Cancer in Families

- Cancer can be ***hereditary*** or ***somatic***. If it is hereditary, the mutation is present in all body tissues, including the tumor tissue, because it is inherited through the germline. In contrast, somatic mutations are confined to the tumor tissue and are absent from most other body tissues.

- 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/ A panel of genes refers to a group of genes that are sequenced because deleterious mutations in these genes are known to increase the risk of cancer.

- 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.

✓ A strong family history with more than one affected individual suggests a hereditary component to cancer. In some patients, the causative gene is known; however, this is not the case for all patients. In many cases, cancer is thought to be multifactorial, involving both environmental factors and genetic predisposition. Multiple genes with low penetrance and small individual effects may collectively contribute to cellular transformation and cancer development. *However, we will talk about genes with high penetrance, since they increase the risk of cancer formation.*

- 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

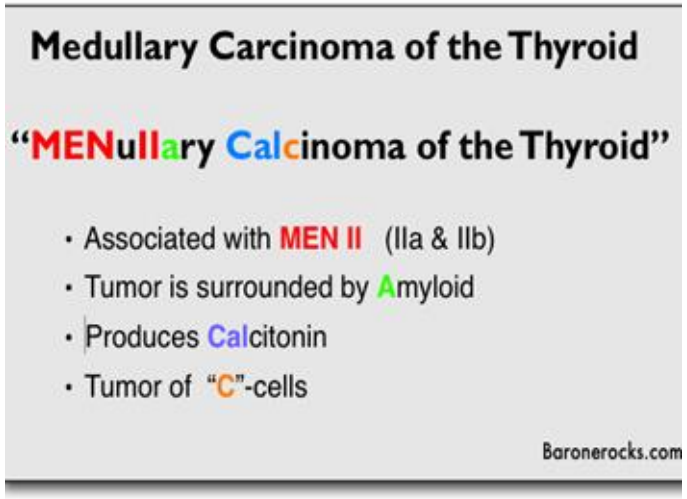
Multiple Endocrine Adenomatosis, Type 2

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

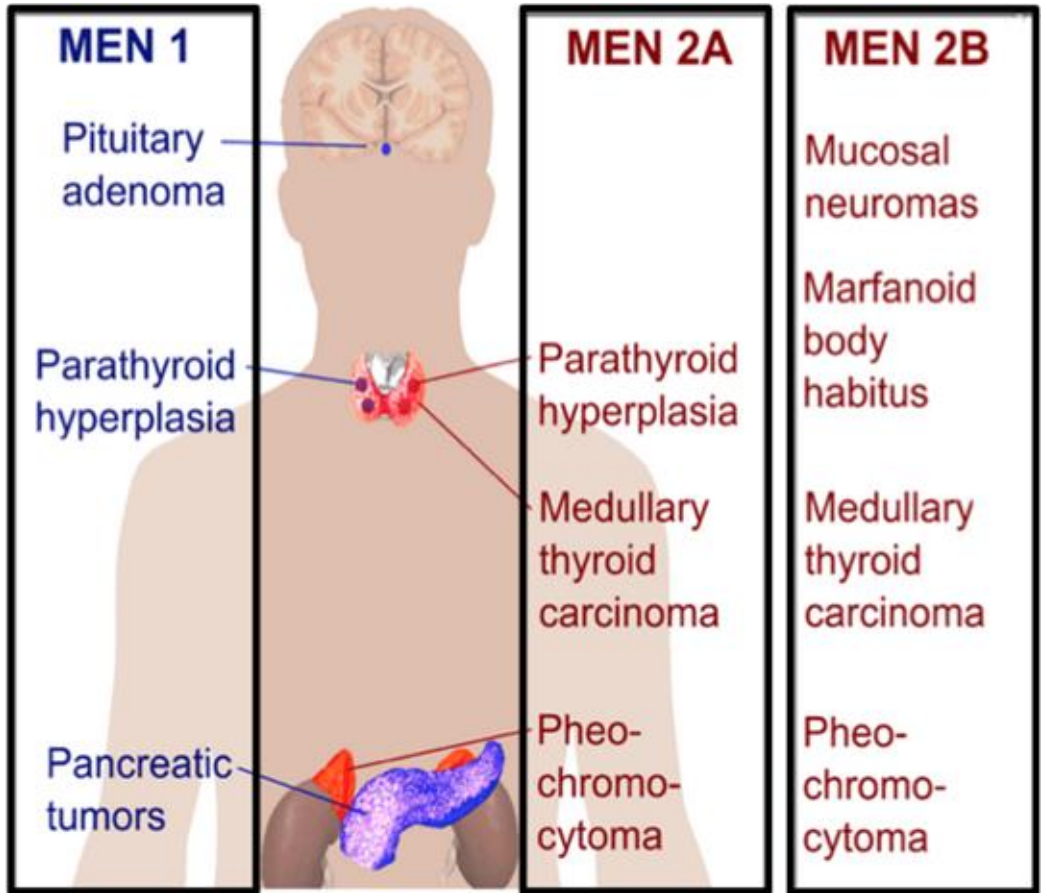
- **MEN2-A** is an AD disorder characterized by:

✓ high incidence of medullary carcinoma of the thyroid that is often but not always associated with pheochromocytoma
benign parathyroid adenomas, or both.

- <u>Pheochromocytoma:</u> is a rare, usually noncancerous (benign) tumor that develops in an adrenal gland.



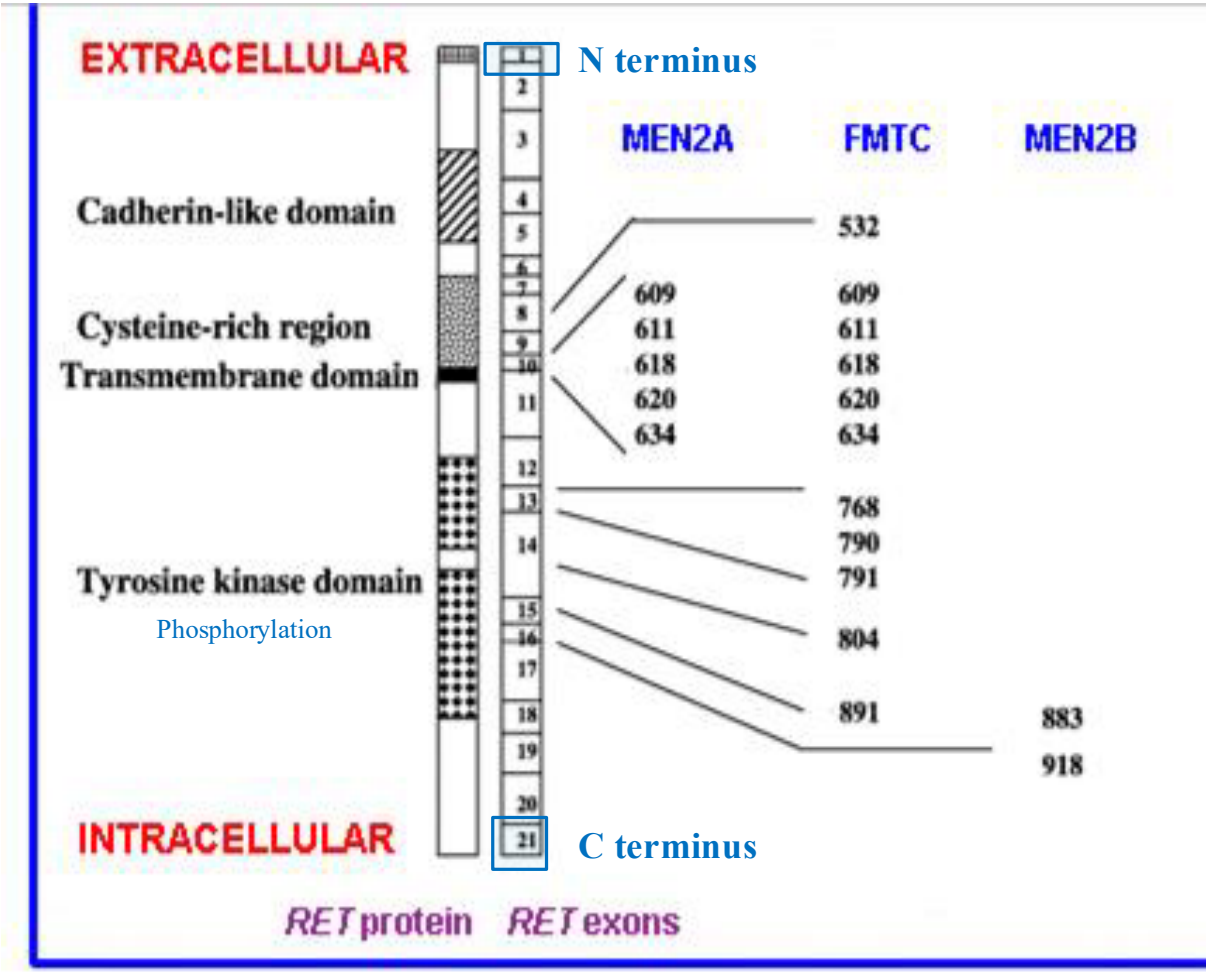
- An adenoma is a benign tumor of epithelial tissue with glandular origin, glandular characteristics, or both. Adenomas can grow from many glandular organs, including the adrenal glands, pituitary gland, thyroid, prostate, and others.
- The suffix ***-osis*** means to be affected with something or can refer to an increase.



- 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 the *RET* gene
- Individuals who inherit an activating mutation in *RET* have a greater than 60% chance of developing a particular type of thyroid carcinoma (medullary)
- More sensitive tests, such as blood tests for thyrocalcitonin or urinary catecholamines synthesized by pheochromocytomas, are abnormal in well above 90% of heterozygotes for MEN2

- RET protein contains different domains; regions of the protein that have different roles, which collectively support the function of the protein.



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 of 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 (hyperactive) even in the absence of its ligand (a state referred to as **constitutive activation** (It remains constitutively active even in the absence of the ligand)

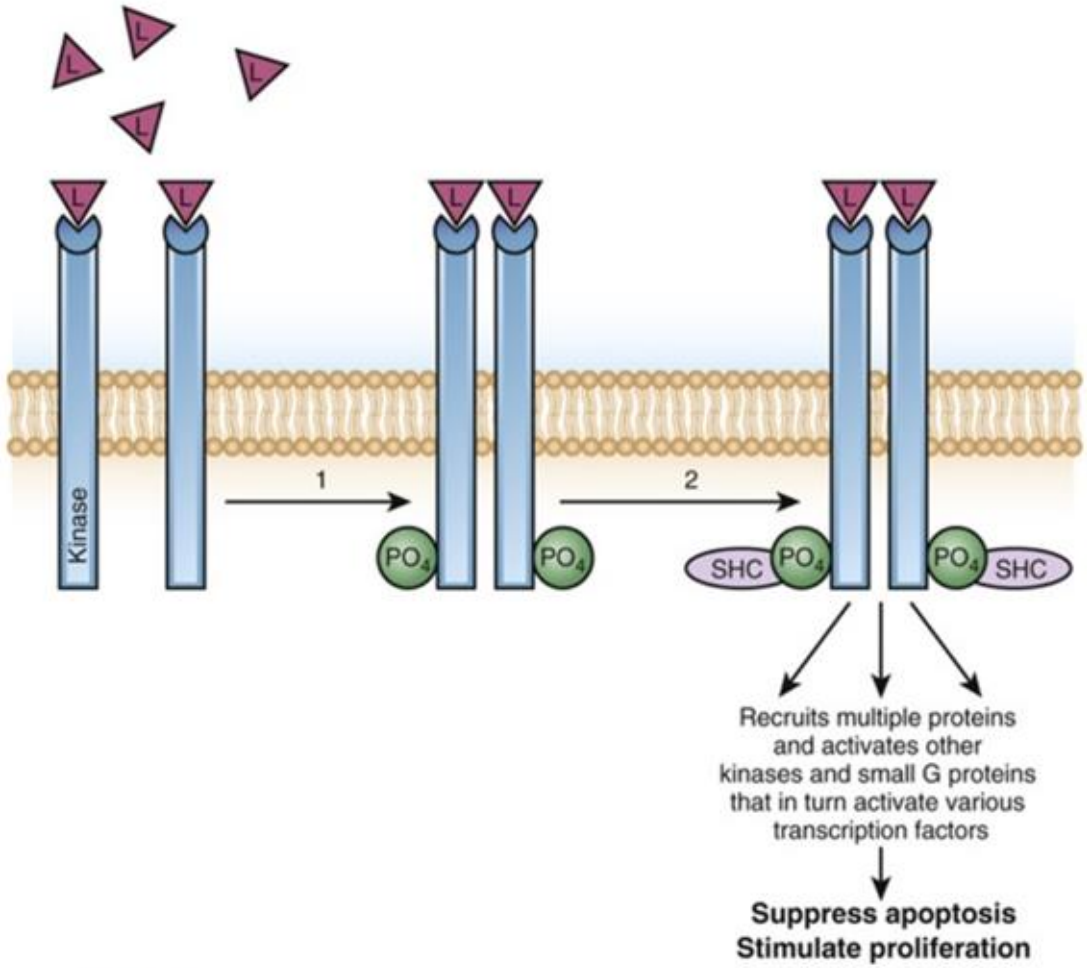


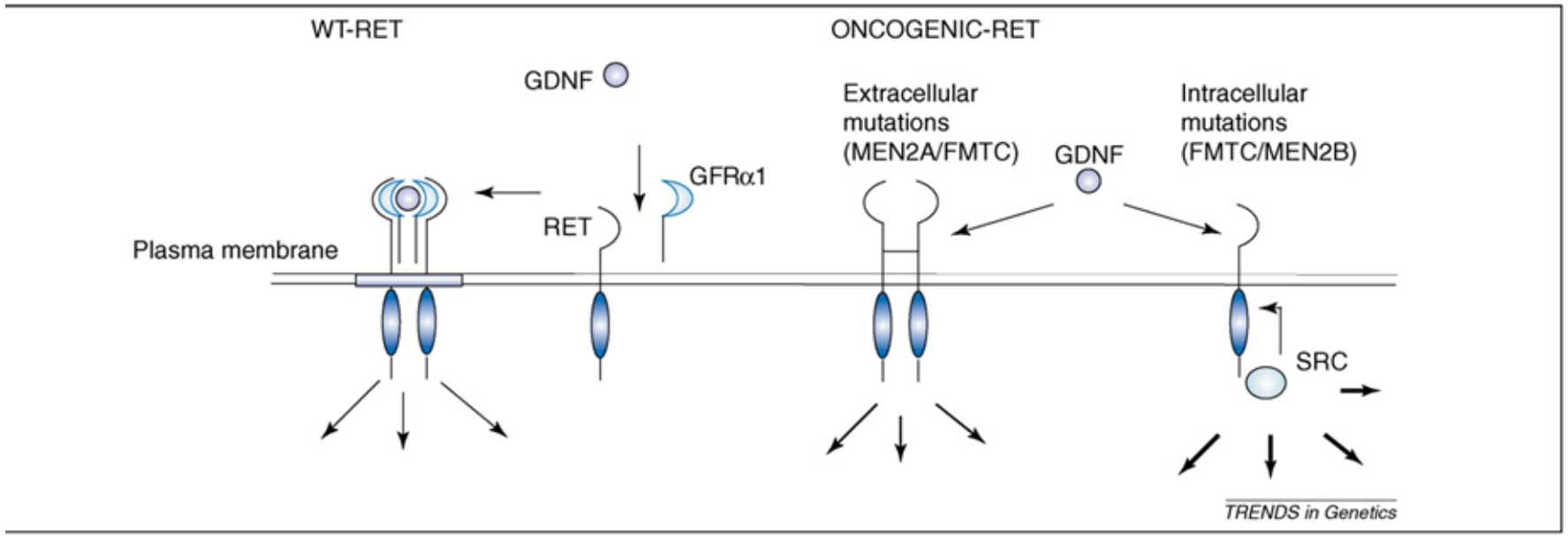
FIGURE 15-5 Schematic diagram of the function of the Ret receptor, the product of the *RET* proto-oncogene. Upon binding of a ligand (L), such as glial-derived growth factor or neurturin, to the extracellular domain, the protein

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 dimerizes and activates its intracellular kinase domain to autophosphorylate specific tyrosine residues. These then bind the SHC adaptor protein, which sets off multiple cascades of complex protein interactions involving other serine-threonine and phosphatidylinositol kinases and small G proteins, which in turn activate other proteins, ultimately activating certain transcription factors that suppress apoptosis and stimulate cellular proliferation. Mutations in RET that result in type A variant of multiple endocrine adenomatosis, type 2 (MEN2A) cause inappropriate dimerization and activation of its own intrinsic kinase without ligand binding.

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

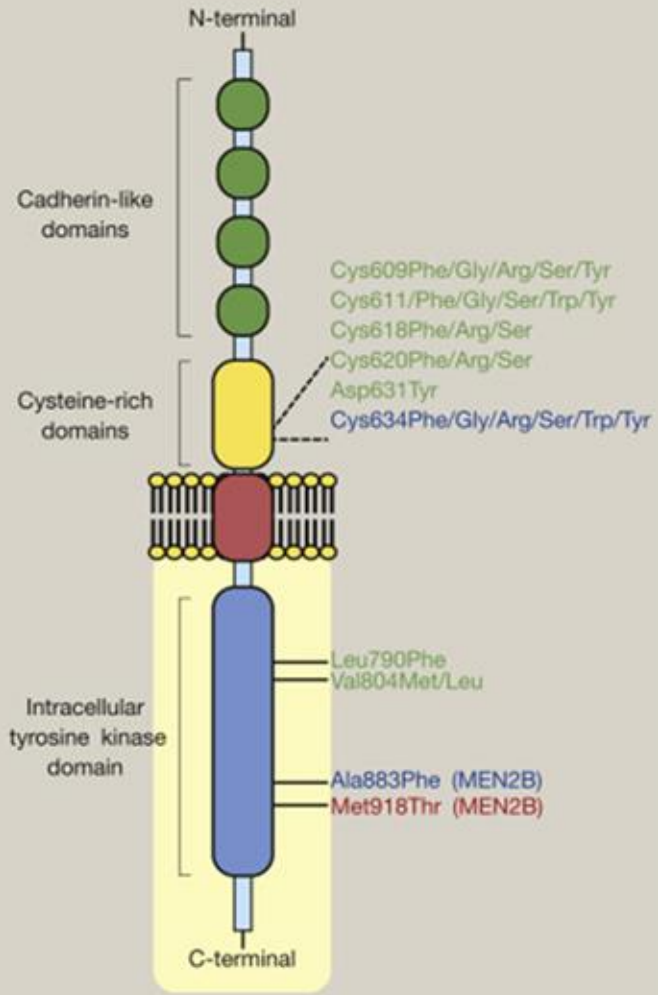
- ***A question may arise:*** if the RET gene is expressed in many tissues, why do RET mutations cause cancer only in certain tissues and not in all tissues where RET is expressed?

✓ It is <u>unclear</u> 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.

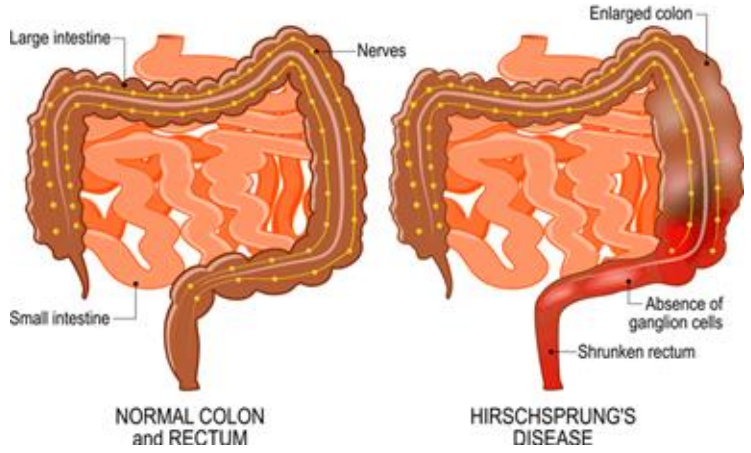
RET receptor structure and location of common MEN2-associated *RET* mutations



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 nomencalture. The American Thyroid Association[5] 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.

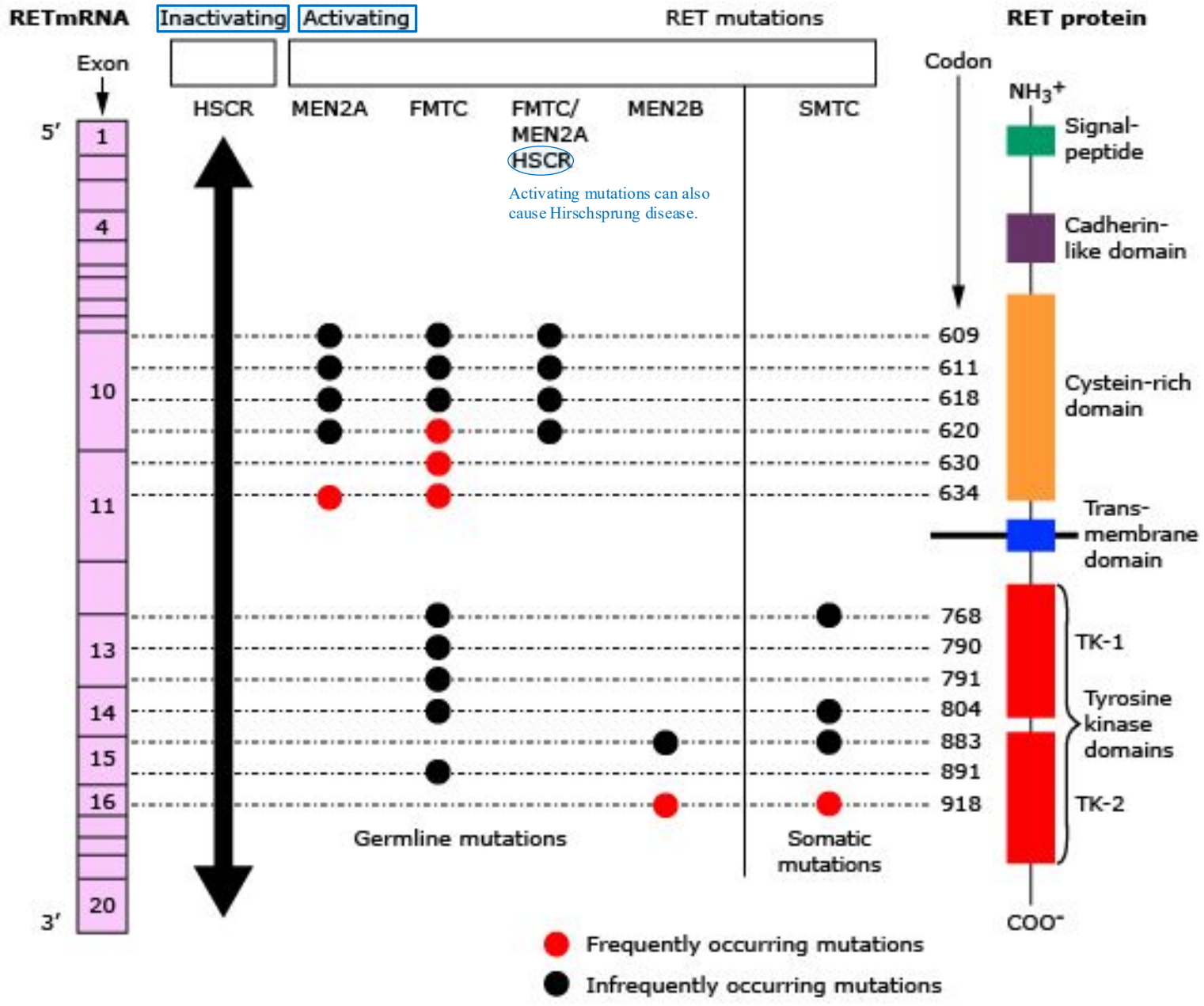


HSCR: Hirschsprung disease (loss of function mutation)

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

(Gain of function mutations)

- What is interesting about RET is that it is not only implicated in MEN2A. In particular, gain-of-function mutations in exons 10 and 11 are commonly identified in patients with MEN2A, while MEN2B is commonly associated with mutations in exons 15 and 16.



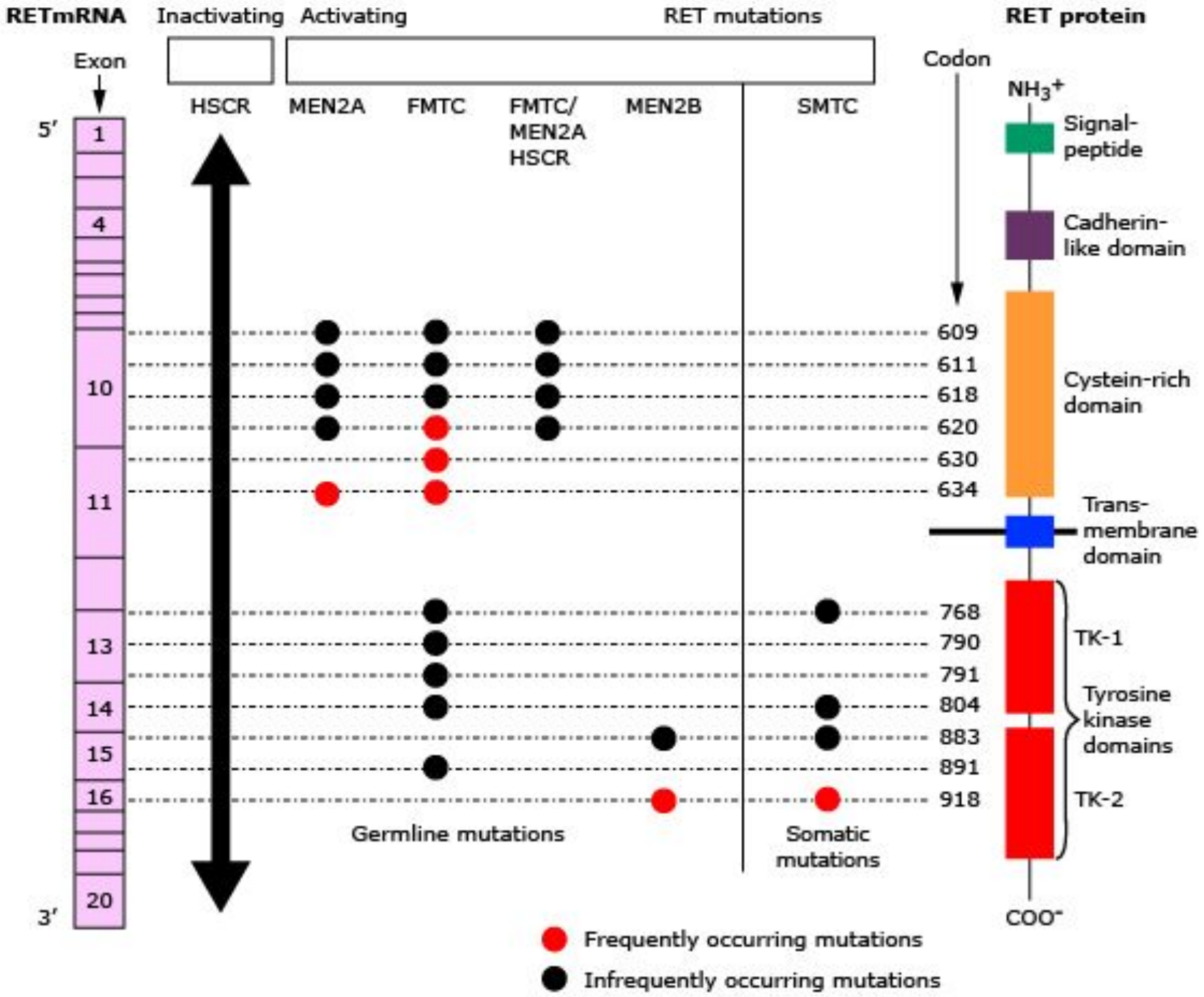
RET tyrosine kinase receptor mutations in MEN2, FMTC and sporadic MTC

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

IMPORTANT

- 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

✓ ***Oncogenes*** *involve activating (gain-of-function) mutations, and mutation in one allele is sufficient (dominant-like effect). In contrast, tumor suppressor genes (**TSGs**) involve inactivating (loss-of-function) mutations, and typically both alleles must be inactivated (recessive-like effect).*

Selected Tumor Suppressor Genes Involved in Human Neoplasms

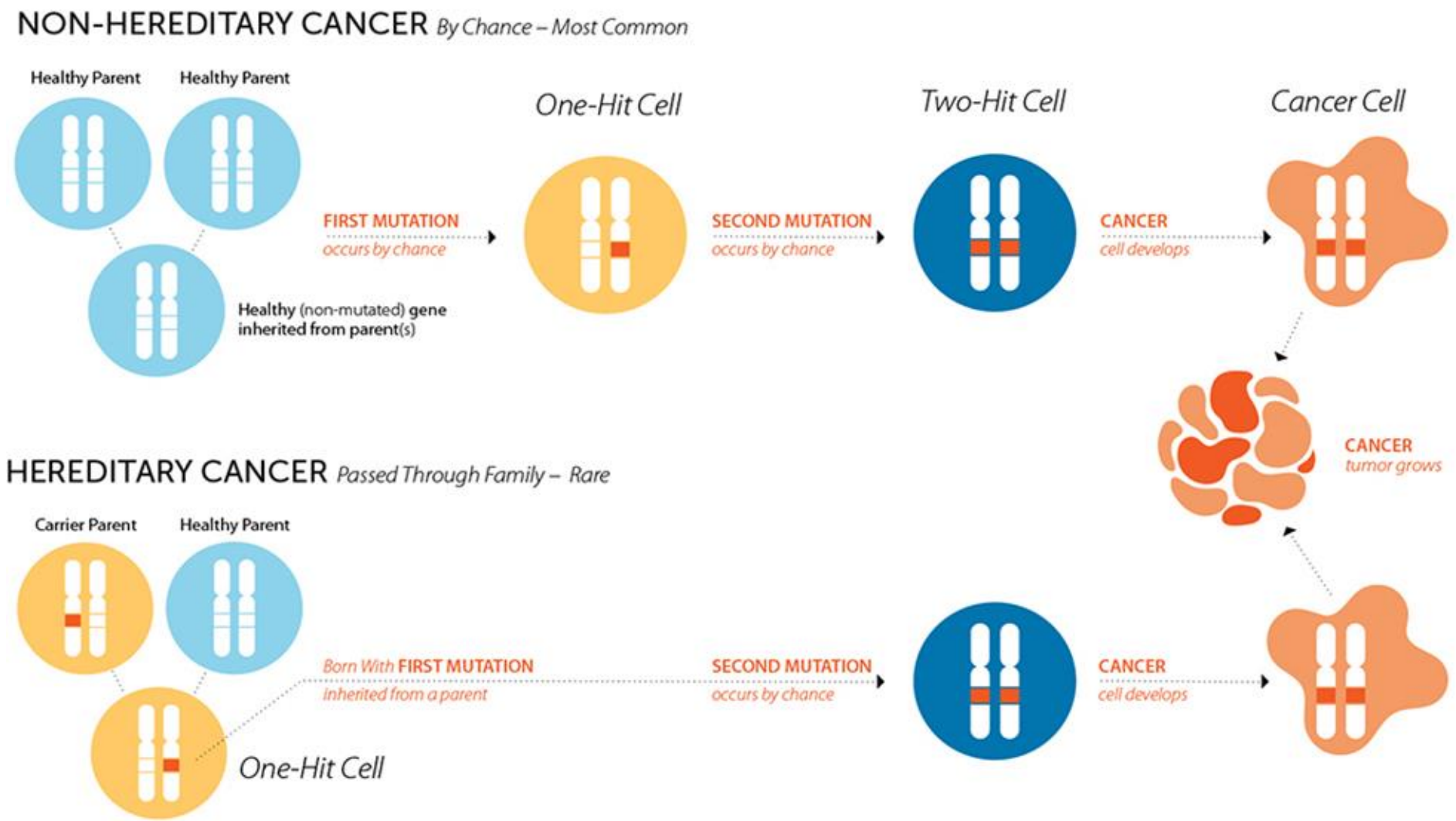
TABLE 7-8 -- Selected Tumor Suppressor Genes Involved in Human Neoplasms

Subcellular Locations	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Assocated with Inherited Mutations Germline
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 Cancer in neurons	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 Cancer in skin
	BRCA1 and *BRCA2*	DNA repair	Unknown	Carcinomas of female breast and ovary; carcinomas of male breast

PI3 kinase, phosphatidylinositol 3-kinase.

The doctor explained the entire table; I recommend watching the lecture.

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



Examine the figure carefully then see the next slide

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

The two-hit theory suggests that for a loss-of-function mutation in a **tumor suppressor gene** to be present, **both alleles must be damaged,** which results in a high risk of **cancer development.** There are two possibilities for tumor suppressor gene mutations: **hereditary (germline) or non-hereditary (non-germline).**

If it is **hereditary**, the mutation is inherited from one parent, either the mother or the father. This inherited pathogenic (disease-causing) mutation affects **one of the two alleles of the tumor suppressor gene** — this is **considered the first hit.**

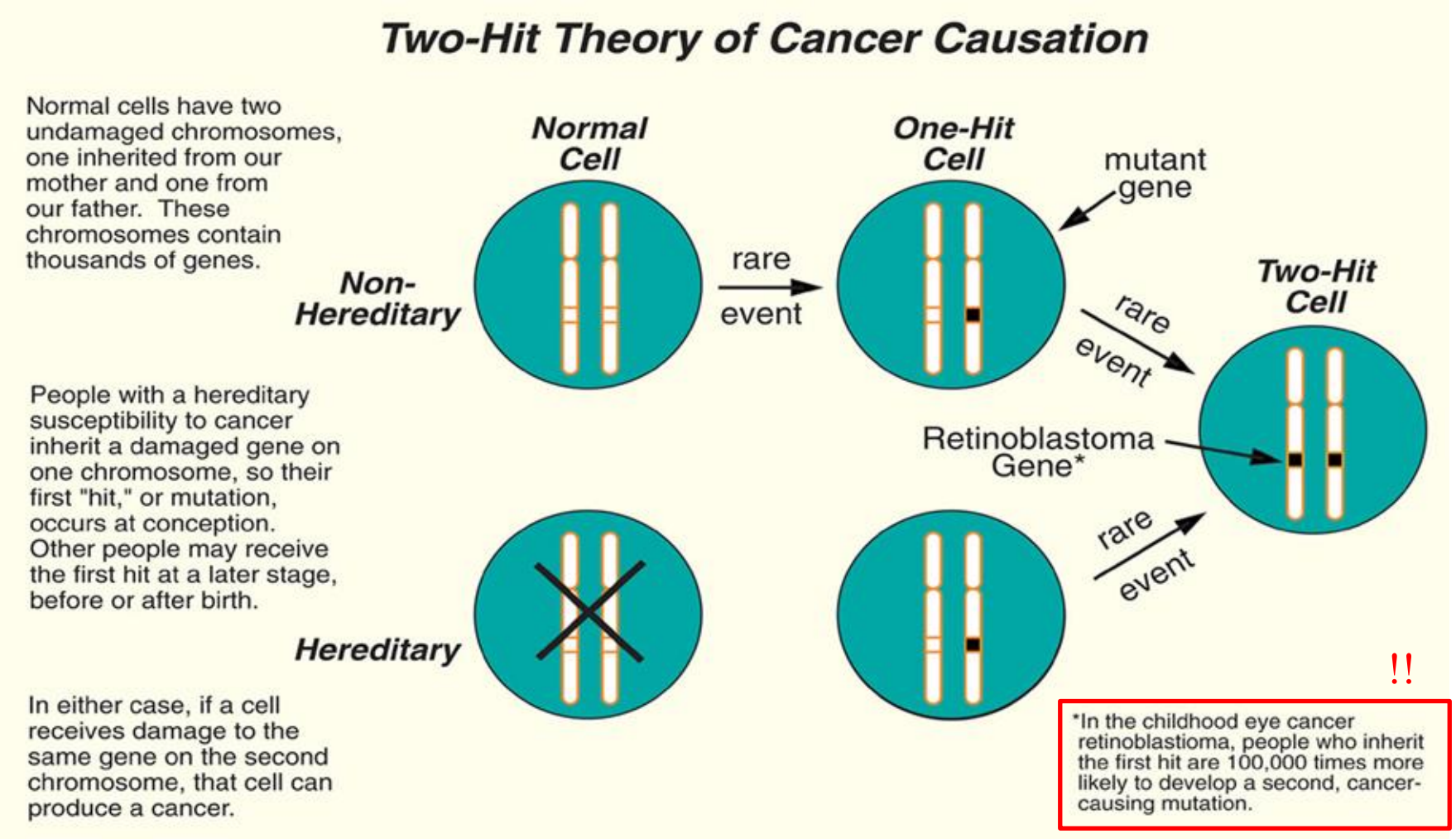
Unlike oncogenes, **tumor suppressor genes require both alleles to be damaged for loss of function to occur**. Therefore, the individual is born with the first hit already present in the **germline**, meaning it exists in the zygote and consequently **in all cells, tissues, and organs of the body.**

Then a **second hit** is acquired randomly later in life. If this second mutation occurs in the **other allele of the same tumor suppressor gene,** then both alleles become **damaged** (loss-of-function mutation), which significantly increases the **risk of cancer development.**

In **non-hereditary** cancer, the **parents are healthy** and do NOT pass a loss-of-function mutation in a tumor suppressor gene to their child. Instead, at some point during life, a **mutation occurs randomly due to DNA damage** — for example during DNA replication, exposure to UV light, or carcinogens such as smoking. This leads to the **first hit.** The mutation can occur in any gene, but if it occurs in a tumor suppressor gene, it becomes a problem.

Later, if another r**andom mutation occurs in the second allele of the same tumor suppressor gene**, this causes the **second hit**. At that point, **both alleles are mutated or damaged,** resulting in loss of function of the tumor suppressor gene, which **increases the risk of cancer development.**

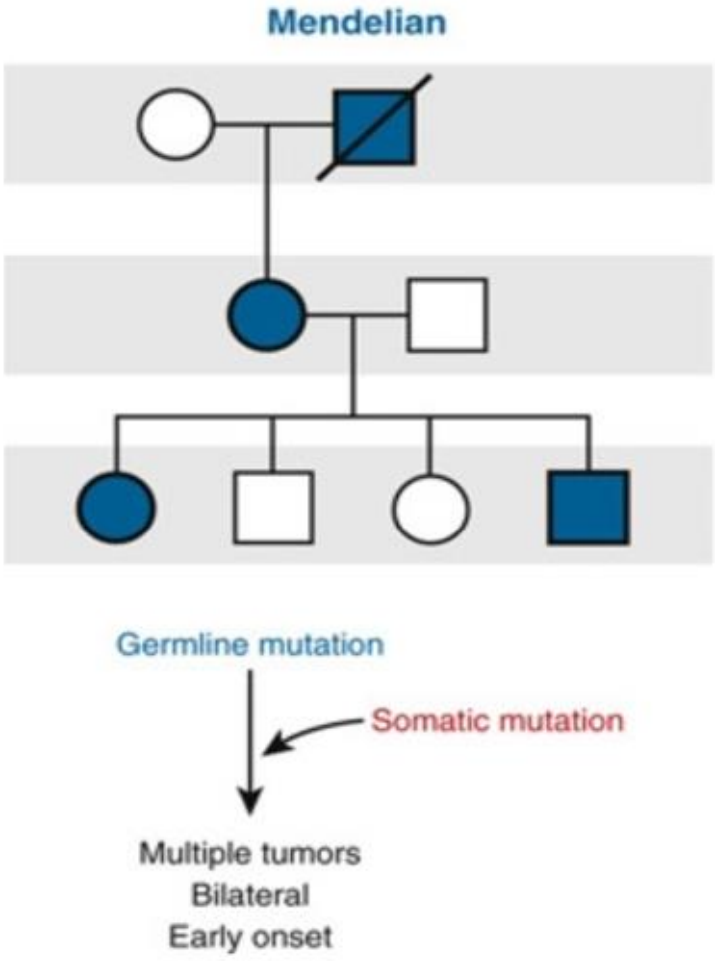
The Two-Hit Theory of Tumor Suppressor Gene Inactivation in Cancer



Examine the figure carefully

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.



- In the pedigree, the affected parent carries one mutant allele and one normal allele, so **each child has a 50% chance of inheriting the mutant allele and a 50% chance of inheriting the normal allele.**

- **Children who inherit the mutant RB allele already have the first hit present in all cells from birth.** They are therefore at high risk of acquiring a second somatic hit in the other RB allele. When this occurs, **both alleles lose function,** which can lead to **retinoblastoma**. **Because only one additional mutation is needed, these patients often develop multiple tumors, usually bilateral (both eyes), with early onset.**

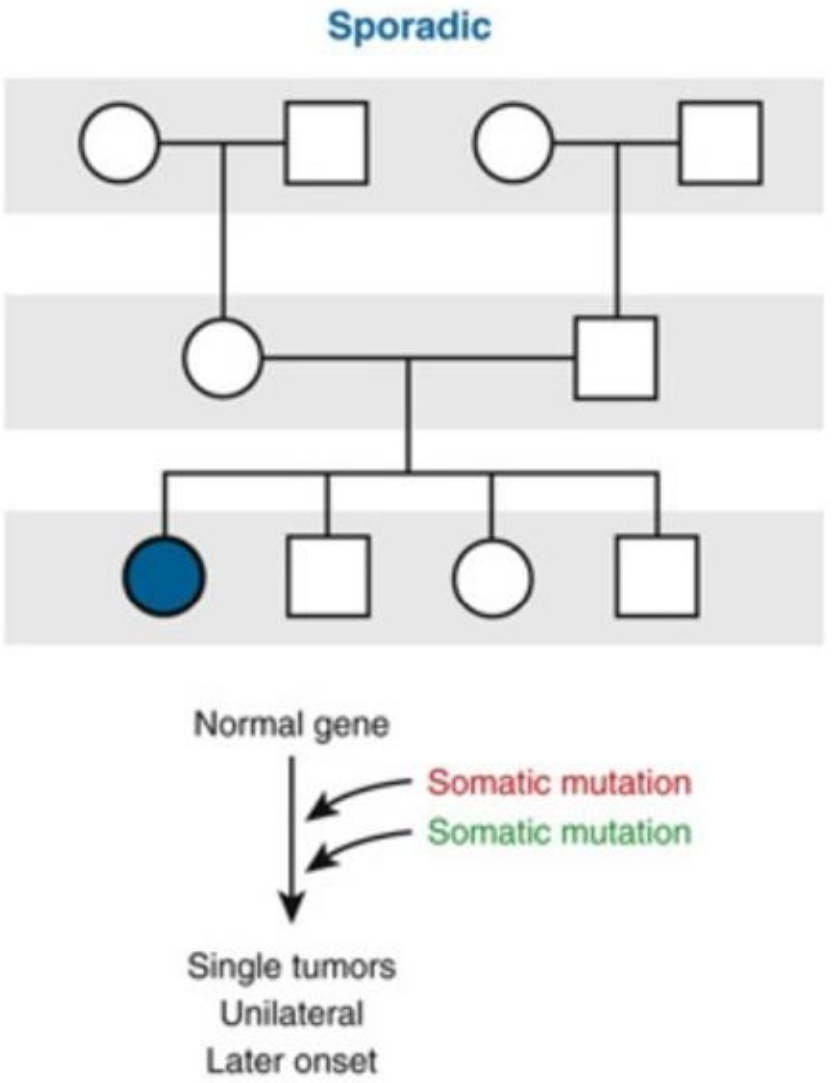
- As a consequence of this second somatic event, the cell loses function of both alleles, giving rise to a tumor.

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.

- In contrast to familial cases, in sporadic retinoblastoma, there is usually **no family history**, meaning only one individual in the family is affected. In this case, **both mutations must occur somatically in the same retinal cell: the first hit occurs after birth, and then a second somatic mutation affects the other allele.**

- Because **two separate somatic mutations are required**, this takes longer. As a result, sporadic retinoblastoma is more commonly associated with **a single tumor, typically unilateral (one eye), and later onset.**



Tow hit hypothesis

- 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.
- Knudson developed the **two-hit hypothesis** based on **statistical analysis of retinoblastoma cases,** before the responsible gene was identified and before the hereditary and somatic mutations were fully understood.

Alfred Knudson

"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

Tow hit hypothesis

- 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

“Most tumor suppressor genes require both alleles to be inactivated to cause a phenotypic change”

Tow hit hypothesis

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
- **At the gene level, tumor suppressor mutations are recessive** because both alleles must be mutated or inactivated for loss of function to occur. However, **at the clinical (phenotypic) level, they often appear dominant** in hereditary cancer syndromes.
- For example, if a child is born with a **heterozygous loss-of-function mutation in the RB1 gene,** one allele is already **mutated** from birth. Although one normal allele is still present, that child has a **high probability of acquiring a second somatic hit in the other allele later in life.** Once the second hit occurs, both alleles lose function and the disease develops.

Tumor Suppressor Genes in Autosomal Dominant Cancer Syndromes

So, **on the gene level it is recessive because both alleles are required to be inactivated, but on the disease level it appears dominant because inheriting just one mutated allele is enough to strongly predispose the person to disease, since the second hit is likely to occur over time.**

A similar example is **BRCA1**. If a girl is born with a **heterozygous pathogenic BRCA1 mutation**, she already carries the first hit in all cells. Even before a second hit occurs, she has a **very high lifetime risk of developing breast cancer because the second hit may happen later in breast tissue cells.** For this reason, some high-risk individuals may choose preventive removal of breast tissue to reduce the risk of cancer development

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.*

Retinoblastoma familial

- 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. Mosaicism means that the mutation is present in only a subset of cells, not in all cells of the body. If the mutation is **present specifically in the gonads (sperm or oocytes),** this is called gonadal/germline mosaicism. In that case, the parent may not have the mutation in most body cells and may even be clinically unaffected, but can still pass the mutation to their children through the gametes.

- 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

Retinoblastoma familial

When an individual is born with a heterozygous germline mutation in the RB1 gene (one mutated allele and one normal allele), **a second hit in tumor suppressor gene may take place.** This can happen through several mechanisms:

- **Epigenetic silencing**, such as abnormal DNA methylation of the normal allele, leading to loss of its expression.
- **A second somatic mutation** that causes another loss-of-function change in the remaining normal allele.
- **Chromosomal loss or recombination,** where the normal allele is lost and the mutant allele is duplicated ("copying" the mutated allele).
- **Deletion of a chromosomal region** containing the normal RB1 allele.

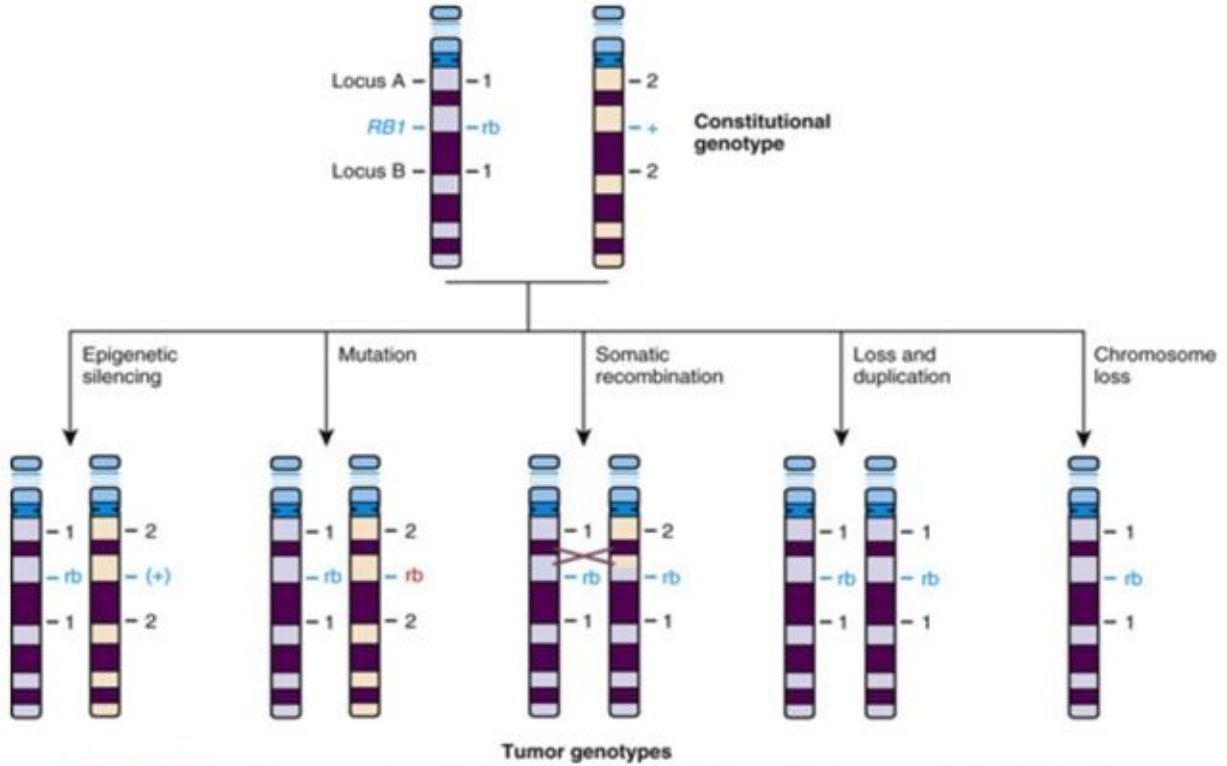


FIGURE 15-8 Chromosomal mechanisms that could lead to loss of heterozygosity for DNA markers at or near a tumor suppressor gene in an individual heterozygous for an inherited germline mutation. The figure depicts the events that constitute the "second hit" that leads to retinoblastoma with loss of heterozygosity (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.

Retinoblastoma familial

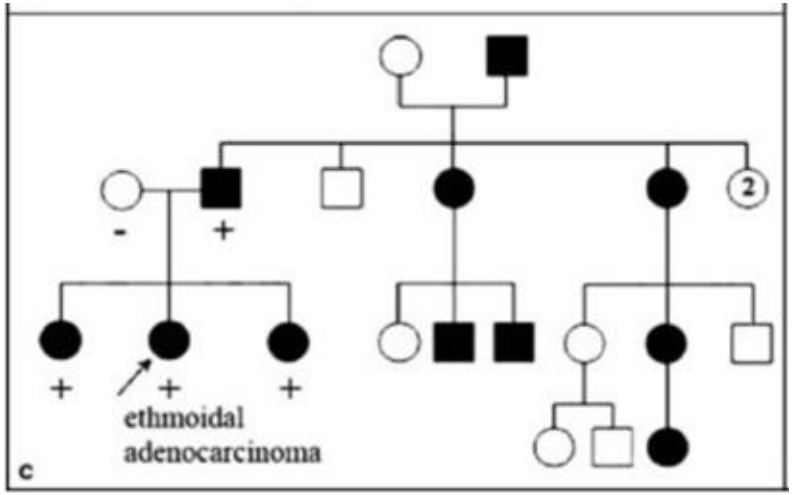
- The disorder appears to be inherited as a dominant trait because the large number of primordial retinoblasts and their rapid rate of proliferation make it very likely that a somatic mutation will occur as a second hit in one or more of the more than10^6 retinoblasts already carrying an inherited RB1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

- Although retinoblastoma is recessive at the gene level (requiring two hits: one inherited and one somatic), it appears as a dominant trait in family pedigrees.

- This is because in hereditary cases, individuals are born with a germline mutation in one RB1 allele, meaning this first hit is present in all retinal and body cells from birth. As a result, many retinal precursor cells that are actively dividing during embryonic development, fetal life, and childhood already carry this first mutation.

- Since these cells are rapidly proliferating, there is a high chance that a second somatic hit will occur in the remaining normal RB1 allele in one of these retinal cells. Once this happens, both alleles become inactivated, leading to tumor formation.

- Therefore, even though two hits are required at the molecular level, the presence of the first germline hit in all cells makes cancer development much more likely and earlier, which creates a dominant inheritance pattern at the clinical (pedigree) level.



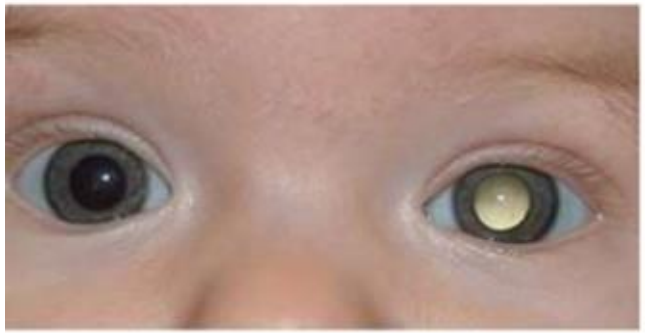


Fig.3 white color in the center circle of the eye

When the eyes are developing, they have progenitor (immature) cells called **retinoblasts**.

Retinoblastoma familial

- 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.

- In a normal healthy individual, a loss-of-function mutation in one of the two RB1 alleles does not by itself cause disease; a second hit in the remaining allele is required for tumor development.

- However, in the inherited form of retinoblastoma, all cells carry a germline mutation in one RB1 allele (first hit). If a somatic mutation (second hit) occurs in the remaining normal allele in one retinal cell, that cell loses both functional copies of RB1.

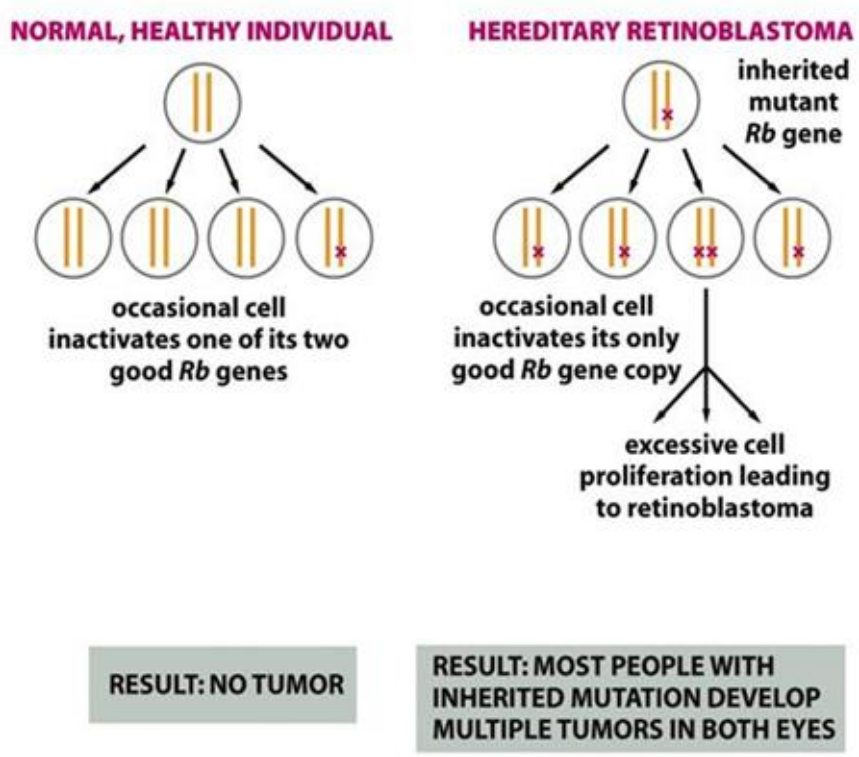
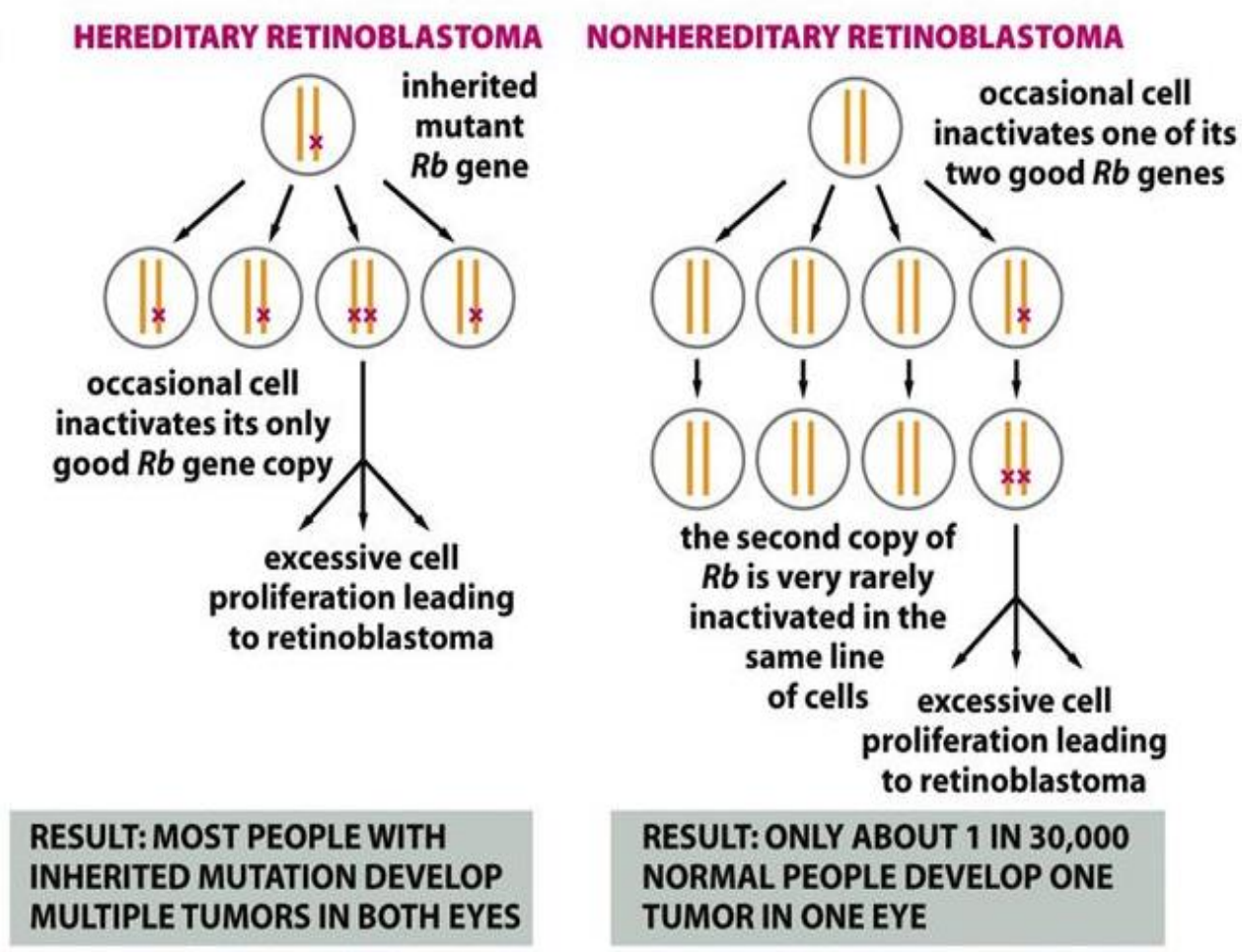


Figure 20-30 *Molecular Biology of the Cell* (© Garland Science 2008)

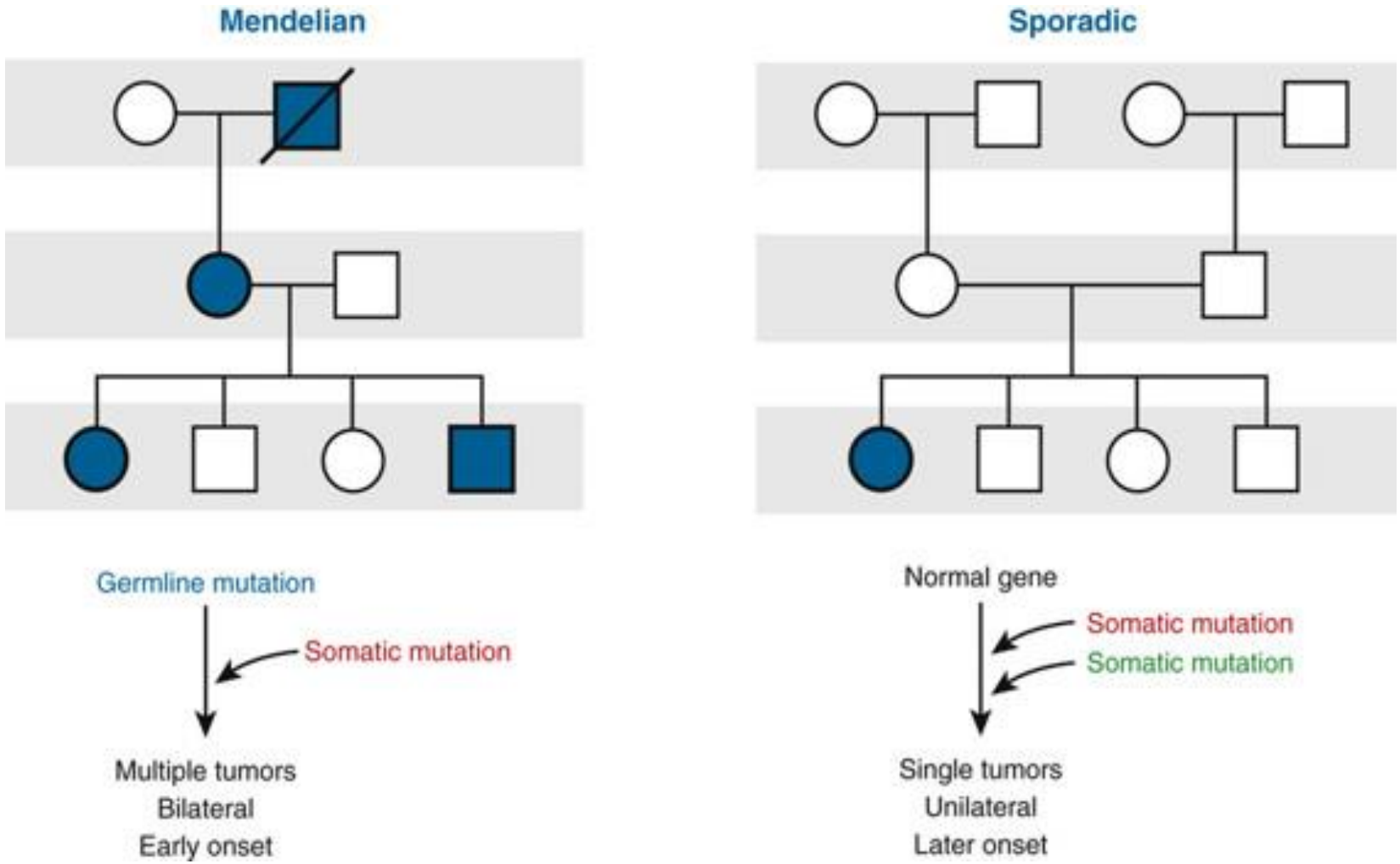
Retinoblastoma sporadic

- 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.
- Because RB1 is a tumor suppressor gene that normally regulates the cell cycle, its loss removes an important control mechanism. As a result, the affected cell gains a proliferative advantage, divides more rapidly than surrounding cells, and undergoes clonal expansion, ultimately leading to transformation into retinoblastoma.



- Another difference between hereditary and sporadic tumors is that the average **age at onset** of the sporadic form is in early <u>childhood</u>, later than in <u>infants</u> 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

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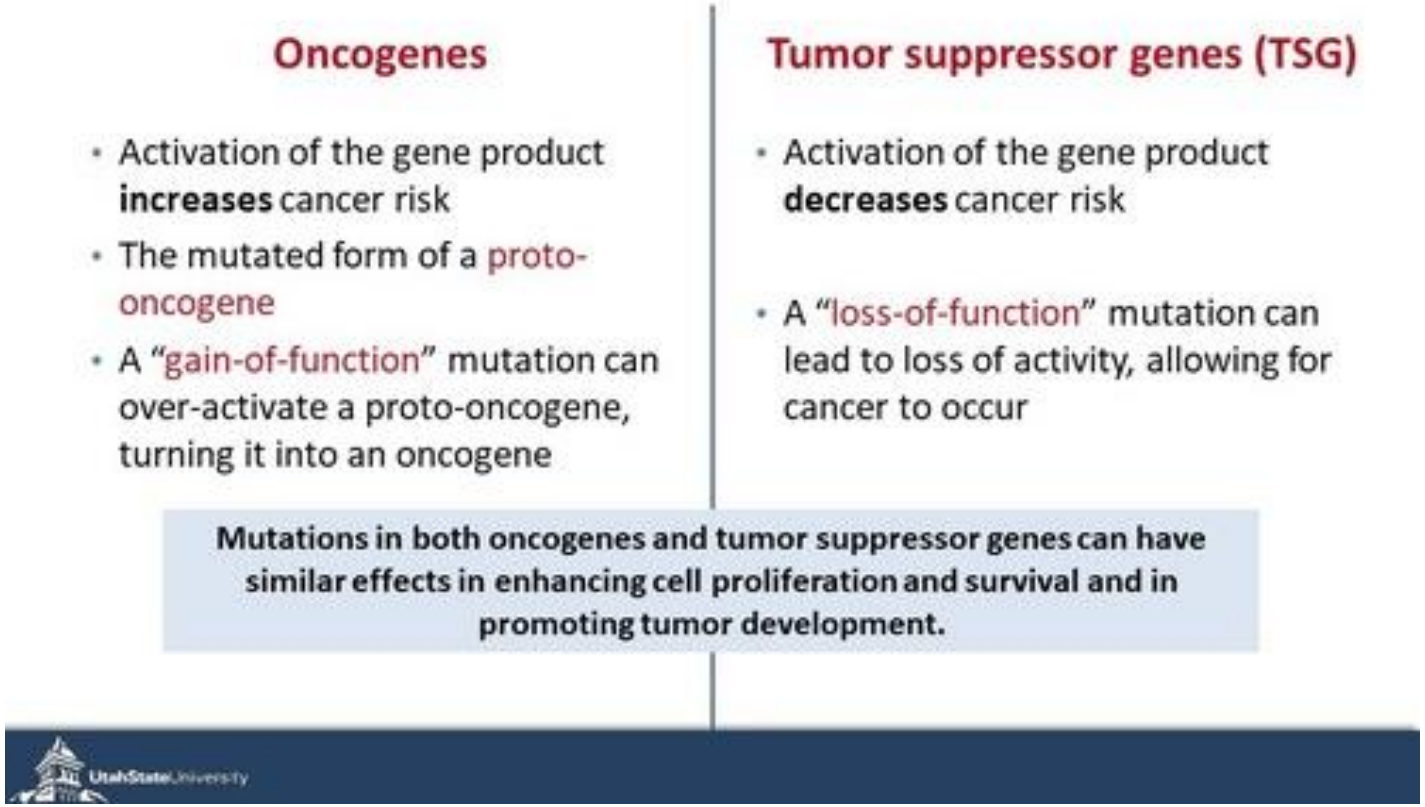


Retinoblastoma gene

- The RB1 gene product, p110 Rb1, is a phosphoprotein that normally regulates entry of the cell into the S phase of the cell cycle.

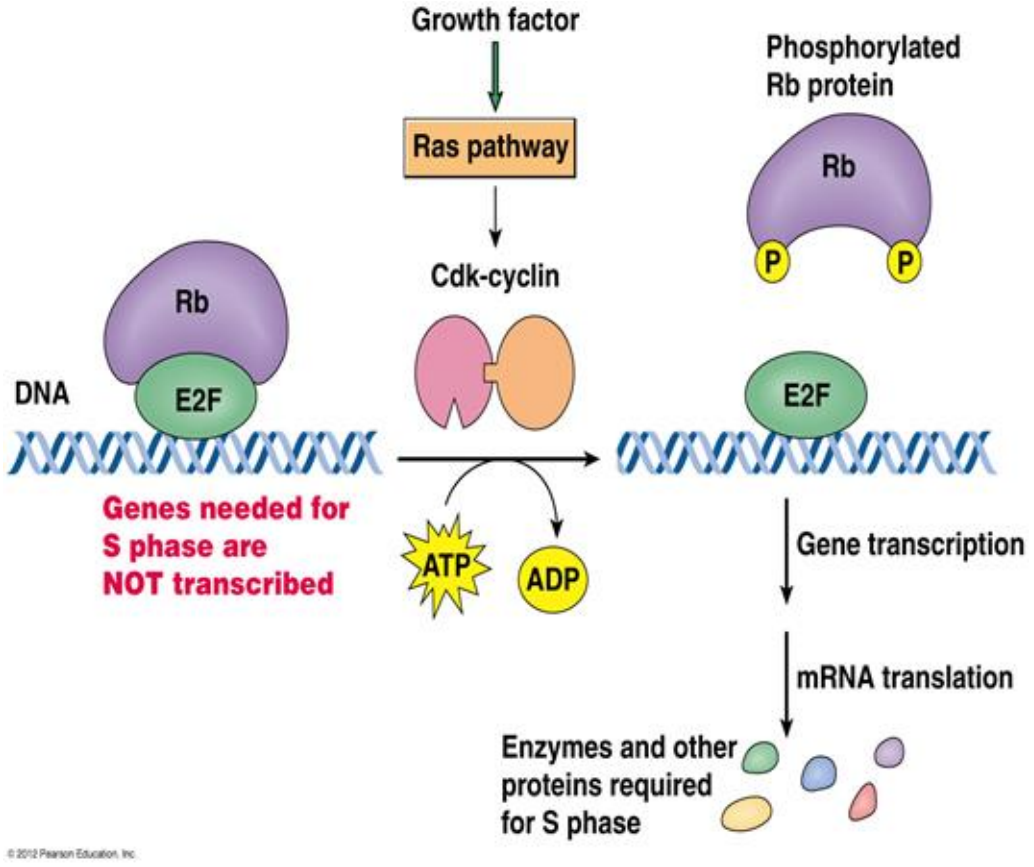
- Thus loss of the RB1 gene and/or absence of the normal RB1 gene product deprives cells of an important checkpoint and allows uncontrolled proliferation.

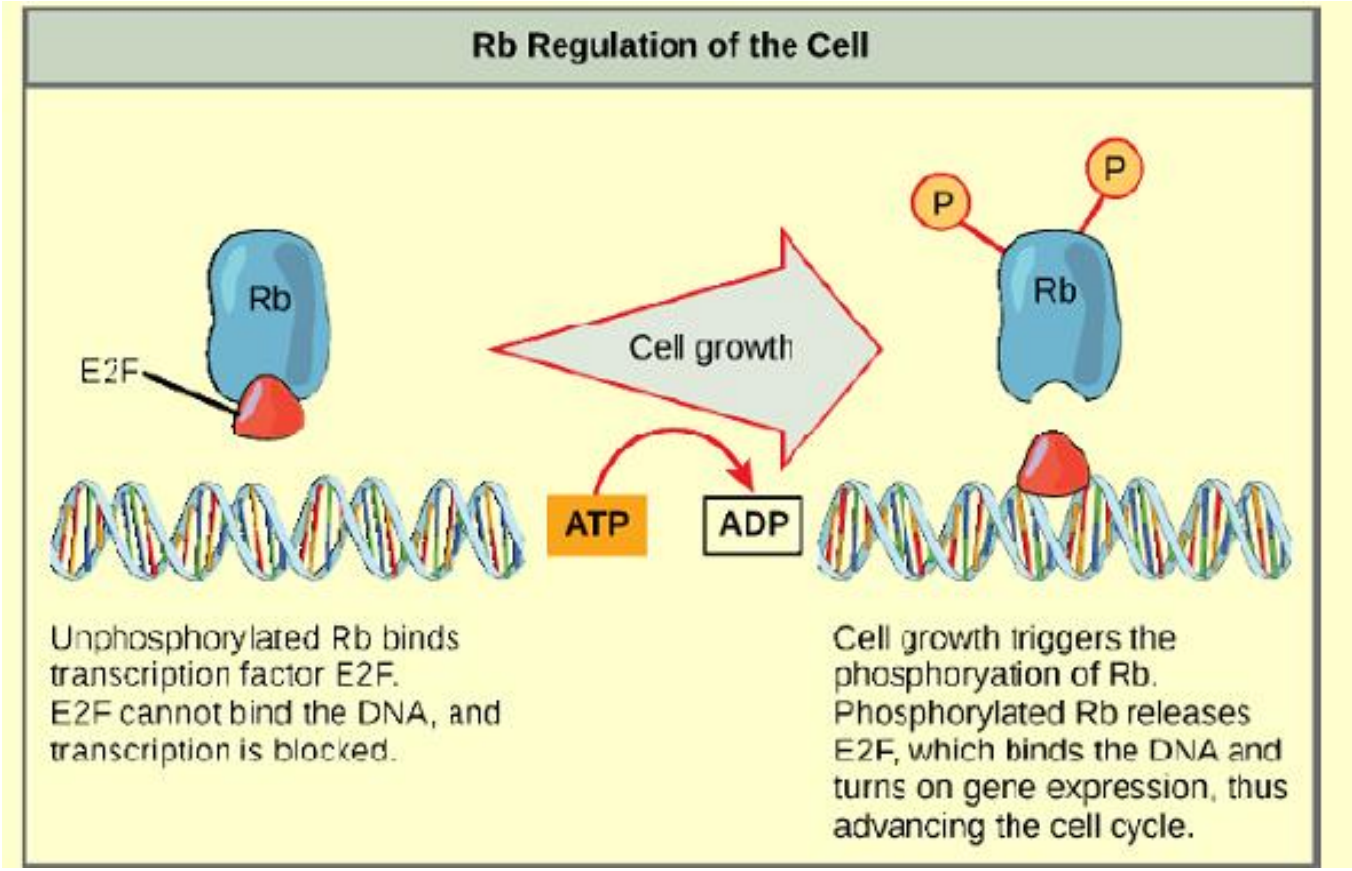
- **RB1 protein normally regulates the G1 → S phase transition in the cell cycle by binding to E2F transcription factors and inhibiting their activity.** When RB1 is bound to E2F, it prevents the expression of genes required for DNA synthesis, thereby blocking entry into the S phase.

- When a **growth factor is present**, it activates signaling pathways such as the **RAS pathway**, which leads to phosphorylation of the RB protein. Once RB is phosphorylated, it changes its conformation and **releases E2F**. Freed E2F then activates transcription of genes necessary for DNA replication, allowing the cell to enter the **S phase**.

- In contrast, when **RB1 is nonfunctional (loss-of-function mutation)**, it cannot bind E2F even in the absence of phosphorylation. As a result, **E2F remains continuously active**, leading to constant expression of S-phase genes and uncontrolled progression of the cell cycle into DNA synthesis.

Retinoblastoma gene

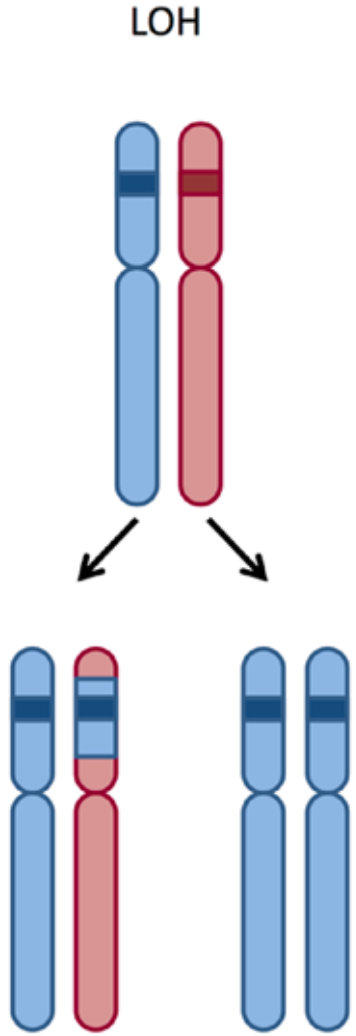




Loss of Heterozygosity

- 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

Loss of heterozygosity (LOH) is another important mechanism of mutation, especially in the **second hit** of tumor suppressor genes like **RB1**. It was discovered through comparisons of DNA polymorphisms at the RB1 locus between normal cells and retinoblastoma tumor cells from the same patient.

Normally, in somatic cells, the two homologous chromosomes are not identical because many regions are **heterozygous** (one allele inherited from the father and one from the mother). Therefore, at the RB1 locus and surrounding regions, there are usually detectable differences between the two chromosomes.

However, in retinoblastoma tumor cells, this **heterozygosity is lost** at the RB1 region, and sometimes across a large portion of chromosome 13. As a result, instead of having two different alleles, the region becomes **homozygous**.

This loss of heterozygosity represents one mechanism of the **second hit** in tumor suppressor gene inactivation. In an individual who already carries one inherited mutated RB1 allele (heterozygous state), the normal allele can be lost and replaced by the mutated one.

This can happen through several mechanisms, including:

- **Copying the mutant allele (gene conversion or mitotic recombination)**, leading to duplication of the mutated allele
- **Loss of the chromosome segment or whole chromosome containing the normal RB1 allele**
- **Deletion of the region containing the normal allele**

Another mechanism leading to loss of heterozygosity (LOH) is the deletion of the normal RB1 allele, followed by replacement through recombination or duplication of the mutant allele. This can result in a copy-neutral LOH, meaning there is no net gain or loss of DNA content; instead, the chromosome structure is maintained but the genetic information becomes homozygous for the mutant allele.

In this situation, the normal allele is lost and the mutant allele is effectively “copied and pasted” to replace it, resulting in two mutant copies.

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.

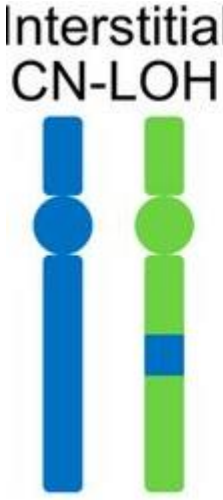
- Thus, in these cases, LOH represents the second hit of the remaining allele.

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

- LOH can occur through different patterns:

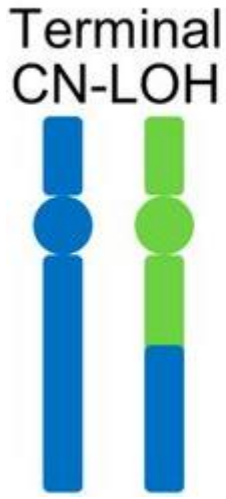
✓ **Interstitial LOH:** where a smaller internal segment containing the normal allele is lost and replaced by a duplicated mutant segment through recombination.

✓ **Terminal LOH:** where a larger distal region of the chromosome (including the RB1 locus on chromosome 13) is lost, and the remaining mutant segment is duplicated or extended to the end of the chromosome.

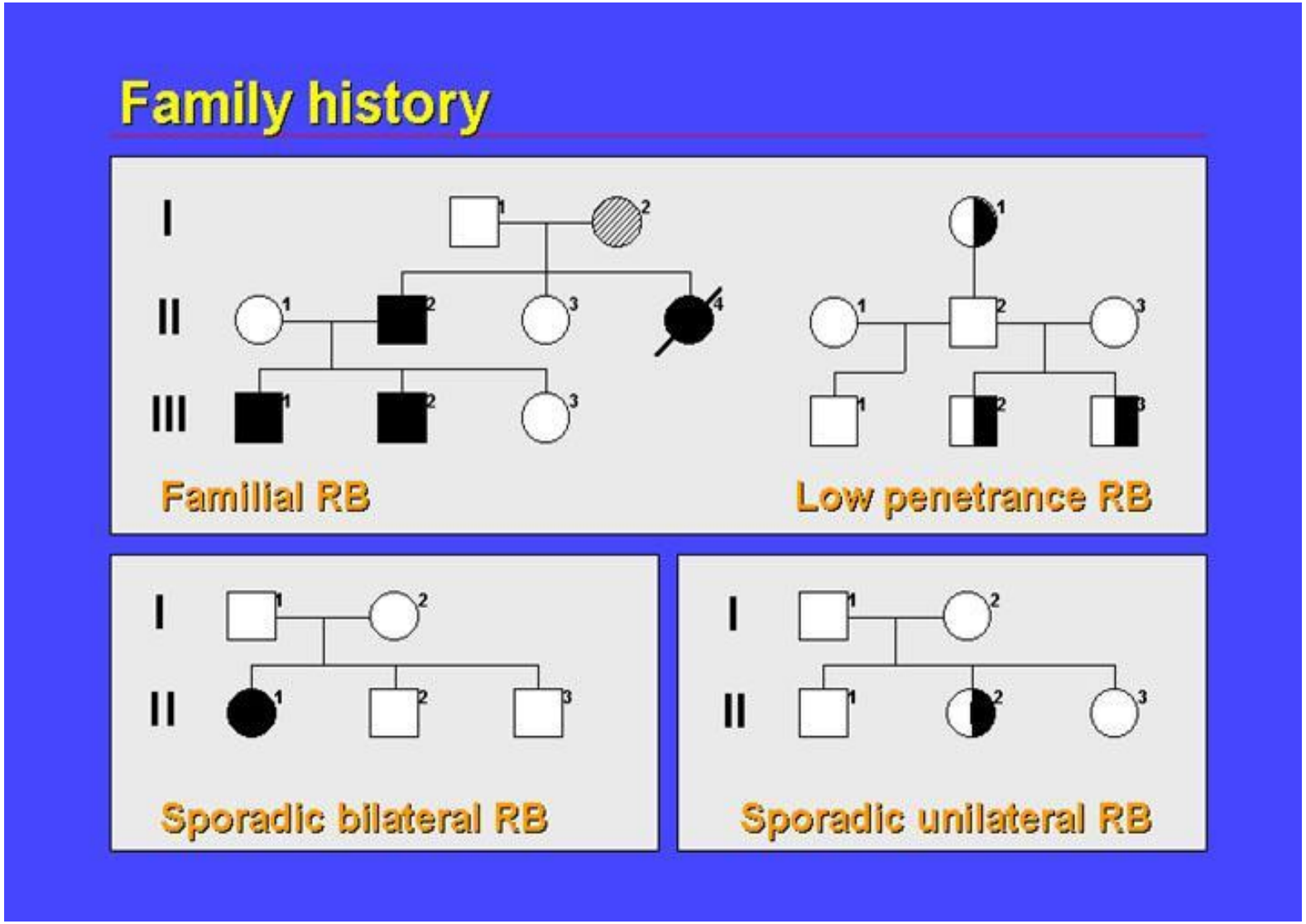


❑ Copy neutral loss of heterozygosity (CN-LOH) is the most common class of structural mutation.

❑ 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.



Please click here and let me know if there's any mistake.
Good Luck ☺