Signaling Pathways and aberrant cell signaling in cancer

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Part I: The Molecular Biology of Cancer

a. aberrant signaling in cancer

Part II: The Cellular Biology of Cancer

b. aberrant cellular behavior in cancer

(i. e., The Hallmarks of Cancer)

Part III: The evolution of cancer treatment

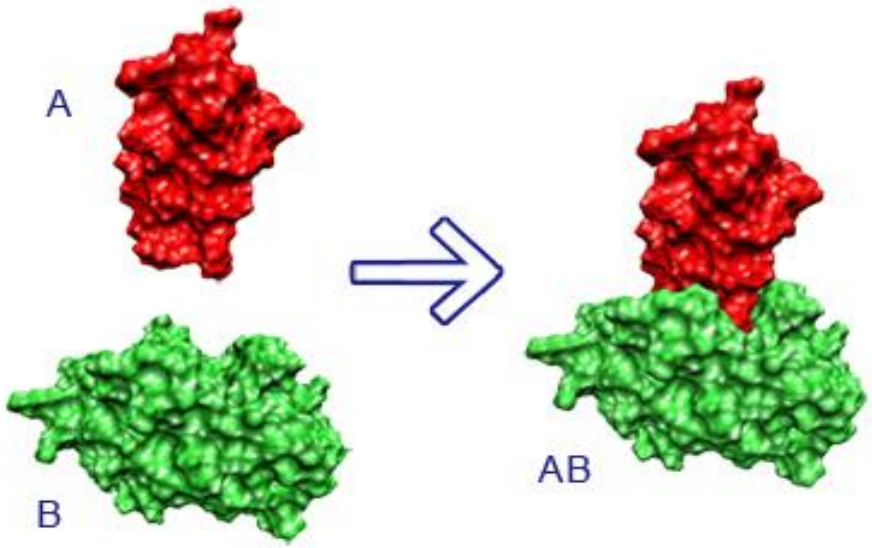
Part I: The Molecular Biology of Cancer

Genetics and Epigenetics of Cancer: Cancer arises due to genetic and epigenetic alterations in cells. These changes dramatically affect cellular function and disrupt cellular control mechanisms.

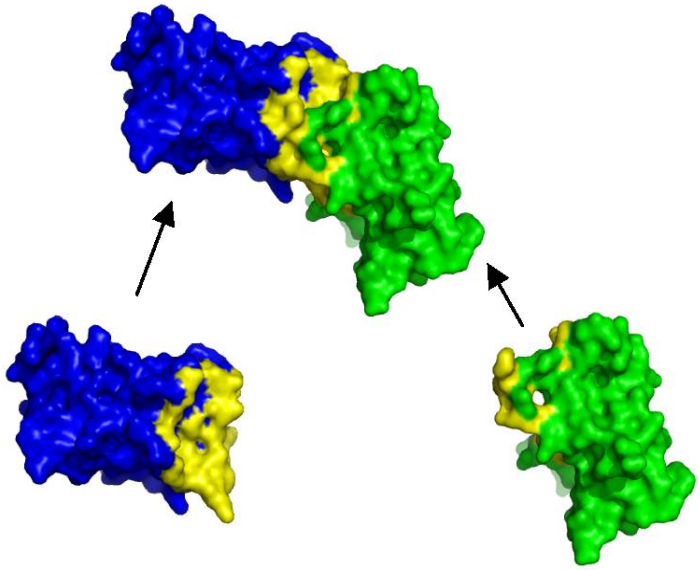
Genetic- alterations in DNA sequence (i.e., mutations) that produce abnormal (mutant) proteins, or no protein at all. These mutant proteins have abnormal shapes or are truncated versions of the normal proteins, and cannot perform their biological roles. They are normally degraded.

Epigenetic- alteration in DNA, mostly structural, that DOES NOT CHANGE the nucleotide sequence, but affects gene expression.

Protein-protein interactions as the basis of biological processes



From http://vred.bioinf.uni-sb.de/DFG-protein-protein-docking/DATA/projectsummary.shtml

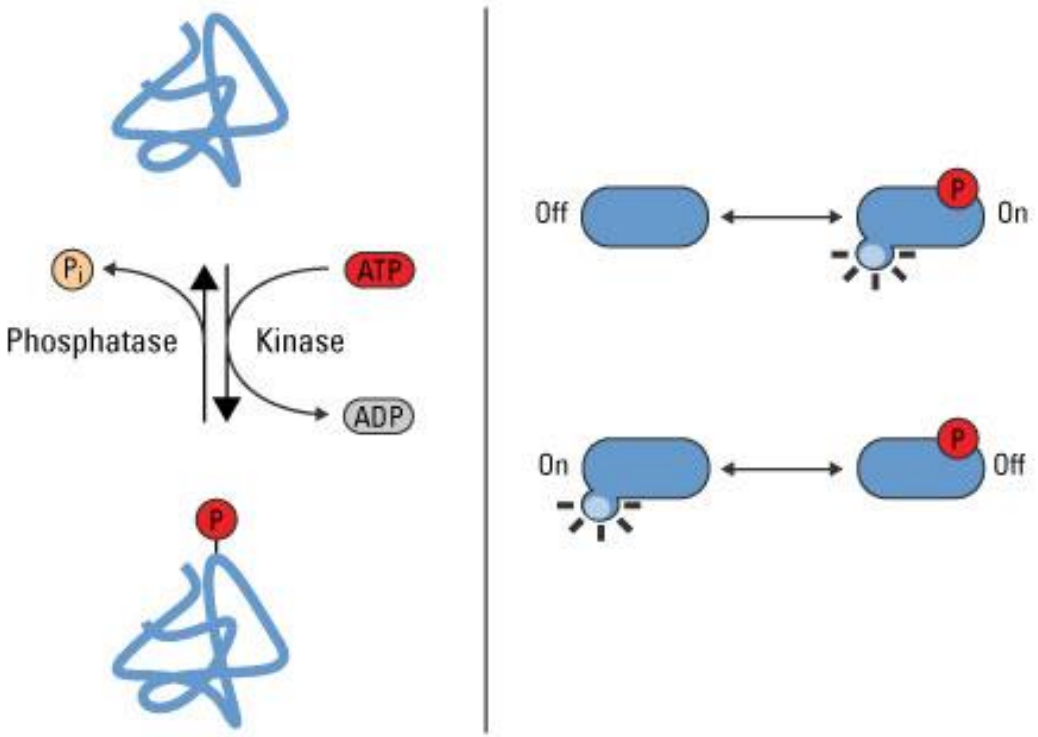


http://www.stats.ox.ac.uk/~hamer/research.html

Cancer-associated mutations sometimes produce proteins with abnormal shapes that are unable to interact with their normal binding partners.

In the case of enzymes, their catalytic activity can also be altered since the enzyme-substrate fit is altered (remember the lock and key model for enzyme-substrate interaction).

Protein phosphorylation as a mechanism to regulate protein structure and function



From: http://www.piercenet.com/method/phosphorylation

Background Mutation Rate:

•Spontaneous changes arising from an inherent error rate in the fidelity of DNA replication and/or repair. It is known that human cells have a background or spontaneous mutation rate of one "mistake" per 10 billion base pairs copied. It has been estimated that a human being can acquire ~2.8 x 10^{15} point mutations in a life time (Loeb, 1991).

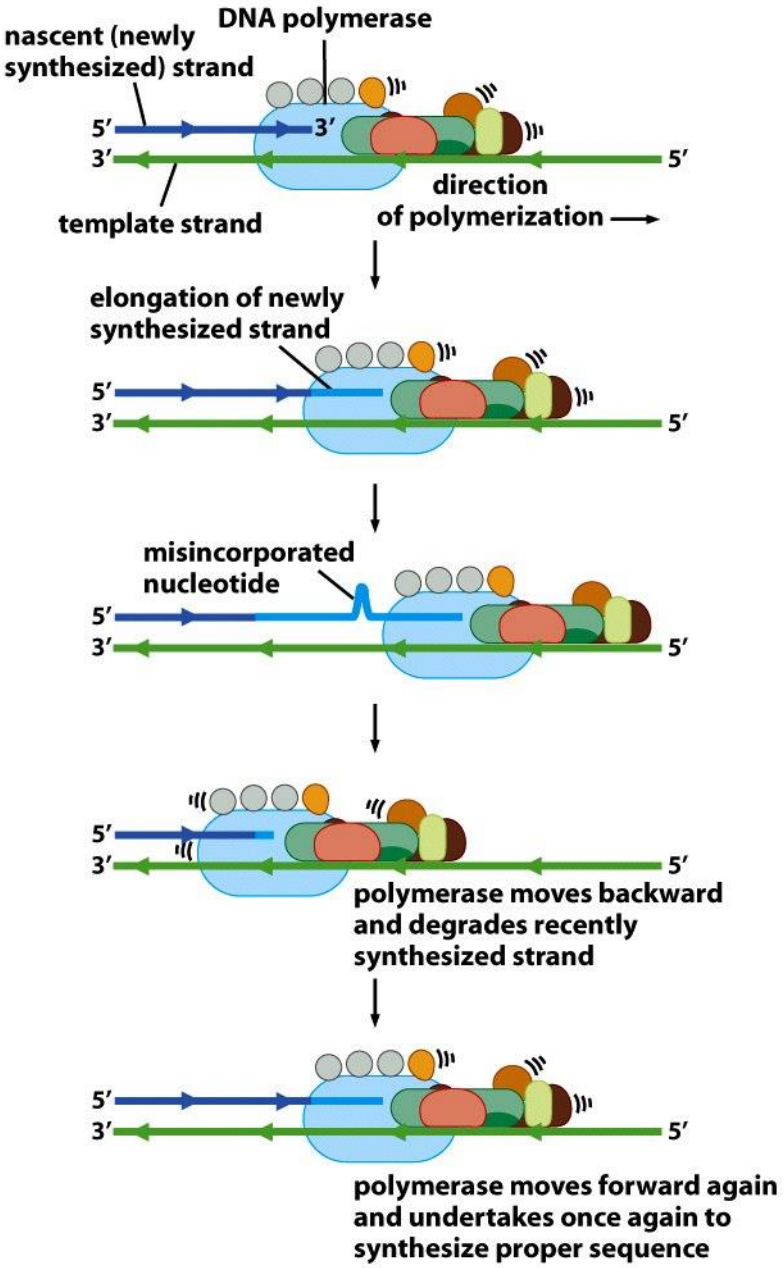


Figure 12.6 *The Biology of Cancer* (© Garland Science 2007)

Oxidative stress and its effect on DNA

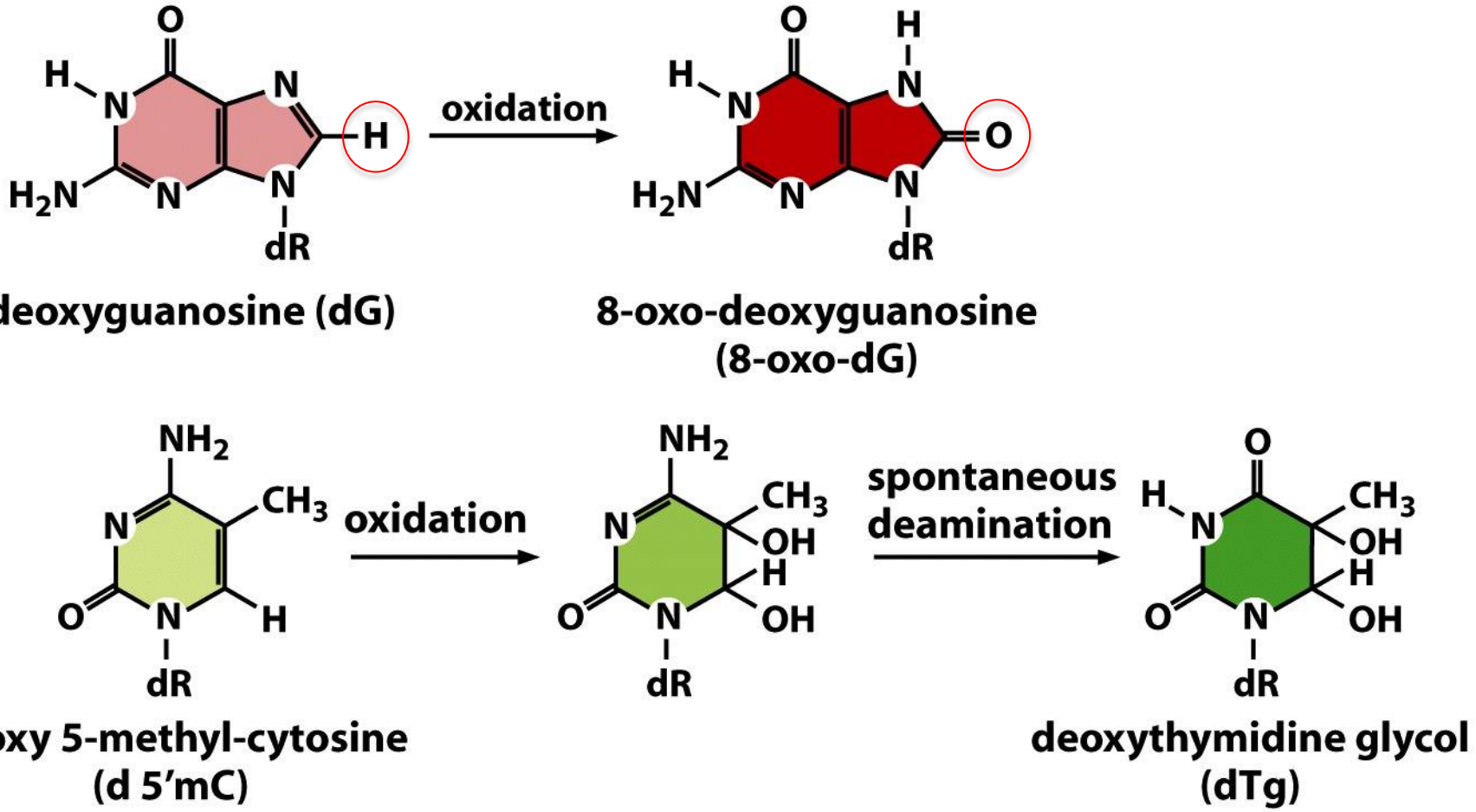
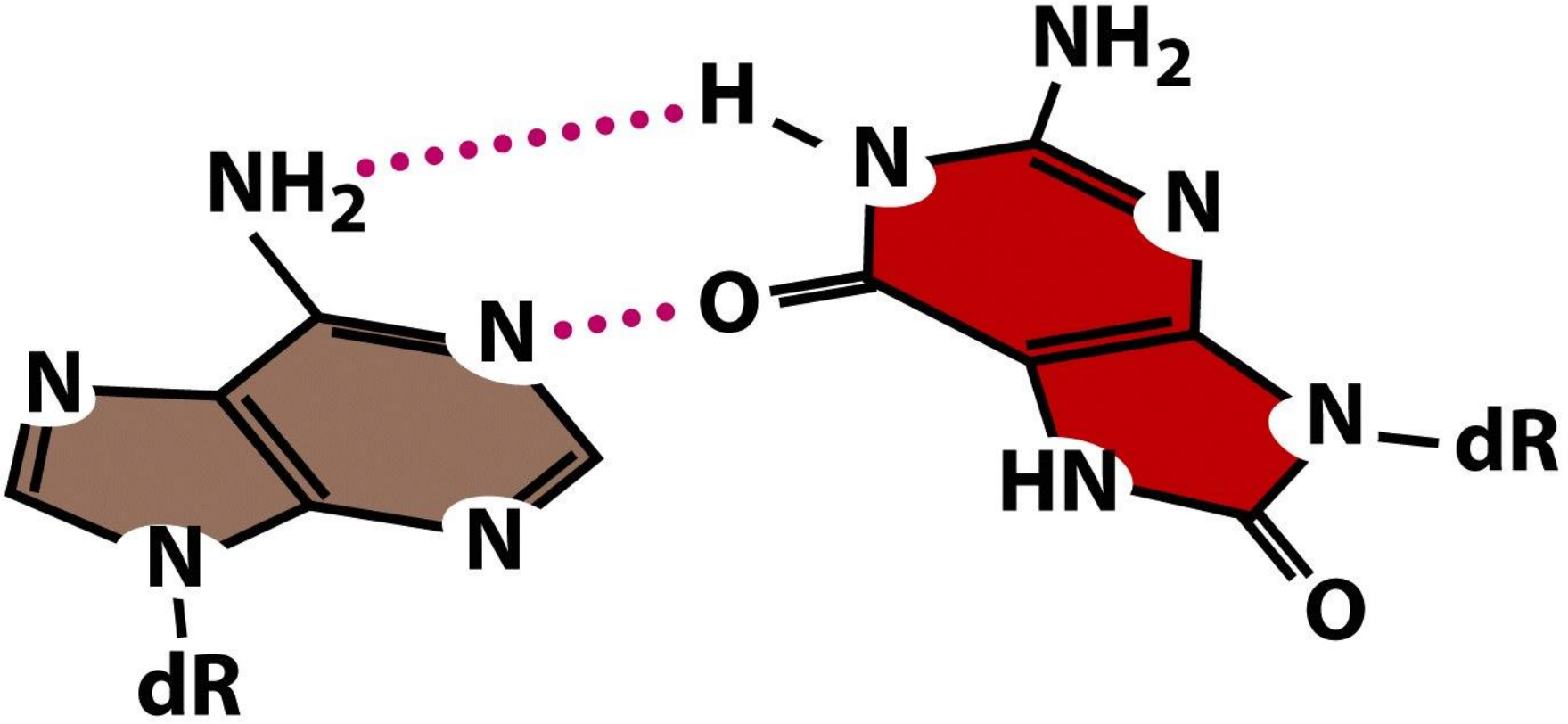


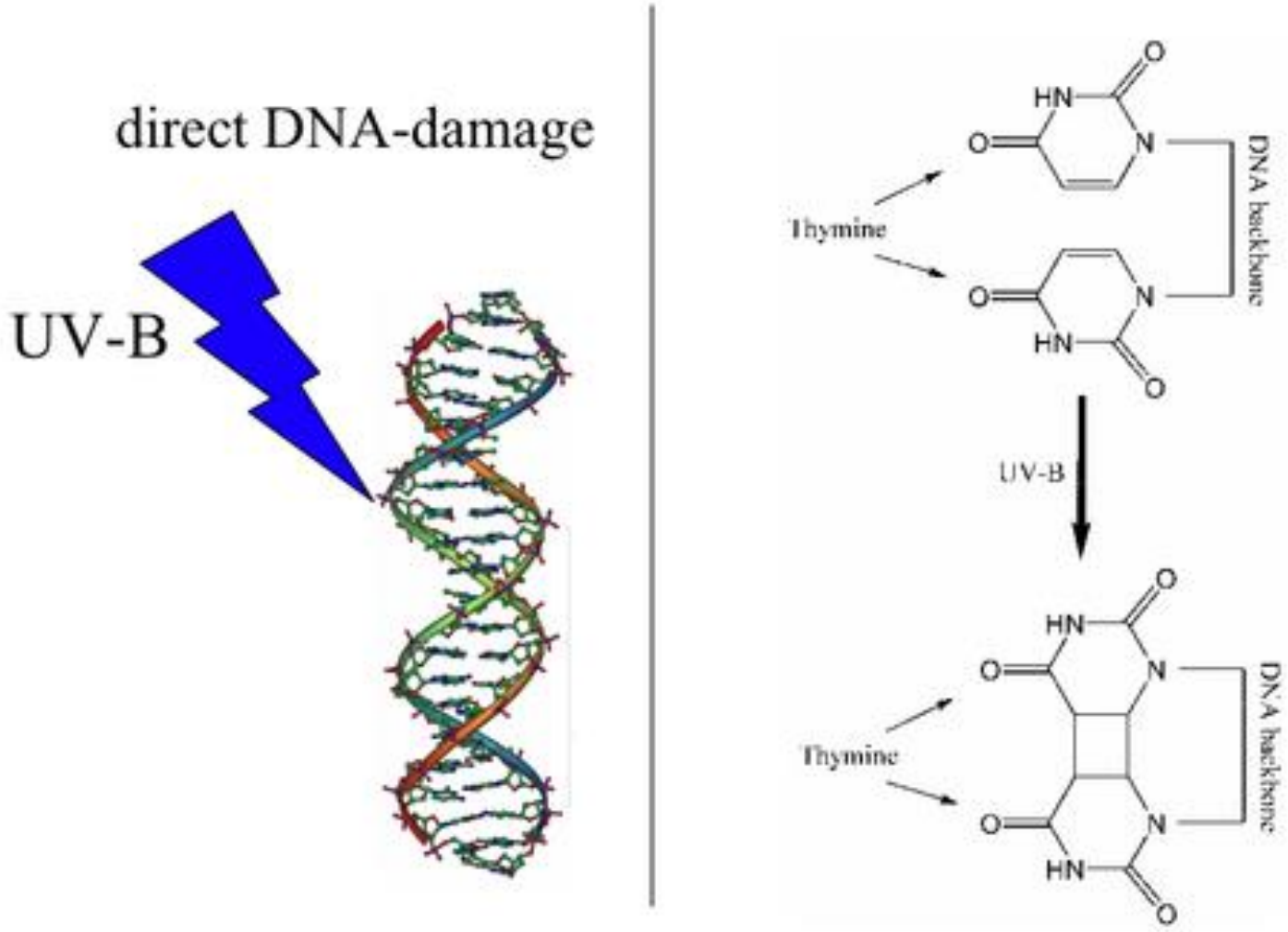
Figure 12.12a *The Biology of Cancer* (© Garland Science 2007)



mispairing of 8-oxo-dG with deoxyadenosine (dA)

Figure 12.12b *The Biology of Cancer* (© Garland Science 2007)

Effect of UV irradiation on DNA structure



Effect of UV irradiation on DNA structure

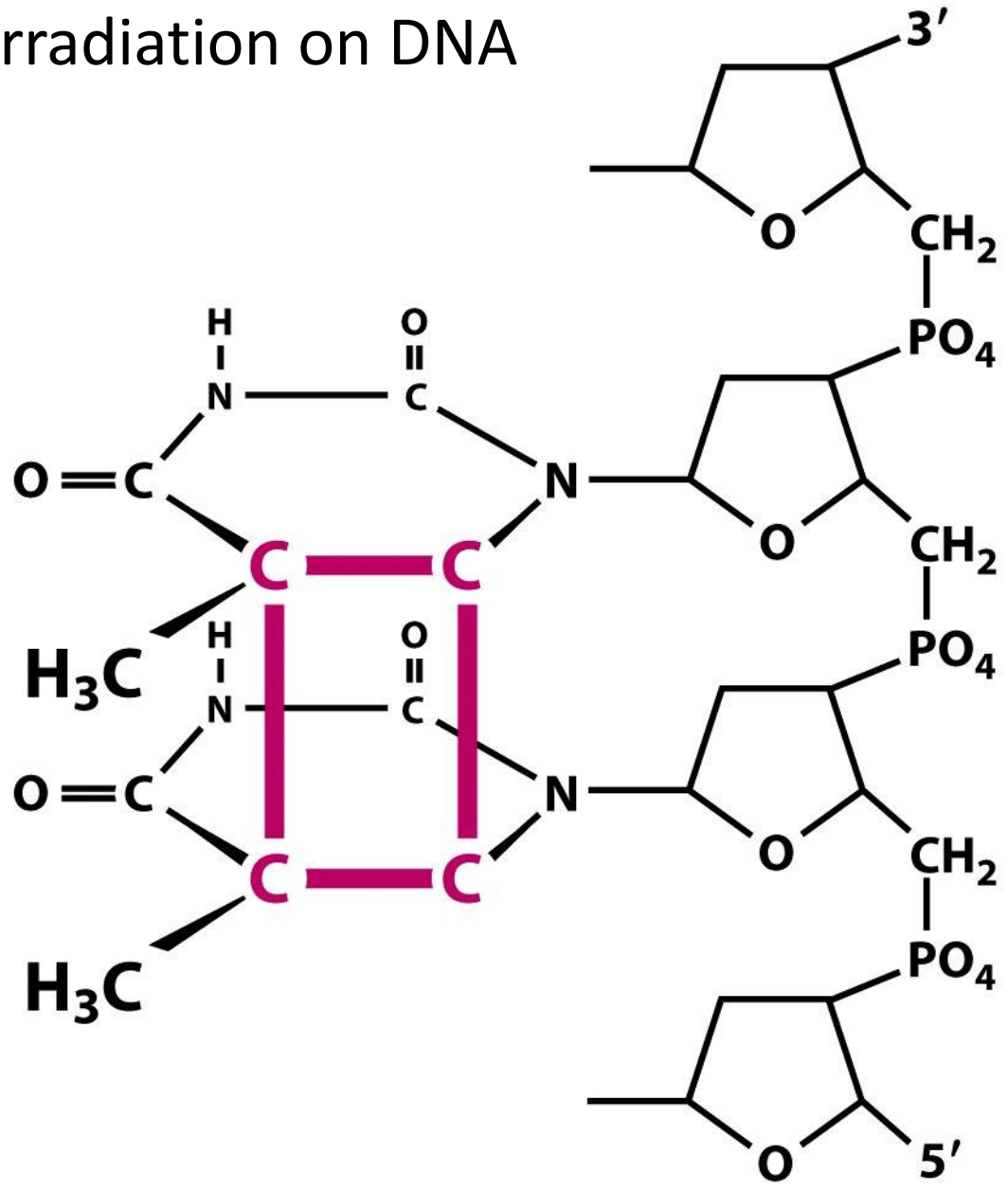
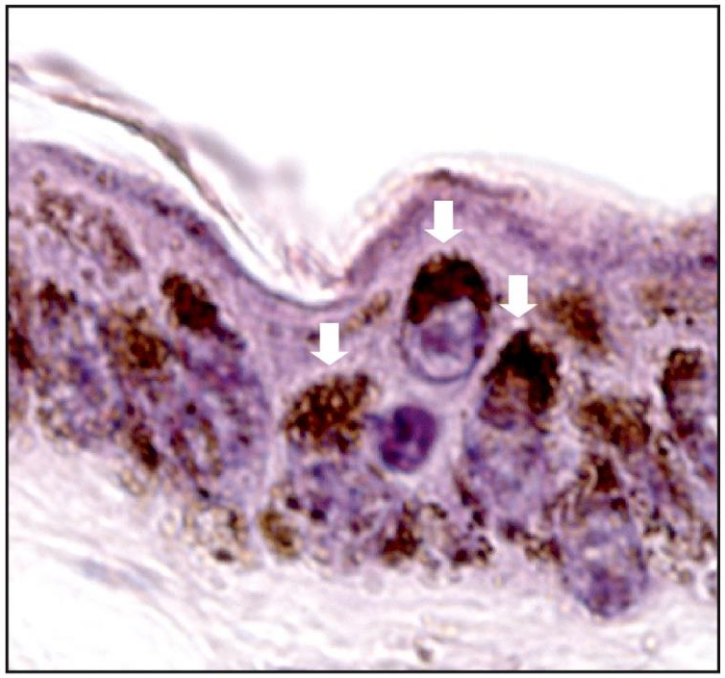
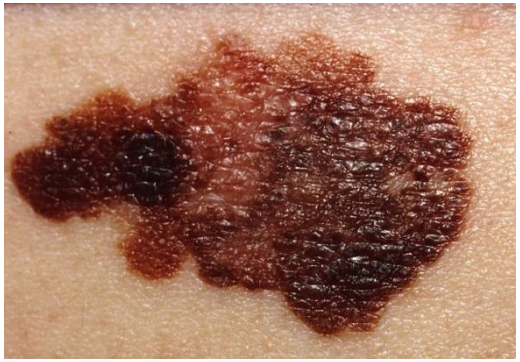


Figure 12.14a *The Biology of Cancer* (© Garland Science 2007)

Melanin protects from UV irradiation





Skin melanoma

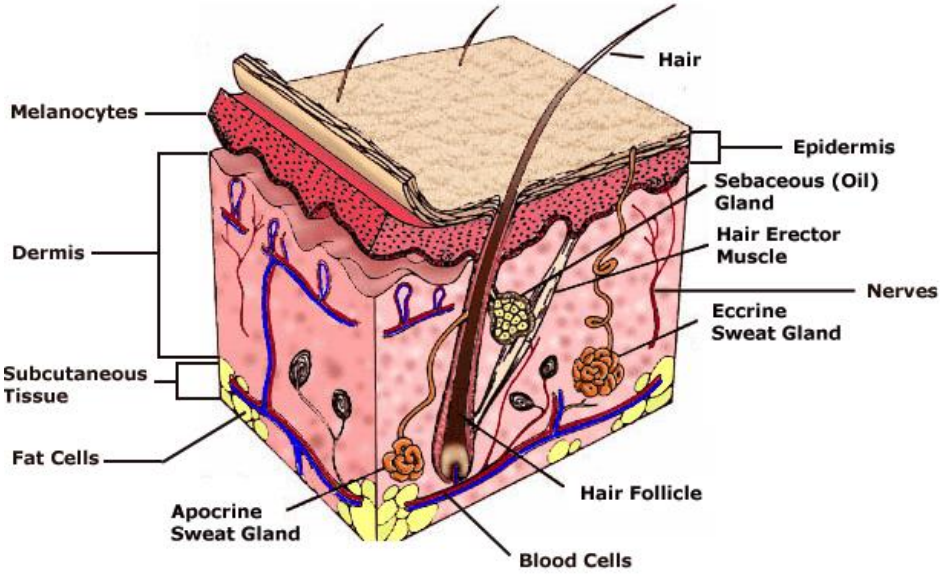


Figure 12.20 *The Biology of Cancer* (© Garland Science 2007)

Mutations and cancer

1. Background mutation rate (replication errors)
    a. Is this enough? Is there a “mutator” phenotype?
2. Oxidative metabolism
3. UV
4. Environmental carcinogens
5. Viral infection

Important risk factor:- age-related decrease in DNA Repair Capacity (DRC)

Epigenetics is important as well!

Current debate on genomic instability

- “Mutator Phenotype”
  - Is this necessary for cancer initiation, promotion or progression?
- Or is the background mutation rate sufficient?

Arguments supporting genomic instability (mutator phenotype) in tumor genesis

- Tumors harbor too many mutations to be explained by anything other than underlying genomic instability. *
- The probability of a tumor acquiring enough mutations for the full, malignant phenotype is too low unless the cells have an unstable genome.
- In some tumors, there is direct evidence that some pathways that are involved in maintaining genomic integrity are defective. *
- Humans and mouse models with inherent genomic instability are prone to tumors.

*Driver or passenger?

Defects in DNA-repair systems perpetuate mutations and are associated with certain cancers

TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication,* 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411:**366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509:**49.

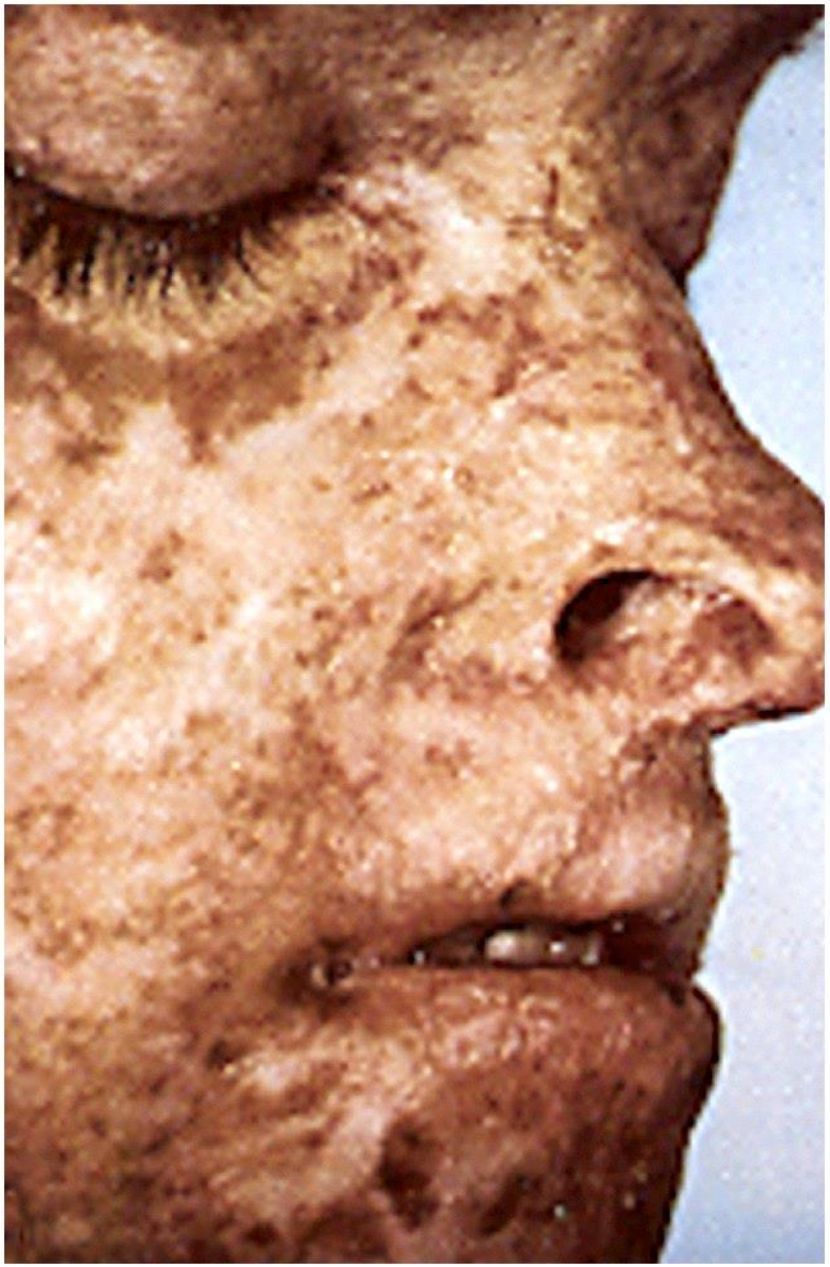
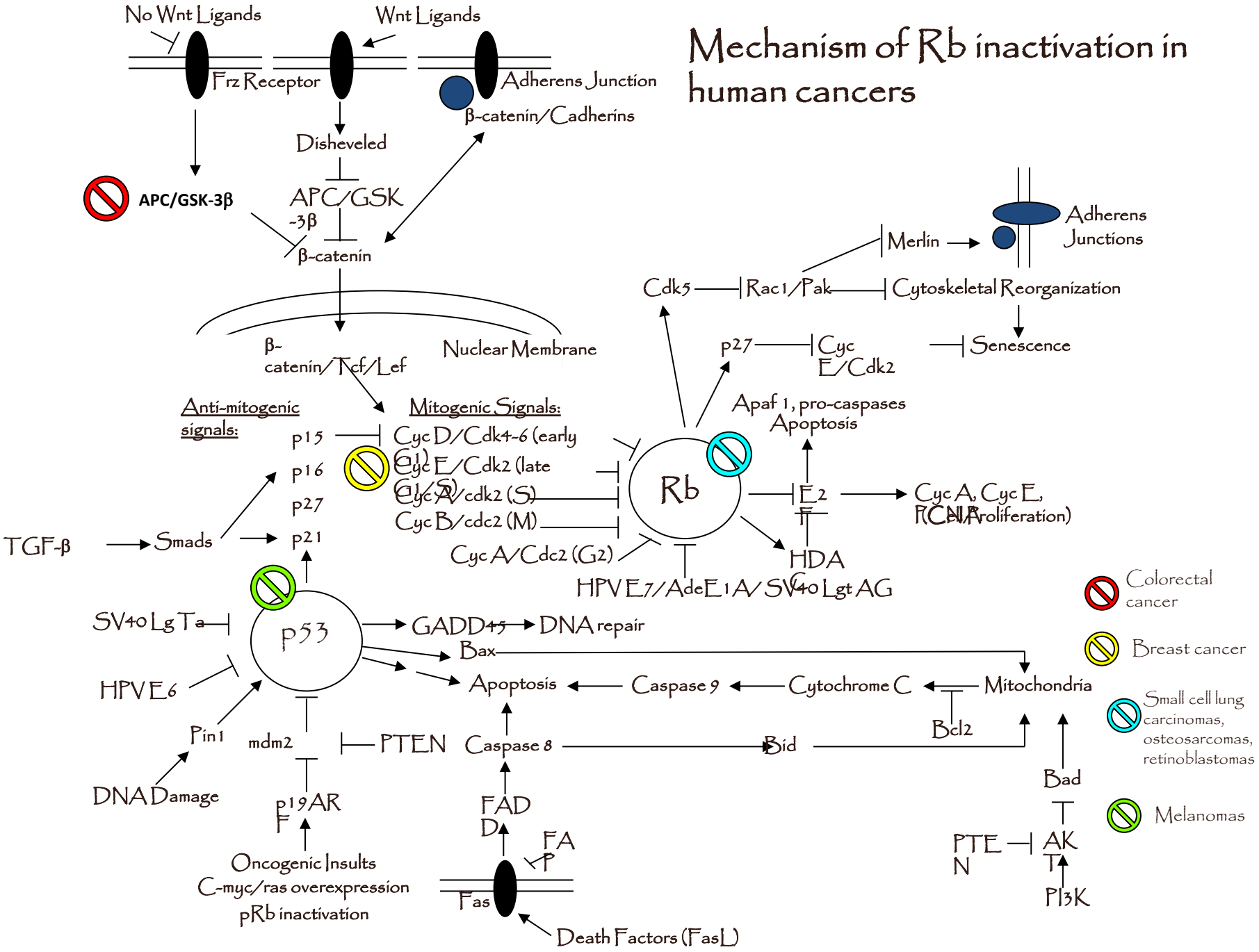
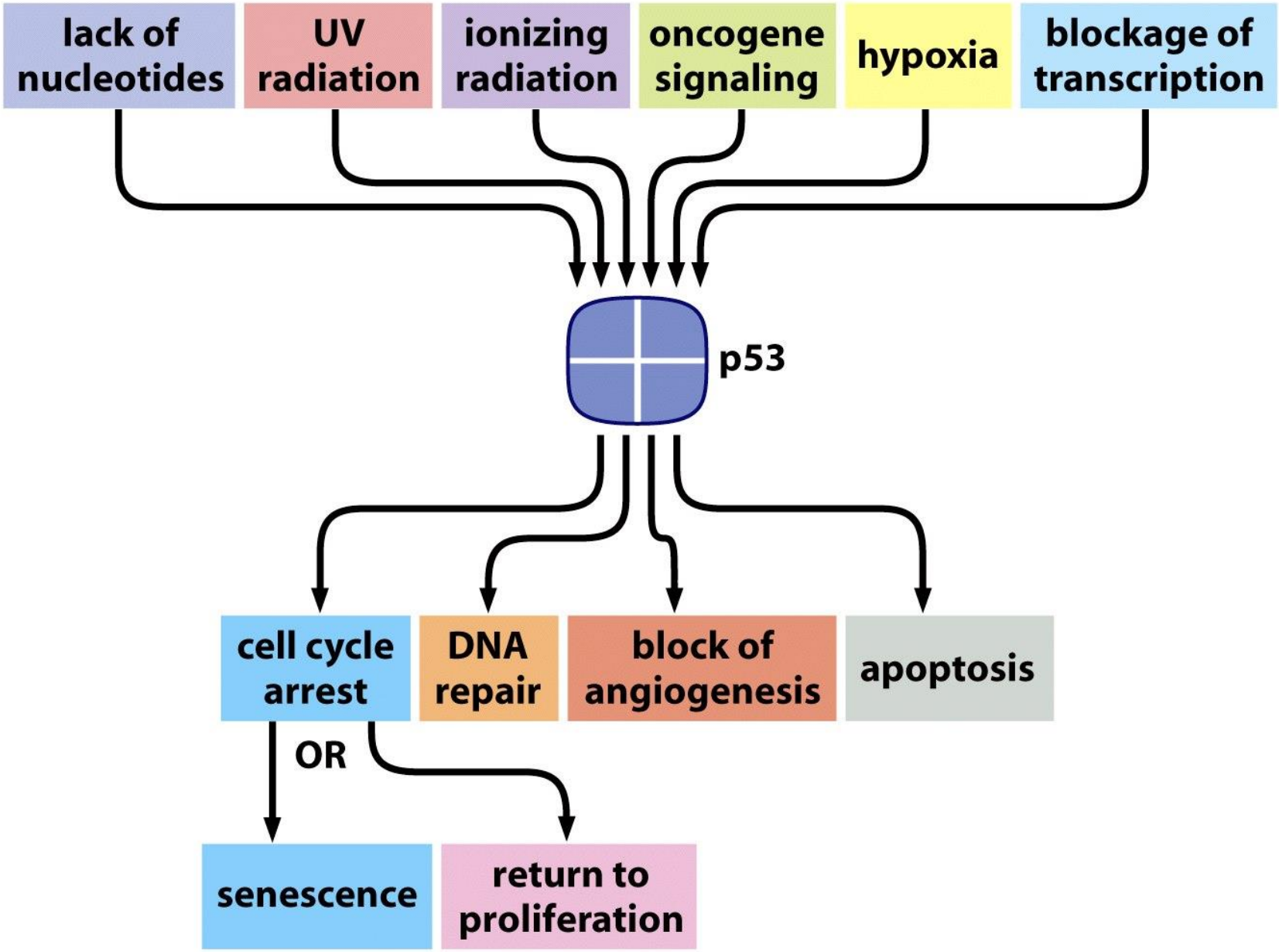


Figure 12.25 *The Biology of Cancer* (© Garland Science 2007)


Mechanism of Rb inactivation in human cancers
No Wnt Ligands
Wnt Ligands
Frz Receptor
Adherens Junction
β-catenin/Cadherins
Disheveled
APC/GSK-3β
APC/GSK -3β
β-catenin
β-catenin/Tcf/Lef
Nuclear Membrane
Merlin
Adherens Junctions
Cdk5
Rac1/Pak
Cytoskeletal Reorganization
Senescence
p27
Cyc E/Cdk2
Anti-mitogenic signals:
Mitogenic Signals:
p15
p16
p27
p21
Cyc D/Cdk4-6 (early G1)
Cyc E/Cdk2 (late G1/S)
Cyc A/cdk2 (S)
Cyc B/cdc2 (M)
Cyc A/Cdc2 (G2)
Rb
Apaf 1, pro-caspases
Apoptosis
E2F
HDAC
Cyc A, Cyc E, PCNA
(Cell Proliferation)
HPV E7/AdeE1A/SV40 Lgt AG
TGF-β
Smads
p53
SV40 Lg Ta
HPV E6
GADD45
DNA repair
Bax
Apoptosis
Caspase 9
Cytochrome C
Mitochondria
Bcl2
Pin1
mdm2
PTEN
Caspase 8
Bid
Bad
DNA Damage
p19ARF
FADD
FAP
Fas
Death Factors (FasL)
Oncogenic Insults
C-myc/ras overexpression
pRb inactivation
PTEN
AKT
PI3K
Colorectal cancer
Breast cancer
Small cell lung carcinomas, osteosarcomas, retinoblastomas
Melanomas

P53-activating signals and p53's downstream effects



Structure of the normal retina

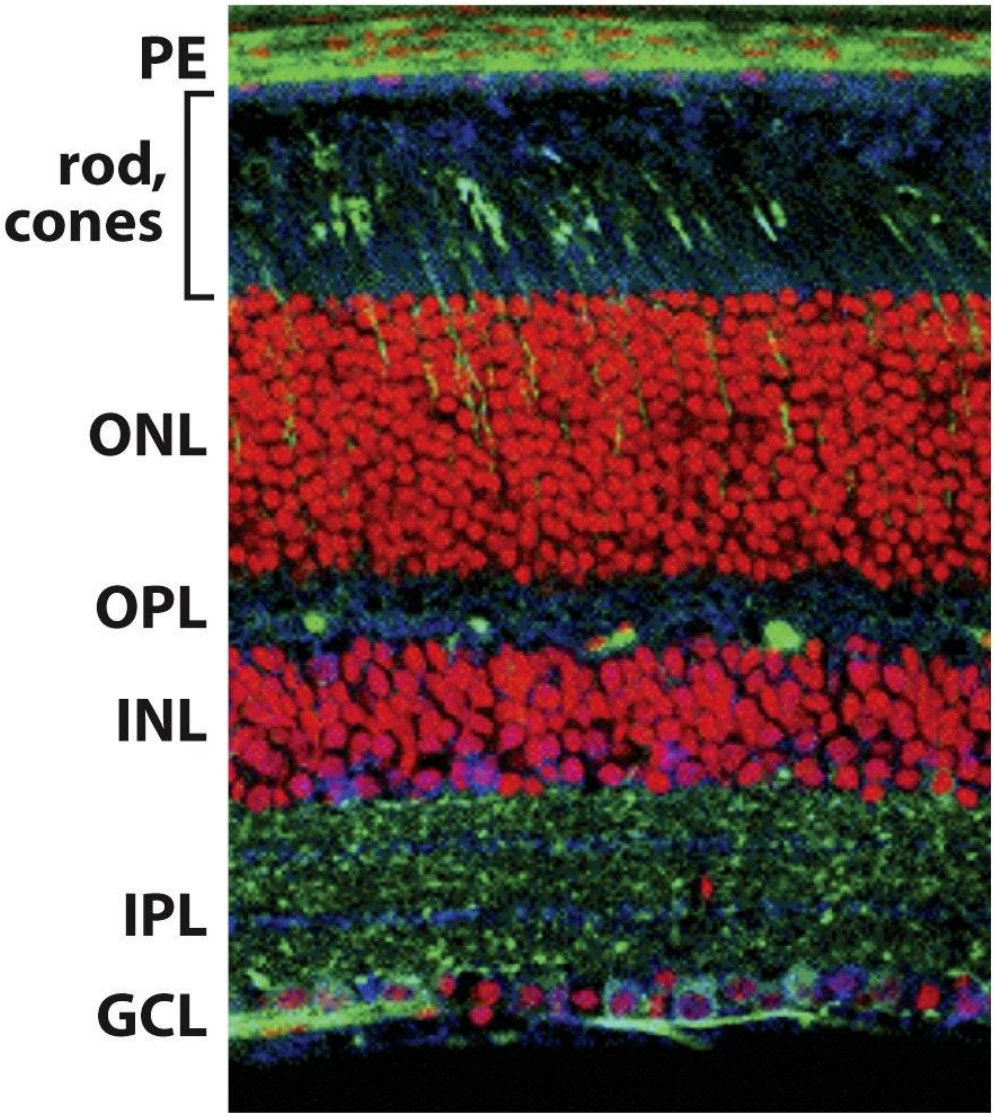


Figure 7.4a *The Biology of Cancer* (© Garland Science 2007)

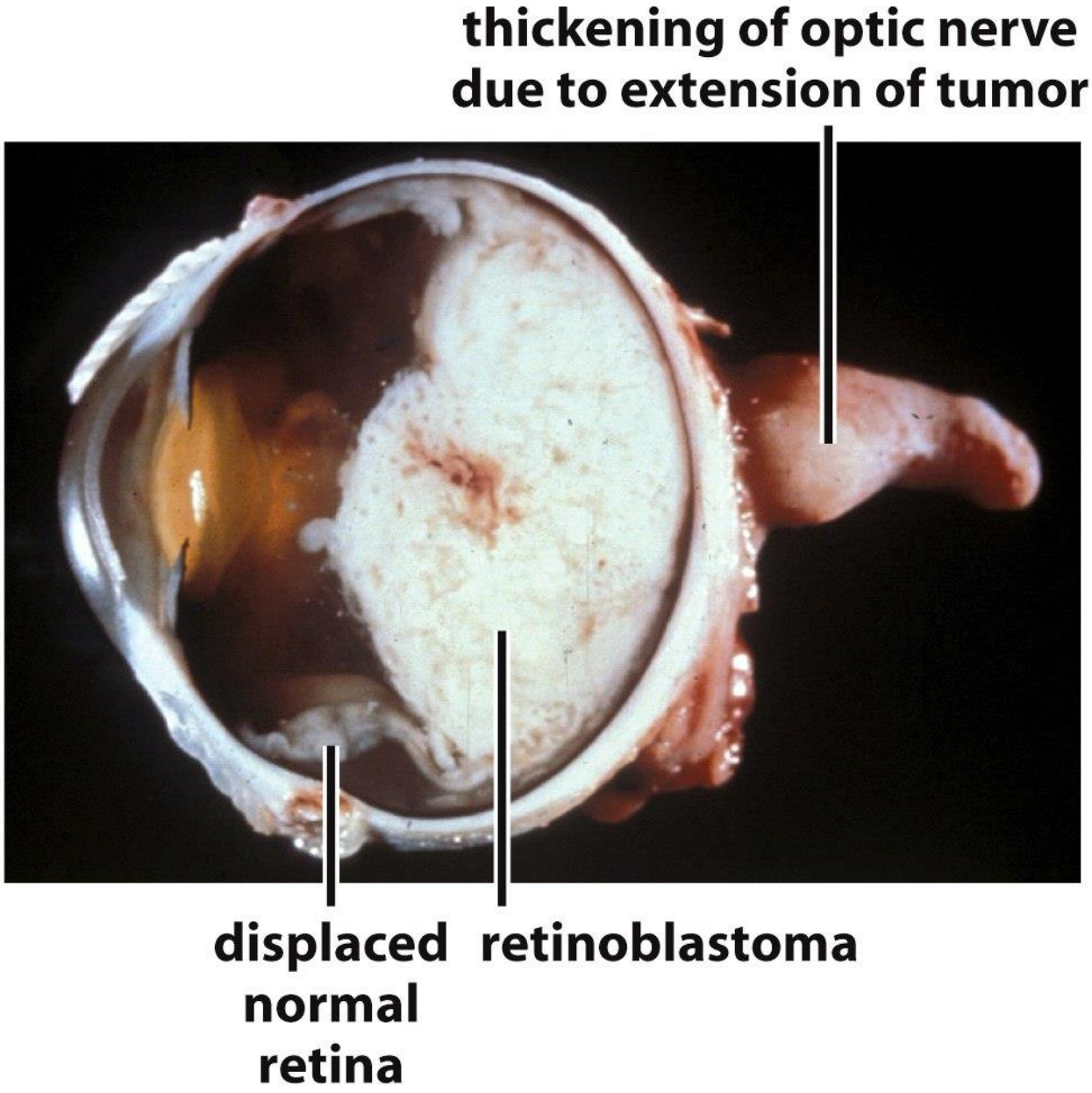


Figure 7.4b *The Biology of Cancer* (© Garland Science 2007)

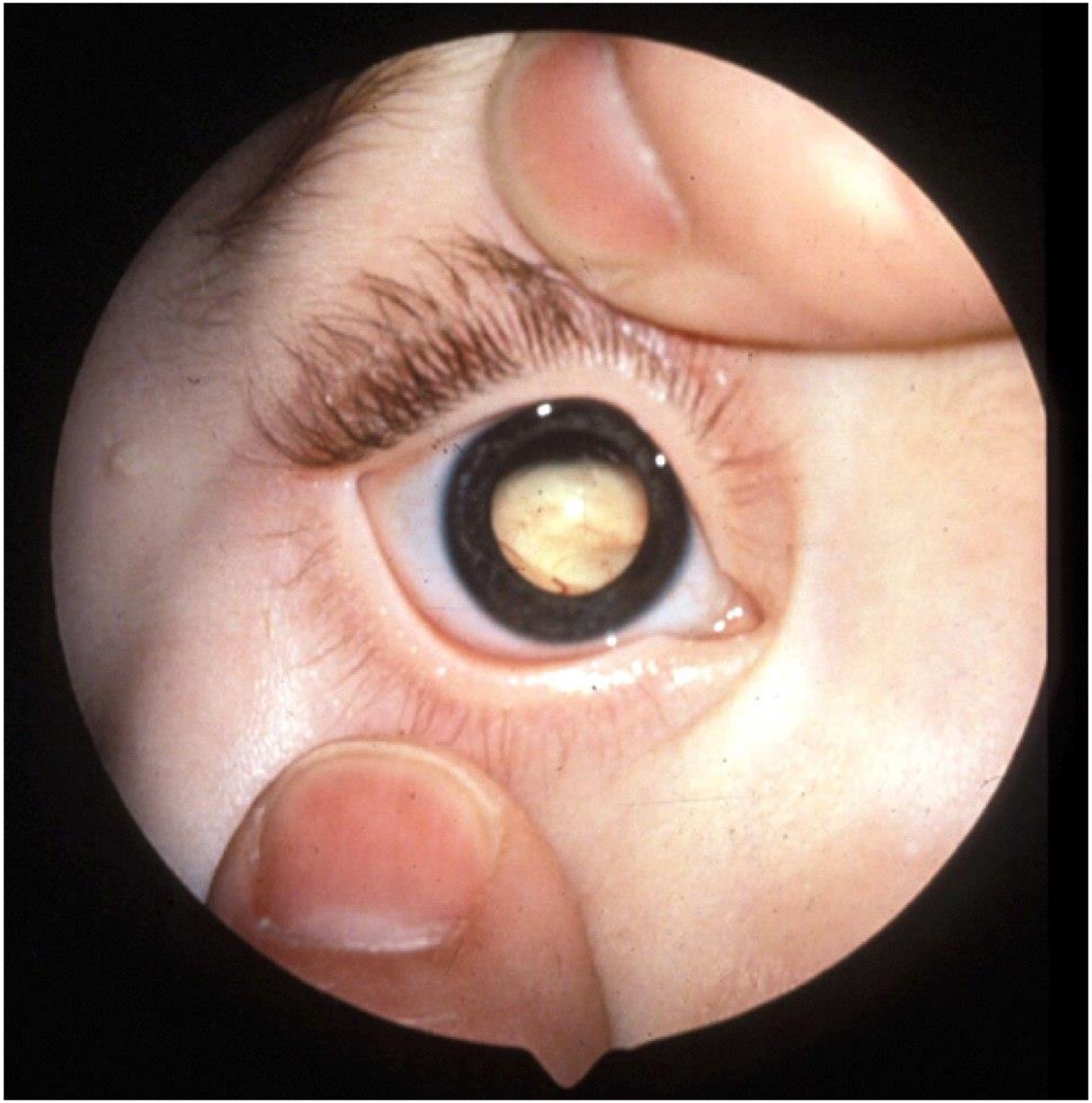
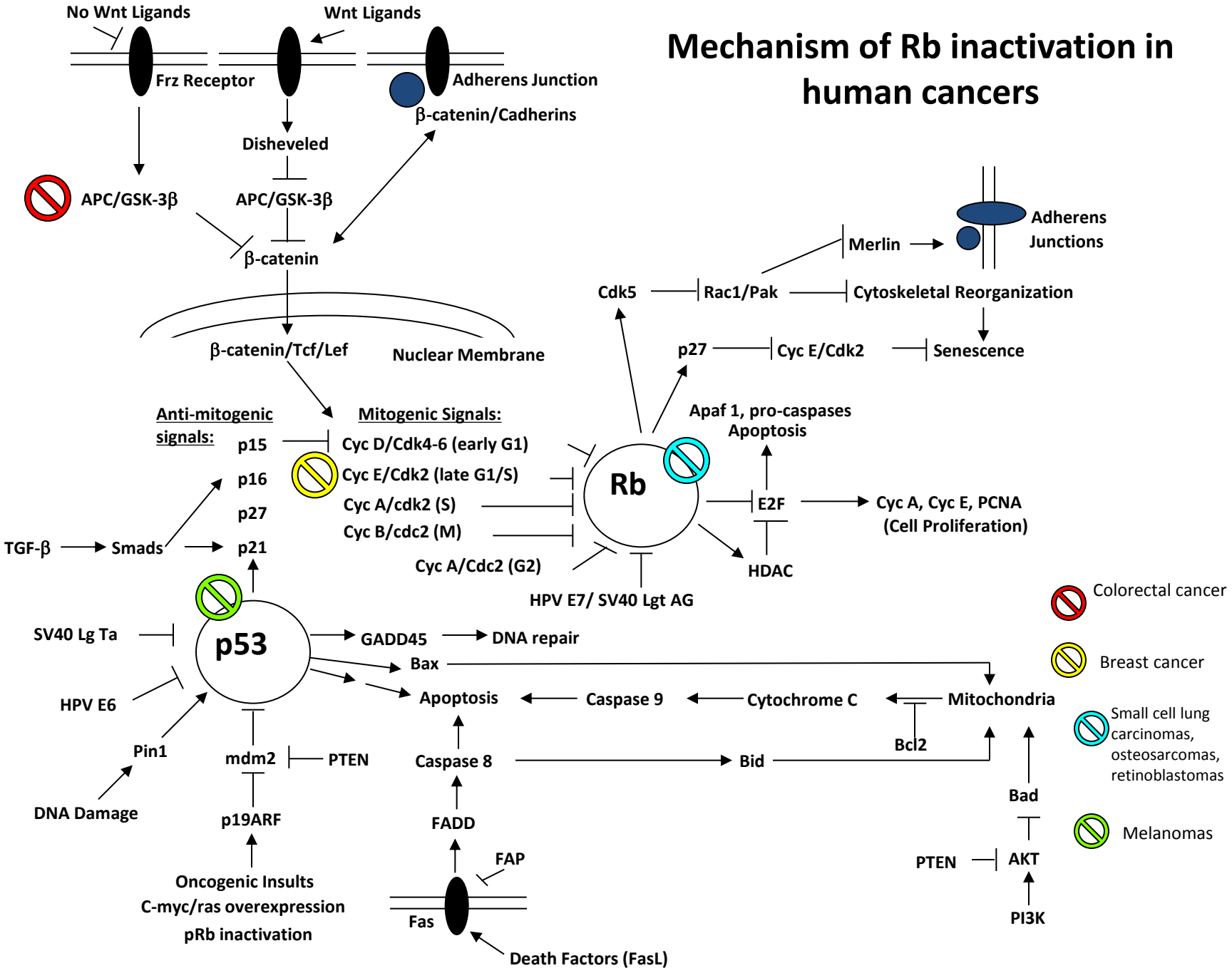


Figure 7.4c *The Biology of Cancer* (© Garland Science 2007)

Mechanism of Rb inactivation in human cancers



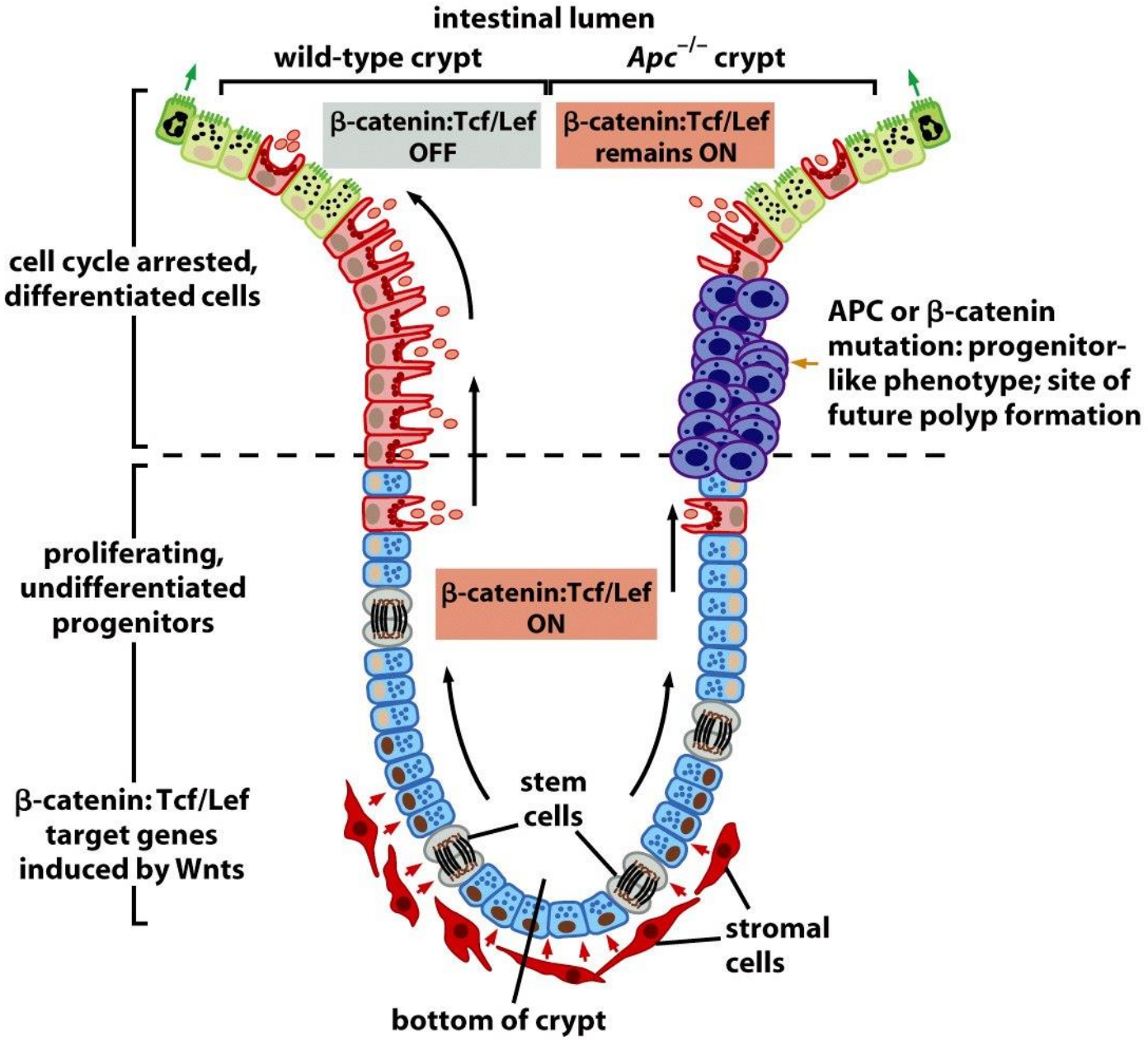


Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)

Figure 7.22 *The Biology of Cancer* (© Garland Science 2007)

p53: The Guardian of the Genome

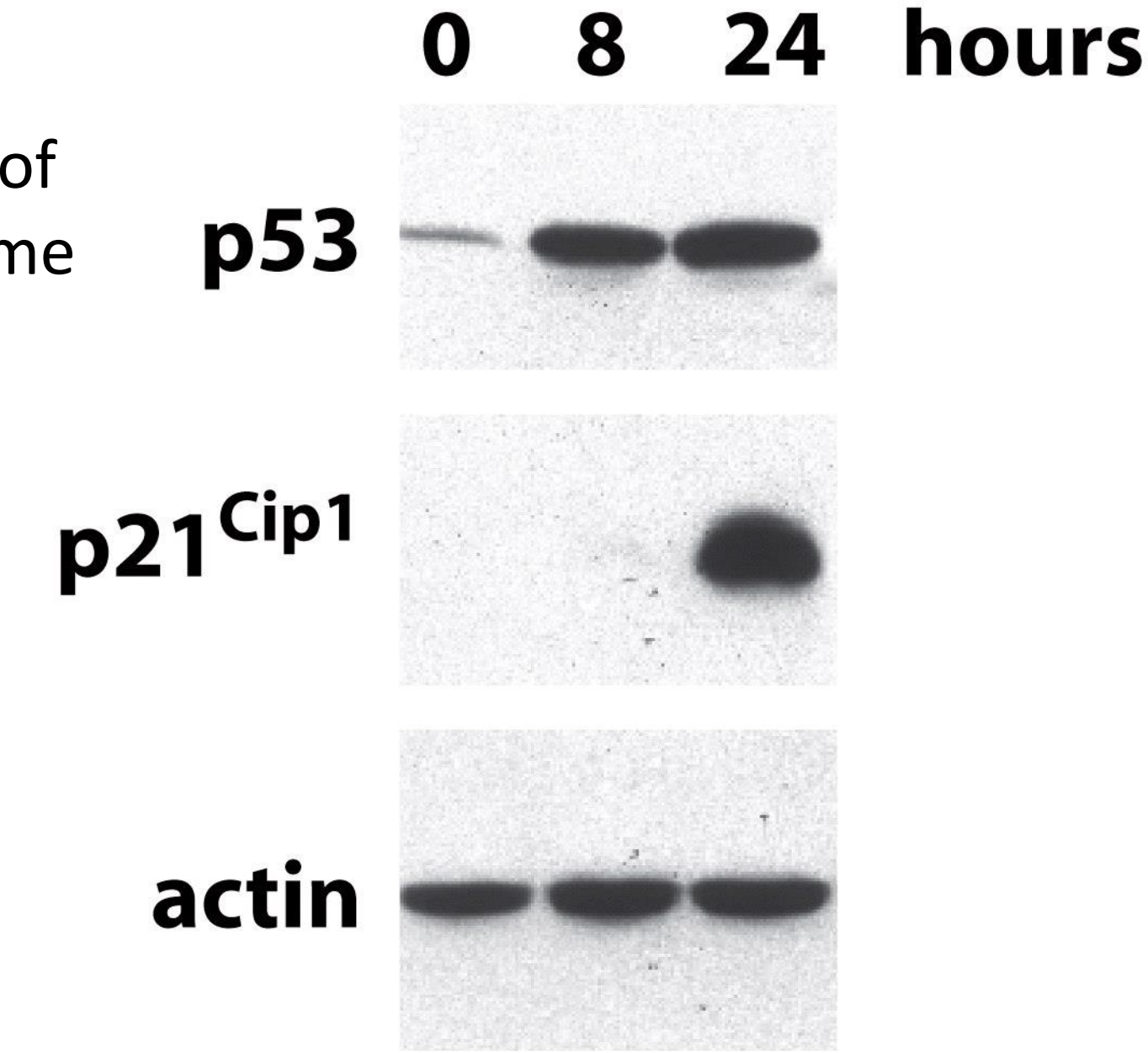


Figure 9.9 *The Biology of Cancer* (© Garland Science 2007)

Science 5 July 1991:
Vol. 253 no. 5015 pp. 49-53
DOI: 10.1126/science.190584

p53 mutations in human cancers

M Hollstein, D Sidransky, B Vogelstein, CC Harris

Mutations in the evolutionarily conserved codons of the p53 tumor suppressor gene are common in diverse types of human cancer. The p53 mutational spectrum differs among cancers of the colon, lung, esophagus, breast, liver, brain, reticuloendothelial tissues, and hemopoietic tissues. Analysis of these mutations can provide clues to the etiology of these diverse tumors and to the function of specific regions of p53. Transitions predominate in colon, brain, and lymphoid malignancies, whereas G:C to T:A transversions are the most frequent substitutions observed in cancers of the lung and liver. Mutations at A:T base pairs are seen more frequently in esophageal carcinomas than in other solid tumors. Most transitions in colorectal carcinomas, brain tumors, leukemias, and lymphomas are at CpG dinucleotide mutational hot spots. G to T transversions in lung, breast, and esophageal carcinomas are dispersed among numerous codons. In liver tumors in persons from geographic areas in which both aflatoxin B1 and hepatitis B virus are cancer risk factors, most mutations are at one nucleotide pair of codon 249. These differences may reflect the etiological contributions of both exogenous and endogenous factors to human carcinogenesis.

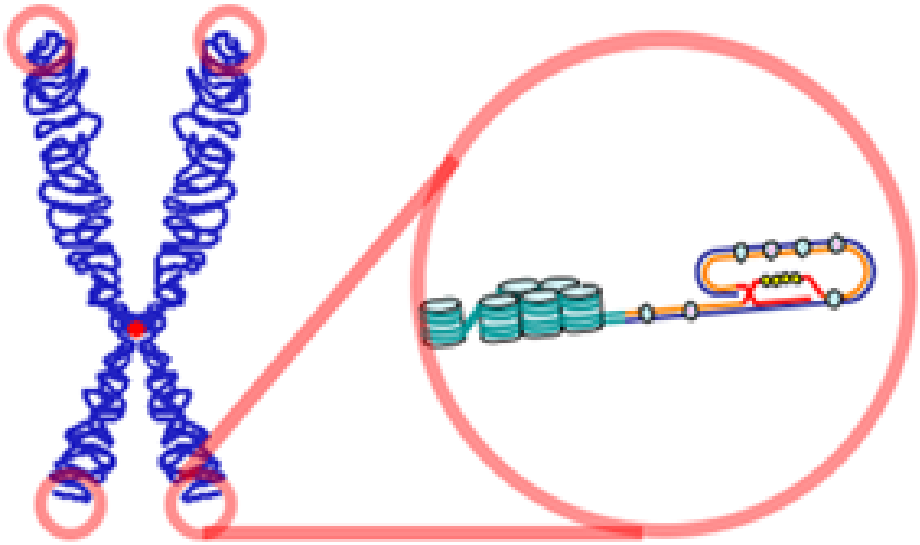
Telomeres protect chromosomal ends…

Chromosome

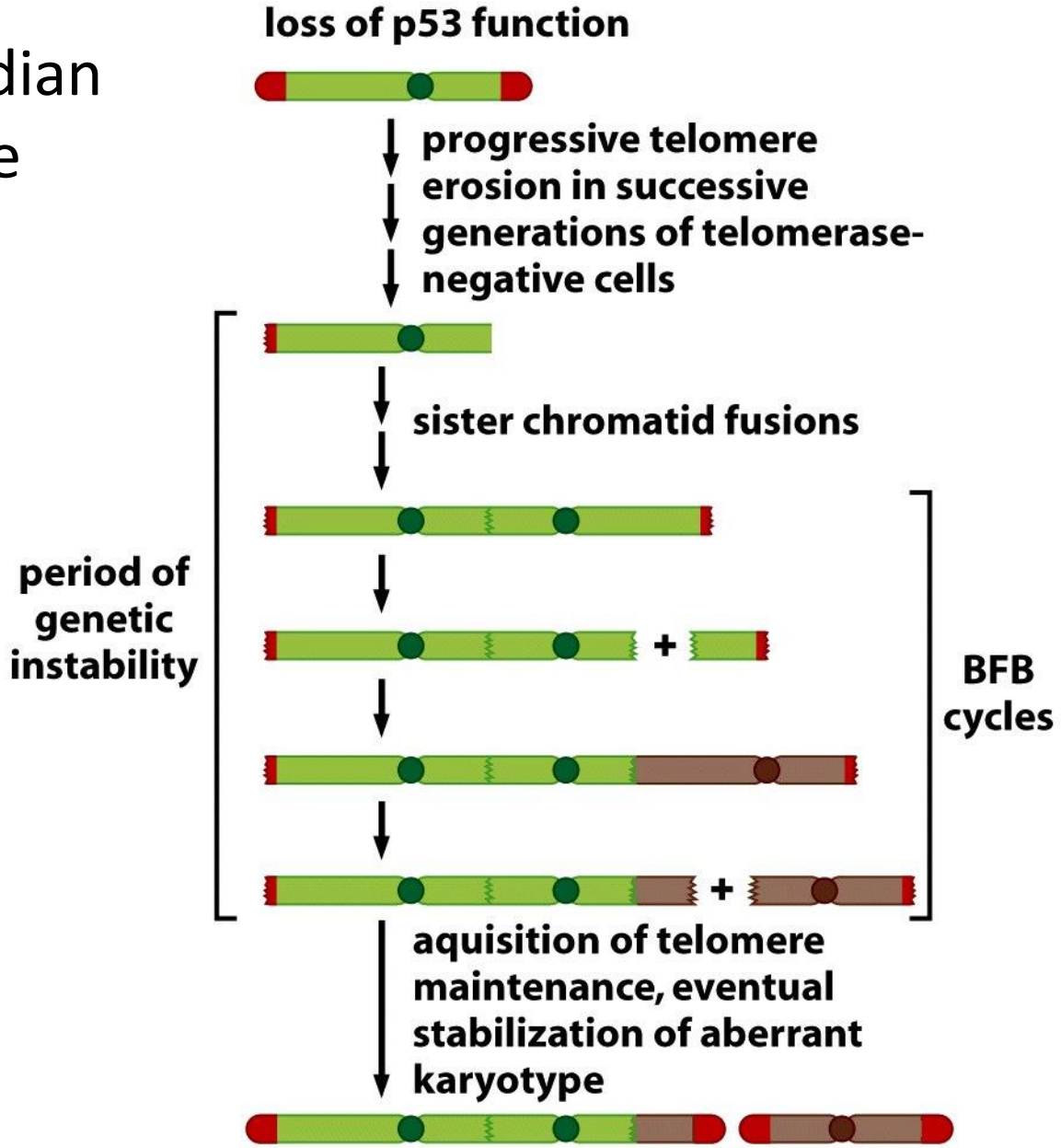
Telomere

Telomere loss

Telomeres protect chromosomal ends…



p53 as a guardian of the genome



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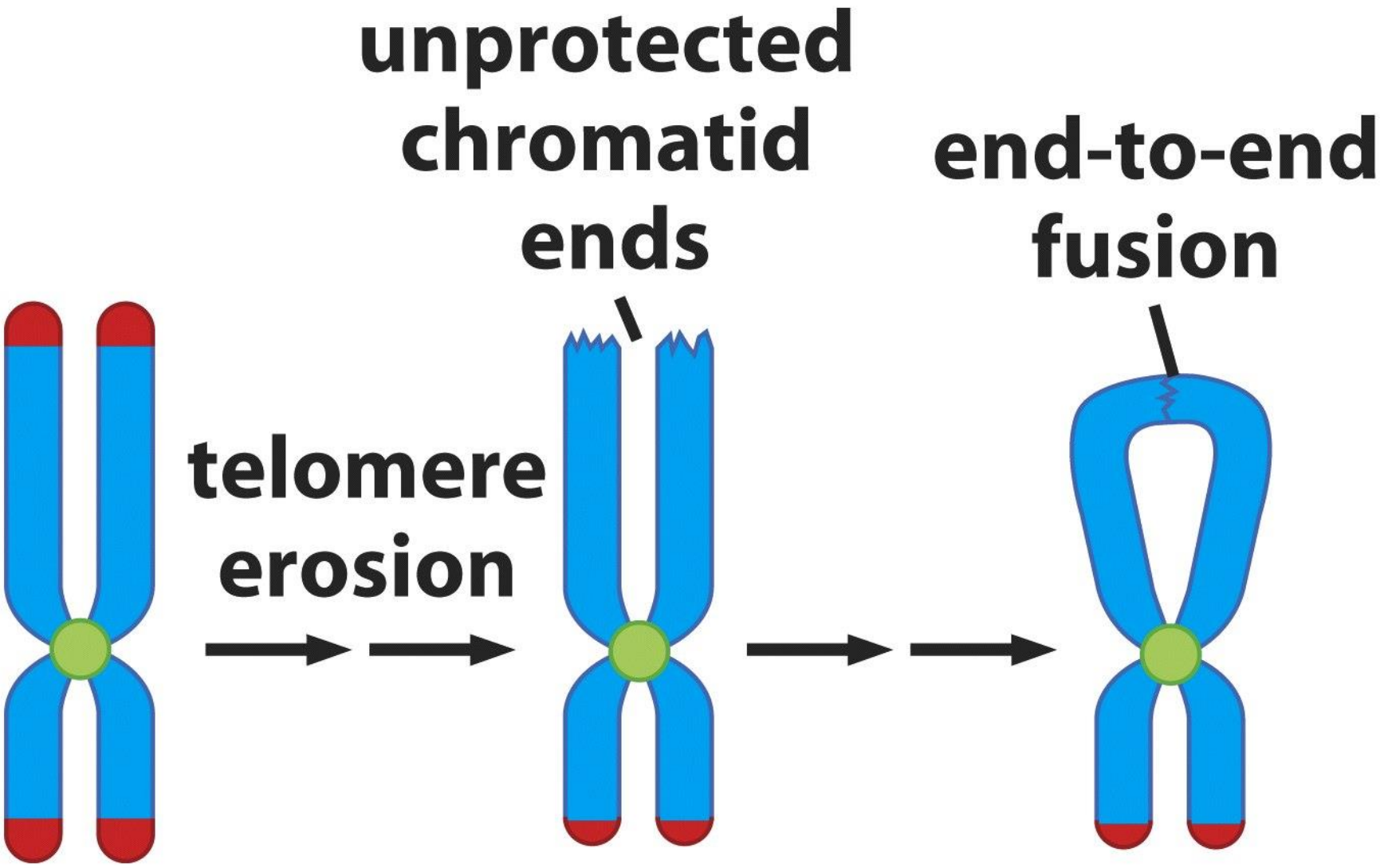
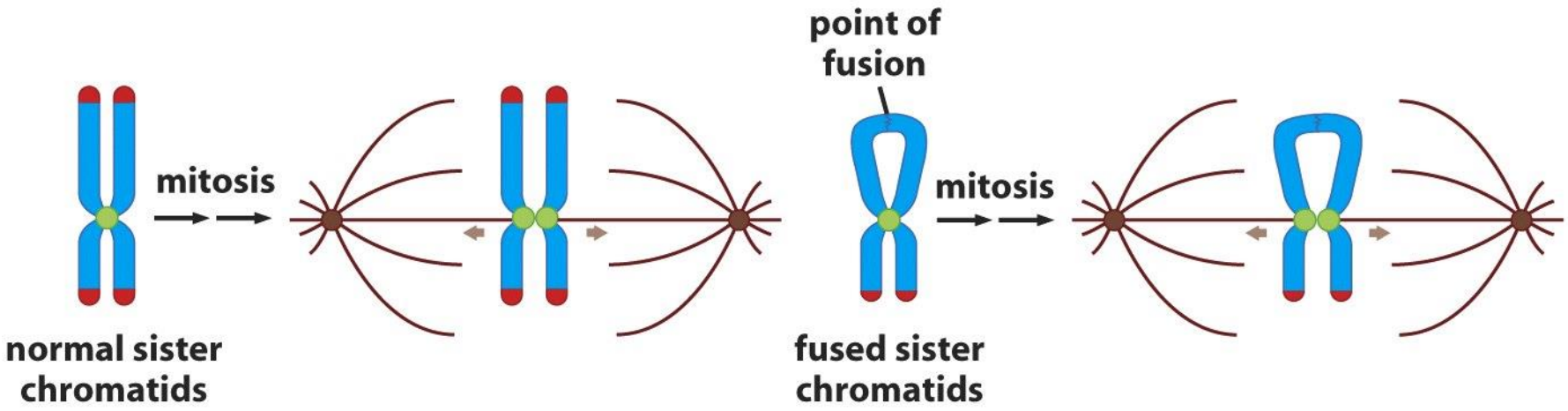


Figure 10.14a *The Biology of Cancer* (© Garland Science 2007)



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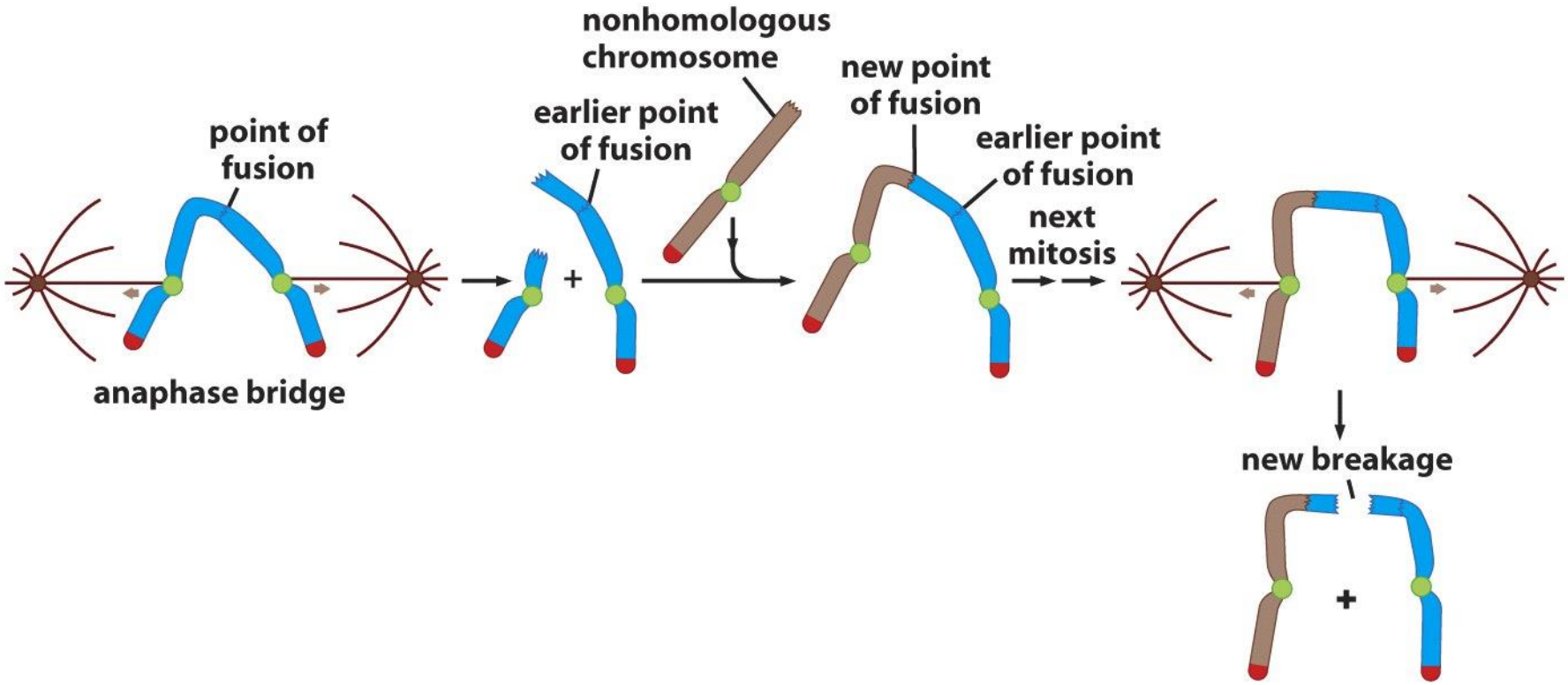

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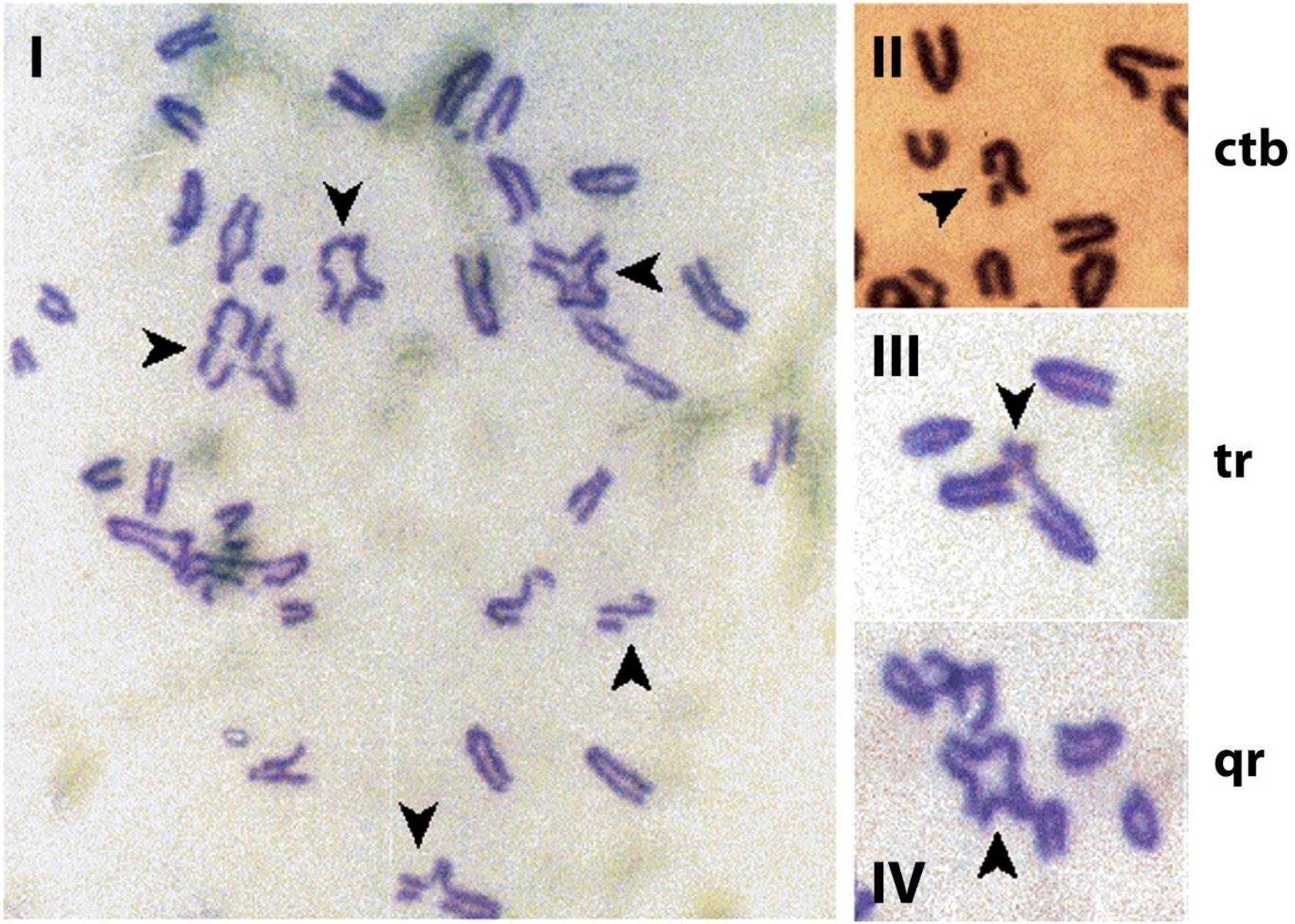

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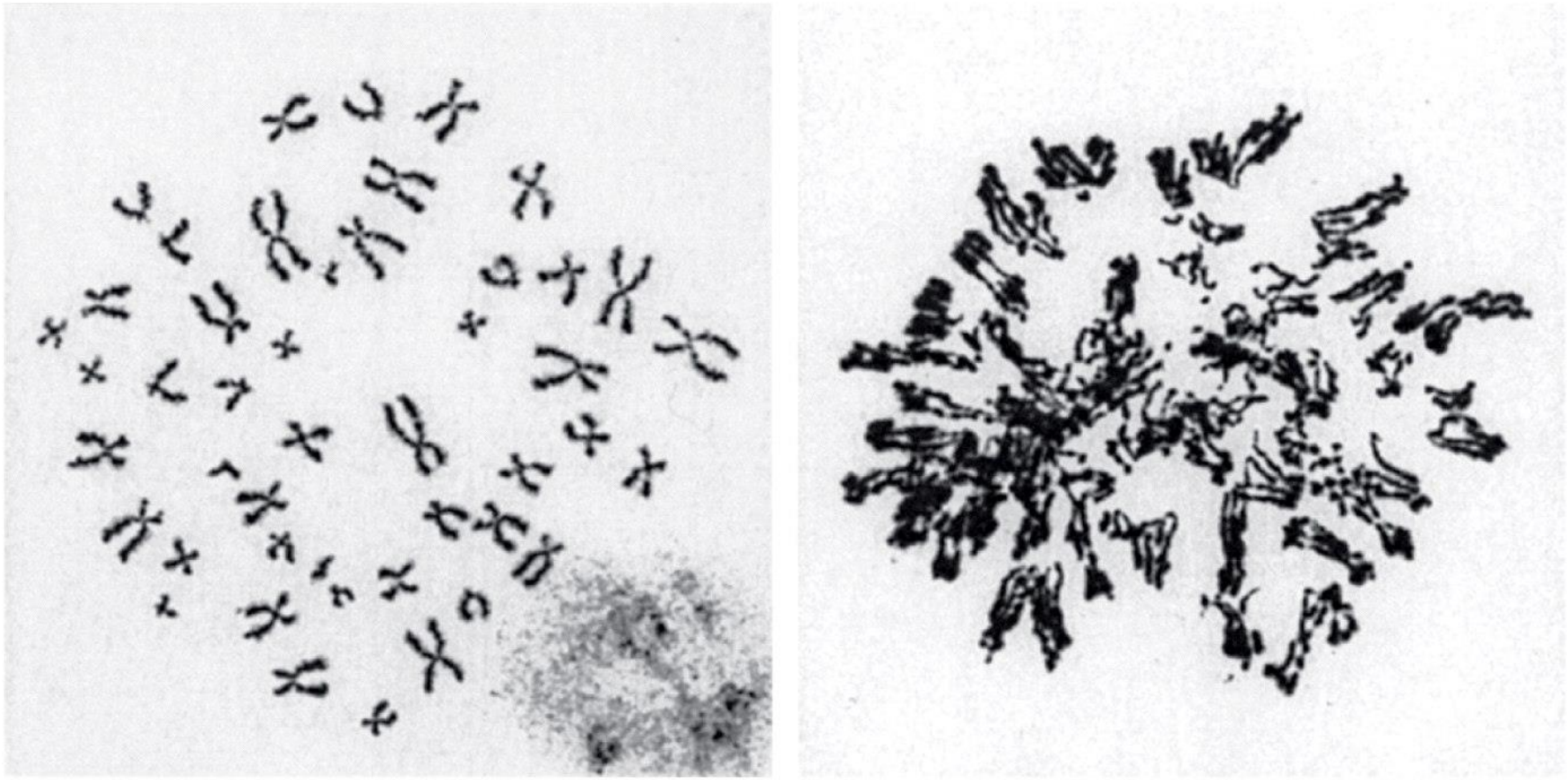


Figure 8.5a *The Biology of Cancer* (© Garland Science 2007)

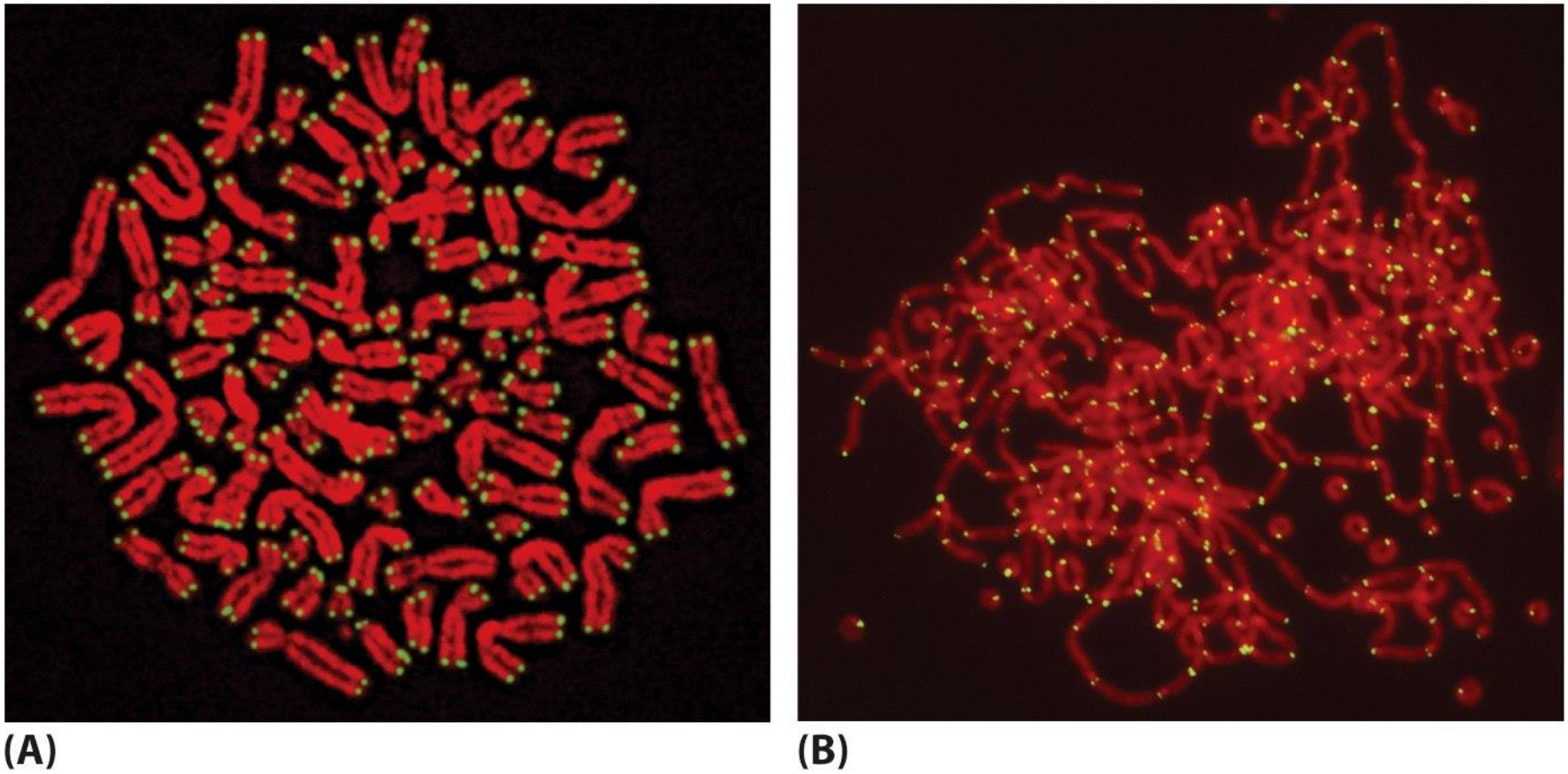
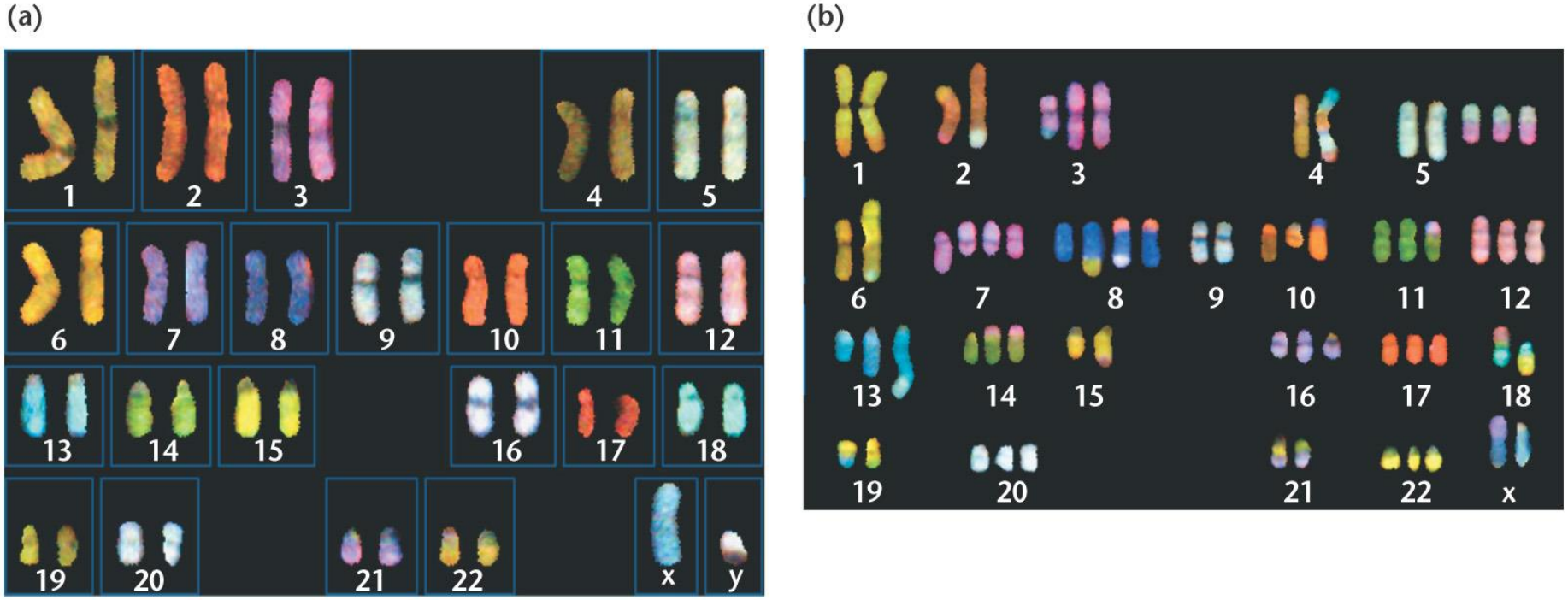


Figure 10.11 *The Biology of Cancer* (© Garland Science 2007)

Normal and Cancer Karyotypes



Normal

Cancer

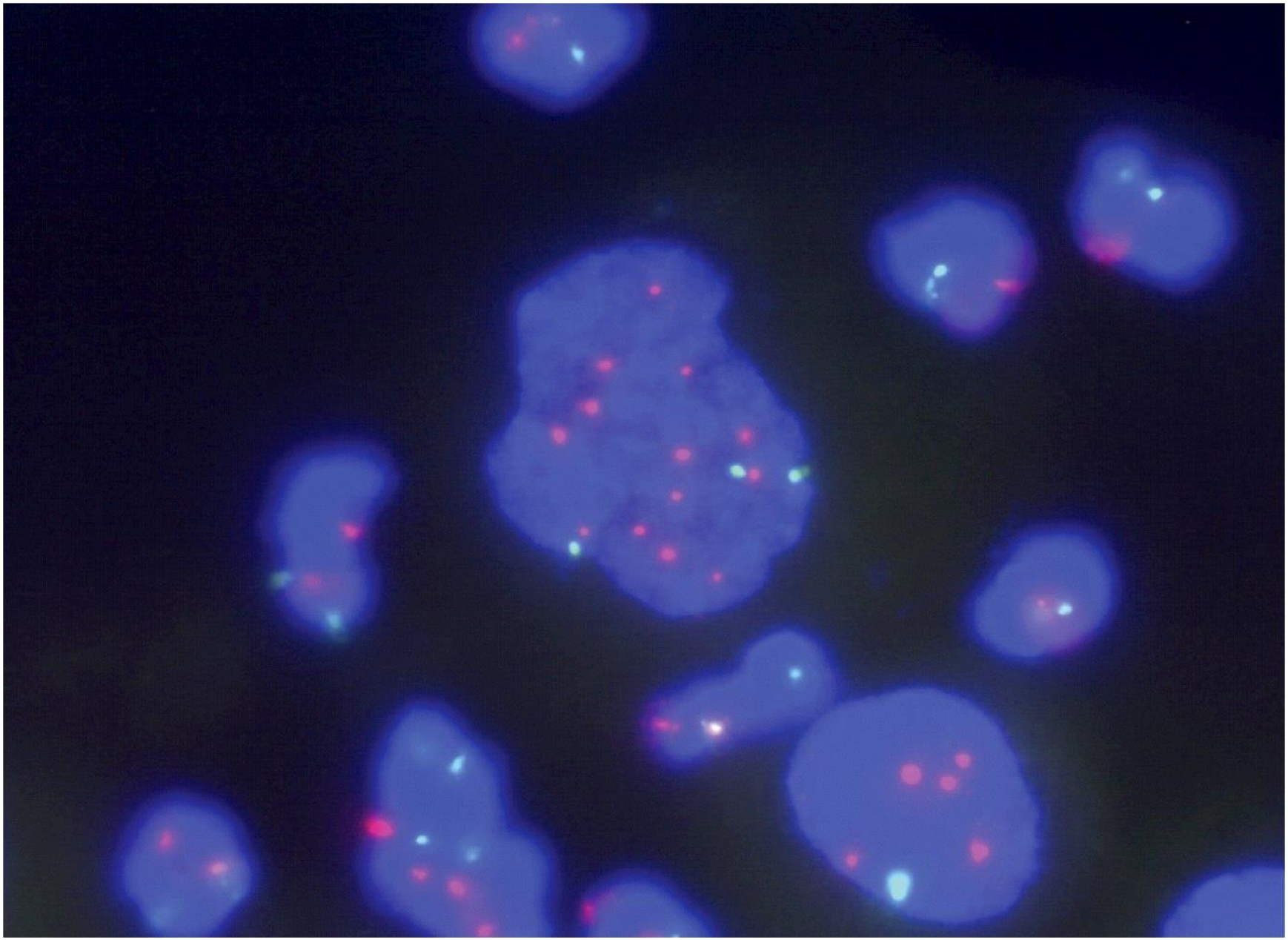


Figure 11.19 *The Biology of Cancer* (© Garland Science 2007)

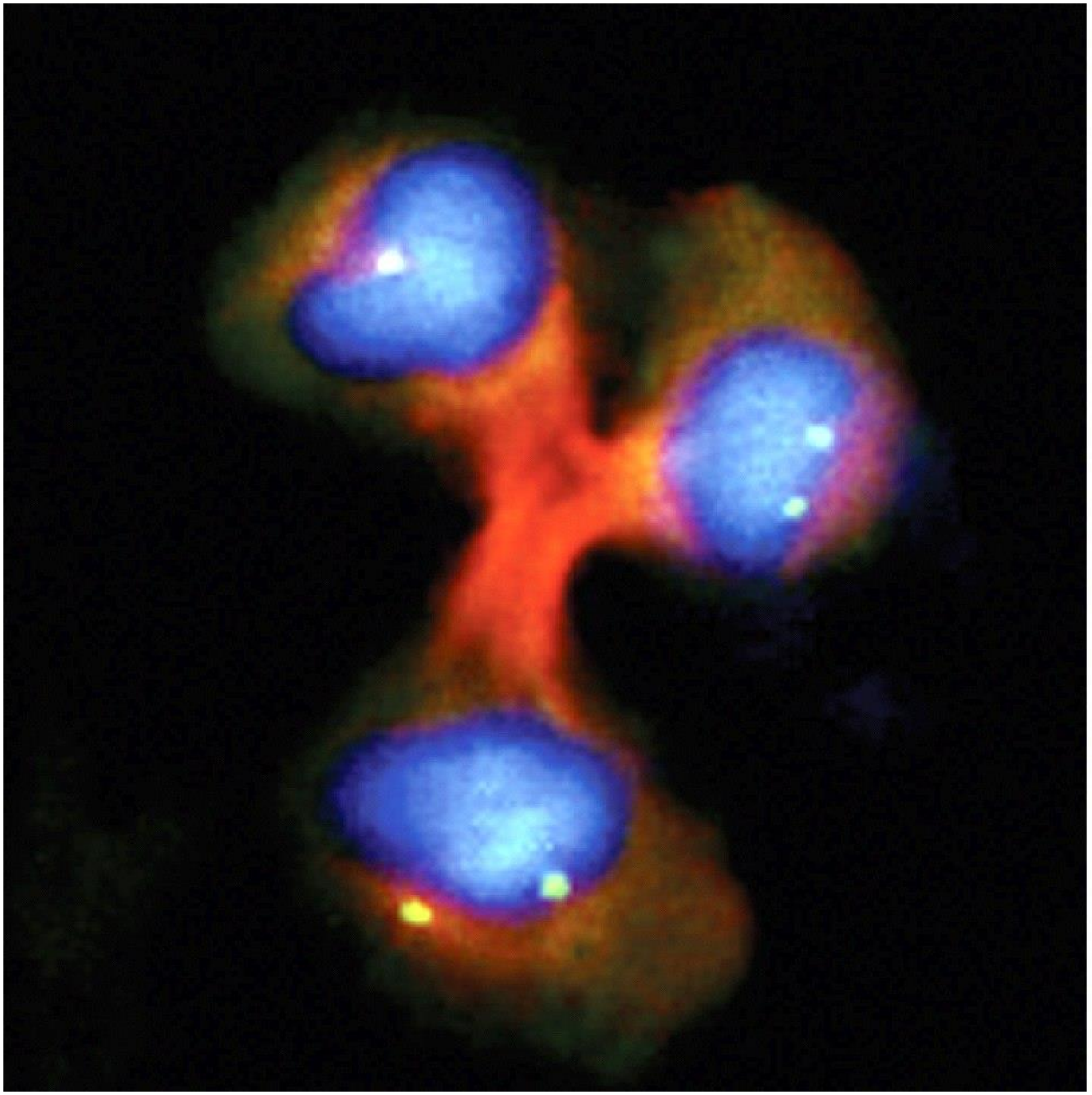
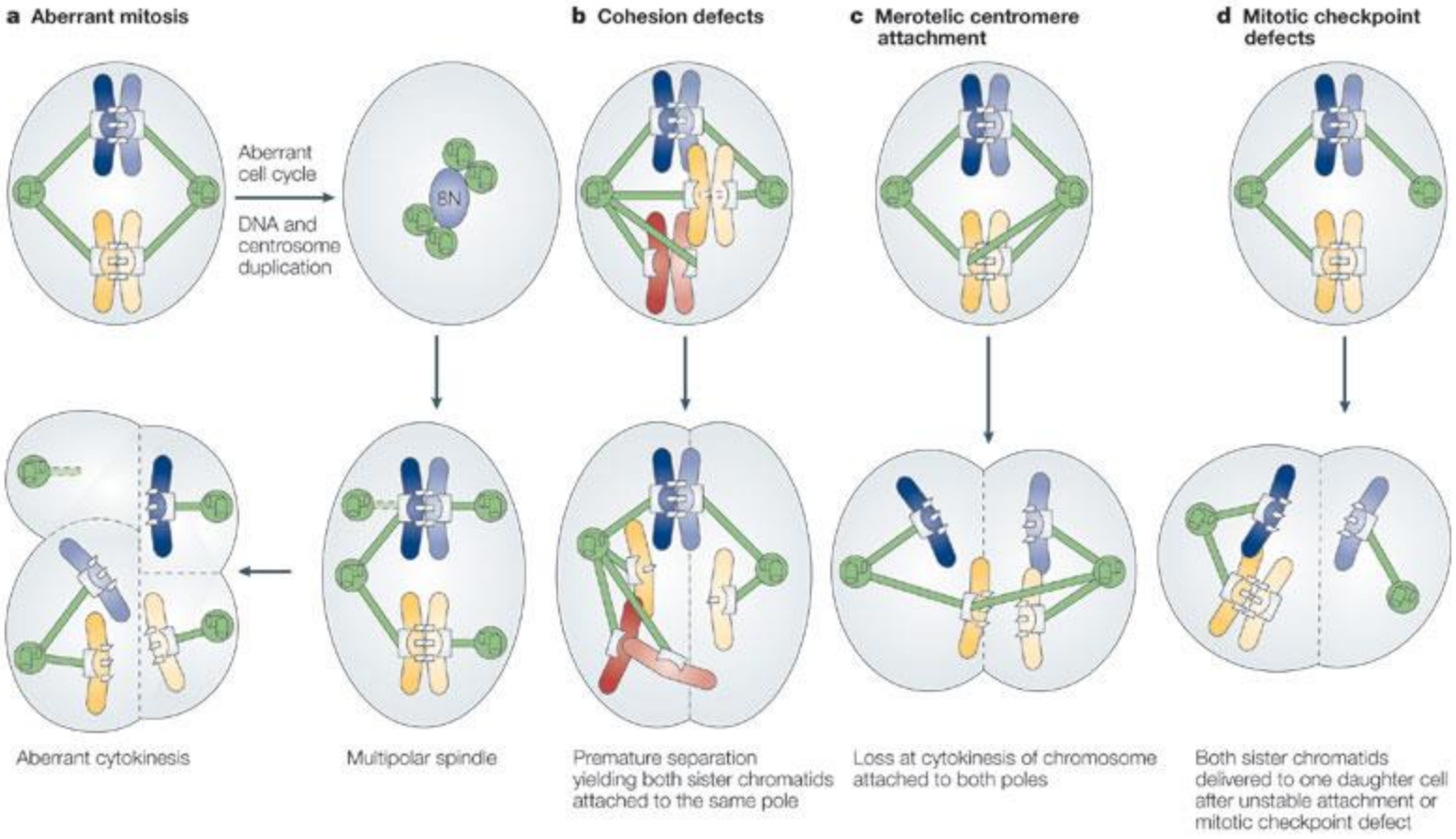


Figure 12.38a *The Biology of Cancer* (© Garland Science 2007)

Aneuploidy and Cancer



Genetic alterations

- Nucleotide sequence alterations
  - Substitution/Insertion/deletion
  - Mutations:
    - silent/missense/nonsense/frameshift
- Gene amplification
  - Oncogenes, metabolism, resistance
  - Defect in DNA damage signaling
- Chromosome translocations
  - Simple (leukemias and lymphomas)
  - Complex (solid tumors)
- Chromosomal aneuploidy
  - Gains or loses
  - Structural aberrations:
    - inversions/deletions/duplications

Chromosomal instability

- Majority of cancers
- Loss of Heterozygosity:
  - Loss of maternal/paternal allele
- Mitotic spindle checkpoint
- DNA-damage checkpoint
- Centrosomes: aneuploidy

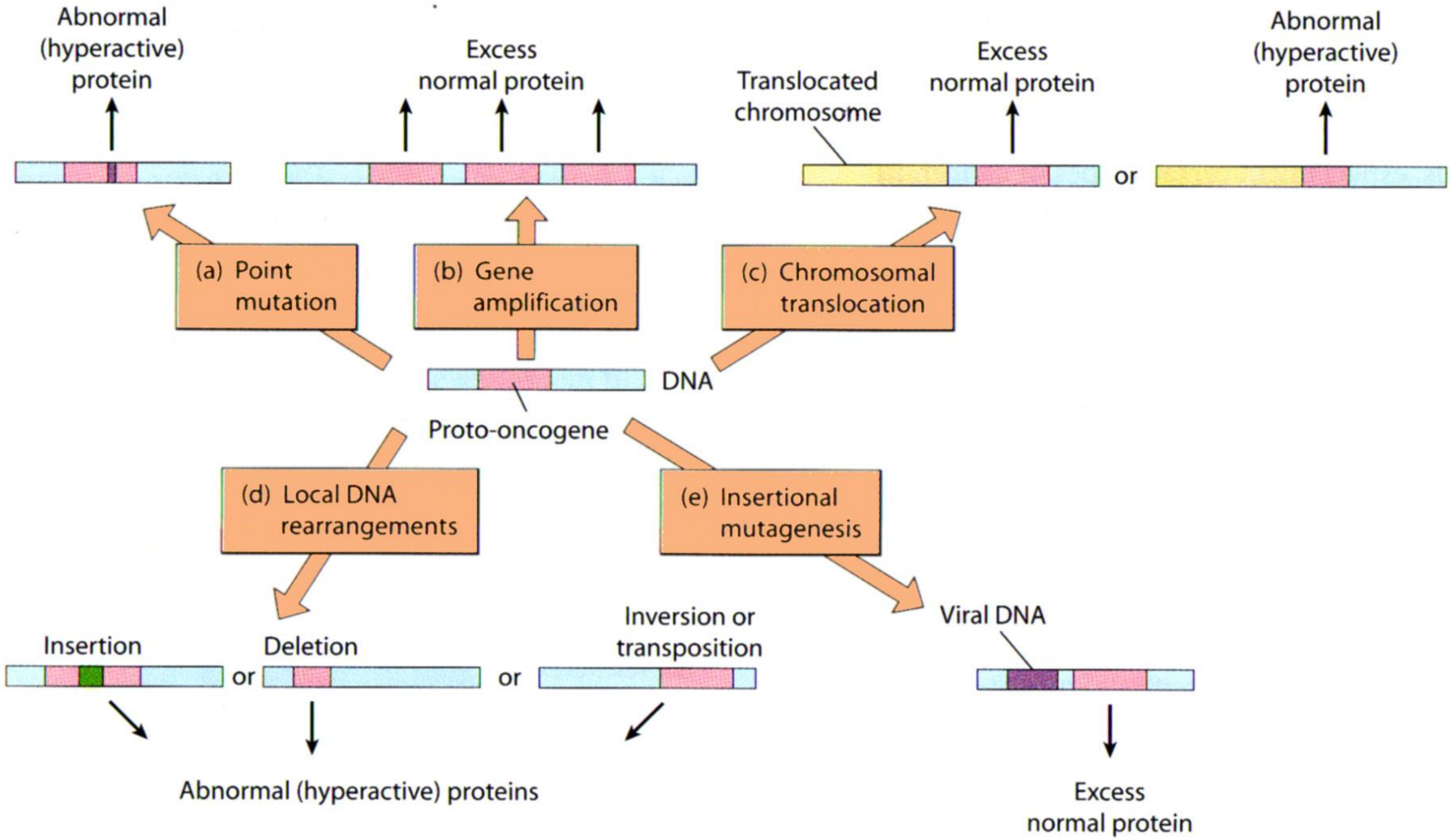
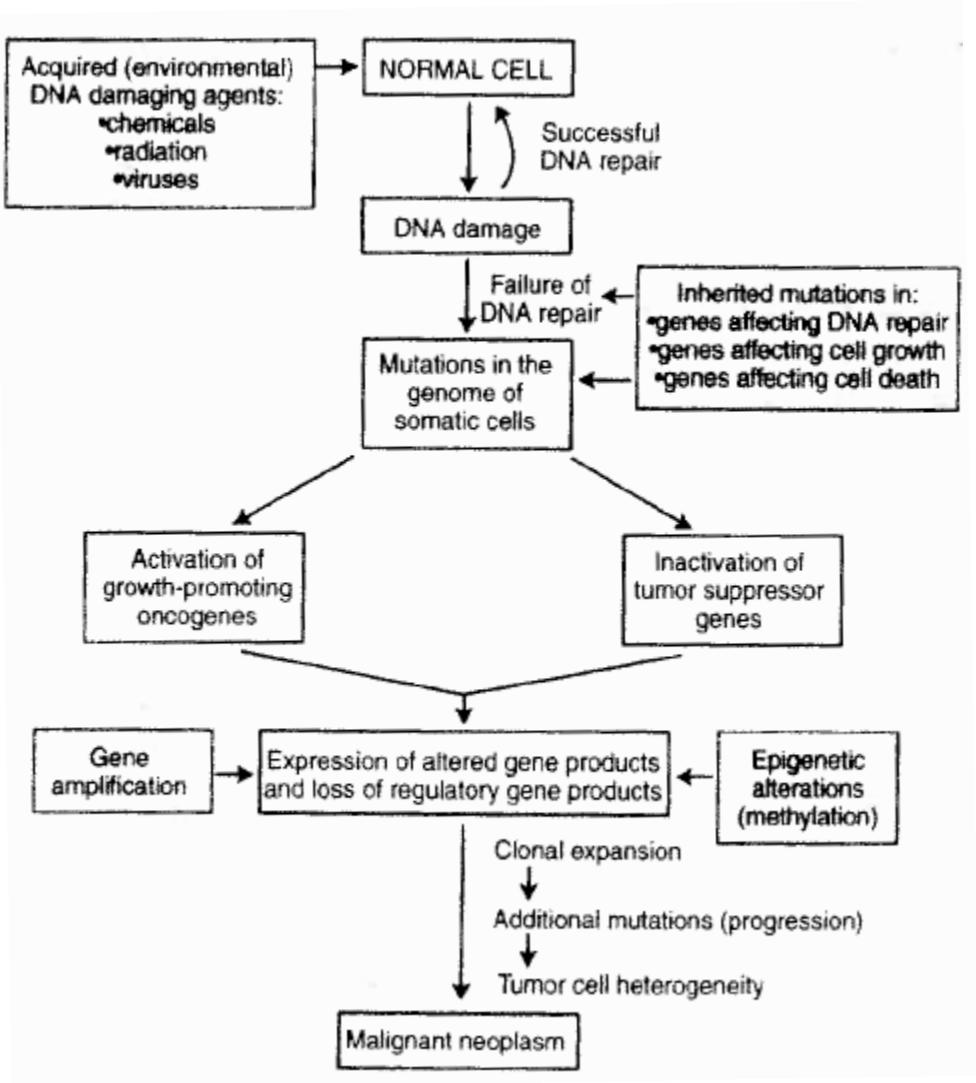


Figure 9-7 Mechanisms for Converting Proto-oncogenes into Oncogenes. In some cases, the structure of a proto-oncogene is altered in a way that causes it to produce an abnormal protein. In other cases, the expression of a proto-oncogene is enhanced, thereby leading to excessive production of a normal protein. (**a**) Point mutation involves a single nucleotide substitution that creates an oncogene coding for an abnormal protein differing in a single amino acid from the normal protein produced by the proto-oncogene. (**b**) Gene amplification involves the production of multiple gene copies that are actively expressed, thereby producing excessive amounts of a normal protein. (**c**) Chromosomal translocations involve the exchange of chromosome segments from one chromosome to another. This exchange may either fuse two genes together to form an oncogene coding for an abnormal protein or it may place a proto-oncogene next to a highly active gene, thereby inducing the translocated proto-oncogene to become more active than normal. (**d**) Local DNA rearrangements such as insertions, deletions, inversions, and transpositions can disrupt the structure of proto-oncogenes and cause them to produce abnormal proteins. (**e**) Insertional mutagenesis occurs when viral DNA is integrated into a host chromosome near a cellular proto-oncogene. The inserted DNA may stimulate the expression of the proto-oncogene and cause it to produce too much protein.

Genetic instability & cancer

Important risk factor: age-related decrease in DNA Repair Capacity



Tumor Suppressors

1. They have anti-oncogenic functions
2. Frequently inactivated in human cancers
3. p53 and the retinoblastoma protein (pRb) are the most important
4. They have anti-proliferative, pro-apoptotic, and pro-DNA repair functions
5. They serve as "guardians" of the genome
6. Inactivated in cancer by "loss-of-function" mutations
7. When inactivated, cells cannot stop their proliferation or repair their DNA or undergo apoptosis

Proto-oncogenes

1. Normal genes that induce cell proliferation in a controlled manner in proliferative cells.

2. Ras is one of the best characterized proto-oncogenes

3. Active in normal proliferative cells

4. They have "shut down" mechanisms that stops proliferation once the cell is ready to differentiate.

5. They are frequently mutated in cancer by "gain-of-function" mutations. When activated by "gain-of-function" they become oncogenes.

6. Oncogenes loose their "shut down" mechanisms, so they are always "on" and therefore induce continuous proliferation, even in the absence of pro-mitogenic stimuli.

TABLE 18.2 SOME PROTO-ONCOGENES AND TUMOR SUPPRESSOR GENES

Proto-oncogene	Normal Function	Alteration in Cancer	Associated Cancers
Ha-ras	Signal transduction molecule, binds GTP/GDP	Point mutations	Colorectal, bladder, many types
c-erbB	Transmembrane growth factor receptor	Gene amplification, point mutations	Glioblastomas, breast cancer, cervix
c-myc	Transcription factor, regulates cell cycle, differentiation, apoptosis	Translocation, amplification, point mutations	Lymphomas, leukemias, lung cancer, many types
c-fos	Transcription factor, responds to growth factors	Overexpression	Osteosarcomas, many types
c-kit	Tyrosine kinase, signal transduction	Mutation	Sarcomas
c-raf	Cytoplasmic serine-threonine kinase, signal transduction	Gene rearrangements	Stomach cancer
RARα	Hormone-dependent transcription factor, differentiation	Chromosomal translocations with PML gene, fusion product	Acute promyelocytic leukemia
E6	Human papillomavirus encoded oncogene, inactivates p53	HPV infection	Cervical cancer
MDM2	Binds and inactivates p53, abrogates cell cycle checkpoints	Gene amplification, over-expression	Osteosarcomas, liposarcomas
Cyclins	Bind to CDKs, regulate cell cycle	Gene amplification, over-expression	Lung, esophagus, many types
CDK2, 4	Cyclin-dependent kinases, regulate cell cycle phases	Overexpression, mutation	Bladder, breast, many types
Tumor Suppressor	**Normal Function**	**Alteration in Cancer**	**Associated Cancers**
p53	Cell cycle checkpoints, apoptosis	Mutation, inactivation by viral oncogene products	Brain, lung, colorectal, breast, many types
RB1	Cell cycle checkpoints, binds E2F	Mutation, deletion, inactivation by viral oncogene products	Retinoblastoma, osteosarcoma, many types
APC	Cell–cell interaction	Mutation	Colorectal cancers, brain, thyroid
Bcl2	Apoptosis regulation	Overexpression blocks apoptosis	Lymphomas, leukemias
XPA–XPG	Nucleotide excision repair	Mutation	Xeroderma pigmentosum, skin cancers
BRCA2	DNA repair	Point mutations	Breast, ovarian, prostate cancers

Gain-of-function mutations in cancer

Proto-Oncogenes (regulated activity)

cancer

Oncogenes (Unregulated activity)

Stimulate Proliferation
Inhibit Differentiation
Inhibit Apoptosis

Consider epigenetics as well!!

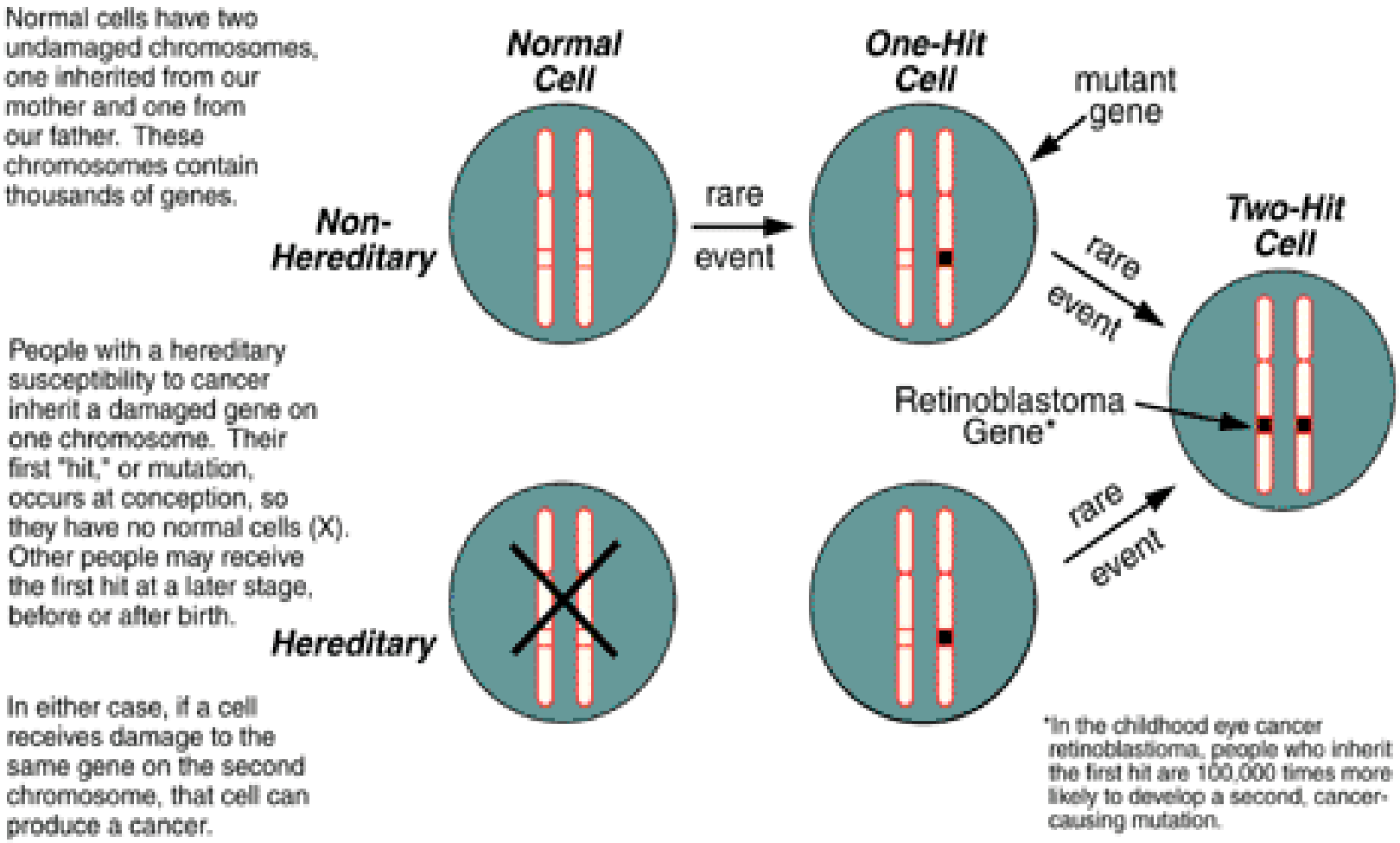
Tumor Suppressor Genes

Inhibit Proliferation
Promote Differentiation
Stimulate Apoptosis

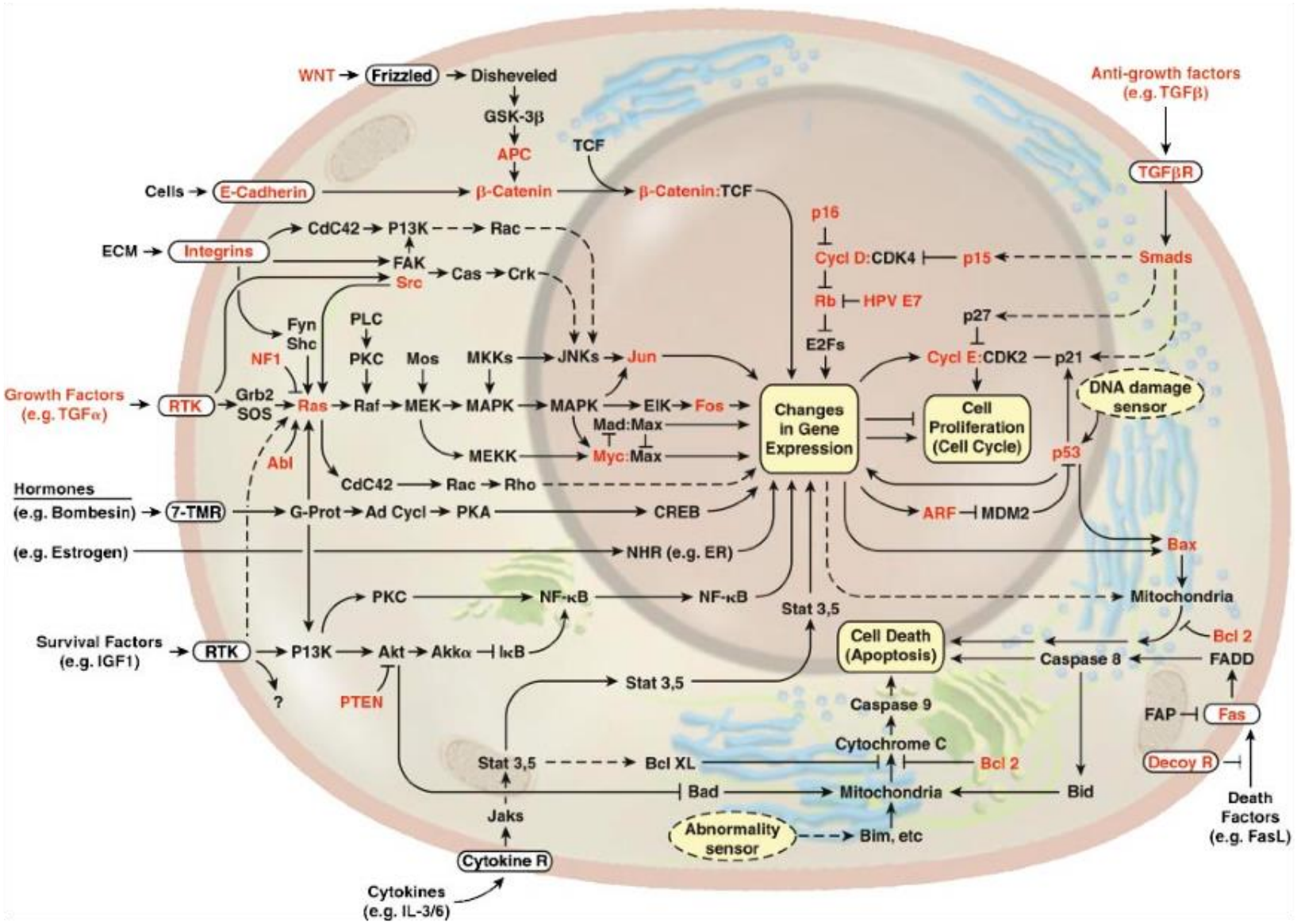
Loss-of-function mutations in cancer

Knudson's Two-Hit Hypothesis

www.fccc.edu



The Molecular Circuitry of the Cell



From Hanahan and Weinberg, 2000

Ras-Raf-MEK-MAPK signaling pathway is central for the processing of pro-mitogenic extracellular signals…

Components of this pathway are **proto-oncogenes**. The protein products of proto-oncogenes induce normal or regulated proliferation. In normal cells they can be turned off to stop proliferation…

In ~25% of human cancers, components of this pathway show dominant "**gain of function" mutation**s. These mutations turn them into oncogenes, which cannot be turned off (they are constitutively activated).

Once they become oncogenes, they trigger a constant mitogenic signal inside the cell without stimulation of their upstream components (Medema and Bos, 1993).

~ 50% of human colon carcinomas have mutant ras oncogenes (Kinzler and Vogelstein, 1996). The remaining 50% have defects in other components of the growth signaling pathways that **phenocopy** ras oncogene activation…

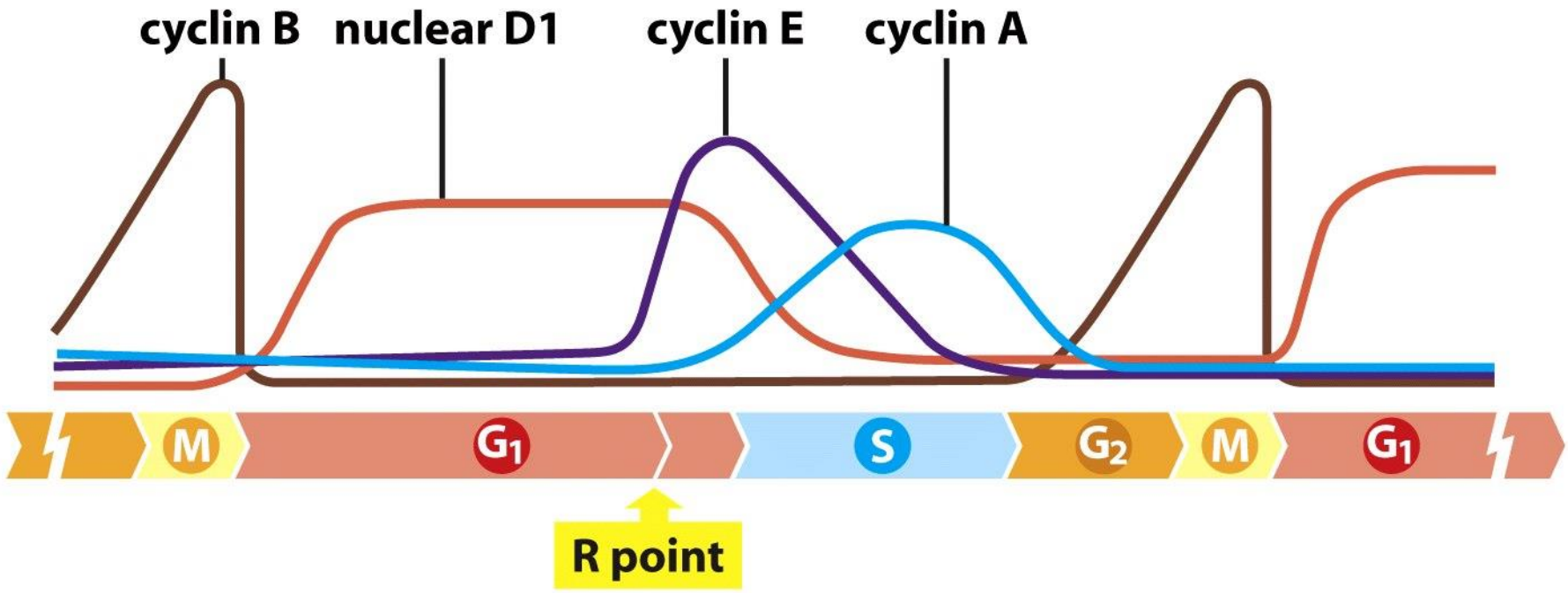


Figure 8.10 *The Biology of Cancer* (© Garland Science 2007)

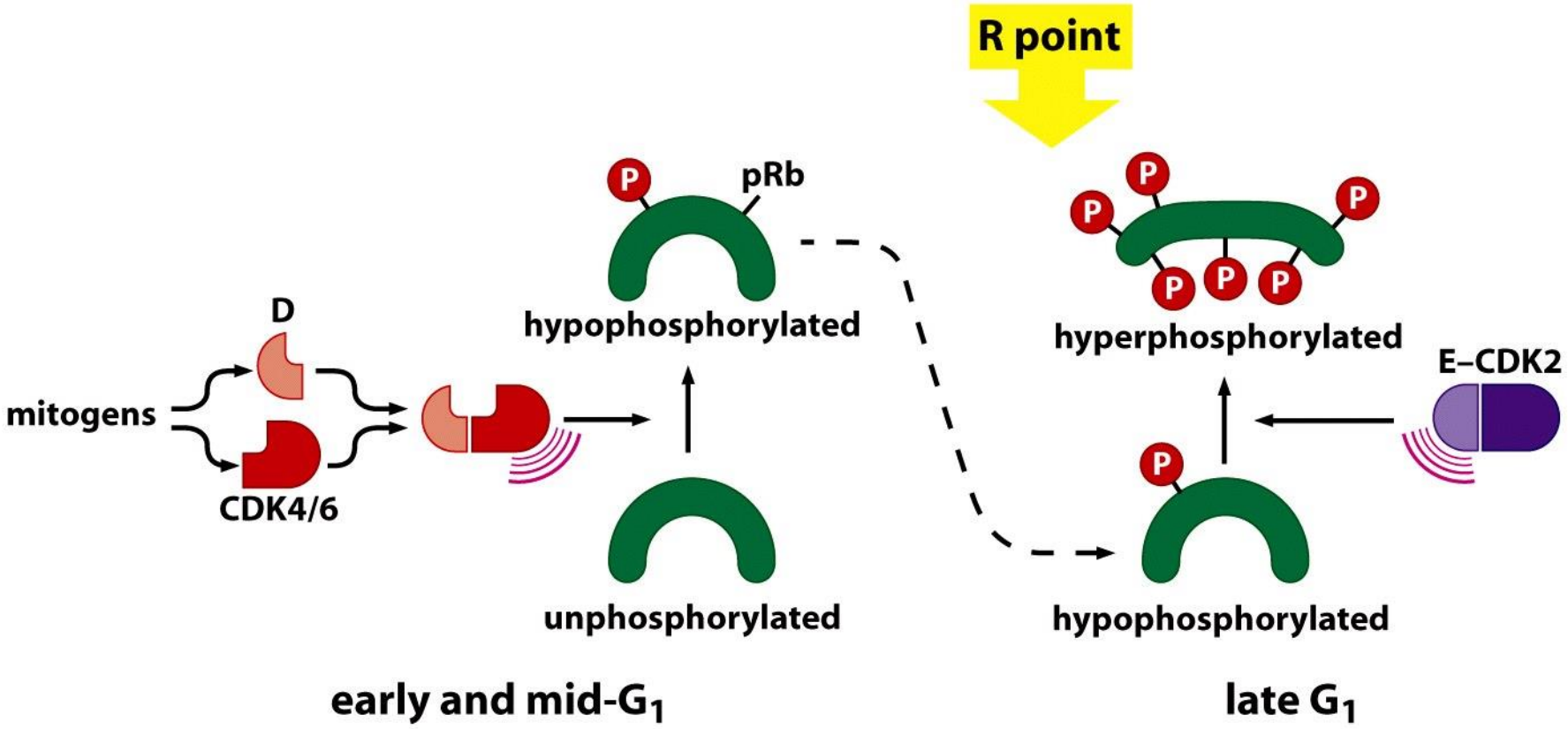


Figure 8.22 *The Biology of Cancer* (© Garland Science 2007)

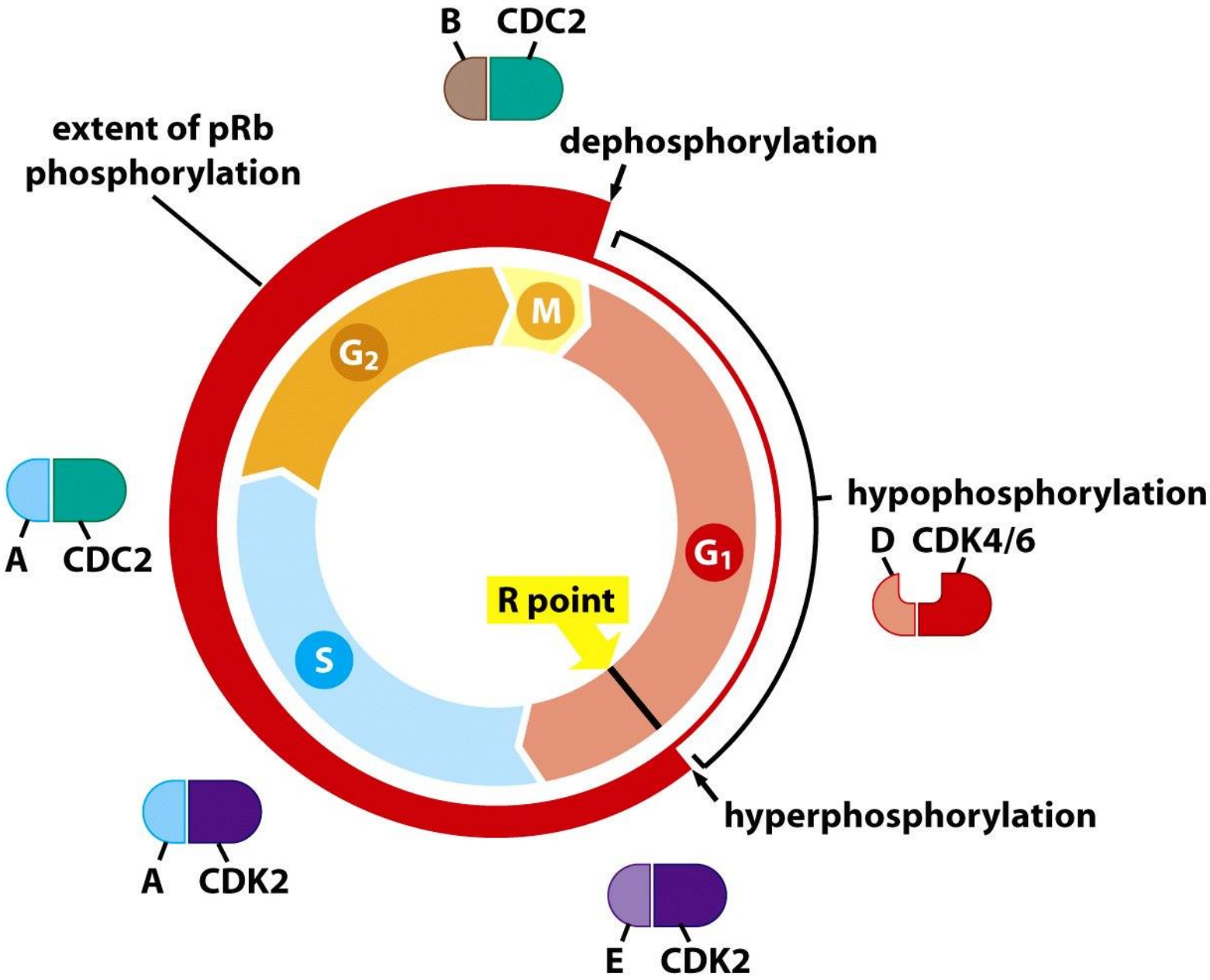


Figure 8.19 *The Biology of Cancer* (© Garland Science 2007)

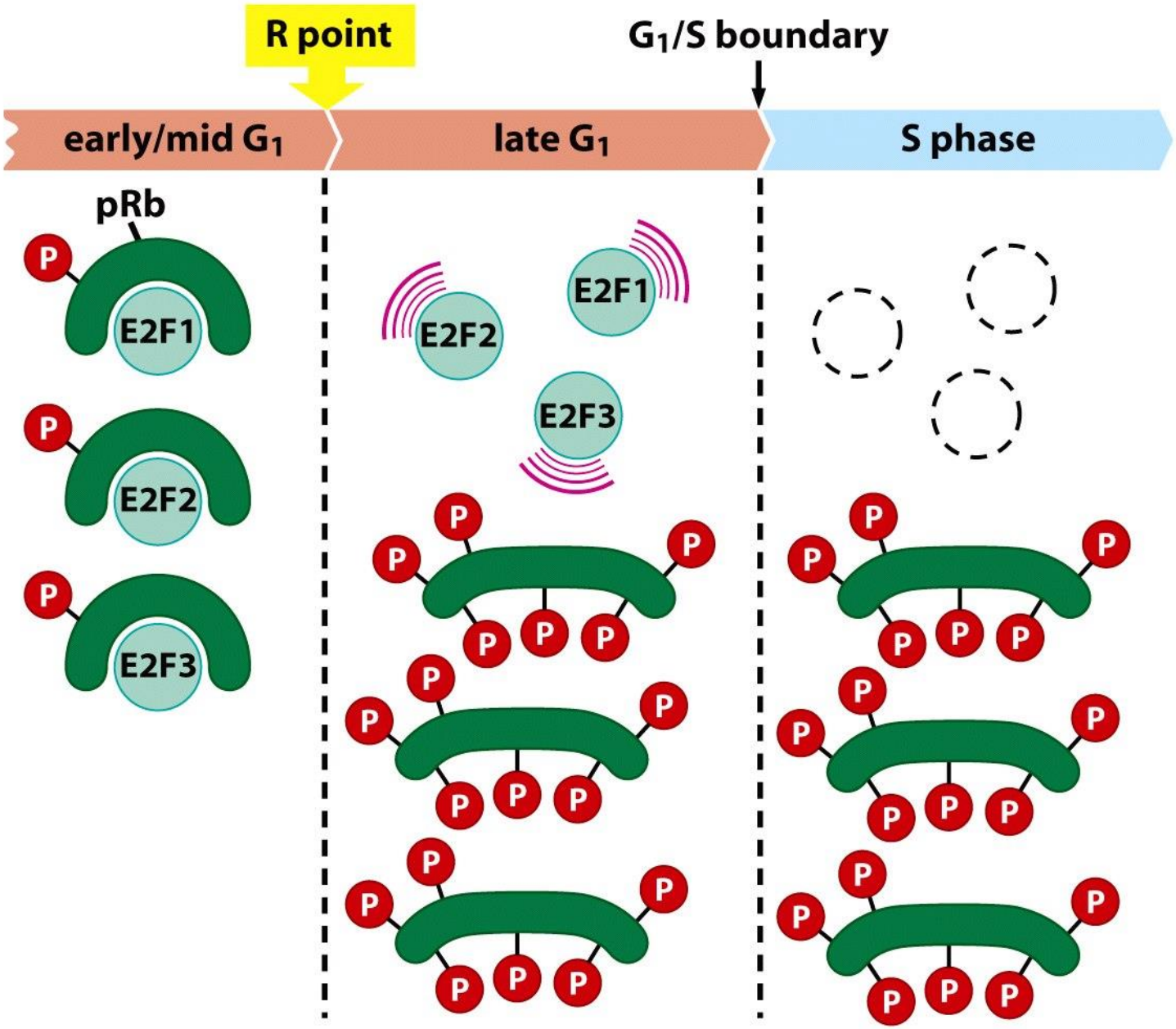


Figure 8.23a *The Biology of Cancer* (© Garland Science 2007)

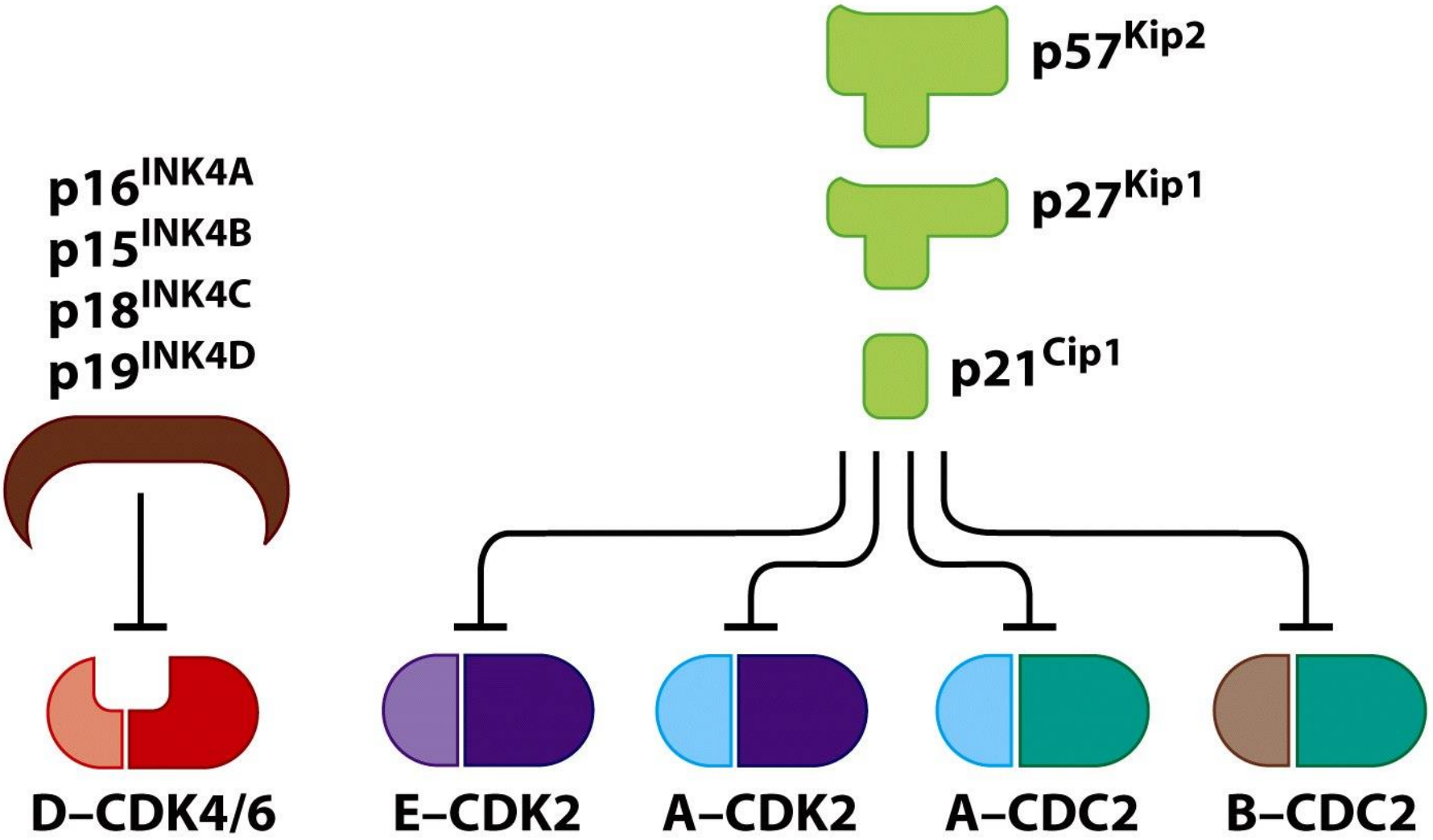
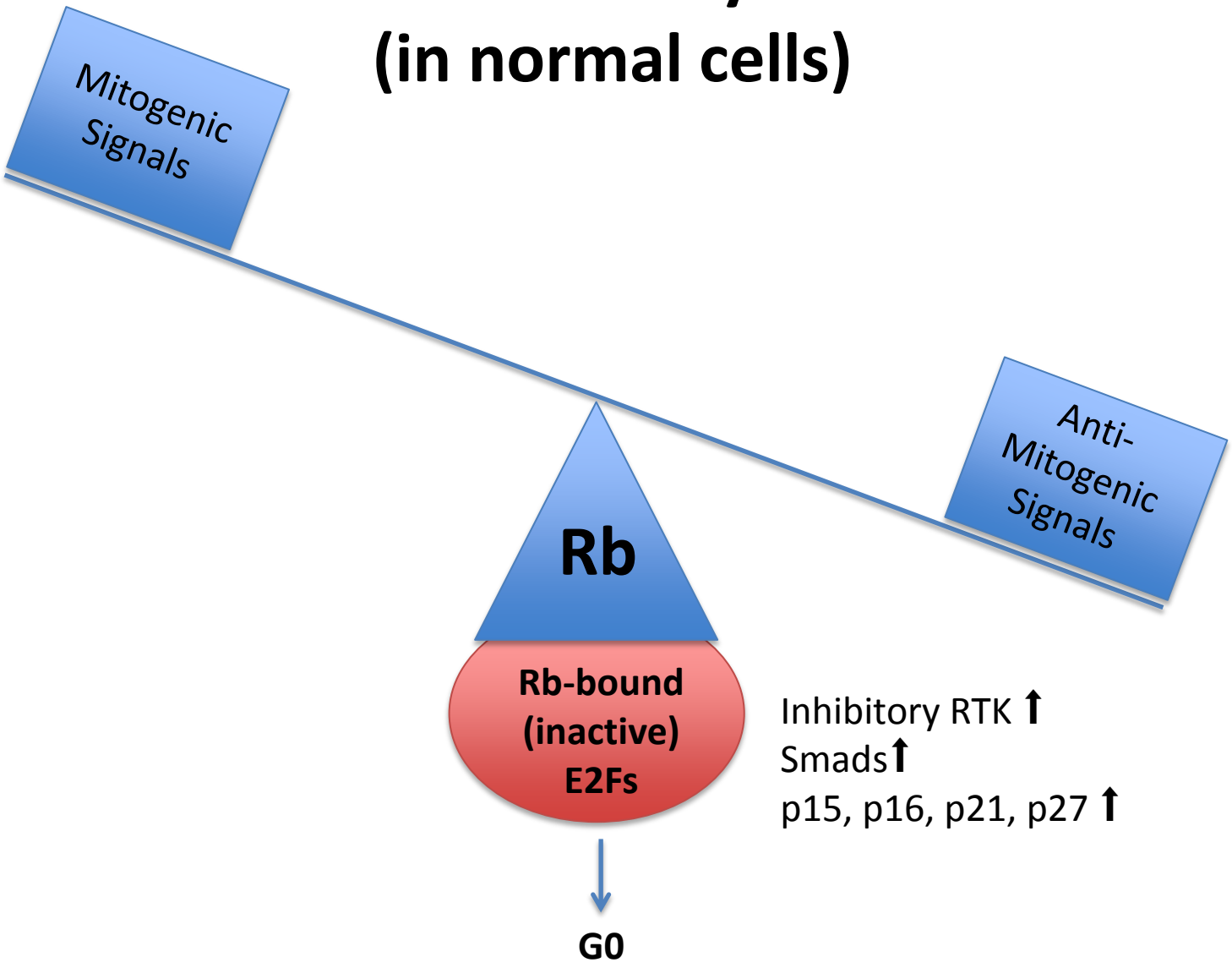
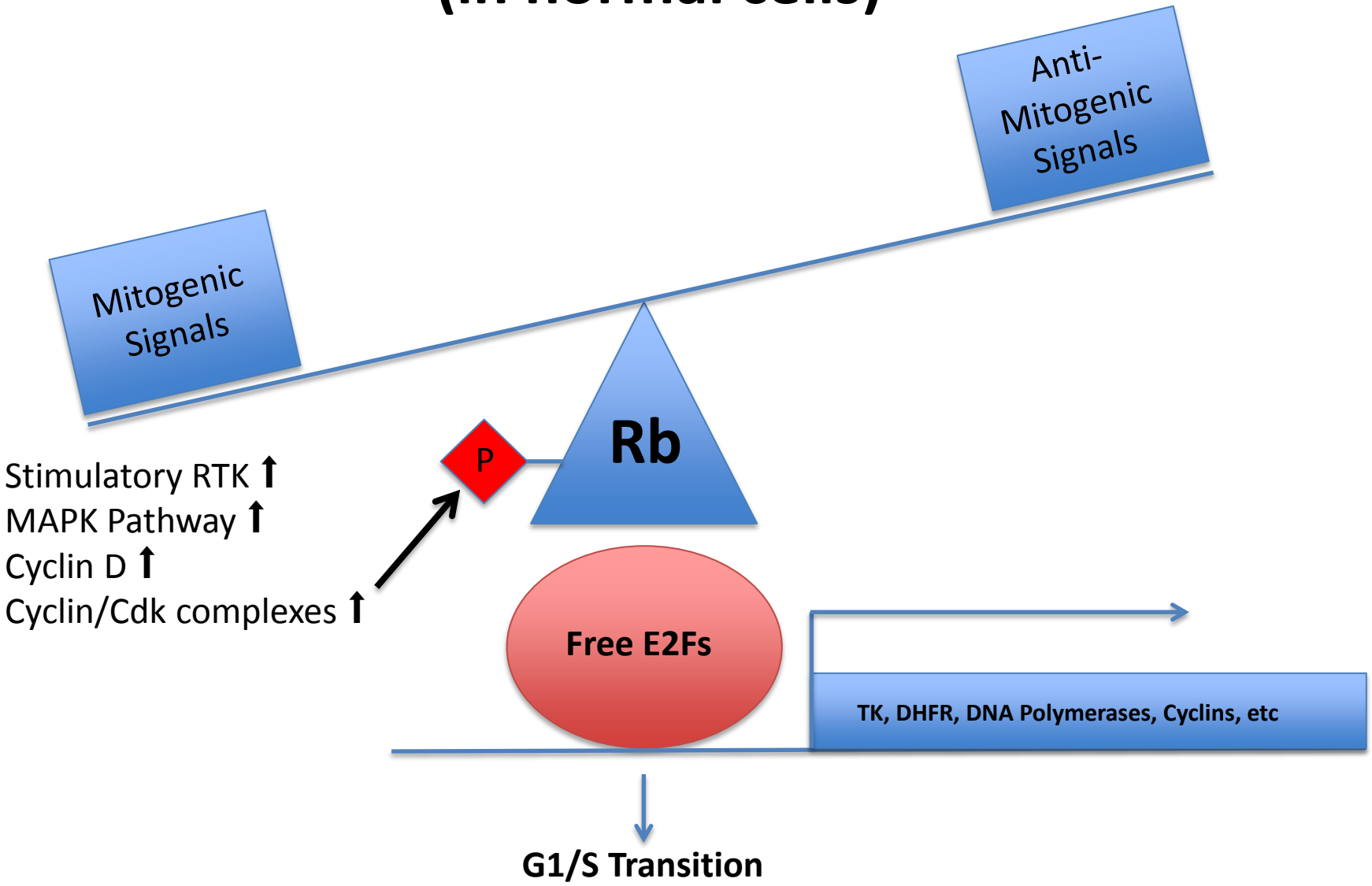


Figure 8.13a *The Biology of Cancer* (© Garland Science 2007)

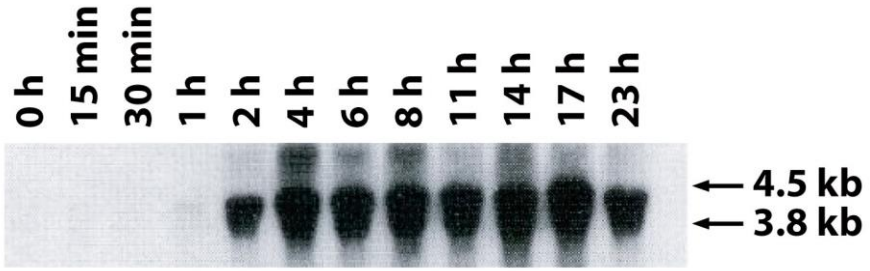
The Basics of Cell Cycle Control (in normal cells)



The Basics of Cell Cycle Control (in normal cells)



Cyclin D1 expression triggers cell proliferation



C

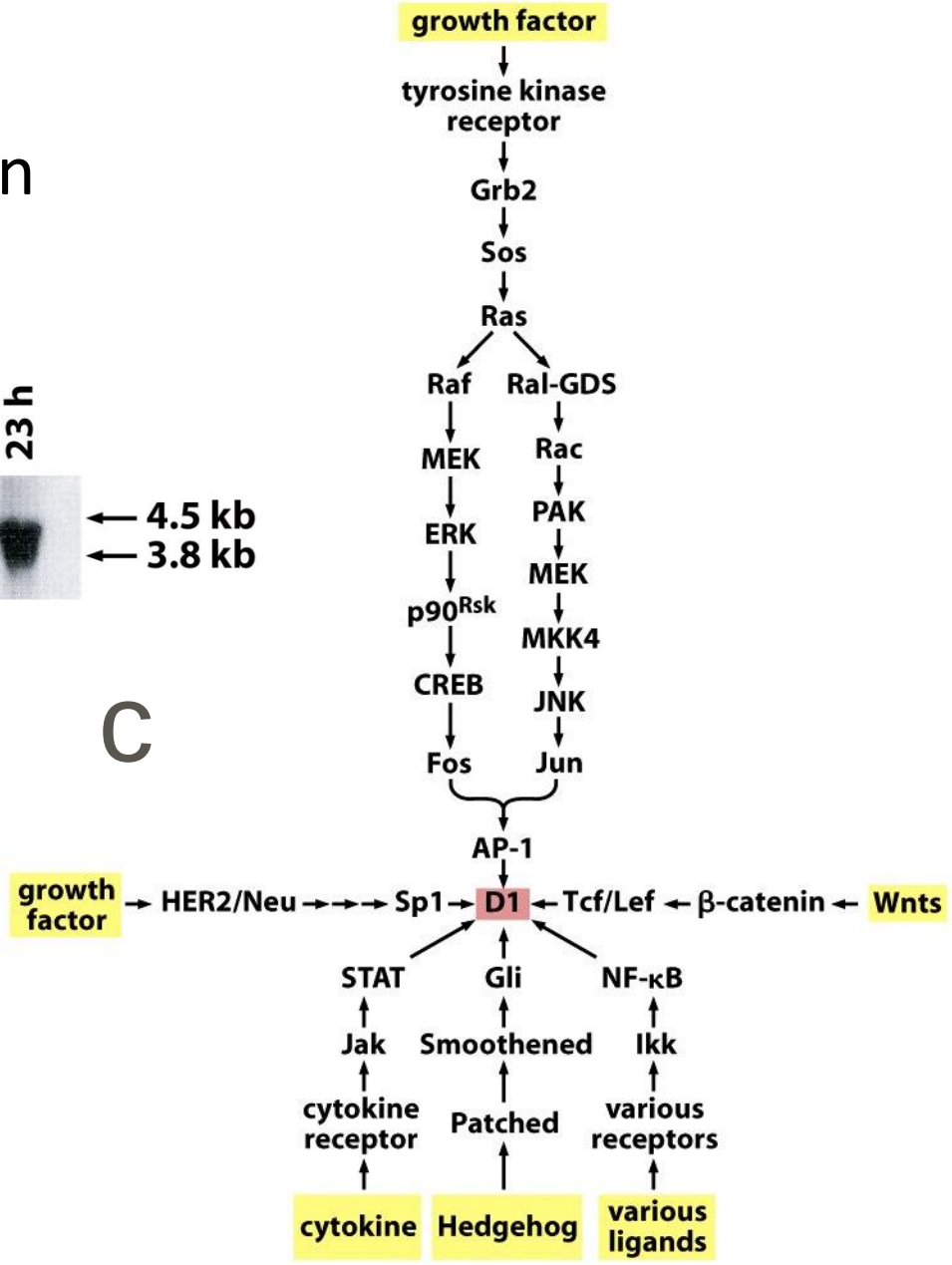


Figure 8.11b *The Biology of Cancer* (© Garland Science 2007)

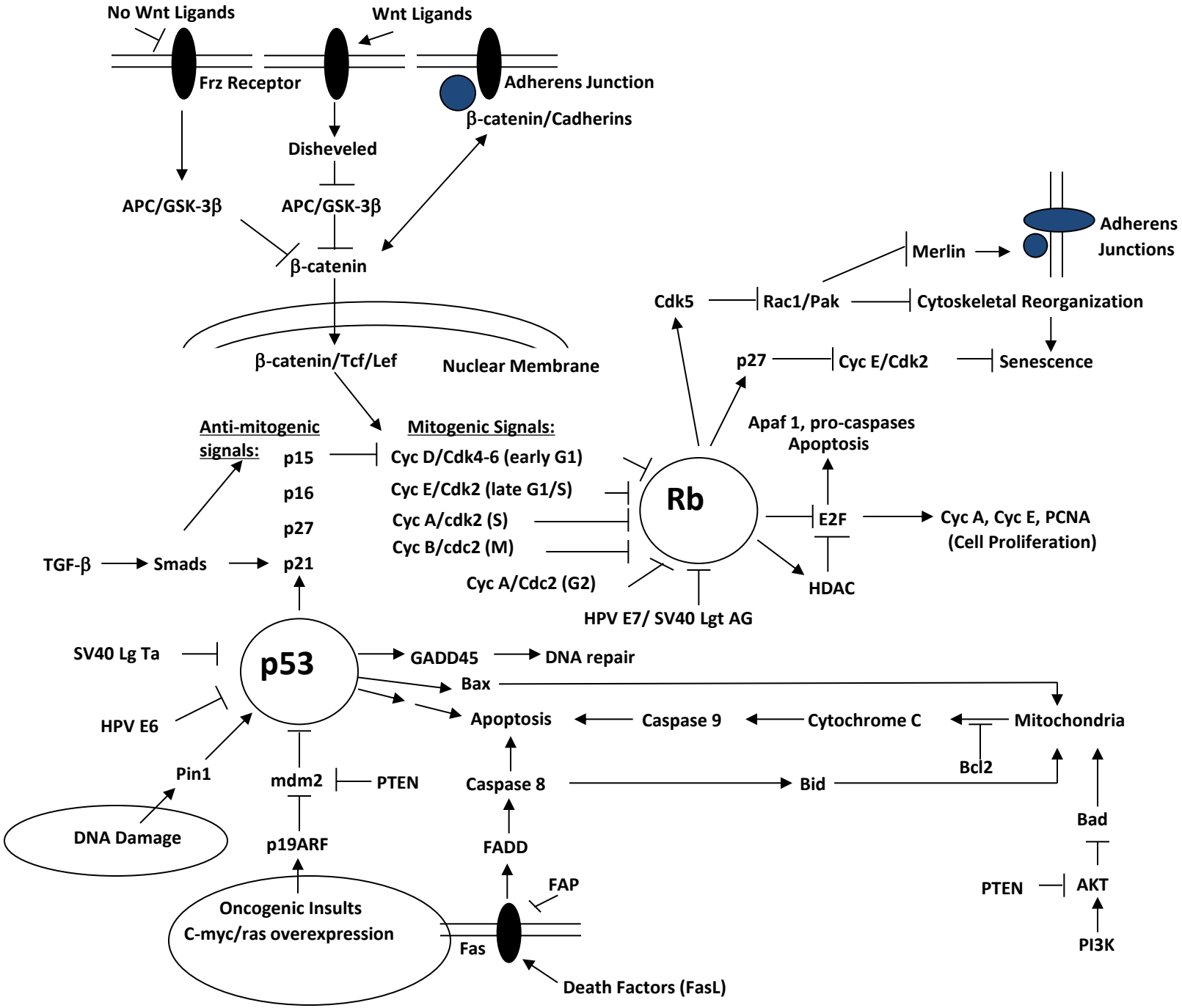

No Wnt Ligands
Wnt Ligands
Frz Receptor
Adherens Junction
β-catenin/Cadherins
Disheveled
APC/GSK-3β
APC/GSK-3β
β-catenin
β-catenin/Tcf/Lef
Nuclear Membrane
Merlin
Adherens Junctions
Cdk5
Rac1/Pak
Cytoskeletal Reorganization
p27
Cyc E/Cdk2
Senescence
Anti-mitogenic signals:
Mitogenic Signals:
p15
p16
p27
p21
Cyc D/Cdk4-6 (early G1)
Cyc E/Cdk2 (late G1/S)
Cyc A/cdk2 (S)
Cyc B/cdc2 (M)
Cyc A/Cdc2 (G2)
Rb
Apaf 1, pro-caspases
Apoptosis
E2F
HDAC
Cyc A, Cyc E, PCNA
(Cell Proliferation)
HPV E7/ SV40 Lgt AG
TGF-β
Smads
SV40 Lg Ta
p53
GADD45
DNA repair
Bax
HPV E6
Apoptosis
Caspase 9
Cytochrome C
Mitochondria
Bcl2
Pin1
mdm2
PTEN
Caspase 8
Bid
Bad
DNA Damage
p19ARF
FADD
FAP
PTEN
AKT
PI3K
Oncogenic Insults
C-myc/ras overexpression
Fas
Death Factors (FasL)

Table 4.4 Translocations in human tumors that deregulate proto-oncogene expression and thereby create oncogenes

Oncogene	Neoplasm
myc	Burkitt's lymphoma; other B- and T-cell malignancies
bcl-2	follicular B-cell lymphomas
bcl-3	chronic B-cell lymphomas
bcl-6	diffuse B-cell lymphomas
hox1	acute T-cell leukemia
lyl	acute T-cell leukemia
rhom-1	acute T-cell leukemia
rhom-2	acute T-cell leukemia
tal-1	acute T-cell leukemia
tal-2	acute T-cell leukemia
tan-1	acute T-cell leukemia

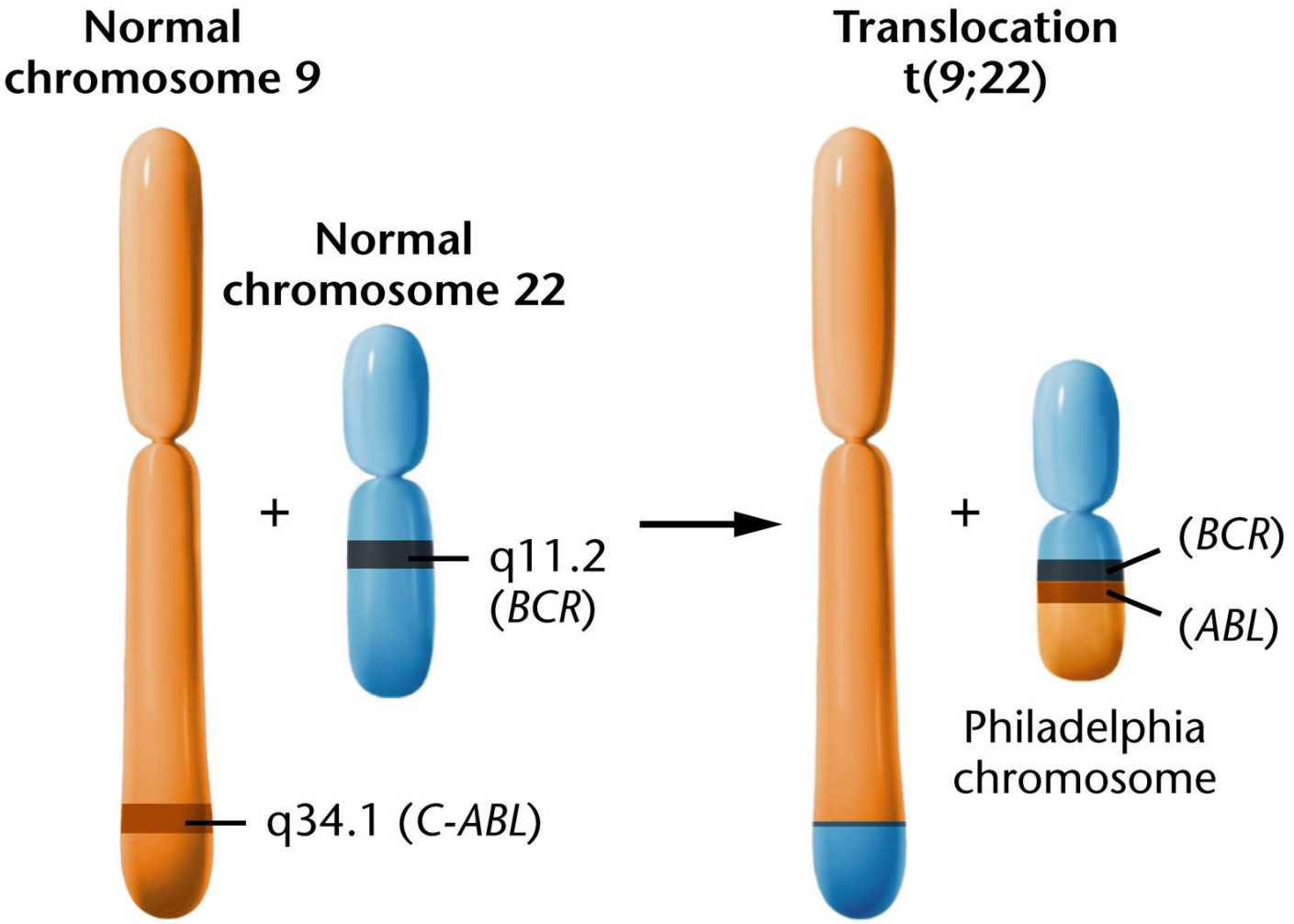
Adapted from G.M. Cooper, Oncogenes, 2nd ed. Boston and London: Jones and Bartlett, 1995.

p53 in chronic myelogenous leukemia (CML) in acute phase

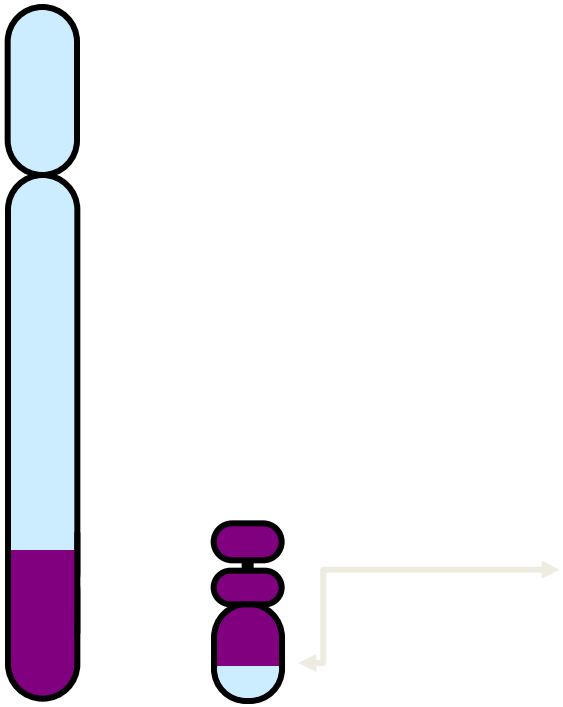
E Feinstein, G Cimino, R P Gale, G Alimena, R Berthier, K Kishi, J Goldman, A Zaccaria, A Berrebi, and E Canaani

All patients with chronic myelogenous leukemia (CML) undergo clinical transition from chronic to acute phase. This transition is often associated with deletion of the short arm of chromosome 17 in the form of the i(17q) aberration. Since the p53 gene is a suppressor gene and is located on 17p13, we examined the possibility that it is inactivated during progression of CML. Therefore, we studied the structure and expression of p53 in the leukemic cells of a large number of CML patients in acute phase. We found that although the gene is rarely rearranged, one p53 allele is completely deleted in patients with the i(17q) aberration as well as in some patients who do not show karyotypic changes. In all of these patients the remaining allele is inactivated through loss of expression, rearrangement, or point mutation. Detailed analysis of some patients who carry both p53 alleles indicated neither loss of expression nor structural alterations.

Chromosomal Translocation in CML



Bcr-Abl has Unregulated Kinase Activity due to Translocation



t(9;22) (q34.1;q11.21)

Philadelphia chromosome

ABL1 (9q34.1)

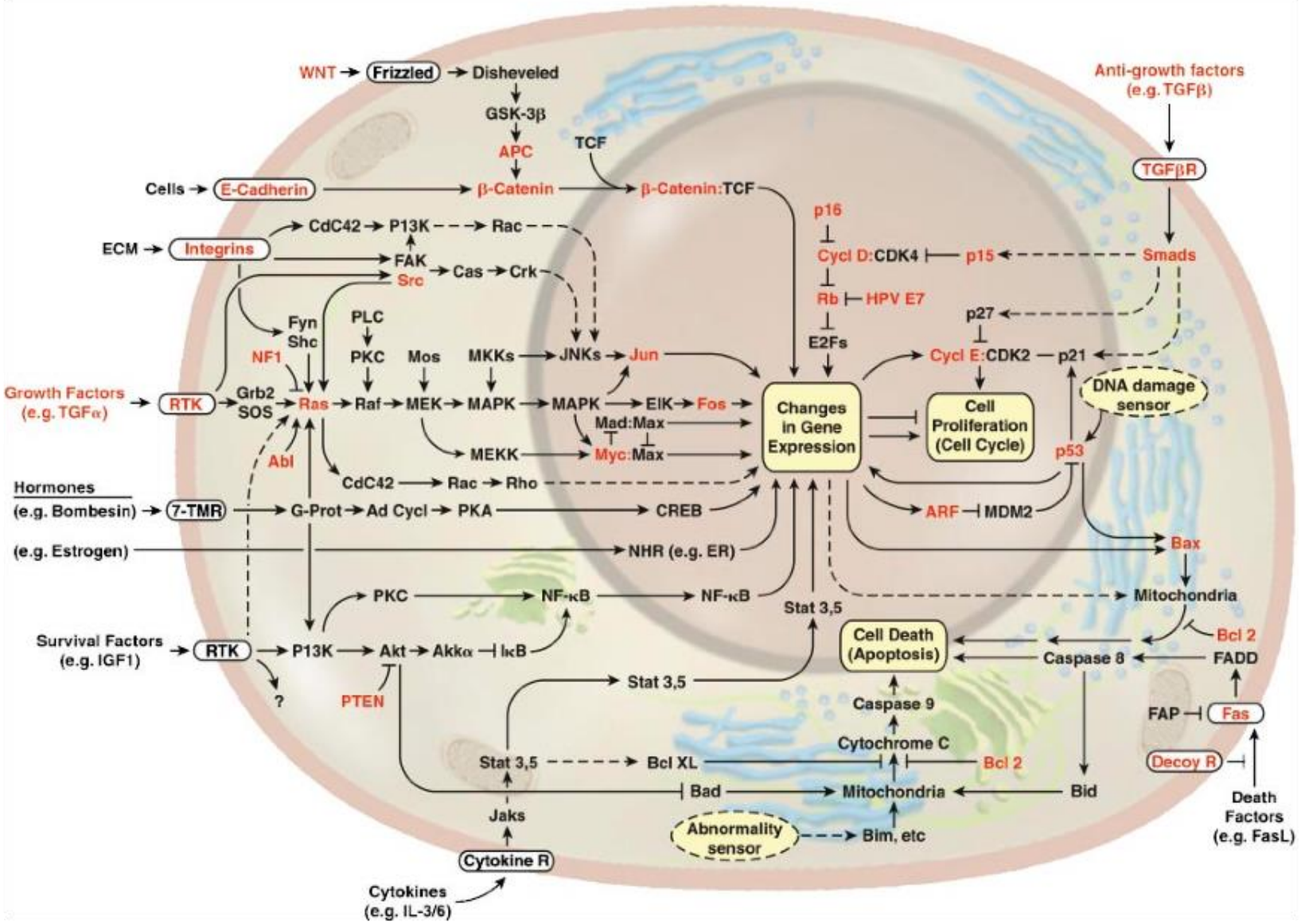
BCR (22q11.21)

translocation

BCR-ABL fusion (Philadelphia chromosome)

fusion protein with
unregulated kinase activity

chronic myelogenous leukemia



From Hanahan and Weinberg, 2000

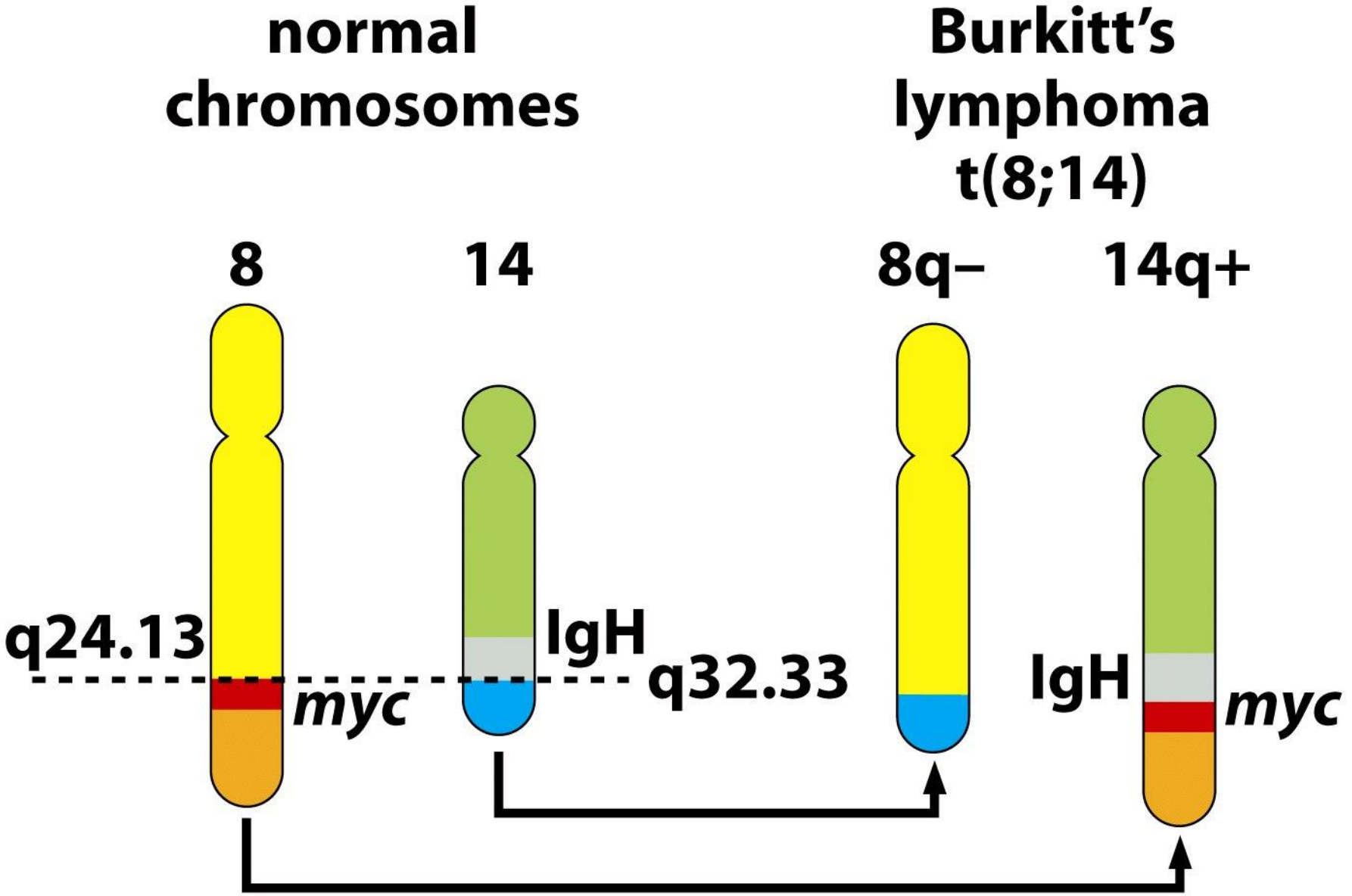


Figure 4.13a *The Biology of Cancer* (© Garland Science 2007)

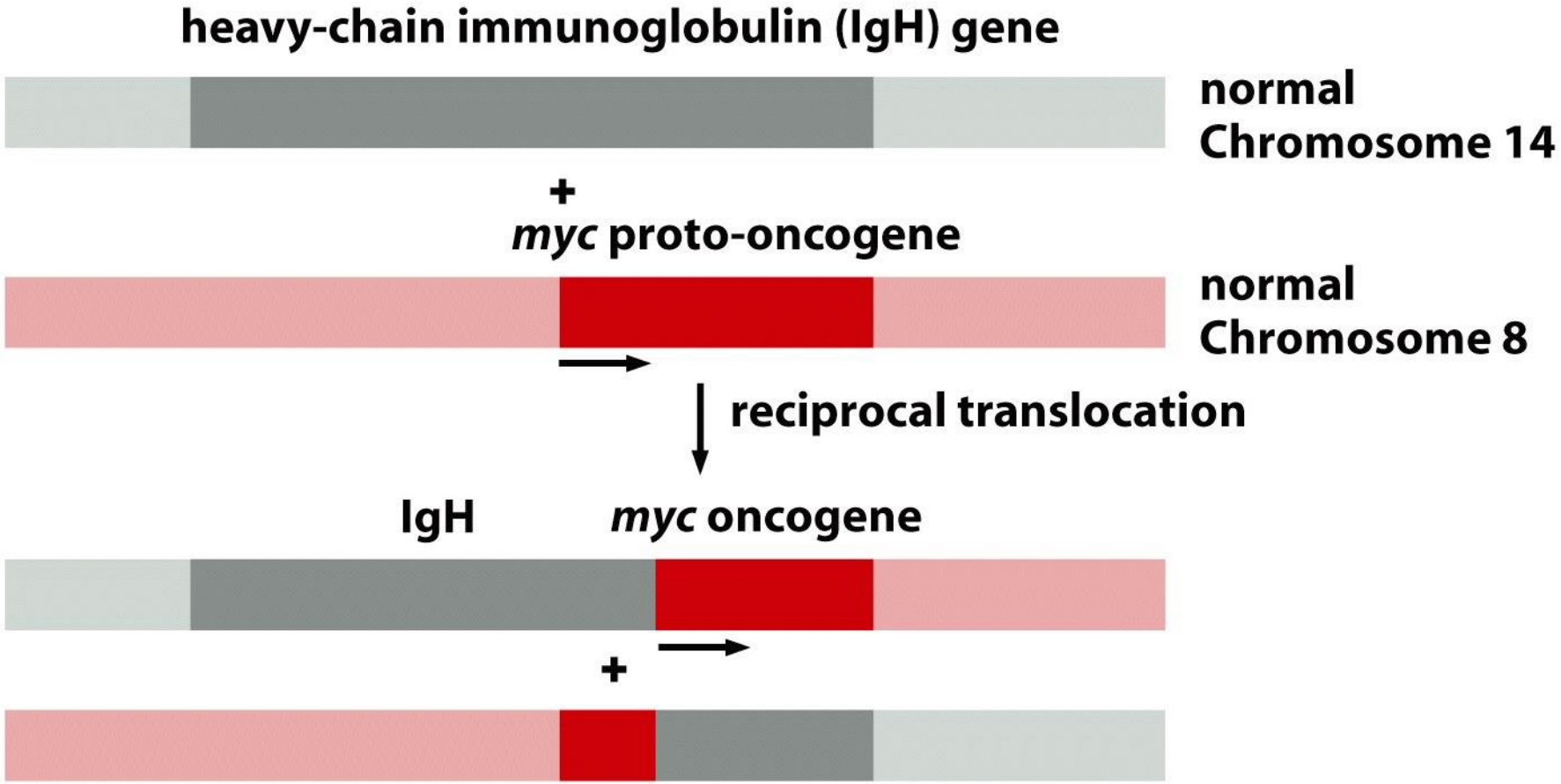


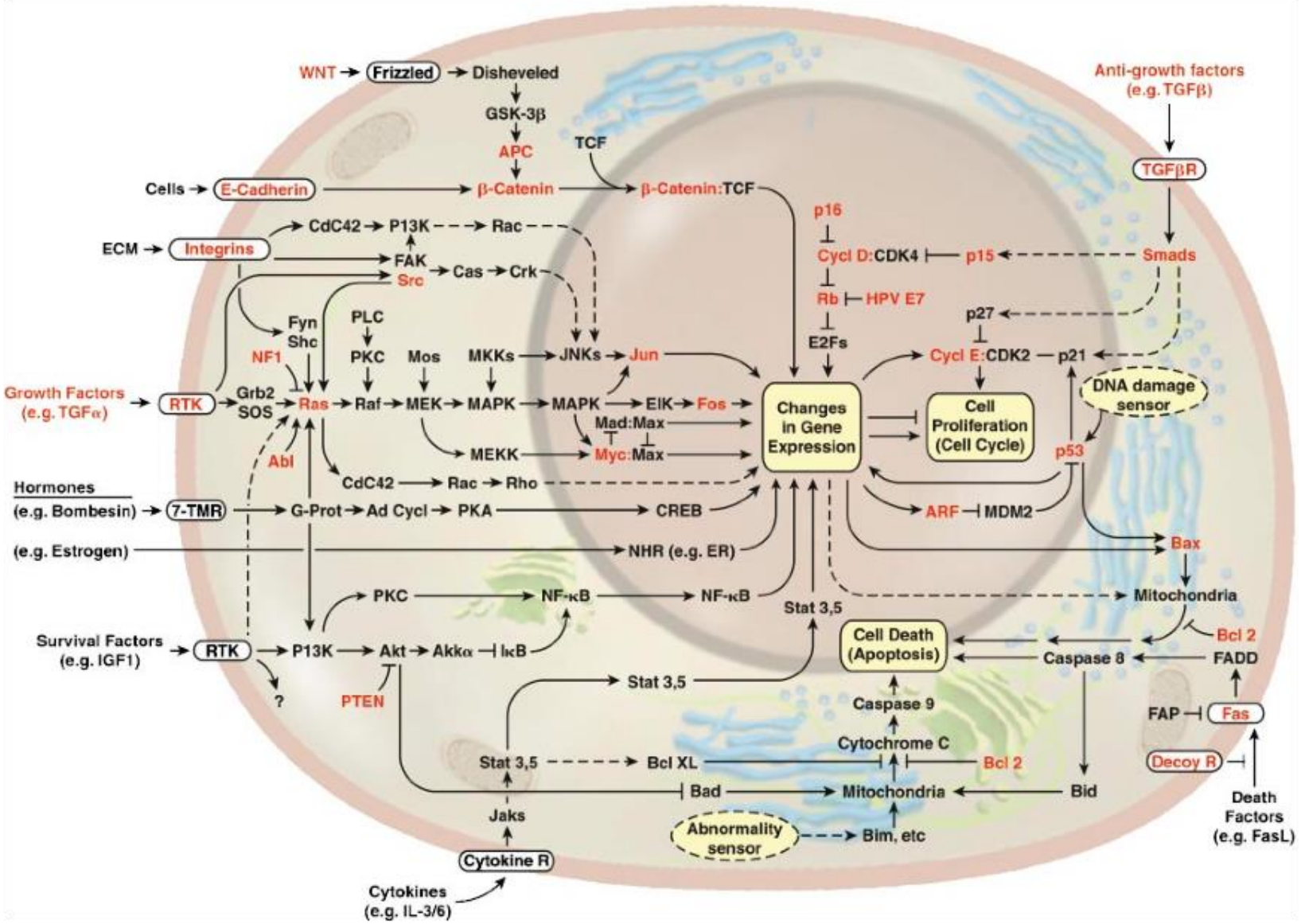
Figure 4.13b *The Biology of Cancer* (© Garland Science 2007)

Table 4.2 A list of point-mutated *ras* oncogenes carried by a variety of human tumor cells

Tumor type	Proportion (%) of tumors carrying a point-mutated *ras* gene[a]
Pancreas	90 K
Thyroid (papillary)	60 (H, K, N)
Thyroid (follicular)	55 (H, K, N)
Colorectal	45 (K)
Seminoma	45 (K, N)
Myelodysplasia	40 (N, K)
Lung (non-small-cell)	35 (K)
Acute myelogenous leukemia	30 (N)
Liver	30 (N)
Melanoma	15 (K)
Bladder	10 (K)
Kidney	10 H

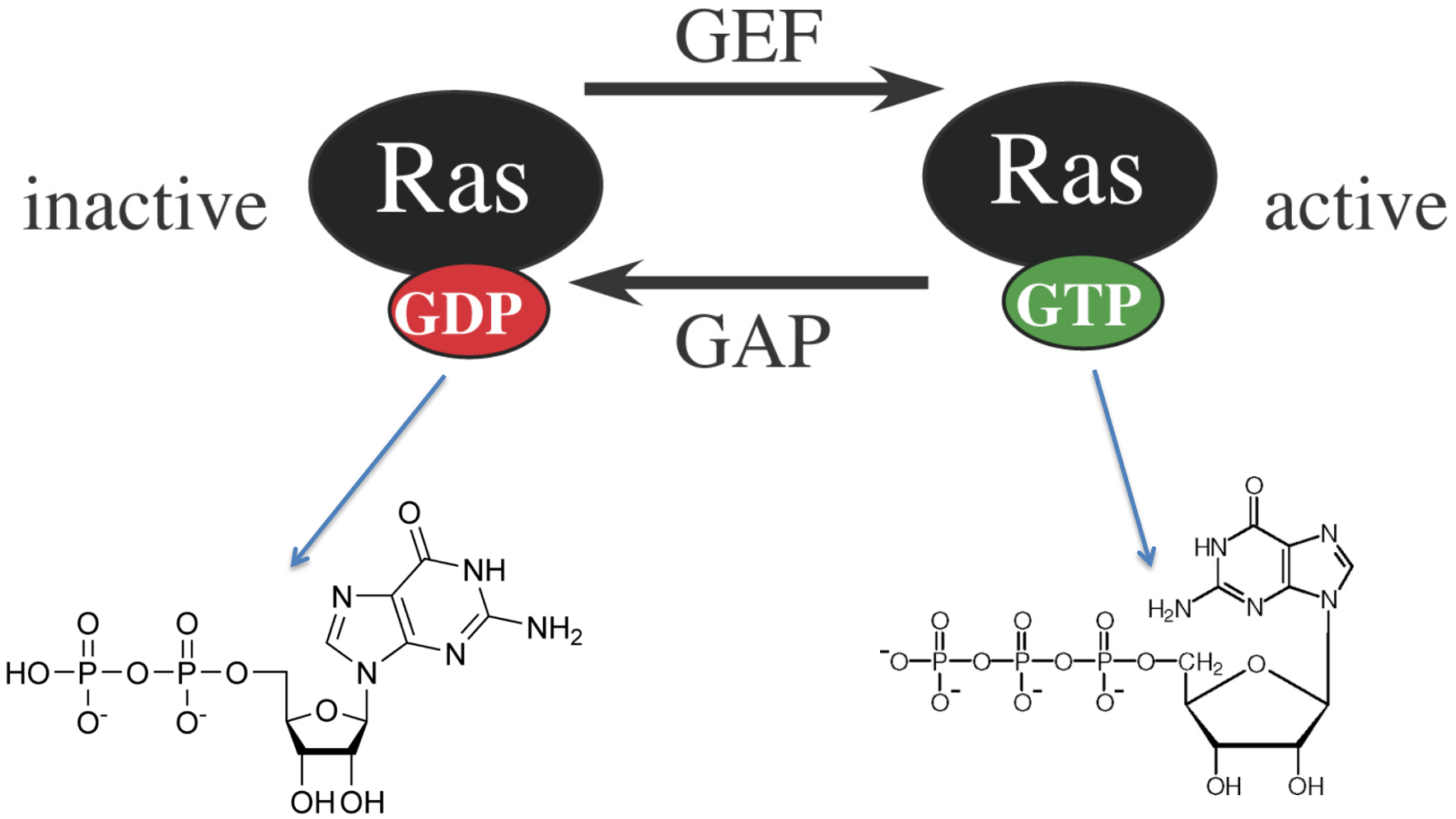
[a]H, K, and N refer to the human *H-RAS*, *K-RAS,* and *N-RAS* genes, respectively.

Adapted from J. Downward, *Nat. Rev. Cancer* 3:11–22, 2003.



From Hanahan and Weinberg, 2000

Regulation of the activity of the Ras GTPase



From: http://www.wormbook.org/chapters/www_RTKRasMAPKsignaling/RTKRasMAPKsignaling.html

Structure of tyrosine kinase receptors...

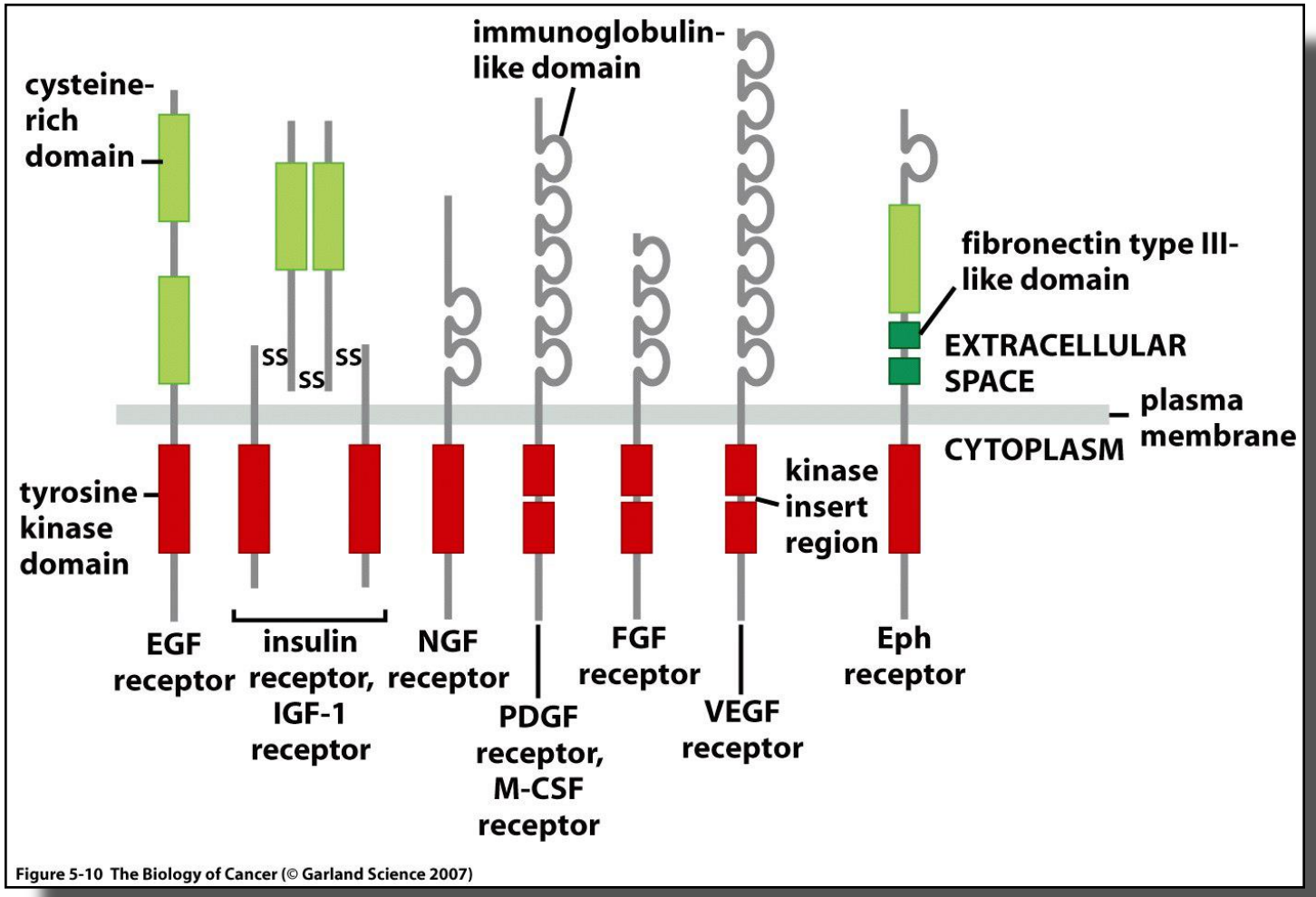


Figure 5-10 The Biology of Cancer (© Garland Science 2007)

Mechanism of activation of growth factor receptors…

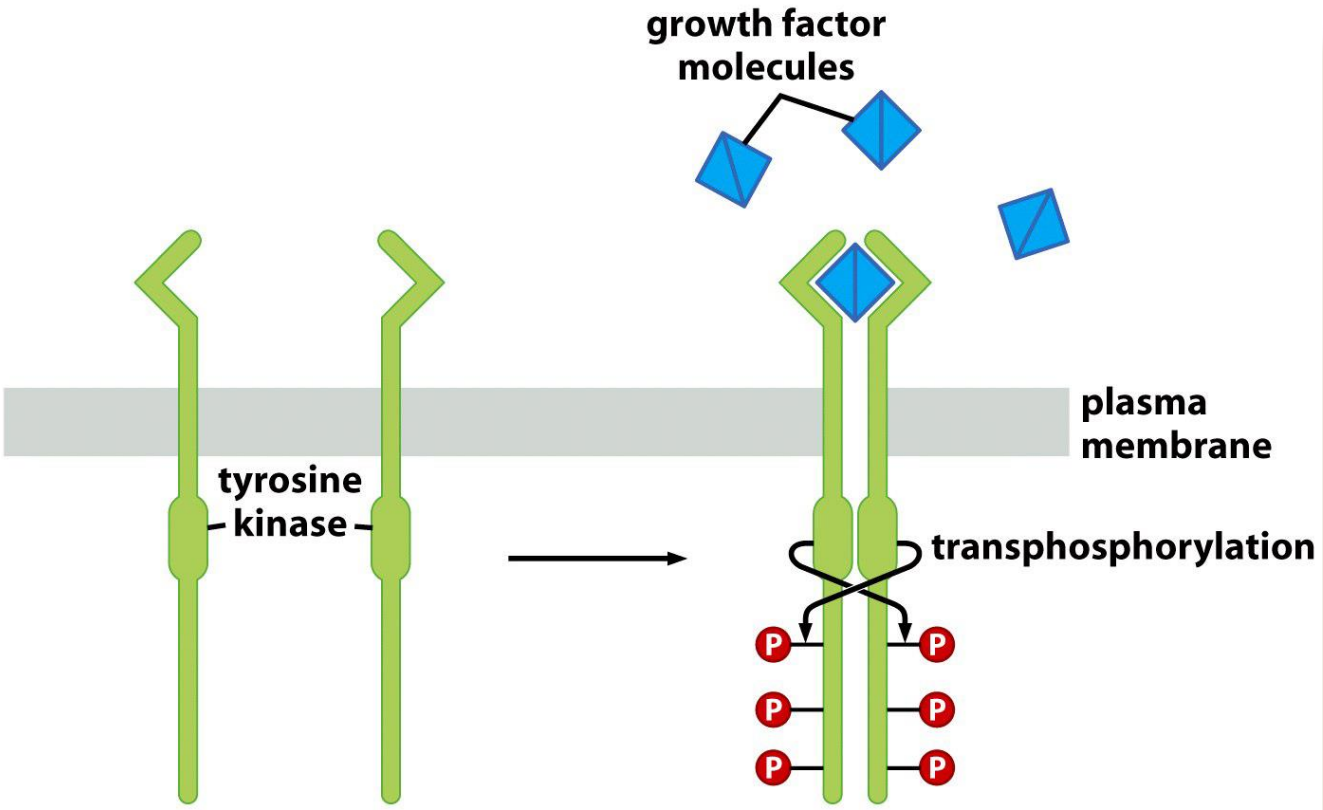
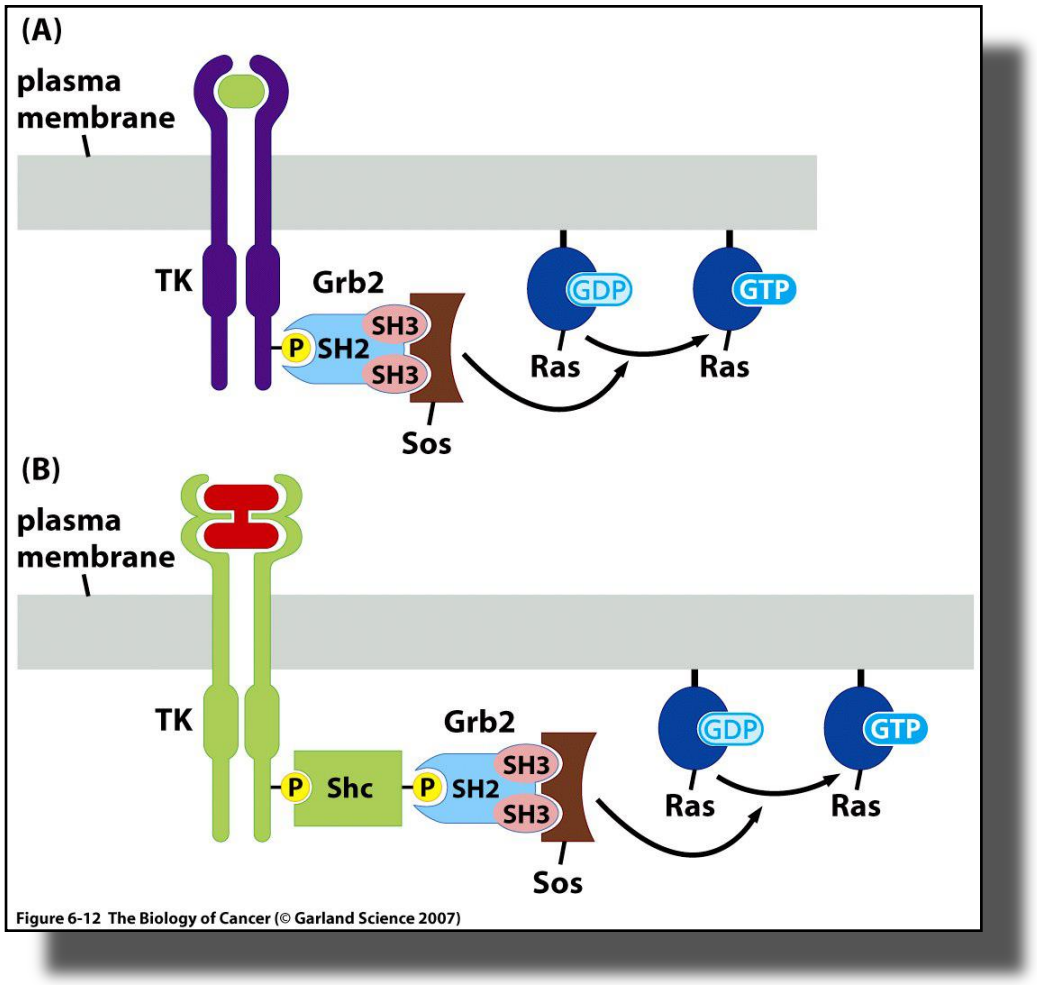


Figure 5-15 The Biology of Cancer (© Garland Science 2007)

Details of the activation of Ras by Grb2, Shc and SOS...



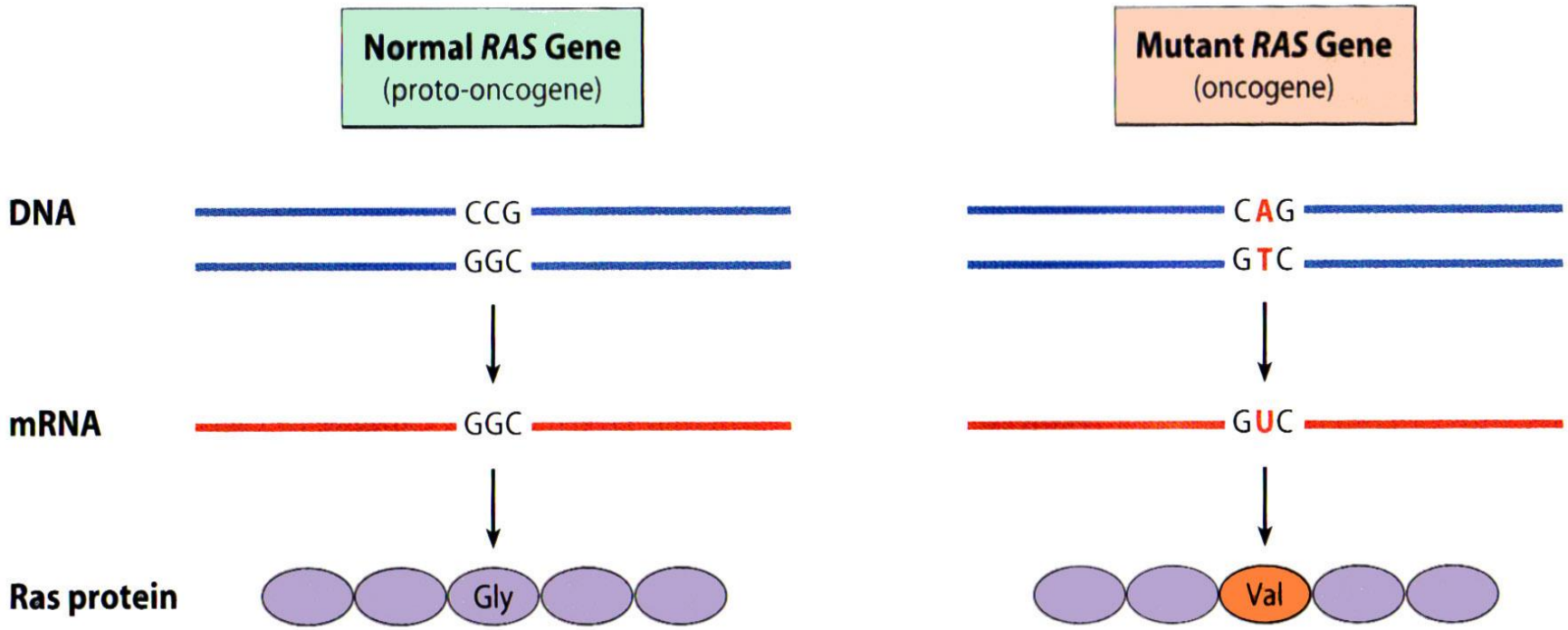


Figure 9-1 Point Mutation in a *RAS* Oncogene. *RAS* oncogenes typically differ from normal *RAS* proto-oncogenes in only a single nucleotide base. In this example, a single nucleotide mutation converts a normal *RAS* proto-oncogene into an oncogene that codes for an abnormal Ras protein in which a single amino acid is converted from glycine (Gly) to valine (Val).

The 3-D structure of the Ras protein...

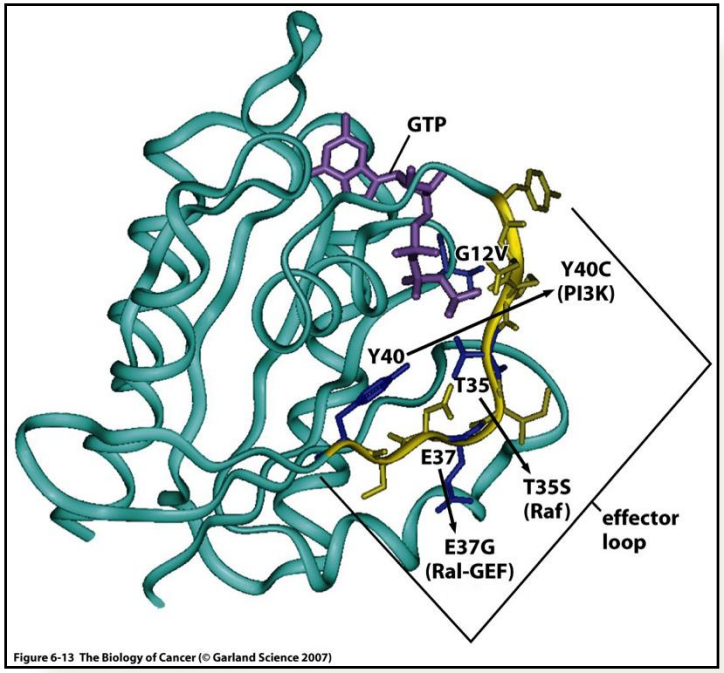


Figure 6-13 The Biology of Cancer (© Garland Science 2007)

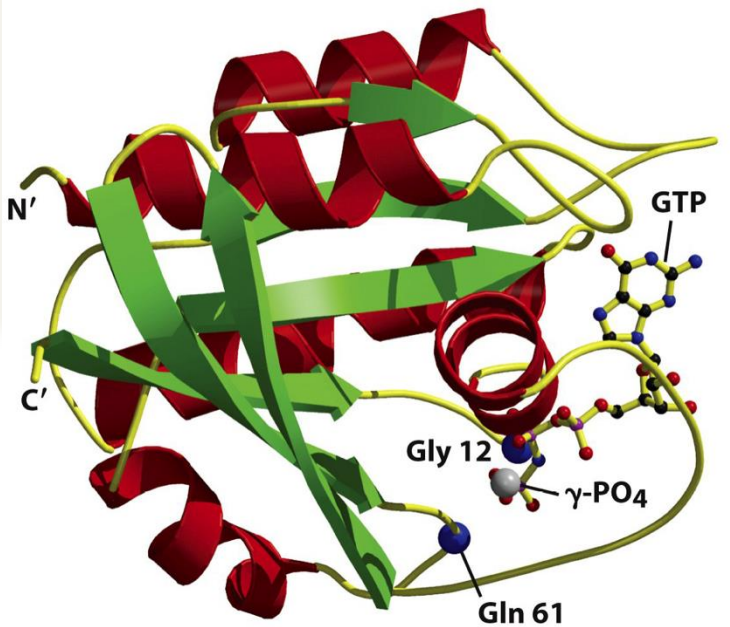
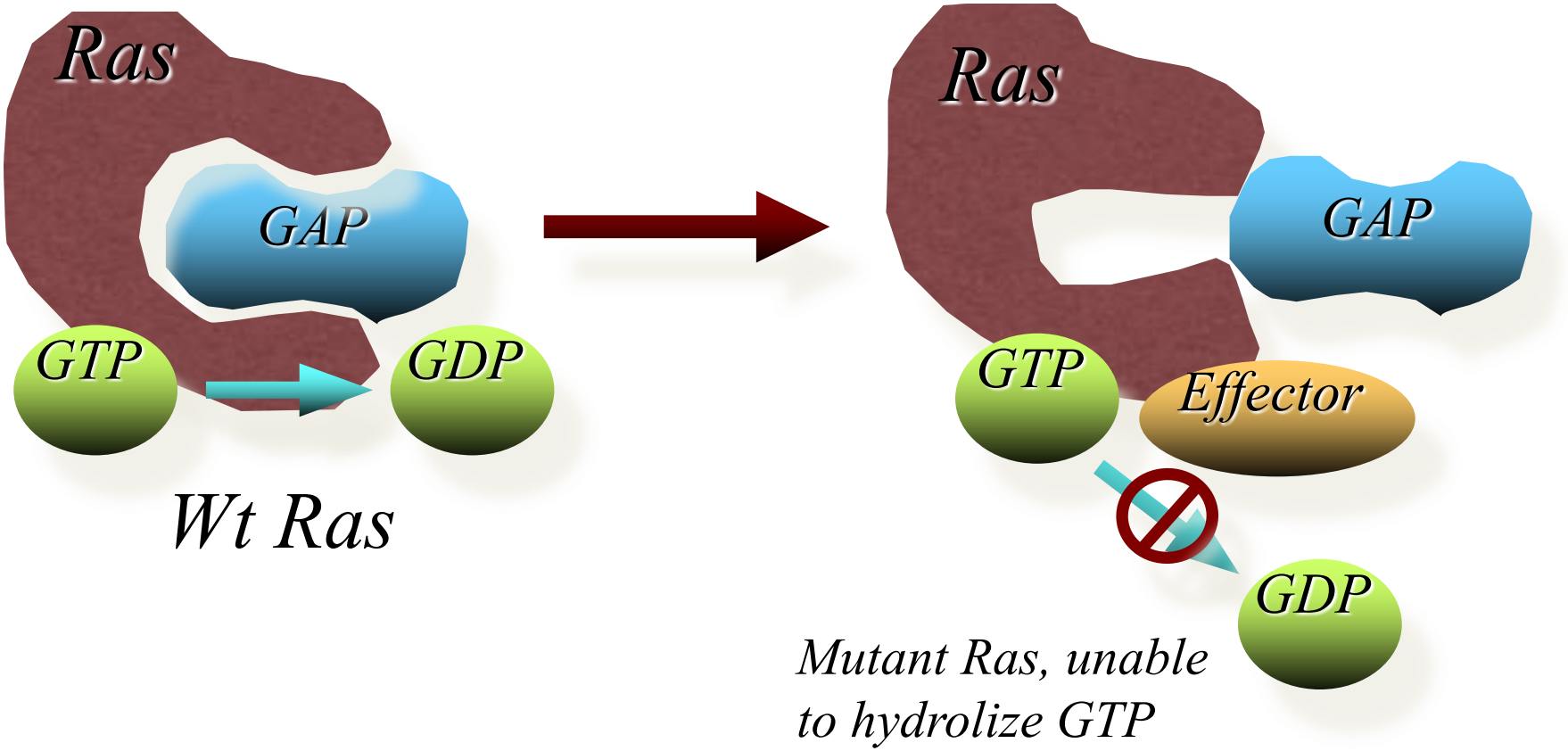
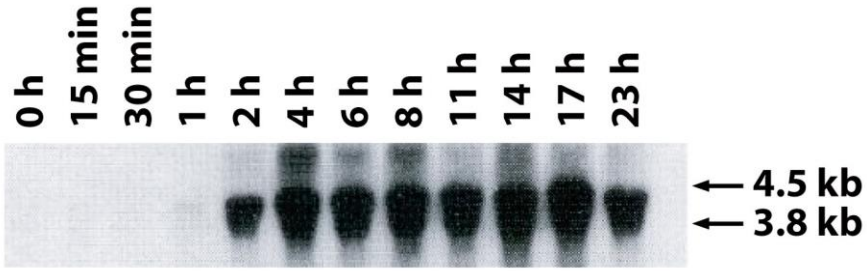


Figure 5-31 The Biology of Cancer (© Garland Science 2007)

Some cancer-associated Ras mutations disrupt the interaction between Ras and GAP





Cyclin D1 expression after mitogenic stimulation or oncogenic insult

C

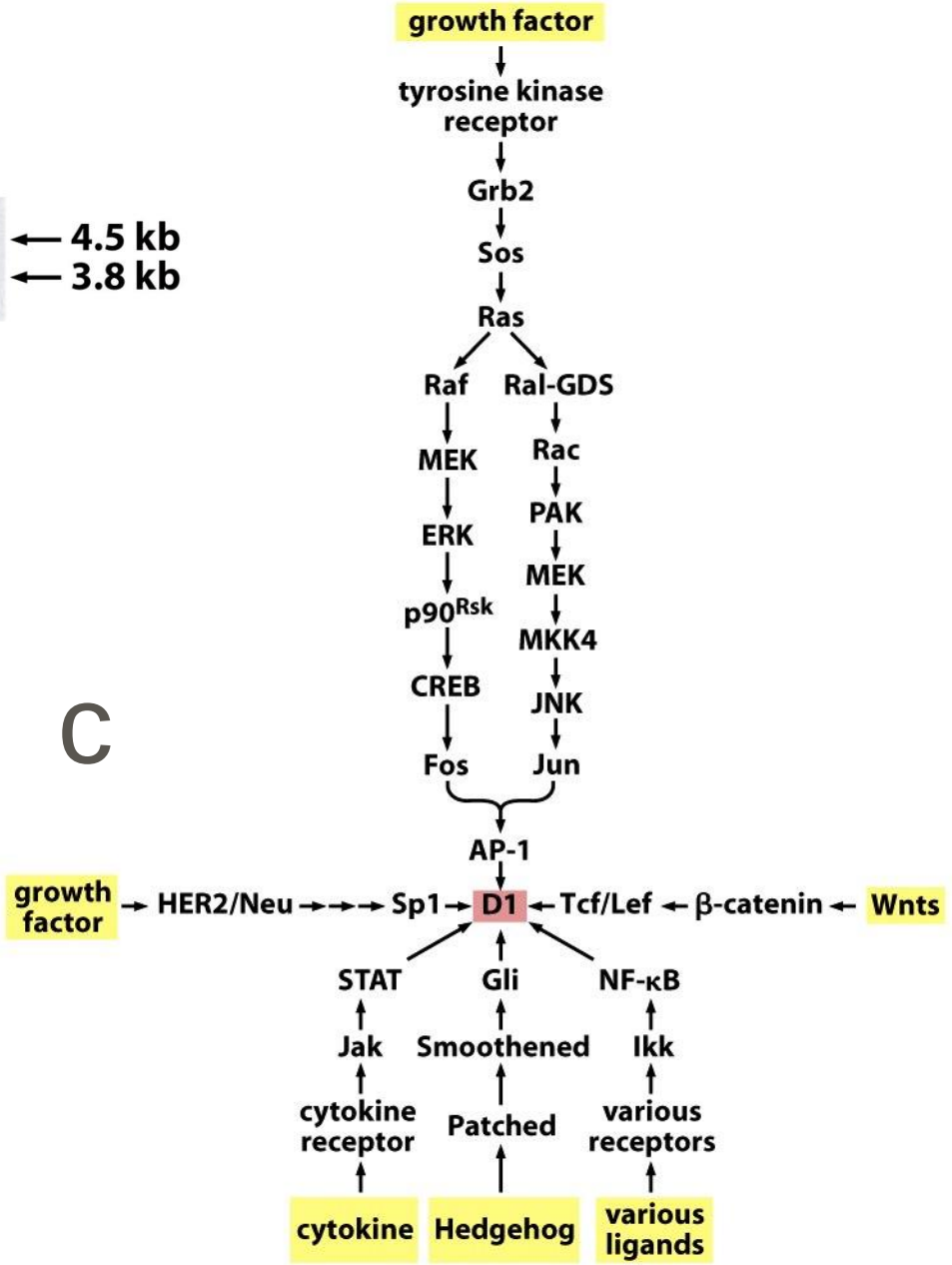
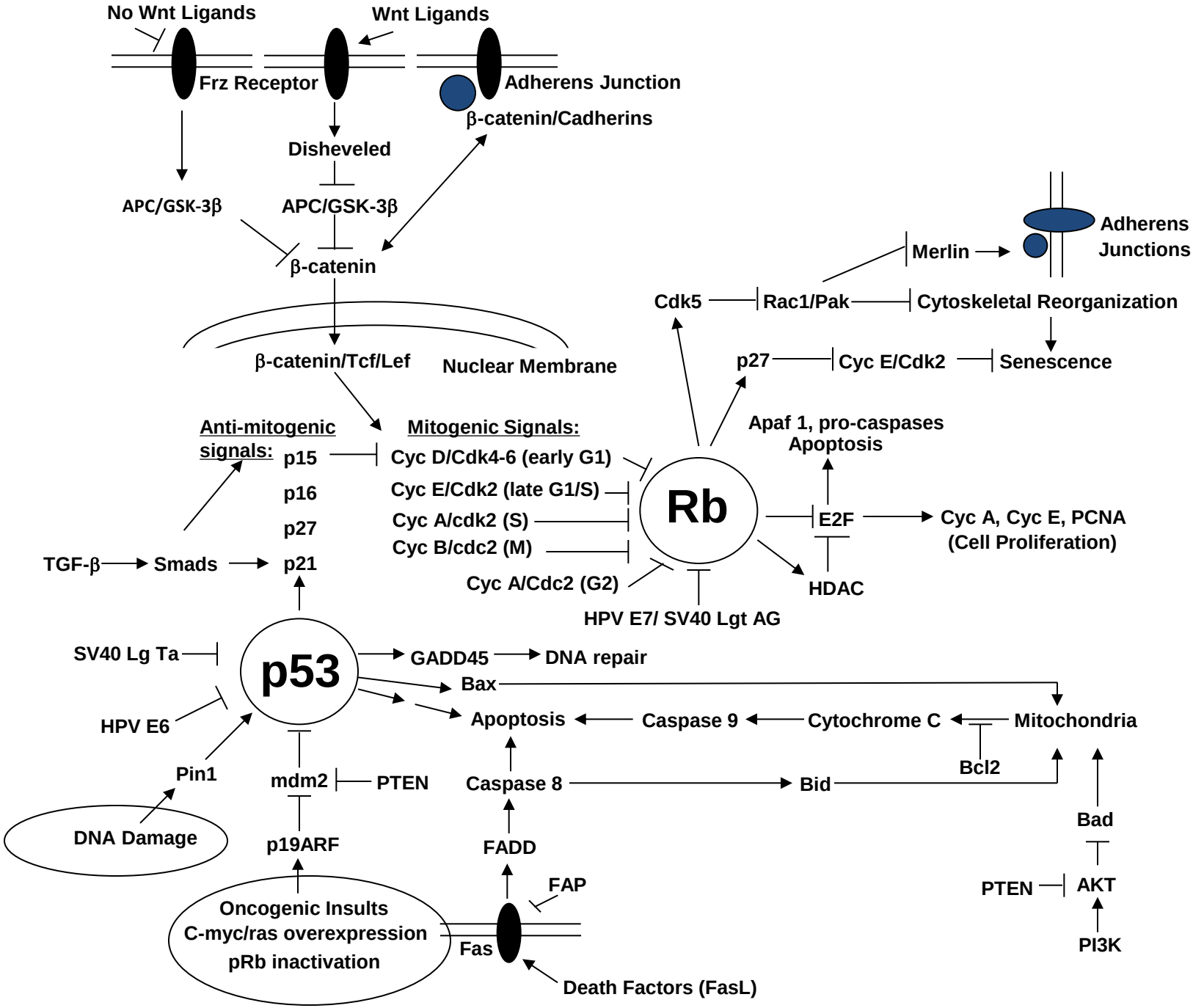


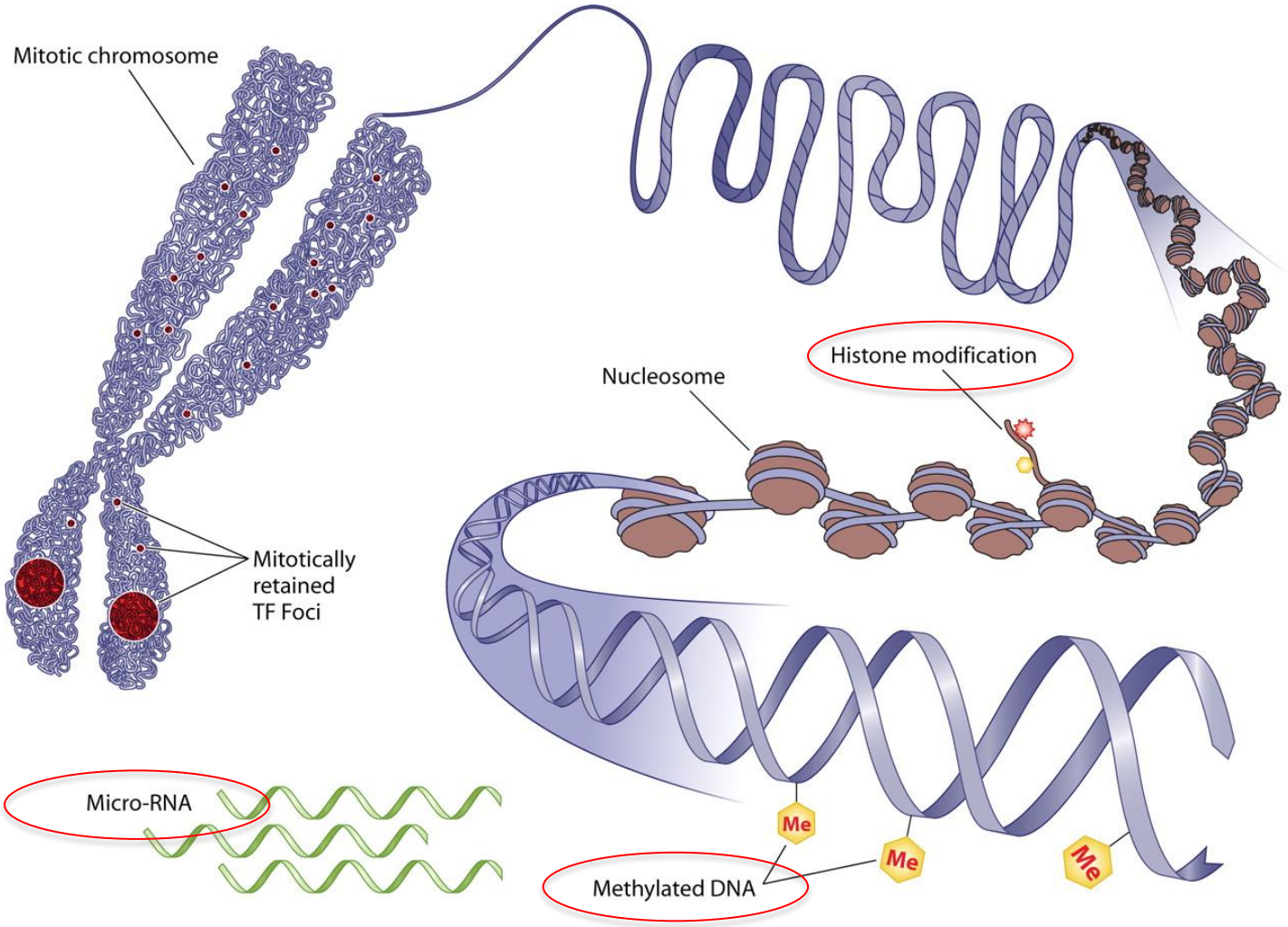
Figure 8.11b *The Biology of Cancer* (© Garland Science 2007)


No Wnt Ligands
Wnt Ligands
Frz Receptor
Adherens Junction
β-catenin/Cadherins
Disheveled
APC/GSK-3β
APC/GSK-3β
β-catenin
Adherens Junctions
Merlin
Cdk5
Rac1/Pak
Cytoskeletal Reorganization
β-catenin/Tcf/Lef
Nuclear Membrane
p27
Cyc E/Cdk2
Senescence
Anti-mitogenic signals:
Mitogenic Signals:
Apaf 1, pro-caspases
Apoptosis
p15
p16
p27
p21
Cyc D/Cdk4-6 (early G1)
Cyc E/Cdk2 (late G1/S)
Cyc A/cdk2 (S)
Cyc B/cdc2 (M)
Rb
E2F
Cyc A, Cyc E, PCNA
(Cell Proliferation)
TGF-β
Smads
Cyc A/Cdc2 (G2)
HDAC
HPV E7/ SV40 Lgt AG
SV40 Lg Ta
p53
GADD45
DNA repair
Bax
HPV E6
Apoptosis
Caspase 9
Cytochrome C
Mitochondria
Pin1
mdm2
PTEN
Caspase 8
Bid
Bcl2
Bad
DNA Damage
p19ARF
FADD
FAP
PTEN
AKT
Oncogenic Insults
C-myc/ras overexpression
pRb inactivation
Fas
PI3K
Death Factors (FasL)

Epigenetics

- DNA methylation
  - Hypomethylation: gene activation (oncogenes)
    - BCL-2: B cell CLL
    - Cyclin D2: gastric cancers
    - K-RAS: lung and colon cancers
  - Hypermethylation: gene silencing (tumor suppressor genes)
    - RB gene
    - Age-related increase in DNA methylation
- Histone modifications
  - Acetylation, methylation, phosphorylation
  - Transcriptional regulation
  - Chromatin stability
  - Chromatin remodeling

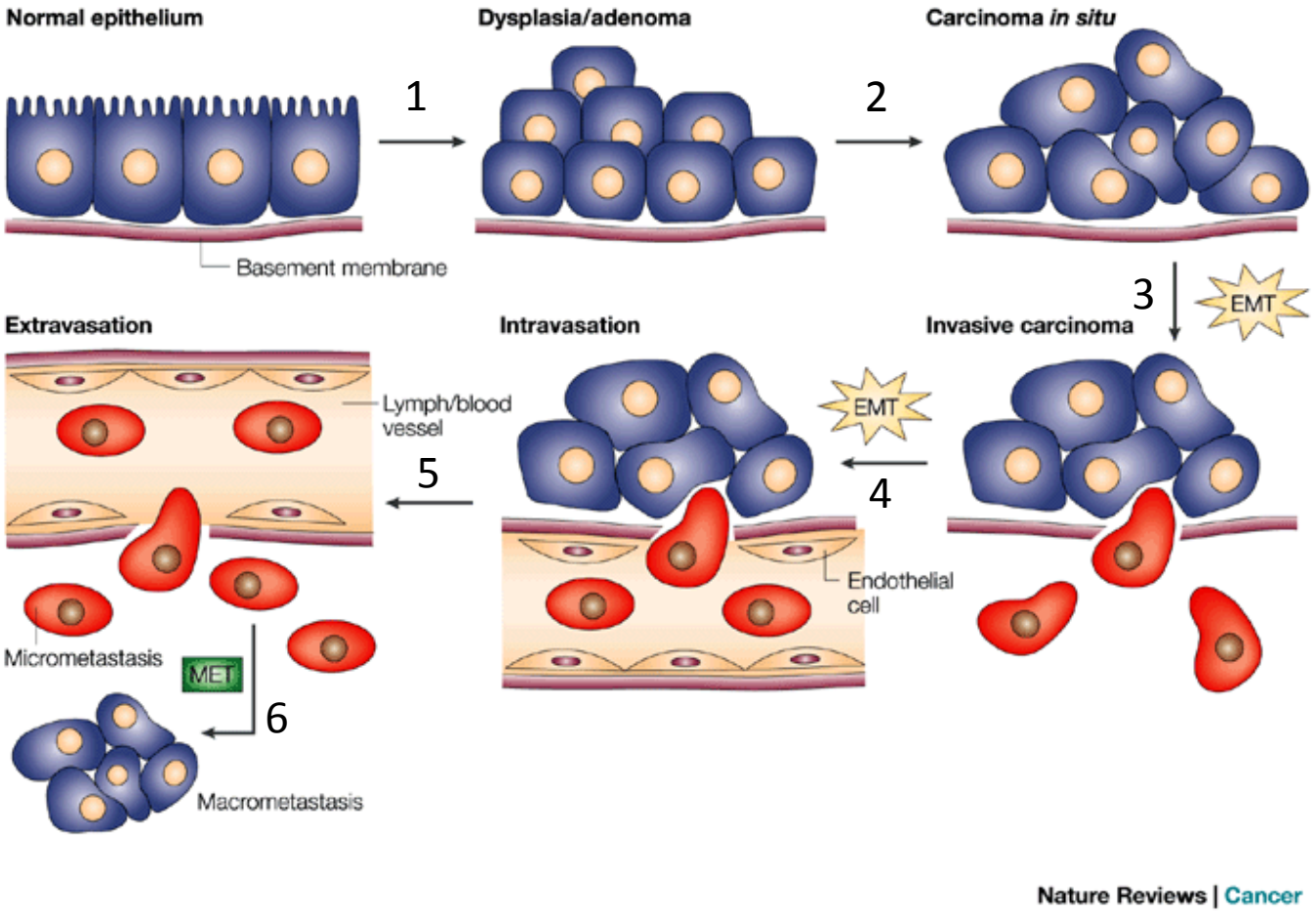
Epigenetic changes in cancer…



From: http://episona.com/short-introduction-epigenetics/

Part II: The Cellular Biology of Cancer
The Hallmarks of Cancer

Model for Carcinoma Progression



Model by Jean Paul Thiery
Nature Reviews Cancer **2**, 442-454 (June 2002)
doi:10.1038/nrc822

A vast catalog of genetic alterations produce a wide spectrum of cancer types…

…but this large number of genetic alterations that drives cancer progression manifests itself at the cellular level as **six** essential alterations in cell function that collectively drive malignant transformation and growth…

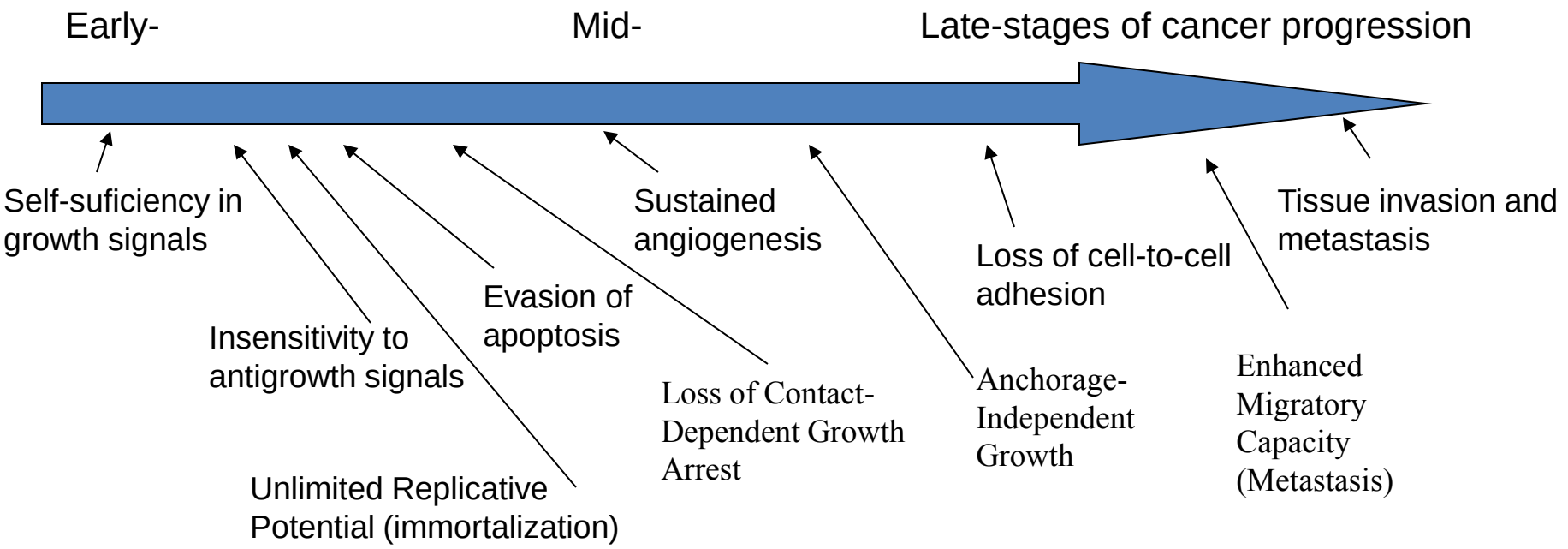
These six alterations in cellular behavior are shared by most, if not all, types of human cancers:

1. Self-sufficiency in growth signals (autocrine mechanisms)
2. Insensitivity of anti-growth signals (Rb inactivation, by-pass of cell cycle checkpoints)
3. Limitless replicative potential
4. Evasion of apoptosis
5. Sustained angiogenesis
6. Tissue invasion and metastasis

(from Hanahan and Weinberg, The Hallmarks of Cancer, 2000)

MUTATIONS ARE THE MOLECULAR BASIS OF THESE ALTERATIONS

Multiple pathways to cancer (but all roads lead to Rome!!!)

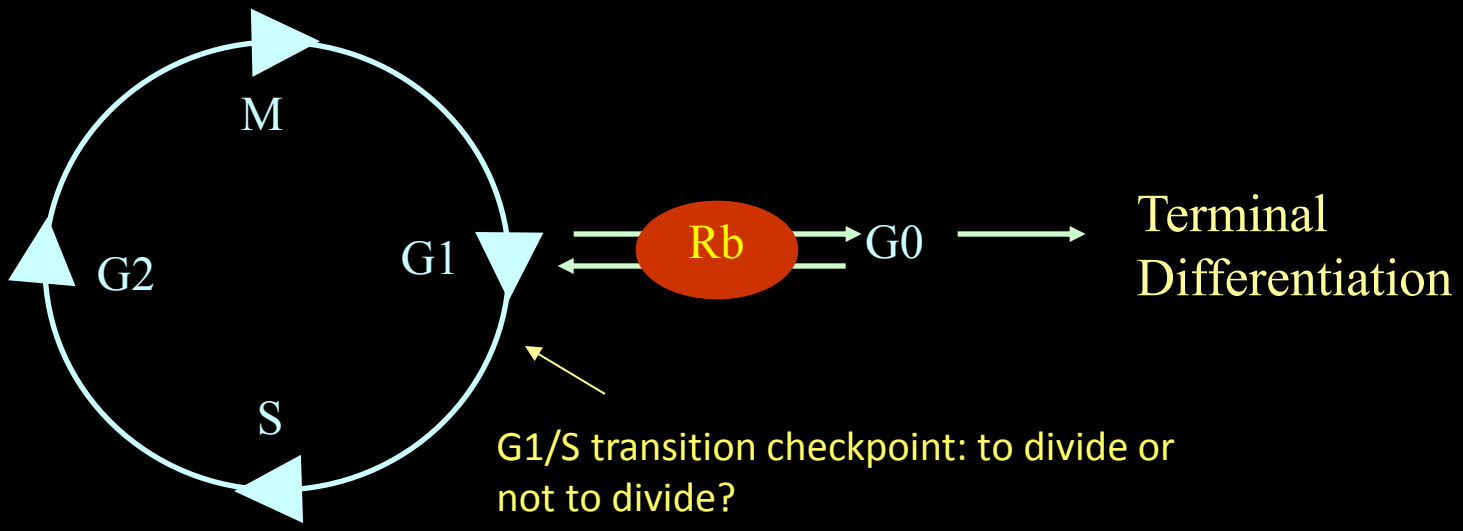


Dozens, maybe even hundreds, of genes are known to be mutated during carcinogenesis. The amount of mutations, the identities of the mutated genes and the order in which they are mutated varies greatly among different tumor types. One specific genetic mutation can contribute to the acquisition of only one trait in one type of tumor while contributing to the acquisition to more that one trait (pleiotropic effect*) in other tumor types. There is great variability in this respect even between histologically identical tumors.

What is important is that the fully transformed phenotype arises as a consequence of the acquisition of all the alterations discussed, independently of the identities of the mutations that led to their acquisition.

*Pleiotropy: a single gene influencing many traits

Alteration #1: Self-sufficiency in growth signals



Final outcome depends on the balance between mitogenic and anti-mitogenic signals

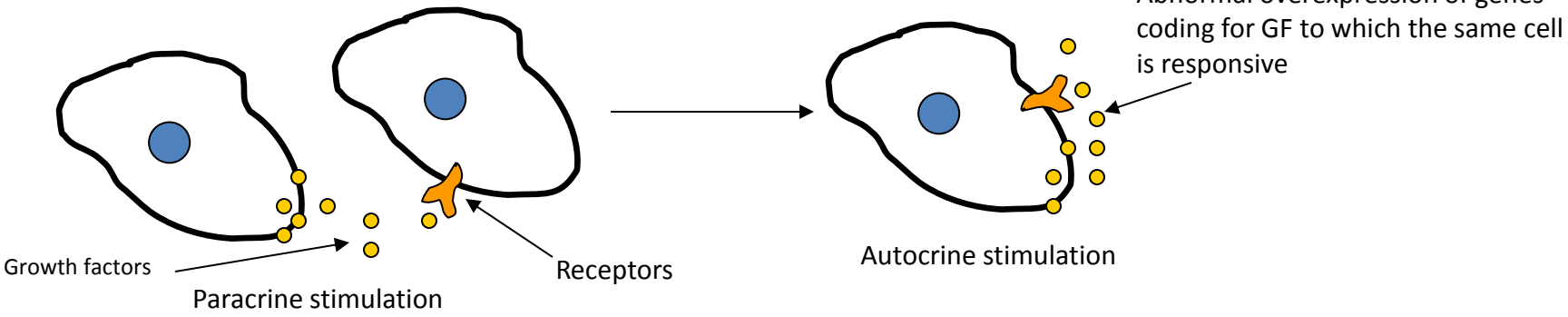
Main mitogenic signals are:

Diffusible growth factors and ECM components. They are received mostly by cell surface receptors coupled to intracellular signaling pathways.

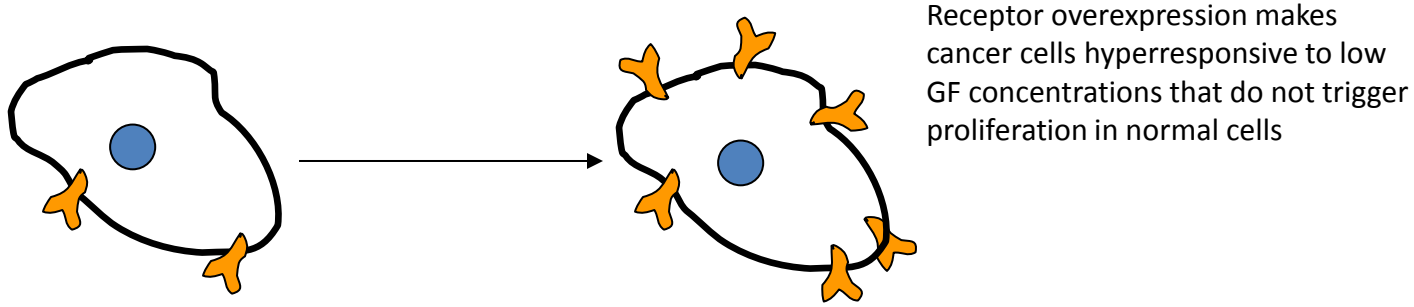
While normal cells **can't** proliferate in the absence of these signals, cancer cells **show a greatly reduced dependence** on them. Cancer cells generate many of their own growth signals, therefore, they have a reduced dependence on stimulation from their normal tissue microenvironment…

How do cancer cells achieve such autonomy?????

Mechanisms a: switch from paracrine to autocrine stimulation



Mechanism b: GF receptor overexpression



Alteration #1. Self-sufficiency in growth signals (autocrine mechanisms)

Other GF receptor-related mechanisms:

a. Gross overexpression of receptors can trigger ligand-independent signaling (DiFiore et al., 1987).

b. Ligand-independent signaling can also be triggered by structural alterations of receptors, e.g. truncated versions of the EGF receptor lacking much of its cytoplasmic domain are constitutively activated (Fedi et al., 1997).

c. Receptor type switch to favor expression of receptors that transmit proliferative signals (Lukashev and Werb, 1998; Giancotti and Ruoslahti, 1999).

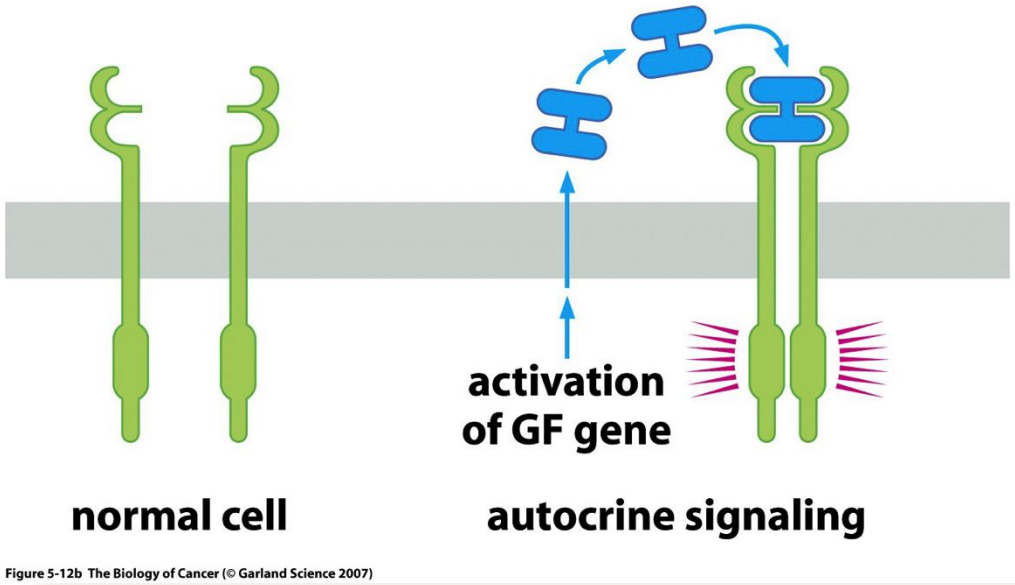


Figure 5-12b The Biology of Cancer (© Garland Science 2007)

Mechanism of loss of receptor function commonly observed in cancer...

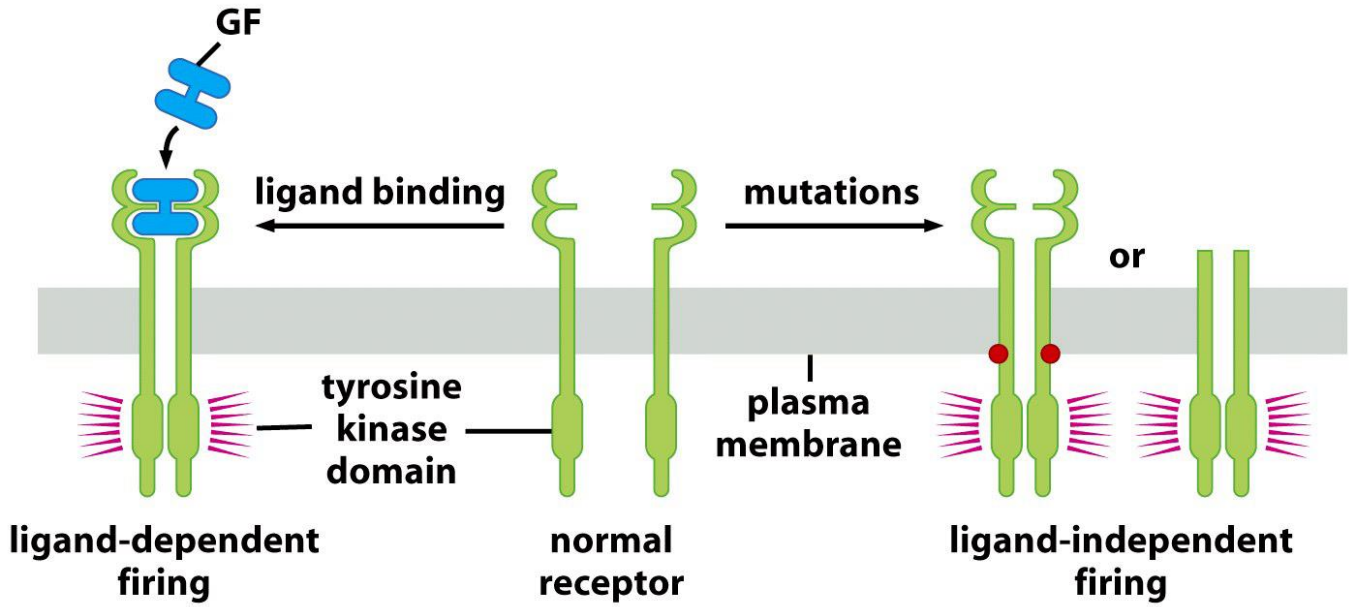
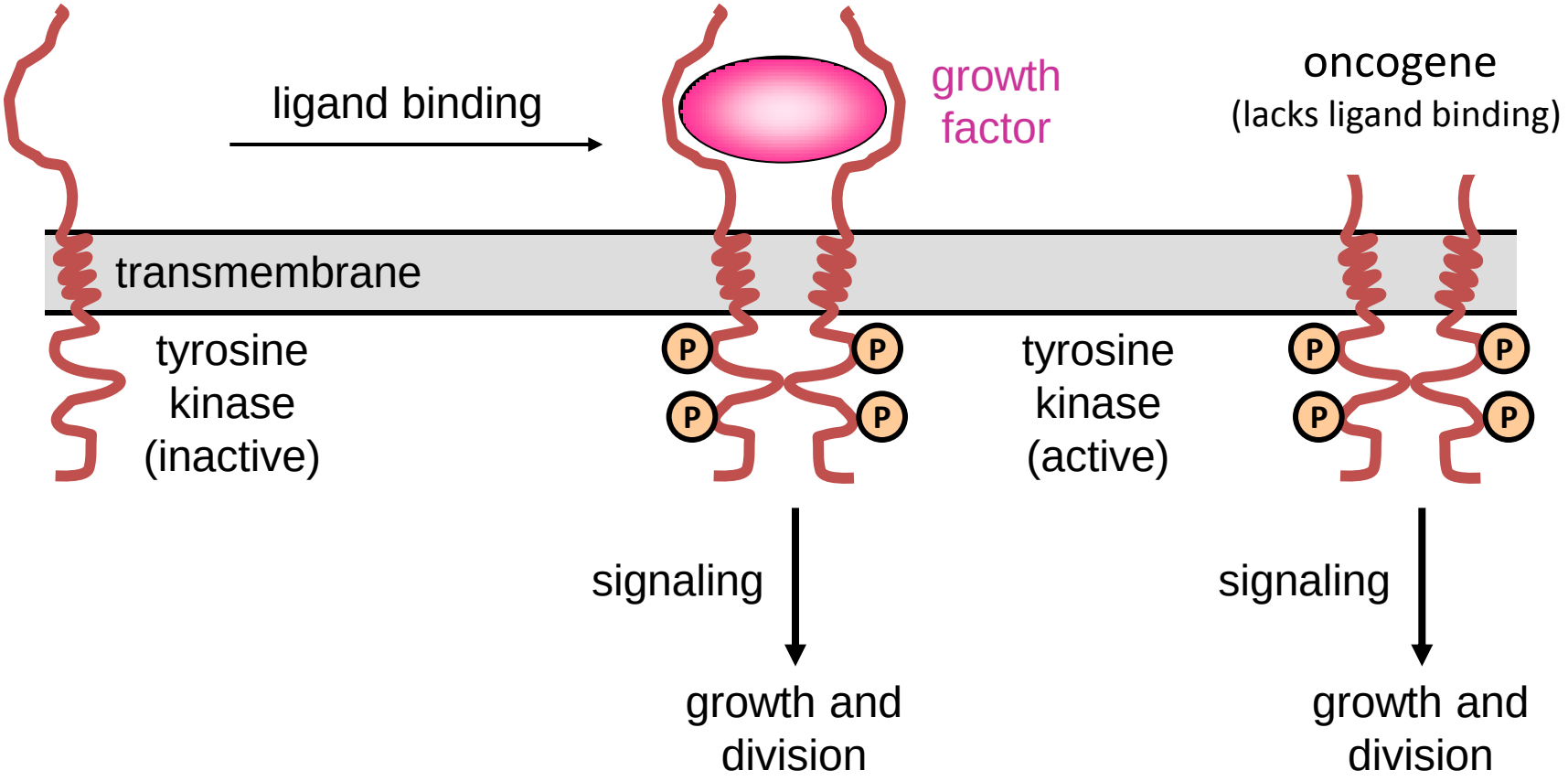


Figure 5-12a The Biology of Cancer (© Garland Science 2007)

Mutation in a Proto-Oncogene can Generate an Oncogene



Some types of human cancers with autocrine growth mechanisms...

Table 5.3 Examples of human tumors making autocrine growth factors

Ligand	Receptor	Tumor type(s)
HGF	Met	miscellaneous endocrinal tumors, invasive breast and lung cancers, osteosarcoma
IGF-2	IGF-1R	colorectal
IL-6	IL-6R	myeloma, HNSCC
IL-8	IL-8R A	bladder cancer
NRG	ErbB2[a]/ErbB3	ovarian carcinoma
PDGF-BB	PDGF-Rα/β	osteosarcoma, glioma
PDGF-C	PDGF-α/β	Ewing's sarcoma
PRL	PRL-R	breast carcinoma
SCF	Kit	Ewing's sarcoma, SCLC
VEGF-A	VEGF-R (Flt-1)	neuroblastoma, prostate cancer, Kaposi's sarcoma
TGF-α	EGF-R	squamous cell lung, breast and prostate adenocarcinoma, pancreatic, mesothelioma
GRP	GRP-R	small-cell lung cancer

[a]Also known as HER2 or Neu receptor.

Table 5-3 The Biology of Cancer (© Garland Science 2007)

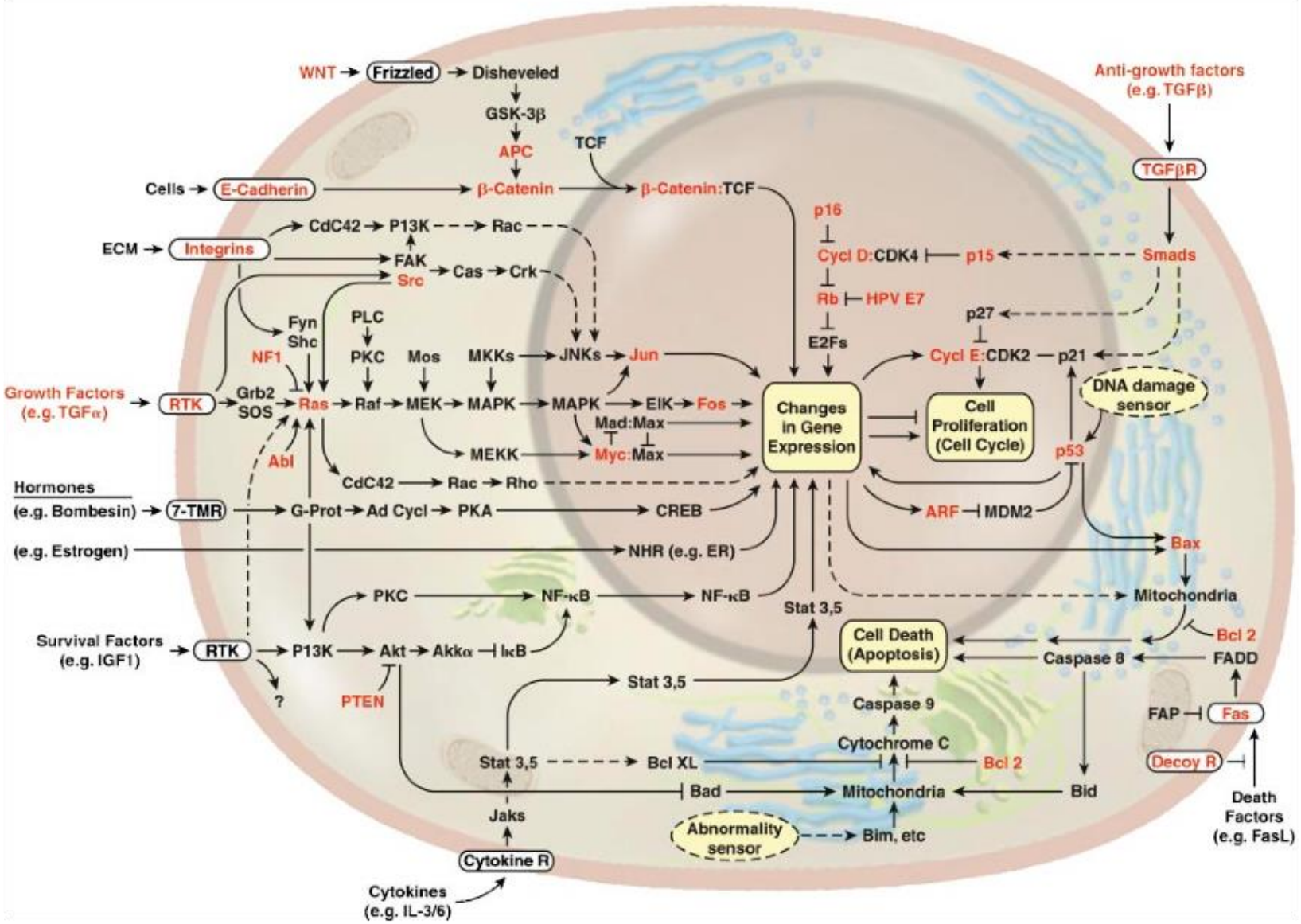
Common receptor alterations in cancer...

Table 5.2 Tyrosine kinase GF receptors altered in human tumors[a]

Name of receptor	Main ligand	Type of alteration	Types of tumor
EGF-R/ErbB1	EGF, TGF-α	overexpression	non-small cell lung cancer; breast, head and neck, stomach, colorectal, esophageal, prostate, bladder, renal, pancreatic, and ovarian carcinomas; glioblastoma
EGF-R/ErbB1		truncation of ectodomain	glioblastoma, lung and breast carcinomas
ErbB2/HER2/Neu	NRG, EGF	overexpression	30% of breast adenocarcinomas
ErbB3, 4	various	overexpression	oral squamous cell carcinoma
Flt-3	FL	tandem duplication	acute myelogenous leukemia
Kit	SCF	amino acid substitutions	gastrointestinal stromal tumor
Ret		fusion with other proteins, point mutations	papillary thyroid carcinomas, multiple endocrine neoplasias 2A and 2B
FGF-R3	FGF	overexpression; amino acid substitutions	multiple myeloma, bladder and cervical carcinomas

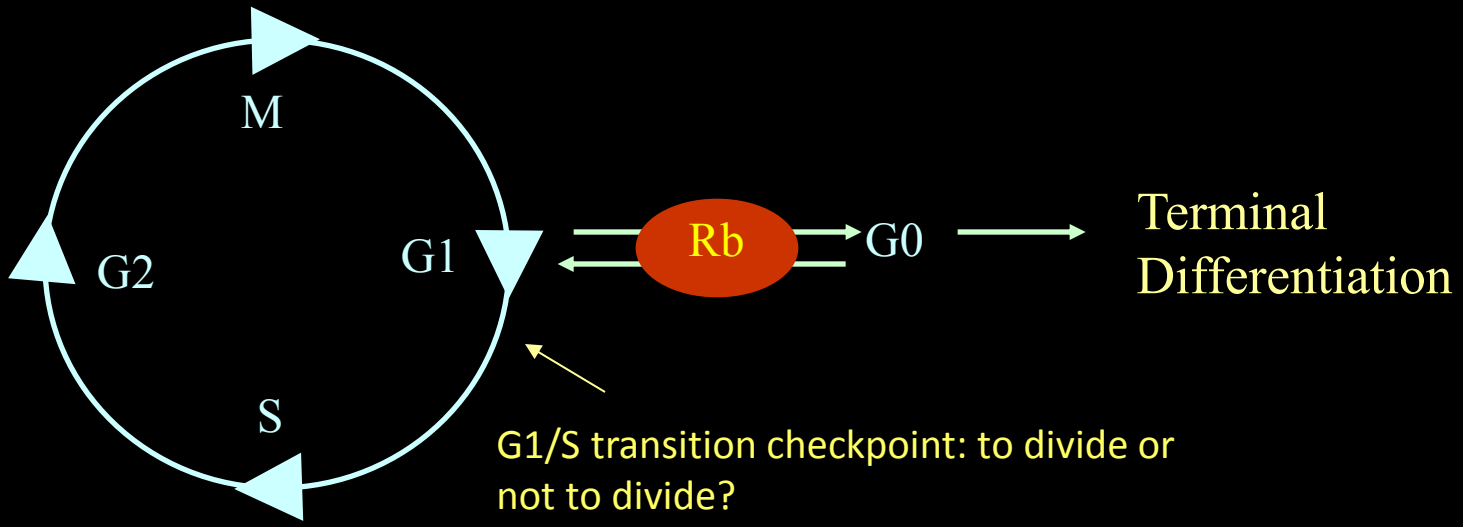
[a]See also Figure 5.17.

Table 5-2 The Biology of Cancer (© Garland Science 2007)



From Hanahan and Weinberg, 2000

Alteration #2: Insensitivity to anti-growth signals (Rb inactivation, by-pass of cell cycle checkpoints)



Final outcome depends on the balance between mitogenic and anti-mitogenic signals

Main anti-mitogenic signals are:

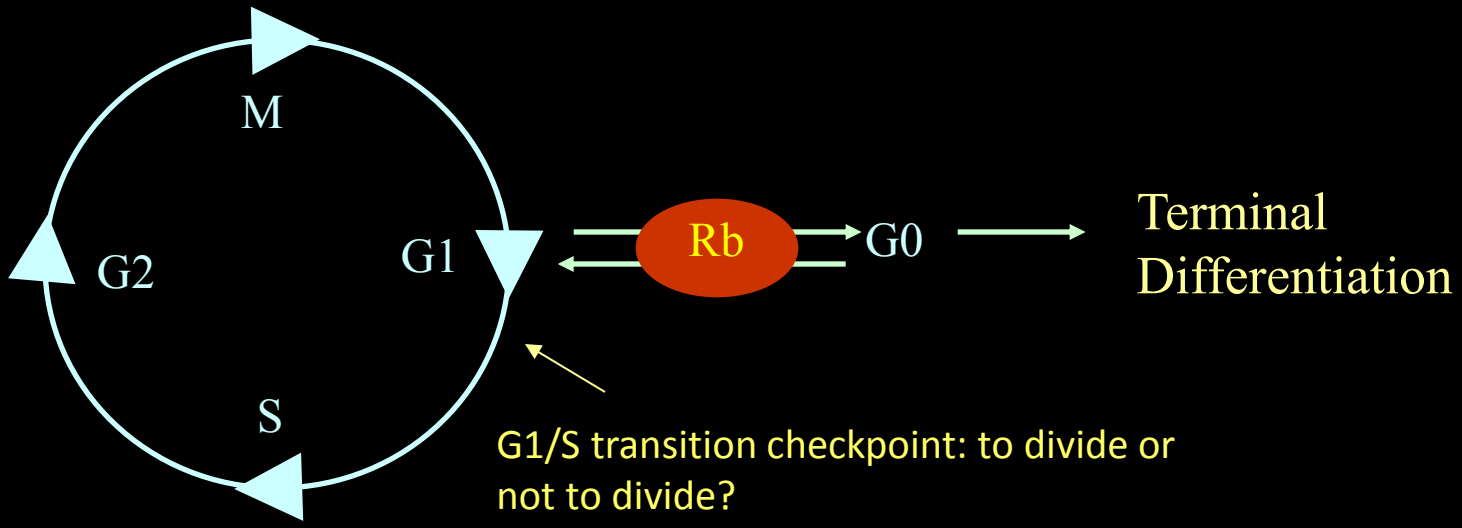
Soluble growth inhibitors, ECM components and cell-cell adhesion. These signals are also received by cell surface receptors coupled to intracellular signaling pathways.

While normal cells **don't** proliferate in the presence of these signals, cancer cells **can ignore them…**

Mechanisms of insensitivity to antigrowth signals…

Main mechanism: inactivation of the Rb (retinoblastoma) pathway.

Almost all, if not all, antiproliferative signals are funneled through the Rb pathway. This pathway is the central and main anti-proliferative cellular circuit!!! Therefore, in order for a normal cell to become a cancer cell **THE RB PATHWAY MUST BE INACTIVATED!!!!** Once this pathway is inactivated, the cell is no longer able to respond to anti-proliferative signals…



Final outcome depends on the balance between mitogenic and anti-mitogenic signals

Mechanisms of inactivation of the Rb pathway commonly found in human cancers…

Abnormal downregulation and/or expression of mutant non-functional of TGFβ receptors (Fynan and Reis, 1993; Markowitz et al., 1995).

Mutational inactivation of Smad4, which transduces signals from the TGFβ receptor to downstream elements in the pathway (Schutte et al., 1996).

Deletion of the locus encoding the p15 gene (Chin et al., 1998).

Mutations that render CDK4 unresponsive to p15 (Zuo et al., 1996).

Rb inactivation by viral proteins coded by oncogenic DNA viruses, such as the Human Papilloma Virus (Dyson et al., 1989).

Abnormal down regulation of integrins and other cell adhesion molecules that send an anti-growth signal through the Rb pathway.

Table 8.3 Molecular changes in human cancers leading to deregulation of the cell cycle clock

Specific alteration	Clinical result
Alterations of pRb	
Inactivation of the *Rb* gene by mutation	retinoblastoma, osteosarcoma, small-cell lung carcinoma
Methylation of *Rb* gene promoter	brain tumors, diverse others
Sequestration of pRb by Id1, Id2	diverse carcinomas, neuroblastoma, melanoma
Sequestration of pRb by the HPV E7 viral oncoprotein	cervical carcinoma
Alteration of cyclins	
Cyclin D1 overexpression through amplification of *cyclin D1* gene	breast carcinoma, leukemias
Cyclin D1 overexpression caused by hyperactivity of *cyclin D1* gene promoter driven by upstream mitogenic pathways	diverse tumors
Cyclin D1 overexpression due to reduced degradation of cyclin D1 because of depressed activity of GSK-3β	diverse tumors
Cyclin D3 overexpression caused by hyperactivity of *cyclin D3* gene	hematopoietic malignancies
Cyclin E overexpression	breast carcinoma
Defective degradation of cyclin E protein due to loss of hCDC4	endometrial, breast, and ovarian carcinomas
Alteration of cyclin-dependent kinases	
CDK4 structural mutation	melanoma
Alteration of CDK inhibitors	
Deletion of 15^{INK4B} gene	diverse tumors
Deletion of 16^{INK4A} gene	diverse tumors
Methylation of $p16^{INK4A}$ gene promoter	melanoma, diverse tumors
Decreased transcription of $p27^{Kip1}$ gene because of action of Akt/PKB on Forkhead transcription factor	diverse tumors
Increased degradation of $p27^{Kip1}$ protein due to Skp2 overexpression	breast, colorectal, and lung carcinomas, and lymphomas
Cytoplasmic localization of $p27^{Kip1}$ protein due to Akt/PKB action	breast, esophagus, colon, thyroid carcinomas
Cytoplasmic localization of $p21^{Cip1}$ protein due to Akt/PKB action	diverse tumors
Multiple concomitant alterations by Myc, N-myc or L-myc	
Increased expression of Id1, Id2 leading to pRb sequestration	diverse tumors
Increased expression of cyclin D2 leading to pRb phosphorylation	diverse tumors
Increased expression of E2F1, E2F2 E2F3 leading to expression of cyclin E	diverse tumor
Increased expression of CDK4 leading to pRb phosphorylation	diverse tumors
Increased expression of Cul1 leading to $p27^{Kip1}$ degradation	diverse tumors
Repression of $p15^{INK4B}$ and $p21^{Cip1}$ expression allowing pRb phosphorylation	diverse tumors

Alteration #3: Limitless Replicative Potential

All mammalian cells have an **intrinsic**, cell autonomous program that limits their multiplication. This program has been compared with a “mitotic clock” which keeps track of the number of times a cell divides. This program operates independently of cell-cell signaling mechanisms.

A normal cell can divide only a finite number of times (~60-80 doublings), and the mitotic clock tracks how many rounds of replication a cell undergoes.

Once a cell has progressed through threshold number of divisions (doublings), the mitotic clock suppresses further cell division. After they stop dividing, cells enter either senescence or quiescence.

Cancer cells have a disrupted mitotic clock, they don’t have any mechanism to keep track of their doublings, therefore they divide without limit. Because of this, cancer cells are said to be **immortal**.

The mitotic clock…

It requires functional pRb and p53!

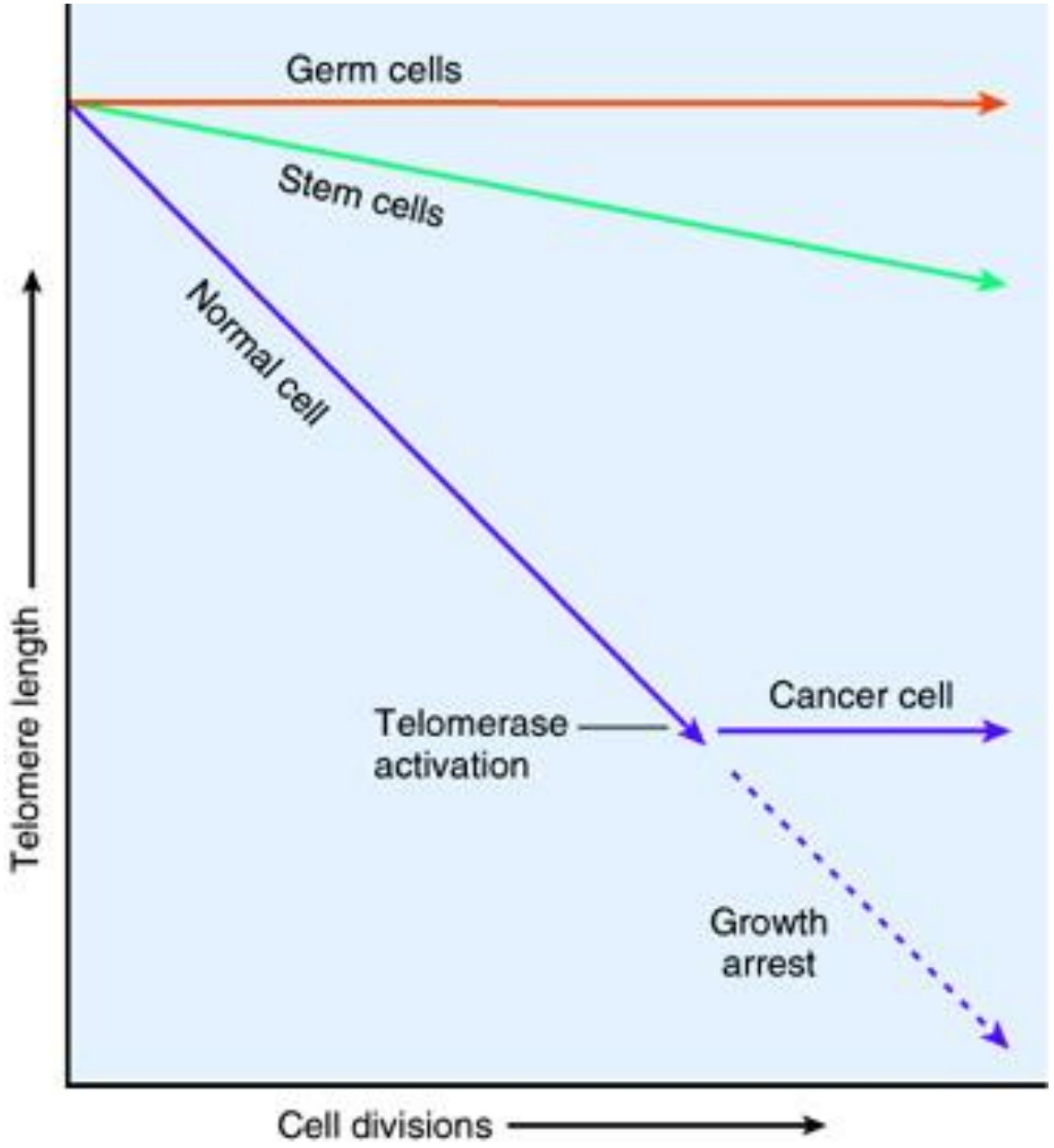
Telomere erosion is its molecular basis (50-100 bp are lost from each telomere during each cell cycle). DNA polymerases responsible for DNA replication cannot replicate telomeres (and the telomerase enzyme, which synthesizes telomeres, is not expressed in differentiated human cells), therefore, telomeres become shorter with each round of division.

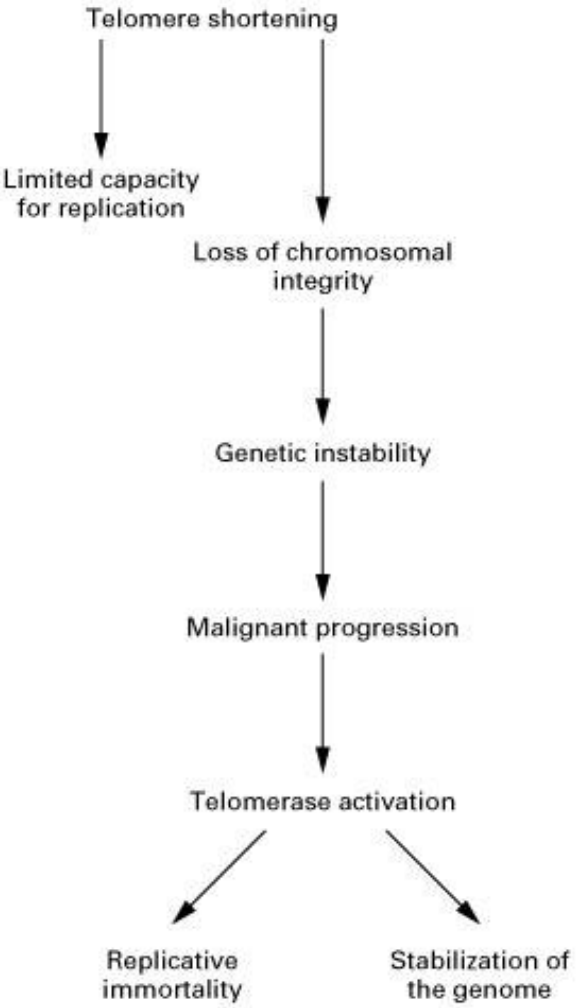
Their progressive erosion limits their capacity to protect chromosome ends, this results in chromosomal abnormalities such as end-to-end chromosomal fusions, etc.

When a minimum critical telomere size is reached, this is interpreted by the mitotic clock as “enough cell division, it is time to stop!!!!”. When this point has been reached, cells withdraw from the cell cycle.

Cancer cells show re-expression of telomerase enzymes or activation of the ALT pathway which maintains telomeres through recombination-based interchromosomal exchanges. Through one or the other of these mechanisms, cancer cells regenerate telomeres at each round of division. Telomere erosion does not occur, the cell can go on dividing way past its allowed number of doublings. **The cell becomes immortal.**

Telomeres, Telomerase & Cancer

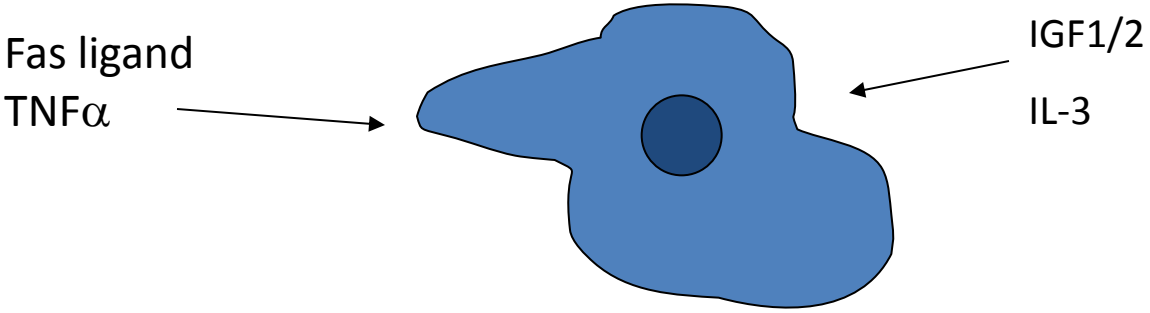




Alteration #4: Evasion of apoptosis

Apoptosis: programed cell death, “cell suicide”, requires activation of certain genes, it is different to necrosis…

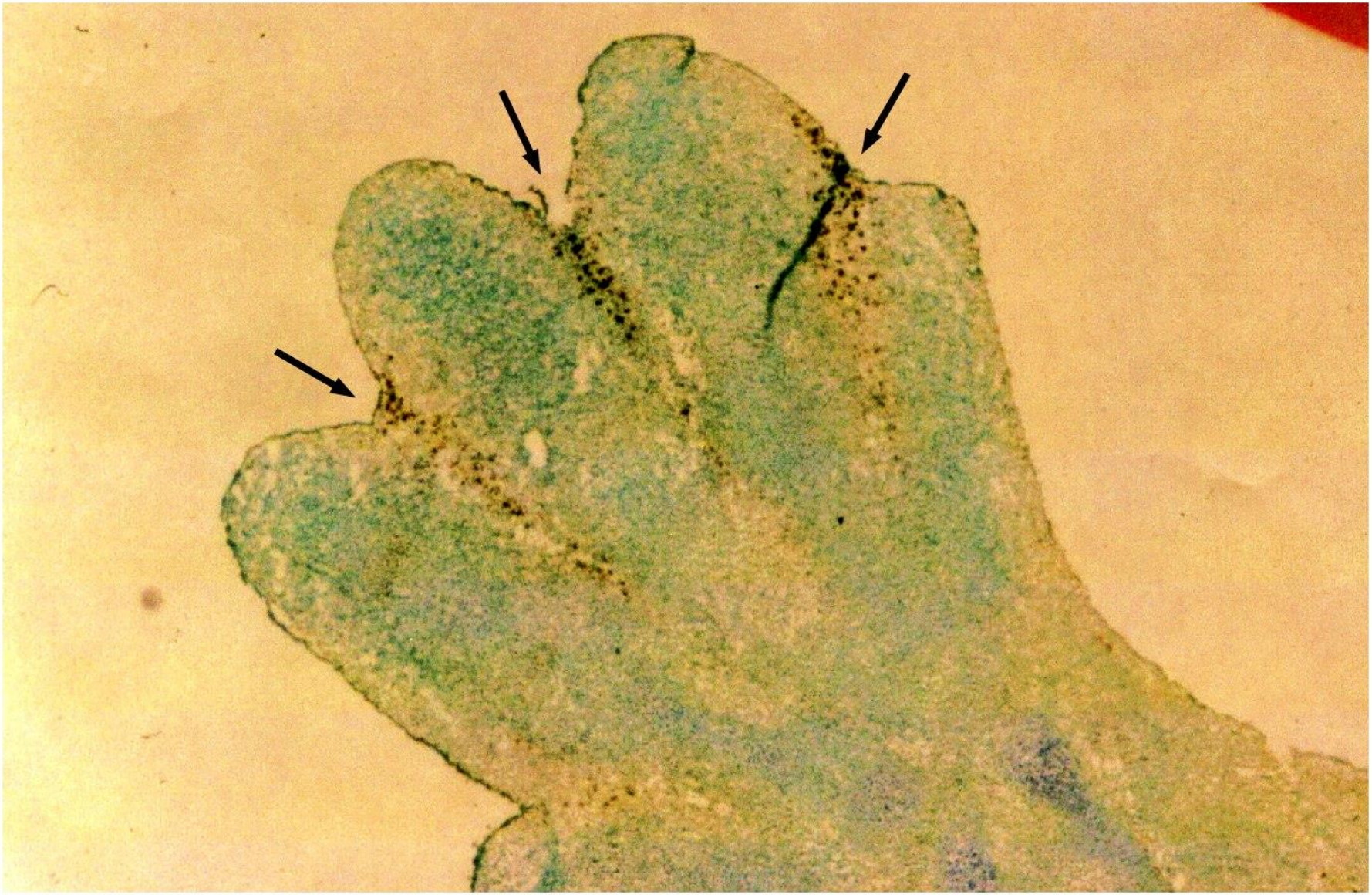
To die or to survive…

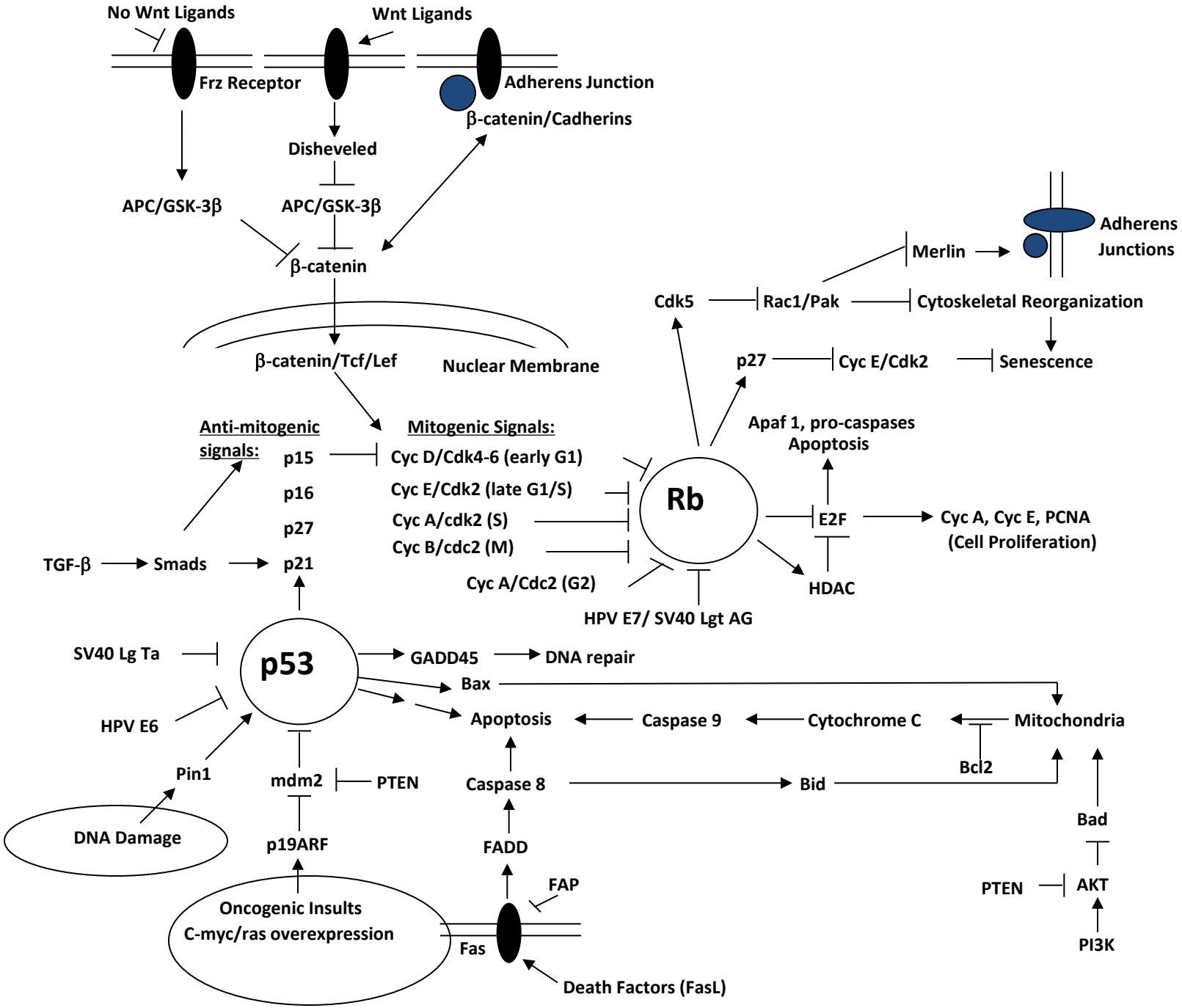


Balance between death vs. survival factors

Apoptosis is triggered by cancer-inducing insults such as oncogene activation, DNA damage, viral infections, etc,

Apoptosis is important in normal morphogenesis




No Wnt Ligands
Wnt Ligands
Frz Receptor
Adherens Junction
β-catenin/Cadherins
Disheveled
APC/GSK-3β
APC/GSK-3β
β-catenin
β-catenin/Tcf/Lef
Nuclear Membrane
Merlin
Adherens Junctions
Cdk5
Rac1/Pak
Cytoskeletal Reorganization
p27
Cyc E/Cdk2
Senescence
Anti-mitogenic signals:
Mitogenic Signals:
p15
p16
p27
p21
Cyc D/Cdk4-6 (early G1)
Cyc E/Cdk2 (late G1/S)
Cyc A/cdk2 (S)
Cyc B/cdc2 (M)
Cyc A/Cdc2 (G2)
Rb
Apaf 1, pro-caspases
Apoptosis
E2F
HDAC
Cyc A, Cyc E, PCNA
(Cell Proliferation)
HPV E7/ SV40 Lgt AG
TGF-β
Smads
SV40 Lg Ta
p53
GADD45
DNA repair
Bax
HPV E6
Apoptosis
Caspase 9
Cytochrome C
Mitochondria
Bcl2
Pin1
mdm2
PTEN
Caspase 8
Bid
Bad
DNA Damage
p19ARF
FADD
FAP
PTEN
AKT
PI3K
Oncogenic Insults
C-myc/ras overexpression
Fas
Death Factors (FasL)

Mechanisms of evasion of apoptosis:

1. p53 inactivation (observed in more than 50% of human cancers)

2. Bcl2 oncogene activation via chromosomal translocation (follicular lymphoma)

3. Disruption of the FAS death signaling circuit (i. e., by mutational inactivation of Fas receptor or by overexpression of mutant non-functional receptors)

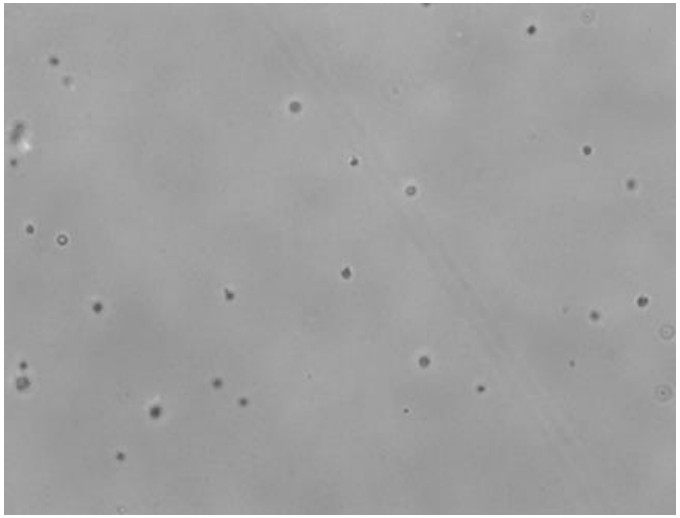
4. Mutations resulting in constitutive activation of the PI3 kinase-AKT/PKB pathway, which transmits anti-apoptosis signals

5. Mutational inactivation of PTEN tumor suppressor

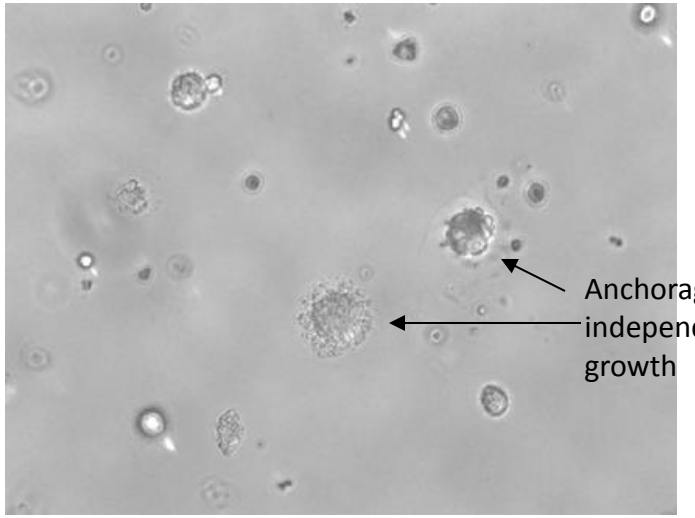
Evasion of Anoikis

Anoikis, from the Greek “homelessness”, is a type of apoptosis that is triggered by loss of contact with a substrate

Normal cells



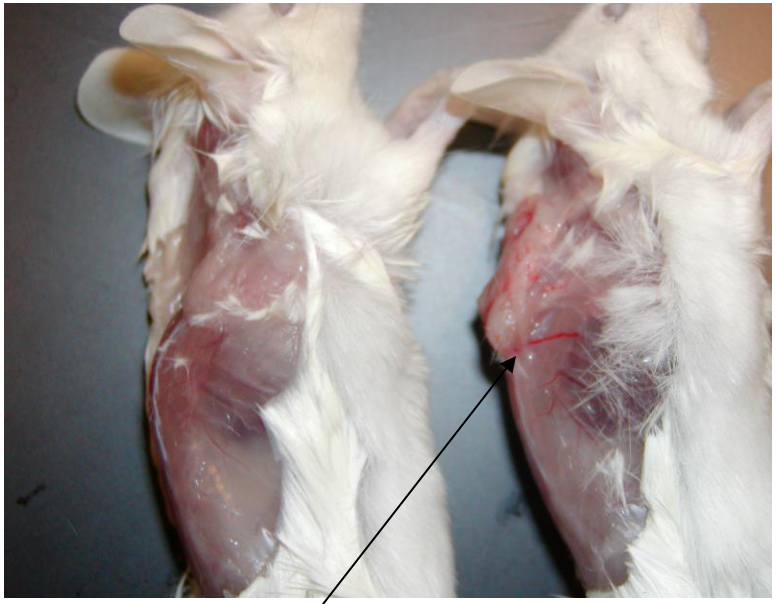
Cancer cells



Loss of anoikis confers cancer cells the capacity to survive and proliferate even when not attached to a substrate (such as the ECM).

Alteration #5: Sustained angiogenesis

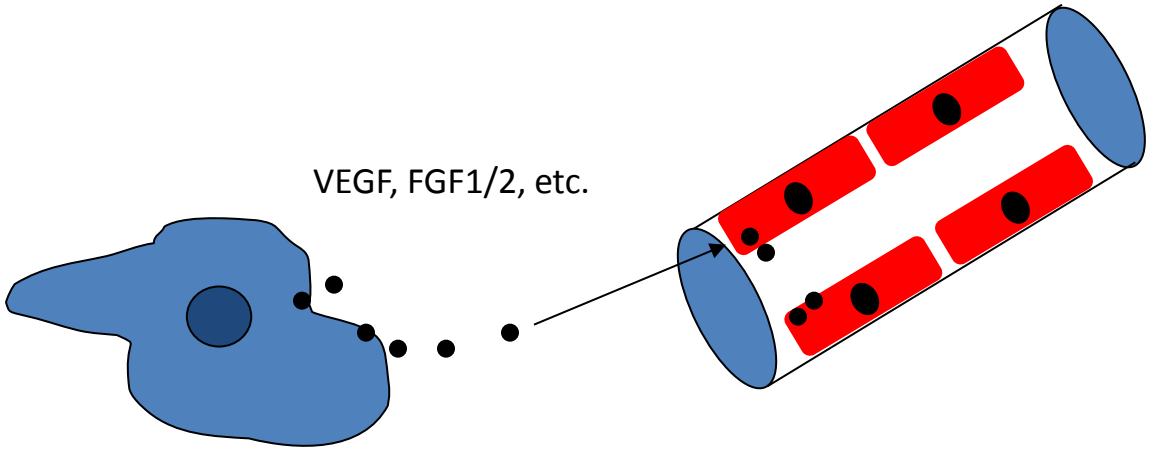
Angiogenesis is the growth of vasculature into tumor masses in order to deliver oxygen and nutrients to tumor cells.



Vascular growth to feed tumor cells

Cancer cells develop angiogenic capacity, i. e., the capacity to attract blood vessels…

Mechanism of angiogenesis: cancer cells attract blood vessels by releasing factors that bind receptors in the surface of endothelial cells…



Cancer cells **overexpress** angiogenesis inducers such as VEGF and FGF1/2, and show down regulation of angiogenesis inhibitors such as thrombospondin - 1 or β-interferon

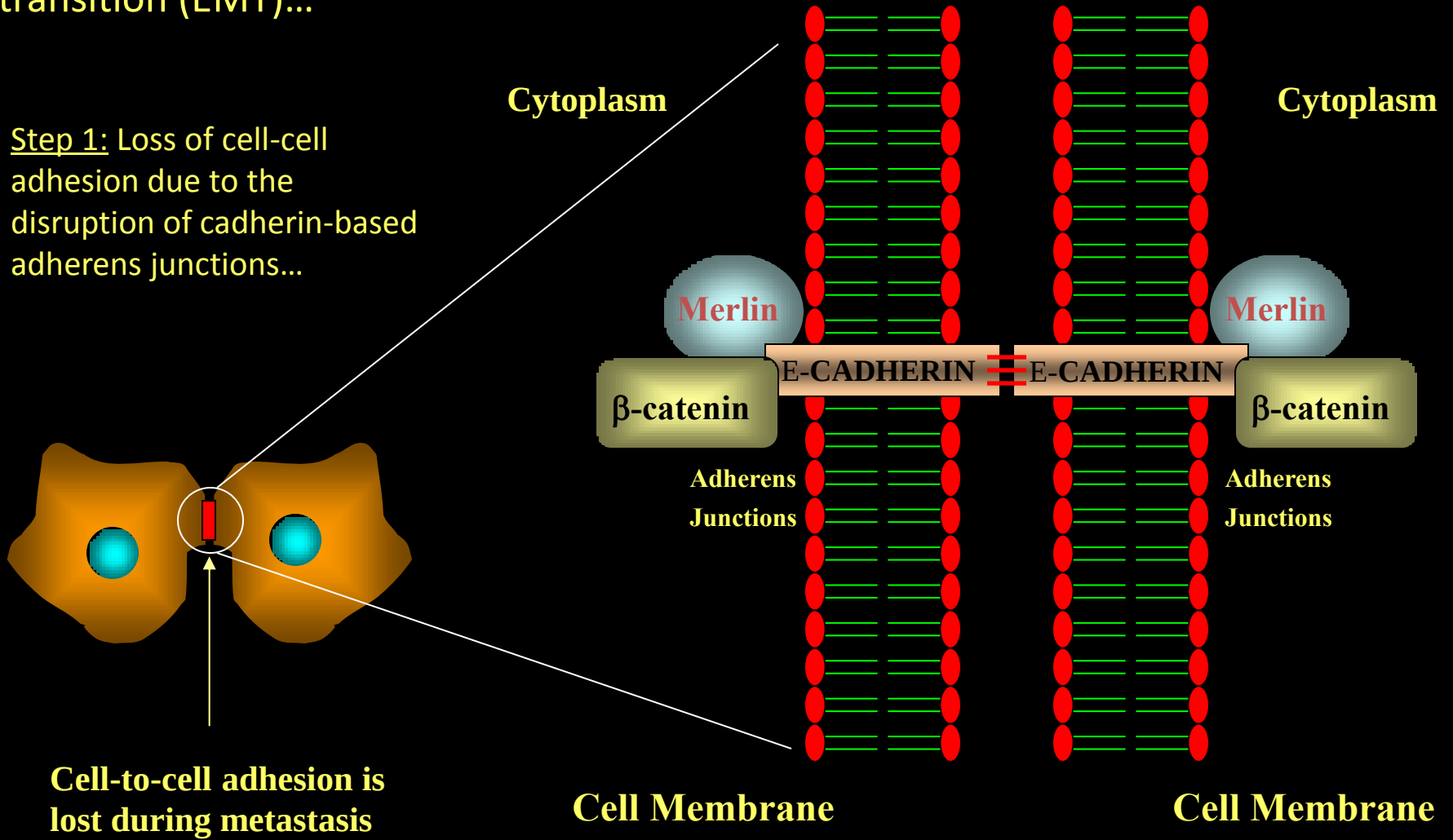
Mechanism of angiogenesis induction by cancer cells…

p53 has been shown to induce thrombospondin - 1 expression. p53 loss during carcinogenesis leads to down regulation of thrombospondin - 1 with consequent increased angiogenesis (Dameron et al., 1994).

Activation of the ras oncogene or loss of the VHL tumor suppressor in certain cell types causes upregulation of VEGF expression (Rak et al., 1995; Maxwell et al., 1999)

Alteration #6: Tissue invasion and metastasis, epithelial to mesenchymal transition (EMT)…

Step 1: Loss of cell-cell adhesion due to the disruption of cadherin-based adherens junctions…

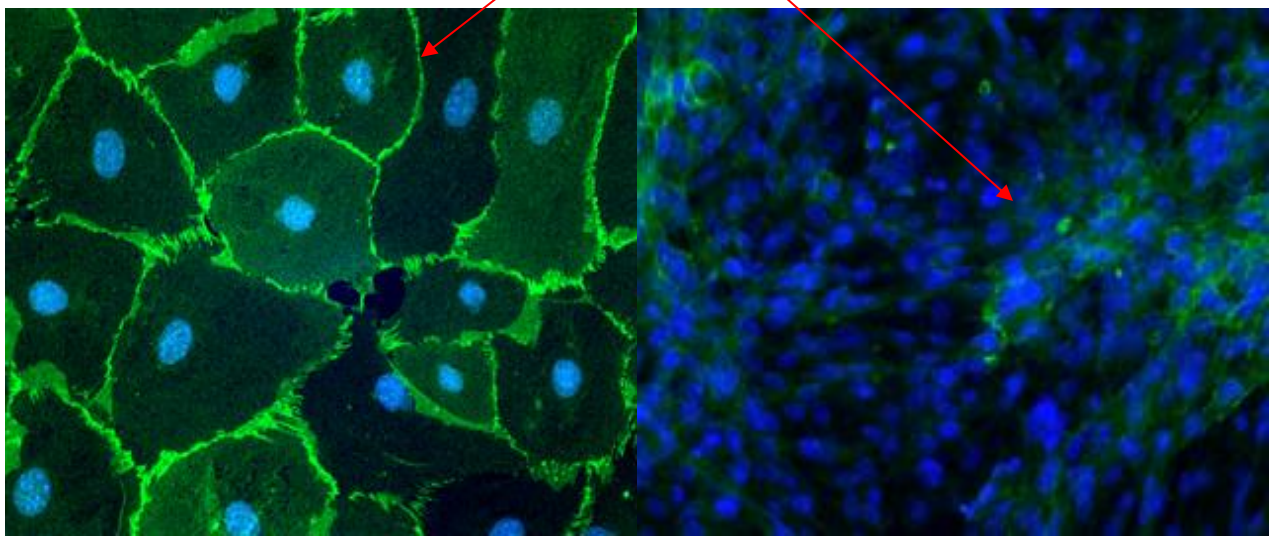


Loss of adherens junctions make cells less adhesive and more metastatic

Alteration #6: Tissue invasion and metastasis, epithelial to mesenchymal transition (EMT)…

Step 1: Adherens junctions are lost during carcinogenesis due to mutational inactivation of cadherin or β-catenin genes, transcriptional repression, or proteolysis of the cadherin extracellular domain (Christofori and Semb, 1999).

Adhrens junctions are lost, cells become less adhesive and escape from contact-dependent growth arrest



Normal cells

Cancer Cells

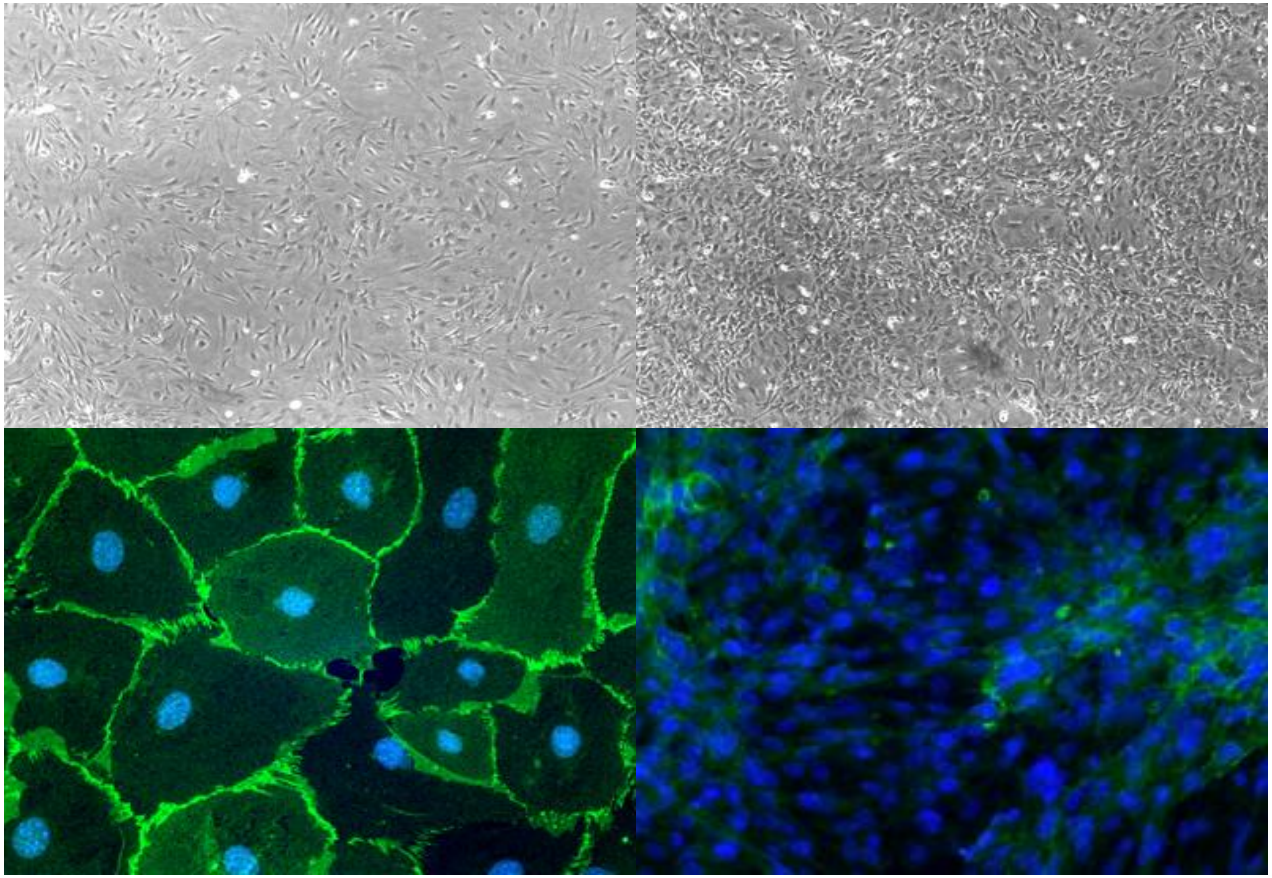
Loss of adherens junctions and cell-to-cell adhesion contribute to EMT!!!!

Loss of contact-dependent growth arrest…

Establishment of cell-cell contact inhibits proliferation of normal cells. Normal cells grow in culture and *in vivo* to form monolayers. Once cell-cell contact has been established, cell proliferation stops.

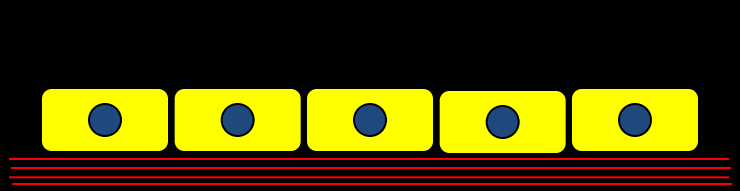
Cancer cells are insensitive to cell-cell contact, they grow in culture to high densities and *in vivo* they grow to form tumors instead of well-organized tissues. Loss of contact-dependent growth arrest is due to the disruption of membrane associated structures involved in cell-cell recognition. These structures are lost or altered during oncogenic transformation.

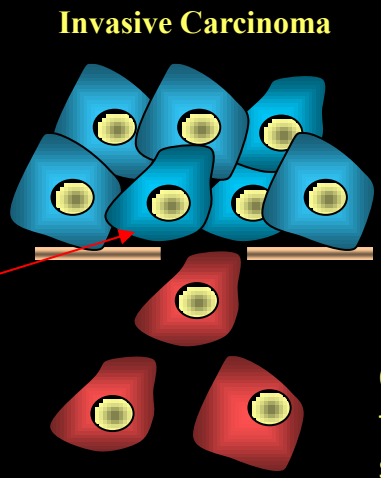
Normal cells | Cancer cells



Alteration #6: Tissue invasion and metastasis, epithelial to mesenchymal transition (EMT)…

Step #2: Degradation of basal lamina enable cancer cells to escape from the tumor, also contributes to EMT…





Tumor is encapsulated in the basal lamina

Production of matrix metalloproteases (MMP) by cancer cells allows them to degrade the basal lamina and escape from the tumor

Once they escape, they invade nearby stroma, lymph nodes and blood vessels

This is achieved by:

1. Upregulation of MMPs
2. Downregulation of MMP inhibitors
3. Increased rate of conversion from inactive zymogen precursor to active enzyme

In many types of carcinomas, matrix-degrading proteases are not produced by the cancer cells themselves, but by conscripted stromal and inflamatory cells (Werb, 1997).

Cancer cell migrating away from tumor...

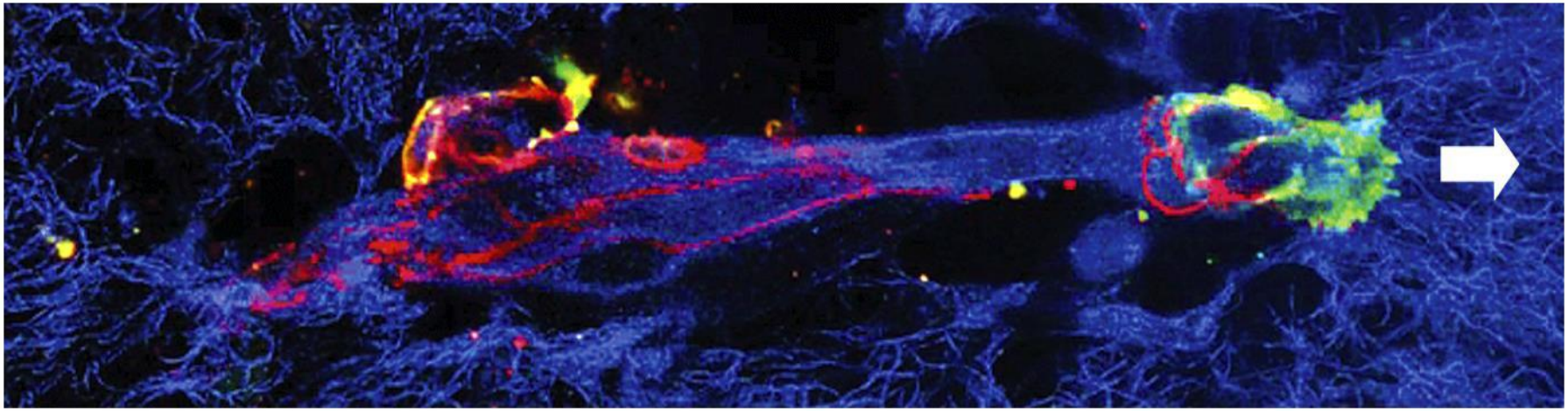
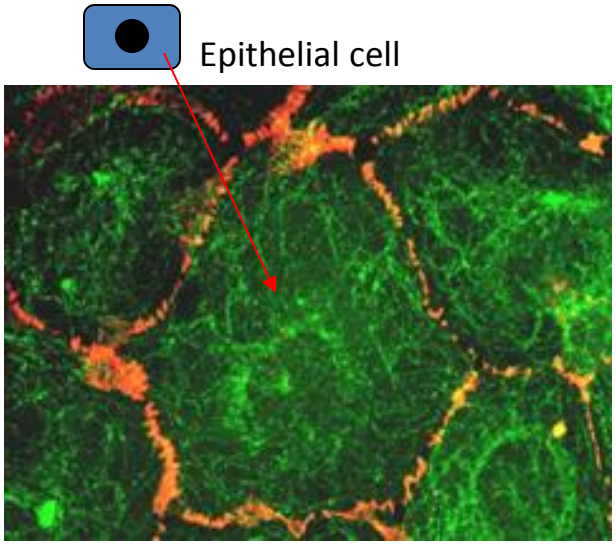


Figure 14-5b The Biology of Cancer (© Garland Science 2007)

Alteration #6: Tissue invasion and metastasis, epithelial to mesenchymal transition (EMT)…

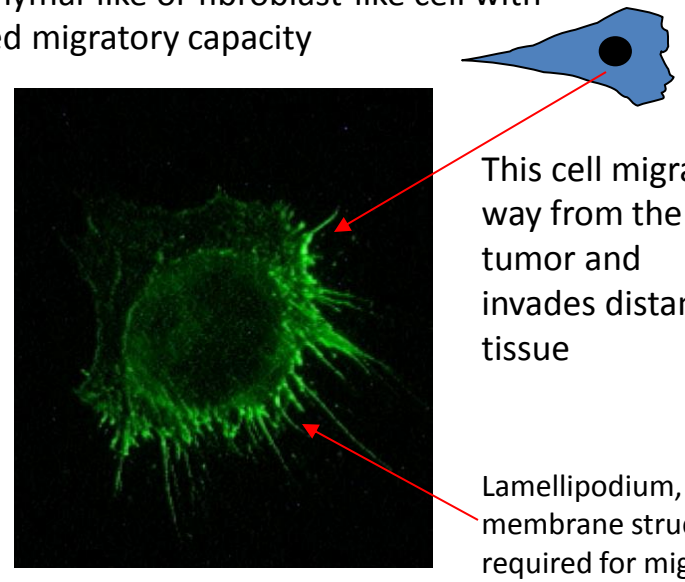
Step #3: enhanced migratory capacity



Cytoskeletal reorganization

Changes in cell shape

Mesenchymal-like or fibroblast-like cell with enhanced migratory capacity



This cell migrates way from the tumor and invades distant tissue

Lamellipodium, membrane structure required for migration

Mechanisms:

The acquisition of this migratory phenotype is due in part **to cytoskeletal reorganization**. This cytoskeletal reorganization promotes changes in cell shape while assisting in the formation of lamellipodia, which are actin-rich specialized areas of the cell membrane involved in migration…

The small Rho GTPases (Rac1, RhoA, Cdc42, etc.) are responsible for controlling cytoskeletal-related processes such as changes in cell shape, migration, cell-to-cell adhesion, etc. As expected, their normal functions are altered during carcinogenesis, and this breakdown in their function can lead to loss of cell-to-cell adhesion and increased migration.

Altered Rho GTPase functions can be the consequence of mutations targeting the genes coding for the GTPases themselves, or genes coding for “upstream” proteins that regulate their activities.

Lamellipodia...

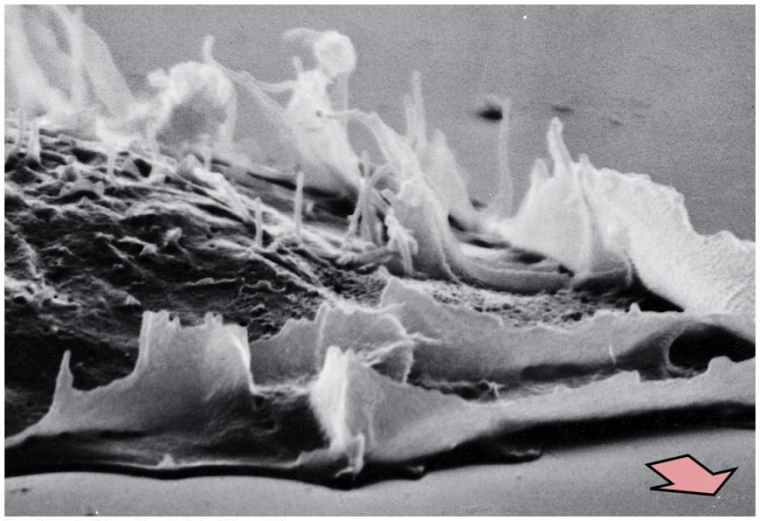
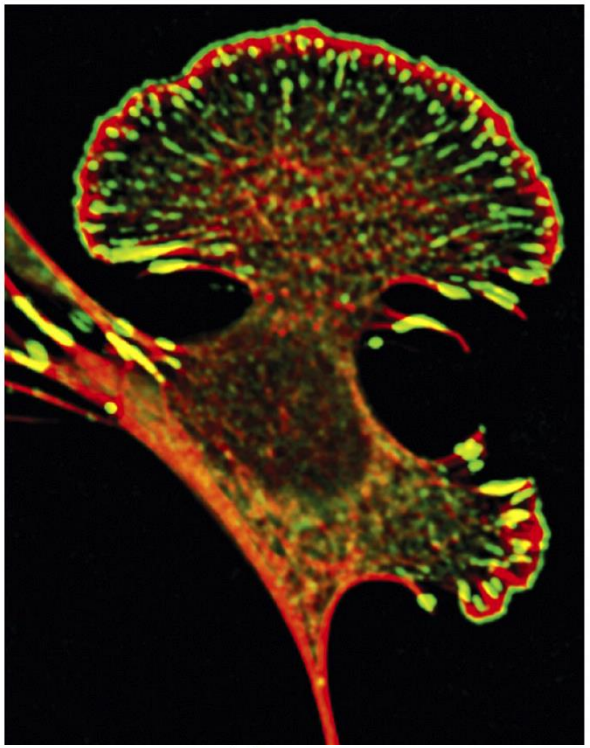


Figure 14-36a The Biology of Cancer (© Garland Science 2007)



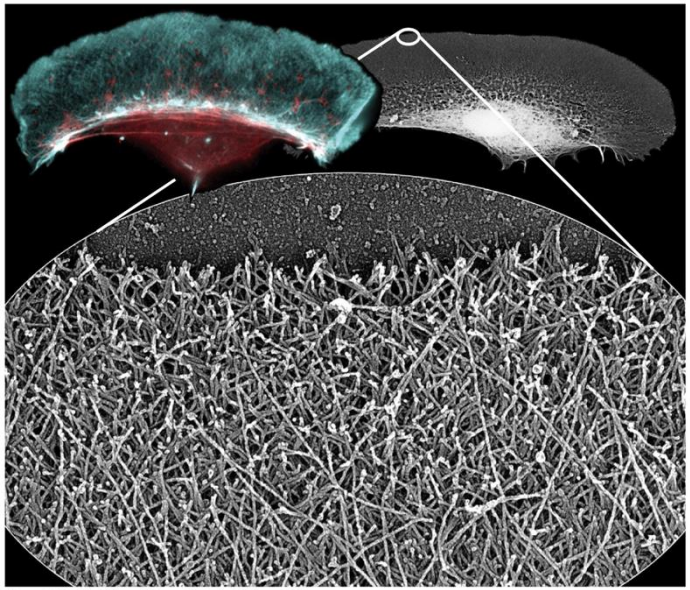


Figure 14-36c The Biology of Cancer (© Garland Science 2007)

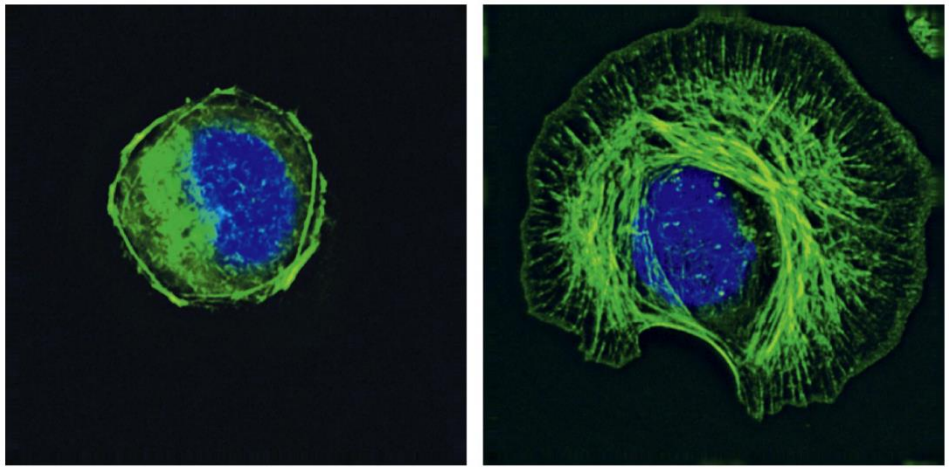
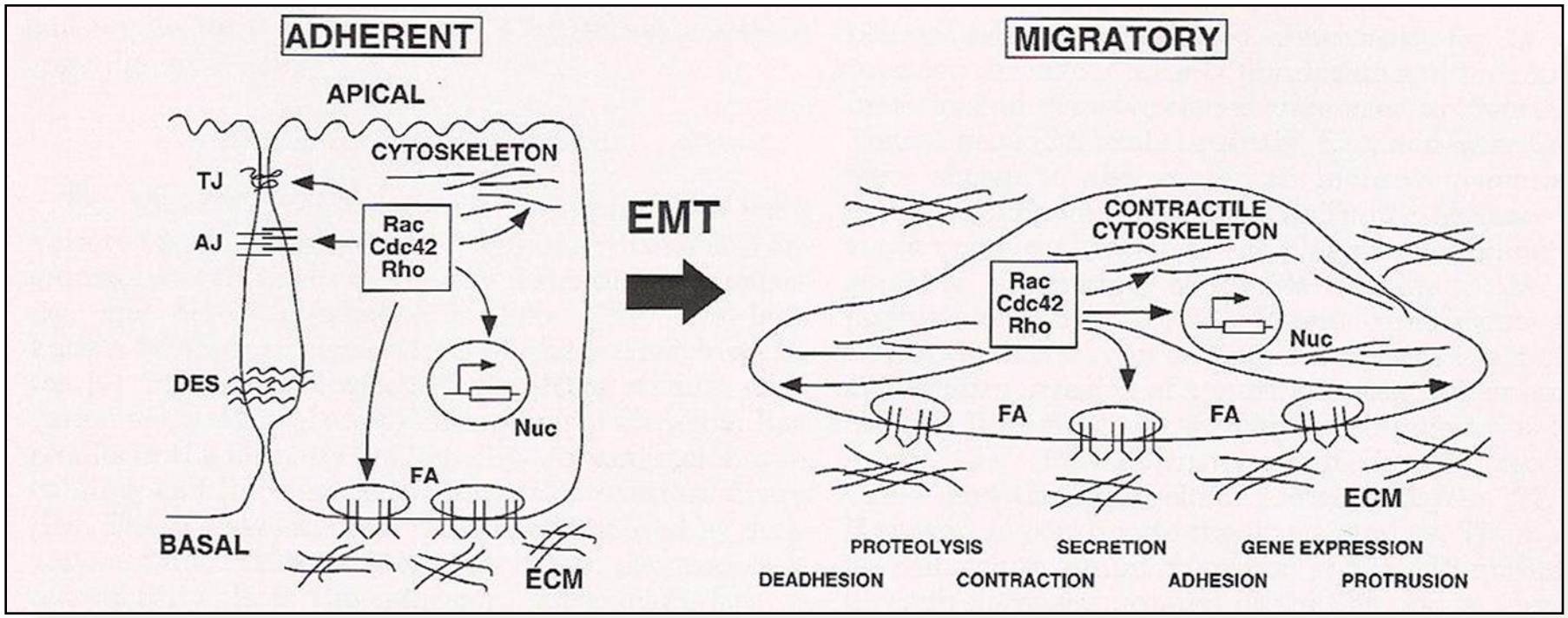


Figure 14-36d The Biology of Cancer (© Garland Science 2007)

Cellular Changes Observed during E

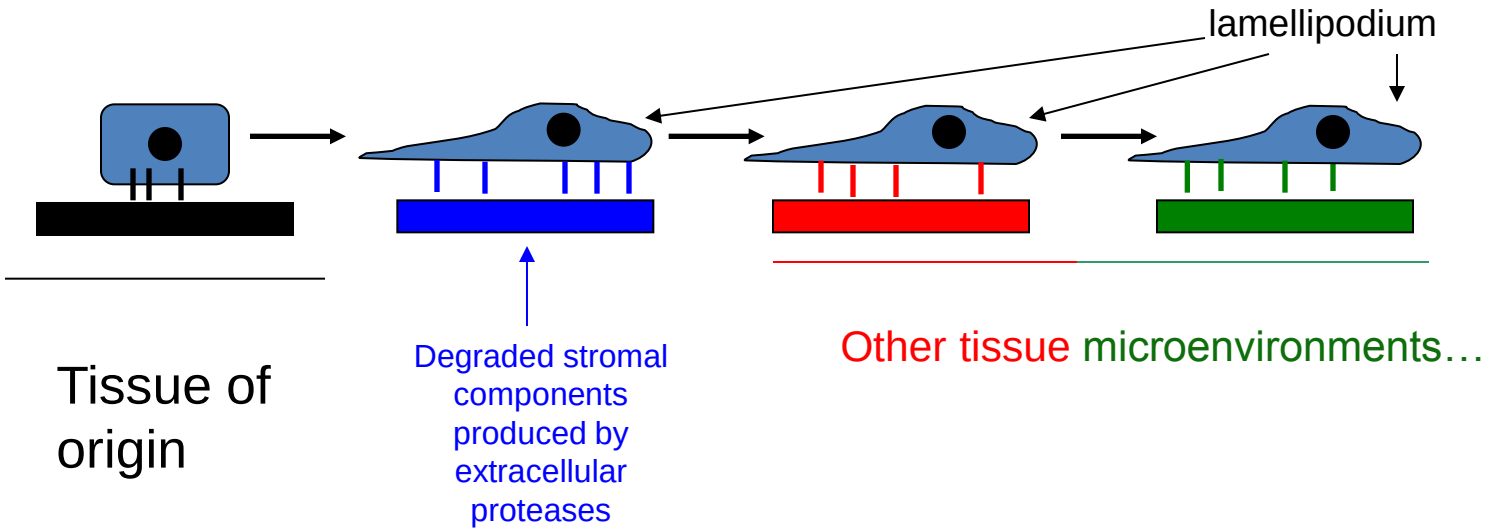


From Schmitz et al., 2000, Exp. Cell Res

Alteration #6: Tissue invasion and metastasis

Step #4: Integrin switch

Invasive and migratory cancer cells experience changing tissue microenvironments and ECM composition during their migrations. They can easily cope with those changes by shifting the spectrum of integrin receptors in their cell membrane. By using this mechanism of integrin shifting, they can invade, colonize and survive in tissues different than their original source…



Mechanisms of integrin switch still await clarification…

The Cellular basis of EMT

Table 14.2 Cellular changes associated with the epithelial–mesenchymal transition

Loss of
Cytokeratin (intermediate filament) expression
Epithelial adherens junction protein (E-cadherin)
Epithelial cell polarity
Acquisition of
Fibroblast-like shape
Motility
Invasiveness
Mesenchymal gene expression program
Mesenchymal adherens junction protein (N-cadherin)
Protease secretion (MMP-2, MMP-9)
Vimentin (intermediate filament) expression
Fibronectin secretion
PDGF receptor expression
αvβ6 integrin expression

Table 14-2 The Biology of Cancer (© Garland Science 2007)

EMT during embryogenesis…

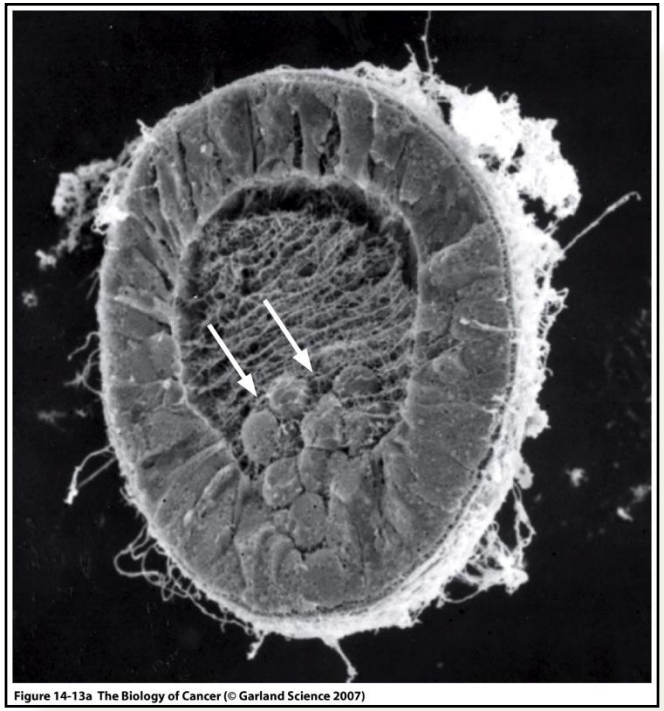


Figure 14-13a The Biology of Cancer (© Garland Science 2007)

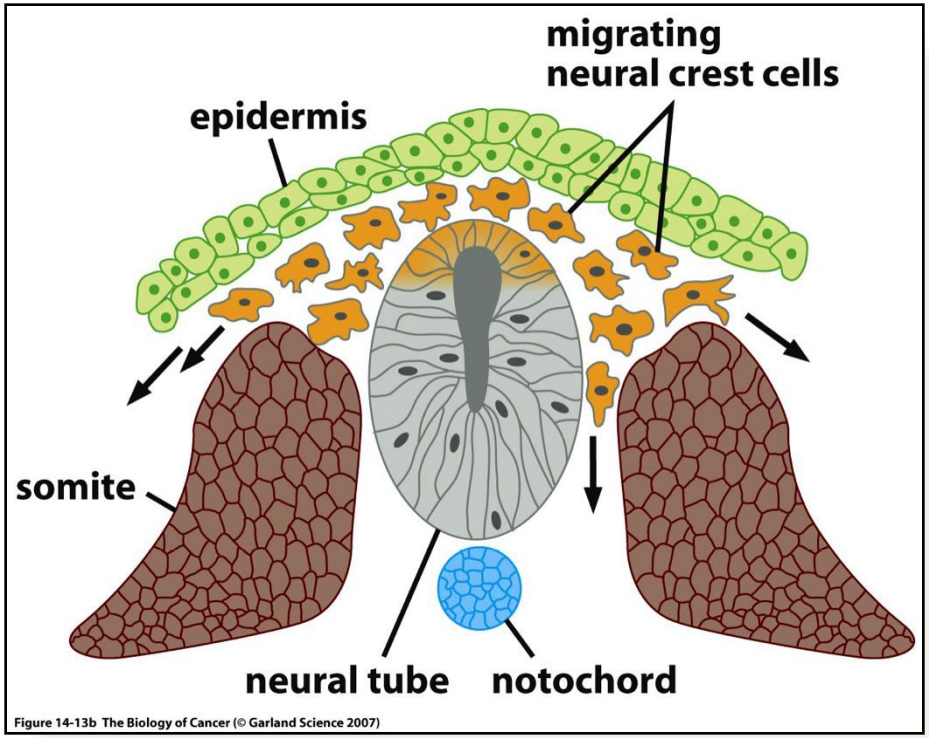


Figure 14-13b The Biology of Cancer (© Garland Science 2007)

Embryonic transcription factors that regulate EMT...

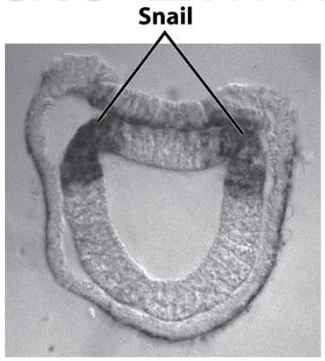


Figure 14-26a The Biology of Cancer (© Garland Science 2007)

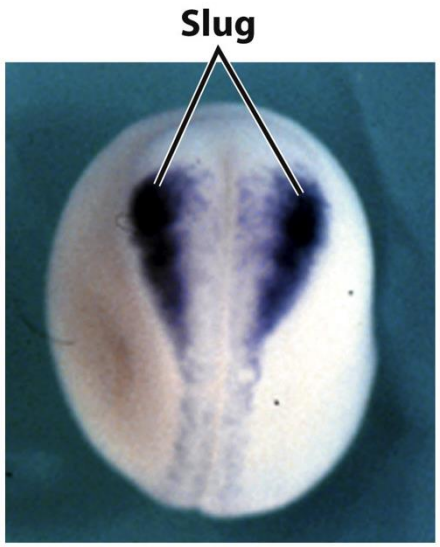


Figure 14-26c The Biology of Cancer (© Garland Science 2007)

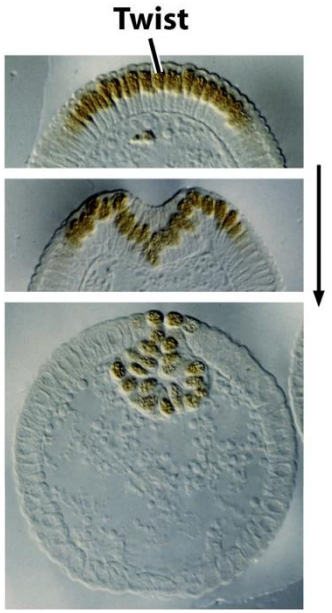


Figure 14-26b The Biology of Cancer (© Garland Science 2007)

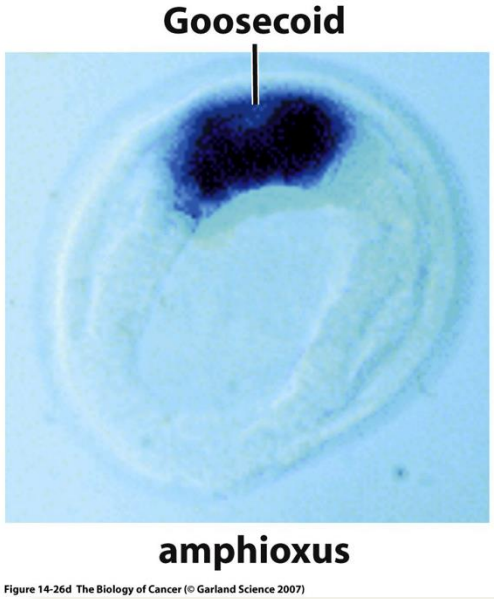


Figure 14-26d The Biology of Cancer (© Garland Science 2007)

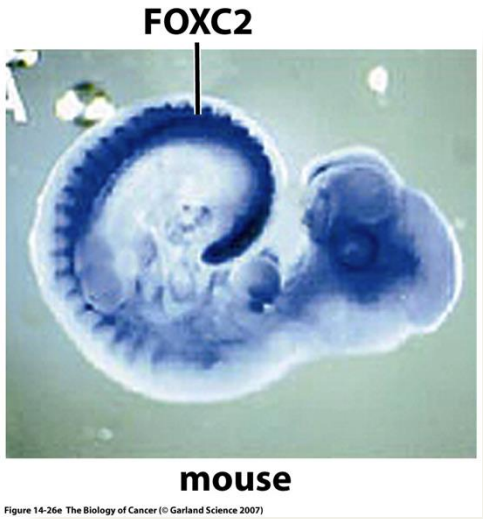


Figure 14-26e The Biology of Cancer (© Garland Science 2007)

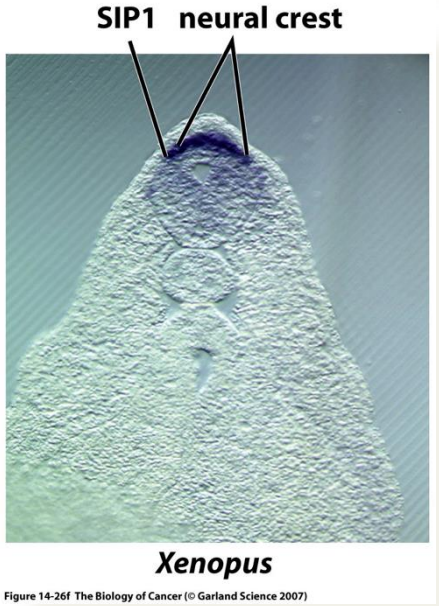


Figure 14-26f The Biology of Cancer (© Garland Science 2007)

Transcription factors involved in development and cancer...

Table 14.3 Transcription factors orchestrating the EMT

Name	Where first identified	Type of transcription factor	Cancer association
E47/E2A	associated with E-cadherin promoter	bHLH	
FOXC2	mesenchyme formation	winged helix/forkhead	basal-like breast cancer
Goosecoid	gastrulation in frog	paired homeodomain	various carcinomas
SIP1	neurogenesis	2-handed zinc finger/homeodomain	ovarian, breast, liver carcinomas
Slug	delamination of the neural crest and early mesoderm in chicken	C2H2-type zinc finger	breast cancer cell lines, melanoma
Snail	mesoderm induction in *Drosophila;* neural crest migration in vertebrates	C2H2-type zinc finger	invasive ductal carcinoma
Twist	mesoderm induction in *Drosophila;* emigration from neural crest	bHLH	invasive lobular breast cancer, diffuse-type gastric carcinoma, high-grade melanoma and neuroblastoma

Table 14-3 The Biology of Cancer (© Garland Science 2007)

Three additional landmarks: The hallmarks of cancer revisited (Weinberg and Hanahan, 2012)

- 1. Metabolic reprogramming (Warburg effect)
- 2. Evasion of the immune system
- 3. Role (recruitment) of the normal stroma-cancer tumors as complex tissues

Tumor dissemination…

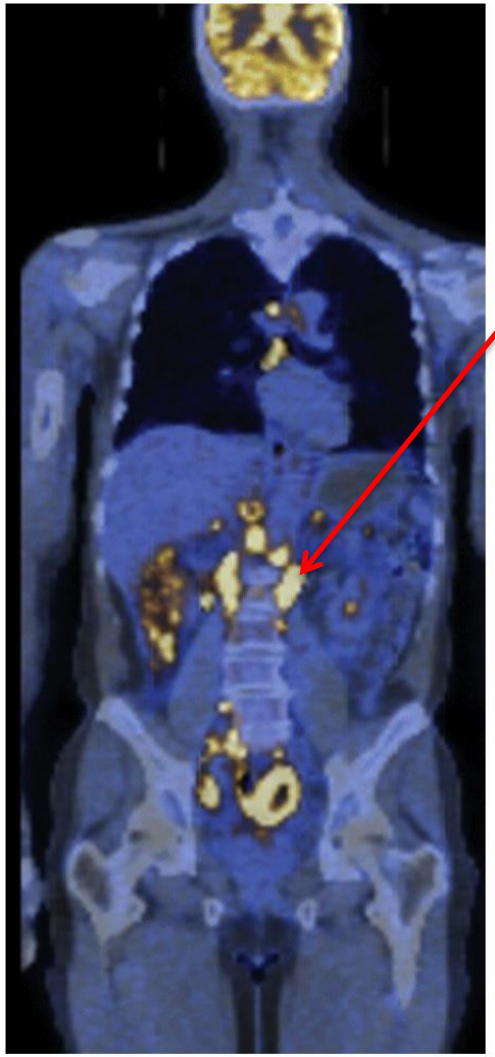


Figure 14-1 The Biology of Cancer (© Garland Science 2007)

Tumors increase their glucose uptake due to the Warburg effect

Primary tumors and their metastatic tropism…

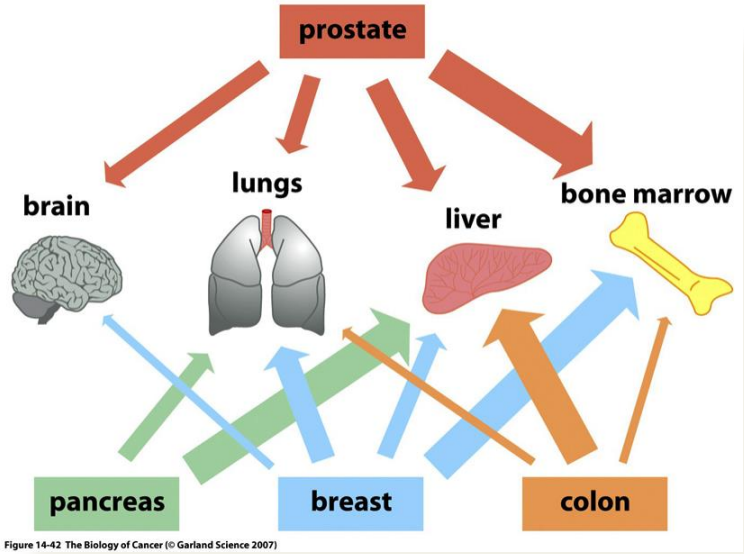


Figure 14-42 The Biology of Cancer (© Garland Science 2007)

Other important aspects of cancer…

Genomic instability: what came first, the chicken or the egg??

Cancer cells are known to harbor lots of genetic changes, ranging from point mutations, deletions and insertions, etc. to to gross changes at the chromosomal level.

Do these genetic changes drive the process of carcinogenesis or are caused by it? Do cancer cells have a higher rate of accumulation of mutations than normal cells (a mutator phenotype)??

Causes of cancer: cancer-associated genetic alterations can have the following causes:

- Spontaneous changes (somatic or germline mutations) arising from an inherent error rate in the fidelity of DNA replication and/or repair. It is known that human cells have a background or spontaneous mutation rate of one "mistake" per 10 billion base pairs copied. It has been estimated that a human being can acquire ~2.8 x 10^{15} point mutations in a life time (Loeb, 1991).

- Chemical carcinogens in the environment (diet, lifestyles, etc.) that directly reacts with DNA damaging or mutating it. Some examples: adduct formation by chemical carcinogens causes distortion of the DNA double helix leading to frame-shift mutations during replication. Alkylated bases in DNA can mispair with the wrong base during replication, etc.

- Infection with oncogenic viruses, such as some strains of the Human Papilloma Virus (HPV).

Things to think about…..

The conversion of a normal cell into a cancer cell is a very unlikely process!!!!

Think about all the obstacles for carcinogenesis to occur…

1. The acquisition of the six alterations observed in cancer cells reflects changes (i.e. mutations) in the genome of cancer cells. The chances that a normal cell will acquire all the mutations in specific genes that will turn it into a cancer cell are very, very, very small !!!!!!!!
2. These is a complex array of DNA monitoring and repairing processes that protect cells from acquiring mutations.
3. If a mutation does "escape" all of the DNA monitoring and repairing processes, cell cycle checkpoint controls are in place to block cell cycle progression. These mechanisms avoid the mutation to be passed on to daughter cells during cell division.
4. When everything else fails, the cell can always commit suicide (apoptosis).
5. Even if apoptosis fails, the immune system can eliminate cancer cells.

Then, how does cancer occur at all!!!!!

The Hallmarks of Cancer (Hanahan and Weinberg, 2000):

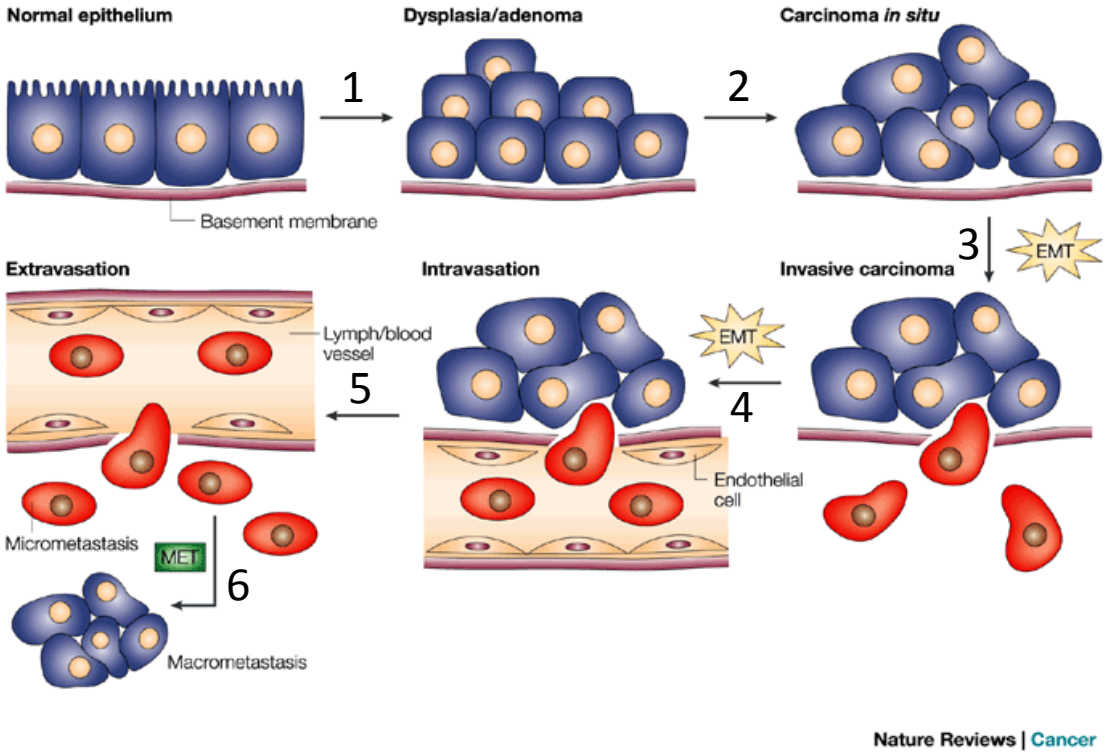
A vast catalog of genetic alterations produce a wide spectrum of cancer types…

…but this large number of genetic alterations that drive cancer progression manifests itself at the cellular level as **six** essential alterations in cell function that collectively drive malignant transformation and growth…

These six alterations in cellular behavior are shared by most, if not all, types of human cancers:

1. Self-sufficiency in growth signals- conversion from proto-oncogenes to oncogenes
2. Insensitivity of anti-growth signals- Rb inactivation
3. Evasion of apoptosis- p53 inactivation
4. Limitless replicative potential- reactivation of telomerases
5. Sustained angiogenesis- VEGF upregulation
6. Tissue invasion and metastasis- Epithelial-to-mesenchymal Transition

Model for carcinoma progression



Mutations and their effects on cell behavior:

1. Confer a **proliferative advantage**- oncogenes and tumor suppressors, e. g., **pRb,** p53, Ras, etc.

2. Help to **evade contact-dependent growth arrest.**

3. & 4. **EMT**- **cadherin switch, loss of cell adhesion**, degradation of basal lamina by **MMP**, acquisition of a **migratory phenotype**.

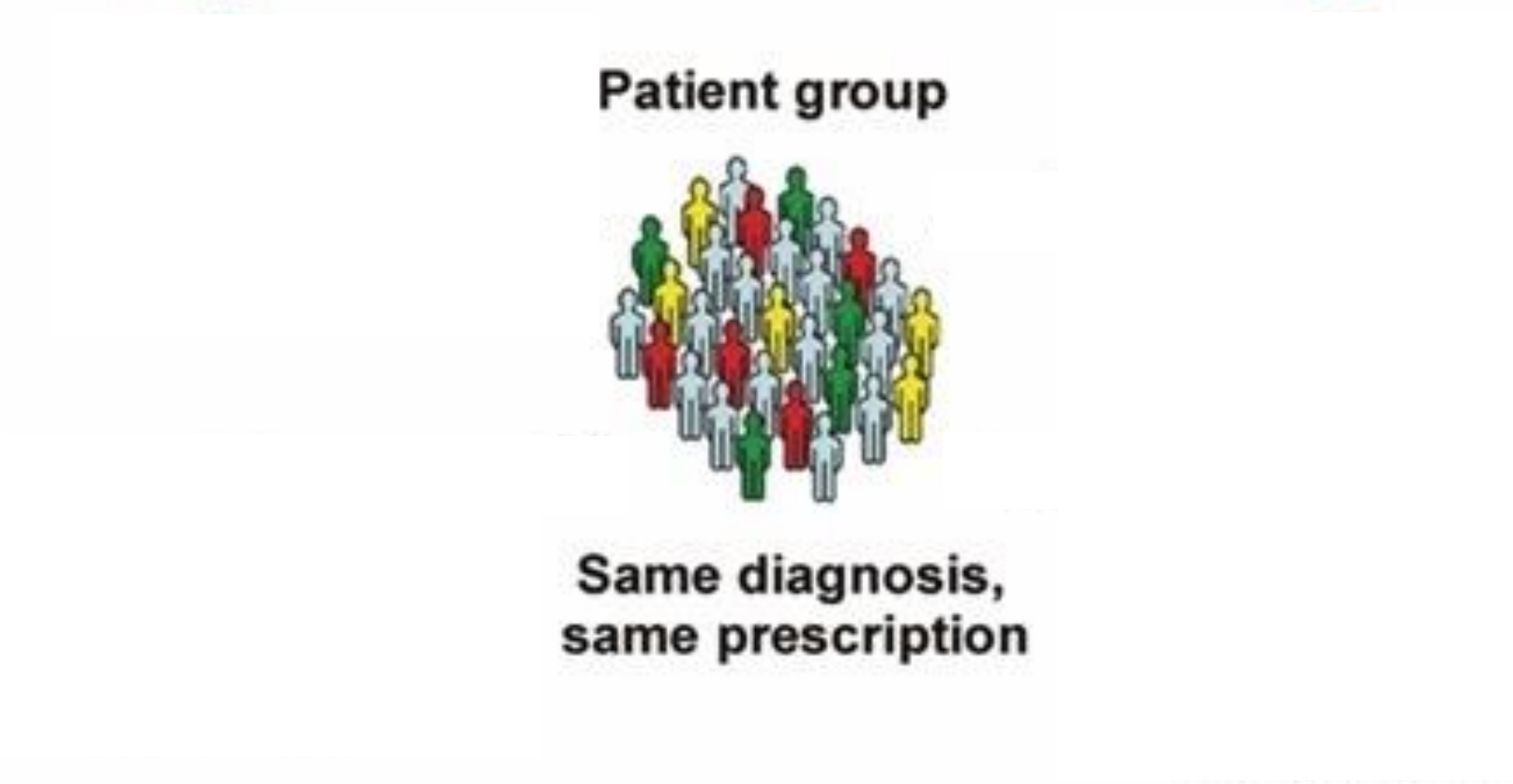
5. **Evasion of anoikis** and acquisition of **anchorage-independent growth.**

6. Change of adhesion molecules, **integrin switch, MET, colonizing and survival** capacities

Model by Jean Paul Thiery
Nature Reviews Cancer **2**, 442-454 (June 2002)
doi:10.1038/nrc822

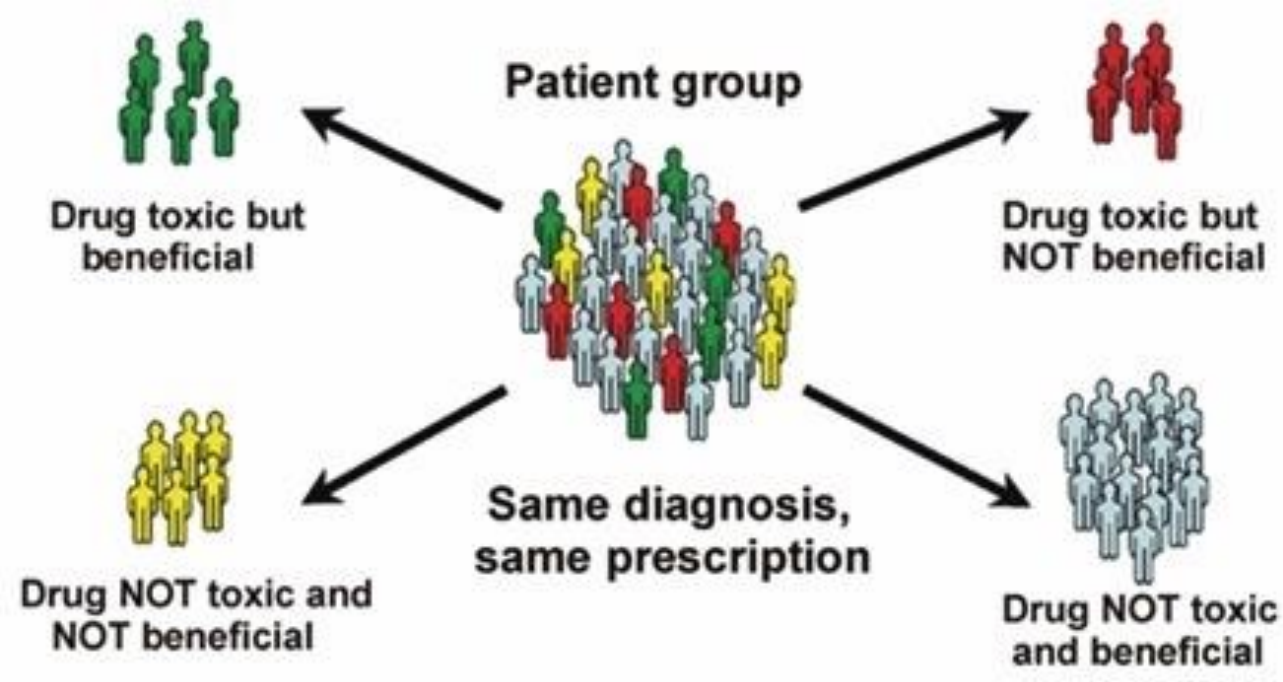
Part III
The Evolution of Cancer Treatment: The Road to ***Personalized Medicine***

Traditional Non-customized Approach: Same disease, same medicine



From: http://mytorontocanadambastudentexperience.blogspot.com/2012/10/personalized-medicine-or-p4-medicine.html

Traditional Non-customized Approach: Same disease, same medicine



From: http://mytorontocanadambastudentexperience.blogspot.com/2012/10/personalized-medicine-or-p4-medicine.html

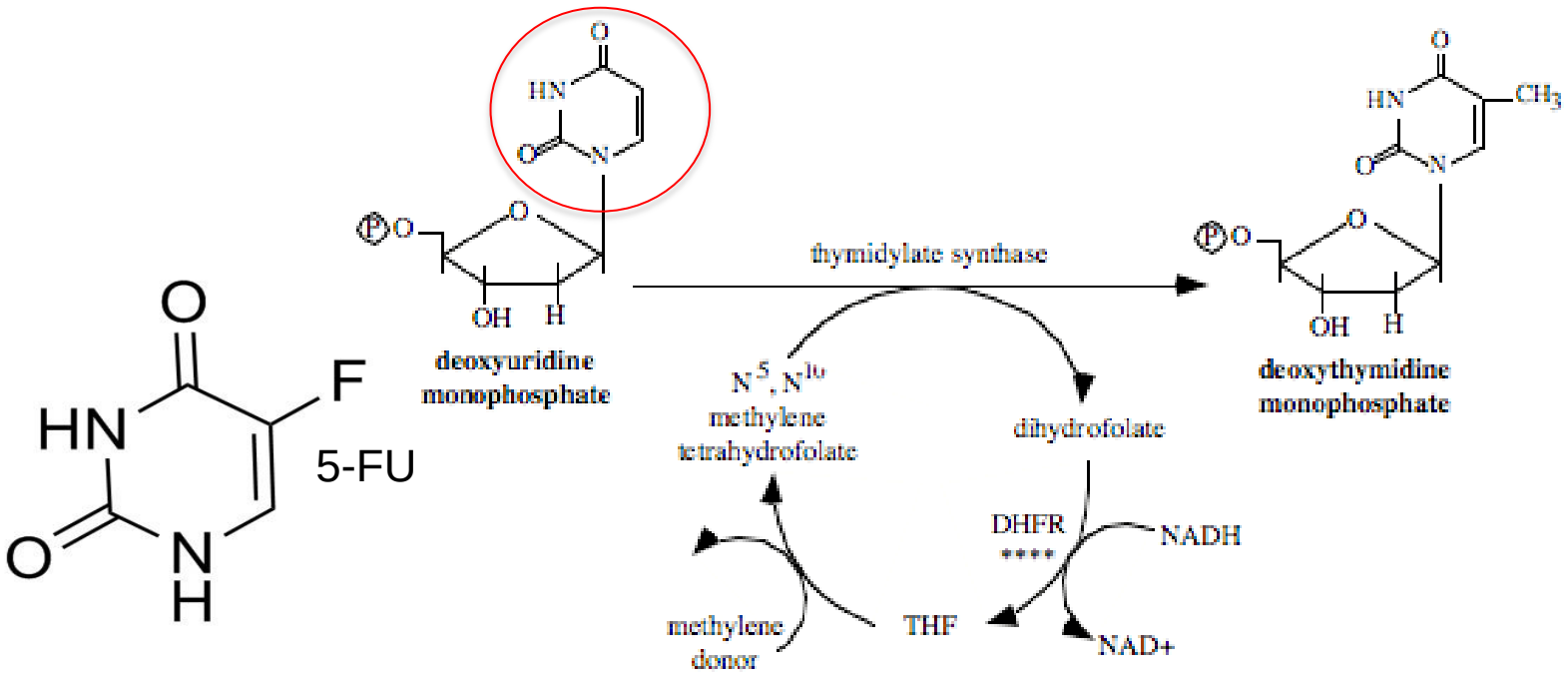
“Classic” Anti-cancer drugs: Proliferation Inhibitors

5-Fluorouracil (5-FU) in cancer treatment:

5-FU was one of the first anticancer drugs to be used in humans, it has been in use for more than 40 years now and still in use today.

It is a pyrimidine analog that works as an irreversible inhibitor of thymidylate synthase (TS), which converts deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP).

dTMP is then phosphorylated to produce the dTTP



- **5-FU common side effects:**

Nausea

GI Ulceration

Vomits

Mucositis

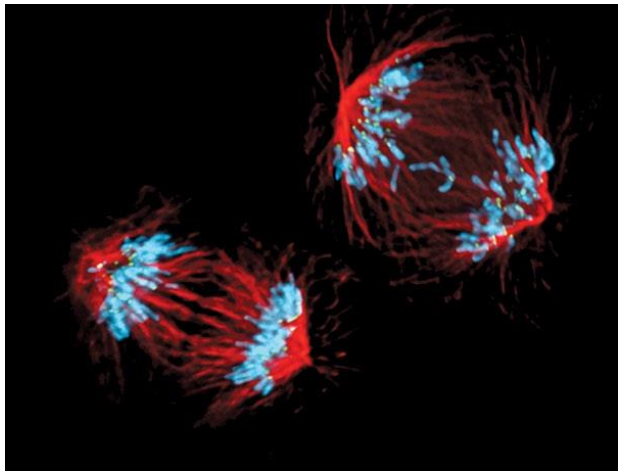
Diarrhoea

Alopecia

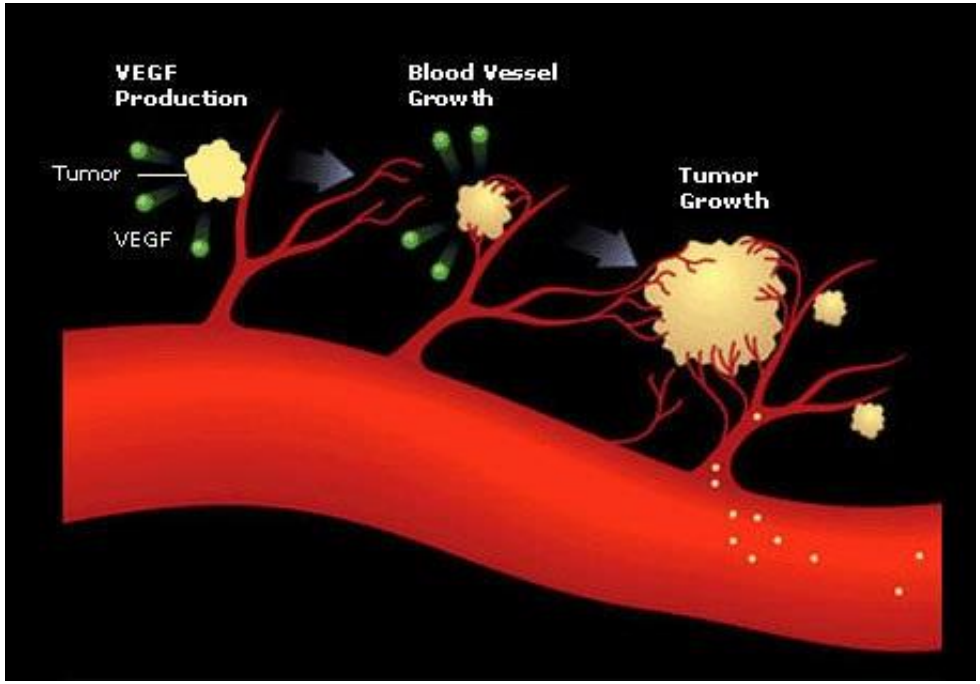
Myelosuppression

Proliferation inhibitors: Taxanes

1. Paclitaxel (taxol), Docetaxel
2. Produced by endophytic fungi in the bark of the Pacific yew tree, *Taxus brevifiora*
3. Microtubule stabilizer that blocks chromosome segregation
4. Severe side effects



By Amos Esty, chttp://dartmed.dartmouth.edu/summer09/html/disc_division.php

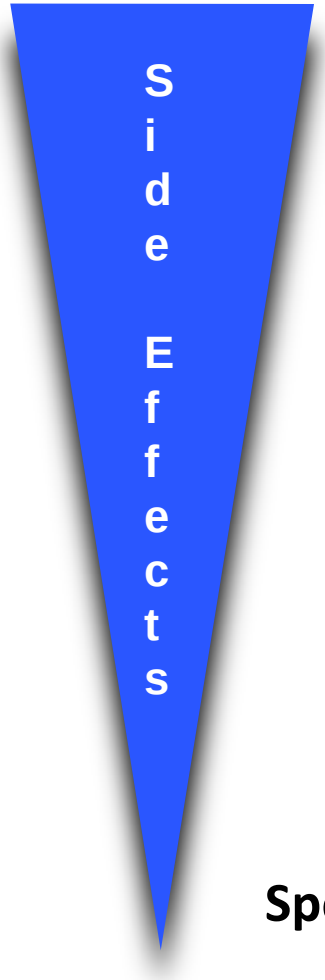


Angiogenesis Inhibitors

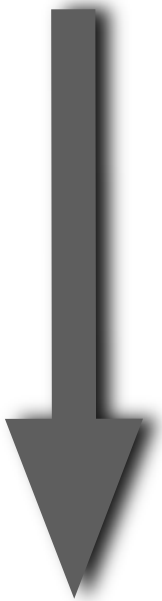
From: http://trialx.com/curetalk/2013/01/anti-angiogenic-therapy-for-treatment-of-cancer/

1. Bevacizumab (Avastin®)
2. Anti-VEGF antibody
3. Side effects: interferes with wound healing and development of co-lateral circulation after blood vessel blockage, worsening of coronary artery disease, hypertension, bleeding, bowel perforations
4. Has been associated with 52 cases of necrotizing fasciitis between 2007 and 2012

The Evolution of Anti-cancer Treatments

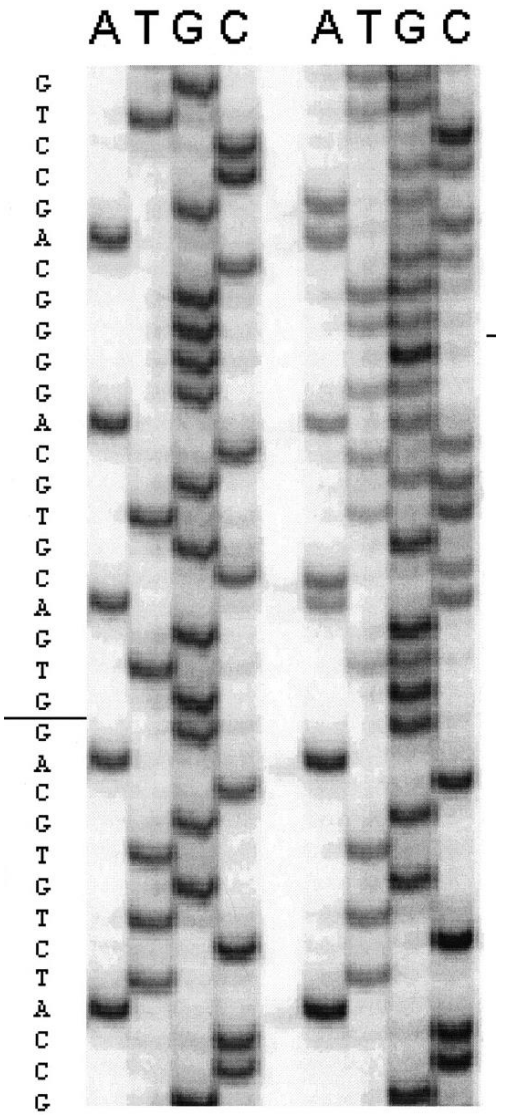


Non-specific inhibition of cellular processes



Specific inhibition of tumor - specific mutant proteins

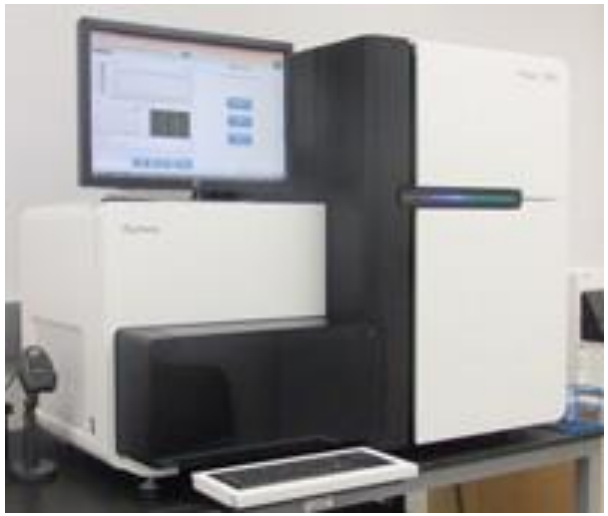
The Evolution of Sequencing

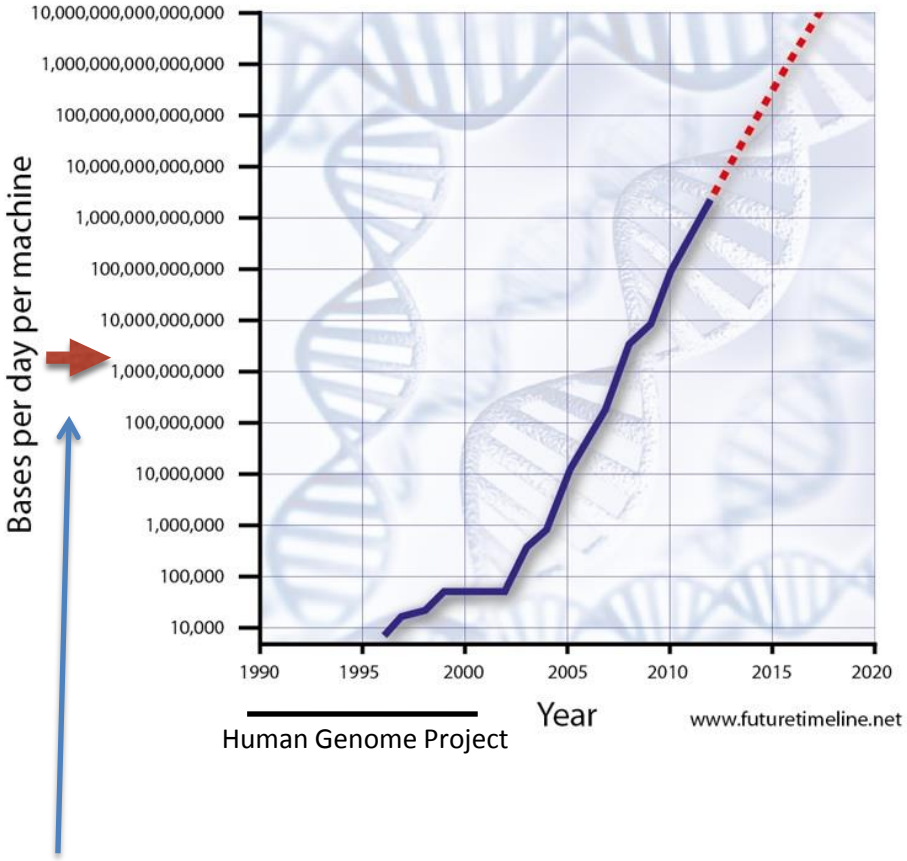


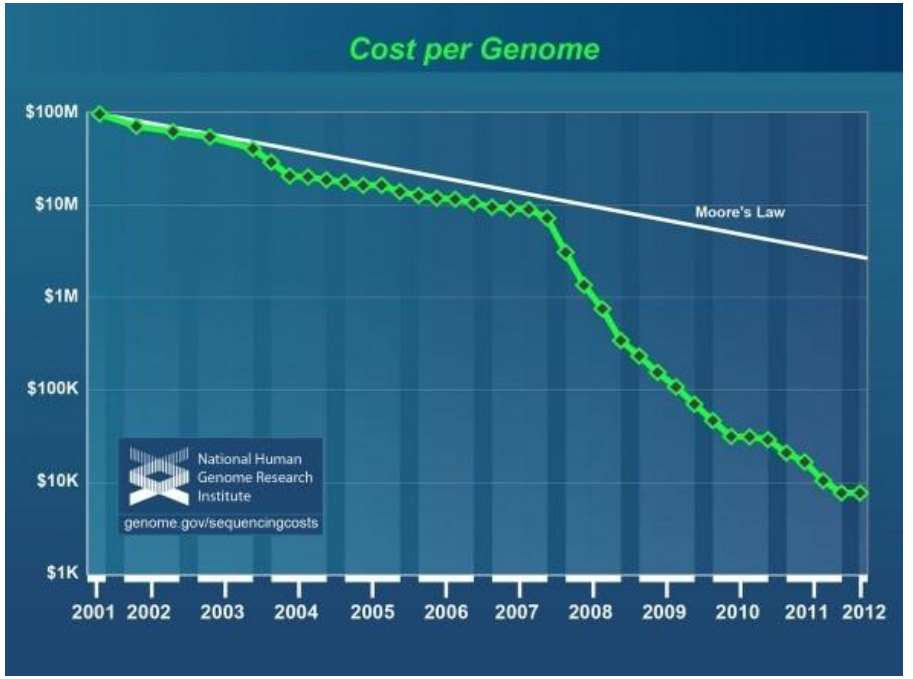
From: Hedrich et al., doi: 10.1212/01.WNL.0000113022.51739.88

Neurology February 10, 2004 vol. 62 no. 3 389-394

The evolution of sequencing: Reading more for less



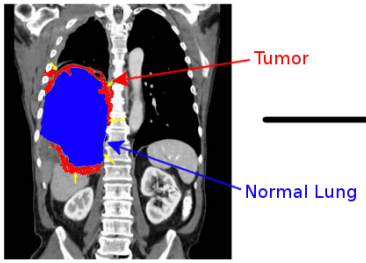




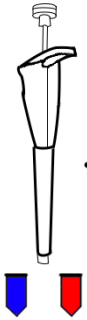
The human haploid genome is 3 billion nucleotides long

Cancer Genome Sequencing Workflow

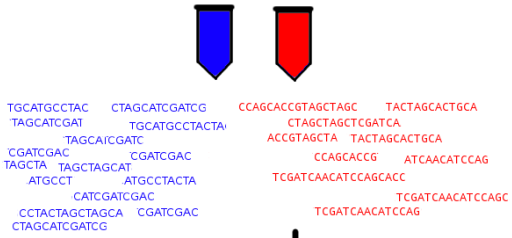
Biopsy, Matched Tissue Collection and Pathology



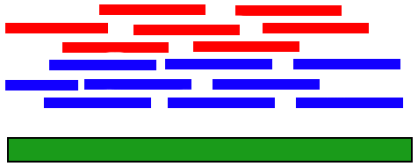
DNA/RNA extraction and Sample Prep



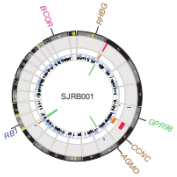
Sequencing



Alignment to Reference Genome



Analysis and Genomic Characterization



Integration and Interpretation

*RB1**

cell proliferation

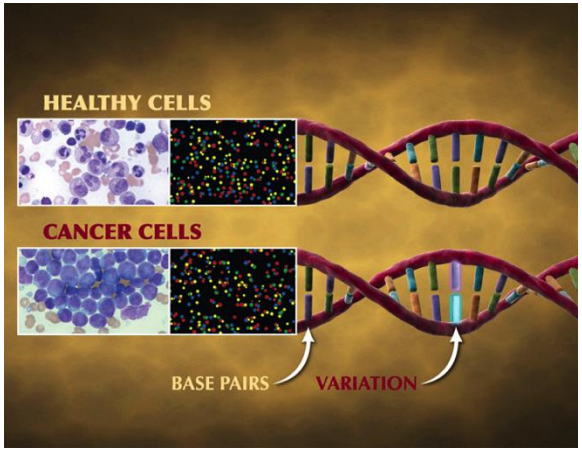
tumor

Conclusion:
A mutation in RB1 caused uncontrolled cell proliferation resulting in the cancerous growth

Treatment Plan

Rx

Information added to database: COSMIC



http://www.pediatriccancergenomeproject.org/site/multimedia

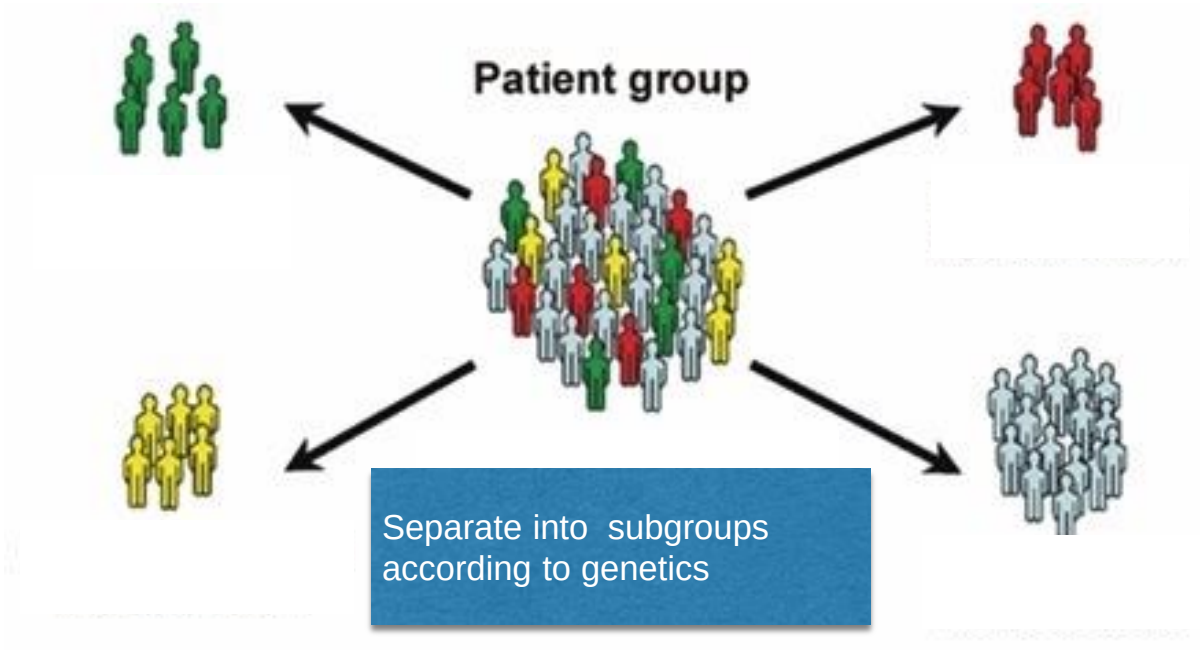
Comprehensive Cancer Genome Projects

Cancer Genome Project: Wellcome Trust Sanger Institute
COSMIC cancer database

Cancer Genome Atlas: NIH NCI

International Cancer Genome Consortium

The Era of Cancer Genomics and Personalized Medicine



Genetic & molecular characterization of each patient´s tumor: WG, exome, transcriptome, RNA sequencing, and micronome sequencing.

Common cancer-associated mutations

TABLE 18.2 SOME PROTO-ONCOGENES AND TUMOR SUPPRESSOR GENES

Proto-oncogene	Normal Function	Alteration in Cancer	Associated Cancers
Ha-ras	Signal transduction molecule, binds GTP/GDP	Point mutations	Colorectal, bladder, many types
c-erbB	Transmembrane growth factor receptor	Gene amplification, point mutations	Glioblastomas, breast cancer, cervix
c-myc	Transcription factor, regulates cell cycle, differentiation, apoptosis	Translocation, amplification, point mutations	Lymphomas, leukemias, lung cancer, many types
c-fos	Transcription factor, responds to growth factors	Overexpression	Osteosarcomas, many types
c-kit	Tyrosine kinase, signal transduction	Mutation	Sarcomas
c-raf	Cytoplasmic serine-threonine kinase, signal transduction	Gene rearrangements	Stomach cancer
RARα	Hormone-dependent transcription factor, differentiation	Chromosomal translocations with PML gene, fusion product	Acute promyelocytic leukemia
E6	Human papillomavirus encoded oncogene, inactivates p53	HPV infection	Cervical cancer
MDM2	Binds and inactivates p53, abrogates cell cycle checkpoints	Gene amplification, over-expression	Osteosarcomas, liposarcomas
Cyclins	Bind to CDKs, regulate cell cycle	Gene amplification, over-expression	Lung, esophagus, many types
CDK2, 4	Cyclin-dependent kinases, regulate cell cycle phases	Overexpression, mutation	Bladder, breast, many types
Tumor Suppressor	**Normal Function**	**Alteration in Cancer**	**Associated Cancers**
p53	Cell cycle checkpoints, apoptosis	Mutation, inactivation by viral oncogene products	Brain, lung, colorectal, breast, many types
RB1	Cell cycle checkpoints, binds E2F	Mutation, deletion, inactivation by viral oncogene products	Retinoblastoma, osteosarcoma, many types
APC	Cell–cell interaction	Mutation	Colorectal cancers, brain, thyroid
Bcl2	Apoptosis regulation	Overexpression blocks apoptosis	Lymphomas, leukemias
XPA–XPG	Nucleotide excision repair	Mutation	Xeroderma pigmentosum, skin cancers
BRCA2	DNA repair	Point mutations	Breast, ovarian, prostate cancers

The Ras GTPase

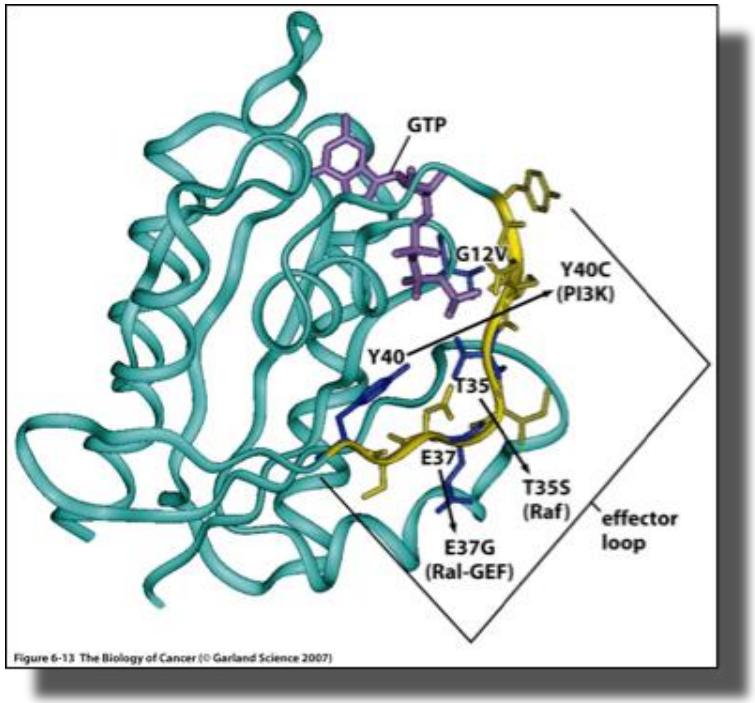


Figure 6-13 The Biology of Cancer (© Garland Science 2007)

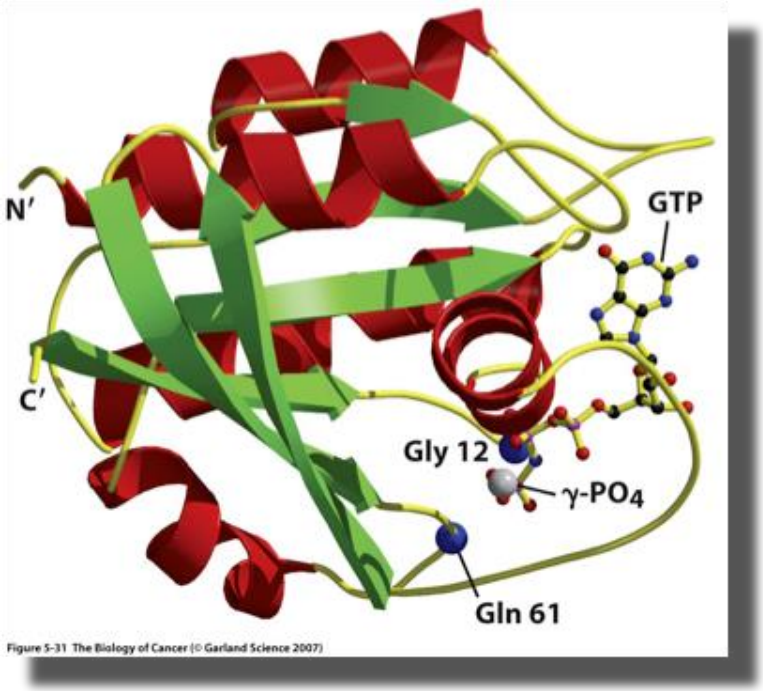


Figure 5-31 The Biology of Cancer (© Garland Science 2007)

A frequent Ras mutation associated with cancer

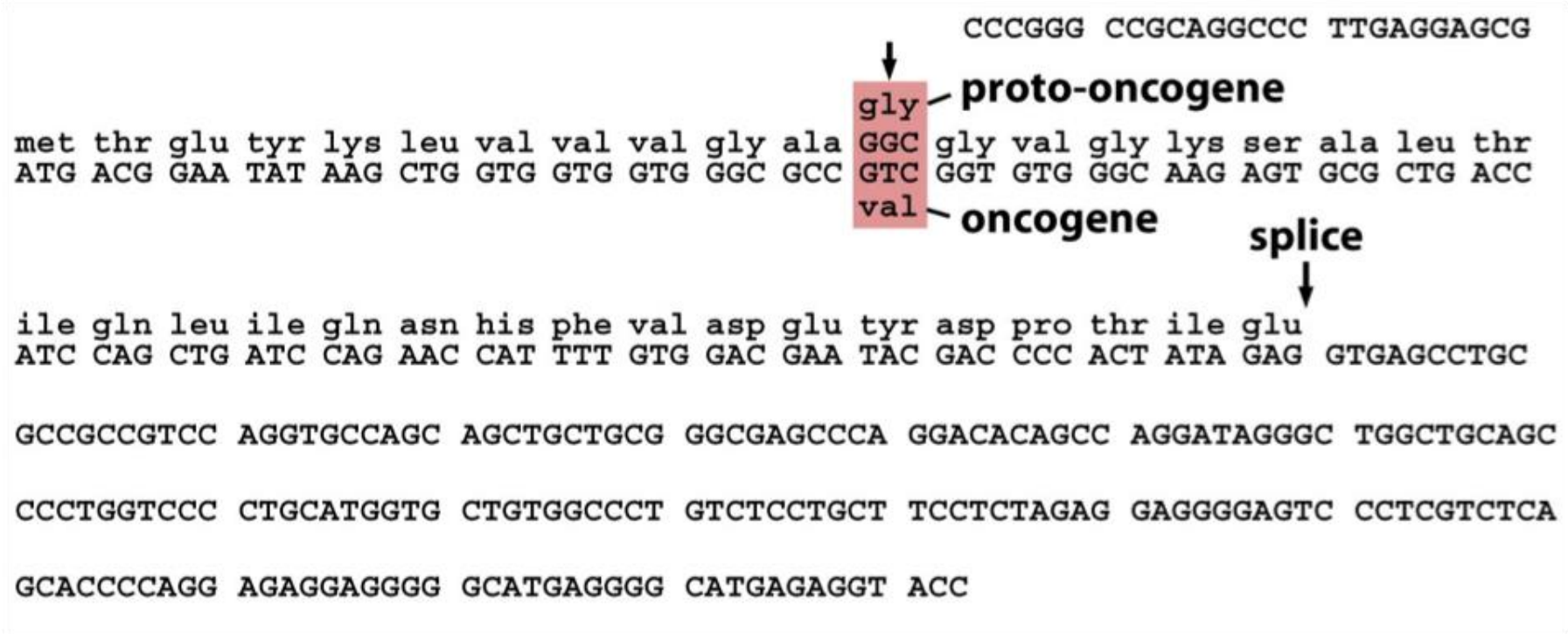
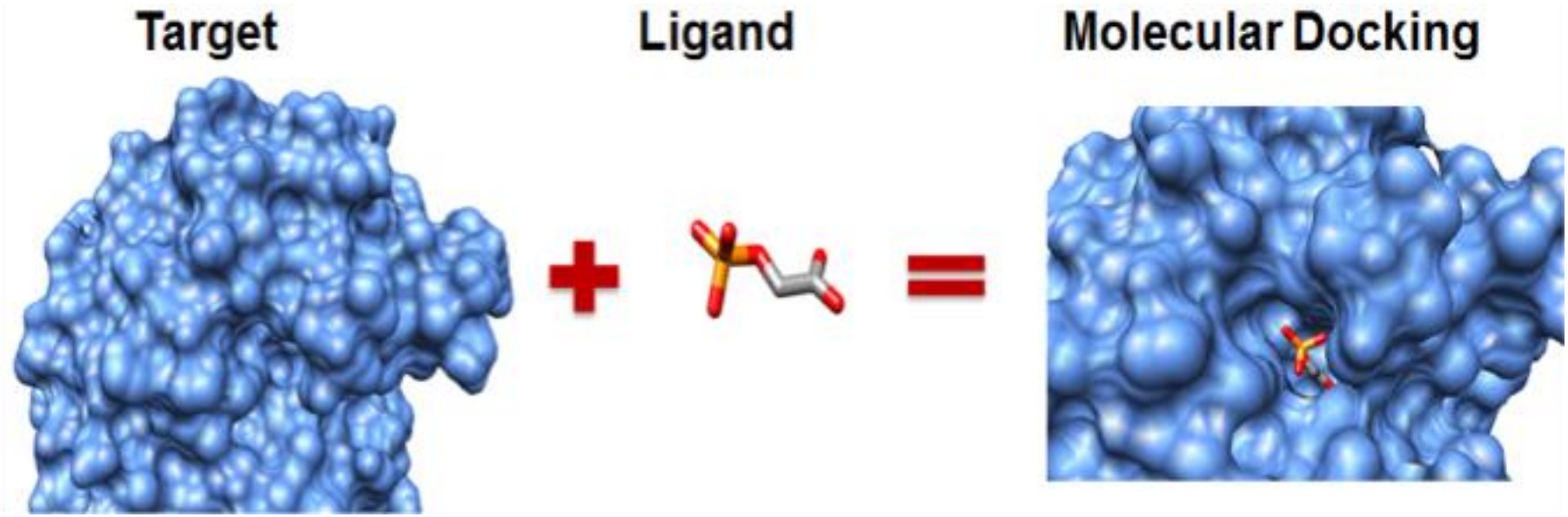


Figure 4.10 *The Biology of Cancer* (© Garland Science 2007)

Molecular docking to identify mutant-specific inhibitors



From: **Protein-Protein and Protein-Ligand Docking**
By Alejandra Hernández-Santoyo, Aldo Yair Tenorio-Barajas, Victor Altuzar, Héctor Vivanco-Cid and Claudia Mendoza-Barrera

Nature. 2013 Nov 28;503(7477):548-51. doi: 10.1038/nature12796. Epub 2013 Nov 20.

K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions.

Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM.

Abstract

Somatic mutations in the small GTPase K-Ras are the most common activating lesions found in human cancer, and are generally associated with poor response to standard therapies. Efforts to target this oncogene directly have faced difficulties owing to its picomolar affinity for GTP/GDP and the absence of known allosteric regulatory sites. Oncogenic mutations result in functional activation of Ras family proteins by impairing GTP hydrolysis. With diminished regulation by GTPase activity, the nucleotide state of Ras becomes more dependent on relative nucleotide affinity and concentration. This gives GTP an advantage over GDP and increases the proportion of active GTP-bound Ras. Here we report the development of small molecules that irreversibly bind to a common oncogenic mutant, K-Ras (G12C). These compounds rely on the mutant cysteine for binding and therefore do not affect the wild-type protein. Crystallographic studies reveal the formation of a new pocket that is not apparent in previous structures of Ras, beneath the effector binding switch-II region. Binding of these inhibitors to K-Ras(G12C) disrupts both switch-I and switch-II, subverting the native nucleotide preference to favor GDP over GTP and impairing binding to Raf. Our data provide structure-based validation of a new allosteric regulatory site on Ras that is targetable in a mutant-specific manner.

Ras mutations in different tumor types

Table 4.2 A list of point-mutated *ras* oncogenes carried by a variety of human tumor cells

	Tumor type	Proportion (%) of tumors carrying a point-mutated *ras* gene[a]
Responsive tumors	Pancreas	90 K
	Thyroid (papillary)	60 (H, K, N)
	Thyroid (follicular)	55 (H, K, N)
	Colorectal	45 (K)
	Seminoma	45 (K, N)
Non-responsive tumors	Myelodysplasia	40 (N, K)
	Lung (non-small-cell)	35 (K)
	Acute myelogenous leukemia	30 (N)
	Liver	30 (N)
	Melanoma	15 (K)
	Bladder	10 (K)
	Kidney	10 H

[a]H, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

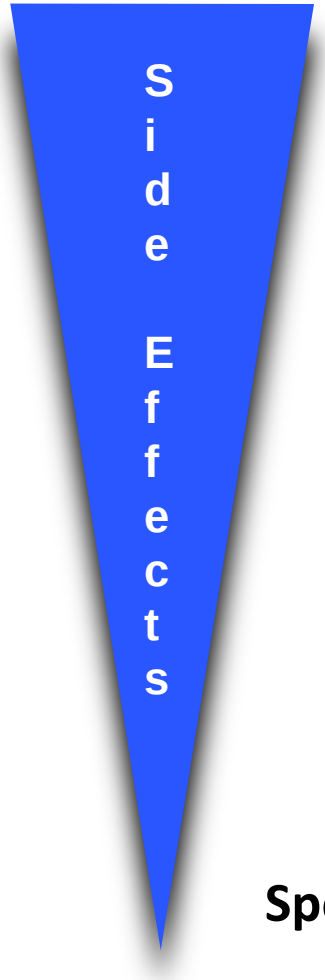
Adapted from J. Downward, *Nat. Rev. Cancer* 3:11–22, 2003.

Breast Cancer Genetics

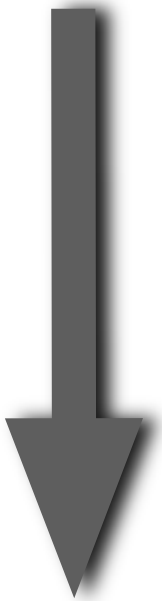
Mutations	Status	Treatment
BRCA1	+ or -	If +, use PARP inhibitors
BRCA2	+ or -	If +, use PARP inhibitors
Estrogen Receptor	+ or -	If +, use Tamoxifen or Aromatase inhibitors
Progesterone Receptor	+ or -	If +, hormone therapy
Her2/neu	+ or -	Trastuzumab*

* works only in cancer cells that over express the receptor

The Evolution of Anti-cancer Treatments

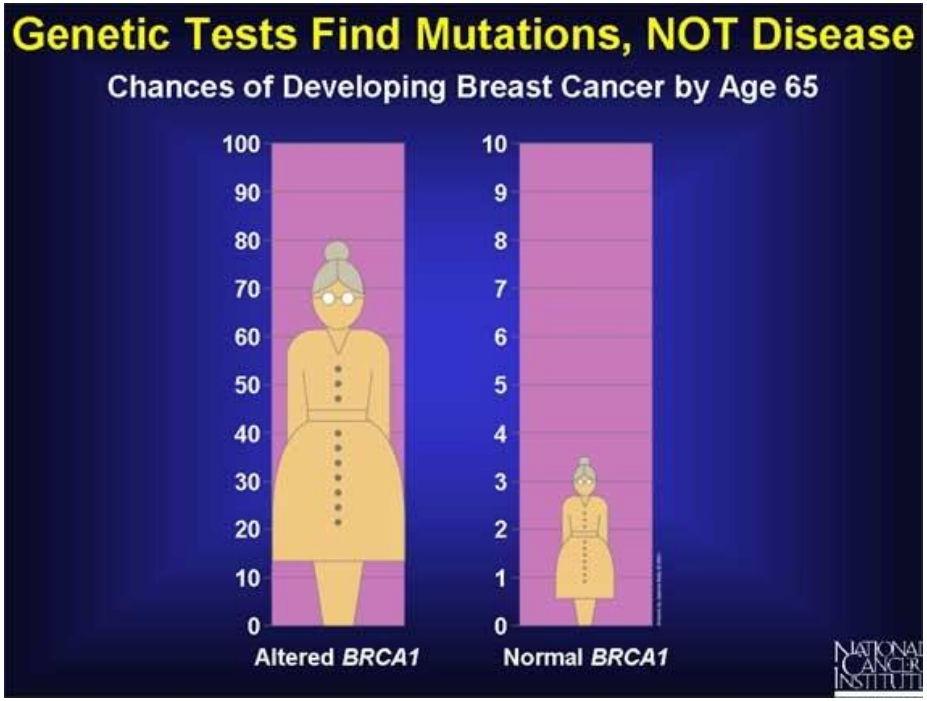


Non-specific inhibition of cellular processes

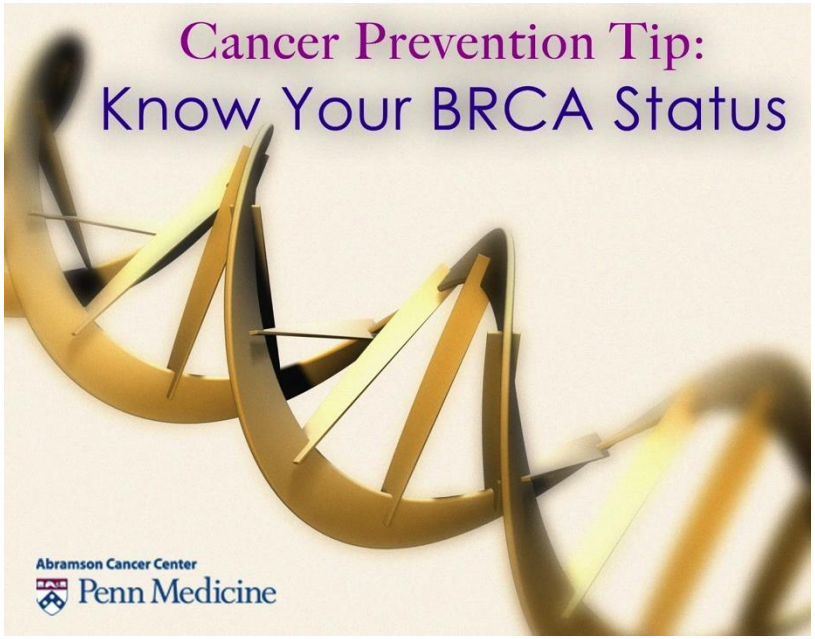


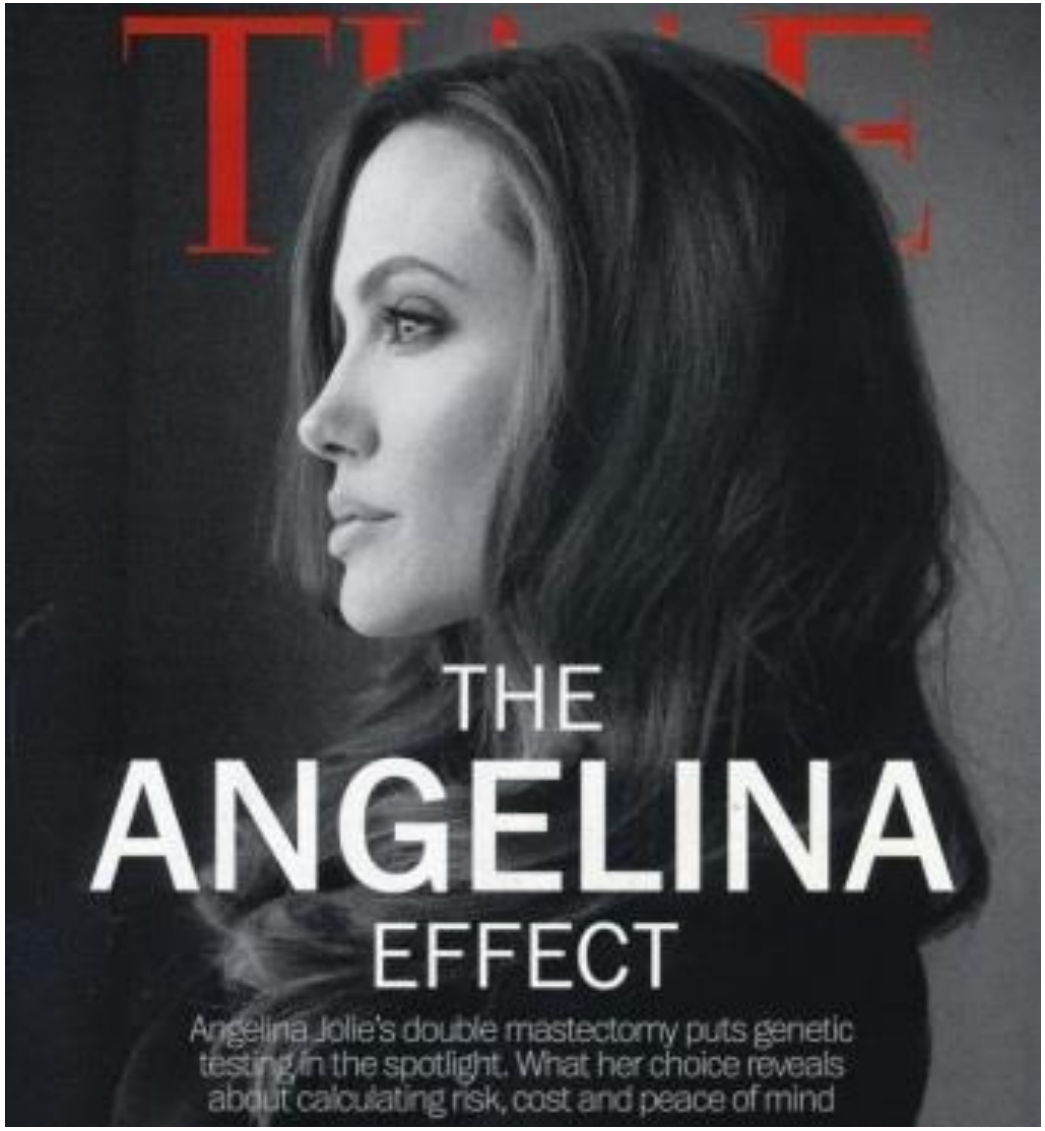
Specific inhibition of tumor - specific mutant proteins

Cancer Genetics and Screening and Prevention



Germ-line mutations as predictors of cancer risks




THE
ANGELINA
EFFECT
Angelina Jolie's double mastectomy puts genetic testing in the spotlight. What her choice reveals about calculating risk, cost and peace of mind

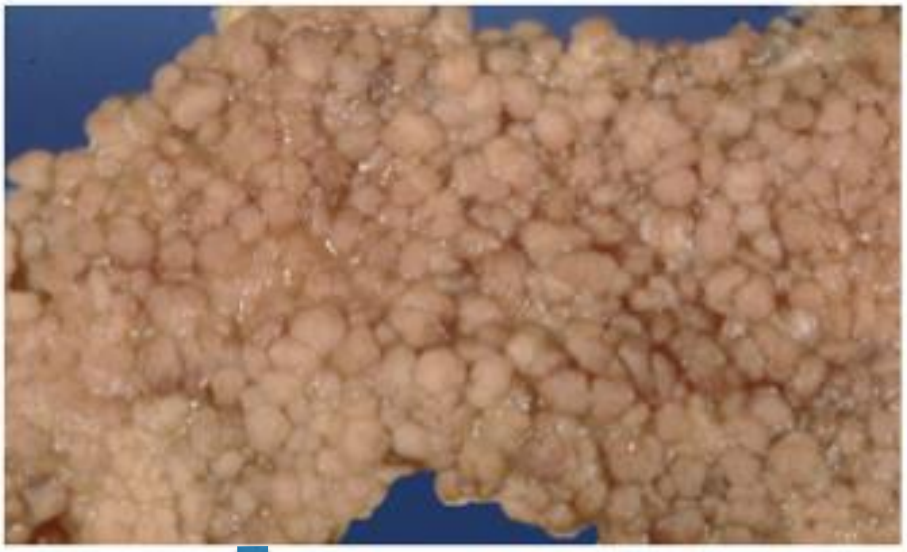
Breast Cancer Genetic Testing

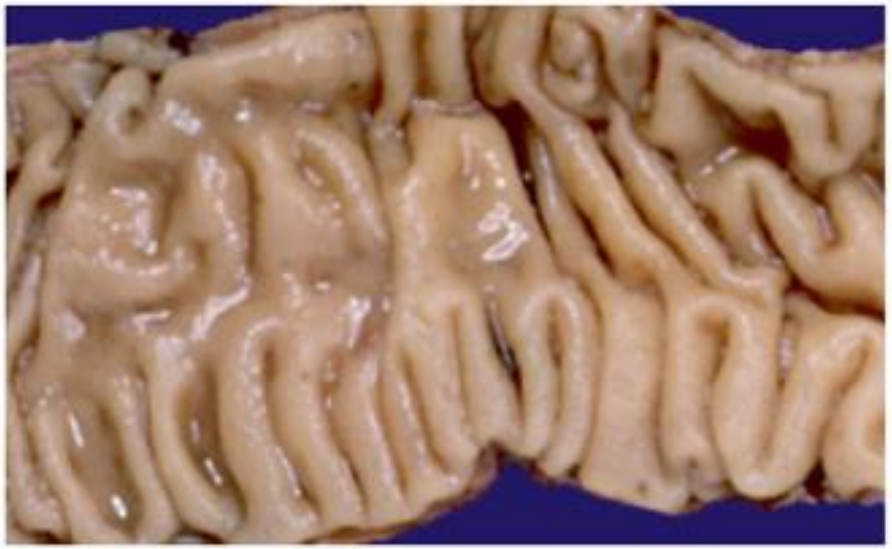
- MammaPrint (FDA approved)
- Oncotype DX
- Around $3,000 each
- Variable health plan coverage
- Recommended if there is family history

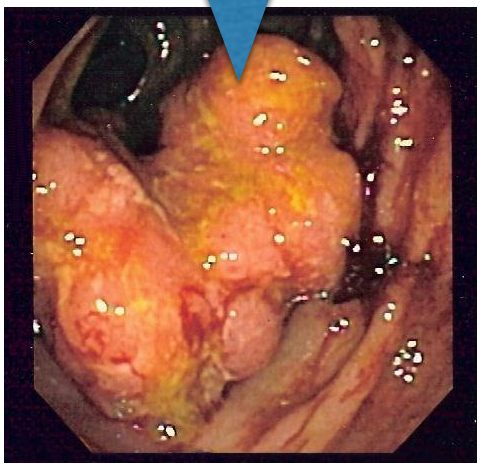
APC Mutations in colorectal cancer

Mutant

WT







Preventive colostomy is recommended

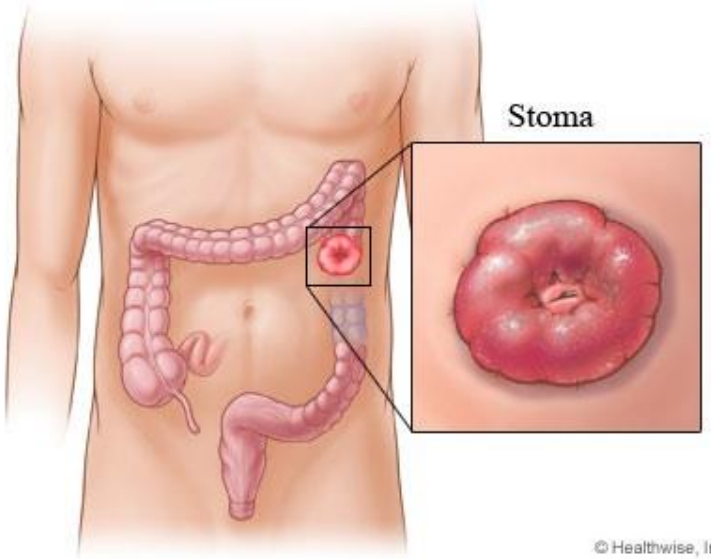


Figure 7.22 *The Biology of Cancer* (© Garland Science 2007)

The End