Oncogenes

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What is the molecular basis of cancer?

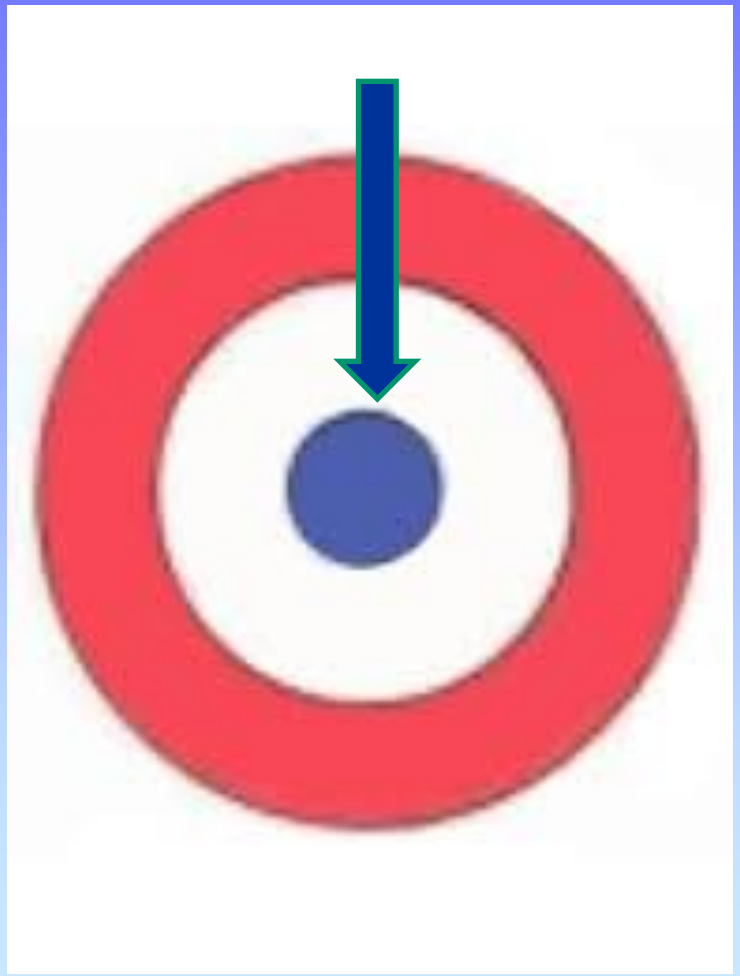
Cancers are formed from repeated rounds of DNA mutation, competition, and natural selection operating with the host.
-arise from a single abnormal cell
-abnormality results from somatic mutation
-development of cancer requires mutations in many cancer critical genes

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

Cancer critical genes: oncogenes and tumor suppressors

LAS TRES ETAPAS DEL CANCER

1) ORIGEN DE LA CELULA TUMORAL



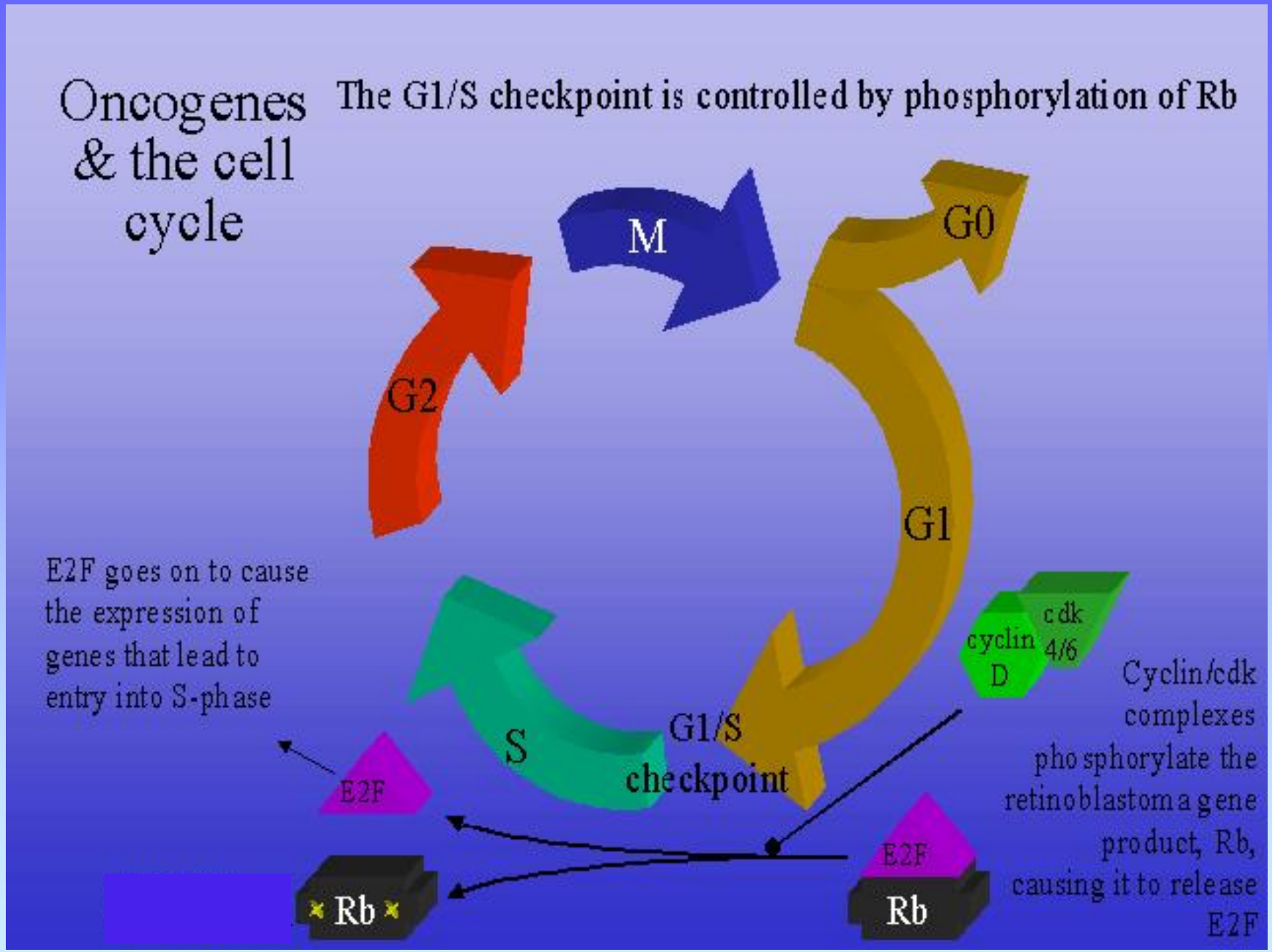

Acelerador
Embrague
Freno


ADAC
LATIN NCAP
GLOBAL NCAP

LOS ACELERADORES

Oncogenes & the cell cycle

The G1/S checkpoint is controlled by phosphorylation of Rb

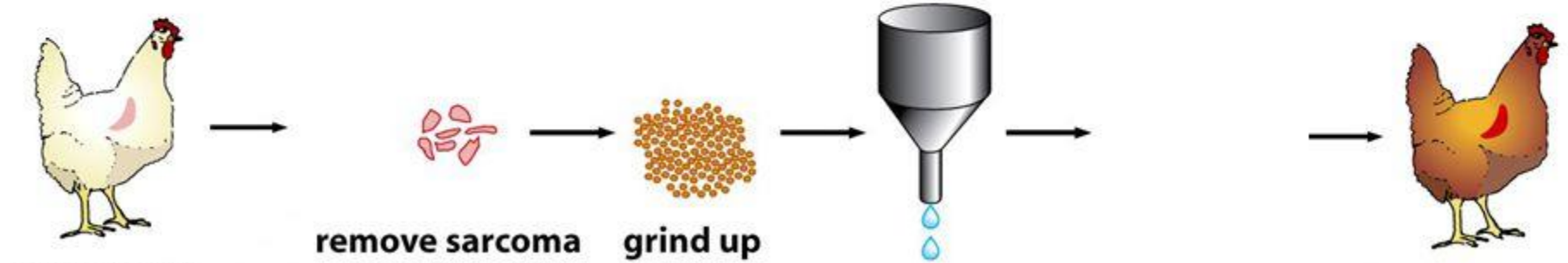


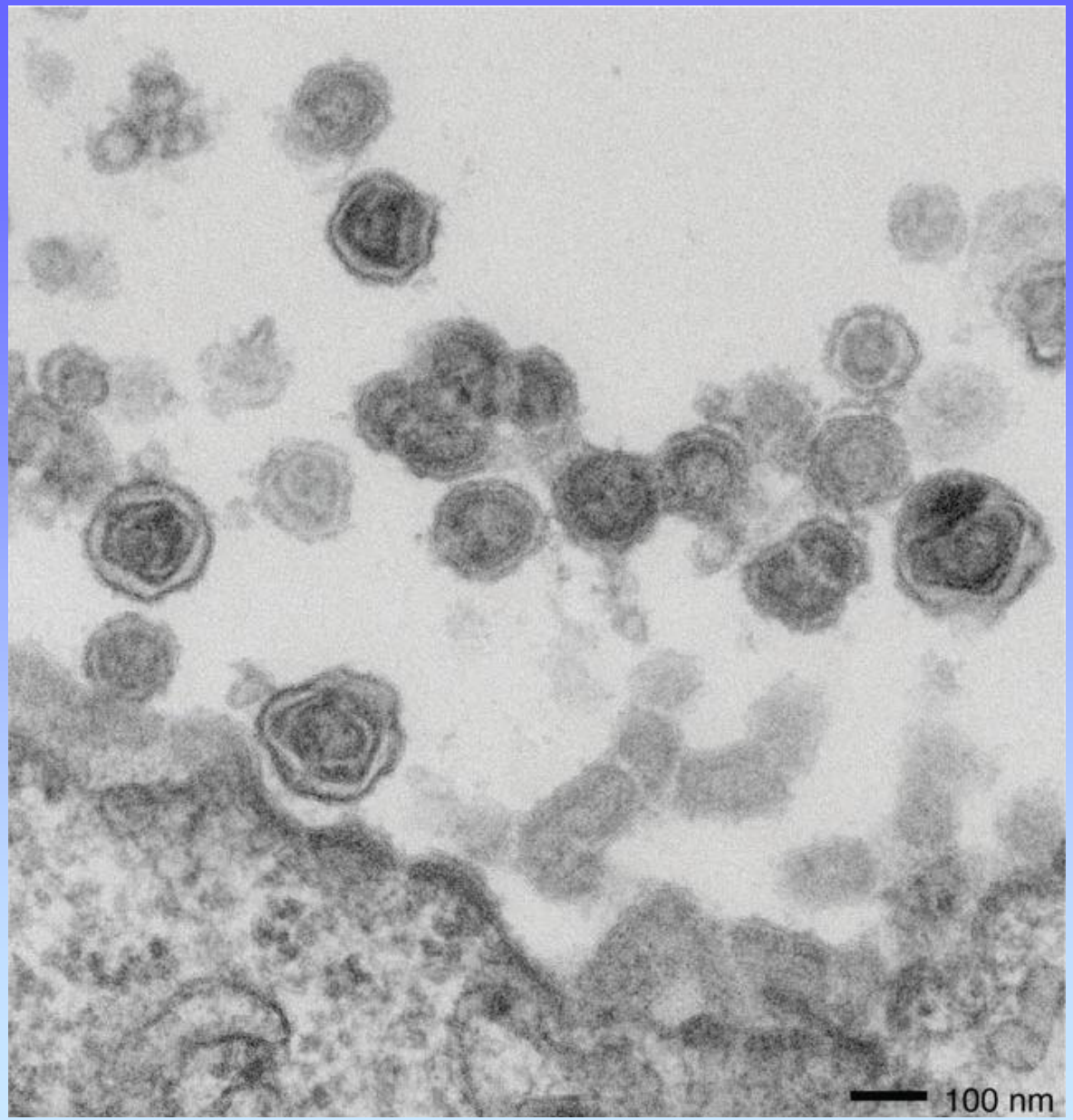
The Rous Sarcoma Virus (RSV)

A virus can transform a normal cell into a tumor

Peyton Rous

Nobel prize in Physiology or Medicine 1966

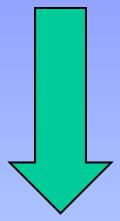




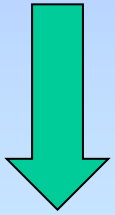
RETROVIRUS LIBERADOS DE CELULA INFECTADA

DOGMA DE LA BIOLOGIA EN LOS AÑOS 60

DNA



RNA

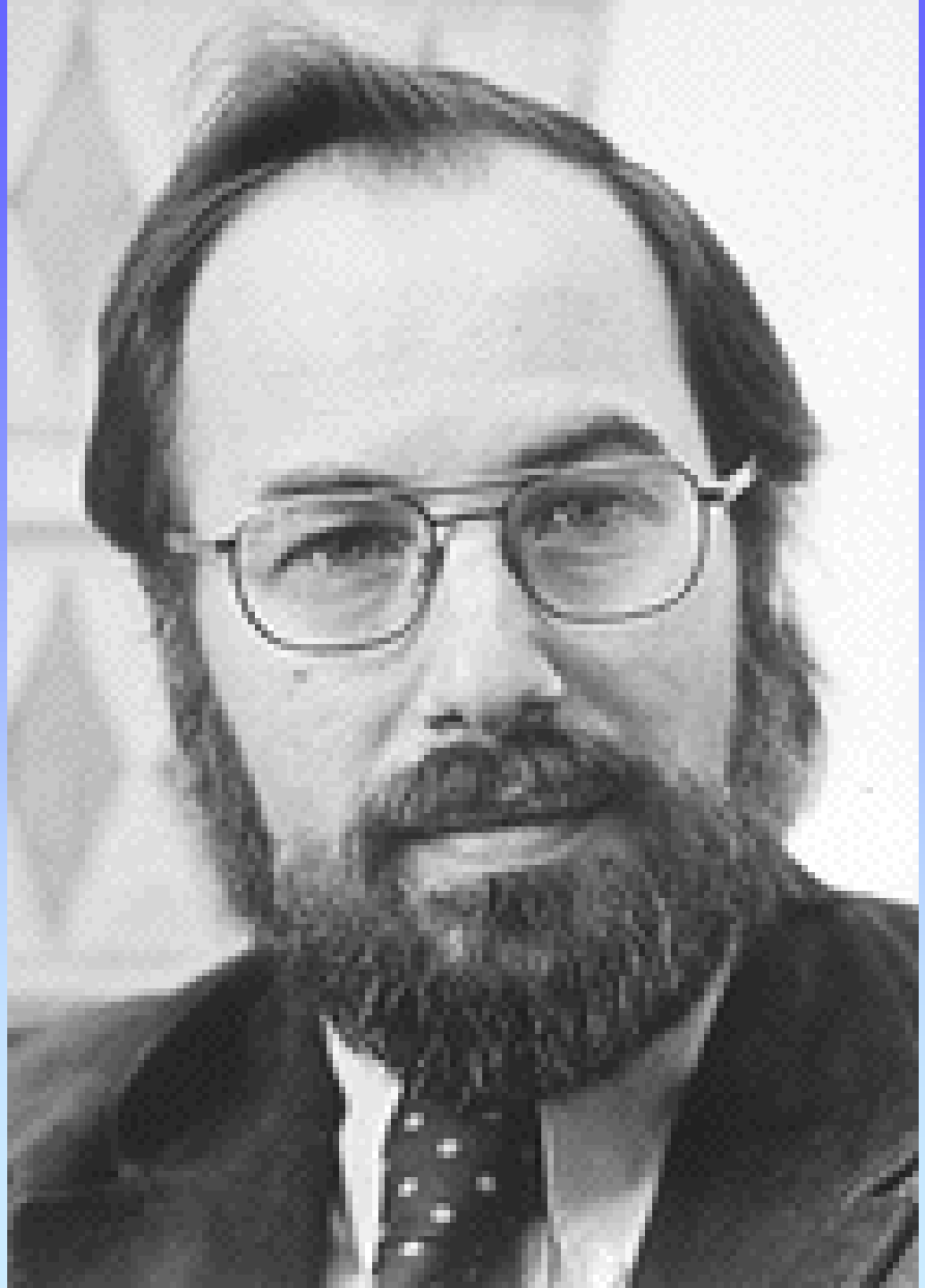


PROTEINA

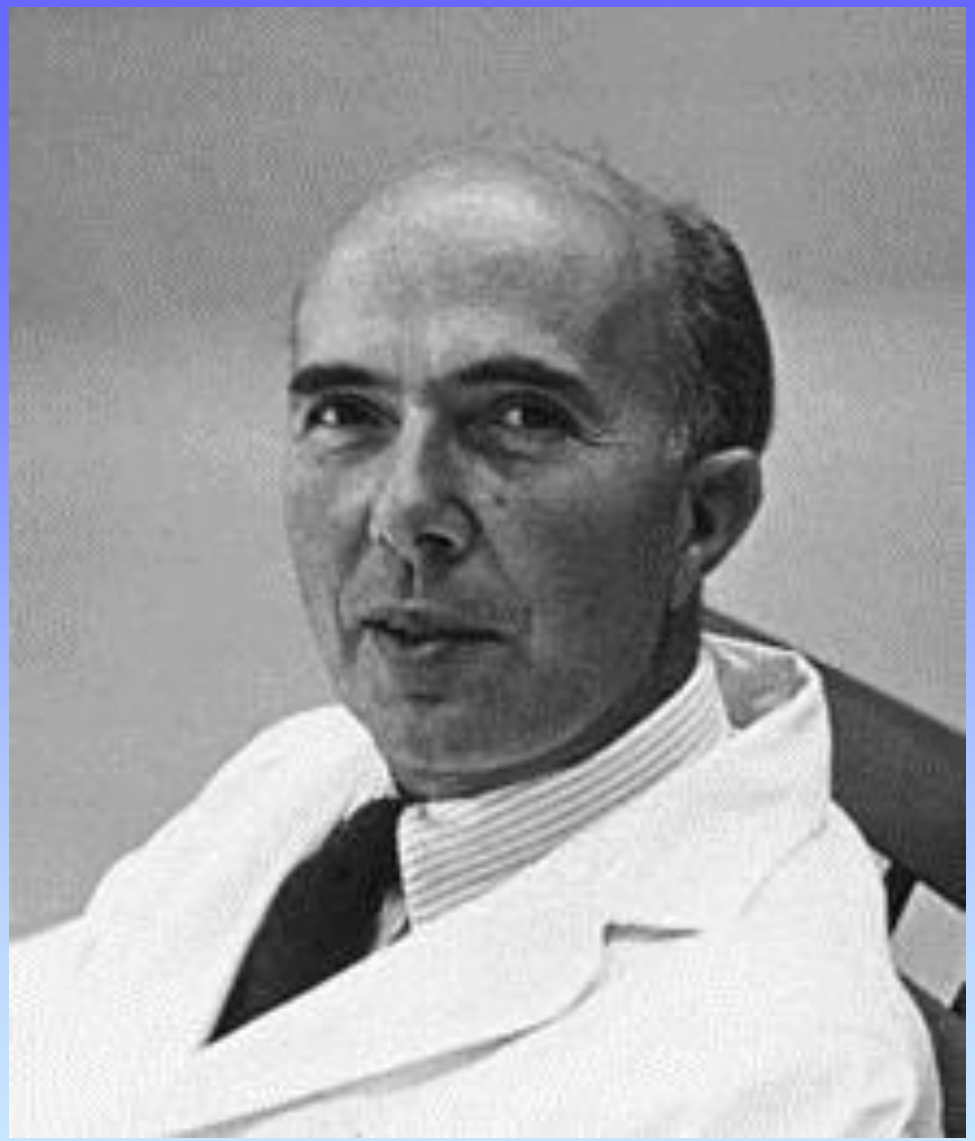
PREGUNTA

- **¿Cómo es posible que un virus que contenga RNA en su genoma se integre en el DNA cromosómico de una célula?**

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



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

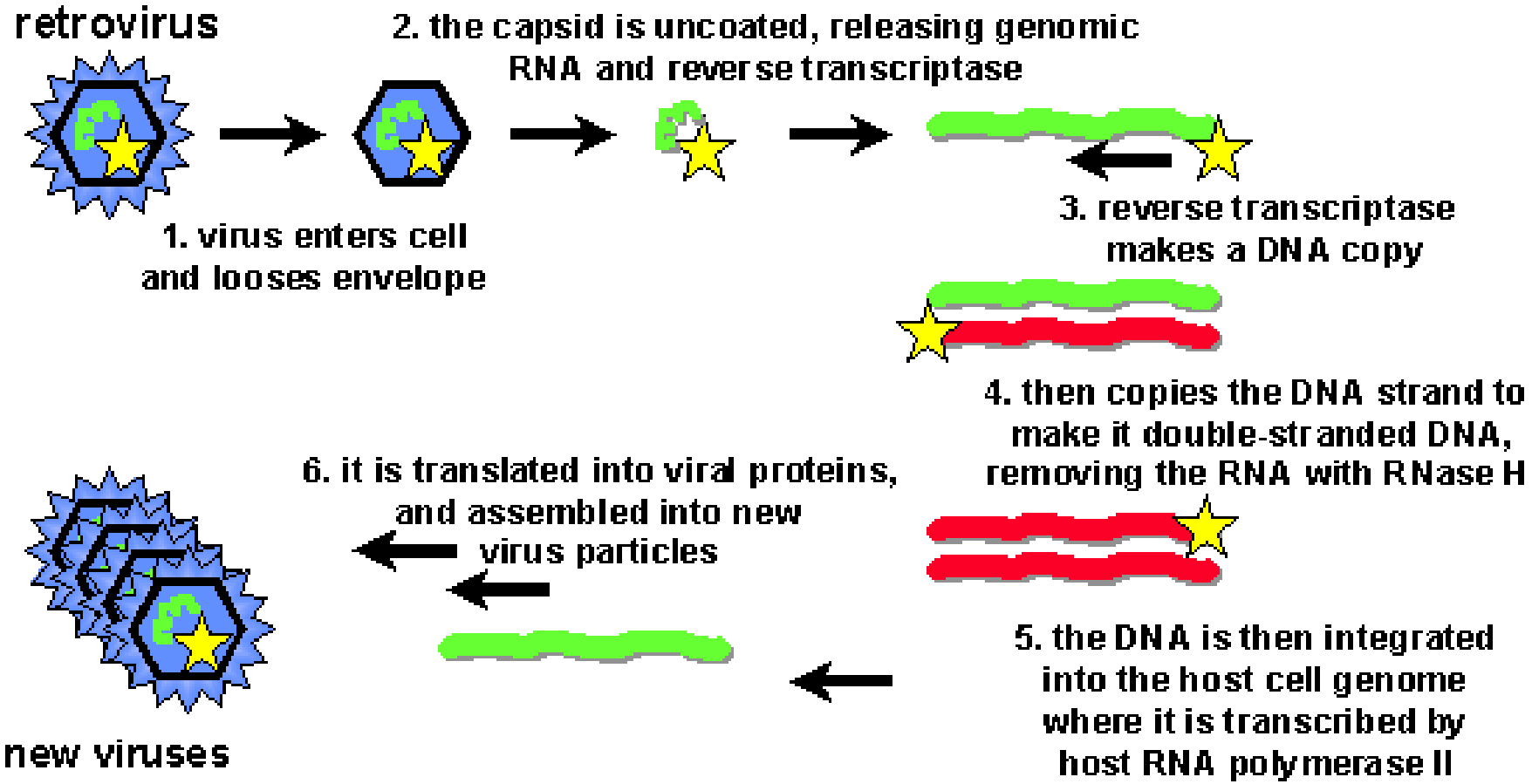


Renato Dulbecco
Premo Nobel de Medicina 1975

Construction of a cDNA library

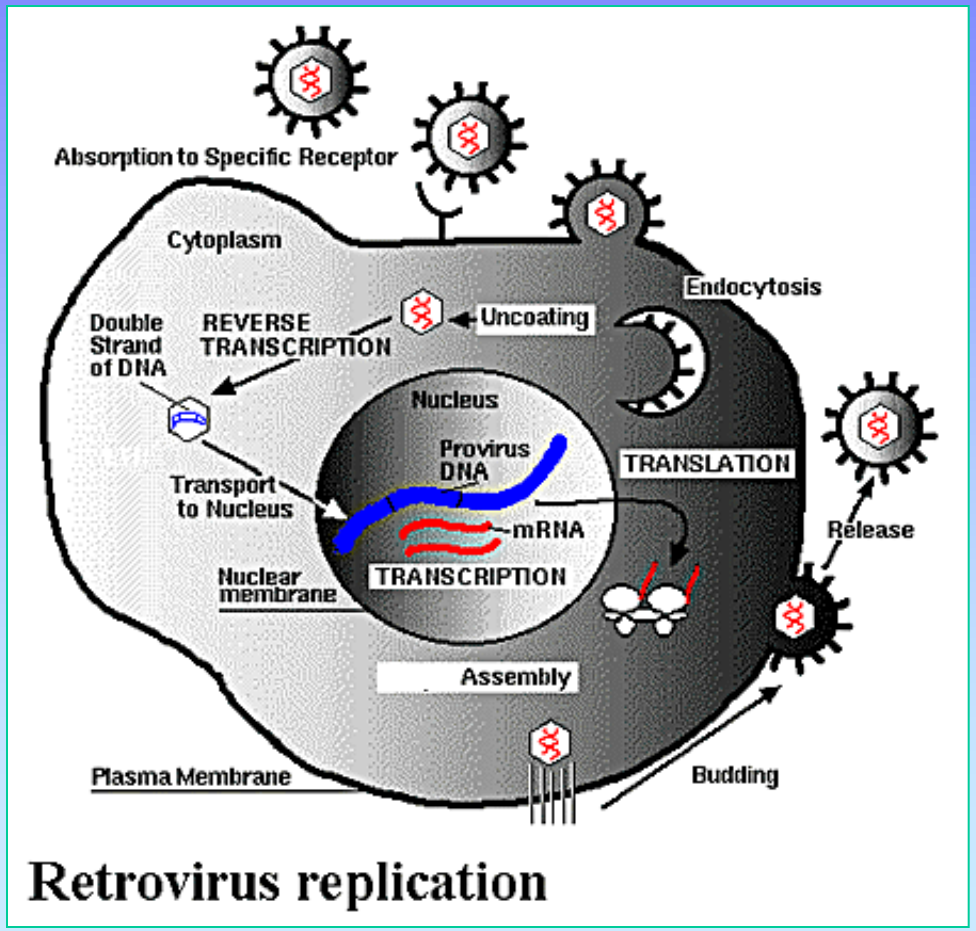
- **reverse transcriptase makes a DNA copy of an RNA**

The life cycle of a retrovirus depends on reverse transcriptase



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.

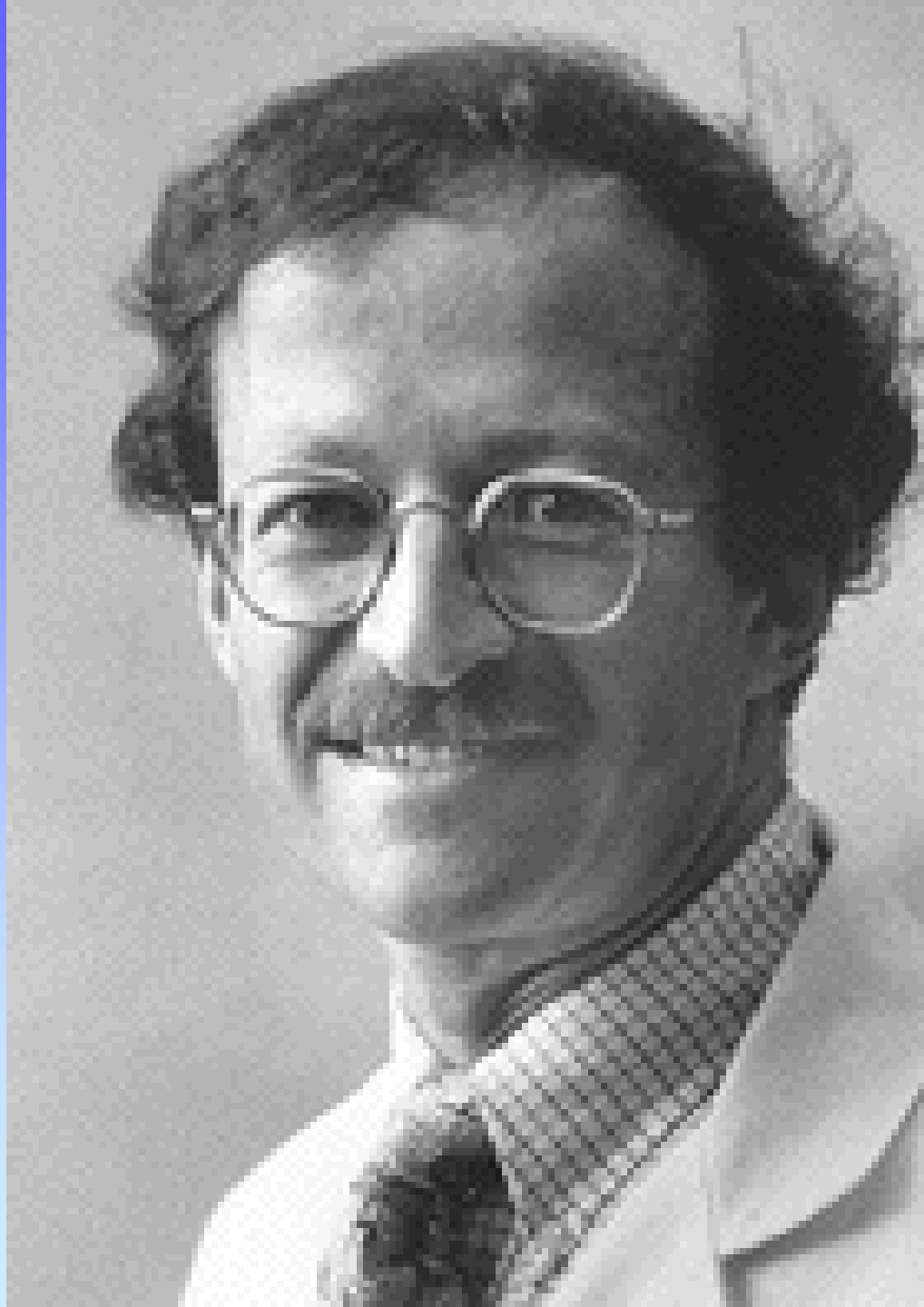


Retrovirus replication

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

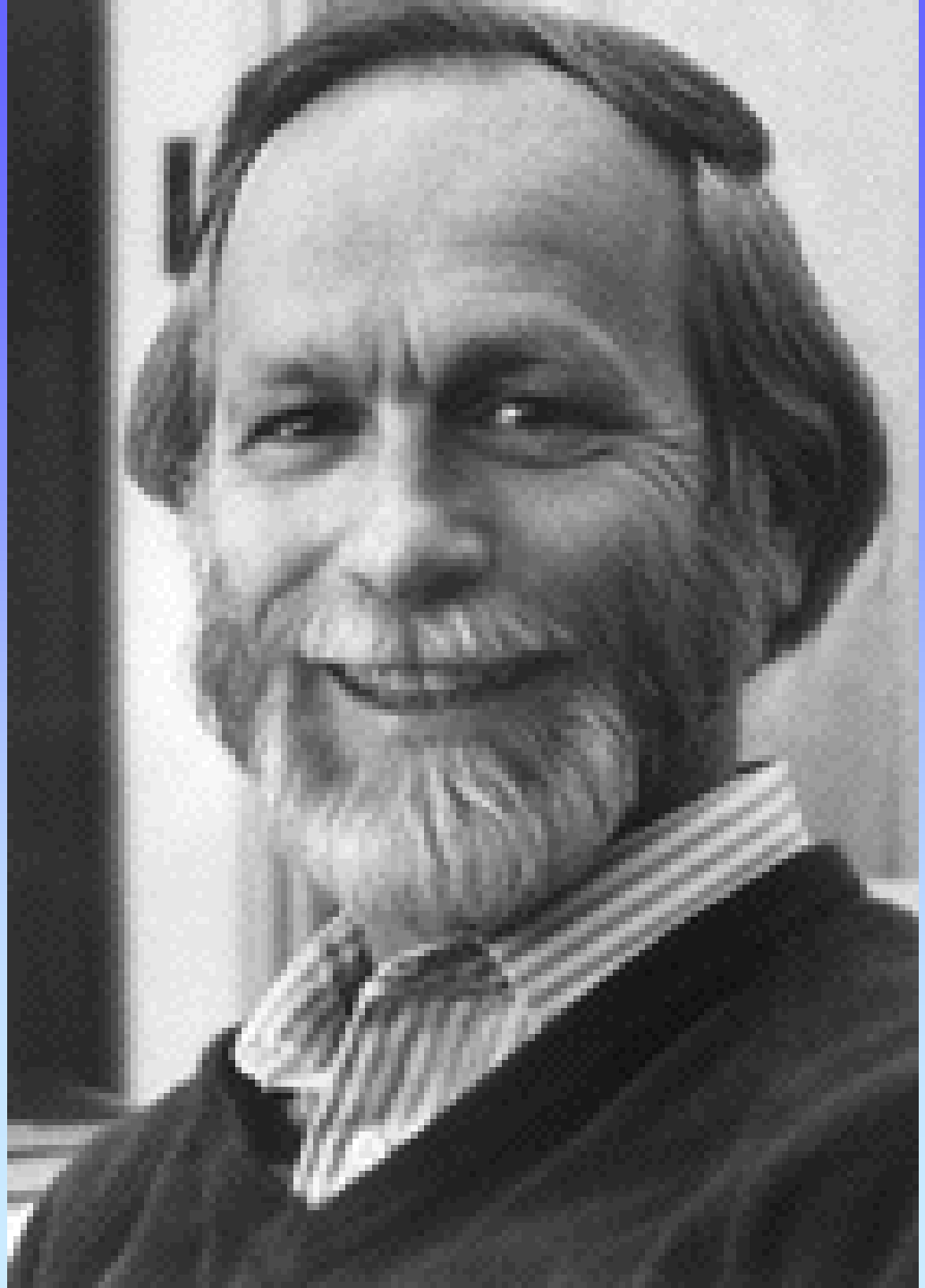
UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO

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



J.MICHAEL BISHOP

Premio Nobel de Medicina 1989



Oncogenes: Historical Considerations-I

- 1911-Peyton Rous discovers a "filterable agent" in extracts of chicken tumors that can induce new tumors when injected into otherwise normal chickens. (Nobel Prize)
- Filterable substance is later identified as a virus that had an RNA genome instead of DNA and is thus termed a "retrovirus".
- Retroviruses have three basic genes: Group Antigen Gene (GAG), a special Polymerase (POL), and viral envelop proteins (ENV).

Oncogenes: Historical Considerations-II

- 1970-Temin and Baltimore discovered that the POL enzyme can "reverse-transcribe" or direct DNA synthesis from an RNA template (Nobel Prize).
- Two types of the retroviruses identified: transforming and non-transforming. 1970-Isolation of an additional genetic element termed SRC from transforming Rous sarcoma viruses that is directly responsible for causing cancer (an oncogene).

Retroviruses

- **Tumor causing retroviruses fall into two groups**
  - Nondefective virus:
    - Activates a cellular proto-oncogene(s)
  - Acute transforming virus:
    - Gain of a new oncogene from the virus
    - Transduction event
- **A transforming retrovirus carries a copy of a cellular sequence in place of its own gene(s)**

Transforming Retrovirus

- **Generation of a fusion protein (*gag*+*v-onc*).**

Original virus

Transforming virus

Transforming Retrovirus

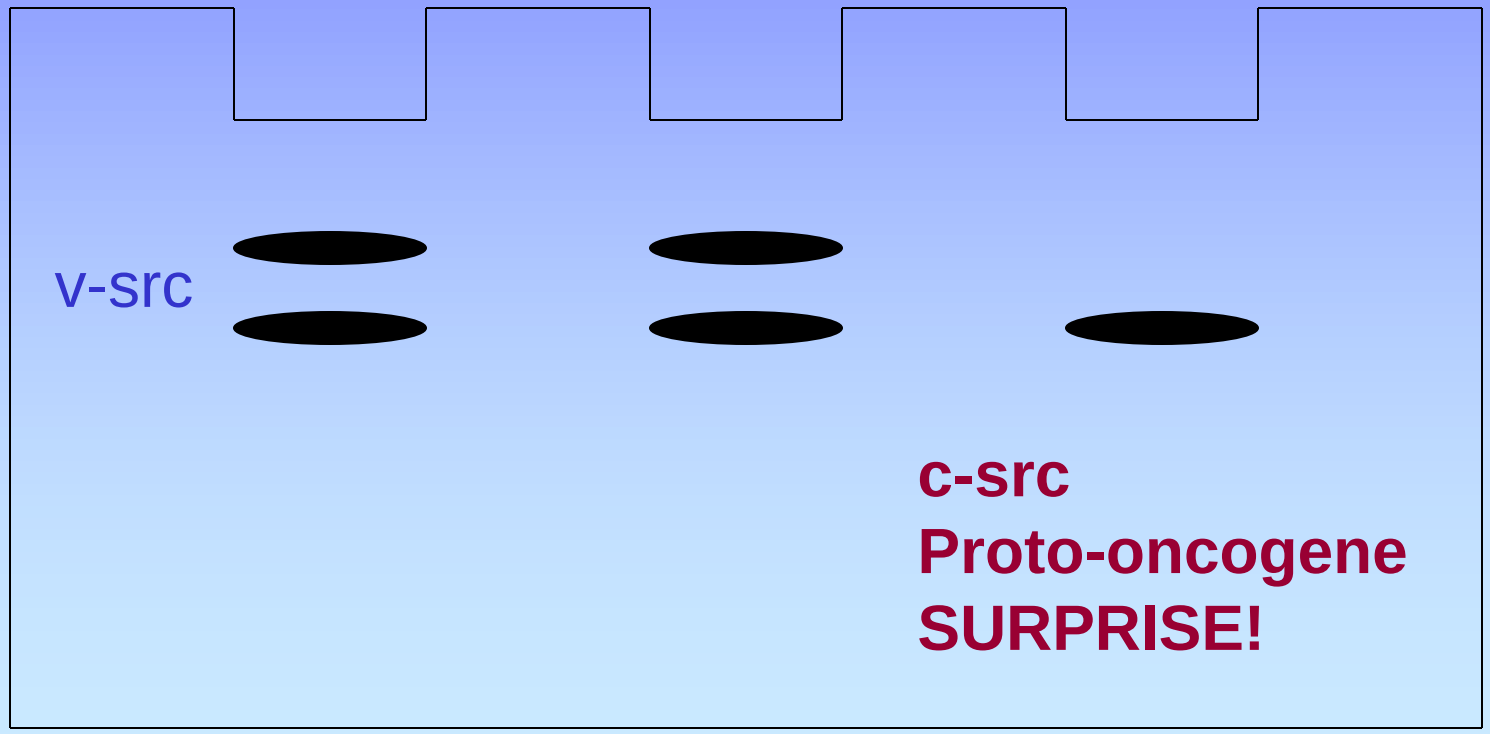
- **Transducing virus (one that has gained cellular genes) has two properties**
  - Cannot replicate by itself
  - Must have a "helper virus"
  - Transducing virus carries cellular genes obtained during recombination
  - Expression of these genes may alter phenotype of infected cell

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

Infected chicken #1

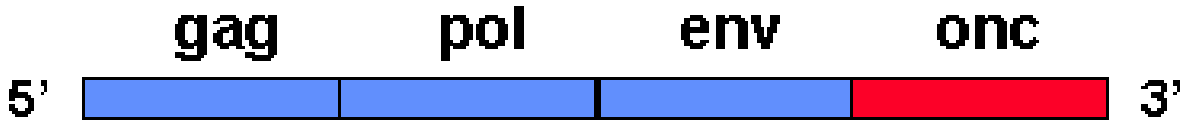
Infected chicken #2

Uninfected chicken (Negative Control)



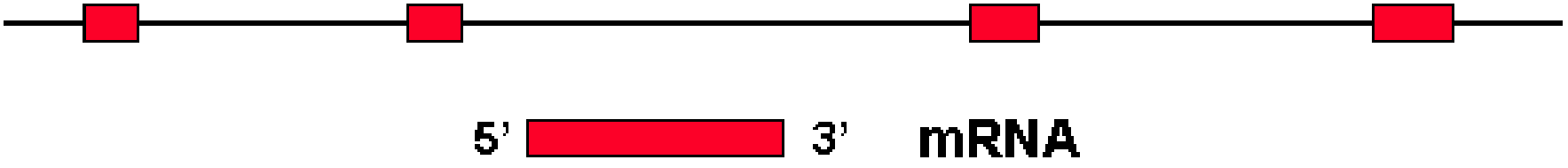
Gene organization of a retrovirus

Gene organization of a transforming retrovirus

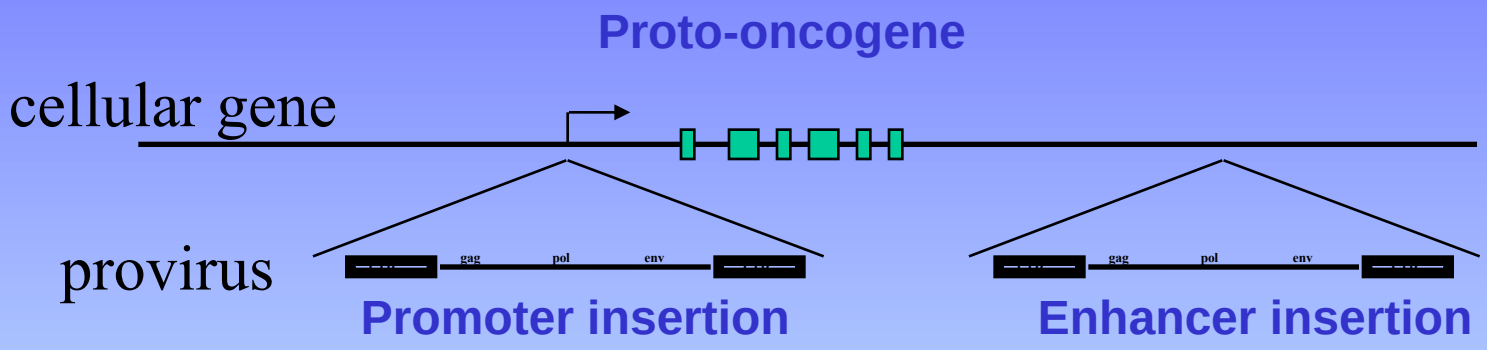


gag = group specific antigen
pol = reverse transcriptase
env = envelope
onc = oncogene

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.

Oncogenes of Acutely Transforming Retroviruses

= Oncogenes of acutely transforming retroviruses important in human cancer

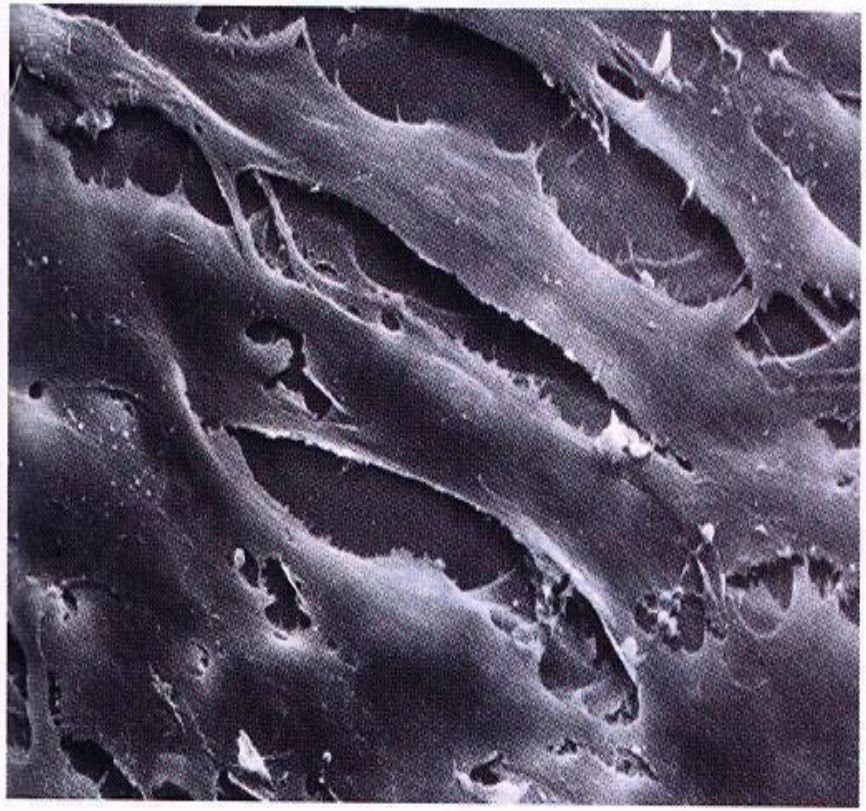
src	Rous sarcoma virus	Chicken
myc	Avian myelocytomatosis virus	Chicken
erb A, erb B	Avian erythroblastosis virus	Chicken
myb	Avian myeloblastosis virus	Chicken
ets	Avian erythroblastosis virus	Chicken
rel	Avian reticuloendotheliosis virus	Turkey
H-ras	Harvey rat sarcoma virus	Rat
K-ras	Kirsten murine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

WHITEHEAD INSTITUTE
FOR BIOMEDICAL RESEARCH

Robert Weinberg

Whitehead Institute-MIT

(a)



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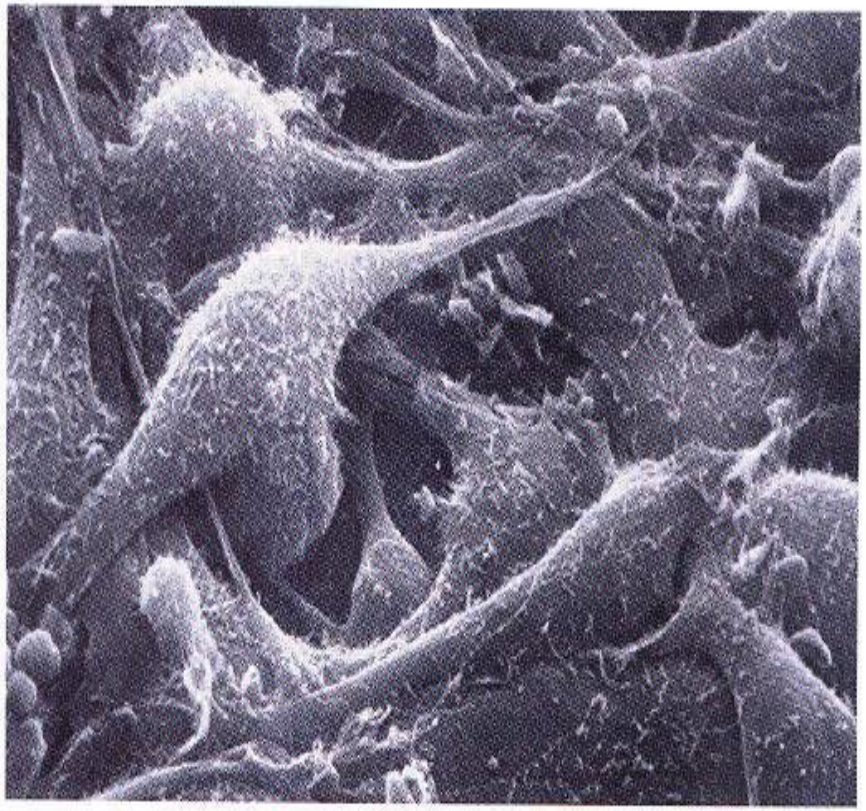
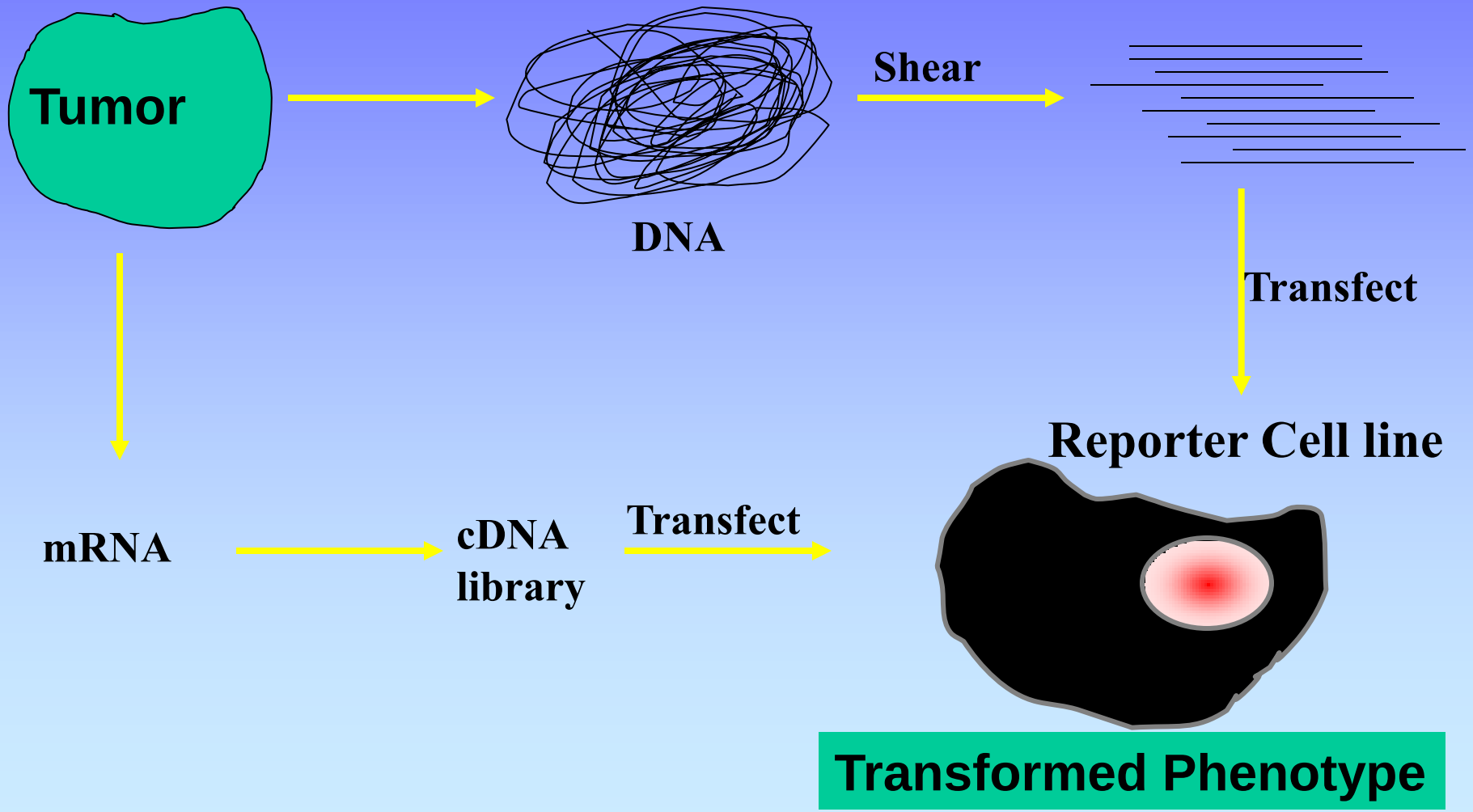
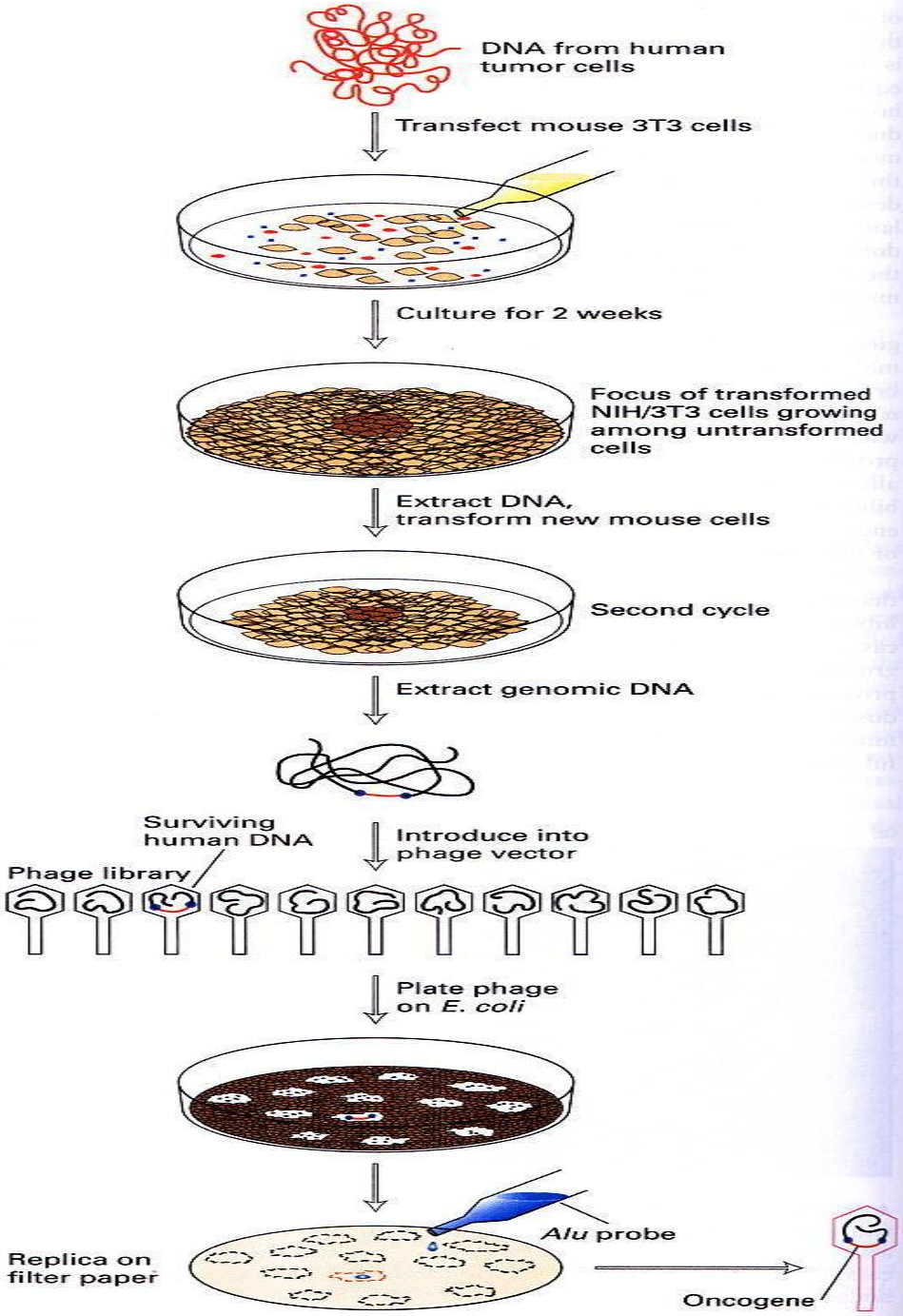


▲ FIGURE 24-3 Scanning electron micrographs of normal and transformed 3T3 cells. (a) Normal 3T3 cells are elongated and are aligned and closely packed in an orderly fashion. (b) 3T3 cells transformed by the v-*src* oncogene encoded by Rous sarcoma virus. The cells are much more rounded, and they are covered with small hairlike processes and bulbous projections. The cells grow one atop the other, and they have lost the side-by-side organization of the normal cells. These transformed cells have many of the same properties as malignant cells. [Courtesy of L.-B Chen.]

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

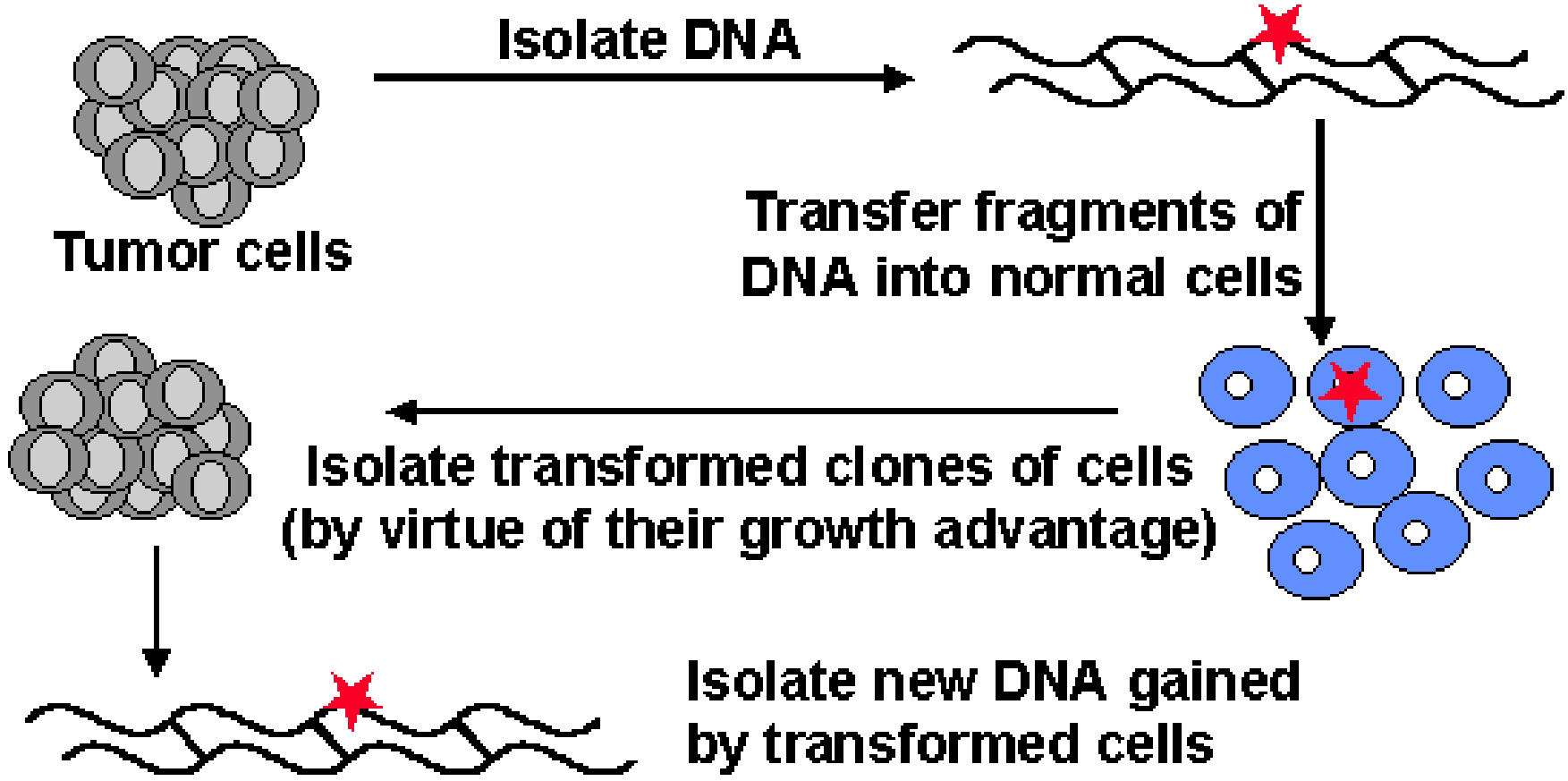




▶ **FIGURE 24-4 The identification and molecular cloning of the *ras*^{D} oncogene.** Addition of DNA from a human bladder carcinoma to a culture of mouse 3T3 cells causes about one cell in a million to divide abnormally and form a focus, or clone of transformed cells. To clone the oncogene responsible 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

LA GRAN SORPRESA !!!

V-ONC Y C-ONC SON IGUALES !!

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

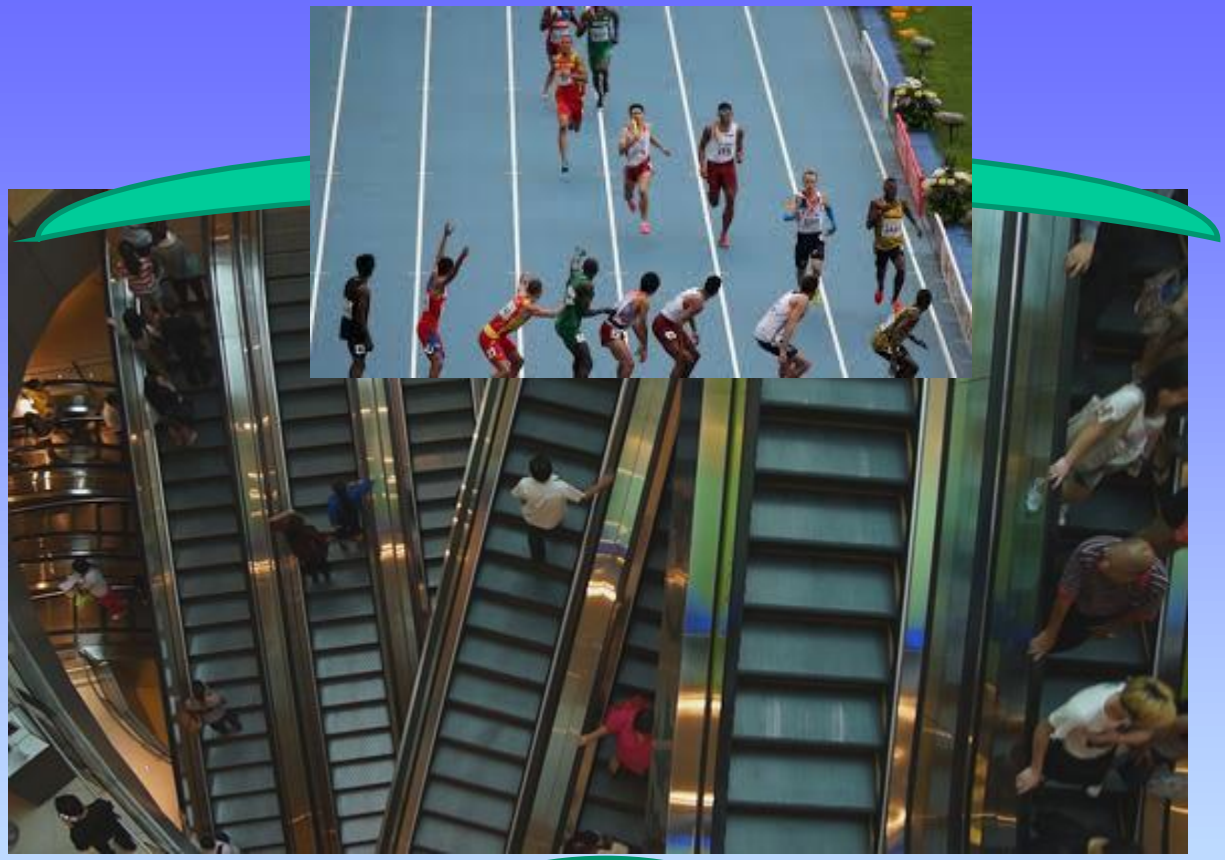
FUNCION DE LOS PROTO-ONCOGENES

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

MEMBRANA CELULAR

Normal

Cáncer



NUCLEO

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

Maestría en Biología Molecular Médica – Dr. José Mordoh 2011

Mecanismos de Transformación de Proto-oncogene a oncogene

1. **Translocación**
2. Amplificación
3. Inserción Viral
4. Mutagénesis

MECANISMOS DE ACTIVACION DE ONCOGENES

2- Traslocaciones cromosómicas: dos mecanismos

- translocación 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.

- **Translocación 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.

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)

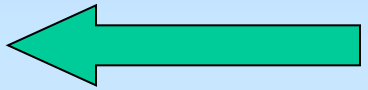
CYTOPLASM

Signal Transducers

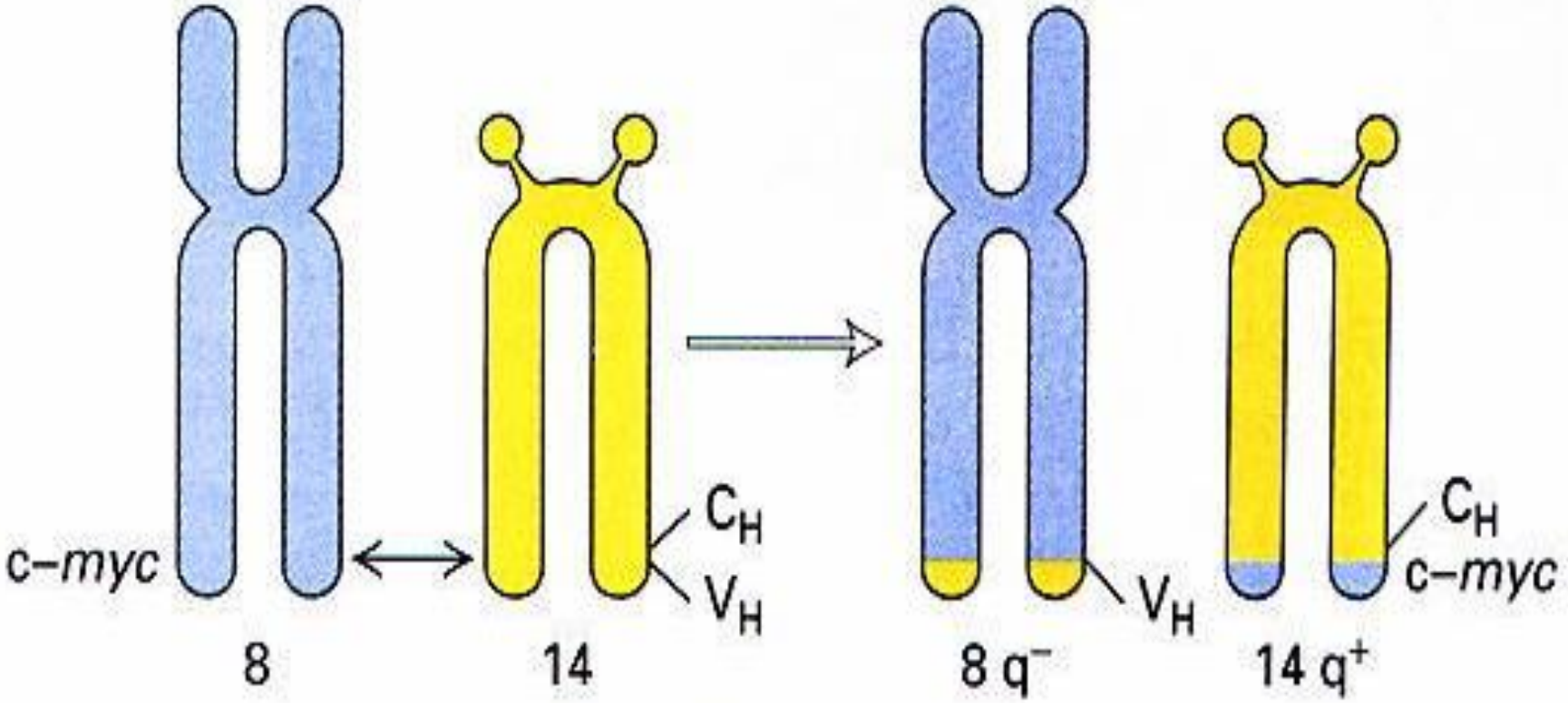
v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

NUCLEUS

Transcription Factors



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



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

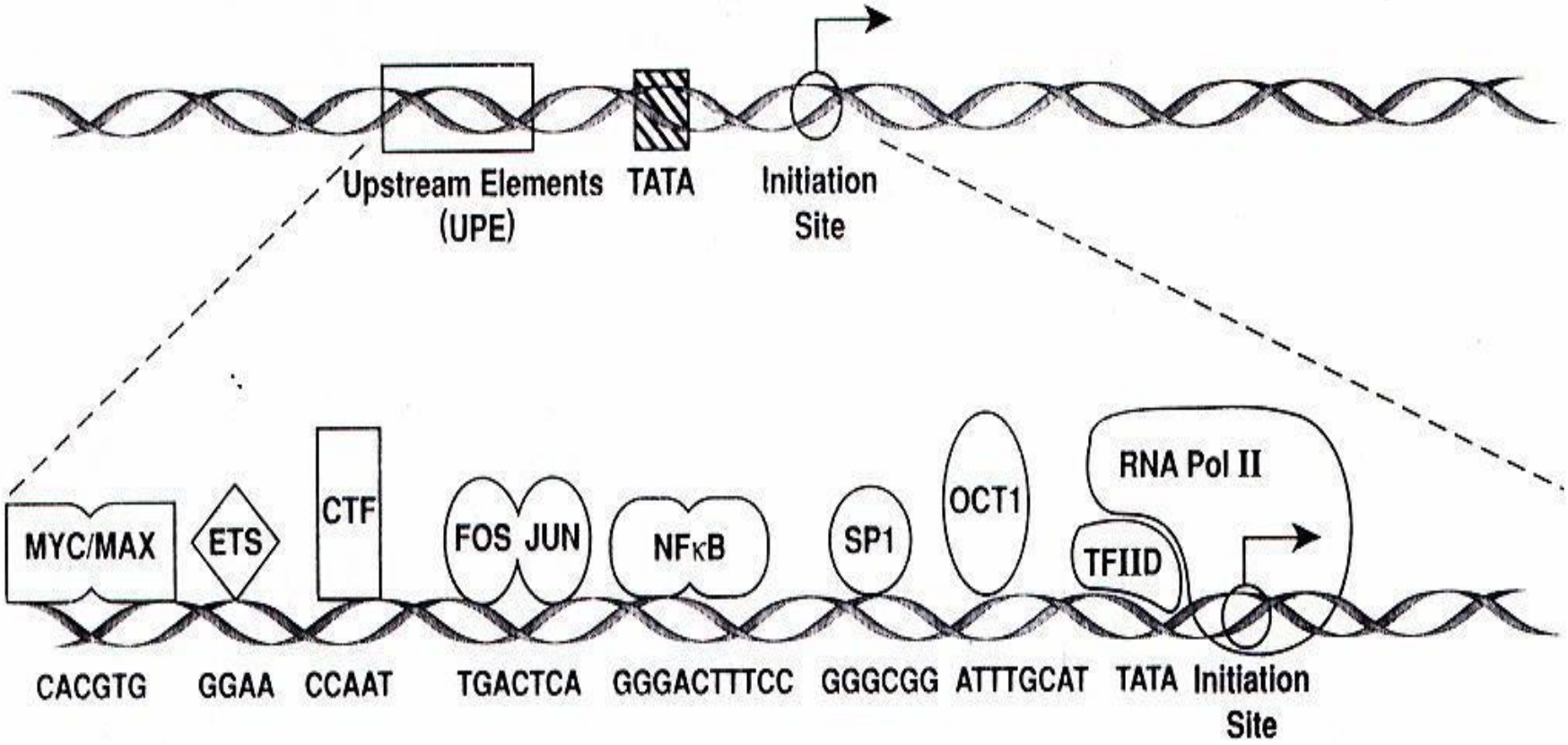
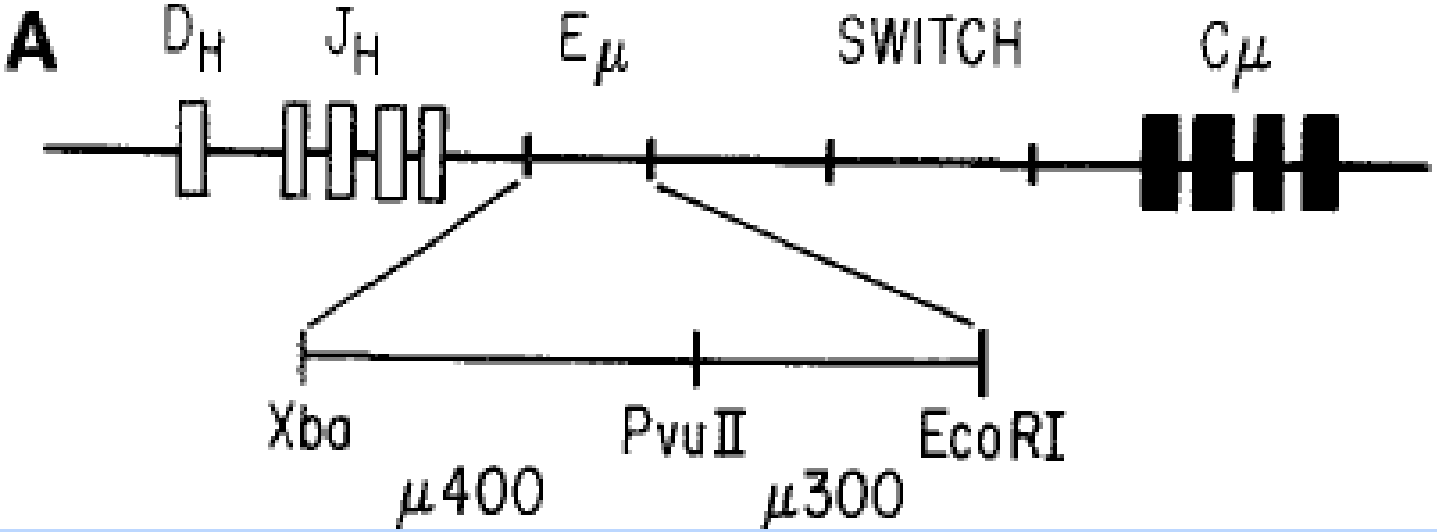
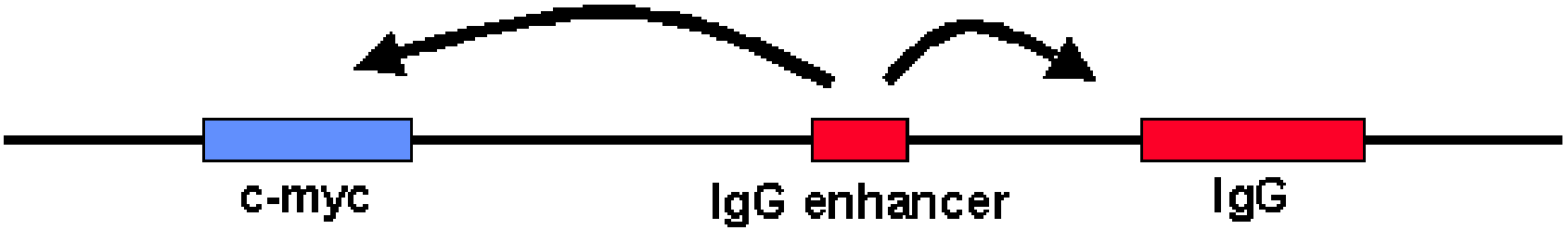


FIGURE 2–1. In the schematic of the transcriptional control region of a eukaryotic gene transcribed by RNA polymerase II, initiation sites (*arrows*), TATA sequences (*hatched boxes*), and upstream elements (*open boxes*) are shown. The transactivating factors that bind to particular DNA sequences are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



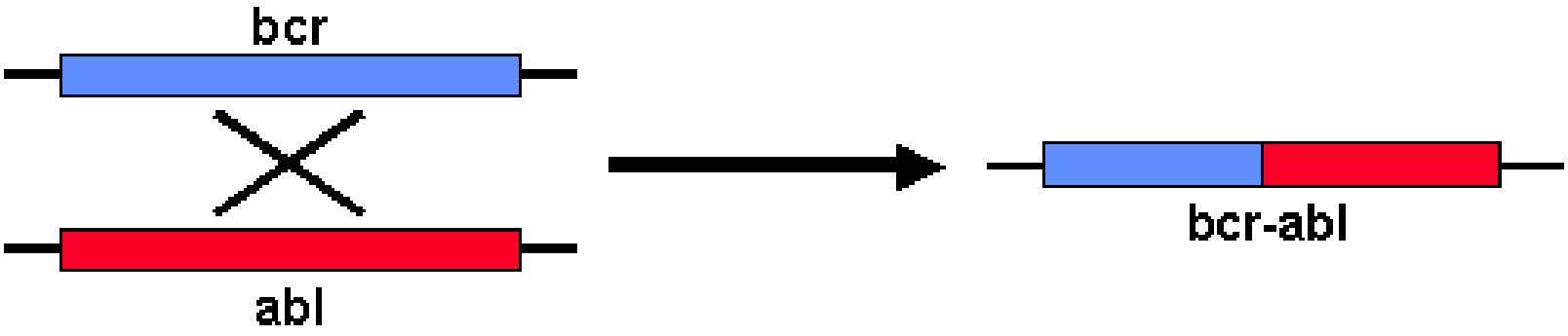
Enhancer cadena pesada Ig (Sen and Baltimore, Cell, 1986)

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

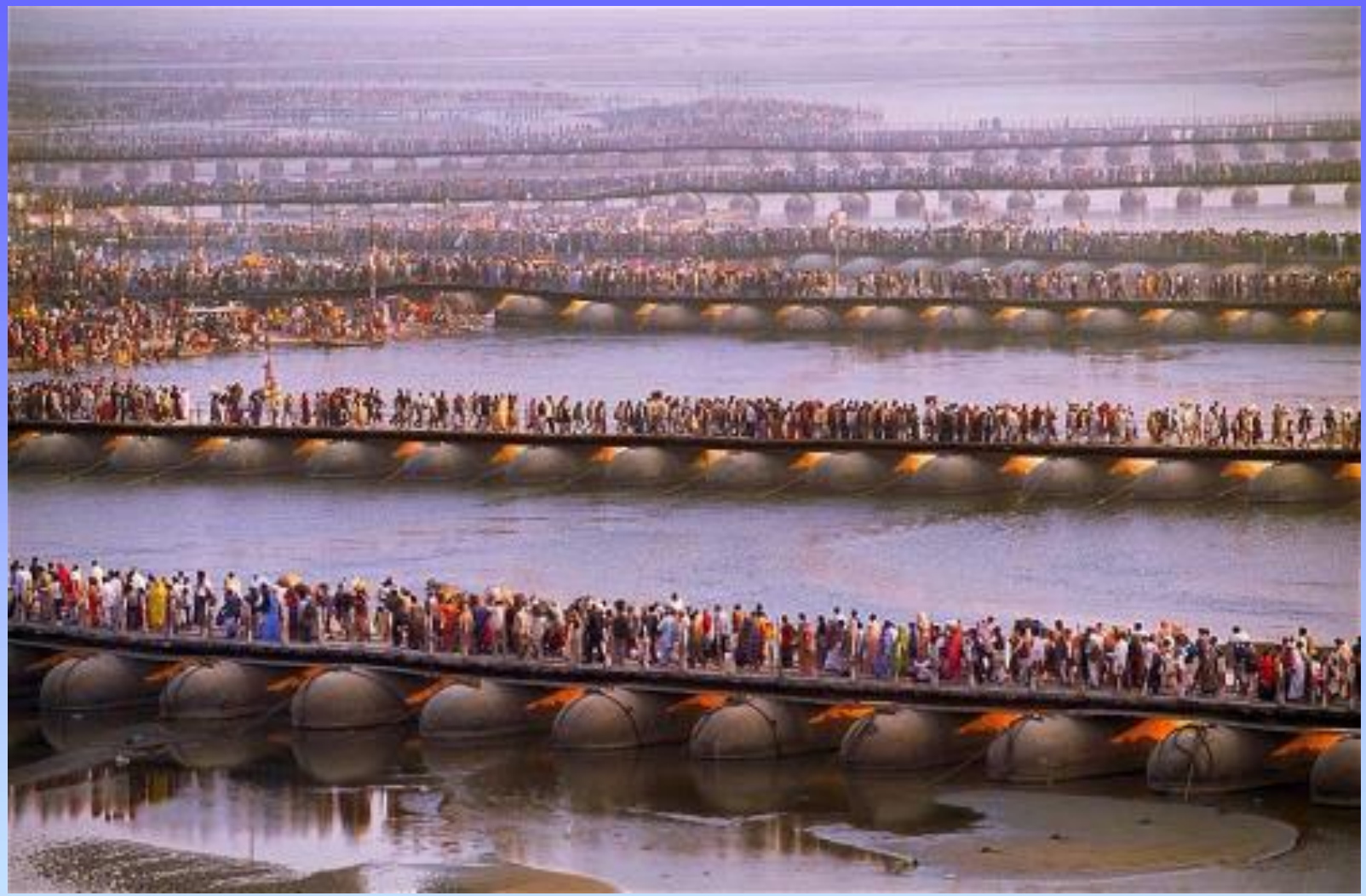


c-myc is activated by the IgG enhancer in lymphocytes

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



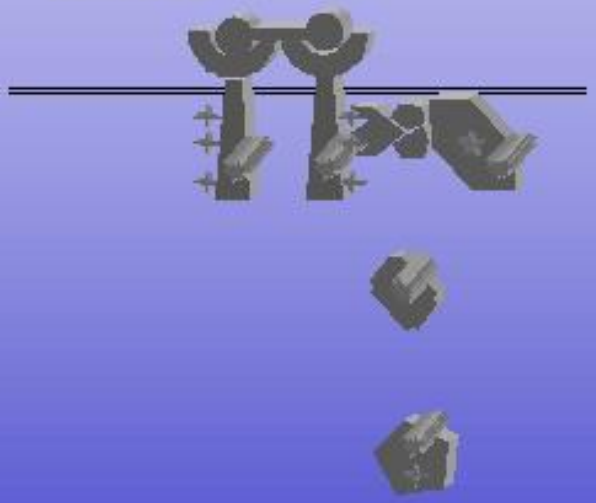

MYC River



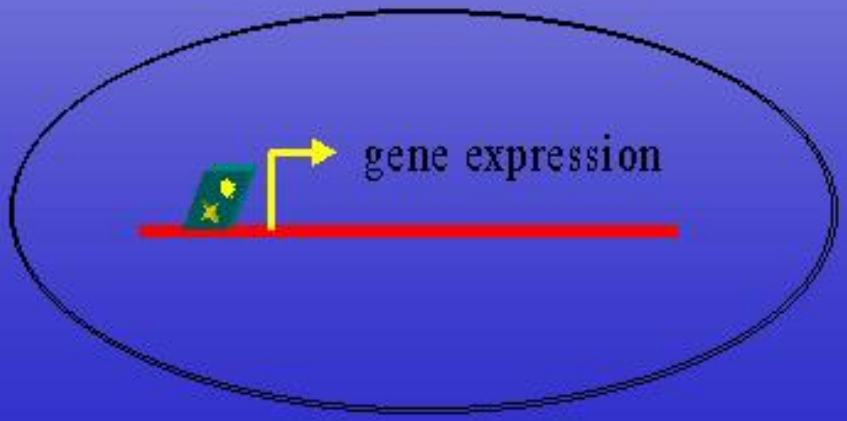
MYC TRANSLOCADO
RIO GANGES

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



✓ It is overexpressed in a large percentage of human tumors, including cancers of lymphoid, mesenchymal and epithelial origin.

✓ **Myc regula la transcripción de entre 10-15% de la totalidad de los genes**

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**

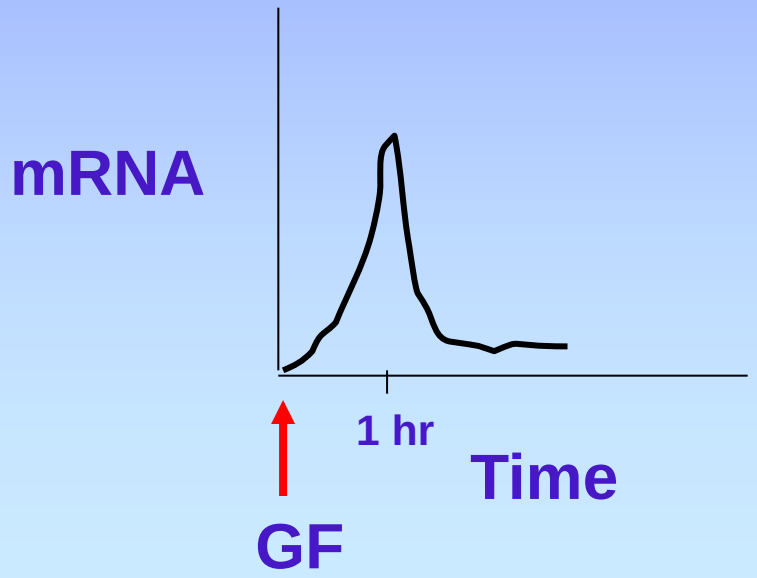
Amplifications of c-myc

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

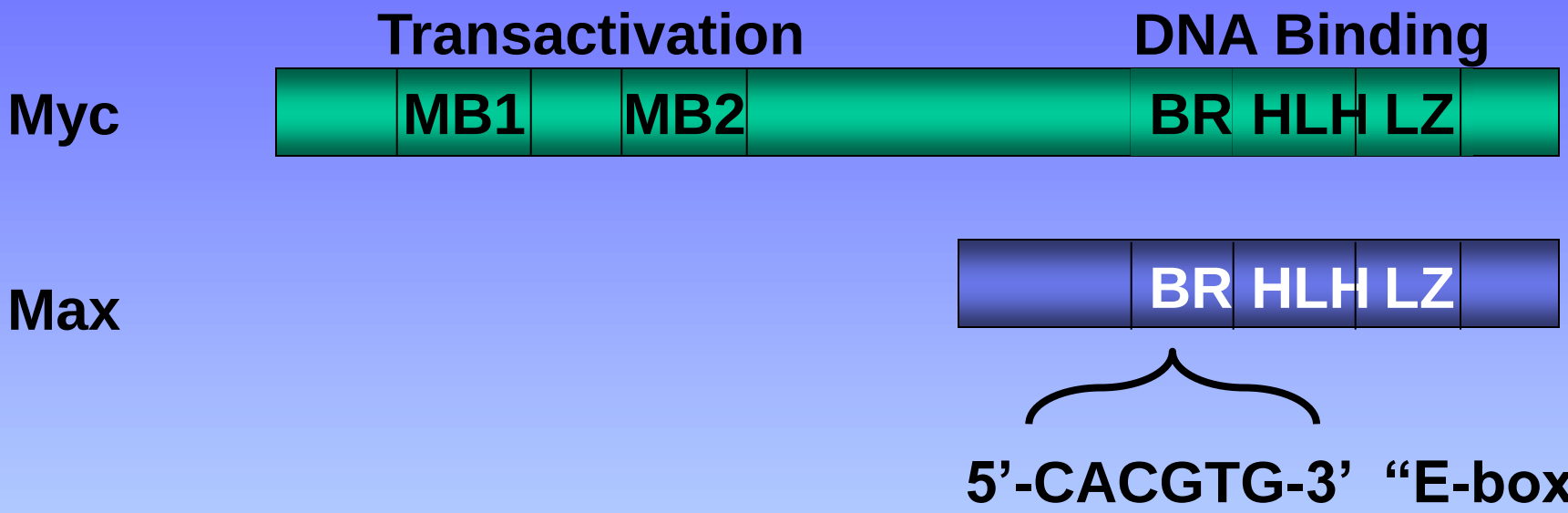
c-Myc is an early response gene (Growth Factor Regulated)

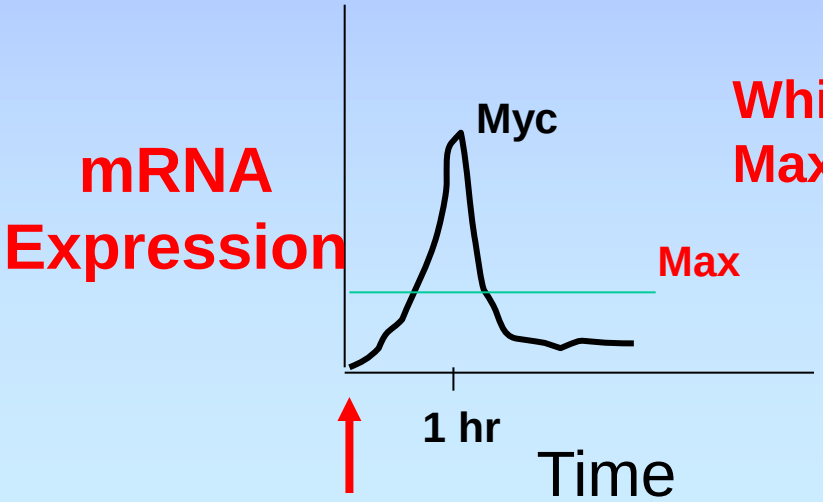
Myc protein has very short half-life <30 min.

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

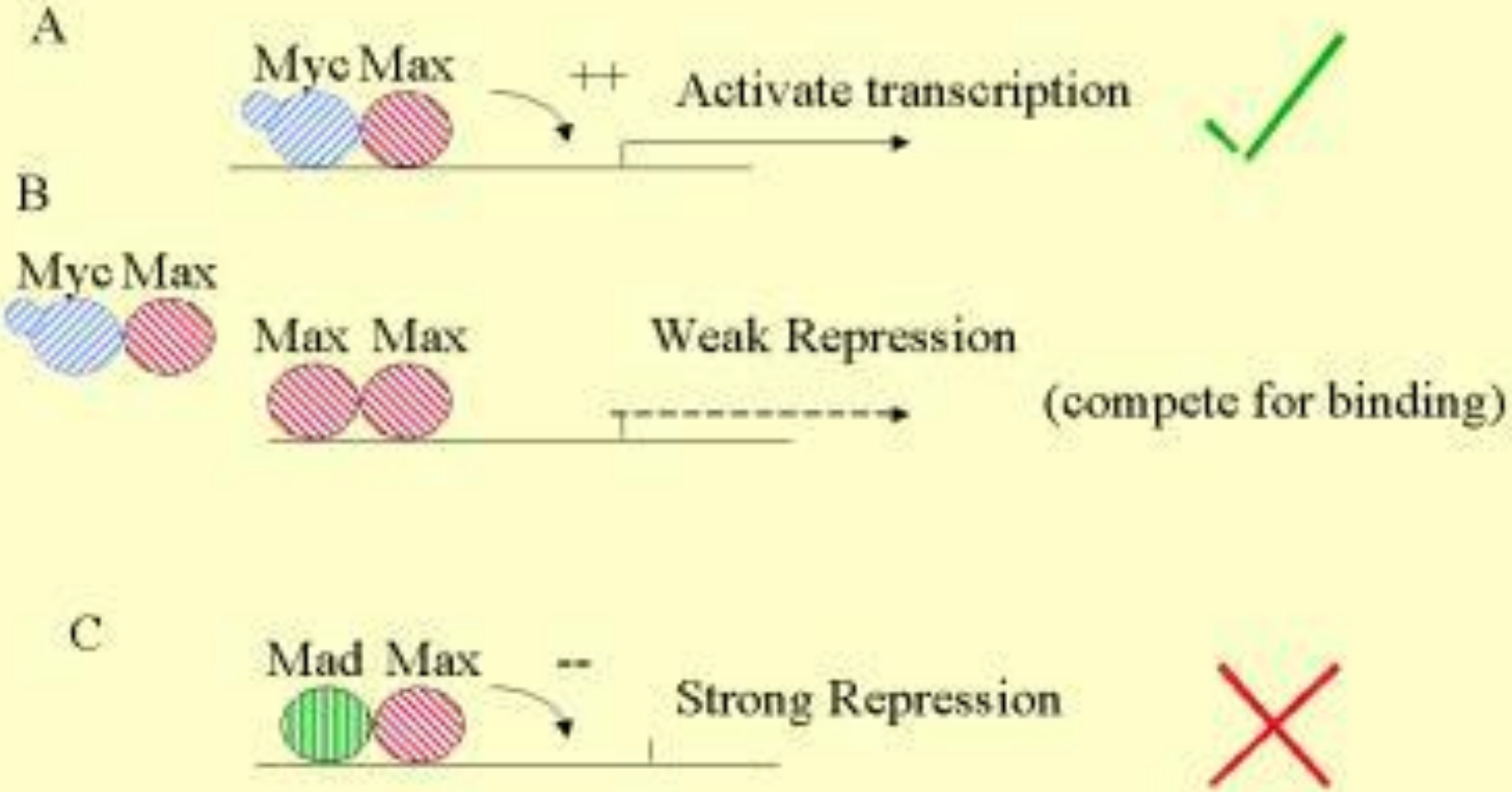
Myc's Associates

- 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



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

MECANISMOS DE ACTIVACION DE ONCOGENES

2- Traslocaciones cromosómicas: dos mecanismos

- translocación 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.

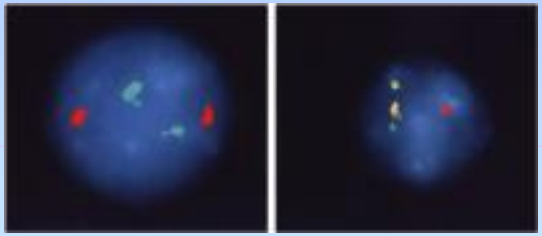
- Translocación y alteración genética de un proto-oncogen:

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.

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).

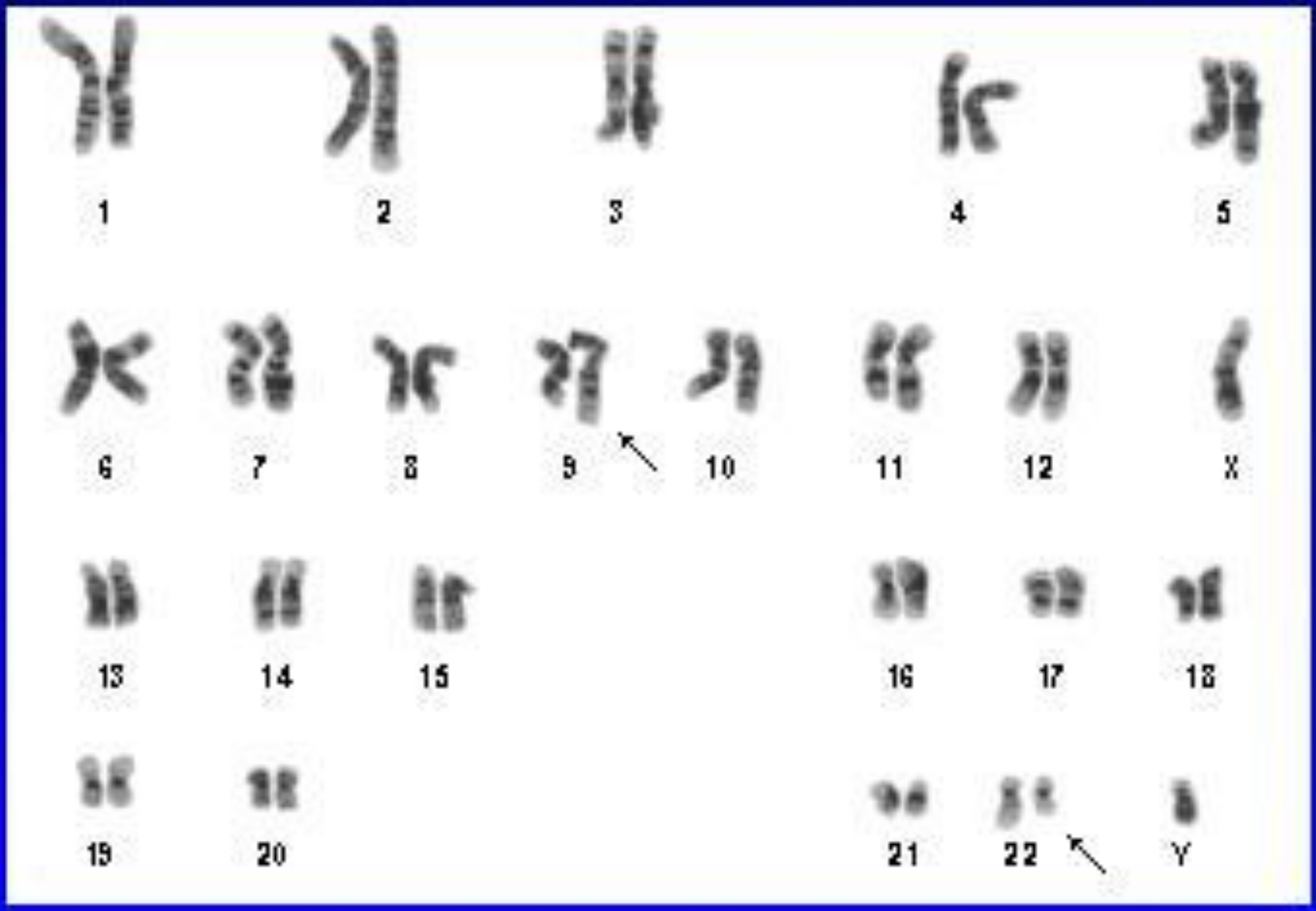
1973: Rowley identified the Ph^1 chromosome as a t(9:22).



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).

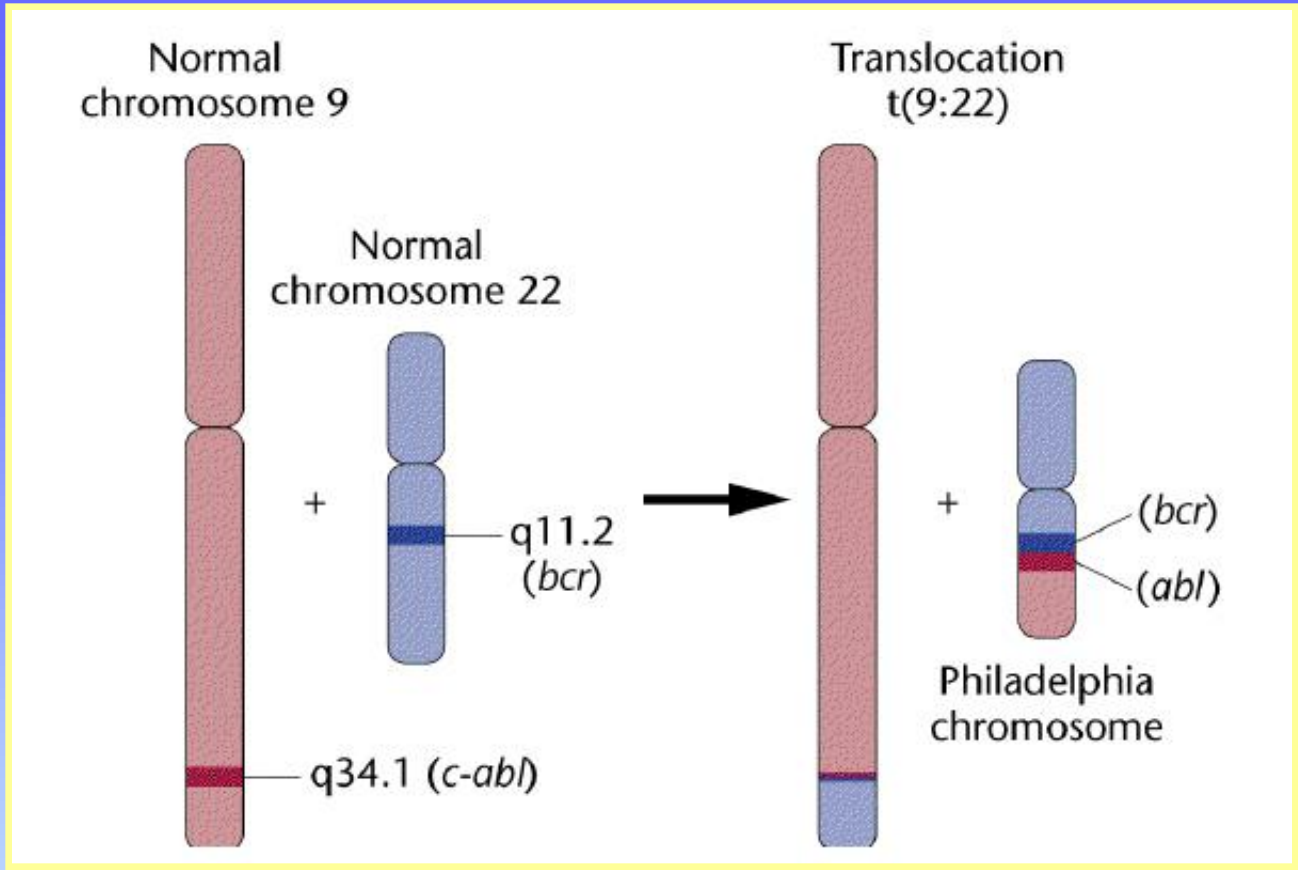
Karyotype Of A Cell With t(9;22) In Current Era



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.

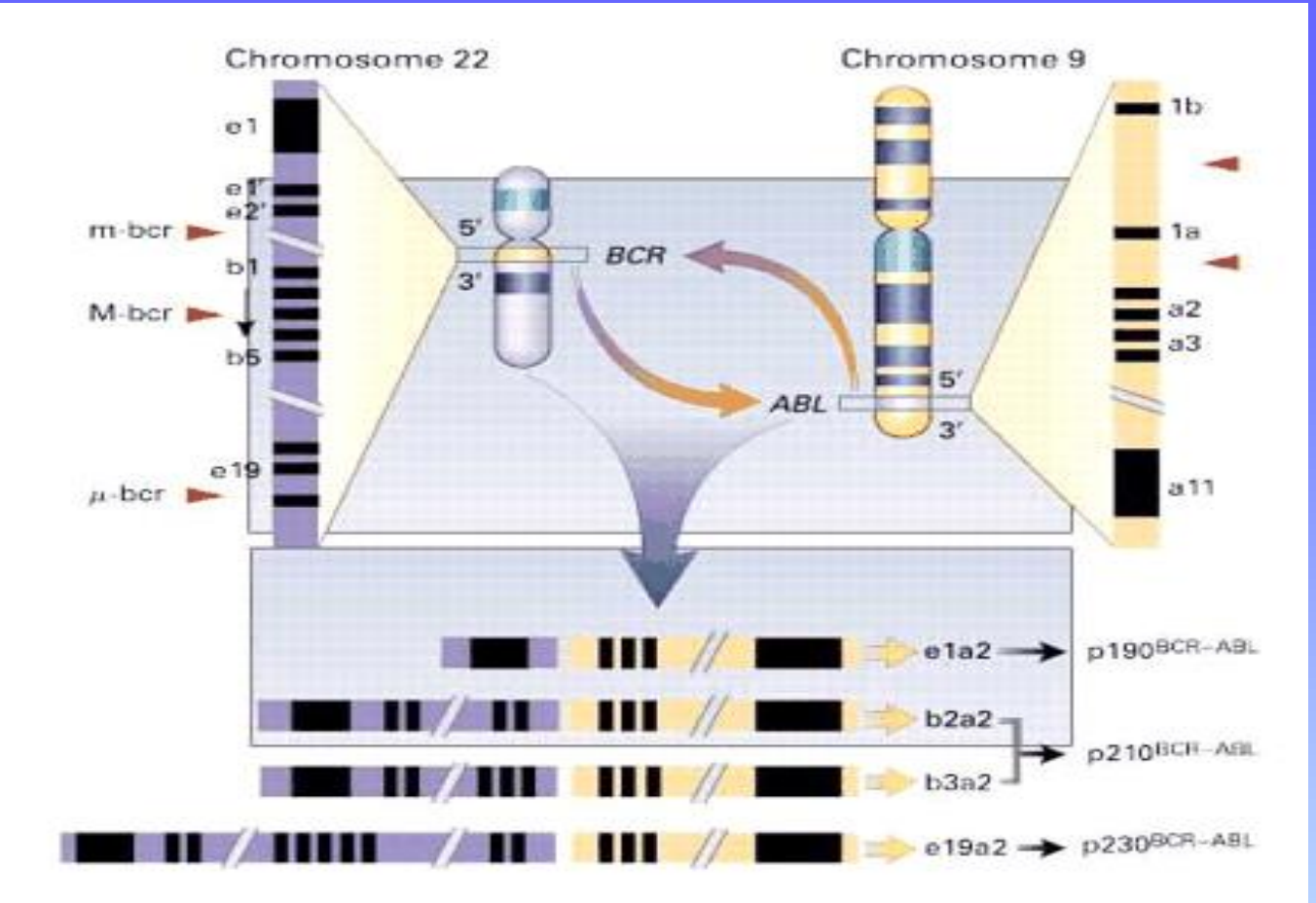
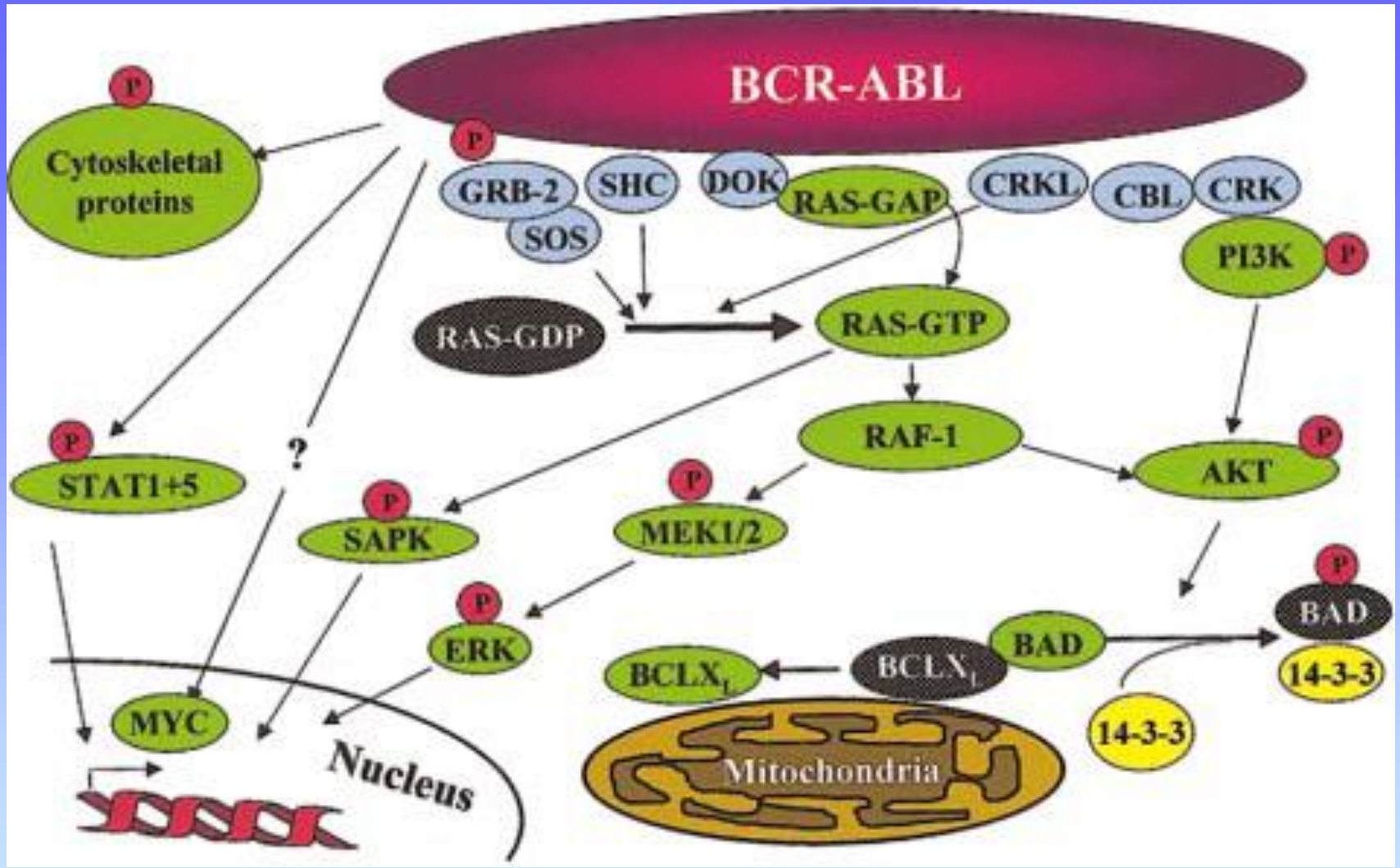


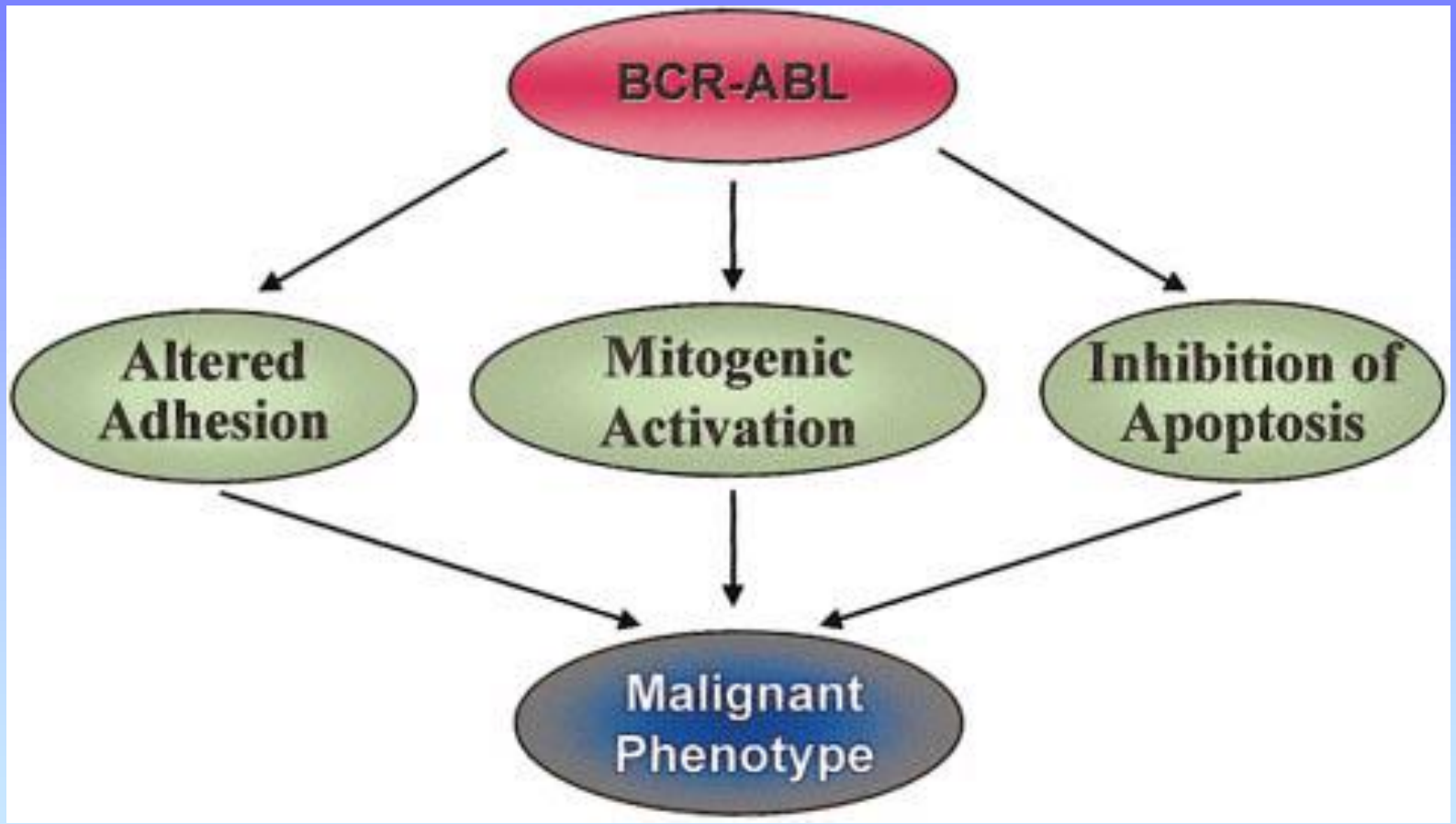
Figure 1. The Translocation of t(9;22)(q34;q11) in CML. The Philadelphia (Ph) chromosome is a shortened chromosome 22 that results from the translocation of 3' (toward the telomere) *ABL* segments on chromosome 9 to 5' *BCR* segments on chromosome 22. Breakpoints (arrowheads) on the *ABL* gene are located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



Signaling pathways activated inBCR-ABL–positive cells. Note that this is a simplified diagram and that many more associations between Bcr-Abl and signaling proteins have been reported.

Deininger et al. BLOOD, 15 NOVEMBER 2000 z VOLUME 96, NUMBER 10

Mechanisms implicated in the pathogenesis of CML



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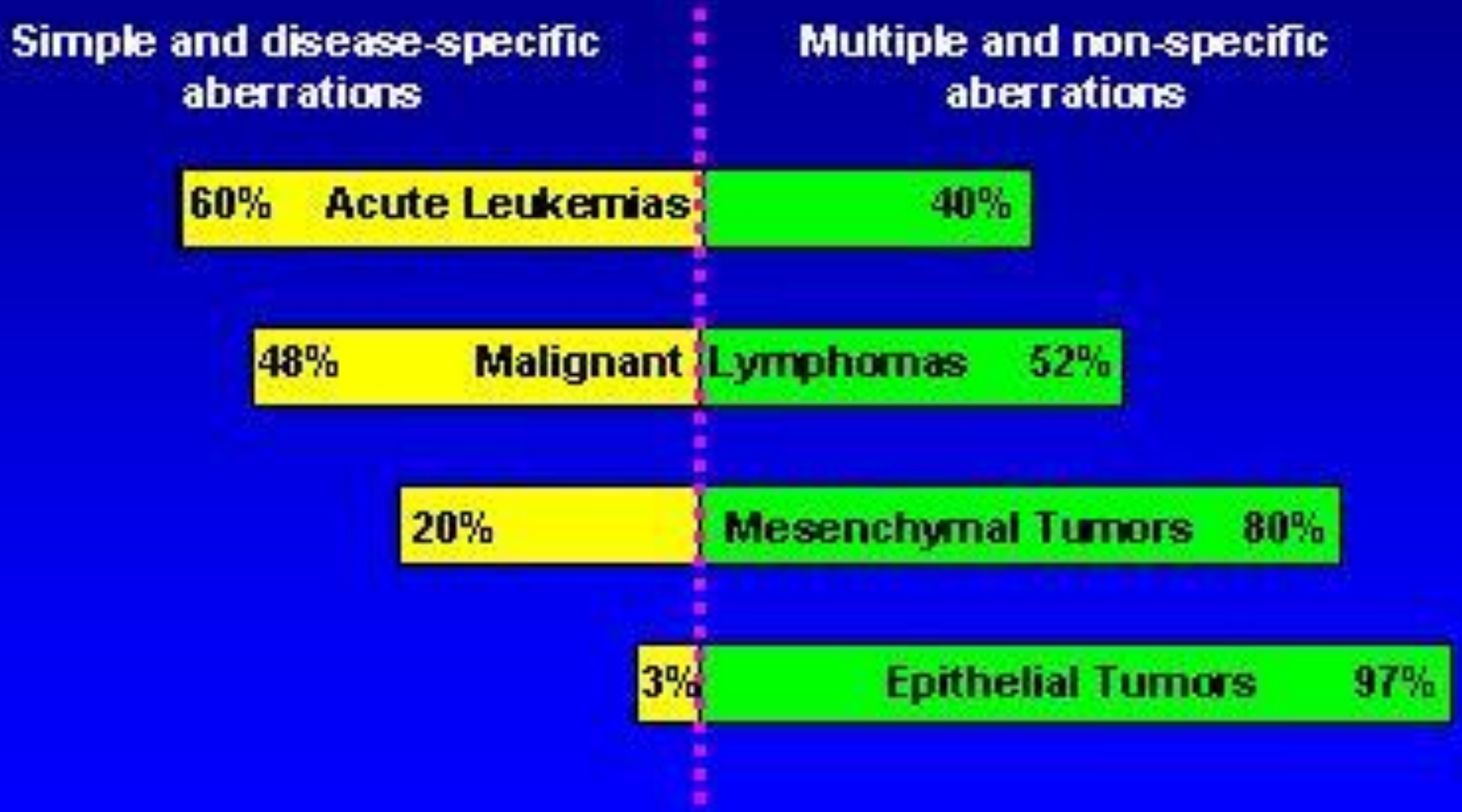
Chromosomal rearrangements or translocations

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

Karyotypic Patterns in Various Neoplasms



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.**

✓**GGC a GTC Glicina a valina en la p21**

✓**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.

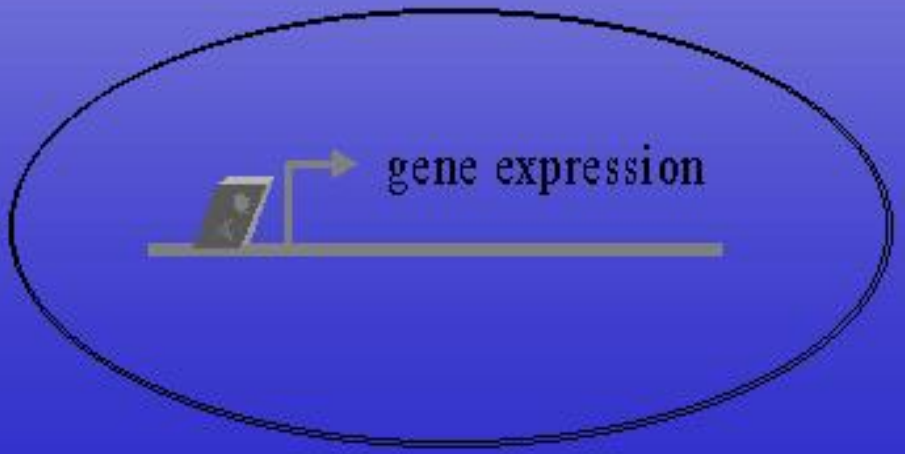
✓**Mutados en el 20-30% de todos los tumores**

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

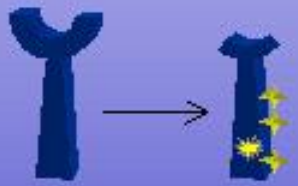


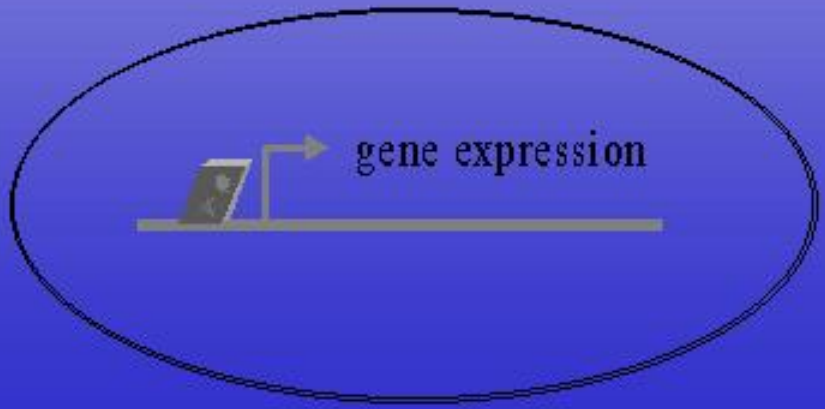
30% de todos los tumores, 90% de cáncer de páncreas, cáncer colorectal, pulmón, ALL

Δ-EGFR

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

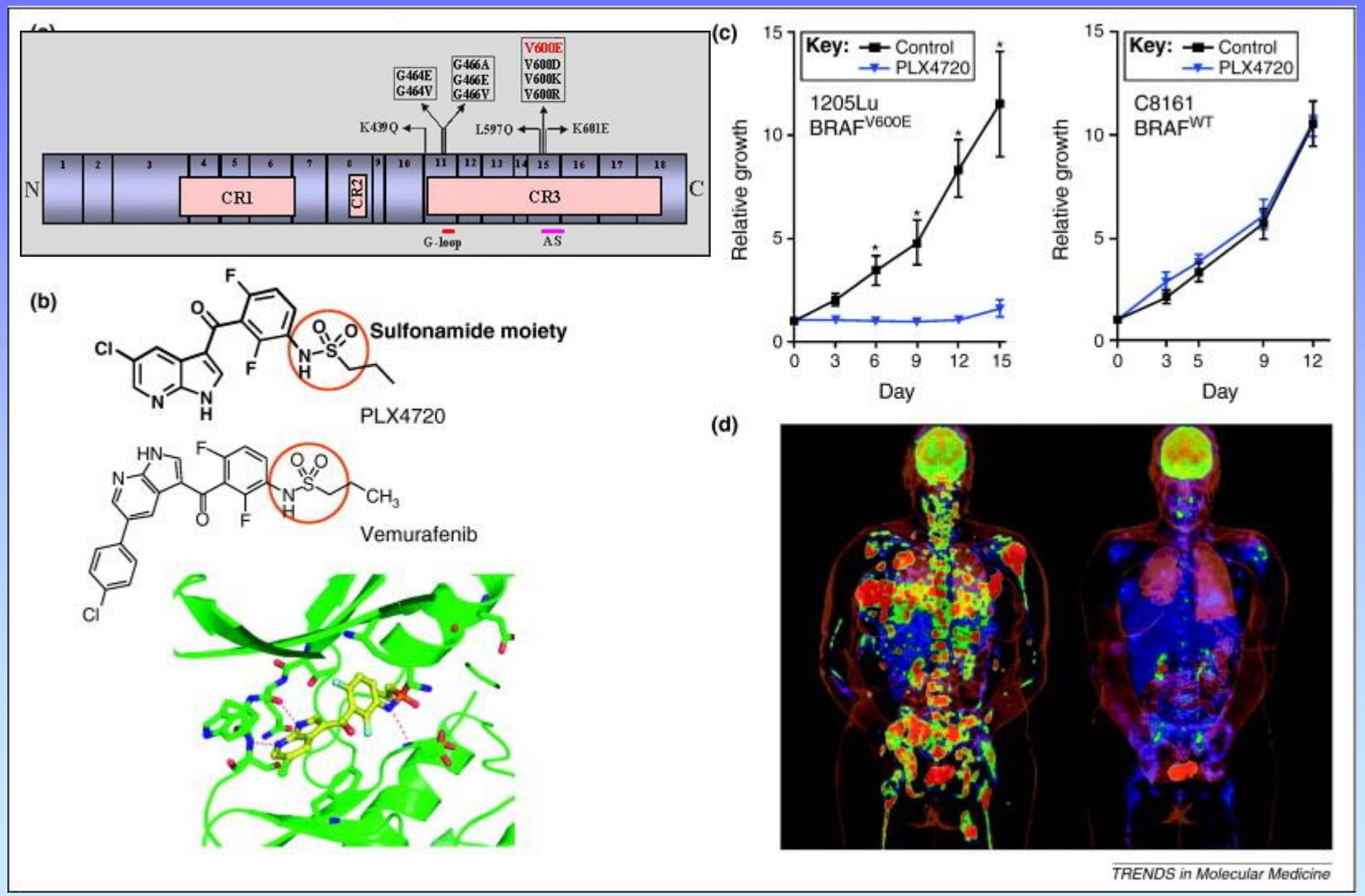
- a deletion mutation eliminates exons 2 to 7 in the extracellular domain, leading to ligand-independent activity
- this oncogene is also often amplified, and occurs in astrocytoma IV/glioblastoma multiforme





NSCLC Cáncer de pulmón de células no pequeñas, glioblastoma

BRAF oncogene

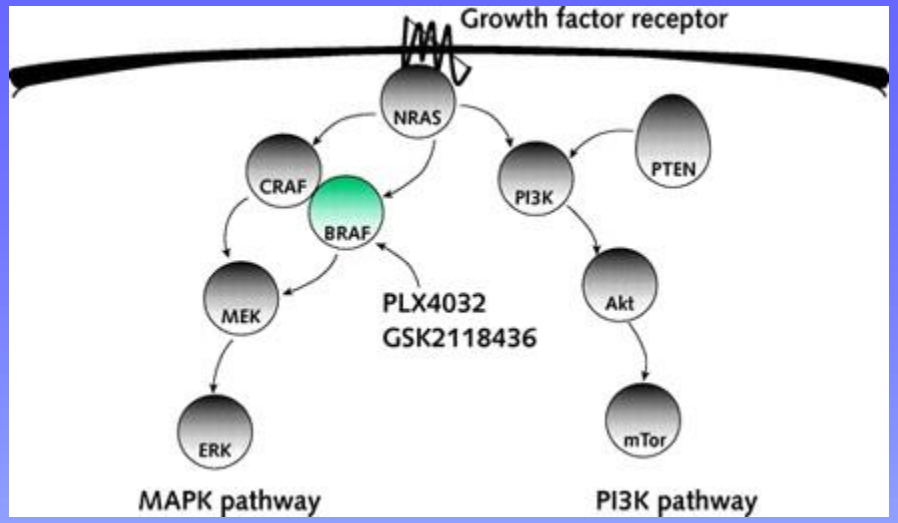


BRAF in melanoma

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. 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. 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.



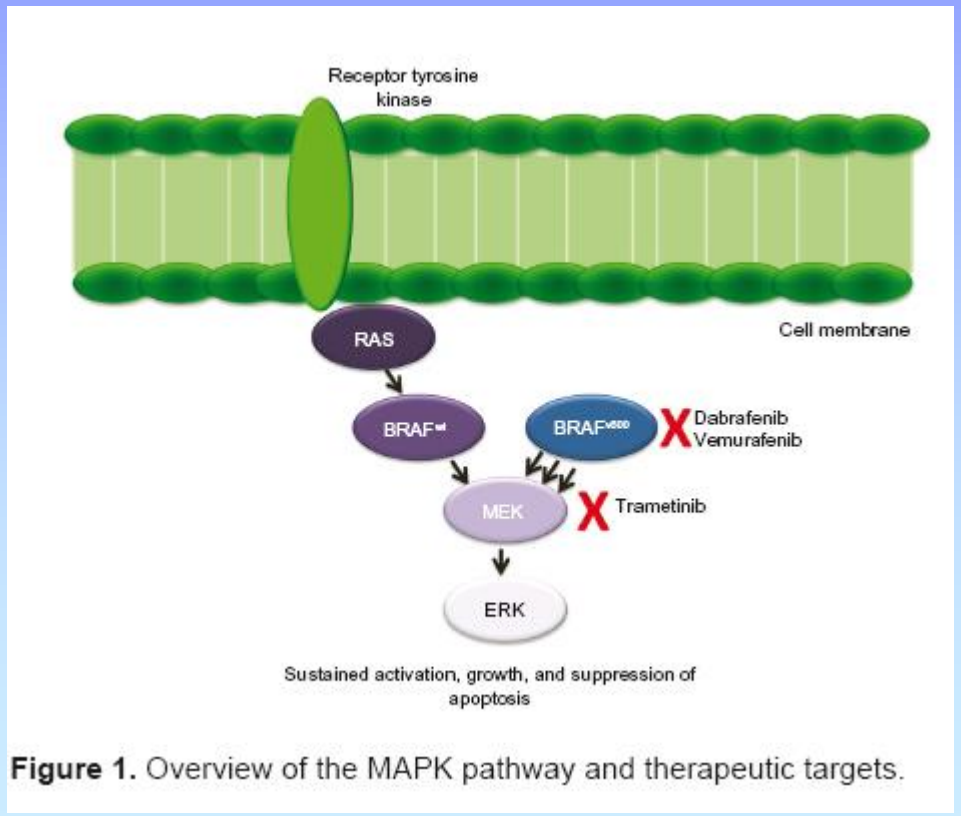
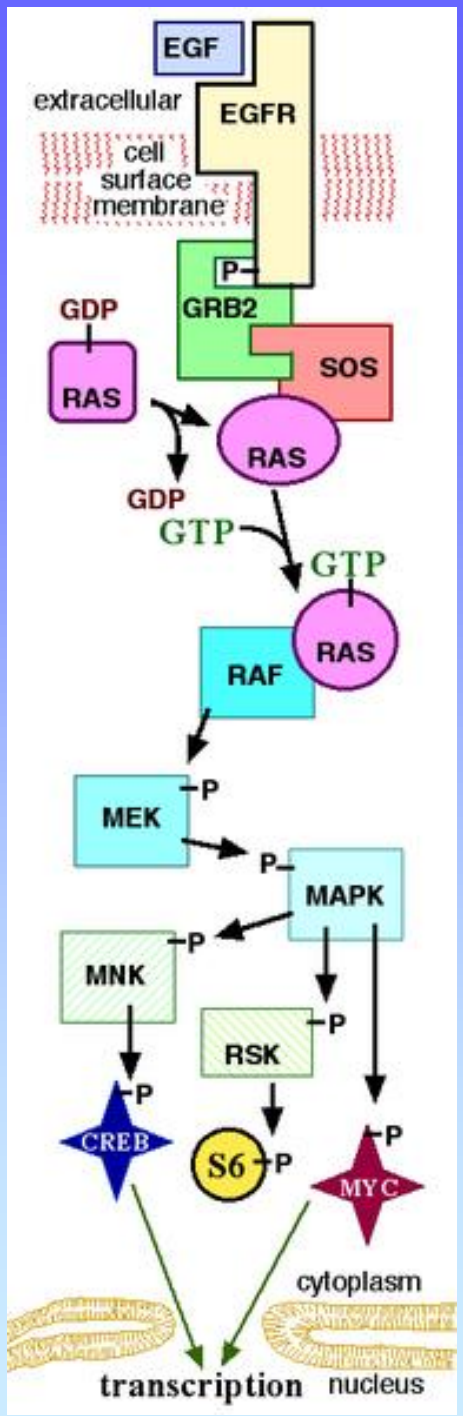


Figure 1. Overview of the MAPK pathway and therapeutic targets.

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

3- Activación por amplificación génica:

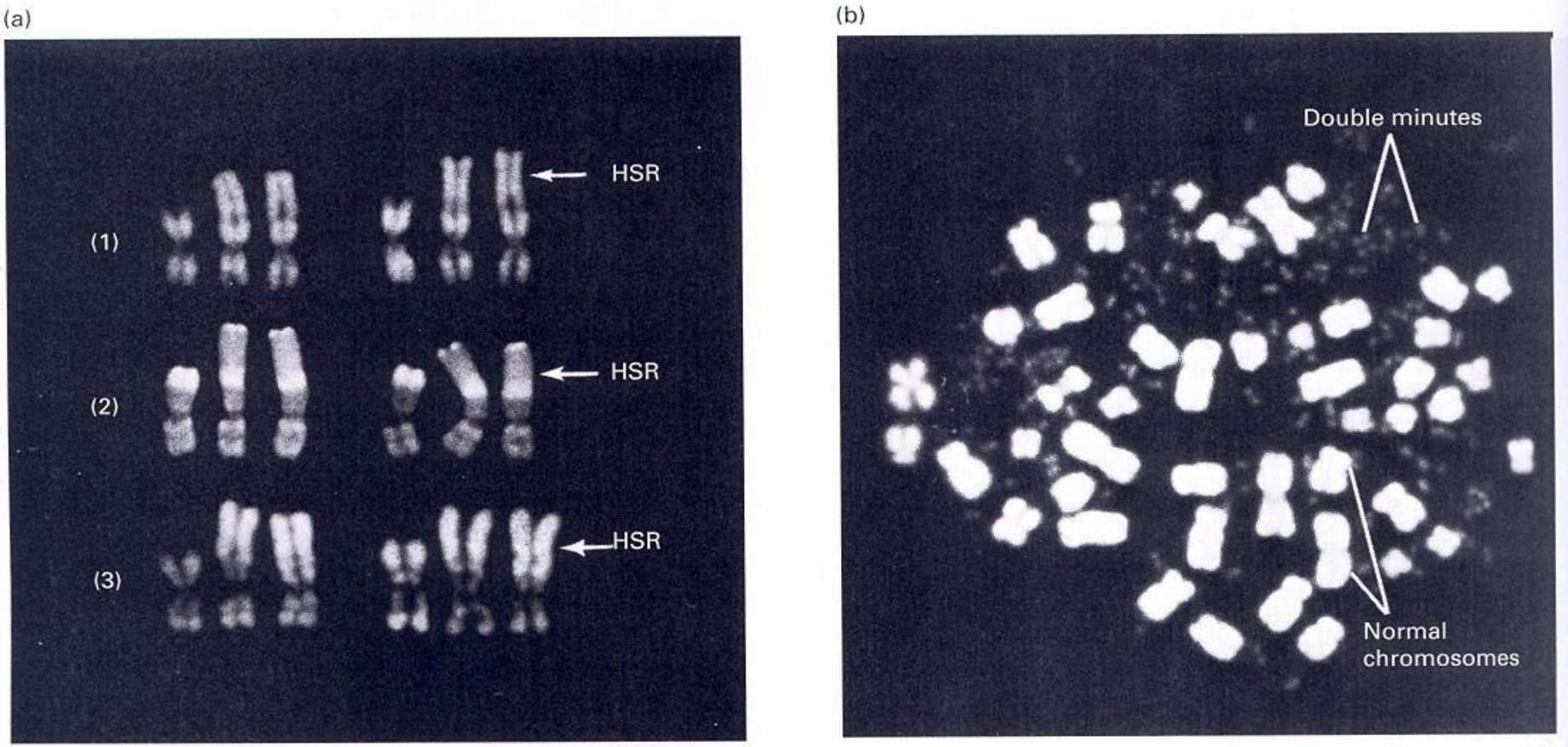
✓**Reduplicación de un proto-oncogen hasta varios cientos de veces en el mismo cromosoma**

✓**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) (minicromosomas sin centromero)**

✓**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)

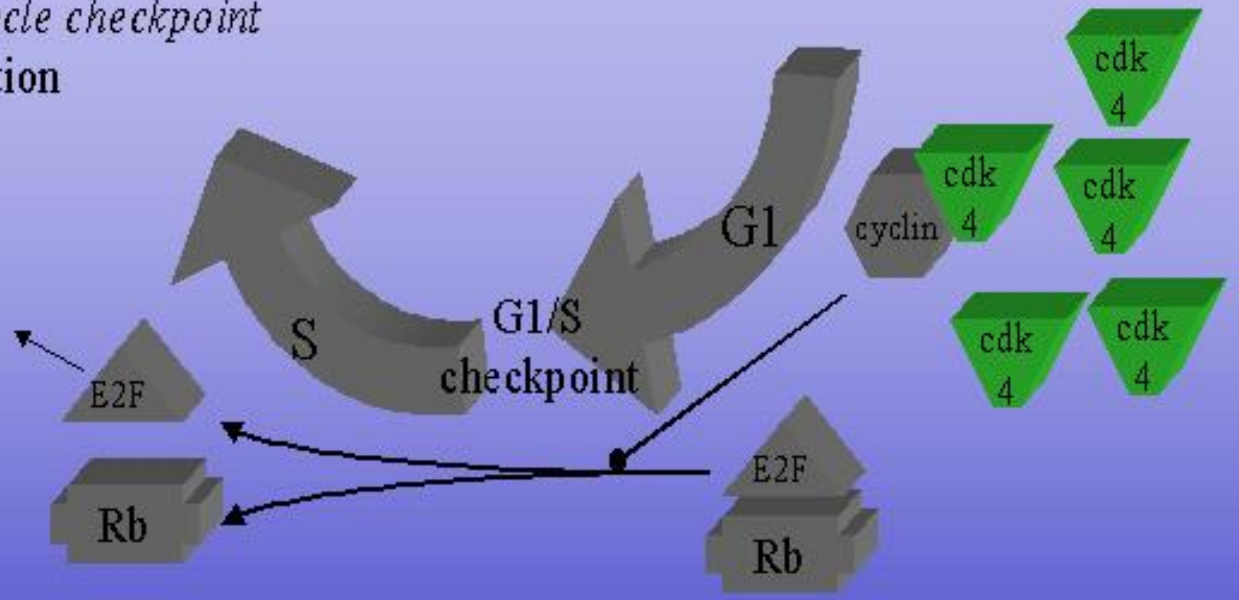
✓**El grado de amplificación incrementa la agresividad de los tumores y puede correlacionar con la sobrevida.**



▲ **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.]

cdk4

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

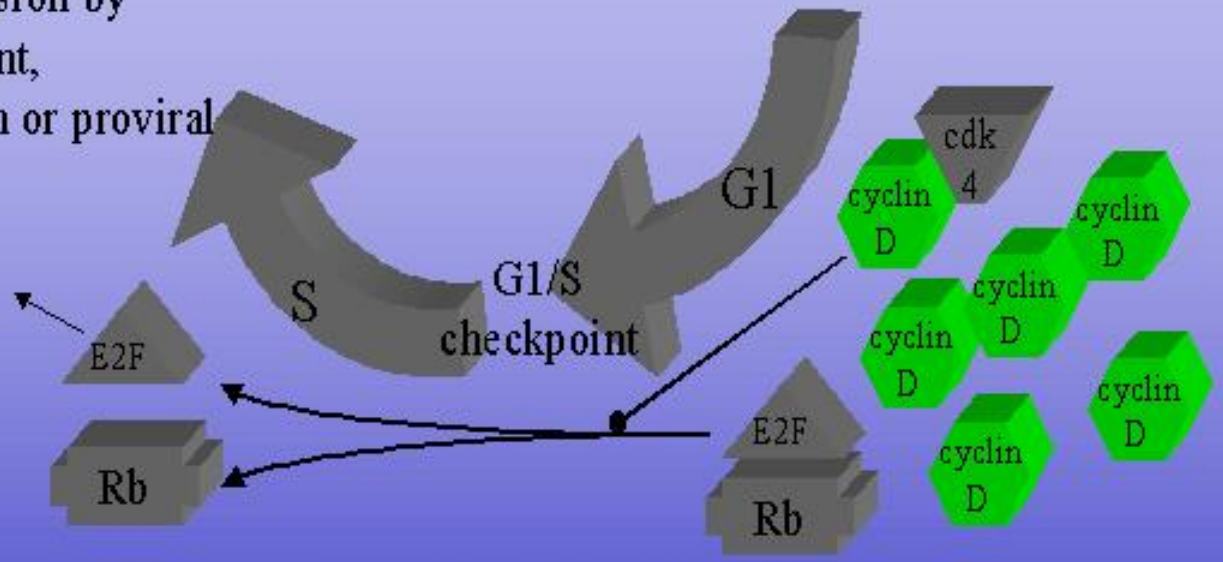


Amplification of the gene for cdk4 at the genomic level can lead to the forcing of the G1 checkpoint by overactivity of the cyclin/cdk complexes.

Cáncer de mama, osteosarcoma, glioblastoma, cáncer de vejiga

cyclin D

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

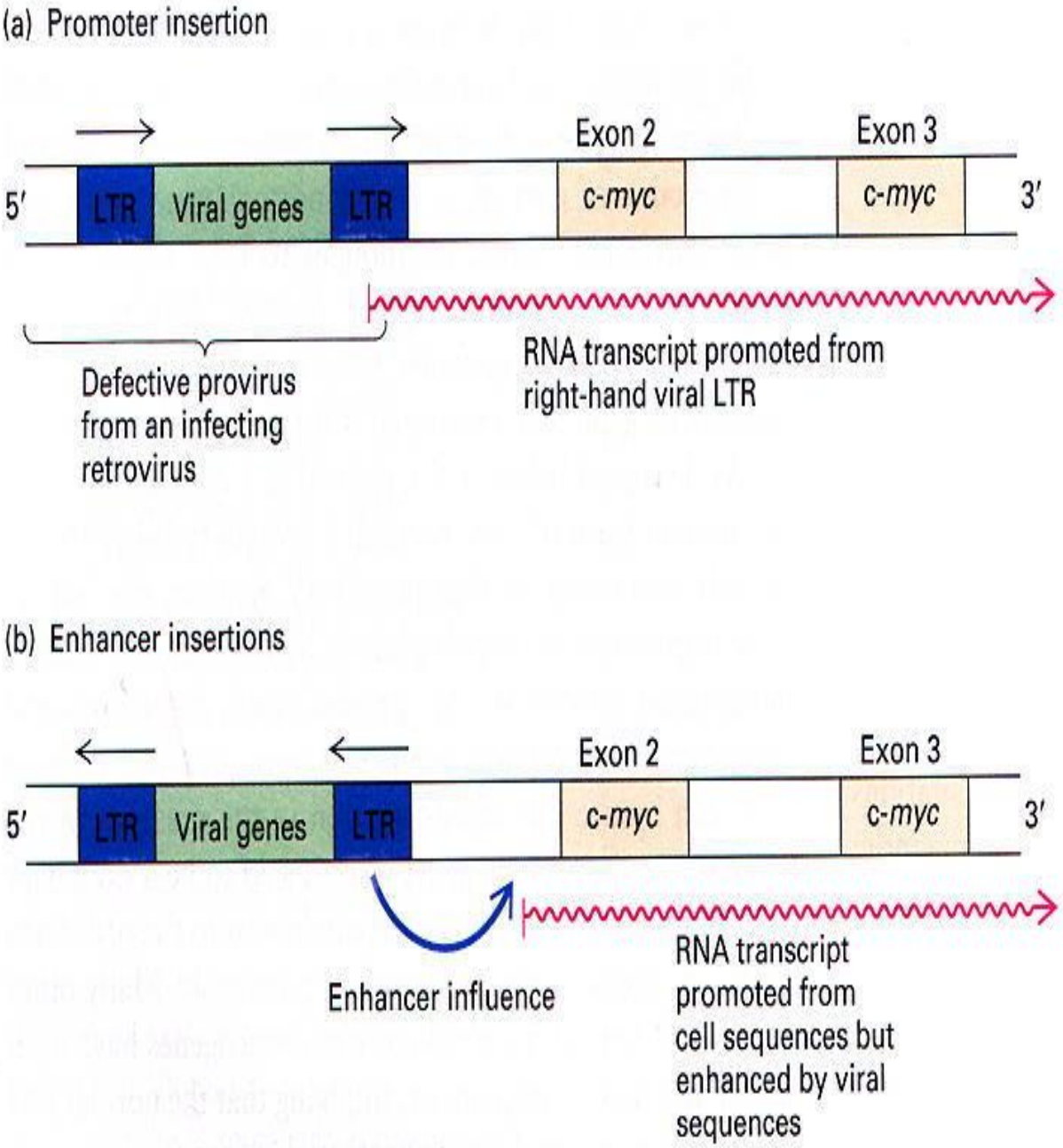


- Overexpression of the cyclin D1 causes deregulation of the G1/S checkpoint.
- It can occur by :
  - rearrangement: PRAD1 in parathyroid carcinomas,
  - amplification in many tumor types
  - or by retroviral insertion: bcl1

Carcinoma de esófago, cáncer de cabeza y cuello, cáncer de mama

Gene amplification

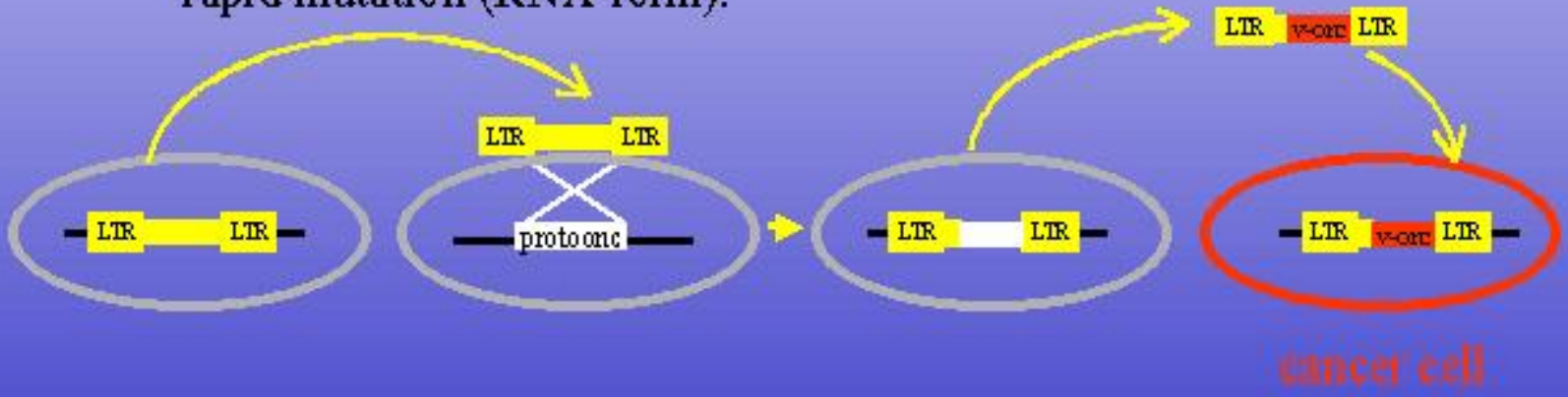
Oncogene	Amplification	Source of tumor
c-myc	~20-fold	leukemia and lung carcinoma
N-myc	5-1,000-fold	neuroblastoma retinoblastoma
L-myc	10-20-fold	small-cell lung cancer
c-abl	~5-fold	chronic myoloid leukemia
c-myb	5-10-fold	acute myeloid leukemia colon carcinoma
c-erbB	~30-fold	epidermoid carcinoma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



◀ 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.]

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).

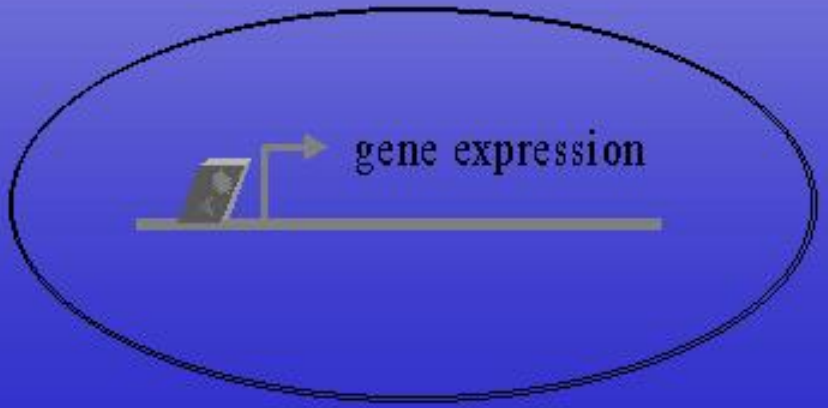


- If this results in an activated v-onc it will provide the virus with a selective advantage by causing its host cell to rapidly divide.

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- Reguladores de apoptosis

1.6- Factores reguladores nucleares

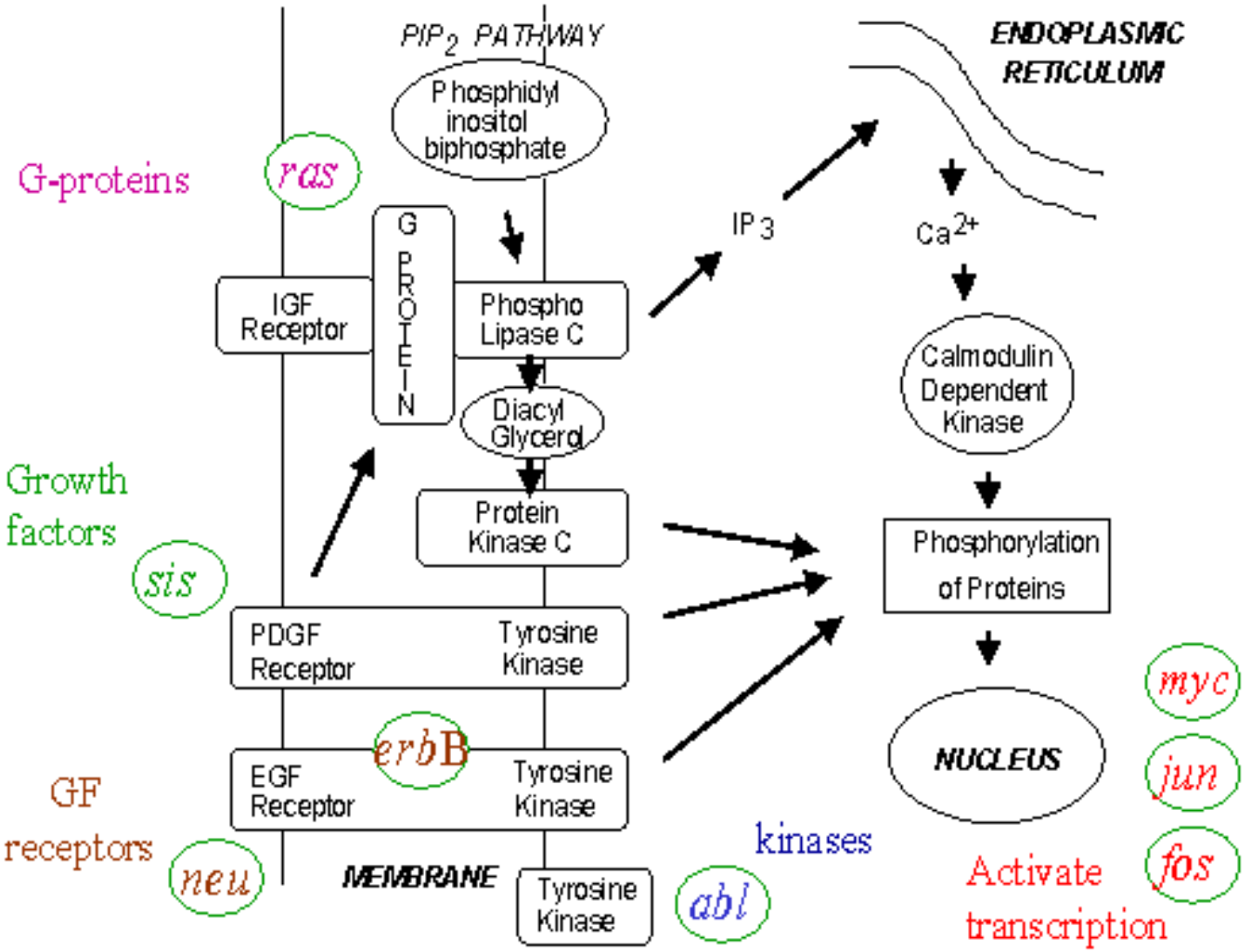
Factores de crecimiento

- ✓ **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



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

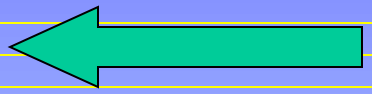
Oncogenes as signal transducers

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

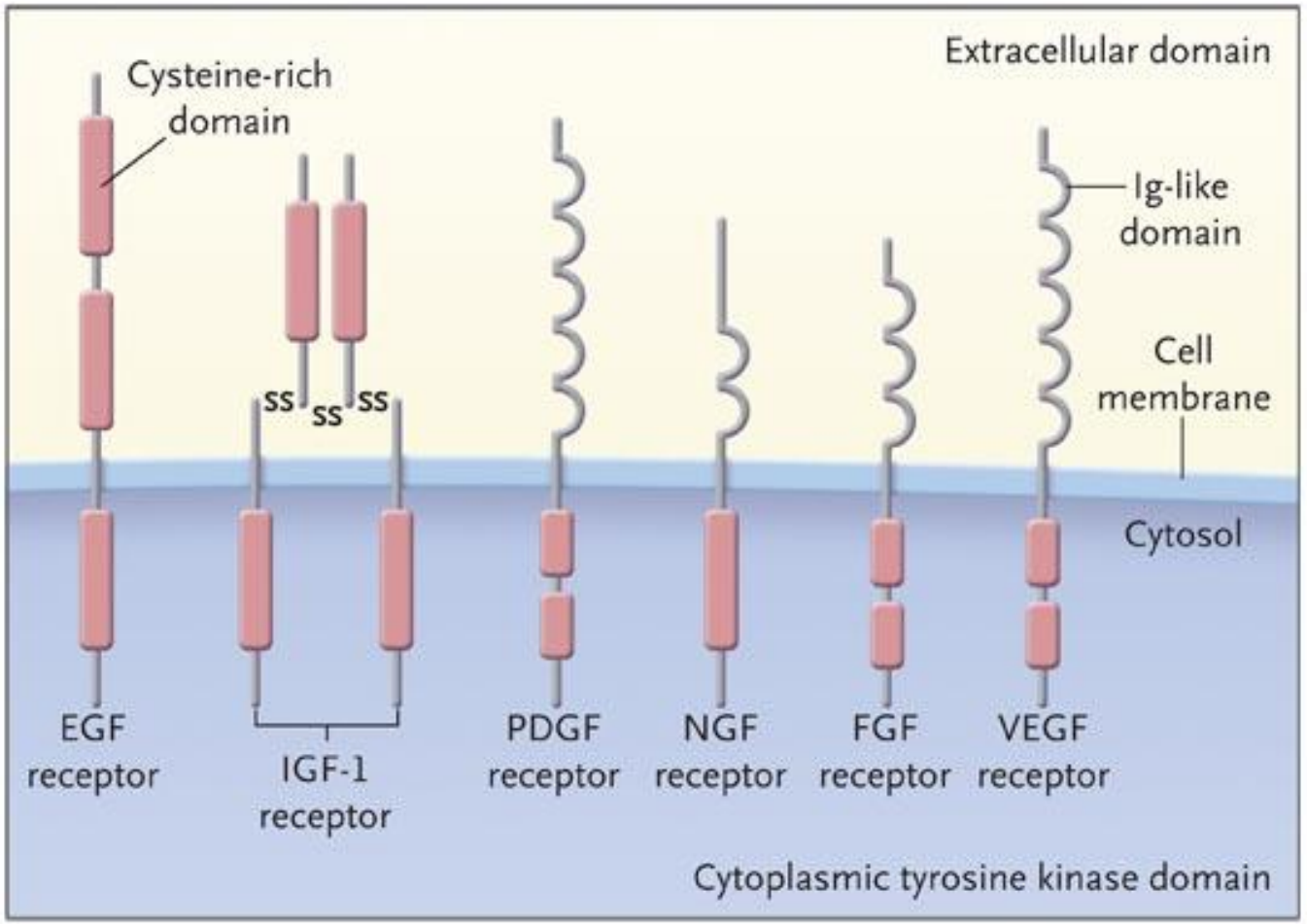
v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

NUCLEUS

Transcription Factors

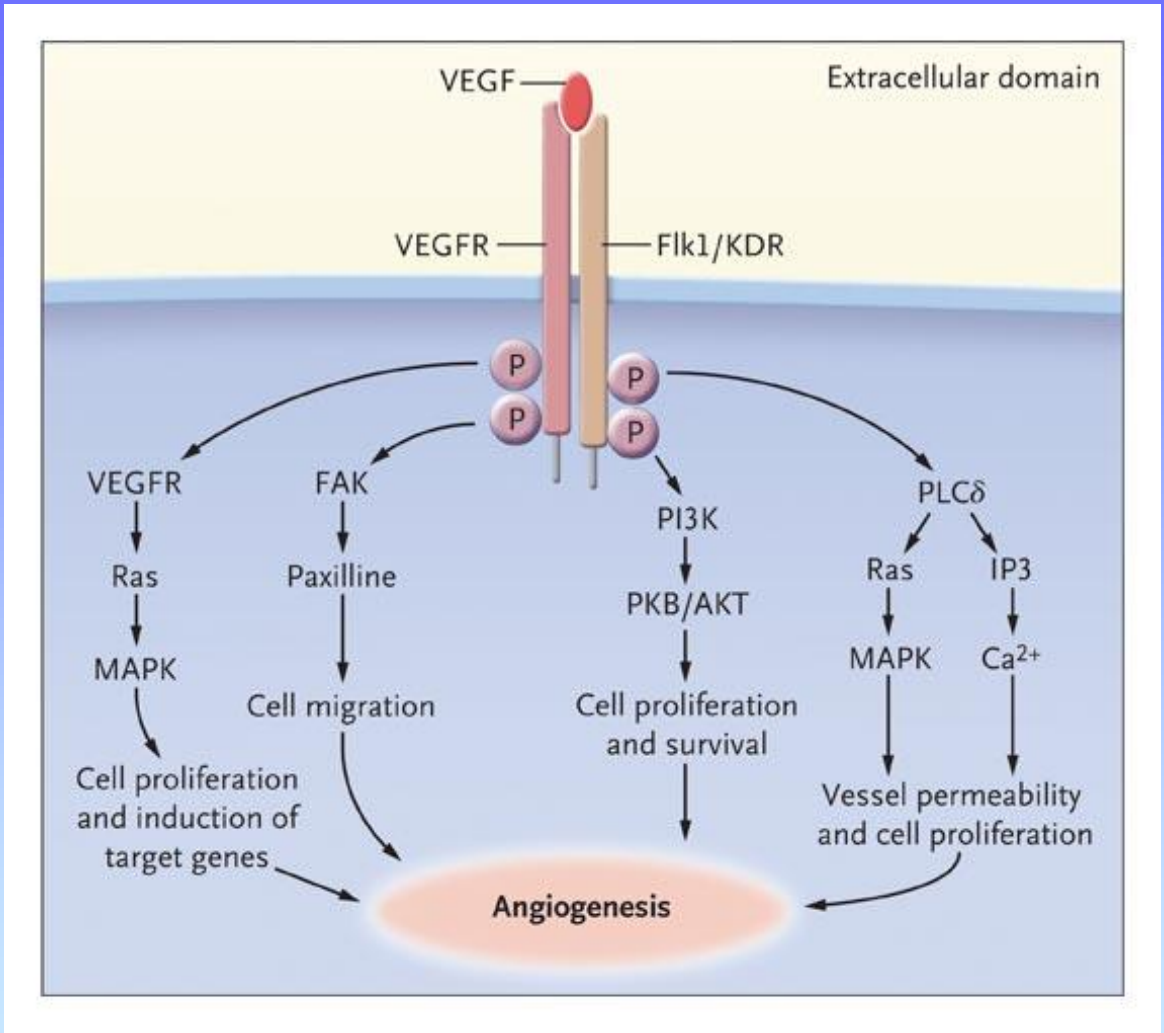
v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

Examples of Receptor Tyrosine Kinases



Croce C. N Engl J Med 2008;358:502-511

Role of VEGF–VEGFR Interaction in Angiogenesis



Croce C. N Engl J Med 2008;358:502-511

HER family receptors

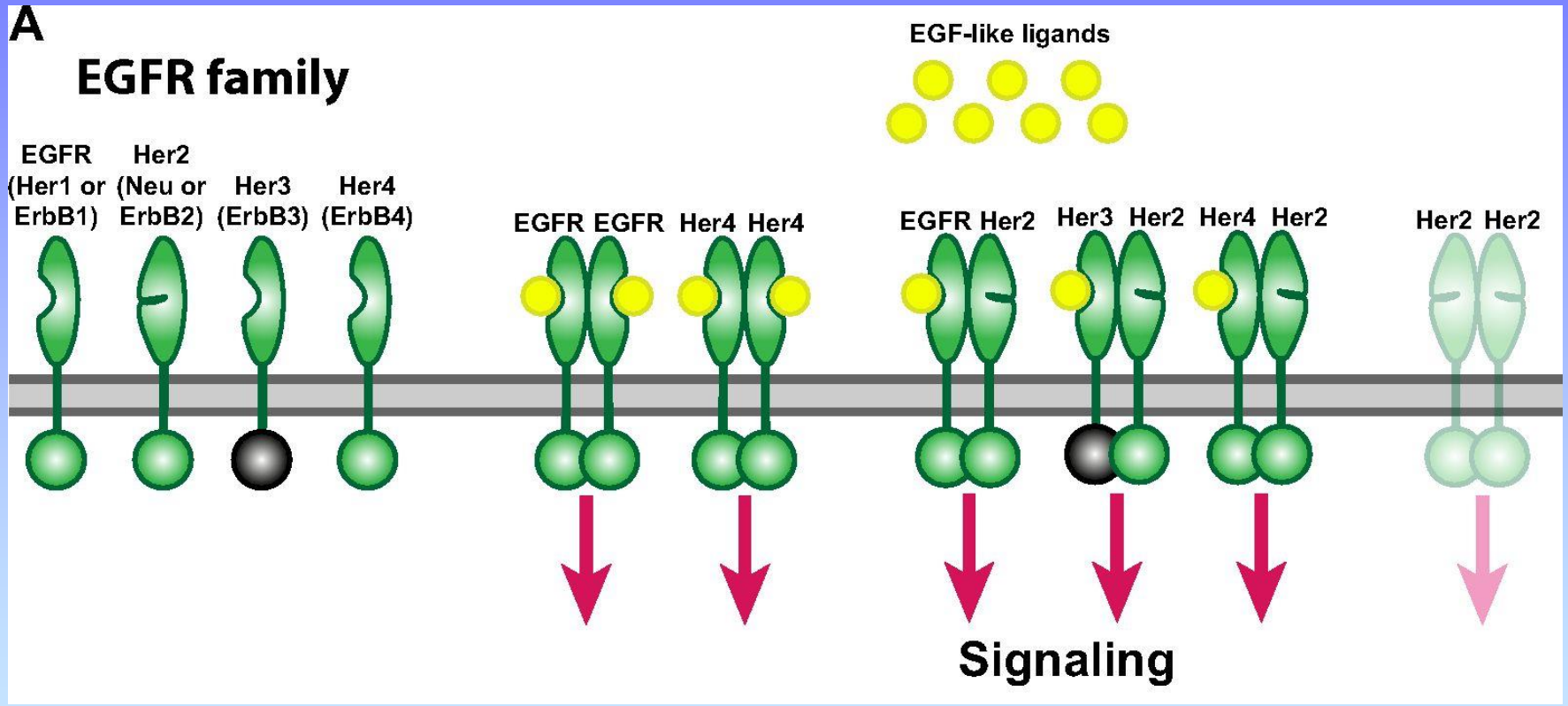
✓HER family receptors are activated by ligand-induced dimerization, or receptor pairing.

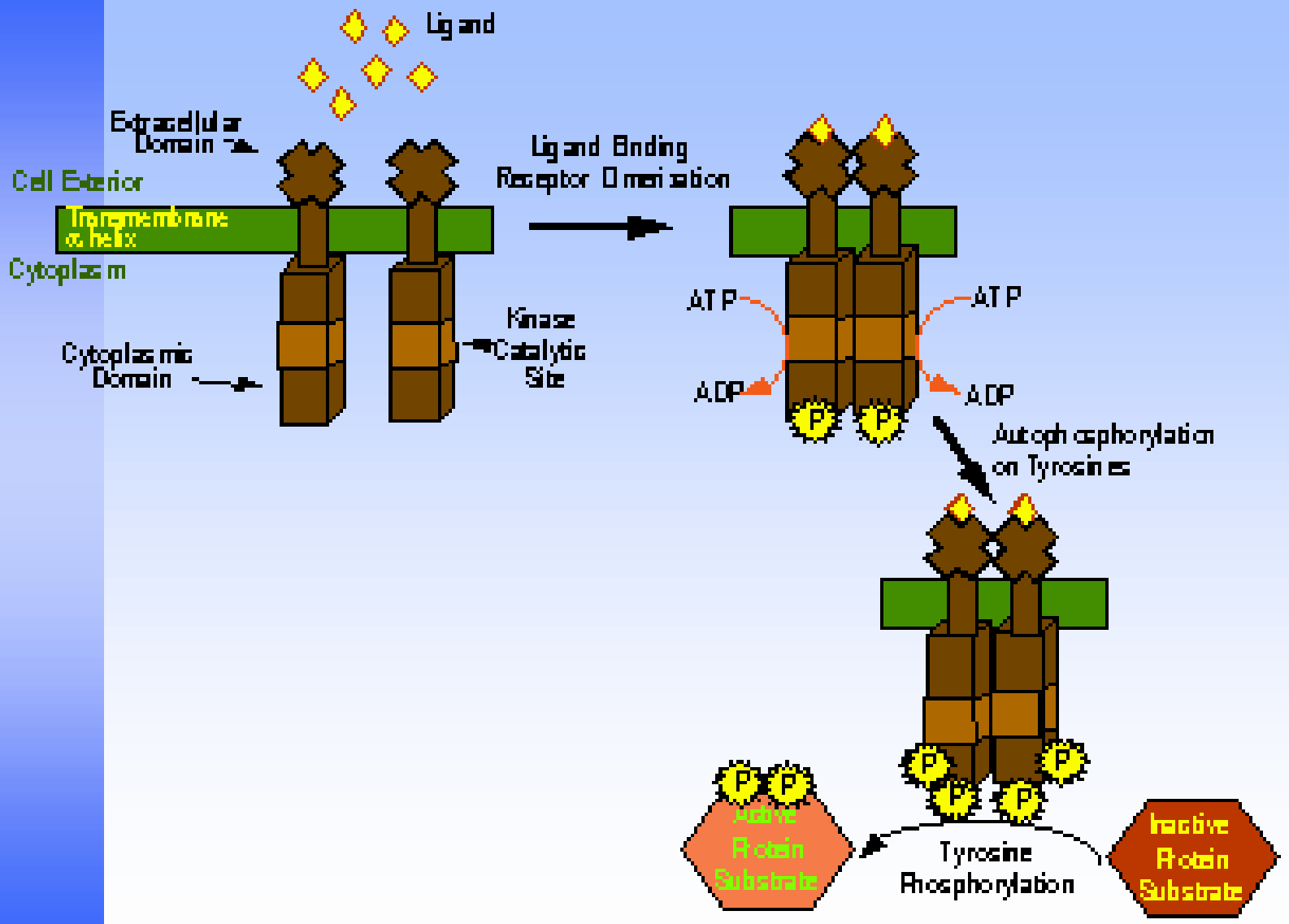
✓Dimerization is a critical step in HER family-mediated signaling, and HER receptors are able to homodimerize or heterodimerize with other HER family members, allowing for multiple receptor combinations.

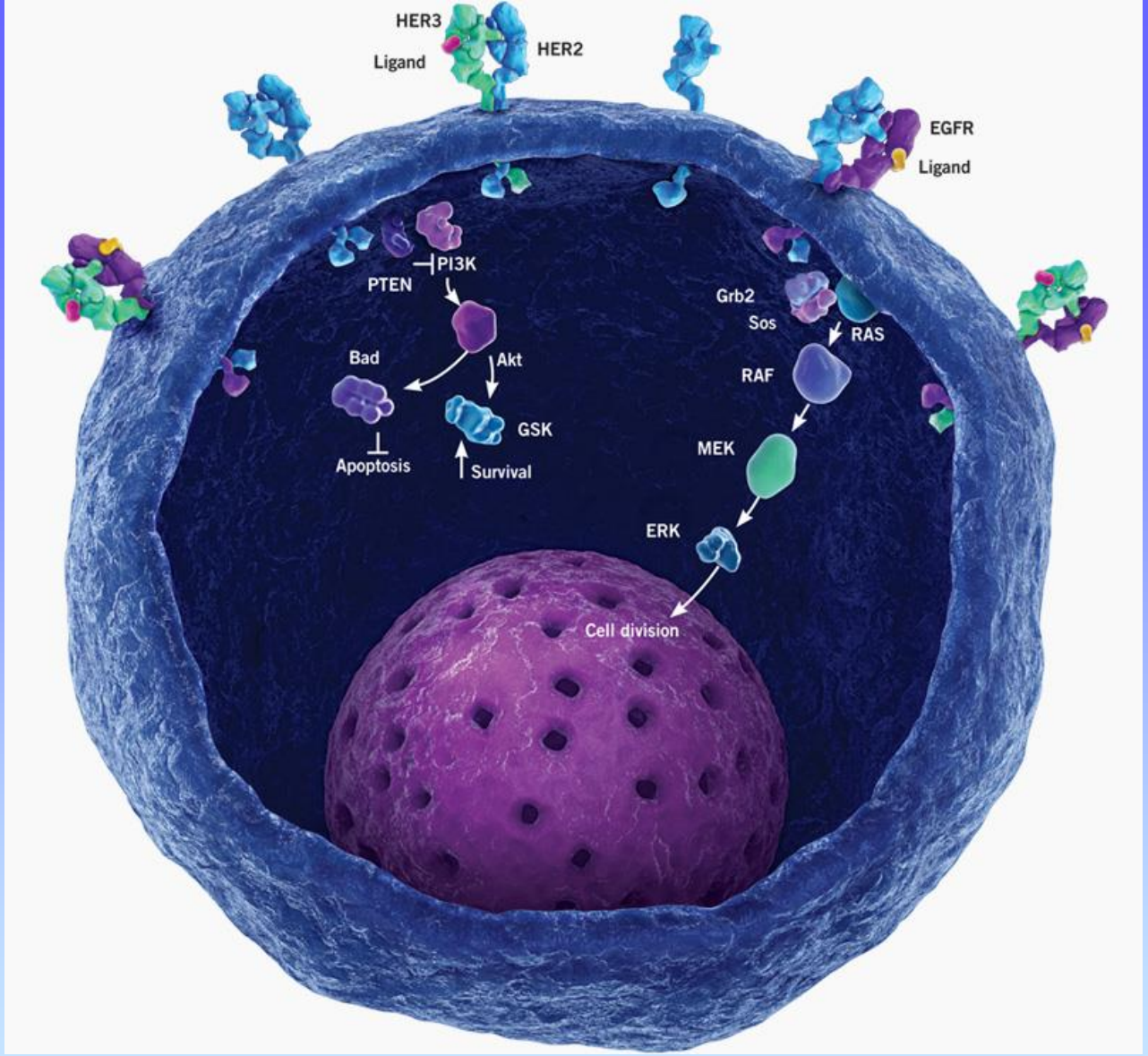
✓The formation of dimers leads to activation of the intrinsic TK domain and subsequent phosphorylation on specific tyrosine residues, which serve as docking sites for a variety of molecules. Recruitment of these molecules leads to the activation of different downstream signaling cascades, including the MAPK proliferation pathway and/or the PI3K/Akt pro-survival pathway.

✓Inappropriate signaling may occur as a result of receptor overexpression or dysregulation of receptor activation, which may lead to:

Increased/uncontrolled cell proliferation
Decreased apoptosis (programmed cell death)
Enhanced cancer cell motility
Angiogenesis


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

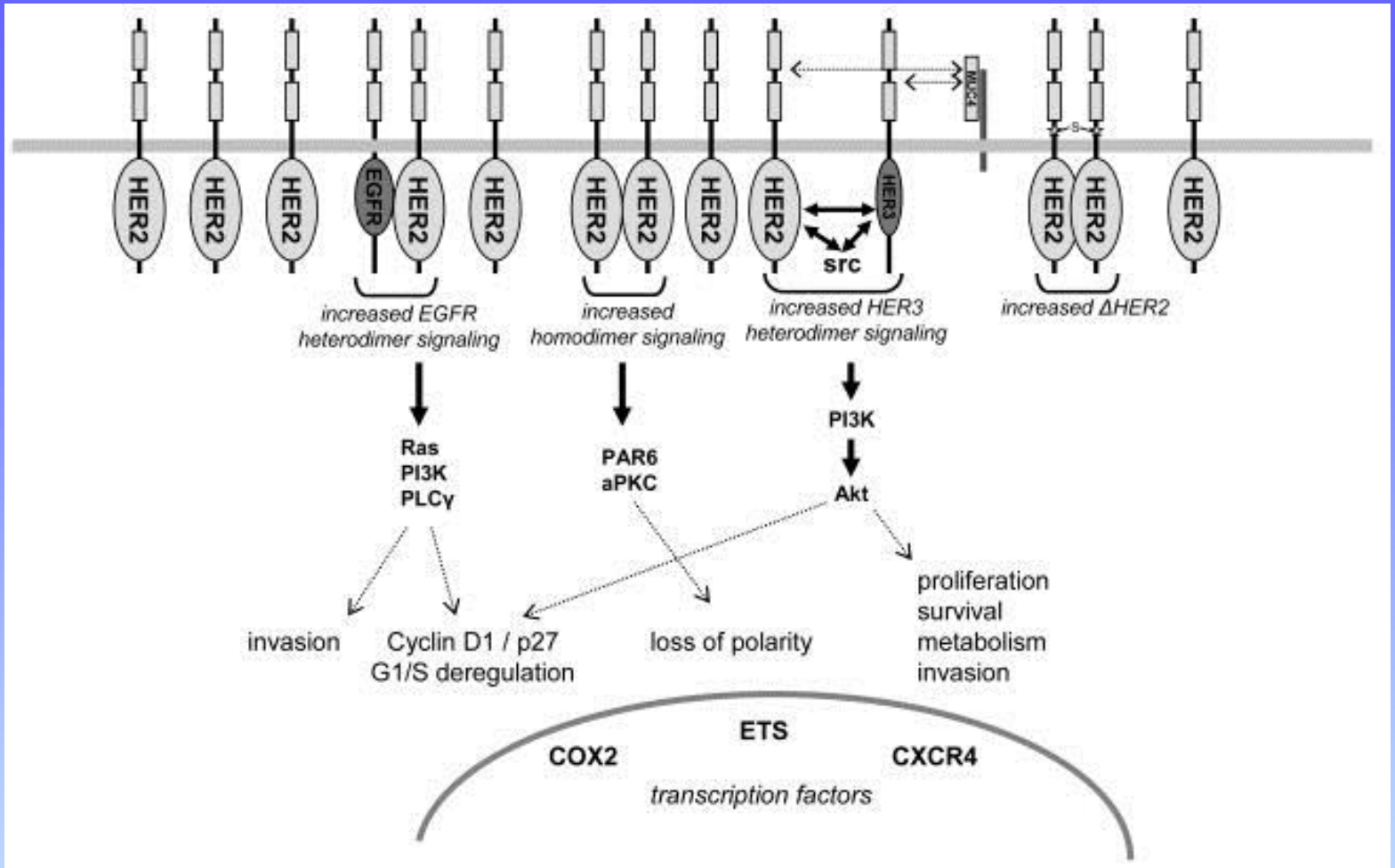

Ligand
Extracellular Domain
Cell Exterior
Transmembrane α helix
Cytoplasm
Cytoplasmic Domain
Kinase Catalytic Site
Ligand Binding Receptor Dimerization
ATP
ATP
ADP
ADP
P
Autophosphorylation on Tyrosines
Active Protein Substrate
Tyrosine Phosphorylation
Inactive Protein Substrate



Dysregulation of HER-mediated signaling pathways results in the growth and spread of cancer cells. The HER family consists of 4 structurally related receptors: HER1 (EGFR), HER2, HER3, and HER4.

HER-2

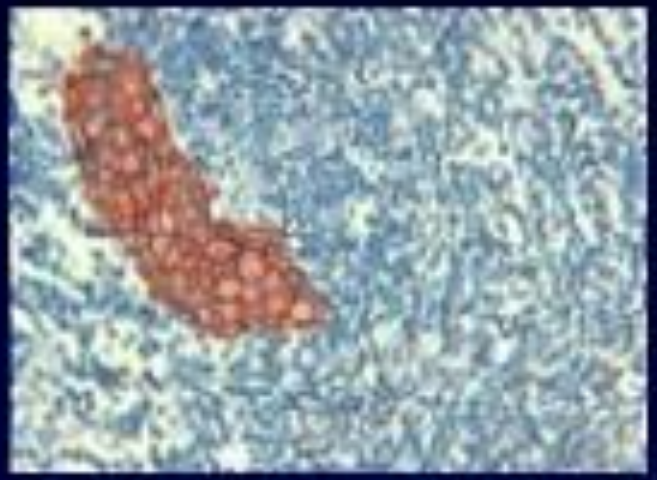
- Human Epidermal Growth Factor Receptor 2
- Also "known as":
  -neu (murine gene) or
  -c-erbB-2
- Member of the type I RTKs which include HER-1 (EGFR), HER-3 and HER-4
- HER-2 protein = p185,000



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

HER-2 Oncogene
Amplification

Breast Cancer



HER-2 Oncoprotein
Overexpression

Shortened Survival

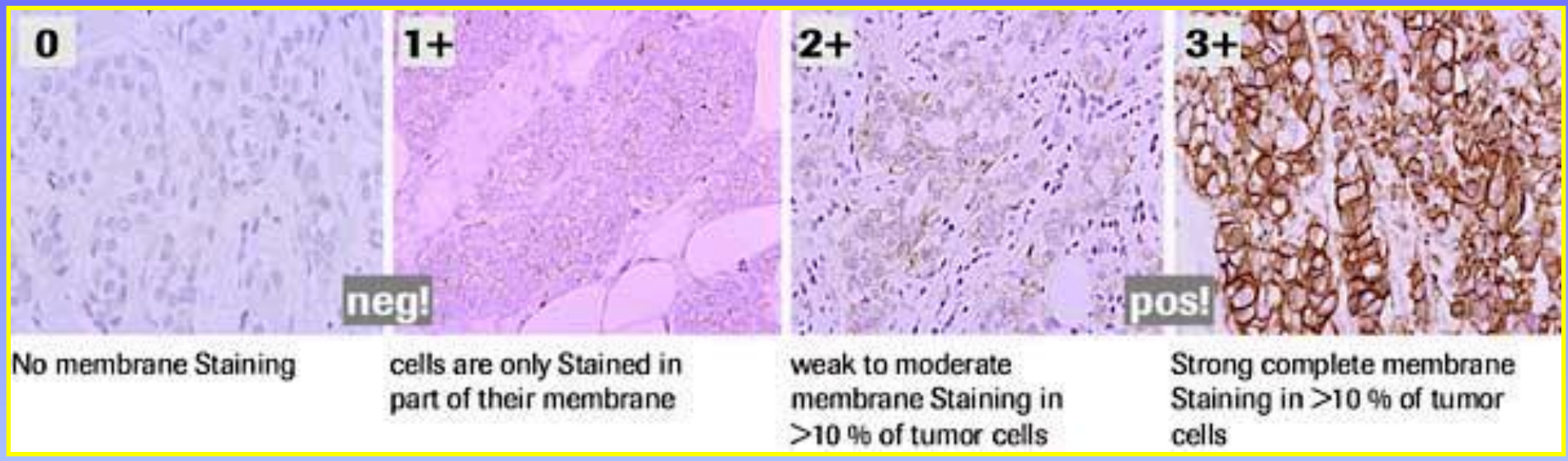
Median Survival from First Diagnosis

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

Slamon et al, 1987

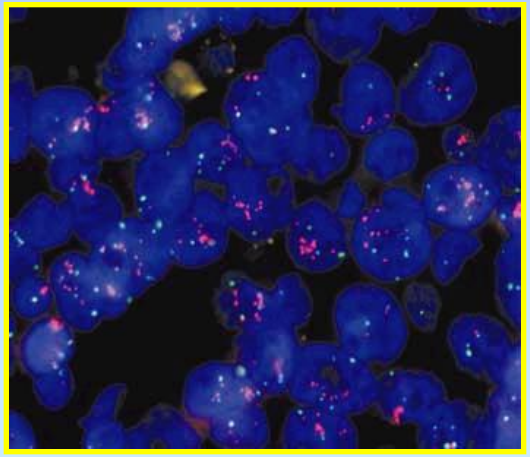
Determination of HER2-protein overexpression

1- semiquantitative DAKO Hercep Test™

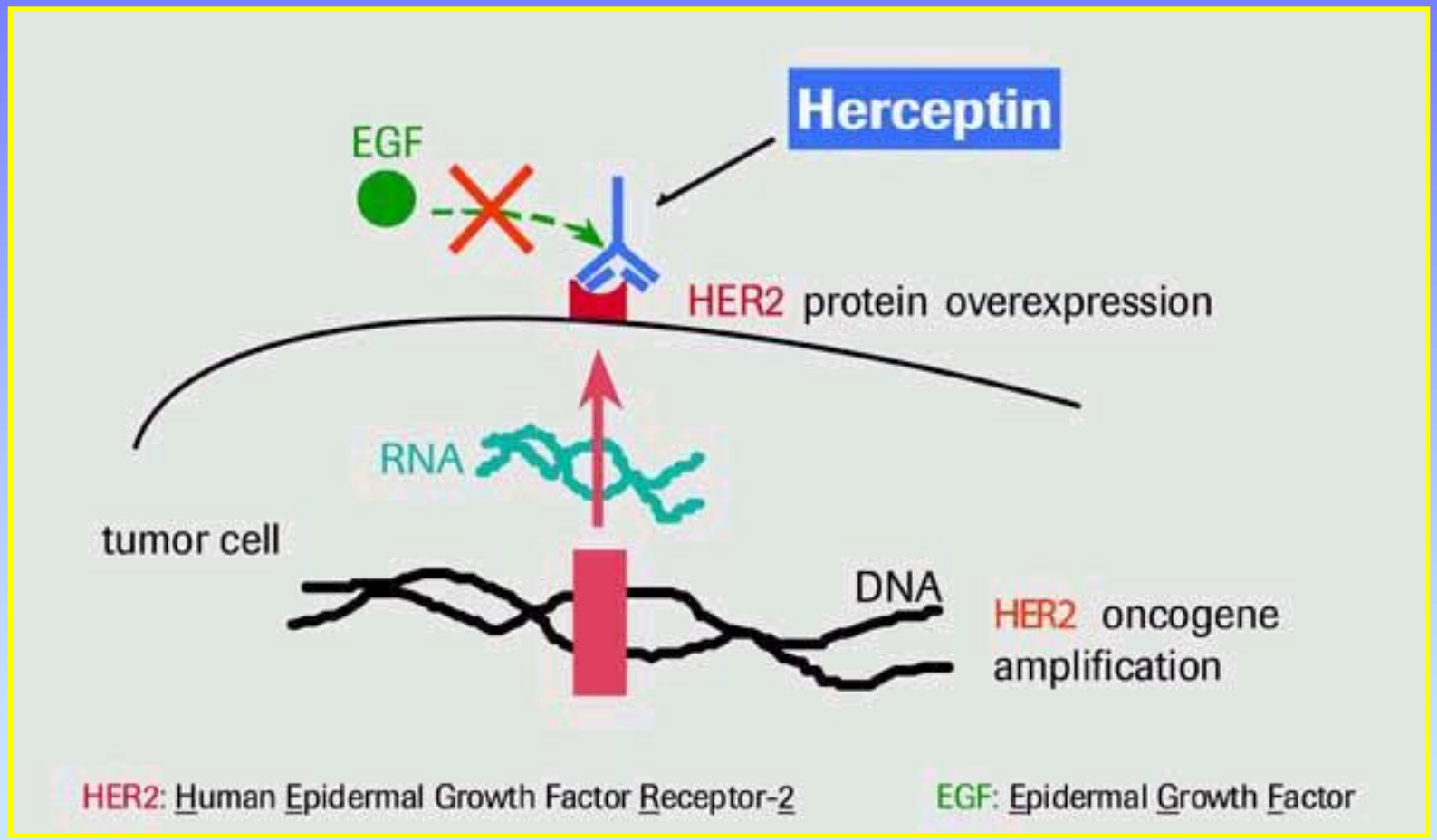


2- Fluorescence in situ hybridisation (FISH)

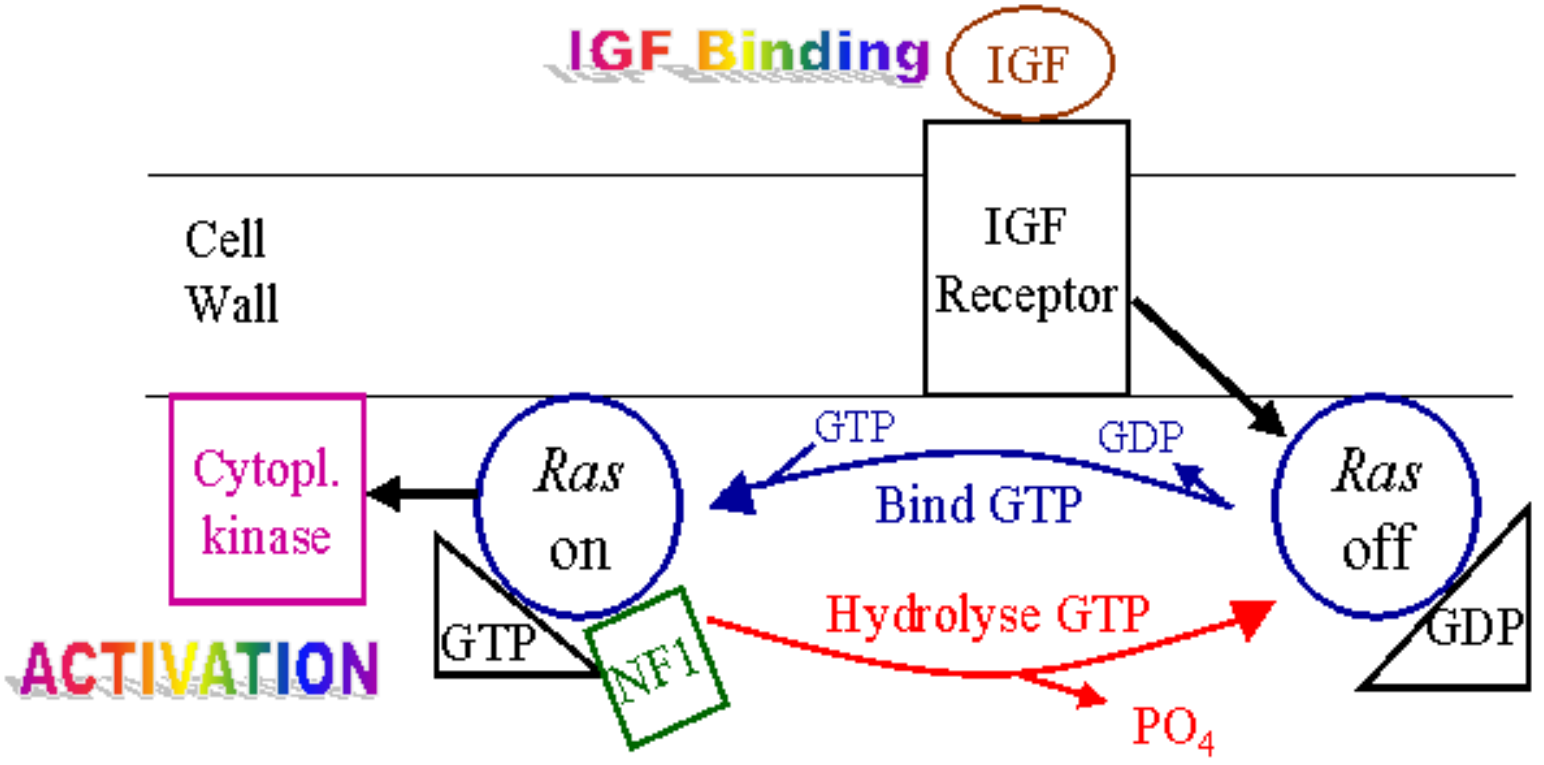
Paraffin section of breast tissue, hybridisation with HER2-specific probe showing *HER2* gene amplification



HERCEPTIN BLOCKS HER2/neu protein



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

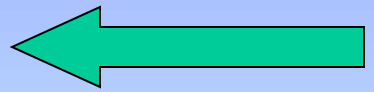
v-sis (PDGF), int-1(WNT-1), int-2(FGF), hst, fgf-5

Growth Factors Receptors

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

CYTOPLASM

Signal Transducers

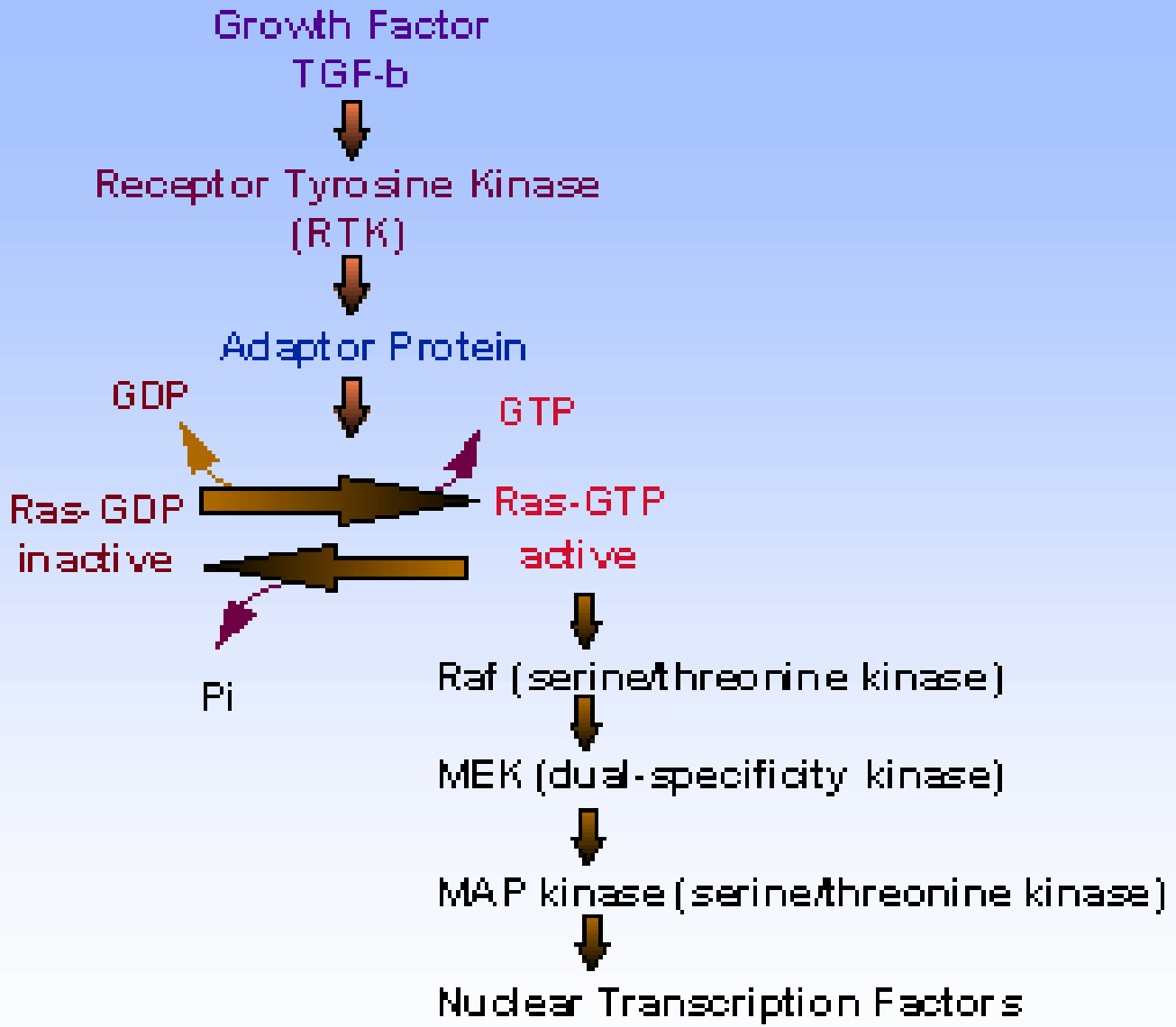


v-ras, v-src, v-raf/mil, v-abl, v-mos, v-crk

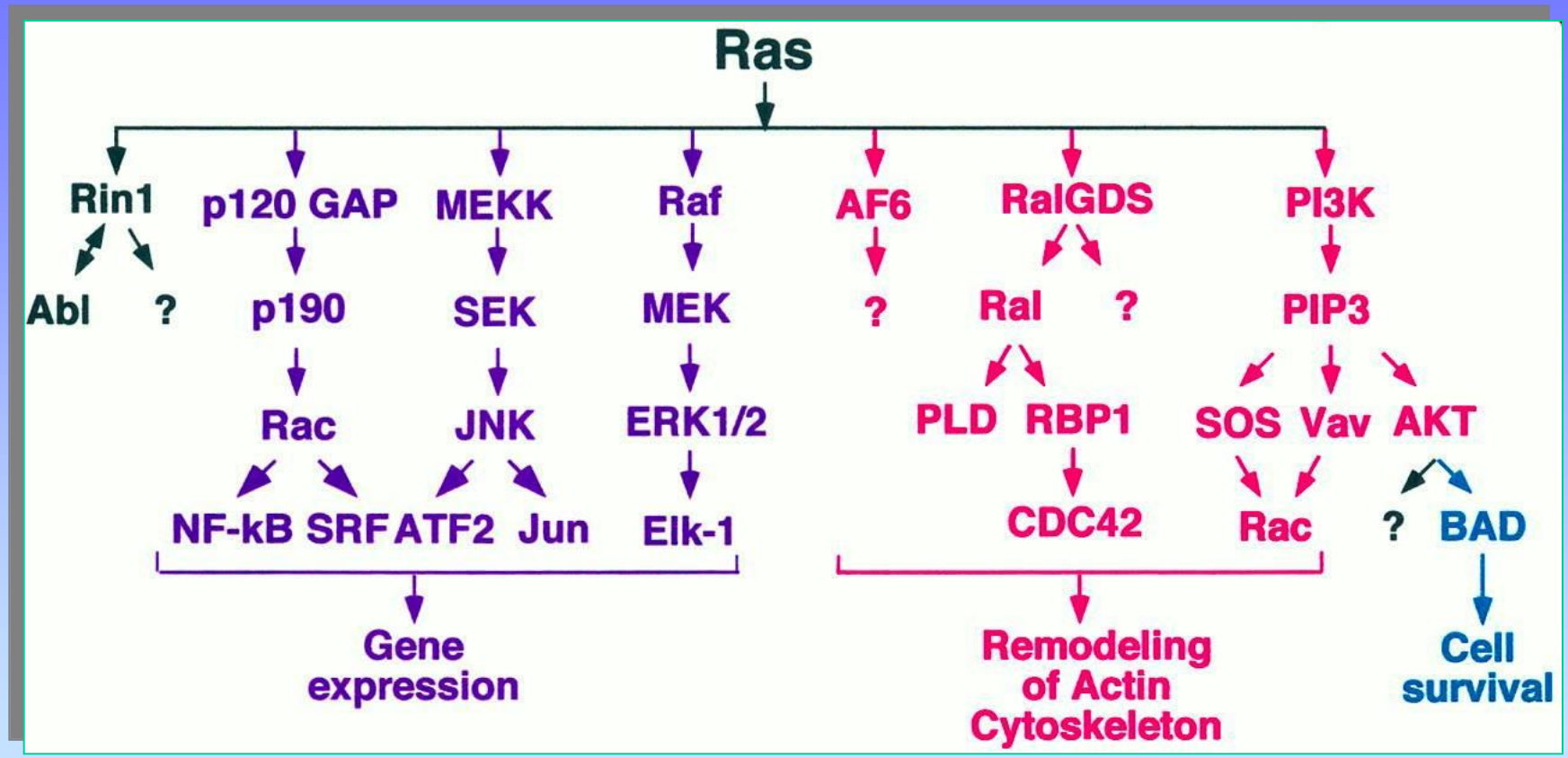
NUCLEUS

Transcription Factors

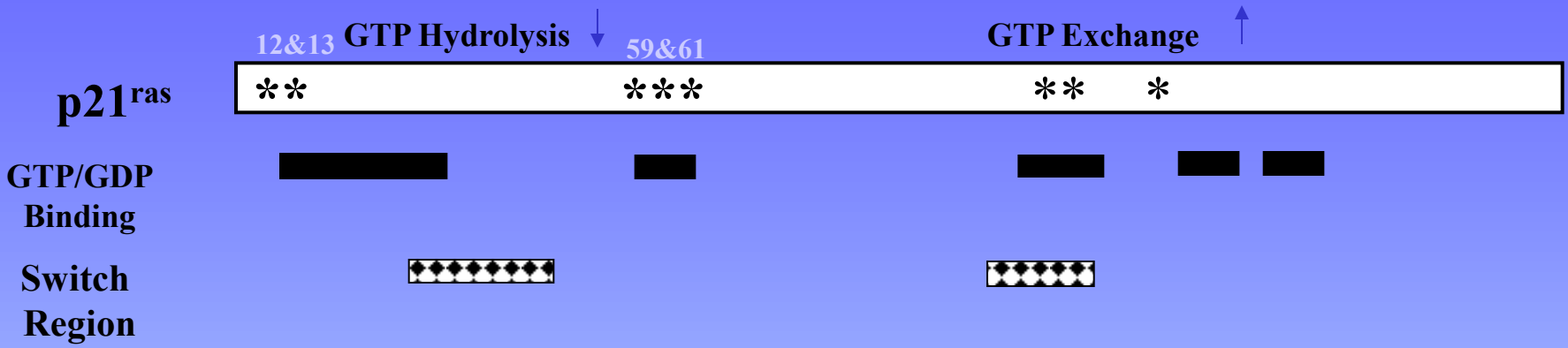
v-ets, v-myc, v-myb, v-rel (NFkB), v-ski, v-erb-A (THR)

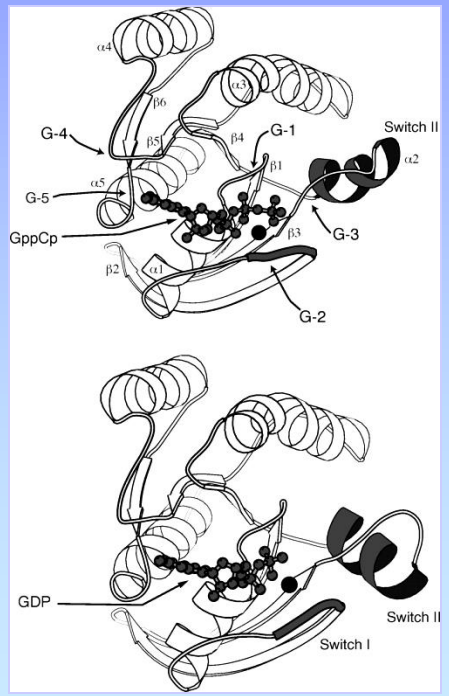

Growth Factor
TGF-b
Receptor Tyrosine Kinase
(RTK)
Adaptor Protein
GDP
GTP
Ras-GDP
inactive
Ras-GTP
active
Pi
Raf (serine/threonine kinase)
MEK (dual-specificity kinase)
MAP kinase (serine/threonine kinase)
Nuclear Transcription Factors

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**

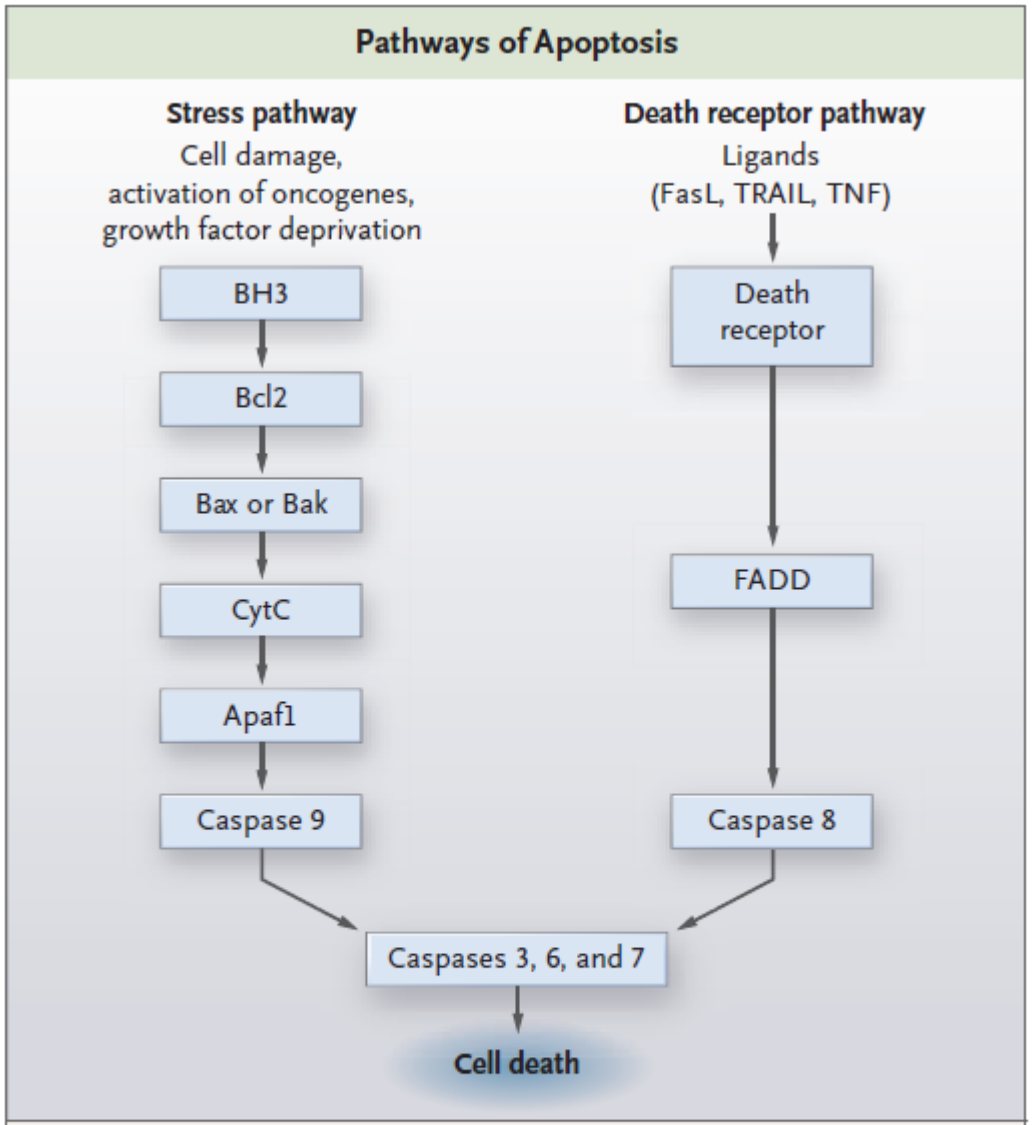


Figure 4. The Two Main Pathways to Programmed Cell Death, or Apoptosis.

The effectors of cell death are the downstream caspases, proteolytic enzymes activated by caspases 8 and 9, which are capable of clearing many of the cellular proteins causing cell death. FADD denotes Fas-associated death domain.

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 (anti-apoptótica).

• *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 (ONCOGEN, activado por translocación y sobre-expresión).

• 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

Cancer Therapies That Target Oncogenic Proteins

Table 1. Cancer Therapies That Target Oncogenic Proteins.*

Anticancer Drug	Target	Disease
Monoclonal antibodies		
Trastuzumab (Herceptin, Genentech)	ERBB2	Breast cancer
Cetuximab (Erbitux, ImClone)	EGFR	Colorectal cancer
Bevacizumab (Avastin, Genentech)	VEGF	Colorectal cancer, non–small-cell lung cancer
Small molecules		
Imatinib (Gleevec, Novartis)	ABL, PDGFR, KIT	Chronic myelogenous leukemia, gastrointestinal stromal tumors, chordoma
Gefitinib (Iressa, AstraZeneca)	EGFR	Non–small-cell lung cancer
Erlotinib (Tarceva, Genentech)	EGFR	Non–small-cell lung cancer
Sorafenib (Nexavar, Bayer/Onyx)	VEGFR, PDGFR, FLT3	Renal-cell carcinoma
Sunitinib (Sutent, Pfizer)	VEGFR, PDGFR, FLT3	Gastrointestinal stromal tumors, renal-cell carcinoma

* EGFR denotes epidermal growth factor receptor, FLT3 FMS-like tyrosine kinase 3, PDGFR platelet-derived growth factor receptor, and VEGF vascular endothelial growth factor.

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