Oncogenes & Tumor Suppressor genes

Sheila Figel

Sheila.figel@roswellpark.org

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

- Genes that 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

- Genes which normally suppress cell growth and/or motility which are frequently downregulated in tumor cells, allowing for unchecked growth/motility

*Cancer is a genetic disease

Gain of oncogene(s)

+

Loss of TSG

=

CANCER

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

Src – the first oncogene

- *The first oncogene was discovered before we understood what oncogenes really are!*

Peyton Rous, 1911 – cell-free extract from chicken tumors can cause new tumors when injected into healthy chickens

Rous Sarcoma – a form of cancer which infects chickens

Rous Sarcoma Virus (RSV) – a retrovirus which generates a cDNA that inserts into the chickens' DNA

v-Src – the gene which gets expressed by RSV

v-Src = oncogenic retrovirus

Other viral oncogenes

• Rous Sarcoma	src
• Abelson leukemia	abl
• Avian erythroblastosis	erbB
• McDonough feline sarcoma	fms
• H-Z feline	kit
• Murine sarcoma 3611	raf
• Simian sarcoma	sis
• Harvey sarcoma	H-ras

- Many oncogenes identified in viruses
- *But…* ***Most human cancers are not viral in origin***

- Cellular versions of oncogenes = **proto-oncogenes**

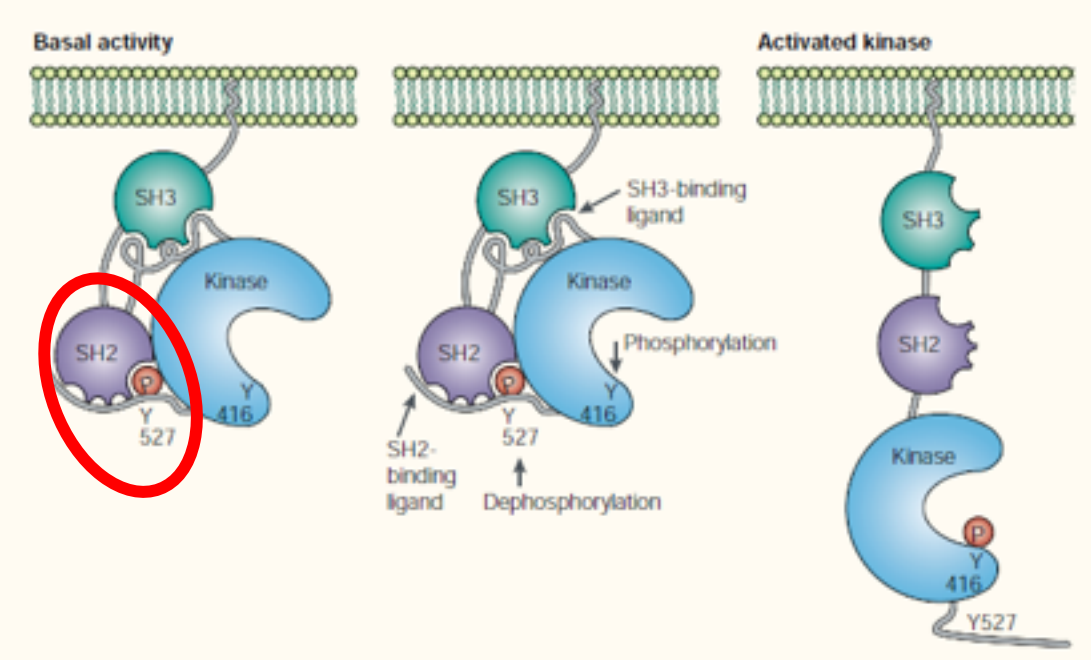
Src in human tumors

Src expression/activity upregulated in:

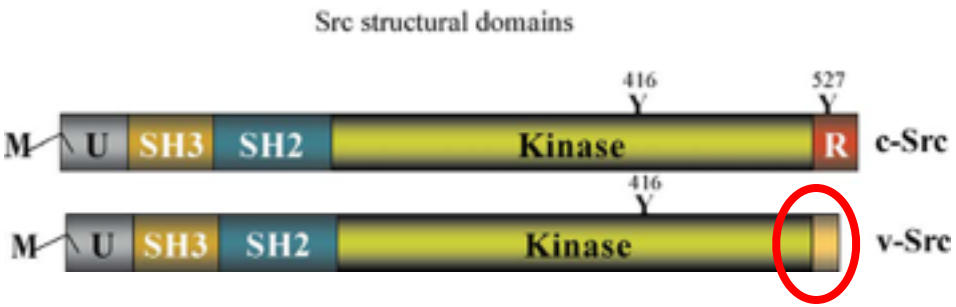
- Breast ca
- Pancreatic ca
- Ovarian ca
- Head & Neck ca
- Lung ca
- Gastric ca
- Colon ca – C-terminal truncation identified

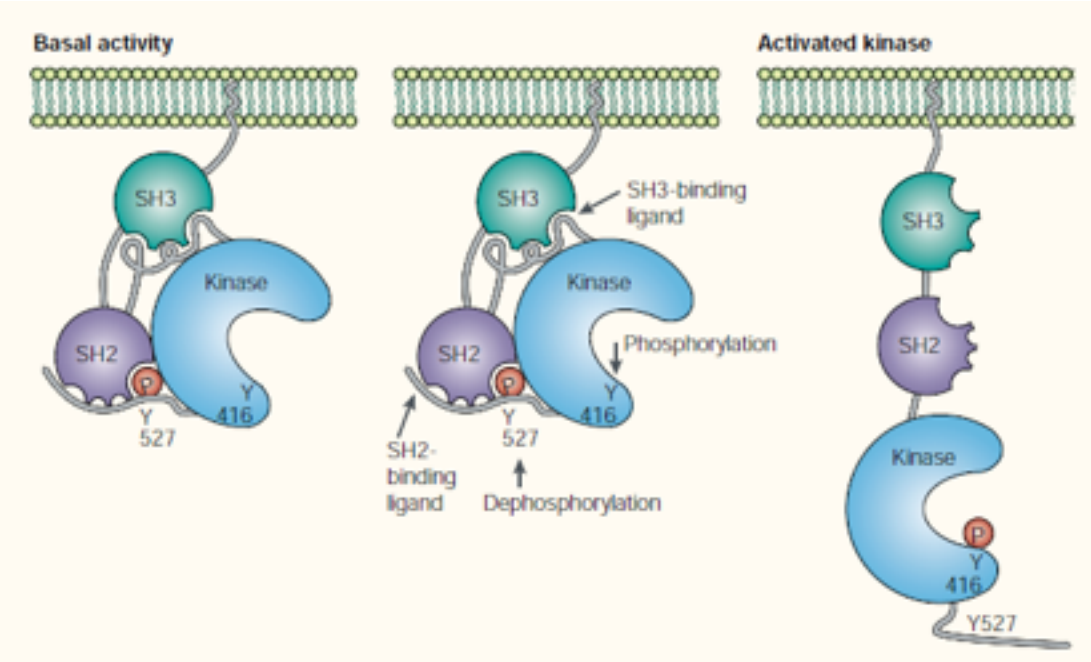
c-Src

- A kinase
- Cell motility, growth, morphology, proliferation
- The first proto-oncogene
- **Activity is tightly regulated**
  - po-Y527



v-Src is a CA version of c-Src

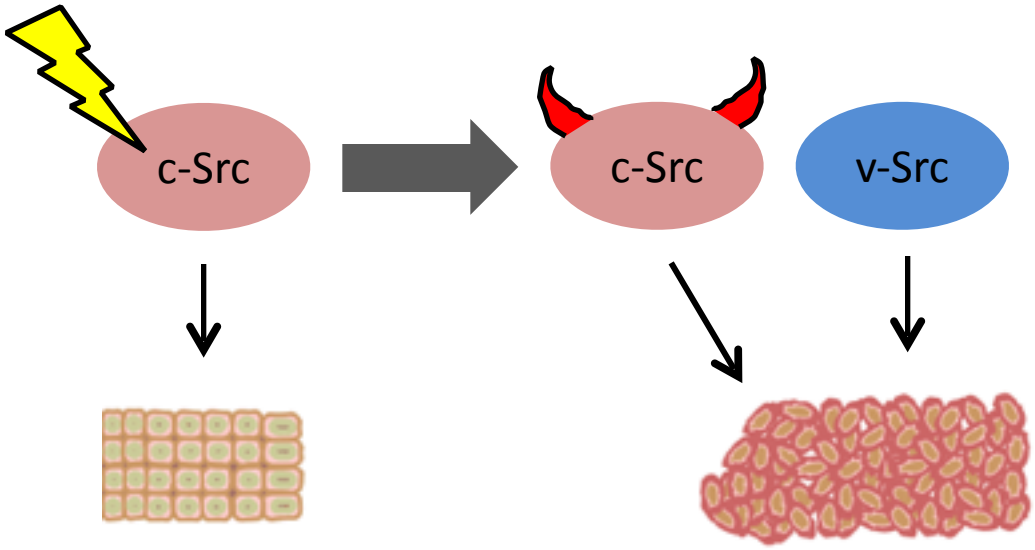




- c-Src – interaction between po-Y527 and SH2 domain hold c-Src in a kinase inactive state
- Dephosphorylation of Y527 releases the interaction
- v-Src lacks regulatory Y527 → constitutively active

Src – the first (proto)oncogene

- 1976 – v-Src is the viral version of **c-Src**
- c-Src is a **proto-oncogene**

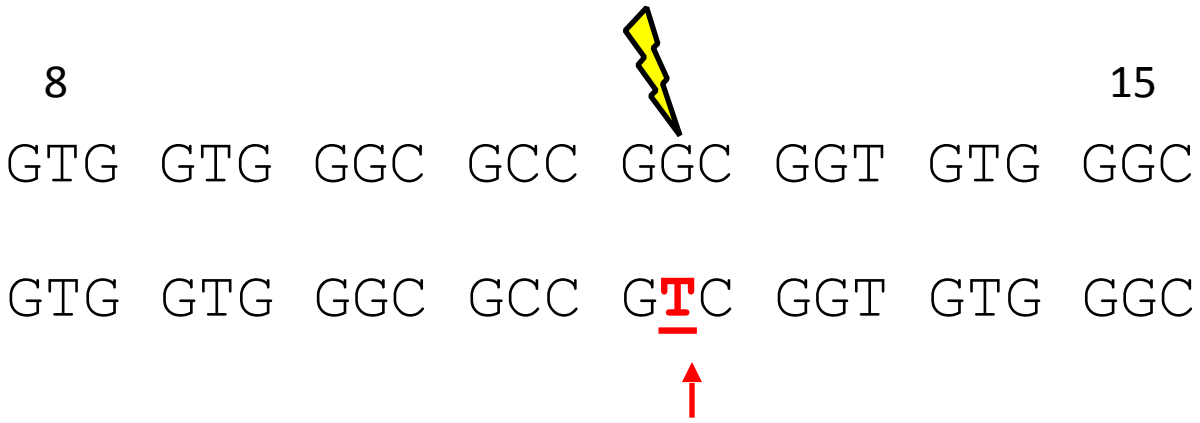


Activation of proto-oncogenes

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

Activation of Ras by mutation



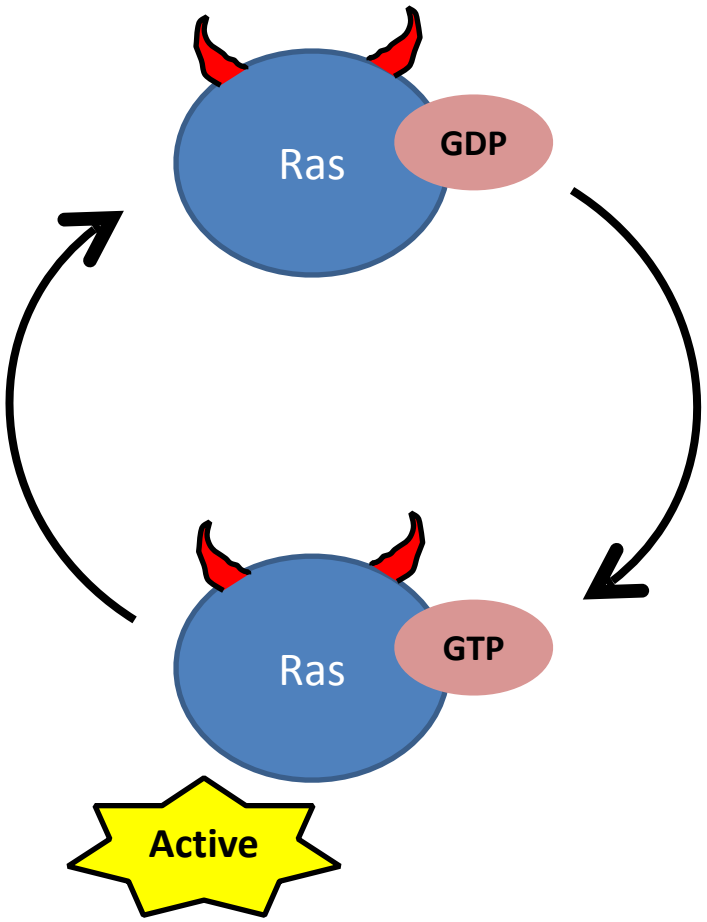
Oncogenic H-Ras mutation in bladder ca**:**

Glycine → Valine (G12V)

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)

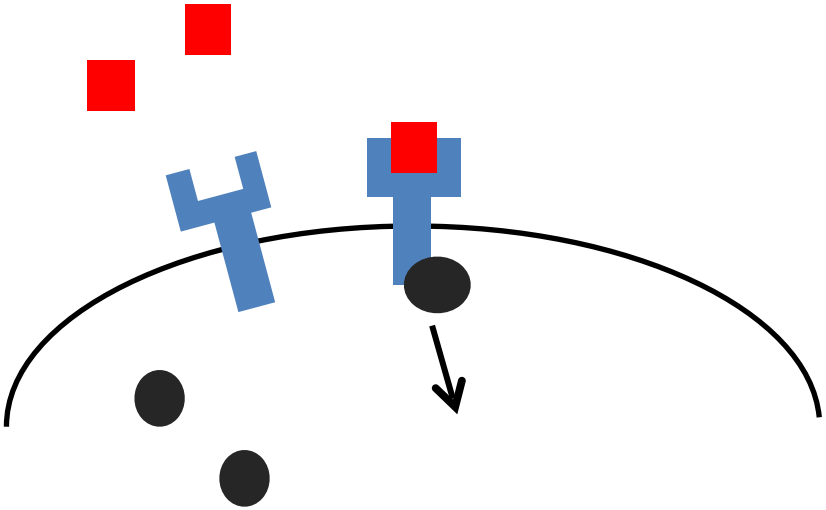
[a]H, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

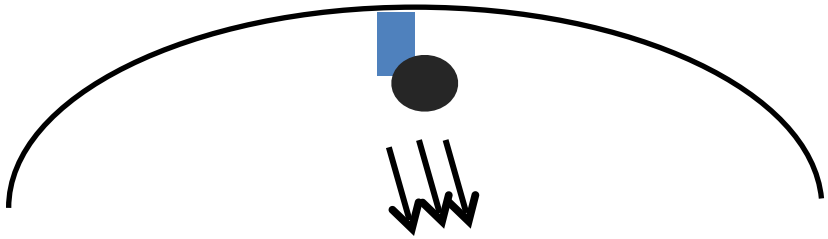
Adapted from J. Downward, *Nature Rev. Cancer* 3:11–22, 2003.

Oncogene activation by mutation

WT EGF Receptor (EGFR)
– ligand binding stimulates activation

Mutant EGFR –
Constitutive activation

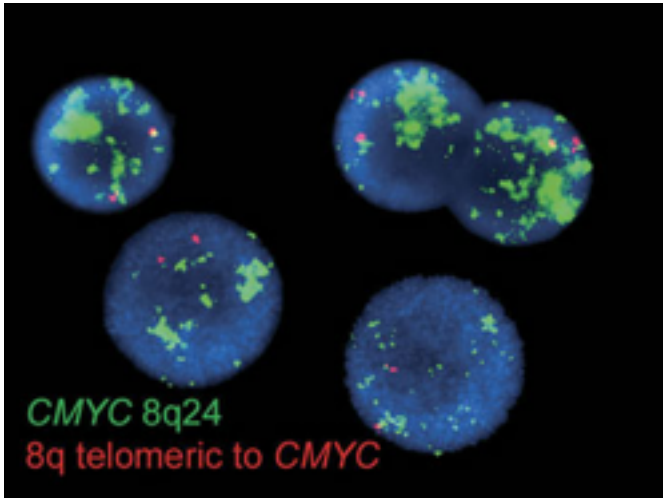


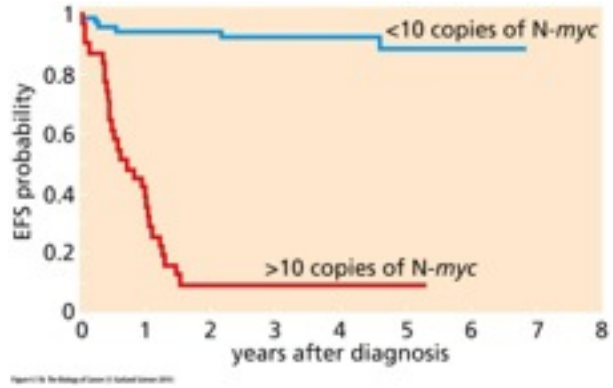


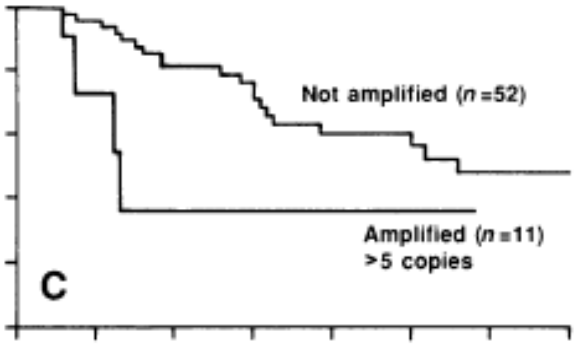
Oncogene activation by gene amplification

- Multiple copies of Myc, ErbB2 → greater expression
- Pro-growth advantage of tumor cells with greater expression

Fluorescence in Situ Hybridization (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

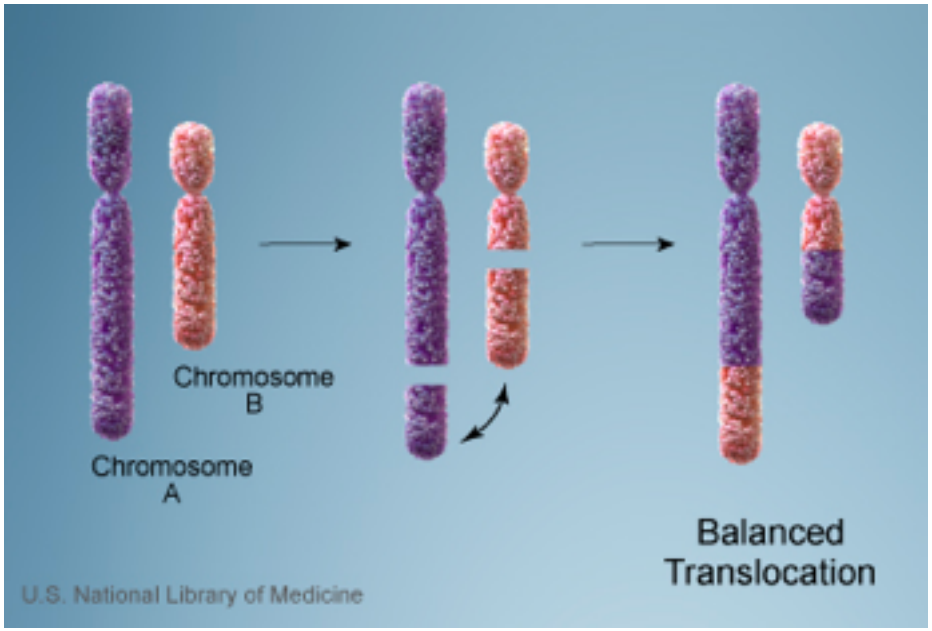
Name of oncogene[a]	Human chromosomal location	Human cancers	Nature of protein[b]
MDM4/MDMX	1q32	breast, colon, lung, pre-B leukemias	p53 inhibitor
PIK3CA	3q26.3	lung SCC, ovarian, breast	PI kinase
erbB1/EGFR	7q12–13	glioblastomas (50%); squamous cell carcinomas (10–20%)	RTK
cab1–erbB2–grb7	17q12	gastric, 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.

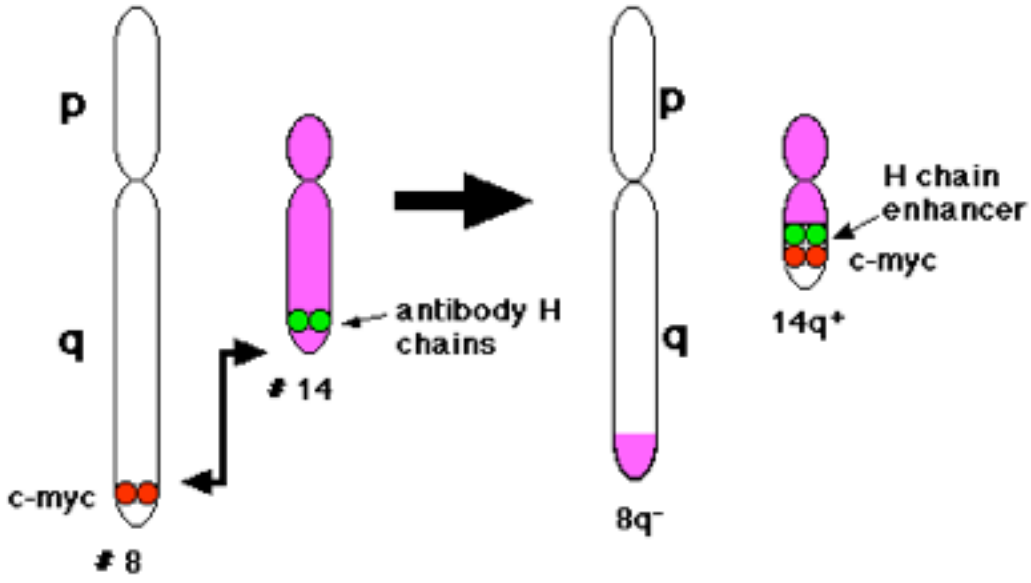
Chromosomal rearrangement

- Genetic instability
  - → Place strong promoter in front of an oncogene
  - → formation of novel hybrid proteins



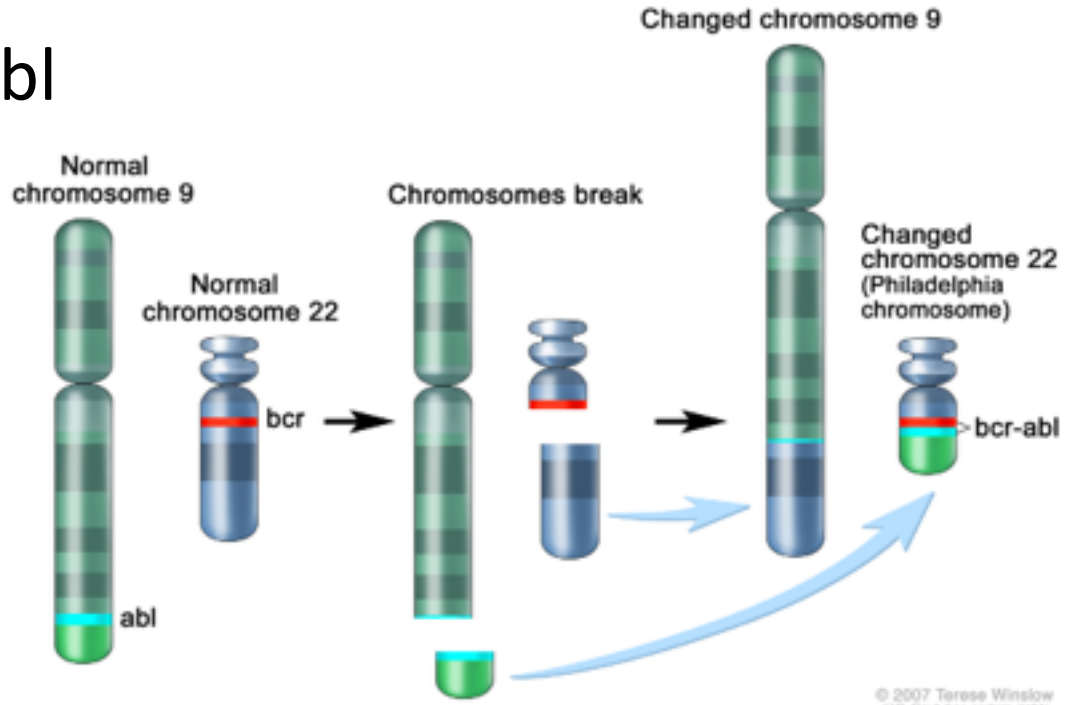
t(8;14)

- Burkitt's Lymphoma
- Fusion of chromosomes 2, 14, or 22 to chromosome 8
- Place Myc under the Ig promoter



Bcr-Abl

- Chronic myelogenous leukemia (CML)
- Abl – oncogene (TK) located on cr 9
- Breakpoint cluster region – cr 22
- t(9;22)
- Result: CA version of Abl

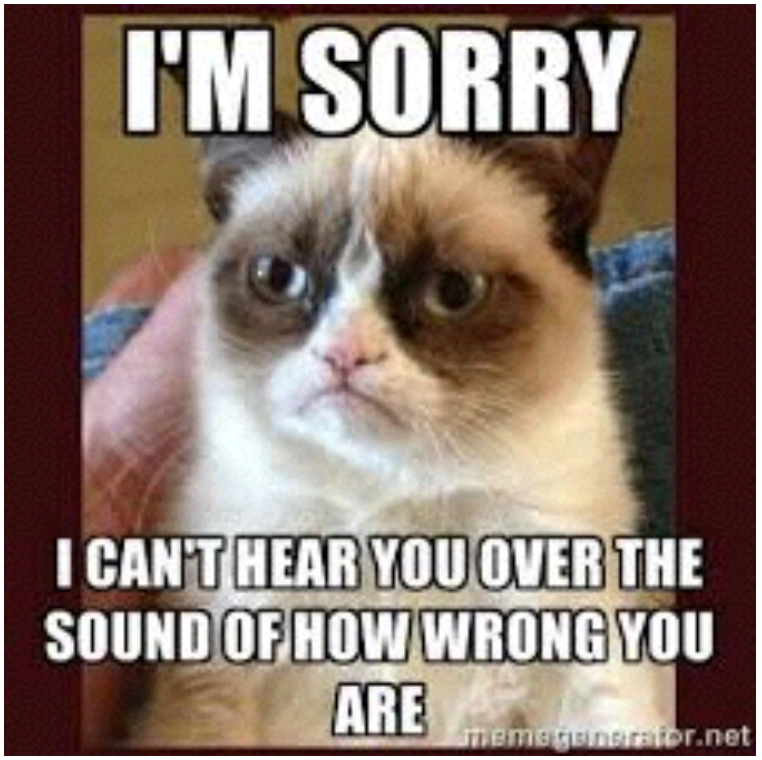


Summary so far

- Oncogenes were first discovered in the DNA of tumor-causing viruses
- Activation of proto-oncogenes drive tumorigenesis
- Oncogenes are activated by gene amplification, activating mutations, and chromosomal rearrangements

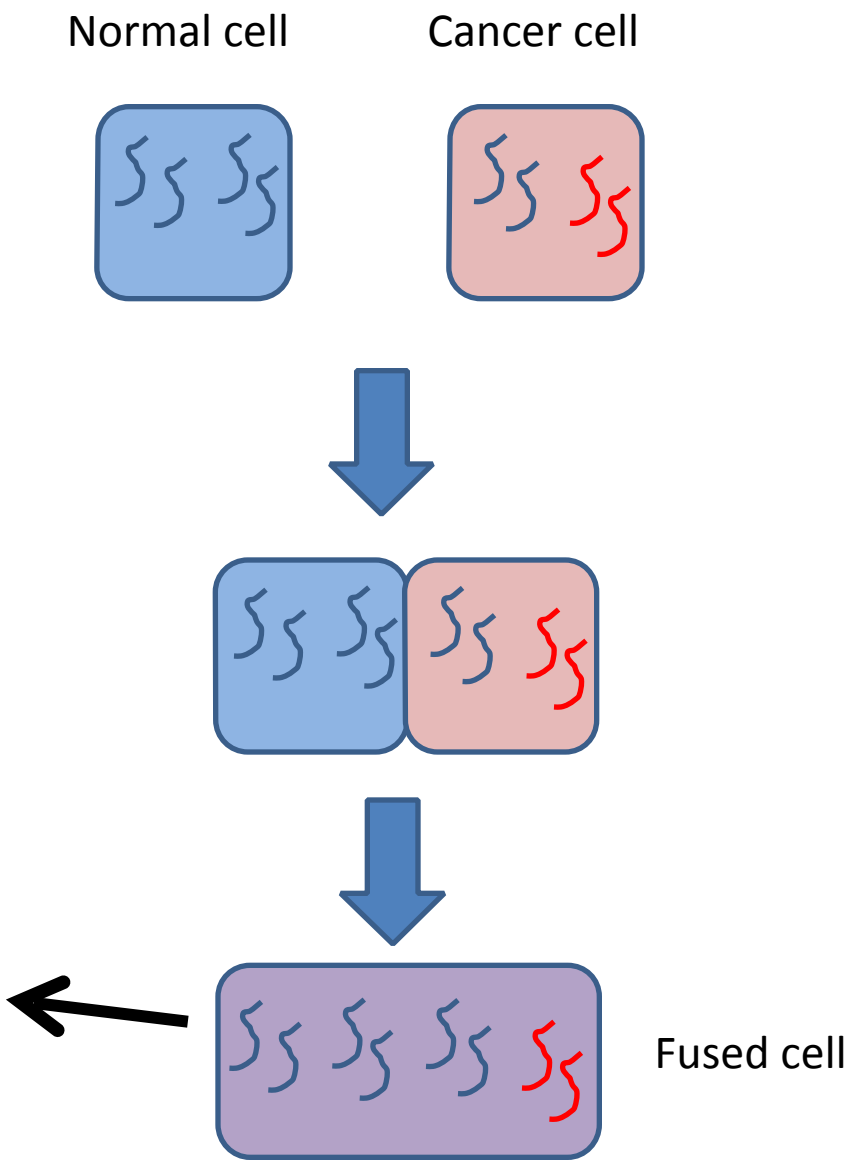
Oncogenes → cancer, end of story, right?!

WRONG



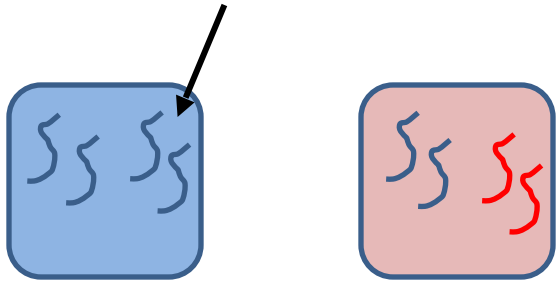
- Scientists expected oncogenes to be genetically dominant

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



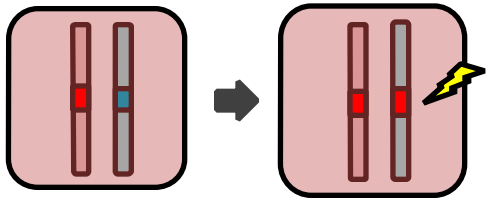
Results of fusion studies

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

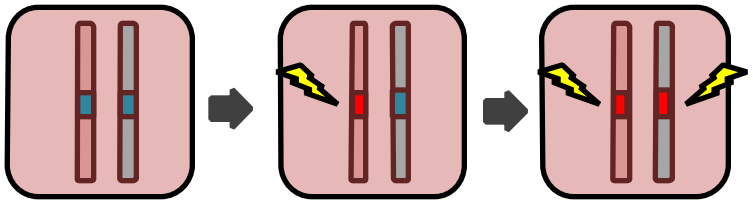


Additional support for TS theory: Retinoblastoma

- **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

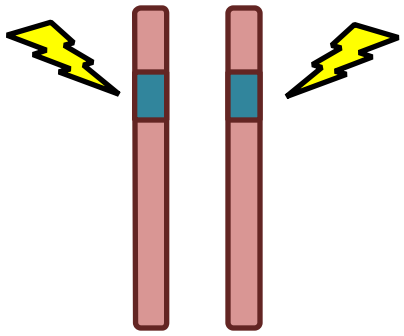


Tumor Suppressor genes

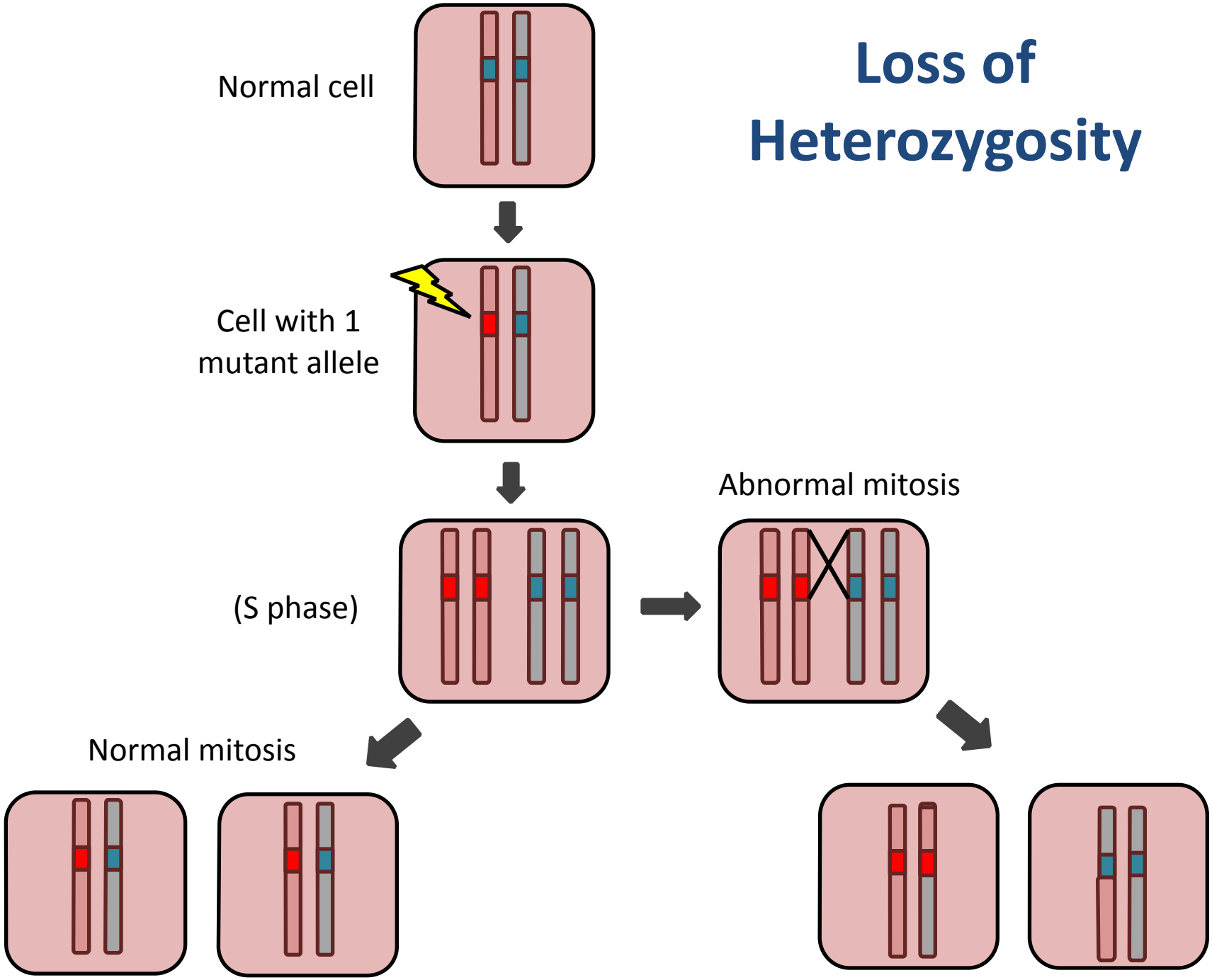
- Tumor suppressor genes function as growth suppressors in healthy cells
- **The loss of tumor suppressor genes causes cancer**
- Cancer = gain of oncogenes + loss of TSG

How to TSG get lost?

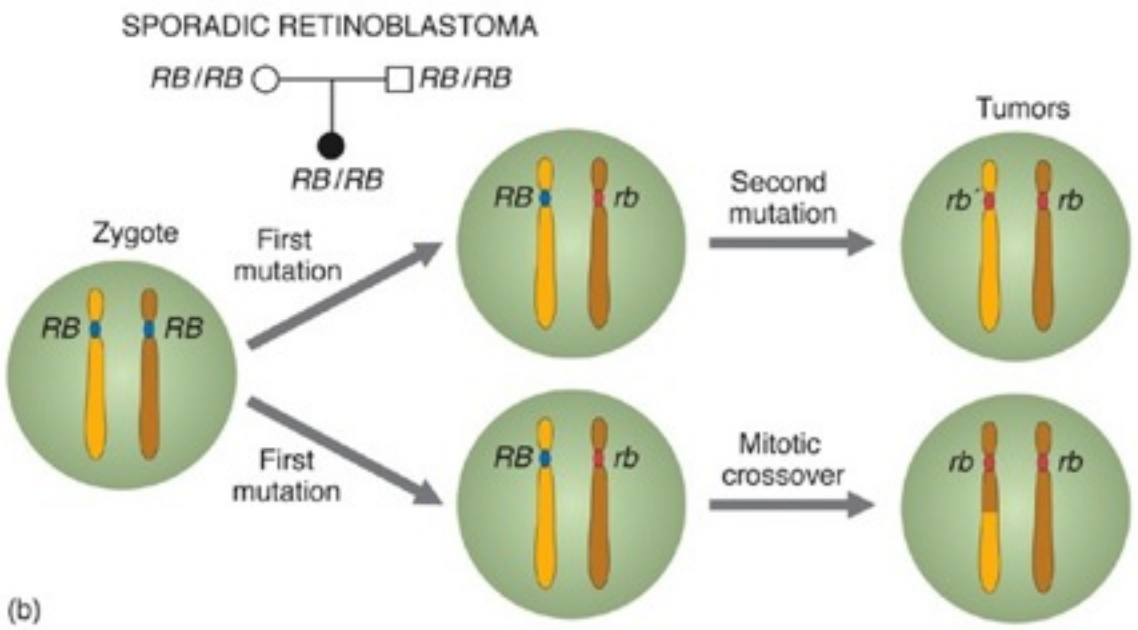
- Direct inactivating mutations
  - Rare (10^{-6} per cell generation)
  - 2 alleles → even more rare (10^{-12} per cell generation)
- Mutations during mitosis
  - Not all that rare
  - **Loss of heterozygosity (LOH)**



Loss of Heterozygosity



Loss of Heterozygosity



2 separate mutations (rare)

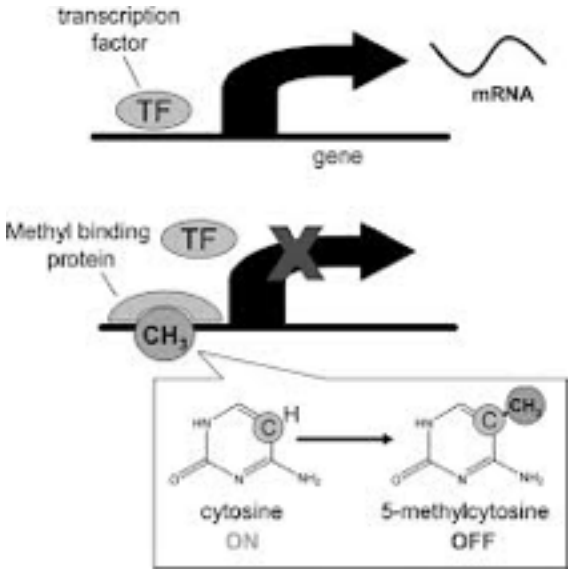
1 mutation + LOH (more likely)

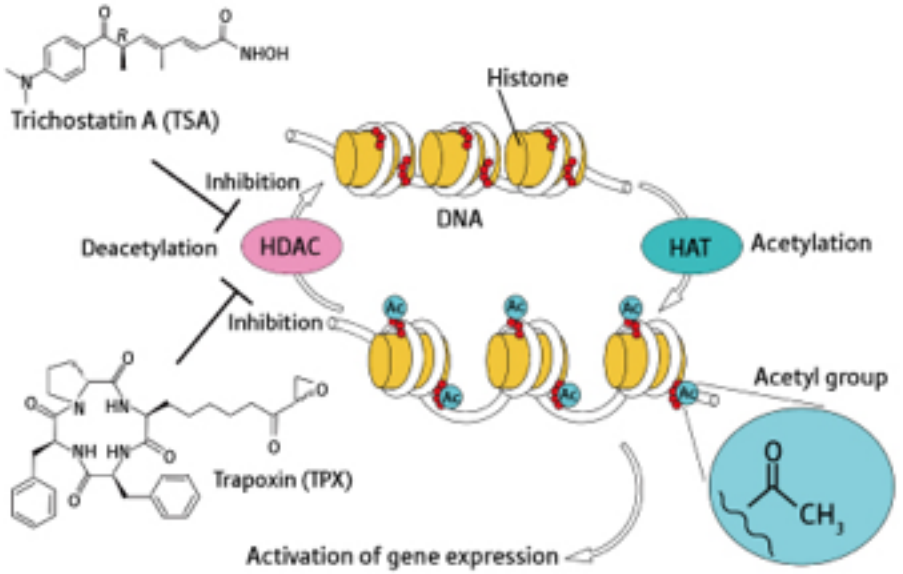
Mechanisms of TSG inactivation

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

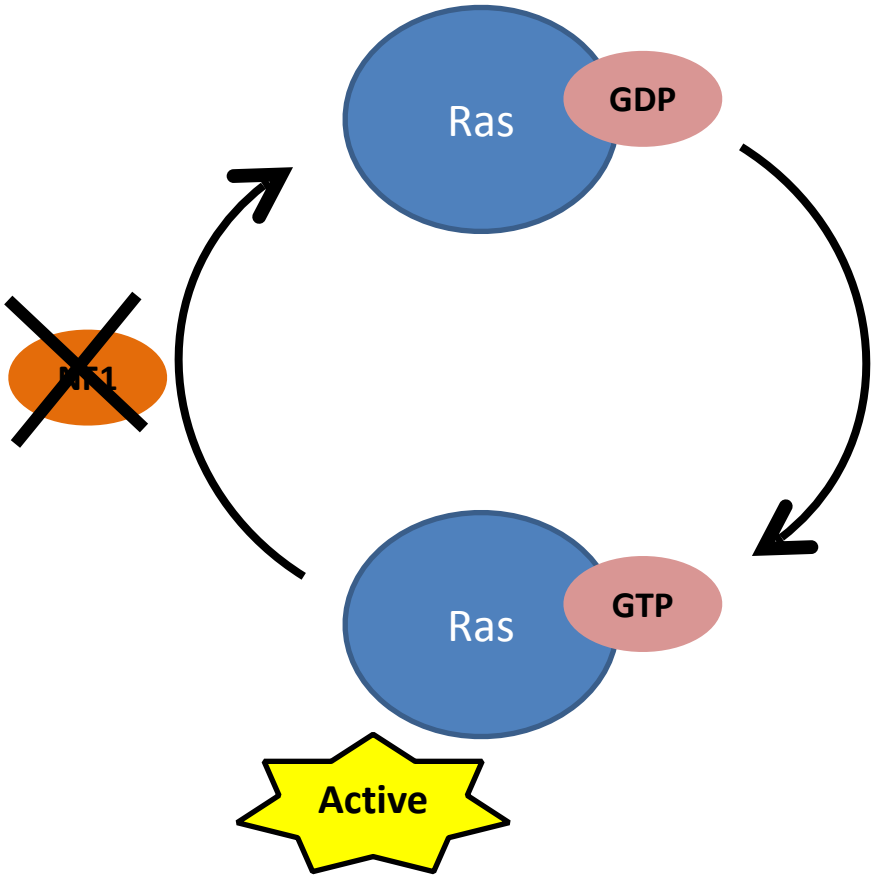
Promoter methylation

- 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
  - Induces hydrolysis of GTP

  →inactive Ras
- Loss of NF1 functionally mimics hyperactivation of Ras

Genetic changes → Cancer

Oncogenes

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

Tumor suppressors

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