Cancer Biology

Lecture 4

Fall 2018

Dr. Dawang Zhou

dwzhou@xmu.edu.cn, E314

ONCOGENES AND CANCER

Outline

- Introduction to cancer and oncogenes
- Compare tumor suppressors and oncogenes
- Tumor progression
- Mechanisms of oncogenes
- Examples of mutations in oncogenes

Definitions

- **Oncogene** – a gene that when mutated or expressed at abnormally high levels contributes to converting a normal cell into a cancer cell
- **Proto-oncogene** – the "normal" cellular progenitors of oncogenes that function to promote the normal growth and division of cells

Proto-oncogene to oncogene

- An alteration occurs in a normal cellular gene (proto-oncogene) that makes the protein hyper-functional (oncogene)

- Proteins involved in the cell signaling pathways are products of proto-oncogenes
  - Proliferative
  - Anti-apoptotic (survival)
  - Angiogenic

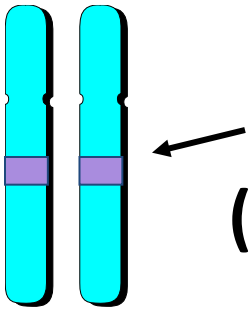
Tumor suppressors

- Normally function to suppress the formation of cancer
  - Growth arrest
  - Apoptosis
  - DNA repair
  - Differentiation
  - Anti-angiogenesis

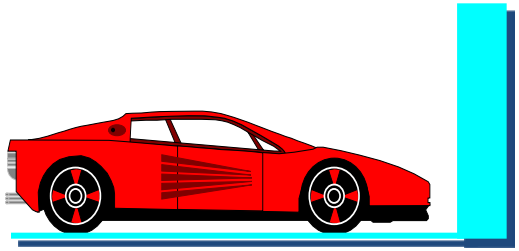
Tumor suppressors are recessive –
require mutation of both alleles

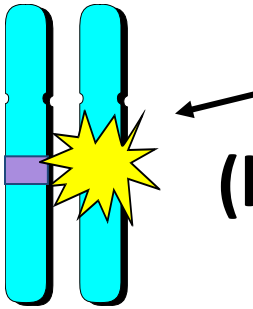
Oncogenes are dominant –
mutation of 1 allele is sufficient

Oncogenes

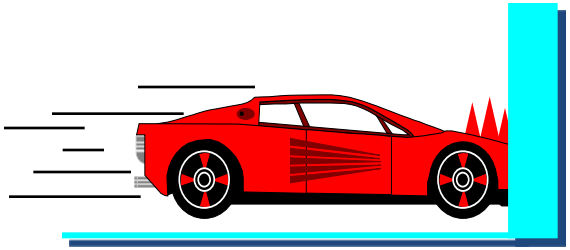


Normal genes
(regulate cell growth)



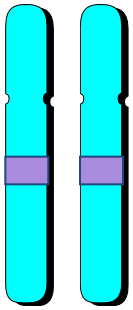


1st mutation
(leads to accelerated cell division)

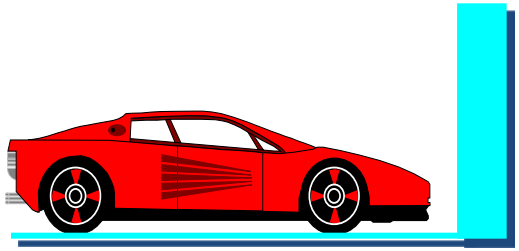


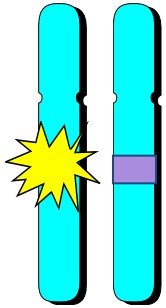
1 mutation is sufficient for a role in cancer development.

Tumor Suppressor Genes

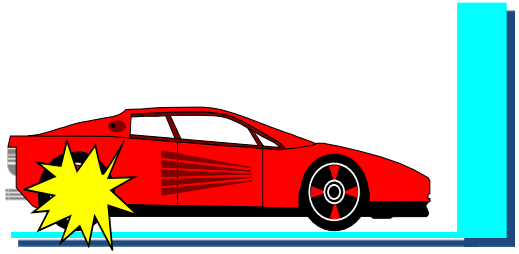


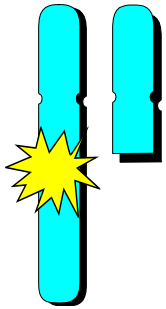
Normal genes (prevent cancer)



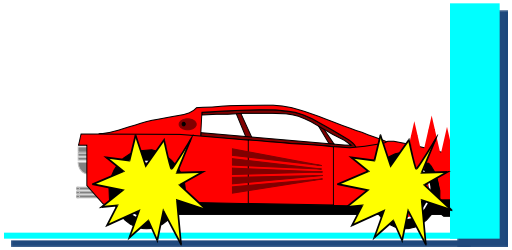


1st mutation (susceptible carrier)





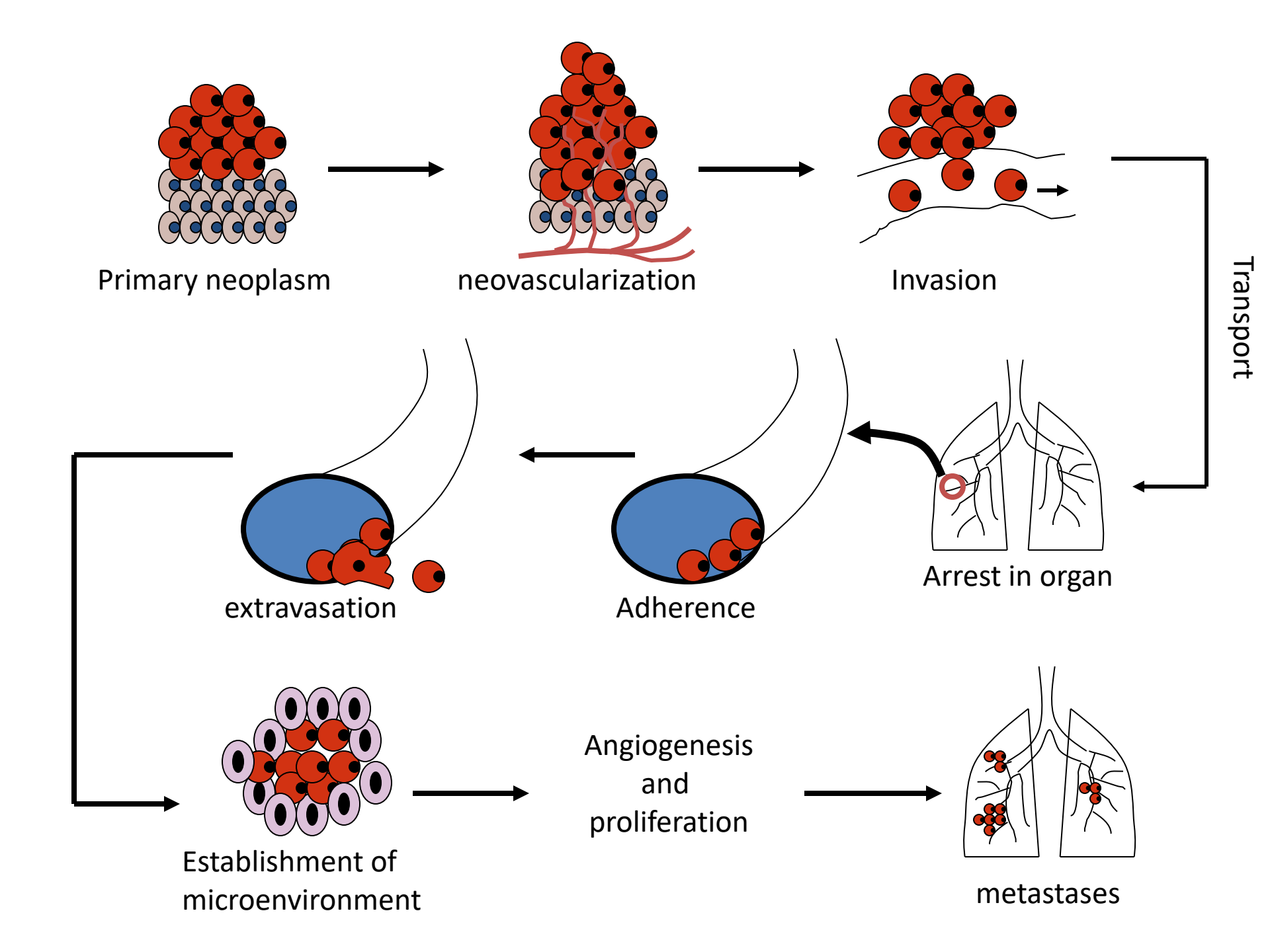
2nd mutation or loss (leads to cancer)



2 mutations are necessary for a role in cancer development.

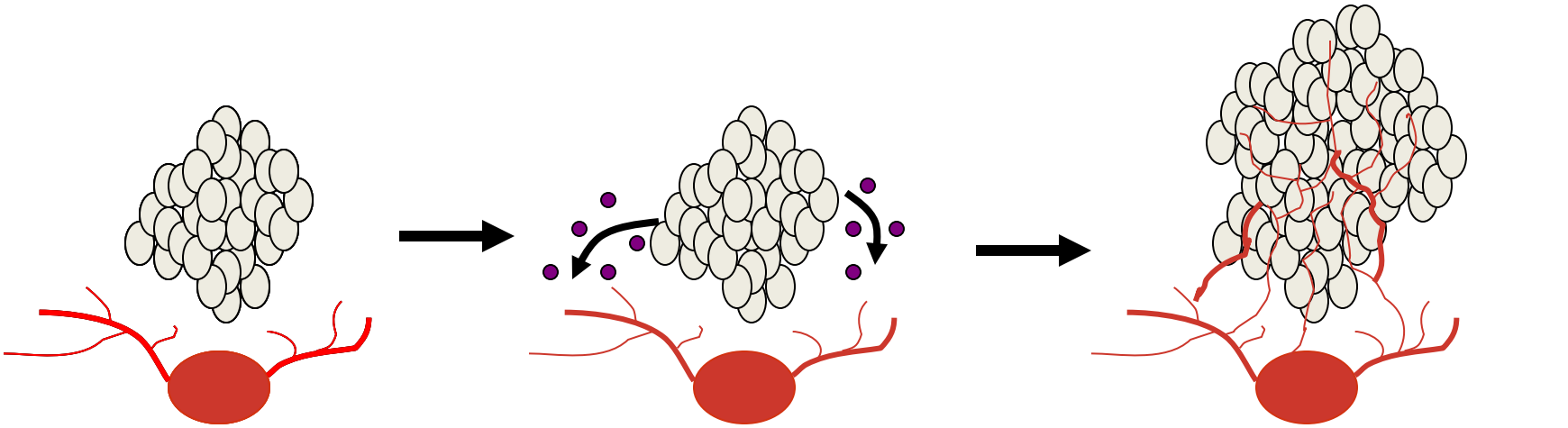
Comparison of Proto-oncogenes and tumor suppressors

Property	Tumor suppressor genes	Proto-oncogenes
Alleles mutated in cancer	Both alleles	One allele
Germ line transmission of mutant allele	frequent	Rare (1 example)
Somatic mutations	yes	yes
Function of mutant allele	Loss of function (recessive allele)	Gain of function (dominant allele)
Effects on cell growth	Inhibit cell growth	Promote cell growth


Primary neoplasm
neovascularization
Invasion
Transport
Arrest in organ
Adherence
extravasation
Establishment of microenvironment
Angiogenesis and proliferation
metastases

Angiogenesis

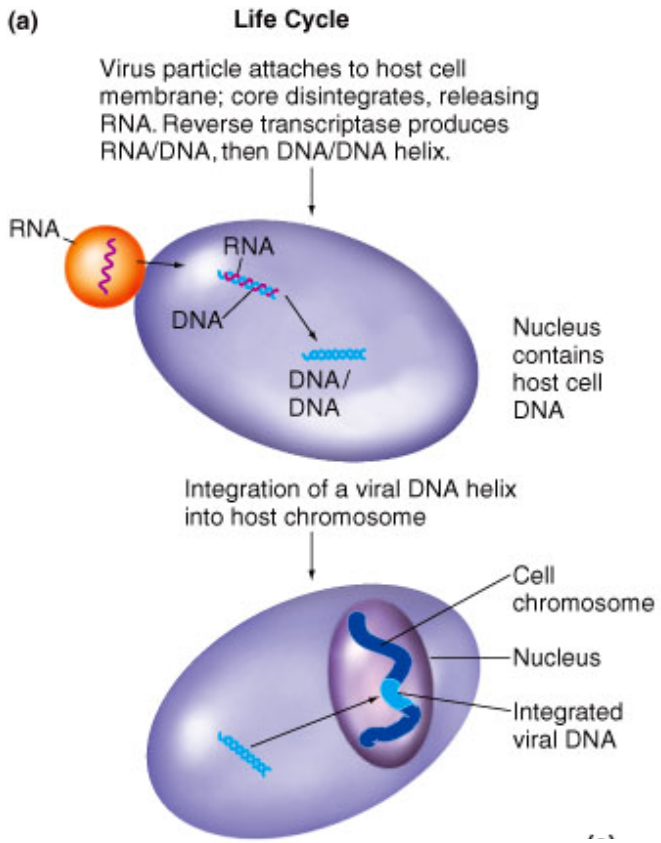
Definition: Process where tumor cells encourage the in-growth of capillaries and vessels from adjacent normal tissue

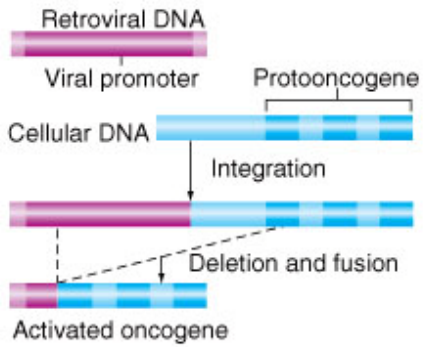


Tumor releases angiogenic factors

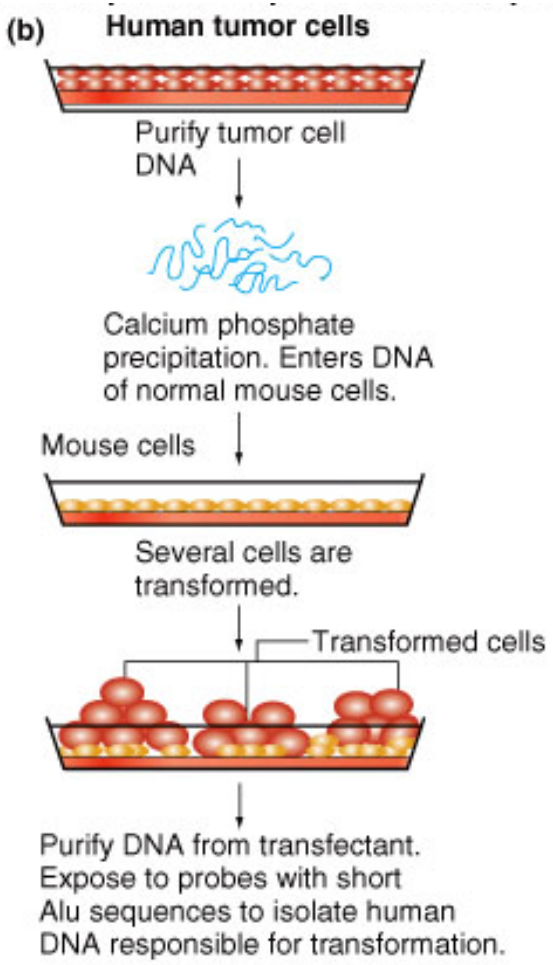
Endothelial cells proliferate
Tumor grows

Two approaches to identifying oncogenes

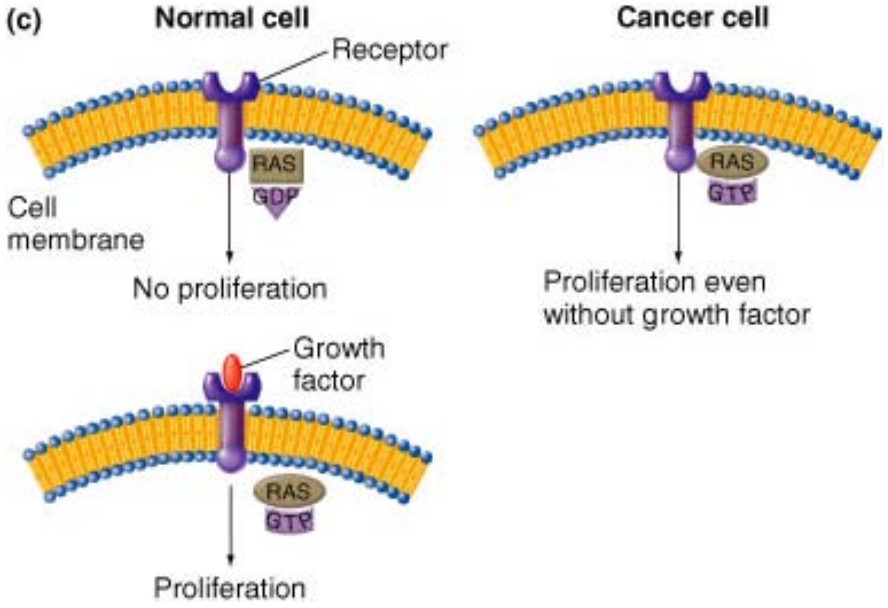




- Analysis of tumor causing retroviruses



- Exposure of noncancerous cells to tumor DNA in culture
  - Human tumor DNA to transform normal mouse cells
  - Human DNA isolated from transformants



Some acronyms

- **Myc** **Myelocytomatosis**
- **Sis** **Simian sarcoma**
- **Erb** **Erythroblastoma**
- **Src** **Rous sarcoma virus**
- **Ras** **Rat sarcoma**
- **Yes** **2 viruses Y73 & ESH sarcoma, isolated from a chicken owned by Mr. Esh**
- **Abl** **Abelson murine leukaemia virus**
- **Fos** **Finkel biskis jinkins reilly mouse sarcoma**
- **jun** **junana**

Immortalized/established cell line

- Anchorage dependence
- Growth factor dependent
- Contact inhibition
- Cytoskeletal organization
- Monolayer

Transformed cell

- Unregulated growth properties
- Serum independence
- Anchorage independent
- No contact inhibition (form foci)
- May induce tumors in vivo

Focus Forming Assay

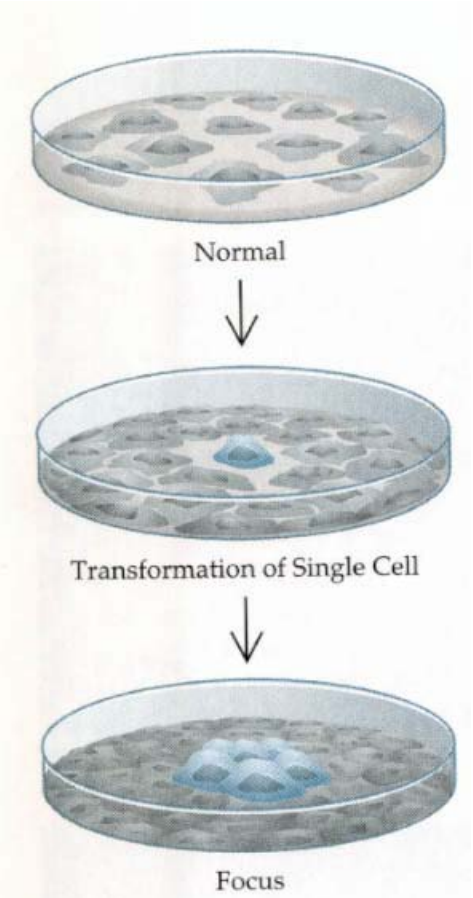
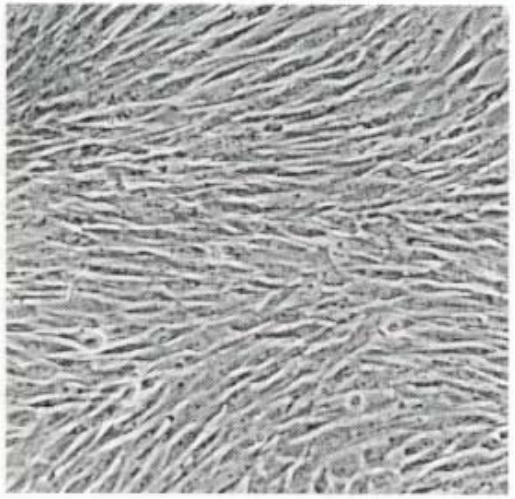
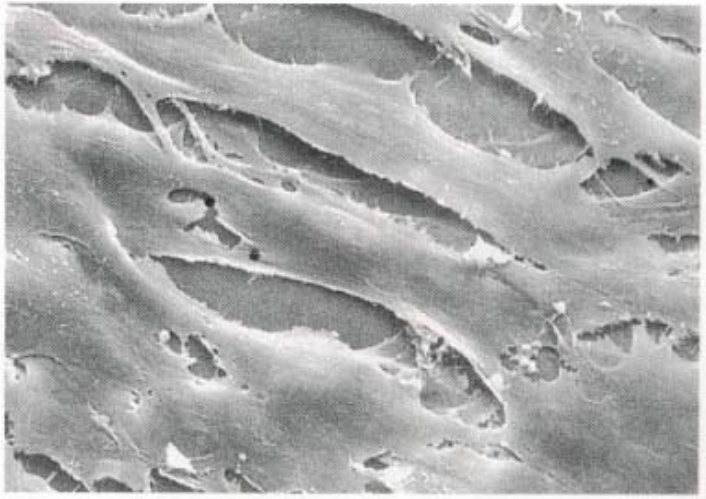


Figure 4 *Side view of cells growing in a Petri dish. Lacking the contact inhibition of normal cells, transformed cells proliferate to form a thick focus that is visible to the naked eye.*

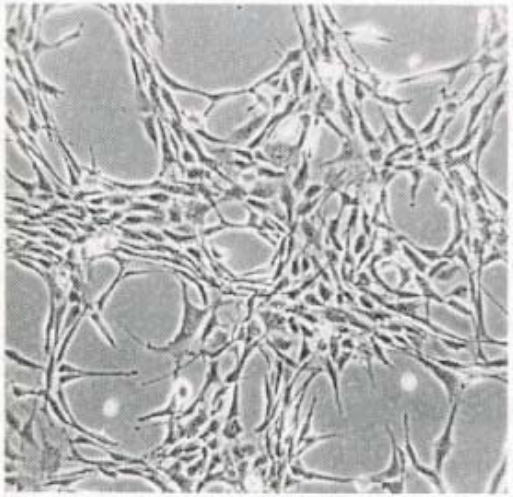
Lacks contact inhibition

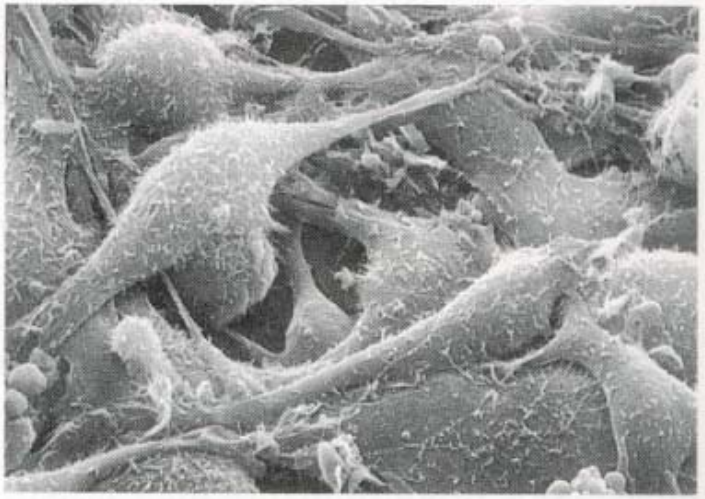
Normal cells



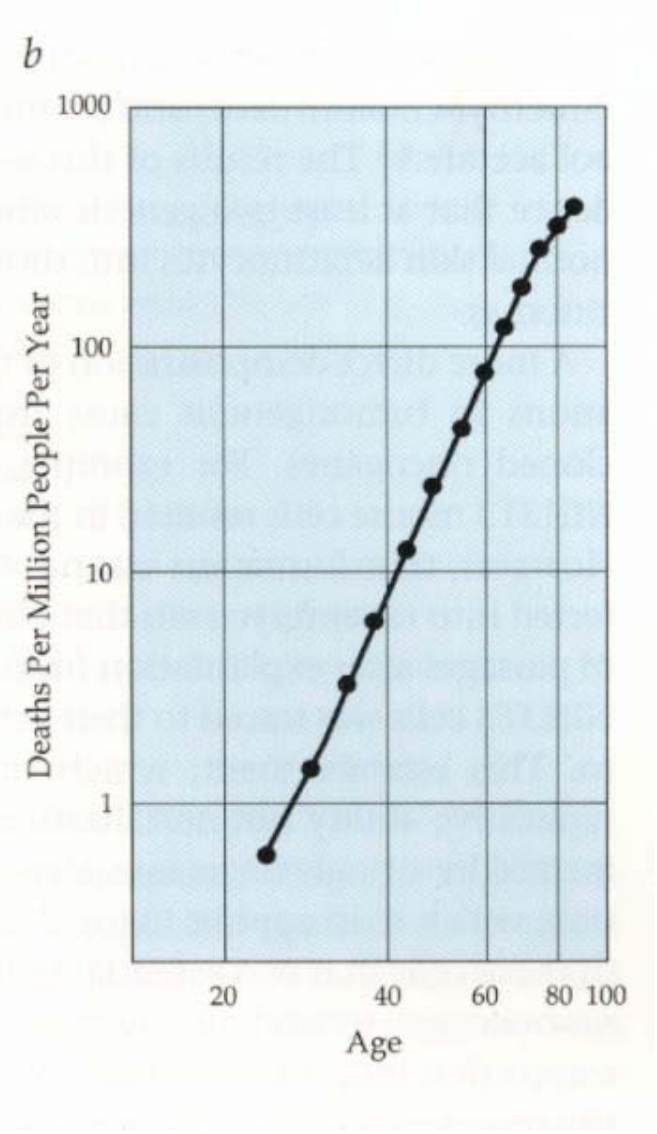


Tumor cells





Evidence for multistep cancer pathogenesis

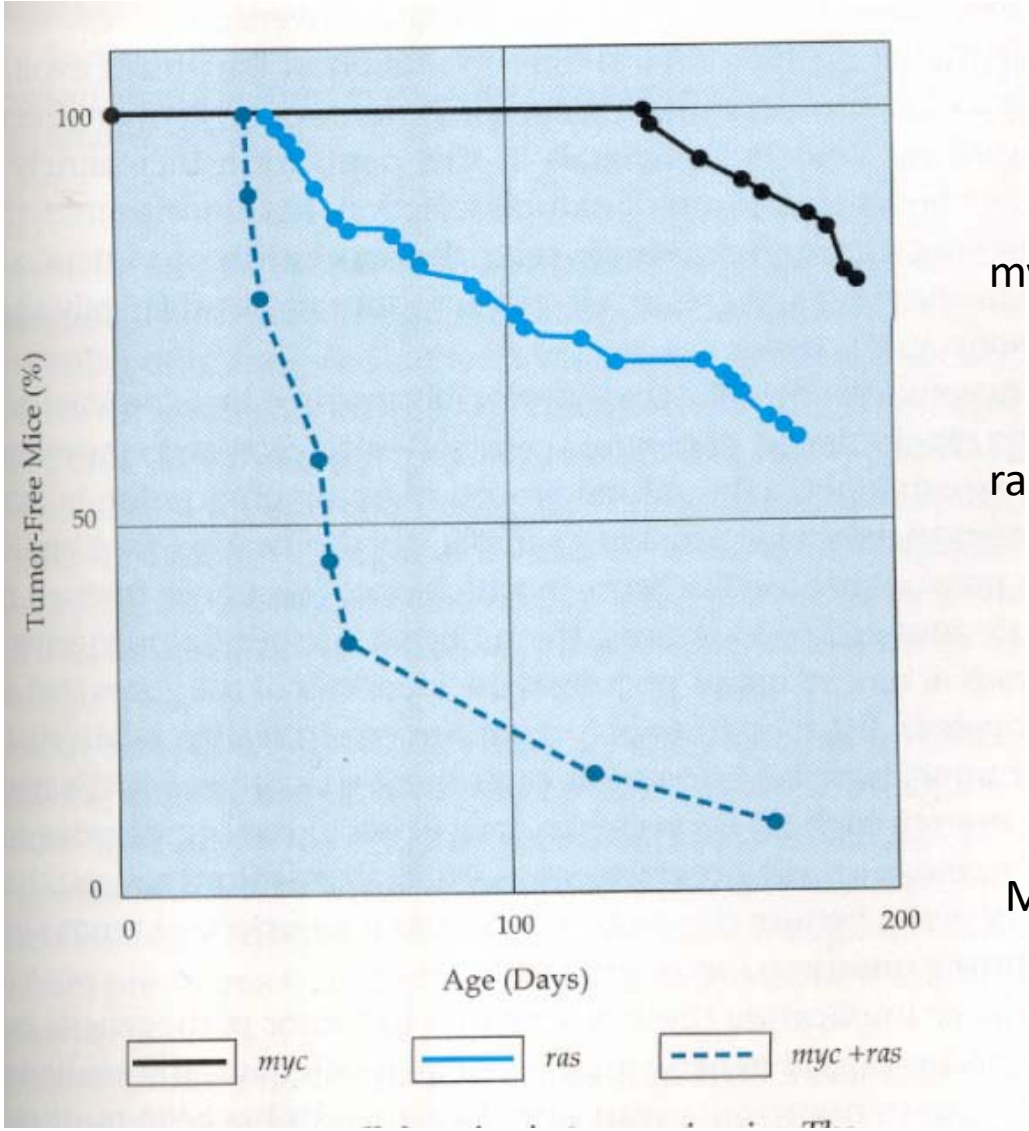


Cooperation between genes

- Myc – few mice with tumors
- Ras – more mice with tumors
- myc + ras – all mice with tumors

Conclusion: Complementary activities of 2 distinct oncogenes function collaboratively to create fully tumorigenic cells

Oncogene Cooperation



myc

ras

Myc + ras

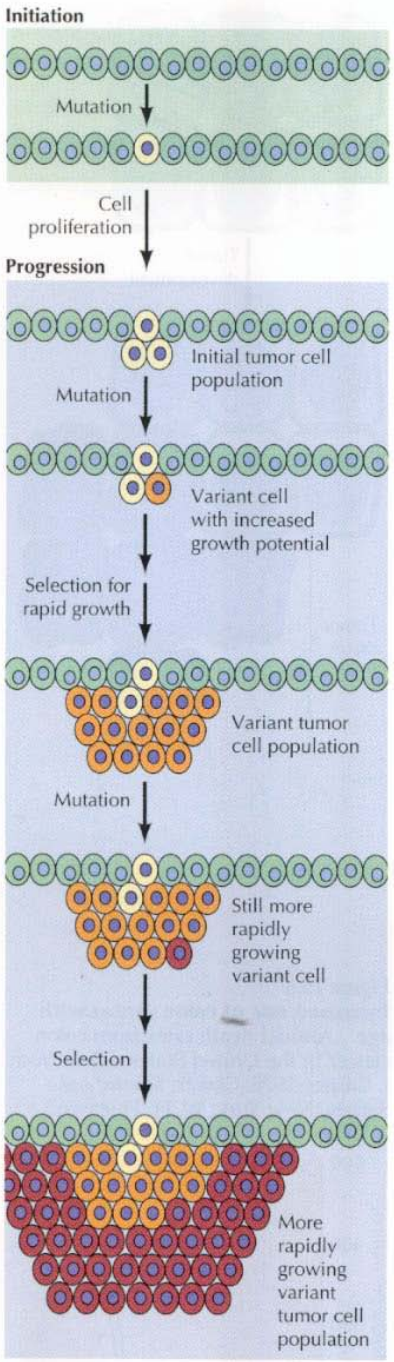
Mechanisms of collaboration

- Multiple mutated genes disrupt multiple control points of anti-cancer mechanism
- Synergistic/complementary activities
- Cell tries to apoptosis but selects for more aggressive cell with increased proliferative abilities

Multistep tumorigenesis

- Initiation
  - 1st mutation
  - Increased proliferation of a single cell
- Progression
  - Additional mutations
  - Selection for more aggressive cells

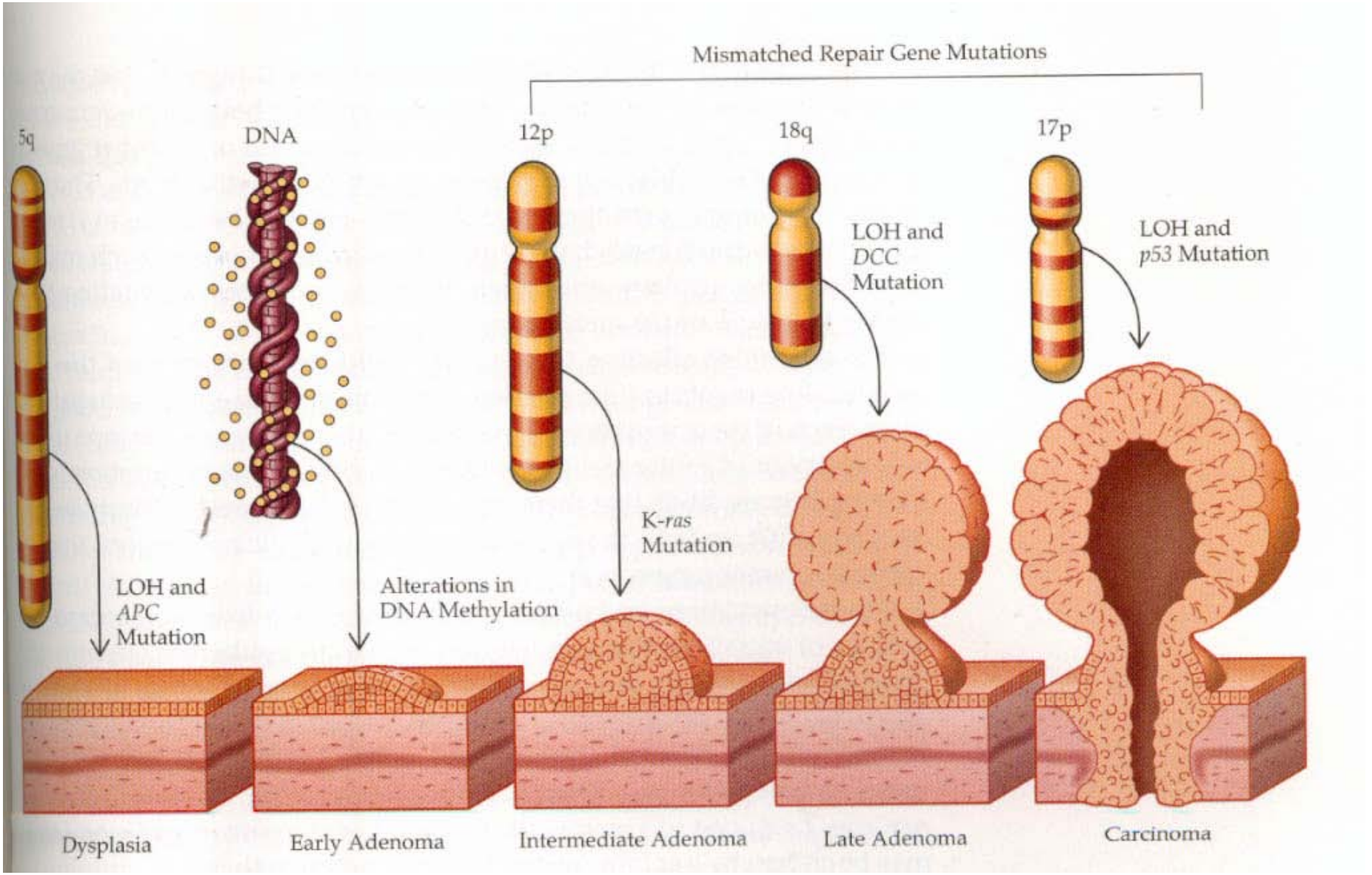
Clonal selection!



Initiation

Progression

Aggressive, rapidly growing tumor



With increase in histopathological abnormalities, there is an increase in the number of mutations at defined genetic loci

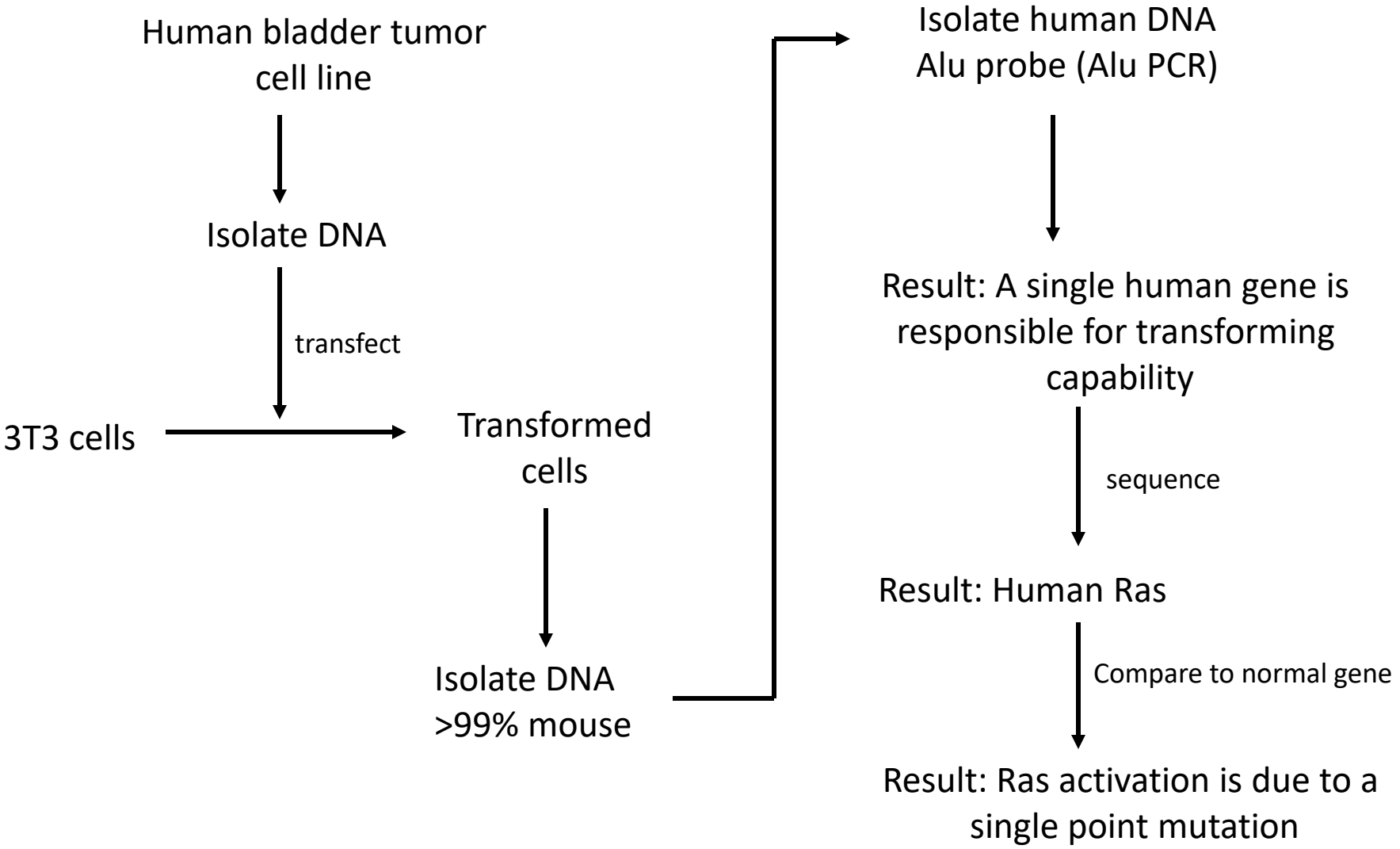
What causes the mutations that lead to cancer?

- Anything that damages DNA
  - Physical agents (radiation)
  - Chemical agents (carcinogens)
- Anything that stimulates the rate of mitosis
  - Viruses
  - **Oncogenes**
  - Tumor suppressor genes

How does damage affect function?

- Quantitative model- increased and sustained activity on a gene or its protein product
  - Altered gene expression
  - Change in protein structure
- Qualitative model- change in the specificity or function of the protein
  - Substrate specificity
  - Transactivation of different genes

Activated oncogenes from DNA transfection



Cancer-critical genes can be divided into 5 classes

4 Classes of oncogenes + tumor-suppressor genes
produce proteins that act as:

1. Mitogen pathway proteins

a) Mitogen – Sis is a PDGF that is secreted by the same cell that has a PDGF receptor and therefore sets up an autocrine stimulation of growth (against the rules).

b) Receptor – Erb B is a truncated EGF receptor that dimerizes two EGF (prolif is growth-factor independent)

c) G-protein – Ras with mutant GTPase activity, so once it binds GTP, always on.

d) Protein kinase – Raf constitutively active

e) Transcription factor – Myc overexpressed

2. Cell cycle proteins - Cyclins A,D, E remain high because mutant cyclin destruction box sequence

3. Immortality Proteins –

a) Telomerase turned back on in adult somatic cell-> unlimited no of proliferations

b) Angiogenesis factors – VEGF secreted by tumor attracts blood vessels to supply the tumor w nutrients

4. Metastatic proteins (allow migration out of tissues)

a) Tyrosine kinase – Src phosphorylates vinculin and other actin-binding proteins to increase cell motility

b) Proteases – type 4 collagenase degrades basal lamena

c) Attachment factors – Fibronectin secreted instead of transmembrane protein reduces attachment to ECMatrix.

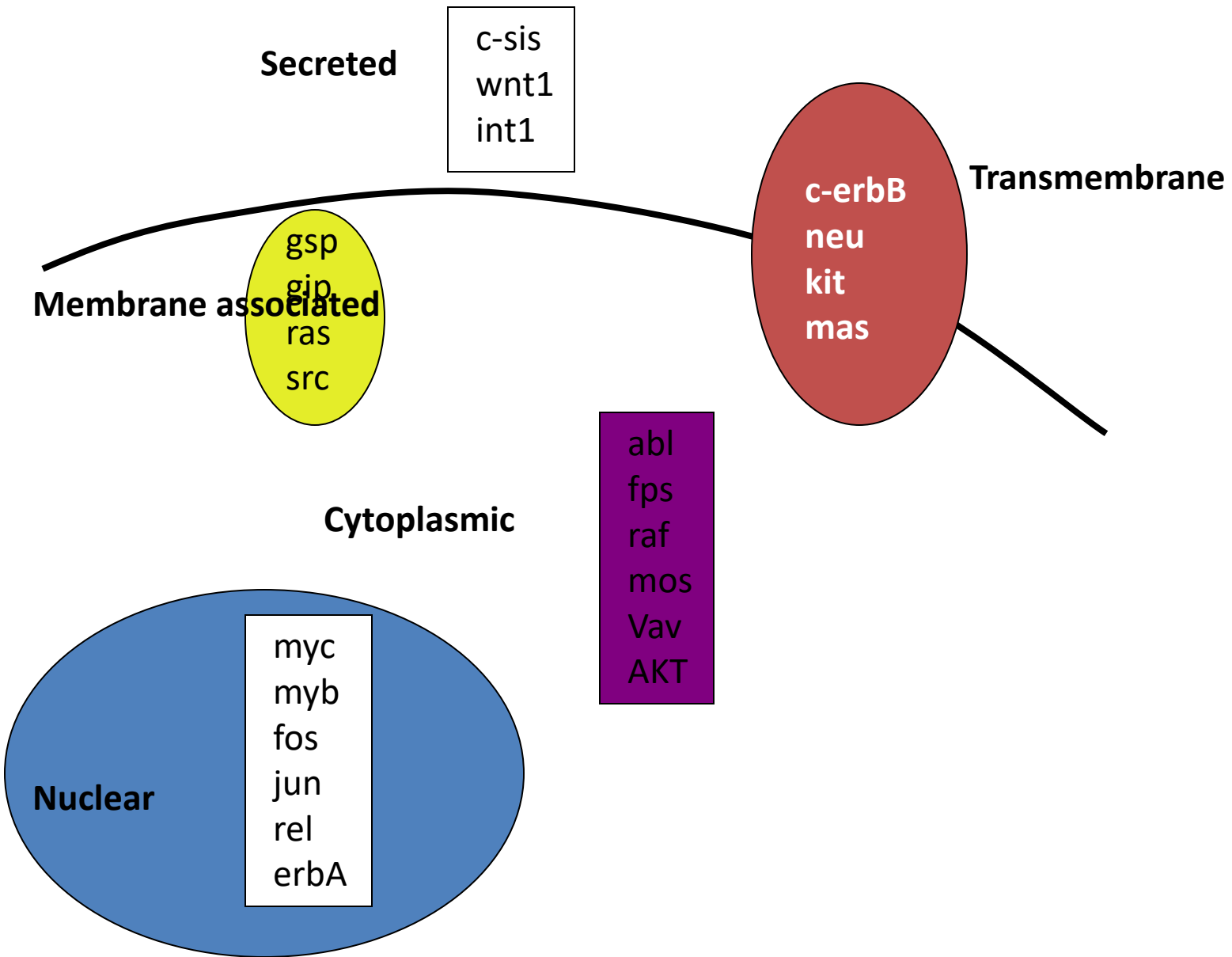
5. Tumor suppressor Proteins

a) Cell cycle “brake” - RB mutant fails to bind E2F

b) Damage contol switch – p53 mutant fails to activate p21 and thus suppress cell cycle; also may fail to activate apoptosis.

c) p15, p16, p21 fail to inhibit Cdks

Oncogenes by location



Oncogenes by function

- Growth factors
- Growth factor receptors
- G proteins
- Intracellular kinases
- Transcription factors

Oncogenic mutations

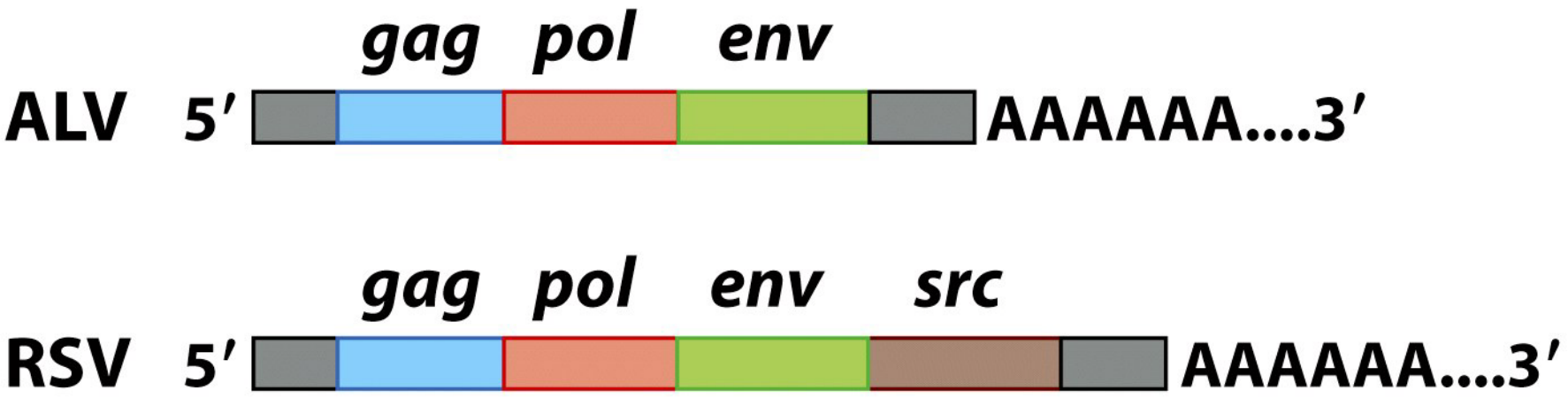
- GF receptors and signaling proteins can exist in active and inactive state
- Active state is rapidly turned over
  - Dephosphorylation of kinases
  - Hydrolysis of GTP to GDP
  - Protein degradation
- Oncogenic mutation alters protein product so that it is locked in the active state
- Interpreted by cell as a continuous and unrestricted growth inducing signal

What about the 1st oncogene: *src*

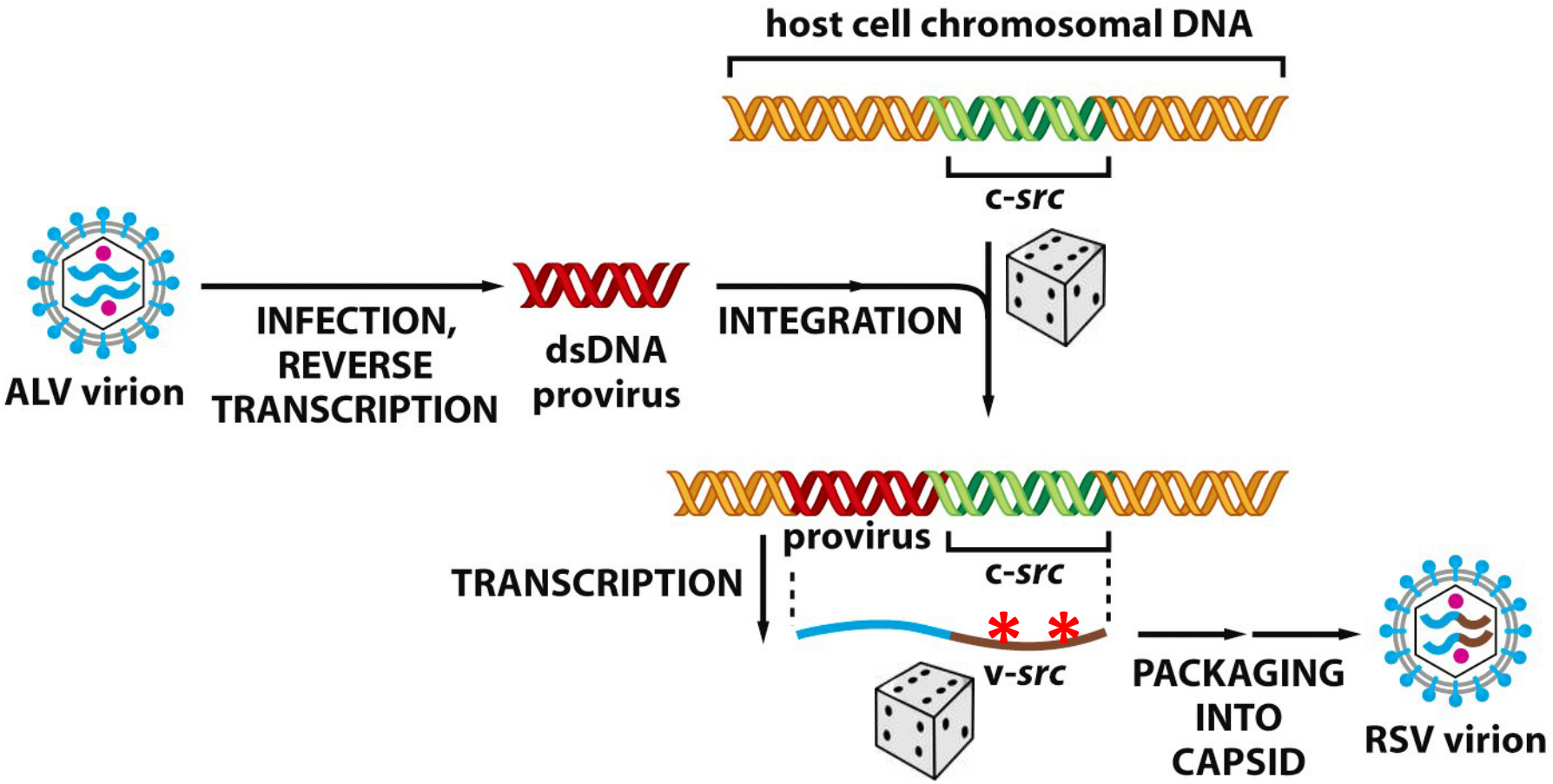
- 1909 hen→ Peyton Rous
- 1975-1976 proto-oncogene
- 1999: ***src*** mutation was found in 12% of advanced human colon carcinomas

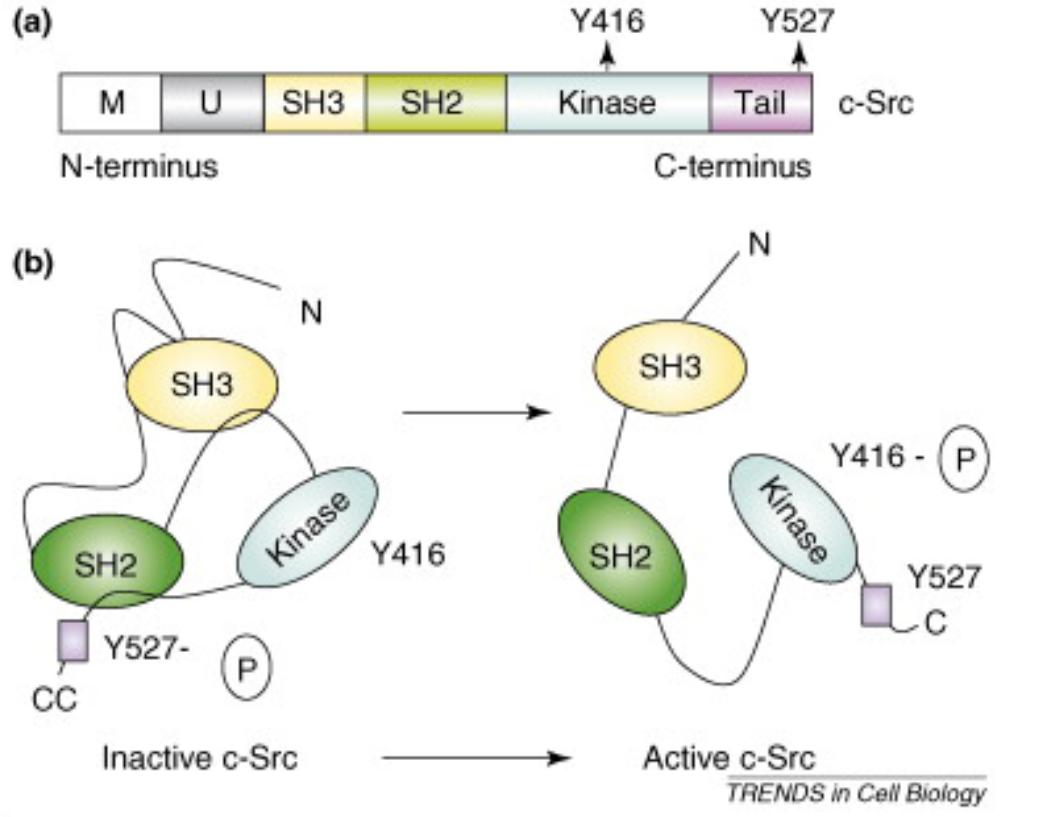
- v-src lacks the C-terminal inhibitory phosphorylation site (tyrosine-527), →constitutively active

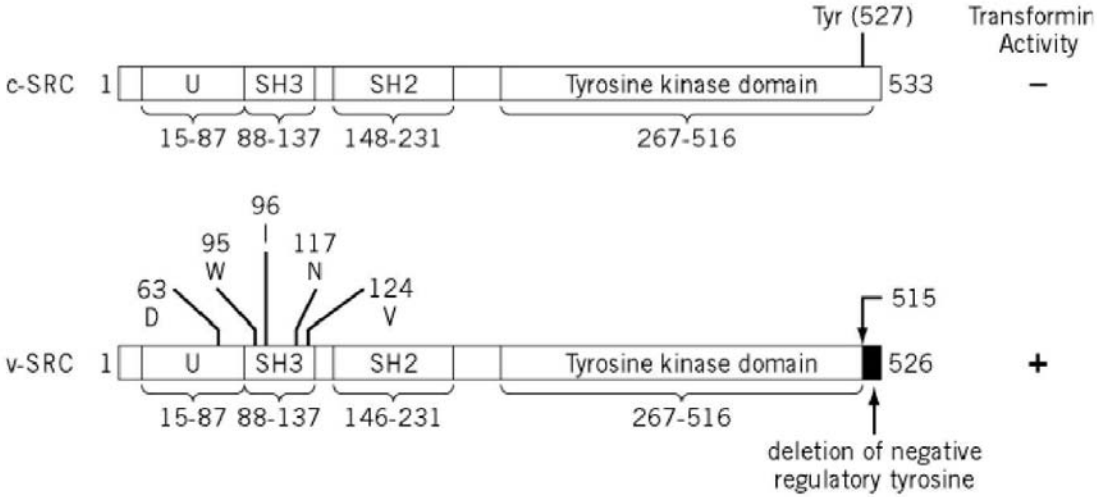
Avian Leukosis Virus: Retrovirus



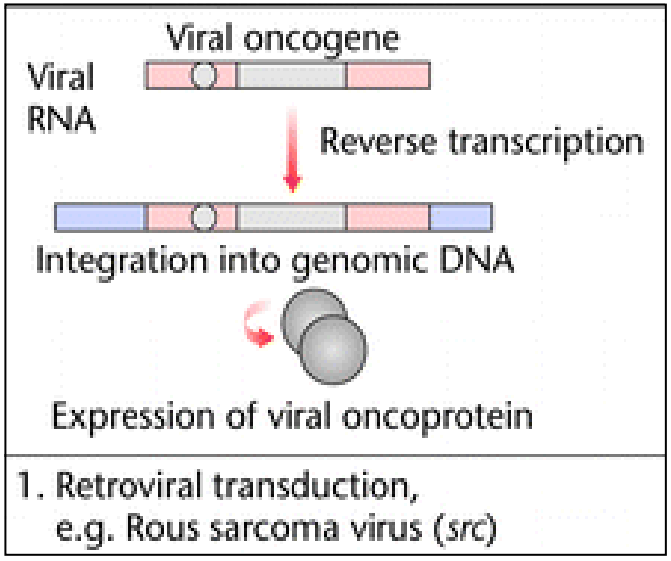
Origin of *v-src*



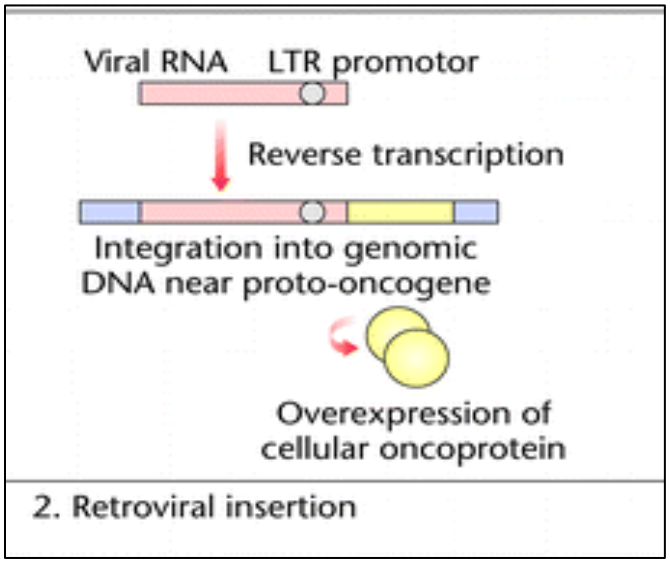

(a)
Y416
Y527
M
U
SH3
SH2
Kinase
Tail
c-Src
N-terminus
C-terminus
(b)
N
SH3
SH2
Kinase
Y416
Y527-
P
CC
Inactive c-Src
N
SH3
Y416 -
P
SH2
Kinase
Y527
C
Active c-Src
TRENDS in Cell Biology


Tyr (527)
Transformin
Activity
c-SRC
1
U
SH3
SH2
Tyrosine kinase domain
533
–
15-87
88-137
148-231
267-516
96
95
W
117
N
63
D
124
V
515
v-SRC
1
U
SH3
SH2
Tyrosine kinase domain
526
+
15-87
88-137
146-231
267-516
deletion of negative
regulatory tyrosine

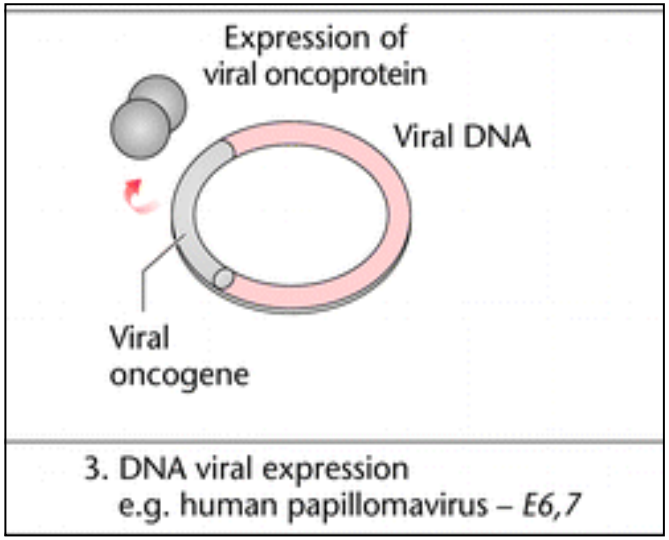
Viral insertion



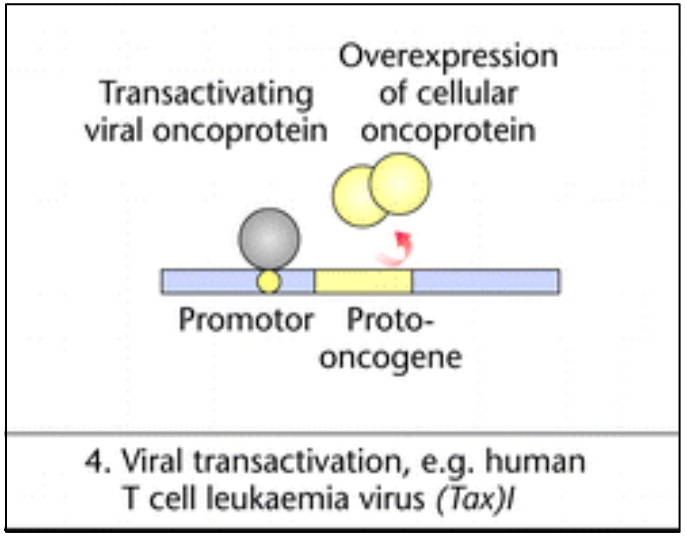
1. Retroviral transduction, e.g. Rous sarcoma virus (*src*)



2. Retroviral insertion



3. DNA viral expression e.g. human papillomavirus – *E6,7*



4. Viral transactivation, e.g. human T cell leukaemia virus *(Tax)I*

3 mechanisms

1. Virus hijacked cellular genes (RSV *src*)

2. Insertional mutagenesis: ALV *myc*

3. Virus gene acts as oncogene: HTLV-I *tax*

Mechanisms of conversion

1. Virus carrying oncogene: V-myc
   Avian myelocytomatosis virus (AMV)

2. ALV: insertional mutation (fusion gene)

3. Gene amplification

4. Chromosomal translocation: fusion gene

5. Point mutation

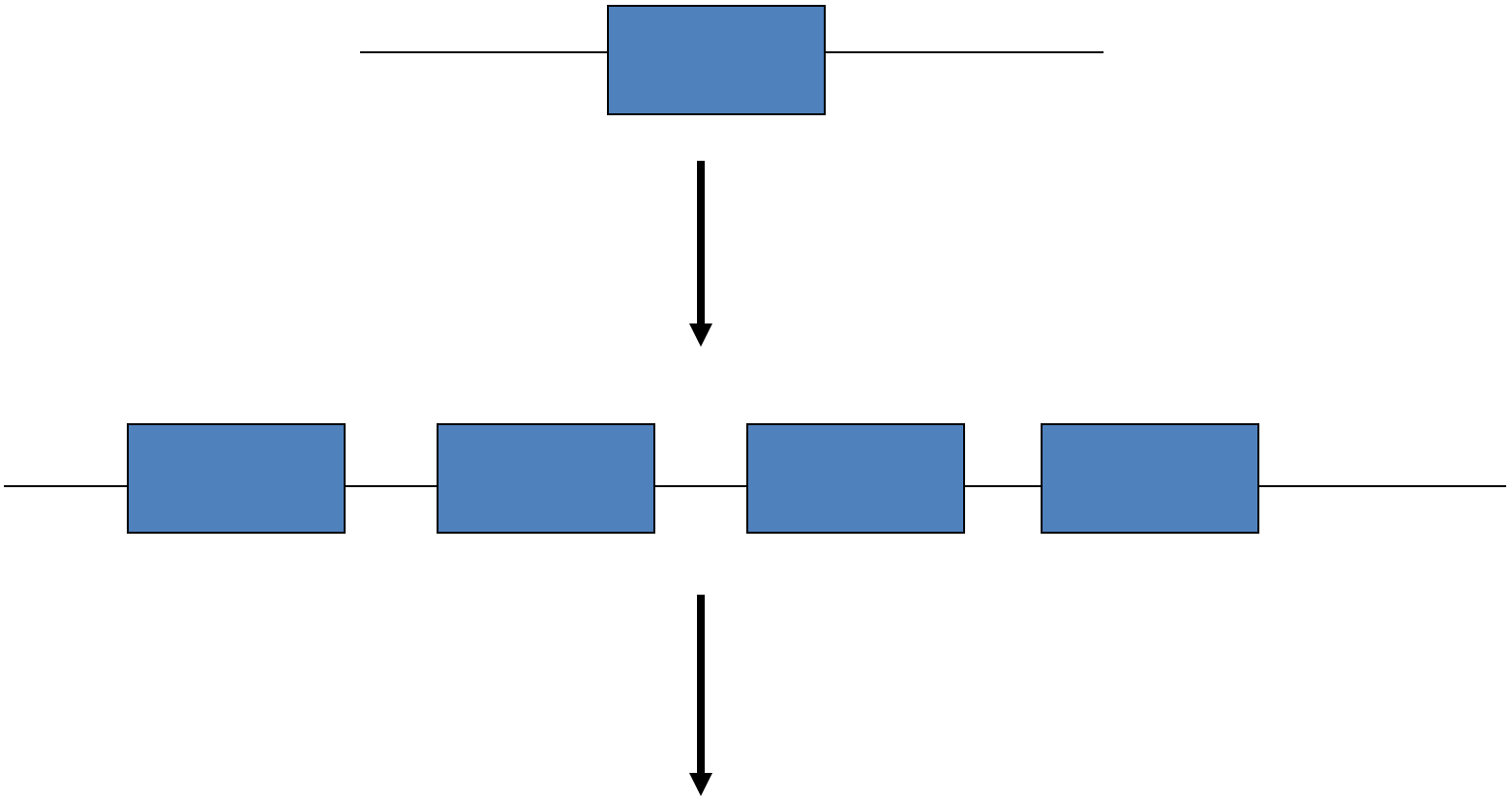
Activation of proto-oncogenes

- Viral insertion
- Chromosomal rearrangements
  - Altered regulation
  - Fusion genes
- Gene amplification
- Point mutations
- Loss of degradation signals

Increased expression of oncogenes

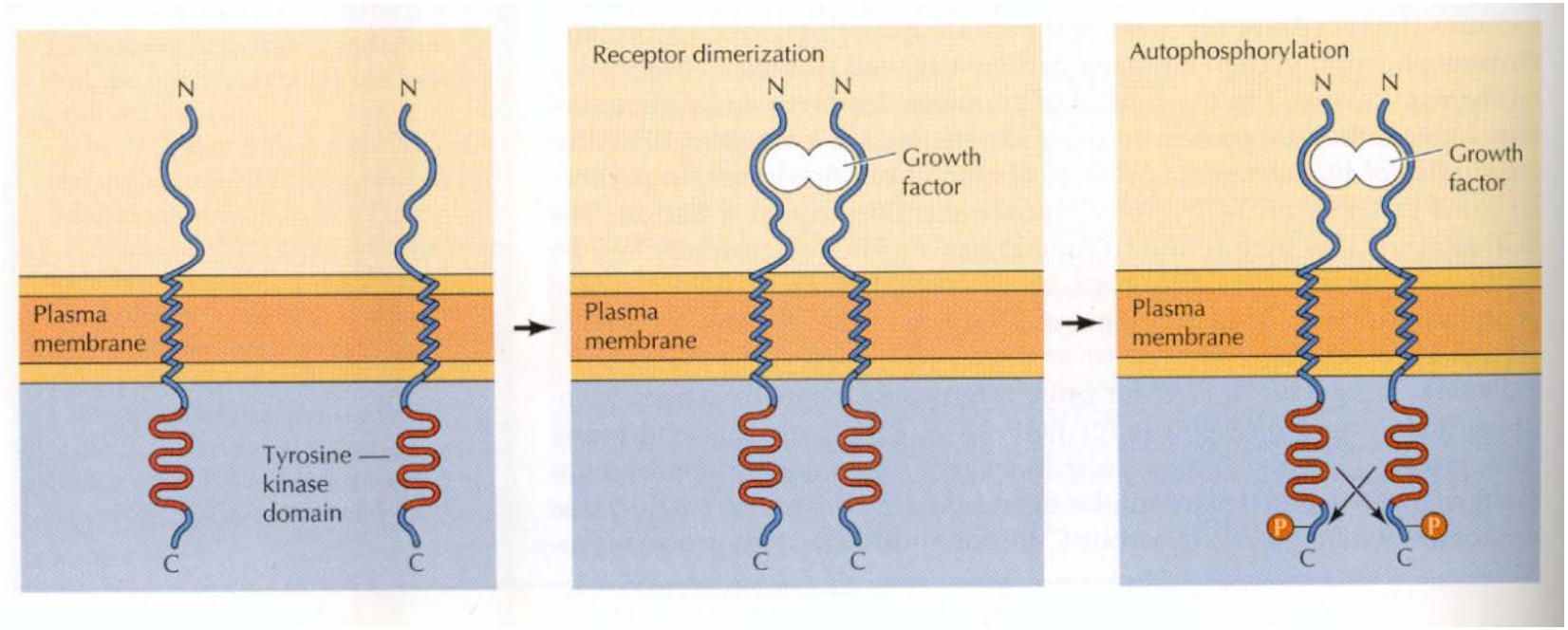
- ***ras*** –first identified in **Harvey rat sarcoma virus**
- ***myc***—first identified in ***ALV*** 10-20 copies per haploid genome in 60 human promyelocytic leukemia cell lines
- ***erbB***—first identified in **avain erythoblastosis virus (AVE)**: human stomach, breast and brain tumors
- **Gene amplification**

Amplification



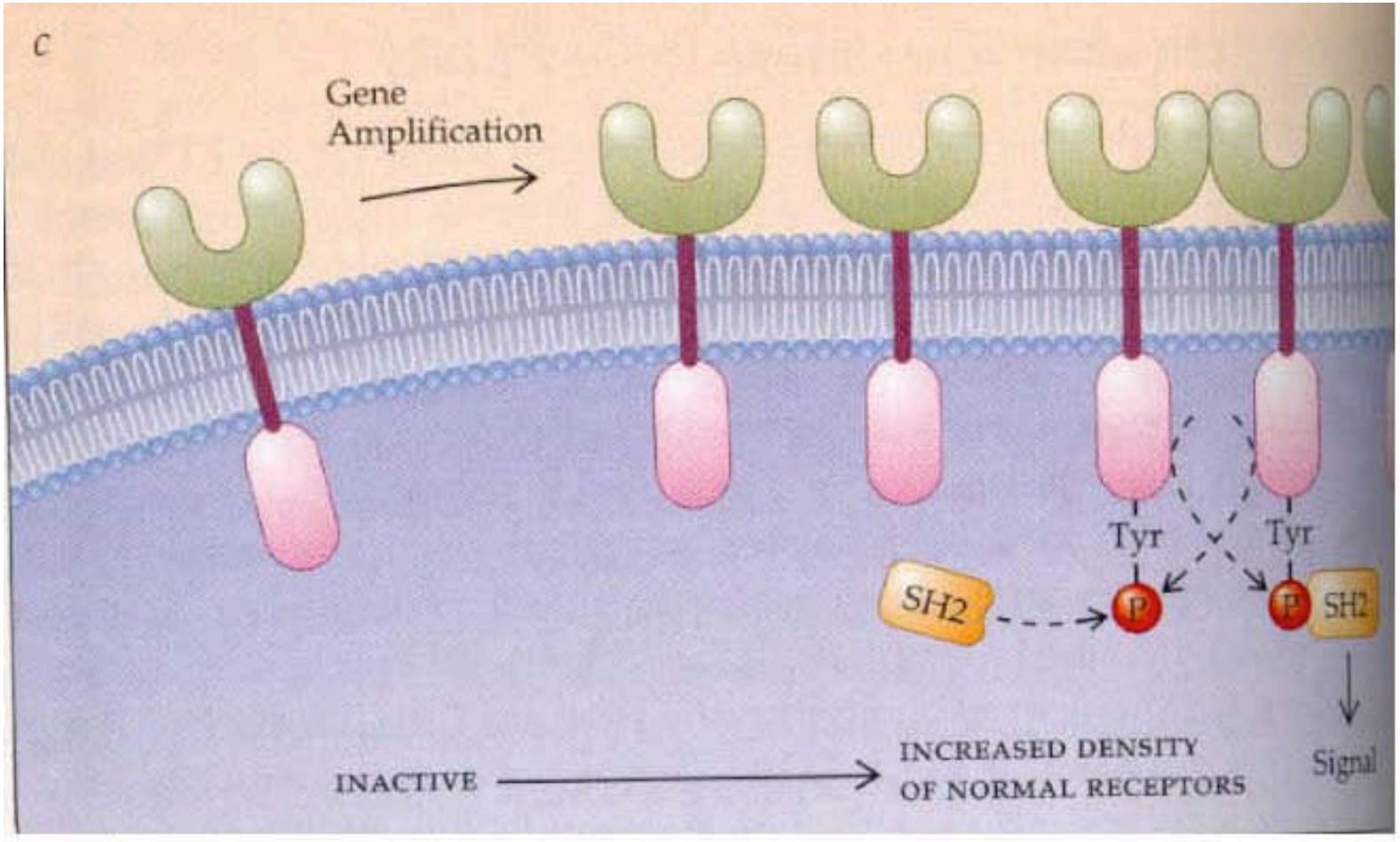
Too much protein

Receptor activation



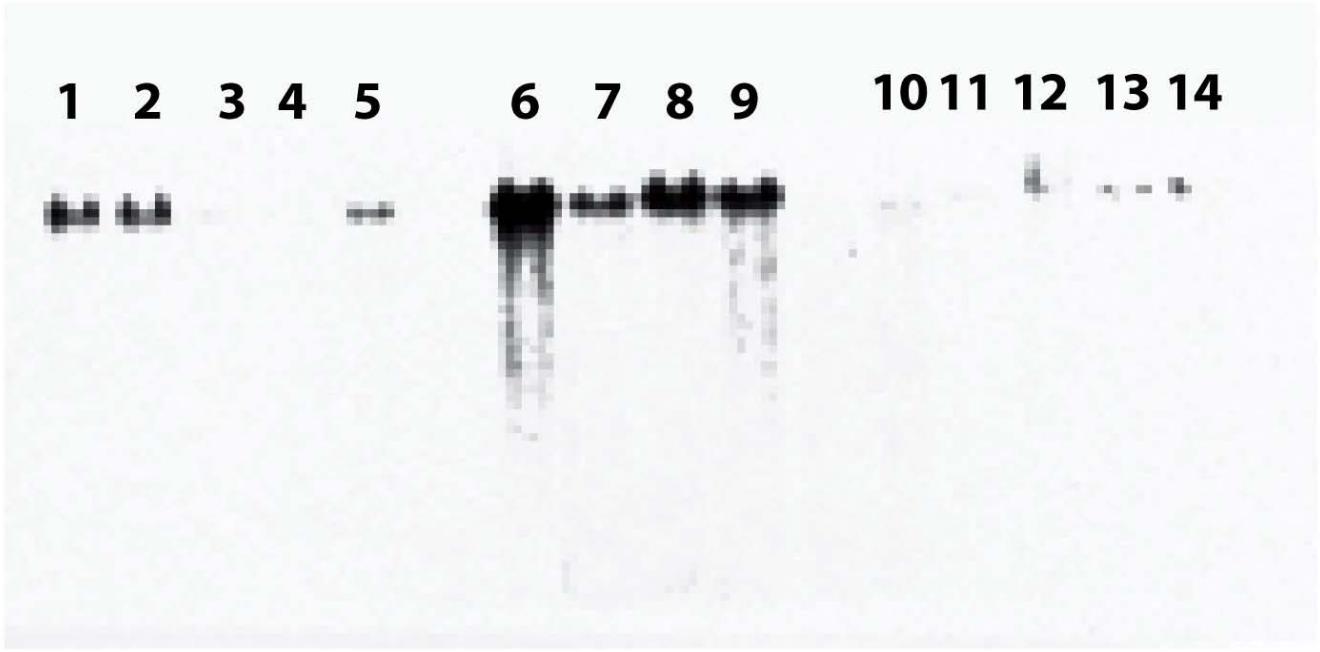
Receptor tyrosine-protein kinase erbB-2

Amplification



Increased density induces dimer formation, autophosphorylation thus constitutively active

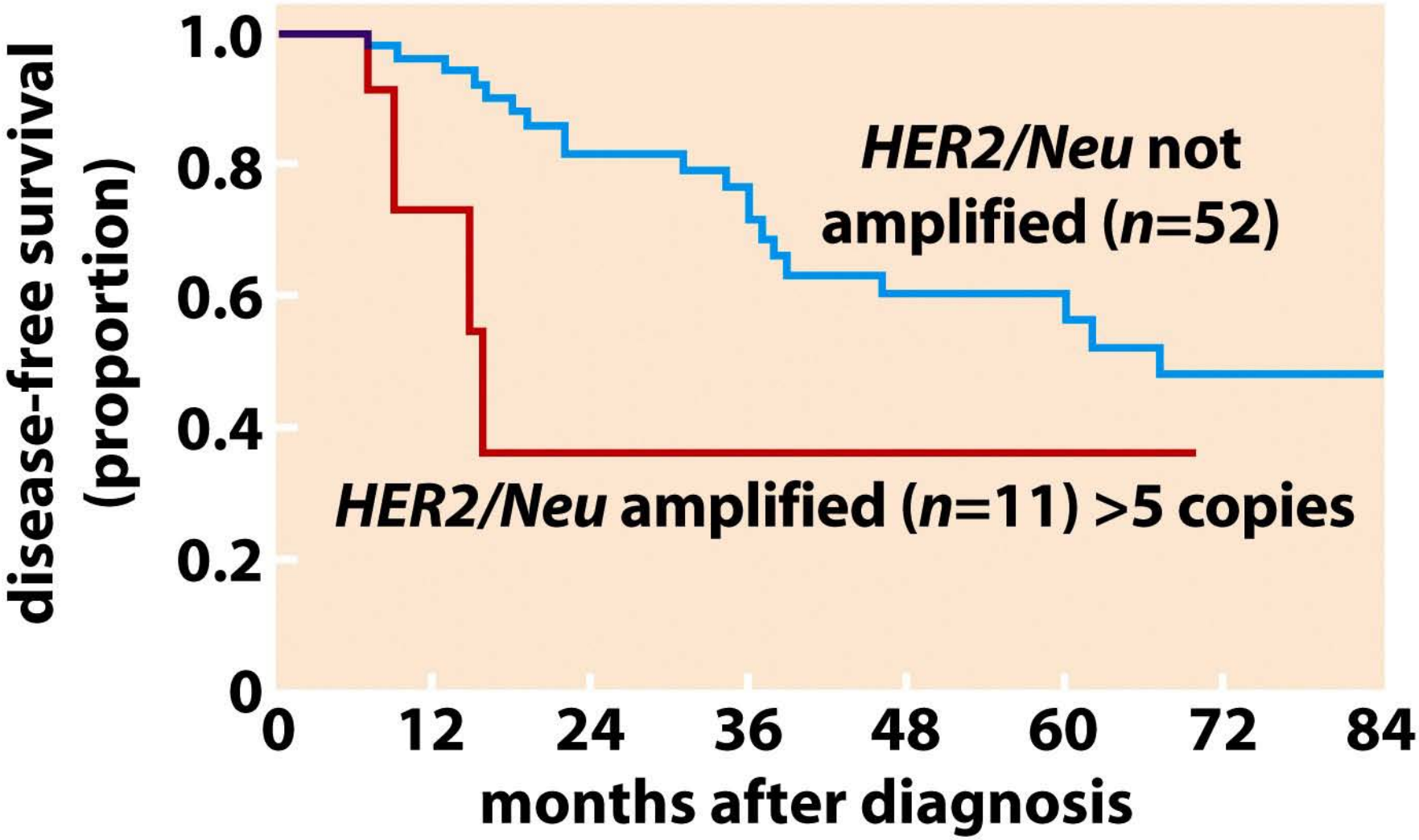
DNA Level of *erb-B/Neu*



Amplified in 30% of breast cancer

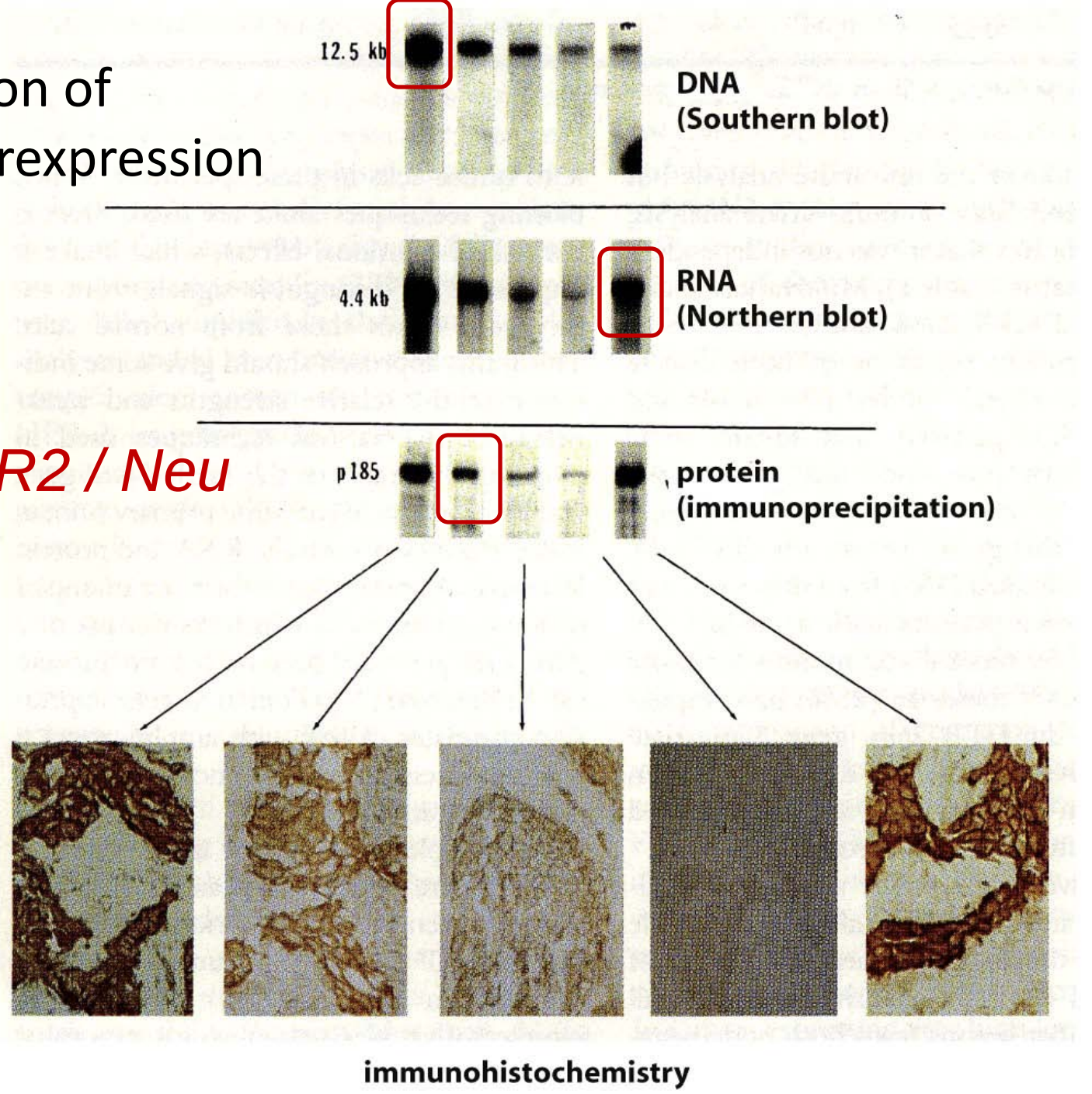
Receptor tyrosine-protein kinase erbB-2

Kaplan-Meier plot of breast cancer

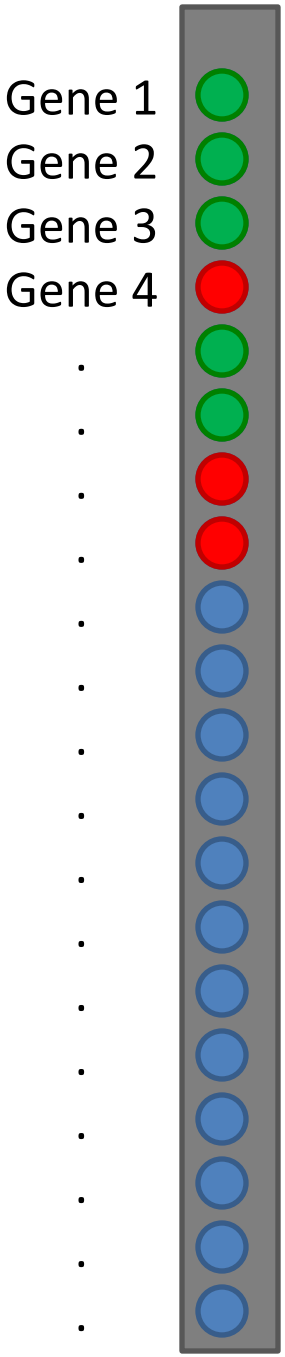


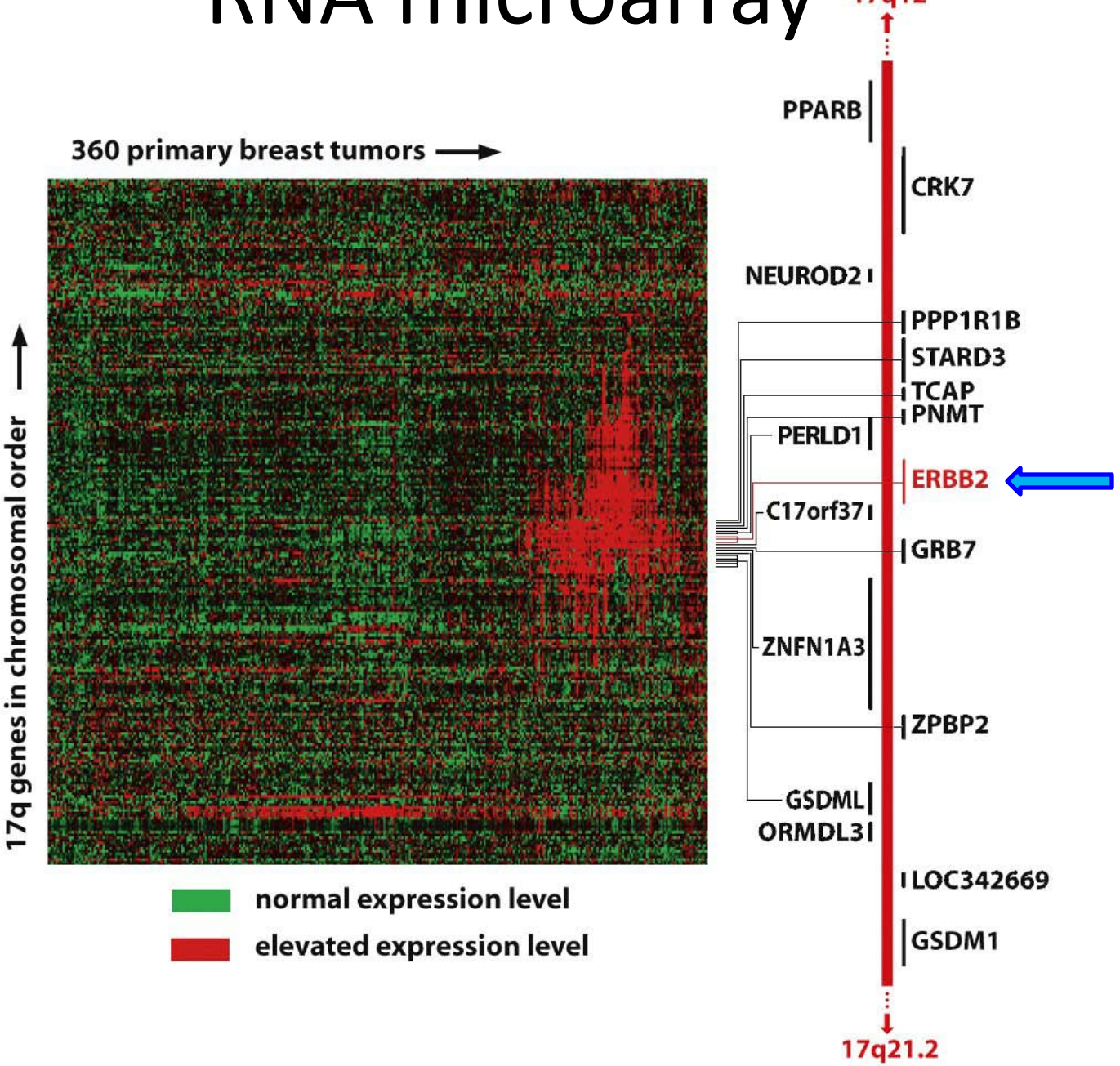
Detection of
oncogene overexpression

erb-B / HER2 / Neu

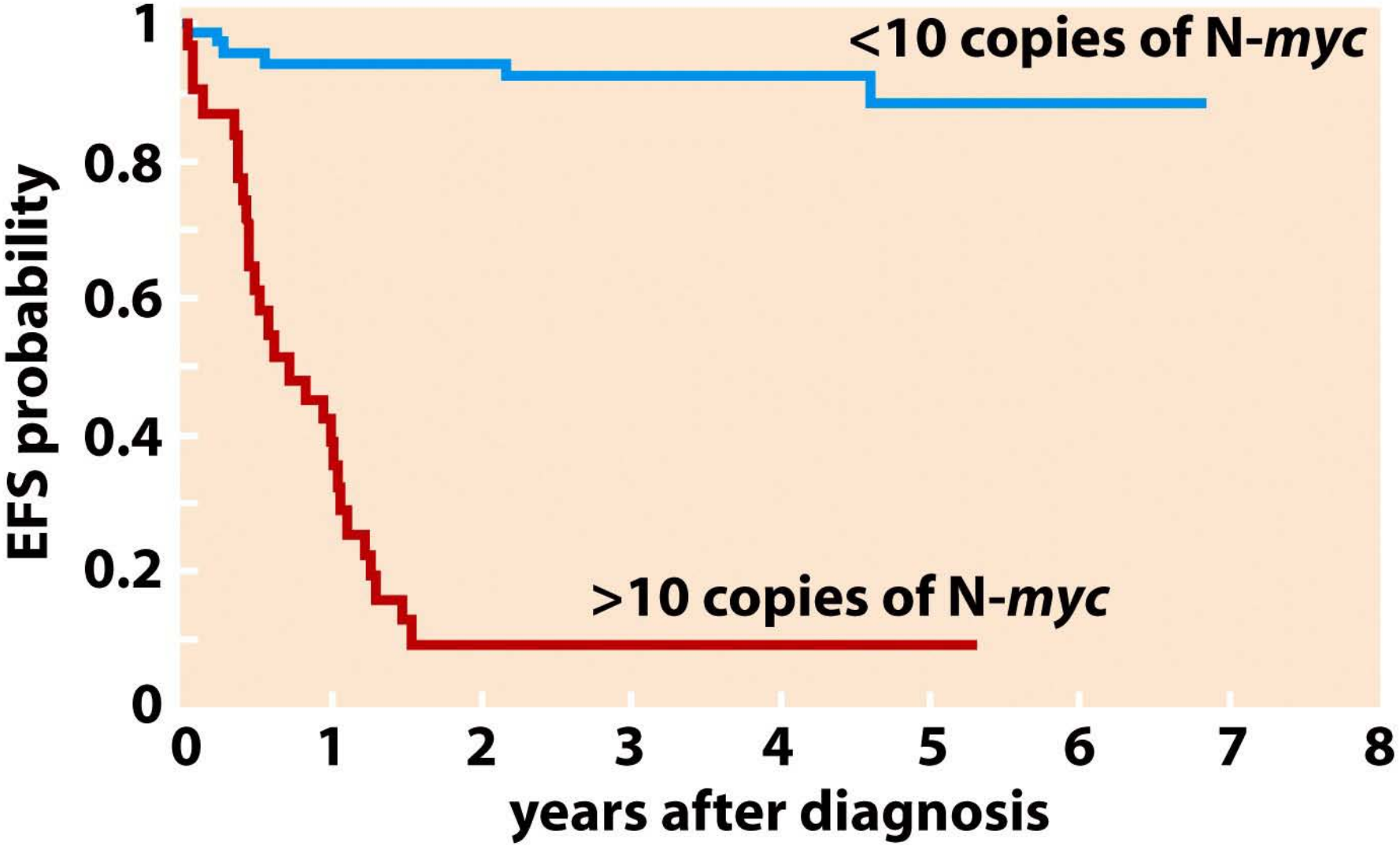


RNA microarray





Kaplan-Meier plot of children's neuroblastoma

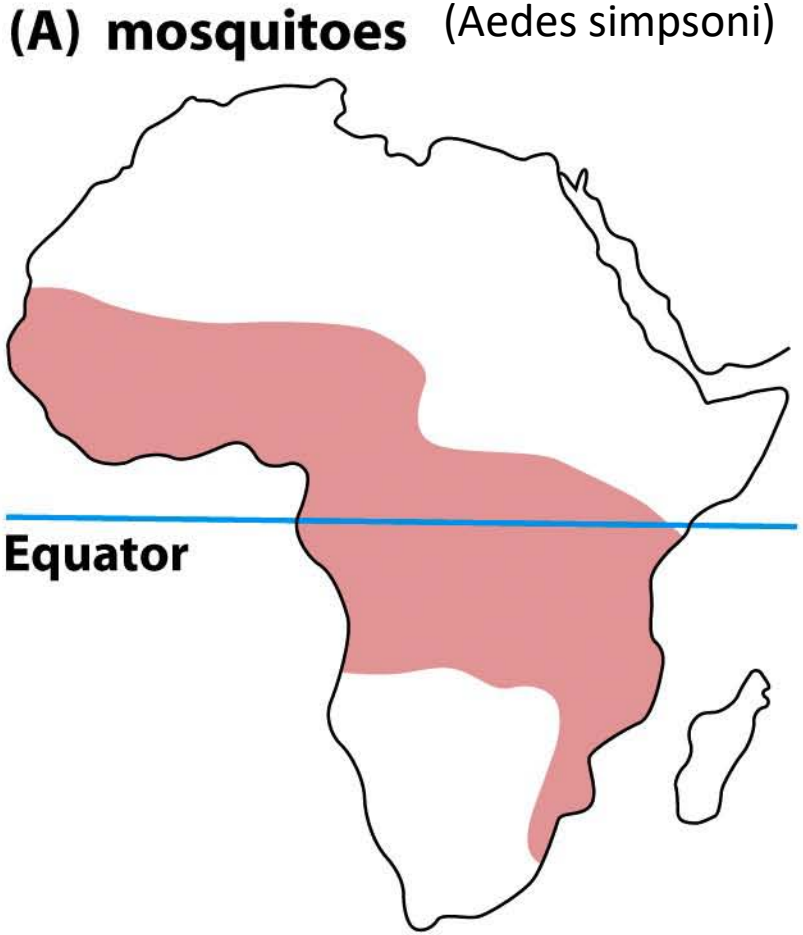


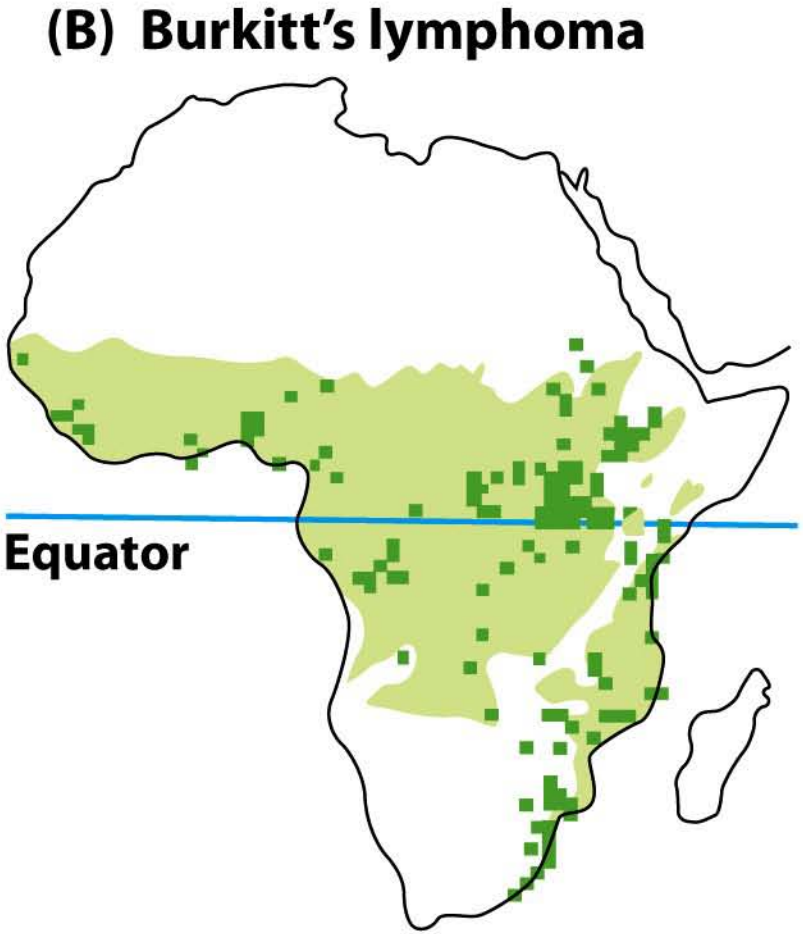
Gene amplification

Oncogene	location	cancer	func
erbB1	7q12-13	Glioblastoma（神经胶质细胞瘤）50% Squamous cell carcinoma 10-20%	RTK
met	7q31	Gastric carcinoma 20%	RTK
K-ras	6p12	Lung, ovarian, bladder carcinomas 5-10%	Small G Protein
N-ras	1p13	Head & neck cancer 30%	
C-myc	8q24	Leukemia, carcinoma 10-50%	TF
L-myc	1p32	Lung carcinoma 10%	
N-myc-ddx1	2p25-25	Neuroblastoma（神经母细胞瘤）, lung carcinoma 30%	
Akt-1	14q32-33	Gastric cance 20%	S/T kinase
Cdk4-mdm2-sas-gli	11q13	Sarcomas 40%	

Chromosomal translocation: fusion gene

Mosquito and tumor

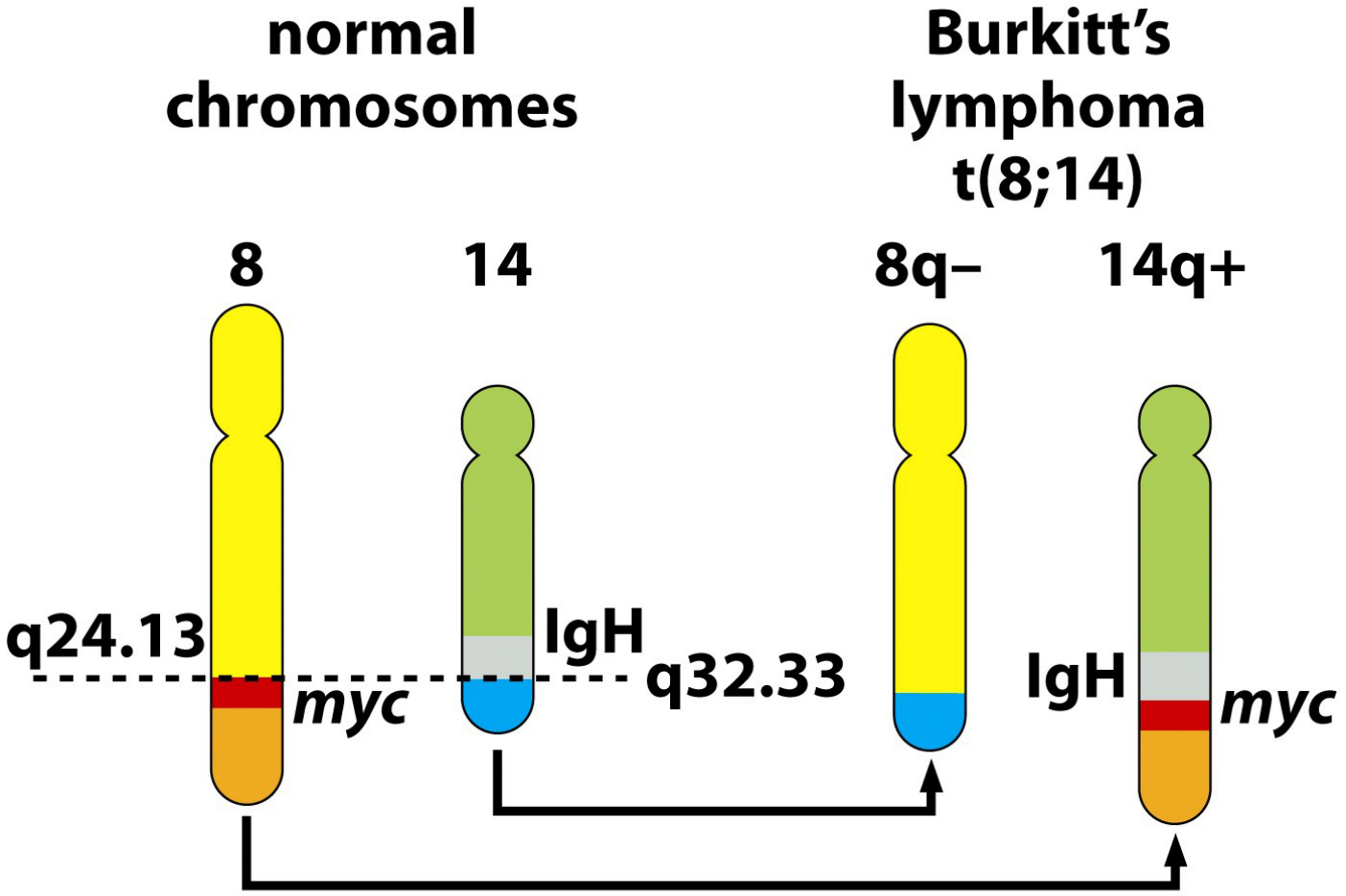




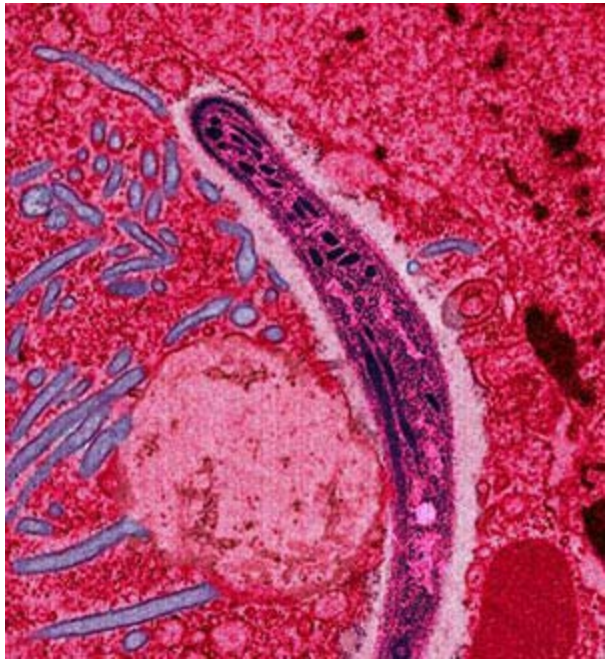
EBV (Epstein-Barr virus) Infect B cells

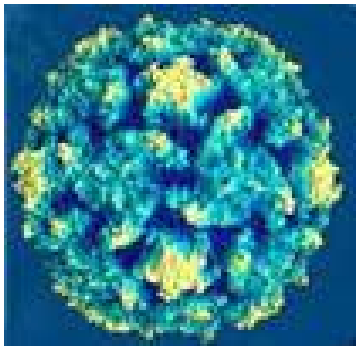
Malarial parasites

Childhood Burkitt's lymphoma



- Malarial parasites





•EBV (Epstein-Barr virus)

• Infected B cells

疱疹病毒

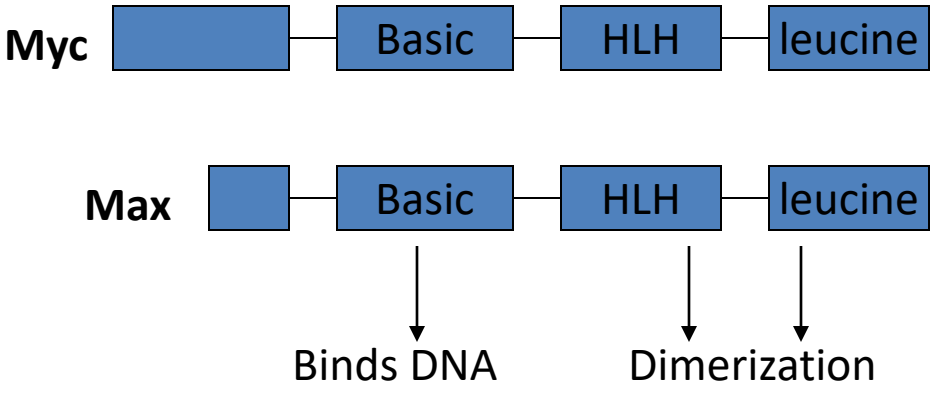
•Large pool of immortalized B cells

•Many Recombination events

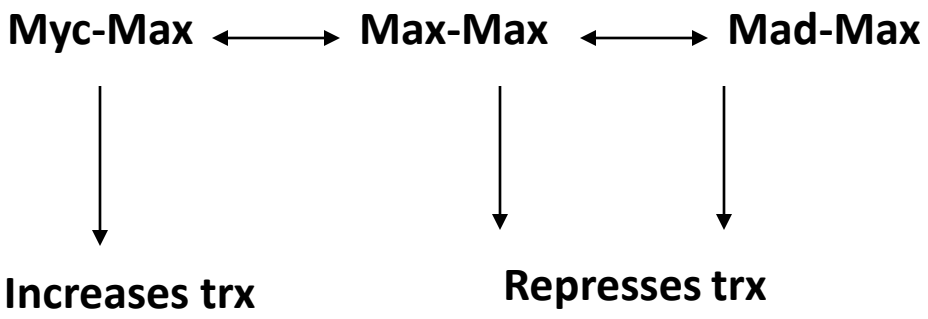
•Proliferation advantage→ tumor

•Rare mistakes—fusion of myc

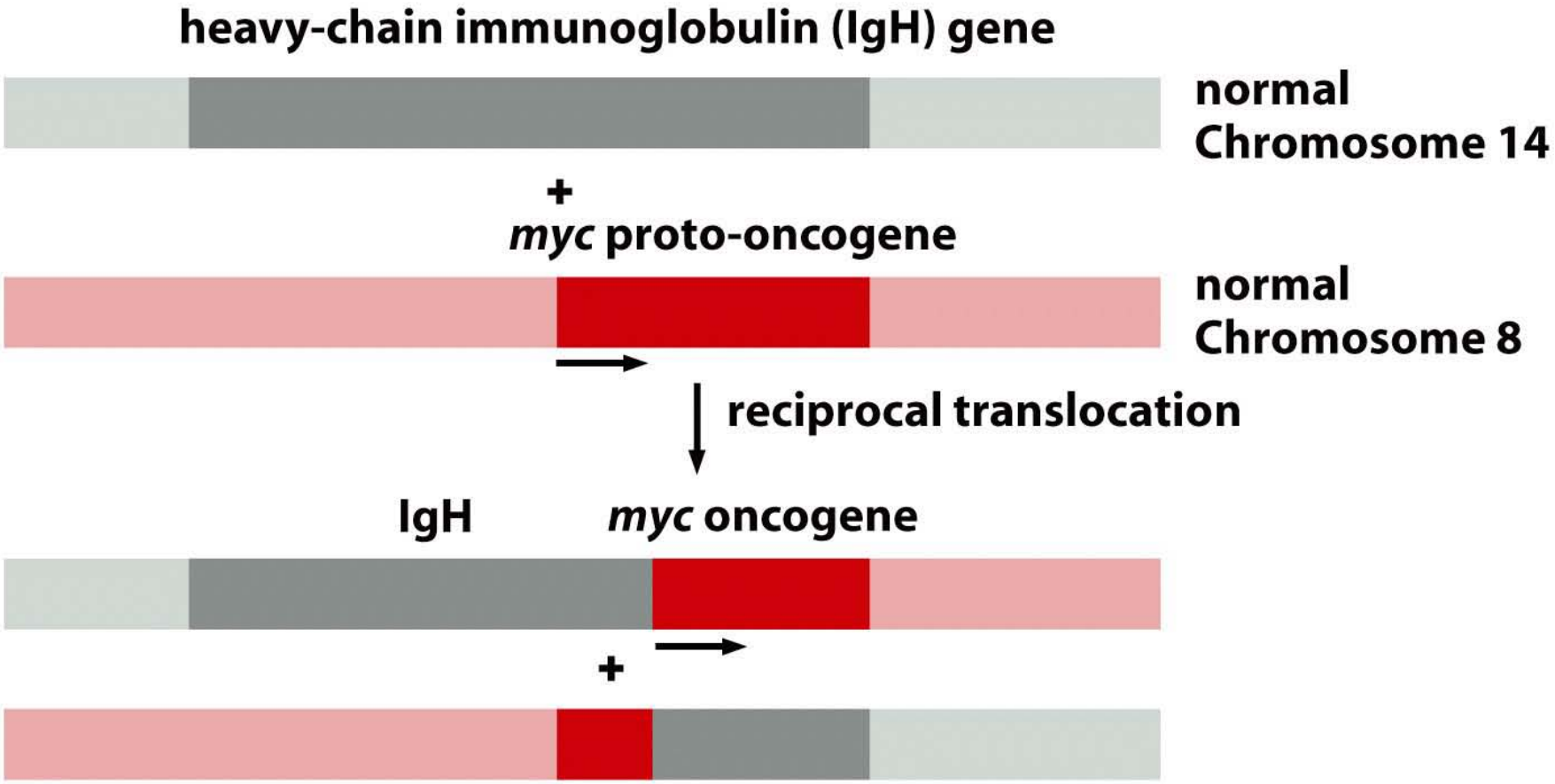
Myc genes



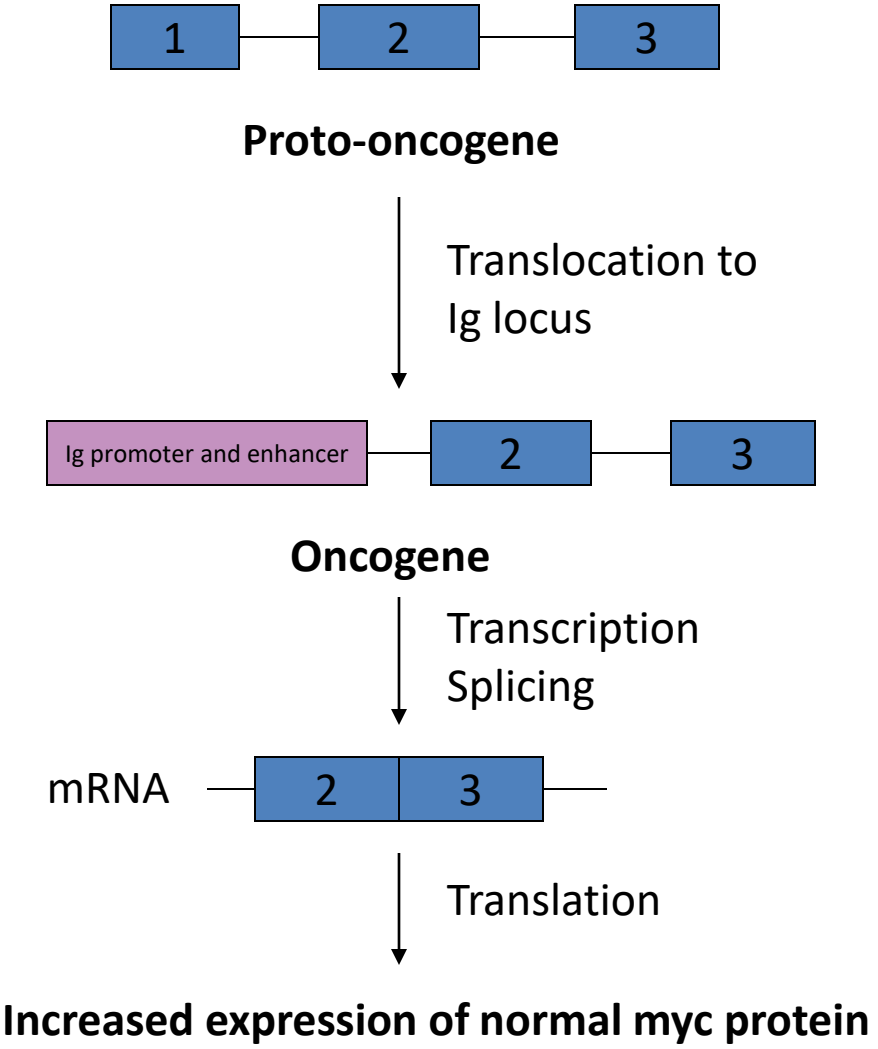
- Increased myc synthesis drives Max to partner with Myc
- Myc has short half life
- Max is stable



Fusion gene

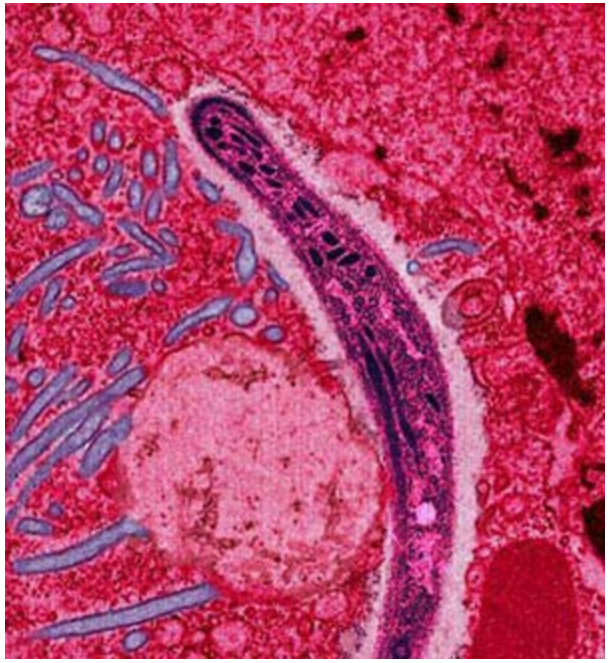

heavy-chain immunoglobulin (IgH) gene
normal
Chromosome 14
+
myc proto-oncogene
normal
Chromosome 8
reciprocal translocation
IgH
myc oncogene
+

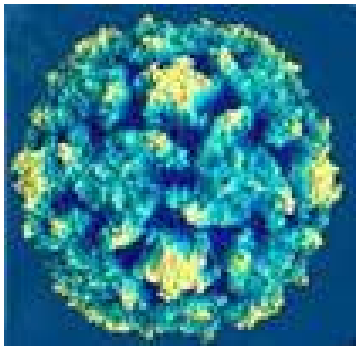
Oncogenic mutation of myc



- Chromosome translocation puts myc under control of strong promoter and enhancer
- Increases the concentration of Myc-Max heterodimers thus increasing cell proliferation
- Burkitt's lymphoma

- Malarial parasites





•EBV (Epstein-Barr virus)

• Infected B cells

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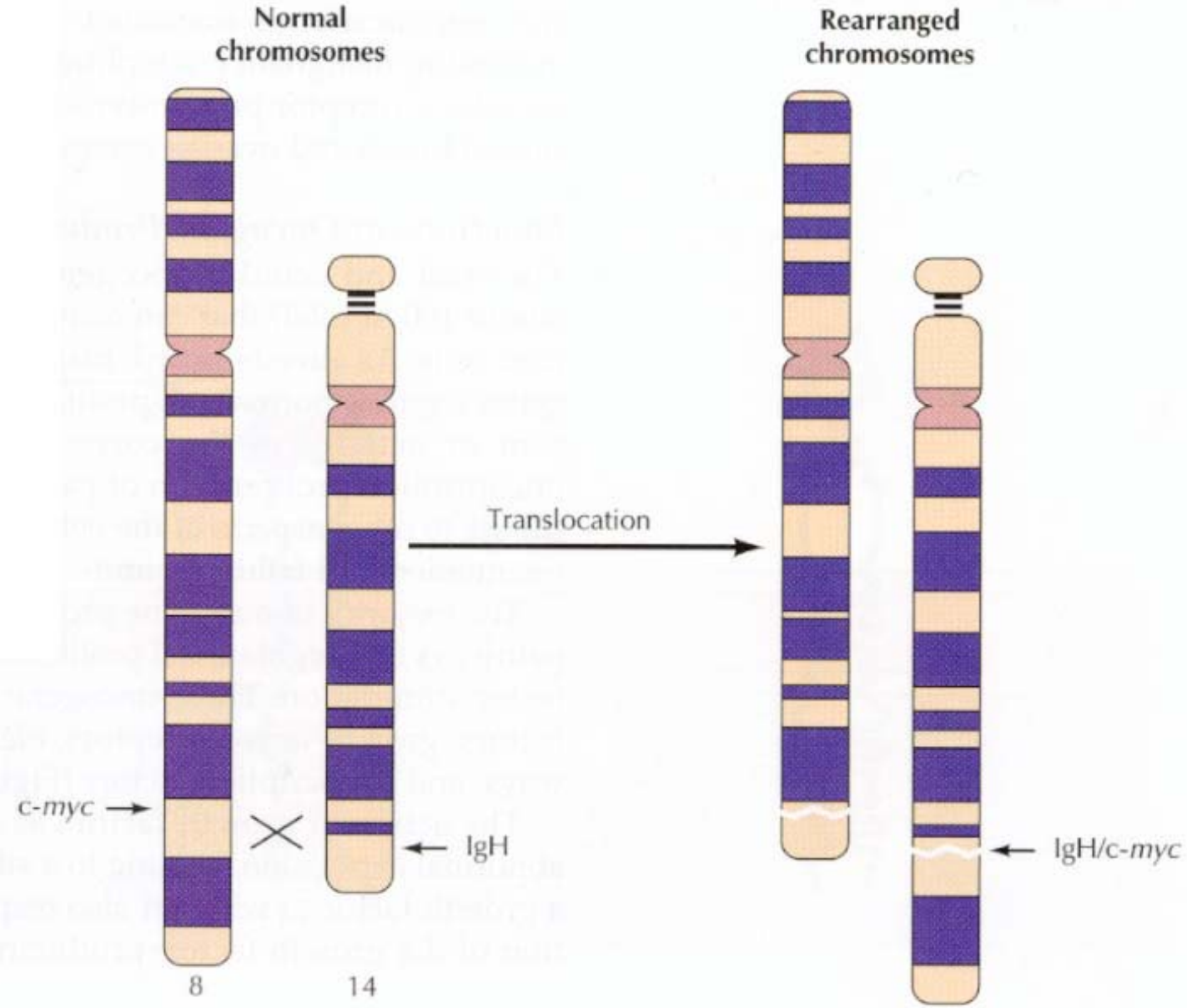
•Large pool of immortalized B cells

•Many Recombination events

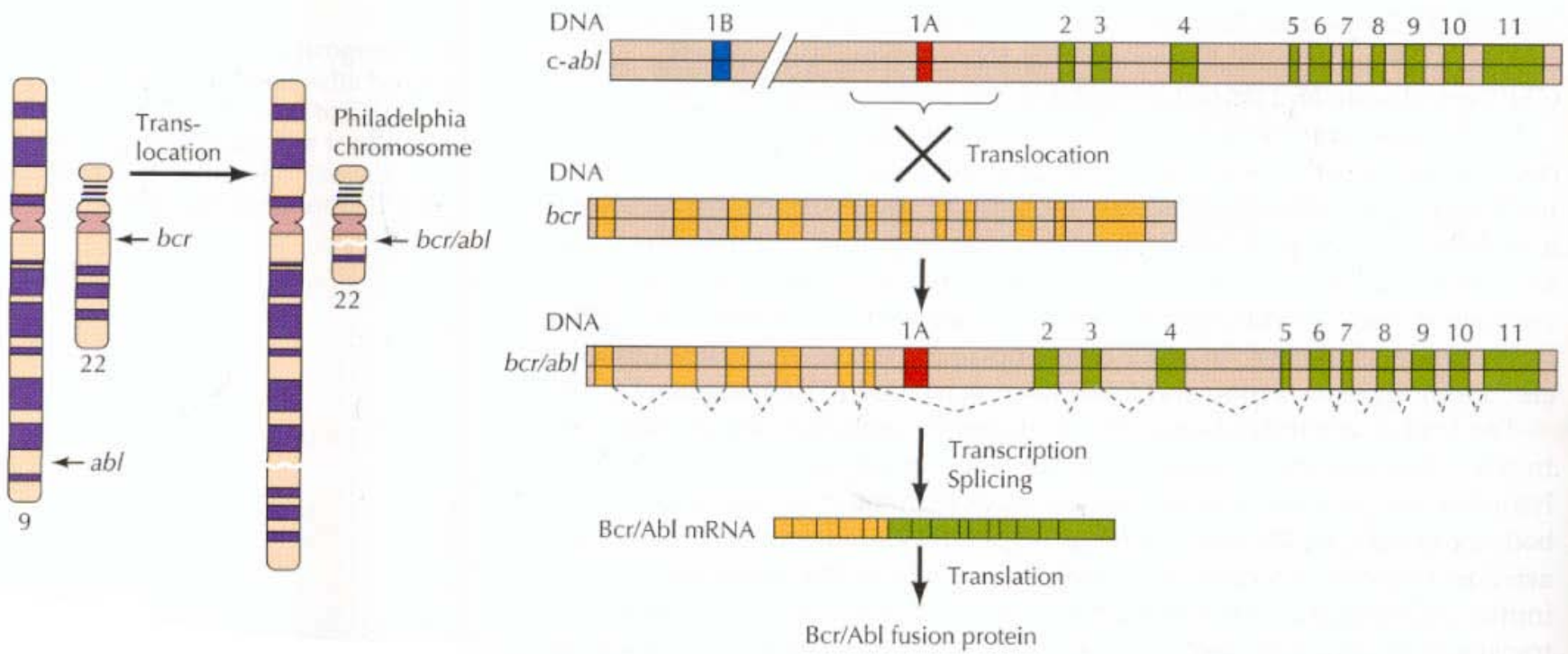
•Proliferation advantage→ tumor

•Rare mistakes—fusion of myc

Chromosomal translocation



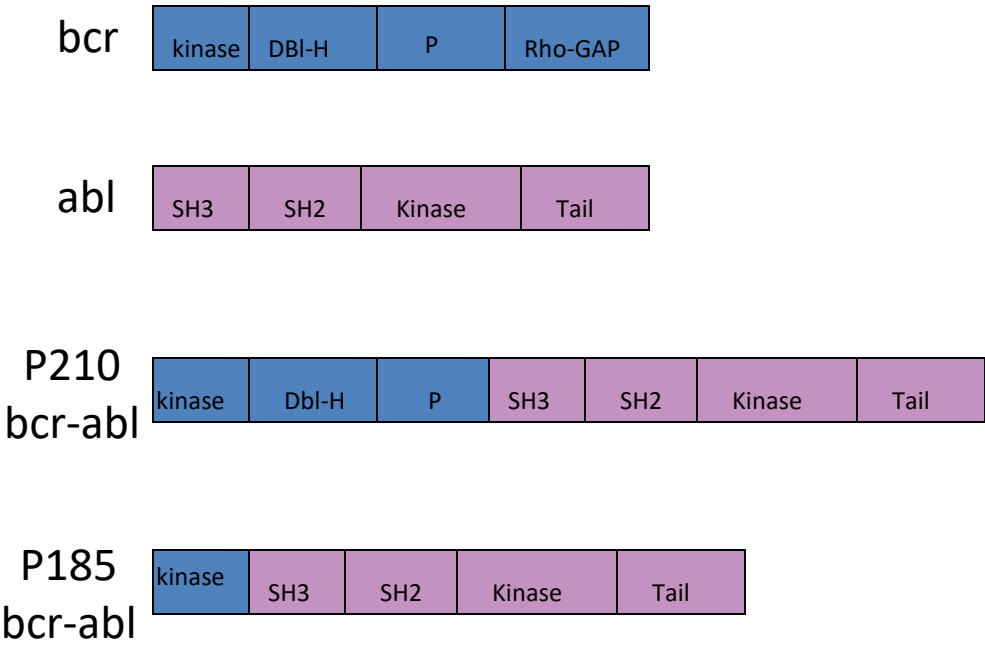
Translocation resulting in fusion of 2 genes



Alters structure of normal c-abl protein

Cytoplasmic tyrosine kinase

- **C-abl** encodes a cytoplasmic tyrosine kinase
- Bcr promotes oligomerization
- Bcr-abl fusion promotes activation of abl by oligomerization induced autophosphorylation
- Philadelphia chromosome – translocation of chr 9 and 22



Gleevec – Specifically inhibits the kinase activity of the Bcr-Abl fusion protein created when chrom 9 & 22 translocations occur to create the Philadelphia chromosome myeloid leukemia.

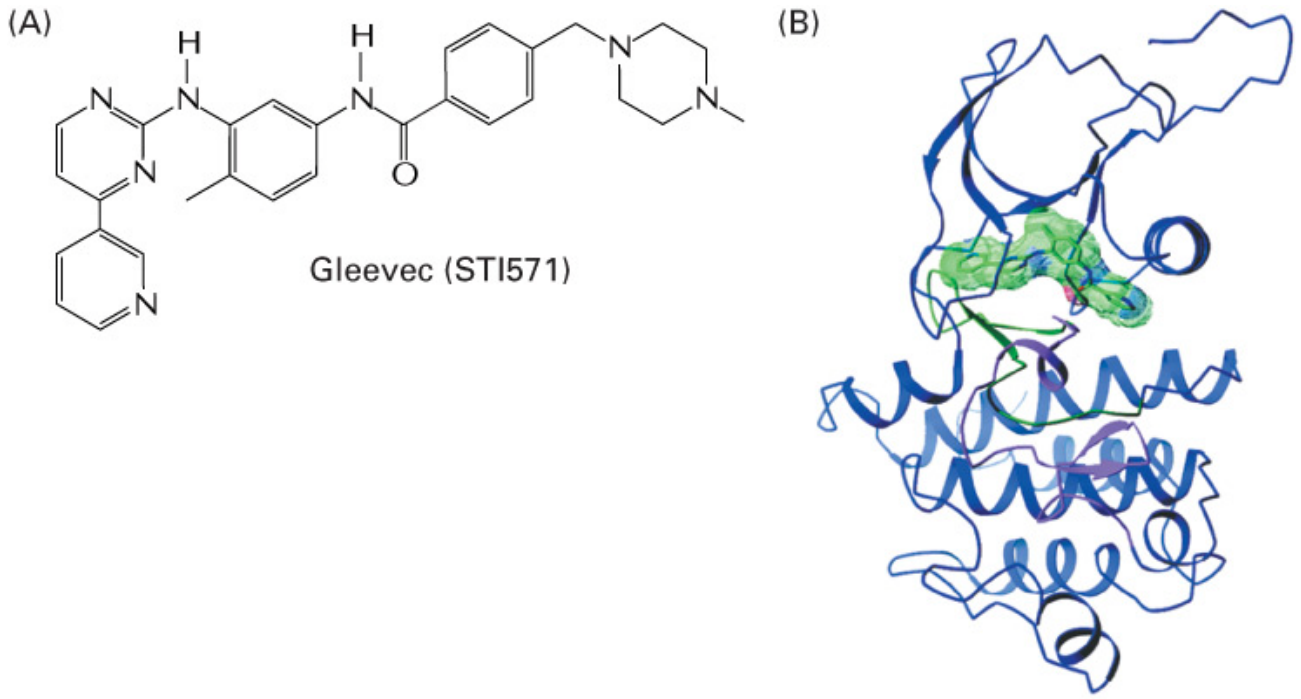


Figure 23–45 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

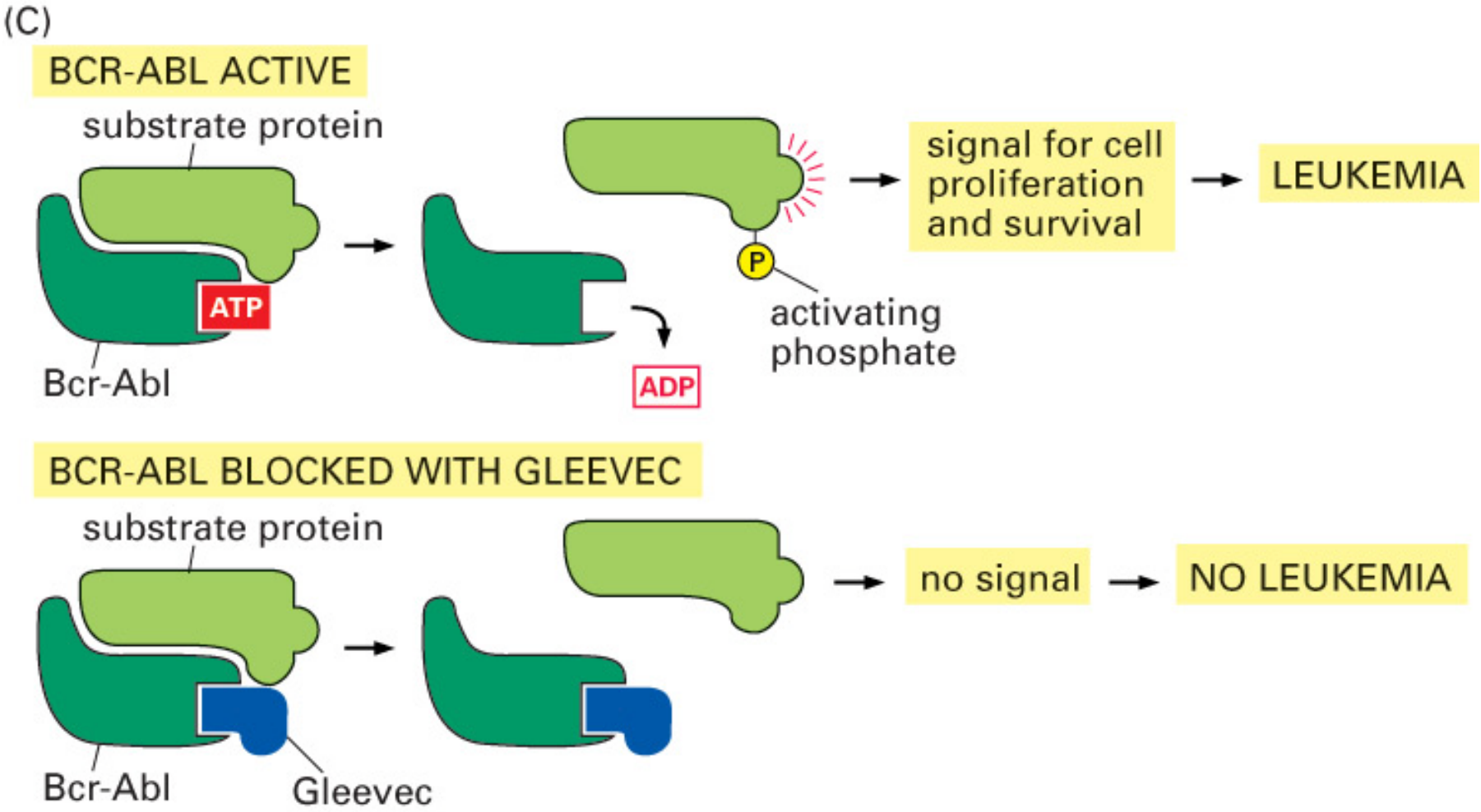
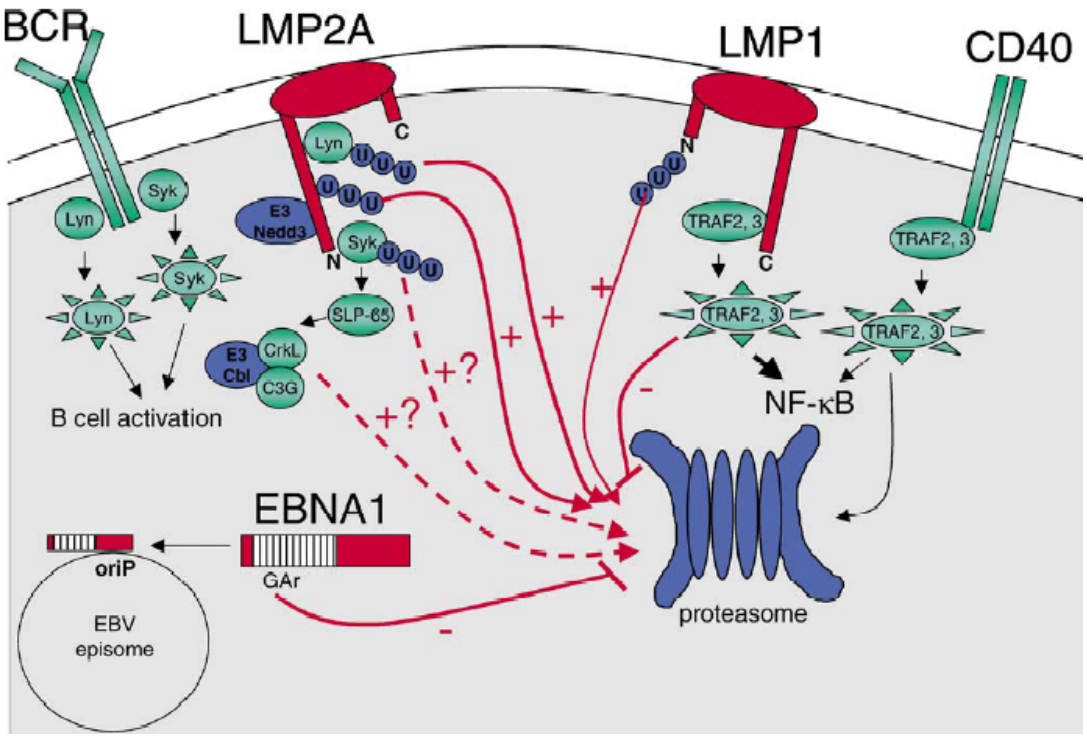


Figure 23–45 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Loss of degradation signals

Epstein–Barr virus (EBV) associated with lymphoid and epithelial malignancies.

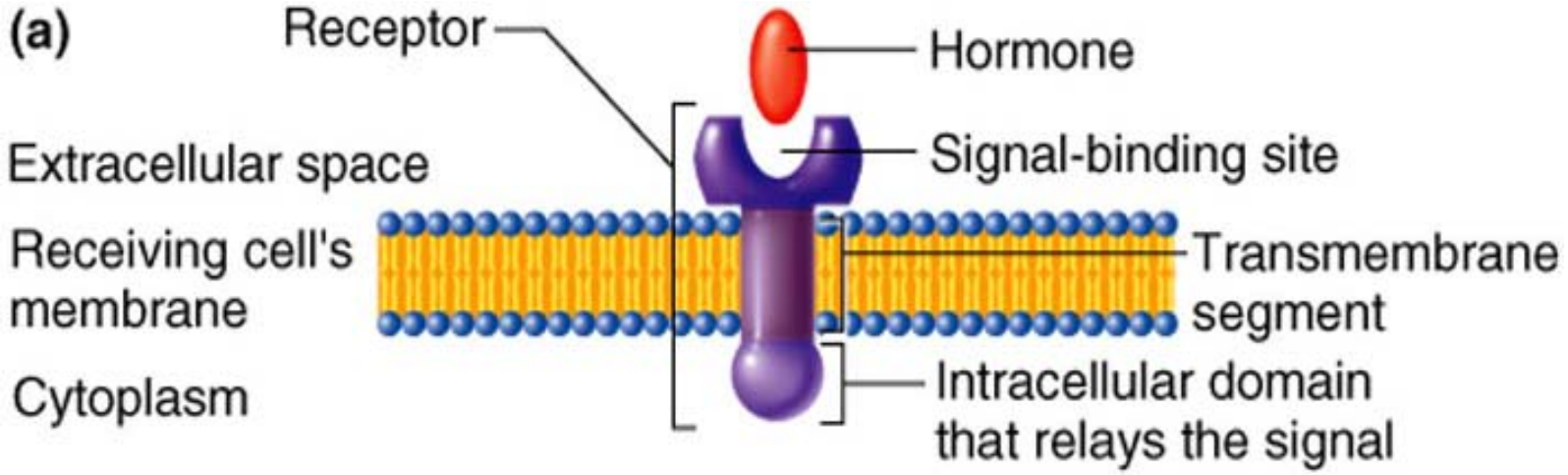
Three viral proteins, EBNA1, LMP-1 and -2A, constitutively activate c-myc oncogene by decreasing ubiquitin-dependent proteolysis of this protein and upregulate compensatory pathways in Burkitt's lymphomas.



Growth factor signalling and oncogenes

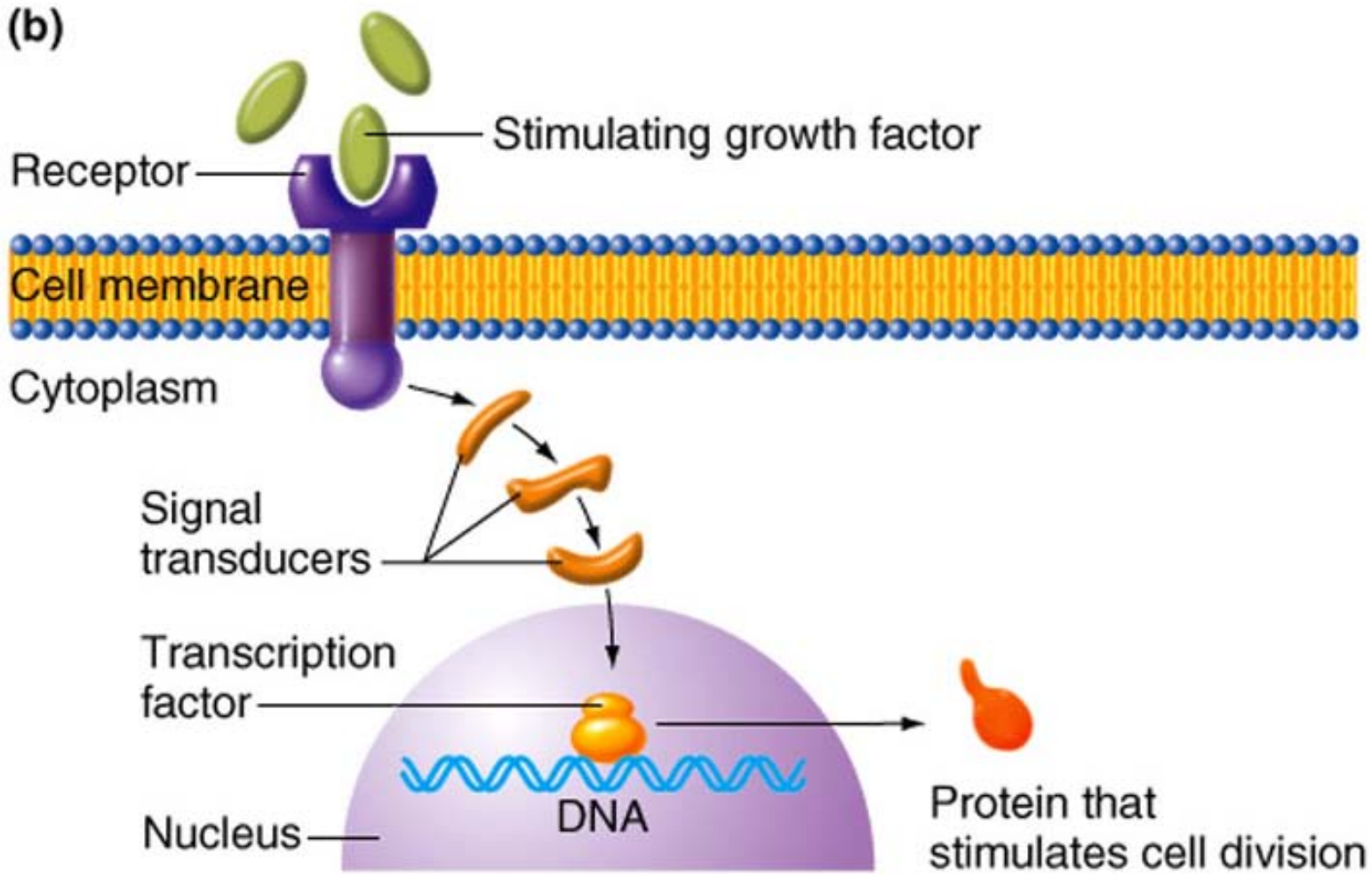
Cell Cycle Control is through the effects of which interact with membrane-bound glycoprotein receptors that transduce the message via a series of intracellular signals that promote or inhibit the expression of specific genes.

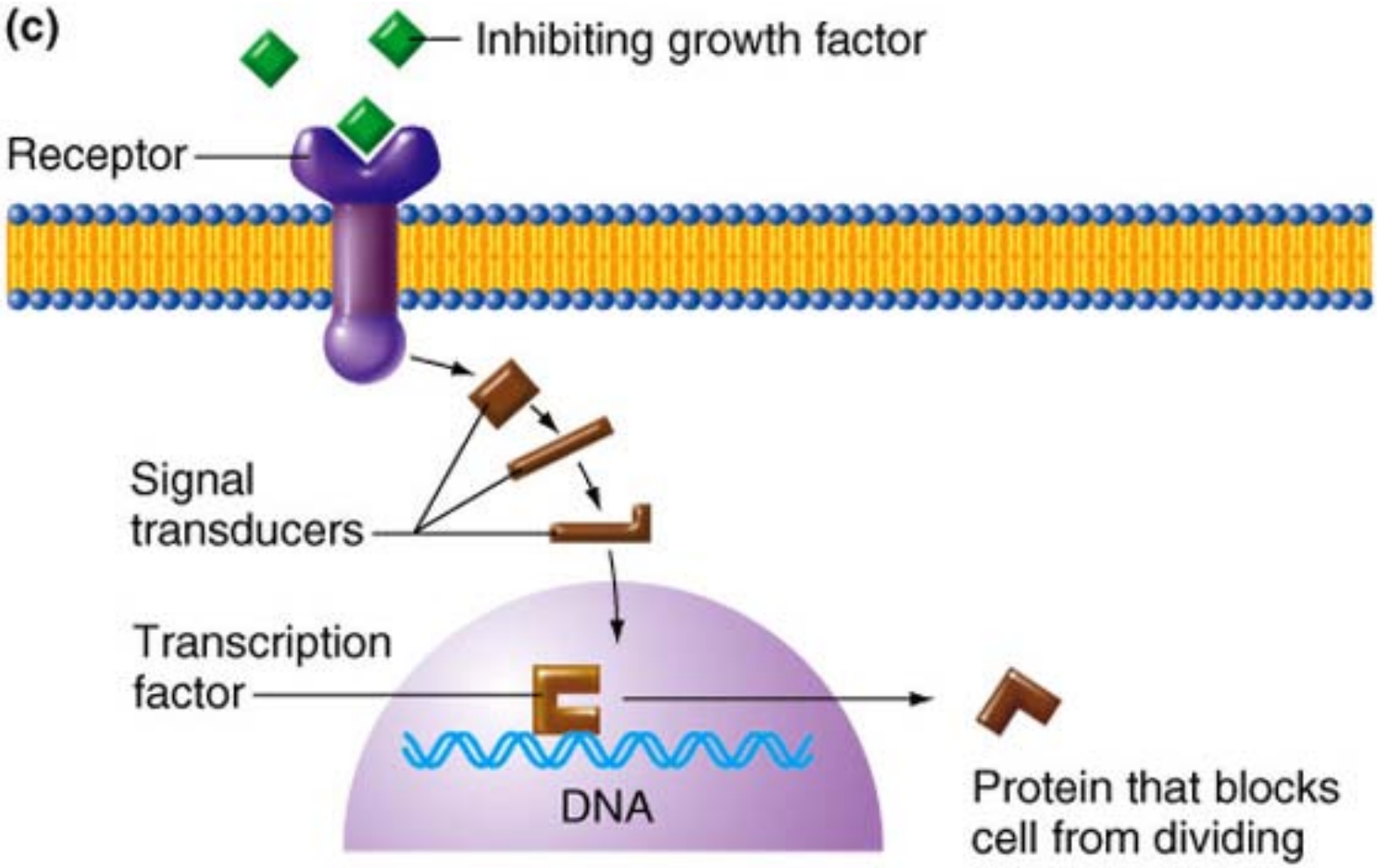
Molecular components of each signaling system



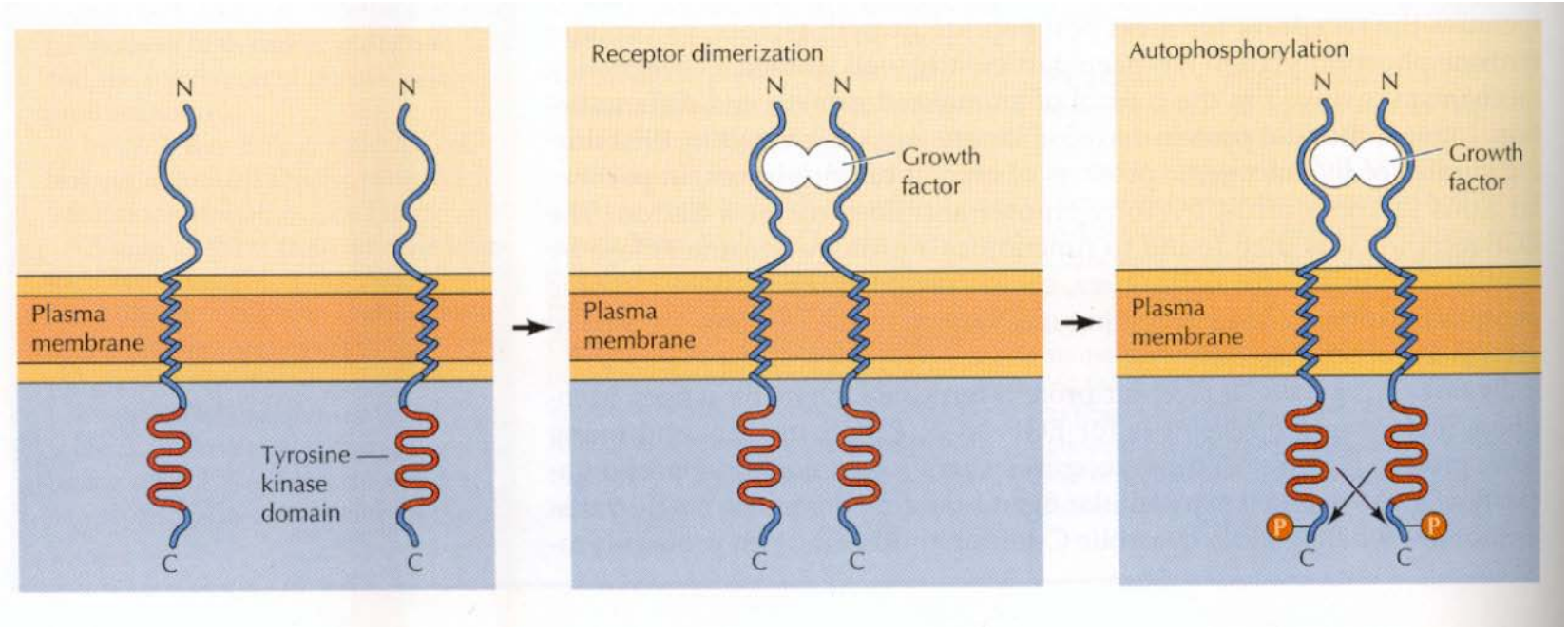
- **Growth factors – hormones and cell-bound signals that stimulation or inhibit cell proliferation**
- **Receptors – membrane bound proteins that accept signals**
  - **signal-binding site**
  - **transmembrane segment**
  - **intracellular domain**

Signal transducers relay messages and transcription factors activate expression of genes

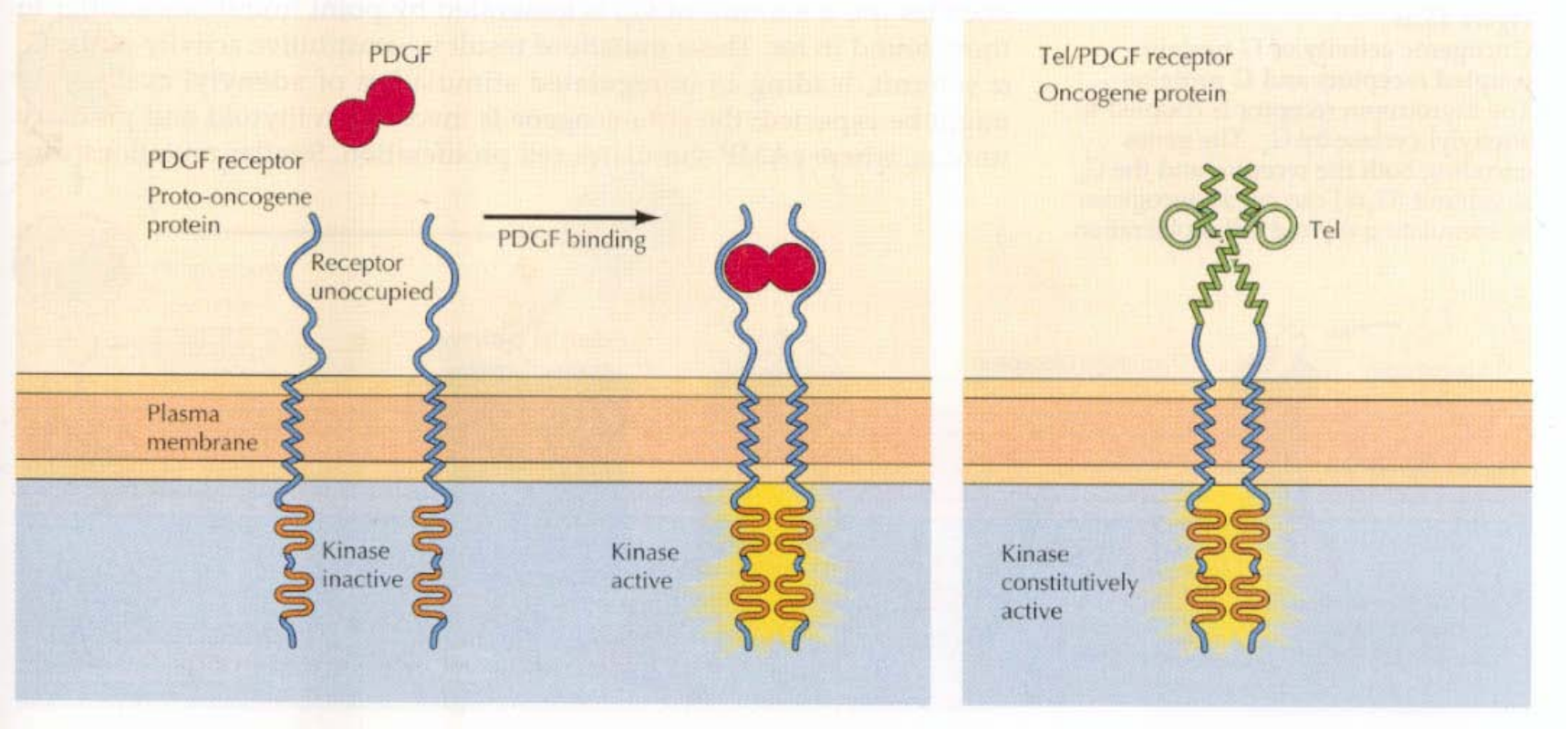



(c)
Inhibiting growth factor
Receptor
Signal transducers
Transcription factor
DNA
Protein that blocks cell from dividing

Receptor activation



Rearrangement



N terminal domain is replaced by a transcription factor that can associate with itself

Mutations in GF receptor can cause ligand-independent activation

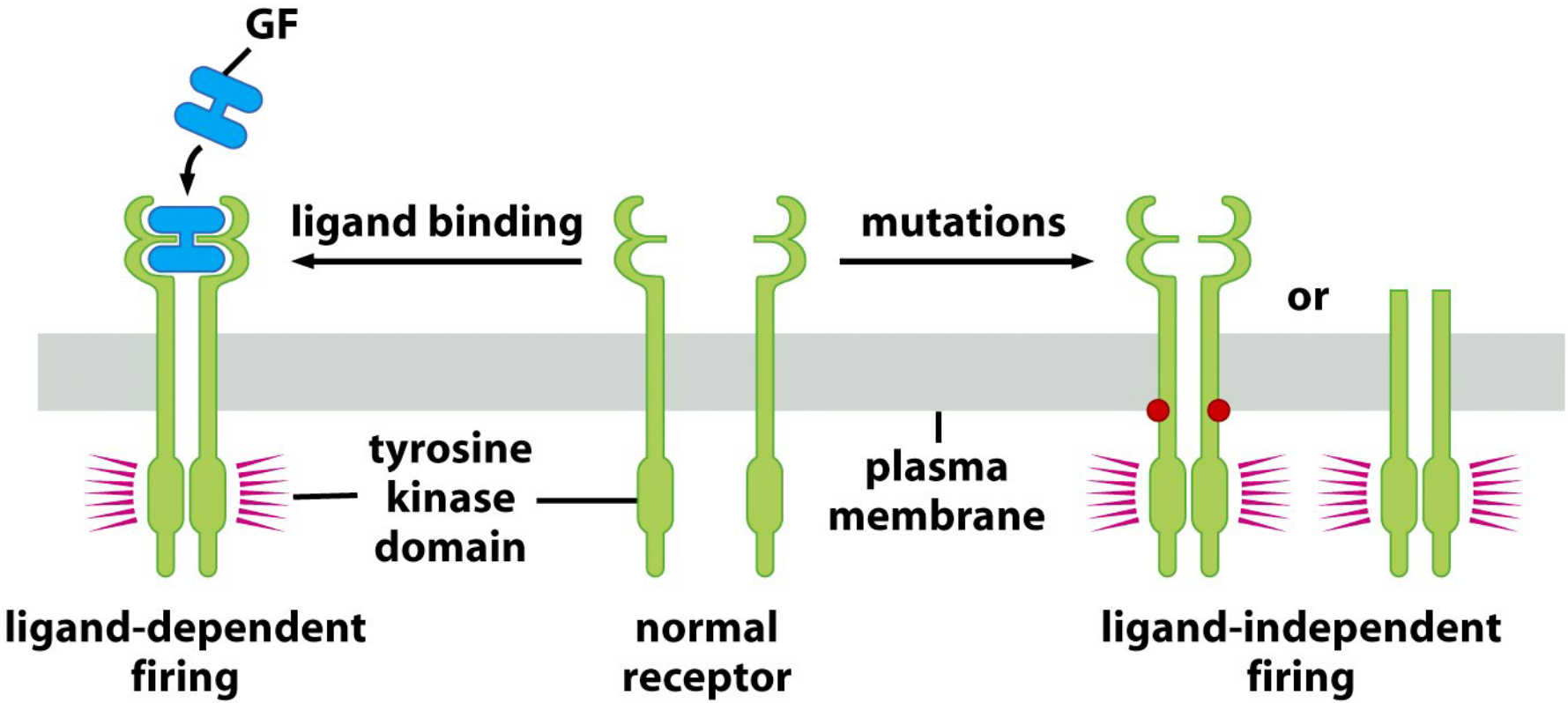


Figure 5.12a *The Biology of Cancer* (© Garland Science 2007)

Other growth factor receptors

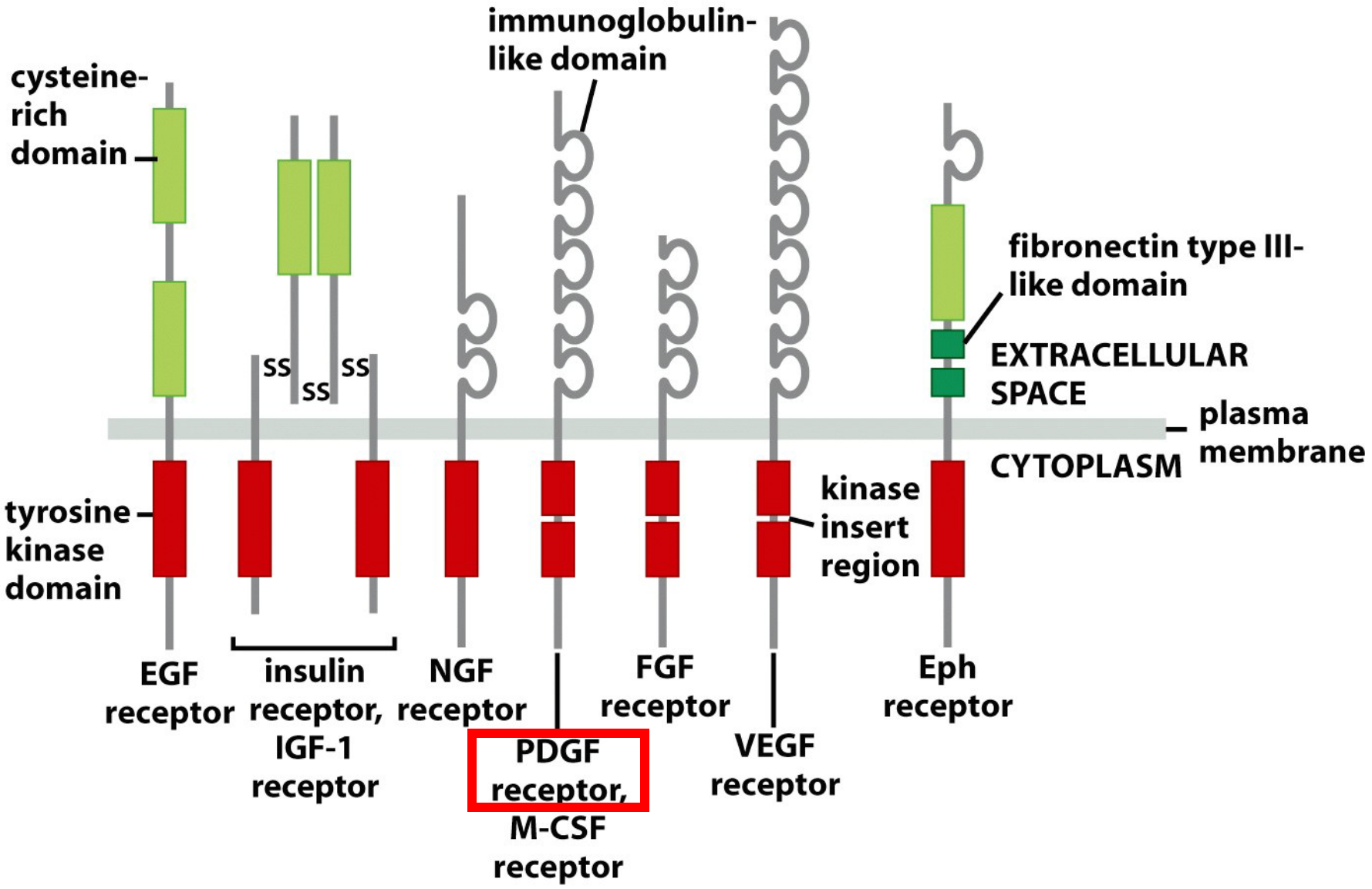


Figure 5.10 *The Biology of Cancer* (© Garland Science 2007)

Growth factor expression

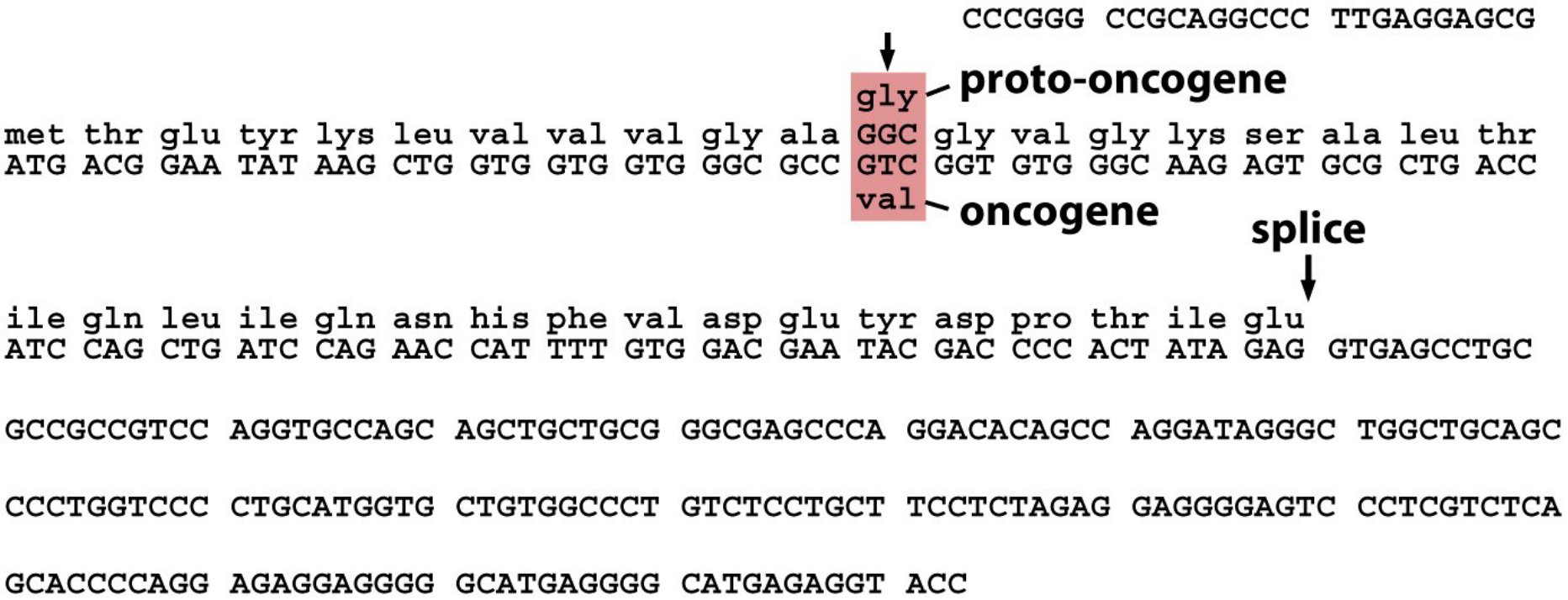
- Controlled at the level of gene expression
  - Autocrine
    - Cell produces a growth factor to which it also responds
    - Sis – encodes a variant form of PDGF
      - Astrocytomas
      - Increases cell growth
  - Paracrine
    - VEGF
    - Increases growth of endothelial cells
    - Secreted by tumor

Point Mutation

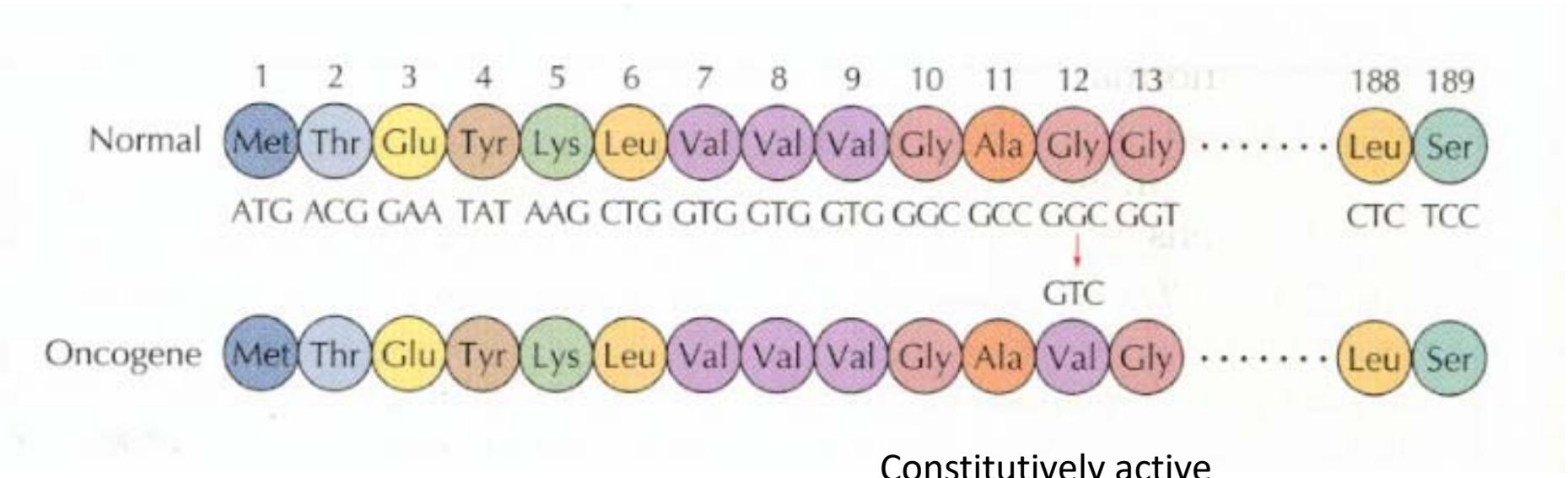
Mechanism of *ras*

- 1982 cloned and sequenced
- H-ras in human bladder carcinoma
- A middle aged man, smoking for 40yrs
- Single copy gene
- Cause transformation of NIH3T3

1st mutation in oncogene
G→T in H-ras

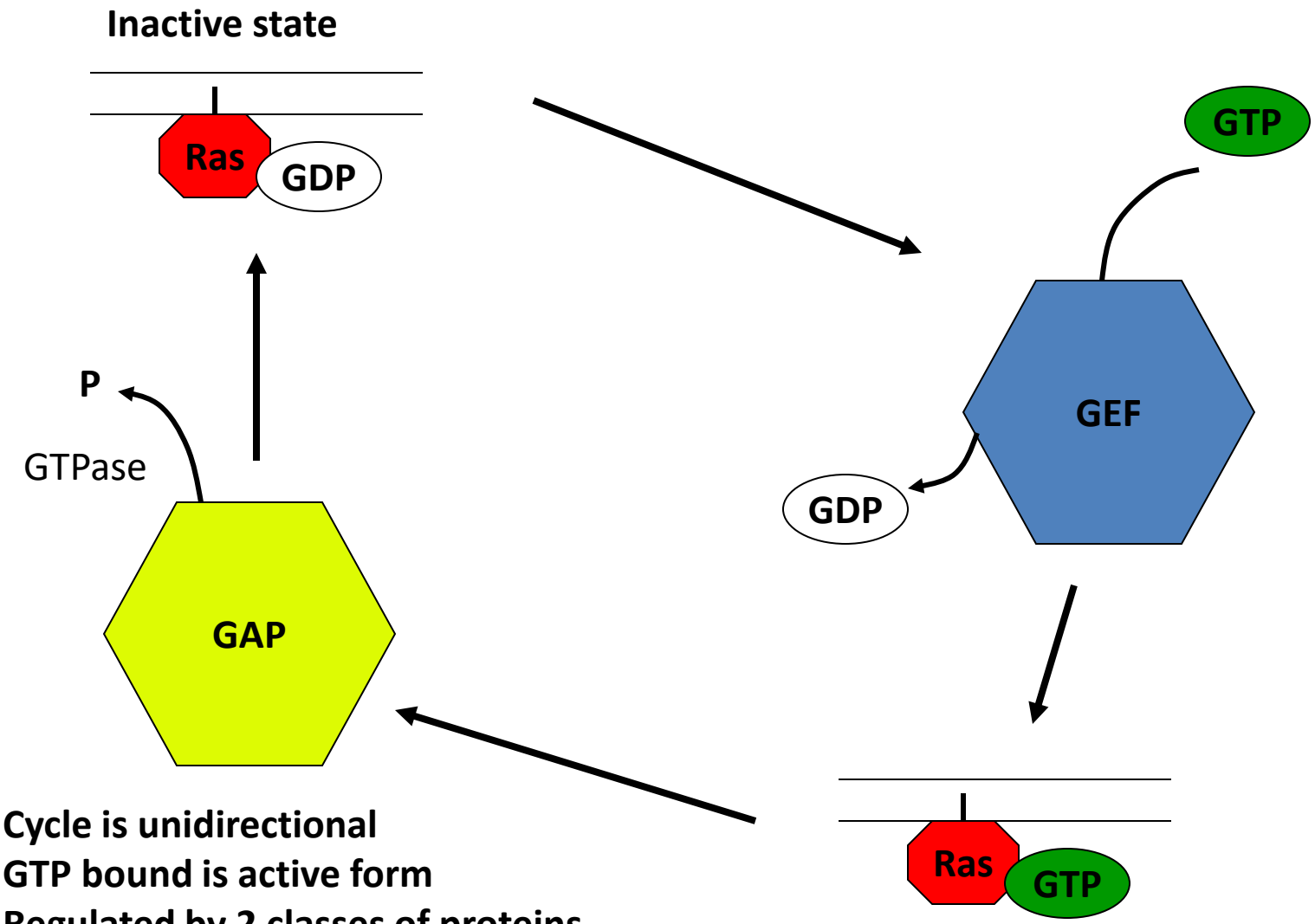


Point mutation

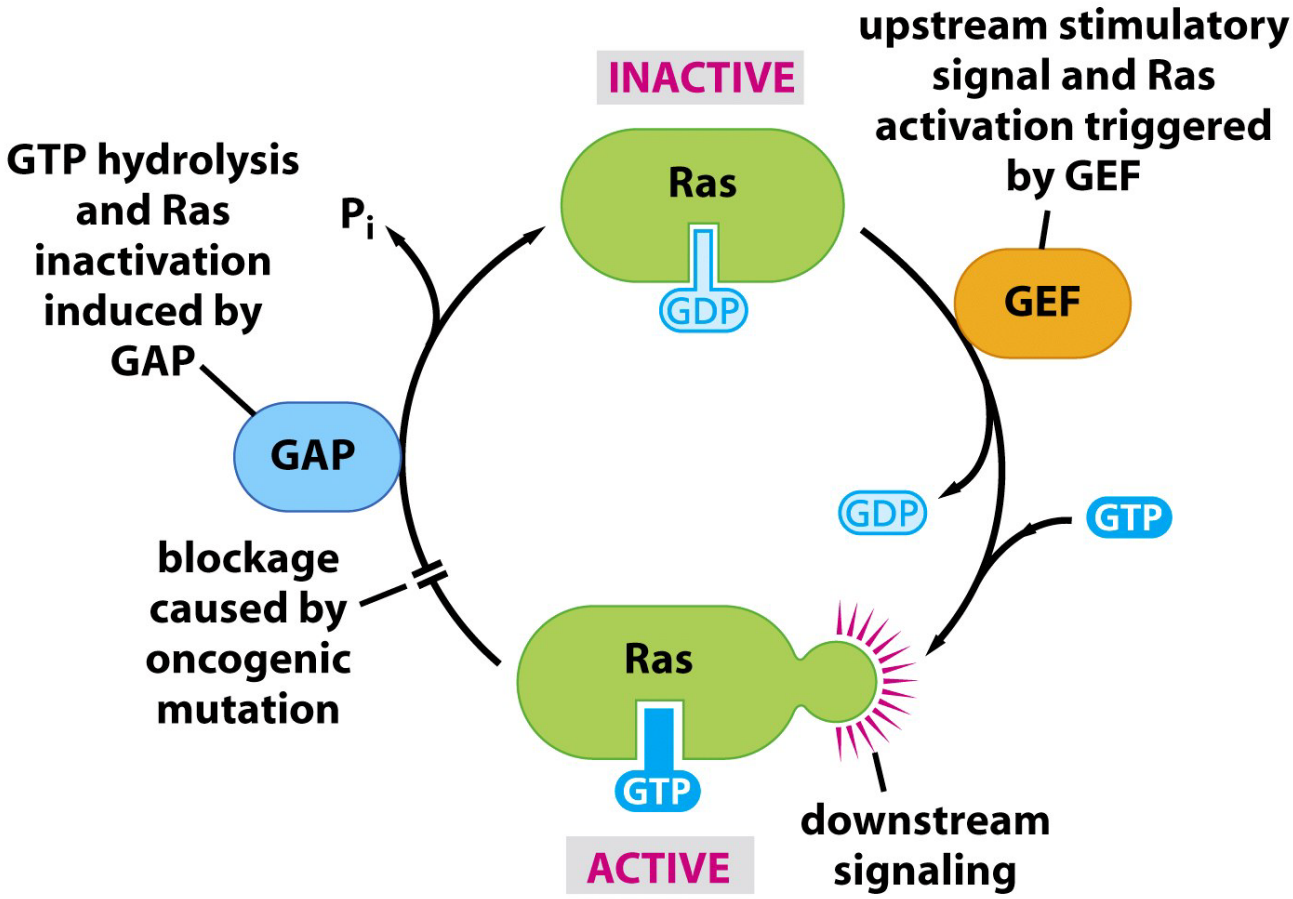


Constitutively active

Regulation of Ras



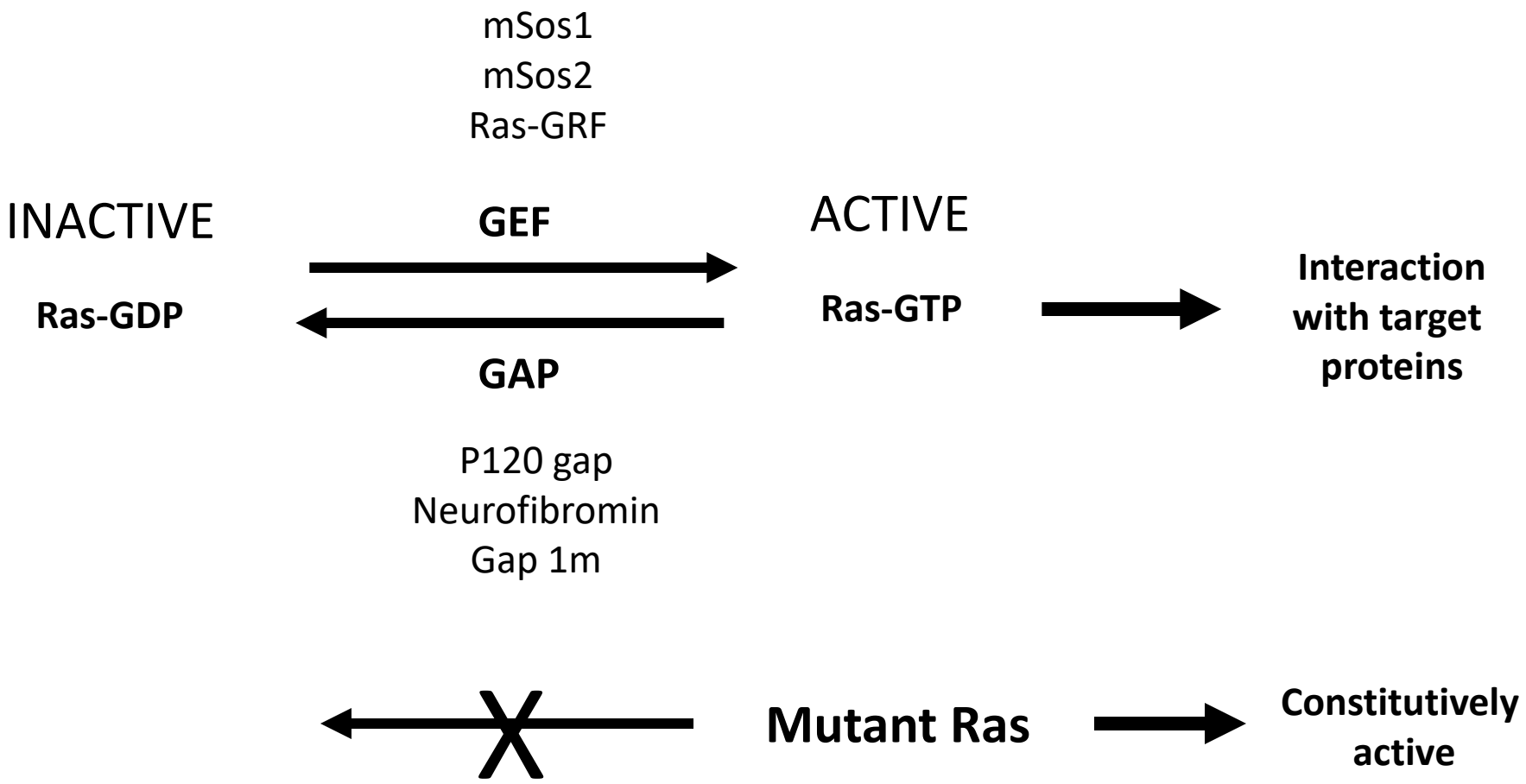
- Cycle is unidirectional
- GTP bound is active form
- Regulated by 2 classes of proteins
  - + regulator
  - -regulator



Guanine nucleotide exchange factor (GEF) - activation by GDP to GTP

GTPPase activation proteins (GAP)

- inactivation by GTP to GDP

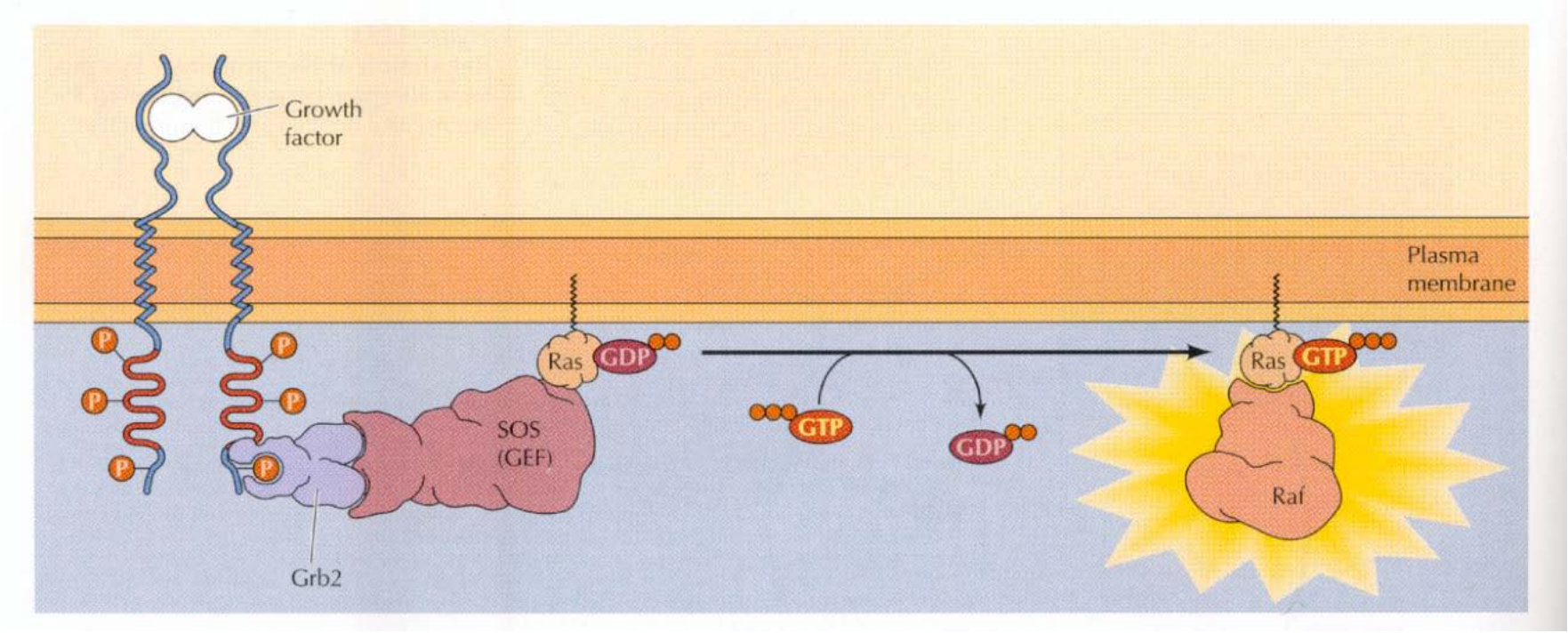

mSos1
mSos2
Ras-GRF
INACTIVE
GEF
ACTIVE
Ras-GDP
Ras-GTP
Interaction with target proteins
GAP
P120 gap
Neurofibromin
Gap 1m
Mutant Ras
Constitutively active

Oncogenic mutations that constitutively activate Ras

- Constitutive activation of GEFs (positive regulator)
- Reduction of GAP activity (negative regulator)
- Mutation of Ras gene
  - Cannot hydrolyze GTP

V-ras or mutated ras has lost the ability to interact with accessory proteins and are either GEF independent or GAP insensitive (GTP state)

Ras proteins

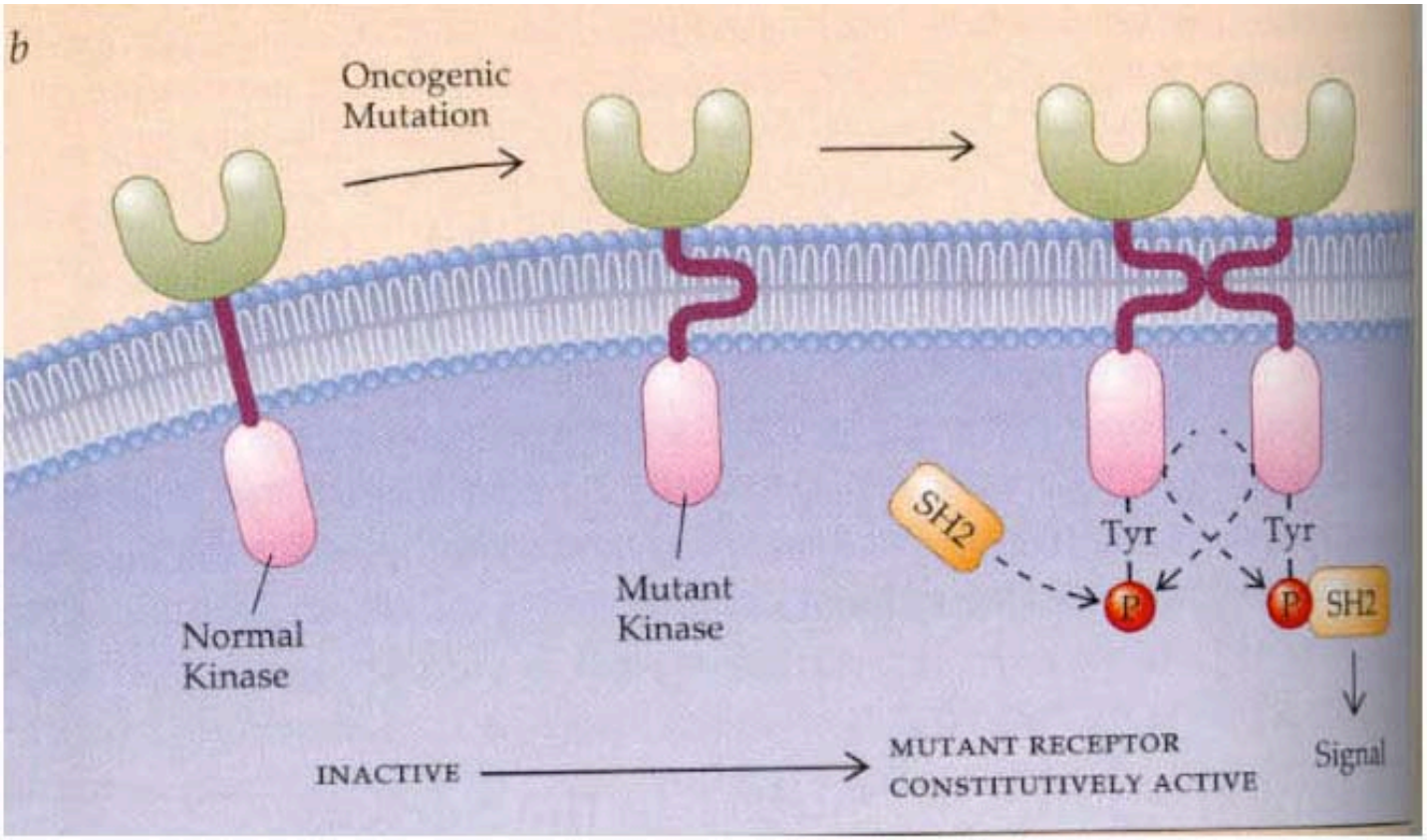


- Activation of PTK receptor by ligand binding
- Receptor associates with adaptor protein (grb2)
- Grb2 SH3 domain binds guanine exchange factor (Sos)
- Sos activates Ras
- Activated Ras interacts with protein kinase (Raf)

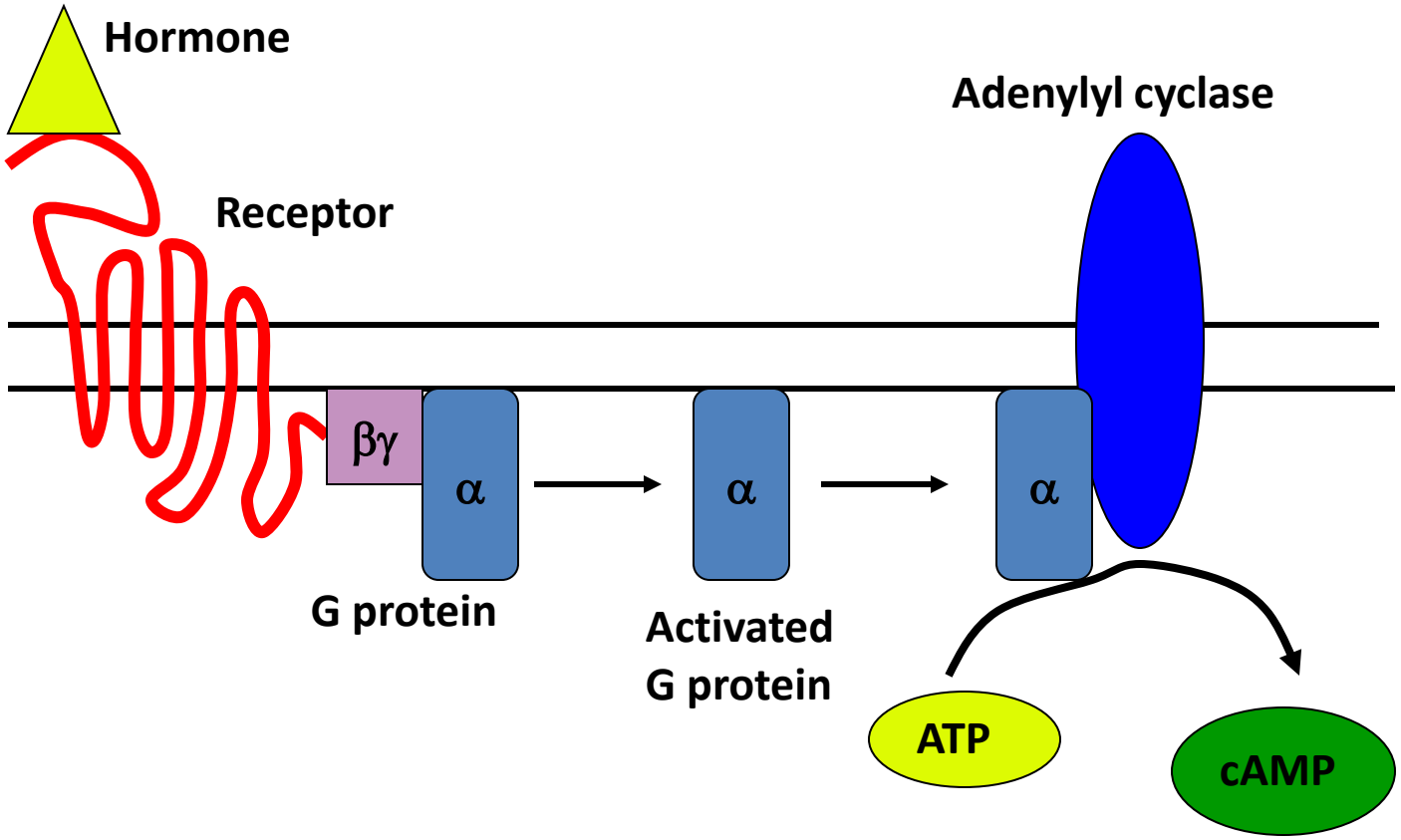
Ras mutations: 20% of human tumor

Tumor type	% of tumor
Pancreas	90% (K-ras)
Thyroid papillary（乳突性）	60% (H-, K-, N-ras)
Thyroid follicular （滤泡性）	55% (H-, K-, N-ras)
Colorectal	45% (K)
Seminoma（精原细胞癌）	45% (K, N)
Myelodysplasia	40% (N, K)
Lung (non-small-cell)	35% (K)
Acute myelogenous leukemia	30% (N)
Liver cancer	30% (N)
Melanoma	15% (K)
Bladder cancer	10% (K)
Kidney cancer	10% (H)

Point mutation of PTK

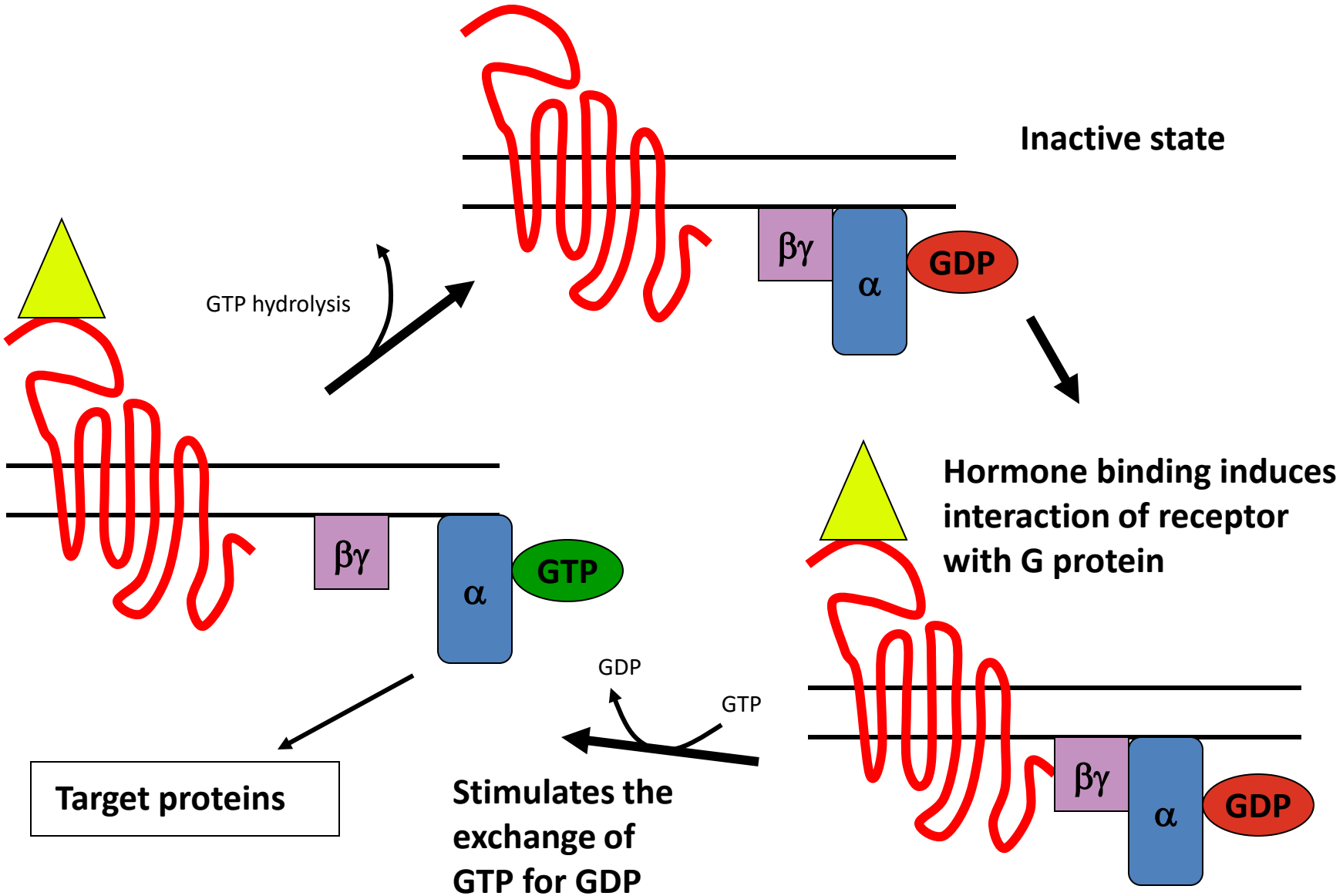


Induces dimerization in absence of ligand



- G protein associated with inner surface of PM
- Hormone bound receptor interacts with G protein
- Stimulates release of GDP and exchange for GTP
- Gα dissociates from complex
- Stimulates production of cAMP by adenylyl cyclase
- cAMP is a second messenger

Regulation of G proteins



Oncogenic mutations

- Locking α subunit in an active state

- Pituitary tumors
  - Gsp – encodes a mutated α subunit that blocks GTPase activity
  - Constitutive production of cAMP
- Thyroid tumors
  - Thyroid receptor mutated
  - Constitutive production of cAMP

Therapeutic implications

- High doses of retinoic acid can induce differentiation
- Block growth factor/receptor interaction with antagonist
- Tyrosine kinase inhibitors
- Block protein interactions in signaling cascade (SH2 domain)
- Block membrane localization of Ras with farnesylation inhibitors