Signaling pathways in Cancer

Understand integrated responses to signaling pathways and their context dependence and relevance to cancer

- Ras, PIK3/AKT
- Wnt
- Shh
- Notch
- YAP/TAZ
- TGFβ/BMP

Signal transduction pathways integrate multiple inputs

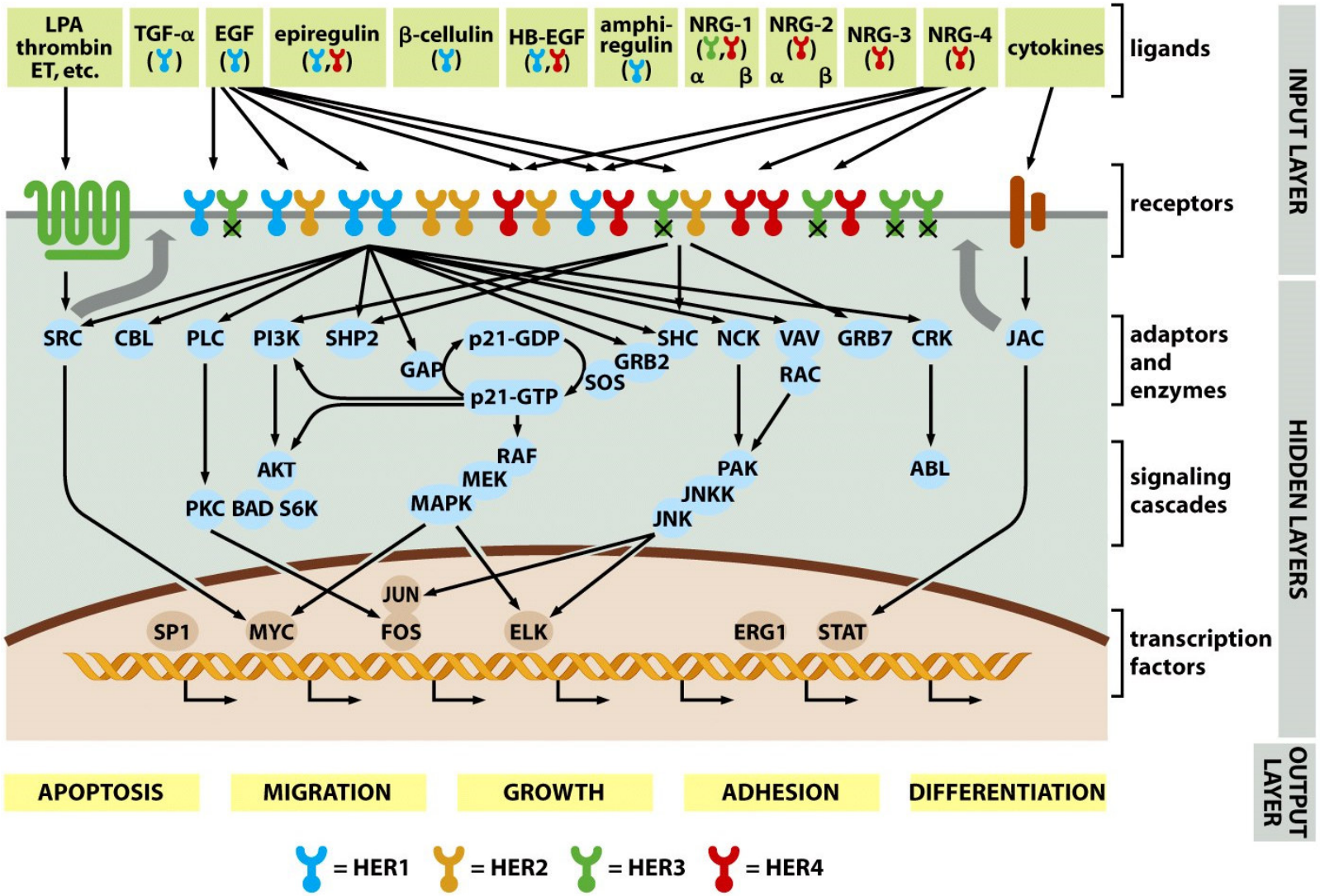
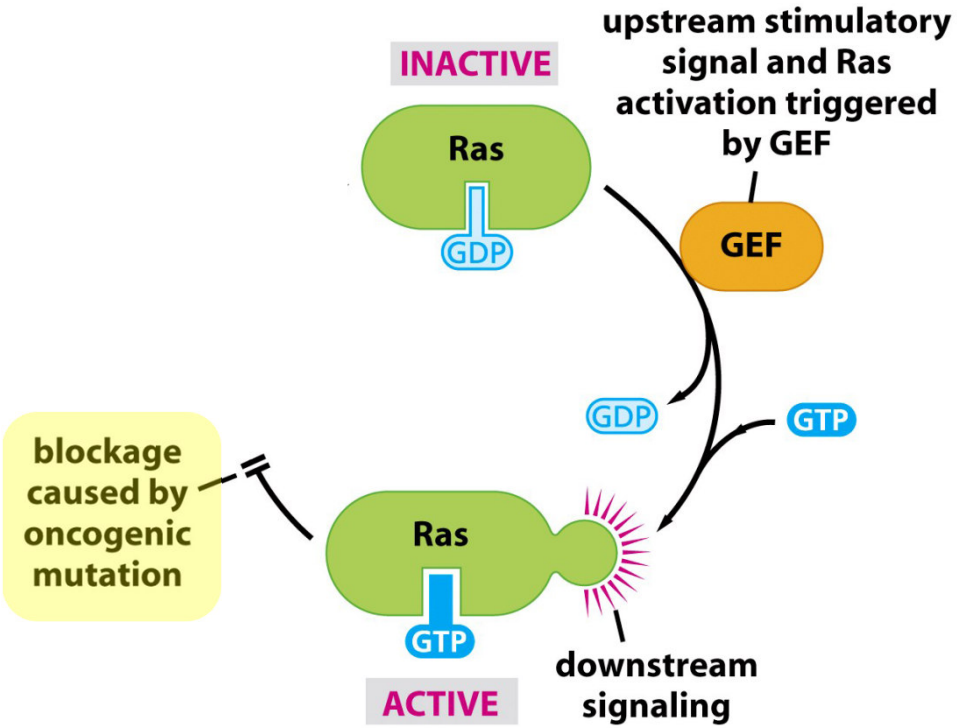


Figure 5-1 The Biology of Cancer (© Garland Science 2007)

Seemingly linear pathways are actually interconnected in signaling networks
=> Output of a given signal can vary substantially in different cell types

Oncogenic mutations promoter the active, GTP-bound form of Ras



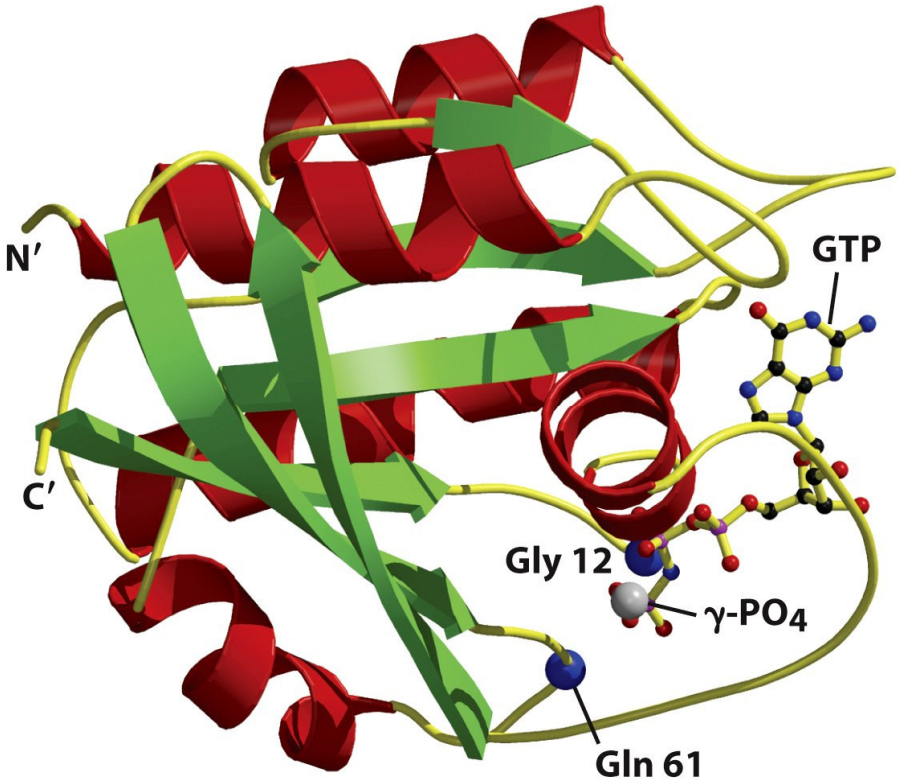
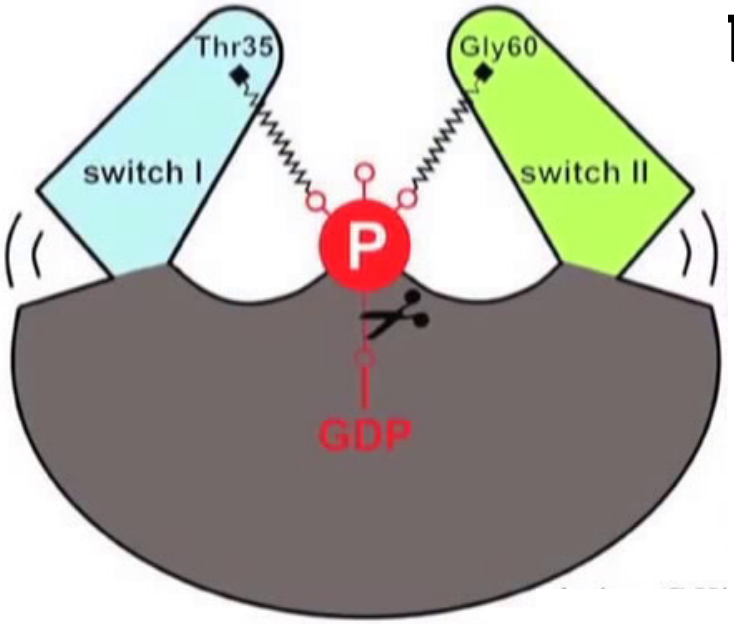


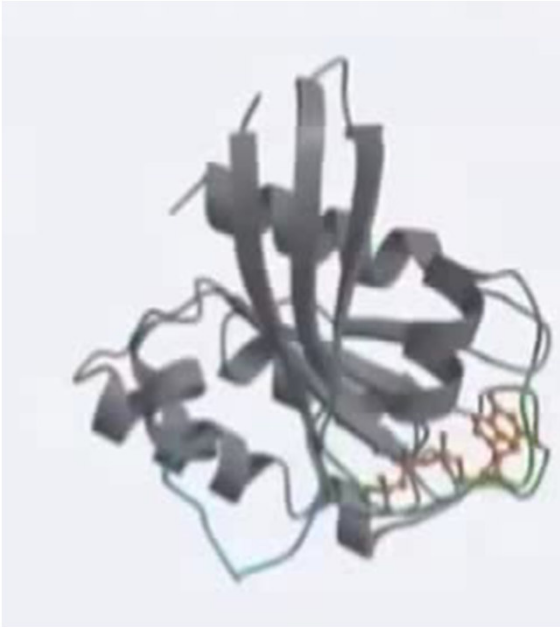
Figure 5-31 The Biology of Cancer (© Garland Science 2007)

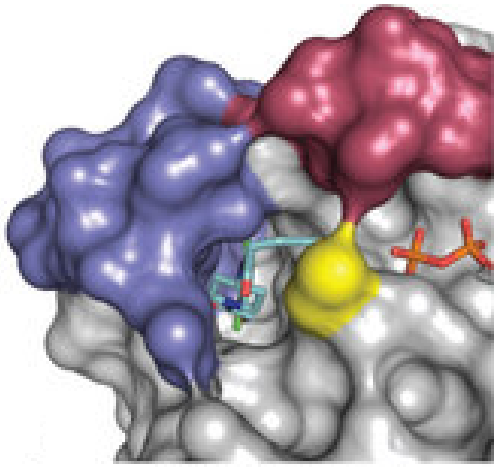
- Gly12 and Gln61 interact with GTP
- mutations in either of these residues block GTP hydrolysis and are oncogenic



Targeting K-Ras

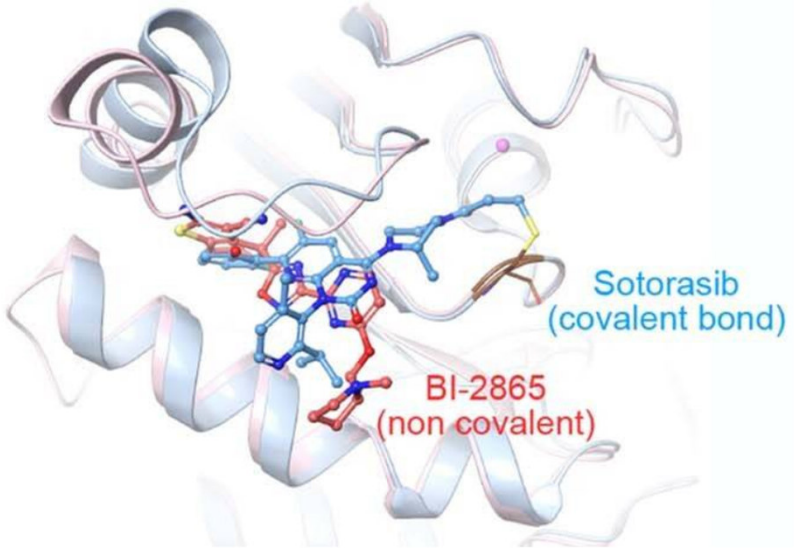
- K-Ras is the most commonly mutated oncogene in human cancers, but drug development was difficult
- The Shokat lab found a new, deep pocket in KrasG12C underneath the effector binding switch-II region
- Sotorasib (FDA approved) targeting this pocket can form a covalent bond with C12 locking KRAS in an inactive GDP-bound state
- based on this design, a new pan-Kras inhibitor was developed by the Lito lab in 2023





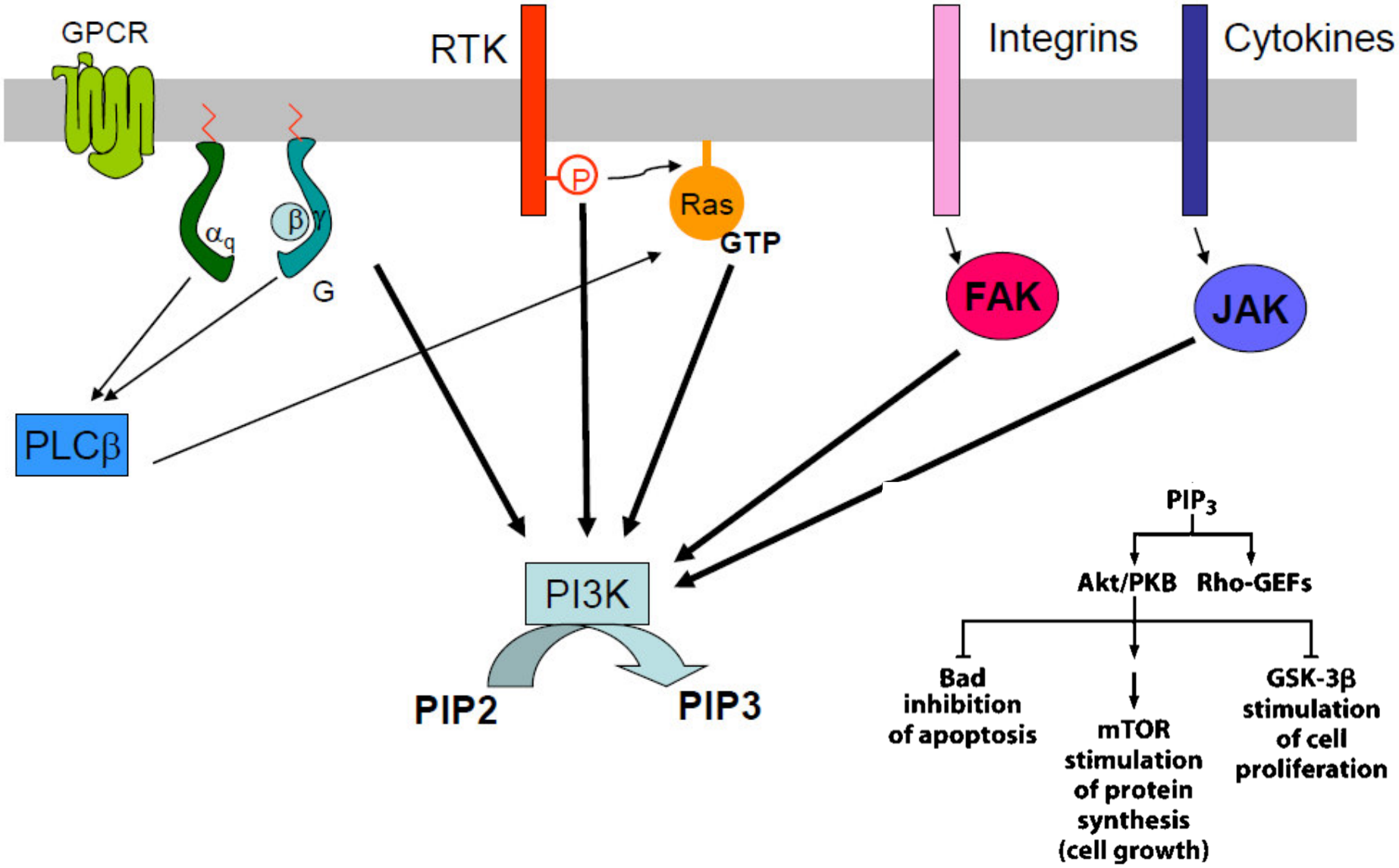
Sotorasib

Ostrem et al., Nature 2013

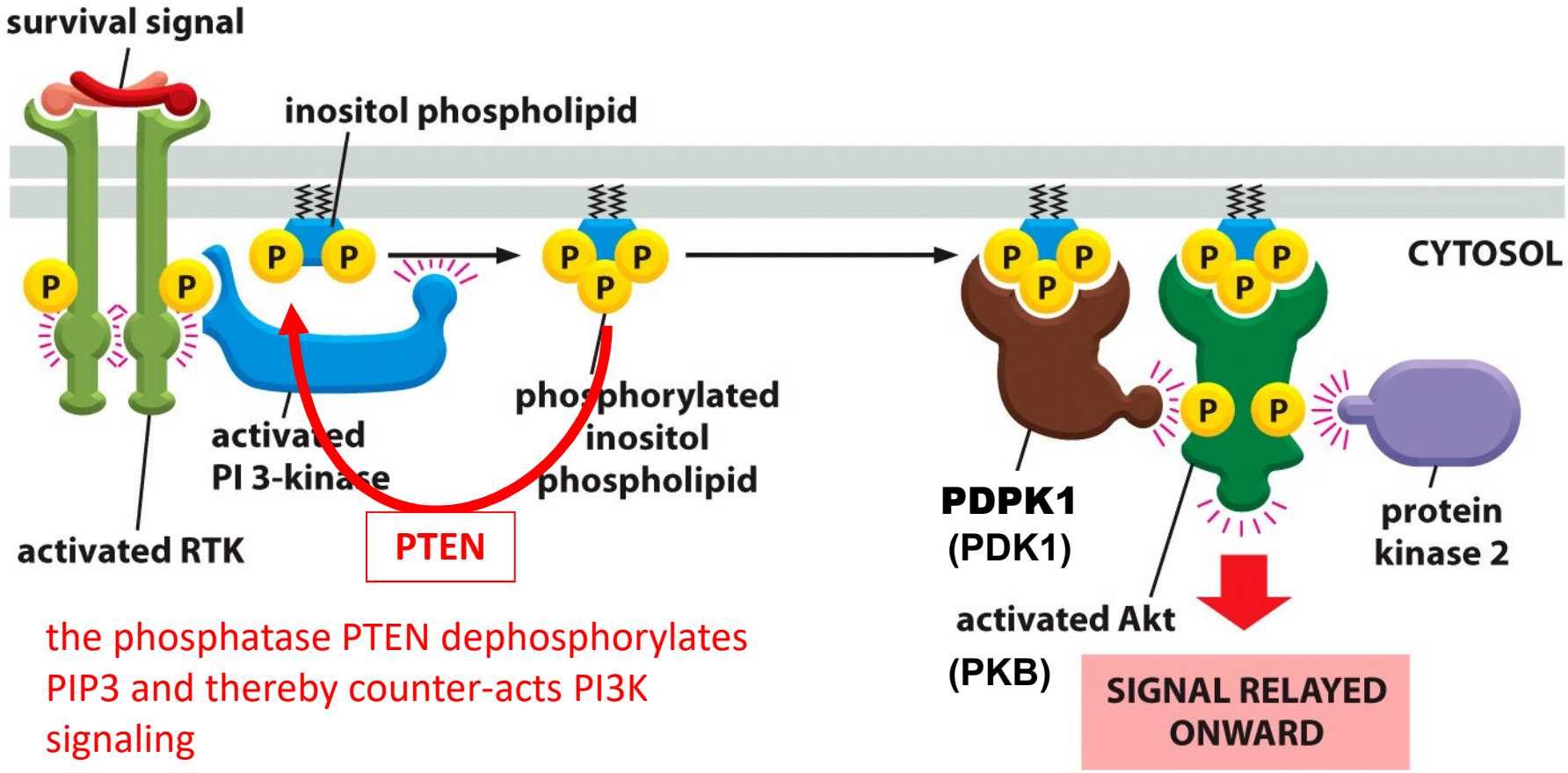


Kim et al., Nature 2023

PI3K is an example of convergence as a signaling node common to multiple upstream signals



Activation of PI3K: downstream effectors

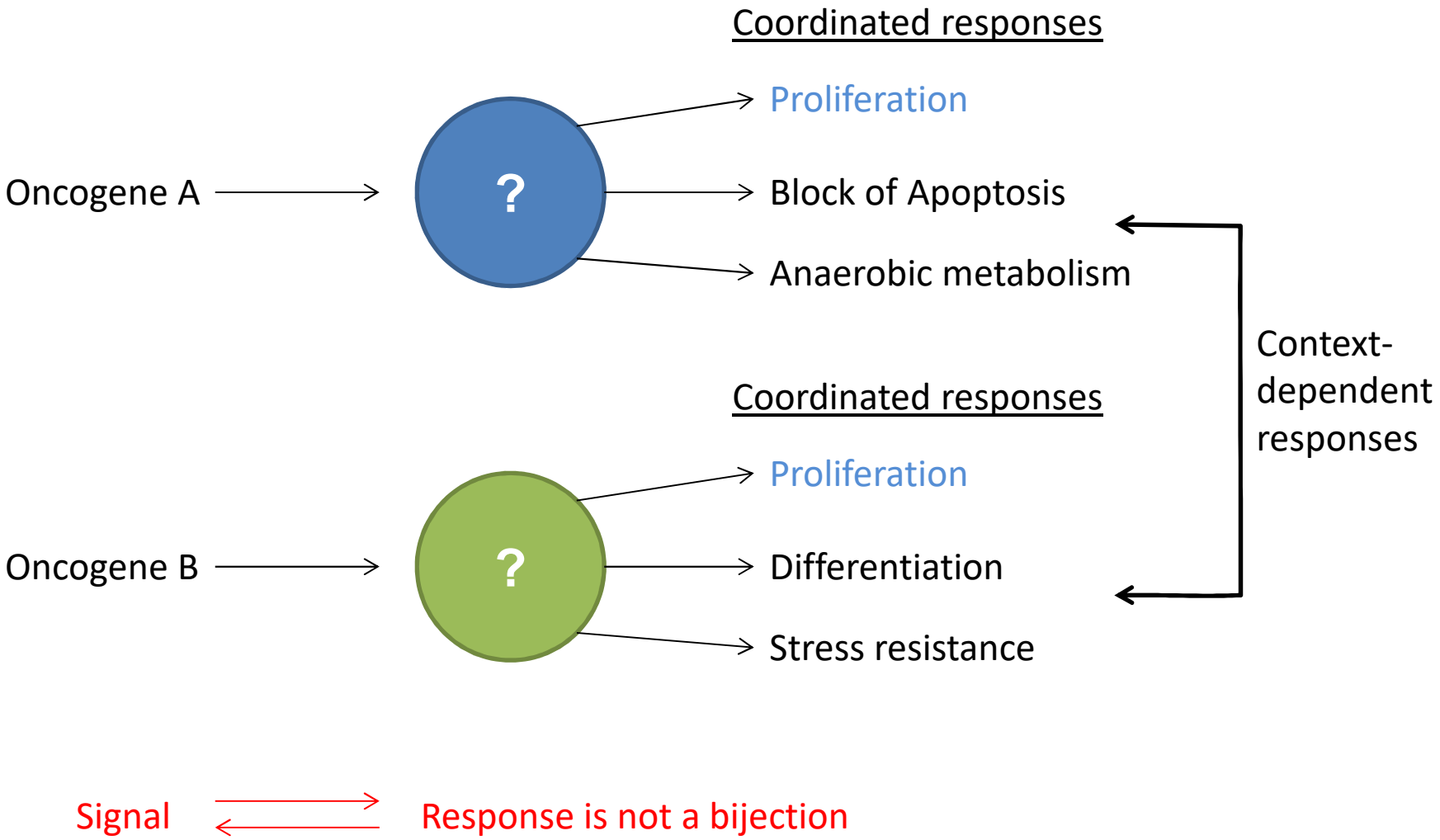


the phosphatase PTEN dephosphorylates PIP3 and thereby counter-acts PI3K signaling

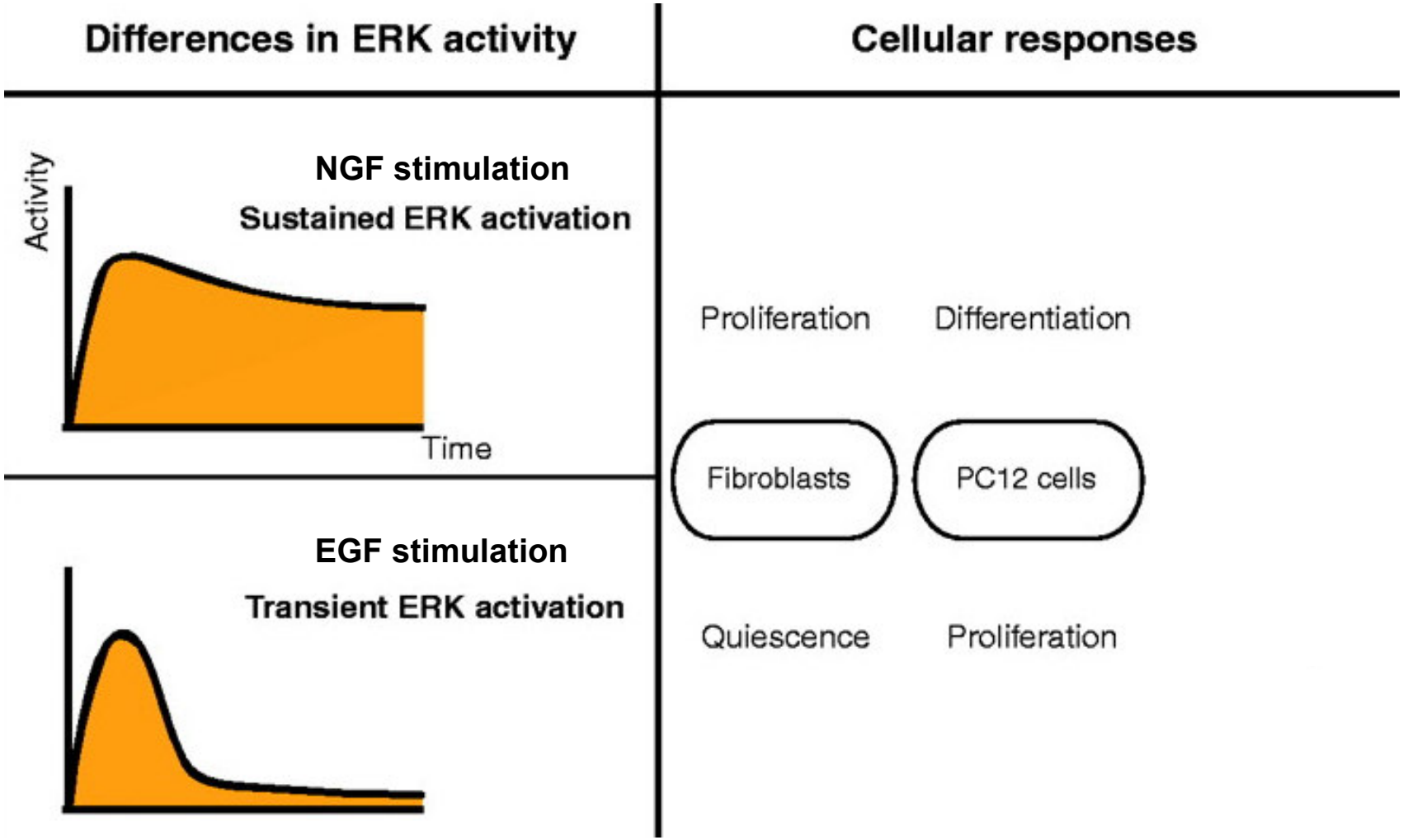
some tumors arise from the absence of such «shut-down» systems:
PTEN in glioblastoma or prostate cancer
PTPN12 (tyrosine phosphatase) in breast cancer

PIP3 then binds to the pleckstrin homology (PH) domain of PDPK1 and Akt inducing Akt phosphorylation and activation by PDPK1

Specificity and coordination of responses to a signal are context dependent

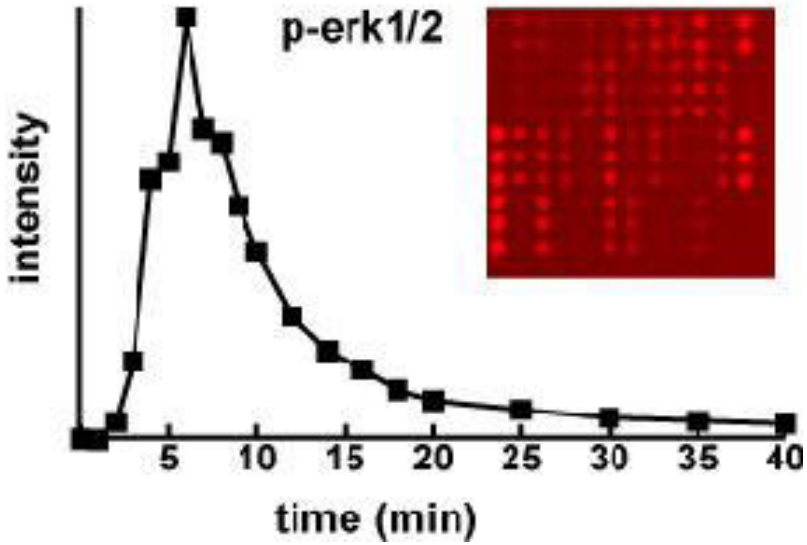


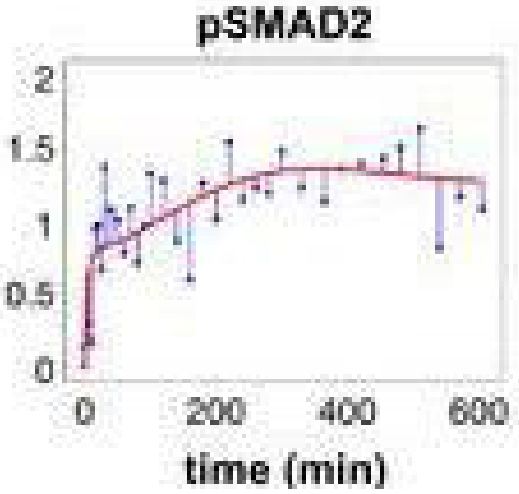
Translating signals into phenotypes: signal duration



Ebisuya et al., J. Cell Science (2005)

Different responses take more or less time





examples of signal duration:

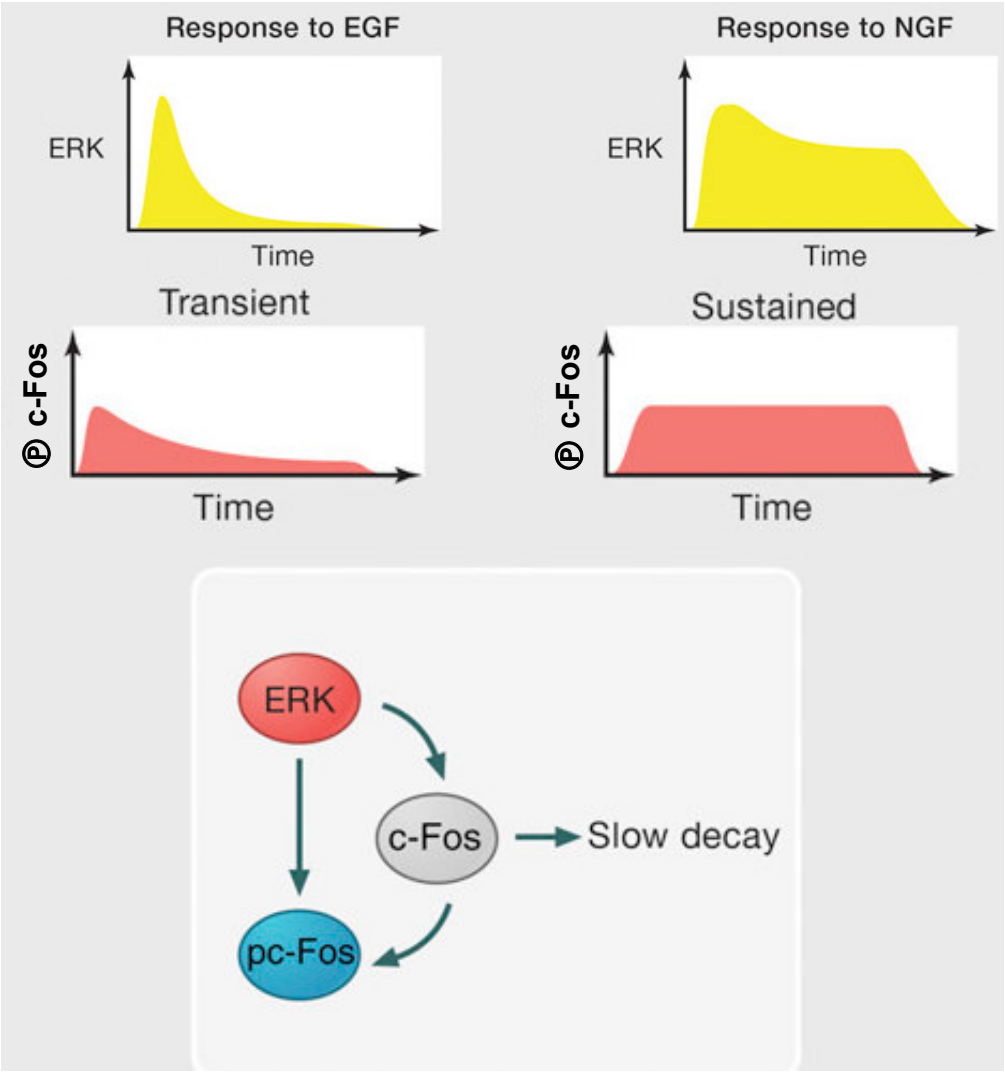
half life SH2-TK interaction: 10 seconds

MAPK activity: 30s to few minuntes

activation of inactive TFs: several minutes

modulation of the concentration of proteins: e.g. β-catenin 15-60 minutes

Signaling dynamics determine outcome



the responding network translates time-dependent changes into different outcomes

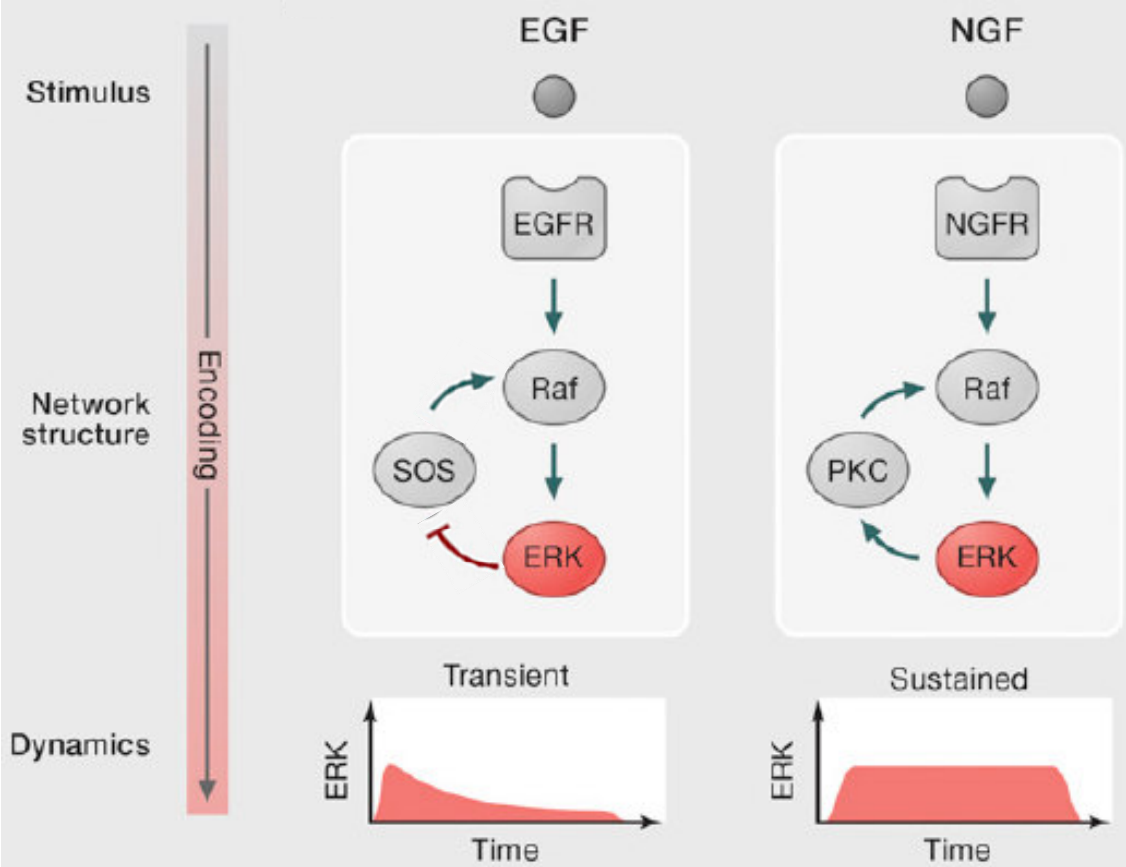
transient and persistent ERK dynamics are distinguished by a set of ‘‘immediate early gene products’’ that accumulate in response to activated ERK

when ERK activation is transient, gene products such as c-Fos are induced but then undergo rapid degradation

when ERK levels are persistent, however, newly synthesized c-Fos is directly phosphorylated by the still-active ERK, which stabilizes c-Fos in the nucleus

=> a feedforward loop comprised of a fast arm (ERK activation) and a slow arm (c-Fos accumulation) serves as a persistence detector for the duration of ERK activation

Signaling networks determine dynamics



differences in ERK dynamics in response to EGF or NGF arise also because of a negative feedback between ERK and SOS in the EGF pathway

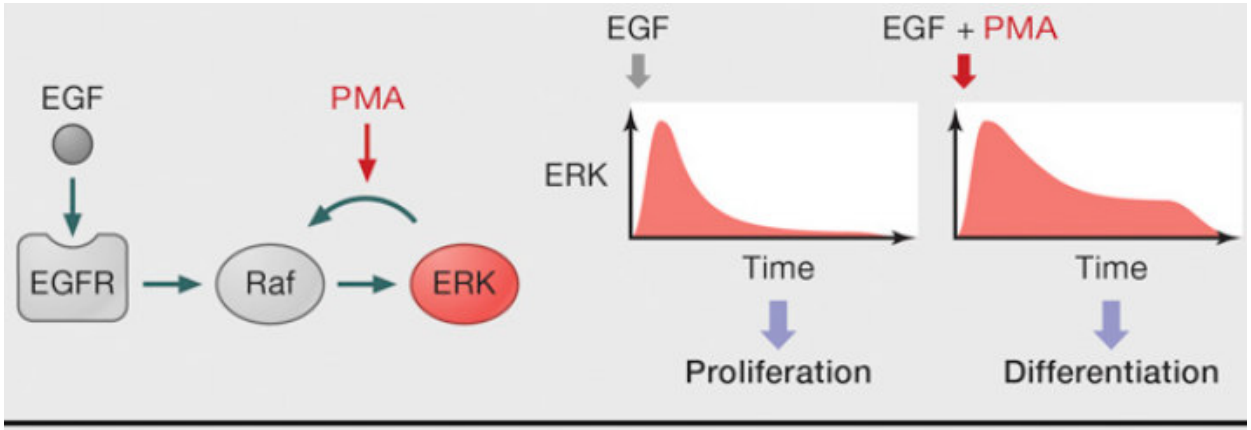
NGF, but not EGF, signaling continues after receptor internalization, which contributes to the sustained activation of ERK

positive feedback on ERK activation by NGF (but not EGF) is provided through PKC

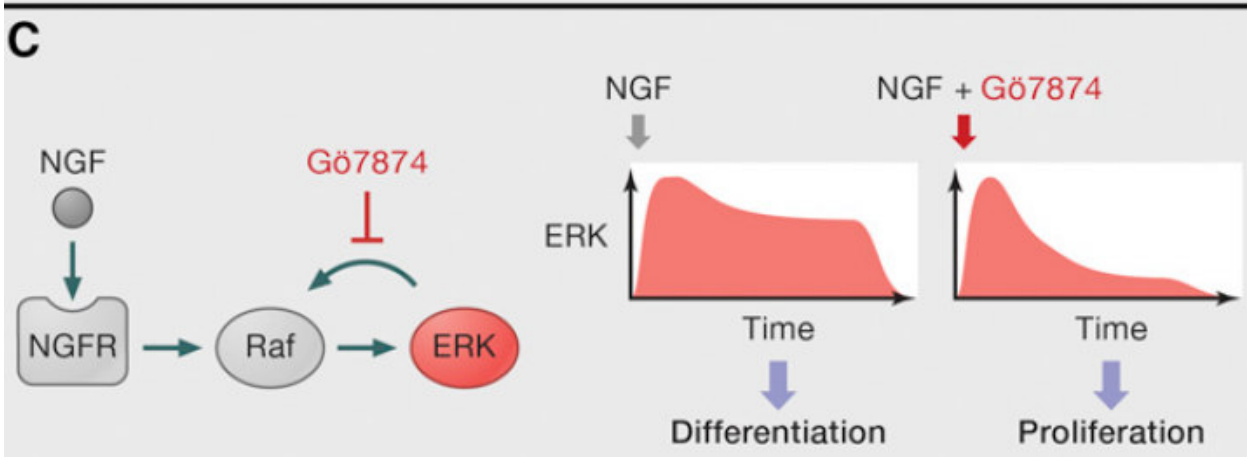
negative feedback on ERK activation by EGF (but not NGF) is provided through SOS (a Ras-GEF) phosphorylation which induces dissociation of SOS from the EGFR and ends signaling

distinct responses to EGF and NGF are due to differences in the identity and connectivity of various pathway components

Perturbation experiments reveal the role of ERK duration

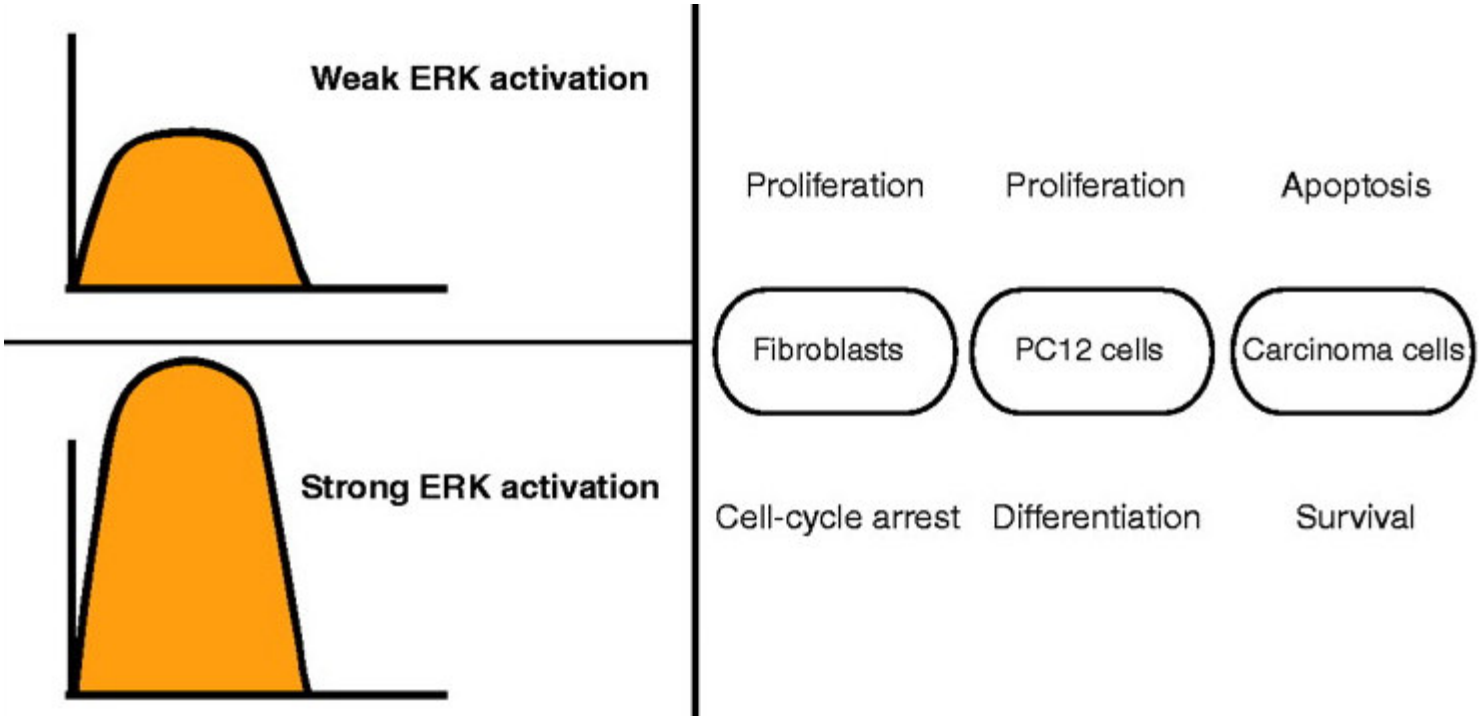


treatment with phorbol-12-myristate-13-acetate (PMA), which stimulates protein kinase C (PKC) and introduces positive feedback from ERK to Raf, results in sustained ERK activation and differentiation in response to EGF



treatment with the PKC inhibitor Gö7874 results in transient ERK activation and increased proliferation following NGF treatment

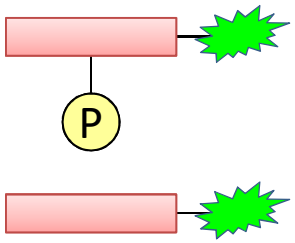
Translating signals into phenotypes: signal strength



Ebisuya et al., J. Cell Science (2005)

Promiscuity of intracellular binding sites

17-19aa peptides (fluorescently labelled) mimicking the phosphorylated tyrosines of EGFR, Erb2 and Erb3 were tested at different concentrations against 160 arrayed SH2 and PTB domains

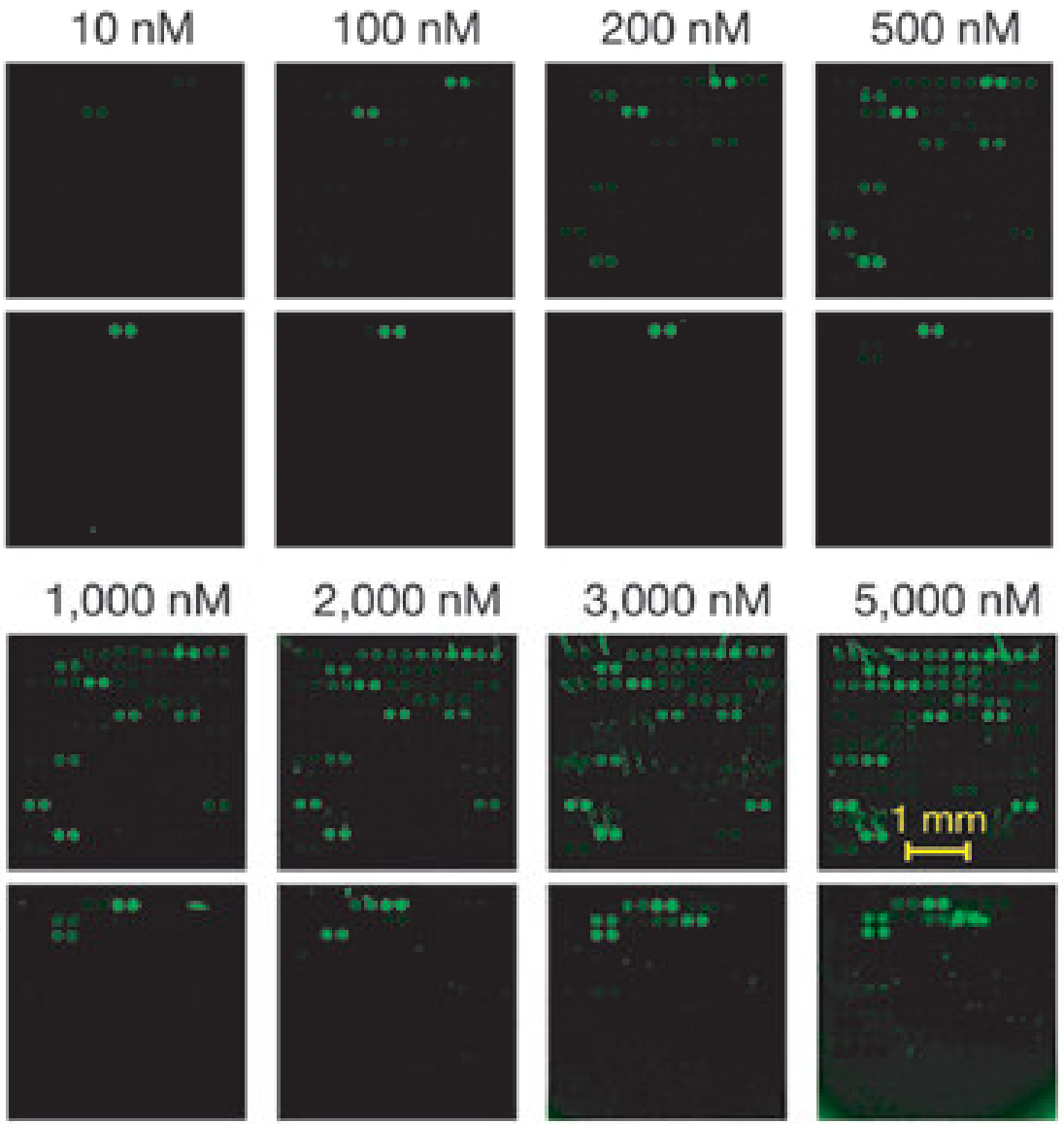


P-Tyr

control

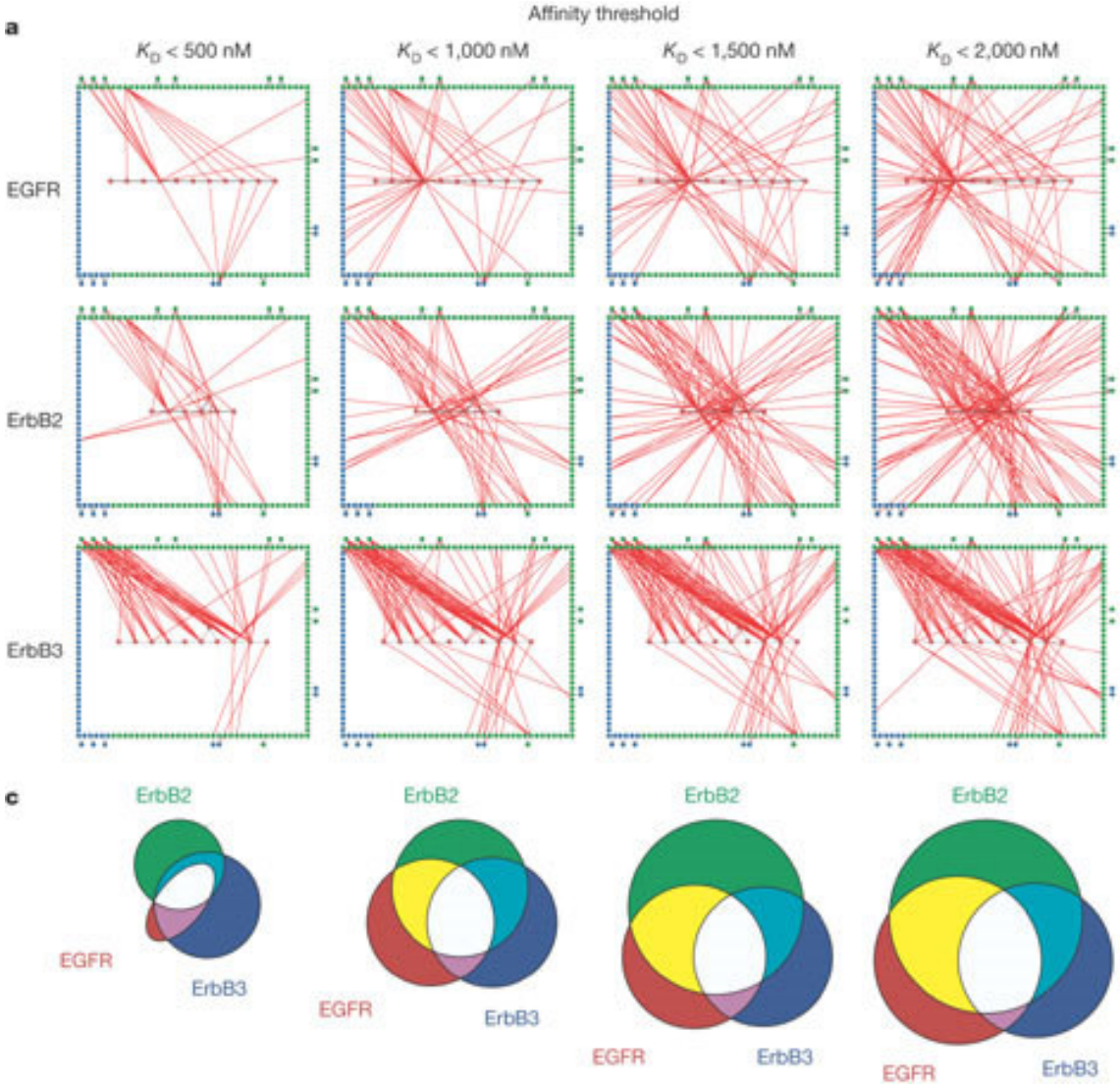
⇒ depending on concentrations, different SH2/PTB adaptor proteins were bound

⇒ affinities are not very high and specificity is limited



Jones et al. Nature. 2006 Jan 12;439(7073):168-74

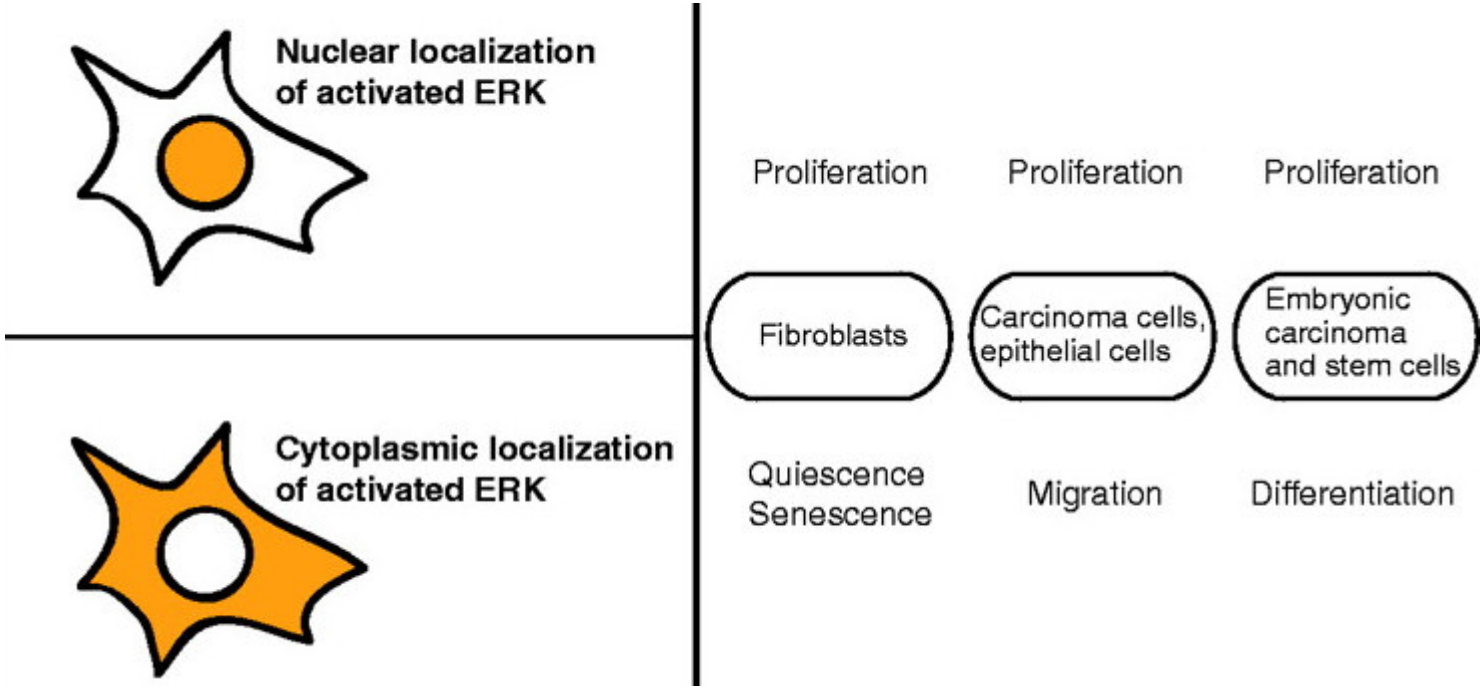
EGFR and Erb2 but not Erb3 bind many more SH2 proteins when their concentration threshold is increased



Biological implications:

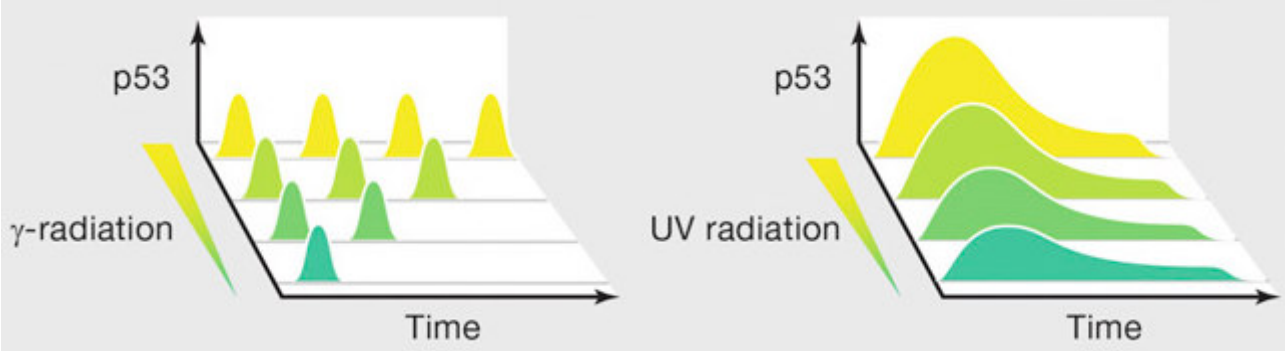
- cells should be less sensitive to changes in the levels of ErbB3 relative to EGFR and ErbB2, indeed ErbB3 is rarely amplified in cancer in contrast to EGFR and ErbB2
- low level activation of EGFR and ErbB2 induces other signals than high level activation

Translating signals into phenotypes: Compartmentalization

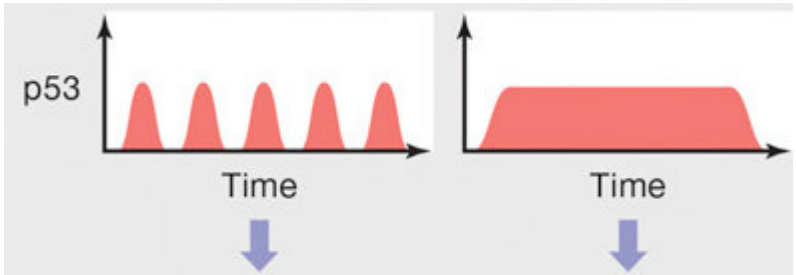


Ebisuya et al., J. Cell Science (2005)

Dynamics of p53 signaling



p53 shows both stimulus- and dose-dependent dynamics



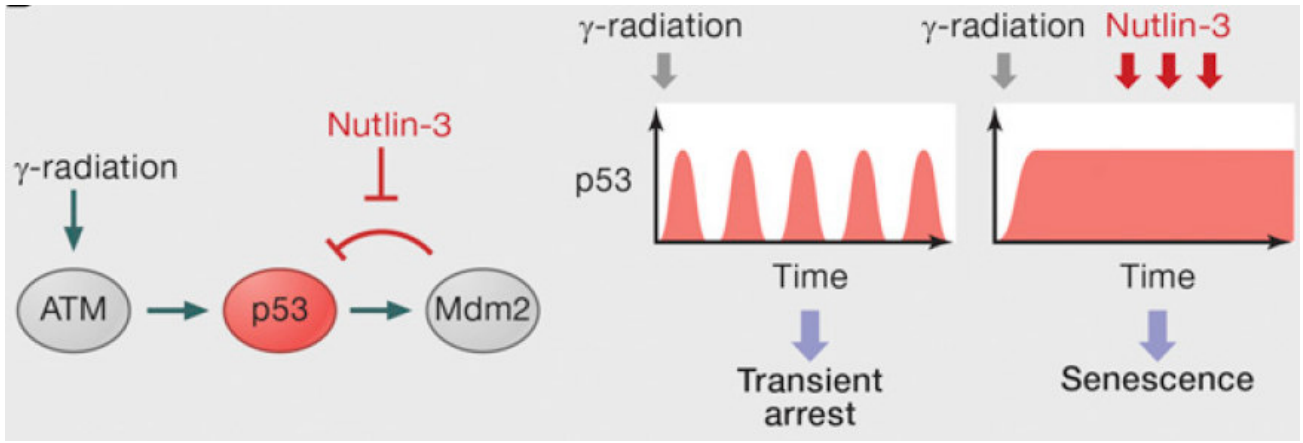
cell cycle arrest

senescence/ apoptosis

double-strand breaks caused by γ-radiation trigger a series of p53 pulses with fixed amplitude and duration

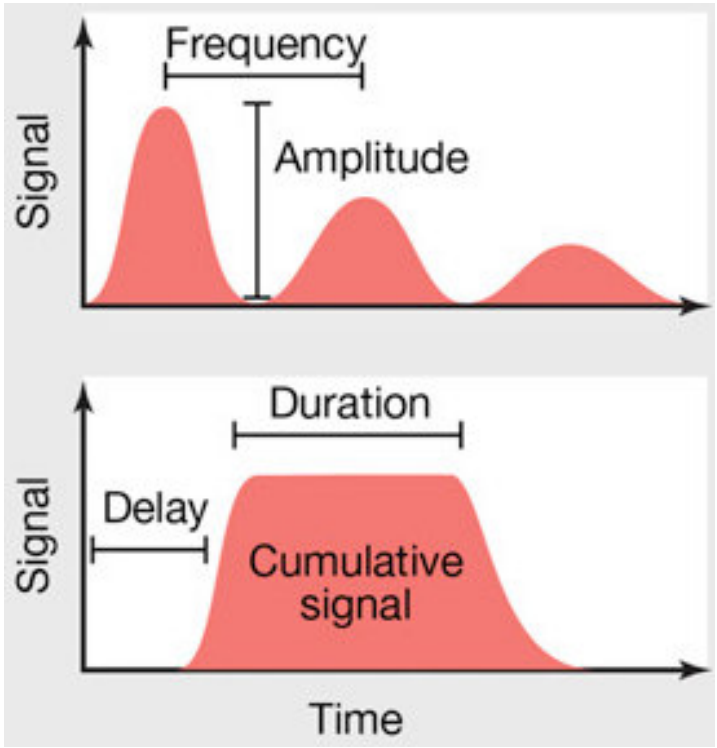
higher doses of radiation increase the number of pulses without affecting their amplitude or duration

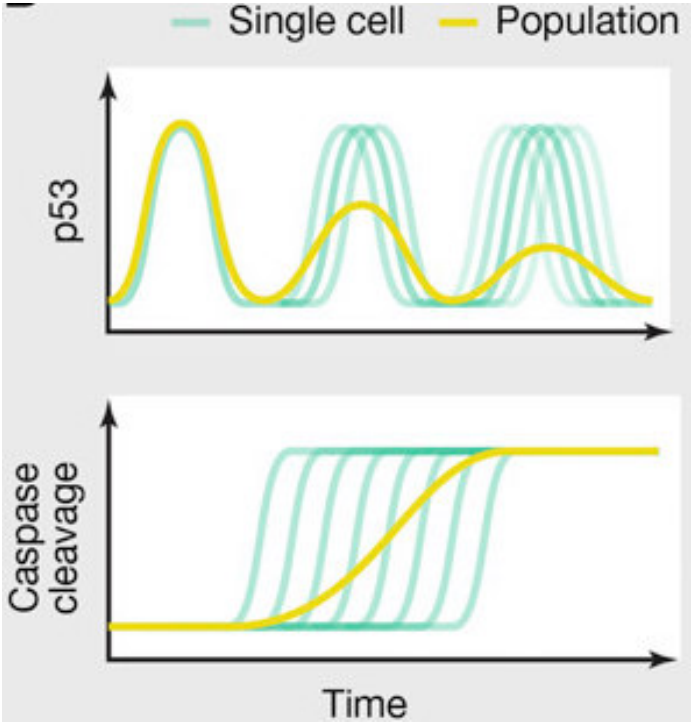
in contrast, UV triggers a single p53 pulse with a dose-dependent amplitude and duration



the small molecule Nutlin-3, which stabilizes p53 levels, can artificially switch p53 dynamics from pulsed to sustained and push cells toward senescence

Dynamics of cellular signaling: the single cell level





transmitting information in cells involves dynamics of signaling molecules with respect to changes of **concentration, activity, modification state,** or **localization** of a molecule over time
thereby signaling encodes information in the **frequency, amplitude, delay** and **duration** of a signal and is therefore more rich and complex than transmitting information at only one point in time

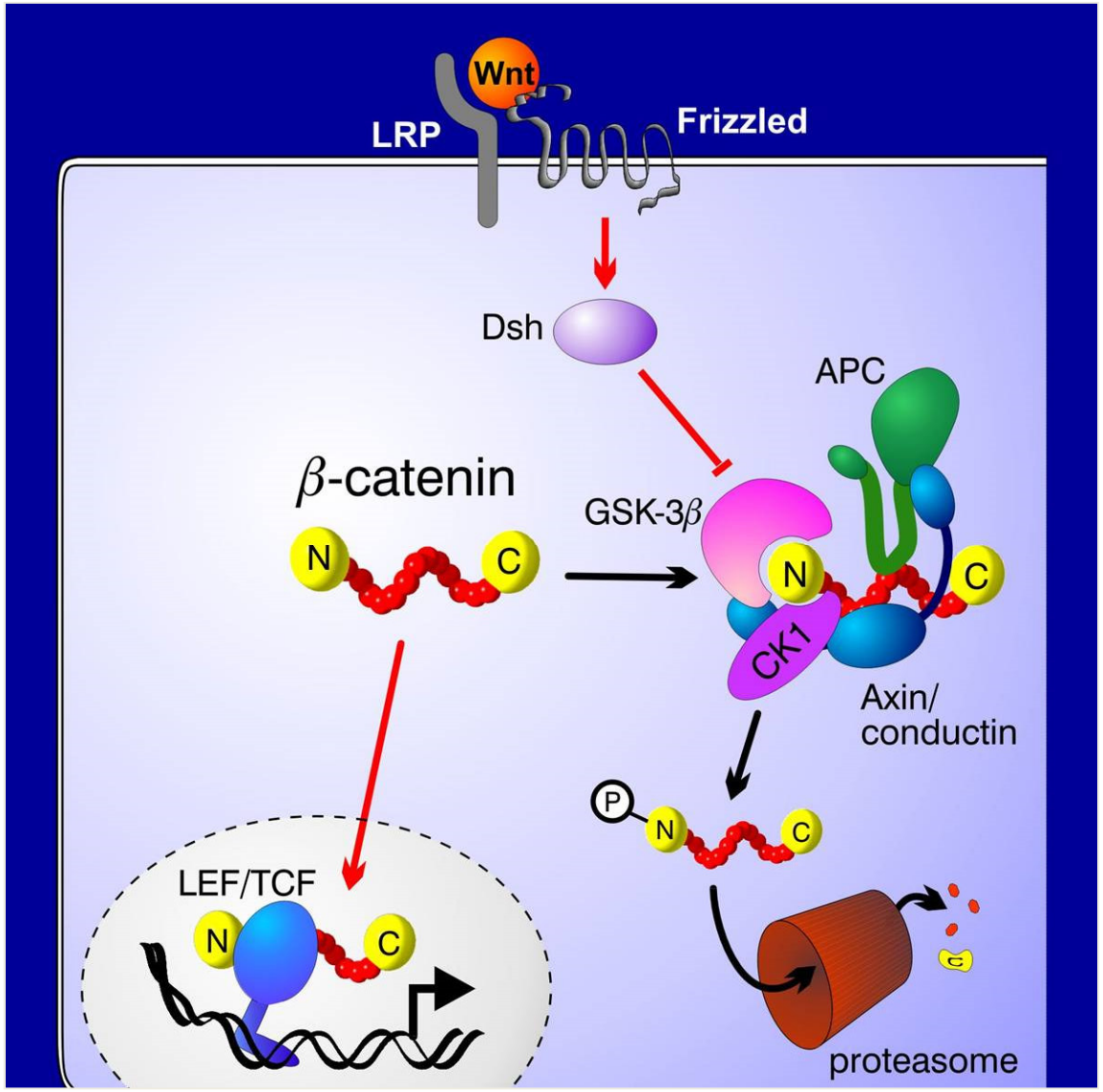
fluorescent sensors allow high-resolution time-lapse imaging in living cells to quantify the dynamics of biological responses in single cells
as a result, the average dynamical behavior of a population often represents a distorted version of individual patterns that can lead to misinterpretations as varying number of pulses and loss of synchronization affects the summary response independent of the cellular mechanism of signaling

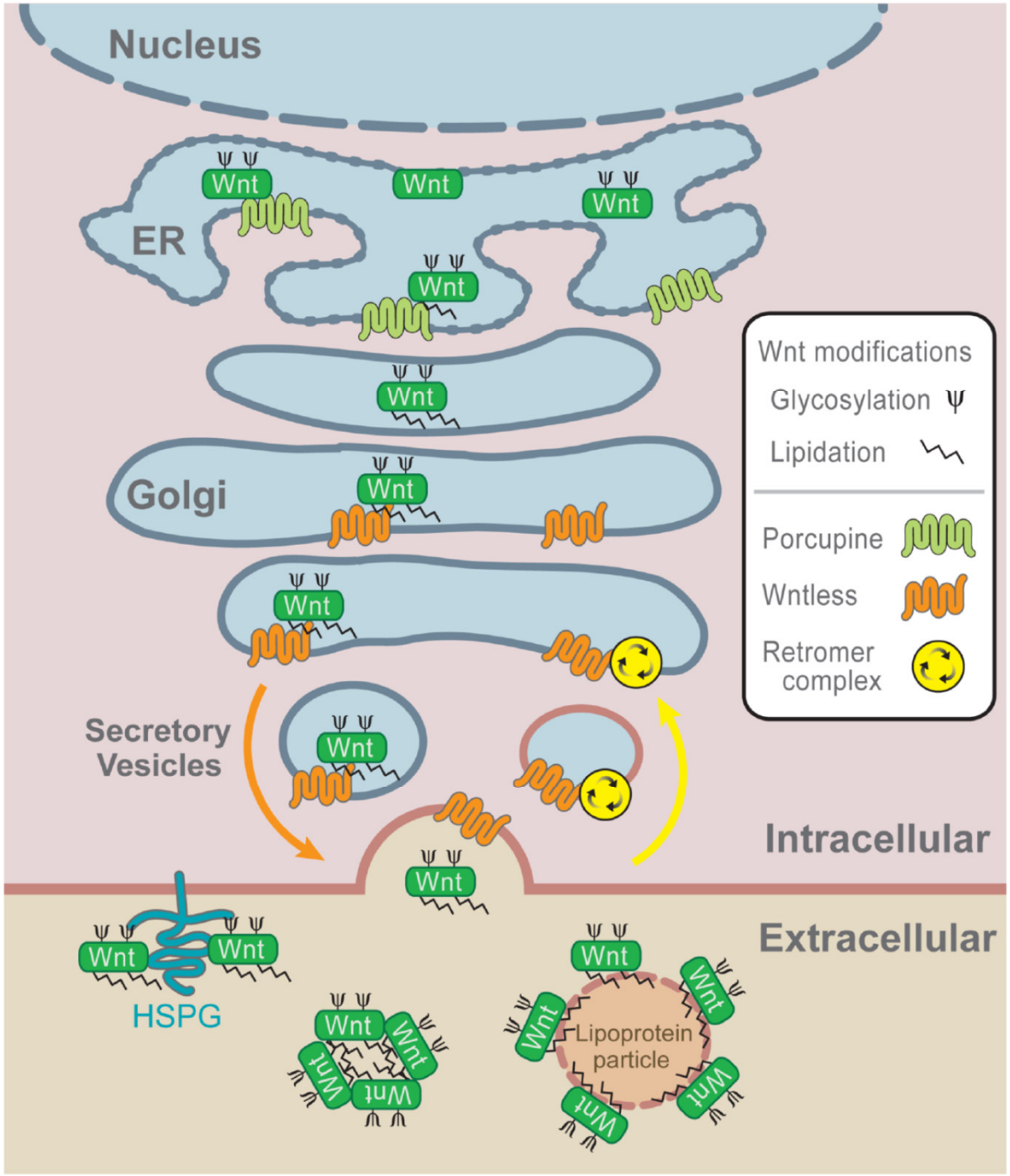
Purvis & Lahav, Cell 2013

Complexity in signaling pathways

- after ligand binding, the modular nature of receptors and signaling components allows multiple responses to occur, sometimes with different timing
- differences in signal strength and/or signal duration can be translated into different responses
- multiple pathways can converge at specific nodes or hubs; these nodes tend to be twice more targeted in cancer than other proteins; convergence of pathways at specific intracellular nodes or at the promoter of genes allows important integration of pathways: summation or subtraction
- use of a specific signaling route depends on the expression of all intermediates in a cell and their availability at the right place (compartmentalization)
- not all known responses to a ligand/receptor occur in the same cell; the above events, although difficult to predict at the moment, make the response cell-specific since they depend on the competence of the cell

The canonical Wnt-signaling pathway (simplified version)

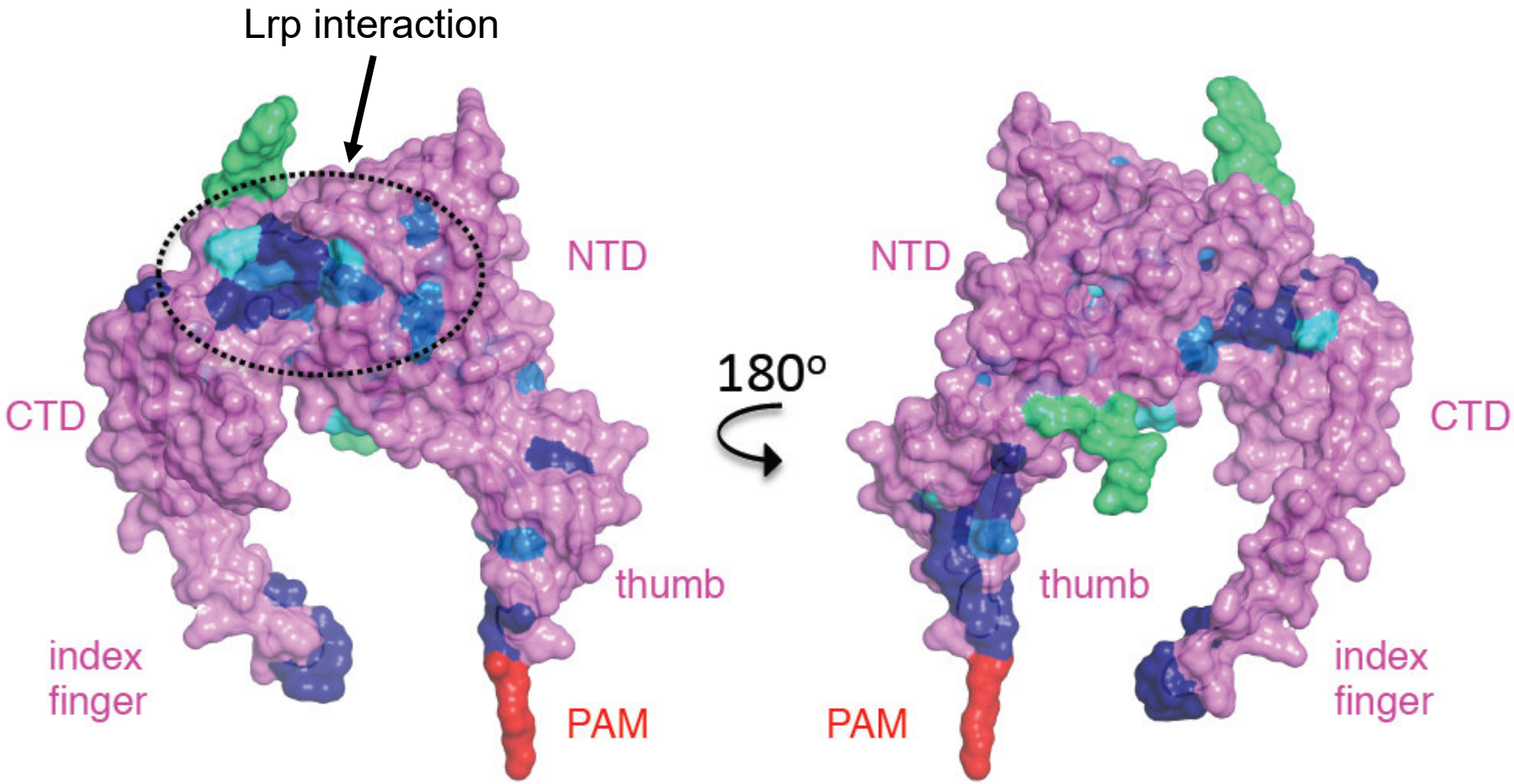




Wnt production

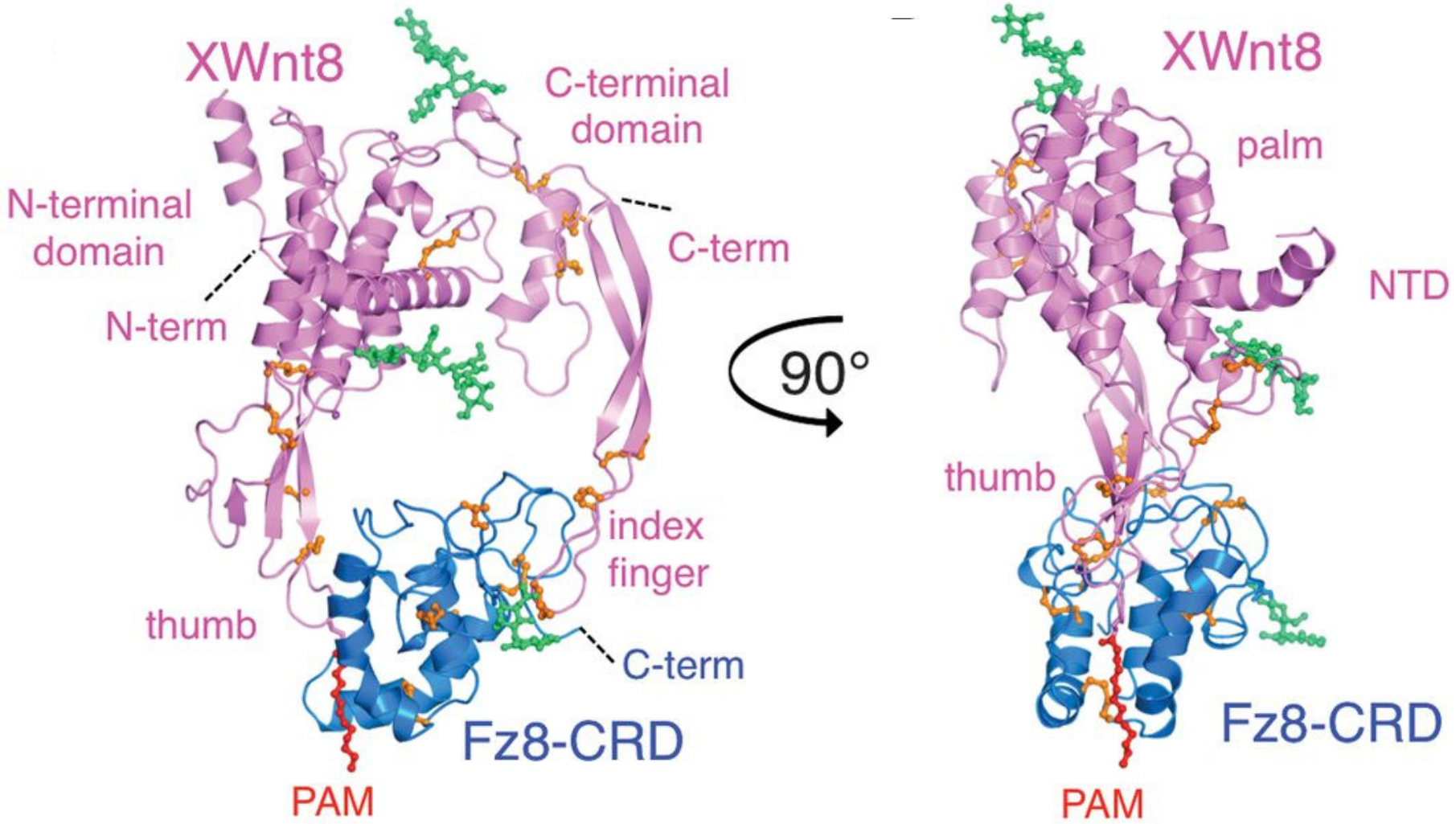
- **Porcupine**, a membrane-bound *O*-acyltransferase, mediates lipid-modification (**S-palmitoylation**, the reversible addition of palmitate to a cysteine via a thioester bond) of Wnt ligands; this modification is required for trafficking
- **Wntless** is involved in vesicle trafficking and secretion of Wnt ligands
- the Retromer complex controls Wntless recycling and is therefore indirectly involved in Wnt secretion

Structure of XWnt8



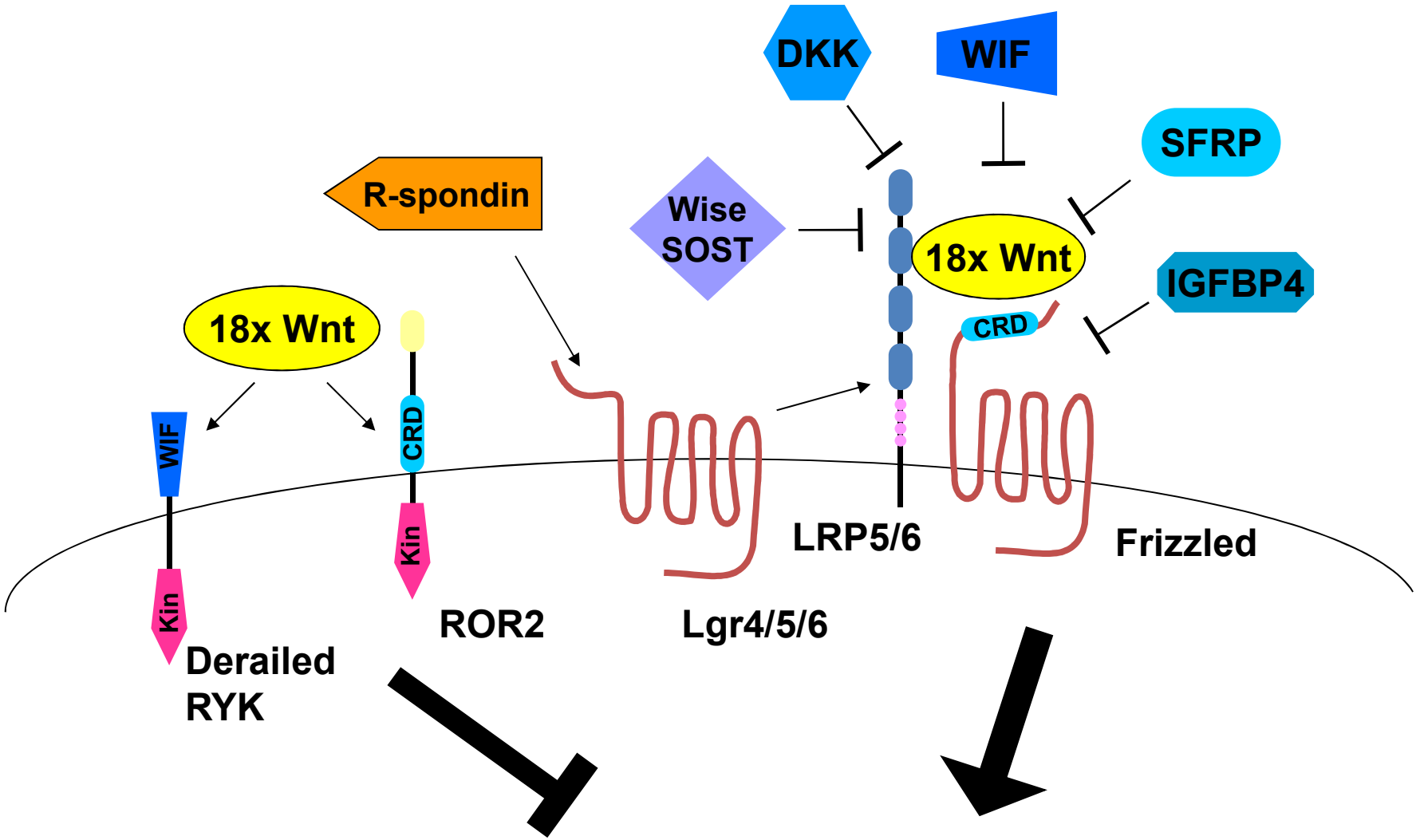
Janda et al., 2012

Structure of Fzd-CRD and the Wnt Interaction Surface

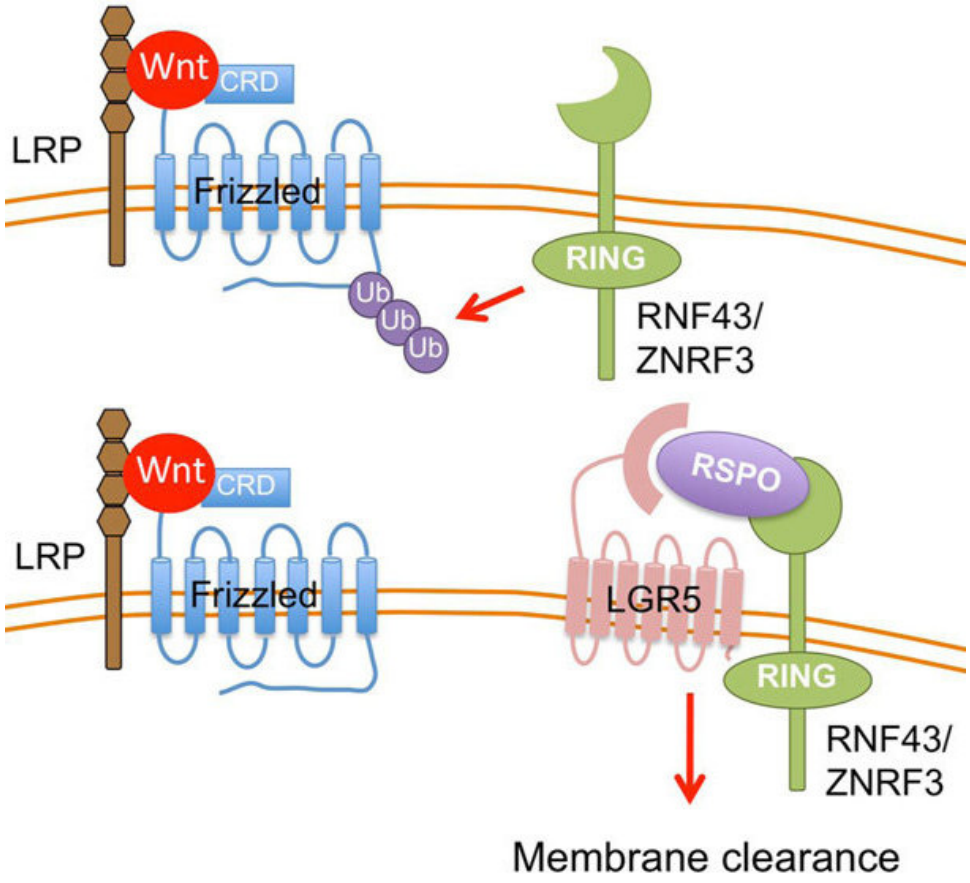


Janda et al., 2012

Wnt signaling: reception



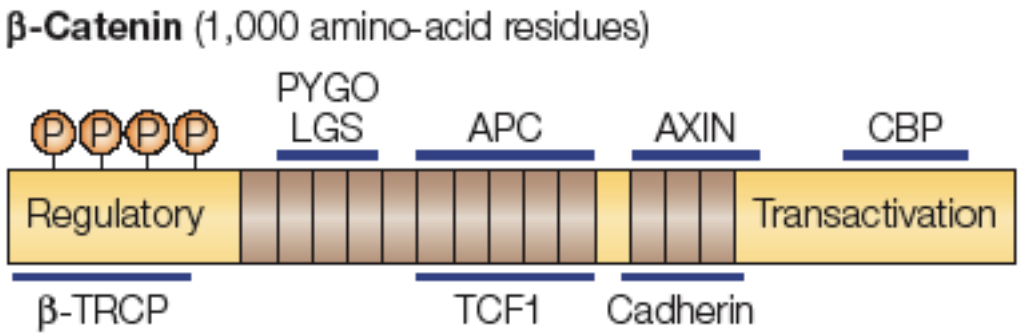
Lgr4/5/6



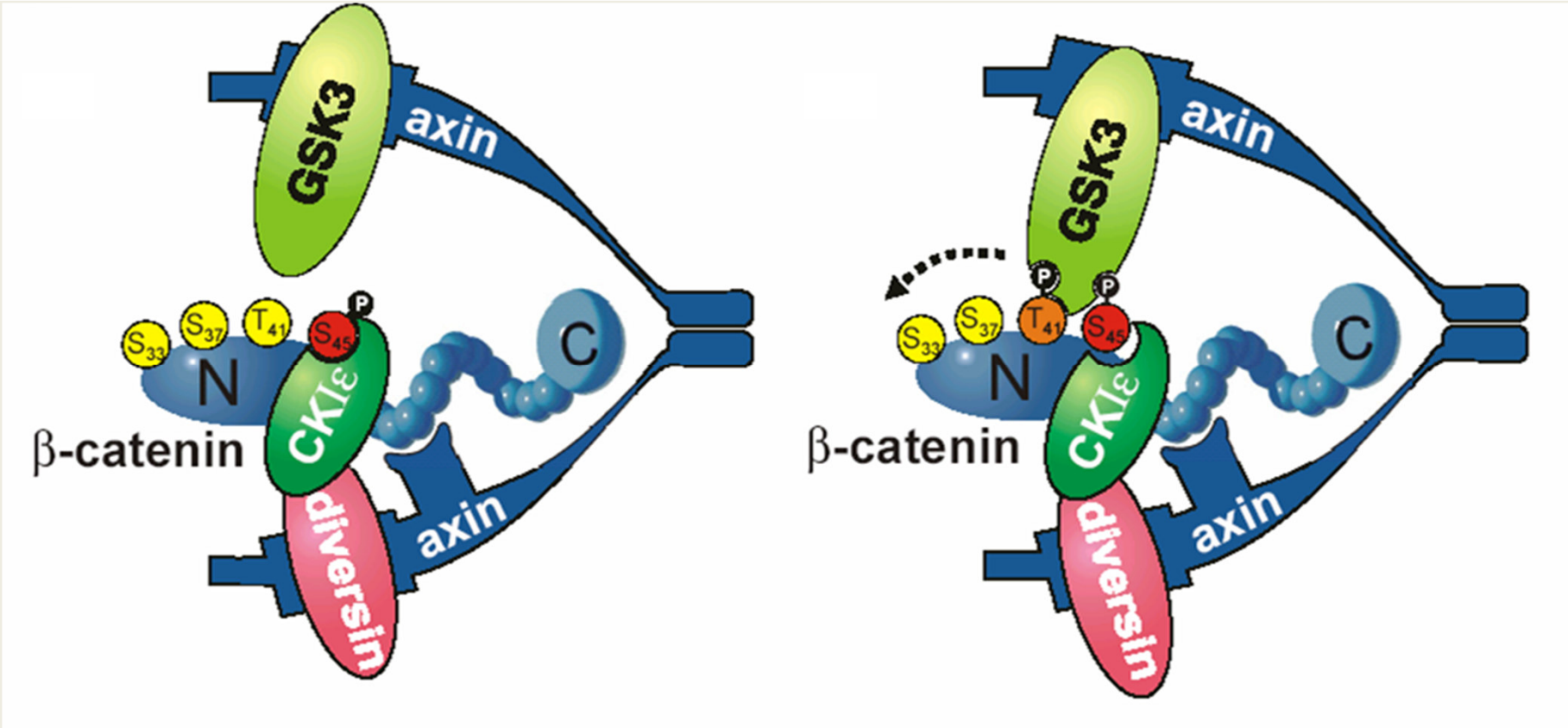
- Lgr4/5/6 comprise a second class of seven-membrane-span receptors which bind to LRPs and are activated by another class of ligands, the **R-spondins** (RSPO)
- Lgr4/5/6 regulate activity of Znrf3 and RNF43, ubiquitin ligases in the membrane, which in turn control Lrp/Fzd stability by ubiquitination
- Lgr signaling can potentiate the canonical pathway which utilizes β-catenin but also the non-canonical pathway
- Lgr receptors have been identified to mark a variety of epithelial stem cells in the adult but appear to have less relevance for embryonic development

β-catenin: a critical component of the canonical Wnt pathway

- the β-catenin protein is the key intracellular mediator of canonical Wnt pathway signals
- its stability and localization is regulated extensively
- it acts as a hub which integrates different signals and forms subcomplexes with specialized functions
- in addition to its role in Wnt signal transduction, β-catenin interacts with cadherins cell adhesion junctions



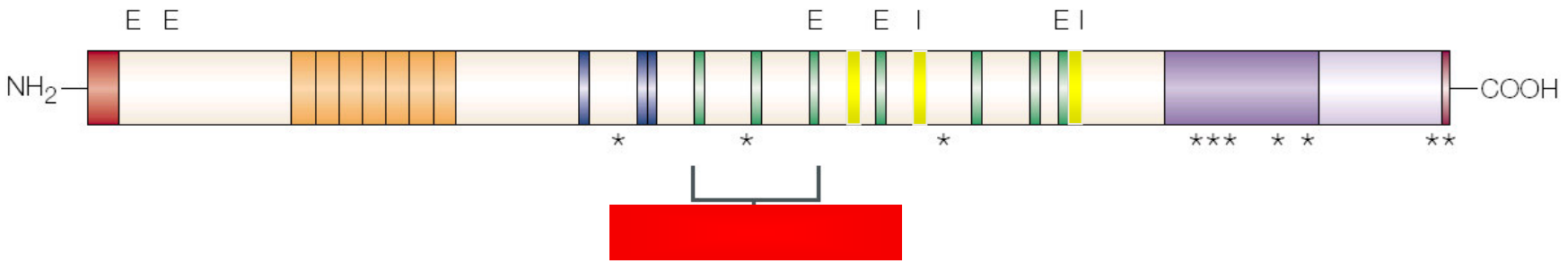
Two step phosphorylation of β-catenin



GSK3β CKI

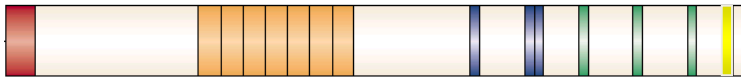
β-catenin QSYLD**S**$_{33}$GIH**S**$_{37}$GAT**T**$_{41}$TAP**S**$_{45}$LSG

Structure and Function of APC



***Apc*$^{+/Min}$** **>100 tumors**

***Apc*$^{+/1638N}$** **5-6 tumors**



***Apc*$^{+/1638T}$** **tumor free**

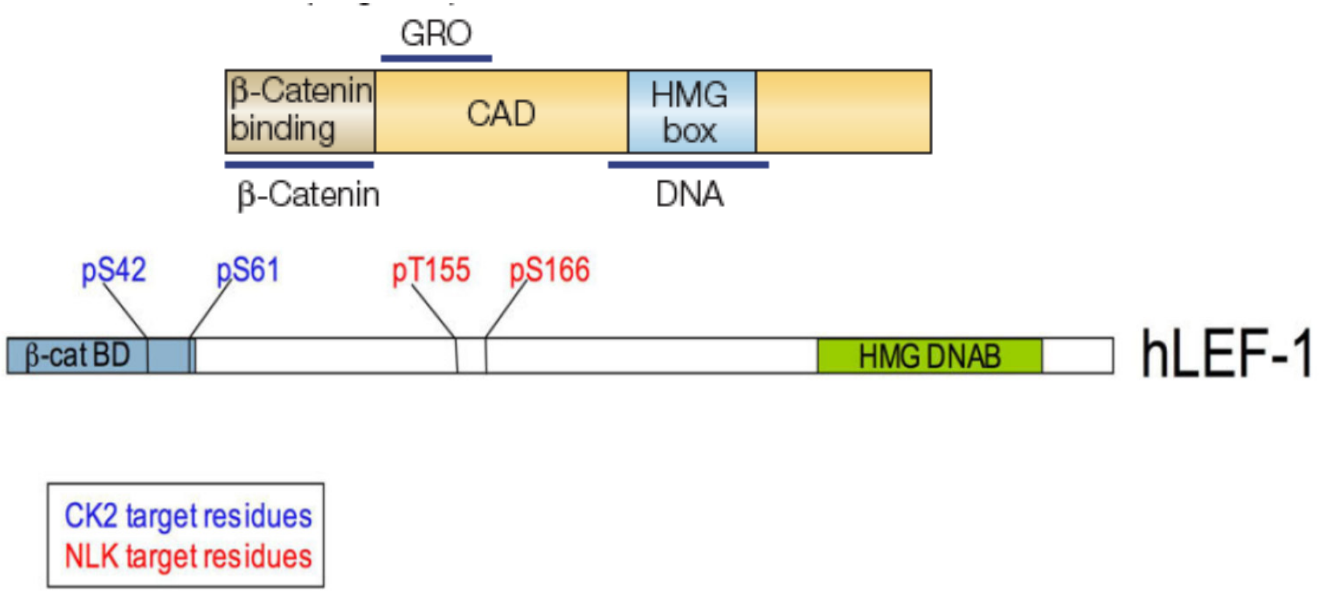
(Fodde & Clevers, 2001)

TCF/LEF1: transcriptional effectors of the canonical Wnt pathway

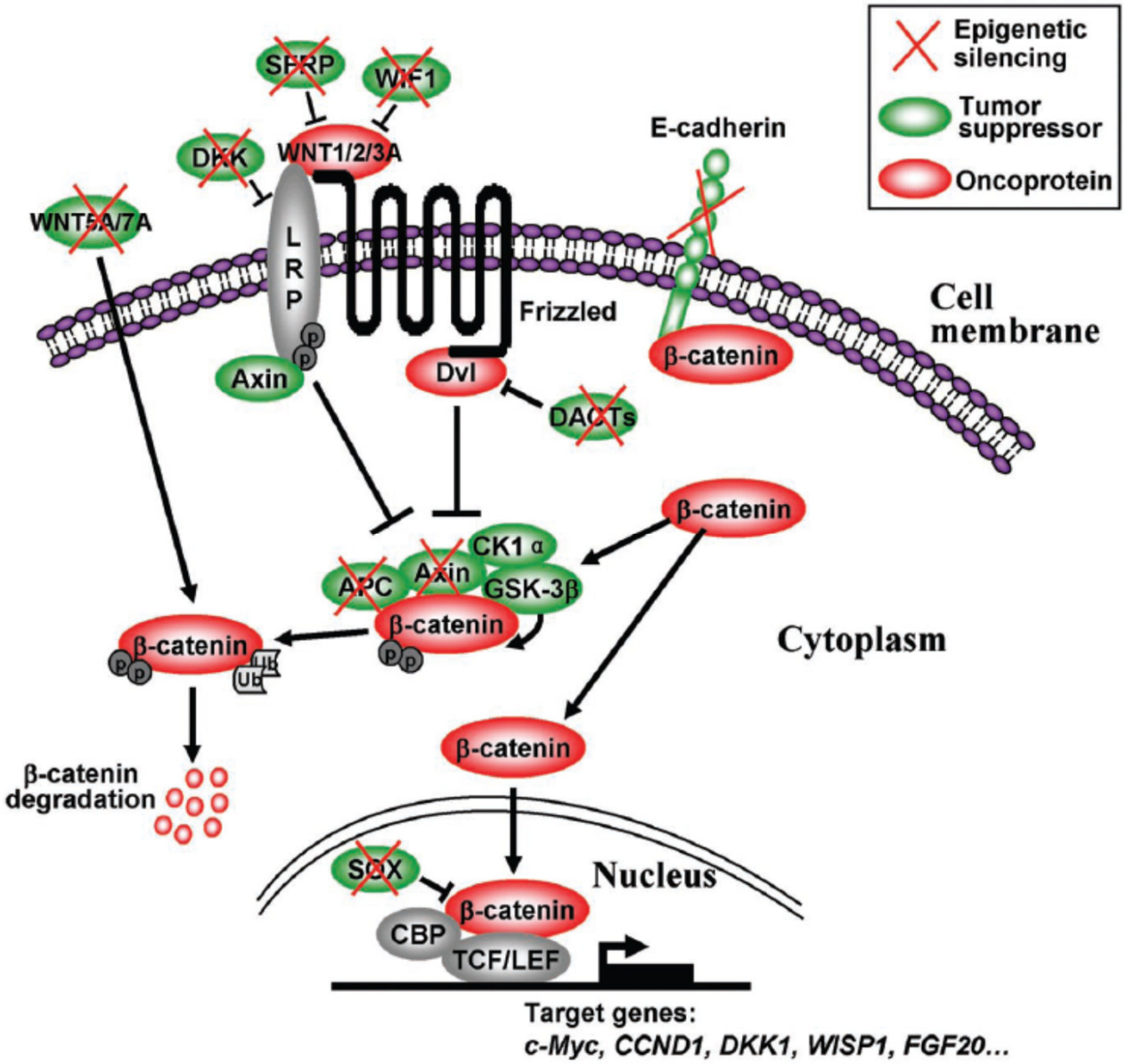
In the Wnt signaling pathway, β-catenin translocates from the cytoplasm into the nucleus where it interacts with members of the LEF/TCF DNA-binding transcription factor family

TCF: T-cell factor, LEF: lymphocyte-enhancer-binding factor

In vertebrates there are 4 family members: TCF1 (*TCF7*), LEF1, TCF3 (*TCF7L1*) and TCF4 (*TCF7L2*).

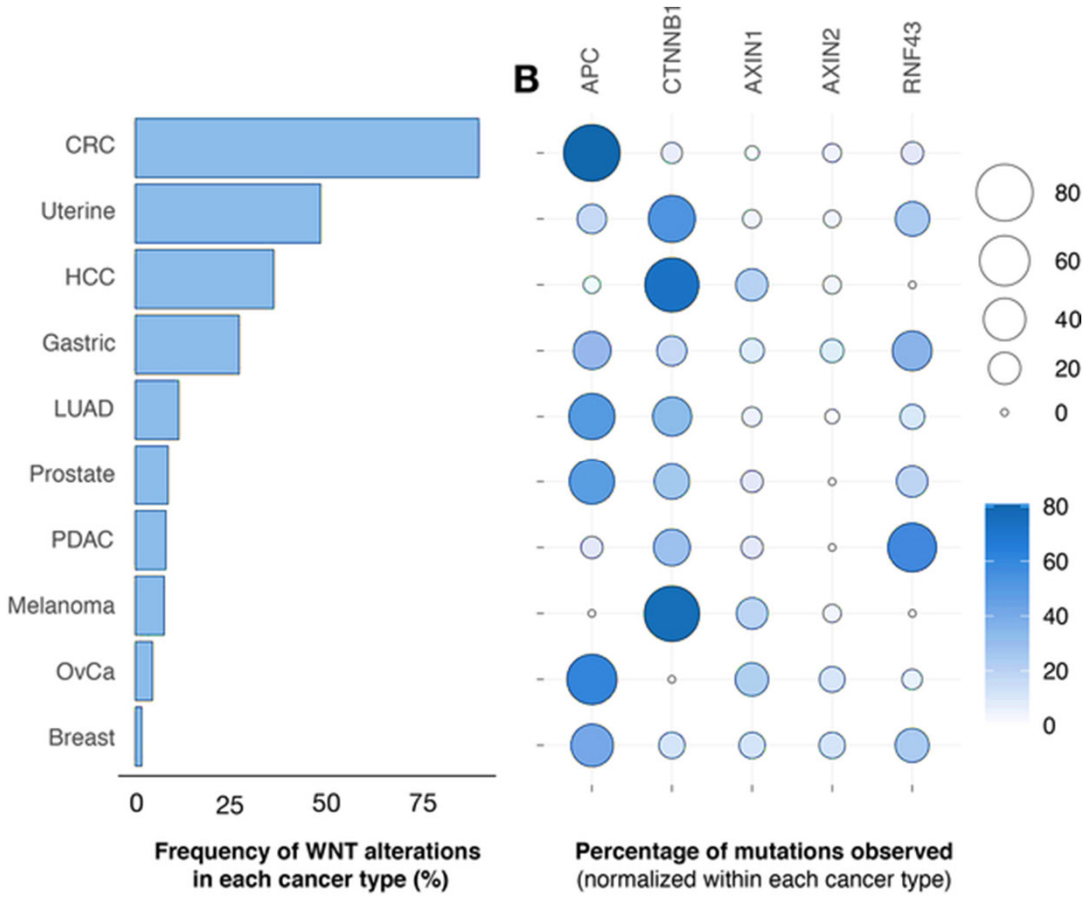


Wnt pathway components affected in cancer



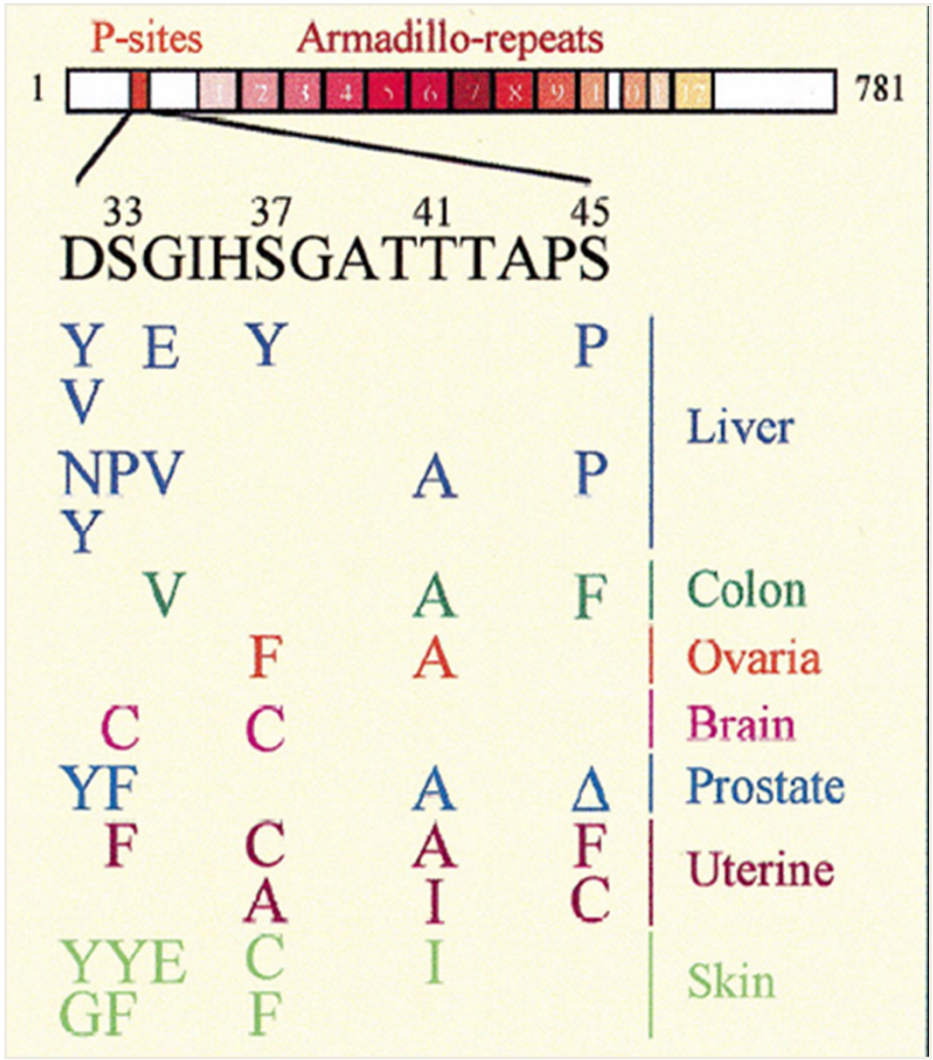
Wnt pathway mutations in human cancer

colorectal (90%)
pilomatricoma (75%)
hepatoblastoma (50%)
hepatocellular (30%)
pancreatic SPN (80%)
anaplastic thyroid (65%)
endometrioid type ovarian (25%)
endometrial (45%)
Wilms tumor (15%)
desmoid tumors (50%)
squamous cell
melanoma
medulloblastoma
prostate
breast
gastric



Parsons et al., Cancer Discov. 2022

β-Catenin Mutations in Cancer



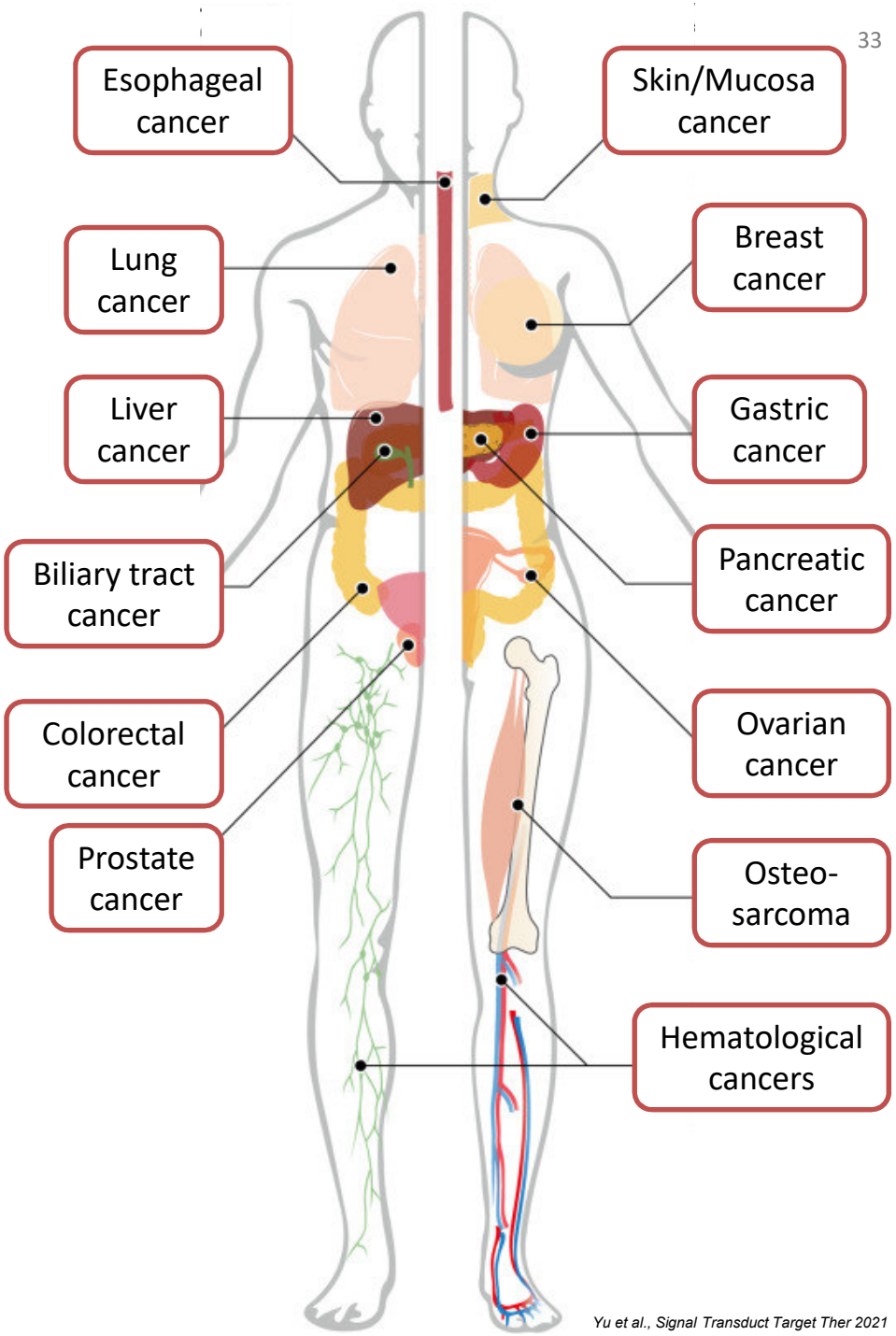
(Roose & Clevers, 1999)

Current clinical trials inhibiting Wnt signalling

several clinical trials in a large variety of cancers are currently underway to test efficacy of Wnt pathway targeting drugs

so far no results have been published which would indicate benefit for patients

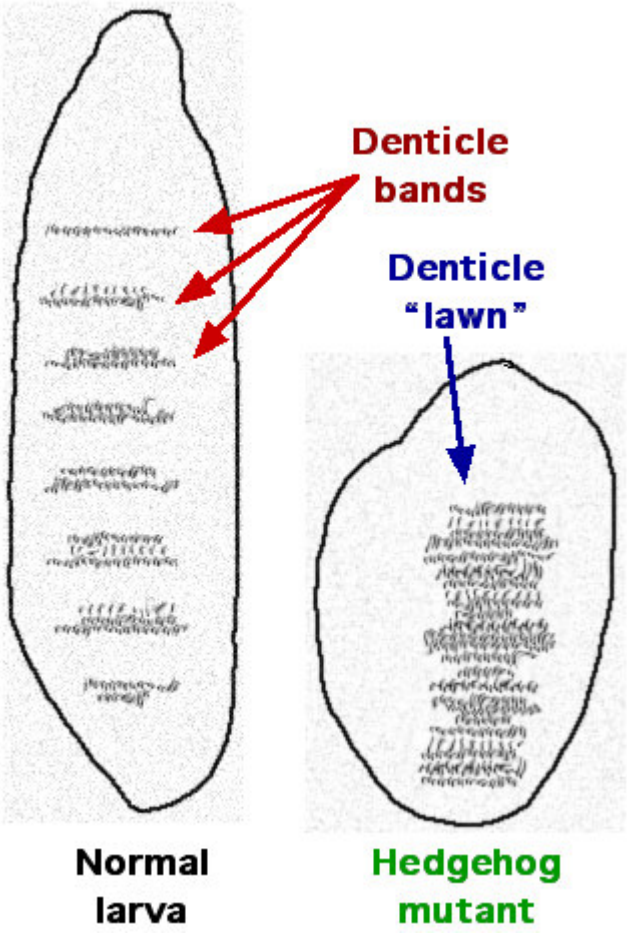
some of the drugs shown on-target toxicity in the bone



Yu et al., Signal Transduct Target Ther 2021

The Hh signaling pathway

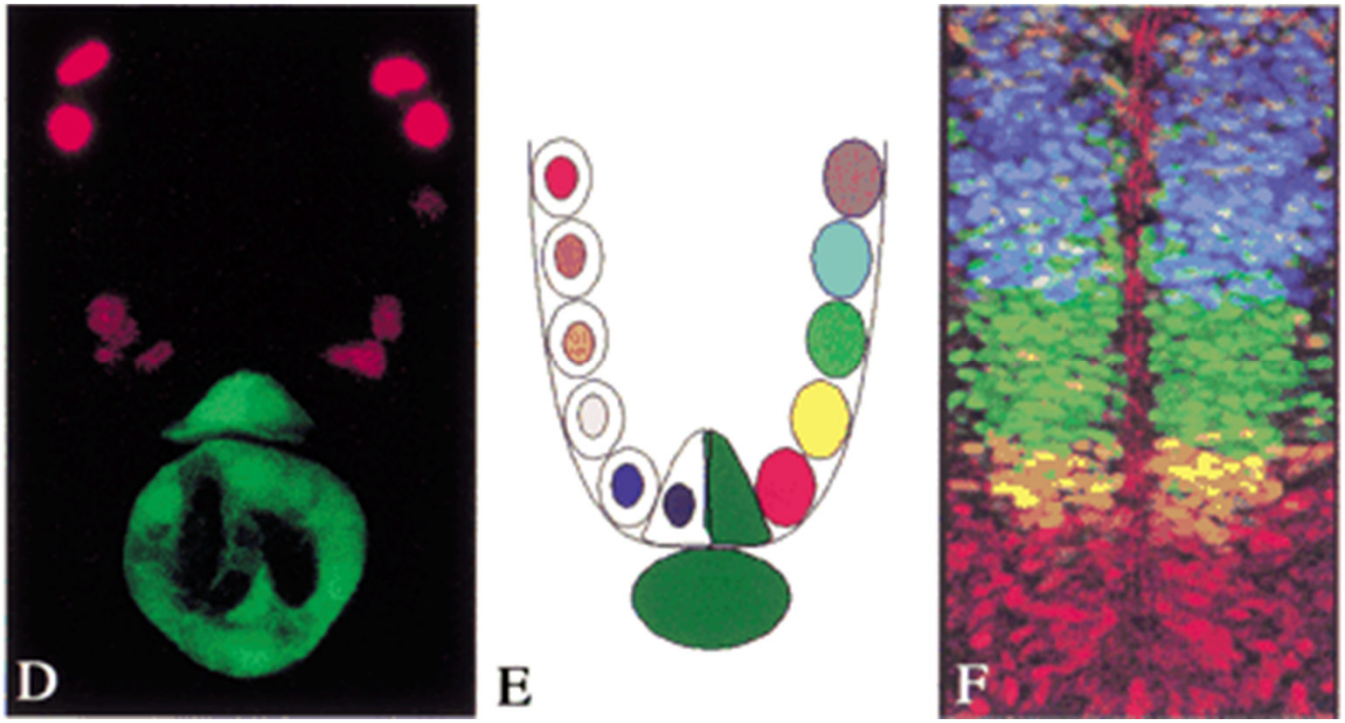
Cuticle patterning defect of hedgehog (Hh) mutants



Ventral denticle belt patterning

Hedgehog mutant phenotype
(Nüsslein-Volhard & Wieschaus 1980 Nature):
Uniform lawn of bristles

Hh morphogen gradients pattern the neural tube

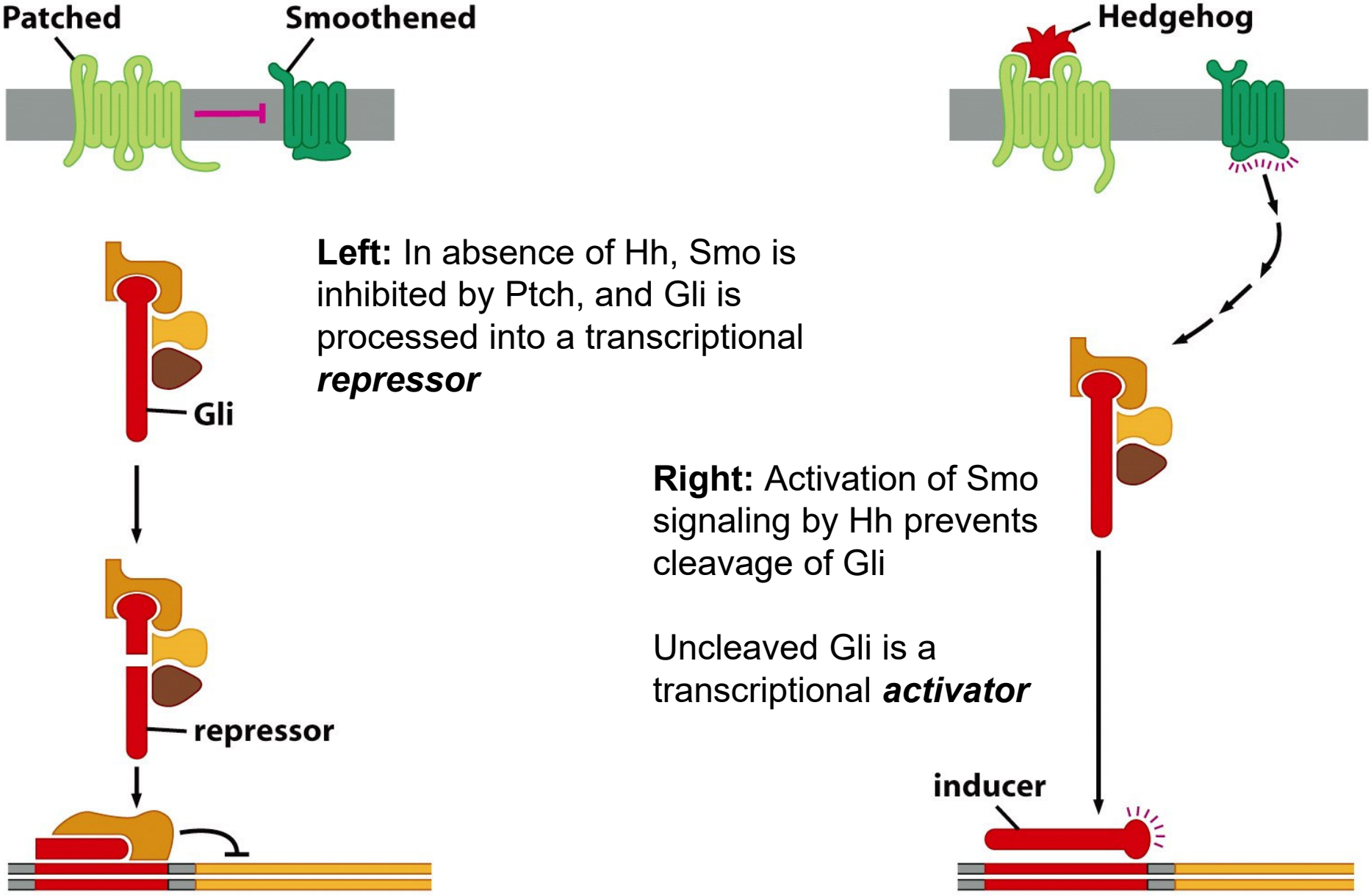


Shh::GFP reporter transgene

SHH morphogen gradient controls localized expression of various homeodomain transcription factors (motor-neuron identity)

Ingham & McMahon, Genes Dev (2001)

The core components of the Hedgehog signaling pathway



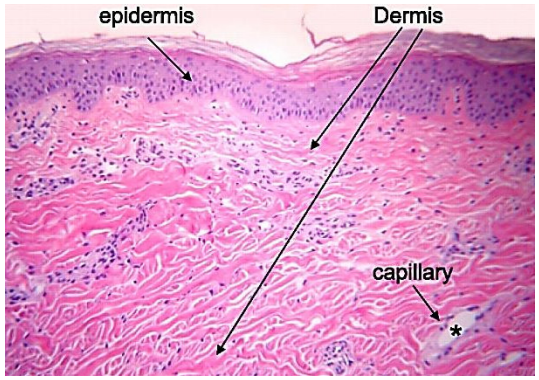
Left: In absence of Hh, Smo is inhibited by Ptch, and Gli is processed into a transcriptional ***repressor***

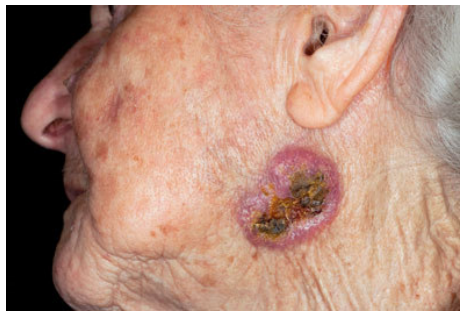
Right: Activation of Smo signaling by Hh prevents cleavage of Gli

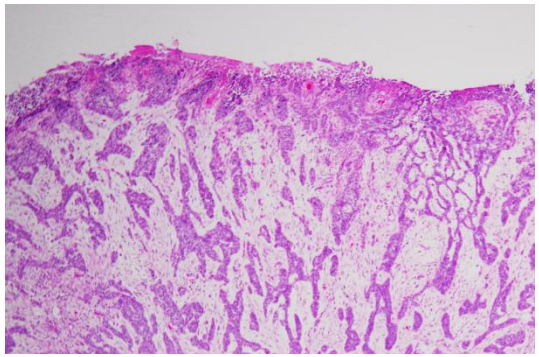
Uncleaved Gli is a transcriptional ***activator***

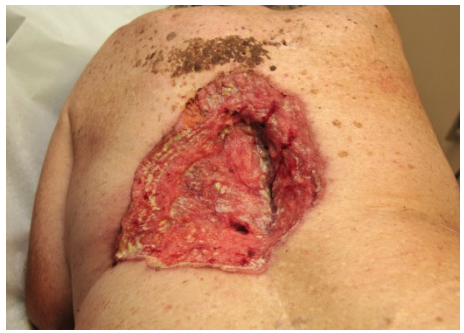
Figure 5-23 The Biology of Cancer (© Garland Science 2007)

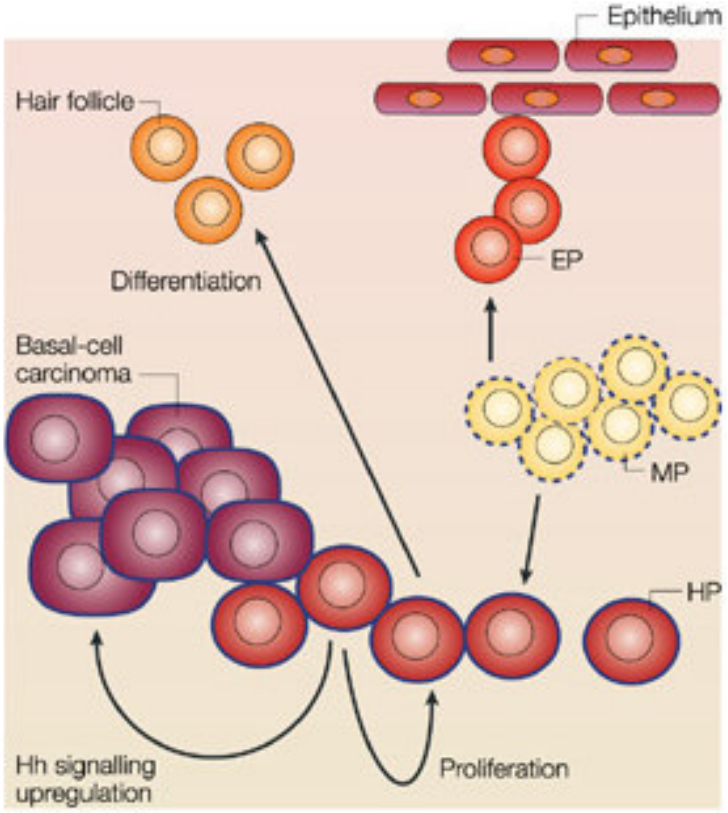
Sustained HH signaling is a cause of Basal Cell Carcinoma











Basal cell carcinoma

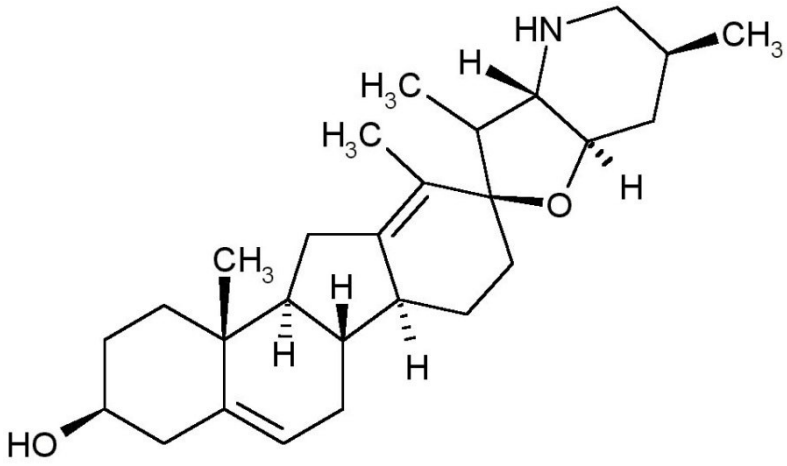
- from keratinocytes in the basal layer (epidermis) of sun-exposed skin
- most common skin cancer (30% of caucasians, male>female)
- rarely metastatic, but malignant because of local invasiveness

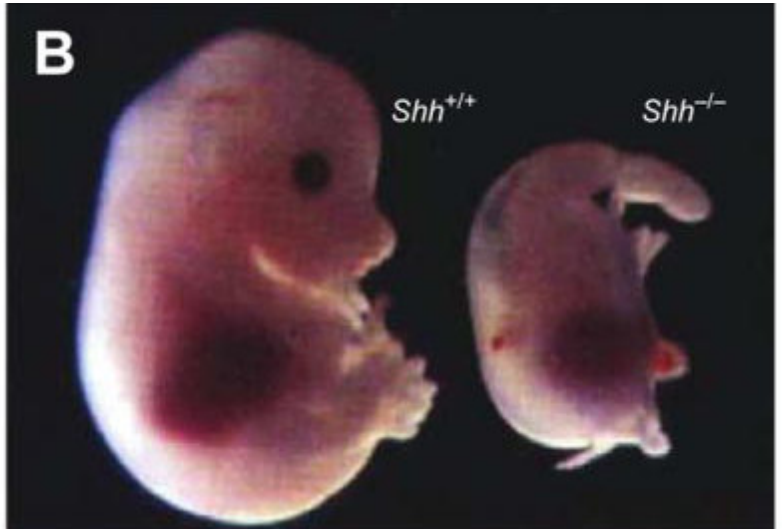
- hair follicle stem cells and multi-potent progenitors (MP) express Ptch transiently when they proliferate
- basal-cell carcinomas derive from loss-of-function mutations in PTCH1 or activating mutations in SMO

Pasca di Magliano & Matthias Hebrok, Nat Rev Cancer (2003)

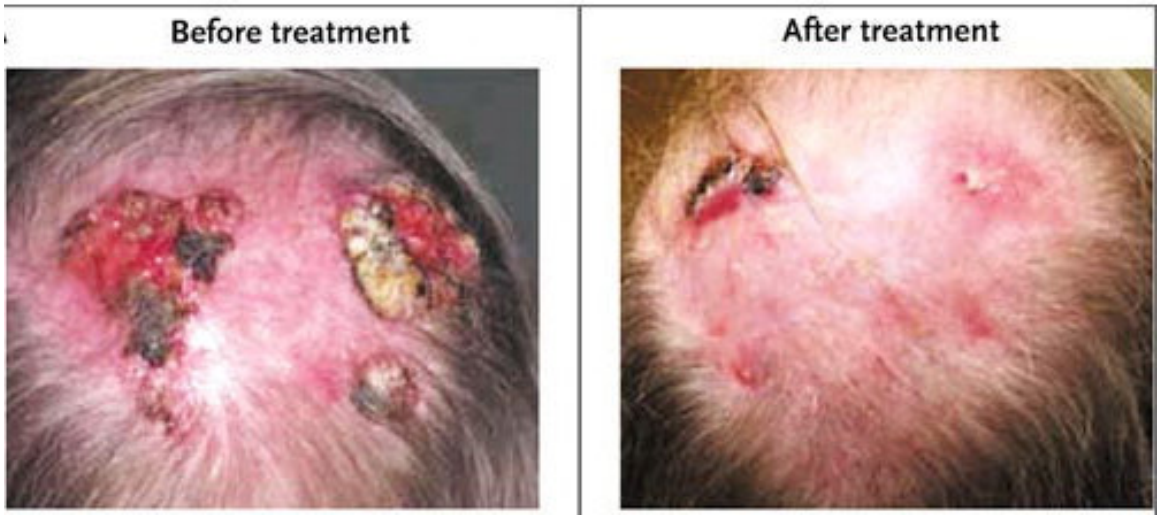
Cyclopamine: A plant alkaloid inhibiting Smo signaling

cyclopamine (a plant alkaloid isolated from corn lilly) as a natural inhibitor of smo

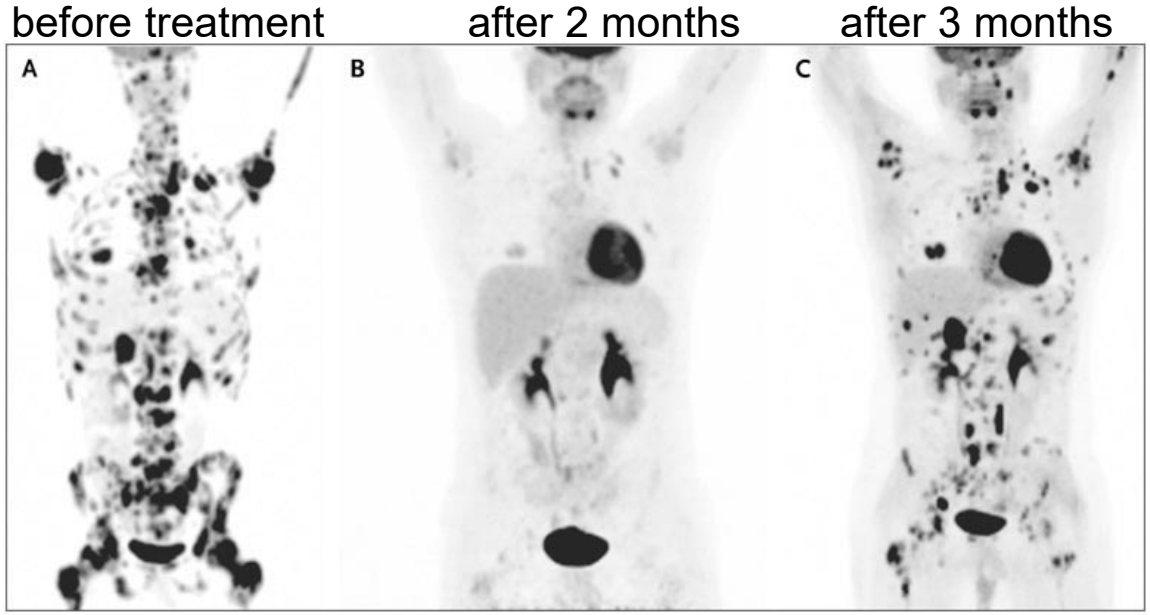




Phase I clinical trial of an orally administered Smo antagonist



Von Hoff et al. N Engl J Med (2009)



Rudin et al. N Engl J Med (2009)

<u>Vismodegib (GDC-0449)</u>

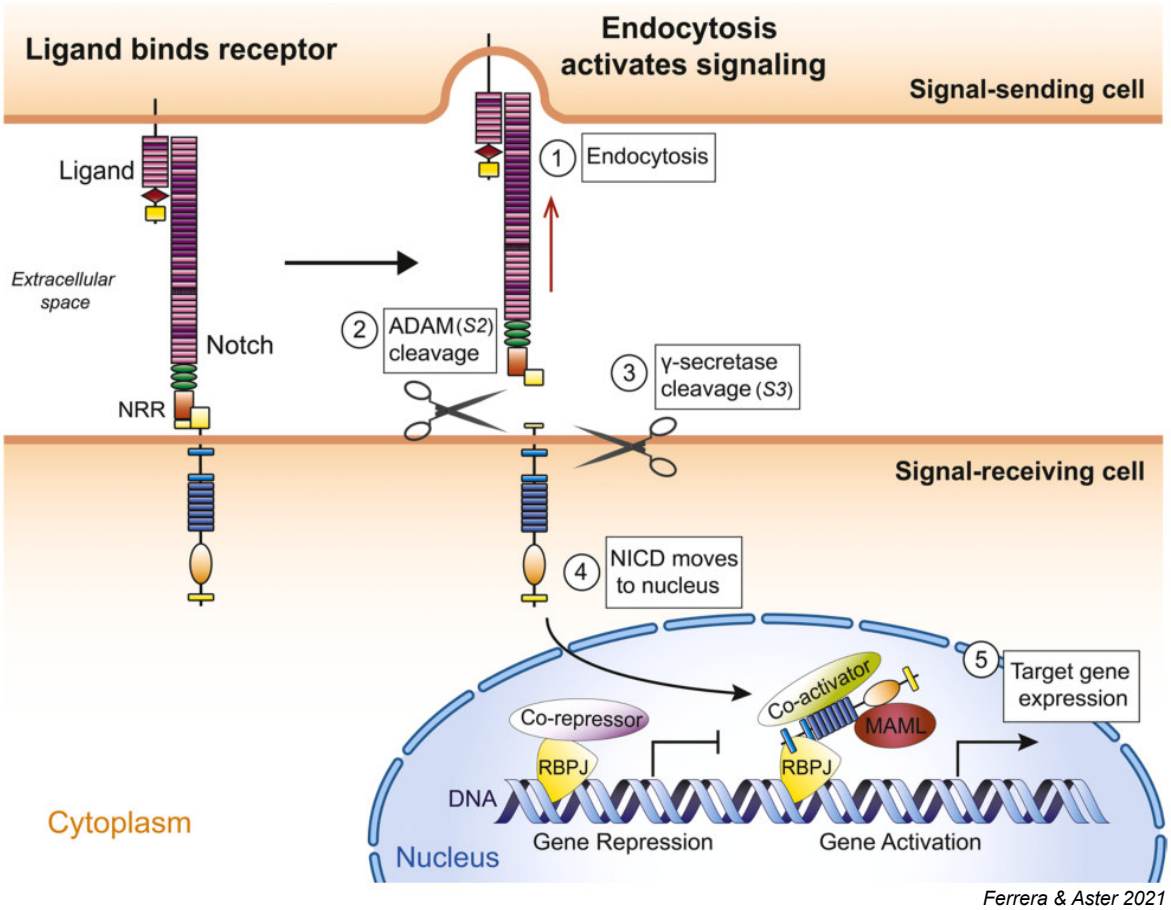
of 33 patients with local BCC, 11 were stabilized and 18 showed at least partial tumor regression

while the drug was FDA approved, many countries decided that the additional benefit for patients wasn't sufficient to support application

in a case report, a 26-yr old patient with metastatic medulloblastoma showed transient response; a mutation in Smo conferred resistance

currently, new inhibitors are developed which still act on mutant forms of Smo

Notch pathway core components

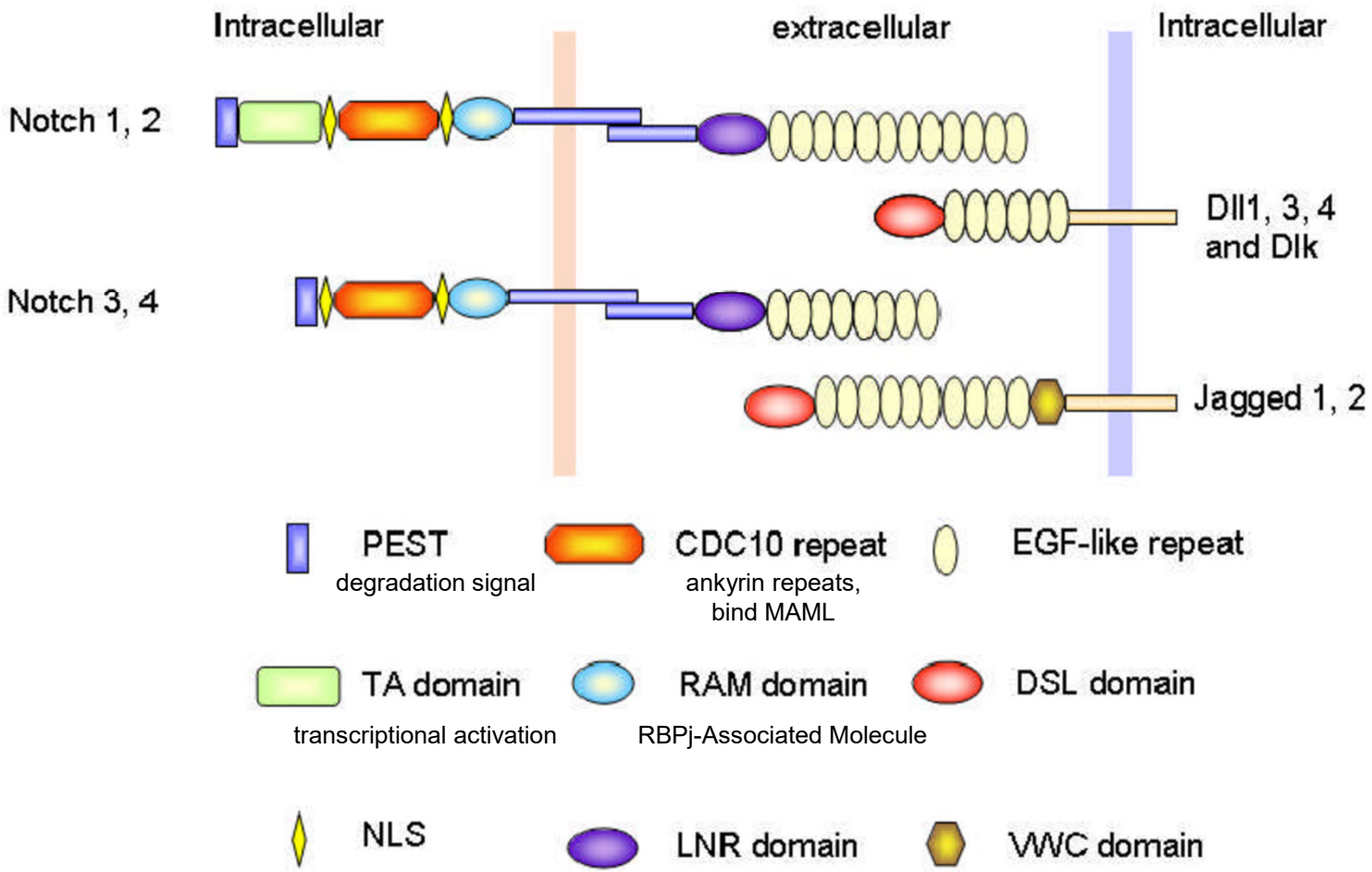


Ferrera & Aster 2021

No catalytic signal amplification step !

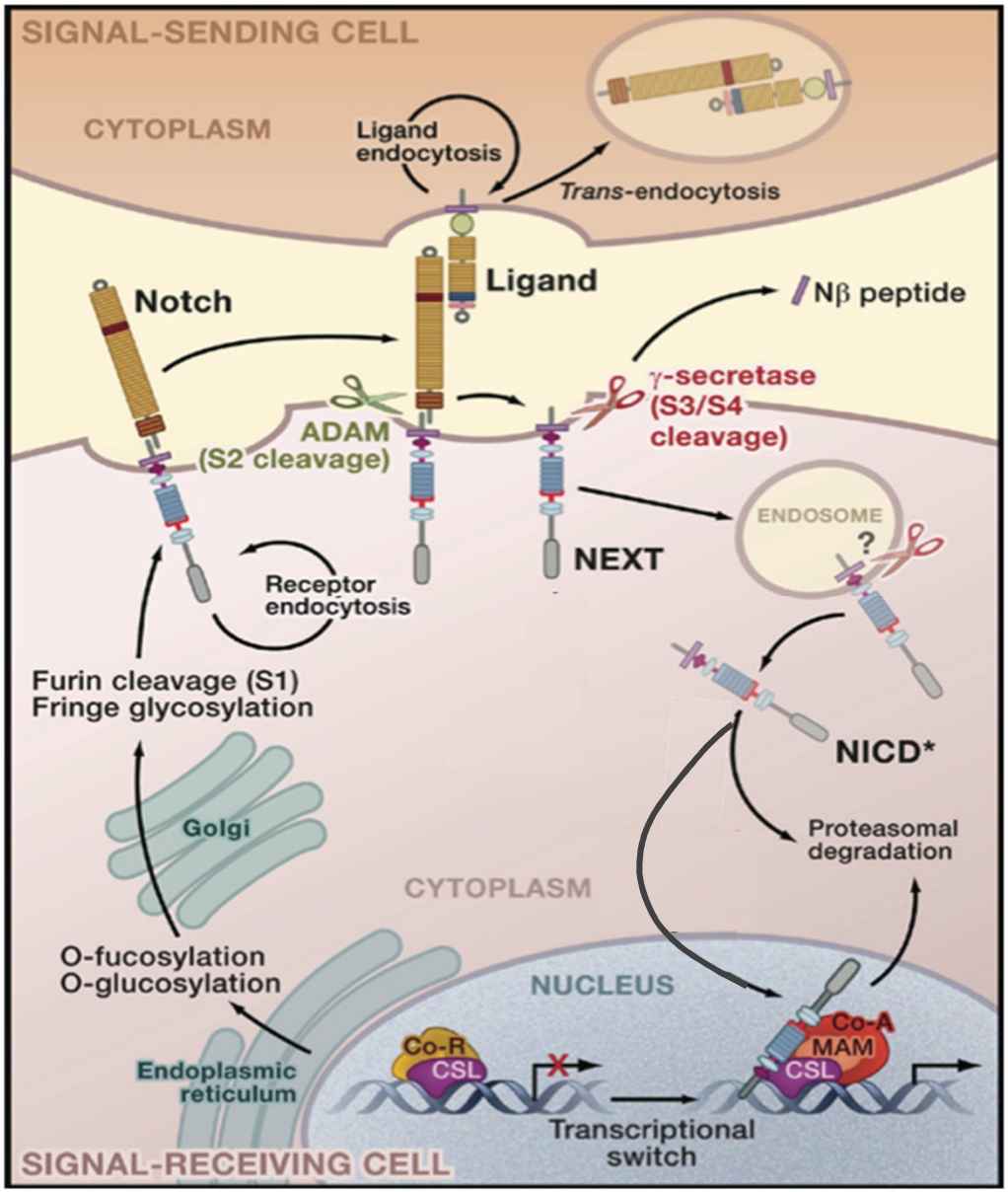
- Notch proteins are transmembrane receptors
- ligand endocytosis triggers cleavage and release of the Notch Intracellular Domain (NICD)
- NICD translocates to the nucleus to assemble a transcription activator complex

DSL ligands interact with four mammalian Notch receptors



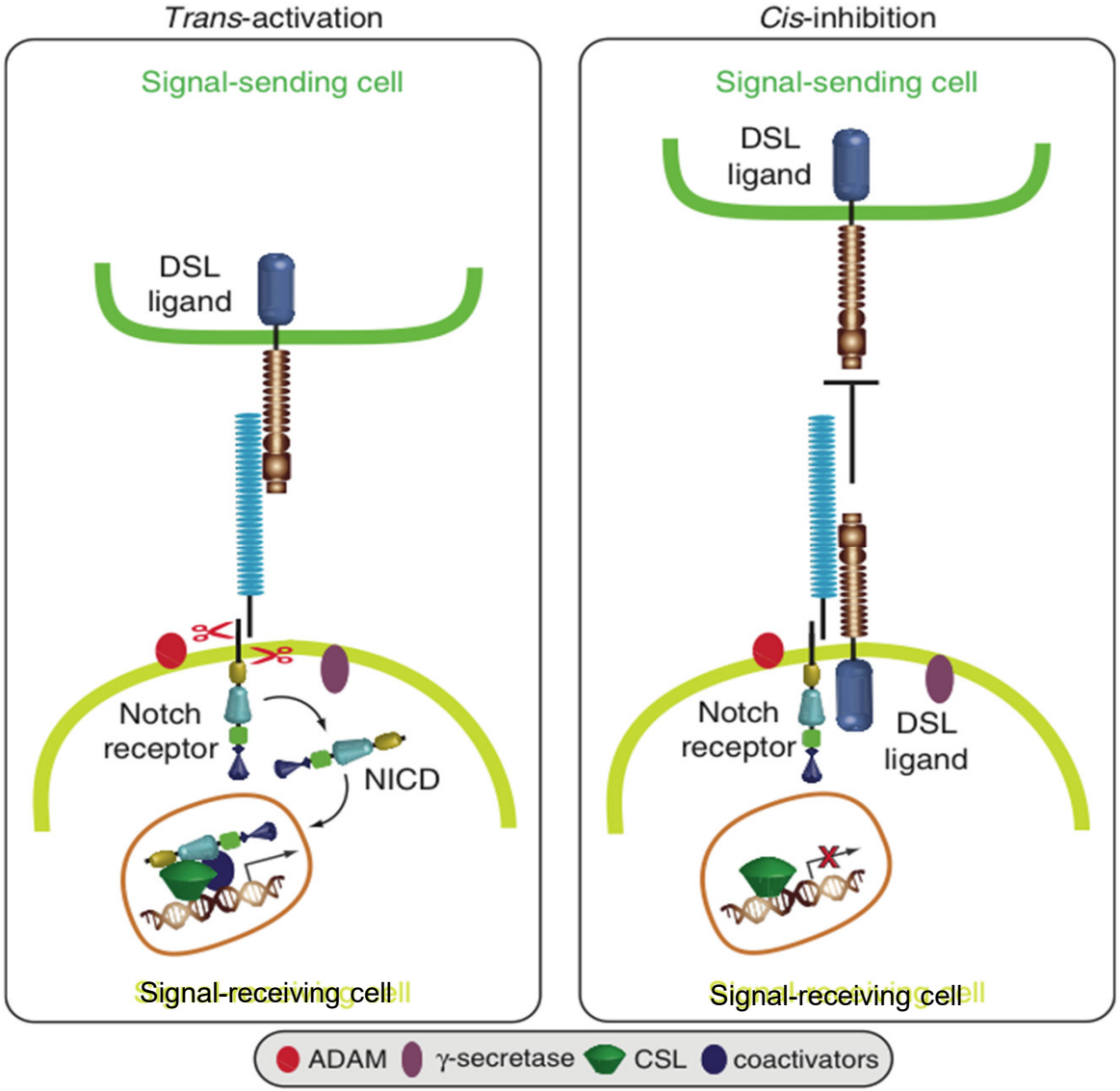
within its ligands, Notch binds the DSL domain (Delta/Serrate/LAG-2)

Notch signalling pathway



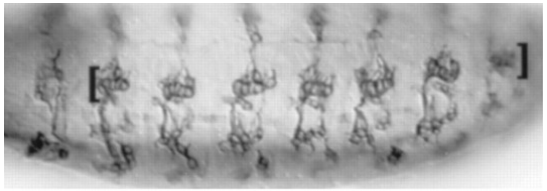
- Ligand binding triggers trans-endocytosis of Notch Extracellular Domain (NEC)
- Removal of NEC unmasks S2 site for ADAM10 and -17
- Endocytosis of Notch Extracellular Truncated (NEXT) unmasks an intramembranous S3 site that is cleaved by γ-secretase
- a fraction of NICD (Notch intracellular domain) moves from endosomes to the nucleus where it interacts with the CSL (CBF1-human, SuH-fly, Lag-worm, RBPj-mouse) transcription factor to exchange co-repressors (Hes, Hey) against the MAML (mastermind-like) co-activator

Notch mediates juxtacrine, but not autocrine signaling

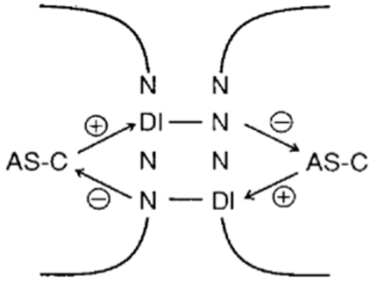


- ligands only signal in *trans*, while inhibiting Notch receptors in *cis*
- small differences in ligand and receptor levels between neighboring cells are amplified by feedback so that one cell sends while the other receives the signal
- for this feedback, Notch signaling represses DSL ligand expression thereby fixing the sender and receiver fate of neighboring cells

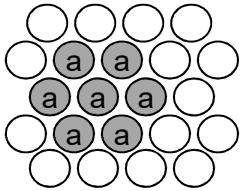
Notch controls a sequence of binary cell fate decisions responsible for sensory organ development in Drosophila



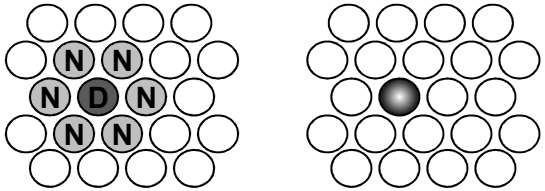
proneural gene clusters:
Ato (chordotonal neurons) or
AS-C (external sensory neurons)

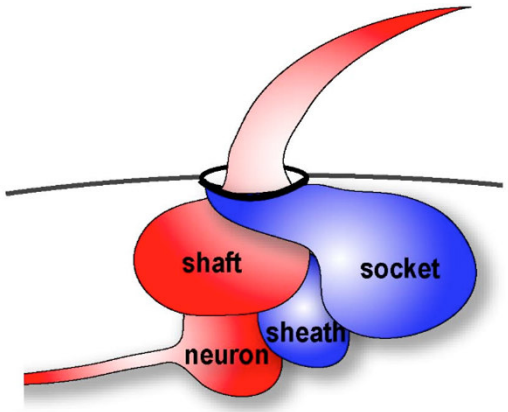


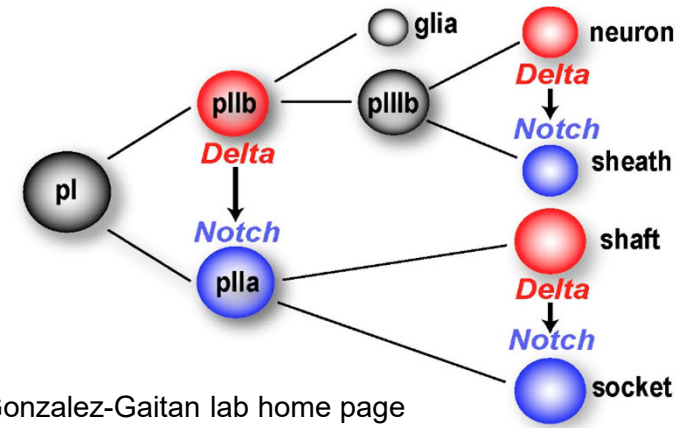
Step1:
Competence



Step 2. Lateral inhibition singles out one SOP:



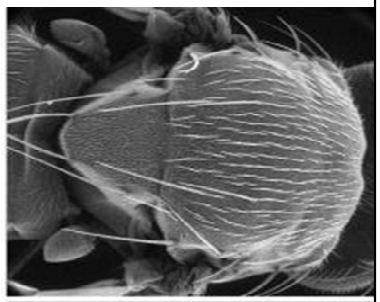




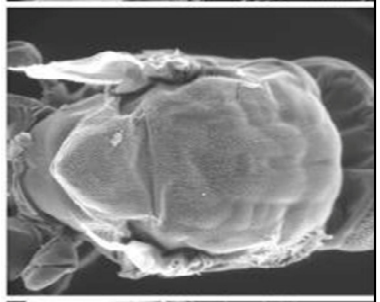
Gonzalez-Gaitan lab home page

sensory bristles

WT



N ↑

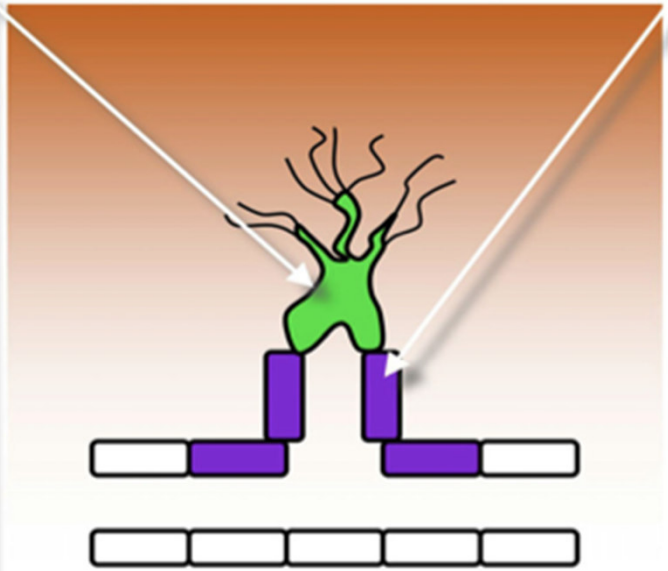


N ↓

Notch signaling in ECs specifies stalk cells of vascular sprouts

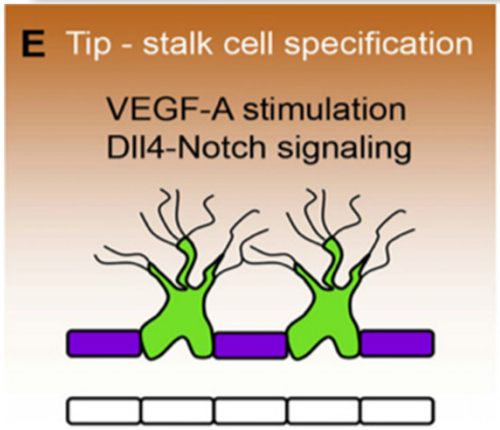
The tip cell: **Dll4**

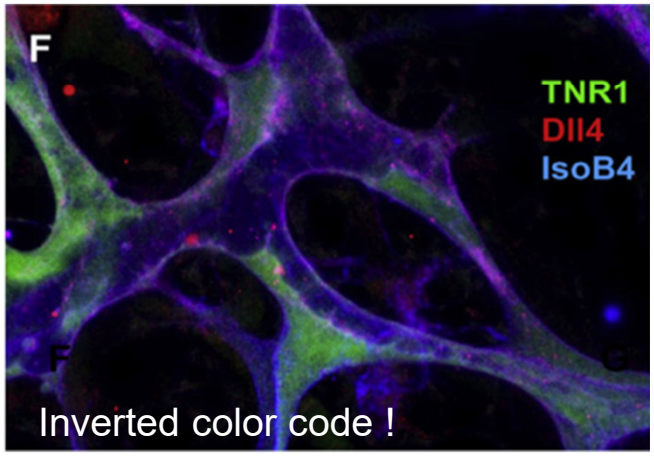
- single highly polarized endothelial cells
- numerous actin rich filopodia protrusions
- induced by VEGF-A
- VEGFR2 on filopodia
- lacks a vascular lumen
- increased mRNA levels of *Pdgfb, Dll4, Unc5b, Kdr Flt4, etc.*
- specialized for guided migration
- rarely proliferates

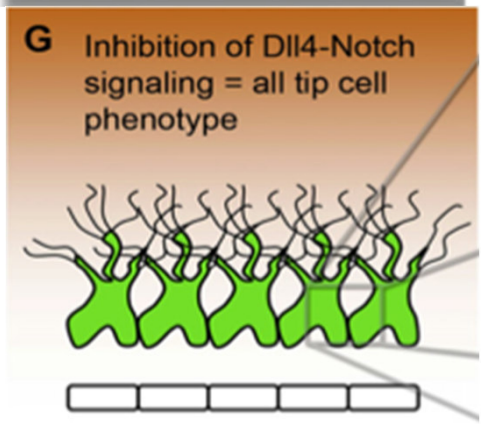


The stalk cells: **Notch1**

- proliferate when stimulated with VEGF-A
- form vascular lumen
- establish firm adherens junctions
- deposit basement membrane
- express higher levels of *Robo4, Jag1, Flt1 etc.*
- can be induced to become new tip-cells



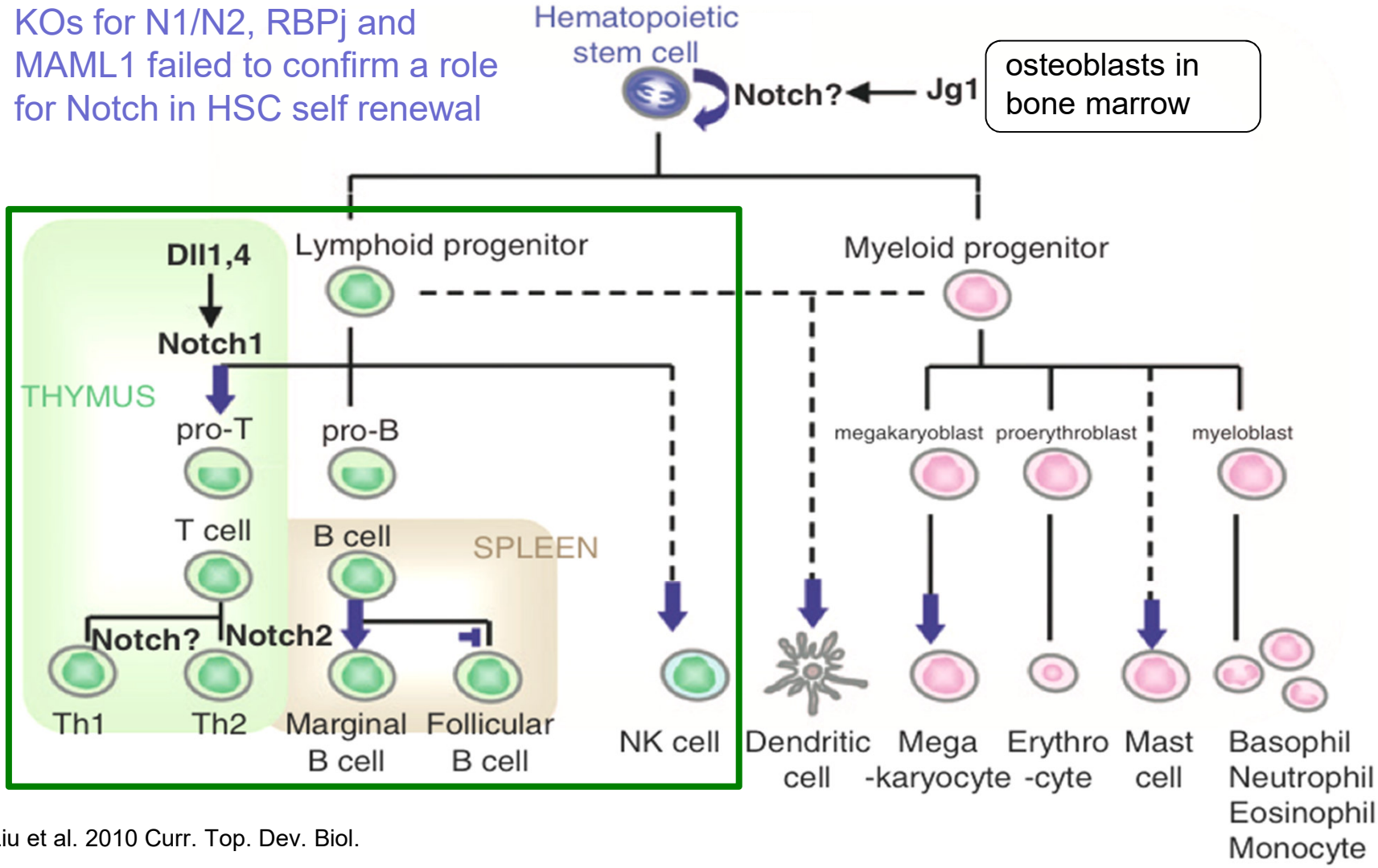




Phng & Gerhardt 2009 Dev. Cell

Sequential Notch-dependent binary fate decisions determine the ratio of T and B cells

KOs for N1/N2, RBPj and MAML1 failed to confirm a role for Notch in HSC self renewal



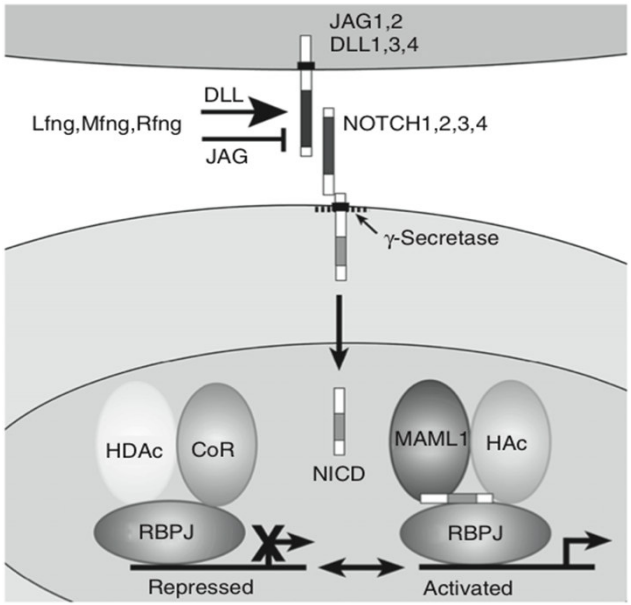
Liu et al. 2010 Curr. Top. Dev. Biol.

The importance of Notch in hematopoietic and solid tumors reflects physiological functions during normal development

endogenous roles:

- Hematopoiesis
- Angiogenesis
- Adult stem cell maintenance (intestine, skin, mammary gland, melanoblasts…)
- Neurogenesis

⇒ observed toxicities: intestine hypertension



main cancer targets:

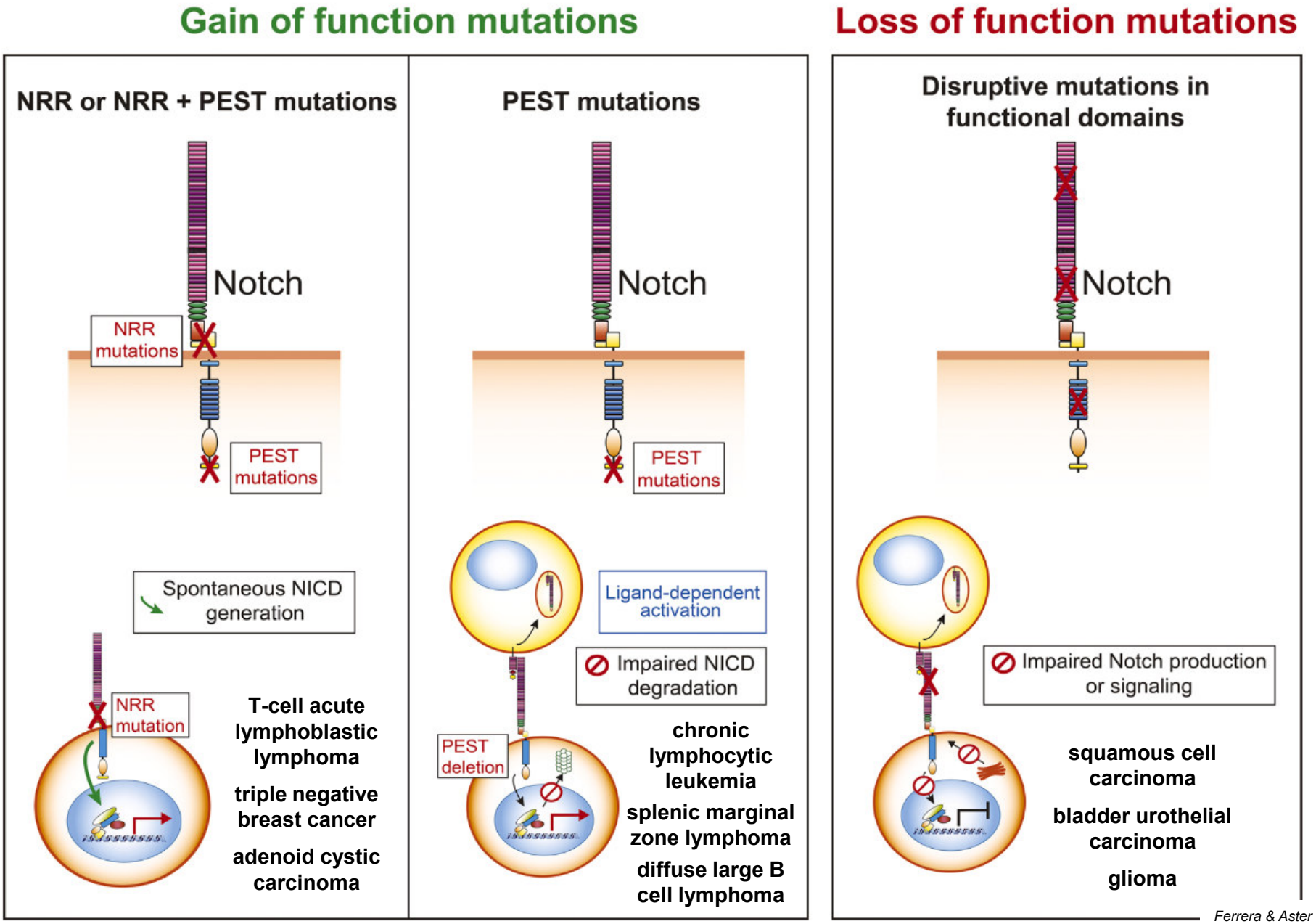
- **T-ALL**
- **Tumor angiogenesis**

other potential targets:

- Breast cancer
- Prostate Cancer
- Colorectal cancer
- Pancreatic cancer
- Melanoma
- Glioblastoma

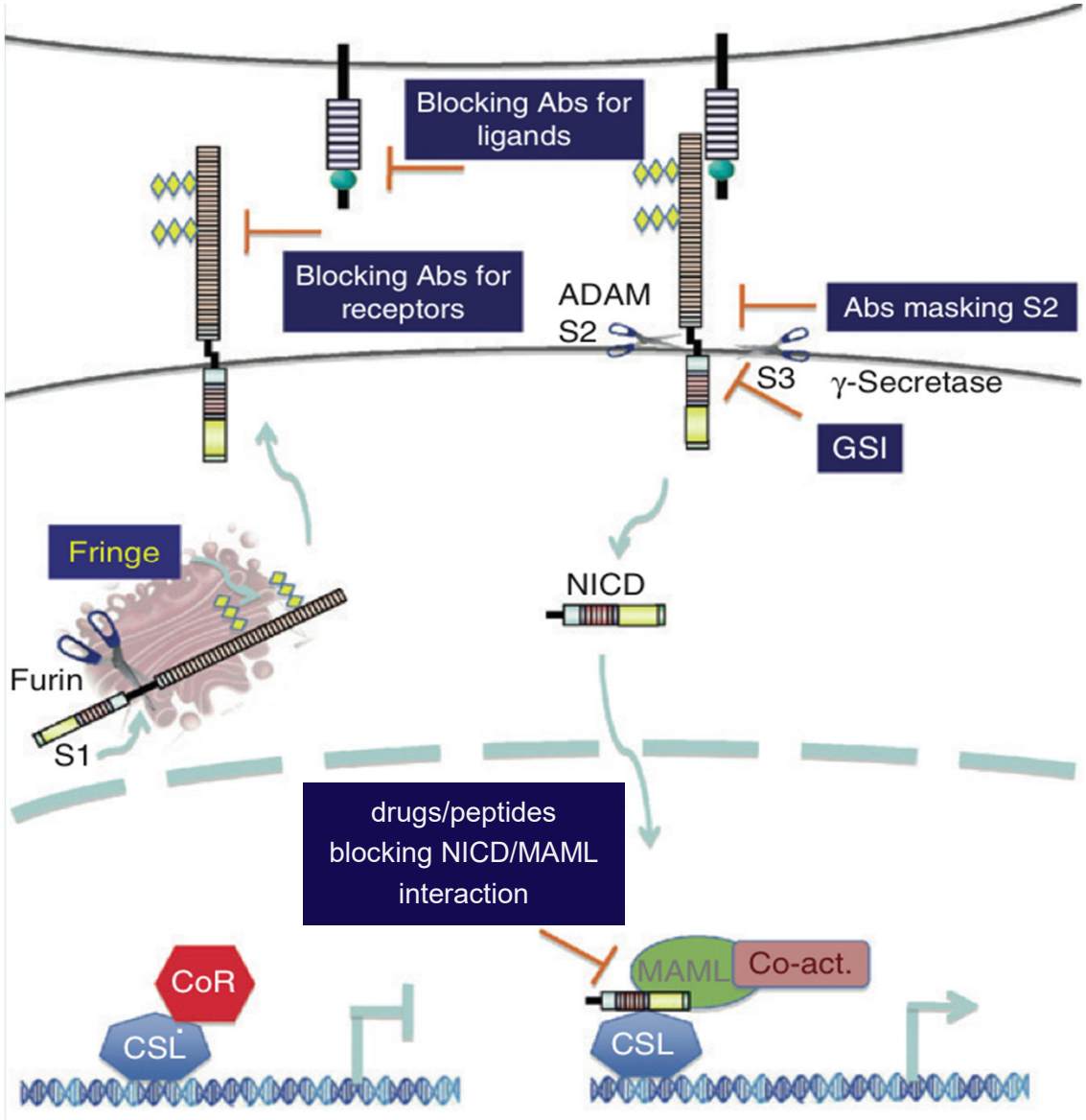
=> NOTCH pathway as a cancer target still awaits clinical demonstration

"Hot spot" mutations activate ligand-independent Notch signaling



Ferrera & Aster 2021

Therapeutic approaches to target the Notch pathway



G-secretase inhibitors (GSIs):

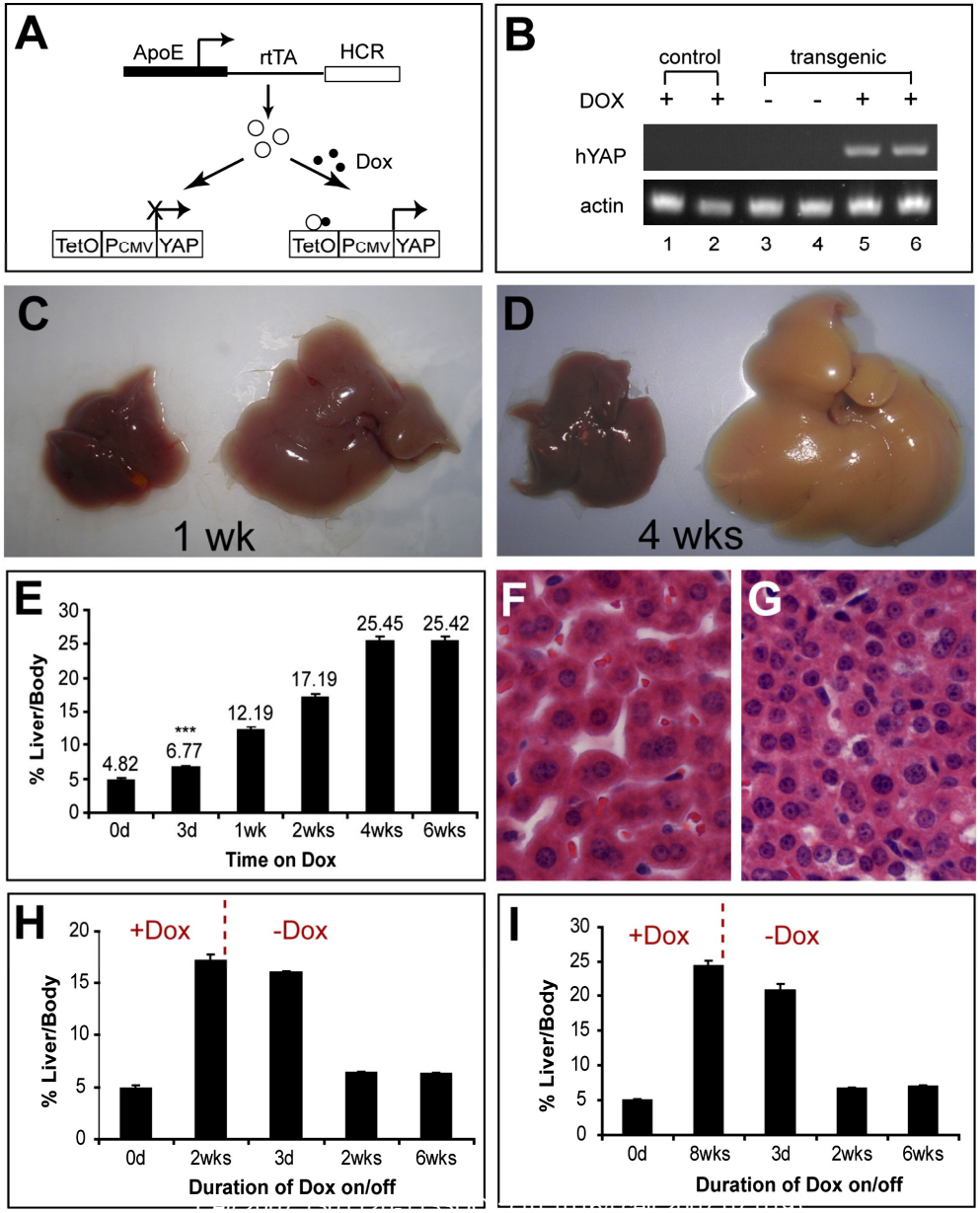
limited selectivity for Notch isoforms

also affect other GS substrates

disappointing clinical results

blocking antibodies also didn't show strong clinical efficacy

YAP/TAZ in organ size control

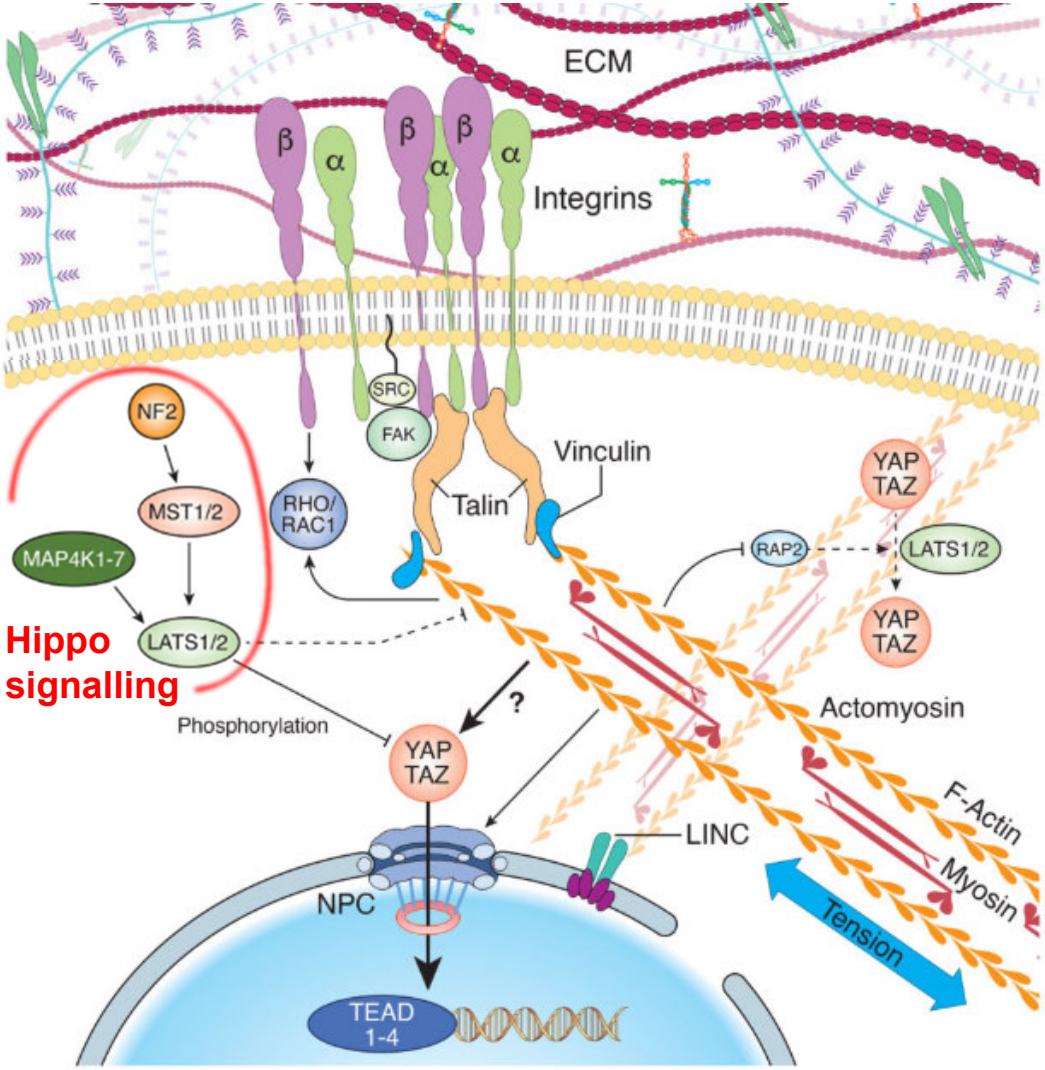


Dong et al., Cell (2007)

- YAP/TAZ are transcriptional co-activators which mediate cell **proliferation**, organ **overgrowth**, **survival** to stress and dedifferentiation of post-mitotic cells into their respective tissue **progenitors** or even embryonic precursors
- YAP and TAZ are required for wound healing and organ **regeneration** but not normal tissue-homeostasis, and are **upregulated in many cancers**

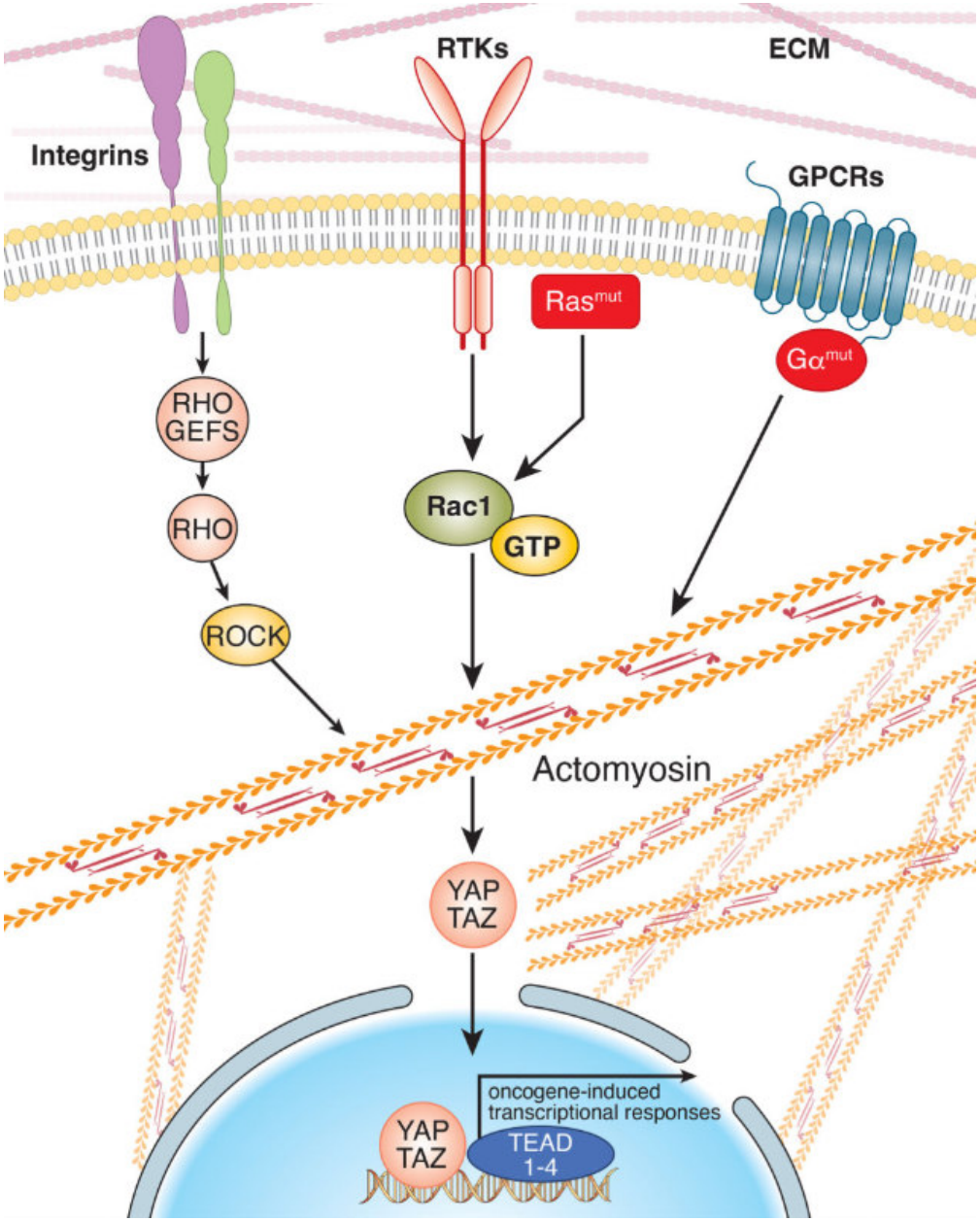
- transgenic mice with tetracycline induced YAP activation in the liver develop massive hepatomegaly
- elevation in liver mass was detectable as early as 3 days after induction, and reached 5x the size of a normal liver
- increase in liver mass was caused by an expansion in cell numbers (hyperplasia) not cell size (hypertrophy)
- within 2 weeks after Dox withdrawal, the enlarged livers had returned to near normal size
- when the mice were exposed to Dox for over 8 weeks, they developed hepatocellular carcinoma

YAP/TAZ in Mechano-sensing



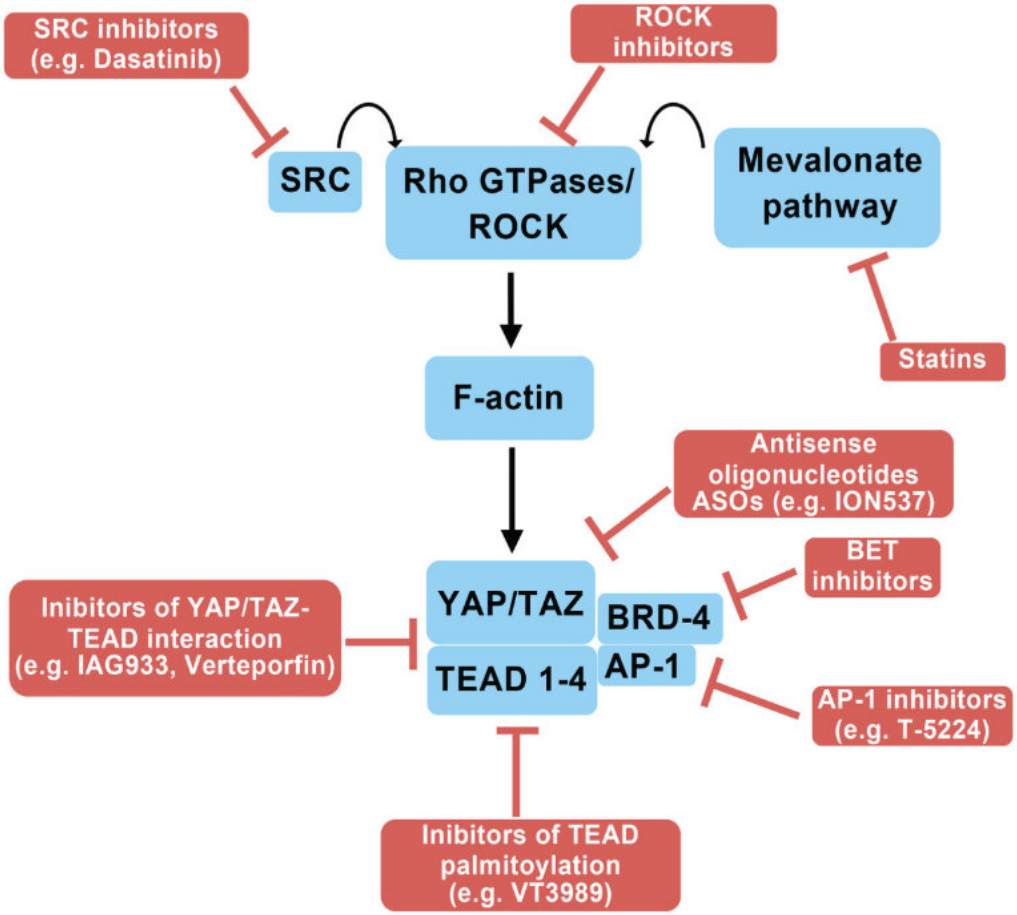
- YAP/TAZ are transcriptional co-factors, which shuttle between the cytoplasm and the nucleus, and are recruited to enhancer elements by TEAD transcription factors
- YAP/TAZ stability and localization is regulated by mechano-transduction based on physical forces between cell-generated traction forces and resisting forces within the extracellular matrix (ECM)
- integrins through focal adhesion junctions connect the ECM with the F-actin cytoskeleton, in a manner involving the ILK, FAK and Src
- the cytoskeleton adapts to forces by regulating F-actin formation and contractility in a process requiring Rho-GTPases (Rho or Rac1), myosin activity and ROCK
- the Hippo pathway inhibits YAP/TAZ through phosphorylation by LATS1/2, contractility can affect LATS1/2 activity trough the GTPase RAP2
- while mechano-signalling enhances YAP/TAZ activity, the exact mechanism is still unclear and likely does not require Hippo signalling

Oncogenic transformation activates YAP/TAZ



- oncogenic mutations in K-Ras or Erbb2 sensitize epithelial cells to ECM stiffness by increasing F-actin stress fibers and contractility through Rac1
- such tumors require YAP/TAZ for their emergence
- YAP/TAZ activation is most prominent in less differentiated and more aggressive tumors
- YAP/TAZ can drive cell fate plasticity and reprogramming, stemness and gain of metastatic abilities, cell proliferation and drug resistance, and accounts for a large fraction of oncogene-induced transcript-tional responses
- experimental gain-of-YAP/TAZ is sufficient to provide full metastatic potential to non-metastatic cells, while YAP/TAZ inactivation impedes metastasis

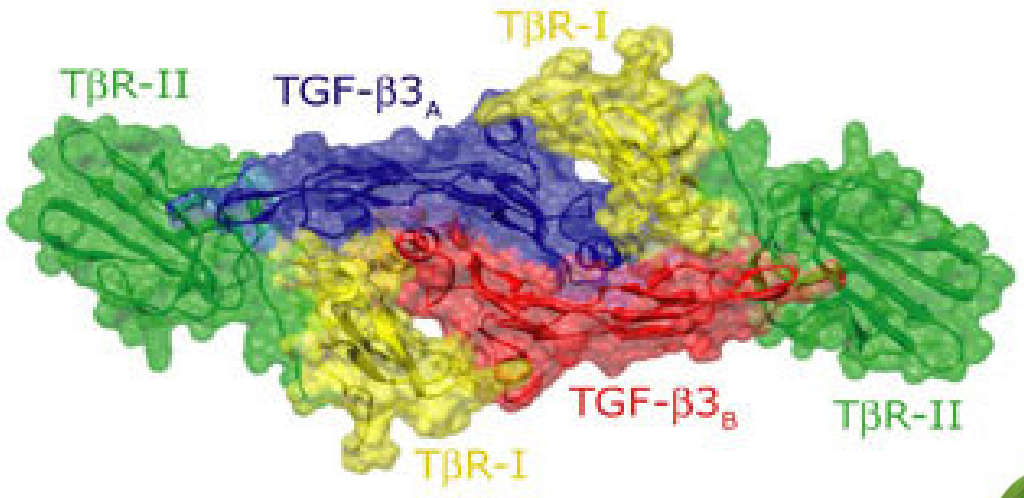
Targeting YAP/TAZ for cancer therapy



Overview of the most promising drugs targeting YAP/TAZ activity

- few human tumor types display genetic alterations in components of the Hippo pathway: mesotheliomas, medulloblastomas and schwannomas (NF2), renal cell carcinomas (NF2, LATS1/2), and cholangiocarcinomas (Sav)
- many other tumor types show widespread YAP/TAZ hyperactivation without genetic alterations in the Hippo pathway or altered LATS1/2 activity
- tumors resemble “chronic wounds” with inflammation and distorted architecture by increased ECM deposition and stiffness contributing to persistent YAP/TAZ hyperactivation in solid tumors

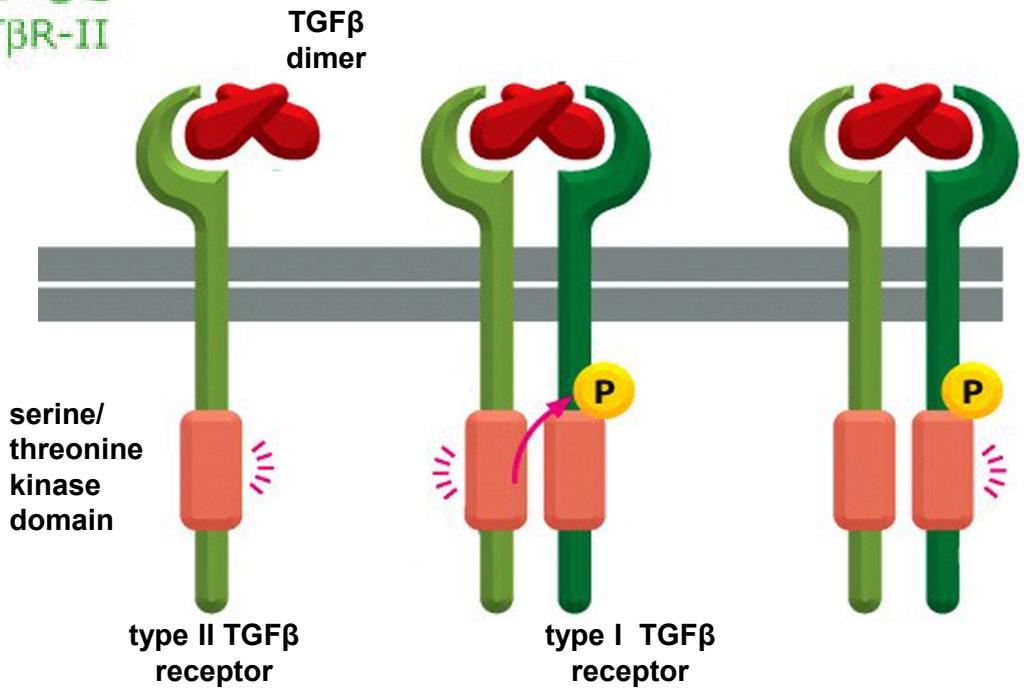
TGFβ/BMP signaling via Receptor Ser/Thr kinases



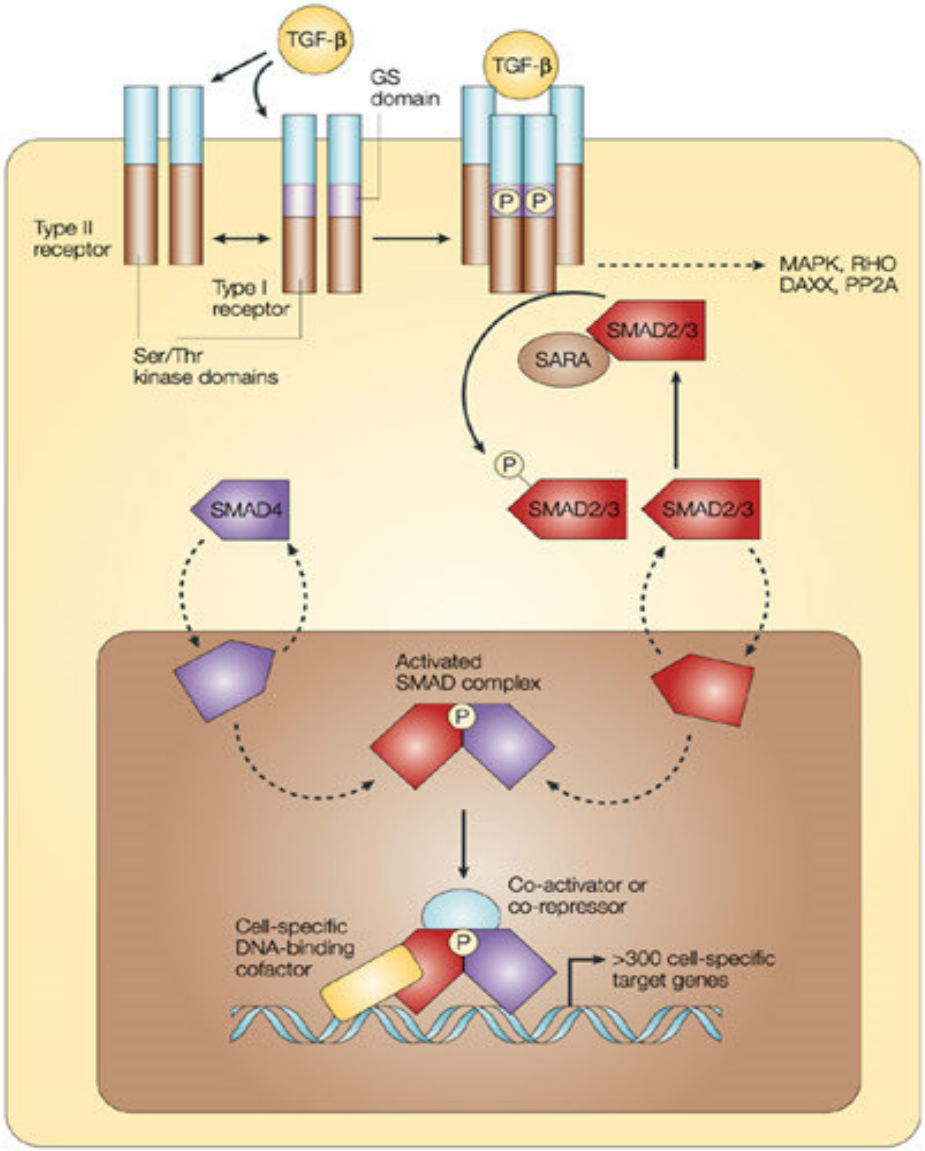
Two subunits of ligand activate
Two TGFβR2 which transphosphorylate
Two TGFβR1 subunits

Ser/Thr phosphorylation by TGFβR2 unmasks the TGFβR1 kinase activity

Activated TGFβR1 emits signals by phosphorylating cytoplasmic effectors



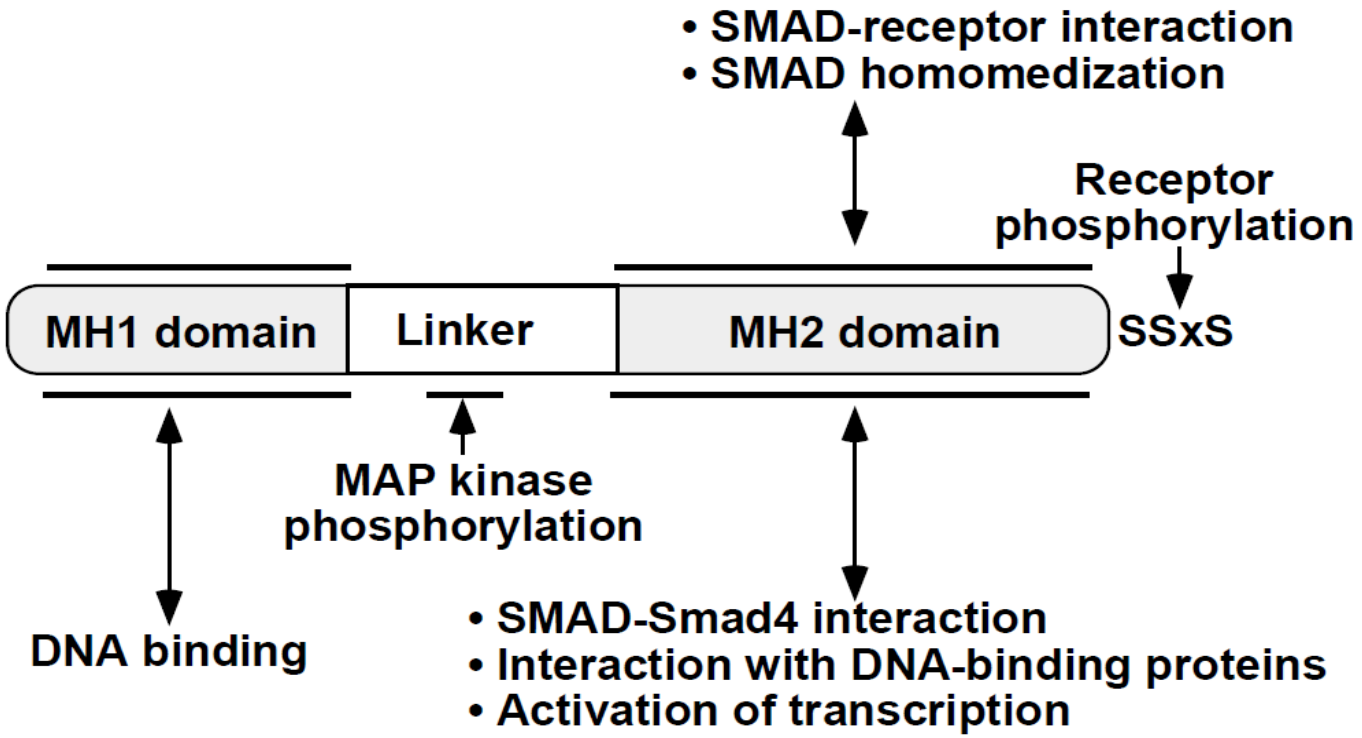
« Canonical » SMAD signal transduction pathway



- Unphosphorylated SMADs shuttle between cytoplasm and nucleus
- SMAD anchor for receptor activation (SARA) localizes phosphorylation events at endosome-limiting membranes
- Regulation of target genes requires binding of phosphorylated R-SMADs to transcriptional co-factors and (in most, but not all cases) Smad4
- SMADs are *not* the only substrates of TGFβRs

Siegel & Massagué Nat Rev Cancer (2003)

R-SMAD structure and function

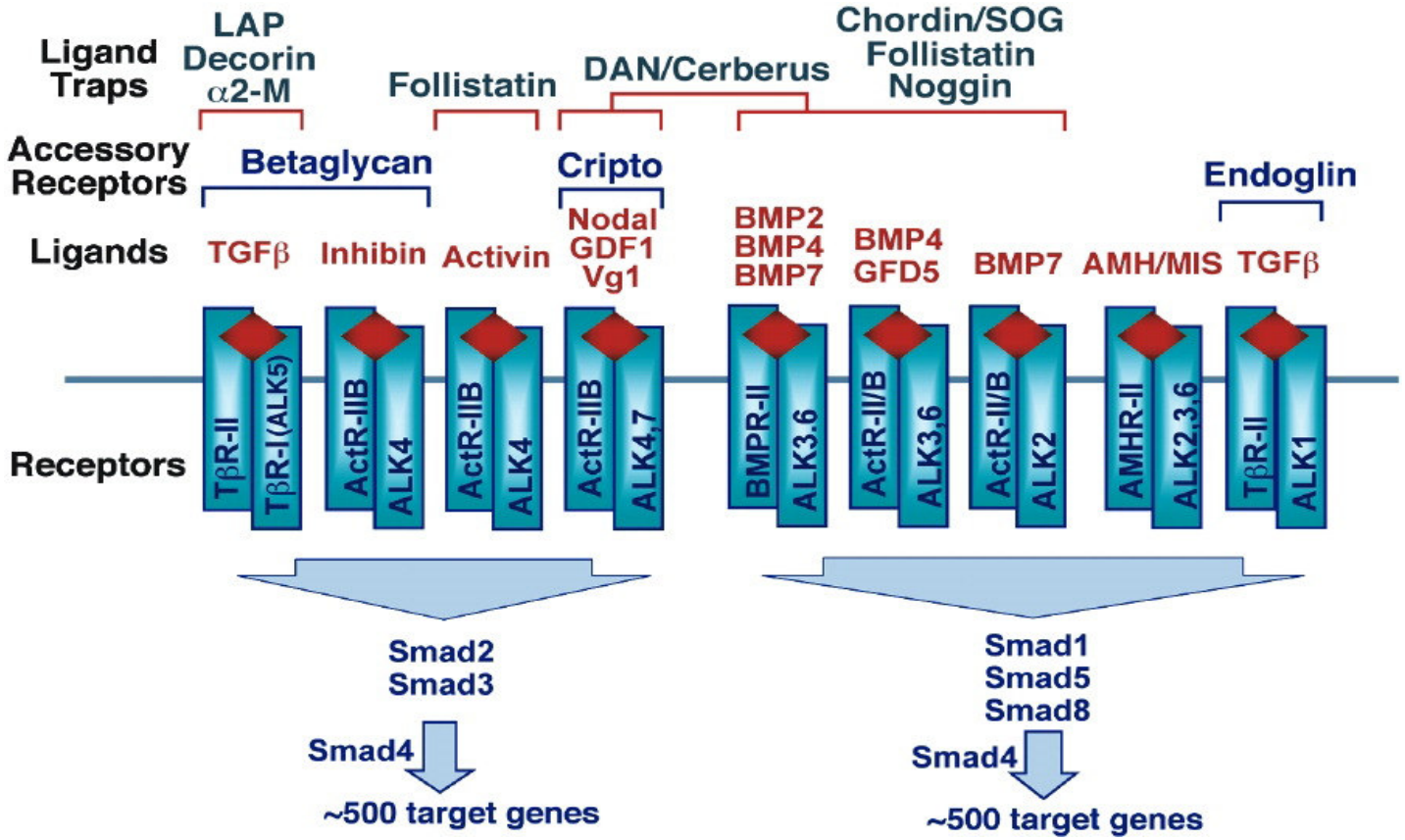


phosphorylation of MH2 domain by type I receptors unmasks DNA binding of the MH1 domain

phosphorylation of linker by other kinases (MAPK, CDK) accelerates SMAD degradation

Massagué Ann. Rev. Biochem. (1998)

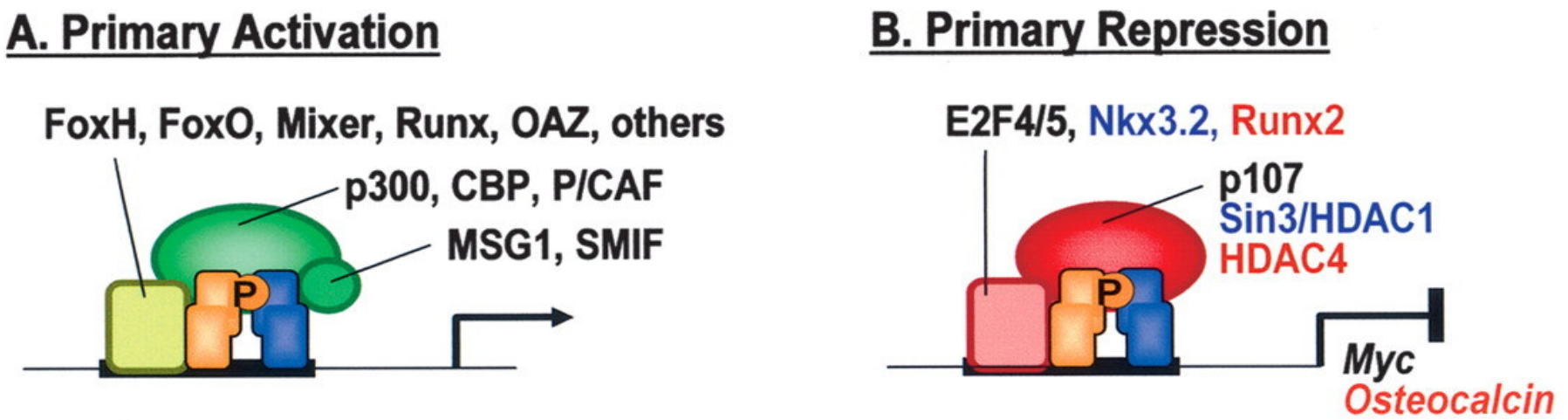
Ligands, ligand traps, two classes of receptors and their R-SMADs



- the seven mammalian R-SMAD proteins are subdivided into two classes
  - Smad1,5,8 are mainly downstream of BMP ligands
  - Smad2,3 are mostly activated by TGFβ, Activin and Nodal
  - specificity is mediated by the type1 receptor

Shi & Massagué Cell (2003)

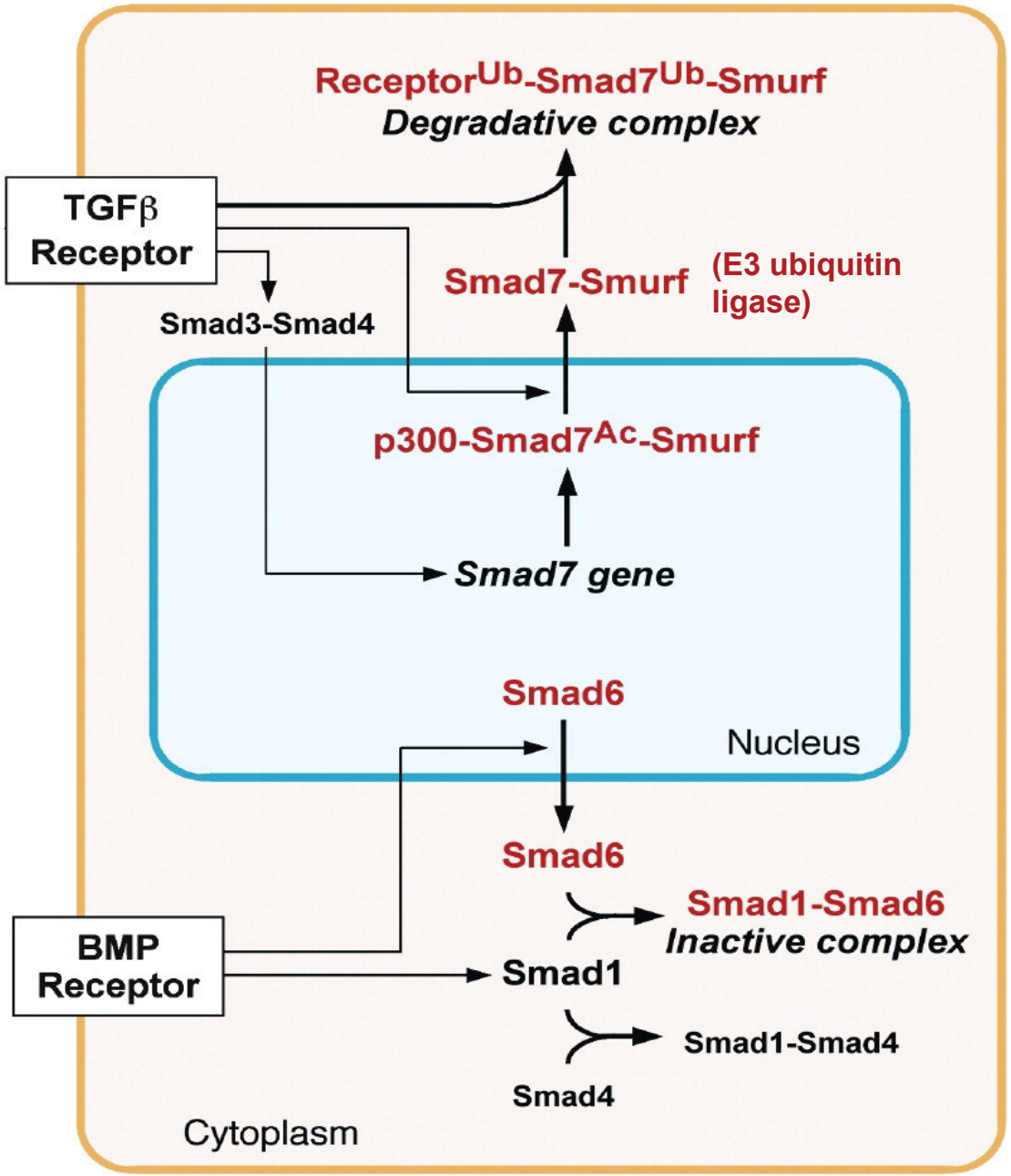
Smad transcription factors act as activators or repressors depending on co-factors



Massagué et al., Genes Dev (2005)

- R-Smad–Smad4 complexes, in combination with different DNA binding cofactors, form assemblies that target specific genes
- FoxH, FoxO, and Mixer are examples of DNA-binding cofactors for Smad2/3–Smad4, and OAZ for Smad1/5–Smad4 which recruit **co-activators** (e.g., p300, CBP, P/CAF)
- R-Smad–Smad4 complexes may also recruit **co-repressors** as a function of the associated DNA-binding cofactor. Smad1–Smad4 with Nkx3.2 recruits Sin3/HDAC1. Smad3–Smad4 with E2F4/5 recruits p107 and with Runx2 recruits HDAC4

TGF-β targets: inhibit cell proliferation, inflammation, immune responses and angiogenesis, activate autophagy and apoptosis
but as well can promote proliferation, plasticity, stemness, and EMT

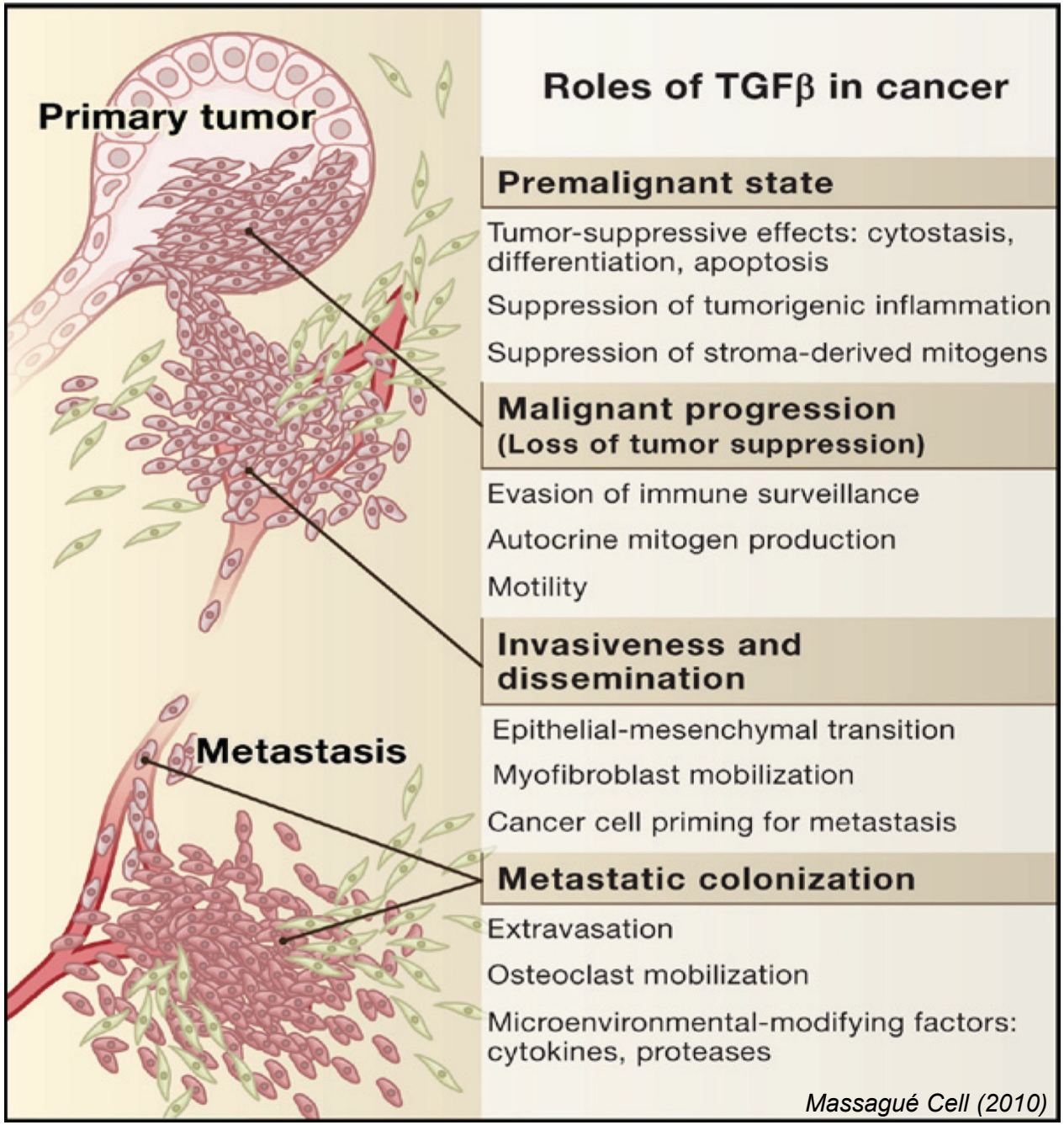


I-SMADs: how to end TGFβ signaling

Shi & Massagué Cell (2003)

Heterogeneous role of TGFβ and BMP signalling in cancer

- mutations in genes encoding members of the TFGβ/BMP pathway have rarely been implicated to cause cancer formation:
  - activating mutations in ACVR1 (encodes ALK2) occur frequently in **Diffuse intrinsic pontine glioma (DIPG)**, a devastating paediatric brainstem tumor, and arrest this glioma at the progenitor cell state by blocking oligodendrocyte differentiation
  - inactivating germline mutations in BMPR1A and SMAD4 are involved in the **familial juvenile polyposis syndrome**, which carries a high lifetime risk of CRC
  - inactivation of SMAD4 increases the risk of **head and neck squamous cell carcinomas**
  - block of signalling by mutations in BMP2, BMP4, GREM1, and SMAD7 increase the **colorectal cancer** risk
- inactivating mutations are however frequently implicated in cancer progression:
  - inactivating SMAD4 or TGFbR2 mutations are often found in advanced **cholangiocarcinoma**, **pancreatic** and **colon cancers** and have been linked to enhanced metastatic potential
- often pathway activity has been observed to preserve progenitor and stem cell phenotypes
  - TGFβ signalling is indispensable to maintain stem cell-like states in **Glioblastoma Multiforme** (GBM)
  - BMP signalling is required for stemness phenotypes in **Acute myeloid leukaemia** (AML), **non-small cell lung cancer** (NSCLC) and **prostate cancer**



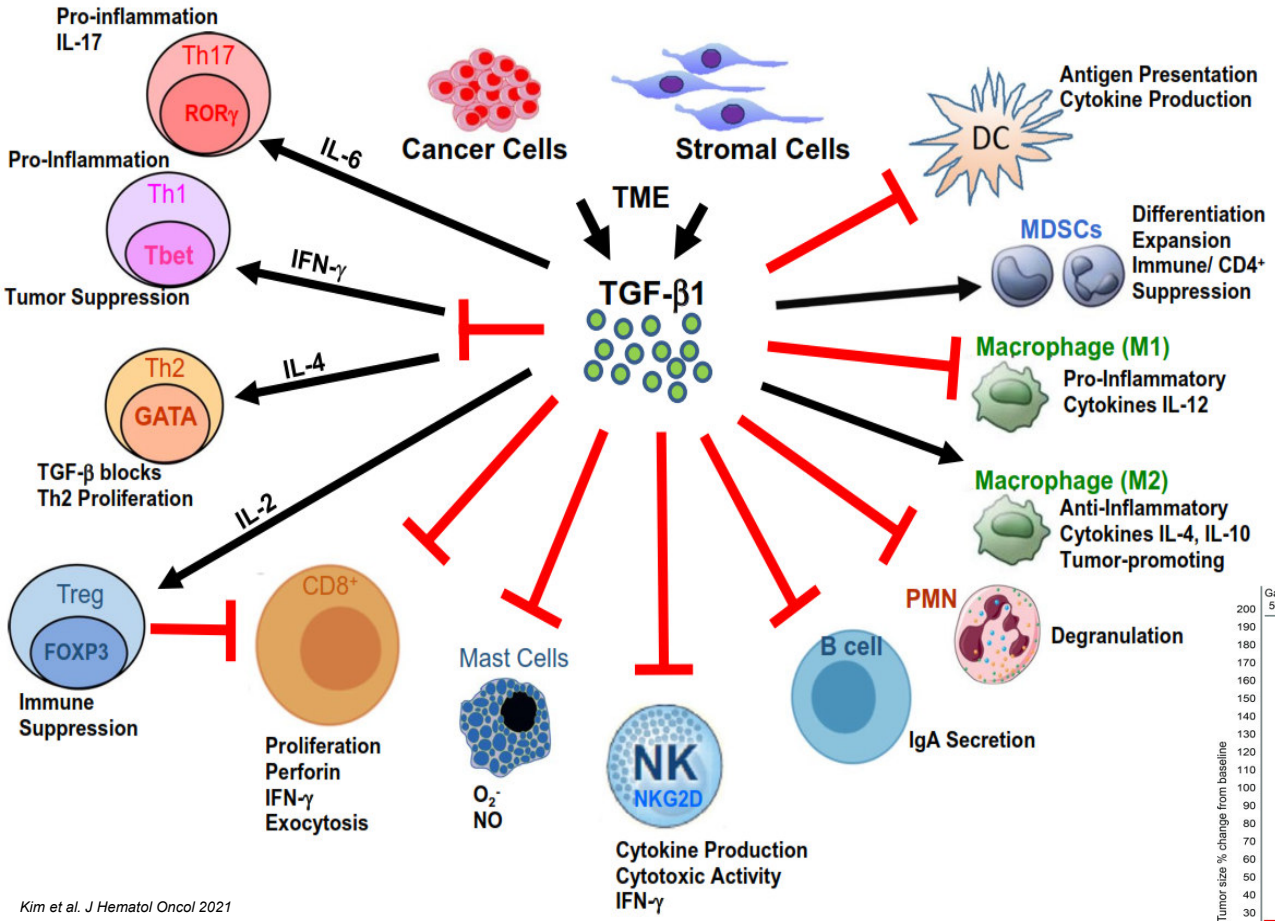
Immune surveillance
potent immune suppressor

Lack of supplies
angiogenesis induction

extracellular matrix barrier
degradation and remodelling of ECM

epithelial phenotype
EMT induction

Immune-suppressive activity of TGFβ and first clinical results

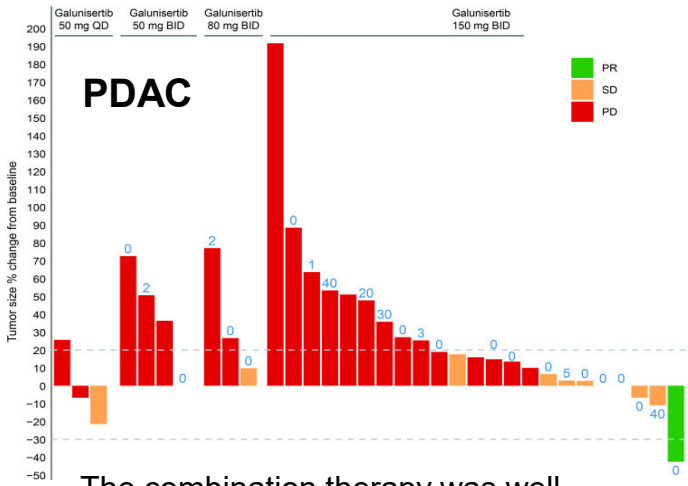


Kim et al. J Hematol Oncol 2021

Combined inhibition of immune checkpoint and TGF-β signaling is a promising therapeutic strategy since these key pathways have independent but complementary immunosuppresssive functions

Safety and efficacy of the TGFβ-RI inhibitor Vactosertib combined with checkpoint blockade against PD-L1 was evaluated in metastatic non-small cell lung cancer (NSCLC) and pancreatic ductal adenocarcinoma (PDAC) patients

The phase 1b trials observed ORRs of 16.7% for NSCLC and 4% for PDAC



The combination therapy was well-tolerated and without safety concerns