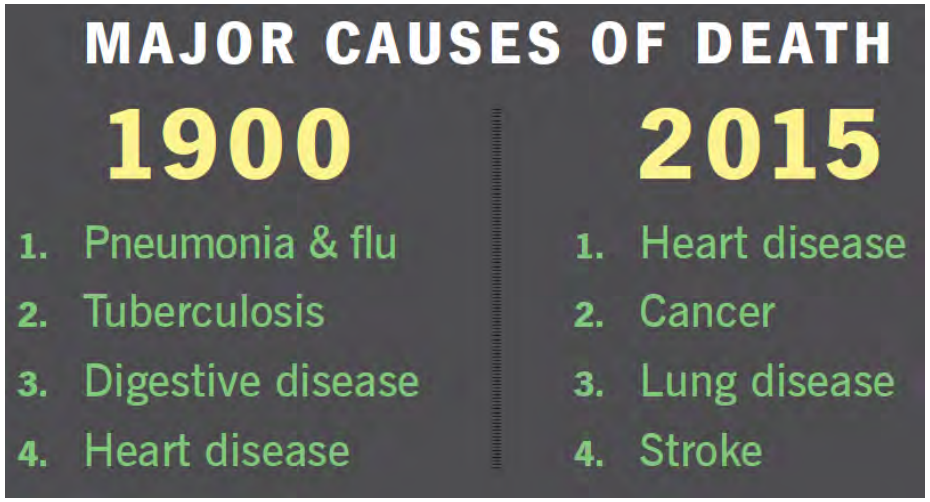
Leading causes of death, United States 2014

- Heart disease: 614,348
- Cancer: 591,699
- Chronic lower respiratory diseases: 147,101
- Accidents (unintentional injuries): 136,053
- Stroke (cerebrovascular diseases): 133,103
- Alzheimer's disease: 93,541
- Diabetes: 76,488
- Influenza and pneumonia: 55,227
- Nephritis, nephrotic syndrome, and nephrosis: 48,146
- Intentional self-harm (suicide): 42,773

http://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm



https://health.clevelandclinic.org

Some terminology

- **Neoplasm**: abnormal mass that exceeds normal bounds and is uncoordinated with normal tissue

- Neoplasm**~tumor**

- **Cancer**: malignant tumor (versus benign tumor)

- **Benign**: "..oma"
  - Lipoma
  - Fibroma
  - Angioma
  - Adenoma (gland)
  - Papilloma (epithelial surface)

- **Malignant**
  - **Sarcoma** (mesenchymal): liposarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma
  - **Carcinoma** (epithelial): adenocarcinoma, papillary carcinoma

Table 6–1. Nomenclature of Tumors

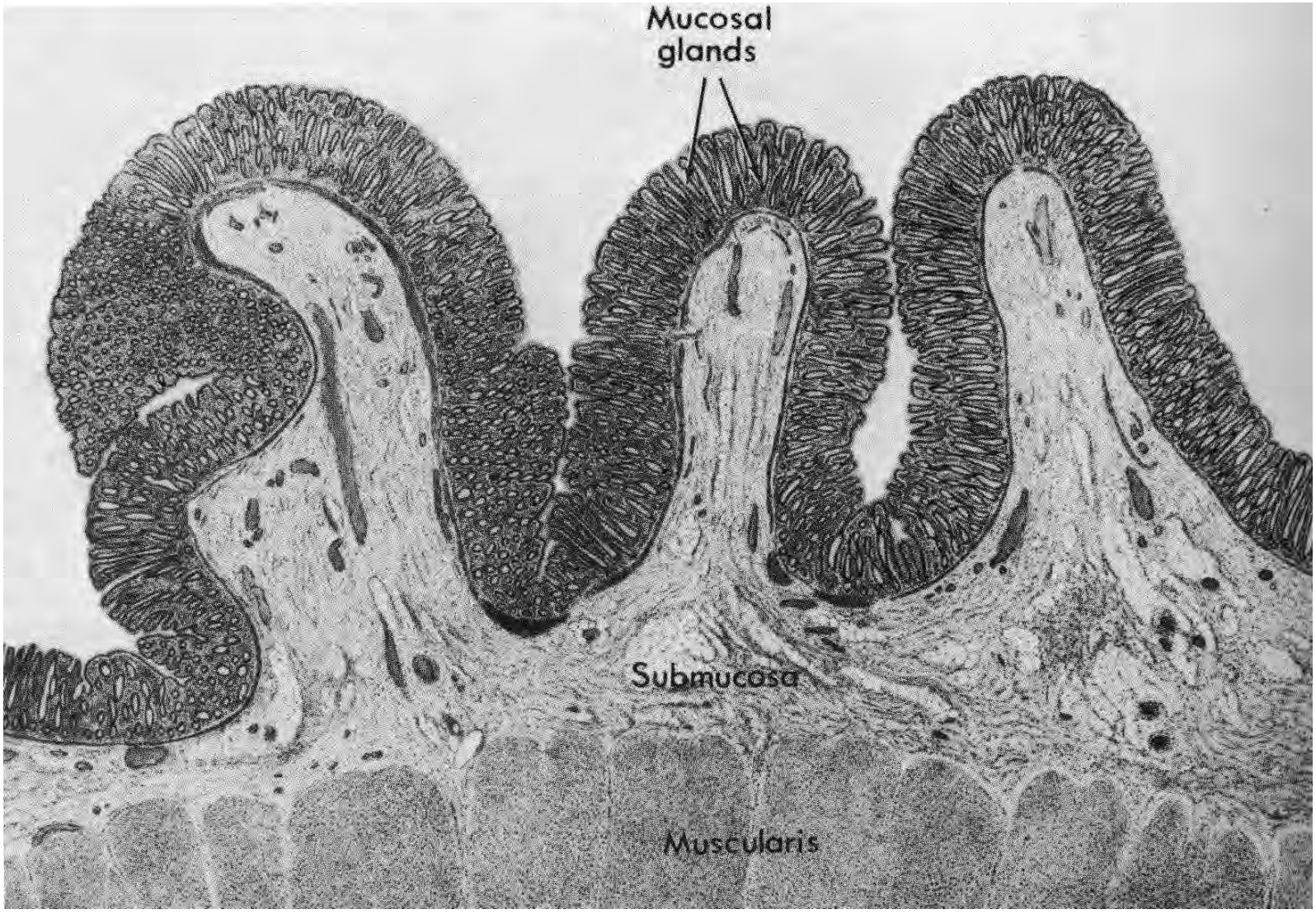
TISSUE OF ORIGIN	BENIGN	MALIGNANT
I. Composed of one parenchymal cell type		
A. Tumors of mesenchymal origin		***Sarcomas***
(1) Connective tissue and derivatives	Fibroma	Fibrosarcoma
	Myxoma	Myxosarcoma
	Lipoma	Liposarcoma
	Chondroma	Chondrosarcoma
	Osteoma	Osteogenic sarcoma
(2) Endothelial and related tissues		
Blood vessels	Hemangioma	Angiosarcoma
	Capillary	
	Cavernous	
Lymph vessels	Lymphangioma	Lymphangiosarcoma
Synovia		Synovioma (synoviosarcoma)
Mesothelium (lining cells of body cavities)		Mesothelioma
Brain coverings	Meningioma	Invasive meningioma
Glomus	Glomus tumor	
(3) Blood cells and related cells		
Hematopoietic cells		Myelogenous leukemia
		Monocytic leukemia
Lymphoid tissue		Malignant lymphomas
		Lymphocytic leukemia
		Plasmacytoma (multiple myeloma)
Langerhans' cells		Histiocytosis X
Monocyte-macrophage		? Histiocytic lymphoma
		? Hodgkin's disease
(4) Muscle		
Smooth muscle	Leiomyoma	Leiomyosarcoma
Striated	Rhabdomyoma	Rhabdomyosarcoma

Cotran et al, Robbins Pathological Basis of Disease

B. Tumors of epithelial origin		***Carcinomas***
(1) Stratified squamous	Squamous cell papilloma	Squamous cell or epidermoid carcinoma
(2) Basal cells of skin or adnexa		Basal cell carcinoma
(3) Skin adnexal glands		
Sweat glands	Sweat gland adenoma	Sweat gland carcinoma
Sebaceous glands	Sebaceous gland adenoma	Sebaceous gland carcinoma
(4) Epithelial lining		
Glands or ducts—well-differentiated group	Adenoma Papilloma Papillary adenoma Cystadenoma	Adenocarcinoma Papillary carcinoma Papillary adenocarcinoma Cystadenocarcinoma
Poorly differentiated group		Medullary carcinoma Undifferentiated carcinoma (simplex)
(5) Respiratory passages		Bronchogenic carcinoma Bronchial "adenoma"
(6) Neuroectoderm	Nevus	Melanoma (melanocarcinoma)
(7) Renal epithelium	Renal tubular adenoma	Renal cell carcinoma (hypernephroma)
(8) Liver cells	Liver cell adenoma	Hepatoma (hepatocellular carcinoma)
(9) Bile duct	Bile duct adenoma	Bile duct carcinoma (cholangiocarcinoma)
(10) Urinary tract epithelium (transitional)	Transitional cell papilloma	Papillary carcinoma Transitional cell carcinoma Squamous cell carcinoma
(11) Placental epithelium	Hydatidiform mole	Choriocarcinoma
(12) Testicular epithelium (germ cells)		Seminoma Embryonal carcinoma

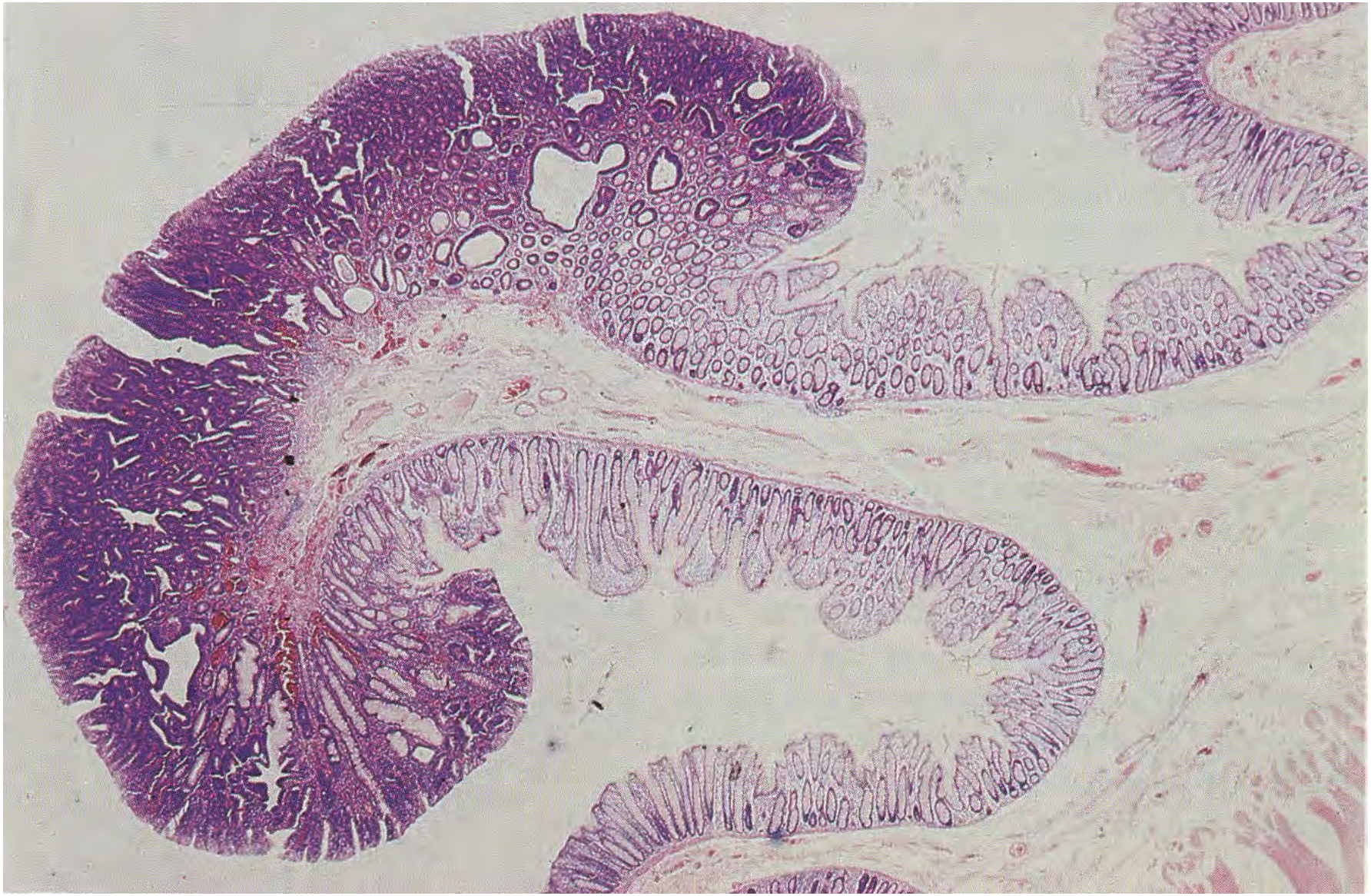
Cotran et al, Robbins Pathological Basis of Disease

Histology of the colon



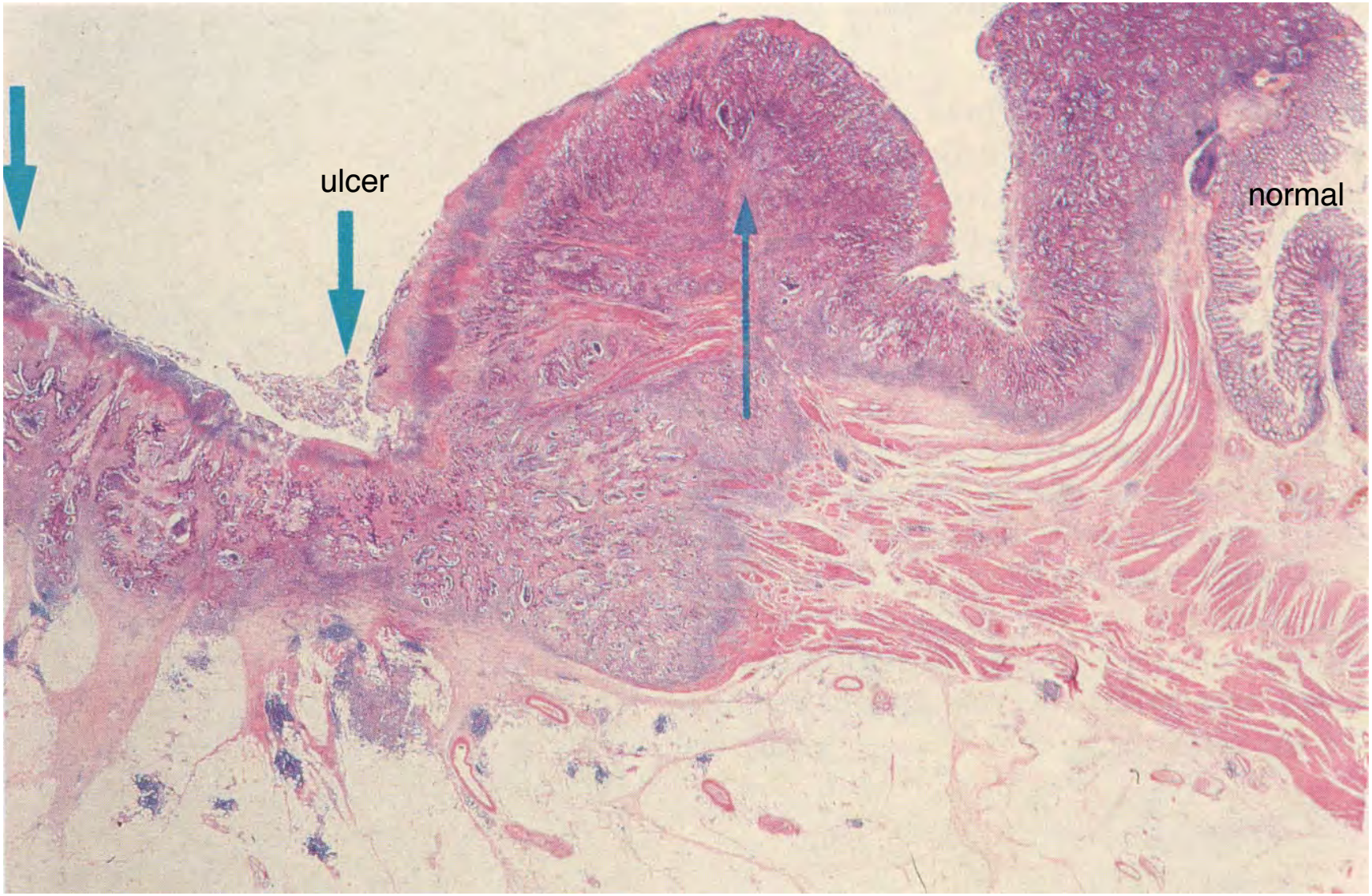
Bloom & Fawcett, 1994

Benign tumor: adenoma (polyp)



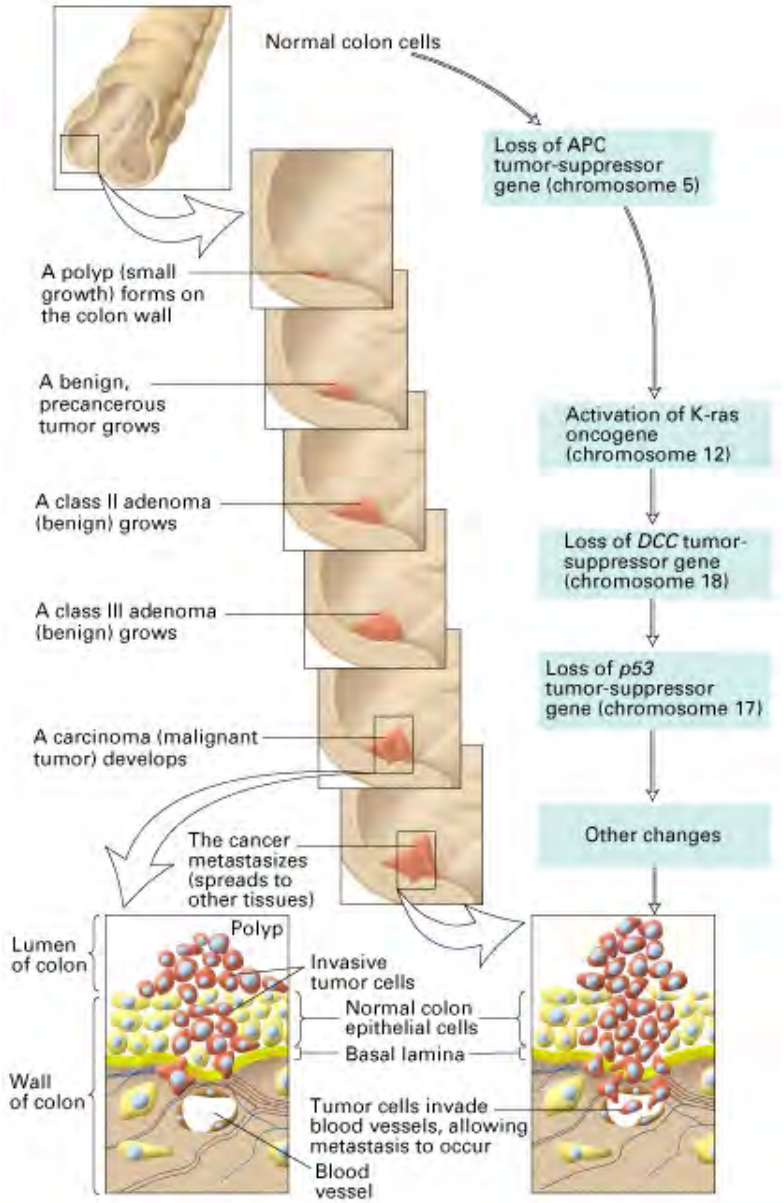
Curran, 1985

Malignant tumor: adenocarcinoma



Curran, 1985

Tumor progression results from accumulation of genetic lesions



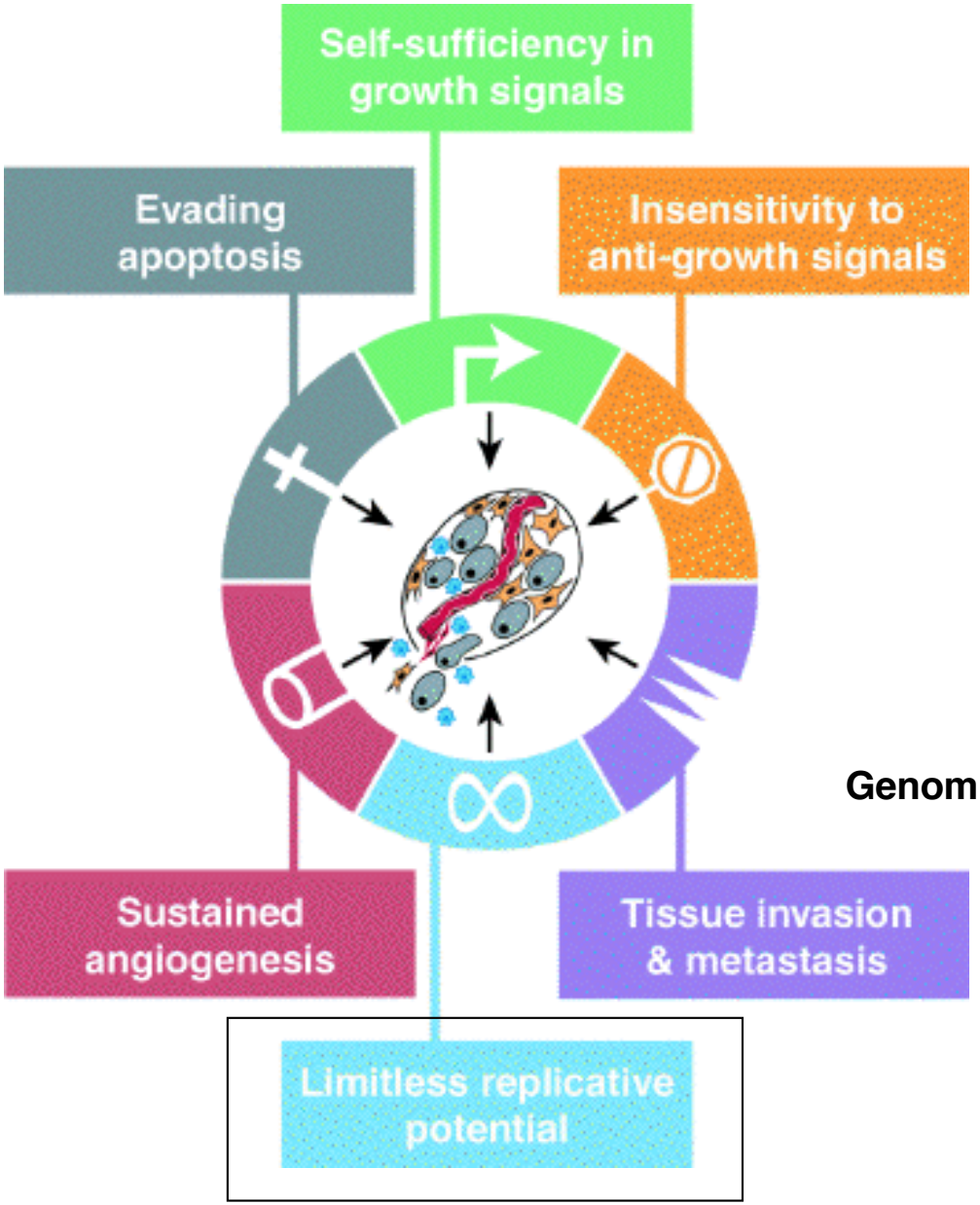
Colon cancer provides evidence for multi-step nature of oncogenesis.

- Tumors can be obtained and accurately staged.
- There appears to be an ordered succession of defined mutations.
- More advanced tumors have more mutations.
- Many colon carcinomas contain 4 common mutations: APC (Adenomatous polyposis coli) , p53, K-ras, and a tumor supressor on chromosome 18.

The role of DCC as tumor suppressor is controversial.

Lodish et al, 2000

Characteristics of malignant tumor cells



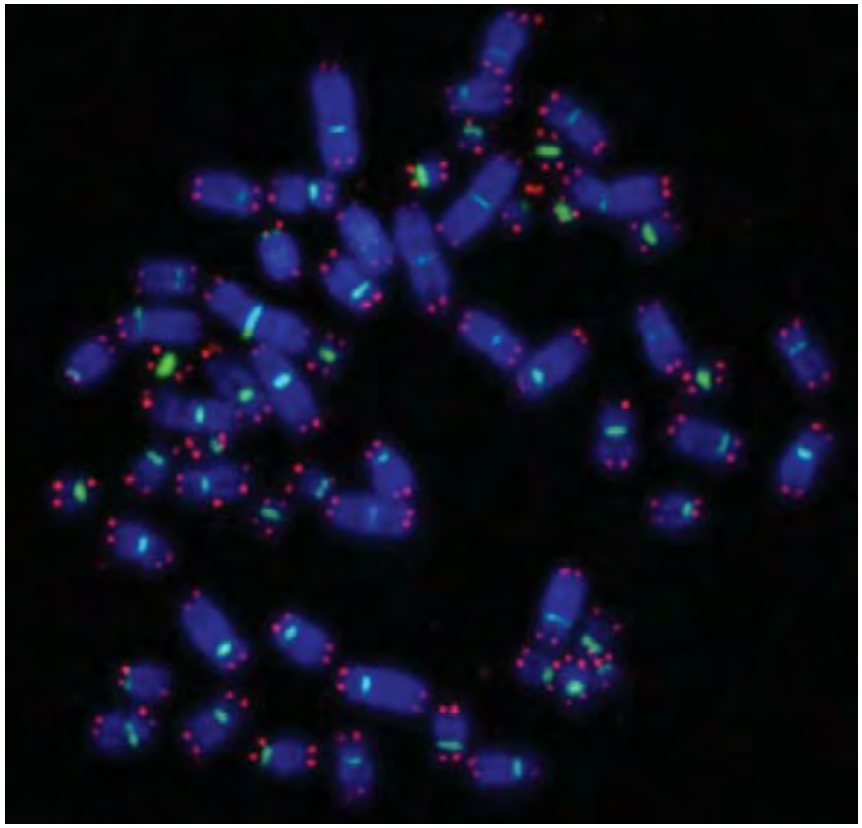
Genomic Instability

Hanahan & Weinberg (2000) Cell 100:57

Telomeres

End of chromosome is capped by telomere.

Telomere=repetitive sequence of noncoding DNA (thousands of repeats of TTAGGG)



FISH localization of centromeres (green) and telomeres (red) in metaphase chromosomes.

Senescence of human cells due to problem of telomere replication

Primary human fibroblast have finite lifespan (~50-100 divisions) before undergoing senescence=Hayflick limit.
tumor suppression
aging?

What is the molecular basis of this clock?

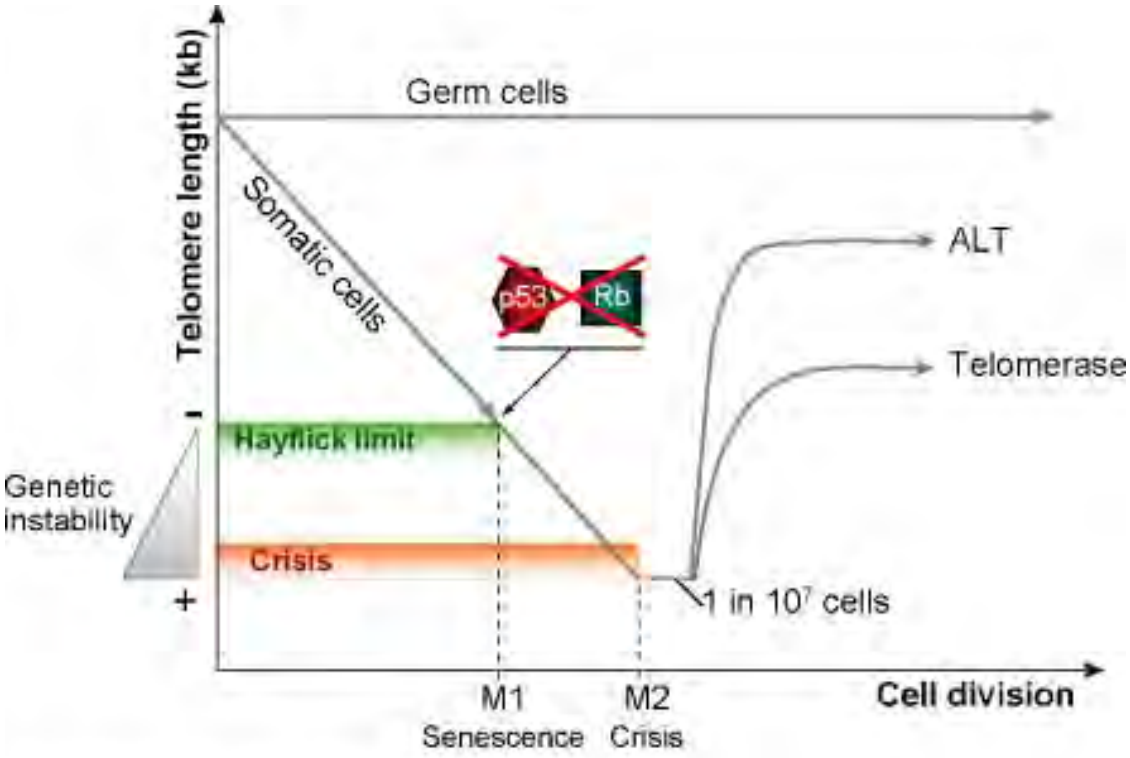
End replication problem: incomplete replication of lagging strand.

Telomeres shorten with cellular lifespan (no telomerase is expressed).

Shortening leads to p53/Rb-dependent growth arrest (senescence).

p53/Rb inactivation can bypass this limit, but further shortening results in “crisis” (massive death due to chromosomal damage and apoptosis).

Rare immortalized clones may arise due to expression of telomerase or an alternative mechanism for telomere maintenance.



Stewart SA, Weinberg RA. 2006.
Annu. Rev. Cell Dev. Biol. 22:531–57

Removal of telomerase activity can reverse immortalization

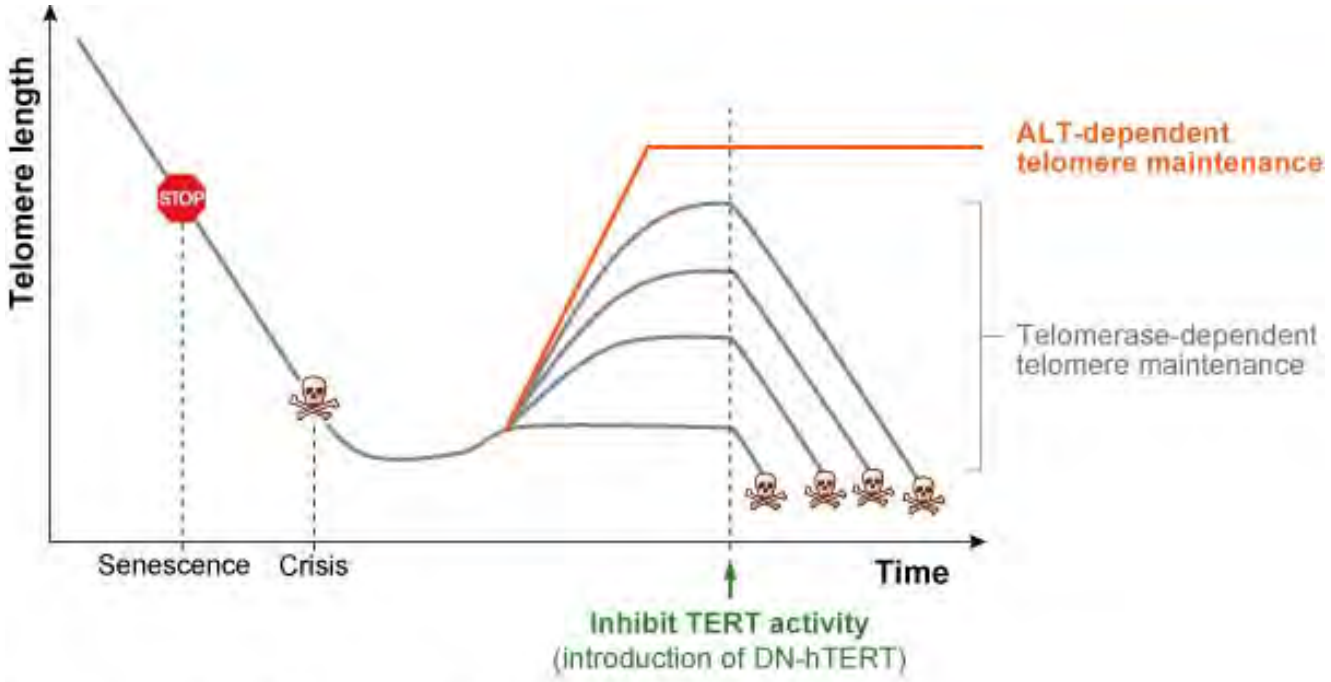
Inhibition of hTERT (human telomerase reverse transcriptase) in immortalized telomerase-positive cells leads to growth arrest and crisis.

Parental clones with longer telomeres take longer to reach crisis in this experiment.

Dominant-negative hTERT used.

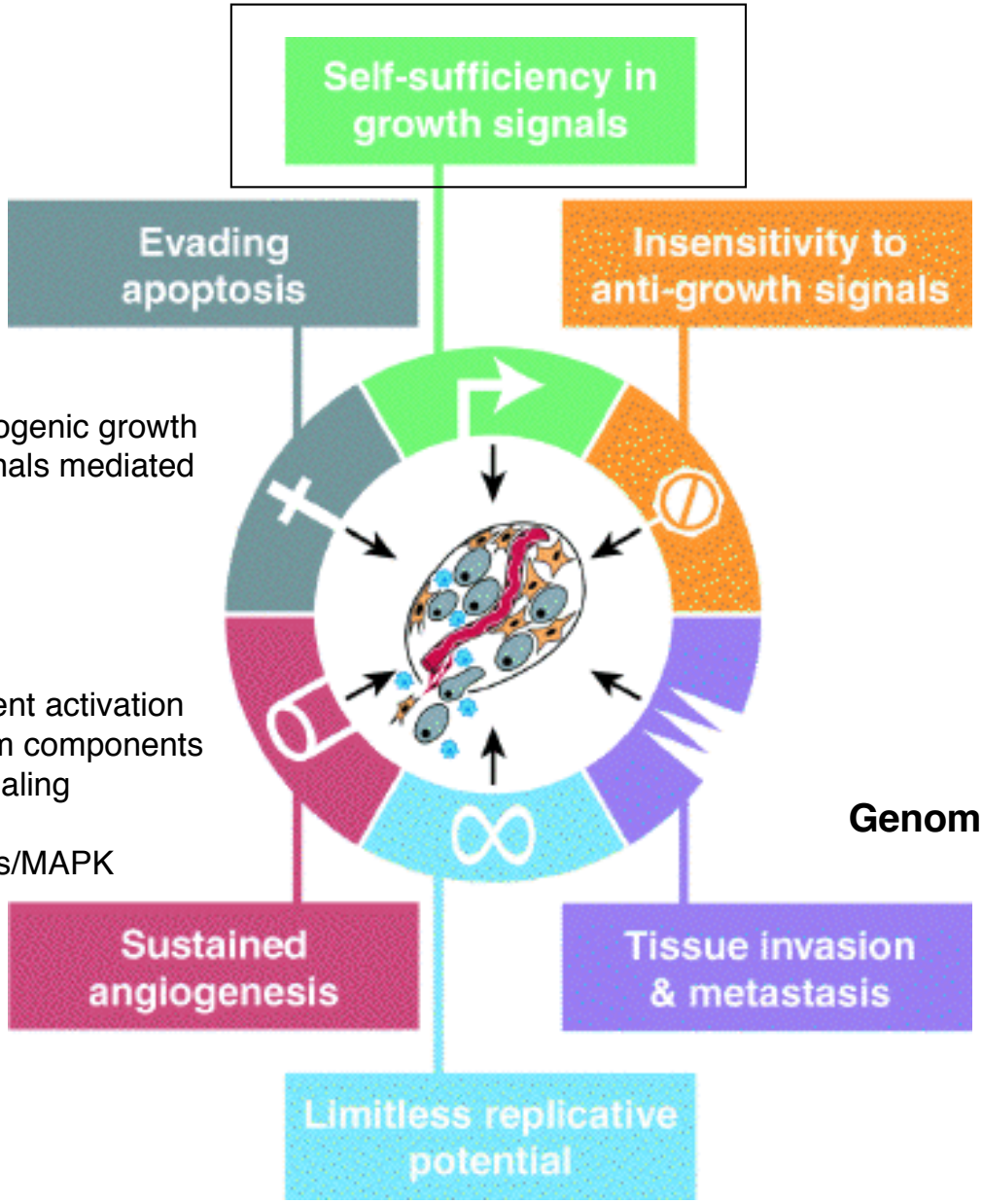
Parental clones that use an alternative mechanism to maintain telomeres are unaffected by inhibition of hTERT.

ALT: Alternative lengthening of telomeres; depends on double-stranded DNA break and recombination.



Stewart SA, Weinberg RA. 2006.
Annu. Rev. Cell Dev. Biol. 22:531–57

Characteristics of malignant tumor cells



- Normal cells require mitogenic growth signals to proliferate; signals mediated by cell surface receptors

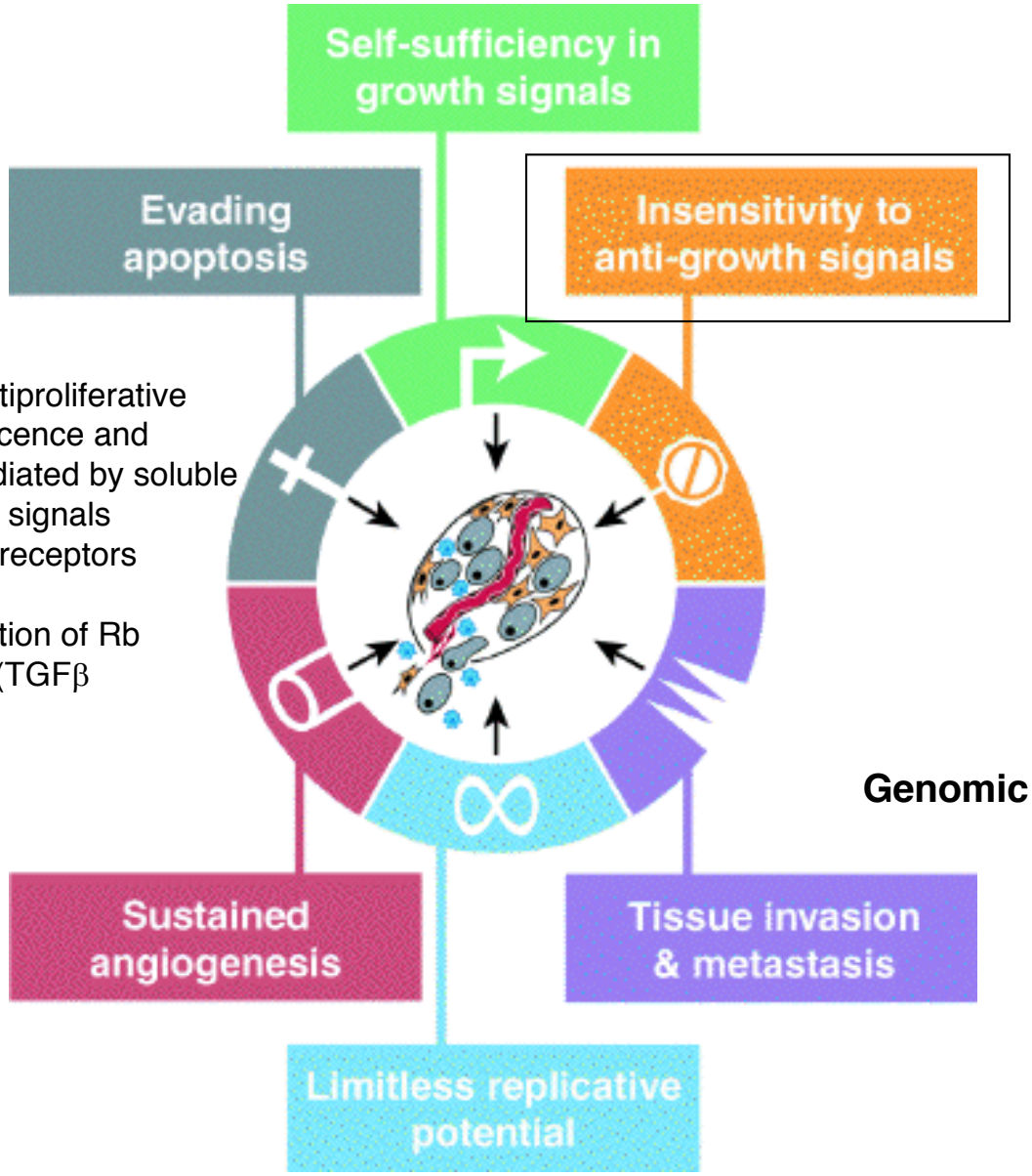
- Autocrine stimulation
- Receptor activation
  - Upregulation
  - Ligand-independent activation
- Activation of downstream components
- Heterotypic cell-cell signaling

- Important pathways: Ras/MAPK

Genomic Instability

Hanahan & Weinberg (2000) Cell 100:57

Characteristics of malignant tumor cells



•Normal cells receive antiproliferative signals, leading to quiescence and tissue homeostasis; mediated by soluble (TGFβ) and immobilized signals received by cell surface receptors

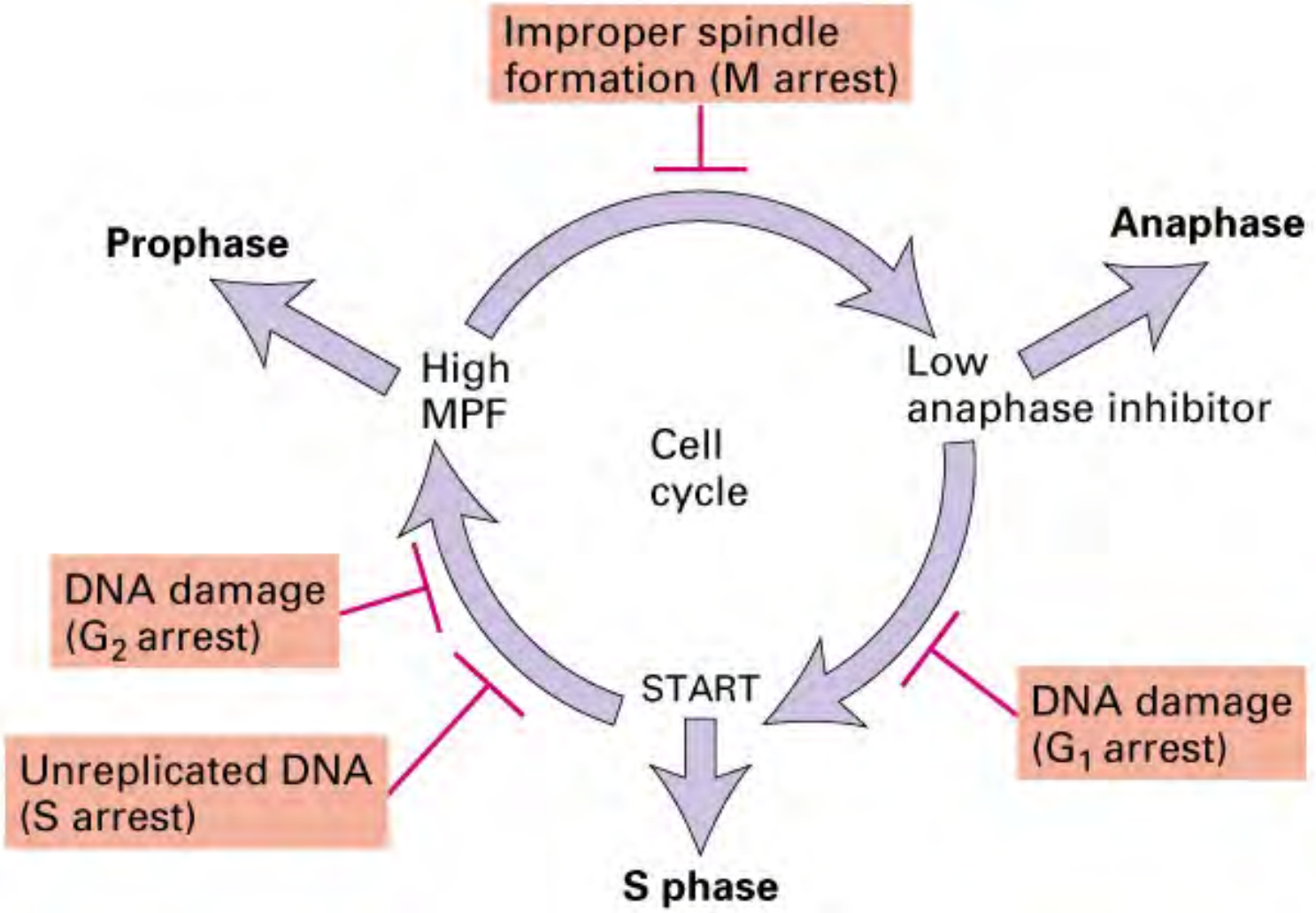
•TGFβ prevents inactivation of Rb
•loss of TGFβ signaling (TGFβ receptors, Smads)

•Rb inactivation
•P53 inactivation

Genomic Instability

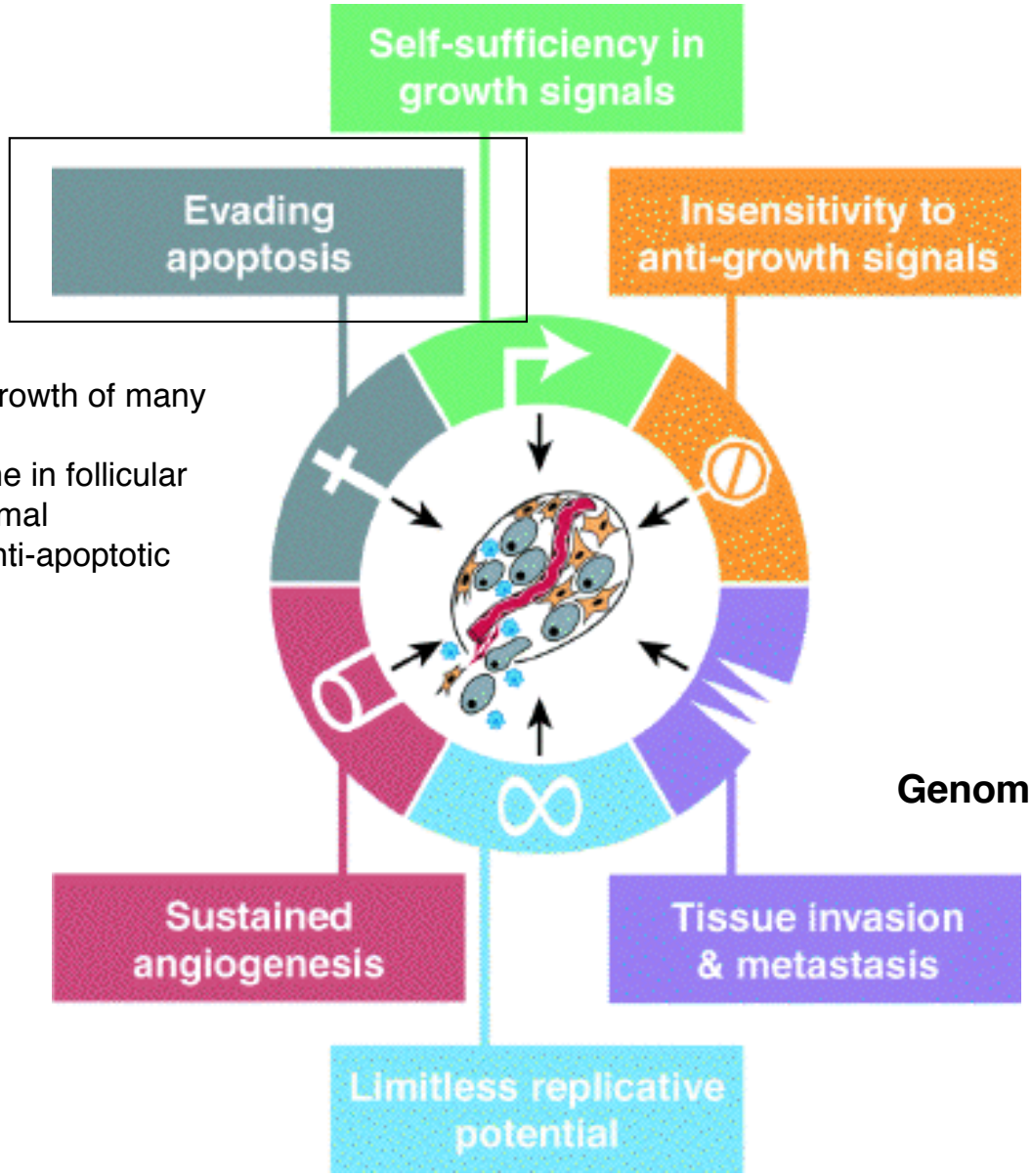
Hanahan & Weinberg (2000) Cell 100:57

Checkpoints arrest the cell cycle in response to various cues



Lodish et al, 2000

Characteristics of malignant tumor cells



- Apoptosis prevents growth of many tumors
- Bcl2 acts as oncogene in follicular lymphoma (chromosomal translocation) by its anti-apoptotic function
- inactivation of p53

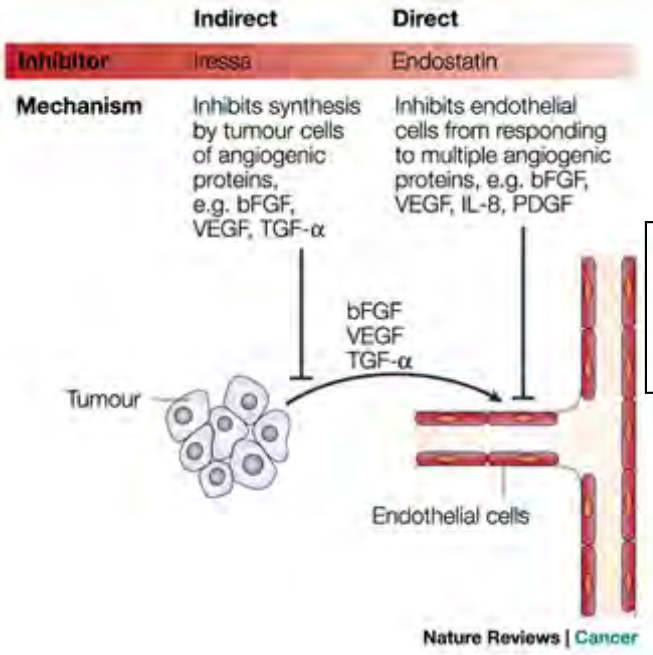
Hanahan & Weinberg (2000) Cell 100:57

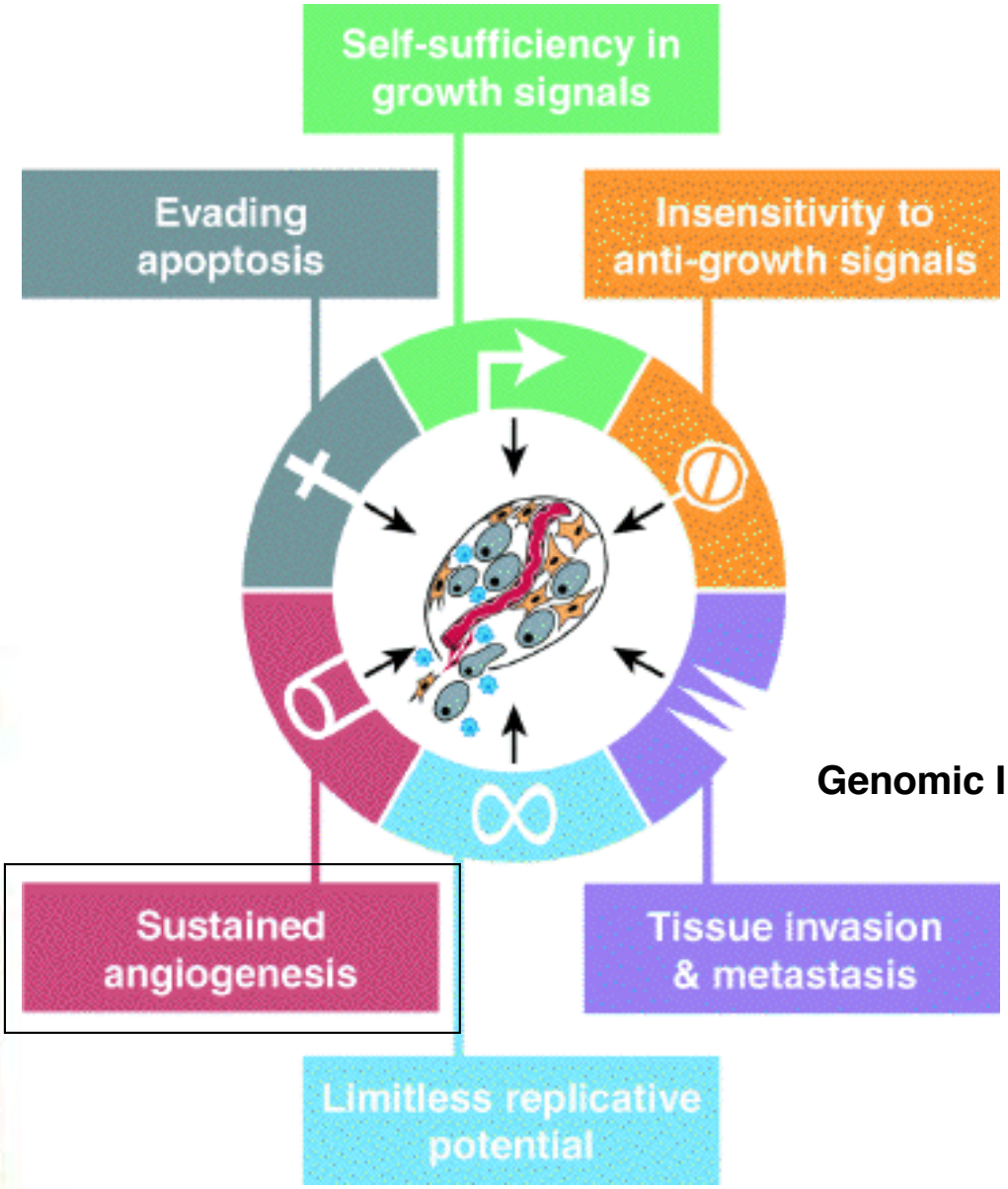
Characteristics of malignant tumor cells

•All cells must be close (100 μm) to blood supply; limits growth of initial neoplasms

•vascular endothelial growth factor (VEGF), FGF

•inhibitors of VEGF prevent growth of tumors in mice

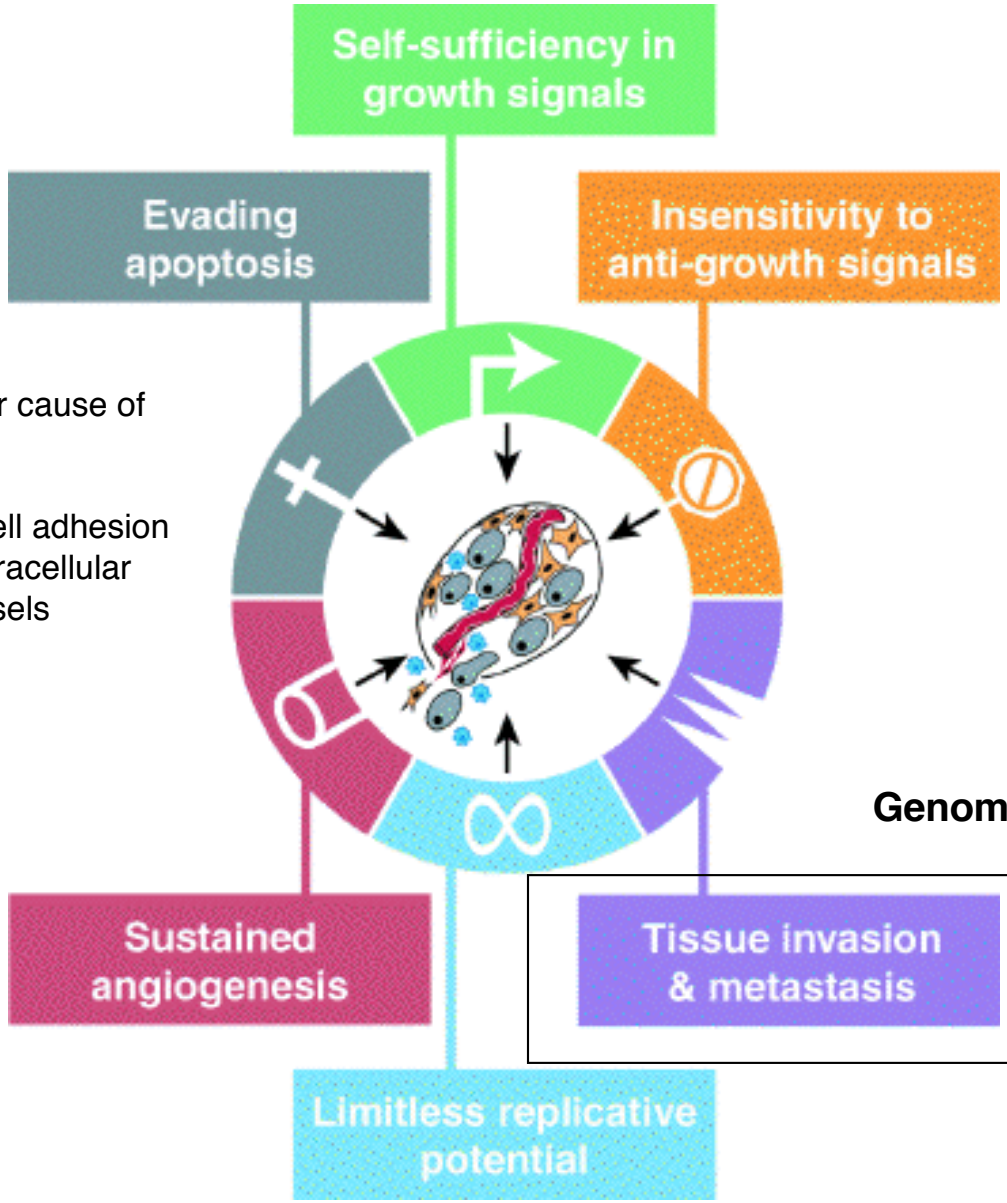




Genomic Instability

Hanahan & Weinberg (2000) Cell 100:57

Characteristics of malignant tumor cells



- metastasis are the major cause of cancer deaths

- requires loss of tumor cell adhesion
- protease to degrade extracellular matrix, across blood vessels
- migration

Genomic Instability

Hanahan & Weinberg (2000) Cell 100:57

Identifying genes involved in cancer

I. Retroviruses
    A. Acute transforming viruses
    B. Slow transforming viruses-- promoter/enhancer insertion
II. Isolation by DNA transfection
III. Chromosomal abnormalities
    A. Amplifications
    B. Translocations
IV. Positional cloning of tumor suppressor genes
V. DNA tumor viruses

TABLE 15.3 Retroviral Oncogenes

	Oncogene	Virus	Species
*	*abl*	Abelson leukemia	Mouse
	akt	AKT8 virus	Mouse
	cbl	Cas NS-1	Mouse
	crk	CT10 sarcoma	Chicken
*	*erbA*	Avian erythroblastosis-ES4	Chicken
*	*erbB*	Avian erythroblastosis-ES4	Chicken
	ets	Avian erythroblastosis-E26	Chicken
	fes	Gardner-Arnstein feline sarcoma	Cat
	fgr	Gardner-Rasheed feline sarcoma	Cat
	fms	McDonough feline sarcoma	Cat
	fos	FBJ murine osteogenic sarcoma	Mouse
	fps	Fujinami sarcoma	Chicken
	jun	Avian sarcoma-17	Chicken
	kit	Hardy-Zuckerman feline sarcoma	Cat
	maf	Avian sarcoma AS42	Chicken
	mos	Moloney sarcoma	Mouse
	mpl	Myeloproliferative leukemia	Mouse
	myb	Avian myeloblastosis	Chicken
*	*myc*	Avian myelocytomatosis	Chicken
	p3k	Avian sarcoma-16	Chicken
	qin	Avian sarcoma-31	Chicken
	raf	3611 murine sarcoma	Mouse
*	*rasH*	Harvey sarcoma	Rat
*	*rasK*	Kirsten sarcoma	Rat
	rel	Reticuloendotheliosis	Turkey
	ros	UR2 sarcoma	Chicken
	sea	Avian erythroblastosis-S13	Chicken
*	*sis*	Simian sarcoma	Monkey
	ski	Avian SK	Chicken
*	*src*	Rous sarcoma	Chicken
	yes	Y73 sarcoma	Chicken

src and abl~tyrosine kinases

erbA~thyroid hormone receptor
erbB~EGF receptor

v-sis~B chain of PDGF

Cooper (2000)

Viruses cause a few human cancers

Hepatitis virus B (DNA virus) and C (Positive-strand RNA virus): Liver cancer (esp. Asia), 5th leading cancer worldwide

Human papilloma virus (DNA virus): Cervical cancer, HPV vaccine now available

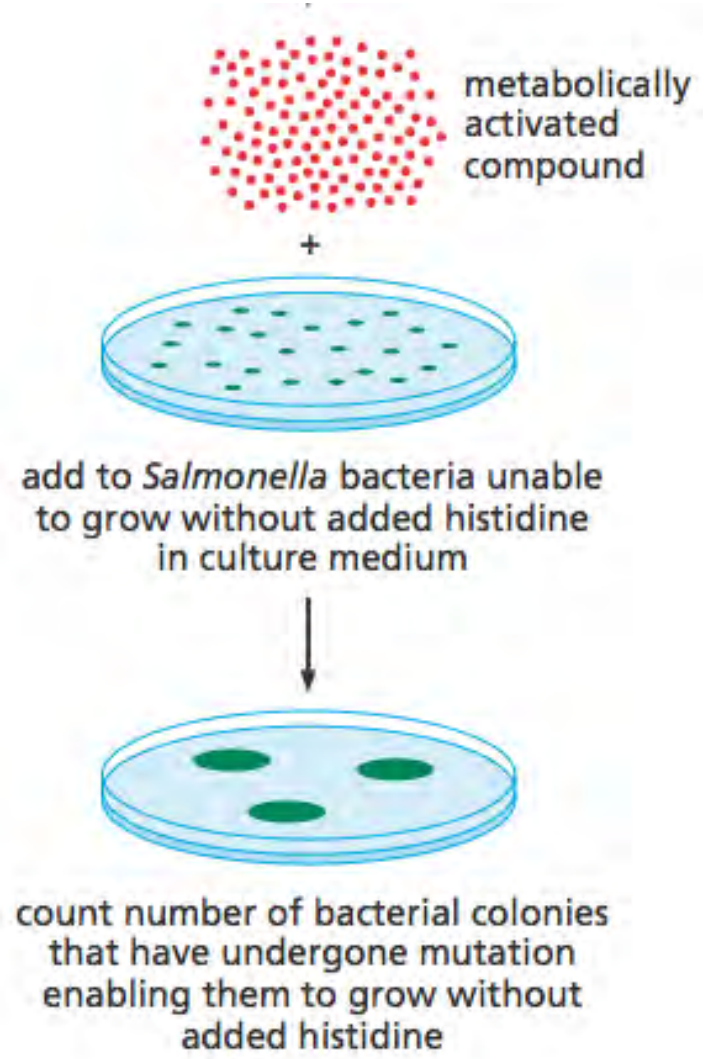
Epstein Barr virus (DNA virus): Burkitt's lymphoma (B cells, Africa); nasopharyngeal cancer (China)

Human T cell leukemia virus (HTLV-I) (retrovirus): Adult T cell leukemia/lymphoma

HIV/Herpes-8 (DNA virus): Kaposi's sarcoma

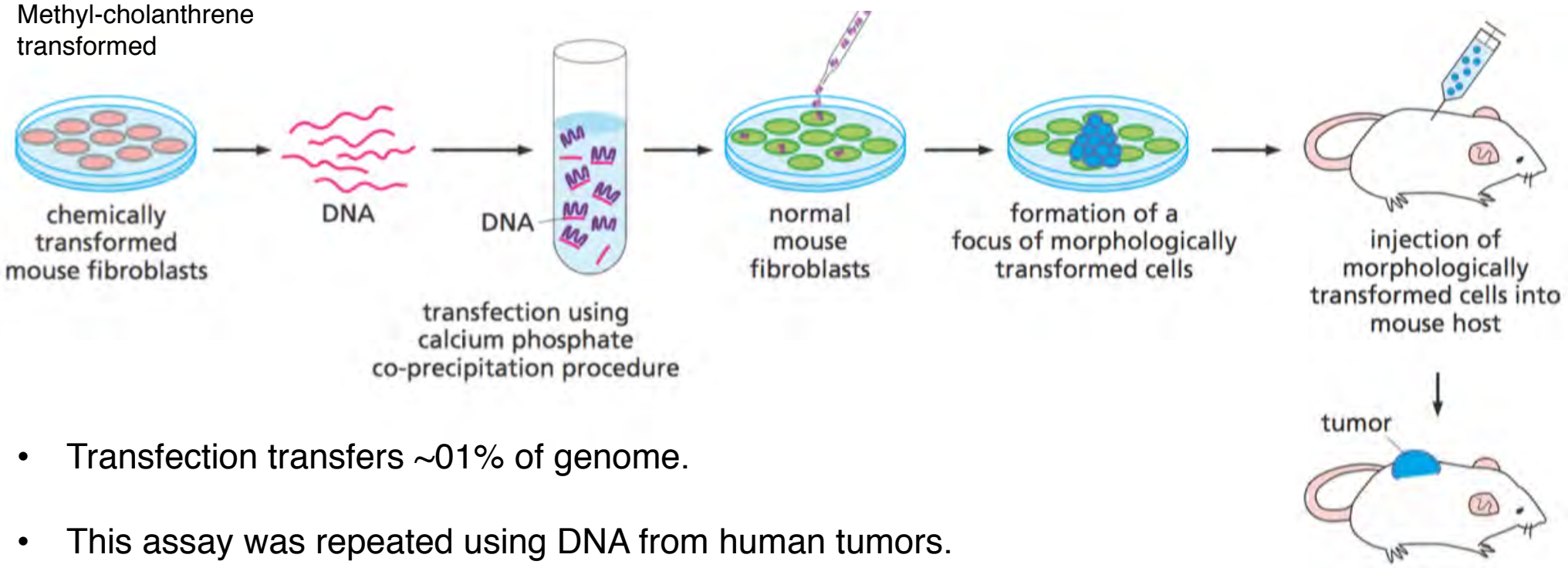
Evidence for a genetic basis for cancer

- Tumor viruses transform cells via viral oncogenes.
- For Rous sarcoma virus, the v-*src* has a normal cellular counterpart, c-*src*. This indicated that mutation of normal cellular genes might also give rise to tumors.
- The Ames test (right) showed a strong correlation between mutagenicity and carcinogenicity. (Though not all carcinogens were mutagenic.)



Weinberg (2013)
The Biology of Cancer

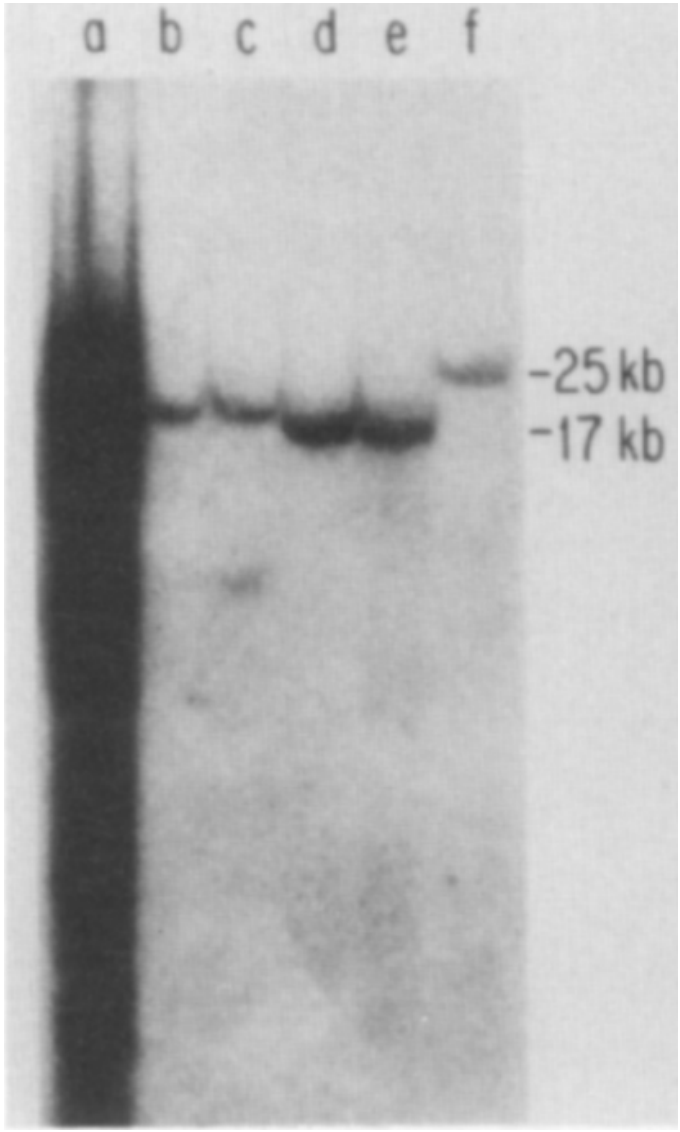
DNA transfection identifies cellular oncogenes



- Transfection transfers ~01% of genome.
- This assay was repeated using DNA from human tumors. The standard assay uses NIH3T3 cells, an immortalized but untransformed cell line.
- Transformation therefore likely due to single genetic locus.
- Oncogenes identified included: H-Ras, K-Ras, N-Ras, Myc, Neu (ErbB2)

Weinberg (2013)
The Biology of Cancer

Identification of transforming sequence



Lane a: Primary focus

Lanes b-f: 5 independent secondary foci

Probe: Alu fragment

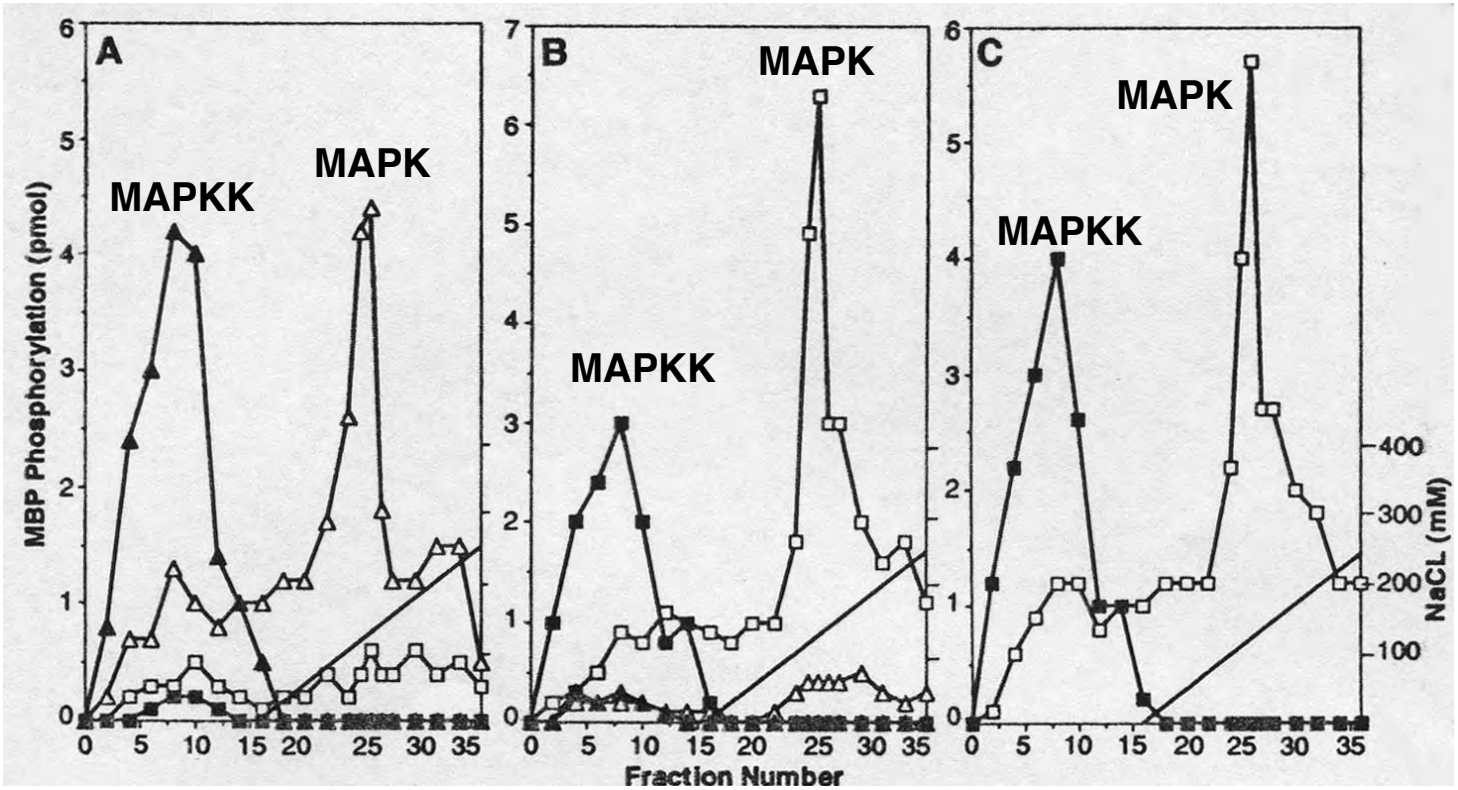
Shih & Weinberg (1982) Cell

DNA transfection identifies cellular versions of viral oncogenes

Name of virus	Species	Oncogene	Type of oncoprotein	Homologous oncogene found in human tumors
Rous sarcoma	chicken	*src*	non-receptor TK	colon carcinoma[a]
Abelson leukemia	mouse	*abl*	non-receptor TK	CML
Avian erythroblastosis	mouse	*erbB*	receptor TK	gastric, lung, breast[b]
McDonough feline sarcoma	cat	*fms*	receptor TK	AML[c]
H-Z feline	cat	*kit*	receptor TK[d]	gastrointestinal stromal
Murine sarcoma 3611	mouse	*raf*	ser/thr kinase[e]	bladder carcinoma
Simian sarcoma	monkey	*sis*	platelet-derived growth factor (PDGF)	many types[f]
Harvey sarcoma	mouse/rat	H-*ras*[g]	small G protein	bladder carcinoma
Kirsten sarcoma	mouse/rat	K-*ras*[g]	small G protein	many types
Avian erythroblastosis	chicken	*erbA*	nuclear receptor[h]	liver, kidney, pituitary
Avian myeloblastosis E26	chicken	*ets*	transcription factor	leukemia[i]
Avian myelocytoma	chicken	*myc*[j]	transcription factor	many types
Reticuloendotheliosis	turkey	*rel*[k]	transcription factor	lymphoma

Weinberg (2013) The Biology of Cancer

Infection of cells with v-Raf virus leads to activation of MAPK and MAPKK



Control virus

No serum: □

Serum: Δ

No serum

No virus: Δ

EC12 virus: □

No serum

22W virus: □

EC12, 22W = Moloney mouse sarcoma viruses containing activated v-Raf (N-term truncations)

The three cellular Raf genes are ser/thr kinases with N-term regulatory domain.

Cell lysates fractionated by ion exchange, and fractions assayed for MAPK and MAPKK activity

MAPK activity: assay phosphorylation of MBP

MAPKK activity: assay phosphorylation of MBP through recombinant inactive MAPK

Control virus expresses a more severely truncated v-Raf that is inactive.
Serum or v-Raf is able to induce MAPKK and MAPK activity in serum-starved cells.

Dent et al. (1992) Science 257:1401

Phosphorylation of “activation lip” activates MAP kinase

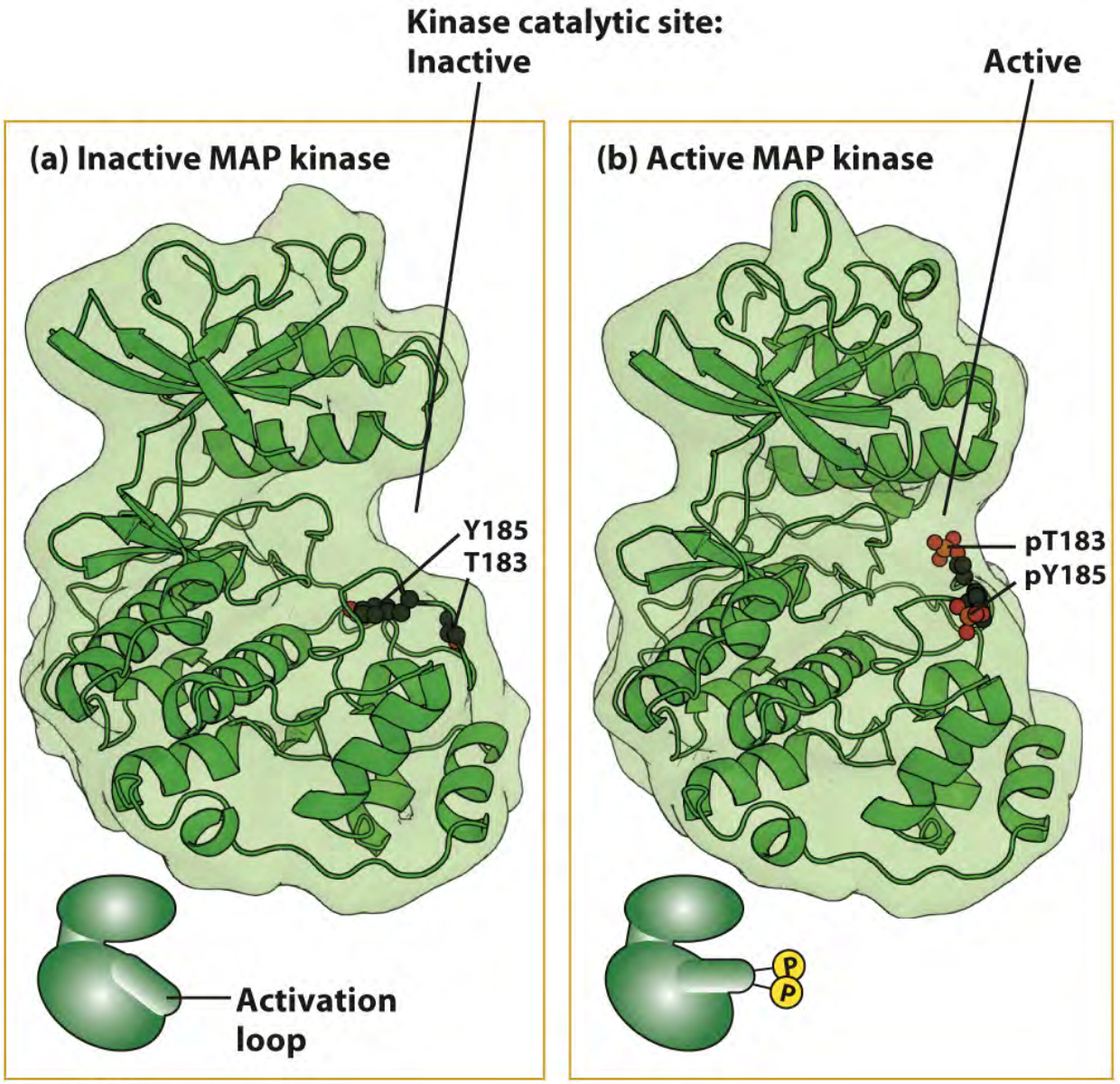
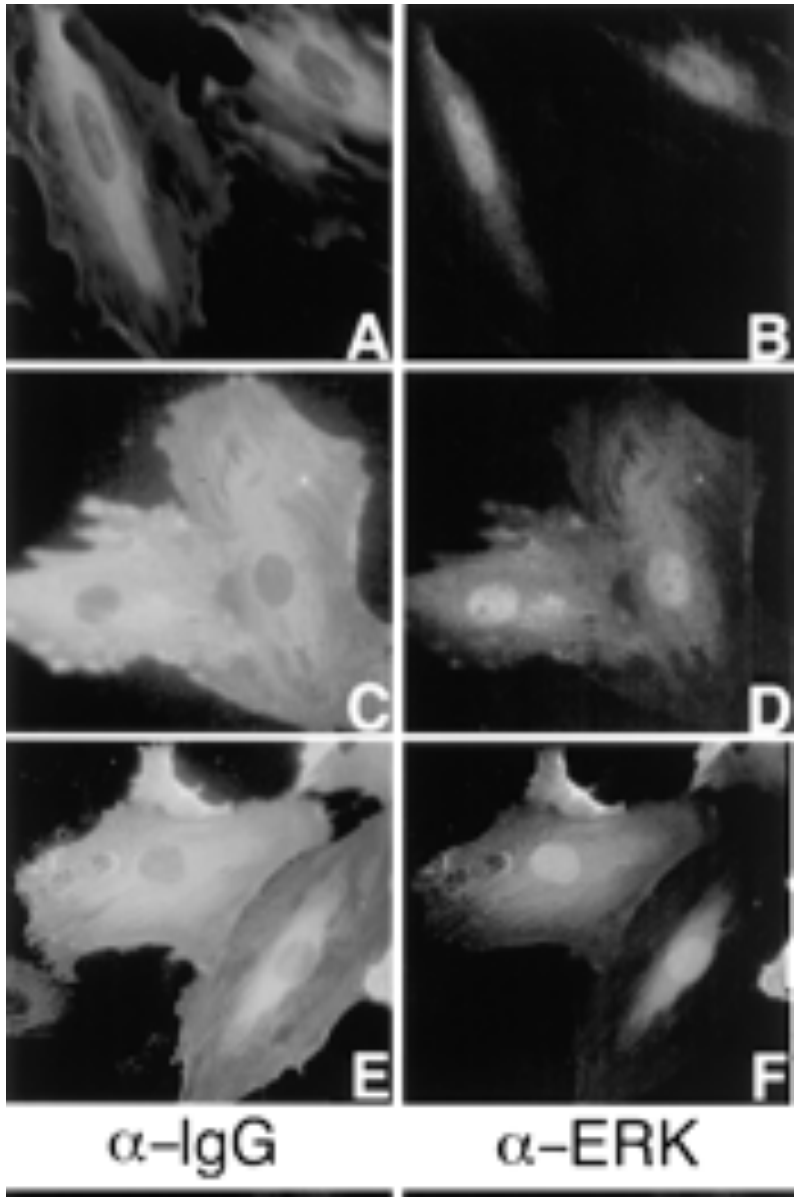


Figure 16-25
Molecular Cell Biology, Eighth Edition

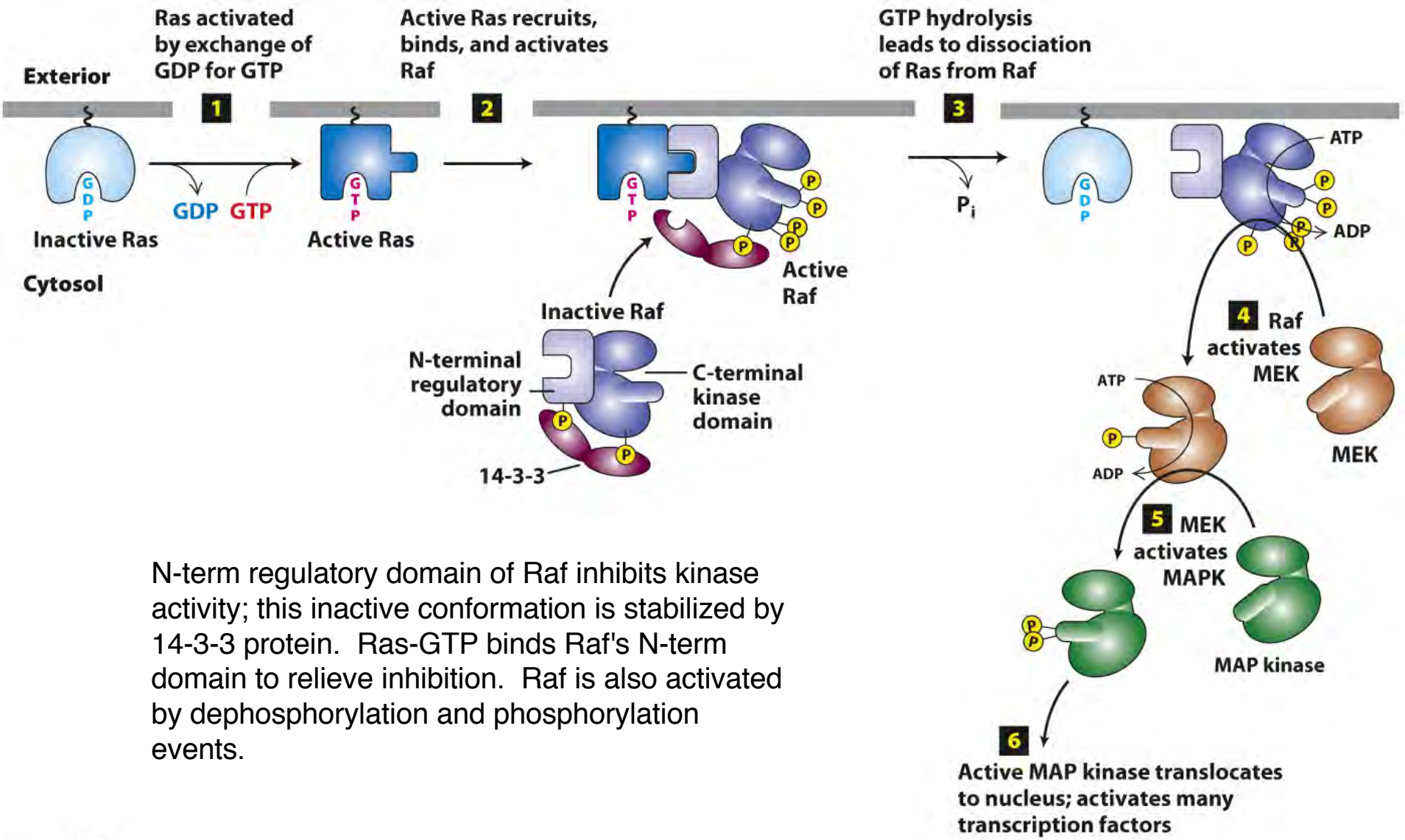
In inactive state, catalytic site is blocked by lip. Phosphorylation of lip by MEK leads to binding of ATP to catalytic site and dimerization, which allow nuclear localization

Phosphorylation of MAPK leads to nuclear translocation



Khokhlatchev et al (1998). Cell 93:605

Summary of mammalian MAP kinase cascade



N-term regulatory domain of Raf inhibits kinase activity; this inactive conformation is stabilized by 14-3-3 protein. Ras-GTP binds Raf's N-term domain to relieve inhibition. Raf is also activated by dephosphorylation and phosphorylation events.

Figure 16-24
Molecular Cell Biology, Eighth Edition

Phosphorylation of MAPK leads to nuclear translocation and gene activation

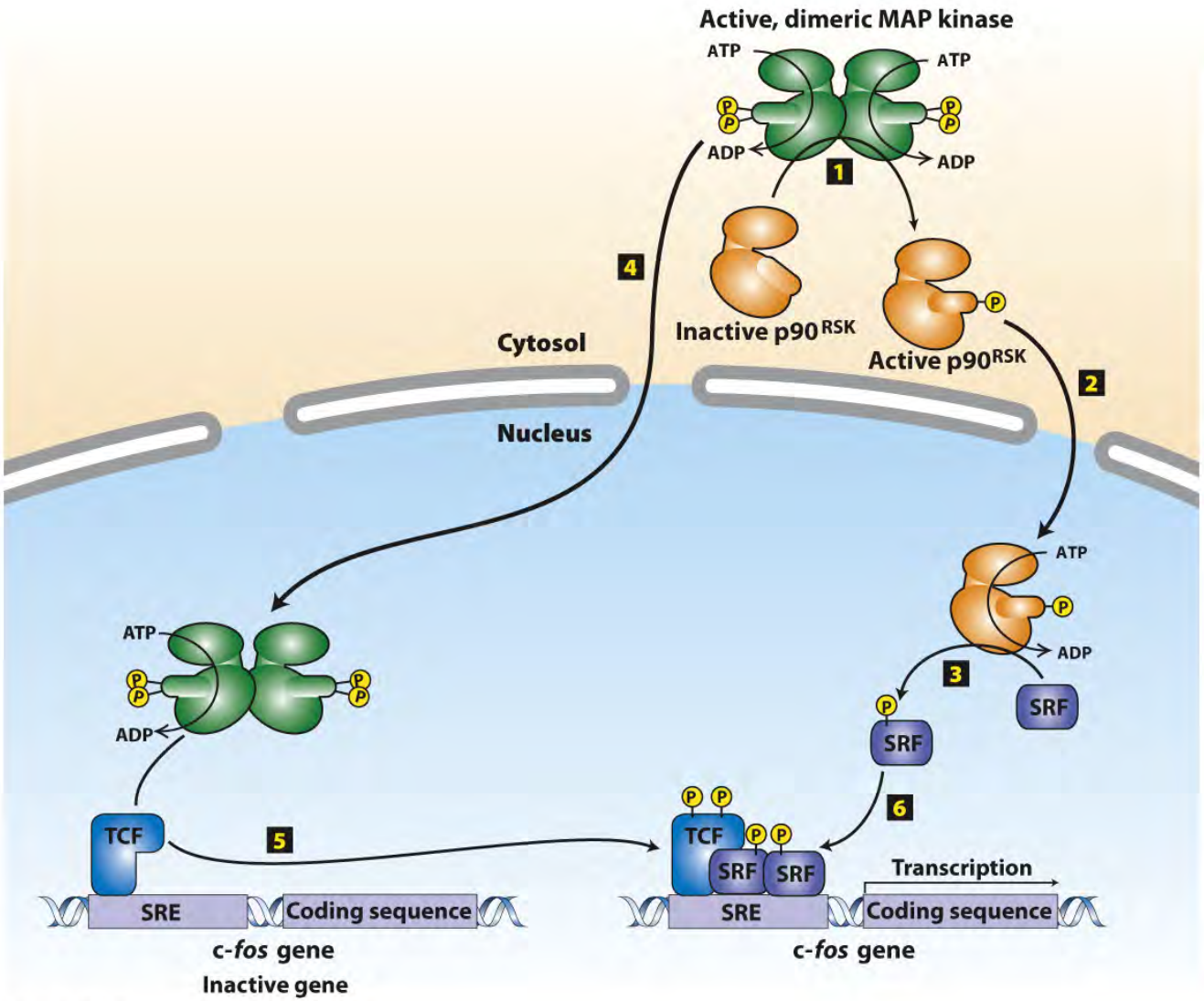
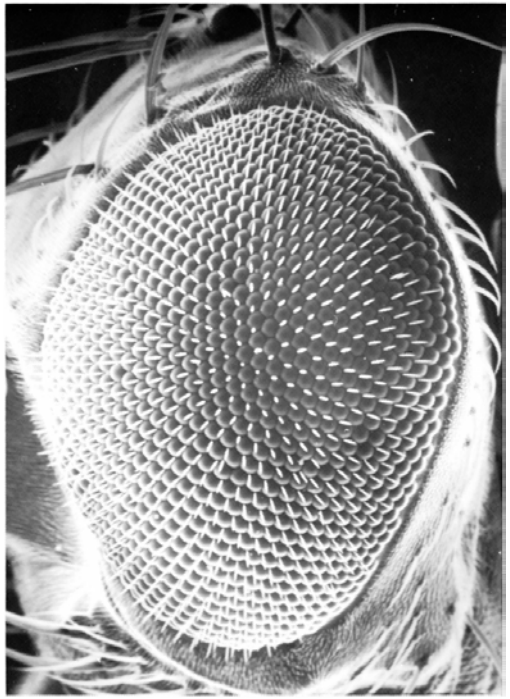


Figure 16-26
Molecular Cell Biology, Eighth Edition

The R7 photoreceptor in *Drosophila* is used to study RTK pathways



Nagaraj &Banerjee (2004) Int J Dev Biol
Gilbert. Developmental Biology, 8th edition

Each ommatidia is an eye that contains 20 cells (8 photoreceptors). R8 differentiates first; R7 last.

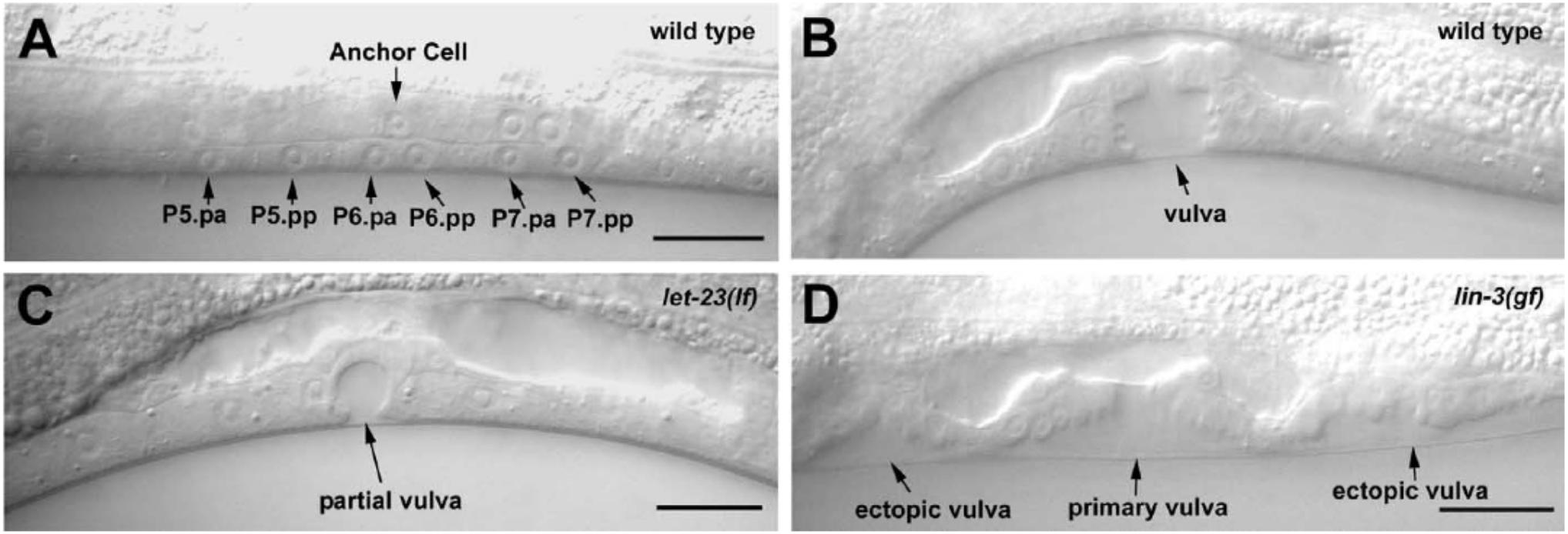
In *sevenless* mutant, R7 has non-neuronal fate, and the fly cannot sense UV light. Mosiac analysis shows *sev* is required cell autonomously in R7.

Boss (*Bride of sevenless*) mutants also lack R7, but the gene product is required in R8.

Sev is a receptor tyrosine kinase; Boss is its ligand.

Screens in this system identified GRB2, SOS (son of sevenless), and Ras.

Genetic dissection of vulva development in *C. elegans*

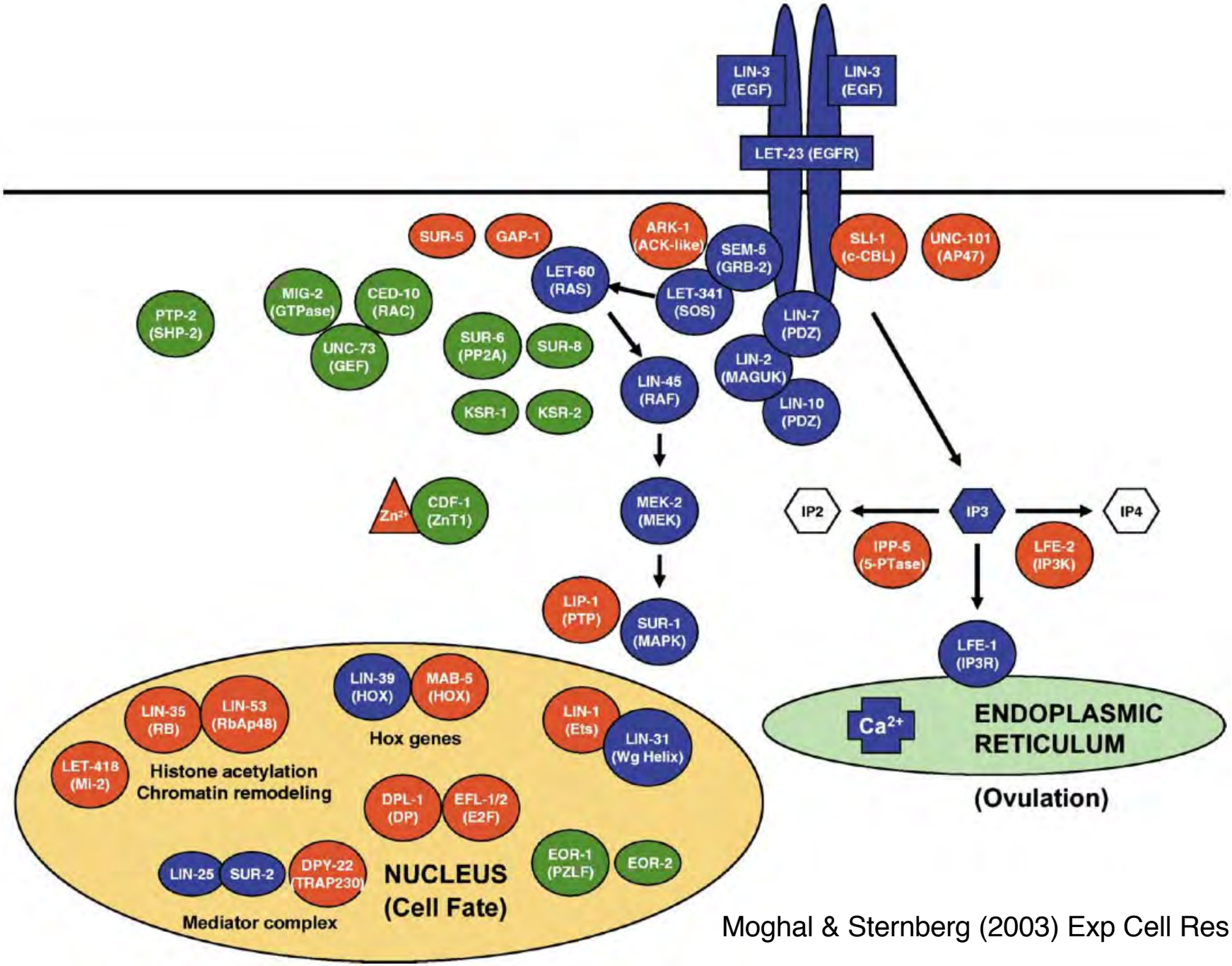


- The gonadal anchor cells sends an inductive signal to ventral precursor cells that generate vulval tissue.

- The anchor cell produces Lin-3 (an EGF molecule). The precursor cells require let-23 (RTK) and Ras (let-60) for vulva devlopment.

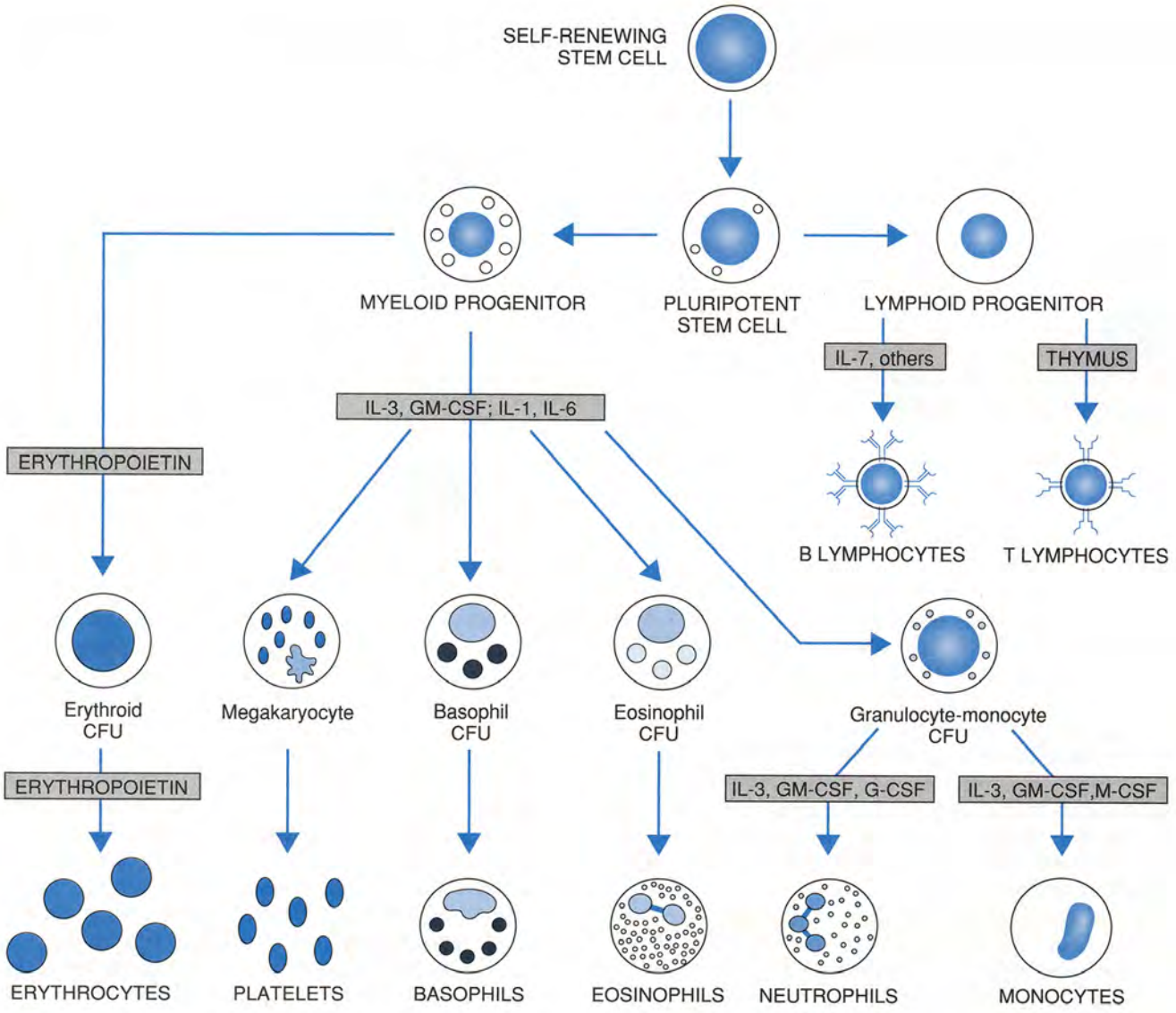
Moghal & Sternberg (2003) Exp Cell Res

The MAPK casade in vulva development



Moghal & Sternberg (2003) Exp Cell Res

Stem cells in the hemapoietic system



Abbas et al. Cellular & Molecular Immunology

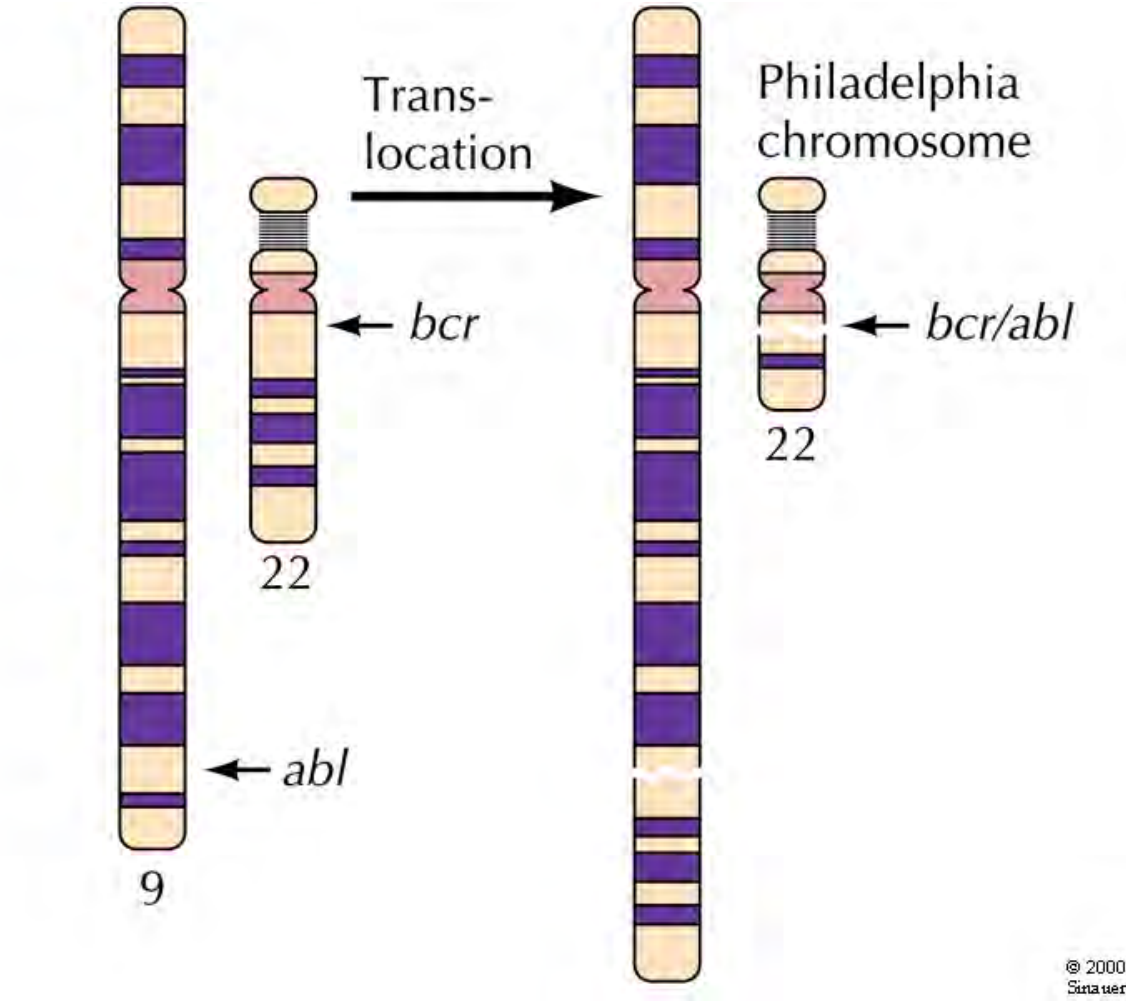
The Philadelphia chromosome in Chronic Myelogenous Leukemia (CML)

- CML: A leukemia due to overgrowth of myeloid cells in the bone marrow.
- Characterized by increased white blood cell count, hepatosplenomegaly, Philadelphia chromosome.
- The first chromosomal abnormality associated with cancer-- 95% of CML patients show the Philadelphia chromosome; also found is smaller fraction of acute lymphoblastic leukemia (ALL).

  Bcr on chromosome 22

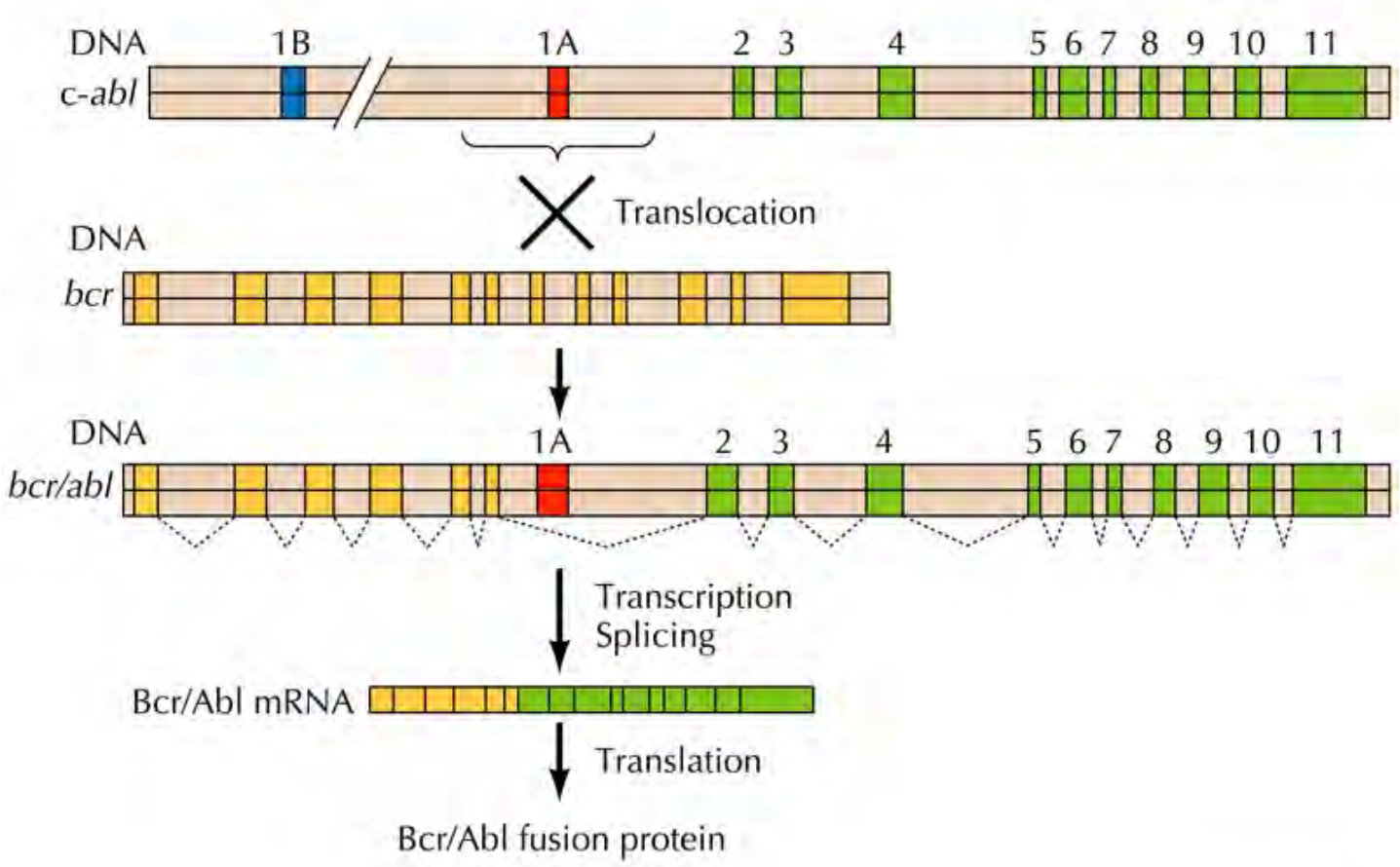
  Abl on chromosome 9

  Philadelphia chromosome due to reciprocal translocation



Cooper, 2000

The Philadelphia chromosome produces a bcr-abl fusion protein with tyrosine kinase activity



Cooper, 2000

- BCR-ABL fusion protein is ~210 kD= cytokine-independent, constitutive proliferation signal
- Expression of BCR-ABL is sufficient to produce myeloproliferative disease in mice

Clinical features of CML

CML: myeloproliferative disease

• median age at diagnosis ~55 years

• often found in asymptomatic patients after routine blood test showing elevated white cells

• chronic-phase CML: elevated white cells with circulating immature precursors, often thrombocytosis (increased platelets) and splenomegaly

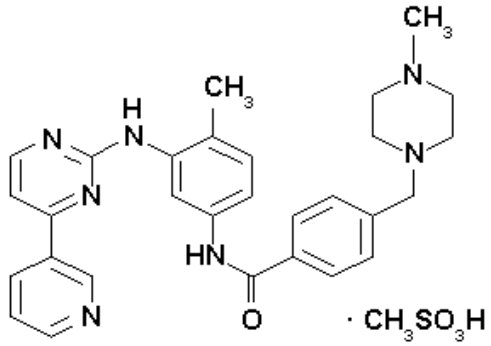
• acquired mutation: Philadelphia chromosome (9;22 translocation)

• If untreated, after 4-5 years, inevitably progresses to more aggressive accelerated and blast phases: patients symptomatic and increase precursors in blood and marrow.

• median survival in blast phase is 6 months, due to infection and hemorrhage

A small molecule inhibitor of the bcr-abl tyrosine kinase is clinically effective

Novartis identified Gleevec=STI-571=Imatinib as a small compound that competitively binds to the abl tyrosine kinase active site and inhibits its phosphorylation of substrates. Highly specific, but also inhibits c-kit and PDGF receptor.



- Gleevec is remarkably effective clinically against CML and ALL.
- Inhibition of BCR-ABL signaling results in apoptosis
- Complete cytogenetic response (i.e., no Ph chromosome positive cells in bone marrow) after 18 months treatment= 76%
- After 5 years, complete cytogenetic response= 87%, progression-free survival=83%
- Success is much lower with patients in accelerated and blast phases of disease.

Imatinib is effective, but issues remain

• Almost all patients relapse after discontinuation of imatinib.

• Therefore, it is recommended that treatment be continued indefinitely. (cost ~$32,000 per year)

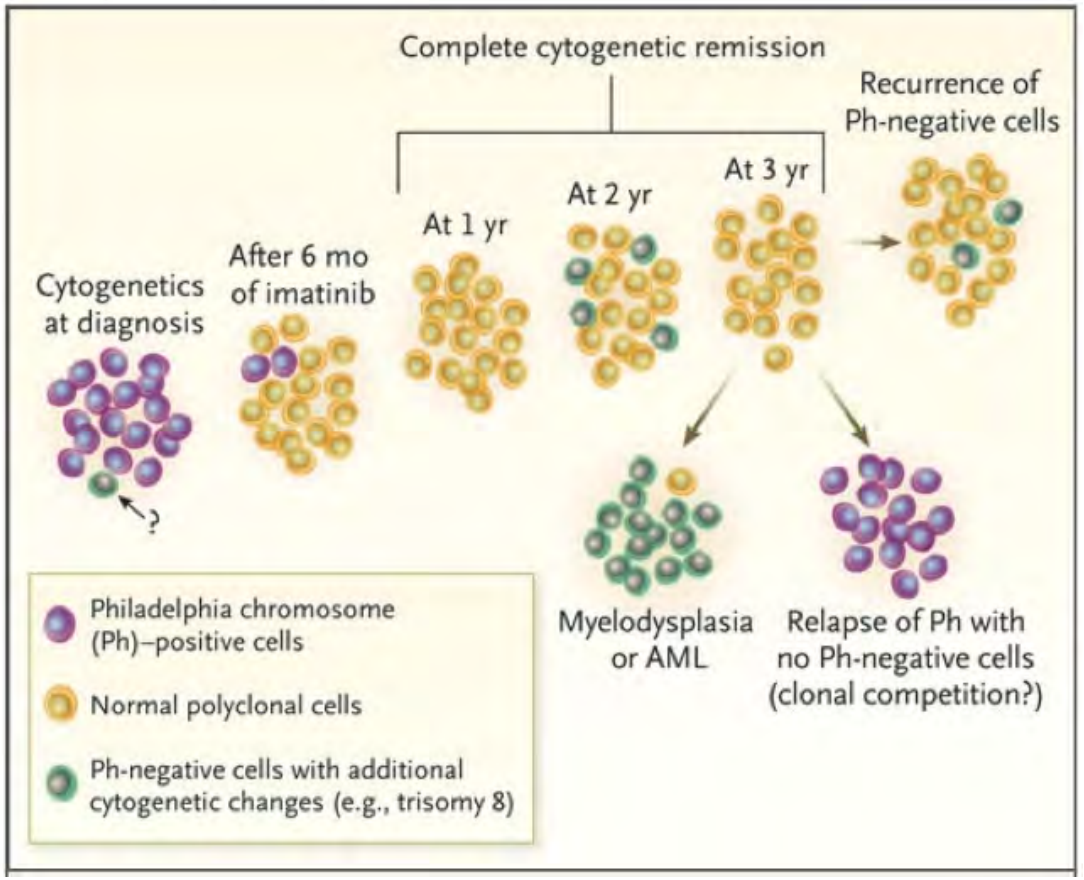
• Side effects associated with drug.

• Imatinib may be teratogenic; both men and women advised to avoid conception; dilemma for young patients.

• 15-20% of patients do not have complete cytogenetic response to CML; others later develop resistance due to additional BCR-ABL mutations.

• Allogeneic stem-cell transplantation is still the only cure for CML (very toxic, and difficult to find matching donors).

• Newer inhibitors of BCR-ABL: dasatinib and nilotinib; more potent than imatinib and reactive against most BCR-ABL mutants.



Schiffer (2011) New England Journal of Medicine

Traditional approaches for management of cancer: breast cancer as an example

1 in 9 lifetime chance of breast cancer in women. Women with BRCA1 or BRCA2 mutations have 60-85% risk for breast or ovarian cancer.

Detection: manual breast exam, mammography, biopsy
Staging: biopsy, histological analysis

Treatment:

- **Surgery**: lumpectomy, mastectomy, lymph nodes (material important for staging also)
- **Radiation**: can be adjunct to surgery
- **Chemotherapy**

Molecular/cellular studies have provided new drugs for breast cancer

Tamoxifen

- estrogen receptor antagonist
- used inhibit growth of estrogen receptor positive breast cancer.

Aromatase inhibitors

- block endogenous estrogen synthesis

Activation of the Neu receptor tyrosine kinase by a point mutation in the transmembrane segment

Neu=ErbB2: Receptor tyrosine kinase of the EGFR (erbB) family

• Isolated from neuronal tumor line (ethylnitrosourea-induced rat neuroglioblastomas) via NIH3T3 focus forming assay
• activated by V664E mutation in the transmembrane segment
• correlated with increased dimerization of the receptor, increased tyrosine kinase activity and autophosphorylation
• N-terminally truncated allele retains transforming activity.

• HER2/Neu amplified in many breast tumors (15-20%)

Herceptin/Trastuzumab

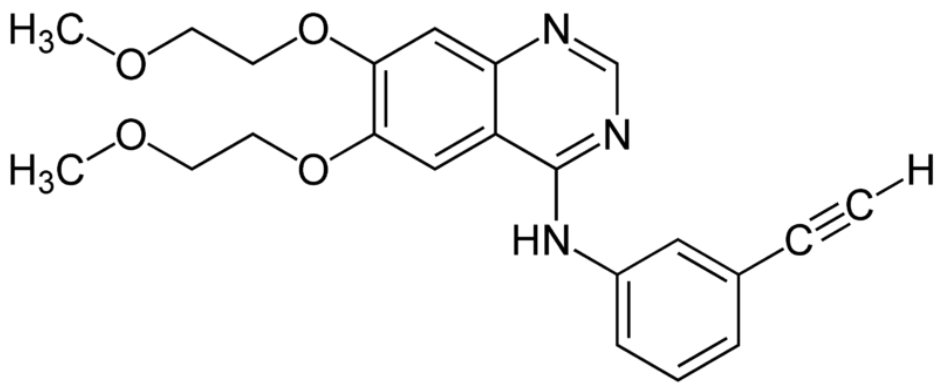
• humanized monoclonal antibody versus HER2/neu/ErbB2 receptor
• used to treat HER2/Neu/ErbB-2 positive tumors
• Treated cells undergo cell cycle arrest

EGFR inhibitor effective for some lung cancers

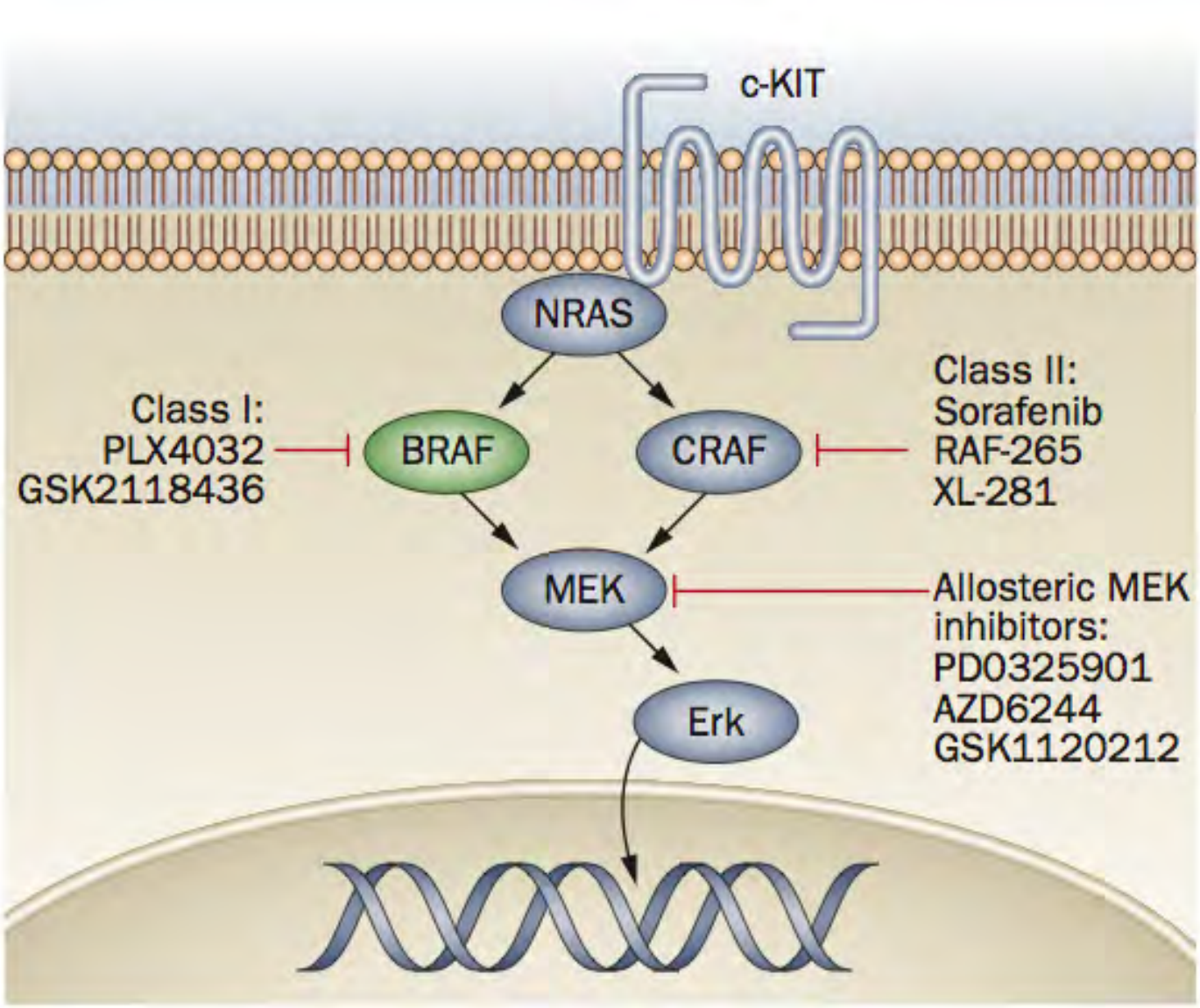
Tarceva/Erlotinib hydrochloride

• Small molecule drug that inhibits tyrosine kinase activity of EGFR; binds to the ATP binding site

• Clinically useful in EGFR-positive non-small cell lung carcinoma and pancreatic cancer.



The MAPK pathway as a drug target



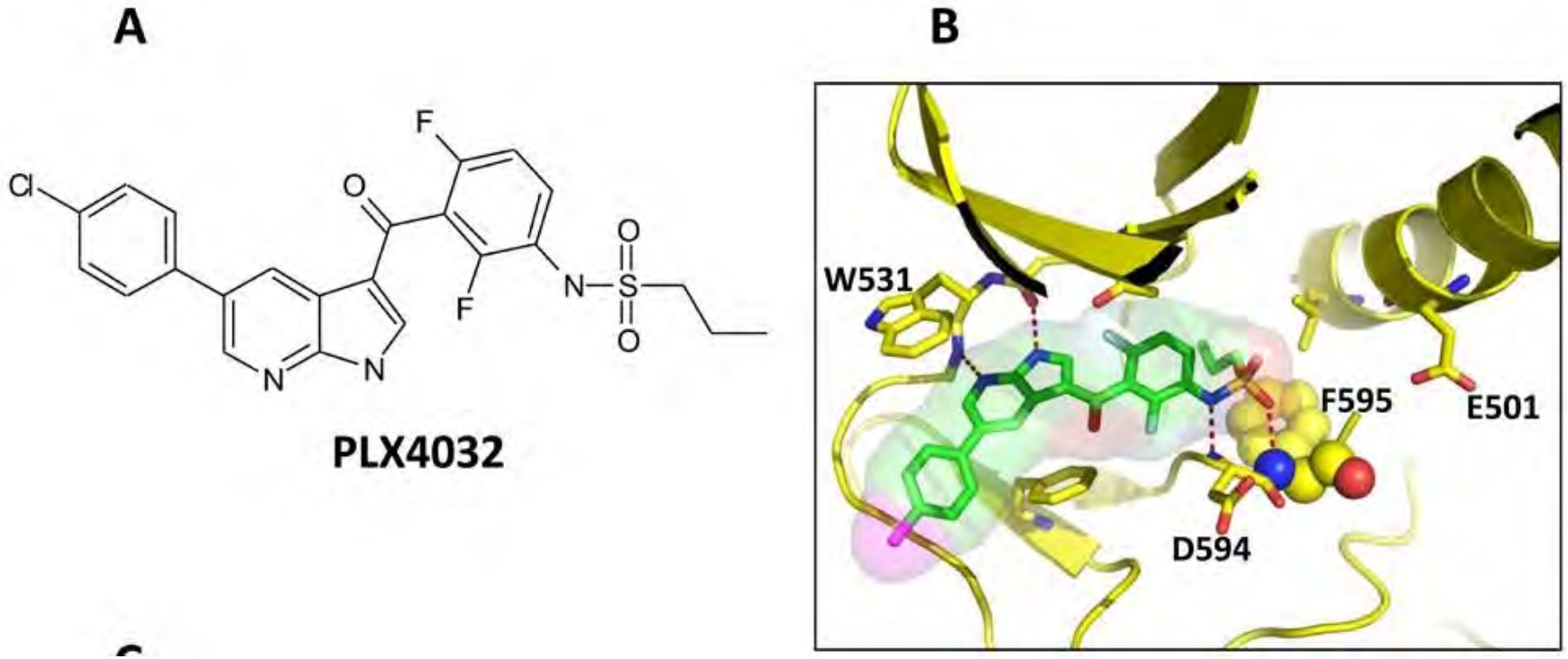
Ribas & Flaherty (2011) Nat Rev Clin Oncol

Melanomas cause the majority of deaths due to skin cancer

- malignant tumor of melanocytes
- Melanomas are the deadliest form of skin cancer
- Incidence increased by UV damage to skin
- Metastatic disease has a median survival of 6-12 months
- >50% of melanomas contain activating mutations in B-Raf (serine/threonine kinase), especially the V600E mutation

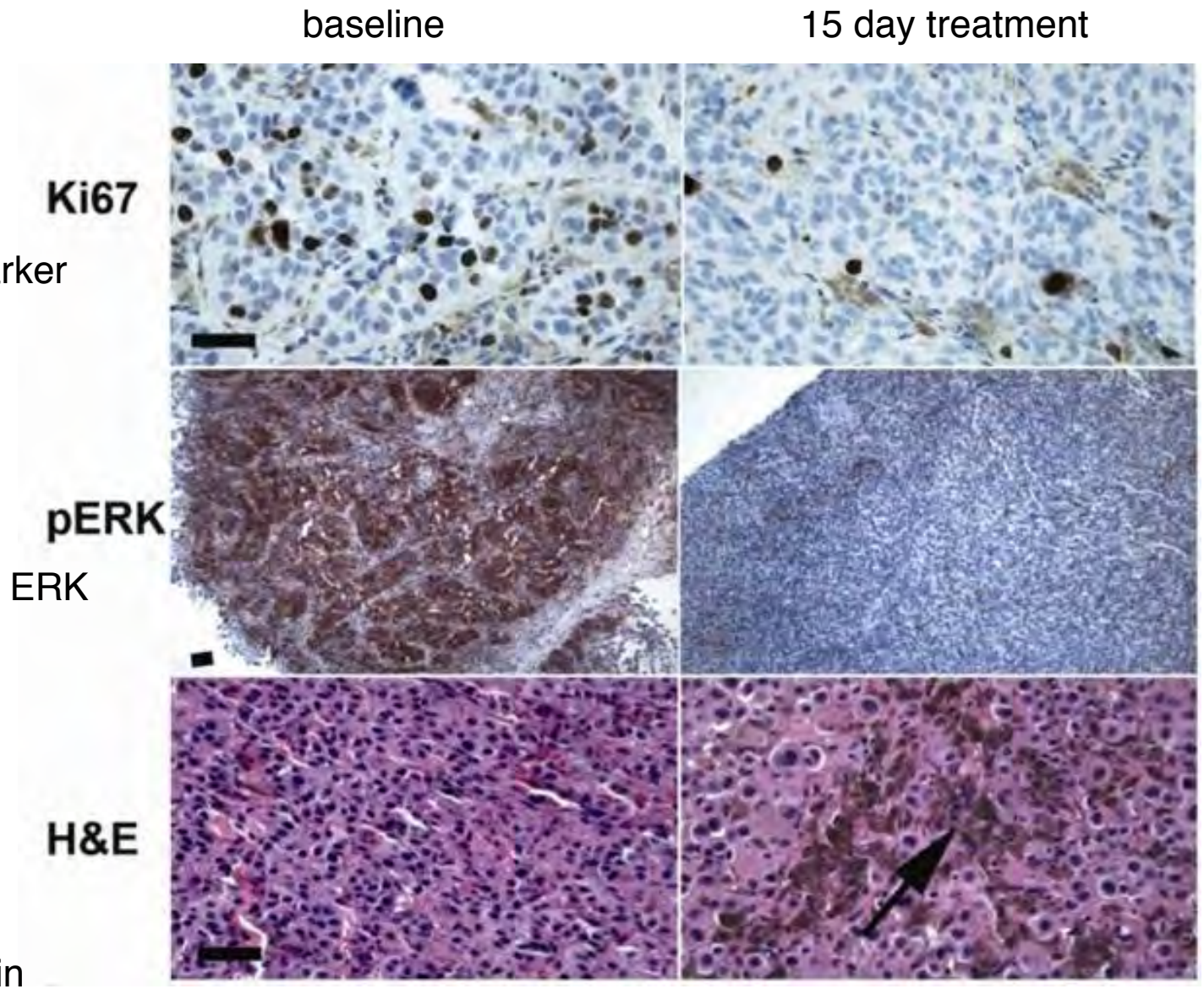
A small molecule inhibitor of B-Raf V600E

- ~50% of melanomas contain mutated B-Raf
- vemurafenib/PLX4032 is a small molecule inhibitor of B-raf V600E; inhibits kinase activity



Bollag et al. (2010) Nature

PLX4032 inhibits downstream signaling



Proliferation marker

Phosphorylated ERK

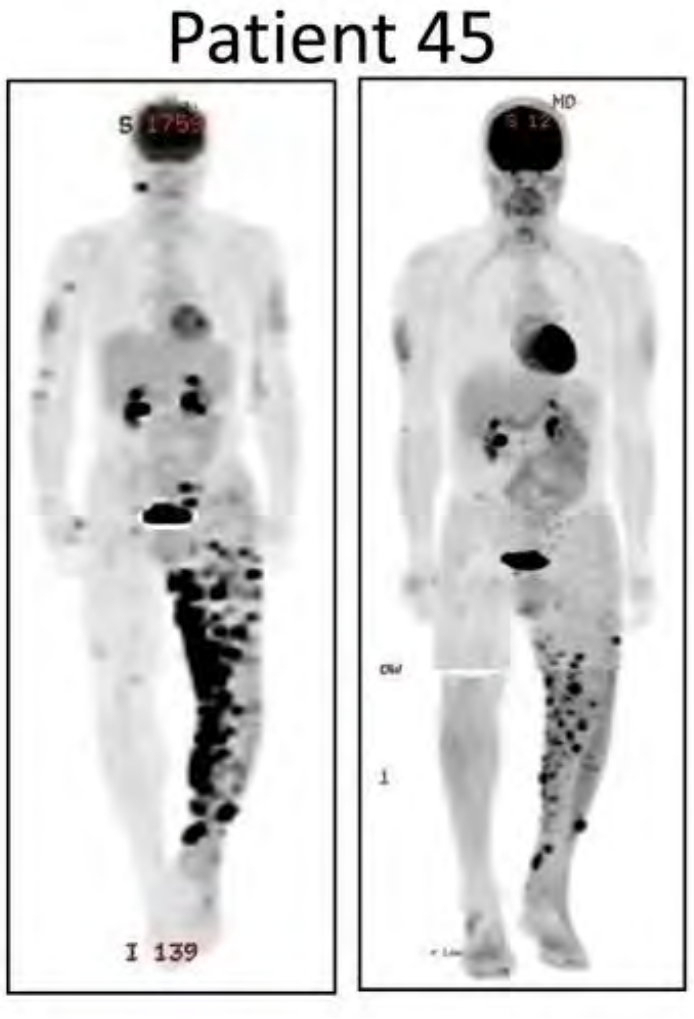
Arrow: tumor breakdown with macrophages engulfing the released melanin

Bollag et al. (2010) Nature

Clinical benefits of PLX4032

- 80% of patients with the B-Raf V600E mutation show reduction in tumor load
- tumor free progression ~6 months

PET scans taken pre-dose and following 2 weeks of dosing with PLX4032; uptake of glucose analog

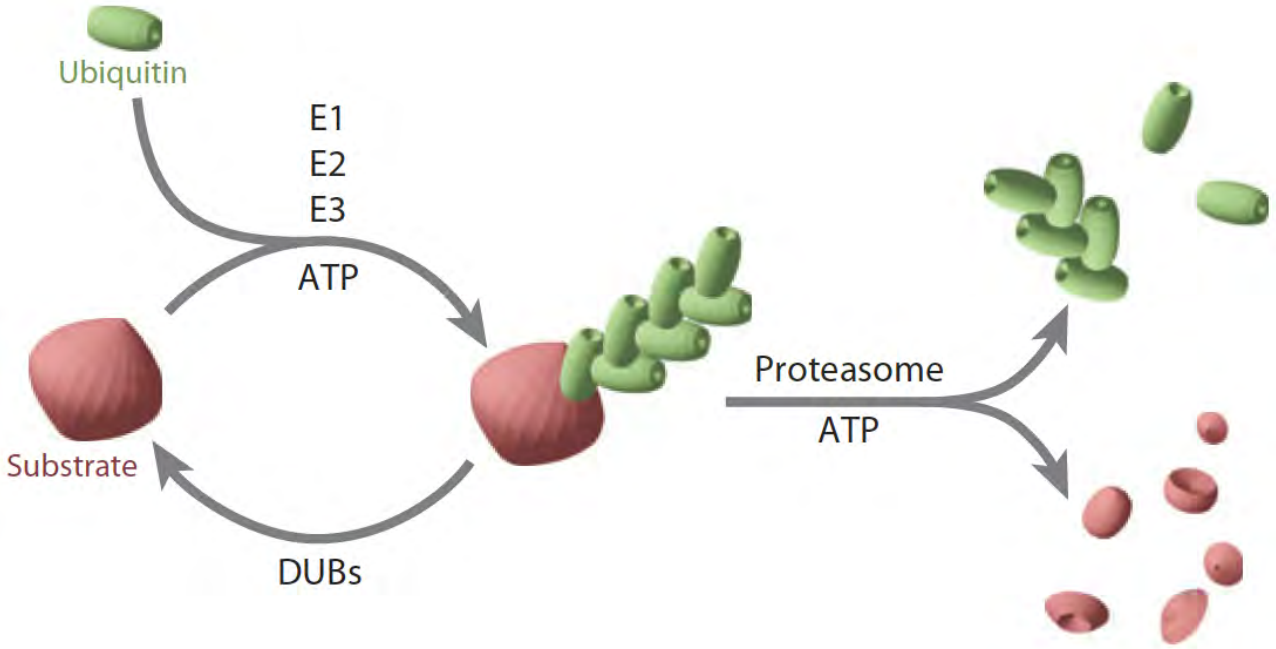


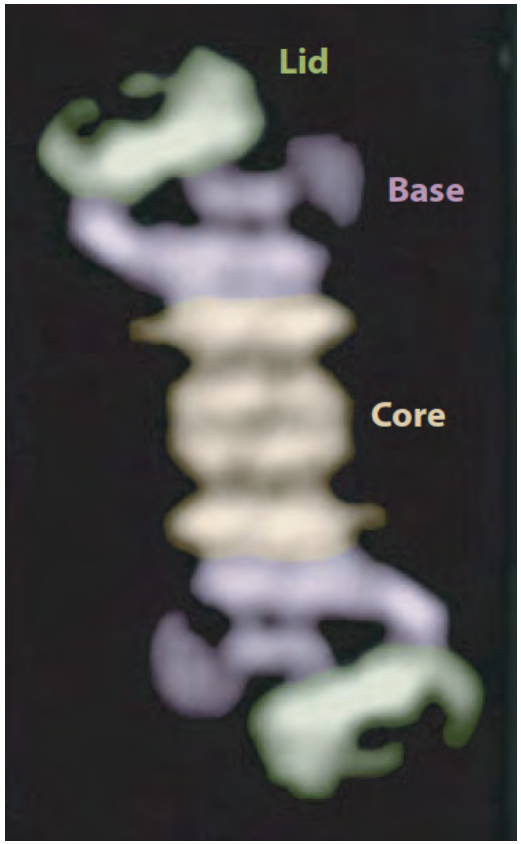
Bollag et al. (2010) Nature

Proteasome inhibitor effect against multiple myeloma

Bortezomib: tripeptide that binds to catalytic site of 26S proteasome; efficacy of drug against multiple myeloma poorly understood.

Multiple myeloma: cancer of plasma cells (differentiated B cells)

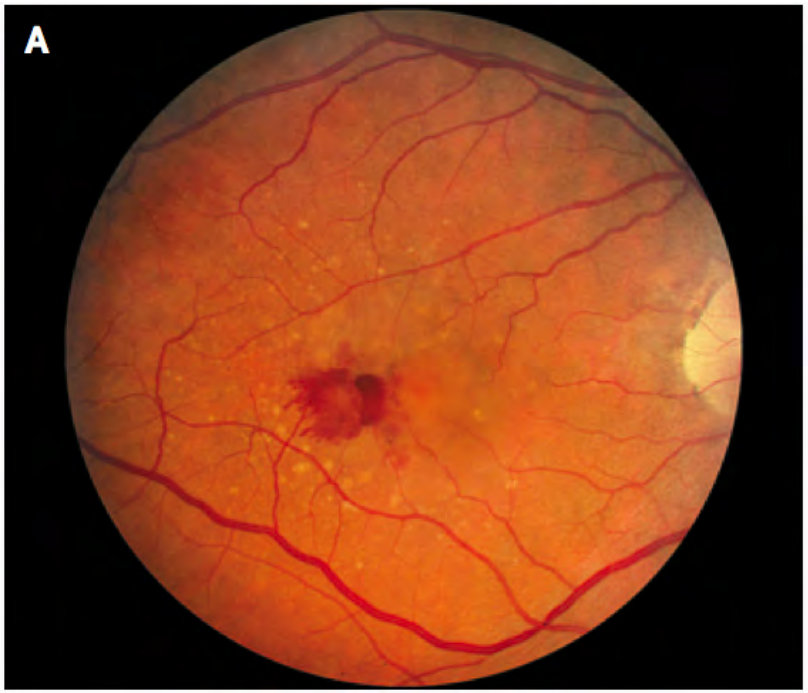




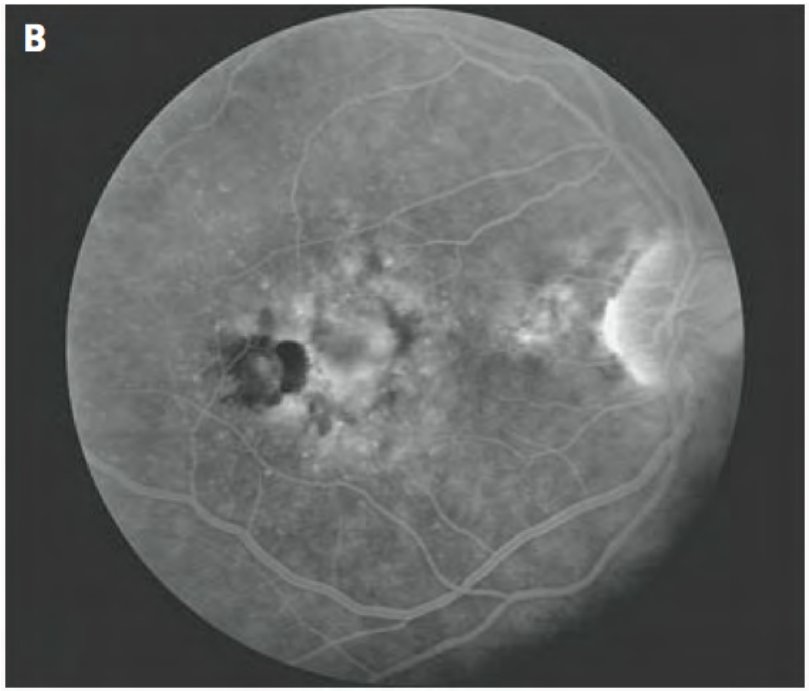
Finley. (2009) Ann Rev Biochem

Abnormal blood vessel growth leads to age-related macular degeneration (AMD)

- AMD is the leading cause of blindness in the US.
- "Wet" AMD is caused by abnormal growth of blood vessels beneath the retinal pigment epithelium and between the retinal pigment epithelium and the overlying retina
- Photoreceptor cells rely on the underlying retinal pigment epithelium for phagocytosis of their outer segments.



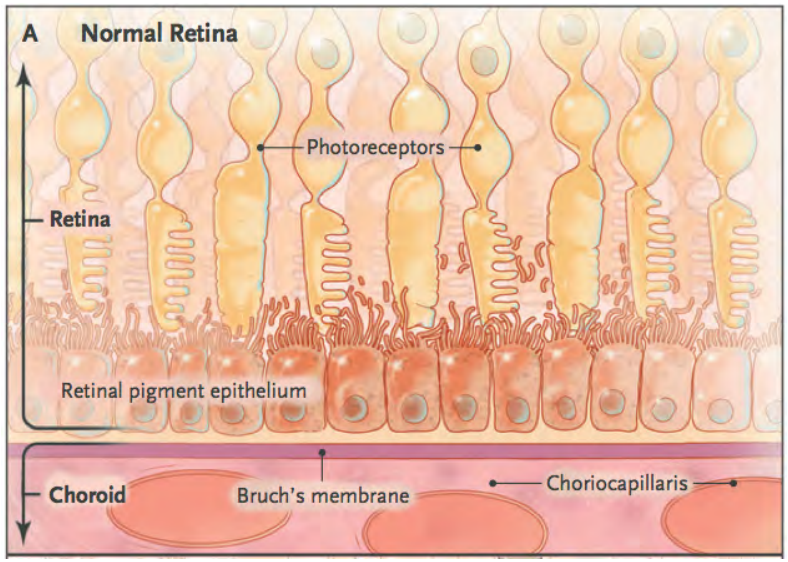
scattered yellow drusen, multilayered hemorrhage, and serous fluid in the macula

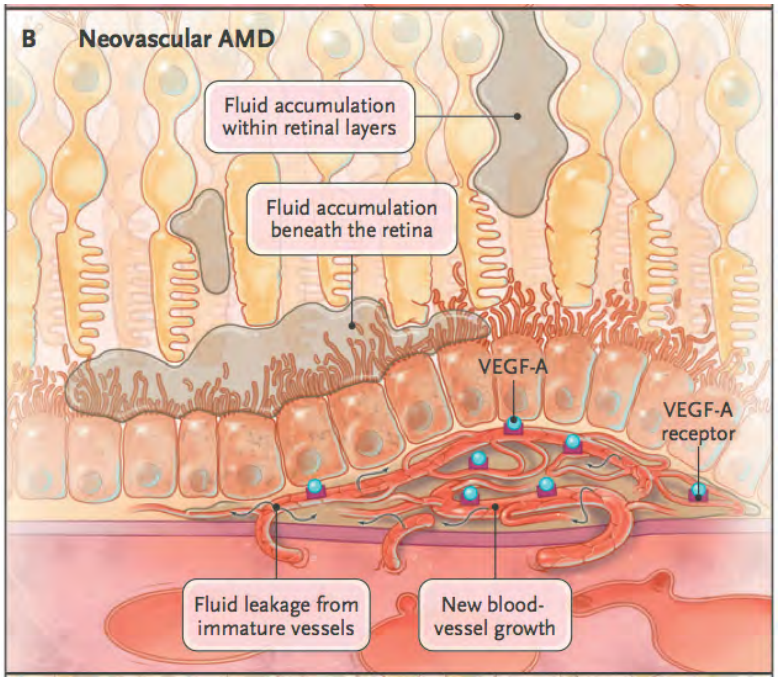


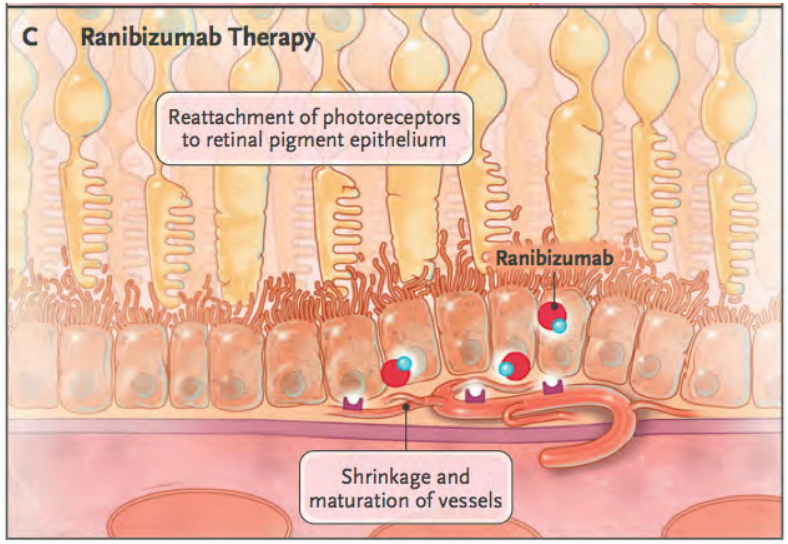
late-phase fluorescein angiogram of the right eye shows hyperfluorescence of occult choroidal neovascularization involving the center of the macula

Folk & Stone(2010) NEJM

Anti-angiogenic therapy for treatment of AMD



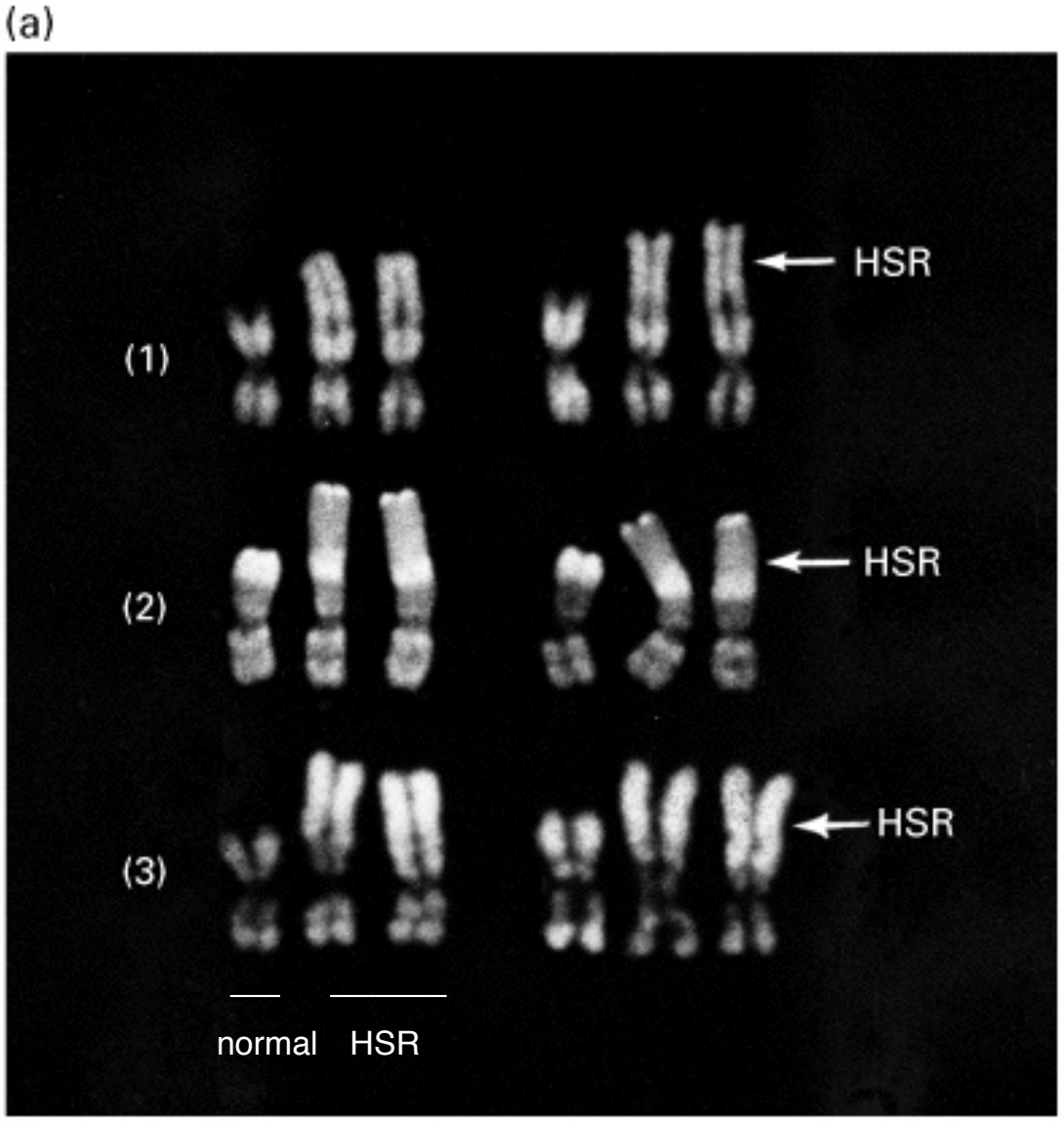




- Ranibizumab and Bevacizumab/Avastin are humanized monoclonal antibodies against VEGF-A
- Limited success in cancer trials
- Clinically effective for AMD via intraocular injection
- Bevacizumab/Avastin approved only for colon cancer, but often used (off-label) for AMD

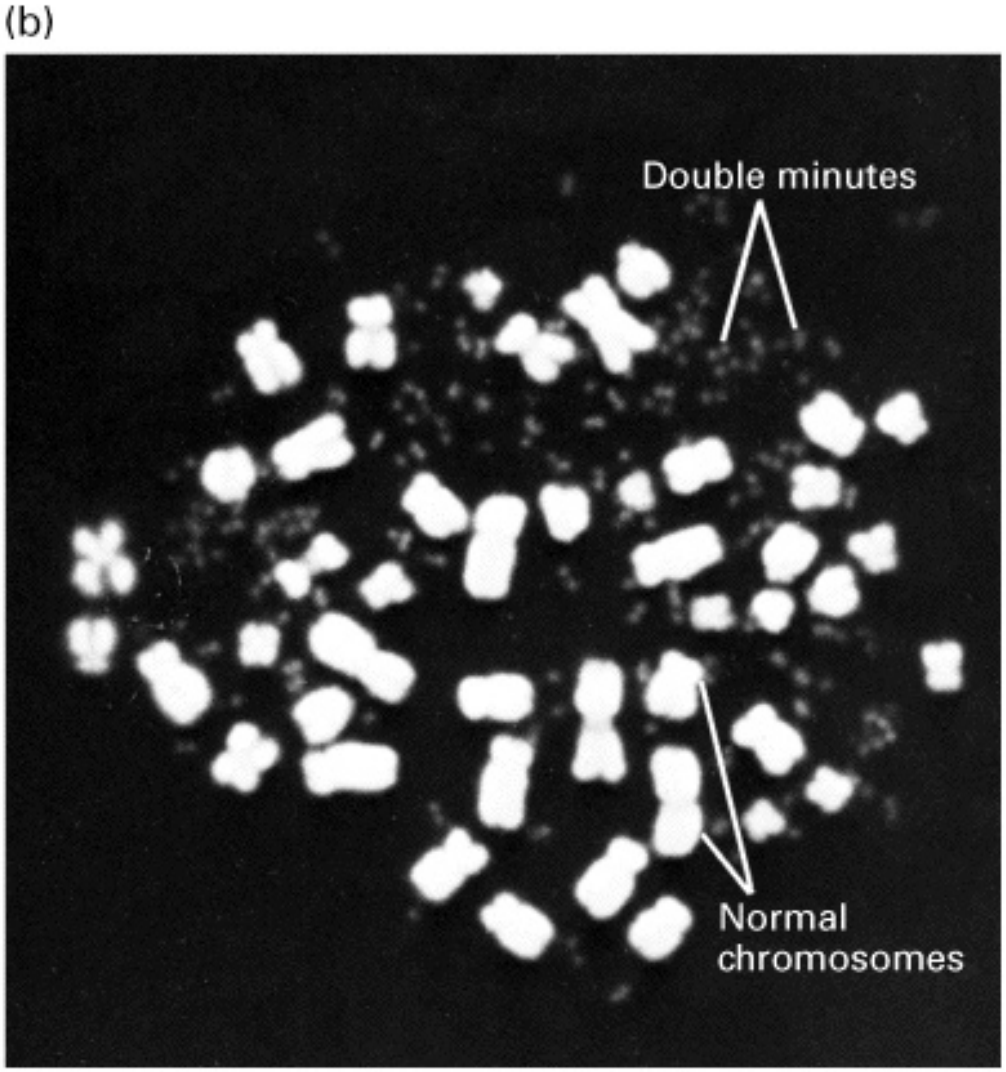
Folk & Stone(2010) NEJM

Oncogene amplification: homogeneous staining regions contain massively duplicated DNA



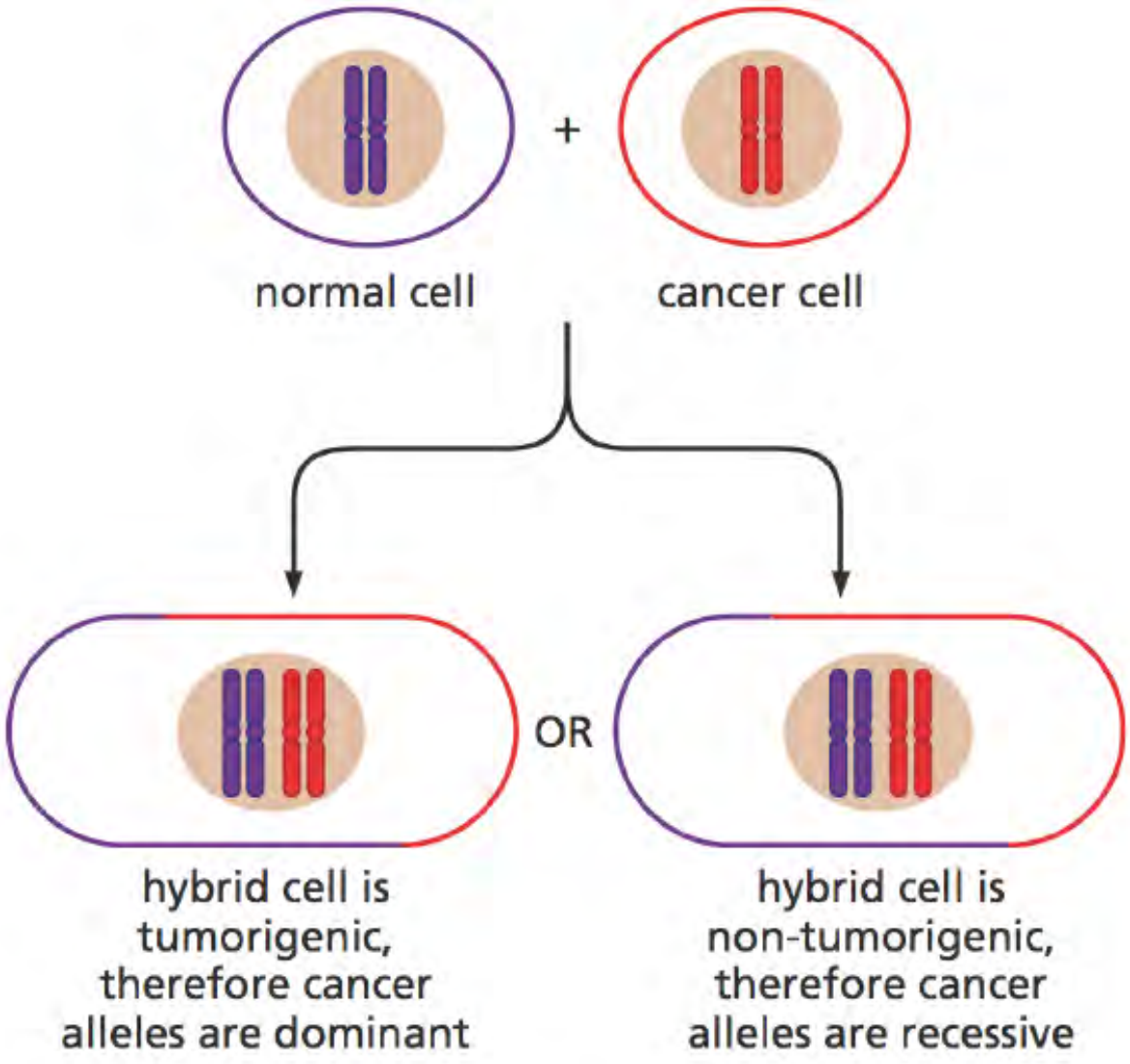
Lodish et al, 2000

Oncogene amplification: double minutes are miniature chromosome-like structures



Lodish et al, 2000

Cell hybrid experiments suggest that the tumor phenotype is usually recessive

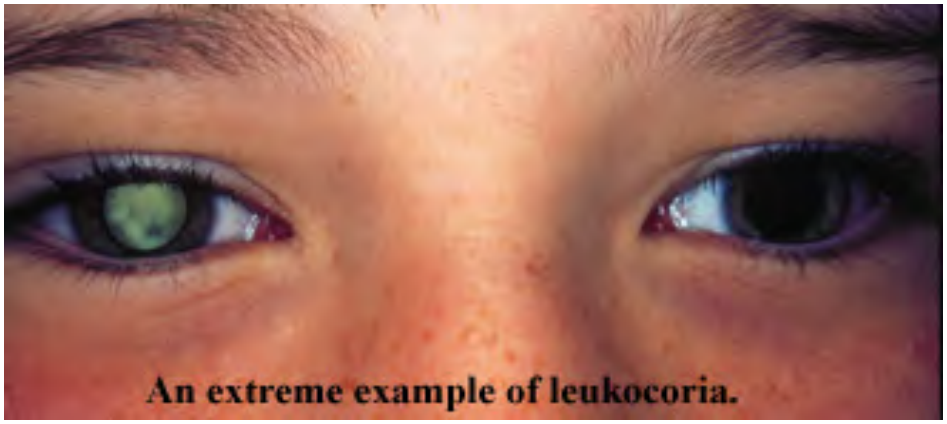


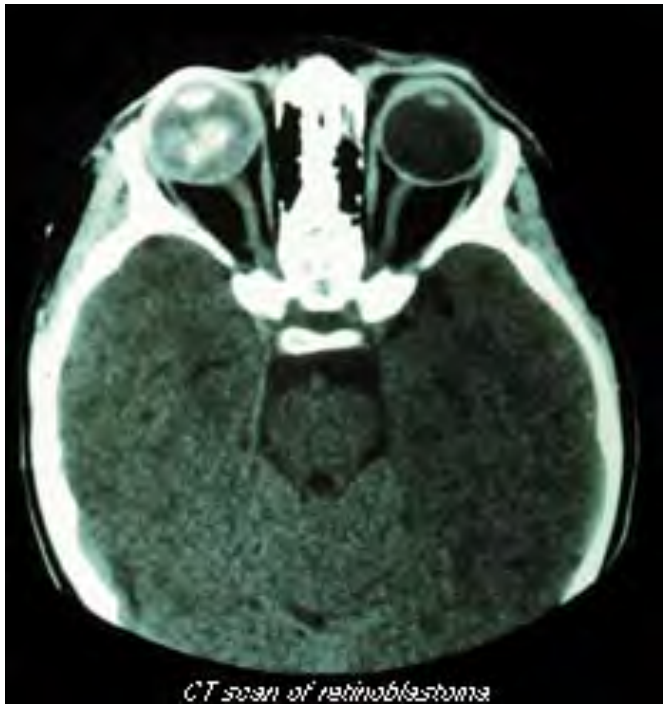
Weinberg (2013) The Biology of Cancer

Retinoblastoma: a childhood malignancy of the retina

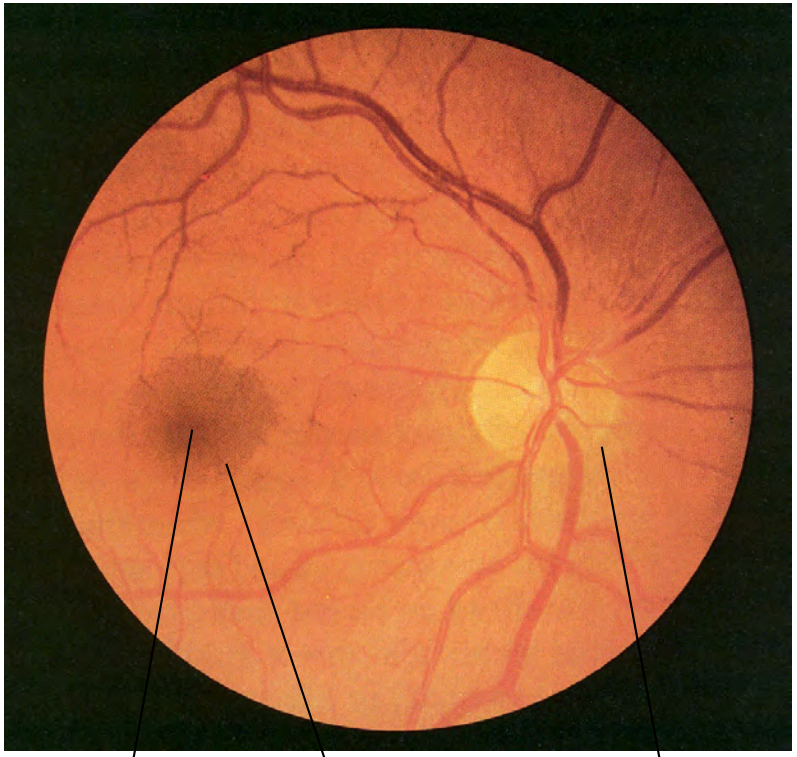
Affected eye does not have red reflex.

Leukocoria: white reflection from eye





Retinoblastoma seen in fundoscopic exam

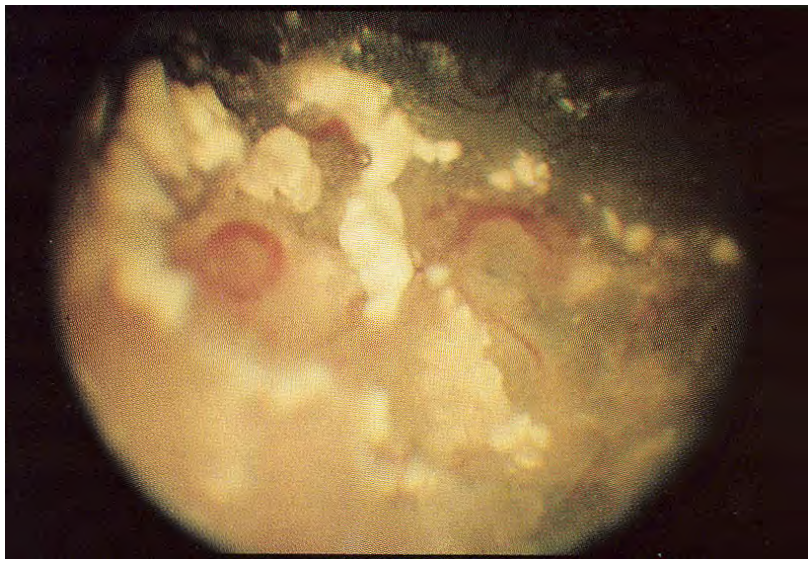


normal

fovea, sharpest vision (center of macula)

macula

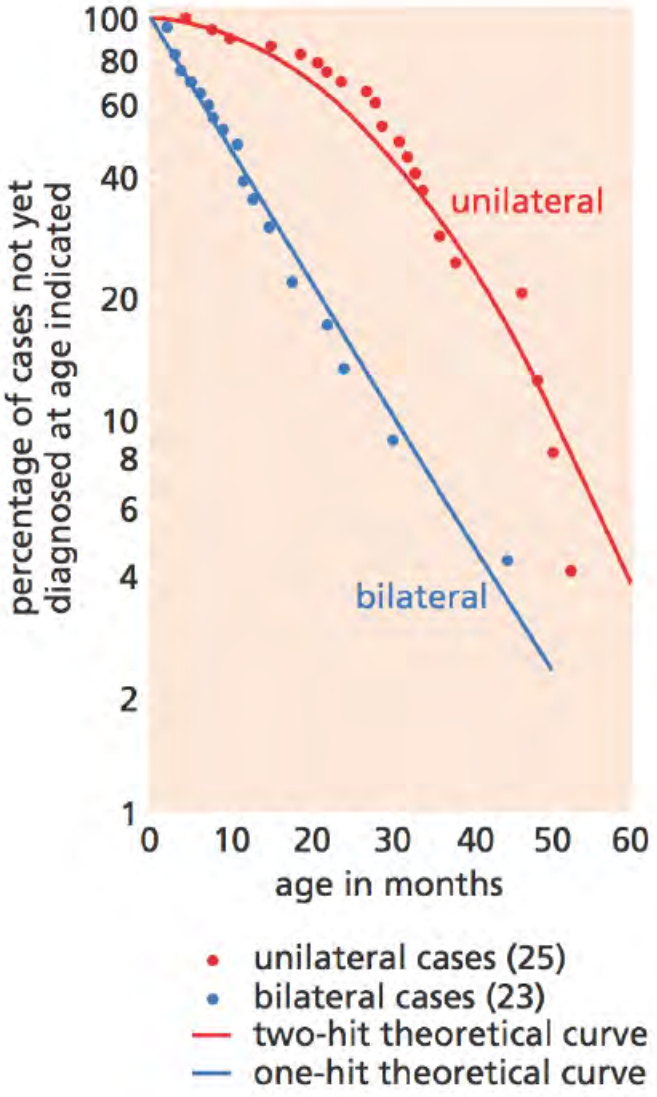
optic dics (where axons of retinal ganglion cells leave eye to form optic nerve)

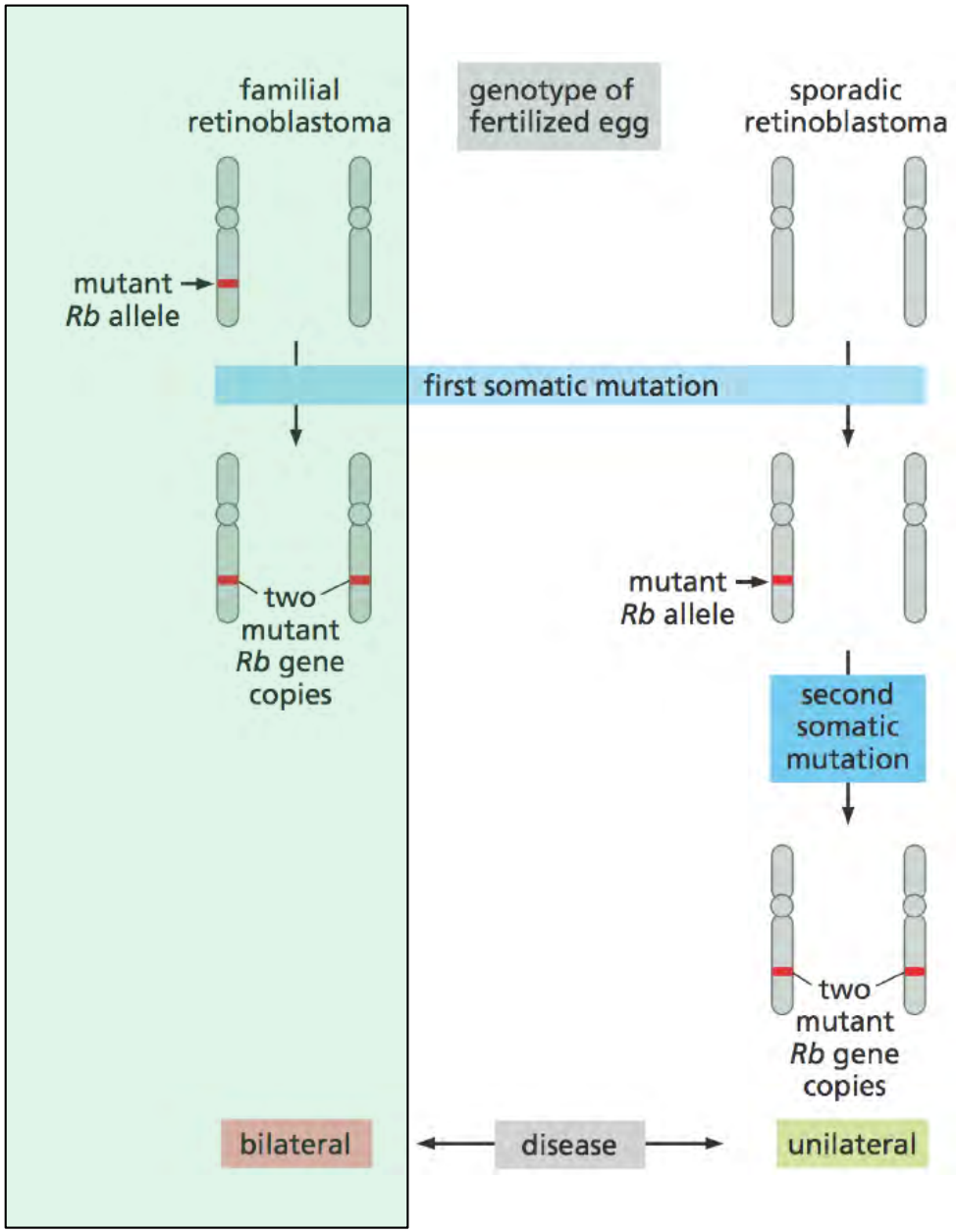


Retinoblastoma with calcification

Paton et al, 1976

Knudson's 2-hit hypothesis





Tumor kinetics argued for a 1-hit model in bilateral Rb, and a 2-hit model in bilateral (sporadic) Rb.

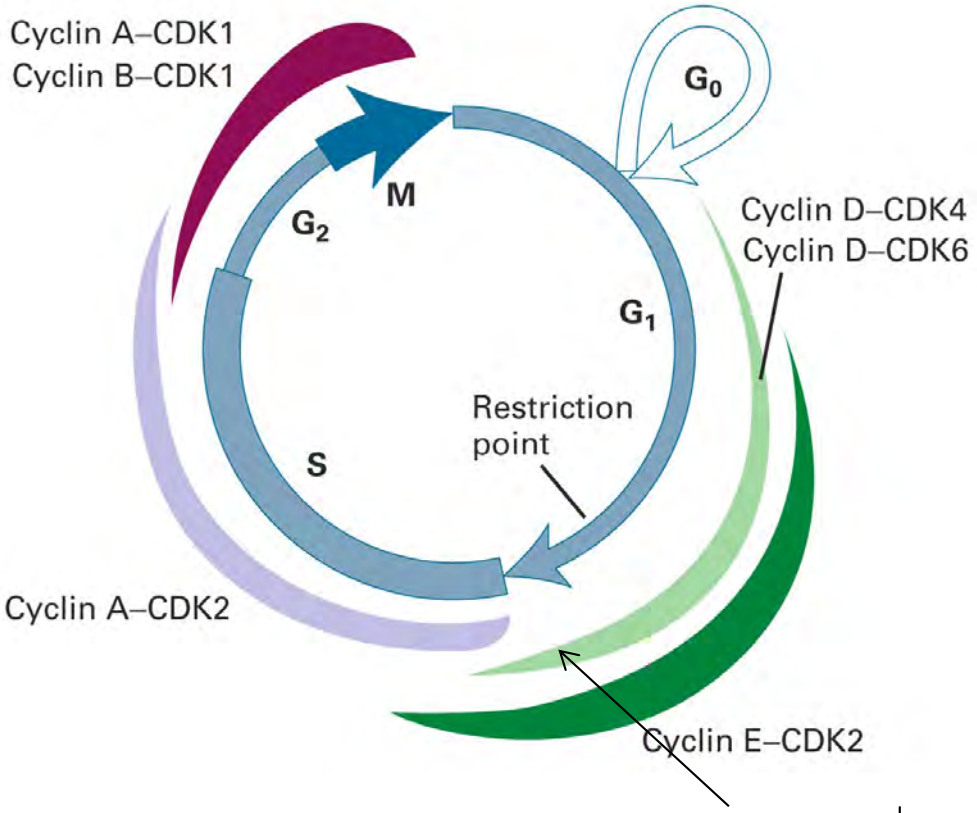
Weinberg (2013) The Biology of Cancer

Rb mutations found in many human tumors

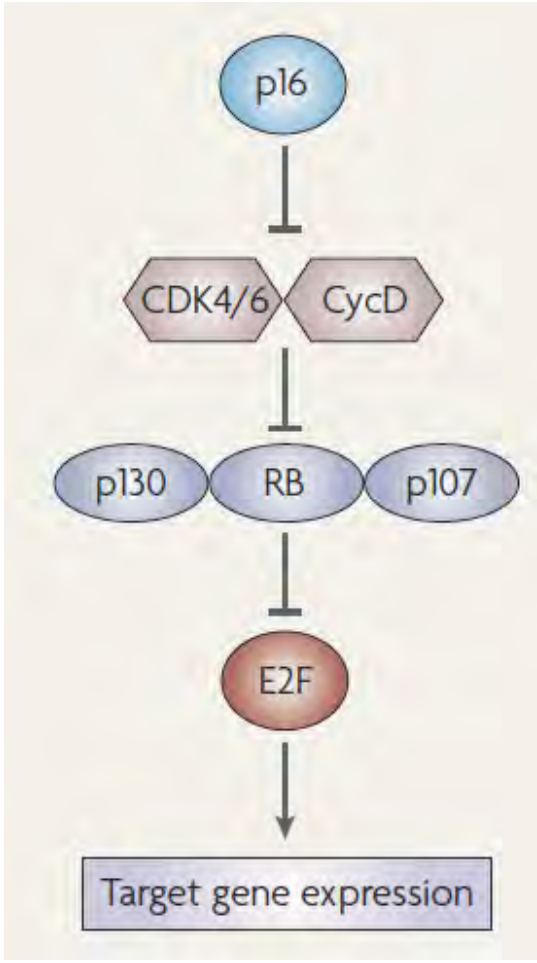
Tumour type	Frequency of *RB* inactivation (genetic or epigenetic)
Lung cancer	Germline *RB* mutations predispose to small cell lung carcinoma (SCLC), and *RB* is inactivated in >90% of sporadic SCLC cases. In contrast, *RB* is mutated in only 15–30% of non-SCLC cases.
Melanoma	*RB* inactivation is rare in sporadic cases, but inherited mutation predisposes to melanoma
Prostate cancer	~20%
Breast cancer	~20%
Bladder cancer	20–50%
Leukaemia	Reduced levels of expression are frequent, but mutations in *RB* are rare in leukaemias, except in 20% of chronic myeloid leukaemia (CML) cases
Brain cancer	*Rb*-mutant mice develop pituitary tumours, but *RB* mutations are rare in human cases. 15–30% of advanced gliomas have *RB* mutations
Oesophageal cancer	*RB* deletion are found in 15–50% of adenocarcinomas or squamous cell carcinomas
Liver cancer	Mutations in *RB* are found in 15–30% of the advanced hepatocellular carcinomas[§]

Burkhart & Sage (2008) Nat Rev Cancer

Rb as a negative regulator of the cell cycle



Lodish et al (2004)



Burkhart & Sage (2008) Nat Rev Cancer

E2F transcription factor family functions in late G1 to activate proteins of S-phase.

E2F acts as tx repressor when complexed with hypo-phosphorylated Rb.

G1 cyclin-CDK phosphorylates Rb.

mid G1: CyclinD/CDK4/4
late G1: Cyclin E/CDK2

S-phase cyclins maintain Rb in inactive phosphorylated form till after mitosis.

E2F ⊣ Rb (hypophosphorylated)

Tumor suppressors in familial cancer syndromes

• **Familial retinoblastoma**: AD disease caused by mutation in Rb; retinal cancers

• **Familial adenomatous polyposis**: AD disease caused by mutation in APC gene; 100s-1000s of adenomatous polyps in colon/rectum; some polyps progress to colorectal carcinoma.

• **Li-Fraumeni syndrome**: AD disease caused by mutation of p53; predisposition to wide range of tumors (sarcomas, breast cancer, brain tumors, leukemia, adrenocortical carcinoma).

• **Wilm's tumor**: nephroblastoma (kidney tumor) mostly in children; mutation in WT1 (zinc finger tx factor)

• **Hereditary breast-ovarian cancer syndrome**: mutations in BRCA1 or BRCA2 genes predispose to breast cancer; both play a role in DNA repair; mutations predispose to genetic instability.

• **Neurofibromatosis**: AD disease with cafe-au-lait spots and neurofibromas (benign Schwann cell tumor of nerve sheath) ; NF1 gene is a RAS-GAP

APC inhibits Wnt signaling

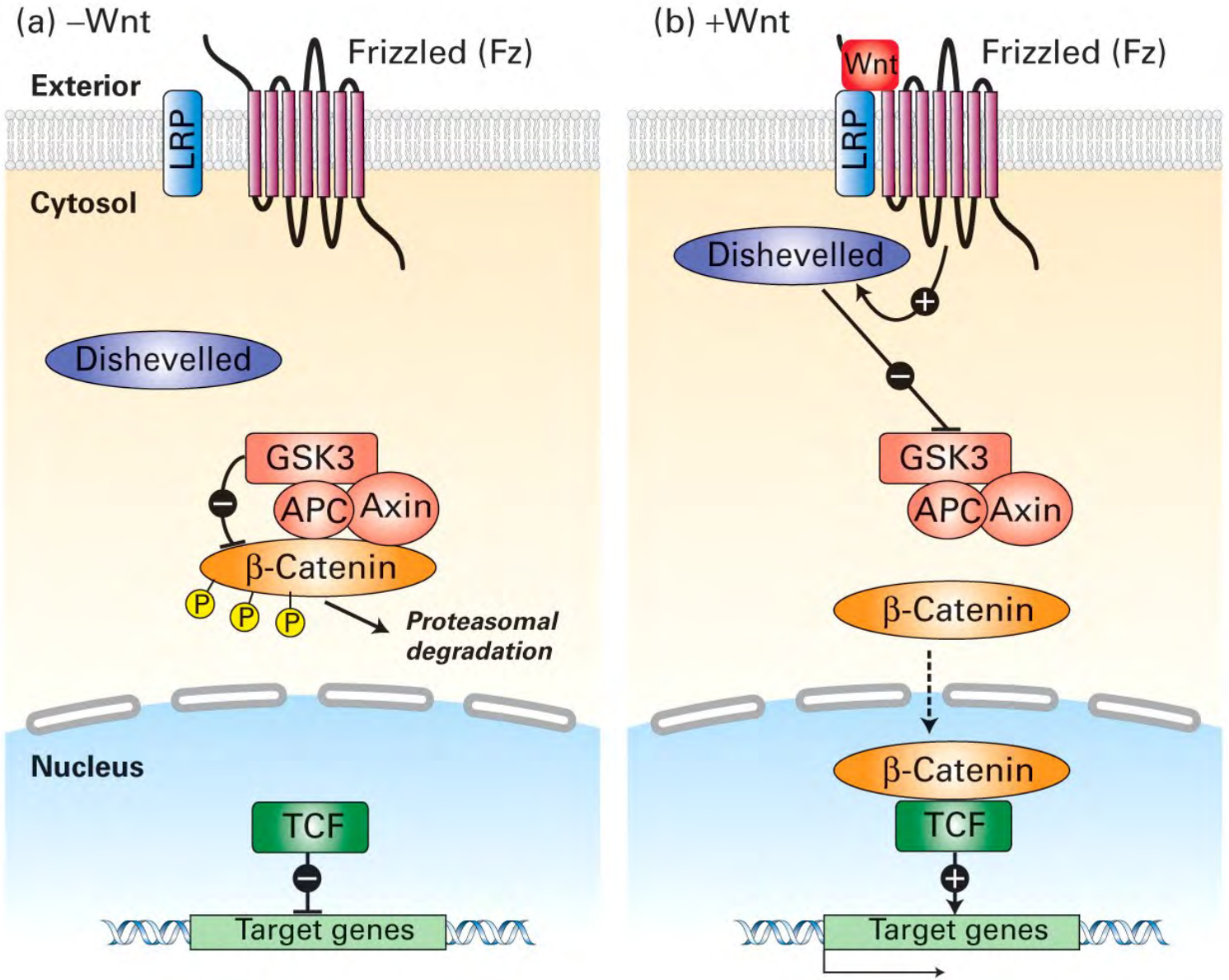
Loss of APC leads to adenomatosis polyposis coli

β-catenin =Armadillo

The complex of GSK3/Axin/APC inhibits β-catenin function by phosphorylation, which promotes ubiqutination and proteolysis.

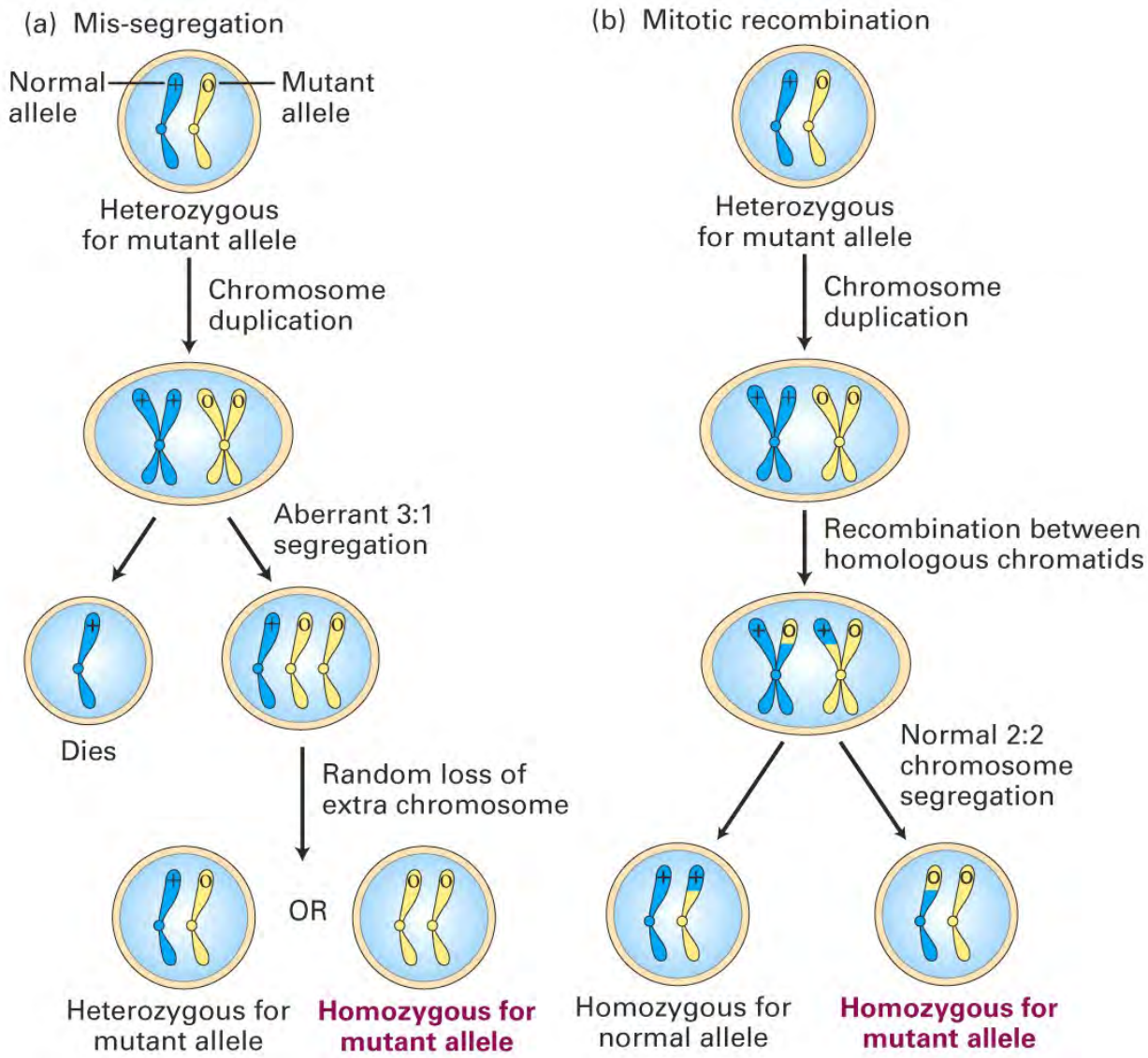
Wnt signaling, via dishevelled, disrupts this inhibitory complex and allows β-catenin to enter nucleus.

Nuclear β-catenin forms a complex with TCF/LEF transcription factors.



Lodish et al, 2004

Mechanisms for loss of heterozygosity: nondisjunction and mitotic recombination



A third mechanism is mutation of the normal allele.

Lodish et al, 2004

Transformation of human cells

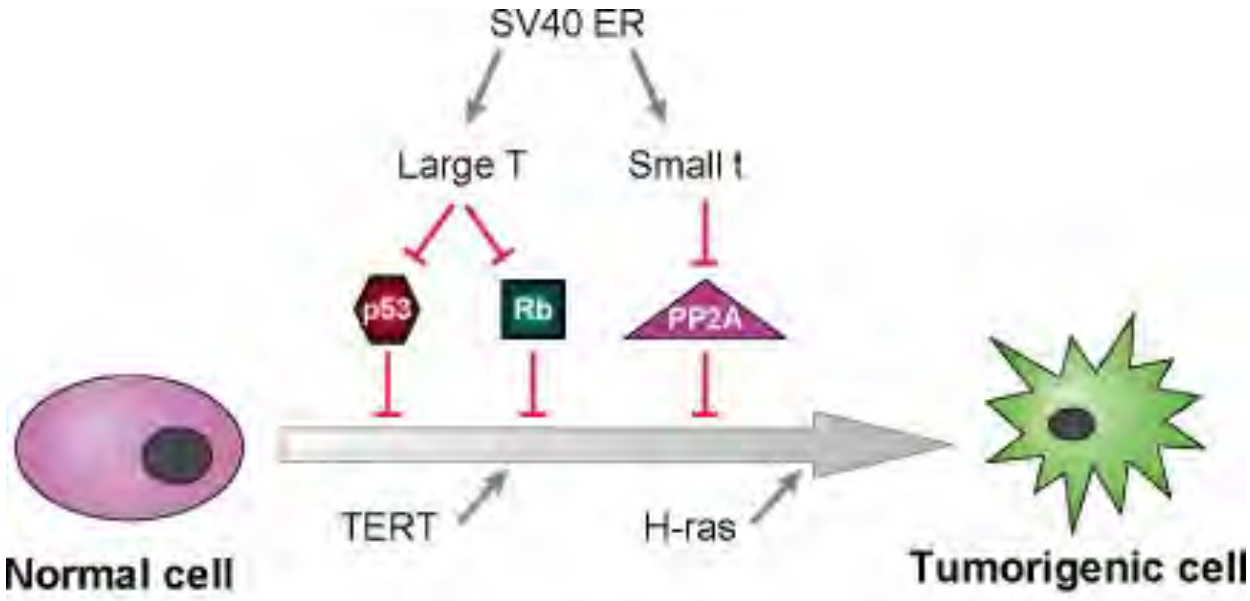
Primary rodent fibroblasts can be transformed with Ras and Myc.

This combination does not transform human cells.

Human cells can be transformed by the following combination:

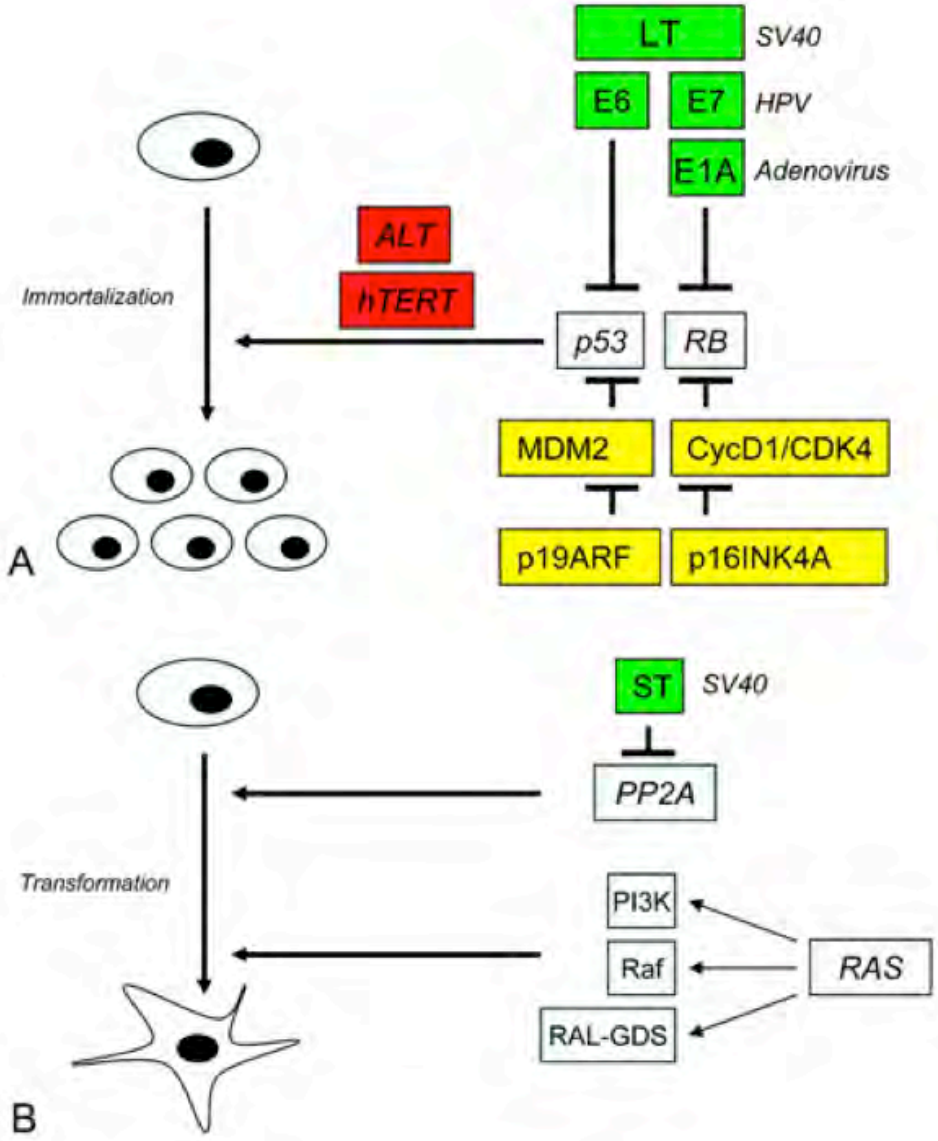
- inactivation of p53 and Rb
- inhibition of protein phosphatase 2A
- H-Ras expression
- hTERT expression

leads to colony formation in soft agar and tumor formation in immunosupressed mice.



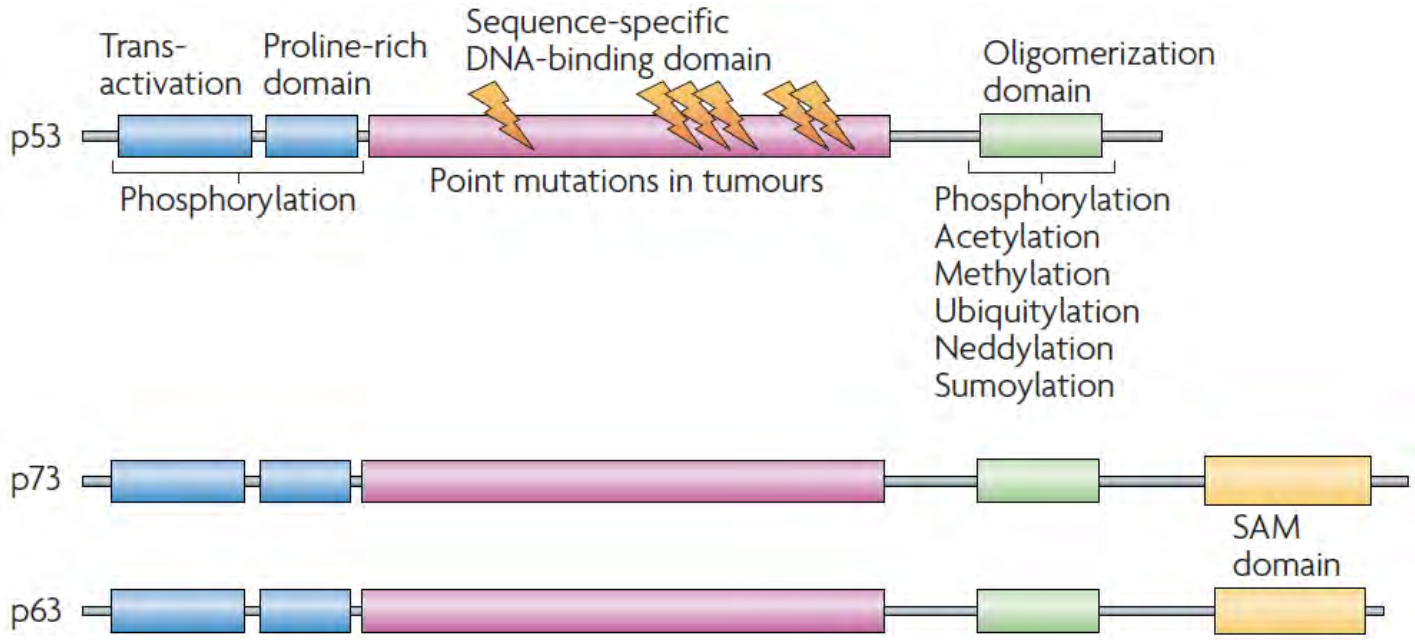
Stewart SA, Weinberg RA. 2006.
Annu. Rev. Cell Dev. Biol. 22:531–57

Pathways to transformation of human cells



Schinzel and Hahn (2008)
Frontiers in Bioscience

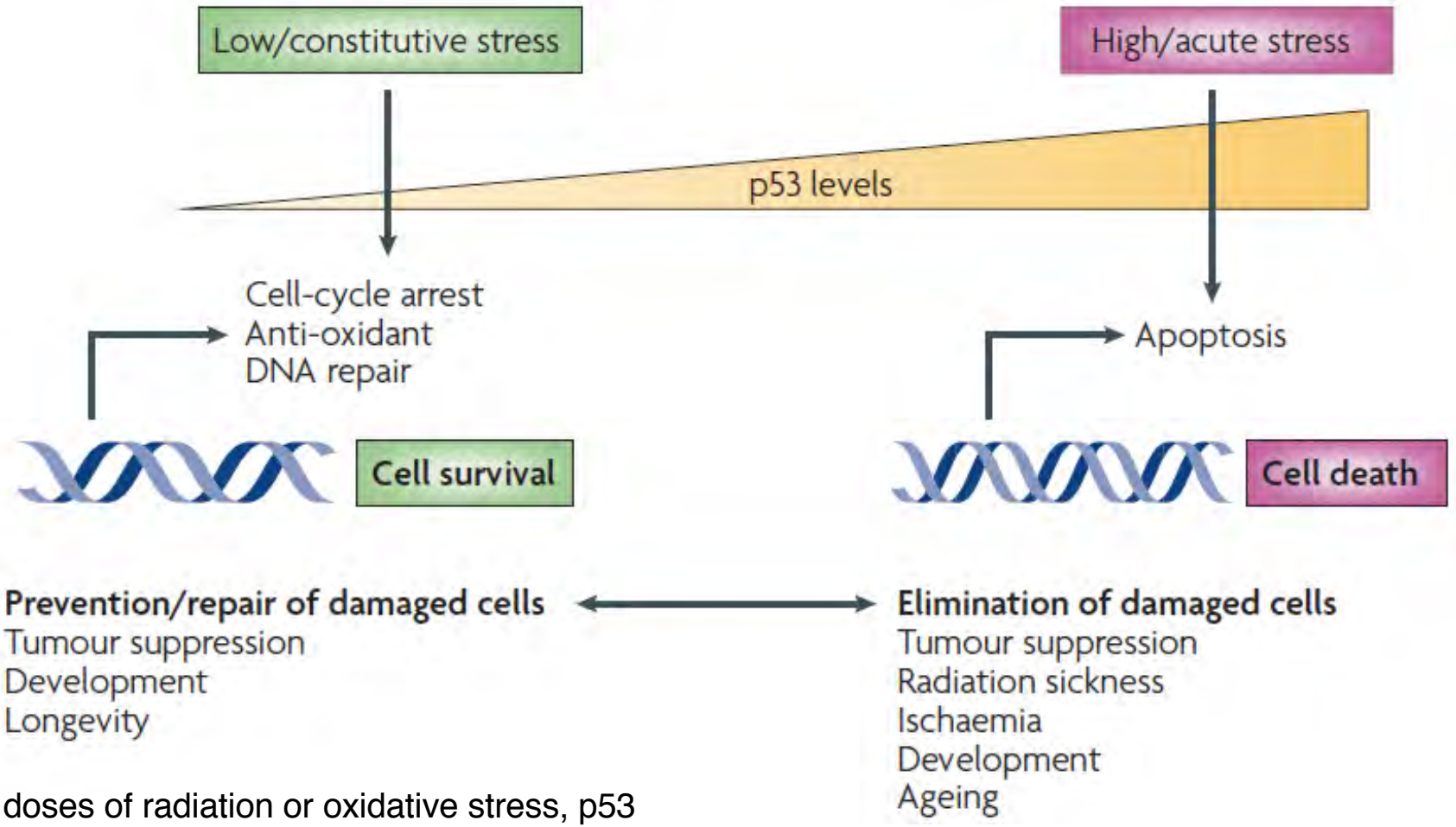
p53 family



•p53 identified as protein bound to large T antigen in SV40-transformed cells. “Tumor antigen”
•Found in high levels in some tumors. p53 expression cooperated with H-Ras; later found to be mutant p53.
•Normal p53 inhibited transformation by E1A or (Myc+H-Ras)
•50% of human tumors contain p53 mutations.

Vousden & Lane (2007) NRMCB

p53 and stress



•With low doses of radiation or oxidative stress, p53 causes cell cycle arrest (G1 or G2), allow DNA repair prior to continuation of cell cycle. Cells without p53 are therefore prone to acquire tumorigenic mutations.
•With high acute stress/damage, more robust activation leads to activation of apoptosis. This prevents survival of cells with tumorigenic mutations.

Vousden & Lane (2007) NRMCB

Models of mutant p53 action

- Point mutations occur in many sporadic tumors.
- Germline p53 mutations cause Li-Fraumeni syndrome (LFS)
- Most LFS alleles contain point mutations in the DNA binding region and are loss-of-function alleles

Dominant-negative model

hetero-oligomerization between mutant and wt p53 results in dominant-negative effect

Gain-of-function model

mutant p53 actively promotes tumorigenesis

Models are not mutually exclusive.

Olive et al (2004) Cell

Mice with p53 null alleles have increased tumors

Mice of p53 null alleles are cancer-prone.

Heterozygous and homozygous p53 mice develop primarily sarcomas and lymphomas. Carcinomas are rare or low-grade; this is different from LFS patients.

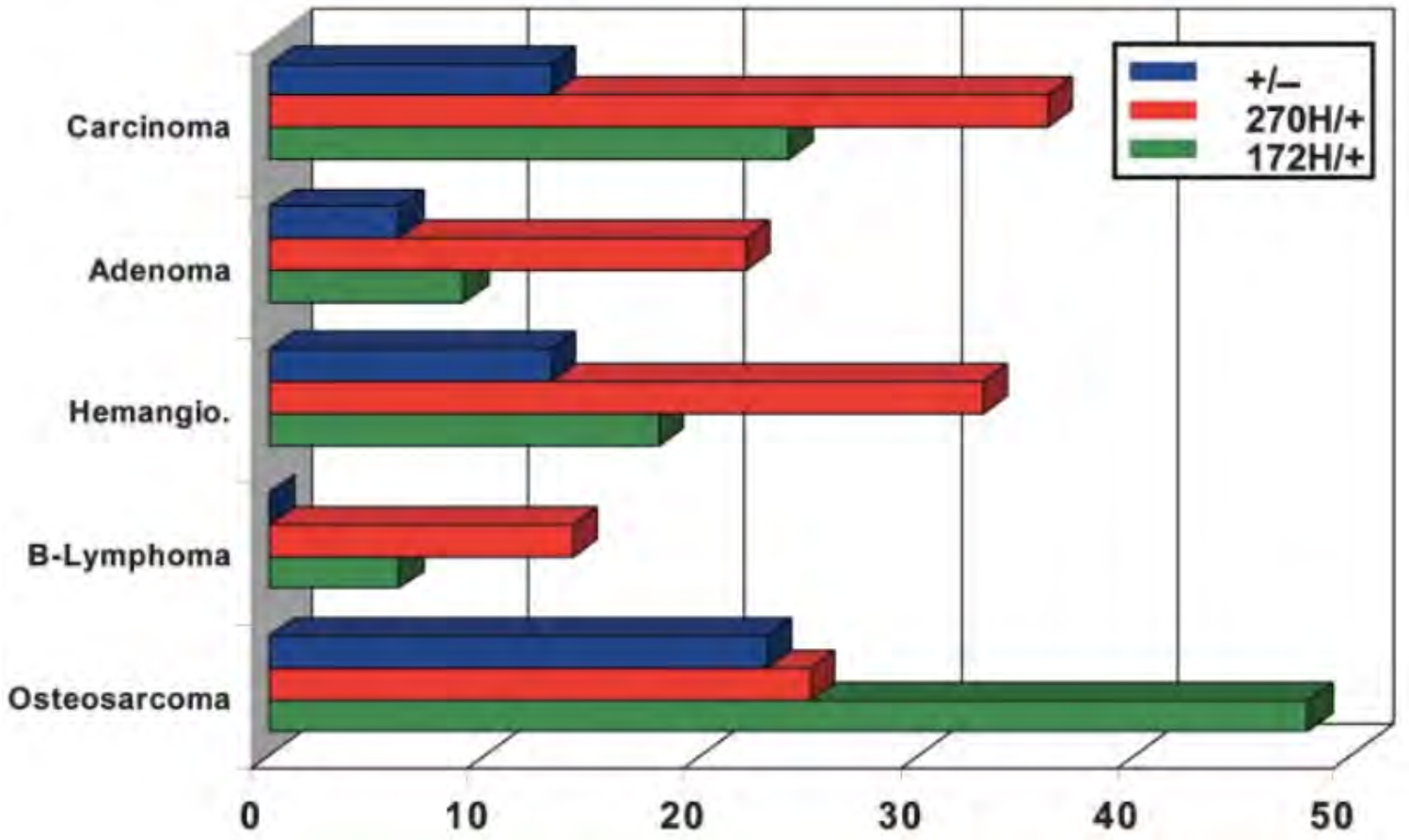
p53 is not essential for development.

Mouse knock-in models of Li-Fraumeni Syndrome

The tumor spectrum of LFS knock-in mice are different from p53 heterozygous mice.

•increase in carcinomas (esp 270/+ line), including lung adenocarcinomas, squamous cell carcinomas, and hepatocellular carcinomas. Many were invasive or metastatic.

•Loss of heterozygosity found in many tumors.



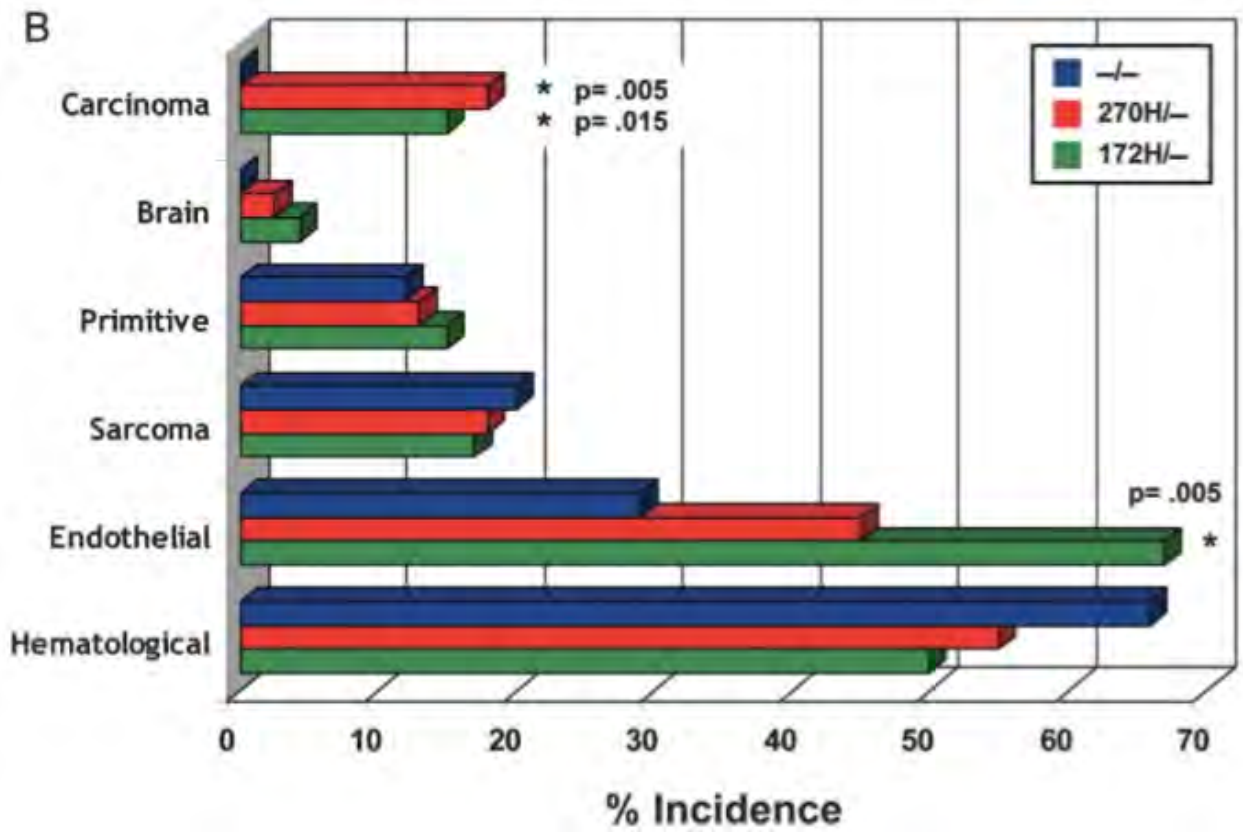
Olive et al (2004) Cell

Mouse knock-in models of Li-Fraumeni Syndrome

p53 null mice developed lymphomas and sarcomas but rarely carcinomas.

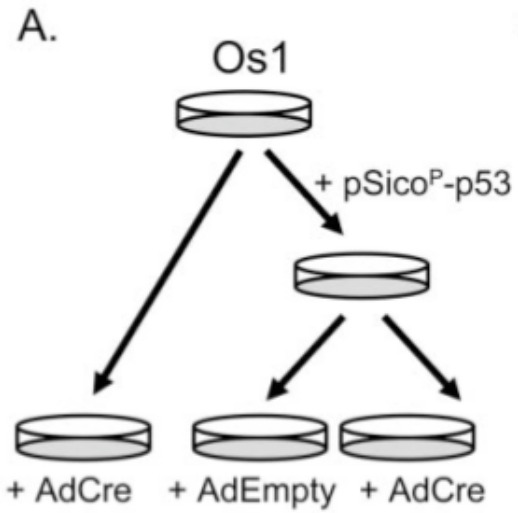
Knockin/null mice had carcinomas that were invasive or metastatic.

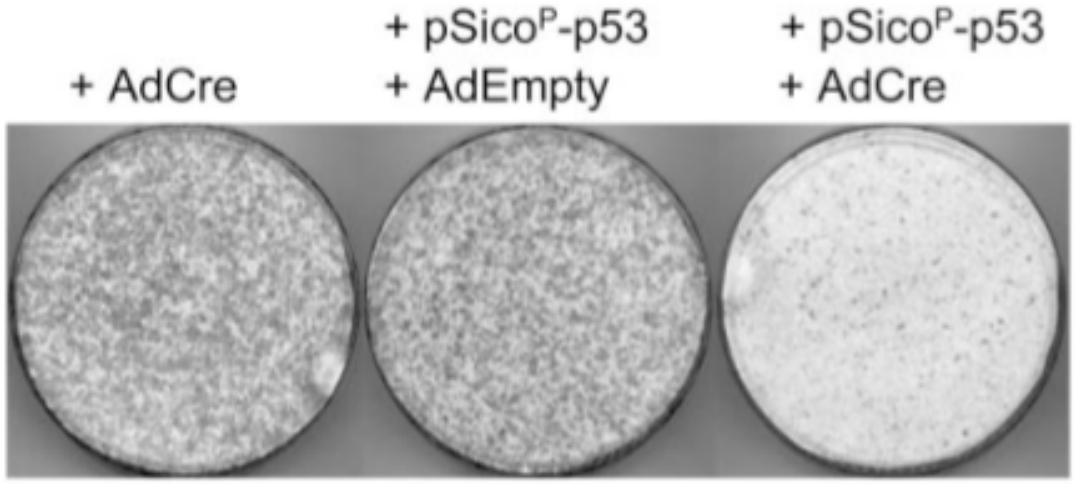
Mutant p53 accumulated in tumor cells, as in LFS tumors.



Olive et al (2004) Cell

Mutant p53 has gain-of-function effect in tumor cells





Os1 is an osteosarcoma cell line from a $p53^{R172H/+}$ metastasis. The WT allele was lost.
pSico is an shRNAi vector that is Cre-dependent.

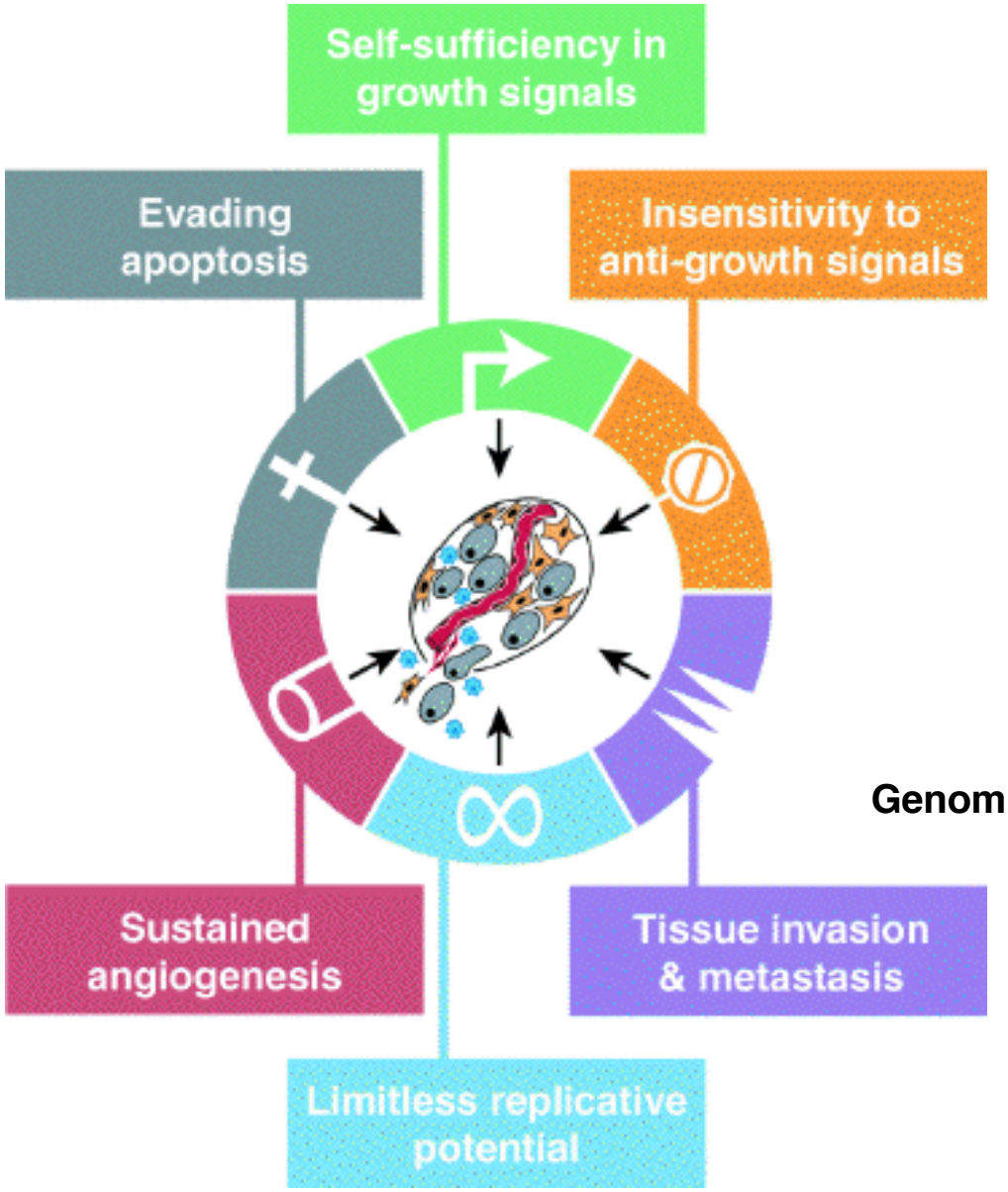
Down-regulation of mutant p53 in tumor cells leads to growth defect. Maybe due to an interaction of mutant p53 with the p53 homologs p63 and p73.

Olive et al (2004) Cell

Identifying genes involved in cancer

I. Retroviruses
    A. Acute transforming viruses
    B. Slow transforming viruses-- promoter/enhancer insertion
II. Isolation by DNA transfection
III. Chromosomal abnormalities
    A. Amplification
    B. Translocation
IV. Positional cloning of tumor suppressor genes
V. DNA tumor viruses

Characteristics of malignant tumor cells



Genomic Instability

Hanahan & Weinberg (2000) Cell 100:57

Defects in DNA repair facilitate oncogenesis

TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411**:366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509**:49.

Lodish et al, 2004

DNA repair by base excision repair

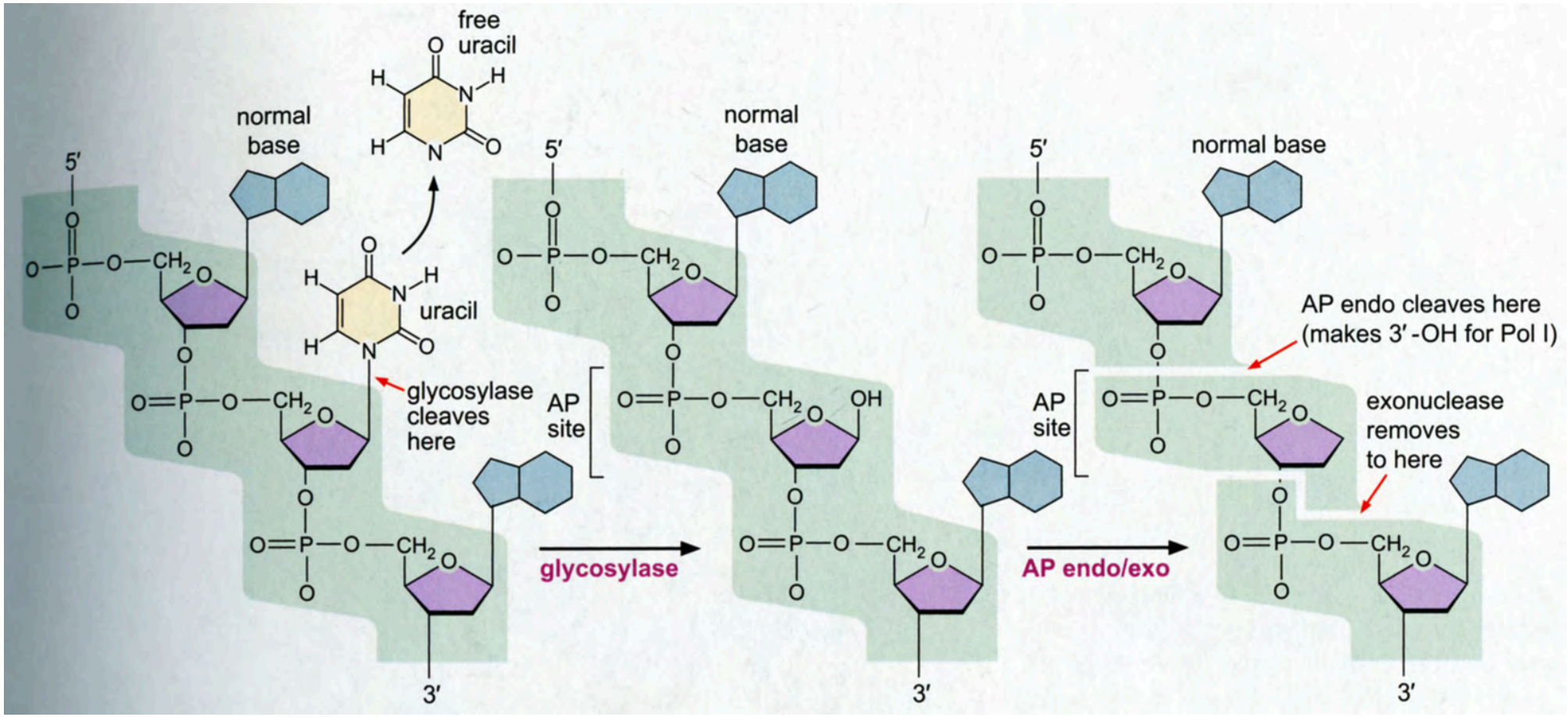
Excision repair systems: used when damage occurs on single strand; damaged nucleotide is removed and the opposite strand is used as a template.

- Base excision repair

- Nucleotide excision repair

Xeroderma pigmentosum

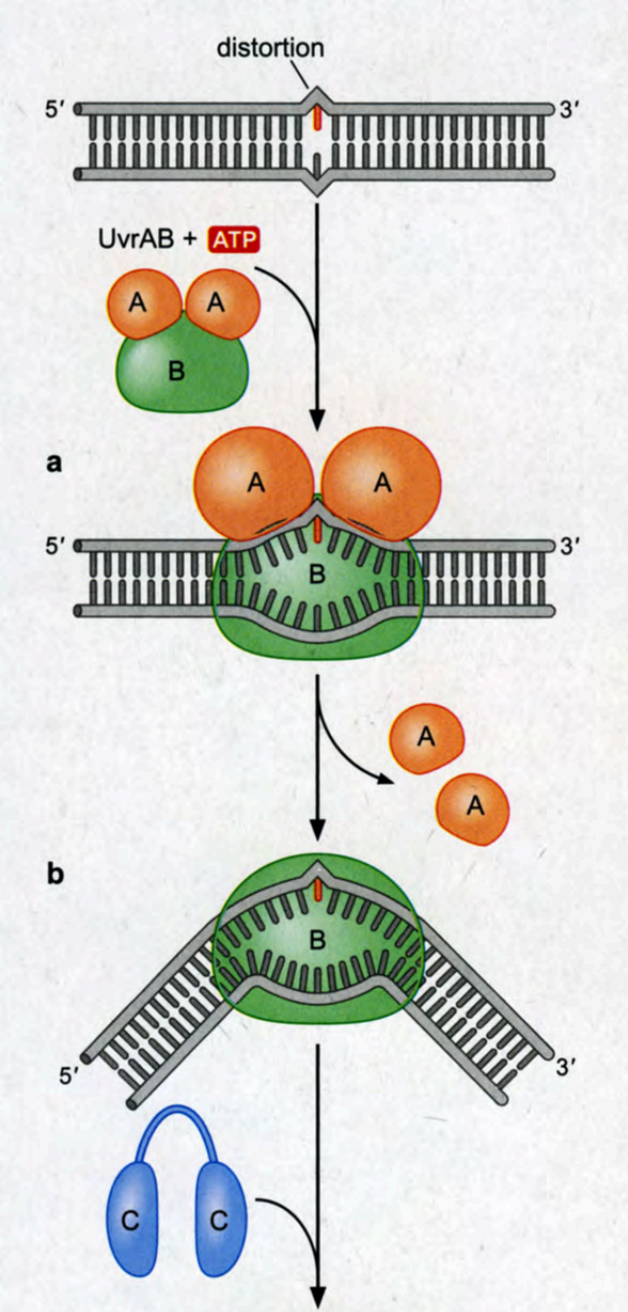
- Autosomal recessive disorder
- Extreme sensitivity to UV light
- Basal cell carcinomas, melanomas, neurological symptoms
- Patient cells have defect in repair of UV-induced DNA damage
- Patient cells placed into >10 genetic complementation groups by cell fusion
- Genes cloned by complementation via genomic DNA transfection. Among cells that have stably incorporated genomic DNA, select for UV-resistant clones.
- XPA complements only group A
- Genes identified function in nucleotide excision repair

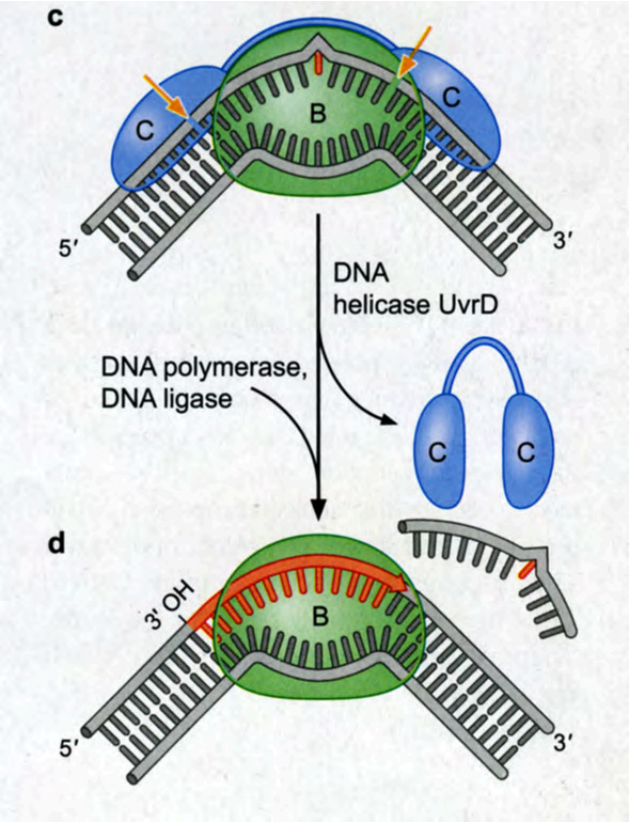


Base excision repair (BER)

- Glycolase recognizes and cleaves damaged base from the sugar, creating AP site (apurinic/apyrimidinic site; abasic site; site in DNA w/o base)
- Specific DNA glycolases recognize specific lesions (e.g., uracil, oxoG; they scan the genome for damage, sometimes using base flipping to assess damage
- AP endonuclease cuts 5'; exonuclease cuts 3', thereby removing nucleotide
- repair DNA polymerase and DNA ligase repairs strand by using opposite strand as template

Watson et al. (2008) Molecular Biology of the Gene



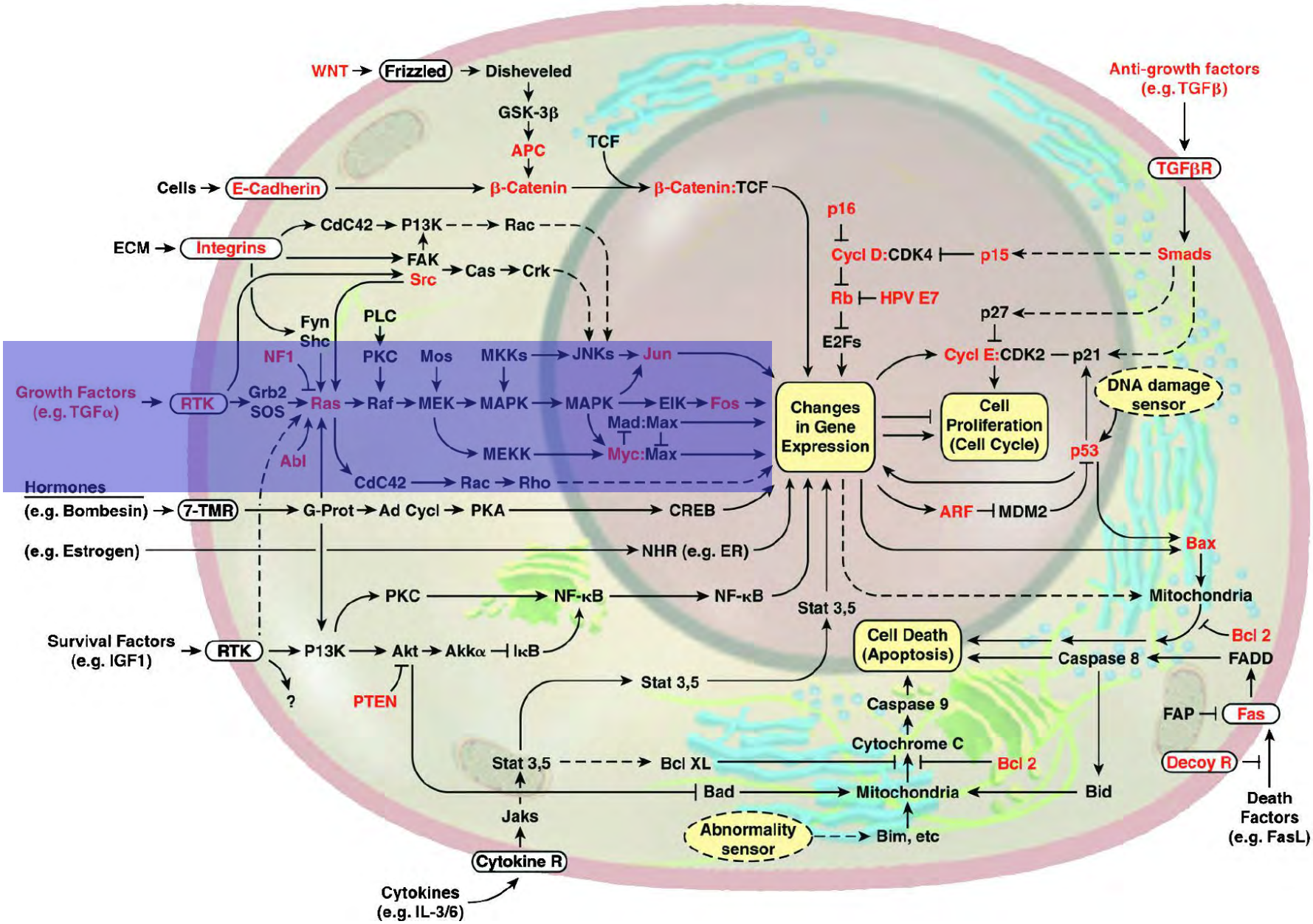


Ultraviolet light is absorbed by DNA; damage includes photochemical fusion of two adjacent pyrimidines on the same polynucleotide strand (e.g., thymine dimer). Such dimers cannot base pair and causes DNA polymerase to stop during replication.

- **Nucleotide excision repair**
  - System recognizes distortions in double helix (thymine dimers or bulky adducts)
  - XP gene products compose many components
  - A single stranded bubble is created around lesion
  - Nucleases cleave 5' and 3', creating a 24-32 nucleotide gap (eukaryotes)
  - Gap filled by DNA polymerase and ligase

Watson et al. (2008) Molecular Biology of the Gene

Intracellular pathways in tumor formation



Hanahan & Weinberg (2000) Cell 100:57