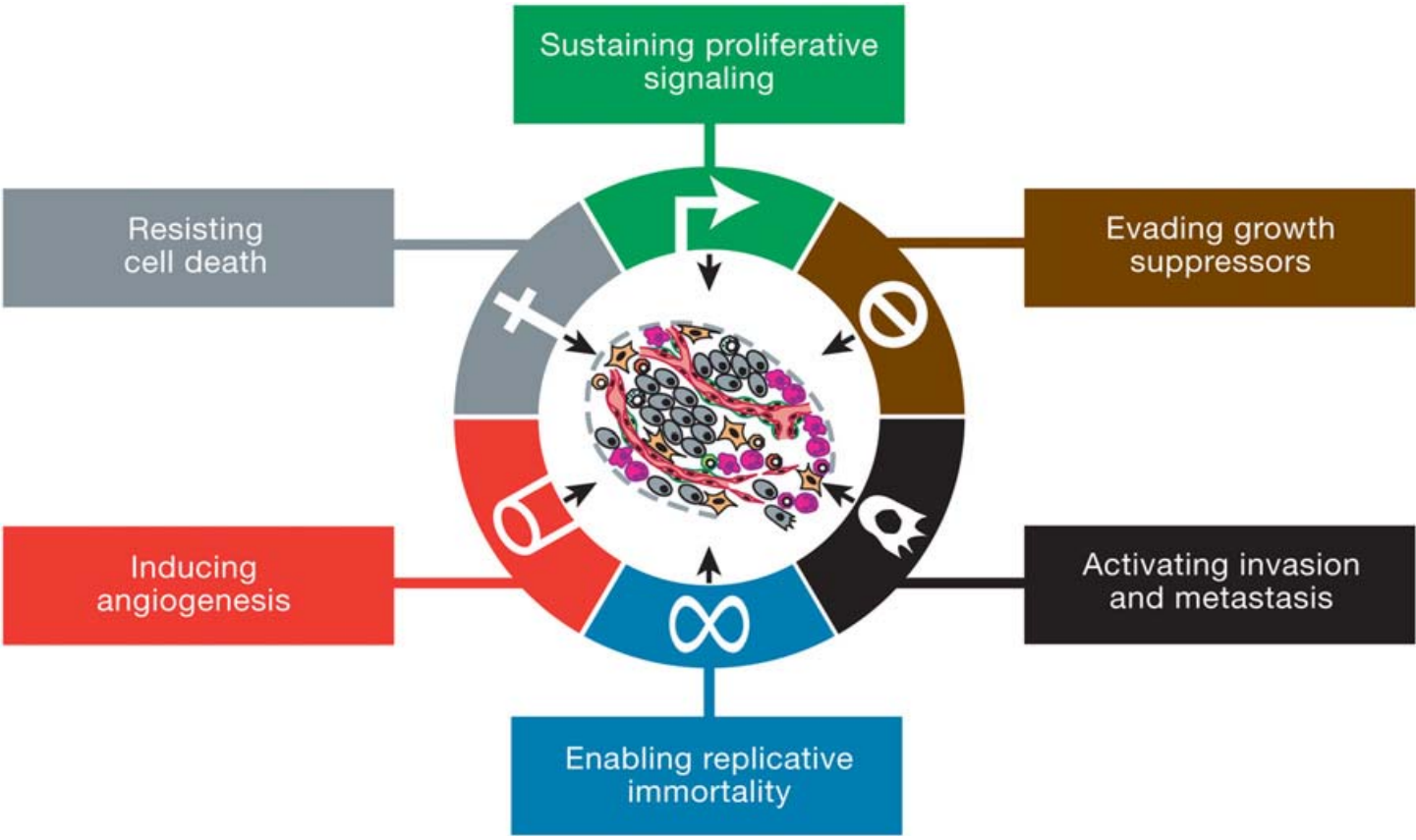
ONCOGENES I and II

Terminology
Viruses and Cancer
RNA tumour viruses
DNA tumour viruses
Genetics and Cancer
Activation of proto-oncogenes
Function of proteins encoded by oncogenes



Hanahan and Weinberg 2000

Search for the cause of cancer

Before 1800 – few cancers (people died of infectious diseases, malnutrition, accidents, etc.)

1761 – snuff linked to nasal tumours

Early 1900's – X-Rays linked to leukemia and skin cancer, discovery of a transmissible agent by Rous

By 1950's, cigarette smokers were showing 20-30 times the lung cancer rate of nonsmokers

1953 – after discovery of the DNA double helix, it was predicted that mutations could occur in genes

1971 – v-src gene discovered (first oncogene)

1976 – cellular src discovered

1982 – discovery of first human oncogene (ras)

1987 – cloning of the first tumour suppressor gene (*RB1*)

TERMINOLOGY

Oncogene = cancer causing gene

Gene product is present and altered in structure and/or amount in such a way as to induce one or more aspects of the cancer phenotype

Acts in a dominant manner

Proto-oncogene = normal gene with potential to become an oncogene

An oncogene is an altered form of a proto-oncogene

Sarcoma – derived from mesodermal cells (muscle, bone, blood vessels, fibroblasts)

Carcinoma – derived from epithelial cells of endodermal (bladder, pancreas, liver, lung, colon) or ectodermal (skin, neuronal cells, glial cells) origin

Leukemia and lymphoma – derived from blood and lymph systems

Genome – genetic material of a cell/organism

Karyotype – set of chromosomes in a cell

TERMINOLOGY- cont'd

Transformation – changes in cell morphology and growth regulation (*in vitro* properties)

Neoplasm/tumour – abnormal growth of cells, benign or malignant

Cancer – abnormal growth of cells, malignant (invasive, metastatic)

Metastatic – tumour spreads to other sites in body, property of malignant cells

GROWTH OF NORMAL AND NEOPLASTIC FIBROBLASTS IN CULTURE

TRANSFORMATION

Growth Characteristic	Normal Cells	Tumour Cells
*Density-dependent inhibition of growth	Present	Absent
Growth factor requirement	High	Low
*Anchorage dependence	Present	Absent
*Proliferative life span	Finite	Immortal
Contact inhibition of movement	Present	Absent
Adhesiveness	High	Low
Morphology	Flat	Rounded

TUMORIGENICITY

Growth Characteristic	Normal Cells	Tumour Cells
Tumour formation in nude or SCID mice	No	Yes

Figure 3-13 The Biology of Cancer (© Garland Science 2007)

VIRUSES AND CANCER

First oncogenic virus described in 1911 by Rous. Cell-free filtrate prepared from a chicken sarcoma tumour produced sarcomas in chicken.

Rous sarcoma virus (RSV) - RNA tumour virus (retrovirus)

1950's - Cultures of chicken fibroblasts + RSV = transformation

Two classes of retroviruses – (i) acute transforming viruses (e.g. RSV) which rapidly induce tumours and efficiently transform cells in culture and (ii) chronic tumour viruses (e.g. ALV – avian leukosis virus) which induce tumours after long latent periods (several months) and don't transform cells in culture

RSV has an extra gene – oncogene "src" not required for viral growth. ALV has no oncogene, induces tumours by inserting themselves next to host genes and altering the expression of these genes.

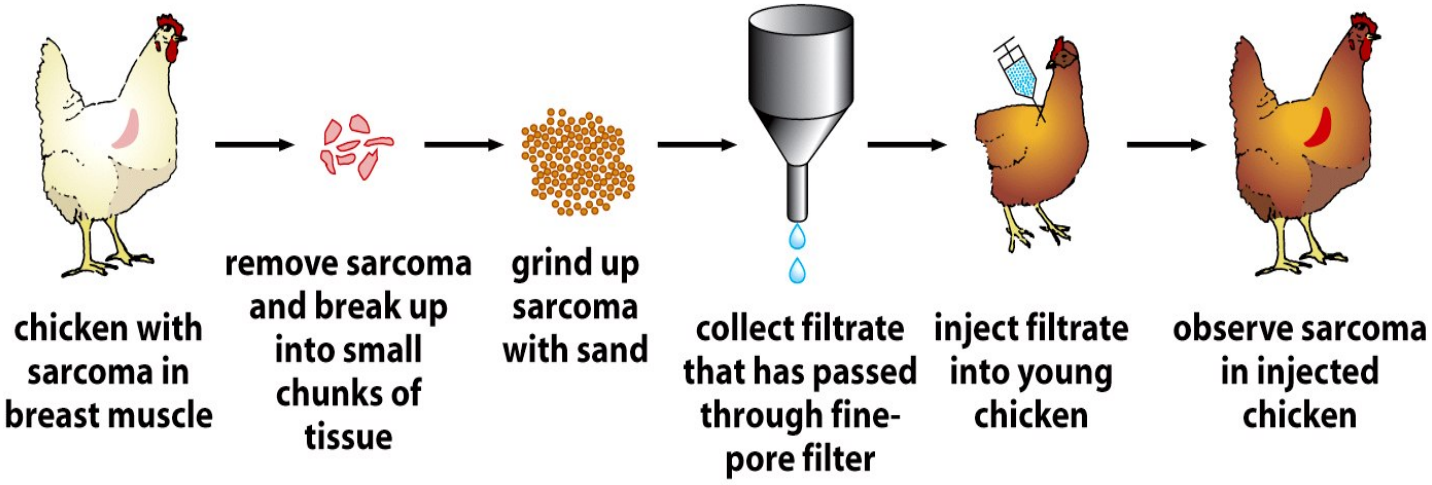


Figure 3-2 The Biology of Cancer (© Garland Science 2007)

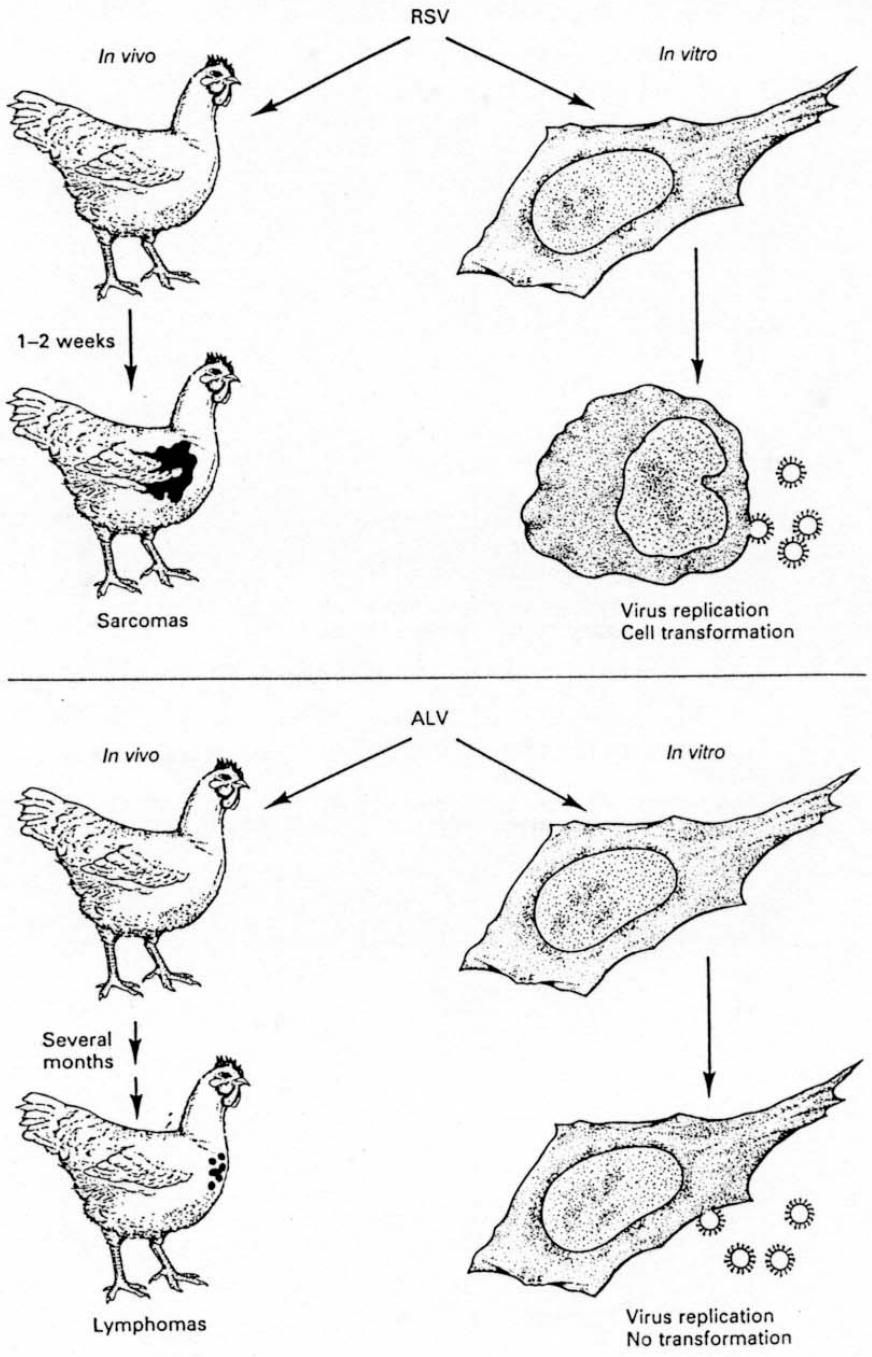


FIGURE 3.1
Neoplasm induction and cell transformation by Rous sarcoma virus (RSV) and a
leukosis virus (ALV). RSV induces sarcomas rapidly in infected chickens and effi
ciently transforms fibroblasts in culture. In contrast, ALV induces lymphomas or
after long latent periods in infected birds and does not transform cells in culture.

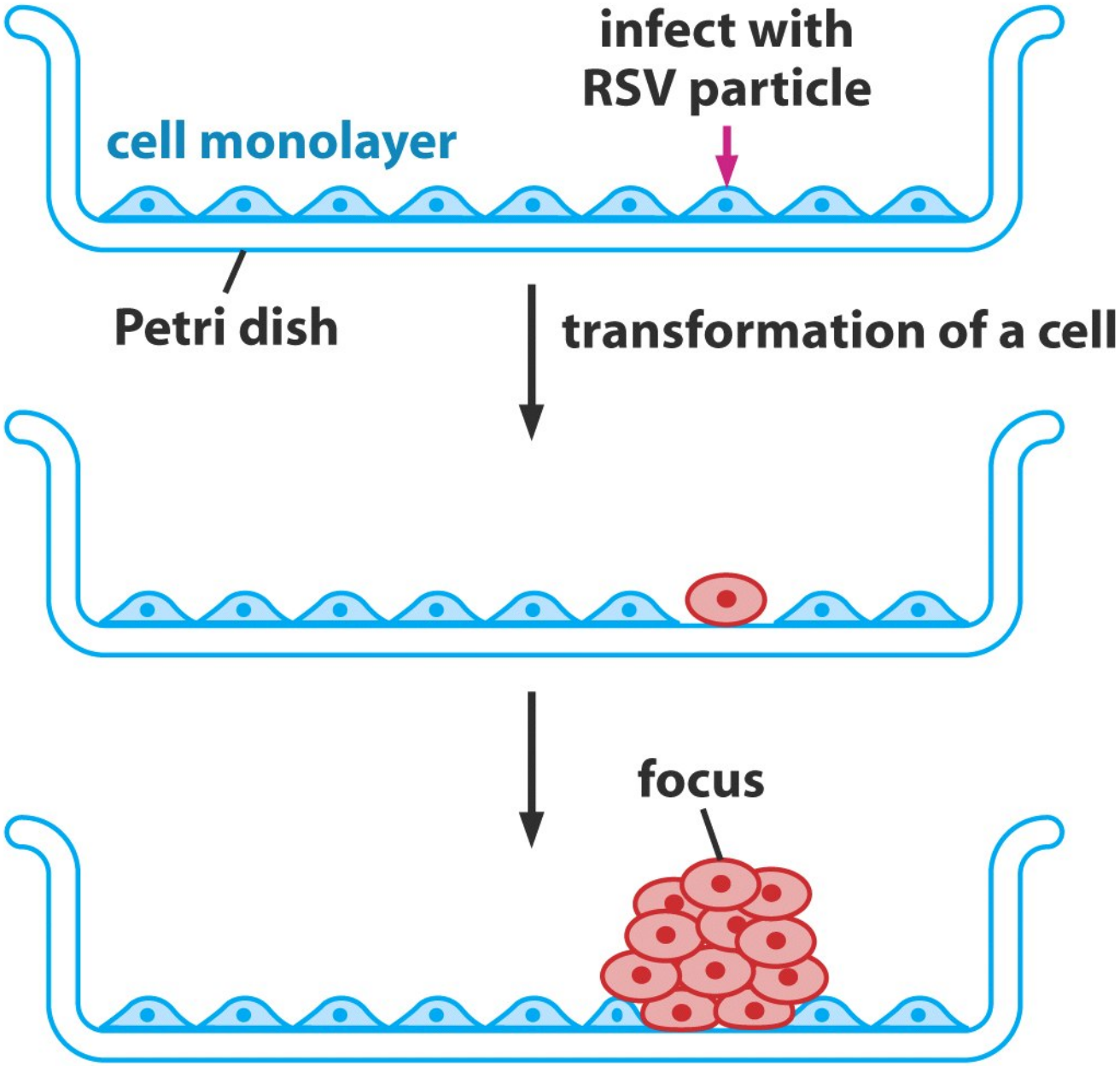


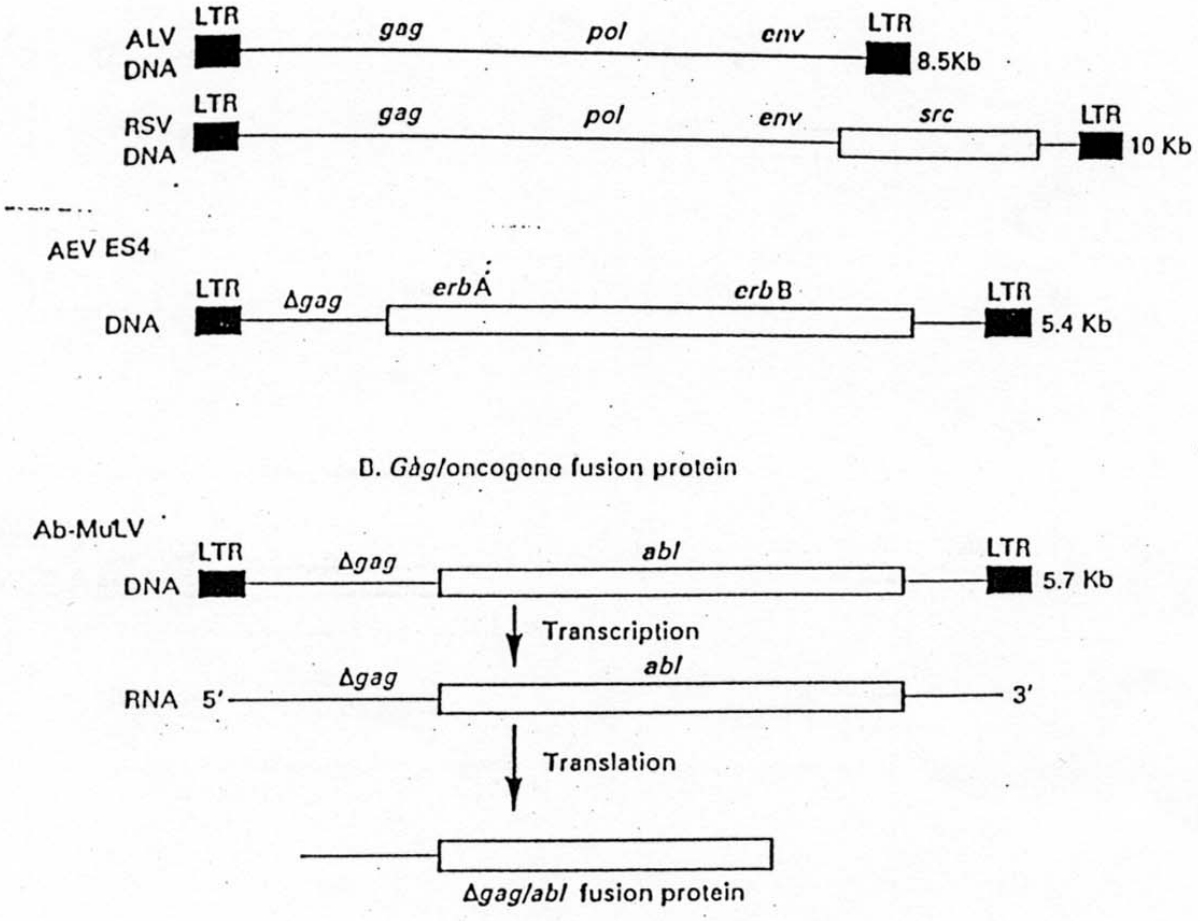
Figure 3-7a The Biology of Cancer (© Garland Science 2007)

Basic retrovirus genome

GAG: internal proteins

ENV: envelope glycoproteins

POL: enzymes, includes reverse transcriptase and integrase


ALV DNA
LTR
gag
pol
env
LTR
8.5Kb
RSV DNA
LTR
gag
pol
env
src
LTR
10 Kb
AEV ES4
DNA
LTR
Δgag
erbA
erbB
LTR
5.4 Kb
D. Gag/oncogene fusion protein
Ab-MuLV
DNA
LTR
Δgag
abl
LTR
5.7 Kb
Transcription
RNA 5'
Δgag
abl
3'
Translation
Δgag/abl fusion protein

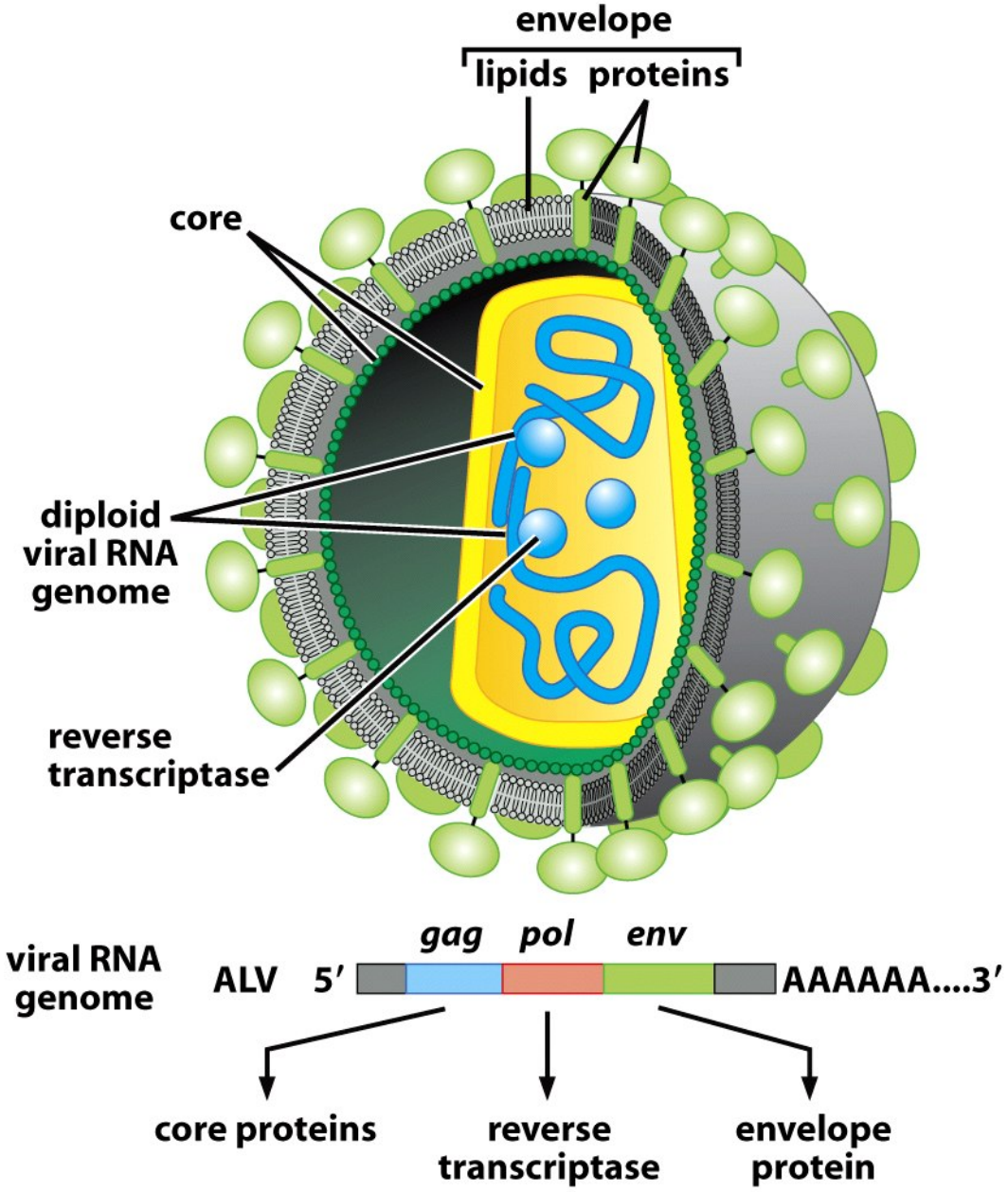
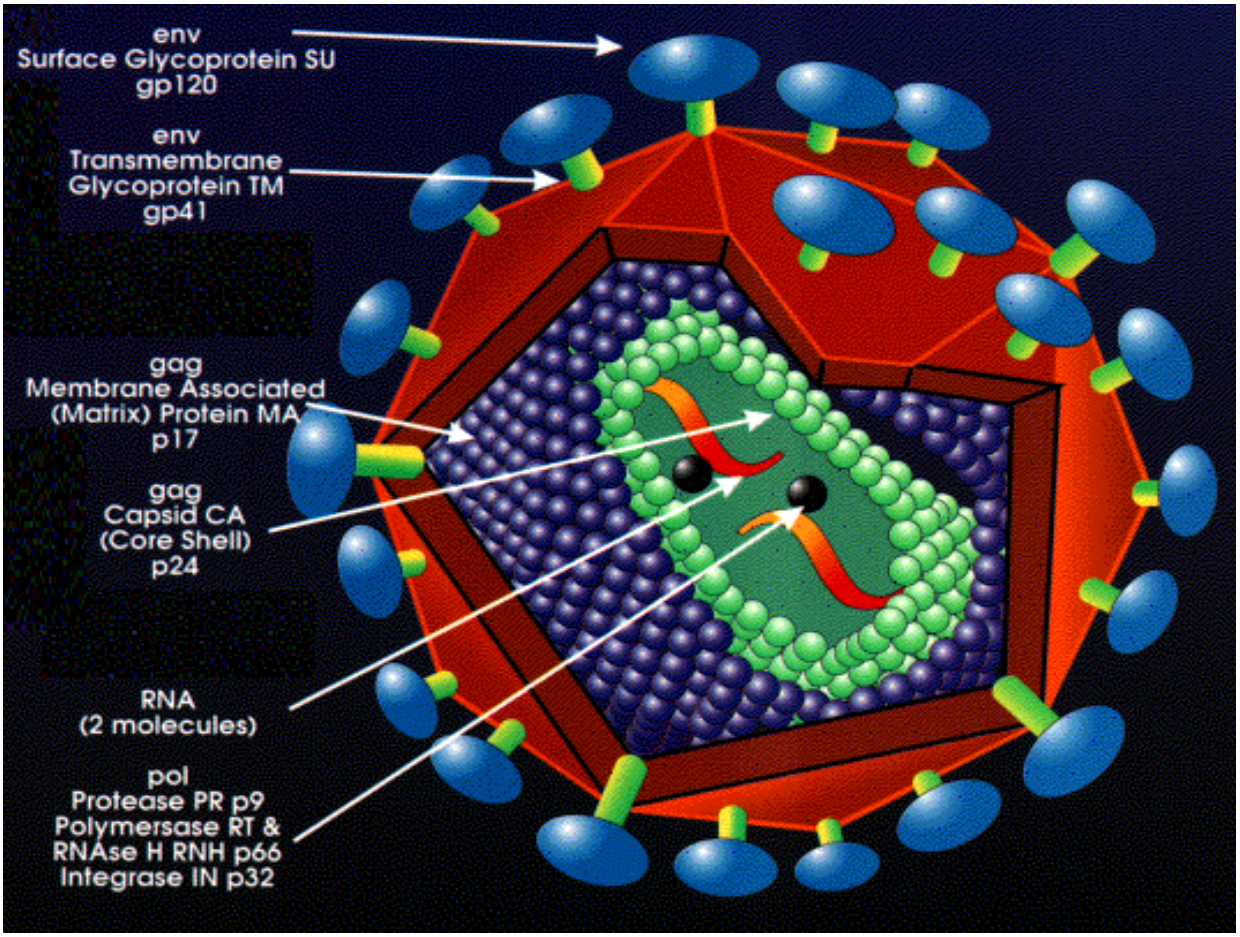


Figure 3-4a The Biology of Cancer (© Garland Science 2007)

Retrovirus


env
Surface Glycoprotein SU
gp120
env
Transmembrane
Glycoprotein TM
gp41
gag
Membrane Associated
(Matrix) Protein MA
p17
gag
Capsid CA
(Core Shell)
p24
RNA
(2 molecules)
pol
Protease PR p9
Polymersase RT &
RNAse H RNH p66
Integrase IN p32

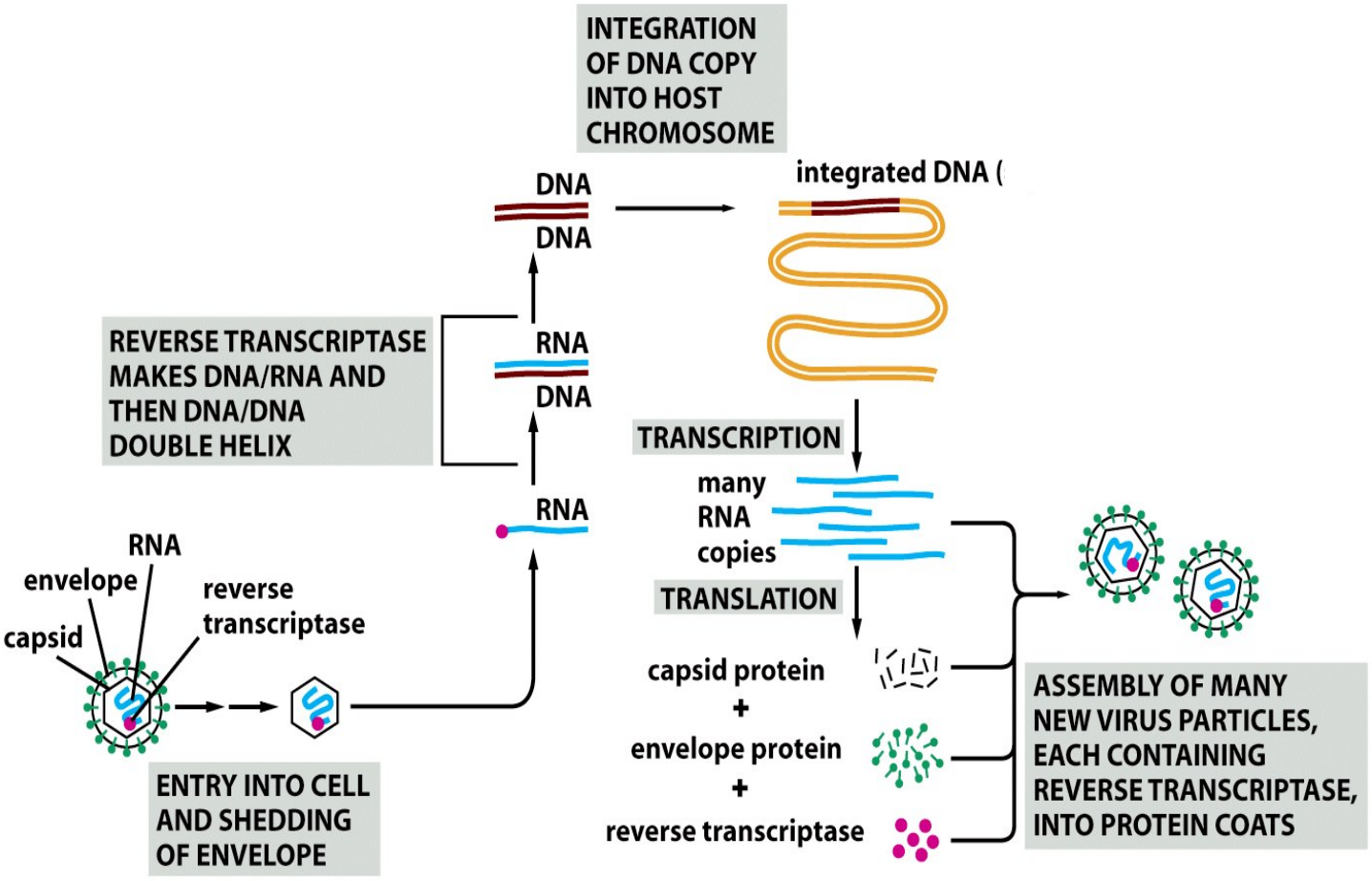


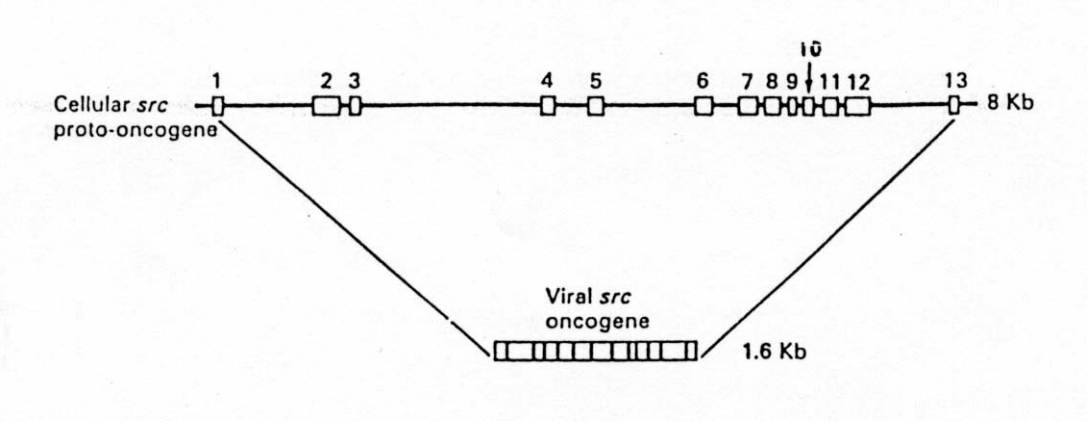
Figure 3-17 The Biology of Cancer (© Garland Science 2007)

Where do oncogenes come from? Virus or cell?

(1) RNA was isolated from RSV and used to probe DNA from various tissues of the chicken. Found sequences homologous to src in the DNA of all tissues of virus-free chickens.

(2) Furthermore, homologous sequences to src are found in yeast, mice, rats, Drosophila, as well as humans.

(3) Eukaryotic genes are composed of coding regions (exons) and non-coding regions (introns). When mRNA is made, the introns are spliced out. Cellular "oncogenes" have exons and introns, while viral oncogenes are mRNA-like. Unlikely that the viruses infected the cells first and then developed introns and exons. Furthermore, size and number of introns and exons in different species are conserved.



Where do oncogenes come from – cont'd

(4) Localization and linkage of proto-oncogenes is conserved in different species. If viral oncogene introduced into cells after speciation, unlikely to find conserved gene structure and linkage.

(5) Animals infected with a RNA tumour virus that doesn't contain an oncogene occasionally develop tumours that produce viruses that now carry an oncogene.

Conclusion – oncogenes are of cellular origin

Proto-oncogenes

>30 viral oncogenes have been identified – each of which has a normal cellular counterpart. In normal cells, these proto-oncogenes are expressed and don't produce tumours.

Proto-oncogenes – normal cellular genes that can be altered to become oncogenes

Table 3.3 Acutely transforming retroviruses and the oncogenes that they have acquired[a]

Name of virus	Viral oncogene	Species	Major disease	Nature of oncoprotein
Rous sarcoma	*src*	chicken	sarcoma	non-receptor TK
Y73/Esh sarcoma	*yes*	chicken	sarcoma	non-receptor TK
Fujinami sarcoma	*fps*[b]	chicken	sarcoma	non-receptor TK
UR2	*ros*	chicken	sarcoma	RTK; unknown ligand
Myelocytomatosis 29	*myc*	chicken	myeloid leukemia[c]	transcription factor
Mill Hill virus 2	*mil*[d]	chicken	myeloid leukemia	ser/thr kinase
Avian myeloblastosis E26	*myb*	chicken	myeloid leukemia	transcription factor
Avian myeloblastosis E26	*ets*	chicken	myeloid leukemia	transcription factor
Avian erythroblastosis ES4	*erbA*	chicken	erythroleukemia	thyroid hormone receptor
Avian erythroblastosis ES4	*erbB*	chicken	erythroleukemia	EGF RTK
3611 murine sarcoma	*raf*[e]	mouse	sarcoma	ser/thr kinase
SKV770	*ski*	chicken	endothelioma (?)	transcription factor
Reticuloendotheliosis	*rel*	turkey	immature B-cell lymphoma	transcription factor
Abelson murine leukemia	*abl*	mouse	pre-B-cell lymphoma	non-receptor TK
Moloney murine sarcoma	*mos*	mouse	sarcoma, erythroleukemia	ser/thr kinase
Harvey murine sarcoma	H-*ras*	rat, mouse	sarcoma	small G protein
Kirsten murine sarcoma	K-*ras*	mouse	sarcoma	small G protein
FBJ murine sarcoma	*fos*	mouse	osteosarcoma	transcription factor
Snyder–Theilen feline sarcoma	*fes*[f]	cat	sarcoma	non-receptor TK
McDonough feline sarcoma	*fms*	cat	sarcoma	CSF-1 RTK
Gardner–Rasheed feline sarcoma	*fgr*	cat	sarcoma	non-receptor TK
Hardy–Zuckerman feline sarcoma	*kit*	cat	sarcoma	steel factor RTK
Simian sarcoma	*sis*	woolly monkey	sarcoma	PDGF
AKT8	*akt*	mouse	lymphoma	ser/thr kinase
Avian virus S13	*sea*	chicken	erythroblastic leukemia[g]	RTK; unknown ligand
Myeloproliferative leukemia	*mpl*	mouse	myeloproliferation	TPO receptor
Regional Poultry Lab v. 30	*eyk*	chicken	sarcoma	RTK; unknown ligand
Avian sarcoma virus CT10	*crk*	chicken	sarcoma	SH2/SH3 adaptor
Avian sarcoma virus 17	*jun*	chicken	sarcoma	transcription factor
Avian sarcoma virus 31	*qin*	chicken	sarcoma	transcription factor[h]
AS42 sarcoma virus	*maf*	chicken	sarcoma	transcription factor
Cas NS-1 virus	*cbl*	mouse	lymphoma	SH2-dependent ubiquitylation factor

Abbreviations: CSF, colony-stimulating factor; EGF, epidermal growth factor; G, GTP-binding; PDGF, platelet-derived growth factor; RTK, receptor tyrosine kinase; ser/thr, serine/threonine; SH, src-homology segment; TK, tyrosine kinase; TPO, thrombopoietin.

[a]Not all viruses that have yielded these oncogenes are indicated here.
[b]Ortholog of the mammalian *fes* oncogene.
[c]Also causes carcinomas and endotheliomas.
[d]Ortholog of the mammalian *raf* oncogene.
[e]Ortholog of the avian *mil* oncogene.
[f]Ortholog of the avian *fps* oncogene.
[g]Also causes granulocytic leukemias and sarcomas.
[h]Functions as a transcriptional repressor.

Adapted in part from S.J. Flint, L.W. Enquist, R.M. Krug et al., Principles of Virology. Washington, DC: ASM Press, 2000. Also in part from G.M. Cooper, Oncogenes. Boston: Jones and Bartlett Publishers, 1995.

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

Four human retroviruses

HTLV-1 – associated with cancer
HTLV-2 – may be associated with cancer
HIV-1 – human immunodeficiency virus - causes AIDS (acquired immunodeficiency syndrome)
HIV-2 – causes AIDS

Human RNA tumour viruses

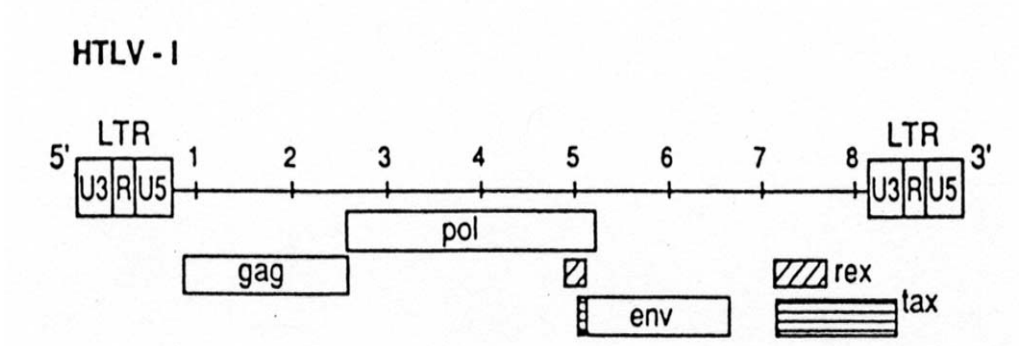
HTLV-1 – human T cell-leukemia virus type 1

Associated with adult T-cell leukemia, a rare cancer found in Southern Japan, Caribbeans

Latency – a few years to >40 years

Does not carry a host derived oncogene

Viral genome



Rex protein is essential for viral replication – enhances expression of incompletely spliced viral transcripts that encode the viral structural proteins

Tax protein is the transforming component of HTLV-1

HTLV-1 – cont'd

Cellular pathways or genes responsive to Tax include:

(i) NF-κB pathway which is activated by Tax resulting in induction of cytokines and genes associated with apoptosis and the cell cycle, and

(ii) DNA polymerase ß, p53 and p16 genes which are repressed by Tax resulting in defects in DNA repair and the cell cycle

DNA tumour viruses

RNA tumour viruses – oncogenes are derived from host; oncoproteins do not elicit immune response; oncogenes are not required for viral replication

DNA tumour viruses – oncogenes are virally encoded; oncoproteins tend to elicit an immune response; often associated with immunocompromised hosts; oncogenes (early genes) are required for viral replication and/or promote cell cycle progression

DNA tumour viruses

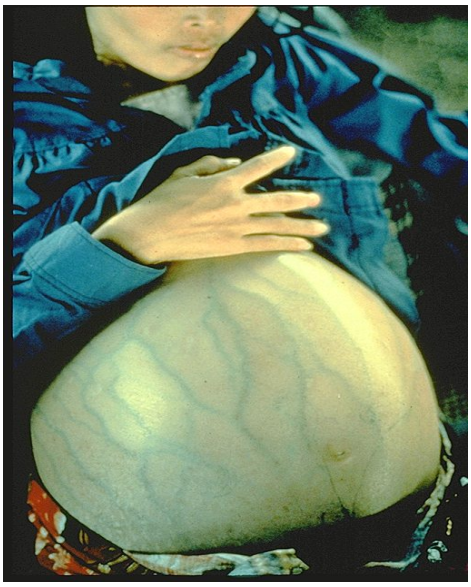
Four families of DNA viruses have oncogenic potential: hepadna-, papova-, adeno-, and herpes-virus.

The transforming proteins of DNA tumour viruses – large T antigen in SV40, E6 and E7 in papillomavirus, E1A and E1B in adenovirus – have no cellular counterparts.

Virus Family		Approximate Genome Size (kb)
Hepadna	Hepatitis B viruses	3
Polyomaviridae	SV40 and Polyomavirus	5
Papillomaviridae	Papillomaviruses	8
Adeno	Adenoviruses	35
Herpes	Herpesviruses	100-200


HBsAg Gene
ORF Pre S1
ORF Pre S2
ORF S
5'
Partially dsDNA
5'
3'
3'
ORF P
ORF Core
Pre C
ORF X
Polymerase Gene

Hepatitis B virus:



- strongly associated with the development of human hepatocellular carcinoma. Frequency of this cancer is increased 100X in individuals chronically infected with HBV. HBV DNA is commonly integrated in the genome of hepatocellular carcinoma cells. Adjacent cellular genes may be activated, increased cell proliferation.

Polyomaviridae – SV40, JC, BK and polyoma virus (not directly linked to human cancer):

Genomes encode 6 to 9 proteins. Contain both early (before viral DNA synthesis) and late (after viral DNA synthesis) genes. Rely on host DNA synthesis machinery to replicate viral DNA. Early proteins trigger cells to enter S phase and are responsible for immortalization and transformation.

Polyomaviridae interact with susceptible cells in two different ways:

(i) in permissive cells, viral DNA is replicated, coat protein made and progeny virion assembled. Virus particles are then released resulting in cell lysis and cell death.

(ii) in non-permissive cells, viral DNA integrates randomly into the host chromosomes. A small proportion of cells with integrated viral DNA will become transformed and tumorigenic.

Simian Virus 40 (SV40) and polyomavirus

- extensively characterized DNA tumour viruses
- 1960: SV40 found to be a contaminant of polio virus vaccine injected into millions of people
- SV40 (permissive in monkey cells) causes tumours in newborn and immunodeficient rodents but has not been directly associated with human cancers
- early proteins are called T antigens (large, middle and small in polyomavirus; large and small in SV40). T antigens are multifunctional proteins that regulate viral DNA replication and transcription
- transgenic mice carrying SV40 large T antigen under the control of tissue-specific promoters develop tumours in the appropriate tissues.
- SV40 large T antigen is used to immortalize rodent cells to establish permanent lines. Although much less efficient, human cells can also be immortalized with SV40 large T antigen.
- large T antigen binds to tumour suppressors p53 and pRB.

Papillomaviruses

- associated with warts and cervical carcinoma (found in 90% of cervical carcinomas). In benign warts, virus is maintained as an episome. In cervical carcinomas, HPV DNA is integrated in the host genome.
- the early genes E6 and E7 encode proteins that have transforming potential. E7 binds to pRB while E6 binds to p53.
- >100 types of papillomaviruses; HPV16 and HPV18 (high-risk HPV) can extend lifespan of human fibroblasts and keratinocytes in culture.
- infection with HPV is not sufficient to cause cancer.
- HPV vaccine made with late proteins (empty capsids) which generate a strong antibody response

Adenovirus

- permissive in humans; most people have antibodies to these viruses
- adenoviruses are not known to cause human cancer
- rodent cells transformed with adenoviruses contain an incomplete viral genome that always includes E1A and E1B. E1A binds RB protein while E1B binds p53.

Herpesvirus

- >60 proteins encoded by viral genome
- EBV (Epstein-Barr virus) infects a large percentage of the population (90%)
- EBV-infected human B lymphocytes become immortalized
- genes required for immortalization: EBNA-2, EBNA-3 and the membrane protein LMP-1.
- in immunocompromised individuals (e.g. AIDS patients, organ transplant recipients), EBV may induce lymphomas.
- EBV has been isolated from several types of cancers: 97% of Burkitt lymphoma (BL) in tropical forest region; 20% of sporadic BL; 30% of AIDS-associated BL; common in nasopharyngeal carcinoma; 50% of Hodgkin's lymphoma.
- HHV-8 is involved in a number of malignancies including Kaposi's sarcoma which is a frequent complication of AIDS

Oncolytic viruses

- oncolytic viruses have intrinsic or engineered tumour selectivity

- oncolytic viruses destroy tumour cells (e.g. by replicating selectively in cancer cells)

- clinical trials have been or are being carried out with oncolytic DNA viruses (including adenovirus and Herpes simplex virus - HSV) and RNA viruses (including reovirus Calgary)

- HSV has the following advantages: can insert a considerable amount of foreign DNA, doesn't integrate in the genome. Disadvantage: hard to work with because of its large genome

- reoviruses are non-pathogenic to humans (cause mild infections of the respiratory or gastrointestinal tracts). Reoviruses replicate selectively in cells with activated Ras pathway (30% of human cancers have activated Ras pathway)

- approx. 300 clinical trials with oncolytic viruses; little toxicity reported; encouraging results in some clinical trials (data not conclusive)

GENETICS AND CANCER

Evidence that cancer is a genetic disease (associated with genetic alterations/mutations) – before the mid 1970s:

(1) Chromosomal abnormalities in tumour cells (translocations, double minute chromosomes)

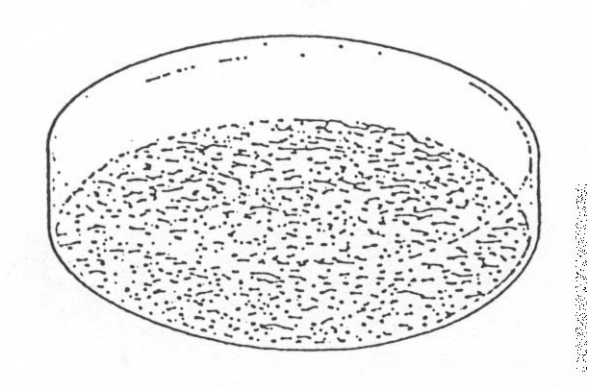
(2) Association of tumour development with DNA-damaging agents (such as chemical carcinogens, e.g., 2 napthyl amine)

(3) Increased incidence of cancer in hereditary diseases such as Ataxia telangiectasia (AT), Xeroderma pigmentosa (XP), retinoblastoma (RB), familial polyposis coli, and other forms of hereditary cancers.

(4) Genetic material from RNA and DNA tumour viruses carry information that allow rapid induction of malignant transformation in tissue culture and in animals.

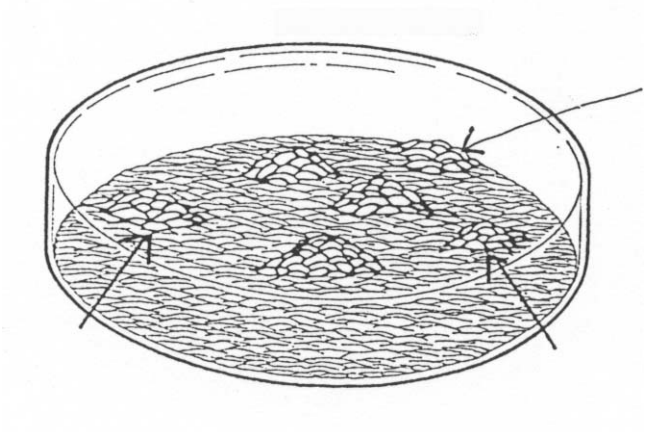
Assay for cell transformation
DNA transfection

DNA of RSV-transformed sarcoma cells



normal chicken embryo fibroblasts

~2 weeks



foci of transformed cells

Can normal cells be transformed with DNA from non-virally-infected cancer cells?

- use “normal” mouse fibroblasts (NIH3T3)
  (normal = immortalized, growth inhibited by contact, anchorage dependent, don’t form tumours in nude mice).
- transfect NIH3T3 cells with genomic DNA from: (a) chemically transformed cells and (b) from a bladder carcinoma cell line (late 1970’s/early 1980’s).
- presence of transformed foci (cells piling up) indicates that the NIH3T3 cells have been transformed by introduced genomic DNA.
- tests for anchorage independence and tumour formation in nude mice provide additional evidence that the foci are transformed and tumorigenic.

If isolate DNA from the transformed cells and use this DNA to transfect NIH3T3 – still works (i.e. obtain transformed foci)

Is the transformed phenotype caused by an oncogene?

Normal cells have proto-oncogenes

Do cancer cells have oncogenes?

Events leading to the identification of cellular oncogenes

In 1980 – identification of Alu repetitive elements (~600,000 repeats = 1/6000 bp). By probing with Alu repetitive DNA, one can detect the human DNA retained in mouse NIH3T3 cells that have been transfected with human tumour DNA.

If there is one Alu sequence/6000 bp of DNA, each intact gene should have multiple Alu sequences. By carrying out secondary and tertiary transfections and selecting for the transformed phenotype, the amount of human material present is reduced but the putative human oncogene is retained. A human oncogene was obtained by: (1) cloning the entire genome, (2) selecting the clones that had human DNA and (3) determining whether this DNA was capable of transforming NIH3T3.

By probing the cloned DNA with viral oncogenes to see if the isolated gene might represent the cellular counterpart of a viral oncogene, the human oncogene in bladder carcinoma cell line was identified as **H-ras** (1982).

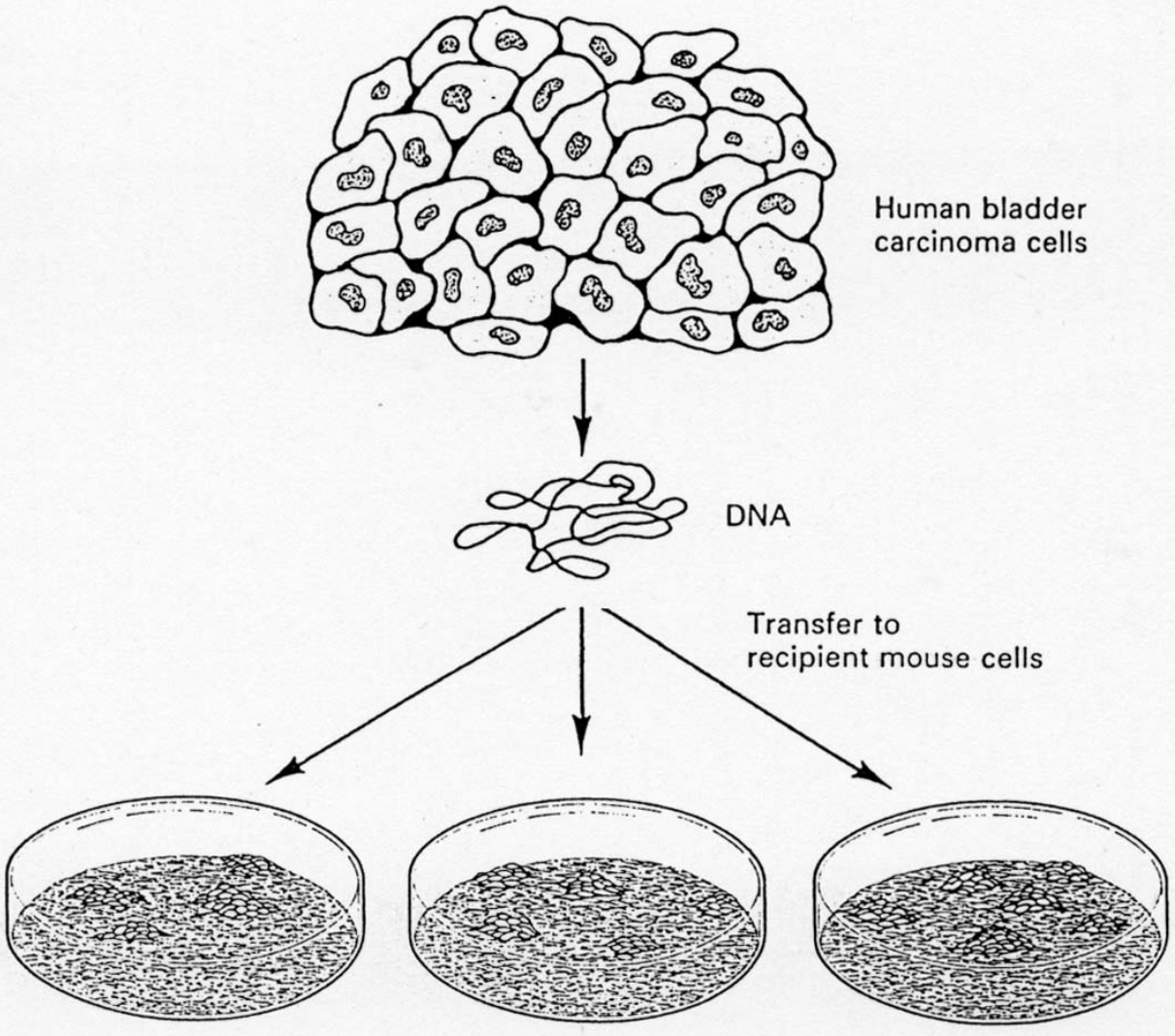
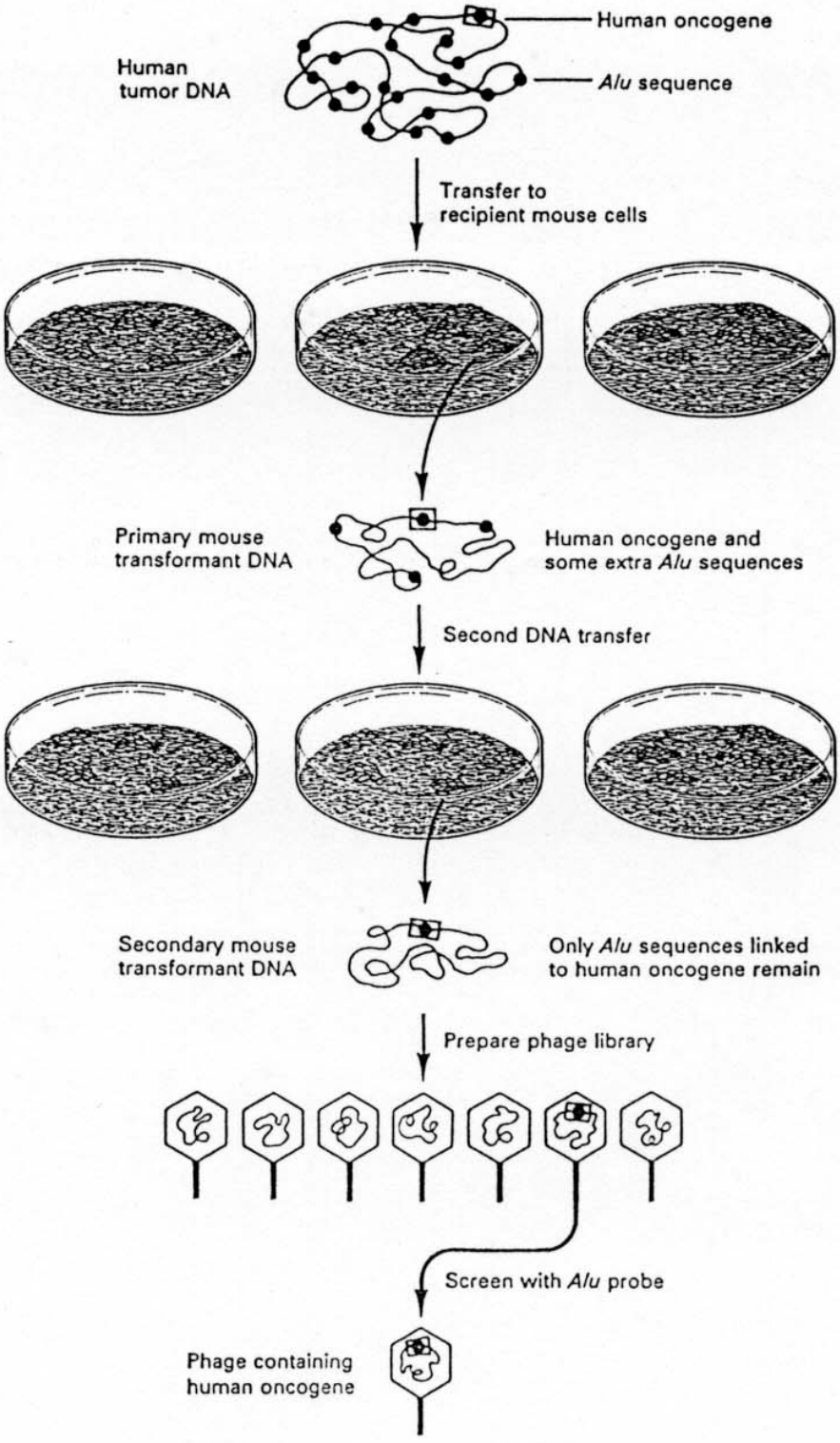


FIGURE 5.2
Detection of the first human oncogene. DNA extracted from the EJ human bladder carcinoma cell line induced high efficiency transformation of recipient mouse cells, indicating that this human tumor contained a biologically active oncogene.


Human oncogene
Human tumor DNA
Alu sequence
Transfer to recipient mouse cells
Primary mouse transformant DNA
Human oncogene and some extra Alu sequences
Second DNA transfer
Secondary mouse transformant DNA
Only Alu sequences linked to human oncogene remain
Prepare phage library
Screen with Alu probe
Phage containing human oncogene

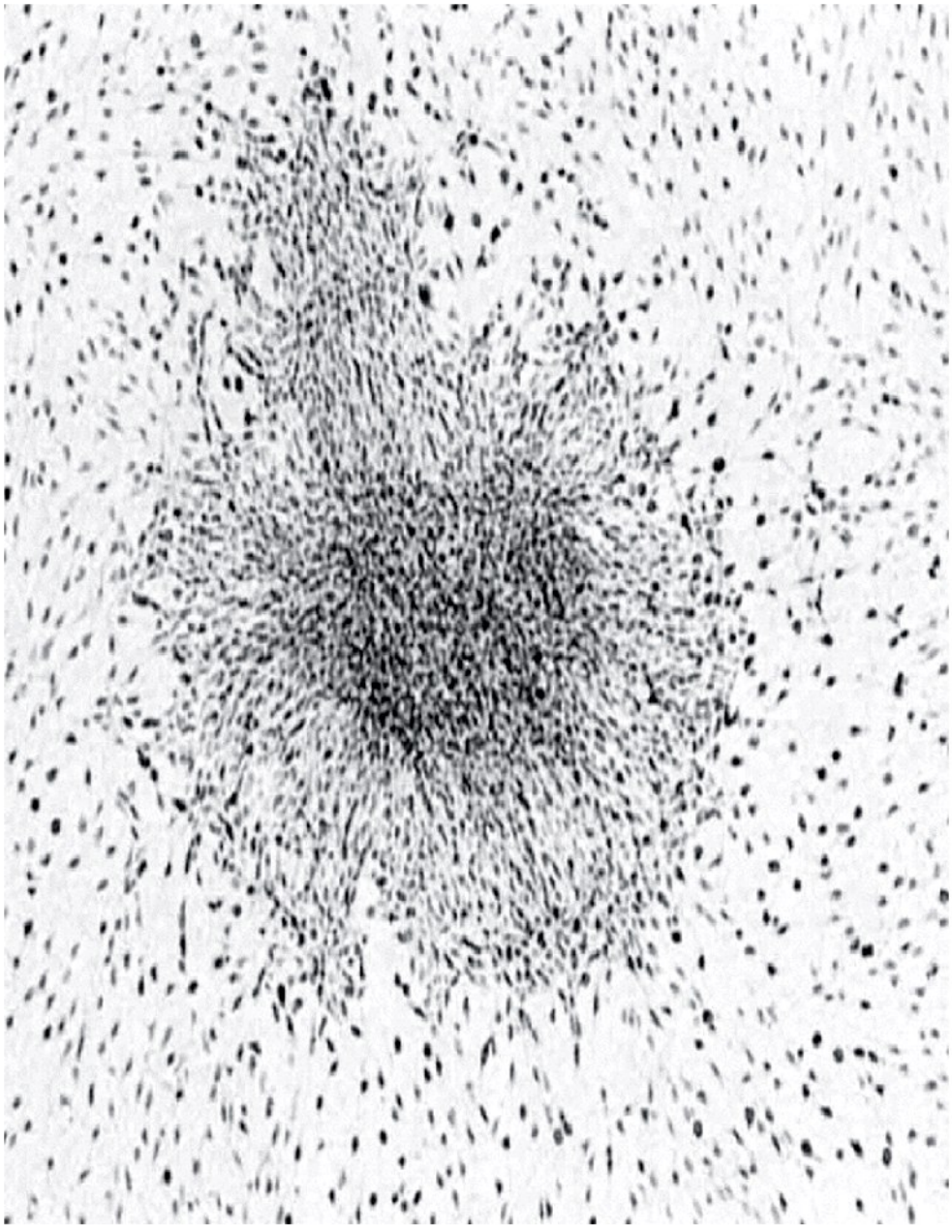


Figure 4-3a The Biology of Cancer (© Garland Science 2007)

Activation of the H-ras proto-oncogene (point mutation)

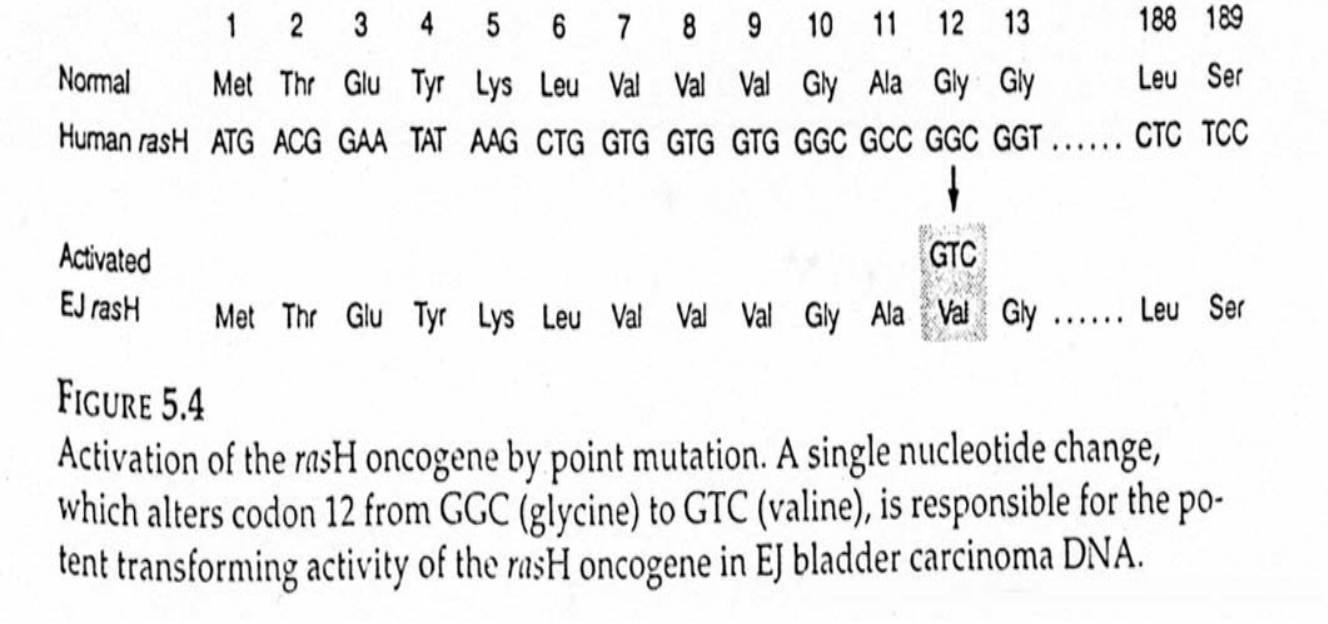


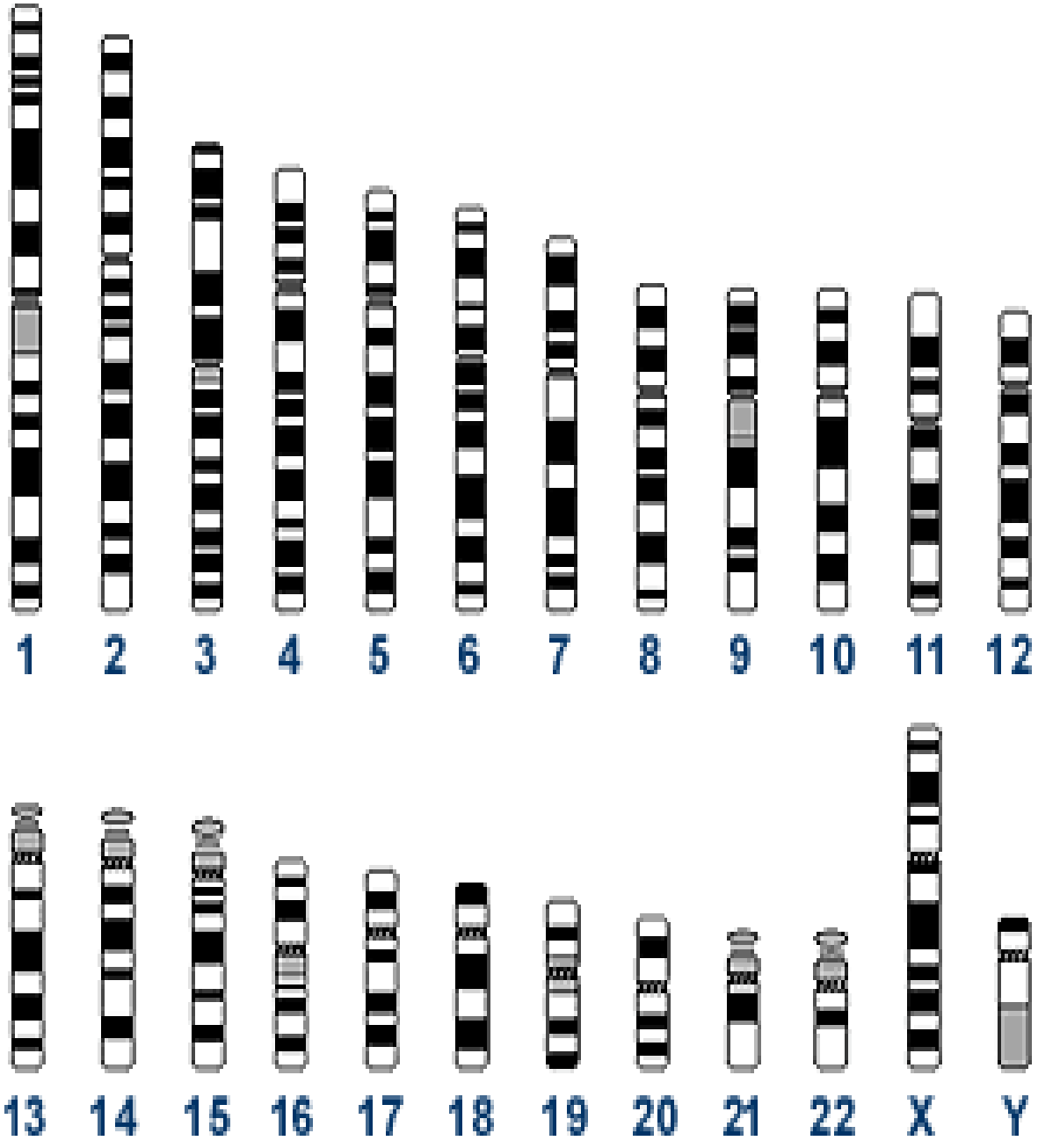
FIGURE 5.4
Activation of the *ras*H oncogene by point mutation. A single nucleotide change, which alters codon 12 from GGC (glycine) to GTC (valine), is responsible for the potent transforming activity of the *ras*H oncogene in EJ bladder carcinoma DNA.

The NIH3T3 DNA transfection assay to identify activated cellular oncogenes was extremely popular in the early 1980's. DNAs from a great variety of tumours were tested.

Assay most efficient at detecting ras oncogenes.

TABLE 5.1
Tumor Oncogenes Detected by Gene Transfer

Oncogene	Tumor	Activation Mechanism
*ras*H, *ras*K, and *ras*N	human and rodent carcinomas, sarcomas, neuroblastomas, leukemias, and lymphomas	point mutation
neu	rat neuroblastomas and glioblastomas	point mutation
met	chemically transformed human osteosarcoma cell line	recombinant fusion protein
ret	human thyroid carcinomas	recombinant fusion protein
trk	human colon carcinoma and thyroid carcinomas	recombinant fusion protein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X
Y

Activation of proto-oncogenes by chromosomal translocation

Burkitt's lymphoma (8:14 translocation)

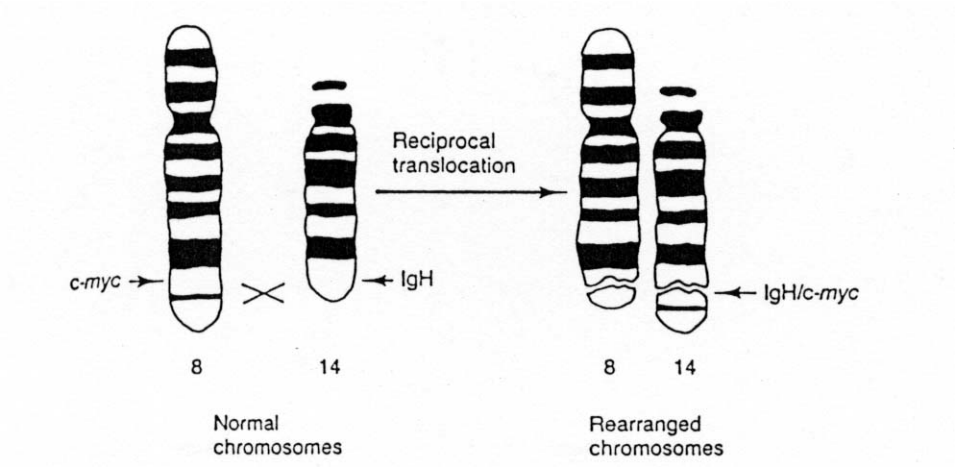


FIGURE 7.1
Translocation of c-*myc* from chromosome 8 to the immunoglobulin heavy-chain (IgH) locus on chromosome 14 in human Burkitt's lymphomas.

Chronic myelogenous leukemia (CML) – Philadelphia Chromosome (9:22 translocation)

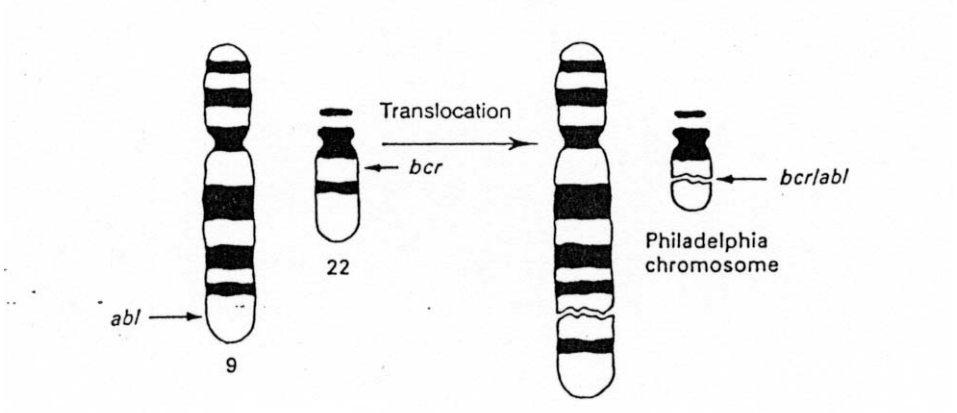


FIGURE 7.3
The Philadelphia chromosome. The *abl* proto-oncogene is translocated from chromosome 9 to the *bcr* locus on chromosome 22.

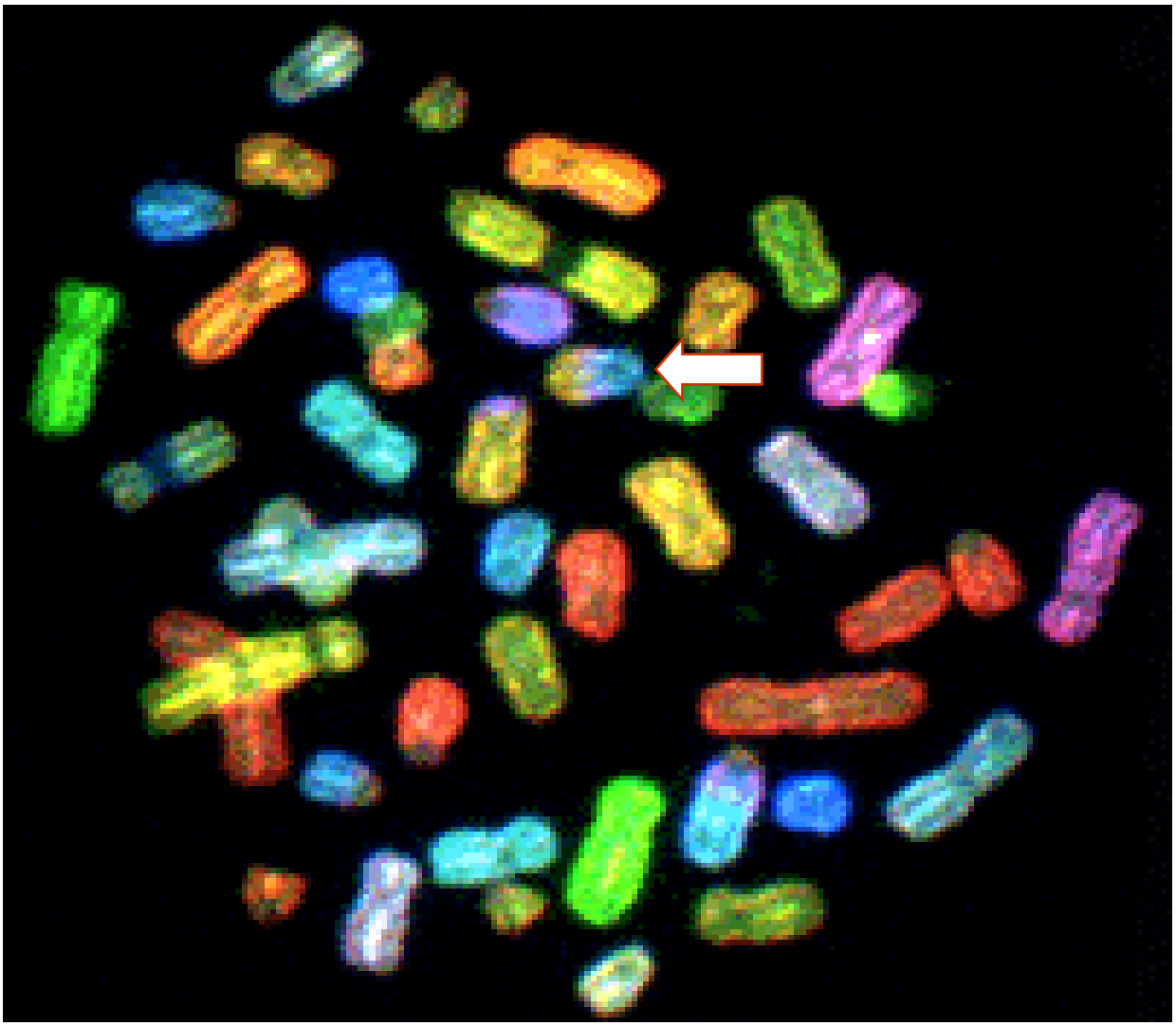


TABLE 7.1
Oncogenes Activated by Translocation

Oncogene	Neoplasm	Activation Mechanism
c-myc	Burkitt's lymphomas and other B- and T-cell neoplasms	aberrant expression
	mouse plasmacytomas	
bcl-2	follicular B-cell lymphomas	
bcl-3	chronic B-cell leukemia	
bcl-6	diffuse B-cell lymphomas	
hox11	acute T-cell leukemia	
IL-3	acute pre B-cell leukemia	
lyl-1	acute T-cell leukemia	
PRAD-1	chronic B-cell leukemia and parathyroid adenoma	
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	
bcr/abl	chronic myelogenous and acute lymphocytic leukemias	fusion proteins
dek/can	acute myeloid leukemia	
E2A/pbx1	acute pre B-cell leukemia	
PML/RAR	acute promyelocytic leukemia	
c16/*erg*[1]	myeloid leukemia	
rel/nrg	B-cell lymphoma	
CBFß/MYH11	acute myeloid leukemia	
ews/fli-1	Ewing sarcoma	
*lyt-10/*Cα_1	B-cell lymphoma	
hrx/enl	acute leukemias	
hrx/af4	acute leukemias	
aml1/mtg8	acute myeloid leukemias	
NPM/ALK	large-cell lymphomas	

[1]The fusion partner of *erg* on chromosome 16 (indicated c16) has not yet been identified.

ACTIVATION OF PROTO-ONCOGENES BY GENE AMPLIFICATION

Homogeneously staining regions (HSRs) and double minutes (DMs)

HSRs and DMs found in a large number of tumours

The first gene identified in HSRs/DMs was c-myc (MYC) (in HL60 – a promyelocytic leukemia)

Neuroblastoma tumours have N-myc (MYCN) amplified in HSRs or DMs. Patients with multiple copies of N-myc (amplification of the N-myc gene) have a poor clinical prognosis.

Table 4.3 Some frequently amplified chromosomal regions and the genes they are known to carry

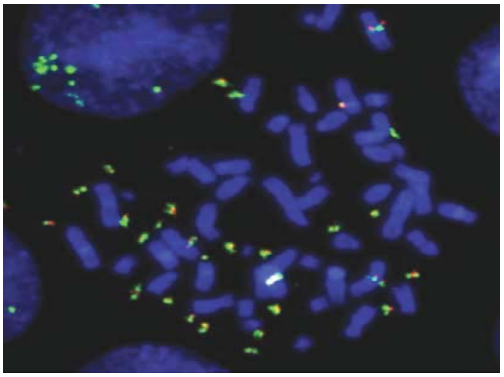
Name of oncogene[a]	Human chromosomal location	Human cancers	Nature of protein
erbB1	7q12–13	glioblastomas (50%); squamous cell carcinomas (10–20%)	RTK
cab1–erbB2–grb7	17q12	gastric, ovarian, breast carcinomas (10–25%)	RTK, adaptor protein
k-sam	7q26	gastric, breast carcinomas (10–20%)	RTK
FGF-R1	8p12	breast carcinomas (10%)	RTK
met	7q31	gastric carcinomas (20%)	RTK
K-*ras*	6p12	lung, ovarian, bladder carcinomas (5–10%)	small G protein
N-*ras*	1p13	head and neck cancers (30%)	TF
c-*myc*	8q24	various leukemias, carcinomas (10–50%)	TF
L-*myc*	1p32	lung carcinomas (10%)	TF
N-*myc–DDX1*	2p24–25	neuroblastomas, lung carcinomas (30%)	TF
akt-1	14q32–33	gastric cancers (20%)	ser/thr kinase
cyclin D1–exp1–hst1–ems1	(11q13)	breast and squamous cell carcinomas (40–50%)	G1 cyclin
cdk4–mdm2–sas–gli	12q13	sarcomas (40%)	CDK, p53 antagonist
cyclin E	19q12	gastric cancers (15%)	cyclin
akt2	(19q13)	pancreatic, ovarian cancers (30%)	ser/thr kinase
AIB1, BTAK	(20q12–13)	breast cancers (15%)	receptor co-activator
cdk6	(19q21–22)	gliomas (5%)	CDK
myb	6q23-24	colon carcinoma, leukemias	TF
ets-1	11q23	lymphoma	TF
gli	12q13	glioblastomas	TF
FGFR2	10q26	breast carcinomas	RTK

[a]The listing of several genes indicates the frequent co-amplification of a number of closely linked genes; only the products of the most frequently amplified genes are described in the right column.

Courtesy of M. Terada, Tokyo, and adapted from G.M. Cooper, Oncogenes, 2nd ed. Boston and London: Jones and Bartlett, 1995.

Gene amplification

Double minutes



HSR

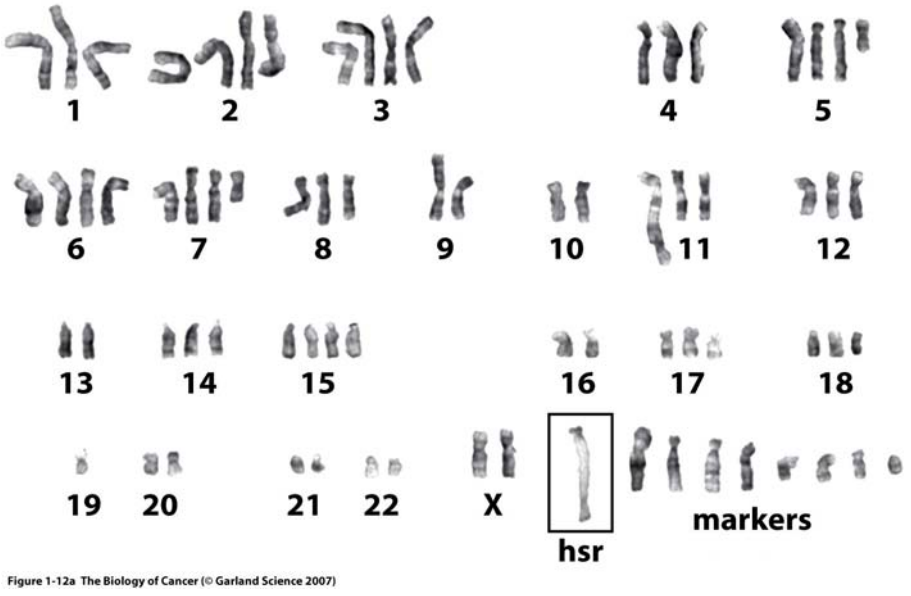


Figure 1-12a The Biology of Cancer (© Garland Science 2007)

HSR

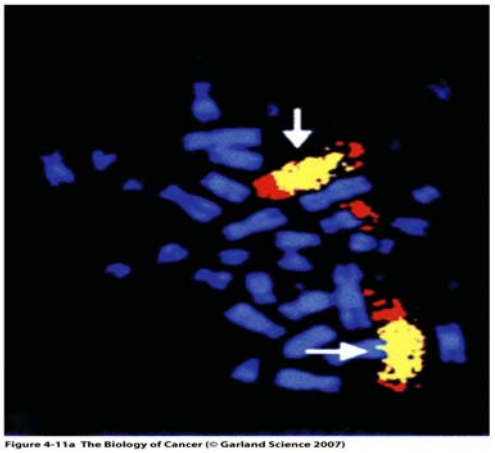
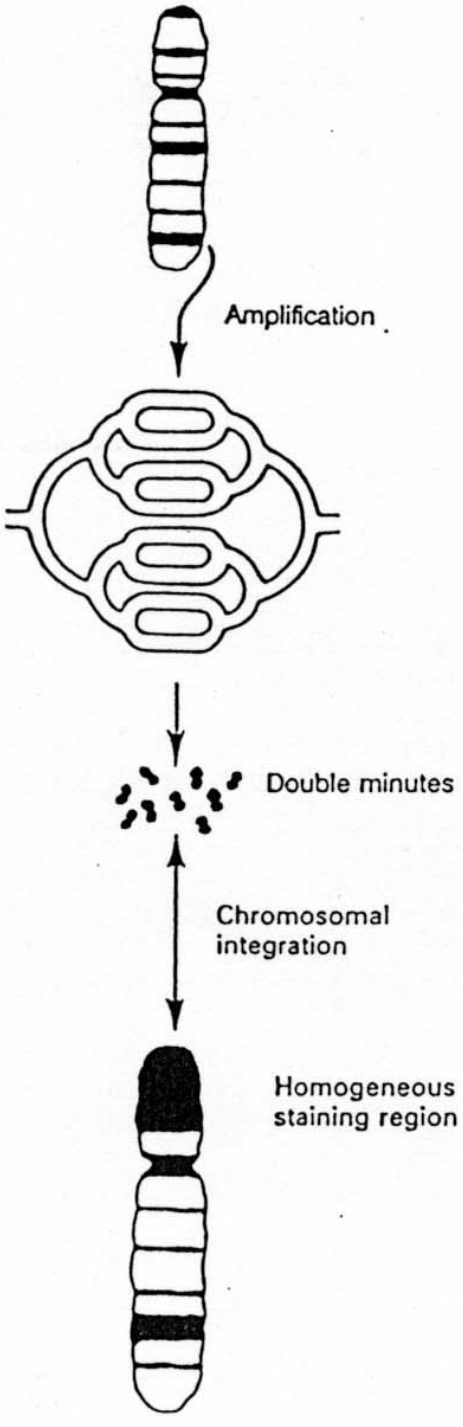


Figure 4-11a The Biology of Cancer (© Garland Science 2007)

MYCN amplification in neuroblastoma



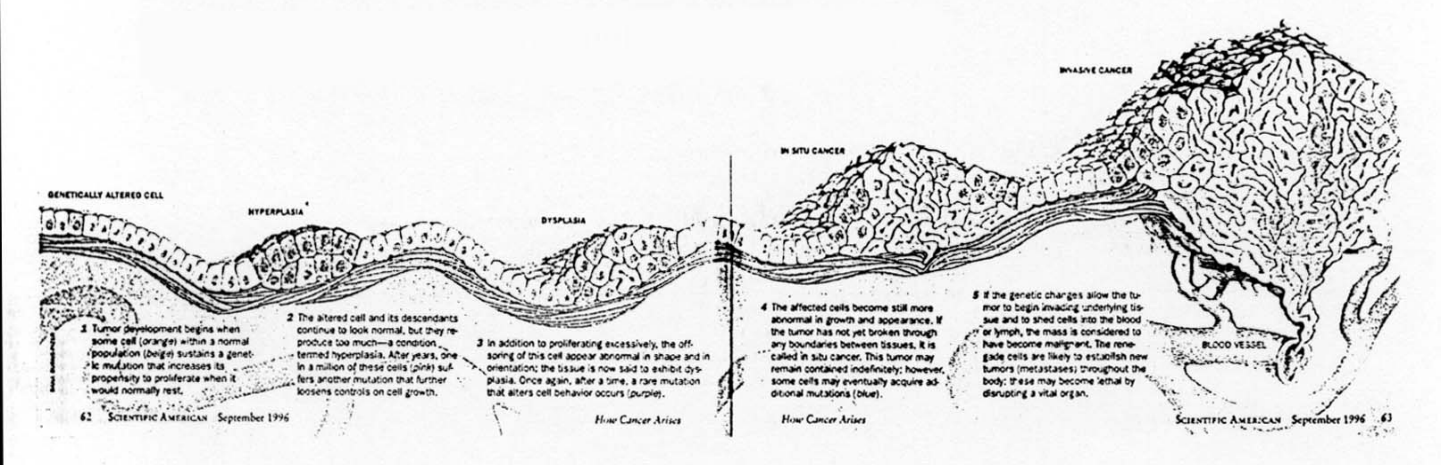
DNA amplification. A locus is amplified by repeated DNA replication. Recombination can generate tandem arrays of the amplified DNA, which can be excised from the chromosome to form double minutes. Double minutes can integrate into another chromosome and form a homogeneous staining region. Because chromosome integration is reversible, double minutes and homogeneous staining regions are interchangeable forms of amplified DNA.

Is cellular proto-oncogene activation sufficient to cause cancer?

Evidence suggesting that multiple hits are required for cancer formation:

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

(2) Some cancers go through several stages



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

Table 5.2. Complementation Groups of Oncogenes

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

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

(4) In some cases, infection of cells with a retrovirus carrying an oncogene suggests that additional events are required. For example, infection of bone marrow cells of mice with a retrovirus carrying the bcr/abl fusion gene found in CML results in CML in some cases only. Furthermore, it takes a long time for CML to develop, suggesting that additional genetic alterations are required

(5) Multiple mutated genes are found in a single cancer cell. E.g., HL60 has both amplified myc gene and amplified N-ras gene. In colon carcinoma, at least 6 different genetic alterations have been identified (Kinsler and Vogelstein Cell 87:159-170 – 1996).

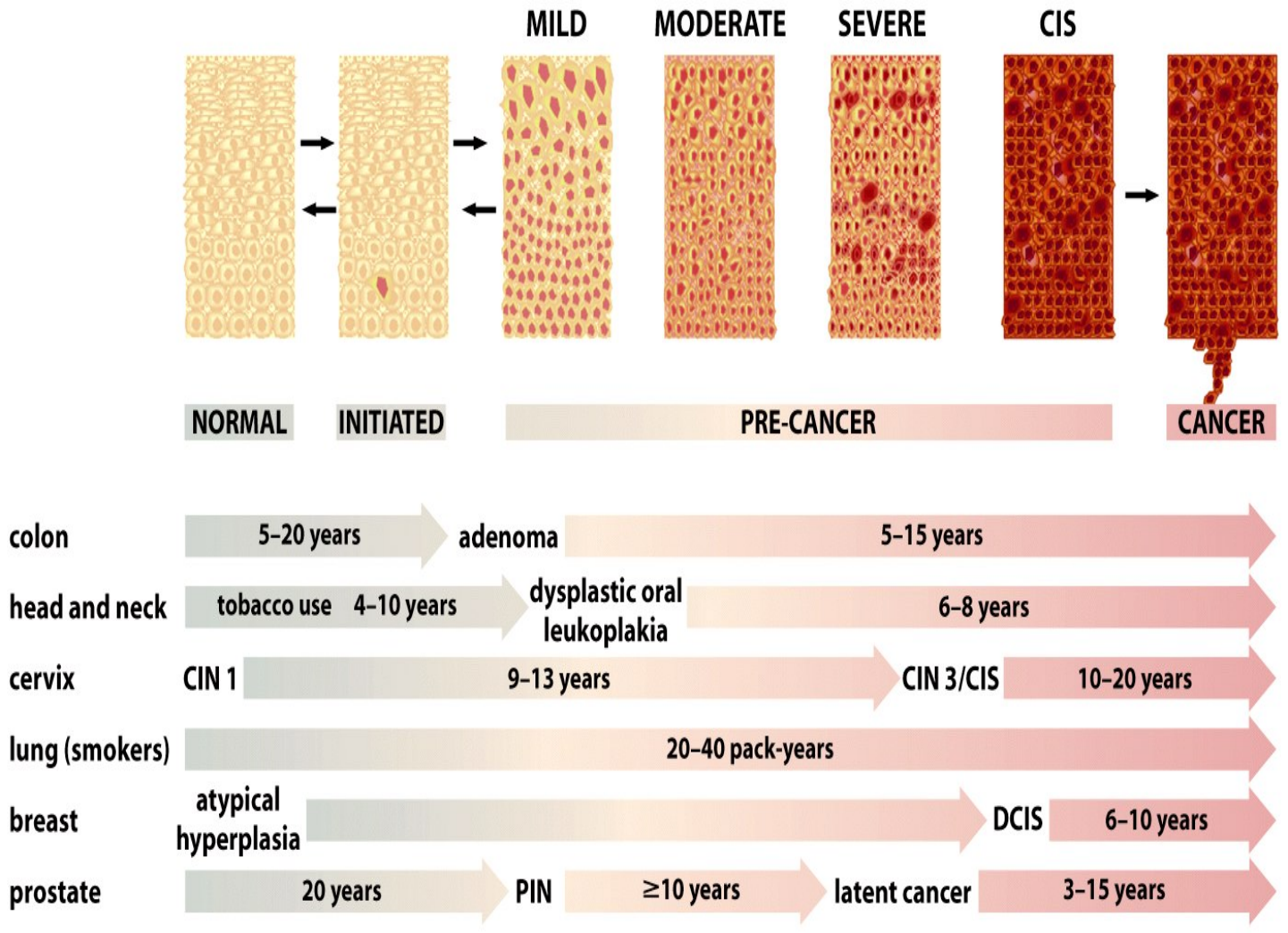


Figure 11-7 The Biology of Cancer (© Garland Science 2007)

CIS = carcinoma *in situ*

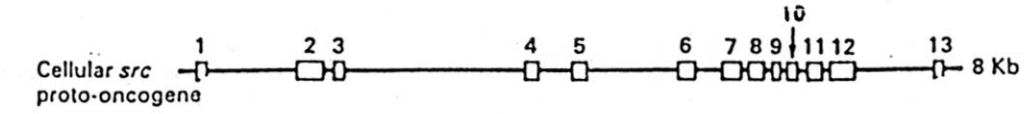
CIN = cervical intraepithelial neoplasia

DCIS = ductal carcinoma *in situ*

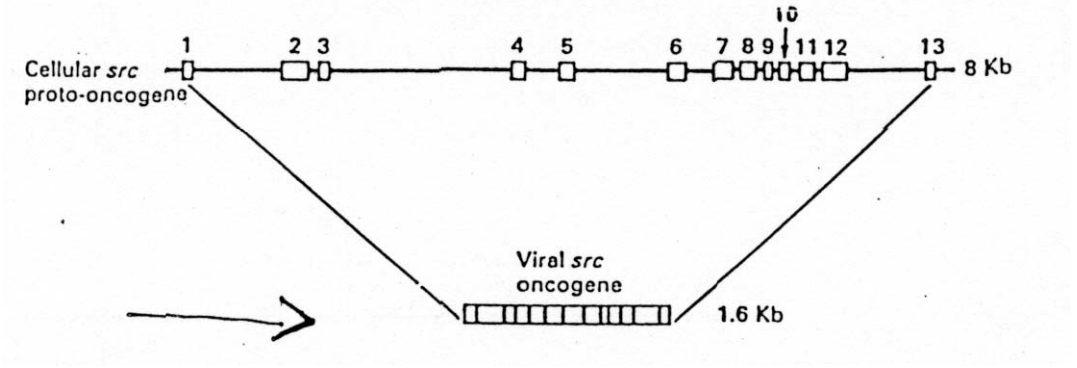
PIN = prostatic intraepithelial neoplasia

Cellular proto-oncogenes, viral oncogenes and cellular oncogenes – e.g. src

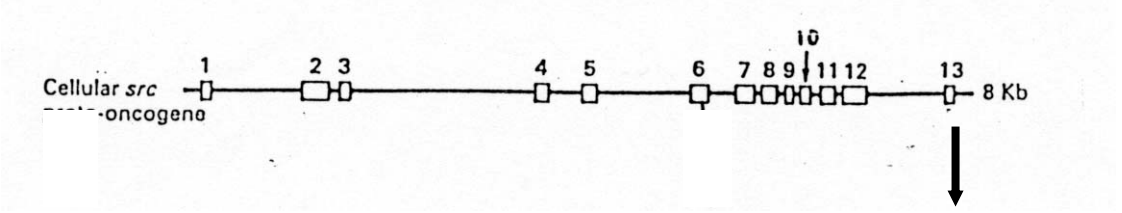
Cellular proto-oncogene



Viral oncogene – multiple mutations, expressed at elevated levels



Cellular oncogene – single base pair substitutions, translocations resulting in fusion proteins or overexpression, gene amplification resulting in overexpression



e.g. point mutation

Table 39.3

The sequences of *v-onc* coding regions are derived from *c-onc* genes by substitutions and (sometimes) loss of terminal regions.

Gene	Changes in corresponding regions	Identity	Region Missing in *v-onc*
mos	11 / 369 substitutions	97%	None
H-ras	3 / 189	98%	None
K-ras	7 / 189	96%	None
sis	18 / 220	92%	None
myc	2 / 417	99%	None
src	16 / 514 substitutions	97%	C-terminal 19 amino acids
fms	20 / 930	98%	C-terminal 50 amino acids
erbB	99 / 600	83%	N-terminal half & C-terminus (610 total)
erbA	22 / 396	95%	N-terminal 12 amino acids
myb	11 / 372	97%	N- & C-termini (268 residues)

NB RSV – 1-2 weeks – sarcoma
other RNA tumour viruses – take longer

RNA tumour viruses do not infect all cell types

Sites of action of oncogenes

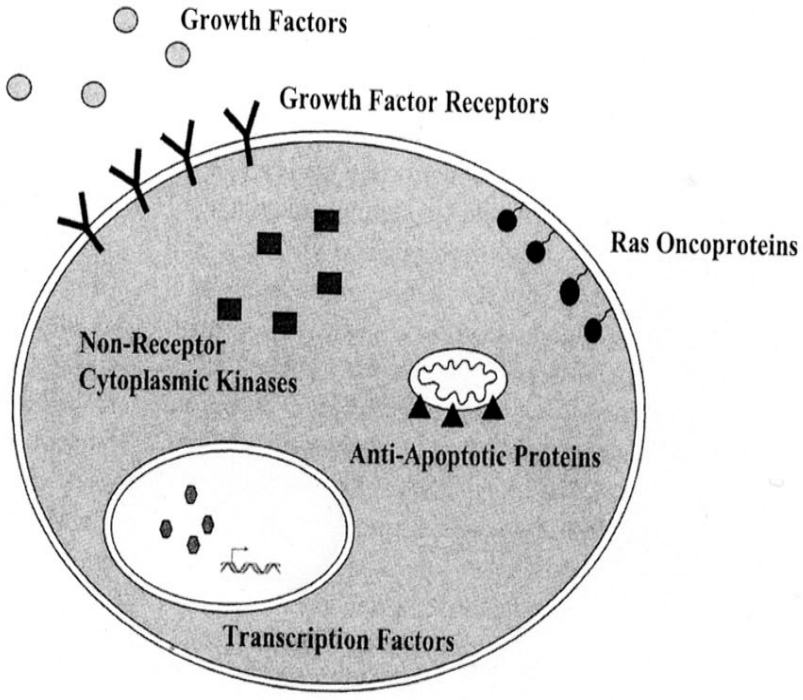


Figure 7.7. Oncogenes are involved in cytoplasmic signaling pathways and nuclear gene regulation. They include growth factors and growth factor receptors, *ras* oncogenes, non-receptor cytoplasmic kinases, anti-apoptotic proteins, and nuclear transcription factors.

Roles of proteins encoded by oncogenes

1. Growth factors
   - sis

2. Growth factor receptors
   - erbB
   - erbB2 (Her-2/neu, HER2)

3. Nonreceptor tyrosine kinases
   - src

4. GTP-binding proteins
   - ras

5. Serine/threonine kinase
   - raf

6. Nuclear transcription factors (DNA binding proteins)
   - myc
   - fos/jun

Table 7.1. Examples of Oncogenes and Their Protein Products

Oncogene/ Protein Product	Function
Growth Factors	
v-sis	Platelet derived growth factor
int-1	Matrix protein
int-2	Fibroblast growth factor-related protein
KS3	Fibroblast growth factor-related protein
Growth Factor Receptors	
FGFR3	Fibroblast derived growth factor receptor
PDGFR	Platelet derived growth factor receptor
IGF-1R	Insulin growth factor receptor
VEGFR	Vascular endothelial growth factor receptor
EGFR	Epidermal growth factor receptor
v-kit	Stem cell growth factor receptor
v-fms	CSF-1 receptor
Her2/NEU	Heregulin receptor
met	Hepatic growth factor receptor
flt3	FLT3 ligand receptor
trk	Nerve growth factor receptor
Ras oncogenes	
H-ras	GTPase
K-ras	GTPase
N-ras	GTPase
Cytoplasmic kinases	
BCR-ABL	Protein tyrosine kinase
src	Protein tyrosine kinase
v-fes	Protein tyrosine kinase
v-fps	Protein tyrosine kinase
v-fgr	Protein tyrosine kinase
hck	Protein tyrosine kinase
pim	Protein serine/threonine kinase
v-raf	Protein serine/threonine kinase
v-mos	Protein serine/threonine kinase
Transcription factors	
c-myc	Transcription factor
N-myc	Transcription factor
L-myc	Transcription factor
v-fos	Transcription factor
v-jun	Transcription factor
v-rel	Transcription factor
v-ets	Transcription factor
Anti-apoptotic proteins	
bcl-2	Anti-apoptotic protein
twist	Anti-apoptotic protein

Modified from BSO 3rd Ed. and Genetic Basis of Human Cancer.

The Basic Science of Oncology, pg126

Functions of proteins encoded by oncogenes

Normal cells – balance between growth-promoting and growth-restraining/inhibiting properties.

Cancer cells – balance is altered so that growth-promoting properties are increased (oncogenes) or growth-inhibiting properties are decreased (tumour suppressors).

1. **Oncogenes and growth factors (sis/PDGF)**

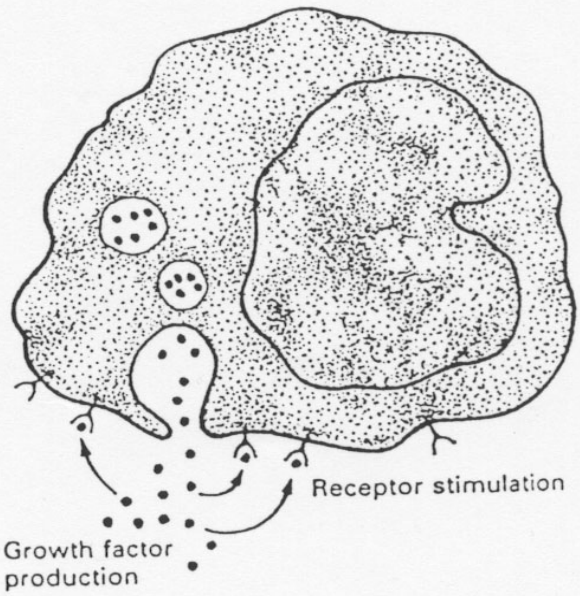
Cell growth and differentiation are triggered by extracellular signals at cell surface. When growth factor binds to cell surface receptor, a cascade of biochemical reactions is initiated that alters protein expression in the cell.

Growth factors are short polypeptides that induce proliferation in appropriate target cells. Normal cell growth and differentiation depends on presence of growth factors. However, if growth factor is always expressed, this can result in continuous stimulation of cell growth (a step towards transformation and cancer).

Growth factors – cont'd

The sis (simian sarcoma virus) oncogene was discovered In 1983 – identified as the B-subunit of the platelet-derived growth factor (PDGF). PDGF is a major growth factor used by fibroblasts and smooth muscle cells. Only cells that are normally responsive to PDGF (i.e. cells that have PDGF receptors) are responsive to sis.

FIGURE 12.1
Autocrine stimulation of cell growth. A cell produces a growth factor to which it also responds, resulting in continuous cell proliferation.



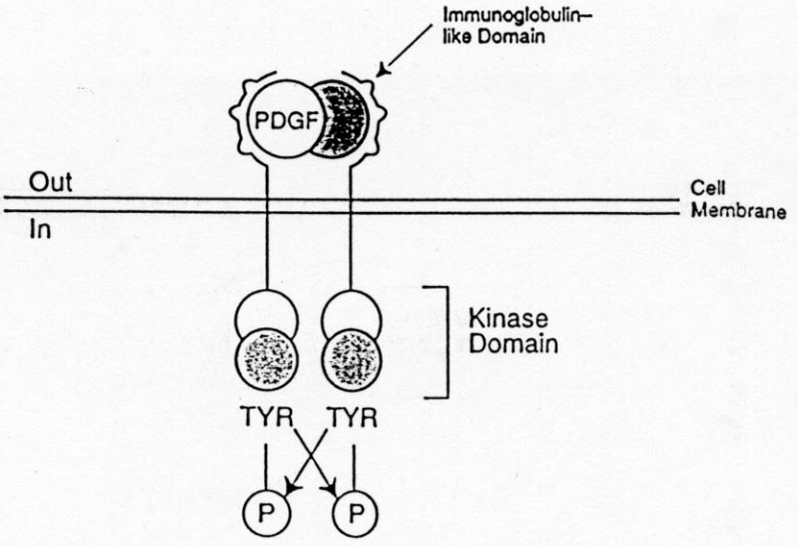


Figure 6.2. Functional significance of PDGF binding to its receptor. PDGF binds two receptor molecules, facilitating phosphorylation of their intracellular components. This allows recruitment of additional intracellular signaling molecules, which bind to phosphotyrosine, thus propagating the signal. TYR = tyrosine, P = phosphate.

Kaposi's sarcoma
(autocrine signaling)

PDGF
TGF-β
IGF-1
Ang2
CC18/14
CXCL11
Endothelin

Plus all receptors for these ligands

Plus HHV-8 produces two more ligands whose receptors are expressed in Kaposi sarcoma cells

2. Oncogenes and growth factor receptors (receptor protein tyrosine kinases)

- a common way of regulating protein function is by phosphorylation. Many oncogenes encode growth factor receptors. Growth factor receptors are often protein tyrosine kinases that can phosphorylate themselves and other proteins at tyrosine residues resulting in the activation of a signaling cascade.

Example 1: EGFR (ErbB)

- the epidermal growth factor (EGF) receptor binds growth factor EGF, resulting in a conformational change that facilitates dimerization of growth factor receptors by exposing a dimerization loop. Receptor dimerization results in intermolecular phosphorylation of tyrosines which in turn allows access to ATP and protein substrates.

- ErbB (is a truncated version of the EGF receptor (contains the PK domain and the transmembrane domain but lacks the extracellular ligand binding domain – so no longer need EGF for stimulation).

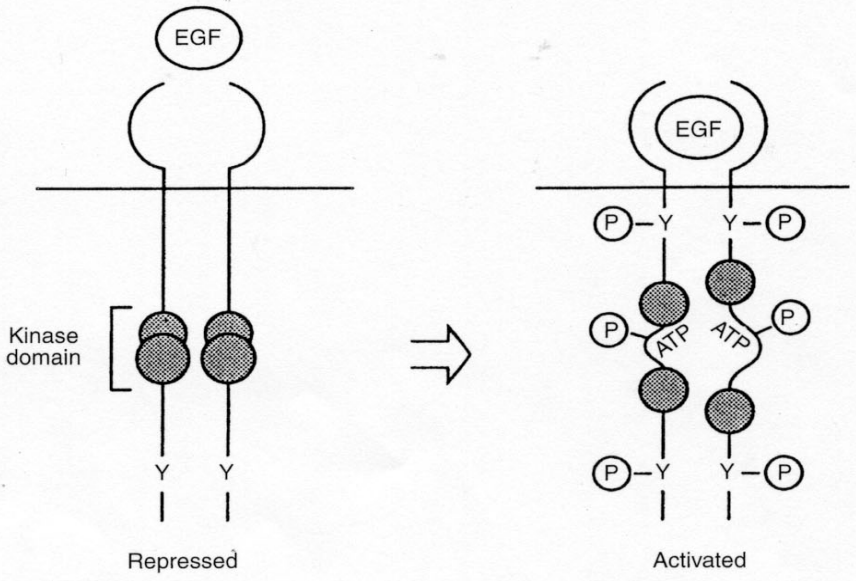


Figure 8.2. Growth factor receptor dimerization and activation. In the absence of ligand (EGF) binding the intracellular kinase domain is inactive, held in a repressed conformation by intramolecular interactions. Ligand binding induces receptor dimerization, relief of inhibitory constraints, and autophosphorylation of the intracellular domains on tyrosine residues. These autophosphorylation sites function to both enhance the catalytic activity and serve as docking sites for intracellular signaling molecules that bind to phosphotyrosine. P, phosphate; Y, tyrosine.

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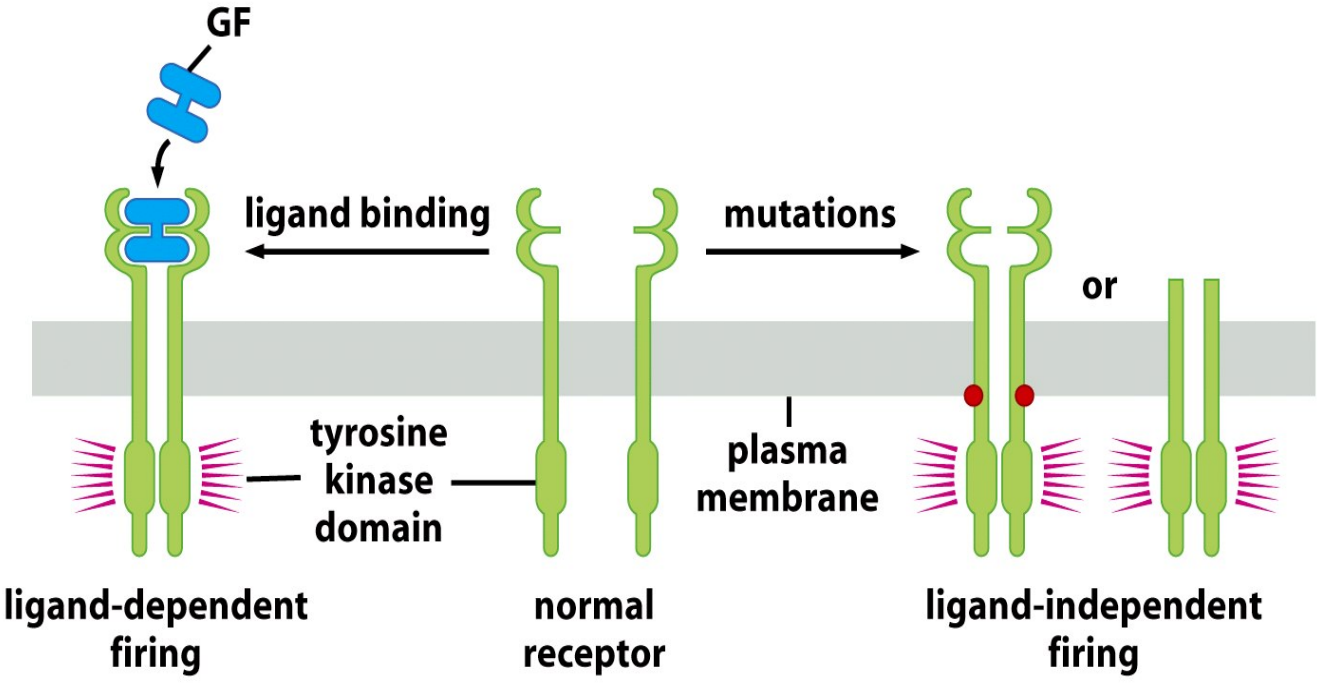


Figure 5-12a The Biology of Cancer (© Garland Science 2007)

Example 2: Her2/neu (ErbB2)

- second family of oncogenes identified using the NIH3T3 assay to analyse rat tumours. Neu protein found in rat tumours is almost identical to c-erbB2 (one amino acid substitution). This amino acid substitution in the transmembrane domain causes ligand-independent dimerization of neu protein.

- in humans, Her2/neu is amplified in many cancers including breast, ovarian, lung, colon, pancreatic. Her2/neu is amplified and over-expressed in ~30% of breast cancers and is associated with metastasis and a poor clinical outcome. As receptor concentration increases, there is a shift from monomeric to dimeric (active) form.

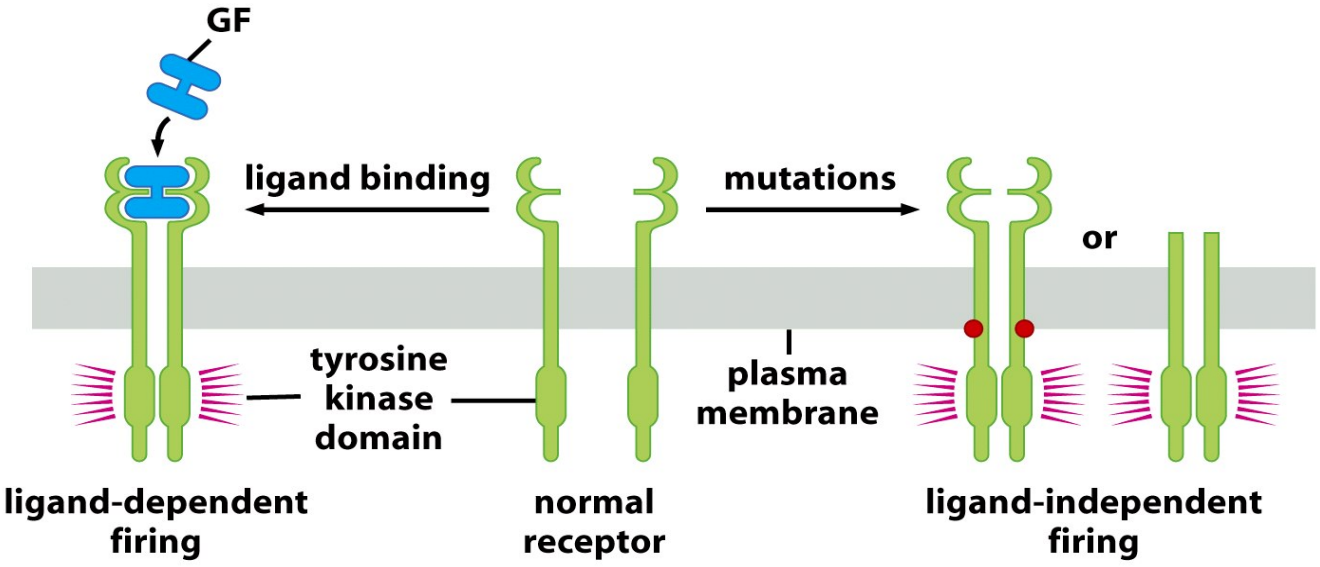


Figure 5-12a The Biology of Cancer (© Garland Science 2007)

TABLE 5.1
Tumor Oncogenes Detected by Gene Transfer

Oncogene	Tumor	Activation Mechanism
*ras*H, *ras*K, and *ras*N	human and rodent carcinomas, sarcomas, neuroblastomas, leukemias, and lymphomas	point mutation
neu (ErbB2, Her2)	rat neuroblastomas and glioblastomas	point mutation
met	chemically transformed human osteosarcoma cell line	recombinant fusion protein
ret	human thyroid carcinomas	recombinant fusion protein
trk	human colon carcinoma and thyroid carcinomas	recombinant fusion protein

Breast cancer – heterogeneous disease

15 distinct forms recognized by the American Joint Committee on Cancer

Four distinct molecular subtypes of locally advanced breast cancer have been identified based on gene expression profiling:

Luminal A (Estrogen receptor (ER) positive – lower percentage of proliferating cells): good prognosis

Luminal B (ER+ve – higher percentage of proliferating cells): intermediate prognosis

Basal-like or triple-negative (ER, PR and HER2 negative): poor prognosis

HER2 positive: poor prognosis

Her2/neu has been targeted for the treatment of Her2/neu-overexpressing metastatic breast cancer

- Trastuzumab (marketed as Herceptin): humanized monoclonal antibody that recognizes Her2/neu protein and inhibits proliferation of Her2/neu-overexpressing breast cancer cells. Trastuzumab has been recommended for the treatment of Her2/neu-overexpressing breast cancer patients.

Problem; resistance to trastuzumab treatment. Need combination therapy.

- other possibilities – drugs that block tyrosine kinases, antisense oligonucleotides. Need specific delivery systems.

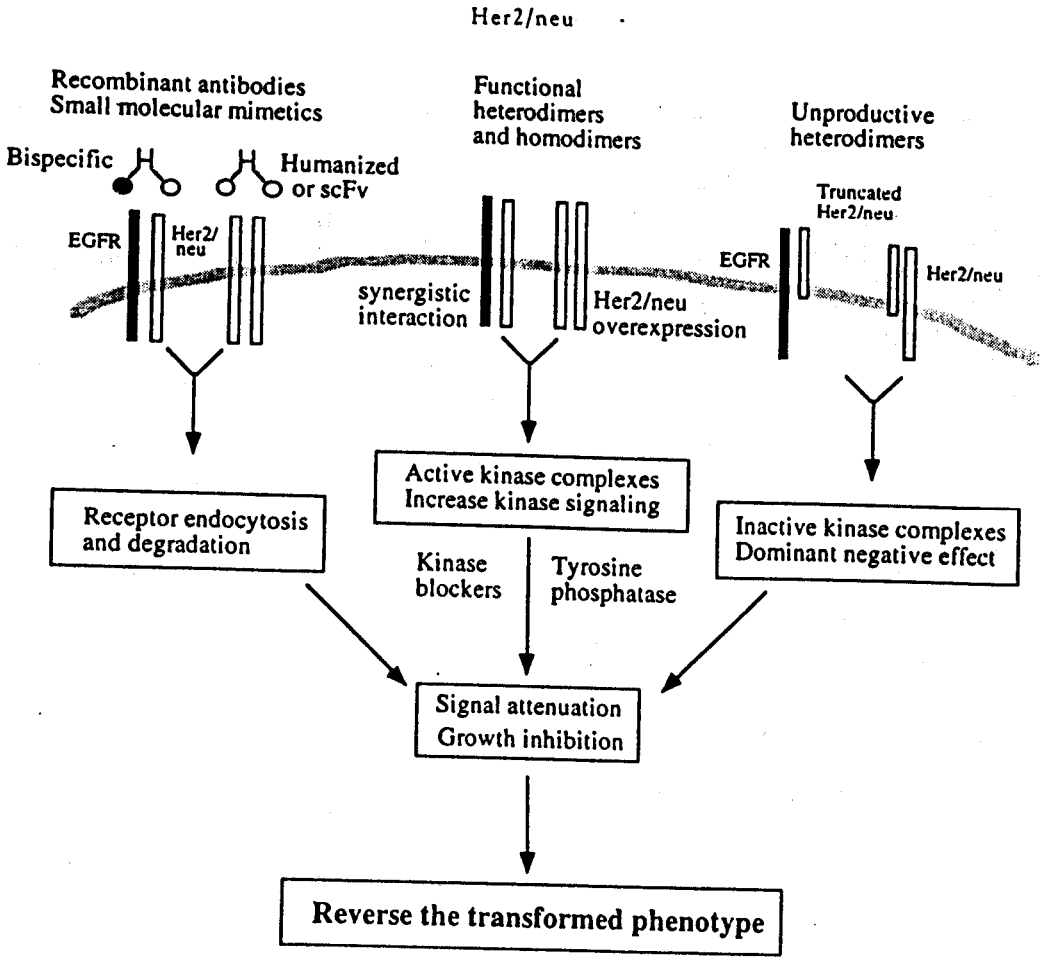


FIGURE 5 Current approaches for the reversion of Her2/neu resultant transformed phenotype (see text for details).

- targets of Her2/neu kinase: phosphatidylinositol-3-kinase (PI3-kinase/Akt), mitogen-activated protein kinase (MAPK), cAMP/protein kinase A. These pathways are involved in cell proliferation and cell survival; e.g. Akt phosphorylates p21 and disrupts its growth-inhibitory activity.

- reduction in Her2/neu receptors on cell surface (after Herceptin treatment) may result in a reduction in PI3-kinase and Akt that protect cells from apoptosis, thus making these cells more sensitive to radiation treatment and chemotherapy.

Junttila et al. Cancer Cell 15, May 5, 2009

ErbB2 does not have a ligand and functions as a co-receptor with other ErbB receptors.

ErbB3 is best suited to activate the PI3K/Akt signaling pathway as it has multiple binding sites for the regulatory subunits of PI3K.

ErbB3 binds heregulins but has impaired kinase activity, and only signals as a complex with another ErbB, preferably ErbB2

Ligand-independent ErbB2/ErbB3/PI3K complexes function as a oncogenic unit in ErbB2-over-expressing breast cancer cells

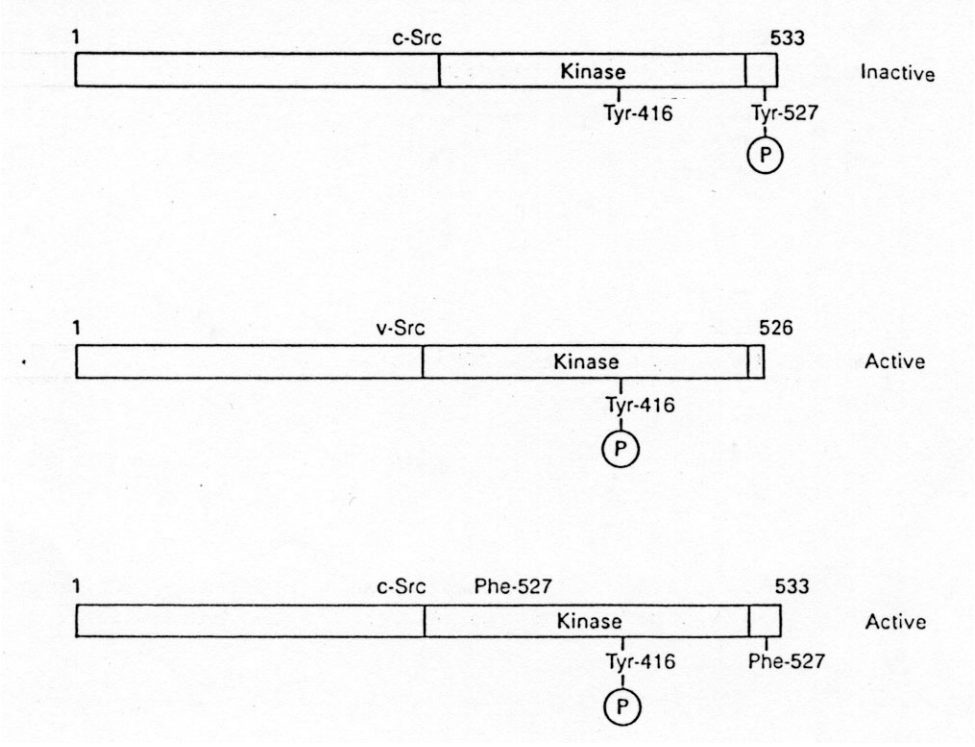
Trastuzumab destabilizes ligand-independent ErbB2/ErbB3/PI3K complexes, uncoupling ErbB3 from ErbB2 and blocking downstream PI3K signaling.

However, PI3K mutations are common in breast cancer. Mutant PI3K remains at cell membrane and activates Akt in the presence of transtuzumab.

Target PI3K pathway in trastuzumab-resistant ErbB2 over-expressing breast cancers.

3. Oncogenes and nonreceptor protein tyrosine kinases

Src was the first oncogene shown to have protein kinase activity. Phosphorylates tyrosine residues. Cytoplasmic (intracellular) protein. A family of 9 closely related tyrosine kinases constitute the Src family. Implicated in signalling through growth factor receptor tyrosine kinases such as receptor for PDGF.

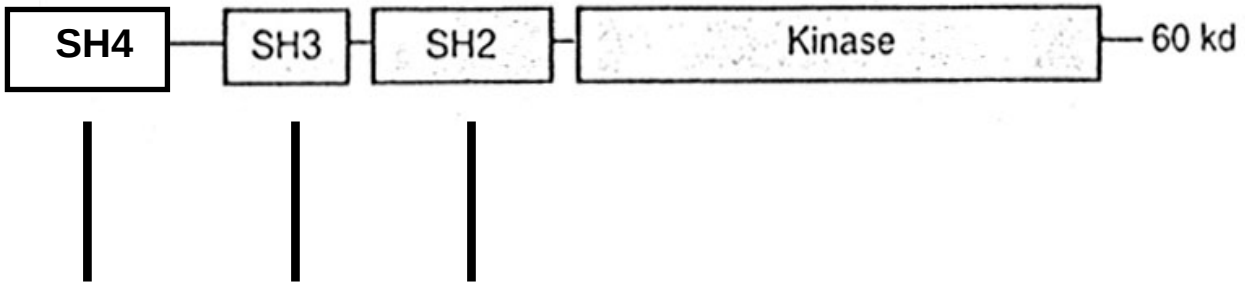


Tyrosine 416 is autophosphorylated by Src – increases kinase activity and transforming potential

Tyrosine 527 is phosphorylated by another protein-tyrosine kinase (e.g. Csk or Chk)

- downregulates kinase activity (tyrosine 527 is not present in viral src protein)

Src homology domains



Myristoy-lation site

Targets Src to cytoplasmic membrane

Binds proline-rich proteins

Binds phosphotyrosine-containing proteins [e.g. PDGF receptor, FAK (focal adhesion kinase)]

Many of Src substrates are part of signaling cascades

Src – cont'd

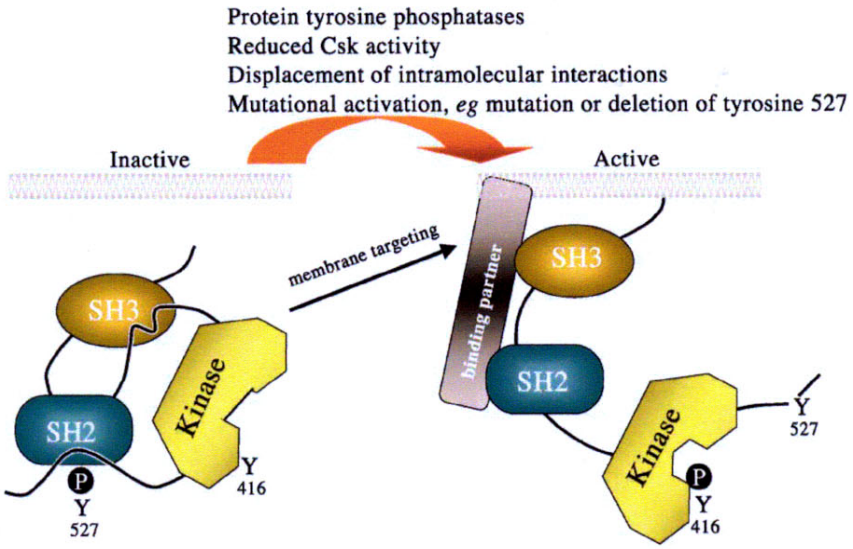


Fig. 2. Shown are the likely regulators, or types of regulation, which can lead to Src's conformational "opening" and catalytic activation that might occur in cancer cells.

BBA 1602: 114-130 (2002)

Src – cont'd

Src is overexpressed in some epithelial cancers (colon, breast)
Src is mutated in some colon cancers [affects Tyr527(530) phosphorylation]

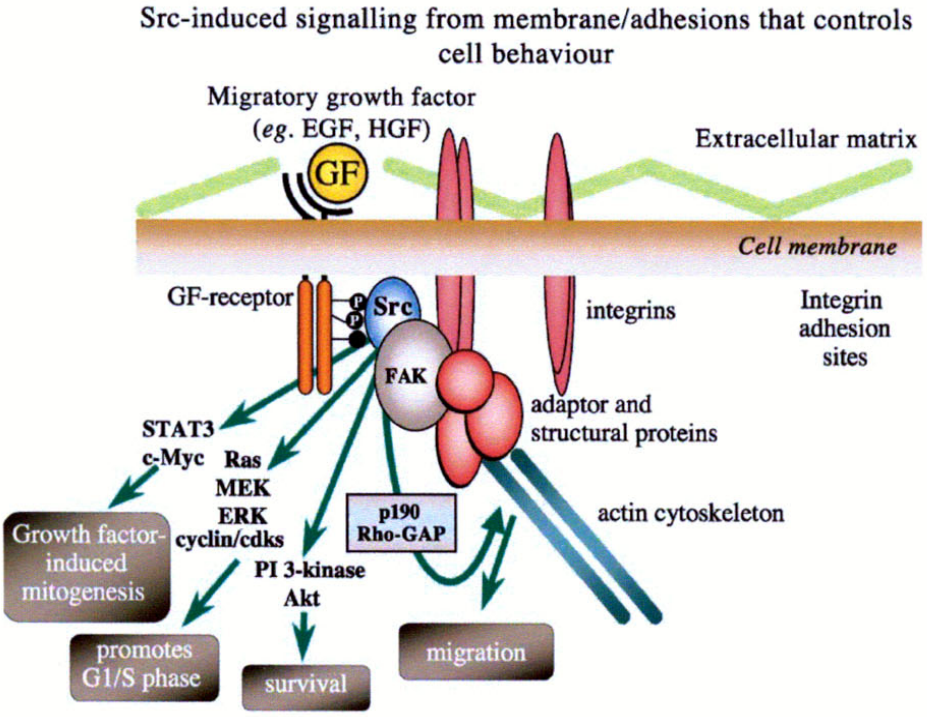


Fig. 3. Src and FAK co-localise at integrin adhesion sites in fibroblasts and cooperates with growth factor (GF) receptors, such as EGF and PDGF to induce signalling pathways that control diverse aspects of cell behaviour, including growth, survival and migration.

BBA 1602: 114-130 (2002)

Src – therapeutic target for cancer?

- Activation of Src is common in solid tumors, whether or not its negative regulator Csk is present

- Activated Src moves to cell membrane

- Lipid rafts are cholesterol-enriched domains believed to be important for protein signaling in cells

Oneyama et al. Molecular Cell 30: 426-436 (2008)

- Csk is a cytoplasmic protein whereas activated Src is anchored to the cytoplasmic membrane

- Csk uses adaptor proteins to access Src

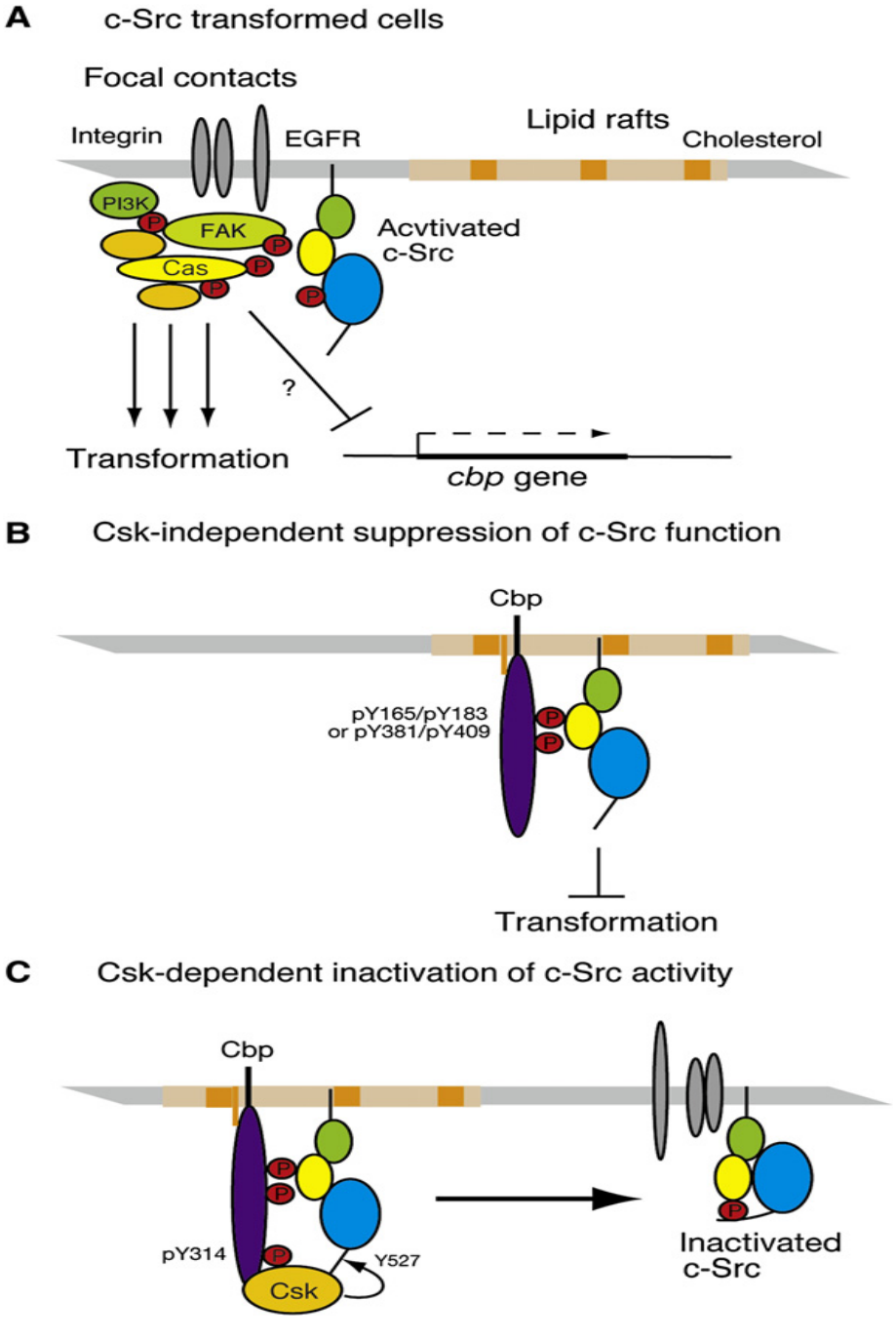
- Cbp is a Csk adaptor protein found in lipid rafts

- In Csk-deficient fibroblasts, over-expression of wild-type Src can induce transformation

- Cbp expression is dramatically downregulated in these transformed fibroblasts

- Re-expression of Cbp suppresses Src transformation (in the absence of Csk); phosphorylated Cbp specifically binds to activated Src and sequesters it in lipid rafts

Conclusion: lipid raft components such as Cbp may inactivate Src and suppress tumour formation


A
c-Src transformed cells
Focal contacts
Integrin
EGFR
Lipid rafts
Cholesterol
PI3K
FAK
Cas
Acvtivated
c-Src
?
Transformation
cbp gene
B
Csk-independent suppression of c-Src function
Cbp
pY165/pY183
or pY381/pY409
Transformation
C
Csk-dependent inactivation of c-Src activity
Cbp
pY314
Y527
Csk
Inactivated
c-Src

4. Oncogenes and guanine nucleotide binding proteins (G proteins) – ras

Cytoplasmic signaling. Regulate activity of target proteins in response to a variety of signals. Links growth factor receptors to cytoplasmic activation pathways. G proteins can bind GDP and GTP and have GTPase activity.

Ras proto-oncogene responds to signals such as PDGF (in fibroblasts) or antigen stimulation (in T cells). In the presence of these growth factors, Ras is converted to active form (Ras-GTP). GTPase activity gradually converts GTP to GDP thereby returning Ras to its inactive form (Ras-GDP). Mutations of Ras at amino acids 12, 13, 61 decrease GTPase activity, thereby increasing the level of active Ras-GTP.

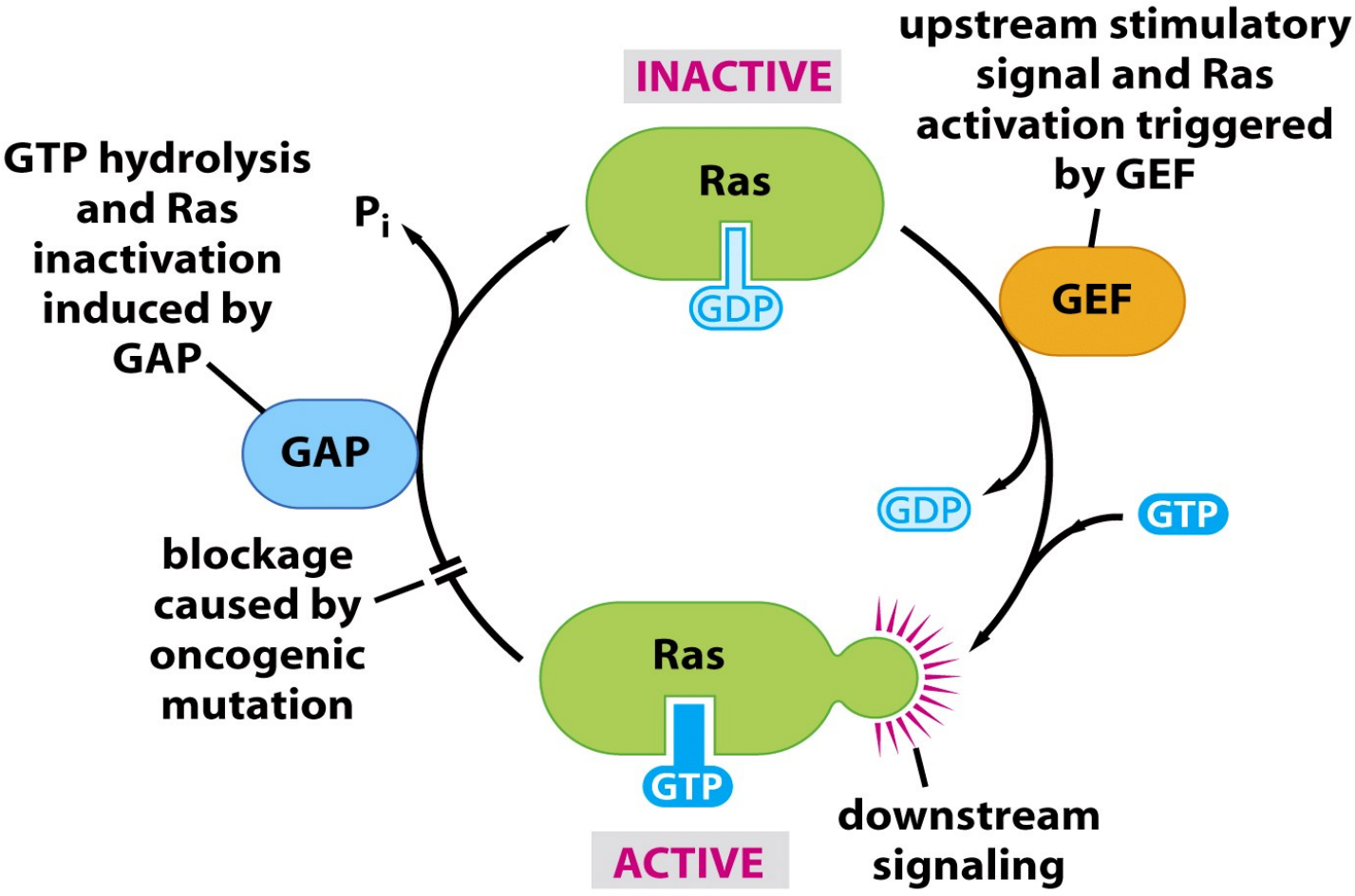


Figure 5-30 The Biology of Cancer (© Garland Science 2007)

Proteins that regulate Ras

GEFs (guanine nucleotide exchange factors) – activation of Ras

GAPs (GTPase activating proteins) – inactivation of Ras – increase GTPase activity of Ras

Mutations affecting Ras-GEFs and Ras-GAPs can also contribute to cell transformation

p120 GAP increases GTPase activity of normal Ras but not mutant Ras

A second protein with GAP activity is the tumour suppressor NF1 neurofibromatosis gene. Lack of activity of this gene results in high levels of normal Ras being in the active GTP bound form

GEFs function as positive regulators of Ras by stimulating the exchange of bound GDP for free GTP. An example of a GEF is Sos (son of sevenless) which can induce transformation of mammalian cells in culture

Sos plays a critical role in activating Ras proteins in response to growth factor stimulation of protein-tyrosine kinases. Sos associates with an adaptor protein called Grb2 which has SH2 and SH3 domains. Grb2 binds Sos through its SH3 domain and phosphotyrosine-containing growth factor receptors through its SH2 domains

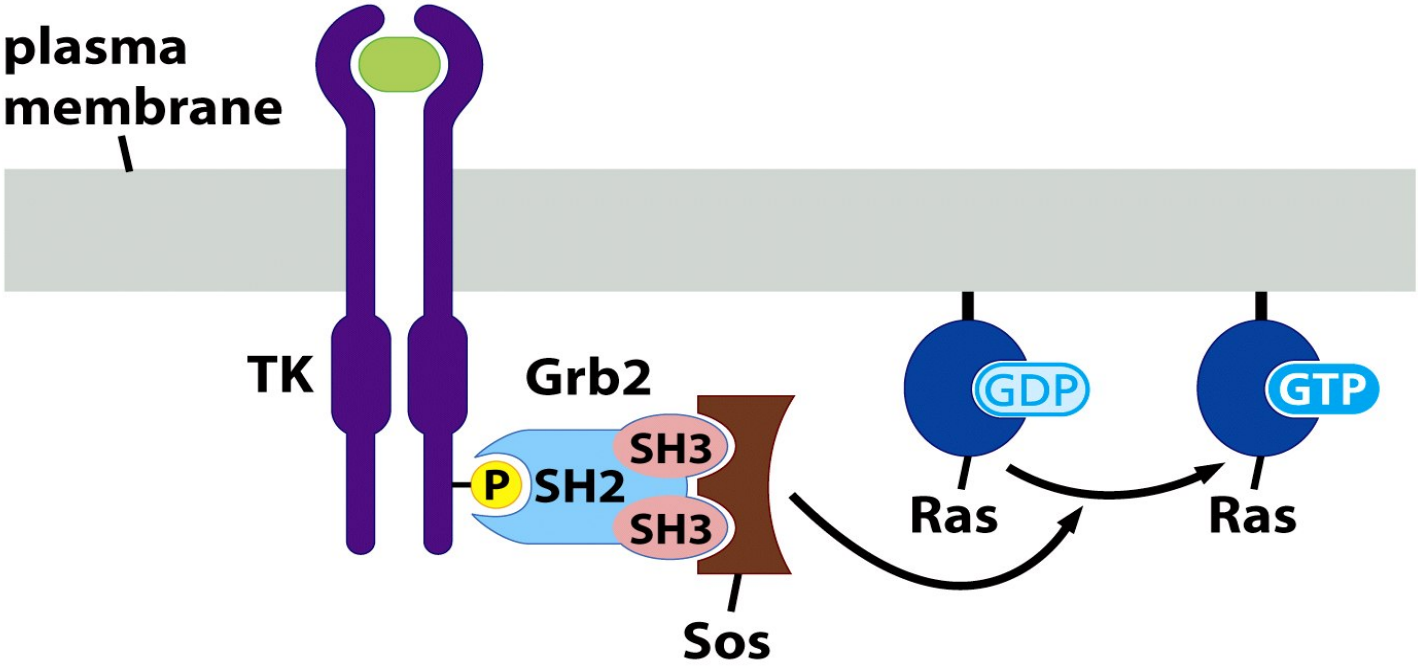


Figure 6-12a The Biology of Cancer (© Garland Science 2007)

Best-studied downstream targets (effectors) of Ras:

(i) Raf kinase – Active Ras binds the protein kinase Raf and activates it. Raf phosphorylates MEK (MAP kinase kinases) which in turn phosphorylate MAP kinases (a.k.a. Erk1 and Erk2). MAP kinase is believed to change the activity of nuclear transcription factors such as Fos, Jun, and Myc. This pathway has been strongly implicated in the transformation of mouse cells.

(ii) PI3-kinase – Ras activates PI3 kinase which results in activation of Akt and Rho-GEF. Activated Akt inactivates Bad (thus suppressing apoptosis), inactivates GSK-3β (thus stimulating cell proliferation) and may activate mTOR (stimulating proliferation). Activated PI3K alone cannot cause transformation of NIH3T3 cells but cooperates with activated Raf to transform cells.

(iii) Ral (protein related to Ras) – Ras communicates with Ral through Ral-GEF. Co-expression of activated Ral-GEF and activated Raf induces foci formation in NIH3T3. Ral-GEF **(and not Raf)** appears to play a key role in transformation of human cells by Ras.

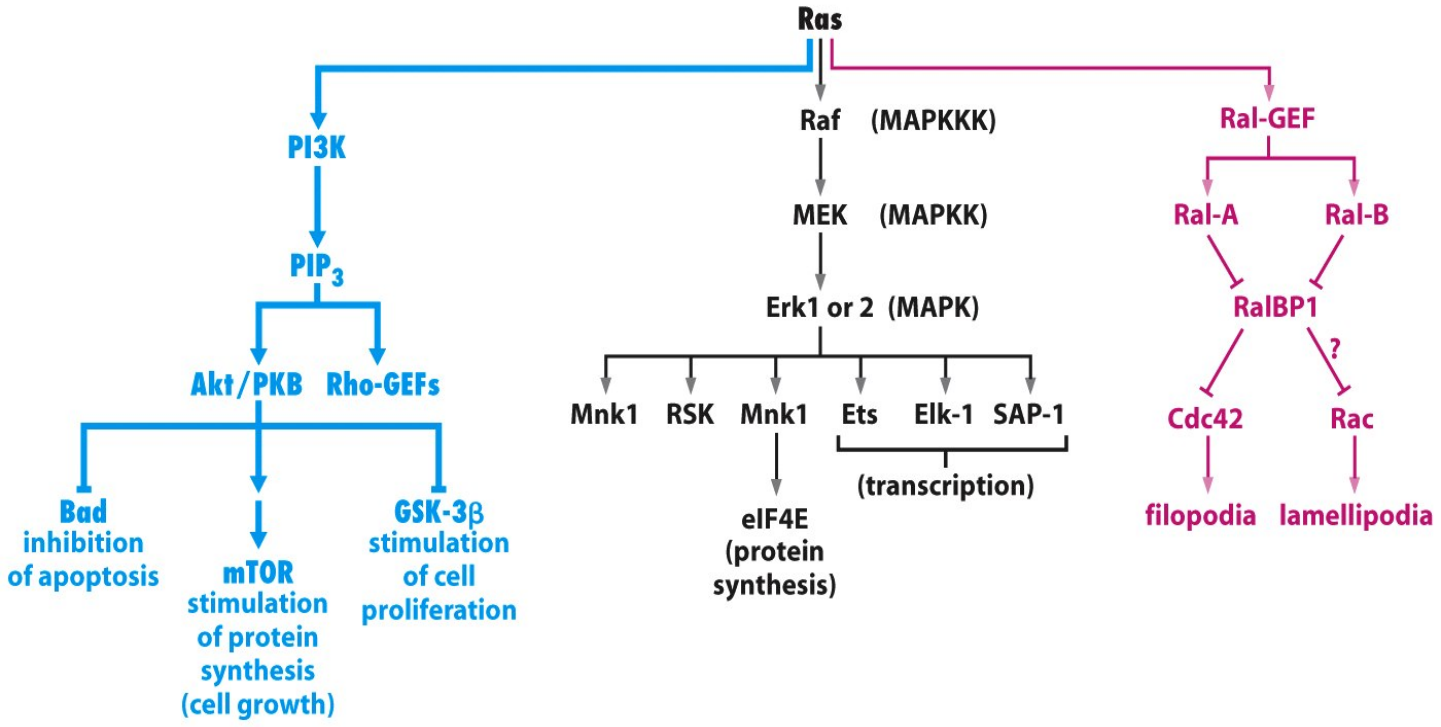


Figure 6-15 The Biology of Cancer (© Garland Science 2007)

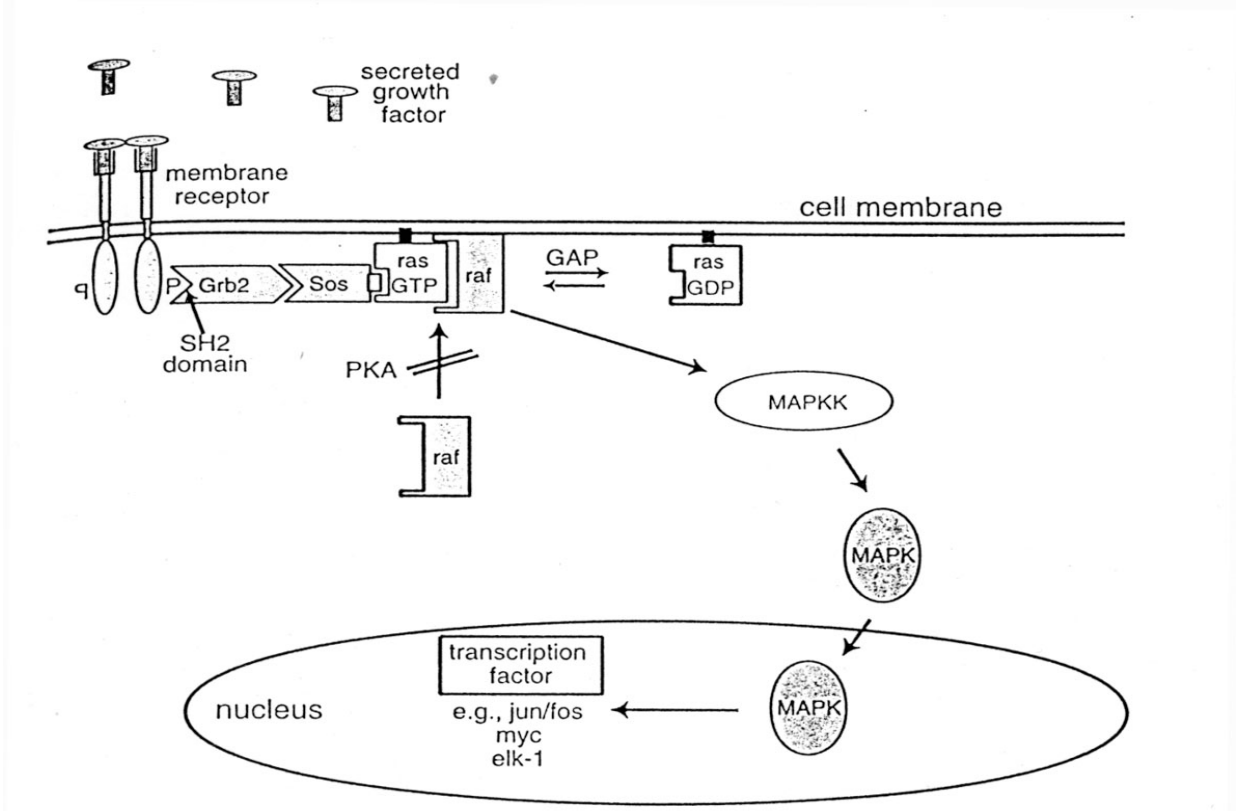
5. Oncogenes and serine threonine kinases (Raf)

- direct binding has been observed between the Ras and Raf proteins

- interaction between Raf and Ras is required to recruit Raf to cell membrane

- once activated, Raf can directly phosphorylate a family of serine/threonine kinases called the mitogen-activated protein kinase kinases (MAPKK or MEK)

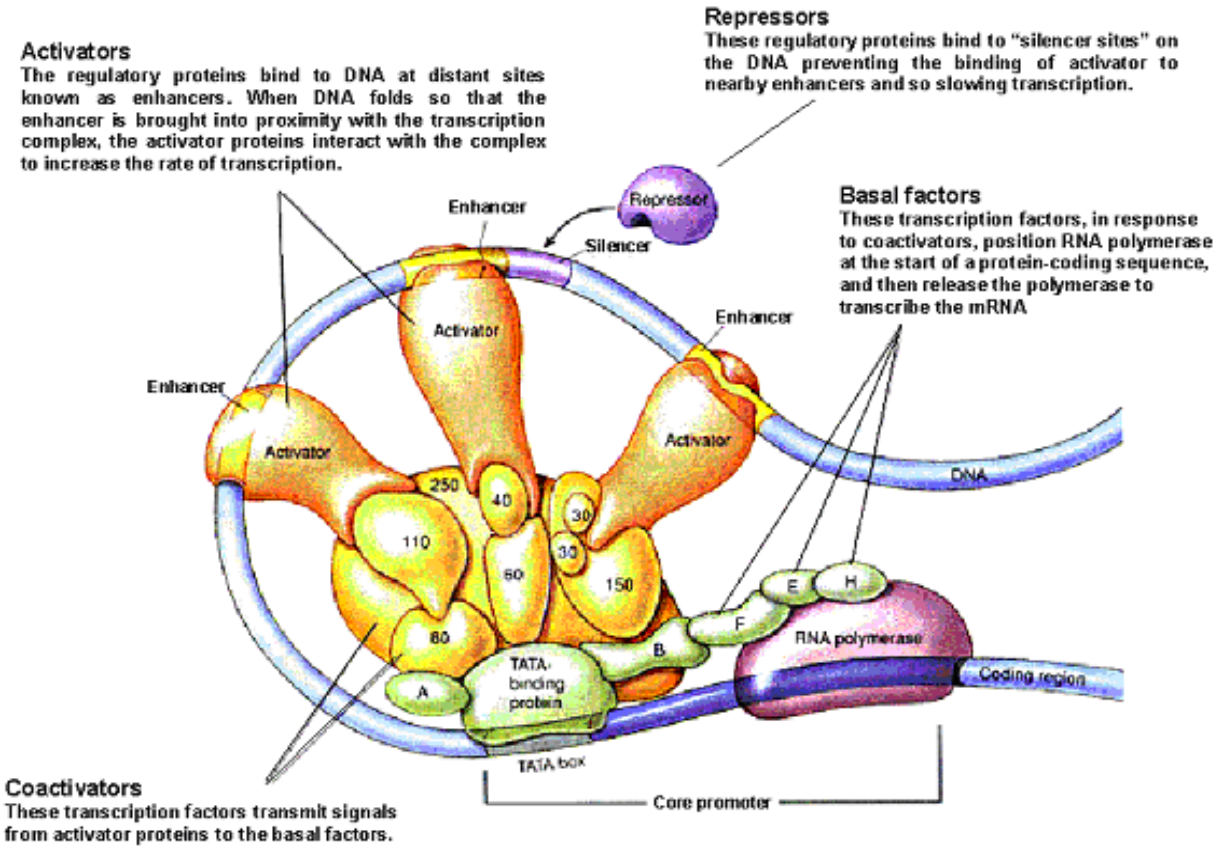
- activated MAPKKs activate MAPKs



The Biological Basis of Cancer (1998)

6. Oncogenes and transcription factors

A number of proteins encoded by oncogenes are located in the nucleus and function as transcription factors. Transcription of genes in the nucleus involves interaction of transcription factors with regulatory elements. Regulatory elements (promoters, enhancers) are usually located upstream of genes.



Myc proteins – c-myc (Myc), N-myc, L-myc

Myc proteins are involved in many cancers (gene amplification, gene translocation). Activation of myc (proto-oncogene to oncogene) is through over-expression of myc protein. Myc proteins are induced rapidly following growth factor stimulation. Myc has a DNA binding domain and functions as a transcription factor.

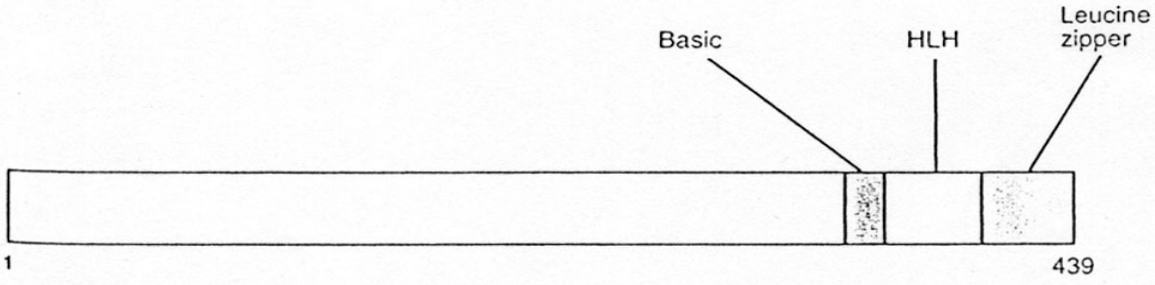


FIGURE 16.7
The Myc DNA-binding domain. The C-terminal region of Myc proteins (human c-Myc is illustrated) has a DNA-binding domain consisting of a region of basic amino acids (designated basic), a helix-loop-helix (HLH) motif, and a leucine zipper.

Table 1 | Myc overexpression in various cancers

Cancerous tissue	Frequency of Myc overexpression
Solid tumours	
Glioblastoma	57–78%
Breast	45%
Colon	67%
Medulloblastoma	31%
Ovarian	66%
Pancreatic	43%
Lymphoma	
Burkitt's	>90%
Diffuse large B cell	10–25%
Transformed follicular	70%
Mantle cell	Up to 100%

Brooks, TA and Hurley, LH. Nature Reviews Cancer 9: 849-861, 2009

Myc – cont'd

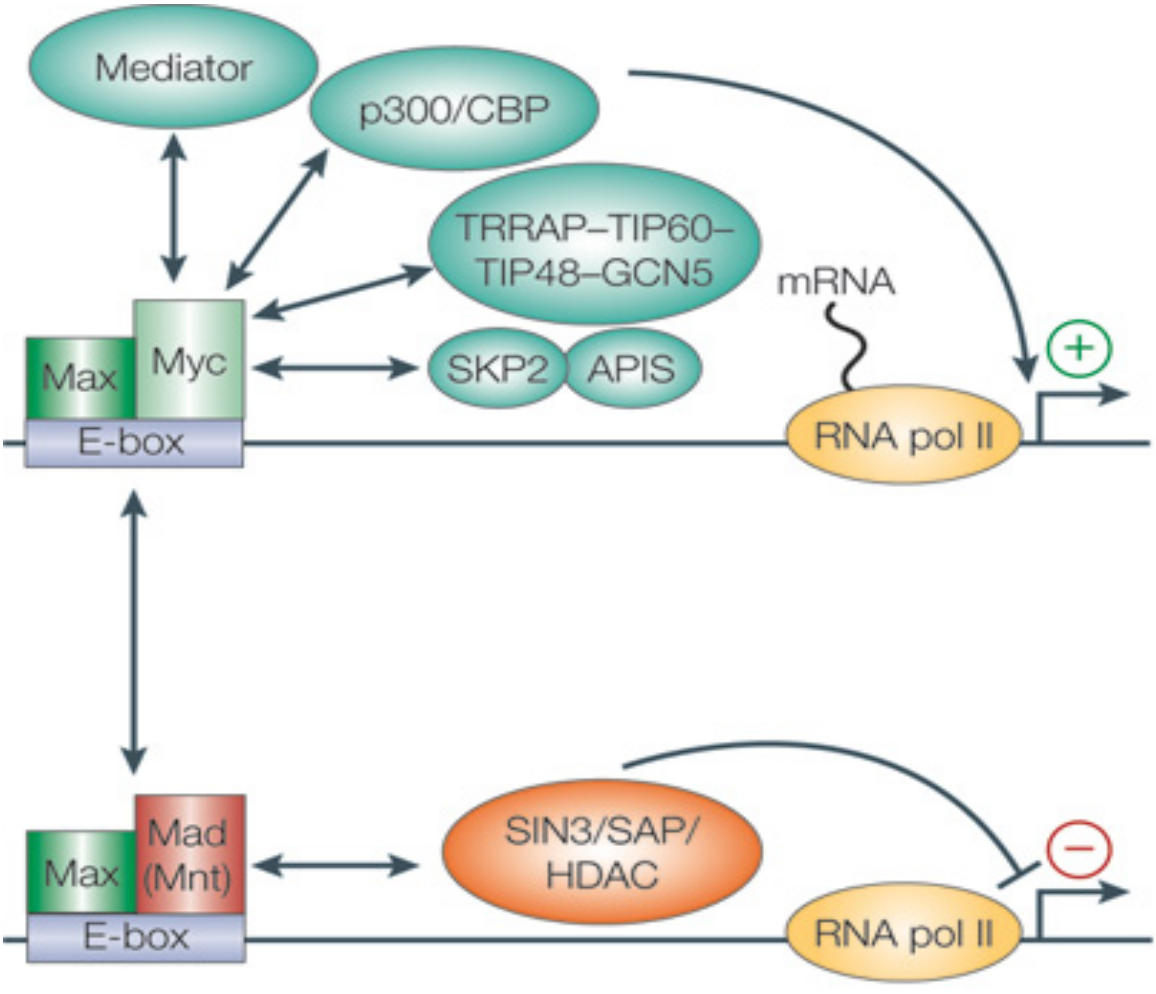
Myc interacts with Max. Like Myc, Max has a DNA binding domain. The Myc-Max complex binds to a specific DNA sequence called the E-box (CACGTG) and activates transcription which results in mitogenic stimulation.

Further regulation is provided by Mnt and Mad which dimerize with Max but not Myc. Max can dimerize with Myc, Mad and Mnt with equal affinity. Max-Mnt and Max-Mad heterodimers repress transcription.

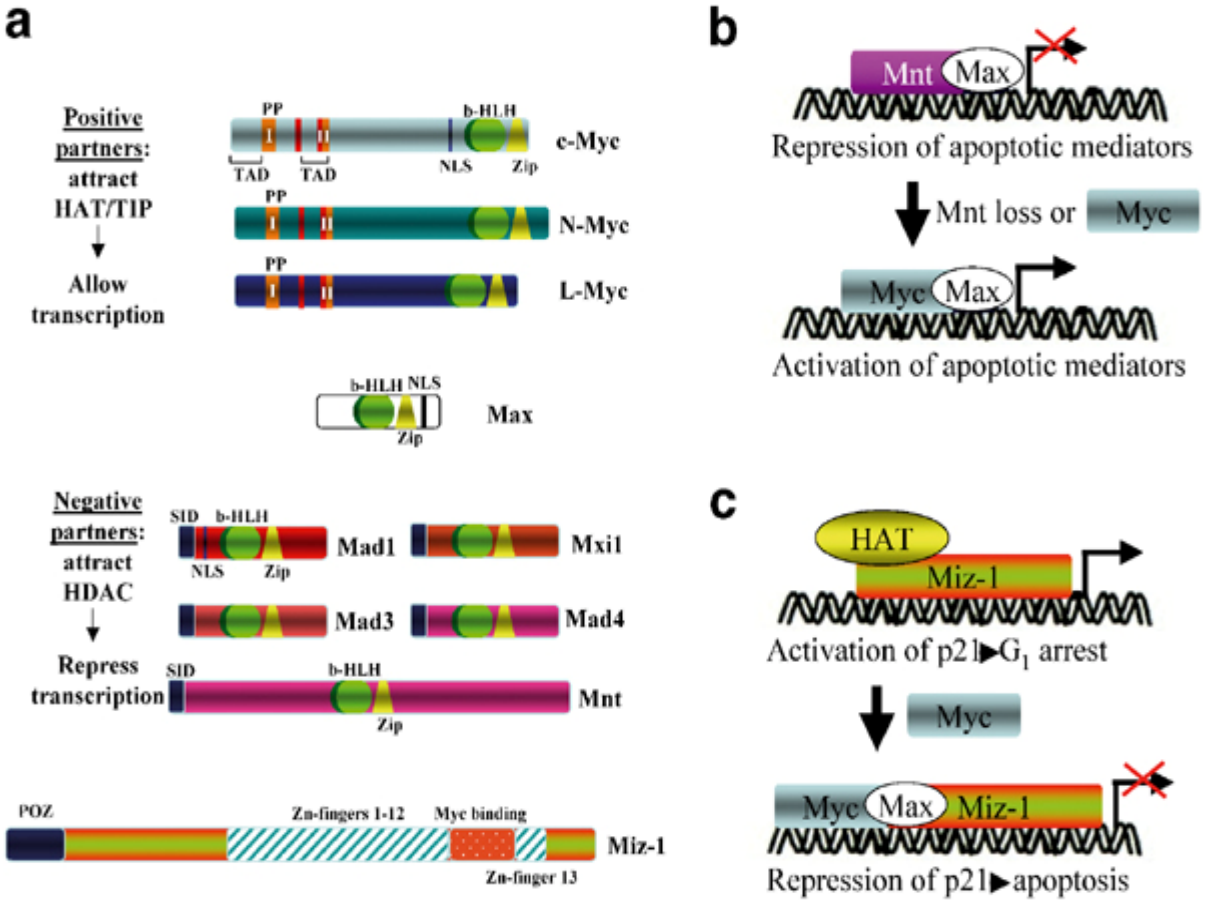
Transcription repression by Mnt-Max and Mad-Max requires interaction with Sin3 which recruits histone deacetylases (HDACs).

Max and Sin3 are constitutively expressed. The dimerization state of Max is determined by the relative levels of Myc and Mnt or Mad.

Target genes for Myc include cyclin D2 and CDK4 involved in cell proliferation and VEGF involved in angiogenesis. Myc also regulates genes involved in glucose metabolism, nucleotide metabolism, and induces microRNAs that affect E2F1 expression.



Myc can also bind to a second transcription factor called Miz-1. When bound to Miz-1, Myc functions as a transcriptional repressor (represses the expression of p21 and p15, cyclin-dependent kinase inhibitors which inhibit CDK4/6 and CDK2 involved in phosphorylating pRB)



Myc pathways
Jonas A Nilsson and John L Cleveland

Is Myc a potential target for cancer treatment?

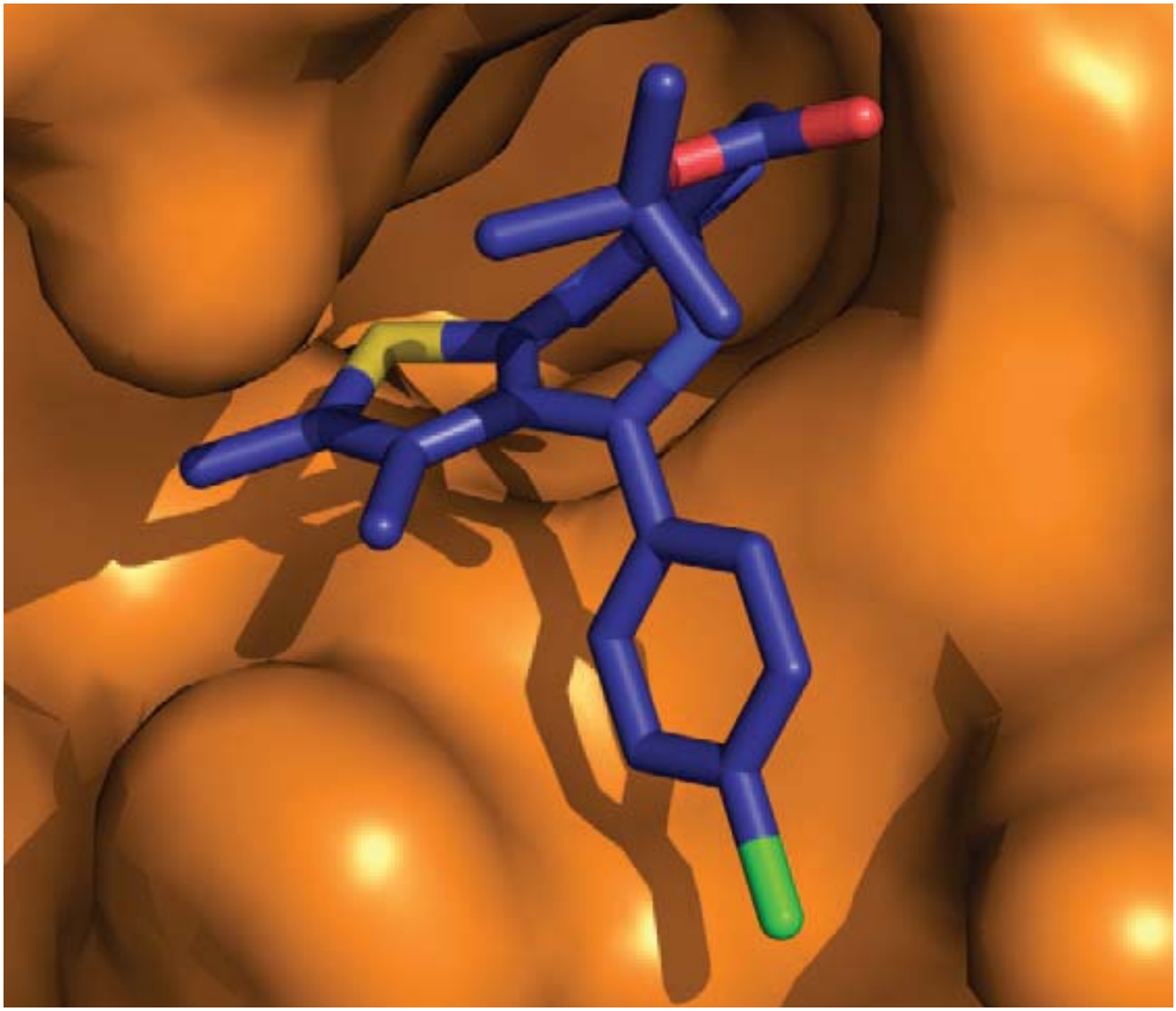
- Myc has a short half-life (20-30 minutes)

- ~30,000 potential Myc binding sites in the human genome

- 10-15% of genes are bound by Myc

- Transcription of the Myc gene induces negative supercoiling of DNA

- Might Myc transcription be controlled through small molecules that target structural elements in the Myc promoter?



Second example of oncogenes that function as transcription factors

Fos (murine osteogenic sarcoma virus) and Jun (avian sarcoma virus)

Members of the Fos and Jun family form dimeric AP-1 transcription factors which bind to TGACTCA

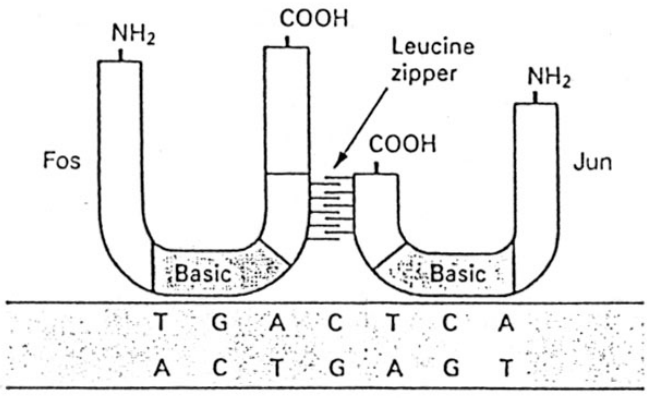
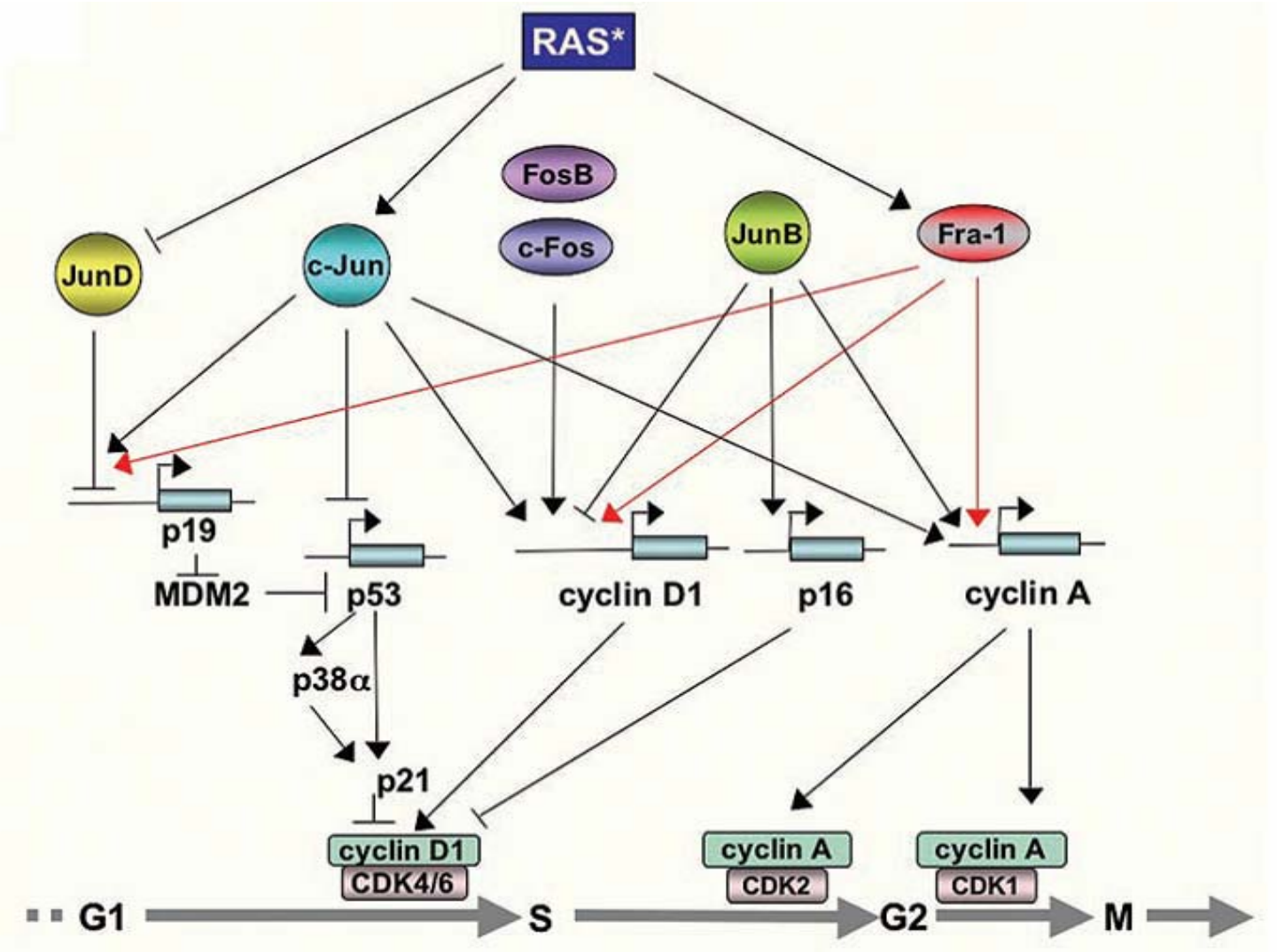


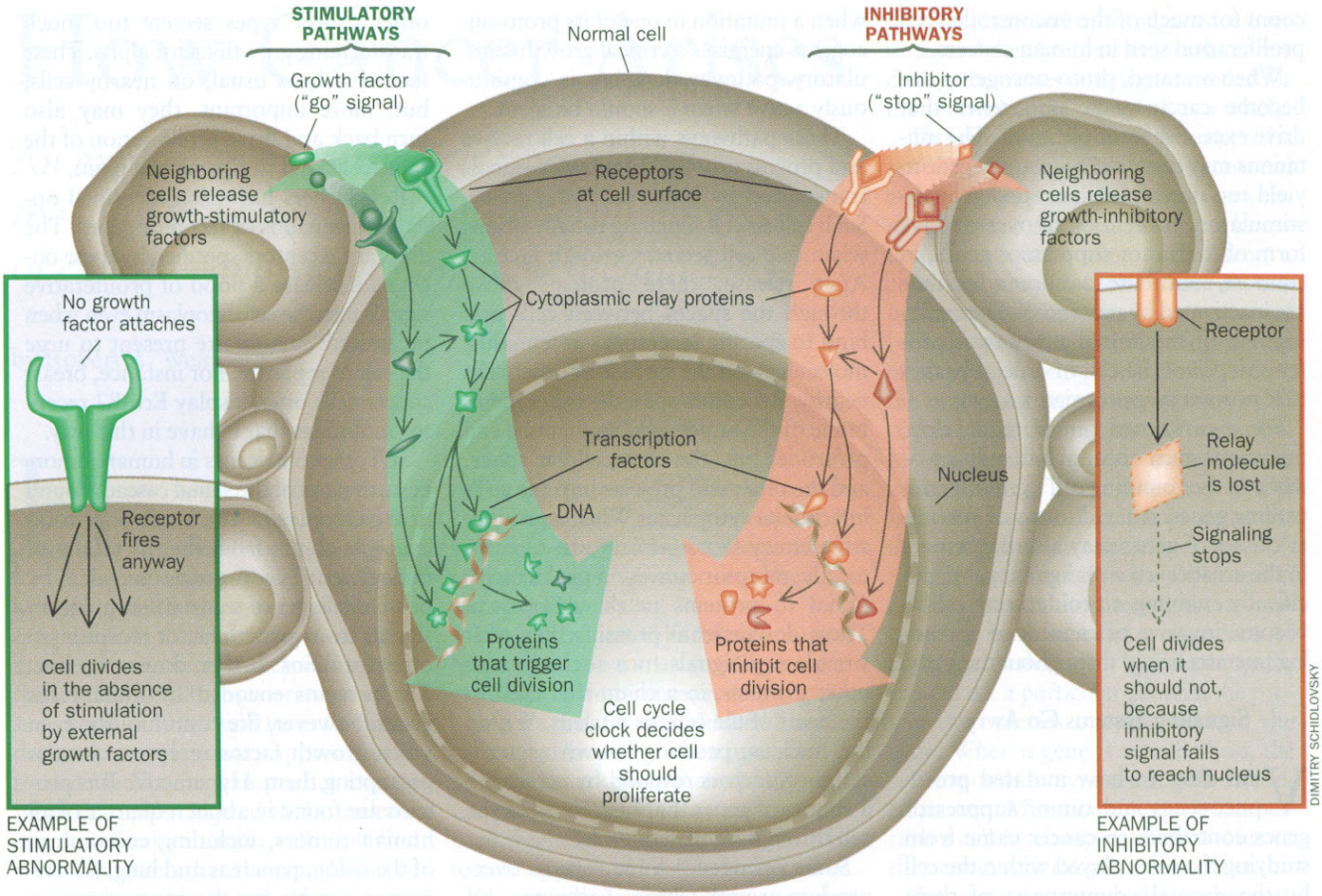
FIGURE 16.6
Binding of a Jun-Fos heterodimer to DNA. Dimerization is accomplished by hydrophobic interactions between leucine side chains to form a "leucine zipper." The basic regions of both Jun and Fos then bind cooperatively to an AP-1 target site, the sequence of which is a symmetrical palindrome.

The levels and/or activity of members of the Fos and Jun family are elevated in the presence of activated Ras and other oncoproteins such as adenovirus E1A



Numerous AP-1 target genes have been identified. Different AP-1 heterodimers may regulate different target genes.

Cell Cycle 6: 2633, 2007



SIGNALING PATHWAYS in normal cells convey growth-controlling messages from the outer surface deep into the nucleus. There a molecular apparatus known as the cell cycle clock collects the messages and decides whether the cell should divide. Cancer cells often proliferate excessively because genetic mutations cause stimulatory pathways (*green*) to issue too many "go" signals or because inhibitory pathways (*red*) can no longer convey "stop" signals. A stimulatory pathway will become hyperactive if a mutation causes any component, such as a growth factor receptor (*box at left*), to issue stimulatory messages autonomously, without waiting for commands from upstream. Conversely, inhibitory pathways will shut down when some constituent, such as a cytoplasmic relay (*box at right*), is eliminated and thus breaks the signaling chain.