Tumour Suppressors

Properties of tumour suppressors

Evidence for the existence of tumour suppressors

Retinoblastoma

Properties and function of the RB protein (pRB, pRb)

- E2F
- pRB phosphorylation
- cell cycle
- proteins that bind pRB
- RB gene knock-outs

p53

- biochemical properties
- transcriptional regulator
- guardian of the genome
- p63, p73

p16 (MTS1, INK4A, CDKN2)

BRCA1, BRCA2

Other Tumour Suppressors (for you information only)

Multiple steps in cancer

Telomere/telomerase

Properties of Tumour Suppressors

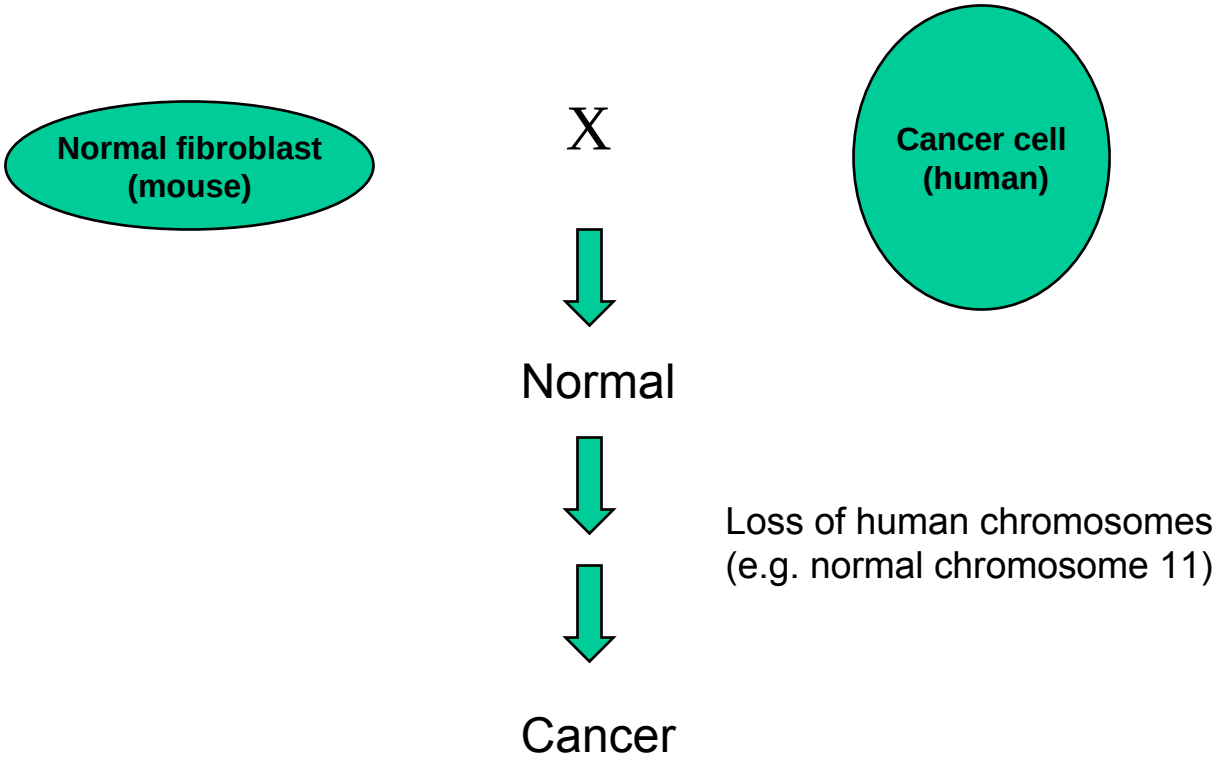
Properties:

- Negative regulation of cell growth and development
- Many tumour suppressors are directly involved in the regulation of the cell cycle
- Mutated in tumours
- Absence or inactivation of the gene product (**absence of function)** is oncogenic
- Mutations are usually recessive; i.e. both copies of the gene must be mutated in order to contribute to tumour formation*

*Two hits are required to inactivate tumour suppressors; therefore one mutated allele can be carried silently in the germline (basis of familial cancer syndromes)

Evidence for the Existence of Tumour Suppressor Genes

(1) **Somatic cell hybridization** (is cancer phenotype dominant or recessive?)

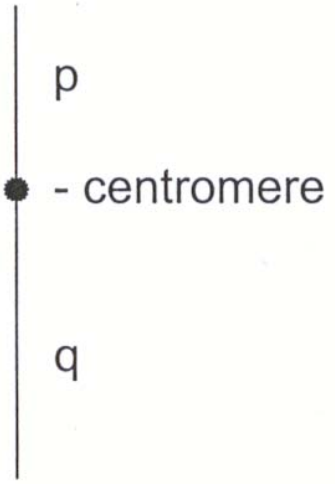


(2) Chromosomal (karyotype) analysis of blood cells of patients with an inherited susceptibility to cancer show chromosome deletions

e.g. Retinoblastoma

5% of patients with the inherited form of retinoblastoma have a germline deletion of part of the long arm of one copy of chromosome 13. In all cases, band13q14 is included in the deletion. (N.B. These patients have many clinical problems in addition to RB tumours.)

Similar deletions are found in tumour cells of patients with normal blood cell karyotypes



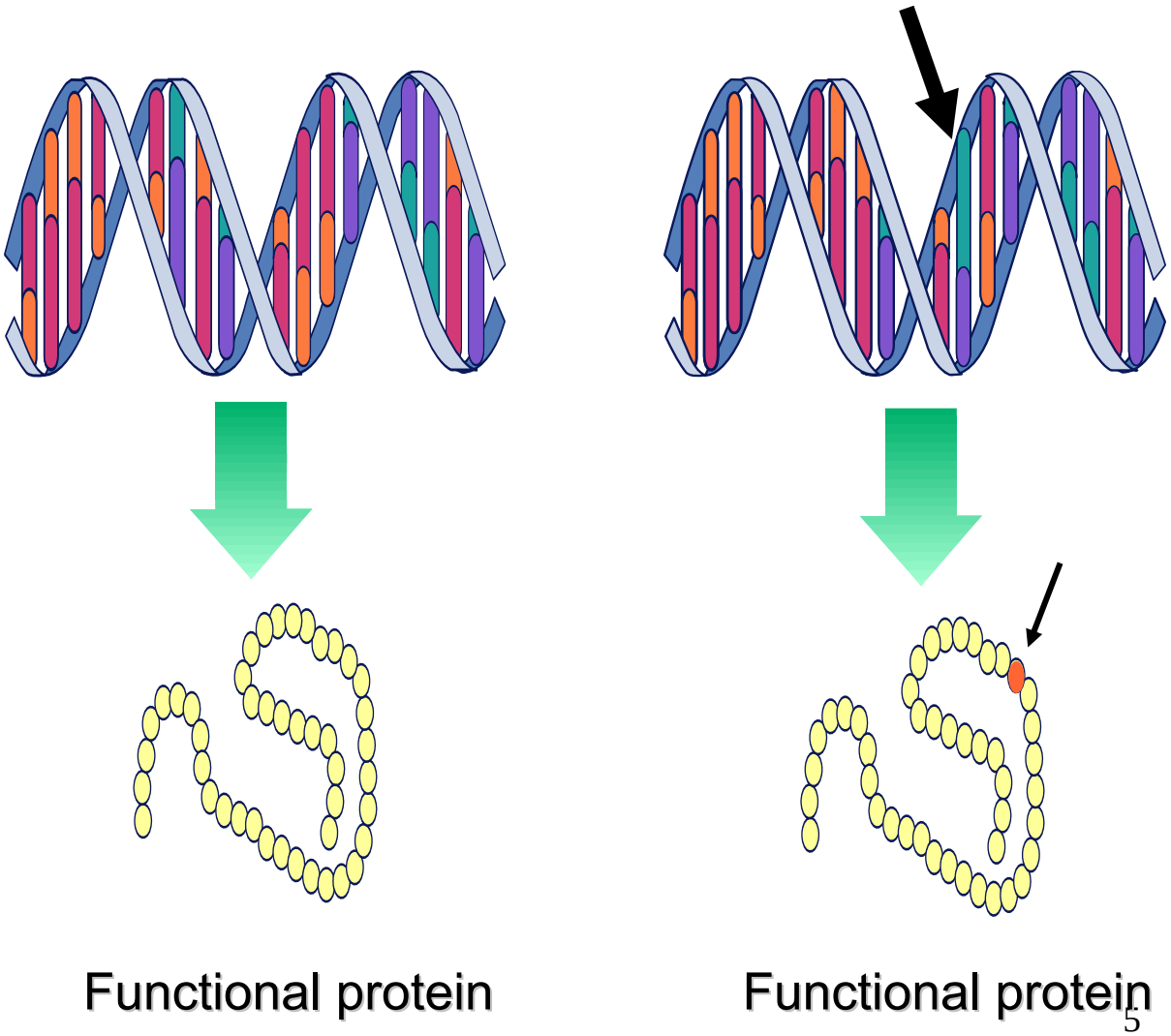
Wilms tumour

Deletions of the short arm of chromosome 11, involving 11p13

(3) Loss of heterozygosity in tumours

DNA Polymorphism = RFLP, SNP

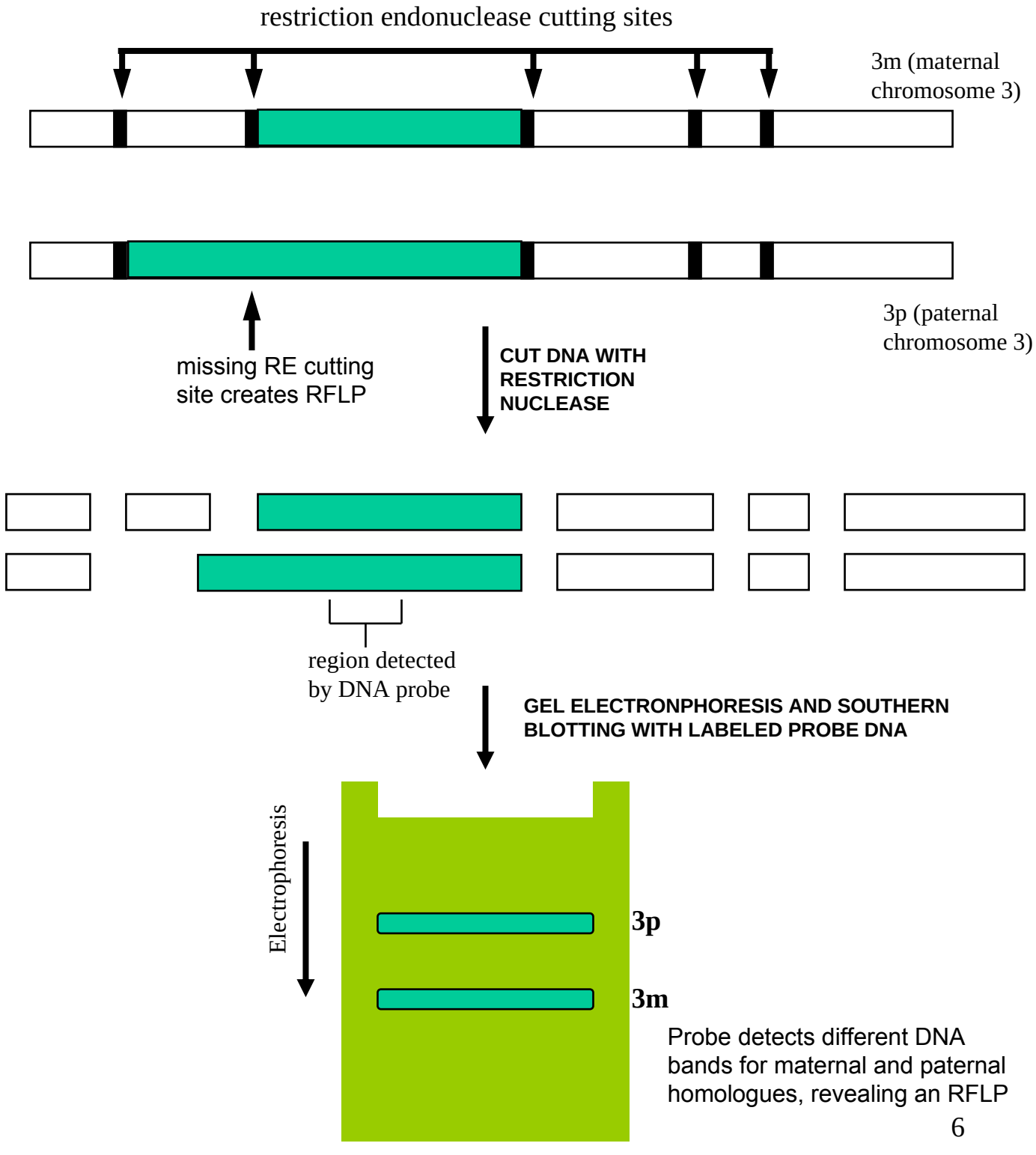
Single nucleotide change in DNA sequence that usually does *not* alter protein function



Functional protein

Functional protein

Restriction fragment length polymorphism (RFLP)



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Loss of heterozygosity (LOH) in tumours – cont'd

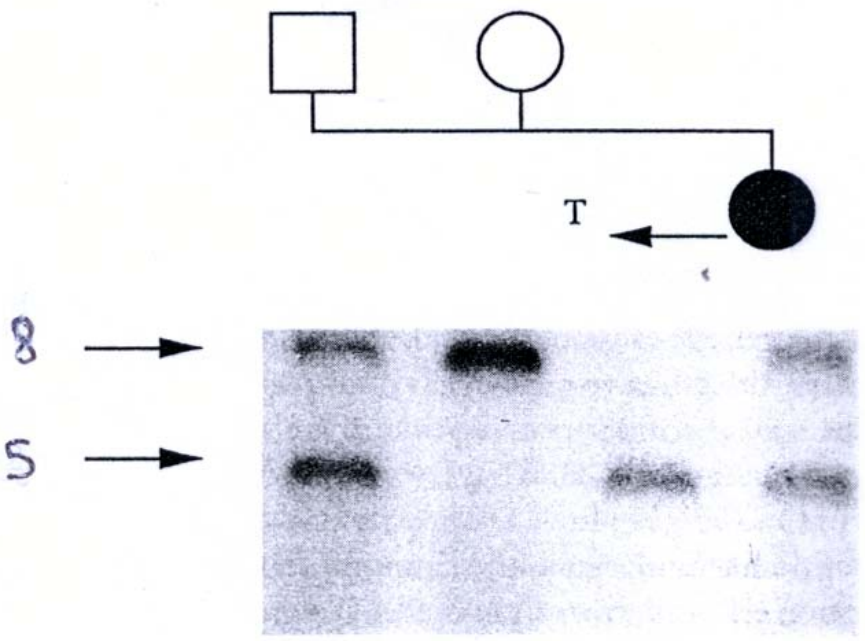


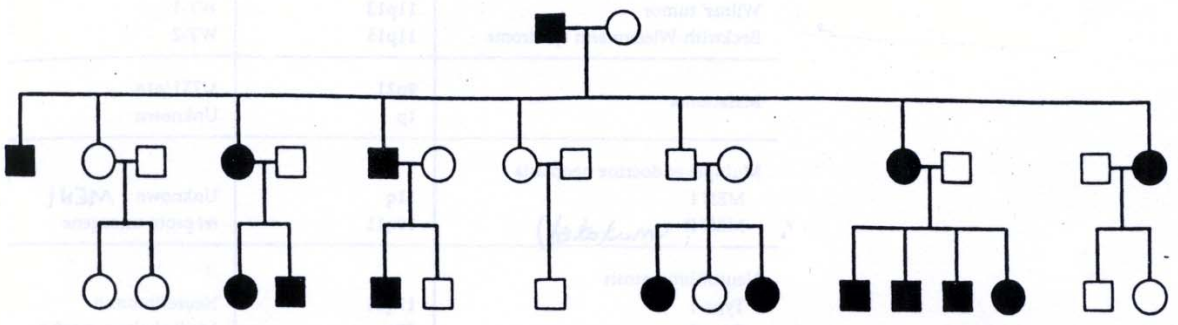
Figure 4.10. The usefulness of restriction-fragment-length polymorphisms (RFLPs) to identify the loss of genetic information in a retinoblastoma tumor is illustrated. In this particular example, DNA probe p88 detects an RFLP (with the restriction enzyme Xba1) in the RB1 gene. As shown in the figure, the mother is homozygous for this marker (lane 2) only having allele 1 and the father heterozygous having both alleles 1 and 2 (lane 1). The daughter inherited one allele from each parent and is also heterozygous in the majority of her cells (lane 4). However, the retinoblastoma which arose in the child (lane 3) shows a loss of the allele (upper band 1) inherited from the mother and retains only the RB1 allele inherited from the father. (Figure courtesy of Xiaoping Zhu, Hospital for Sick Children, Canada)

Table 2 Examples of Loss of Heterozygosity in Human Tumours

Tumour type/Syndrome	Chromosomal Region	Tumour Suppressor Gene Involved
Breast cancer	13q14 17p13 17q21 13q12 – 13q13 1p, 1q, 11p	Rb p53 BRCA1 BRCA2 Unknown
Colorectal cancer	2p 3p21 5q21 17p13 18q21	HMSH2 HMLH1 APC p53 DCC
Retinoblastoma	13q14	Rb
Wilms' tumour Beckwith-Wiedemann syndrome	11p13 11p15	WT-1 WT-2
Melanoma	9p21 1p	MTS1/p16/INK4A/CDKN2 Unknown
Multiple endocrine neoplasia MEN I MEN II	 11q 10q11	 MEN1 Ret proto-oncogene*
Neurofibromatosis Type 1 Type 2	 17q11 22q	 Neurofibromin NF1 Merlin/schwannomin NF2
Renal cell carcinoma	3p25 3p14	VHL Unknown
Lung cancer	13q14 17p13 3p14-3p21	Rb p53 Unknown
Prostate cancer	8p22, 10q24	Unknown
Neuroblastoma	1p36, 14q	Unknown
Brain tumours and gliomas Meningiomas	9p21 17p13 1p, 14q, 17, 22	MTS1/p16/INK4A, CDKN2 p53 Unknown
Pancreatic cancer	18q21.1	Smad4/DPC4

Retinoblastoma

- Childhood ocular tumour
- Incidence 1/20,000
- Occurs in both sporadic (nonhereditary) (60%) and familial (hereditary) (40%) forms
- Sporadic - one focus of tumour growth (unilateral)
- Familial - 3-4 foci of tumour growth (usually bilateral)
- Familial tumours appear earlier than sporadic tumours



PEDIGREE of a family with familial retinoblastoma was published by Thaddeus P. Dryja and his collaborators. Affected members are indicated by solid circles (females) or squares (males). Five children in the second generation developed the tumor. One son who was unaffected had nonetheless inherited a mutated chromosome 13: two of his daughters were affected.

- Inherited as an autosomal dominant trait
- Patients with familial RB commonly develop other tumours with the most common being osteosarcoma

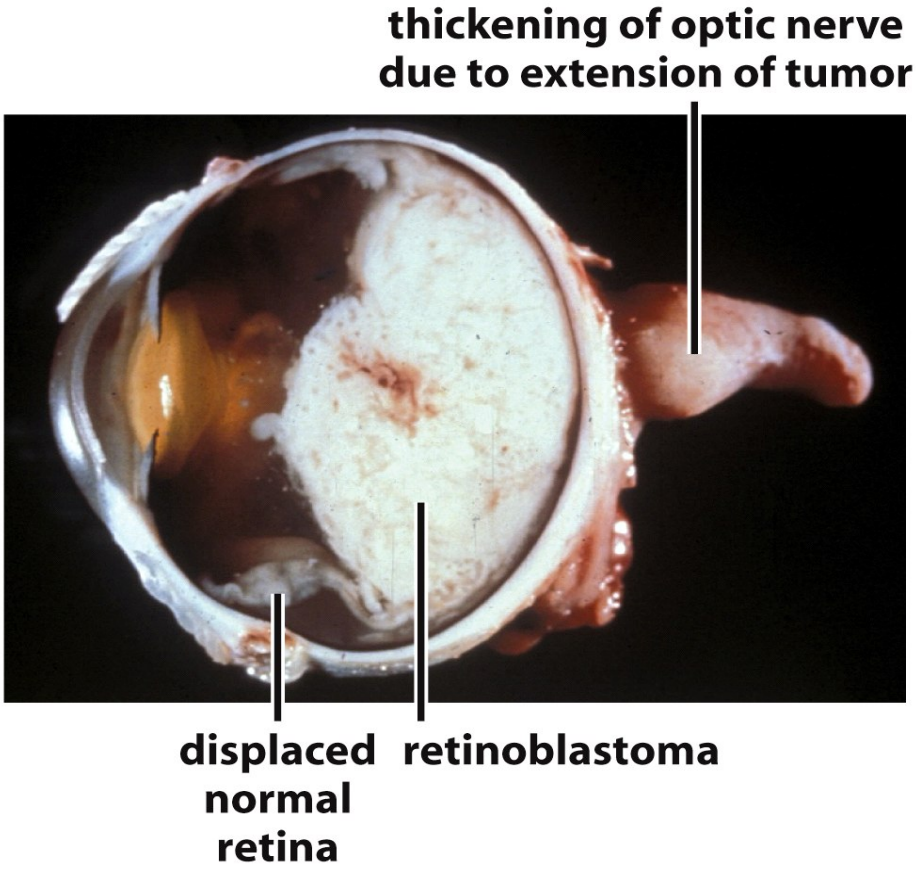


Figure 7-4b The Biology of Cancer (© Garland Science 2007)

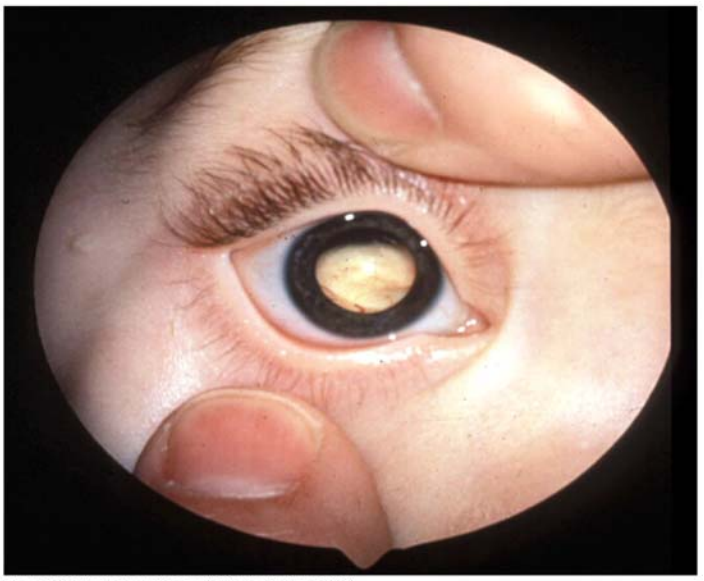


Figure 7-4c The Biology of Cancer (© Garland Science 2007)

Inherited Cancer

A family history with many affected close relatives

An early age of onset compared to sporadic cancer of the same type

Multiple primary tumours

Two mutation model of cancer

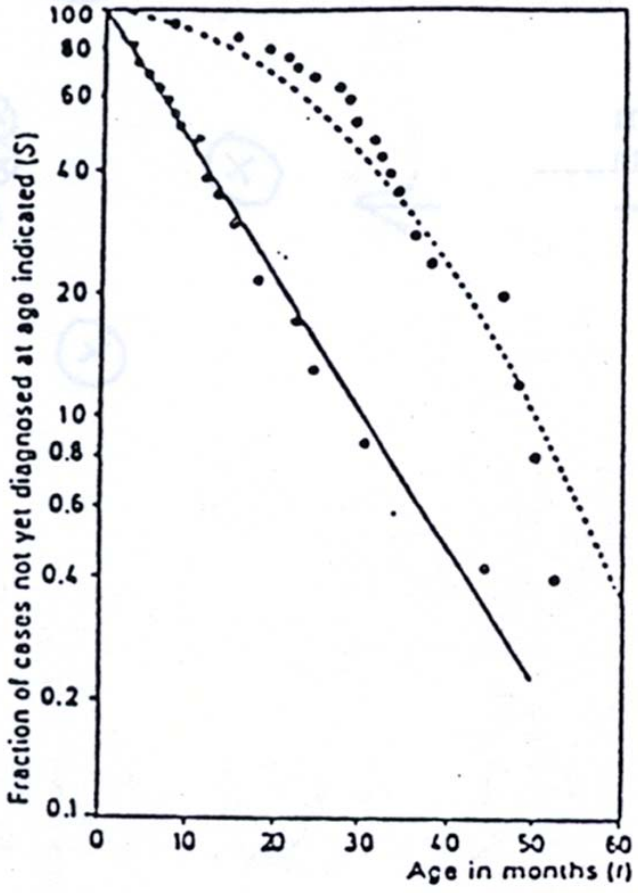
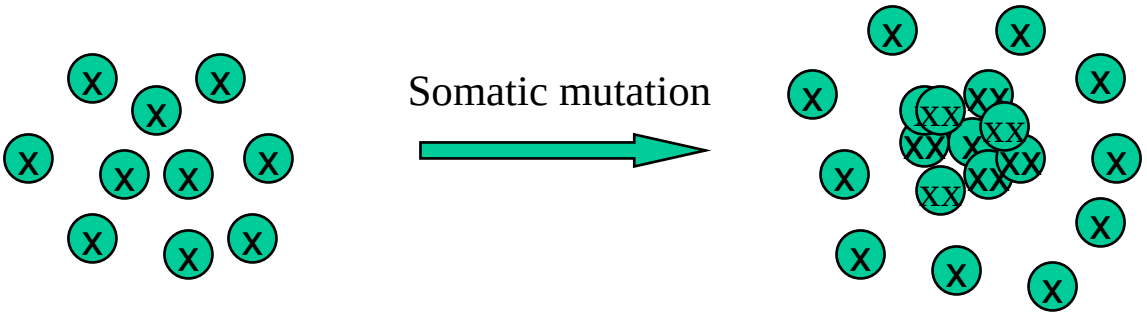
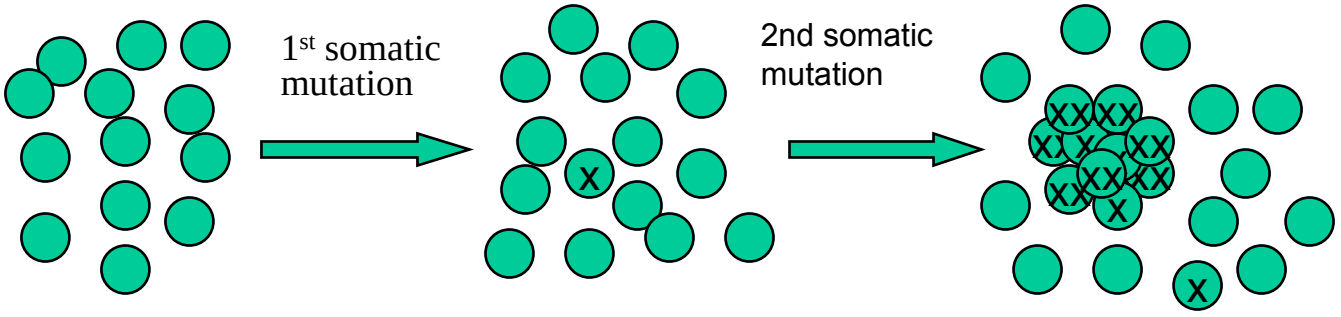


Fig. 5. Semilogarithmic plot of fraction of cases of R not yet diagnosed (S) vs age in months (t). The one-hit curve was calculated from $\log S = -4 \times 10^{-4} t^2$. Apparently, bilateral cases (n = 23: —•—) follow one-hit kinetics, unilateral ones (n = 25: – –o– –) follow two-hit kinetics. (From Knudson, 1971)

Hereditary Cases



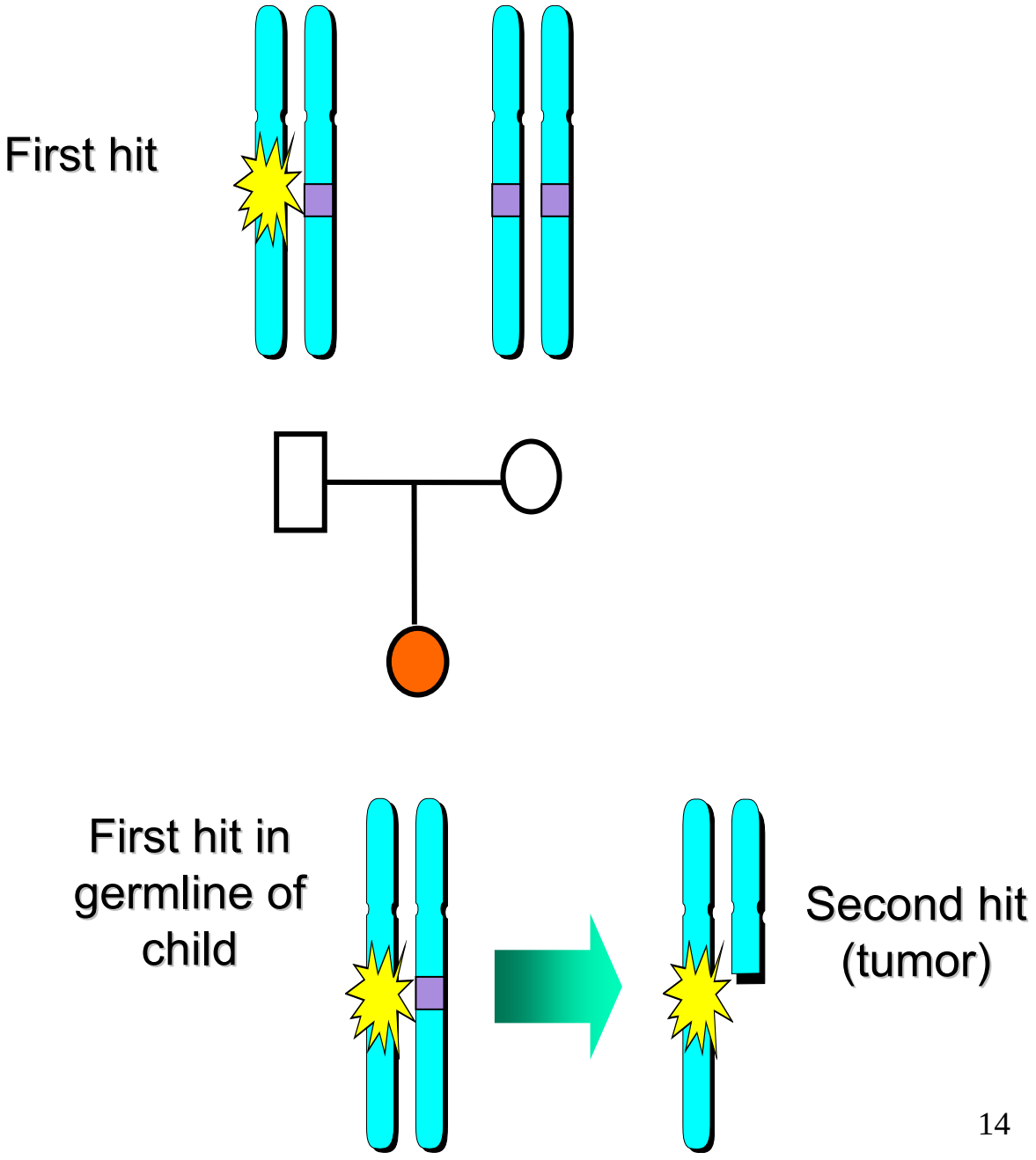
Nonhereditary Cases



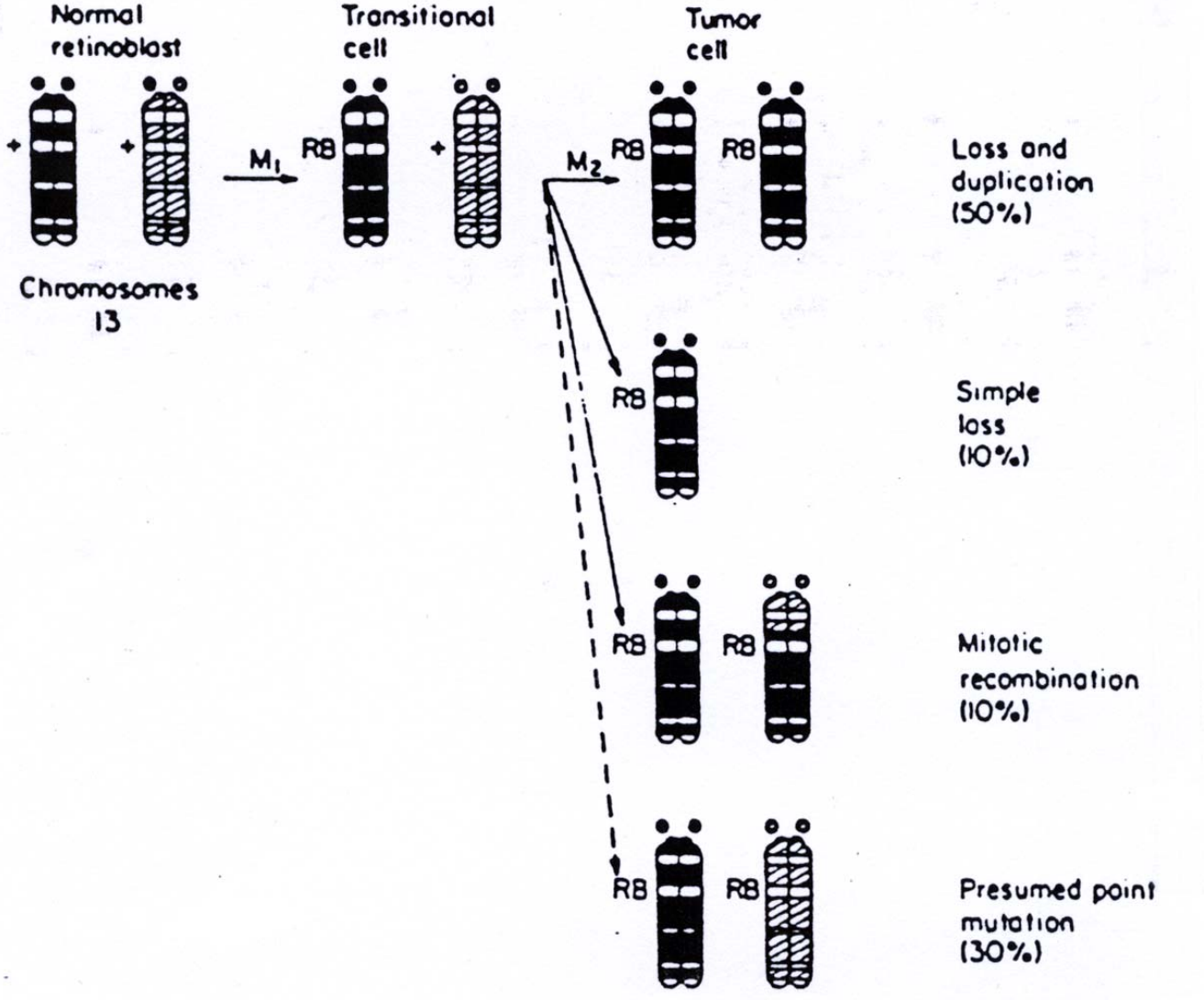
In a small percentage of cases - constitutional deletion of part of chromosome 13

- common band missing (13q14)

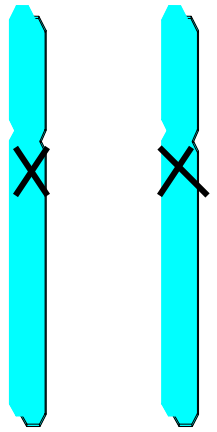
The Two-Hit Hypothesis



Analysis of DNA polymorphic markers specific to chromosome 13 indicate the following mechanisms for loss of gene function (in both hereditary and nonhereditary RB tumours):



Family studies indicate that the duplicated chromosome 13 originates from the affected parent - therefore suggesting that RB tumours arise as the result of complete loss of function at a locus on chromosome 13.



13p **13m**

Conclusion - The RB gene is a “recessive” tumour suppressor gene. Both alleles have to be mutated for tumour formation. Absence or inactivation of the RB gene product leads to tumour formation.

However, RB is inherited as an autosomal “dominant” trait because the chance of getting RB if you inherit a mutated RB gene from an affected parent is close to 100%.

The RB gene was cloned in 1986

RB is inherited as an autosomal dominant trait

RB gene is a recessive tumour suppressor gene

Classic recessive genetic disease:

25% of children from two heterozygote parent will have the disease (every cell in the child's body is -/- for a particular genetic mutation)

+/- X +/-

¼ +/+ : ½ +/- : **¼ -/-**

Retinoblastoma:

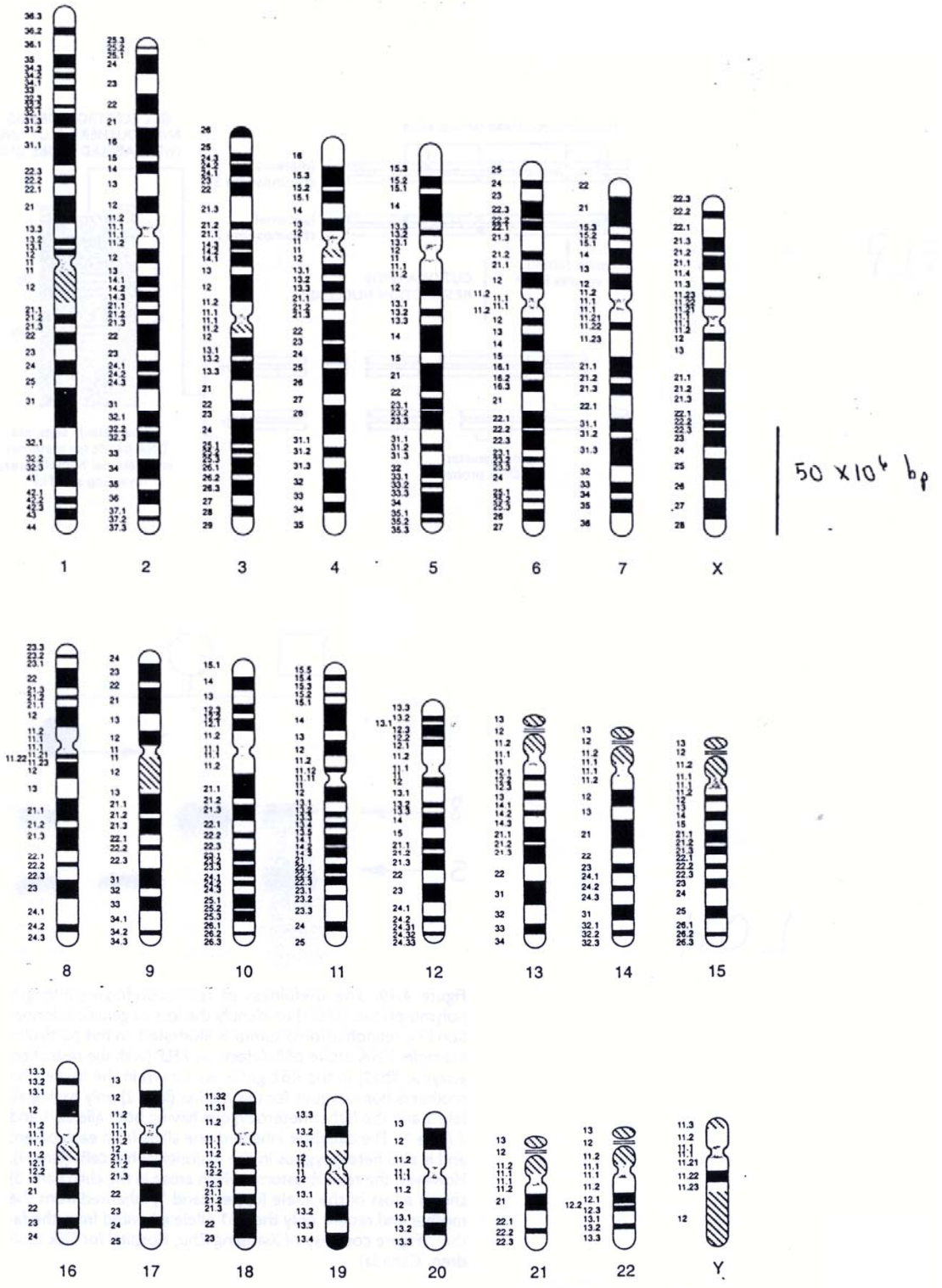
50% of children from an affected (+/-) parent will inherit the first RB mutation (every cell in the child's body is +/-). Because of mutation rates and number of target cells, an average of 3-4 retinal cells will get the second mutation (resulting in 3-4 tumours).

+/- X +/+

½ +/+ ; ½ +/-

chromosome - $1.5X10^8$ bp

chromosome band - 10^7 bp



The RB (RB1) gene cloned in 1987

Lee et al. Science 235: 1394

Recent evidence indicates the existence of a genetic locus in chromosome region **13q14** that confers susceptibility to retinoblastoma, a cancer of the eye in children. A gene encoding a messenger RNA (mRNA) of 4.6 kilobases (kb), located in the **proximity of esterase D**, was identified as the retinoblastoma susceptibility (RB) gene on the **basis of chromosomal location, homozygous deletion, and tumor-specific alterations in expression**. Transcription of this gene was abnormal in six of six retinoblastomas examined: in two tumors, RB mRNA was not detectable, while four others expressed variable quantities of RB mRNA with decreased molecular size of about 4.0 kb. In contrast, full-length RB mRNA was present in human fetal retina and placenta, and in other tumors such as neuroblastoma and medulloblastoma. DNA from retinoblastoma cells had a homozygous gene deletion in one case and hemizygous deletion in another case, while the remainder were not grossly different from normal human control DNA. The gene contains at least 12 exons distributed in a region of **over 100 kb**. Sequence analysis of complementary DNA clones yielded a single long open reading frame that could encode a hypothetical protein of 816 amino acids.

Mutation or absence of the RB protein has been reported in :

Retinoblastoma

Osteosarcoma

Small cell lung carcinoma

Breast carcinoma

Bladder carcinoma

Prostate carcinoma

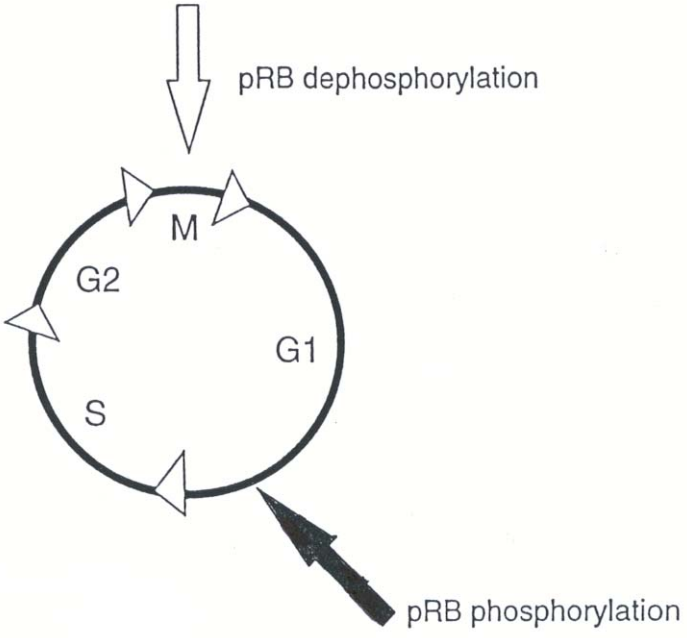
Malignant glioma

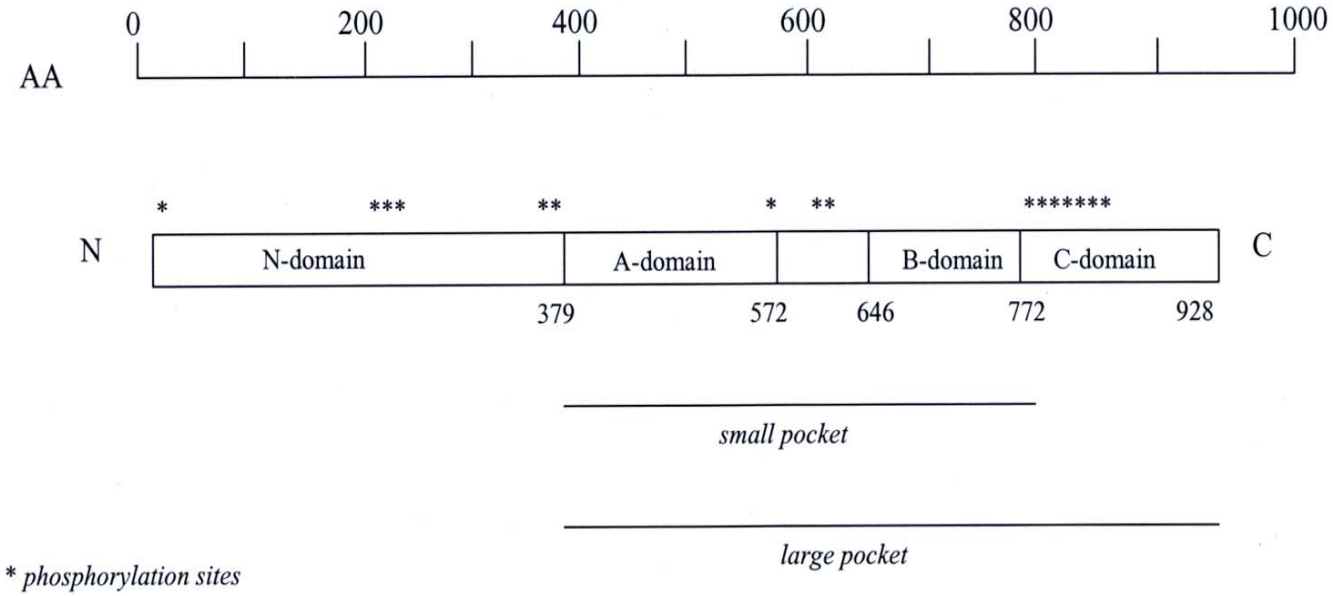
In RB tumours, mutation of both copies of the RB gene appear to be sufficient to cause cancer (two rate-limiting steps)

In other tumours, additional (rate-limiting) events are required

Properties of the RB protein

- Molecular weight of 105,000 (p105, pRB, pRb)
- Found in the nucleus of the cell
- Phosphorylated from S to M phase of the cell cycle and dephosphorylated in G_1
- Hypophosphorylated **(active)** RB protein suppresses cell proliferation
- Binds to: E1A protein of adenovirus
  Large T antigen of SV40, polyoma
  E7 protein of papillomavirus
- These viral oncoproteins bind to hypophosphorylated **(active)** pRB
- Binding region - pocket domain - residues aa 379 to 772





pRB protein

Morris and Dyson, 2001

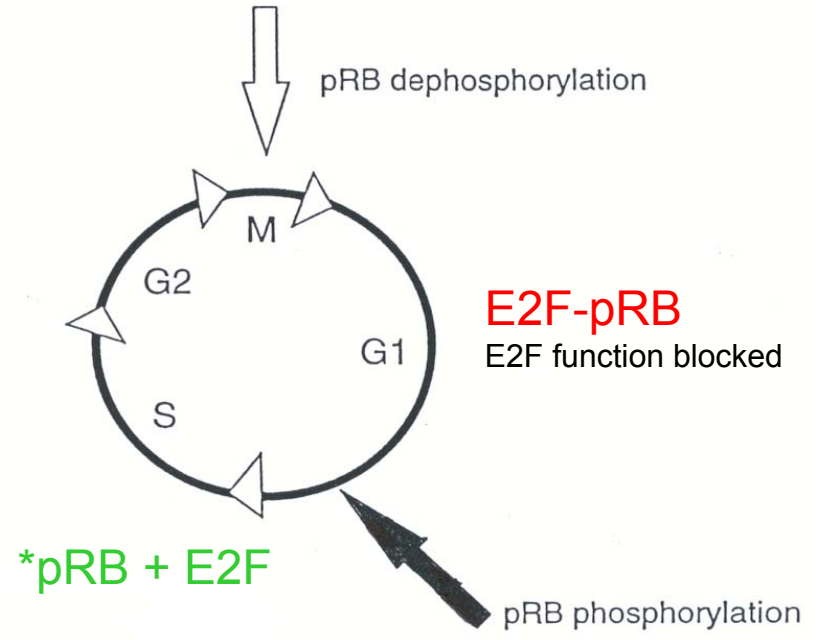
Advances in Cancer Research

E2F - transcription factor

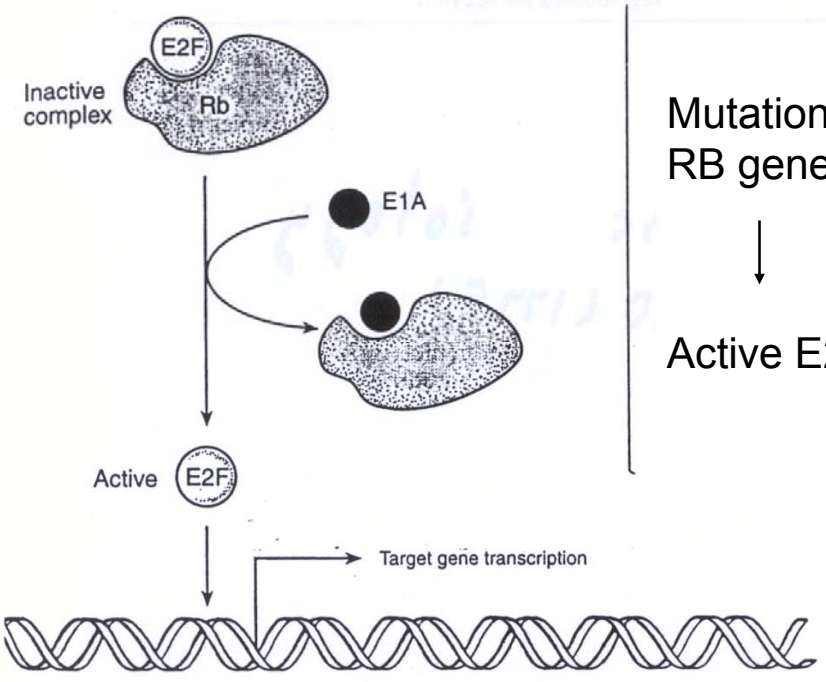
- Originally identified as a transcriptional activator of the adenovirus E2 gene
- E2F is of cellular origin
- E2F function requires heterodimerization with members of the DP family (e.g. DP-1)
- E2F binding sites have been found in cellular genes (c-myc, N-myc, c-myb, DHFR, thymidine kinase, DNA polymerase α, EGF receptor)
- Many of these genes are expressed at the G_1 transition and encode proteins essential for DNA synthesis

pRB-E2F

- E2F binds to hypophosphorylated pRB in G_1
- Blocks function of E2F as a transcriptional activator
- Cell does not progress beyond G_1



Inactivation of pRB = activation of E2F



Mutation of RB gene

↓

Active E2F

Phosphorylation of pRB

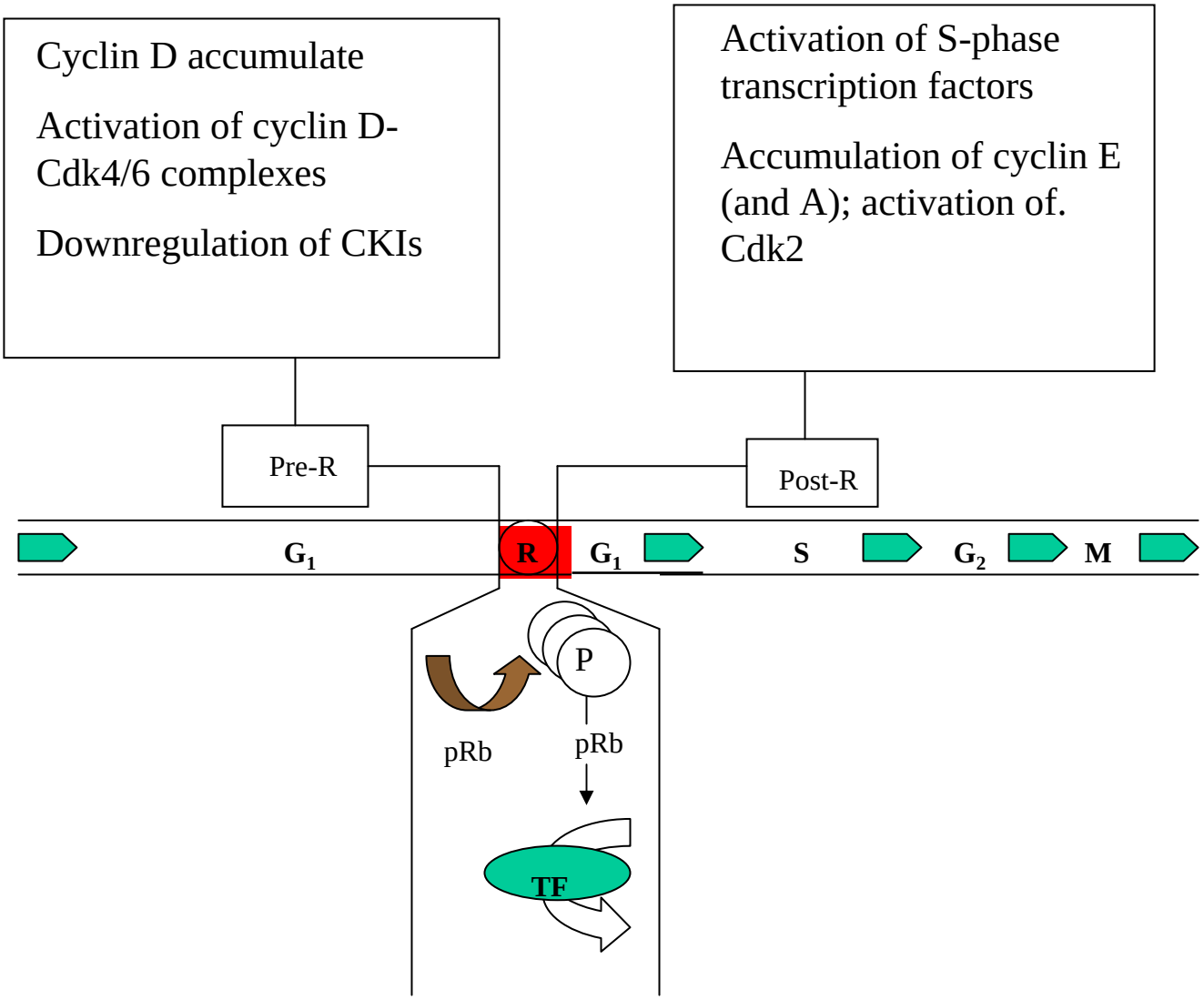
↓

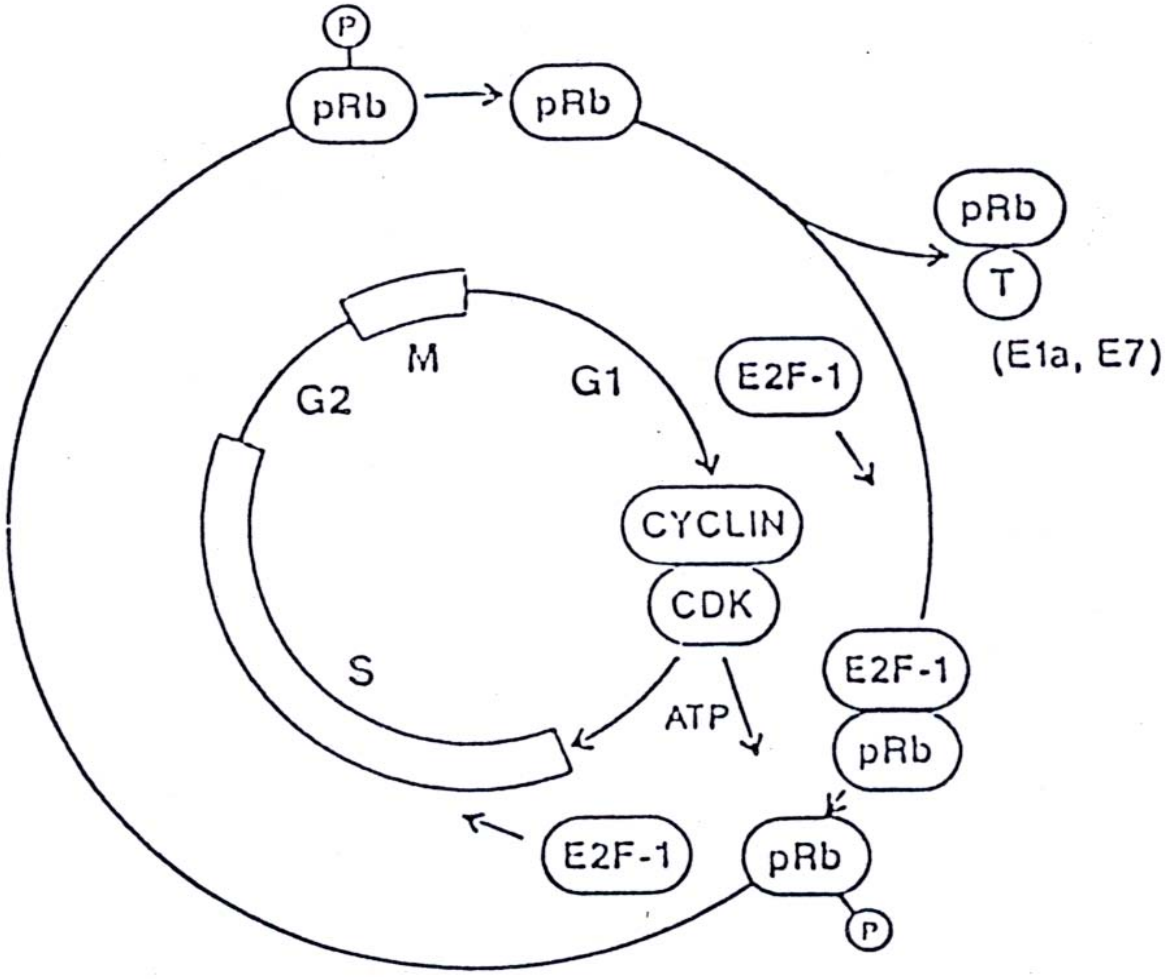
Active E2F

Analysis of the structure of pRB bound to adenovirus E1A indicates that E1A and E2F bind to the same region of the pRB pocket domain

R point (restriction point) - point in mid-to-late G_1 when the cell makes the decision to either progress through cell cycle or go to G_0 quiescent state

pRB protein is phosphorylated at R point




P
pRb
pRb
pRb
T
(E1a, E7)
M
G1
G2
S
E2F-1
CYCLIN
CDK
ATP
E2F-1
pRb
E2F-1
pRb
P

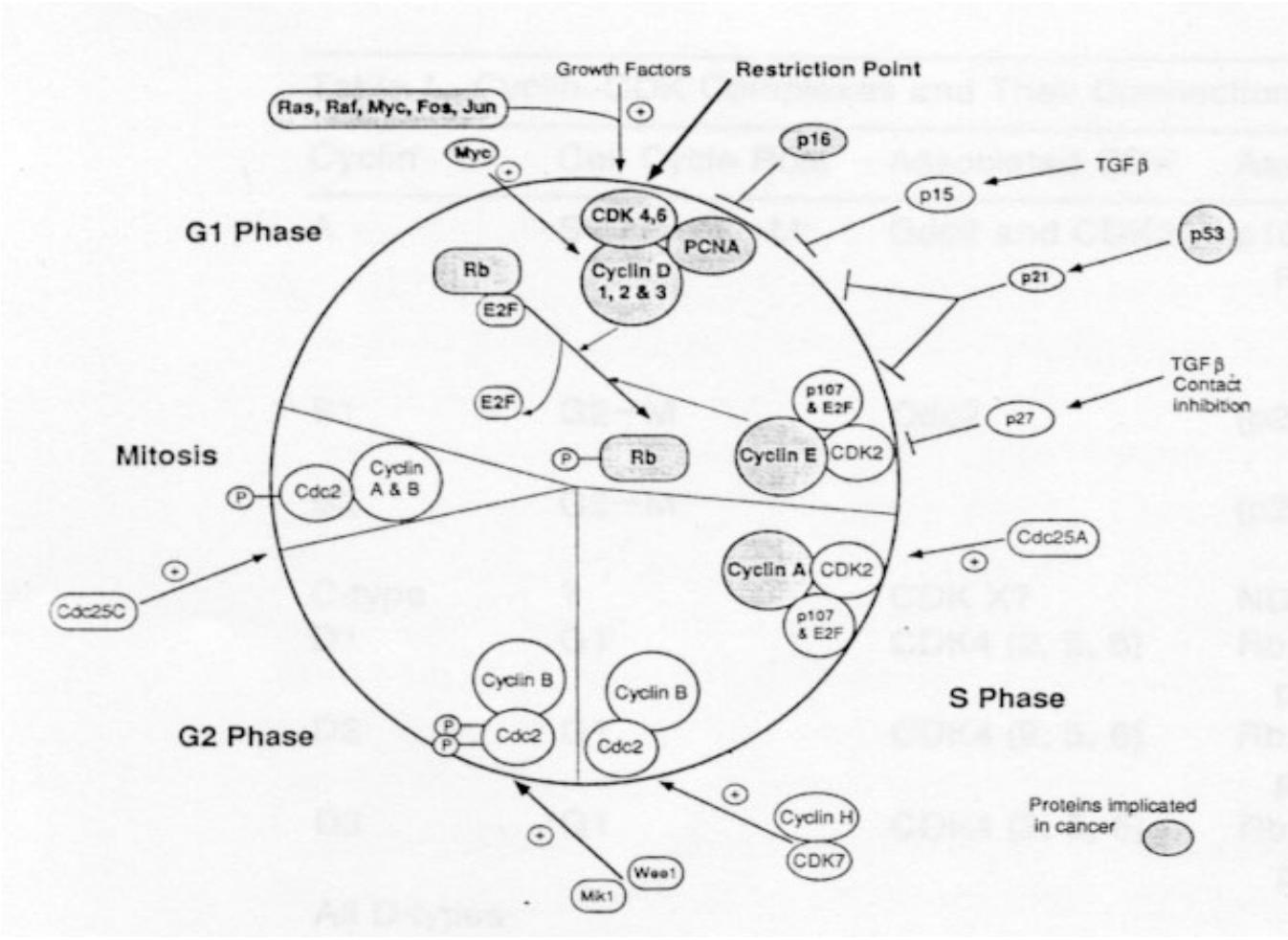
Phosphorylation of pRB is controlled by kinases, phosphatases and kinase inhibitors:

• Cyclins D1, D2, D3 (cyclin E, cyclin A) are implicated in pRB phosphorylation

• These cyclins are regulators of CDK4, CDK6 (cyclin D) and CDK2 (cyclins A and E) which phosphorylate (inactivate) pRB

• CDK inhibitors (p16, p21) inhibit CDK activity; pRB is not phosphorylated and remains active (i.e. bound to E2F)

CDKs have oncogenic (growth promoting) functions while pRB, CDK inhibitors, protein phosphatases (e.g. PP1) have tumour suppressor (growth suppression) functions



Cell 79: 573-582 (1994)

pRB and E2F

pRB is a member of a family of proteins that also includes p107 and p130

There are 7 different E2F genes, encoding E2F-1, E2F-2, E2F-3, E2F-4, E2F-5, E2F-6, E2F-7

E2F1, E2F2, E2F3 are usually associated with active promoters in S phase

E2F4, E2F5, E2F6, E2F7 are usually associated with repressed promoters in G0 or G1 phase

pRB associates with E2F1, E2F2 and E2F3

p107 associates with E2F4

p130 associates with E2F5

pRB and family members interact with many other proteins (including transcription factors)

Table 1. Cellular pRB-family binding proteins [TIG 14:223-229 (1998)]

Binding protein	Putative function	pRB	p107	p130
E2F1 – E2F3	Regulate transcription: growth control	+	-	-
E2F4	Regulate transcription: growth control	+	+	+
E2F5	Regulate transcription: growth control	-	+	+
MYC	Regulate transcription: growth control	-	+	-
BRG family	Remodel chromatin	+	+	+
HDAC1	Remodel chromatin	+	-	ND
MDM2	Inhibit p53 (TP53)	+	-	ND
ABL	Tyrosine kinase	+	ND	ND
TFIIIB	Regulate transcription: RNA Pol III	+	ND	ND
UBF	Regulate transcription: RNA Pol I	+	ND	ND
TAF250	Regulate transcription: RNA Pol II	+	ND	ND
Cyclin A, E	Regulate CDK activity	-	+	+
D-type cyclins	Regulate CDK activity	+	-	-
HBP1	Regulate transcription: repression	+	-	+
ELF1	Regulate transcription: lymphoid	+	ND	ND
PU.1	Regulate transcription: lymphoid	+	ND	ND
C/EBP family	Regulate transcription: lineage specific	+	ND	ND
MYOD1, myogenin	Regulate transcription: myogenic control	+	+	ND
ATF2	Regulate transcription	+	ND	ND

* A more complete list of pRB interacting proteins can be found in "Retinoblastoma Protein Partners" by Morris and Dyson, Advances in Cancer Research 2001

For your information only

Functions of pRB

(1) pRB is a negative regulator of the cell cycle

Mitogens and growth factors induce cyclin-dependent kinases (CDK) to phosphorylate pRB

E2F is released

E2F responsive genes are expressed

Cell progresses to S phase of the cell cycle

Differentiation factors and growth inhibitory factors block CDKs, maintain pRB in hypophosphorylated form

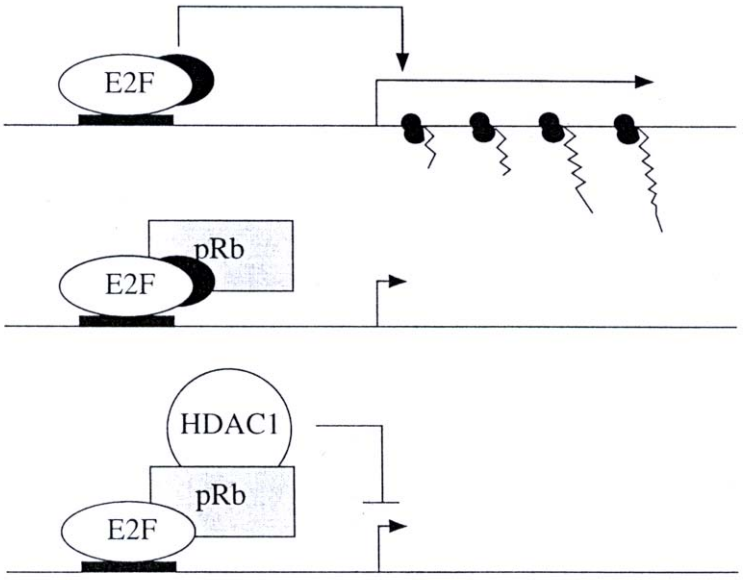
Functions of pRB

(2) pRB - transcriptional regulator of Pol II genes: repression of E2F-dependent promoters by pRB

E2F-pRB complexes bind to E2F DNA binding sites within the promoter regions of E2F target genes required for progression to S phase of the cell cycle. E2F-pRB complexes actively repress transcription by:

(1) Preventing interaction of adjacent transcription activators with the basal transcription machinery

(2) pRB recruits a co-repressor such as histone deacetylase I, RPB1, RbAp48



Source: Tumor suppressor genes in human cancer (2001), pg. 137

Functions of pRB

(3) pRB – transcriptional regulator of Pol II genes: activation of transcription by pRB

Transcriptional activation of some myogenic genes by MyoD requires pRB

pRB cooperates with C-EBP to promote adipocyte differentiation

pRB cooperates with transcription factor Pax8 to activate Pax8 target genes (Pax8 is a transcription factor crucial for differentiation of thyroid follicular cells)

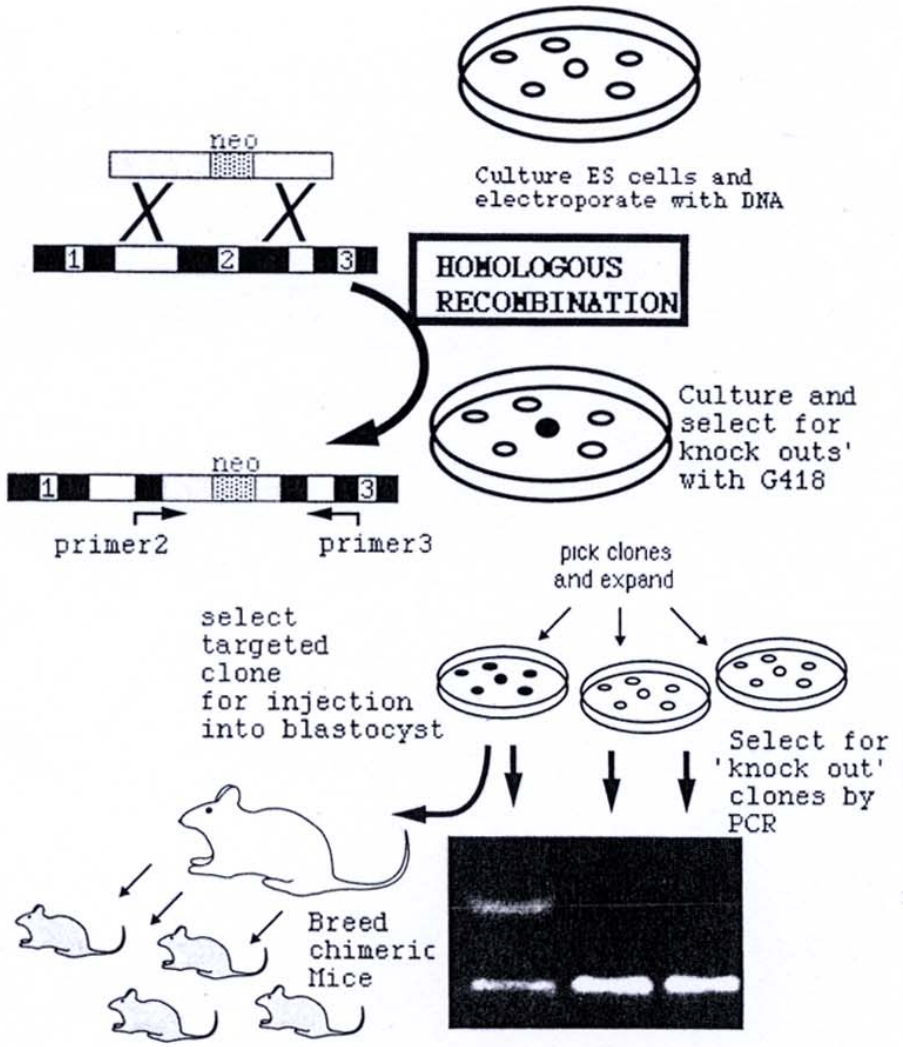


Figure 3.13. Disruption of a gene by homologous recombination in embryonic stem (ES) cells. Exogenous DNA is introduced into the ES cells by electroporation or by one of the methods described in Sec. 3.3.10. The homologous region on the exogenous DNA is shown in gray, the selectable gene neomycin (neo) is speckled, and the target exons are black. The two recombination points are shown by X's and the exogenous DNA replaces some of the normal DNA of exon 2, thereby destroying its reading frame by inserting the small "neo" gene. ES cells that have undergone a successful homologous recombination are selected as colonies in G418 because of the stable presence of the neo gene. PCR primers for exons 2 and 3 are used to identify colonies in which a homologous recombination event has taken place. ES cells from such positive cells (*dark colony*) are injected into blastocysts, which are implanted into foster mothers (*white*). If germ line transmission has been achieved, chimeric mice are bred to generate homozygotes for the "knocked out" gene.

Knock-out of RB gene in mice

- One RB allele was disrupted in ES cell using a targeting vector.
- Chimeric mice were created by aggregating ES cells with embryonic cells at blastocyst stage. Chimeric animals able to transmit the disrupted RB allele to their offspring were identified.
- Generated mice heterozygous for the disrupted RB gene (i.e. RB+/-). Heterozygous mice don't get retinoblastoma tumours but have pituitary gland tumours later in life.
- Homozygous mice (RB-/-) all die before birth (14.5-15.5 gestation). These mice have a defect in erythropoiesis and show a significant amount of neuronal cell death. Eye development is normal; however, eye is still at a very early stage of development.
- Retinal cells expressing E7 in transgenic mice undergo apoptosis at a time when they would normally be undergoing terminal cell differentiation.

Why don't RB-/- mice get retinoblastoma tumours?

During G_1 decision is made to:
- proliferate
- growth arrest
- differentiate
- die (apoptose)

Normally, if hypophosphorylated (active) pRB is present, it binds to E2F, and prevents progression of the cell to the S phase. When pRB is phosphorylated, cell progresses to the S phase.

In human retinal cells (RB-/-): Absence of pRB results in active (free) E2F, the cell immediately proceeds to S phase - no control.

In mouse retinal cells expressing E7 (RB-/-): If pRB is inactivated in mouse retinal cells by E7, these cells undergo apoptosis. The latter may represent a safety mechanism to protect the cell in case of pRB inactivation. Deregulation of pRB in relation to E2F may be recognized by most cell types as a potentially dangerous state (continuous cell proliferation, increased rate of mutation); therefore the cell initiates programmed cell death (apoptosis).

RB-/- cell: deregulation of E2F -- programmed cell death

RB-/- cell which has additional mutations (e.g. loss of p53) -- continuous cell proliferation

Human retinoblastoma appears to be an exception to this rule in that pRB inactivation is sufficient to induce tumour formation

pRB and apoptosis

Loss of pRB can trigger a p53-dependent apoptotic pathway

Release of E2F as the result of loss of pRB triggers p53-dependent apoptosis

- a possible target of free E2F is ARF (alternate reading frame encoded by p16INK4a)

- ARF appears to inhibit the MDM2-mediated turnover of p53

- increase in p53 leads to increase in apoptosis

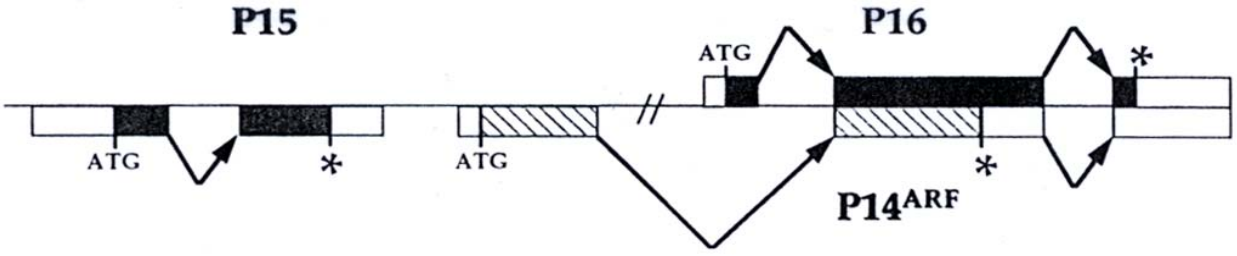


Fig. 3. *p16* locus. Asterisks indicate translation termination sites; ATG indicates translational initiation. Distances between genes and exons not drawn to scale.

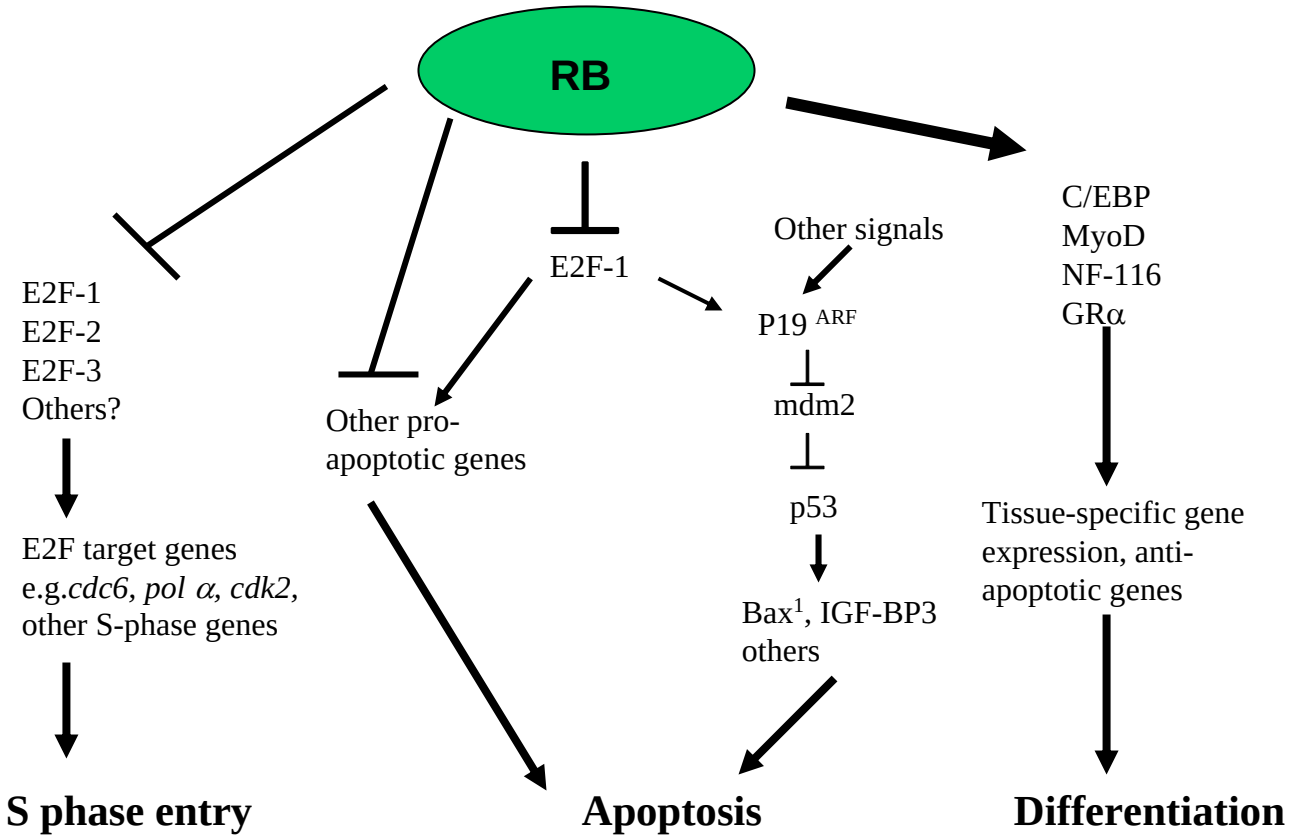
Tumor suppressor genes in human cancer (2001), pg.191

Table 2. Murine retinal abnormalities associated with inactivation of pRB-family proteins

pRB-family mutations	Retina phenotype	Ref.
Retinal transgene (promoter)		
SV 40 large T antigen (luteinizing hormone)	Retinoblastoma	43
HPV E7 (interstitial retinol binding protein)	Retinal degeneration; apoptosis	44
HPV E7 + E6 (interstitial retinol binding protein)	Retinoblastoma	44
HPV E7 (IRBP); $p53^{-/-}$	Retinoblastoma	44
pRB family mutant mouse strain		
$Rb^{+/-}$		36
$p107^{-/-}$		40
$p130^{-/-}$		40
$p130^{-/-}$ $p107^{-/-}$		40
$Rb^{+/-}$ $p107^{-/-}$	Retinal dysplasia	41
$Rb^{+/-}$ $p130^{-/-}$		a
$p107^{+/-}$ $p130^{-/-}$		a
$p107^{-/-}$ $p130^{+/-}$		a
$p53^{-/-}$		75
$Rb^{+/-}$ $p53^{-/-}$	Retinal dysplasia (40% of mice)	75

[a]G. Mulligan and T. Jacks, unpublished.

This summarizes various murine studies of tumor suppressor gene function specifically with regard to the induction of retinal abnormalities. Transgenic studies utilizing viral oncoproteins and their specific promoters are shown at top. In the T antigen study low level expression was detected in the pituitary, but this line also exhibited high level retinal specific expression of T antigen. Approximately 40% of $Rb^{+/-}$ $p53^{-/-}$ animals exhibited dysplasia, occasionally bilateral, with a mean number of lesions per animal (both eyes) of 3–4. All $Rb^{+/-}$ $p107^{-/-}$ animals exhibited bilateral dysplasia with a mean number of lesions approximately 8–10.



p53

p53 – second tumour suppressor gene to be identified (1989); oncogene (1984)

p53 gene is the most commonly altered gene in human tumours (50%)

p53 was initially thought to be an oncogene: overexpression of p53* induced immortalization in the DNA transfection assay (primary mouse fibroblasts). However, the transfected p53* was a mutant form of p53 (single amino acid change). Wild-type (normal) p53 does not have transforming ability - overexpression of normal p53 inhibits transformation.

Loss of normal p53 function through deletion, truncation or mutation. Mutant and truncated proteins have **dominant negative function** - i.e., the mutant p53 interferes with the function of normal p53. p53 proteins binds to DNA as a tetramer.

Is cellular proto-oncogene activation sufficient to cause cancer?

Evidence suggesting that multiple hits are required for cancer formation:

(1) Estimates based on age at which cancer appears and incidence of cancer indicate that 3 to 8 mutations (2 mutations in the case of retinoblastoma) are required for cancer formation

(2) Some cancers go through several stages

(3) Multiple oncogenes are required to transform cells in culture

Table 5.2. Complementation Groups of Oncogenes

Immortalizing Function	Morphologic Transformation
myc	H-*ras*
myb	N-*ras*
p53*	*src*
EIA of adenovirus	
Large T of SV40	

*The p53 used in these studies contained a point mutation, which converts it from a suppressor gene (see chapter 6, section 6.3.6) to an oncogene.

p53 – cont'd

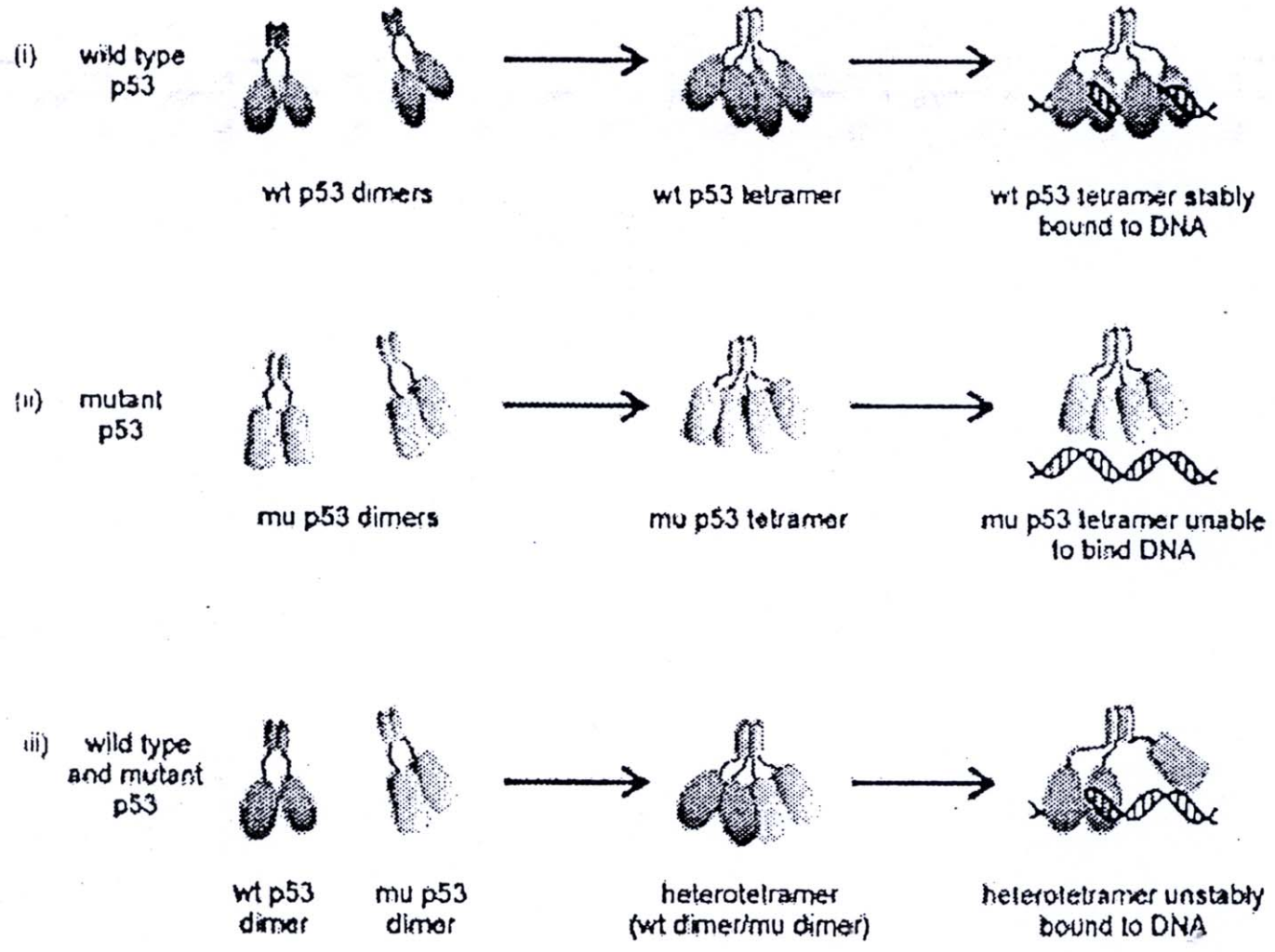
First example of p53 gene inactivation in a human tumour - colon carcinoma. 17p13 (to which the p53 gene has been mapped) is frequently deleted in colon carcinoma (on one chromosome). The remaining p53 gene is mutated - both copies of p53 need to be inactivated.

Mutation of both copies of the p53 gene (TP53) is common in many cancers.

Mutation of a single p53 allele is found in some cancers. Incompetent partner model: poor binding of p53 tetramer to target sites (Nicholls et al. J. Biol. Chem. 277:12937, 2002).

Germ line mutation of p53 is found in a rare disease called Li-Fraumeni cancer family syndrome. Children who inherit the mutated copy of the p53 gene have a wide variety of tumours at a very early age.

B. Binding of p53 homotetramers and heterotetramers to DNA



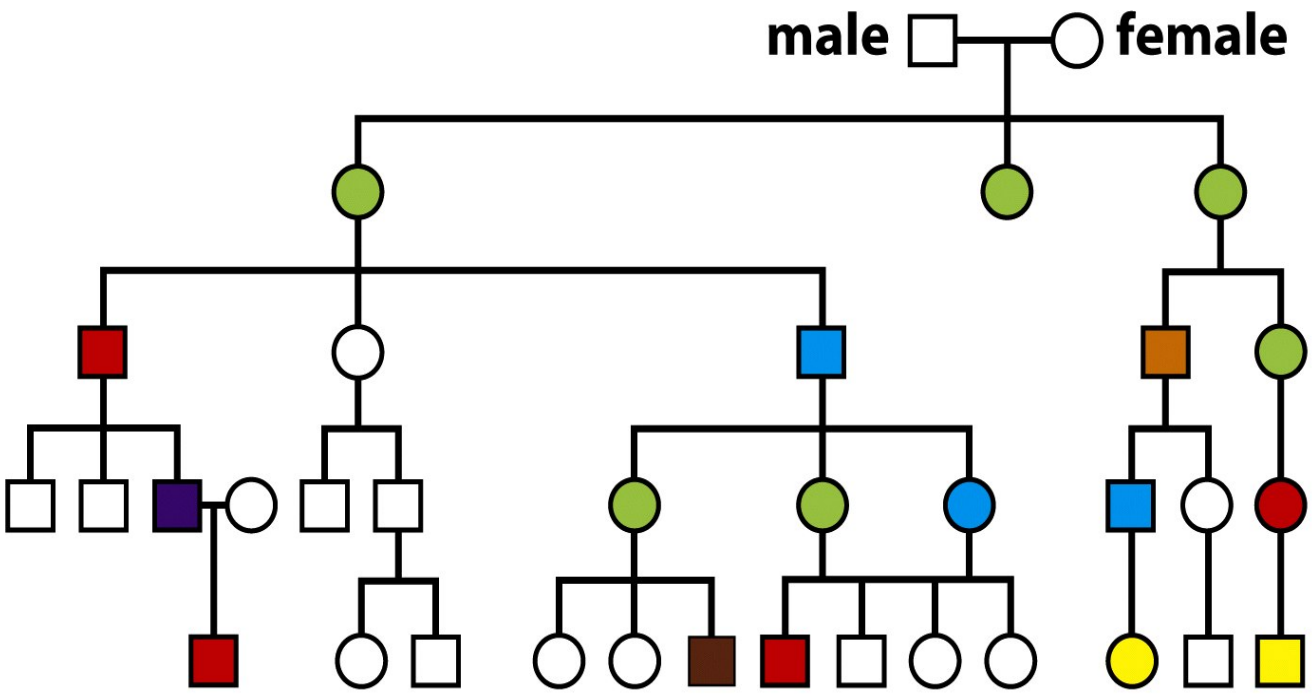


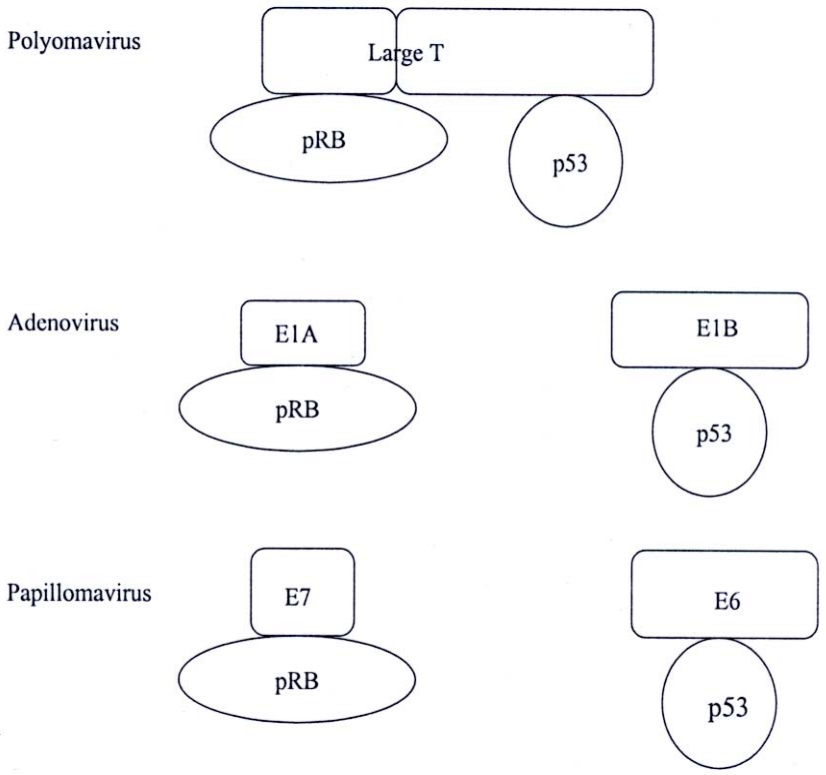
Figure 9-20 The Biology of Cancer (© Garland Science 2007)

Li-Fraumeni syndrome – affected individuals have a high incidence of cancer

Green – breast cancer
Yellow – glioblastoma
Purple – leukemia
Blue – lung cancer
Orange- pancreatic cancer
Red – sarcoma
Brown – Wilms tumour

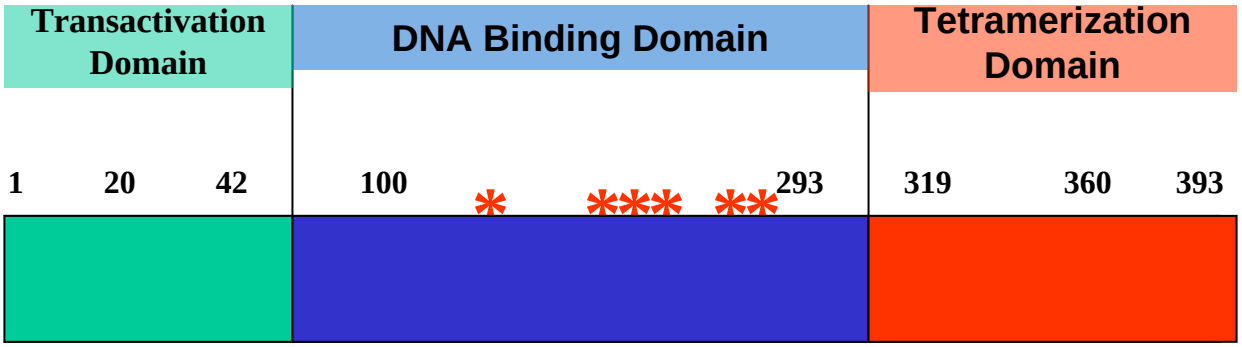
Biochemical properties of p53

- 375 aa, nuclear, phosphorylation
- Complexes with SV40 large T antigen, adenovirus E1B and papilloma virus E6



p53
Transcription Factor

p53 domains



p53 binds to specific nucleotide sequences (called p53-responsive elements) located within the promoter of target genes

p53 target genes

- MDM2 (gene over-expressed in some tumours). Oncogene that confers enhanced tumorigenic potential when it is over-expressed. The MDM2 protein binds p53, abolishing its transcriptional activation function. MDM2 binding also targets p53 for degradation. MDM2 is a ubiquitin E3 ligase that targets p53 for nuclear export (mono-ubiquitinated) or degradation (poly-ubiquitinated). MDM2 serves as a negative feed-back control for p53.

- p21 (Waf1/Cip1) (cyclin-dependent kinase inhibitor). N.B. p21 can inhibit CDKs required for pRB phosphorylation (inhibits proliferation).

Table 9.2 **Examples of p53 target genes according to function** The expression of genes in this table is induced by p53 unless otherwise indicated.

Class of genes	Name of gene	Function of gene product
p53 antagonist	*MDM2/HDM2*	induces p53 ubiquitylation
Growth arrest genes	*$p21^{Cip1}$*	inhibitor of CDKs, DNA polymerase
	Siah-1	aids β-catenin degradation
	14-3-3σ	sequesters cyclin B–CDC2 in cytoplasm
	Reprimo	G_2 arrest
DNA repair genes	*p53R2*	ribonucleotide reductase—biosynthesis of DNA precursors
	XPE/DDB2	global NER
	XPC	global NER
	XPG	global NER, TCR
	GADD45	global NER ?
	DNA pol κ	error-prone DNA polymerase
Regulators of apoptosis	*BAX*	mitochondrial pore protein
	PUMA	BH3-only mitochondrial pore protein
	NOXA	BH3-only mitochondrial pore protein
	p53AIP1	dissipates mitochondrial membrane potential
	Killer/DR5	cell surface death receptor
	PIDD	death domain protein
	PERP	pro-apoptotic transmembrane protein
	APAF1	activator of caspase-9
	NF-κB	transcription factor, mediator of TNF signaling
	Fas/APO1	death receptor
	PIG3	mitochondrial oxidation/reduction control
	PTEN	reduces levels of the anti-apoptotic PIP_3
	Bcl-2	(repression of) its expression
	IGF-1R	(repression of) its expression
	IGFBP-3	IGF-1–sequestering protein
Anti-angiogenic proteins	*TSP-1* (thrombospondin)	antagonist of angiogenesis

Table 9-2 The Biology of Cancer (© Garland Science 2007)

p53 - Guardian of the genome

In response to ionizing or ultraviolet irradiation, p53 levels and p53 activity increase: p21 accumulates, CDKs are inhibited, pRB is not phosphorylated, E2F is not released, cell cycle does not proceed to S

Induction of p53 can result in:

G_1 arrest - allows the cell to repair DNA damage before proceeding to S phase

Apoptosis (depends on extent of damage, cell type, environment)

p53-/- cells do not arrest. They replicate damaged DNA resulting in an increased number of mutations and genomic instability - cancer

p53 gene knock-out mice -developmentally normal

- dramatic increase in incidence of cancer with age

Other members of the p53 family -- p73, p63

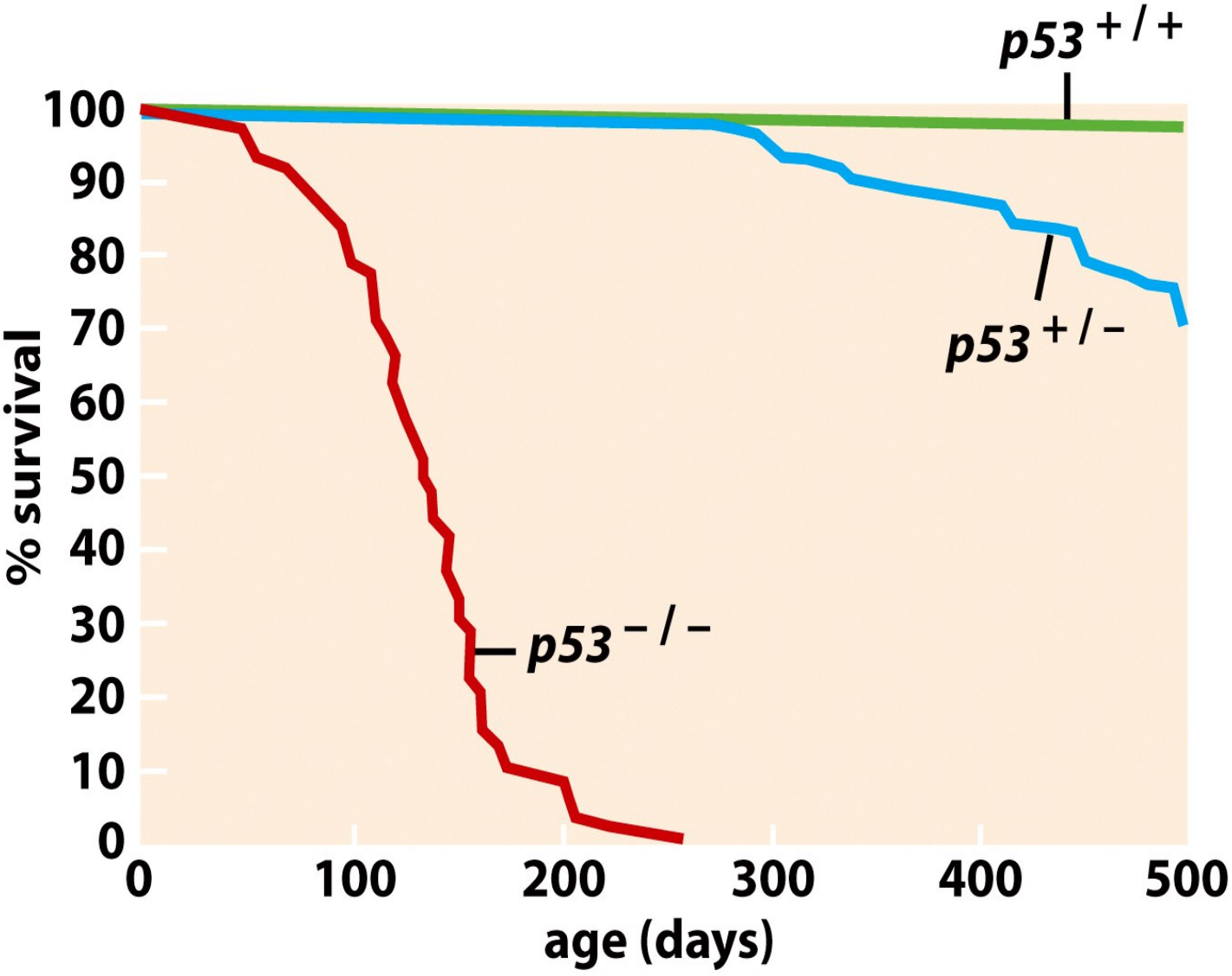


Figure 9-5 The Biology of Cancer (© Garland Science 2007)

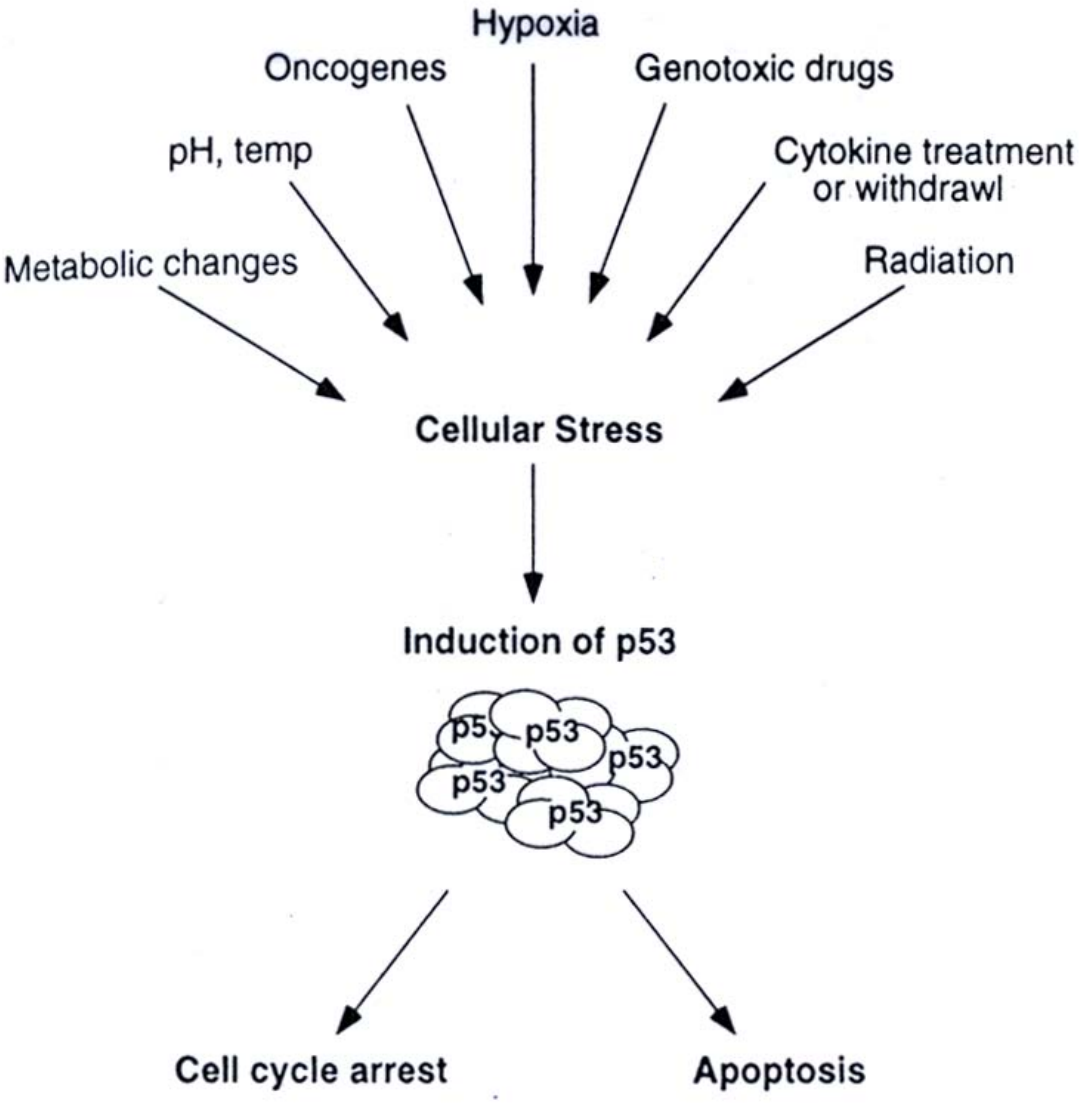


Fig. 2. Multiple pathways lead to the stabilization of p53.

Tumour suppressor genes in human cancer (2001), pg. 167

p53 and Cancer Therapy – read Chen F., Wang W and El-Deiry, WS. Biochem Pharmacol. 80:724-730 (2010) for update on new strategies to target p53 in cancer

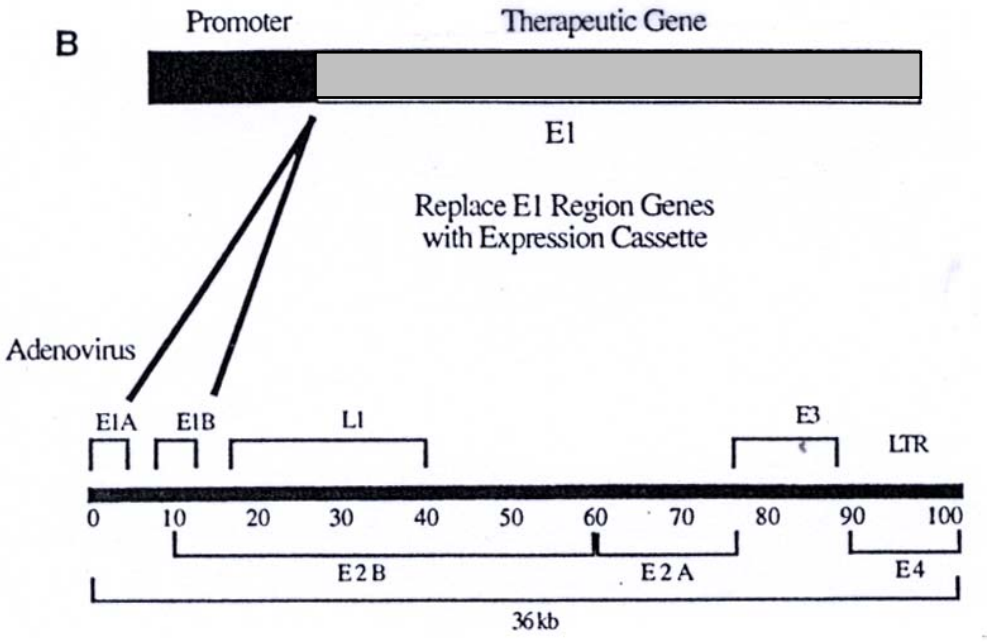
~50% of all breast cancers, 60% of lung cancers, 70% of colorectal cancers have p53 mutations

Phase I-III trials involving p53 gene therapy:

Lung non-small cell carcinoma: adenovirus vector expressing p53 injected directly in tumour nodules (plus cisplatin)

Head and neck squamous cell carcinoma: same treatment as above

Hepatocellular carcinoma and liver metastases: hepatic artery infusion of p53



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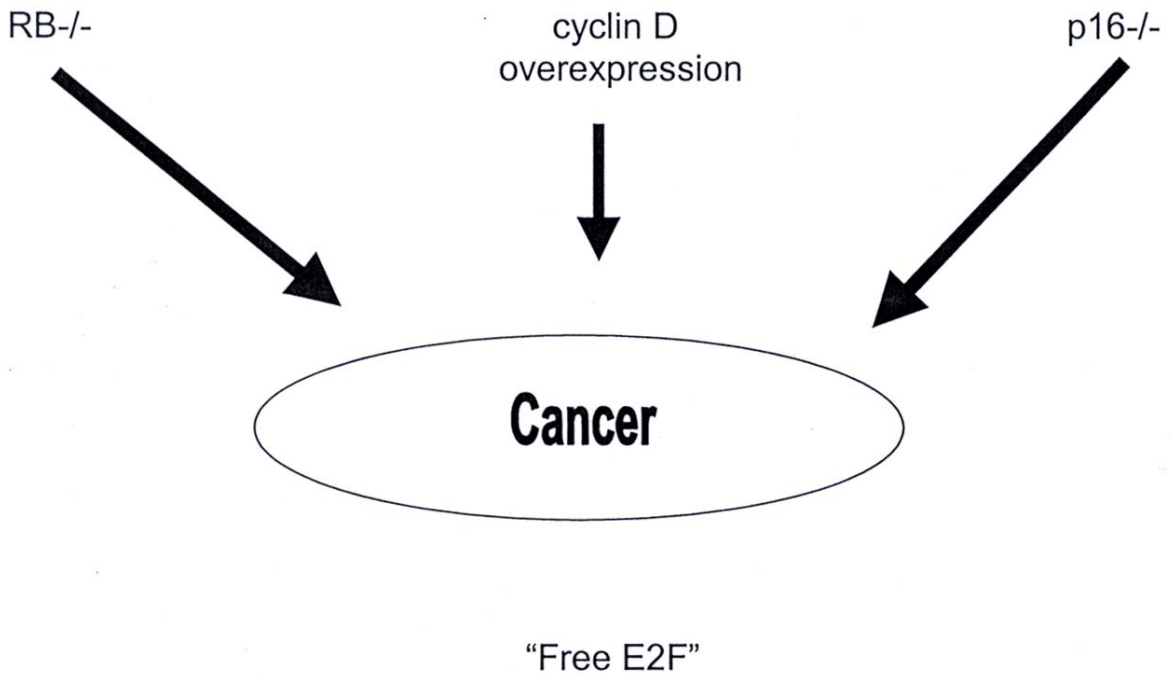
p16 (MTS1, INK4A, CDKN2)

Cytogenetic abnormalities at 9p21 noted in a variety of tumours

p16 is a CDK4/6 inhibitor, inhibits phosphorylation of pRB by cyclin D/CDK. If pRB is hypophosphorylated, it is in active state (bound to E2F) and the cell does not proceed to S phase of cell cycle until pRB becomes phosphorylated and releases E2F

In a p16-negative cell, phosphorylation of pRB is not inhibited



pRB, p53, ARF and apoptosis

- Loss of proper pRB control can trigger a p53-dependent apoptotic pathway
- Release of E2F as the result of loss of pRB triggers p53-dependent apoptosis
- A possible target of free E2F is ARF (alternate reading frame encoded by p16INK4a)
- ARF inhibits MDM2-mediated turnover of p53
- Increase in p53 leads to apoptosis

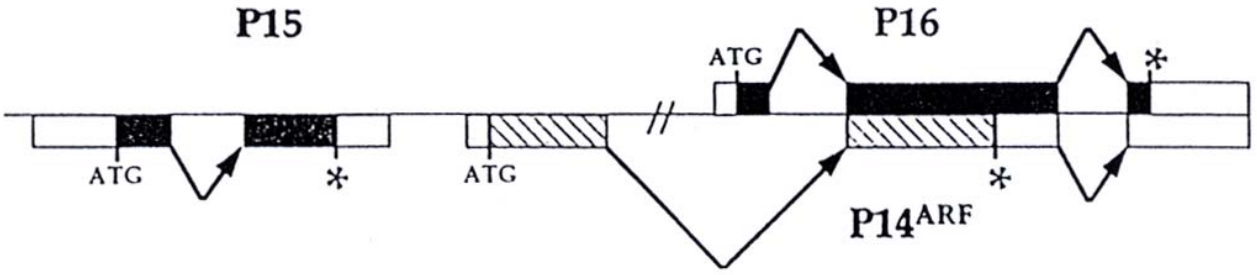


Fig. 3. *p16* locus. Asterisks indicate translation termination sites; ATG indicates translational initiation. Distances between genes and exons not drawn to scale.

Tumor suppressor genes in human cancer (2001), pg.191

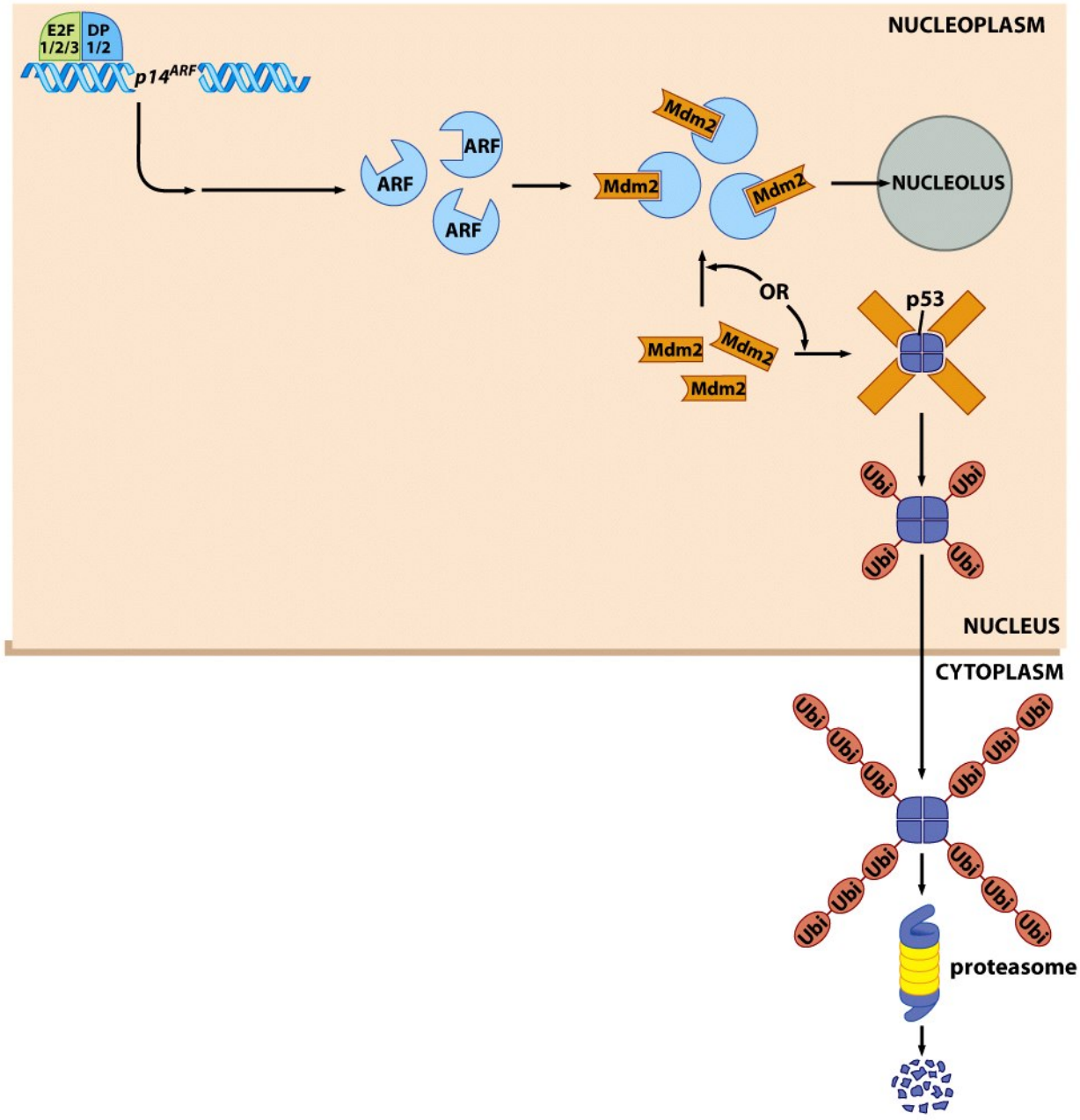


Figure 9-15a The Biology of Cancer (© Garland Science 2007)

Breast cancer susceptibility genes

(review Rosen et al. J. Cell Physiol. 196:19-41, 2003)

5-10% of **all** breast cancers result from inherited genetic mutations. 25% of breast cancers diagnosed before age 30 result from inherited genetic factors.

Three well-characterized predisposition genes: p53, BRCA1, BRCA2.

BRCA1 and BRCA2 identified by positional cloning: (1) linkage analysis using polymorphic probes to study large families, (ii) identification of transcripts located in this region and (iii) determining whether mutations are found in possible breast cancer genes.

Breast cancer susceptibility genes – cont'd

BRCA1 [Science 266:66-71 (1994)]

BRCA1 gene mutations in one allele were found in the germline of 4 of the 8 families used in the linkage analysis

In each of the four families, at least one woman >80 years carried the mutation without ever having had breast cancer - other factors are involved.

BRCA1 gene mutations are found in ~40-45% of hereditary breast cancer cases and 80% of families predisposed to breast and ovarian cancer. In contrast to the RB gene, BRCA1 mutations are rarely found in sporadic breast and ovarian cancers.

BRCA1 – maintenance of genomic integrity

Proposed functions for BRCA1:

BRCA1 is a large nuclear protein, contains two BRCT motifs - motif found in DNA repair proteins.

BRCA1 may function as a transcription factor.

Expression of BRCA1 in MCF7 breast cancer cells decreased their capacity for tumour formation in nude mice.

BRCA1 interacts with DNA repair protein RAD51. BRCA1-/- breast cancer may arise because of inadequate DNA repair response.

Breast cancers from patients with mutations in BRCA1 have 2 to 3 times more chromosome abnormalities than sporadic cancers.

Some of the BRCA1 functions may be mediated by interaction with p53 and pRB.

BRCA1-/- mice die at day 7.5-8.5 gestation. BRCA1 is important for early development.

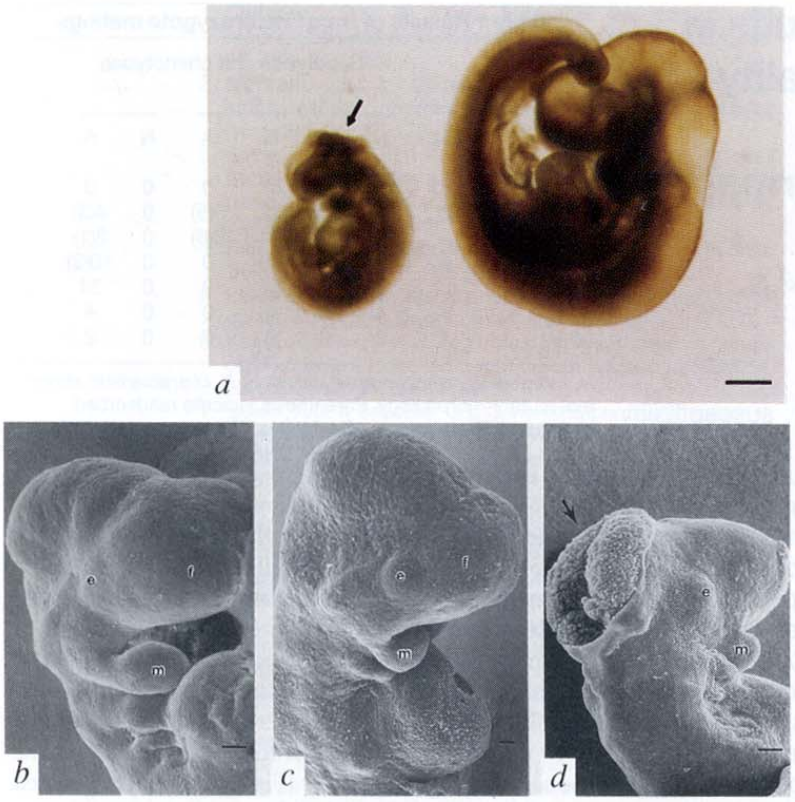


Fig. 2 External appearance of E10.5 embryos isolated from intercrosses of mice heterozygous for the mutant *Brca1* allele. *a*, Developmental delay caused by *Brca1* deficiency is indicated by the size difference between the *Brca1* –/– embryo and the control, as well as by the fact that the *Brca1* –/– embryo has approximately 20 somite pairs while the normal embryo has approximately 29. The cranial neural tube in the *Brca1* –/– embryo has also failed to close (arrow). Scale bar, 500 μm. Scanning electron micrographs of E10.5 *Brca1* –/– embryos (*c*,*d*) and a comparable E9.5 *Brca1* +/+ control embryo (*b*). Of particular note in *Brca1* –/– embryos is reduction in the size of the forebrain region in an embryo that has achieved closure of its neural tube (*c*) and failure of closure of the neural tube at the level of the midbrain (arrow) in the other *Brca1* –/– embryo (*d*). e, eye; m, mandibular prominence of the first pharyngeal arch; f, forebrain region. Scale, 100 μm.

Nature Genetics 12: 191-194, 1996

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BRCA2 [Nature 378: 789-792 (1995)]

BRCA2 implicated in ~35% of breast cancer-only families, as well as male breast cancer.

BRCA2 is a large nuclear protein. Like BRCA1, BRCA2 interacts with RAD51.

BRCA2-/- mice start dying after day 8.5 gestation. Phenotype similar to that of BRCA1-/- mice, although abnormalities are not massive as observed in BRCA1-/- mice.

Myriad Genetics holds the patents on BRCA1 and BRCA2.

ON MAY 12, 2009, THE ACLU AND THE PUBLIC PATENT FOUNDATION (PUBPAT) FILED A LAWSUIT CHARGING THAT PATENTS ON TWO HUMAN GENES ASSOCIATED WITH BREAST AND OVARIAN CANCER ARE UNCONSTITUTIONAL AND INVALID. THE SUIT CHARGES THAT THE PATENTS STIFLE DIAGNOSTIC TESTING AND RESEARCH THAT COULD LEAD TO CURES AND THAT THEY LIMIT WOMEN'S OPTIONS REGARDING THEIR MEDICAL CARE.

ON MARCH 29, 2010 A NEW YORK FEDERAL COURT RULED THAT THE PATENTS ON THE BRCA1 AND BRCA2 GENES ARE INVALID. THE U.S. COURT OF APPEALS FOR THE FEDERAL CIRCUIT HEARD MYRIAD'S APPEAL OF THAT RULING IN APRIL 2011.

IN JULY 2011, THE APPEALS COURT RULED THAT COMPANIES CAN OBTAIN PATENTS ON THE GENES BUT CANNOT PATENT METHODS TO COMPARE THOSE GENE SEQUENCES.

NF1 and NF2

Neurofibromatosis type 1 (LOH at 17q11) - autosomal dominant disorder that affects 1/3500 people: benign neurofibromas (90% of cases) and malignant neurofibrosarcomas, abnormal eye development and cafe-au-lait skin spots in second decade of life.

All cases of NF1 result from the inheritance of a mutant allele (equal frequency of predisposing alleles from an affected parent versus new germline mutations).

NF1 gene identified by linkage analysis of affected families and positional cloning. Confirmed by sequencing the DNA of NF1 patients (and finding mutations in NF1 gene). Mutations in NF1 gene found in sporadic tumours including colon cancer and melanoma.

NF1 and NF2 – cont'd

NF1 is a tumour suppressor because: (i) NF1 mutations are inactivating (no functional product), (ii) LOH in malignant neurofibrosarcomas is common (nonmutated allele is deleted and mutated allele is retained), (iii) LOH has been reported in tumours arising in heterozygous NF1+/- mice, (iv) introduction of normal NF1 gene into tumour cells of NF1 patients results in reversion of the tumour phenotype.

Function of NF1: GAP protein (down-regulates ras activity)

NF1 and NF2 – cont'd

Neurofibromatosis type 2 (LOH at 22q) - autosomal dominant disorder, much rarer than NF1, generally leads to acoustic nerve tumours and meningiomas.

NF2 gene was also isolated by linkage analysis and positional cloning. Same criteria as described above were used to confirm that NF2 is a tumour suppressor.

NF2 gene encodes a protein called merlin. Merlin is proposed to interact with a signal transduction pathway to convey messages between the cell membrane and cytoskeleton (review article: Xiao et al. Genes Chromosomes Cancer 38:389, 2003)

Recently, merlin has been shown to increase the stability of p53 by inhibiting MDM2-mediated degradation of p53.

PTEN
(phosphatase and tensin homologue)

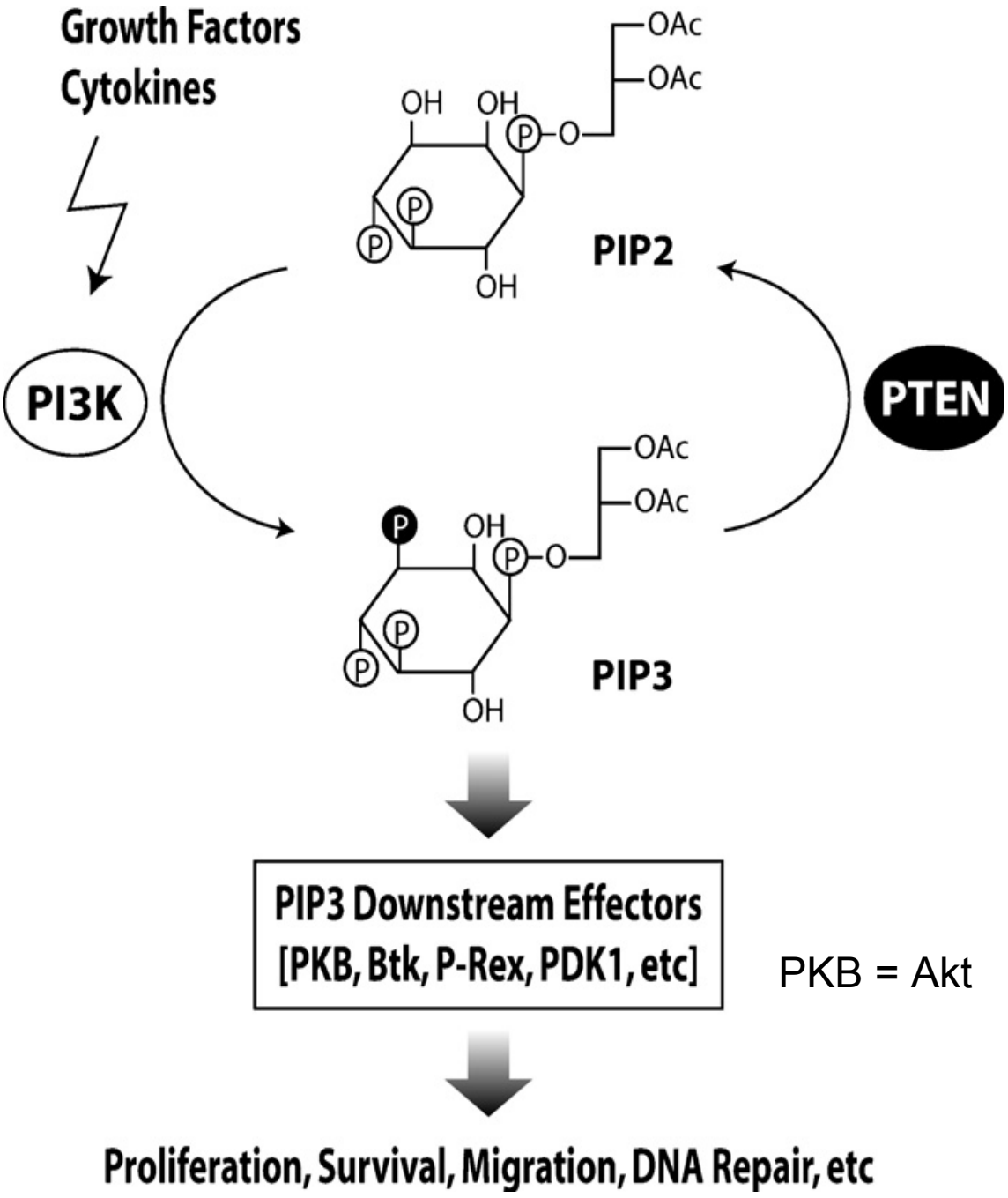
- Tumour suppressor associated with deletion at 10q23 in glioblastoma (brain tumour), prostate, breast, thyroid, skin cancers

- Germline mutation of PTEN causes Cowden syndrome (high incidence of benign tumour-like nodules early in life and increased incidence of breast, thyroid, brain tumours later in life)

- PTEN functions by dephosphorylating PIP3, the primary product of PI3-kinase function (PIP3→PIP2), and by antagonizing PI3-kinase function

- PI3-kinase implicated in regulation of cell proliferation and survival; activated by growth factor receptors such as Her2/neu, EGFR, PDGFR

PIP3 =
Phosphatidylinositol (3,4,5)-trisphosphate
(PtdIns(3,4,5)*P*3)



PKB = Akt

Maehama, T. Biol. Pharm. Bull. 30: 1624, 2007

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- PI3K-Akt-PTEN signaling pathway – target for cancer therapy? E.g. for treatment of glioblastoma?

- Control of PTEN function is very complicated. PTEN is positively and negatively regulated at the transcriptional level, and is also regulated by post-translational modification (phosphorylation, ubiquitylation, oxidation and acetylation). Furthermore, the subcellular distribution of PTEN at the plasma membrane, in nucleus and cytoplasm, is strictly controlled (reviewed in Tamguney, T and Stokoe, D. J. Cell Sci. 120: 4071, 2007).

Table 6.4 Alteration of the PI3K pathway in human tumors

Cancer type	Type of alteration
Glioblastoma (25–50%)	*PTEN* mutation
Ovarian carcinoma	*PTEN* mutation; *AKT2* amplification; *PI3K* amplification; PI3K *p85α* mutation
Breast carcinoma	increased Akt1 activity; *AKT2* amplification; *PTEN* mutation
Endometrial carcinoma (35%)	*PTEN* mutation; *PTEN* methylation[a]
Hepatocellular carcinoma	*PTEN* mutation
Melanoma	*PTEN* mutation; *PTEN* methylation[a]
Lung carcinoma	*PTEN* mutation
Renal cell carcinoma	*PTEN* mutation
Thyroid carcinoma	*PTEN* mutation; Akt/PKB overexpression
Lymphoid	*PTEN* mutation
Prostate carcinoma (40–50%)	*PTEN* mutation
Colon carcinoma (>30%)	Akt/PKB overexpression; *PI3K* mutation

[a]Methylation refers to repression of transcription of a gene through methylation of cytidines in its promoter; see Section 7.8.

Adapted from I. Vivanco and C.L. Sawyers, The phosphatidylinositol 3-kinase-AKT pathway in human cancer, *Nat. Rev. Cancer* 2:489–501, 2002.

GENES IN INHERITED CANCER SYNDROMES

Syndrome	Gene	Types of Tumours
Retinoblastoma	RB1	retinoblastoma osteosarcoma
Li-Fraumeni syndrome	TP53	soft tissue sarcoma breast cancer CNS-tumors adrenocortical cancer
Familial polyposis	APC	colorectal cancer
Wilms Tumor	WT1	Wilms tumor
von Hippel-Lindau Syndrome	VHL	renal cell cancer hemangioblastoma retinal angioma pheochromocytoma
Neurofibromatosis 1	NF1	neurofibroma
Neurofibromatosis 2	NF2	acoustic neuroma meningioma schwannoma
Familial breast cancer/ breast-ovarian cancer	BRCA1	breast cancer ovarian cancer
Familial breast cancer/ breast-ovarian cancer	BRCA2	breast cancer ovarian cancer male breast cancer
Juvenile polyposis	SMAD4	polyps colorectal cancer
Familial gastric cancer	E-cadherin	gastric cancer breast cancer

Oncogenes

Syndrome	Gene	Types of Tumours
Multiple endocrine neoplasia type 2 (MEN2)	RET	medullary thyroid cancer pheochromocytoma
Familial renal cancer	MET	papillary renal cancer

Table 7.1 Human tumor suppressor genes that have been cloned

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

[a]Familial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.
[b]Also known as *MTS1*, *CDKN2*, and *p16*.
[c]The human homolog of the murine $p19^{ARF}$ gene.
[d]Also called *MMAC* or *TEP1*.
[e]*SDHS* encodes the succinate–ubiquinone oxidoreductase subunit D, a component of the mitochondrial respiratory chain complex II.
[f]mTOR is a serine/threonine kinase that controls, among other processes, the rate of translation and activation of Akt/PKB. TSC1 (hamartin) and TSC2 (tuberin) control both cell size and cell proliferation.
[g]The *CBP* gene is involved in chromosomal translocations associated with AML. These translocations may reveal a role of a segment of CBP as an oncogene rather than a tumor suppressor gene.
[h]Also termed Carney complex.
[i]Encodes the Smad4 TF associated with TGF-β signaling; also known as *MADH4* and *SMAD4*.
[j]The human SNF5 protein is a component of the large Swi/Snf complex that is responsible for remodeling chromatin in a way that leads to transcriptional repression through the actions of histone deactylases. The rhabdoid predisposition syndrome involves susceptibility to atypical teratoid/rhabdoid tumors, choroid plexus carcinomas, medulloblastomas, and extra-renal rhabdoid tumors.

Adapted in part from E.R. Fearon, *Science* 278:1043–1050, 1997; and in part from D.J. Marsh and R.T. Zori, *Cancer Lett.*181:125–164, 2002.

Table 7-1 part 1 of 2 The Biology of Cancer (© Garland Science 2007)

Table 7.1 Human tumor suppressor genes that have been cloned

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

[a]Familial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.
[b]Also known as *MTS1, CDKN2,* and *p16.*
[c]The human homolog of the murine $p19^{ARF}$ gene.
[d]Also called *MMAC* or *TEP1.*
[e]*SDHS* encodes the succinate–ubiquinone oxidoreductase subunit D, a component of the mitochondrial respiratory chain complex II.
[f]mTOR is a serine/threonine kinase that controls, among other processes, the rate of translation and activation of Akt/PKB. TSC1 (hamartin) and TSC2 (tuberin) control both cell size and cell proliferation.
[g]The *CBP* gene is involved in chromosomal translocations associated with AML. These translocations may reveal a role of a segment of CBP as an oncogene rather than a tumor suppressor gene.
[h]Also termed Carney complex.
[i]Encodes the Smad4 TF associated with TGF-β signaling; also known as *MADH4* and *SMAD4.*
[j]The human SNF5 protein is a component of the large Swi/Snf complex that is responsible for remodeling chromatin in a way that leads to transcriptional repression through the actions of histone deactylases. The rhabdoid predisposition syndrome involves susceptibility to atypical teratoid/rhabdoid tumors, choroid plexus carcinomas, medulloblastomas, and extra-renal rhabdoid tumors.

Adapted in part from E.R. Fearon, *Science* 278:1043–1050, 1997; and in part from D.J. Marsh and R.T. Zori, *Cancer Lett.*181:125–164, 2002.

Table 7-1 part 2 of 2 The Biology of Cancer (© Garland Science 2007)

Multi-step cancer – Familial adenomatous polyposis

FAP occurs in 1/10,000 people and is characterized by the development of thousands of polyps in the colon during the second or third decade of life.

A small percentage of these polyps will develop into carcinomas of the colon.

At least three tumour suppressors and one oncogene in FAP:

APC (adenomatous polyposis coli) – mutations in APC found in polyps (predisposes to colon cancer) (5q21); mutations in APC result in constitutive activation of Wnt signaling pathway

K-ras - 40% of large polyps

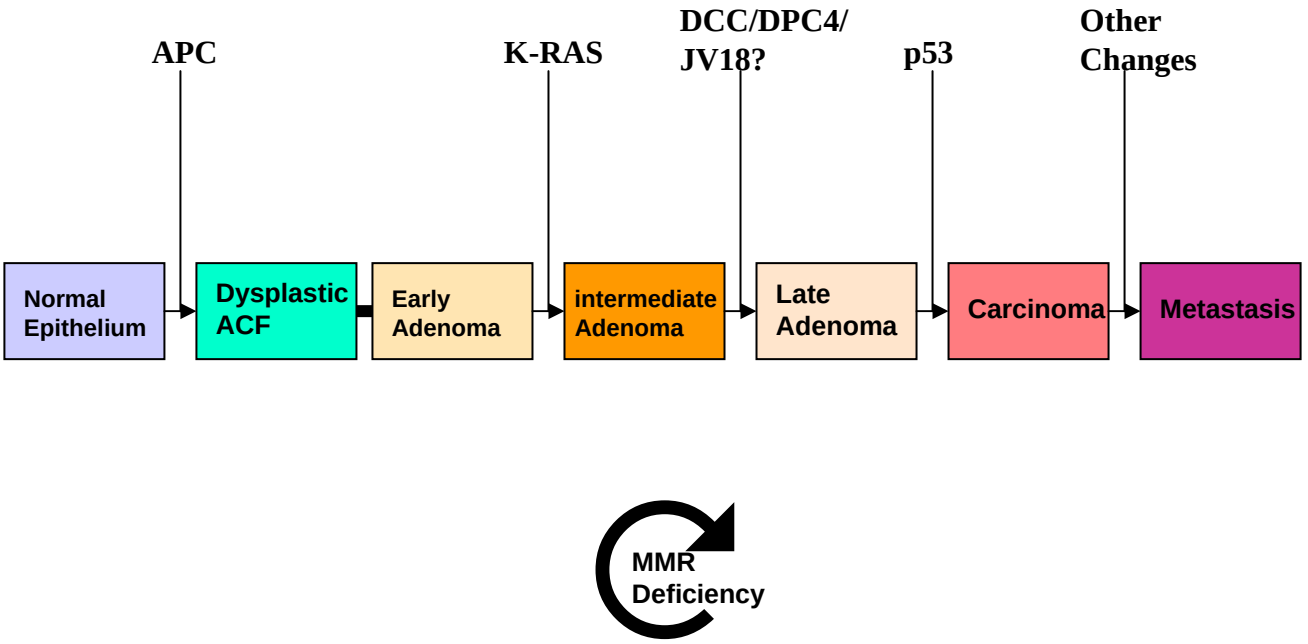
p53, DCC - (deleted in colon cancer; 18q) - uncommon in polyps, found in the majority of colon cancers

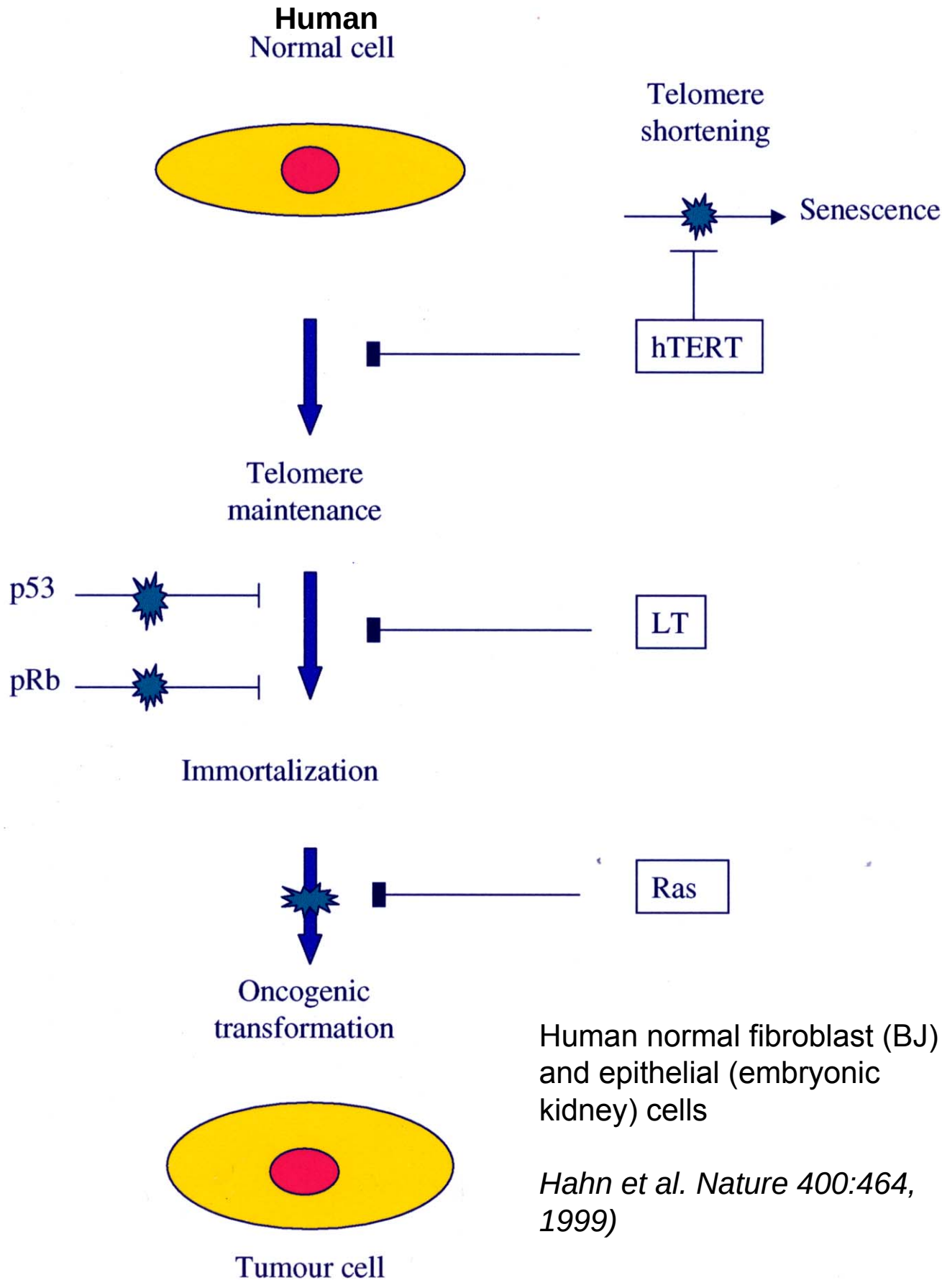
Wall of the colon from an individual with FAP

Figure 7-22 The Biology of Cancer (© Garland Science 2007)

FAP

normal


APC
K-RAS
DCC/DPC4/
JV18?
p53
Other
Changes
Normal
Epithelium
Dysplastic
ACF
Early
Adenoma
intermediate
Adenoma
Late
Adenoma
Carcinoma
Metastasis
MMR
Deficiency



Human normal fibroblast (BJ) and epithelial (embryonic kidney) cells

Hahn et al. Nature 400:464, 1999)

Telomeres and telomerases

Telomeres are located at the ends of chromosomes

- tandem hexameric repeats
- 10-15 kb of TTAGGG repeats
- telomere length in primary human cells progressively gets shorter with age

Telomerase activity maintains telomere length, synthesizes telomeric DNA

- telomerase active in ~85% of human cancers
- important for tumour progression and tumour maintenance

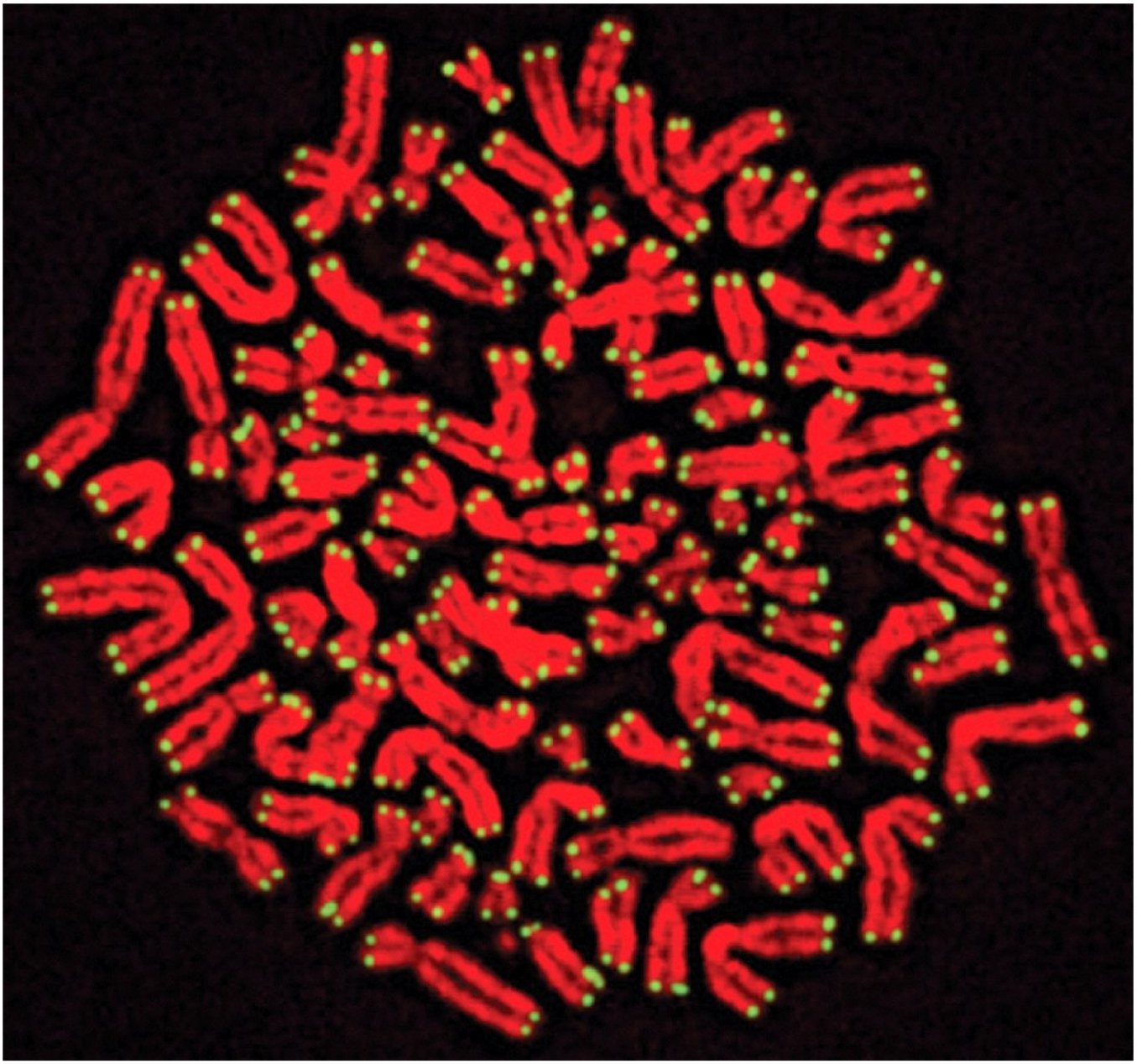


Figure 10-11a The Biology of Cancer (© Garland Science 2007)