Oncogenes

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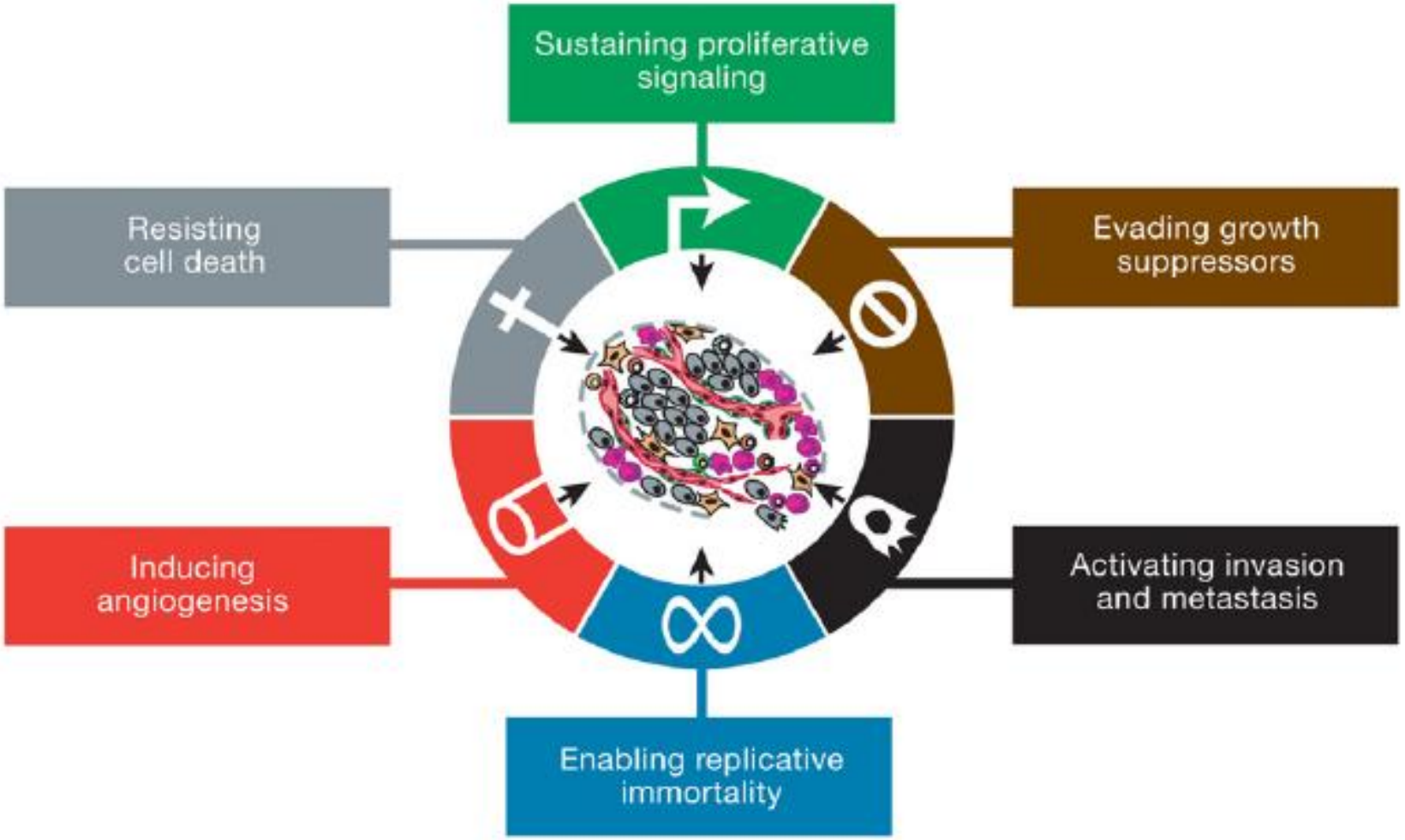
What is the molecular basis of cancer?

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

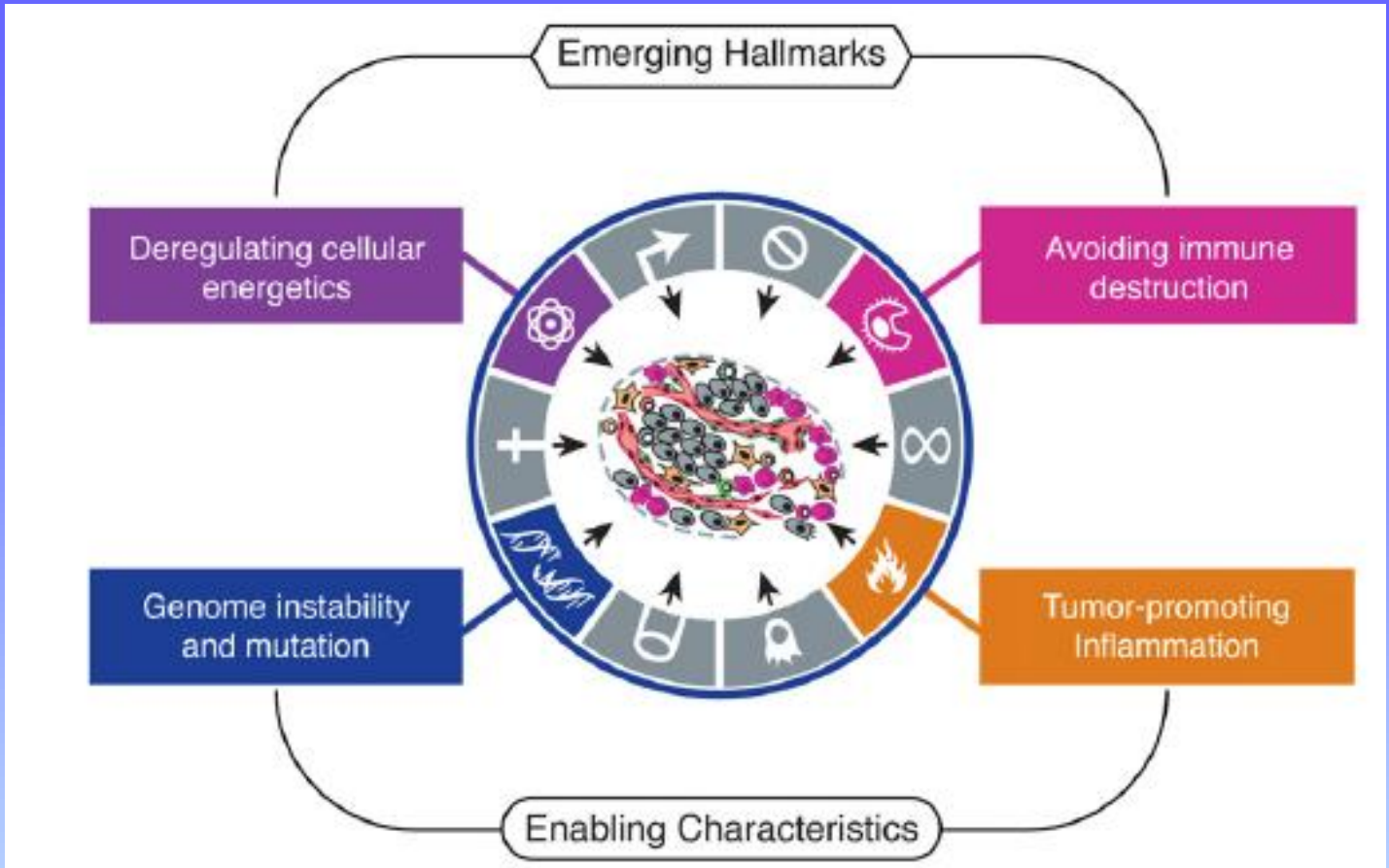
For a cancer cell to be successful the mutations must…

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

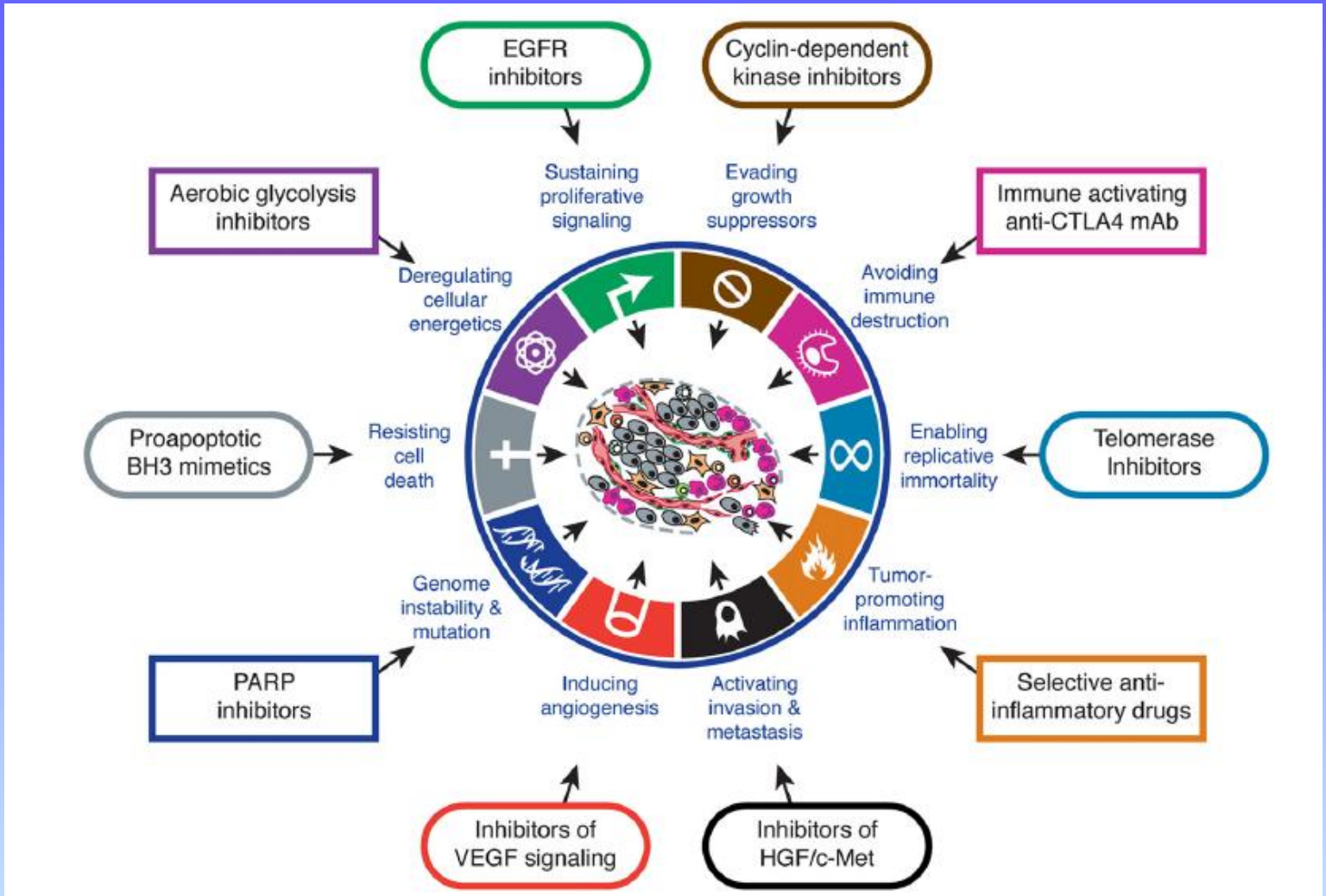


Hallmarks of Cancer: The Next Generation. Cell 144, 646-, March 4, 2011
Douglas Hanahan and Robert A. Weinberg



Emerging Hallmarks and Enabling Characteristics: An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or reprogram, cellular metabolism in order to most effectively support neoplastic proliferation. The second allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells. Because neither capability is yet generalized and fully validated, they are labeled as emerging hallmarks.

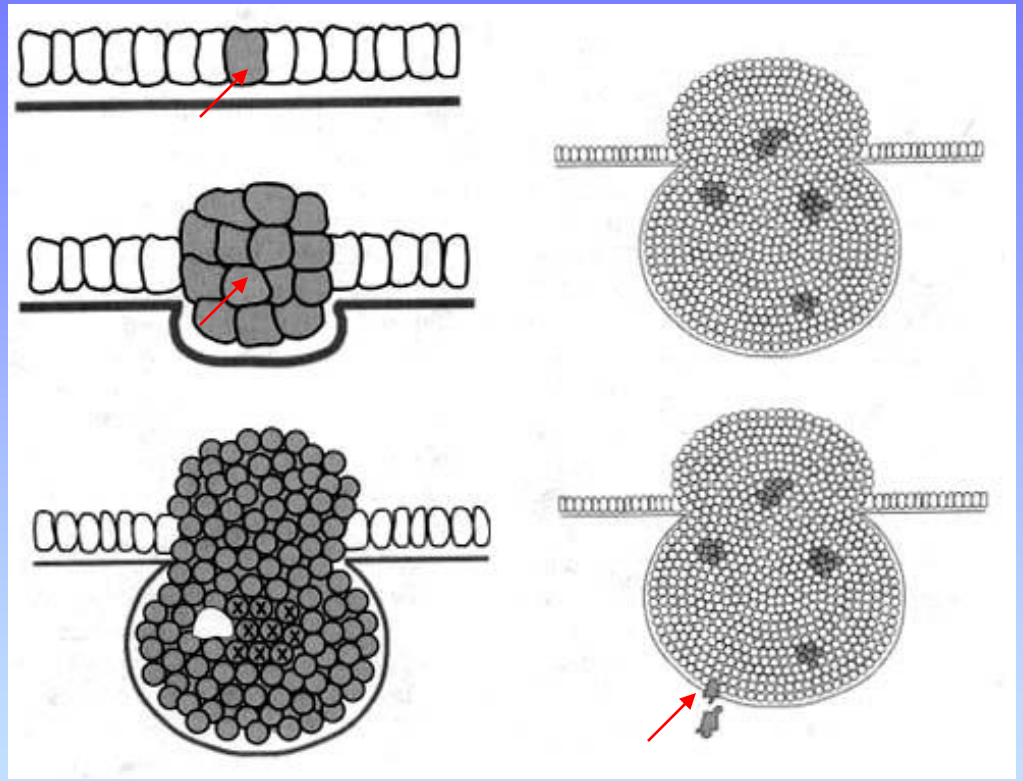
Additionally, two consequential characteristics of neoplasia facilitate acquisition of both core and emerging hallmarks. Genomic instability and thus mutability endow cancer cells with genetic alterations that drive tumor progression. Inflammation by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumor-promoting consequences of inflammatory responses.



Therapeutic Targeting of the Hallmarks of Cancer

Drugs that interfere with each of the acquired capabilities necessary for tumor growth and progression have been developed and are in clinical trials or in some cases approved for clinical use in treating certain forms of human cancer. Additionally, the investigational drugs are being developed to target each of the enabling characteristics and emerging hallmarks depicted in the Figure, which also hold promise as cancer therapeutics. The drugs listed are but illustrative examples; there is a deep pipeline of candidate drugs with different molecular targets and modes of action in development for most of these hallmarks.

Tumor Progression: Evolution at the Cellular Level



Benign tumor (polyp in epithelial cells) is confined by basal lamina; then additional mutation occurs.

Malignant tumor (carcinoma in epithelial cells) grows very fast, becomes invasive, and metastasizes.

Ciclo celular y cáncer

✓ **El ciclo celular normal está controlado por transducción de señales.**

✓ **Los factores de crecimiento se unen a sus receptores en la superficie celular; proteínas transmembrana liberan señales hacia el interior de las células.**

✓ **<u>Existen dos tipos de factores de crecimiento</u>:**

1. **Factores estimulatorios de crecimiento → Estimulan la división celular**

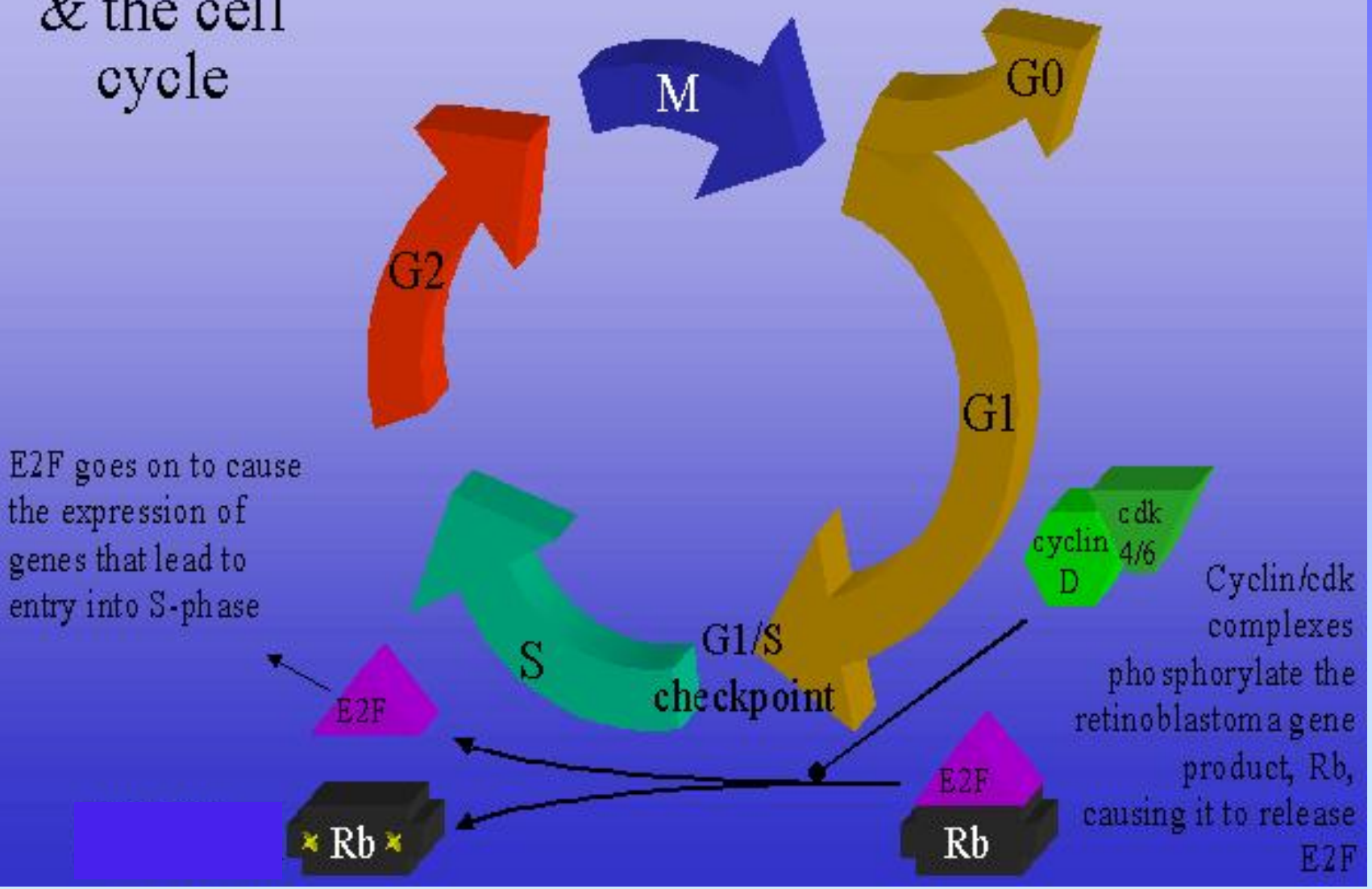
2. **Factores inhibitorios del crecimiento → Inhiben la división celular**

✓ **Las células sanas se dividen solo cuando el balance entre factores de crecimiento y de inhibición de crecimiento favorece la división celular.**

✓ **Las células tumorales se dividen sin restricciones (mutaciones en los genes de factores de crecimiento e inhibidores del crecimiento).**

Oncogenes & the cell cycle

The G1/S checkpoint is controlled by phosphorylation of Rb



E2F goes on to cause the expression of genes that lead to entry into S-phase

Cyclin/cdk complexes phosphorylate the retinoblastoma gene product, Rb, causing it to release E2F

Review of the neoplastic phenotype

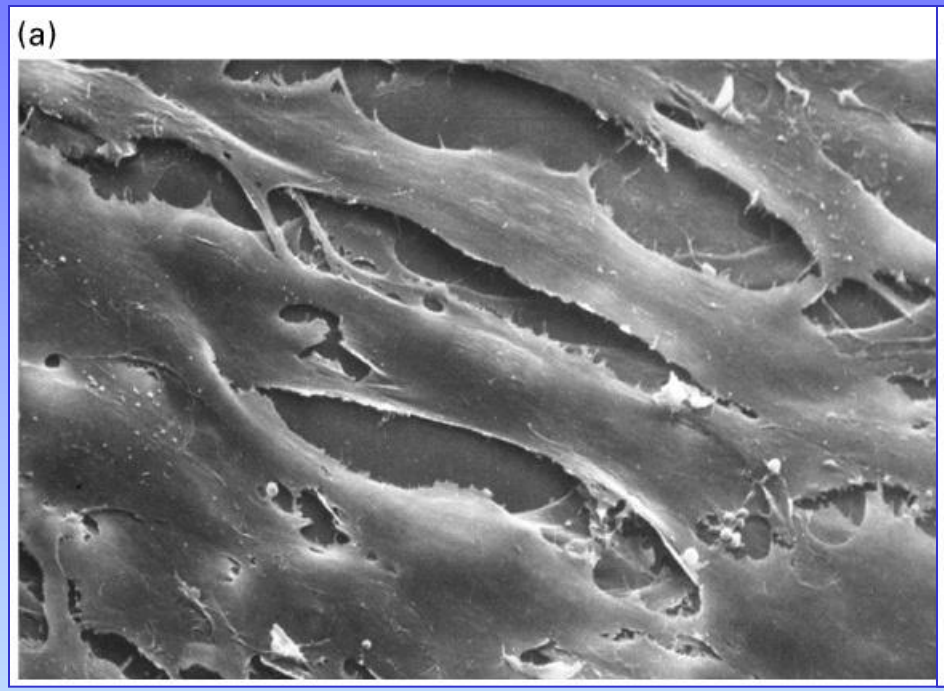
Growth of Normal and *Neoplastic fibroblasts in culture

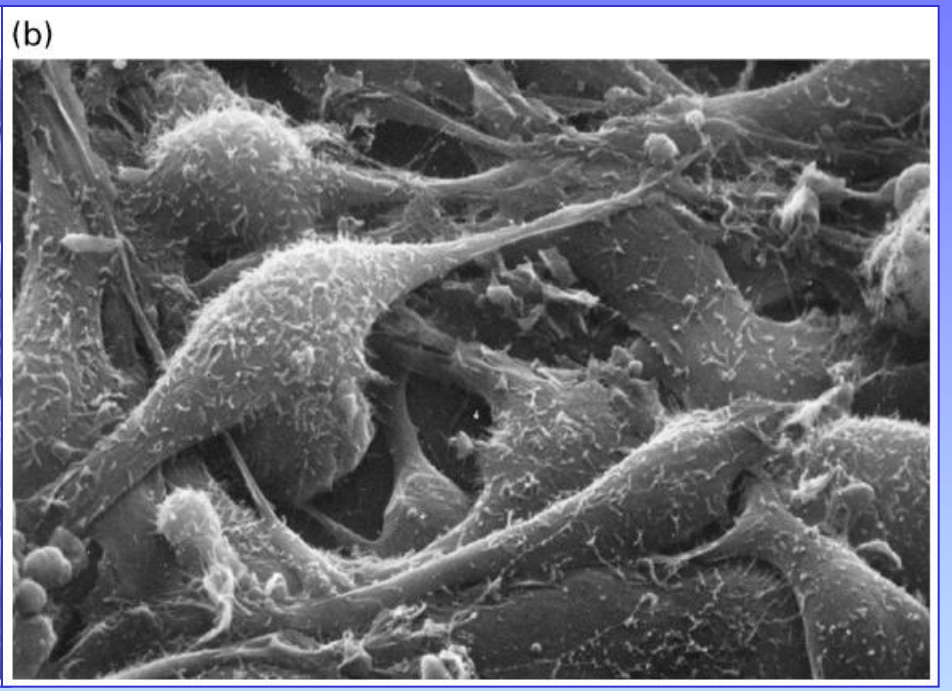
Growth Characteristics	Normal	Tumor
Density dependent inhibition of growth	present	absent
Growth factor requirements	high	low
Anchorage dependence	present	absent
Proliferative life span	finite	indefinite
Contact inhibition	present	absent
Adhesiveness	high	low
Morphology	flat	rounded

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

Review of the neoplastic phenotype

Normal and transformed NIH3T3 cells





Normal NIH3T3
(immortal)

Transformed NIH3T3

Oncogene Discovery

I. Tumor Viruses

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

II. Genomic Rearrangements

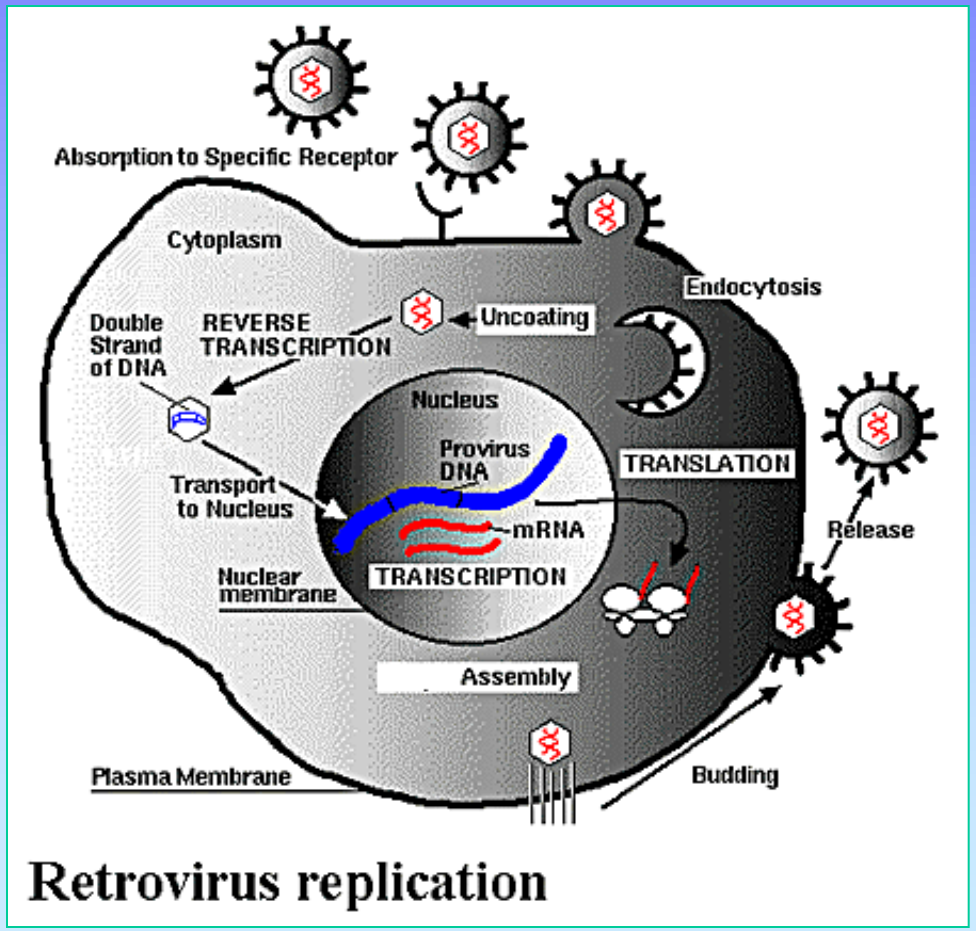
- **Translocations/Inversions**
- **Amplifications/Minute Chromosomes**

III. Functional Assay

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

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.

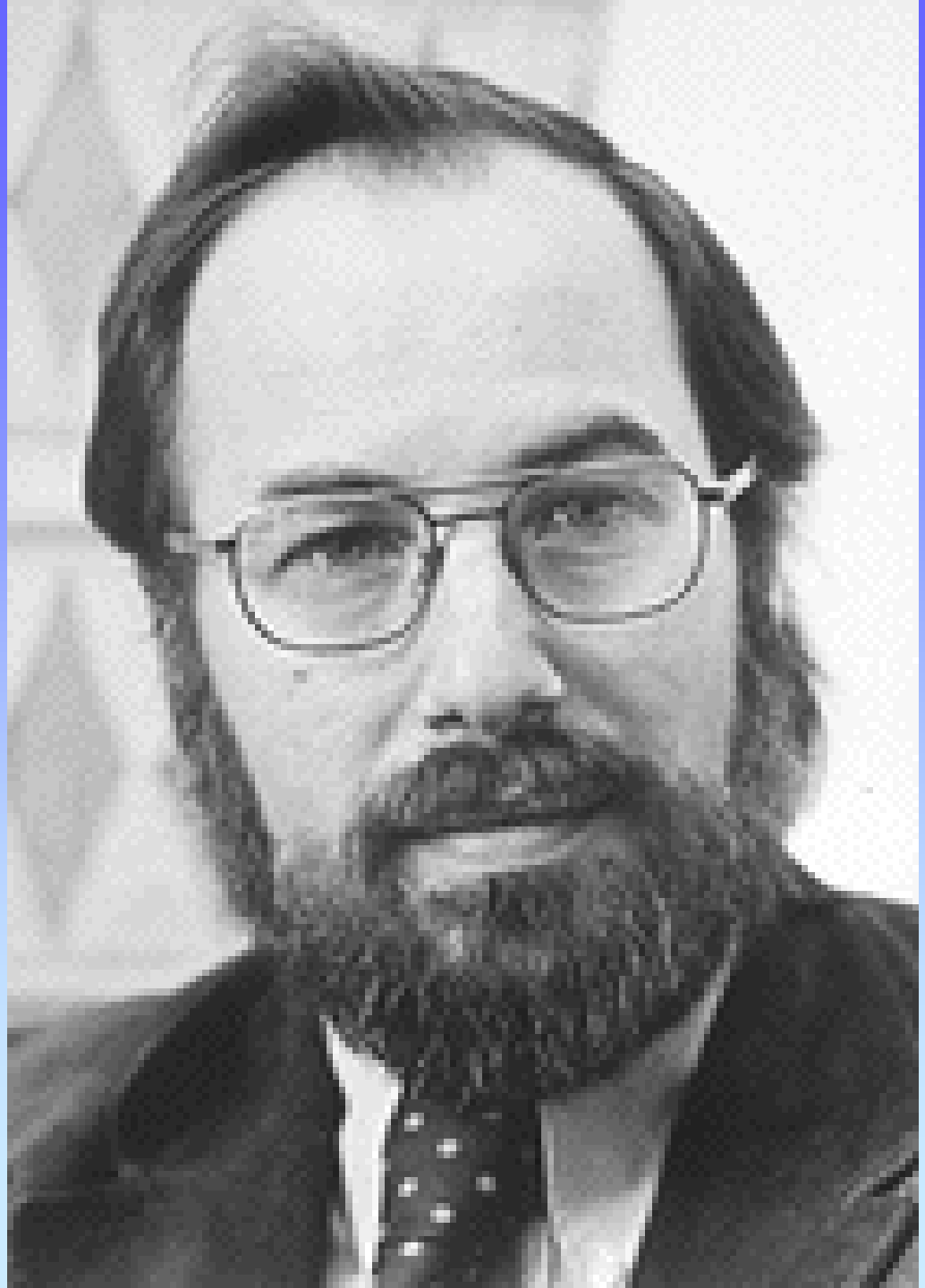


Retrovirus replication

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

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

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



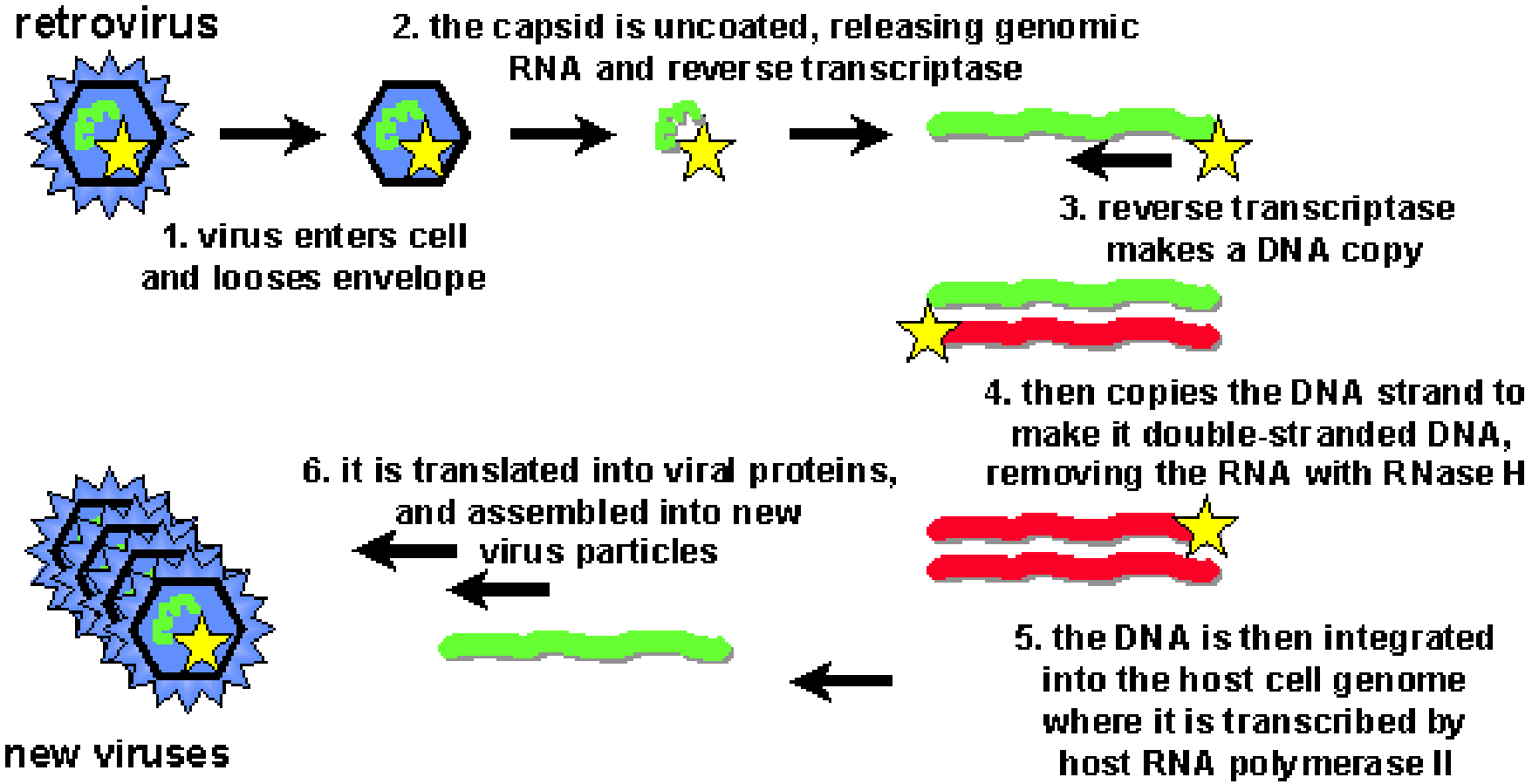
- **RENATO DULBECCO**
- **Premio Nobel de Medicina 1975**

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

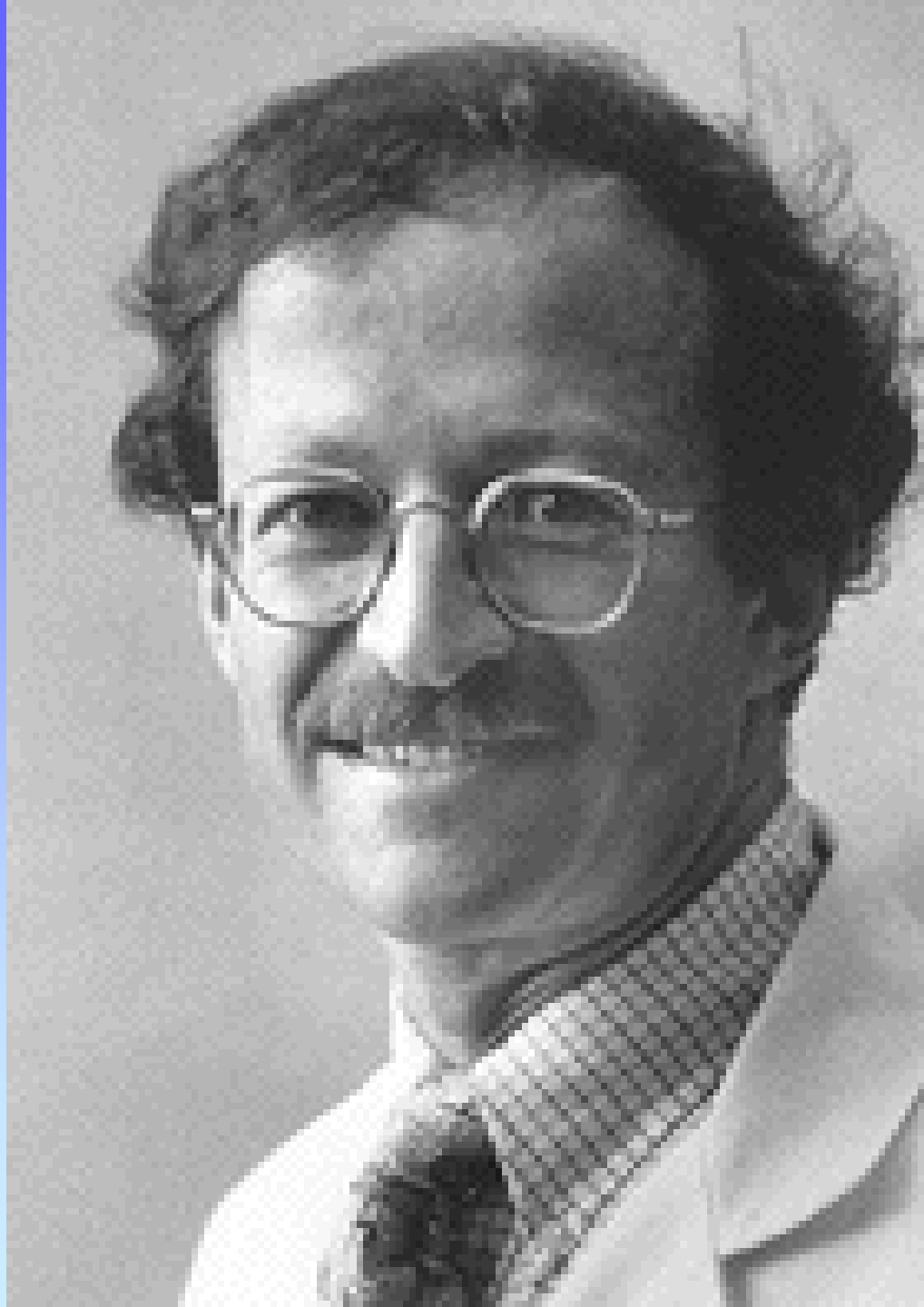
Construction of a cDNA library

- **reverse transcriptase makes a DNA copy of an RNA**

The life cycle of a retrovirus depends on reverse transcriptase

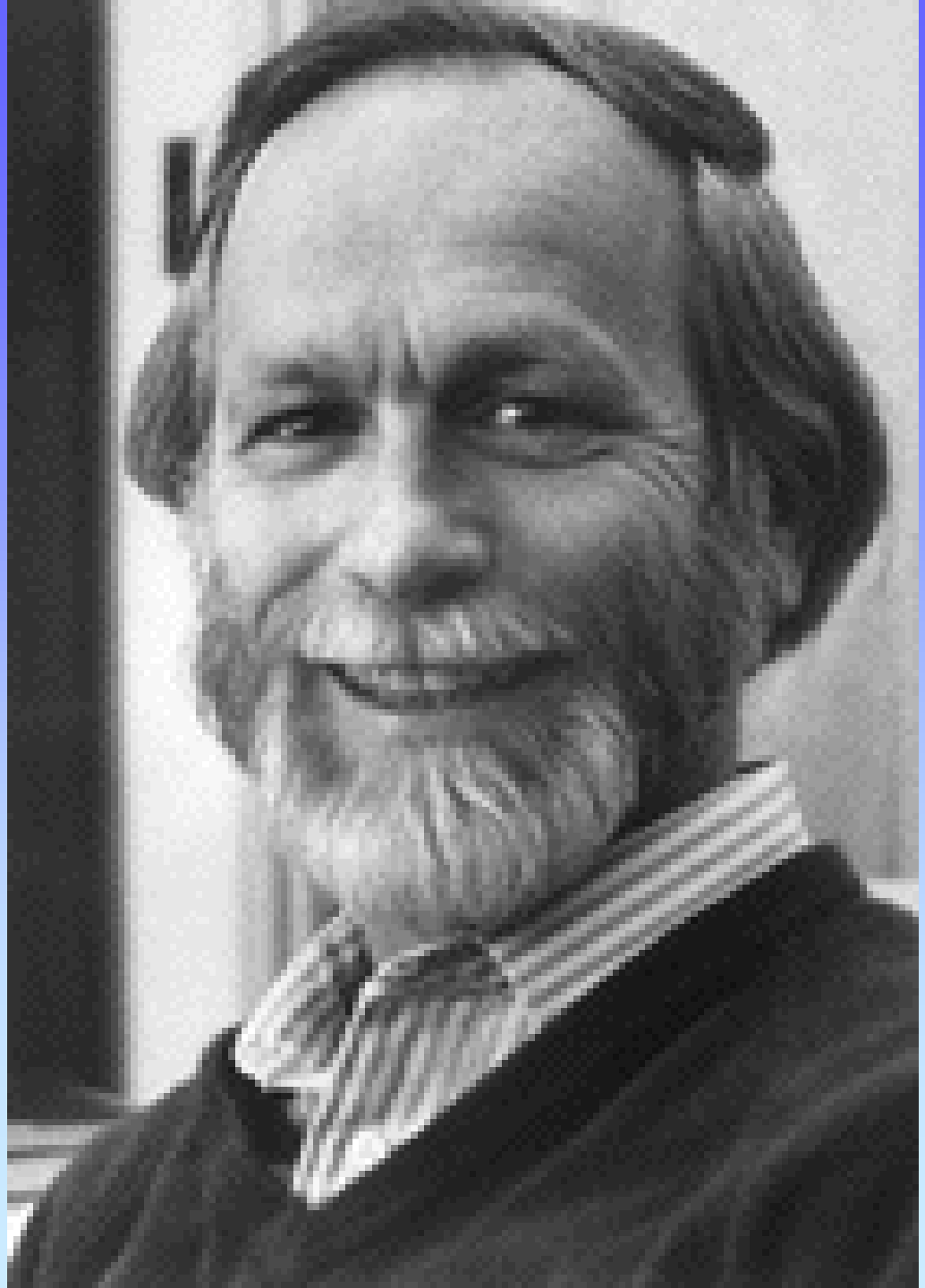


- **HAROLD VARMUS**
- **Premio Nobel de Medicina 1989**



J.MICHAEL BISHOP

Premio Nobel de Medicina 1989



Retroviral Transduction

Acutely Transforming Retroviruses encode an onc gene.

Retrovirus normal

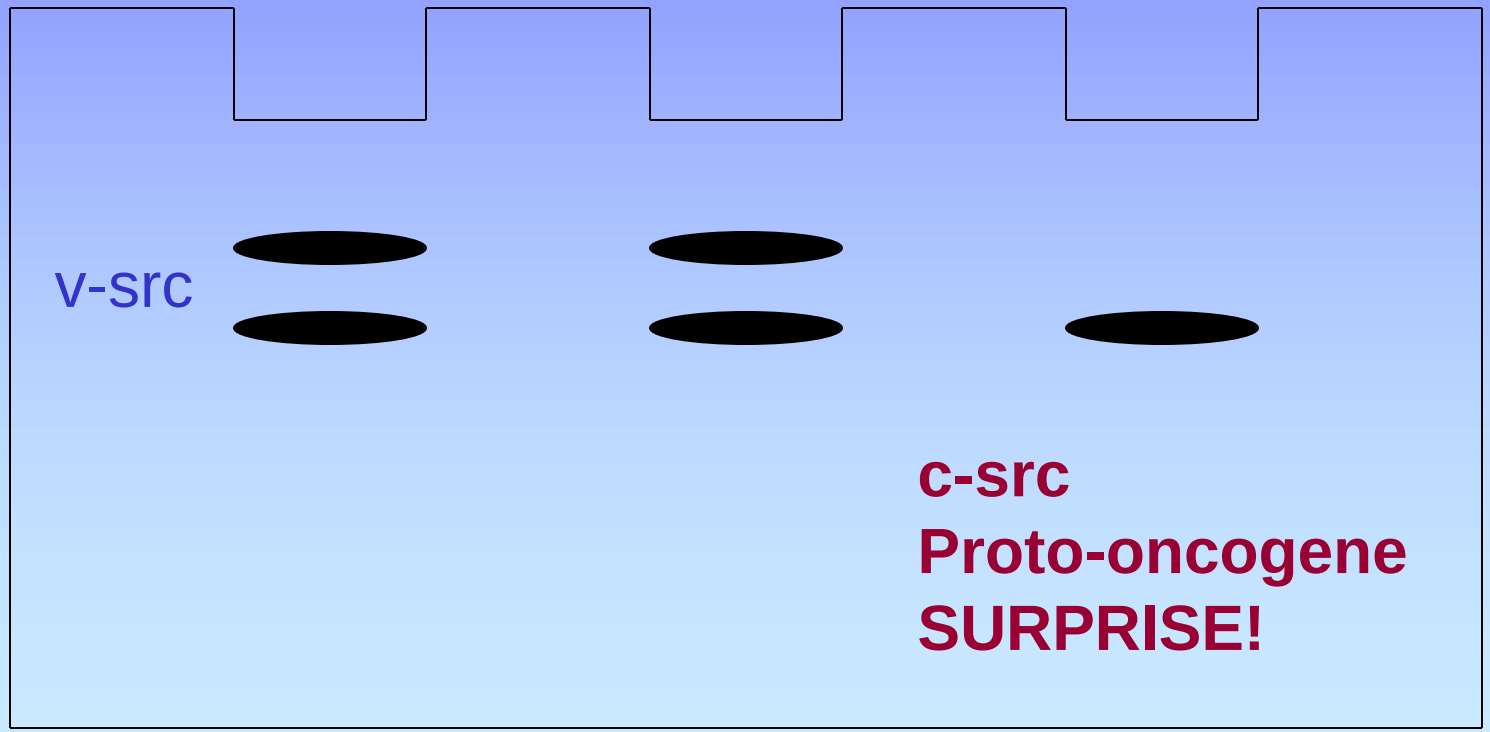
RSV has a env-onc fusion

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

Infected chicken #1

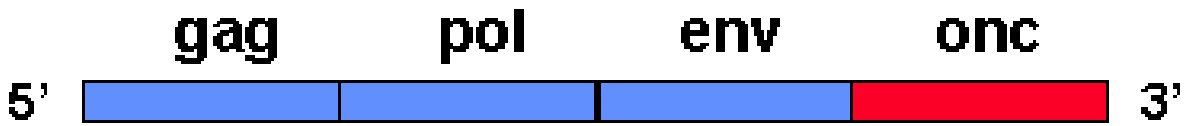
Infected chicken #2

Uninfected chicken (Negative Control)



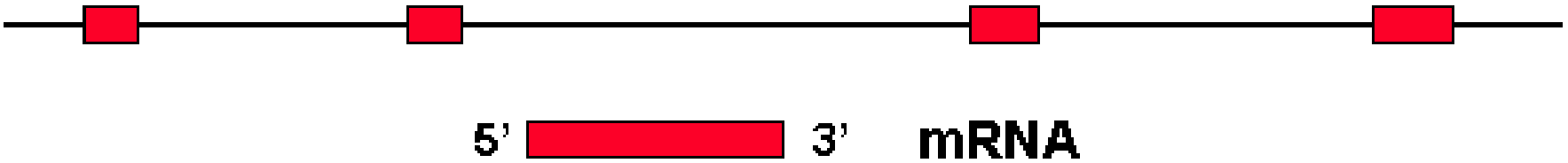
Gene organization of a retrovirus

Gene organization of a transforming retrovirus

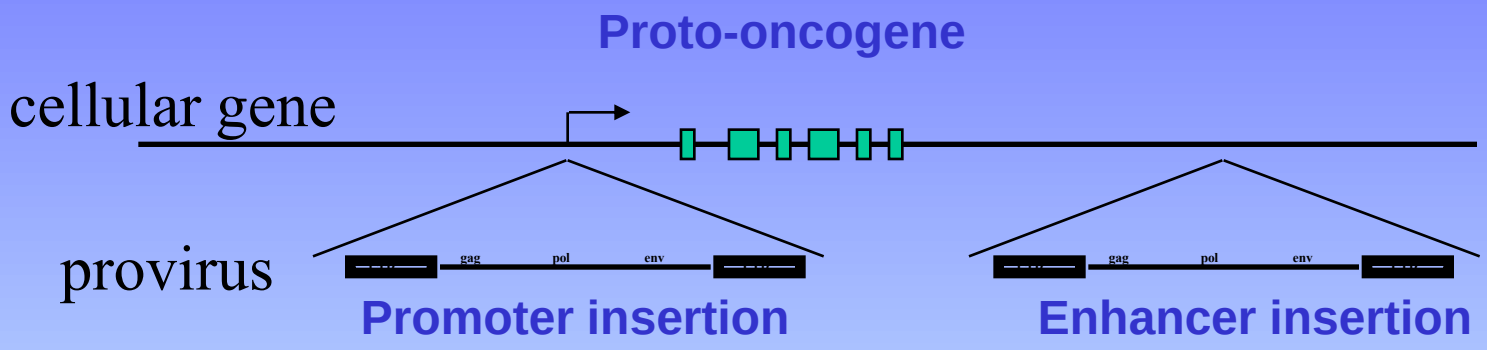


gag = group specific antigen
pol = reverse transcriptase
env = envelope
onc = oncogene

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.

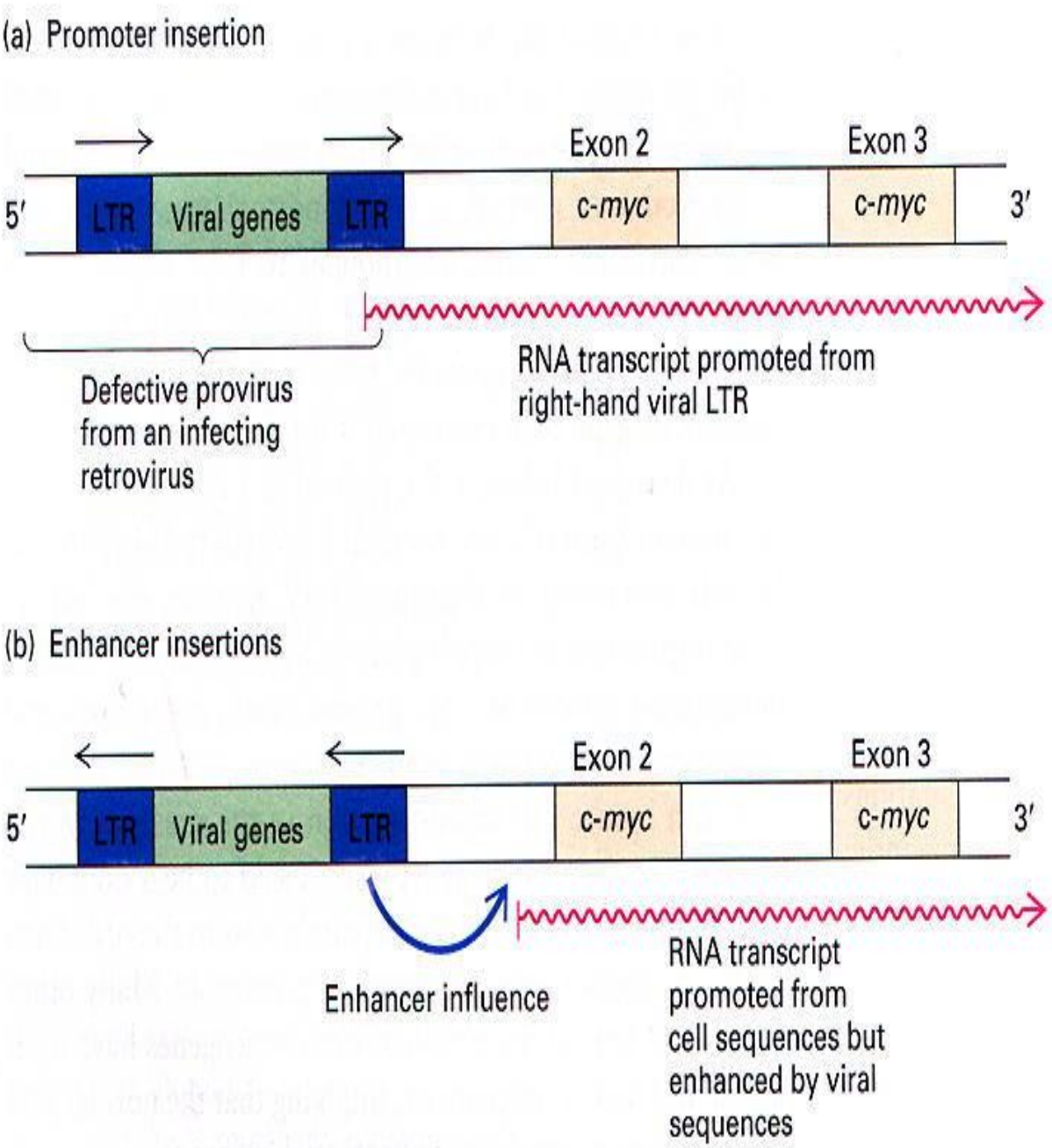
An oncogene is:

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.



◀ 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

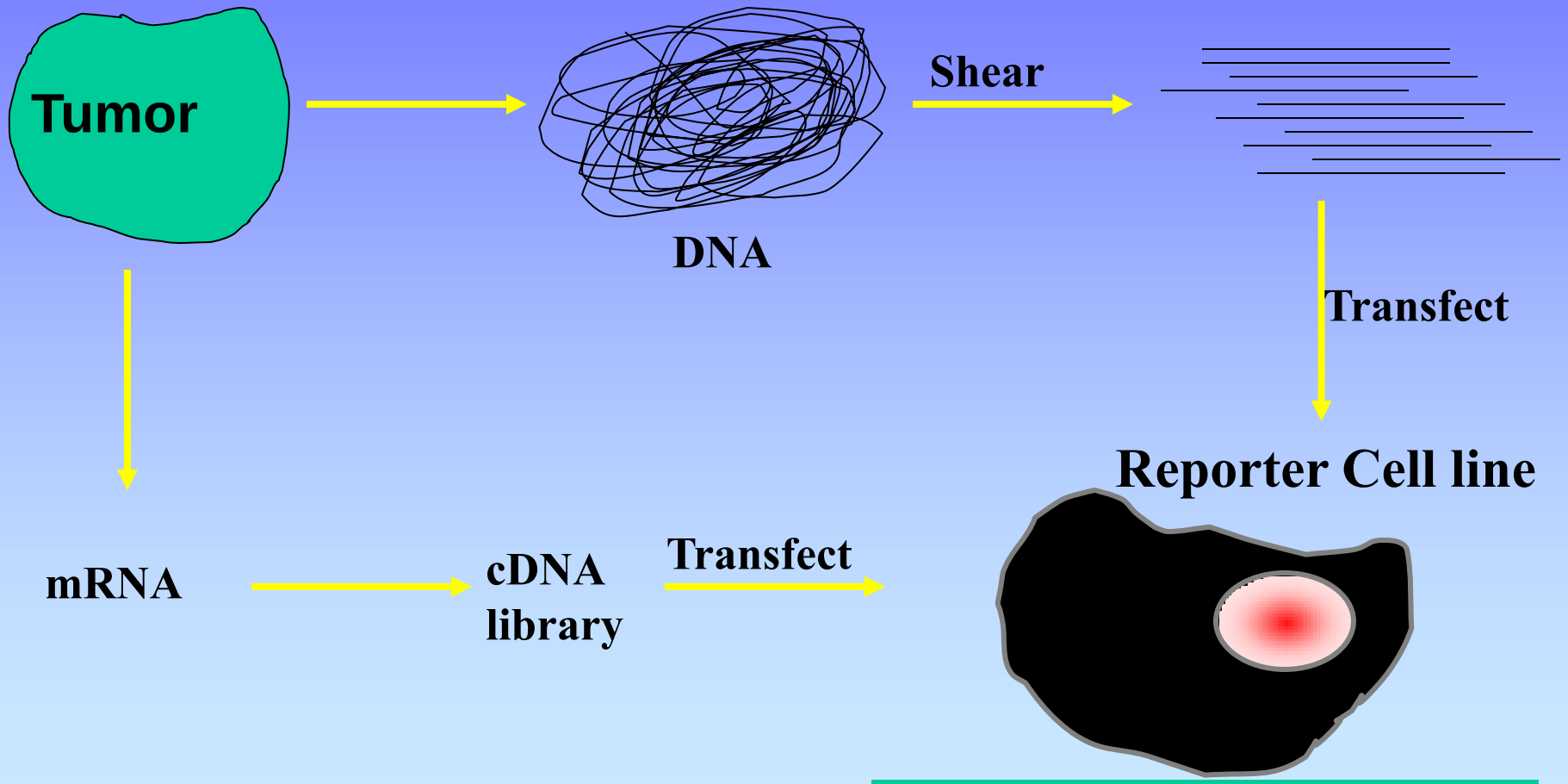
src	Rous sarcoma virus	Chicken
myc	Avian myelocytomatosis virus	Chicken
erb A, erb B	Avian erythroblastosis virus	Chicken
myb	Avian myeloblastosis virus	Chicken
ets	Avian erythroblastosis virus	Chicken
rel	Avian reticuloendotheliosis virus	Turkey
H-ras	Harvey rat sarcoma virus	Rat
K-ras	Kirsten murine 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

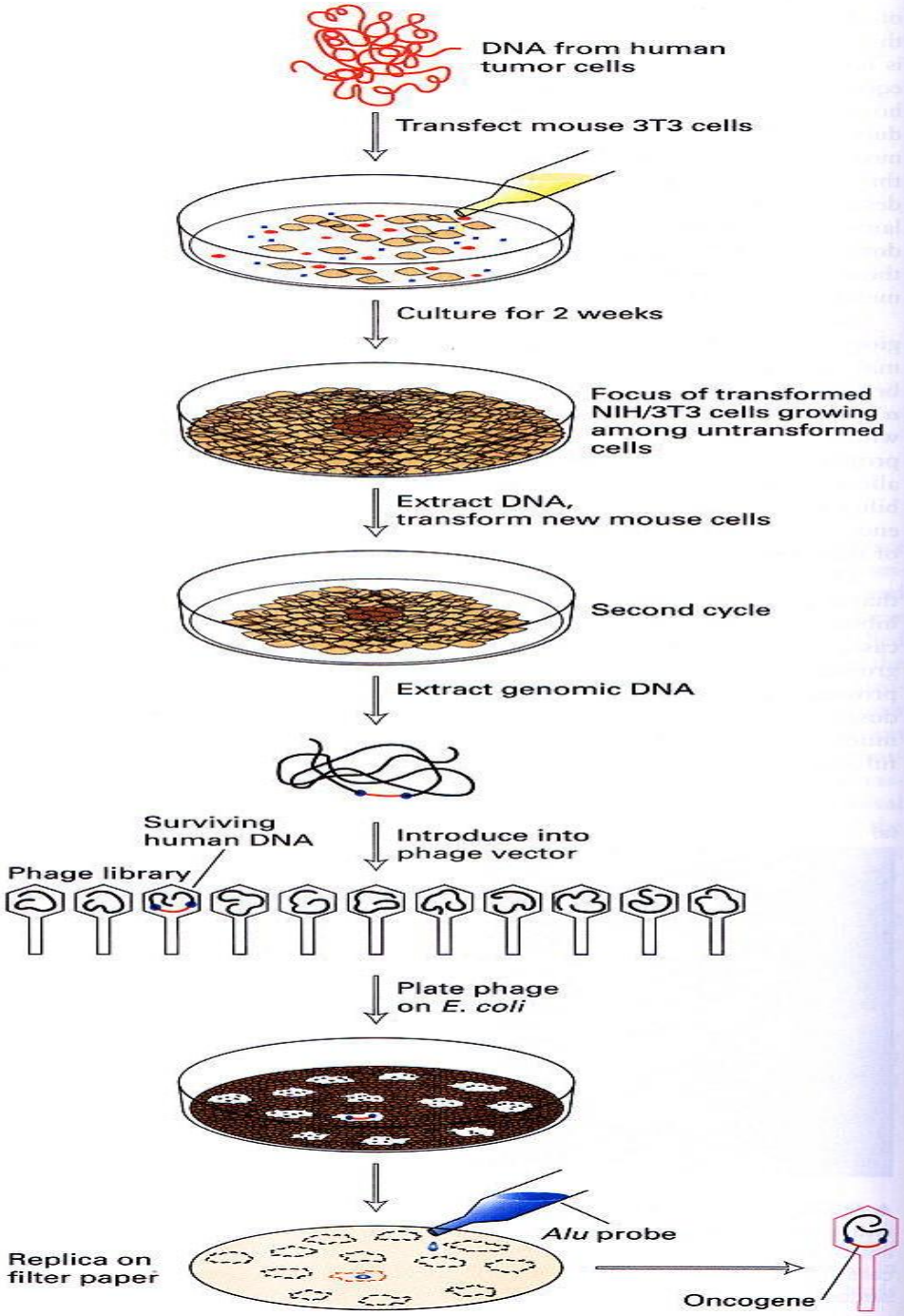
Robert Weinberg

Whitehead Institute-MIT

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



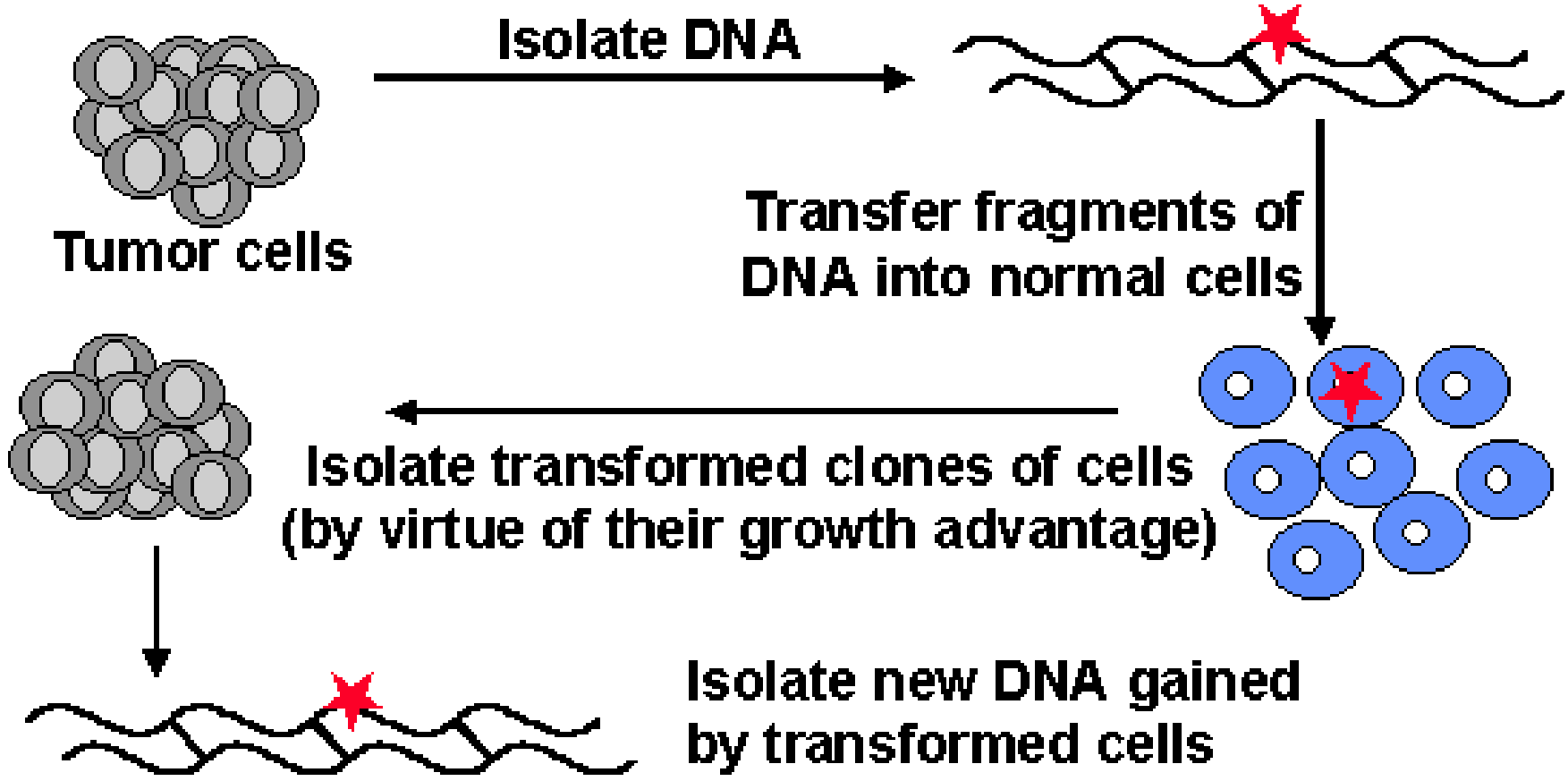
*transfection: putting foreign DNA into mammalian cells.



▶ **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.**

Oncogene co-operativity

- One assay used to characterize a gene as an oncogene is to transfect it into normal fibroblasts and look for the formation of foci - groups of dense growing cells - so called *transformed cells*.
- Such transfection studies showed that often one oncogene was not enough to yield full cellular transformation. It was found that a "nuclear" and a "membrane" oncogene was necessary.
  - For example v-ras + c-myc
- cancer is a multigene disease

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

ONCOGENES PROTOTIPICOS= PROPIEDADES

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

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Transformación neoplásica

- ✓ **Resulta de la acumulación de daños genéticos, típicamente involucrando por lo menos 6 mutaciones**

- ✓ **Genes mutados durante la transformación neoplásica:**

1- Mutación de Proto-oncogenes, resultando en un estímulo proliferativo para la célula (c-erb-B)
2- Inactivación de genes supresores de tumor (p53)
3- Mutación de genes que regulan la apoptosis (bcl-2)
4- Mutación de genes que codifican para enzimas de reparación del ADN

- ✓ **Proto-oncogenes pueden convertirse en oncogenes como resultado de:**

1- mutaciones heredadas
2- Factores ambientales tales como químicos, radiación y virus.

FUNCION DE LOS PROTO-ONCOGENES

- - Transductores de señales
- - Factores de transcripción
- - Receptores de factores de crecimiento
- - Factores de crecimiento
- - Reguladores de Apoptosis
- - Remodeladores de cromatina

MECANISMOS DE ACTIVACION DE ONCOGENES

1- Mutaciones puntuales:

✓ **Ejemplos: *ras*, *erb*-B, *fms***

✓ **En *ras* (proteína G), un cambio en un único aminoácido inhibe la hidrólisis del GTP, prolongando el estado activado, independiente de factores de crecimiento, inhibiendo la interacción de *ras* con GAPs.**

✓ **Funciona como un interruptor molecular en la vía de transducción de señales que conecta los factores de crecimiento con la expresión de genes que controlan la proliferación celular.**
GF → receptor → → Ras → → → FT → genes target → división celular.

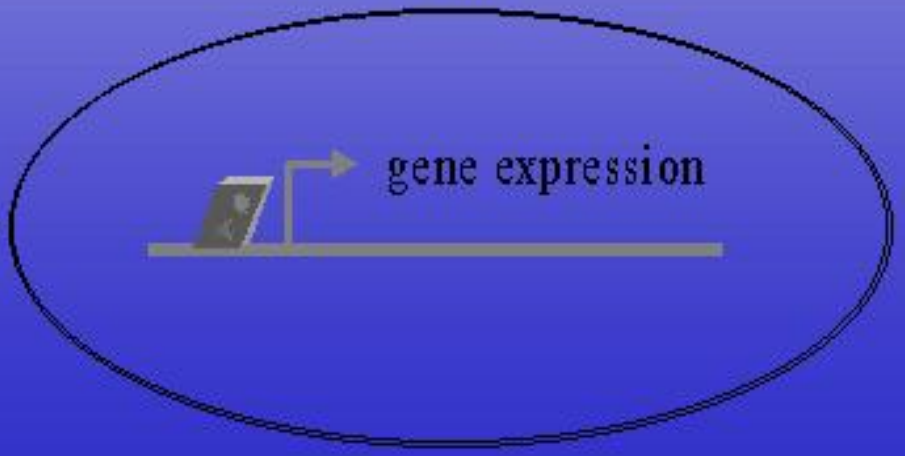
✓ **Mutados en el 30% de todos los tumores**

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



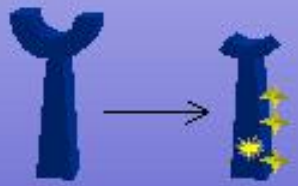
Mutational mechanisms of oncogenes: intragenic deletion

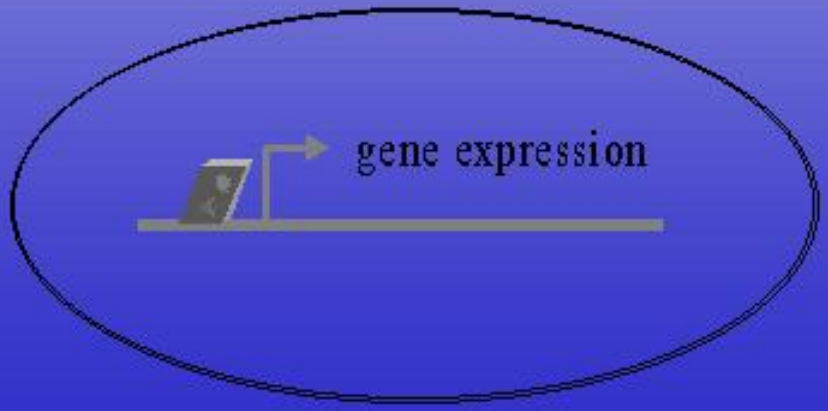
- genomic deletions can selectively delete portions of a genes coding region, leading to loss of an inhibitory/regulatory domain
- this is akin to non-sense mutations causing truncations, but can occur within the gene

Δ-EGFR

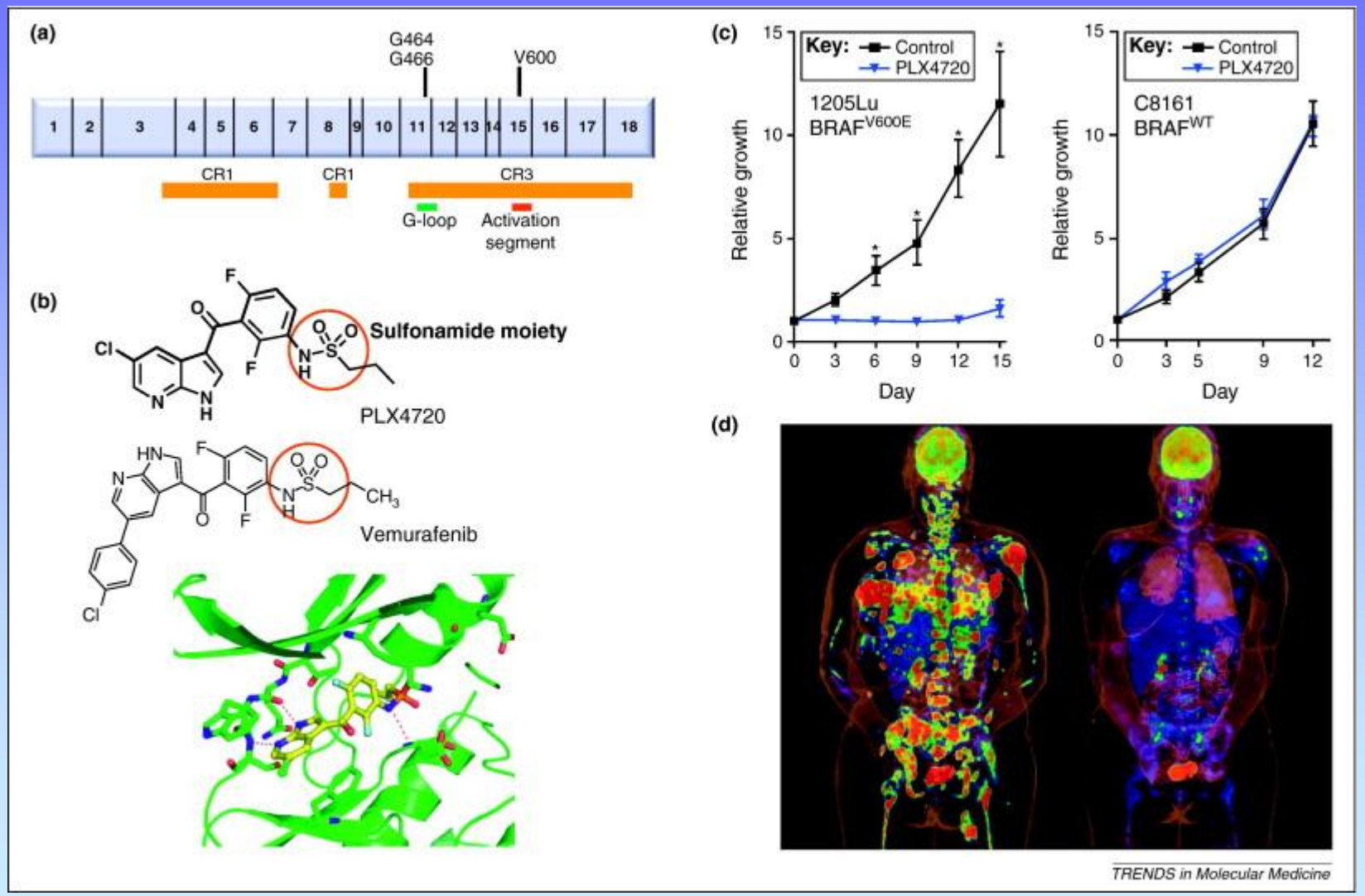
- *growth factor receptor*
- **intragenic deletion mutation**

- a deletion mutation eliminates exons 2 to 7 in the extracellular domain, leading to ligand-independent activity
- this oncogene is also often amplified, and occurs in astrocytoma IV/glioblastoma multiforme





BRAF oncogene

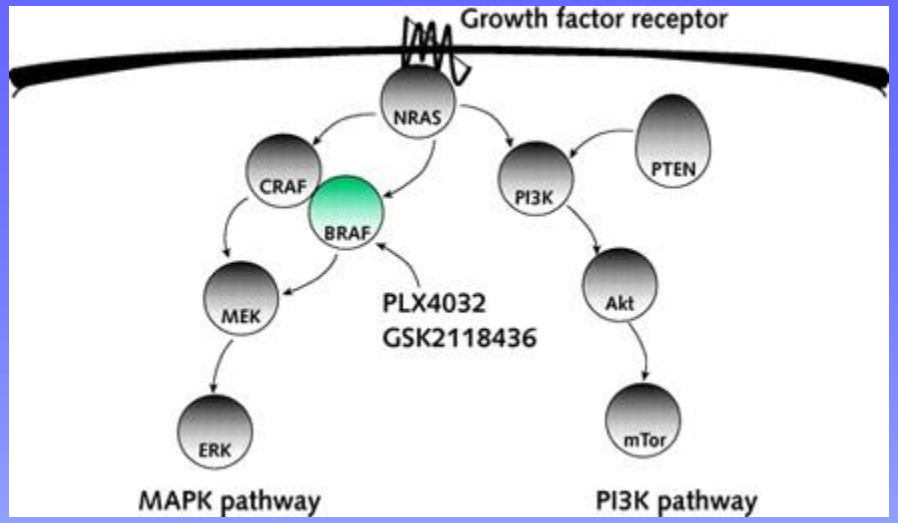


BRAF in melanoma

The BRAF mutation was identified as an oncogene in melanoma in 2002. Scientists soon worked out the mechanics of the pathway and its key role in melanoma. BRAF is a version of RAF in the MAP kinase signaling pathway of RAS-RAF-MEK-ERK (see diagram).

The early growth and survival of about half of all melanomas seems to depend upon a BRAF mutation that dials up the activity of the protein, pumping up activity at each next step, MEK and then ERK, which directs cell proliferation and survival, among other things. About 90 percent of BRAF mutations are in one spot: V600E, a substitution of one amino acid for another that renders BRAF deaf to the molecules that normally turn down its volume. However, in intact cells, vemurafenib only blocks MEK activation in cells that harbor the activating BRAF mutations. In BRAF wild-type cells, vemurafenib paradoxically increases MEK activation by stimulating the kinase activity of BRAF dimers.[5-7] In the setting of activating mutations, BRAF can phosphorylate MEK as a monomer and its activity inhibited as the concentration of vemurafenib is increased. Only cancer cells that have activating BRAF mutations are growth-inhibited or undergo cell death upon vemurafenib exposure.[4] However, increased MEK activation in normal cells appears to underlie some of the toxicities observed with vemurafenib treatment in patients.

In healthy cells, BRAF is found in the testes, some hematopoietic precursors, and some brain cells (which develop from the same embryonic tissue as melanocytes). In contrast, BRAF's better-known cousin CRAF is essential to the daily function of most other cells. Researchers hope highly selective inhibition of BRAF will translate to fewer debilitating toxicities for patients.



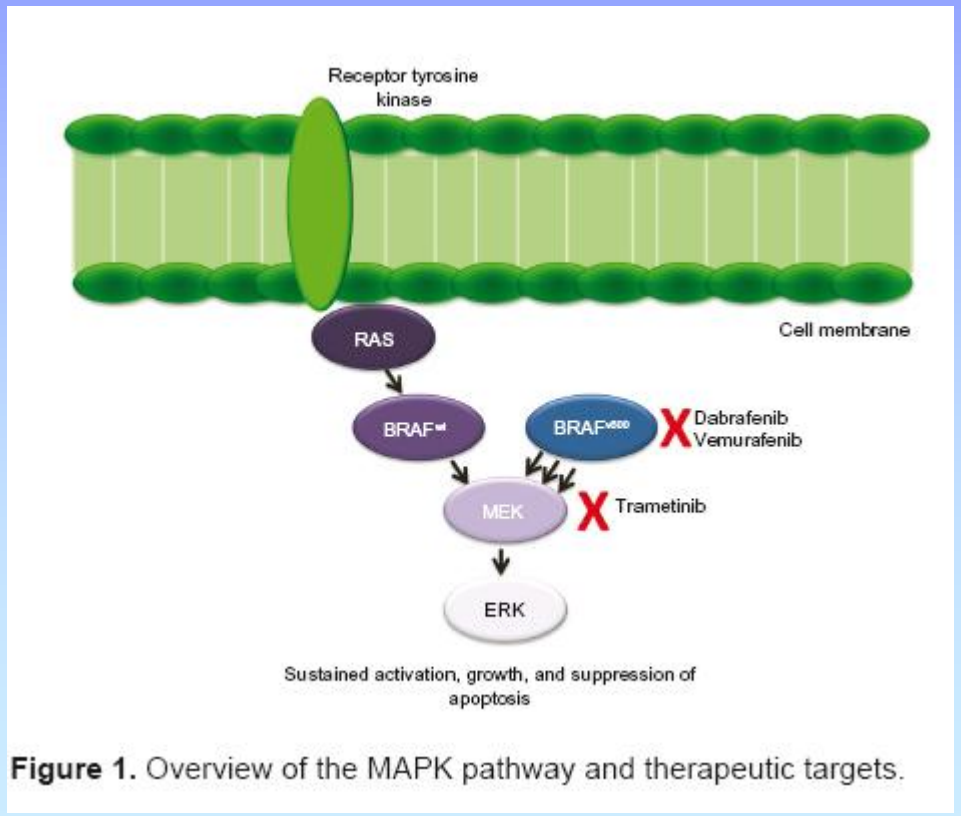
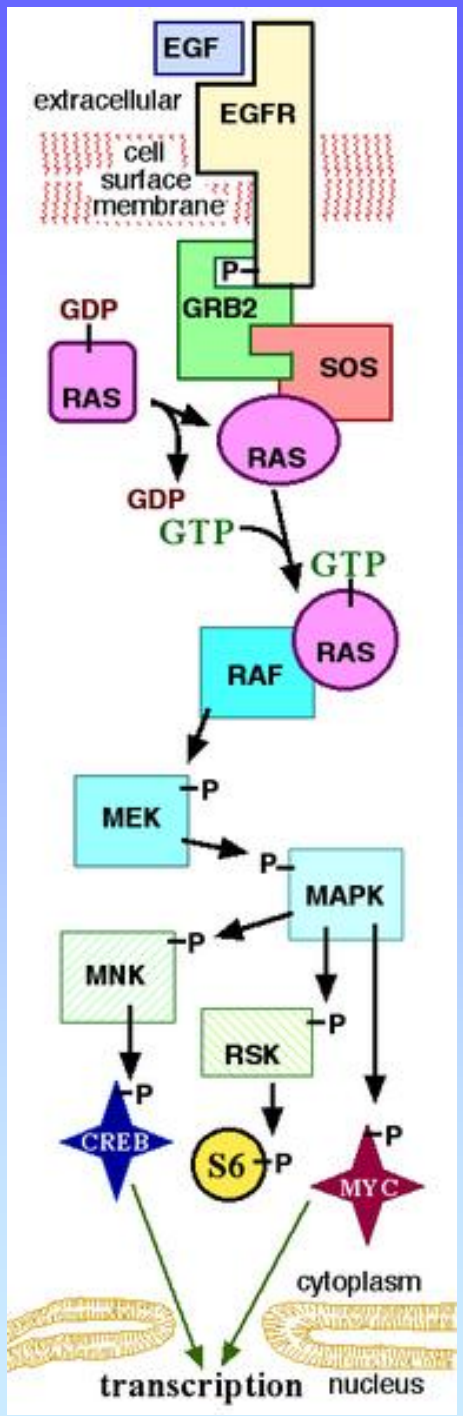


Figure 1. Overview of the MAPK pathway and therapeutic targets.

About half of all melanomas are "addicted" to an activating mutation in BRAF, which fuels cancer growth by constituently activating the kinases MEK and ERK. To overcome drug resistance to the selective BRAF inhibitors (RG7204/PLX4032, Roche) (GSK2118436, GlaxoSmithKline), researchers are testing the addition of a MEK inhibitor and are eyeing other targets in the same pathway and in the PI3K pathway. Courtesy of Keith Flaherty/Annals of Internal Medicine



MAPK pathway

Key components of the MAPK/ERK pathway. "P" represents **phosphate,** which communicates the signal. Top, epidermal growth factor (EGF) binds to the EGF receptor (EGFR) in the cell membrane, starting the cascade of signals. Further downstream, phosphate signal activates MAPK (also known as ERK). Bottom, signal enters the cell nucleus and causes transcription of DNA, which is then expressed as protein.

MECANISMOS DE ACTIVACION DE ONCOGENES

2- Traslocaciones cromosómicas: dos mecanismos

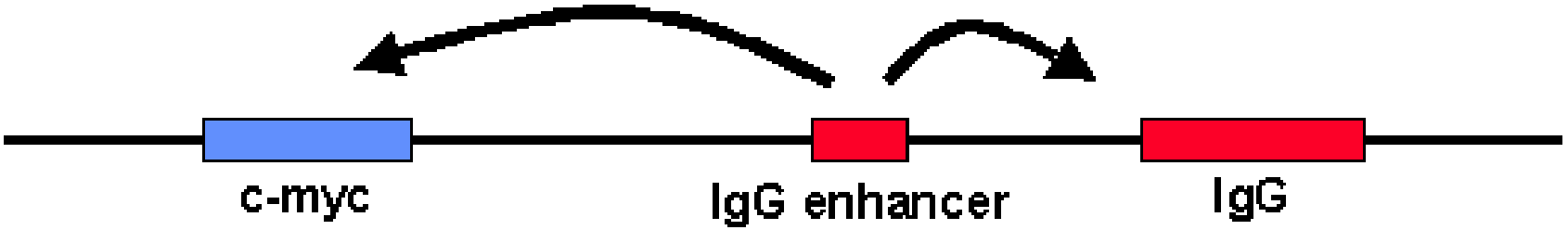
- traslocación que conduce a la sobreexpresión de un proto-oncogen:

Ej: Linfoma de Burkitt → c-myc de cromosoma 8 es traslocado al cromosoma 14 cerca del gen de cadena pesada de Ig, una región sujeta a gran actividad transcripcional, llevando a la sobreexpresión de la proteína myc normal.

- **Traslocación y alteración genética de un proto-oncogen:**

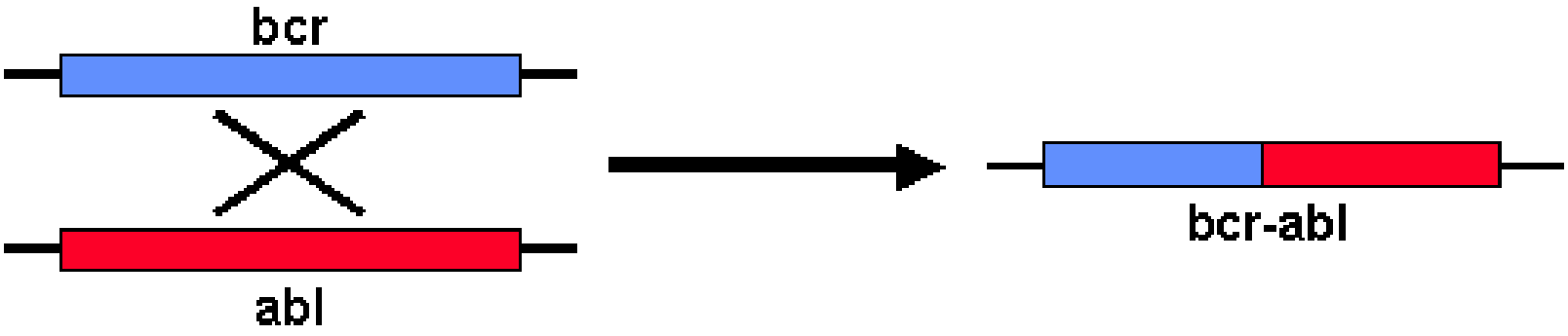
Ej: Cromosoma Philadelphia en Leucemia Mieloide Crónica (CML) → parte del gen *abl* (tirosin quinasa) en cromosoma 9 trasloca al cromosoma 22 para formar una proteína híbrida (quimera) con el gen *bcr* (breakpoint cluster region). La quimera *abl-bcr* de 210 kDa tiene potente actividad tirosin quinasa constitutiva.

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



c-myc is activated by the IgG enhancer in lymphocytes

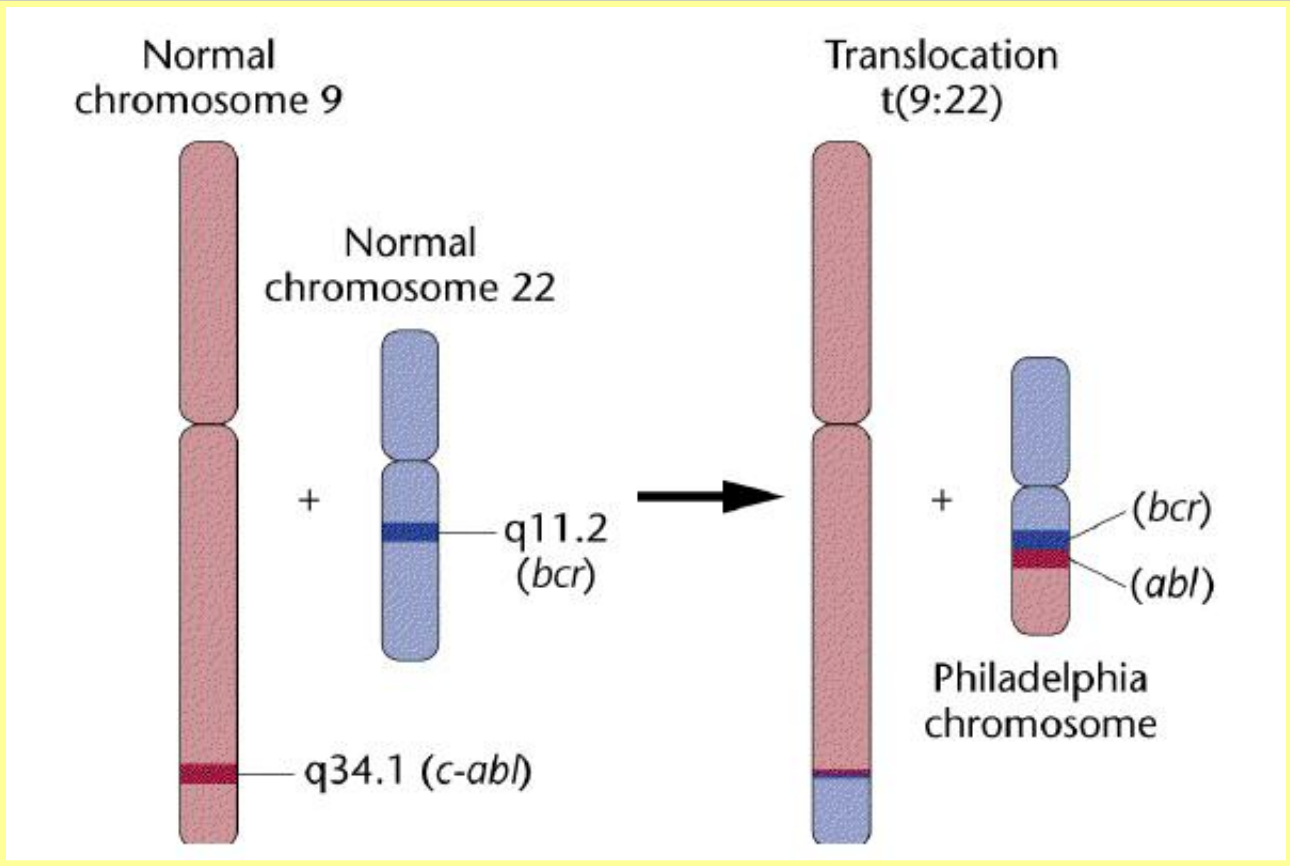
bcr-abl fusion protein is produced, which results in a constitutively active abl kinase



abl

- *cytoplasmic protein kinase*
- **fusion**
  - protooncogene is a non-receptor tyrosine kinase
  - it is activated by fusion with other proteins following chromosomal breaks

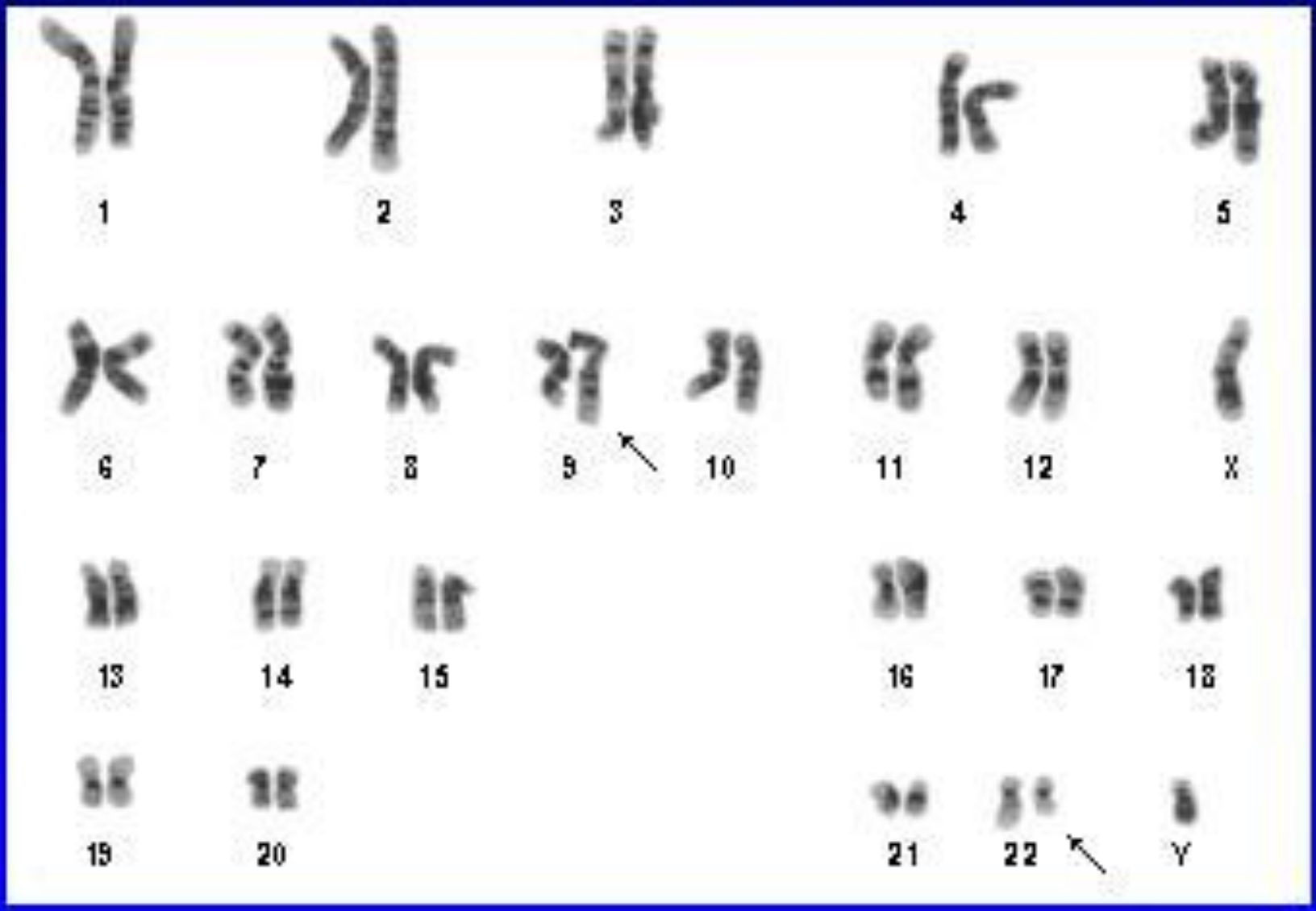
Philadelphia chromosome:
an abnormal chromosome t(9:22) resulting in creation of bcr-abl fusion protein, with enhanced tyrosine kinase activity conferring growth factor independent growth.
This oncogene is common in adult chronic myelogenous leukemia.



**-translocación recíproca entre crom. 9 y 22

El protooncogen c-abl se fusiona con el gen bcr y el oncogen híbrido resultante bcr/c-abl es transcripcionalmente activo; el ciclo celular se desregula - se produce la leucemia mieloide crónica. Leucocitos únicos portadores del evento de translocación actuarían como origen de la patología.

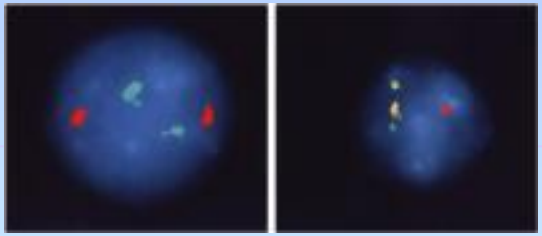
Karyotype Of A Cell With t(9;22) In Current Era



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).

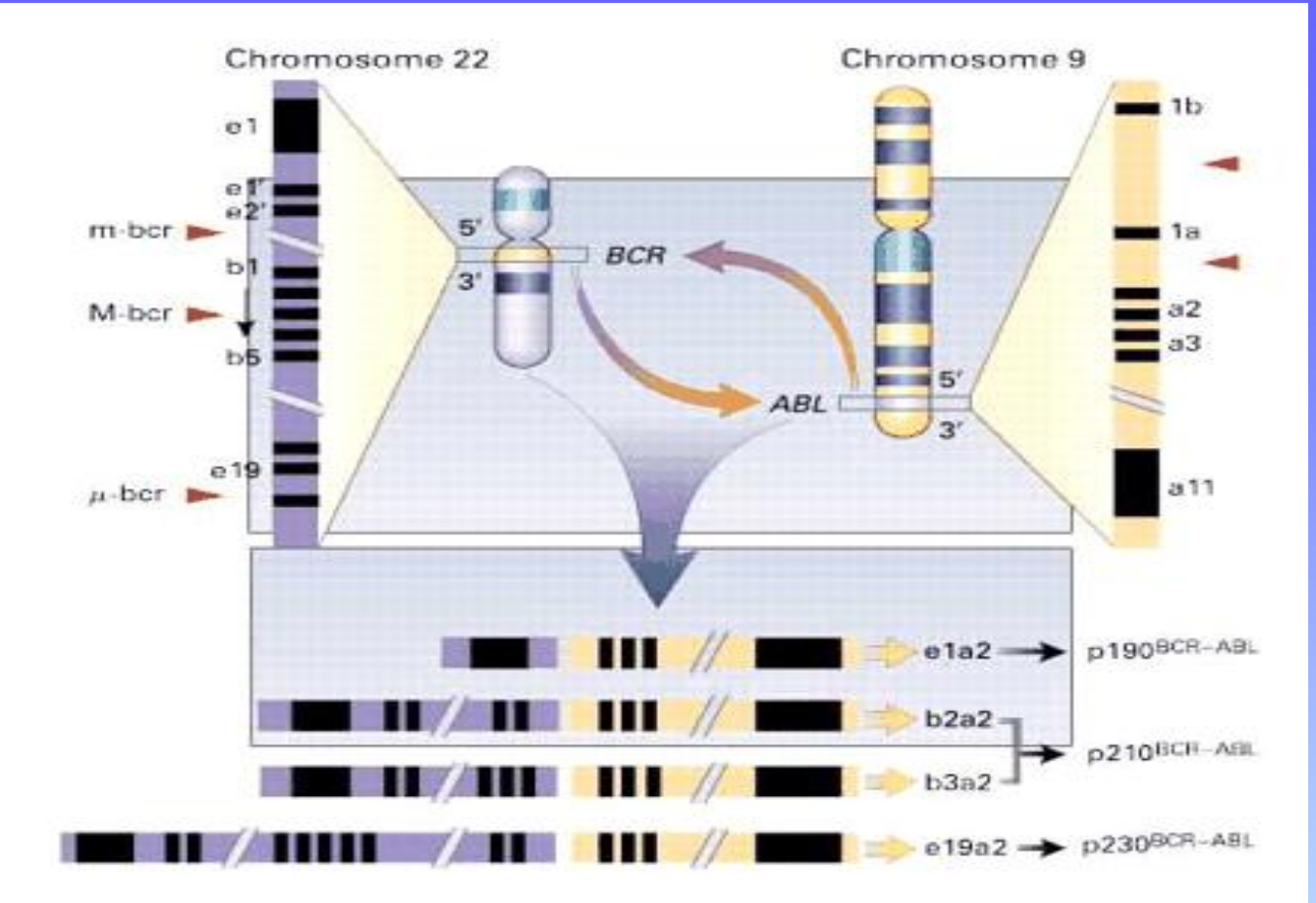


Figure 1. The Translocation of t(9;22)(q34;q11) in CML. The Philadelphia (Ph) chromosome is a shortened chromosome 22 that results from the translocation of 3' (toward the telomere) *ABL* segments on chromosome 9 to 5' *BCR* segments on chromosome 22. Breakpoints (arrowheads) on the *ABL* gene are located 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.

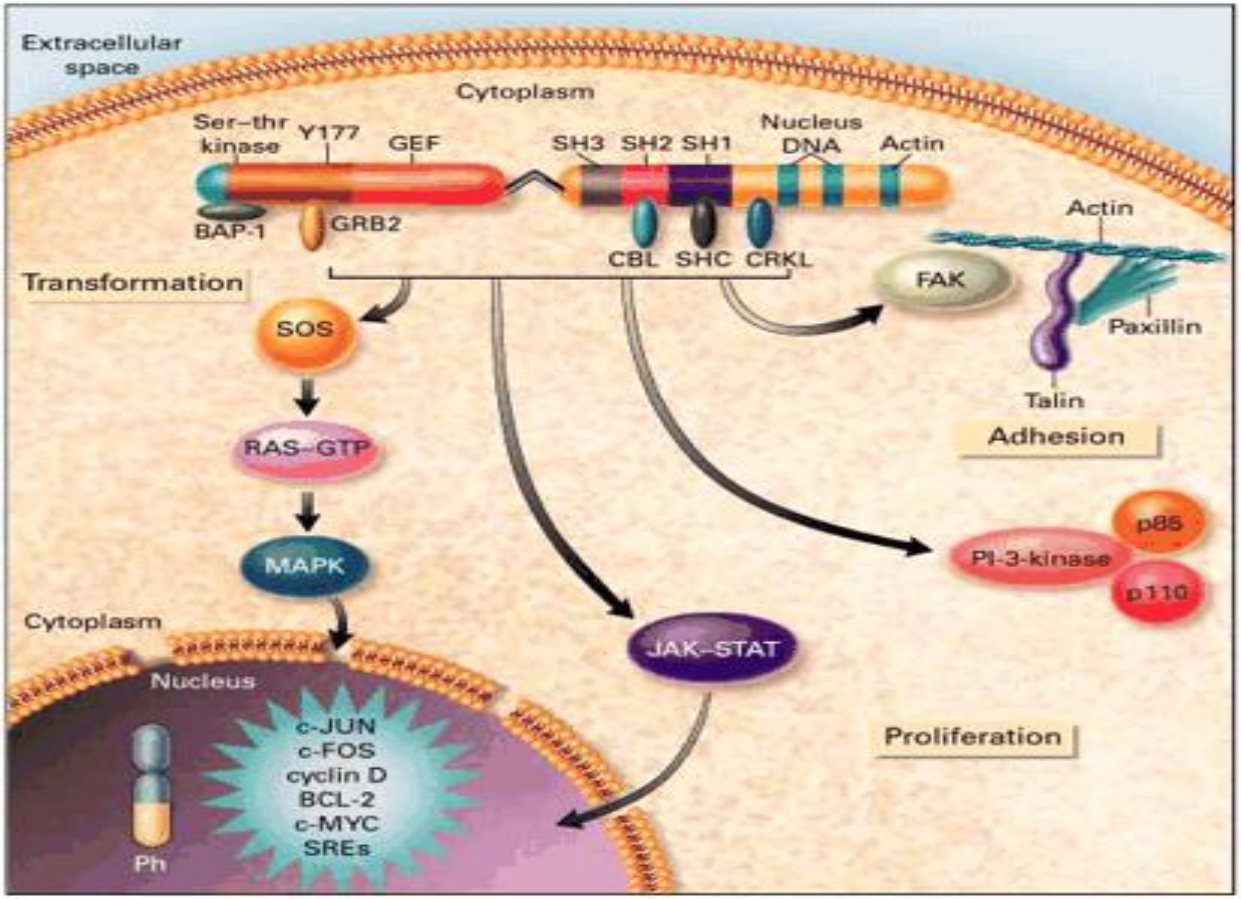
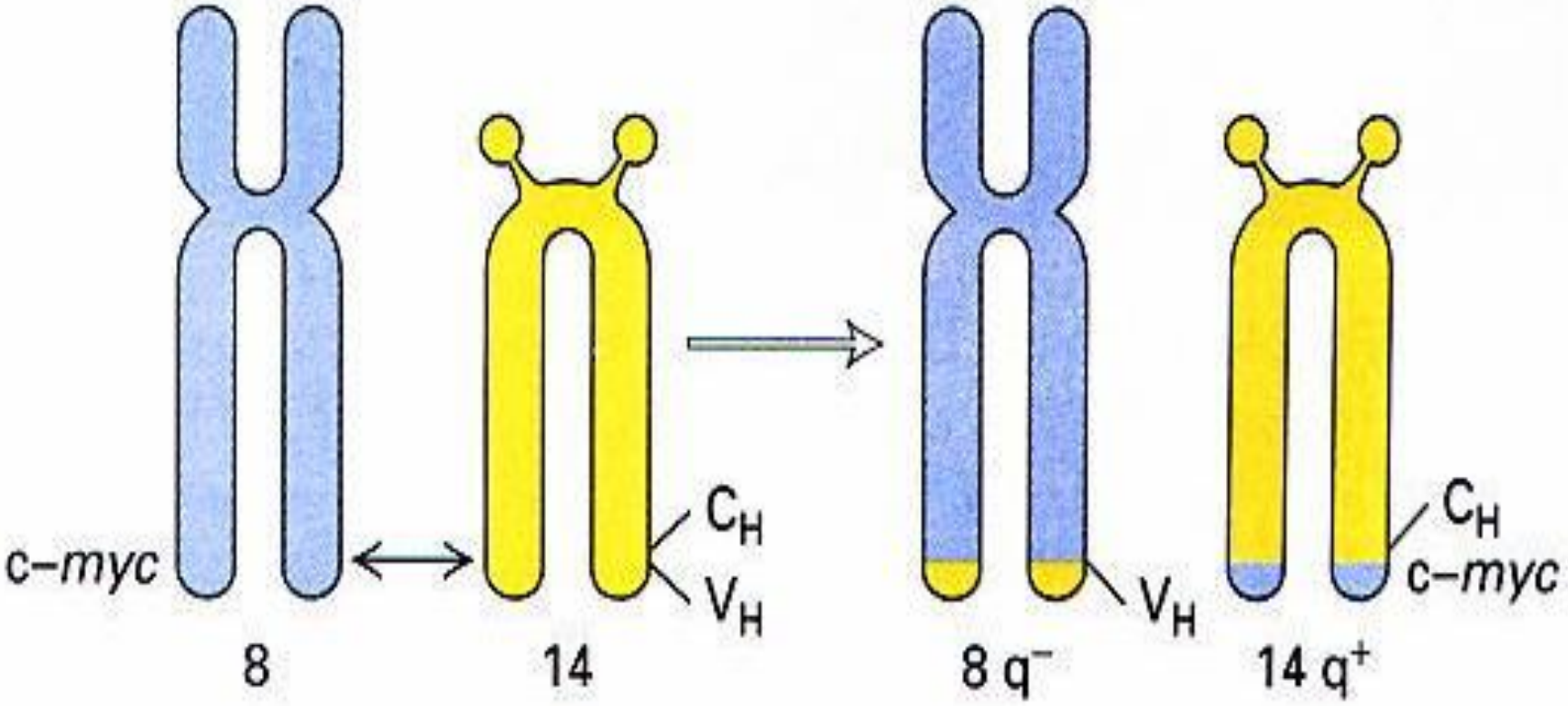


Figure 3. Signaling Pathways of $p210^{BCR–ABL}$. Several regions of BCR–ABL serve as important control elements for RAS, which is at the center of the most prominent signaling pathways in CML (see Fig. 2 and Table 2). Activation of RAS is mediated through a series of adapter proteins, such as GRB2, 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.

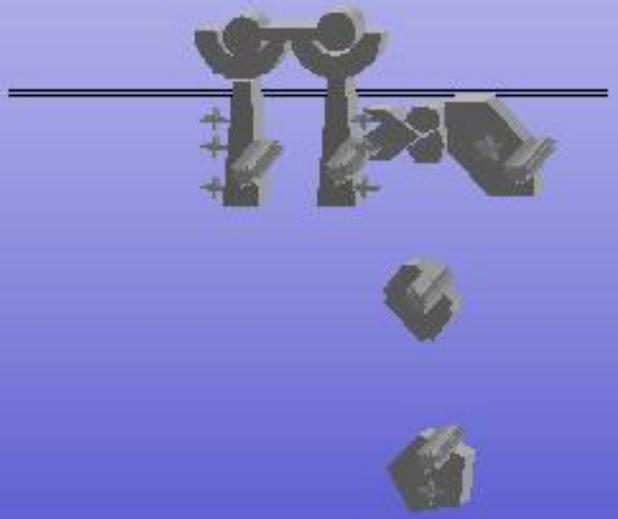
BAP-1 denotes BCR-associated protein 1, GRB2 growth factor receptor–bound protein 2, CBL casitas B-lineage lymphoma protein, SHC SRC homology 2–containing protein, CRKL CRK-oncogene–like protein, JAK–STAT Janus kinase–signal transducers and activators of transcription, FAK focal adhesion kinase, SOS 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.



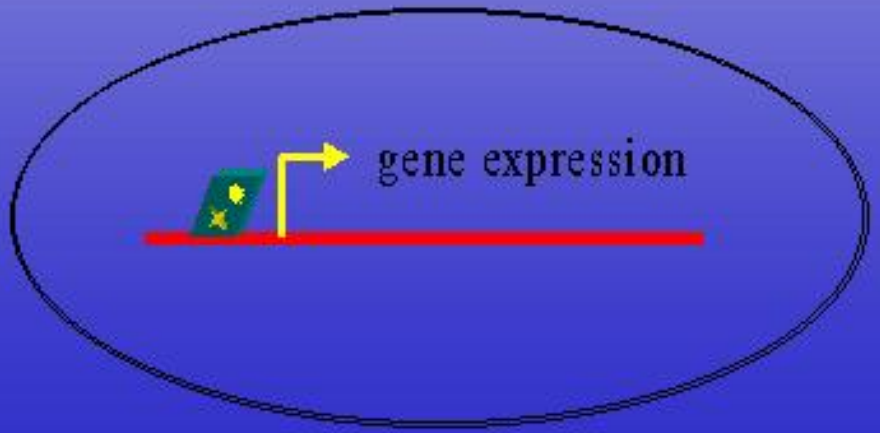
▲ FIGURE 24-22 Chromosomal translocation in Burkitt's lymphoma. This leads to overexpression of the Myc transcription factor.

myc

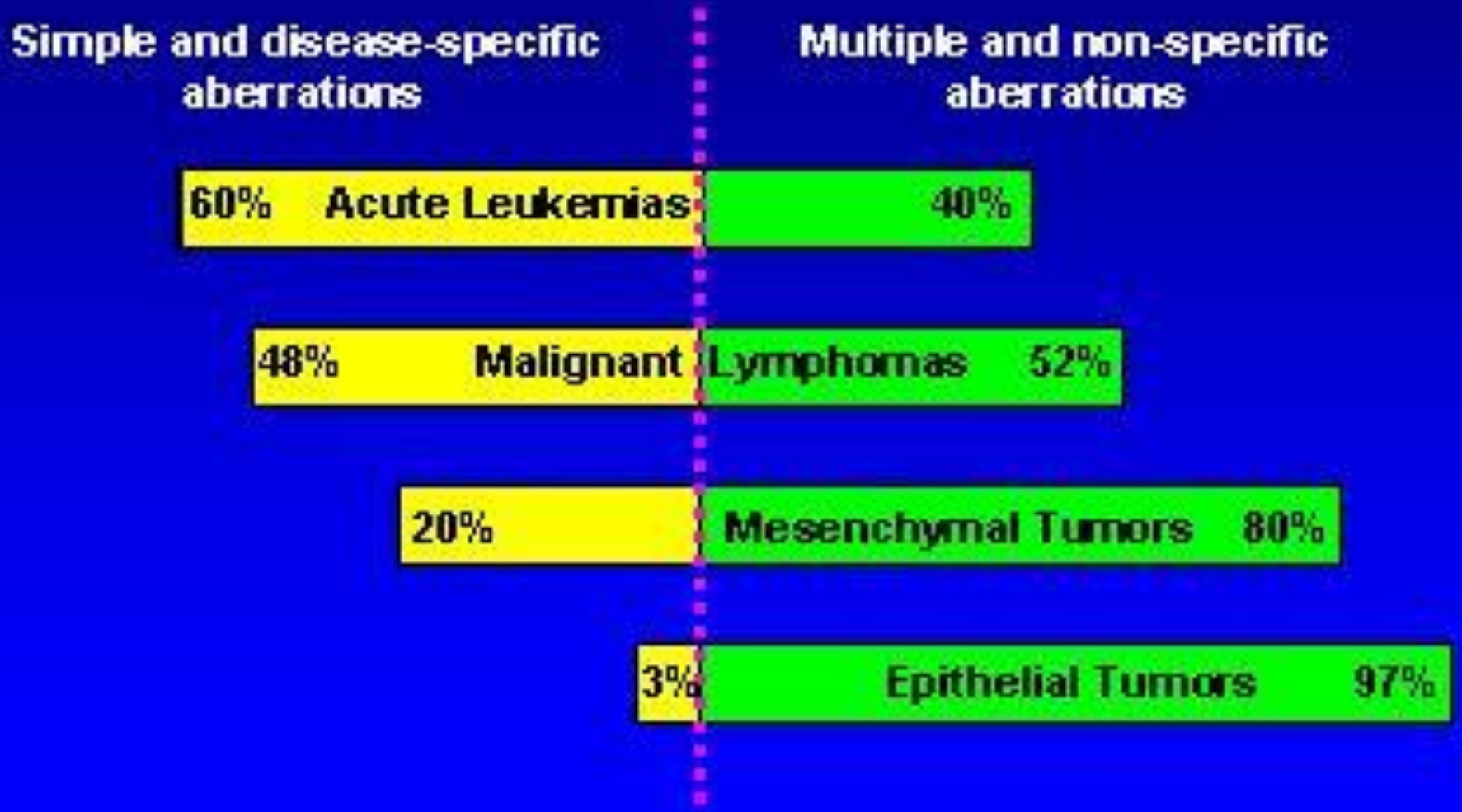
- *transcription factor*
- amplification, retroviral insertion



- transcription factors such as myc or jun can have oncogenic activity by mutation or misexpression
- the mechanism of oncogenesis is not clear for many of these genes, but is likely to involve misregulation of downstream genes



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

MECANISMOS DE ACTIVACION DE ONCOGENES

3- Activación por amplificación génica:

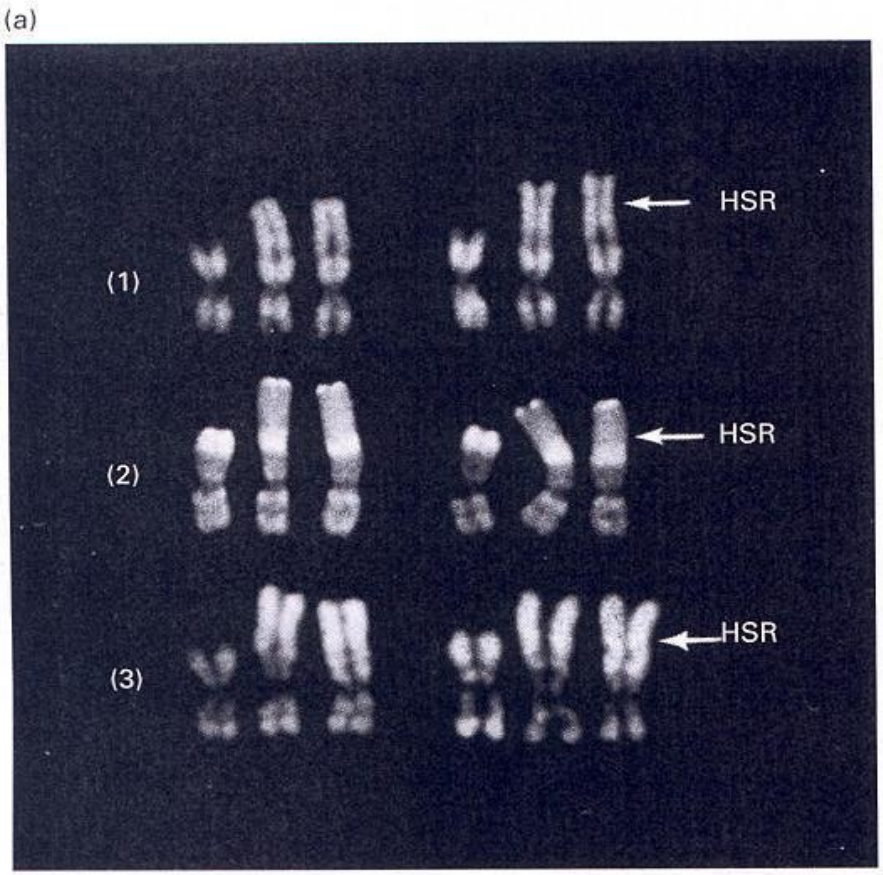
✓ **Reduplicación de un proto-oncogen hasta varios cientos de veces en el mismo cromosoma**

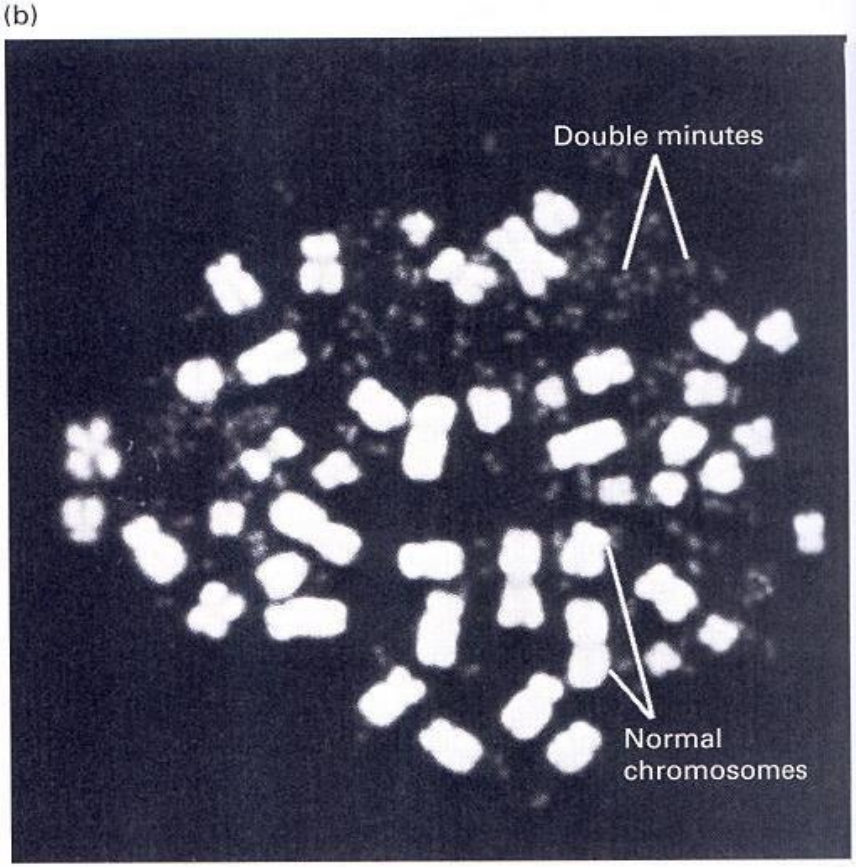
✓ **Resulta en la aparición de regiones de tinción homogéneas (HSR's) en los cromosomas, y /o la presencia de pequeñas porciones de DNA llamadas double minutes (DM's)**

✓ **La amplificación de un protooncogén resultará en incremento de la expresión de la proteína, predisponiendo a la transformación neoplásica.**

Ej: myc (neuroblastoma y cáncer de pulmón de células pequeñas) y neu (c-erb-B2) (cáncer de mama)

✓ **El grado de amplificación incrementa la agresividad de los tumores y puede correlacionar con la sobrevida.**





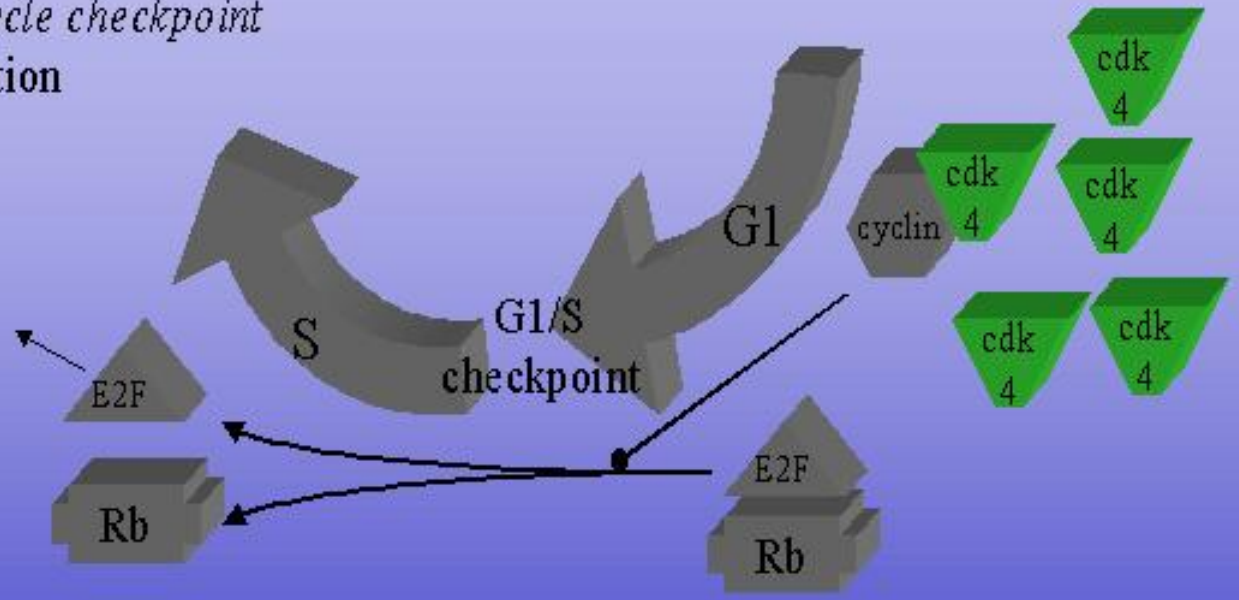
▲ **FIGURE 24-23 Visible DNA amplifications.** (a) Homogeneously staining regions (HSRs) in chromosomes from two neuroblastoma cells. In each set of three chromosomes, the left-most one is a normal chromosome 1 and the other two are HSR-containing chromosomes. The three preparations (1, 2, and 3) represent 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.]

Mutational mechanisms of oncogenes: overexpression/amplification

- the simple overexpression, in the absence of alterations in sequence, is sufficient to activate some oncogenes
- chromosomal amplifications can lead to an increase in gene dosage and so increase gene expression levels
  - amplicons are typically 200 to 2000 kb in size, and so can contain many genes
  - amplicons can be present in dozens to hundreds of copies
- chromosomal breaks and fusions can bring genes under the control of transcriptionally active regions of DNA.
  - for example translocations can bring genes into the Ig locus in B-lymphocytes.

cdk4

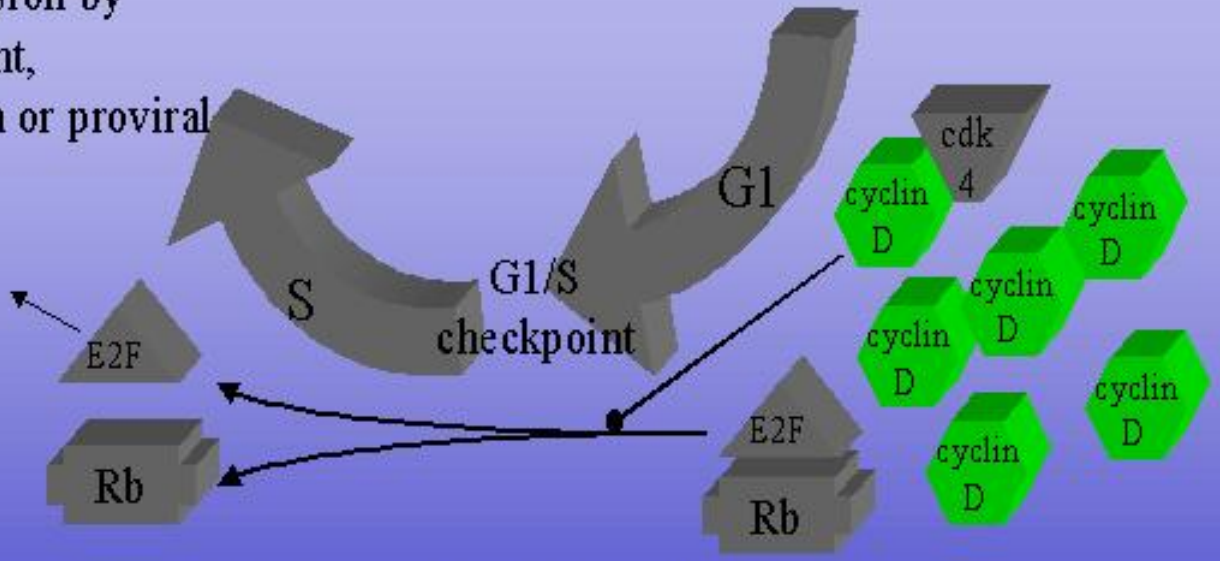
- *G1 cell cycle checkpoint*
- **amplification**



Amplification of the gene for cdk4 at the genomic level can lead to the forcing of the G1 checkpoint by overactivity of the cyclin/cdk complexes.

cyclin D

- *G1 cell cycle checkpoint*
- overexpression by rearrangement, amplification or proviral insertion



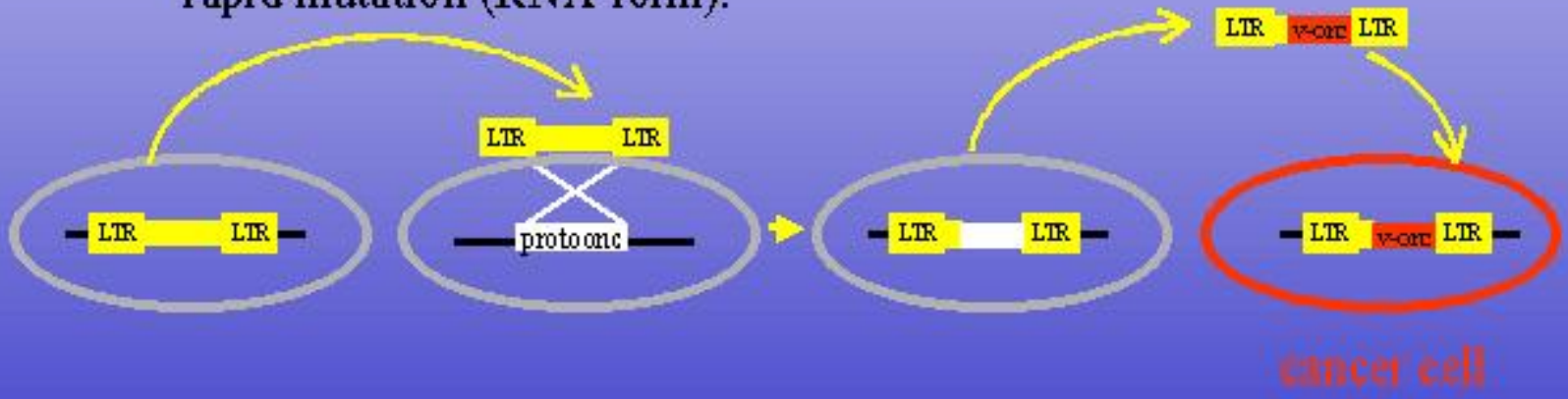
- Overexpression of the cyclin D1 causes deregulation of the G1/S checkpoint.
- It can occur by :
  - rearrangement: PRAD1 in parathyroid carcinomas,
  - amplification in many tumor types
  - or by retroviral insertion: bcl1

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

Mutational mechanisms of oncogenes: viral transduction

- The retroviral life cycle includes a DNA pro-viral stage that is incorporated into the genome. Transcripts generated from this pro-virus are packaged and shed. If these transcripts incorporate (by virtue of recombination events during insertion) cellular genes, then these genes are transduced into the virus, where they can undergo rapid mutation (RNA form).

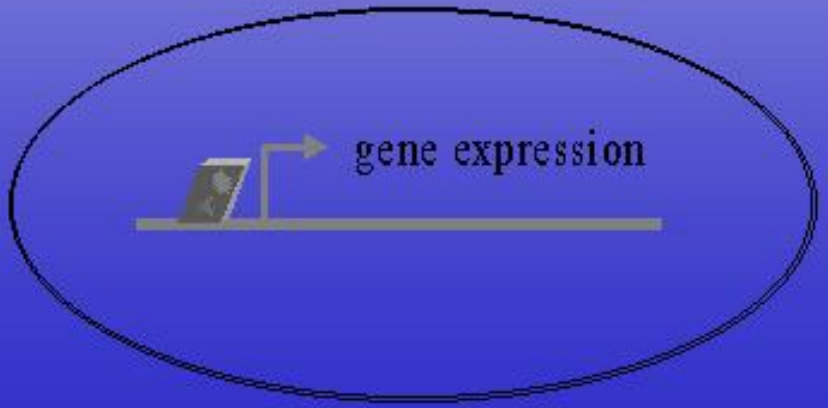


- If this results in an activated v-onc it will provide the virus with a selective advantage by causing its host cell to rapidly divide.

v-sis

- *growth factor*
- overexpression by viral transduction

- c-sis is the cellular protooncogene encoding PDGF-B chain growth factor.
- v-sis is a virally encoded oncogene. Cells that are infected with viruses carrying v-sis overproduce PDGF like growth factors, causing constitutive growth stimulation
- c-sis can also become overexpressed in some tumors in the absence of viral involvement
- occurs in sarcomas and astrocytomas



1- Mutaciones en proto-oncogenes resultando en un estímulo proliferativo para la célula

Se pueden identificar 5 categorías:

1.1- Factores de crecimiento

1.2- Receptores de factores de crecimiento

1.3- Proteinas transductoras de señales (no receptores) con actividad kinasa

1.4- Proteínas G transductoras de señales

1.5- Factores reguladores nucleares

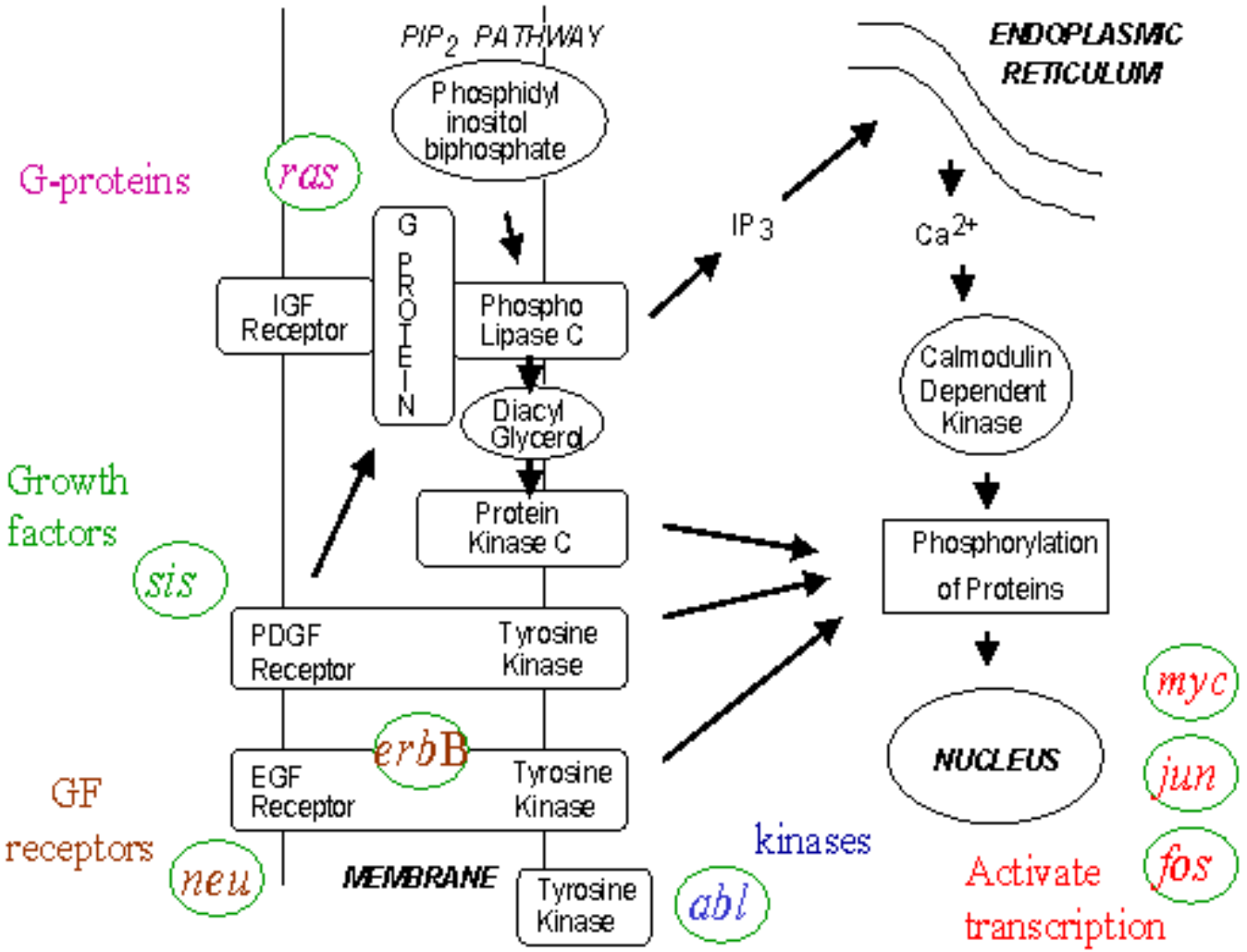
Factores de crecimiento

- ✓ **Por ejemplo PDGF, FGF .**
- ✓ **Sobreexpresión de PDGF, exceso de secreción por la célula, resulta en proliferación celular por mecanismo de feed-back autócrino.**
- ✓ **Asociado con astrocitomas y osteosarcomas humanos.**
- ✓ **Similarmente hst-1 y hst-2 sobreexpresan FGF. Asociado con cáncer de estómago, vejiga y mama y con melanoma.**

Receptores de Factores de crecimiento

- ✓ **Receptores para EGF y CSF-1 han sido implicados en neoplasia.**
- ✓ **Estos receptores son normalmente receptores de transmembrana y poseen una kinasa en su cara citoplasmática**

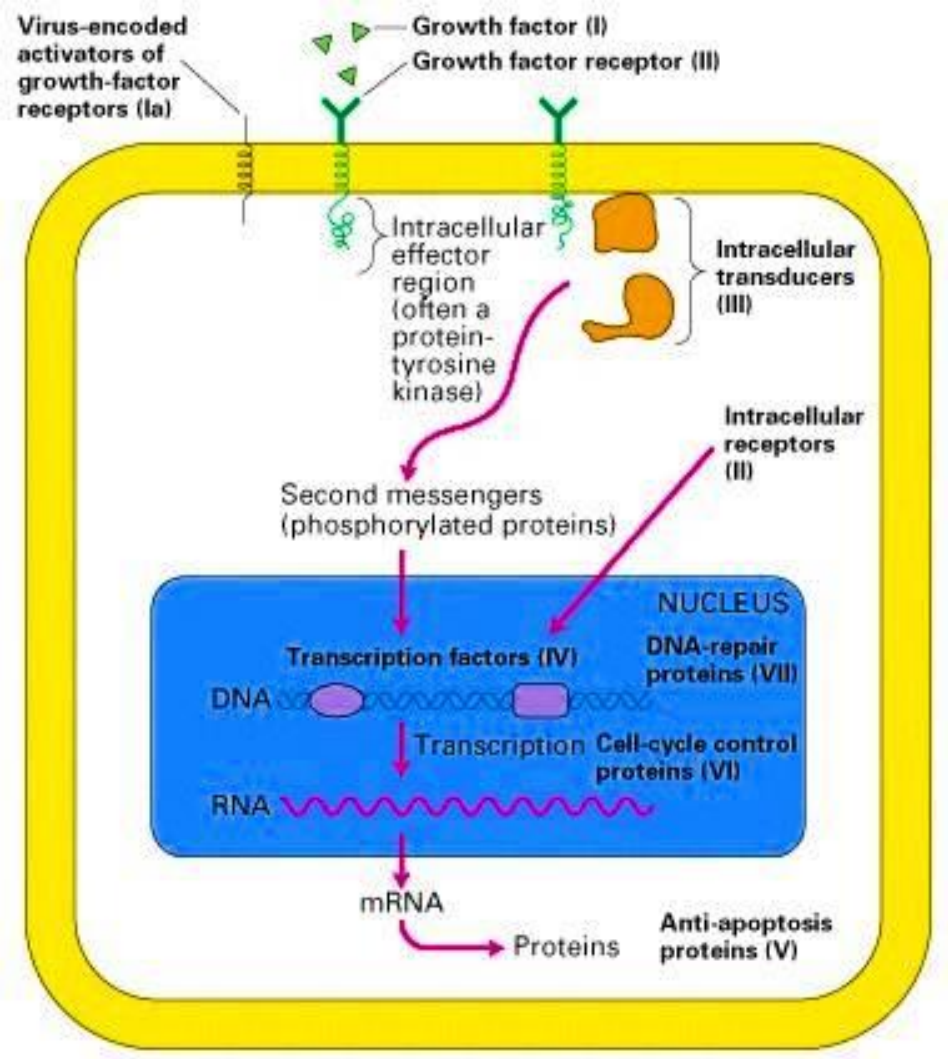
PROLIFERATIVE ONCOGENES



ONCOGENES PROTOTIPICOS= PROPIEDADES

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

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**

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

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

CYTOPLASM

Signal Transducers

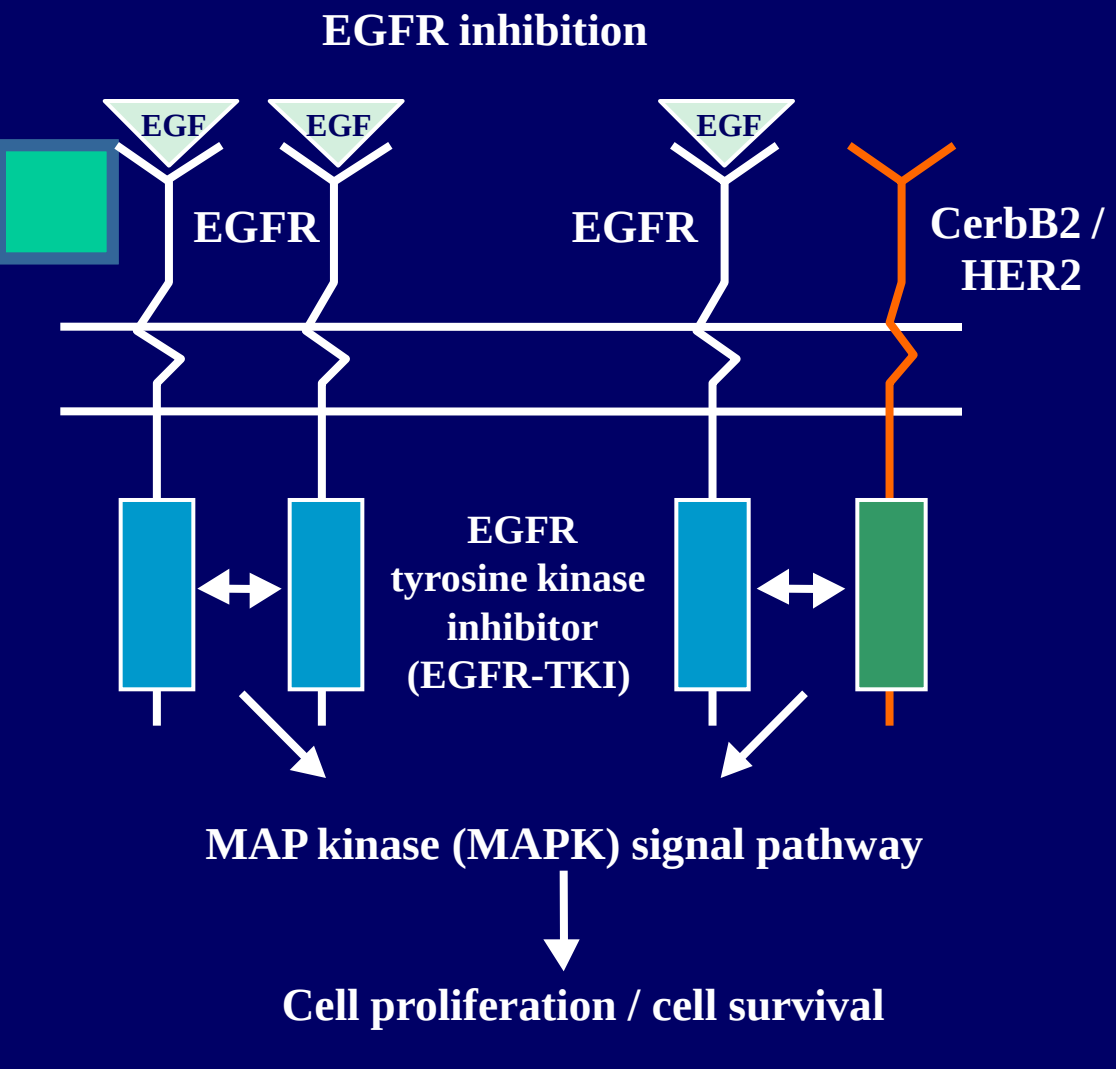
v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

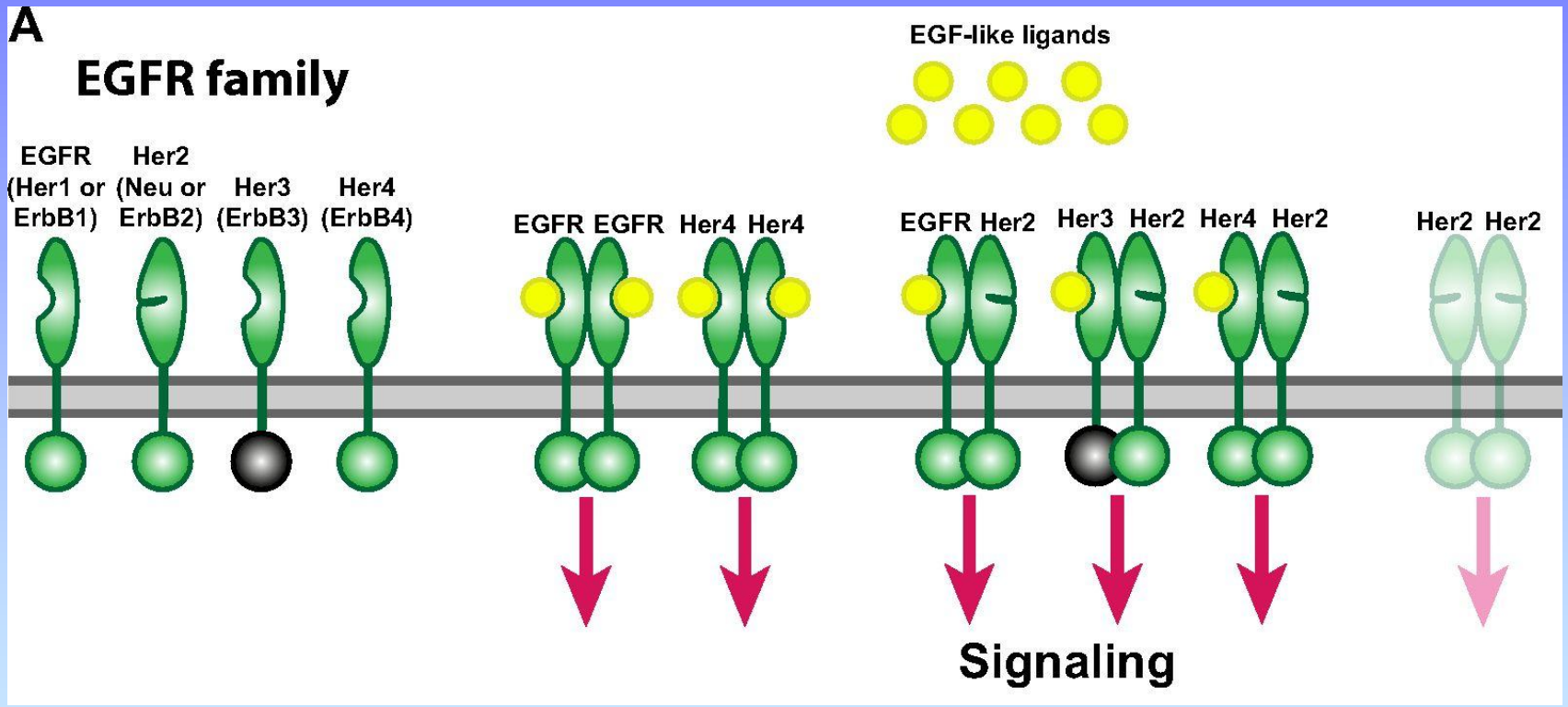
NUCLEUS

Transcription Factors

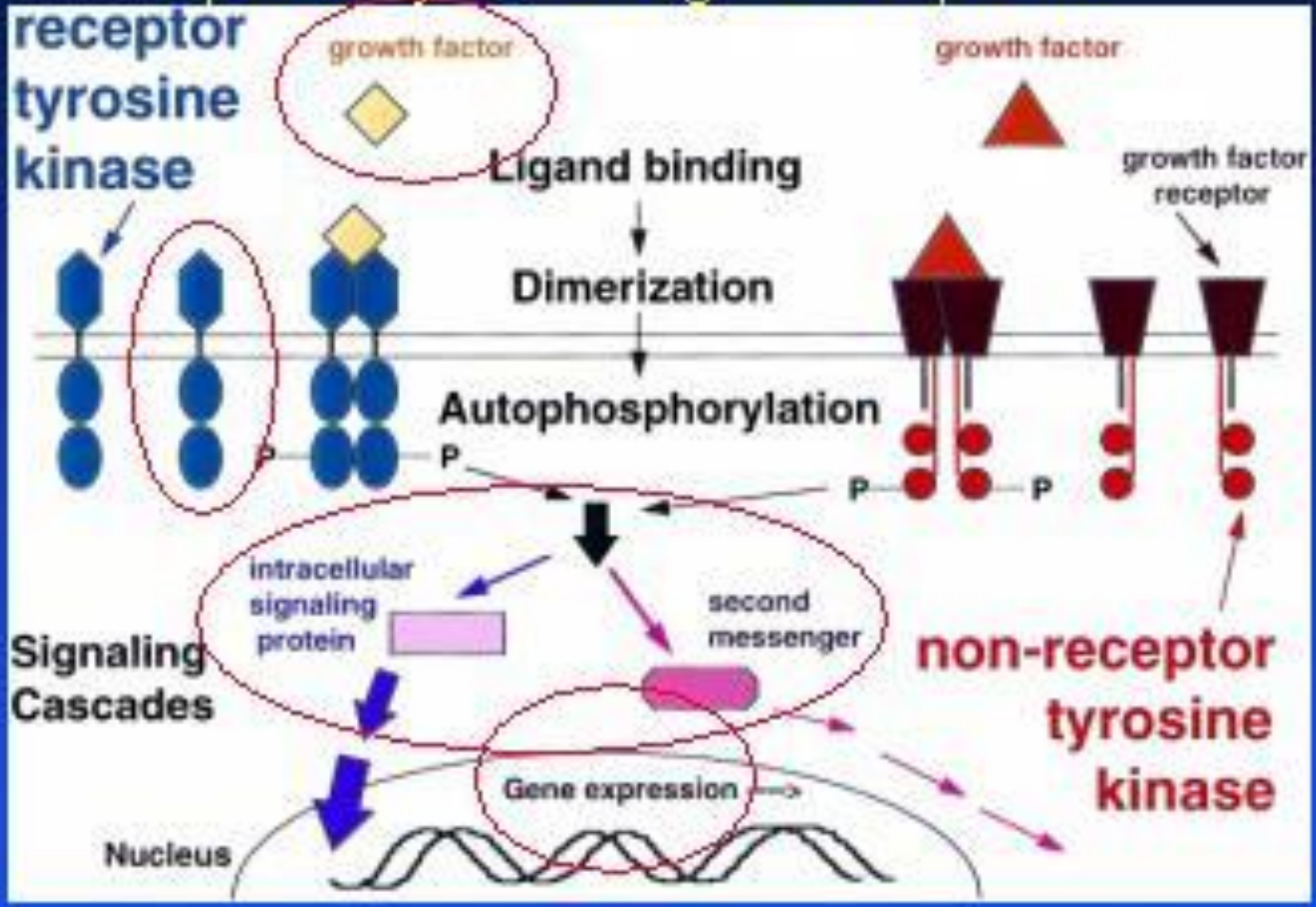
v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

Epidermal growth factor receptor (EGFR)

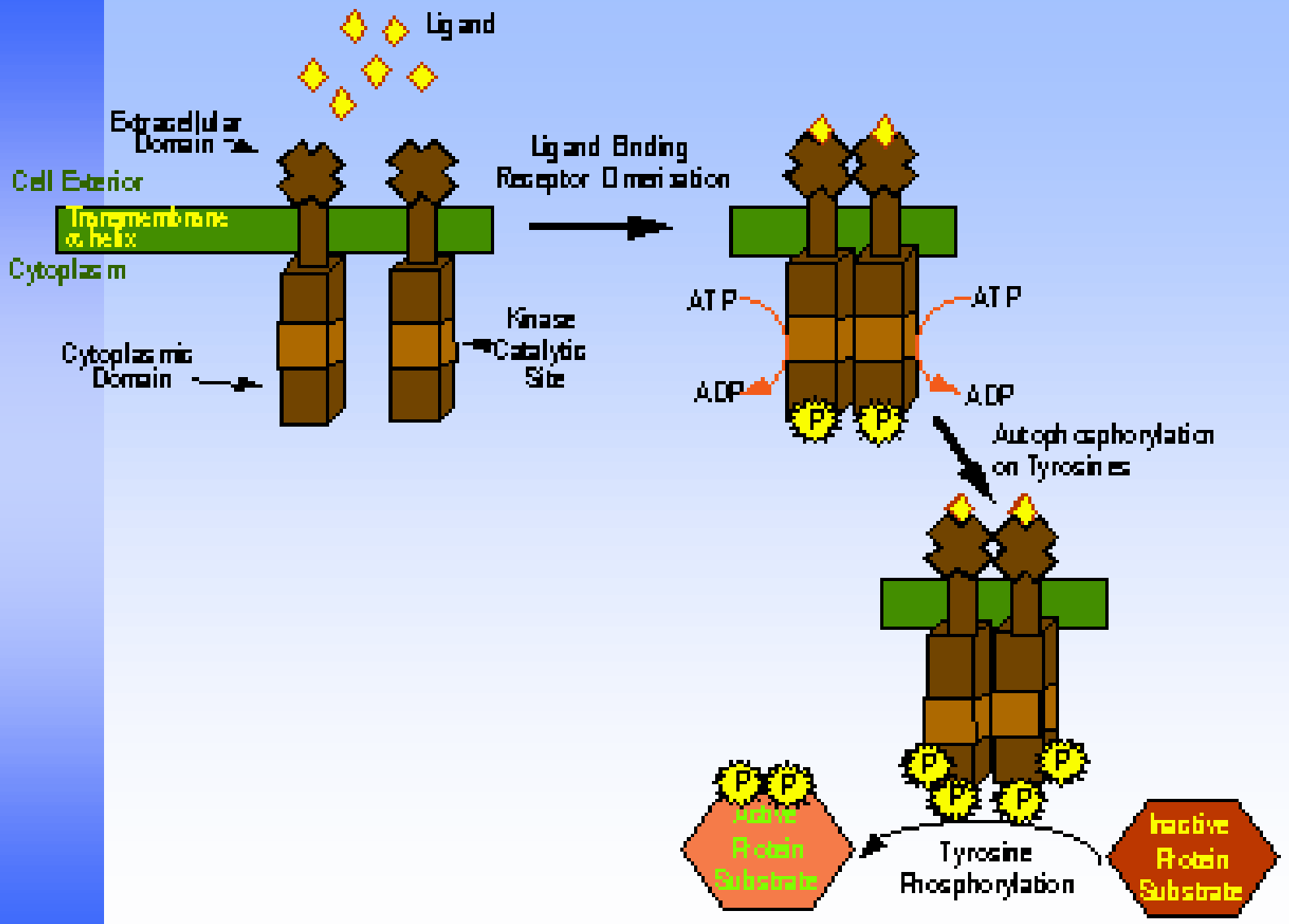



A
EGFR family
EGF-like ligands
EGFR
(Her1 or
ErbB1)
Her2
(Neu or
ErbB2)
Her3
(ErbB3)
Her4
(ErbB4)
EGFR EGFR
Her4 Her4
EGFR Her2
Her3 Her2
Her4 Her2
Her2 Her2
Signaling

Receptor Tyrosine Kinases: Determinants of Specificity of Biological Response



Kolibaba & Druker BBA 1333:F217, 1997


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

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

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

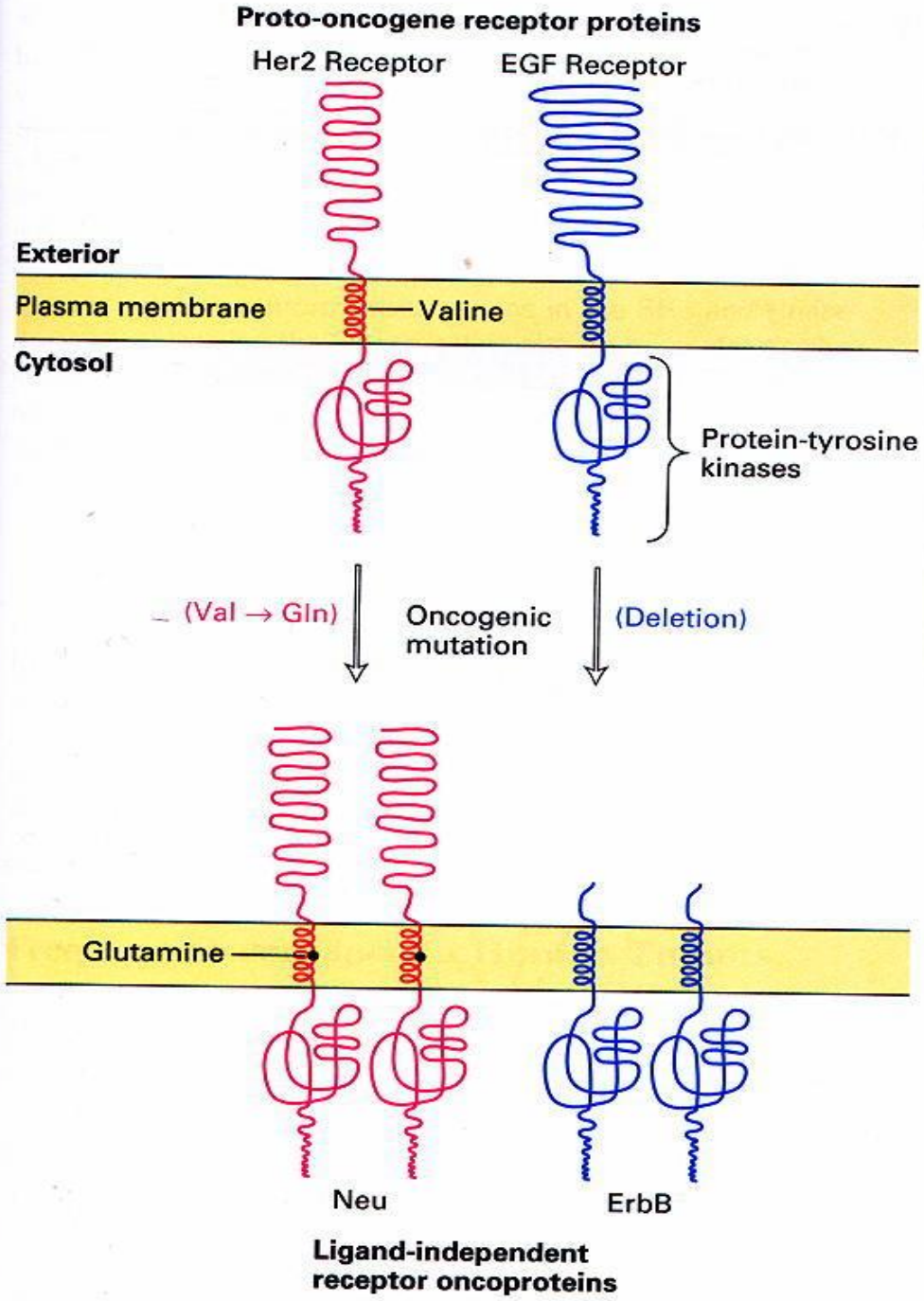
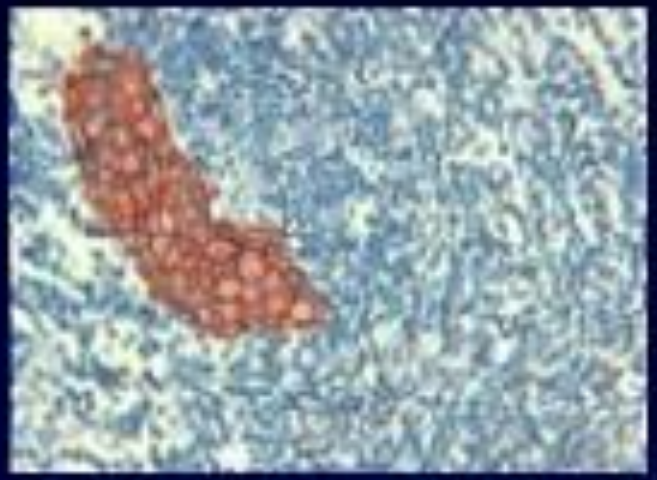


FIGURE 24-15 Effects of oncogenic mutations in proto-oncogenes that encode cell-surface receptors. *(Left)* A mutation that alters a single amino acid (valine to glutamine) in the transmembrane region of the Her2 receptor causes dimerization of two receptor proteins in the absence of the normal EGF-related 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.

HER-2 Oncogene
Amplification

Breast Cancer



HER-2 Oncoprotein
Overexpression

Shortened Survival

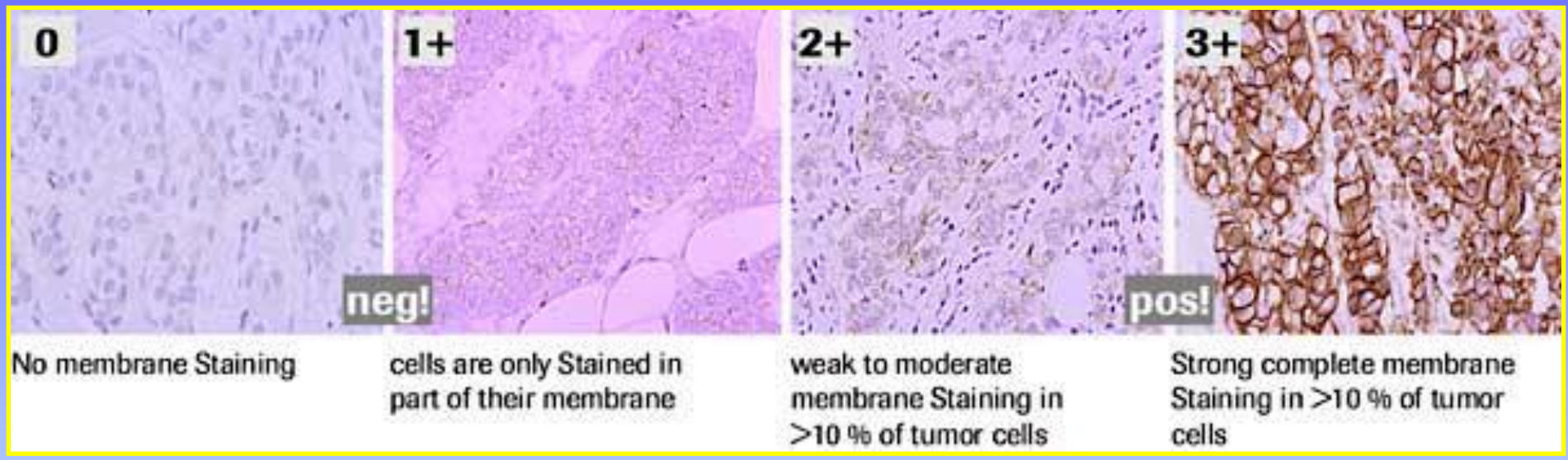
Median Survival from First Diagnosis

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

Slamon et al, 1987

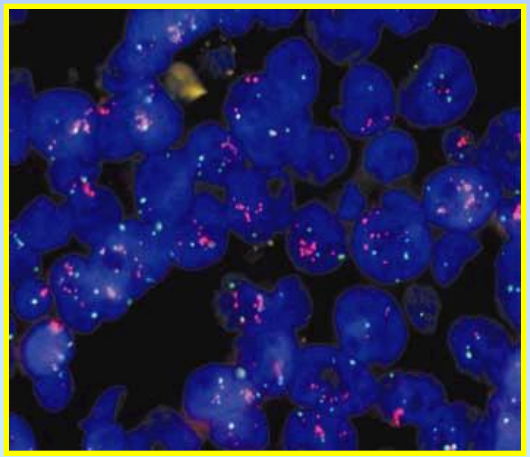
Determination of HER2-protein overexpression

1- semiquantitative DAKO Hercep Test™



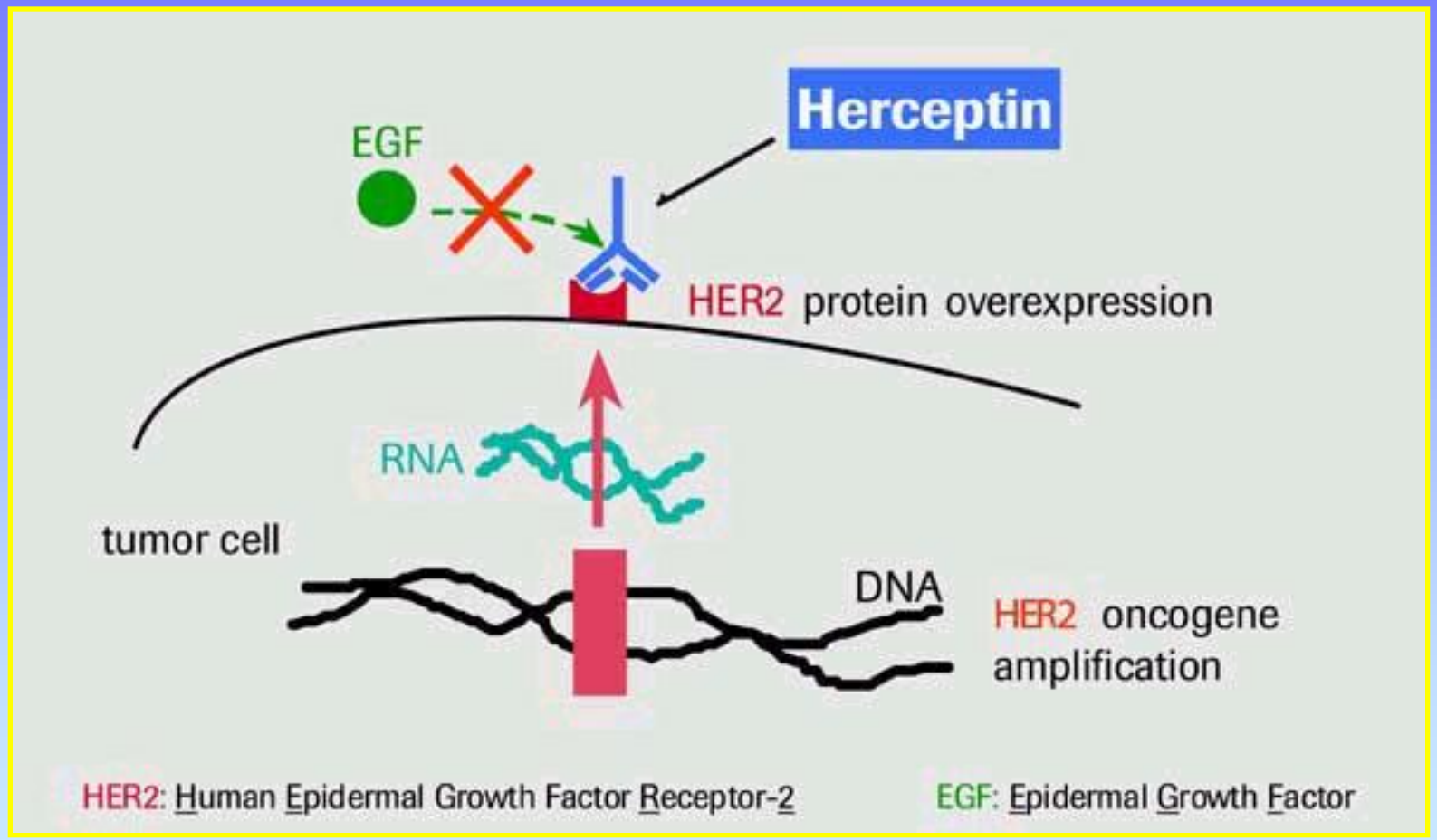
2- Fluorescence in situ hybridisation (FISH)

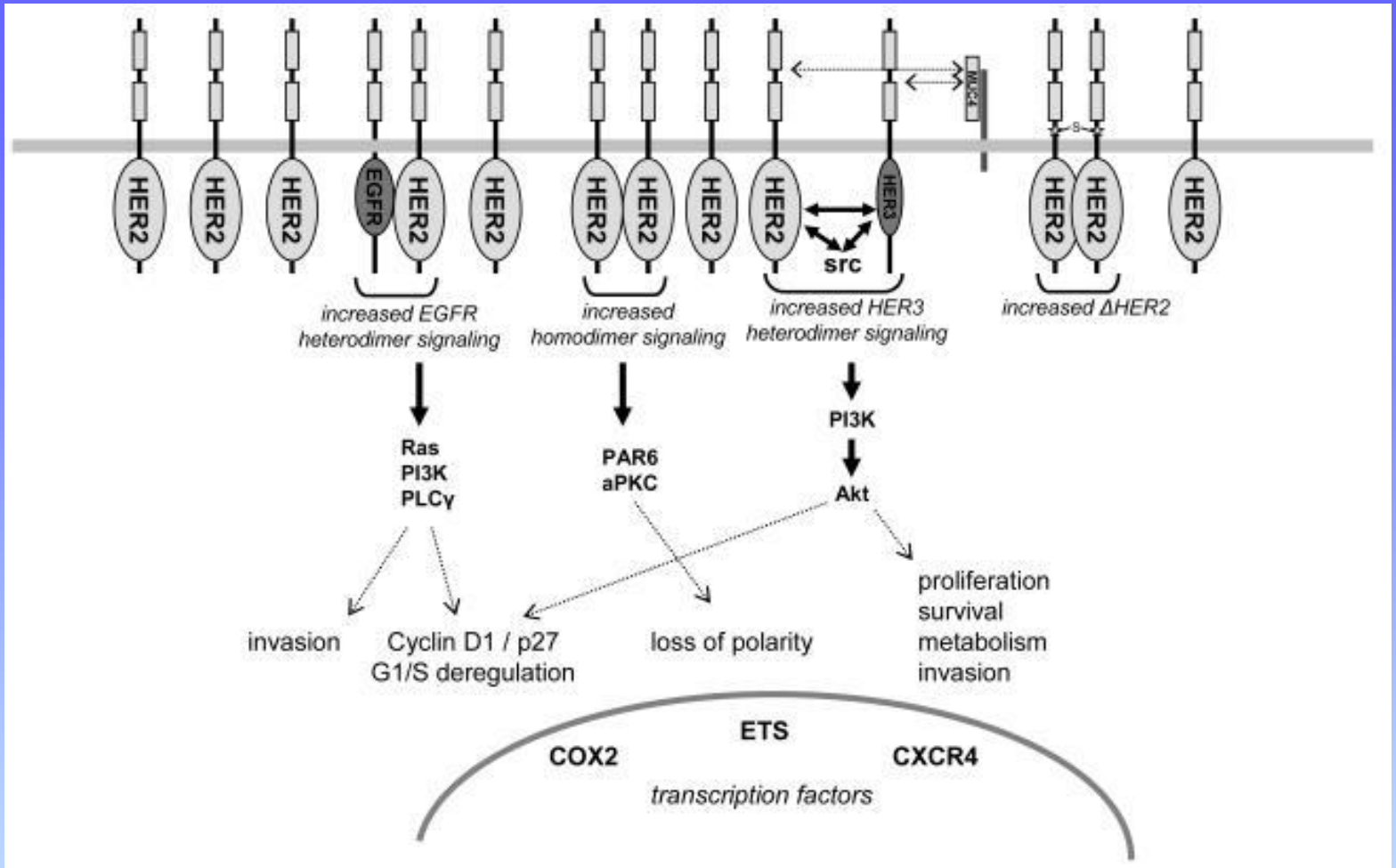
Paraffin section of breast tissue, hybridisation with HER2-specific probe showing *HER2* gene amplification



CIO-FUCA

HERCEPTIN BLOCKS HER2/neu protein





Schematic of the signaling abnormalities resulting from HER2 overexpression that are felt to contribute to tumorigenesis. HER2 overexpression results in increased HER2 containing dimers of all kinds. Increased HER2-EGFR dimers drive proliferative and invasive functions. Increased HER2 homodimers disrupt cell polarity. Increased HER2-HER3 dimers drive proliferative, survival, invasive, and metabolic functions. Increased HER2 expression results in an increase in the rare ΔHER2 isoform with more potent signaling characteristics. Several transcription factors are induced in HER2 overexpressing cells resulting in a plethora of gene expression changes

Mechanism of action: Oncogenes as signal transducers

EXTRACELLULAR

Growth Factors

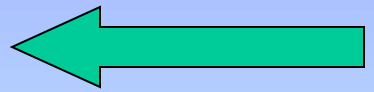
v-sis (PDGF), int-1(WNT-1), int-2(FGF), hst, fgf-5

Growth Factors Receptors

CYTOPLASM

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

Signal Transducers

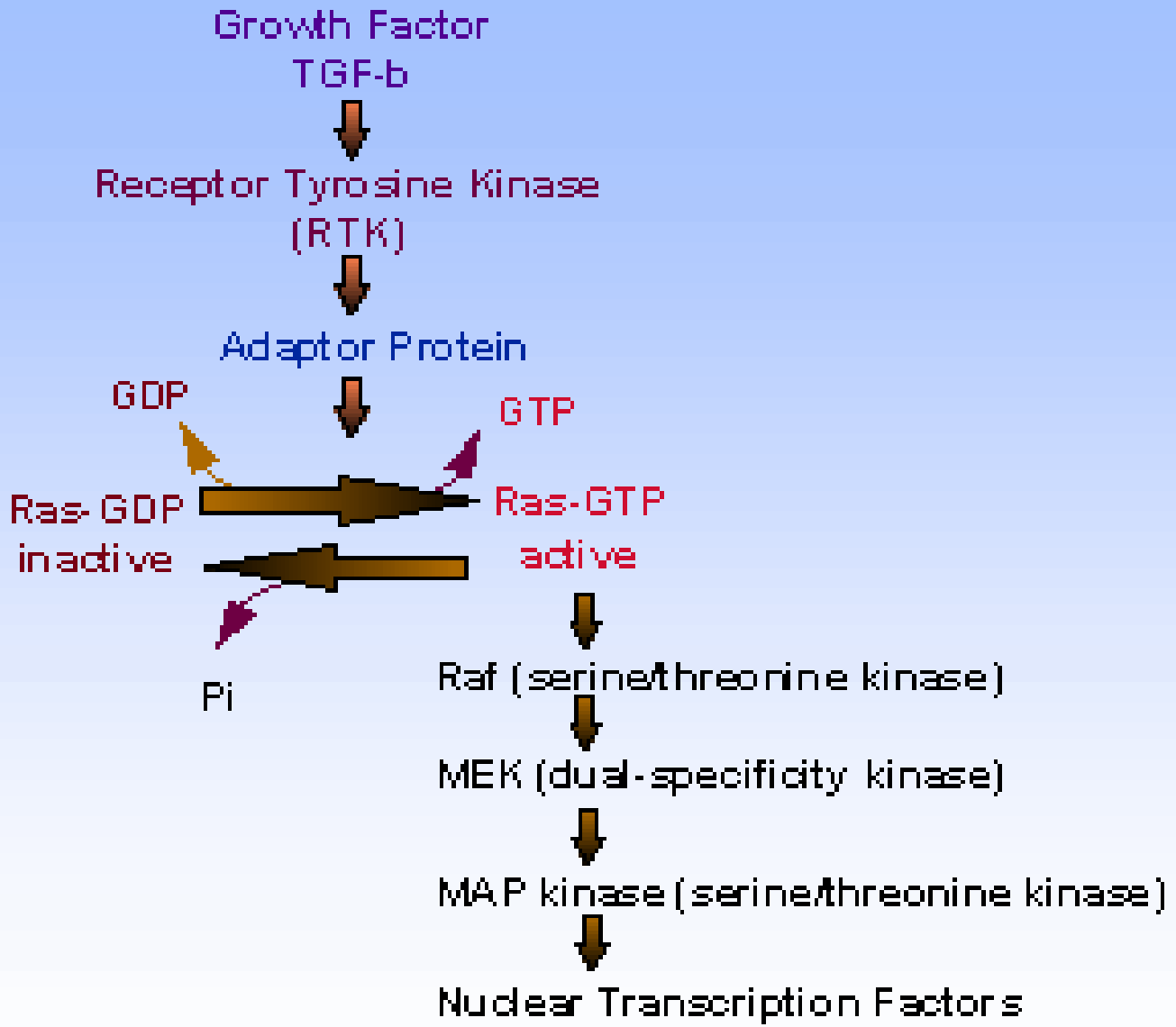


v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

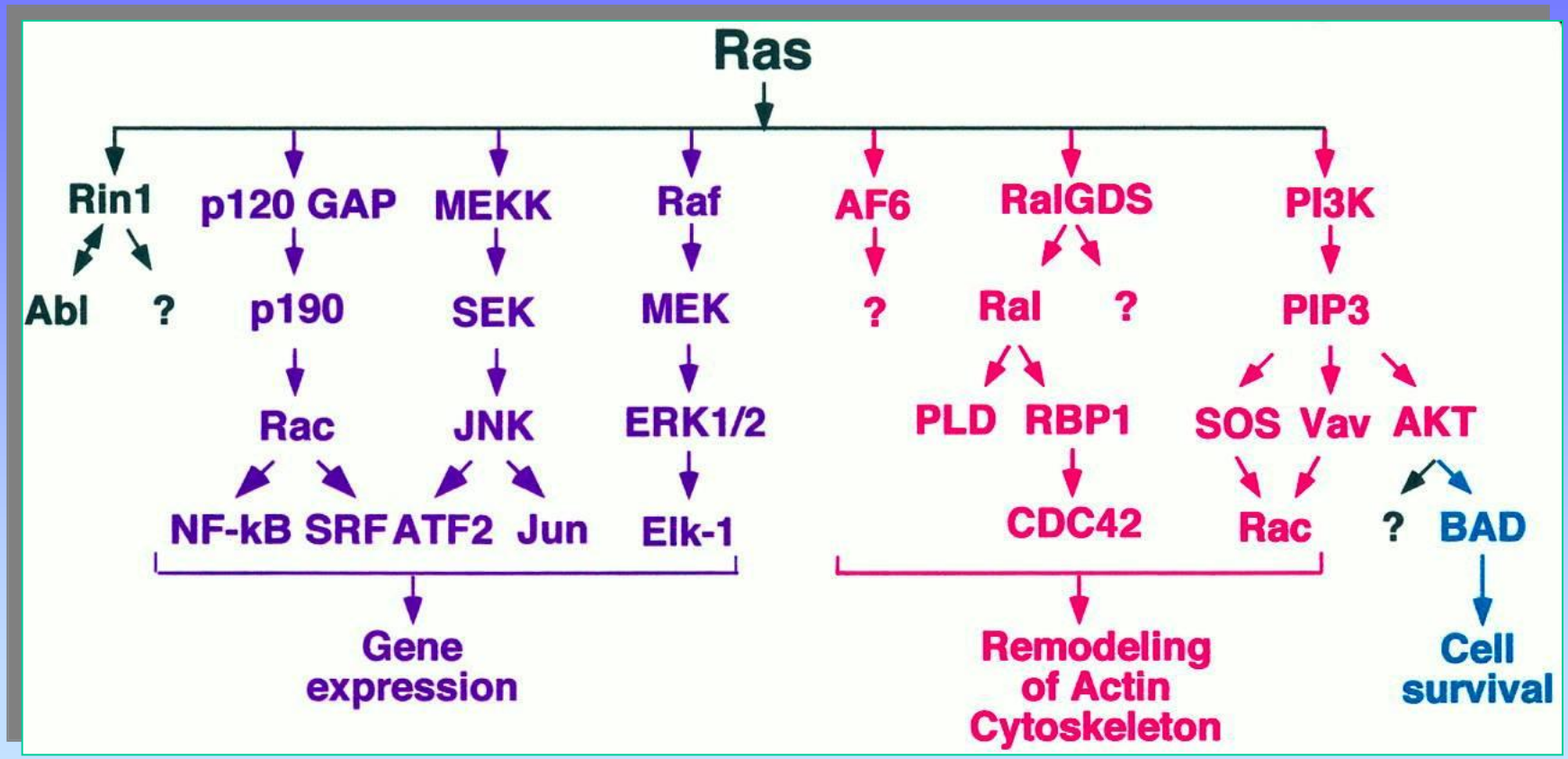
NUCLEUS

Transcription Factors

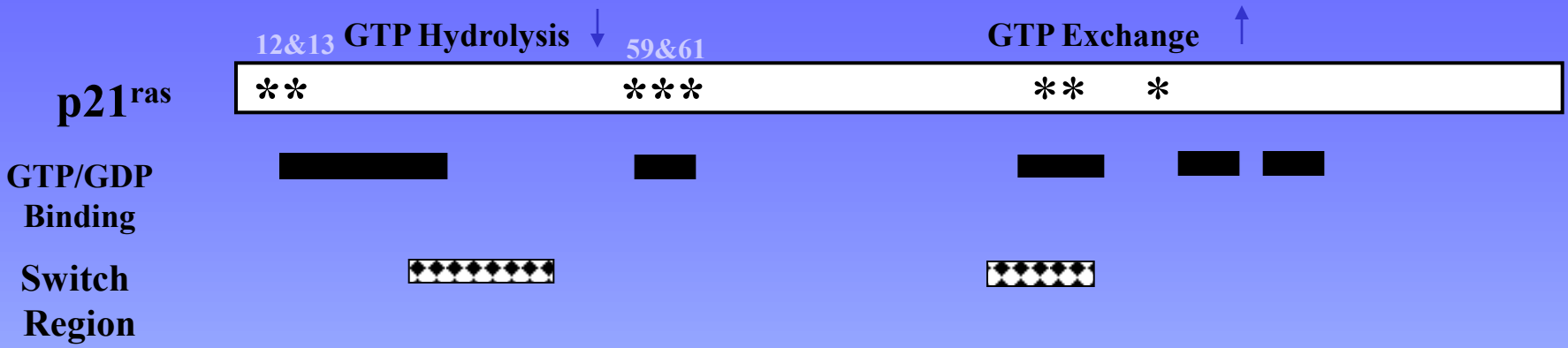
v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

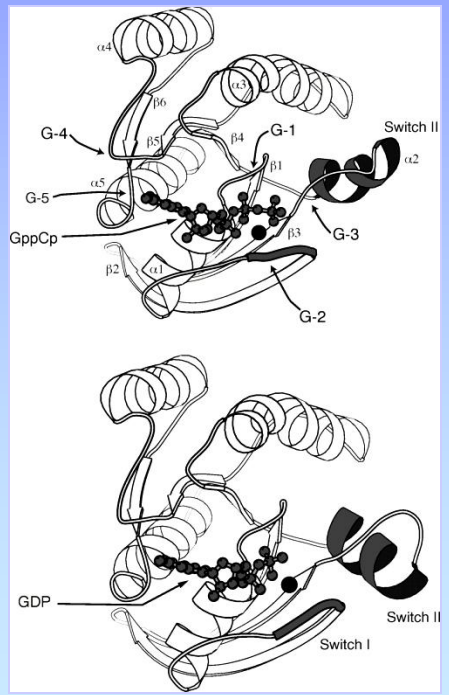

Growth Factor
TGF-b
Receptor Tyrosine Kinase
(RTK)
Adaptor Protein
GDP
GTP
Ras-GDP
inactive
Ras-GTP
active
Pi
Raf (serine/threonine kinase)
MEK (dual-specificity kinase)
MAP kinase (serine/threonine kinase)
Nuclear Transcription Factors

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.

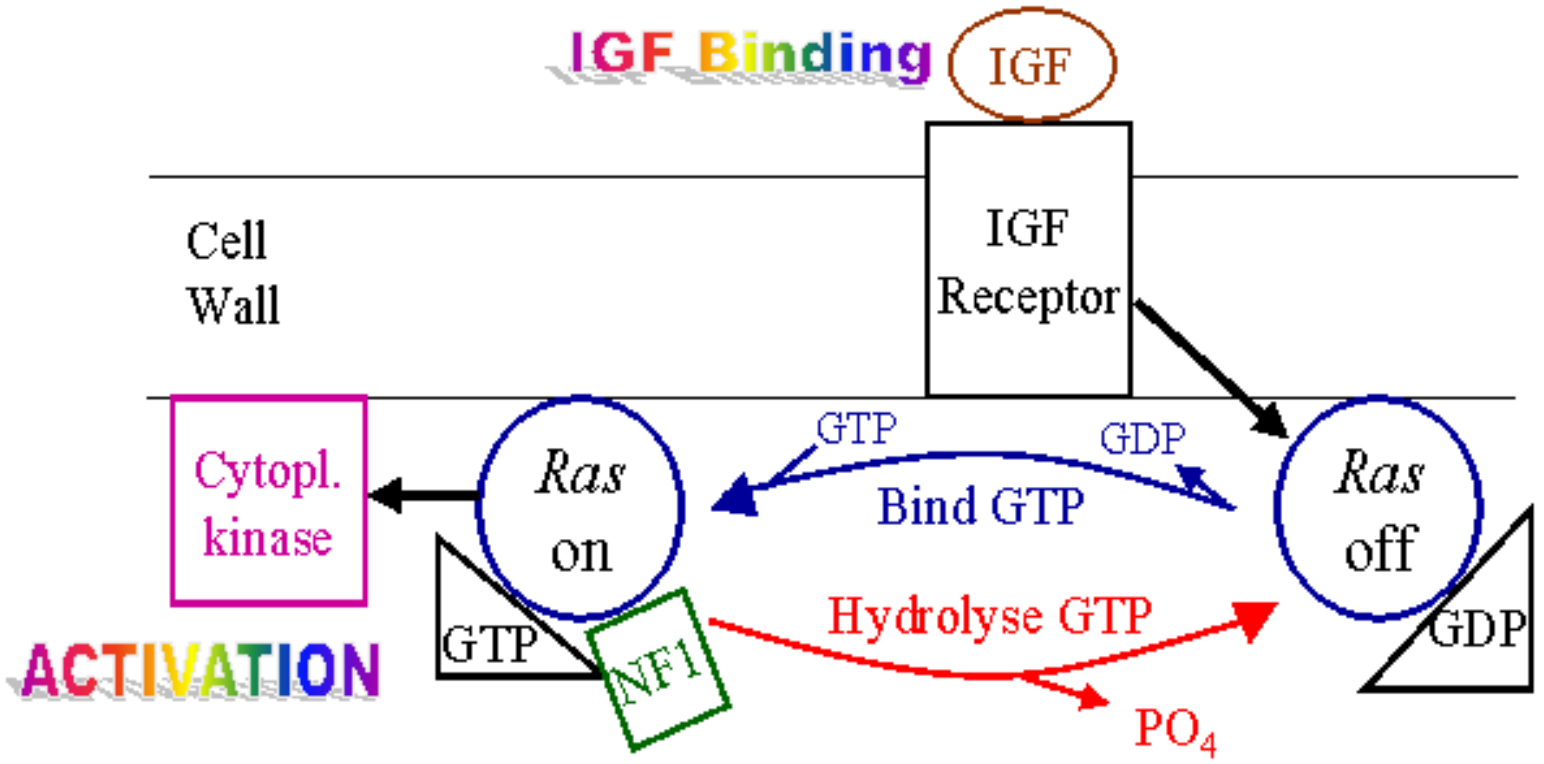
Activated in 90% of pancreatic adenocarcinomas and 50% of colon adenocarcinomas and leukemias.

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

Proteínas G transductoras de señales

- ✓ **Ligandos externos se unen a receptores de la superficie los cuales activan proteínas G (familias de proteínas intermediarias con la superficie celular, ej: ras)**
- ✓ **Las proteínas G se unen al GTP lo cual activa efectores específicos generando segundos mensajeros (ej: PLC o adenilato ciclasa)**
- ✓ **Segundos mensajeros (ej cAMP, cGMP, Ca^{++}, IP, DG)**
- ✓ **Activación de quinasas**
- ✓ **Las proteínas G hidrolizan GTP a GDP desactivamdo la proteína G**
- ✓ **La proteína G cicla otra vez si un complejo ligando-receptor está todavía presente en la superficie celular.**
- ✓ **Proteínas activadoras de GTPasa (GAPs) aceleran la velocidad de la GTPasa (x 1000), actuando como frenos que evitan la actividad descon-trolada de ras.**
- ✓ **Por lo tanto las GAPs normales son genes supresores de tumor**

Proteínas G transductoras de señales



GTPase activating proteins (GAPs) eg NF1 increase GTPase rate

Mutant *ras* slow to hydrolyse GTP

Mechanism of action: Oncogenes as signal transducers

EXTRACELLULAR

Growth Factors

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Growth Factors Receptors

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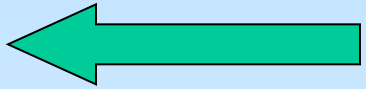
CYTOPLASM

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)

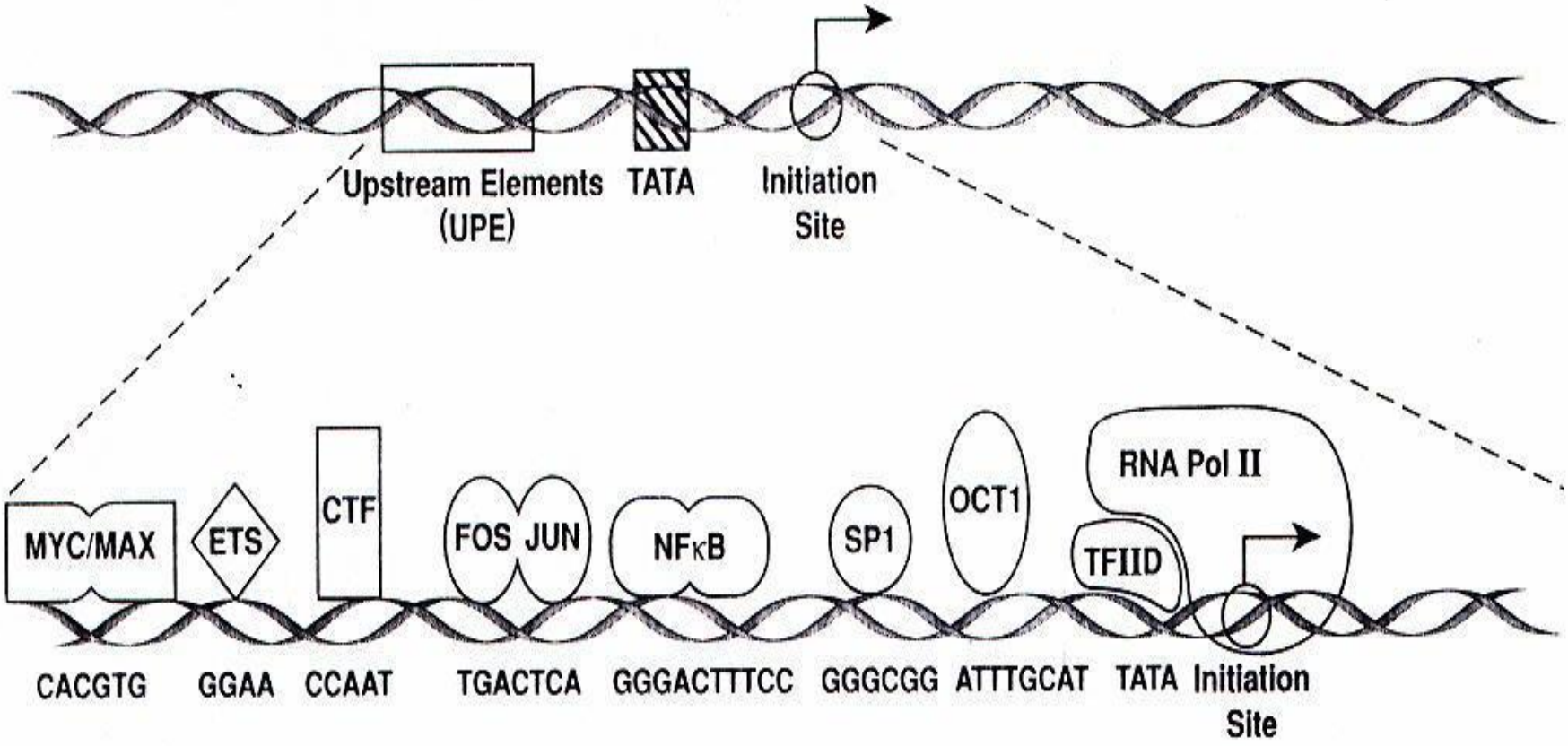


FIGURE 2–1. In the schematic of the transcriptional control region of a eukaryotic gene transcribed by RNA polymerase II, initiation sites (*arrows*), TATA sequences (*hatched boxes*), and upstream elements (*open boxes*) are shown. The transactivating factors that bind to particular DNA sequences are 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.

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**

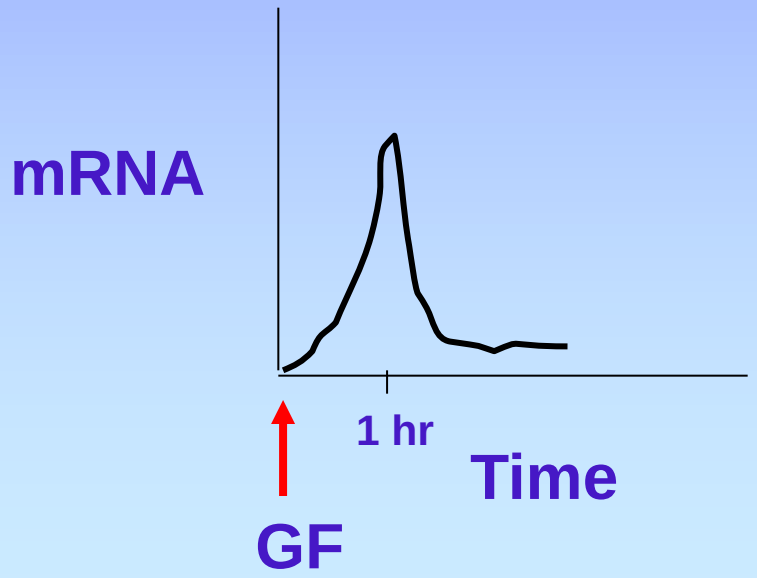
Amplifications of c-myc

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

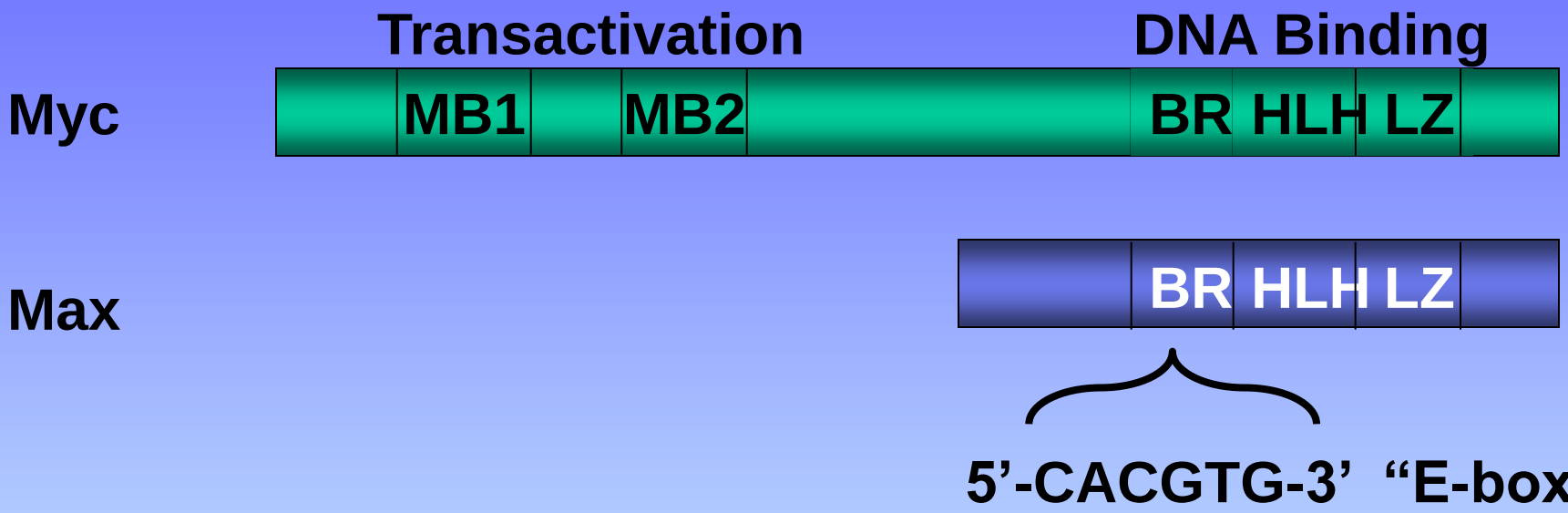
c-Myc is an early response gene (Growth Factor Regulated)

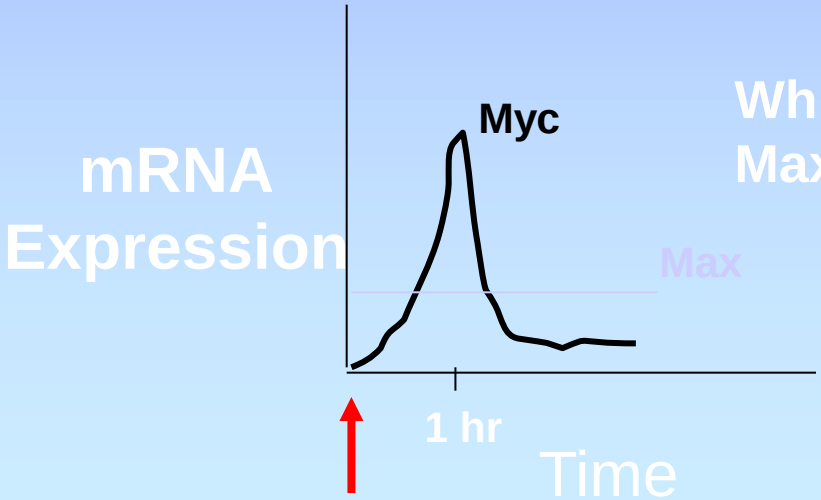
Myc protein has very short half-life <30 min.

Transcription regulates Myc protein levels



Myc has a partner called Max





While Myc is GF inducible,
Max is constitutively expressed

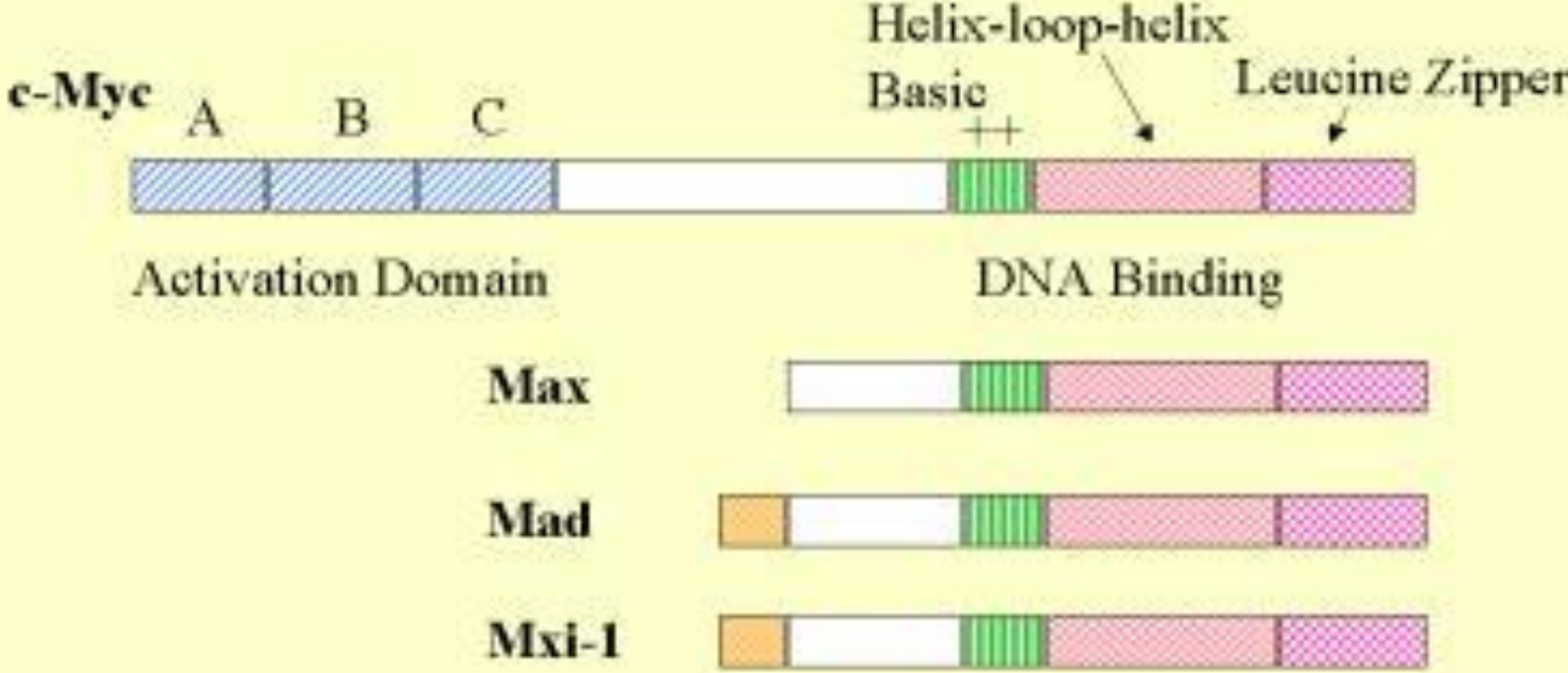
What does Myc Bind to?

- The E-Box - a sequence in DNA:

CACGTG

- Found upstream of Myc target genes

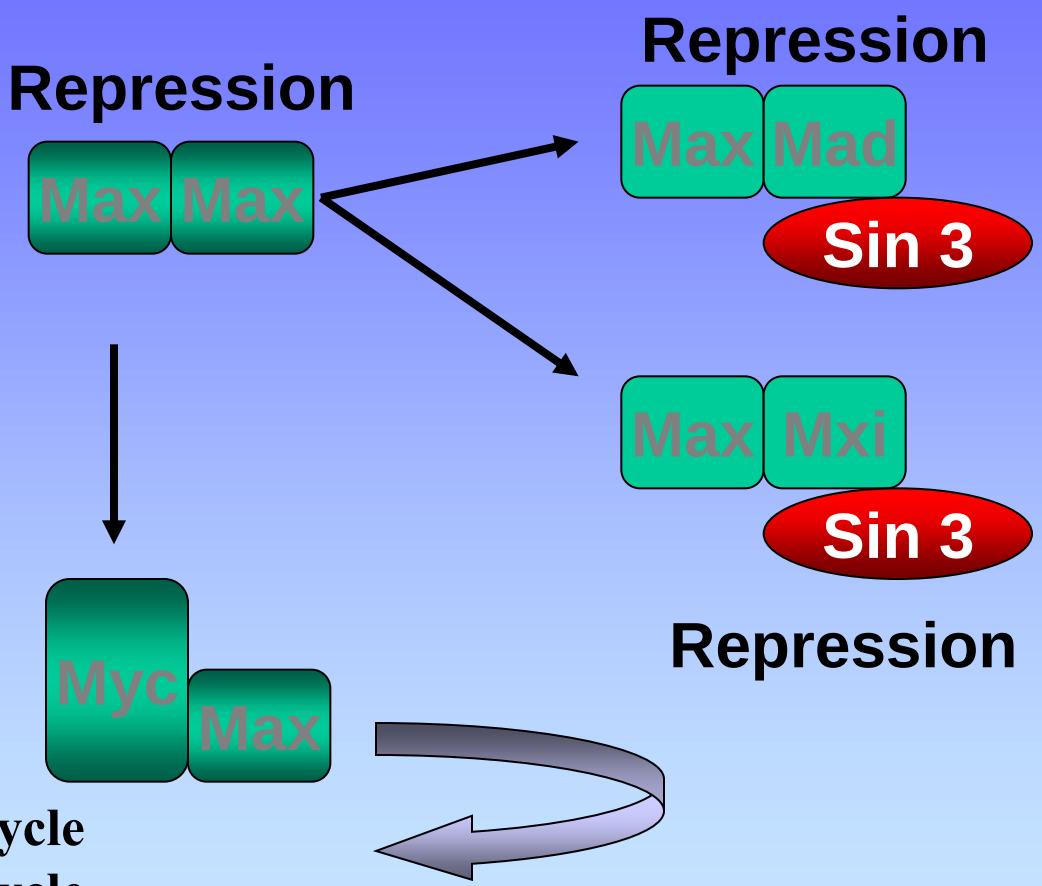
Structure of Myc and Associates



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

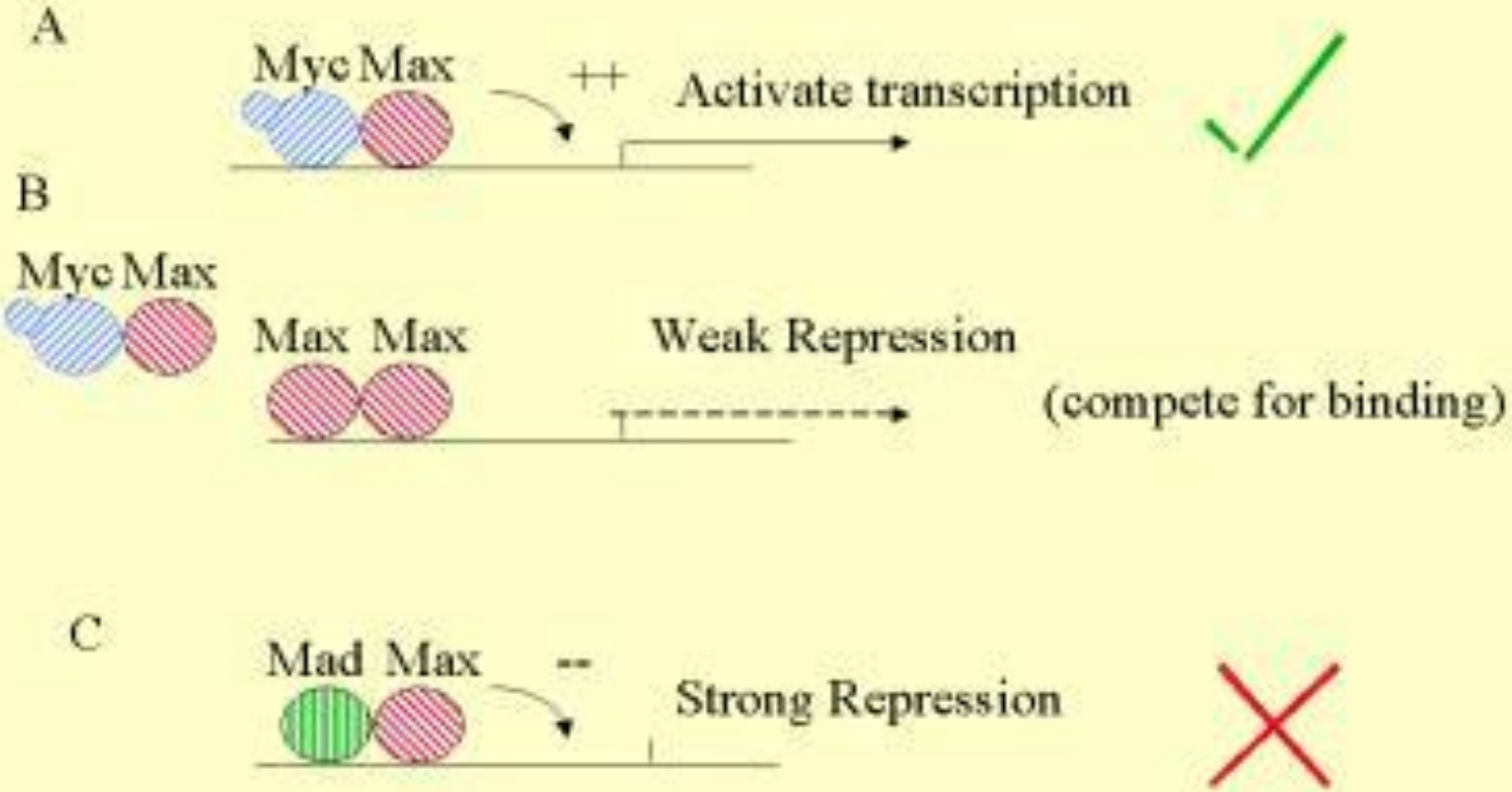
Myc's Associates

- Myc dimerizes with Max - another transcription factor

- Max can dimerize with Mad and Mxi1

BUT Mad and Mxi1 CANNOT dimerize with Myc!

Regulation of Transcription



Apoptosis Regulators

• The *BCL2* gene, which is involved in the initiation of almost all follicular lymphomas and some diffuse large B-cell lymphomas encodes a cytoplasmic protein that localizes to mitochondria and increases cell survival by inhibiting apoptosis.

• *BCL2* is also important in chronic lymphocytic leukemia and lung cancer.

•The BCL2 family members BCL-XL and BCL2 inhibit apoptosis and are up-regulated in many cancers.

• Two main pathways lead to apoptosis:
1- the stress pathway: triggered by proteins that contain the BCL2 homology 3 domain; this domain inactivates BCL2 and BCL-XL (which normally inhibit apoptosis) and thereby activates the caspases that induce apoptosis.

Drugs that mimic the BCL2 homology 3 domain and can bind to BCL-XL or BCL2 (peptides or small organic molecules that bind in a groove of these proteins) are under development. This approach has attracted considerable attention because many tumors overexpress BCL2 or related proteins

2- the death-receptor pathway: is activated by the binding of Fas ligand, TRAIL, and tumor necrosis factor α, to their corresponding (death) receptors on the cell surface. Activation of death receptors activates caspases that cause cell death