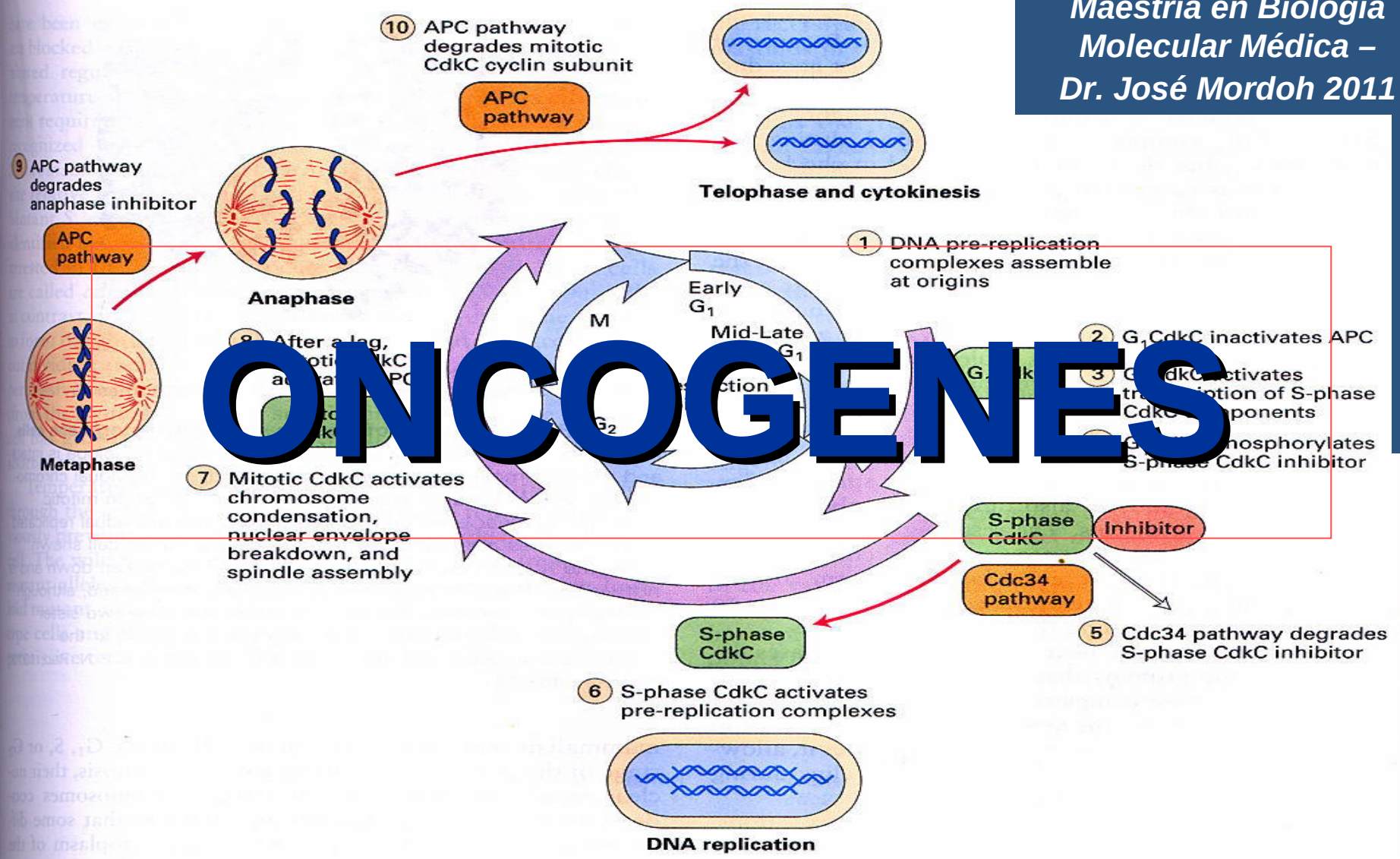
ONCOGENES



▲ **FIGURE 13-2 Current model for regulation of the eukaryotic cell cycle.** Passage through the cycle is controlled by G_1, S-phase, and mitotic cyclin-dependent kinase complexes (CdkCs) highlighted in green. These are composed of a regulatory cyclin subunit and a catalytic cyclin-dependent kinase subunit. Protein complexes (orange) in the Cdc34 pathway and APC pathway polyubiquitinate specific substrates including the S-phase inhibitor, anaphase inhibitor, and mitotic cyclins, marking these substrates for degradation by proteasomes (see Figure 3-18). These pathways thus drive the cycle in one direction because of the irreversibility of protein degradation. Proteolysis of anaphase inhibitors inactivates the protein complexes that connect sister chromatids at metaphase (not shown), thereby initiating anaphase.

What is the molecular basis of cancer?

Cancers are formed from repeated rounds of DNA mutation, competition, and natural selection operating with the host.

-arise from a single abnormal cell

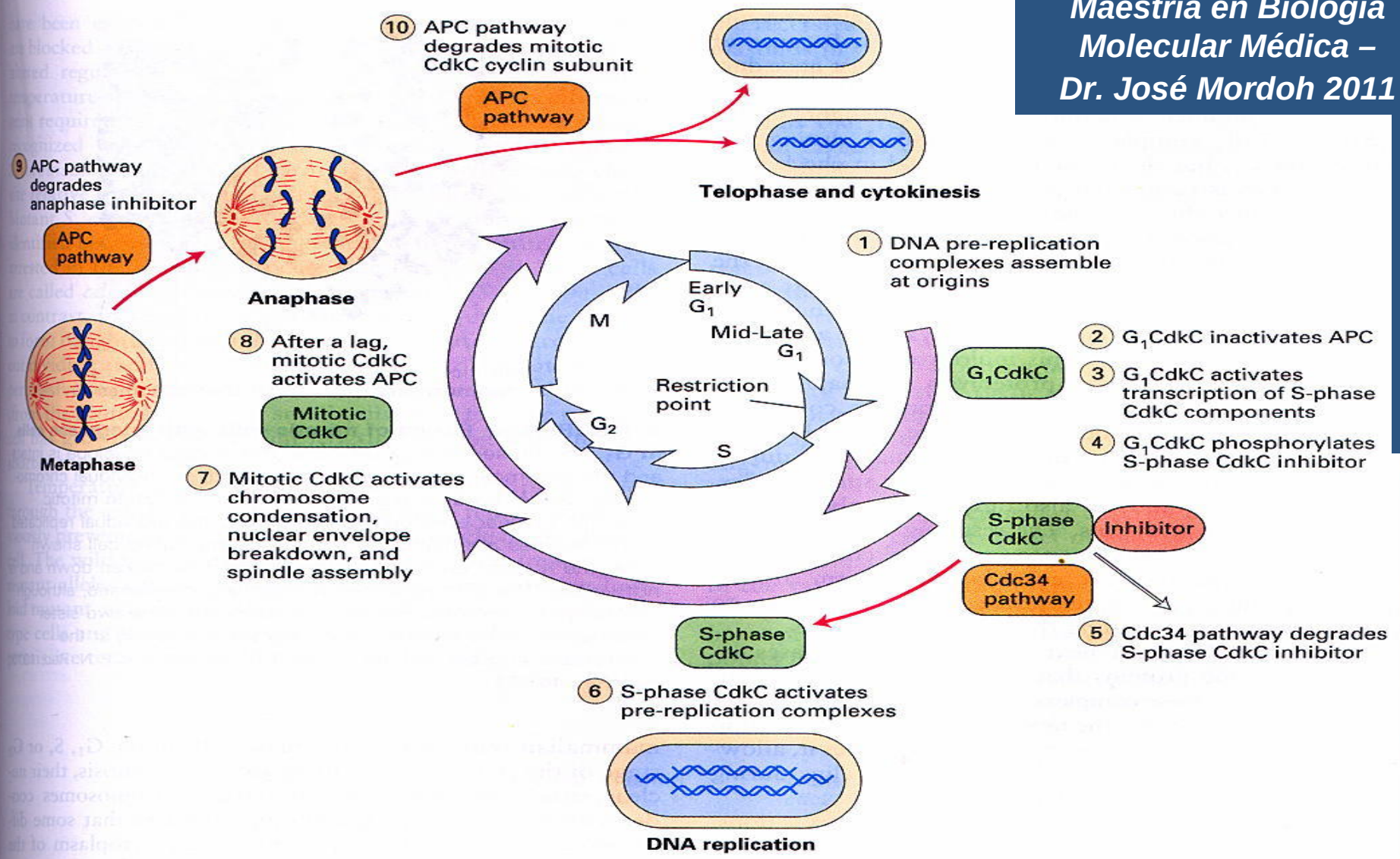
-abnormality results from somatic mutation

-development of cancer requires mutations in many cancer critical genes

For a cancer cell to be successful the mutations must…

1. **Allow the cells to disregard the external and internal signals that regulate proliferation**
2. **Allow the cells to avoid apoptosis and escape programmed limitations to proliferation including differentiation.**
3. **Allow the cells to escape from their tissue of origin**
4. **Allow the cells to survive and proliferate in foreign sites**
5. **Allow further genetic instability (but not too much!)**

Cancer critical genes: oncogenes and tumor suppressors



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Oncogene Discovery

I. Tumor Viruses

- **RNA Tumor Virus**
  - **Acutely Transforming**
  - **Slow Transforming**
- **DNA Tumor Viruses**

II. Genomic Rearrangements

- **Translocations/Inversions**
- **Amplifications/Minute Chromosomes**

III. Functional Assay

- **Transfection of Tumor DNA**
- **Transfer of cDNA libraries**

- **PEYTON ROUS**
- **Premio Nobel de Medicina 1966**

1.Review of the neoplastic phenotype

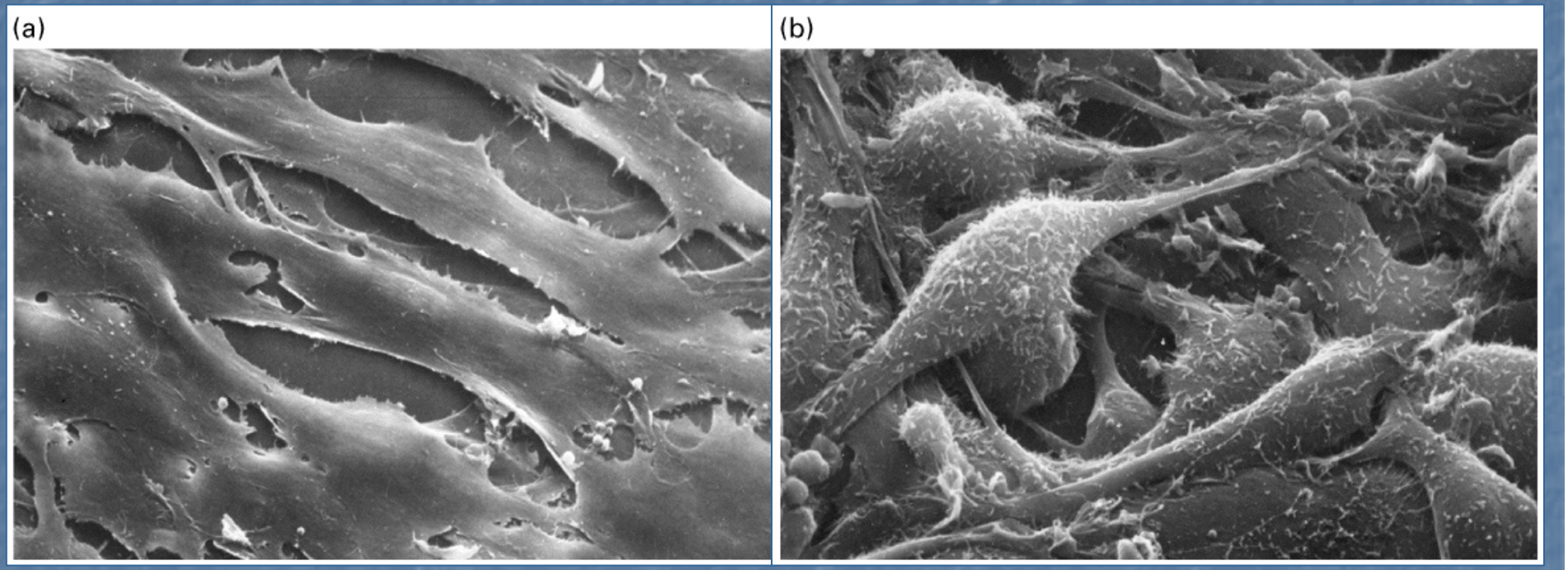
Growth of Normal and *Neoplastic fibroblasts in culture

Growth Characteristics	Normal	Tumor
Density dependent inhibition of growth	**present**	**absent**
Growth factor requirements	**high**	**low**
Anchorage dependence	**present**	**absent**
Proliferative life span	**finite**	**indefinite**
Contact inhibition	**present**	**absent**
Adhesiveness	**high**	**low**
Morphology	**flat**	**rounded**

***Neoplastic: new shape; any new or abnormal growth: specifically a new growth of tissue in which the growth is uncontrolled or aggressive.**

1.Review of the neoplastic phenotype

Normal and transformed NIH3T3 cells



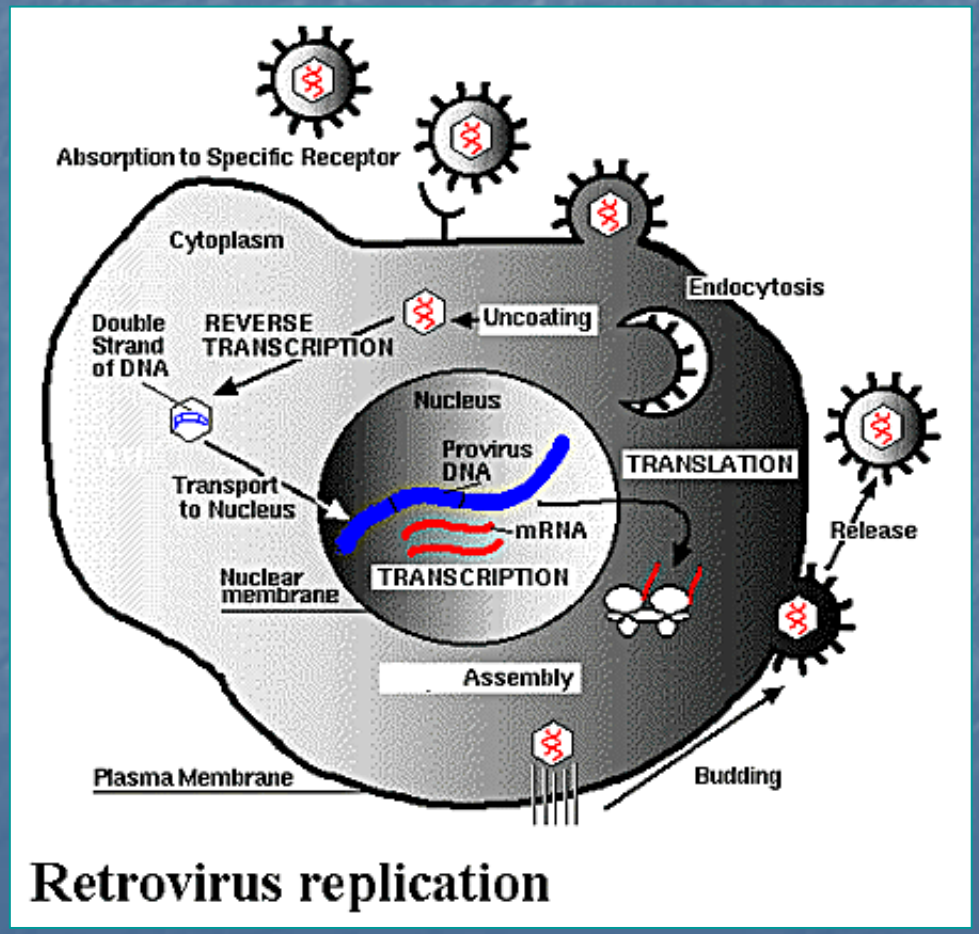
Normal NIH3T3 (immortal)

Transformed NIH3T3

Density dependent inhibition of growth
Growth factor requirements
Anchorage dependence
Proliferative life span
Contact inhibition
Adhesiveness
Morphology

Discovery I. Tumor Viruses; RNA

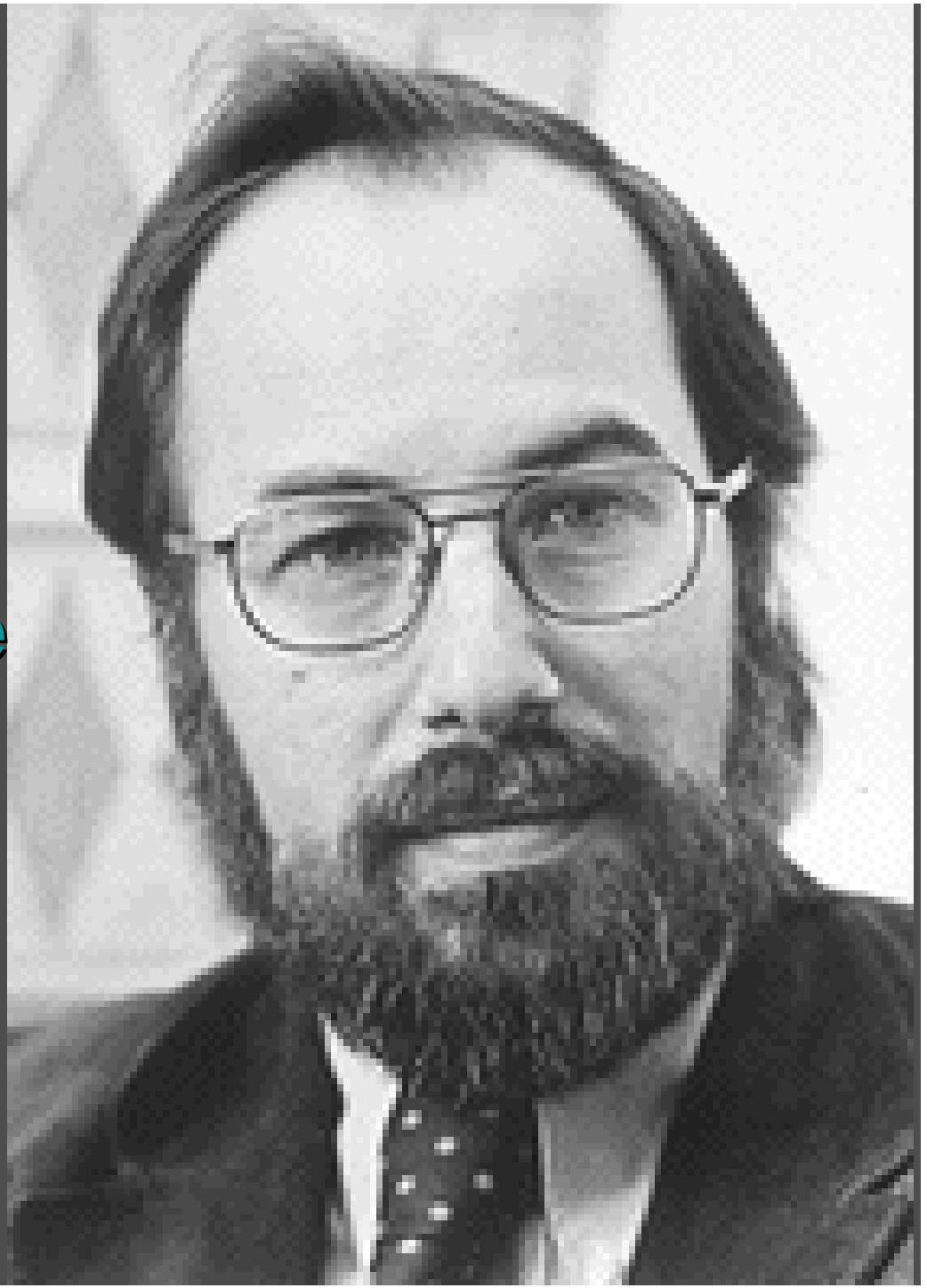
Retrovirus: RNA genome reversed transcribed into proviral DNA which integrates randomly into the host cell genome.
Productively infects only proliferating cells.



Retrovirus replication

Peyton Rous:
1st evidence that viruses could cause cancer (1911).
-Chickens
--fibrosarcoma
--Rous Sarcoma virus
-*Nobel prize 1966

- **DAVID BALTIMORE**
- **Premio Nobel de Medicina 1975**



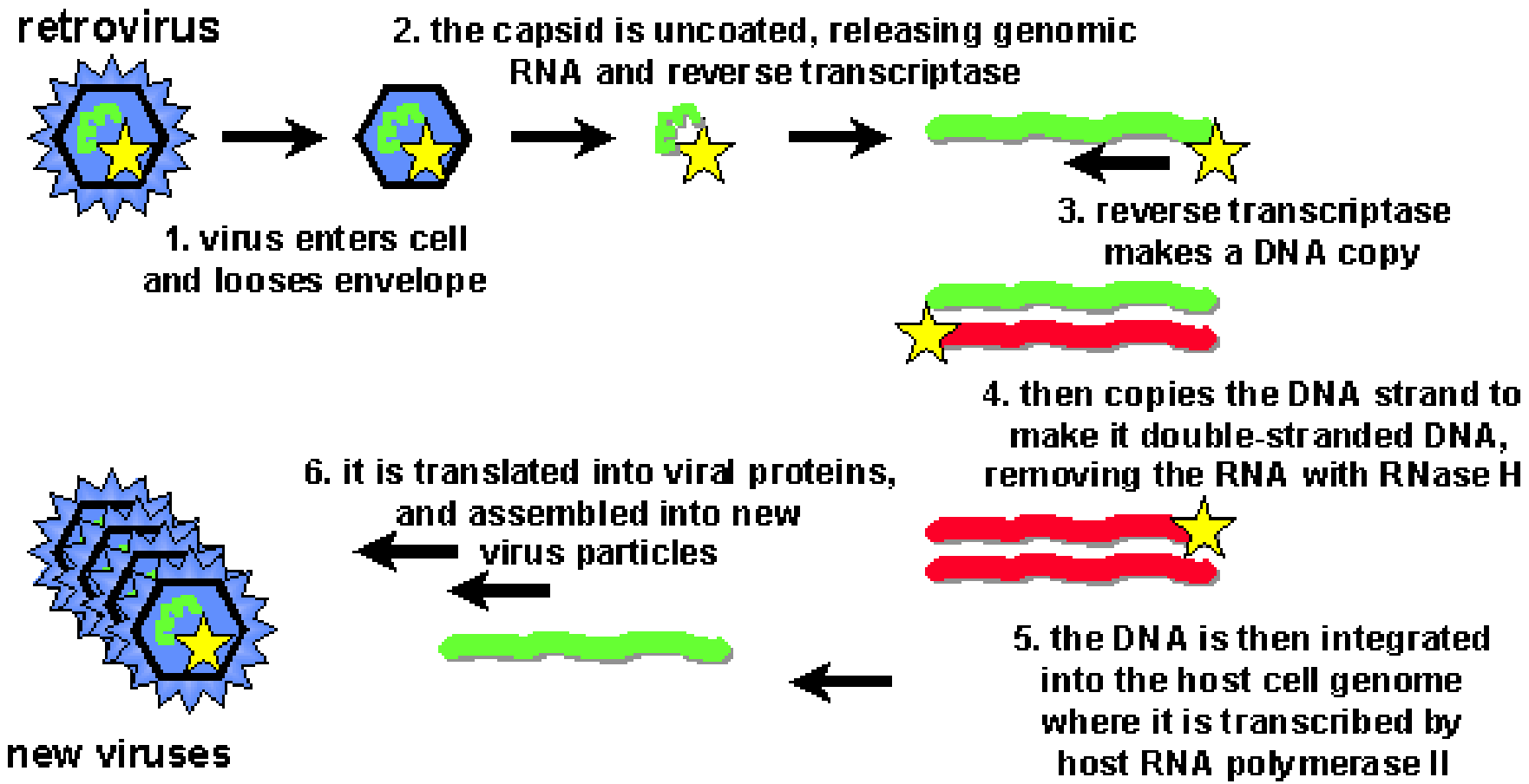
- **RENATO DULBECCO**
- **Premio Nobel de Medicina 1975**

- **HOWARD TEMIN**
- **Premio Nobel de Medicina 1975**

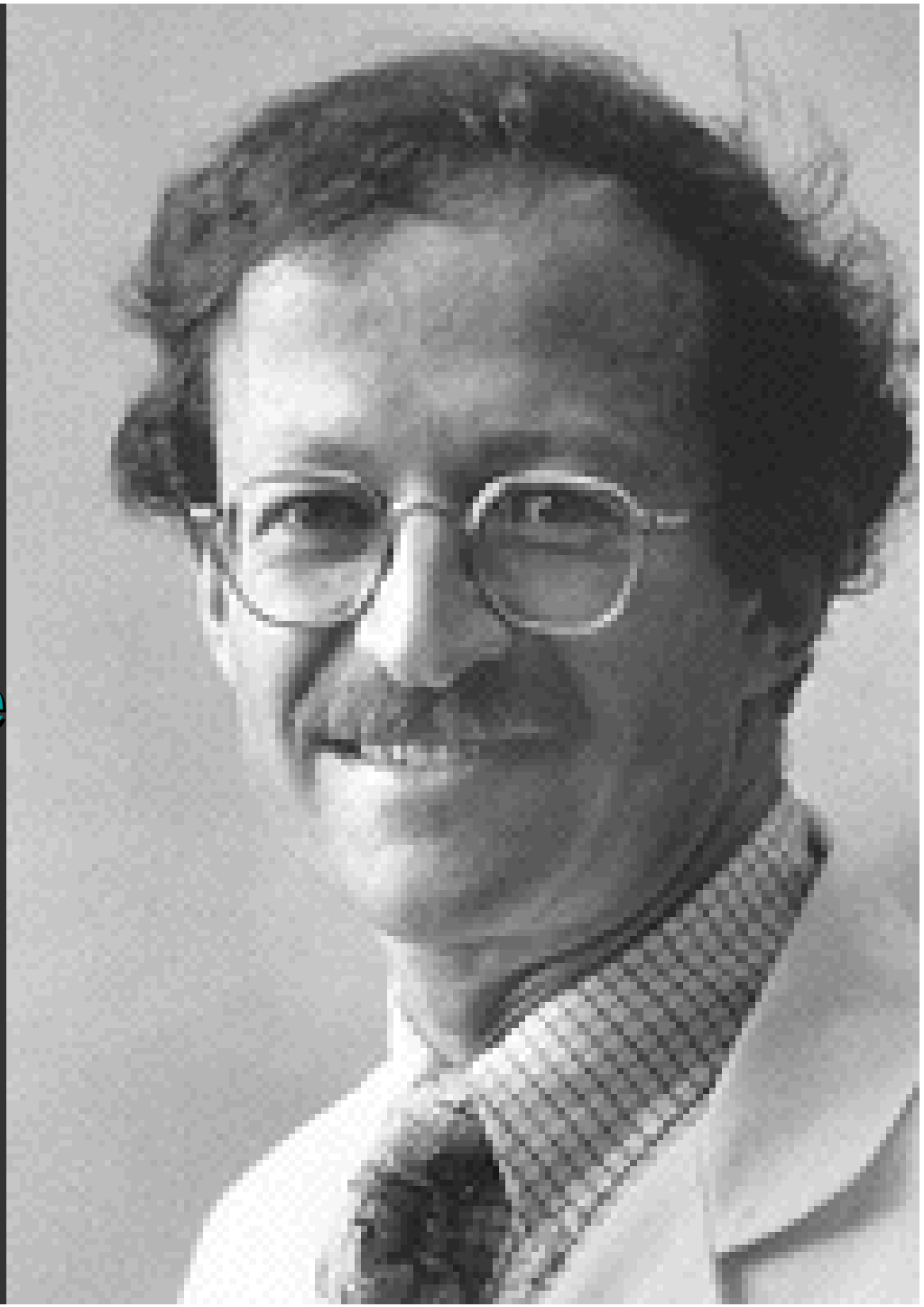
Construction of a cDNA library

- reverse transcriptase makes a DNA copy of an RNA

The life cycle of a retrovirus depends on reverse transcriptase

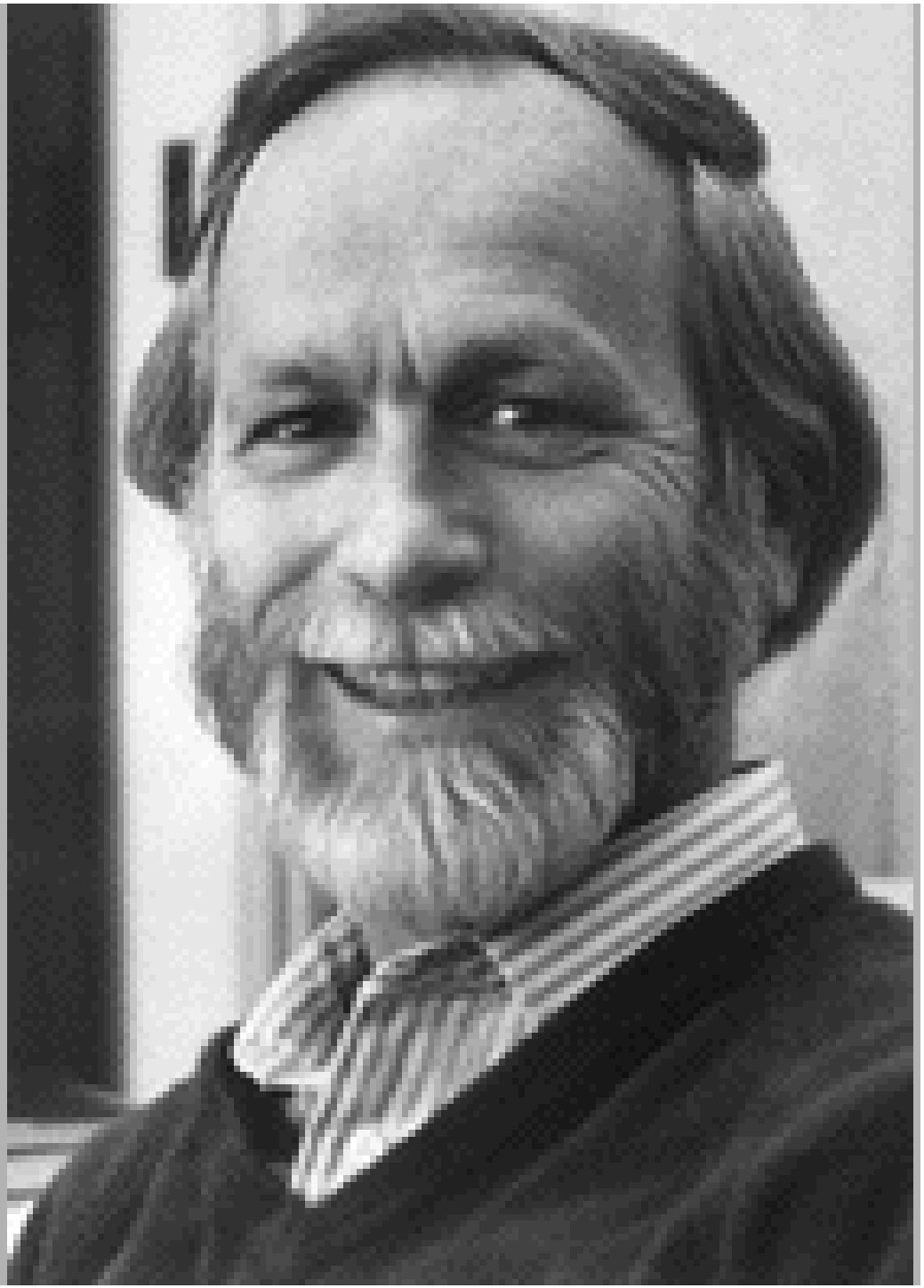


- **HAROLD VARMUS**
- **Premio Nobel de Medicina 1989**



J.MICHAEL BISHOP

Premio Nobel de Medicina 1989



Retroviral Transduction

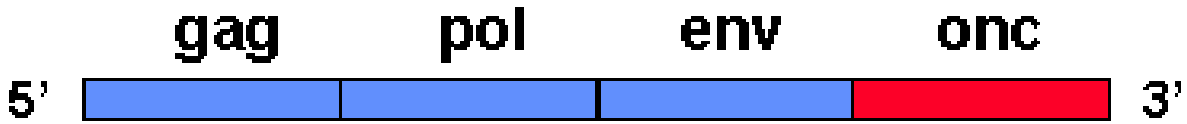
Acutely Transforming Retroviruses encode an onc gene.

Retrovirus normal

RSV has a env-onc fusion

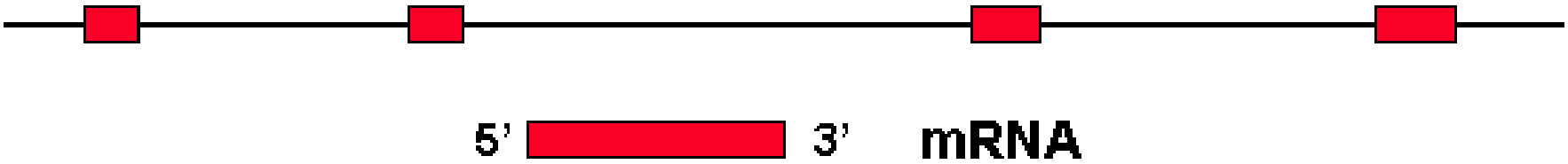
Gene organization of a retrovirus

Gene organization of a transforming retrovirus

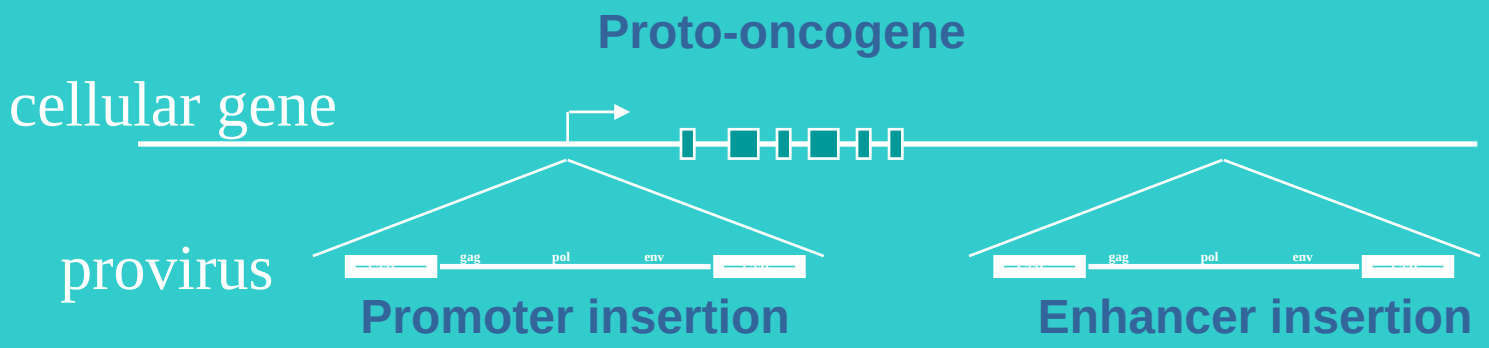


gag = group specific antigen
pol = reverse transcriptase
env = envelope
onc = oncogene

Gene organization of a cellular proto-oncogene



Slow transforming retroviruses



May be 5’ or 3’ in either orientation.

Slow transforming retroviruses activate proto-oncogenes by insertional mutagenesis.

Dysregulated expression occurs after insertion of strong promoters or enhancers into the genetic loci.

An oncogene is:

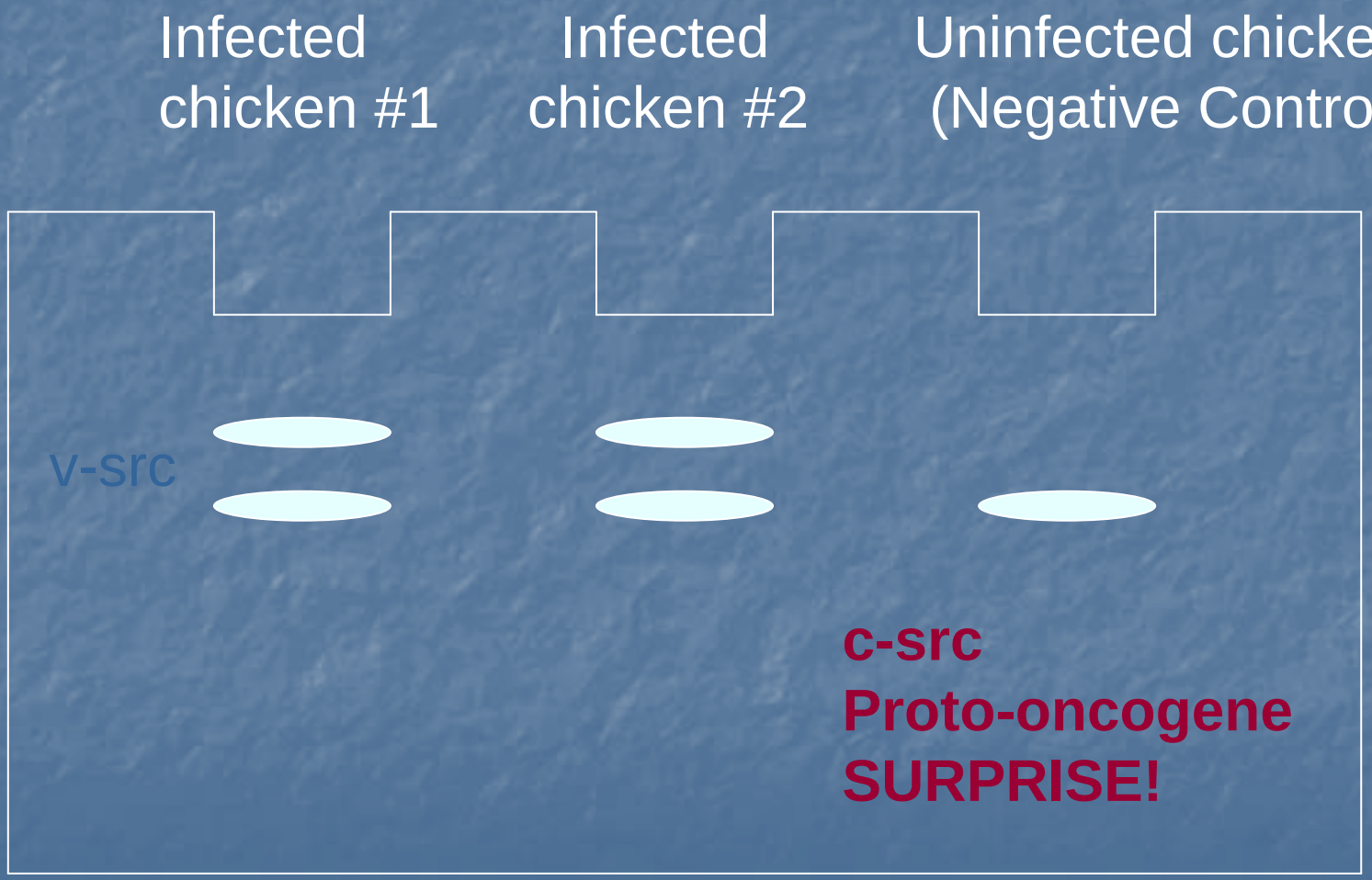
Mutant or overactive form of a normal gene (normal gene is referred to as a proto-oncogene)

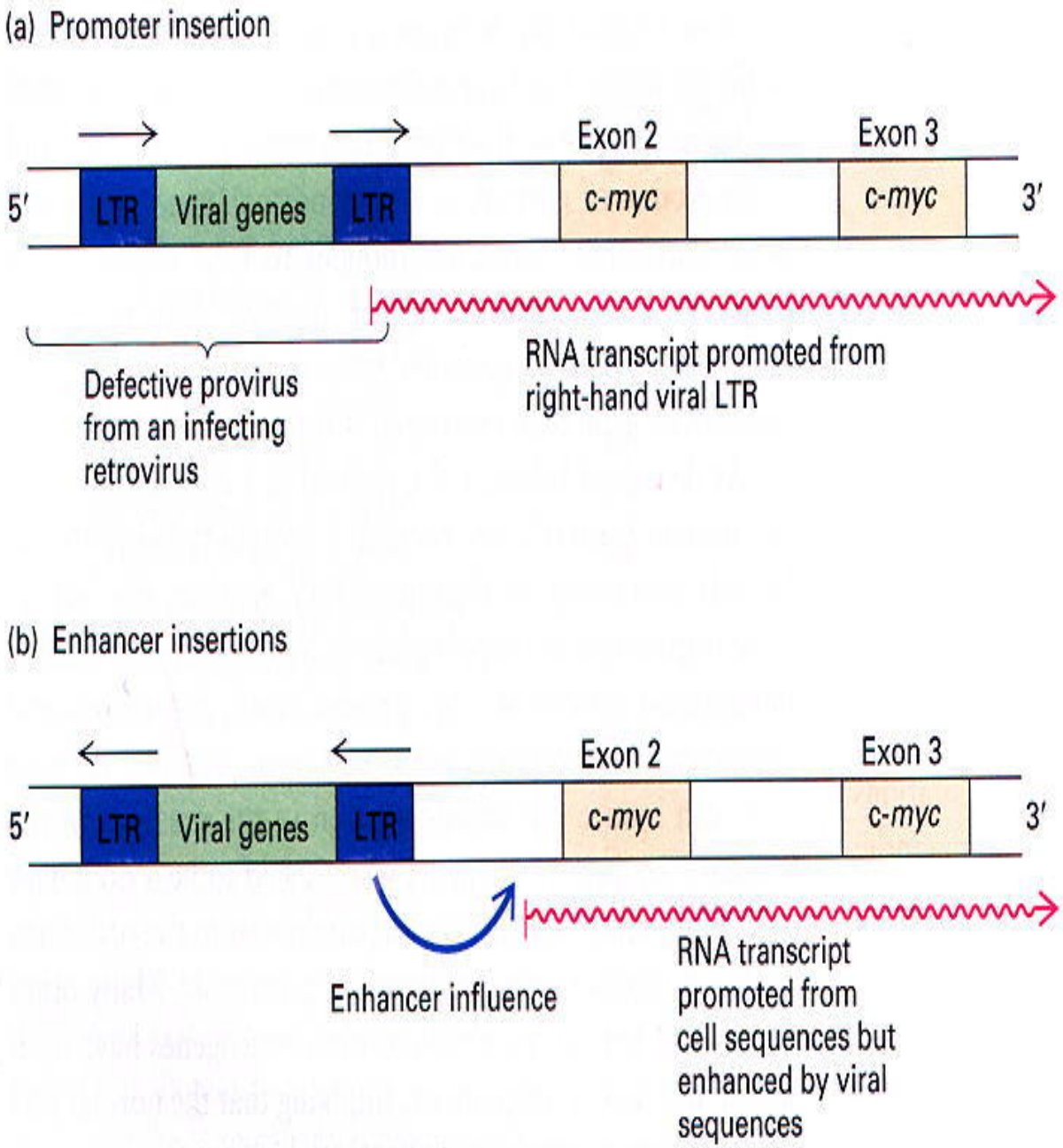
A gene capable of inducing cancer.

Any gene which produces a “malignant phenotype” when introduced into a “normal cell”.

A gene intimately associated with a particular malignant disease such as a specific chimera in a particular leukemia.

Southern Blots Probed with viral *src* Gene Revealed Cellular Origin of Oncogenes





◀ FIGURE 24-10 Activation of the *c-myc* proto-oncogene by retroviral promoter and enhancer insertions. (a) The promoter can be activated when the retrovirus inserts upstream (5′) of the *c-myc* exons. The right-hand LTR may then act as a promoter if the provirus has a defect preventing transcription through to the right-hand LTR. The *c-myc* gene is shown as containing two exons; there is a further upstream exon but it has no coding sequences. (b) The *c-myc* gene can also be activated when a retrovirus inserts upstream of the *c-myc* gene in the opposite transcriptional direction; a viral LTR acts as an enhancer, activating transcription from the *c-myc* promoter sequence. [Modified from actual cases of retroviral insertion described in G. G. Payne et al., 1982, *Nature* **295**:209.]

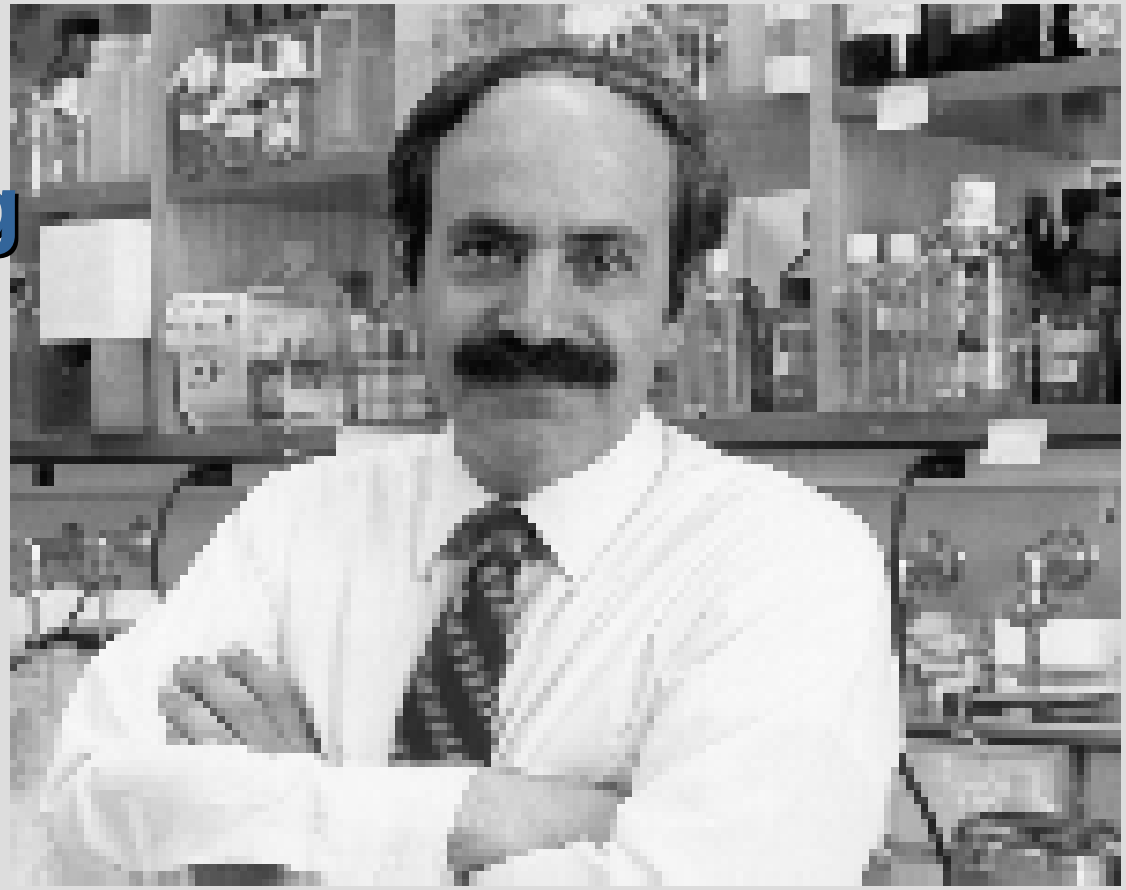
Oncogenes of Acutely Transforming Retroviruses

src	Rous sarcoma virus	Chicken
myc	Avian myelocytomatosis virus	Chicken
erb A, erb B	Avian erythroblastosis virus	Chicken
myb	Avian myeloblastosis virus	Chicken
ets	Avian erythroblastosis virus	Chicken
rel	Avian reticuloendotheliosis virus	Turkey
H-ras	Harvey rat sarcoma virus	Rat
K-ras	Kirsten murine sarcoma virus	Mouse
abl	Abelson murine leukemia virus	Mouse
raf	Murine sarcoma virus	Mouse
fos	Mouse osteosarcoma virus	Mouse
fms	Feline sarcoma virus	Cat
fes	Feline sarcoma virus	Cat
sis	Simian sarcoma virus	Monkey

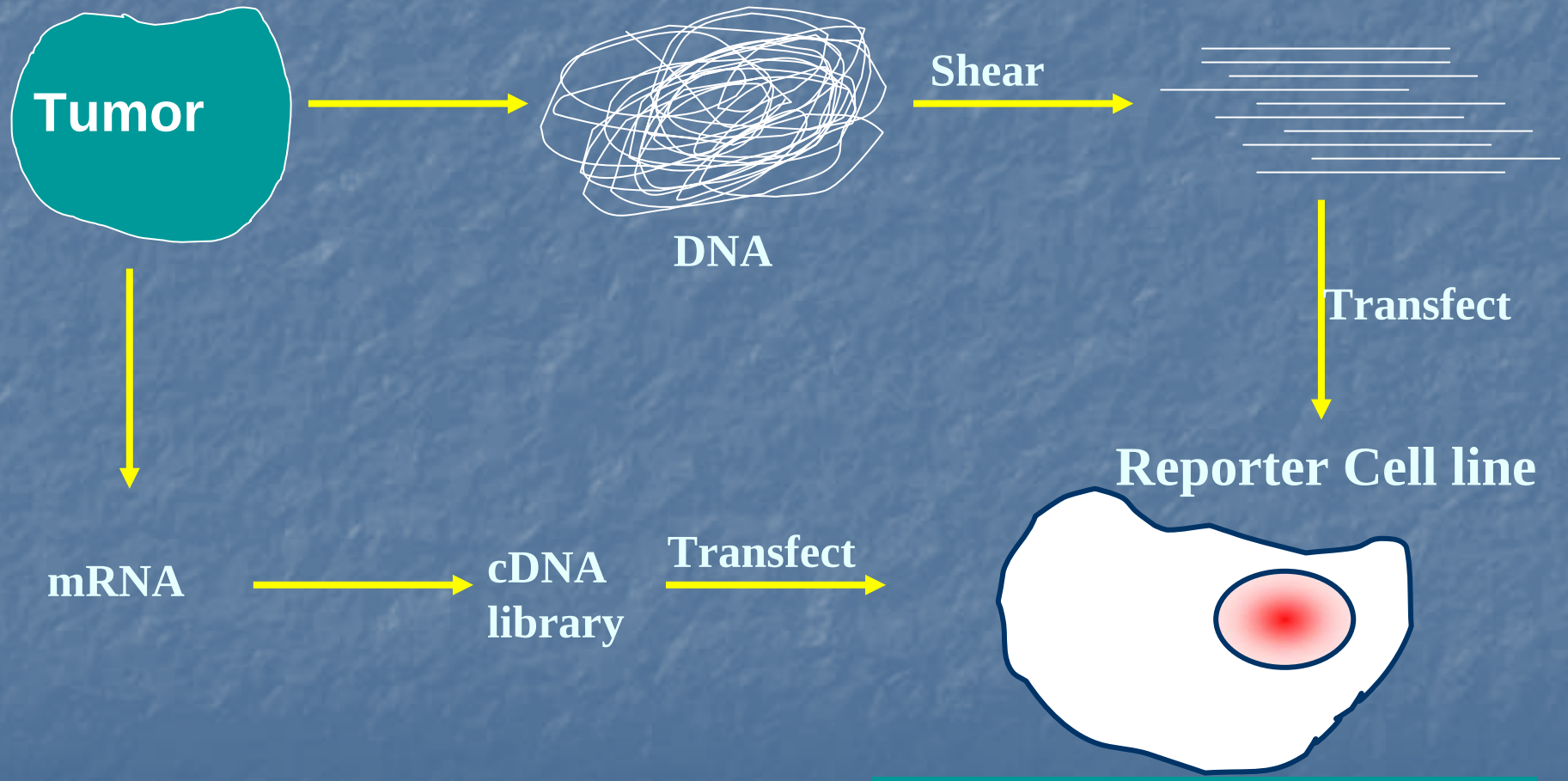
= Oncogenes of acutely transforming retroviruses important in human cancer

Robert Weinberg

Whitead Institute-MIT



Discovery III. Identification of Oncogenes by functional assays; *Transfection



*transfection: putting foreign DNA into mammalian cells.

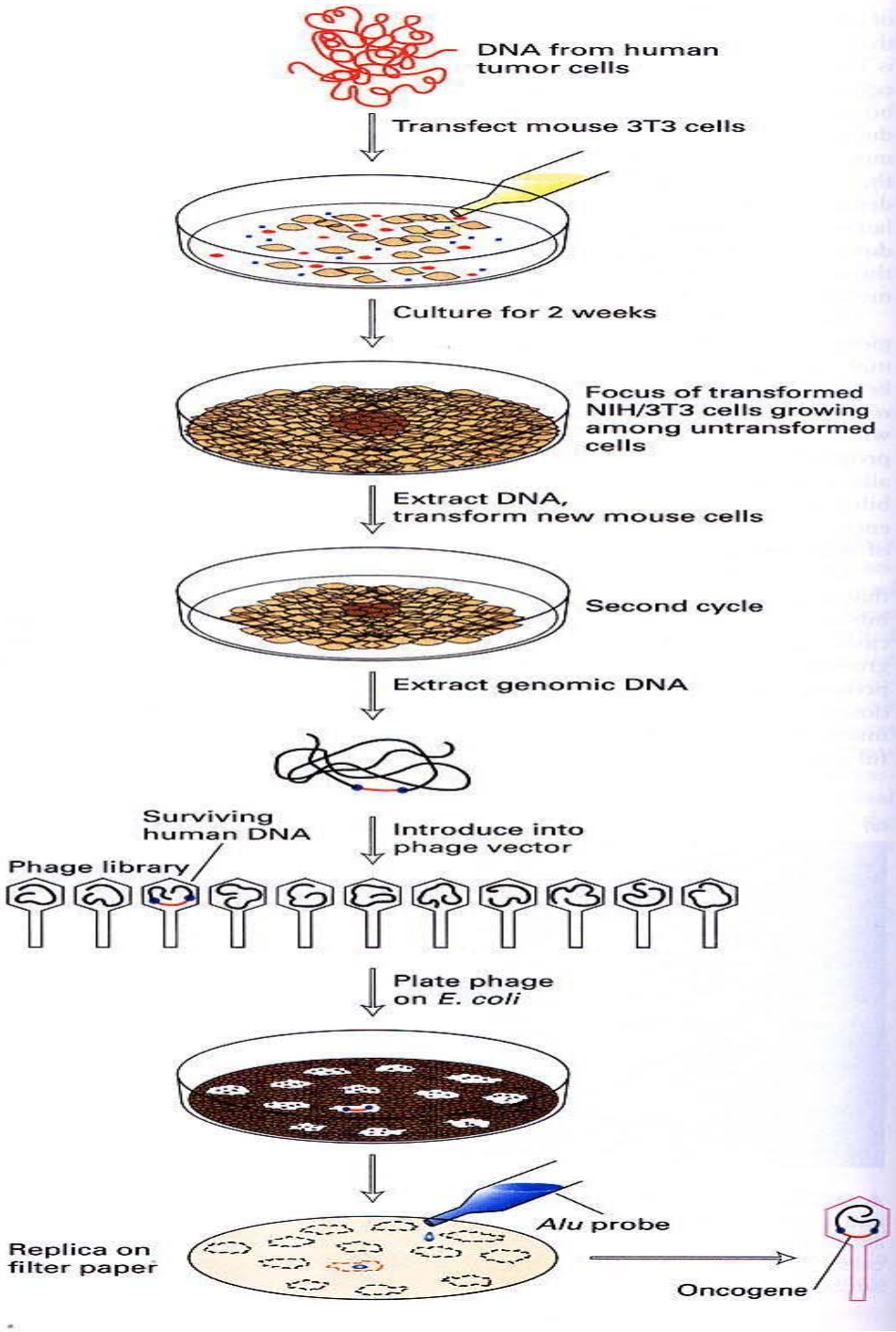
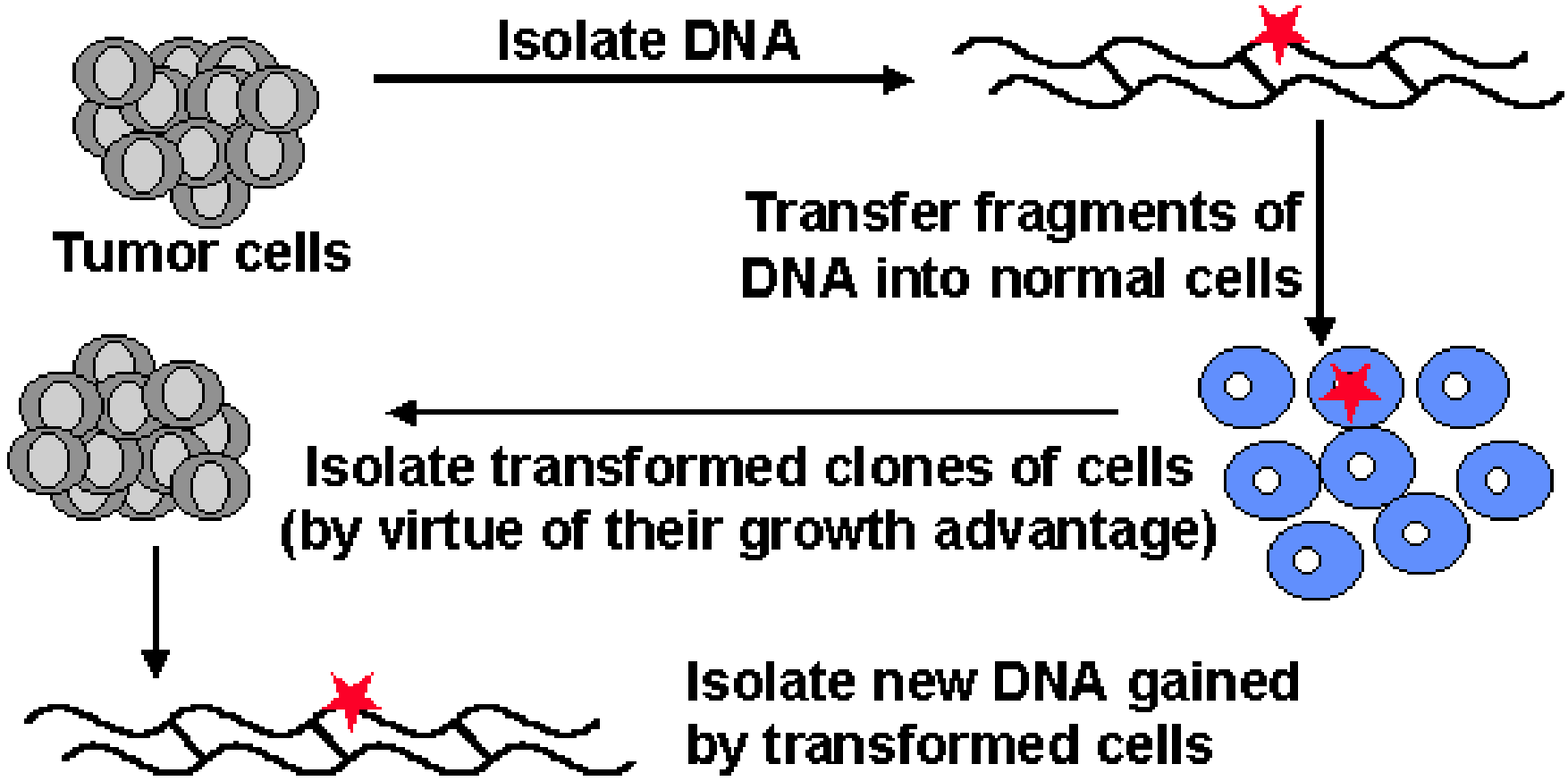


FIGURE 24-4 The identification and molecular cloning of the *ras*^{D} oncogene. Addition of DNA from a human bladder carcinoma to a culture of mouse 3T3 cells causes about one cell in a million to divide abnormally and form a focus, or clone of transformed cells. To clone the oncogene responsible for transformation, advantage is taken of the fact that most human genes have nearby repetitive DNA sequences called *Alu* sequences. DNA from the initial focus of transformed mouse cells is isolated, and the oncogene is separated from adventitious human DNA by secondary transfer to mouse cells. The total DNA from a secondary transfected mouse cell is then cloned into bacteriophage λ; only the phage that receives human DNA hybridizes with an *Alu* probe. The hybridizing phage should contain part or all of the transforming oncogene. This expected result can be proved by showing either that the phage DNA can transform cells (if the oncogene has been completely cloned) or that the cloned piece of DNA is always present in cells transformed by DNA transfer from the original donor cell.

Identification of oncogene mutations in human tumors

- most human tumors contain mutated or "activated" proto-oncogenes
- demonstrated by isolating the mutated genes from human tumors



10-20% of spontaneous human tumors have DNA that will transform cells in culture; most are due to ras gene mutations

Some Oncogenes identified by Transfection

Weinberg- activated *ras* from bladder carcinoma.

Vande Woude- *met* oncogene which is hepatocyte growth factor receptor from a chemically transformed cell line.

***hst* is a FGF-related gene identified from a human stomach carcinoma.**

Retrovirus oncogenes derived from normal cellular genes

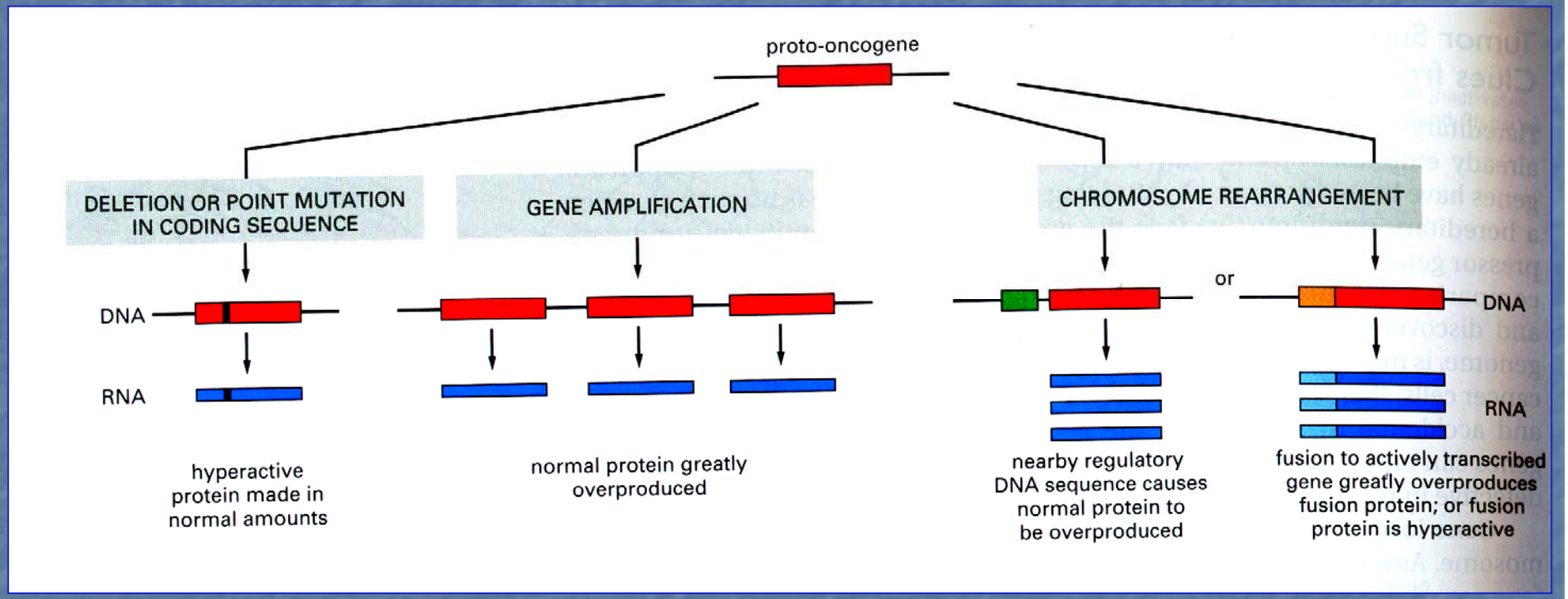
Retrovirus	Viral oncogene	Cellular proto-oncogene
Rous sarcoma virus	v-src	c-src (src)
Simian sarcoma	v-sis	c-sis (sis)
Harvey murine sarcoma	v-H-ras	c-H-ras (H-ras)
Kirsten murine sarcoma	v-K-ras	c-K-ras (K-ras)
FBJ murine osteosarcoma	v-fos	c-fos (fos)
Avian myelocytomatosis	v-myc	c-myc (myc)
Abelson leukemia virus	v-abl	c-abl (abl)
Avian erythroblastosis	v-erbB	c-erbB (erbB)

- viral oncogenes are ~80-99% homologous to cellular proto-oncogenes
- viral oncogenes in general are copies of cellular mRNA and lack introns

FUNCION DE LOS PROTO-ONCOGENES

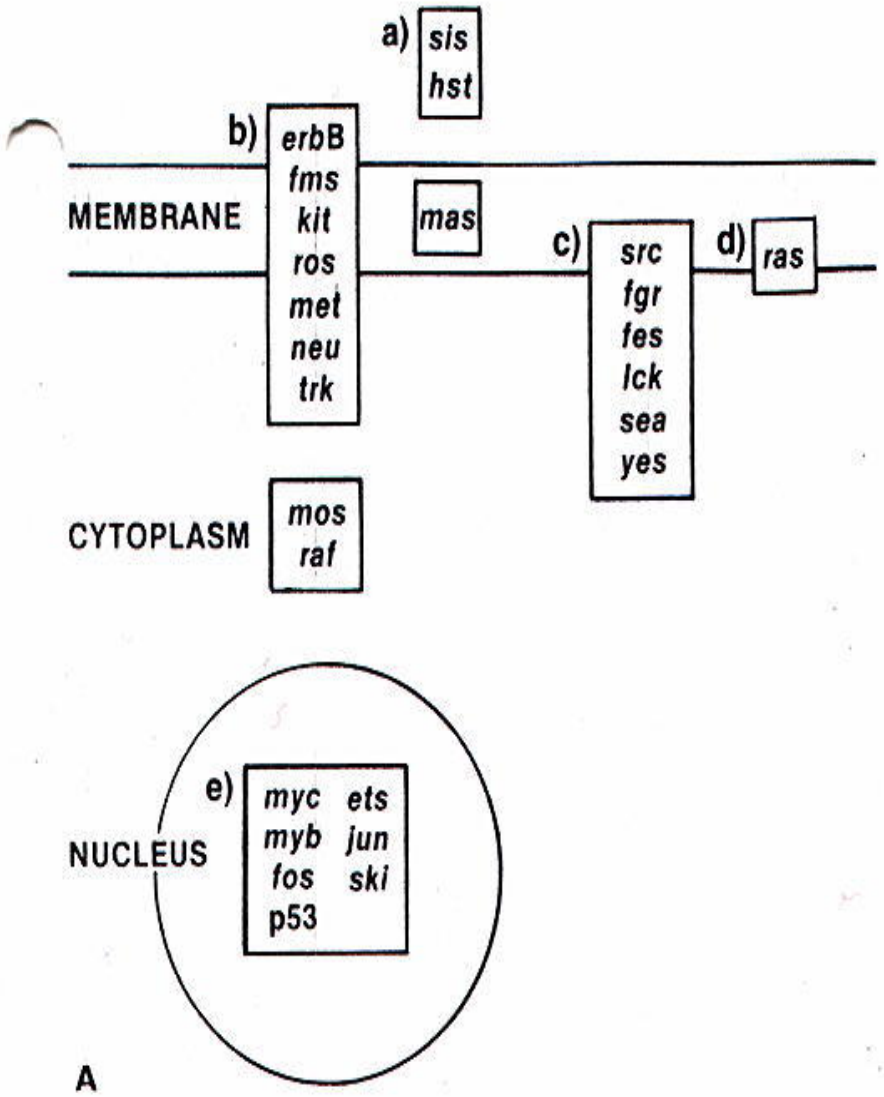
- - Transducción de señales
- - Factores de transcripción
- - Receptores de factores de crecimiento
- -Factores de crecimiento

Three ways in which a proto-oncogene can be converted into an oncogene.



Oncogenes: Mechanisms of Action

1. Oncogenes in Signal Transduction
2. Oncogenes in Cell Cycle
3. Oncogenes in Cell Survival



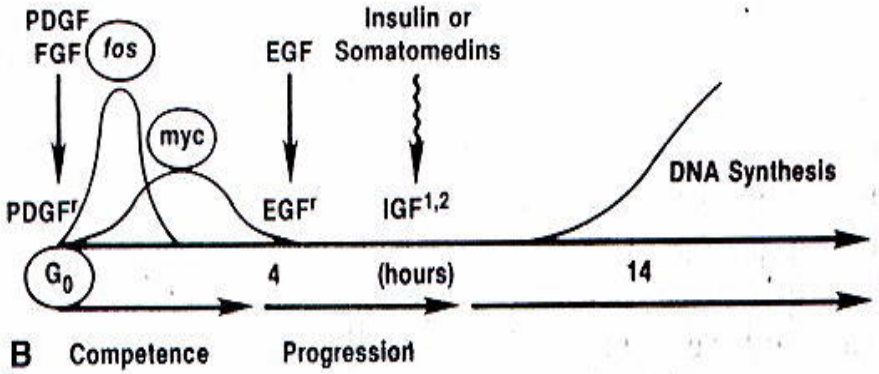


FIGURE 3–1. (A) Schematic presentation of the cellular compartments in which oncogene or protooncogene products are localized. The cell cycle is regulated by growth factors (external mitogenic signals), transmembrane tyrosine kinase growth factor receptor membranes, nonintegral membrane-associated proteins of the *SRC* gene family and *RAS* gene family, and oncogenes localized in the nucleus. **(B)** Stimulation of quiescent murine fibroblasts to enter the G_1 phase of growth by addition of platelet-derived growth factor (PDGF) or fibroblast growth factor (FGF). A transient increase in the expression of both C-*FOS* and C-*MYC* follows PDGF or FGF stimulation or treatment of cells with phorbol ester TPA plus a calcium ionophore. Cells rendered competent require epidermal growth factor and insulin-like growth factors to progress through DNA synthesis and the cell cycle.

ONCOGENES PROTOTIPICOS= PROPIEDADES

Función	Oncogene	Propiedades
Tirosina-Quinasas Integrales de membrana	V-ERB B	
	HER 2-NEU	RECEPTOR FACT. CRECIMIENTO
	c-Kit (PDGFR)	
Tirosina-Quinasas Asociadas a membrana	V-SRC	TRANSDUCCION
	V-ABL	
Serina-Treonina Quinasas	V-MOS	
	RAF	TRANSDUCCION
Familia Fact. Crecimiento	V-SIS (PDGF)	
Familia Ras	V-H-RAS	TRANSDUCCION
	V-K-RAS	
	N-RAS	
Familia Proteínas Nucleares	V-MYC	UNION DNA
	N-MYC	
	V-MYB	
	V-FOS	
	V-JUN	

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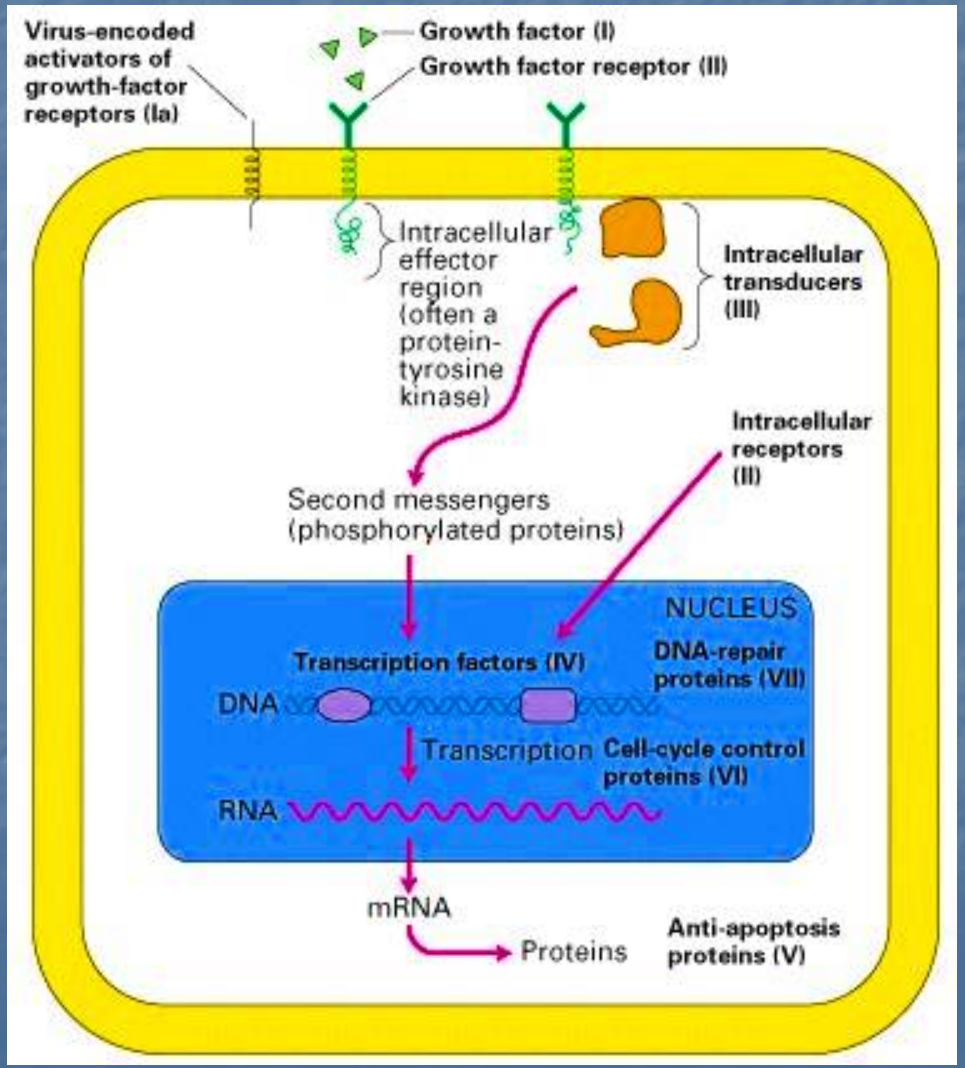
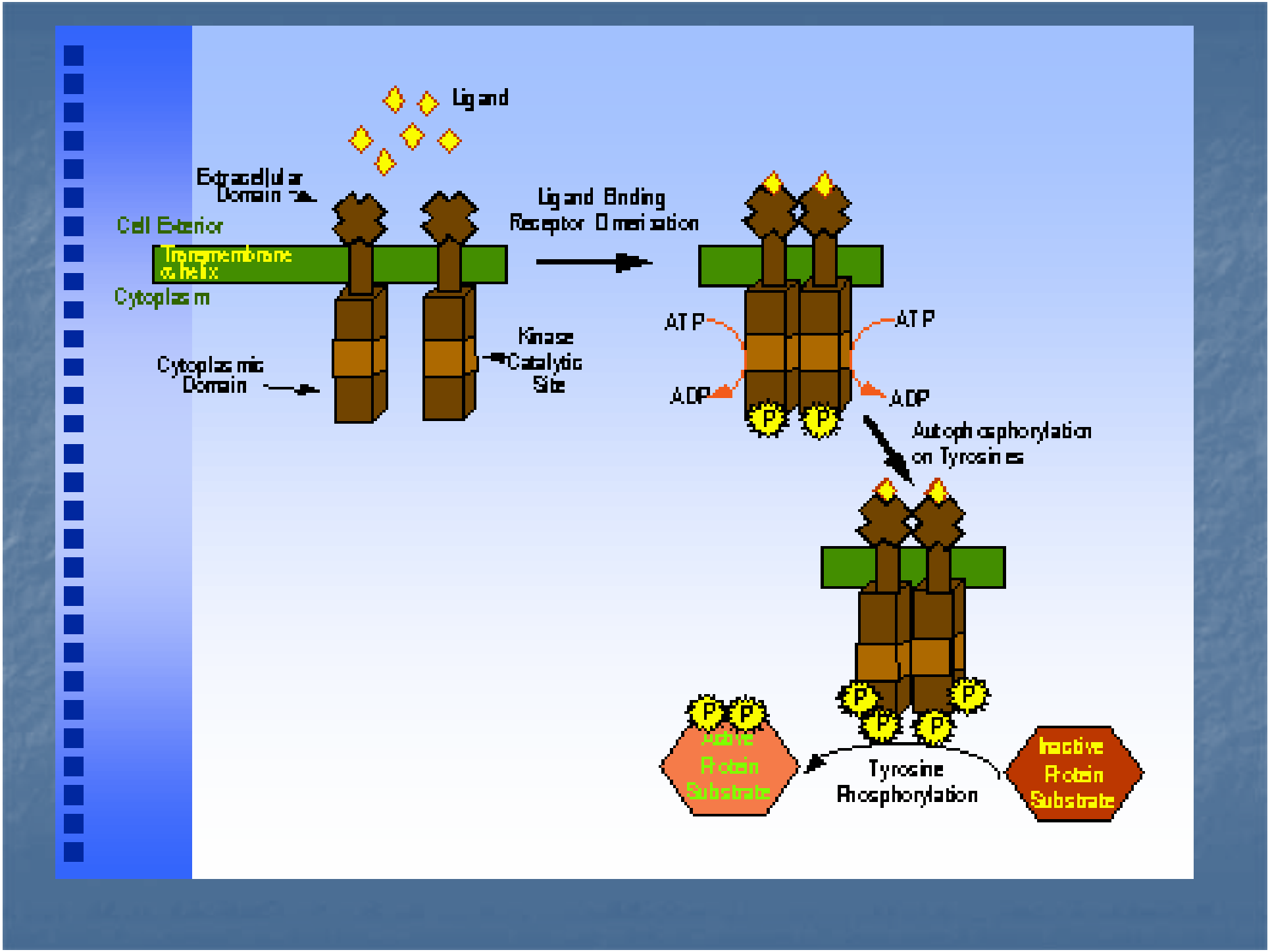
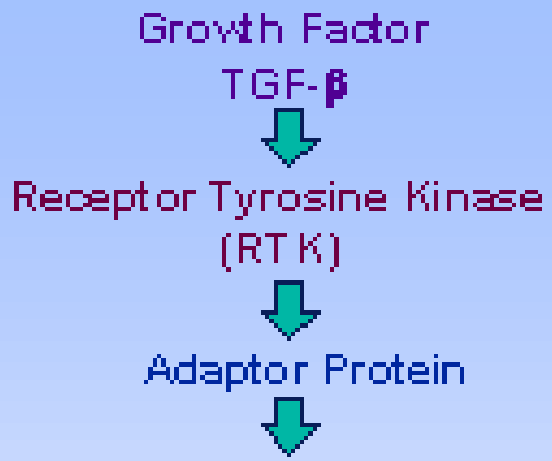
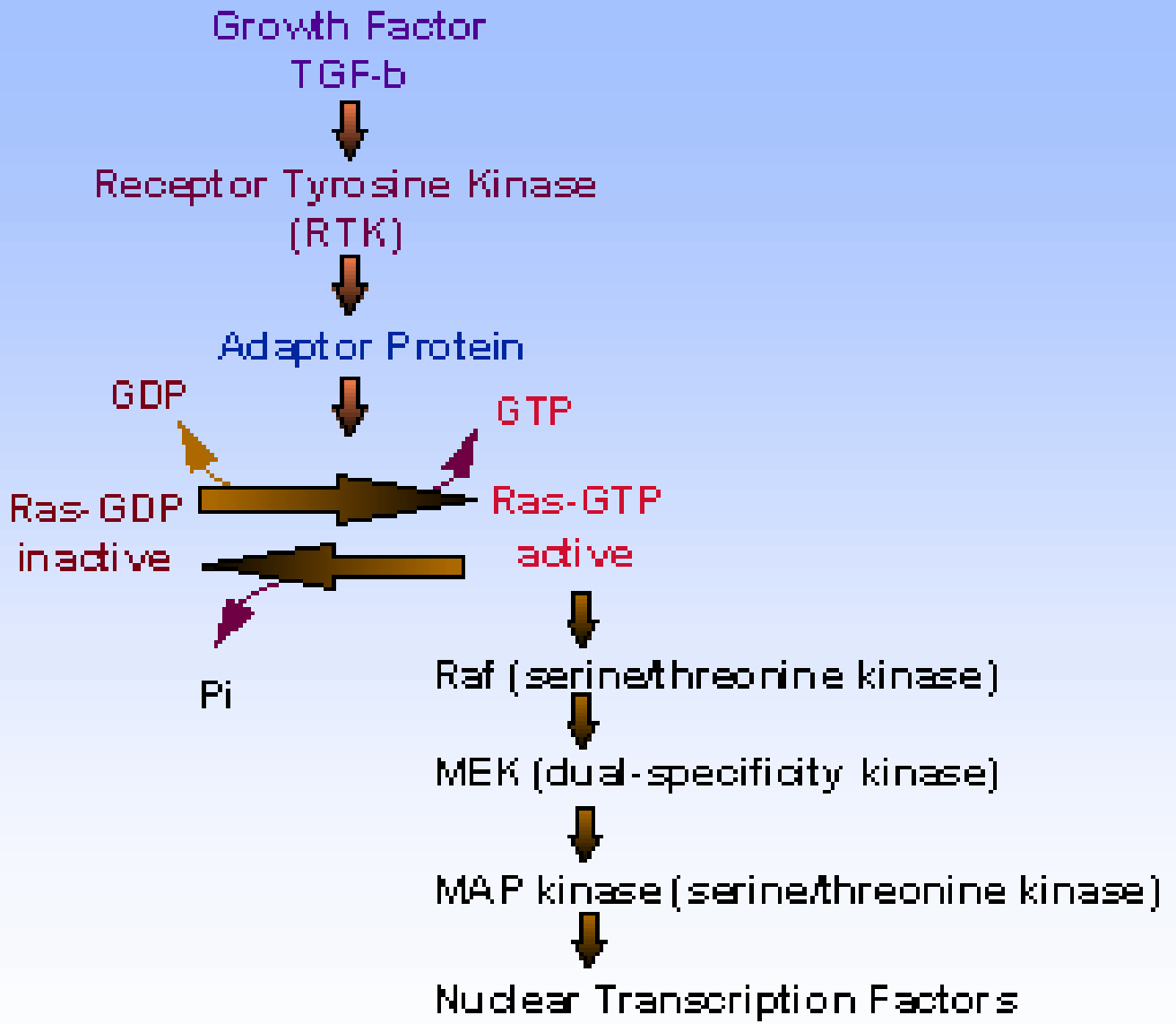
Cancer results from the mutant/aberrant expression of proteins that control cell growth and death



1. Growth factors
2. Receptors
3. Signal-transduction molecules
4. Transcription factors
5. Proteins controlling apoptosis
6. Cell-cycle proteins (pRB pathway)
7. DNA repair proteins


Ligand
Extracellular Domain
Cell Exterior
Transmembrane α helix
Cytoplasm
Cytoplasmic Domain
Kinase Catalytic Site
Ligand Binding Receptor Dimerization
ATP
ATP
ADP
ADP
P
P
Autophosphorylation on Tyrosines
P
P
P
P
P
P
Active Protein Substrate
Tyrosine Phosphorylation
Inactive Protein Substrate

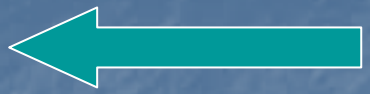

Growth Factor
TGF-β
Receptor Tyrosine Kinase
(RTK)
Adaptor Protein


Growth Factor
TGF-b
Receptor Tyrosine Kinase
(RTK)
Adaptor Protein
GDP
GTP
Ras-GDP
inactive
Ras-GTP
active
Pi
Raf (serine/threonine kinase)
MEK (dual-specificity kinase)
MAP kinase (serine/threonine kinase)
Nuclear Transcription Factors

Mechanism of action: Oncogenes as signal transducers

EXTRACELLULAR

Growth Factors



v-sis (PDGF), int-1(WNT-1), int-2(FGF), hst, fgf-5

Growth Factors Receptors

CYTOPLASM

v-erb-B (EGFR), v-fms (CSF-1R), v-kit (KIT)

Signal Transducers

v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

NUCLEUS

Transcription Factors

v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

Mechanism of action: Growth Factors as Oncogenes

Growth Factors affect:

- **Proliferation- autocrine loop**
  c-sis (PDGF) and PDGFR in glioblastoma.
  EGF and TGF-α and -EGFR in non-small cell lung carcinoma.

- **Neovascularization**
  VEGF,
  FGF family members

- **Invasion**
  scatter factor/HGF (Met ligand)

- **Evasion of Immunosurveillance**
  TGF-β

Oncogenes as signal transducers

EXTRACELLULAR

Growth Factors

v-sis (PDGF), int-1(WNT-1), int-2(FGF), hst, fgf-5

Growth Factors Receptors

CYTOPLASM

v-erb-B (EGFR), v-fms (CSF-1R), c-kit (KIT)

Signal Transducers

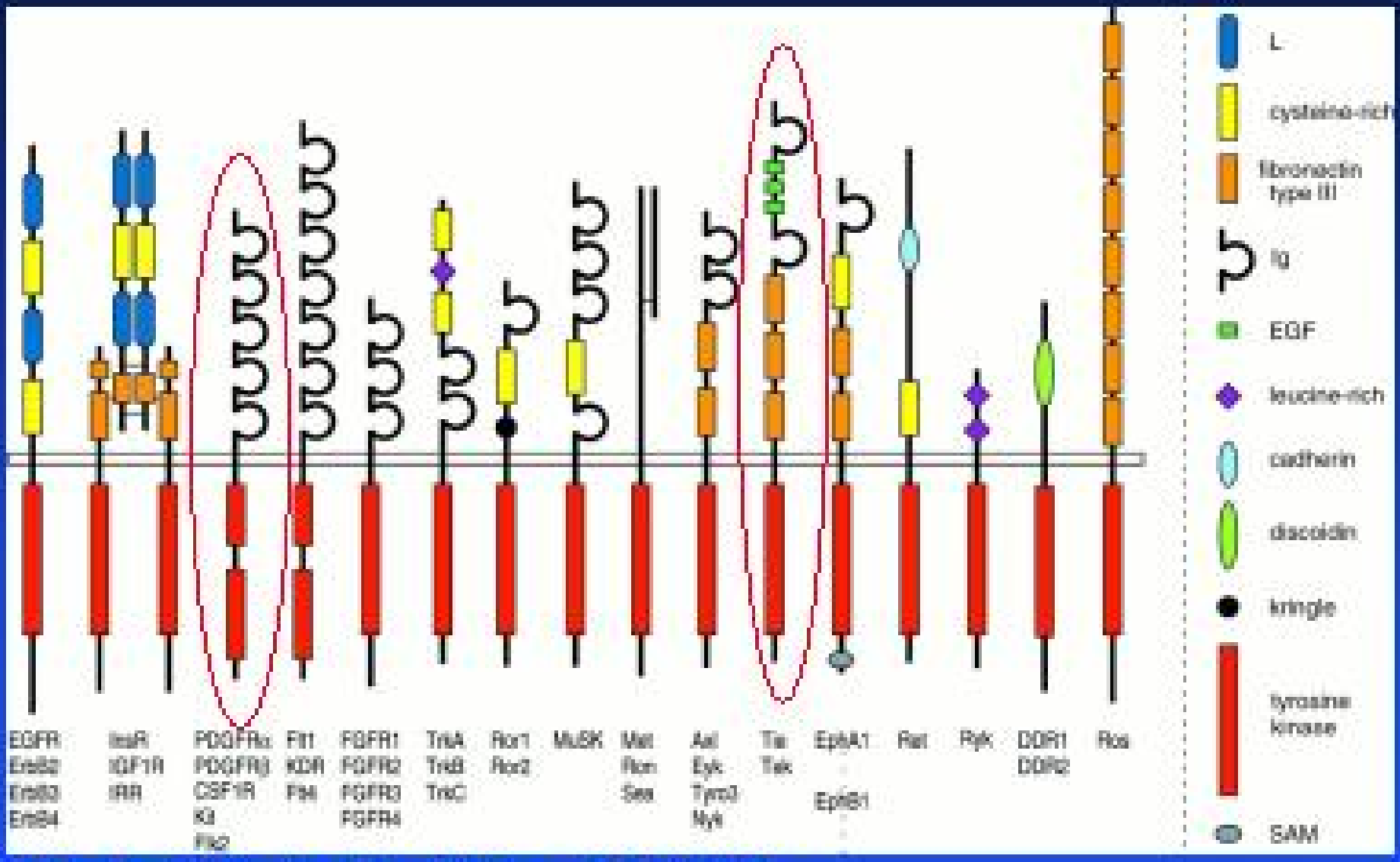
v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

NUCLEUS

Transcription Factors

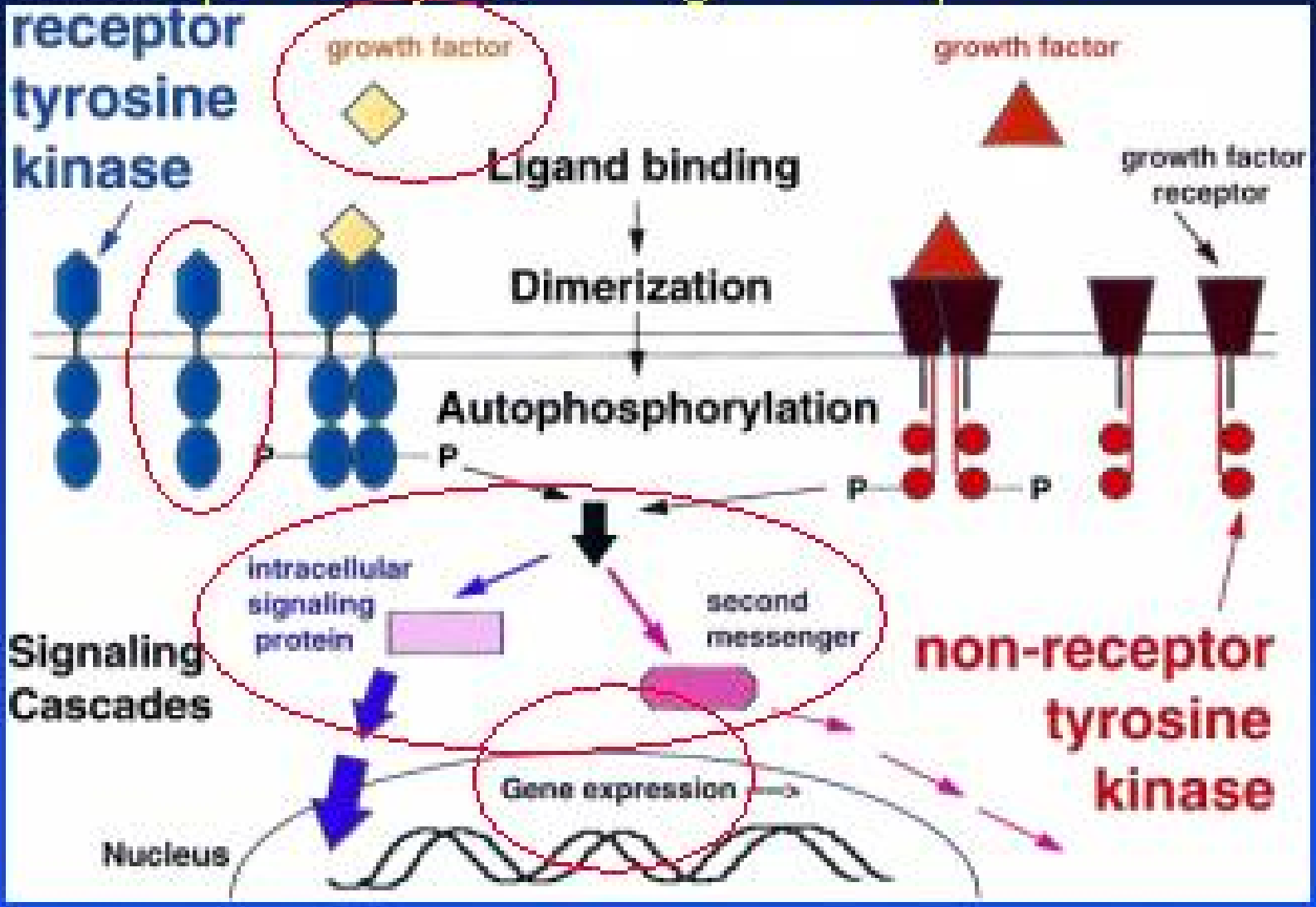
v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

RTKs



Hubbard and Till, Ann. Rev. Biochem. 69:373, 2000

Receptor Tyrosine Kinases: Determinants of Specificity of Biological Response



Kolibaba & Druker BBA 1333:F217, 1997

Growth Factor Receptors in Human Disease

ErbB-2/HER2/Neu in breast carcinoma.

EGFR truncations in glioblastoma mutliforme.

C-kit (PDGFR) in GIST (gastrointestinal sarcoma)

TPR-TRK fusion in papillary thyroid carcinomas

Translocated promoter region and TRK is Nerve Growth Factor Receptor (another RTK).
TPR-Met (RTK) found in gastric cancers.

Chimeric Growth Factor receptors in leukemias
NPM-ALK and TEL-PDGFR

Mechanism of action: Oncogenes as signal transducers

EXTRACELLULAR

Growth Factors

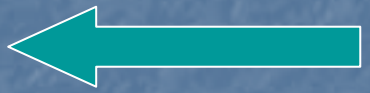
v-sis (PDGF), int-1(WNT-1), int-2(FGF), hst, fgf-5

Growth Factors Receptors

v-erb-B (EGFR), v-fms (CSF-1R), v-kit (KIT)

CYTOPLASM

Signal Transducers



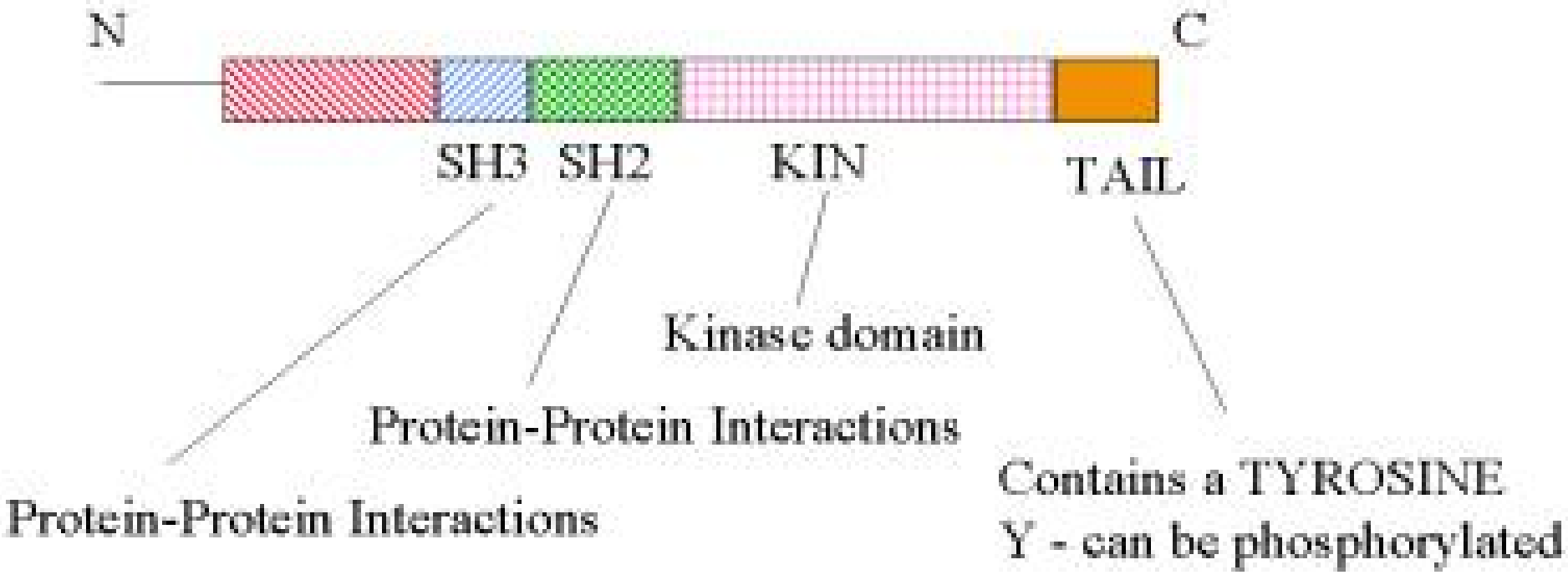
v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

NUCLEUS

Transcription Factors

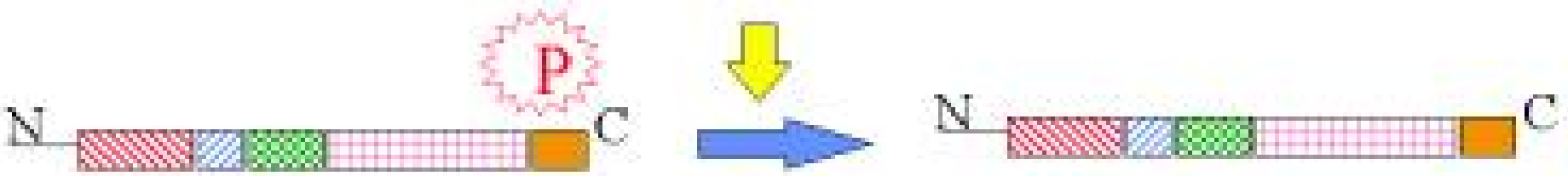
v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

Src Protein Structure



Src Activity is tightly Regulated

in vivo



Phosphorylation

Low kinase activity

Dephosphorylation

High kinase activity

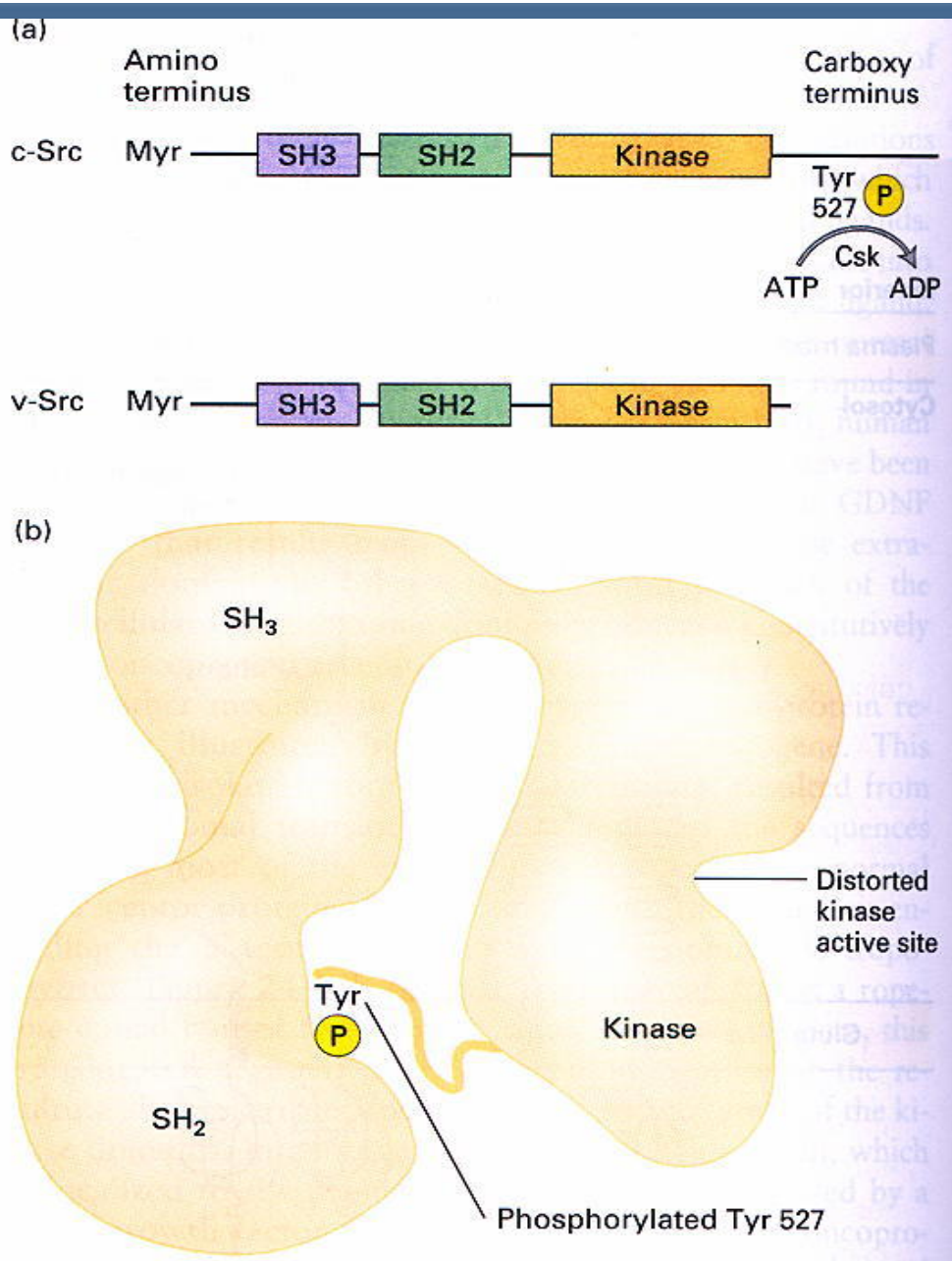
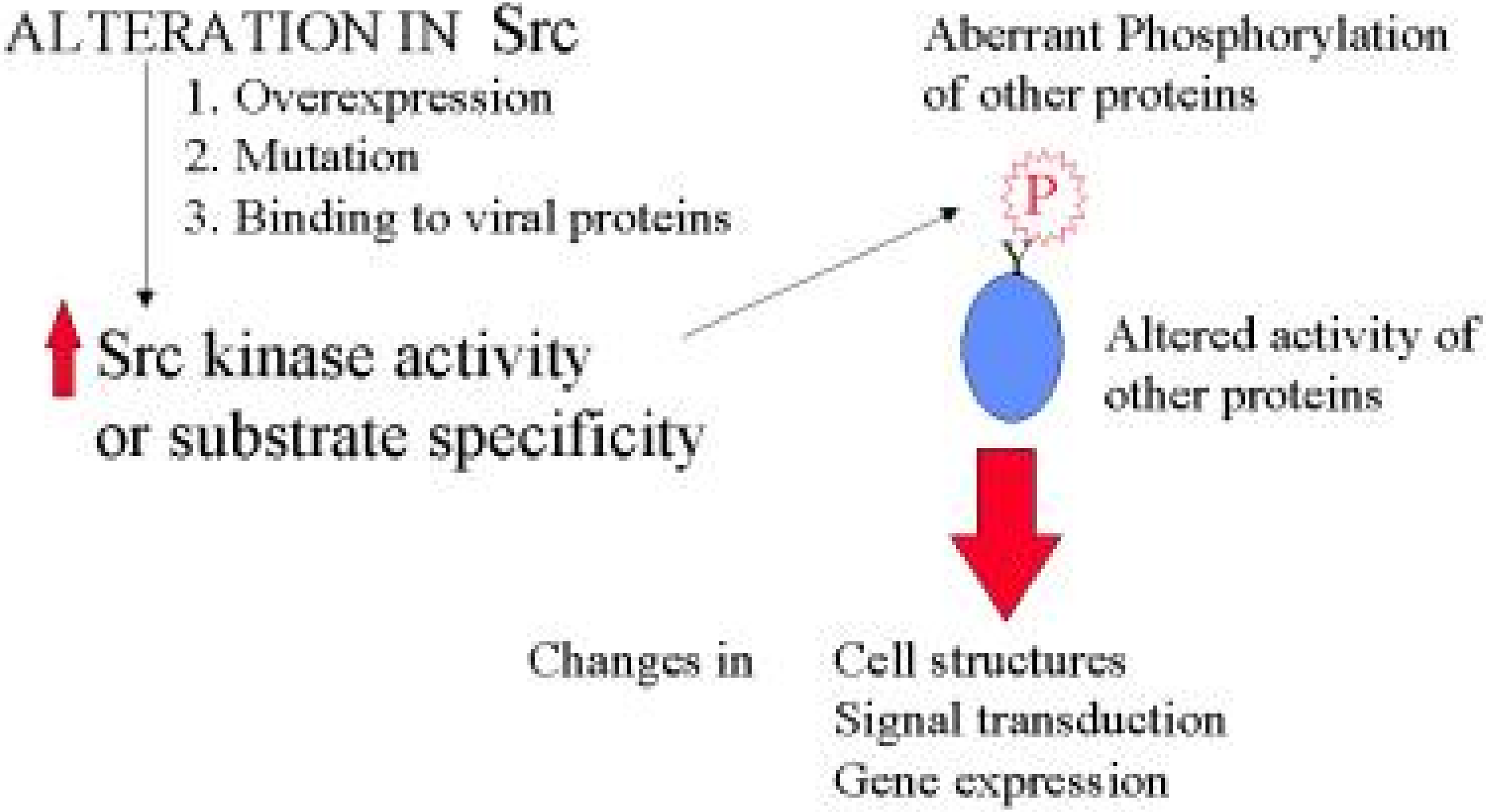


FIGURE 24-17 Regulation of Src activity and its activation by an oncogenic mutation. (a) Domain structure of c-Src and v-Src. Phosphorylation of tyrosine 527 by Csk, another cellular tyrosine kinase, inactivates the Src kinase activity. The transforming v-Src oncoprotein encoded by Rous sarcoma virus is missing the C-terminal 18 amino acids including tyrosine 527 and thus is constitutively active. (b) Effect of phosphorylation on c-Src conformation. Binding of phosphotyrosine 527 to the SH2 domain induces conformational strains in the SH3 and kinase domains, distorting the kinase active site so it is catalytically inactive. The kinase activity of c-Src is normally activated by removing the phosphate on tyrosine 527. [Adapted from T. Pawson, 1997, *Nature* **385**:582. See also W. Xu et al., 1997, *Nature* **385**:595; and F. Sichrei et al., 1997, *Nature* **385**:602.]

Src in Cancer

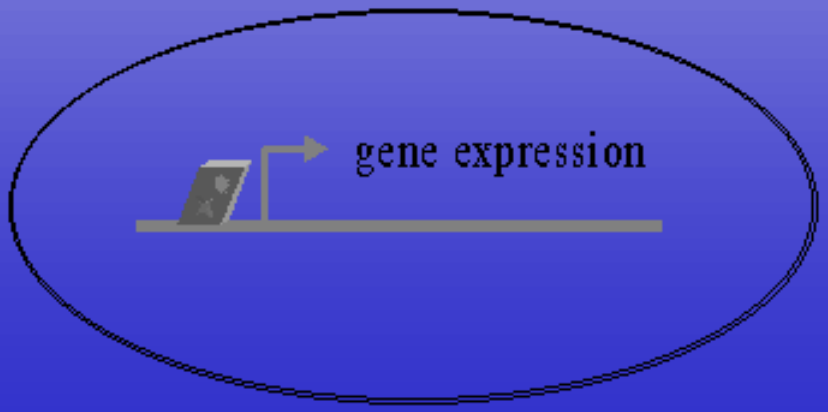


ras

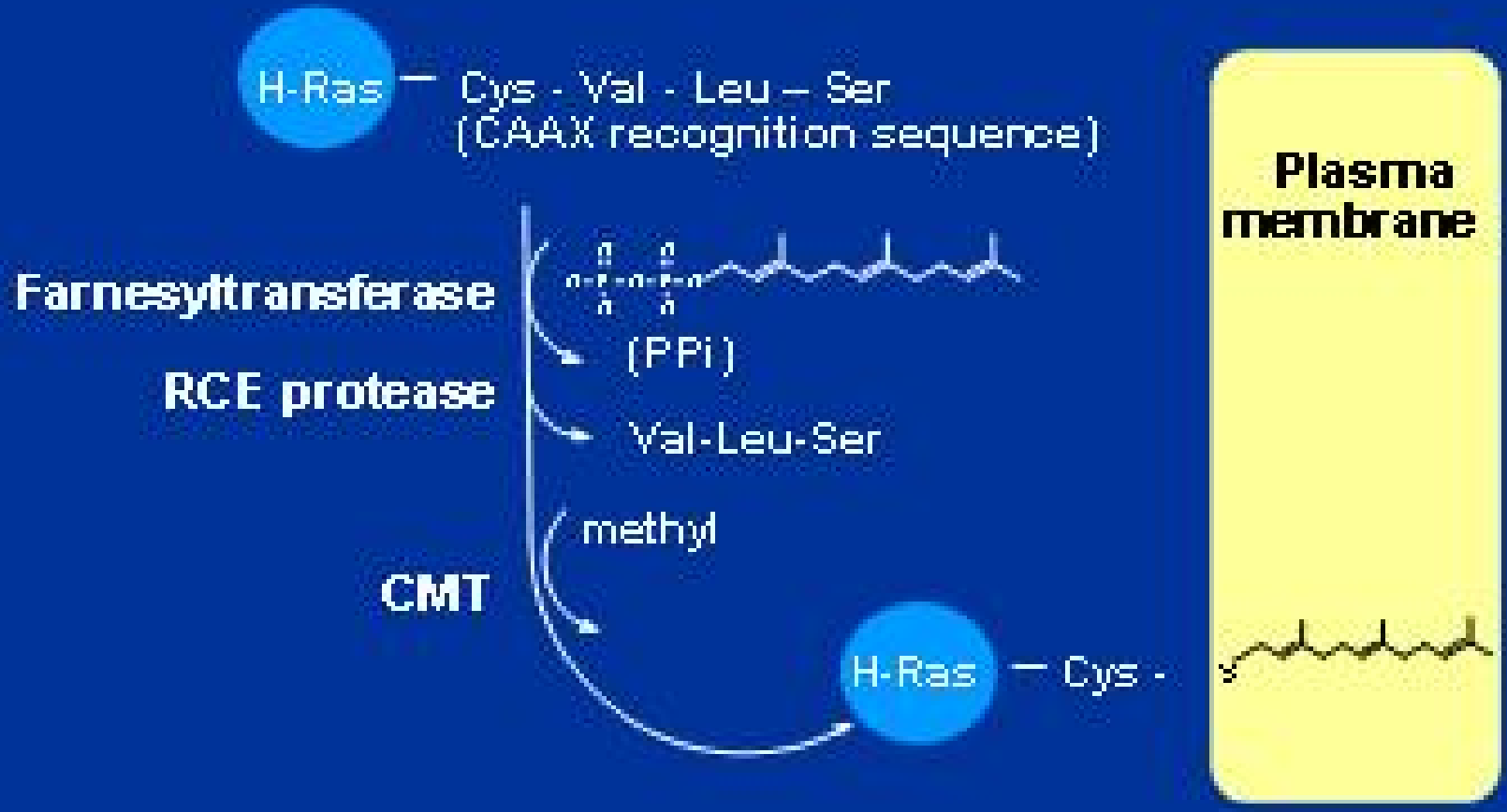
- *small GTP binding signaling molecule*
- **point mutation by viral transduction and chemical carcinogenesis**

- Various viral forms identified:
  - v-Ha-ras
  - v-Ki-ras
  - N-ras
- chemical carcinogenesis results in characteristic mutations at residues 12, 13, 59 and 61.

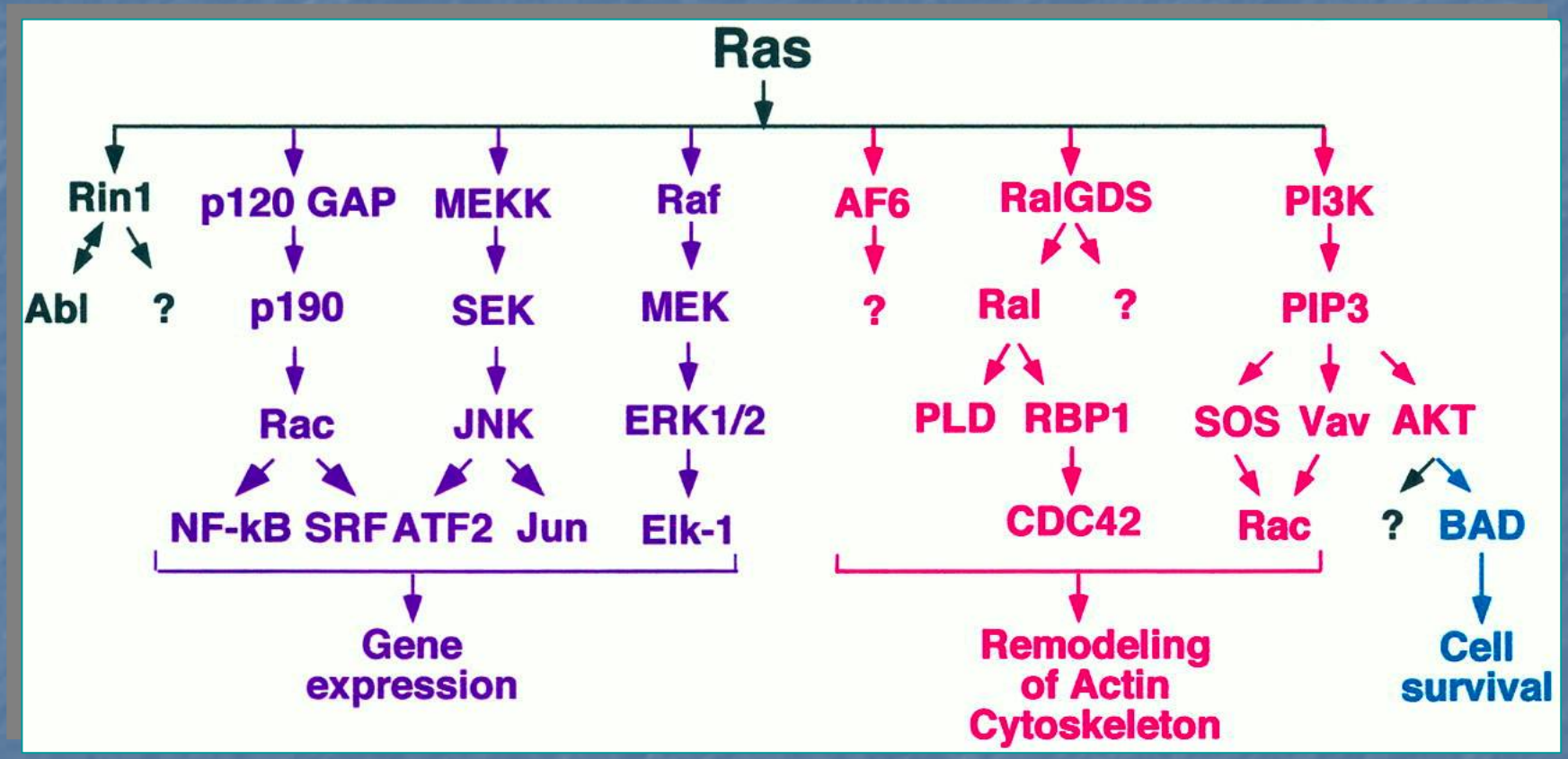
- Cellular ras is only active when GTP is bound. It cleaves GTP to GDP + Pi, switching itself off. These transitions are catalysed by accessory proteins:
  - guanine nucleotide exchange factors that cause the GDP -> GTP transition
  - GTPase activating proteins that cause the GTP->GDP transition
- **v-ras** or mutated cellular ras protein has lost the ability to interact with either accessory factors, and so are either
  - GEF independent, and so constitutively activated
  - or, GAP insensitive, and so remain in the GTP state



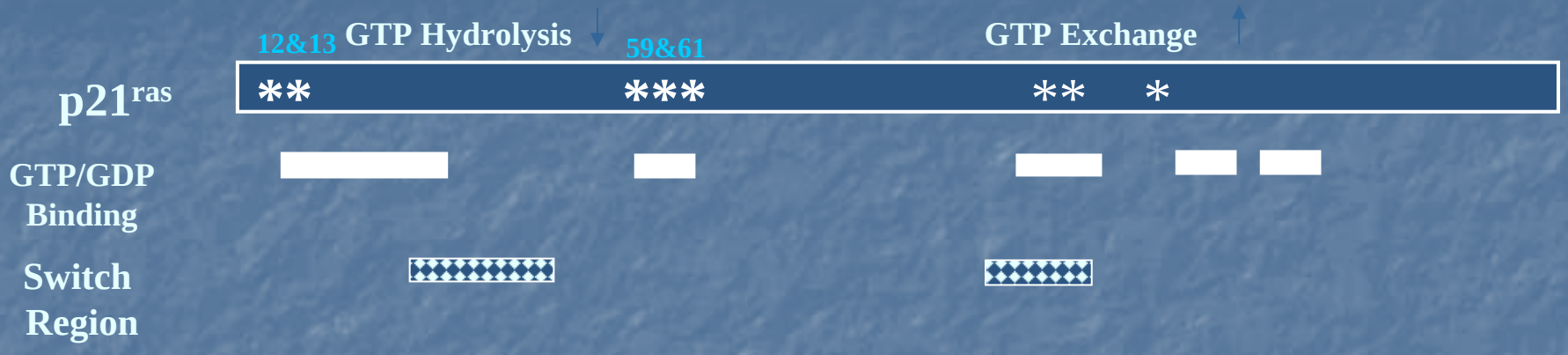
Ras processing and membrane association

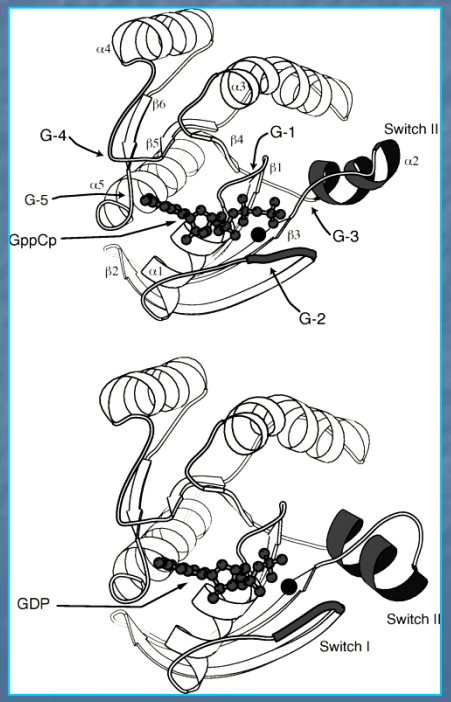


Ras Effectors



Oncogenes as Signal Transducers; Ras is altered in many human cancers





GTP

GDP

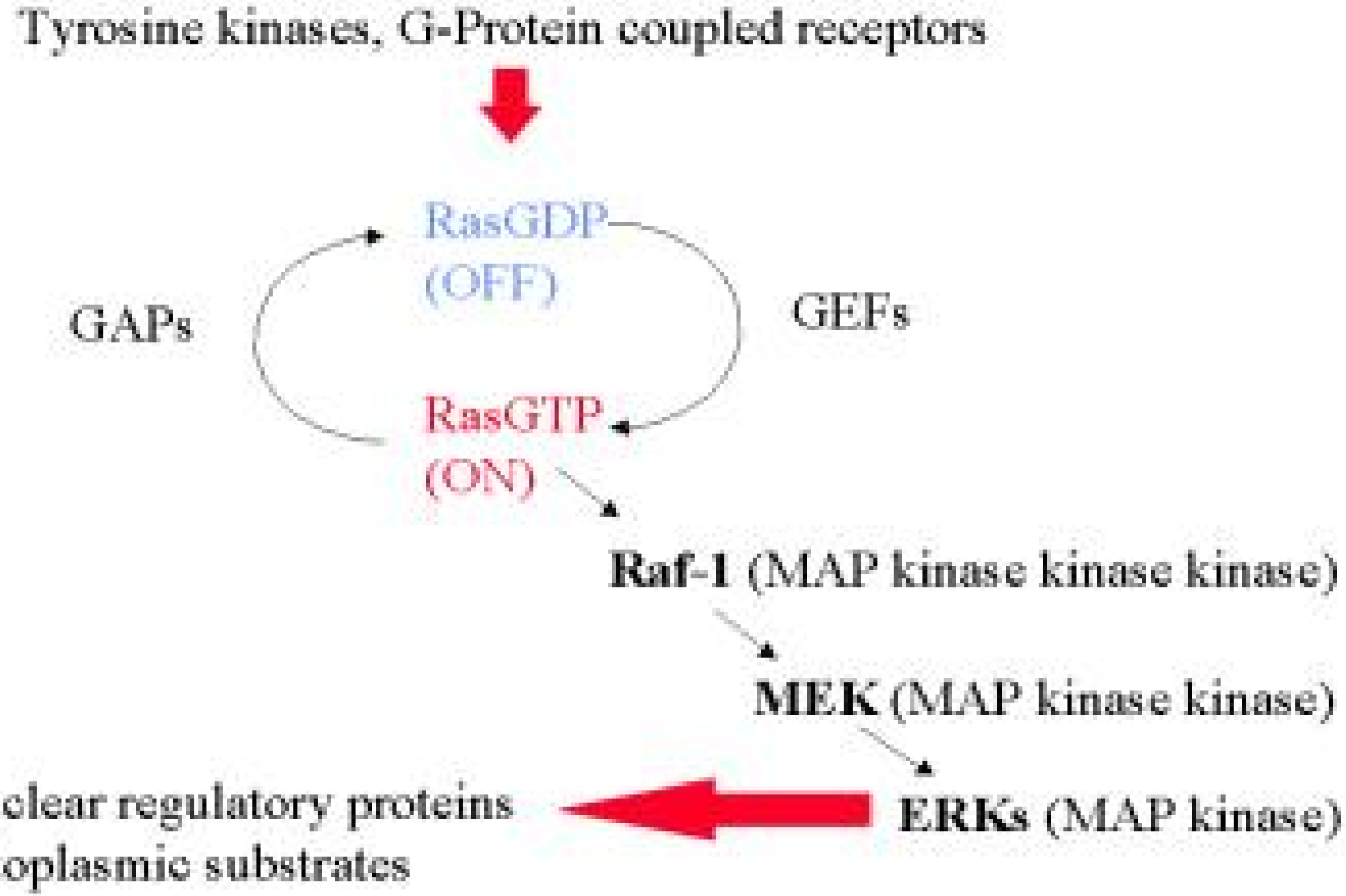
p21ras refers to three closely related proteins
H-Ras (Harvey),
K-Ras (Kirsten)
N-Ras (neuronal).

p21ras activating mutations lock Ras in a GTP-bound state.

Activated in 90% of pancreatic adenocarcinomas and 50% of colon adenocarcinomas and leukemias.

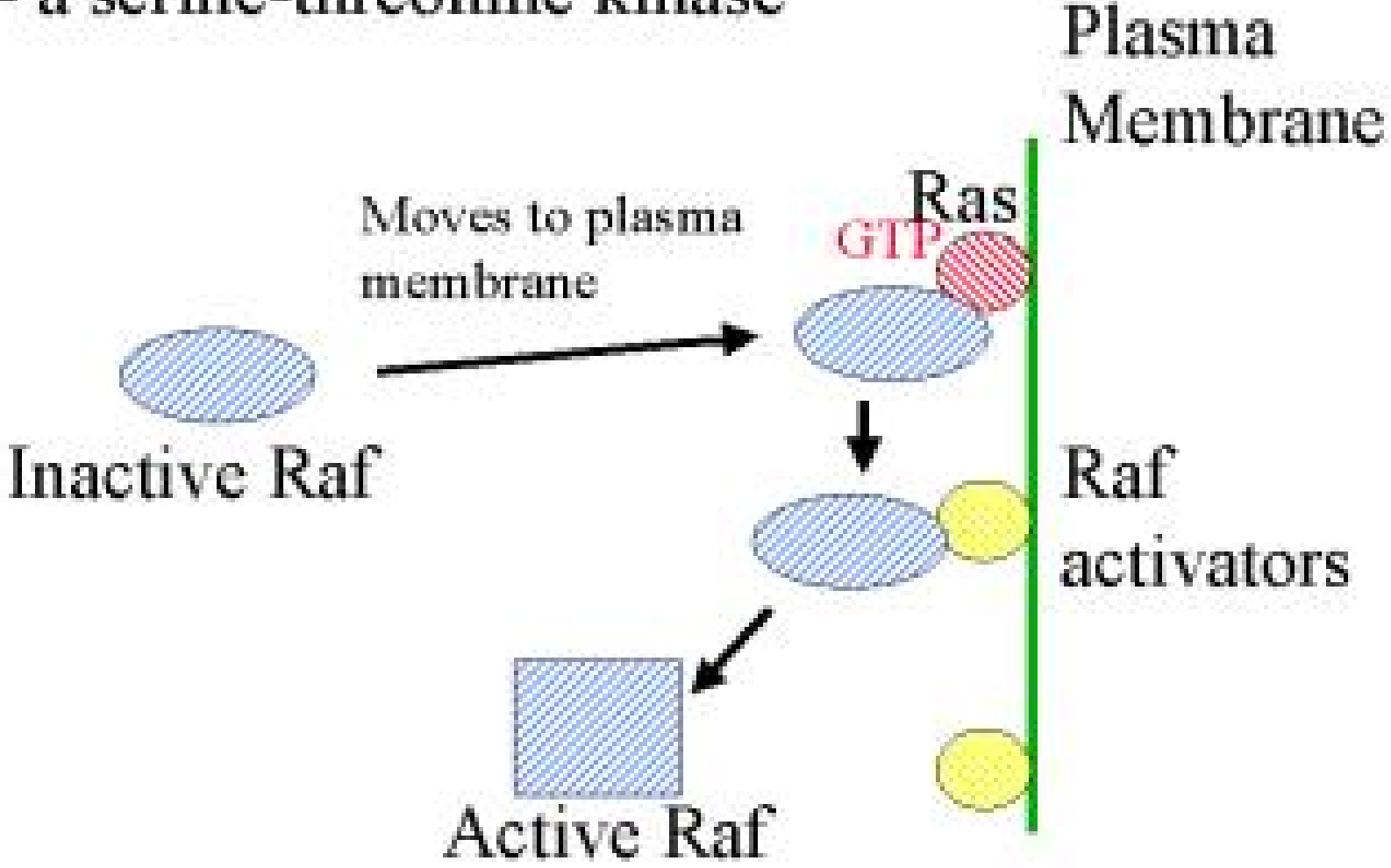
Structures from
Sprang S.R.,
Annu. Rev. Biochem 1997. 66:639-78

Ras Activation and the MAP Kinase Cascade



What does Ras interact with?

Raf - a serine-threonine kinase



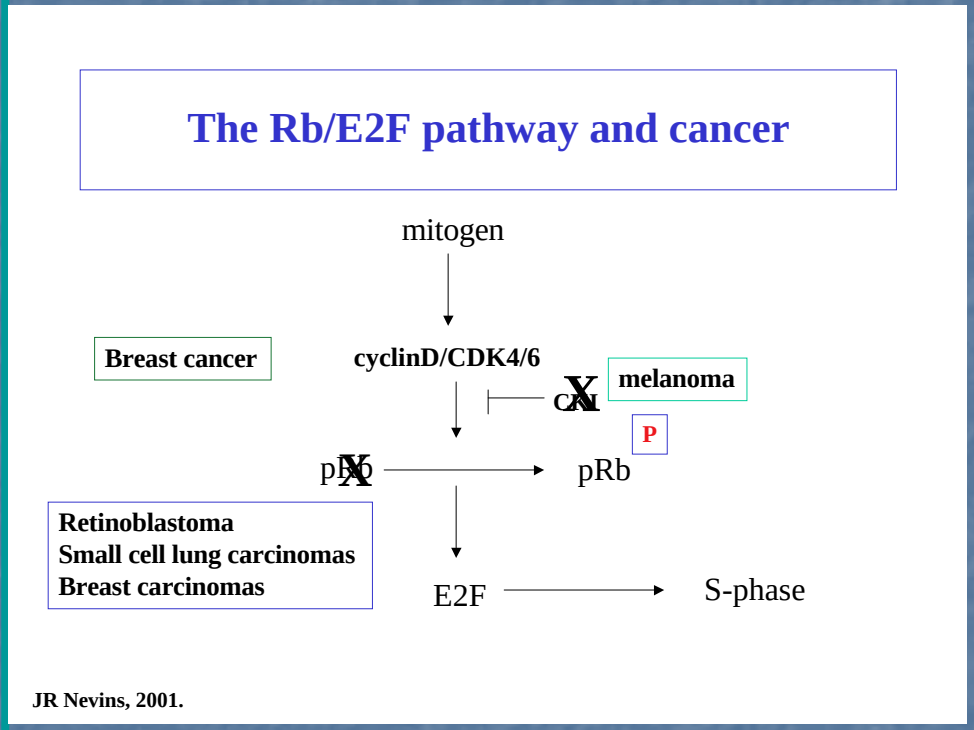
Oncogenes involved in cell cycle / the pRB pathway

Cyclin D1 was isolated as -

PRAD1
-target of t(11;11)
-benign parathyroid adenoma
-translocated to parathyroid hormone promoter.

BCL1- t(11;14)
centrocytic lymphomas
Ig heavy chain enhancer is inserted in BCL-1 loci.

Cyclin D1
Shown to be in amplicons containing hst and int-2
in breast carcinoma.



Mechanism of action: Oncogenes as signal transducers

EXTRACELLULAR

Growth Factors

v-sis (PDGF), int-1(WNT-1), int-2(FGF), hst, fgf-5

Growth Factors Receptors

v-erb-B (EGFR), v-fms (CSF-1R), v-kit (KIT)

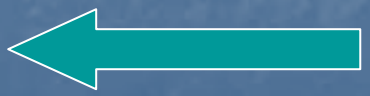
CYTOPLASM

Signal Transducers

v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

NUCLEUS

Transcription Factors



v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

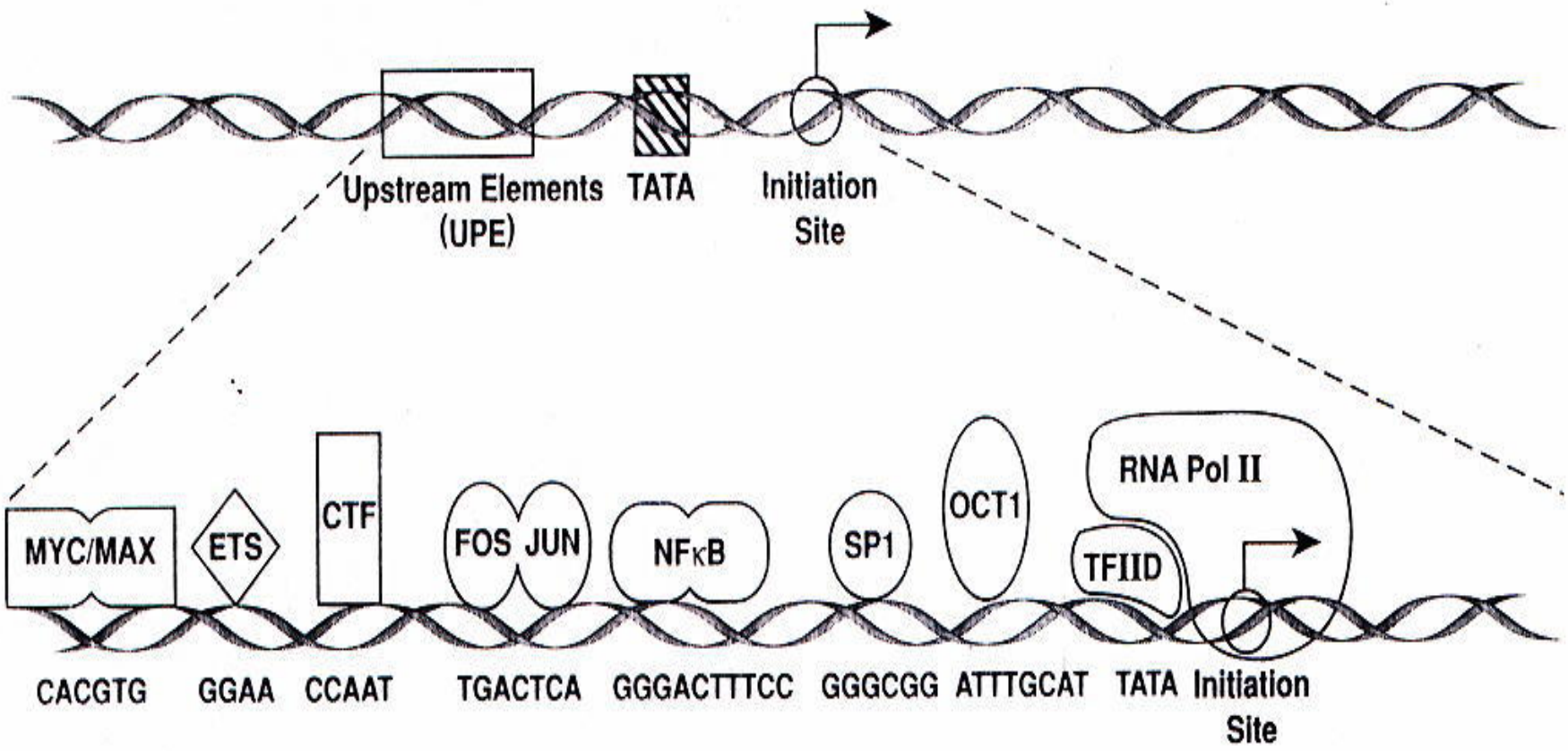
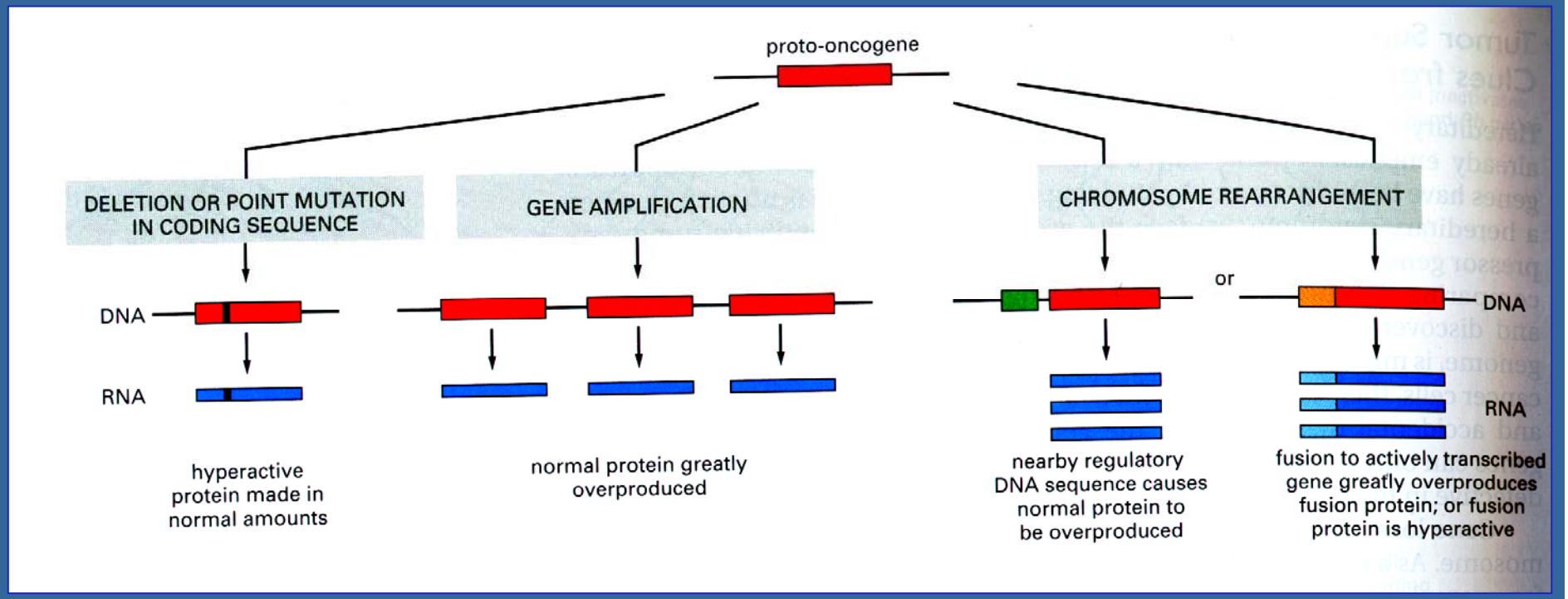


FIGURE 2–1. In the schematic of the transcriptional control region of a eukaryotic gene transcribed by RNA polymerase II, initiation sites (*arrows*), TATA sequences (*hatched boxes*), and upstream elements (*open boxes*) are shown. The transactivating factors that bind to particular DNA sequences are indicated symbolically. The upstream elements that are essential for transcriptional activation may contain binding sites for various factors, some of which are depicted. The diagram is somewhat speculative, and all of the binding sites shown here may not be present within the transcriptional control region of a single gene. During the transactivation process, factors may shift their positions to interact with other factors or with RNA POLII.

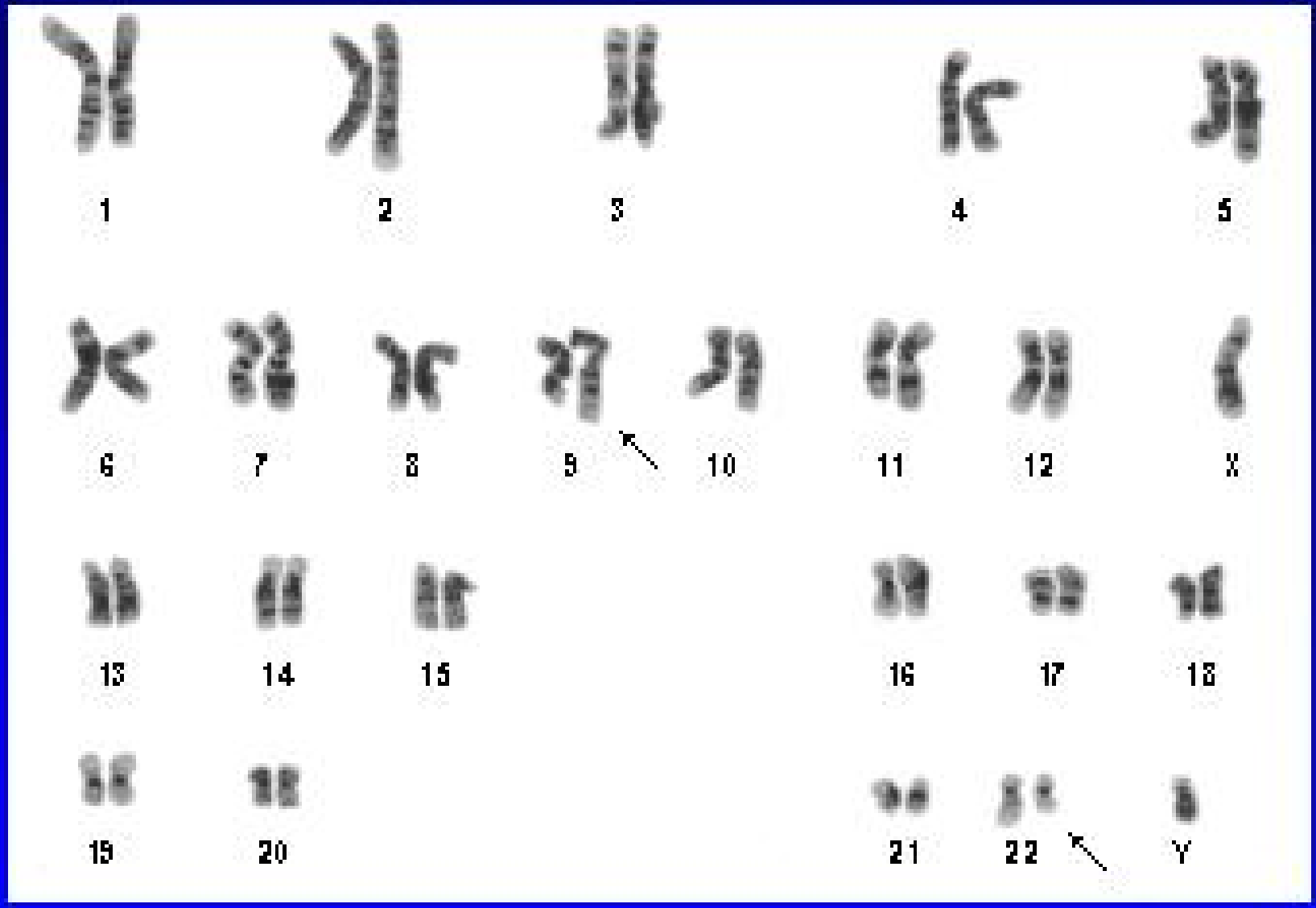
Three ways in which a proto-oncogene can be converted into an oncogene.



ALTERACIONES GENETICAS

TRANSLOCACION

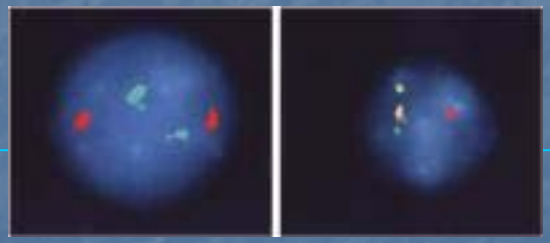
Karyotype Of A Cell With t(9;22) In Current Era



Identification of Oncogenes by mapping Chromosomal Rearrangements; description of the philadelphia chromosome

1960: Nowell and Hungerford showed novel chromosome in cells of CML patients. Later termed the Philadelphia chromosome (Ph^1).

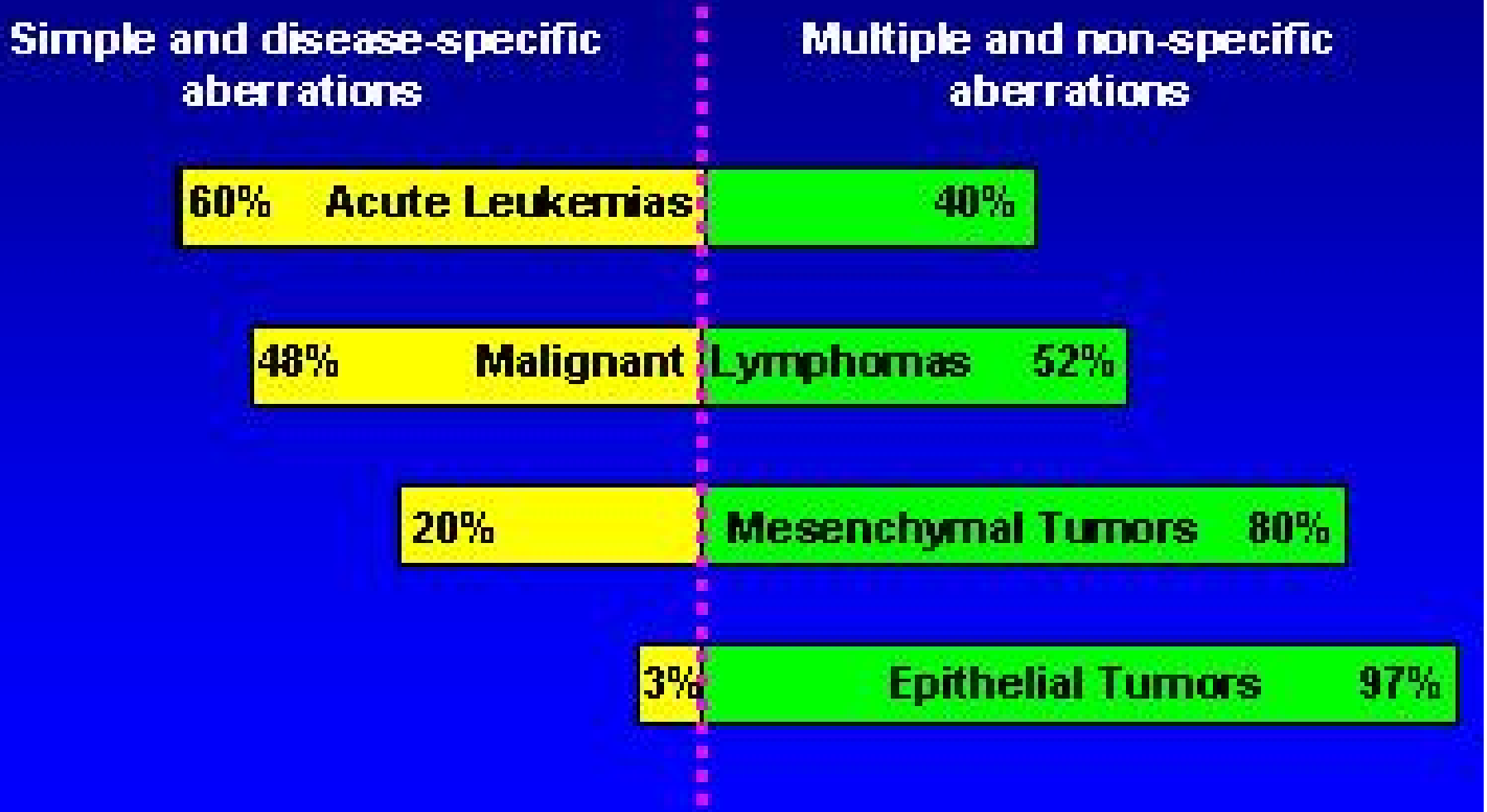
1973: Rowley identified the Ph^1 chromosome as a t(9:22).



ID of oncogenes + chomosomal mapping = ID of targets

(FISH) using unique-sequence double-fusion DNA probes for *BCR* (22q11.2) in red color and *c-abl* (9q34) gene regions in green. The abnormal *BCR/abl* fusion present in positive Philadelphia chromosome cells demonstrates the presence of yellow color (right panel) compared to control (left panel) (used with permission, copyright, Emmanuel C. Besa, MD).

Karyotypic Patterns in Various Neoplasms



Chromosomal rearrangements or translocations

Neoplasm	Translocation	Proto-oncogene
Burkitt lymphoma	t(8;14) 80% of cases t(8;22) 15% of cases t(2;8) 5% of cases	c-myc[1]
Chronic myelogenous leukemia	t(9;22) 90-95% of cases	bcr-abl[2]
Acute lymphocytic leukemia	t(9;22) 10-15% of cases	bcr-abl[2]

[1]c-myc is translocated to the IgG locus, which results in its activated expression
[2]bcr-abl fusion protein is produced, which results in a constitutively active abl kinase

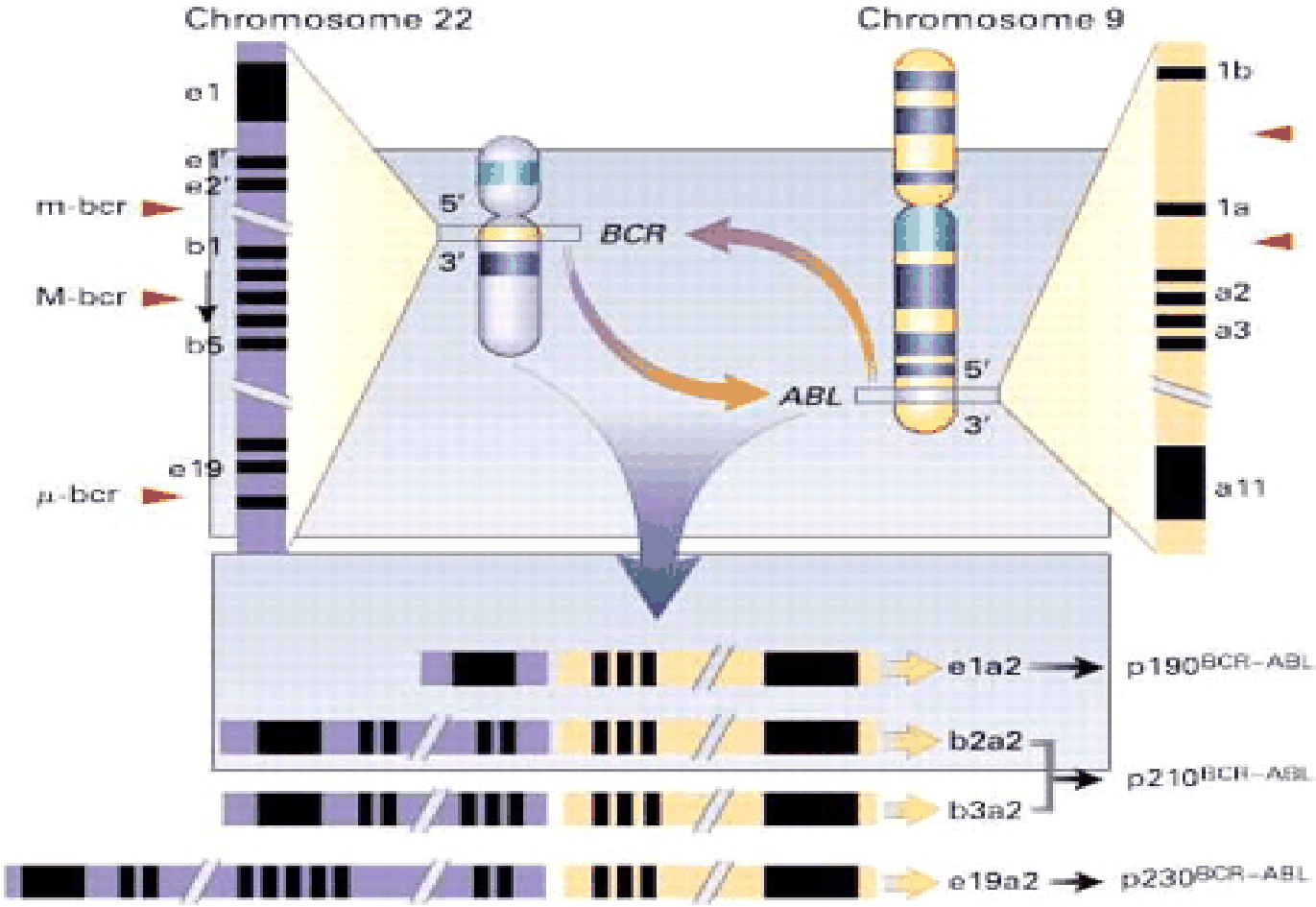


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Figure 1. The Translocation of t(9;22)(q34;q11) in CML. The Philadelphia (Ph) chromosome is a shortened chromosome 22 that results from the translocation of 3' (toward the telomere) *ABL* segments on chromosome 9 to 5' *BCR* segments on chromosome 22. Breakpoints (arrowheads) on the *ABL* gene are located 5' (toward the centromere) of exon a2 in most cases. Various breakpoint locations have been identified along the *BCR* gene on chromosome 22. Depending on which breakpoints are involved, different-sized segments from *BCR* are fused with the 3' sequences of the *ABL* gene. This results in fusion messenger RNA molecules (e1a2, b2a2, b3a2, and e19a2) of different lengths that are transcribed into chimeric protein products (p190, p210, and p230) with variable molecular weights and presumably variable function. The abbreviation m-bcr denotes minor breakpoint cluster region, M-bcr major breakpoint cluster region, and μ-bcr a third breakpoint location in the *BCR* gene that is downstream from the M-bcr region between exons e19 and e20.

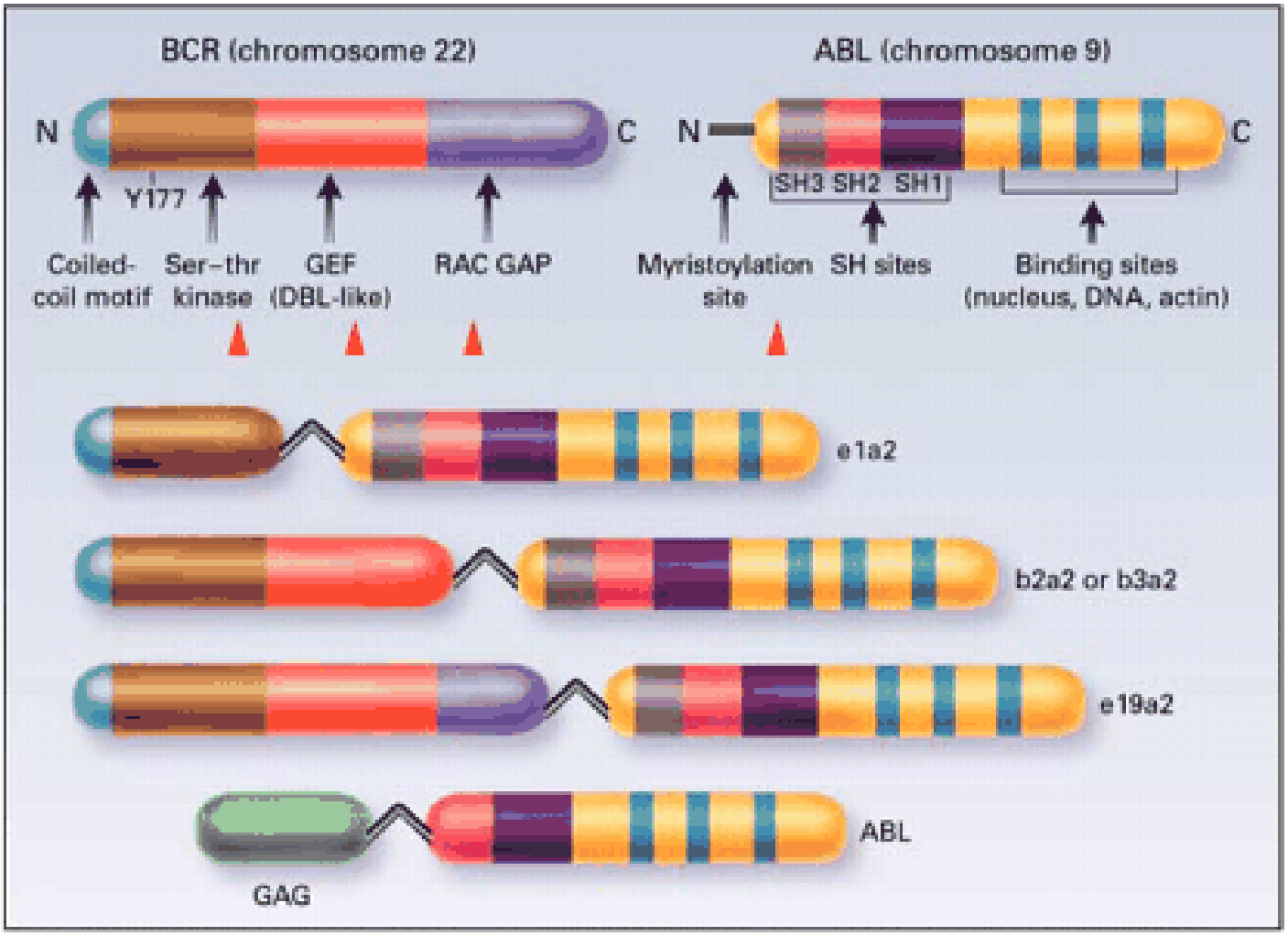

Figure 2. Functional Domains of $p160^{BCR}$, $p145^{ABL}$, and $p210^{BCR–ABL}$.
Important functional domains of the *BCR* and *ABL* gene products as well as of the different fusion-protein products are shown. Breakpoints are indicated by arrowheads (see Table 2 and the text for details). N denotes N-terminal amino acid sequence, C C-terminal amino acid sequence, Ser–thr serine–threonine, GDP guanosine diphosphate, GTP guanosine triphosphate, GEF GDP–GTP exchange factor, DBL diffuse B-cell lymphoma oncogene, RAC a RAS-like GTPase, GAP guanosine triphosphatase–activating function, and SH *SRC* homology domain.

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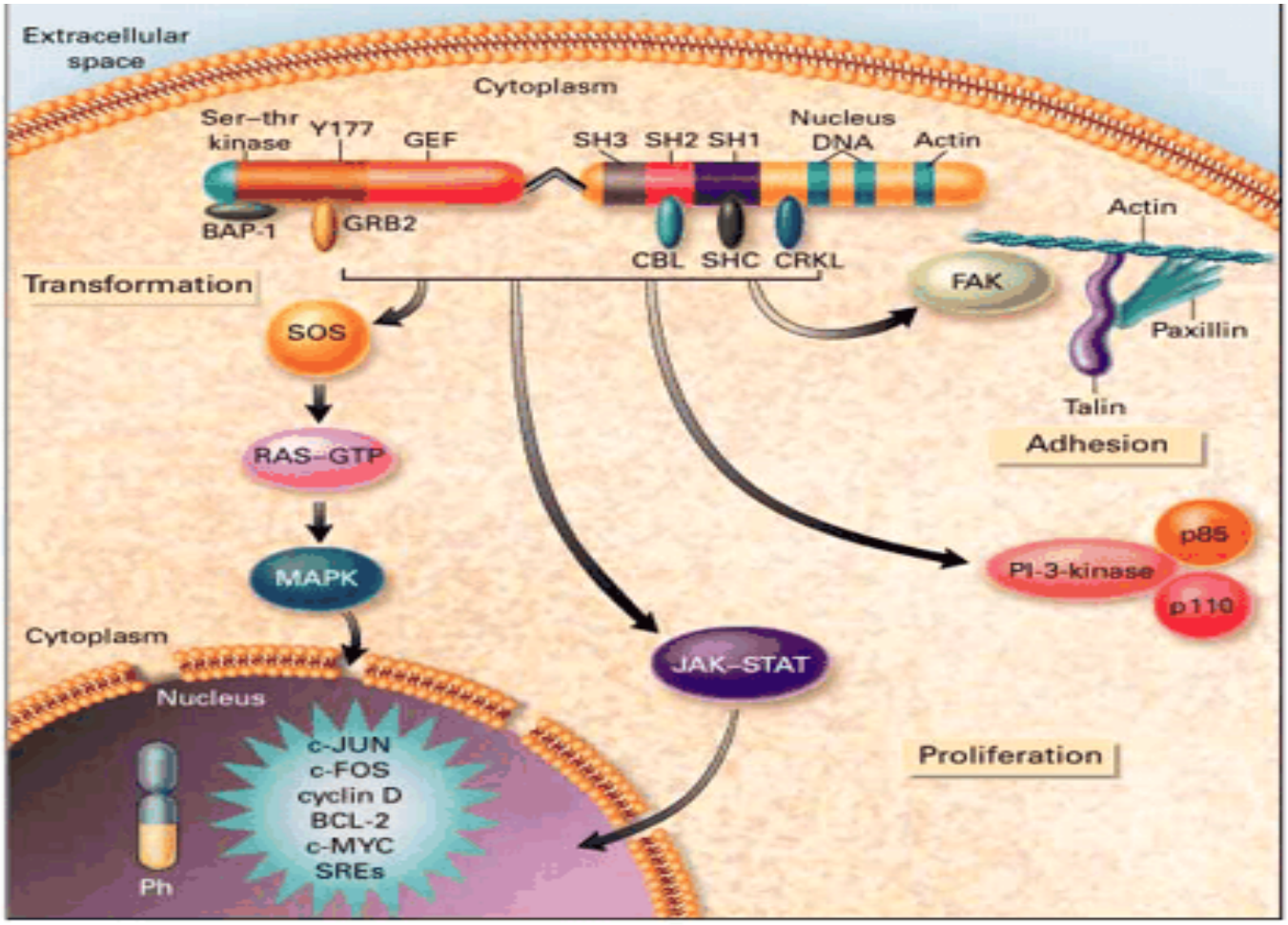


Figure 3. Signaling Pathways of $p210^{BCR–ABL}$. Several regions of BCR–ABL serve as important control elements for RAS, which is at the center of the most prominent signaling pathways in CML (see Fig. 2 and Table 2). Activation of RAS is mediated through a series of adapter proteins, such as GRB2, CBL, SHC, and CRKL. Adapter proteins also connect $p210^{BCR–ABL}$ to focal adhesion complexes, PI-3 kinase, and other messenger systems such as JAK–STAT kinases. Signaling events downstream of RAS are less well characterized. They appear to involve mainly mitogen-activated protein kinases (MAPKs), preferably the JUN kinase (JNK) pathway. BAP-1 denotes BCR-associated protein 1, GRB2 growth factor receptor–bound protein 2, CBL casitas B-lineage lymphoma protein, SHC SRC homology 2–containing protein, CRKL CRK-oncogene–like protein, JAK–STAT Janus kinase–signal transducers and activators of transcription, FAK focal adhesion kinase, SOS son-of-sevenless, GDP guanosine diphosphate, GTP guanosine triphosphate, SRE stimulated response element, Ser–thr serine–threonine, Y177 a conserved tyrosine residue, GEF GDP–GTP exchange factor, and SH *SRC* homology domain.

Oncogenes and Signal Transduction: Transcription Factors-Myc

c-Myc plays a role in many human cancers; over-expression.

Translocations: c-myc and Ig genes

- -Burkitt´s Lymphoma
- -Low-grade follicular lymphomas (sometimes with BCL-2)
- -Diffuse large cell lymphomas

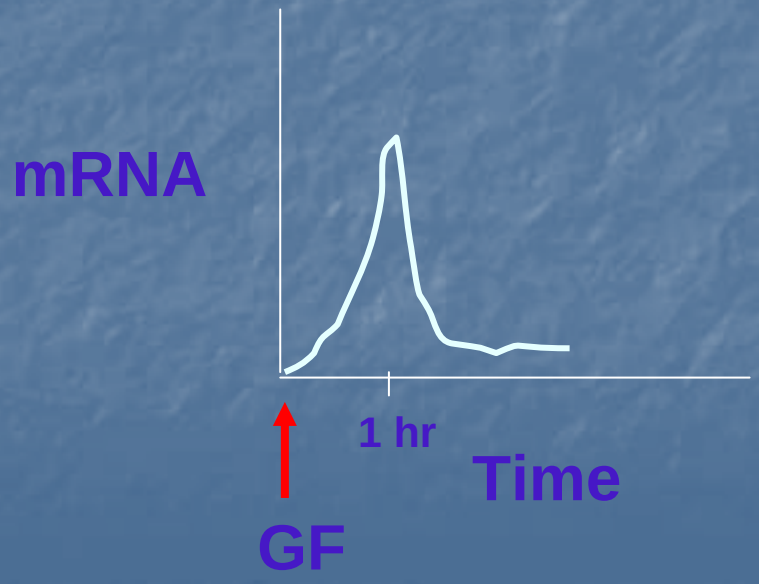
Amplifications of c-myc

- -Breast carcinoma
- -neuroblastoma (involves the related N-myc gene)
- -Small cell lung cancer (involves the related L-myc gene)

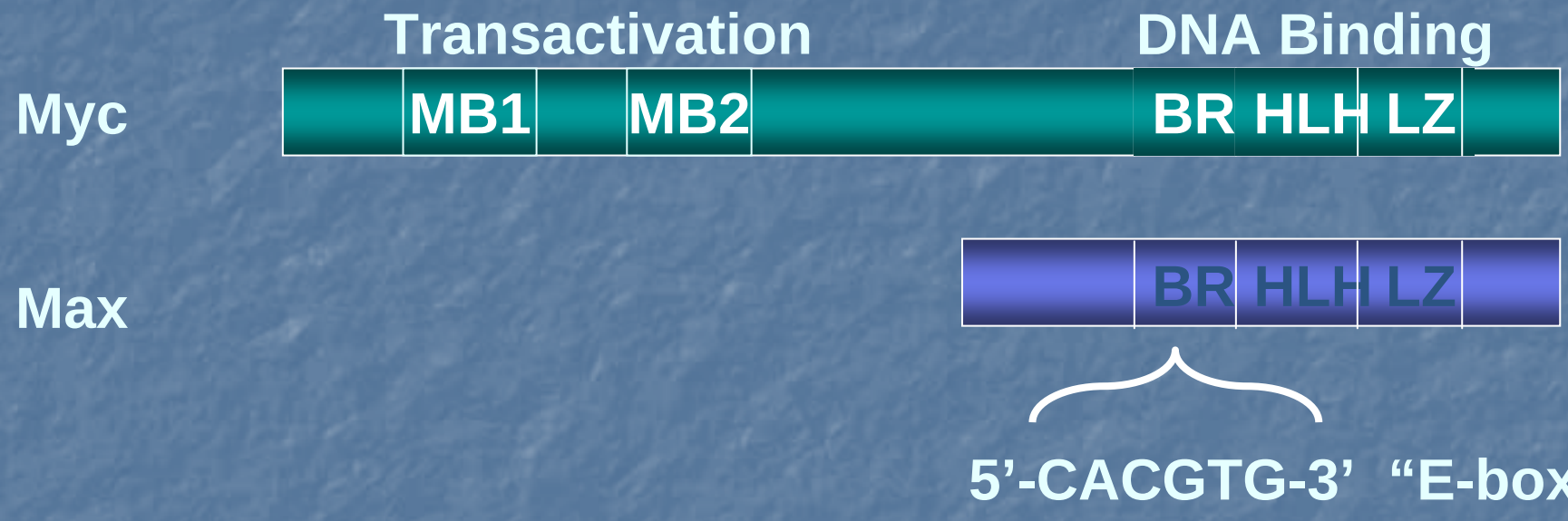
c-Myc is an early response gene (Growth Factor Regulated)

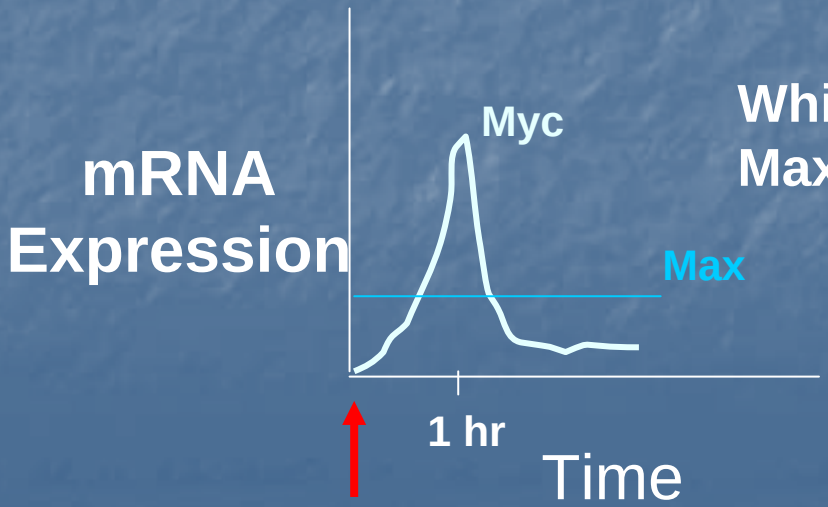
Myc protein has very short half-life <30 min.

Transcription regulates Myc protein levels



Myc has a partner called Max





While Myc is GF inducible,
Max is constitutively expressed

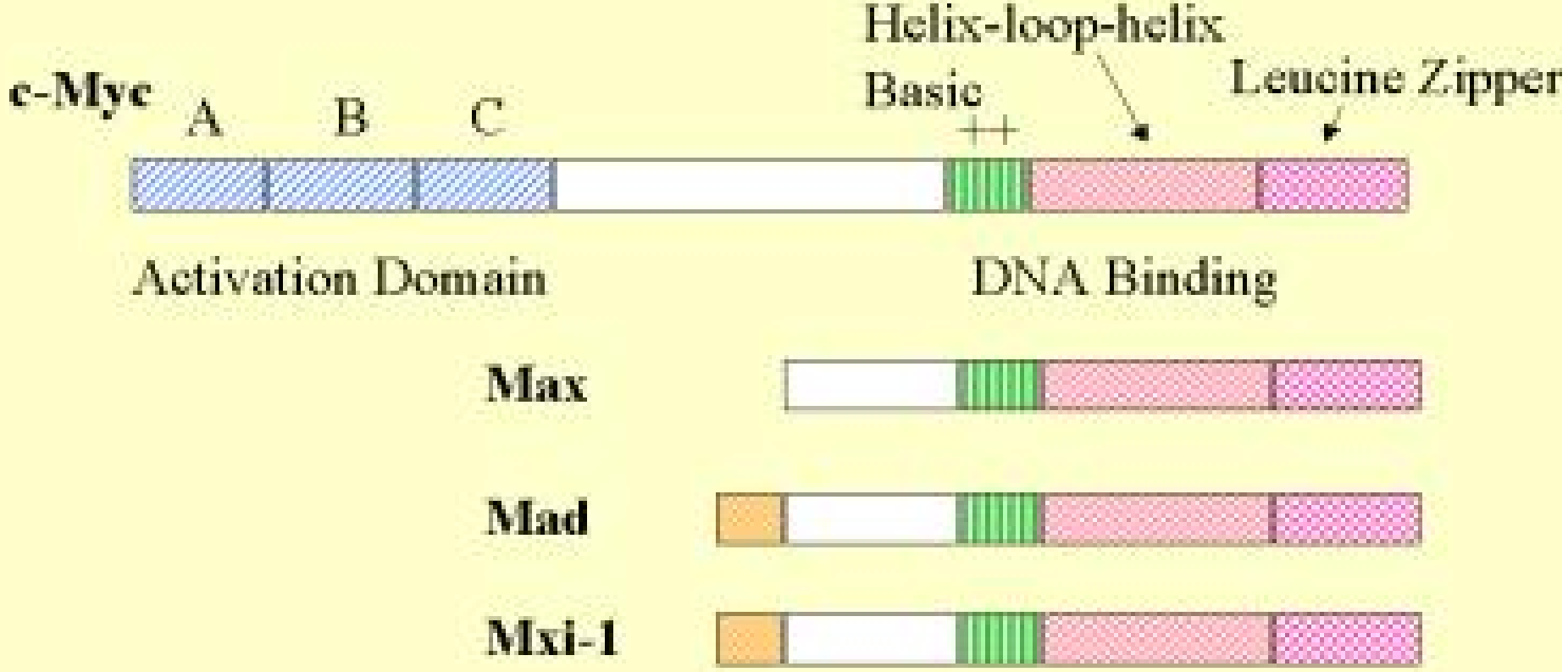
What does Myc Bind to?

- The E-Box - a sequence in DNA:

CACGTG

- Found upstream of Myc target genes

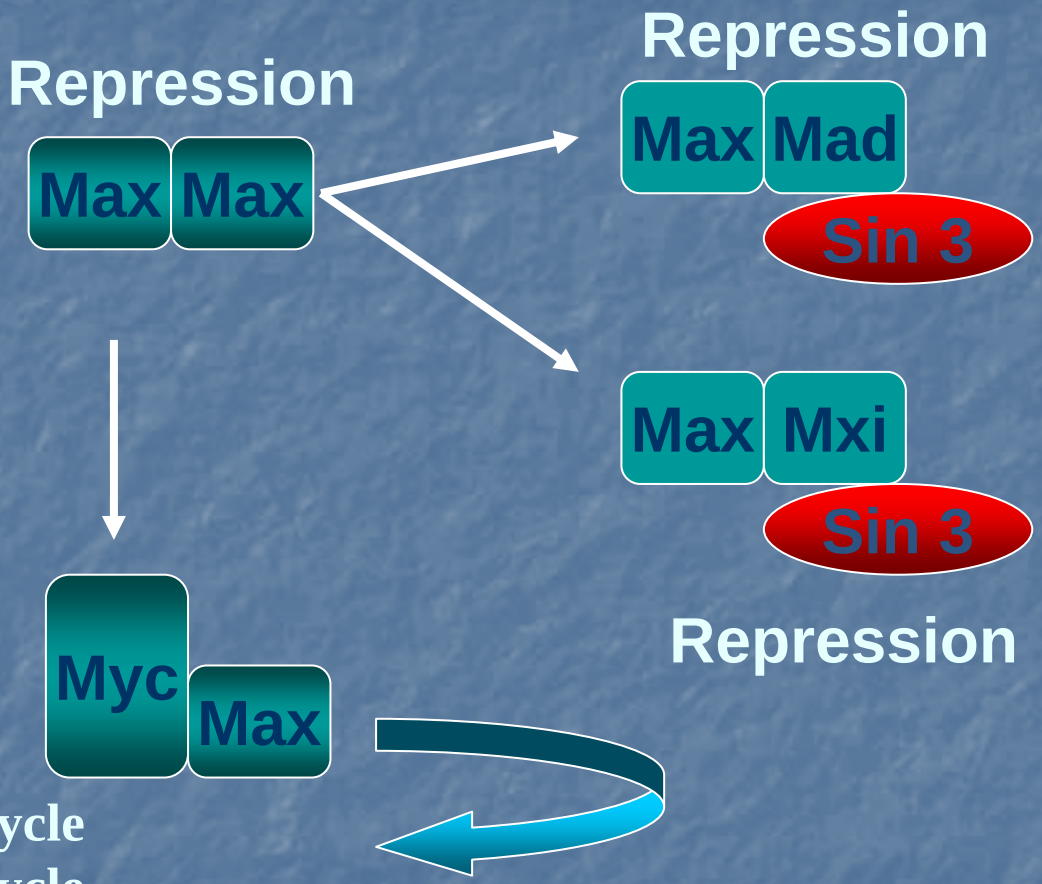
Structure of Myc and Associates



Mechanism of action: Dimerization Regulates Myc

Growth factors induce c-Myc expression leading to target gene activation.

Over-expression or amplification mimics growth factor.



Activation of target genes:

Cdc25A	Cell cycle
Cyclin D1	Cell cycle
ODC	Polyamine biosynthesis
Cyclin A	Cell cycle
Cyclin E	Cell cycle

How does this go wrong in Cancer?

Myc expression is increased 

More Myc-Max heterodimers

than Max-Max homodimers

or Mad-Max heterodimers

Hence INCREASED Expression of Cdc25A

Myc's Associates

- Myc dimerizes with Max - another transcription factor

Myc Max

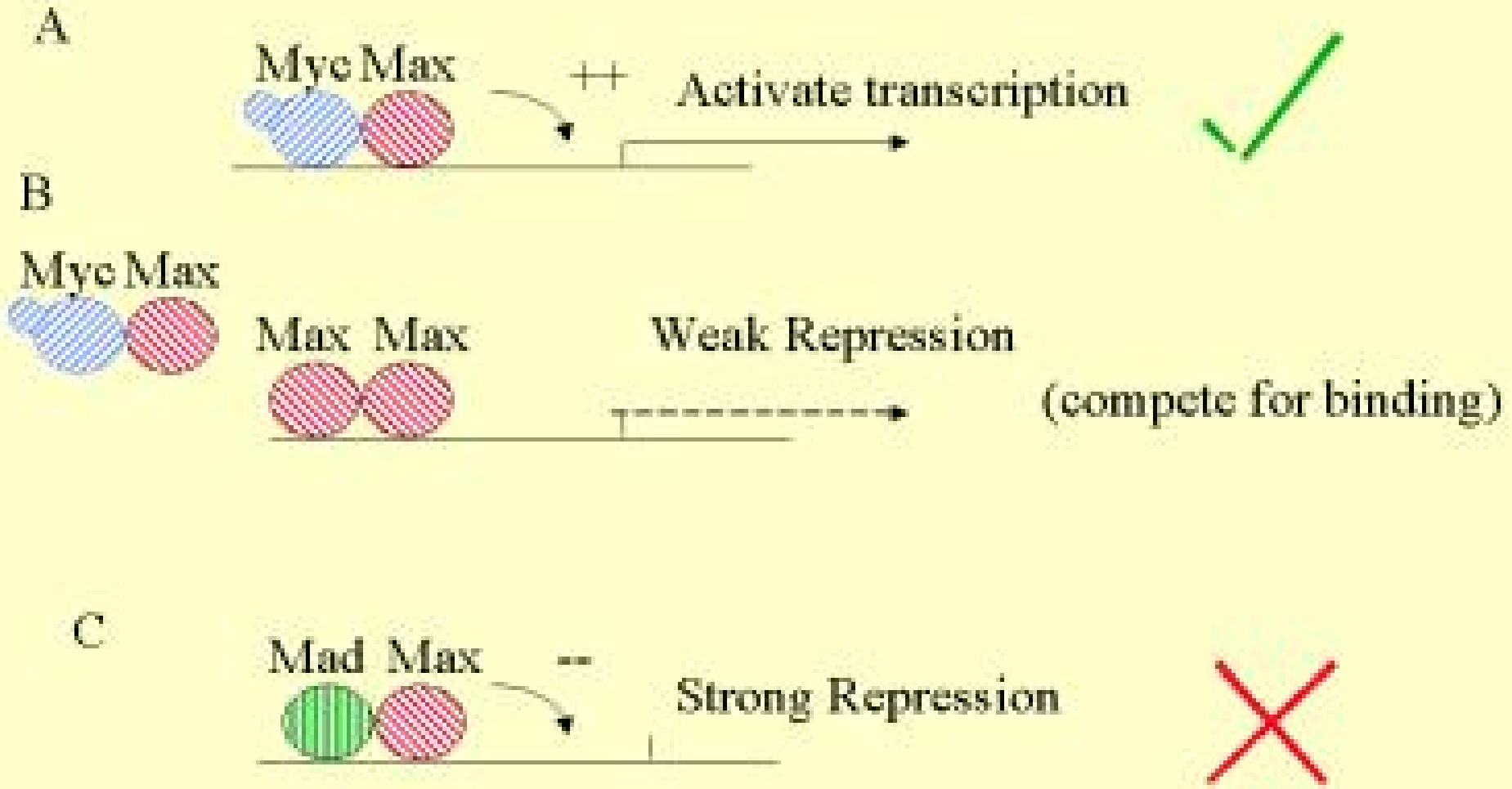
- Max can dimerize with Mad and Mxi1

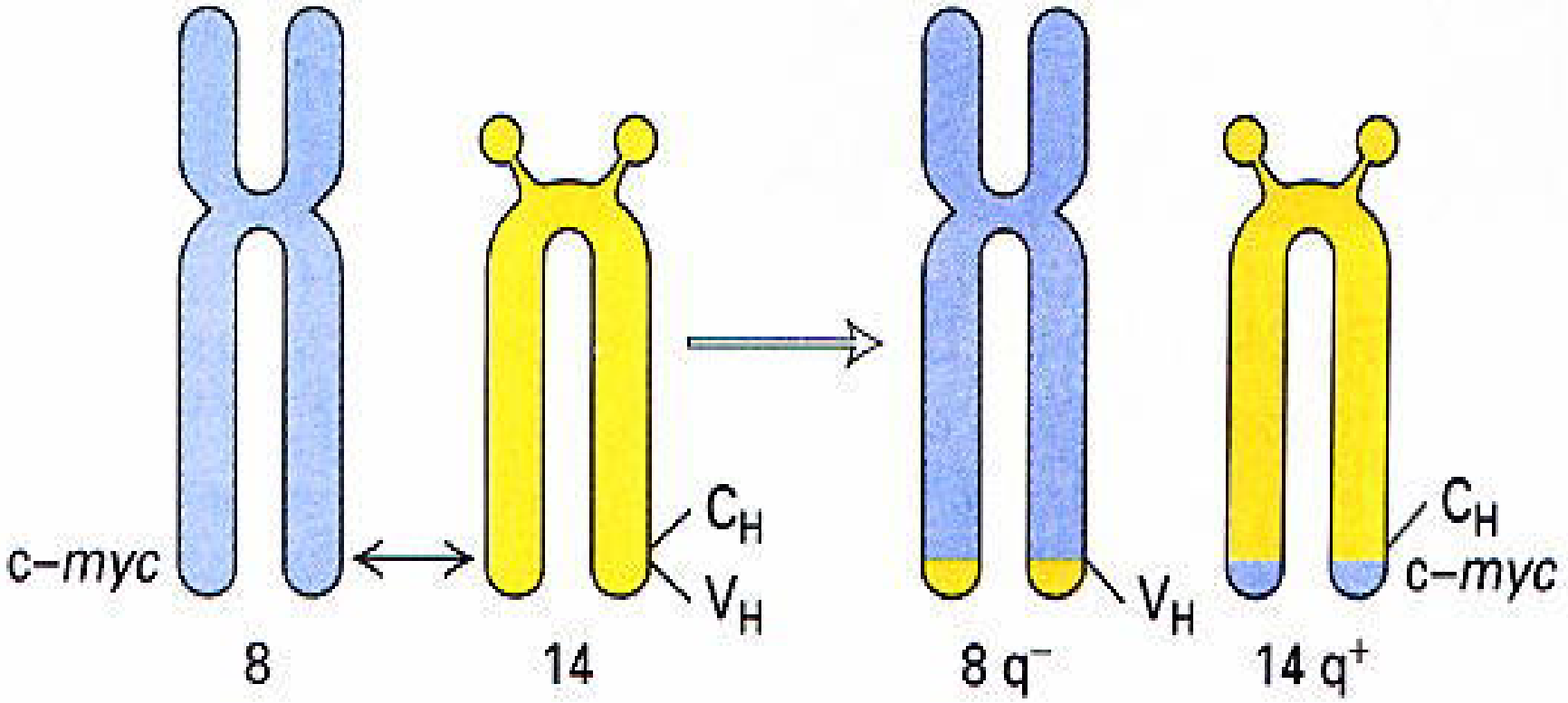
Mad Max

Mxi1 Max

BUT Mad and Mxi1 CANNOT dimerize with Myc!

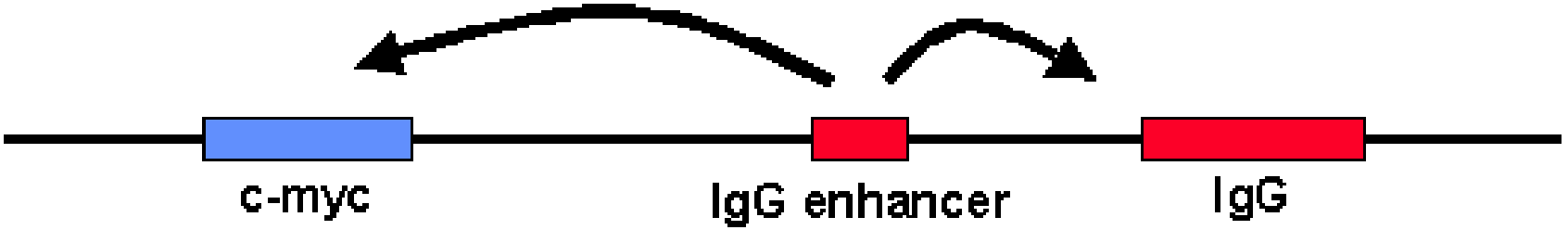
Regulation of Transcription





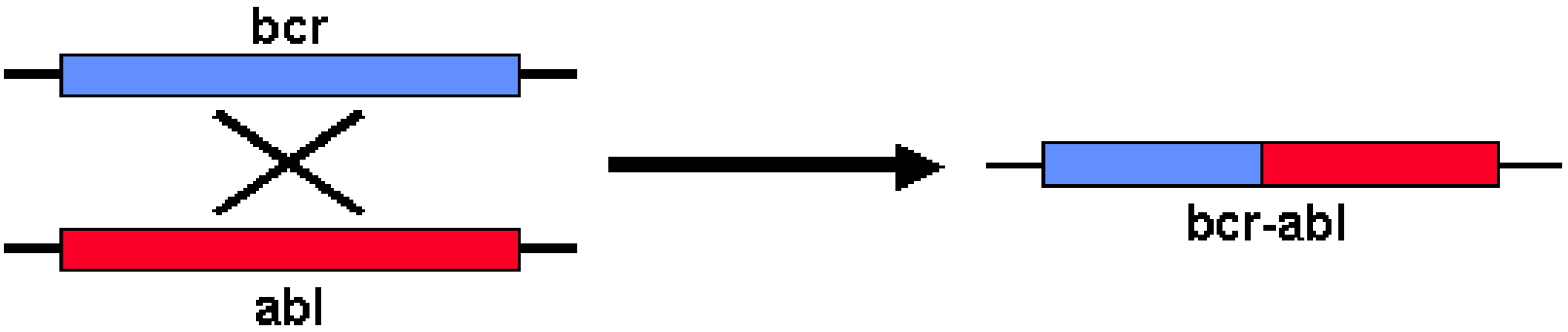
▲ **FIGURE 24-22 Chromosomal translocation in Burkitt's lymphoma.** This leads to overexpression of the Myc transcription factor.

c-myc is translocated to the IgG locus, which results in its activated expression



c-myc is activated by the IgG enhancer in lymphocytes

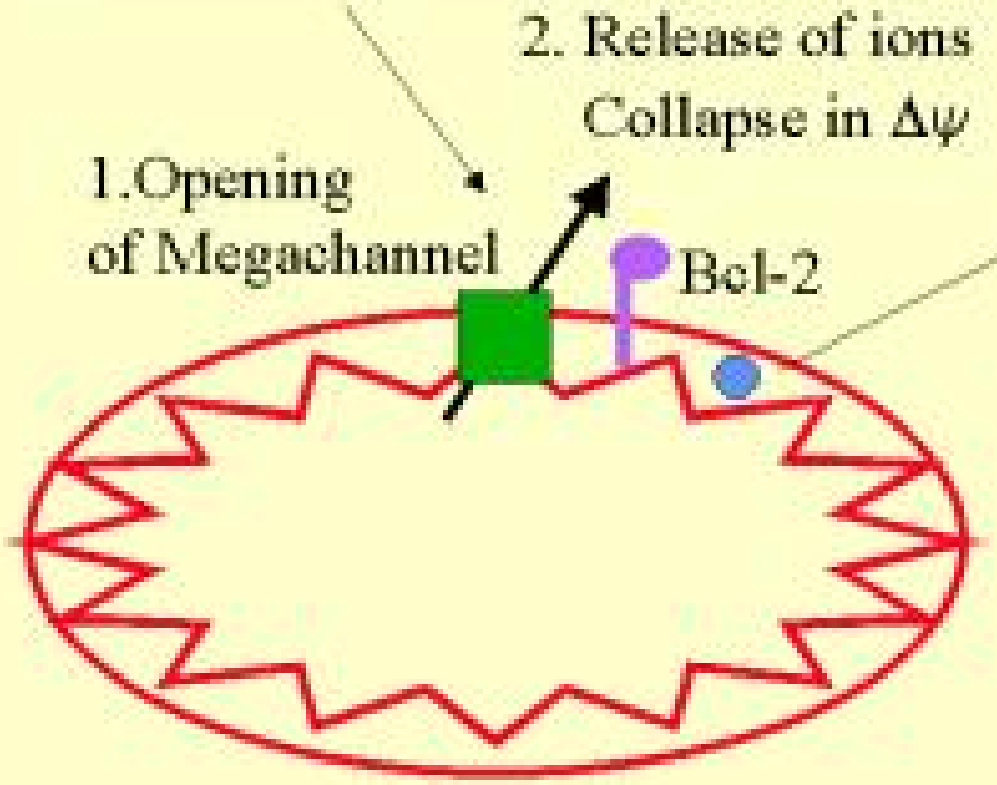
bcr-abl fusion protein is produced, which results in a constitutively active abl kinase



Events in Apoptosis

DAMAGE

Bcl-2 can prevent the release of ions



3. Release of
cytochrome C, Apaf-1

Activation of caspases
(cysteine proteases)
Degradation of proteins

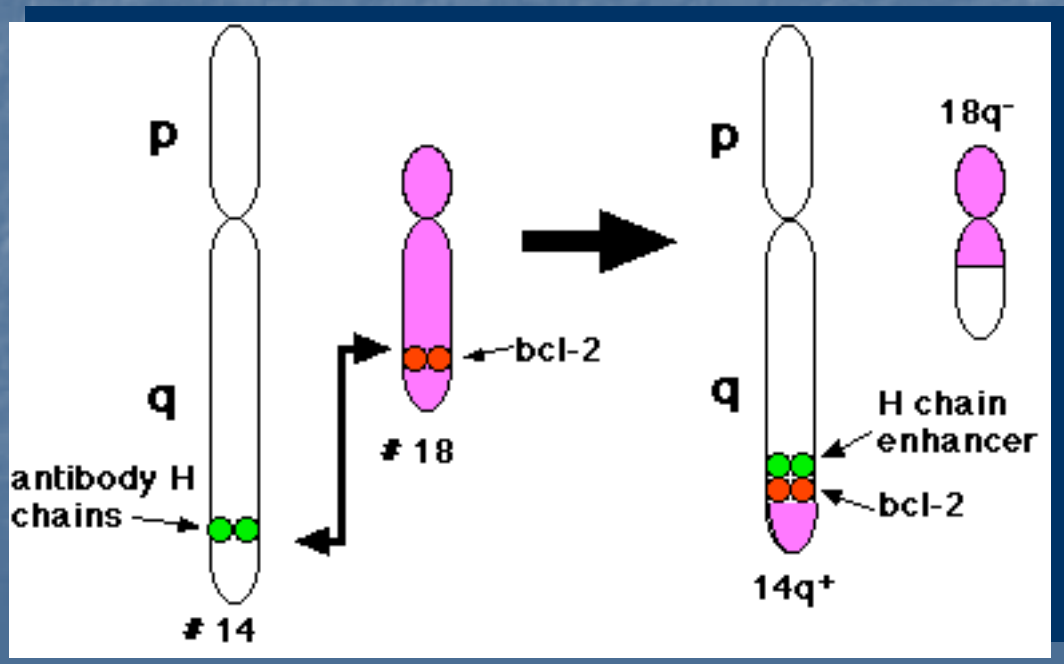
Apoptosis

Oncogenes Involved in Cell Survival

t(14;18)

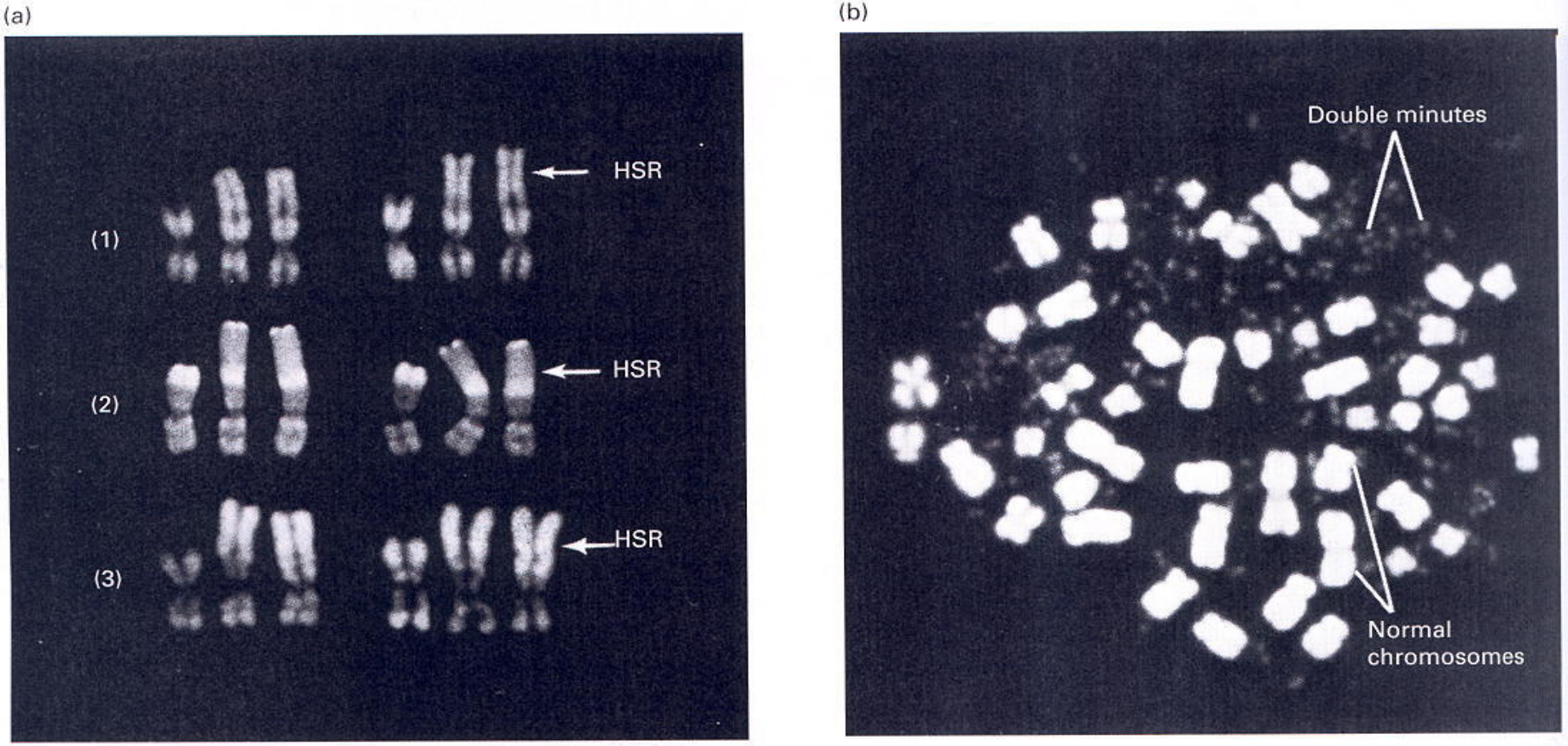
BCL-2 (B-cell lymphoma)
follicular center B cell lymphoma.
Ig heavy chain gene enhancer moved to the bcl-2 locus.

BCL-2 likely inhibits checkpoint dependent apoptosis allowing cells to survive.



ALTERACIONES GENETICAS

AMPLIFICACION



▲ **FIGURE 24-23 Visible DNA amplifications.** (a) Homogeneously staining regions (HSRs) in chromosomes from two neuroblastoma cells. In each set of three chromosomes, the left-most one is a normal chromosome 1 and the other two are HSR-containing chromosomes. The three preparations (1, 2, and 3) represent three different methods of staining the chromosomes. Method 1 is quinacrine staining, which highlights AT-rich regions; method 2 is staining with chromomycin A3 plus methyl green, which highlights GC-rich areas; and method 3 is 33258 Hoechst staining after a pulse of bromodeoxyuridine late during the S phase, which highlights the early replicating regions. In all three cases the HSRs stain homogeneously whereas the rest of the chromosomes are somewhat banded. (b) Quinacrine-stained double minute chromosomes from a human neuroblastoma cell. The normal chromosomes are the large white structures; the double minute chromosomes are the many small paired dots. Both the HSRs and the double minute chromosomes shown here contain the N-*myc* oncogene. [Part (a) see S. Latt et al., 1975, *Biopolymers* **24**:77; part (b) see N. Kohl et al., 1983, *Cell* **35**:359; photographs courtesy of Dr. S. Latt.]

Gene amplification

Oncogene	Amplification	Source of tumor
c-myc	~20-fold	leukemia and lung carcinoma
N-myc	5-1,000-fold	neuroblastoma retinoblastoma
L-myc	10-20-fold	small-cell lung cancer
c-abl	~5-fold	chronic myoloid leukemia
c-myb	5-10-fold	acute myeloid leukemia colon carcinoma
c-erbB	~30-fold	epidermoid carcinoma
K-ras	4-20-fold 30-60-fold	colon carcinoma adrenocortical carcinoma

HER-2

- Human Epidermal Growth Factor Receptor 2
- Also "known as":
  -neu (murine gene) or
  -c-erbB-2
- Member of the type I RTKs which include HER-1 (EGFR), HER-3 and HER-4
- HER-2 protein = p185,000

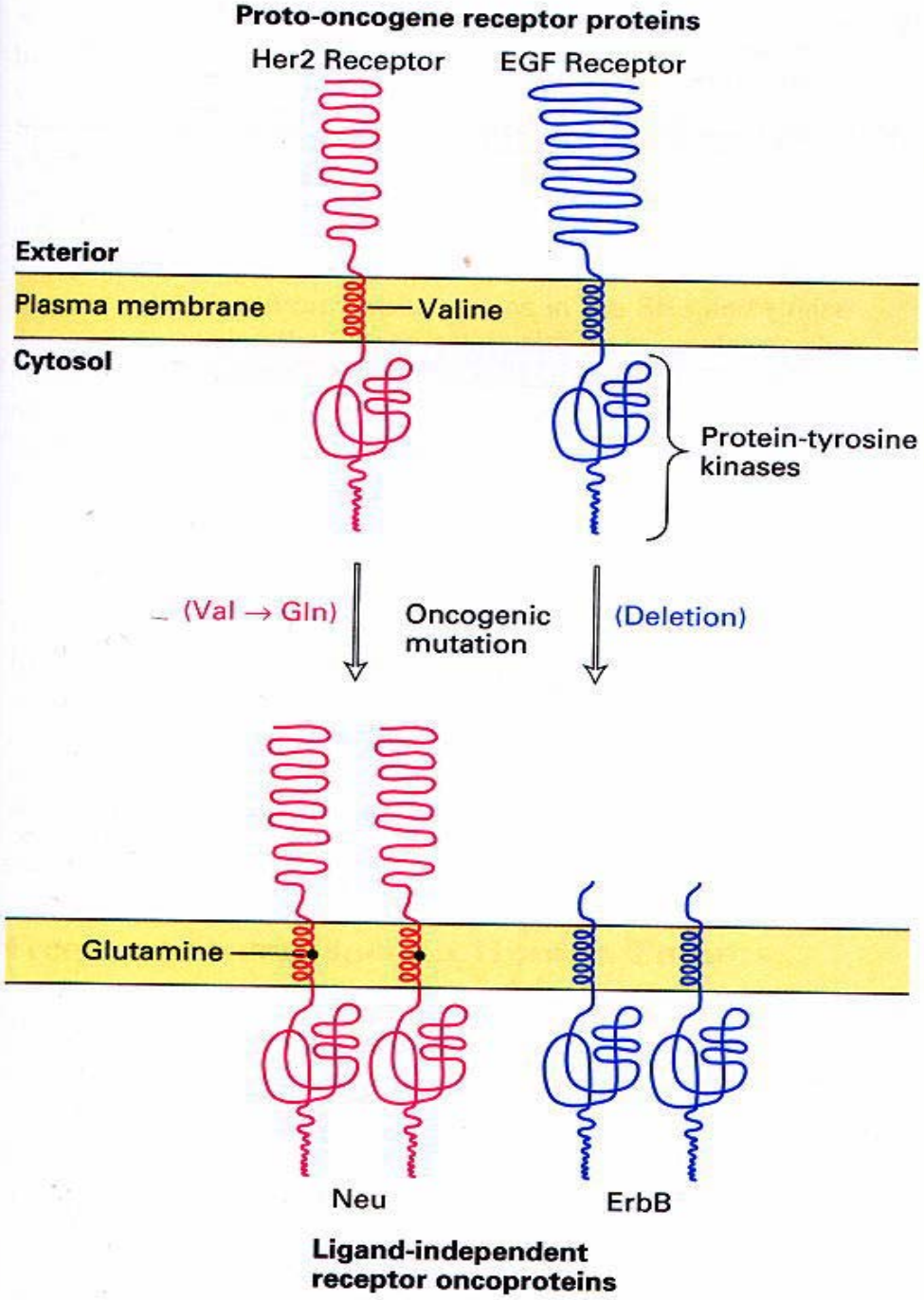


FIGURE 24-15 Effects of oncogenic mutations in proto-oncogenes that encode cell-surface receptors. *(Left)* A mutation that alters a single amino acid (valine to glutamine) in the transmembrane region of the Her2 receptor causes dimerization of two receptor proteins in the absence of the normal EGF-related ligand, making the protein constitutively active as a kinase. *(Right)* A deletion that causes loss of the extracellular ligand-binding domain in the EGF receptor leads, for unknown reasons, to constitutive activation of the protein kinase.

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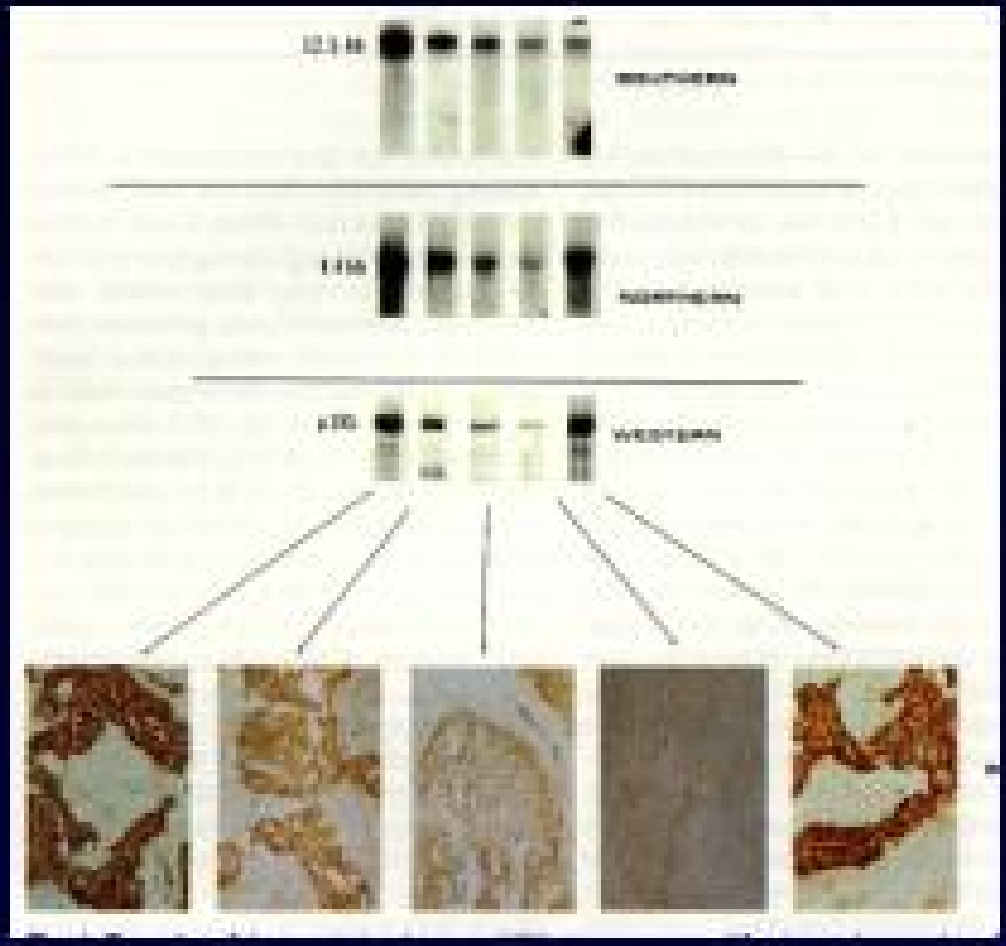
The HER2 Alteration



Southern

Northern

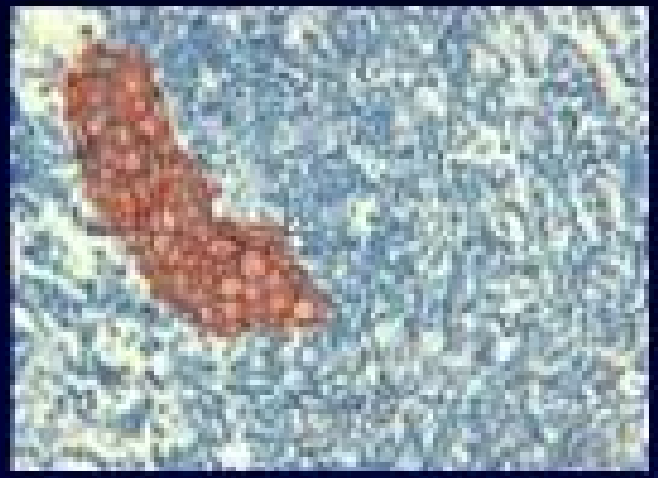
Western

IHC

Slamon et al. *Science* 1989

HER-2 Oncogene
Amplification

Breast Cancer



HER-2 Oncoprotein
Overexpression

Shortened Survival

Median Survival from First Diagnosis

HER-2 overexpressing	3 yrs
HER-2 normal	6 - 7 yrs

Slamon et al, 1987