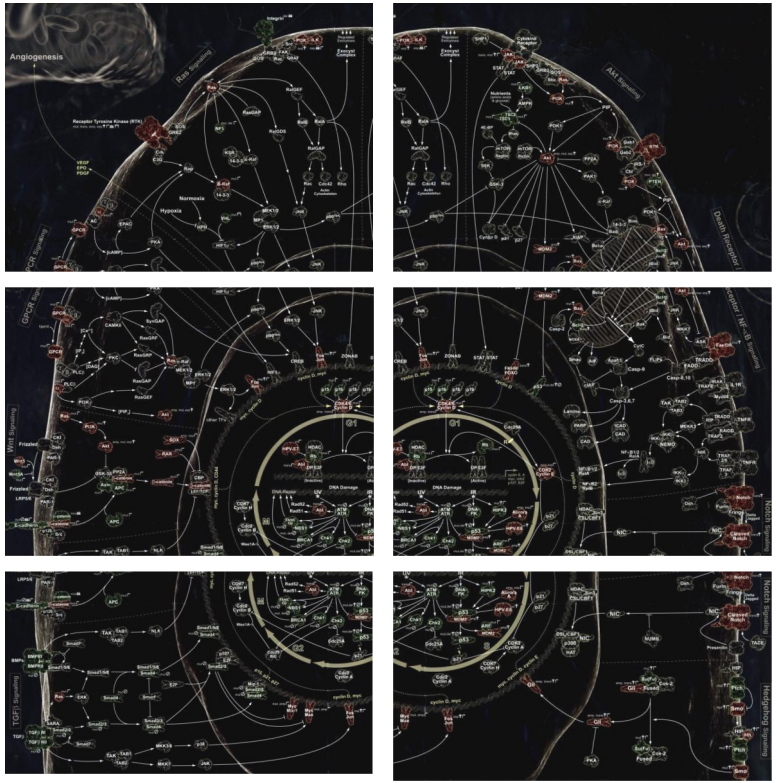


FREQUENTLY ALTERED PATHWAYS IN CANCER

CANCER BIOLOGY

AGENDA

Nov 5th: Cancer genomics- mutations

Nov 11th: Cancer genomics-copy number alteration, heterogeneity, evolution *(recording)*

Nov 18th: Cancer Epigenetics- chromatin 3D structure, cell plasticity

Nov 25th: – Major signaling pathways leading to cancer

Dec 2th: Cancer Therapies – chemo and targeted therapies

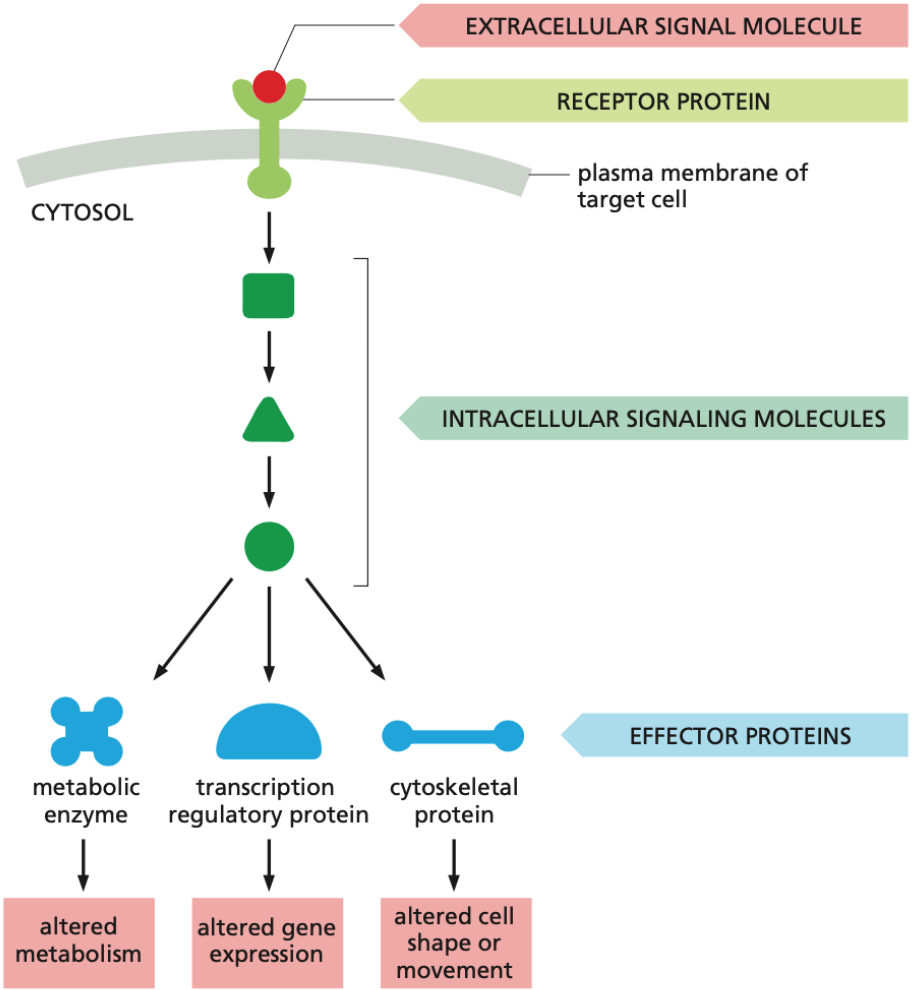
Dec 9th: Introduction to immunotherapies –

Dec 16th: questions for the exam

Dec 18th: Exam 1.30 PM-3.30 PM

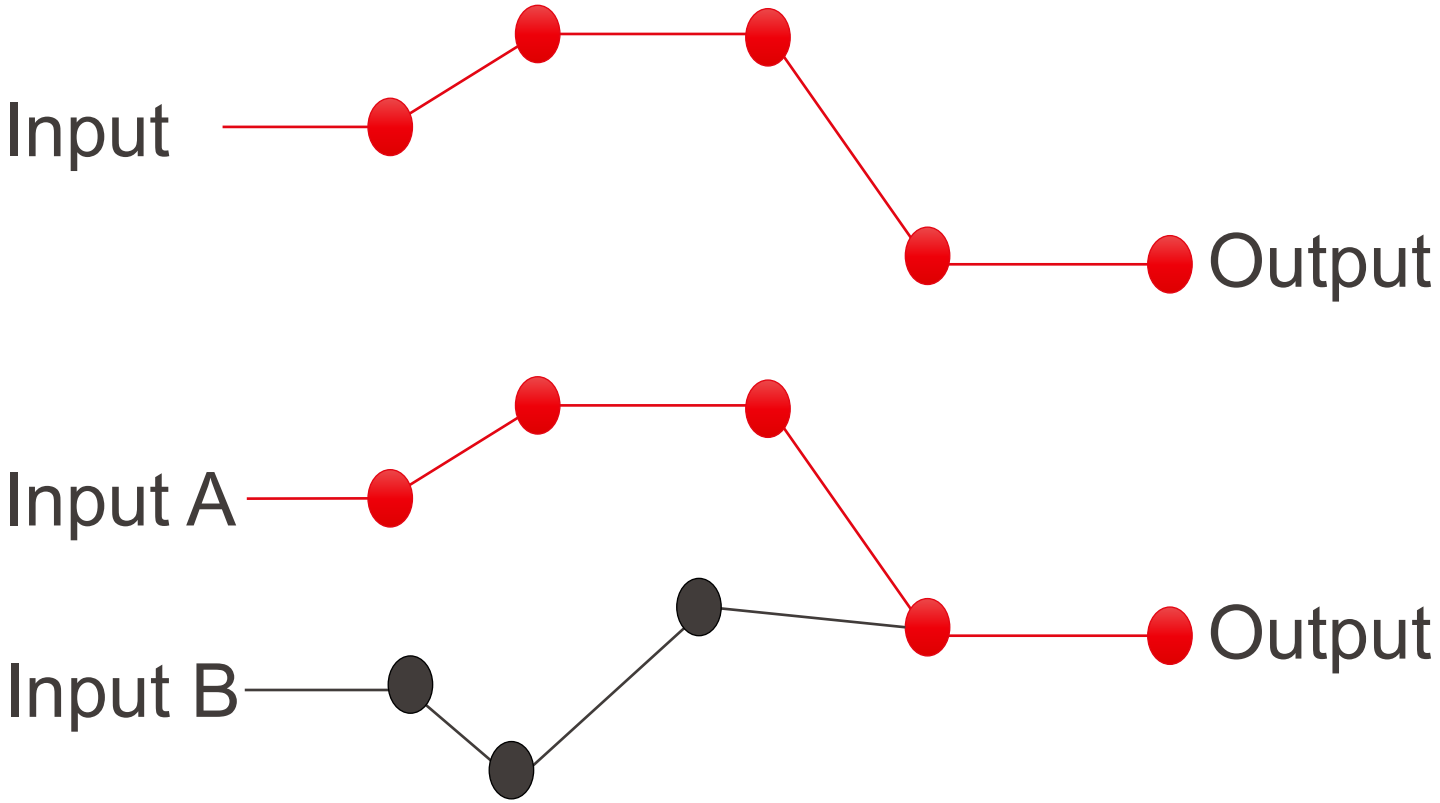
What is a pathway

- A group of molecules that work together to coordinate a response
- The pathway works as a cascade signals
- The first molecule of the pathway receives the input and starts a cascade of events that produces output (response)

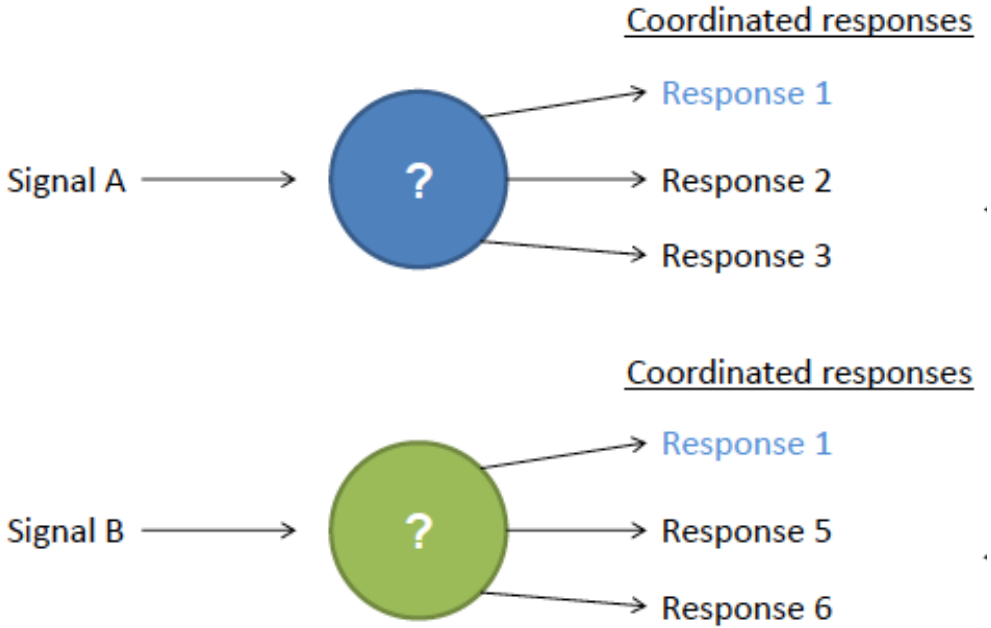


Alberts et al., Molecular Biology of the Cell, 7th ed

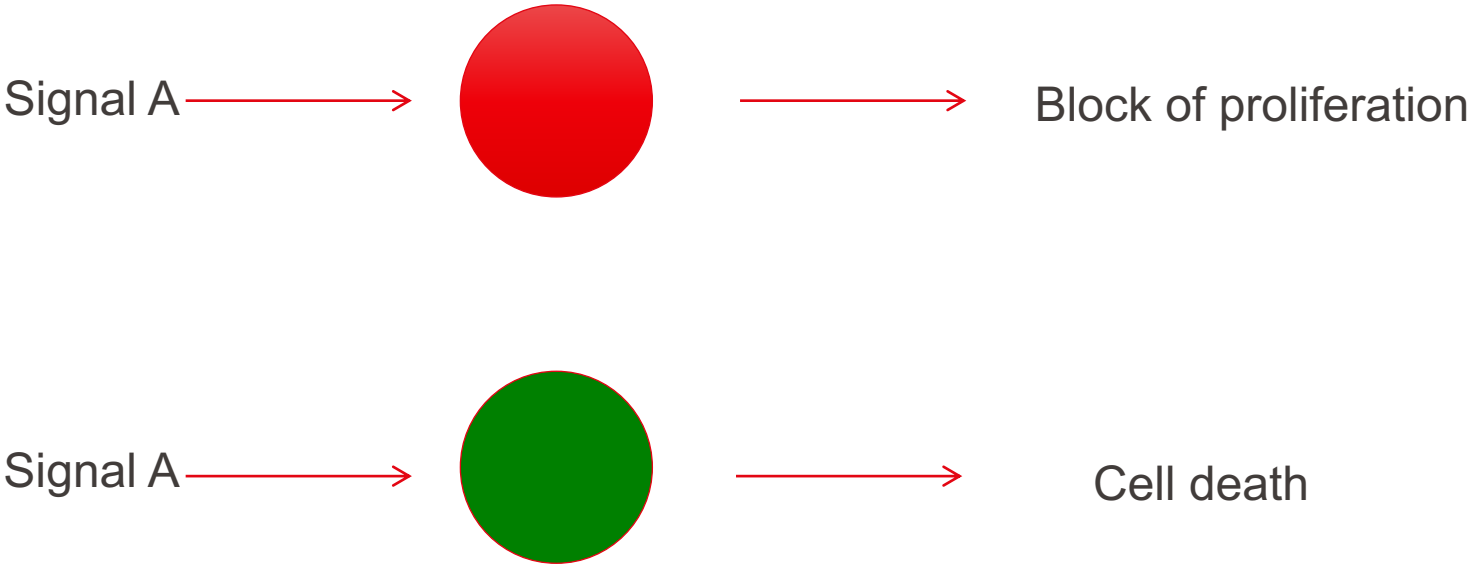
Input and Output are coordinated



Signal can coordinate multiple responses

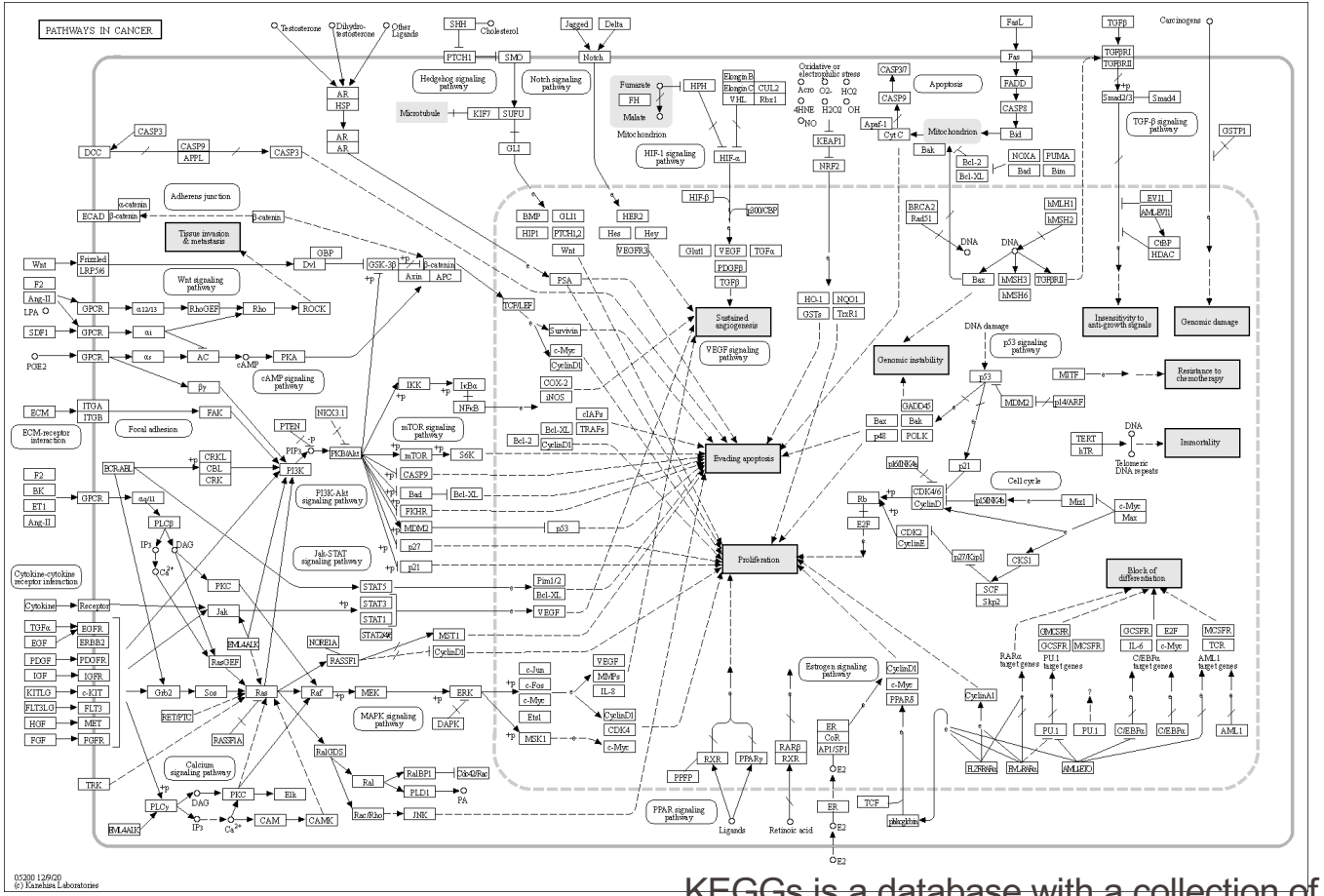


Response can be context dependent



Difference in response can be linked to mutations in the pathway and parallel signals

Complex circuits



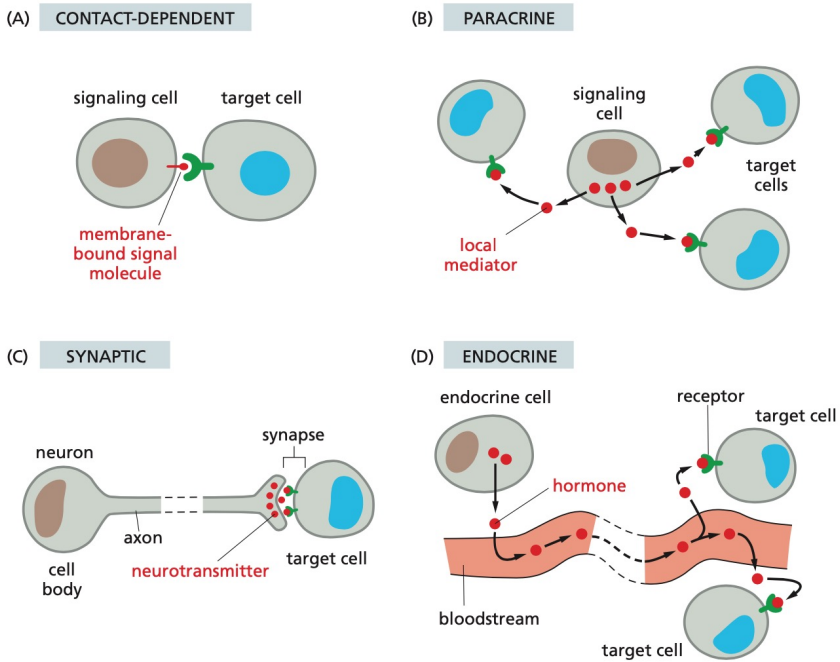
KEGGs is a database with a collection of the pathways

Major signaling pathways in cancer

- *Ras Signaling*
- *AKT Signaling*
- *Notch Signaling*
- *Hedgehog Signaling*
- *TGF-β Signaling*
- *WNT Signaling*
- *Cell Death Signaling*

Where does the signal (=input) come from?

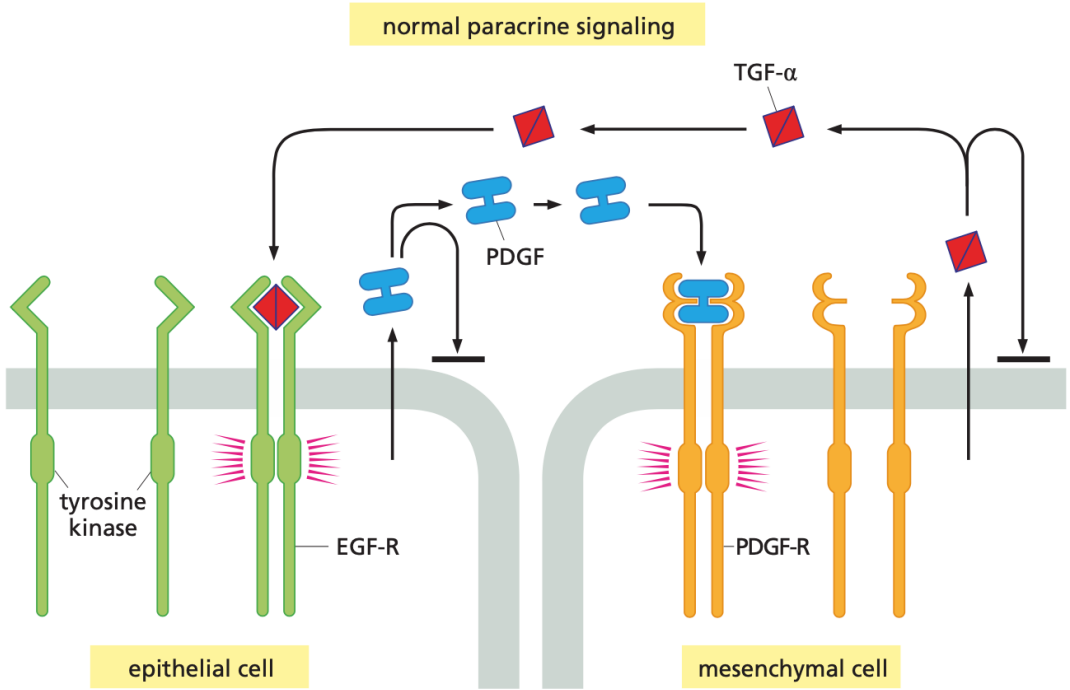
- *Endocrine signaling (long distances)*
- *Paracrine signaling (local mediators)*
  - *Autocrine signaling*
- *Gap junction signaling (contact-dependent)*
- *Synaptic signaling (in neurons)*



Alberts et al., Molecular Biology of the Cell, 7th ed

Paracrine signals

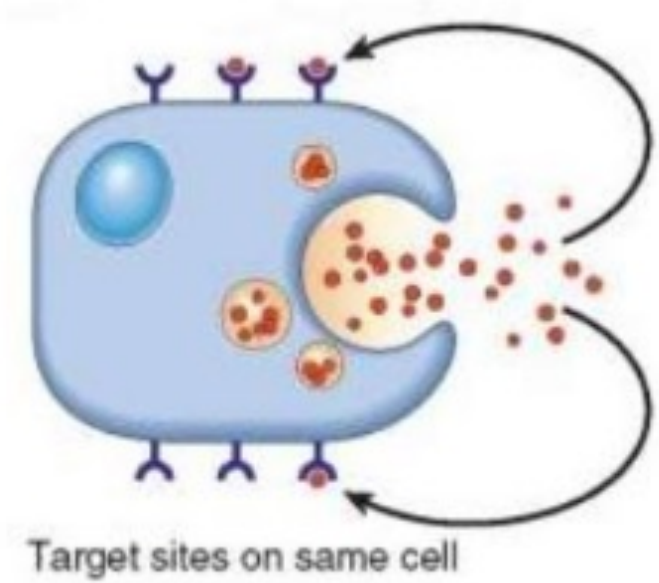
The signal molecule (e.g. interleukins, hormones) is produced by different cell types



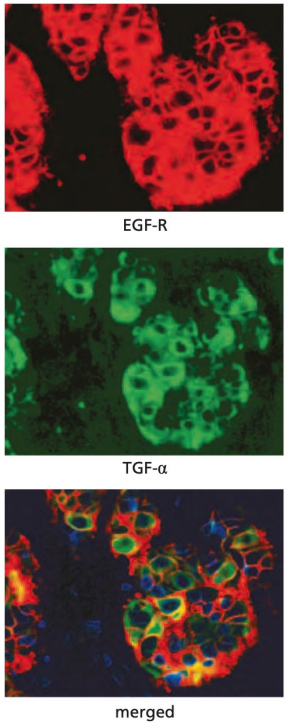
Weinberg, The Biology of Cancer 2nd ed.

Autocrine signals

The signal molecule is produced by the same cells that received the signal



An example
of autocrine signaling in
successive sections of an
invasive human breast
carcinoma.



Weinberg, The Biology of Cancer 2nd ed.

Autocrine molecules

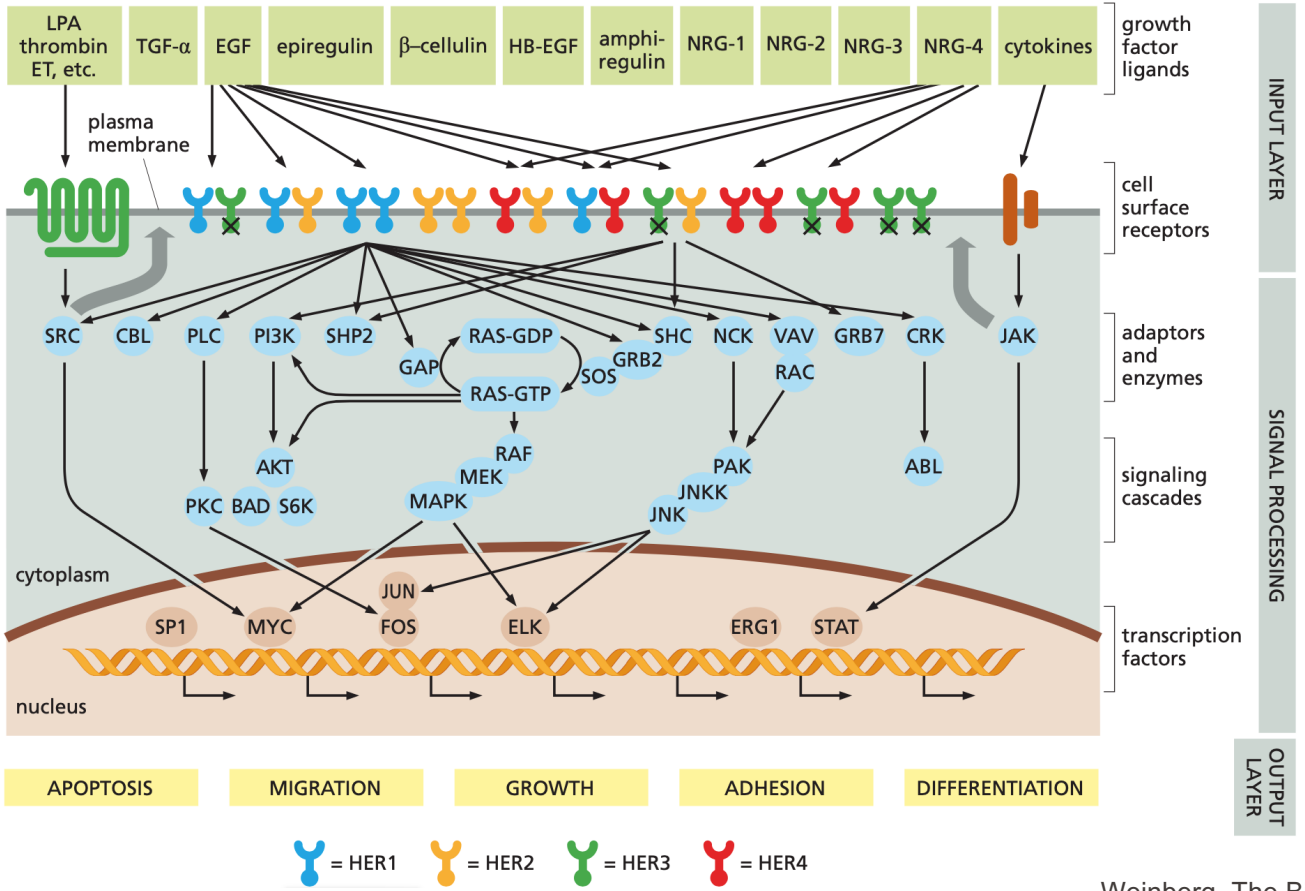
Table 5.3 Examples of human tumors making autocrine growth factors

Ligand	Receptor	Tumor type(s)
HGF	Met	miscellaneous endocrinal tumors, invasive breast and lung cancers, osteosarcoma
IGF-2	IGF-1R	colorectal
IL-6	IL-6R	myeloma, HNSCC
IL-8	IL-8R A	bladder cancer
NRG	ErbB2[a]/ErbB3	ovarian carcinoma
PDGF-BB	PDGF-Rα/β	osteosarcoma, glioma
PDGF-C	PDGF-Rα/β	Ewing's sarcoma
PRL	PRL-R	breast carcinoma
SCF	Kit	Ewing's sarcoma, SCLC
VEGF-A	VEGF-R (Flt-1)	neuroblastoma, prostate cancer, Kaposi's sarcoma
TGF-α	EGF-R	squamous cell lung, breast and prostate adenocarcinoma, pancreatic, mesothelioma
GRP	GRP-R	small-cell lung cancer

[a]Also known as HER2 or Neu receptor

Weinberg, The Biology of Cancer 2nd ed.

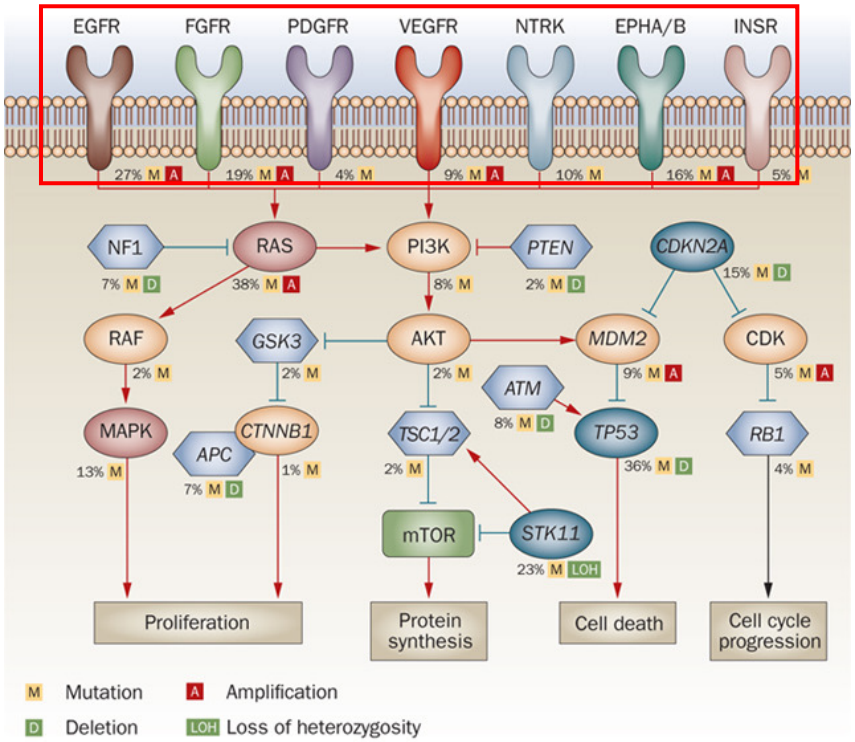
One ligand can bind to multiple receptors



Weinberg, The Biology of Cancer 2nd ed.

Receptor Tyrosine Kinases

- **Cell growth**



Epidermal growth factor

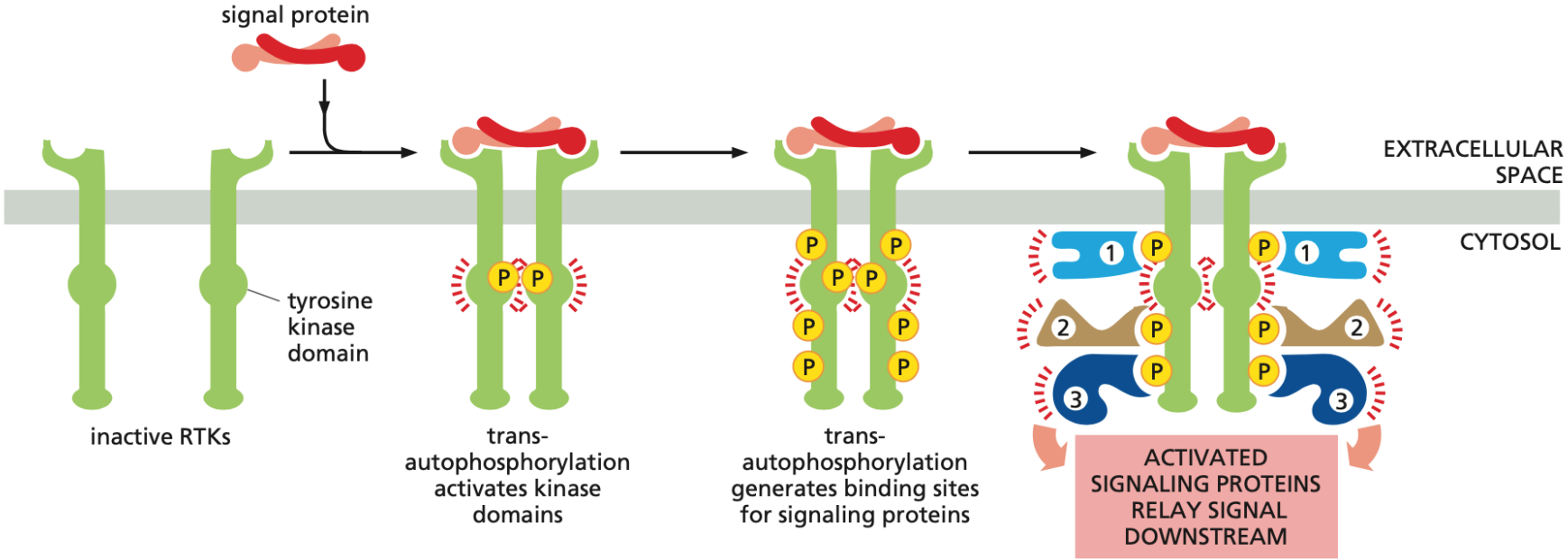
Fibroblast growth factor

Platelet-derived growth factor

Vascular endothelial growth factor

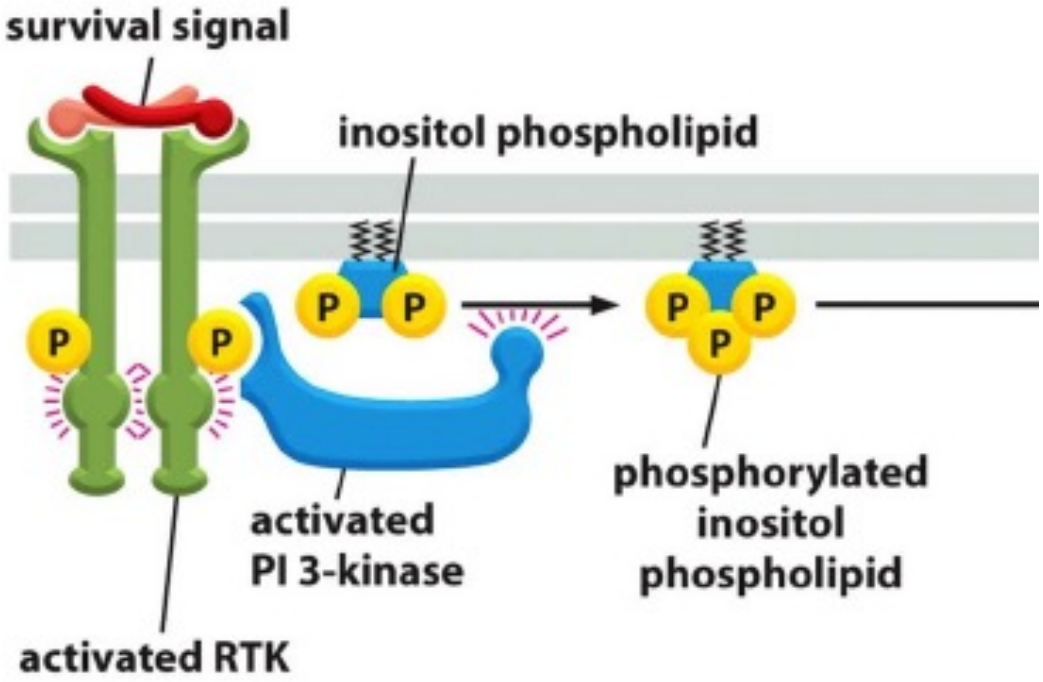
Insulin growth factor

Receptor Tyrosine Kinases dimerization

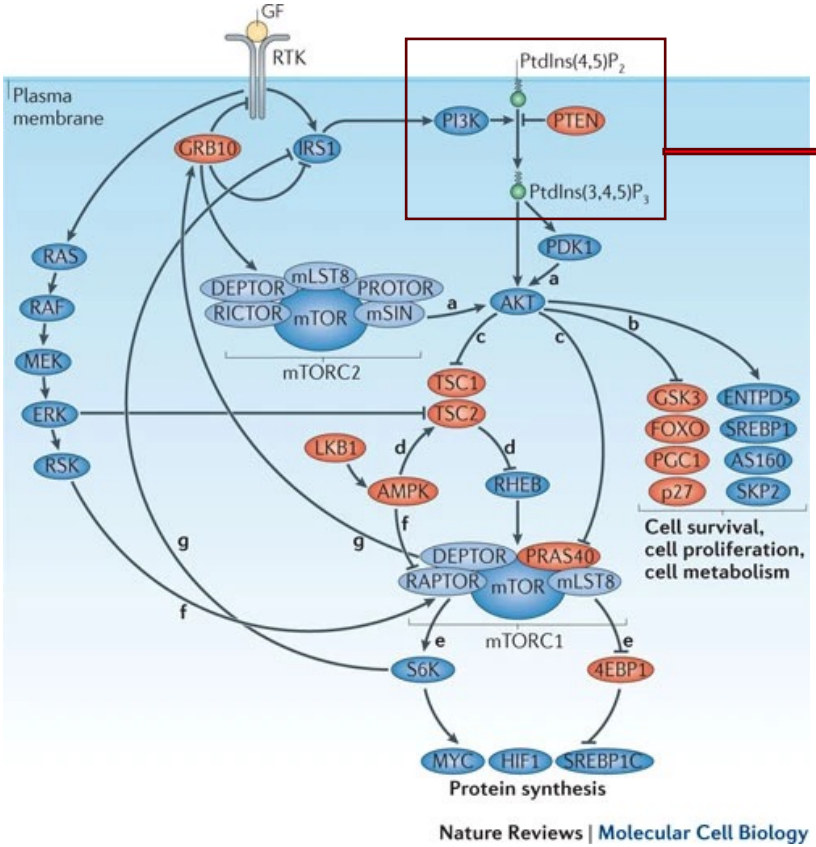


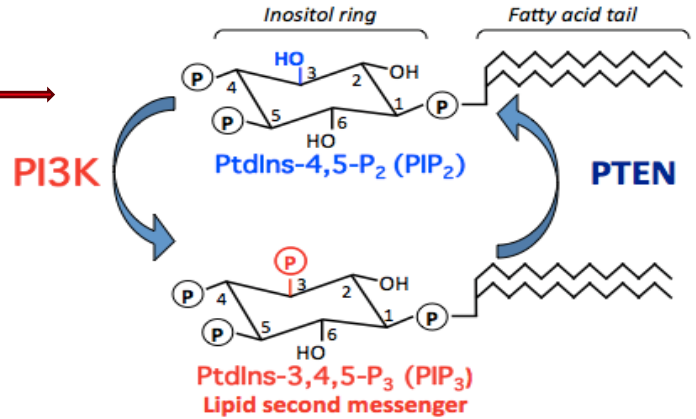
Alberts et al., Molecular Biology of the Cell, 7th ed

Downstream RTK proteins: the case of PI3K



PI3K activity is controlled by PTEN

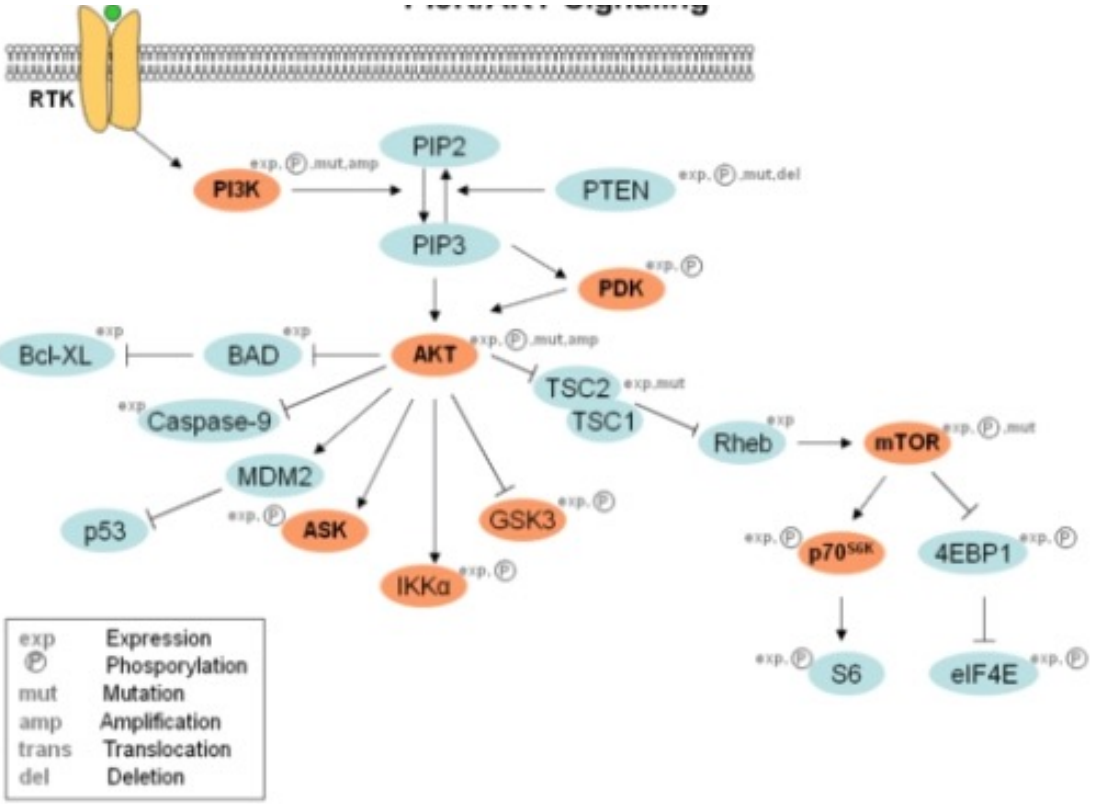




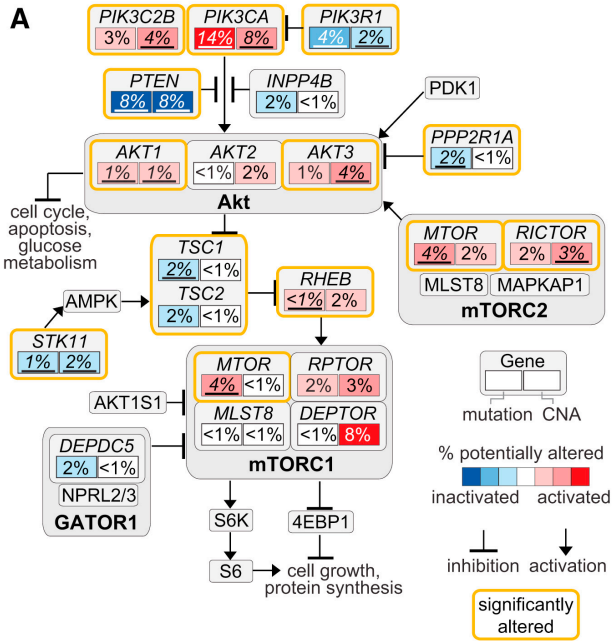
PTEN: Phosphatase and tensin homolog

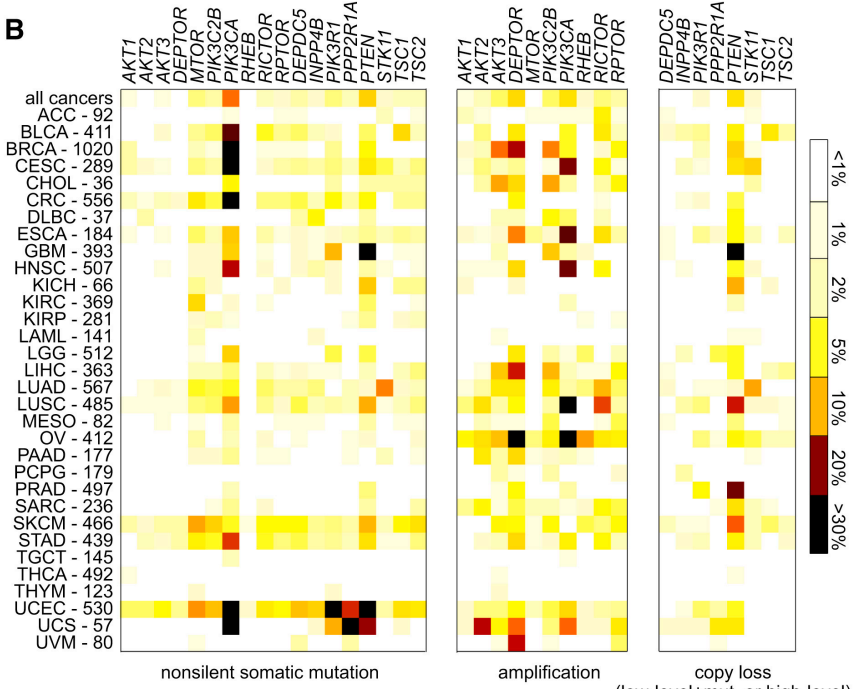
Song et al., 2012

PI3-kinase activates Akt



PI3K/Akt pathway is frequently altered across tumors

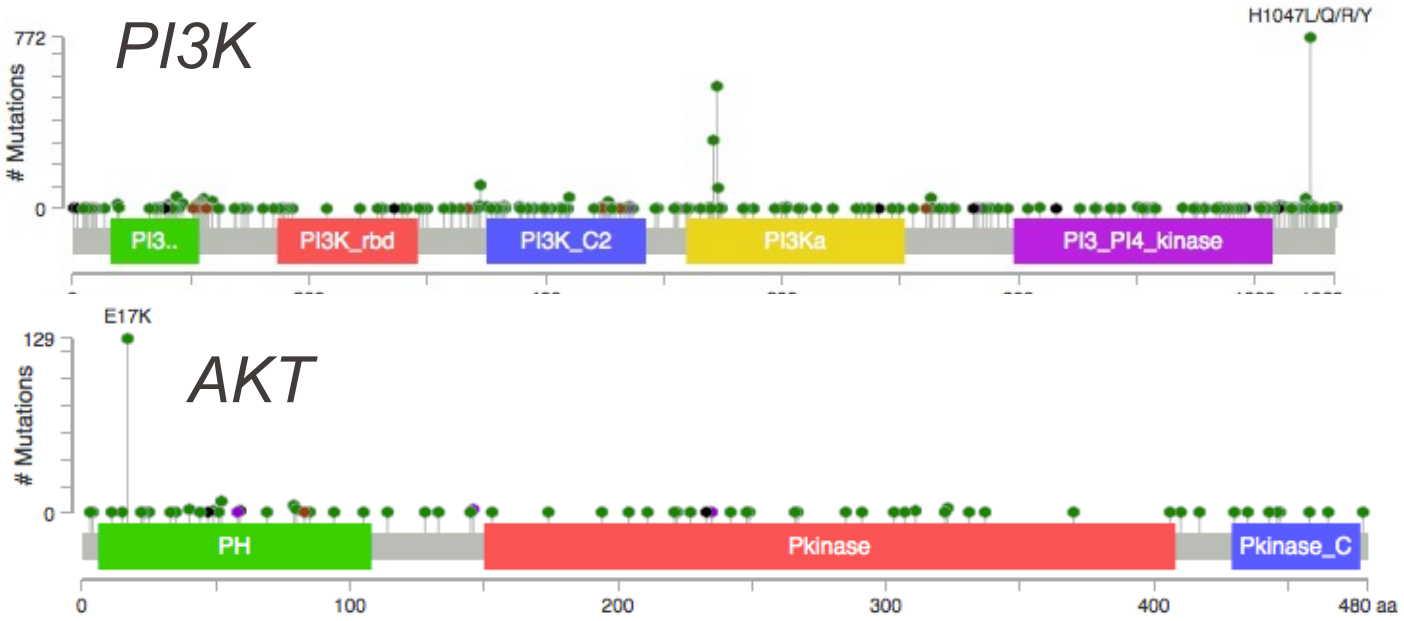




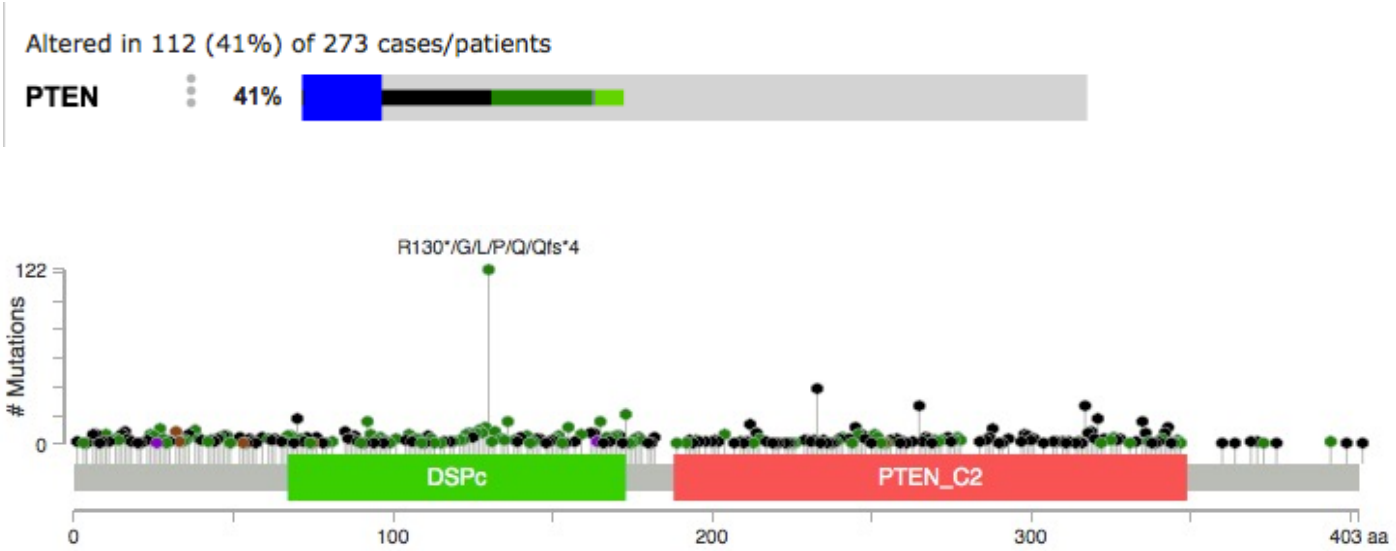
LAML, Acute Myeloid Leukemia; ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; LGG, Brain Lower Grade Glioma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; CRC, Colorectal adenocarcinoma (combining COAD and READ projects); ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THYM, Thymoma; THCA, Thyroid carcinoma; UCS, Uterine Carcinosarcoma; UCEC, Uterine Corpus Endometrial Carcinoma.

Zhang et al., 2017

Hot-spot mutations

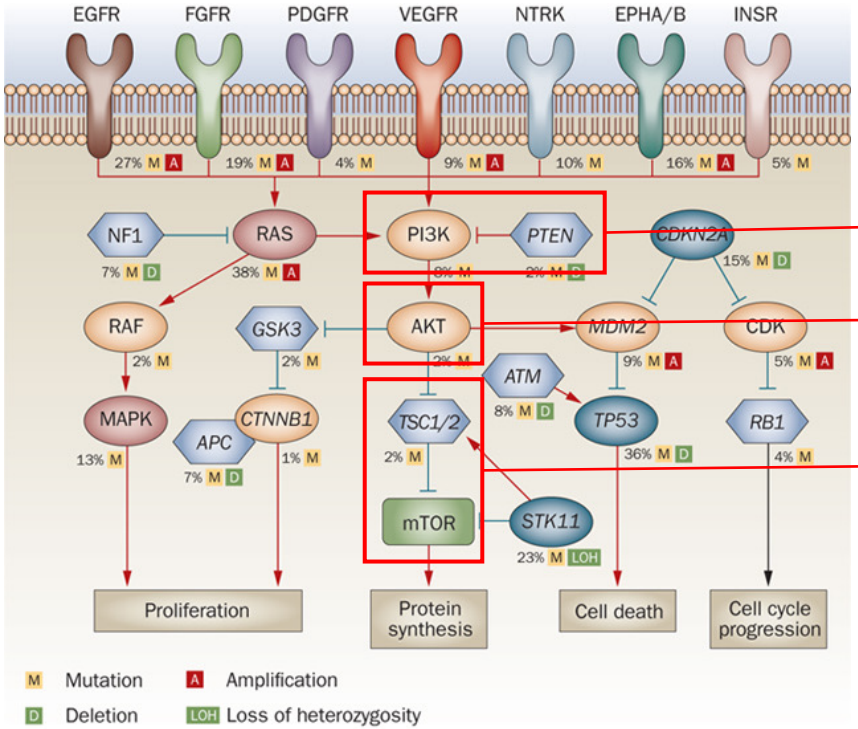


PTEN is deleted or mutated in several cancers



PI3K/Akt pathway

- **Survival & Translation**



PIK3CA, PIK3R1, PTEN

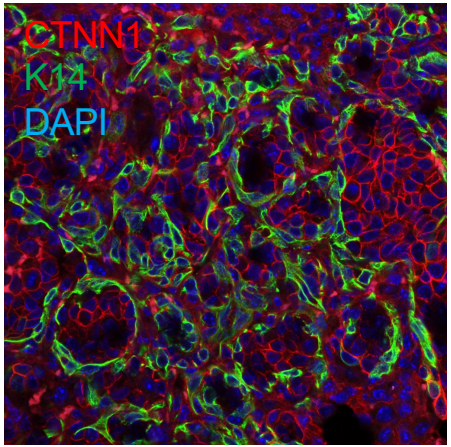
AKT1

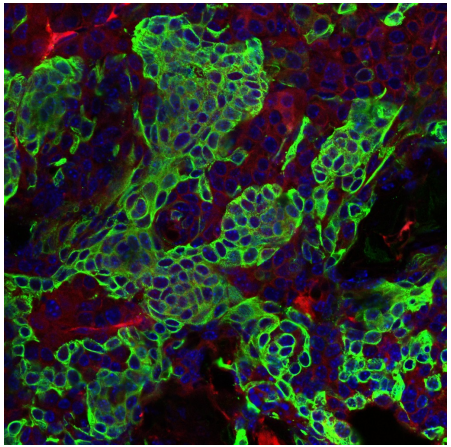
MTOR

The Wnt pathway

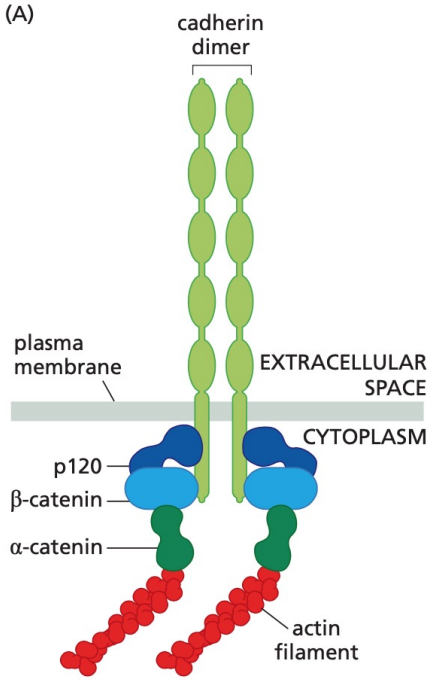
- Important during development (patterning decisions).
- 19 ligands (Wnt members)
- More than 15 receptors: Frizzled, LRP5, LRP6, RYK,…
- Canonical and non-canonical signaling.

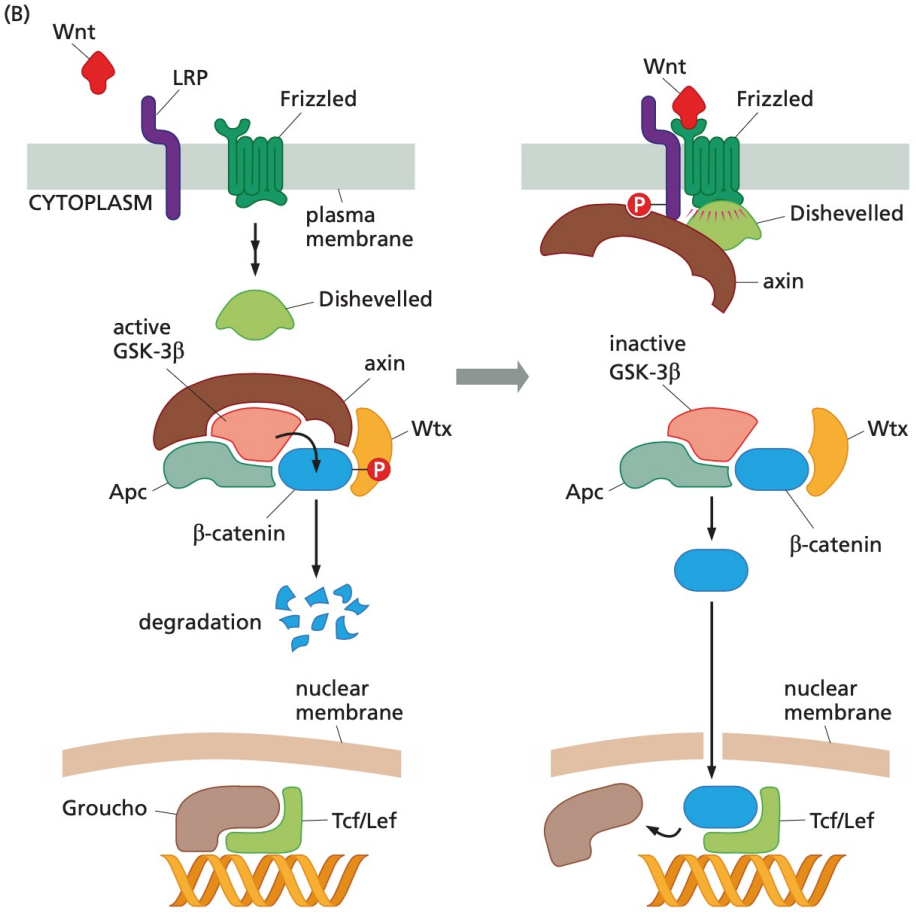
- Roles in cancer:
  - Cancer stem cells: e.g. self-renewal, TERT expression.
  - Invasion and metastasis through EMT.





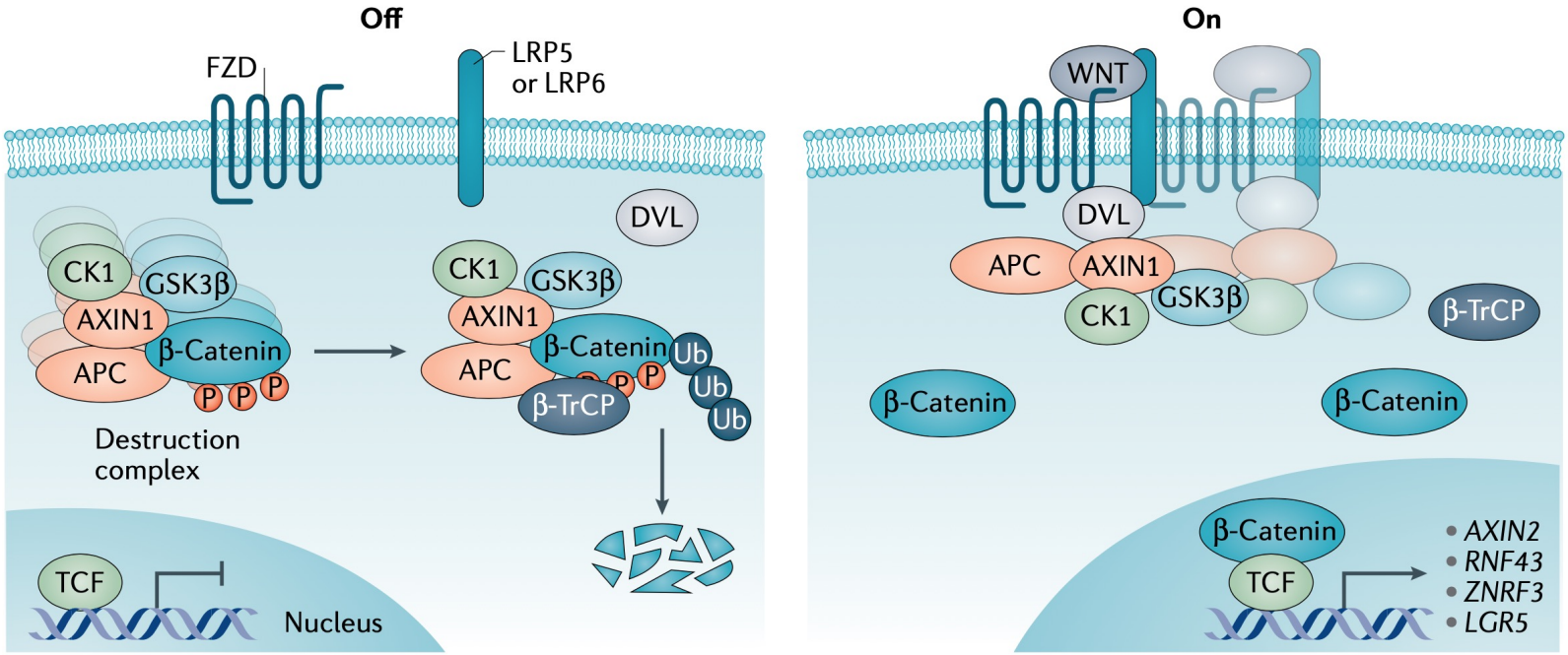
The Wnt pathway





Weinberg, The Biology of Cancer 2nd ed.

Canonical Wnt signaling

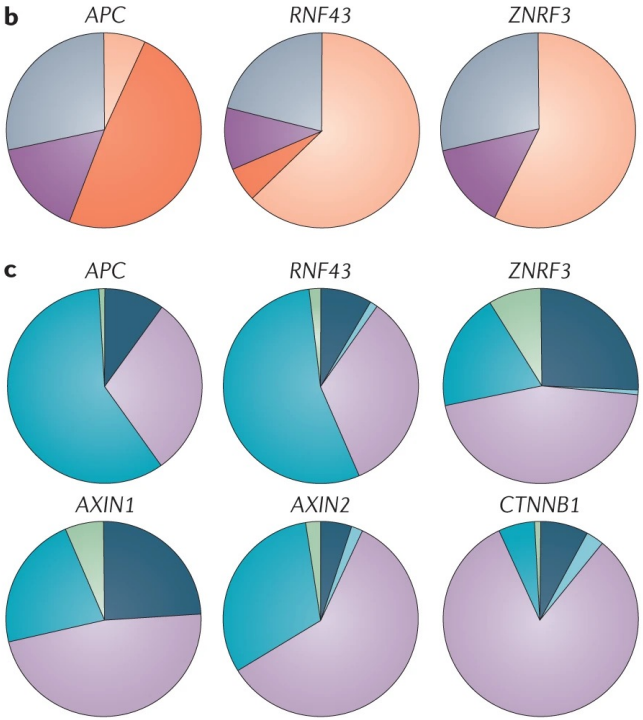


Bugter et al., 2021

Alterations of the Wnt pathway in cancer

a

APC	Colorectal adenocarcinoma	66.55%
	Uterine corpus endometrial carcinoma	14.34%
	Skin cutaneous melanoma	14.25%
	Stomach adenocarcinoma	13.38%
	Bladder urothelial carcinoma	7.04%
RNF43	Uterine corpus endometrial carcinoma	15.28%
	Stomach adenocarcinoma	10.20%
	Colorectal adenocarcinoma	7.90%
	Pancreatic ductal adenocarcinoma	5.95%
ZNRF3	Adenocortical carcinoma	20.43%
	Uterine corpus endometrial carcinoma	6.23%
	Skin cutaneous melanoma	5.35%
AXIN1	Liver hepatocellular carcinoma	7.77%
	Uterine corpus endometrial carcinoma	6.42%
AXIN2	Uterine corpus endometrial carcinoma	7.36%
	Colorectal adenocarcinoma	5.04%
CTNNB1	Liver hepatocellular carcinoma	25.47%
	Uterine corpus endometrial carcinoma	25.47%
	Adenocortical carcinoma	15.05%
	Skin cutaneous melanoma	6.68%
	Stomach adenocarcinoma	6.68%
	Colorectal adenocarcinoma	5.71%

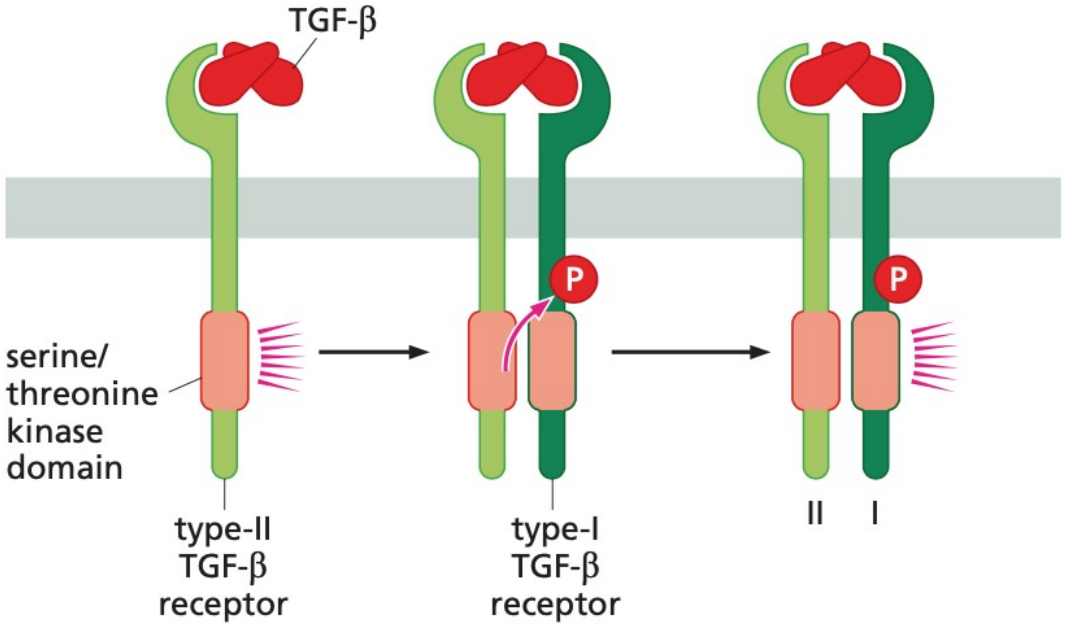


Bugter et al., 2021

The TGFβ pathway

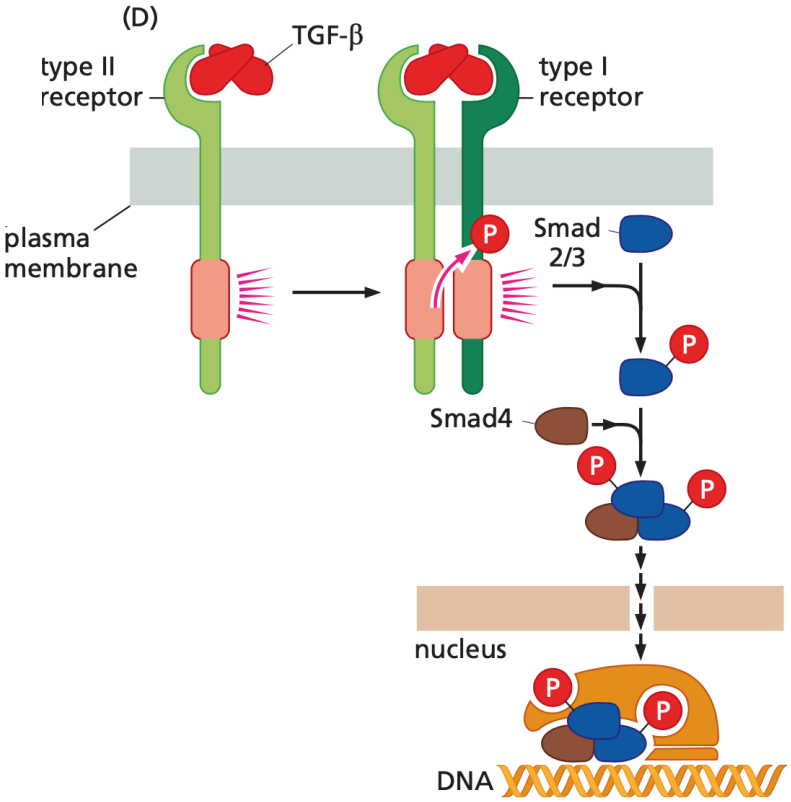
- Controls: cell cycle, metastasis, invasion, angiogenesis, wound healing, cancer stem cells,…
- Cell- and context-dependent.
- Integrated in many other signaling pathways (Wnt, Notch, Ras, etc).
- Pleiotropic effects: can exert cytostatic and tumor-suppressive effects in early stage tumors, but can also induce proliferation, invasion, and angiogenesis in advanced tumors.

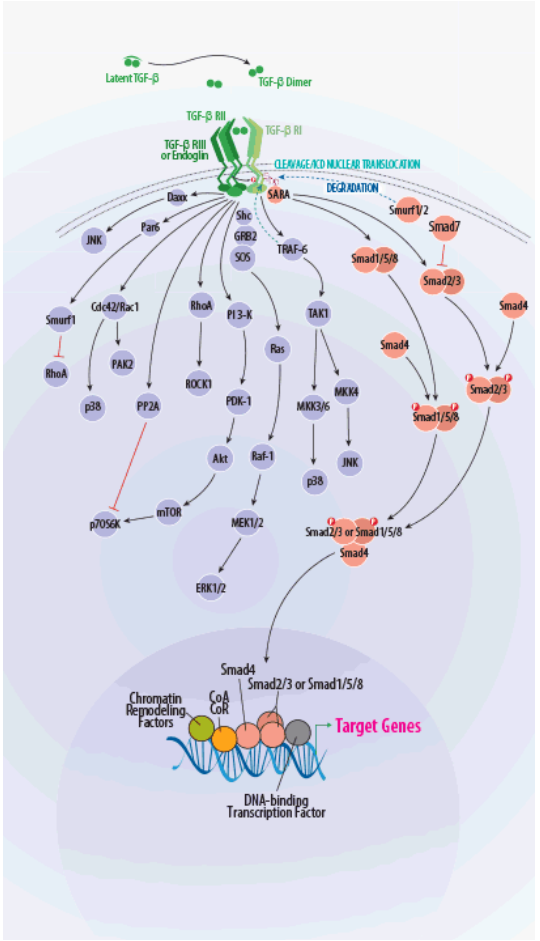
The TGFβ pathway



Weinberg, The Biology of Cancer 2nd ed.

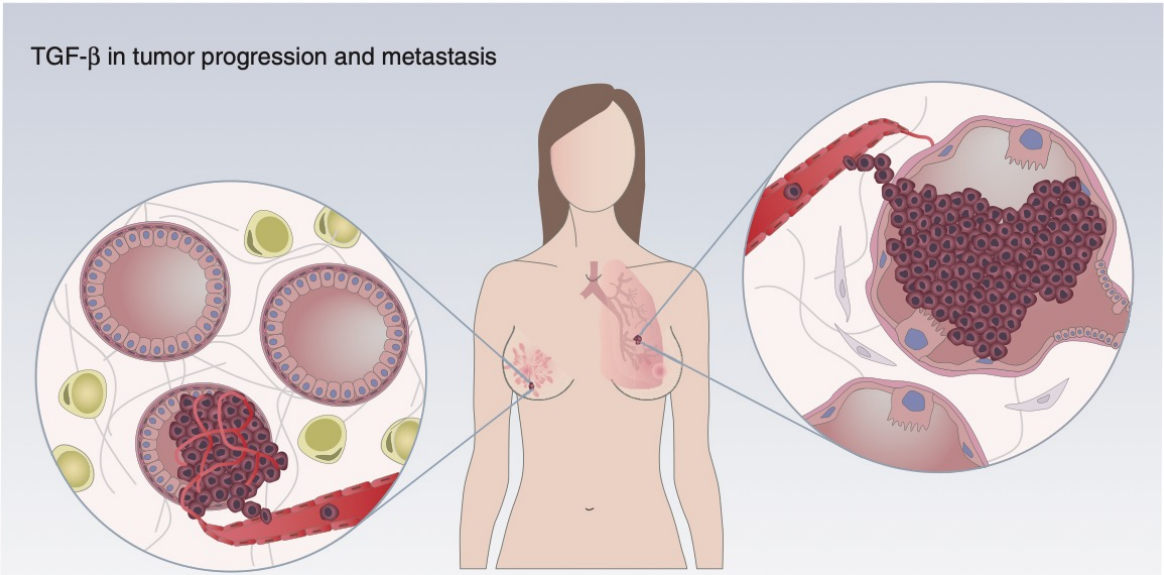
The TGFβ pathway





Weinberg, The Biology of Cancer 2nd ed.

A case of pleiotropism: The TGFβ pathway



Premalignant	Malignancy	Dissemination	Metastasis
Tumor-suppressor signals:	Evasion of immunity	EMT	Extravasation
Cytostasis Differentiation Apoptosis	Evasion of cytostasis	Stromal recruitment: Fibroblasts BMD cells	BMD cells mobilization
Inflammatory suppressor	Autocrine mitogenic signals	Metastasis endowing properties	Stroma-modifying factors
Stroma response suppressor	Motility and migration	Niche preparation	

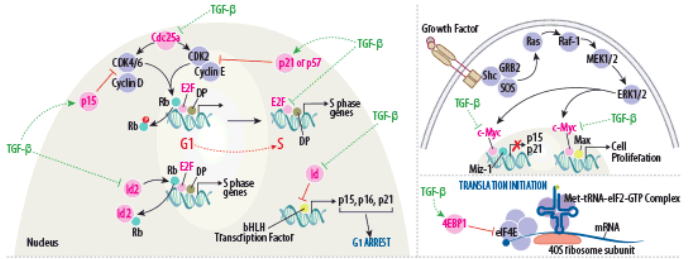
Seoane & Gomis, 2017

TGFβ target genes

Tumor-Suppressing Effects of TGF-β

Inhibits Cell Proliferation	Induces Apoptosis	Activates Autophagy	Inhibits Growth Factors in the Tumor Stroma	Inhibits Angiogenesis	Suppresses Inflammation
TGF-β Target Genes → p15, p21, p57, 4EBP1	TGF-β Target Genes → BIK, BIM, DAPK, Fas, GADD45β	TGF-β Target Genes → ATG5, ATG7, Beclin 1/ATG6, DAPK		TGF-β Target Genes → Thrombospondin	TGF-β Target Genes → FoxP3
TGF-β Target Genes ✗ CDC25A, E2F-1, Id1-3, c-Myc	TGF-β Target Genes ✗ Bcl-xL, Bcl-2		TGF-β Target Genes ✗ HGF, MSP, TGF-α		TGF-β Target Genes ✗ GATA-3, T-bet

Inhibits Cell Proliferation

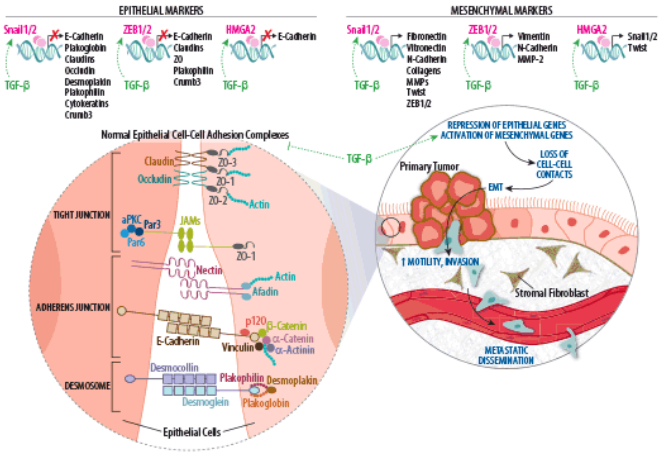


TGF-β inhibits cell proliferation by inducing the expression of 4EBP1 and the cyclin-dependent kinase (CDK) inhibitors, p15, p21, and p57. 4EBP1 binds to eukaryotic initiation factor 4E (eIF4E) and inhibits protein translation (bottom right panel), while p15, p21, and p57 prevent cell cycle progression by inhibiting the activities of CDK-cyclin complexes that are required for the G1/S transition (left panel). Additionally, TGF-β represses the expression of CDC25A phosphatase, which is also required for CDK-cyclin activation (left panel), and negatively regulates the expression of multiple other factors involved in driving cell cycle progression and cell proliferation, including the Id proteins (left panel), E2F (left panel), and c-Myc (top right panel).

Tumor-Promoting Effects of TGF-β

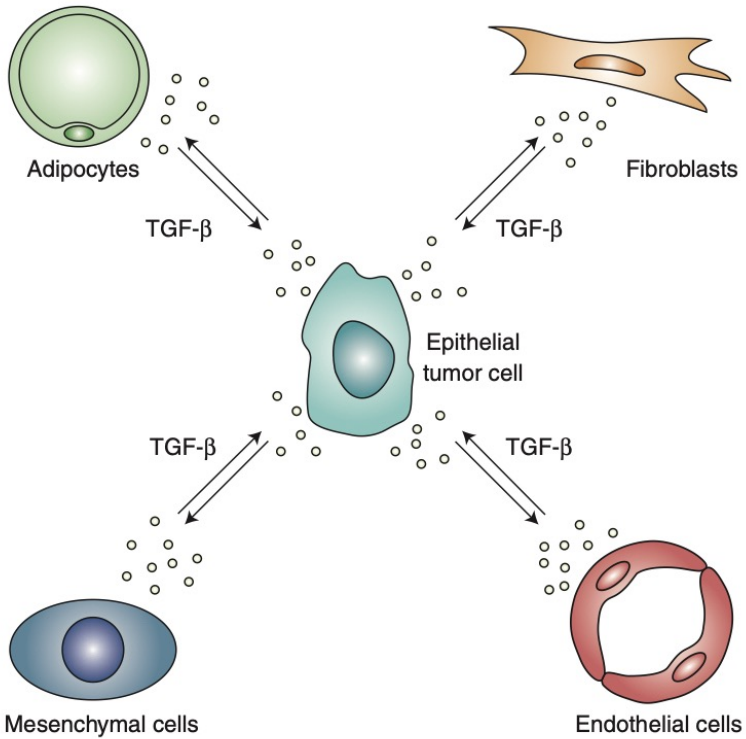
Promotes Cell Proliferation	Suppresses the Immune Response	Promotes Angiogenesis	Promotes Cancer Stem Cell Self-Renewal	Promotes the Epithelial-to-Mesenchymal Transition	Promotes Metastasis
TGF-β Target Genes → PDGF-B	TGF-β Target Genes → FoxP3	TGF-β Target Genes → VEGF, MMP-2, MMP-9	TGF-β Target Genes → LIF, SOX4	TGF-β Target Genes → Snail1/2, ZEB1/2, HMGA2	TGF-β Target Genes → HDM2, MMP-9
	TGF-β Target Genes ✗ Fas Ligand, GATA-3, Granzyme A/B, IFN-γ, MICA, NKG2D, Nkp30, Perforin, T-bet	TGF-β Target Genes ✗ TIMP			

Promotes the Epithelial-to-Mesenchymal Transition



TGF-β signaling in epithelial tumor cells promotes an epithelial-to-mesenchymal transition by inducing the expression of transcription factors, such as Snail1/2, ZEB1/2, and HMGA2, which repress the expression of epithelial cell adhesion proteins and induce the expression of mesenchymal proteins. These changes promote the loss of cell polarity and cell-cell contacts and lead to the acquisition of a migratory, invasive phenotype that may allow cancer cell dissemination.

The TGFβ pathway



Seoane & Gomis, 2017

The TGFβ pathway

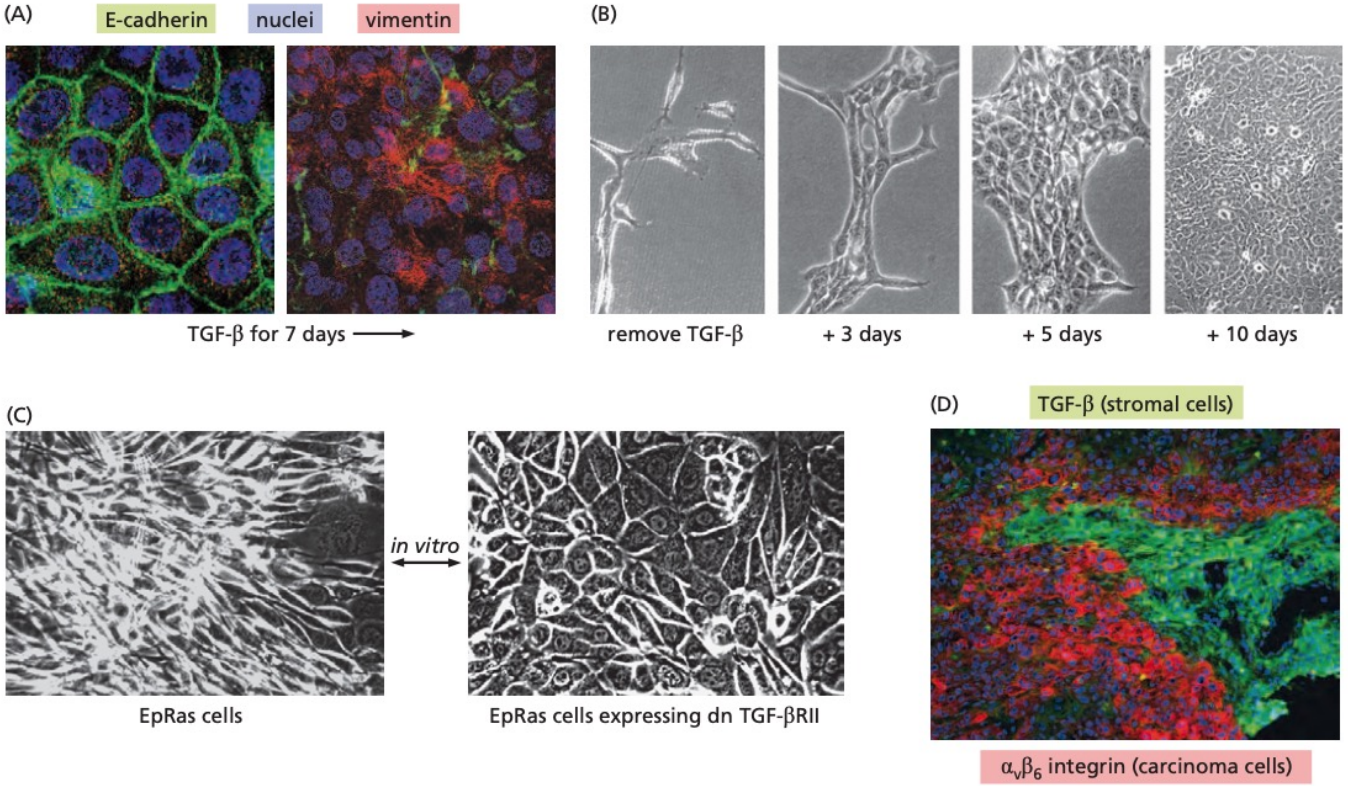
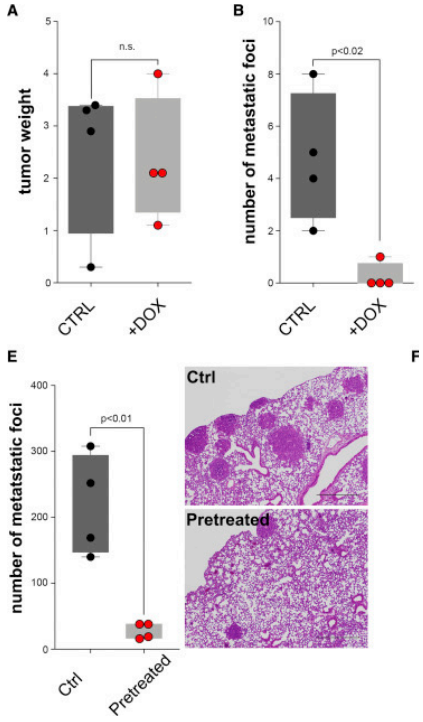
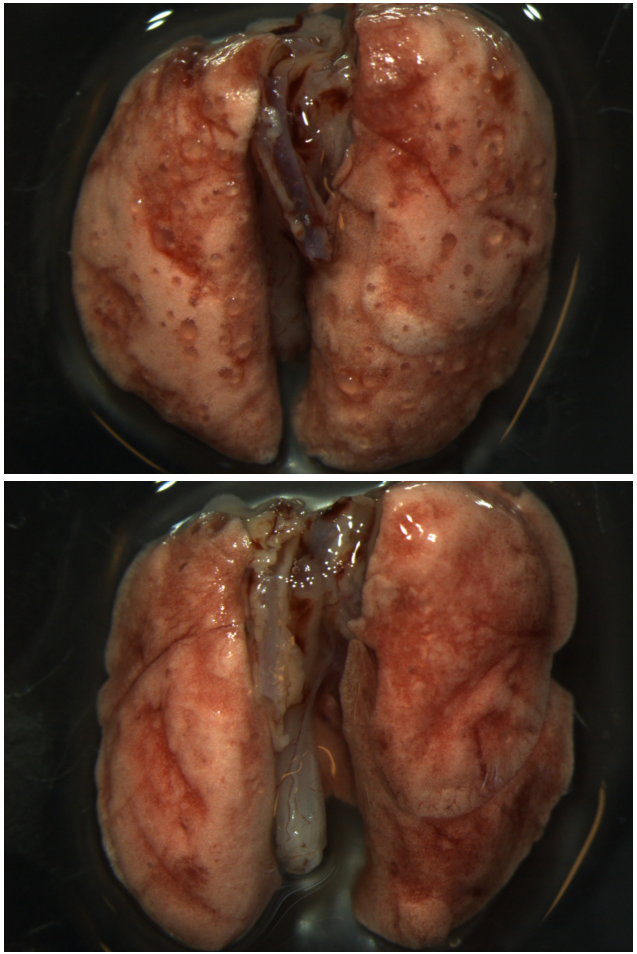


Figure 14.19 Control of the EMT by TGF-β and its effects on tumorigenic cells Immortalized mouse mammary epithelial cells epithelial transition (MET). (C) Use of a dominant-negative (dn) type II TGF-β receptor (which effectively blocks autocrine TGF-β

Weinberg, The Biology of Cancer 2nd ed.

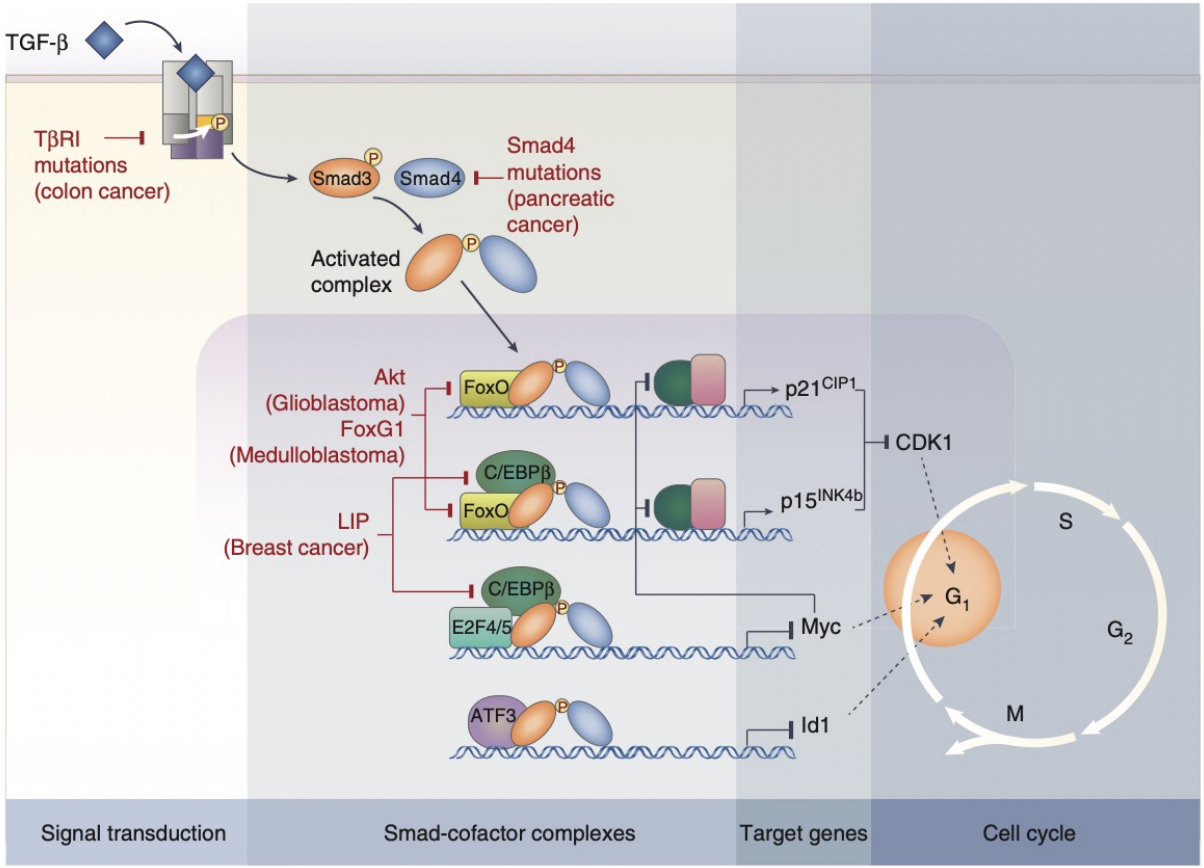
The TGFβ pathway





Fico et al., 2019

The TGFβ pathway: mutations in cancer



Seoane & Gomis, 2017

Metabolic alterations

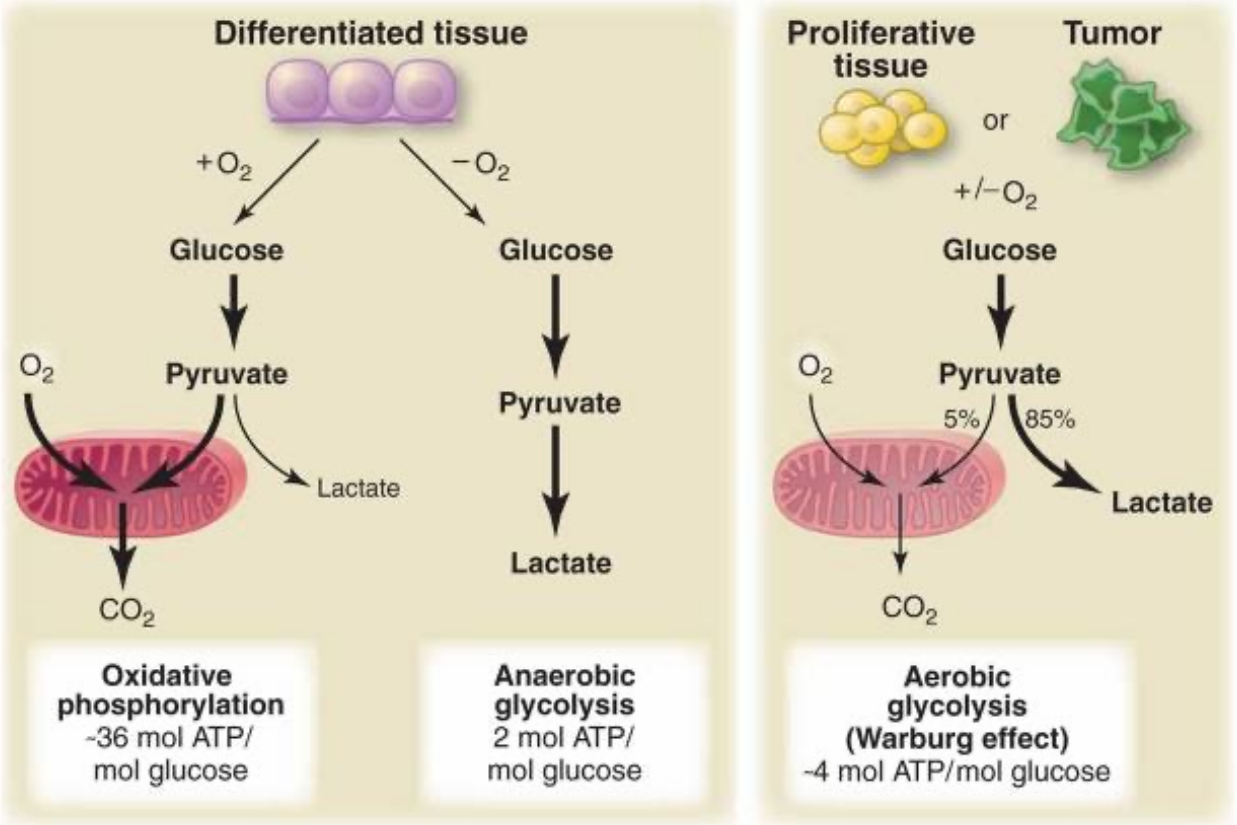
- Change the use of metabolites (associated with cancer cell proliferation)

- Synthesis of new metabolites (associated with mutation in some genes)

Metabolic alterations

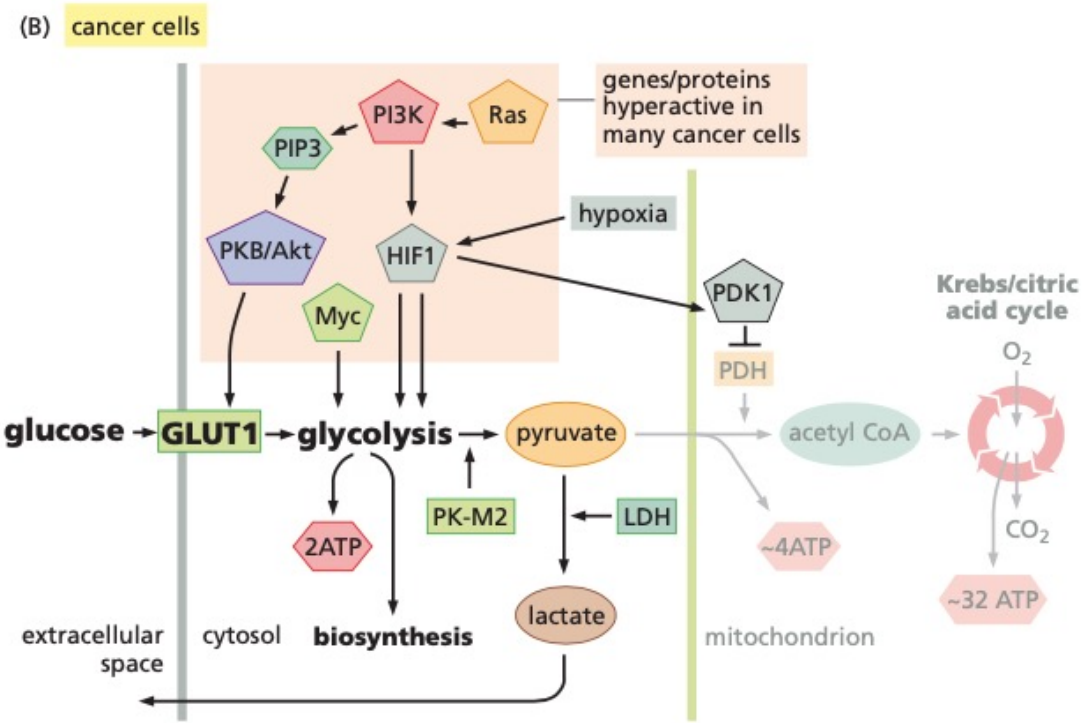
Cancer cells have a different diet compared to the normal cells

The Warburg effect

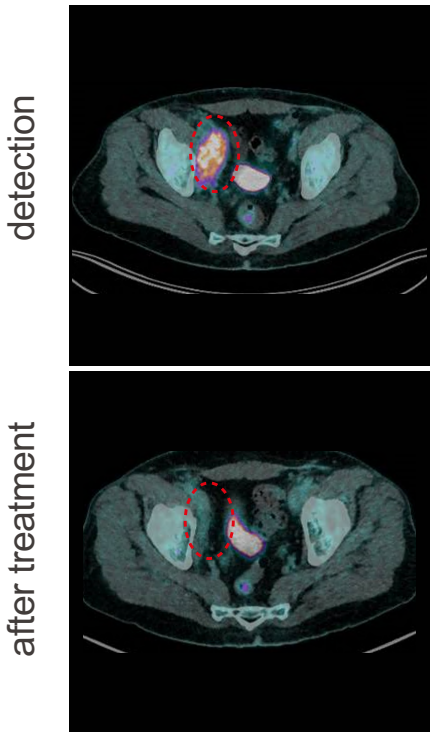


Heiden et al., 2009

The Warburg effect



PET-CT



Weinberg, The Biology of Cancer 2nd ed.

Production of new metabolites

↓

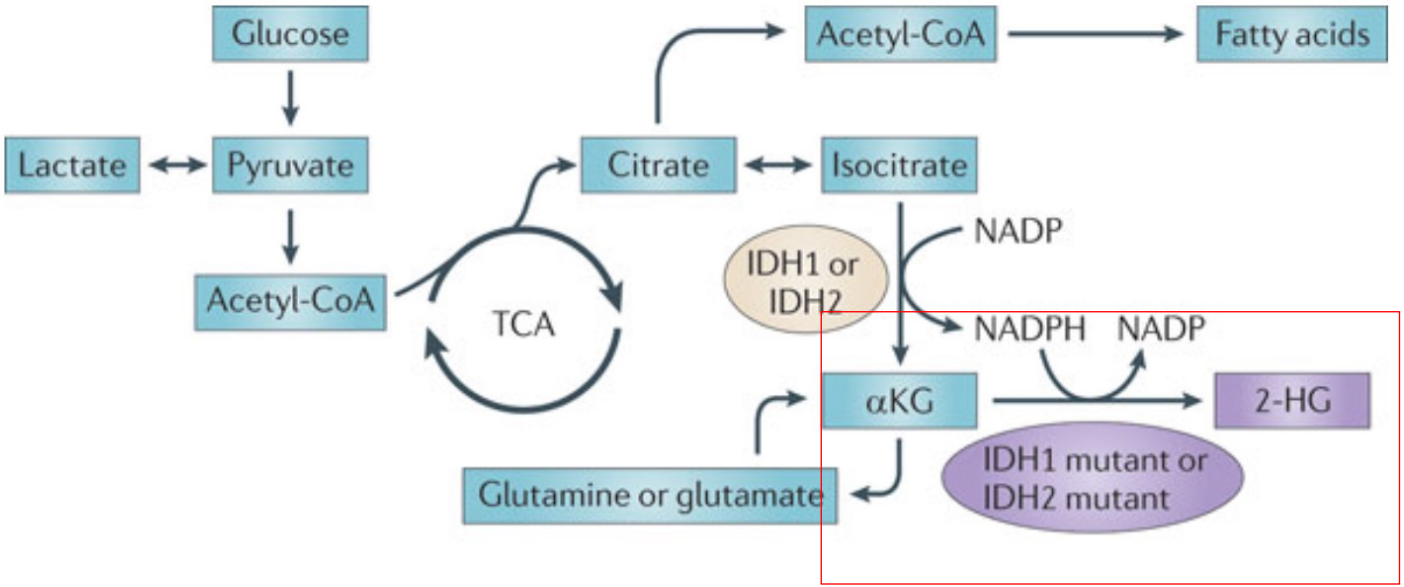
Associated with gene mutations

Mutation of IDH1 and IDH2 genes

IDH1 R132X (predominant in glioma; arginine R at position 132 is replaced by another amino acid, giving a missense mutation)

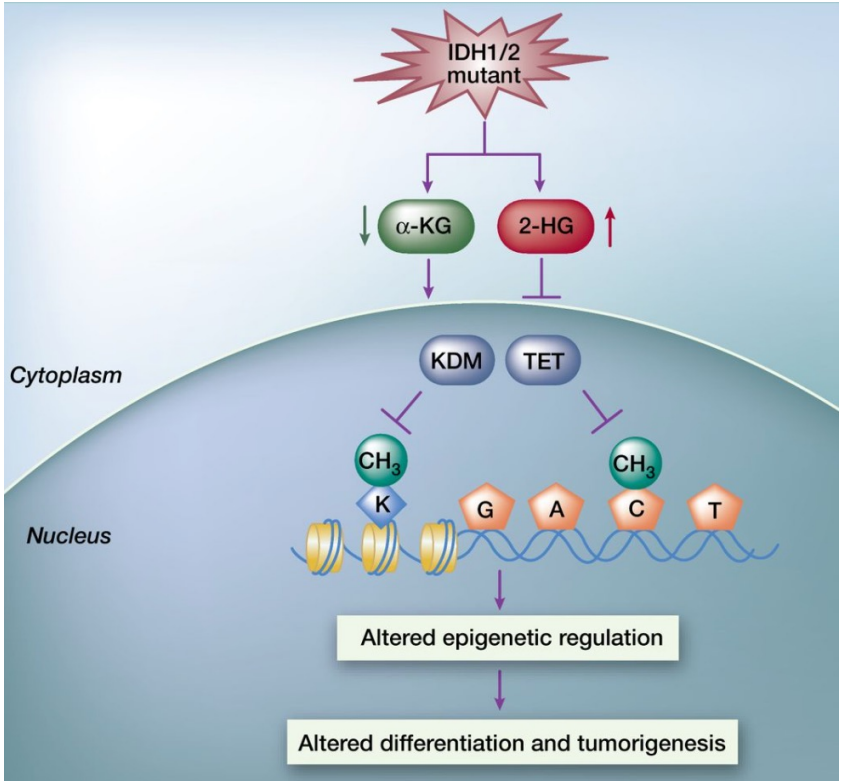
IDH2 R140X (predominant in leukemia; arginine R at position 140 is replaced by another amino acid, giving a missense mutation)

Production of new metabolites: 2-hydroxyglutarate (2-HG)



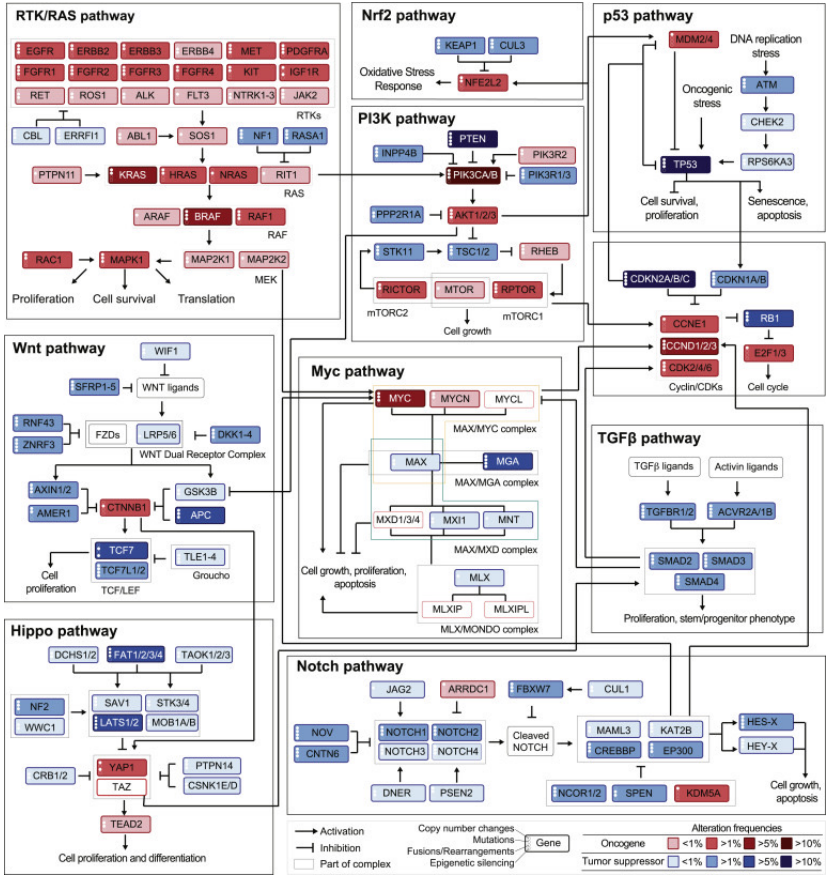
Cairns et al., 2011

Production of new metabolites: 2-HG influence DNA methylation

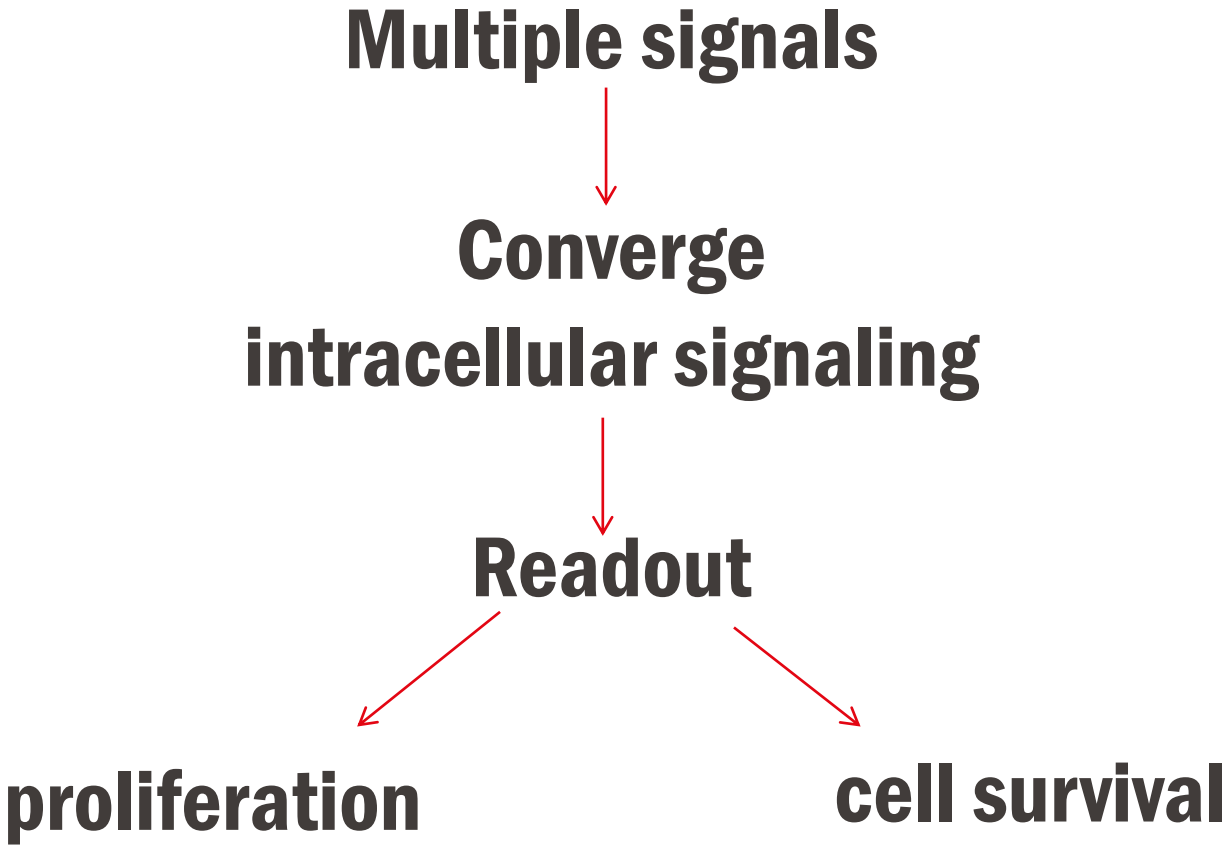


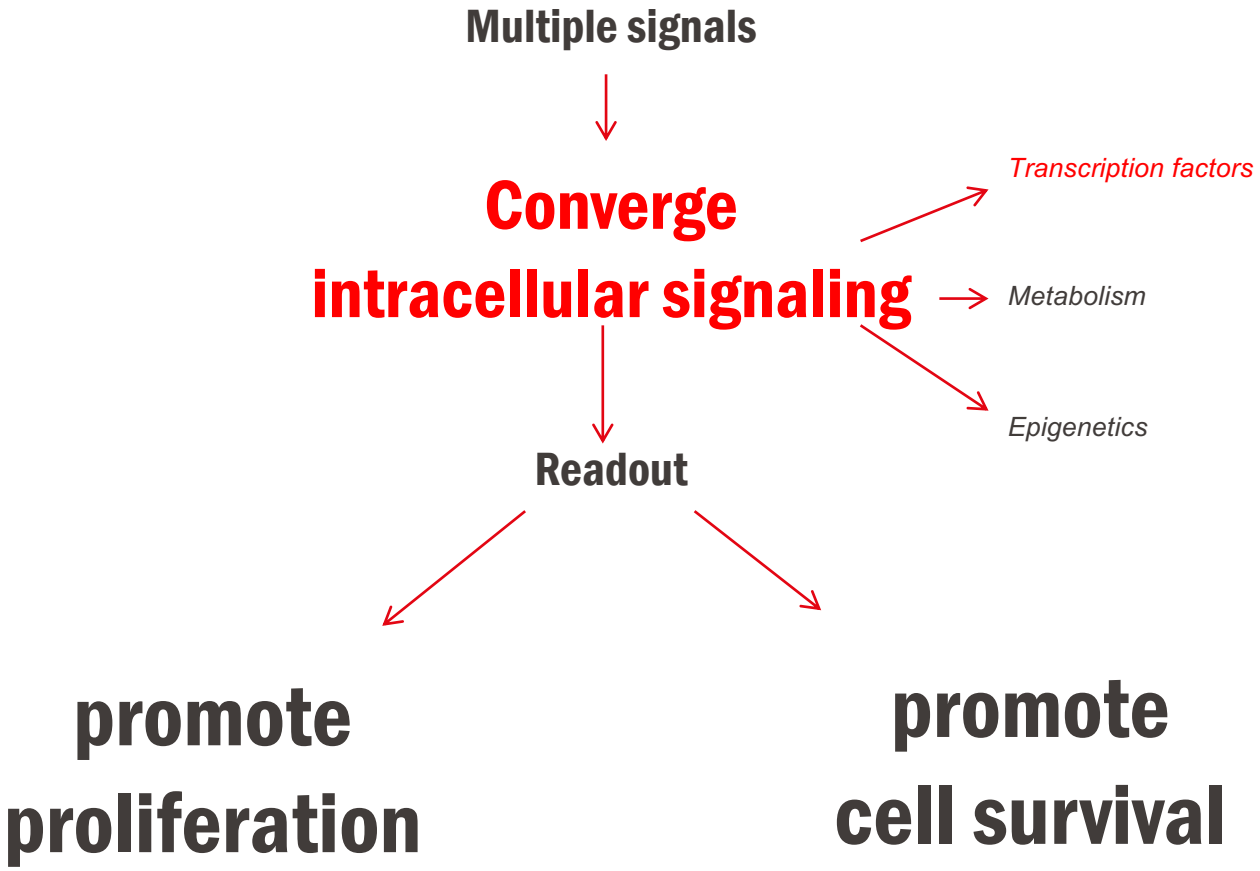
Yang et al., 2012

Many pathways are interconnected (network)

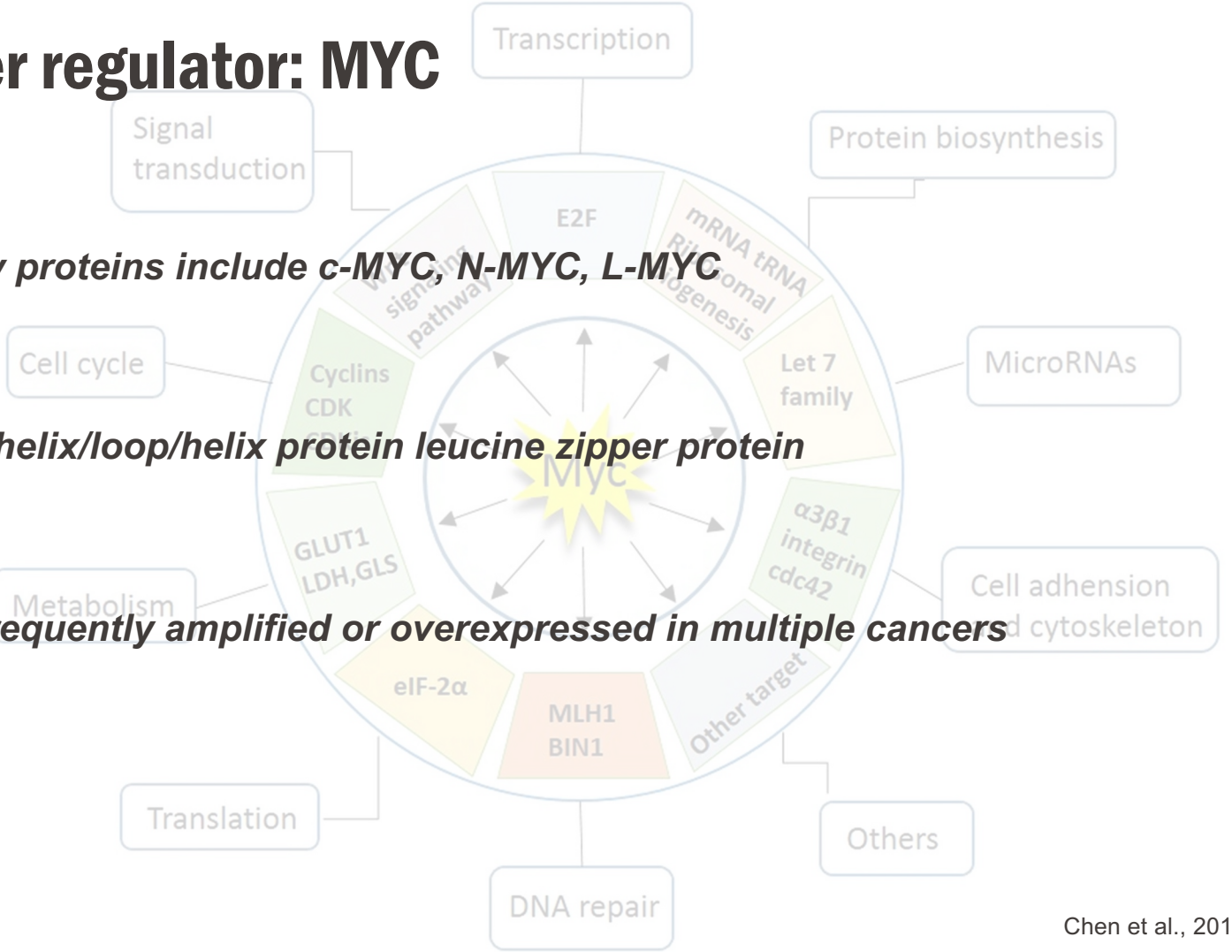


Sánchez-Vega et al., 2018


Multiple signals
Converge
intracellular signaling
Readout
proliferation
cell survival


Multiple signals
Converge
intracellular signaling
Transcription factors
Metabolism
Epigenetics
Readout
promote
proliferation
promote
cell survival

A master regulator: MYC



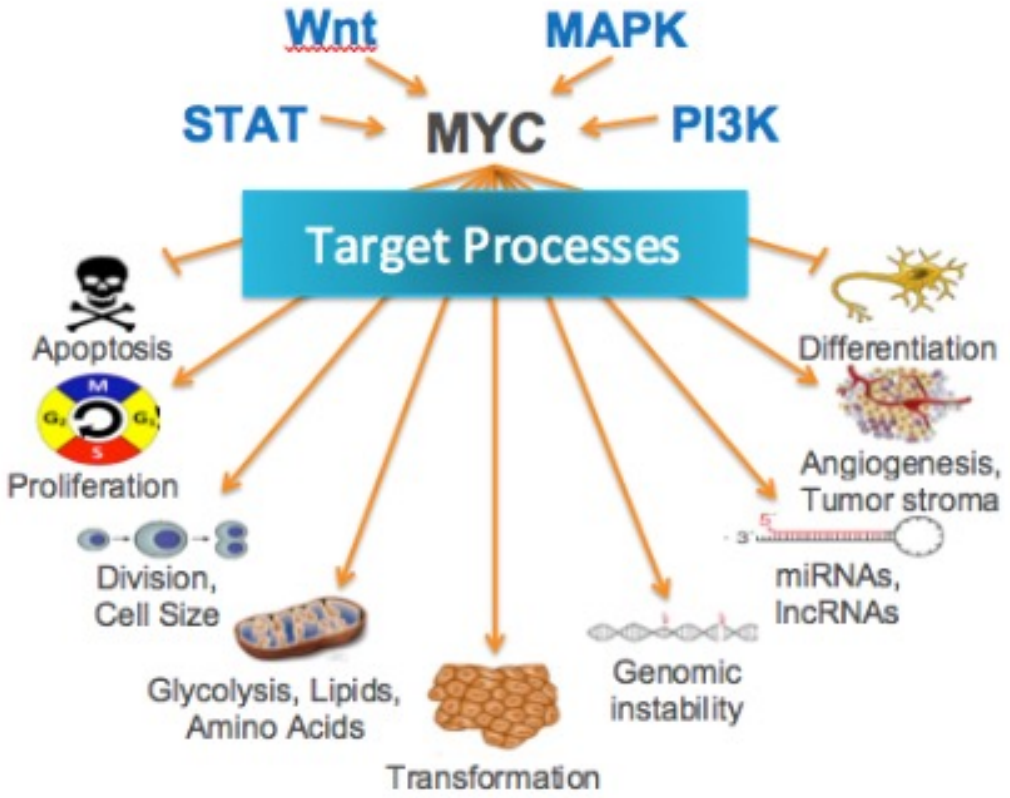
MYC family proteins include c-MYC, N-MYC, L-MYC

MYC is an helix/loop/helix protein leucine zipper protein

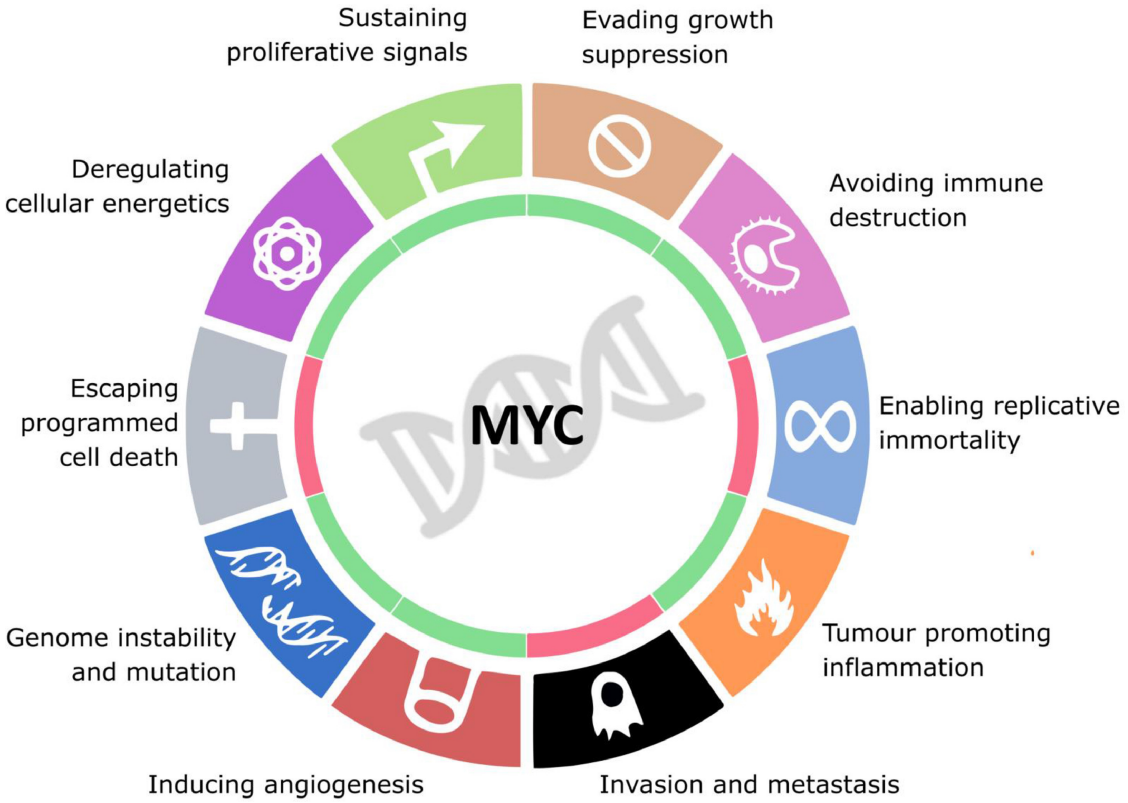
c-MYC is frequently amplified or overexpressed in multiple cancers

Chen et al., 2018

MYC is a transcription factor and potent oncogene

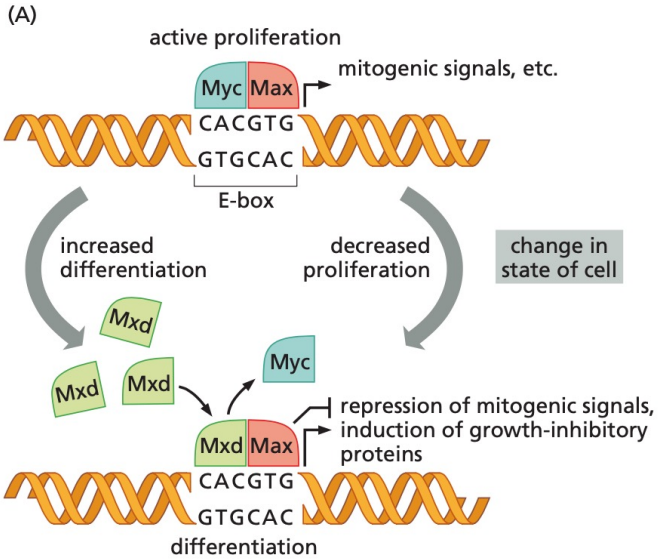


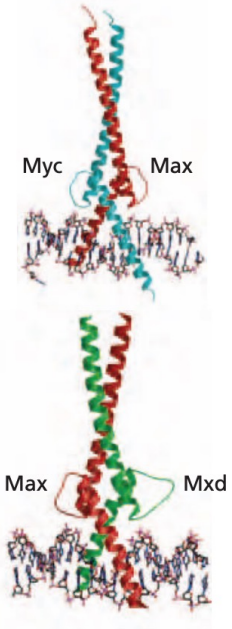
MYC regulates all the hallmarks of cancer



Llombart & Mansour., 2021

How does MYC control several biological processes?





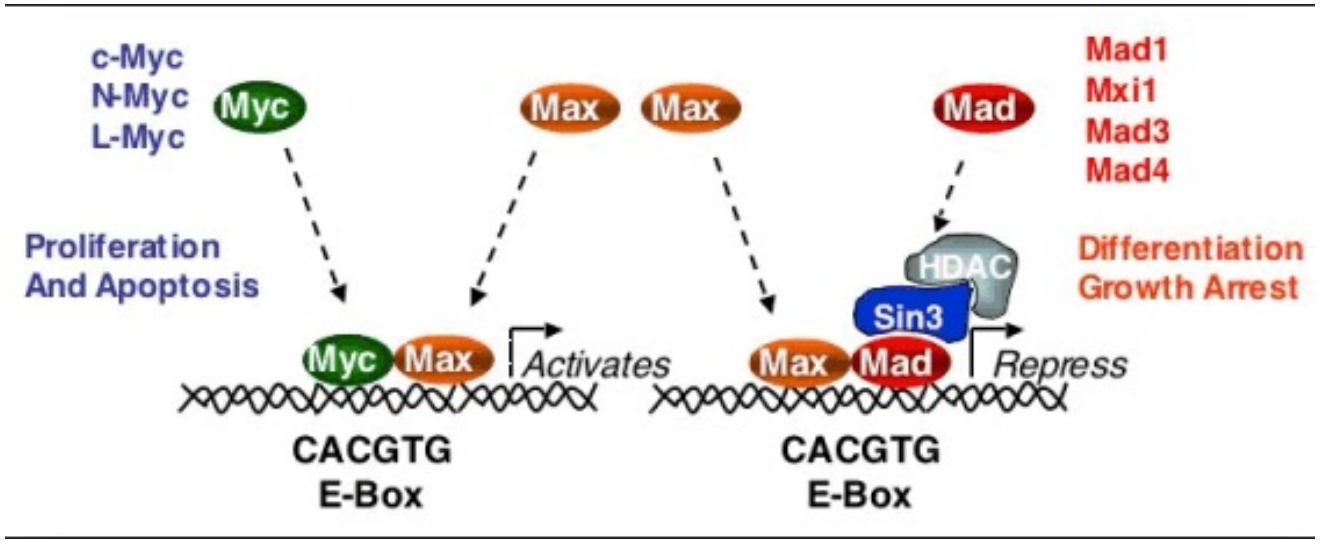
MYC forms dimer with MAX

MYC/MAX complex binds specific DNA sequence (E-BOX)

MYC activates the expression of many genes

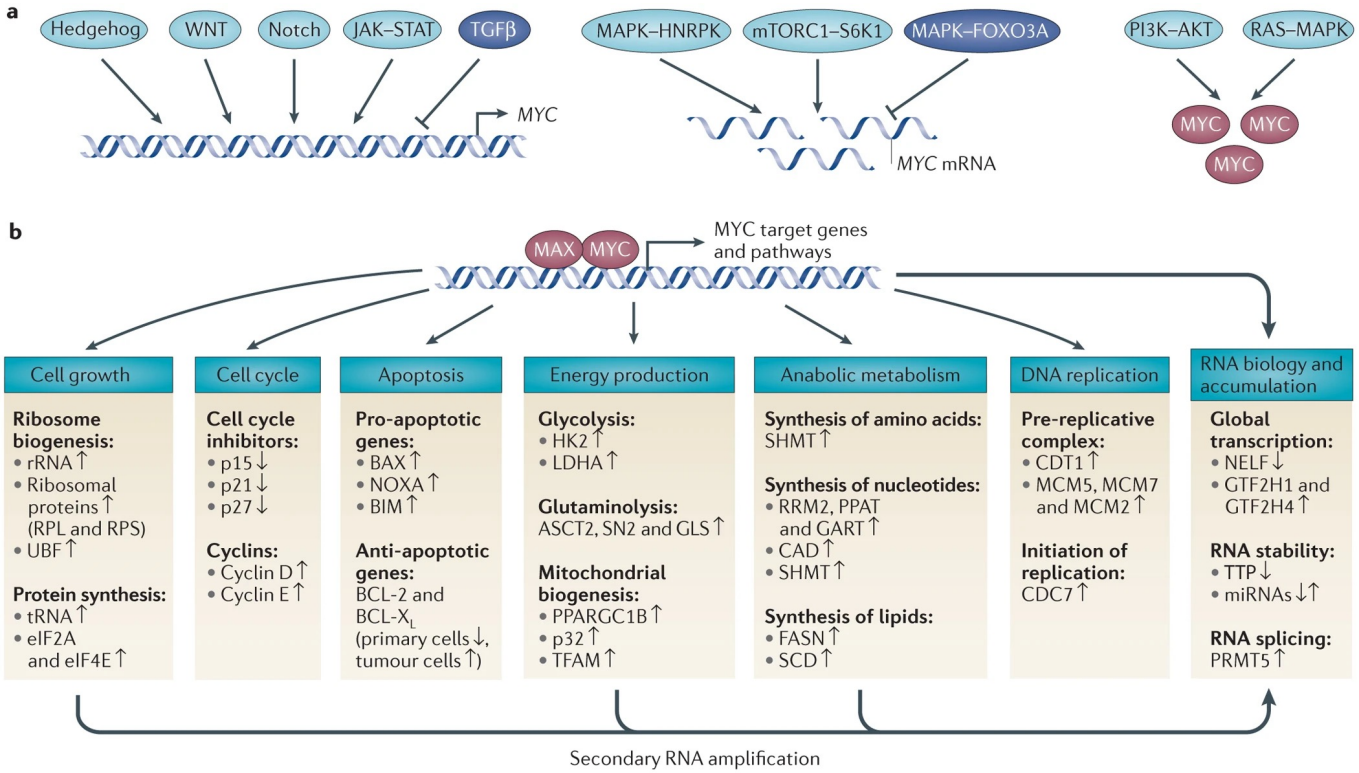
Weinberg, The Biology of Cancer 2nd ed.

MYC regulates gene expression



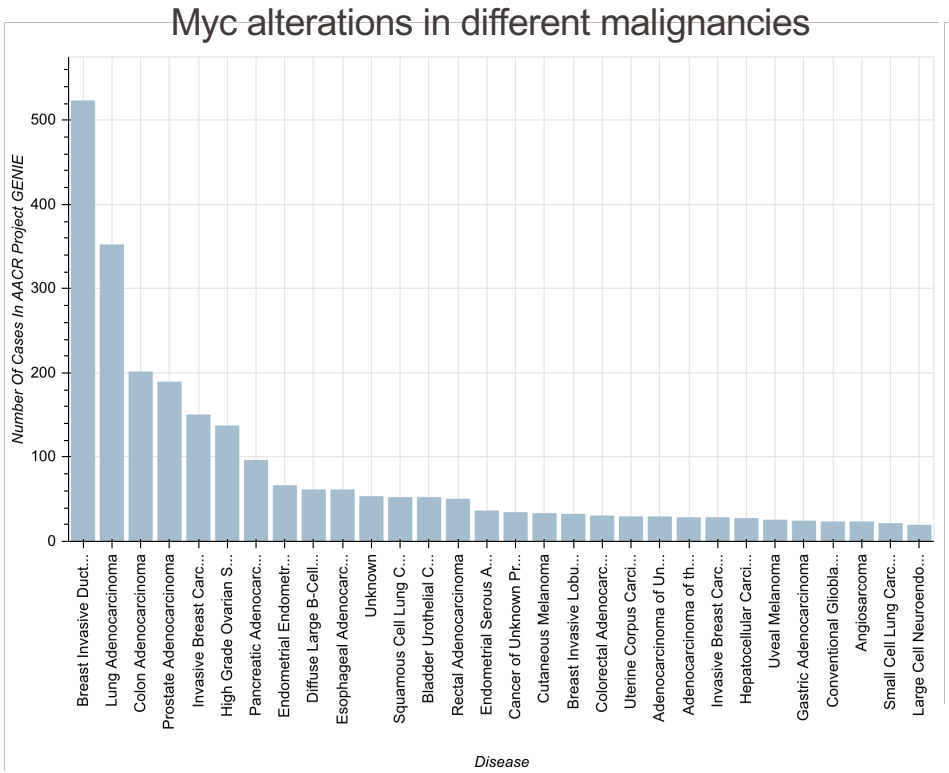
Antagonistic effects of Max partners

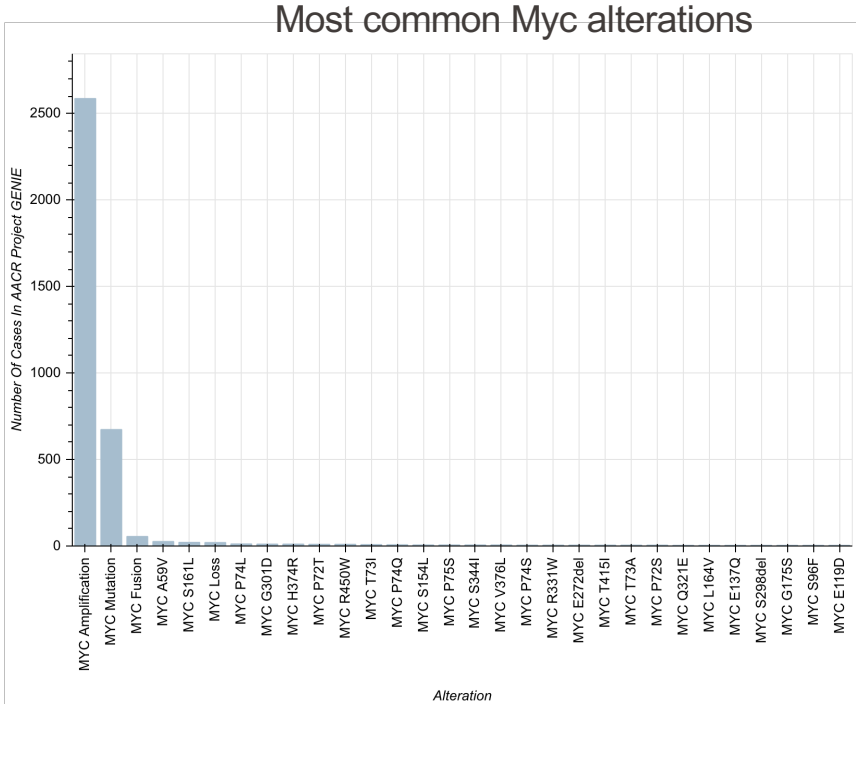
MYC targets



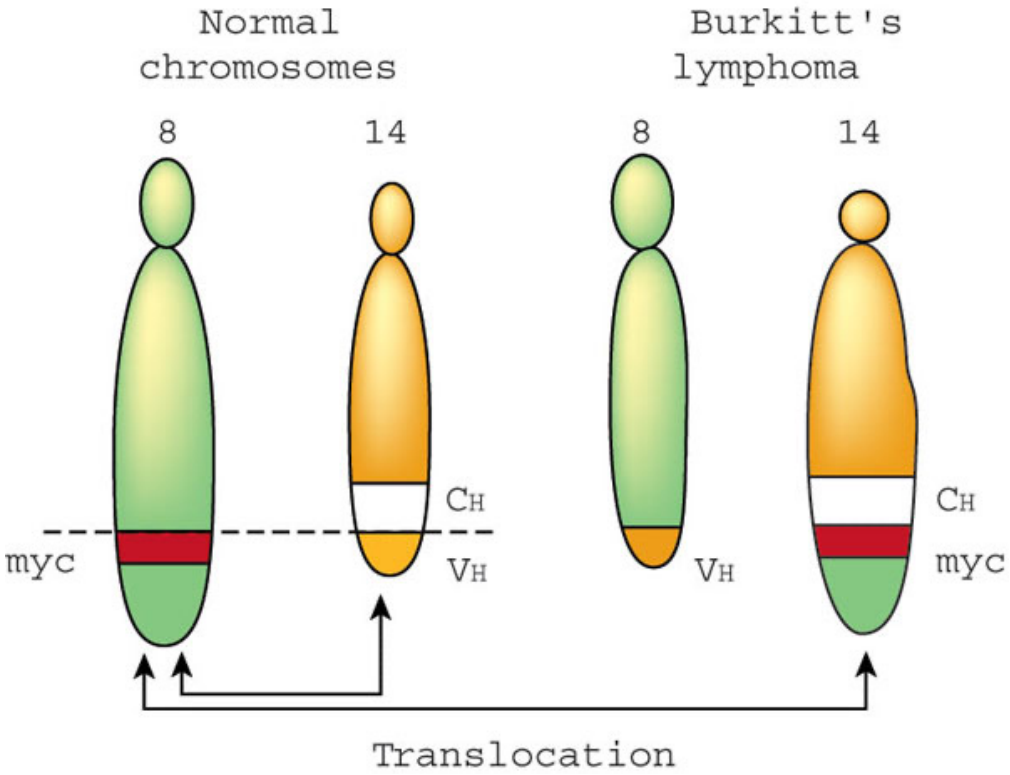
Kress et al., 2015

MYC alterations in cancer

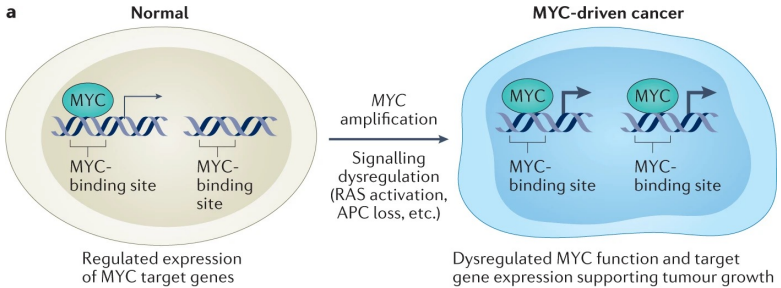


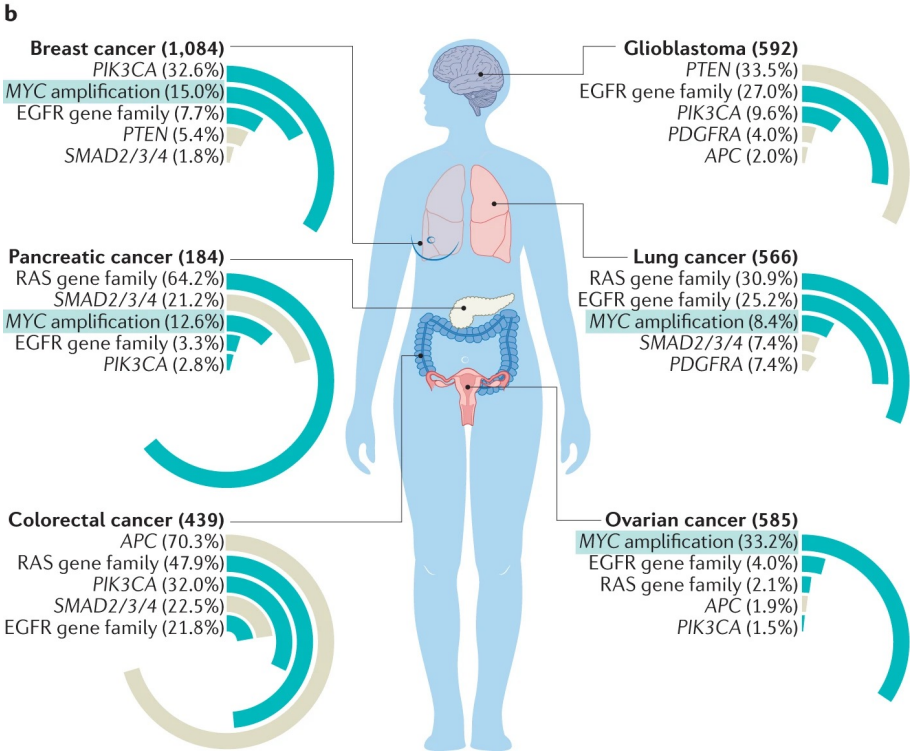


MYC translocation in Burkitt lymphoma



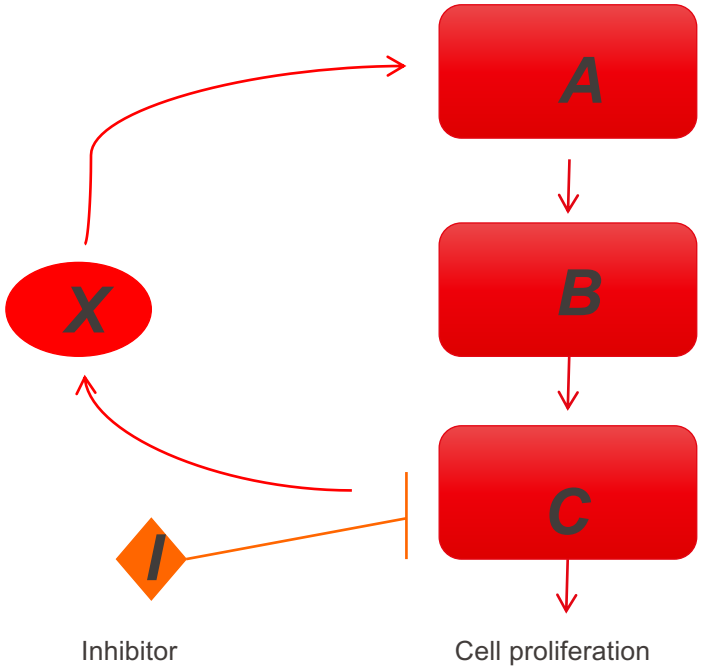
MYC amplification (copy number increase) in cancer



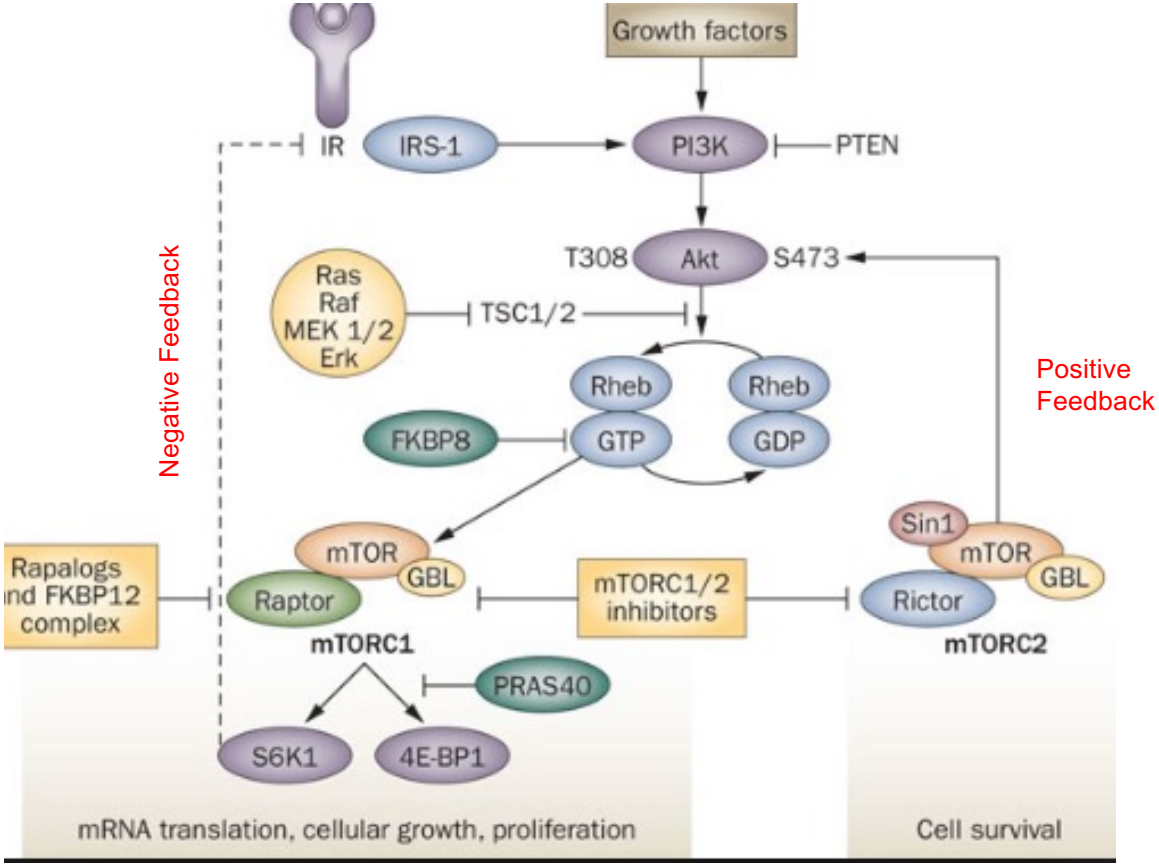


Lourenco et al., 2021

The pathway signal is not linear but can be supported by feedback activations



mTOR re-activates AKT



How can we analyze a pathway's activity?

Visualizing the signaling:

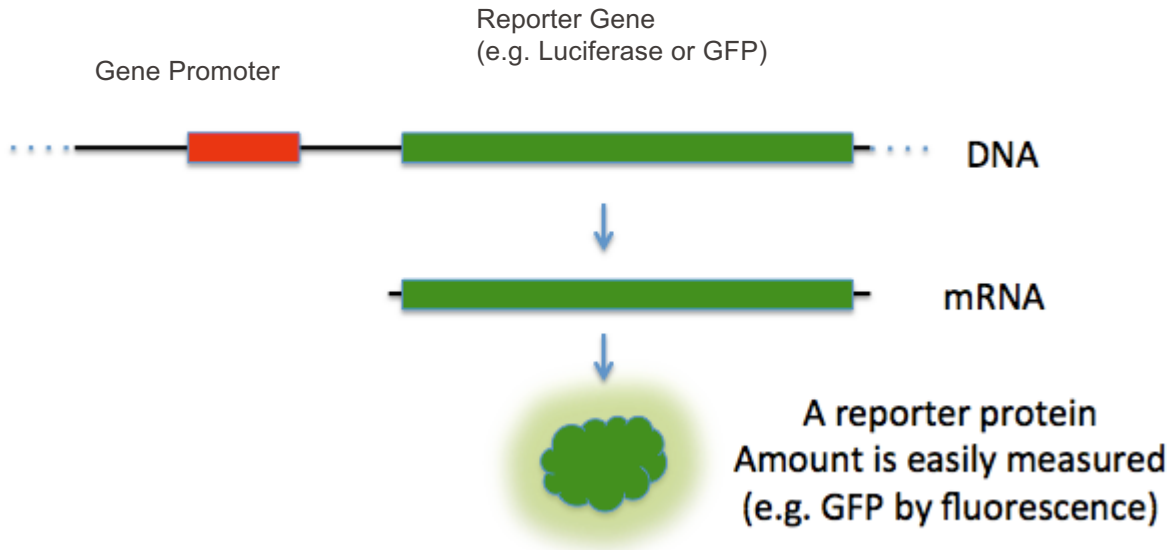
Reporter constructs
Protein-protein interactions (FRET)

Understand the signaling connection:

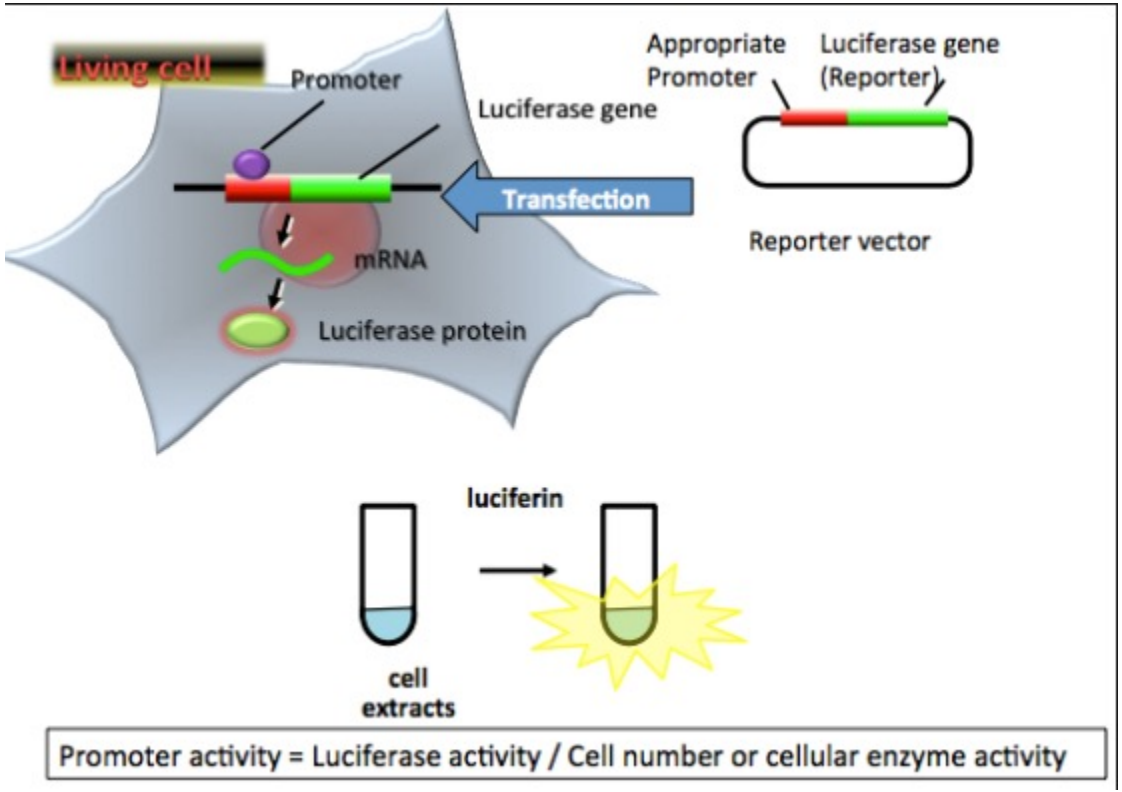
yeast two hybrid screen/affinity purification/mass spectrometry
Mass-cytometry (Cytof)

Reporter Construct

To understand if a transcription factor regulates the expression of specific genes



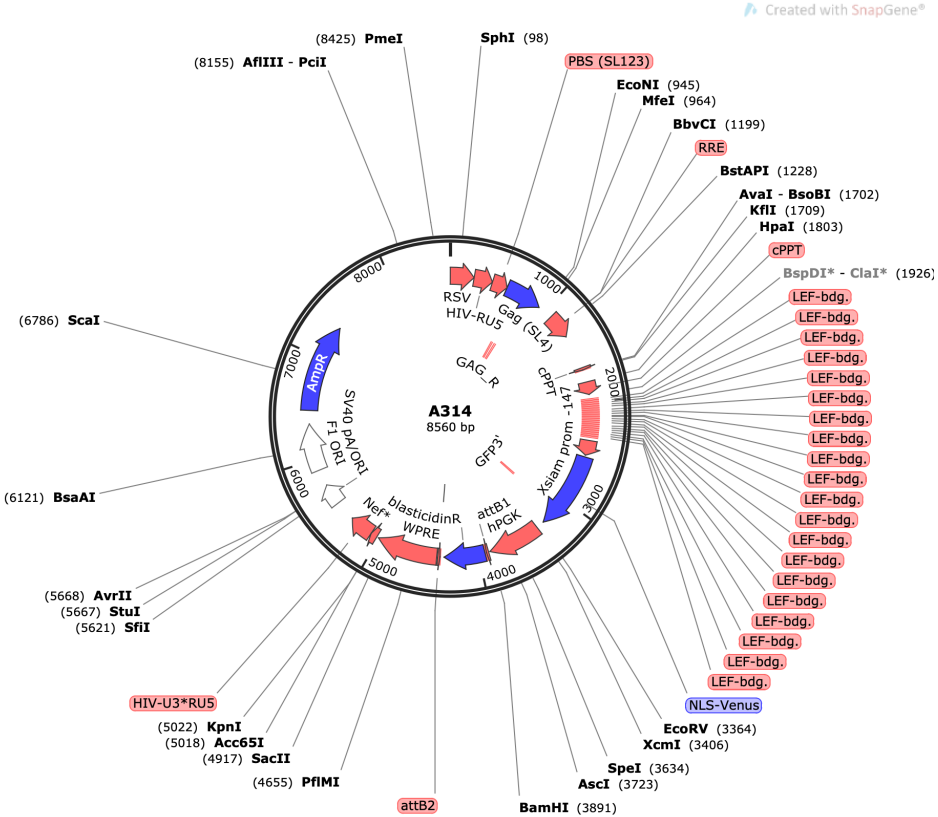
Reporter Construct



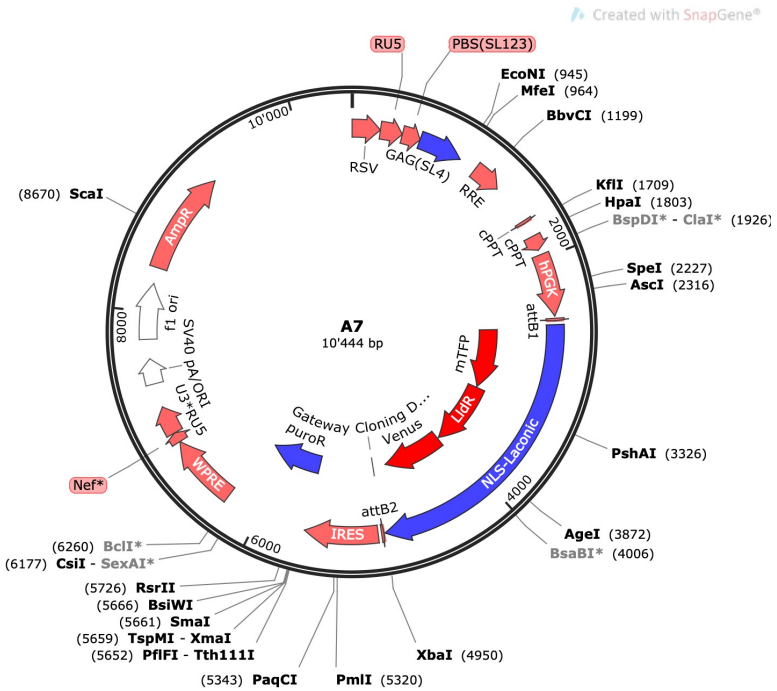
Reporter Construct: examples

CANCER BIOLOGY / FREQUENTLY ALTERED PATHWAYS IN CANCER

Wnt reporter



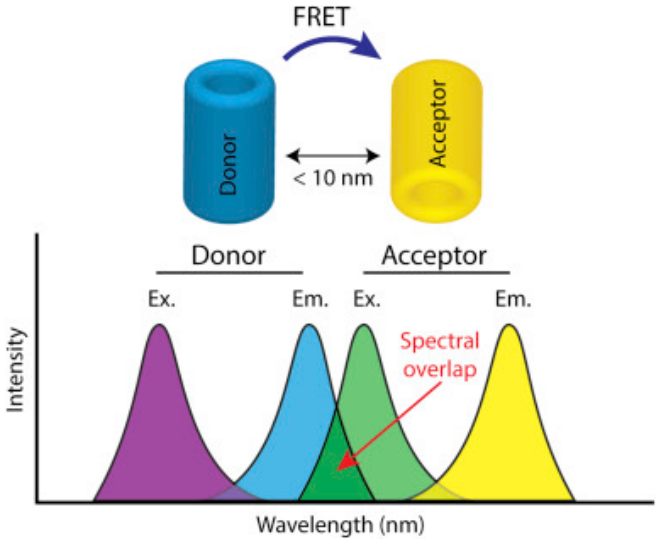
lactate reporter

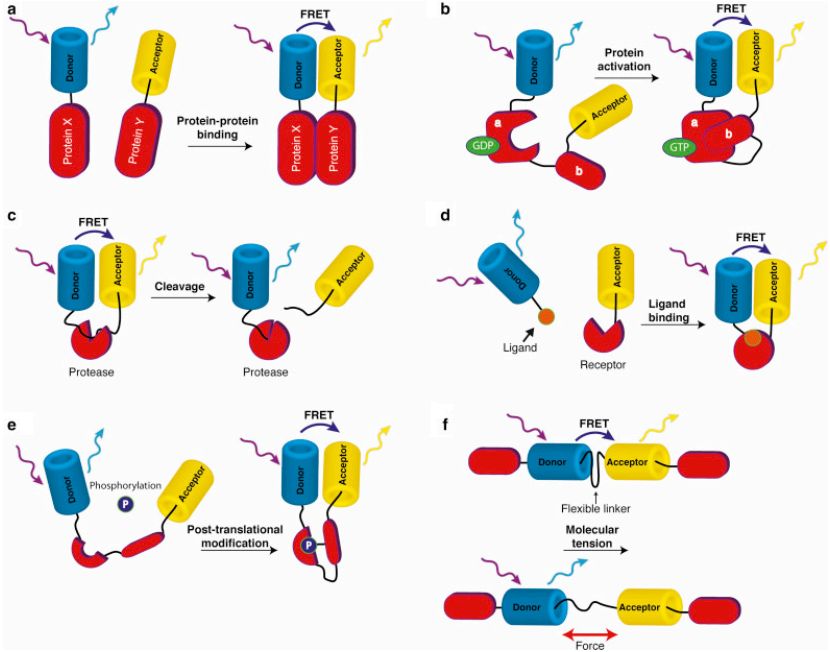


FRET (Förster Resonance Energy Transfer)

Used to study (in living cells):

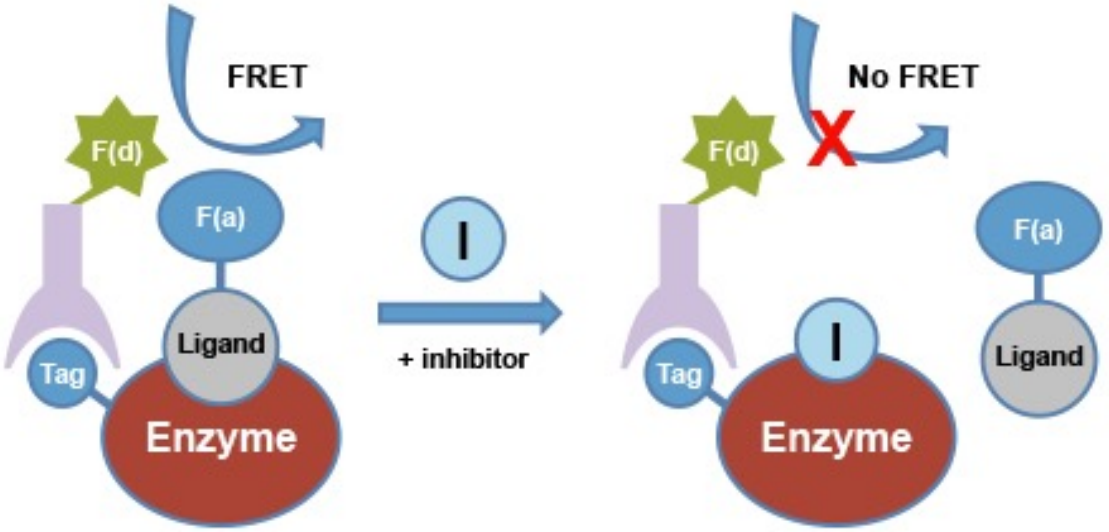
Protein-protein interactions
Ligand binding to a receptor
Molecular dimarization





Broussard & Green, 2017

FRET with specific inhibitors



How can we analyze the pathways activity?

Visualizing the signaling:

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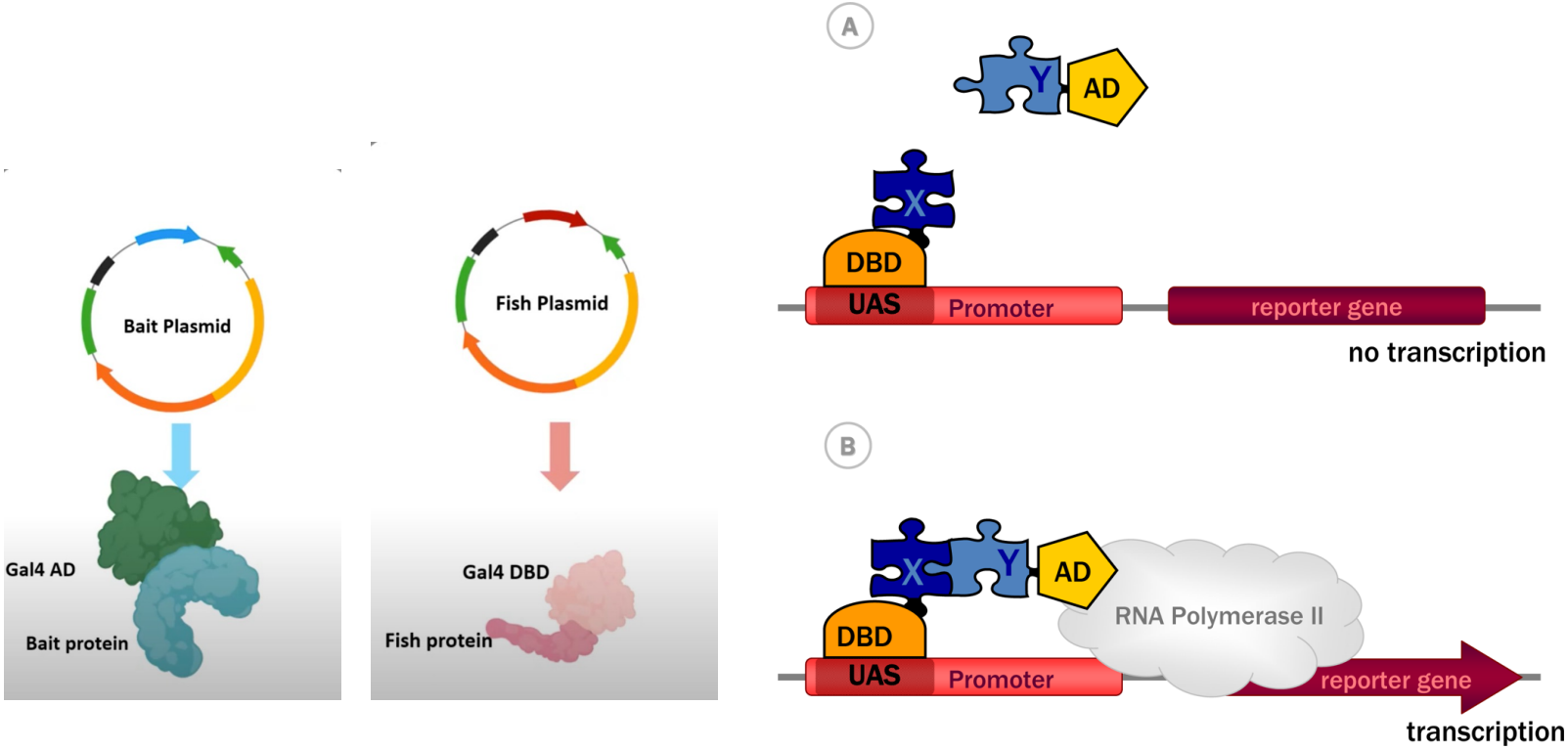
Understand the signaling connection:

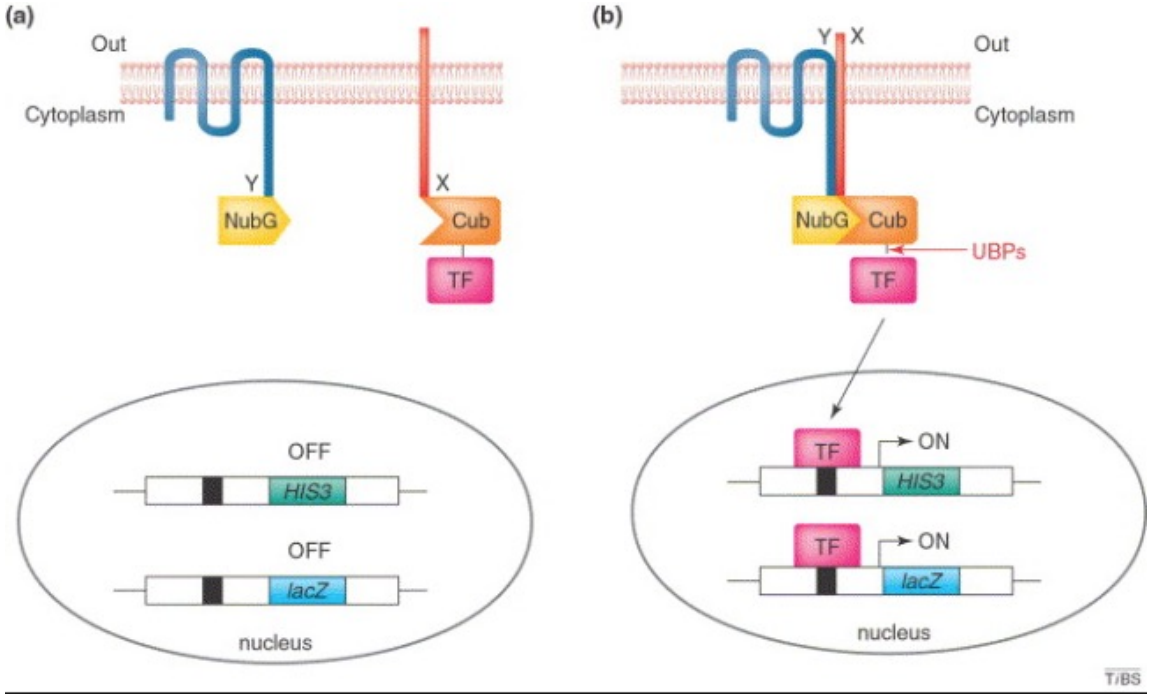
yeast two hybrid screen/affinity purification/mass spectrometry
Mass-cytometry (CyTOF)

Yeast two hybrid screen (to discover protein-protein interactions)

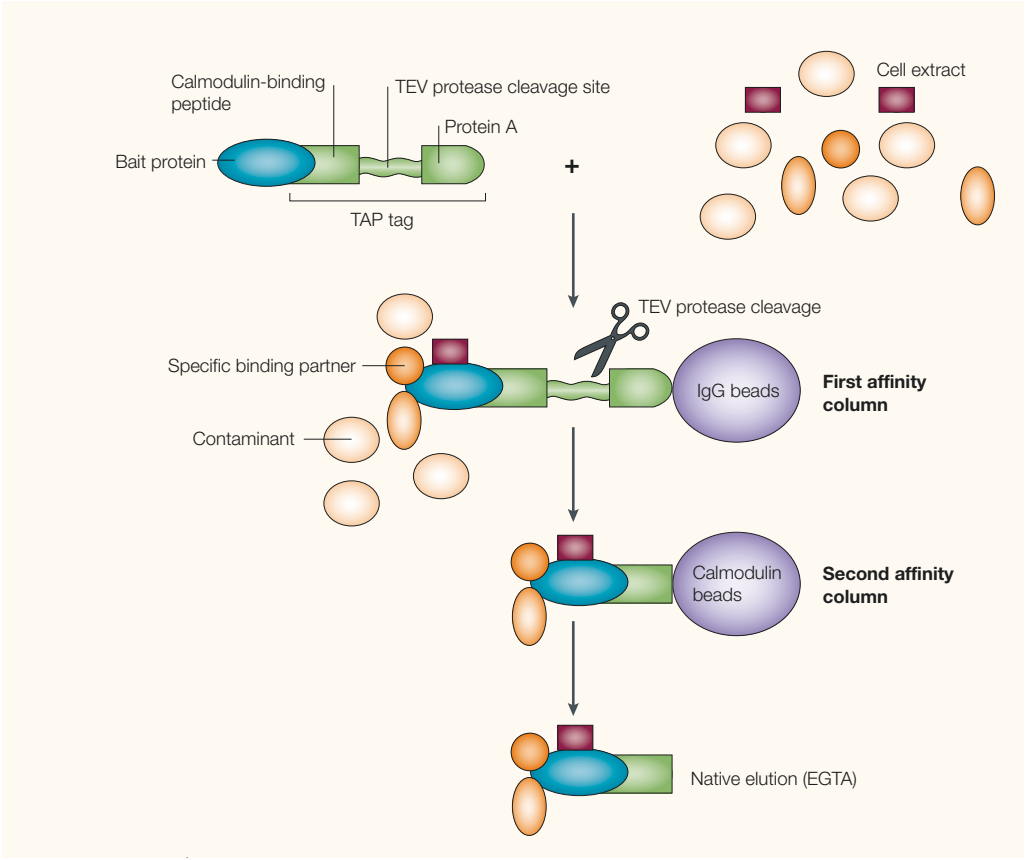


Brückner et al., 2009

Yeast two hybrid screen (transcription activation)



Protein-protein interaction by affinity purification



Huber, 2003

Frequently altered pathways in Cancer

https://www.pnas.org/content/pnas/105/2/692.full.pdf