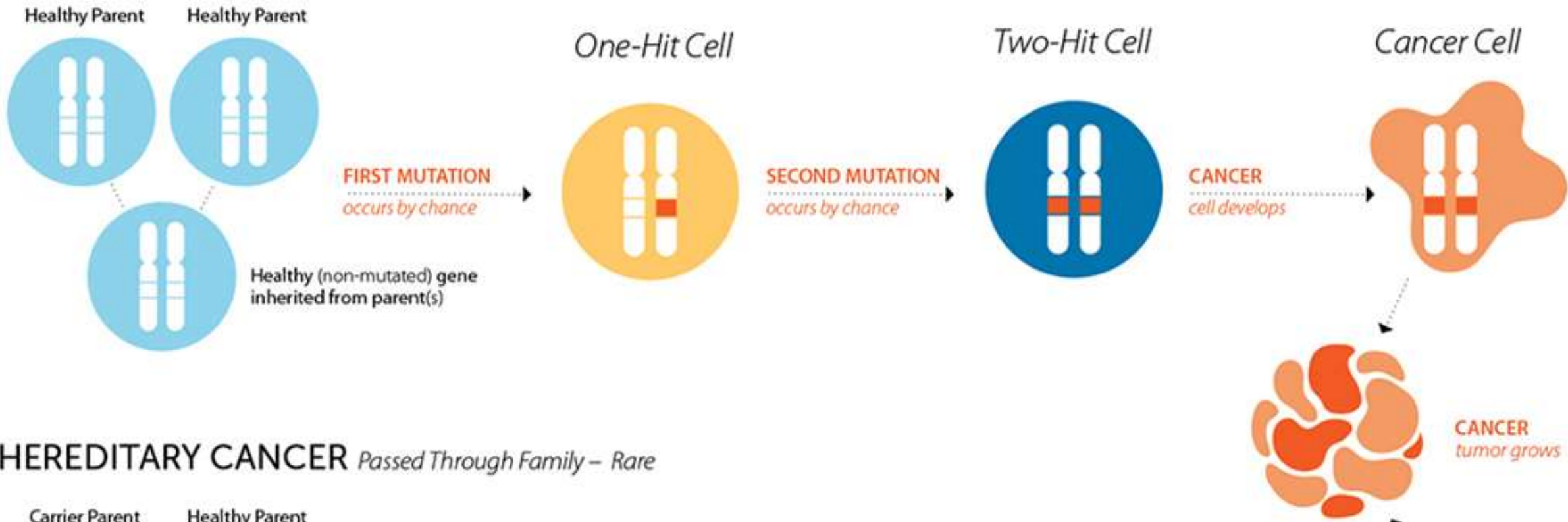
The products of many TSGs have now been isolated and characterized

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

Subcellular Locations	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Associated with Inherited Mutations
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	<i>NF1</i>	Inhibition of RAS signal transduction and of p21 cell cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	<i>NF2</i>	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromatosis type 2, acoustic schwannomas, and meningiomas
Cytosol	<i>APC</i> / β -catenin	Inhibition of signal transduction	Carcinomas of stomach, colon, pancreas; melanoma	Familial adenomatous polyposis coli/colon cancer
	<i>PTEN</i>	PI3 kinase signal transduction	Endometrial and prostate cancers	Cowden syndrome
	<i>SMAD2</i> and <i>SMAD4</i>	TGF- β signal transduction	Colon, pancreas tumors	Unknown
Nucleus	<i>RB1</i>	Regulation of cell cycle	Retinoblastoma; osteosarcoma carcinomas of breast, colon, lung	Retinoblastomas, osteosarcoma
	<i>p53</i>	Cell cycle arrest and apoptosis in response to DNA damage	Most human cancers	Li-Fraumeni syndrome; multiple carcinomas and sarcomas
	<i>WT1</i>	Nuclear transcription	Wilms' tumor	Wilms' tumor
	<i>P16/INK4a</i>	Regulation of cell cycle by inhibition of cyclindependent kinases	Pancreatic, breast, and esophageal cancers	Malignant melanoma
	<i>BRCA1</i> and <i>BRCA2</i>	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*



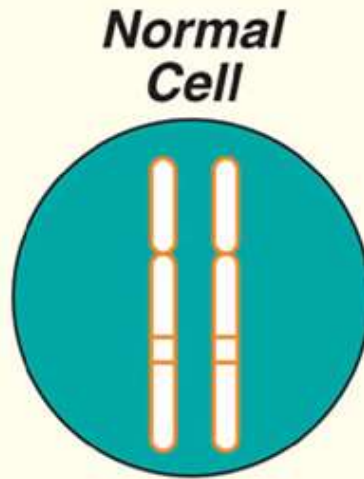
HEREDITARY CANCER *Passed Through Family – Rare*



Two-Hit Theory of Cancer Causation

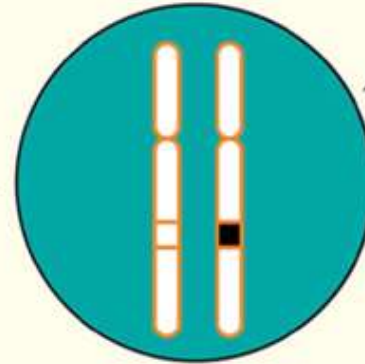
Normal cells have two undamaged chromosomes, one inherited from our mother and one from our father. These chromosomes contain thousands of genes.

Non-Hereditary



rare event

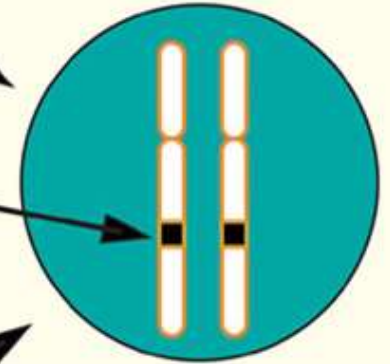
One-Hit Cell



mutant gene

rare event

Two-Hit Cell

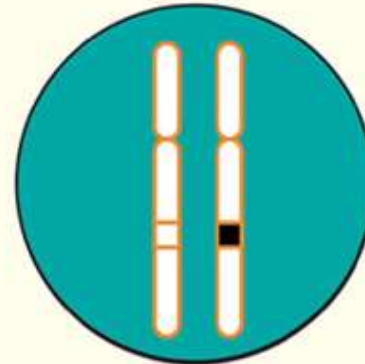
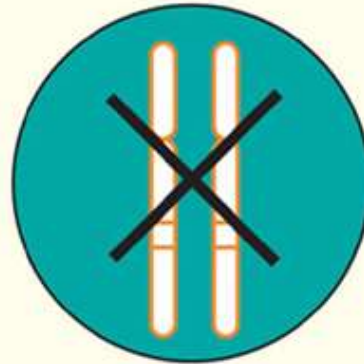


Retinoblastoma Gene*

rare event

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.

Hereditary



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 retinoblastoma, people who inherit the first hit are 100,000 times more likely to develop a second, cancer-causing mutation.

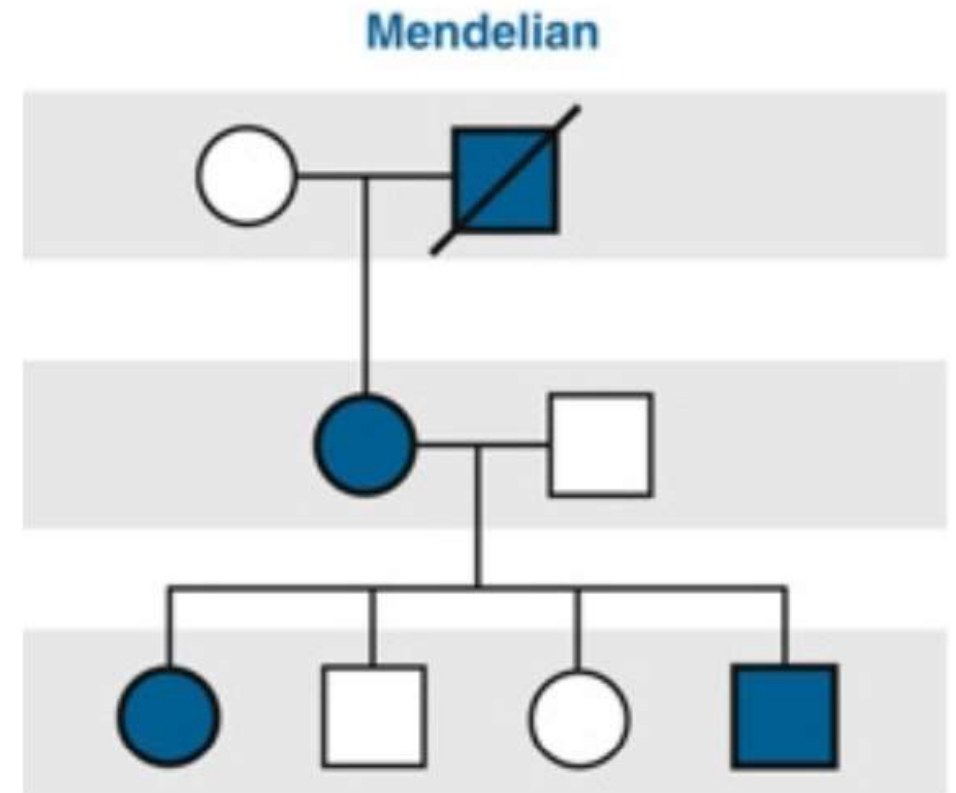
Retinoblastoma familial

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.



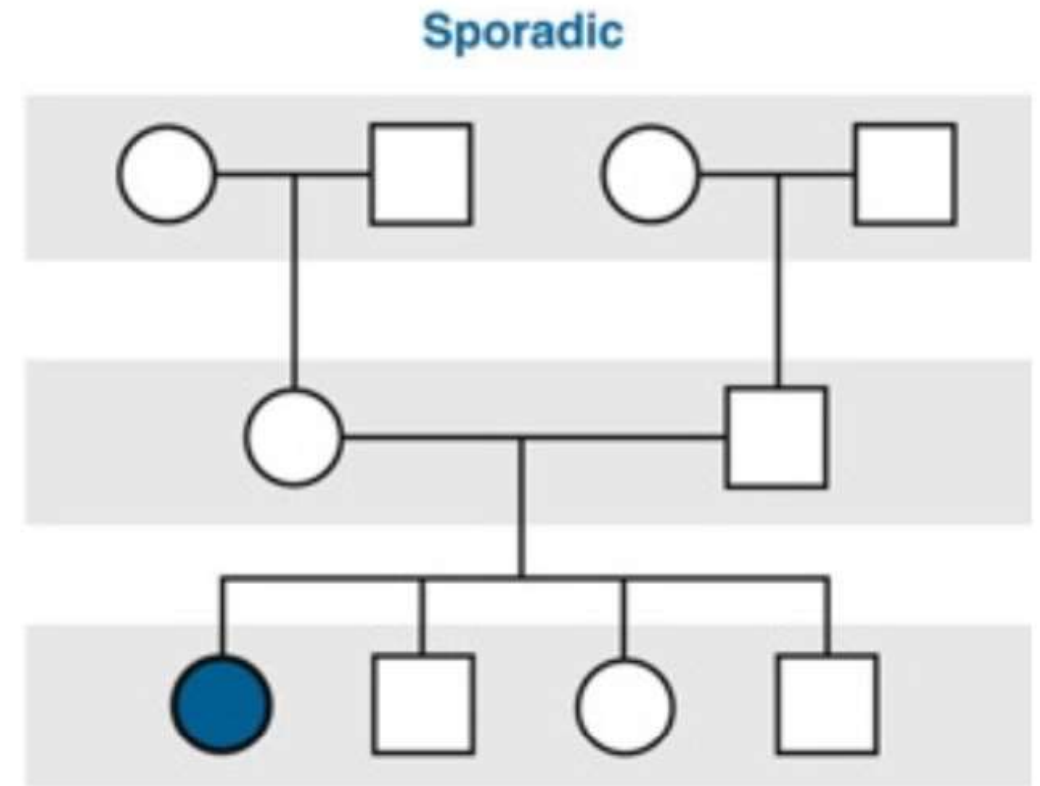
Germline mutation

Somatic mutation

Multiple tumors
Bilateral
Early onset

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.



Normal gene



Somatic mutation

Somatic mutation

Single tumors

Unilateral

Later onset

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

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

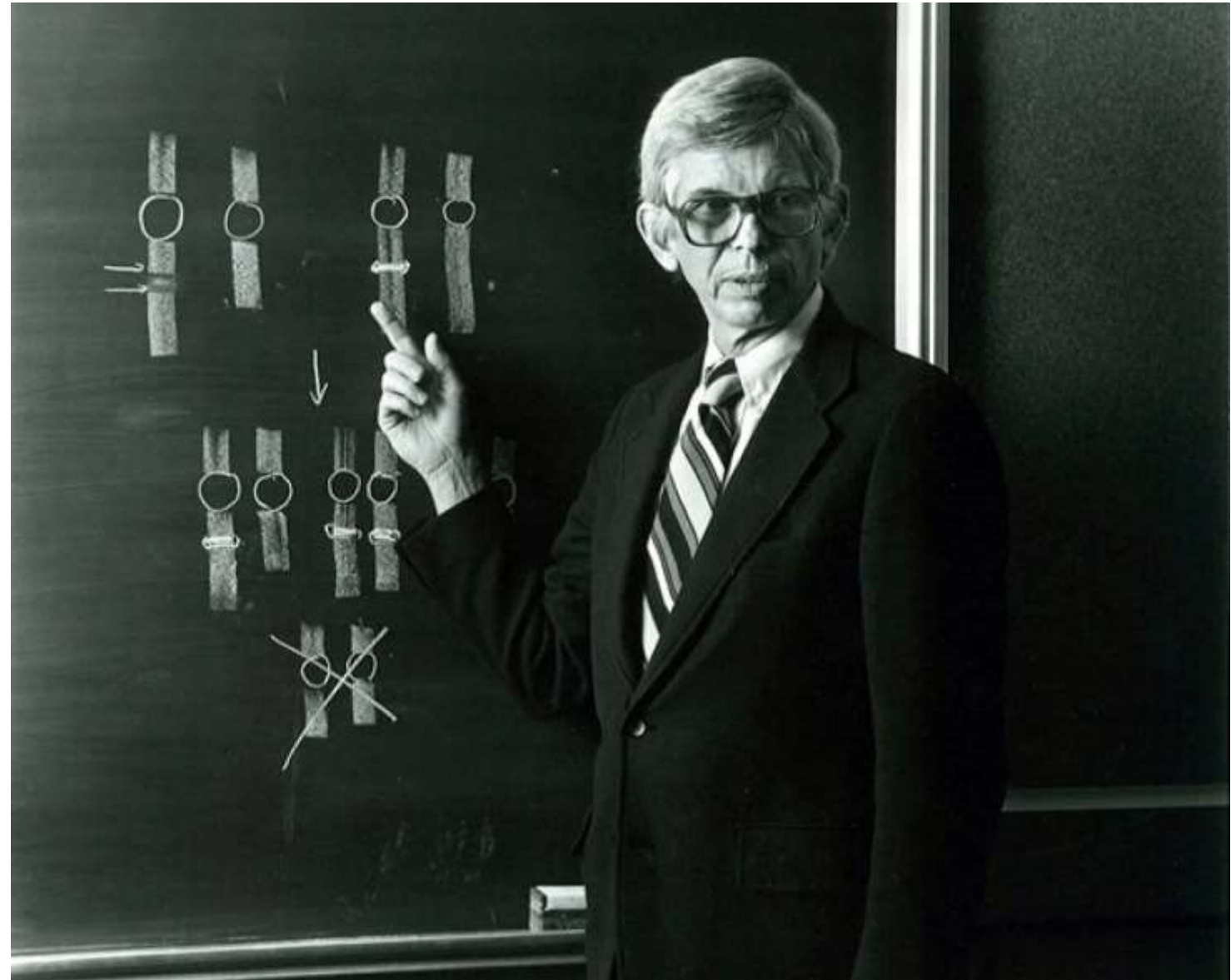
S

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=51

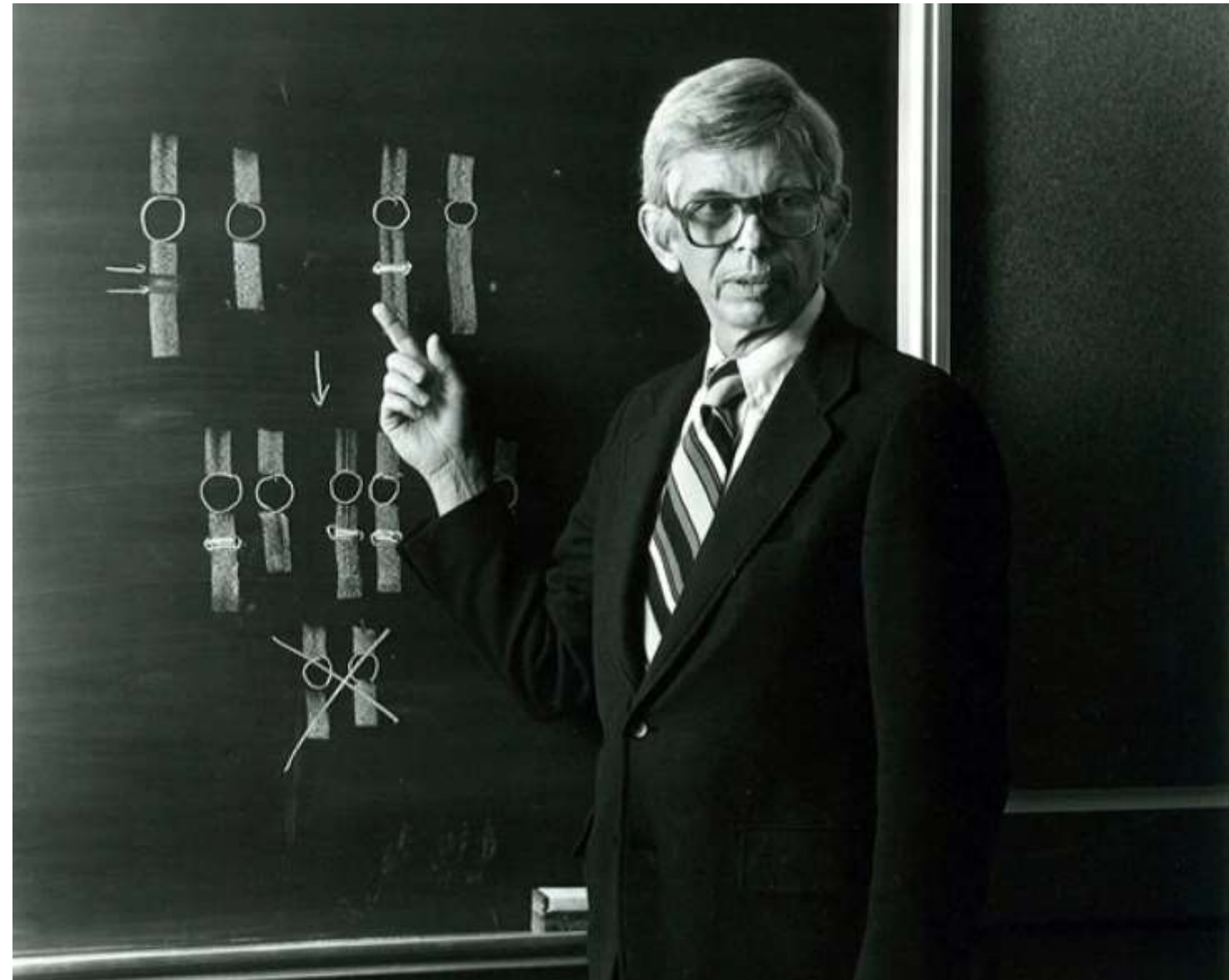
S

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

familial polyposis coli

familial breast cancer

neurofibromatosis type 1 (NF1)

Lynch syndrome

Li-Fraumeni



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

Tumor Suppressor Genes in Autosomal Dominant Cancer Syndromes

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

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

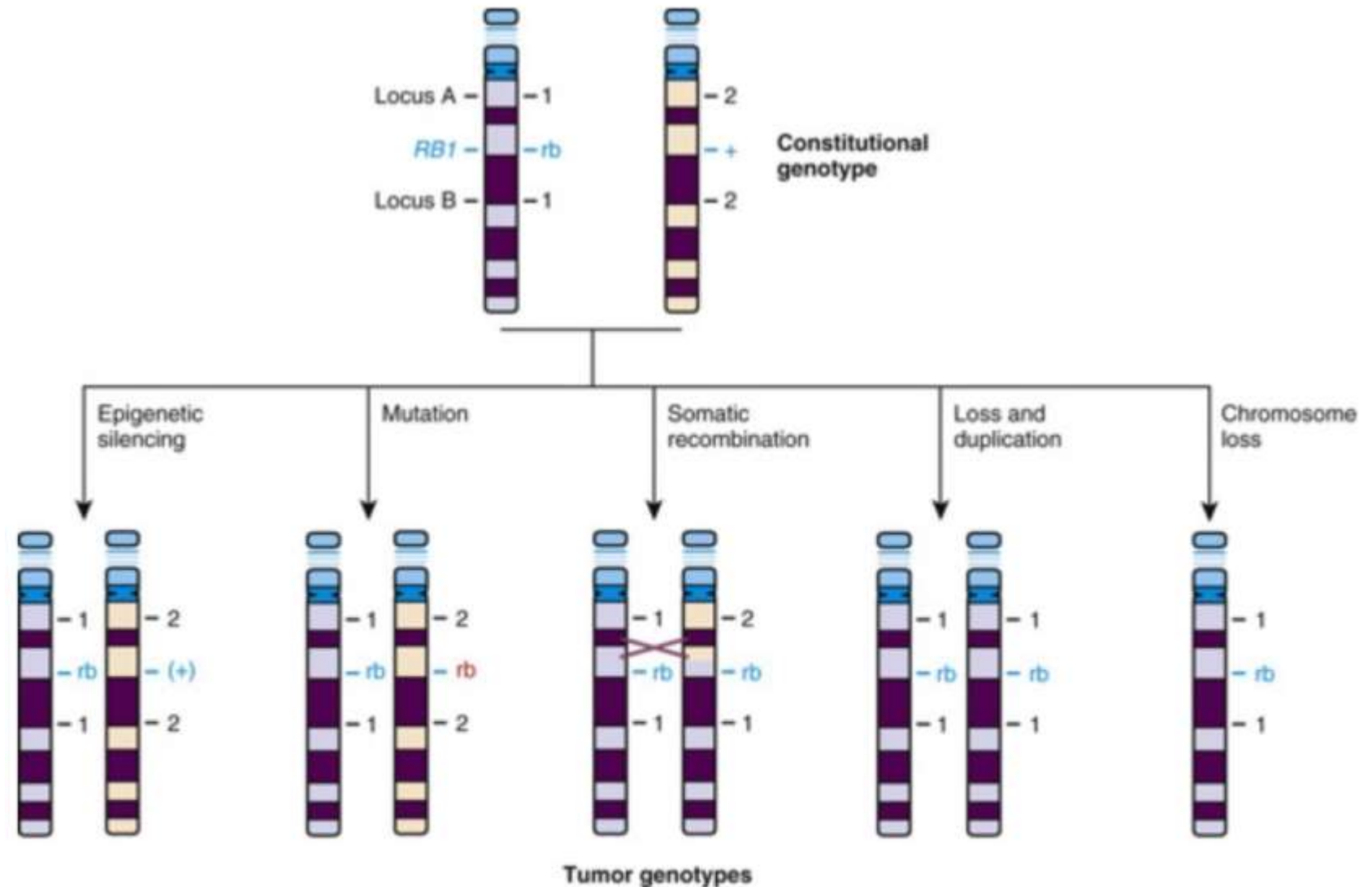


FIGURE 15-8 Chromosomal mechanisms that could lead to loss of heterozygosity for DNA markers at or near a tumor suppressor gene in an individual heterozygous for an inherited germline mutation. The figure depicts the events that constitute the “second hit” that leads to retinoblastoma with loss of heterozygosity (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 of the more than 10^6 retinoblasts already carrying an inherited RB1 mutation.

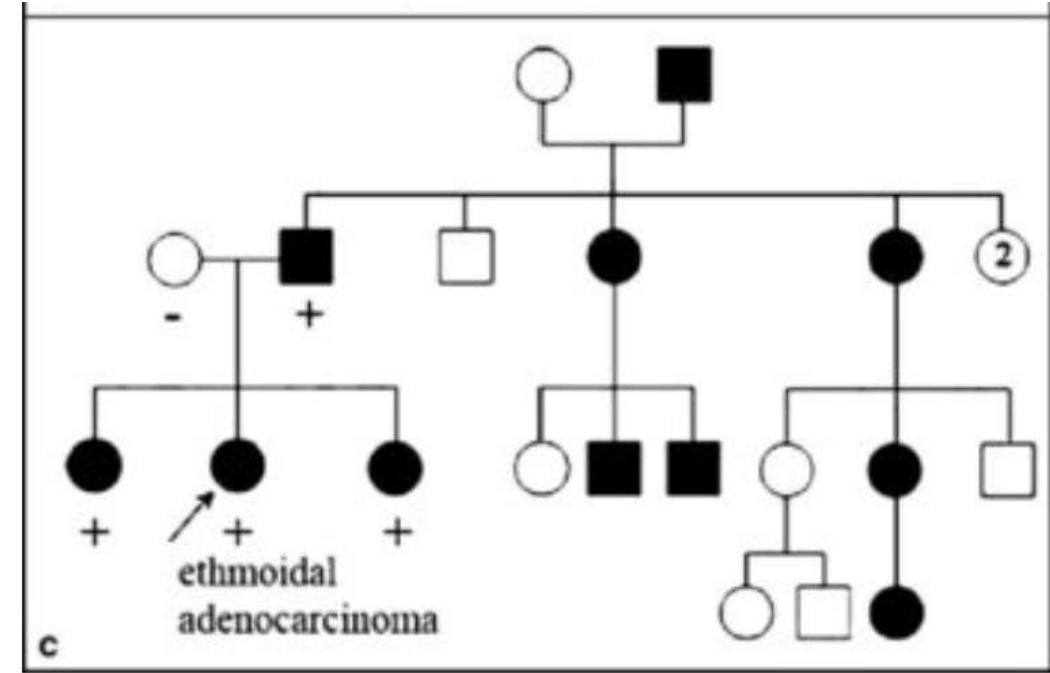


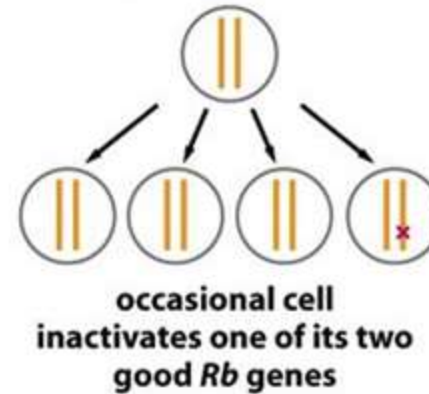
Fig.3 white color in the center circle of the eye

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).

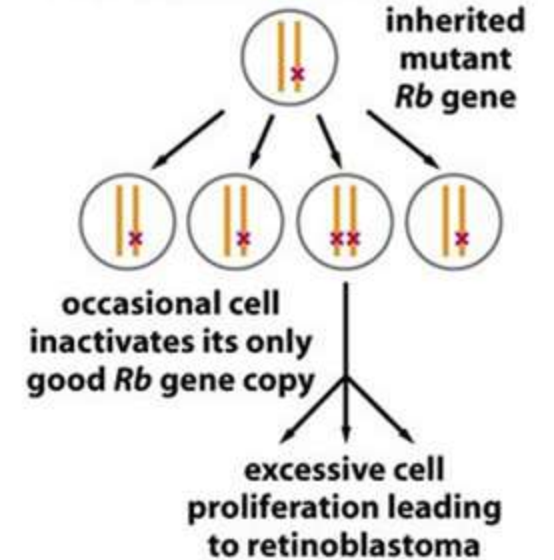
The occurrence of a second hit is a matter of chance and does not occur 100% of the time; the penetrance of retinoblastoma therefore, although greater than 90%, is not complete.

NORMAL, HEALTHY INDIVIDUAL



RESULT: NO TUMOR

HEREDITARY RETINOBLASTOMA



RESULT: MOST PEOPLE WITH
INHERITED MUTATION DEVELOP
MULTIPLE TUMORS IN BOTH EYES

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

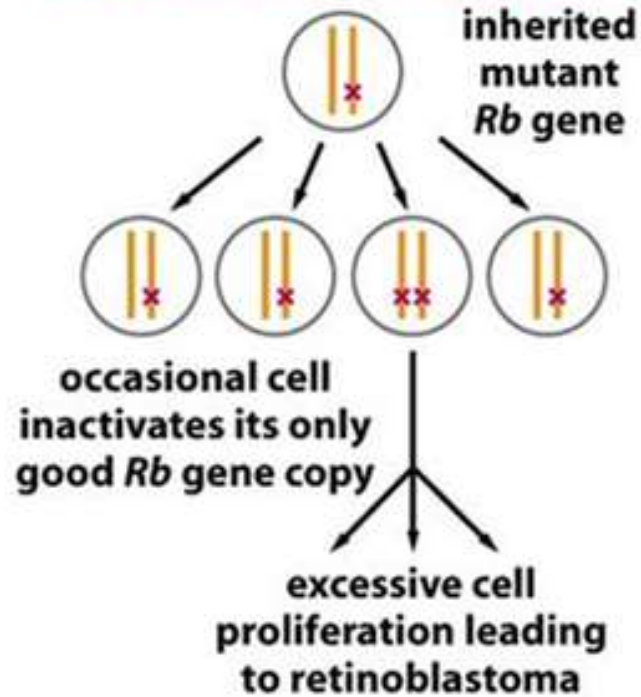
The other 60% of cases of retinoblastoma are nonhereditary

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.

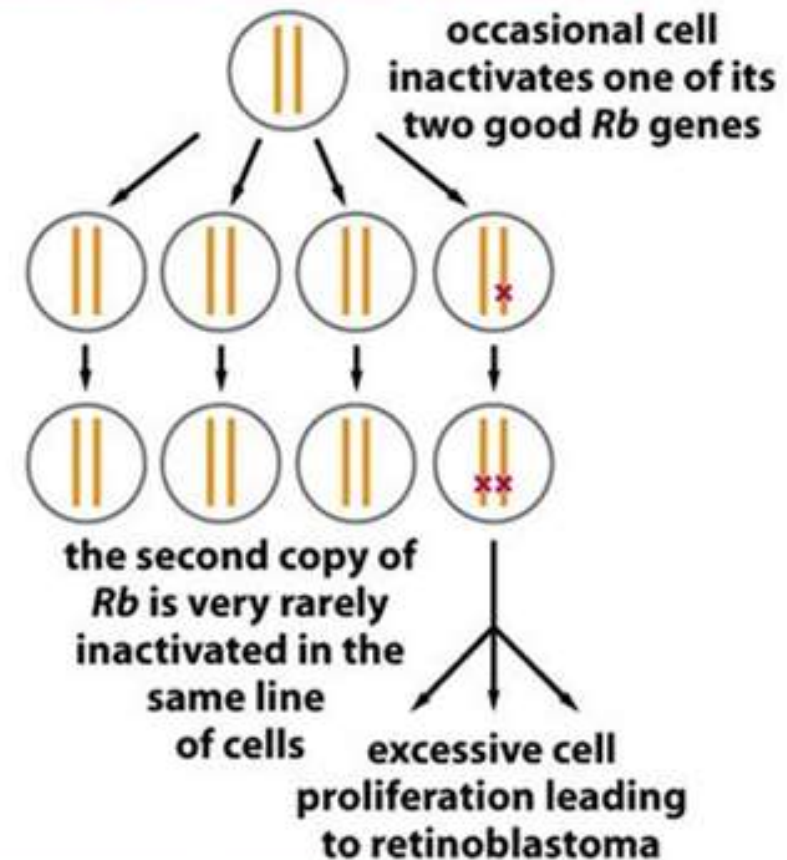
Unilateral tumor is no guarantee that the child does not have the heritable form of retinoblastoma, however, because 15% of patients with the heritable type develop a tumor in only one eye.

HEREDITARY RETINOBLASTOMA



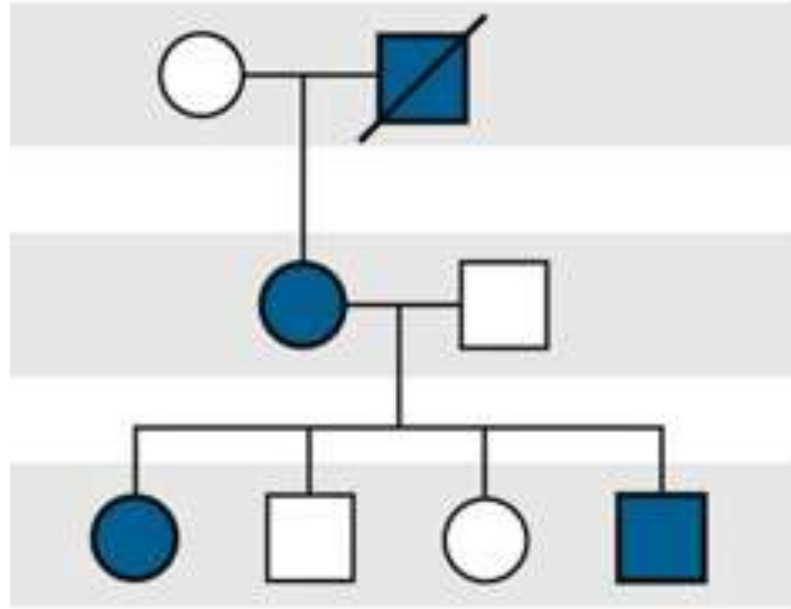
RESULT: MOST PEOPLE WITH INHERITED MUTATION DEVELOP MULTIPLE TUMORS IN BOTH EYES

NONHEREDITARY RETINOBLASTOMA



RESULT: ONLY ABOUT 1 IN 30,000 NORMAL PEOPLE DEVELOP ONE TUMOR IN ONE EYE

Mendelian



Germline mutation

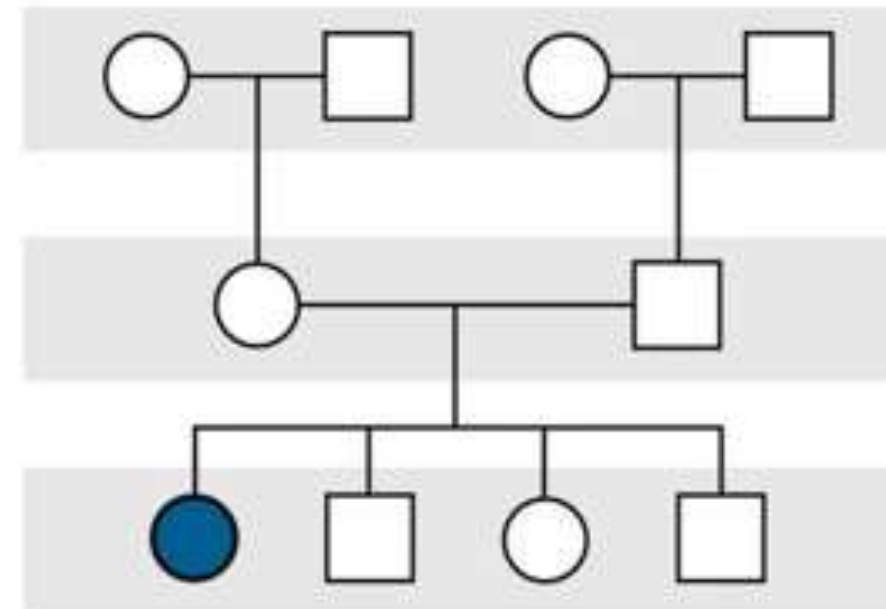


Somatic mutation



Multiple tumors
Bilateral
Early onset

Sporadic



Normal gene



Somatic mutation

Somatic mutation



Single tumors
Unilateral
Later onset

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.

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

Oncogenes

- Activation of the gene product **increases** cancer risk
- The mutated form of a **proto-oncogene**
- A **“gain-of-function”** mutation can over-activate a proto-oncogene, turning it into an oncogene

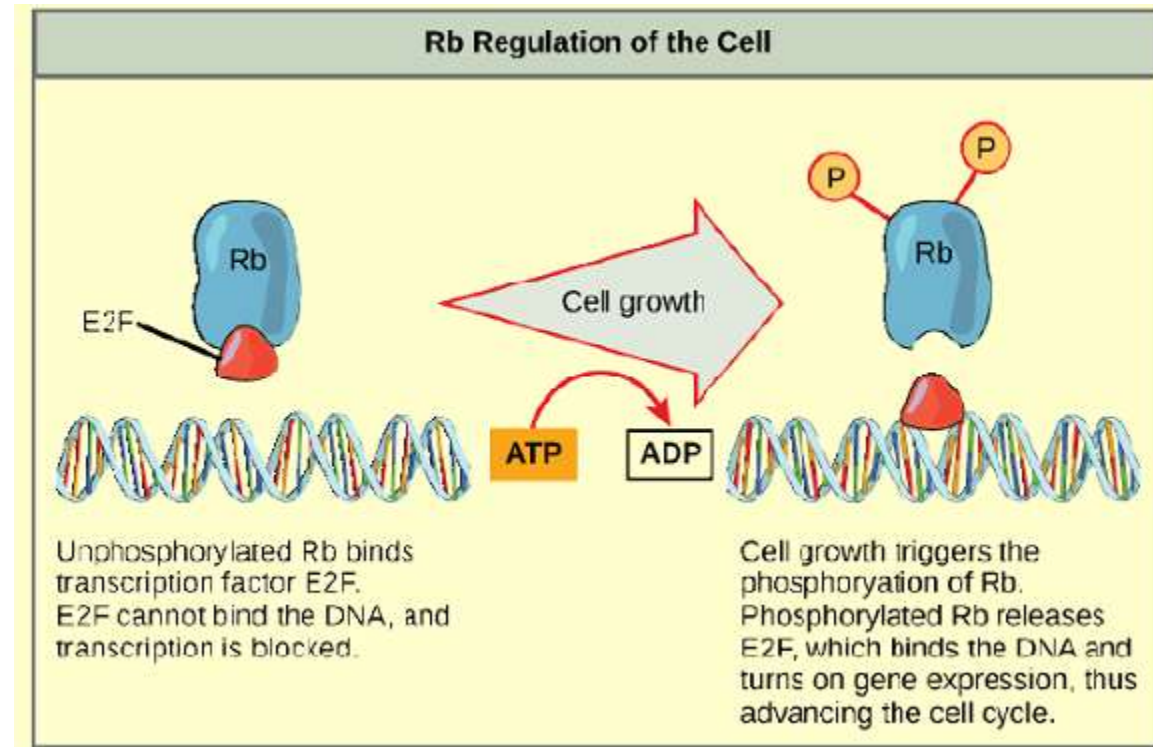
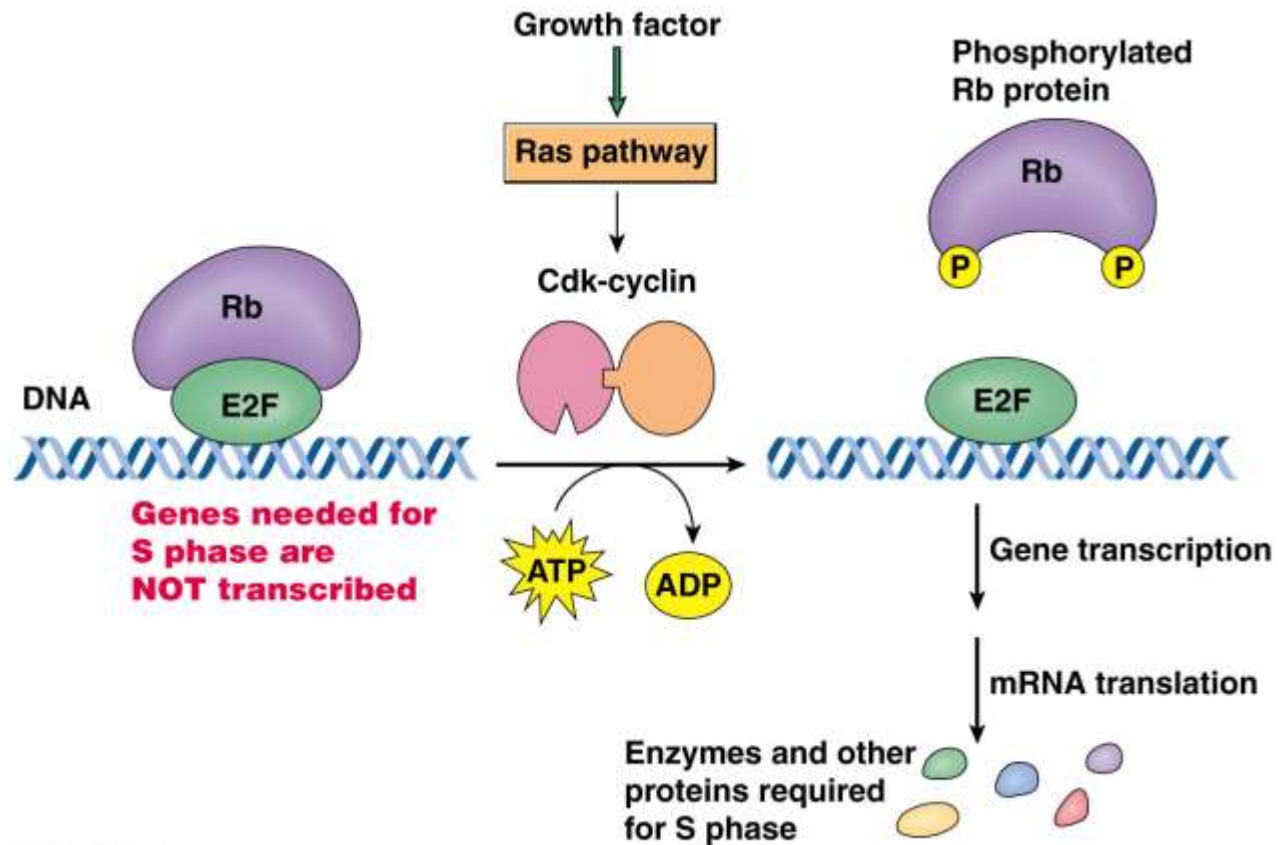
Tumor suppressor genes (TSG)

- Activation of the gene product **decreases** cancer risk
- A **“loss-of-function”** mutation can lead to loss of activity, allowing for cancer to occur

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.

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.

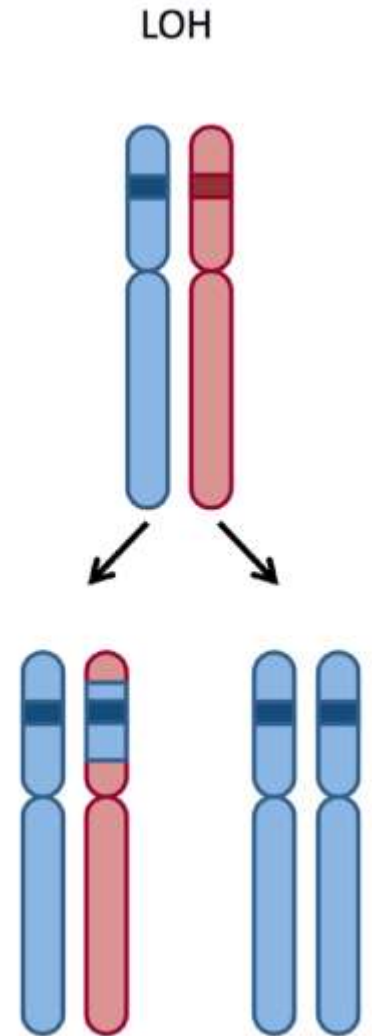


Loss of Heterozygosity

In addition to mutations and epigenetic silencing

a novel genomic mechanism was uncovered when geneticists made an unusual but highly significant discovery when they **compared DNA polymorphisms at the RB1 locus in DNA from normal cells to those in the retinoblastoma tumor from the same patient.**

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

Interstitial
CN-LOH



❑ 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.

Terminal
CN-LOH

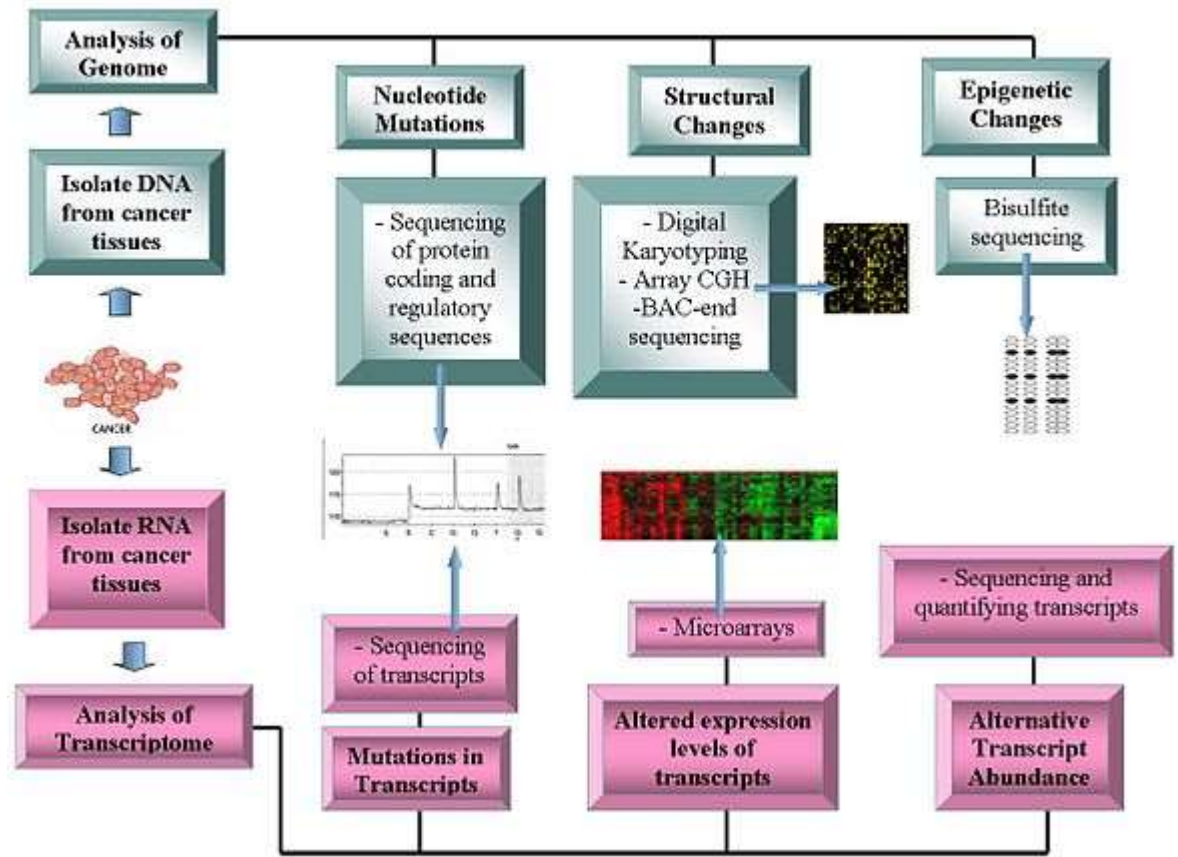


❑ CN-LOH mutation mechanisms are universal to diploid genomes, and play a key role in humans, both in cancer tumor suppressor loss and somatic mosaicism.

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.



Family history

