Oncogenes and Tumor Suppressors

Oncology for Scientists
RPN 530

Irwin H. Gelman, Ph.D.

Lecture overview

- What are oncogenes
- How do oncogenes function in cancer cells?
- How are oncogenes “turned on”?
- What are tumor suppressor genes?
- How do TSG function in cancer cells?
- How is TSG function lost?

Oncogenes and Tumor Suppressor genes

Oncogenes

Promote cell growth and/or motility which, when upregulated or deregulated, imbue cells with the cancerous properties of unregulated growth and motility

Tumor suppressor genes

Normally suppress cell growth and/or motility which are frequently downregulated in

tumor cells, allowing for unchecked

growth/motility

Oncogenes

- The term “oncogene” was coined in 1969 by R. Huebner & G. Todaro
- Genes that have the potential to cause cancer (proto-oncogenes)
- Transform healthy cells – cause them to gain “hallmarks of cancer”
- **First discovered in viruses**, later in cells

Peyton Rous

J. Exp. Med., 1991

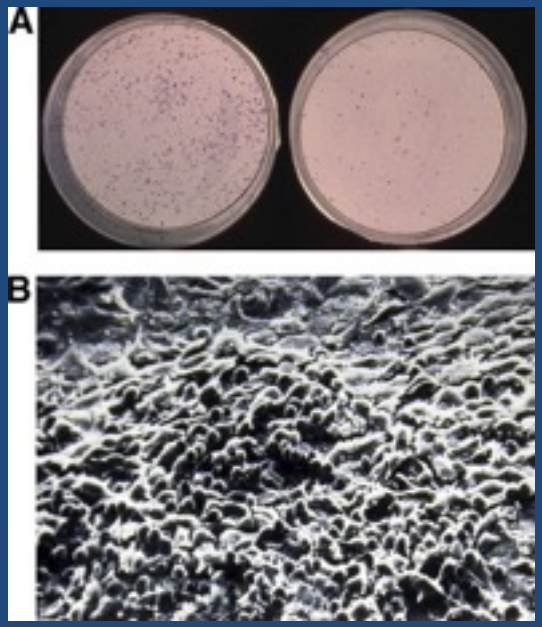
A SARCOMA OF THE FOWL TRANSMISSIBLE BY AN AGENT SEPARABLE FROM THE TUMOR CELLS.*

By PEYTON ROUS, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

PLATES XLVII–LII.

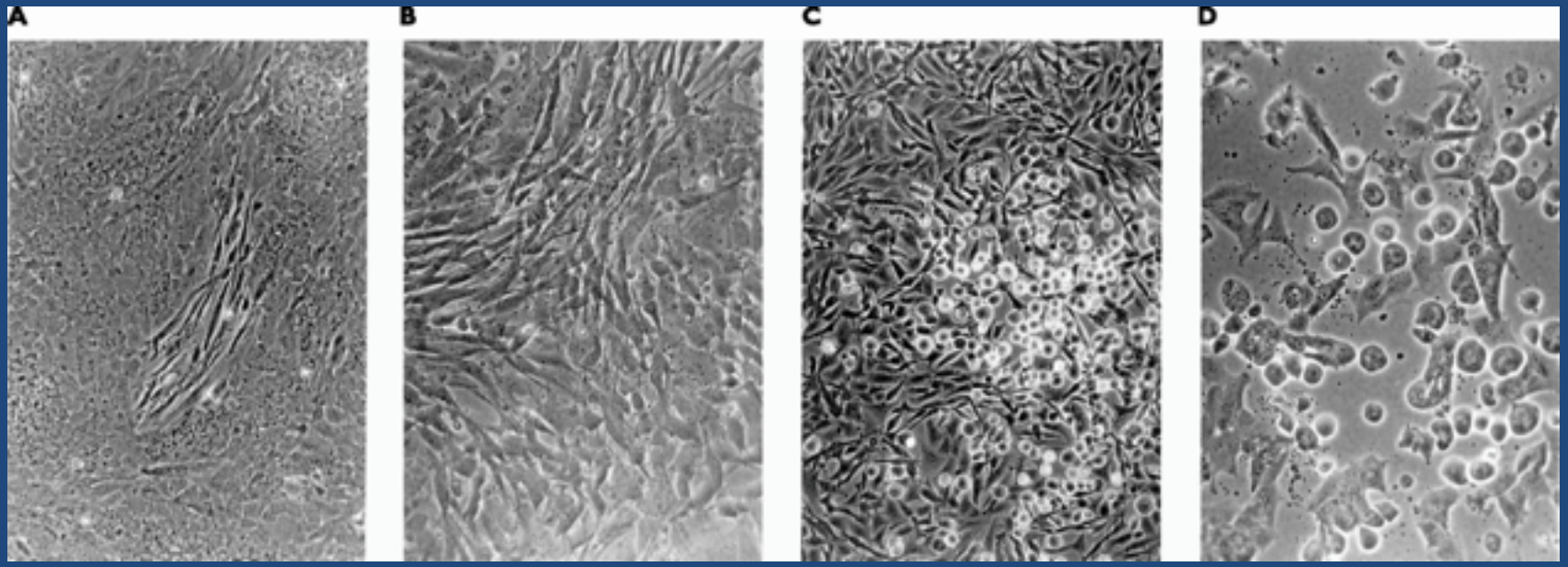
A transmissible sarcoma of the chicken has been under observation in this laboratory for the past fourteen months,[1] and it has assumed of late a special interest because of its extreme malignancy and a tendency to wide-spread metastasis.[2] In a careful study of the growth, tests have been made to determine whether it can be transmitted by a filtrate free of the tumor cells. Attempts to so transmit rat, mouse, and dog tumors have never succeeded; and it was supposed that the sarcoma of the fowl would not differ from them in this regard, since it is a typical neoplasm. On the contrary, small quantities of a cell-free filtrate have sufficed to transmit the growth to susceptible fowls.



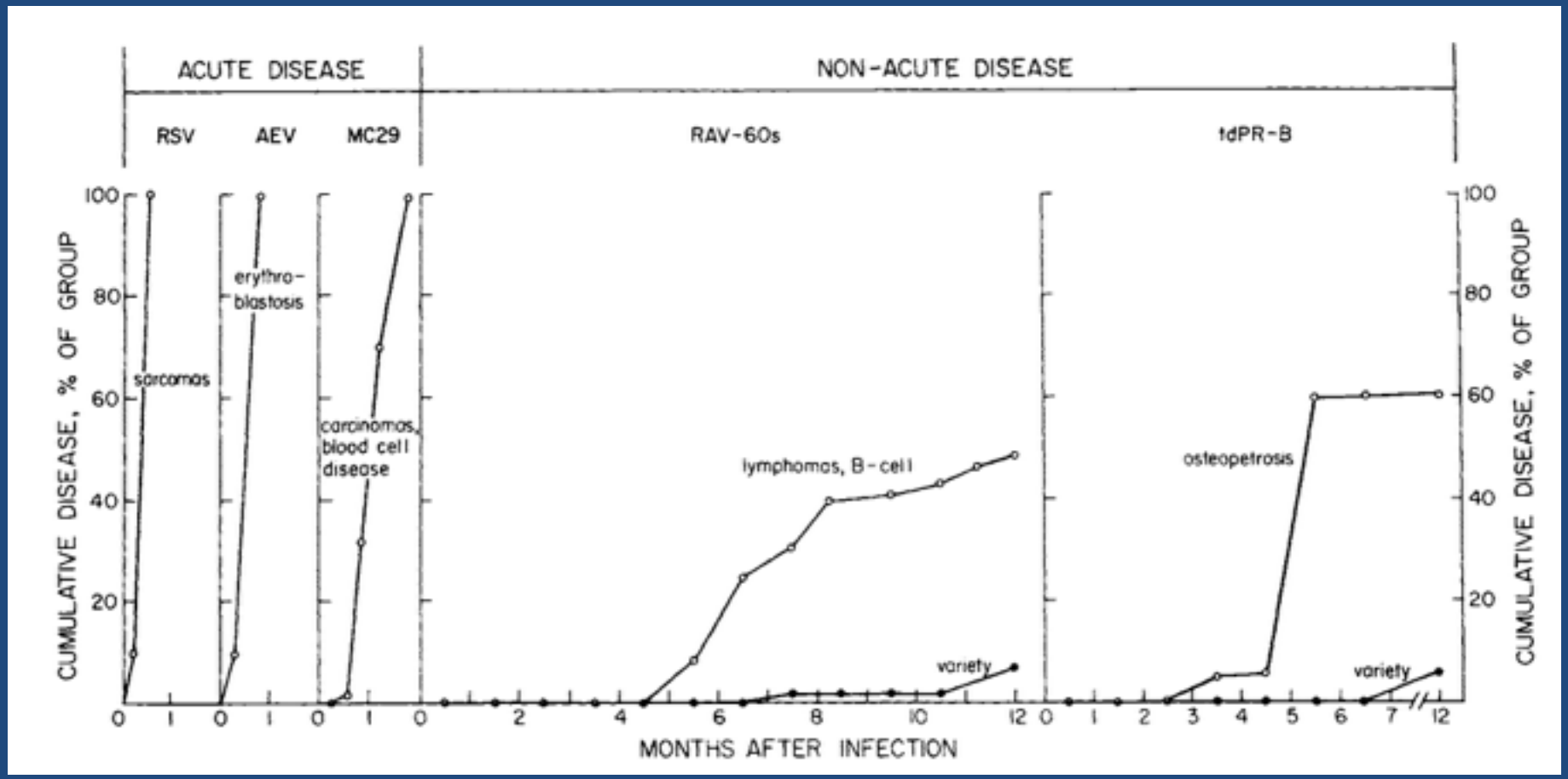
Weiss R A , and Vogt P K J
Exp Med 2011;208:2351-2355

Oncogenic Transformation by RSV:

Loss of contact inhibition (focus formation) and normal cell morphology

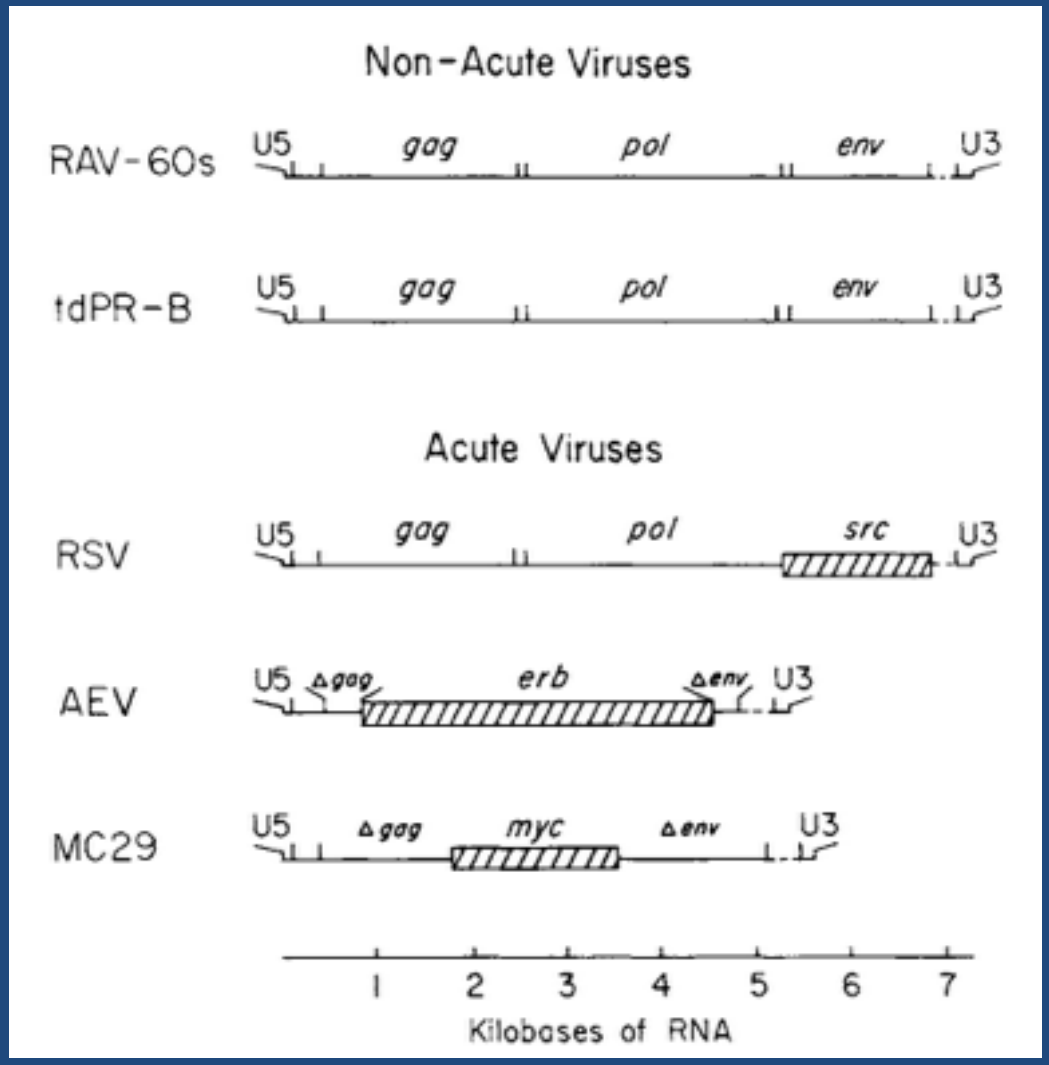


Acute vs. Chronic Transforming Retroviruses



Robinson, Rev. Infectious Diseases, 1982.

Acute vs. Chronic Transforming Retroviruses, con't.



Robinson, Rev. Infectious Diseases, 1982.

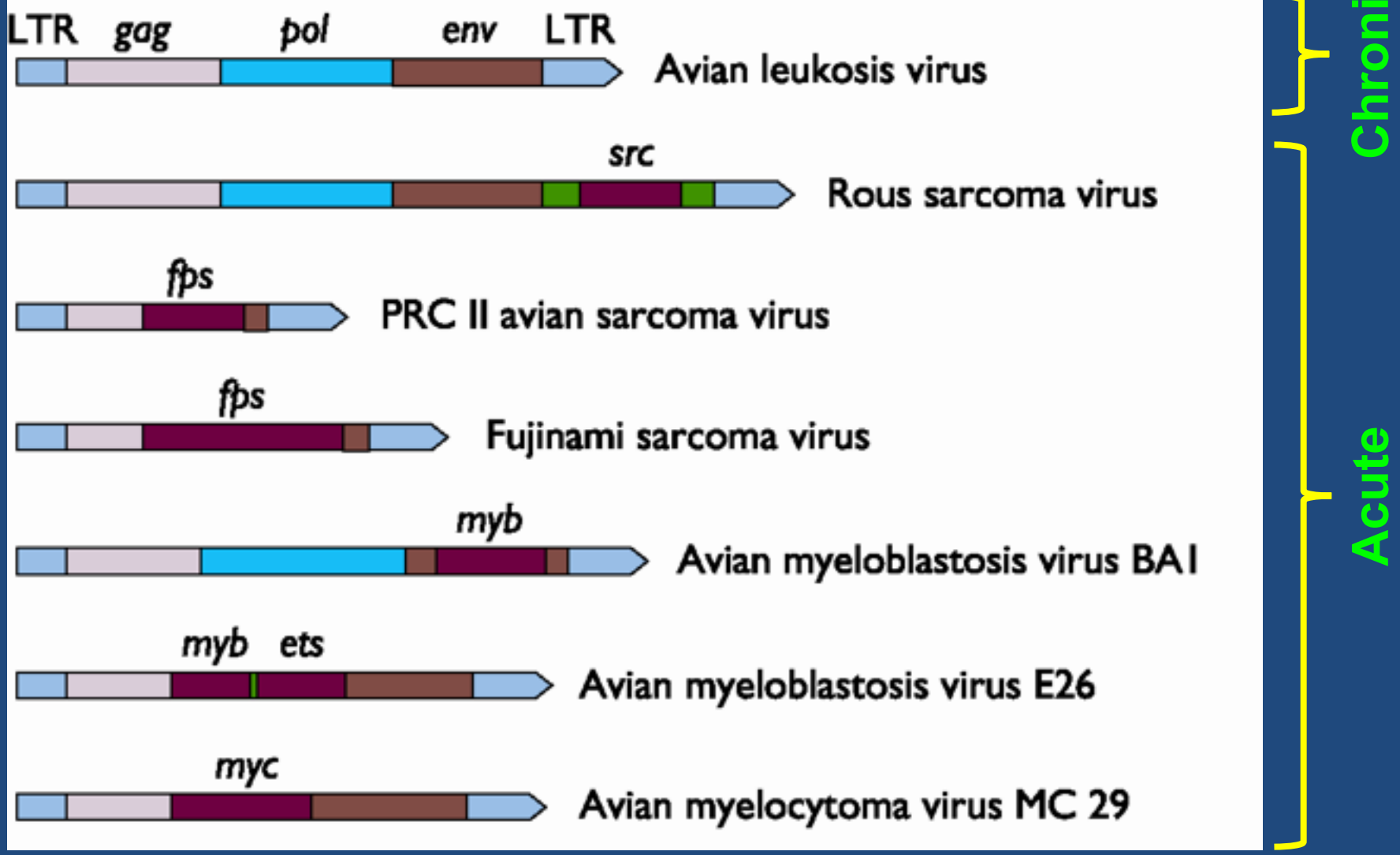
Acute Transforming Retroviruses:

-"extra gene" not encoding Gag, Pol or Env.

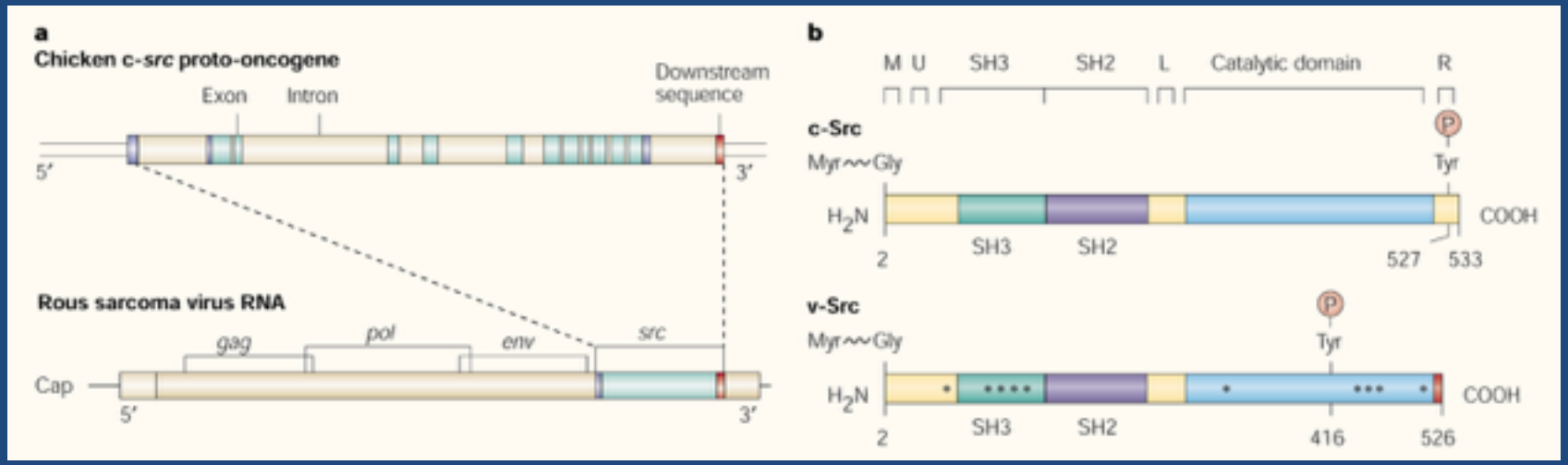
tsRSV:

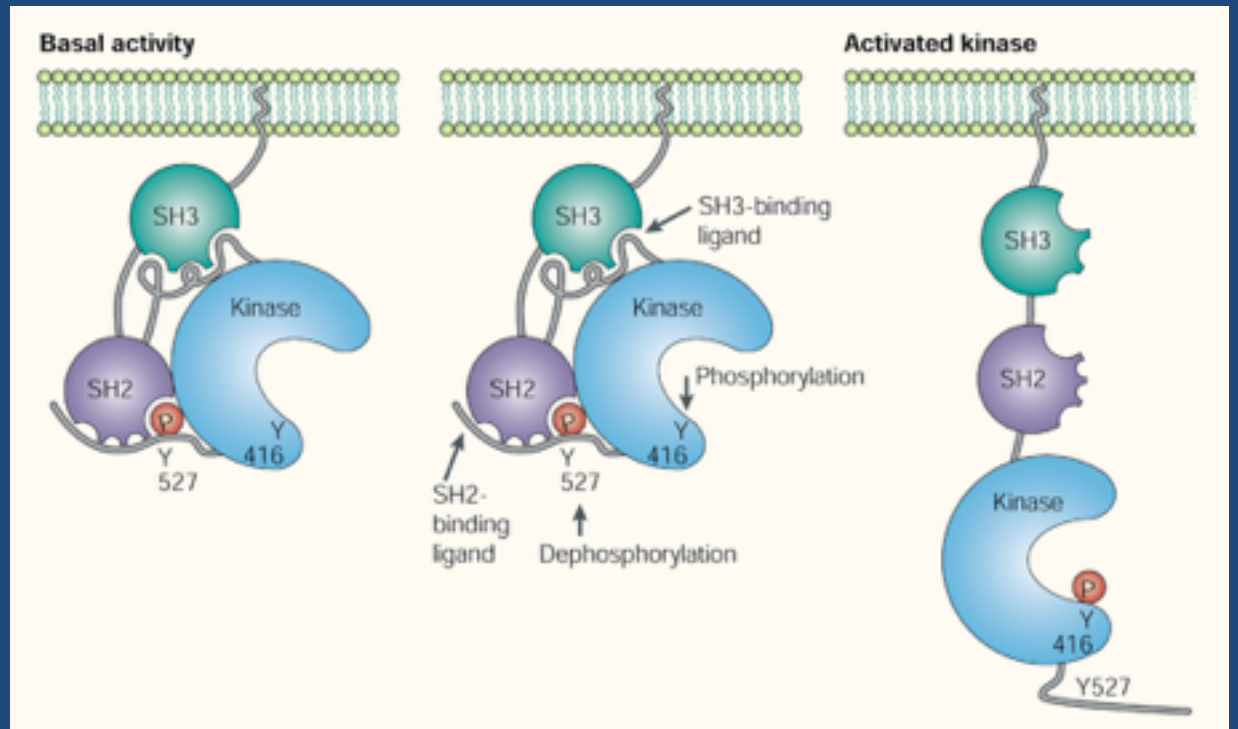
-causes transformation at the permissive temp. (35ºC) but not at the non-permissive temp. (39.5ºC). First proof of a transforming gene ("oncogene") not required for virus replication.

Avian transducing retroviruses



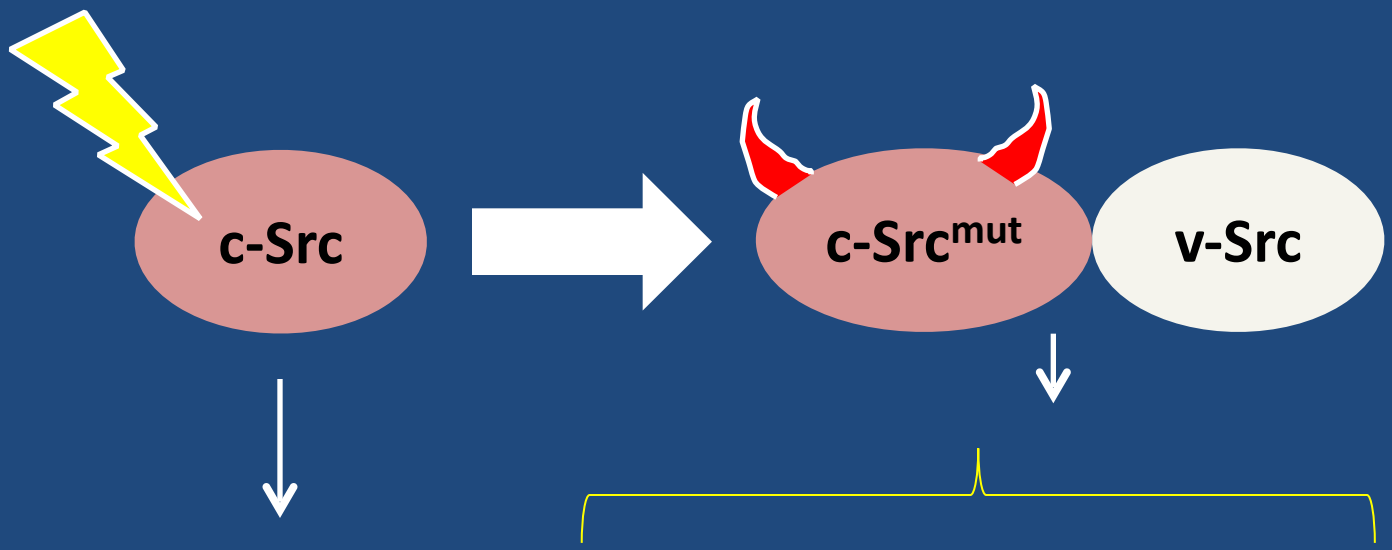
Src: The Prototypic Oncogene

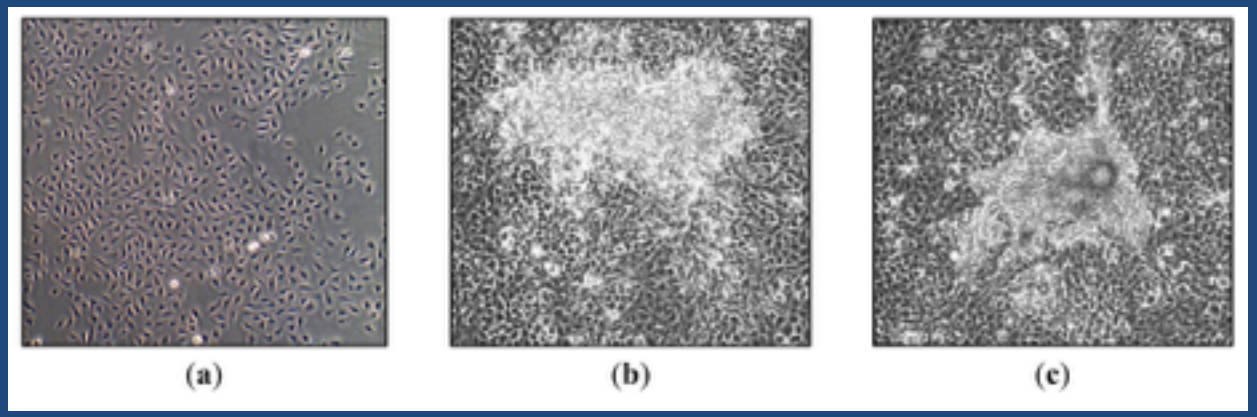




Martin, G.S., *Nature Rev. Mol.Cell. Biol.*, 2003

Src – the first (proto)oncogene





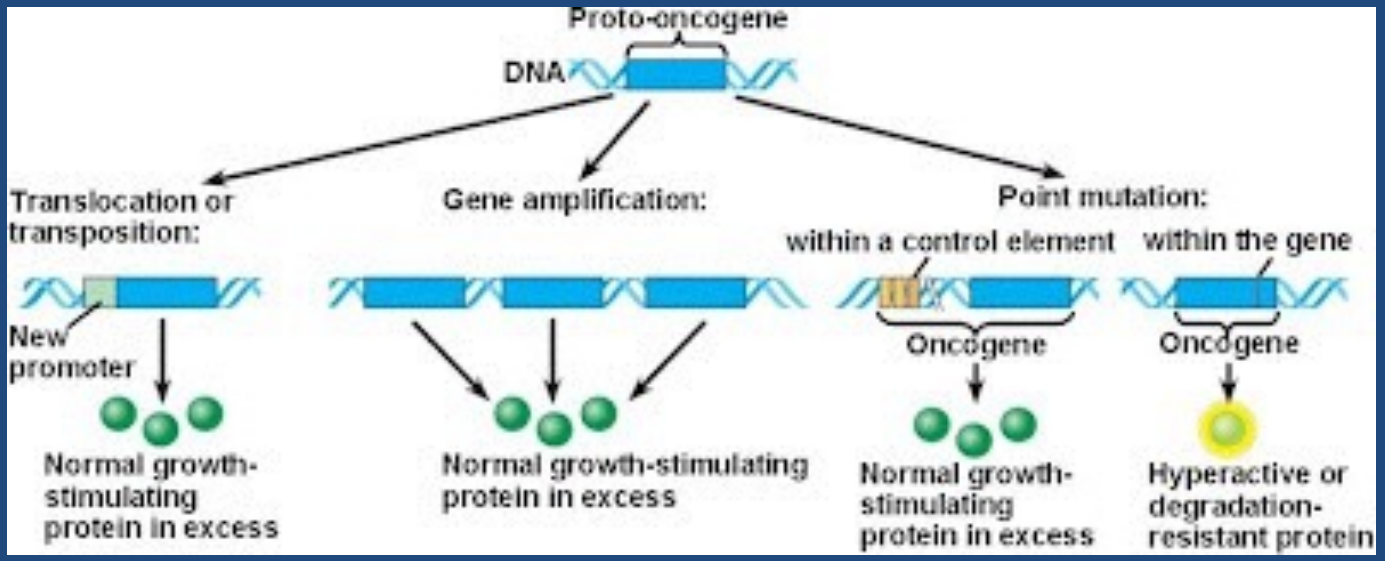
Growth factors	Transcription factors	Membrane receptors	Signal molecules
sis,/PDGF	bZIP: fos, jun	RTK:	src
int-2/FGF-F	bHLH: myc,	erb-B/EGF-R	yes
hst (KS3)	N-myc, L-myc	Neu, ROS,	fps
int-1/GF	lyl-1, fal, scl	Fms/CSF-1	abl
	ZF: myl/RARA,		met
	erb-A, vav, gli-1	Non-RTK:	mos
	HD: pbx, Hox-2,	mas	raf
	Other: myb, rel,		ras
	est-1, est-2,		crk
	spi-1		

Mechanisms to Activate the Oncogenic Functions of Proto-Oncogenes

Activation of a proto-oncogene into an oncogene: generally involves a *gain-of-function* mutation.

Three major mechanisms for activation of proto-oncogenes:

1) Point mutations in a proto-oncogene that result in a constitutively - acting protein product
2) Localized reduplication (gene amplification) of a DNA segment that includes a proto-oncogene, leading to overexpression of the encoded protein
3) Chromosomal translocation that brings a growth-regulatory gene under the control of a different promoter: unregulated gene expression

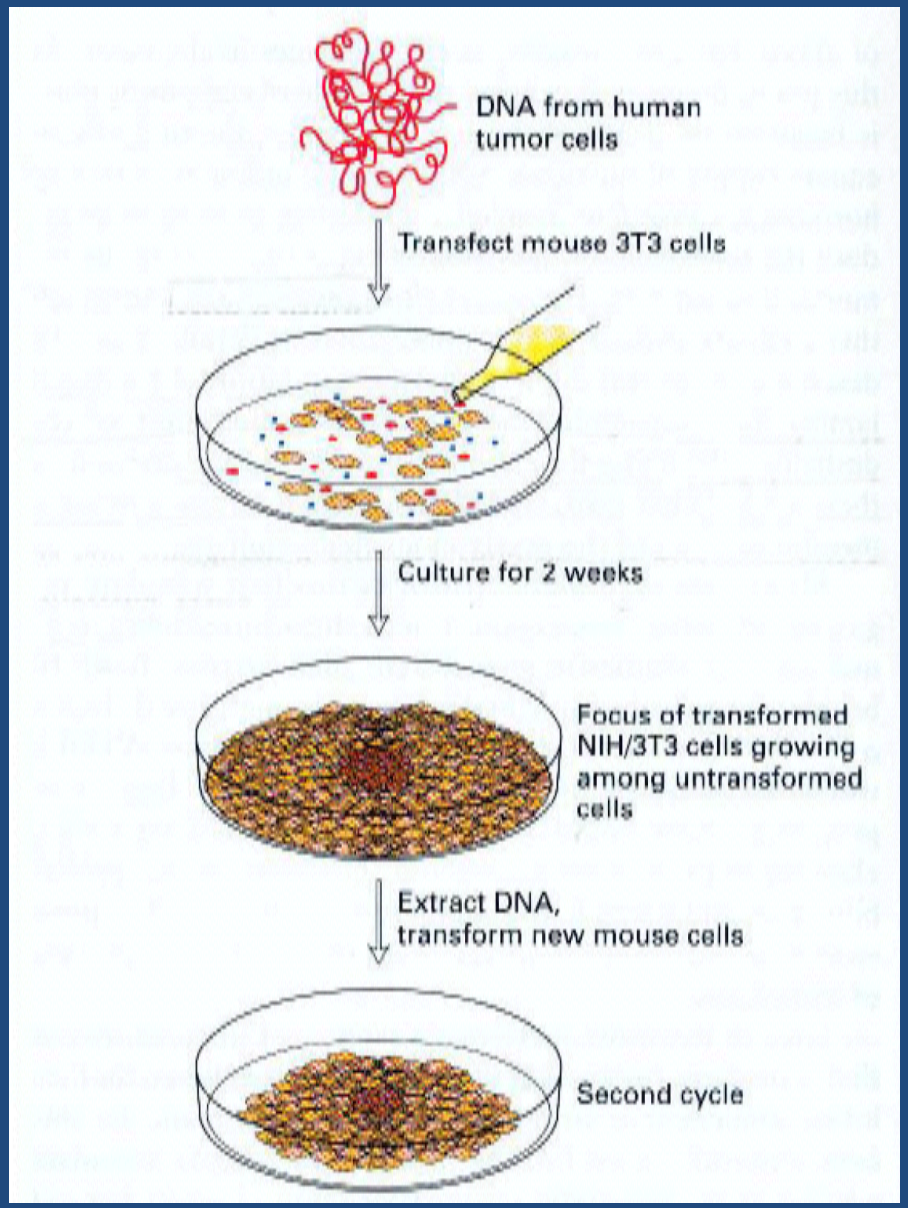


http://www.cancer-biomarkers.com/2012/06/proto-oncogenes.html

Activation of proto-oncogenes

Proto-oncogenes are tightly regulated in healthy cells

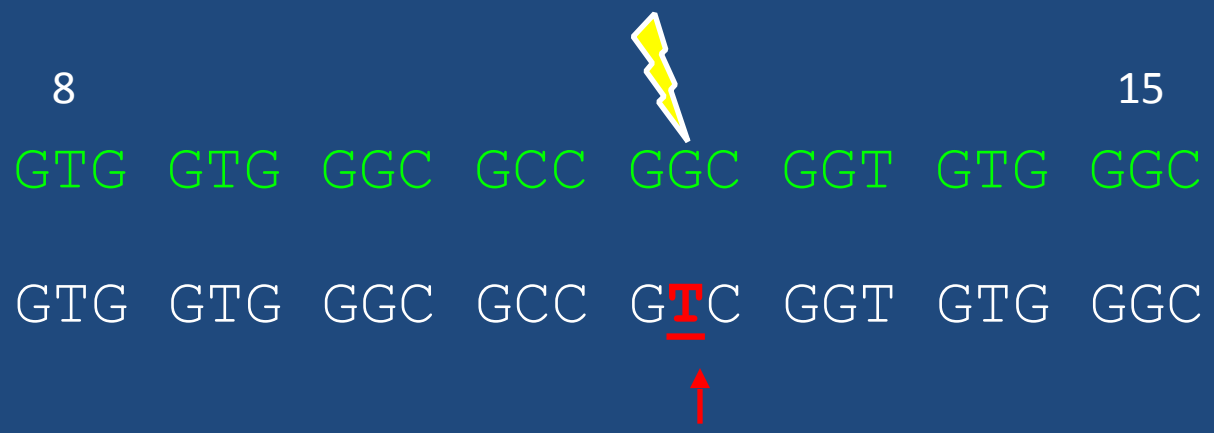
- **Mutation**
  - H-ras, K-ras, N-ras
  - EGFR
- **Gene amplification**
  - Myc
  - ErbB2/HER2
- **Chromosomal translocations**
  - Myc
  - Bcr/Abl



Robert Weinberg

Identification of non-viral oncogenes by transfection

Activation of Ras by mutation



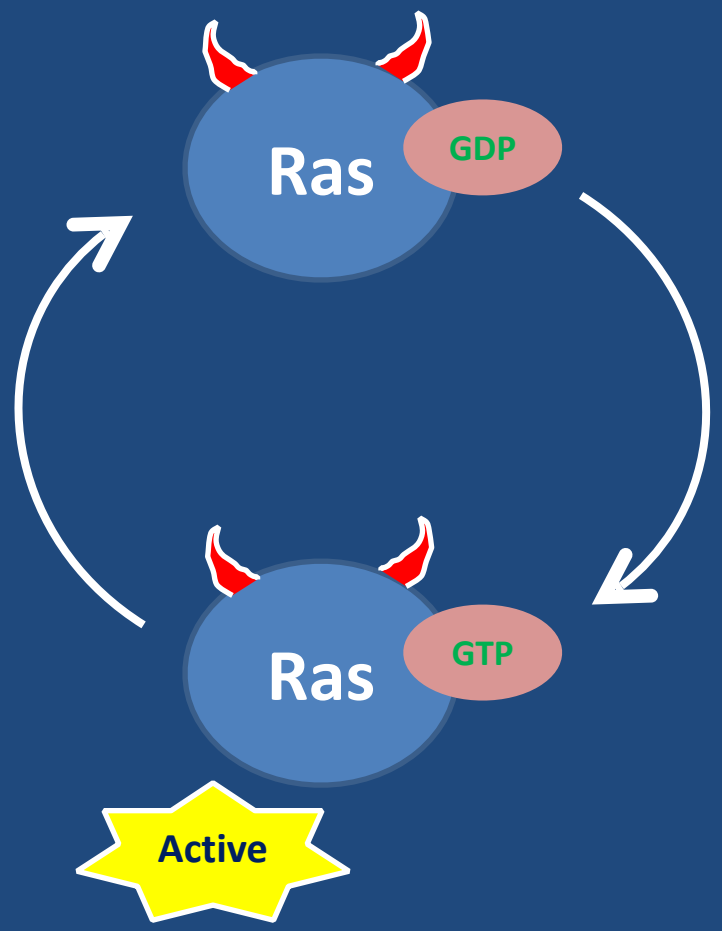
Glycine → Valine
(G12V)

H-Ras: bladder cancer
K-Ras: pancreatic & lung cancer

Common point mutations found in oncogenic Ras:

- **aa 12**
- **aa 13**
- **aa 61**

The effect of oncogenic point mutations on Ras signaling



- Ras is a GTPase – binds to and hydrolyzes GTP
- In the GTP-bound form, Ras is active
- Hydrolysis of GTP kills Ras activity
- G12V mutant Ras loses GTPase activity, remains active

Table 4.2 A list of point-mutated *ras* oncogenes carried by a variety of human tumor cells

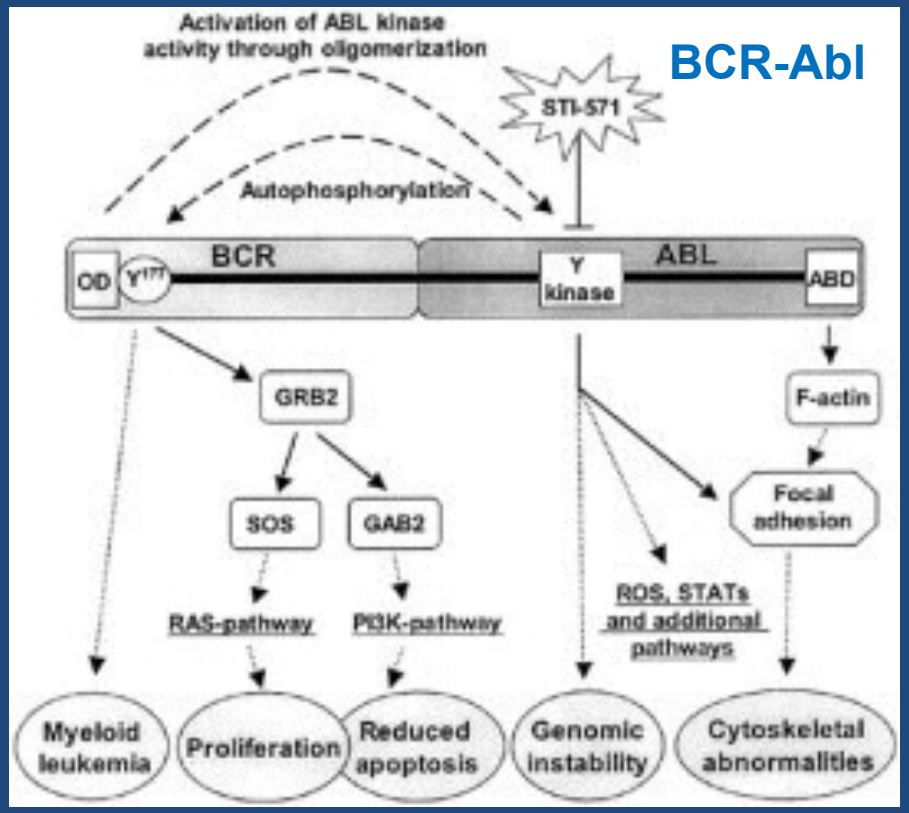
Tumor type	Proportion (%) of tumors carrying a point-mutated *ras* gene[a]
Pancreas	90 (K)
Thyroid (papillary)	60 (H, K, N)
Thyroid (follicular)	55 (H, K, N)
Colorectal	45 (K)
Seminoma	45 (K, N)
Myelodysplasia	40 (N, K)
Lung (non-small-cell)	35 (K)
Acute myelogenous leukemia	30 (N)
Liver	30 (N)
Melanoma	15 (N)
Bladder	10 (H, K)
Kidney	10 (H)

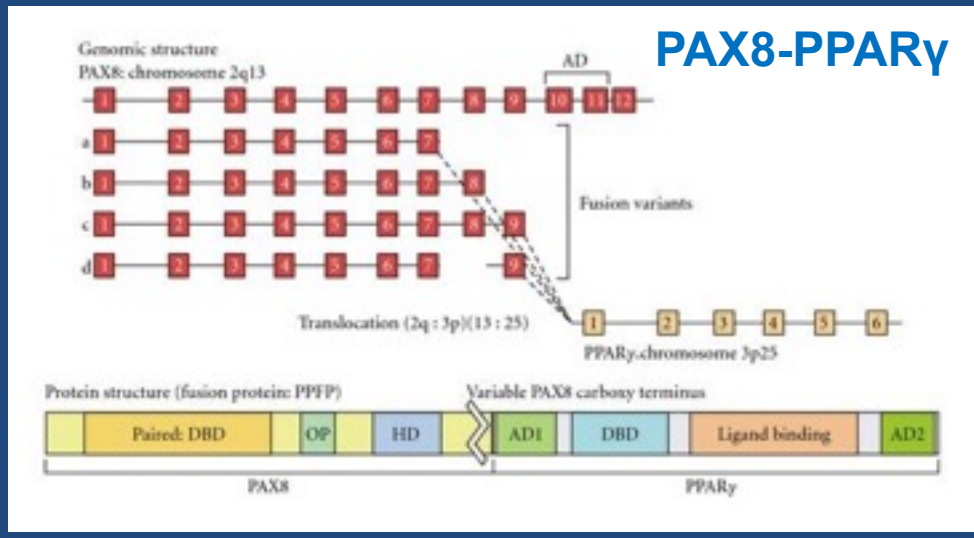
[a]H, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

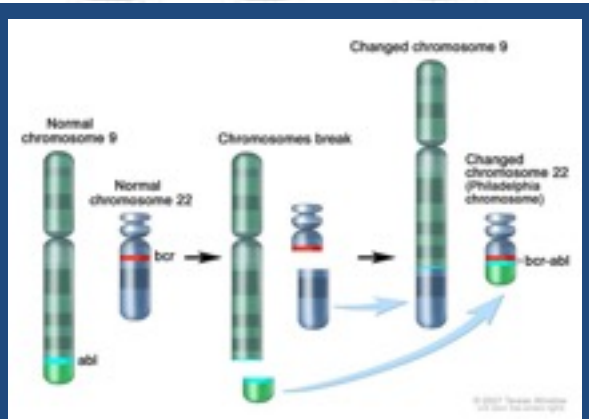
Adapted from J. Downward, *Nature Rev. Cancer* 3:11–22, 2003.

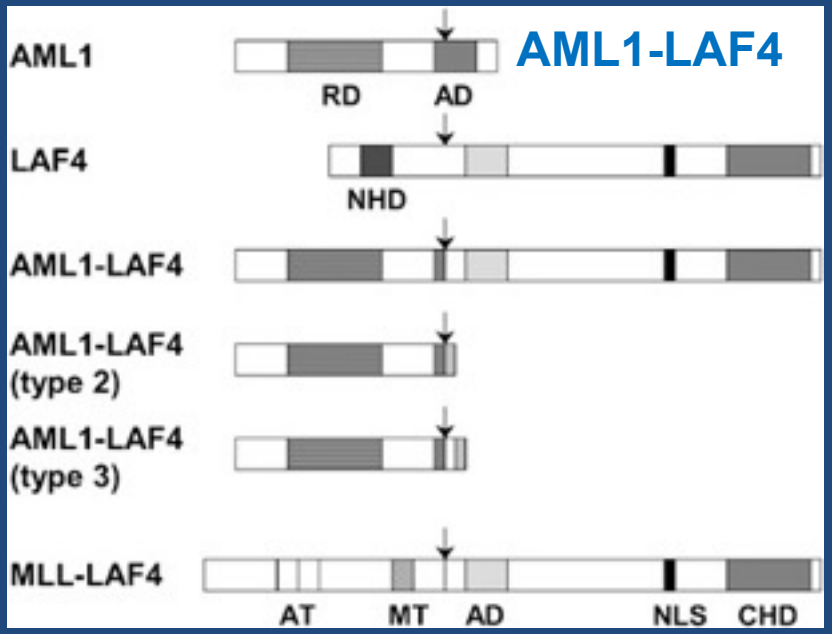
Table 4.2 The Biology of Cancer (© Garland Science 2014)

Fusion Proto-oncogenes, Chromosome Rearrangements





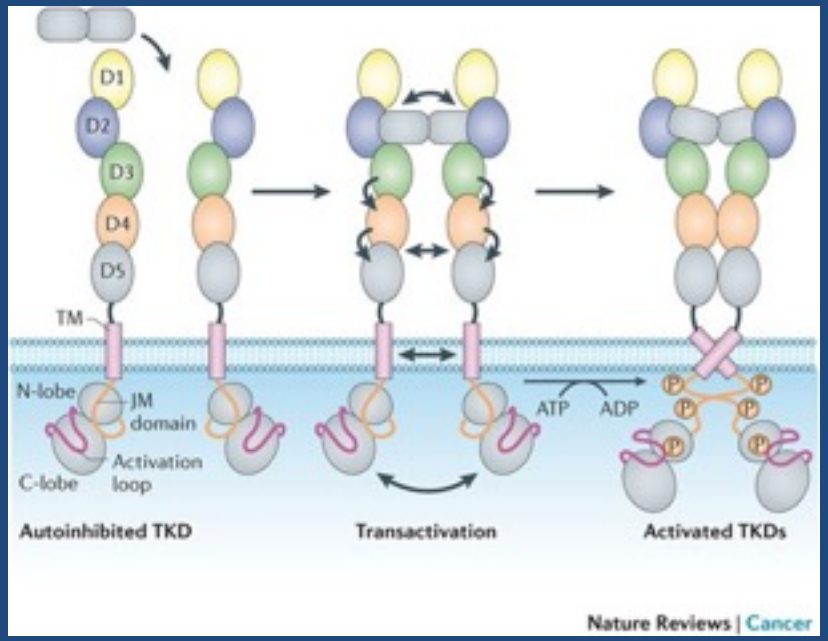


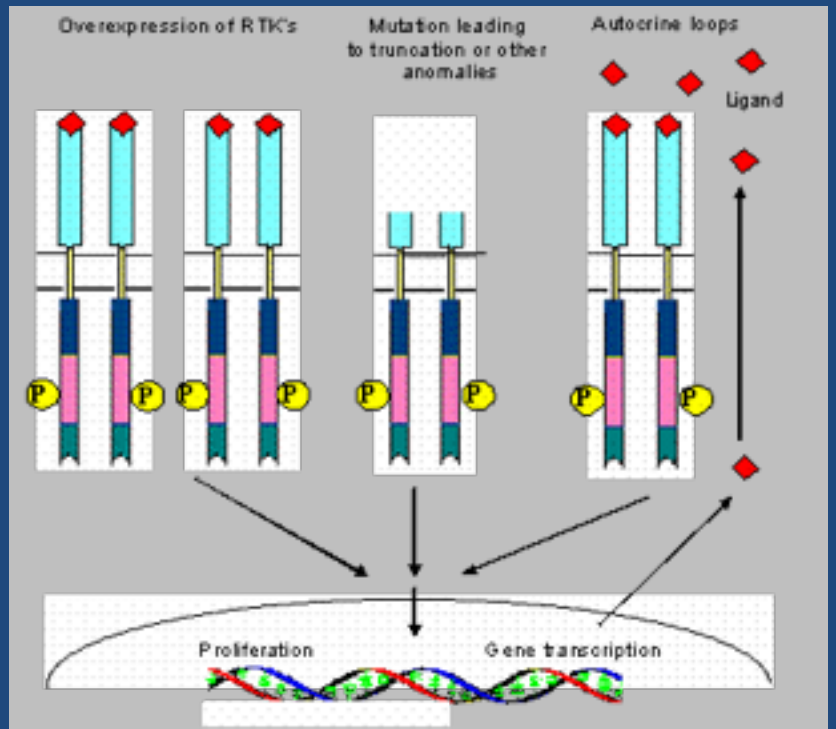


Childhood T-cell ALL with t(2;21)(q11;q22)

Truncation Proto-oncogenes:

Generally growth-factor receptors.
Truncations → CA

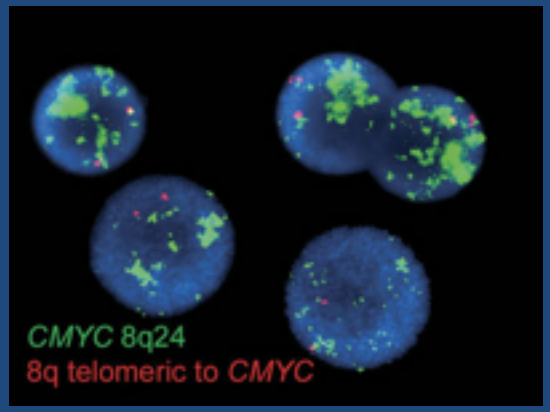


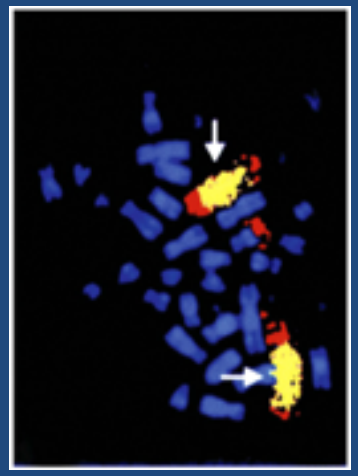


Oncogene activation by gene amplification

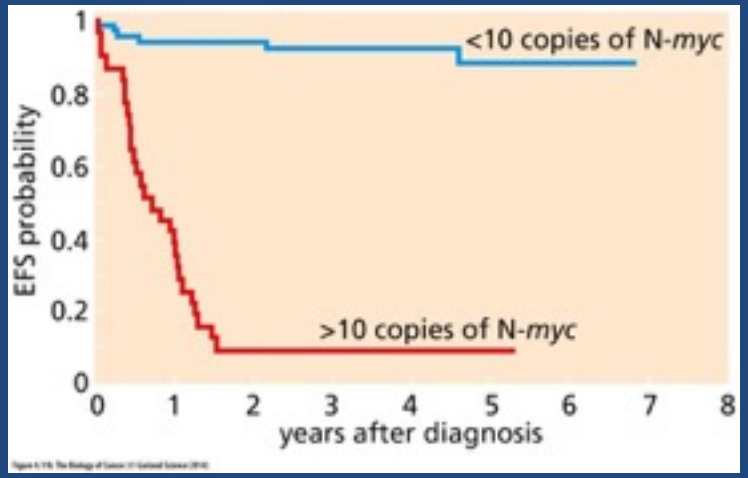
- Multiple copies of Myc, Her2→ greater expression
- Pro-growth advantage of tumor cells with greater expression

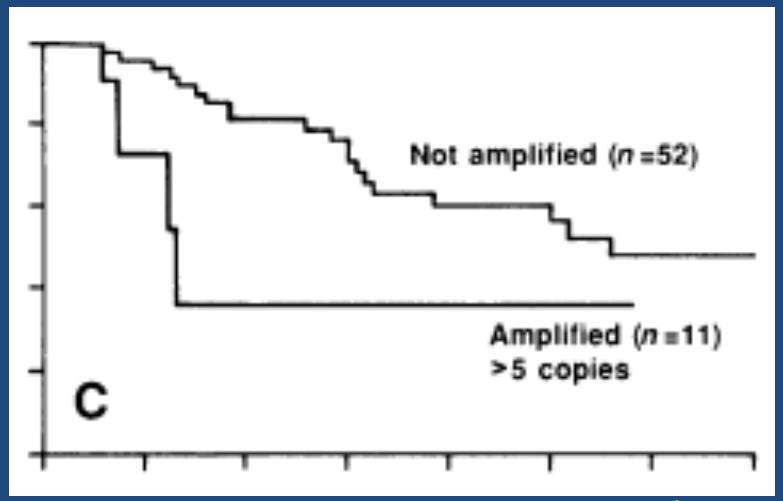
Fluorescence *in situ* Hybridization (FISH)





CMYC FISH





Breast ca survival based on Her2/neu expression (Slamon et al, 1987)

Table 4.3 Some frequently amplified chromosomal regions and the genes they are known to carry

Name of oncogene[a]	Human chromosomal location	Human cancers	Nature of protein[b]
MDM4/MDMX	1q32	breast, colon, lung, pre-B leukemias	p53 inhibitor
PIK3CA	3q26.3	lung SCC, ovarian, breast	PI kinase
erbB1/EGFR	7q12–13	glioblastomas (50%); squamous cell carcinomas (10–20%)	RTK
cab1–erbB2–grb7	17q12	gastric, ovarian, breast carcinomas (10–25%)	RTK, adaptor protein
k-sam	7q26	gastric, breast carcinomas (10–20%)	RTK
FGF-R1	8p12	breast carcinomas (10%)	RTK
met	7q31	gastric carcinomas (20%)	RTK
K-*ras*	12p12.1	lung, ovarian, colorectal, bladder carcinomas (5–20%)	small G protein
N-*ras*	1p13	head and neck cancers (30%)	small G protein
H-*ras*	11p15	colorectal carcinomas (30%)	small G protein
c-*myc*	8q24	various leukemias, carcinomas (10–50%)	TF
L-*myc*	1p32	lung carcinomas (10%)	TF
N-*myc–DDX1*	2p24–25	neuroblastomas, lung carcinomas (30%)	TF
akt-1	14q32–33	gastric cancers (20%)	ser/thr kinase
akt-2	19q13	ovarian carcinomas	ser/thr kinase
cyclin D1–exp1–hst1–ems1	(11q13)	breast and squamous cell carcinomas (25–50%)	G1 cyclin
cdk4–mdm2–sas–gli	12q13	sarcomas (10–30%), HNSCC (40%), B-cell lymphomas (25%)	CDK, p53 antagonist
cyclin E	19q12	gastric cancers (15%)	cyclin
akt2	(19q13)	pancreatic, ovarian cancers (30%)	ser/thr kinase
AIB1, BTAK	(20q12–13)	breast cancers (15%)	receptor co-activator
cdk6	(19q21–22)	gliomas (5%)	CDK
myb	6q23–24	colon carcinoma (5–20%), leukemias	TF
ets-1	11q23	lymphoma	TF
gli	12q13	glioblastomas	TF

[a]The listing of several genes indicates the frequent co-amplification of a number of closely linked genes; only the products of the most frequently amplified genes are described in the right column.
[b]Abbreviations: TF, transcription factor; RTK, receptor tyrosine kinase; CDK, cyclin-dependent kinase; G protein, guanine nucleotide-binding protein; HNSCC, head-and-neck squamous cell carcinomas.

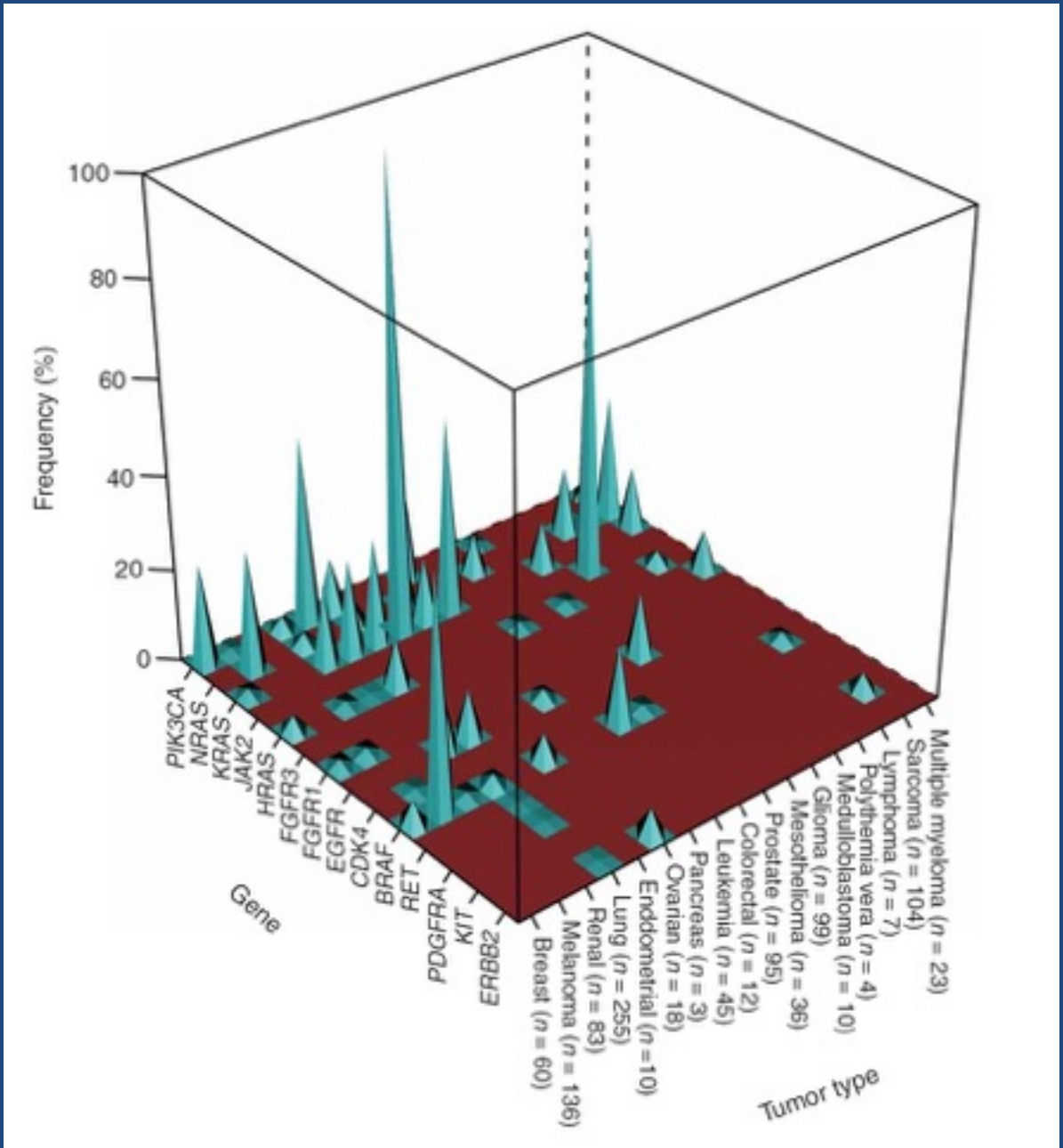
Courtesy of M. Terada, Tokyo, and adapted from G.M. Cooper, Oncogenes, 2nd ed. Boston and London: Jones and Bartlett, 1995.

Table 4.3 The Biology of Cancer (© Garland Science 2014)

Oncogene	Function/Activation	Cancer*
abl	Promotes cell growth throughtyrosine kinase activity	Chronic myelogenous leukemia
Af4/hrx	Fusion affects the hrx transcription factor/methyltransferase. *hrx is also called MLL, ALL1 and HTRX1*	Acute leukemias
akt-2	Encodes a protein-serine/threonine kinase	Ovarian cancer
alk	Encodes a receptor tyrosine kinase	Lymphomas
alk/npm	Translocation creates fusionprotein with nucleophosmin(npm)	Large cell lymphomas
aml1	Encodes a transcription factor	Acute myeloid leukemia
aml1/mtg8	New fusion protein created by translocation	Acute leukemias
axl	Encodes a receptor tyrosine kinase	Hematopoietic cancers
bcl-2, 3, 6	Block apoptosis (programmed cell death)	B-cell lymphomas and leukemias
bcr/abl	New protein created by fusion of bcr and abl triggers unregulated cell growth	Chronic myelogenous and acute lymphotic leukemia
c-myc	Transcription factor that promotes cell proliferation andDNA synthesis	Leukemia; breast, stomach, lung, cervical, and colon carcinomas; neuroblastomas and glioblastomas
dbl	Guanine nucleotide exchange factor	Diffuse B-cell lymphoma
dek/can	New protein created by fusion	Acute myeloid leukemia
E2A/pbx1	New protein created by fusion	Acute pre B-cell leukemia
egfr	Cell surface receptor that triggers cell growth through tyrosine kinase activity	Squamous cell carcinoma
enl/hrx	Fusion protein created by a translocation t(11;19).	Acute leukemias
erg/TLS	Fusion protein created by t(16:21) translocation. The erg protein is a transcription factor	Myeloid leukemia
erbB	Cell surface receptor that triggers cell growth through tyrosine kinase activity	Glioblastomas, and squamous cell carcinomas
erbB-2	Cell surface receptor that triggers cell growth through tyrosine kinase activity; also known as HER2 or neu	Breast, salivary gland, and ovarian carcinomas
ets-1	Transcription factor	Lymphoma
ews/fli-1	Fusion protein created by t(11:22) translocation.	Ewing Sarcoma
fms	Tyrosine kinase	Sarcoma
fos	Transcription factor for API	Osteosarcoma
fps	Tyrosine kinase	Sarcoma
gli	Transcription factor	Glioblastoma
gsp	Membrane associated G protein	Thyroid carcinoma
HER2/neu	overexpression of signaling kinase due to gene amplification	Breast and cervical carcinomas
hox11	Transcription factor	Acute T-cell leukemia
hst	Encodes fibroblast growth factor	Breast and squamous cell carcinomas
IL-3	Cell signaling molecule	Acute pre B-cell leukemia
int-2	Encodes a fibroblast growth factor	Breast and squamous cell carcinomas
jun	Transcription factor for API	Sarcoma
kit	Tyrosine kinase	Sarcoma
KS3	Herpes virus encoded growth factor	Kaposi's sarcoma
K-sam	Fibroblast growth factor receptor	Stomach carcinomas

Lbc	Guanine nucleotide exchange factor	Myeloid leukemias
lck	Tyrosine kinase	T-cell lymphoma
lmo1, lmo2	Transcription factors	T-cell lymphoma
L-myc	Transcription factor	Lung carcinomas
lyl-1	Transcription factor	Acute T-cell leukemia
lyt-10	Transcription factor. Also called NFκB2	B-cell lymphoma
lyt-10/C alpha1	Fusion protein formed by the (10:14)(q24:q32) translocation of lyt-10, next	
MYH11/CBFB	New protein created by fusion of transcription factors via an inversion in chromosome 16.	Acute myeloid leukemia
neu	Tyrosine kinase. Also called erbB-2 or HER2	Glioblastomas, and squamous cell carcinomas
N-myc	Cell proliferation and DNA synthesis	Neuroblastomas, retinoblastomas, and lung carcinomas
ost	Guanine nucleotide exchange factor	Osteosarcomas
pax-5	Transcription factor	Lympho-plasmacytoid B-cell lymphoma
pbx1/E2A	Fusion protein formed via t(1:19). Transcription factor	Acute pre B-cell leukemia
pim-1	Serine/threonine kinase	T-cell lymphoma
PRAD-1	Encodes cyclin D1. Involved in cell cycle regulation.	Breast and squamous cell carcinomas
raf	Serine/threonine kinase	Many cancer types
RAR/PML	Fusion protein caused by t(15:17). Retinoic acid receptor.	Acute premyelocytic leukemia
rasH	G-protein. Signal transduction	Bladder carcinoma
rasK	G-protein. Signal transduction	Lung, ovarian, and bladder carcinoma
rasN	G-protein. Signal transduction	Breast carcinoma
rel/nrg	Fusion protein formed by deletion in chromosome 2. Transcription factor.	B-cell lymphoma
ret	Cell surface receptor. Tyrosine kinase	Thyroid carcinomas, multiple endocrine neoplasia type 2
rhom1, rhom2	Transcription factors	Acute T-cell leukemia
ros	Tyrosine kinase	Sarcoma
ski	Transcription factor	Carcinomas
sis	Growth factor	Glioma, fibrosarcoma
set/can	Fusion protein formed by rearrangement of chromosome 9. Protein localization	Acute myeloid leukemia
src	Tyrosine kinase	Sarcomas
tal1, tal2	Transcription factor.TAL1 is also called SCL	Acute T-cell leukemia
tan-1	Altered form of Notch (a cellular receptor) formed by t(7:9)	Acute T-cell leukemia
Tiam1	Guanine nucleotide exchange factor	T-lymphoma
TSC2	GTPase activator	Renal and brain tumors
trk	Receptor tyrosine kinase	Colon and thyroid carcinomas

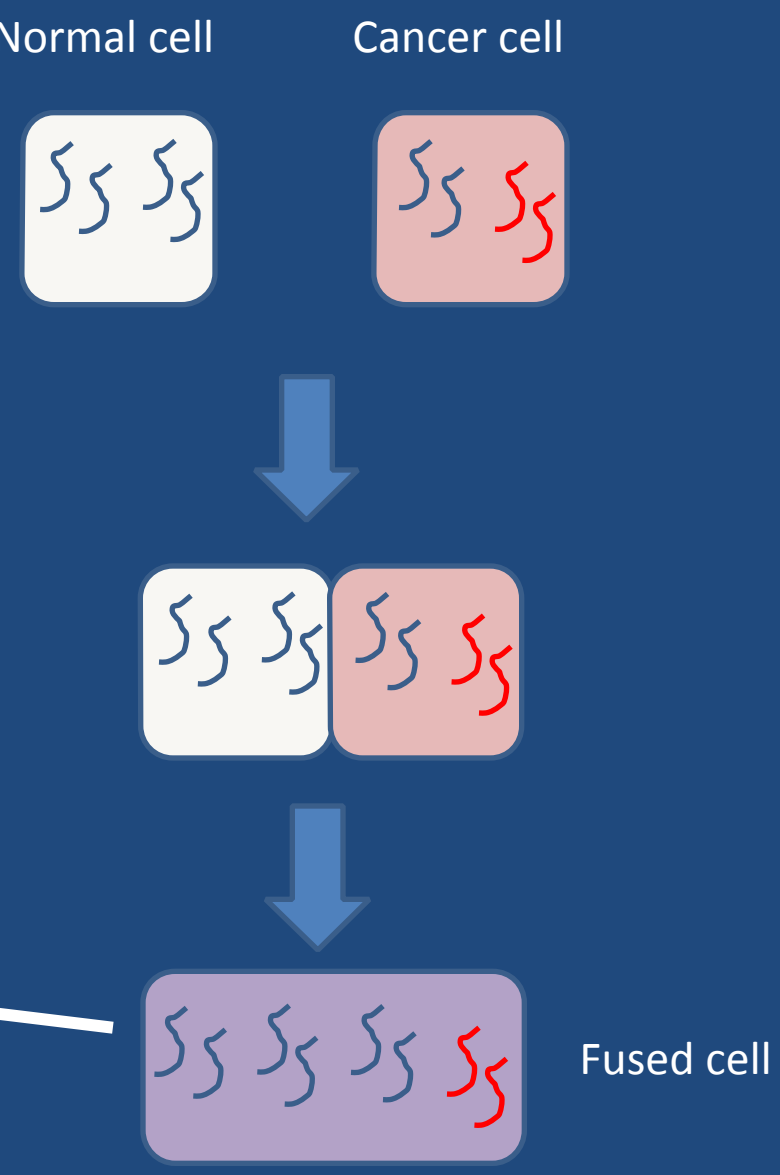
Oncogenes and Human Cancer



Chial, *Nature Education*, 2008

- Scientists expected oncogenes to be genetically dominant

- BUT – fusion of cancer and normal cells create non-tumor forming hybrid

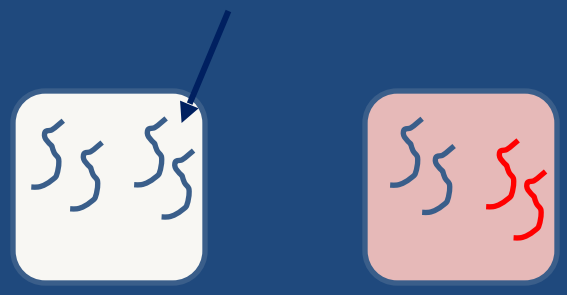


Inject into mouse

NO TUMOR!

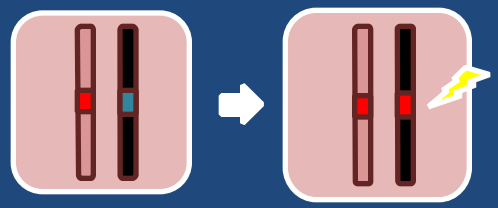
Results of fusion studies

- Tumor phenotype is recessive to normal phenotype
- Normal cells have properties which suppress tumorigenesis
- **Tumor suppressor genes**

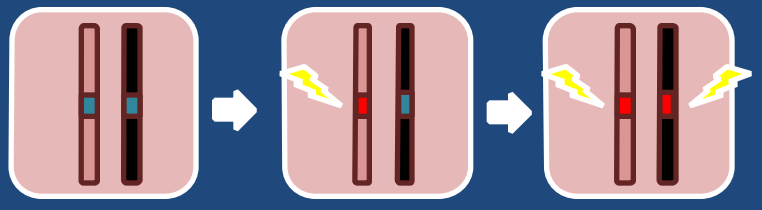


Additional support for TS theory: Retinoblastoma

- **Familial**
  - Parent previously had the disease (carry one disease allele)
  - More likely to get sporadic "second hit"
  - Presents in both eyes



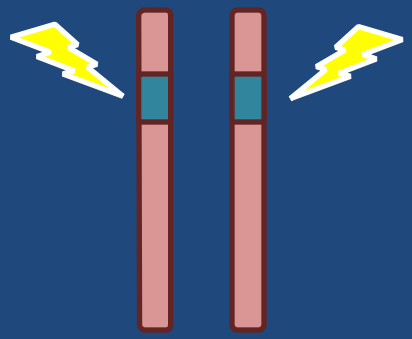
- **Sporadic**
  - Requires two hits per cell (one per allele)
  - Less frequent
  - Presents in one eye



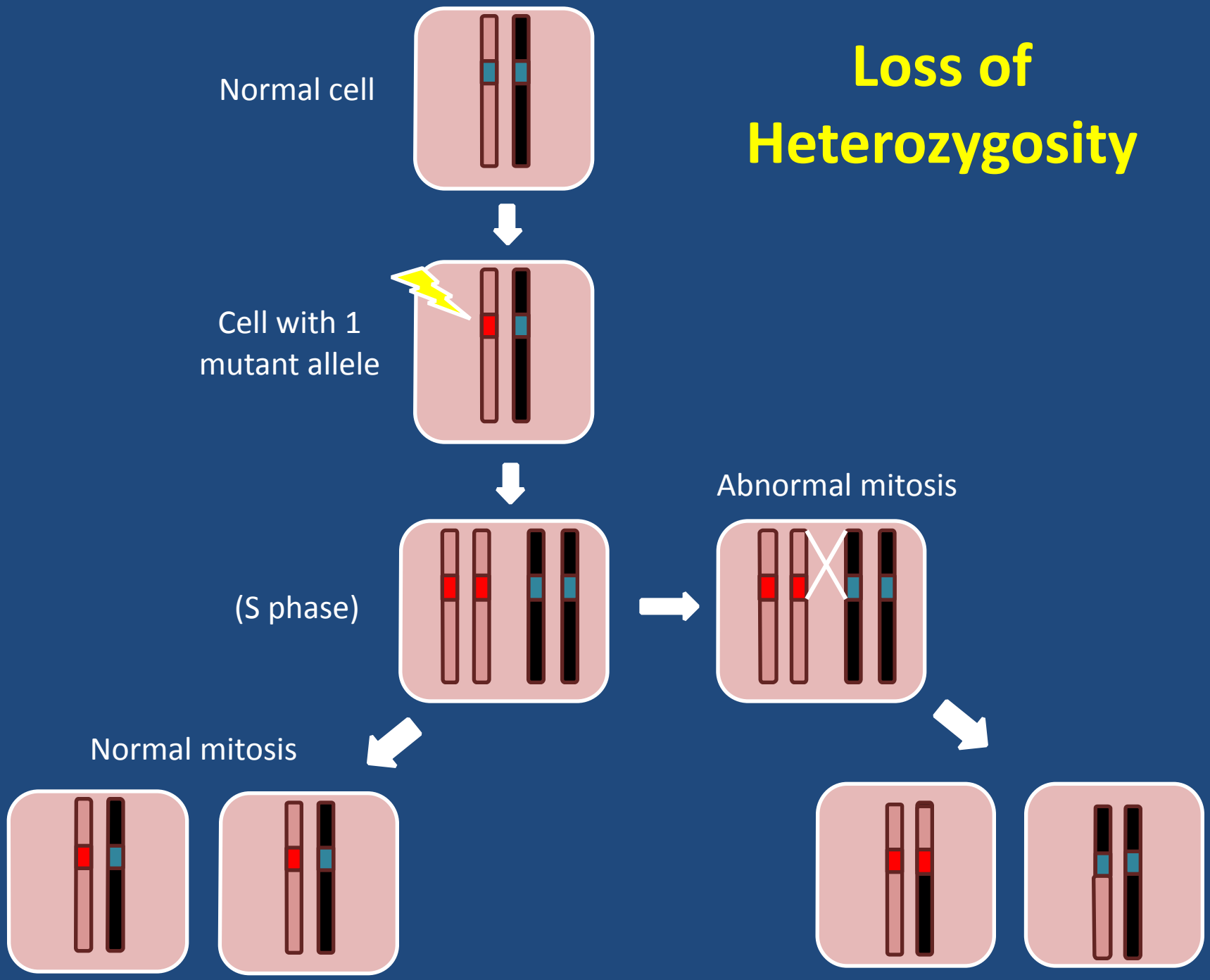
Alfred Knudsen: "2-hit" hypothesis- need gain 2 events (gain of oncogenes and/or loss of TS)

How to TSG get lost?

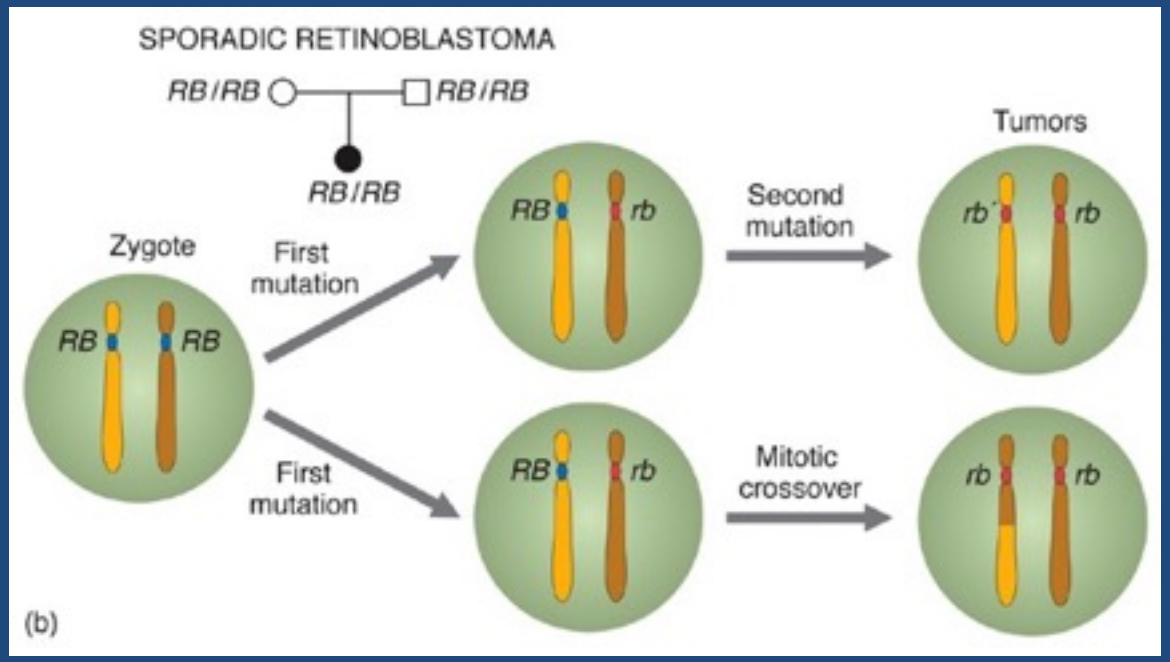
- Direct inactivating mutations
  - Rare (10^{-6} per cell generation)
  - 2 alleles → even more rare (10^{-12} per cell generation)
- Mutations during mitosis
  - Not all that rare
  - **Loss of heterozygosity (LOH)**



Loss of Heterozygosity



Loss of Heterozygosity



2 separate mutations (rare)

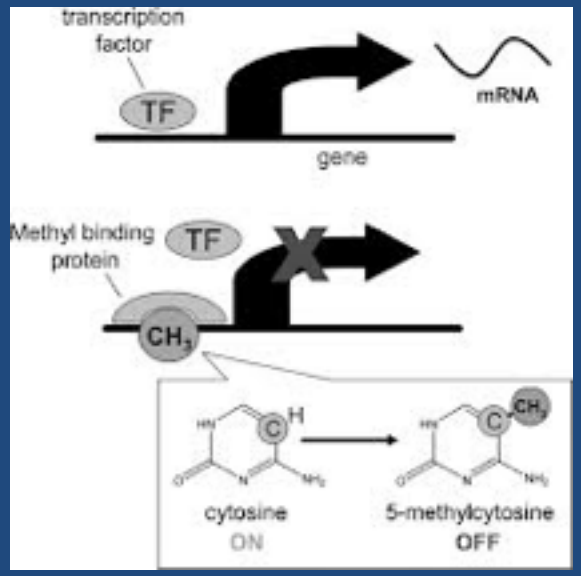
1 mutation + LOH (more likely)

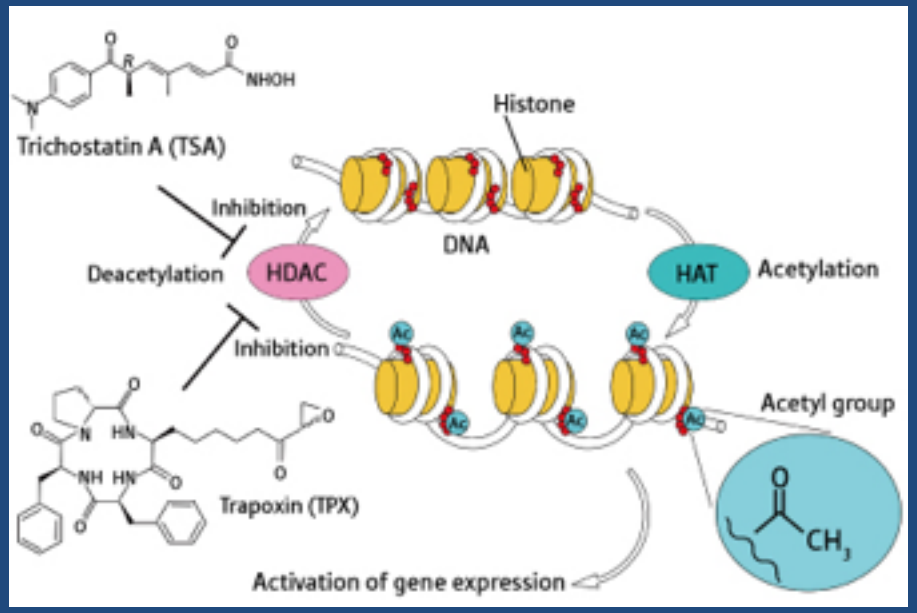
Mechanisms of TSG inactivation

- Gene deletion
- Direct mutation
- Loss of Heterozygosity
- **Epigenetic silencing (promoter methylation)**

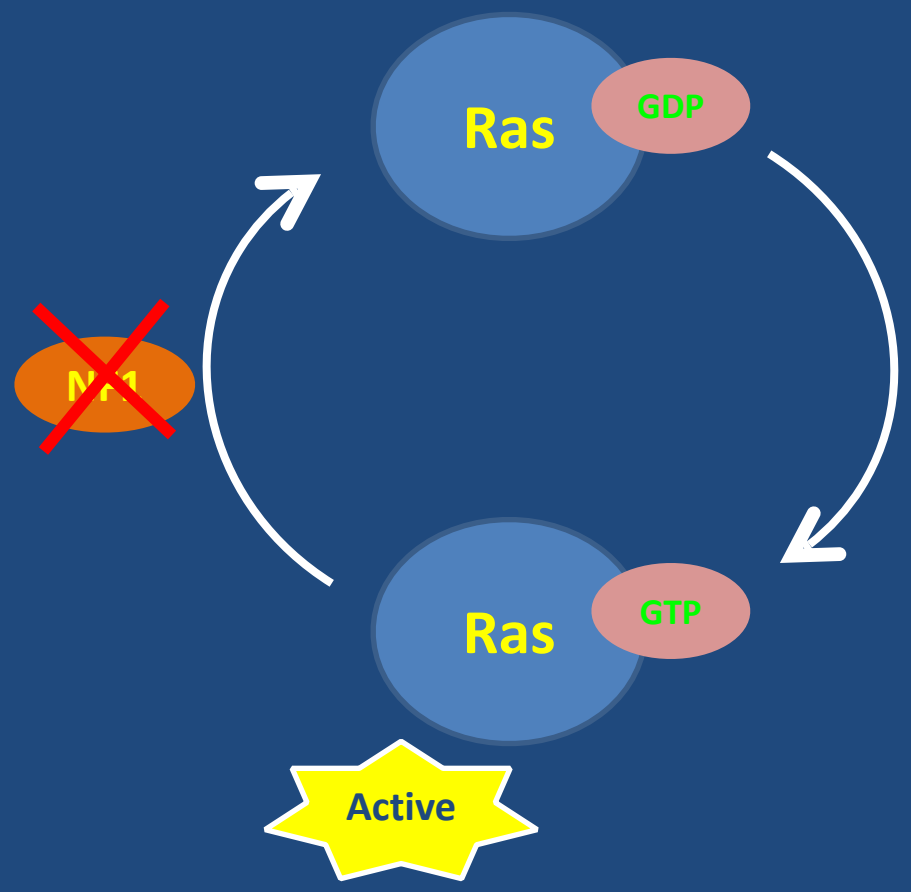
Promoter hypermethylation

- Promoters rich in the sequence cytosine-guanosine (CpG)
- Cytosine gets methylated
- HDAC protein complexes recognize methyl-CpG
- HDAC removes histone acetylations
- Histones instigate "closed" DNA conformation
- → turn off transcription





NF1 as a tumor suppressor



NF1

- Lost in neurofibromatosis
- A GTPase Activating Protein (**GAP**)
  - induces hydrolysis of GTP
  - →inactives Ras
- Deletion of NF1 functionally mimics hyperactivation of Ras

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
RUNX3	1p36	—	gastric carcinoma	TF co-factor
HRPT2	1q25–32	parathyroid tumors, jaw fibromas	parathyroid tumors	chromatin protein
FH	1q42.3	familial leiomyomatosis[a]	—	fumarate hydratase
FHIT	3p14.2	—	many types	diadenosine triphosphate hydrolase
RASSF1A	3p21.3	—	many types	multiple functions
TGFBR2	3p2.2	HNPCC	colon, gastric, pancreatic carcinomas	TGF-β receptor
VHL	3p25	von Hippel–Lindau syndrome	renal cell carcinoma	ubiquitylation of HIF
hCDC4	4q32	—	endometrial carcinoma	ubiquitin ligase
APC	5p21	familial adenomatous polyposis coli	colorectal, pancreatic, and stomach carcinomas; prostate carcinoma	β-catenin degradation
NKX3.1	8p21	—	prostate carcinoma	homeobox TF
p16^{INK4A} [b]	9p21	familial melanoma	many types	CDK inhibitor
p14^{ARF} [c]	9p21	—	all types	p53 stabilizer
PTC	9q22.3	nevoid basal cell carcinoma syndrome	medulloblastomas	receptor for hedgehog GF
TSC1	9q34	tuberous sclerosis	—	inhibitor of mTOR[f]
BMPR1	10q21–22	juvenile polyposis	—	BMP receptor
PTEN[d]	10q23.3	Cowden's disease, breast and gastrointestinal carcinomas	glioblastoma; prostate, breast, and thyroid carcinomas	PIP_3 phosphatase
WT1	11p13	Wilms tumor	Wilms tumor	TF
MEN1	11p13	multiple endocrine neoplasia	—	histone modification, transcriptional repressor

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
BWS/CDKN1C	11p15.5	Beckwith–Wiedemann syndrome	—	$p57^{Kip2}$ CDK inhibitor
SDHD	11q23	familial paraganglioma	pheochromocytoma	mitochondrial protein[e]
RB	13q14	retinoblastoma, osteosarcoma	retinoblastoma; sarcomas; bladder, breast, esophageal, and lung carcinomas	transcriptional repression; control of E2Fs
TSC2	16p13	tuberous sclerosis	—	inhibitor of mTOR[f]
CBP	16p13.3	Rubinstein–Taybi	AML[g]	TF co-activator
CYLD	16q12–13	cylindromatosis	—	deubiquitinating enzyme
CDH1	16q22.1	familial gastric carcinoma	invasive cancers	cell–cell adhesion
BHD	17p11.2	Birt–Hogg–Dube syndrome	kidney carcinomas, hamartomas	unknown
TP53	17p13.1	Li–Fraumeni syndrome	many types	TF
NF1	17q11.2	neurofibromatosis type 1	colon carcinoma, astrocytoma	Ras-GAP
BECN1	17q21.3	—	breast, ovarian, prostate	autophagy
PRKAR1A	17.q22–24	multiple endocrine neoplasia[h]	multiple endocrine tumors	subunit of PKA
DPC4[i]	18q21.1	juvenile polyposis	pancreatic and colon carcinomas	TGF-β TF
LKB1/STK11	19p13.3	Peutz–Jegher syndrome	hamartomatous colonic polyps	serine/threonine kinase
RUNX1	21q22.12	familial platelet disorder	AML	TF
SNF5[j]	22q11.2	rhabdoid predisposition syndrome	malignant rhabdoid tumors	chromosome remodeling
NF2	22q12.2	neurofibroma-position syndrome	schwannoma, meningioma; ependymoma	cytoskeleton–membrane linkage

Name of gene	Nature of protein function	Type of tumor
RARβ2	nuclear receptor for differentiation	breast, lung
$p57^{Kip2}$	CDK inhibitor	gastric, pancreatic, hepatic; AML
TIMP3	inhibitor of metalloproteinases	diverse tumors
IGFBP	sequesters IGF-1 factor	diverse tumors
$CDKN2A/p16^{INK4A}$	inhibitor of CDK4/6	diverse tumors
$CDKN2B/p15^{INK4B}$	inhibitor of CDK4/6	diverse tumors
$p14^{ARF}$	inhibitor of HDM2/MDM2	colon, lymphoma
APC	inducer of β-catenin degradation	colon carcinomas
p73	aids p53 to trigger apoptosis	diverse tumors
GSTP1	mutagen inactivator	breast, liver, prostate
MGMT	DNA repair enzyme	colorectal
CDH1	cell–cell adhesion receptor	bladder, breast, colon, gastric
DAPK	kinase involved in cell death	bladder
MLH1	DNA mismatch repair enzyme	colon, endometrial, gastric
TGFBR2	TGF-β receptor	colon, gastric, small-cell lung
THBS1	angiogenesis inhibitor	colon, glioblastoma
RB	cell-cycle regulator	retinoblastoma
CASP8	apoptotic caspase	neuroblastoma, SCLC
APAF1	pro-apoptotic cascade	melanoma
CTMP	inhibitor of Akt/PKB	glioblastoma multiforme

Genetic changes → Cancer

Oncogenes

- Gene amplification
- Insertion of powerful (viral) promoters
- Activating mutations
- Fusion genes

Tumor suppressors

- Gene deletion
- Silencing mutations
- Loss of heterozygosity
- Promoter hypermethylation