Cancer

When good cells go bad

What is cancer?

- Caner is defined as the continuous uncontrolled growth of cells.
- A tumor is a any abnormal proliferation of cells.
- Benign tumors stays confined to its original location
- Malignant tumors are capable of invading surrounding tissue or invading the entire body
- Tumors are classified as to their cell type
- Tumors can arise from any cell type in the body

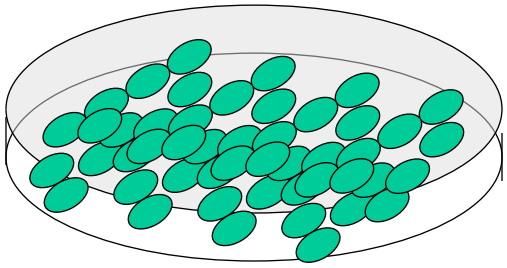
Cancer is an umbrella term covering a plethora of conditions characterized by unscheduled and uncontrolled cellular proliferation.

- Almost any mammalian organ and cell type can succumb to oncogenic transformation, giving rise to a bewildering array of clinical outcomes.
- The causes of cancer are many and varied, and include genetic predisposition, environmental influences, infectious agents and ageing. These transform normal cells into cancerous ones by derailing a wide spectrum of regulatory and downstream effector pathways. It is just this complexity that has hampered the development of effective and specific cancer therapies.
- Any attempt to provide a comprehensive overview of cancer-related knowledge would be futile — therefore the next two lectures will focus on topics undergoing particularly rapid progress.

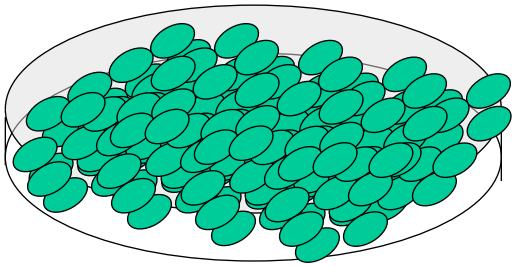
Cancer continued; three cancer types

- Carcinomas; constitute 90% of cancers, are cancers of epithelial cells
- Sarcomas; are rare and consist of tumors of connective tissues (connective tissue, muscle, bone etc.)
- Leukemias and lymphomas; constitute 8% of tumors. Sometimes referred to as liquid tumors. Leukemias arise from blood forming cells and lymphomas arise from cells of the immune system (T and B cells).

Properties of cancer cells



Normal cells show contact inhibition



Cancer cells lack contact inhibition

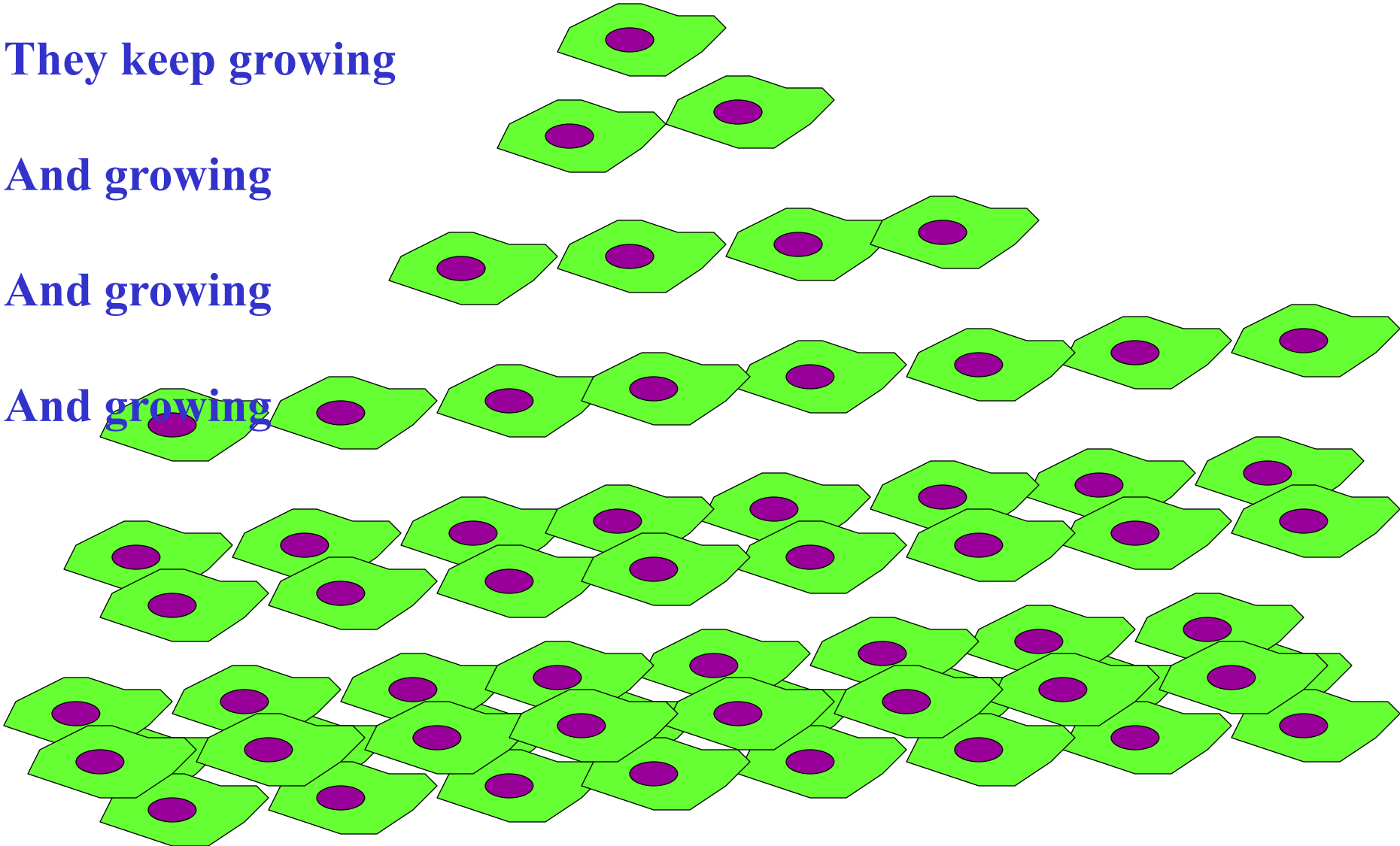
Properties of cancer cells

They keep growing

And growing

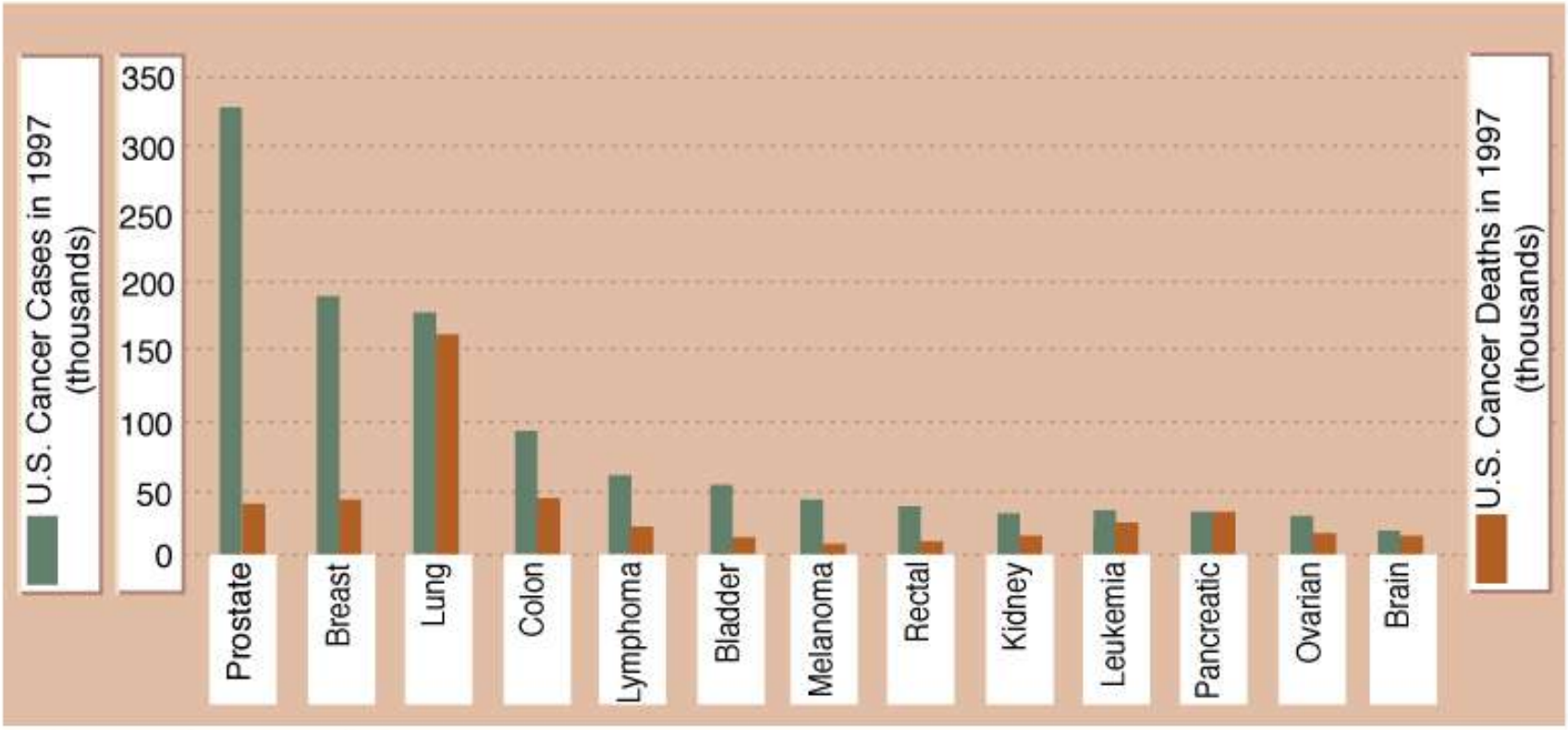
And growing

And growing



Cancer Incidence and Death Rate

Fig. 16.2



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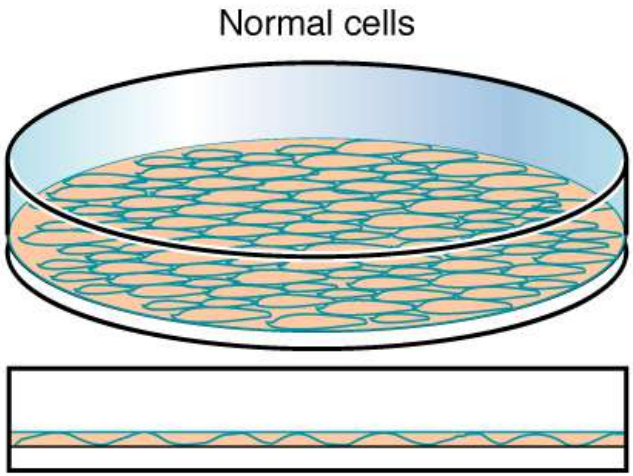
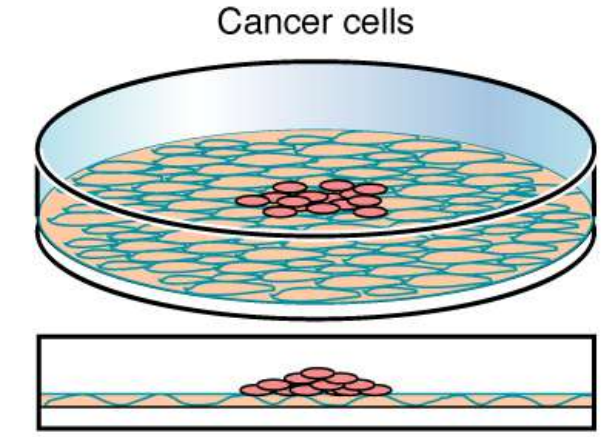
Cancer Fig 16.3



(a)



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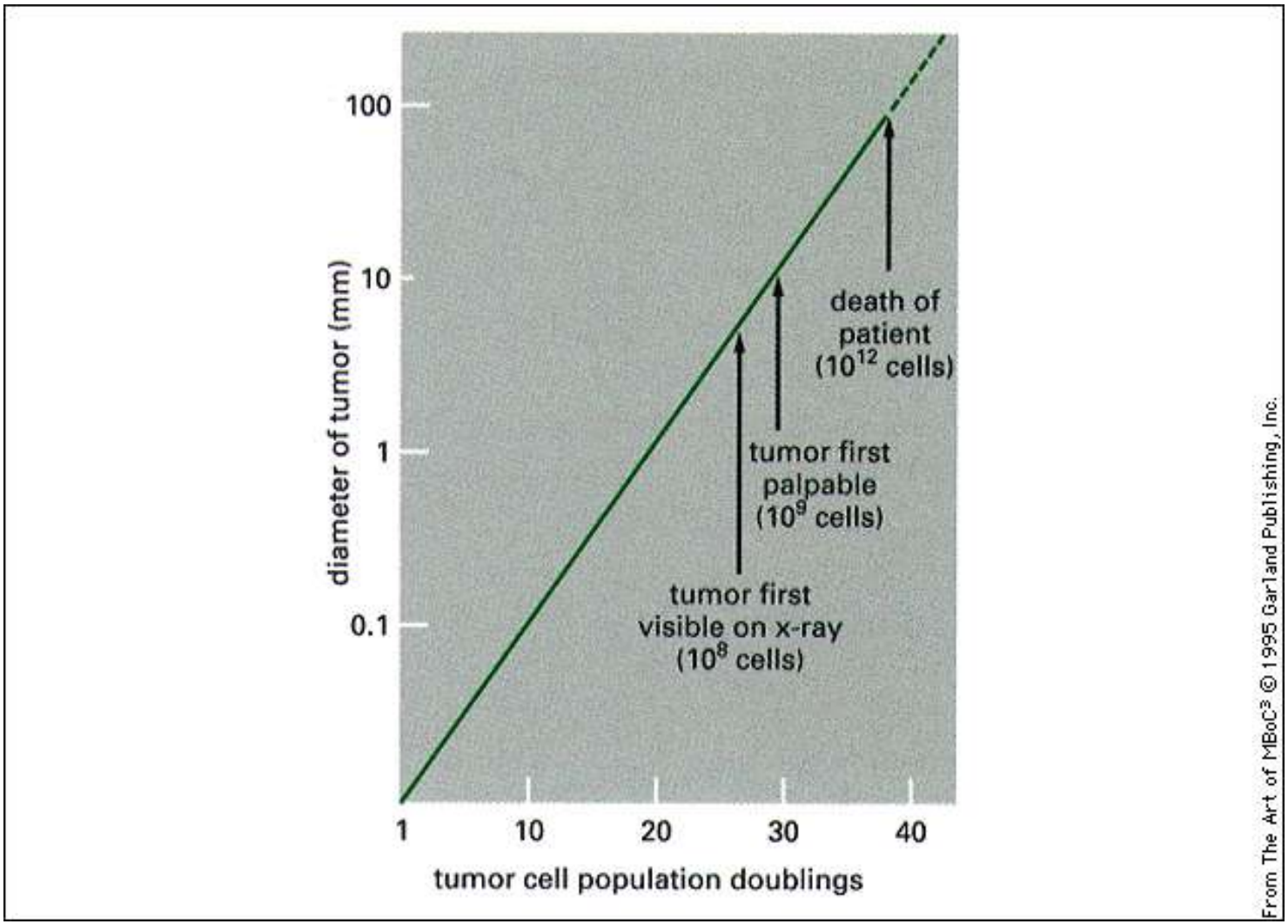
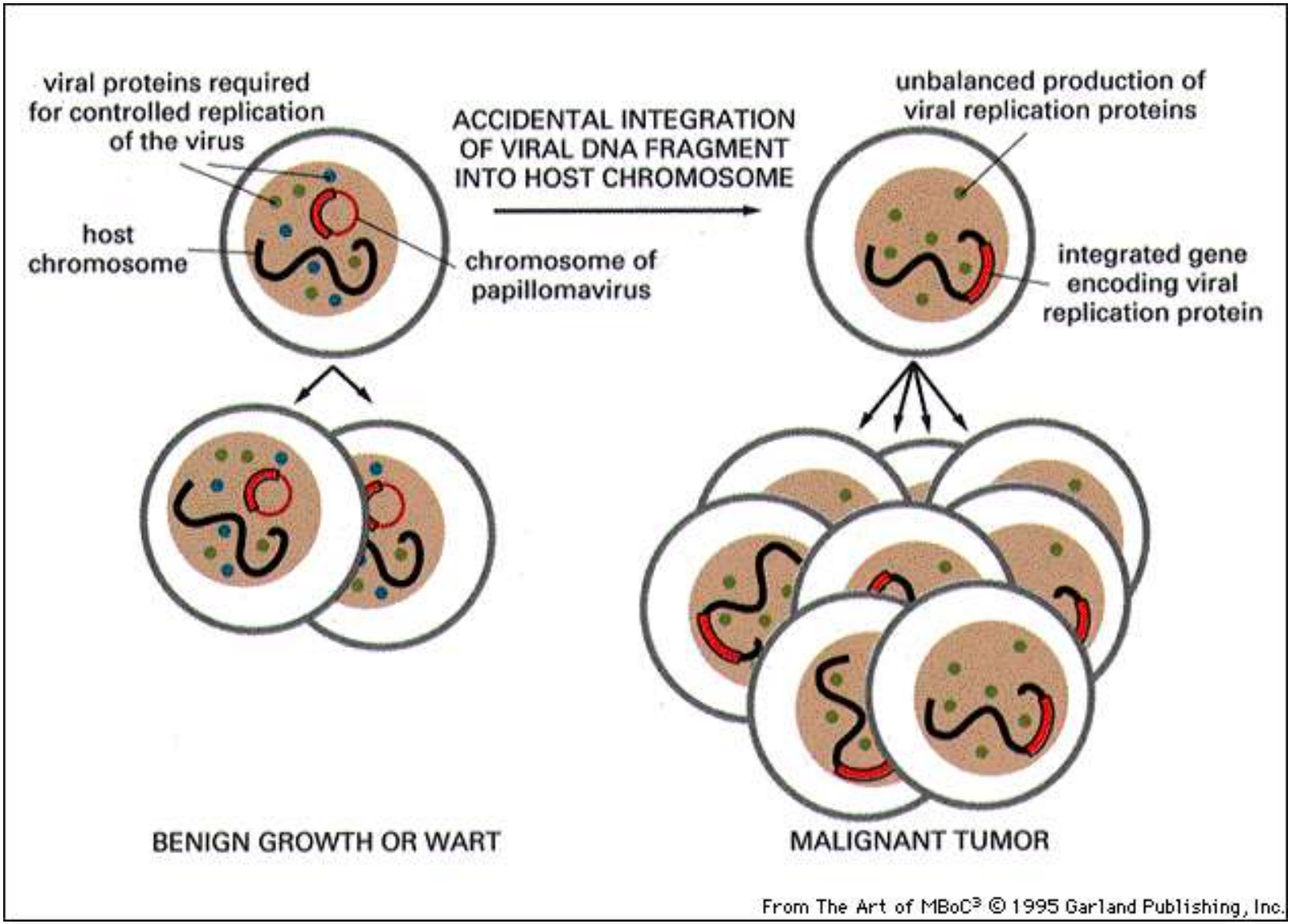
- Cells in culture and in vivo exhibit contact-inhibition
- Cancer cells lack contact inhibition feedback mechanisms. Clumps or foci develop.

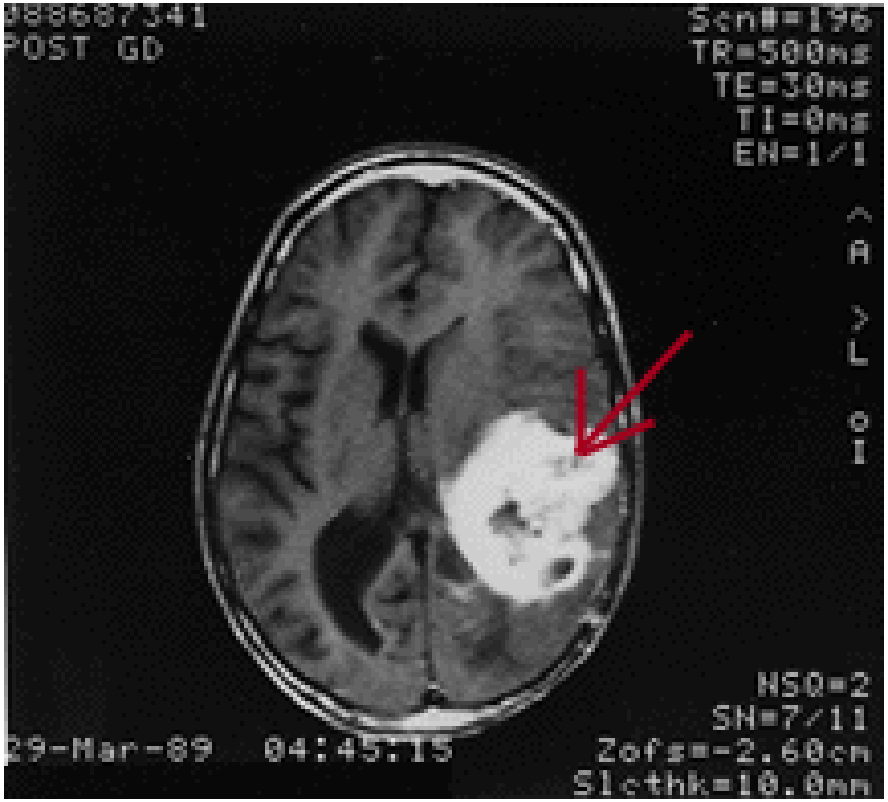
Early detection is the key!



What causes Cancer?
Genetic mutations

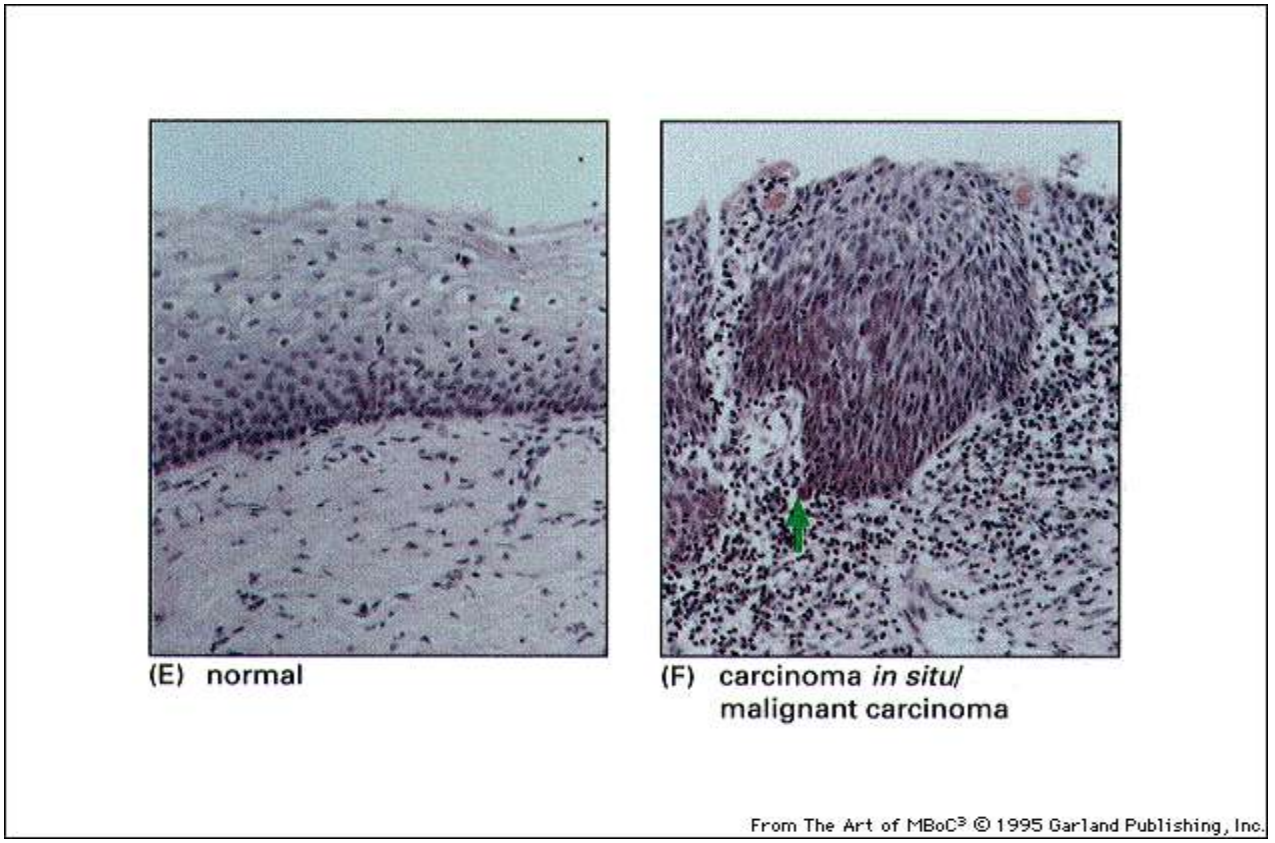


Cancer: Benign



- **Benign**: localized and of small size
- Cells that closely resemble, and may function, like normal cells
- May be delineated by a fibrous (Basal lamina) capsule
- Become problems due to sheer bulk or due to secretions (e.g. hormones)

Cancer : Malignant

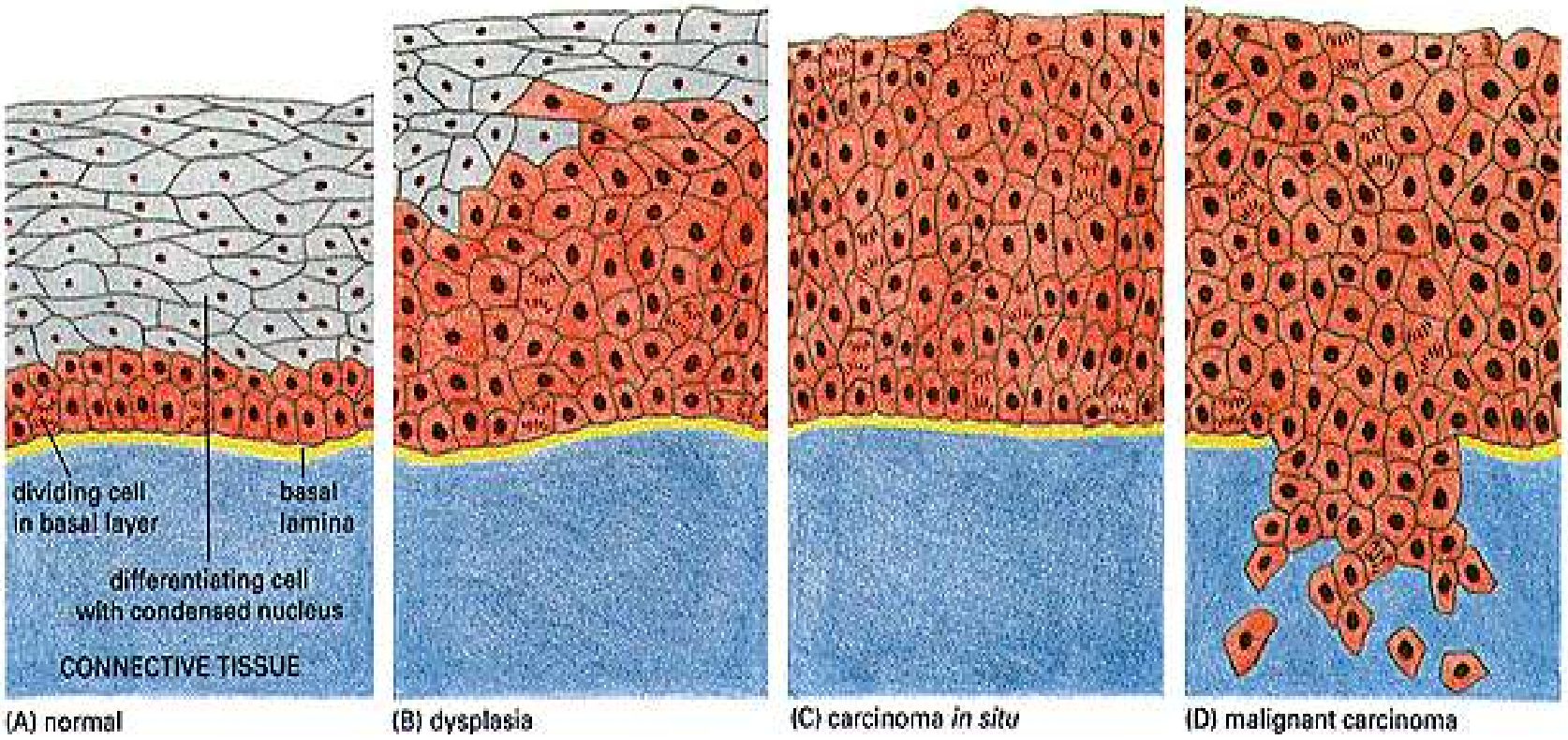


Malignant tumors: high rate of division, properties may vary compared to cells of origin. *Most malignant cells become metastatic*

Invade surrounding tissue and establishment of secondary areas of growth: **Metastasis**

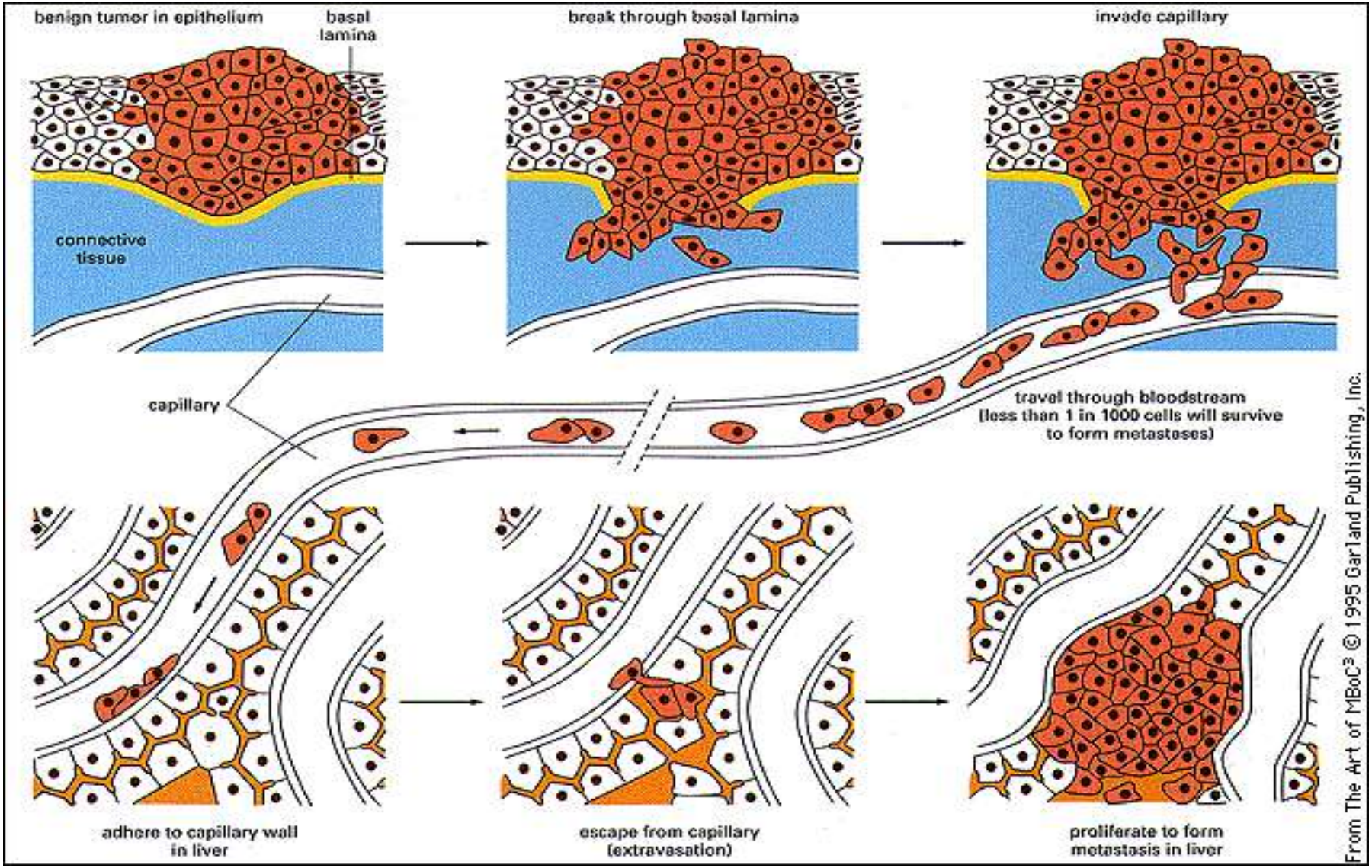
Metastasis

Carcinoma: derived from endoderm or ectoderm



From The Art of MBoC[3] © 1995 Garland Publishing, Inc.

Events in metastasis.



ASSOCIATION WITH HUMAN CANCERS

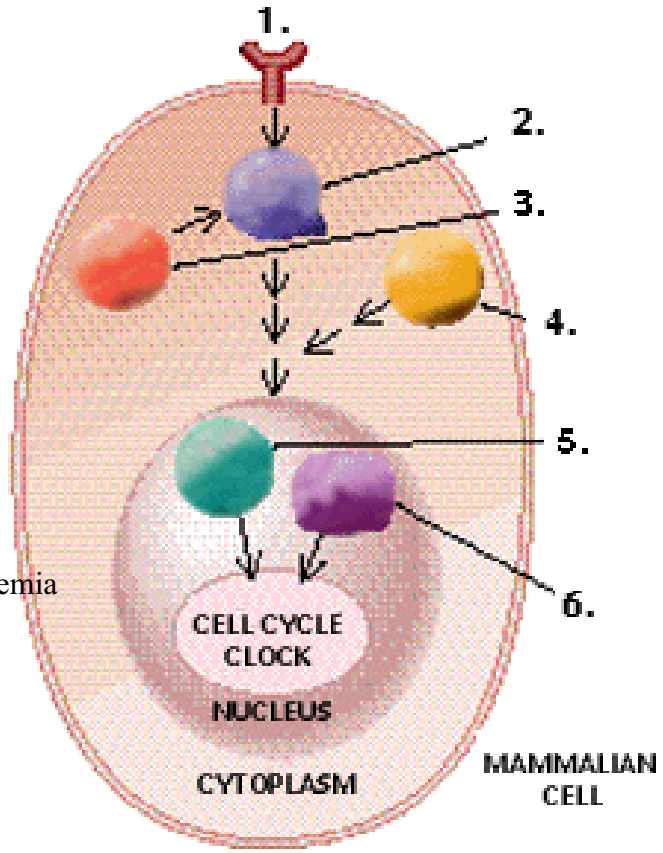
1. Growth Factor Receptor Increased numbers in 20 percent of breast cancers

2. Ras Protein Activated by mutations in 20 to 30 percent of cancers

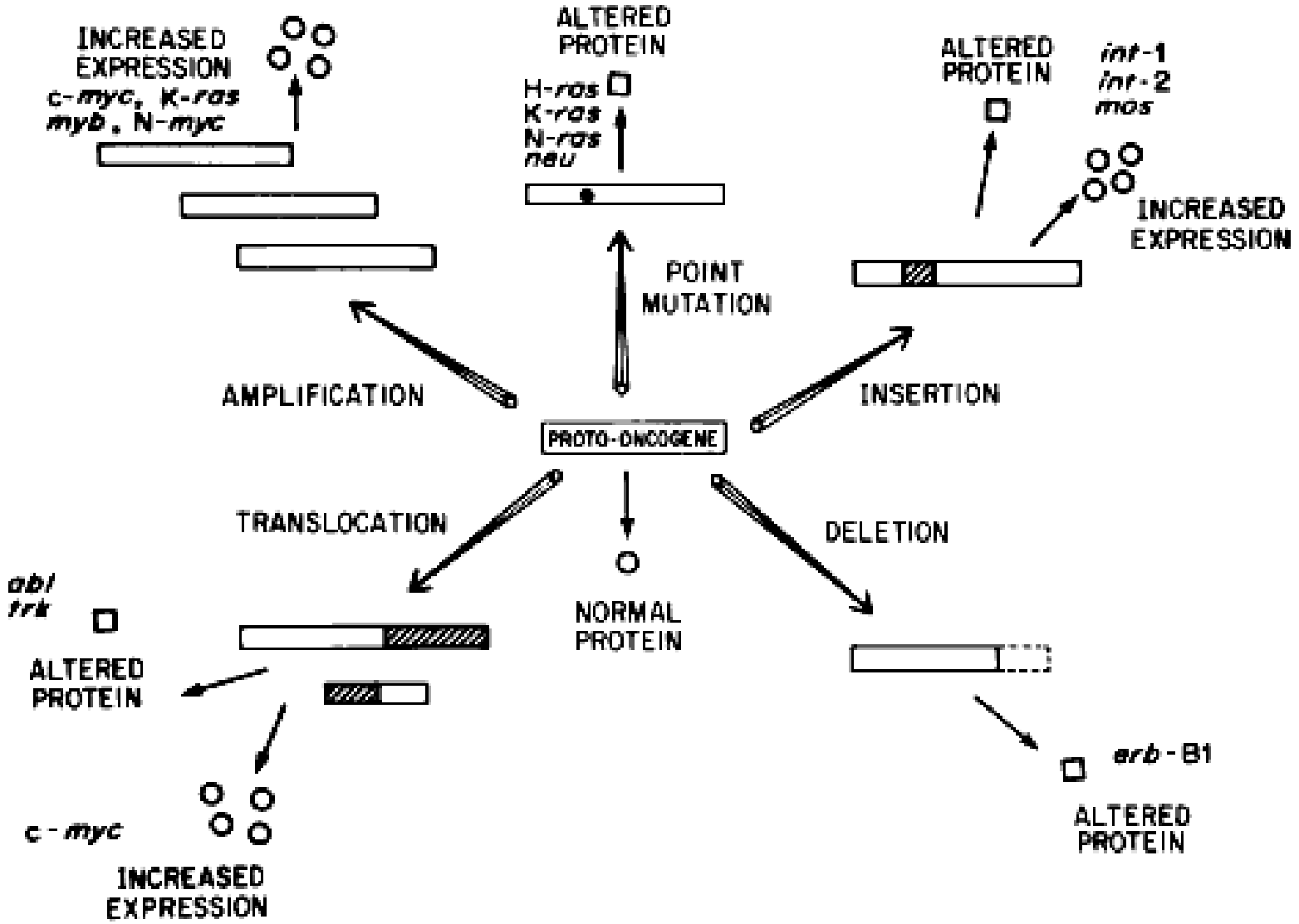
3. Abl Kinase Activated by abnormal chromosomes in chronic myelogenous leukemia

4. Src Kinase Activated by mutations in 2 to 5 percent of cancers

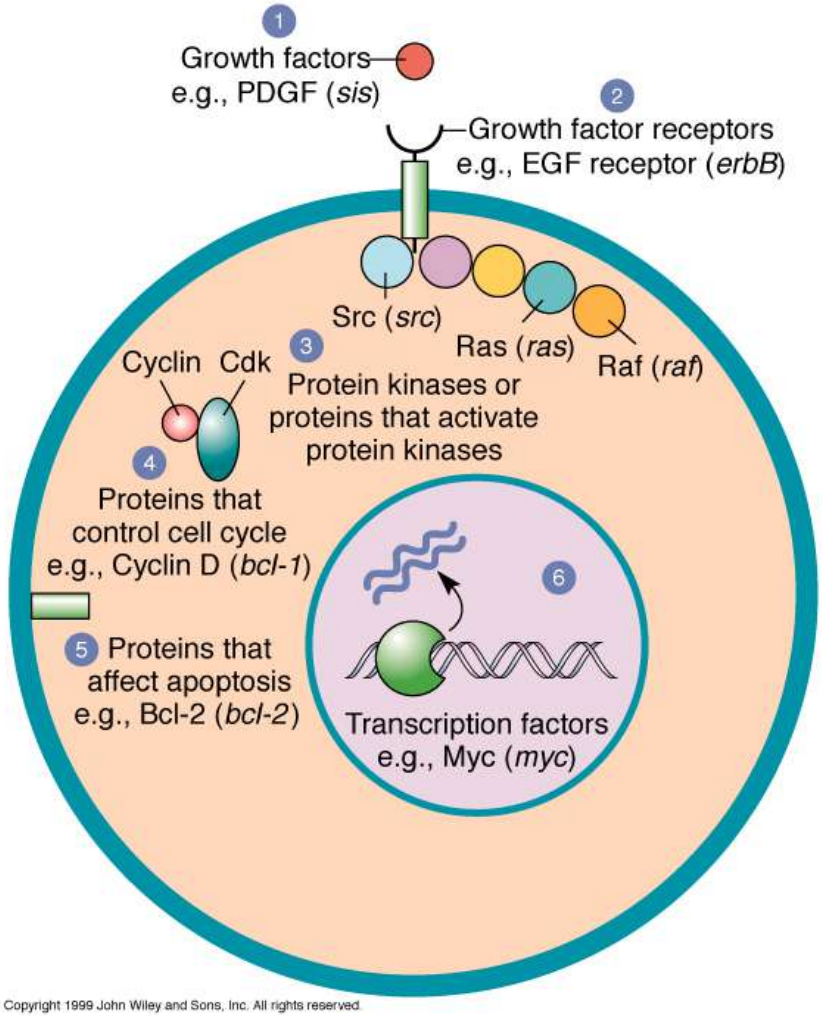
5. p53 Protein Mutated or deleted in 50 percent of cancers



Mechanisms of oncogene activation



Types of proteins encodes by oncogenes



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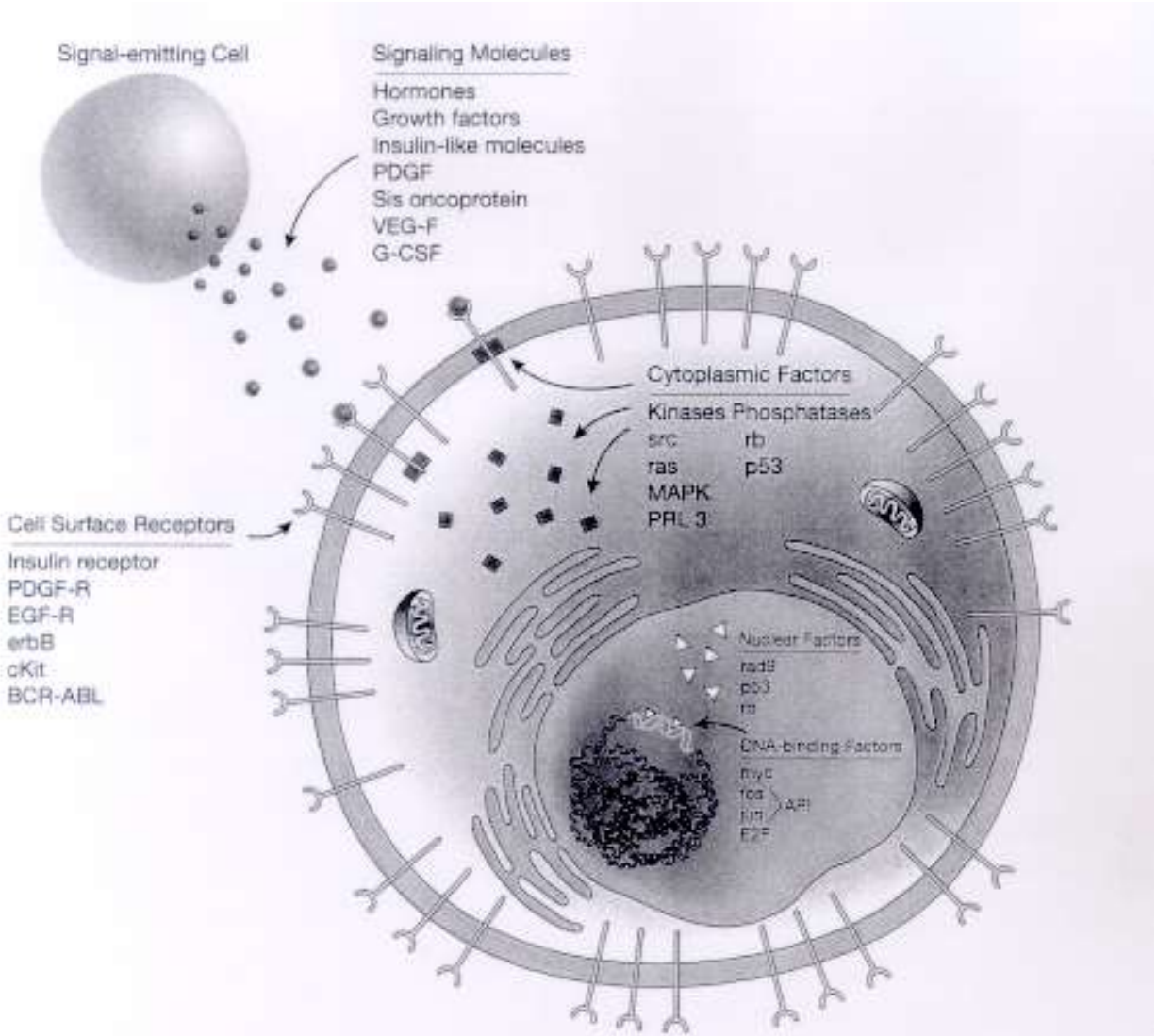
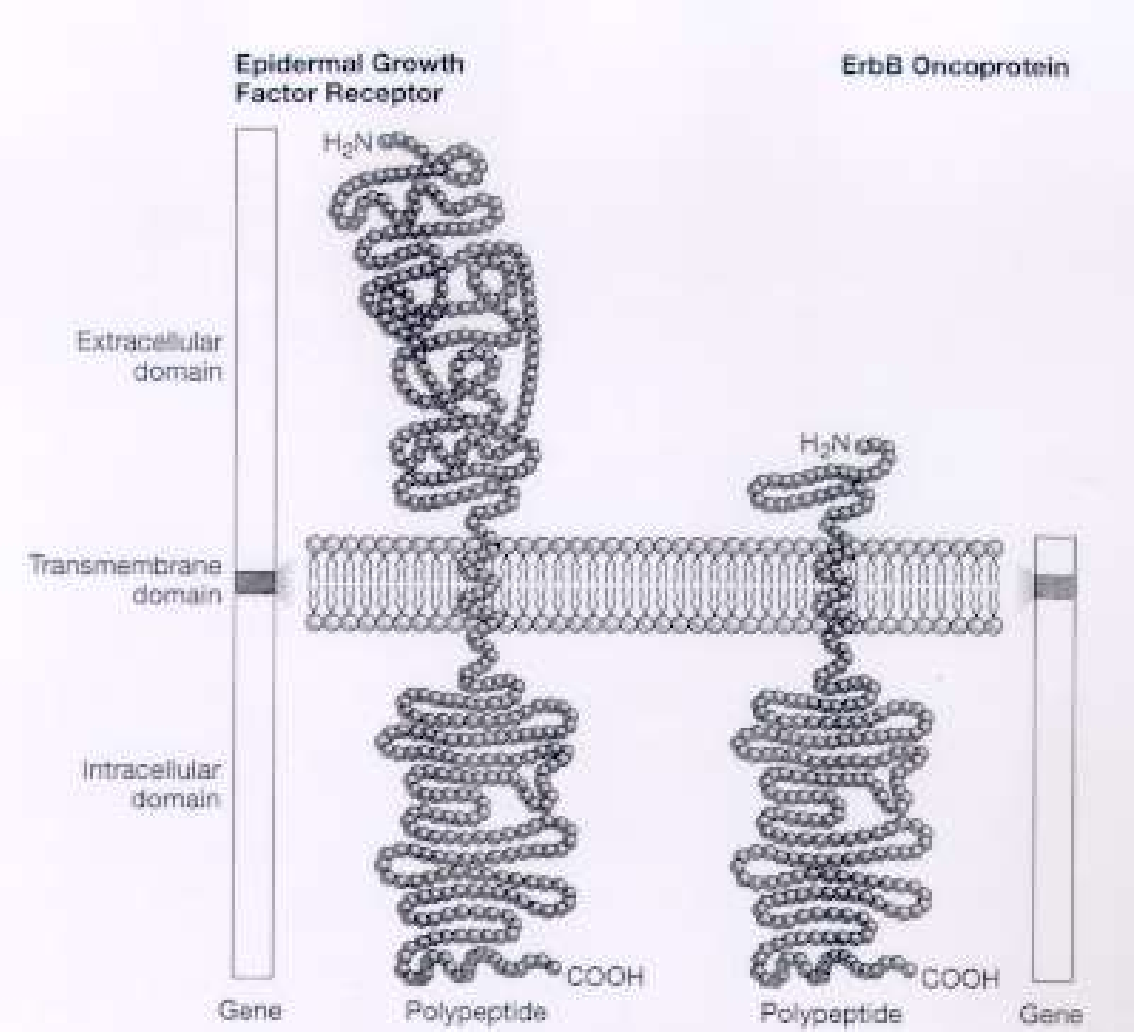
Cancer has a lot to do with cell signaling for growth



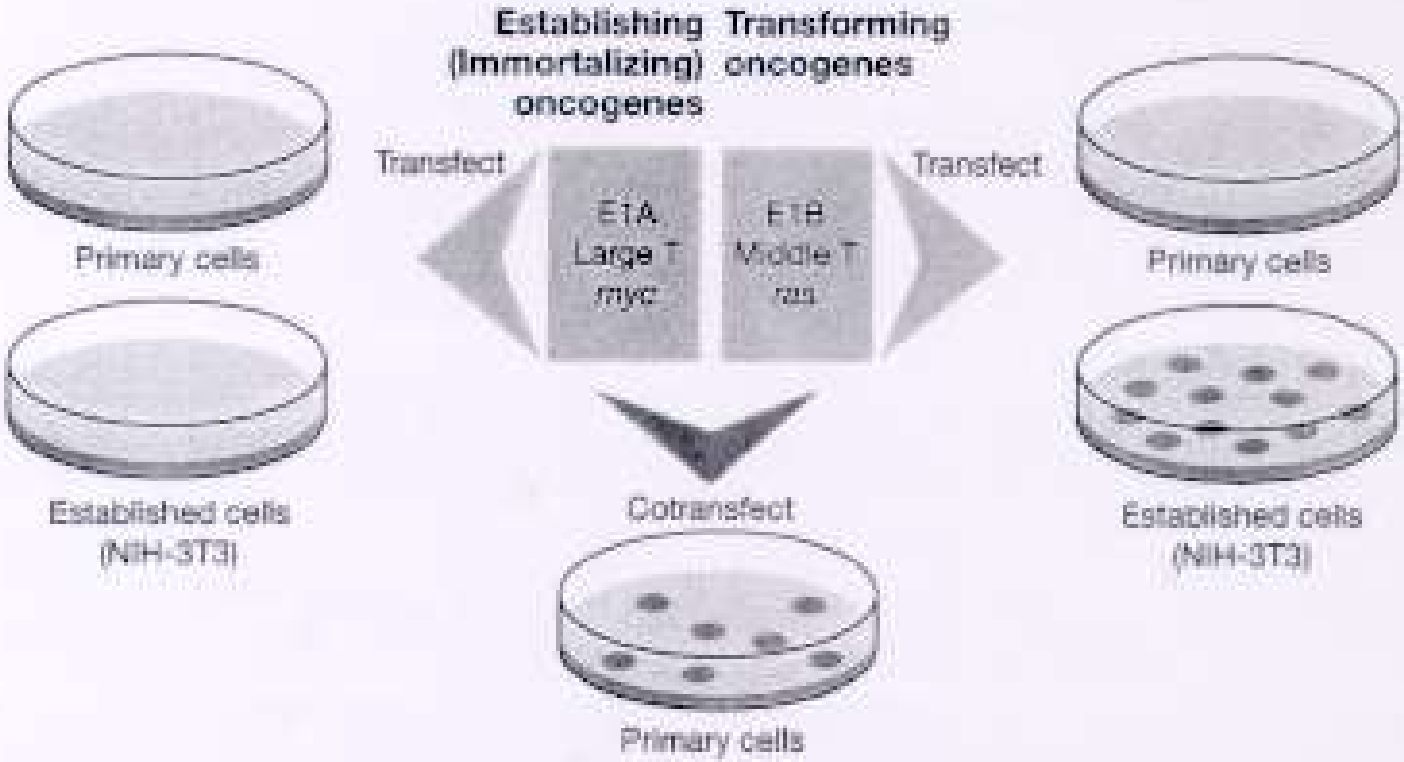
Signal Transduction Proteins That Have Roles in Cancer

ErbB is mutant EGFR



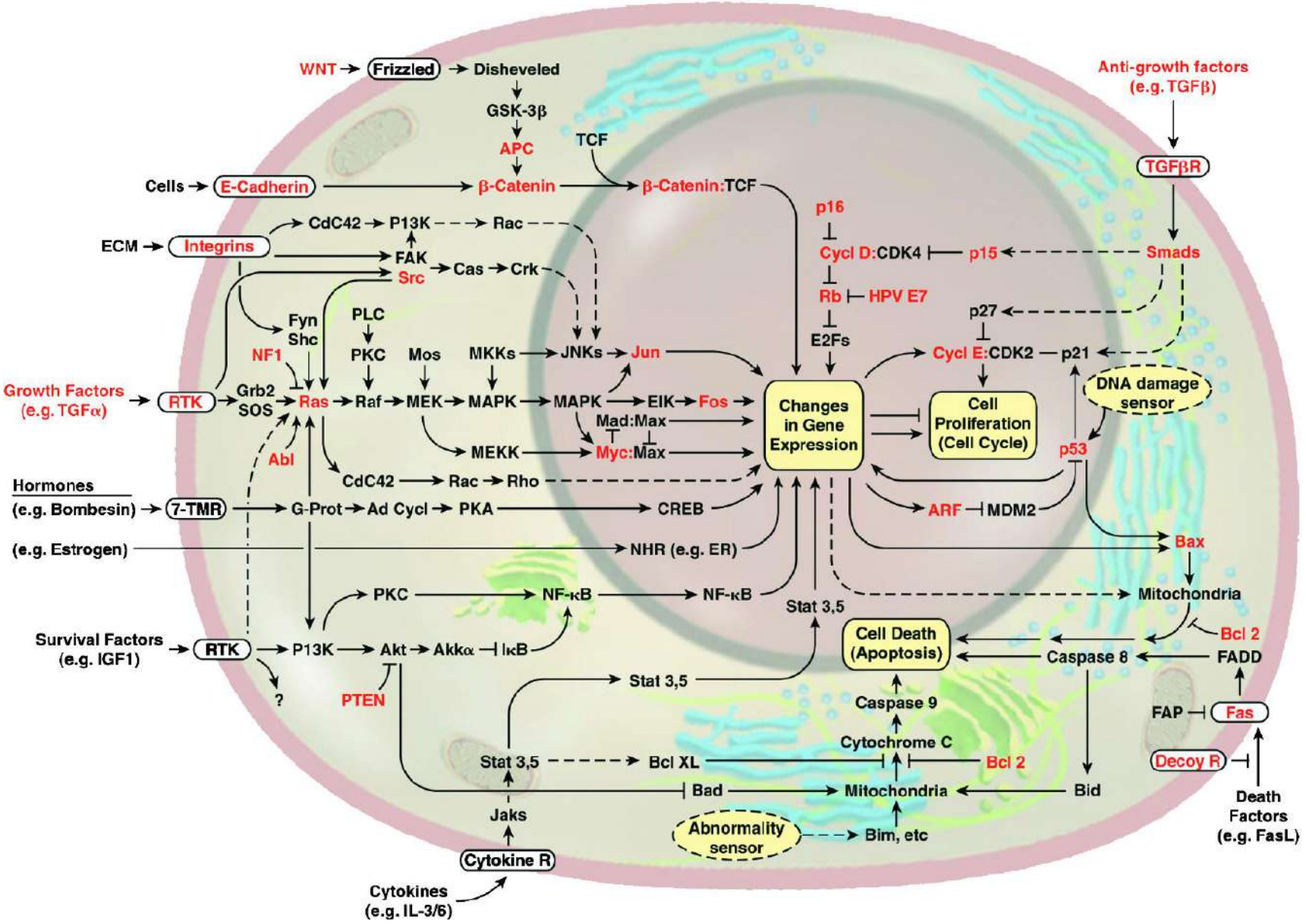
Comparison of Epidermal Growth Factor Receptor and ErbB Oncoprotein

It takes two or more

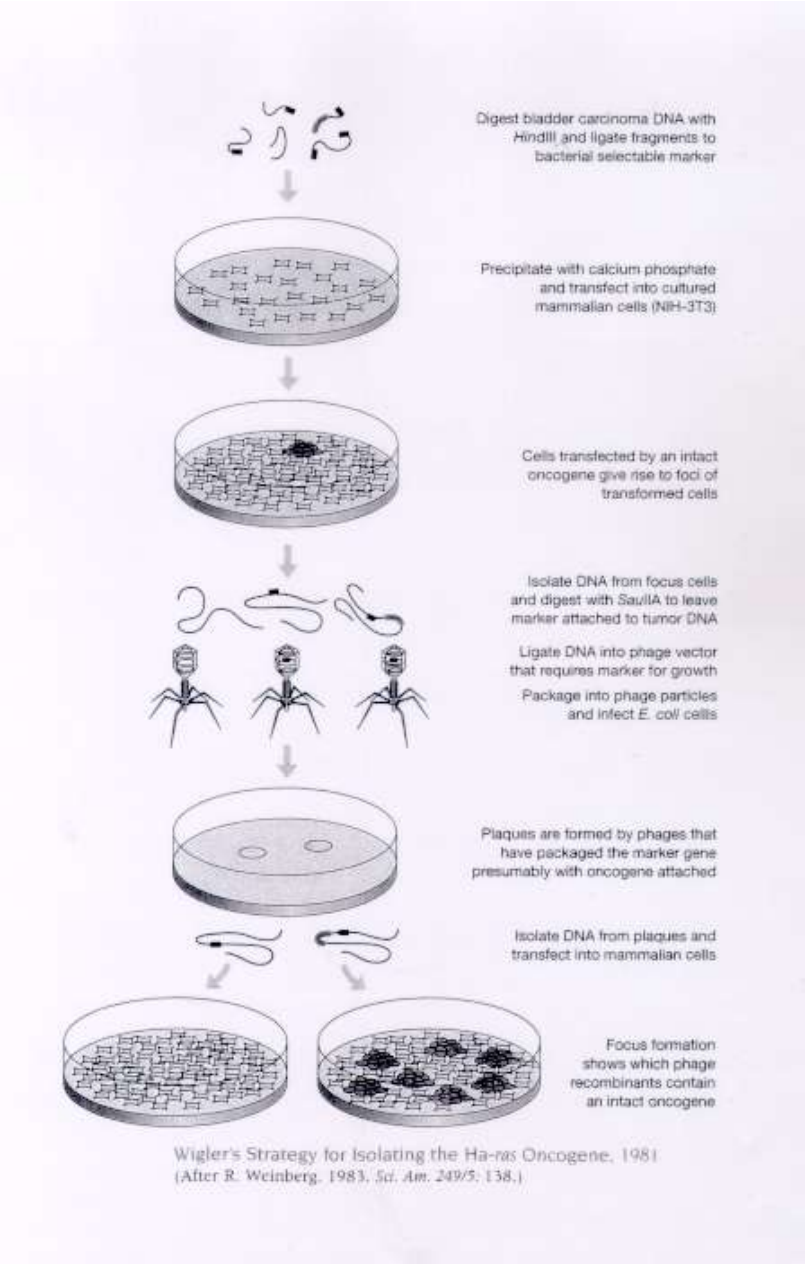


Cooperative Transformation of Primary Cells by Two Oncogenes
Transfected alone, neither immortalizing nor transforming oncogenes transform primary cells. However, cotransfection of both types of oncogenes causes foci of transformed cells. Established cells, such as NIH-3T3, require only a transforming oncogene to form foci.

Pathways leading to cancer

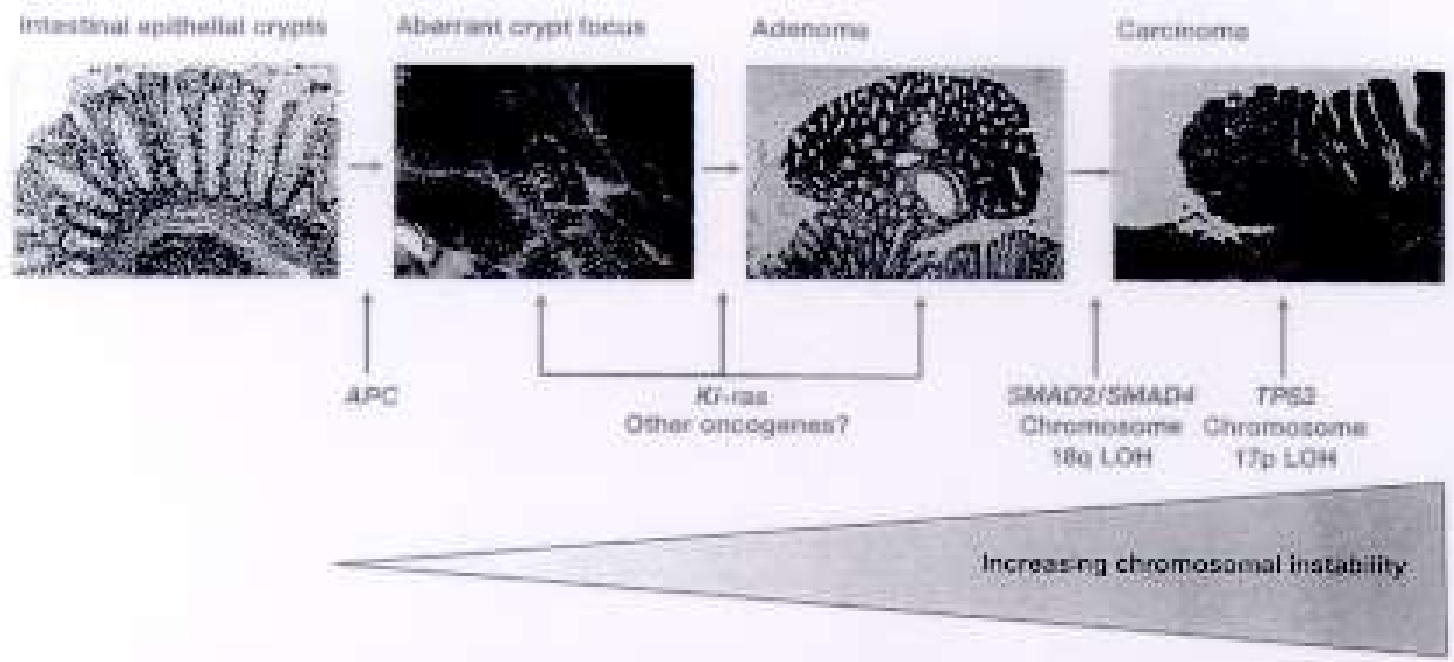


Cloning human ras

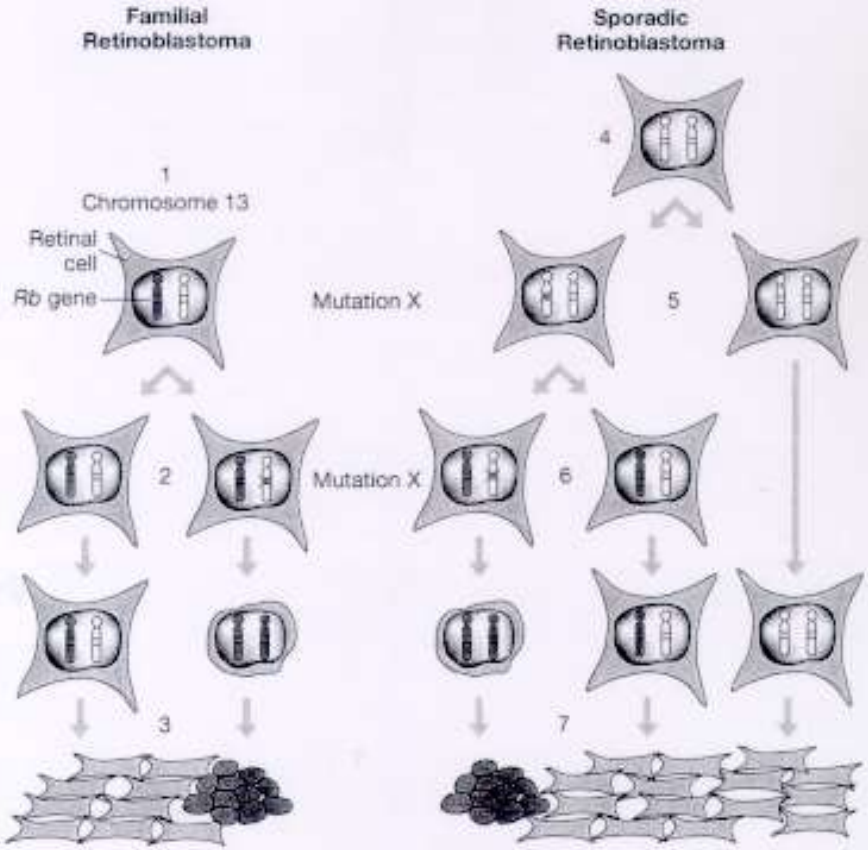


Wigler's Strategy for Isolating the Ha-*ras* Oncogene, 1981
(After R. Weinberg, 1983, *Sci. Am.* 249/5: 138.)

Cancer is a multi-step process



Loss of Rb and cancer



Rb Gene Mutations in Familial and Sporadic Retinoblastoma
In familial retinoblastoma, one normal and one mutated *Rb* gene are inherited (*1*). (*2*) Subsequent mutation in *any* retinal cell inactivates remaining *Rb* gene, (*3*) leading to loss of growth control in a clone of tumor cells. In sporadic retinoblastoma, two normal *Rb* genes are inherited (*4*). (*5*) First mutation inactivates one copy of *Rb* gene; (*6*) subsequent mutation within *same* retinal cell inactivates remaining copy of *Rb* gene, (*7*) leading to loss of growth control in a clone of tumor cells.

B. Virology

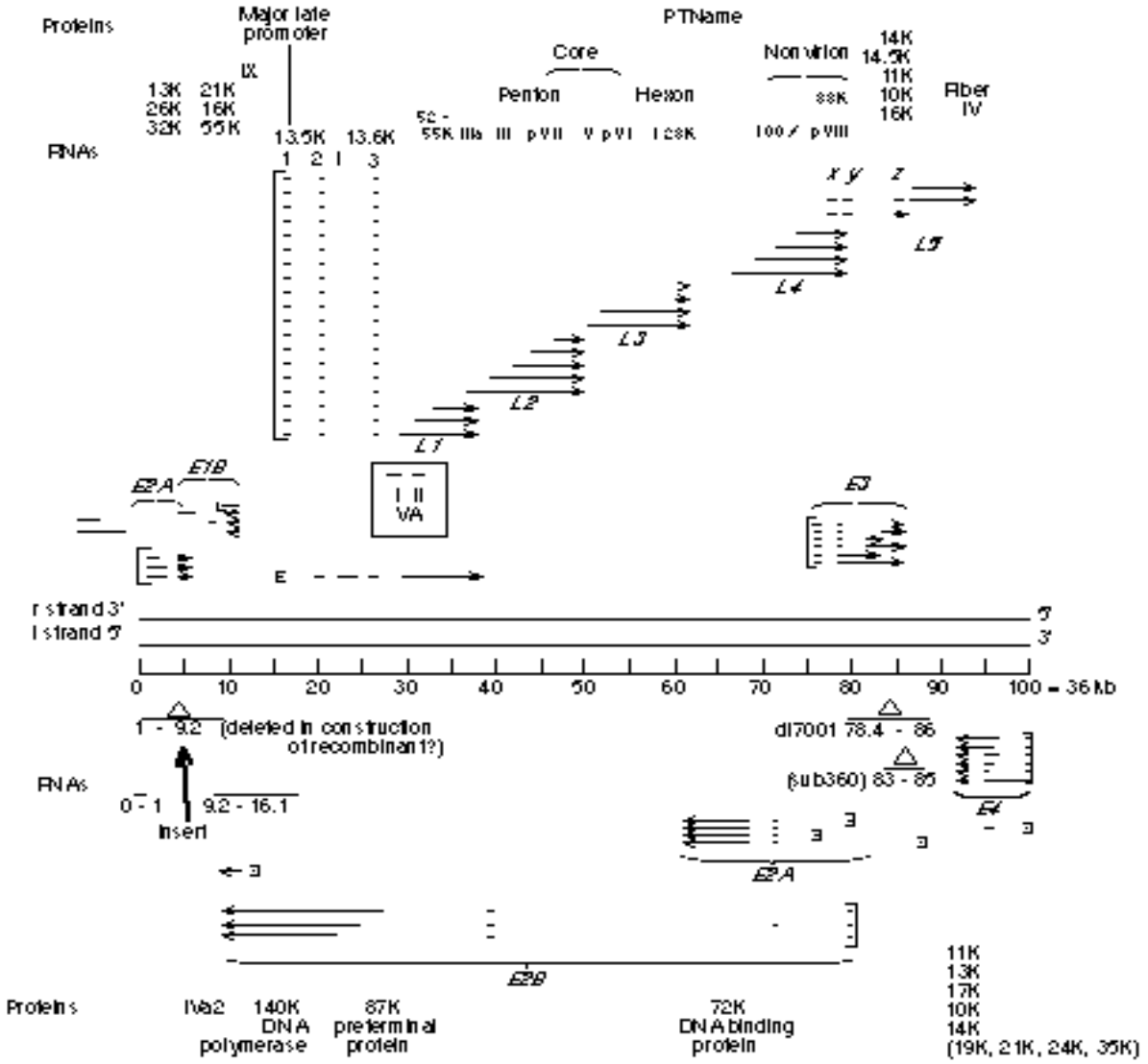
DNA tumor viruses- subvert cellular machinery for replication

Adenovirus:
Early: dedicated to replication of genome.
Triggered by E1A
Need host cell to be in S-phase, and E1A does the job
Uses host cell factors to activate transcription of essential early viral genes.

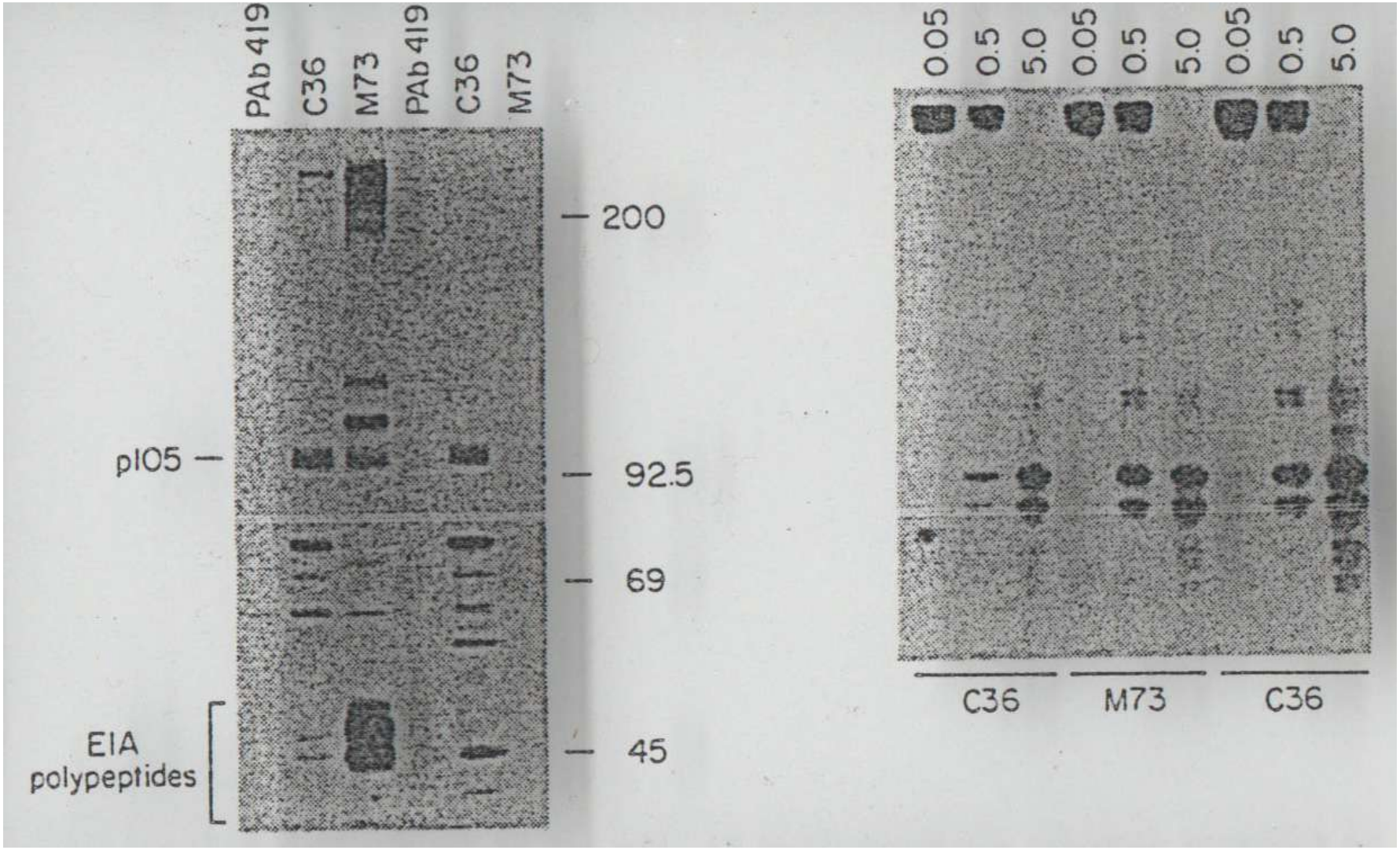
(late: viral capsid/packaging proteins)

Immortalization characterized by increased S-phase entry- overcome a G1 block

Adenovirus genome



How DNA TV cause cancer



What cellular proteins bind E1A and SV40 Large T??
(Harlow, Livingston)

Objective: provide clues into the cellular pathway.

What kinds of proteins co-IP's with E1A?

(anti E1A IP from 35S-cells)

<u>RB Family</u>: p105, p107, p130
<u>Cell Cycle</u>: Cyclin A, CDK2

For E1A and Lg.T:

<u>Model</u>

E1A neutralized RB growth arrest to enhance S-phase.

RB PATHWAY

The Retinoblastoma Family: pRB, p107, p130

Focus mainly on RB

(Merger of virology, genetics, and cell biology)

A. Genetics/Tumor Suppressors

The concept of tumor suppressor protein came from studies of retinoblastomas--tumors of the eye.

Found loss of heterozygosity in a particular position in the chromosome.

When gene was cloned-p105-110

A. highly mutated in retinoblastomas
B. many other tumors have mutations.

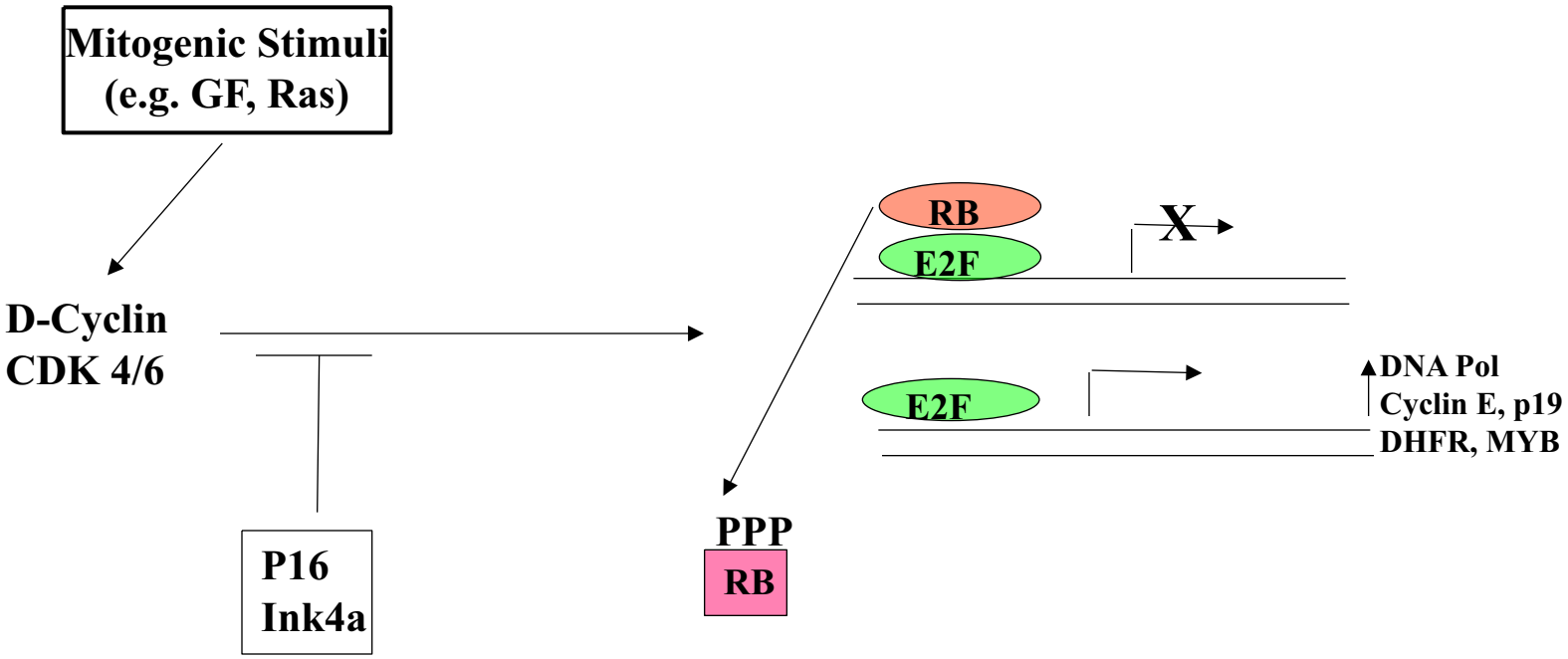
Mutations in tumors: in a pocket region.

Led to the idea that the normal function is the suppression of cell growth.

Over expression leads to suppression of growth.

Nuclear phosphoprotein

pRB Pathway



Tumor Suppressor Genes
RB, p16

Oncogenes
Cyclin D1

From Sharpless and DePinho (1999)
Current Opinions in Genetics and Dev. 9:22

Cancer Fig. 16.13

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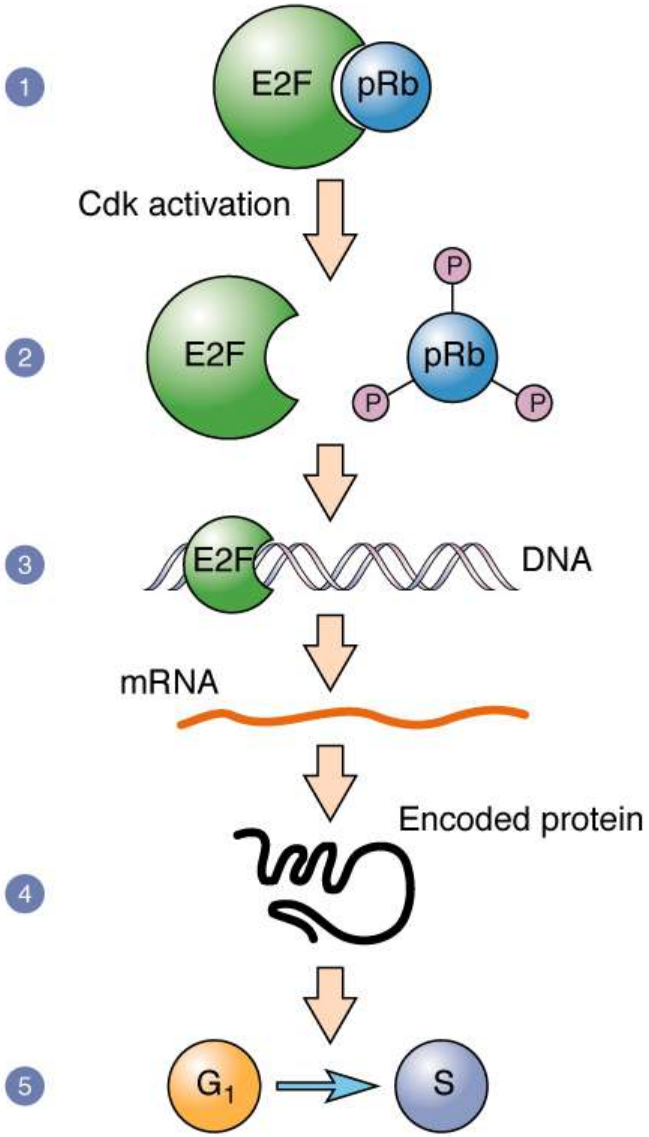

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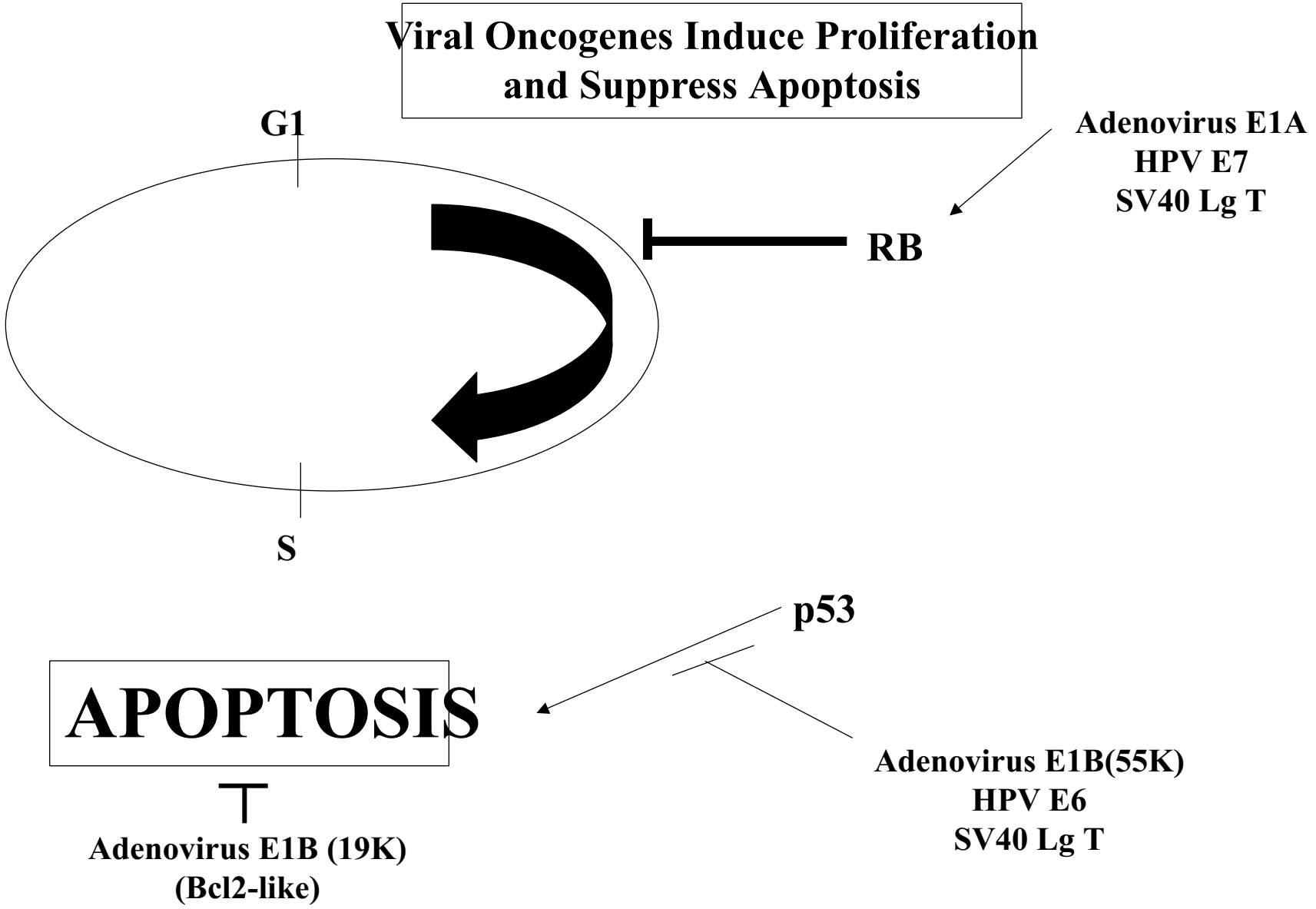

Viral Oncogenes Induce Proliferation
and Suppress Apoptosis
G1
S
Adenovirus E1A
HPV E7
SV40 Lg T
RB
p53
APOPTOSIS
Adenovirus E1B (19K)
(Bcl2-like)
Adenovirus E1B(55K)
HPV E6
SV40 Lg T

Apoptosis Vs Programmed Cell Death

- Apoptosis is a morphological description of dying cells which contrast with necrosis.
- Programmed Cell Death (PCD) is a term originally used to describe cells that die at at predictable time and places during development.
- Since nearly all PCD is apoptotic these terms are sometimes used interchangeably.

Features of Apoptosis Vs Necrosis

1972 Kerr Wyllie Currie

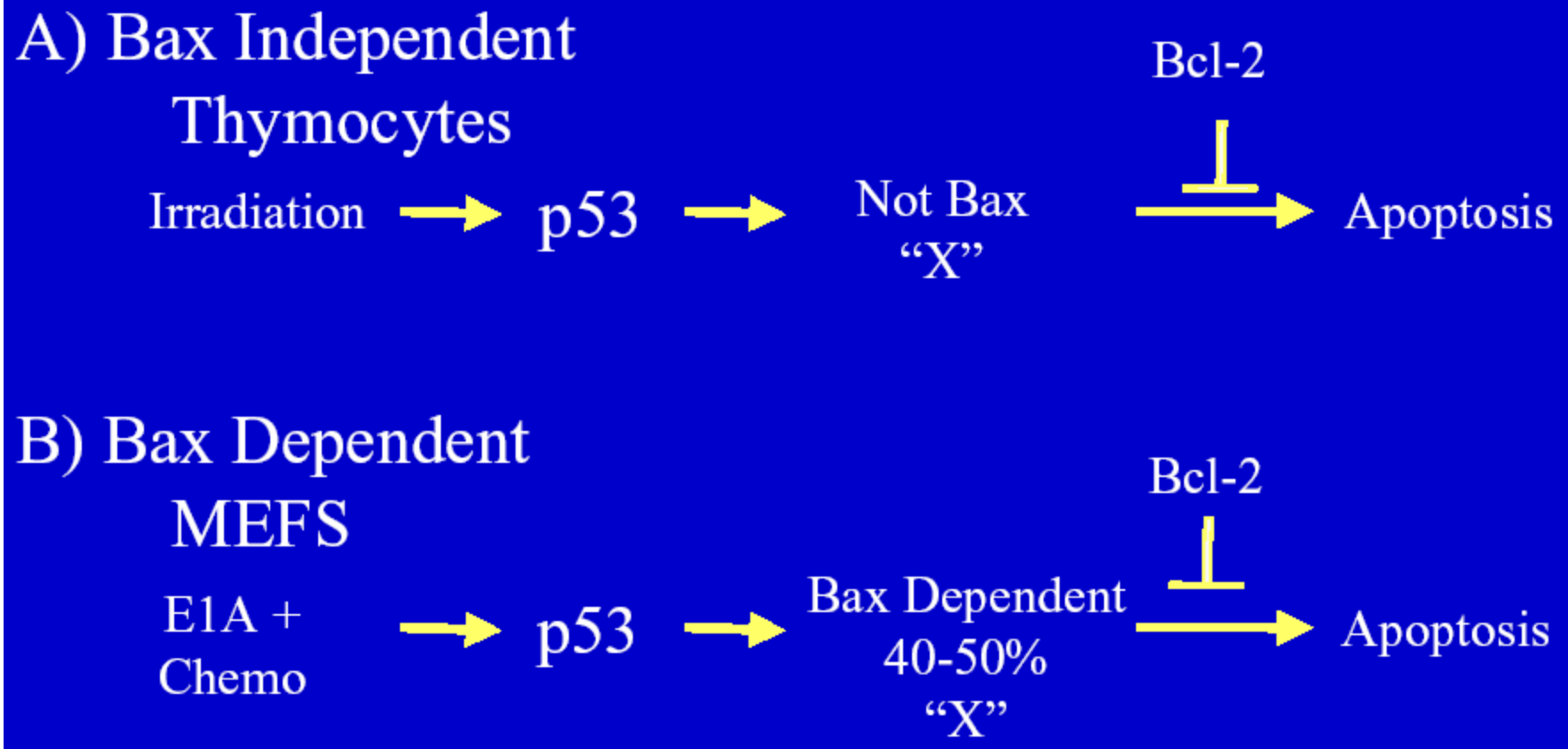
Apoptosis

- Chromatin condensation
- Cell Shrinkage
- Preservation of Organelles and cell membranes
- Rapid engulfment by neighboring cells preventing inflammation

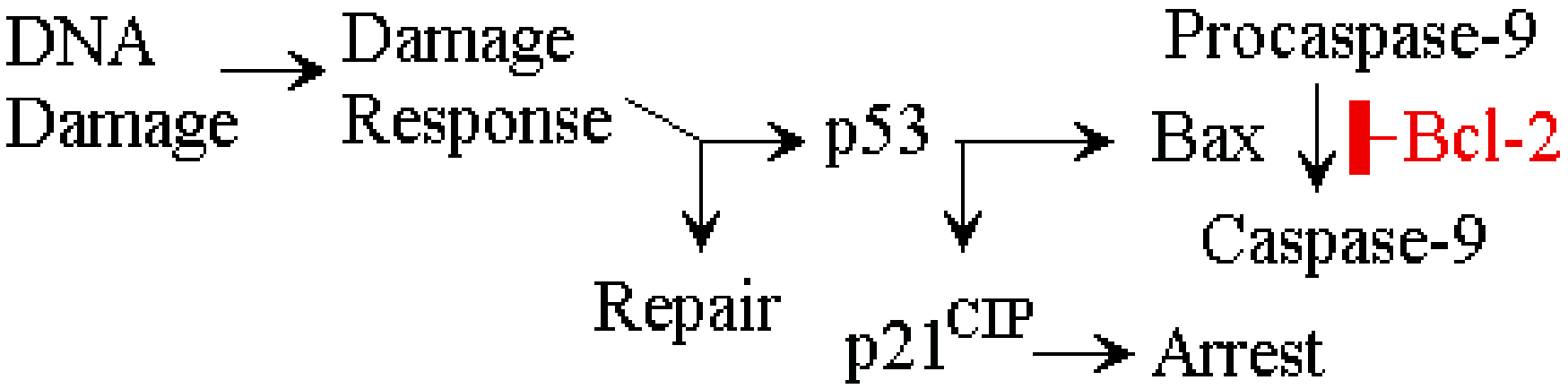
Necrosis

- Nuclear swelling
- Cell Swelling
- Disruption of Organelles
- Rupture of cell and release of cellular contents
- Inflammatory response

2 Pathways for p53 Induced Apoptosis



p53 in apoptosis



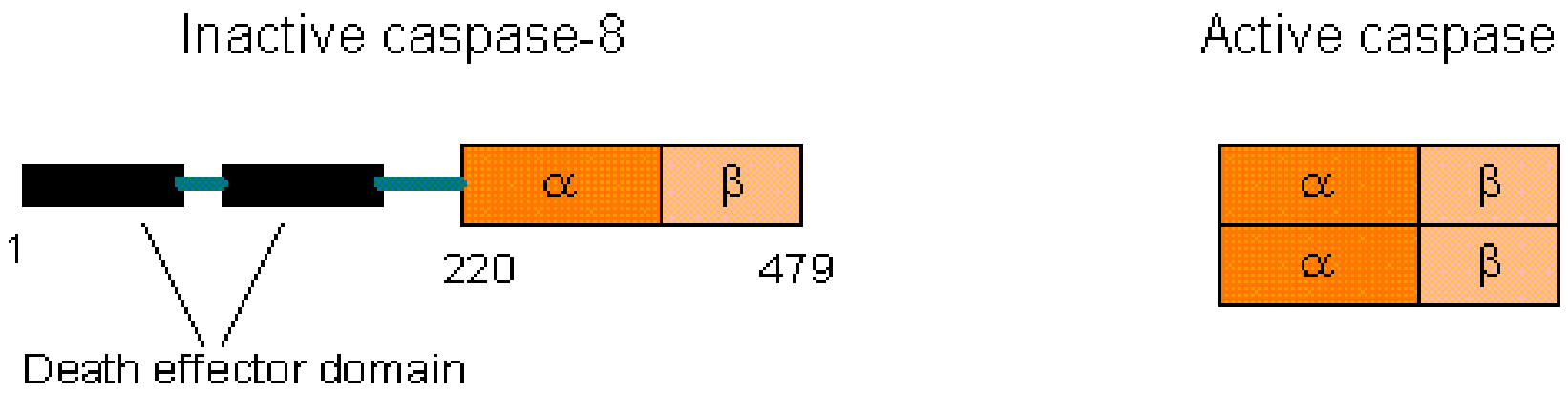
Following DNA damage, e.g. by radiation, p53 levels rise, and proliferating cells arrest in G1. This allows time for DNA repair prior to the next round of replication. This arrest is mediated by stimulation of expression of p21CIP1, the cyclin kinase inhibitor. Very high p53 levels, or susceptible cell types, e.g. lymphocytes, are triggered to undergo apoptosis. Bcl-2 acts between p53 and the caspase:

Apoptosis

- **Apoptosis** is a tightly regulated form of cell death, also called the programmed cell death. Morphologically, it is characterized by chromatin condensation and cell shrinkage in the early stage. Then the nucleus and cytoplasm fragment, forming membrane-bound apoptotic bodies which can be engulfed by phagocytes. In contrast, cells undergo another form of cell death, **necrosis**, swell and rupture. The released intracellular contents can damage surrounding cells and often cause inflammation.

Capsase activation

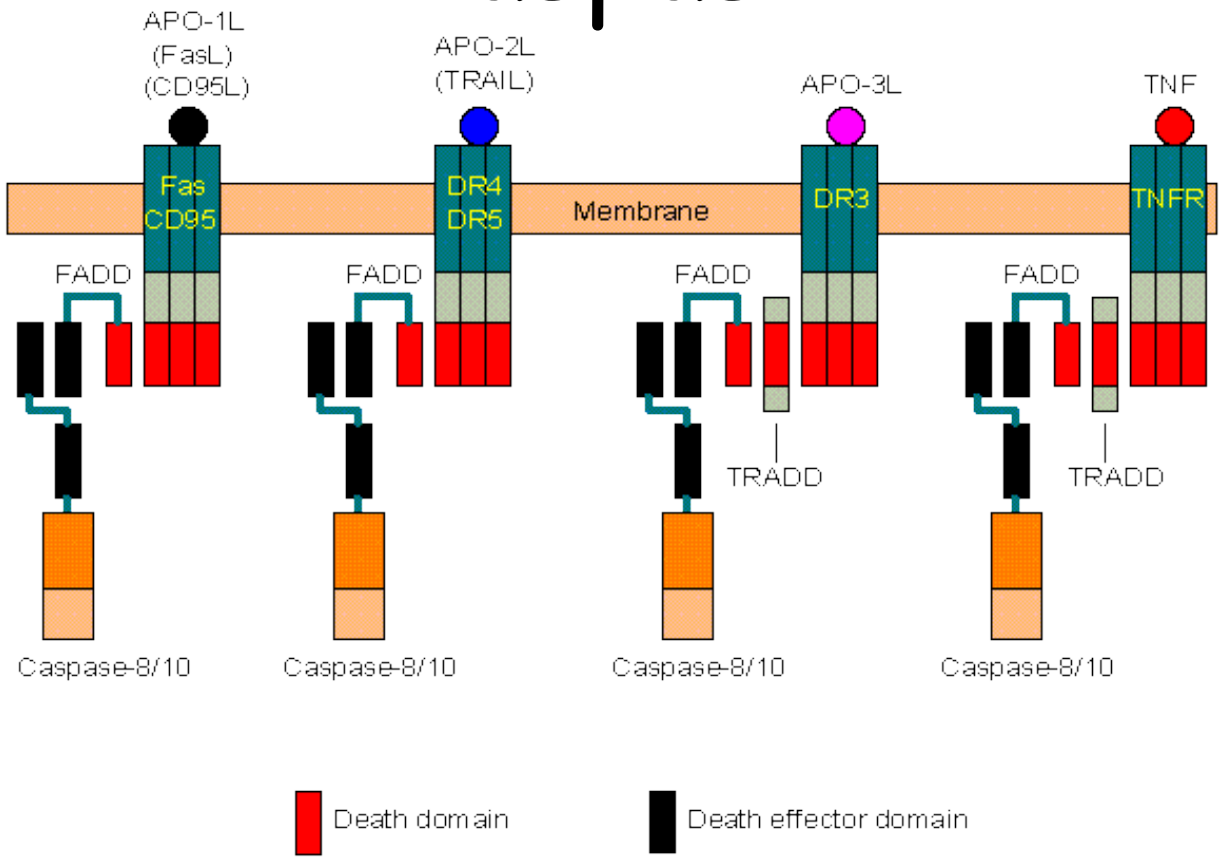
- Comparison between active and inactive forms of caspases. Newly produced caspases are inactive. Specifically cleaved caspases will dimerize and become active.



The role of caspase

- During apoptosis, the cell is killed by a class of proteases called **caspases**. More than 10 caspases have been identified. Some of them (e.g., caspase 8 and 10) are involved in the initiation of apoptosis, others (caspase 3, 6, and 7) execute the death order by destroying essential proteins in the cell. The apoptotic process can be summarized as follows:
- Activation of initiating caspases by specific signals
- Activation of executing caspases by the initiating caspases which can cleave inactive caspases at specific sites.
- Degradation of essential cellular proteins by the executing caspases with their protease activity.

Caspase



- As shown in the above figure, a variety of death ligands (FasL/CD95L, TRAIL, APO-3L and TNF) can induce apoptosis. It is natural to see if they can kill cancer cells without affecting normal cells. TNF was first investigated in the 1980s for cancer therapy, but with disappointing results. Then CD95L (FasL) was tested in the 1990s. The results were still not satisfactory. Recently, TRAIL has been demonstrated to be highly selective for transformed cells, with minimal effects on normal cells. It could be an effective drug for both cancer and AIDS.

Mechanisms: What are the cellular targets?

E1A: CR1 and CR2; In IPs

p105, p107, p130--all pocket proteins

RB protein.

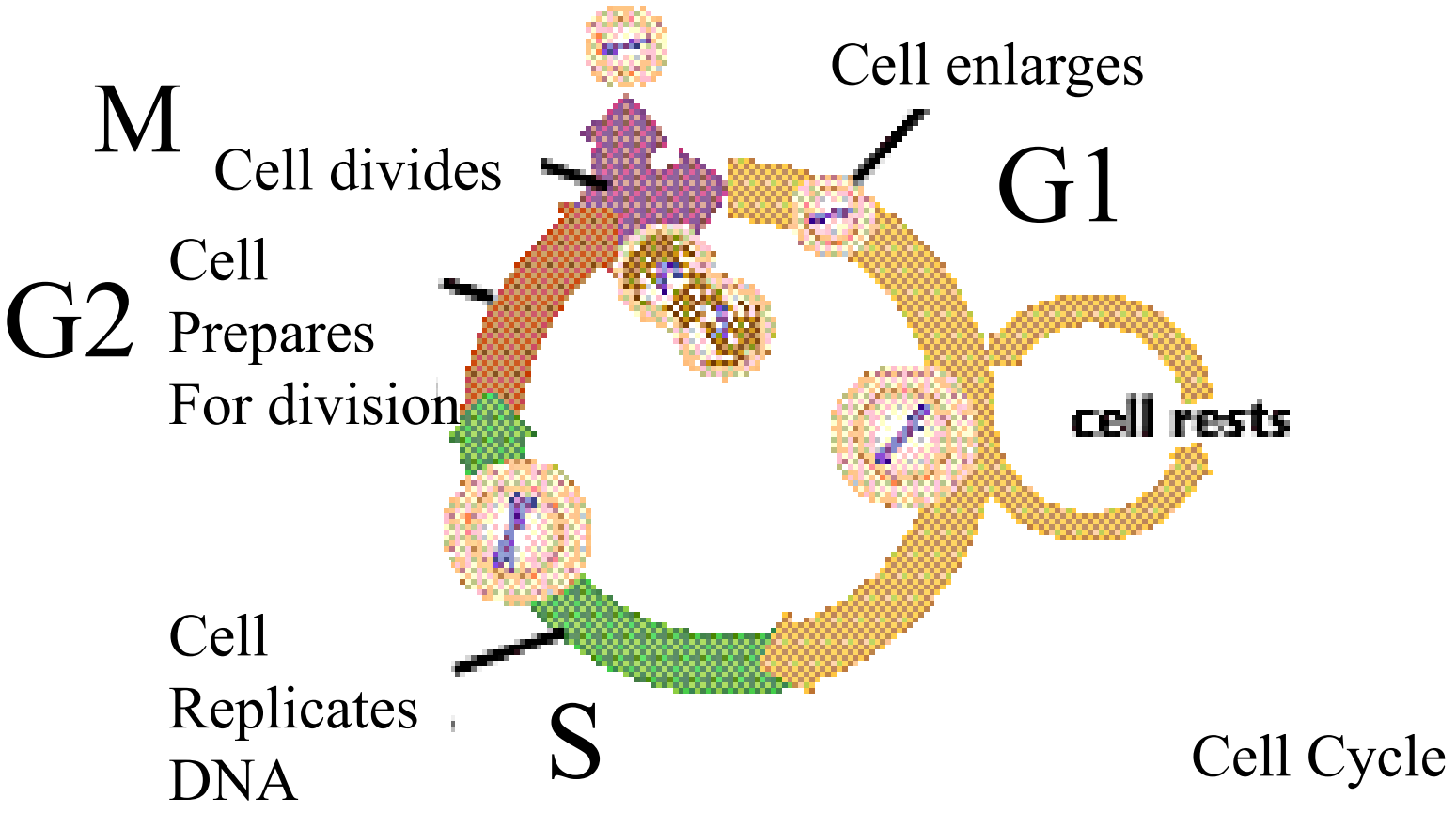
Binds in pocket

Model: neutralization of RB leads to G1/S progression.

(Aside p300 family of co-activators-through CR1; CBP, p300)

P53 as a transcription factor which exerts its effect by regulating other genes

Kinases in checkpoints must be
Activated for cell to proceed through cell cycle

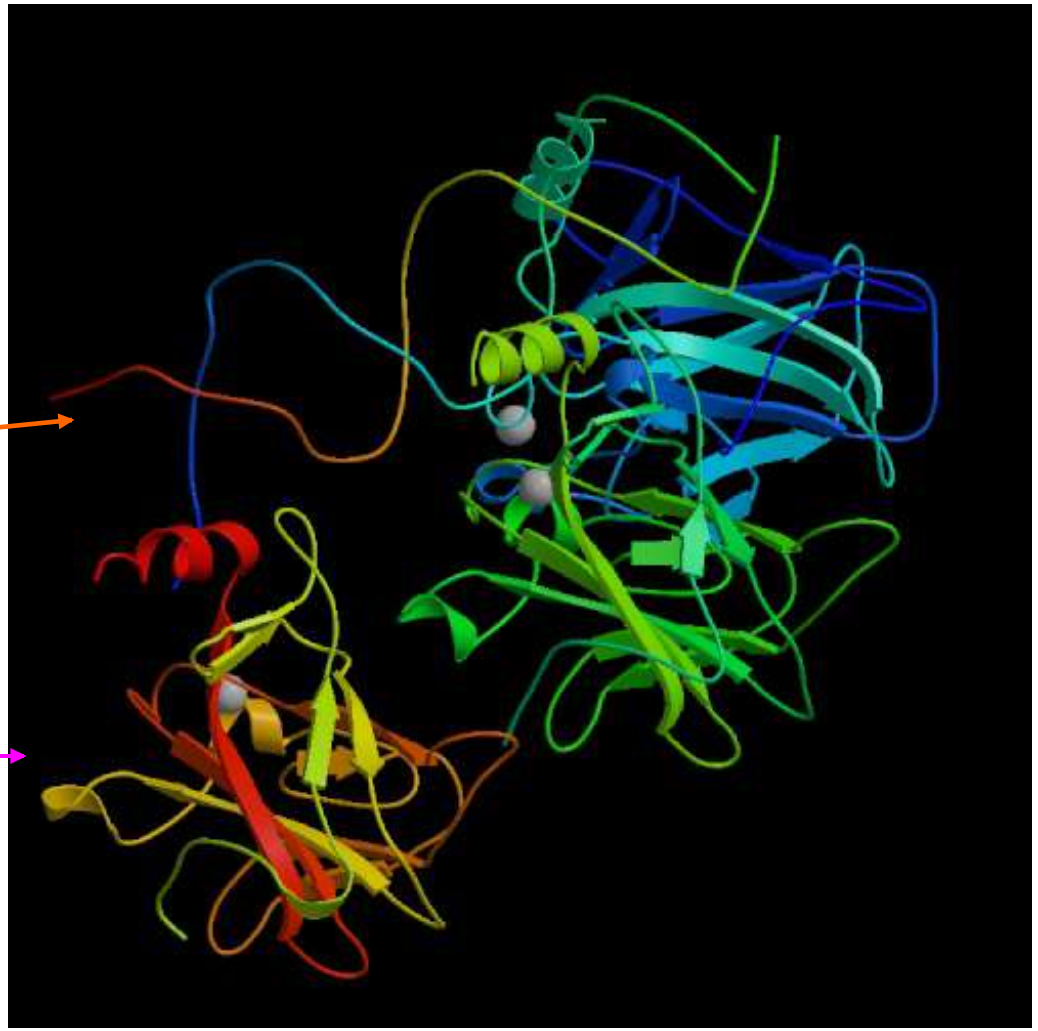


P53 can bind to DNA!

Stabilized by Zn^{2+}.

DNA

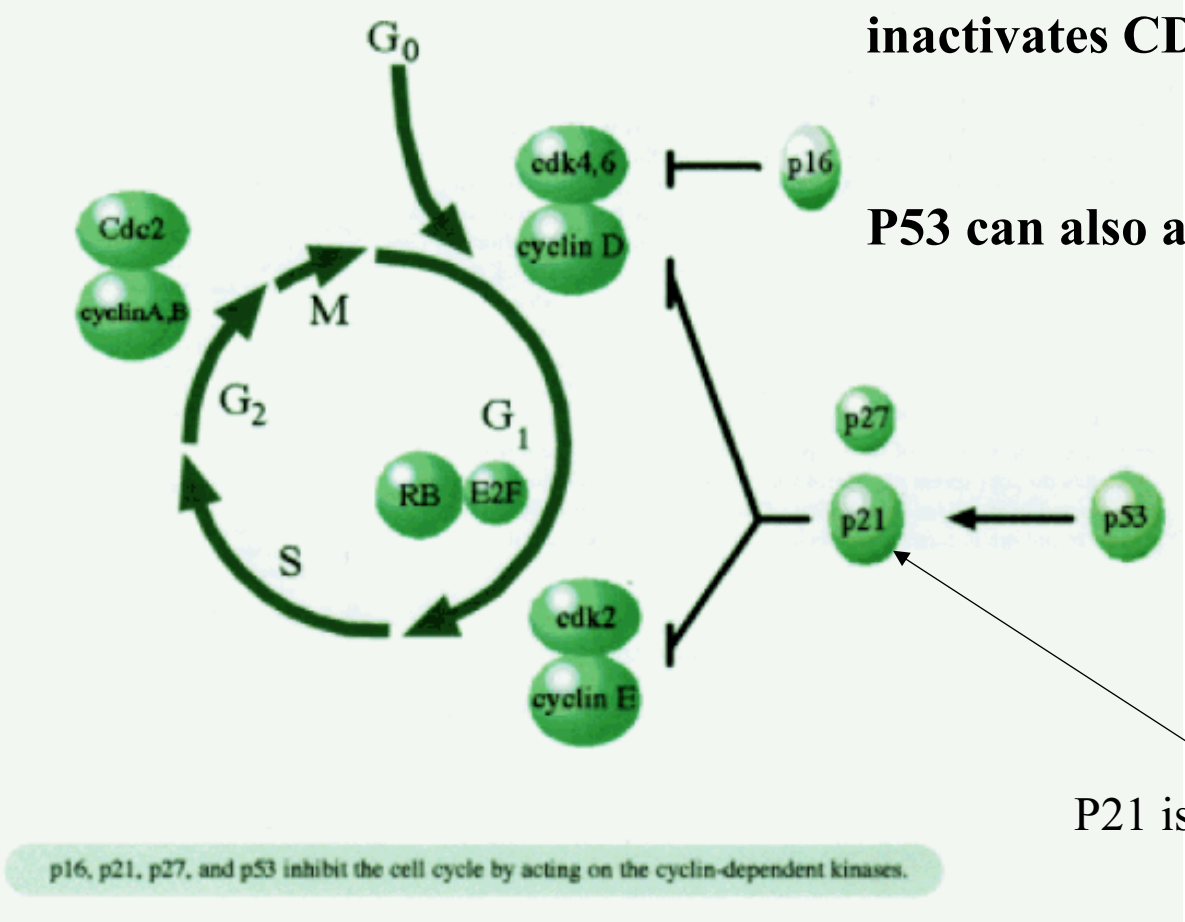
p53



P53 and the cell cycle

P53 arrests the cell cycle primarily by upregulating p21 (Cip1/Waf-1), which inactivates CDK/cyclin

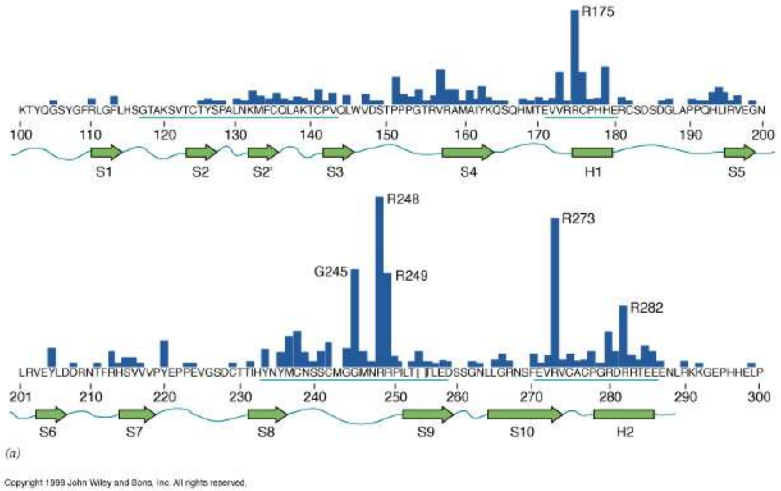
P53 can also activate apoptosis



p16, p21, p27, and p53 inhibit the cell cycle by acting on the cyclin-dependent kinases.

P21 is a kinase inhibitor

Cancer Fig. 16.14
p53 and tumor formation



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- The P53 tumor suppressor gene is the most frequently mutated gene in human cancer

What does p53 do?

- Suppresses tumors in response to DNA by inducing cell cycle arrest or apoptosis

How can you inhibit gene expression?

How is p53 Activated?

- Regulation of p53 by MDM2

P53 tumor suppressor protein can be stabilized and activated by two separate mechanisms in response to DNA-damage-induced phosphorylation.

2) p53 nuclear export is inhibited, to ensure that it is activated in response to DNA damage.

Mouse double minute 2

- The mdm2 gene encodes a zinc finger protein that negatively regulates p53 function by binding and masking the p53 transcriptional activation domain. Two different promoters control expression of mdm2, one of which is also transactivated by p53.

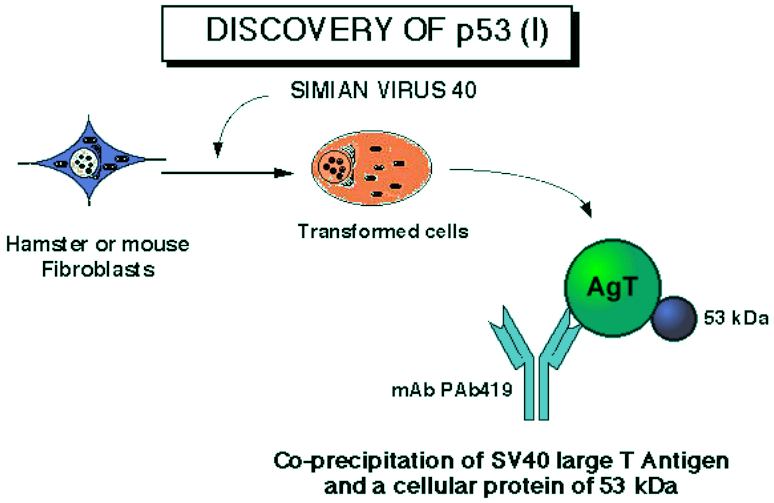
- What does negative regulation mean? MDM2 protein inhibits p53 activity during normal cell growth.

- How: Inhibits p53 transcriptional activity
- Targets p53 for ubiquitylation and degradation.

- This inhibition is inhibited by p53 is phosphorylated.

- MDM2 has been shown to be overexpressed in sarcomas and more recently was implicated in the pathogenesis of carcinomas.

The discovery of p53

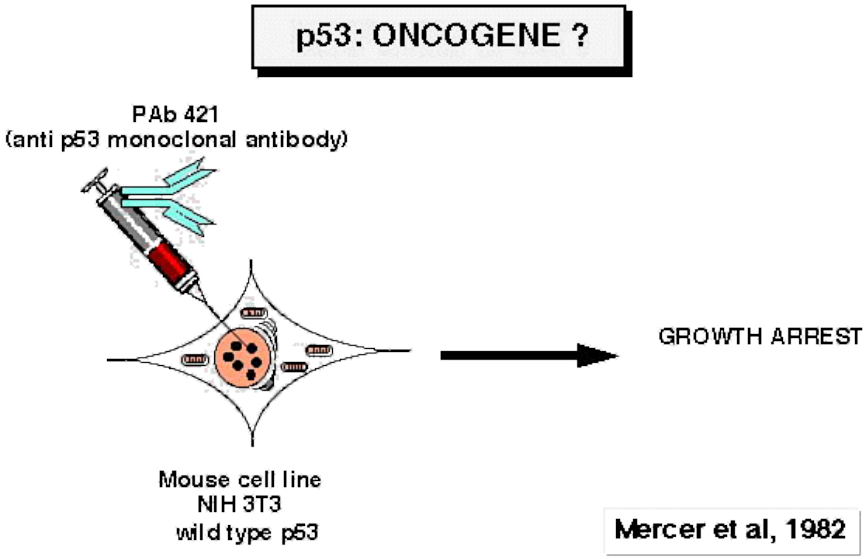


Co-precipitation of SV40 large T Antigen and a cellular protein of 53 kDa

- **Studies of SV40-transformed cells show that a 55-kDa protein is coprecipitated with the large-T antigen (Chang et al. 1979; Kress et al. 1979; Lane and Crawford 1979; Linzer and Levine 1979; Melero et al. 1979). This association was shown to be the result of an in vivo association between the two proteins (Lane and Crawford 1979). It was then postulated that this protein could be encoded by the cellular genome. (It should be kept in mind that no middle-T was found for SV40 and that the molecular weight of this protein was similar to that of polyoma middle-T antigen). Linzer and Levine (Linzer and Levine 1979) found that the 54-kDa protein was overexpressed in a wide variety of murine SV40 transformed cells, but also in uninfected embryonic carcinoma cells. A partial peptide map from this 54-kDa protein was identical among the different cell lines, but was clearly different from the peptide map of SV40 large-T antigen (Kress et al. 1979; Linzer and Levine 1979). It was then postulated that SV40 infection or transformation of mouse cells stimulates the synthesis or stability of a cellular 54-kDa protein.**

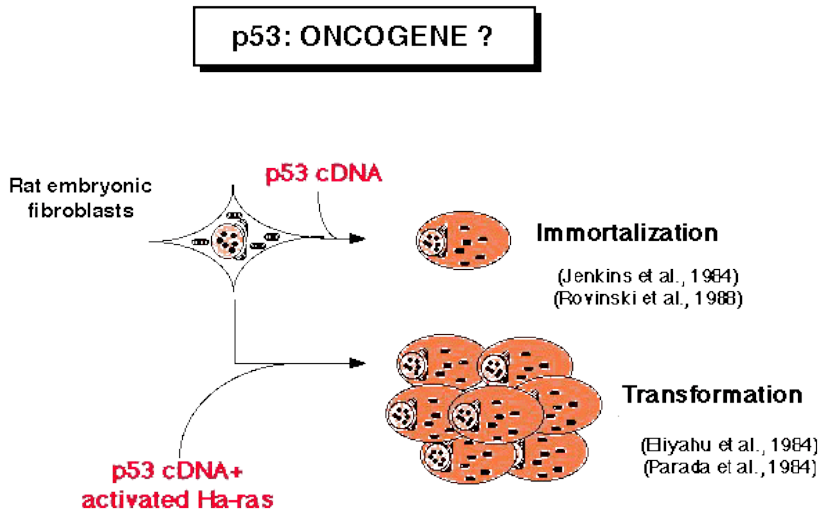
p53 as a positive cell regulator

An oncogene?



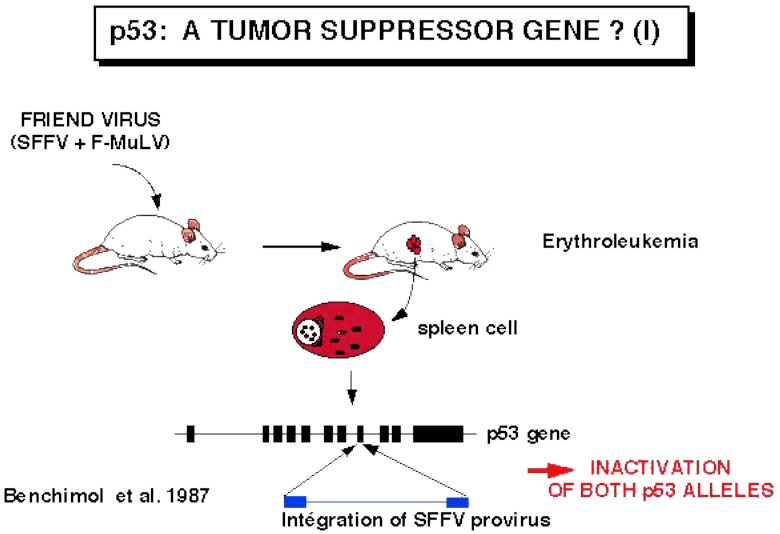
- **Early work on p53 suggested that it may be implicated in the promotion of cell proliferation. Earlier experiments by Reich and Levine (Reich and Levine 1984) showed that mouse 3T3 cell growth, when arrested by serum deprivation, exhibited very low levels of p53 mRNA and protein. When the cell was induced to grow by serum stimulation, the level of p53 mRNA and the rate of p53 protein synthesis increased markedly, reaching a peak near the G1/S boundary just prior to initiation of DNA replication (Reich and Levine 1984). Similar experiments performed with normal resting T lymphocytes (Milner and McCornick 1980) and normal diploid fibroblasts (Mercer et al. 1984) showed that p53 expression is always concomitant with induction of cell growth. The level of p53 mRNA and protein is somewhat constant throughout the cell cycle when the cells are growing exponentially(Calabretta et al. 1986).**
- **This observation, added to other characteristics of the p53 protein (short half life, nuclear localization), led to the notion that wild type p53 could play a positive role in cell proliferation. This idea was strengthed by the work of Mercer and collaborators (Mercer et al. 1984; Mercer et al. 1982). Microinjection of p53 antibody (200.47 and PAb122) into the nucleus of quiescent Swiss 3T3 mouse cells inhibited the subsequent entry of the cell into the S phase after serum stimulation. This inhibition was effective only when microinjection was performed at or around the time of growth stimulation, suggesting that p53 is critical for G0/G1 transition (Mercer et al. 1984; Mercer et al. 1982). Recently, similar results were obtained using methylcholanthrene-transformed mouse cells which express mutant p53 (Deppert et al. 1990; Steinmeyer et al. 1990). Also consistent with these results is an antisense experiment which showed that inhibition of p53 expression prevented cell proliferation in both non-transformed NIH3T3 cells and transformed cells (Shohat et al. 1987). All of these observations led to the notion that wild type p53 is a positive regulator of cell proliferation.**

53 cooperate with Ha-ras



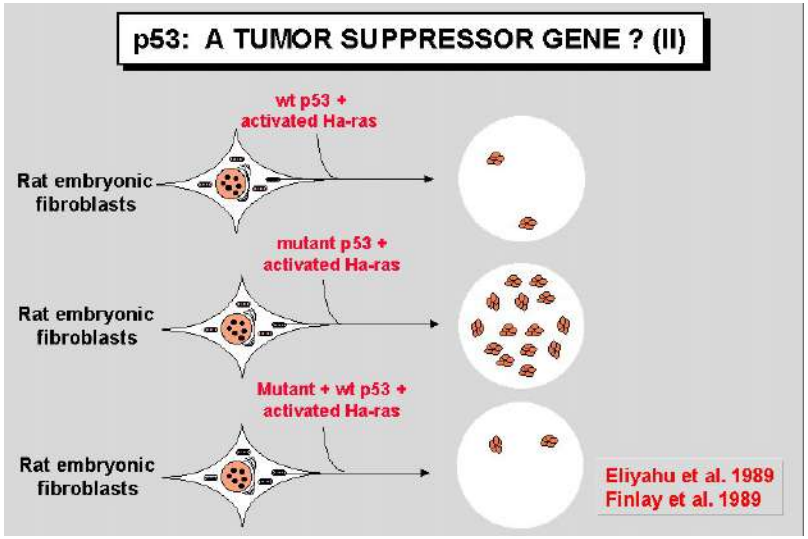
- **In 1984 two groups reported that cotransfection of murine p53 with plasmids encoding an activated c-Ha-ras oncogene could transform REF cells in a manner similar to that observed with proto-oncogenes such as myc or E1A (Eliyahu et al. 1984; Jenkins et al. 1984; Parada et al. 1984). These observations resulted in the classification of p53 as a nuclear dominant oncogene. A third group, demonstrate that murine p53 could imortalized normal rat chondrocytes leading to cells sensitive to ras transformation (Jenkins et al. 1985; Jenkins et al. 1984).**

Inactivation of p53 in Friend murine erythroleukemia



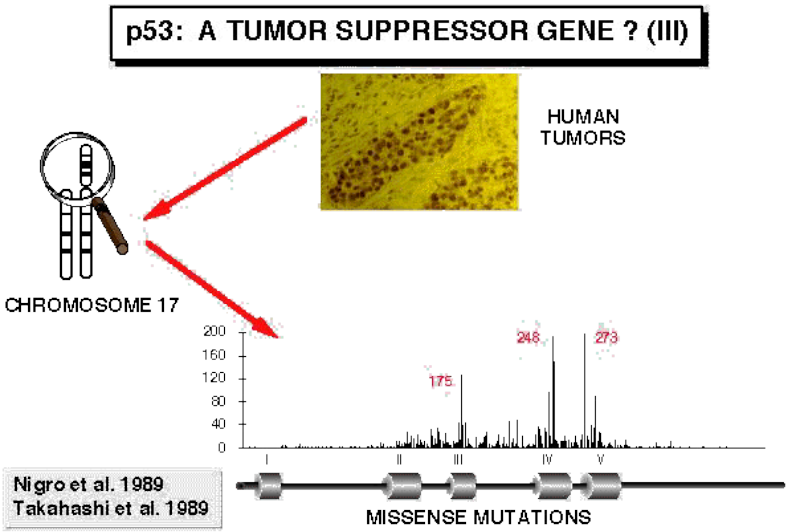
- **In these tumors induced by the Friend virus, the p53 gene found in the tumor cells is very often rearranged, leading to an absence of expression or the synthesis of a truncated or mutant protein (Mowat et al. 1985) The mutation often affects one of the conserved blocks of the protein (Munroe et al. 1988). In all cases studied, the second allele is either lost through loss of the chromosome, or inactived by deletion. In this tumor model, functional inactivation of the p53 gene seems to confer a selective growth advantage to erythroid cells during the development of Friend leukemia in vivo.**

Wild type p53 has antiproliferative properties and does not cooperate with Ha-ras



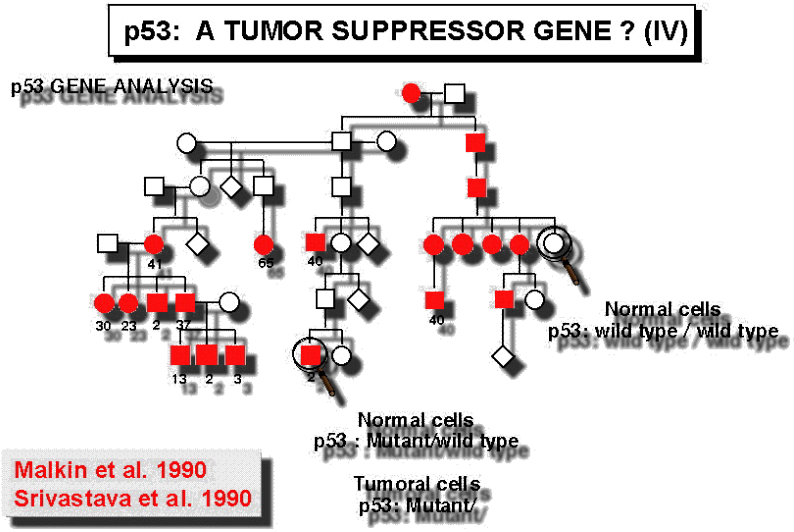
- **A new set of experiments has shown that cotransfection of a plasmid encoding wild type p53 reduced the transformation potential of plasmids encoding p53 and an activated Ha-ras gene (Eliyahu et al. 1989; Finlay et al. 1989). Furthermore, wild type p53 was shown to suppress transformation by a mixture of E1A or myc and an activated Ha-ras gene. These transformation experiments indicate that wild type p53 is a suppressor of cell transformation in vitro.**

p53 gene is mutated in a wide variety of human cancer



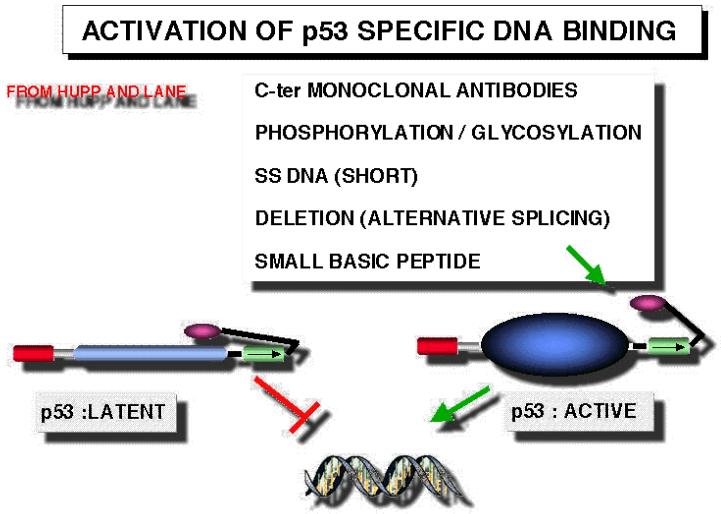
- **The expression of p53 in different human cancers or in tumor cell lines has long been under study by several different investigators. This expression is often high, but no precise explanations exist for this phenomenon because apart from the case of several osteosarcomas, no gene rearrangements, detectable by Southern blotting, have been detected. Genetic analysis of colorectal cancer reveals a very high rate of heterozygous loss of the short arm of chromosome 17, which carries the p53 gene (Vogelstein et al. 1988). PCR analysis and sequencing of the remaining p53 allele shows that it often contains a point mutation (Baker et al. 1989). Similar observations have been made in the case of lung cancer (Takahashi et al. 1989). On the heels of these initial observations have come several hundred reports of alterations of the p53 gene in all types of human cancer (see below). In many cases these mutations are accompanied by a heterozygous loss of the short arm of chromosome 17**

Germline mutation of the p53 gene are found in Li-Fraumeni patients



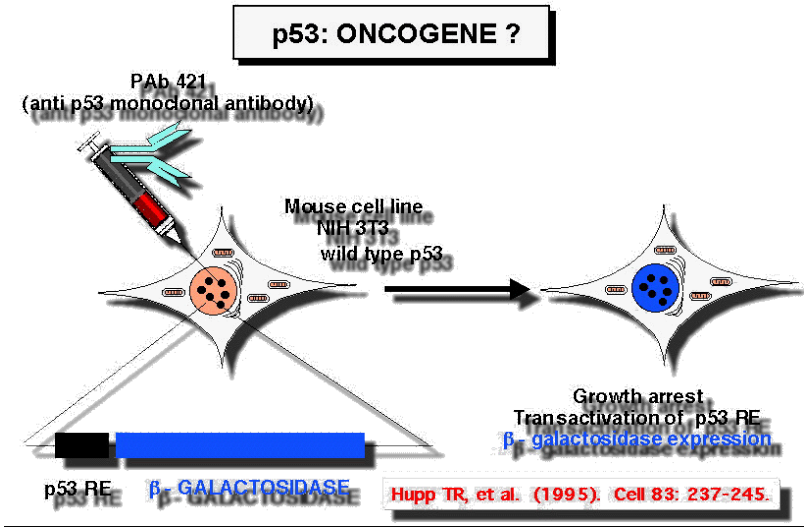
- **Transgenic mice carrying a mutant p53 gene develop many types of cancer, with a high proportion of sarcomas (Lavigueur et al. 1989). This observation led various authors to study patients with Li-Fraumeni syndrome. This syndrome presents as a familial association of a broad spectrum of cancers including osteosarcomas, breast cancer, soft tissue sarcoma and leukemias, appearing at a very early age. Statistical analysis predicts that 50 % of these individuals will have a tumor before the age of 30, and 90 % before the age of 70. Germ-line mutations in the p53 gene have been found in several families with this syndrome (Malkin et al. 1990; Srivastava et al. 1990). In all cases there is a strict correlation between transmission of the mutant allele and development of a cancer.**

Why p53 micro-injection of monoclonal antibodies induces a growth arrest ?



- The carboxy-terminus of Hp53 has been shown to play an important role in controlling the specific DNA binding function. Wils type p53 is found in a latent form whicvh does not bind to DNA. The specific DNA binding activity was shown to be activated by various pathways: phosphorylation (Hupp et al., 1992), antibody specific for the carboxy-terminus of the protein (Hupp et al., 1992), small peptides which could mimic the carboxy-terminus of the p53 (Hupp et al., 1995), short single stranded DNA (Jayaraman & Prives, 1995), deletion of the last 30 amino-acids (Hupp et al., 1992) and the interaction with a cellular protein (Jayaraman et al., 1997).
- This observation suggest that micro-injection of antibodies such as PAb421 induces an activation of the transcriptional activity of p53. Such hypothesis have been confirmed (Hupp et al., 1995)

Wild type p53 as a tumor suppressor gene and mutant p53 as a dominant oncogene ?



- Taken together, these data made it possible to define the p53 gene as a tumor suppressor gene. Yet unlike the Rb gene, which is the archetype of the tumor suppressor genes, the p53 gene has some original features. In particular, more than 95 % of alterations in the p53 gene are point mutations that produce a mutant protein, which in all cases has lost its transactivational activity (see above). Nevertheless, the synthesis of these mutant p53 proteins is not harmless for the cell. In paticular, it has been shown that some p53 mutants (depending on the site of mutation) exhibit a transdominant phenotype and are able to associate with wild-type p53 (expressed by the remaining wild-type allele) to induce the formation of an inactive heteroligomer (Milner and Medcalf 1991). Moreover, cotransfection of mutant p53 with an activated ras gene shows that some p53 mutants have high, dominant oncogenic activity (Halevy et al. 1990). These observations led to the proposal that several classes of mutant p53 exist, according to the site of mutation and its phenotype (Michalovitz et al. 1991): i) null mutations with totally inactive p53 that do not directly intervene in transformation; ii) dominant negative mutations with a totally inactive p53 that is still able to interfere with wild-type p53 expressed from the wild-type allele, and iii) positive dominant mutations where the normal function of p53 is altered but in this case the mutant p53 acquires an oncogenic activity that is directly involved in transformation.

Suppression of Oncogene

To suppress oncogene expression:

•(1) transcriptional level: deliver a transcriptional repressor acting on the promoter of oncogene, e.g. adenovirus E1A gene products can repress the neu promoter or truncation protein of SV40 Large T antigen

•(2) post-transcriptional level: deliver ribozyme, antisense, dominant negative molecule. e. g. ribozyme for activated ras (point mutation)

FEBS Letters Volume 493 30 March 2001 Nuclear and mitochondrial apoptotic pathways of p53 Ute M. Moll, and Alex Zaika

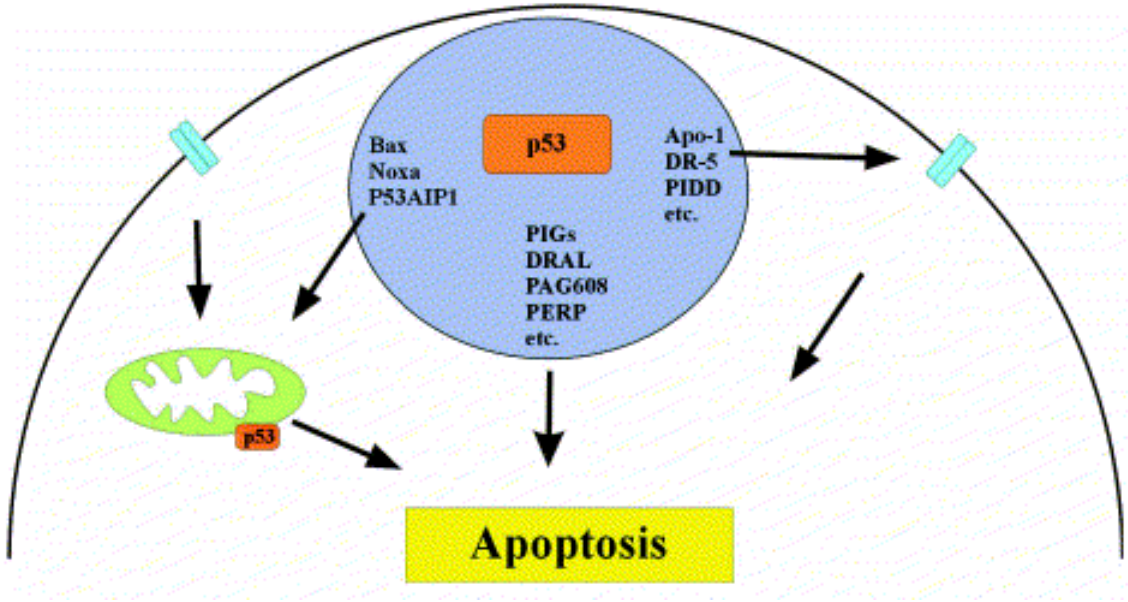
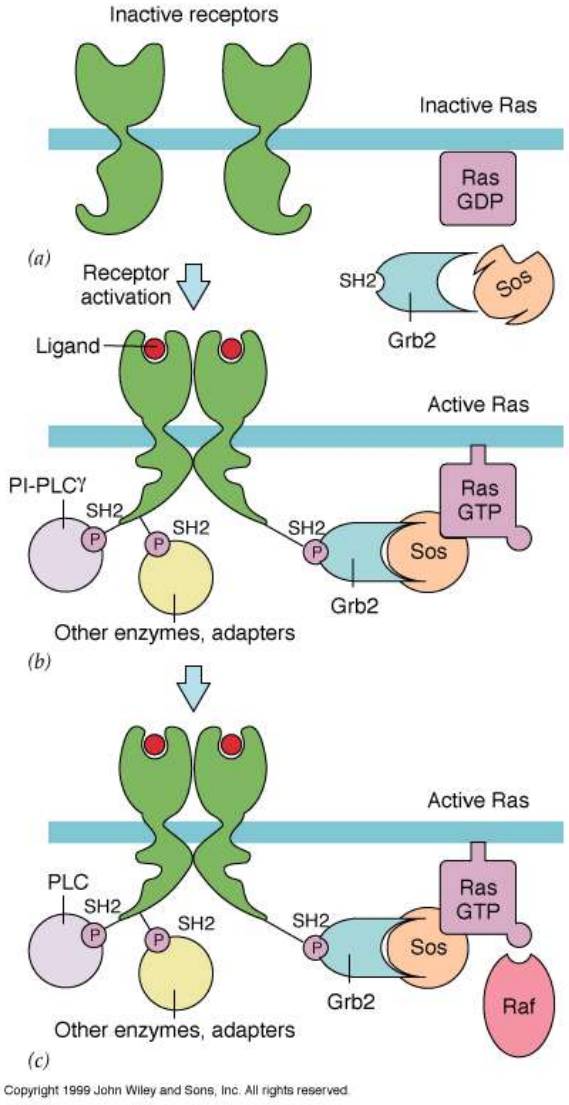


Fig. 1. Transcriptionally dependent and independent mechanisms of p53-mediated apoptosis both activate the mitochondrial pathway of cell death. Alterations in mitochondrial membrane potential, mitochondrial ROS production and/or cytochrome *c* release can result from p53-mediated transcriptional activation of mitochondrial proteins such as Noxa, p53 AIP1 and Bax. Moreover, the rapid translocation of p53 protein directly to mitochondria occurs in a broad spectrum of cell types and death signals and enhances the apoptotic potency of p53. In addition, p53 can enlist a multitude of other p53-induced effector genes. p53 can activate the death receptor pathway via death receptor target genes (DR-5, Apo-1) or

death-domain-containing proteins (PIDD). Other target genes operate through unknown apoptotic pathways (PIGs, DRAL, PAG608, PERP).

Steps in the activation of Ras by RTKs.

Fig. 15.24



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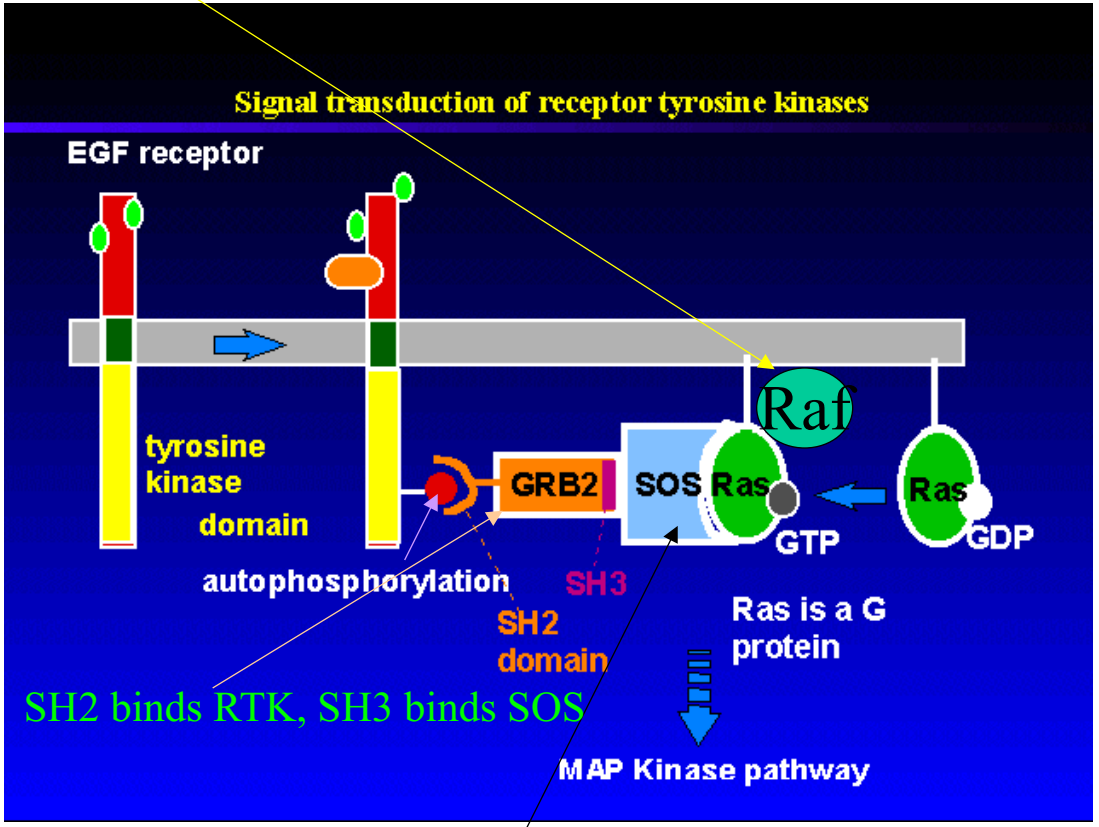
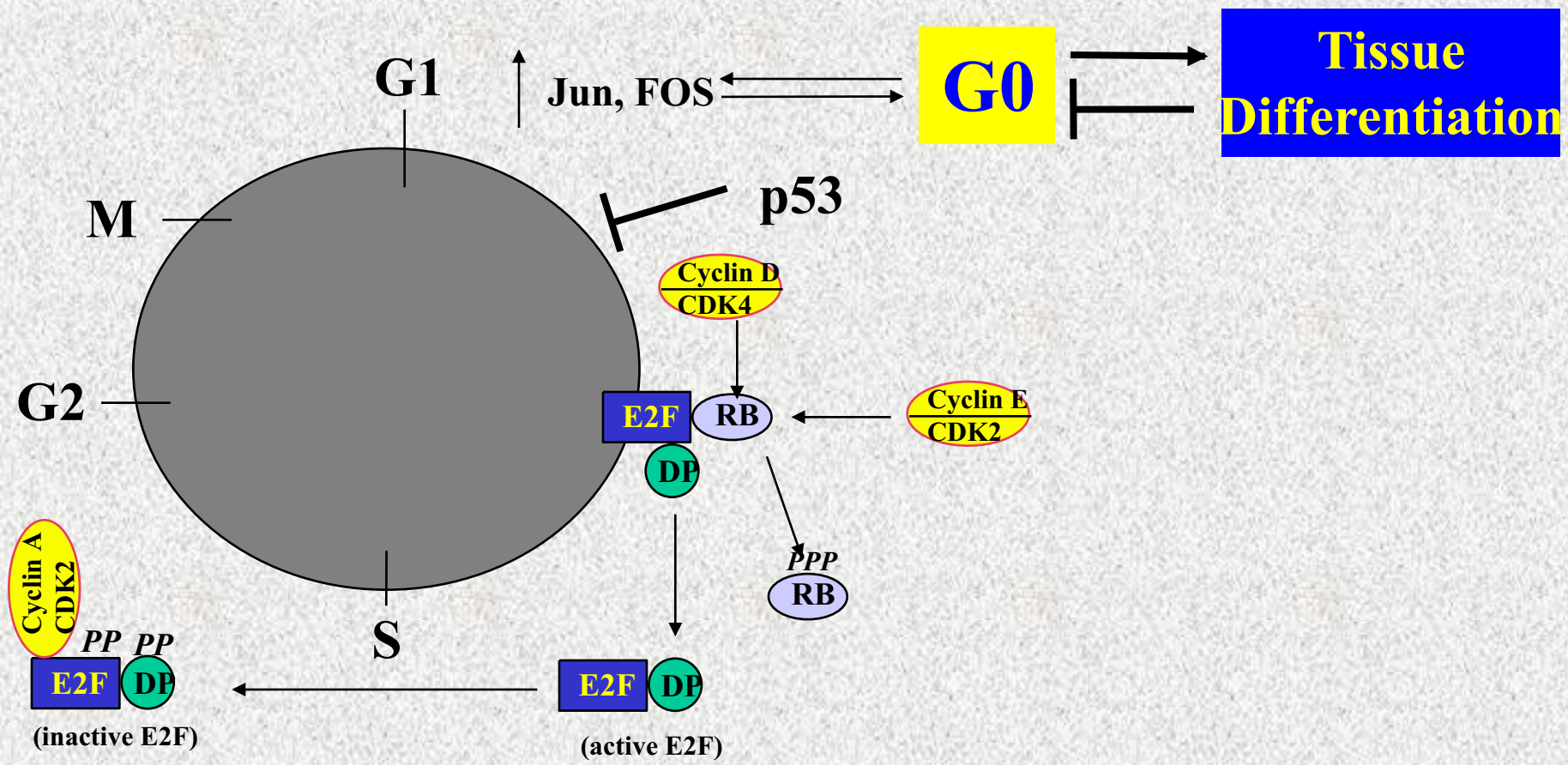
Raf is a PK that triggers MAP-K pathway



c-fos, c-jun
Cell proliferation

Ras-GEF


G1
Jun, FOS
G0
Tissue
Differentiation
M
p53
Cyclin D
CDK4
G2
E2F
RB
Cyclin E
CDK2
DP
PPP
RB
Cyclin A
CDK2
PP PP
E2F
DP
S
E2F
DP
(inactive E2F)
(active E2F)