Genes Supresores de Tumor

Dra. María Marcela Barrio
Centro Investigaciones Oncológicas – FUCA

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“Gatekeeper” genes

- **The “gatekeeper” gene monitor cell proliferation and death. They include:**

-”oncogenes” which are cell growth promoters, where dominant mutations lead to uncontrolled growth, and resistance to apoptosis.

- “tumor suppressor” genes which are negative regulators of cell growth, where recessive mutation can lead to uncontrolled cell growth (e.g., p53).

“Caretaker” genes

- **The “caretaker” genes include genes that regulate DNA repair and chromosome segregation in mitosis. Mutation of these genes can cause abnormal “gatekeeper” function because of mutation or abnormal gene content. The “caretaker” genes include:**

- DNA repair genes, whose mutations lead to genetic instability (more mutations).

- genes that regulate chromosome segregation, whose mutations lead to abnormal chromosome content, or chromosomal instability.

Tumor suppressor genes

- First discovered in 1960s by Henry Harris.
- Harris fused tumor cells with normal cells and discovered some of the hybrid cells were normal.
- Harris hypothesized that the normal cells contained gene products that suppressed uncontrolled cell proliferation.
- Some cancers show deletions of specific sites (tumor repressor genes) that normally inhibit cell growth and division.

e.g., breast cancer, colon cancer, lung cancer

- Two mutations (one on each allele) are required to inactivate tumor suppressor genes.

Tumor suppressor genes

Definition:

1- its function is lost in the development of the tumor

2- its in vivo inactivation enhances initiation, growth, or progression of a tumor

Tumor suppressor genes

Properties of "classic" tumor suppressor genes

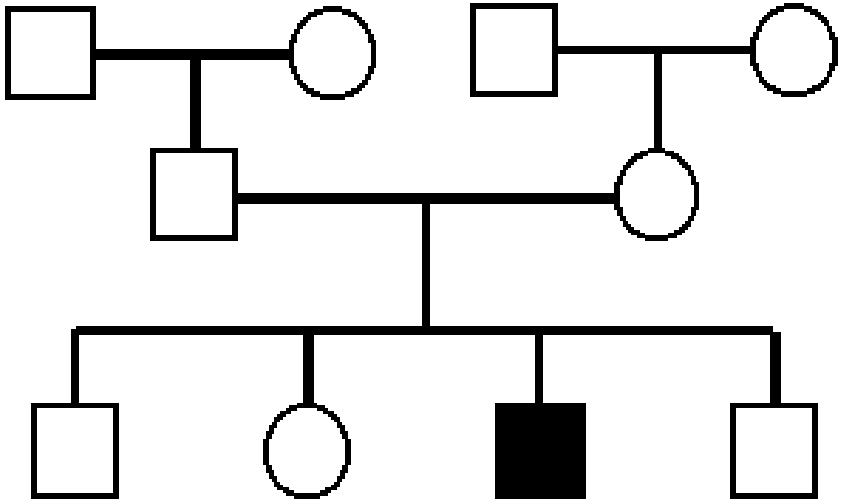
1- They are recessive and undergo biallellic inactivation

2- The inheritance of a single mutant allele accelerates tumor susceptibility, because only one additional mutation is required for complete loss of gene

3- The same gene is frequently inactivated in sporadic cancers

Sporadic and familial (Mendelian) forms of cancer
Knudson's two-hit hypothesis

Sporadic



Normal tumor suppressor gene

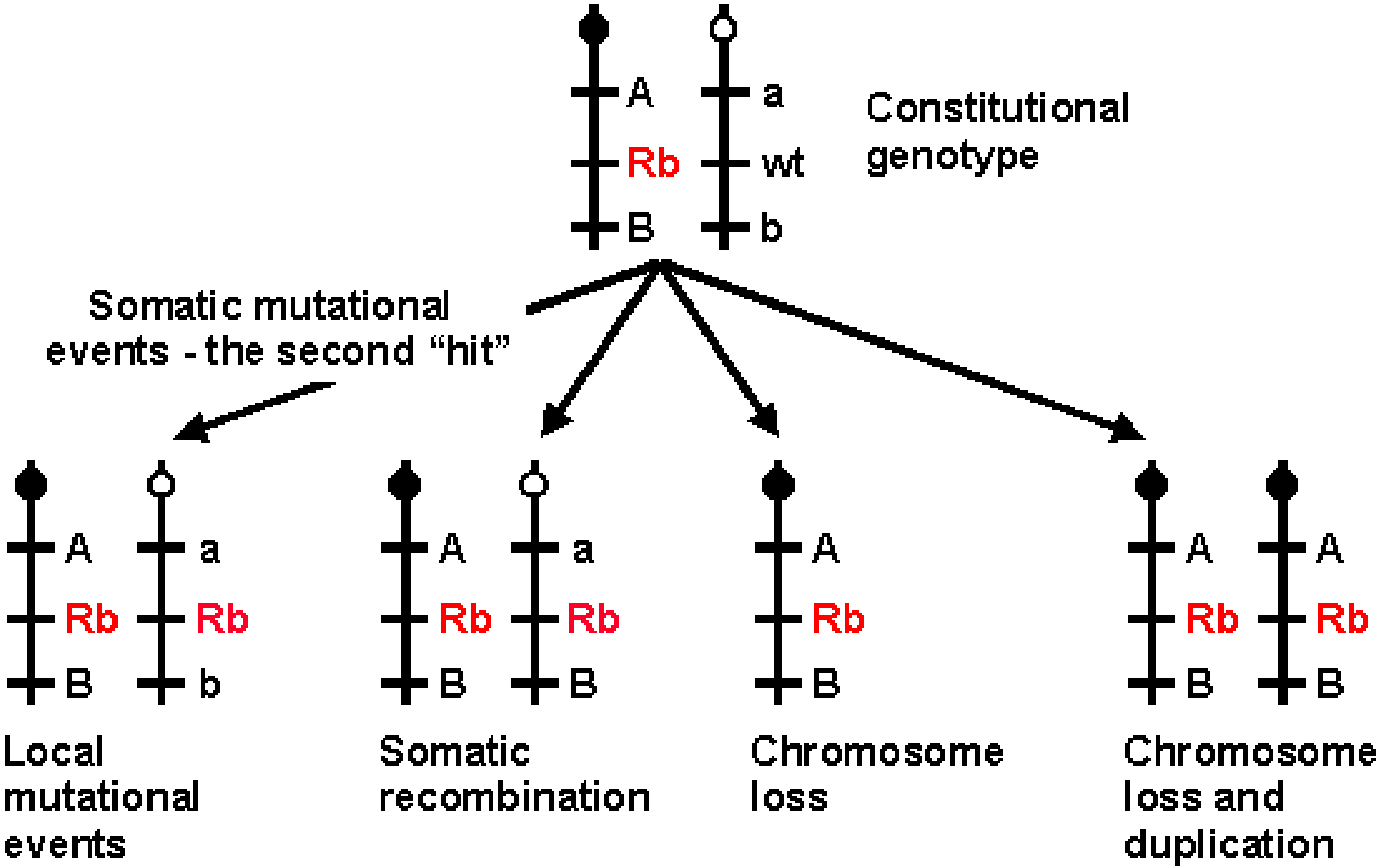
Somatic mutation in one allele

Somatic mutation in other allele

Single tumors, unilateral, later-onset

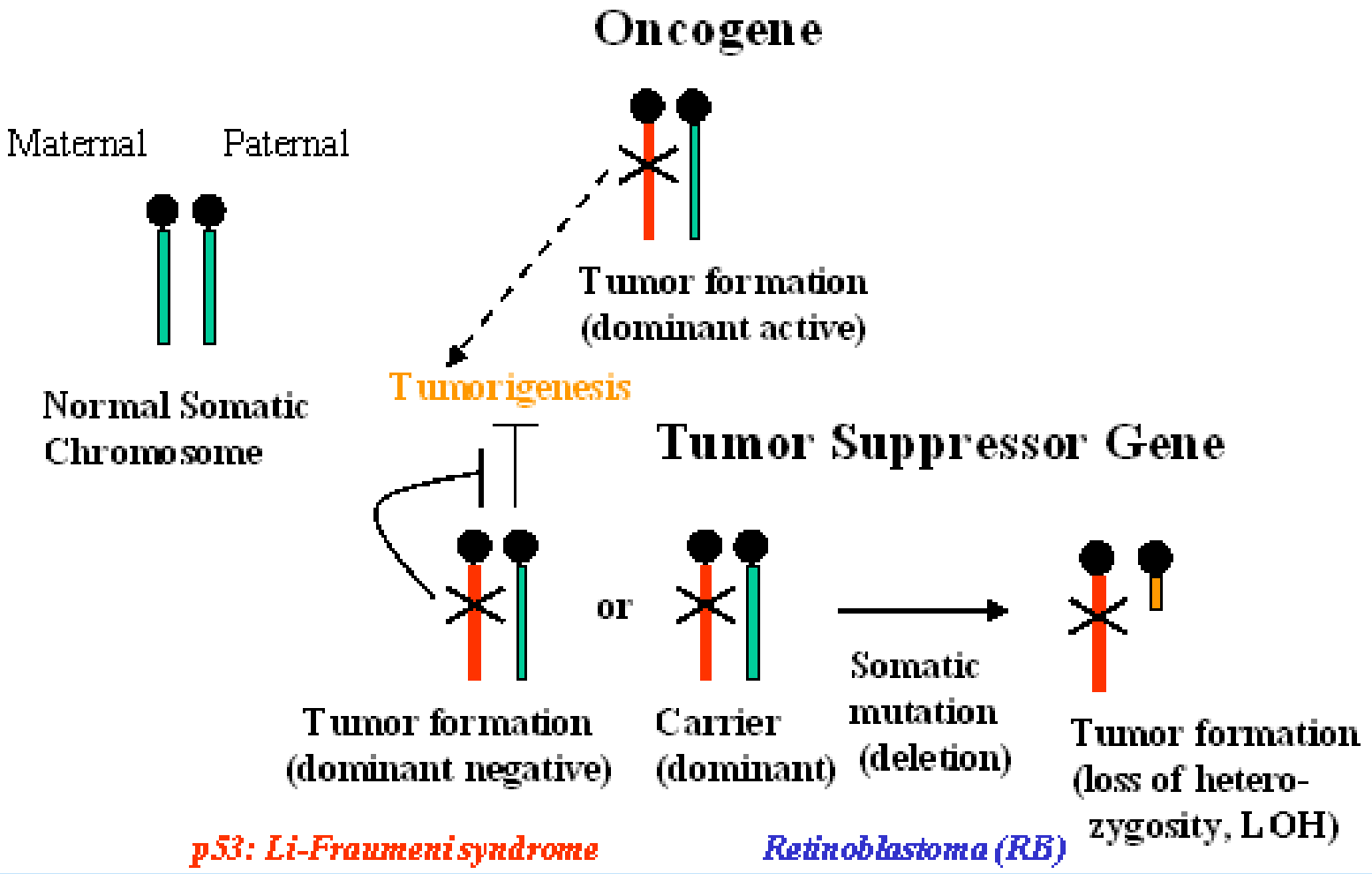
- two mutations (two hits) are required for loss of tumor suppressor function

Chromosomal mechanisms that could lead to loss of function due to loss of heterozygosity



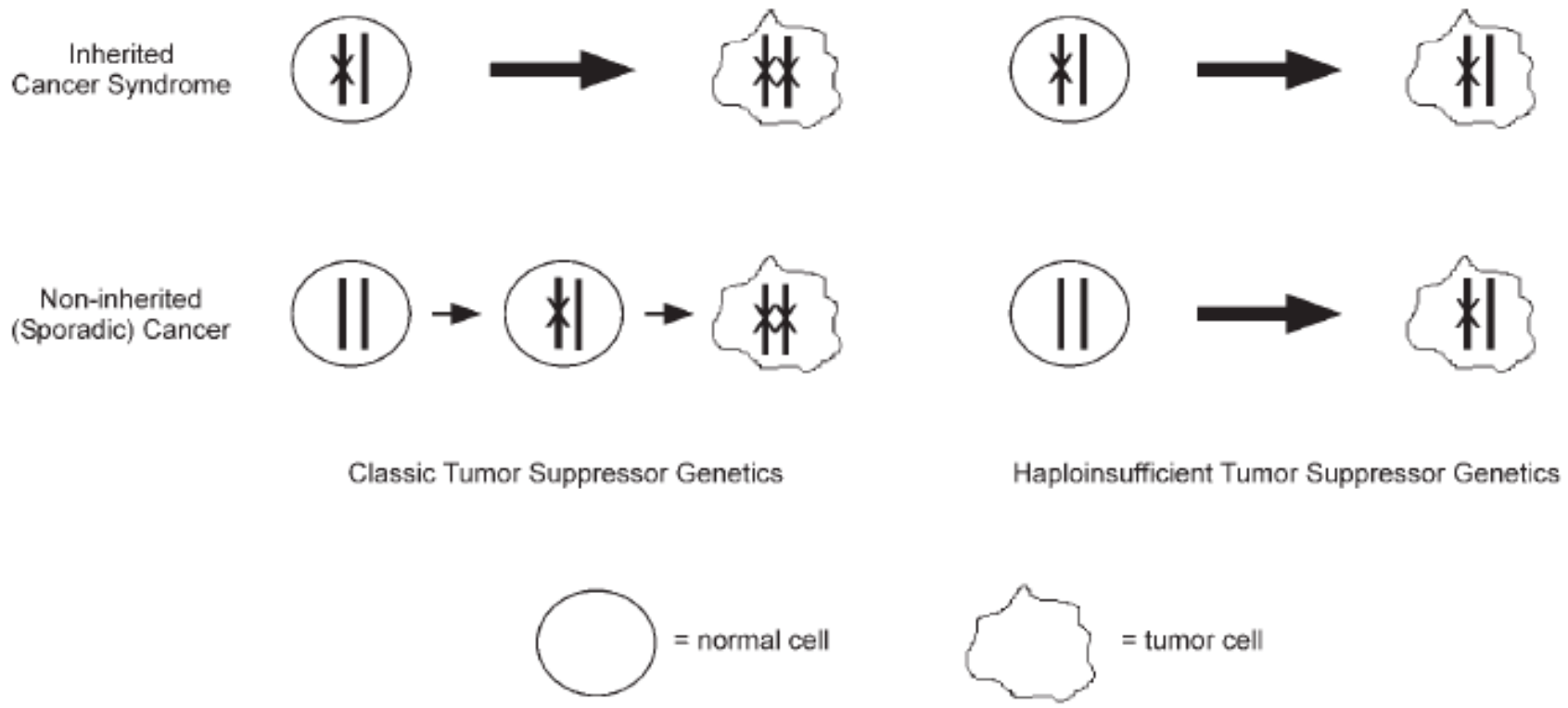
Tumor genotypes in retinoblastoma

Theory of Tumor-prone Autosomal Dominatnt Mutations (Knudson's "two-hit" theory)



A mutation in one of two copies (alleles) of an oncogene may be sufficient to cause the cell to lose growth control, both copies of a tumor suppressor gene must be knocked out to induce the same effect

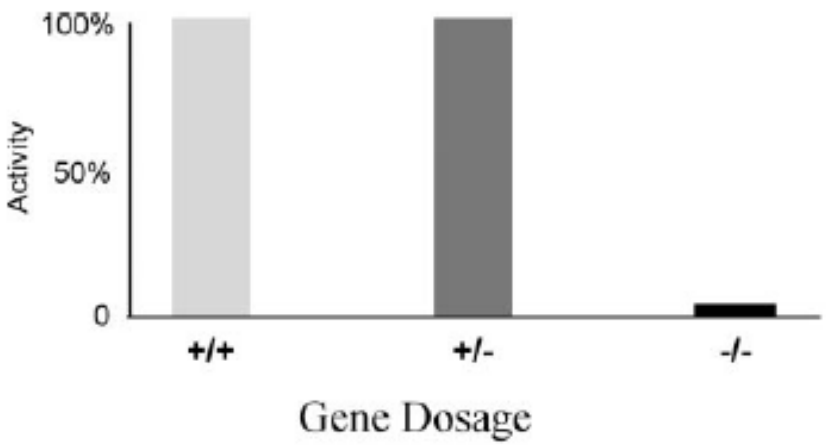
Inactivation of Tumor Suppressor Genes.



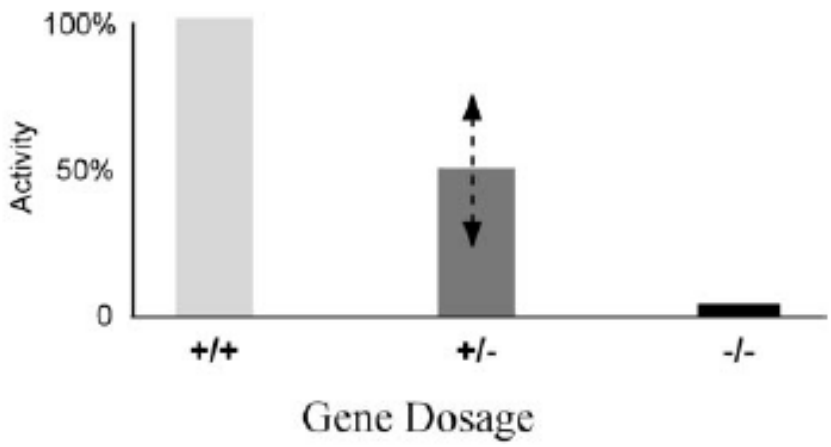
Classic tumor suppressor genes are inactivated via 'two hits'. In the case of inherited cancer susceptibility, one of these 'hits' is acquired in the germline with the second 'hit' being acquired somatically during tumor development.

Haploinsufficient tumor suppressor genes are compromised by a single 'hit', obviating the need to sustain 'two hits' during the course of tumor development.

Tumor suppressor activitiy versus gene dosage



"Two Hit" Tumor Suppressor



Haploinsufficient Tumor Suppressor

Wild type (+/+) activity represents 100% of diploid gene function and true null (-/-) represents complete loss of functionality.

Haploinsufficient tumor suppressors exhibit a continuum of activity based on gene dosage with even 50% reduction sufficient for phenotypic manifestation, i.e. accelerated tumorigenesis.

While some genes may be less dosage-sensitive than others (in which a true threshold of close to 0% of normal gene product is required in order to detect a phenotype), we predict that most genes will be sensitive to dosage with some threshold that varies on a continuum between 0 and 100%.

Definition of Penetrance:

Complete Penetrance: Every individual who inherits a mutated gene develops the related disease

100% Incidence of Huntington Disease

Ex: Huntington disease

100% Normal Individuals

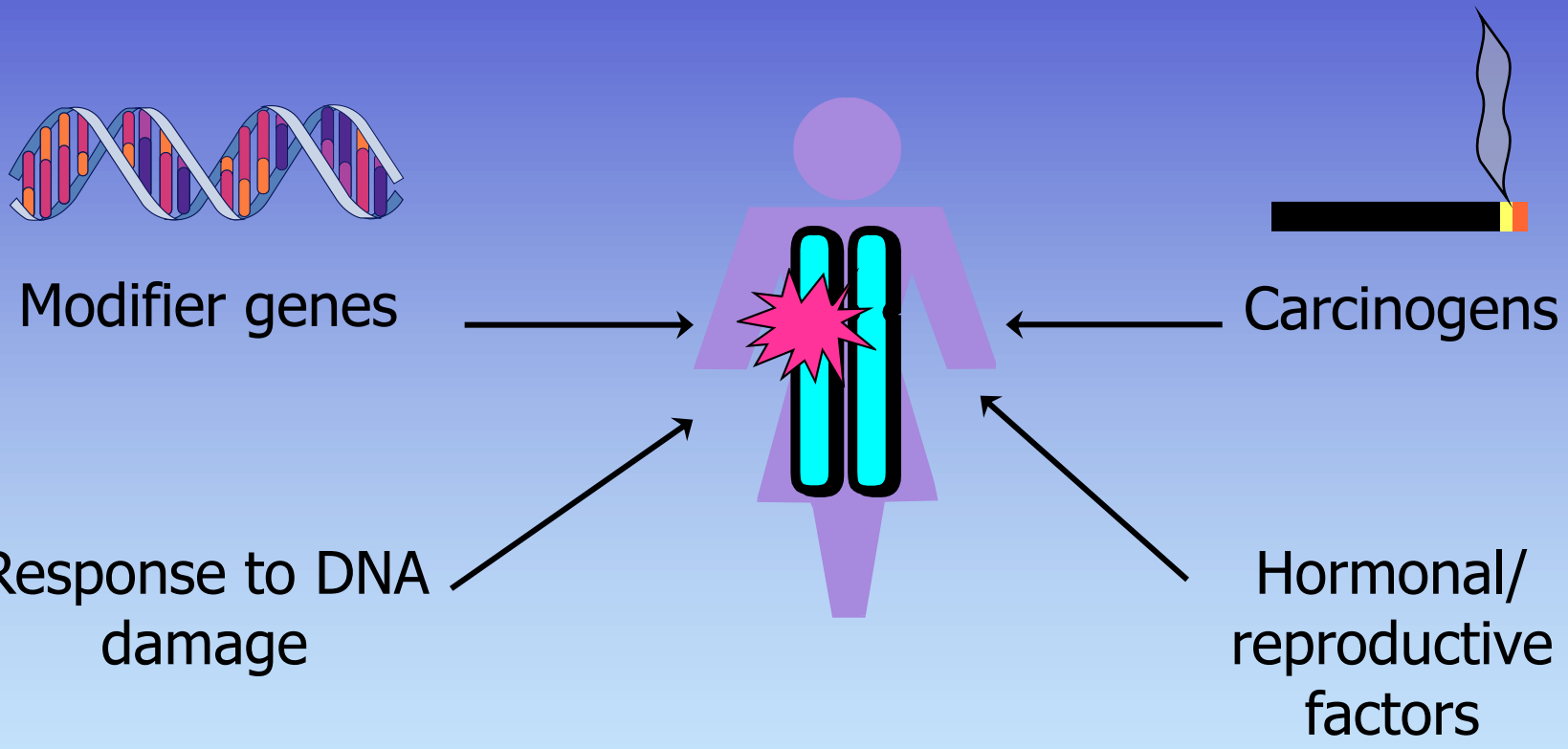
Incomplete Penetrance: Less than 100% of the carriers of the mutation develop the disease

Greater probability of developing breast cancer, but another mutant factor (from environment, other genes) is also needed

Ex: BRCA1 Mutation:

May develop sporadic breast cancer

Factors Affecting Penetrance



Not everyone with an altered gene develops cancer

Loss of Heterozygosity

- oncogene activation at one allele --> e.g. activity increase 50 fold --> provide growth advantage --> second allele activation --> activity increase two fold further --> probably provide no further selection advantage. 2 => 51 => 100 activity change
- tumor suppressor gene inactivation at first allele --> activity decrease half --> possibly provide no growth advantage --> inactivation of second allele --> complete loss of activity --> selection favored. 2=> 1 => 0 activity change.

Mechanisms of Tumor Suppression

- **Suppression of Cell Division**

Ej: retinoblastoma protein (Rb), adenomatosis polyposis coli (APC), alternate reading frame (ARF), RIZ1, p15, p16, p18, p19, p21, p27 and p53

- **Induction of Apoptosis**

Ej: p53, APC, cluster of differentiation 95 (CD95), bridging integrator 1 (Bin1), Deleted in colorectal carcinoma (DCC) and phosphatase and tensin homolog (PTEN)

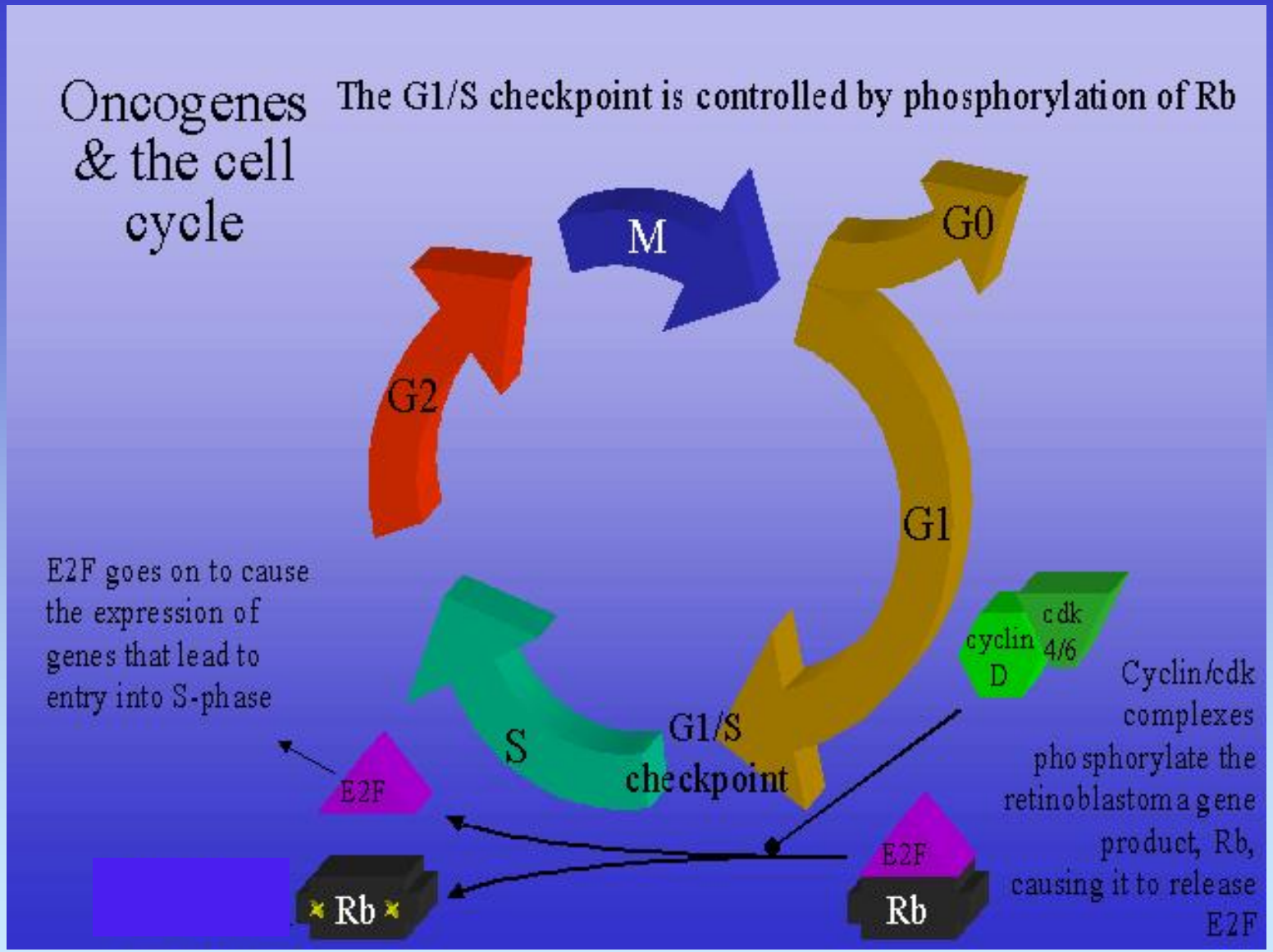
- **DNA Damage Repair**

Ej: mutS homolog 2 (MSH2), mutL homolog 1 (MLH1), Ataxia-telangiectasia Mutated gene product (ATM), breast cancer proteins (BRCA 1-2), Nijmegen breakage syndrome 1 (NBS1), Fanconi-Anemia–related tumor suppressor (FA) And p53

- **Inhibition of Metastasis**

Ej: metastin, breast cancer metastasis suppressor 1 (BRMS1), tissue inhibitor of metalloproteinase (TIMP), cofactor required for specificity protein 1 activation (CRSP) and KAL1/CD82

Oncogenes & the cell cycle



Mechanisms of Tumor Suppression

Suppression of Cell Division

❖ is the main mechanism for most tumor suppressors (Rb, APC, ARF, RIZ1, p15, p16, p18, p19, p21, p27 and p53)

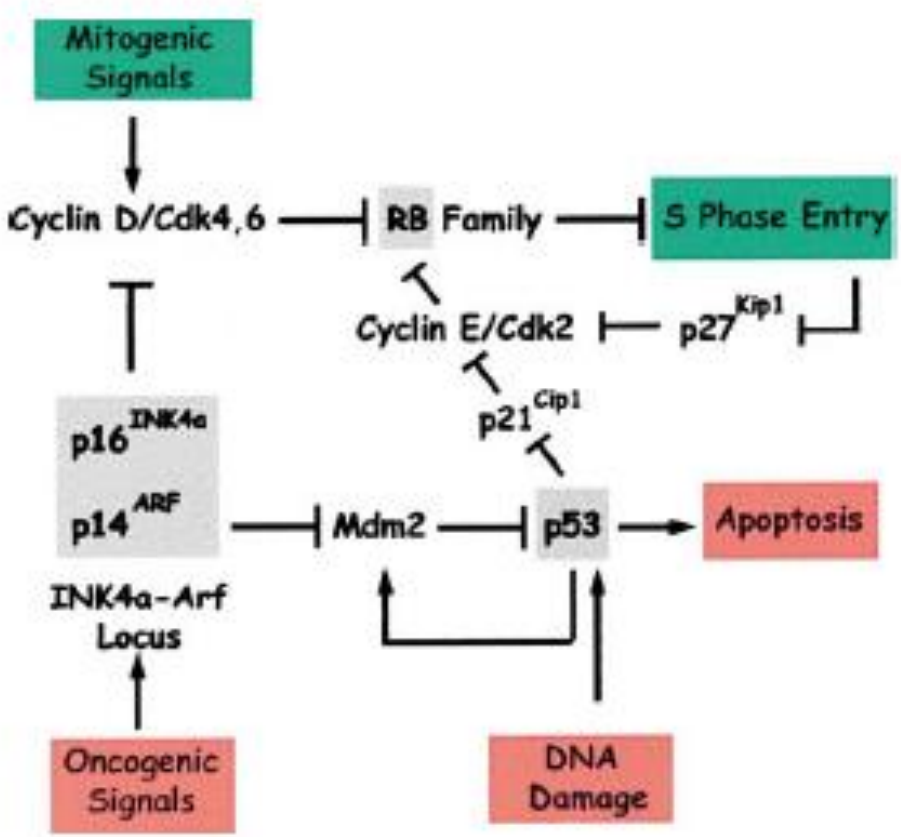


Figure 1. RB and p53 Regulate Cell Cycle Checkpoint Controls

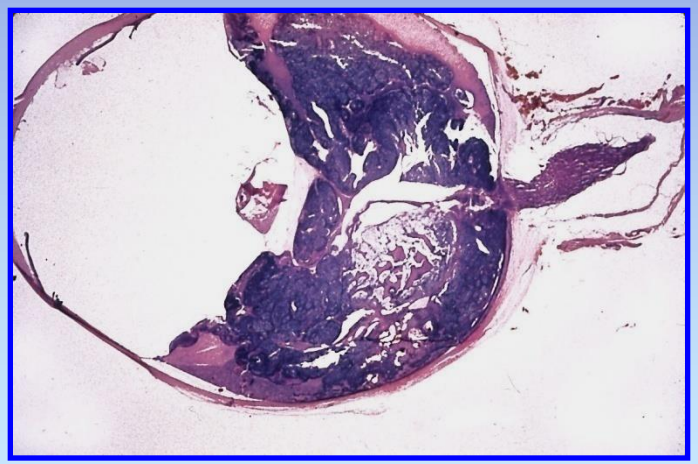
Mitogenic signals activate cyclin D-dependent kinases, which phosphorylate RB and RB family proteins (p107 and p130) to facilitate entry into S phase (top). The Cdk2 inhibitor, $p27^{Kip1}$, expressed at high levels in quiescent cells, is phosphorylated by cyclin E-Cdk2 in late G1 phase and degraded as cells enter S phase. Constitutive oncogenic signals can activate the *INK4a/ARF* locus. By antagonizing the activity of cyclin D-dependent kinases, $p16^{INK4a}$ activates RB and prevents entry into S phase. Mdm2 is a p53-inducible gene that normally acts to terminate the p53 response. The $p14^{ARF}$ protein inhibits Mdm2 to induce p53, leading either to p53-dependent apoptosis or to induction of the Cdk2 inhibitor $p21^{Cip1}$, inhibition of cyclin E/Cdk2, and RB-dependent cell cycle arrest. As cells exit the division cycle, $p27^{Kip1}$ is stabilized and reaccumulates. DNA damage signals activate p53 via ARF-independent pathways.

Retinoblastoma tumor suppressor gene (Rb)

- ✓ **Mapped to gene chromosome 13q14 and sequenced.**
- ✓ **180 kb; codes a 4.7 kb mRNA that produces a 928 amino acid nuclear phosphoprotein, pRB.**
- ✓ **pRB is expressed in every tissue that has been examined and regulates the cell cycle.**
- ✓ **Retinoblastoma tumor cells possess point mutations or deletions in RB**
- ✓ **In hereditary retinoblastoma, second RB mutation often is identical to the inherited one (a possible example of gene conversion).**

Retinoblastoma

- ✓ **Rare malignant tumor arising from immature neurons in the retina**
- ✓ **About 40% of cases are associated with a germline mutation (60% are sporadic)**
- ✓ **5-10% of germline cases are inherited germline mutations**
- ✓ **20-30% are NEW germline mutations**



Inherited germline Rb

- ✓ **Affected child inherits one defective Rb allele, together with one normal gene**
- ✓ **If the remaining normal Rb allele is inactivated by deletion or mutation, the loss of its suppressor function leads to development of a retinoblastoma**
- ✓ **Thus, susceptibility to Retinoblastoma is inherited in a dominant fashion**
- ✓ **At the cell level TSG is recessive to show the phenotype**

Sporadic retinoblastoma

- ✓ **Child is homozygous for two normal Rb Alleles**
- ✓ **Both alleles must be inactivated in a retinal cell for disease to develop**
- ✓ **Sporadic disease is rare (1/30,000)**

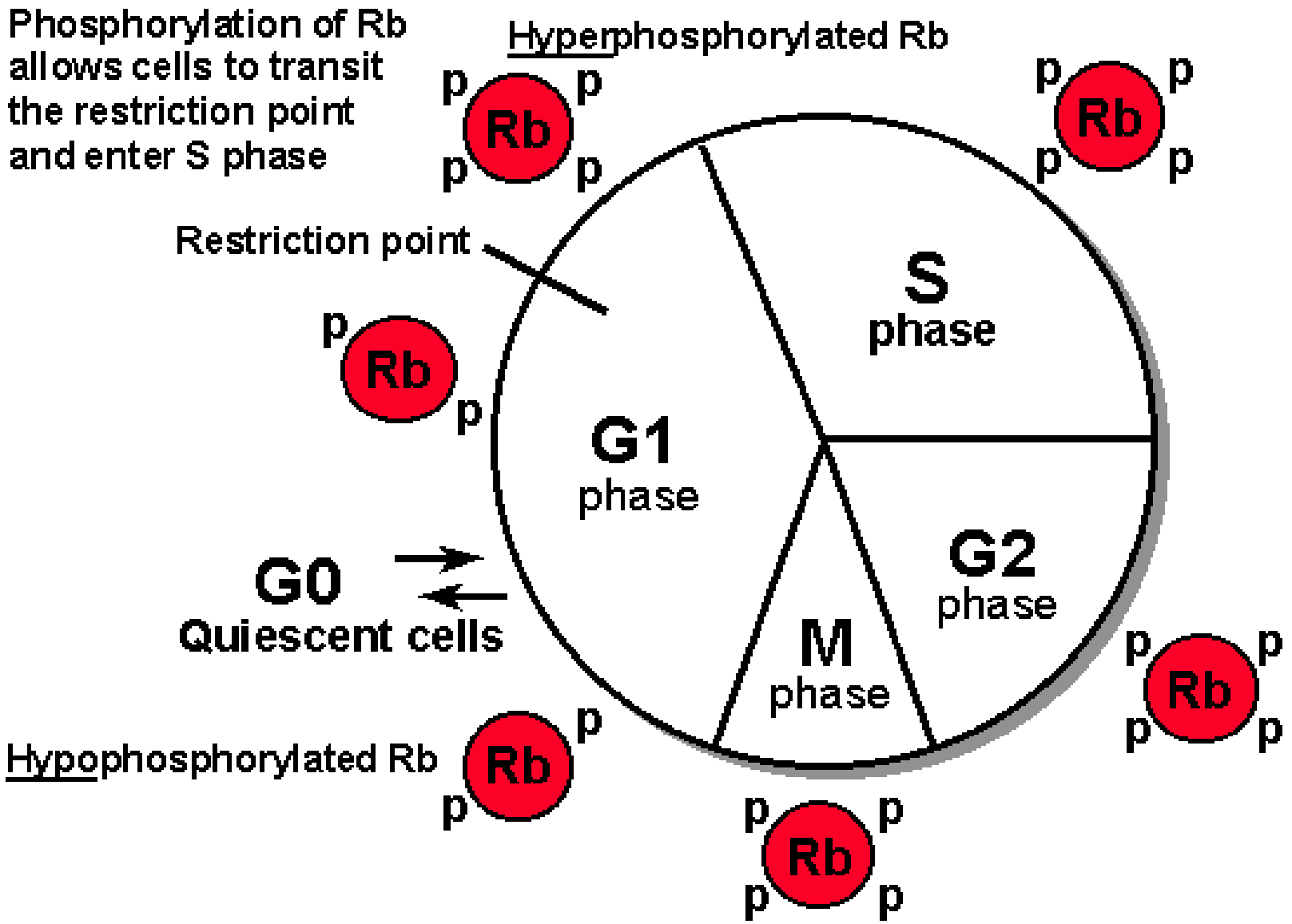
Retinoblastoma

- pRb; tumor suppressor

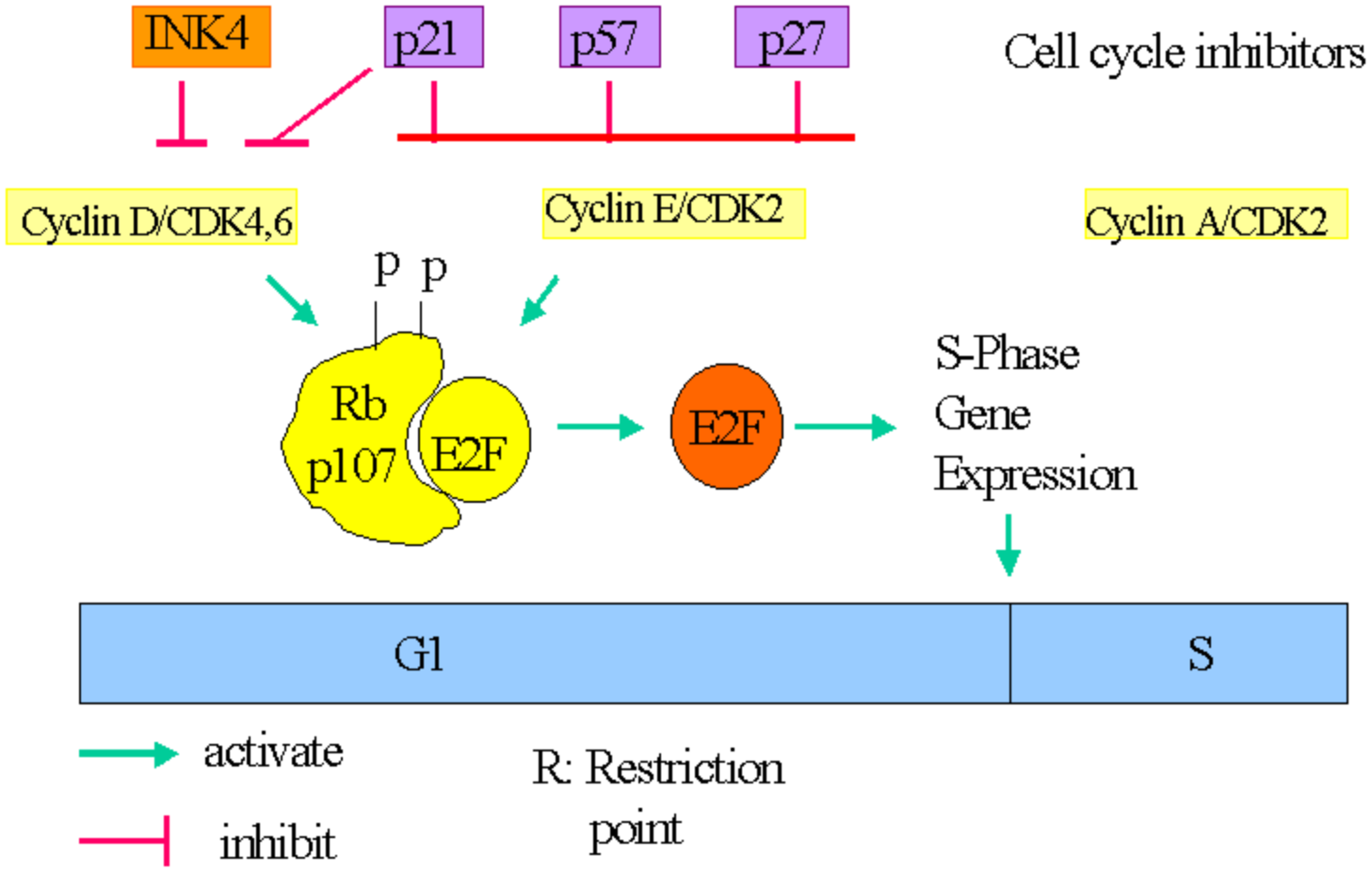
- deleted in retinoblastoma
  plays a pivotol role in mammalian cell cycle
  represses transcription - E2F
  regulated by phosphorylation

- -underphosphorylate: growth suppressive
- -hyperphosphorylated: growth promoting

Cell-cycle dependent phosphorylation of Rb

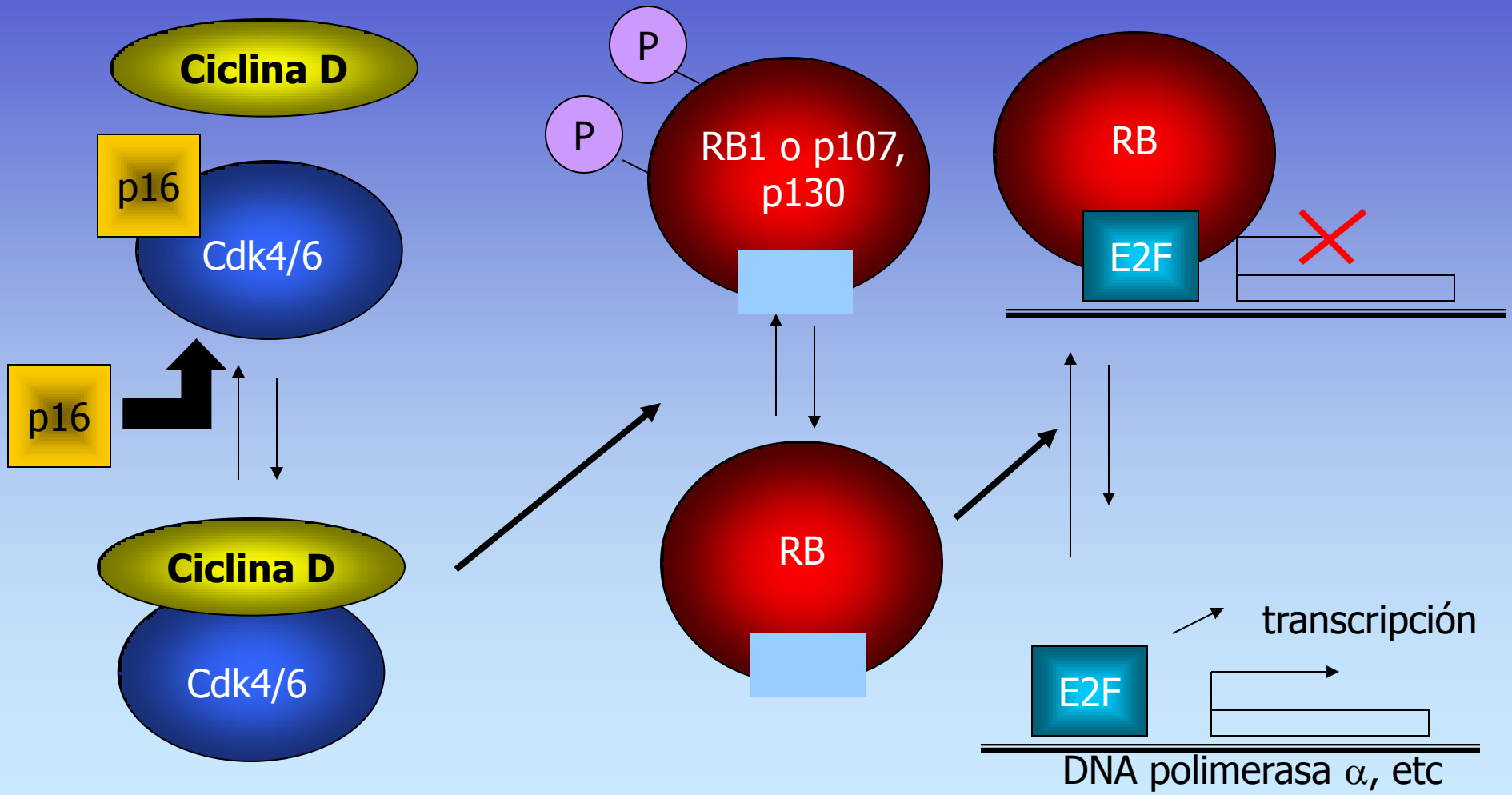


S-Phase Entry

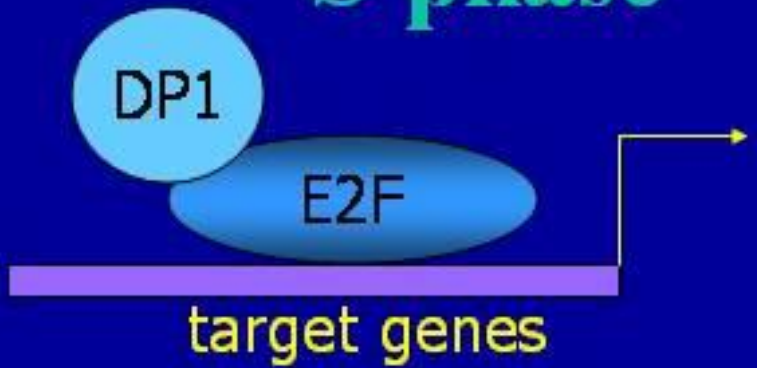


Inhibidores de Cdk4 y Cdk6 (INK4)

- p14, p15, p16, p18 → bloquean actividad de las kinasas dependientes de ciclina D produciendo el arresto del ciclo celular en G1.



E2F regulates genes required for S-phase



Target genes:

Genes encoding proteins involved in DNA synthesis

-DNA polymerase a

-thymidine kinase

-thymidylate synthase

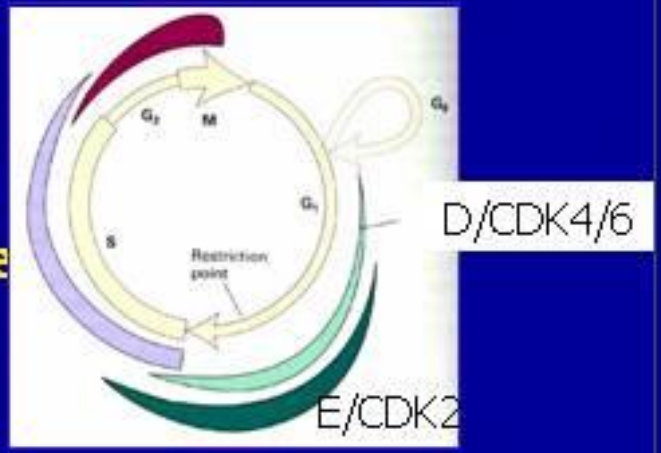
-ribonucleotide reductase

DNA repair proteins

-RAD51

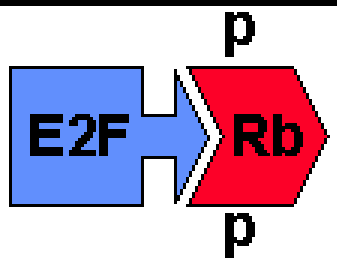
Cell cycle regulators

- Cdk2 and cyclins A and E.



Function of the Rb protein

Growth suppression

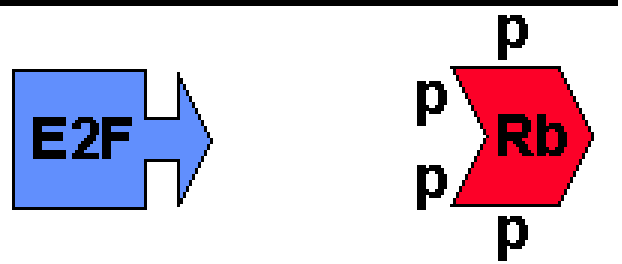


- E2F is a transcription factor that mediates growth-dependent activation of genes required to make the transition into and through S phase

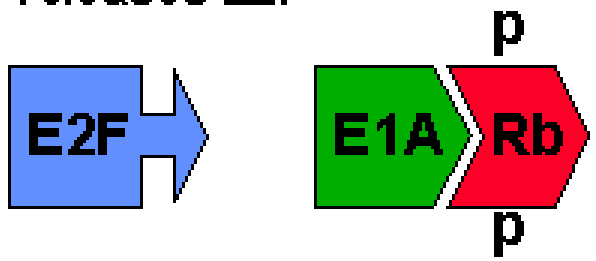
- Rb binds and inactivates E2F under conditions of growth suppression

- There are several ways to alleviate growth suppression resulting in controlled or uncontrolled cell growth

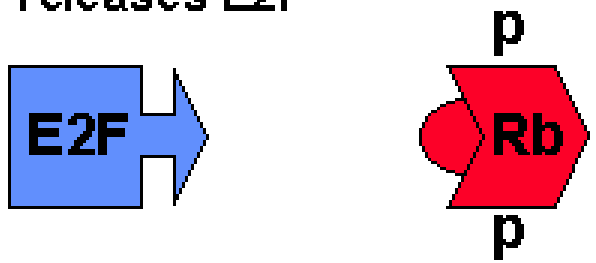
Relief of growth suppression



G1 phase phosphorylation releases E2F

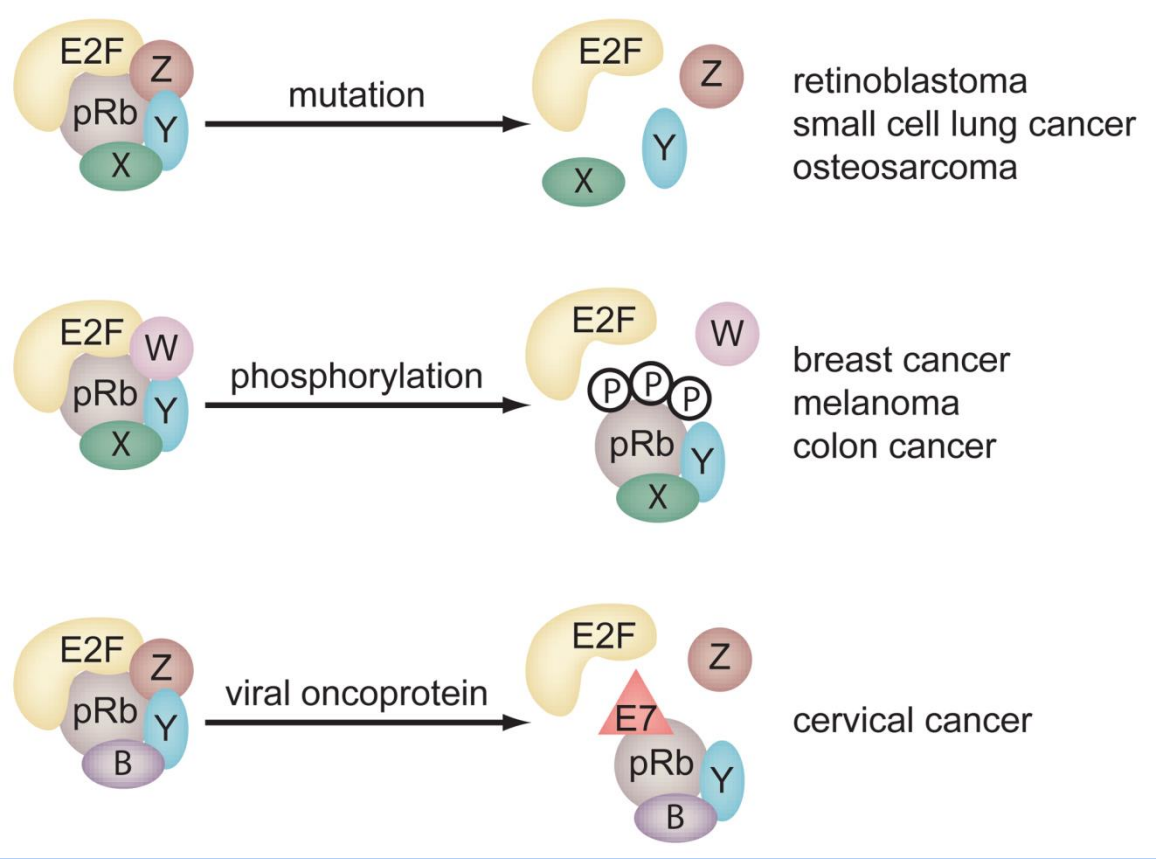


Adenovirus E1A oncoprotein binding releases E2F



Gene mutation affecting binding pocket releases E2F

Mechanisms of pRb inactivation in human cancer



-Inactivation by deregulated pRb phosphorylation is common in a distinct subset of human cancers, but Rb1 mutation is rarely observed in this subset.

- The model proposes that each mechanism of inactivation has different molecular and functional consequences.

-Deregulated pocket protein phosphorylation is proposed to abrogate some protein interactions but not others.

- Different cell types may select for different mechanisms of pRb inactivation because the molecular consequences are more favorable for Carcinogenesis.

CIO-FUCA

Tumor suppressor genes

Gene (locus)	Function	Disorders in which gene is affected	
		Familial	Sporadic
DCC (18q)	cell surface interactions	unknown	colorectal cancer
WT1 (11p)	transcription	Wilm's tumor	lung cancer
Rb1 (13q)	transcription	retinoblastoma	small-cell lung carcinoma
p53 (17p)	transcription	Li-Fraumeni syndrome	breast, colon, & lung cancer

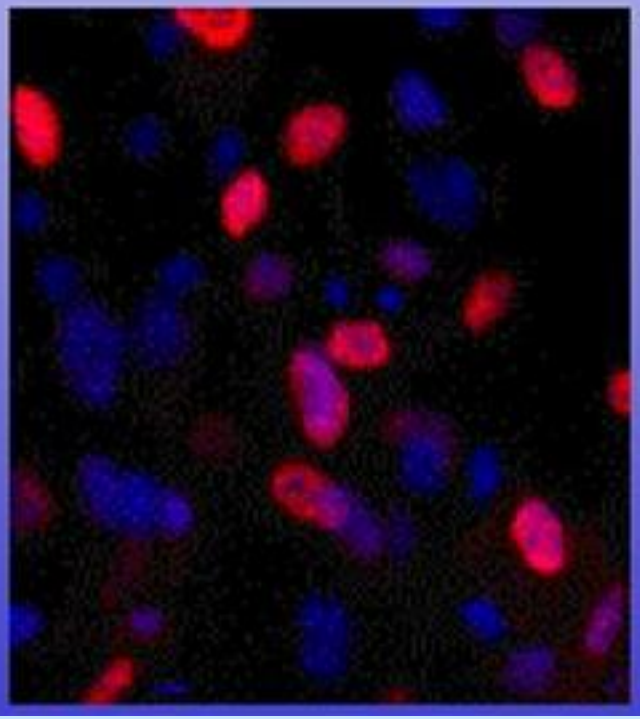
Genes supresores en tumores humanos

GEN	Localización	Cánceres asociados
RB	13p14	Retinoblastoma, osteosarcoma, leucemias linfoides pulmón, mama ovario, próstata, vejiga.
p53	17p13	Li-Fraumeni síndrome, mama, próstata, pulmón, colon, vejiga, hígado, linfomas/leucemias, cerebro, adrenal.
WT1	11p13-15	Tumor de Wilm's (cáncer renal infantil).
NF-1	17q11.2	Neurofibromatosis, neurofibromas.
NF-2	22q	Meningioma, mama, colon.
VHL	3p26	Células renales. Hemangioblastoma de cerebro.
APC	5q21	Colon (FAP)
MSH-2	2p16	Colon (HNPCC, proximal colon), endometrial, estómago.
MLH-1 PMS 1 PMS 2	3p21.3-23 2q31-33 7p22	Páncreas, vejiga, ovario, mama.
MTS1(p16) MTS2(p15)	9p21	Familial melanoma, glioblastoma,T-ALL, mesotelioma. NSCLC, vejiga.
BRCA-1	17q21	Ca. de mama temprano, ovario, próstata, colon.
BRCA-2	13q	Ca. de mama temprano (Ca.de mama masc.)

P53 tumor suppressor gene

P53 as tumor antigen and oncogene

- P53 was was initially identified as a tumor specific nuclear antigen of molecular weight 53kDa
- isolation of the gene from tumor cells yielded an *oncogene*: when it was transfected into normal cells it could transform them (together with ras)
- a variety of different sequences were identified raising the question of which sequence represented the wild-type



P53 as tumor suppressor gene

- When the p53 gene was isolated from the genomic DNA of normal cells, it was discovered that the tumor derived gene was mutated - still consistent with the characteristics of an oncogene
- when the wild-type p53 gene was transfected into tumor cells it stopped their growth, a key characteristic of a *tumor suppressor gene* - wild-type dominant over mutant
- genetic analysis of p53's genomic region 17p suffered loss of heterozygosity as a follow up to mutation

P53 central to cancer

- **p53**: the most significant cancer gene has properties of both an oncogene and a tumor suppressor gene
- it is mutated in about half of all human cancers

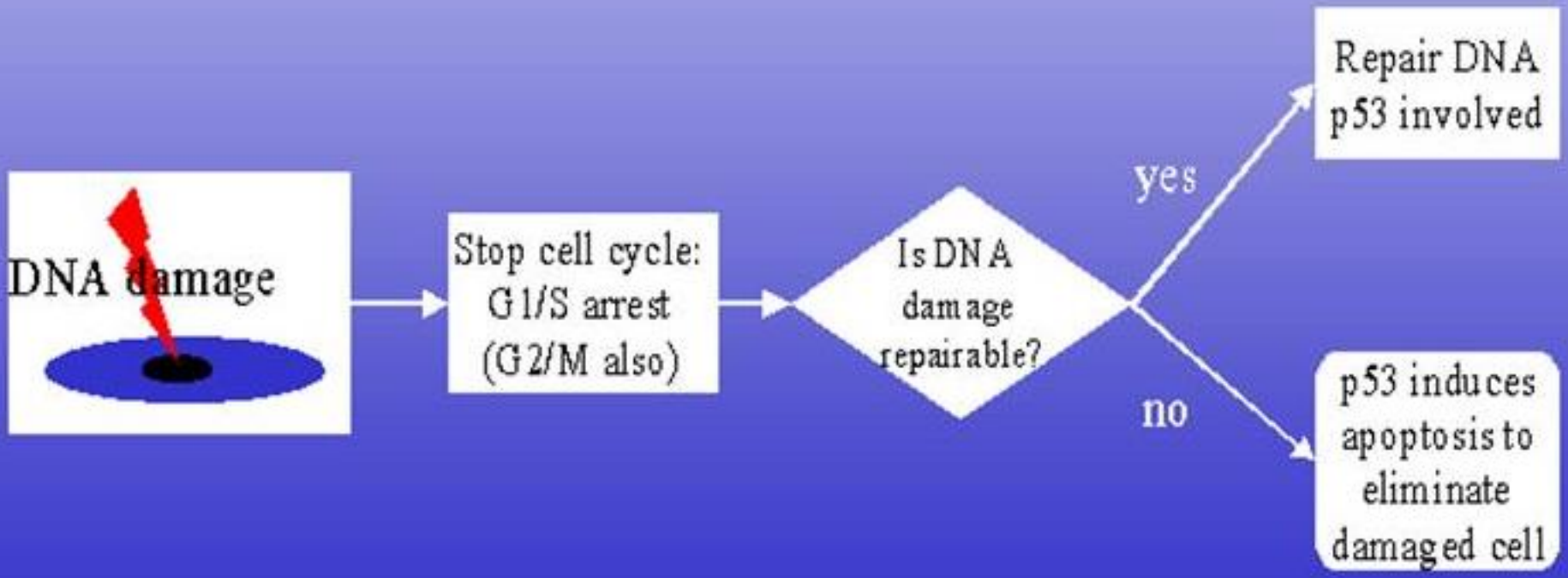
- A note on viral tumor antigens:
  - DNA tumor viruses (Adenovirus, SV40, polyoma virus) all encode proteins that interact with cellular proteins in the viruses' bid to take over the cell's metabolism
  - in all viruses one protein binds and inactivates p53, further evidence that it is central to the biology of cancer (the other popular cellular protein target is Rb)

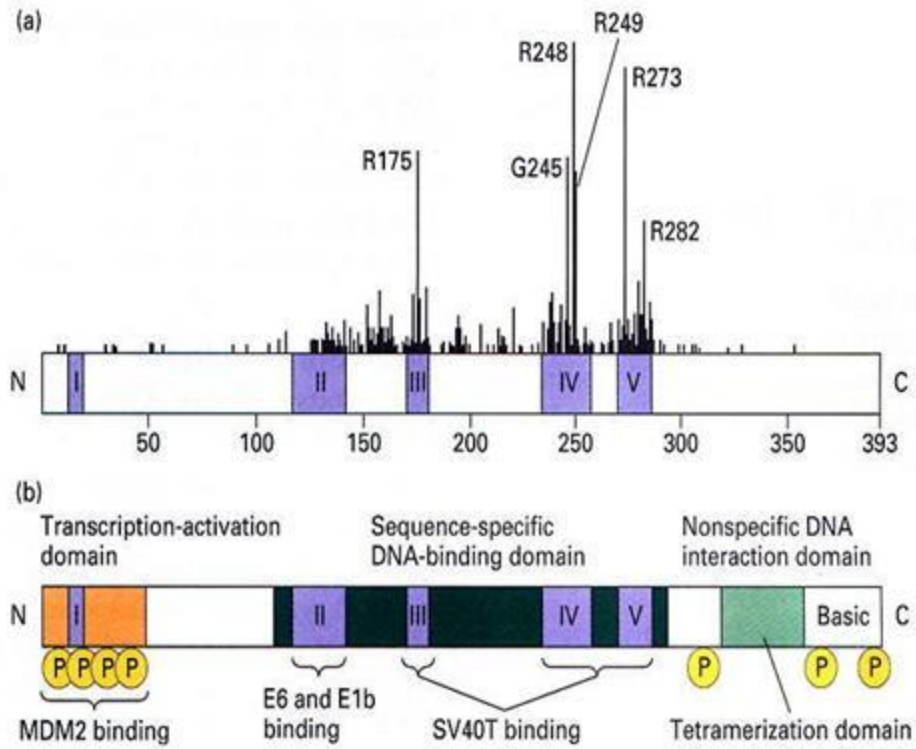
p53 tumor suppressor gene

- 53 kDa nuclear phosphoprotein
- the most commonly altered gene identified in human cancers to date
- functions as a tumor suppressor when normal and a dominant oncogene when mutant
- many mutations result in a prolonged protein half-life and/or overexpression

Functions of p53: simple

- One way of thinking about p53 is that it is a "guardian of the genome": it protects the cells DNA from damage

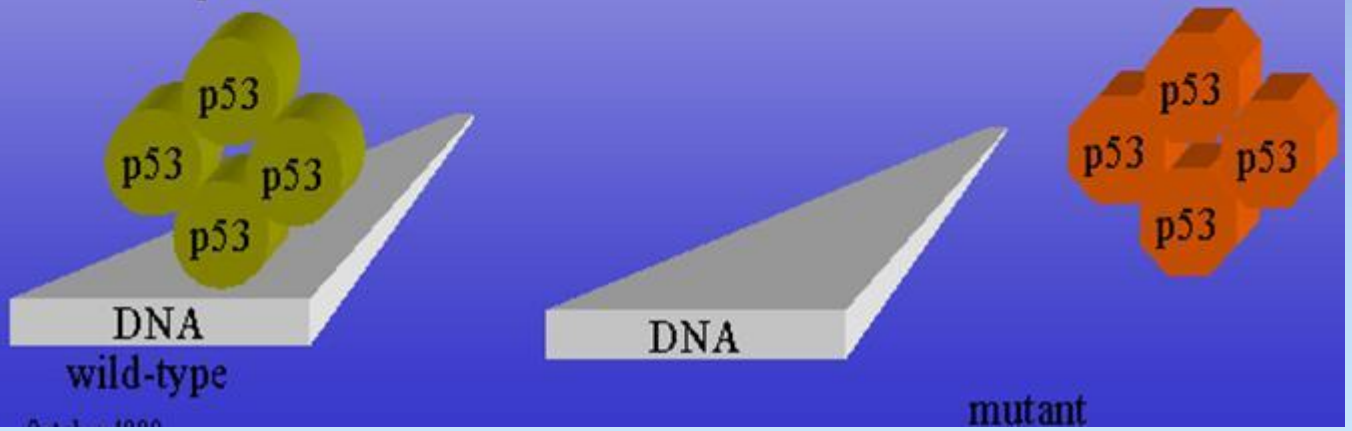




▲ **FIGURE 24-21 The human p53 protein.** (a) Mutations in human tumors that inactivate the function of p53 protein. Hatched boxes represent sequences highly conserved in evolution. Vertical lines represent the frequency at which mutations are found at each residue in various human tumors. These mutations are clustered in conserved regions II–V. (b) Structural organization of the p53 protein. Phosphorylation by various kinases at the sites indicated by (P) stabilize p53. MDM2 protein binds at the indicated site and represses transcription activation by p53 as part of the normal control of p53 function. The activity of p53 also is inhibited by binding of viral proteins such as E6 from human papillomavirus and E1b from adenovirus. [Adapted from C. C. Harris, 1993, *Science* **262:**1980; and L. Ko and C. Prives, 1996, *Genes & Develop.* **10:**1054.]

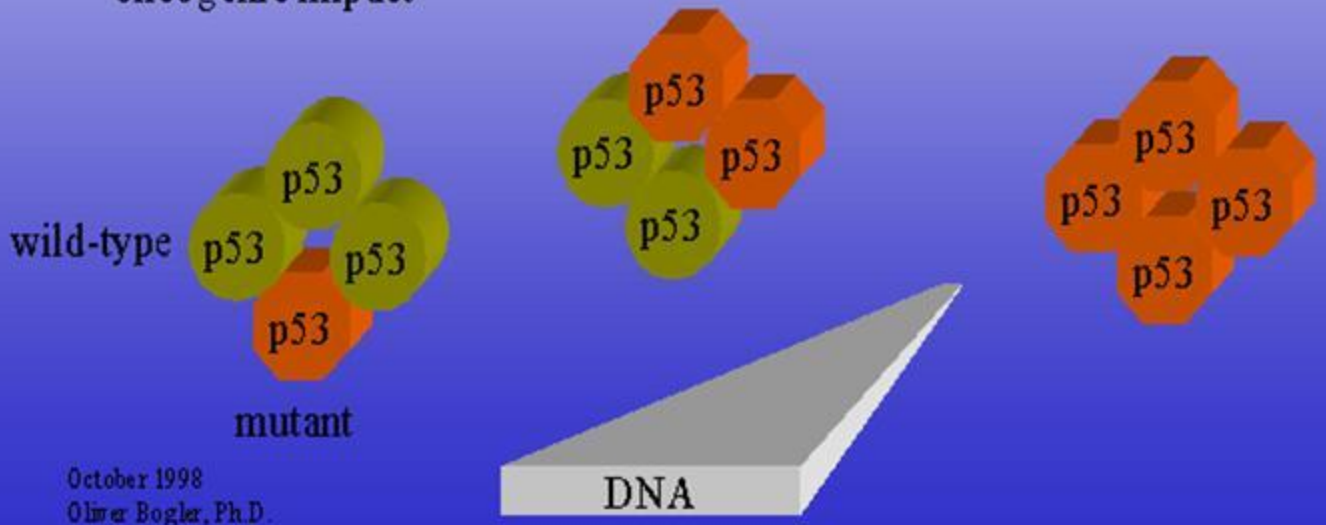
P53 mutations: loss of function

- 80% point mutations, 20% truncations
- almost all mutants have an abnormal structure, leading to loss of DNA binding capability and so loss of normal function
- loss of wild-type p53 function leads to loss of cell cycle arrest in response to DNA damage ie tumor suppression
- loss of p53 function leads to increase in mutation rate



P53 mutations: dominant negative

- P53 acts as a tetramer
- heterotetramers containing mutant p53 are non functional, and so the presence of mutant p53 can prevent wild-type from functioning properly
- explains why introduction of mutant p53 into normal cells has oncogenic impact



p53 tumor suppressor gene

- ✓ **Mutations in p53 are implicated in ~50% of human cancers, including cancers of the:**

  breast, brain, liver, lung,
  colorectal,bladder, and blood

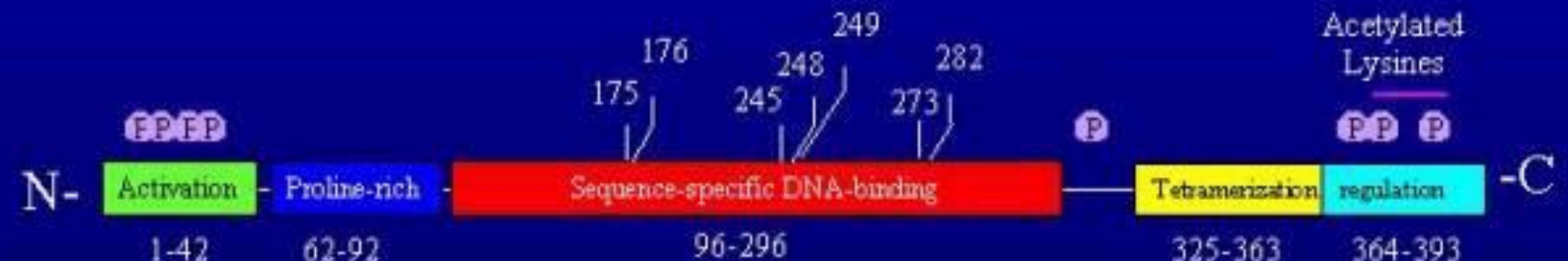
- ✓ **Development of tumors requires mutations on two p53 alleles.**

- ✓ **Codes a 393 amino acid protein involved in transcription, cell cycle control, DNA repair, and apoptosis (programmed cell death).**

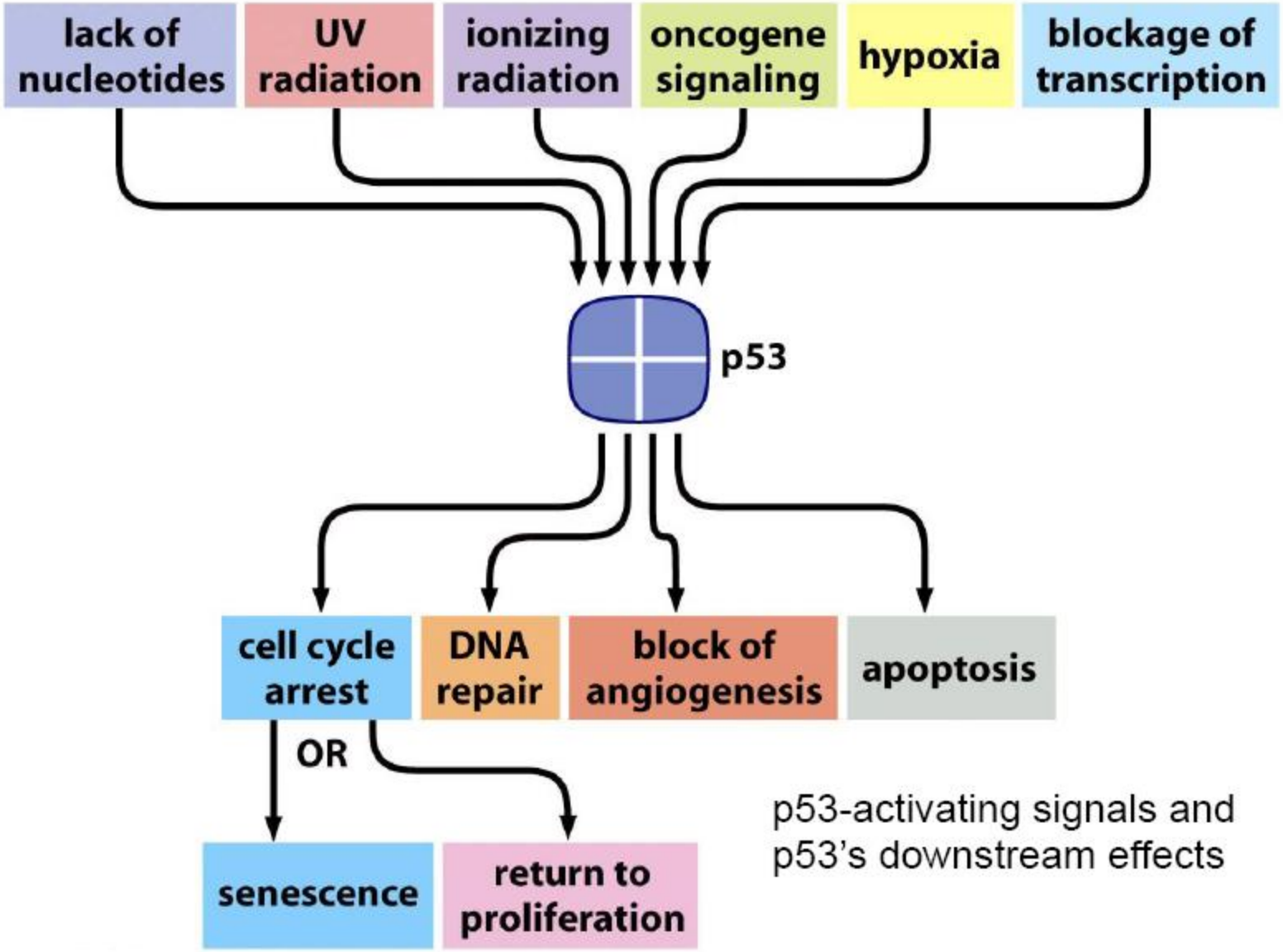
- ✓ **p53 binds to several genes, including WAF1, and interacts with at least 17 cellular and viral proteins.**

- ✓ **Transgenic mice with deletions of both p53 alleles are viable, but 100% develop cancer by ten months of age.**

Mutations in the p53 coding sequence



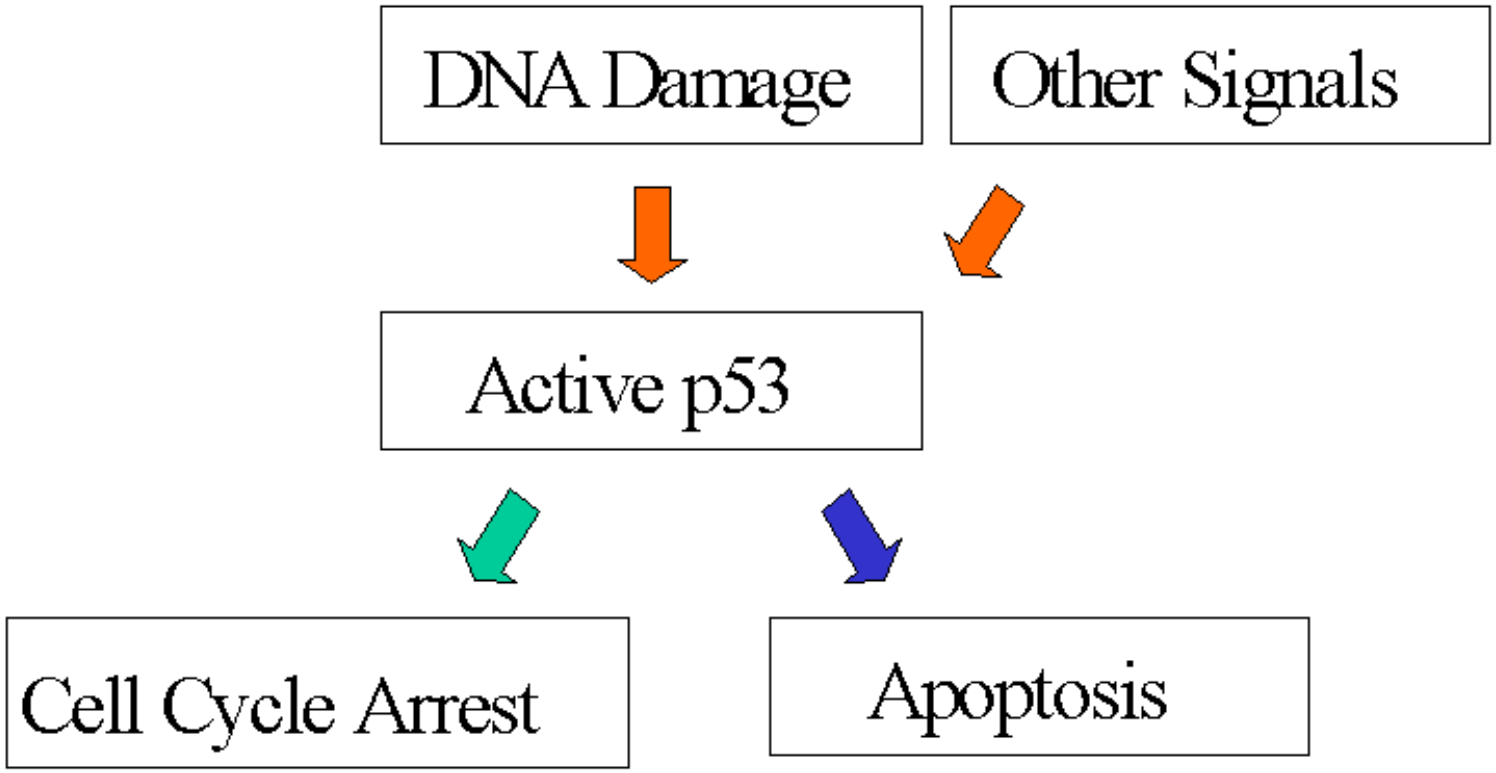
	N-terminus		Core domain		C-terminus	
Missense	14 (66%)	41 (48.2%)	7122	(77.7%)	32 (33.7%)	2 (33.3%)
Nonsense	0	13 (15.3%)	535	(5.8%)	37 (38.9%)	0
Silent	1 (4.8%)	1 (1.2%)	381	(4.2%)	3 (3%)	1 (16.6%)
Other	6 (28.6%)	30 (28.6%)	1085	(12.3%)	23 (24.2%)	3 (50%)
Total	21	85	9123		95	6

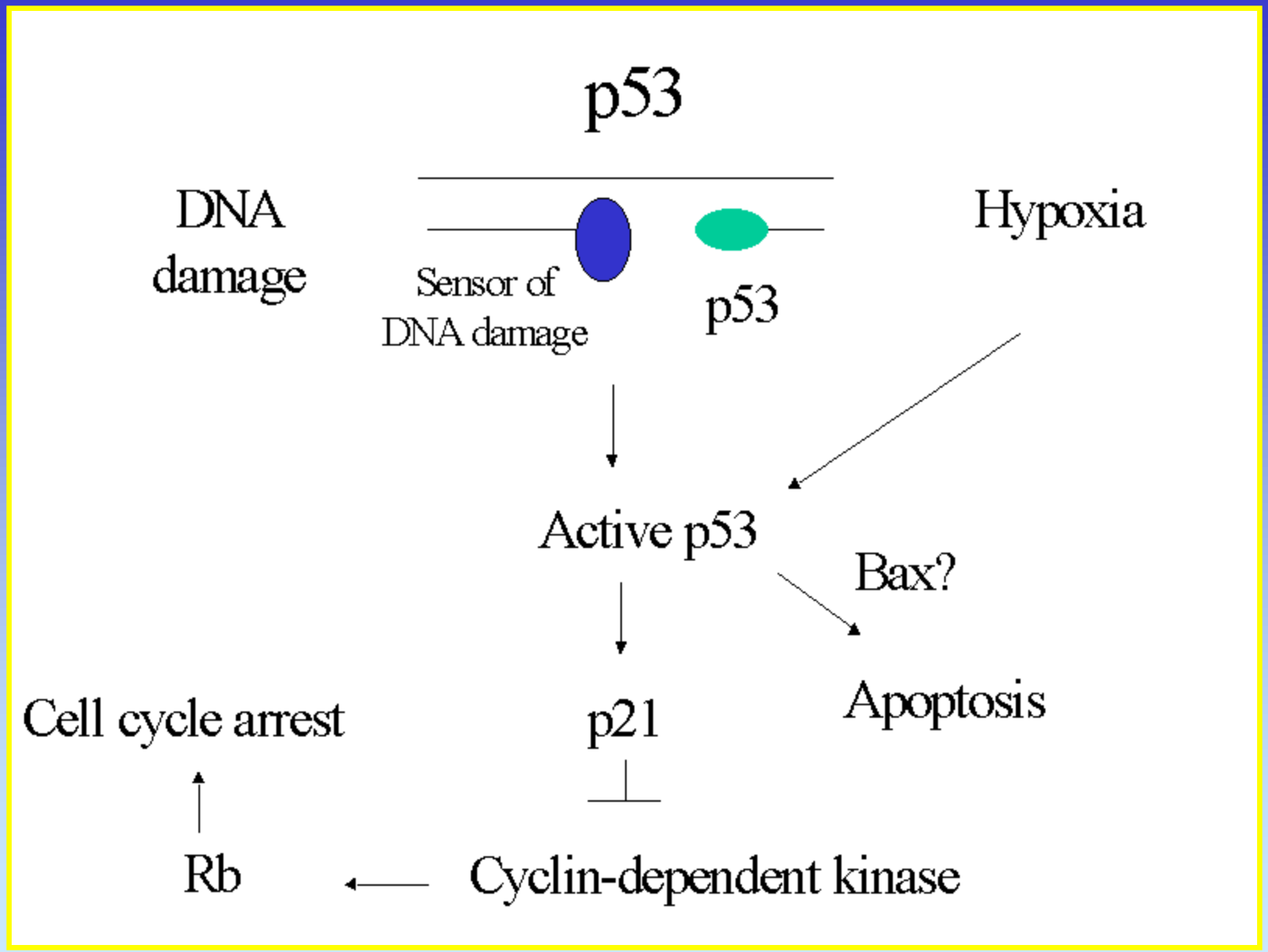


p53-activating signals and p53's downstream effects

Figure 9-8 The Biology of Cancer (© Garland Science 2007)

p53 Signaling




p53
DNA damage
Sensor of DNA damage
p53
Hypoxia
Active p53
Bax?
Apoptosis
p21
Cell cycle arrest
Rb
Cyclin-dependent kinase

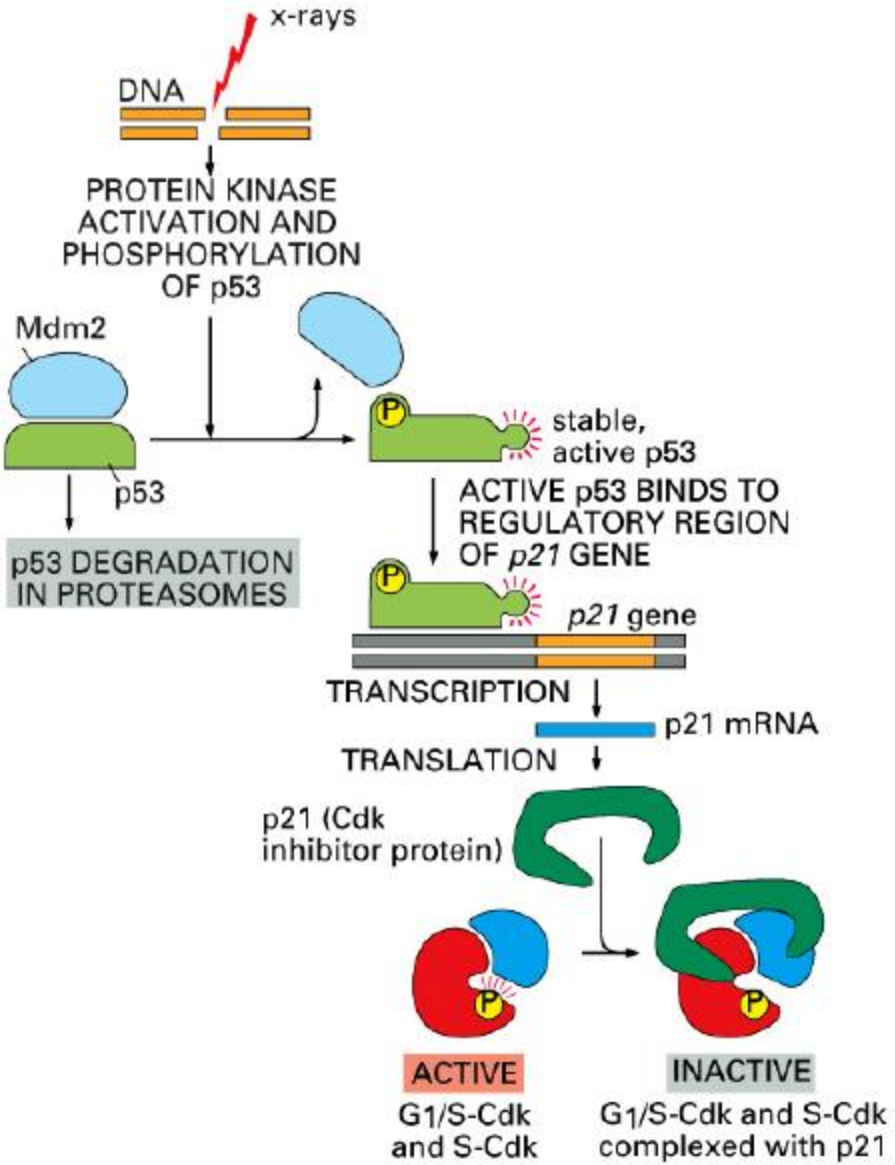
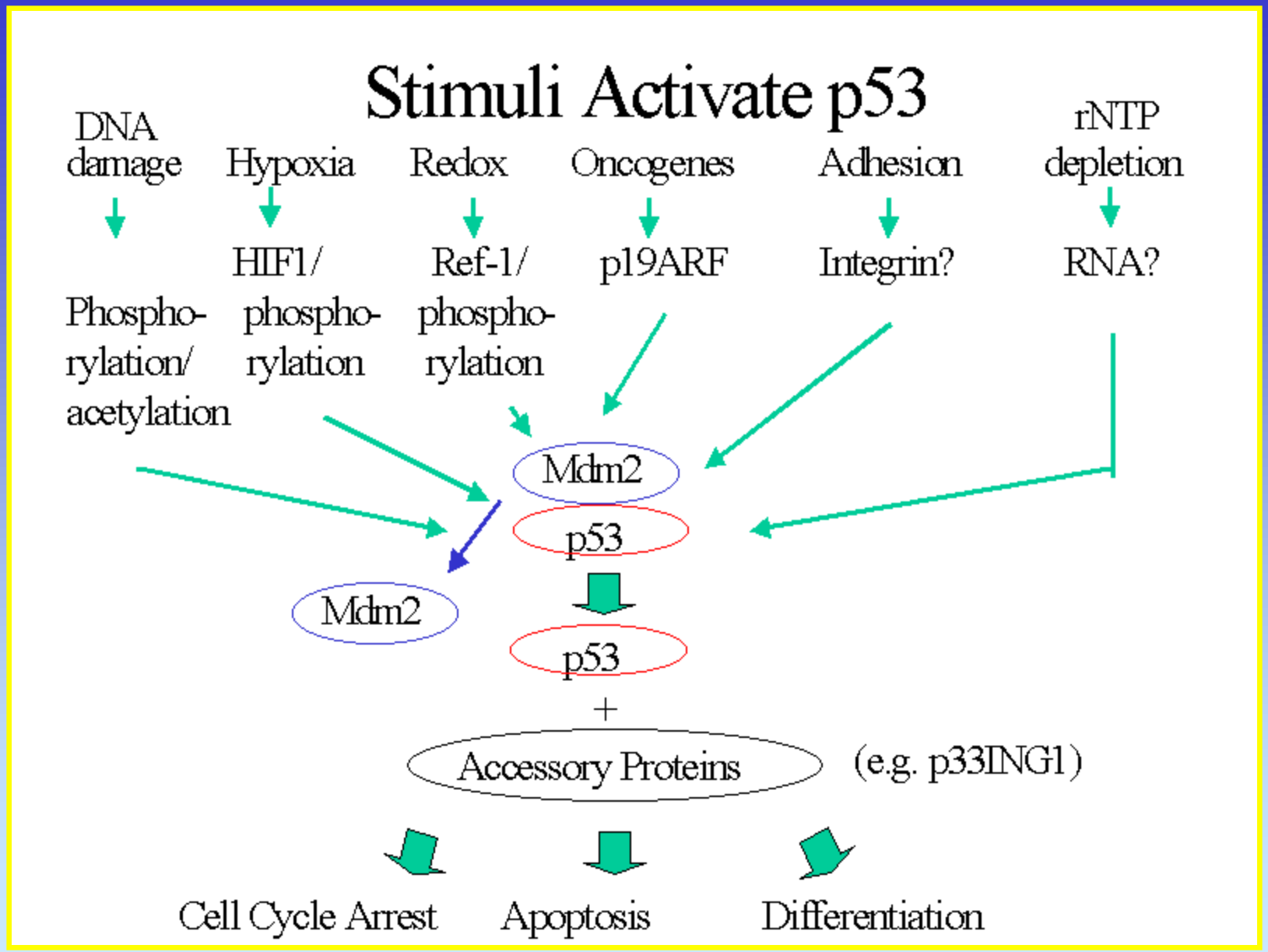
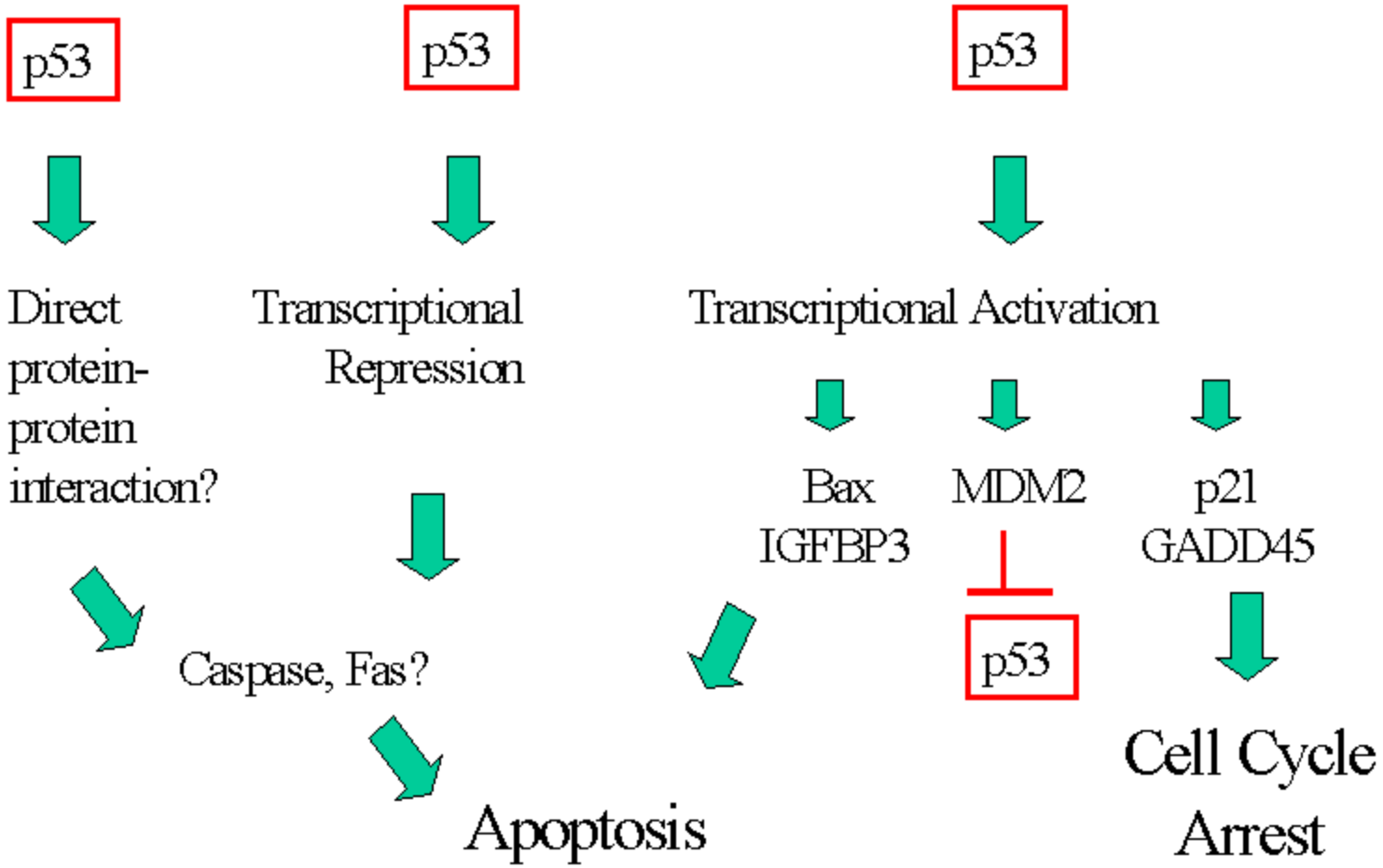


Figure 17–33. Molecular Biology of the Cell, 4th Edition.


Stimuli Activate p53
DNA damage
Hypoxia
Redox
Oncogenes
Adhesion
rNTP depletion
Phospho-rylation/ acetylation
HIF1/ phospho-rylation
Ref-1/ phospho-rylation
p19ARF
Integrin?
RNA?
Mdm2
p53
Mdm2
p53
+
Accessory Proteins
(e.g. p33ING1)
Cell Cycle Arrest
Apoptosis
Differentiation

Functions of Activated p53



Algunos genes target de p53…

-P21, GADD45: Se une a CDK2 ⇨ inhibe fosforilación del RB ⇨ arresto del ciclo celular

-BAX, PUMA, NOXA: mitocondria ⇨ liberan citocromo c ⇨ activan caspasas – apoptosis
P53AIP1

-MDM2: ejerce loop feedback negativo autoregulatorio de p53 por ubiquitinación y degradación en el proteasoma de p53

-P53R2: Induce el arresto en el checkpoint G2/M y participa en la reparación al daño del DNA

-Existen 4000 genes con sitios potenciales de regulación por p53

-También p53 controla la transcripción de múltiples miRNA (miR-34, miR-192/215, miR-107) y de otros que actúan como supresores de tumor. Como resultante p53 puede controlar la progresión tumoral, invasión y metastasis, entre otras funciones.

Control de la estabilidad de p53 y modificaciones post-traduccionales en p53

- MDM2 ubiquitina el extremo COOH-ter para enviar a p53 al proteasoma para su degradación

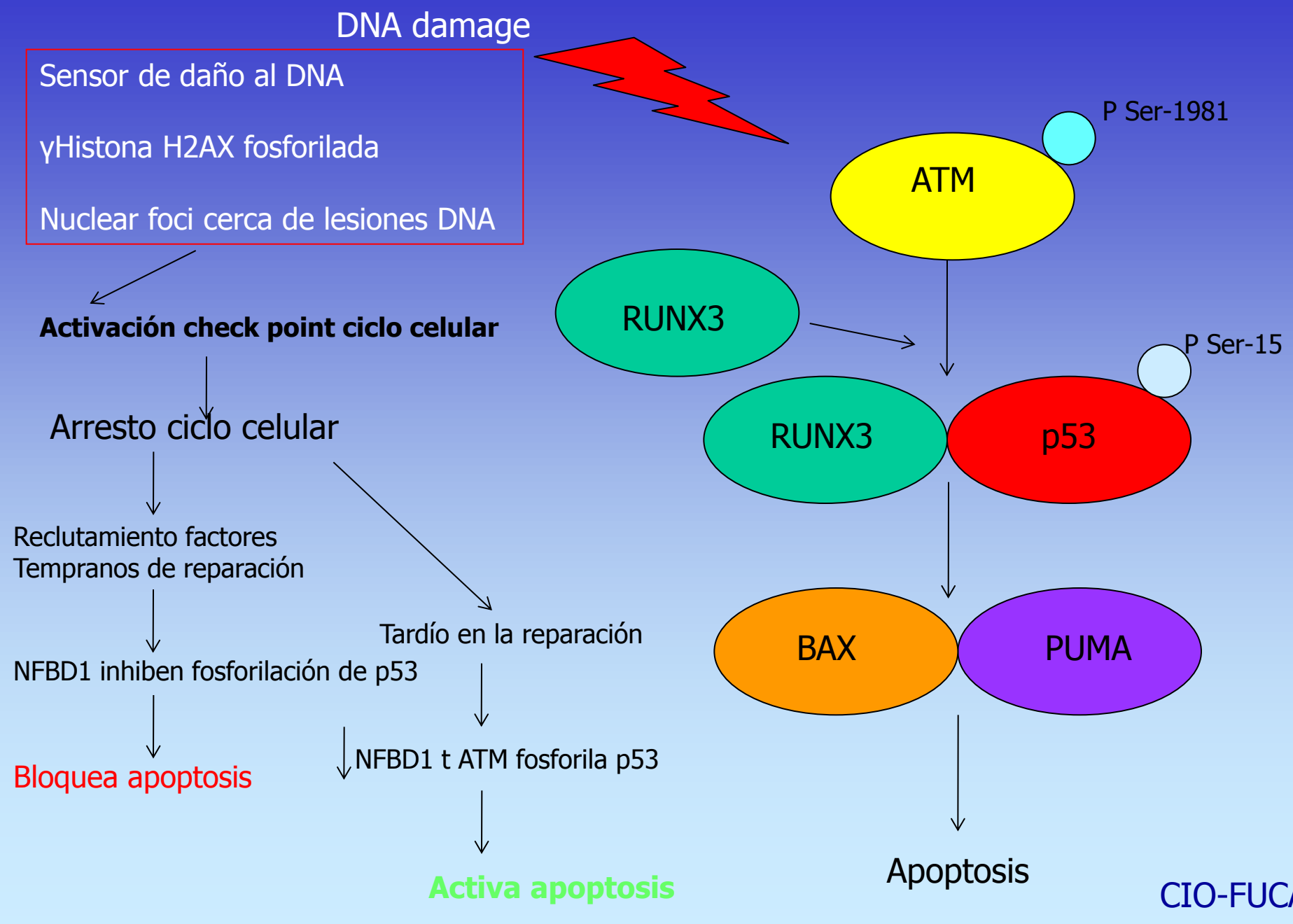
-MDM2 además impide la unión al DNA para la transcripción de genes target al unirse al dominio de trasactivación NH_2-ter

-MDM2 asistiría en el reclutamiento físico de p53 ubiquitinada hacia el proteasoma, contribuyendo a su degradación

La inducción y activación de p53 en respuesta al stress está sujeta a regulación por Modificaciones post-traduccionales:

- acetilación
- sumoilación
- fosforilación
- o-glicosilación

DNA damage response



CIO-FUCA

P53 como target terapéutico

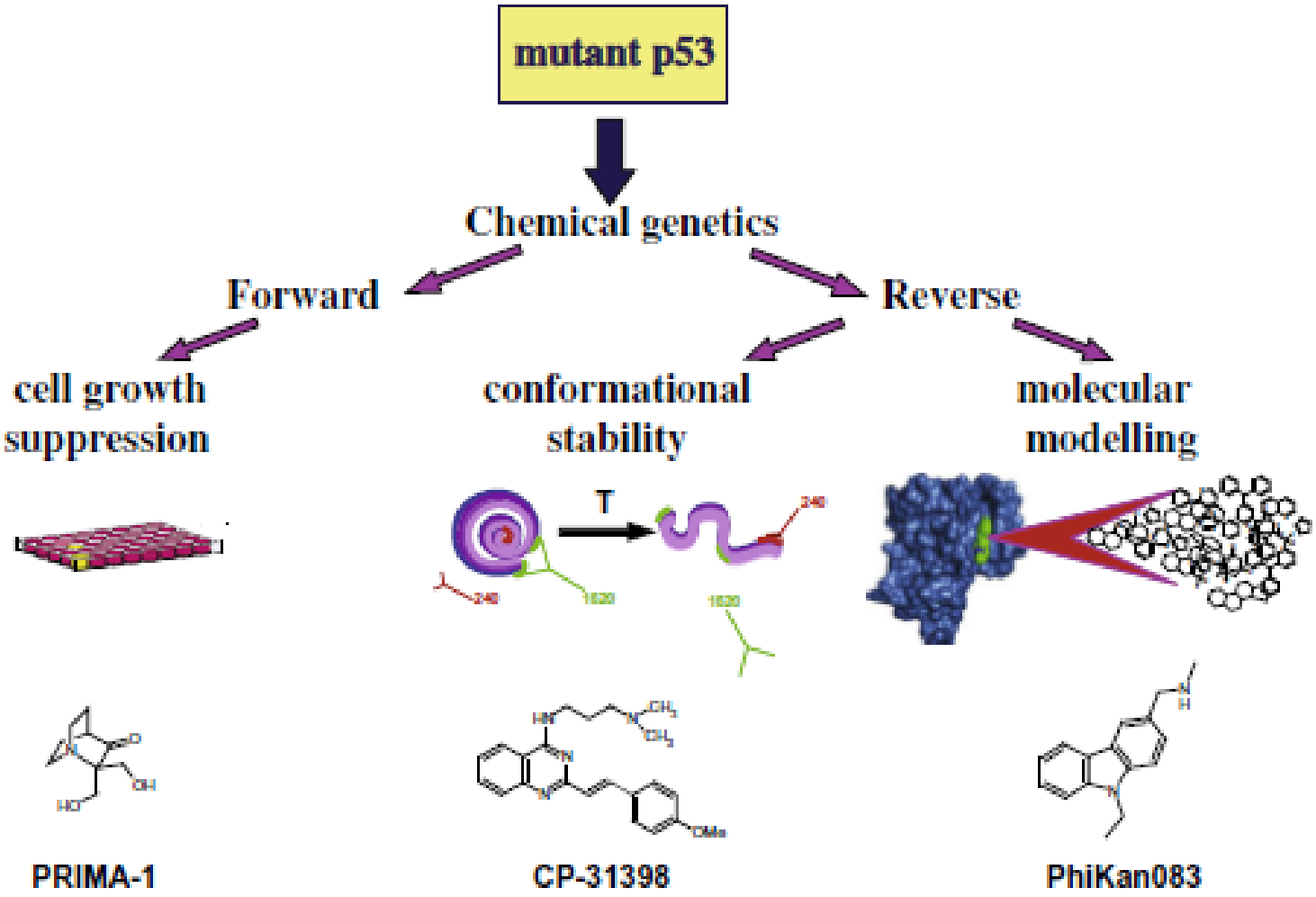
-P53 mutada debe ser reactivada y no inhibida como otros targets mutados (oncogenes)

-Múltiples mutaciones lo convierten en un target estructural heterogéneo

- CP-31398 re-pliega p53 mutante dandole estabilidad conformacional activa, restaurando el fenotipo wild type
- PhiKan083 se diseñó por moldeado molecular para unirse a residuos críticos que confieren estabilidad conformacional activa a p53 mutada
- PRIMA-1 es un compuesto que restaura la conformación wild type de p53 mutante e inhibe el crecimiento de muchas líneas tumorales en forma dependiente de p53. (in vitro e in vivo)

Se están realizando ensayos clínicos con PRIMA-1 en ptes con ca. próstata y cánceres hematológicos- Se vio respuesta clínica en pts con p53 mutado

P53 como target terapéutico



Adenomatous polyposis coli (APC)

- ✓ **Tumor suppressor gene**
- ✓ **First characterized because of its association with familial adenomatous polyposis**
- ✓ **Further study proved its role in sporadic cases of colon cancer**

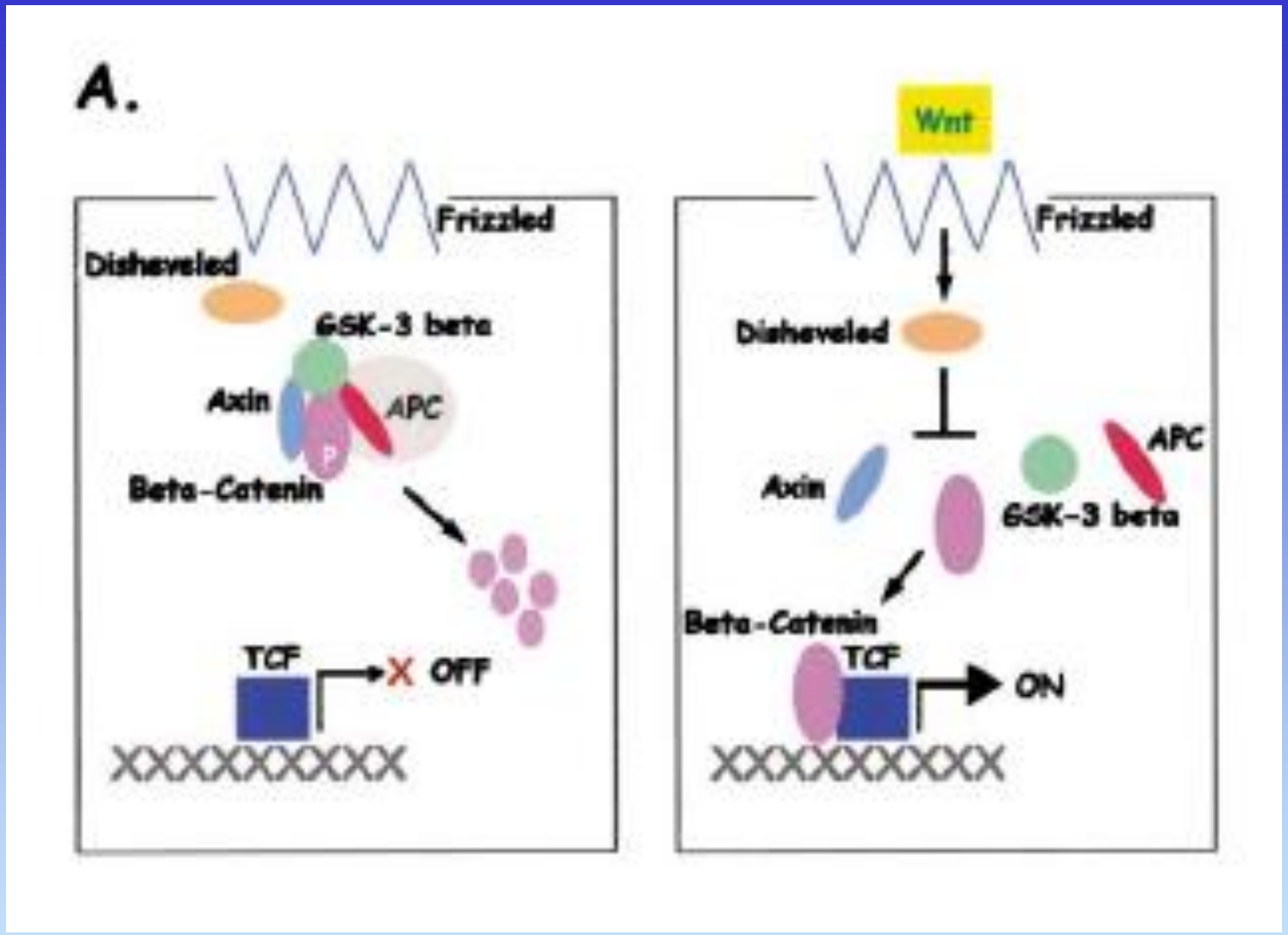
Familial adenomatous polyposis

- ✓ An autosomal dominant disease
- ✓ Occurs in 1 in 10,000 individuals in U.S.
- ✓ Accounts for 10% of colorectal cancer
- ✓ APC gene function is lost or defective

APC gene product

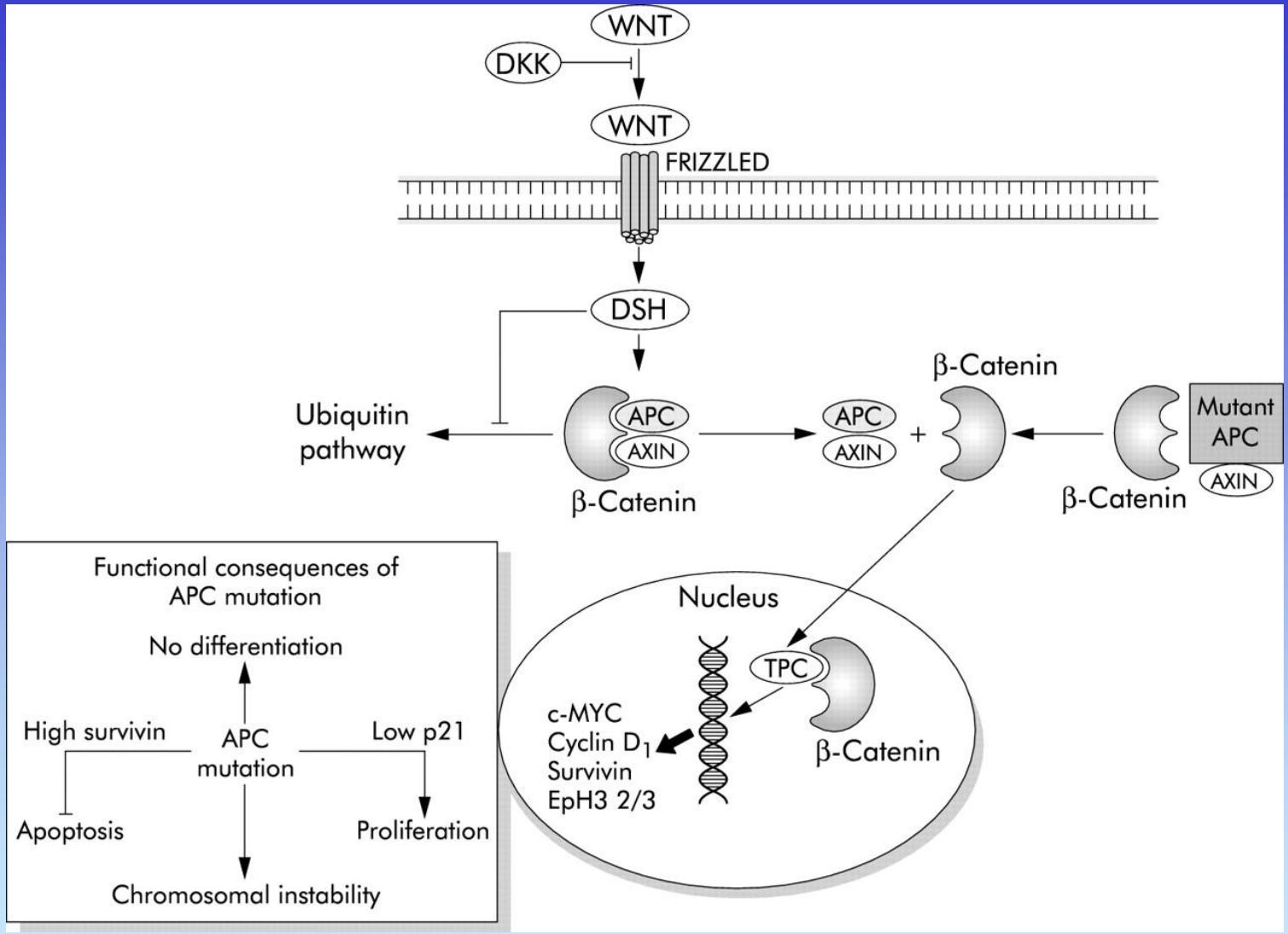
- ✓ Cytoplasmic protein
- ✓ Interacts with and promotes degradation of Beta-catenin (transcriptional activator of growth promoting genes – Wnt pathway)
- ✓ Loss of APC = up-regulation of cell proliferation

APC



Targets of TCF: c-myc, cyclin D1 (cell proliferation)

(A) Wnt signaling. In the absence of Wnt ligand (left image), β-catenin binds to a destruction complex containing APC, Axin, and GSK-3β. Phosphorylation of β-catenin facilitates its recognition by a unbiquitin-conjugating E3 ligase (SCF^{TRCP}) that targets it for proteasomal degradation. When Wnt binds to its receptor (right image), signaling via Frizzled and Disheveled prevents β-catenin phosphorylation and destruction. Import of β-catenin and its binding to TCF/LEF transcription factors induces expression of Wnt target genes (adapted from Polakis, 1997; Fearnhead et al., 2001). Inactivation of APC mimics the effects of the Wnt signal.


WNT
DKK
WNT
FRIZZLED
DSH
Ubiquitin pathway
APC
AXIN
β-Catenin
APC
AXIN
+
β-Catenin
β-Catenin
Mutant APC
AXIN
Functional consequences of APC mutation
No differentiation
High survivin
APC mutation
Low p21
Apoptosis
Proliferation
Chromosomal instability
Nucleus
TPC
c-MYC
Cyclin D1
Survivin
EpH3 2/3
β-Catenin

BRCA 1 Y BRCA 2
CANCER DE MAMA Y OVARIO

Breast Cancer Tumor Suppressors

- **Breast cancer affects 1 in 10 women and represents 31% of cancers in women (~215,990 women diagnosed each year, 2004 estimate).**

- **~5-10% of breast cancers are hereditary; age of onset for hereditary breast cancer is earlier than other forms (mutations at 2 alleles).**

  - **A small proportion of breast cancer is heritable. Two genes are associated with predisposition to developing breast cancer.**
    - **BRCA1 on chromosome 17**
    - **BRCA2 on chromosome 13**

  - **Normal function of both is in repair of ds DNA breaks.**

BRCA 1

- ✓ A tumor suppressor gene for breast and ovarian cancer
- ✓ Localized on chromosome 17q21
- ✓ Approximately 5-15% of breast Ca is inherited - 85% is sporadic
- ✓ BRCA1 is responsible for approximately HALF of the INHERITED breast cancers

BRCA 2

- ✓ **Chromosome 13q12-13**
- ✓ **Germline mutations in BRCA2 gene increase risk of breast (male and female) and ovarian cancer**

CANCER DE MAMA HERED.

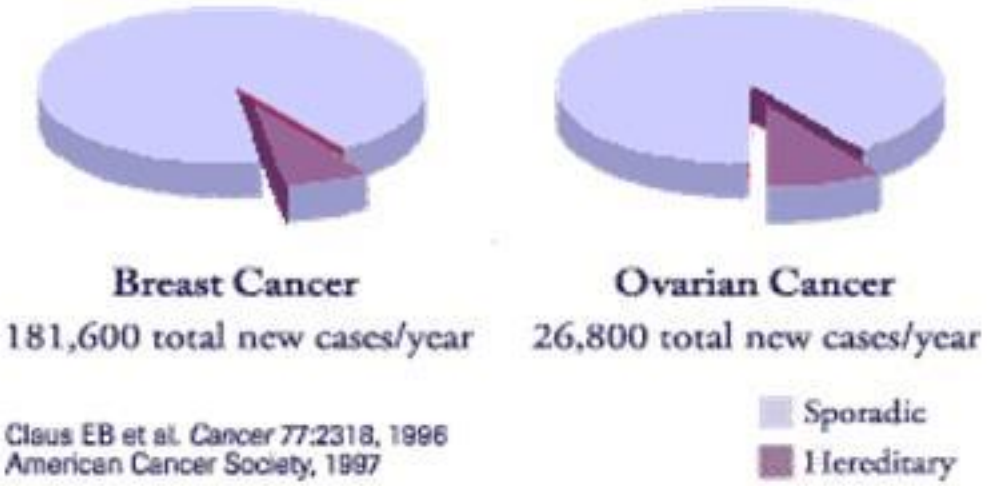
- **ANÁLISIS: 237 FAMILIAS**

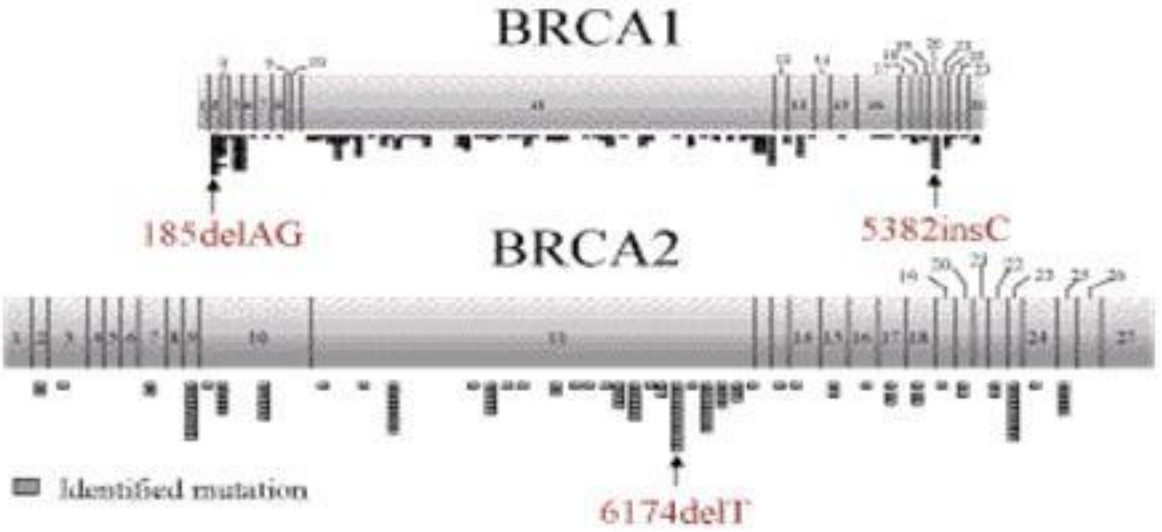
- **BRCA 1: 52 %**
- **BRCA 2: 32 %**
- **INDETERM.: 16 %**

- **Familias con:**
- **Ca. mama y ov.: BRCA1 >> BRCA2**
- **Ca. mama fem.y masc. BRCA2>>BRCA1**

Ford et al.Am.J.Hum.Genet.62, 676, 1998

CÁNCER DE MAMA Y OVARIO





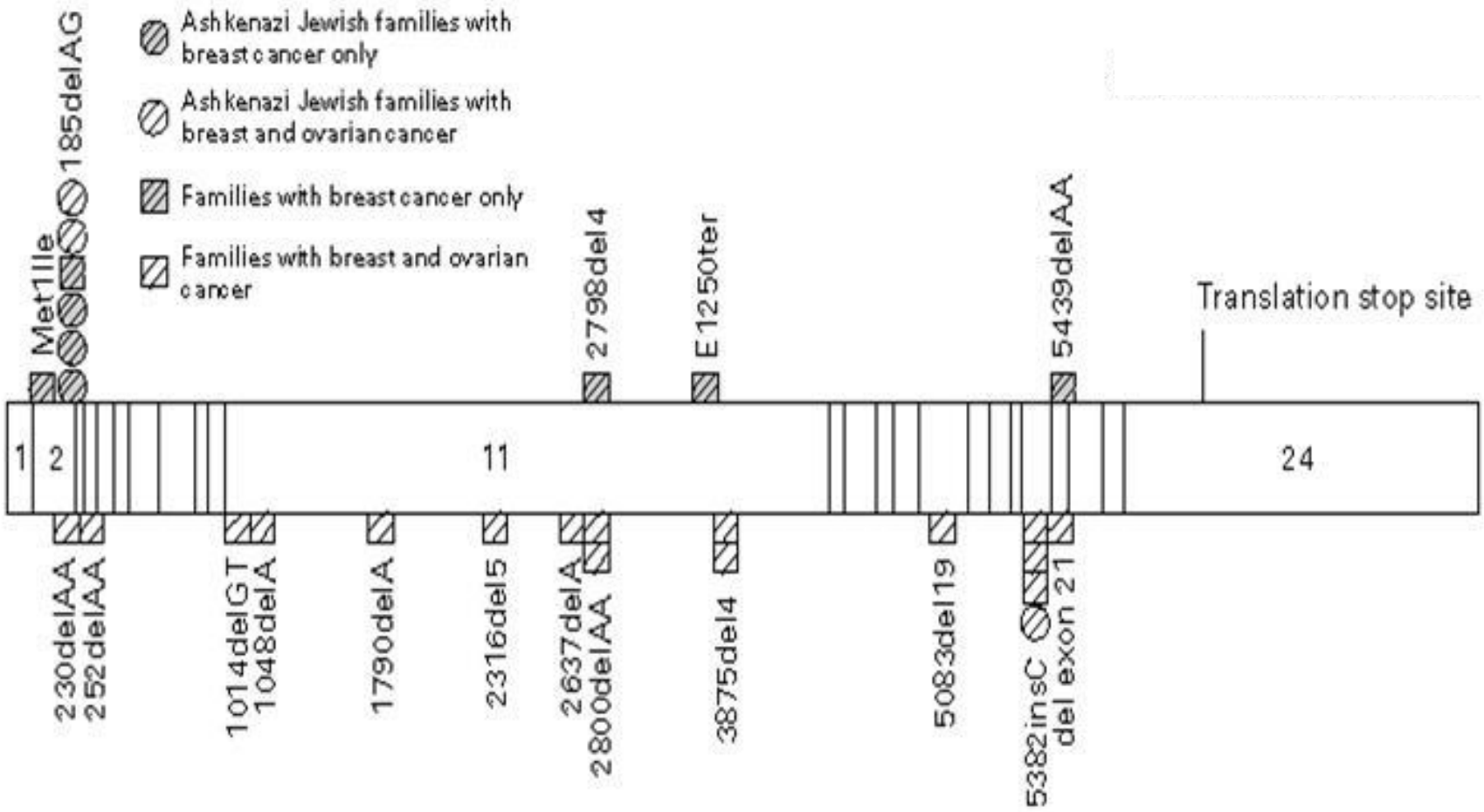


Figure 1. Location and Tumor Specificity of the 27 *BRCA1* Mutations Identified in Families with Breast Cancer.
Exon 1 and the coding region of *BRCA1* are depicted, with exons 1, 2, 11, and 24 included for reference. The translation start site is located at the mutation Met1Ile.

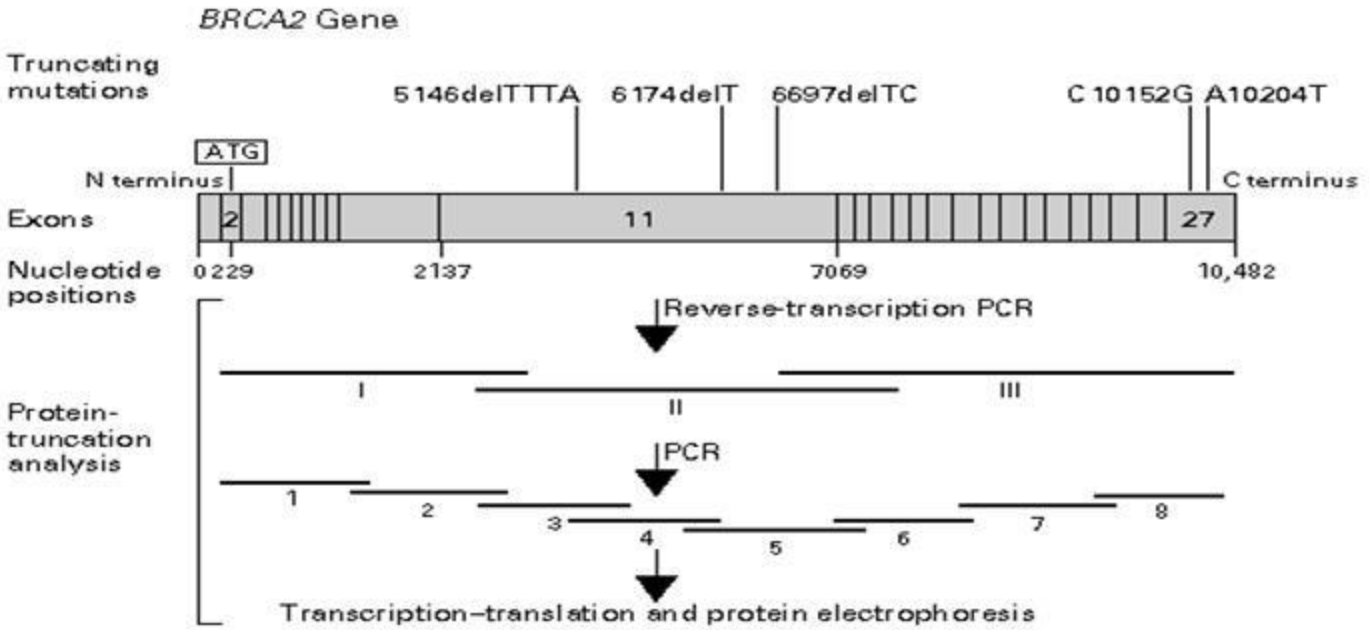


Figure 1. Identification and Location of *BRCA2* Mutations That Truncate the Polypeptide Chain. The *BRCA2* gene is encoded by 27 exons, the second of which contains the initiator codon (ATG) at nucleotide position 229. The coding sequence spans 10,254 nucleotides, encoding 3418 amino acids. Each truncating mutation results in the insertion of a premature stop codon in the sequence. Detecting these mutations by a protein-truncation assay involves making a complementary DNA copy of the *BRCA2* transcript, performing two rounds of nested PCR amplification, and synthesizing messenger RNA and protein in vitro from the PCR-generated templates. Abnormally shortened protein products are created when there is a truncating mutation in one of the two *BRCA2* alleles; these products can be detected by electrophoresis.

Dominios de BRCA1

N-ter Ring domain:

E3 ubiquitin ligase activity and facilitates protein ubiquitination

C-ter BRCT domain:

Phospho-protein binding domain, required for BRCA1 translocation and accumulation at DNA damage sites

BRCA1 is a key player in the DNA damage response

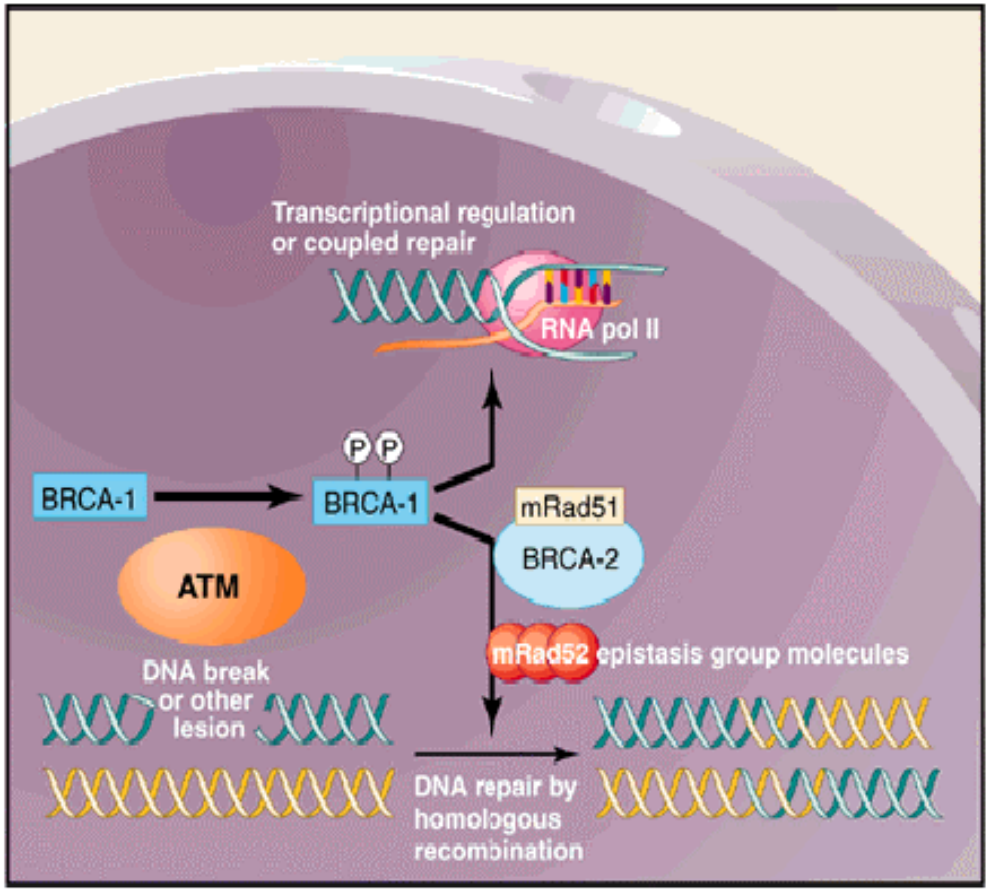
BRCA1 relocates to DNA damage sites and form nuclear loci following DNA double- strand breaks

ESTRUCTURA Y FUNCION DE BRCA 1

- **Fosfoproteína nuclear de 220 kDa en células normales.**
- **Los niveles de mRNA de BRCA1 disminuyen con la transición CIS a carcinoma invasor.**
- **Como BRCA1 y RAD51, BRCA2 relocaliza a sitios de replicación en fase S luego de exposición a OH-urea ó luz UV.**
- **INTERVIENE EN LA REPARACION DEL DNA**

Breast Cancer genes and DNA Repair

Repairing damaged DNA. BRCA1 is phosphorylated by the ATM protein kinase in response to DNA damage induced by g radiation. Phosphorylated BRCA1 activates DNA repair through homologous recombination, in cooperation with BRCA2, mRad51, and other molecules related to members of the yeast RAD52 epistasis group. Phosphorylated BRCA1 may also regulate transcription and transcription-coupled DNA repair.



Science (1999) 285: 747-750;
286: 1100-1102, 1162-1166

Functions of Non-mutant BRCA1 Protein

- Suppressor of tumors
  - How we know: when normal, it constrains cell division, but when mutated, inappropriate cell division results
- Facilitator of DNA repair
  - How we know:
    - BRCA1 has been found at replication forks just after a cell has been exposed to UV radiation
    - BRCA1 contains 2 BRCT domains—found in proteins involved in DNA repair
    - BRCA1 is phosphorylated after DNA is damaged and BRCA1 is relocated from cytosol into nucleus
- Factor in cell degradation
  - How we know:
    - BRCA1 contains a RING finger domain near the N-terminus.
      - RING fingers: cysteine-rich sequences that facilitate ubuquitination

BRCA1 in DNA damage-induced cell cycle checkpoints activation

G1/S checkpoint:

p53, control via p21

BRCA1 facilitates p53 phosphorylation by ATM

S-phase checkpoint:

ATM and ATR via Chk1 and 2 regulate cyclins and cyclin-dependent kinases activity during S phase

BRCA1 participates in this signal transduction by regulating Chk1 kinase and phosphorylation of BRCA1 is required for the S-phase checkpoint following irradiation Suggesting phospho-BRCA1 may recruit functional partners for regulating this signal Cascade.
Additionally BRCA1 may also regulate ATM phosphorylation after DNA damage in S phase.

G2/M checkpoint:

ATM and ATR phosphorylates Chk1 and 2 ⟶ Phosphorylate mitotic kinase Weel and complex Cdc25A/B/C

This suppresses the activuty of cyclin B abd Cdc and blocks mitosis

Loss of BRCA1 abolishes G2/M checkpoint;
Like in S-phase checkpoint , BRCA1 regulates Chk1 kinase activity during G2/M checkpoint.

ATM phosphorylates BRCA1 in a different position than in S-phase checkpoint, Implicating that BRCA1 may have different functional partners in G2/M checkpoint

BRCA1

-BRCA1 activa los 3 check points del ciclo celular (G1/S, S y G2/M) a través de distintas fosforilaciones que le confieren funciones reguladoras distintas

-Interacciona con p53 facilitando la fosforilación de p53 por ATM en respuesta al daño

1) P53 –P → ↑P21 ^{WAF1} → Arresto en G1/S

2) BRCA1 detiene la replicación del DNA (mecanismo desconocido)

3) Detiene la mitosis para permitir reparación del DNA regulando la actividad de ChK1 quinasa

TIPOS DE BRCA- ANÁLISIS

1- Total: para determinar mutación por primera vez se secuencia todo el gen.

2- Sitio Único: para pacientes con parientes cuya mutación BRCA1/BRCA2 es conocida.

3- Multisitios: para individuos Ashkenazi.
Detecta la presencia de mutaciones en tres sitios diferentes.
185 del AG, 5382 insC y 6174 del T

Tumor suppressor genes

Gene	Contribution to Hereditary Breast Cancer
BRCA1	**20%–40%**
BRCA2	**10%–30%**
TP53	**<1%**
PTEN	**<1%**
CHK2	**<1%**
Undiscovered genes	**30%–70%**

PTEN Tumor suppressor gene

- Mapea en cromosoma 10q23
- Es fundamental para el desarrollo embrionario
- Está mutado y delecionado en múltiples cánceres (mama, pulmón) y desórdenes linfoproliferativos letales
- Presenta haploinsuficiencia (no todos los tumores pierden el alelo normal)

-Pérdida de PTEN en línea germinal genera sindromes hereditarios (PHTS):

múltiples Hamartomas

Alta susceptibilidad al cáncer

Desórdenes neurológicos

-Biopsias de cánceres esporádicos tienen menor expresión de PTEN pero el segundo alelo no está mutado

PTEN Tumor suppressor gene

Función:

1- Fostasa de lípidos: defosforila PIP3 (3' fosfoinosítido-3,4,5 trifosfato)

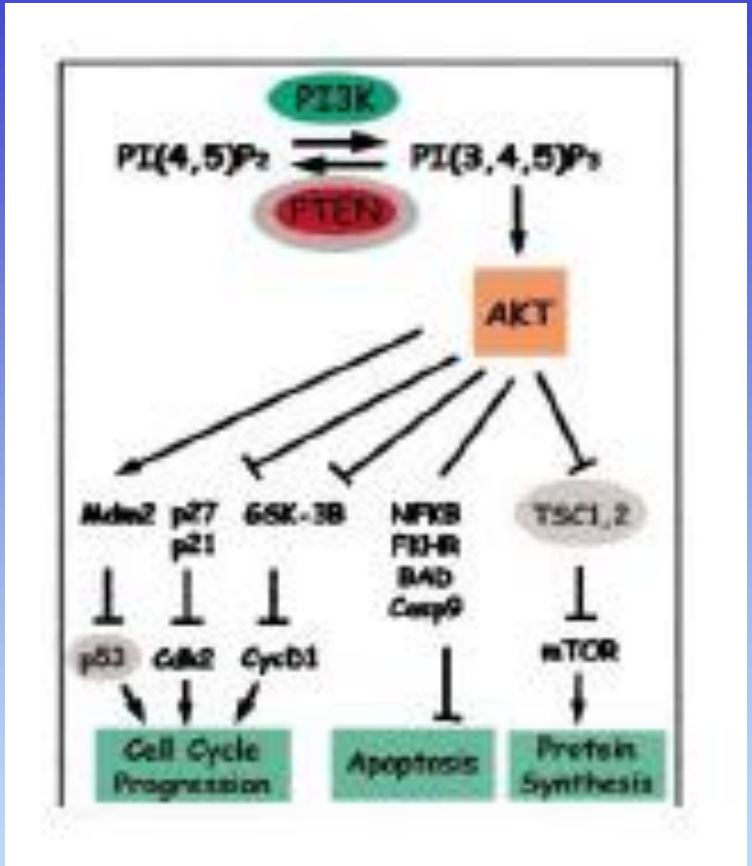
PIP3 activa la vía PI3K ∴ proliferación celular- inhibición apoptosis

PTEN es un regulador negativo de vía PI3K

2- Actividad supresora tumoral nuclear directa sin actividad fosfatasa:

- regula inestabilidad genómica
- Progresión en el ciclo celular
- diferenciación celular
- expresión génica

PTEN



(D) Phosphoinositide 3-kinase (PI3K) signaling. PI3K, which is activated via many growth factor receptors, catalyzes the conversion of phosphatidylinositol (4,5) bis-phosphate [PI(4,5)P_2] to PI (3,4,5)P_3. The activity of PI3K is opposed by the PTEN lipid phosphatase. PI(3,4,5)P_3 recruits the AKT (PKB) kinase to the plasma membrane where it undergoes phosphorylation by PDK1 (not shown) and activation. AKT phosphorylates substrates that foster cell cycle progression, cancel apoptosis, and facilitate translation of capped mRNAs. The tuberous sclerosis complex [TSC1 (hamartin) and TSC2 (tuberin)] antagonizes the function of a G protein (Rheb, not shown) whose activity is required for activity of the mTOR kinase and its ability to promote translation (adapted from Sulis and Parsons, 2003; Tee et al., 2003). Loss of PTEN upregulates signaling.

PTEN Tumor suppressor gene

- Ratones heterocigotas (penetrancia incompleta) desarrollan ca. próstata intraepitelial e in situ

- Ratones con fenotipo hypo/-, presentan reducción de proteína PTEN de 70-80% y forman tumores de próstata con 100% penetrancia, invasivos y metastásicos

- Ratones hyper/+ , presentan reducción de proteína PTEN del 20% y forman tumores de mama, ganglios linfáticos, pulmón. No poseen mutación en el alelo wt.

Existe una exquisita sensibilidad tejido específica a la disminución de los niveles de PTEN con tejidos muy poco sensibles (próstata) y otros muy sensibles (mama y ganglio)

Haploinsuficiencia convierte a PTEN en TSG

(ca. mama, reducción del 35%, asociados a signature génica ; reducción del 15 % en sanas, predisposición?)

PTEN Tumor suppressor gene

¿Qué factores pueden disminuir los niveles de PTEN?

- factores del ambiente (dieta, zinc, drogas)

- eventos genéticos (mutaciones, deleciones)

- Control transcripcional de PTEN por vía de MAPK y c-Jun (ras mutado resulta en reducción de PTEN)

- Control post-transcripcional (miRNA, pérdida de ceRNA (competitivo endógeno)

- Control post-traduccional (drogas, E3-ligasas, enzimas deubiquitinantes, DUB)

PTEN

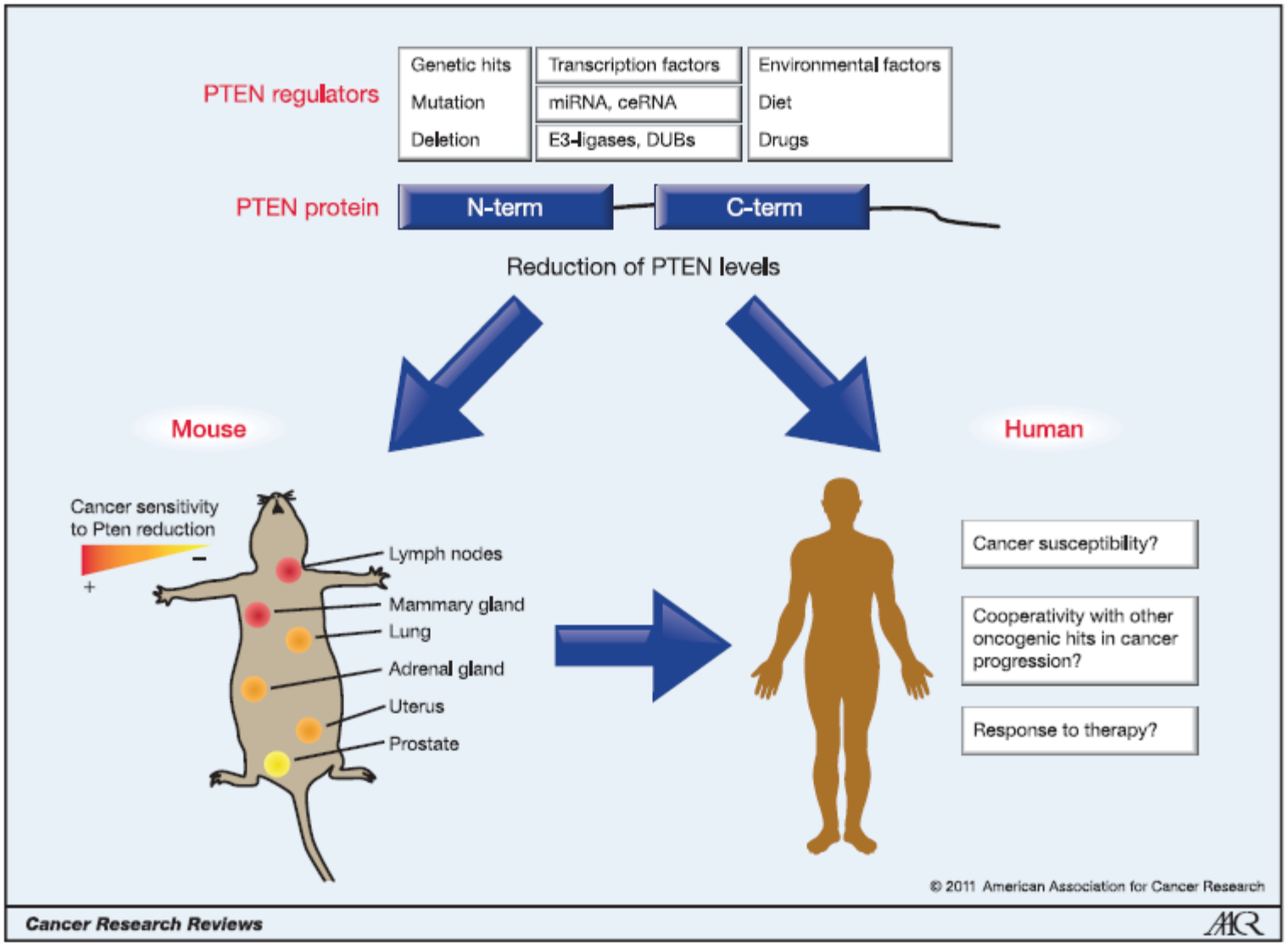


Figure 1. Impact of PTEN regulatory cues in tumorigenesis. Schematic of the tissue-specific cancer sensitivity to PTEN downregulation and the regulatory factors that could lead to the reduction of PTEN levels. PTEN expression is regulated at multiple levels, genomic (mutation and deletions), transcriptional (transcription factors), post-transcriptional (miRNAs, ceRNAs, such as the PTEN pseudogene), and post-translational [drugs, E3-ligases, deubiquitinating enzymes (DUB)]. In turn, the reduction of PTEN levels has a gradual and tissue-specific impact on cancer initiation in the mouse, with the mammary tissue being among the most sensitive tissues to PTEN level reduction. These data suggest that, in humans, subtle reduction of PTEN levels may lead to cancer susceptibility, cooperate with other oncogenic events in cancer progression, or affect the response to therapy.

CIO-FUCA

Control epigenético de la supresión tumoral

-Modificaciones epigenéticas son cambios heredables en la expresión génica que ocurren sin alterar la secuencia de DNA

(ej: metilación del DNA y modificaciones en las histonas)

- Metilación del DNA : 1- preserva la integridad del DNA
2-silenciamiento de genes a nivel transcripcional

- CpG son targets de metilación (CpG islands en promotores, DNA repetitivo, regiones pericentroméricas, y non-CpG islands)

-Metilación en promotores y cuerpo de los genes tiene función regulatoria

- Metilación fisiológica para imprinting de genes e inactivación de X en hembras

Control epigenético de la supresión tumoral

-Maquinaria metilante fisiológica está regulada por el ciclo celular para mantener la metilación y preservar los patrones durante la replicación

- En tumores todo lo contrario: existe deregulación de la maquinaria demetilante y una gradual de-metilación del genoma así como la adquisición de hipermetilación aberrante en CpG islands (puede reprimir TSG).

- Hipometilación puede activar oncogenes

- Hipoacetilación de histonas también resultar en silenciamiento transcripcional de TSG ; también fosforilación, sumoilación, ubiquitinación, biotinilación, etc)

- Hipermetilación de promotores interfiere con la unión de factores de transcripción o alterando la cromatina, existiendo cooperatividad con la marcas de histonas acetiladas

-Se pueden usar como target, tratamiento con agentes de-metilantes o inhibidores de la HDAC para re-expresar los genes.
(ej: 5-azadC, tricostatina)

TABLE 2
Partial Listing of Proven and Putative TSGs Exhibiting Epigenetic Modifications in Different Tumor Types

Gene	Pathway	Cancer type(s)	Initial report(s)
p15^{INK4B}	Cell cycle	Hematologic, hepatic, lung, colorectal	210
p16^{INK4A}	Cell cycle	Hematologic, lung, gliomas, head and neck, squamous cell	211, 212
p14^{ARF}	Cell cycle	Breast, lung, gastrointestinal, hepatocellular, salivary gland	97
RB	Multifunctional, cell cycle	Retinoblastoma, lung, bladder, pancreatic, pituitary adenoma, hepatic, glioblastoma	213
MLH1	DNA repair	Colorectal, endometrial, gastric, squamous cell	214–216
MGMT	DNA repair	Colorectal, medulloblastoma, melanoma, testicular, esophageal	217, 218
BRCA1	DNA repair	Breast, ovarian, cervical	219
DAPK	Apoptosis	Cervical, lung, hematologic, glioma, laryngeal, esophageal, mesothelioma	220, 221
TMS1	Apoptosis	Breast, neuroblastoma, cervical, prostate	222
CDH1	Cell adhesion	Breast	
GSTP1	Carcinogen detoxification	Prostate, breast, renal	223
SERPINB5 (*maspin*)	Cell adhesion, invasion, metastasis	Breast, thyroid, prostate, melanoma	224
MDG1	Cell growth and proliferation	Breast, gastric	225
RIZ1	Transcriptional regulation	Gall bladder, gastric, thyroid	226
VHL	Transcriptional regulation	Esophageal, renal, oral	227
DIRAS3	Growth suppression	Ovarian, breast, thyroid	113
SFRP1	Cell adhesion, migration	Colorectal, breast	228
APC	Nuclear export	Colorectal, endometrial, breast, lung, stomach, melanoma	229
FHIT	Proliferation, survival	Esophageal; hematologic, lung	230
HIC1	Transcriptional regulation	Glioblastoma, hematologic, cervical	231–233
RASSF1A	DNA repair, cell cycle	Gastric, lung, breast, kidney, medulloblastoma, esophageal	234
RARB2	Signal transduction	Esophageal, breast	235
GADD45G	DNA damage, apoptosis	Lymphoma, nasopharyngeal, esophageal, cervical, lung	236
14-3-3σ	DNA damage	Breast, gastric, liver, lung skin, ovarian, neuroblastoma	237
CST6	Metastasis suppressor	Breast, glioma	238–240
BEX1	Unknown	Glioma	120
BEX2	Putative transcriptional regulator	Glioma	120
CEBPA	Transcription factor	Endometrial, lung, hepatoma	241
p27^{KIP1}	Cell cycle	Melanoma	242
p21$^{WAF1/CIP1}$	Cell cycle	Prostate	243
RUNX3	Transcription factor, apoptosis	Yolk sac, colon, nasopharyngeal, lung	244

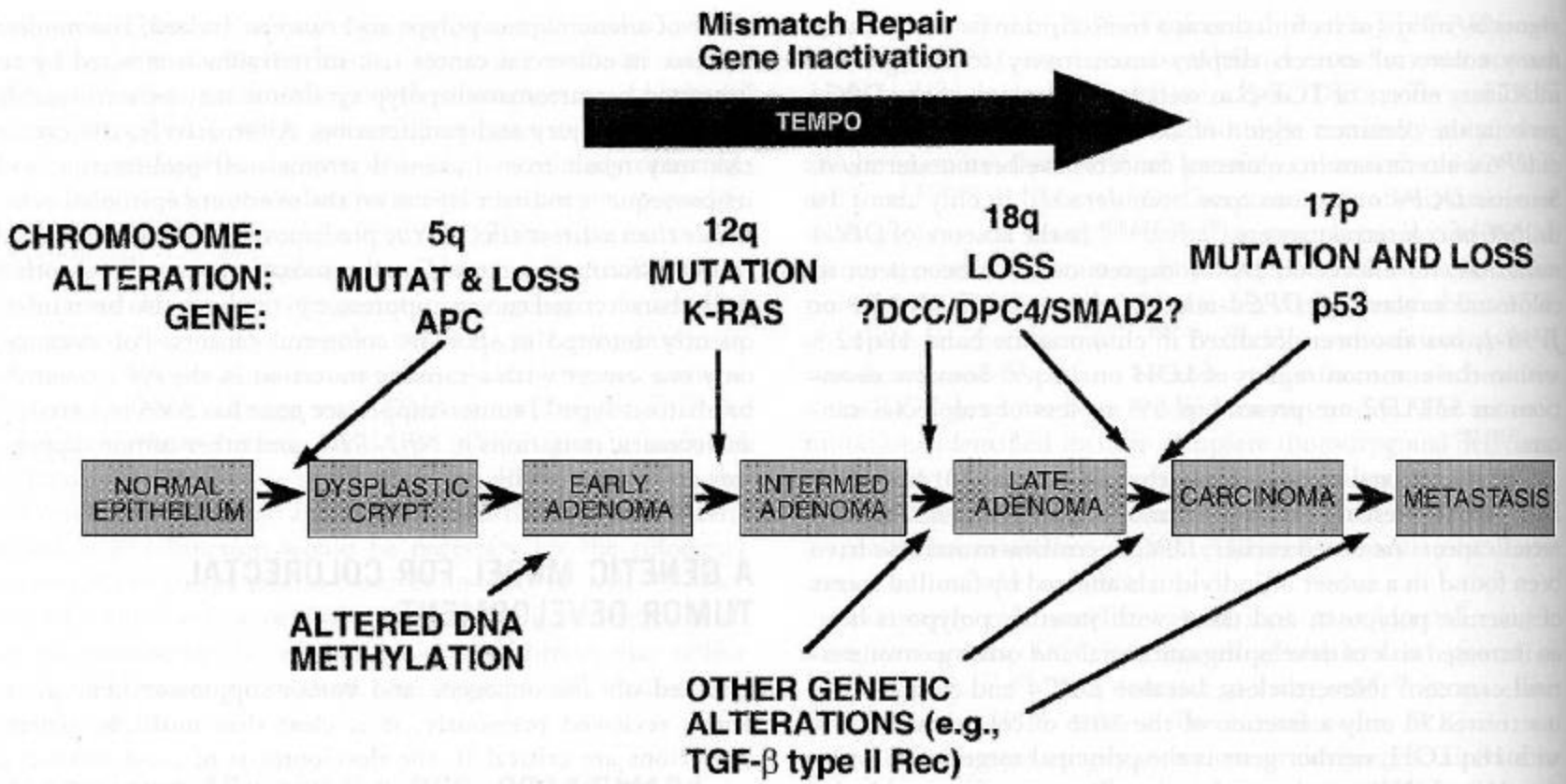
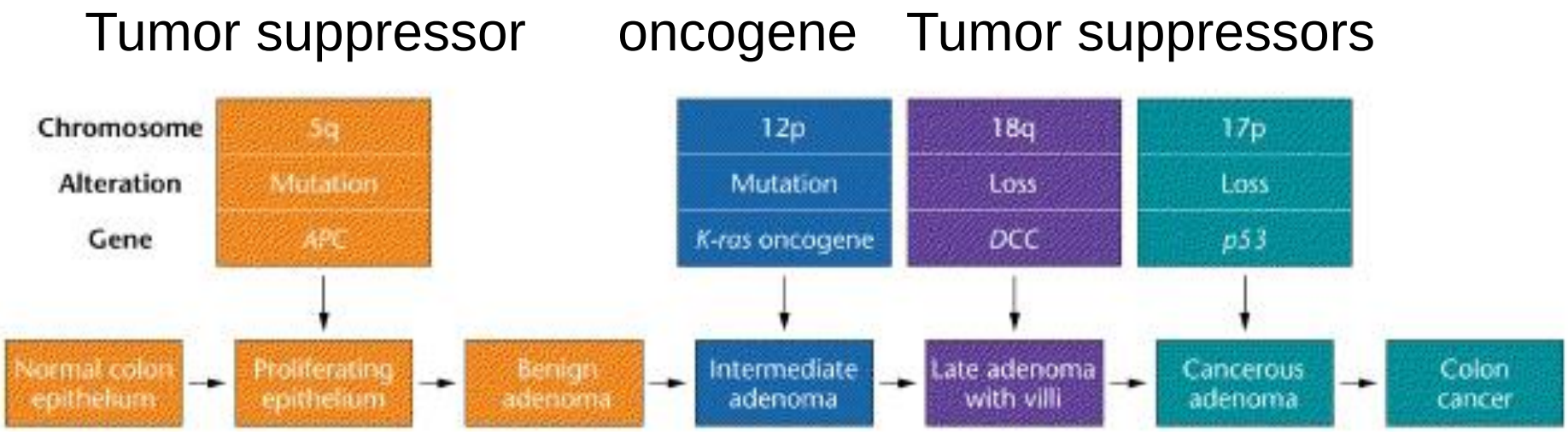


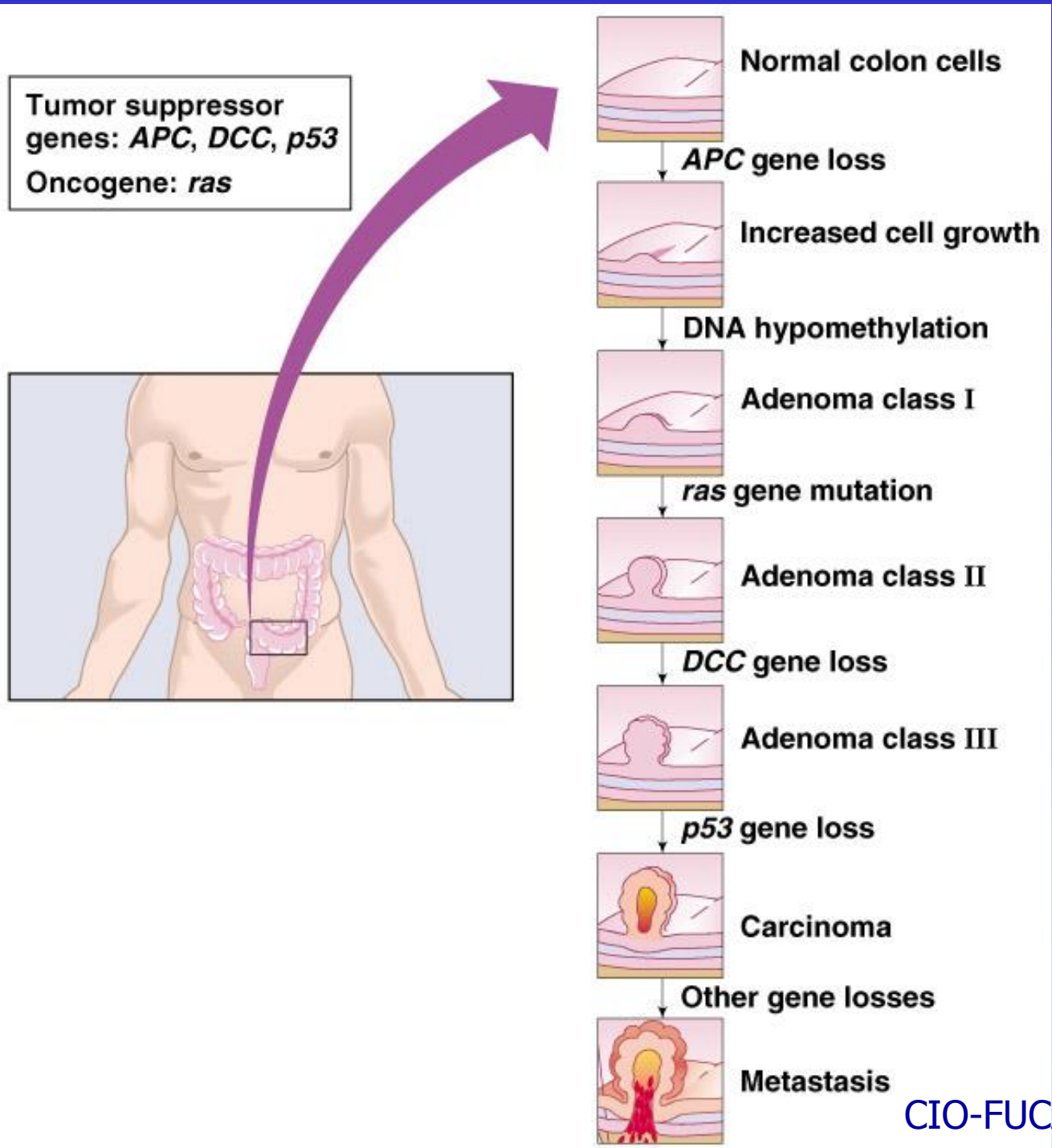
FIGURE 15–12. Genetic model of colorectal cancer. The majority of colorectal cancers are believed to arise from adenomatous polyps over a period of years or even decades. The inherited and somatic genetic alterations believed to underlie tumor initiation and progression are indicated and are discussed in detail in the text. Although their order is not invariant, the mutations show strong association with particular stages of tumorigenesis. In about 15% of colorectal cancers, germline and somatic mutations as well as epigenetic mechanisms inactivate mismatch-repair gene function. Cells with mismatch-repair gene inactivation manifest a mutator phenotype, and the mutation rate and tempo of tumor pregression are clearly altered. Mutations inactivating the transforming growth factor-β type II receptor (TGF-β type II Rec) appear to be restricted to those tumors with mismatch-repair gene inactivation (i.e., tumors with the MSI^+ phenotype). (Modified from Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61:759, 1990.)

Cancers Usually Result from a Series of Mutations in a Single Cell



Normal -> proliferating -> benign -> intermed. -> late -> cancerous -> colon
epithelium adenoma adenoma adenoma with villi adenoma cancer

Bert Vogelstein's model of colorectal cancer OMIM-175100



CIO-FUCA