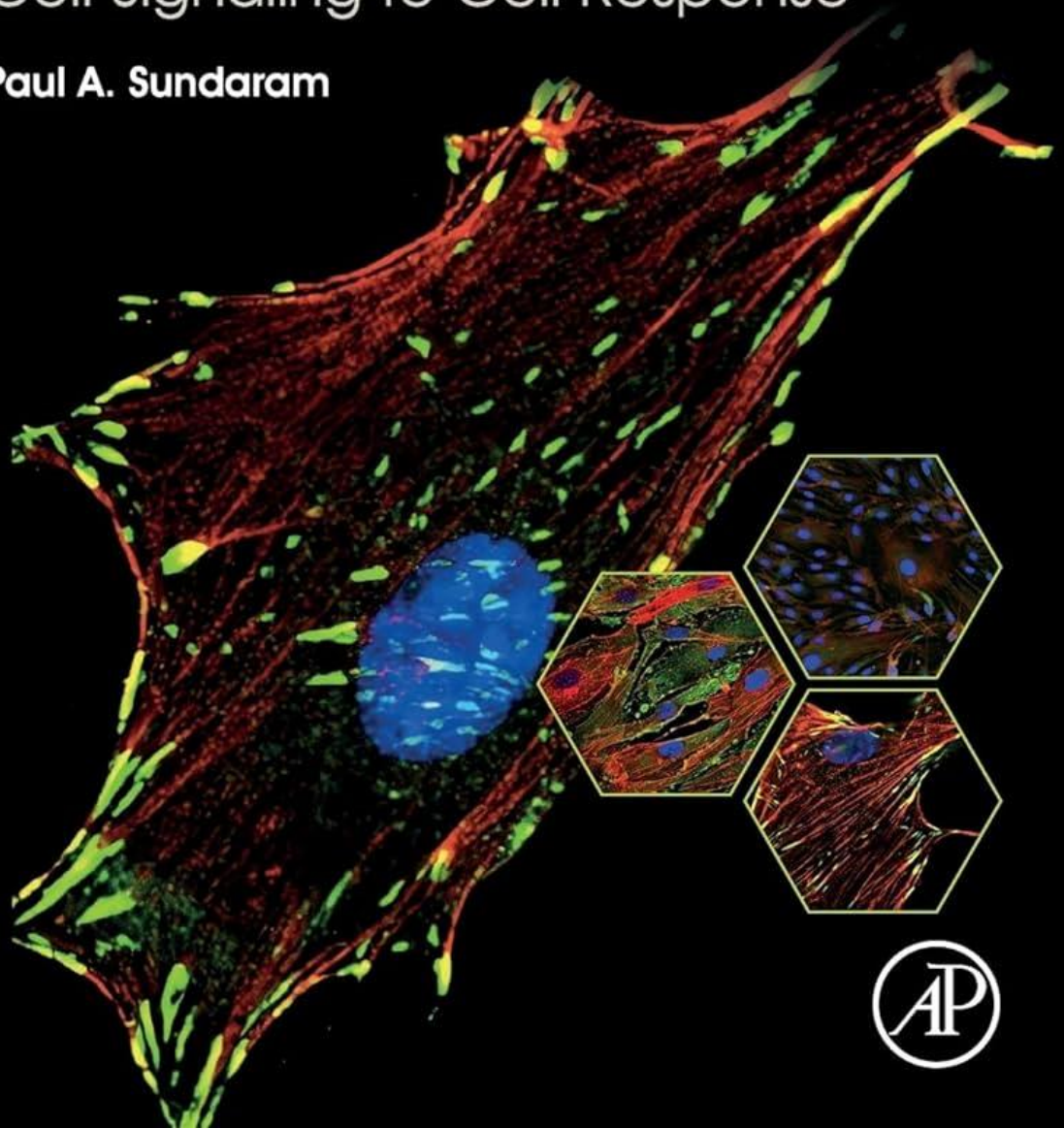
Mechanotransduction

Cell Signaling to Cell Response

Paul A. Sundaram

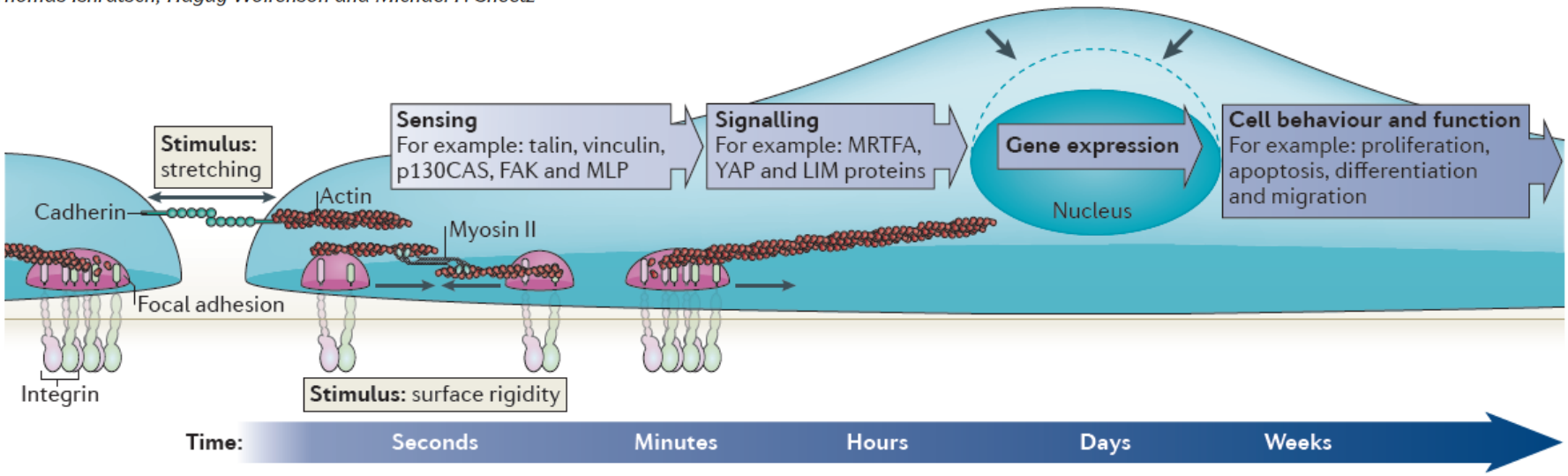


Nature Reviews Molecular Cell Biology

TIMELINE

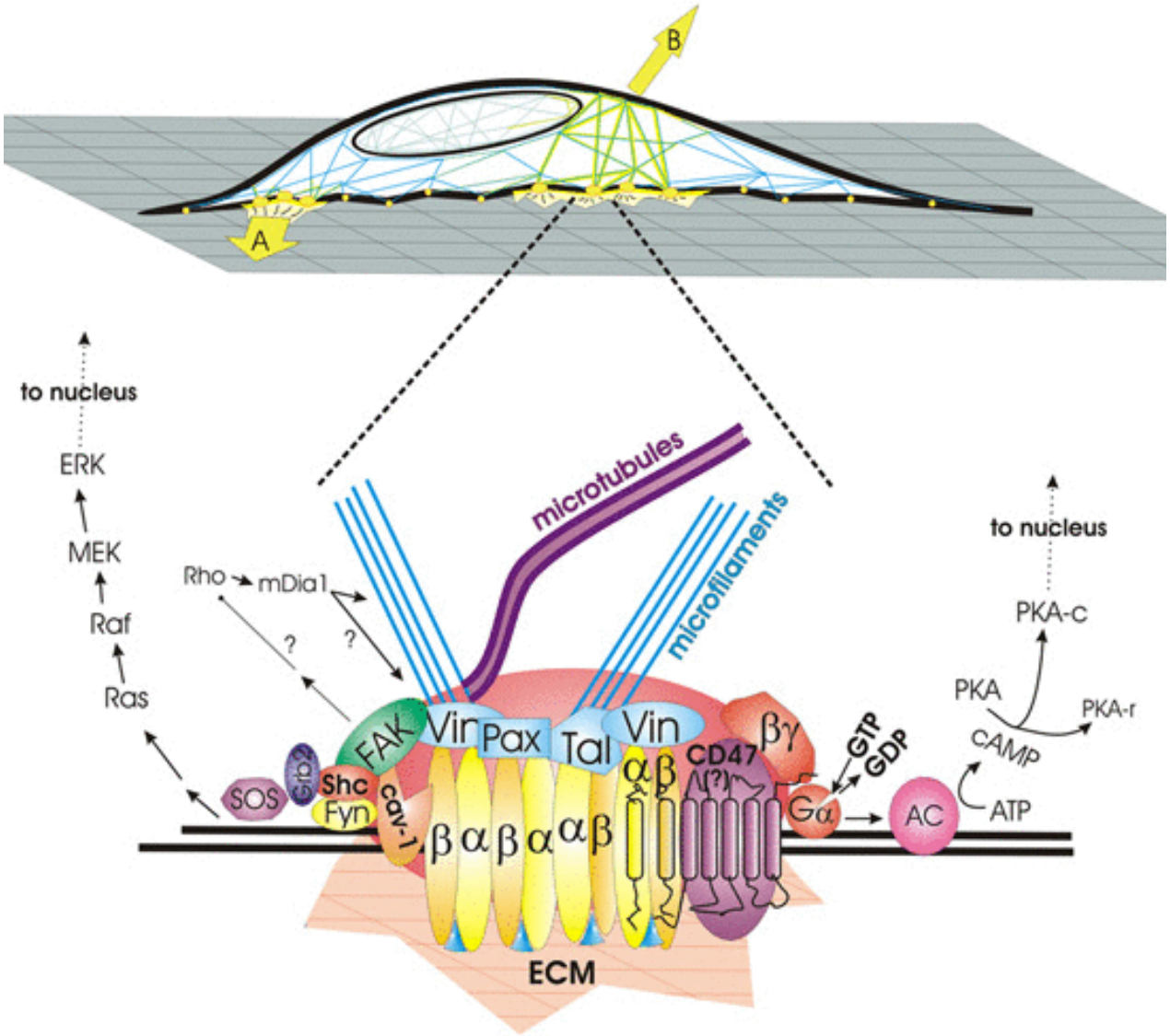
Appreciating force and shape — the rise of mechanotransduction in cell biology

Thomas Iskratsch, Haguy Wolfenson and Michael P. Sheetz



Mechanotransduction converts mechanical stimuli into chemical signals to regulate cell behaviour and function. Typically, the pathway involves receptors at focal adhesions or cell–cell contacts (integrins and cadherins), mechanosensors (stretchable proteins such as talin and p130CAS) and nuclear signalling factors to change gene and protein expression profiles. Nuclear deformation can also lead to changes in gene expression patterns. The timescale of these events ranges from milliseconds to seconds for the stretching of mechanosensors, hours for altered gene expression, days for changes in cell behaviour and function, and weeks for tissue development.

A very well known paradigm…

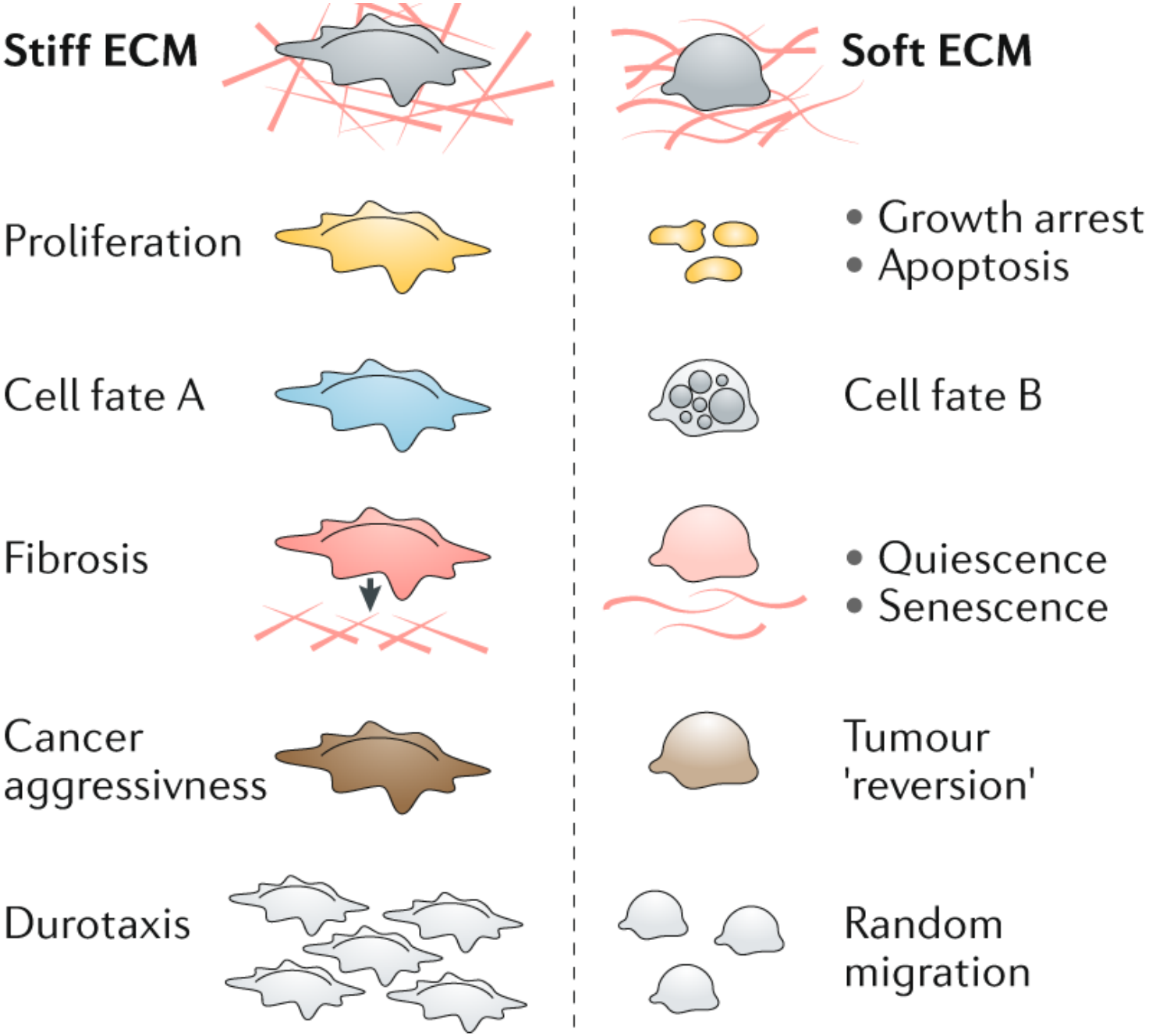


Nuclear mechanotransduction

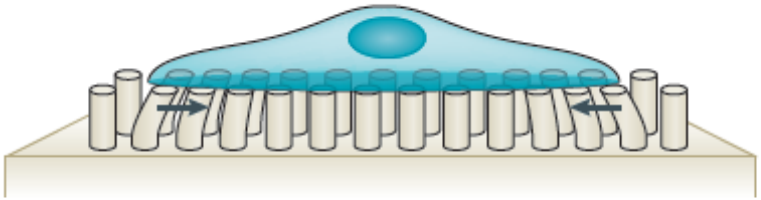
Cytoskeletal mechanotransduction

FA Mechanosensing

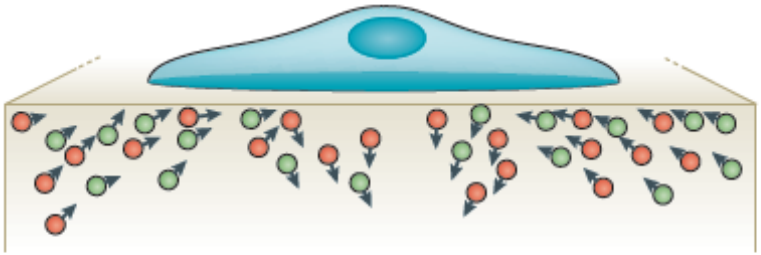
Alenghat, F. J.; Ingber, D. E. (2002). *Mechanotransduction: All Signals Point to Cytoskeleton, Matrix, and Integrins. Science Signaling, 2002(119)*


Stiff ECM
Soft ECM
Proliferation
• Growth arrest
• Apoptosis
Cell fate A
Cell fate B
Fibrosis
• Quiescence
• Senescence
Cancer aggressivness
Tumour 'reversion'
Durotaxis
Random migration

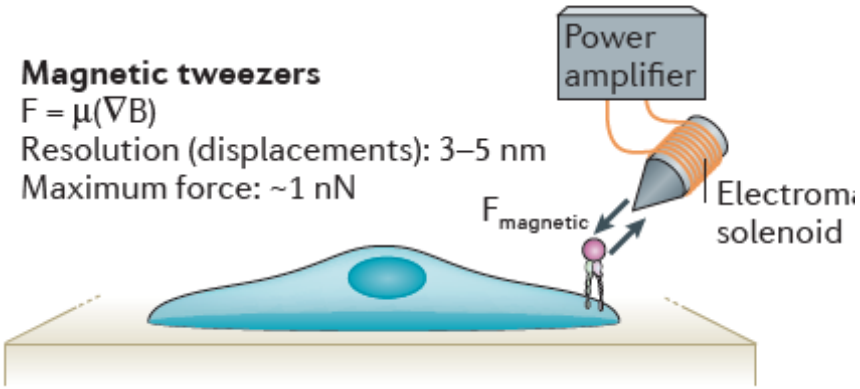
Pillar arrays
F = k*d
Resolution (pillar displacement): <1 nm
Pillar size: >100 nm
Maximum force: >50 nN



Traction force microscopy
F from finite element analysis
Resolution: 1–3 μm
Maximum force: indeterminant



Magnetic tweezers
F = μ(∇B)
Resolution (displacements): 3–5 nm
Maximum force: ~1 nN



Experimental tools in mechanobiology

Pillar arrays allow to check of substrate rigidity and force resolution. Pillar displacement is measured in live cells and is used to determine cellular forces applied to the substrate.

Traction force microscopy uses embedded fluorescent beads to measure substrate deformations by the cell.

Magnetic tweezers create magnetic fields that cause magnetic beads to apply forces to molecules *in vitro* or *in vivo*.

METHODS TO STUDY MECHANOSENSING

Several tools were developed over the years to measure mechanosensing-related forces. The first demonstration of cellular traction forces on matrices was the wrinkling of elastic silicone surfaces by adherent cells (123). Later, in order to quantify the forces more precisely, this technique was modified into the widely used traction force microscopy method, which utilizes tracking the movements of fluorescent beads embedded in elastic gels (124) whose rigidities could be modulated by changing the cross-linking properties of its different components (125). Later, the flexible pillar array system was developed to track forces (126), in which the effective rigidity of the pillars is modulated by changing their height or width. In this method, live-cell brightfield or fluorescent imaging tracks the movements of the pillars as cells are moving on top of them (33, 49); this system has allowed improved resolution of cellular forces (32). At the molecular level, atomic force microscopy has played a crucial role in elucidating the submolecular mechanisms of mechanosensitive protein unfolding, in particular talin (127) and titin (128). More recently, fluorescence resonance energy transfer paired within force-bearing proteins enabled measurement of the approximate forces on those proteins (75).

CELL STRETCHING AND EXTERNAL FORCE EFFECTS

In addition to the cell testing the matrix, the matrix can also stimulate the cell by pulling or pushing on it. Cells within tissues are stretched periodically as part of normal blood flow and breathing, and changes in physical activity alter the lengths and frequencies of this stretch with consequences for cell expression patterns (129). Furthermore, periodic stretching of soft pillar arrays showed that forces from soft surfaces can stimulate growth (130). There has been considerable interest in characterizing cell responses to external mechanical perturbations (reviewed in 3, 131). Stretching experiments have shown that endothelial cells and fibroblasts will orient relative to uniaxial stretches dependent upon the strain and the frequency (132–135). This has been explained theoretically by the dependence on the dynamics of deformation of the matrix through the adhesions, which then affect the cytoskeletal organization (136). The important parameters are the time constants for the actin filament turnover and adhesion dynamics. In addition, actin filaments are broken easily by bending forces but can support much higher axial forces. Thus, once again, the interplay between mechanical forces and actomyosin dynamics strongly affects the shape of the cell.

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A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes

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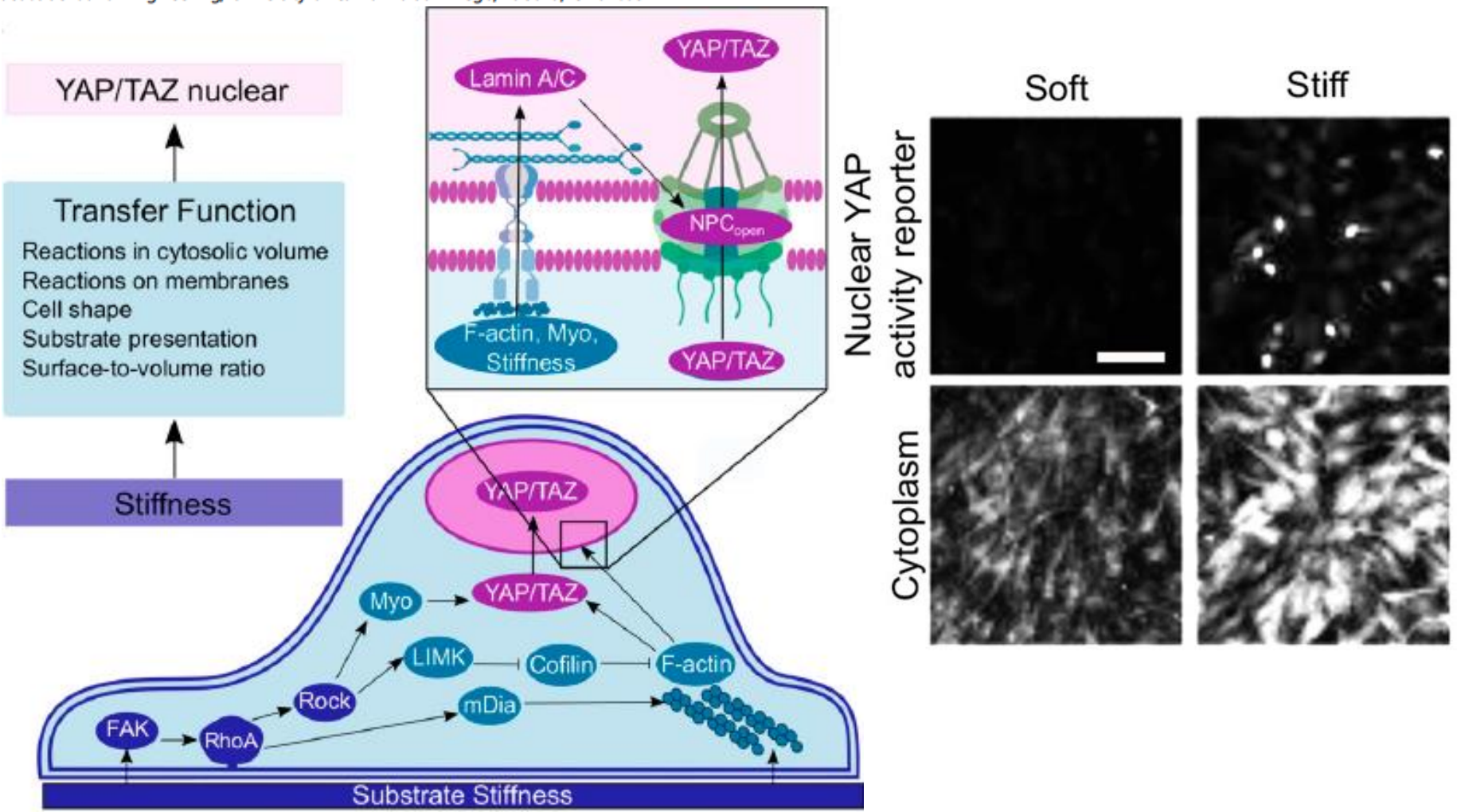


ARTICLE

9 JUNE 2011 | VOL 474 | NATURE | 179

doi:10.1038/nature10137

Role of YAP/TAZ in mechanotransduction

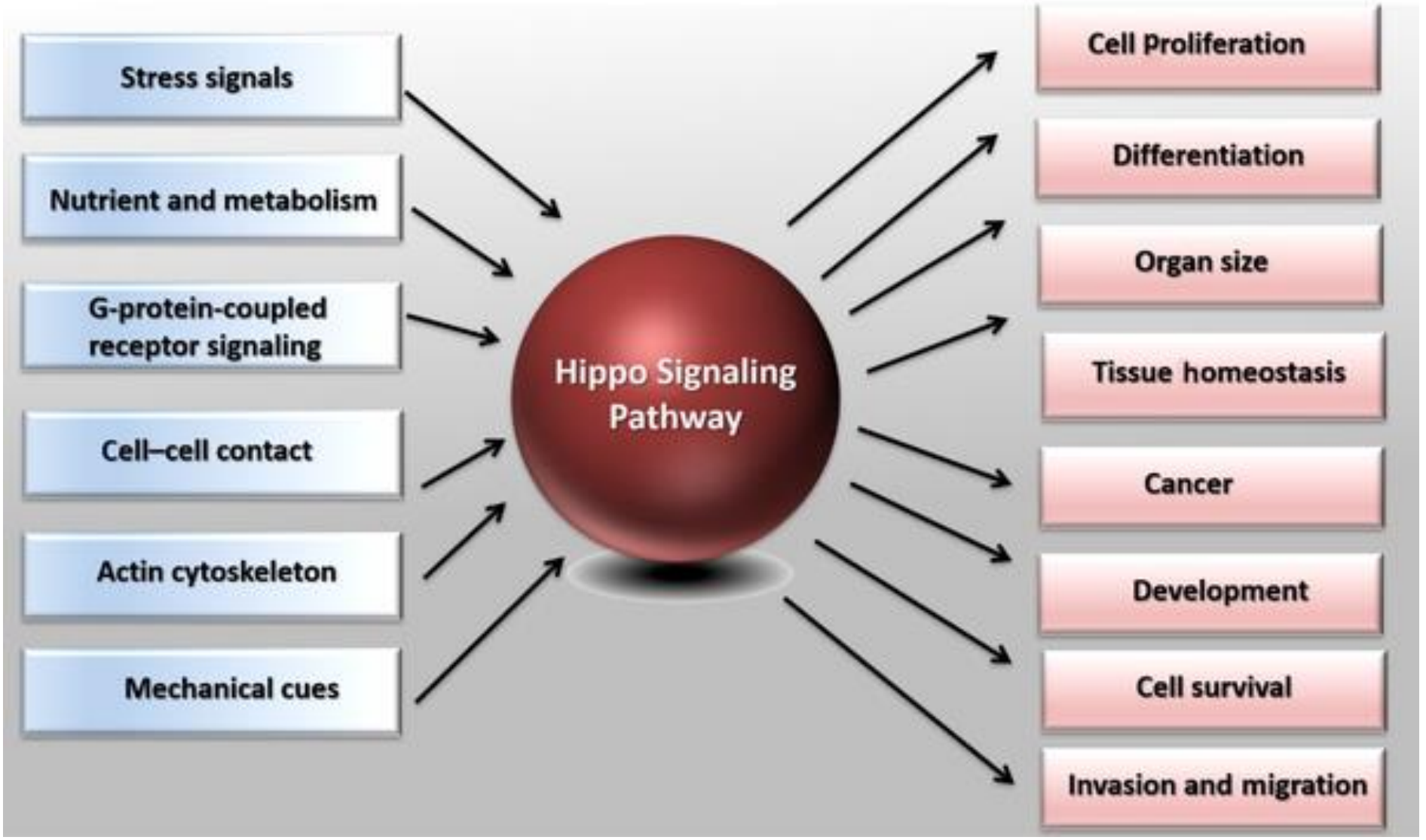
Sirio Dupont[1]*, Leonardo Morsut[1]*, Mariaceleste Aragona[1], Elena Enzo[1], Stefano Giulitti[2], Michelangelo Cordenonsi[1], Francesca Zanconato[1], Jimmy Le Digabel[3], Mattia Forcato[4], Silvio Bicciato[4], Nicola Elvassore[2] & Stefano Piccolo[1]

REVIEWS

Mechanobiology of YAP and TAZ in physiology and disease

Tito Panciera, Luca Azzolin, Michelangelo Cordenonsi and Stefano Piccolo

Hippo/YAP Signaling Pathway

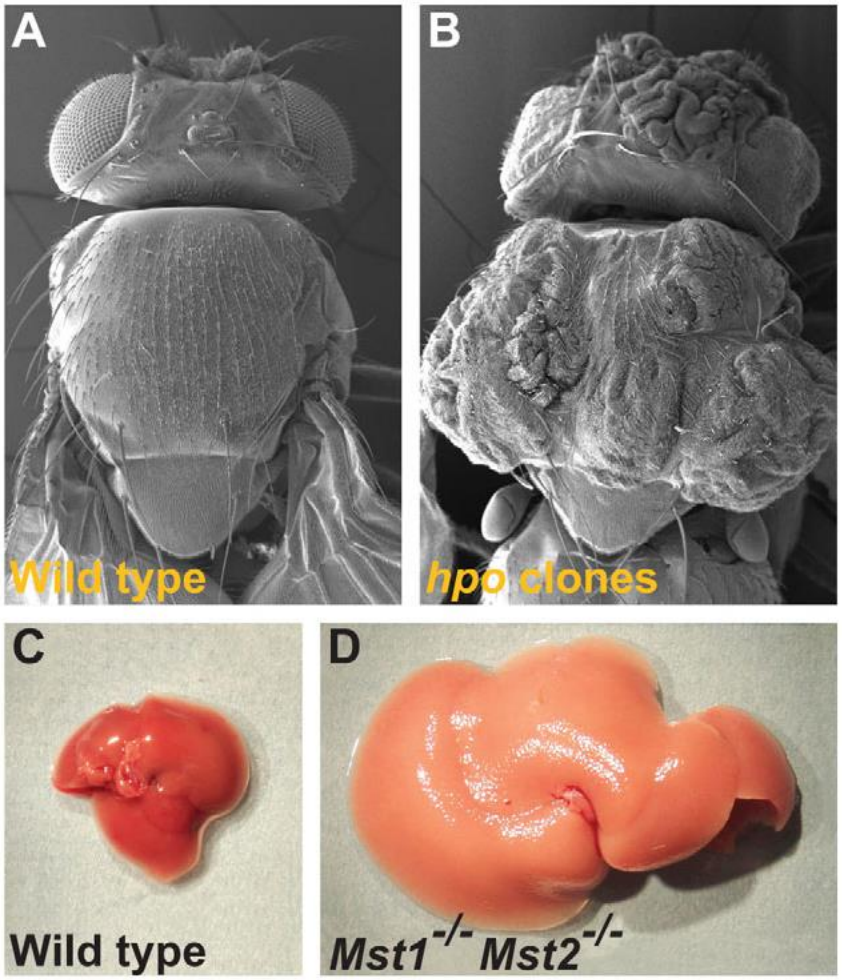


REVIEW

Development 138, 9-22 (2011) doi:10.1242/dev.045500

Hippo signaling: growth control and beyond

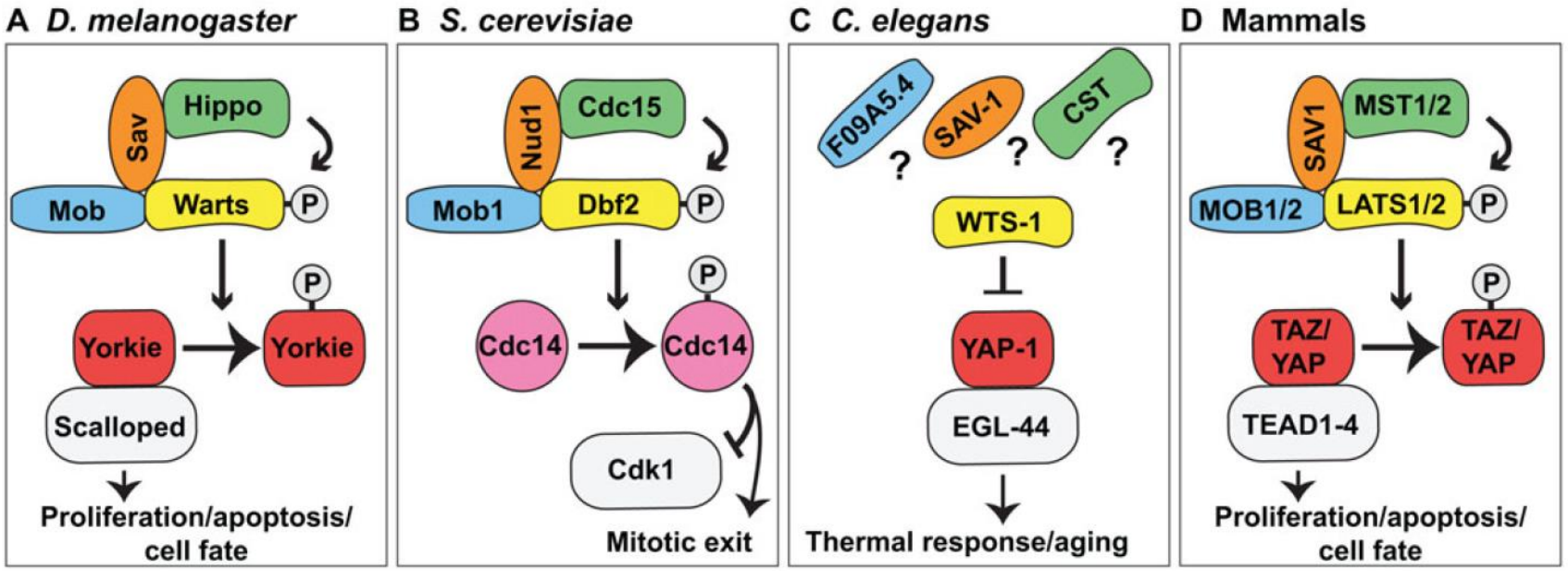
Georg Halder[1,2,3,*] and Randy L. Johnson[1,2,3,*]



- Wts, Hpo, Mats and Sav KO mice show an identical phenotype, characterized by a massive tissue hyperproliferation, due to an increase of cellular proliferation and diminished apoptosis

- All these genes are connected in a signaling cascade, whose main target is the transcription factor Yorkie (Yki)

The Hippo pathway is quite conserved throughout evolution



The core components of the Hippo signaling pathway: the functionally conserved factors are matched by color.

In S. cerevisiae these signals are known as the mitotic exit network, which controls mitotic exit and cytokinesis.

In C. elegans these signals control transcriptional events important for thermal response and aging, whereas in D. melanogaster and mammals this network controls transcriptional events that direct proliferation, apoptosis and cell fate.

Signal Transduction and Targeted Therapy www.nature.com/sigtrans

REVIEW ARTICLE OPEN

The Hippo signalling pathway and its implications in human health and diseases

Minyang Fu[1], Yuan Hu[2], Tianxia Lan[1], Kun-Liang Guan[3], Ting Luo[1]✉ and Min Luo[1]✉

Received: 24 June 2022 Revised: 9 September 2022 Accepted: 9 September 2022

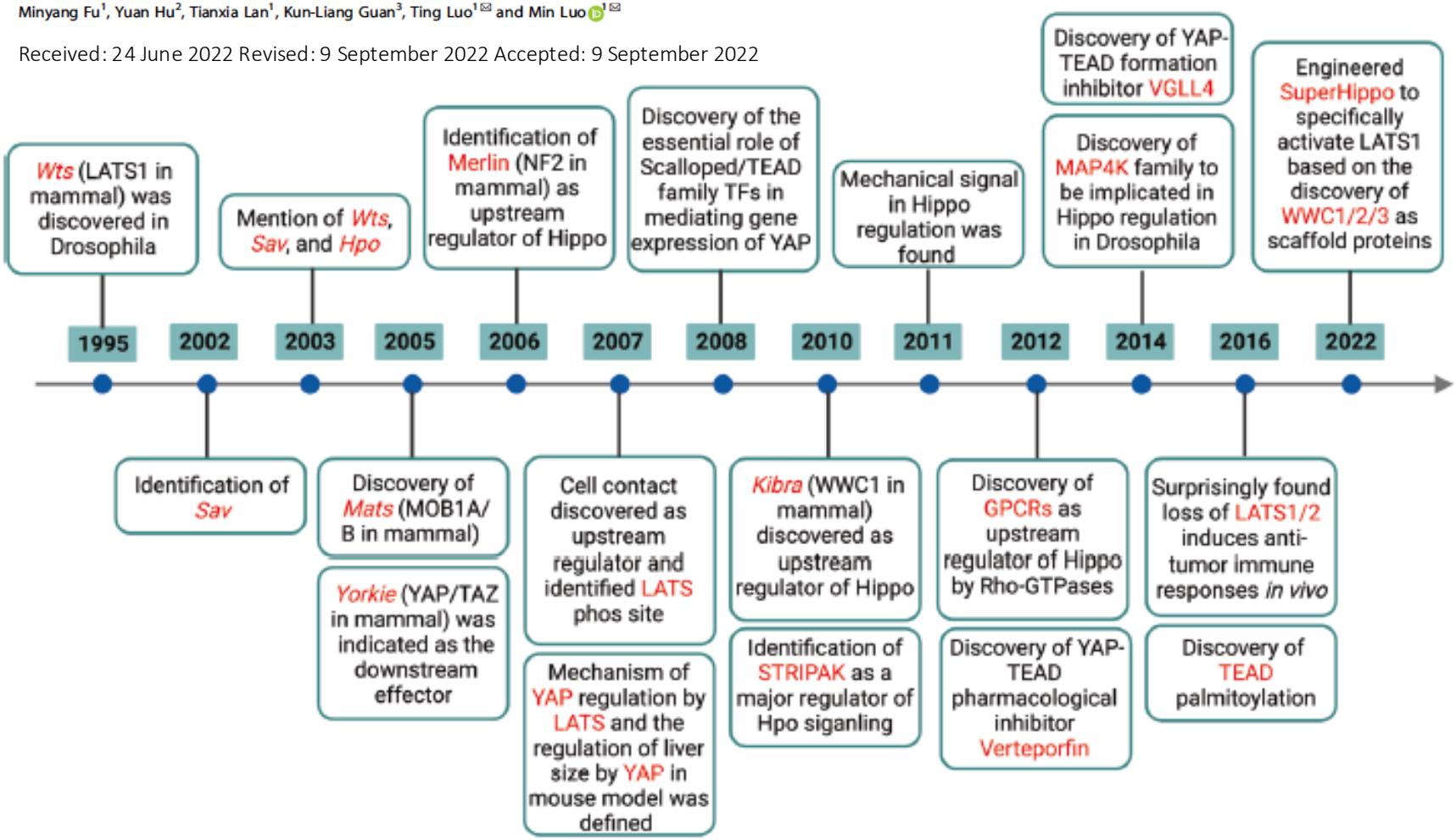
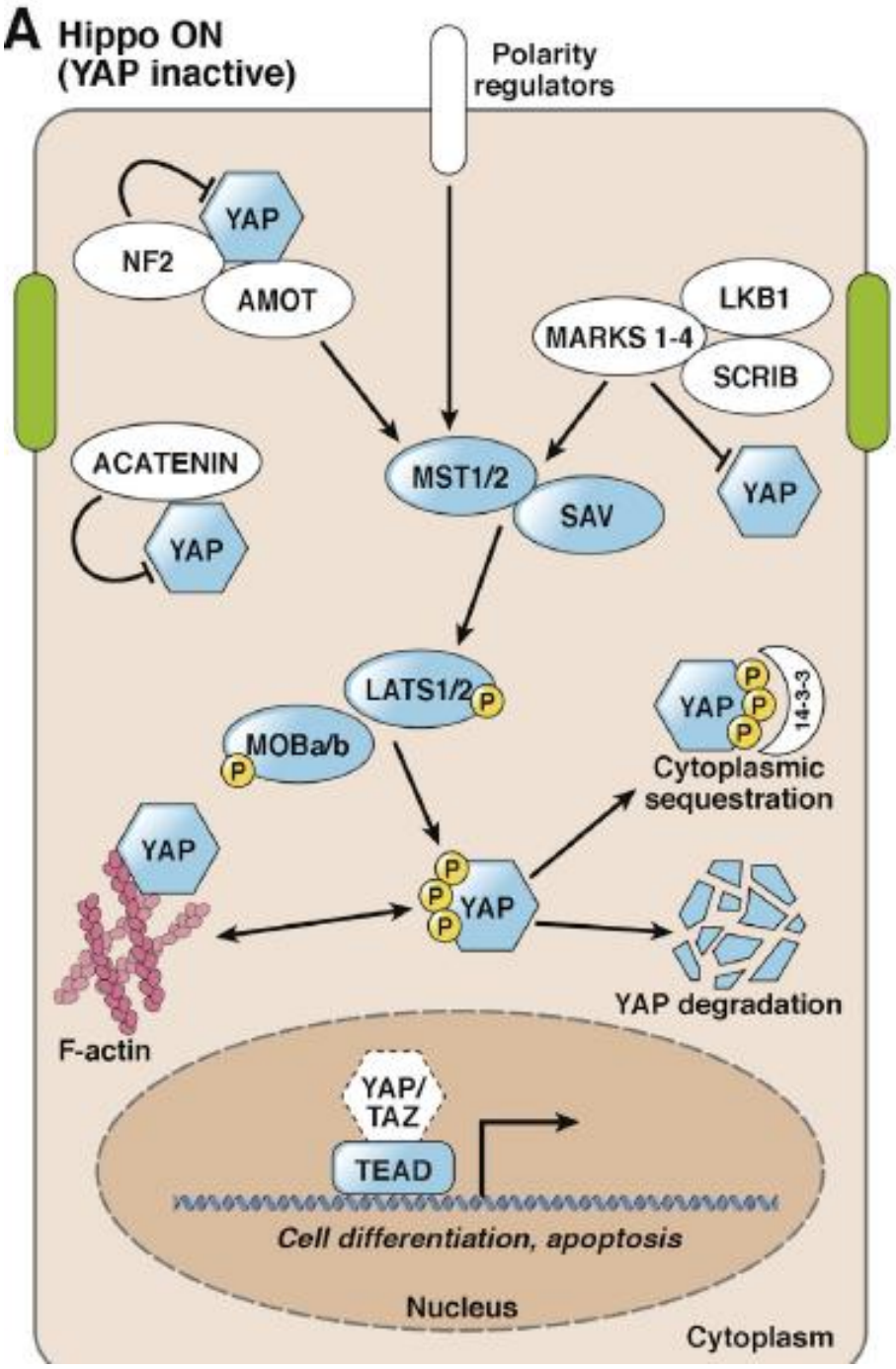


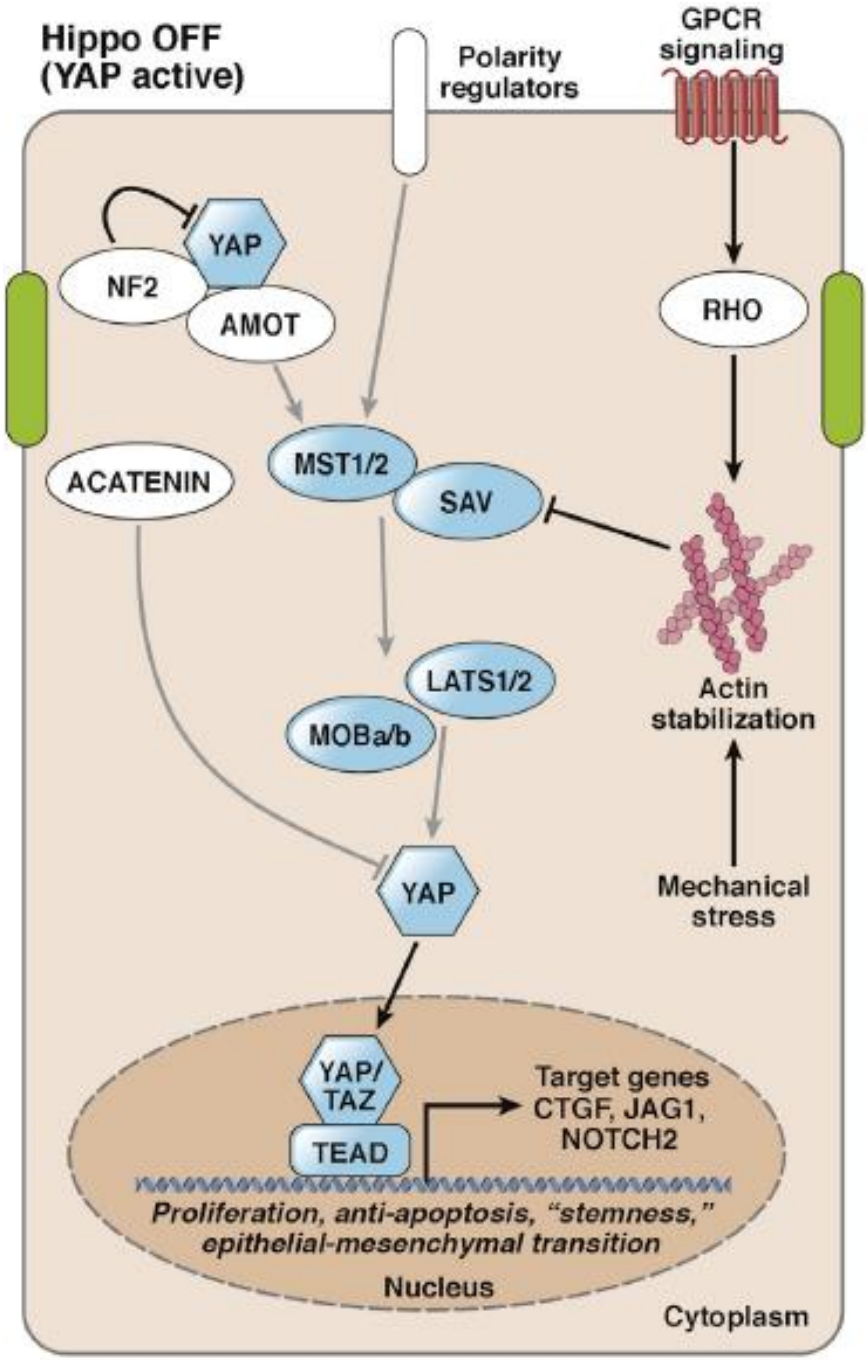
Fig. 1 A timeline of essential discoveries and processes of the Hippo pathway. These discoveries were made initially in 1995 and then gradually to the present. The discoveries mainly focus on two aspects, including the components and processes of Hippo pathway and the function of Hippo pathway in physiological and pathological conditions



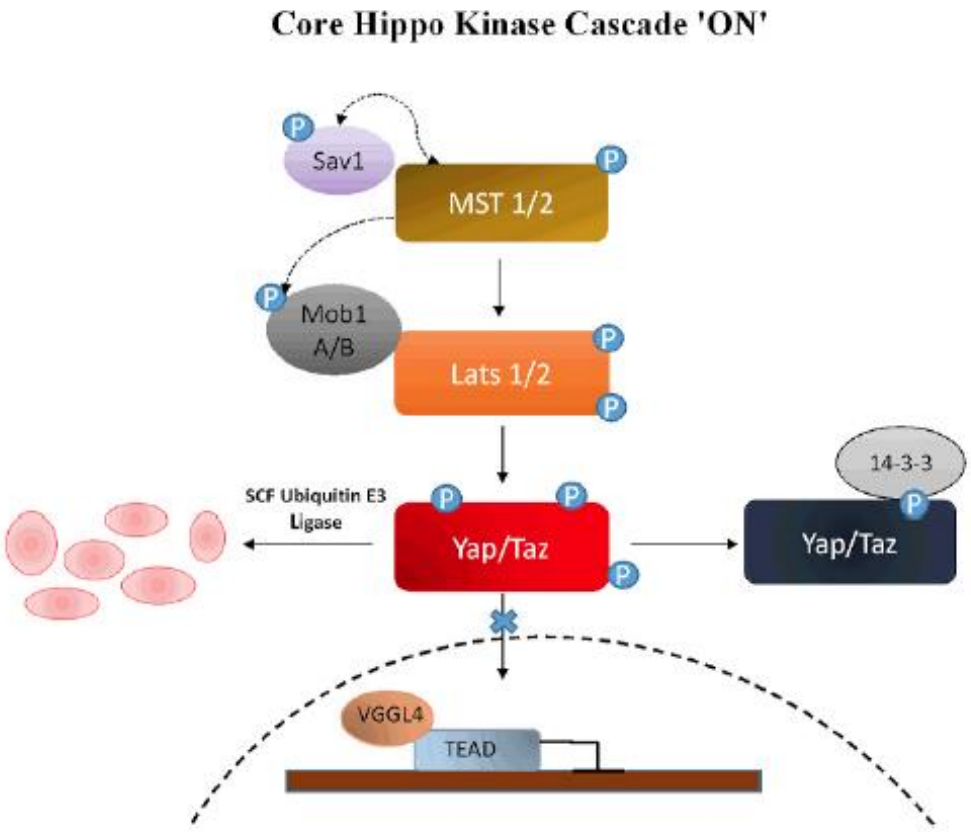
Hippo signalling

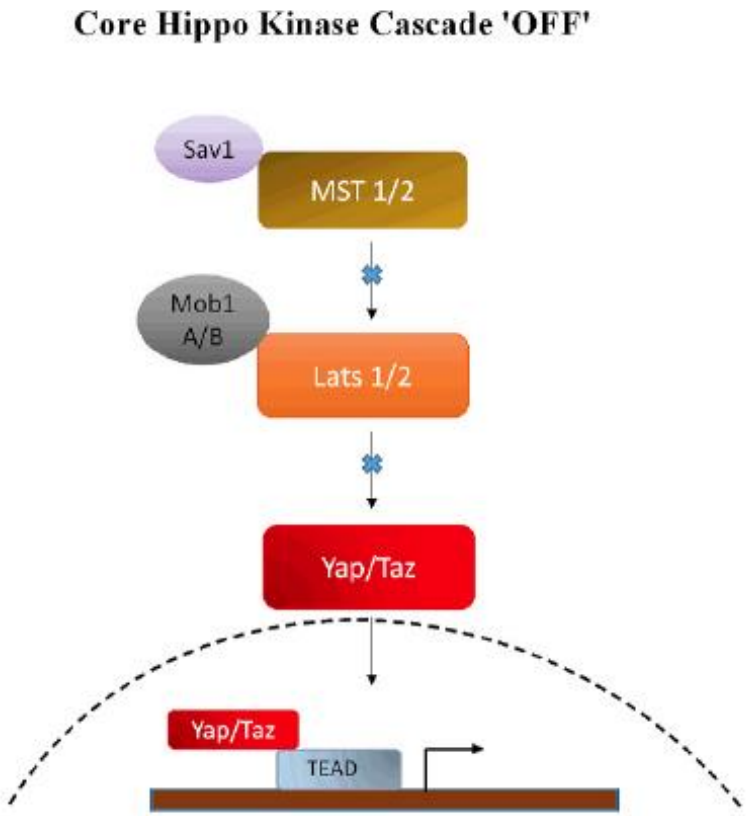
The core Hippo pathway signaling cascade in mammals is comprised of a serine/threonine kinase cascade consisting of MST1/2 (homologs of the Drosophila kinase Hippo), interacting with the scaffolding proteins Salvador homolog 1 (SAV1) and neurofibromatosis type 2 (NF2/Merlin), as well as LATS1/2, which interact with MOB kinase activator 1A and B (MOB1A and B).

In the canonical Hippo pathway, MST1/2 interact with SAV1 and phosphorylate LATS1/2, which are activated and phosphorylate YAP/TAZ on five (YAP) and four (TAZ) conserved serine residues. These inhibitory phosphorylations of YAP and its paralog TAZ is a signal for the cytoplasmic retention and YAP/TAZ binding to 14-3-3 protein or YAP/TAZ degradation. This activation of MST1/2 and LATS1/2 denotes the Hippo pathway on state, where YAP/TAZ are inactive.

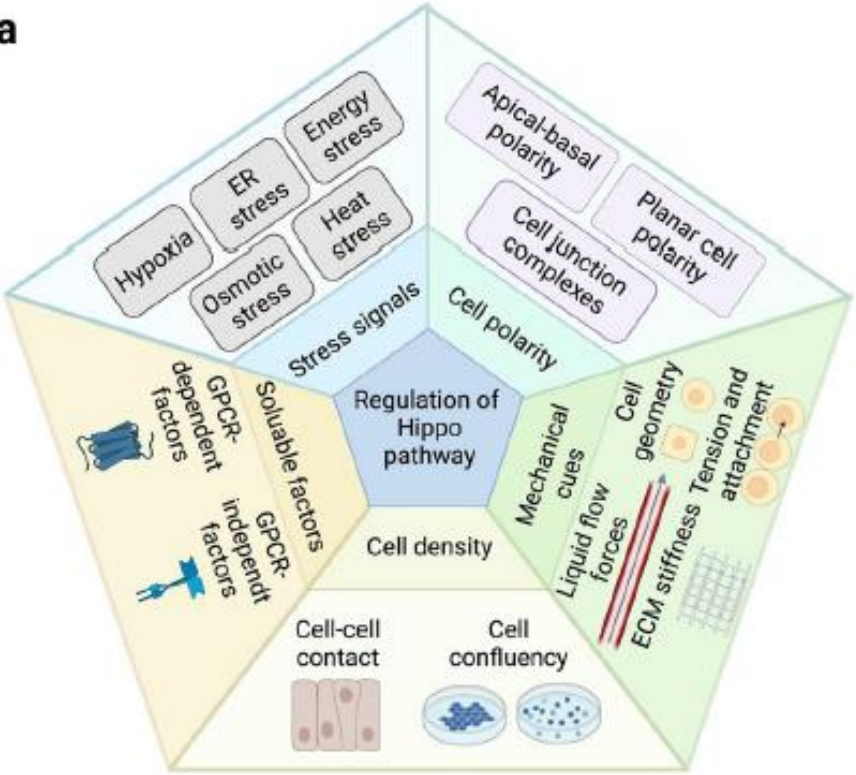


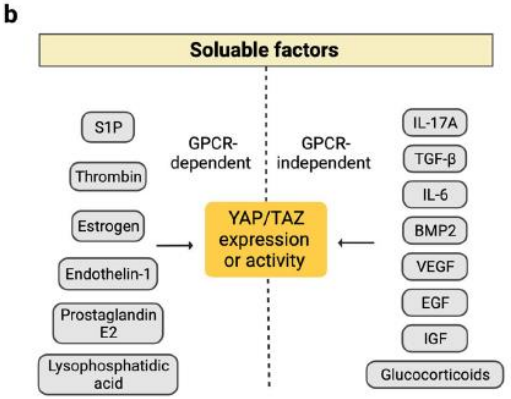
When Hippo is **OFF**, YAP translocates to the nucleus and binds to the TEAD family of transcription factors, leading to the transcription of genes involved in cell survival, growth, and proliferation.

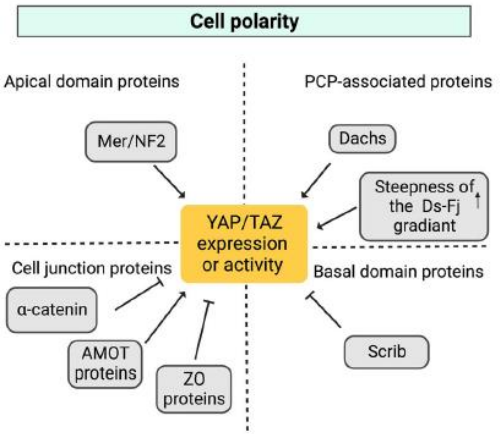

Core Hippo Kinase Cascade 'ON'
Sav1
MST 1/2
Mob1 A/B
Lats 1/2
14-3-3
SCF Ubiquitin E3 Ligase
Yap/Taz
Yap/Taz
VGGL4
TEAD

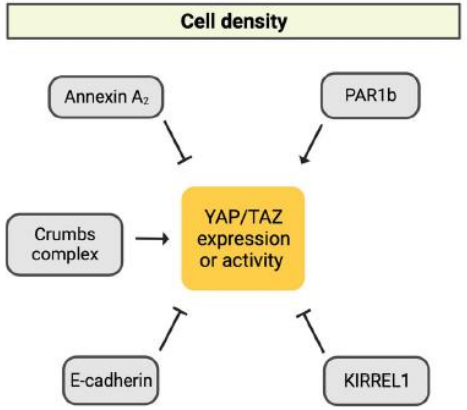

Core Hippo Kinase Cascade 'OFF'
Sav1
MST 1/2
Mob1 A/B
Lats 1/2
Yap/Taz
Yap/Taz
TEAD

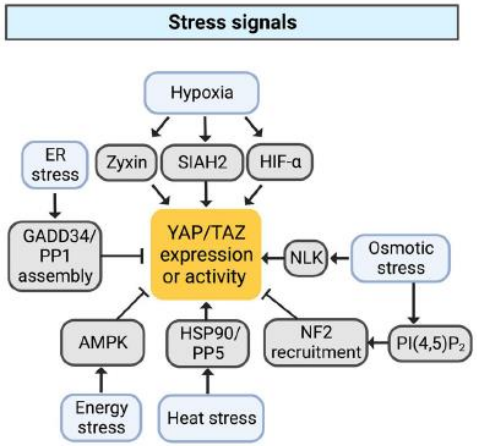
Regulation of the Hippo pathway by upstream signals.

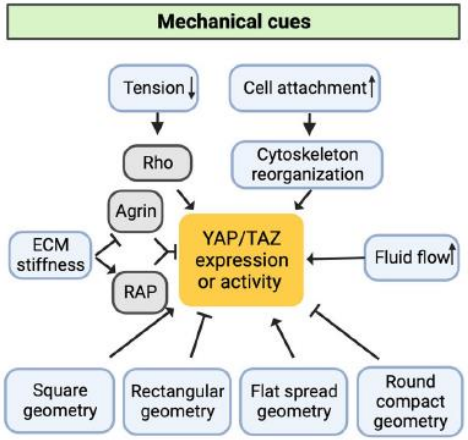


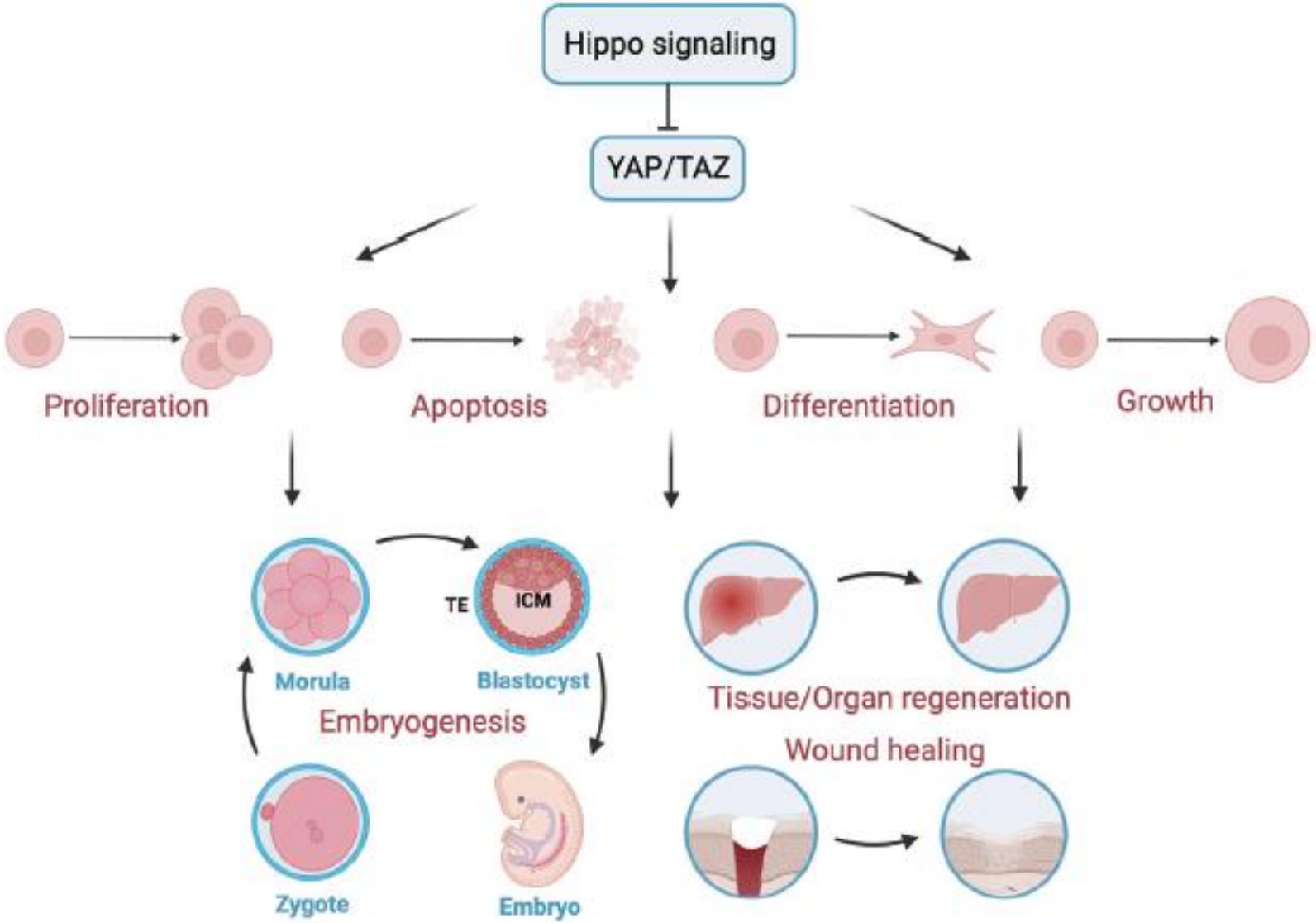




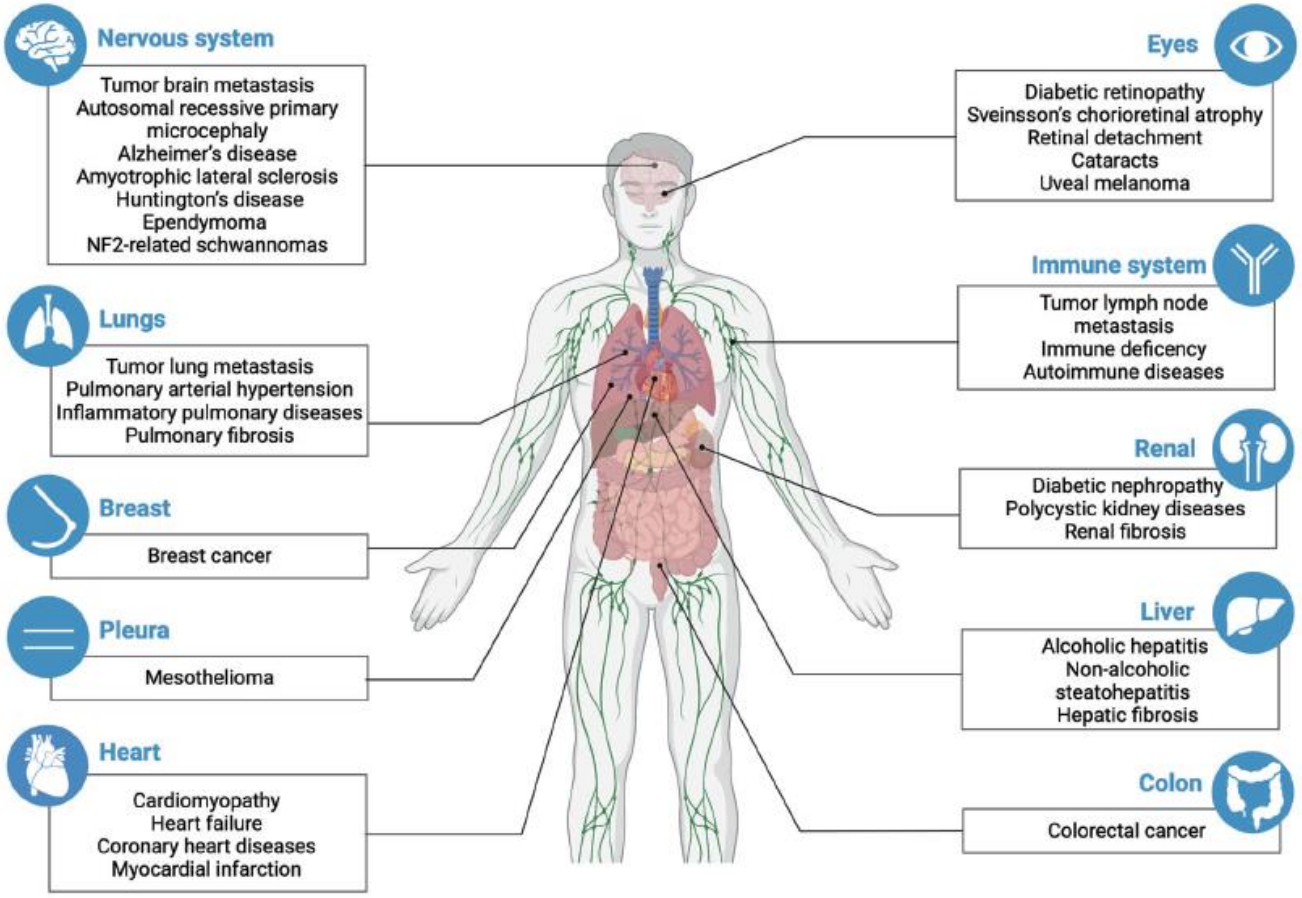








The essential physiological function of Hippo pathway. The Hippo pathway effectors YAP/TAZ can take part in the modulation of multiple cell events, including proliferation, apoptosis, differentiation and growth, thereby participating in the physiological processes of embryogenesis and development, as well as tissue/organ regeneration and wound healing



Hippo pathway dysregulation has been found to be present in a variety of organs or systems diseases and involved in the regulation of occurrence or progression of these diseases.

Table 2. The drugs targeting Hippo pathway in clinical trials

Mechanism	Name (sponsor)	Phase	Indications	ClinicalTrials.gov Identifier
TEAD palmitoylation inhibition	VT3989 (Vivace Therapeutics)	Phase 1	Solid Tumour Mesothelioma	NCT04665206
	IK-930 (Ikena Oncology)	Phase 1	Solid Tumours Mesothelioma Epithelioid Hemangioendothelioma NF2 Deficiency YAP1 or TAZ Gene Fusions	NCT05228015
YAP antisense oligonucleotide	ION537 (Ionis Pharmaceuticals)	Phase 1	Advanced solid tumours	NCT04659096
Not been disclosed	IAG933 (Novartis)	Phase 1	Mesothelioma	NCT04857372

YAP and TAZ

YAP conservation through evolution

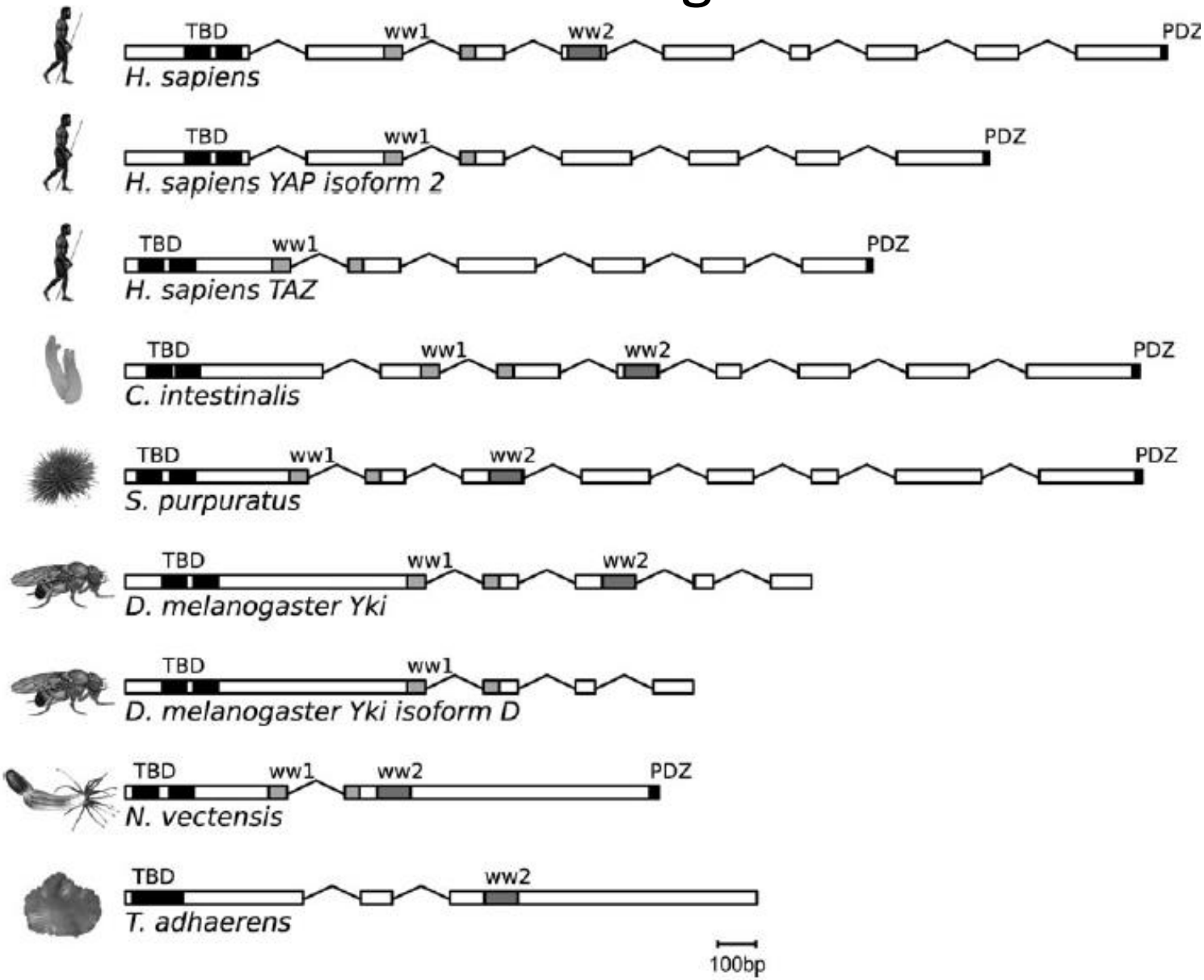
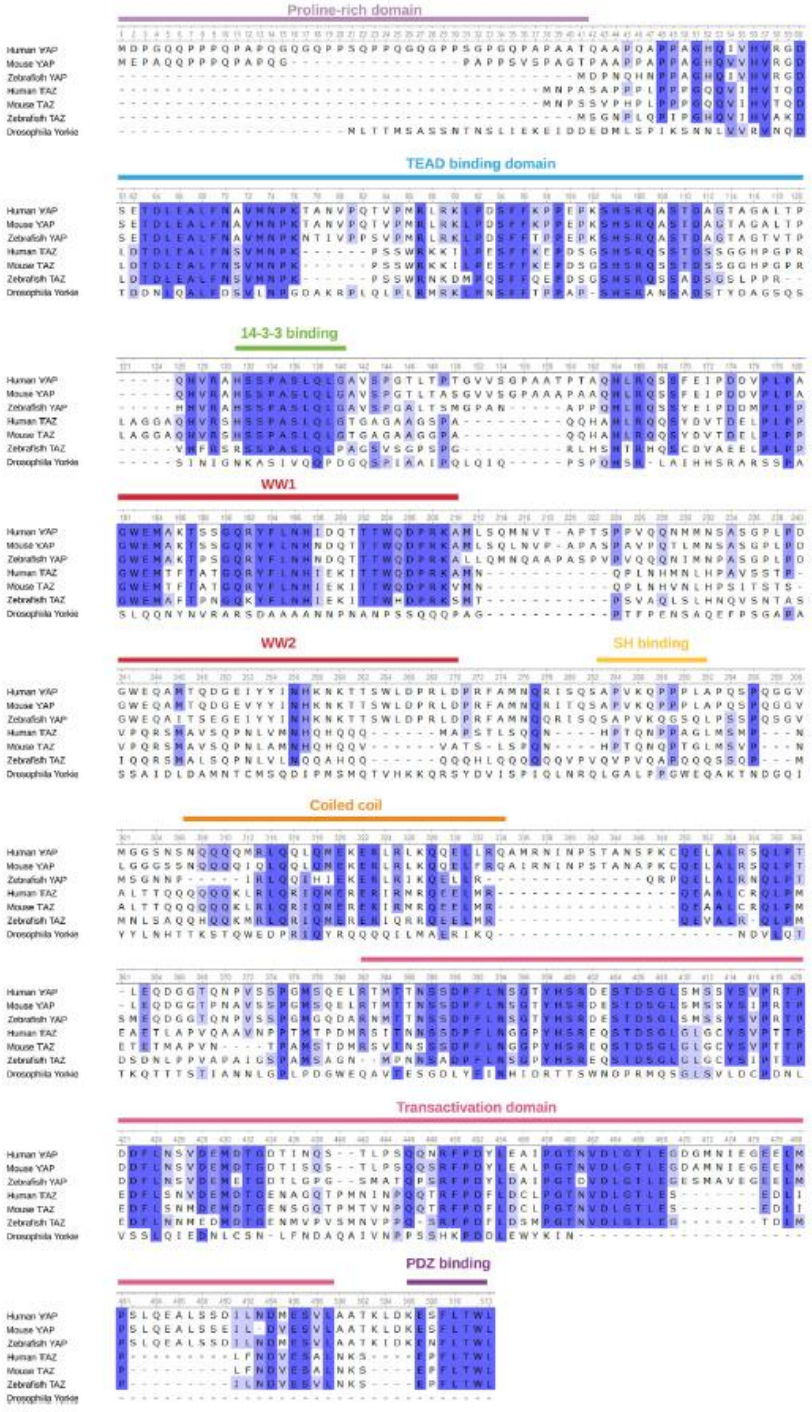
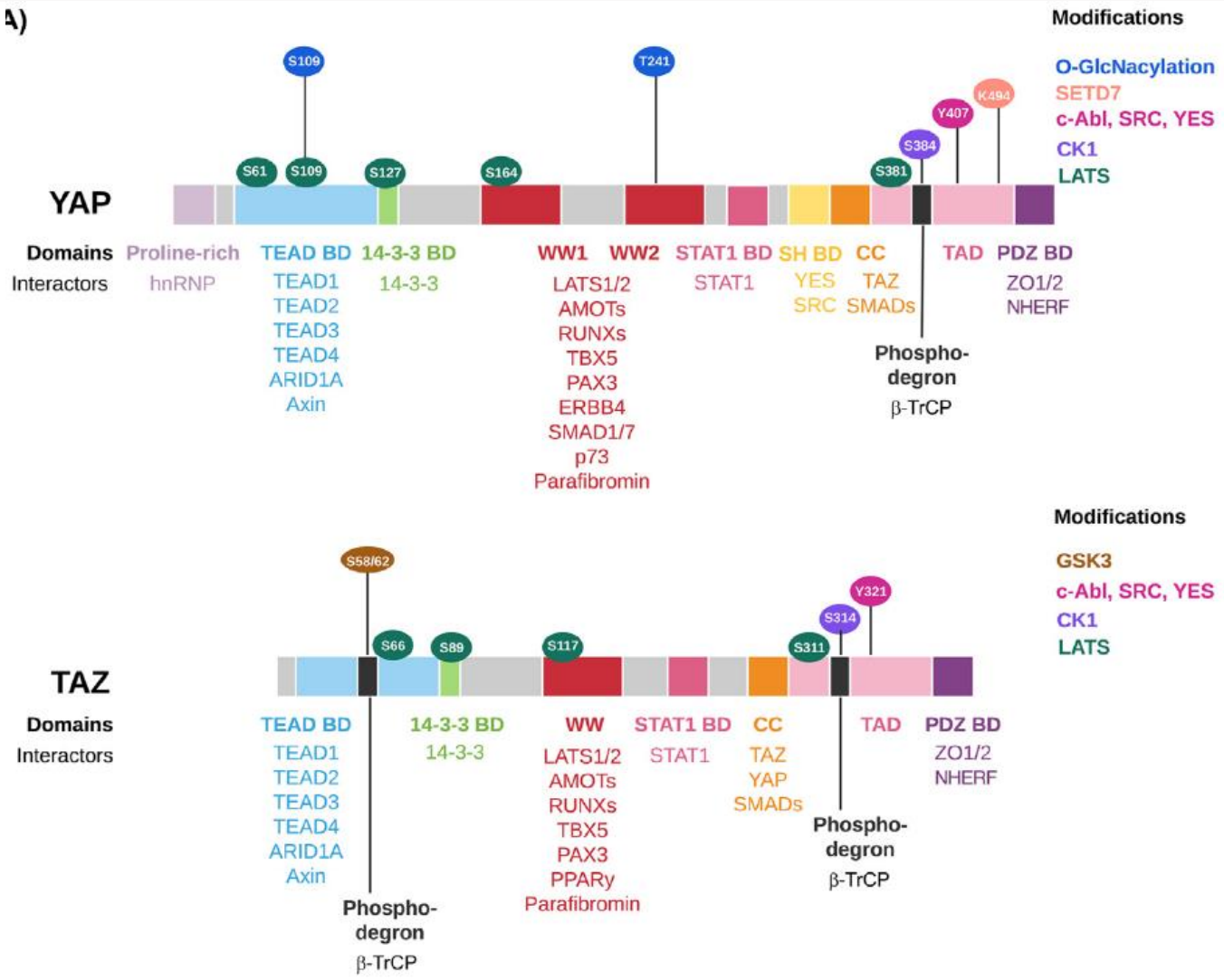


FIG. 4. Genomic structure of YAP in representative metazoans. Major domains are marked with rectangles. The transcript coding region is drawn to scale and introns positions are illustrated but their size is not drawn to scale.


Proline-rich domain
TEAD binding domain
14-3-3 binding
WW1
WW2
SH binding
Coiled coil
Transactivation domain
PDZ binding
Human YAP
Mouse YAP
Zebrafish YAP
Human TAZ
Mouse TAZ
Zebrafish TAZ
Drosophila Yorkie

Regulatory domains of the Hippo pathway effectors TAZ/YAP.

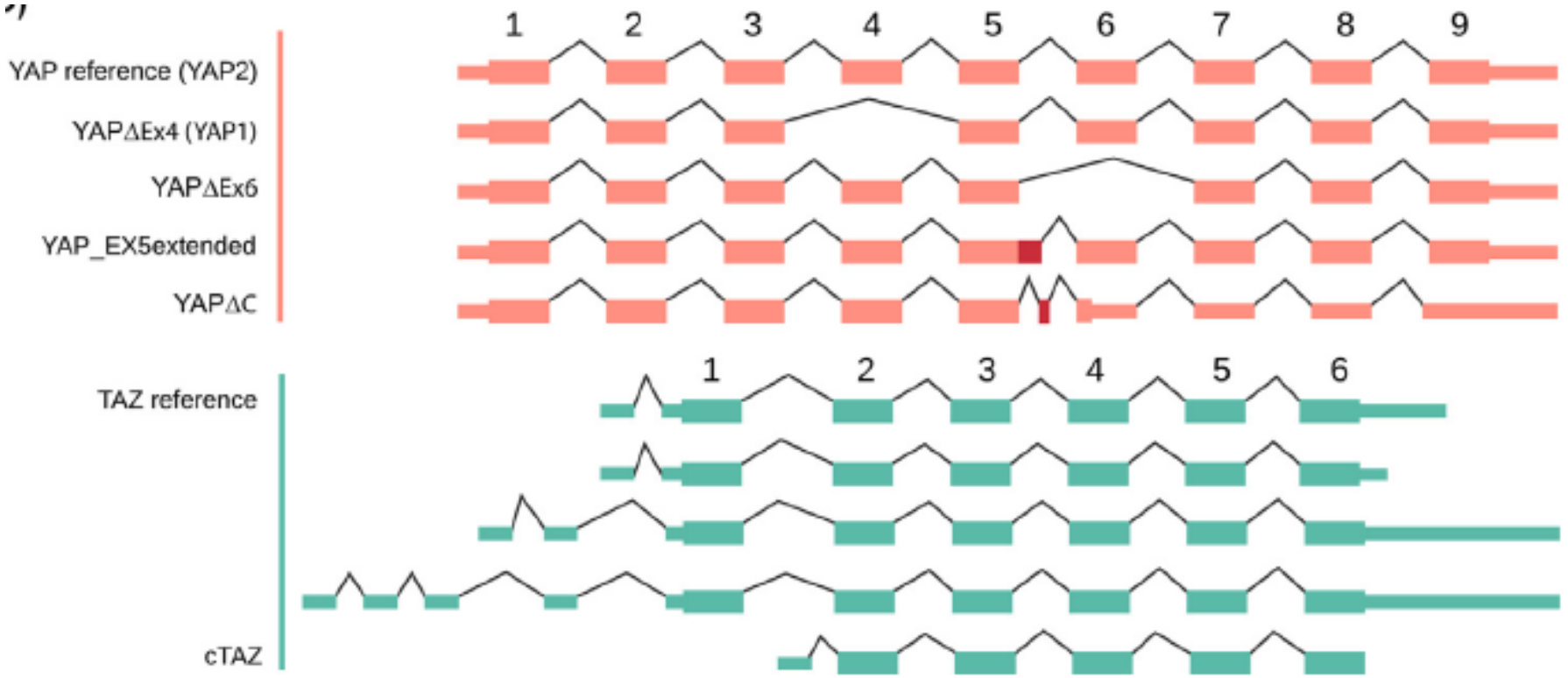


The five serines of YAPand the corresponding four serines of TAZ that are targeted by LATS1/2 phosphorylation are shown in green, the CK1 phosphorylation sites on both proteins are shown in gray, and the c-Abl phosphorylation site on YAPis shown in cyan. The lysine residue of YAP targeted for methylation by Set7 is also shown. TEAD BD is theTEAD binding domain. 14–3-3 BD is the domain that binds 14–3-3 proteins upon phosphorylation byLATS1/2. TAD is the transcriptional activation domain. PDZ BD is the small COOH-terminal domain able tointeract with proteins bearing PDZ domains.

Review
Trends in Biochemical Sciences, February 2021, Vol. 46, No. 2

YAP and TAZ Are Not Identical Twins

Francesca Reggiani,[1] Giulia Gobbi,[1] Alessia Ciarrocchi,[1] and Valentina Sancisi[1,*]



Schematic representation of YAP (pink) and TAZ (green) differential transcripts. Exons are represented as boxes and introns as lines. 5′ UTR and 3′ UTR regions are represented as lower boxes. Exon 5 extended region and additional nucleotides inserted between

Complementary Functions of YAP and TAZ in Development

Differences in phenotypes of KO mice support distinctive functions of the two paralogs during development.

KO of YAP *leads to embryonal lethality with yolk sac vasculogenesis defects and embryonic axis abnormalities.*

TAZ KO mice *are viable, but develop kidney disease and lung emphysema.*

The finding that YAP KO mouse embryos show yolk sac vasculogenesis defects that cannot be compensated by TAZ, points to a differential role of YAP and TAZ in regulating blood vessels formation.

In endothelial cells, ***YAP*** *mainly affects EC proliferation whereas* ***TAZ*** *promotes migration.*

A similar situation, in which both paralogs are involved in organ development but with complementary functions, occurs in ***lung****.*

YAP *is required for bronchial morphogenesis at embryonic stage, whereas* ***TAZ*** *ablation leads to abnormal alveolarization, mimicking lung emphysema.*

YAP and TAZ exert differential or even opposite roles in tumor progression, suggesting that tumor genetic background, cell and tissue contexts may affect YAP and TAZ functions.

YAP/TAZ functions in organs and tissues

B. Liver

The simple overexpression of YAP in the liver of transgenic animals is sufficient to induce a fourfold increase in liver mass caused by proliferation of mature hepatocytes (20, 49); this also leads to the acquisition of biliary duct/liver progenitor cell traits by the hepatocytes (242). This over-

C. Heart

Conditional deletion of YAP in embryonic cardiomyocytes affects their proliferation leading to severe heart hypoplasia, and a similar phenotype has been reported in TEAD1 knockouts (34, 182, 216, 232). Consistently, overall heart size is increased by YAP overexpression, in a TEAD-dependent manner. *Salvador/WW45*, *Mst1/2*, and *Lats2* inactivation in developing mouse hearts also caused severe heart enlargement (84, 188).

D. Intestinal Epithelium

YAP overexpression in transgenic mice by means of an inducible and ubiquitous promoter potently expands intestinal cell proliferation at the expense of differentiation, without affecting whole organ size (20). Intriguingly, nuclear YAP is endogenously restricted to intestinal progenitor cells at the bottom of intestinal crypts, and this cell population expands up to the tip of the villus after YAP overexpression.

I. Early Embryonic Development

YAP/TAZ double null mutants die before implantation (156). *YAP−/−* embryos die shortly after gastrulation, at stage E8.5 (150). Embryos display a shortened and highly

E. Epidermis

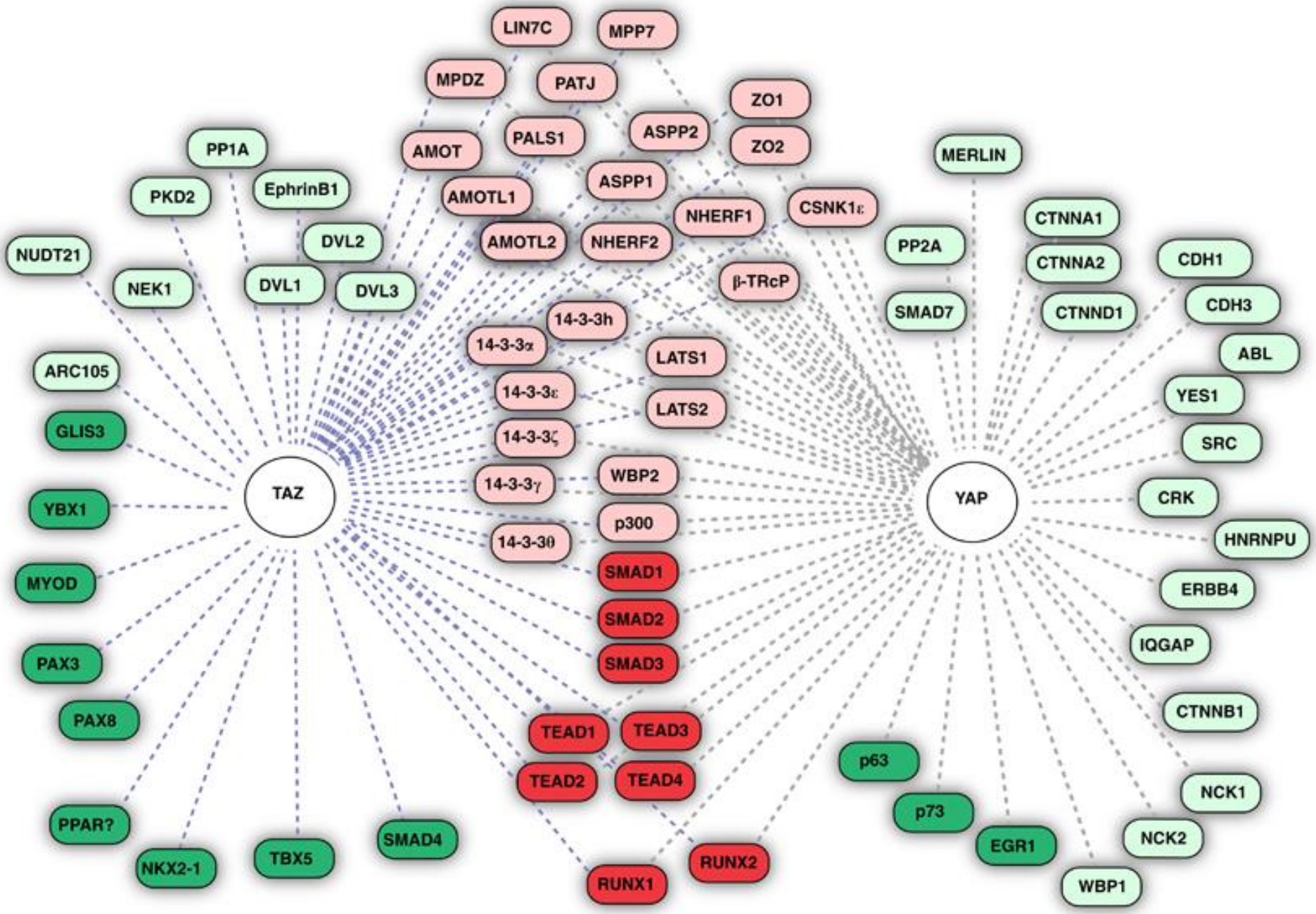
YAP/TAZ play important roles in skin homeostasis: overexpression of activated YAP in the basal layer of the epidermis causes thickening and increased proliferation of keratinocytes, with defective stratification and reduced terminal differentiation. Gain of YAP can specifically expand the

F. Nervous System

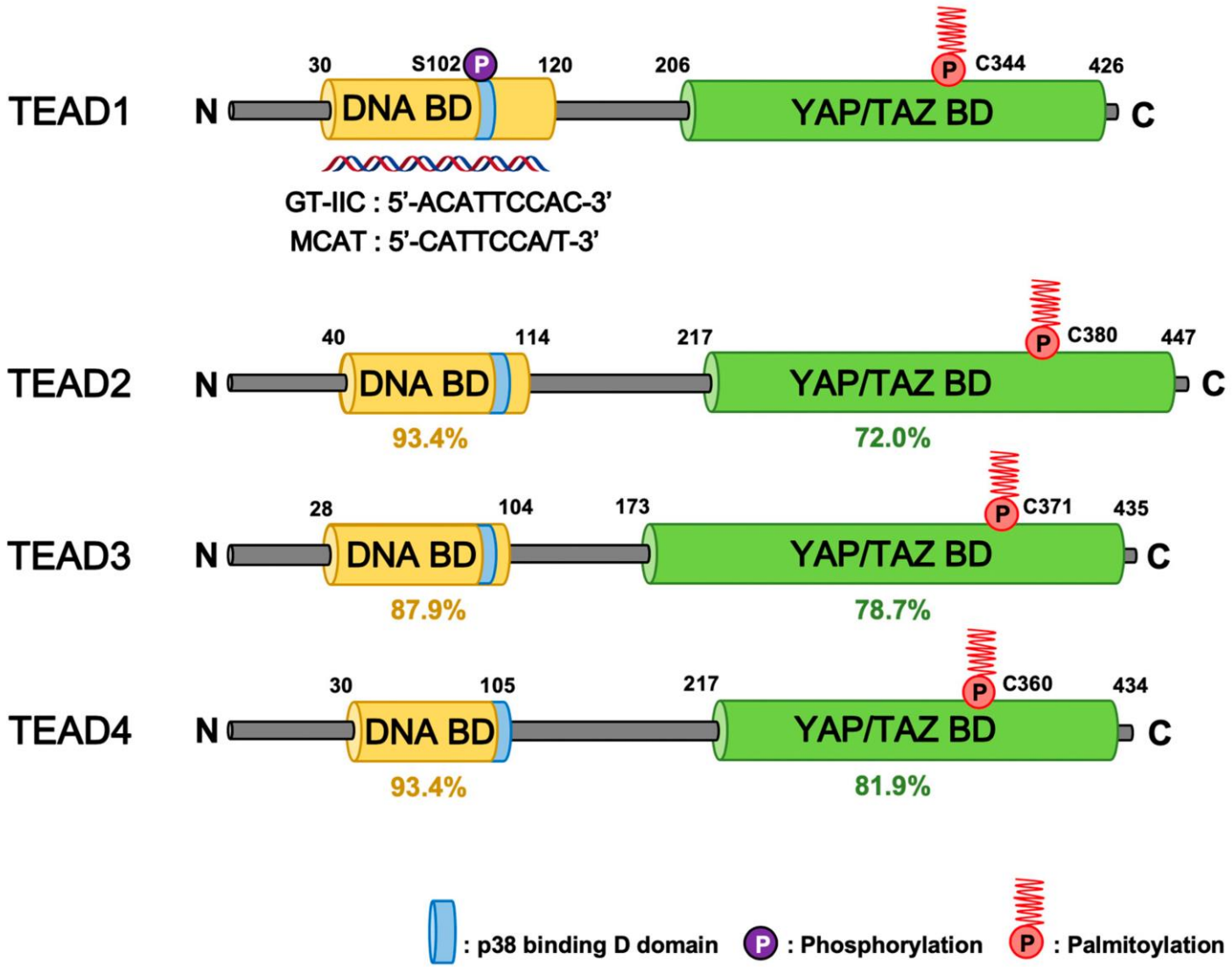
Evidence for the involvement of YAP in brain development comes from inactivation of *NF2* in the dorsal telencephalon, causing severe malformations due to expansion of neural progenitor cells (NPC) in the cortical hem, hippocampus, and neocortex. Transgenic overexpression of YAP induces a hippocampal phenotype similar to *NF2* inactivation, while combined loss of *NF2* and *YAP* rescues this phenotype (110).

H. Kidney

During organogenesis, inactivation of *YAP* or *TAZ* in kidney precursor cells (metanephric mesenchyme) produces very different phenotypes: *YAP* is required for efficient nephron morphogenesis, while *TAZ* inactivation causes polycystic kidney disease (90, 130, 176). This clearly indicates that, at least in this tissue, YAP and TAZ have distinct and specific functions. A link between YAP and kidney

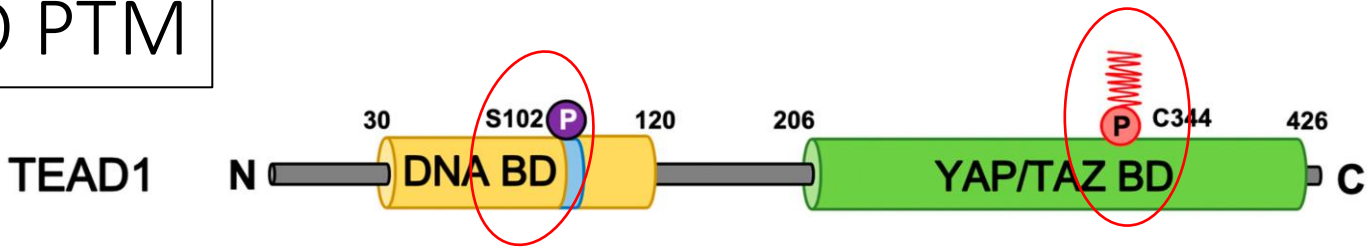

LIN7C
MPP7
MPDZ
PATJ
ZO1
ZO2
ASPP2
PALS1
AMOT
PP1A
PKD2
EphrinB1
ASPP1
AMOTL1
NHERF1
CSNK1ε
DVL2
AMOTL2
NHERF2
NUDT21
NEK1
DVL1
DVL3
β-TRcP
14-3-3h
14-3-3α
LATS1
14-3-3ε
LATS2
ARC105
GLIS3
14-3-3ζ
14-3-3γ
WBP2
TAZ
YBX1
p300
14-3-3θ
SMAD1
MYOD
SMAD2
SMAD3
PAX3
PAX8
TEAD1
TEAD3
TEAD2
TEAD4
PPAR?
NKX2-1
TBX5
SMAD4
RUNX1
RUNX2
MERLIN
PP2A
SMAD7
CTNNA1
CTNNA2
CTNND1
CDH1
CDH3
ABL
YES1
SRC
CRK
YAP
HNRNPU
ERBB4
IQGAP
CTNNB1
p63
p73
EGR1
NCK1
NCK2
WBP1

TEAD TRANSCRIPTION FACTORS



TEAD post-translation modifications include palmitoylation and PKA-, PKC-mediated phosphorylation that occur in the YAP/TAZ-BD and DNA-BD, respectively. Palmitoylation is required for proper TEAD functions.

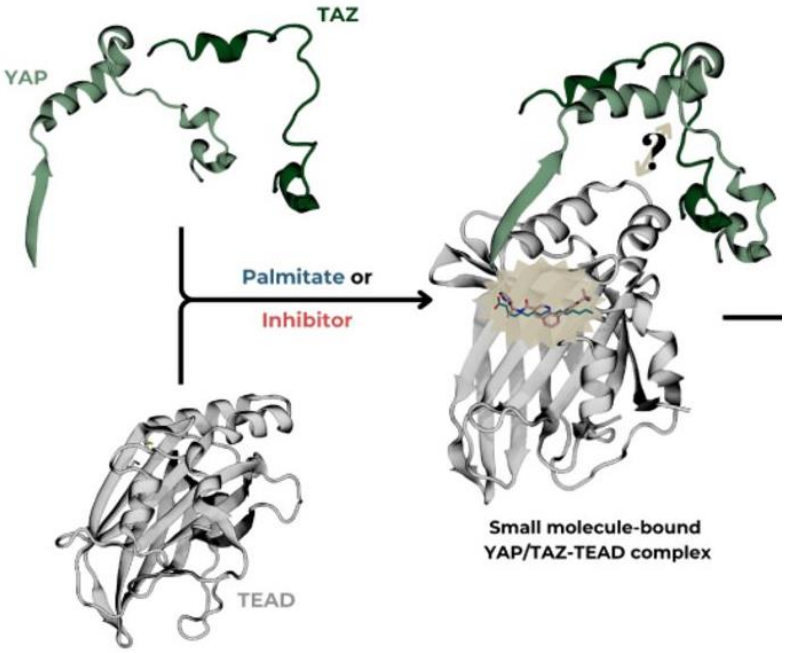
TEAD PTM



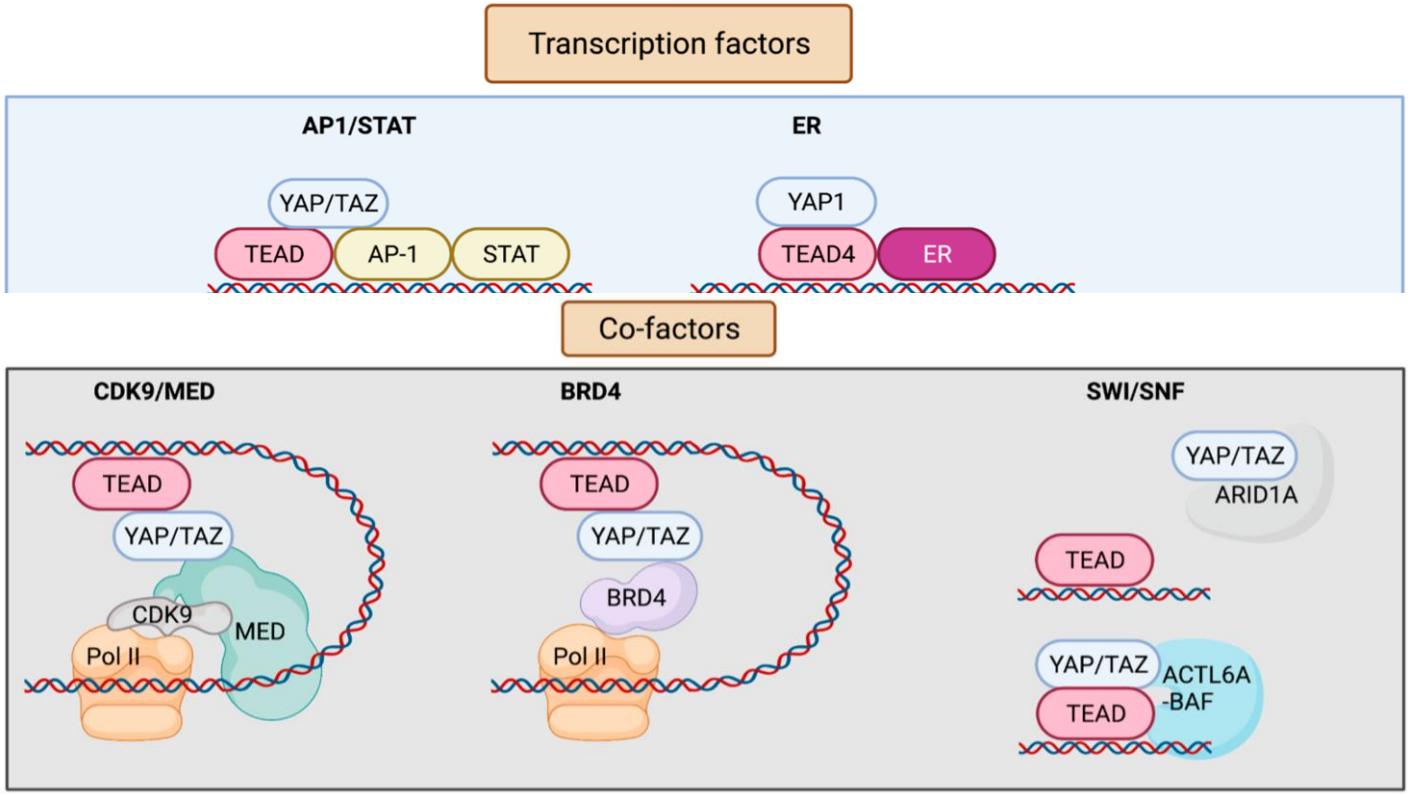
TEAD is phosphorylated by protein kinaseA (PKA) and protein kinase C (PKC), which have been shown to inhibit TEAD by disrupting its DNA-binding.

Protein palmitoylation is important for protein trafficking and membrane localization, for proper TEAD folding, protein stability and for YAP/TAZ interaction, playing important roles in regulating its binding to the transcriptional coactivators.

Interestingly, environmental stresses, such as osmotic stress, high cell density, and cell suspension, promote TEAD cytoplasmic translocation.
Because TEADs are the major effectors that dictate the transcriptional output of the Hippo-YAP/TAZ pathway, physiologic and pathologic conditions affecting TEAD localization significantly impact the functional output of the Hippo pathway.



YAP forms a heterodimer with TEAD, while TAZ–TEAD complexes can exist as both heterodimers or heterotetramers

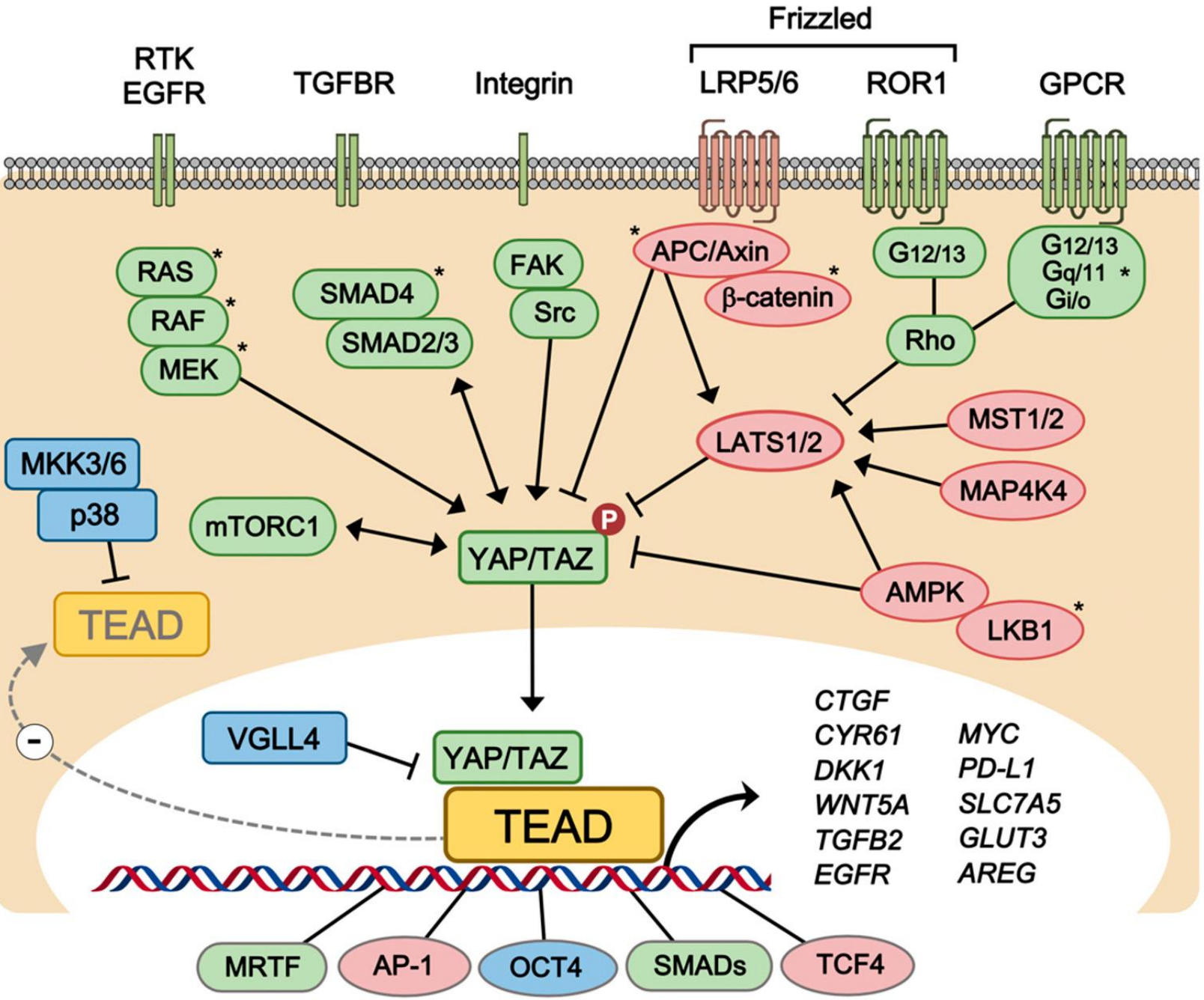


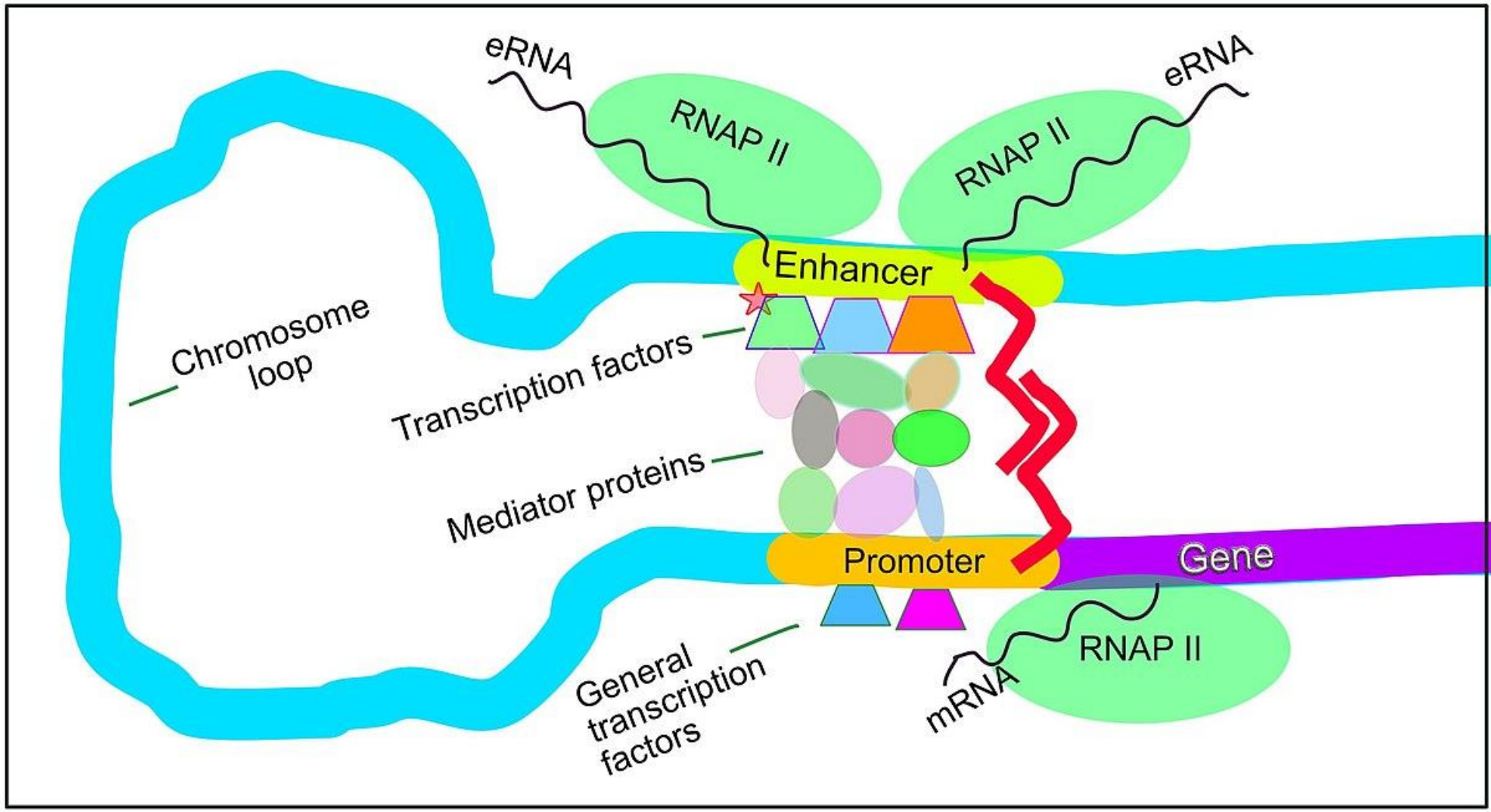
TEADs bind to the DNA but are barely known to exert any transcriptional activity by themselves.

Table 1. YAP and TAZ Transcriptional Partners

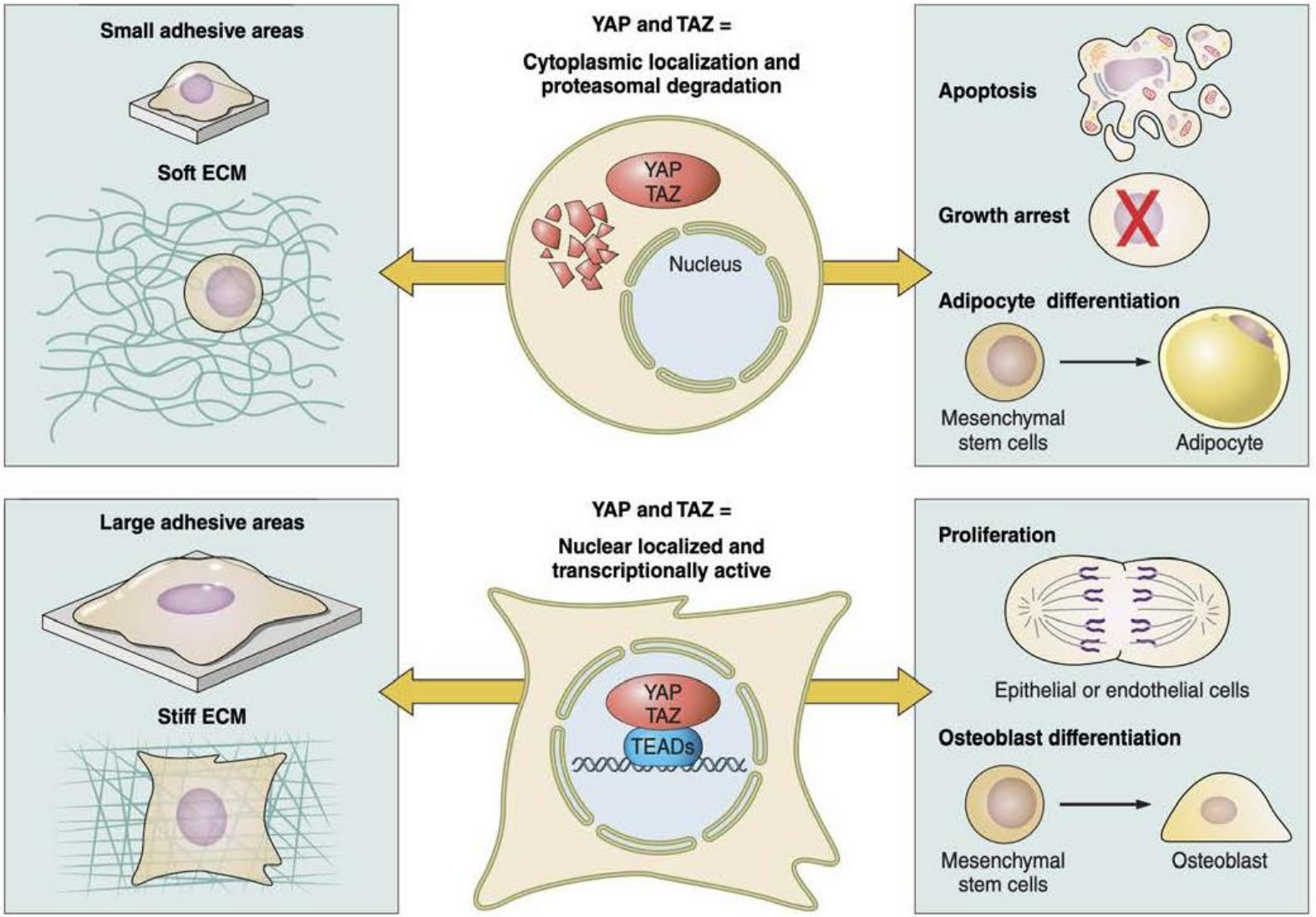
Partner	Interaction domain	Function	YAP/TAZ	Refs
TEAD1–4	TEAD binding domain	Promoting transcription	YAP, TAZ	[97]
RUNX1/2	WW	Promoting transcription	YAP, TAZ	[55–57]
PAX3	WW	Promoting transcription	YAP, TAZ	[58,59]
TBX5		Promoting transcription	YAP, TAZ	[17,28]
AP-1		Promoting transcription	YAP, TAZ	[60]
SMAD2–4	CC	Nuclear shuttling; promoting transcription	YAP, TAZ	[38,62]
SMAD1	WW	Promoting transcription	YAP	[67]
SMAD7	WW	Promoting inhibitory function	YAP	[68]
p73	WW	Promoting transcription	YAP	[70–72]
ERBB4	WW	Promoting transcription	YAP	[74,75]
PPARγ	WW	Suppressing transcription	TAZ	[56]
NFATC5	pY316	Suppressing transcription	TAZ	[26]
STAT1	STAT1 binding motif	Inhibiting STAT1/2 dimerization	YAP, TAZ	[21]

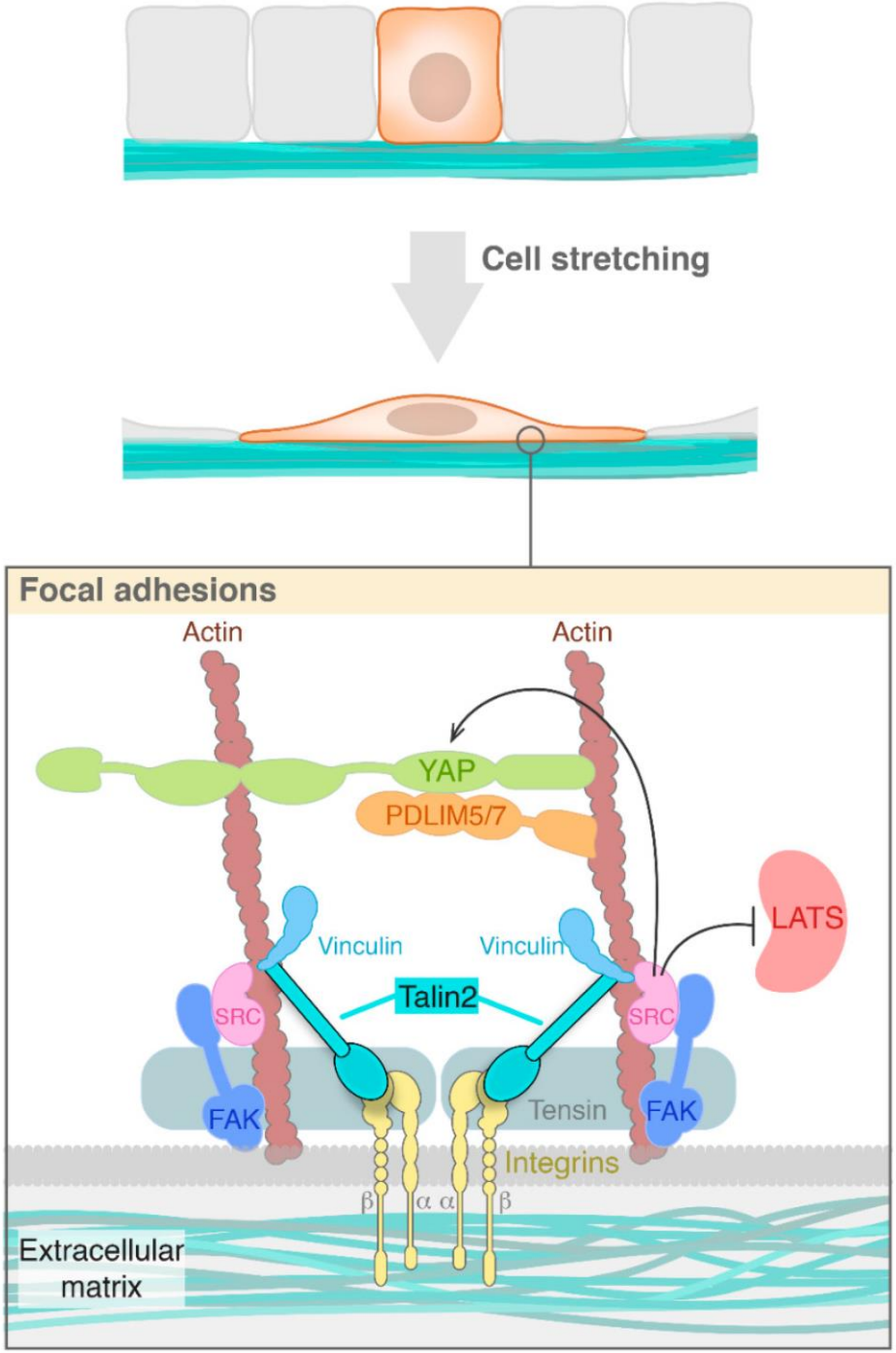
They form complexes with multiple TFs, coactivators, and chromatin remodelers to regulate gene expression in diseases and cancers.


Frizzled
RTK
EGFR
TGFBR
Integrin
LRP5/6
ROR1
GPCR
RAS
RAF
MEK
SMAD4
SMAD2/3
FAK
Src
APC/Axin
β-catenin
G12/13
Rho
G12/13
Gq/11
Gi/o
MST1/2
LATS1/2
MAP4K4
MKK3/6
p38
mTORC1
YAP/TAZ
P
AMPK
LKB1
TEAD
VGLL4
YAP/TAZ
TEAD
CTGF
CYR61
DKK1
WNT5A
TGFB2
EGFR
MYC
PD-L1
SLC7A5
GLUT3
AREG
MRTF
AP-1
OCT4
SMADs
TCF4


eRNA
RNAP II
eRNA
RNAP II
Enhancer
Chromosome loop
Transcription factors
Mediator proteins
Promoter
Gene
General transcription factors
mRNA
RNAP II

Biological responses triggered by activation of YAP and Taz by high levels of mechanical signalling





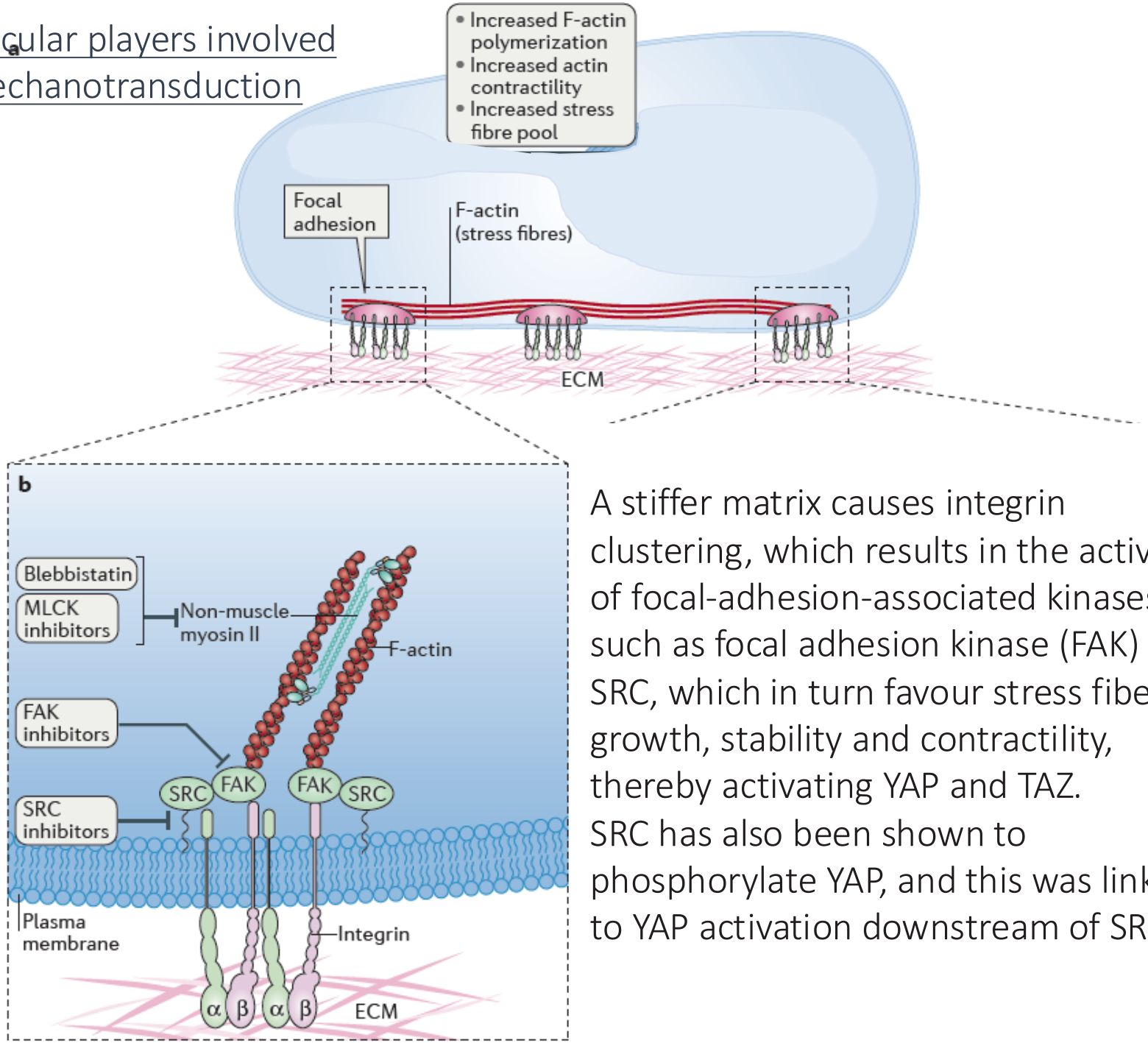
The key to the mistery

The adaptor proteins Talin and Vinculin, which link integrins to F-actin at the focal adhesions, effect the localization of YAP/TAZ.

Secondary to the forces generated above a certain "stiffness threshold", Talin unfolds, binds to Vinculin, and stabilizes the attachment of actin filaments. In this context, YAP/TAZ nuclear translocation is enhanced.

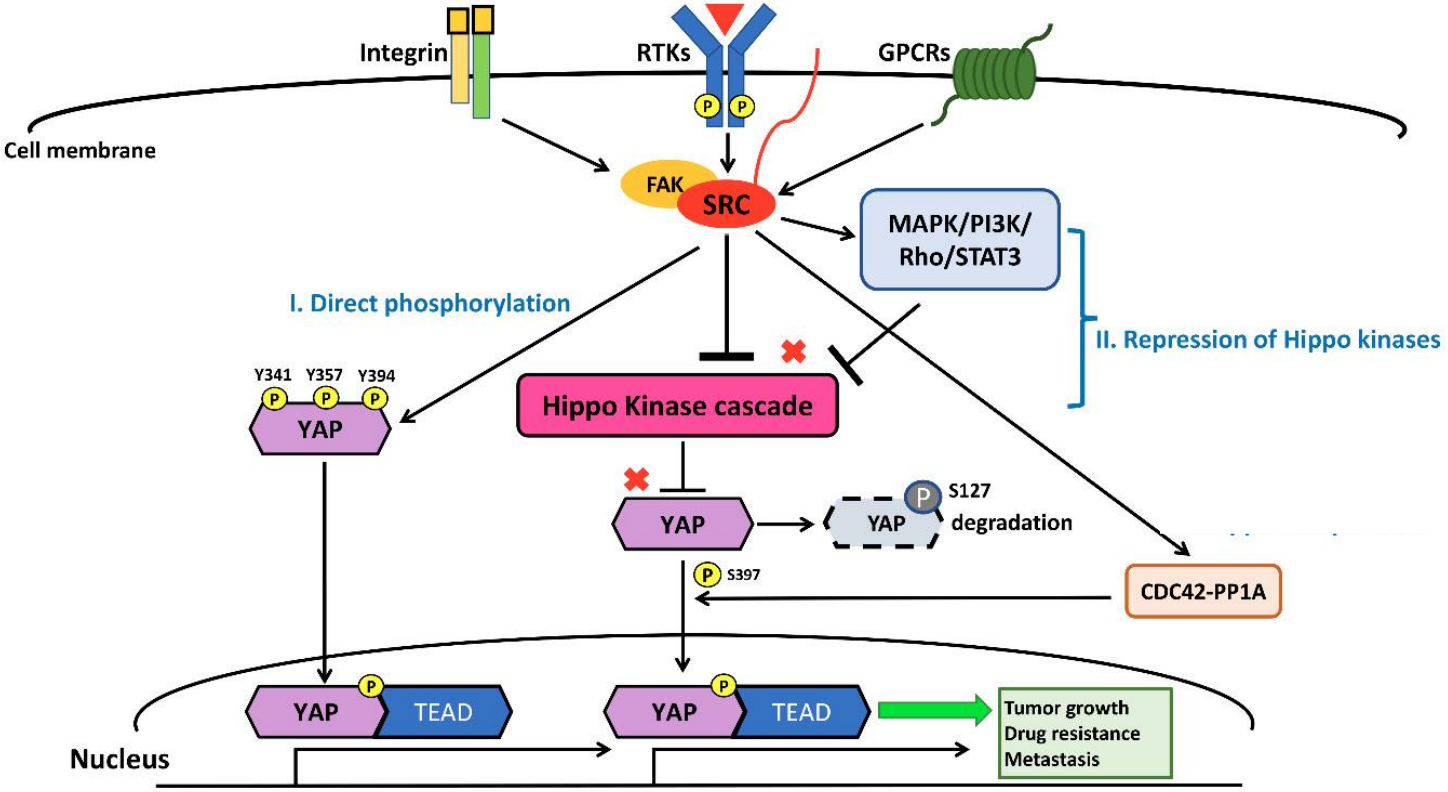
HOW?

Molecular players involved in mechanotransduction



A stiffer matrix causes integrin clustering, which results in the activation of focal-adhesion-associated kinases, such as focal adhesion kinase (FAK) and SRC, which in turn favour stress fiber growth, stability and contractility, thereby activating YAP and TAZ.
SRC has also been shown to phosphorylate YAP, and this was linked to YAP activation downstream of SRC.

Src kinase modulates YAP signalling



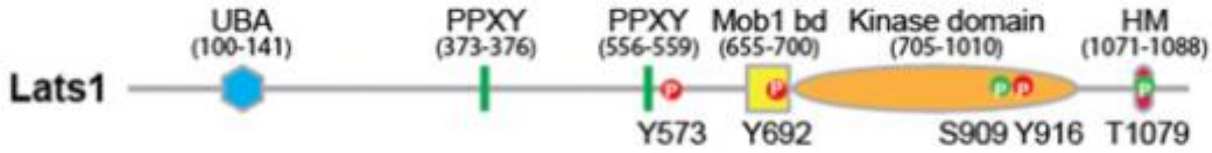
Transmembrane cell surface growth factor receptors activate YAP by Src kinase through three mechanisms:

(1) direct phosphorylation: Src phosphorylates YAP at the site of tyrosine 357 (Y357) to activate it, and Y357 phosphorylation of YAP is required for Wnt/β-catenin signaling to maintain survival and tumorigenesis.

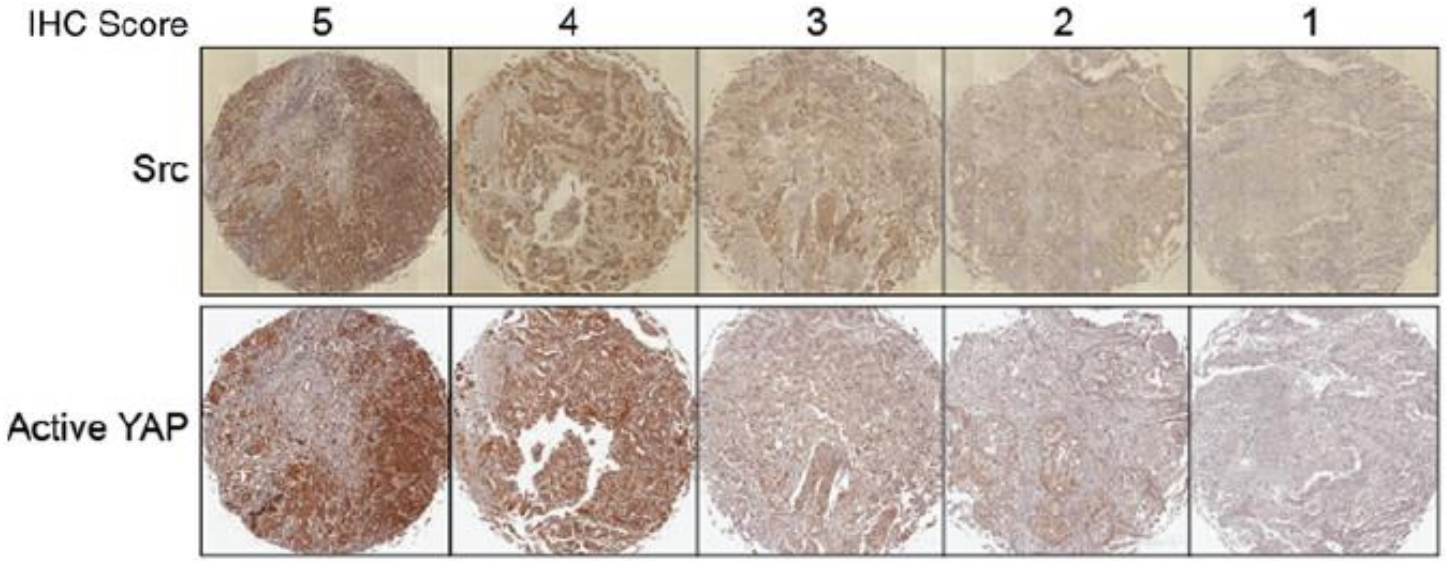
(2) the activation of pathways repressing Hippo kinases: *SRC-inhibitory phosphorylation of LATS facilitate YAP nuclear localization and induction of gene transcription*

Src Inhibits the Hippo Tumor Suppressor Pathway through Tyrosine Phosphorylation of Lats1

Yuan Si[1], Xinyan Ji[1], Xiaolei Cao[1], Xiaoming Dai[1], Lingyi Xu[1], Hongxia Zhao[1], Xiaocan Guo[1], Huan Yan[1], Haitao Zhang[1], Chu Zhu[1], Qi Zhou[1], Mei Tang[1], Zongping Xia[1], Li Li[2], Yu-Sheng Cong[2], Sheng Ye[1], Tingbo Liang[3], Xin-Hua Feng[1], and Bin Zhao[1,2]



Tyrosine phosphorylation on multiple residues of the Hippo pathway tumor suppressor LATS1 by Src is a mechanism underlying its regulation by cell adhesion.



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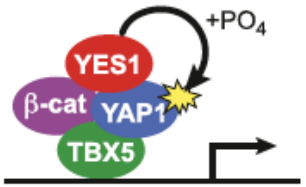
β-Catenin-Driven Cancers Require a YAP1 Transcriptional Complex for Survival and Tumorigenesis

Joseph Rosenbluh,[1,3,5] Deepak Nijhawan,[1,3,5] Andrew G. Cox,[3,4,6] Xingnan Li,[7] James T. Neal,[7] Eric J. Schafer,[1,3,5] Travis I. Zack,[2,5,8] Xiaoxing Wang,[1,3,5] Aviad Tsherniak,[5] Anna C. Schinzel,[1,3,5] Diane D. Shao,[1,3,5] Steven E. Schumacher,[2,5] Barbara A. Weir,[1,5] Francisca Vazquez,[1,5] Glenn S. Cowley,[5] David E. Root,[5] Jill P. Mesirov,[5] Rameen Beroukhim,[2,3,5] Calvin J. Kuo,[7] Wolfram Goessling,[1,3,4,6] and William C. Hahn[1,2,3,5,*]

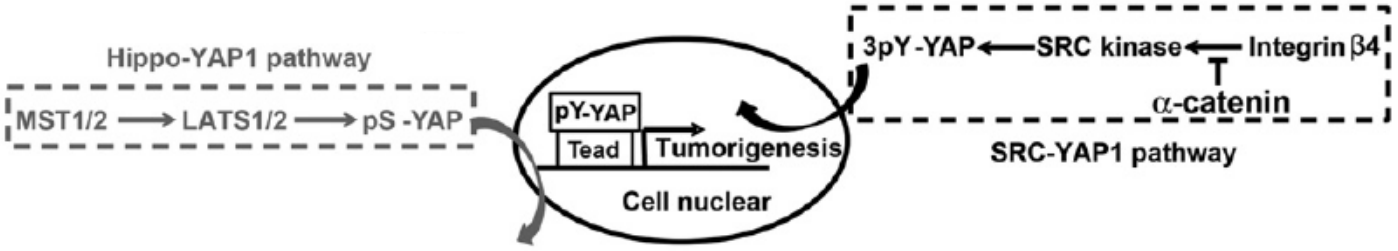
The Src kinase YES1 phosphorylates YAP at the site of tyrosine 357 (Y357); Y357 phosphorylation is required for Wnt/ b-catenin signaling to maintain survival and tumorigenesis in human colorectal cancer cells.



GENES & DEVELOPMENT 30:798–811

αE-catenin inhibits a Src–YAP1 oncogenic module that couples tyrosine kinases and the effector of Hippo signaling pathway

Peng Li,[1,4] Mark R. Silvis,[1,4] Yuchi Honaker,[1,4] Wen-Hui Lien,[1,3] Sarah T. Arron,[2] and Valeri Vasioukhin[1]



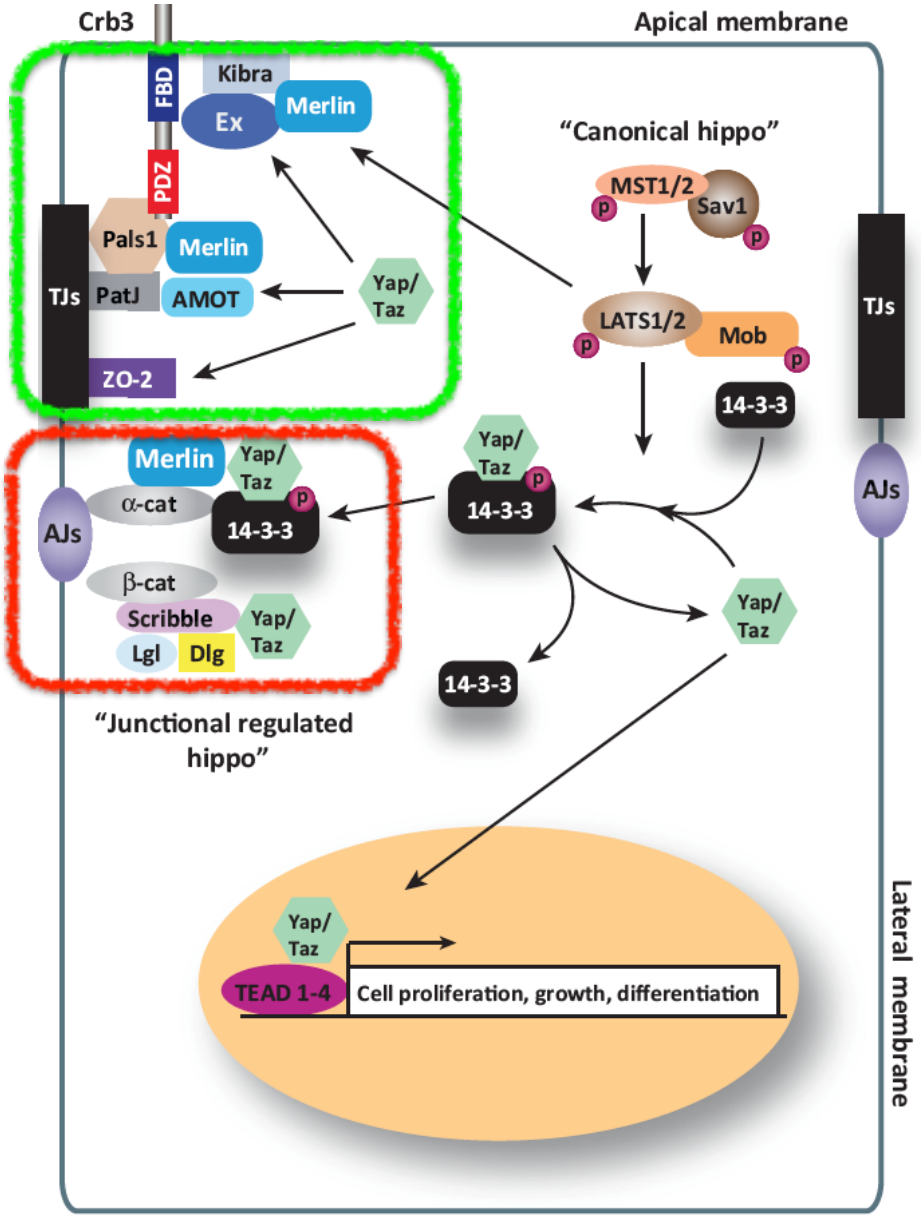
Concurrent tyrosine phosphorylation at sites 341, 357, and 394 by Src kinases is essential for YAP transcriptional activity, nuclear localization, and interaction with TEAD in skin squamous cell carcinomas

Box 1 | **YAP and TAZ mechanotransduction drives organoid biology**

Organoids are 3D cultures of primary cells whose cellular composition and spatial organization closely recapitulate those of the tissue from which they derive (reviewed in REF 133). YAP and TAZ are essential for the maintenance and self-renewal of organoids derived from distinct tissues[65]; regulation of organoids by cell mechanics is also instrumental for their 'self-organizing' and 'self-renewing' properties, that is, to preserve their stem cells and, at the same time, allow differentiation. Accordingly, YAP and TAZ are genetically required for the maintenance of intestinal stem cell function in organoids derived from adult intestinal crypts[45,64,134], and YAP was reported to be active in the growing (that is, 'crypt-like') compartment of the organoids, whereas it is confined to the cytoplasm of their more differentiated compartment[45]. Typically, organoids are grown in Matrigel, a natural basement membrane substrate that is chemically and physically heterogeneous, making it impossible to dissect the relative contributions of distinct inputs on organoid biology. Intriguingly, when intestinal organoids are embedded in fully defined synthetic hydrogels, their growth is inhibited in soft substrates but dramatically fostered by more rigid matrices, a condition accompanied by, and requiring, nuclear accumulation of YAP and TAZ[45] (FIG. 1). As cells accumulate and the organoid expands, increased cell crowding progressively limits YAP and TAZ activity (FIG. 1), promoting differentiation. As such, to support long-term organoid expansion, colonies must be transferred into a cleavable, and thus mechanically dynamic, artificial matrix that allows budding of 'crypt-like' finger-shaped protrusions at whose tips stem cells are preserved by nuclear (active) YAP and TAZ[45].

As another example, in human airway organoids, YAP and TAZ are nuclear in basal (progenitor) cells and absent in differentiated cells. Similarly to intestinal organoids, mechanoactivation of YAP and TAZ by raising tension in the F-actin cytoskeleton is essential for branching morphogenesis of these lung organoids *in vitro*[135].

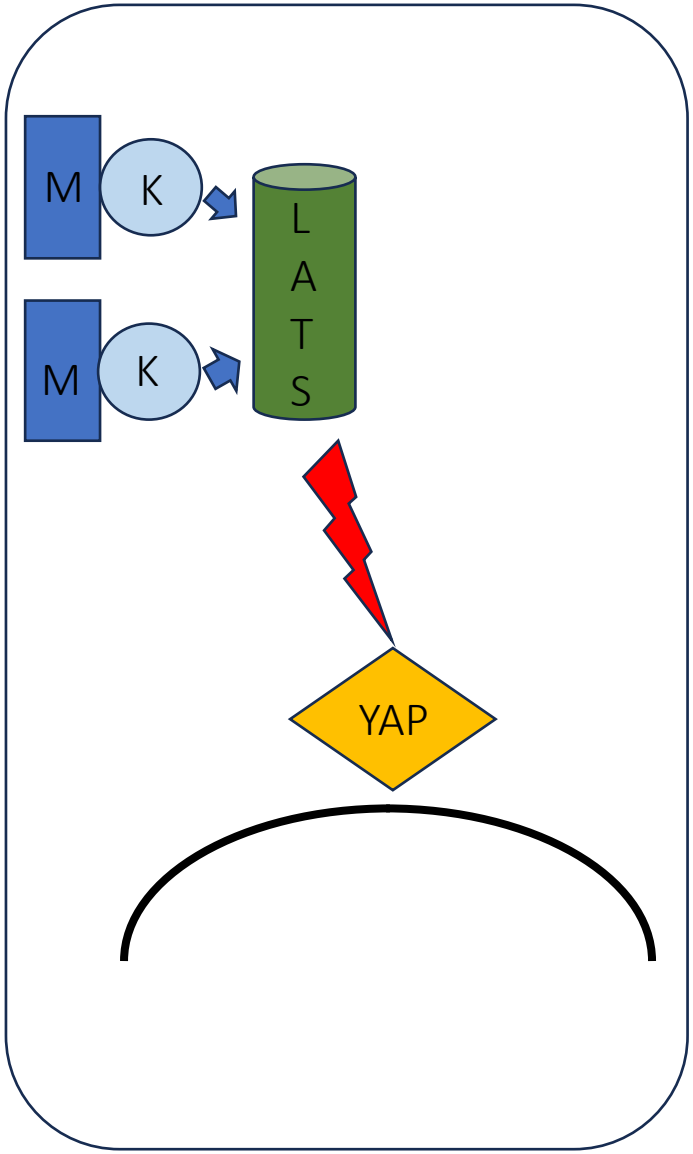
Regulation of Yap/Taz by the canonical Hyppo pathway and cellular junction sequestration



"Adherens junctions" and "tight junctions" represent the main structures by which epithelial cells are bound together via protein complexes.

Key junctional proteins regulate the activity of YAP/TAZ: bind and detain YAP/TAZ at cell junctions, thus suppressing their nuclear entry and activity.

Regulation of Yap/Taz by the canonical Hyppo pathway and cellular junction sequestration

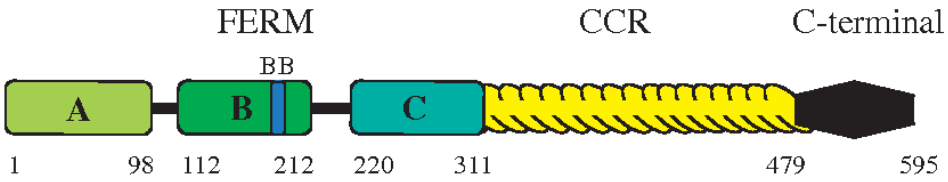


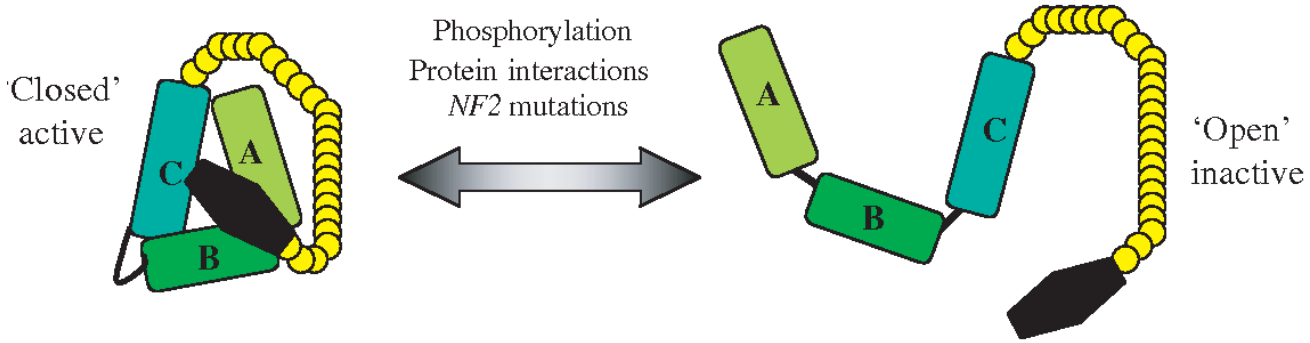
Merlin is encoded by the NF2 (neurofibromatosis type 2) tumor suppressor: in confluent monolayers of mammalian epithelial cells, Merlin/NF2 is preferentially localized in adherens and tight junctions.

Merlin is an important inhibitor of YAP/TAZ: at cell-cell junctions, Merlin promotes the assembly of the appropriate protein scaffolds that allow LATS activation and YAP phosphorylation.
The WW-domain containing protein **Kibra** may serve as a bridge between LATS and Merlin at AJ.

- Moreover, in Drosophila and mammalian cells, Merlin directly binds to LATS, recruiting it to the cell membrane where it gets synergistically activated by the Hippo/Sav kinase complex.

MERLIN





Spatial Organization of Hippo Signaling at the Plasma Membrane Mediated by the Tumor Suppressor Merlin/NF2

Feng Yin,[1] Jianzhong Yu,[1] Yonggang Zheng,[1] Qian Chen,[1] Nailing Zhang,[1] and Duojia Pan[1,*]
[1]Department of Molecular Biology and Genetics, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
*Correspondence: djpan@jhmi.edu
http://dx.doi.org/10.1016/j.cell.2013.08.025

Merlin directly binds and recruits the effector kinase Wts/ Lats to the plasma membrane. Membrane recruitment, in turn, promotes Wts phosphorylation by the Hpo-Sav kinase complex. Disruption of the actin cytoskeleton promotes Merlin-Wts interactions, which implicates Merlin in actin-mediated regulation of Hippo signaling.

The plasma membrane is a critical subcellular compartment for Hippo signal transduction.

Yurika Matsui[1], Zhi-Chun Lai[1,2,3]

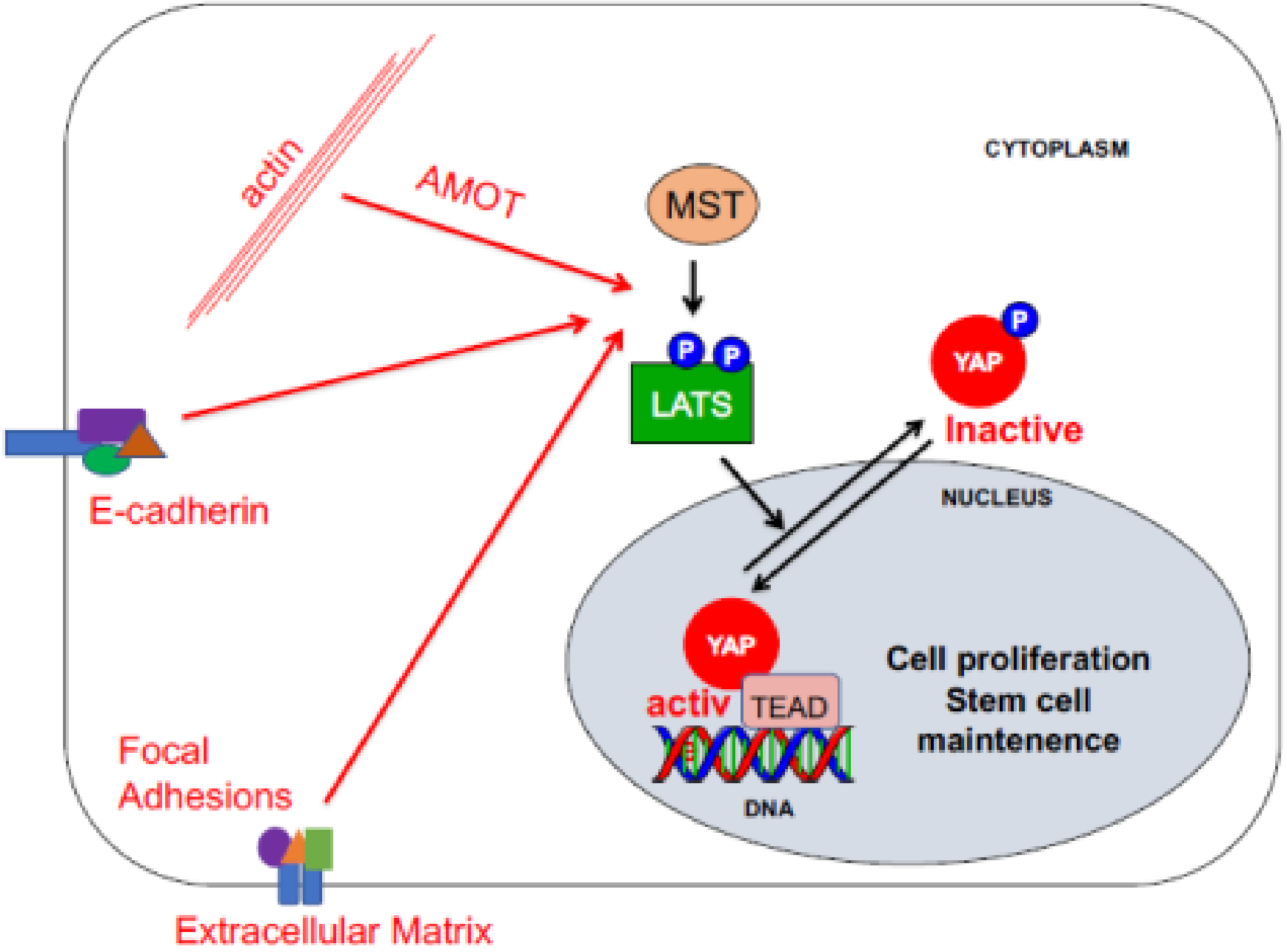
REVIEW

Mutual regulation between Hippo signaling and actin cytoskeleton

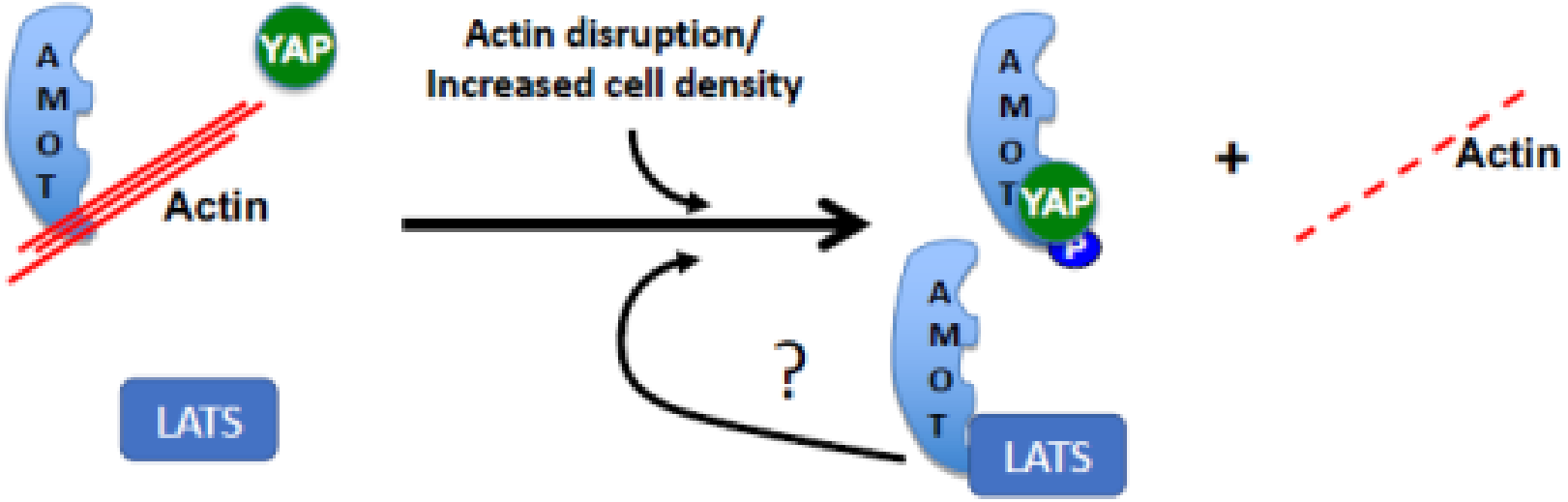
ABSTRACT

Hippo signaling plays a crucial role in growth control and tumor suppression by regulating cell proliferation, apoptosis, and differentiation. How Hippo signaling is regulated has been under extensive investigation. Over the past three years, an increasing amount of data have supported a model of actin cytoskeleton blocking Hippo signaling activity to allow nuclear accumulation of a downstream effector, Yki/Yap/Taz. On the other hand, Hippo signaling negatively regulates actin cytoskeleton organization. This review provides insight on the mutual regulatory mechanisms between Hippo signaling and actin cytoskeleton for a tight control of cell behaviors during animal development, and points out outstanding questions for further investigations.

Mechanical Regulation of Hippo Signaling



ANGIOMOTINS: sensors for F-actin levels



Angiomotins are novel Hippo pathway scaffolding proteins able to interact with both F-actin and multiple core components of the signaling network.

Angiomotins **inhibit** YAP through two mechanisms:
1- directly binding YAP and sequestering it in the cytoplasm,
2- by activating the YAP inhibitory kinase LATS.

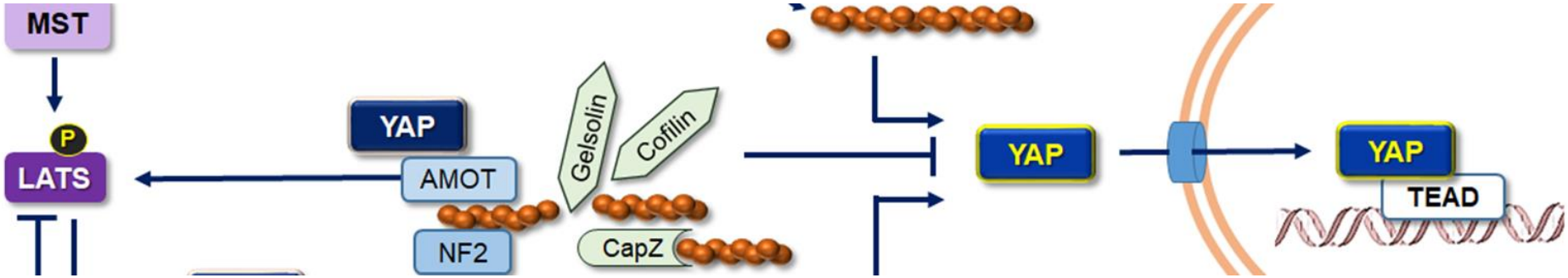
F-actin and YAP compete for binding to angiomotins rendering angiomotin inhibition of YAP sensitive to F-actin levels.

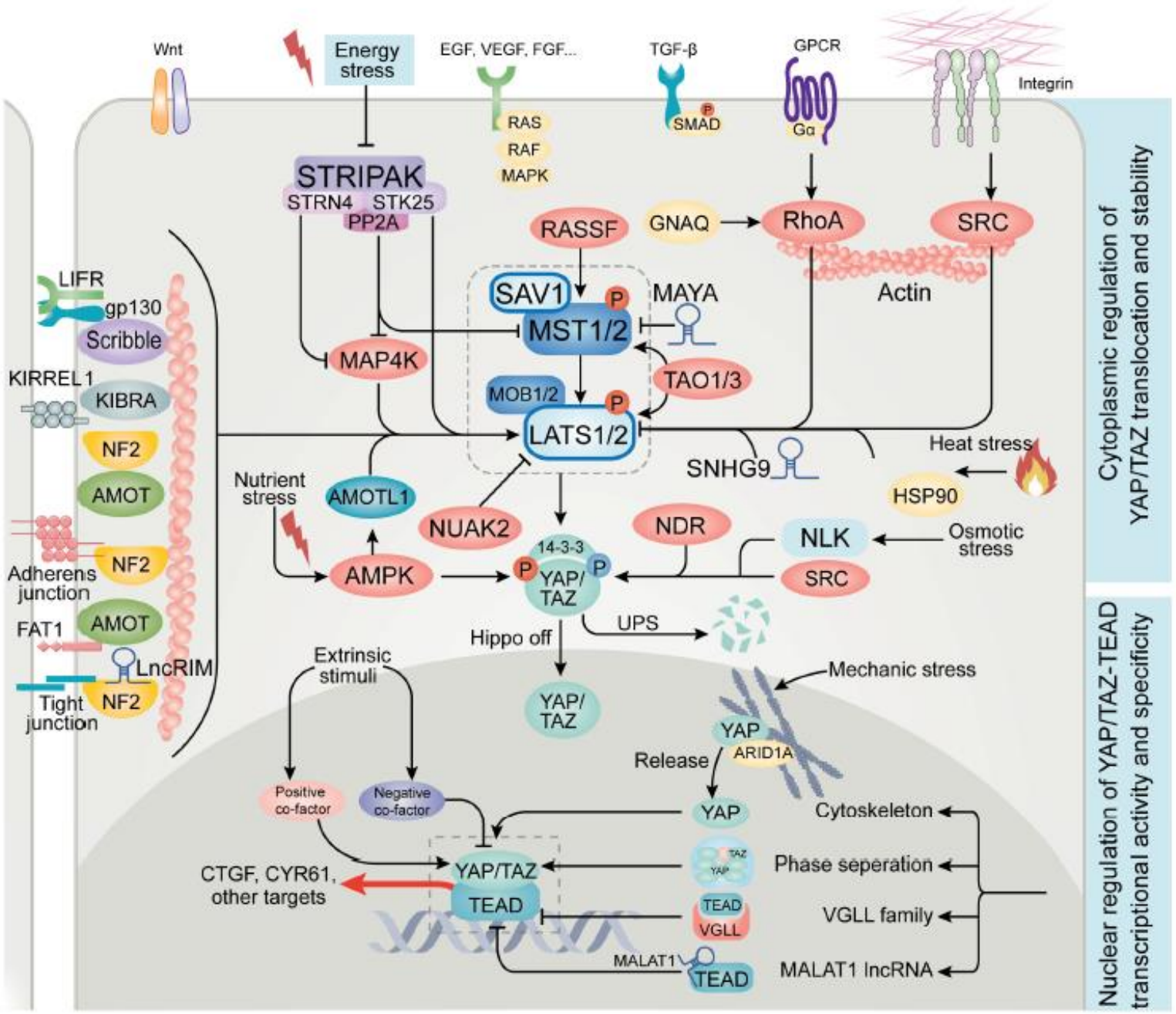
F-actin capping

Actin-interacting proteins that restrict actin polymerization are involved in the regulation of actin dynamics, adjusting cell shape and motility in response to environmental factors.

The F-actin-capping protein CapZ and the F-actin-severing proteins Cofilin and Gelsolin have been identified as crucial negative regulators of YAP/TAZ activity.

- Knockdown of Cofilin, Gelsolin, or CapZ increases F-actin levels and rescues the expression of YAP/TAZ target genes.

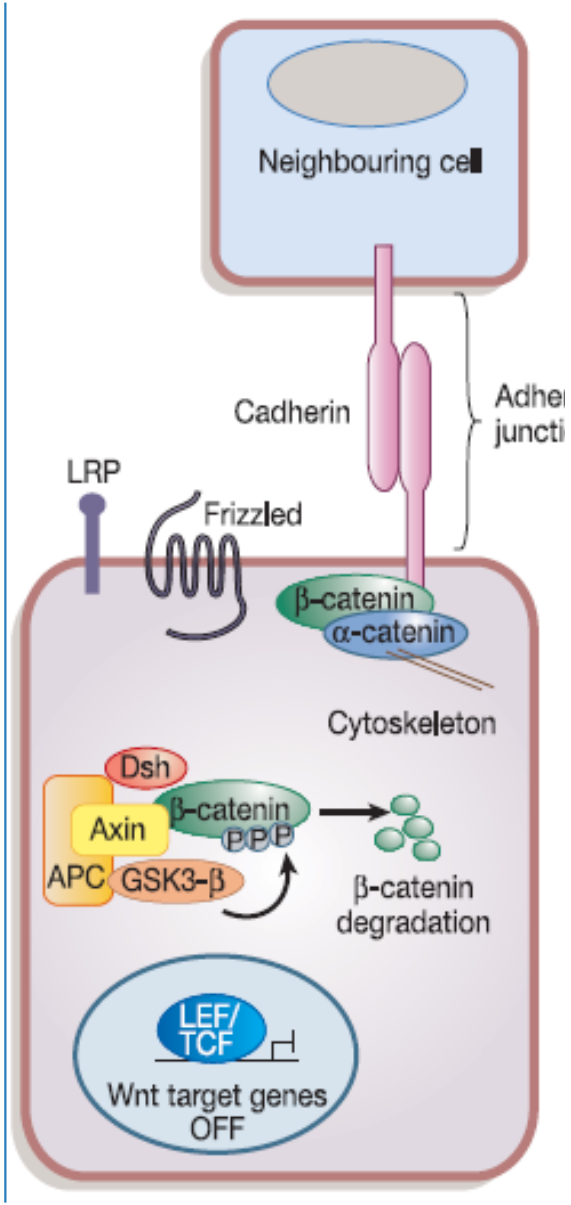


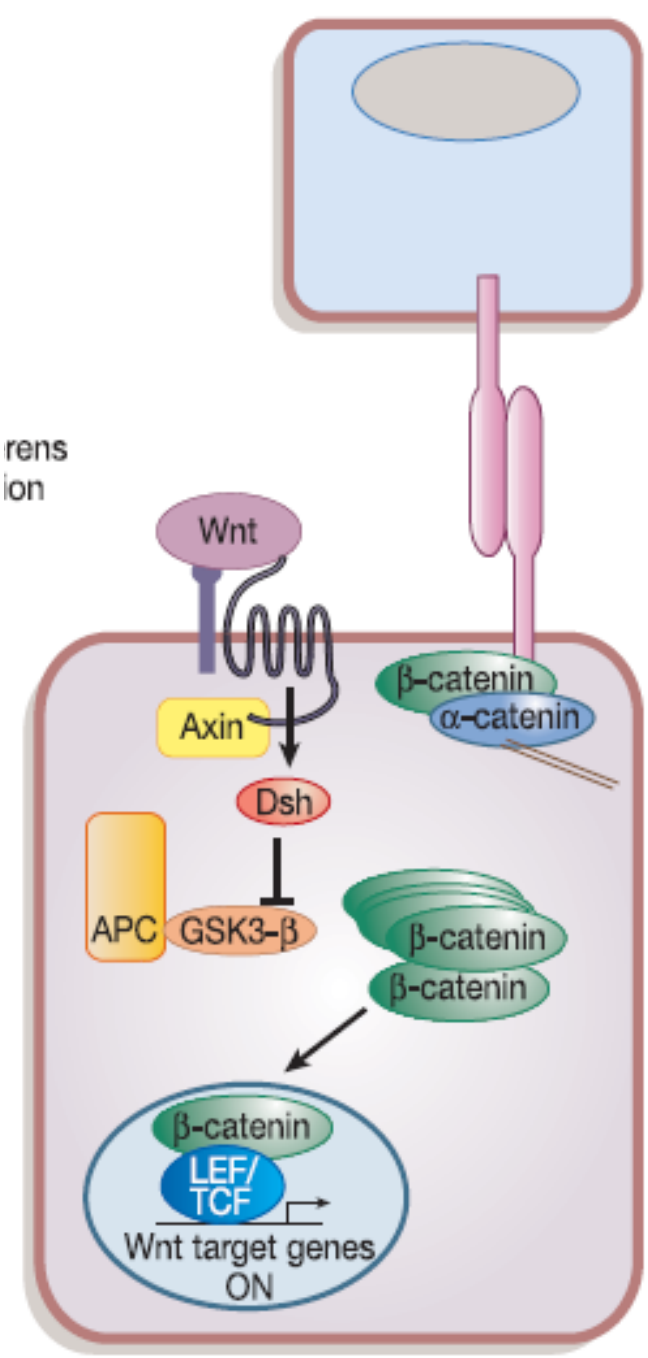

Wnt
Energy stress
EGF, VEGF, FGF...
TGF-β
GPCR
Integrin
RAS
RAF
MAPK
SMAD
Gα
STRIPAK
STRN4
STK25
PP2A
RASSF
GNAQ
RhoA
SRC
Actin
LIFR
gp130
Scribble
KIRREL1
KIBRA
NF2
AMOT
Adherens junction
NF2
FAT1
AMOT
LncRIM
Tight junction
NF2
SAV1
MST1/2
MAYA
MAP4K
TAO1/3
MOB1/2
LATS1/2
SNHG9
Heat stress
HSP90
Nutrient stress
AMOTL1
NUAK2
NDR
NLK
Osmotic stress
AMPK
14-3-3
YAP/TAZ
SRC
UPS
Hippo off
Extrinsic stimuli
Mechanic stress
YAP/TAZ
YAP
ARID1A
Release
Positive co-factor
Negative co-factor
YAP
Cytoskeleton
CTGF, CYR61, other targets
YAP/TAZ
TEAD
TAZ
YAP
Phase seperation
TEAD
VGLL
VGLL family
MALAT1
TEAD
MALAT1 lncRNA
Cytoplasmic regulation of YAP/TAZ translocation and stability
Nuclear regulation of YAP/TAZ-TEAD transcriptional activity and specificity

The Wnt/β-catenin signalling pathway

In the absence of Wnt ligand, b-catenin is sequestered in a multiprotein degradation complex containing the scaffold protein Axin, APC, casein kinase I (CKI) and glycogen synthase kinase 3b (GSK3b).

Upon phosphorylation b-catenin is ubiquitinated and subsequently degraded by the proteasome machinery. As a consequence, there is no transcription of Wnt target genes.



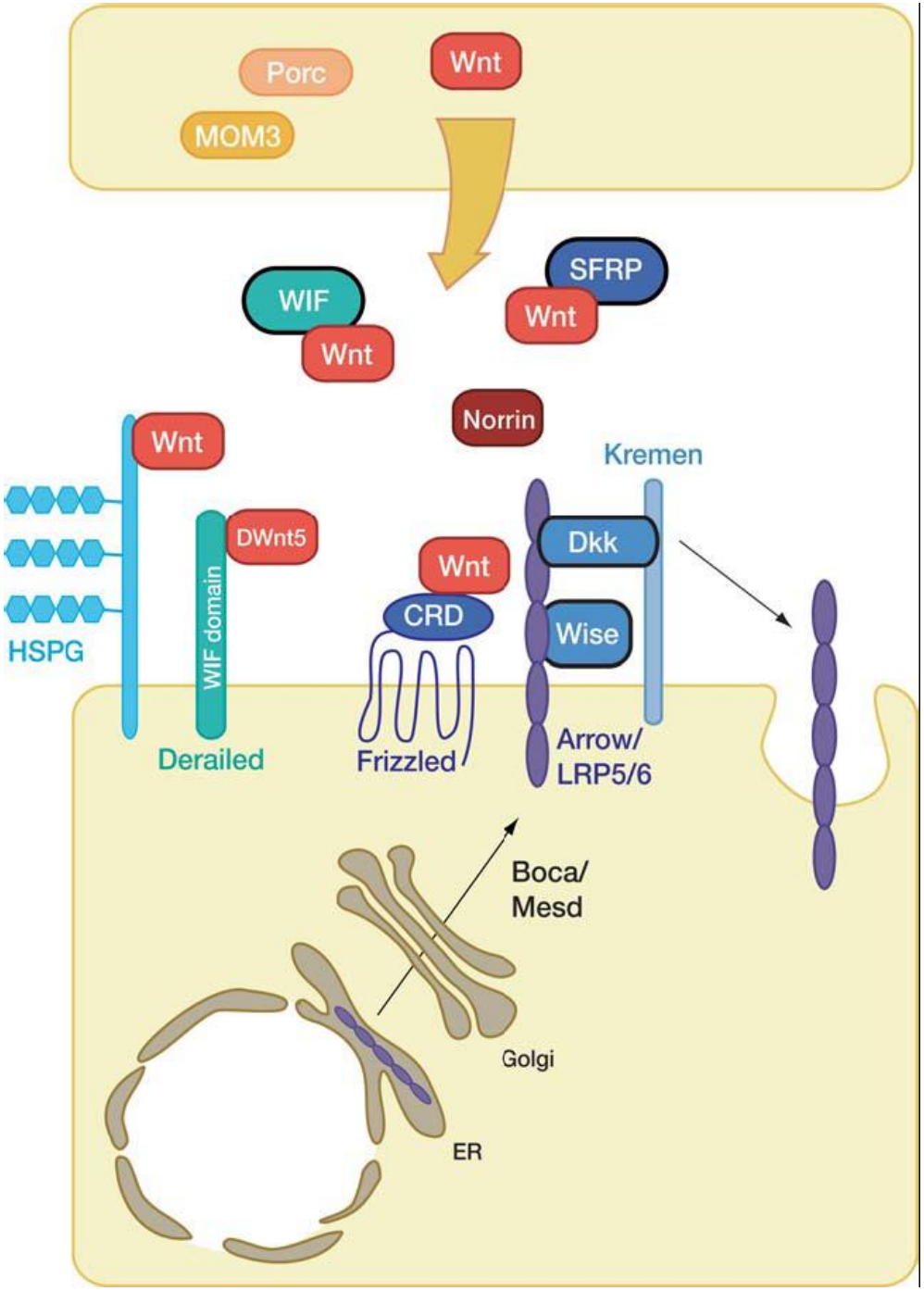


In the presence of Wnt ligand, Axin is recruited to the plasma membrane. b-catenin is then released from the multiprotein degradation complex and accumulates in the cytoplasm in a stabilized non-phosphorylated form.

As a consequence, b-catenin is translocated into the nucleus, where it associates with transcription factors of the T-cell factor/lymphoid enhancing factor (TCF/LEF) family leading to the transcription of Wnt target genes, such as the c-myc oncogene and cyclin D1.

- In vertebrates, Wnt proteins are inhibited by direct binding to either secreted frizzled-related protein (SFRP) or Wnt inhibitory factor (WIF).

- SFRP is similar in sequence to the cysteine-rich domain (CRD) of Frizzled, one of the Wnt receptors.

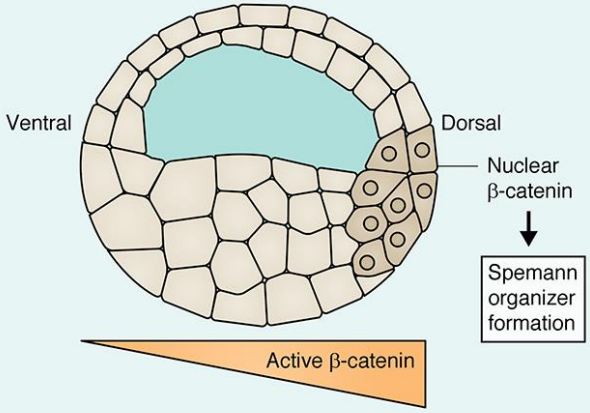


Wnt signalling is essential in early embryonic development

Wnt signaling in embryonic development

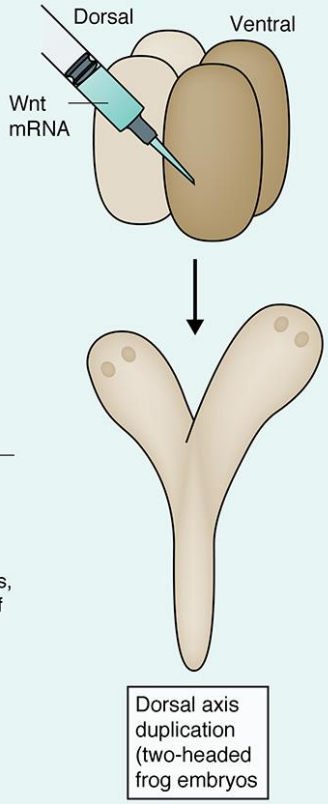
Blastula

Early during *Xenopus* embryo development, cortical rotation leads to dorsal enrichment of maternal factors, which in turn leads to nuclear accumulation of β-catenin. A gradient of nuclear β-catenin and signaling activity is therefore found across the blastula dorsal-ventral axis. High levels of nuclear β-catenin are observed on the dorsal side, where they promote the transcriptional program required for formation of the Spemann organizer.



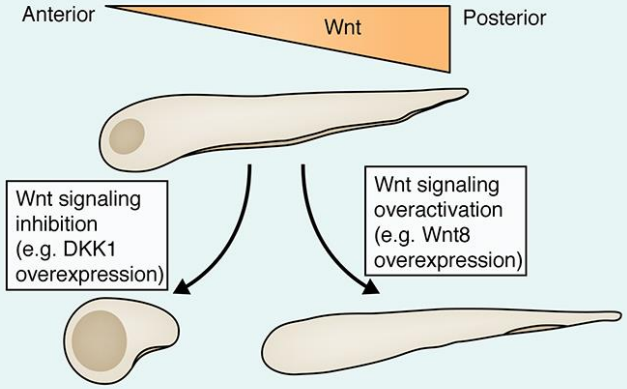
Four-cell embryo

Injection of Wnt mRNA into the future ventral cells of the *Xenopus* four-cell embryo leads to development of a secondary dorsal axis, resulting in two-headed embryos.



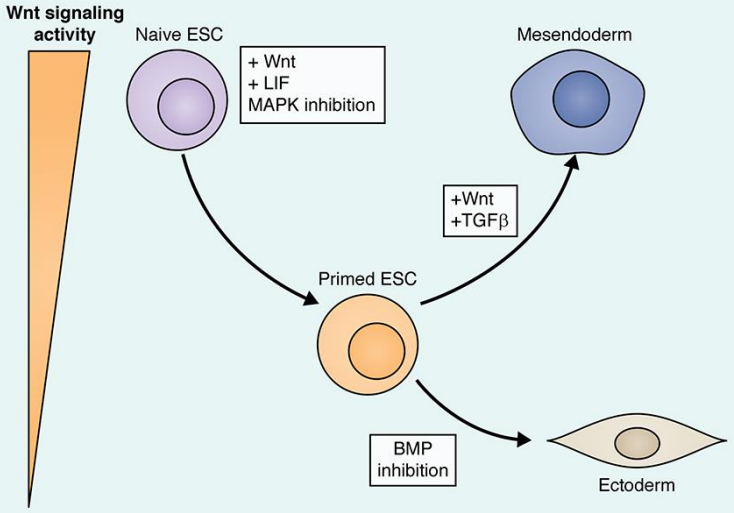
Post-gastrulation

In post-gastrulation vertebrate embryos, a Wnt-β-catenin signaling gradient is formed across the anteroposterior axis, with high Wnt-β-catenin signaling activity on the posterior side. The overexpression of DKK1 (which inhibits Wnt signaling) at this stage leads to anteriorization of the AP axis and loss of posterior structures, whereas overexpression of Wnt8 causes posteriorization and loss of anterior structures.



Wnt signaling in pluripotent cells

The *ex vivo* culture of mammalian ESCs in the naive state requires high Wnt signaling, mediated by GSK3 inhibition, for self-renewal, along with LIF activation and MAPK pathway inhibition. In the primed pluripotent ESC state, however, Wnt activity is not required. Differentiation of ESCs into mesendodermal lineages requires high Wnt-β-catenin and TGFβ signaling, whereas directed differentiation into ectodermal lineages requires BMP inhibition and low Wnt-β-catenin activity.



Wnt1 Deficiency in neural crest derivatives, reduction in dorsolateral neural precursors in the neural tube (with Wnt3A KO)
Decrease in thymocyte number (with Wnt-4 KO)

Wnt3 Early gastrulation defect, perturbations in establishment and maintenance of the apical ectodermal ridge (AER) in the limb

Wnt3a Paraxial mesoderm defects, tailbud defects, deficiency in neural crest derivatives, reduction in dorsolateral neural precursors in the neural tube (with Wnt1 KO)
Loss of hippocampus
Somitogenesis defects

Wnt5a Truncated limbs and AP axis
Defects in distal lung morphogenesis
Chondrocyte differentiation defects, perturbed longitudinal skeletal outgrowth
Inhibits B cell proliferation, produces myeloid leukemias and B-cell lymphomas in heterozygotes

Wnt7a Female infertility; in males, Mullerian duct regression fails
Delayed maturation of synapses in cerebellum

Wnt7b Placental development defects
Respiratory failure, defects in early mesenchymal proliferation leading to lung hypoplasia

Wnt11 Ureteric branching defects and kidney hypoplasia

Mapping Wnt/β-catenin signaling during mouse development and in colorectal tumors

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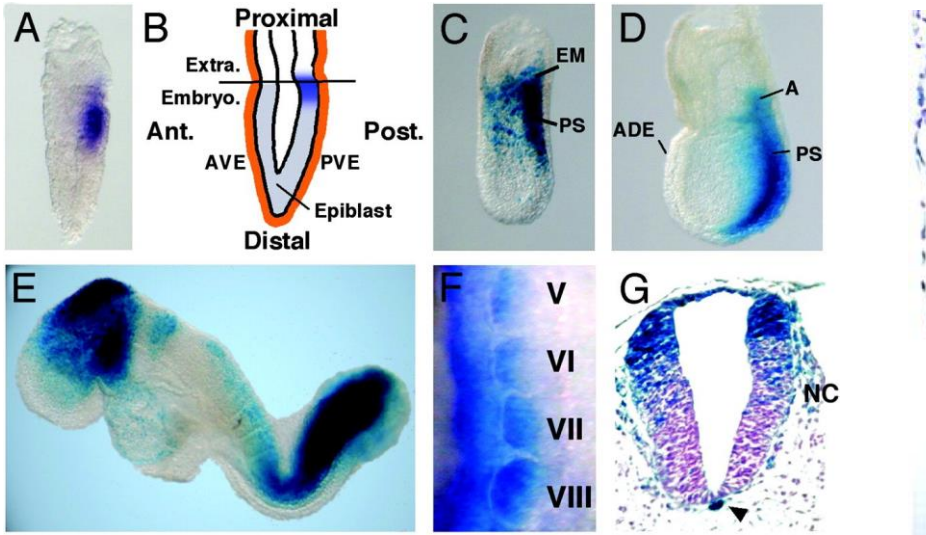
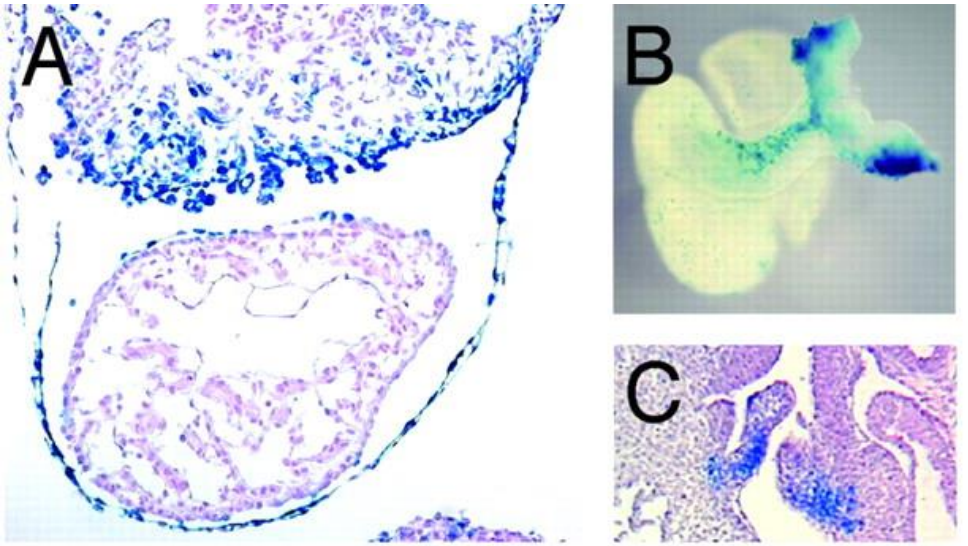
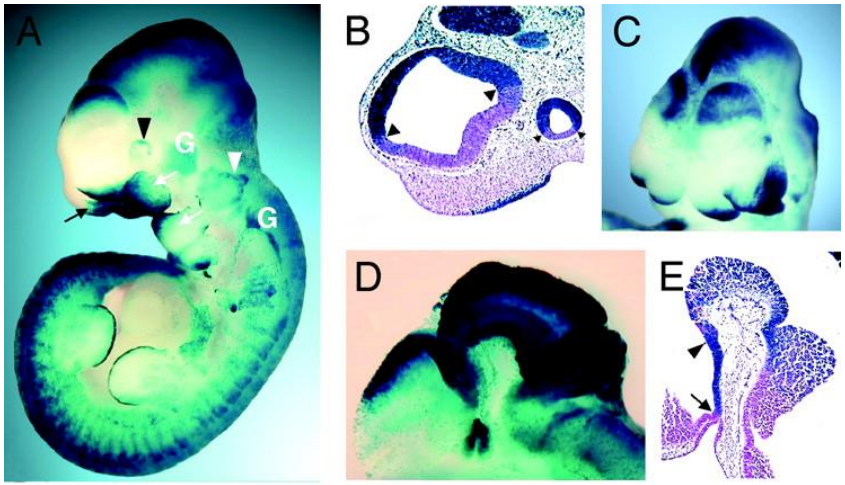
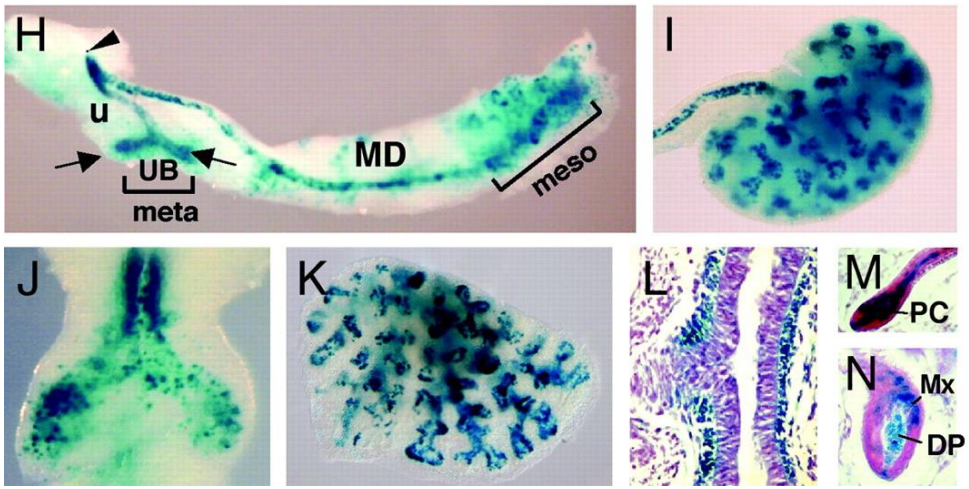
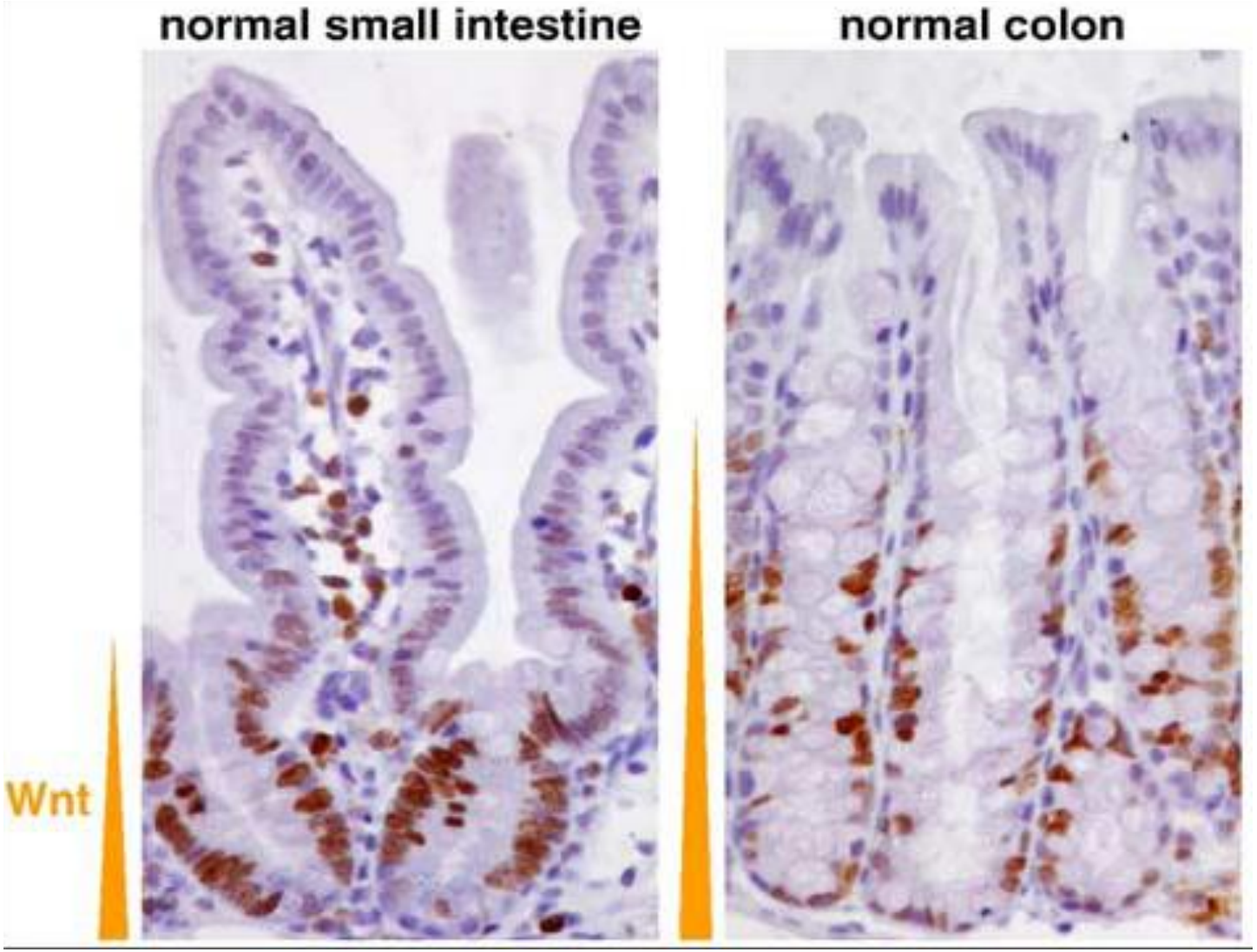


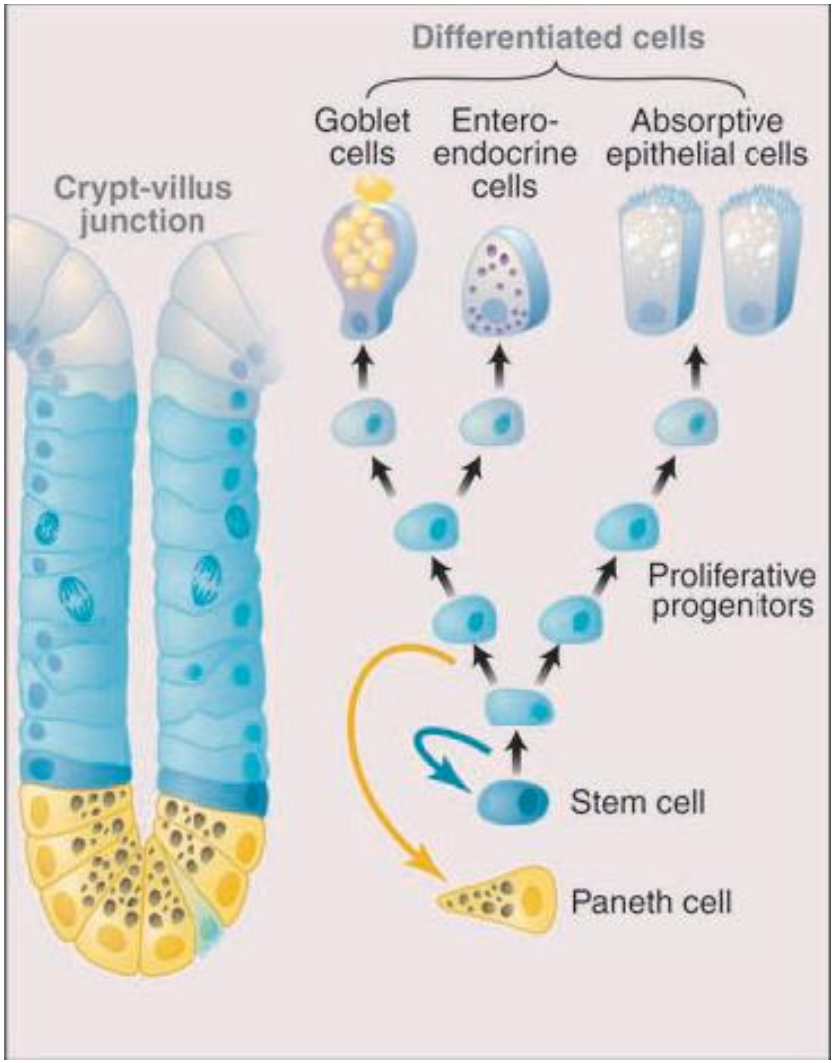






The Wnt cascade is the dominant force in controlling cell fate along the crypt-villus axis.





Slowly dividing multipotent stem cells are anchored at the base of each crypt.

Stem cells give rise to an intermediate cell population referred to as transit amplifying (TA) cells: they undergo rapid proliferation (approx. every 12 h) and expands into a population of non-proliferating daughter cells.

These daughter cells gradually differentiate into 4 epithelial lineages:

1- absorptive cells or enterocytes;

2- mucus-producing goblet cells;

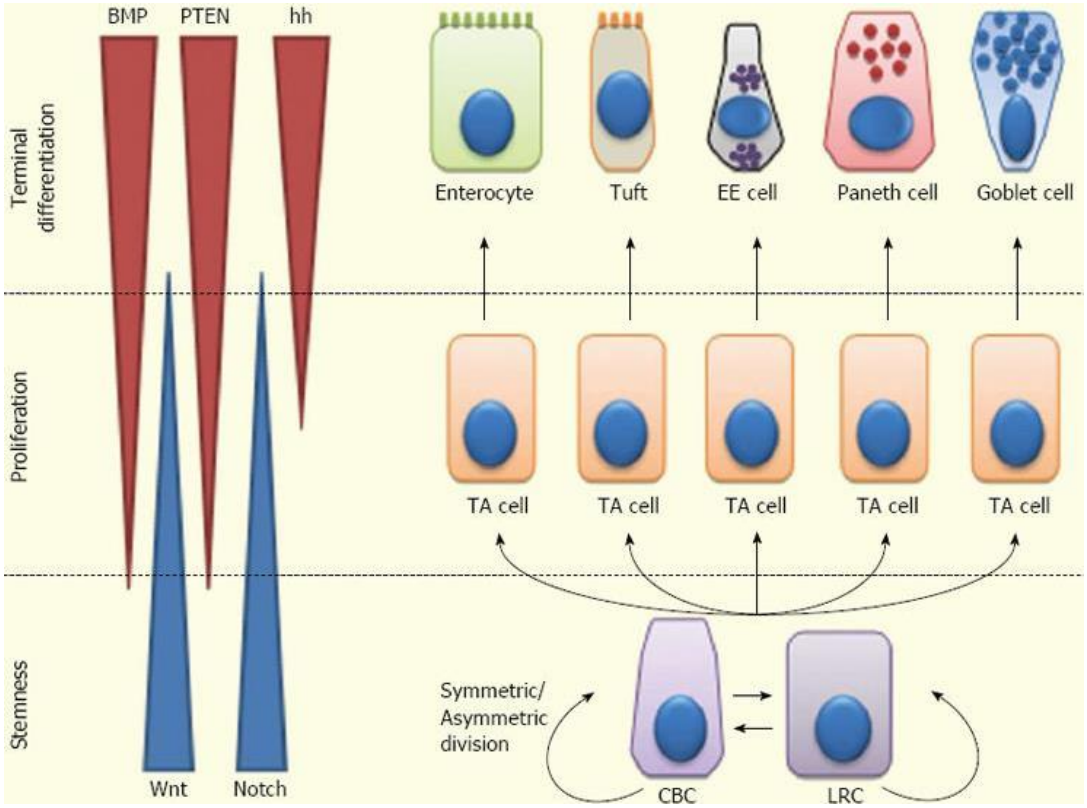
3- enteroendocrine cells, secreting hormones such as serotonin or secretin;

4- Paneth cells, secreting antimicrobial peptides such as cryptidins, defensins and lysozyme.

A sheath of specialized fibroblasts is apposed to the epithelial crypt cells.

These so-called myo-epithelial fibroblasts are critical to the establishment of the crypt niche, sending signals which regulate the whole differentiation program.

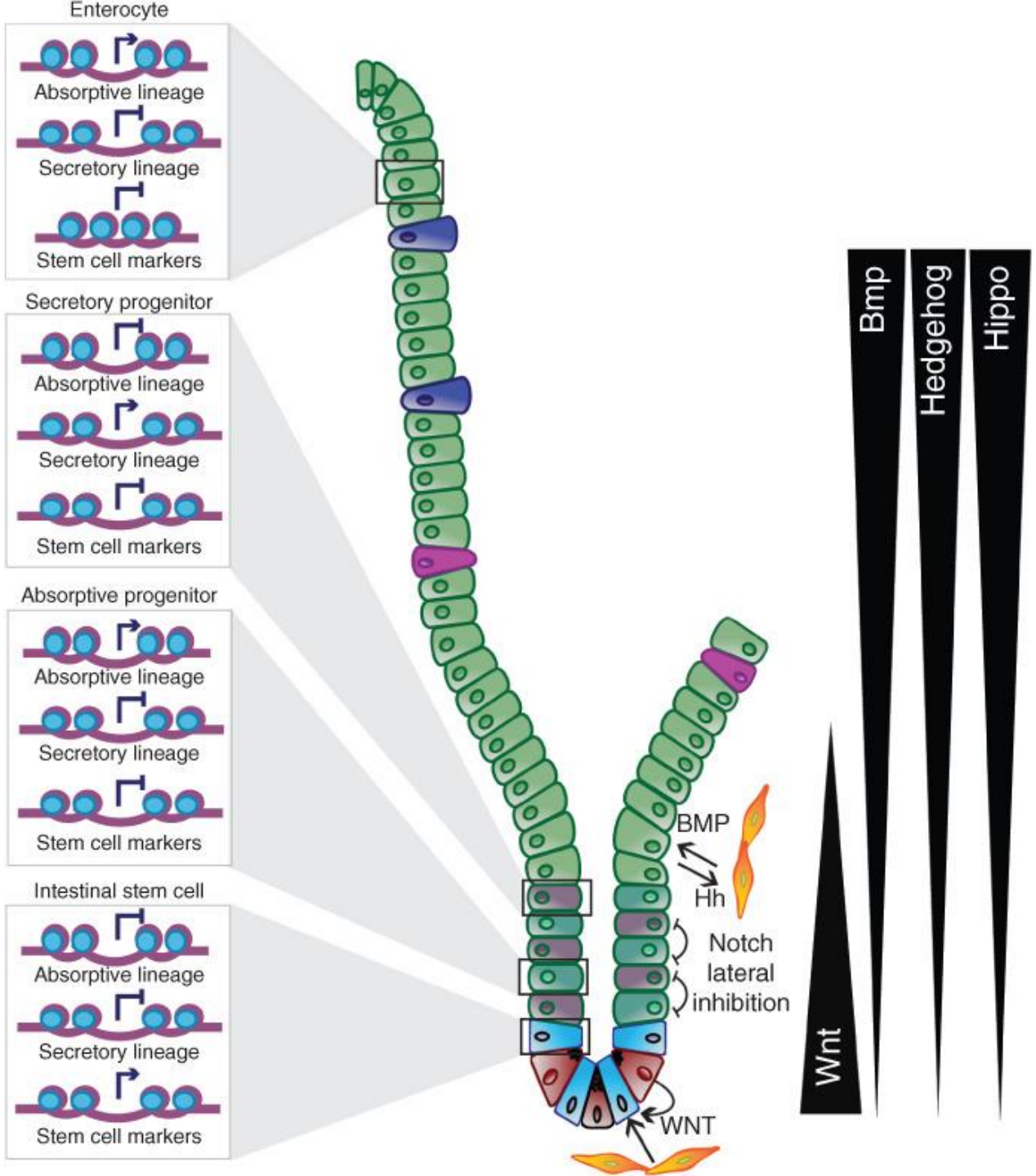
Lineage specification of intestinal stem cells



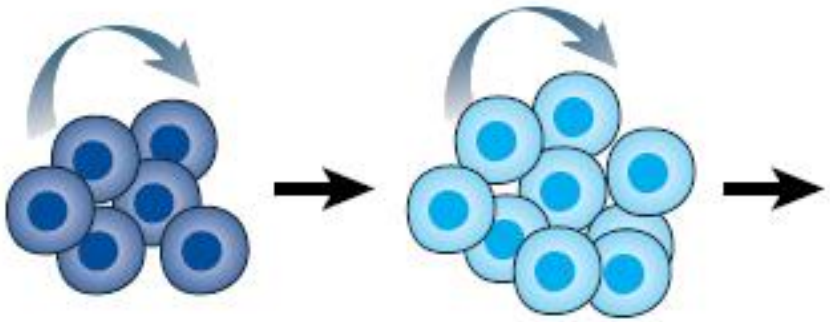
Intestinal stem cells divide asymmetrically or symmetrically to maintain the stem cell compartment. ISCs give rise to Transit Amplifying (TA) cells which actively proliferate and can further differentiate into enterocytes, tuft cells, enteroendocrine (EE) cells or goblet cells.

Wnt signaling maintains the stem-like phenotype of ISCs, while Notch signaling maintains the proliferation of progenitor cells.

In the upper crypt region, hedgehog (hh) triggers BMP expression in stromal cells which activates PTEN expression; all these factors inhibit Wnt signaling in the ISC niche

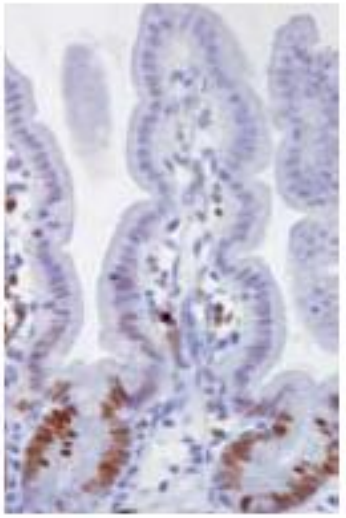

Enterocyte
Absorptive lineage
Secretory lineage
Stem cell markers
Secretory progenitor
Absorptive lineage
Secretory lineage
Stem cell markers
Absorptive progenitor
Absorptive lineage
Secretory lineage
Stem cell markers
Intestinal stem cell
Absorptive lineage
Secretory lineage
Stem cell markers
BMP
Hh
Notch
lateral
inhibition
WNT
Bmp
Hedgehog
Hippo
Wnt

Normal Wnt signalling



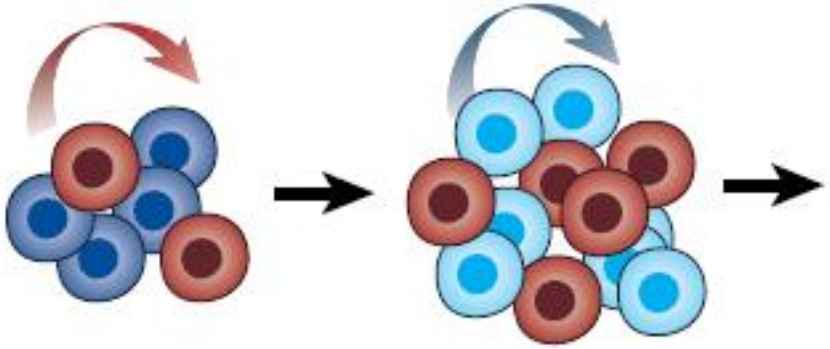
Stem cells

Progenitors

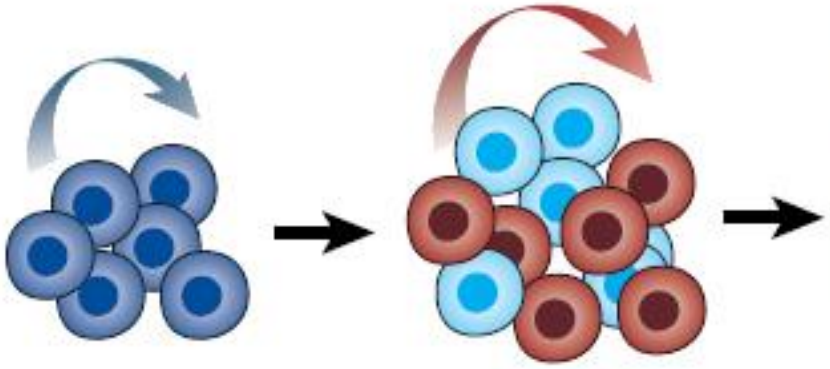


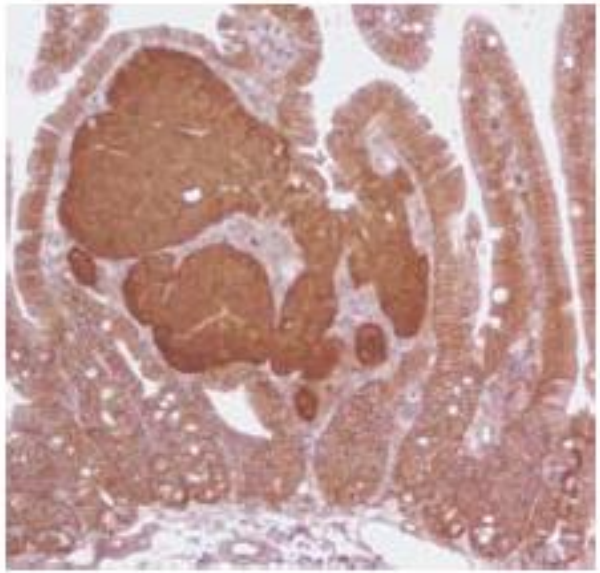
Normal tissue

Dysregulated Wnt signalling in stem cells



Dysregulated Wnt signalling in progenitor cells





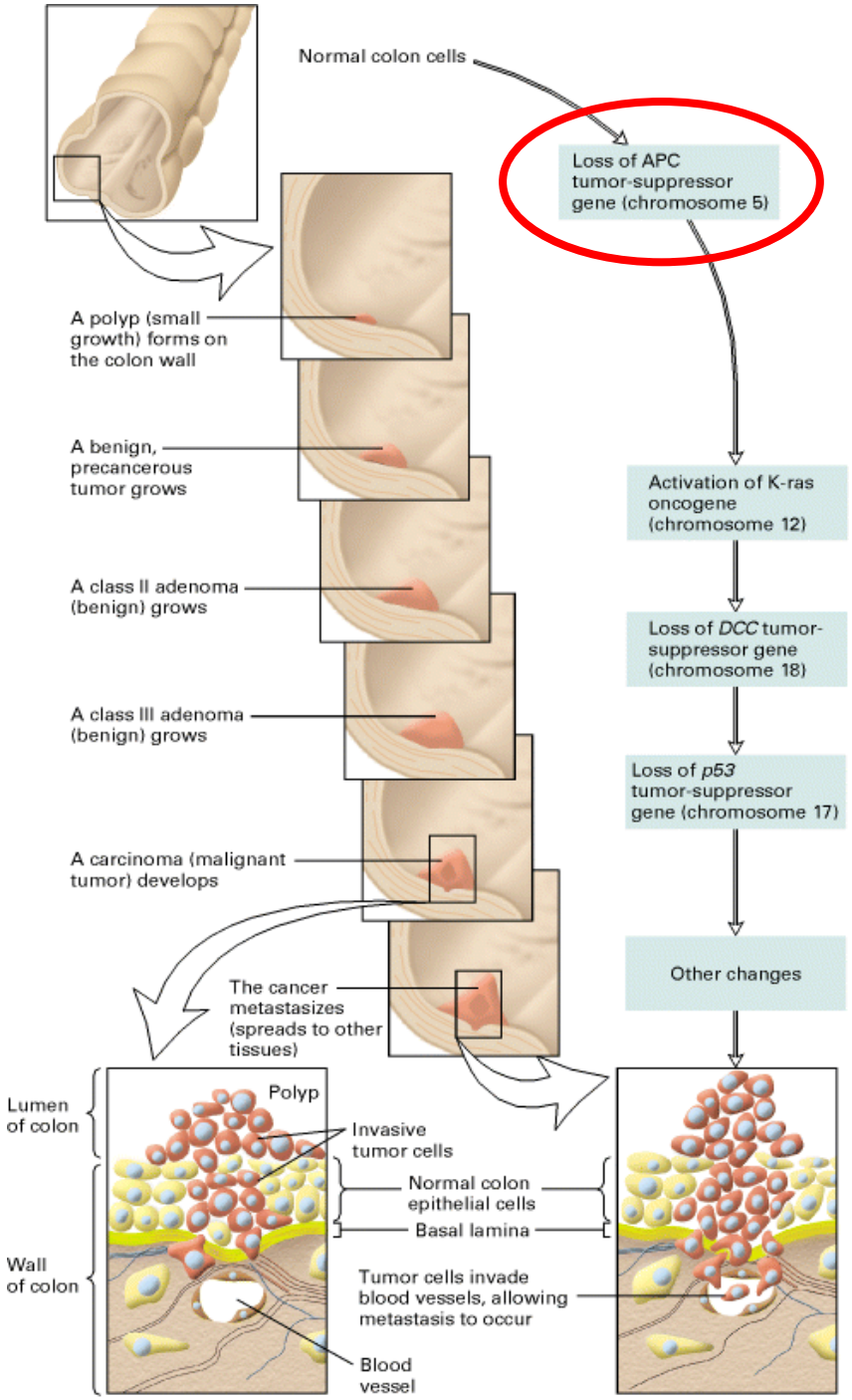
Cancer

The multistep evolution of cancer (Fearon & Vogelstein, 1990)

(i) colorectal tumors result from mutational activation of oncogenes combined with the inactivation of tumor-suppressor genes,

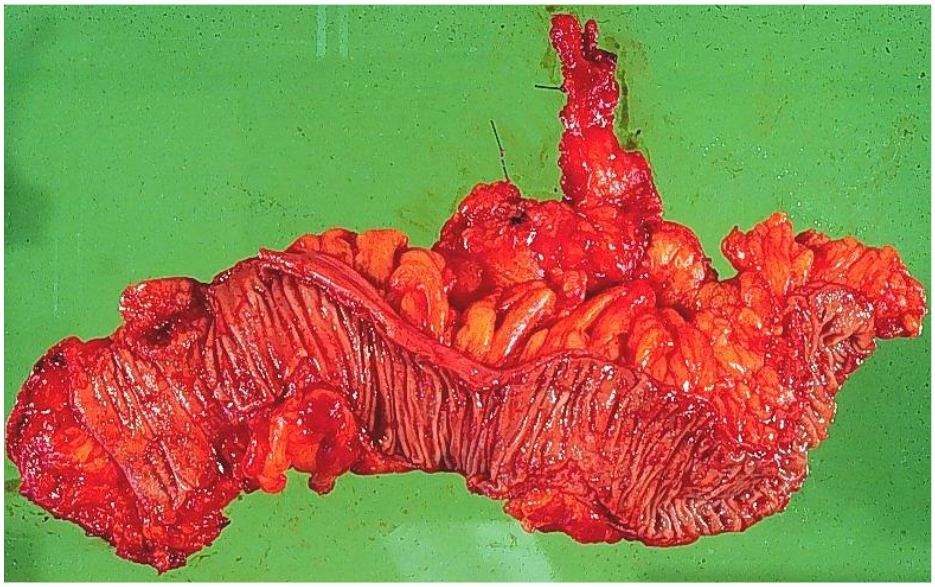
(ii) multiple gene mutations are required to produce malignancies, and

(iii) genetic alterations may occur in a preferred sequence, yet the accumulation of changes rather than their chronologic order determines histopathological and clinical characteristics of the colorectal tumor.



The Wnt pathway in colon cancer

The APC gene was originally discovered to be the culprit in a hereditary cancer syndrome termed familial adenomatous polyposis (FAP).

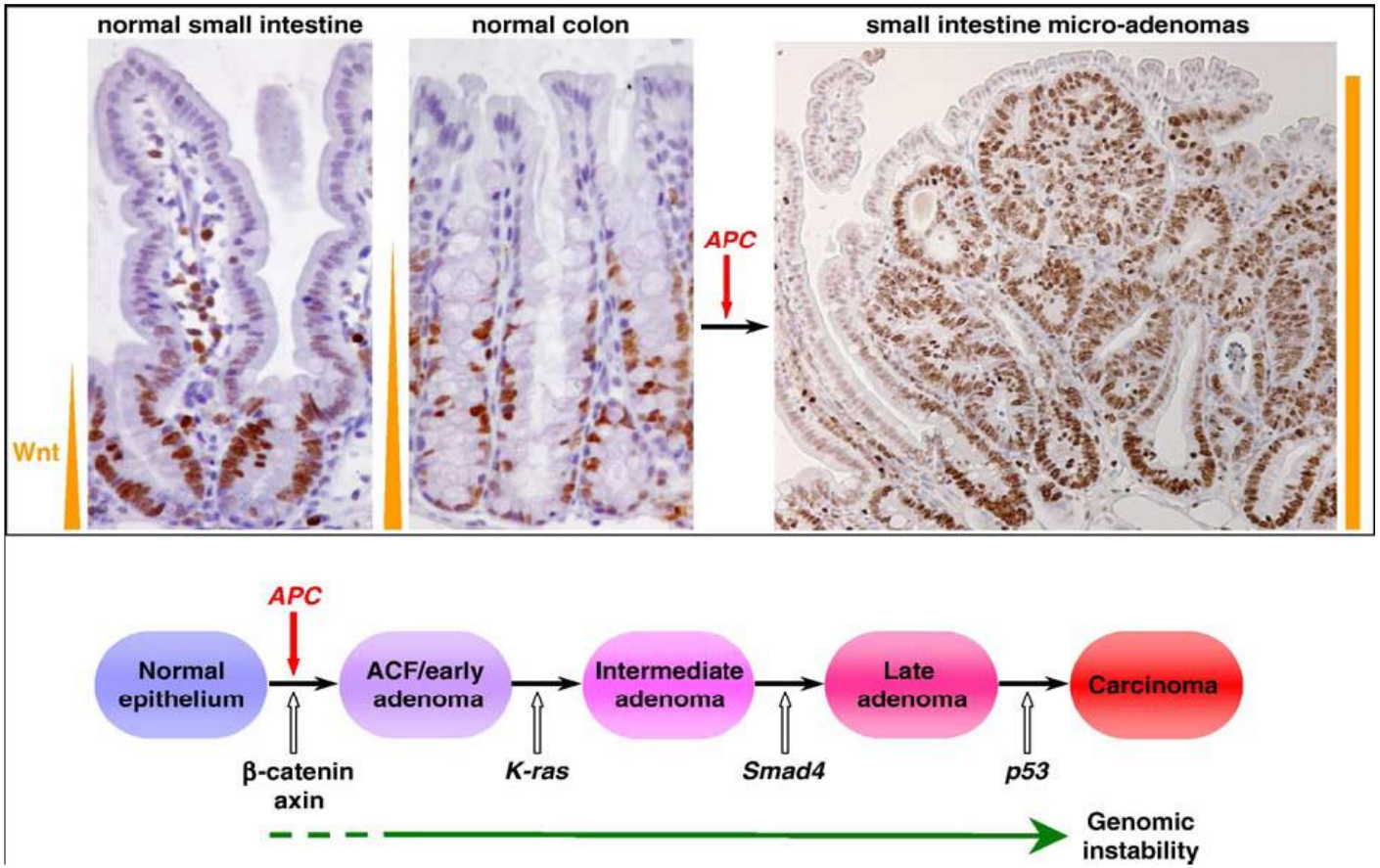


In FAP, as in most sporadic CRCs, tumorigenesis occurs incrementally. The earliest lesions in the colon or the rectum are “aberrant crypt foci” which progress to benign tumors termed adenomas or adenomatous polyps. Colorectal polyps can eventually develop into malignant tumor stages termed adenocarcinomas.

FAP patients develop hundreds to thousands of adenomatous polyps in the colon and rectum at an early age, of which a subset inevitably progresses to carcinomas if not surgically removed.

Germline (loss-of-function) mutations in the APC gene were found to be the essential genetic event responsible for FAP.

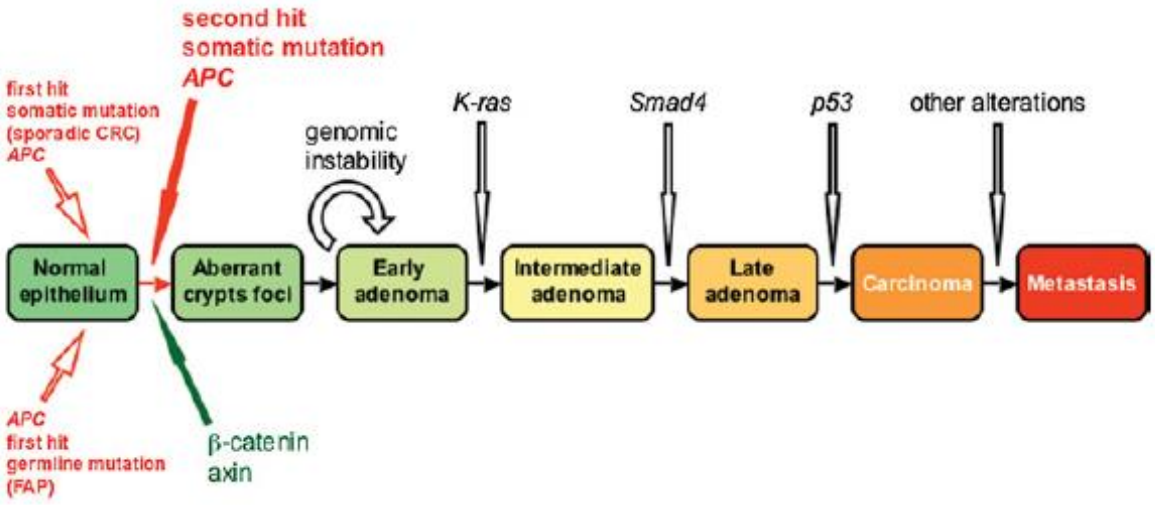
The Wnt/β-catenin signalling pathway controls the homoeostasis of the intestinal epithelium



•Inactivating mutations in the APC gene (that selectively disable binding to b-catenin) or activating mutations in b-catenin (that remove the regulatory phosphorylation sites) lead to nuclear accumulation of b-catenin .

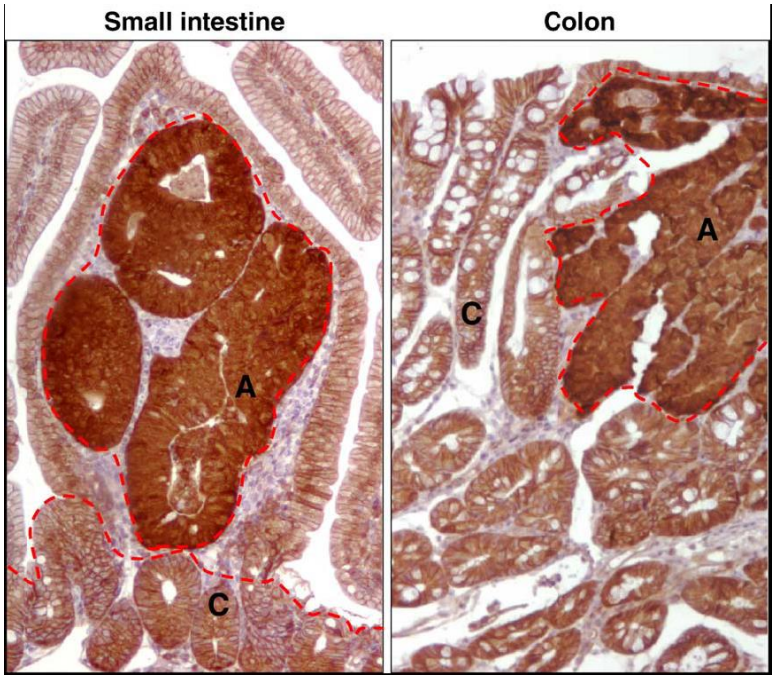
•Any mutational event stabilizing nuclear b-catenin in the intestinal epithelium, which leads to constitutively activated canonical Wnt signaling, represents the initiating event of intestinal tumorigenesis.

- Mutational inactivation of APC leads to the inappropriate stabilization of β-catenin, implying that the absence of functional APC transforms epithelial cells through activation of the Wnt cascade.
- In some cases of colorectal cancer in which APC is not mutated, the scaffolding protein axin 2 is mutant, or activating (oncogenic) point mutations in β-catenin remove its N-terminal Ser/Thr destruction motif.



1- nuclear accumulation of β-catenin is a hallmark of activated canonical Wnt signaling;

2- APC (and Axin) is critical for β-catenin degradation and thus considered a key negative regulator of the Wnt/β-catenin signaling cascade.



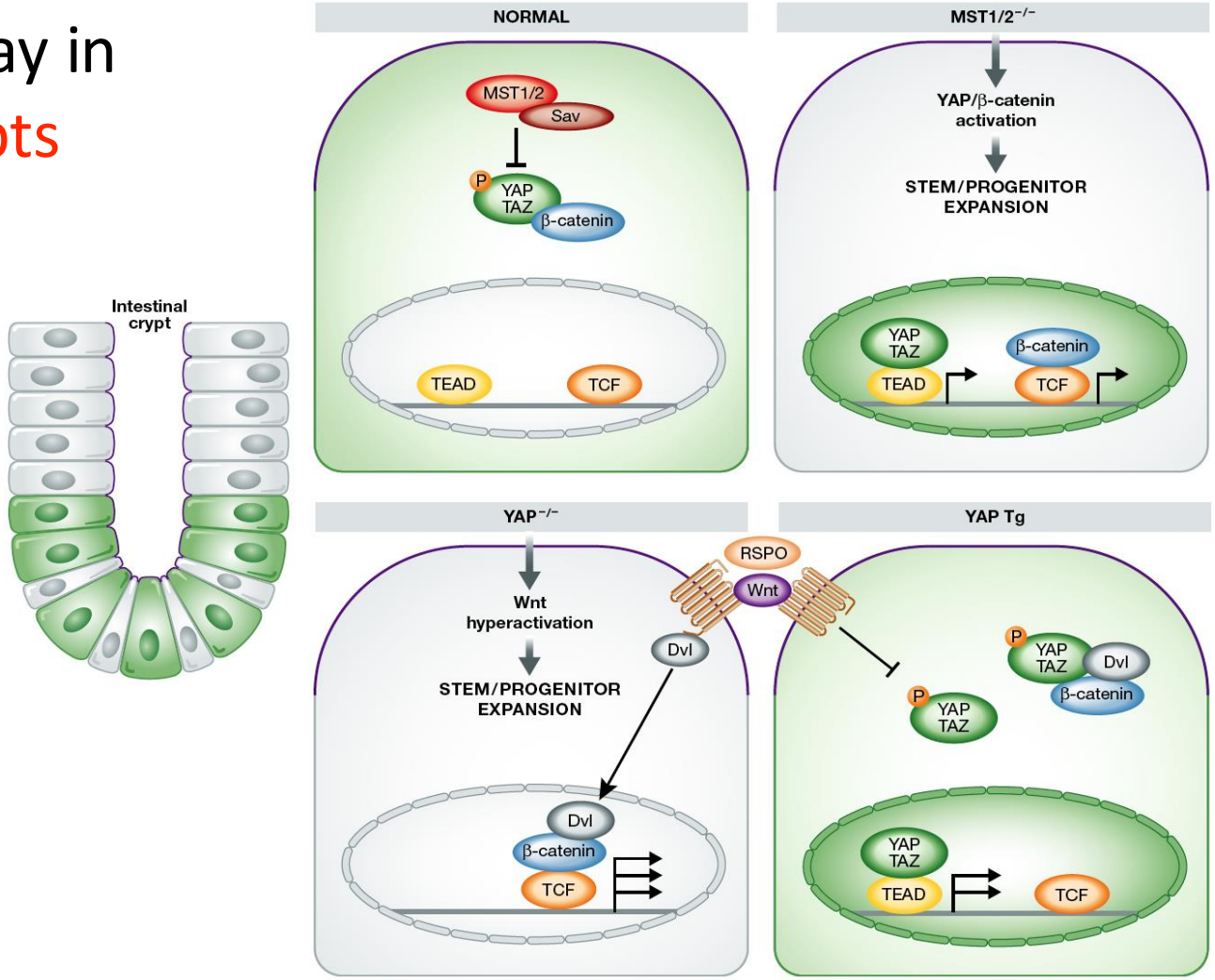
•Nuclear β-catenin accumulates in the crypt stem cell/progenitor compartments in small intestine and colon;

•Transgenic expression in the intestine of adult mice of the Wnt inhibitor Dkk- 1 results in greatly reduced epithelial proliferation coincident with the loss of crypts;

•Inducible inactivation of APC in the intestine of adult mice results within days in the entire repopulation of villi by crypt-like cells, which accumulate nuclear β-catenin and fail to migrate and differentiate.

Wnt signaling is absolutely required for driving and maintaining crypt stem cell/progenitor compartments, and, thus, is essential for homeostasis of the intestinal epithelium.

The Hippo pathway in the intestinal crypts

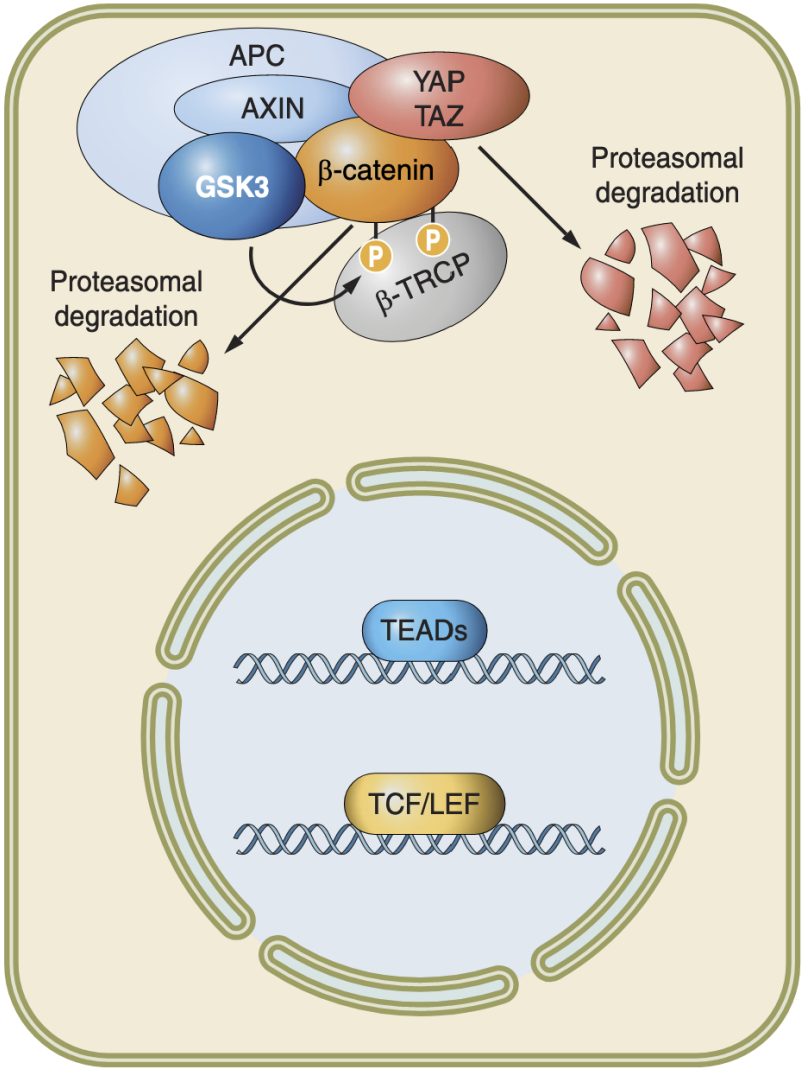


In the intestinal stem cells (ISC), the Hippo pathway inhibits YAP activity by phosphorylation and cytosolic retention of YAP. The cytosolic YAP directly binds to β-catenin and subsequently inhibits the canonical Wnt signaling. In Mst1/2$^{-/-}$ intestinal epithelia, loss of Hippo pathway regulation promotes dephosphorylation and nuclear translocation of YAP/β-catenin and induces their target gene expression. Activation of YAP/β-catenin results in the expansion of ISC. However, a controversial role of YAP has been demonstrated in the context of Wnt-induced intestinal regeneration. In YAP$^{-/-}$ intestinal epithelia, hyperactivation of Wnt/β-catenin signaling results in ISC expansion, whereas YAP overexpression represses Wnt/β-catenin signaling, which leads to the loss of ISC and epithelial self-renewal. In this context, YAP functions to inhibit the nuclear translocation of disheveled (Dvl).

YAP/TAZ orchestrate the Wnt response

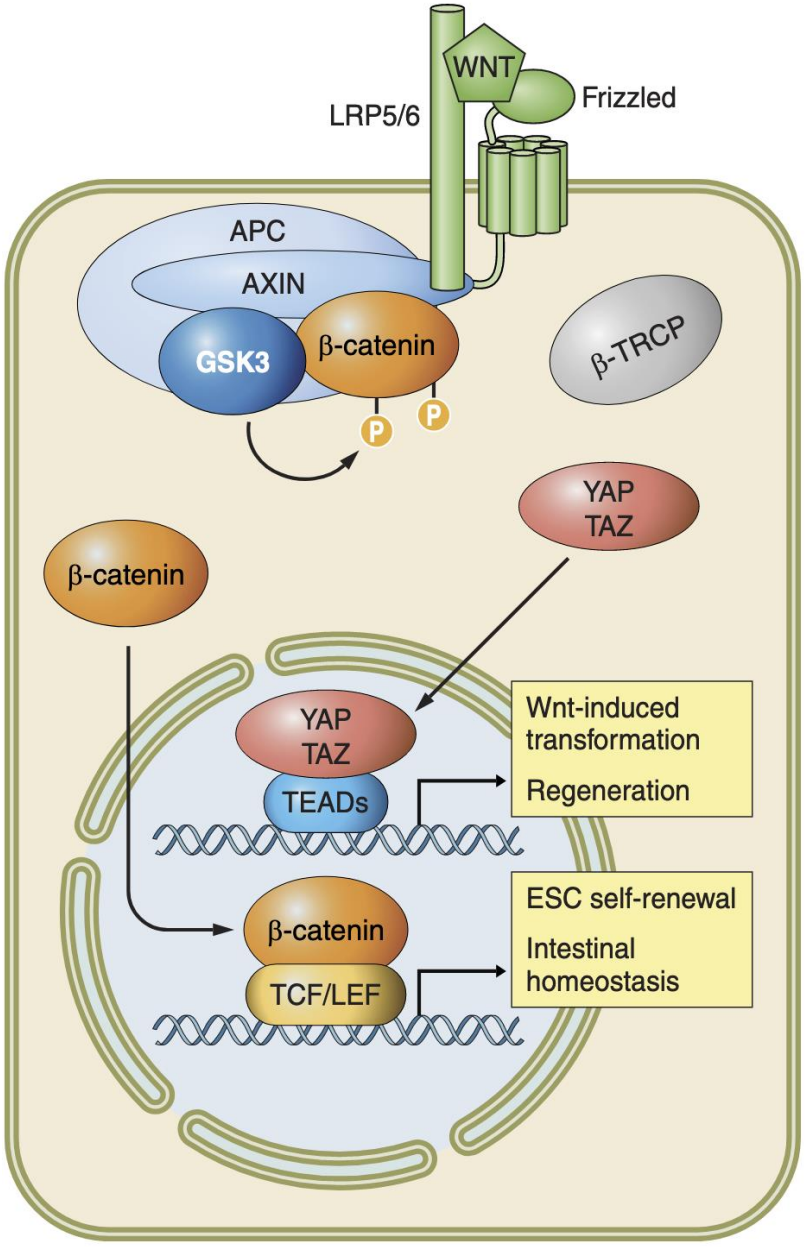
YAP/TAZ are not only messengers of the cell's structural features, but also of Wnts, a leading family of growth factors involved in cell proliferation, stem cell expansion, regeneration, and tumorigenesis (36, 154). Recent work highlighted a deep integration of YAP/TAZ in the Wnt pathway that mechanistically explains the extensive overlaps between Wnt and YAP/TAZ biology

Azzolin et al. (8) discovered that YAP and TAZ are components of the β-catenin destruction complex. The significance of this is twofold: *1*) YAP/TAZ are sequestered in the cytoplasm in the destruction complex, and *2*) cytoplasmic YAP/TAZ associate to Axin and are required for recruitment of β-TrCP to the complex. As such, in "Wnt OFF" cells, YAP/TAZ are critical for β-catenin degradation, and depletion of YAP/TAZ leads to the activation of β-catenin/TCF transcriptional responses

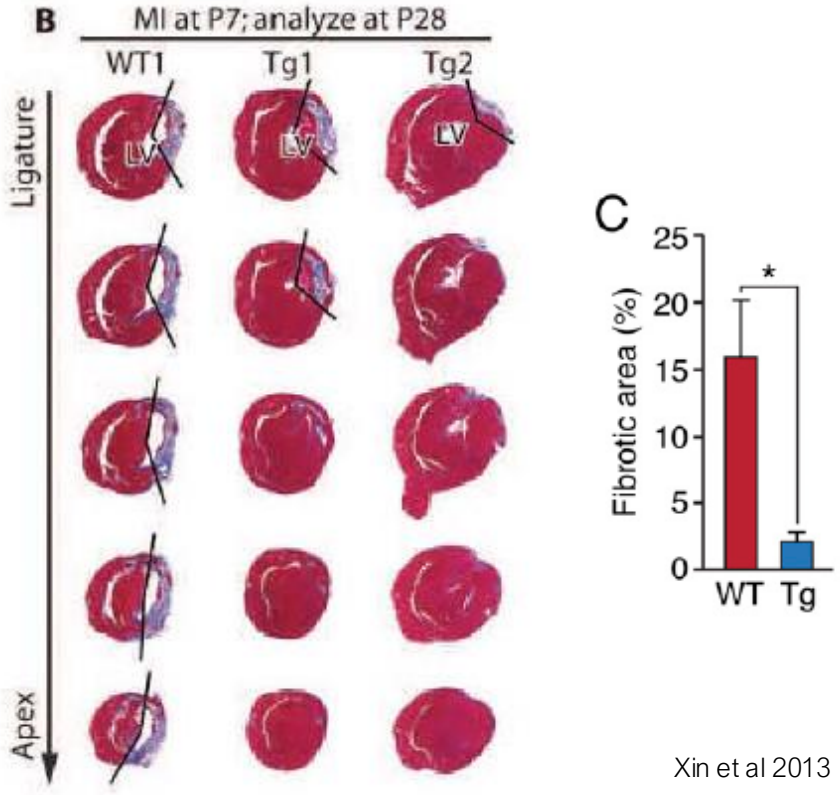


The arrival of a Wnt ligand triggers the association between the Wnt receptor LRP6 and Axin with concomitant release of YAP/TAZ from the destruction complex (8). The consequence of such release is again two-fold: *1*) without YAP/TAZ, the destruction complex is now "invisible" to β-TrCP, favoring β-catenin accumulation; and 2) YAP/TAZ can now accumulate in the nucleus leading to the activation of Wnt-induced, YAP/TAZ-dependent transcriptional responses

As such, YAP/TAZ can serve either as nuclear, transcriptional mediators of Wnt signaling or as antagonists of Wnt/β-catenin signaling in the cytoplasm. Such duality is reinforced by additional regulatory mechanisms: on the one hand, cytoplasmic YAP/TAZ can inhibit β-catenin nuclear entry, and oppose phosphorylation of the Wnt transducer Dvl (94, 206). On the other hand, the destruction complex assembles a phospho-β-catenin/TAZ/β-TrCP association that leads to TAZ (but not YAP) degradation (9). In other words, the presence of YAP/TAZ and phospho-β-catenin in the destruction complex allows β-TrCP recruitment leading to TAZ and β-catenin inhibition. By disassembling that complex, Wnt does not only promote nuclear accumulation of YAP/TAZ but also TAZ stabilization.



The Hippo pathway in the heart



Xin et al 2013

Costitutive expression of YAP in the heart after MI

YAP S112A transgenic mice heart regenerate with an increase of proliferation rather than hypertrophy.

YAP deletion in the heart

Lethal cardiomyopathy