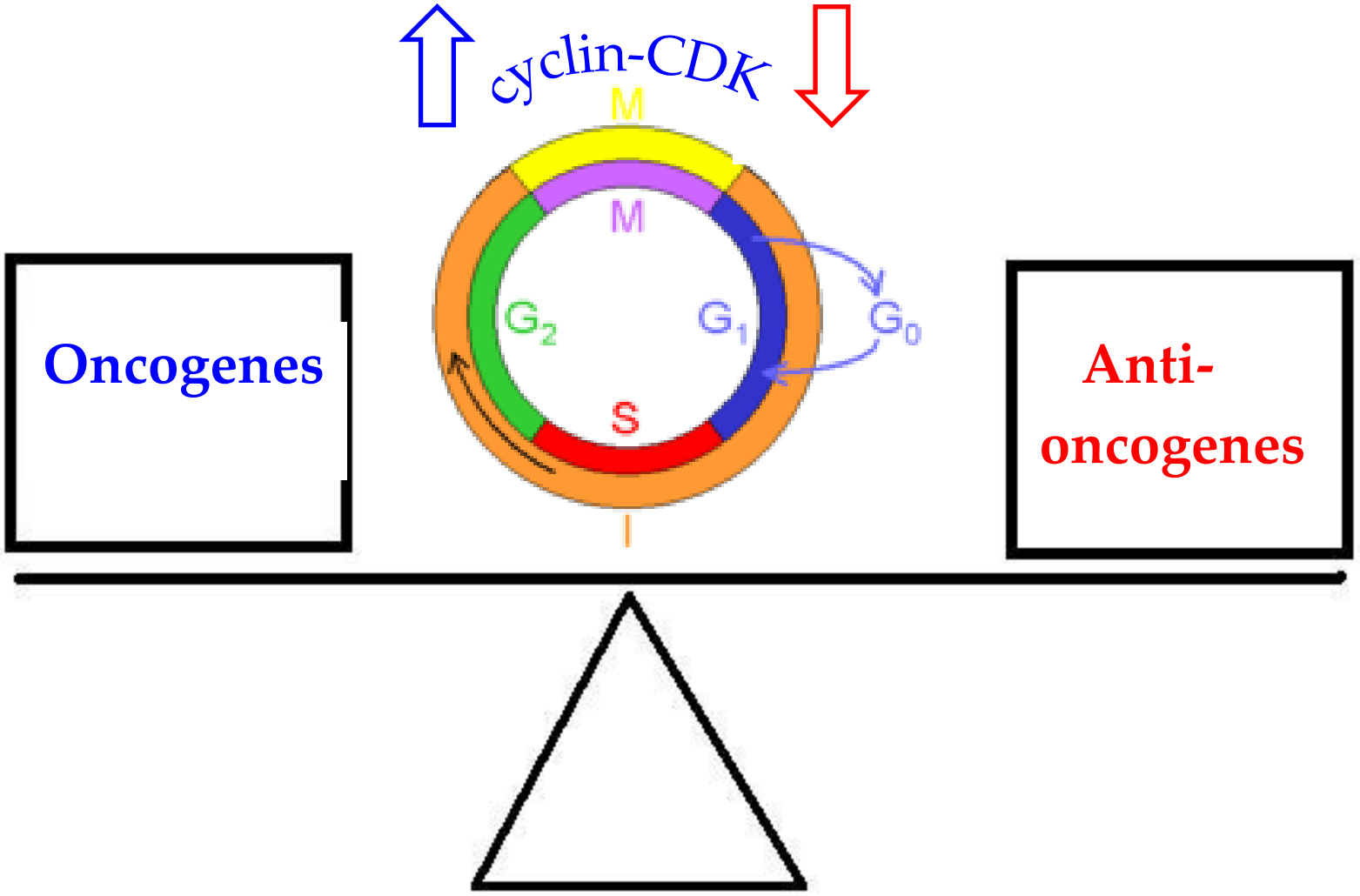
Teaching unit 05

TUMOR SUPPRESSOR GENES 1

let us remind ourselves...



Tumor phenotype

- A viral oncogene has the ability to dictate cell behavior despite the function of "opposing" genes, which have a role to ensure normal cell proliferation.
- Syncytium
- Heterokaryon

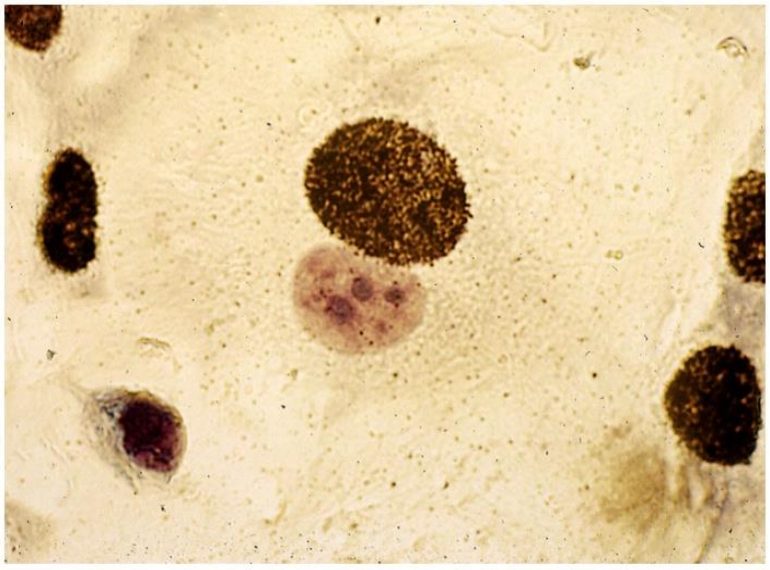


Figure 7.1b The Biology of Cancer (© Garland Science 2014)

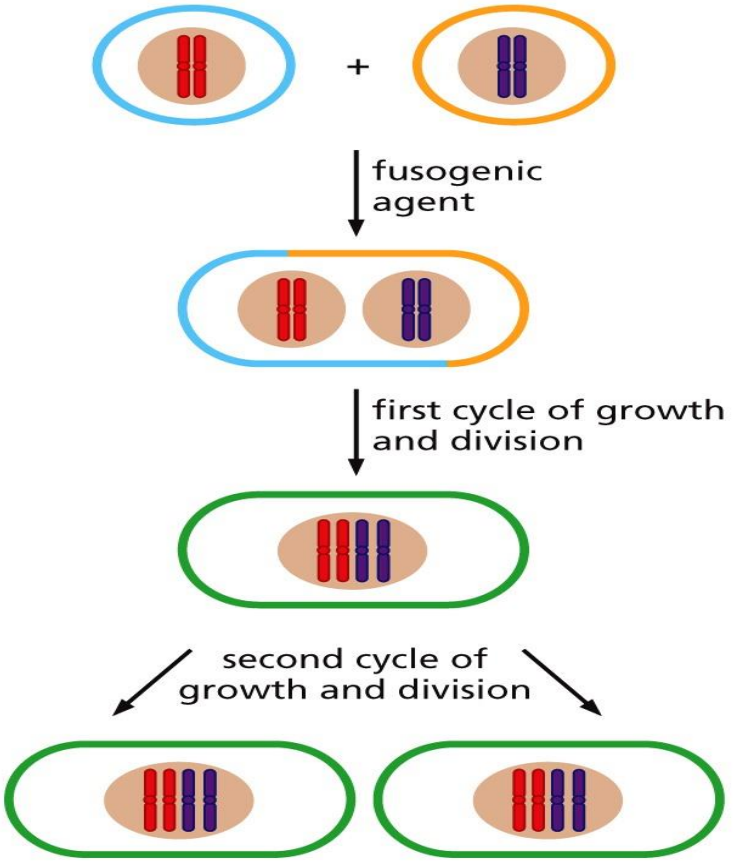


Figure 7.1a The Biology of Cancer (© Garland Science 2014)

Dominance and recessiveness of the tumor phenotype

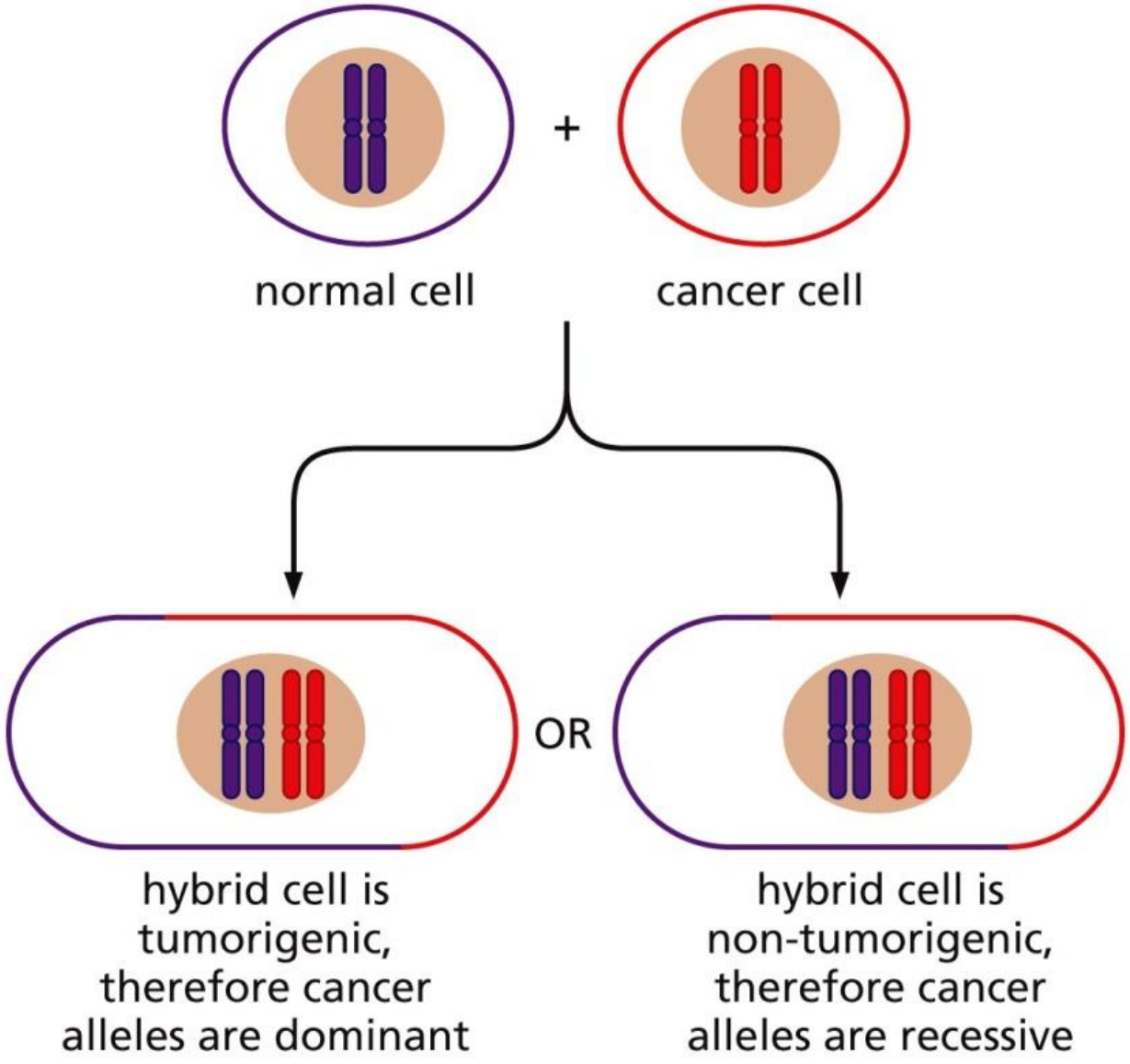


Figure 7.2 The Biology of Cancer (© Garland Science 2014)

Tumor phenotype

- A normal cell carries genes that suppress its proliferation.
- During tumor development, cancer cells lose completely or functionally one or more of these genes.
- Once the growth-suppressing genes are lost, the cancer cells are no longer under their control, and their proliferation accelerates.

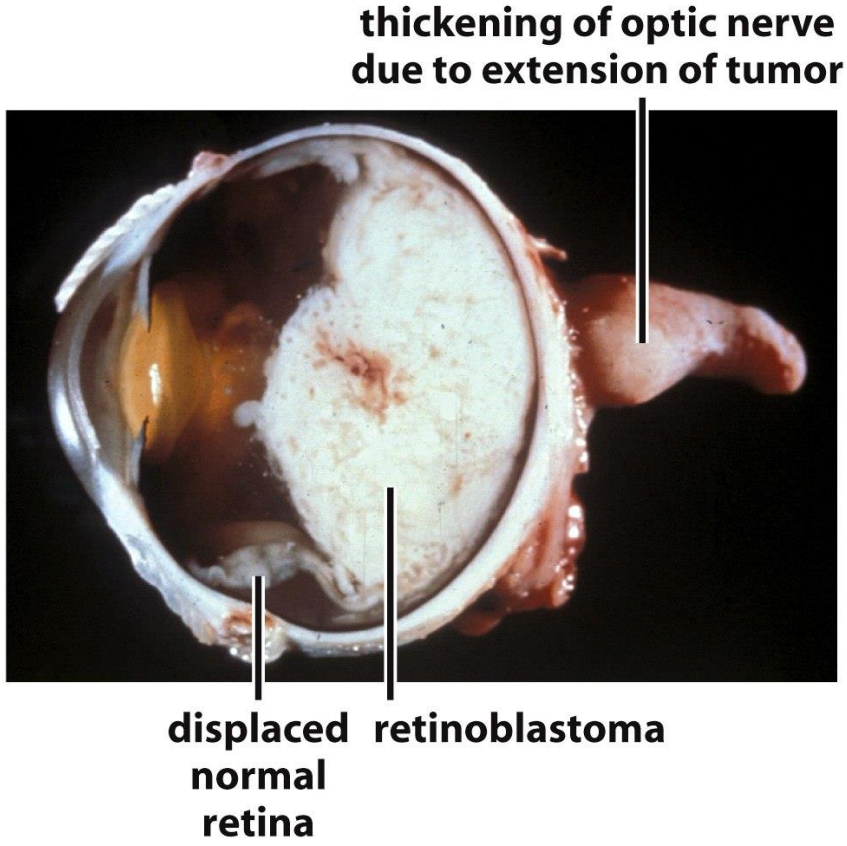
Tumor suppressor gene

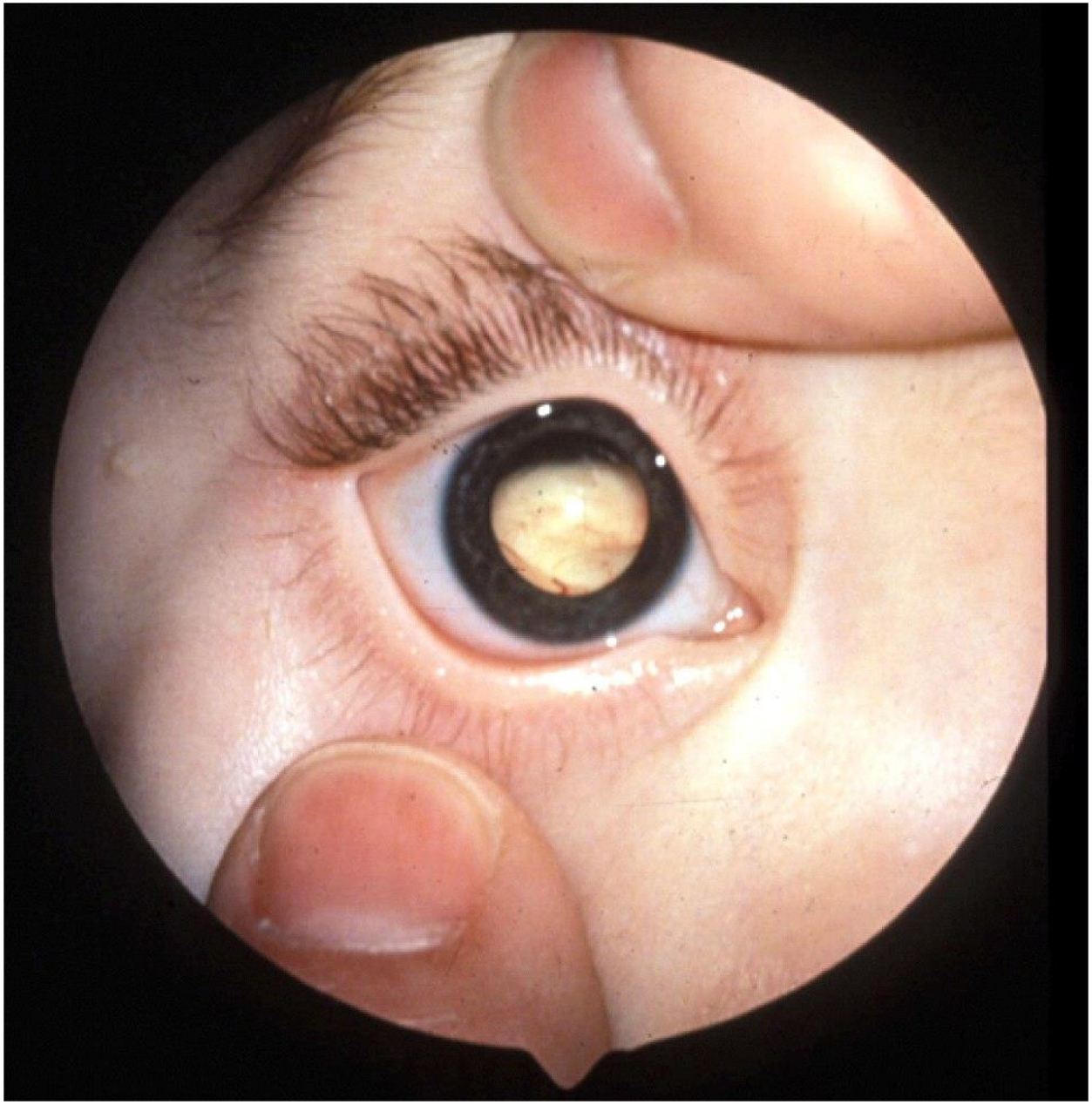
- Every gene, which, by losing its function, contributes to tumor development.

- Inactivation of tumor suppressor genes plays a role in the pathogenesis of tumors as important as the activation of oncogenes, for many tumors and more important.

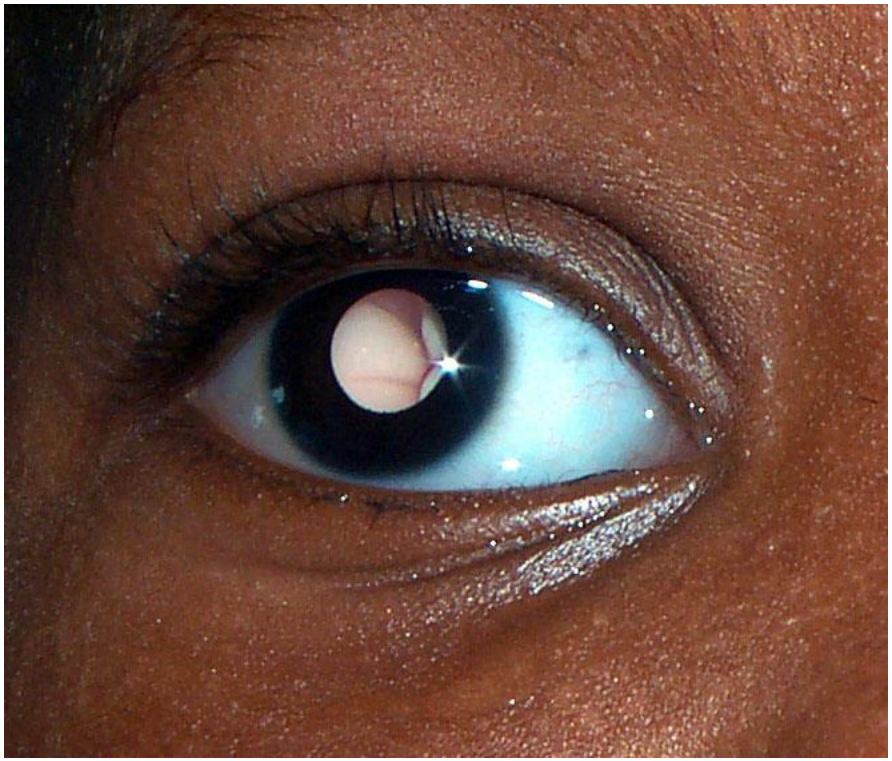
Children's eye tumor - Retinoblastoma

- Retinal tumor
- Arises from precursors of photoreceptor cells - occurs in 1 in 20,000 children.
- Diagnosed from
  birth to 6-8 years
  life, rarely later.





Rb; first tumor suppressor gene



From the Eye Cancer Network

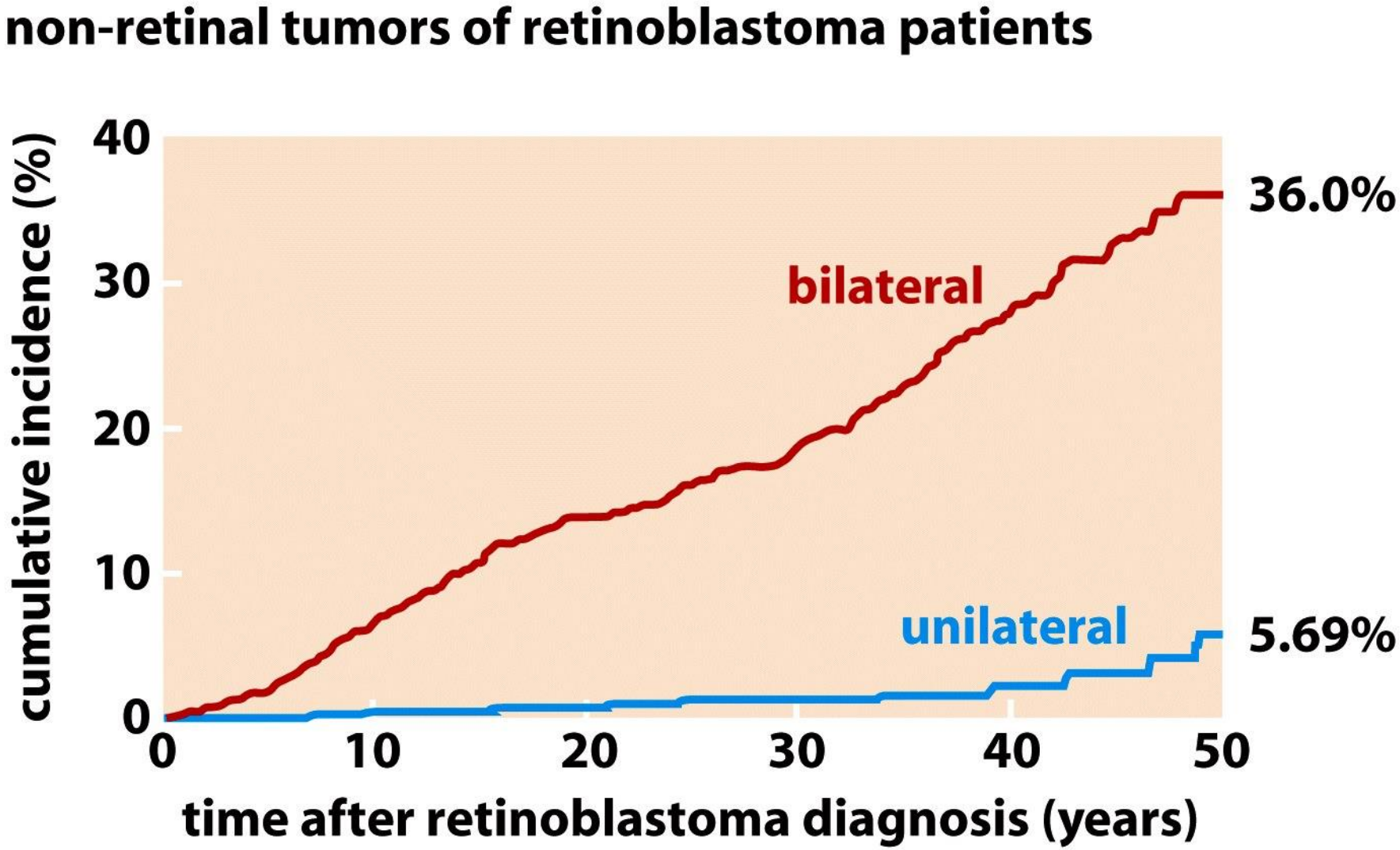
http://www.eyecancer.com/conditions/Cases/Case13/Case13.html

Children's eye tumor - Retinoblastoma

This "**tumor syndrome**" occurs in 2 forms:

- Some children (with no family history of retinoblastoma) have one tumor in one eye. If the tumor is removed, with radiation or surgery, these children have no further risk of retinoblastoma or another tumor. Since the tumor occurs in children without a family history of this disease, it is considered a sporadic form of the disease, and since it occurs in one eye, it is also called unilateral retinoblastoma.

- The familial form of retinoblastoma occurs in children whose parents were also affected and cured in childhood. In this case, multiple tumors are registered in both eyes (that's why it's called bilateral retinoblastoma). Curing eye tumors, with radiation or surgical intervention, does not protect children from a high risk (500 times higher than normal children) of bone tumors (osteosarcoma) during adolescence nor from an increased susceptibility to developing many other tumors during life.



The Biology of Cancer (© Garland Science 2007)

Retinoblastoma gene-1 (Rb-1)

- Mutation responsible for childhood retinoblastoma.
- Mutations of the Rb gene make it inactive - recessive.

- its product (pRb):
  - Cell cycle regulation
  - Differentiation
  - DNA replication
  - Repair of damaged DNA
  - Inhibition of apoptosis.

pRb

- Nuclear transcription factor.
- Phosphorylation through the cell cycle.

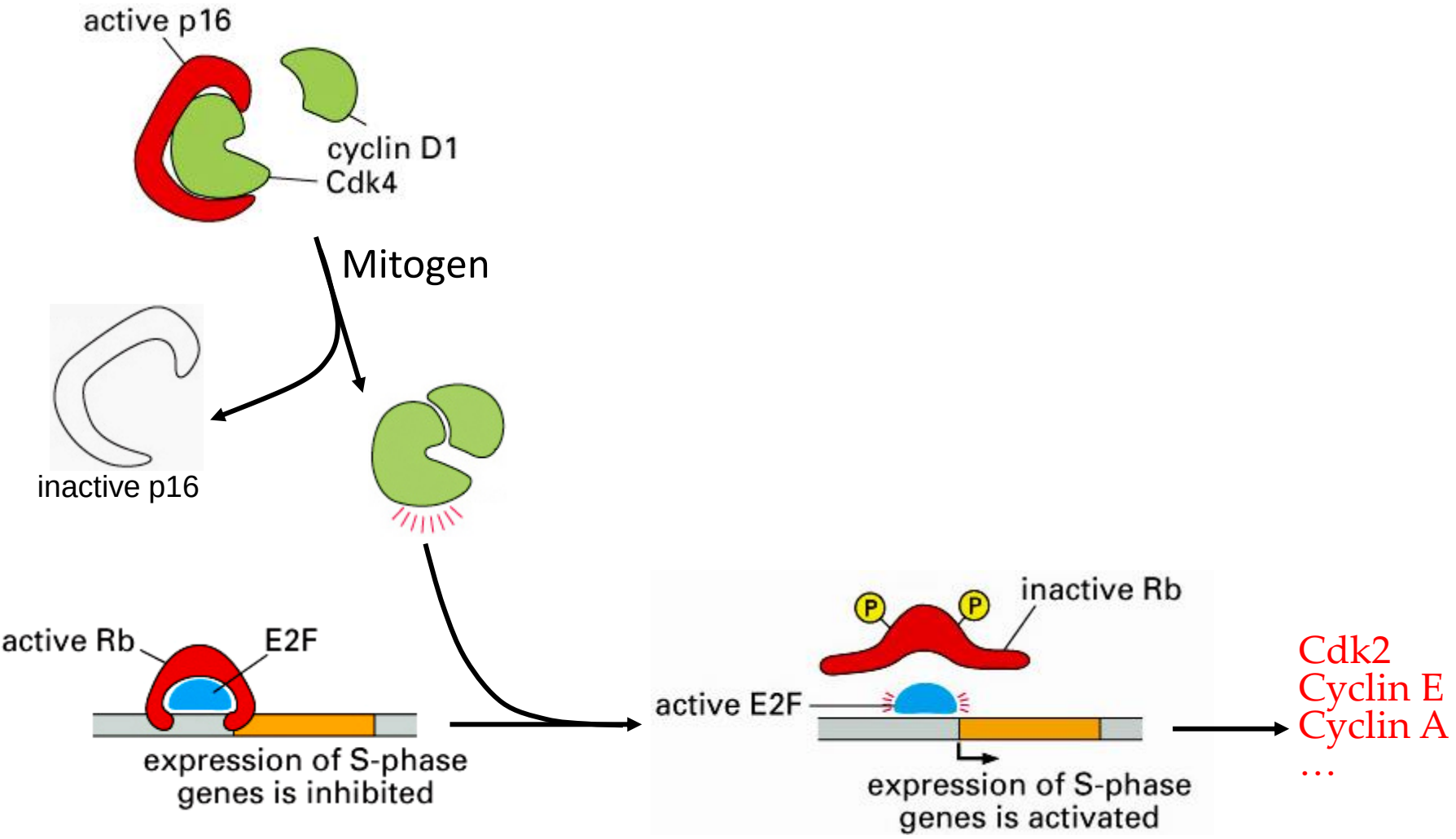
- Hypophosphorylated pRb → p105 nuclear protein → inhibits transcription factor E2F, important for cell cycle control.

- Cdk + cyclin → phosphorylated Rb → inactive → E2F.

E2F

- Starts the transcription of cyclin E, a protein necessary for the transition of the cell to the S phase, as well as cyclin A important in the G2 phase.
- Increases the activity of histone acetyl transferase, which enables the separation of histone proteins from the DNA chain.
- Accelerates DNA replication and helps in its repair by binding polymerase to DNA.
- Enables transcription of dihydrofolate reductase and thymidine kinase - DNA synthesis.

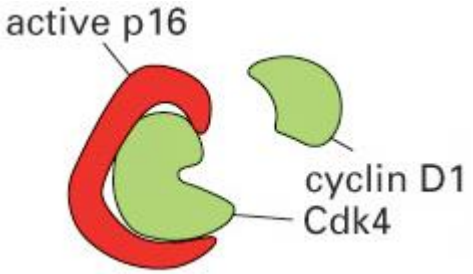
pRb inhibits transcription of E2F



Adapted from Albert's et al. Figure 23-32

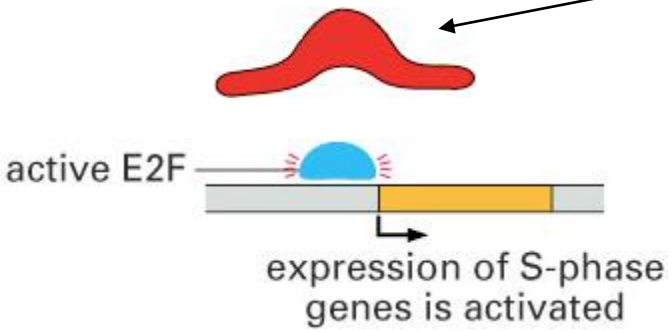
pRb inhibits transcription of E2F

Mutation of pRb enables transition to S-phase, without mitogen



Cells without pRb function
they do not control the G1 phase
- **constant E2F-dependent transcription**
- **bypass Cdk4/6**
- **proliferation even without mitogen**

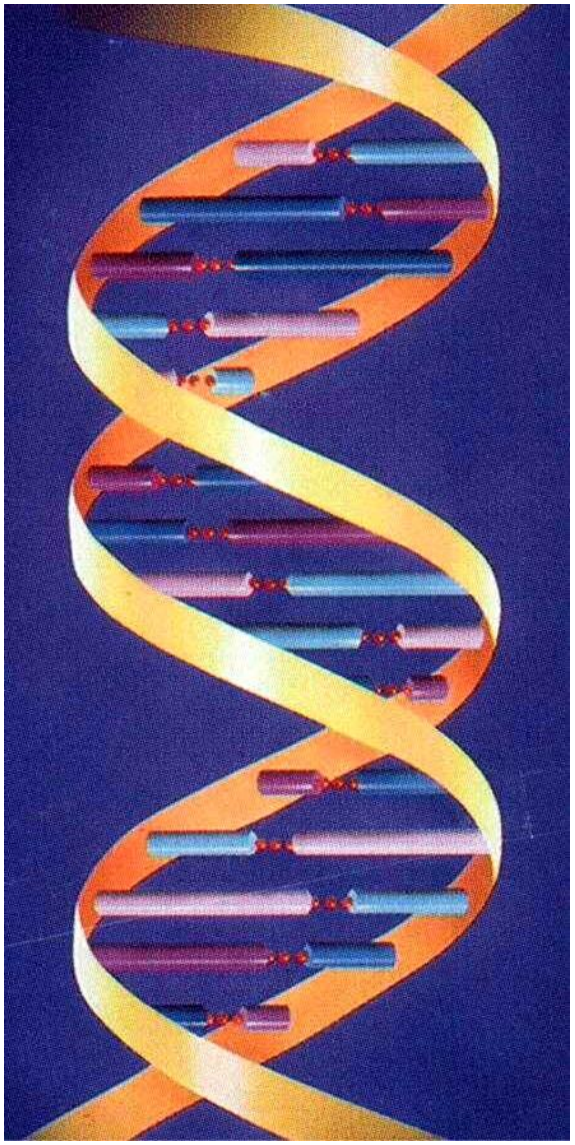
Mutated pRB:

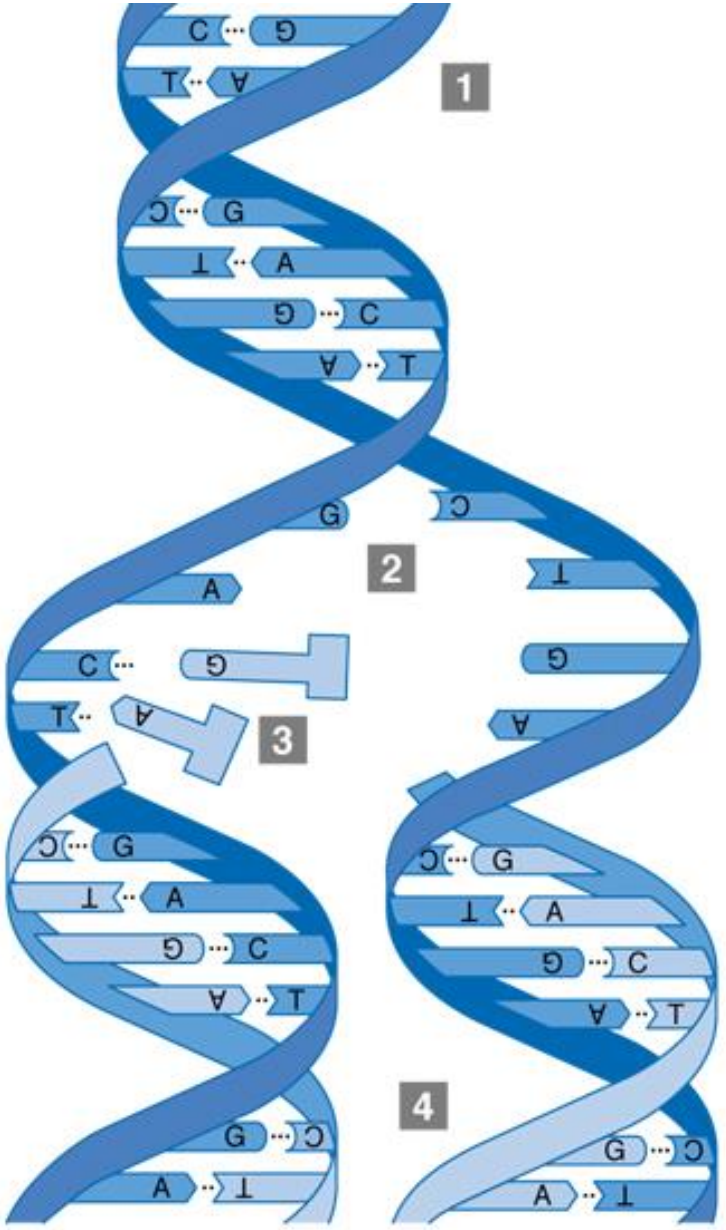


Cdk2
Cyclin E
Cyclin A
etc…

Adapted from Albert's et al. Figure 23-32

http://www.youtube.com/watch?v=l--sYuM1iTs

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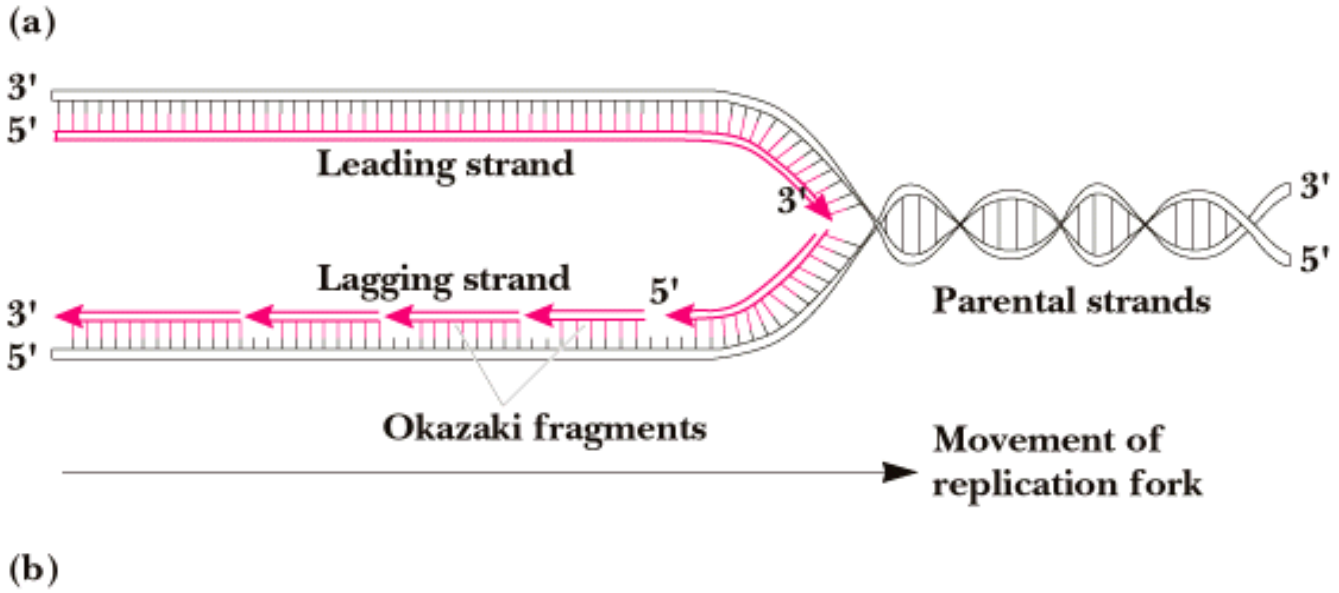
Garrett & Grisham: Biochemistry, 2/e
Figure 30.6



"On the Heredity of Tumors... If tumors can arise in this way, the homologous elements on both chromosomes must be damaged in the same way“

Theodore Boveri, pathologist 1914.

- The importance of Rb-1 and p53 genes in the genesis of tumors has been proven by the identification of mutations of the same genes in people with tumor predisposition syndromes, such as congenital retinoblastoma (Rb-1) and Li Fraumeni multicancer syndrome (p53).
- Inactivation or mutation of one allele of the tumor suppressor gene is not sufficient for tumor development, a change in both loci is required - loss of heterozygosity.

- How is it possible for both copies of a tumor suppressor gene to become nonfunctional, one after the other, during the development of sporadic retinoblastoma, if the probability of such a combination of mutations occurring is extremely low?
- It is almost impossible for successful mutations to occur that will eliminate the functions of both alleles of a tumor suppressor gene.

How retinoblastoma occurs

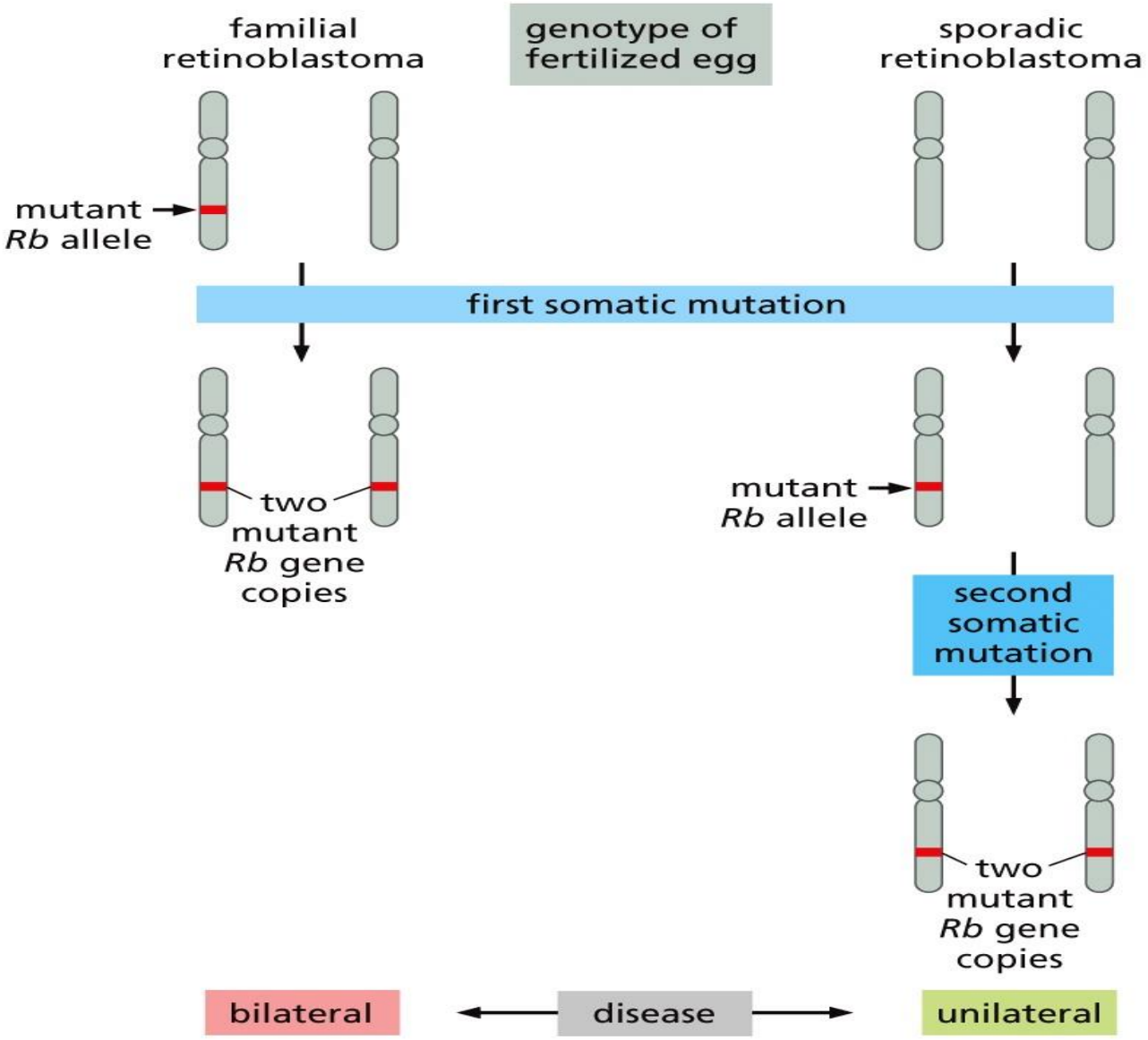
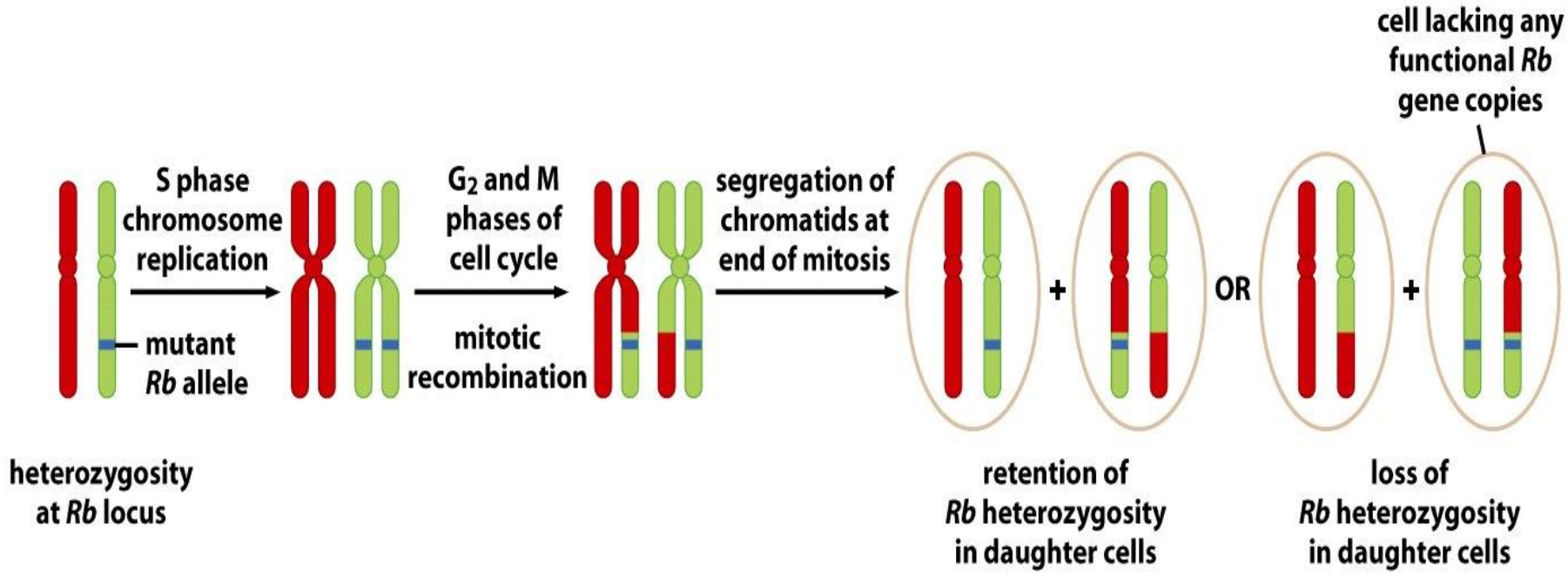


Figure 7.6 The Biology of Cancer (© Garland Science 2014)

- Some geneticists have offered an explanation for this phenomenon:
- If the cell "suffered" a mutation of one of the alleles of the Rb gene, then it is in a "heterozygous conformation", with one wild type and one defective gene - Rb+/-.
- As the mutated allele is recessive, the heterozygous cell retains the normal wild type phenotype.
- It is then possible to exchange genetic information between paired homologous chromosomes, on which there are wild type and defective Rb genes.
- This recombination occurs during proliferation and is called mitotic recombination, to distinguish it from recombination in meiosis.

Elimination of the wild type Rb allele



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Response to extracellular signals during the cell cycle

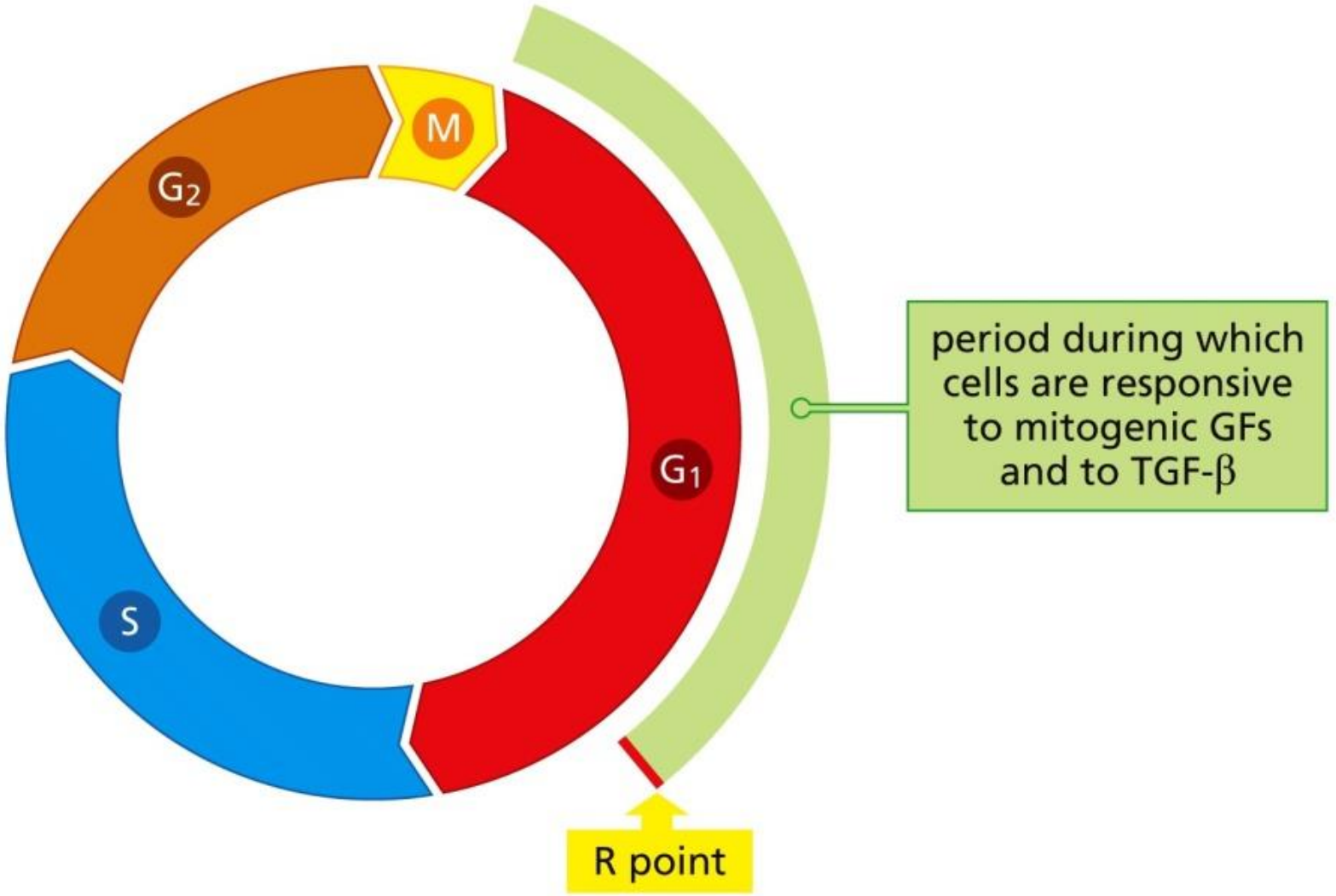


Figure 8.6 The Biology of Cancer (© Garland Science 2014)

Cell cycle progression depends on pRb phosphorylation

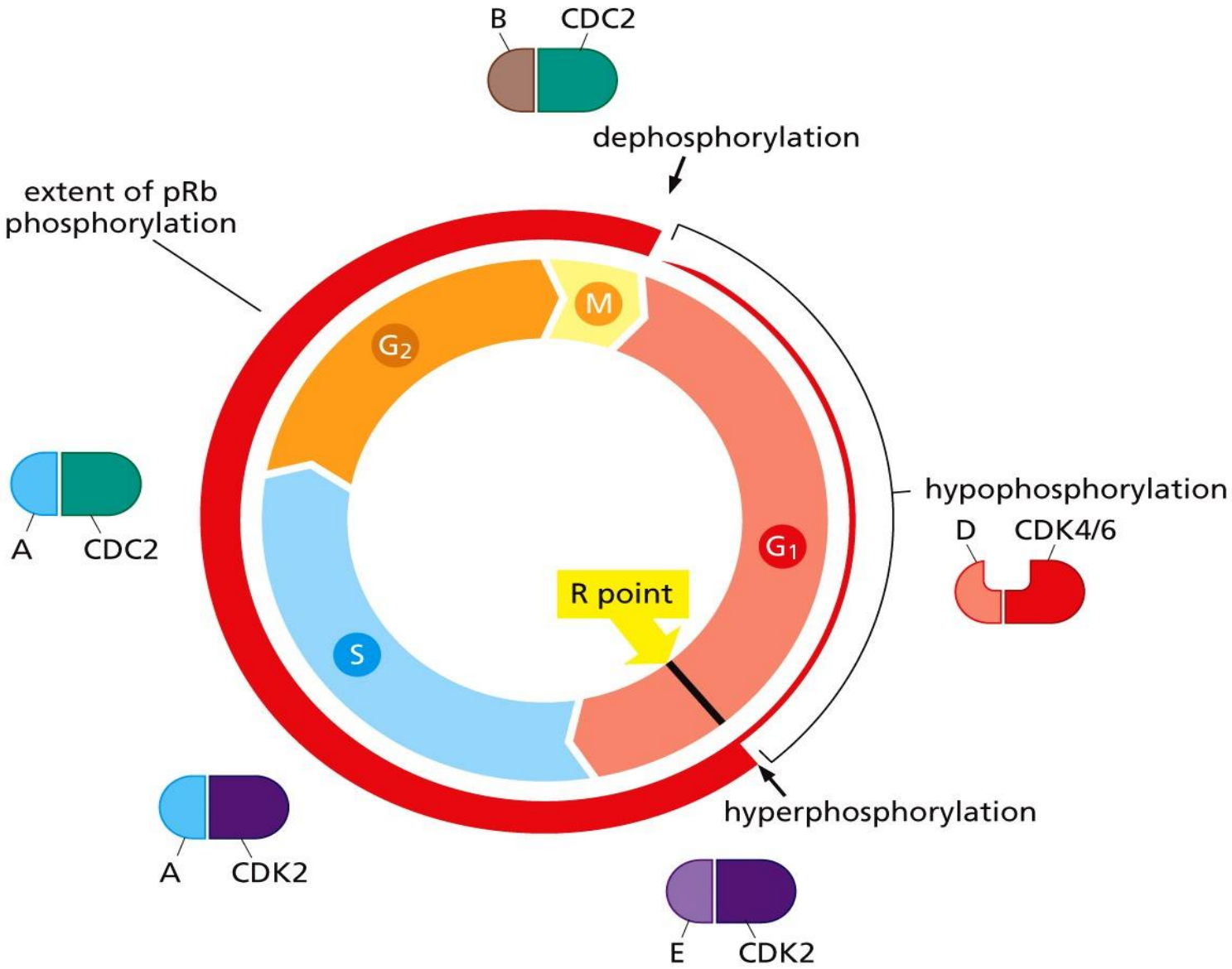


Figure 8.19 The Biology of Cancer (© Garland Science 2014)

Control of transition through the R point by mitogens

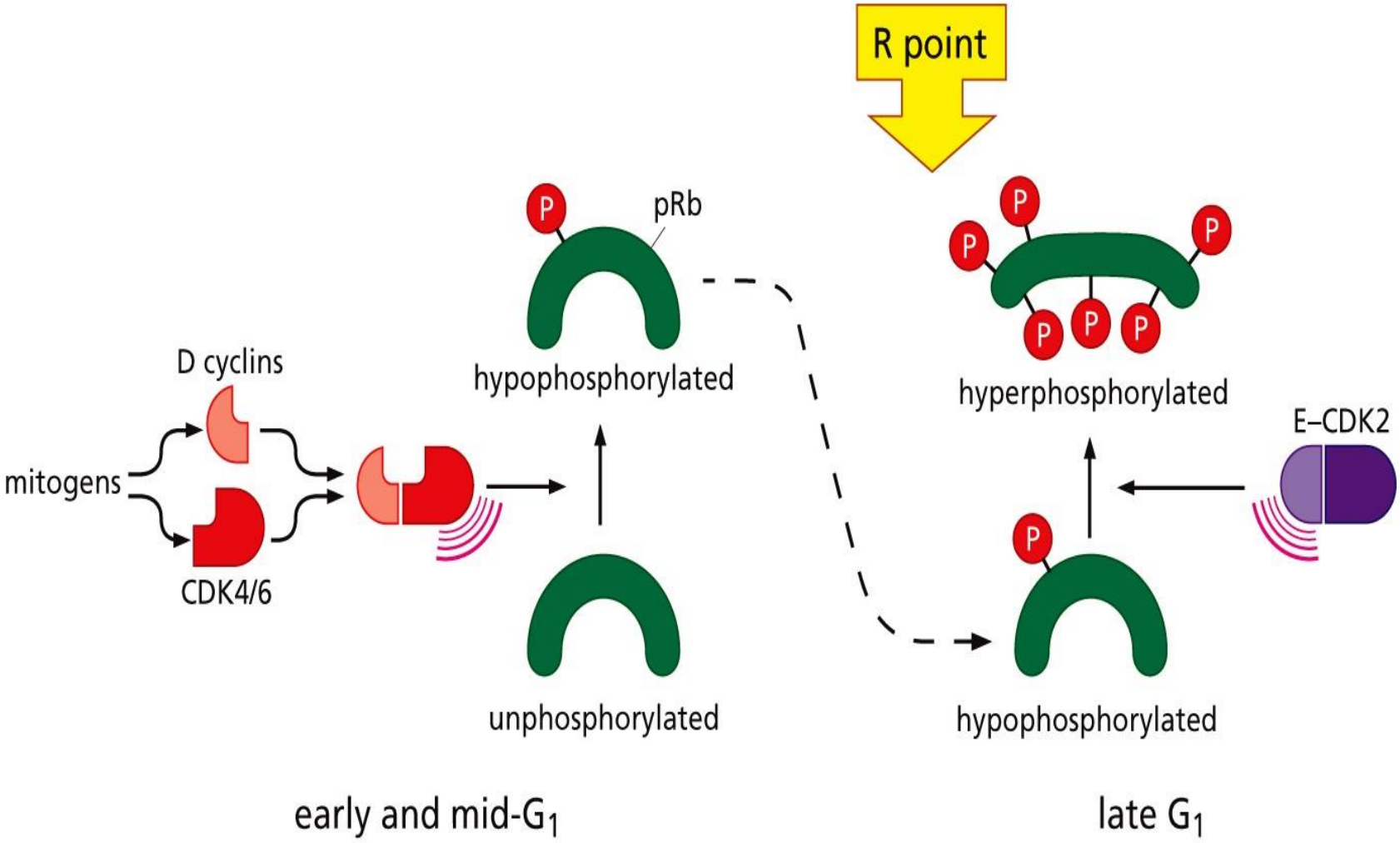


Figure 8.22 The Biology of Cancer (© Garland Science 2014)

Neurofibromatosis

- First described by Friedrich von Recklinghausen in 1862.

- Neurofibromatosis type 1 relatively common familial tumor syndrome, with an incidence of 1 in 3,000 people
- Development of benign tumors of neuron sheath cells, peripheral nervous system. Progression to neurofibrosarcoma, malignant tumors
- Increased risk of glioblastoma (tumor of astrocytes in the CNS), pheochromocytoma and myeloblastic leukemias.

NF1

- Familial tumor syndrome neurofibromatosis type 1 has a genetic background, a mutated allele of a gene called NF1.
- Genetic changes in NF1 correspond to changes in the Rb gene
- Mutated, inactivated allele of the NF1 gene creates the characteristic phenotype of this syndrome
- Heterozygous conformation of the NF1+/- gene converts to the homozygous NF1-/- state in tumor cells, with the loss of heterozygosity.
- Since half of the patients have no family history of the disease, mutations of both NF1 alleles arise de novo.

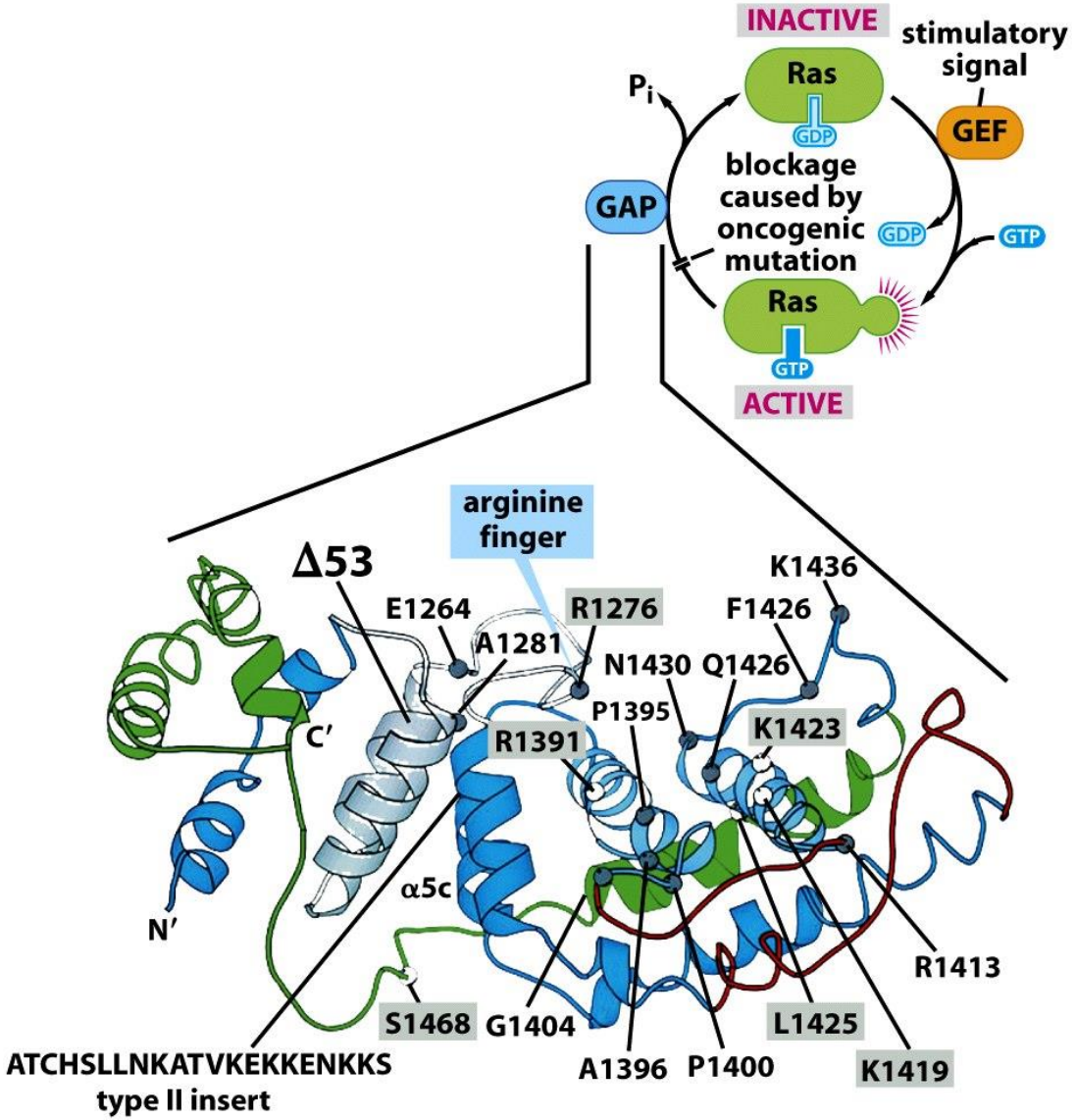
NF1- protein

- Similarity to the IRA protein
- Functions as a GAP for the Ras molecule
- In a growing cell (proliferation), Ras proteins regulate important metabolic and proliferative processes.
- By inducing GTPase activity, IRA converts Ras from an activated to an inactive state, GDP-Ras.
- NF1 in peripheral and CNS cells

NF1- protein

- After growth factor stimulation, NF1 is degraded in the cell, which enables activation of the Ras signaling pathway.
- After 60-90 minutes, the level of cellular NF1 returns to normal and blocks the Ras signaling pathway, in a negative feedback loop.
- In neuroectodermal cells in which NF1 is not functional, the Ras protein is in the active GTP-Ras form significantly longer than in normal cells.

Ras signaling pathway



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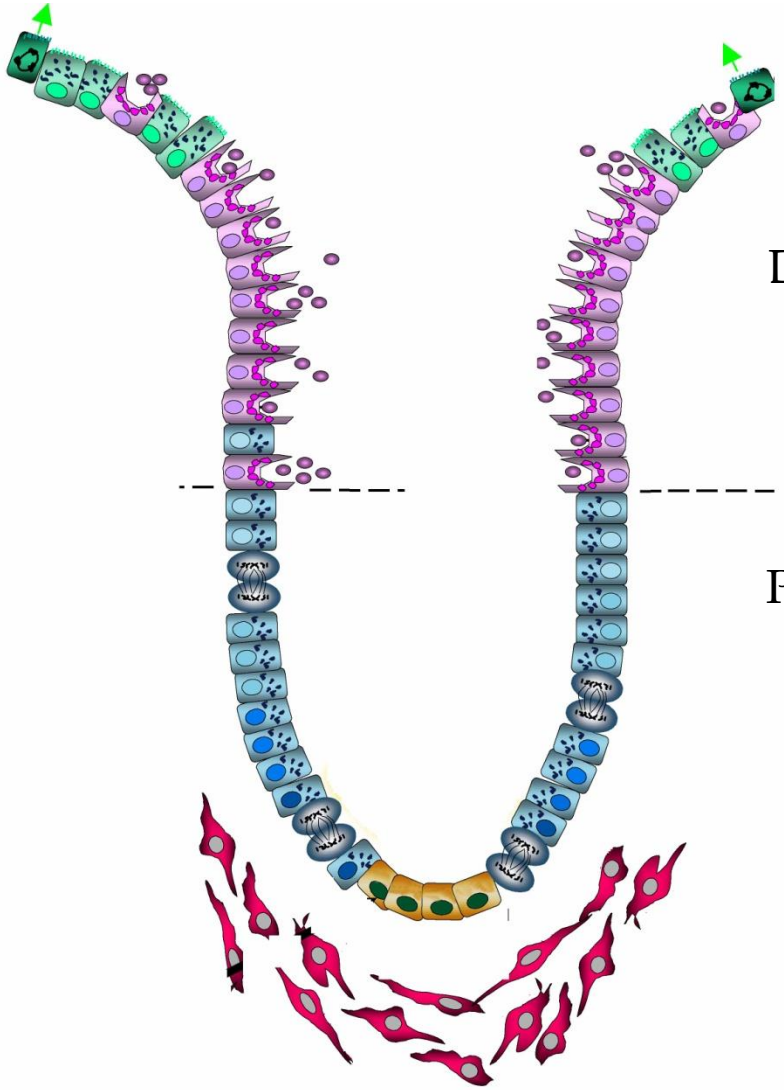
Localization of stem cellsand cells in differentiation in the GIT

- Stem cells are housed and protected at the bottom of recesses called crypts.

- While some of the progenitor cells remain at the bottom of the crypts where they maintain a constant number of stem cells, most of them separate and migrate from the crypts to the surface of the intestinal lumen, where they form the intestinal wall, perform their function, then die by apoptosis and separate into the lumen of the colon.

- The whole process from emergence, through migration, effector function and death takes 3-4 days.

Localization of stem cells and a cells in differentiation



Differentiated cells

villi

Proliferating cells

crypt

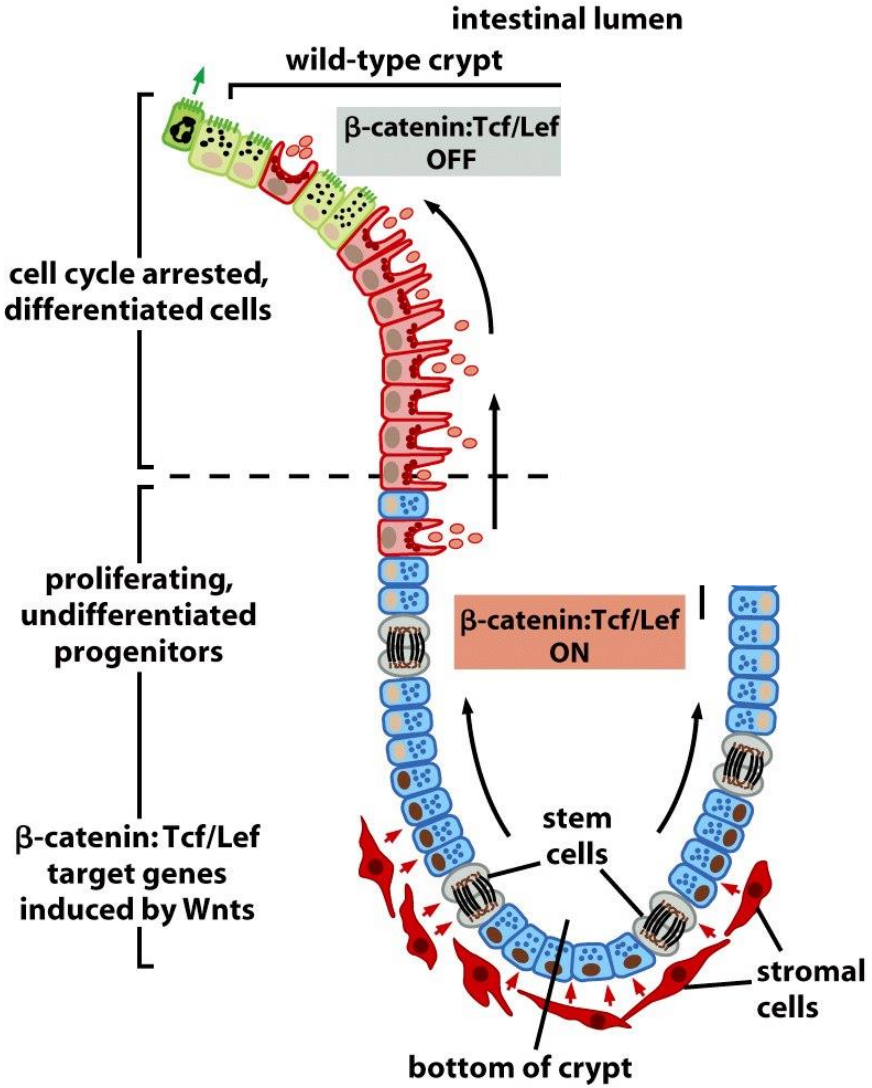
Localization of stem cells and a cells in differentiation

- Cells die within a few days of their formation.
- It is clear that the mutations that lead to the formation of tumors are those that block the migration of epithelial cells from the crypts and cell death.
- Enterocytes whose mutations have enabled them to remain in the crypts will live significantly longer.

Molecular mechanisms controlling the migration of enterocytes from the crypts

- They depend on the expression of intracellular β-catenin.
- Enterocyte stem cells have high levels of intracellular β-catenin.
- These cells maintain the "stem cell state" by maintaining the concentration of intracellular β-catenin.
- When one of the progenitor cells begins to migrate, the amount of β-catenin decrease.
- These cells then lose their stem cell phenotype, exit the cell cycle and differentiate into functional enterocytes.

Molecular mechanisms controlling the migration of enterocytes from the crypts



Adenomatous polyposis coli- Apc

- Apc is the product of the colonic adenomatous polyposis gene and is responsible for the negative control of β-catenin levels in the cytosol.

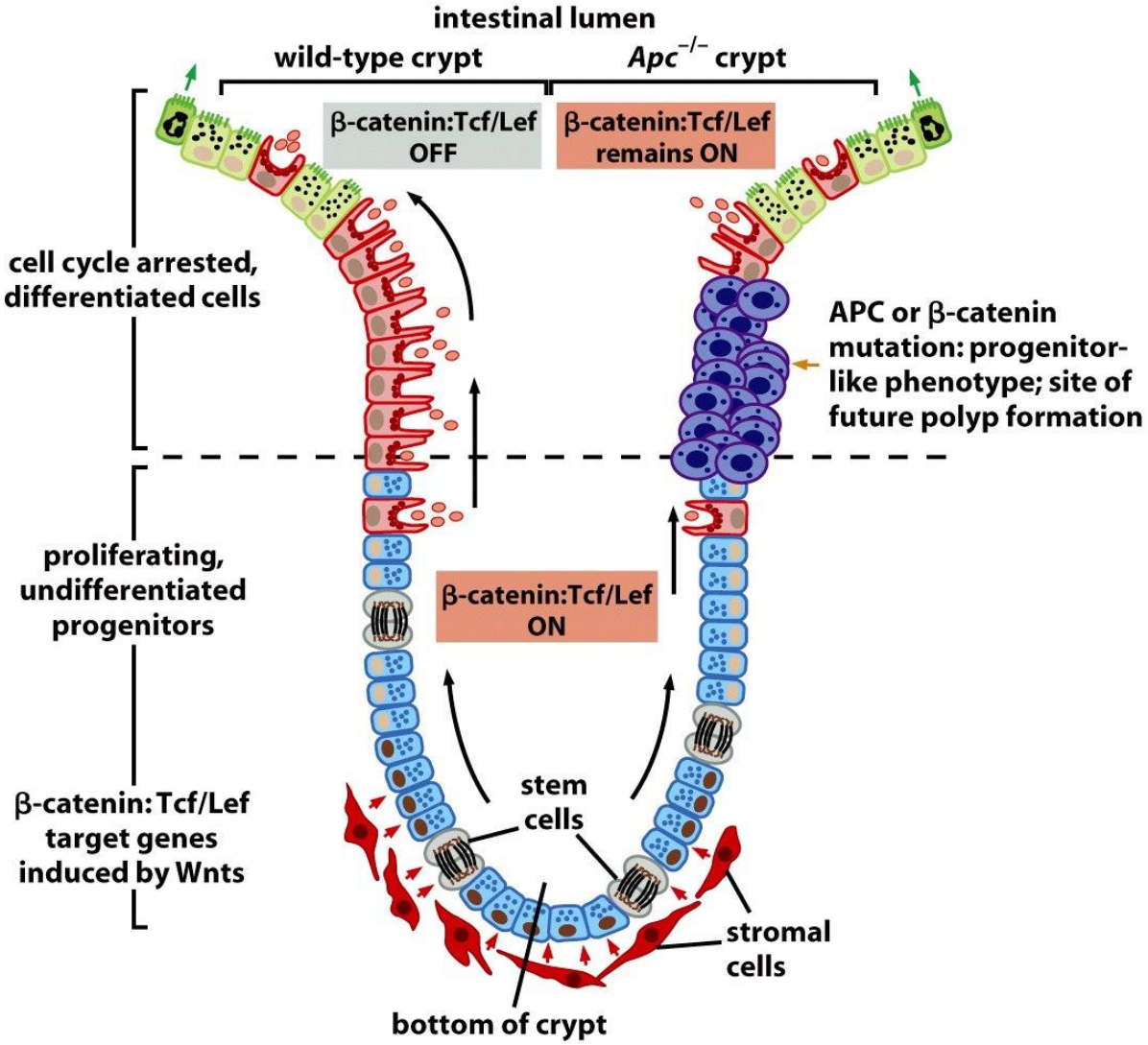
- In cells at the base of normal crypts, Apc genes are not expressed and β-catenin is present in large amounts.

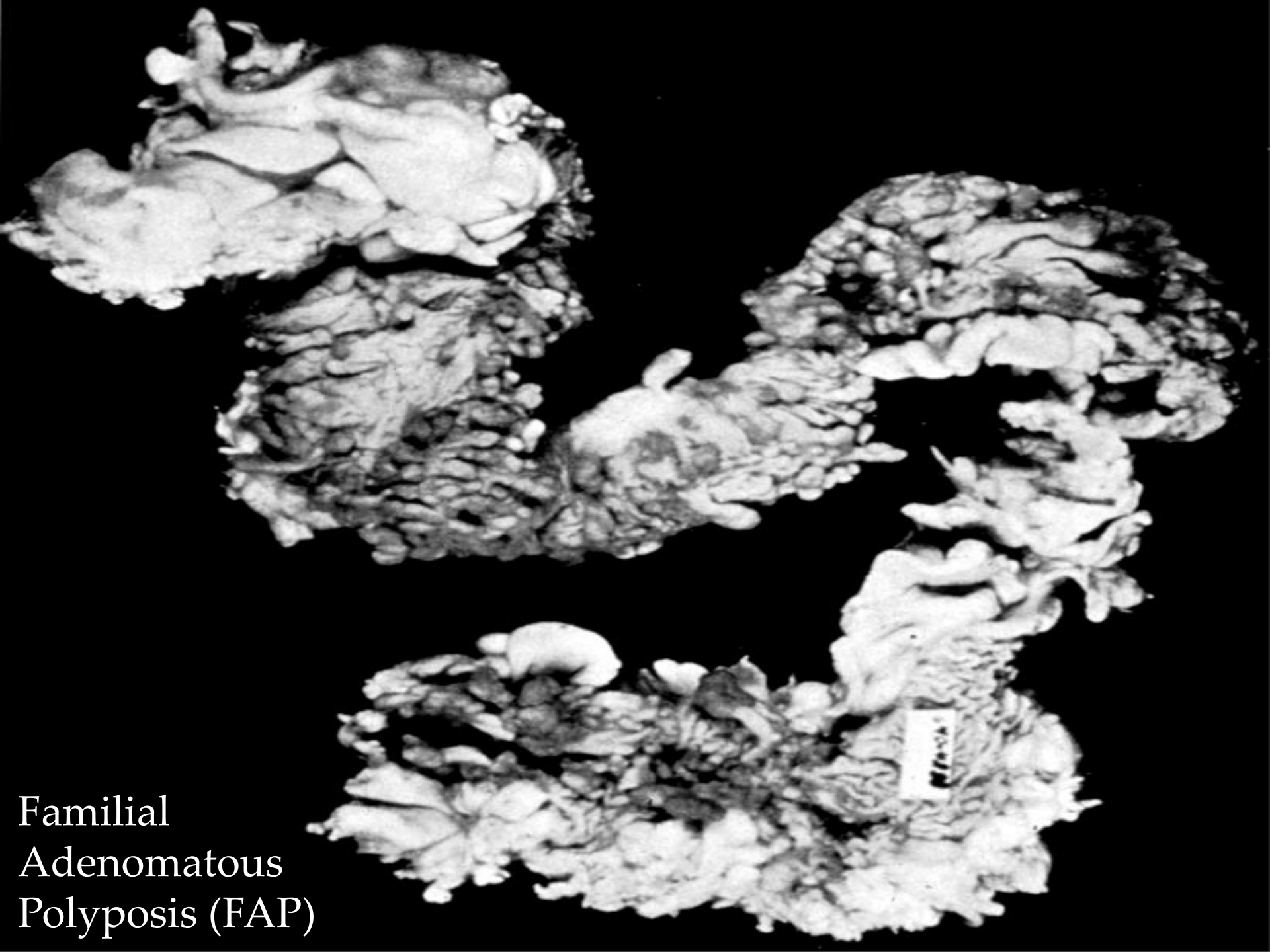
- As the cell migrates out of the crypt, the level of Apc gene expression increases, and the Apc protein decreases the level of β-catenin.

Adenomatous polyposis coli- Apc

- Accumulation of β-catenin is the most significant consequence of Apc gene inactivation, 90% of all sporadic colon cancers.
- When β-catenins accumulate in enterocyte precursors as a consequence of Apc gene inactivation or by some other mechanism, these cells retain a stem cell-like phenotype, which keeps them in the crypts.
- The first of the mutations is the inactivation of Apc.
- Such a cell is "trapped" in the crypt and accumulates mutations of other genes, such as K-ras, which enables faster growth.
- This causes the accumulation of large numbers of relatively undifferentiated cells in the crypts of the colon, which sometimes form adenomatous polyps. Mutations can accumulate in these cells that allow them to form more "aggressive" polyps or cancers.

Molecular mechanisms controlling the migration of enterocytes from the crypts



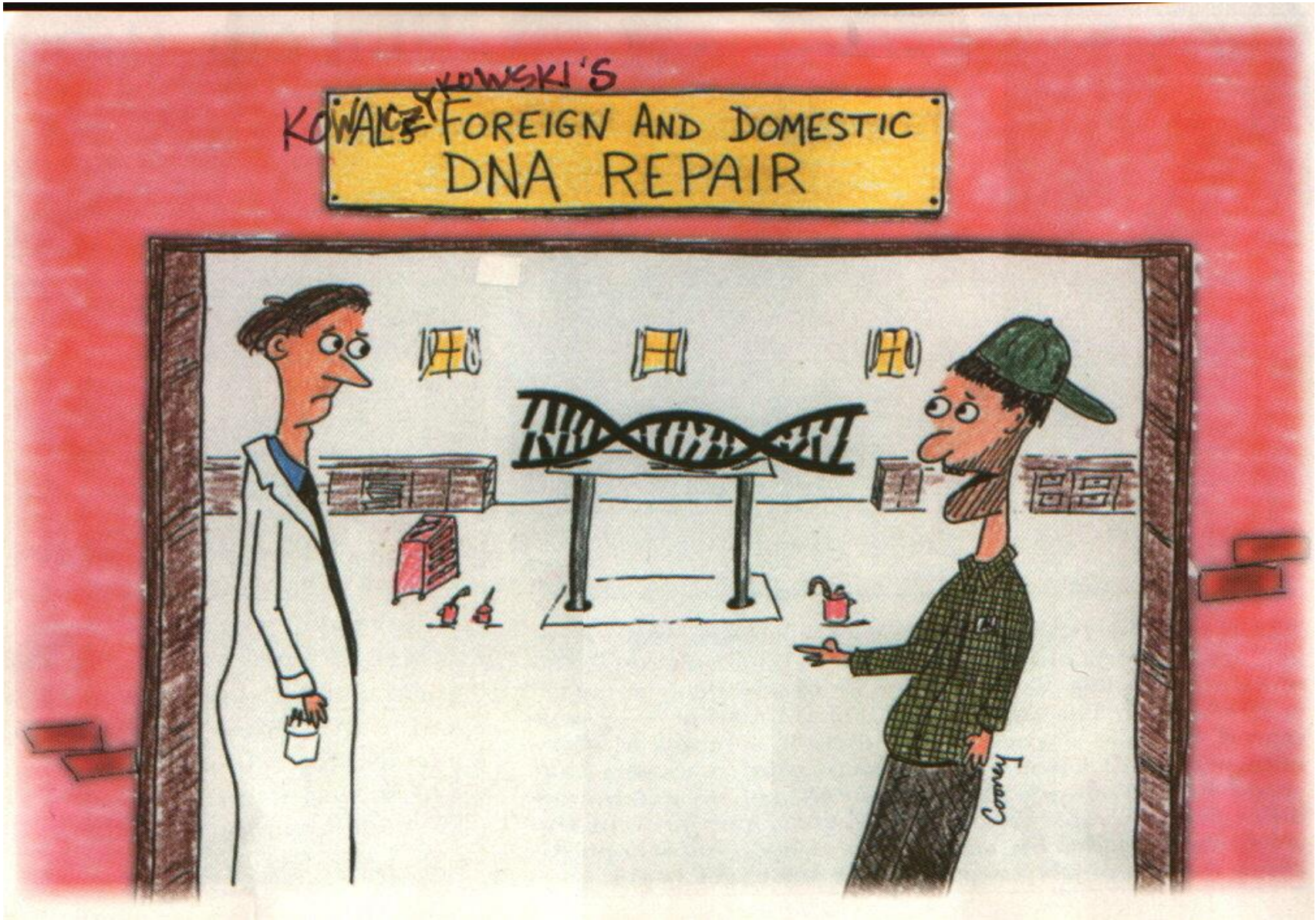


Familial
Adenomatous
Polyposis (FAP)

Adenomatous polyposis coli- Apc

- Apc molecules bind to the microtubules that form the division spindle and are responsible for the separation of chromatids during anaphase and telophase of mitosis.
- Cells without functional Apc are characterized by significantly increased chromosomal instability, accompanied by an increased or decreased number of chromosomes.
- Resulting aneuploidy changes the relative number of critical tumor-promoting and tumor-inhibiting genes.

DNA repair system



BRCA1 and BRCA2

- **BRCA1 (breast cancer 1) is expressed in the cells of the mammary gland but also in other tissues where it helps to repair damaged DNA or to destroy the cell if the DNA cannot be repaired.**

- **Repairs DNA double helix breaks. Most often, DNA strand breaks are single (only one of the 2 strands), but sometimes the break is complete (both strands).**

- **BRCA1 forms part of a protein complex that repairs DNA when both strands are broken. When the DNA is completely broken it is difficult to insert the appropriate nucleotides (no missing sequence is recorded). Repair by the BRCA1 protein involves homologous recombination. The repair system uses the sequence of nucleotides from the sister chromatid (homologous chromosome).**

BRCA1 and BRCA2

- ATM detects DNA damage → BRCA ½phosphorylation by ATM-kinase → p53 → p21 → cell cycle arrest.

- BRCA 1/2 also interact with estrogen receptors (suppressing their function) as well as with the promoter of the c-Mys oncogene (suppressing the expression of this oncogene).

BRCA1 and BRCA2

- Although the structures of the BRCA1 and BRCA2 genes are different, the functions of the proteins they encode are similar - repairing damaged DNA.

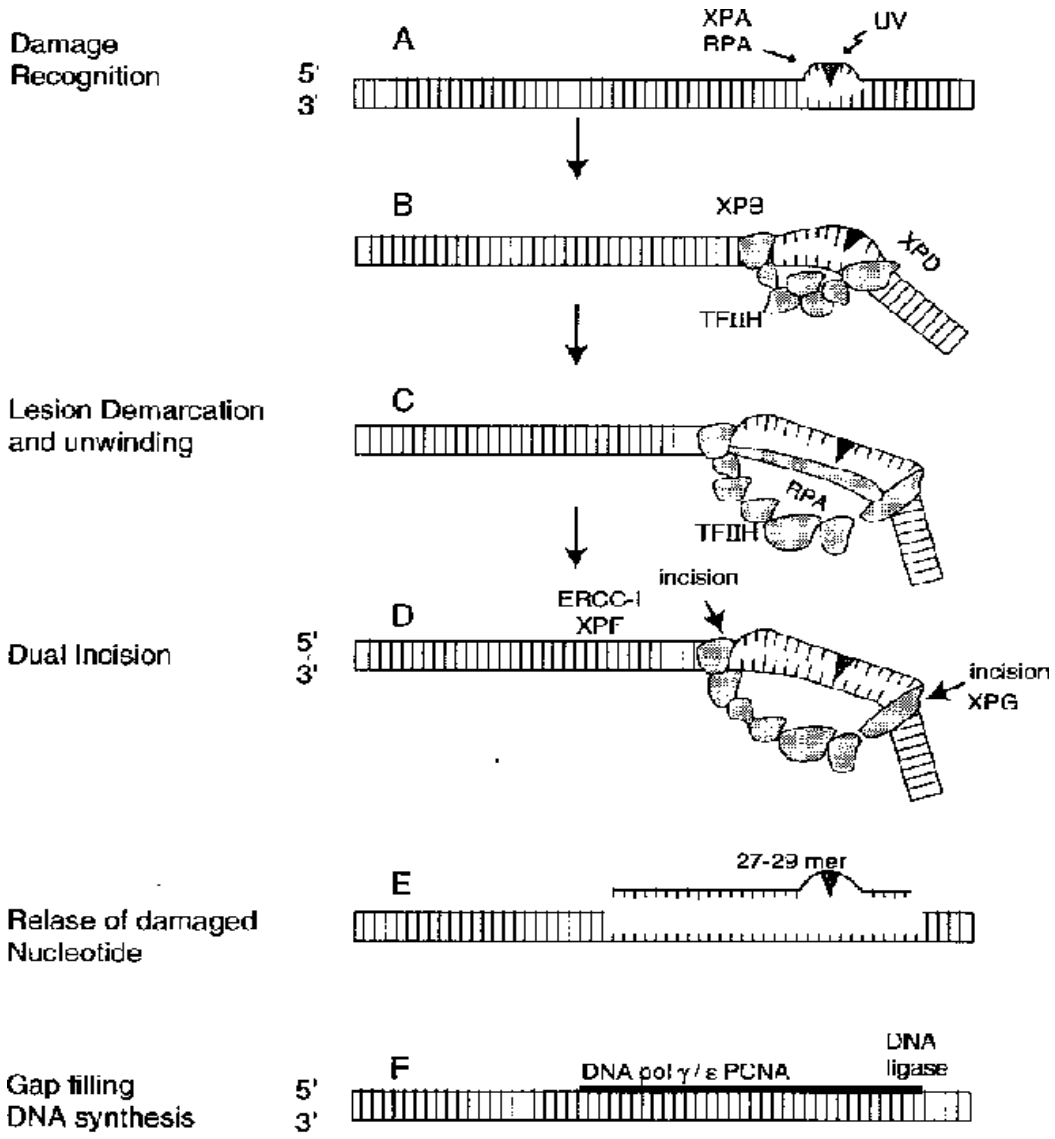
- If BRCA1 and BRCA2 are damaged and dysfunctional, the damaged DNA cannot be repaired, which increases the risk of tumor formation.

- Mutations of these genes increase the possibility of breast and ovarian cancer.

BRCA1 and BRCA2

- 50-65% of women born with a BRCA 1/2 gene mutation will develop breast cancer by age 70
- 35-46% will get ovarian cancer
- Breast cancers associated with BRCA gene dysfunction are much more aggressive than normal breast cancers.
- Most often, they are negative for hormone receptors
- They appear on average 20 years earlier than other forms of breast cancer.

DNA repair system



Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
RUNX3	1p36	—	gastric carcinoma	TF co-factor
HRPT2	1q25–32	parathyroid tumors, jaw fibromas	parathyroid tumors	chromatin protein
FH	1q42.3	familial leiomyomatosis[a]	—	fumarate hydratase
FHIT	3p14.2	—	many types	diadenosine triphosphate hydrolase
RASSF1A	3p21.3	—	many types	multiple functions
TGFBR2	3p2.2	HNPCC	colon, gastric, pancreatic carcinomas	TGF-β receptor
VHL	3p25	von Hippel–Lindau syndrome	renal cell carcinoma	ubiquitylation of HIF
hCDC4	4q32	—	endometrial carcinoma	ubiquitin ligase
APC	5p21	familial adenomatous polyposis coli	colorectal, pancreatic, and stomach carcinomas; prostate carcinoma	β-catenin degradation
NKX3.1	8p21	—	prostate carcinoma	homeobox TF
$p16^{INK4A}$ [b]	9p21	familial melanoma	many types	CDK inhibitor
$p14^{ARF}$ [c]	9p21	—	all types	p53 stabilizer
PTC	9q22.3	nevoid basal cell carcinoma syndrome	medulloblastomas	receptor for hedgehog GF
TSC1	9q34	tuberous sclerosis	—	inhibitor of mTOR[f]
BMPR1	10q21–22	juvenile polyposis	—	BMP receptor
PTEN[d]	10q23.3	Cowden's disease, breast and gastrointestinal carcinomas	glioblastoma; prostate, breast, and thyroid carcinomas	PIP_3 phosphatase
WT1	11p13	Wilms tumor	Wilms tumor	TF
MEN1	11p13	multiple endocrine neoplasia	—	histone modification, transcriptional repressor

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
BWS/CDKN1C	11p15.5	Beckwith–Wiedemann syndrome	—	$p57^{Kip2}$ CDK inhibitor
SDHD	11q23	familial paraganglioma	pheochromocytoma	mitochondrial protein[e]
RB	13q14	retinoblastoma, osteosarcoma	retinoblastoma; sarcomas; bladder, breast, esophageal, and lung carcinomas	transcriptional repression; control of E2Fs
TSC2	16p13	tuberous sclerosis	—	inhibitor of mTOR[f]
CBP	16p13.3	Rubinstein–Taybi	AML[g]	TF co-activator
CYLD	16q12–13	cylindromatosis	—	deubiquitinating enzyme
CDH1	16q22.1	familial gastric carcinoma	invasive cancers	cell–cell adhesion
BHD	17p11.2	Birt–Hogg–Dube syndrome	kidney carcinomas, hamartomas	unknown
TP53	17p13.1	Li–Fraumeni syndrome	many types	TF
NF1	17q11.2	neurofibromatosis type 1	colon carcinoma, astrocytoma	Ras-GAP
BECN1	17q21.3	—	breast, ovarian, prostate	autophagy
PRKAR1A	17.q22–24	multiple endocrine neoplasia[h]	multiple endocrine tumors	subunit of PKA
DPC4[i]	18q21.1	juvenile polyposis	pancreatic and colon carcinomas	TGF-β TF
LKB1/STK11	19p13.3	Peutz–Jegher syndrome	hamartomatous colonic polyps	serine/threonine kinase
RUNX1	21q22.12	familial platelet disorder	AML	TF
SNF5[j]	22q11.2	rhabdoid predisposition syndrome	malignant rhabdoid tumors	chromosome remodeling
NF2	22q12.2	neurofibroma-position syndrome	schwannoma, meningioma; ependymoma	cytoskeleton–membrane linkage