ONCOGENES

Molecular Oncology - 2013

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ONCOGENES - Lecture Outline

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INTRODUCTION

The term oncogene is given to genes whose expression causes cells to exhibit the properties of cancer cells. Most known oncogenes are derived from normal cellular genes. The precursor genes are best described as protooncogenes but they are sometimes loosely referred to as oncogenes. Activation of a single oncogene is not usually sufficient for cell transformation

IDENTIFICATION OF ONCOGENIC GENES IN RETROVIRUSES

Oncogenes were first identified in some RNA viruses. The oncogenic RNA viruses contain reverse transcriptase and are described as retroviruses. Retroviruses that possess oncogenes are a rare and highly oncogenic group. While most viral oncogenes are believed to have originated from rare transductions of cellular sequences, there are exceptions. Thus in human T-cell leukemia viruses (HTLV) there is a 1.5 kilobase region described as the X region which lies between the env gene and the 3'LTR and is believed to mediate the transformation properties of the HTLV family. This region does not appear to have a cellular homolog.

HOMOLOGOUS SEQUENCES ARE IDENTIFIED IN DNA OF TRANSFORMED CELLS

Hybridization experiments revealed homology between viral oncogenes and DNA sequences in normal cellular DNA. This was first established for the src gene. The viral and cellular genes were distinguished as v-src and c-src, respectively. It appears that some viruses have acquired cellular genes and that changes in the nucleotide sequences have caused the genes to have transforming properties. The acquired genes or oncogenes are not required for the virus to be infective or for viral replication. Transformation-defective variants which lack the oncogene can replicate.

METHODS FOR TRANSFORMING CELLS WITH ONCOGENES

In addition to viral infection, oncogenes may be introduced into cells by any of the standard techniques for introducing DNA into mammalian cells such as calcium phosphate precipitation, electroporation and lipofection. These procedures permit one to test the transforming potential of a gene. Transfection of DNA into the immortalized NIH 3T3 mouse fibroblast cell line has been a useful model to examine the transforming potential of genes.

chemically
transformed
mouse fibroblasts

DNA

DNA

transfection using
calcium phosphate
co-precipitation procedure

normal
mouse
fibroblasts

formation of a
focus of morphologically
transformed cells

injection of
morphologically
transformed cells into
mouse host

tumor
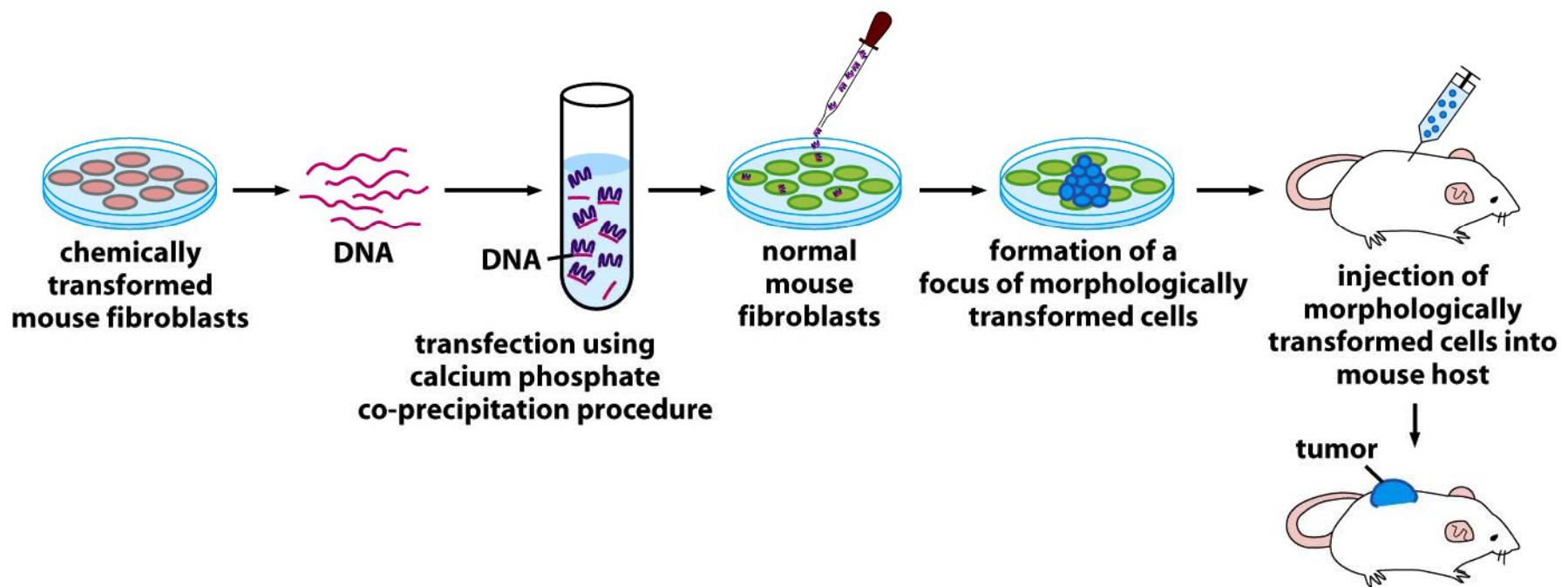
Figure 4-2 The Biology of Cancer (© Garland Science 2007)

MECHANISMS FOR THE ACTIVATION OF PROTOONCOGENES

1. <u>Mutations</u> in the gene sequence may cause either a change in activity or an alteration in the function of the gene protein product e.g. ras genes.

2. <u>Chromosomal translocation</u> may put the gene in a different regulatory environment e.g. myc. The first chromosomal translocation to be consistently associated with malignancy in humans was termed the Philadelphia chromosome. This occurs in many cases of chronic myelocytic leukemia and results from a translocation from chromosome 9 to chromosome 22. Other frequently observed chromosomal translocations occur near the sites for c-myc and c-mos. These are 8:14 in 90% of Burkitt's lymphoma and 8:21 in acute myeloid leukemia, respectively.

normal chromosomes

Burkitt's lymphoma t(8;14)

8   14   8q−   14q+

q24.13   *myc*   IgH   q32.33   IgH   *myc*
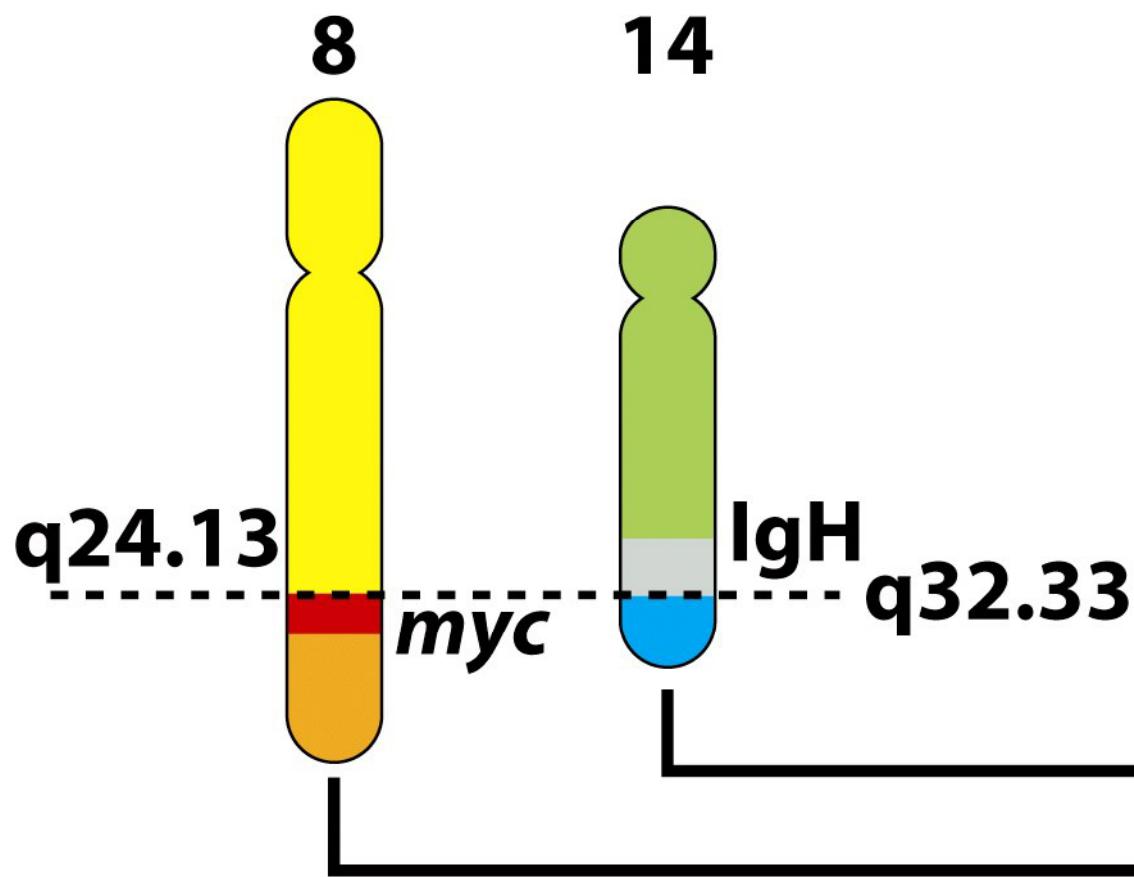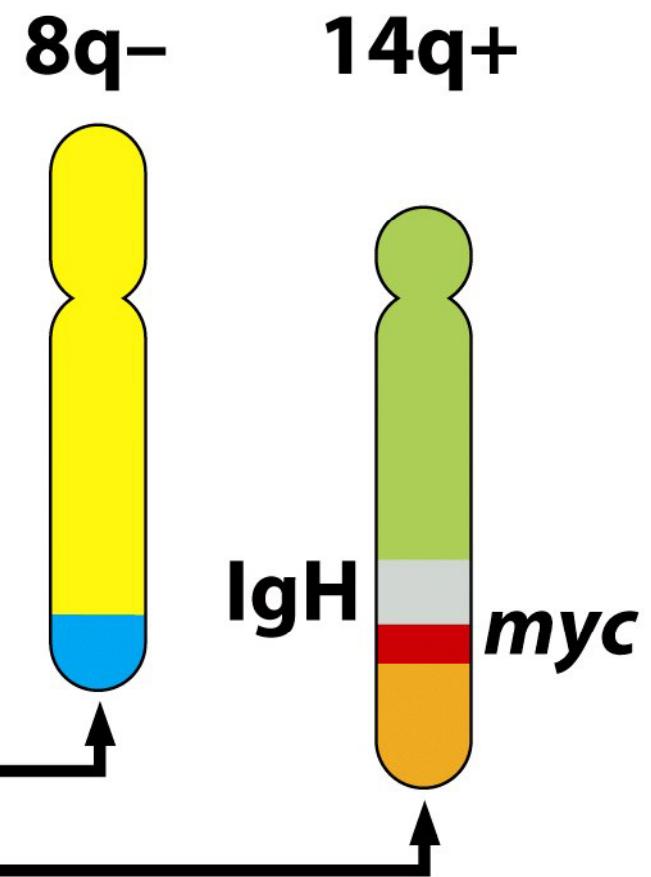
Figure 4-13a The Biology of Cancer (© Garland Science 2007)

heavy-chain immunoglobulin (IgH) gene

normal Chromosome 14

+

myc proto-oncogene

normal Chromosome 8

reciprocal translocation

IgH   *myc* oncogene

+
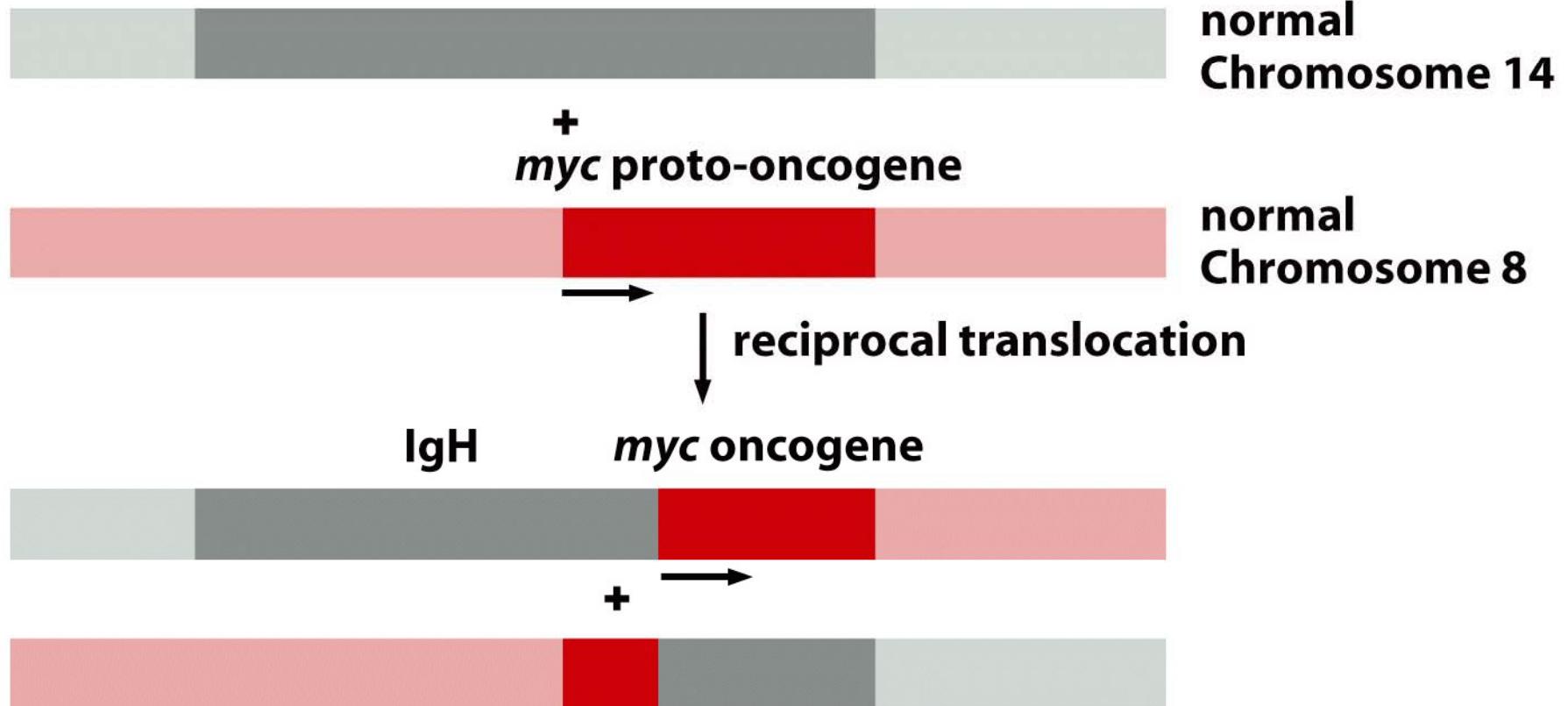
Figure 4-13b The Biology of Cancer (© Garland Science 2007)

Translocation of chromosomes 9 and 22 yielding the Philadelphia chromosome seen in most cases of Chronic myelogenous leukemia
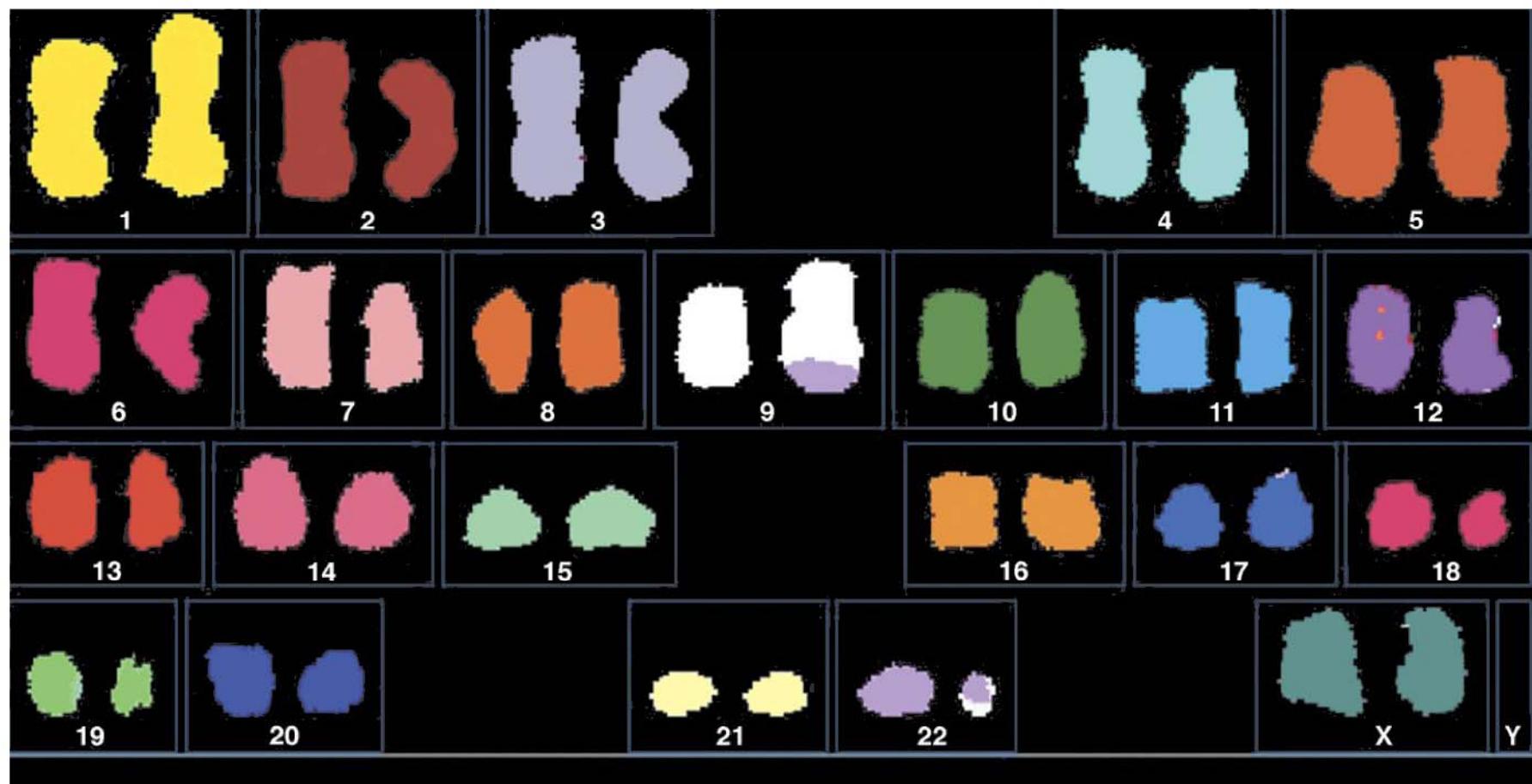

Figure 2-23b The Biology of Cancer (© Garland Science 2007)

site of future breakpoint

abl

Chromosome 9

sites of alternative breakpoints

bcr

Chromosome 22

one possible translocation

breakpoint

+

hybrid Bcr–Abl protein
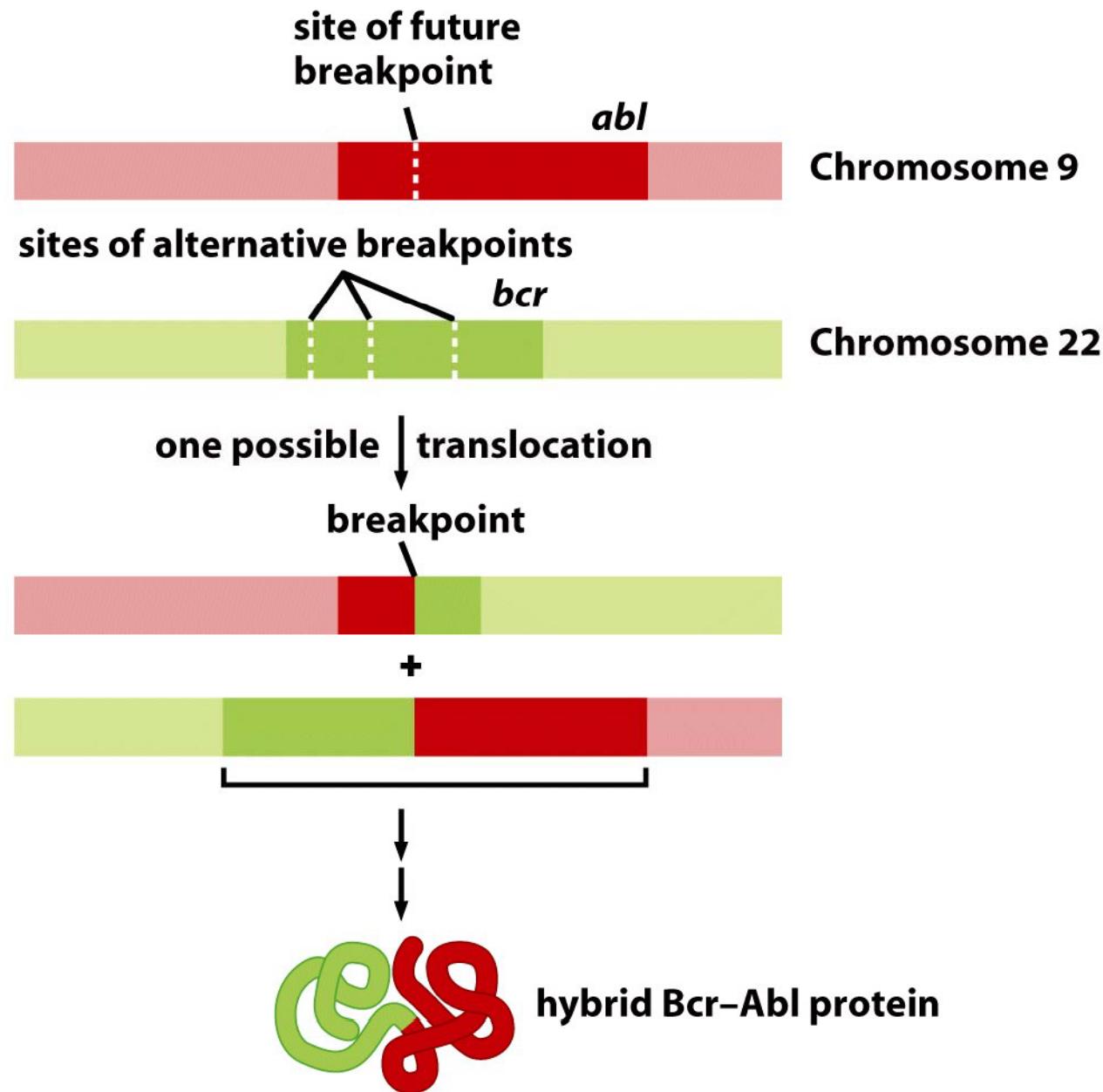
Figure 4-15a The Biology of Cancer (© Garland Science 2007)

MECHANISMS FOR THE ACTIVATION OF PROTOONCOGENES

3. <u>Gene amplification</u> is accompanied by two cytogenetic changes: double minute chromosomes and homogeneously staining regions (HSRs). Changes of this type are seen infrequently in tumors in vivo but are more frequent in cultured cells. N-myc and c-myc are the oncogenes which have been found most frequently to be amplified. Other genes which have been found to be amplified in some tumor cells include c-Ki-ras, c-myb, c-abl and c-erb B.

Oncogene amplification may be associated with tumor progression as seen with N-myc in neuroblastomas and with c-myc in small cell lung carcinomas. In the case of c-myc the gene product may antagonize cell differentiation. Amplification of genes is accompanied by roughly proportional increase in the number of transcripts. In the human promyelocytic leukemia cell line, HL60, the amplification of c-myc may decrease the tendency of promyelocytic leukemia cells to differentiate in culture. In HL60 cells, differentiating agents such as 1,25-dihydroxyvitamin D3 can cause down-regulation of the amplified c-myc genes and this is accompanied by differentiation.

MECHANISMS FOR THE ACTIVATION OF PROTOONCOGENES

Oncogene amplification can be accompanied by gene rearrangement but most amplified oncogenes are apparently normal on the basis of restriction endonuclease mapping. Gene amplification arises from a segment of DNA replicating more than once during a single cell cycle. There is evidence that there are preferred chromosomal positions for amplification of cellular genes and chromosomal rearrangements may facilitate gene amplification by placing a gene at a more favorable site.

4. Insertional mutagenesis may operate if regulatory elements of a virus are inserted in proximity to a cellular protooncogene. This type of mechanism may occur with avian leukosis virus which does not have an identified oncogene. There is evidence that proviral integration in lymphomas can occur in the region of c-myc. Avian retroviruses possess a well defined sequence which is responsible for the control of viral gene transcription. This long terminal repeat sequence (LTR) is located both upstream and downstream of the viral structural genes in the integrated provirus. The LTR contains potential transcription regulatory signals including a TATA box, a polyadenylation-termination signal and the initiation signal (cap site). There is evidence that the downstream LTR is unable to act as an efficient promoter of transcription when a transcriptionally active upstream LTR is present. This transcriptional interference may explain the observation that only deleted proviruses have been observed adjacent to c-myc in lymphomas of chickens induced by the leukosis virus.

NAMING OF ONCOGENES

Most of the oncogenes have been given three letter names which are often derived from the virus bearing the viral oncogene. The following list is taken from Table 7-1 of Ruddon (3rd edition):

_____

Oncogene	Retrovirus	Species of Origin
abl	Abelson murine leukemia virus	Mouse
erb B	Avian erythroblastosis virus	Chicken
fos	FBJ osteosarcoma virus	Mouse
myb	Avian myeloblastosis virus	Chicken
myc	MC29 myeloblastosis virus	Chicken
Ha-ras	Harvey murine sarcoma virus	Rat
Ki-ras	Kirsten murine sarcoma virus	Rat
N-myc	None	Human
sis	Simian sarcoma virus	Monkey
src	Rous sarcoma virus	Chicken

NORMAL ROLE OF ONCOGENES

The cellular protooncogenes have shown great evolutionary stability and presumably have an important role in normal development and function. ras genes have been identified in yeast. There is activation of c-myc and ras genes in liver regeneration after partial hepatectomy. Protooncogene expression is not necessarily associated with growth. Differentiation of HL60 cells is accompanied by a decrease in c-myc expression but there is an increase in the expression of c-fos.

CELLULAR LOCATIONS AND FUNCTIONS OF ONCOGENE PRODUCTS

The protein products of oncogenes differ in their cellular locations. They may be associated with the cell membrane, the cytoplasm or the nucleus. Oncogene products have been described with one of the following functions

Growth factor

Growth factor receptor

Signal transduction factor

GTP-binding plasma membrane protein

Tyrosine-specific protein kinase

Serine/threonine-specific protein kinase

Transcription factor

CELLULAR LOCATIONS AND FUNCTIONS OF ONCOGENE PRODUCTS

The most intensively studied oncogenes include:

bcl 2	Mitochondrial membrane-associated protein. Inhibits apoptosis
erb B	Truncated EGF receptor protein - tyrosine kinase
fos	Forms a dimer with c-jun to act as the AP-1 transcription factor
jun	
met	Soluble truncated receptor-like protein-tyrosine kinase
mos	Cytoplasmic protein-serine kinase
myc	Sequence-specific DNA-binding protein
raf	Cytoplasmic protein-serine kinase
ras	Membrane associated GTP binding/GTPase
sis	PDGF B-chain growth factor
src	Membrane associated non-receptor protein-tyrosine kinase

GROWTH FACTORS WITH ONCOGENIC POTENTIAL

Cooper (Oncogenes, 2nd edition, 1995) has summarized growth factors with oncogenic potential as follows:

<u>Proto-oncogene product</u>

PDGF family: A chain, B chain (sis)

FGF family: acidic FGF, basic FGF, int-2, hst, FGF-5

EGF family; EGF, TGF-alpha

Wnt family wnt-1 (int-1), wnt-3

Hematopoietic growth factors

  interleukin-2

  interleukin-3

  M-CSF

  GM-CSF

Biologically active PDGF is a dimer (A:A, A:B or B:B). The c-sis gene encodes a polypeptide precursor of the B chain of platelet derived growth factor (PDGF). The v-sis gene product may have an intracellular action and not require binding to the surface receptor.

ONCOGENES CODING FOR TRUNCATED GROWTH FACTOR RECEPTORS

The most extensively studied oncogene corresponding to a truncated growth factor receptor is erb B. The proto-oncogene product consists of an extracellular receptor domain, a transmembrane domain and an intracellular tyrosine kinase domain. In the activated oncogene the extracellular domain has been lost and there is a constitutively active tyrosine kinase domain.

Other growth factor receptors that are proto-oncogenes are the hepatocyte growth factor receptor (met), stem cell receptor (kit), NGF receptor (trk), CSF-1 receptor (fms). There are other genes for receptor like proteins for which the ligand is unknown and which can be activated to form oncogenes. These include ret, ros, sea and neu.

Some receptors lack tyrosine kinase activity and may act through nonreceptor protein kinases, particularly of the Jak family. Others may act through GTP-binding proteins. An example is the product of the mas oncogene which corresponds to the angiotensin receptor.

ONCOGENES CODING FOR NON-RECEPTOR TYROSINE KINASES

The src gene was first identified in the Rous sarcoma virus where the gene sequence is:

LTR-gag-pol-env-src-LTR

The src gene product pp60c-src encodes a non-receptor tyrosine kinase. Phosphorylation of a tyrosine residue near the C terminus (Tyr 527) is inhibitory. pp60c-src kinase activity is stimulated by the action of phosphoprotein phosphatase. One mechanism of activation of src is mutation of the Tyr 527 to give a constitutively active tyrosine kinase.

Src homology domains (SH2 and SH3) are found in several proteins that interact with growth factor receptors. The SH2 domain recognizes phosphotyrosine. SH3 domains recognize peptides that are rich in proline residues. The location of these domains in pp60c-src is as follows:

-SH3-SH2-protein kinase-

The src protein binds to activated growth factor receptors e.g. the PDGF receptor. Some members of the src family such as lck bind to cell surface receptors that lack protein kinase activity. lck binds to CD4 and CD8 receptors on T cells. The src family of genes includes lck, fgr and yes.

ONCOGENES CODING FOR GUANINE NUCLEOTIDE BINDING PROTEINS

The most frequently activated oncogenes in human tumors belong to the ras family of GTP-binding proteins. The protein products of these genes have a molecular weight of 21 kd. This is a property that they share with the p21WAF1/Cip1 tumor suppressor gene and may give rise to some confusion when reading the old literature. The ras gene products can be activated by point mutations particularly at codons 12, 13 or 61. The ras gene product is active in signal transduction when it binds GTP and inactive when it binds GDP. GTPase activating proteins (GAPs) cause inactivation whereas guanine nucleotide exchange proteins (GEFs) are activating for ras. GEFs catalyze the exchange of GTP for GDP bound to the ras protein. The c-ras protein has GTPase activity and this activity can be diminished when the gene is activated to form an oncogene.

The active form of ras activates the raf gene product which is part of a protein kinase cascade that results in the activation of transcription factors and changes in gene transcription. Some of the cytoplasmic protein kinases involved in signal transduction have specificity for serine and threonine residues. These include the raf and mos gene products.

The NF1 tumor suppressor gene product down regulates ras by stimulating its GTPase activity.

INACTIVE

GTP hydrolysis and Ras inactivation induced by GAP

P_i

Ras

GDP

upstream stimulatory signal and Ras activation triggered by GEF

GEF

GAP

GDP

GTP

blockage caused by oncogenic mutation

Ras

GTP

ACTIVE

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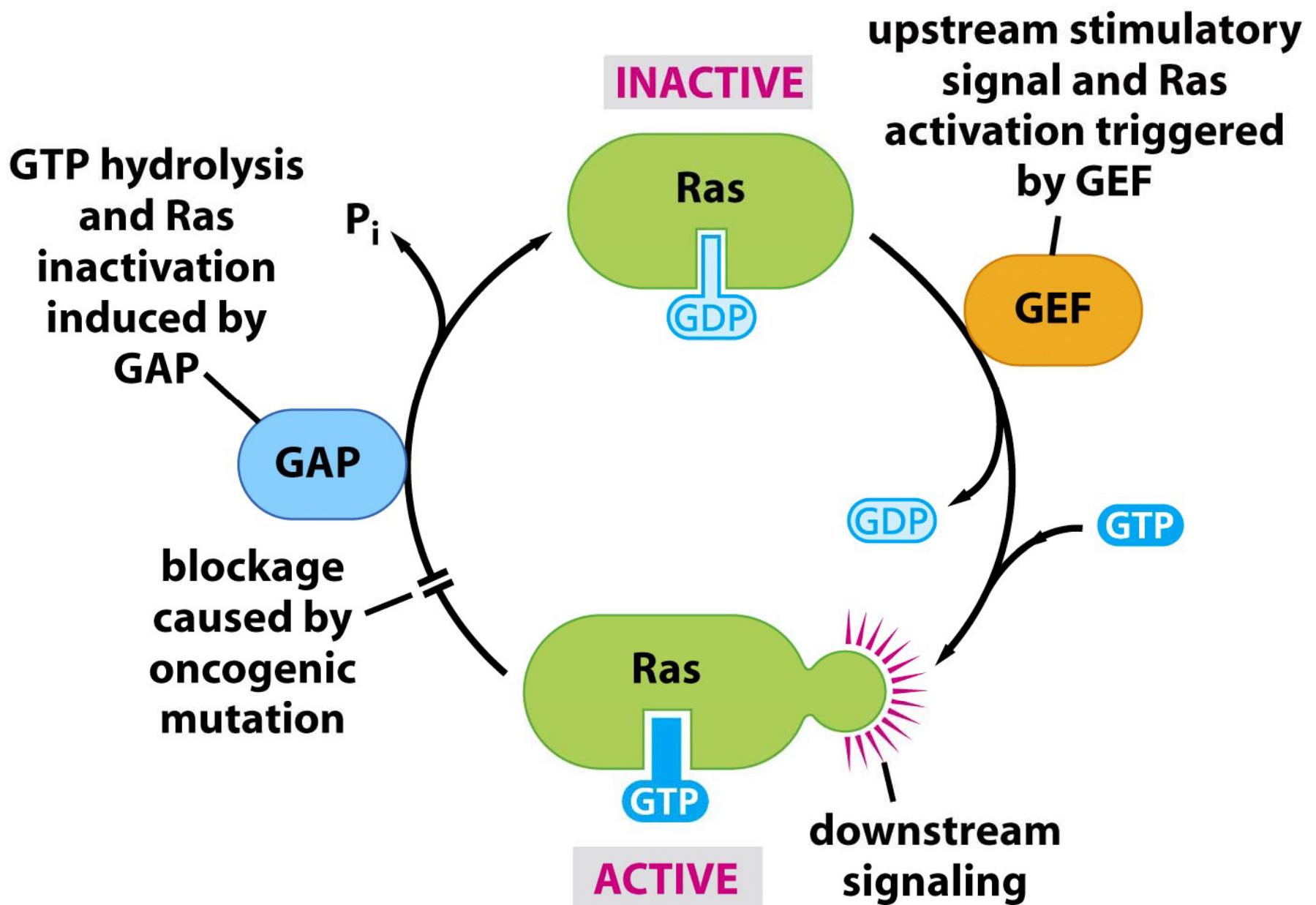
Figure 5-30 The Biology of Cancer (© Garland Science 2007)

signal molecule

EXTRACELLULAR SPACE

inactive Ras protein

active Ras protein

CYTOSOL

P P P P P P P P

GDP

GDP

GTP

GTP

activated receptor tyrosine kinase

Sos

ONWARD TRANSMISSION OF SIGNAL ALONG MULTIPLE PATHWAYS

Grb-2 adaptor protein

Ras GEF
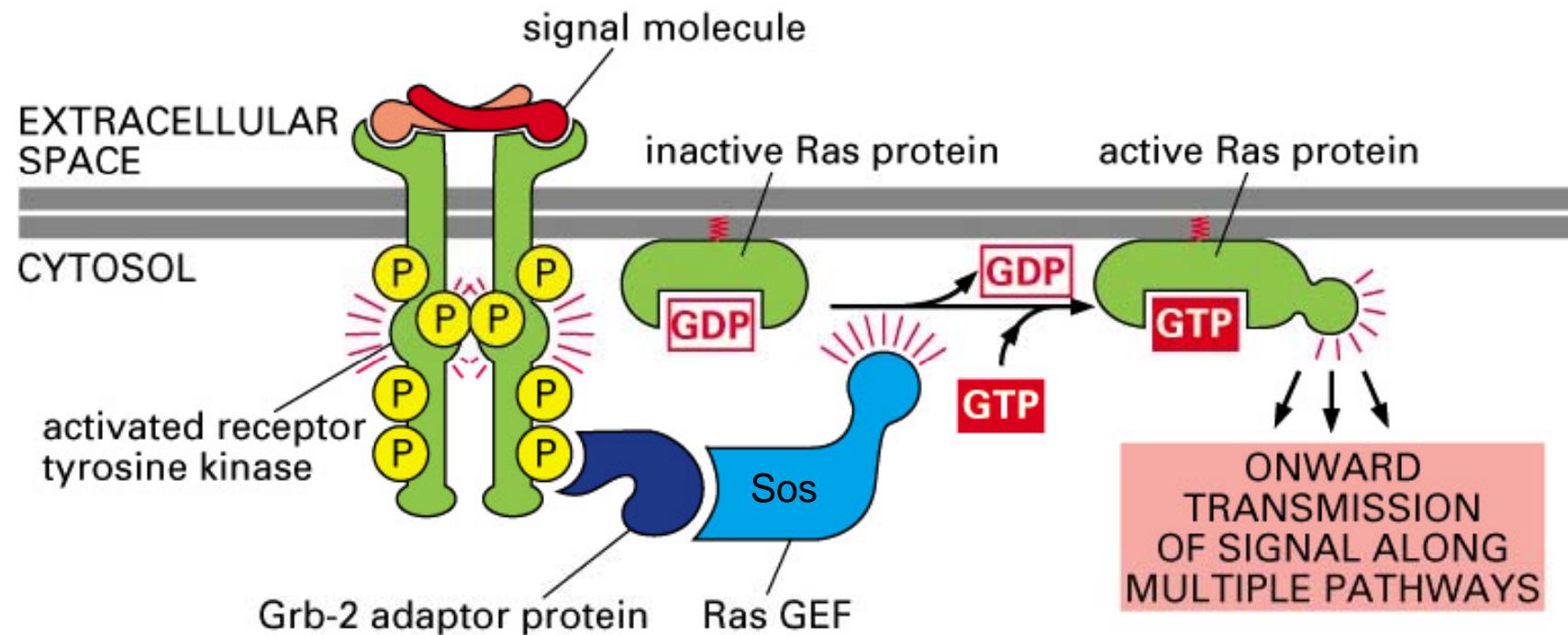
Figure 15–55. Molecular Biology of the Cell, 4th Edition.

plasma membrane

CYTOSOL

GTP

active Ras protein

MAP-kinase-kinase-kinase
RAF

ATP
ADP

P P

MAP-kinase-kinase
MEK

ATP
ADP

P P

MAP-kinase
ERK

ATP
ADP

P protein X   P protein Y   P gene regulatory protein A   P gene regulatory protein B

changes in protein activity   changes in gene expression
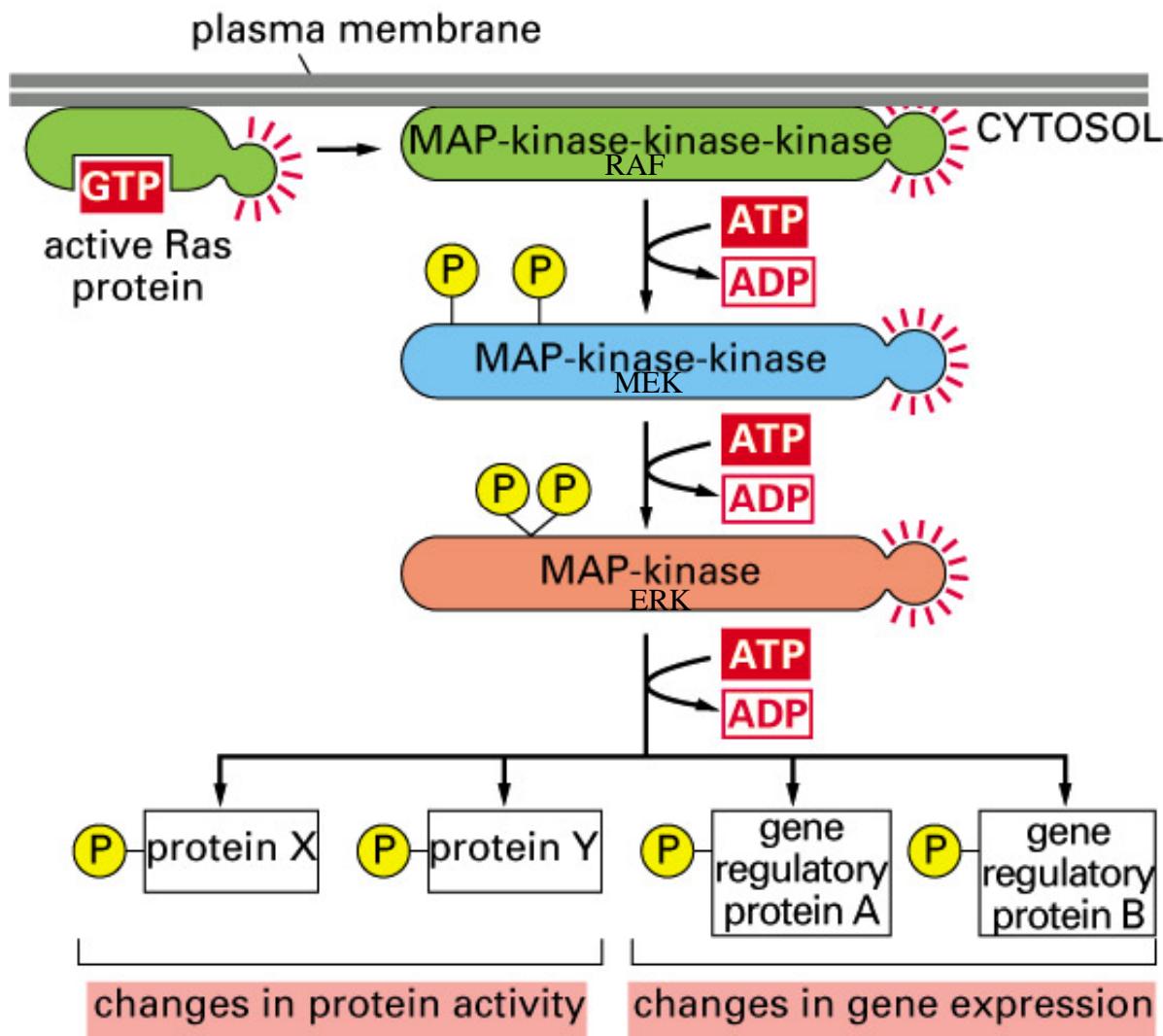
Figure 15–56. Molecular Biology of the Cell, 4th Edition.

THE MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY

Activation of ras occurs in a broad spectrum of tumors and leads to activation of the mitogen-activated protein kinase signaling (MAPK) pathway. The mitogen activated protein kinase is also known as ERK.

The sequence can be expressed as:

RAS -> RAF (MAPKKK) -> MEK (MAPKK) -> ERK (MAPK)

Inhibition of this pathway offers chemotherapeutic potential. The most promising drugs now undergoing clinical trials are directed against RAF and MEK

Reference: J.S. Sebolt-Leopold and R. Herrera. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nature Reviews Cancer 4: 937-947, 2004.

ONCOGENES CODING FOR DNA BINDING PROTEINS

Nuclear proteins that bind to DNA and affect transcription typically have separate DNA binding and transcriptional activation domains. There are frequently domains that are important for dimer formation such as leucine zippers. Some oncogenes are related to well known transcription factors such as erb A to the thyroxine receptor and rel to NF-κB. The fos and jun gene products form a dimer that constitutes the AP-1 transcription factor and is important for the activation of several immediate/early response genes. Such genes are expressed early when cells are stimulated to divide as in regenerating liver and in serum-fed cells in culture. The myc gene product forms dimers with the max gene product. Max dimerizes with the products of the three members of the myc family of genes, c-myc, L-myc and N-myc. Max may also dimerize with another protein called Mad so that Mad may competitively antagonize myc function. The function of myc may also be antagonized by the formation of max-max homodimers which antagonize transcription.

Other oncogenes coding for sequence-specific DNA binding proteins include myb and ets.

GENES ASSOCIATED WITH APOPTOSIS

The bcl-2 gene is associated with cell membranes particularly mitochondrial membranes. The bcl-2 gene is located on human chromosome 18. It is frequently translocated in non-Hodgkin's lymphoma. Activation of bcl-2 can block apoptosis in some cell types including B cells. The bcl-XL gene is a related gene that also inhibits apoptosis. There are related genes that counter the effect of bcl-2 and promote apoptosis. These include the bcl-XS and Bax genes. The bcl-2 gene product may function in an anti-oxidant pathway but the exact mechanism is uncertain.

GENES OF DNA VIRUSES

Some DNA viruses produce proteins that bind with the products of tumor suppressor genes that are DNA binding proteins. These include the large T antigen of SV40, the polyoma middle T antigen and the E1A protein of adenoviruses. In addition, these T antigens can serve as transcriptional activators for some growth related genes.

The HBVx gene in hepatitis B virus codes for a gene that has been implicated in the induction of liver cancer because it is often expressed in human liver cancer and it is able to induce liver cancer in transgenic mice.

hTERT

Cancer cells have the ability to replicate without reaching a state of senescence. In many cancers the ability to divide without limit is achieved by the production of telomerase. Telomerase maintains the ends of chromosomes so that they do not shorten. Telomerase is a normal protein that is present in cells during fetal development. In most cells of an adult human, telomerase is not present as the gene for the enzyme is not being expressed (transcribed and translated). However, in some cancer cells the necessary task of achieving unlimited replication is made possible by the reactivation of the gene that codes for telomerase.

In cancer cells that do not possess telomerase activity, shortening of chromosomes is thought to be prevented by other mechanisms. The maintenance of telomere length allows for unlimited cell division. The gene that codes for the telomerase enzyme, hTERT, is considered a proto-oncogene because abnormal expression contributes to unregulated cell growth.

Reference: http://cancerquest.org/index.cfm?page=565

MICRO RNAs

The work of Carlo Croce and collaborators has been important in revealing the importance of micro RNAs in the development of cancer. They found that a deletion on chromosome 13 in chronic lymphocytic leukemia involves the location of two micro RNAs (mir15 and mir16). There are no open reading frames for conventional genes in the region deleted. Expression of the anti-apoptotic gene Bcl-2 is regulated by mir15 and mir16.

Loss of mir29 was important in aggressive CLL and mir 155 was overexpressed in some lymphomas.

In solid tumors more miRNAs were upregulated than down regulated and there were tissue specific patterns.

MAJOR GENE MUTATIONS REQUIRED FOR PRIMARY TUMORS AND METASTASIS

Robert Weinberg has suggested that different gene mutations are important for the establishment of primary tumors and the later progression to a metastatic tumor.

Genes mutations important for primary tumors:

Ras, Rb, p53, hTERT, PP2A

Gene mutations important for the development of metastases:

Twist, Goosecoid, Slug, FOXC2. These genes code for transcription factors that have a role in normal embryological development.

The interaction of tumor cells with stromal cells may be critical for the establishment of metastases. An important protein is SDF-1 (stroma derived factor). Twist is expressed in more aggressive tumors. It is an inducer of epithelial-mesenchymal transition.

SUMMARY OF HUMAN CANCER GENES

As of March 2004, 291 cancer genes had been reported, about 1% of all the genes in the human genome.

90% of cancer genes show somatic mutations in cancer, 20% show germline mutations and 10% show both.

The most common mutation class is a chromosomal translocation that creates a chimeric gene or apposes a gene to the regulatory elements of another gene.

Many more cancer genes have been found in leukemias, lymphomas and sarcomas than in other types of cancer, despite the fact that they represent only 10% of human cancer. These genes are usually altered by chromosomal translocation.

The most common domain that is encoded by cancer genes is for protein kinase. Several domains involved in DNA binding and transcriptional regulation are common.

Reference: P.A. Futreal et al., A census of human cancer genes. Nature Reviews Cancer 4: 177183, 2004.

"STUDY FINDS MANY NEW CANCER GENES"

Scientists have found many more than 100 new genes that can cause cancer if they become mutated. The discovery was part of the largest survey of the human genome to date, which also suggests that the number of cancer genes is far larger than previously thought. "Thus far, there are approximately 350 genes in the human genome that have been shown to be cancer genes," said Mike Stratton, co-leader of the Cancer Genome Project at the Wellcome Trust Sanger Institute, Cambridge.

Reference: Guardian Weekly, March 16-22, 2007

SUGGESTED READING

M.A. Pierotti, M. Frattini, G. Sozzi and C.M. Croce. *In* Holland-Frei Cancer Medicine - 8th Ed. Edited by D.W. Kufe et al., Part II - Scientific Foundations, Section 1 - Cancer Biology, 6. Oncogenes, 2010.

R.A. Weinberg. The Biology of Cancer, Chapter 4, Garland Press, 2007.