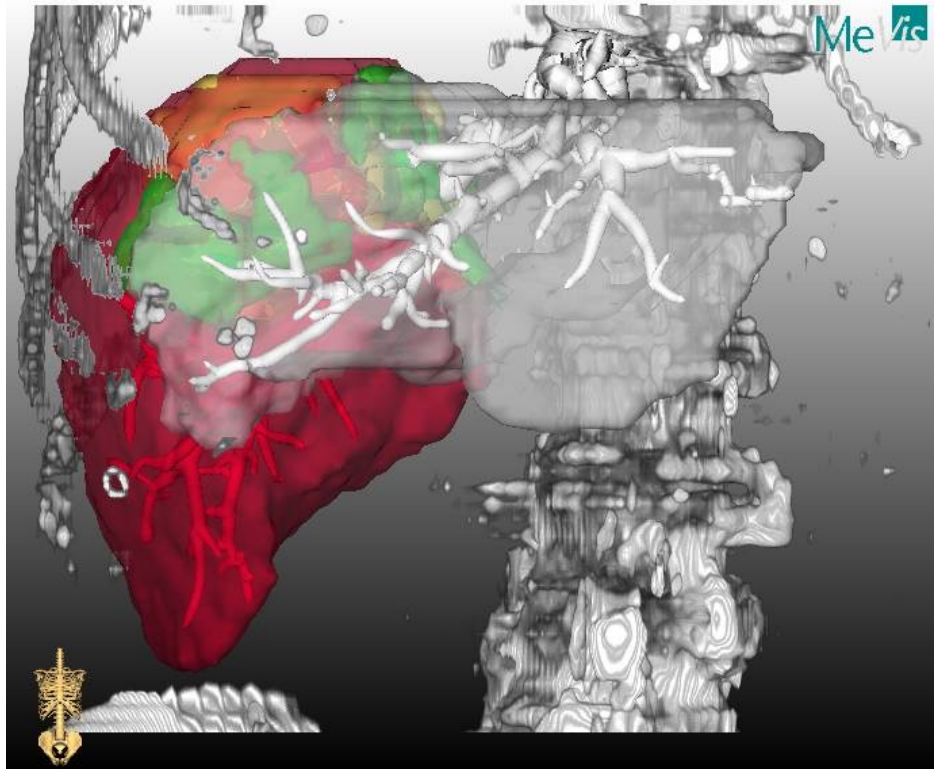
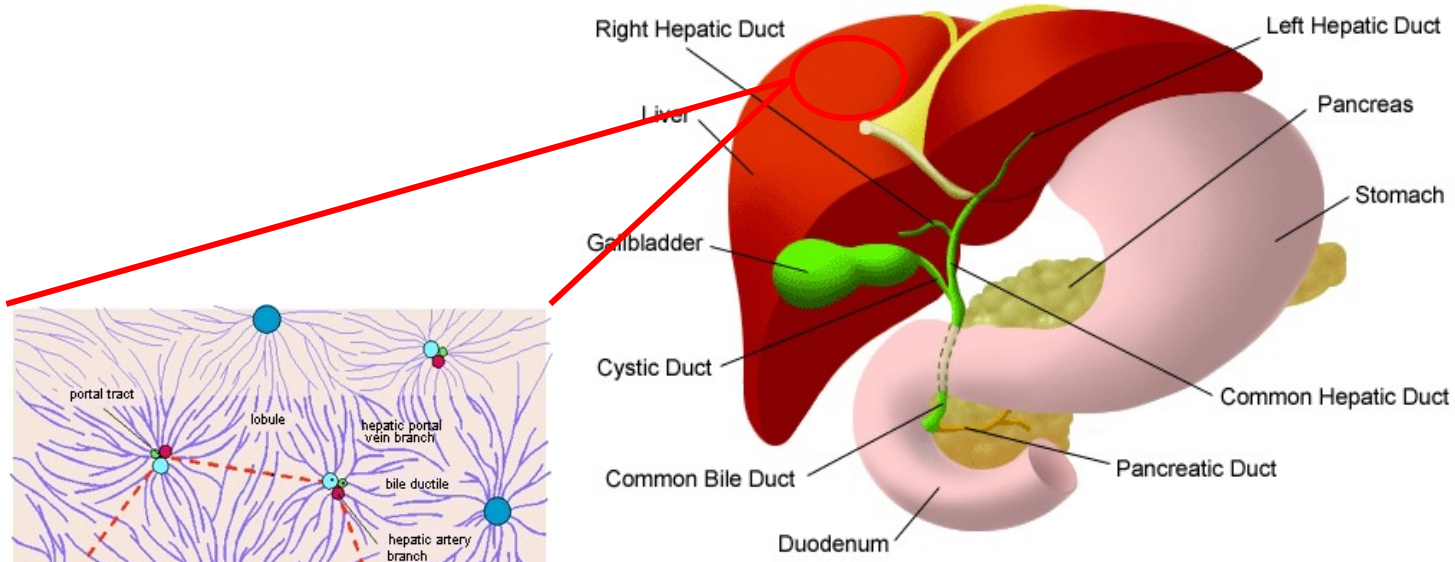
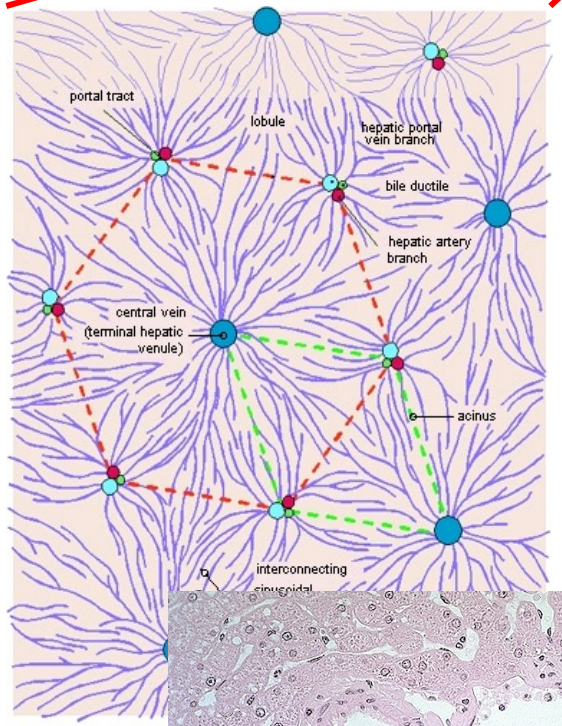
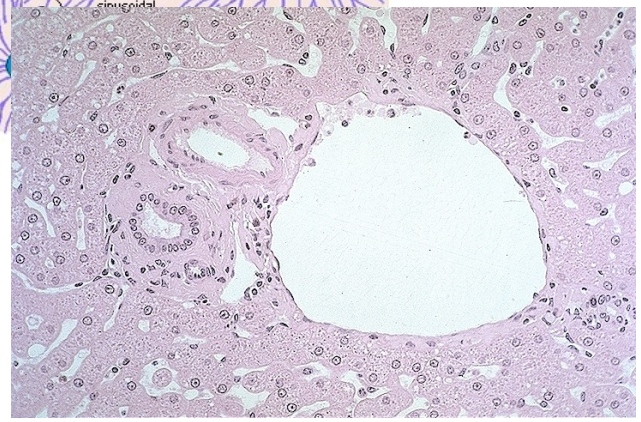
LIVER REGENERATION: FROM MYTH TO MECHANISM









- Il fegato e' irrorato da sangue proveniente dall'intestino (vena porta) e dal cuore (arteria epatica).
- La vena epatica portale e l'arteria epatica si ramificano nel parenchima per dar luogo ad un intimo contatto con gli epatociti.
- Ramificazioni dell'arteria epatica corrono negli spazi portali
- Il sangue portale defluisce nelle vene centrolobulari e di qui si riversa nelle vene intercalari, nella vena epatica che drena nella vena cava inferiore.
- La bile è secreta in *canalicoli biliari,* posti tra epatociti adiacenti, che drenano in dotti collettori negli spazi portali.

LIVER CELL TYPES AND FUNCTIONS

Hepatocytes: Parenchymal cells of the liver that comprise 70% of the liver cells and 90% of the liver volume; organized in single-cell plates; perform metabolic and detoxification function; maintain metabolic function while replicating during liver regeneration; can secrete HGF, IL-6, proteases and protease inhibitors.

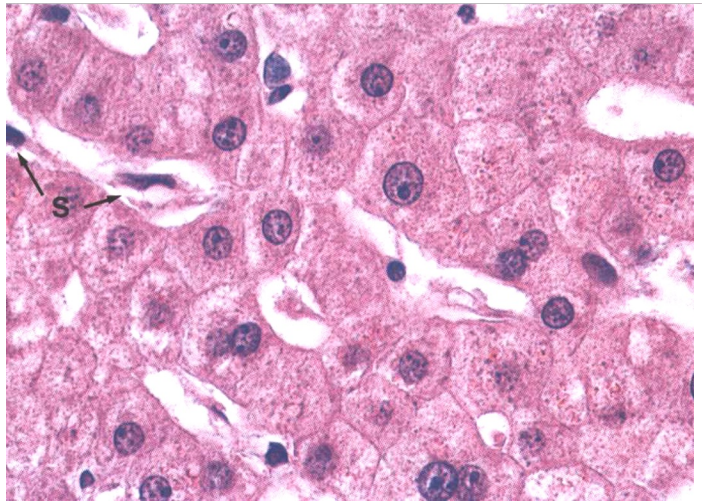
Sinusoidal endothelial cells: Line liver capillaries that separate hepatocytes from sinusoidal blood; pathogenic barrier and general selective barrier; involved in endocytosis and metabolism of molecules including glycoproteins, lipoproteins, ECM components; can produce TGFβ, HGF, IL-6 and nitric oxide.

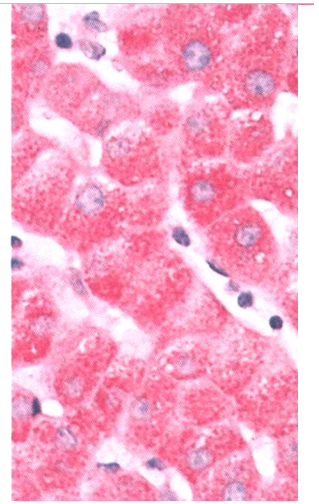
Biliary epithelial cells: Line bile ducts in hepatic portal triads; can secrete cytokines such as monocyte chemotactic protein-1 (MCP-1) and IL-6; promote fibrogenesis by attraction of hepatic stellate cells.

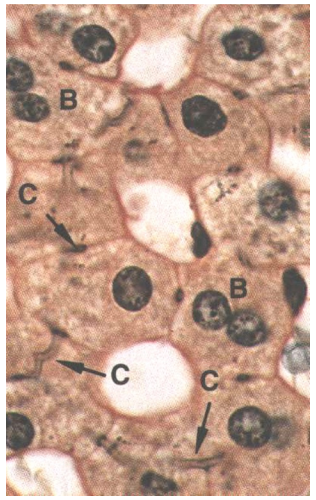
Hepatic stellate cells: Found within the peri-sinusoidal space; store vitamin A, secrete ECM proteins, including laminins, collagens and proteoglycans, growth factors such as HGF, FGF and TGFβ and cytokines such as IL-6; produce some MMPs and TIMPs.

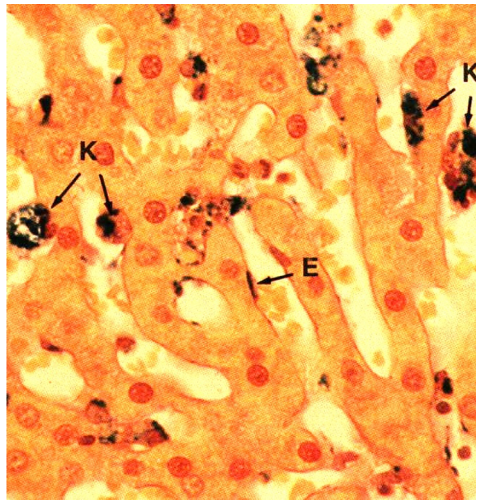
Kupffer cells: Resident liver macrophages found in sinusoids; phagocytosis of foreign particles and bacteria; major producers of cytokines including TNF and IL-6.

Oval cells: Hepatic progenitor cells; able to differentiate towards the biliary and the hepatocytic lineage through intermediate progeny; implicated in liver regeneration and hepatocarcinogenesis.









Higgins, G. M. & Anderson, R. M.

Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal.

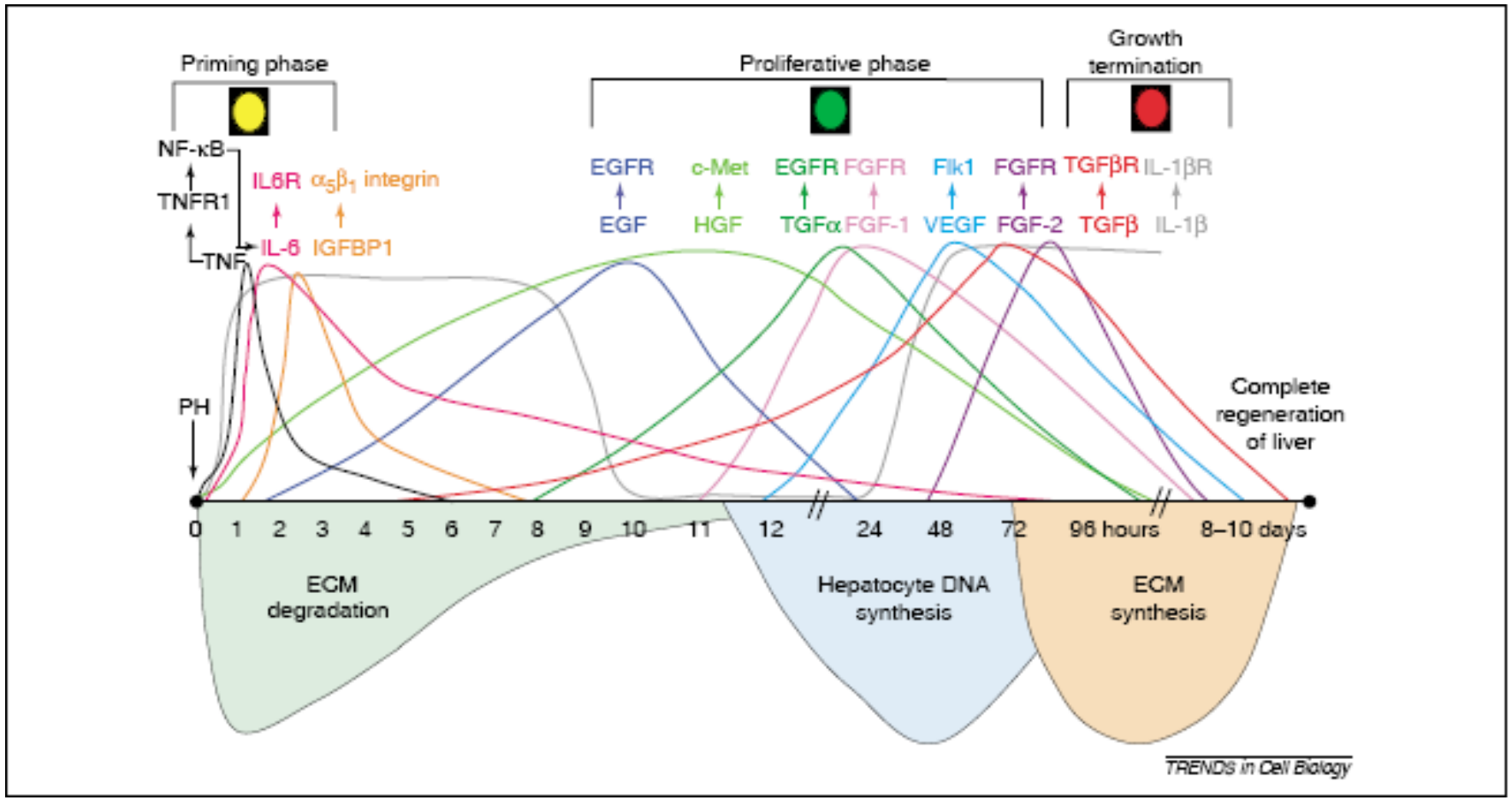
Arch. Pathol. **12**, 186–202 (1931).

In this experimental system there is a hyperplastic response:

liver regeneration does not require the recruitment of liver stem cells or progenitor cells, but involves replication of the mature functioning liver cells.

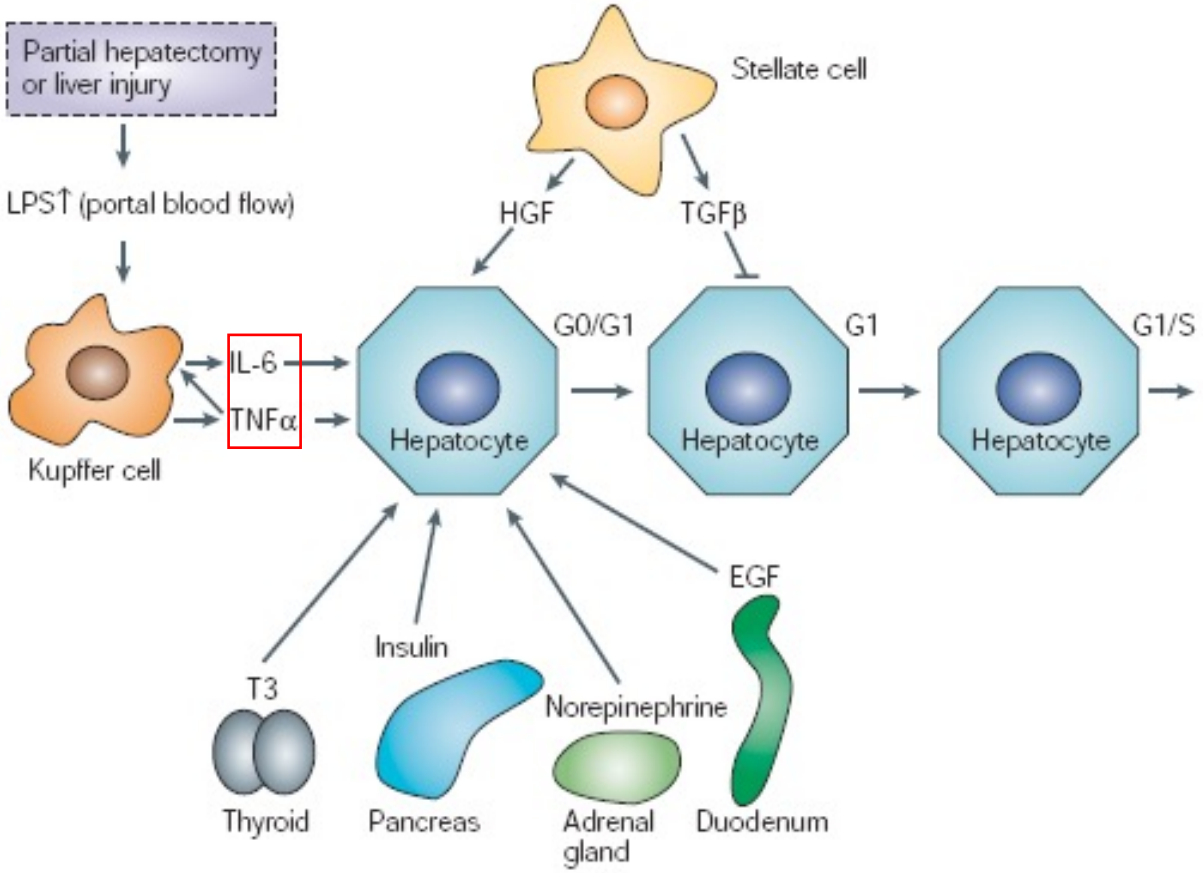
The regenerative process is compensatory: the size of the resultant liver is determined by the demands of the organism, and, once the original mass of the liver has been re-established, proliferation stops.

Liver regeneration: the timely sequence of morphological events



Liver regeneration proceeds along a sequence of distinctive phases:

1. An ***initiation*** or ***priming*** phase, rendering hepatocytes in a state of replicative competence
2. A ***proliferation*** phase, where expansion of the entire population takes place
3. A ***termination*** phase, where cell proliferation is switched off

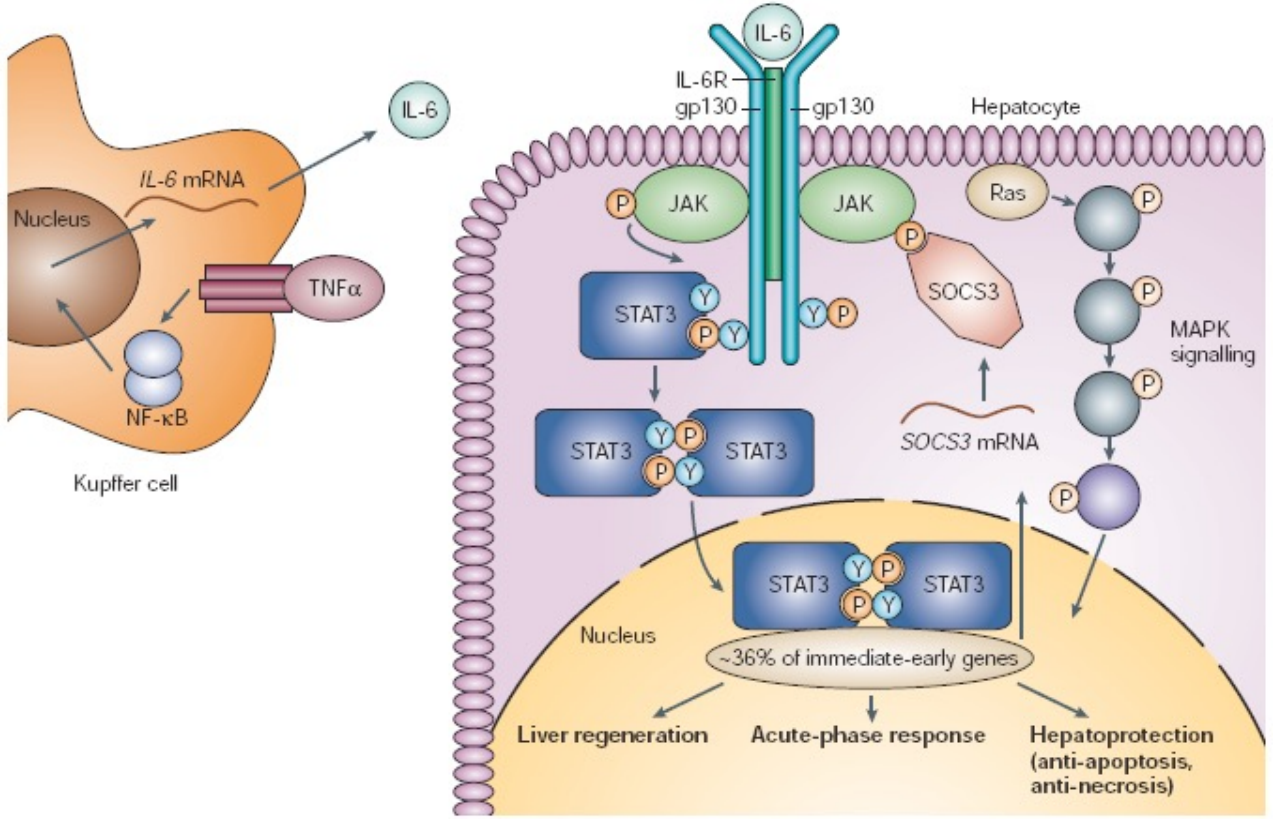


Gut-derived factors, such as lipopolysaccharide (LPS), are upregulated and reach the liver through the portal blood supply. They activate hepatic non-parenchymal cells (including Kupffer cells and stellate cells) and increase the production of tumour necrosis factor TNFα.

Other factors are released from the pancreas (insulin), duodenum (EGF), adrenal gland (norepinepherine), thyroid gland (triodothronine; T3) and stellate cells (HGF).

These cooperative signals allow hepatocytes to move from G0, through G1, to the S phase of the cell cycle. This leads to DNA synthesis and hepatocyte proliferation. TGFβ signalling, which inhibits hepatocyte DNA synthesis, is blocked during the proliferative phase but is restored at the end of the process of regeneration, helping hepatocytes to return to the quiescent state.

1- Initiation phase

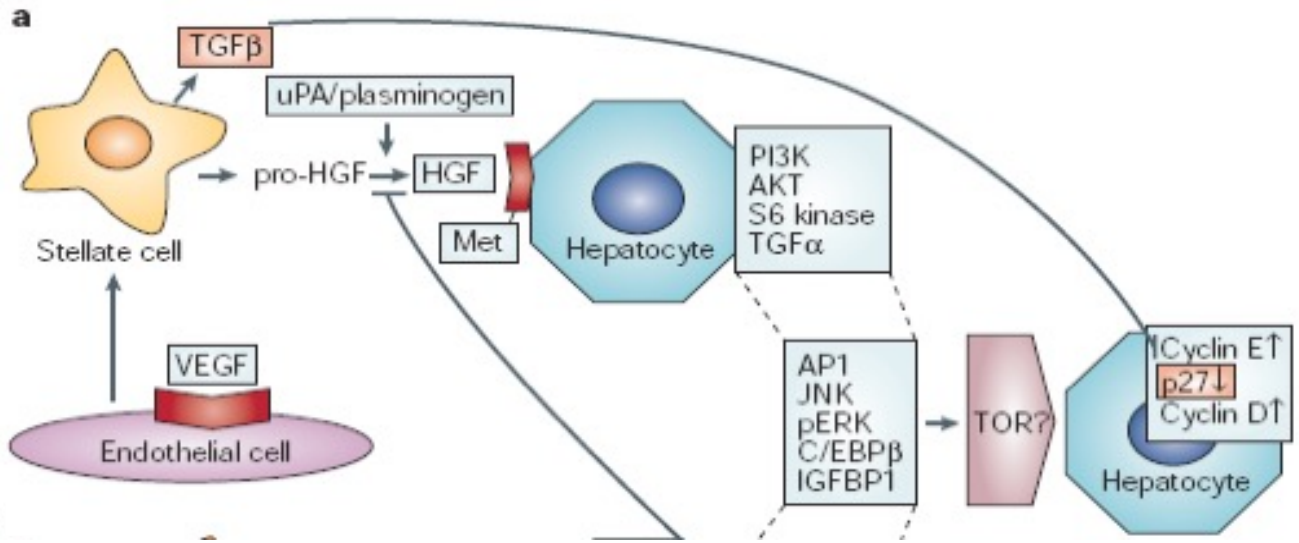


Tumour necrosis factor α binds to its receptor on Kupffer cells, which results in the upregulation of interleukin-6 (*IL-6*) transcription by the nuclear factor (NF)-κB pathway. IL-6 binds to its receptor on hepatocytes and activates Janus kinase (JAK). Activated JAK triggers two pathways:

1) The MAPK pathway, activated by SHP2–GRB2-SOS–Ras signal transduction;

2) The STAT3 pathway, activated through JAK-mediated tyrosine phosphorylation. STAT3 transcription factor activates transcription of ~36% of immediate-early target genes.

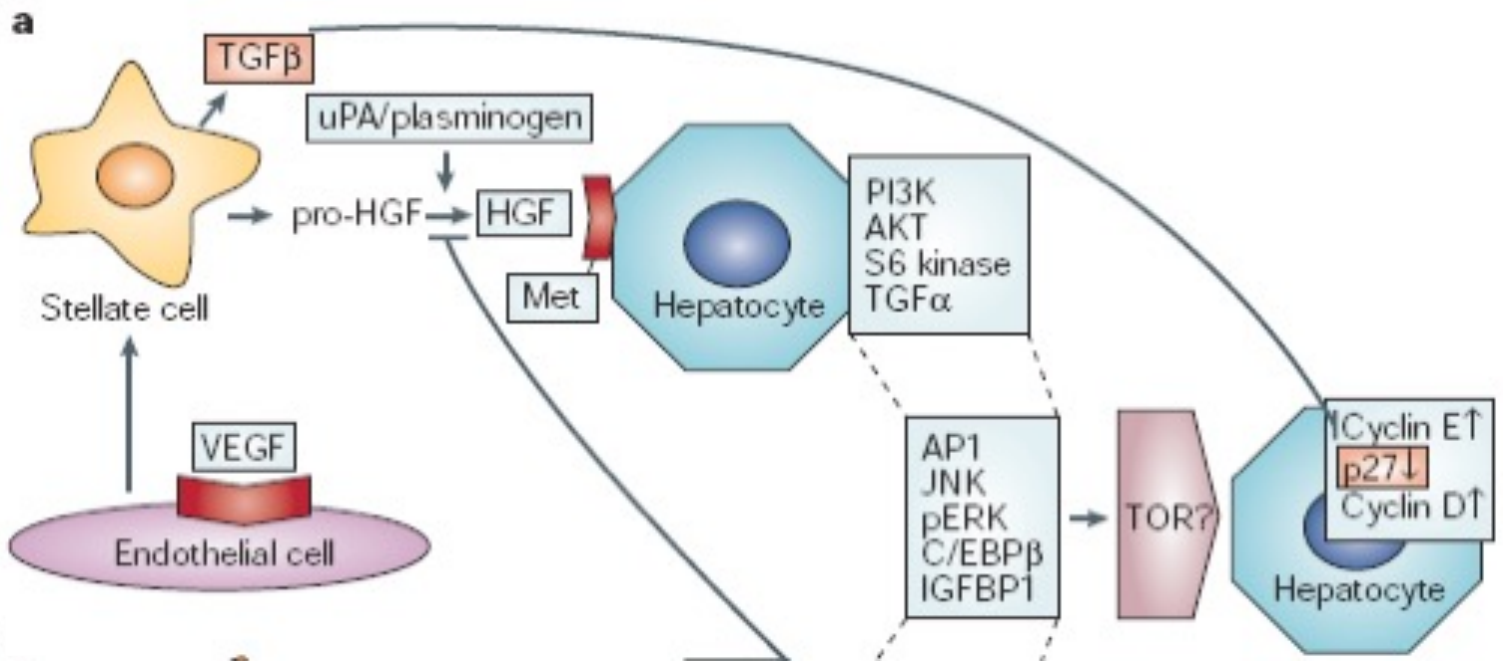
2- Proliferation phase



Progression of primed/competent hepatocytes throught G1 and subsequent replicative cycling is dependent on hepatocyte growth factor (HGF) and Transforming growth factor-α signalling; afterwards the proliferation process proceeds autonomously under the control of cyclins and cyclin-dependent kinases

Vascular endothelial growth factor (VEGF) binds to endothelial cells, triggering the release of the HGF precursor, pro-HGF, from stellate cells. The urokinase-type plasminogen activator (uPA) and plasminogen proteases cleave pro-HGF to HGF. HGF binds to the Met receptor on hepatocytes to activate the phosphatidylinositol 3-kinase (PI3K), AKT and S6 kinase signal-transduction pathways. HGF signalling releases transforming growth factor (TGF)α and triggers other downstream signals such as AP1, Jun amino-terminal kinase (JNK), phosphorylated extracellular signal-regulated kinases (pERKs), CCAAT-enhancer-binding protein (C/EBP)β and insulin-like-growth-factorbinding protein (IGFBP)1. These factors lead to cell-cycle transition by increasing the expression of cyclins D and E and reducing p27 levels.

3- Termination phase



Subsequent to the expansion phase, the growth response must be terminated: major factors involved in this step are members of the TGFβ superfamily, which includes TGFβ1, 2 and 3, activins and inhibins, all signaling through TGFβ receptors. They regulate hepatic mass, inhibit DNA synthesis in hepatocytes and induce apoptosis via a c-Jun dependent mechanism.

WHAT ABOUT THE OTHER CELL TYPES??

…in liver regeneration and repair

Hepatocytes are themselves the functional stem cells of the liver.

More severe liver injury can activate a potential stem cell compartment located within the intrahepatic biliary tree, giving rise to cords of bipotential transit amplifying cells (oval cells), that can ultimately differentiate into hepatocytes and biliary epithelial cells.

Table 1. Origin of Hepatocytes in Liver Regeneration and Repair

Growth processes that depend of the replication of differentiated hepatocytes
Liver regeneration after partial hepatectomy[2]
Hepatocyte regeneration after carbon tetrachloride and acetaminophen (centrolobular) injury[131]
Conditions in which oval cells proliferate and generate hepatocytes
Experimental
Injury caused by galactosamine[132]
Choline-deficient diet combined with ethionine or AAF[133,134]
Partial hepatectomy combined with AAF or Dipin[135,136]
Carbon tetrachloride combined with AAF[137]
3,5-dietoxycarbonyl-1-1, 4-dihydrocollidine (DCC)[138]
Allyl alcohol[9]
Human disease
Atypical ductular reactions in advanced stages of cirrhosis of various etiologies
Fatty liver disease
Small cell dysplasias
Massive hepatocyte necrosis[17,52,68,139]
Conditions in which small hepatocyte precursor cells (SHPC) represent a large fraction of the proliferating cells
Injury caused by retrorsine[61,62] and galactosamine[63]

Abbreviation: AAF, N-2-acetylaminofluorene.
NOTE. Only representative publications are listed.

What then is the evidence that the adult liver has any stem cells at all?

The only general principle underlying oval cell activation is liver injury combined with an inability of hepatocytes to divide in response to the damage.

OVAL CELLS

Analysis of expression markers suggests that proliferating oval cells constitute a heterogeneous cell compartment containing cells that may differ in their differentiation capacity and stage of differentiation. Some of these cells may function as hepatocyte progenitors (expressing AFP and albumin), whereas others may be indistinguishable from cholangiocytes (expressing cytokeratins 7 and 19). Oval cells also express markers of ematopoietic stem cells. Among these are Thy-1, CD34, CD45, Sca-1, c-Kit, and flt-3.

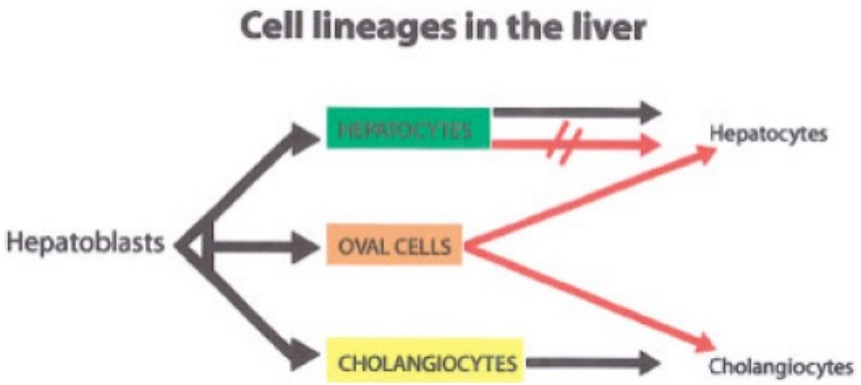


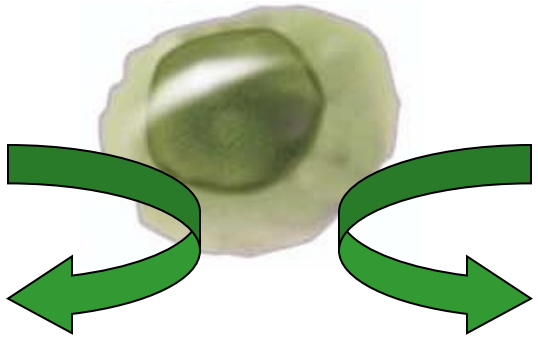
Table 1. Marker genes commonly used to identify oval cells in adult liver

Marker	Oval cells	Hepatocytes	Bile duct cells	Refs.
Albumin	+	+	–	86,87
AFP	+	Fetal	+	86–88
π-GST	+	Fetal	–	89
M2-PK	+	Fetal	+	90
CK7	+	–	+	91
CK8	+	+	+	91,92
CK14	+/–	–	–	93
CK18	+	+	–	91
CK19	+	–	+	91,92
OV-6	+	–	+	94
A6	+	–	+	95
Thy-1	+	–	–	82
c-kit	+	–	–	96
SCF	+	–	–	96
Sca-1	+	–	–	97
Dlk	+	–	–	98

AFP, alpha-fetoprotein; GST, glutathione s transferase; PK, pyruvate kinase; CK, cytokeratin; SCF, stem cell factor.

1. They are scarce in healthy liver, appearing near the portal triad, adjacent to the terminal ducts of the biliary tree, during chronic liver injury,
2. They express markers in common with bile duct cells, fetal and adult hepatocytes
3. They are basophilic, possess ovoid nuclei and scant cytoplasm, and are substantially smaller than adult hepatocytes (approximately 10μm in diameter, versus 50 for hepatocytes),
4. They are immature, and possess a high turnover rate and proliferative capacity
5. They are involved in the regeneration of liver following injury, but are also a cellular precursor to hepatocellular carcinoma (HCC).

OVAL CELLS



Dr Jekyll

During liver regeneration, oval cells give rise to both hepatocytes and bile duct cells. This pathway can be viewed as a 'second line of defence' following liver injury, when hepatocyte-mediated repair cannot be accomplished.

Mr Hyde

Years of experimental evidences have shown a second role for these cells during liver injury: carcinogenesis. The flip-side of regenerative oval cell proliferation is the increased probability of liver tumour formation.

Unanswered questions:

- how does the regenerating liver stop proliferating when appropriate mass is restored?

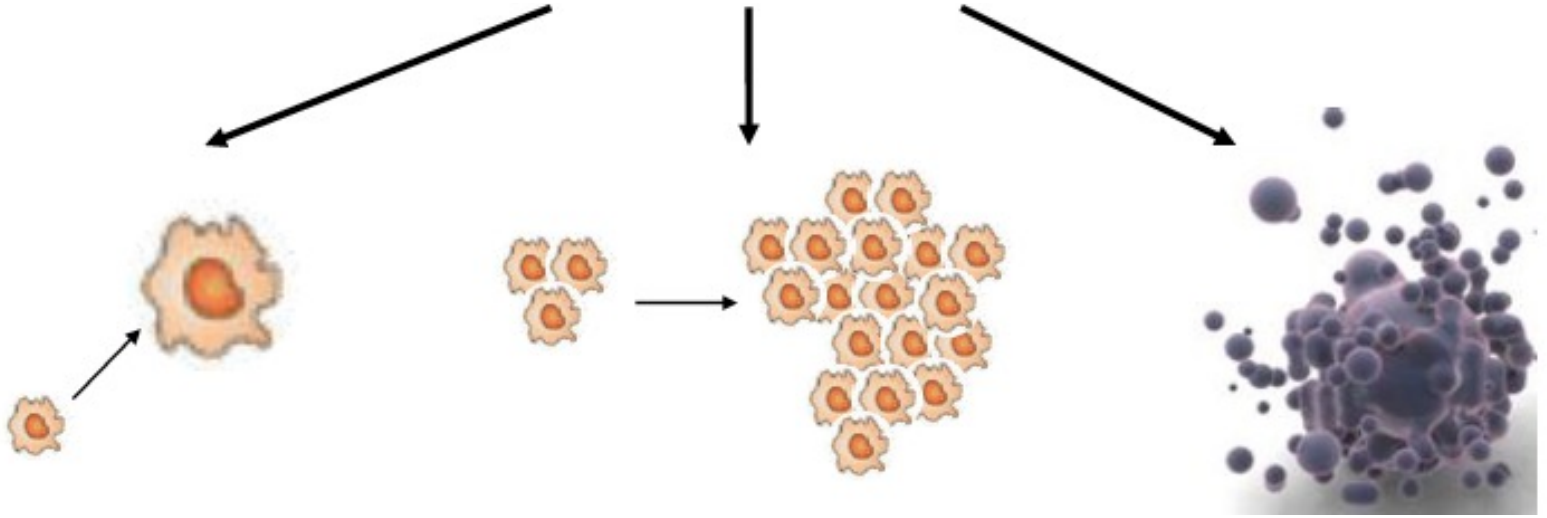
- how do these mechanisms relate to normal regulation of organ size during development?

Organ size control

Most organs have an intrinsic genetic program regulating the final size to be achieved during the embryonic development

In case of damage, the size genetic program is reactivated, therefore the regenerated organ maintains its original size

How is the organ size regulated?

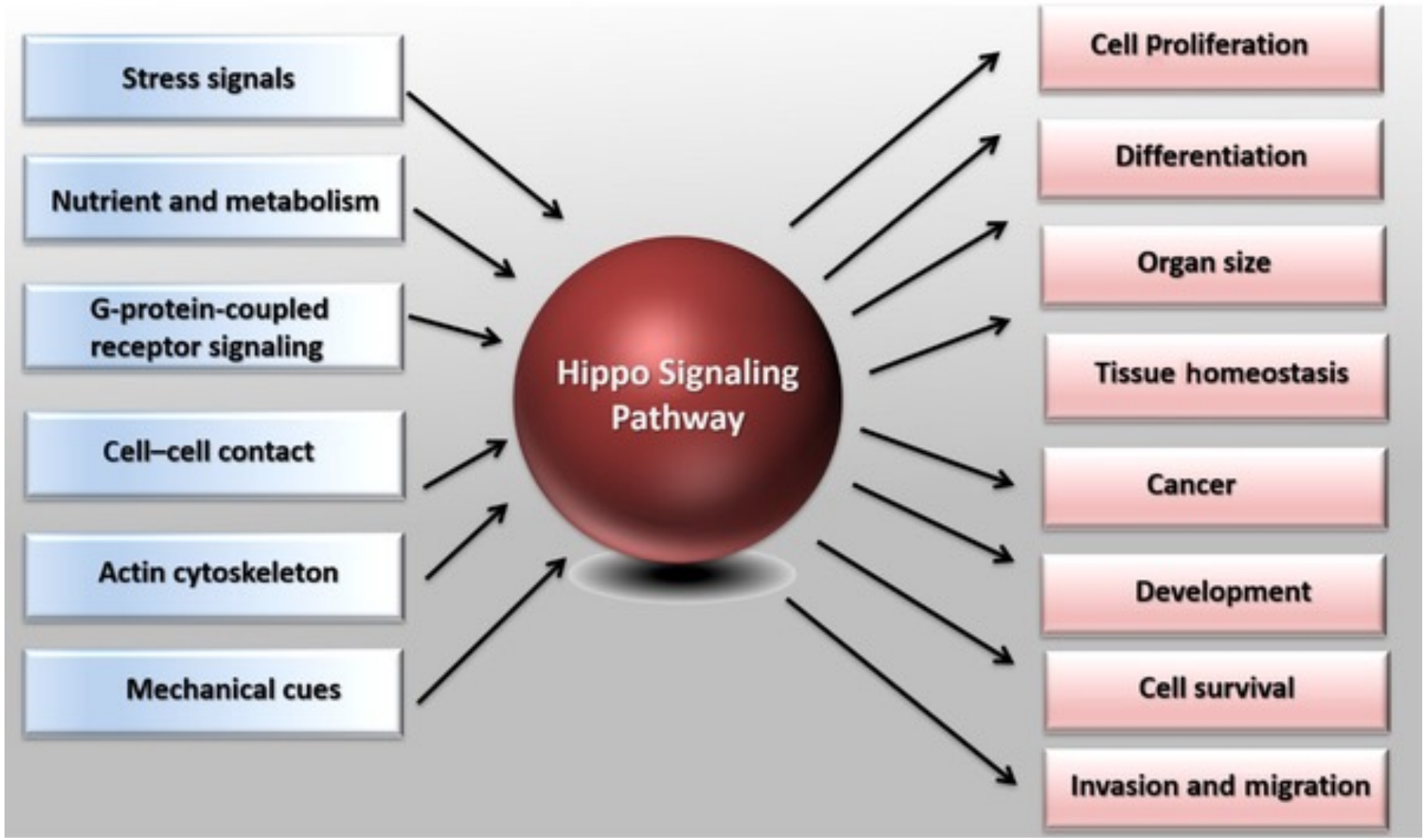


Hypertrophic growth

Proliferation

Apoptosis

Hippo/YAP Signaling Pathway

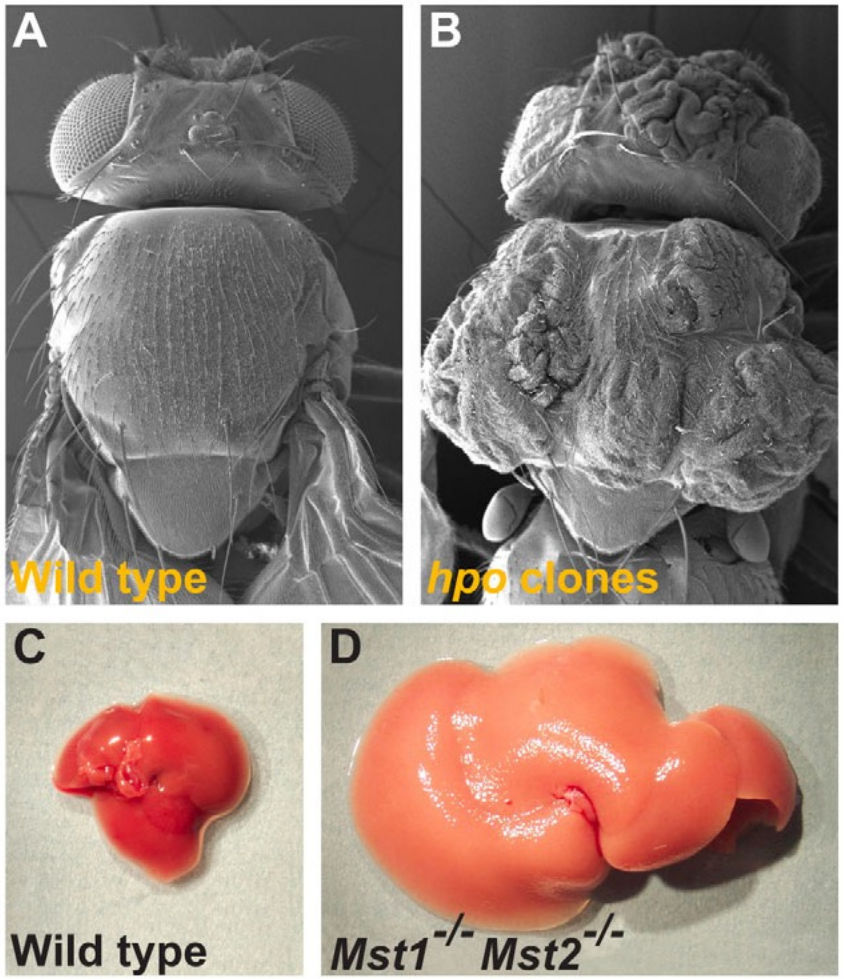


REVIEW

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

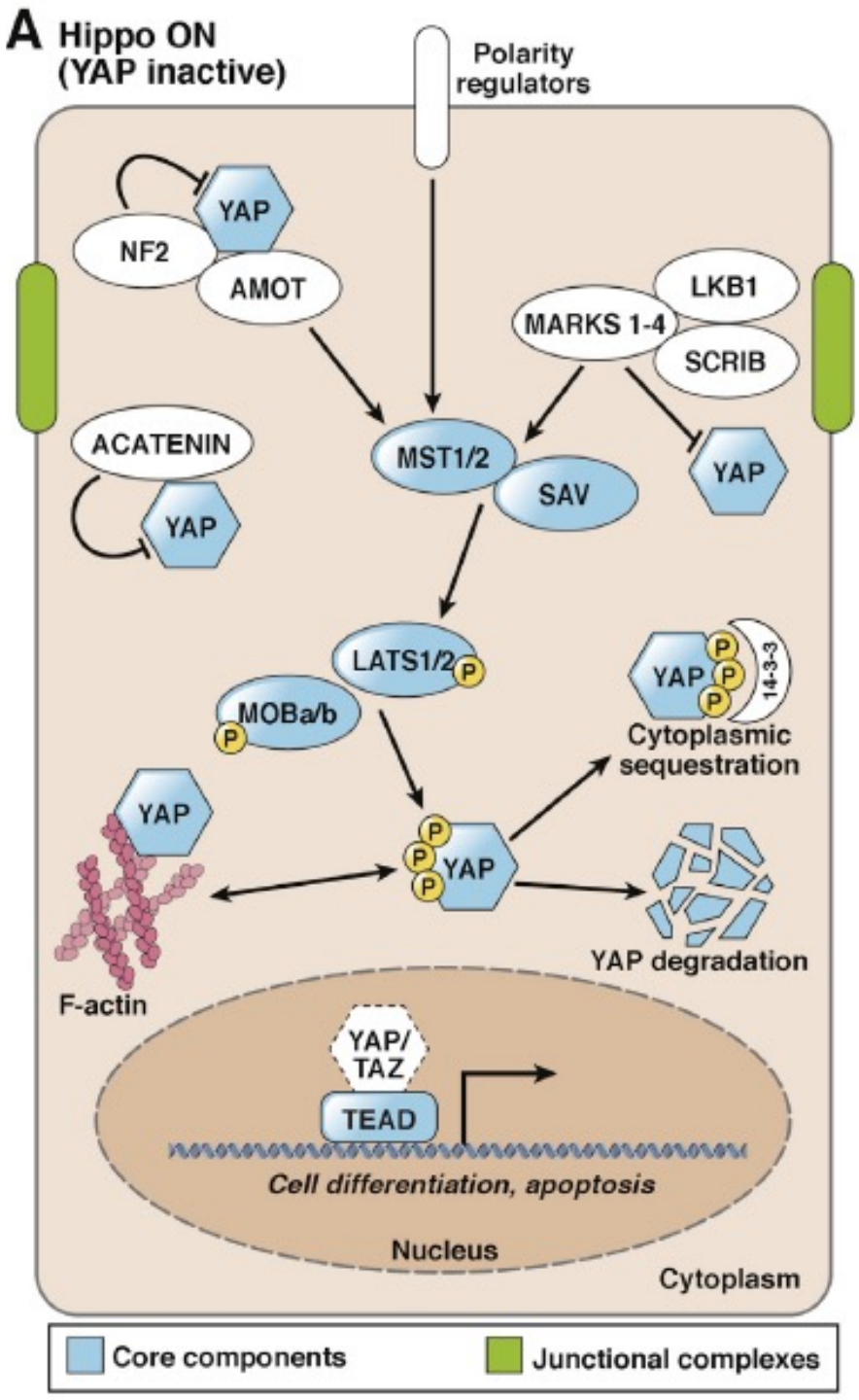
Hippo signaling: growth control and beyond

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)

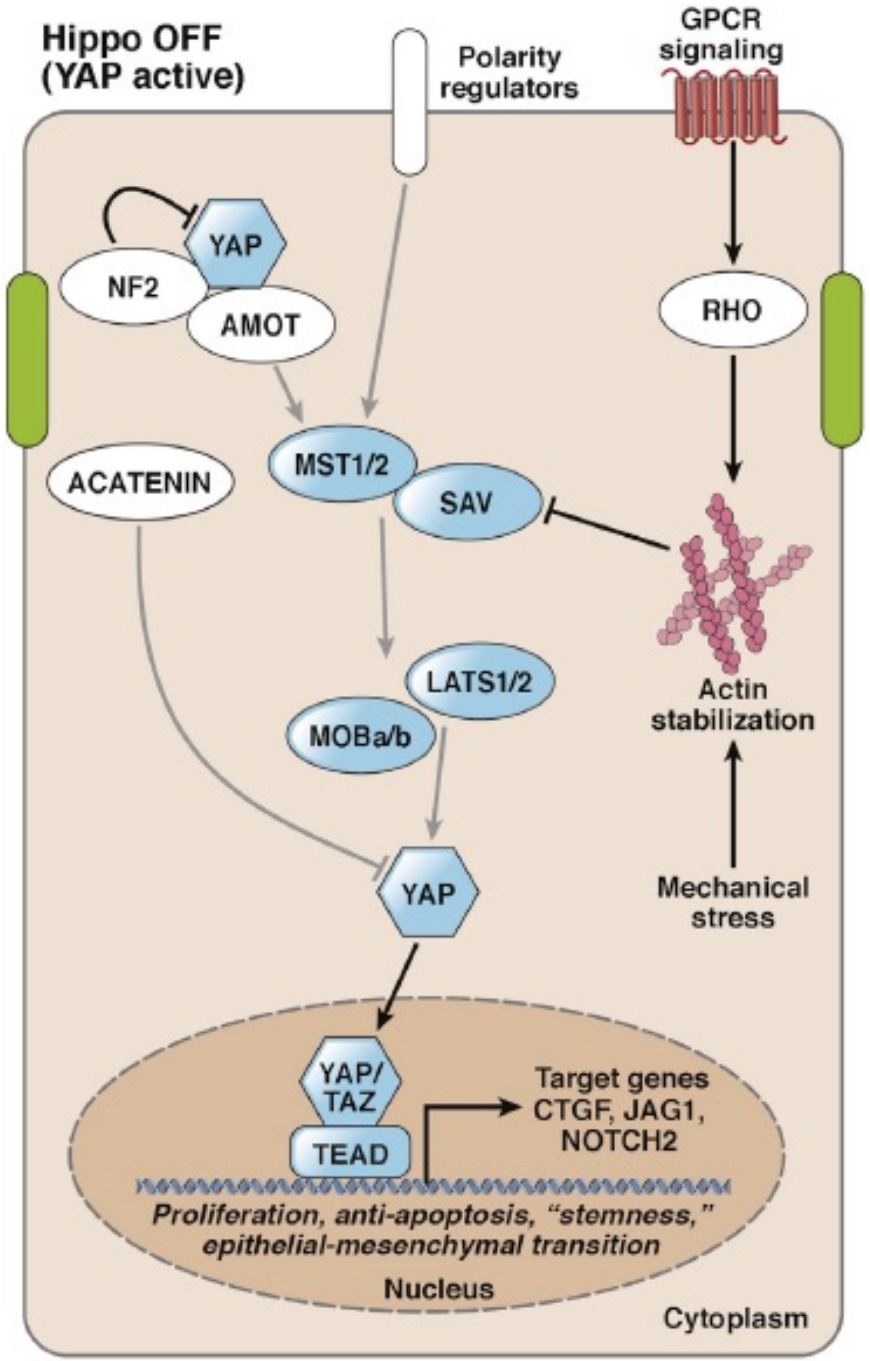


The Hippo pathway (mammalian) consists of the core components MST1 and MST2, SAV, LATS1 and 2, MOB1A and B, YAP, TAZ, and TEAD.

Upon activation of the **canonical** Hippo pathway, MST1/2 phosphorylates and activates LATS, which subsequently phosphorylates cytoplasmic YAP.

During homeostasis, Hippo signaling is on resulting in YAP phosphorylation (S112 in mice, S127 in humans) causing 14-3-3 binding and cytoplasmic sequestration.

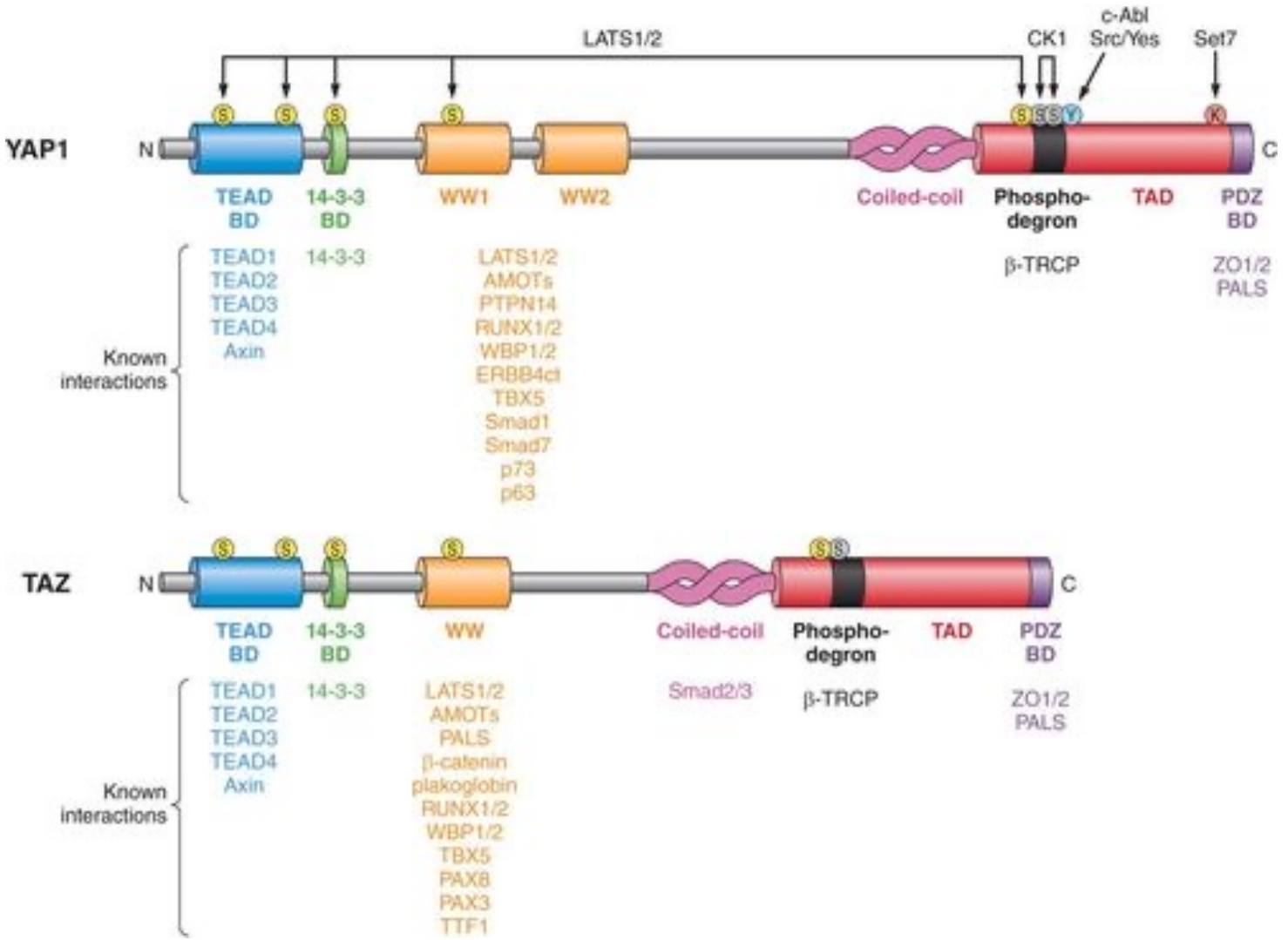
Phosphorylation of YAP can also lead to proteasomal degradation.



When Hippo is **OFF**, YAP (in the unphosphorylated form) 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.

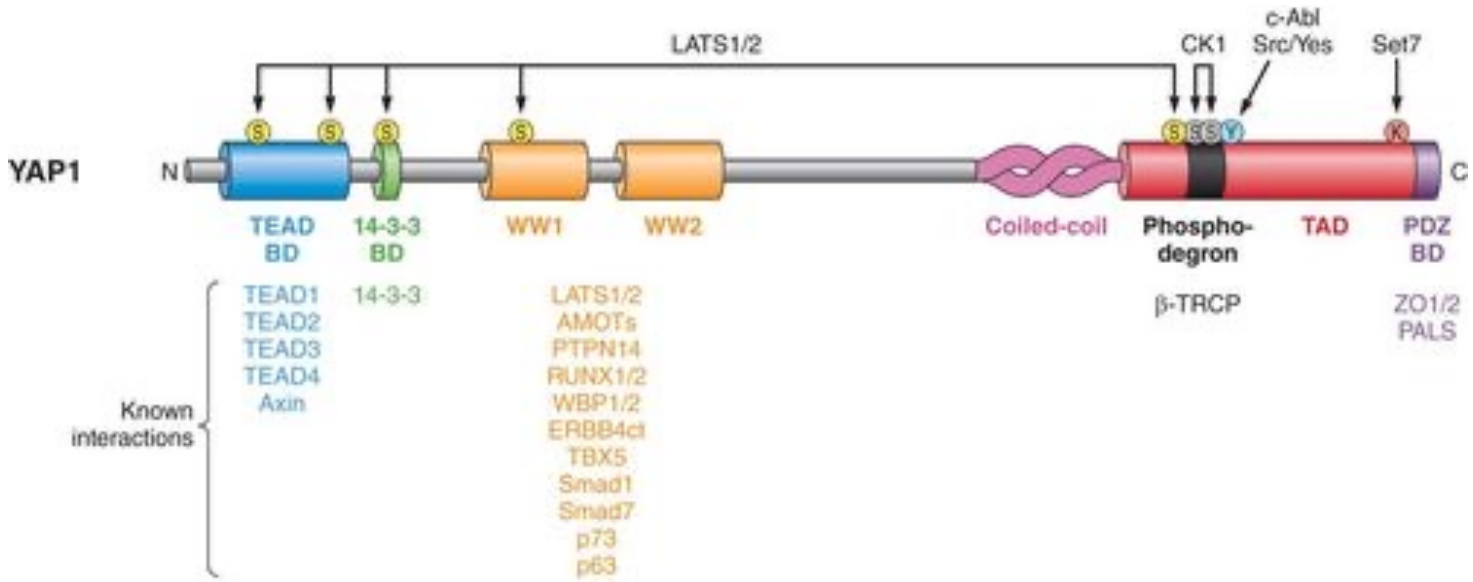
YAP and TAZ

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 yellow, 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.

Regulatory domains of the Hippo pathway effectors TAZ/YAP.



Apart from serine phosphorylation by LATS and other kinases such as AKT and JNK (14, 42), YAP1 can be also targeted by tyrosine phosphorylation triggered by the Yes/Src and c-Abl kinases (117, 180). In these cases, however, tyrosine phosphorylation is a positive trigger of YAP1 activity; for example, Src-mediated phosphorylation is essential for growth of colon cancer cells. Moreover, YAP1 is a

YAP conservation through evolution

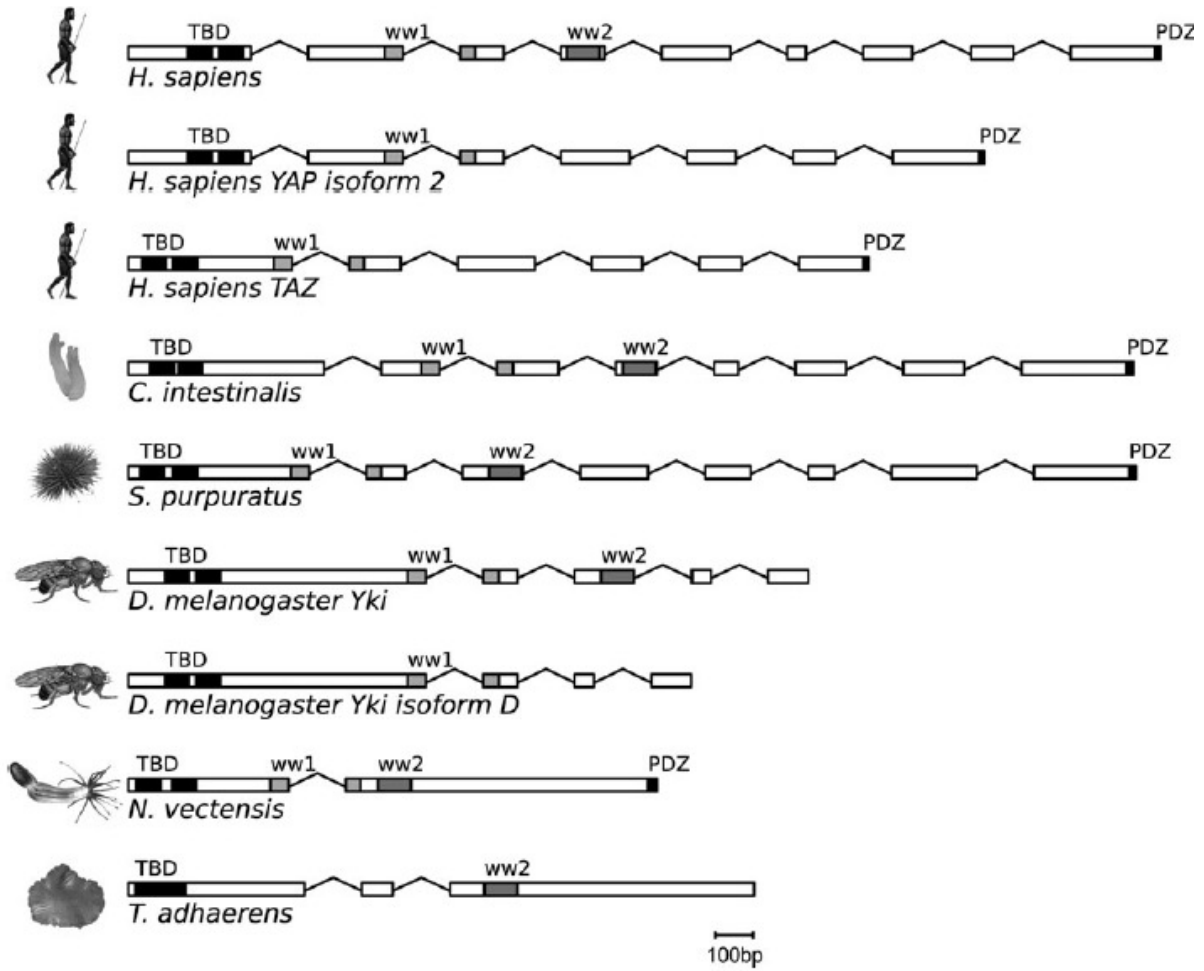
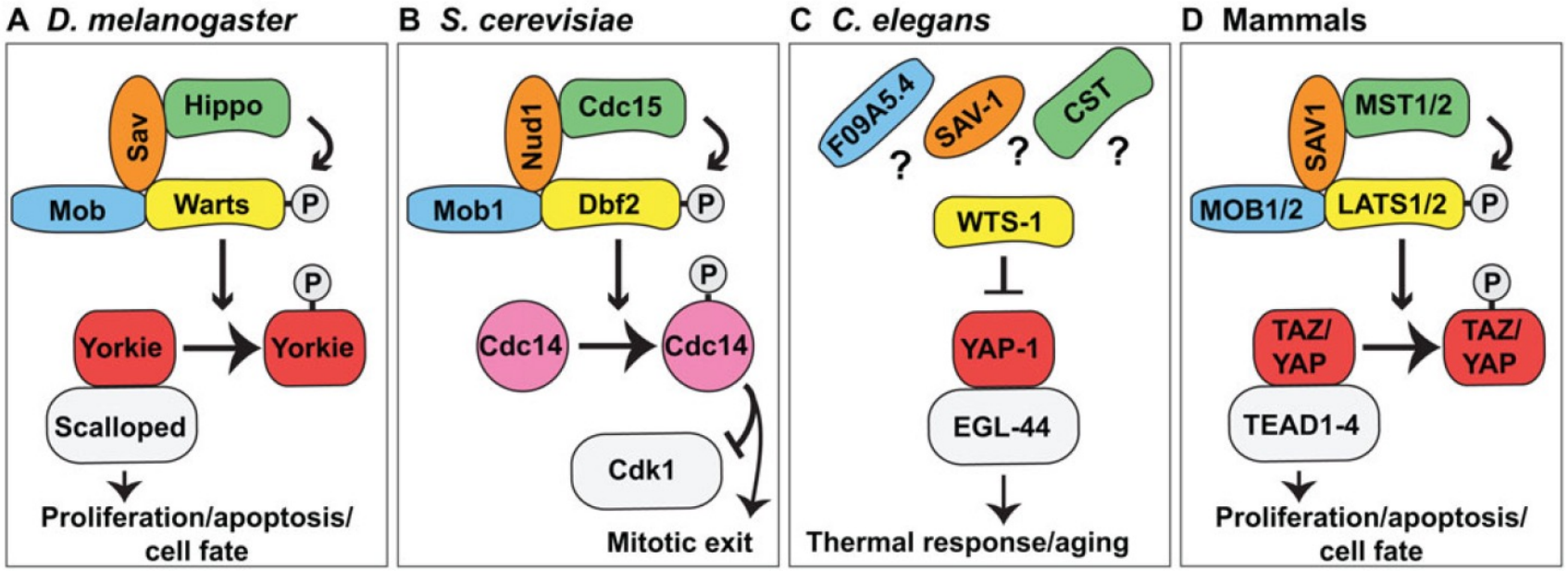


FIG. 4. Genomic structure of YAP in representative metazoans. Major domains are marked with rectangles. The transcript coding region is drawn to scale and introns positions are illustrated but their size is not drawn to scale.

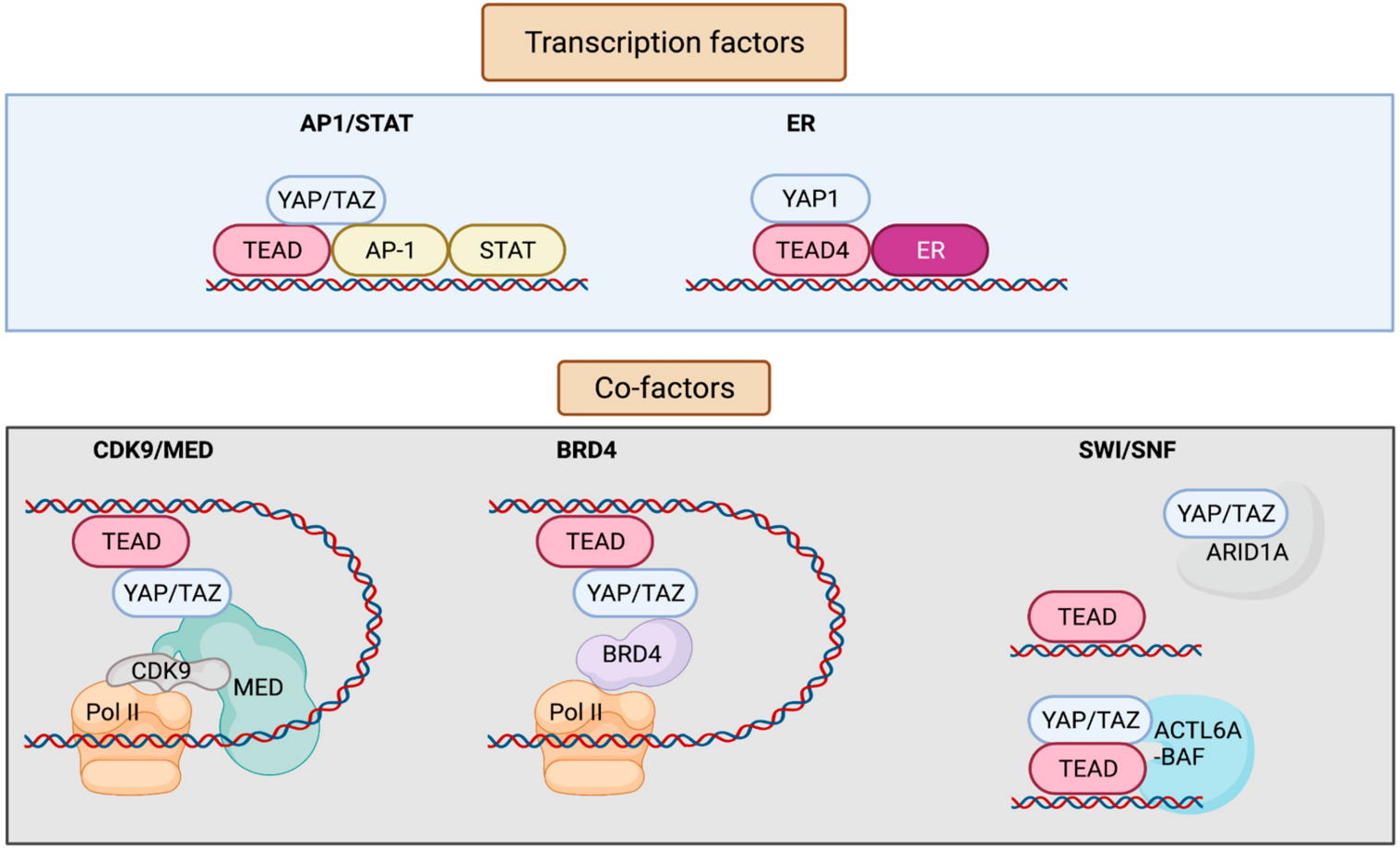
The Hippo pathway is quite conserved throughout evolution



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.



TEAD TFs (TEAD1-4) are evolutionarily conserved from Drosophila to humans. They regulate developmental processes transcending a wide variety of tissue types, from the formation of neural tubes to the development of heart, brain, and skeletal muscle.

Several chromatin immunoprecipitation (ChIP) studies reveal that TEADs serve as the primary effectors of the YAP/TAZ signaling: (1) 78% of the TEAD4-bound promoters and enhancers are co-occupied by YAP/TAZ.

TEADs bind to the DNA but are barely known to exert any transcriptional activity by themselves. They form complexes with multiple TFs, coactivators, and chromatin remodelers to regulate gene expression in diseases and cancers.

They are also not known to harbor any oncogenic mutations.

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

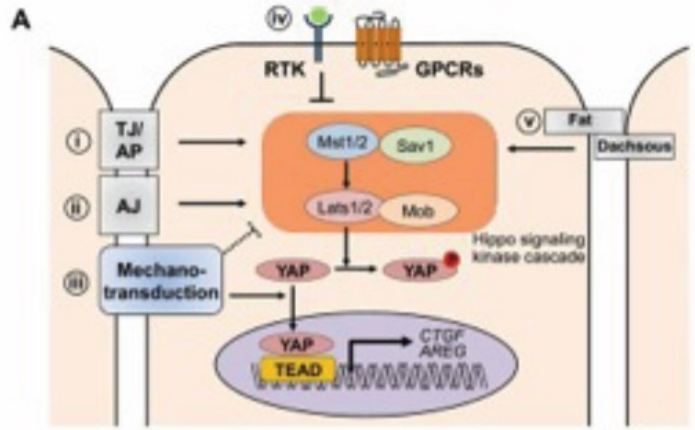
Hippo Pathway plays a key role in lots of different cellular processes

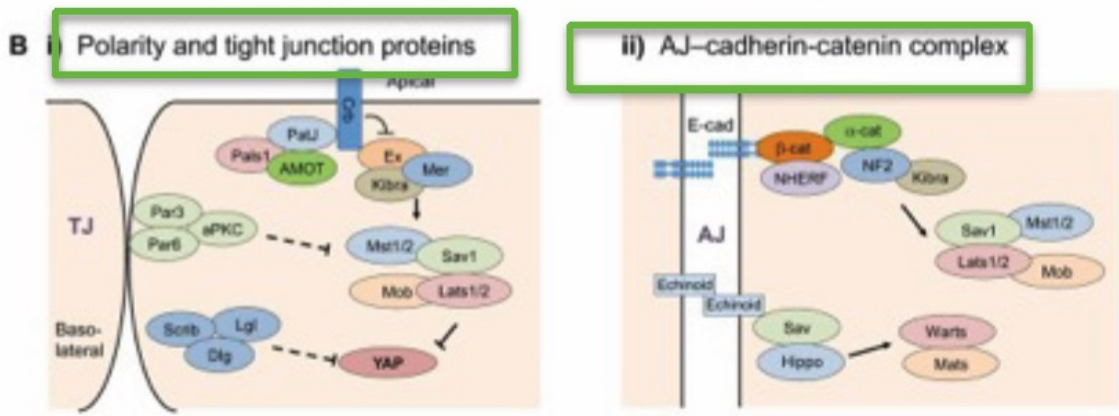
GPCR mediated signaling

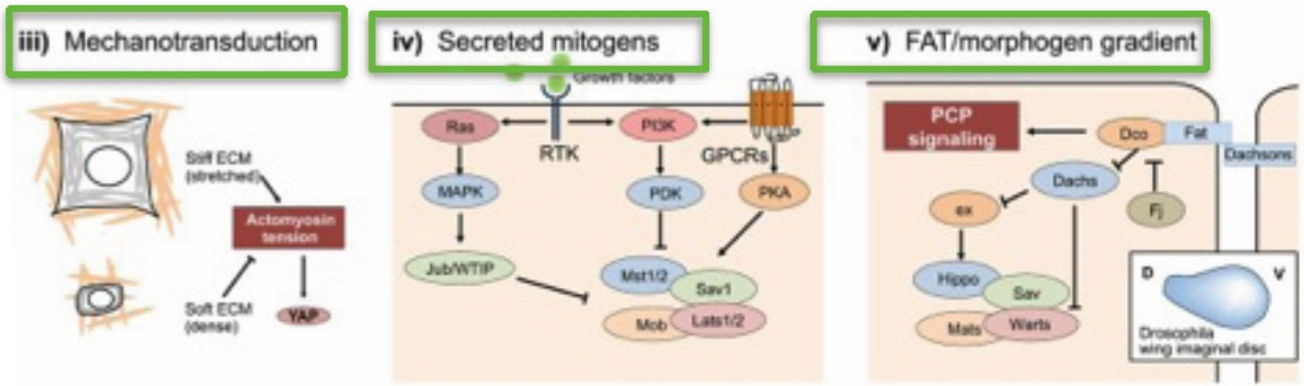
external signals: hormones and growth factors

cell-cell contact
cell polarity

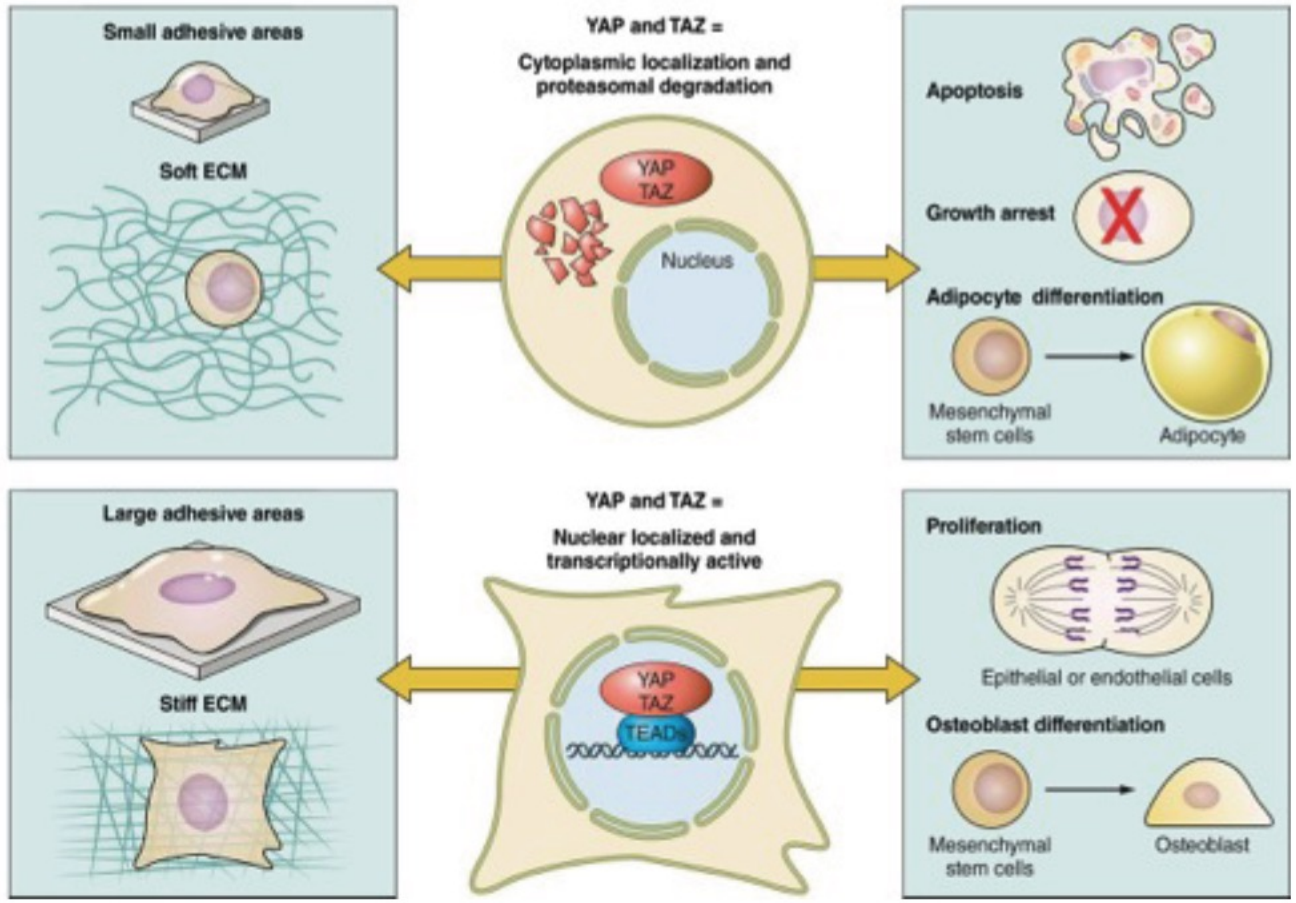
external signals: ECM, secreted factors



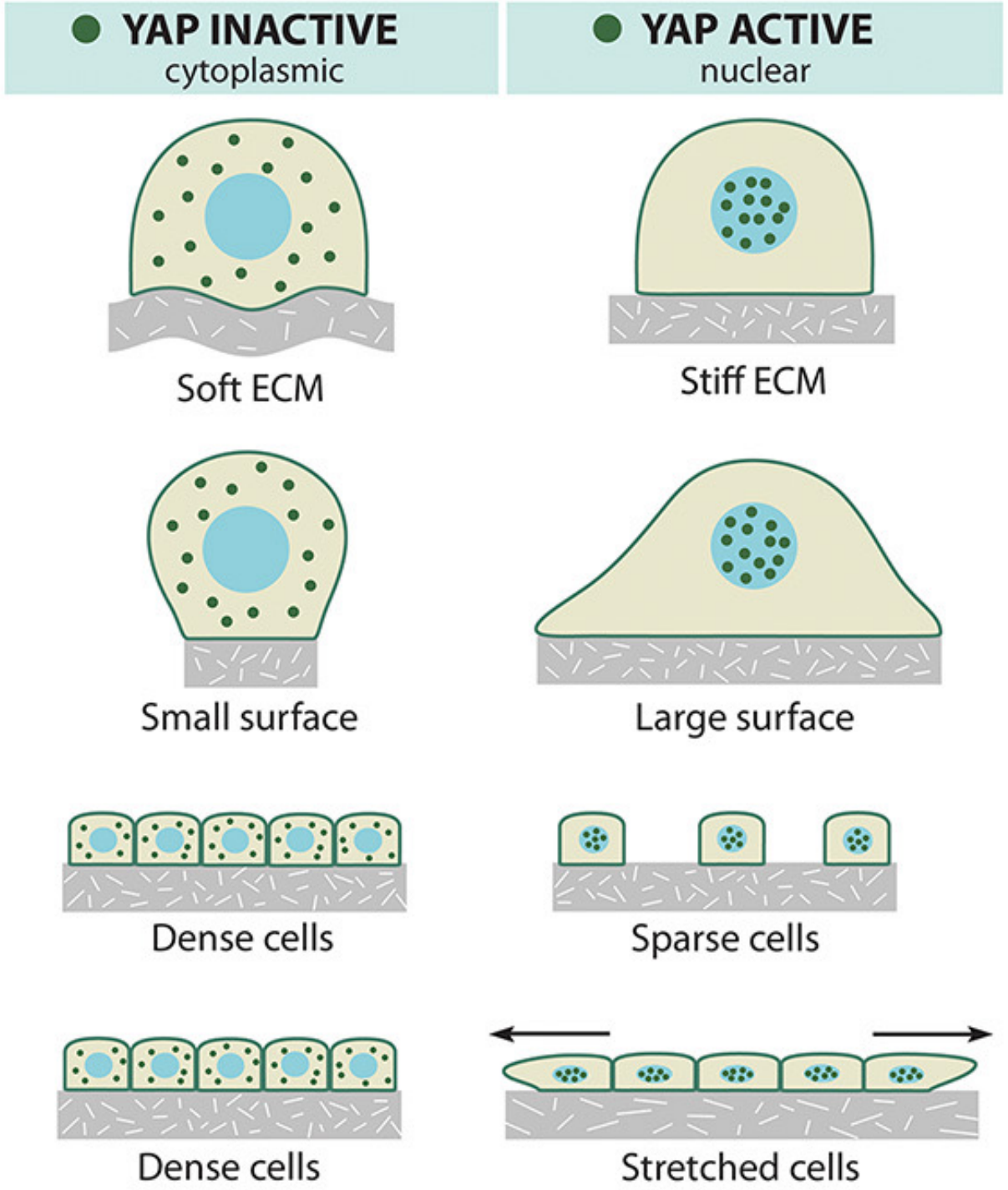




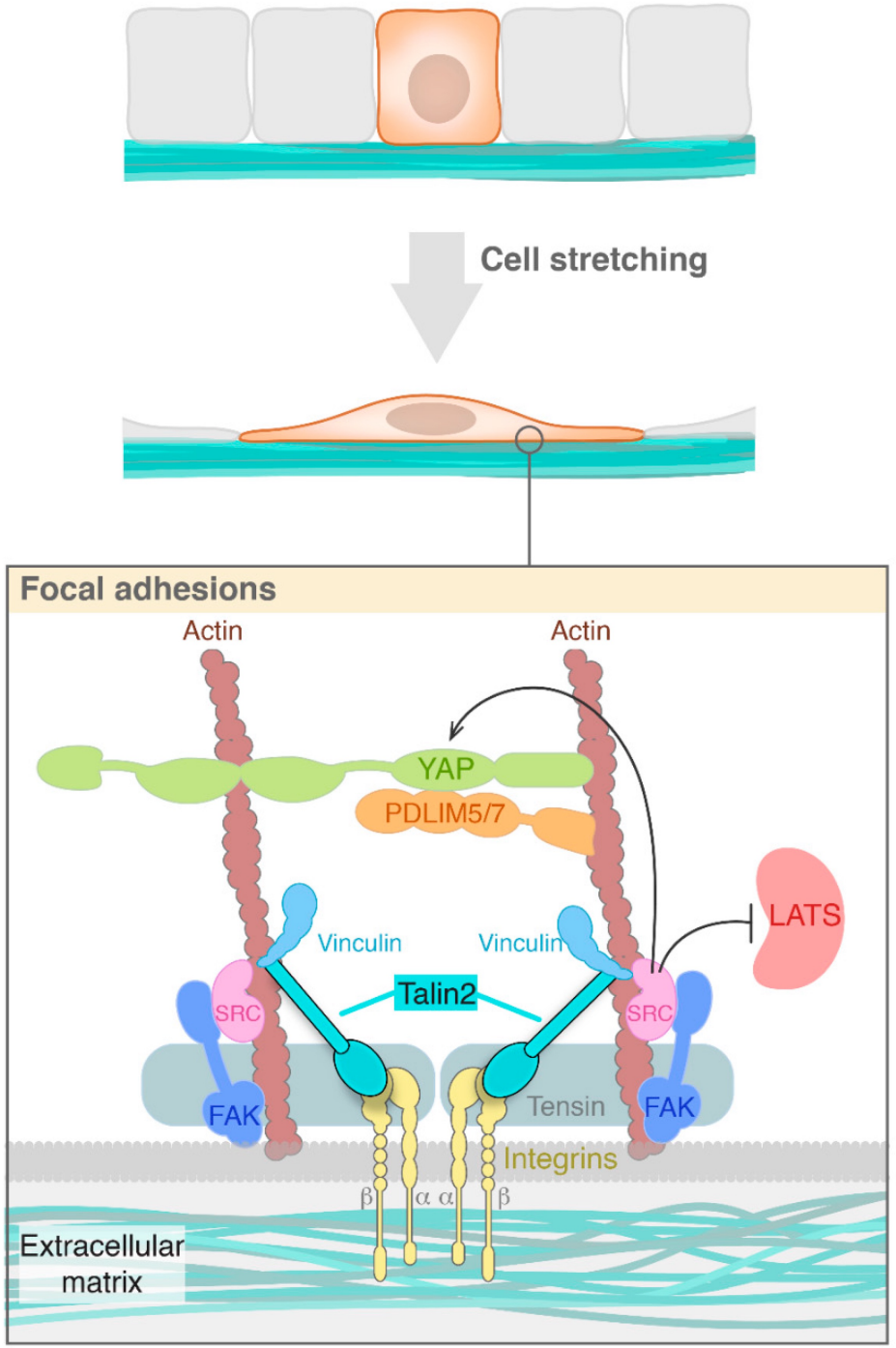
YAP/TAZ in mechanotransduction



Dupont et al 2014



YAP and TAZ are activated by a stiff ECM and stretched cell shape in contrast to inhibition by a soft ECM environment and round cell shape.
Cells cultured on highly stiff ECM display activated YAP/TAZ with high nuclear localization and subsequent transcriptional activity, whereas cells arrest when YAP/TAZ is inhibited and relocalised to the cytoplasm on a soft ECM.
In stiffer ECM, cells continue to proliferate and execute invasive phenotypes by increasing the expression and activity of adhesion receptors and thereby mechano-transduction pathways.
Round and compact cell geometry presents with cytoplasmic localization of YAP/TAZ, while cells undergoing spreading exhibit nuclear enrichment of YAP/TAZ. YAP, which is normally cytoplasmic in cells grown at high density, can re-enter the nucleus upon stretching on an elastic substrate. YAP is also activated by fluid share stress in osteoblasts and by disturbed flow in endothelial cells.

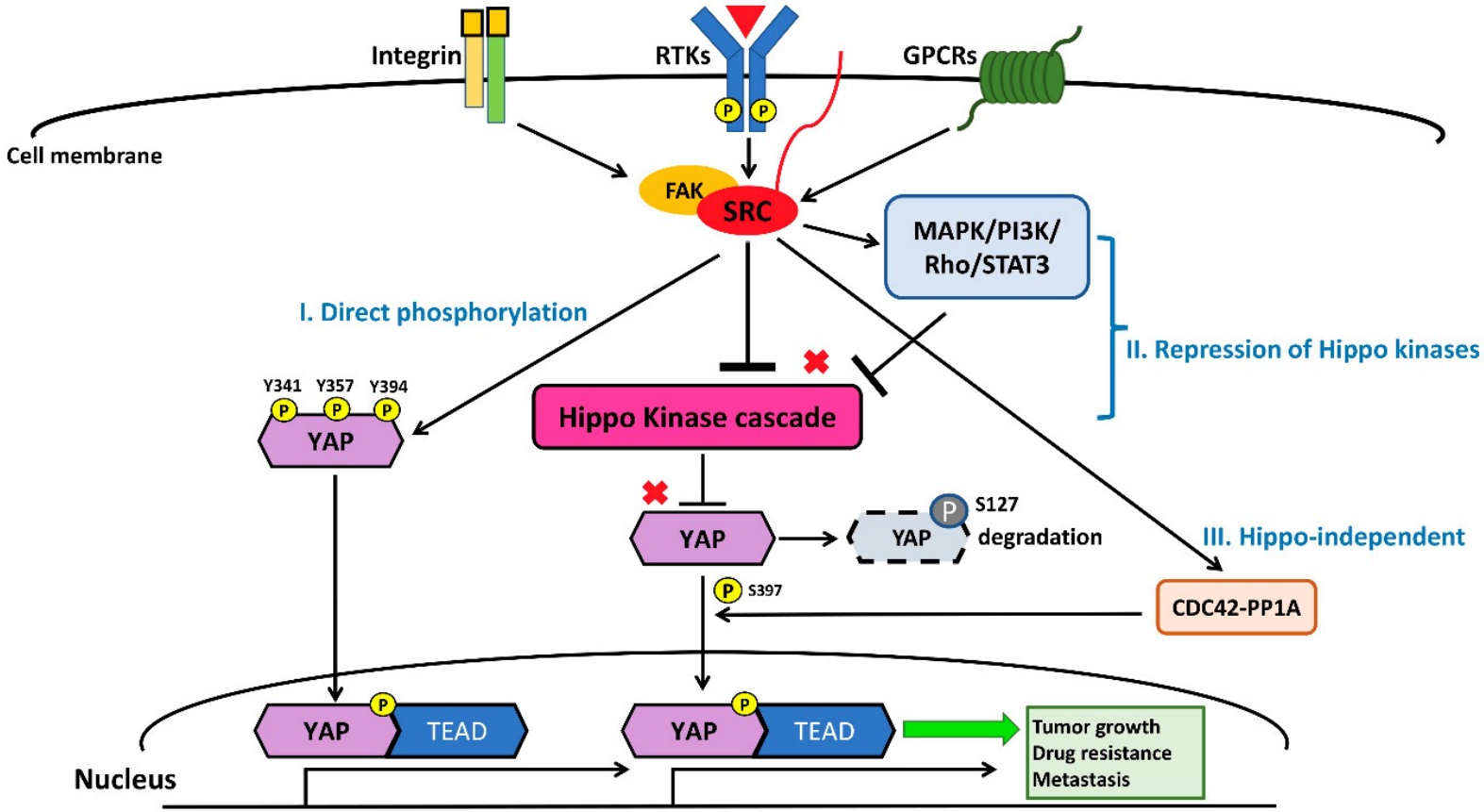


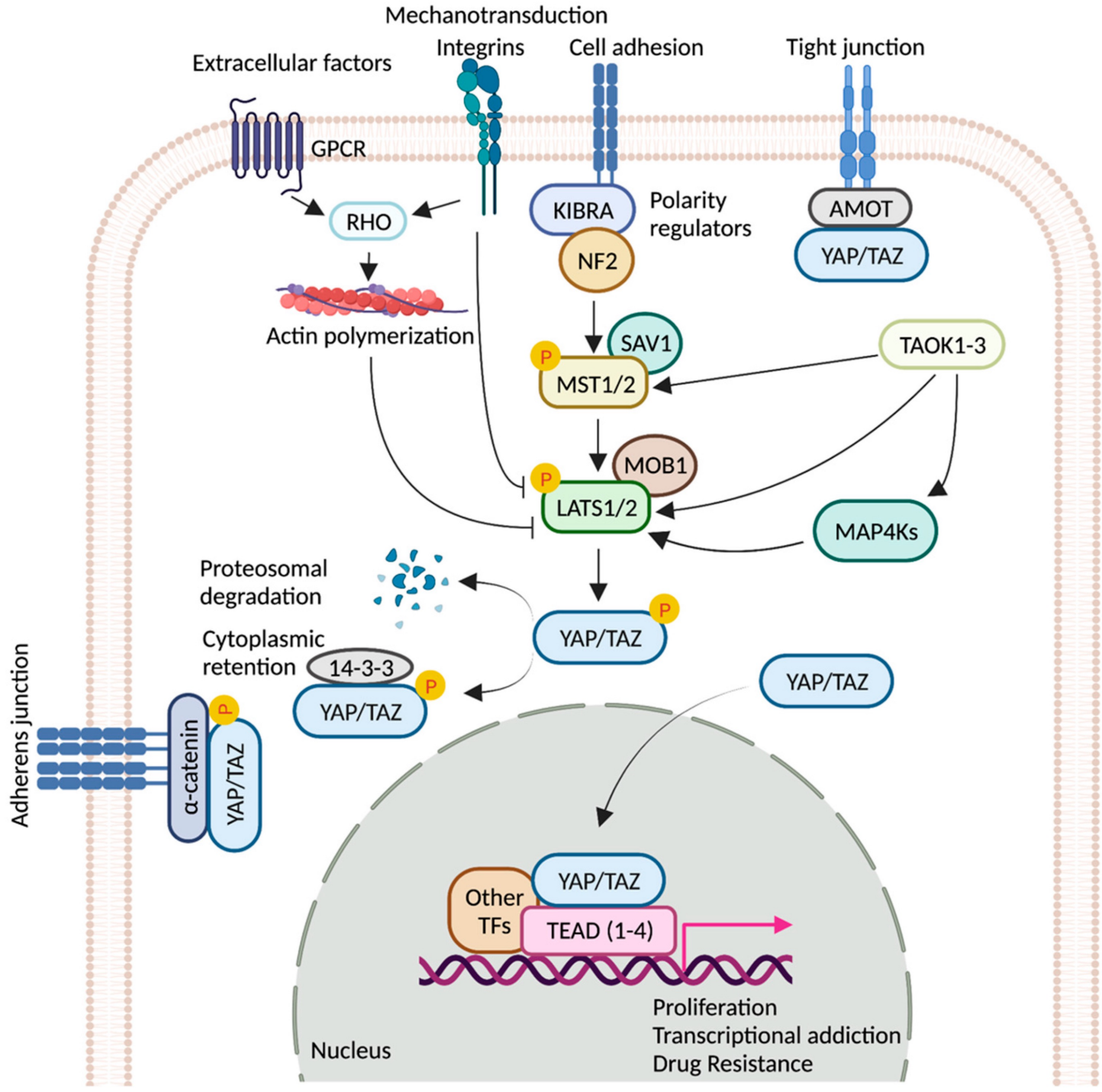
The adaptor proteins Talin and Vinculin, which link integrins to F-actin at the focal adhesions, effect the localization of YAP/TAZ.
Secondary to the forces generated above a certain "stiffness threshold", Talin unfolds, binds to Vinculin, and stabilizes the attachment of actin filaments. In this context, YAP/TAZ nuclear translocation is enhanced.

HOW?

In cancer cells, transmembrane cell surface growth factor receptors activate YAP by Src kinase through three mechanisms: (1) direct phosphorylation; (2) the activation of pathways repressing Hippo kinases; and (3) Hippo-independent mechanisms.

The Src kinase YES1 phosphorylates YAP at the site of tyrosine 357 (Y357) to activate YAP, and Y357 phosphorylation of YAP is required for Wnt/β-catenin signaling to maintain survival and tumorigenesis in human colorectal cancer cells. The activation of YAP through the phosphorylation of integrin-Src signaling is crucial for controlling skin homeostasis. In addition, 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.




Mechanotransduction
Extracellular factors
Integrins
Cell adhesion
Tight junction
GPCR
RHO
KIBRA
NF2
Polarity regulators
AMOT
YAP/TAZ
Actin polymerization
SAV1
MST1/2
TAOK1-3
MOB1
LATS1/2
MAP4Ks
Proteosomal degradation
Cytoplasmic retention
14-3-3
YAP/TAZ
YAP/TAZ
Adherens junction
α-catenin
YAP/TAZ
YAP/TAZ
Other TFs
TEAD (1-4)
Proliferation
Transcriptional addiction
Drug Resistance
Nucleus

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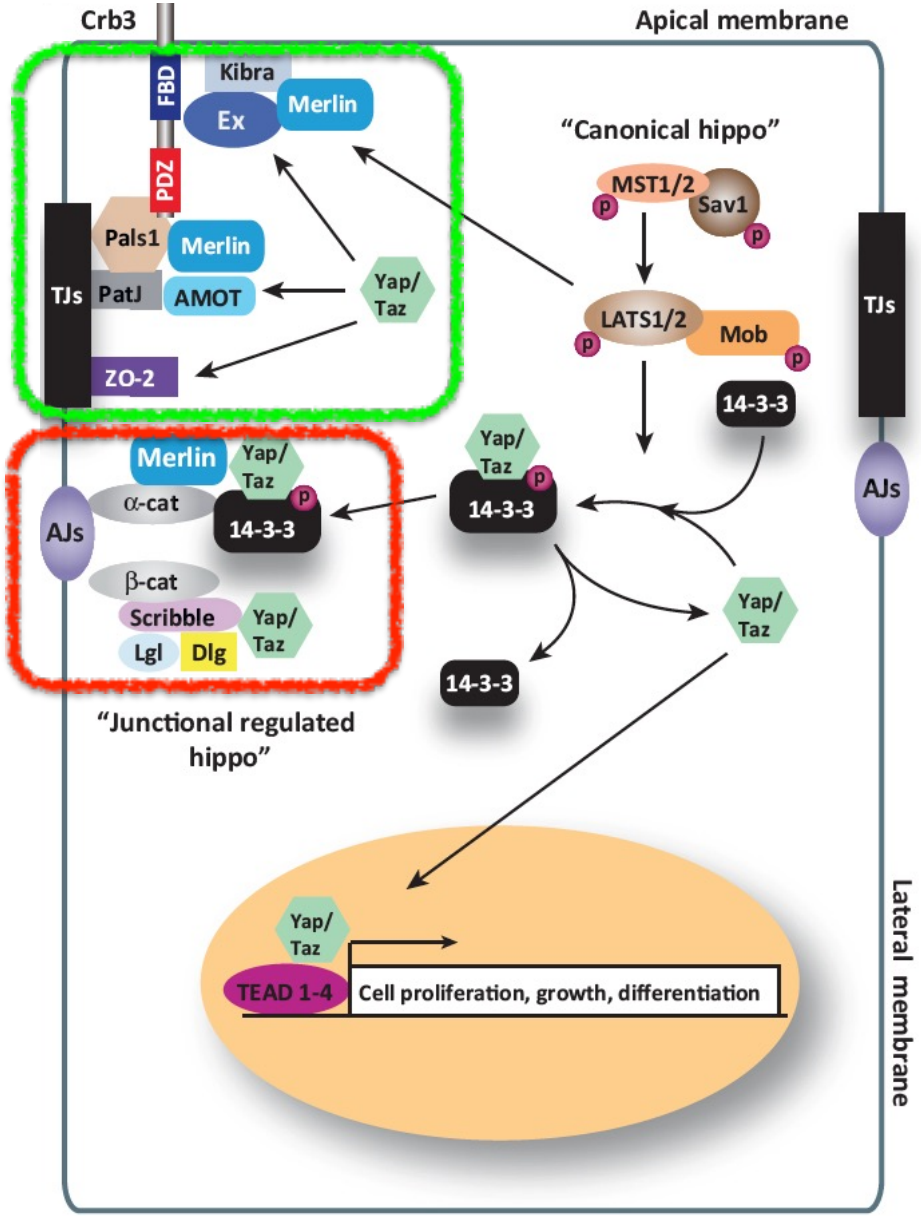
doi:10.1152/physrev.00005.2014

THE BIOLOGY OF YAP/TAZ: HIPPO SIGNALING AND BEYOND

Stefano Piccolo, Sirio Dupont, and Michelangelo Cordenonsi

Department of Molecular Medicine, University of Padua School of Medicine, Padua, Italy

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

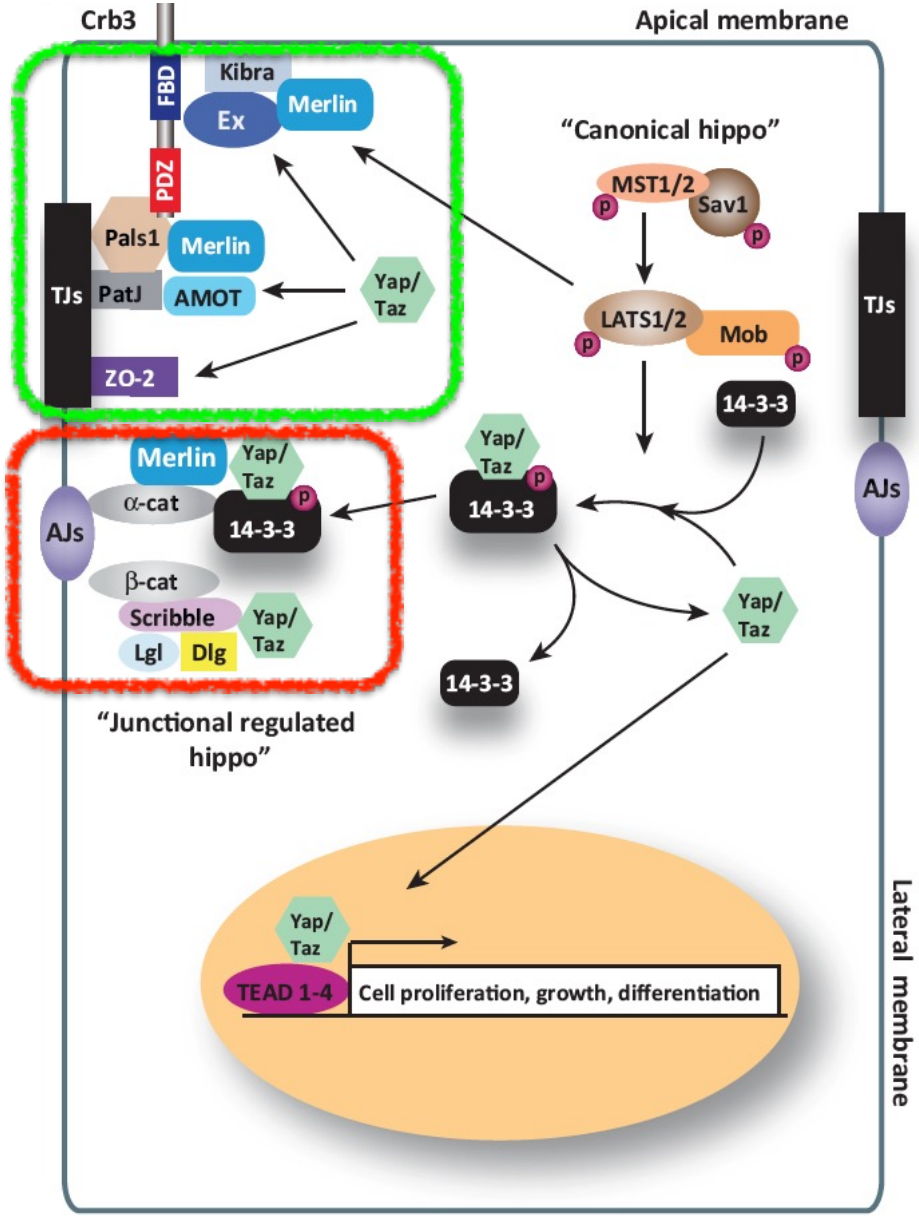


"Adherens junctions" and "tight junctions" represent the main structures by which epithelial cells are bound together via protein complexes.

In the skin, adherens junctions (AJs) control the Hippo pathway through the interaction of phospho-Yap1/14-3-3 with merlin and a-catenin.

Key junctional proteins (such as Crumbs, PATJ, PALS, α-catenin, and E-cadherin) can regulate the activity of YAP/TAZ. These junctional proteins bind and detain YAP/TAZ at cell junctions, thus suppressing their nuclear entry and activity.
It is also likely that junctional proteins affect the stability of YAP/TAZ by regulating the access of specific phosphatases or kinases.

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



Merlin is encoded by the NF2 (neurofibromatosis type 2) tumor suppressor locus.
In confluent monolayers of mammalian epithelial cells, Merlin/NF2 is preferentially localized in adherens and tight junctions (AJs and TJs).
Merlin is an important inhibitor of YAP/TAZ, acting upstream of the Hippo kinases. This inhibition is entirely dependent on LATSl/2 and YAP/TAZ phosphorylation.

- At cell-cell junctions, Merlin may promote 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.
- NF2/Merlin also operates in the nucleus: it binds and inhibits the nuclear E3 ubiquitin ligase CRL4DCAFl. Loss of NF2/Merlin unleashes this enzyme that ubiquitinates nuclear LATSl/2 fostering YAP/TEAD-dependent transcription.

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

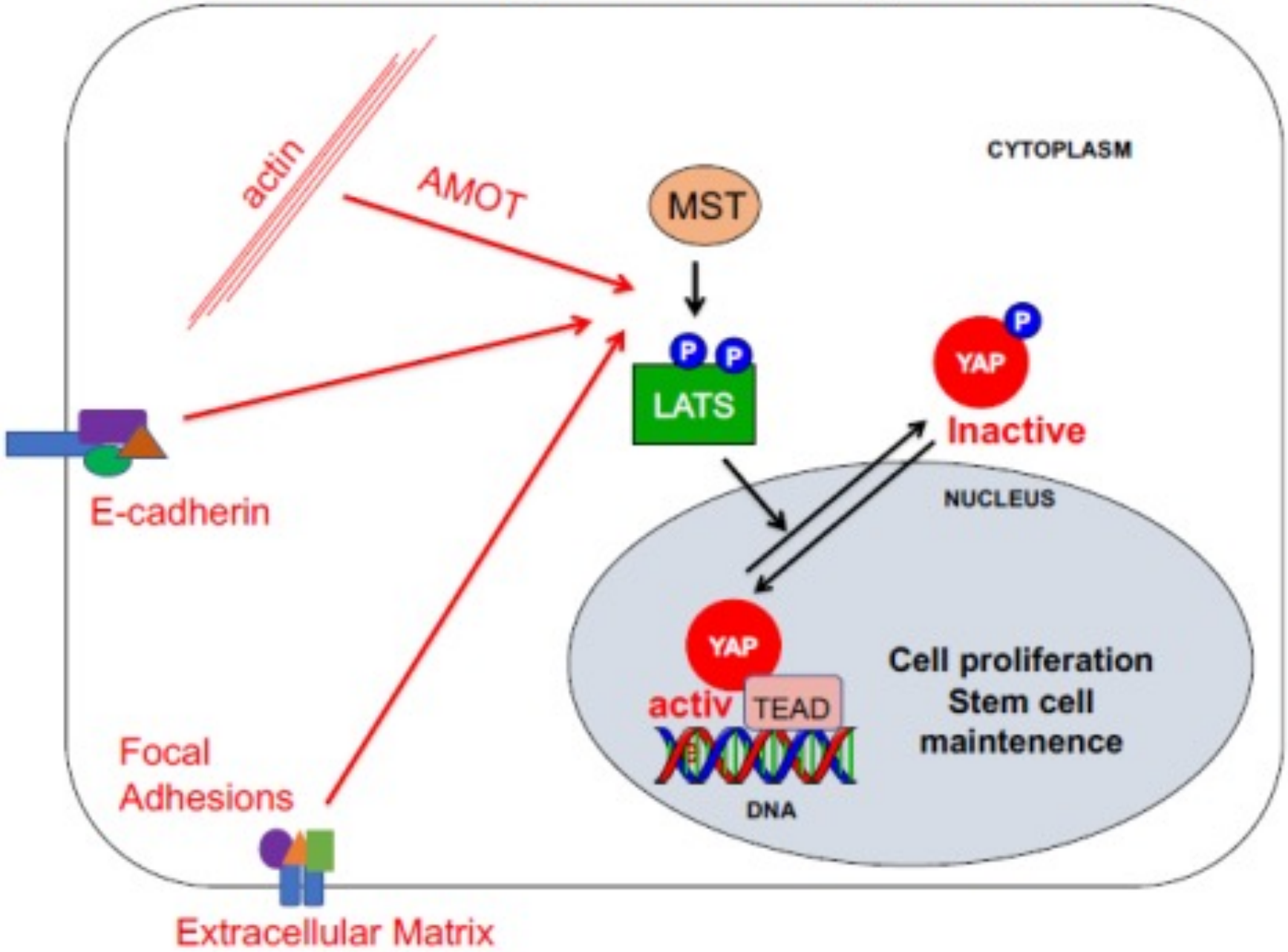
REVIEW

Mutual regulation between Hippo signaling and actin cytoskeleton

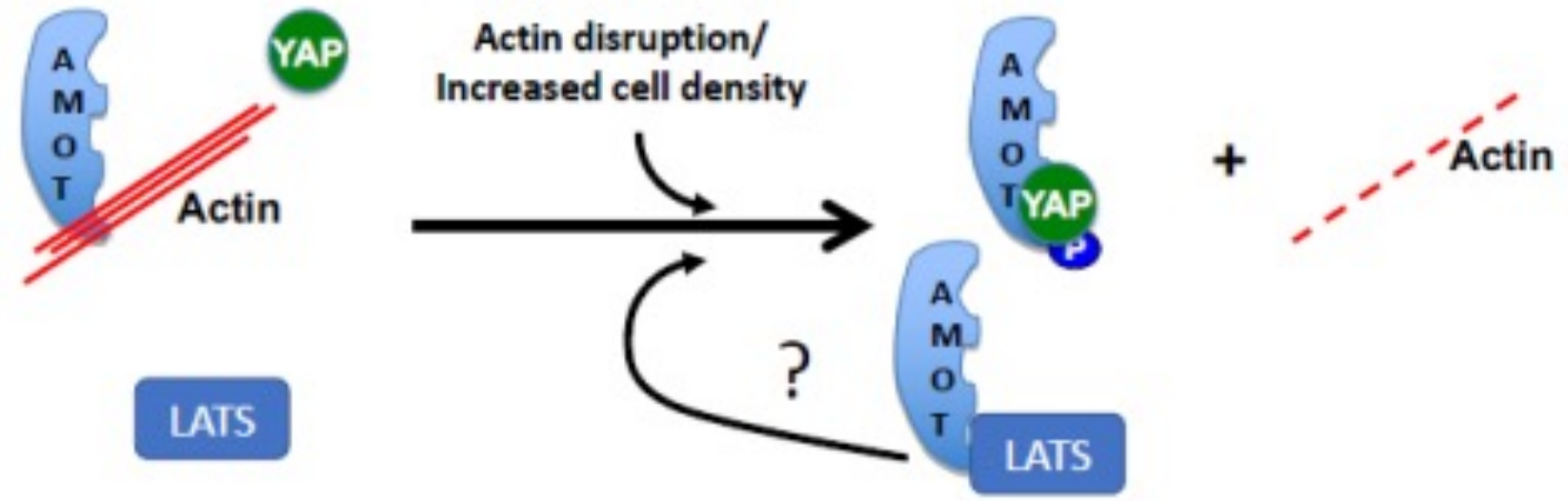
ABSTRACT

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



McCollum lab at UMass identified the **angiomotin proteins** as Hippo pathway sensors for F-actin levels and are currently studying how they control Hippo signaling.



AMOT regulates Hippo signaling in response to changes in F-actin.

One way that the Hippo pathway senses the mechanical environment appears to be through monitoring the actin cytoskeleton.

Angiomotins are novel Hippo pathway scaffolding proteins that are capable of interacting with both F-actin and multiple core components of the signaling network.

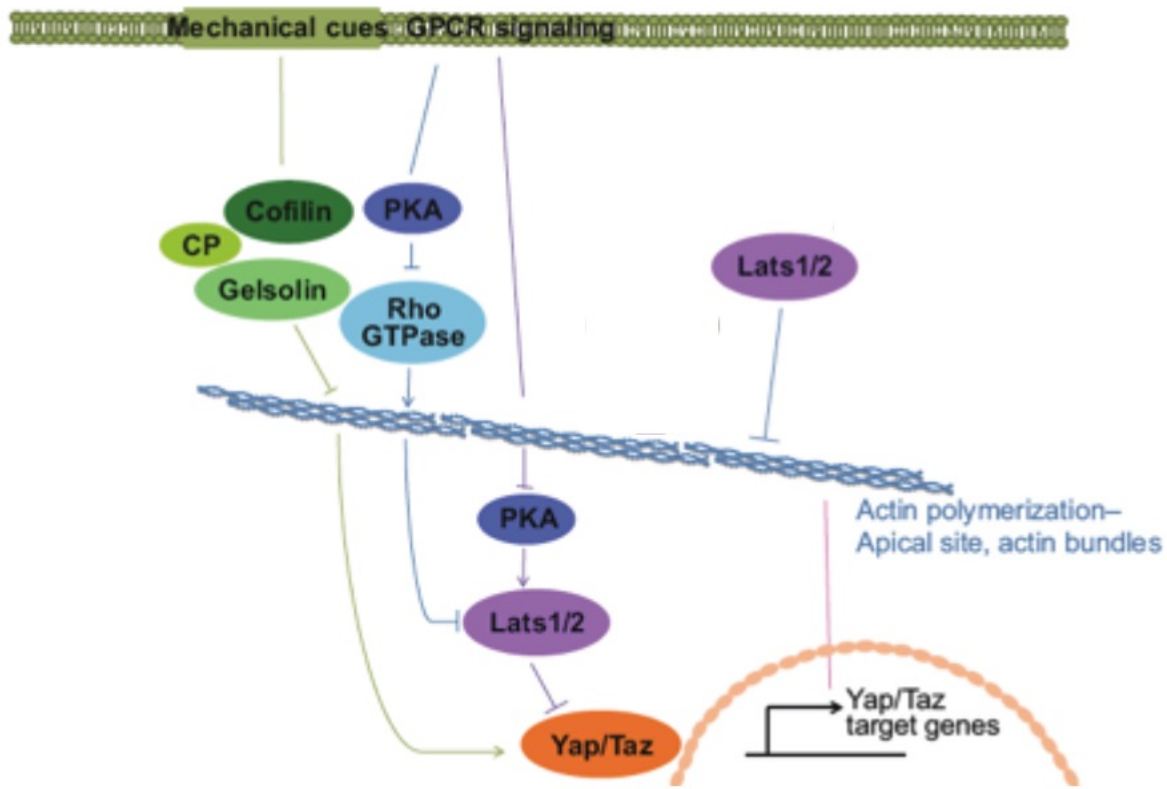
Angiomotins inhibit YAP through two mechanisms:

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.

Mutual regulation between actin cytoskeleton and the Hippo pathway in mammalian cells



In mammalian cells, mechanical cues control actin cytoskeleton and Yap/Taz activity **independently from the Hippo pathway.**

- Negative regulators of F-actin, such as CapZ, Cofilin, and Gelsolin, are required in this regulation.
- GPCR signaling also influences actin cytoskeleton and the activity of Yap/Taz, but in a Lats1/2-dependent manner. Involvement of Rho GTPase and PKA is reported in this regulation. Lats1/2 proteins can directly bind to β-actin, suppressing F-actin polymerization.

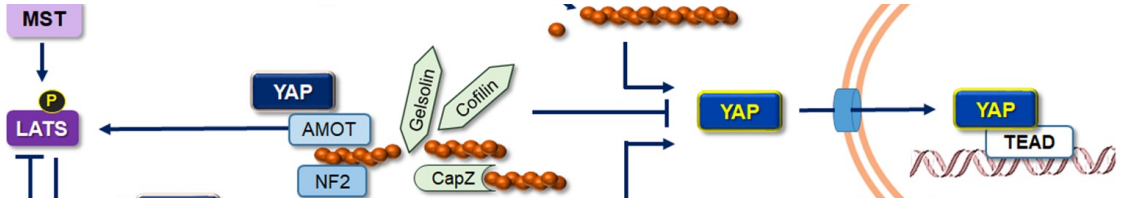
F-actin capping

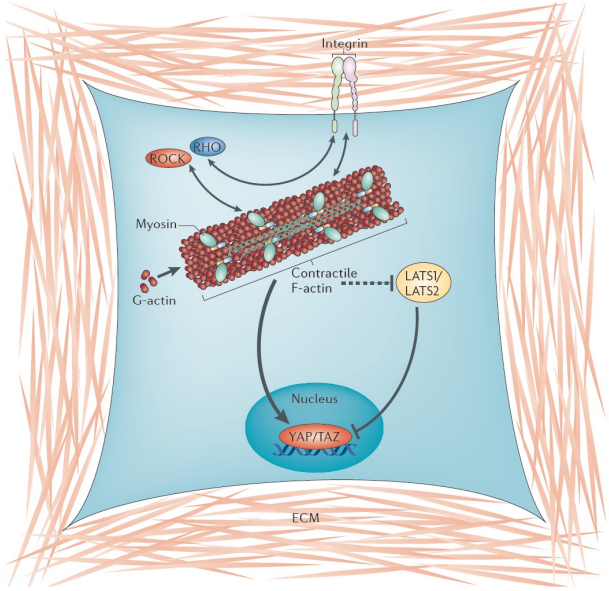
Actin-interacting proteins that restrict actin polymerization are also involved in the regulation of actin dynamics, adjusting cell shape and motility in response to environmental factors.

Studies in Drosophila have showed that inactivation of actin-capping proteins, which results in abnormal accumulation of F-actin, leads to Yki activation and cell proliferation.

In human mammary epithelial cells, 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. Interestingly, knockdown of CapZ does not affect the level of YAP phosphorylation, suggesting that the actin cytoskeleton has the capacity to regulate YAP/TAZ activity, independent of the core Hippo kinases.





The two faces of the Hippo signaling pathway:

1.Regeneration

2. Cancer

Am J Physiol Gastrointest Liver Physiol 307: G196–G204, 2014.
First published May 29, 2014; doi:10.1152/ajpgi.00077.2014.

Dynamic alterations in Hippo signaling pathway and YAP activation during liver regeneration

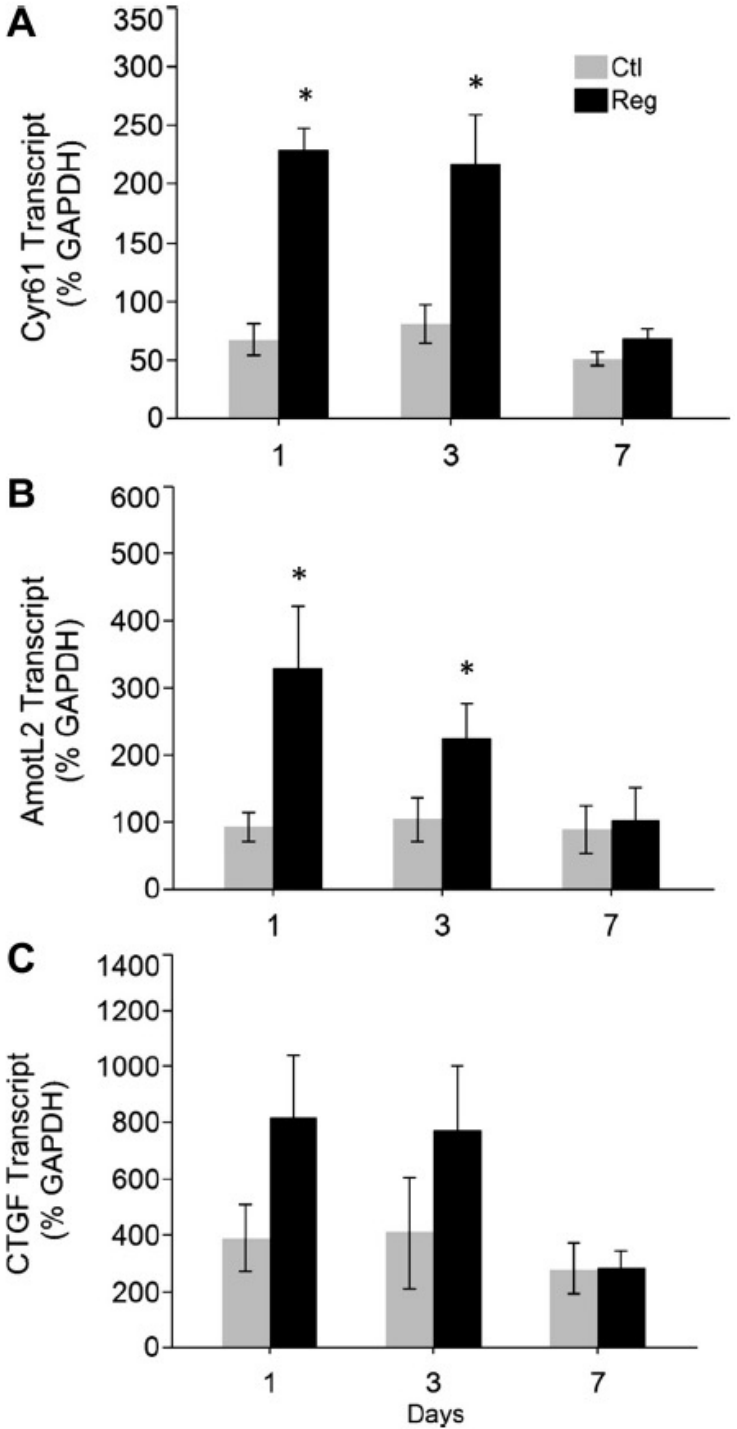
James L. Grijalva,[1] Megan Huizenga,[2] Kaly Mueller,[1] Steven Rodriguez,[2] Joseph Brazzo,[1] Fernando Camargo,[3] Ghazaleh Sadri-Vakili,[2] and Khashayar Vakili[1]

[1]Department of Surgery, Boston Children's Hospital, Boston, Massachusetts; [2]MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Boston, Massachusetts; and [3]Department of Stem Cell and Regenerative Biology, Boston Children's Hospital, Boston, Massachusetts

While Hyppo pathway is activated in quiescent livers, its inhibition leads to liver overgrowth and tumorigenesis

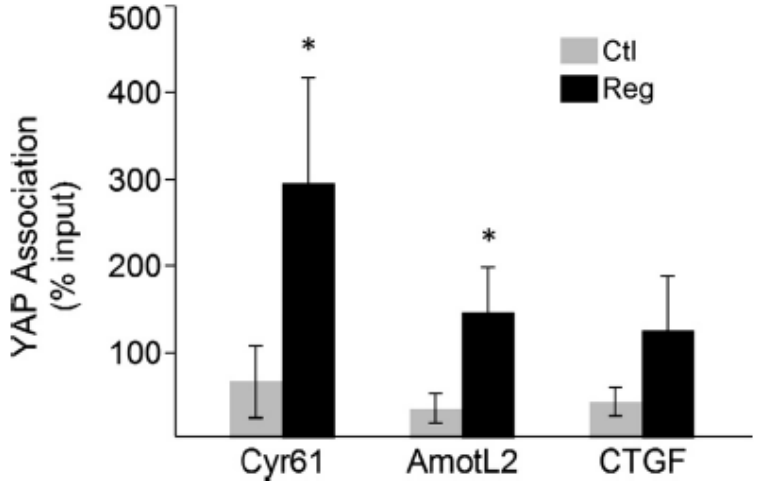
- 70% partial hepatectomy (PH) rat model.
- Increase in YAP activation by 1 day following PH.
- Decrease in the activation of core kinases Mst1/2 by 1 day as well as Lats1/2 by 3 days following PH.
- **Liver reaches its near normal size by 7 days following PH, which correlated with a return to baseline YAP nuclear levels and target gene expression, indicating reactivation of the Hippo signaling pathway.**

Dynamic changes in the Hippo signaling pathway and YAP activation during liver regeneration, which stabilize when the liver-to-body weight ratio reaches homeostatic levels



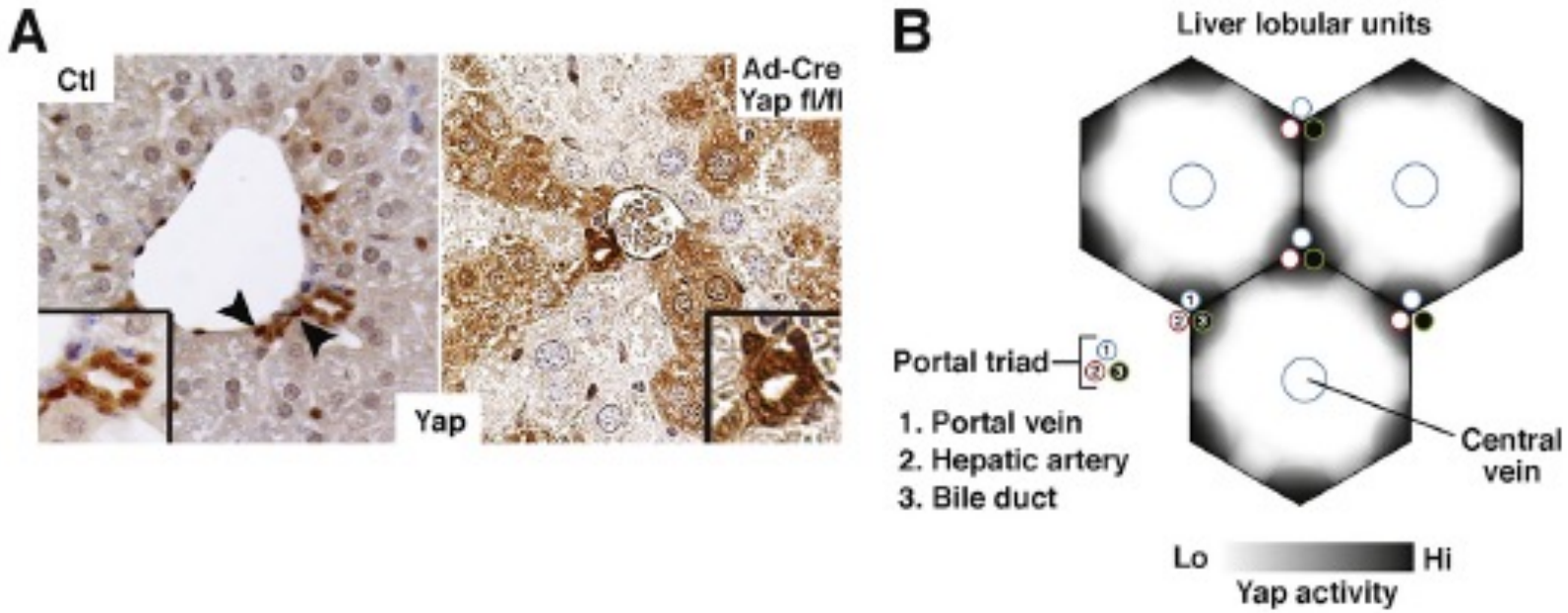
Expression of YAP target genes is increased during liver regeneration.

YAP is activated during liver regeneration.
The pattern of activation of YAP and inactivation of associated Mst1/2 and Lats1/2 kinases correlates with liver growth following PH.



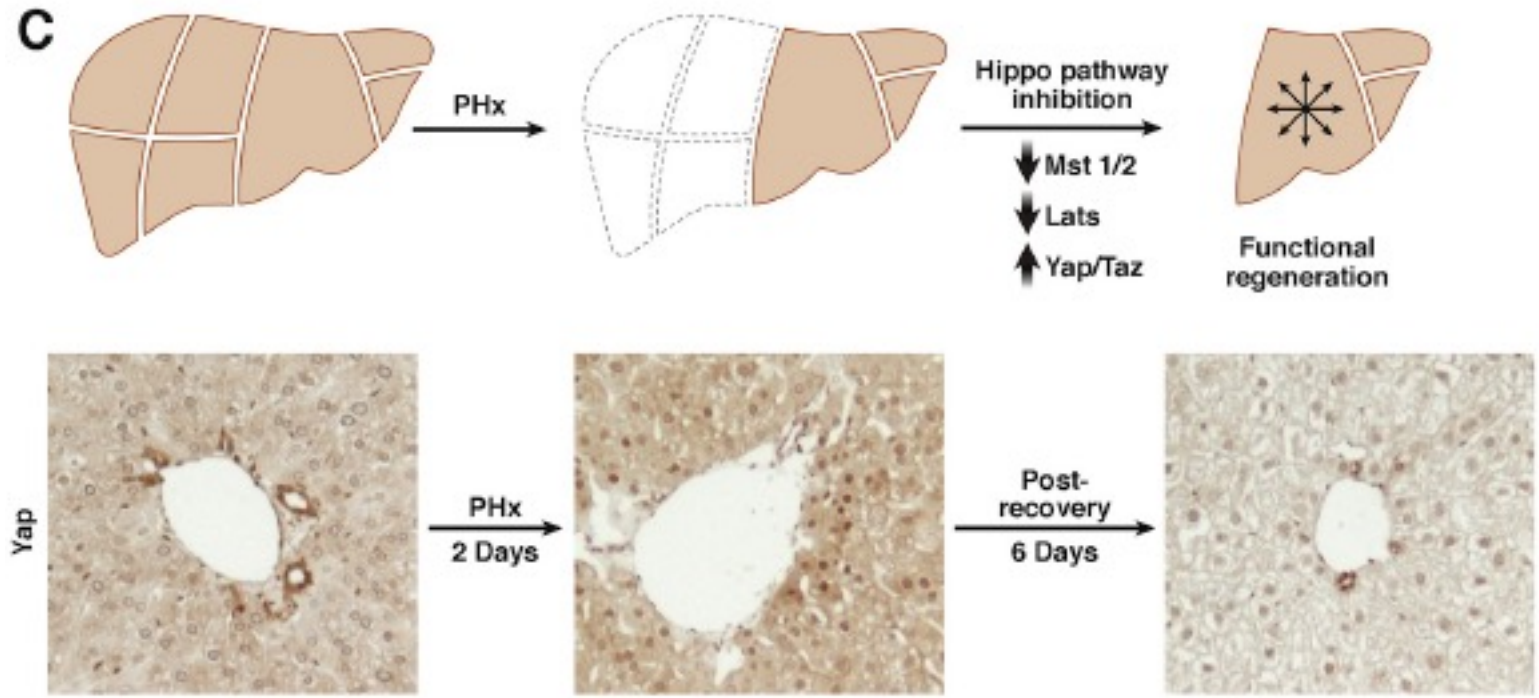
YAP associates with Cyr61 and AmotL2 promoter during liver regeneration.

YAP expression during homeostasis and regeneration.



(A) YAP is present in the epithelial cells of mouse liver (hepatocytes and biliary cells).
YAP expression and nuclear localization is more prominent in biliary cells (arrowhead) as compared with hepatocytes.

(B) Schematic of YAP activity in the liver. YAP activity is highest in the biliary cells/portal hepatocytes, diminishing in the hepatocytes toward the central vein.



Hippo/Yap activity dynamically changes after partial hepatectomy.
Yap levels increase with an associated decrease in MST1, MST2, LATS1, and LATS2 activity. These return to their normal levels as the liver reaches its appropriate size.

Partial hepatectomy in mice results in YAP enrichment and an increase in nuclear localization (day 2).
After 8 days of recovery, YAP expression is reduced to below baseline levels.

REVIEW Open Access

Role of the Hippo pathway in liver regeneration and repair: recent advances

Monica Pibiri* and Gabriella Simbula

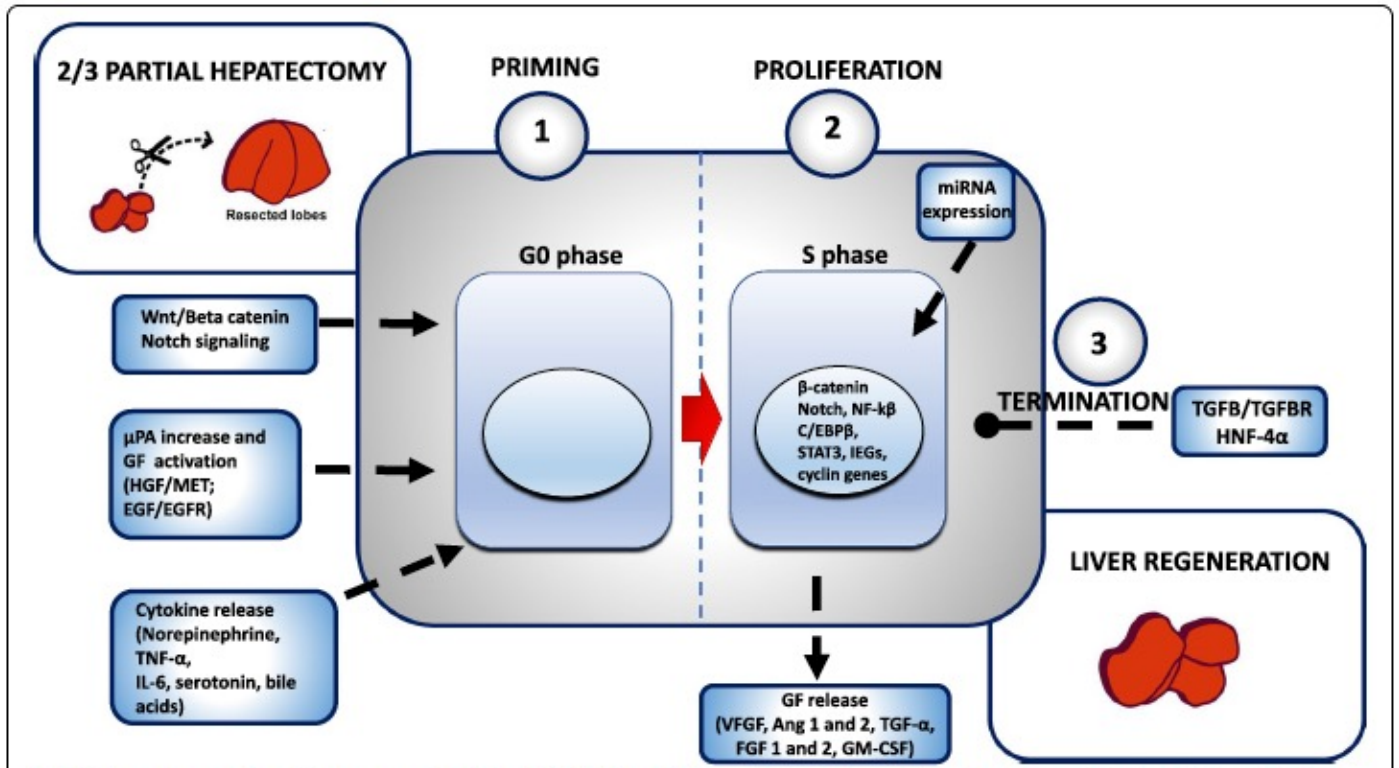
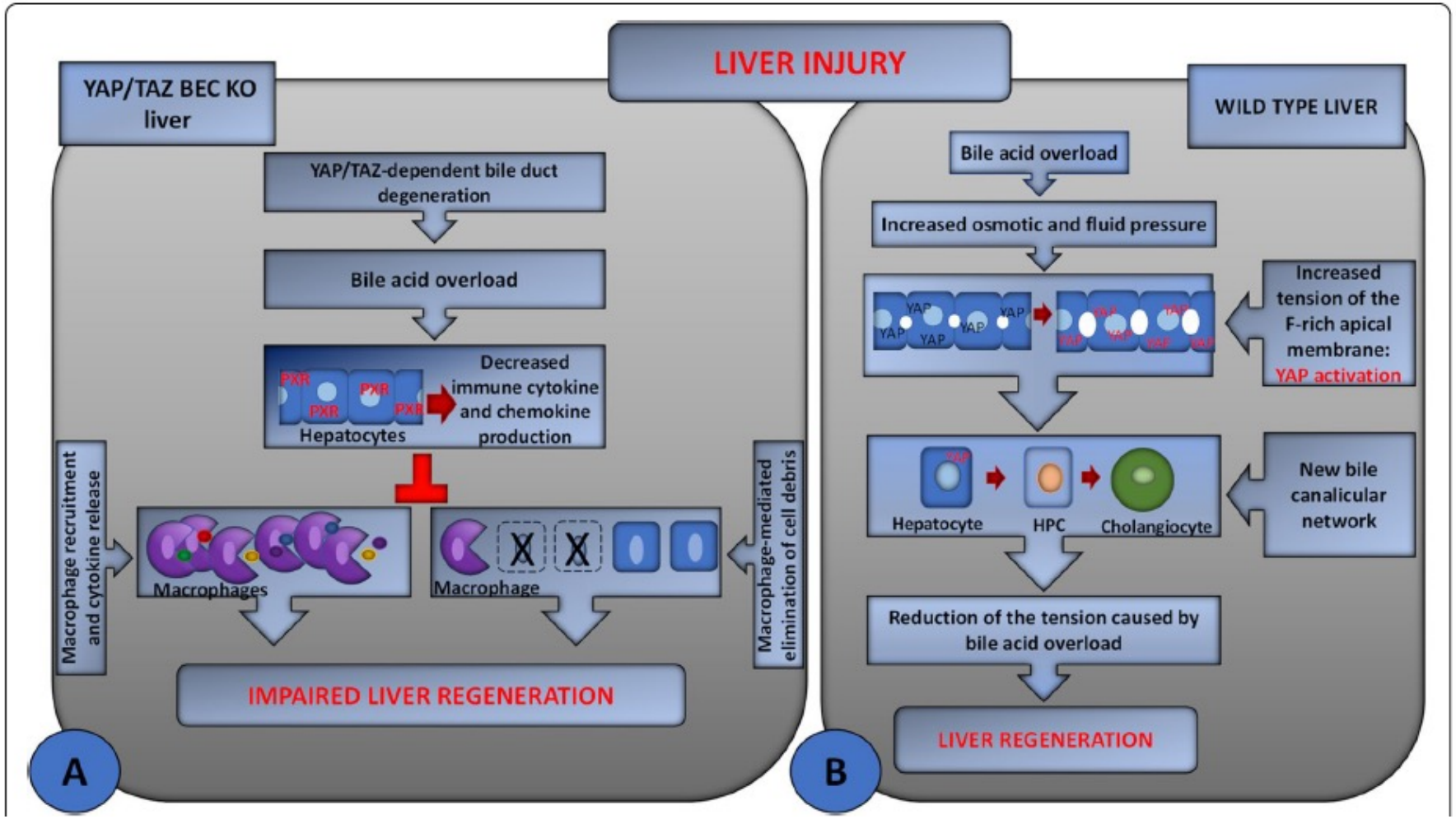


Fig. 1 Schematic overview of liver regeneration after 2/3 PH. After partial hepatectomy, the remaining liver cells proliferate until the original organ size is restored. Three phases of liver regeneration have been identified: (1) priming, (2) proliferation, and (3) termination. The priming phase (1) is related to the activation of growth factors (HGF and EGF, which are ligands of MET and EGF receptors, respectively), induced by the 2/3 PH-induced increase in µPA and the nuclear translocation of Notch1 and beta-catenin into hepatocytes, as well as by the release of cytokines (TNF-α; norepinephrine, bile acids, IL-6, serotonin) that modulate hepatocyte proliferation and the interaction between hepatocytes and non-parenchymal cells. The proliferation phase (2) is preceded by activation and nuclear translocation of transcription factors such as STAT3, C/EBPβ, and NFκB. Increased expression of IEGs (c-Fos, c-Jun, and c-Myc) is also observed. All these factors promote the proliferation phase, leading to the transcription of cyclin genes. Proliferating hepatocytes release many growth factors that stimulate the proliferation of non-parenchymal cells: VEGF and Ang1 and 2, mitogenic for LSECs; TGF-α, mitogenic for endothelial cells, LSECs, and HSCs; FGF1 and 2, mitogenic for HSCs and LSECs; and GM-CSF, which stimulates the proliferation of KCs. In addition, microRNAs (miRNAs) were also found to be involved in the regulation of hepatocyte DNA synthesis in mouse models of liver regeneration. The termination phase (3) is likely promoted by activation of signal transduction pathways that suppress cell growth, such as that mediated by TGF-β/TGFβ receptor and HNF-4α



In YAP /TAZ BEC-KO liver severe degeneration of bile ducts causes cholestasis and has secondary effects on hepatocytes and macrophages, resulting in impaired liver regeneration.
More specifically, bile acid overload is responsible for PXR-mediated suppression of cytokine production in hepatocytes, which impairs phagocytic macrophage recruitment and activation.
Decreased macrophage function impairs the tissue regeneration process by reducing the clearance of cellular debris from the injury site.

In chronically diseased livers, YAP/TAZ activation results in a reparative process characterized by liver fibrosis. In this condition, YAP/TAZ activation in parenchymal and non-parenchymal cells results in (i) differentiation of quiescent HSCs into myofibroblastic HSCs; (ii) recruitment of macrophages releasing inflammatory cytokines; (iii) polarization of macrophages toward the M2 phenotype.

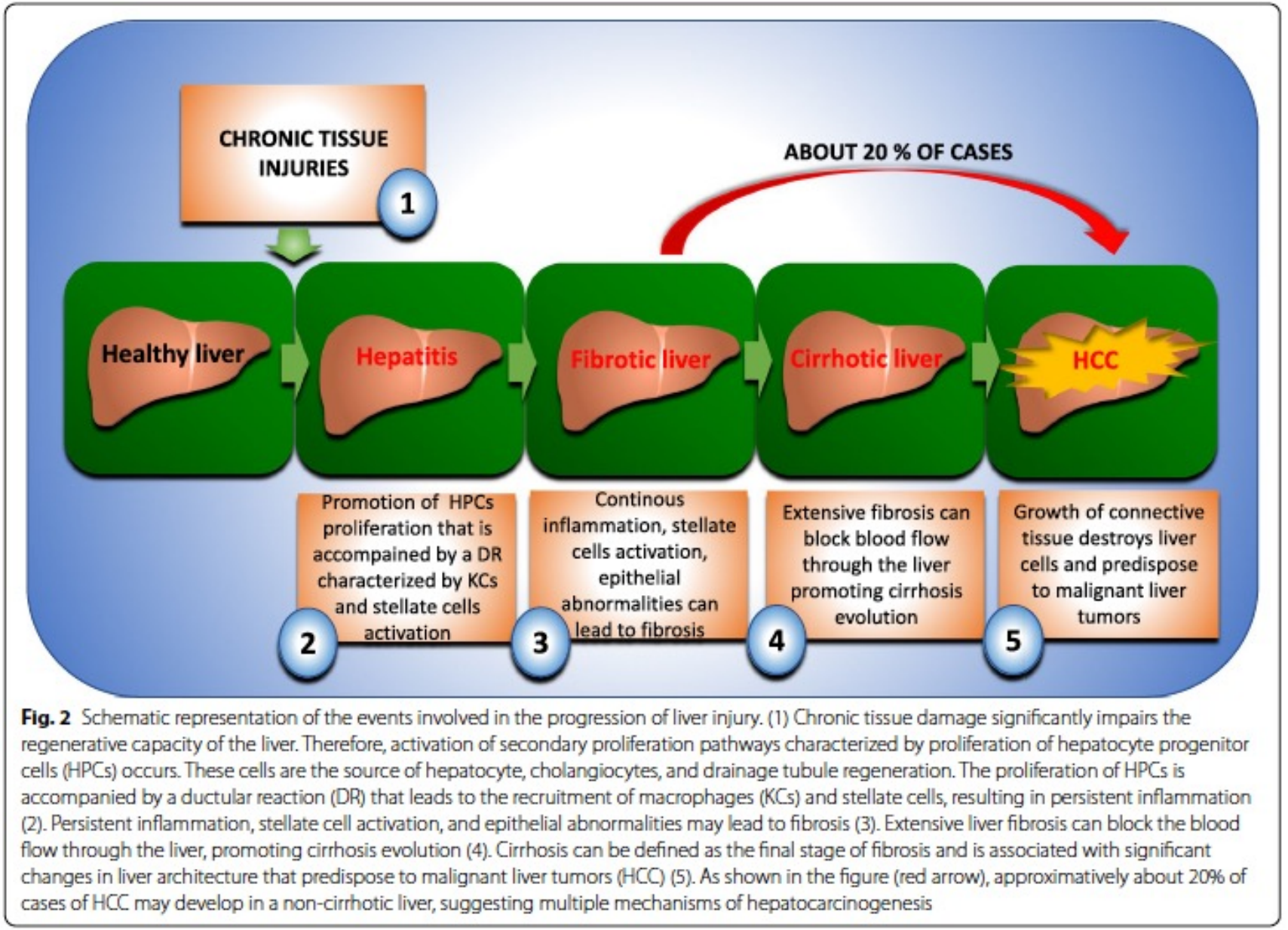


Fig. 2 Schematic representation of the events involved in the progression of liver injury. (1) Chronic tissue damage significantly impairs the regenerative capacity of the liver. Therefore, activation of secondary proliferation pathways characterized by proliferation of hepatocyte progenitor cells (HPCs) occurs. These cells are the source of hepatocyte, cholangiocytes, and drainage tubule regeneration. The proliferation of HPCs is accompanied by a ductular reaction (DR) that leads to the recruitment of macrophages (KCs) and stellate cells, resulting in persistent inflammation (2). Persistent inflammation, stellate cell activation, and epithelial abnormalities may lead to fibrosis (3). Extensive liver fibrosis can block the blood flow through the liver, promoting cirrhosis evolution (4). Cirrhosis can be defined as the final stage of fibrosis and is associated with significant changes in liver architecture that predispose to malignant liver tumors (HCC) (5). As shown in the figure (red arrow), approximatively about 20% of cases of HCC may develop in a non-cirrhotic liver, suggesting multiple mechanisms of hepatocarcinogenesis

Hippo Signaling in the Liver Regulates Organ Size, Cell Fate, and Carcinogenesis

Gastroenterology 2017;152:533–545

Sachin H. Patel,[1] Fernando D. Camargo,[1,2,3] and Dean Yimlamai[1,4]

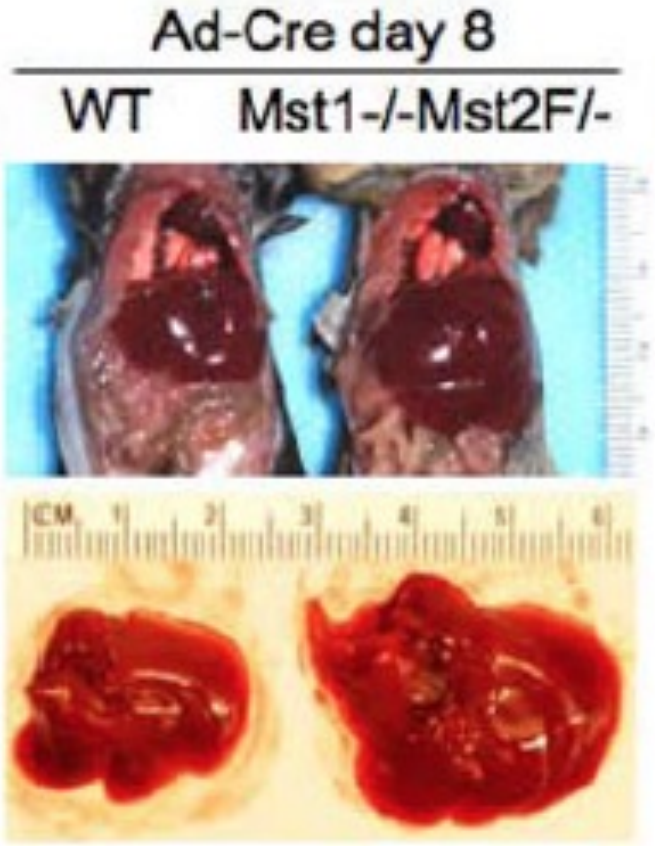
The two faces of the Hippo signaling pathway:

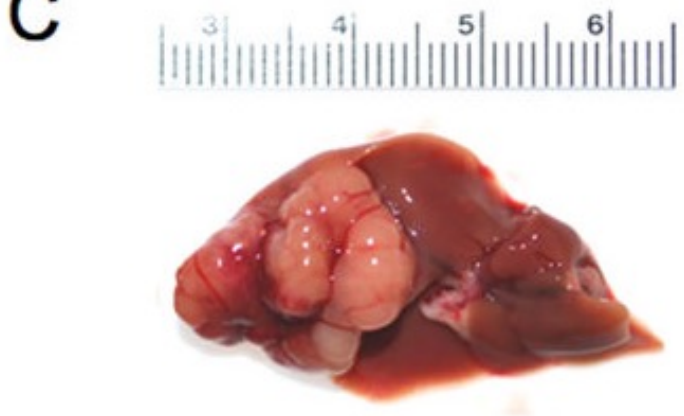
1.Regeneration

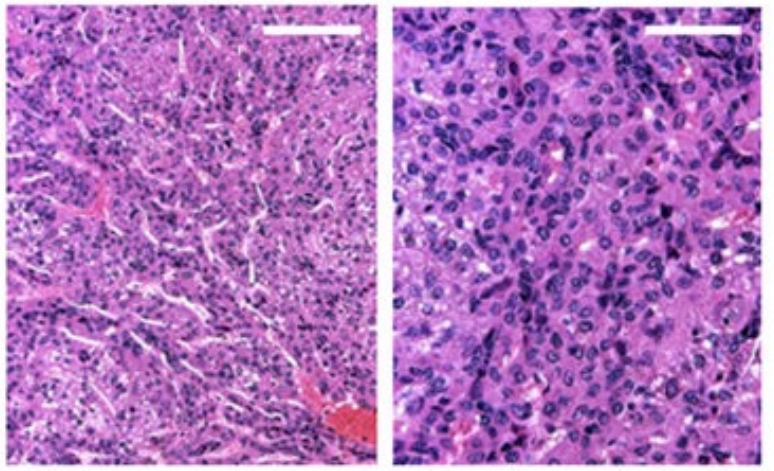
2. Cancer

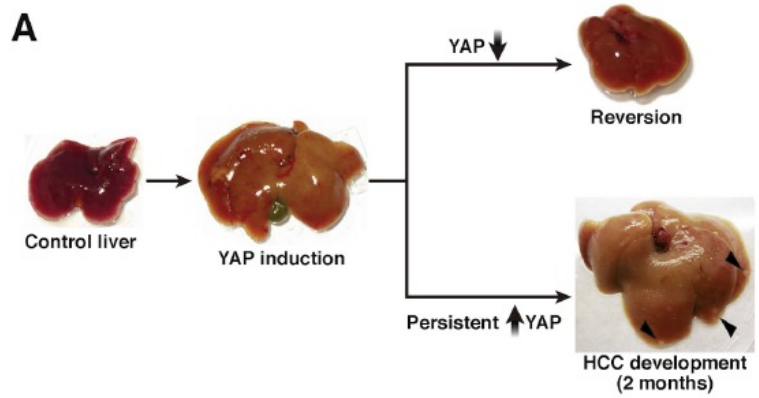
The Hippo pathway in HCC

K.O. mice for the upstream regulators of YAP and TAZ lead to an tumorigenic overgrowth of the liver

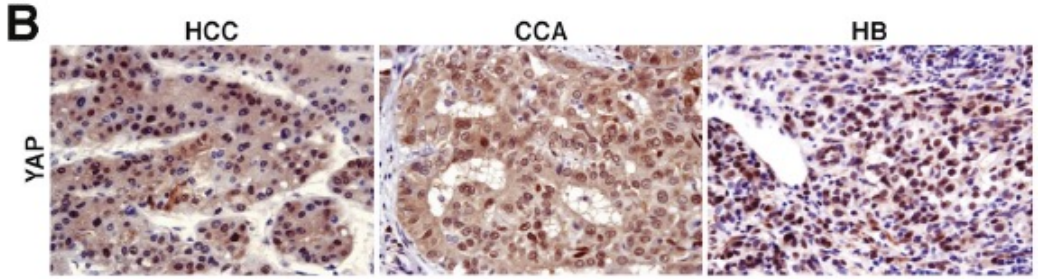






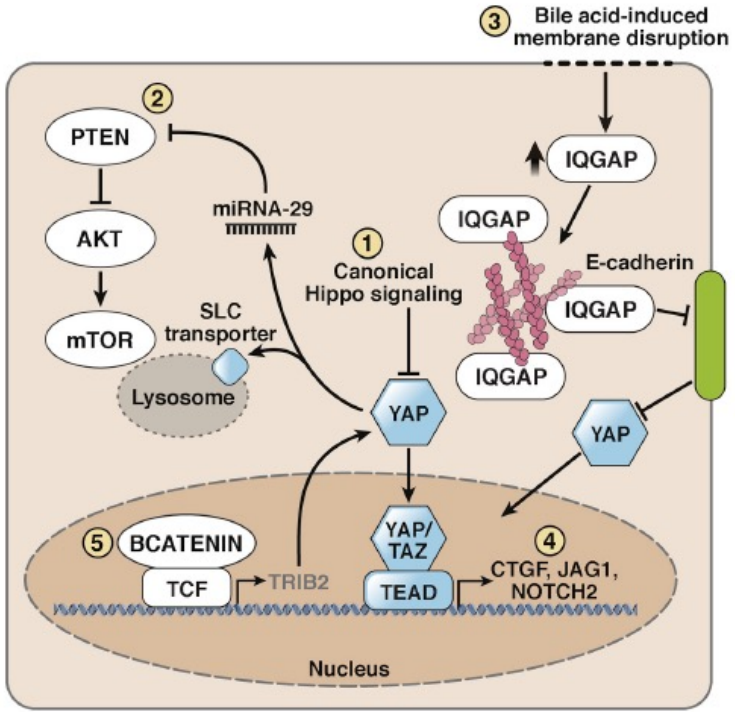


Liver-specific overexpression of YAP leads to massive hepatomegaly with livers approaching 4-5X their original size. Upon restoration of endogenous levels of YAP, the liver returns to its usual size. Persistent YAP activation for 2 months frequently results in HCC development (arrowheads).



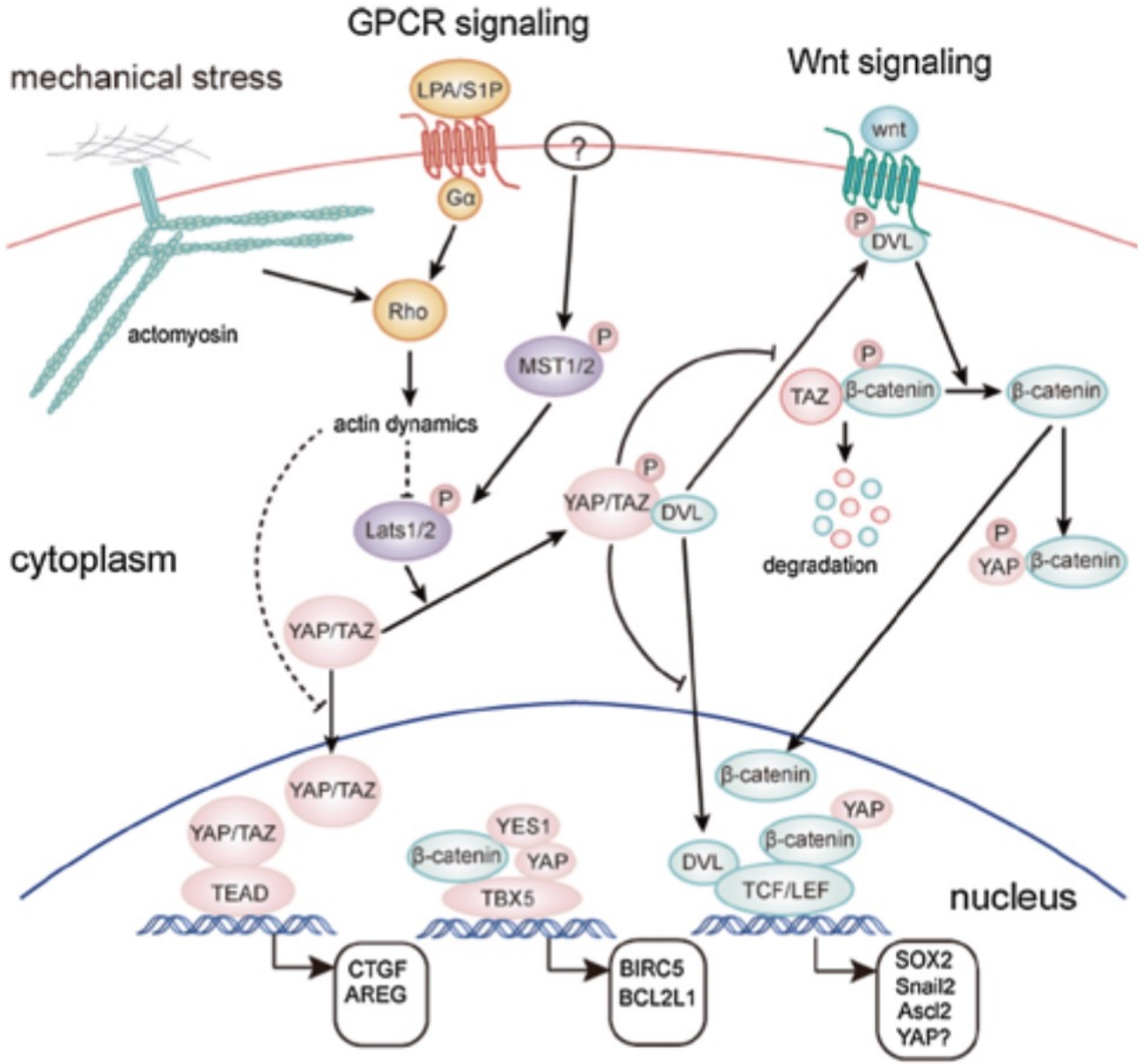
Increased overall YAP and nuclear YAP is a feature of several liver cancers.

C



YAP can mediate its tumorigenic effects either autonomously or through synergy with other pathways. YAP can be activated through canonical Hippo inactivation (1) or noncanonical membrane associated signaling (2).
YAP can also interact with the PI3KAkt mammalian target of rapamycin pathway through a microRNAmediated mechanism.
Finally, YAP can interact with the NOTCH and Wnt pathways, as evidenced through upregulation of NOTCH ligands and receptors (4) and YAP's stabilization by the Wnt target gene TRIB2 (5).

YAP/TAZ are effectors of mechanical stress, GPCR signaling, and the Wnt signaling pathway



Mechanisms of YAP and TAZ regulation by mechanical stress, GPCR signaling, and the Wnt pathway as well as YAP/TAZ as modulators of the Wnt pathway are shown.