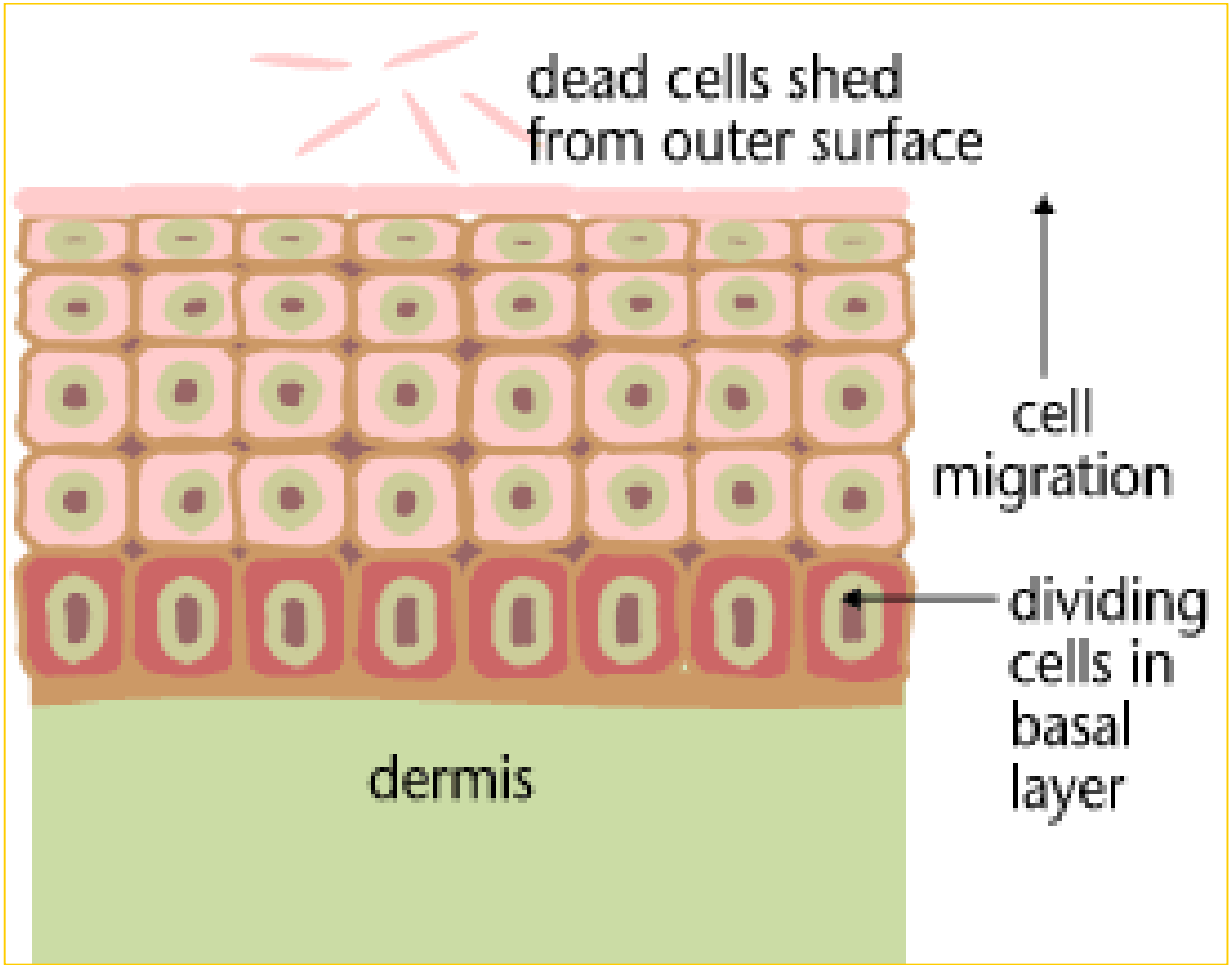
ONCOLOGIA MOLECULAR

Maestría en Biología

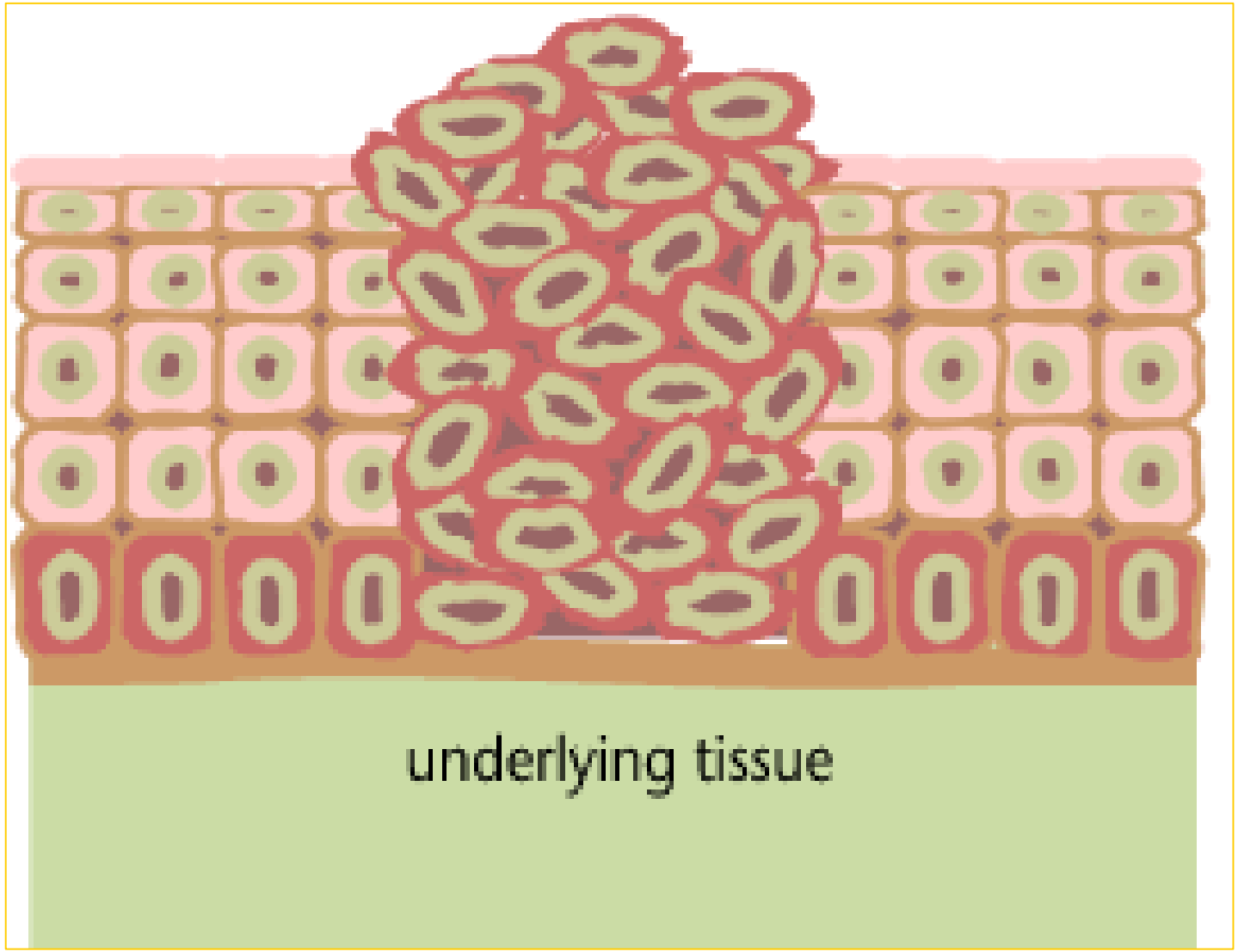
Molecular Médica –2008

¿QUE ES EL CANCER?

1) **Proliferación ilimitada**
2) **Capacidad de dar metástasis**



Normal cell division: enough to replace



Excessive proliferation …….

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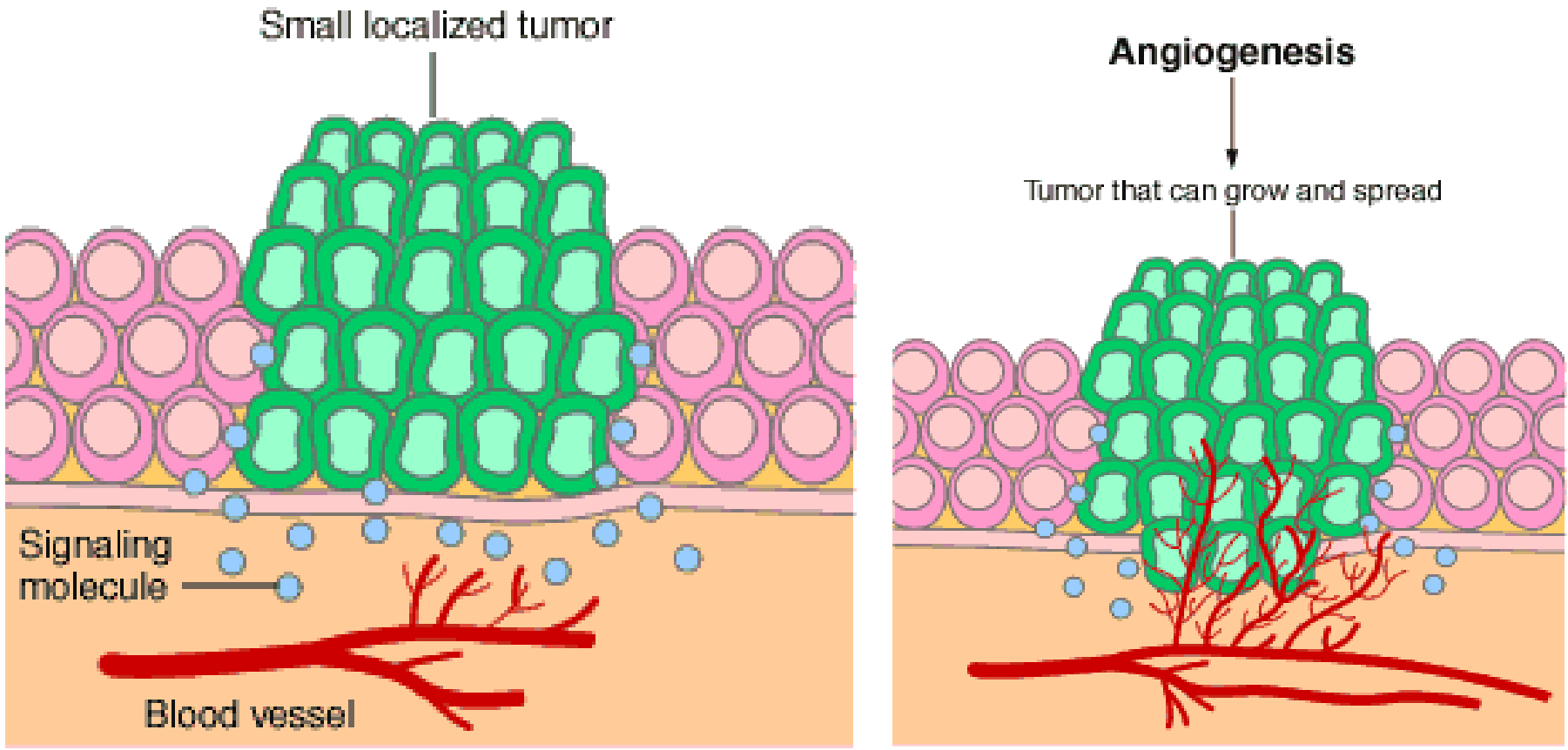
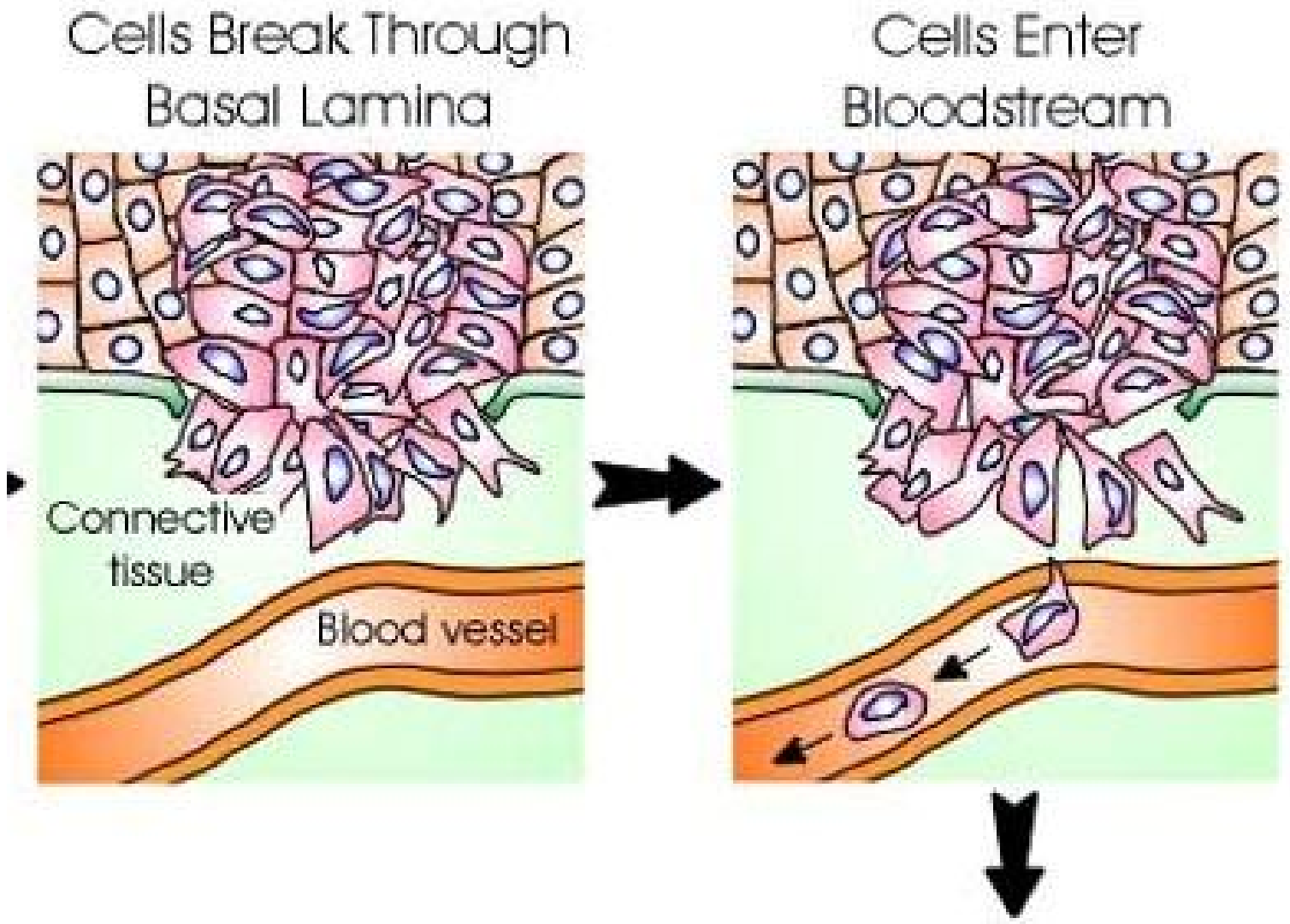
Tumours attract a blood supply



By secreting tumour angiogenesis factors

(Misuse of a normal mechanism)

Metastasis: formation of secondary tumours



NB: This requires survival without anchorage

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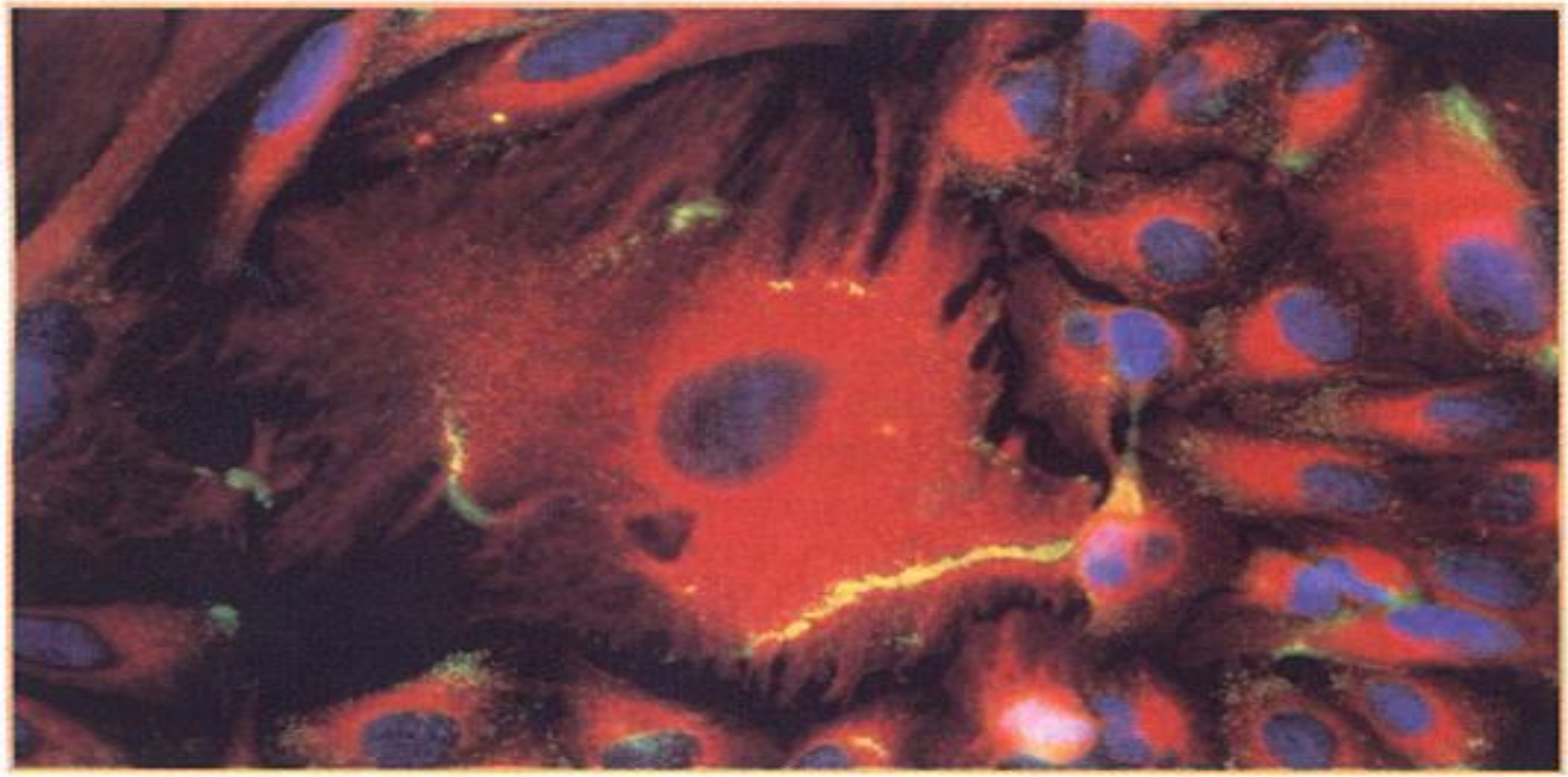
¿COMO SE PRODUCE EL CANCER?

ONCOGENES



Human melanoma cells stained for a melanoma-specific cell-surface glycoprotein (green), and counterstained for myosin (red) and for DNA (blue).

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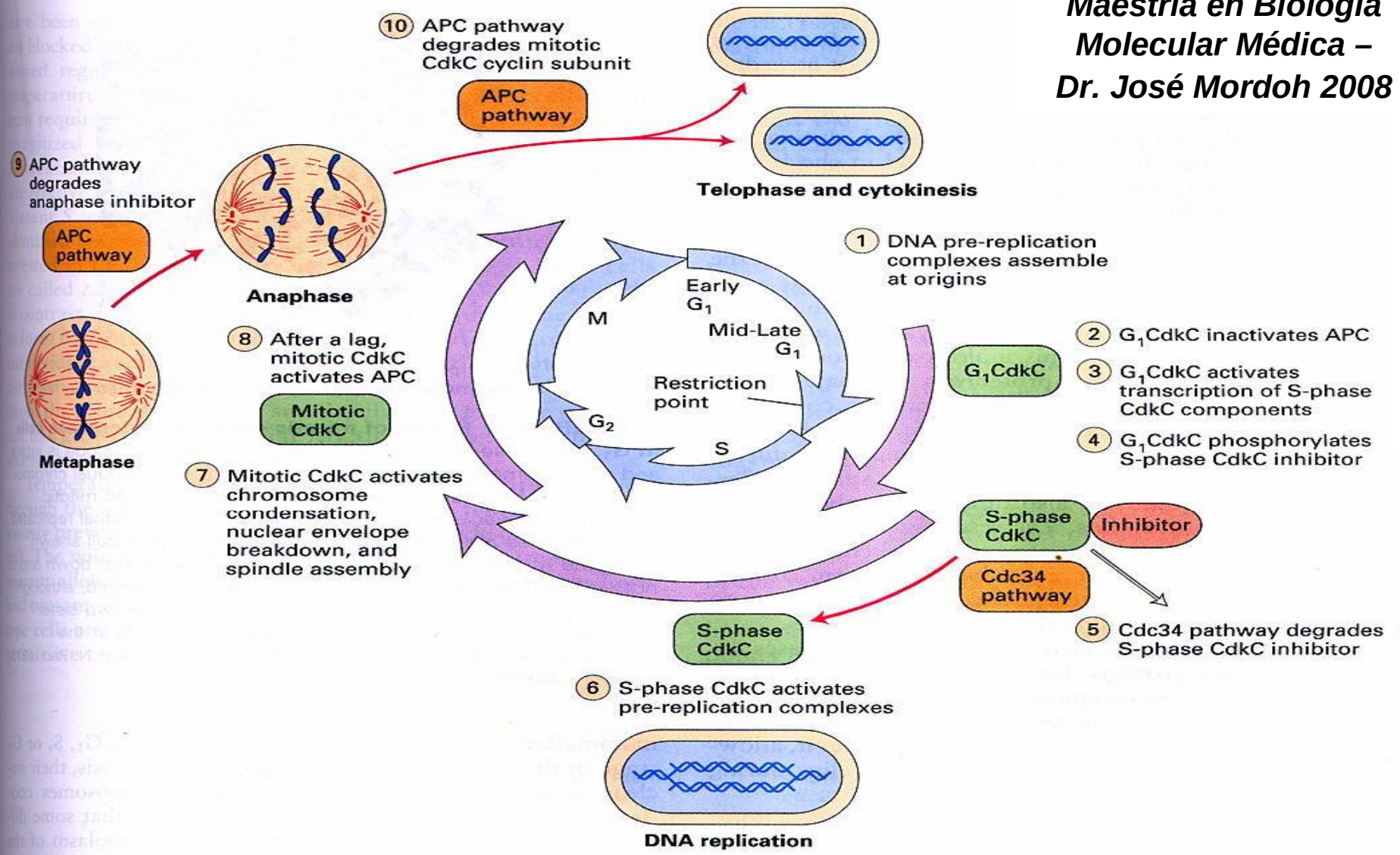
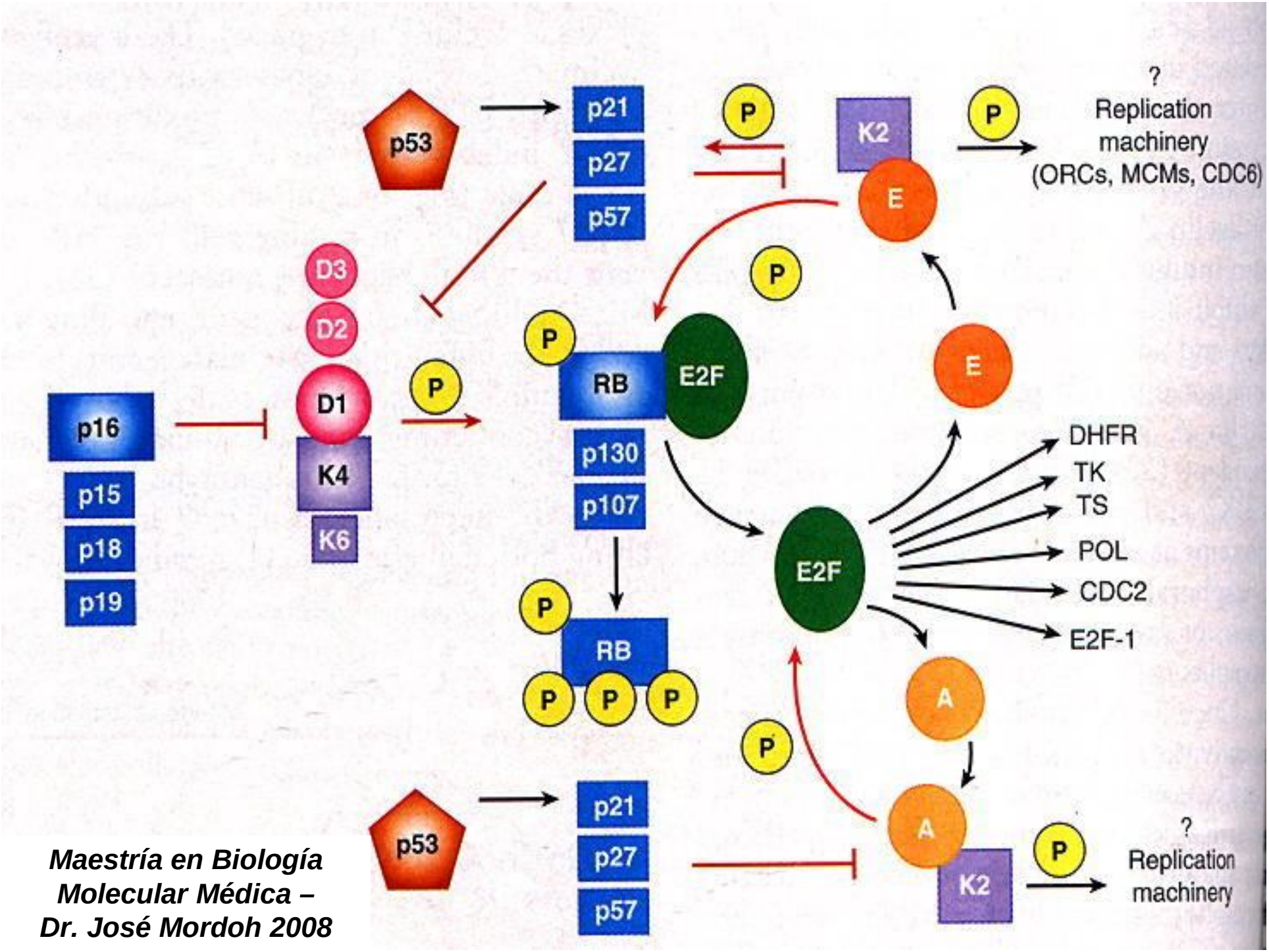


▲ **FIGURE 13-2 Current model for regulation of the eukaryotic cell cycle.** Passage through the cycle is controlled by G_1, S-phase, and mitotic cyclin-dependent kinase complexes (CdkCs) highlighted in green. These are composed of a regulatory cyclin subunit and a catalytic cyclin-dependent kinase subunit.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



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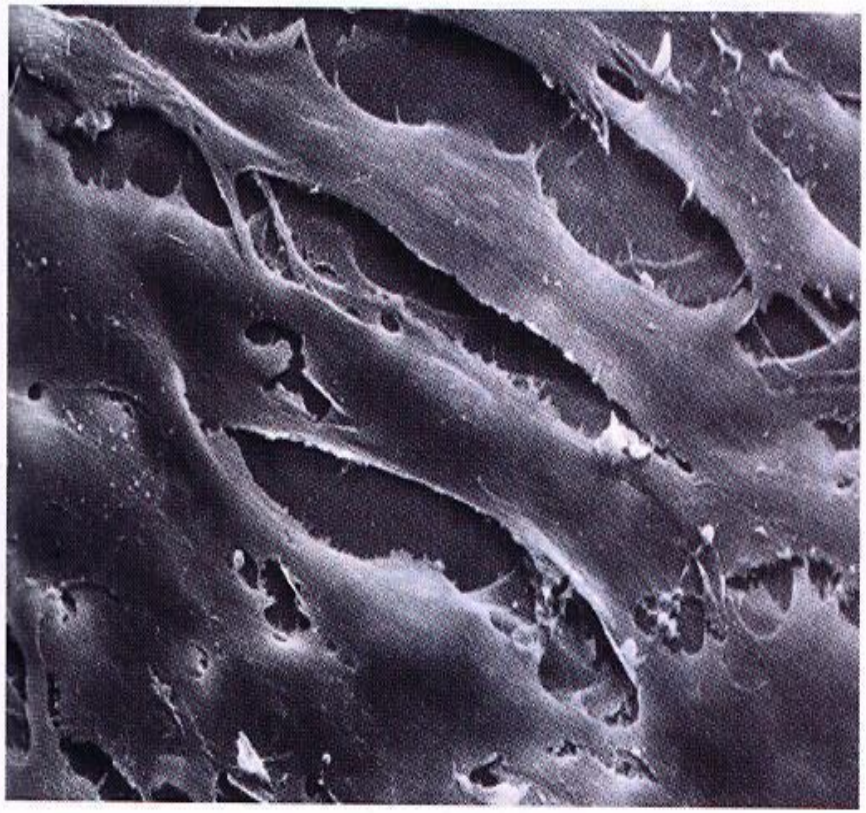
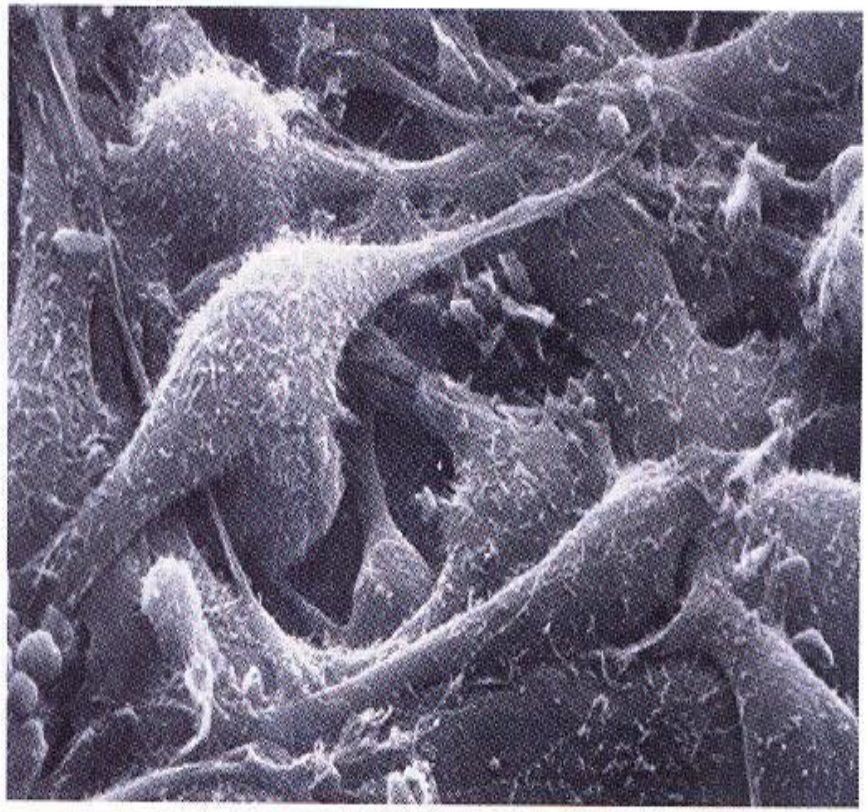
Oncogenes: Historical Considerations-I

- 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).

(a)



(b)



▲ **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.]

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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

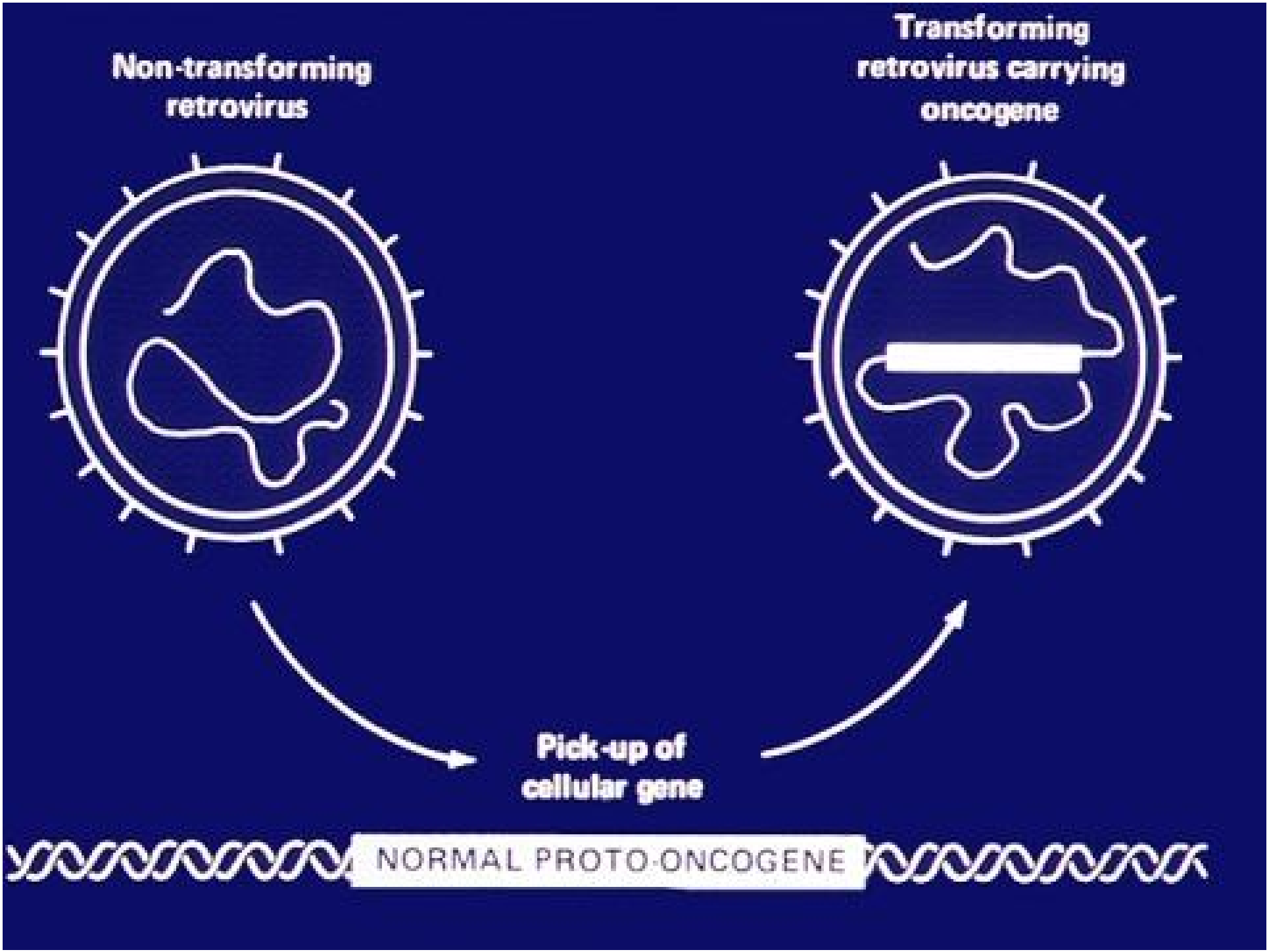
- **Generation of a fusion protein (*gag*+*v-onc*).**

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


Non-transforming retrovirus
Transforming retrovirus carrying oncogene
Pick-up of cellular gene
NORMAL PROTO-ONCOGENE

Oncogenes: Historical Considerations-IV

- 1982-Weinburg transforms mouse fibroblasts with human DNA derived from bladder carcinoma cells. Those cells that become transformed have a human gene homologous to v-ras. First demonstration that an oncogene can cause human cancer.
- Over 50 oncogenes and proto-oncogenes have been identified by studying oncogenic viruses and transforming genes from human and animal tumors.

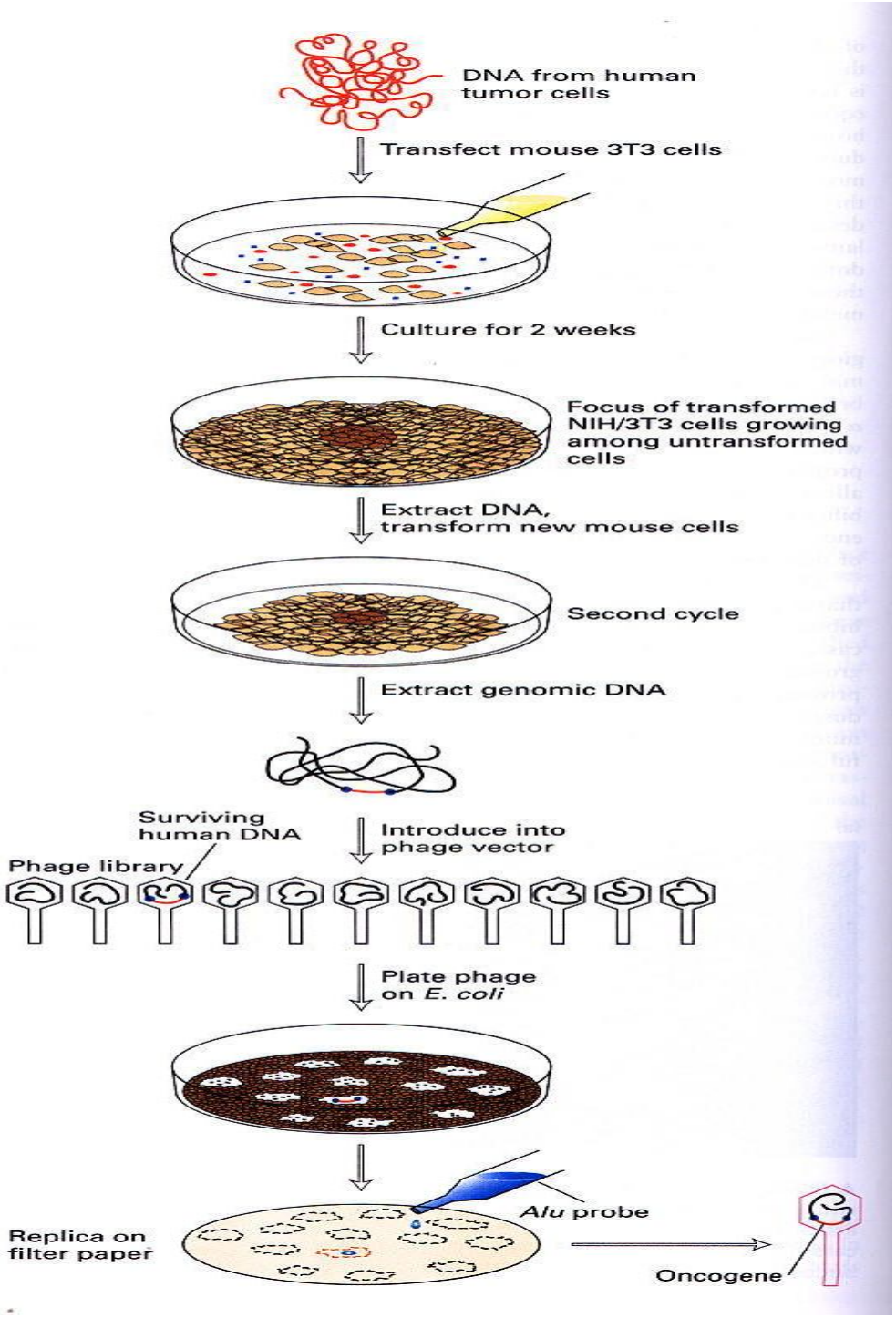


FIGURE 24-4 The identification and molecular cloning of the *ras*^{D} oncogene. Addition of DNA from a human bladder carcinoma to a culture of mouse 3T3 cells causes about one cell in a million to divide abnormally and form a focus, or clone of transformed cells. To clone the oncogene responsible for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

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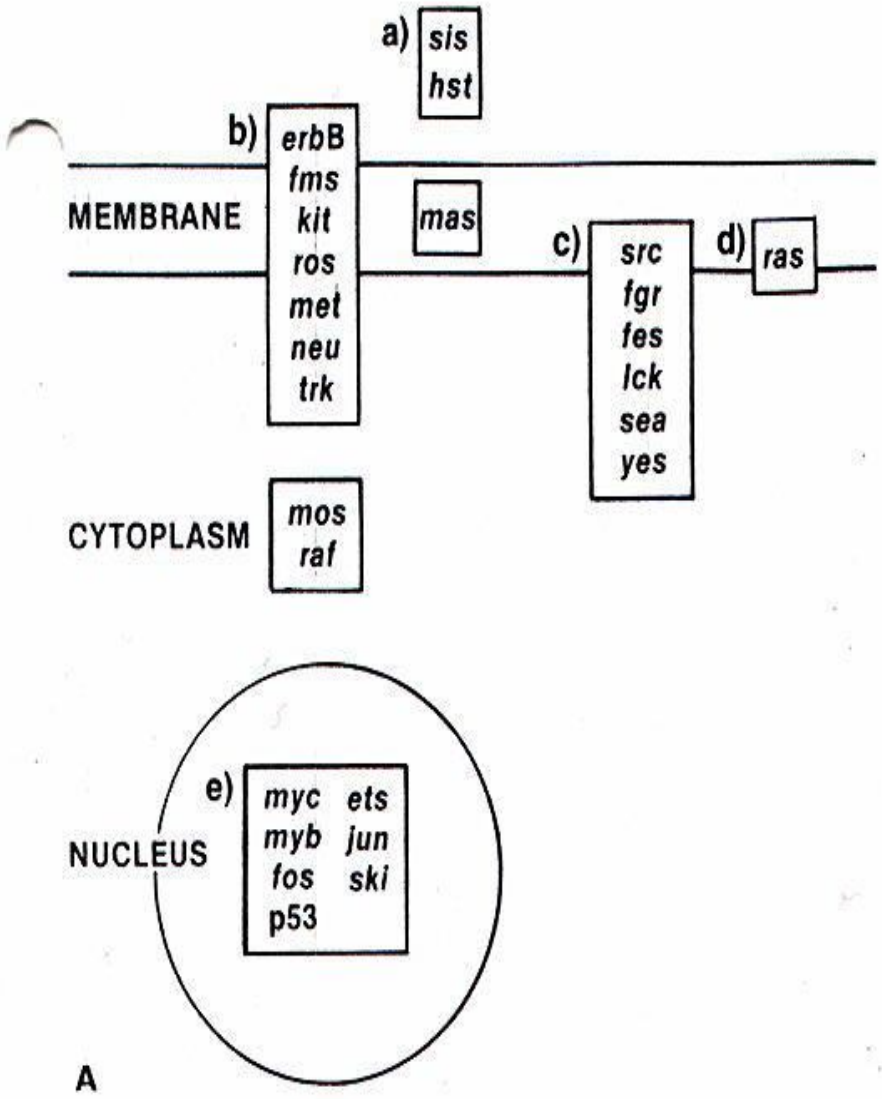
- **PROTO-ONCOGENE = GEN NORMAL**
- **SUSCEPTIBLE DE TRANSFORMACIÓN**

- **ONCOGENE = GEN MUTADO**



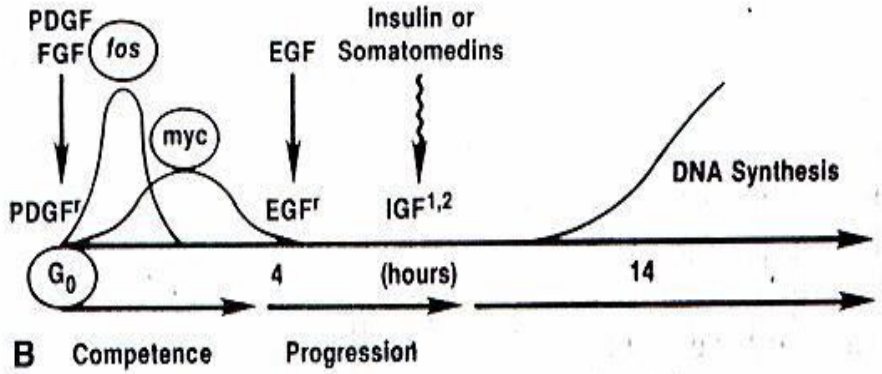


FIGURE 3–1. (A) Schematic presentation of the cellular compartments in which oncogene or protooncogene products are localized. The cell cycle is regulated by growth factors (external mitogenic signals), transmembrane tyrosine kinase growth factor receptor membranes, nonintegral membrane-associated proteins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

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ONCOGENES PROTOTIPICOS= PROPIEDADES

Función	Oncogene	Propiedades
Tirosina-Quinasas Integrales de	V-ERB B	EGFR-SIMIL
Membrana	HER 2-NEU	RECEPTOR
Tirosina-Quinasas Asociadas a	V-SRC	TRANSDUCCION
Membrana	V-ABL	
Serina-Treonina Quinasas	V-MOS	
	RAF	TRANSDUCCION
Familia Factores Crecimiento	V-SIS	PDGF
Familia Ras	V-H-RAS	TRANSDUCCION
	V-K-RAS	
	N-RAS	
Familia Proteínas Nucleares	V-MYC	UNION DNA
	N-MYC	
	V-MYB	
	V-FOS	
	V-JUN	
Varios	INT-1	

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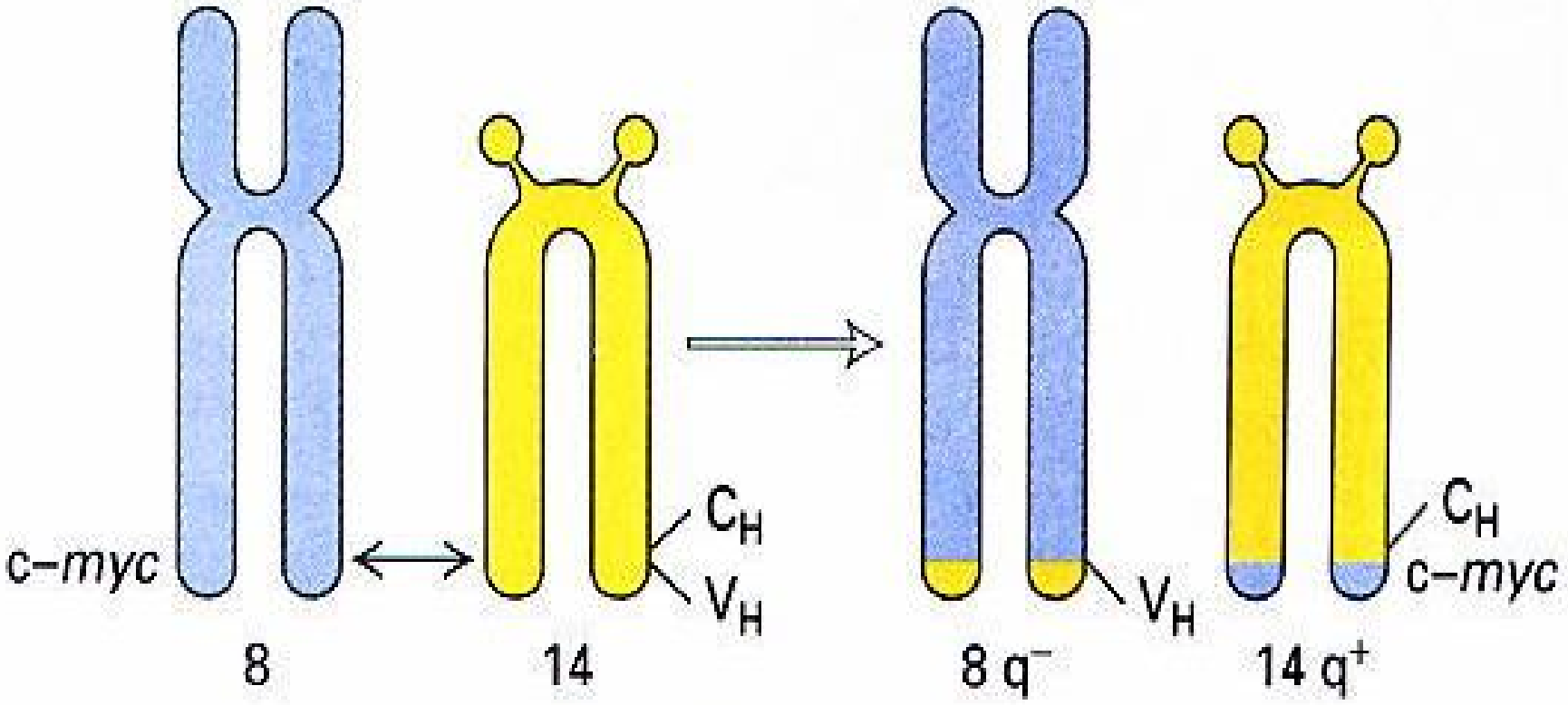
Mecanismos de Transformación de Proto-oncogene a oncogene

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

Insertion, Translocation, or Amplification

- **Some proto-oncogenes are activated by changing expression**
  - non-altered coding sequences
- ***Example***
  - *c-myc*: Differences in Insertion



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

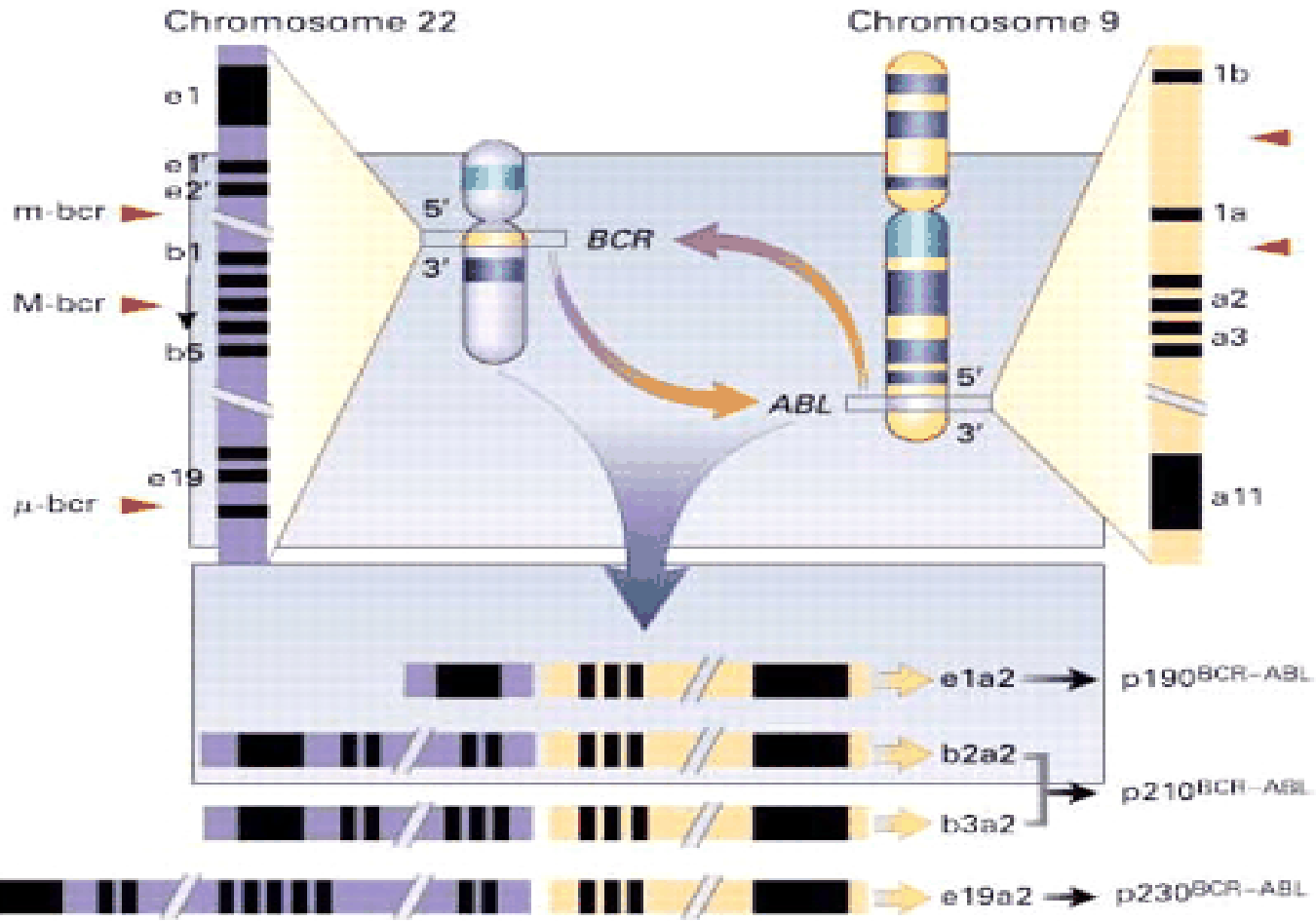


Figure 1. The Translocation of t(9;22)(q34;q11) in CML. The Philadelphia (Ph) chromosome is a shortened chromosome 22 that results from the translocation of 3' (toward the telomere) *ABL* segments on chromosome 9 to 5' *BCR* segments on chromosome 22. Breakpoints (arrowheads) on the *ABL* gene are located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

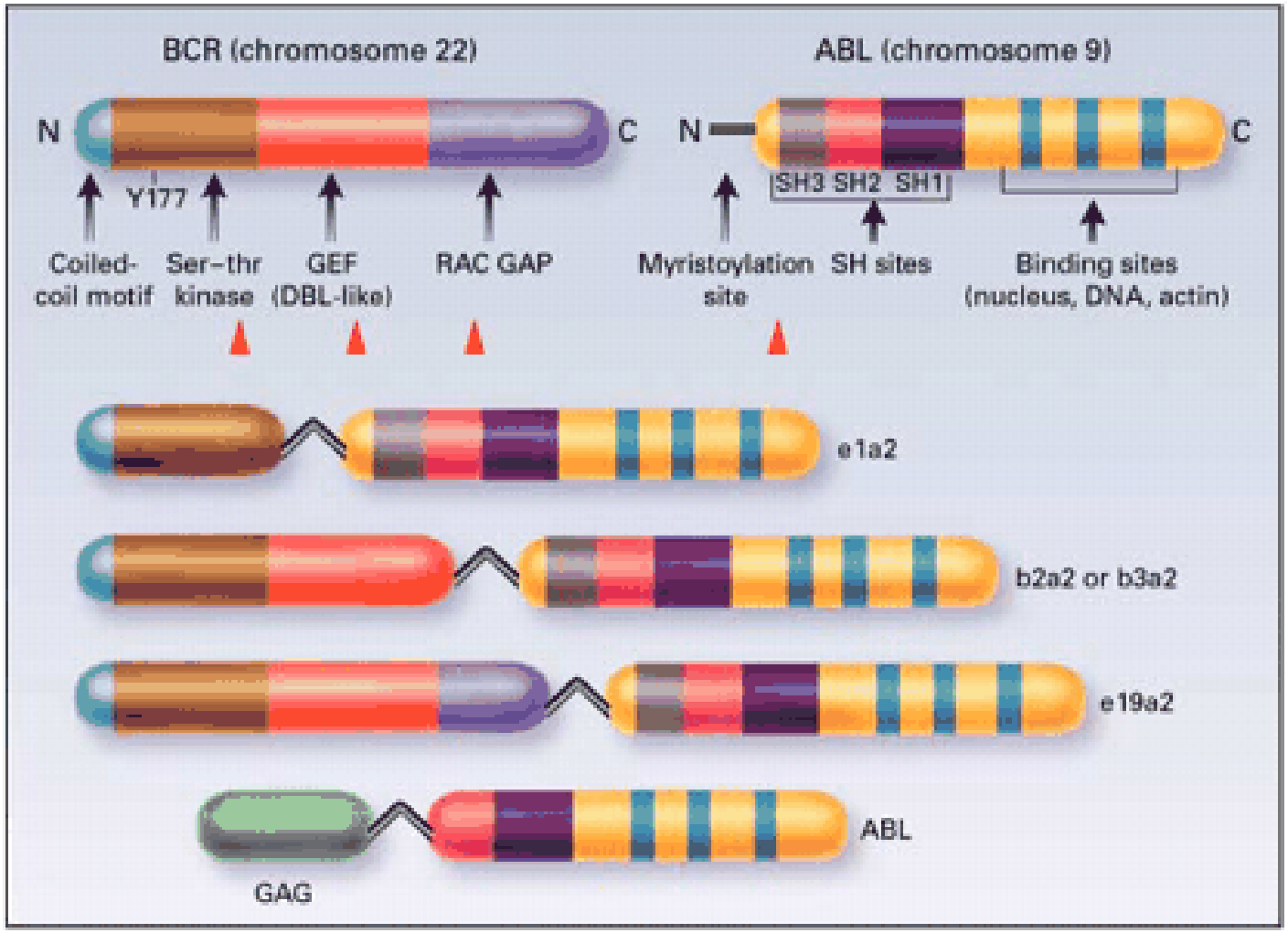


Figure 2. Functional Domains of $p160^{BCR}$, $p145^{ABL}$, and $p210^{BCR–ABL}$.
Important functional domains of the *BCR* and *ABL* gene products as well as of the different fusion-protein products are shown. Breakpoints are indicated by arrowheads (see Table 2 and the text for details). N denotes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

Mecanismos de Transformación de Proto-oncogene a oncogene

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

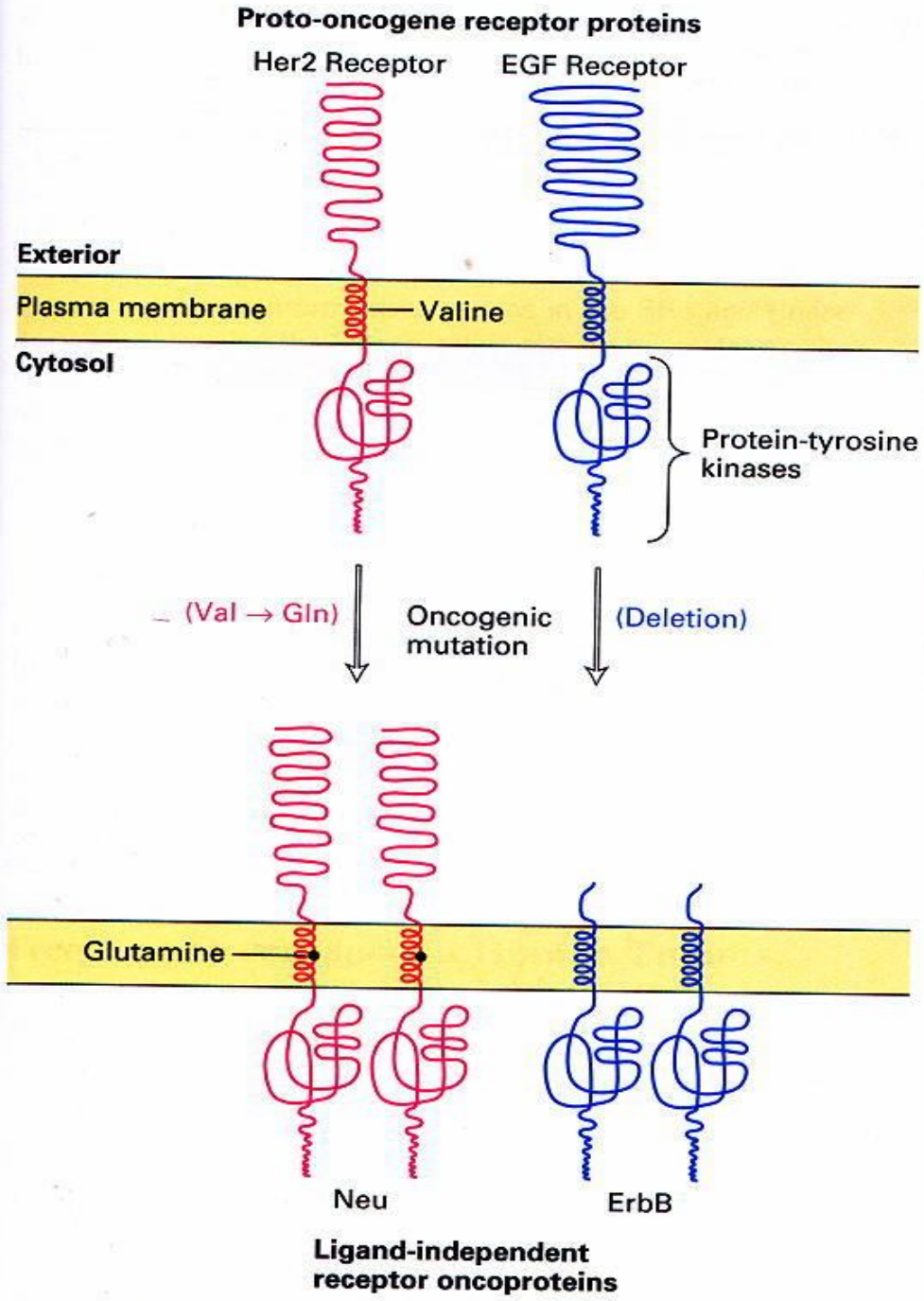


FIGURE 24-15 Effects of oncogenic mutations in proto-oncogenes that encode cell-surface receptors. *(Left)* A mutation that alters a single amino acid (valine to glutamine) in the transmembrane region of the Her2 receptor causes dimerization of two receptor proteins in the absence of the normal EGF-related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

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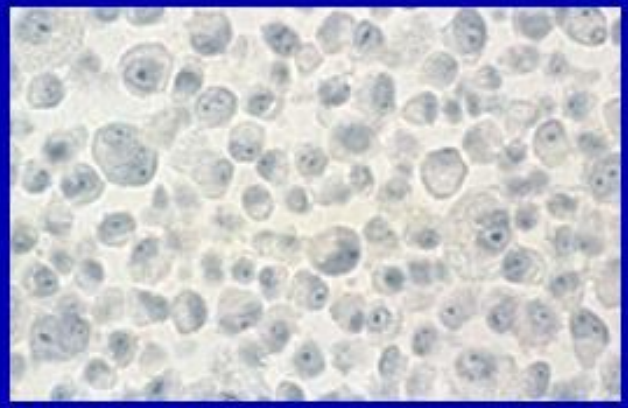
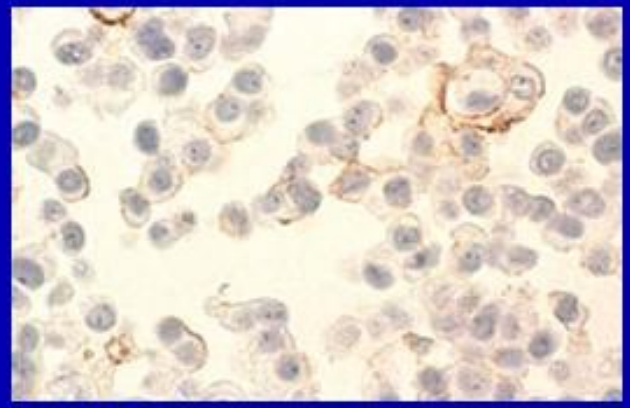
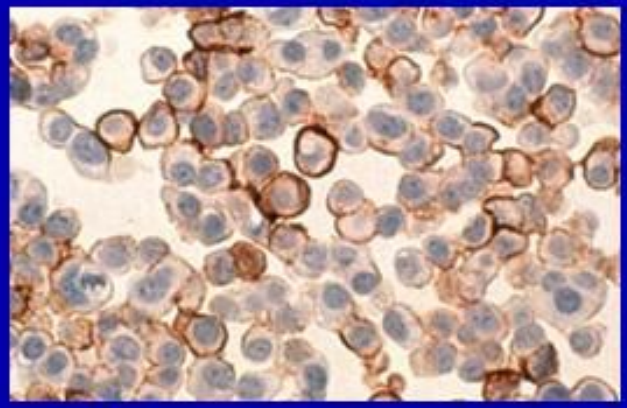
HER2/neu Expression Evaluated by Immunohistochemistry



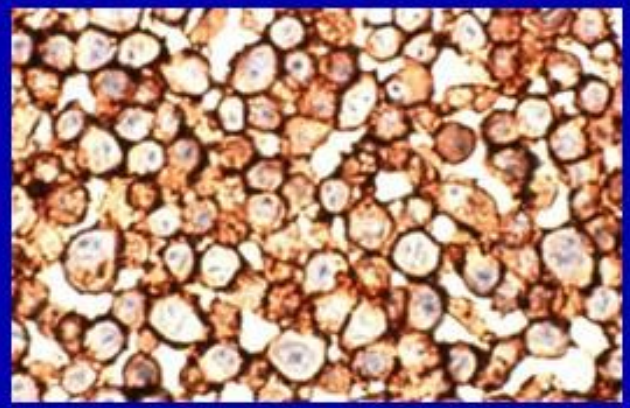
Negative (overexpression negative)



1 (+) (overexpression negative)



2 (+) (overexpression positive)



3 (+) (overexpression positive)

Mecanismos de Transformación de Proto-oncogene a oncogene

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

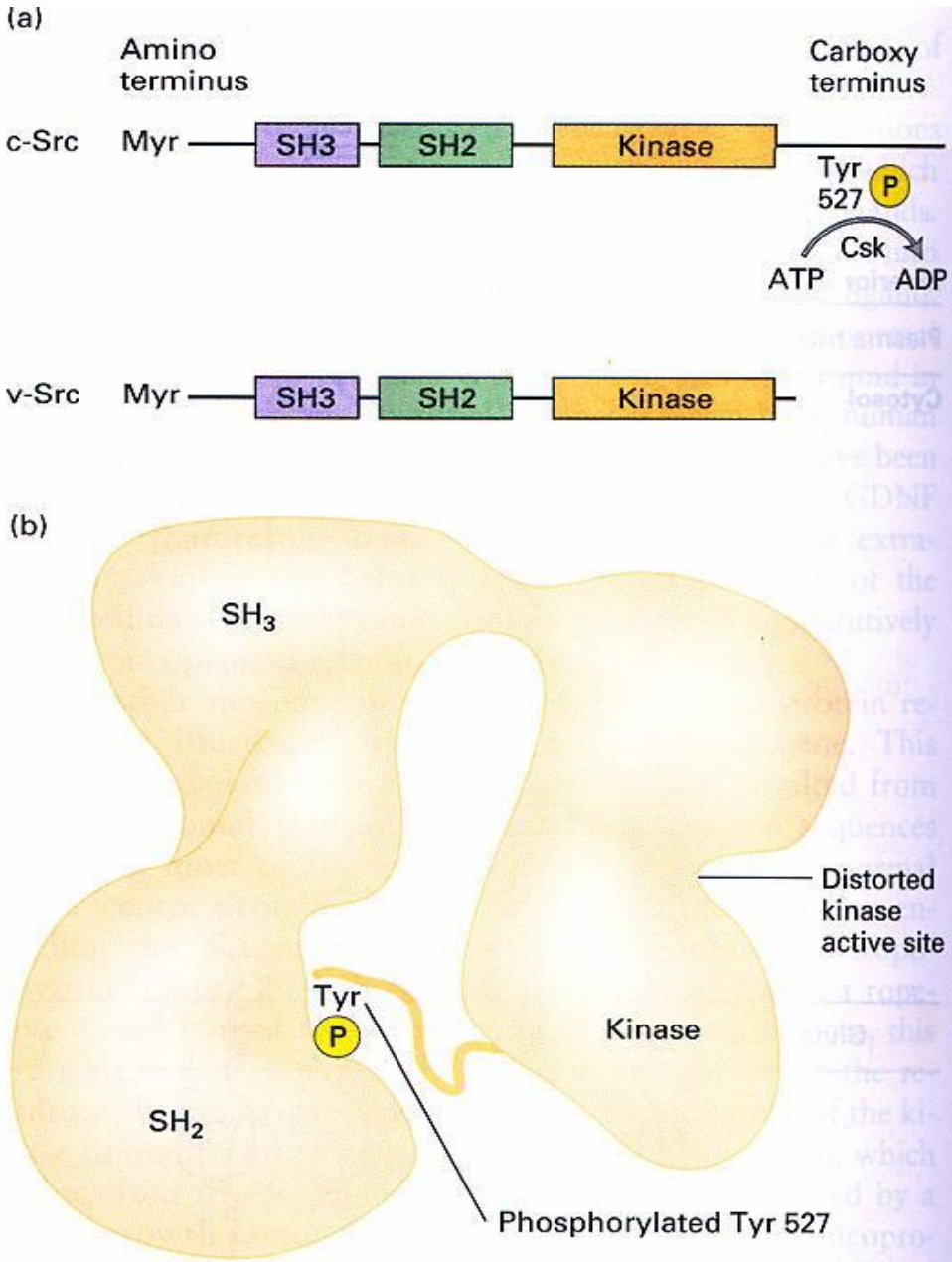


FIGURE 24-17 Regulation of Src activity and its activation by an oncogenic mutation. (a) Domain structure of c-Src and v-Src. Phosphorylation of tyrosine 527 by Csk, another cellular tyrosine kinase, inactivates the Src kinase activity. The transforming v-Src oncoprotein encoded by Rous sarcoma virus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

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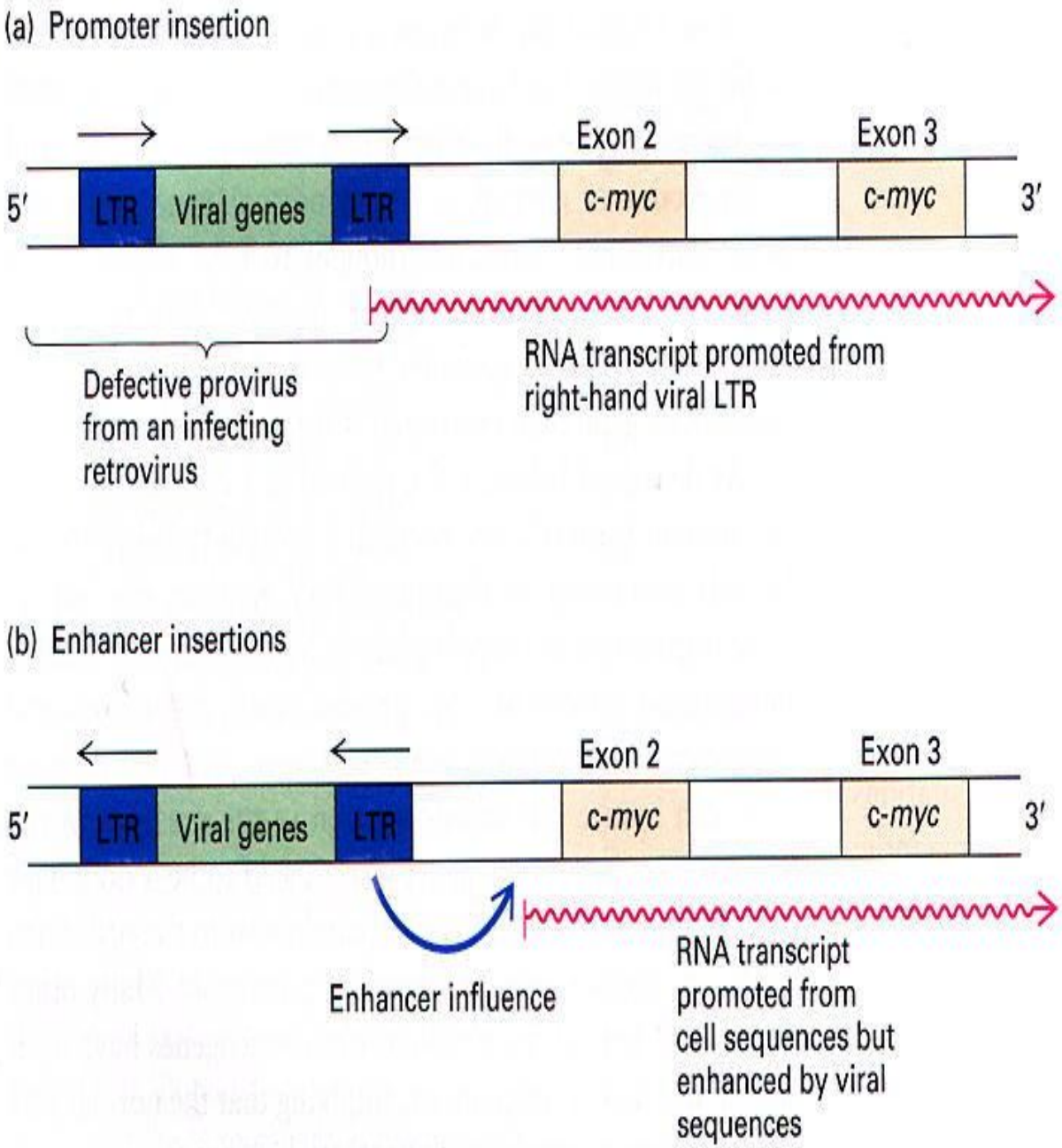

◀ FIGURE 24-10 Activation of the *c-myc* proto-oncogene by retroviral promoter and enhancer insertions. (a) The promoter can be activated when the retrovirus inserts upstream (5′) of the *c-myc* exons. The right-hand LTR may then act as a promoter if the provirus has a defect preventing transcription 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.]

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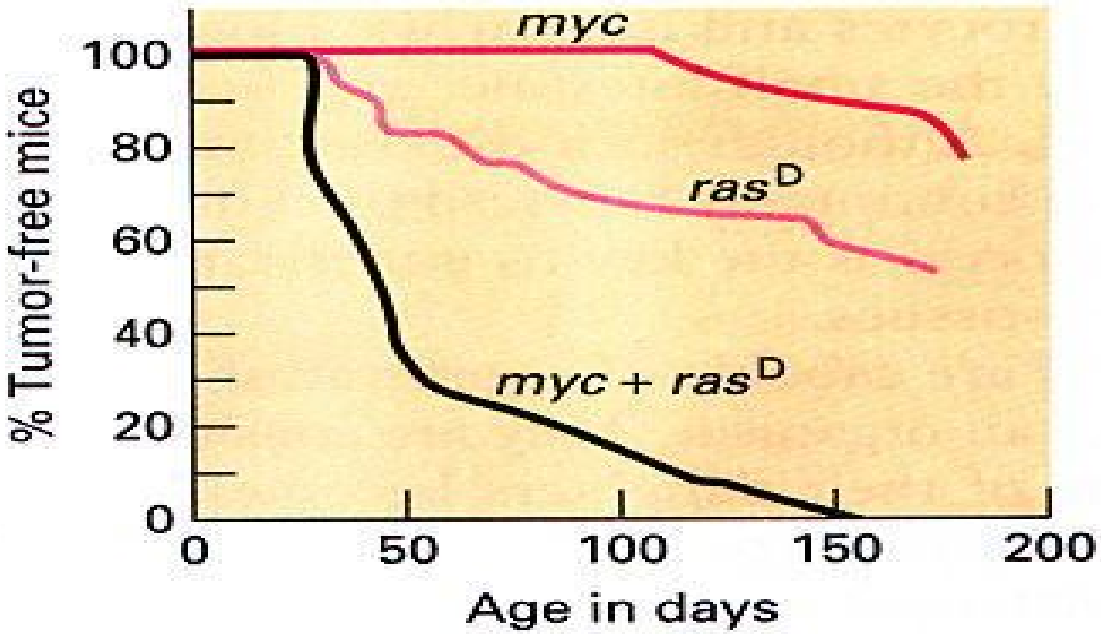
Mecanismos de Transformación de Proto-oncogene a oncogene

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

Ras proto-oncogenes can be activated by mutation

- **Cellular *ras* genes** (*H-ras* & *K-ras*) **have counterparts in murine sarcoma virus** (*v-ras*)
- **Point mutation**
  - single amino acid change converts cellular proto-oncogene into oncogene
  - spontaneous tumor in organism
  - can be carried by retrovirus
- **Fine balance**
  - increased expression of c-*ras* = cancer



▲ FIGURE 24-7 Kinetics of tumor appearance in female transgenic mice carrying transgenes driven by the mouse mammary tumor virus (MMTV) breast-specific promoter. Shown are results for mice carrying either myc or *ras*^{D} transgenes as well as for the progeny of a cross of *myc* carriers with *ras*^{D} carriers that contain both transgenes. The percentage of tumor-free mice graphically depicts the time course of tumorigenesis. Females were studied because the hormonal stimulation of pregnancy activates expression of the MMTV-driven oncogenes. [See E. Sinn et al., 1987, *Cell* **49**:465.]

GENES SUPRESORES

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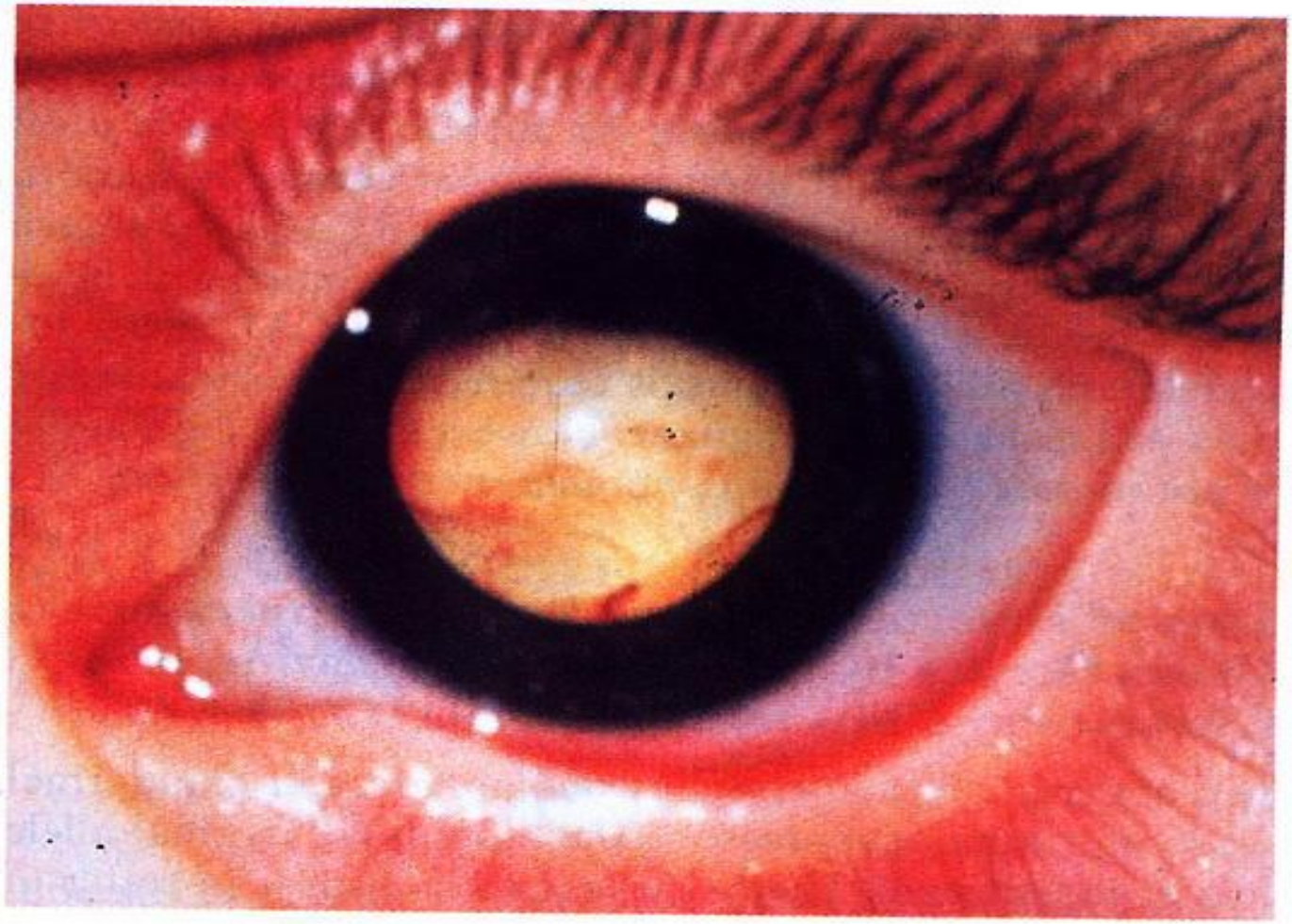
Medica 2008



▲ **FIGURE 24-11 Children with hereditary retinoblastoma develop retinal tumors early in life and generally in both eyes.** They inherit one mutant allele of the *RB* gene. Somatic mutation of the other allele coupled with oncogenic mutations in other genes leads to tumor development. [Courtesy of T. Dryja.]

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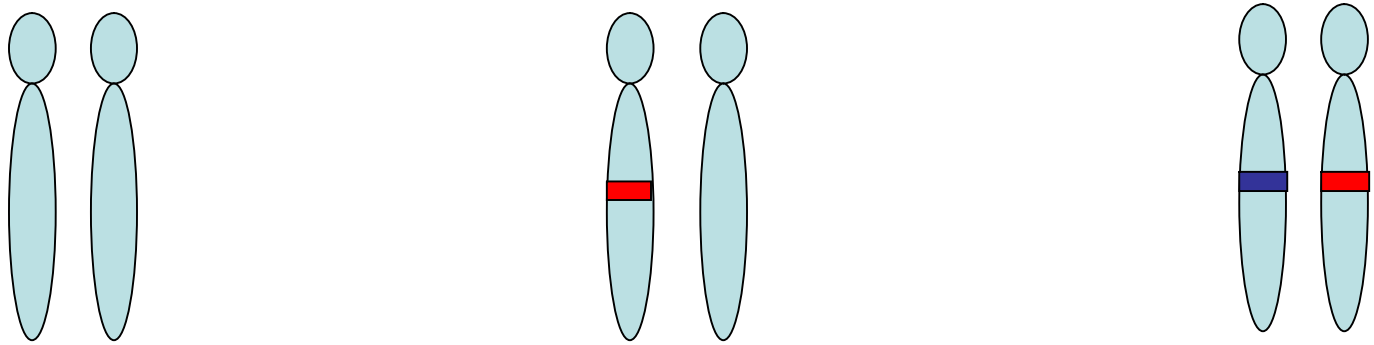
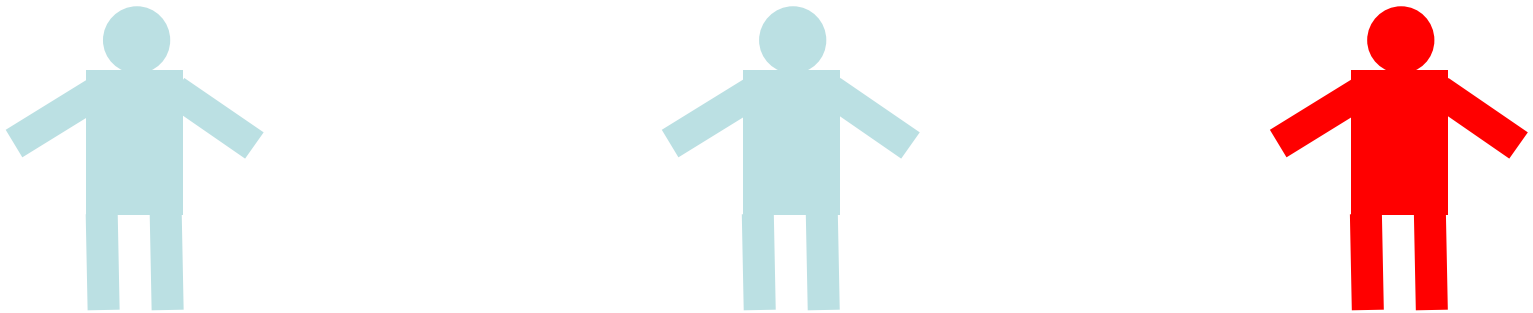
ENFERMEDAD GENÉTICA RECESIVA
HIPÓTESIS DE KNUDSON



Mutación somática

Mutación germinal

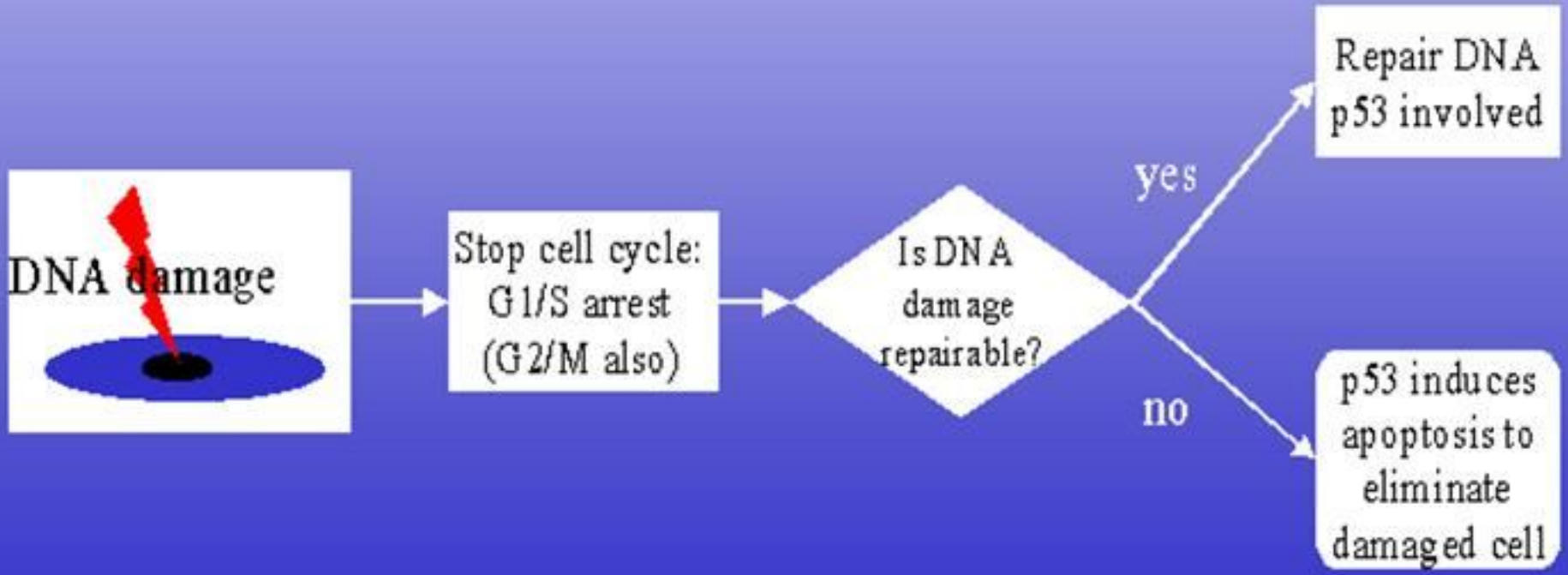


Fenotipo normal

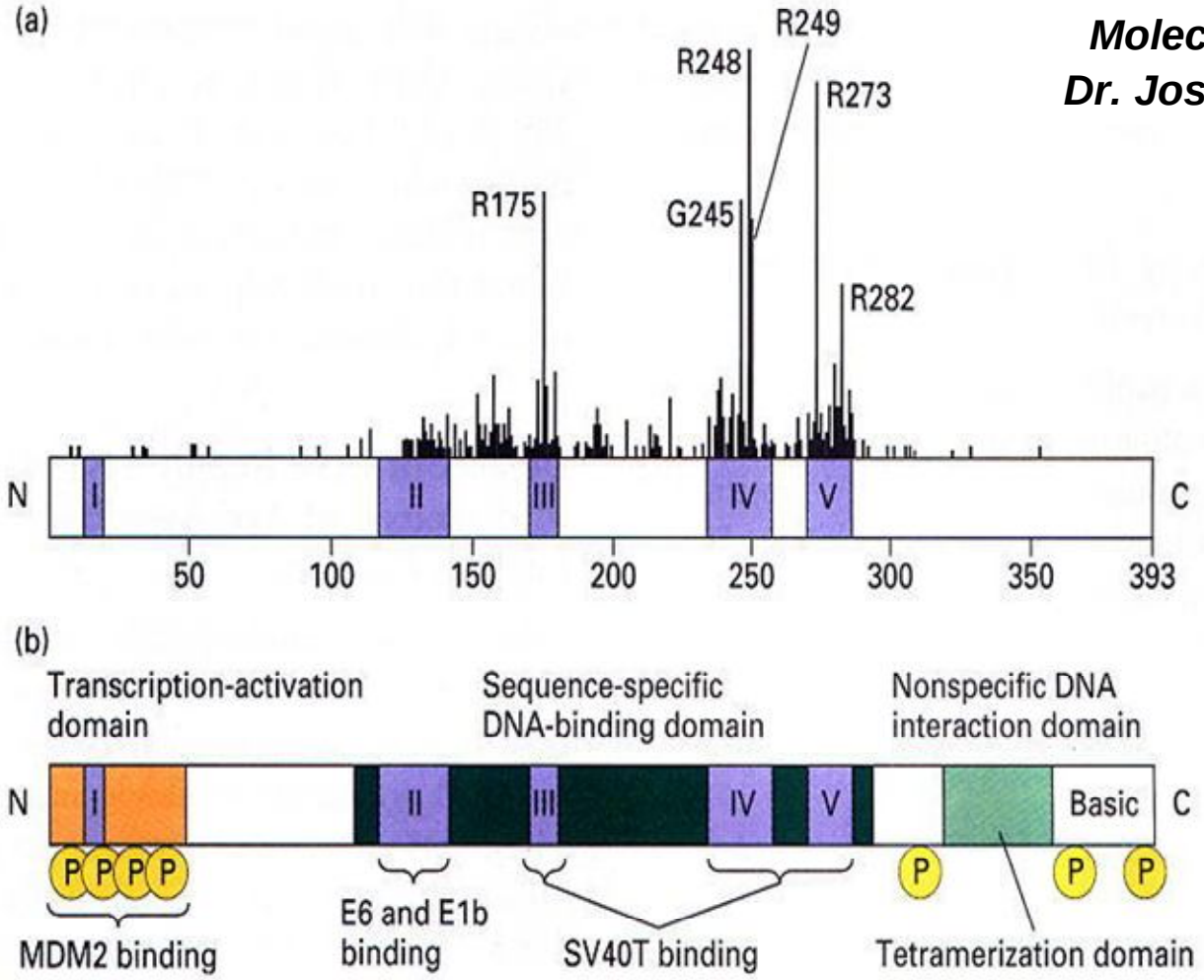
Fenotipo neoplásico

Functions of p53: simple

- One way of thinking about p53 is that it is a "guardian of the genome": it protects the cells DNA from damage



October 1998
Oliver Bogler, Ph.D.



▲ FIGURE 24-21 The human p53 protein. (a) Mutations in human tumors that inactivate the function of p53 protein. Hatched boxes represent sequences highly conserved in evolution. Vertical lines represent the frequency at which mutations are found at each residue in various human tumors. These mutations are clustered in conserved regions II–V. (b) Structural organization of the p53 protein. Phosphorylation by various kinases at the sites indicated by (P) stabilize p53. MDM2 protein binds at the indicated site and represses transcription activation by p53 as part of the normal control of p53 function. The activity of p53 also is inhibited by binding of viral proteins such as E6 from human papillomavirus and E1b from adenovirus. [Adapted from C. C. Harris, 1993, *Science* **262:**1980; and L. Ko and C. Prives, 1996, *Genes & Develop.* **10:**1054.]

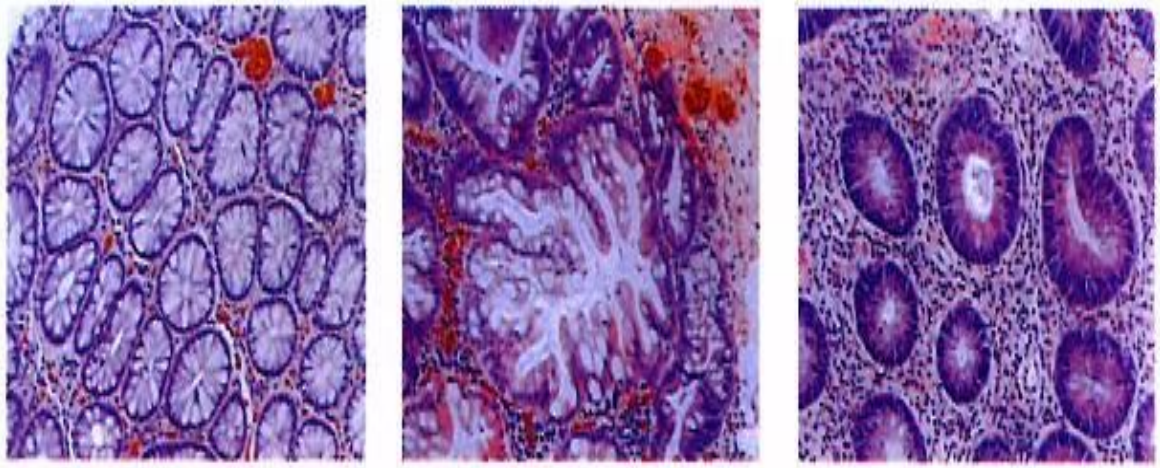
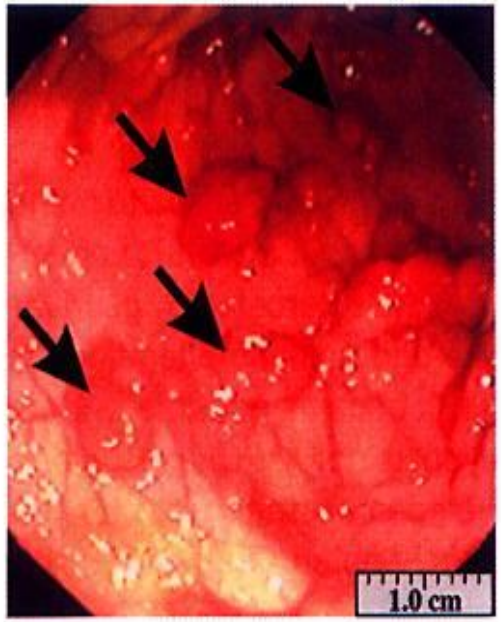
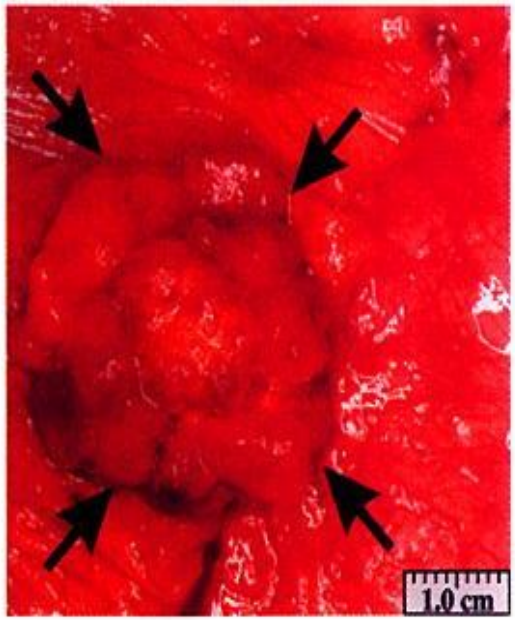


Figure 4. Histology of Normal and Neoplastic Colonic Epithelium

The left panel shows a group of normal colonic crypts in cross section. Note that the epithelial cells are precisely lined up along the basement membrane and that there is great uniformity among the glands. The center panel shows the morphology typical of a hyperplastic lesion. Individual cells are morphologically normal, but the increased number of cells in the crypts promotes crowding and mucosal folding, resulting in a saw-toothed appearance. The right panel shows morphology typical of a dysplastic ACF or adenoma. Note the increased nuclear/cytoplasmic ratio, the lack of uniform architecture, and the many nuclei that are no longer lined up along the basement membrane.



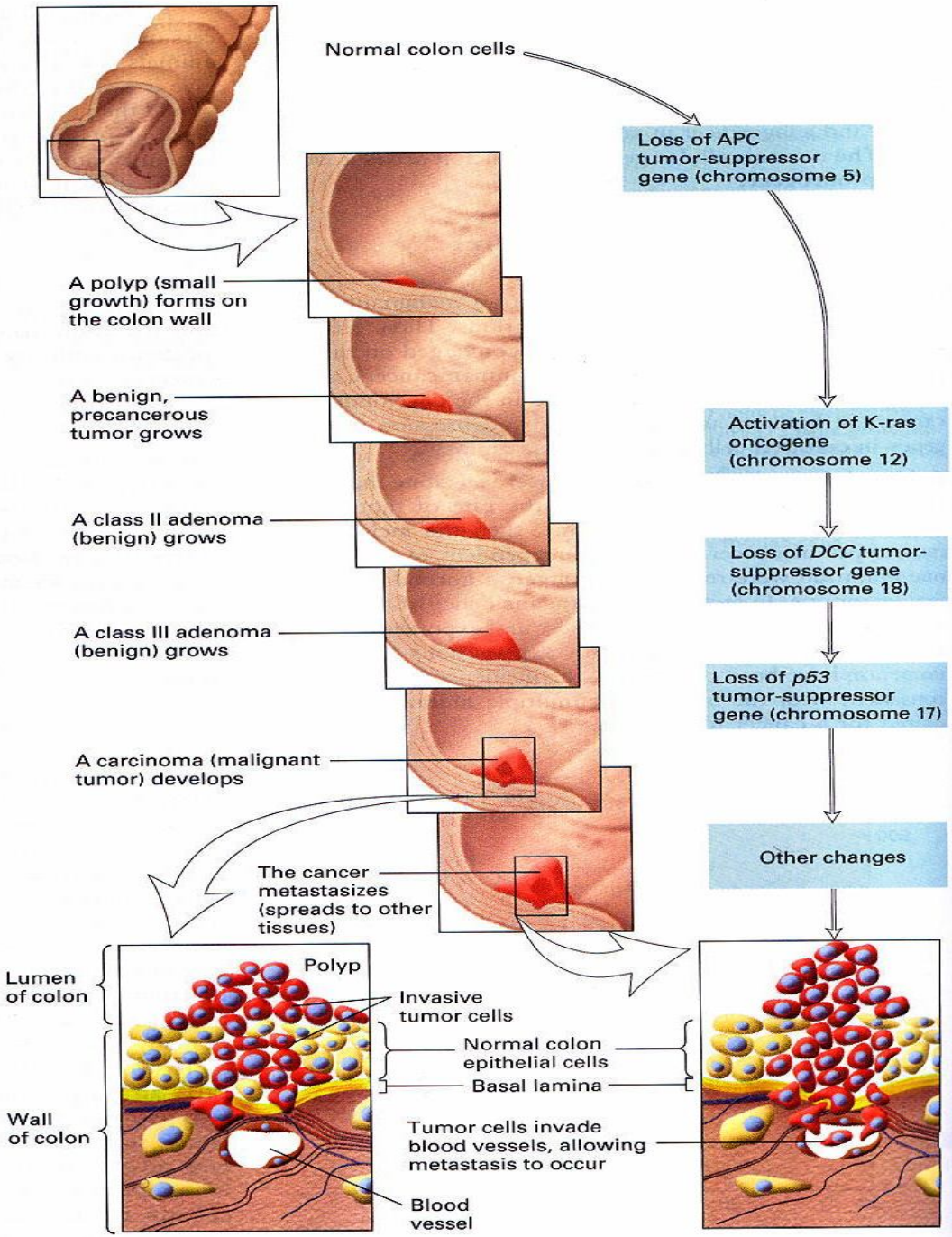
FAP



HNPCC

Figure 1. Examples of Colorectal Tumors Arising in FAP and HNPCC Patients

The left panel is a small portion of the colon from an FAP patient as viewed through the colonoscope, illustrating the multiple benign tumors (adenomas) characteristic of FAP (arrows). The right panel shows a single cancer from an HNPCC patient after surgical resection.



▶ **FIGURE 24-6 The development and metastasis of human colorectal cancer and its genetic basis.** A mutation in the *APC* tumor-suppressor gene in a single epithelial cell causes the cell to divide, although surrounding cells do not, forming a mass of localized benign tumor cells called a *polyp*. Subsequent mutations leading to expression of a constitutively active Ras protein and loss of two tumor-suppressor genes, *DCC* and *p53*, generates a malignant cell carrying all four mutations; this cell continues to divide and the progeny invade the basal lamina that surrounds the tissue. Some tumor cells spread into blood vessels that will distribute them to other sites in the body. Additional mutations cause exit of the tumor cells from the blood vessels and growth at distant sites; a patient with such a tumor is said to have cancer. [Adapted from B. Vogelstein and K. Kinzler, 1993, *Trends Genet.* **9**:101.]

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Table 1. APC Mutations in Colorectal Neoplasia

	FAP	Sporadic Adenomas	Sporadic Cancers
Population incidence	1 in 7000	1 in 2	1 in 20
APC mutation prevalence	>85%[b] (Germline Mutations)	>80%[c] (Somatic Mutations)	>80%[d] (Somatic Mutations)
Nature of mutations[a]			
Truncating	96%[e]	89%[f]	98%[g]
Missense	4%[e]	11%[f]	2%[g]

[a] Based on APC mutations that could be precisely defined at the nucleotide level. For the purposes of this table, frameshift, nonsense, and splice site mutations were considered "truncating".

[b] Based on 62 kindreds (Powell et al., 1993).

[c] Based on analysis of 12 colorectal polyps (Jen et al., 1994).

[d] Based on analysis of 23 colorectal cancer cell lines (Smith et al., 1993).

[e] Based on 174 mutations (summarized in Nagase and Nakamura, 1993).

[f] Based on 19 mutations (Miyoshi et al., 1992; Powell et al., 1992).

[g] Based on 56 mutations (Miyoshi et al., 1992; Powell et al., 1992).

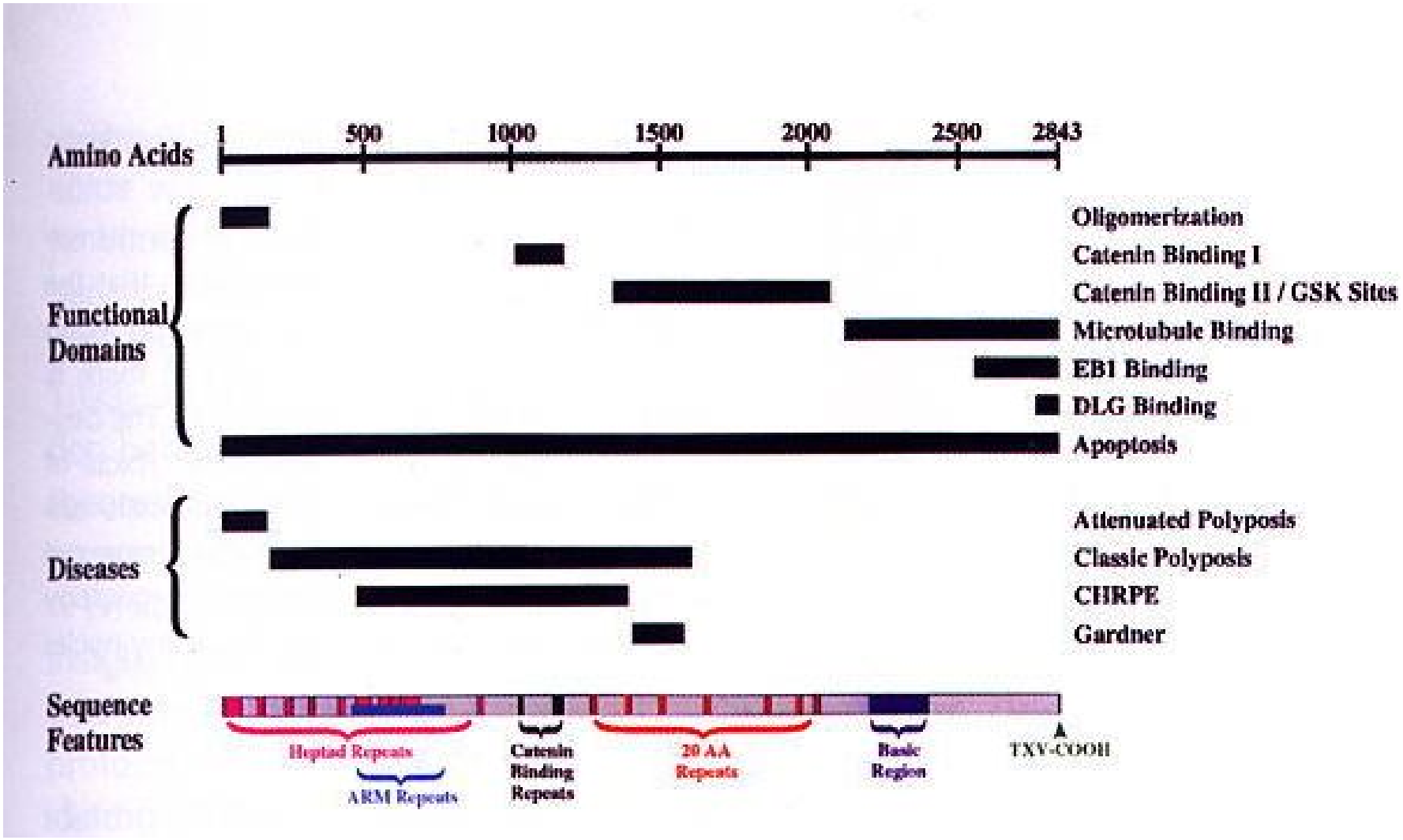

Figure 2. Functional and Pathogenic Properties of APC

CADHERINS AND THEIR ROLE IN CANCER METASTASIS

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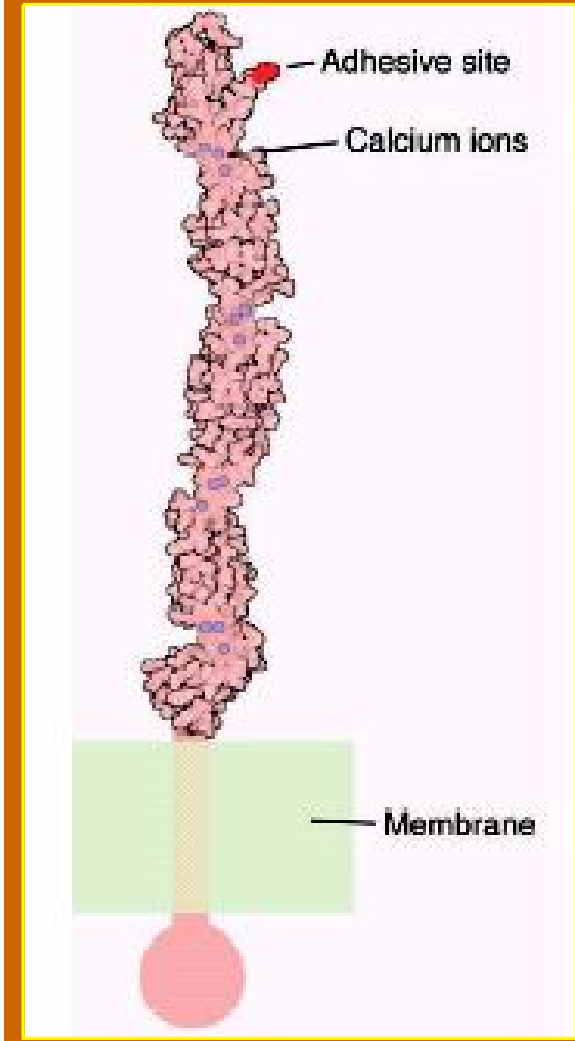


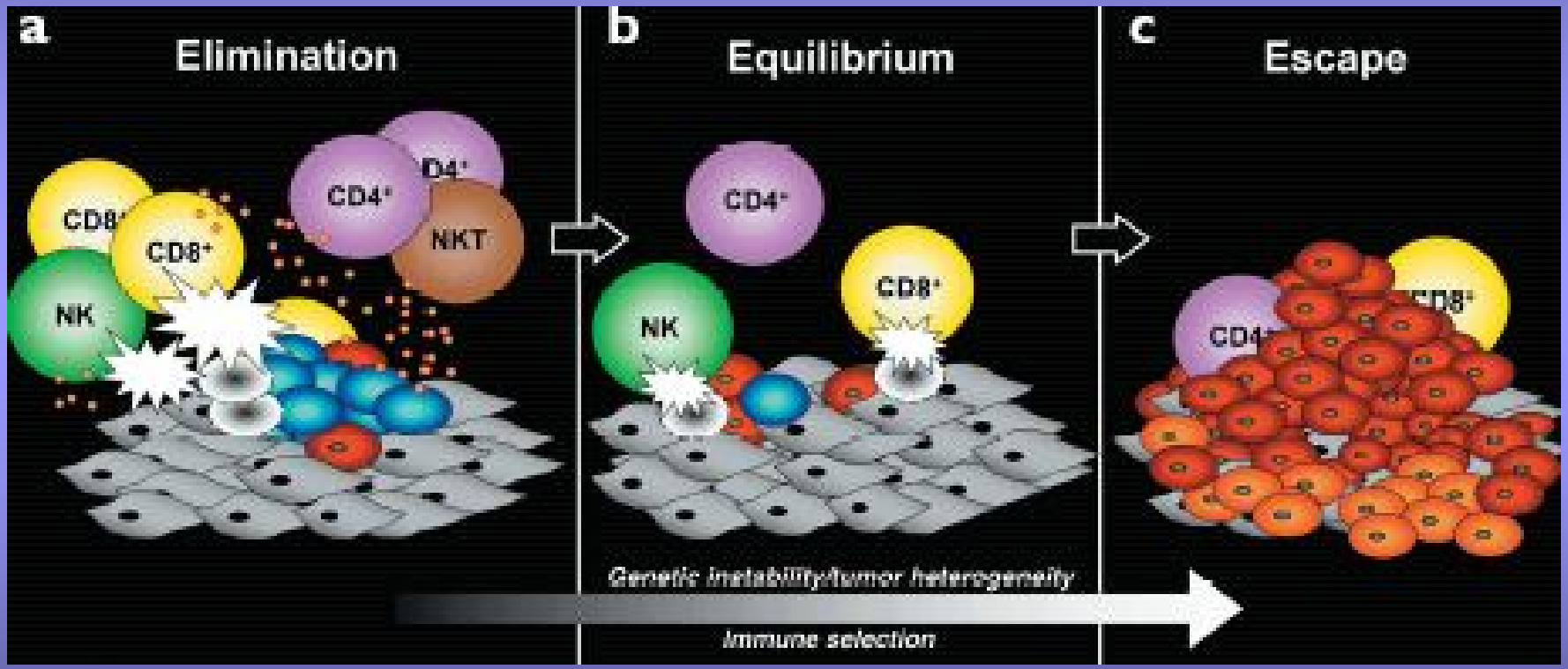
Cadherin is composed of a single protein chain that folds into a series of domains. On the outside of the cell, there are five compact domains. Calcium ions, shown here as blue spheres, bind at the junction between the domains. They provide stability and are essential for proper adhesive function. The adhesive site is in the uppermost domain and relies on a key tryptophan amino acid, shown here in red. Inside the cell, there is a small domain that interacts with catenins and other proteins that bind to the cytoskeleton. This region is shown as a schematic here, with the cell membrane in green. Coordinates for the extracellular region were taken from entry 1l3w at the Protein Data Bank (www.pdb.org).

MBMM 2004

HIPOTESIS DE LA VIGILANCIA INMUNOLÓGICA

RATONES KNOCK – OUT PARA RECEPTOR DE IFN-γ Y STAT GENERAN ESPONTÁNEAMENTE TUMORES

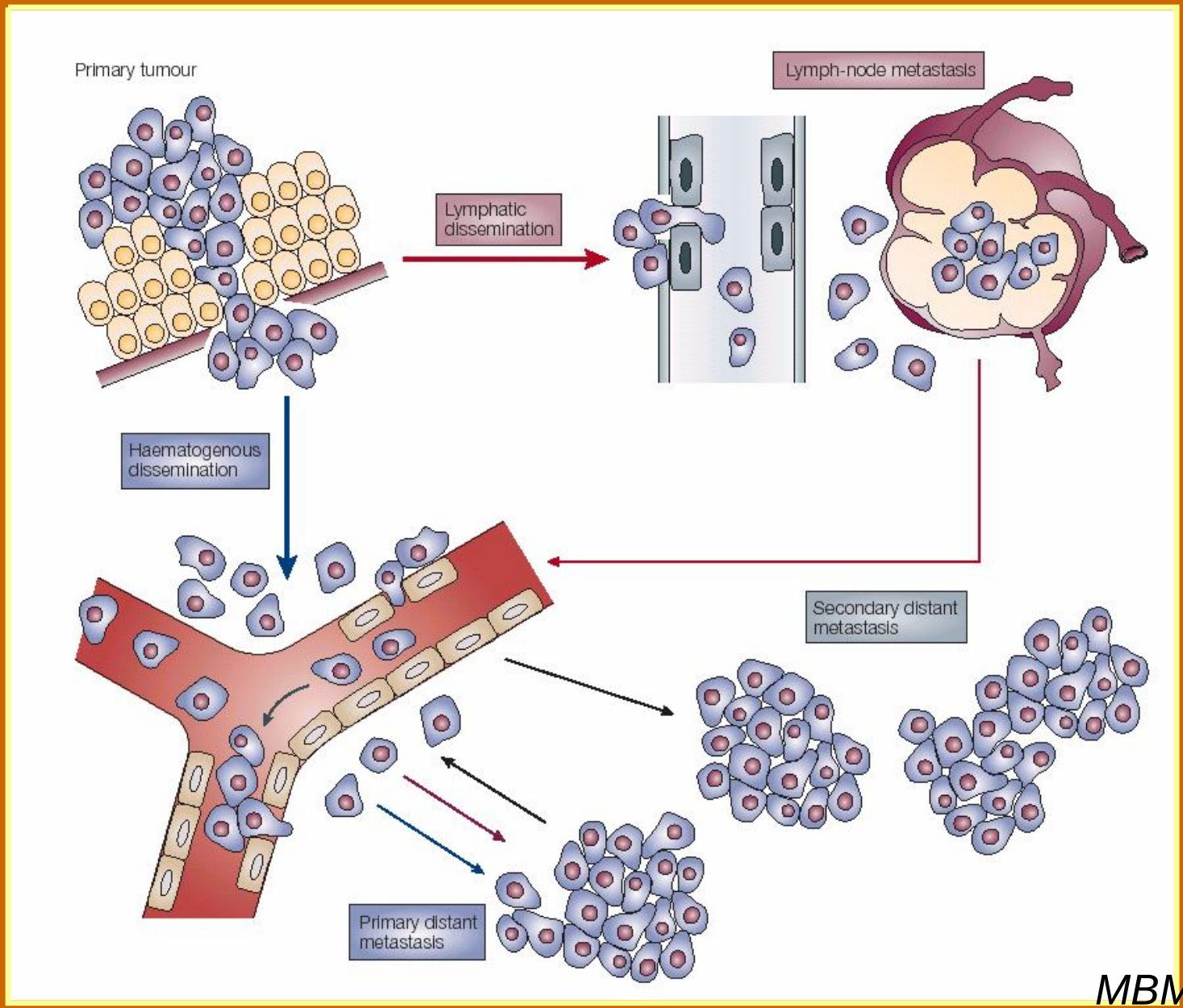
Las “tres E” de la inmunoedición



Nature Immunol 2002; 3:991.

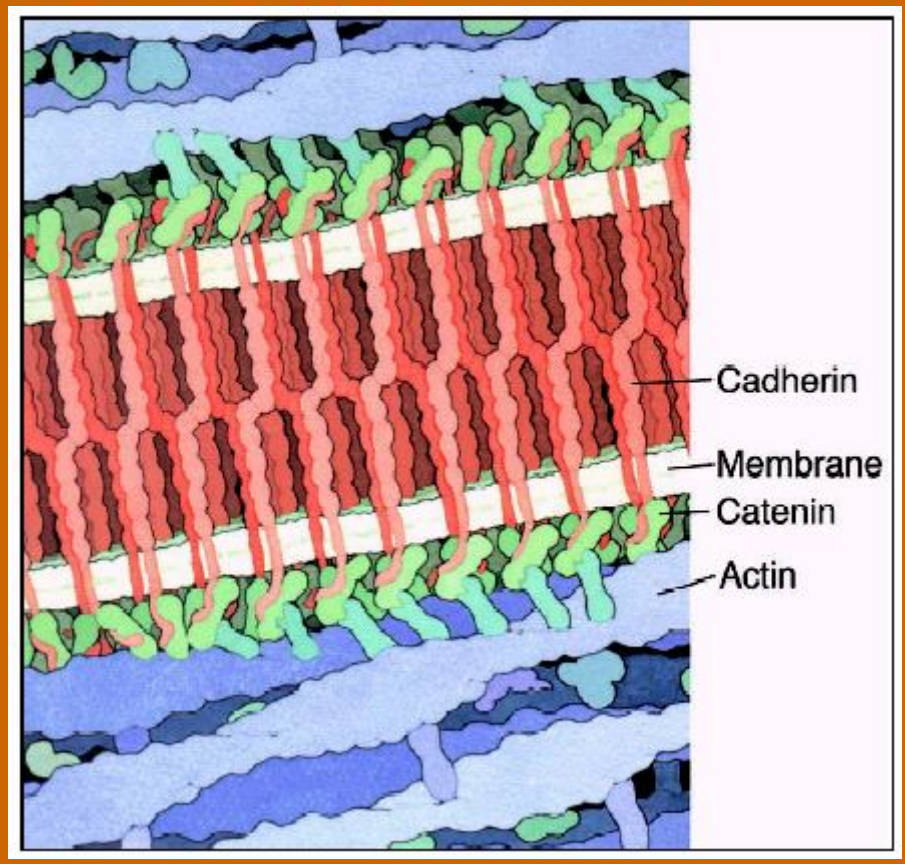
METASTASIS

MODELS OF THE METASTATIC CASCADE



Proteases in metastasis

- **Urokinase-like plasminogen activator**
  - Serine protease
  - Attached to invasive cell surface.
  - Activates tissue plasminogen to plasmin, which is not specific.
- **Matrix metalloproteinases**
  - Zinc metalloproteinase
  - >27 family members
  - Important in tissue remodeling
- **Cathepsins**
  - cysteine proteases (B,S,L,O,W,F,C,H,Z)
  - Aspartic proteases (D)
  - Lysosomal, released to ECM, or degrades MMP framents
  - B, D and L important in colon, prostate and breast cancer progression



Cadherins extend from the surface of the cell and attach to cadherins on a neighboring cell. *Here, a portion of an adherens junction is shown, with cadherin molecules in red. The two cell membranes are shown in light green, and the cadherin molecules are linked in the space between the two cells. Just inside each cell, catenin molecules (in green) link the cadherins to actin filaments (in blue).*

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PATOLOGIA MOLECULAR DE LA METASTASIS

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Diagnóstico molecular de diseminación linfática

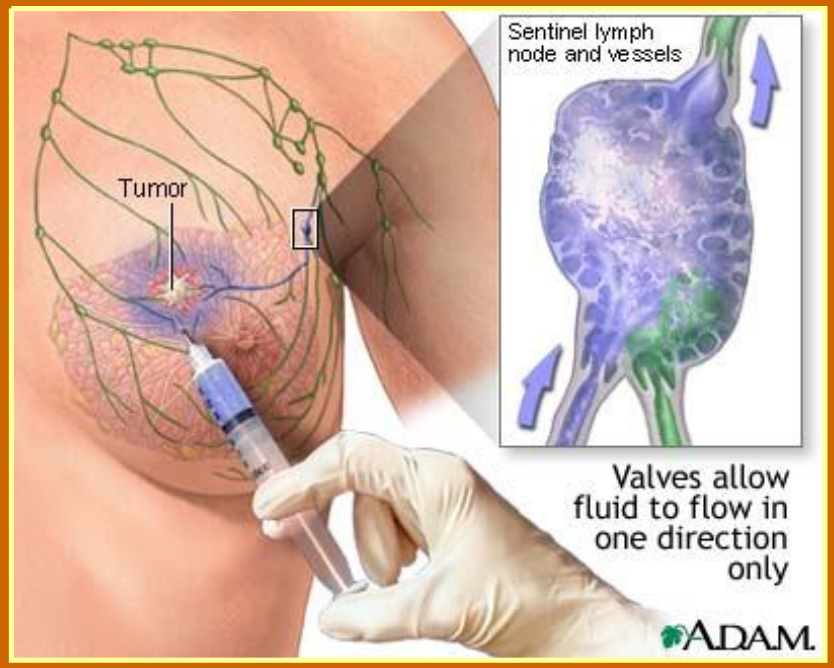
√ **Lymph node involvement is indicative of poor prognosis in several cancer types, because it indicates clear evidence of metastatic disease.**

√ **A subset of patients with histologically node negative disease will develop metastatic disease with subsequent reduced survival.**

√ **Using molecular staging techniques micrometastases are defined as single disseminated tumor cells or small clusters of neoplastic cells which can only be detected by immunohistochemical techniques or assays based on PCR**

√ **With these techniques, lymph-node micrometastases can be detected in up to 40% of nodenegative patients with breast cancer, and non-small-celllung cancer**

Principio de ganglio centinela



Sentinel node biopsy is a minimally invasive technique to select patients with occult lymph node metastases who may benefit from further regional or systemic therapy.

The sentinel node is the first lymph node reached by metastasising cells from a primary tumour.

The development of the dynamic technique of intraoperative lymphatic mapping in the 1990s resulted in general acceptance of the sentinel node concept.

This hypothesis of sequential tumour dissemination seems to be valid according to numerous studies of sentinel node biopsy with confirmatory regional lymph node dissection.

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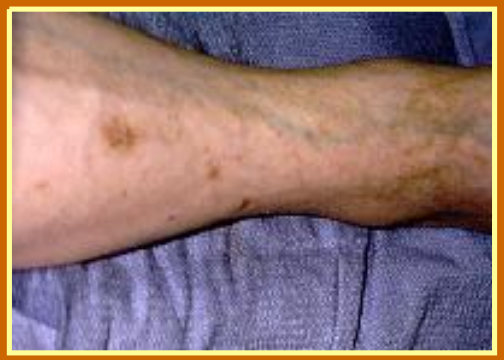
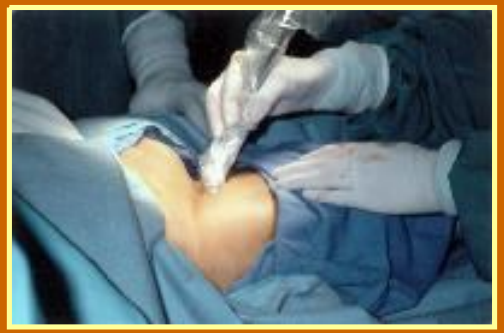
Procedimiento de ganglio centinela

The surgeon injects a small dose of radioactive tracer (technetium-99) into the breast in the region of the patient's tumor.

Next, the surgeon will wait for the technetium-99 to travel from the tumor region to the sentinel lymph node(s). Depending on the protocol followed, the surgeon usually waits between 45 minutes to 8 hours after injection before performing the biopsy.



At some point during the procedure, a small amount of blue dye (isosulfan blue) will also be injected near the area of the tumor.



Once the technetium-99 tracer and dye have reached the nodes, the surgeon will scan the area with an electric, hand-held gamma ray counter attached to a small probe which the surgeon traces over the axilla or ingle to locate the sentinel node(s).

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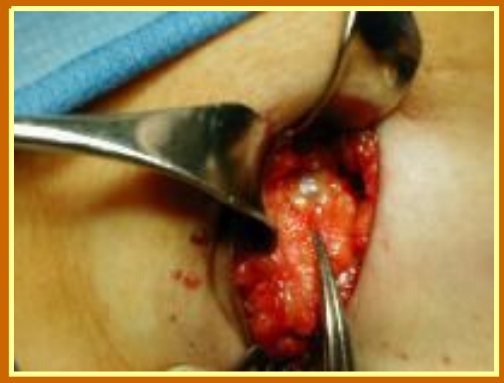

The surgeon will make a small incision (usually one-half inch) and remove the sentinel node(s) for a pathologist to examine under a microscope. The blue dye provides additional visual confirmation of the sentinel node's location during surgical removal.

The sentinel lymph nodes will be classified as negative (no cancer), positive (contain cancer), or indeterminate. However, this preliminary report is followed by close examination and the final pathology report.

If the sentinel node is determined to be cancerous while the patient is still in surgery, the surgeon will usually remove additional lymph nodes in the axilla.

However, the final pathology report is not available until after the surgery has been completed, and patients should schedule a follow-up visit with the surgeon to discuss the final report.

Sometimes, the final report indicates a positive (cancerous) sentinel node that was not seen on preliminary review. If this occurs, then additional surgery may be necessary to remove more nodes for examination.

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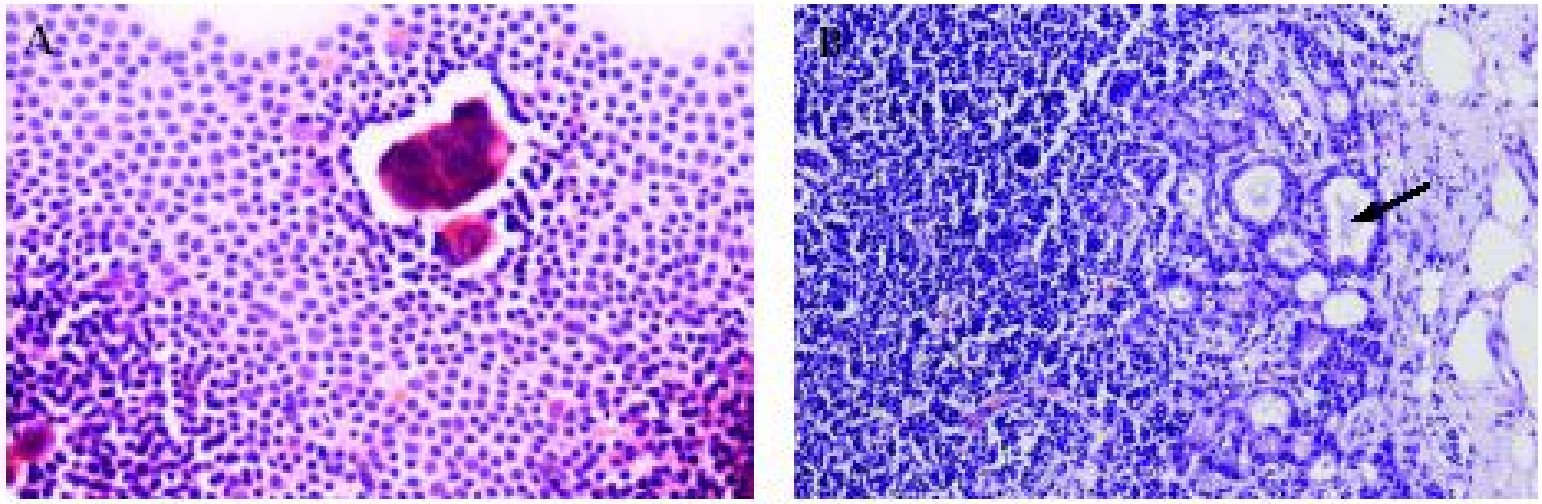
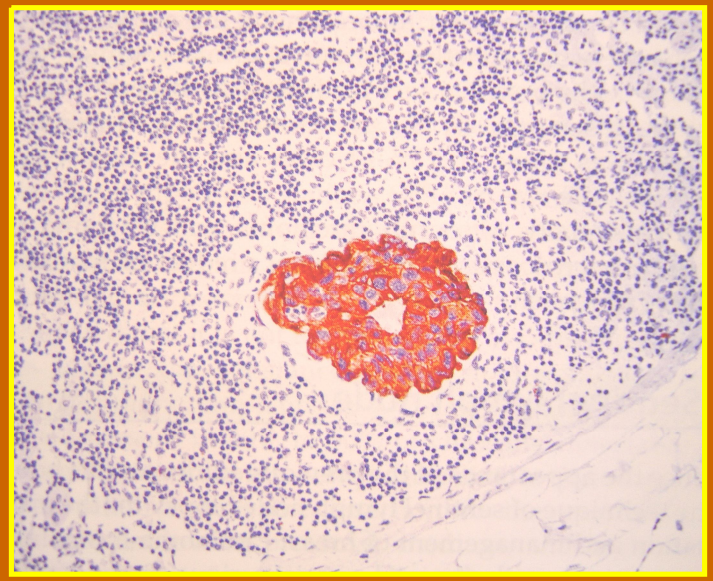
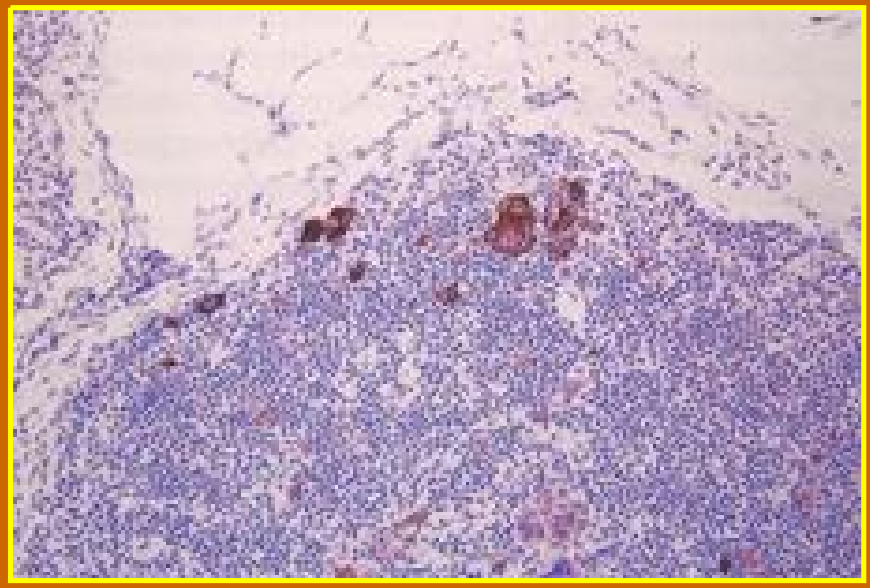


Figure 1. *Pathology of sentinel lymph node in breast cancer A: Intraoperative imprint cytology of a sentinel lymph node of an invasive ductal cancer of the breast. Note the large epithelial cell-aggregate in the lymphoid background. H&E staining. B: Histology of a sentinel lymph node in a case of invasive ductal cancer of the breast. Note the subcapsular micrometastasis (arrow). H&E staining.*





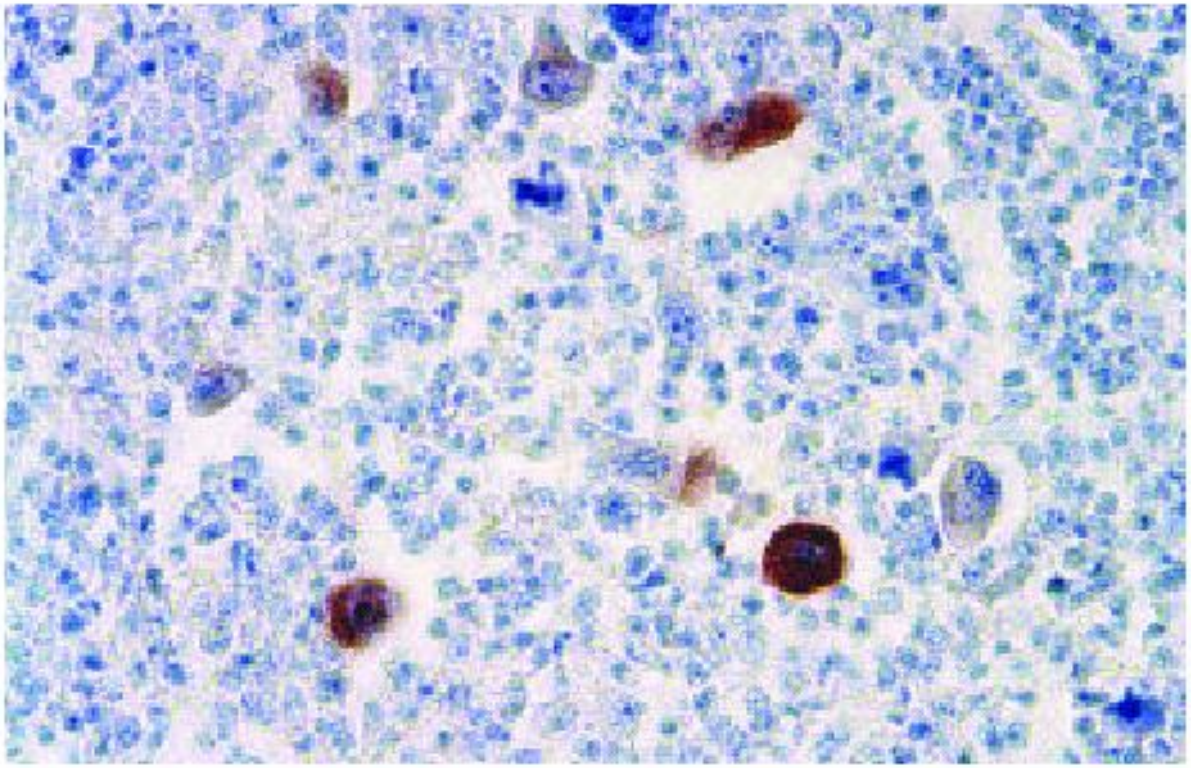
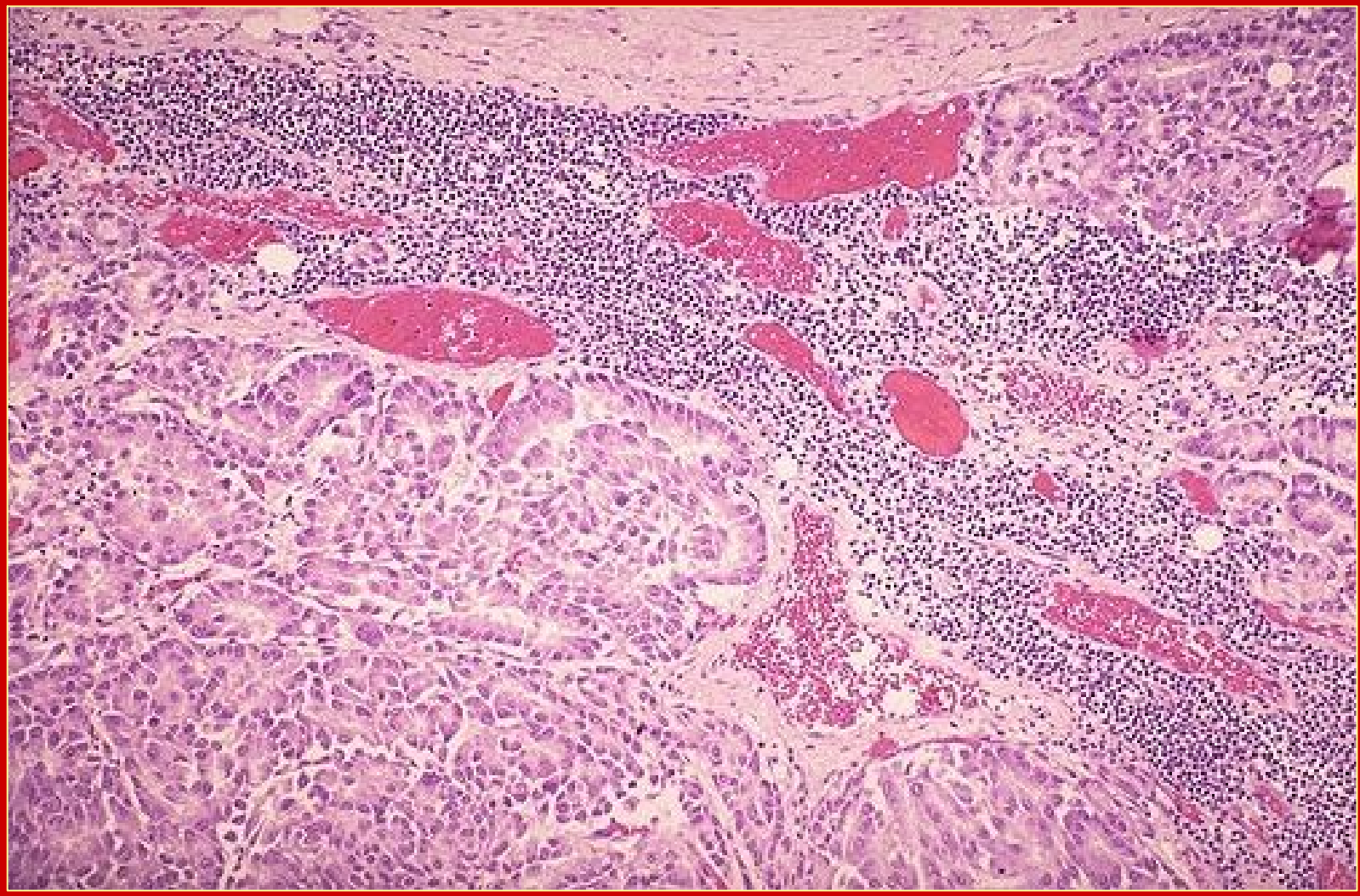


Figure 2. *Identification of melanoma cells in sentinel lymph node of melanoma malignum of the skin using MART-1 immunohistochemistry. Note the redish-brown staining in the cytoplasm of anaplastic tumor cells. (chromogen: AEC)*



Metastatic adenocarcinoma is seen in a lymph node here.
It is common for carcinomas to metastasize to lymph nodes.

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