Molecular and Cell Biology of Tumors

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3. Mitogenic signaling II

Radiation induced thyroid cancer

Why thyroid cancer?

The thyroid has a unique ability to concentrate and bind radioactive iodine, so that it receives a dose 500–1000 times higher than the rest of the body.

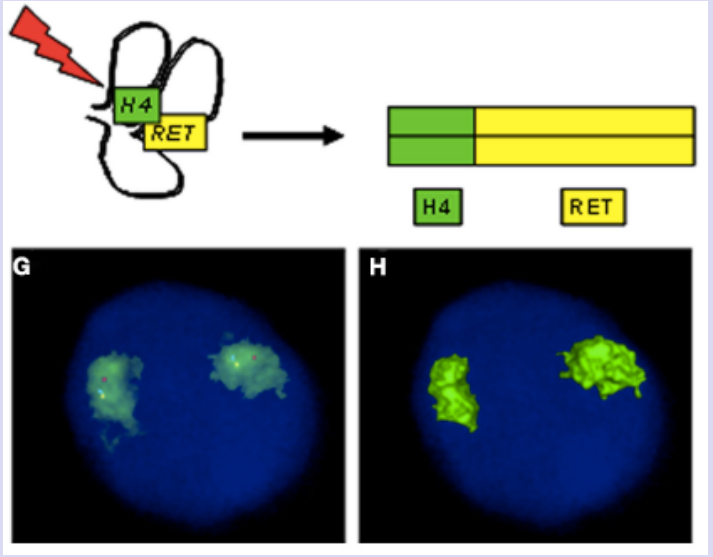
Why higher risk for children exposed to radiation?

The thyroid grows relatively rapidly during development; by the end of adolescence the growth rate is very low = higher mutation rate

Why RET oncogene-driven thyroid cancerogenesis?

RET expressed in nervous cells, not thyroid. By chromosomal rearrangements its kinase domain fuse with a thyroid-expressed gene (general name PTC- papillary thyroid carcinoma 1-, whose product homodimerizes − ligand-independent mitogenic signaling

RET and its most common fusion partners (*CCDC6=PTC1* and *NCOA4=PTC3*) seem to be particularly susceptible to breakage (DNA fragile sites), also CCDC6, NCOA4, and RET loci display close proximity specifically in thyroid follicular cell chromatin



Menicali E et al. Front Endocrinol (Lausanne). 2012 May 22;3:67.

Radiation induced thyroid cancer

Why RET/PTC fusions present also in sporadic (non-radiation-induced) thyroid cancer?

H_2O_2-induced breaks

H_2O_2 is produced in large amounts by thyrocytes during the process of thyroid hormone biosynthesis

Suzuki K et al. Cancers (Basel). 2019 Sep; 11(9): 1290.

Table 1

Oncogenic rearrangements in childhood thyroid cancers related to the Chernobyl accident.

Oncogenes	Rearrangement Partners	Chromosome Location	Type of Rearrangements
RET rearrangements			
RET/PTC1	*CCDC6* (also *H4*)	10q11.21/10q21	Inversion
RET/PTC2	*PRKAR1A*	10q11.21/17q24.2	Translocation
RET/PTC3	*NCOA4* (also *Ele*)	10q11.21/10q11.22	Inversion
RET/PTC4	*NCOA4* (also *Ele*)	10q11.21/10q11.22	Inversion
RET/PTC5	*GOLGA5* (also *RFG5*)	10q11.21/14q32.12	Translocation
RET/PTC6	*TRIM24*	10q11.21/7q32-q34	Translocation
RET/PTC7	*TRIM33* (also *RFG7*)	10q11.21/1p13.1	Translocation
RET/PTC8	*KTN1*	10q11.21/14q22.1	Translocation
RET/PTC9	*RFG9* (also *MBD1*)	10q11.21/18q21	Translocation
SPECC1L-RET	SPECC1L	22q11.23/10q11.21	Translocation
SQSTM1-RET	SQSTM1	5q35.3/10q11.21	Translocation
BRAF rearrangements			
AKAP9/BRAF	*AKAP9*	7q21.2/7q34	Inversion
AGK/BRAF	*AGK*	7q34/7q34	Inversion
SND1-BRAF	SND1	7q32.1/7q34	Inversion
MBP-BRAF	MBP	18q23/7q34	Translocation
POR-BRAF	POR	7q11.23/7q34	Inversion
ZBTB8A-BRAF	ZBTB8A	1p35.1/7q34	Translocation
MACF-BRAF	MACF1	1p34.3/7q34	Translocation
NTRK rearrangements			
TPR/NTRK1	*TPR*	1q31.1/1q23.1	Inversion
BANP-NTRK1	BANP	16q24.2/1q23.1	Translocation
ETV6/NTRK3	*ETV6*	12p13.1/15q25.3	Translocation
PPARg rearrangements			
PAX8/PPARg	*PAX8*	2q14.1/3p25.2	Translocation
CREB3L2/PPARg	*CREB3L2*	7q33/3p25.2	Translocation
Other rearrangements			
STRN-ALK	*ALK*	2p22.2/2p23.2-p23.1	Inversion
THADA-IGF2BP3		2p21/7p15.3	Translocation

Other types of receptors

- Receptor tyrosine kinases

- Receptors recruiting Janus kinases
- TGF-β receptors
- Serpentine receptors
  - GPCRs
  - Frizzled (Wnt-1/β-catenin)
  - Patched
- Notch
- Integrins
- NF-κB

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- **TGF-β receptors**
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- **Integrins**
- NF-κB

TGF-β family

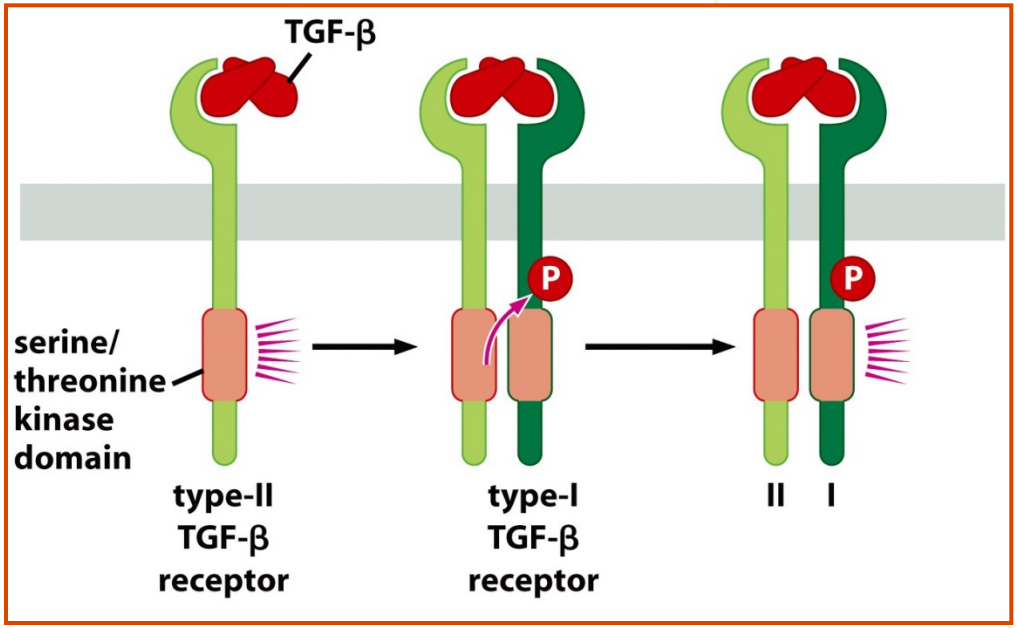
TGF-β (*transforming growth factor*) – in human genome 3 main isoforms: TGFβ1, 2 a 3

- Cytokine, produces by all types of leukocytes
- **Inhibitory** for most cells: inhibits growth of normal epithelial, endothelial, neuronal, lymphoid and haematopietic cells, stimulates differentiation and induces apoptosis
- But **elevates** invasive features of already transformed cancer cells
- Represents **anti-mitogenic** signaling, it functions as a tumor suppressor
- Inactivated by somatic mutations or gene deletions mainly in colorectal and stomach cancer

TGF-β receptors

- **TGF-βR** (type I and II) functions as **heterodimer**
- They have catalytic **serine/threonine** kinase domain

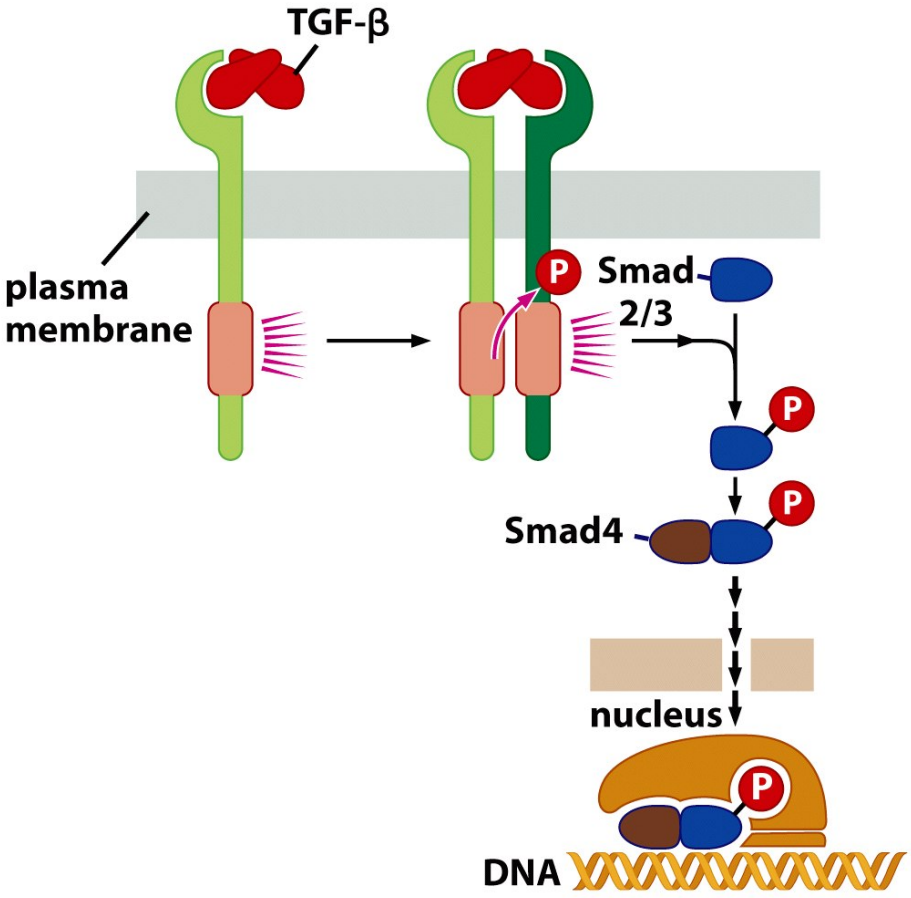
➢ Upon ligand binding subunit TGF-βRII (with constitutively active Ser/Thr kinase) recruits TGF-βRI that is phosphorylated thereby activated; that in turn phosphorylates cytosolic proteins that are translocated to the nucleus



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

TGF-β signaling pathway

- Binding of TGF-β to the receptor leads to the phosphorylation of **SMAD2** and/or **SMAD3** that form a complex with **SMAD4** and this complex migrates to the nucleus. There, in cooperation with other TFs, transactivates TGF-β-target genes (e.g. **p21^{CIP1}**, **p15^{INK4B}**, etc).



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TGF-β pathway and cancer

- In colorectal cancer ***smad2*** frequently **mutated**
- In 50% of pancreatic carcinoma and 25% of colorectal cancer **Smad4** is **inactive**
- Most of the MSI (microsatelite instable) colorectal cancers have **mutations inactivating TGF-βII receptor**

TGF-β pathway and cancer

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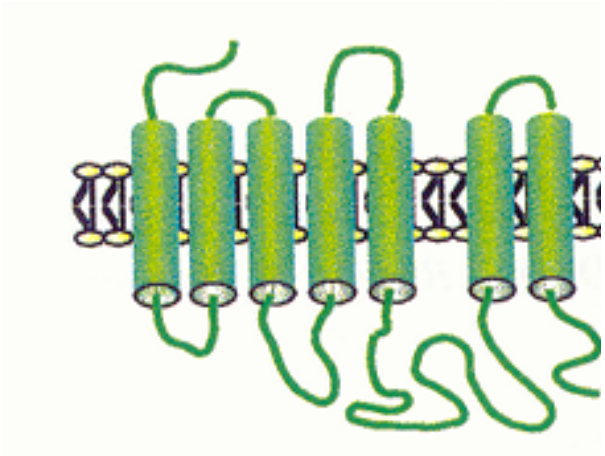
Serpentine receptors

- typical structure - 7 hydrofobic transmembrane domains: they pass through the cell membrane seven times in form of six loops

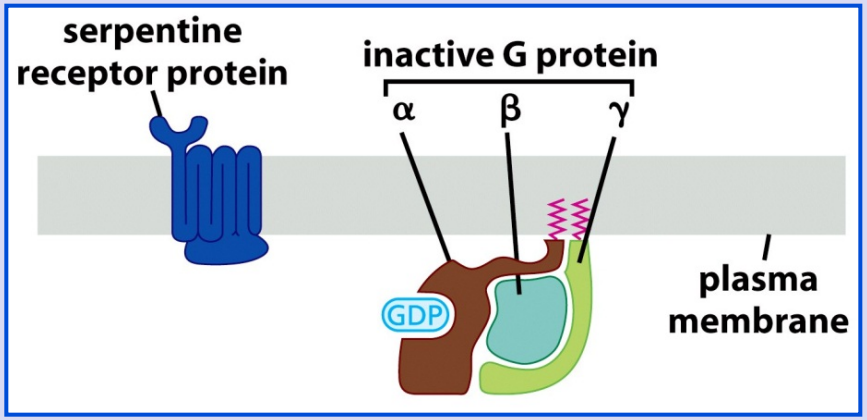
Include:

- G protein-coupled receptors
- Frizzled
- Patched/Smoothened



G protein-coupled receptors (GPCRs)

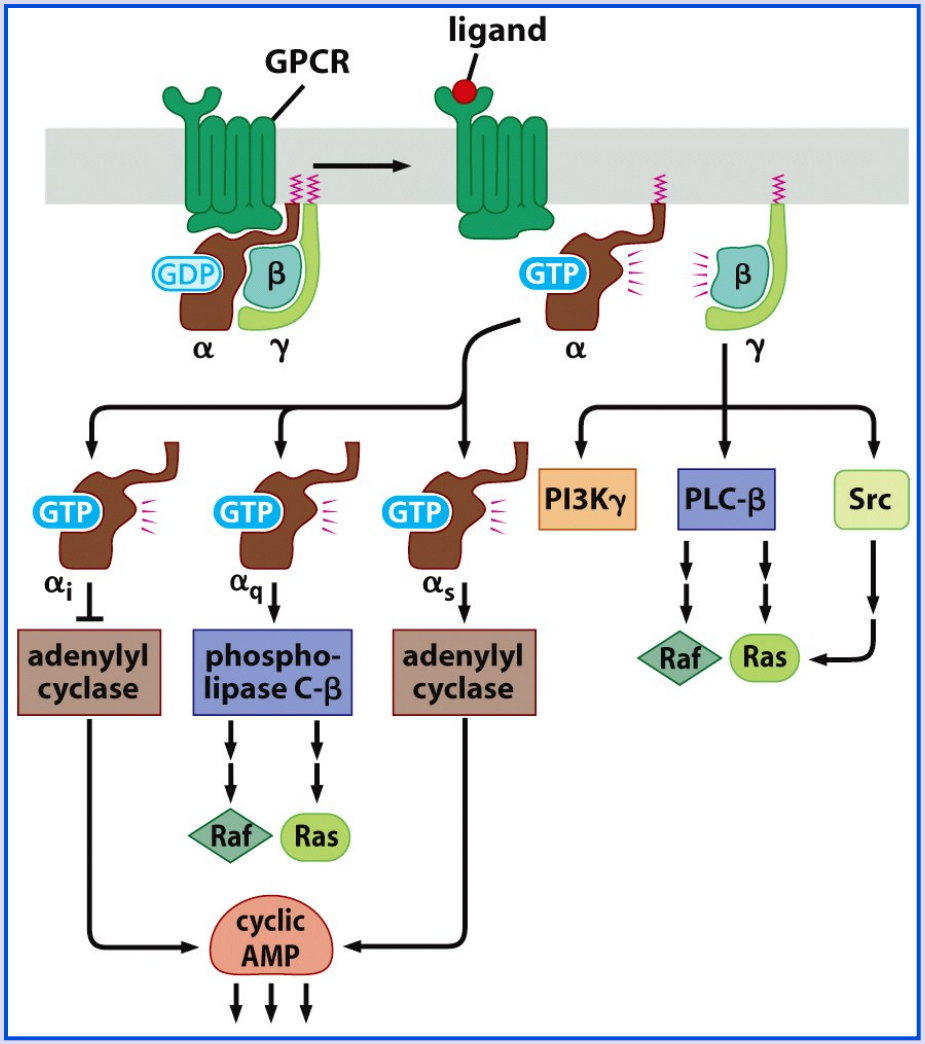
- GPCRs are the largest group of surface (more than 1000 in mammals – represent almost 5% of genes in human genome!)
- Activated upon binding of respective ligand
- Ligands for GPCRs are various extracellular molecules: growth factors, hormones (serotonin, epinephrine, glucagon, thyrotropin), phospholipids, neurotransmitters, and others.



Activation of GPCRs

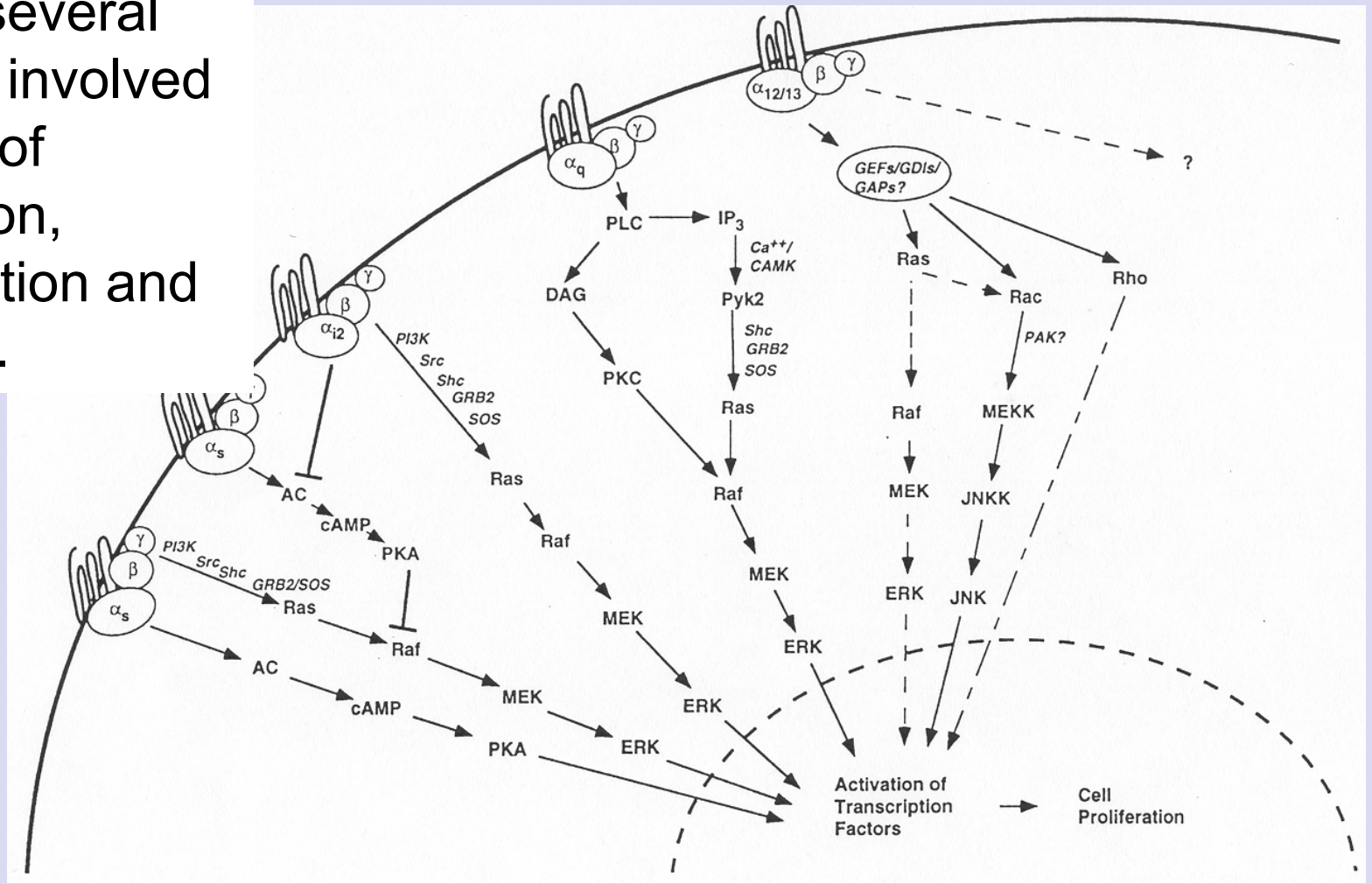
- Dissociated subunit **α-GTP** activates range of cytosolic enzymes:
  - **Adenylyl cyclase** (ATP → cAMP)
  - **phospholipase C-β** (cleaves PIP_2)
  - **Src**
- Complexes of **β+γ** subunits might stimulate **PI3K**, **Src**

⇒ GPCRs may trigger mitogenic signaling; potential involvement in cancer development



GPCR signaling pathways

GPCRs transduce signal to several pathways involved in control of proliferation, differentiation and apoptosis.



Signaling pathways: Wnt/β-catenin Sonic hedgehog/Gli NOTCH

- regulate pivotal cell-fate choices, self-renewal/ maintanance of tissue specific stem cells and their commitment to different lineages

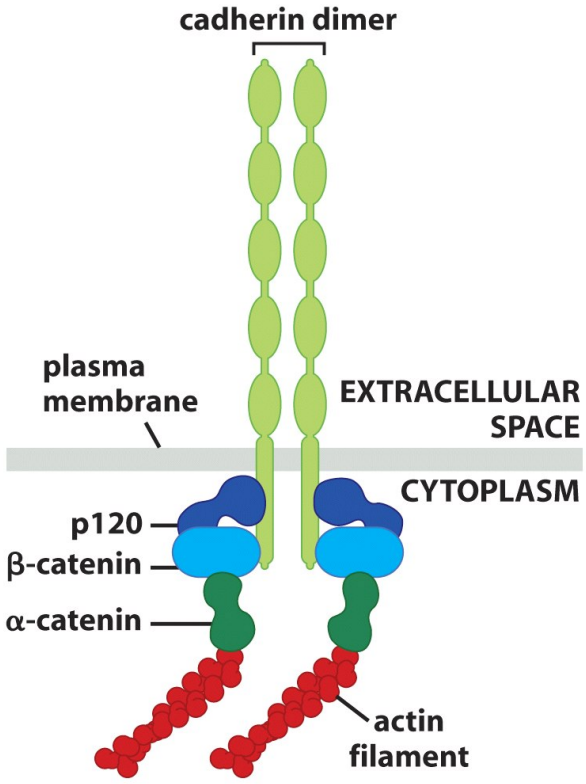
- Important pathways for cancer cells as well

➢ For most cell the dominant pathway mediating mitogenic signals is cascade RTKs → Ras, but it is not the only one

Signaling pathways Wnt/β-catenin

β-catenins plays two distinct functions in cells and both may participate in cancerogenesis; may have **3 different forms/ localisations**:

1. Molecules of β-catenin may be integral structural components of **adherens junctions** (mediating interaction between cadherins and actin filaments of cytoskeleton). Changes in cell-cell adhesion precede metastatic dissemination.

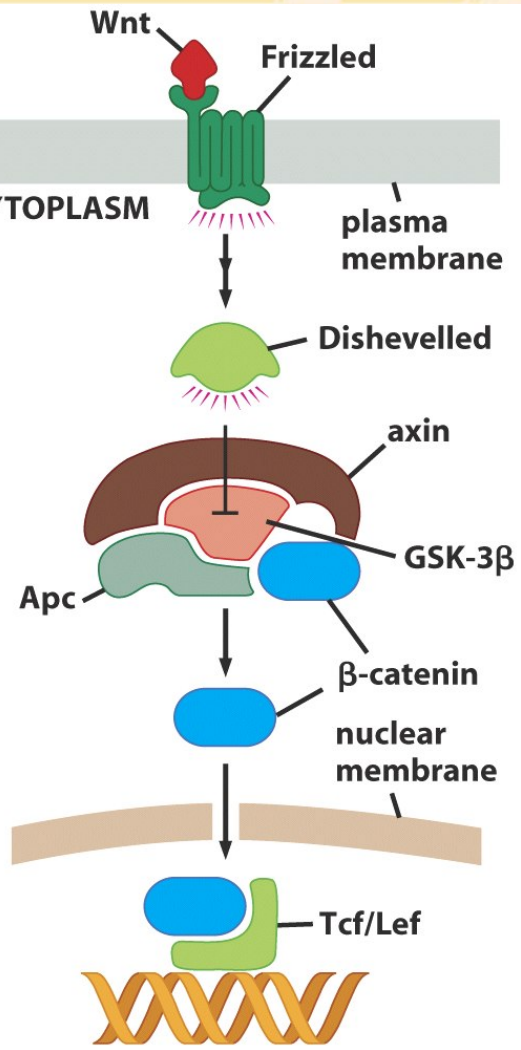


Signaling pathway Wnt/β-catenin

2A. **Free** excess β-catenin in cytosol (half-life approx 20 mins − phosphorylated and degraded), functions as a signaling molecule of Wnt pathway.

2B. May be transported to the **nucleus** (after Wnt blocks destruction complex) and functions in complex with other proteins as **transcription factor**.

Target genes of β-catenin are involved in proliferation (↑) and apoptosis (↓)

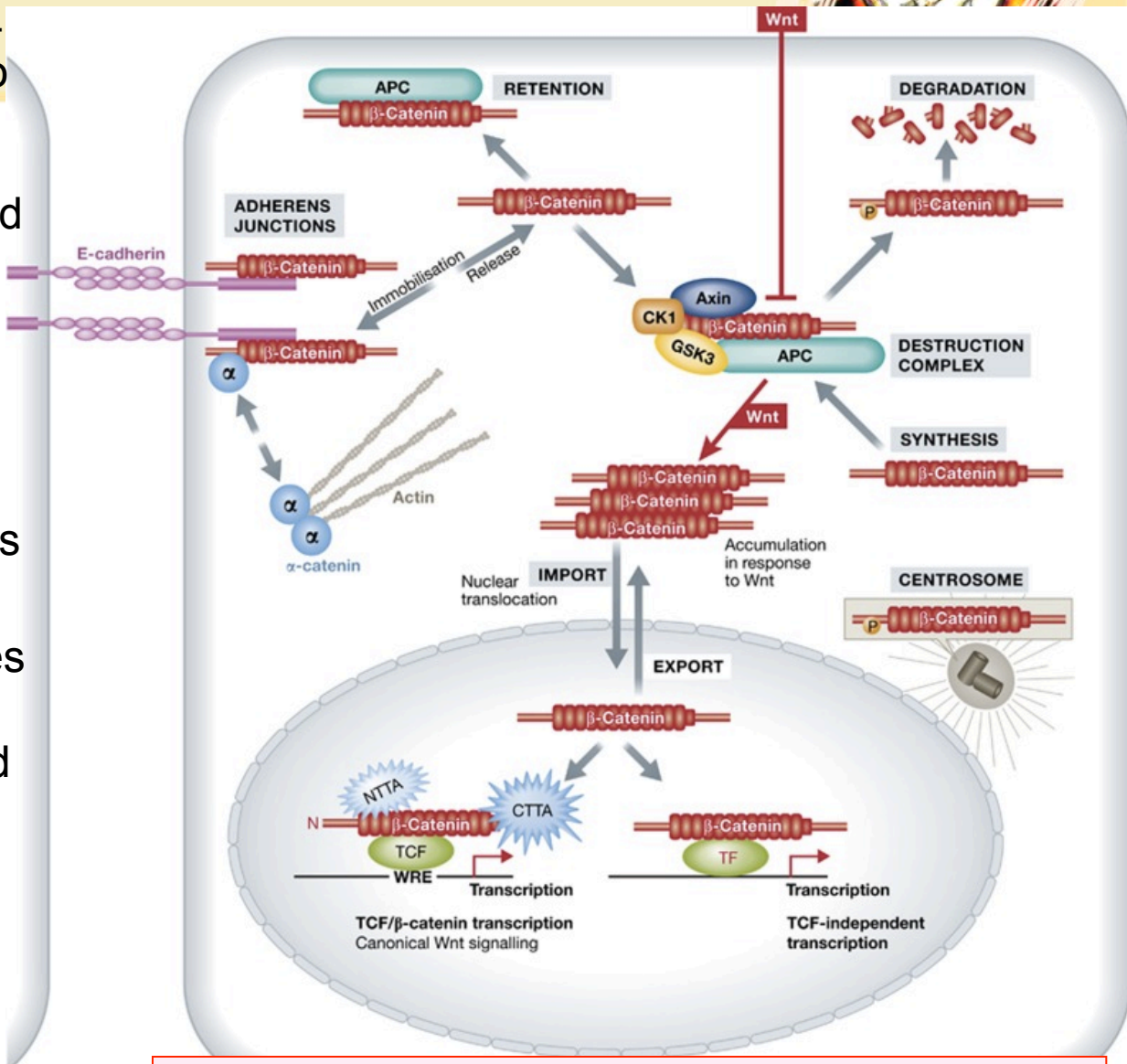


Regulation of β-catenin

- Levels of **free** cytosolic β-catenin is controlled by so called **destruction complex**. It consists of proteins: **GSK-3**, **Axin** and **APC**.

Axin and **APC** function as a scaffold of destruction complex, contain binding sites for all its components

GSK-3 serine/threonine kinase that phosphorylates β-catenin (S33, S37, T47 and S45). Phosphorylated β-catenin is marked for ubiquitinylation and proteosomal degradation.



Valenta et al. EMBO J. 2012 31(12): 2714–2736.

Regulation of β-catenin

- Activation of Wnt/β-catenin pathway − i.e. binding of **Wnt*** glycoprotein to respective **Frizzled** receptor leads to the activation of protein **Dsh** (disheveled). Dsh interacts with destruction complex and blocks activity of **GSK-3β**. Thus phosphorylation of β-catenin is inhibited and its half-life is prolonged to 1-2 hours. Outcome is an increase in levels of **free** β-catenin.
- That is transported to the nucleus where it interacts with other proteins including **Tcf** („T cell factor“) and **Lef** („lymphoid enhancer factor“) and this multiprotein complex transactivates target genes.

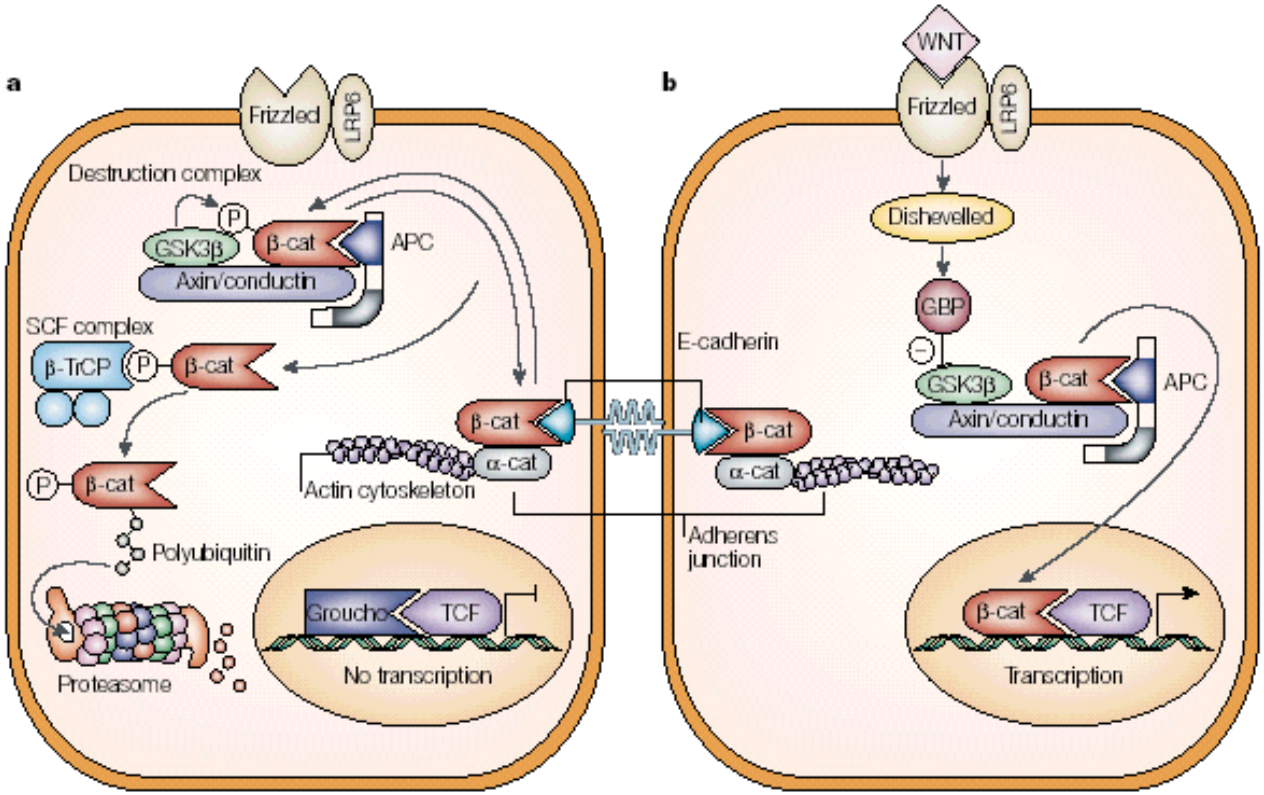
* Wnt ligands are extracellular, secreted, but as the lipid-modified (palmitoleoylation) proteins are hydrophobic thereby unable of free diffussion. Extracellular transport mediated by different carriers.

Wnt/β-catenin signaling pathway

- Target genes of β-catenin/Tcf/Lef are (among others) **c-*myc*** and gene encoding **cyclin D1** (***CCND1***). These are resposible for the increased cell proliferation

- Wnt/β-catenin signaling pathway participates in embryonic development, it is critical for cell fate specification, cell proliferation (fluctuation of β-catenin during cell cycle) and migration.

Wnt/β-catenin signaling pathway

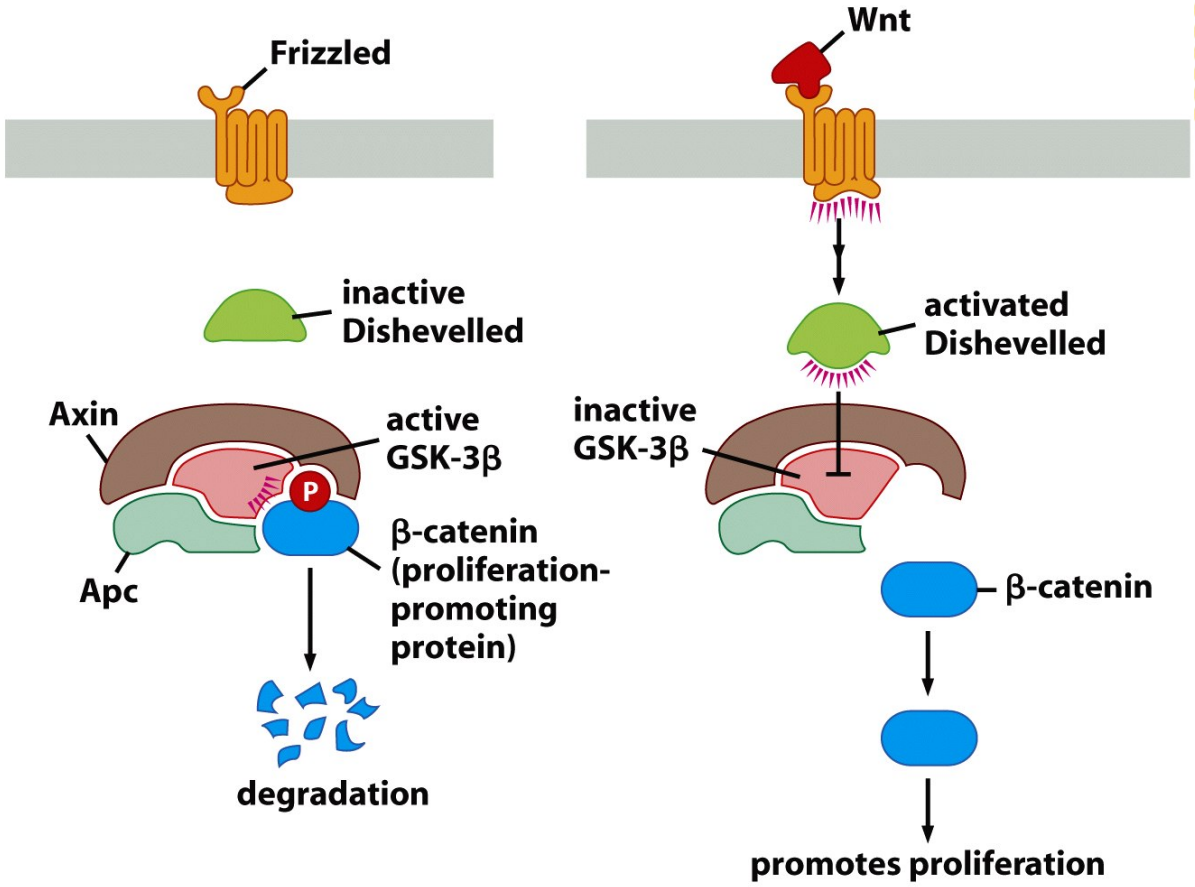


Excess of free β-catenin is degraded (after phosphorylation by GSK-3) by proteasome.

Fodde R et al, *Nat Rev Cancer* 1: 55-66, 2001

Wnt/β-catenin signaling pathway

- Wnt/β-catenin signaling pathway maintains appropriate levels of free β-catenin
- Crucial is GSK-3β kinase activity



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Wnt/β-catenin signaling pathway

- **GSK-3β** phosphorylates other targets, e.g. **cyclin D1**. Phosphorylated cyclin-D1 is marked for degradation as well
- Wnt/β-catenin controls cyclin D1 abundance at two levels: **transcriptional** (via β-catenin) and **posttranslational** (via GSK-3β kinase activity).

- Wnt ligand is morphogenic and mitogenic factor

Wnt/β-catenin signaling pathway **and cancer**

- Overexpression of **Wnt-1** is associated with breast cancer development
- Mutations of **APC** (tumor suppressor) blocking binding of APC to β-catenin or to axin result in constitutive β-catenin activation
  - Mutations of APC (including germinal) are early events during colorectal cancer development (growth advantage)
- Mutations of **axin** (tumor suppressor) that disable binding of axin to β-catenin are frequent in some hepatocellular carcinomas
- Mutations of **β-catenin**: that remove or replace serine for other AA. That prevents phosphorylation by GSK-3β and degradation of β-catenin; found in prostate, colorectal, endometrial and ovarian cancer and melanomas
- Mutations of β-catenin also occur in colorectal cancer, but usually not concomitantly with APC mutations (**mutually exclusive**)

Regulation of β-catenin by Siah-1

Independent mechanism of β-catenin degradation: mediated by protein **Siah-1** **(ubiquitin ligase)**

.

- By this route also mutated oncogenic form of β-catenin (GSK3 phosphorylation-"resistant") may be degraded

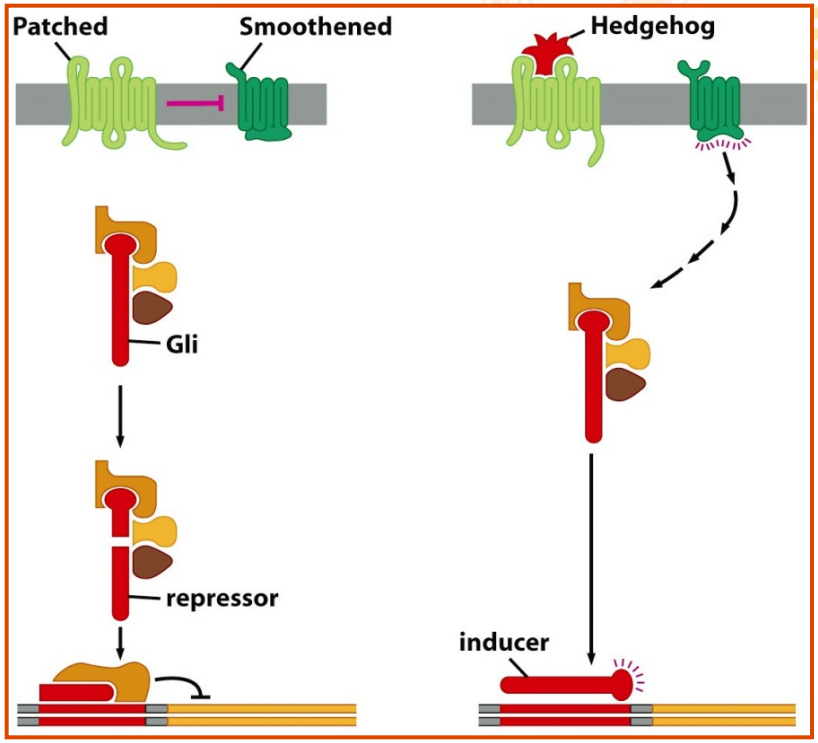
- This pathway is dependent on functional APC.
- **!!** However, most of APC mutations are deletion of the sequence resposible to binding of Axin and Siah-1**!!**

- This pathway represents a crosstalk between **p53** a β-catenin: p53 activates expression of Siah-1 (overexpression of p53 cause downregulation of β-catenin). However, dysregulation of β-catenin is not the key outcome of p53 inactivation.

Hedgehog/Patched/Gli signaling pathway

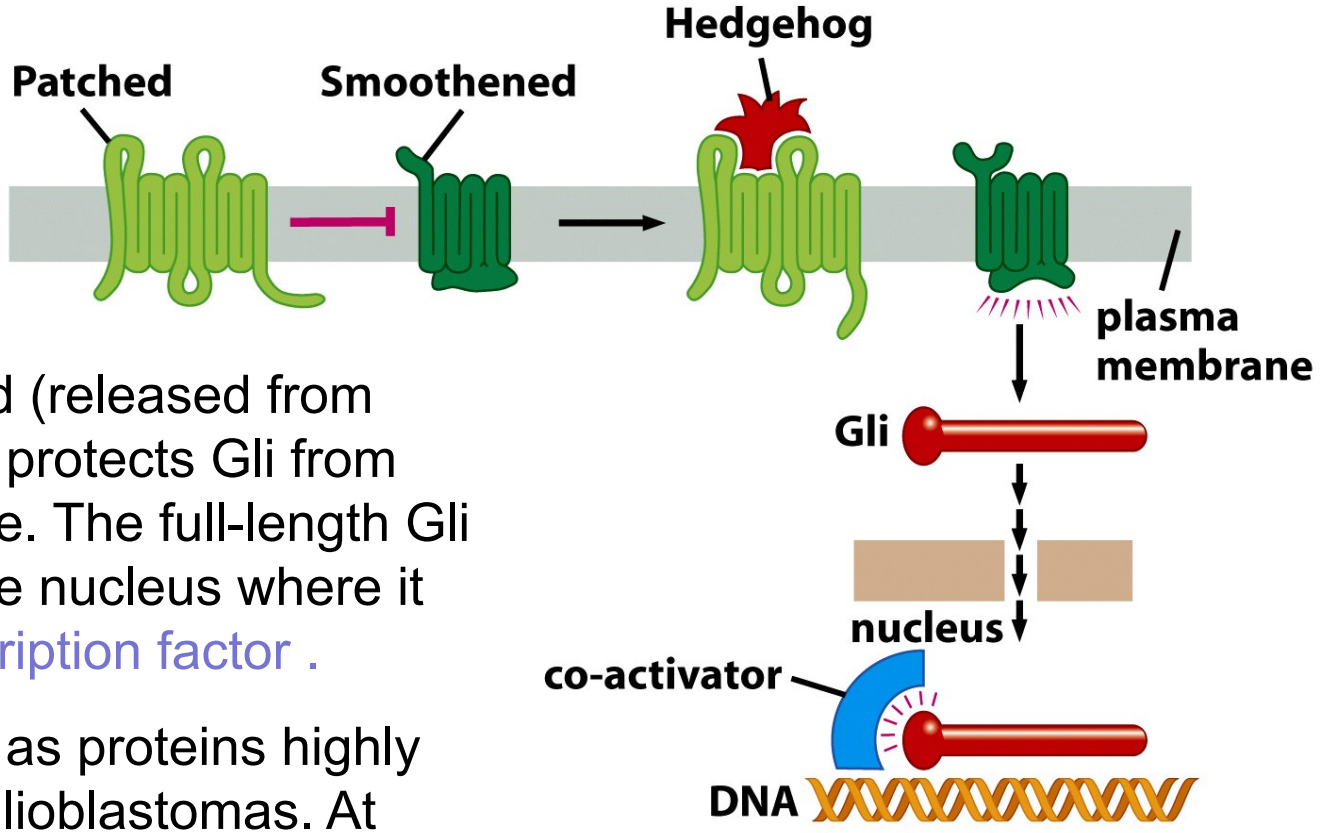
Hedgehog are secreted glycoproteins that interact with surface receptors that transduce signal to TF Gli.

SHH (**S**onic **h**edge**h**og) inhibits receptor **Patched** (**Ptc** − tumor suppressor), that otherwise inhibits receptor complex **Smoothened** (**Smo** - oncogene). Inhibition of Ptc thereby activates Smo and that in turn activates TF **Gli**.



Normally SHH/Gli is inactive, activation tightly controlled at spacio-temporal level (mediate essential tissue-patterning events during embryonic development).
3 types of Hedgehog proteins in mammals: Sonic (SHH), Desert, Indian.

Signaling pathway Patched



Active Smoothened (released from Patched inhibition) protects Gli from proteolytic cleavage. The full-length Gli is transported to the nucleus where it functions as transcription factor .

Gli first discovered as proteins highly overexpressed in glioblastomas. At high levels function as oncoproteins.

Transcription factors Gli

- **Gli** are long (around 1000 AA), versatile TF
- **Gli1**, **2** and **3** − have distinct biological properties, they are partially redundant, and probably function context-dependently

- Present in nucleus and in cytosol

➢ In absence of SHH, transcription factors Gli are cleaved and C-terminal carboxyl fragment is transported to nucleus, where it functions as a dominant-negative **transcription repressor** (expression of HH targets OFF)

➢ If SHH signal is present (Smoothened activated), the production of the repressor is inhibited and full-length protein functions as **transactivator** (expression of HH targets ON)

Hedgehog/Patched/Gli and cancer

- Loss of function (**inactivation**) of SHH-Gli causes **developmental defects** including holoprosencephaly

- pathological **activation** of the pathway may cause **cancer** development, both sporadic and hereditary

- 40 % of sporadic basal cell carcinomas carry **mutations of PTCH** or **SMO**; mutations of PTCH are frequent also in meduloblastomas, meningiomas, breast and esophageal carcinomas

- In esophagus, stomach, bile duct and pancreatic cancer is often found **high expression** of **Patched** receptor and **ligands** (Sonic Hedgehog, Indian Hedgehog); resulting in high levels of full-length Gli in nuclei of cancer cells

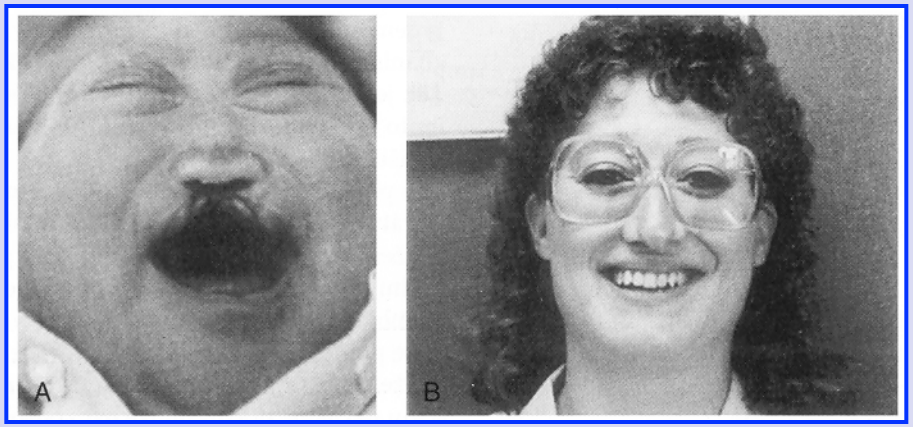
Basal cell carcinomas

- The most common form of skin cancer
- Grow usually very slowly and rarely metastasize, but belong to malignant tumors
- Often appears as non-healing growth or nodule that may be quite stable over time (removed by surgery), sometimes may spread fast and damage skin (radiotherapy and targeted therapy)
- Most often located at the skin exposed to weather conditions (80% cases face and neck)
- Hereditary form is know as Gorlin syndrome (mutations in the PTCH1 gene)
- Incidence of this tumor increases, there is a causative link between the basal cell carcinoma and indoor tanning (especially at young age)

Intermezzo: Holoprosencephaly

- **SHH** functions as a **morphogen** during the development = paracrine signal with local action that control switching ON/OFF genes and trigger various responses based on its concentration along the gradient (non-uniform distribution of the morphogen in embryo/tissue)
- SHH forms gradient by diffussion, and different local concentrations determine different fates of affected cells
- H**oloprosencephaly** is caused by **inactivating mutations of SHH** (dominant inheritance – 50% downregulation of gene expression is enough to cause the defect) – the embryonic forebrain fails to sufficiently divide into the double lobes of the cerebral hemispheres − skull and facial malformations
- Symptoms of holoprosencephaly range from mild (no facial/ organ defects, or only a single central incisor) to moderate to severe (cyclopia).

Holoprosencephaly



Variable expressivity of SHH mutation: mother and her daughter:

A: daughter with severe microcephaly, hypotelorism, cleft lip

B: mother – only a single central incisor

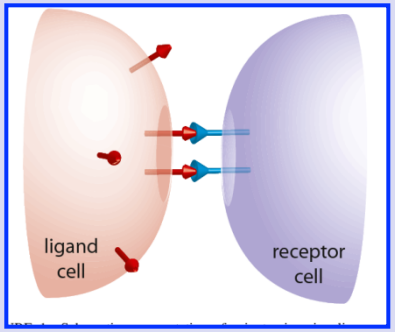
Notch signaling pathway

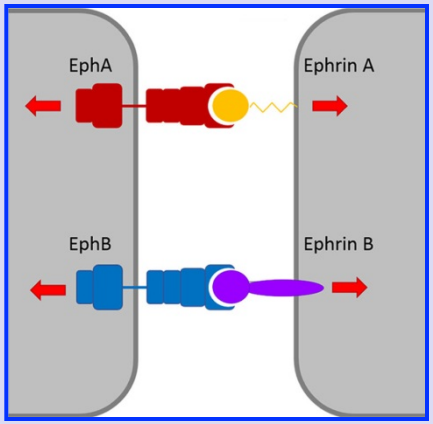
- plays a major role in the regulation of embryonic development. (neurogenesis, angiogenesis, etc)
- (1919 – noticed the appearance of a notch in the wings of the fruit fly *Drosophila melanogaster*)

- **Notch** − transmembrane receptors
- 4 variants encoded by 4 different genes in mammals genome: *NOTCH1, 2, 3, 4*.
- Ligands are **Notch L**, **Delta**, **Jagged1**, **Jagged2** (also membrane proteins)
- Signal is triggered by interaction between ligand and receptor on neighbouring cells (endothelial – cancer – stromal cells → tumor is complex tissue) − importnant for cell-cell communitation
- Example of juxtacrine signaling

Juxtacrine signaling

- signaling between neighbouring cells: „***contact-dependent***“



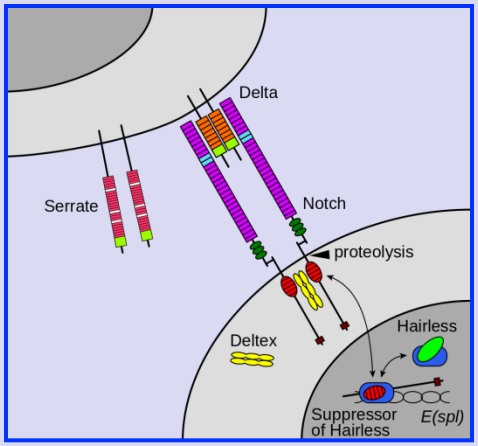


➢ Ephrins and receptors EphB

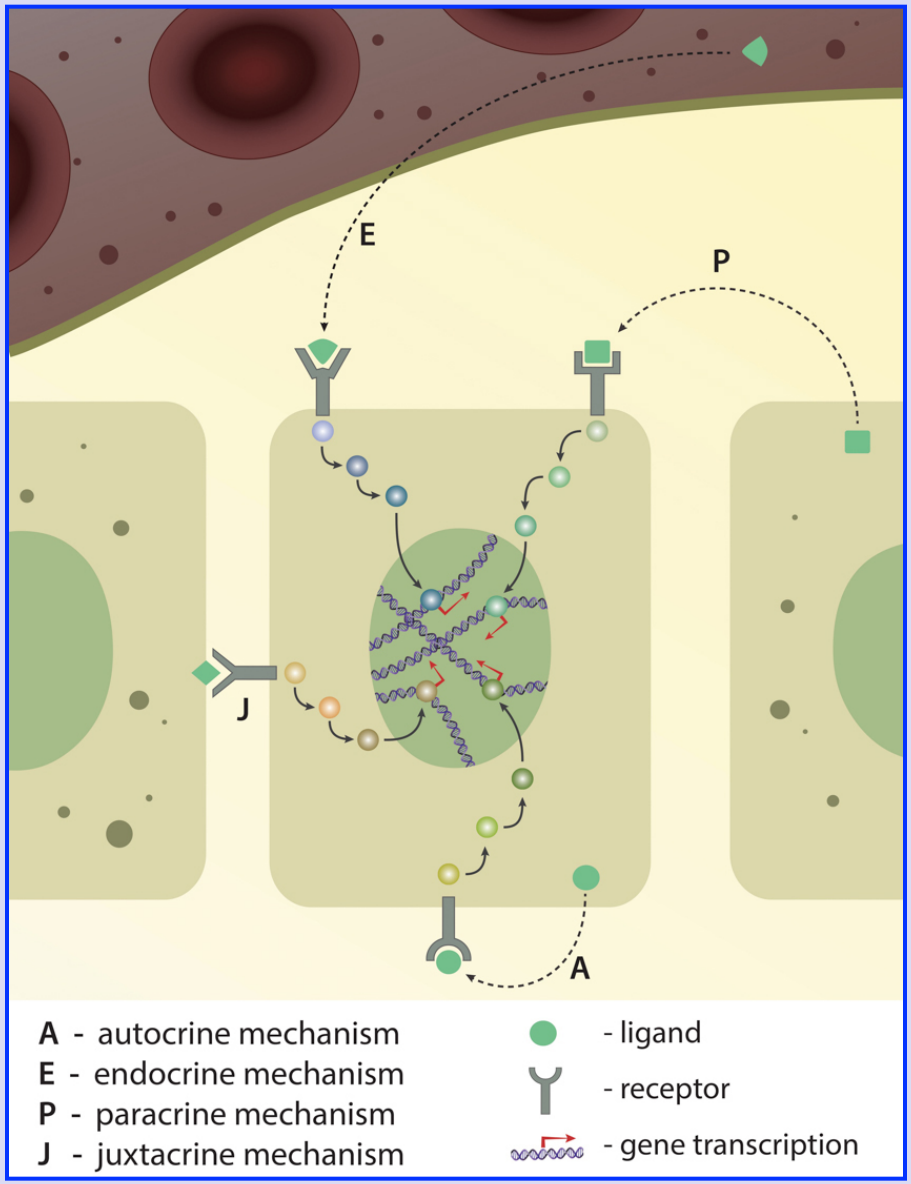
- Ephrins – unlike most of ligands must be <u>bound to the cell surface</u> to activate their receptors

➢ NOTCH signaling pathway

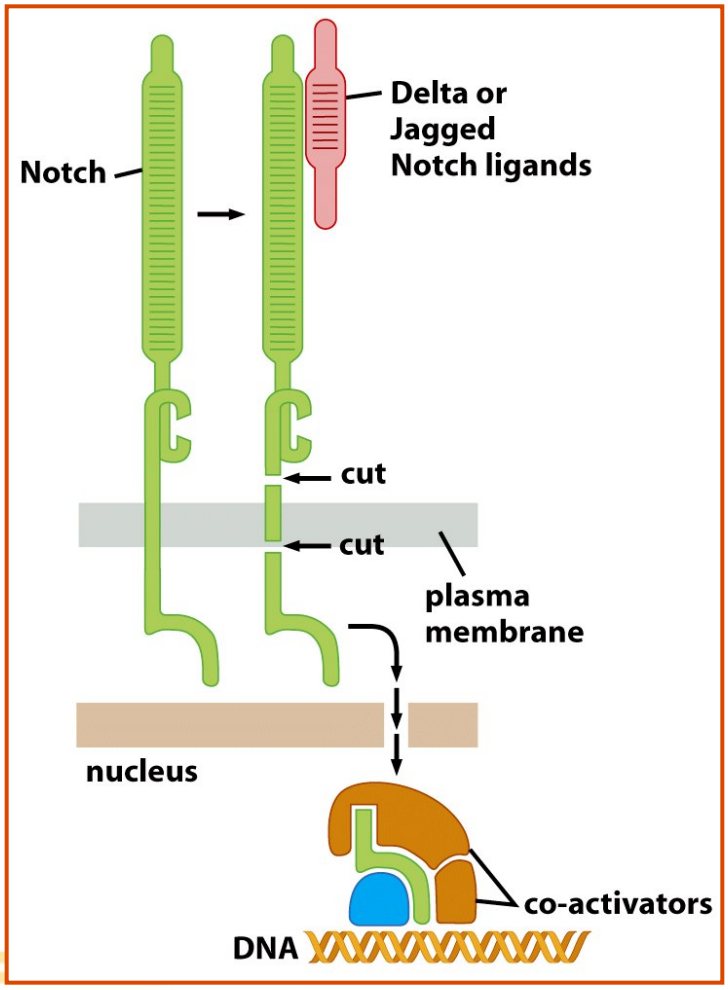
- Initiated by interaction of Notch L and Notch on **neighbouring cells** (e.g. Endothelial cell – cancer cell – stromal cell)



Juxtacrinne signaling



Notch signaling pathway



- Upon interaction with ligand (Notch L, Delta, Jagged) Notch receptor is twice proteolytically cleaved : 1x in extracellular domain, 1x in transmembrane domain

- Cytoplasmatic fragment is thus released from membrane and migrates into nucleus where it functions (in complex with other proteins) as a transcription factor

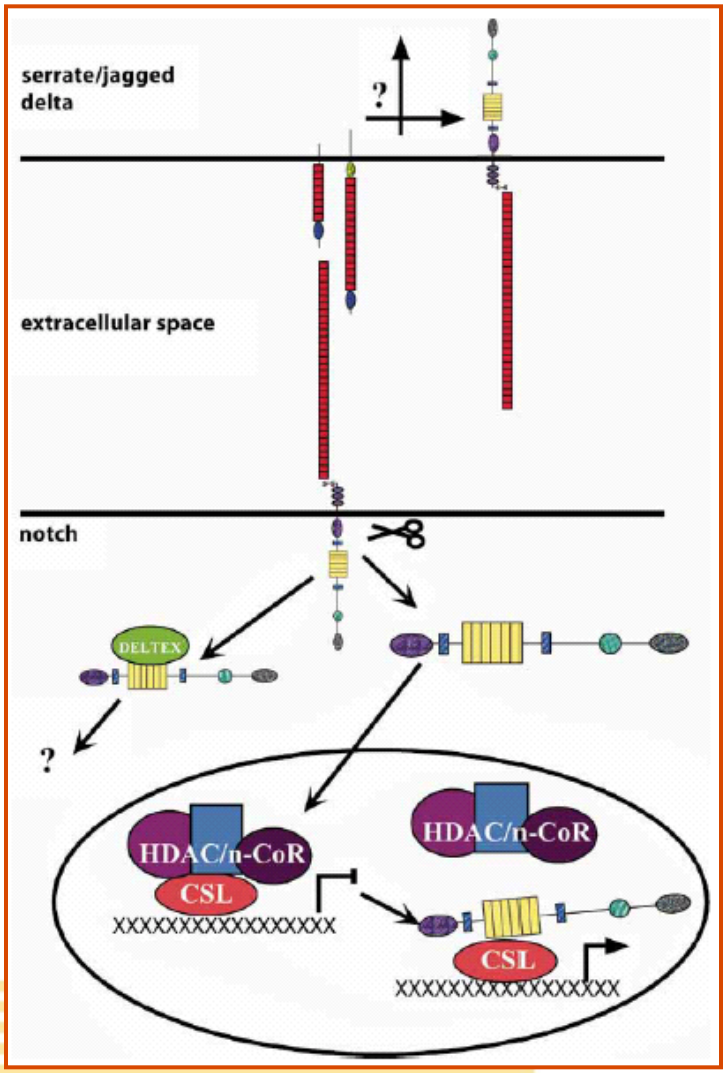
Notch receptors

* Notch receptors have different mode of function compared to RTK:

- Receptor in complex with ligand is **irreversibly** changed (proteolytic cleavage) ⇒ each molecule of receptor signals **only 1x**

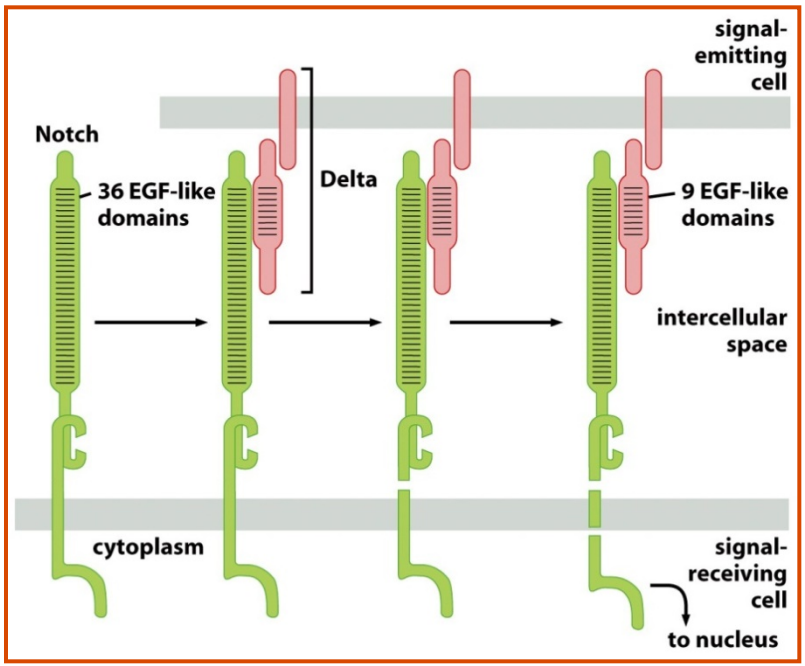
- Notch receptors **cannot amplify signal** (unlike RTK)

NOTCH signaling pathway



Cell expressing ligand emits the signal − cell expressing receptor receives the signal

⇒ **Tumors are complex tissues**



Notch signaling pathway and cancer

- In most cervical cancers and some colorectal and lung cancer there is **overexpression of Notch**, associated with its **nuclear localization**

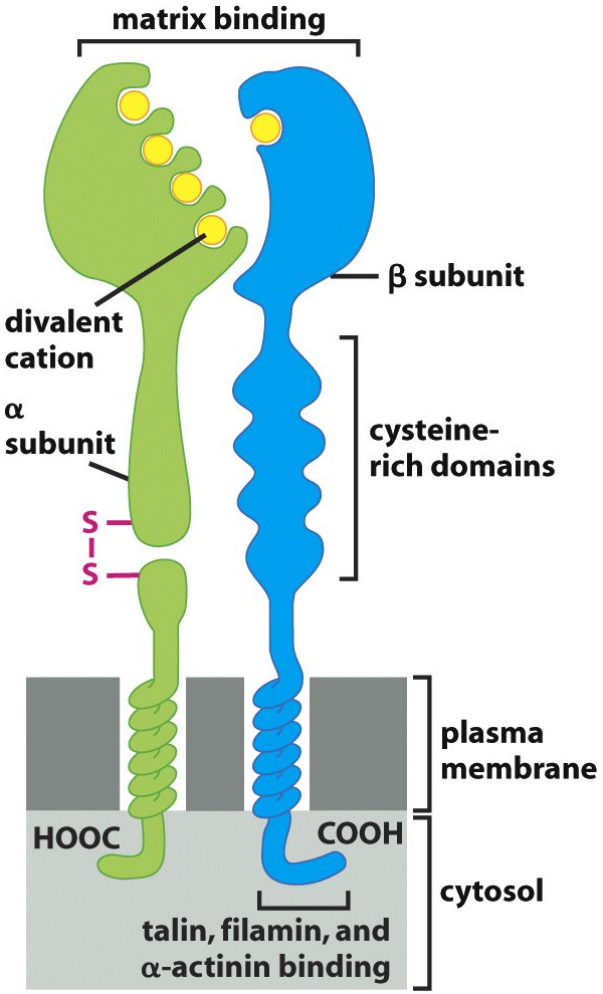
- **Overexpression of ligands** (Notch L, Jagged and Delta) detected in cervival and prostate carcinomas

- 10 % of ALL have constitutively active Notch because of a **deletion** of part of *NOTCH-1* gene encoding **extracellular domain of the receptor**

Integrins

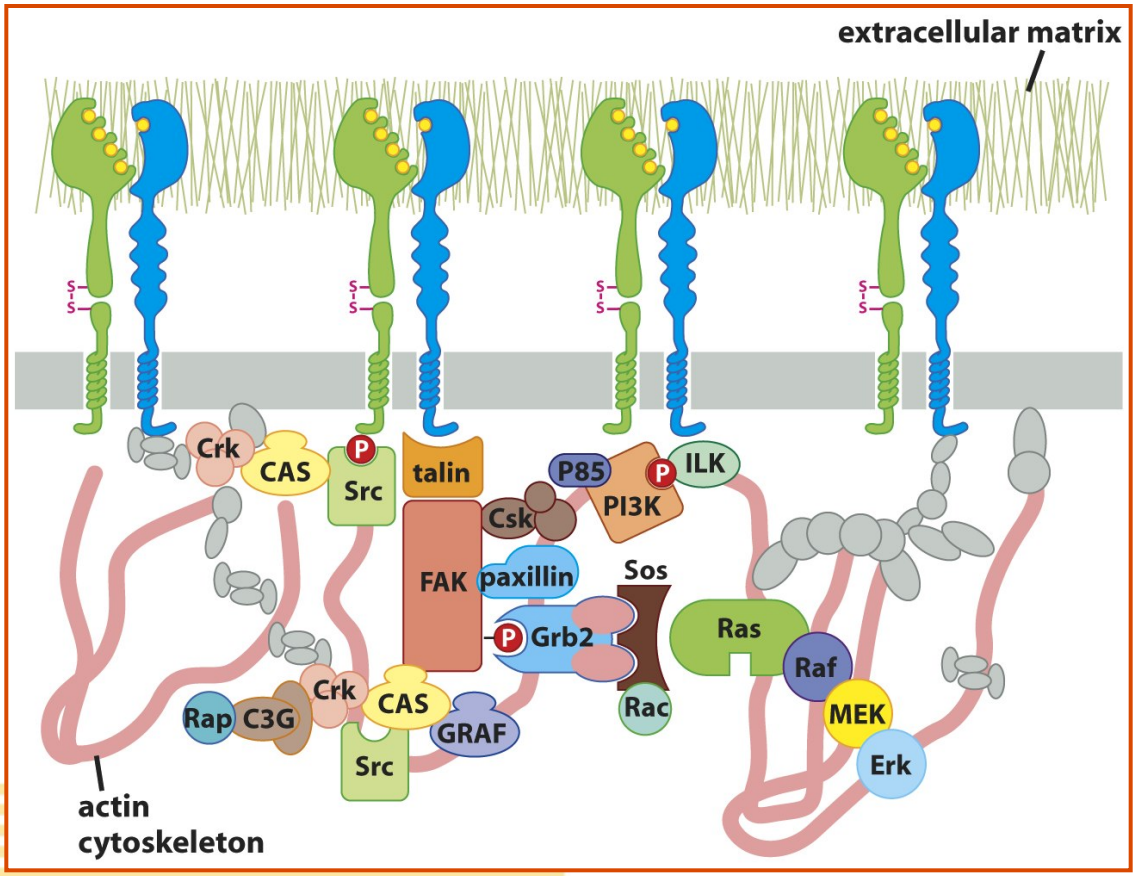
Integrins are surface receptors mediating:

- (1) cell-cell and cell-extracellular matrix adhesion
- (2) pro-survival and proliferation stimulating signaling
- **Heterodimers** formed by non-covalently associated subunits **alfa** and **beta**; 18 subunits alfa and 8 subunits beta identified, may form 24 different heterodimers with different specificity
- Subunits consist of size extensive extracellular domain (binding of ligand), short transmembrane and variable cytoplasmatic C-terminal domains



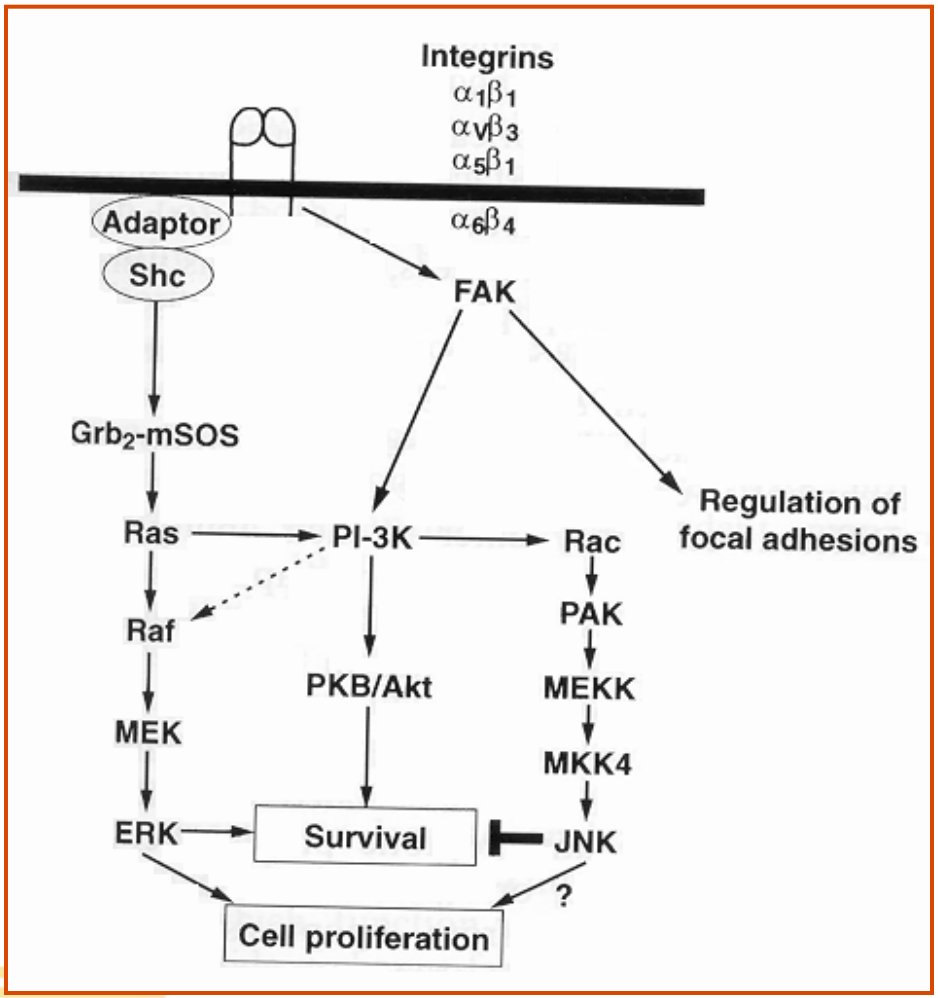
Integrins' functions

Cells continuously monitor their attachement to ECM (if it is lost - **anoikis**).



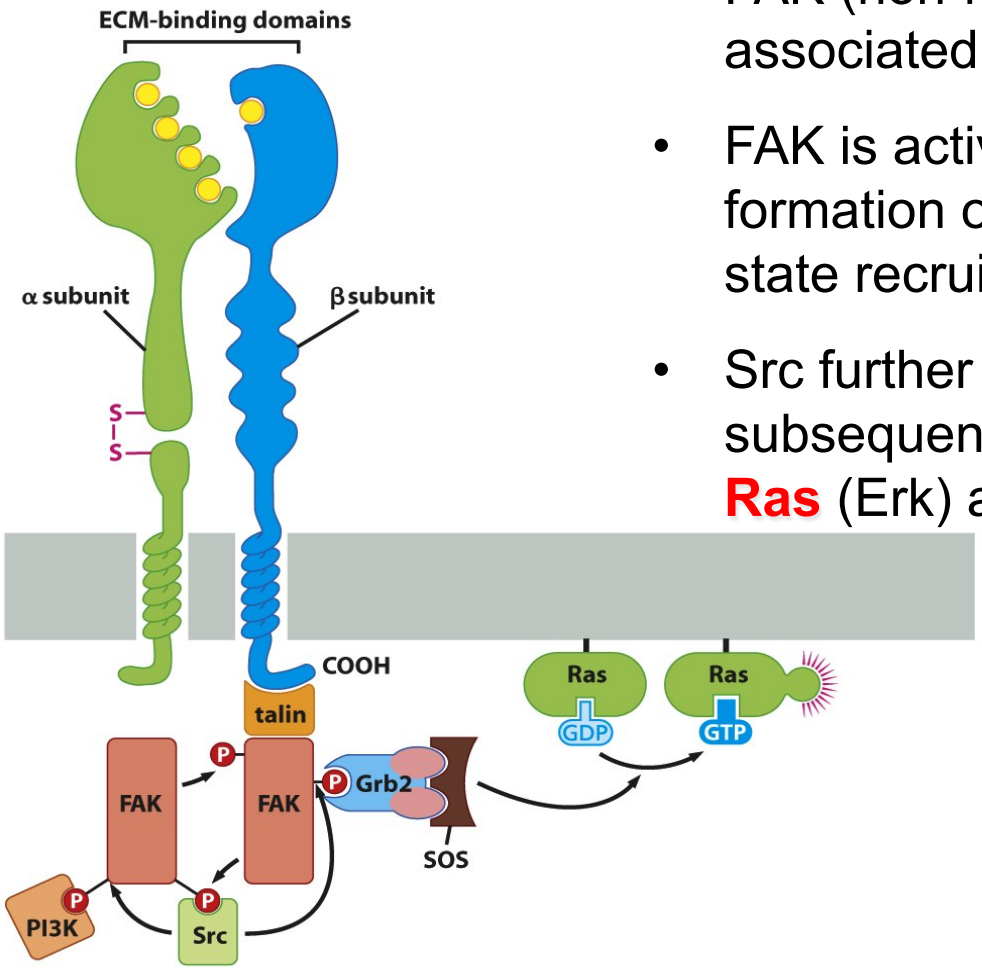
- Interact with ECM
- Modulate cytoskeleton
- Signal outside−in (cellular responses of the integrin expressing recipient cell − spreading, retraction, migration, etc)
- Signal inside-out (signaling activates the ligand binding function of integrins − bind different ECM components)
- Enable cell movement

Integrins



- Crucial aspect of integrin function is ability to activate **FAK** - „focal adhesion kinase“.
- Integrins (bound to ECM) form clusters and thereby so called focal adhesion. These activate FAK.
- FAK is involved in signaling towards cell adhesion, migration, spreading.

Integrins‘ signaling pathway



- FAK (non-receptor tyrosine kinase) is associated with β subunit of integrin
- FAK is activated by phosphorylation (after formation of focal adhesion) and in this state recruits **Src**
- Src further phosphorylates FAK and subsequently other substrates including **Ras** (Erk) and **PI3K**

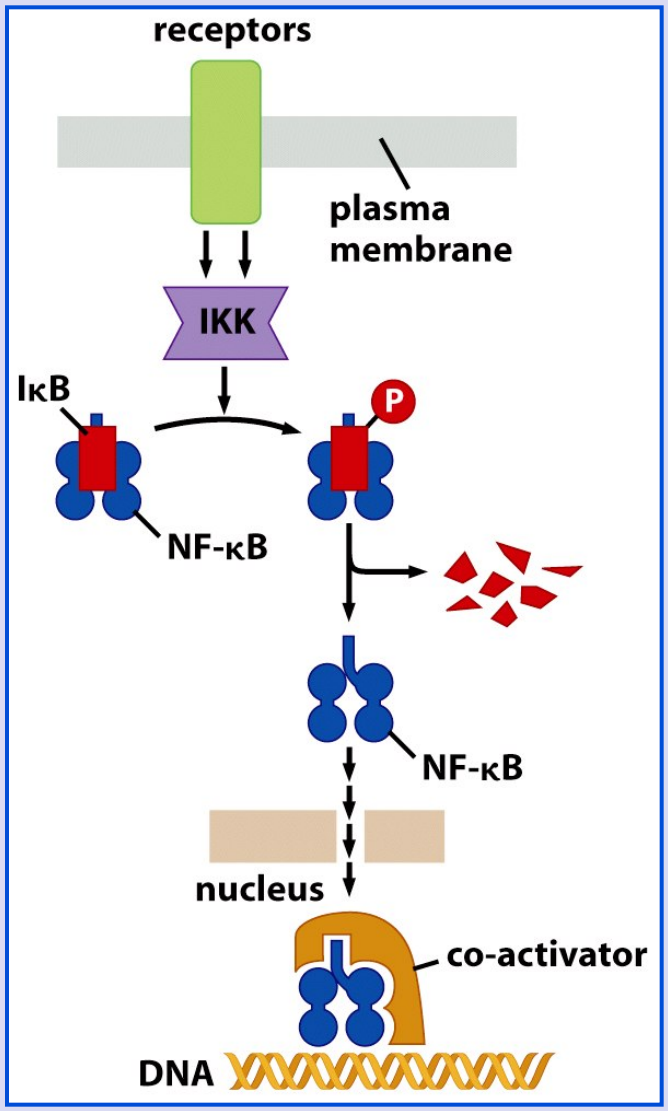
ECM → integrins → Sos → Ras → Erk

- Knowledge about the functional cross-talk between these signaling molecules helped to elucidate significant aspect of **Ras** onco-protein transforming capacity: it enables cell survival without attachment to ECM. Normally survival is dependent on signals transduced from integrins to Ras. Without this signaling cell cannot proliferate and programmed cell death (anoikis) is induced
- Oncogenic Ras activation thus mimics the situation where cell receive information about its successful anchorage in ECM. Even without the anchorage.
- Ability to evade anoikis seems to be a key for development of some cancers, e.g. breast (and also critical for metastasis)

NF-κB signaling pathway

- First link between NF-kB and cancer: discovery of the **v-*rel*** oncogene in avian acute transforming virus causing reticuloendotheliosis (B-cell lymphoma).
- **Rel** belongs to NF-κB family.
- Members of NF-kB family form in cytosol homodimers and heterodimers. Most common is dimer **p65-p50**.
- Dimers are kept in cytosol bound to protein **IκB**. In this state pathway is inactive.
- In response to various signals IκB is phosphorylated and degraded and NF-κB is released ad translocated to nucleus. It functions as TF with more than 150 target genes.
- Kinase phosphorylating IκB (**IKK = IκB kinase**) is induced e.g. by TNF-α, interleukin-1β, LPS, reactive oxygen species (ROS), chemotherapy,…

NF-κB signaling pathway



Target genes of NF-κB:

- Anti-apoptotic: e.g. ***bcl*-2**, **IAP-1**, **IAP-2** and others.
- Proliferation-stimulating: e.g. ***myc***, **cyclin D1** and others.

- **NF-κB is critical for regulation of inflammation!**

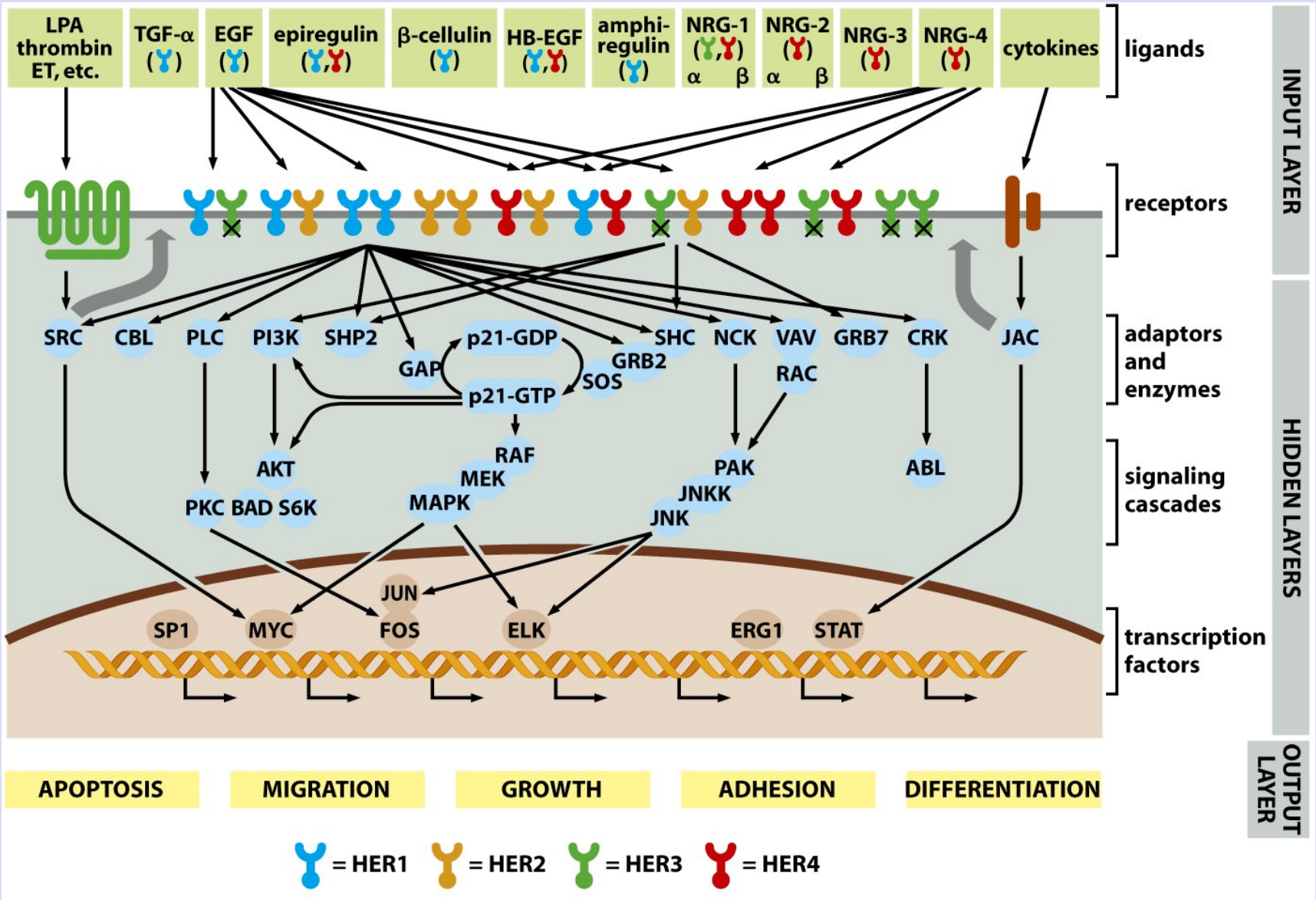
NF-κB and cancer

- Mutations of individual components of NF-κB pathway are very rare, but frequently **constitutive activation** of the NF-κB pathway is seen, e.g. in breast cancer.

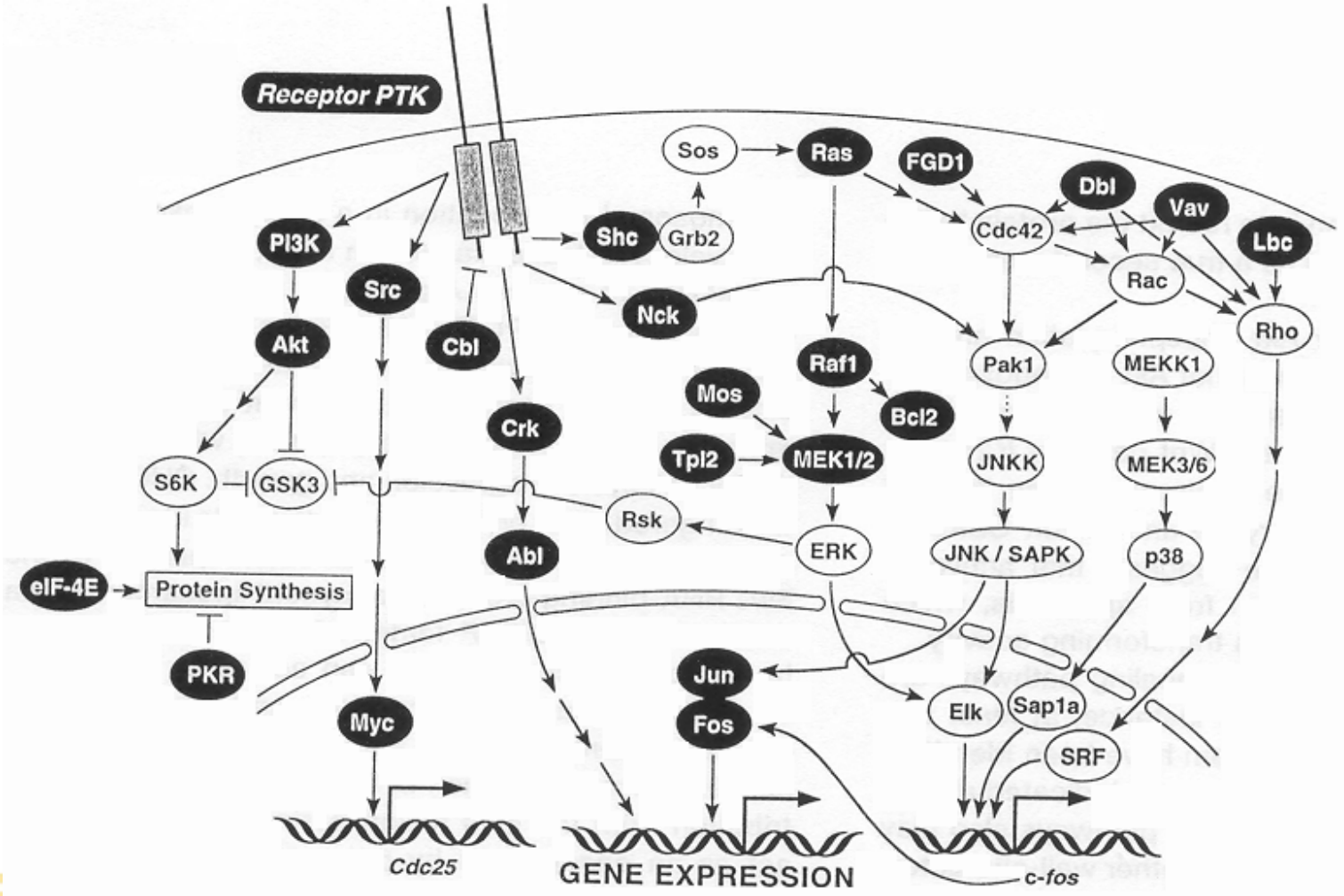
Most significant is NF-kB pathway in lymphomas:

- Approx 1/4 of DLBCL (Diffuse large B-cell lymphoma) have **amplification of *REL* gene**, leading to 4x to 35x increase in Rel protein levels
- **translocations affecting *NFKB2*** gene are common in B- and T-cell lymphomas and myelomas

Signaling networks…



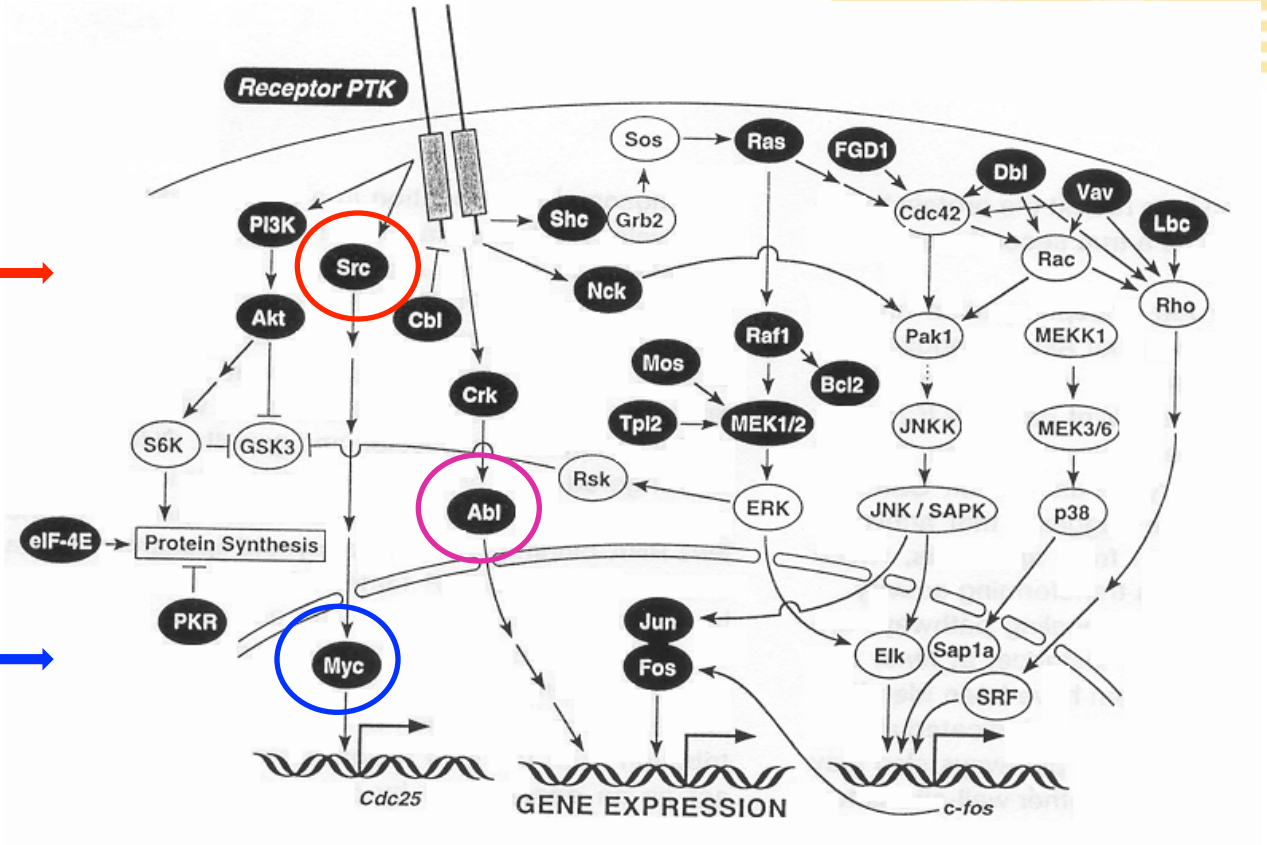
Signaling networks…



Signaling networks…

Cytosolic transducer

Transcription factor

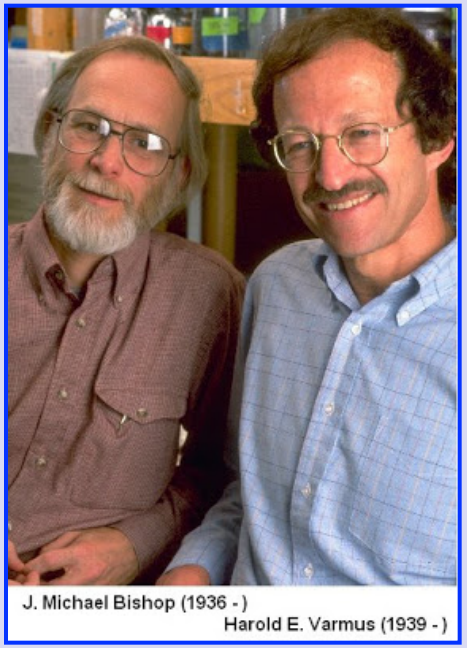


Protooncoprotein Src - pp60$^{c\text{-}src}$

- **first** described cellular counterpart of viral oncoprotein v-Scr. v-Src is a gene found in **Rous** sarcoma **virus** (RSV) that causes a type of cancer in chickens (1909 - Peyton Rous) - pp60$^{v\text{-}src}$;
- One of the first identified proto-oncogenes, but no sooner than 1999 (more than 25 years after its discovery) mutant forms identified (in 12% of CRC)
- Non-receptor (cytoplasmatic) tyrosine proteinkinase, signal transducer
- Involved in several cellular processes − proliferation, differentiation, movement, adhesion
- Normally in inactive state, may be transiently activated e.g. during mitosis
- Associated with membranes, mostly with cytoplasmatic membrane and endosomal membranes
- Src family has many members: **Fyn**, **Yes**, **Lck**, **Hck**, **Blk**, **Fgr**, **Lyn**, **Yrk**.

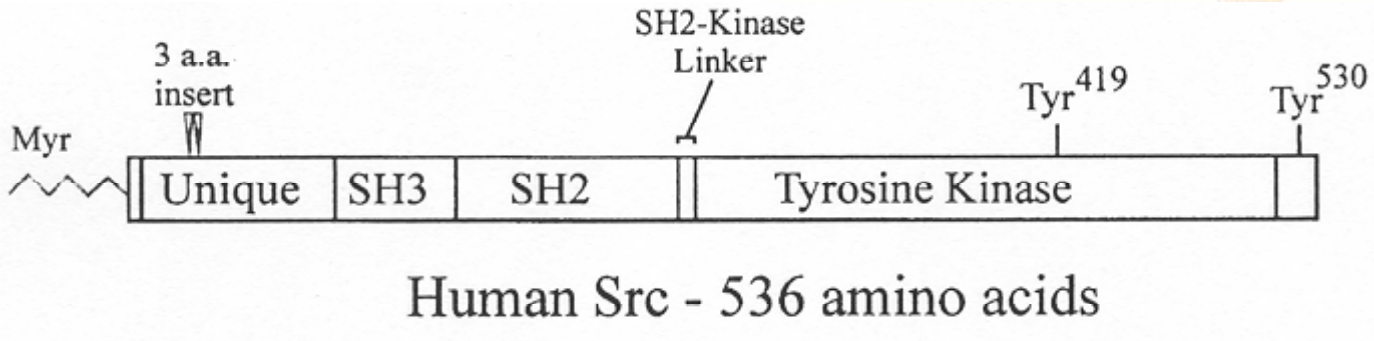
Discovery of c-*src* protooncogene

- 1979: J. Michael **Bishop** and Harold E. **Varmus** discovered that chicken genome contains a gene structurally similar to v-scr oncogene
- Denoted as cellular – c-*src*
- 1989 – Nobel prize



J. Michael Bishop (1936 -)
Harold E. Varmus (1939 -)

Structure of c-Src



- c-Src molecule has 536 AA (pp60)
- N-terminus myristoylated
- Central domains SH3, SH2 and SH2 linker
- Two main phosphorylation sites:

  1. **Tyr 530** (527) **negative** regulatory site (at the C-terminus)

  2. **Tyr 419** (416) **positive** autoregulatory site in catalytical domain

Myristoylation

- posttranslalational modification
- the addition of a 14-carbon unsaturated fatty acid, myristic acid, to the N-terminal glycine of a subset of proteins.

  CH_3-$(CH_2)_{12}$-COOH
- This modification enables membrane anchorage of proteins

Regulation of c-Src

Main regulatory site on c-Scr molecule is **Tyr**(**530**/527): If phosphorylated it interacts with SH2 domain of c-Scr and thereby inhibits tyrosine kinase activity of c-Scr.

Tyr(**419**/416) in catalytic domain. Its phosphorylation opens Scr for binding to its substrates

Other regulatory phospho-residues on c-Scr, but less important e.g. Thr34, Thr46, Ser72, etc).

c-Src activation

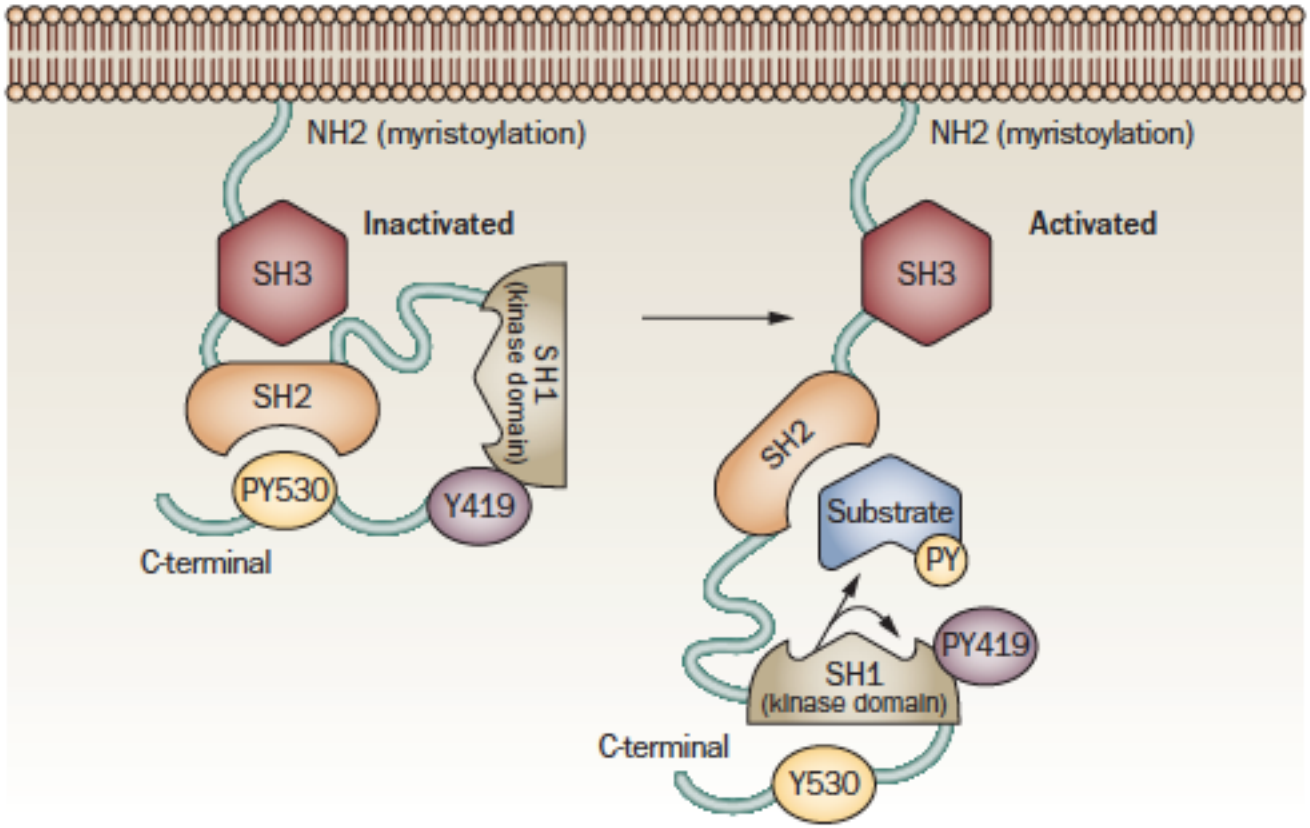
In inactive state (Tyr 530/527 phosphorylated) there is a physical interaction between C-terminus and SH2 domain and also between SH3 domain and SH2 linker, Tyr 419/416 is dephosphorylated.

1. Dephosphorylation of Tyr 530/527 releases C-terminus from SH2 domain and Tyr 419/416 may be auto-phosphorylated and thus activated.

2. Binding of Src (via SH2 domain) to phosphorylated RTK releases C-terminus and allows phosphorylation of Tyr 419/416 and thus activation of Src.

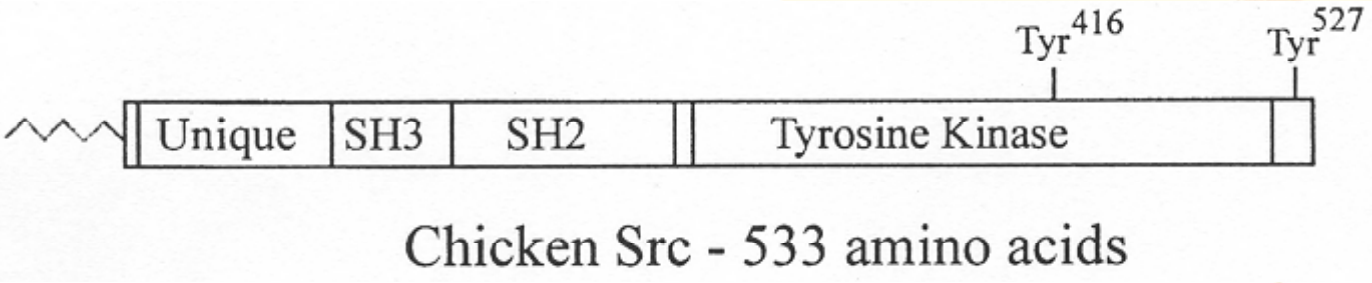
In addition a range of kinases and phosphatases may alter (phopho-)modified Scr

c-Src activation

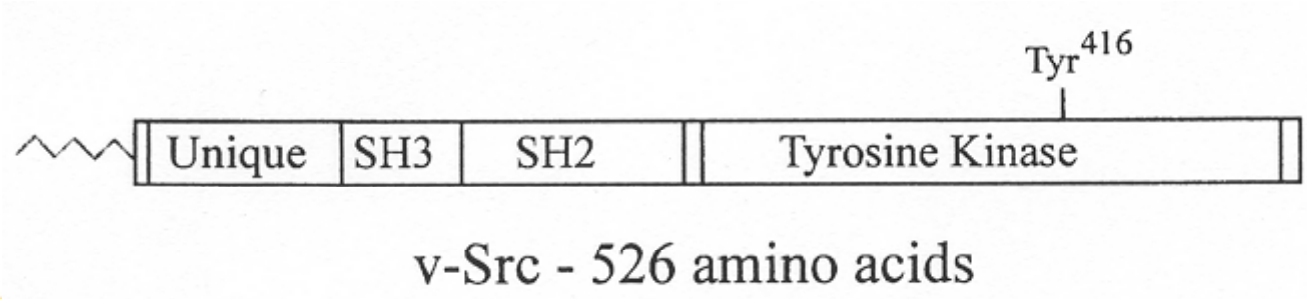


Kim et al. Nat Rev Clin Oncol. 2009;6(10):587-95.

Comparison of chicken c-Src and v-Src of RSV



- Several missense mutations
- 19 AA at the C-terminus of c-Src is replaced by different 12 AA



Regulation of c-Src by subcellular localization

Src is associated with membranes: in part due to the myristoylation at the N-terminus, and also mediated by specific AA sequences at the N-terminus

Localisation affects Src function:

- cytoplasmatic membrane (focal adhesions, cytoskeleton, adherens junctions...) → mitogenic signaling via RTKs and GPCRs; cell adhesion, migration, cell-cell interactions
- cytosol and perinuclear space (Golgi, endosomes, synaptic vesicules,...) → transport of proteins, cell cycle progression
- nucleus → cell cycle regulation

c-Src and cancer

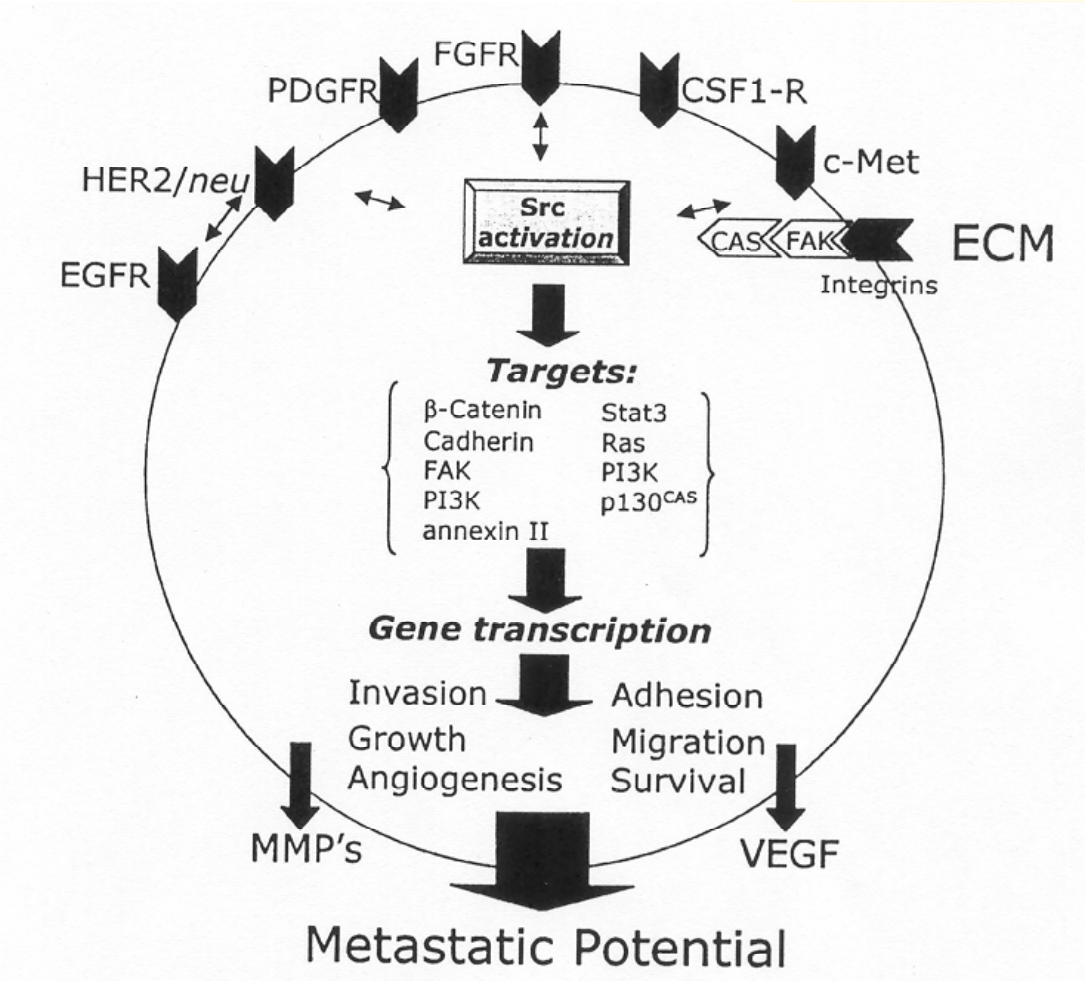
Protein levels and **activity of Src** are **increased** in cancer cells of various origin compared to normal cells and is correlated with degree of malignant progression

Src kinase activity is 4-20x higher in breast neoplastic compared to normal tissues. One potential mechanism is connected with activation of phosphatase removing phosphate group from Tyr530 (Protein Tyrosine Phosphatase Non-Receptor Type 1).

In most colorectal tumors is Src activity enhanced 5-8x, it is early event in cancerogenesis (occurs in premalignant stages) and further increases with tumor progression. (Probably also dictates localization of metastases.)

truncating mutation in Src at codon 531 in some advanced human colon cancer

Role of Src in cancerogenesis



Proto-oncoprotein ABL

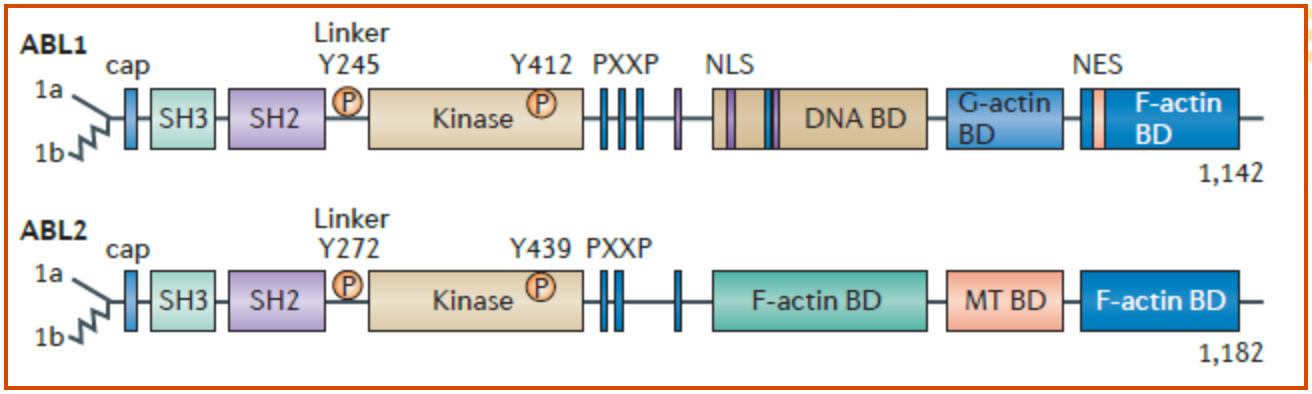
- 1980: discovered viral oncogene (**v-*abl***) in Abelson murine leukemia **virus**
- Ubiquitous **non-receptor tyrosine kinase**
- human (mammals): 2 genes *ABL1* and *ABL2* (more isoforms), approx 90% homology of N-terminus

➢ Integrate different extracellular and intracellular signals and activate pathways regulating cell growth, survival, invasion, adhesion and migration

➢ some specialized functions of ABL: signaling downstream antigen receptors in lymphocytes, formation of neural synapses, adhesion of microbiota to intestinal mucosa

➢ ⇒ many tissue-specific and context-dependent functions

Structure of ABL protein



N-terminus (kinase assembly):

- SH3 and SH2 domains
- kinase domain

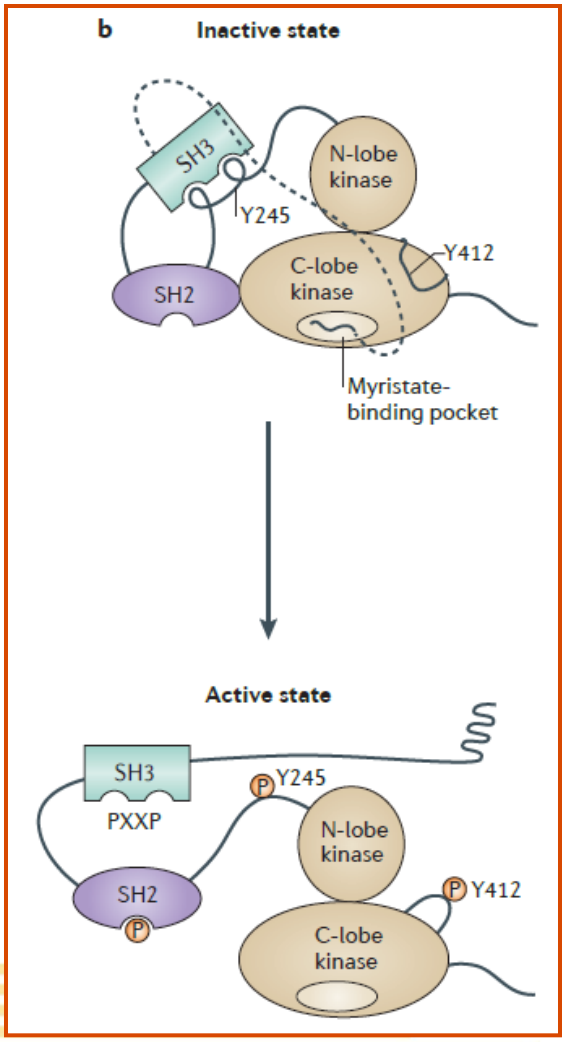
C-terminus (location cues):

- Actin-binding domain (ABD)
- 3 NLS – nuclear import (only ABL1; ABL2 is in cytosol)
- NES – nuclear export (via binding to exportin-1)

→ *shuttling*

Greuber EK et al, *Nat Rev Cancer* 13: 559-71, 2013

Regulation of ABL

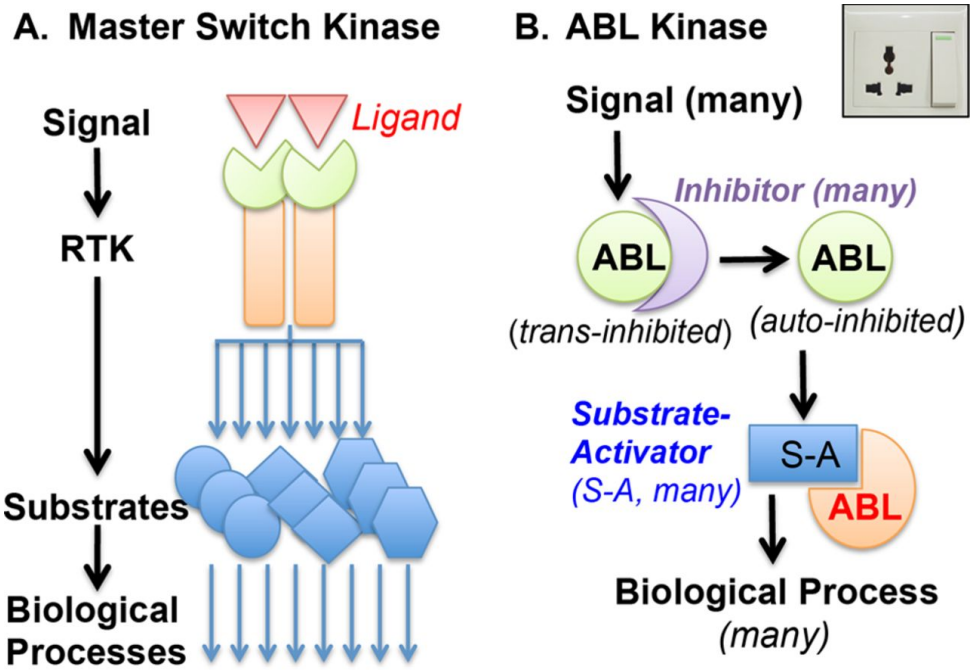


Catalytic activity is regulated by:

- Intra-molecular interactions: autoinhibition involving SH2 and SH3 domains (SH3 bound to internal PXXP motif), locking kinase in an inactive conformation
- Inter-molecular interactions: Activating interactions with substrates of Abl: works as allosteric activators (binding to SH3 and SH2), e.g. RIN1. *Vice versa* trans-inhibitors, such as Rb, F-actin)
- posttranslational modification (phosphorylation of Tyrosines $412^{ABL1}/439^{ABL2}$ and $245^{ABL1}/272^{ABL2}$ – prevent autoinhibiton via disruption of SH3-PXXP motif interaction − increased kinase activity)

Greuber EK et al, *Nat Rev Cancer* 13: 559-71, 2013

Functions of ABL



ABL kinase (B):

- Activated by **many different signals** both external and internal (DNA damage, stress,..)
- each signal may activate only a fraction of cellular ABL pool and phosphorylate specific substrates

„Classical“ master switch kinases (A):

- RTKs: after interaction with specific ligand (external) autophosphorylation and phosphorylation of other target intracellular proteins
- Activation via cAMP or PIP3

Wang JYJ. *Mol Cell Biol*34: 1188-1197, 2014

Functions of ABL

- ABL proteins *shuttle* between nucleus and cytosol
- A wide range of ABL substrates: both nuclear (regulating transcription, DNA repair and chromatin remodelling), and cytosolic (regulating actin polymerization and biologic functions related to cytoskeleton)

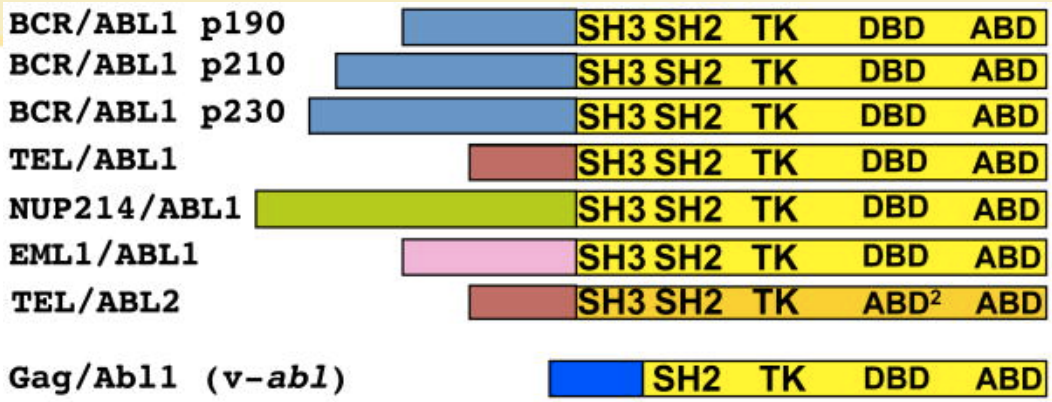
- activated ABL regulate function/formation of invadopodias (actin-rich protrusions of the plasma membrane that are associated with degradation of the extracellular matrix during cancer invasion and metastasis), epithelial polarity

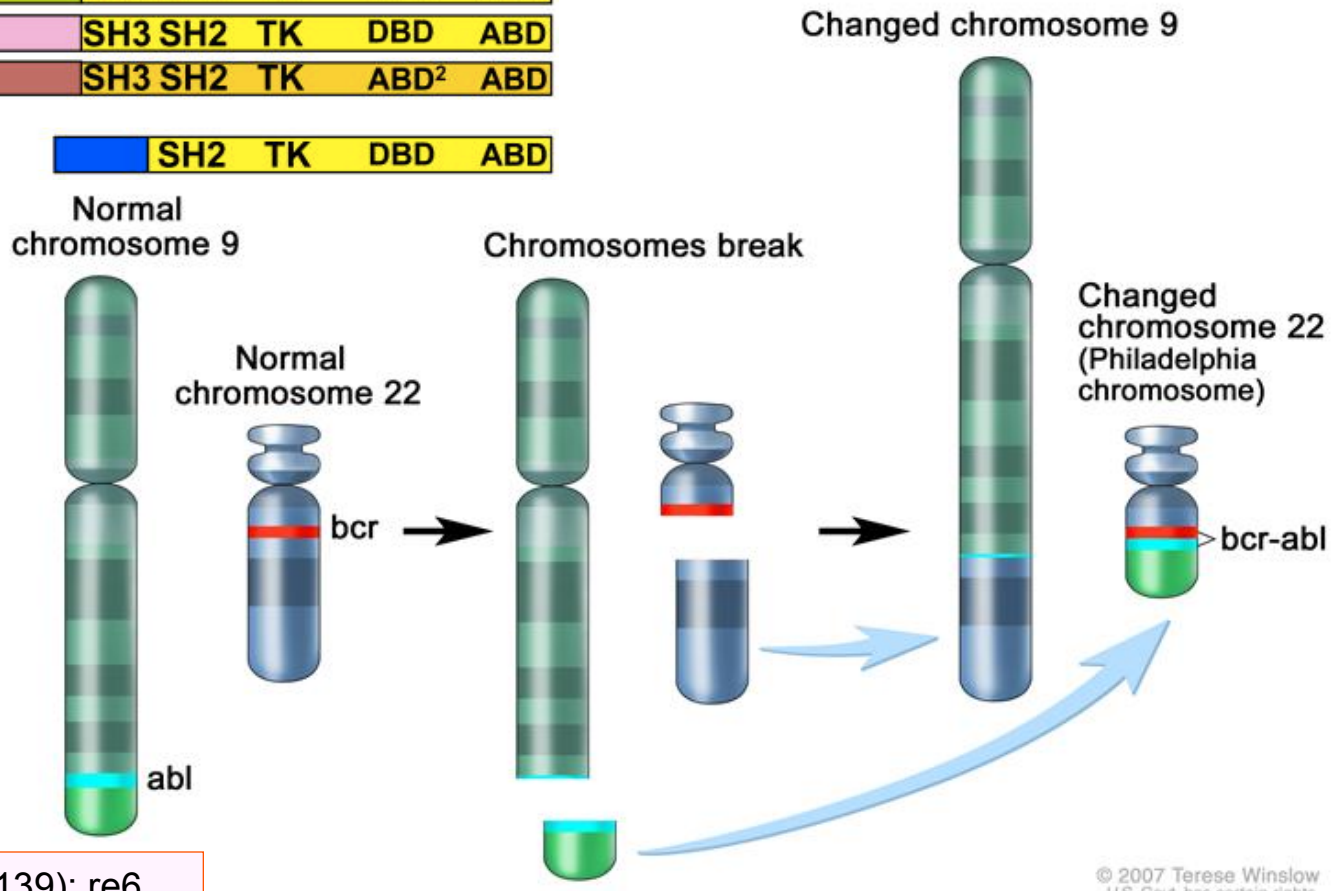
Wang JYJ. *Mol Cell Biol*34: 1188-1197, 2014

Oncoprotein BCR/ABL

- 1986: **Philadelphia chromosome:** result of reciprocal translocation t(9;22) − proto-oncogene ***ABL*** (9q34.12) fused with gene ***BCR*** (22q11.21, breakpoint cluster region).
- ABL breakpoint position consistently leads to removal of the amino-terminal Cap peptide. N-terminal sequences normally stabilizes inactive conformation
- chimeric protein (p190 −ALL or p210 CML) is strong oncoprotein, constitutively active Abl with reduced nuclear localization
- Philadelphia chromosome is present in 95% of pacients with chronic myeloid leukemia **CML** and some (i.e. Ph^+) ALL.
- BCR-ABL protein is sensitive to inhibition by TKIs, e.g. **imatinib** (Gleevec), dasatinib, nilotinib; Imatinib: first and most successful therapy with TKIs!!
- aberant ABL also in solid tumors, (mostly amplifications and/or overexpression, mainly ABL2), rather rare

Philadelphia chromosome





Colicelli *Sci Signal.* 2010; 3(139): re6.

Proto-oncoprotein Myc

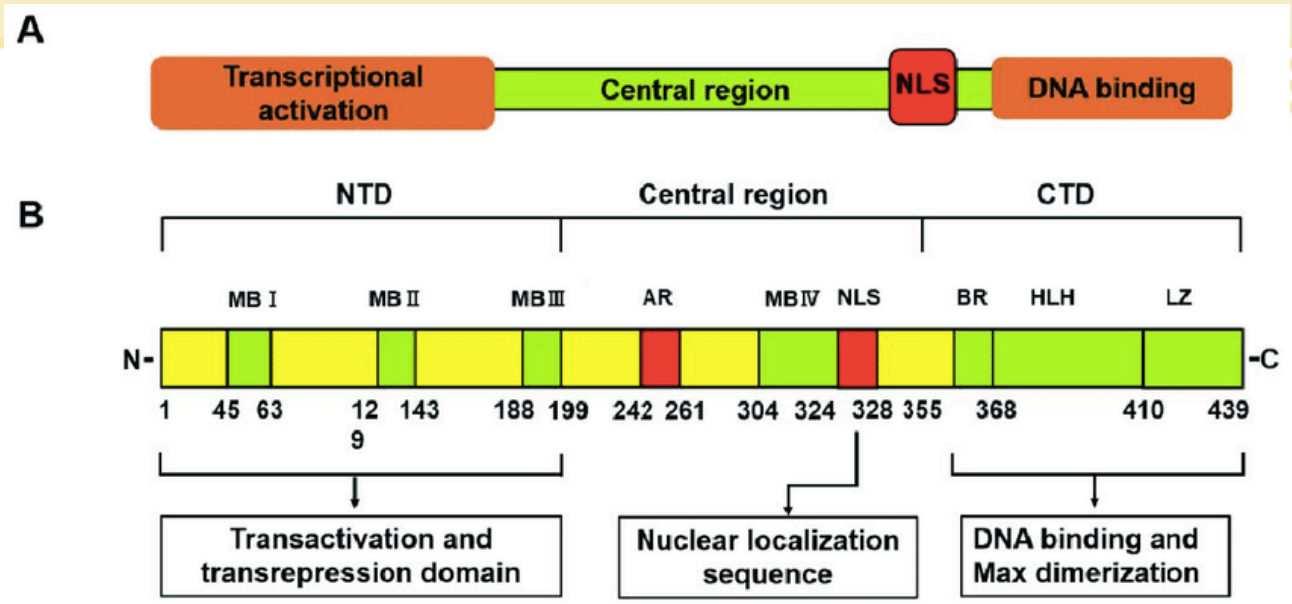
- Originally described as cellular counterpart of v-*myc* transduced by avian myelocytomatosis virus
- Myc protein family consists of 3 members (all involved in cancerogenesis): **c-Myc**, **N-Myc** and **L-Myc**

- Myc proteins are nuclear phosphoproteins (430 AA) functioning as transcription factors

- Myc proteins form homodimers and heterodimers
- In promoters of target genes they recognize **E boxes** (containing sequence CACGTG)

- For biological function of Myc proteins are required highly conserved regions at the N-terminus **MBI** and **MBII** („Myc Boxes I and II“)

Structure of Myc proteins



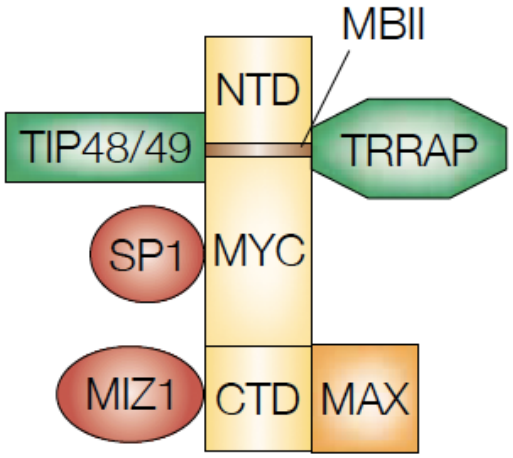
Elbadawy et al. *Int J Mol Sci*. 2019;20(9):2340

- MBI a II – required for transactivation and contain several **phosphorylation sites**
- Mutations of **phosphorylation sites** (mainly threonine 58) increase tranforming potential of Myc, found in some tumors; T58 phosphorylation leads to the proteosomal degradation od Myc ⇒ mutation of T58 increase the protein levels of Myc

Myc/Max transactivator

- **Myc** forms heterodimers with protein **Max**. Together they function as TF and activate transcription of their target genes (involved in proliferation).
- Myc protein levels are increased in response to mitogenic signals, while Max protein levels are relatively constant

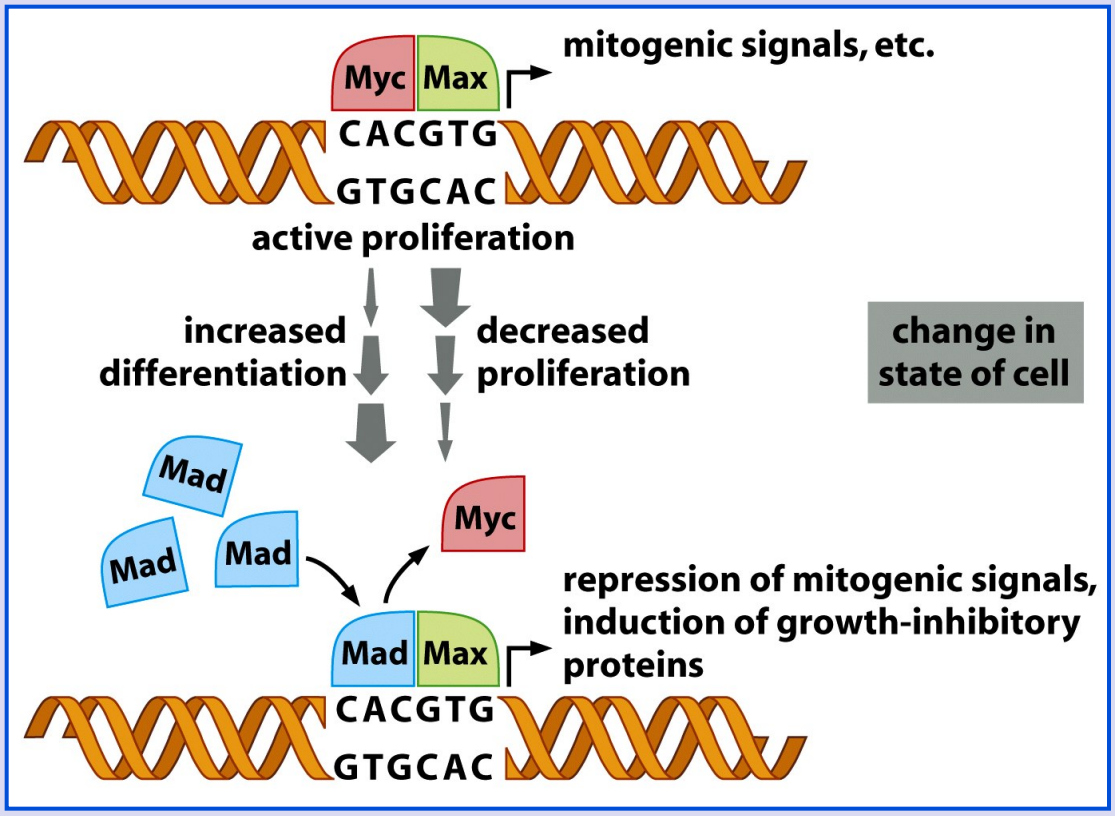
- *Transactivation of target genes by Myc/Max dimers is regulated by other cofactors that bind N-terminal sequences of Myc;* e.g. *TRRAP mediates interaction of Myc with HAT (histon acetyl transferases) – nucleosomal remodelling - transactivation*



Pellegaris. *Nat Rev Cancer*. 2002;2(10):764-76.

Max/Mad

- Protein **Mad** competes with Myc for binding to Max. Mad/Max form repressor, negatively regulating transcription of target genes.
- *Mad proteins not only replace Myc from dimer, but also recruit **Sin3/HDAC** complex to promoters and thus induce chromatin remodelling to repress transcription.*



- With decresed proliferation there is downregulation of Myc and upregulation of Mad.

Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Myc as transactivator

Target genes of Myc/Max dimer are involved in control of cell proliferation :

- **cyclin D2** (***CCND2***)
- **CDK4**
- **Cul1**, that is responsible for **$p27^{Kip1}$** degradation

- **E2F1**, **E2F2** and **E2F3**

- Myc/Max promotes telomerase activity by transactivation of catalytic subunit **hTERT**

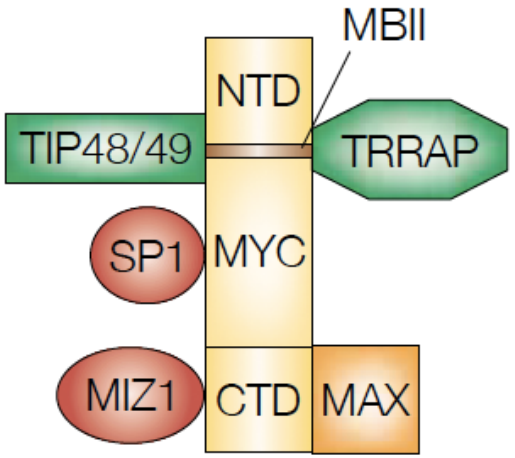
- Myc/Max induces transcription of **Bim**; Bim binds and inactivates Bcl-2 ($\Rightarrow$ wt Myc induces apoptosis)

Myc as repressor

C-terminus of Myc is a binding site for other proteins such as TF **Miz-1**.

Miz-1 in complex with Myc/Max cannot activate its target genes:

- Genes involved in negative regulation of cell cycle **p15^{INK4B}** and **p21^{WAF1}**
- **Myc** - Negative feedback regulation
- Genes involved in cell adhesion − encoding subunits of integrins **αLβ2** and **α3β1**
- Genes involved in differentiation: ***mim*-1**, **lysosyme** and **C/EBPα**

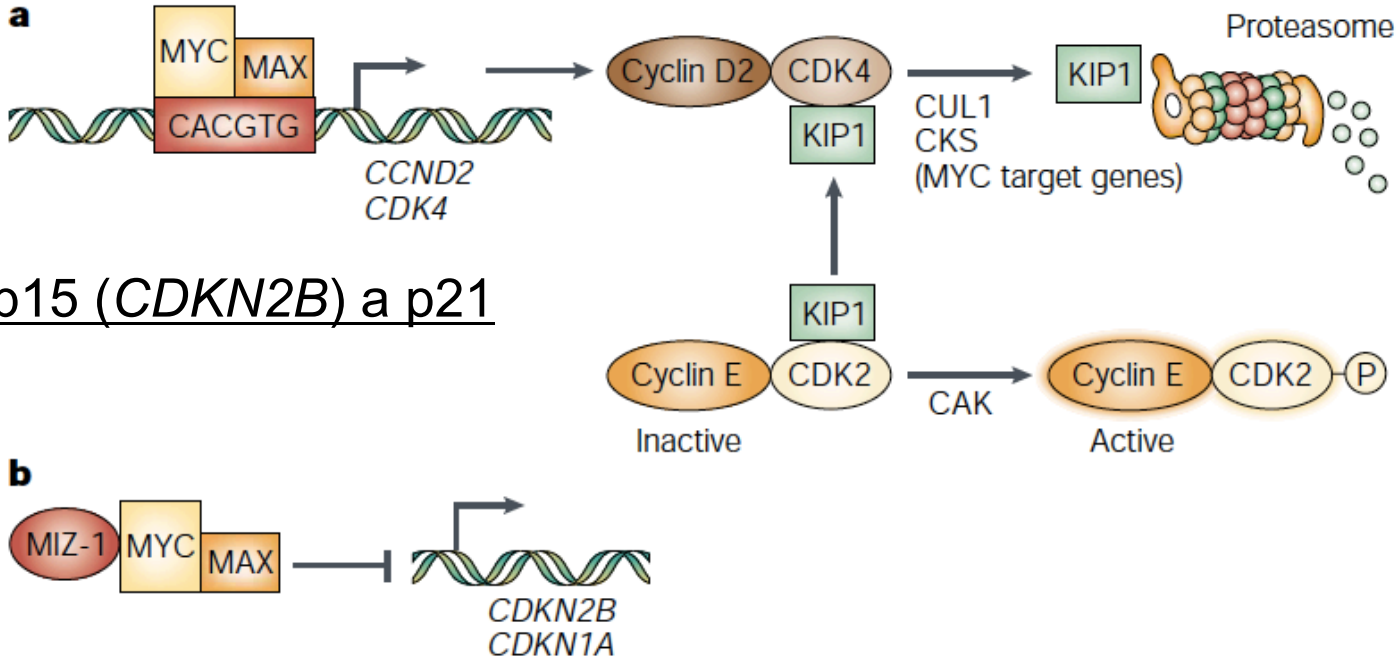


Pellegaris. *Nat Rev Cancer*. 2002;2(10):764-76.

Myc regulates progression G1-S both by transactivation and repression

Activation of cyclin D2 a CDK4

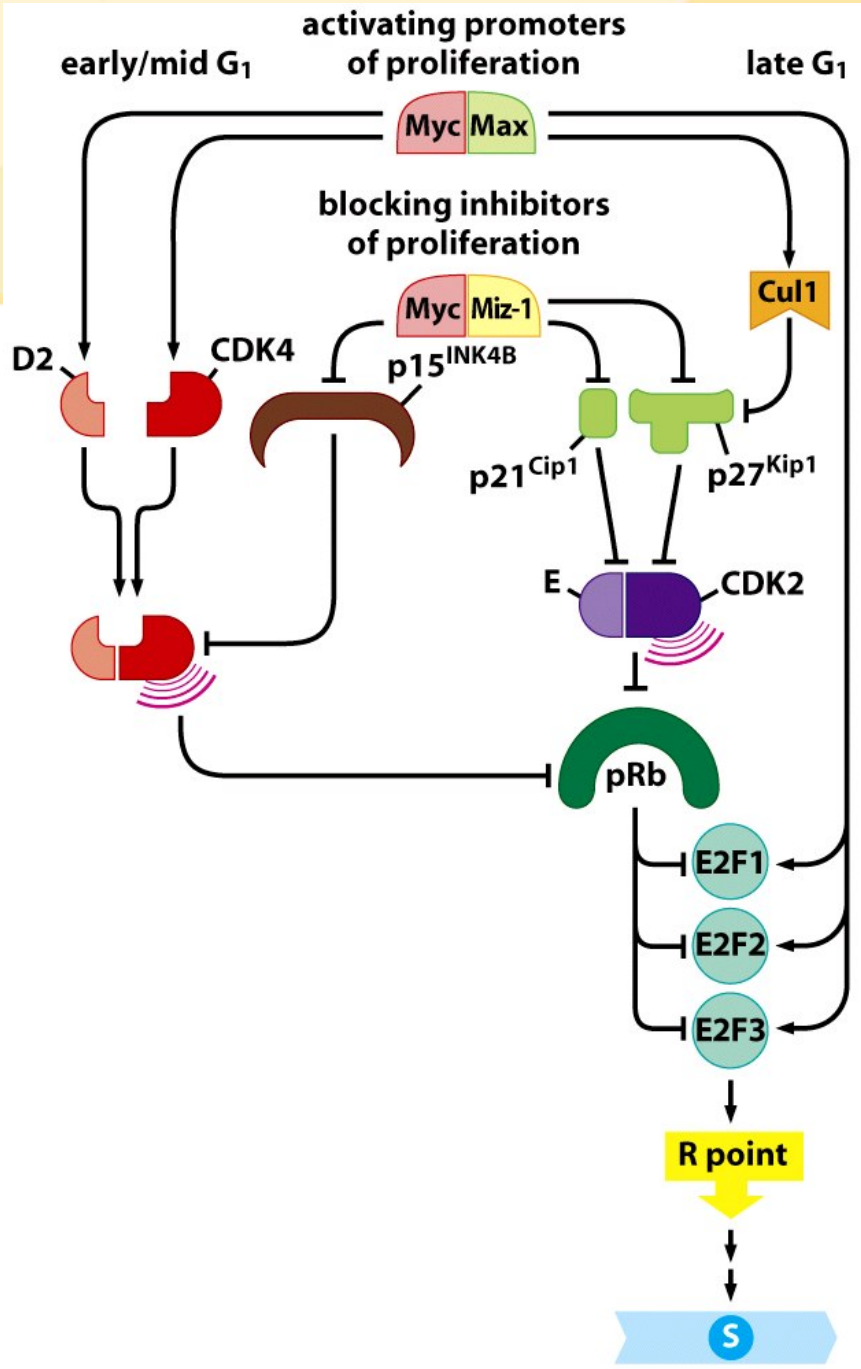
Repression of p15 (*CDKN2B*) a p21 (*CDKN1A*)



Pellegaris. *Nat Rev Cancer*. 2002;2(10):764-76.

Myc effects on cell cycle

- Dimer Myc-Max induces expression of cyclin D2 and CDK4 → ↑**BC** (G1)
- Dimer Myc-Max induces expression of Cul1 that is responsible for degradation of p27 (KIP1) → ↑**BC** (G1)
- Dimer Myc-Max induces expression of E2Fs → ↑**BC** (S)
- Dimer Myc-Miz-1 represses expression of p15, p21 a p27 → ↑**BC** (G1)



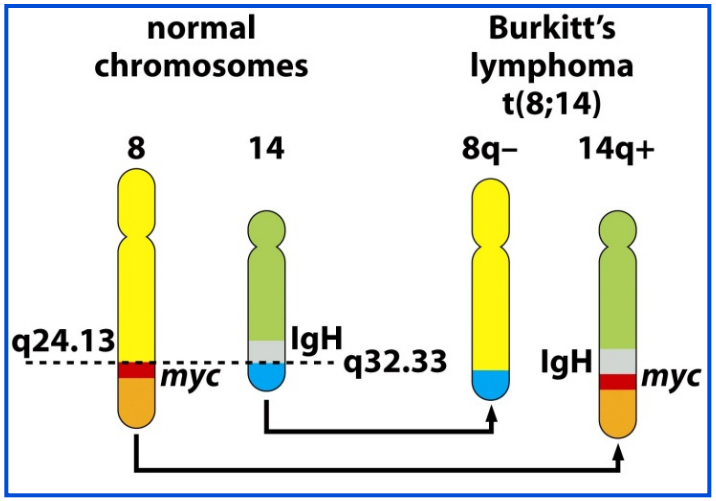
Myc and cancer

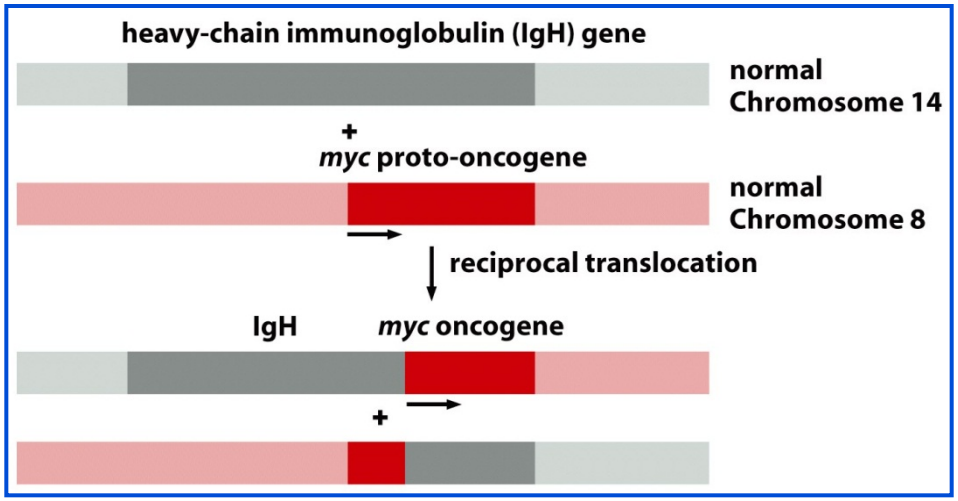
- Myc family:
  c-Myc, **N-Myc**, **L-Myc**
- Similar in protein structure and mechanism of action
- Different in expression levels during development, regulate (in the same cell type) different sets of genes and thus modulate different cellular programs
- Participate in development of different tumors

c-*myc* and cancer

Burkitt lymphoma:

Almost all cases of Burkitt lymphoma are linked to **translocation** of c-*myc* gene (chromosome **8**). Fusion partner is either immunoglobuline heavy chain μ or light chain λ or κ (located on chromosomes **14**, 22 a 2).





Translocations in Burkitt lymphoma

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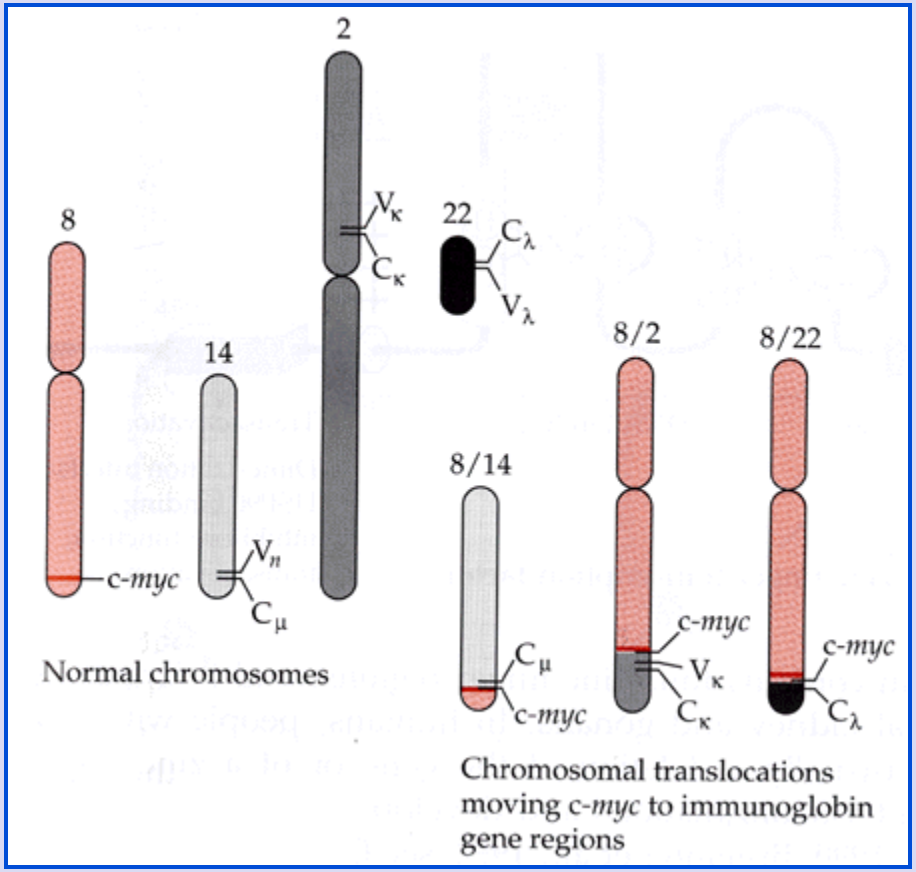
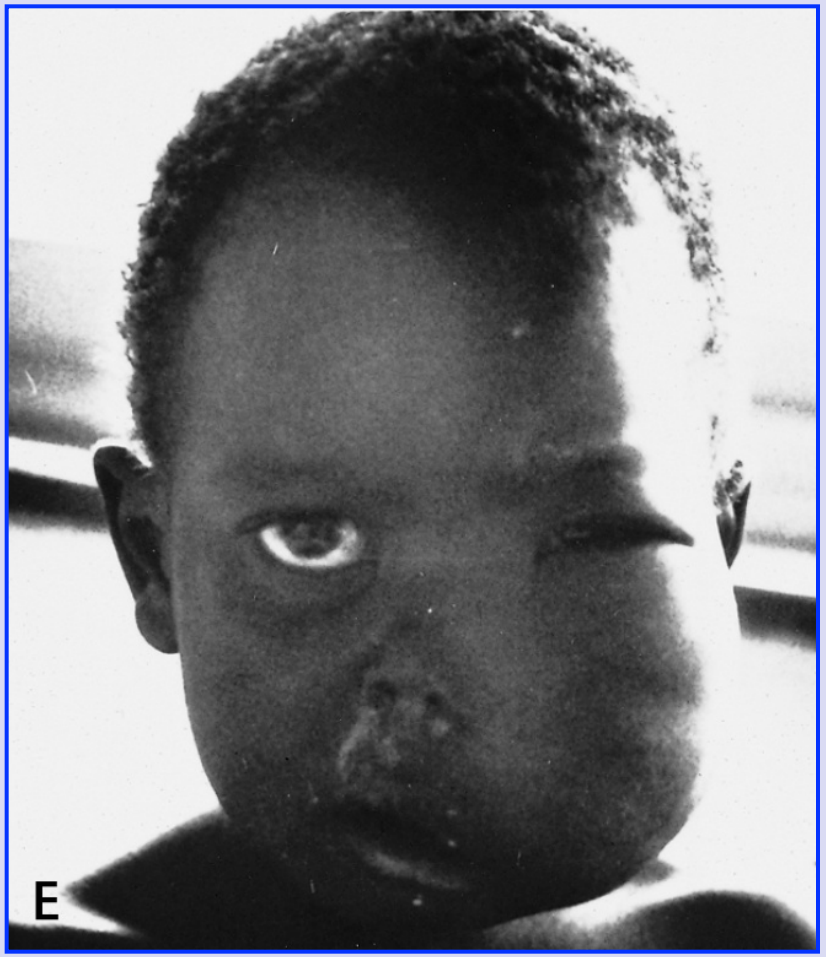
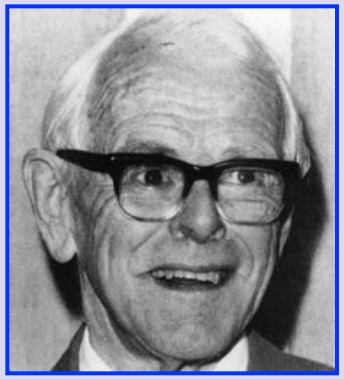


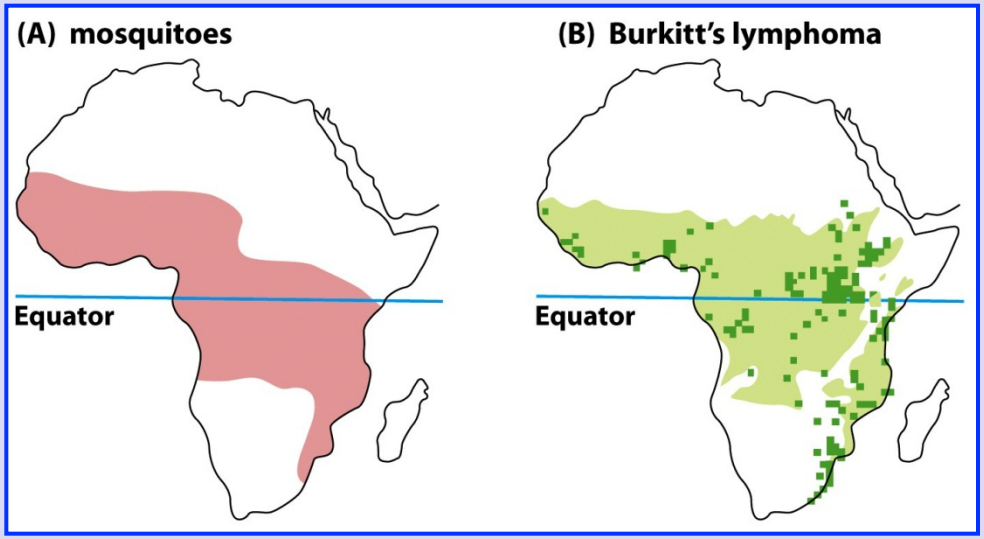
Burkitt lymphoma



Burkitt lymphoma



The endemic variant (also called "African variant") most commonly occurs in children living in malaria-endemic regions of the world. Epstein–Barr virus (EBV) infection is found in nearly all patients. The disease characteristically involves the jaw or other facial bone.



Pink: geographic distribution of *Aedes simpsoni*, malaria vector.

Green: geographic distribution of Burkitt lymphoma (described by Dennis Burkitt)

Possible explanation

Chronic malaria weaken immune system ⇒ more sensitive to **EBV** ⇒ leading to the accumulation of immortalized (EBV+) B lymphocytes: in these lymphocytes is present enzymatic machinery for DNA rearrangement of immunoglobulin loci → it may happen that errors in rearrangements involve c-Myc locus → translocations.

c-*myc* and cancer

Other lymphomas:

- Low-grade follicular lymphom is usually associated with translocation Ig/Bcl-2 - t(14;18), only rarely transcolations of c-*myc.* But if these lymphomas progress into an agressive form they usually have also translocations of Ig/c-*myc*: t(8;14), t(2;8), t(8;22)

- Diffuse large B-cells lymphomas are very heterogenous. 50% of patients have *Ig* translocation either with *BCL2*, *BCL6* or c-*myc*.

c-*myc* and cancer

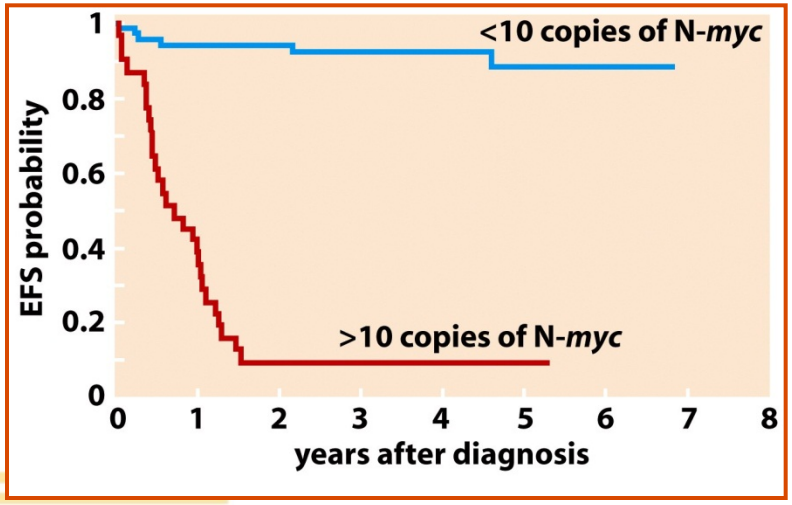
Solid tumors:

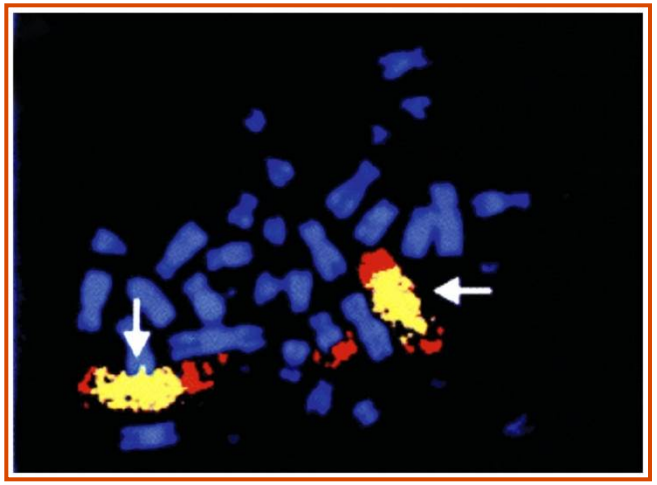
Amplification and/or **overexpression** of c-*myc* occur in a large subset of invasive ductal breast carcinomas (associated with worse prognosis), in some prostate cancer and gastrointestinal tumors (association of c-Myc with APC:β-catenin: nuclear β-catenin is co-activator of TF Tcf-4 that transactivates c-*myc*), in some melanomas and multiple myelomas (correlates with s disease aggressiveness).

N-*myc* and cancer

Amplifications of N-*myc* occur in approx 30% (in 40% of advanced) neuroblastomas (tumor of periferal NS) and is associated with poor prognosis. Some neuroblastomas have 5 to 30 copies of N-*myc*, some approx 100-150 of copies (yellow marked FISH probe).

Overexpression of N-*myc* was found in a subset of small cell lung carcinomas and some cases of medullary thyroid carcinoma, retinoblastoma, rhabdomyosarcoma and astrocytomas.





Weinberg RA. The Biology of Cancer. *GS* **2007**

L-*myc* and cancer

Amplification and **overexpression** of L-*myc* (but also c-*myc* and N-*myc*) found in some cases of small cell lung carcinoma.

Thank you for your attention!