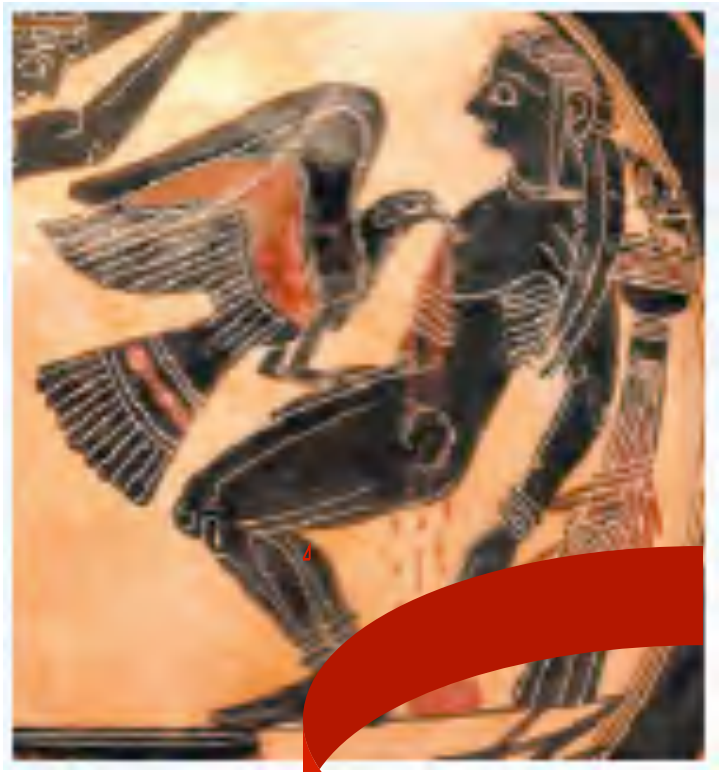
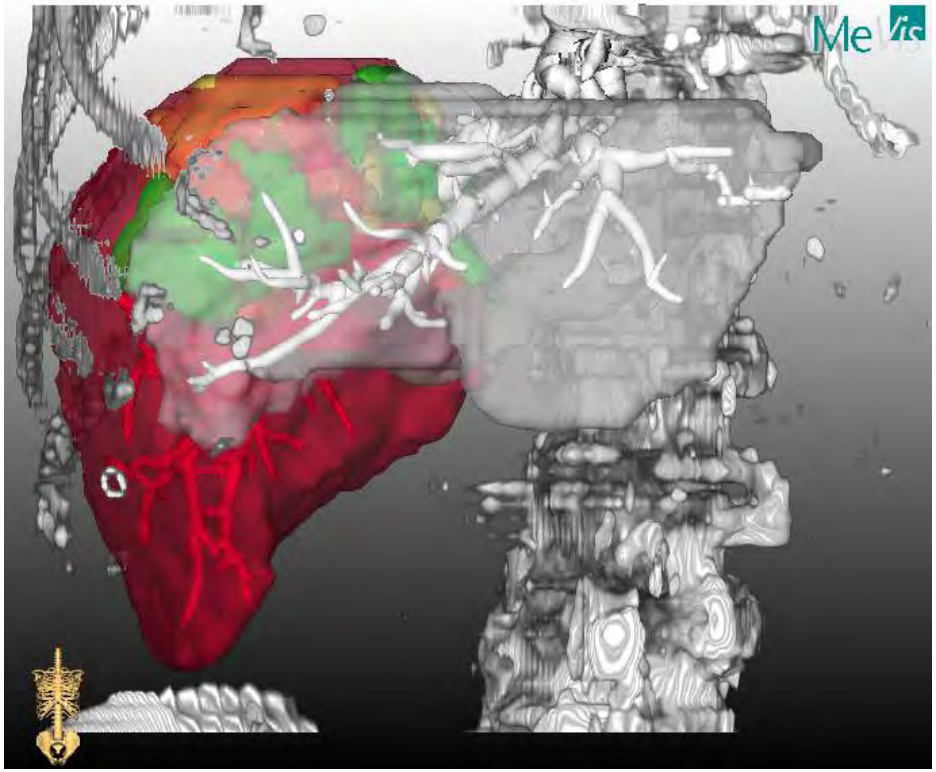
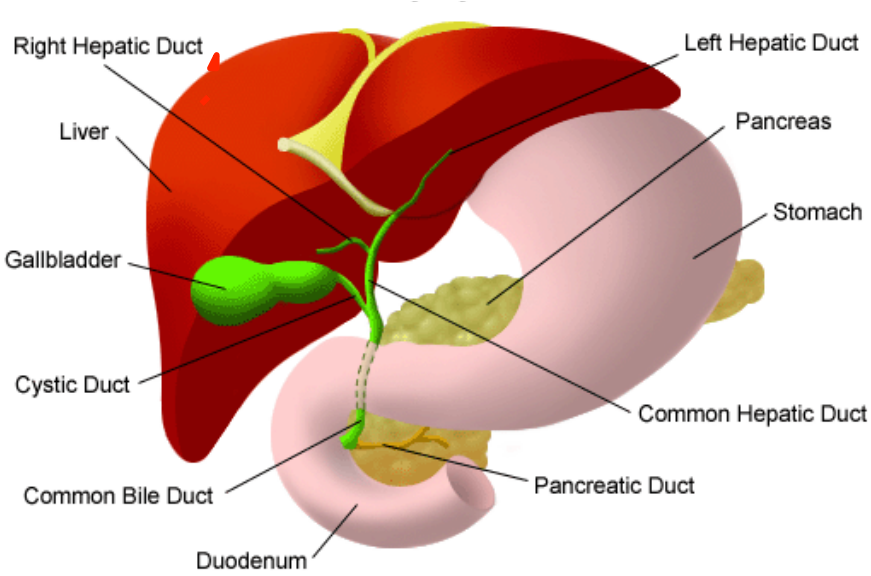
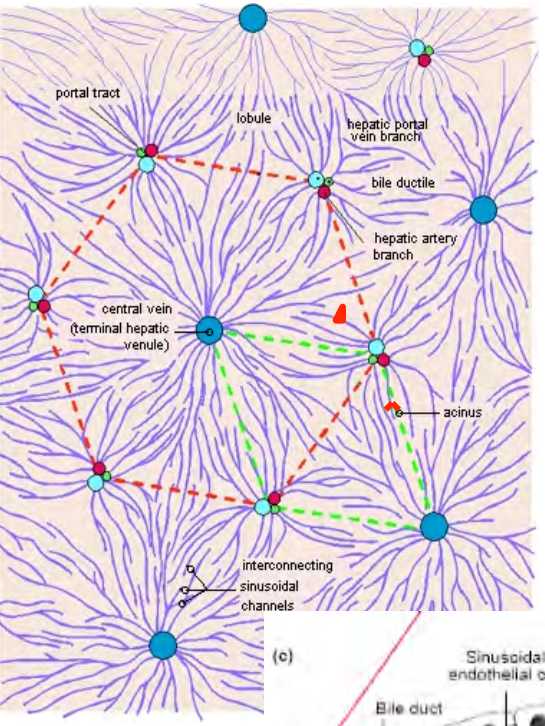
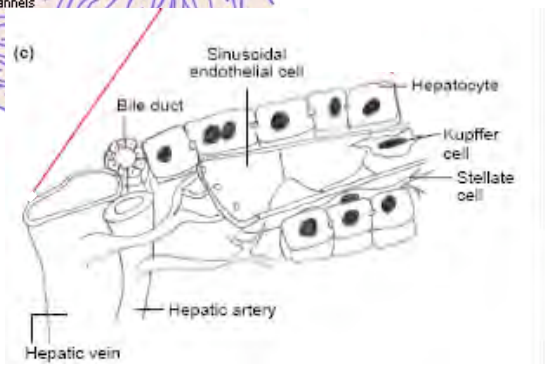
LIVER REGENERATION: FROM MYTH TO MECHANISM



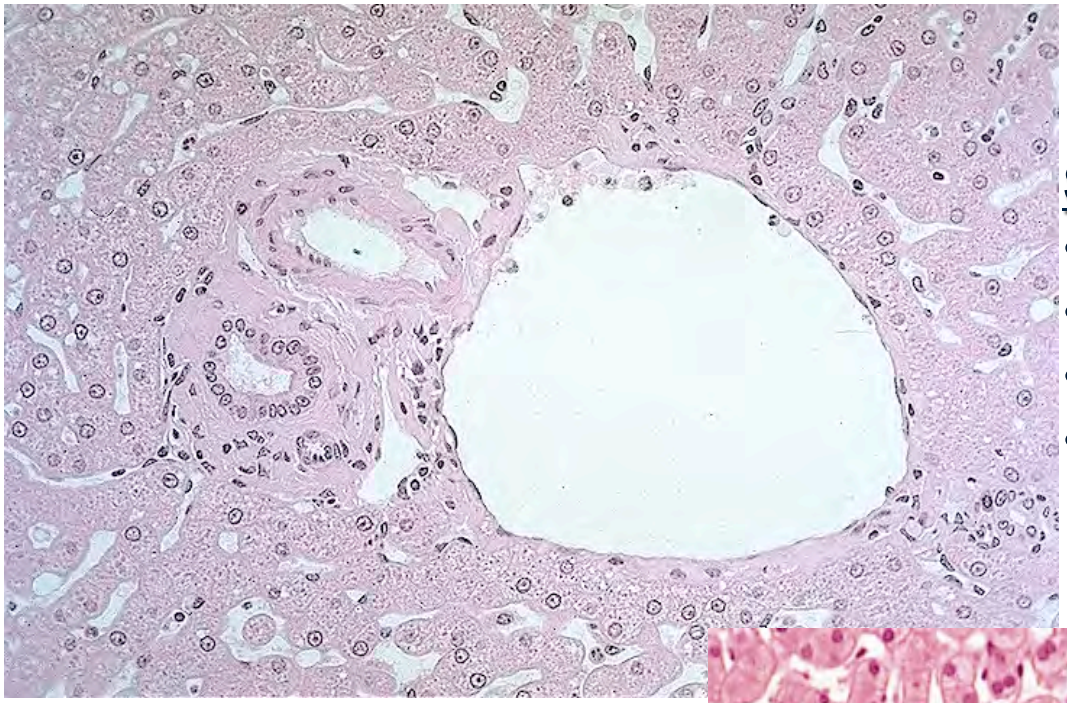






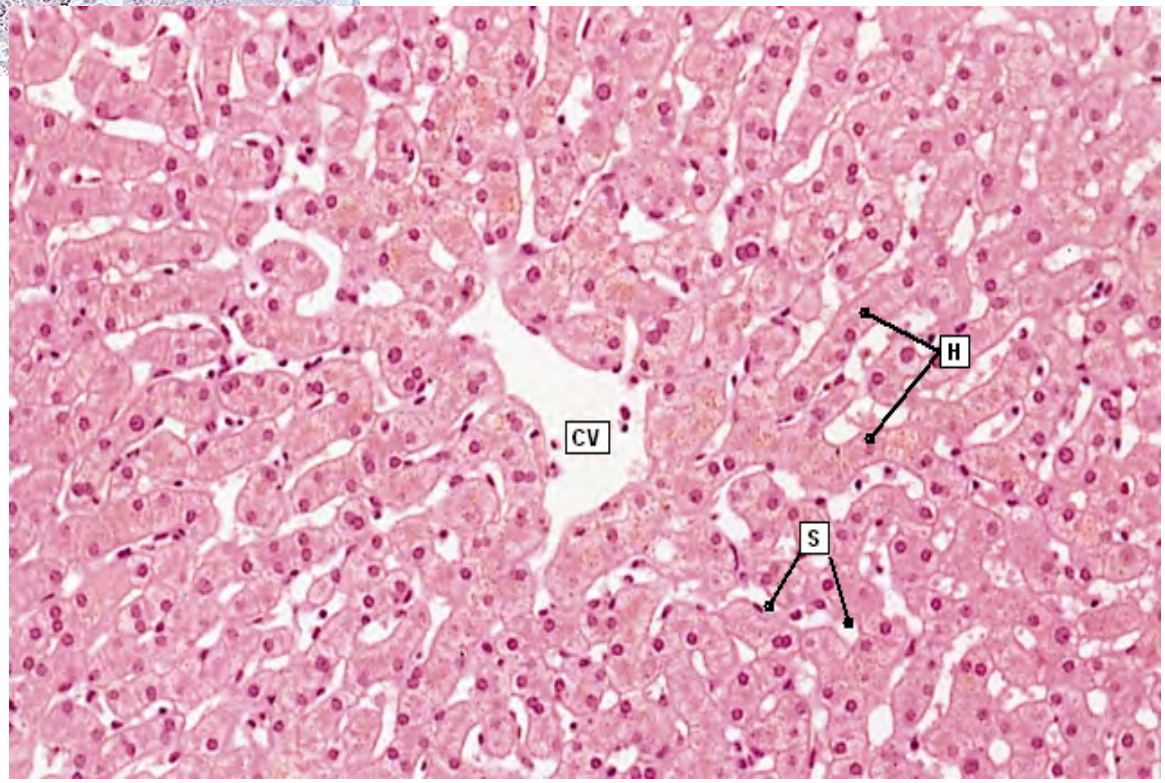


- Il fegato e' irrorato da sangue proveniente dall'intestino (vena porta) e dal cuore (arteria epatica).
- La vena epatica portale e l'arteria epatica si ramificano nel parenchima per dar luogo ad un intimo contatto con gli epatociti.
- Ramificazioni dell' arteria epatica corrono negli spazi portali
- 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.



SPAZIO PORTALE

- VENULA PORTALE TERMINALE
- ARTERIOLA EPATICA
- VASO LINFATICO
- DUTTULO BILIARE



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.

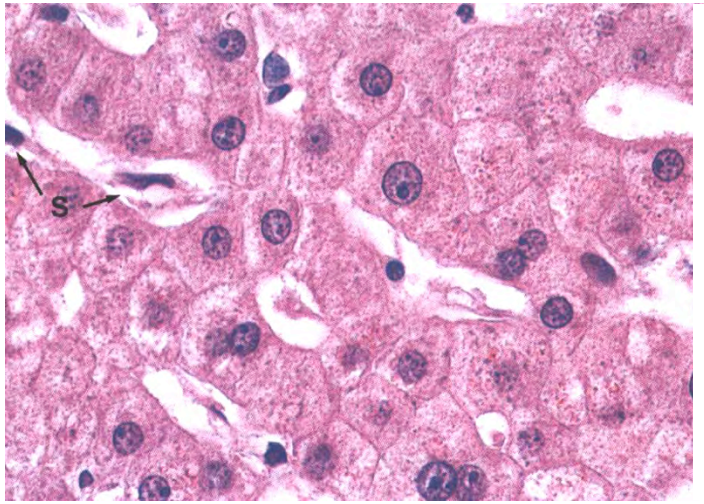
Sinusoidal endothelial cells: Line liver capillaries that separate hepatocytes from sinusoidal blood; pathogenic barrier and general selective barrier; involved in endocytosis and metabolism of molecules including glycoproteins, lipoproteins, ECM components; can produce TGFβ, HGF, IL-6 and nitric oxide.

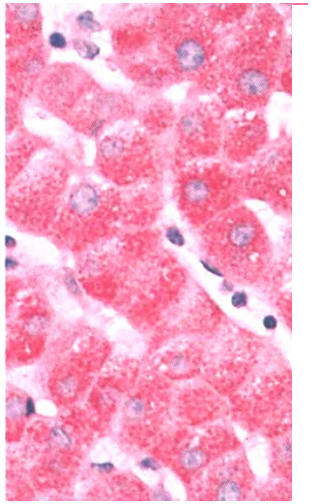
Biliary epithelial cells: Line bile ducts in hepatic portal triads; can secrete cytokines such as monocyte chemotactic protein-1 (MCP-1) and IL-6; promote fibrogenesis by attraction of hepatic stellate cells.

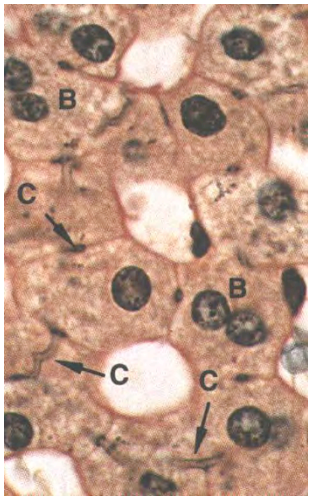
Hepatic stellate cells: Found within the peri-sinusoidal space; store vitamin A, secrete ECM proteins, including laminins, collagens and proteoglycans, growth factors such as HGF, FGF and TGFβ and cytokines such as IL-6; produce some MMPs and TIMPs.

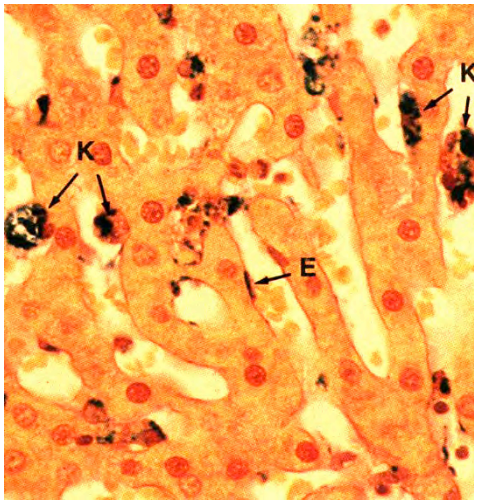
Kupffer cells: Resident liver macrophages found in sinusoids; phagocytosis of foreign particles and bacteria; major producers of cytokines including TNF and IL-6.

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.

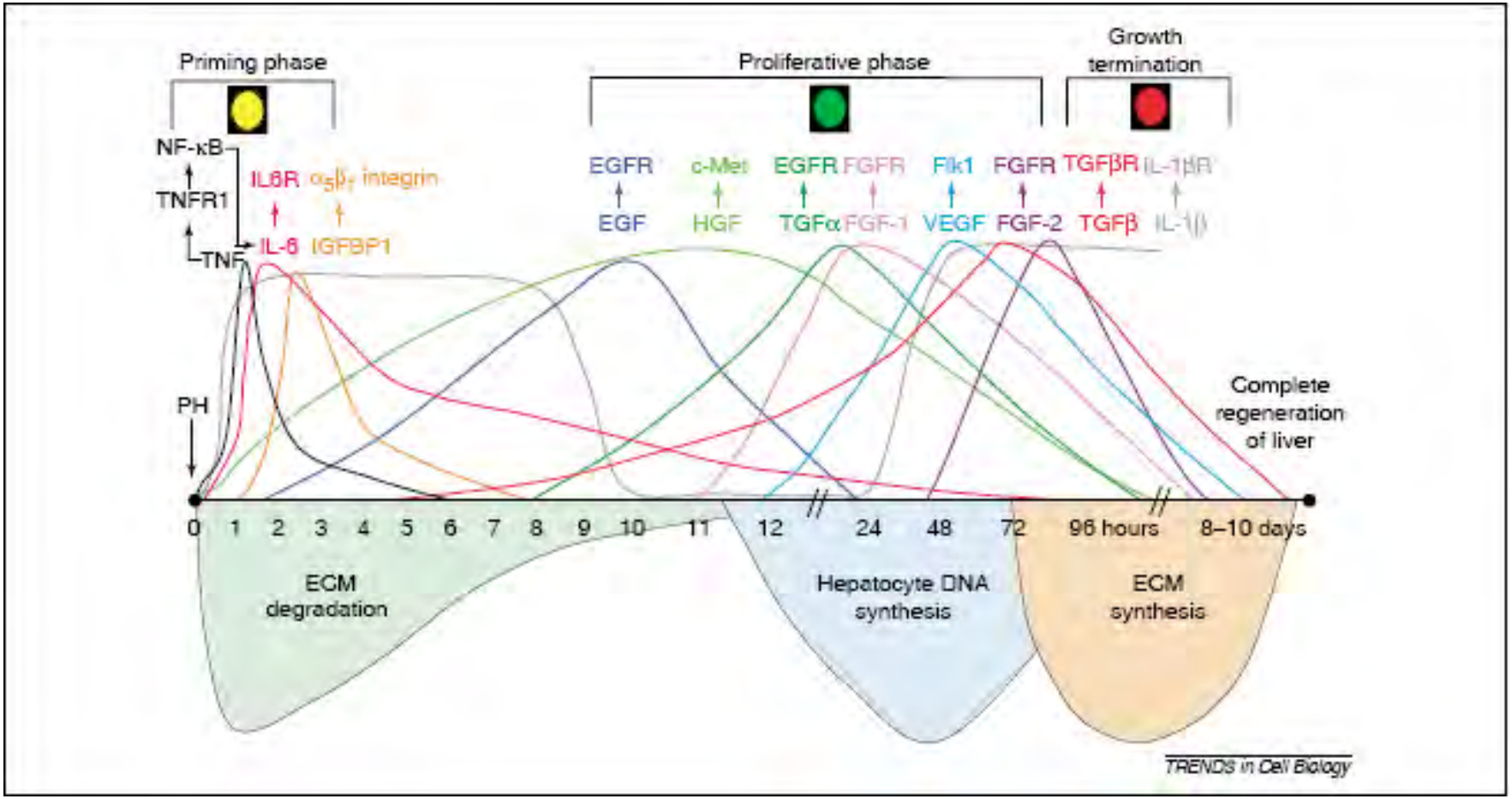
Arch. Pathol. **12**, 186–202 (1931).

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.

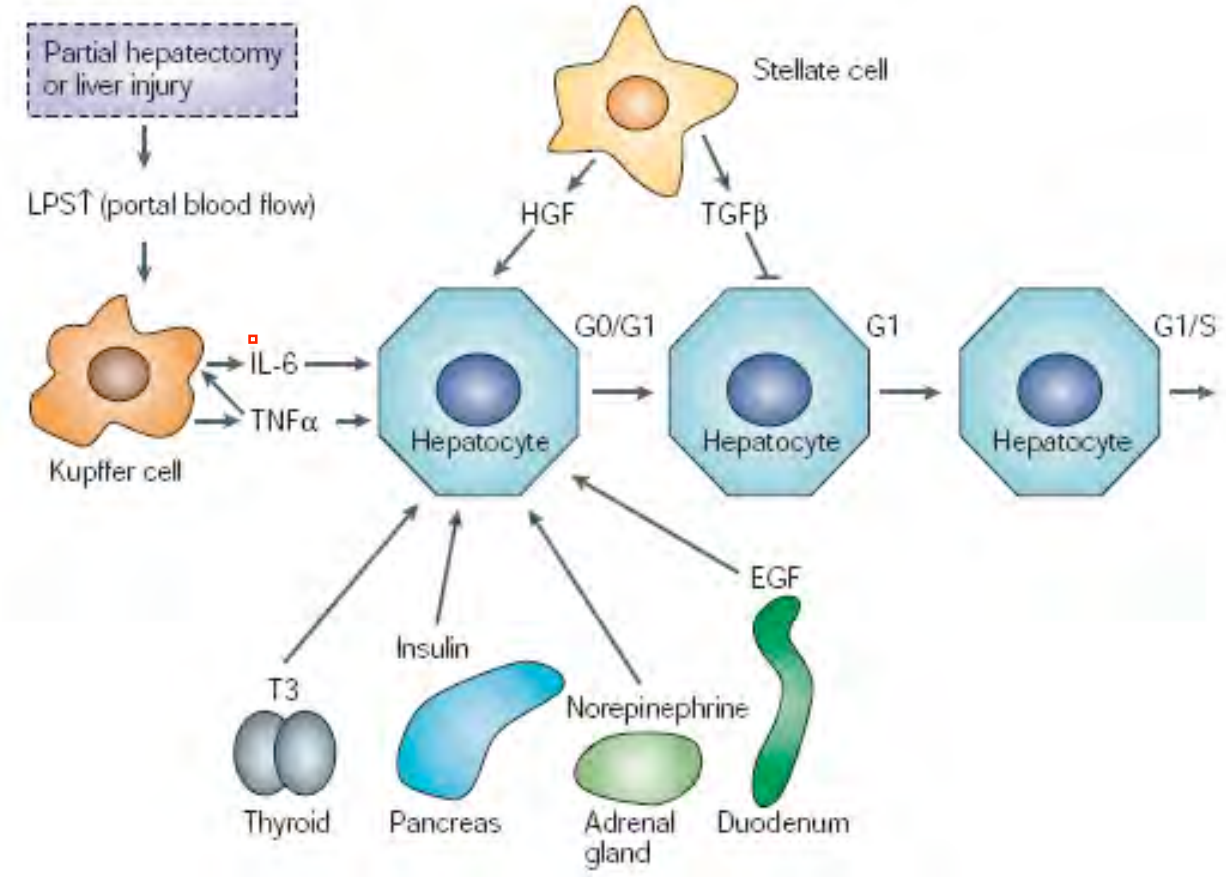
The regenerative process is compensatory: the size of the resultant liver is determined by the demands of the organism, and, once the original mass of the liver has been re-established, proliferation stops.

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

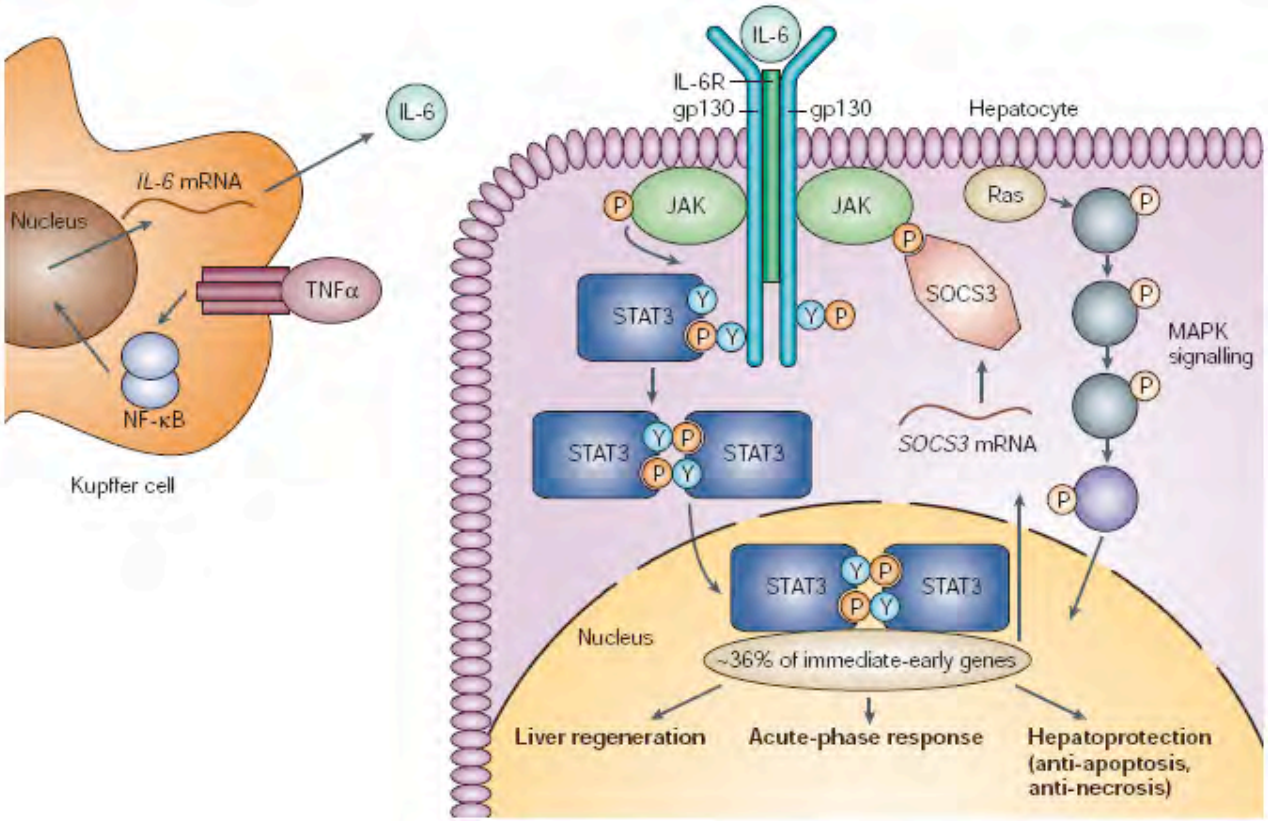


Gut-derived factors, such as lipopolysaccharide (LPS), are upregulated and reach the liver through the portal blood supply. They activate hepatic non-parenchymal cells (including Kupffer cells and stellate cells) and increase the production of tumour necrosis factor TNFα and interleukin IL-6.

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

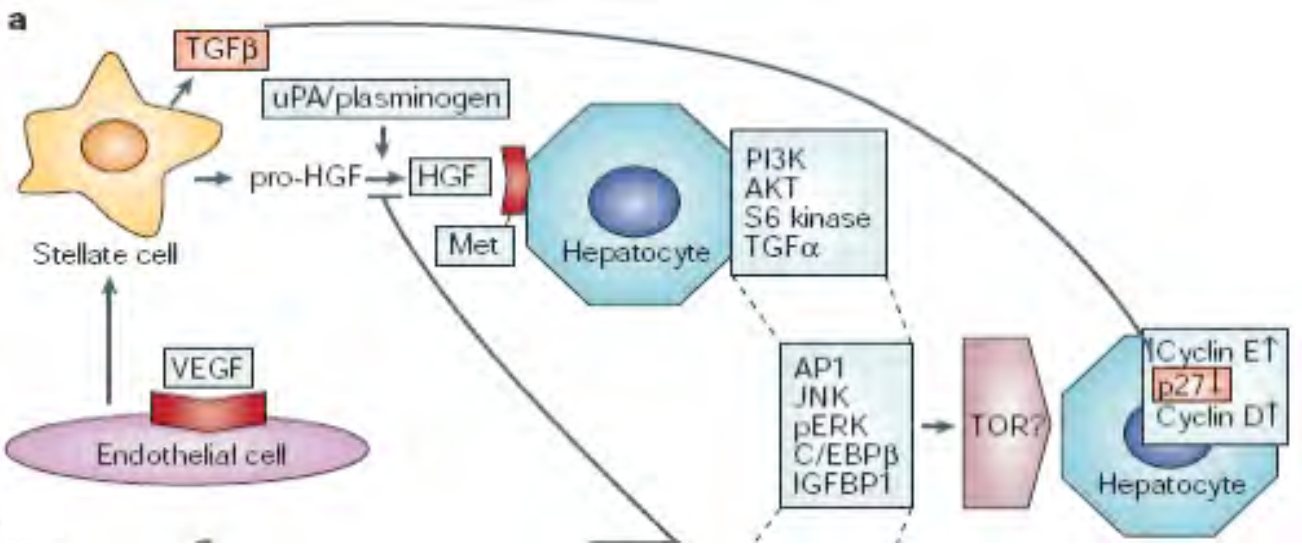


Tumour necrosis factor α binds to its receptor on Kupffer cells, which results in the upregulation of interleukin-6 (*IL-6*) transcription by the nuclear factor (NF)-κB pathway. IL-6 binds to its receptor on hepatocytes and activates Janus kinase (JAK). Activated JAK triggers two pathways:

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.

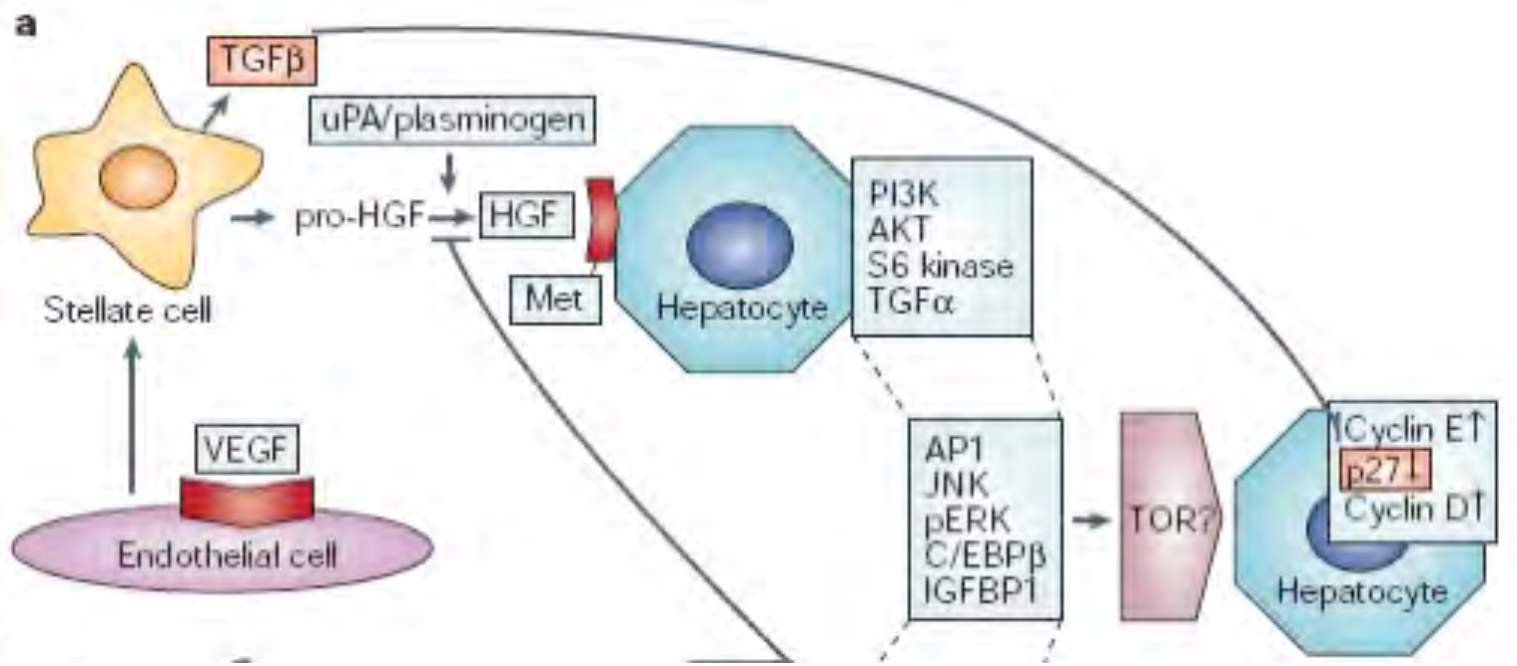
2- Proliferation phase



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



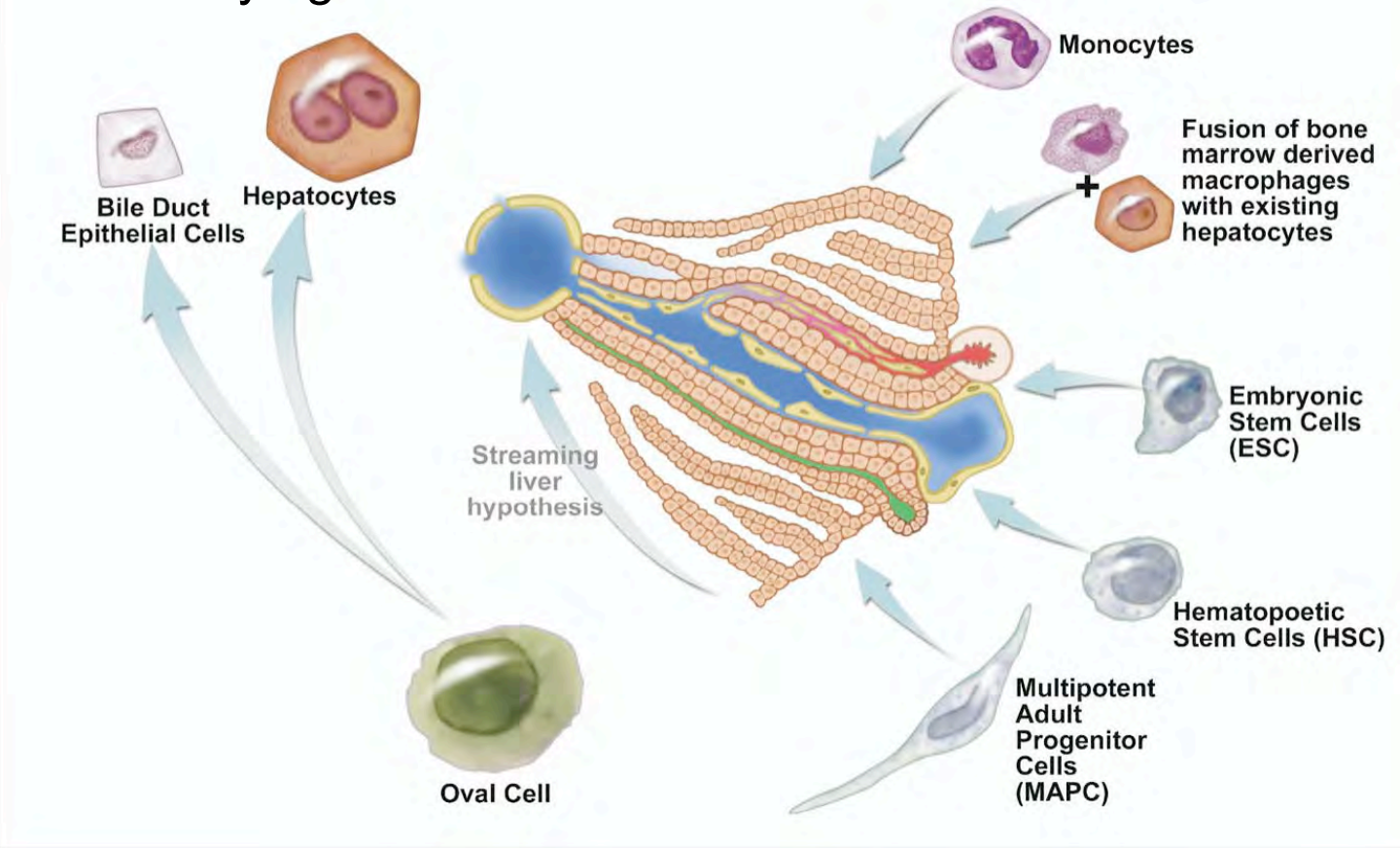
Subsequent to the expansion phase, the growth response must be terminated: major factors involved in this step are members of the TGFβ superfamily, which includes TGFβ1, 2 and 3, activins and inhibins, all signaling through TGFβ receptors. They regulate hepatic mass, inhibit DNA synthesis in hepatocytes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

WHAT ABOUT THE OTHER CELL TYPES??

The origin of hepatocytes…

Unlike blood, skin, and intestine, tissue maintenance in the liver is driven division of the mature epithelial cells.

The "streaming liver hypothesis" that suggested that the liver lobule was similar to the intestinal crypt was disproved. According to this model, young hepatocytes were born from periportal stem cells and then streamed to the central vein as they aged.



…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.

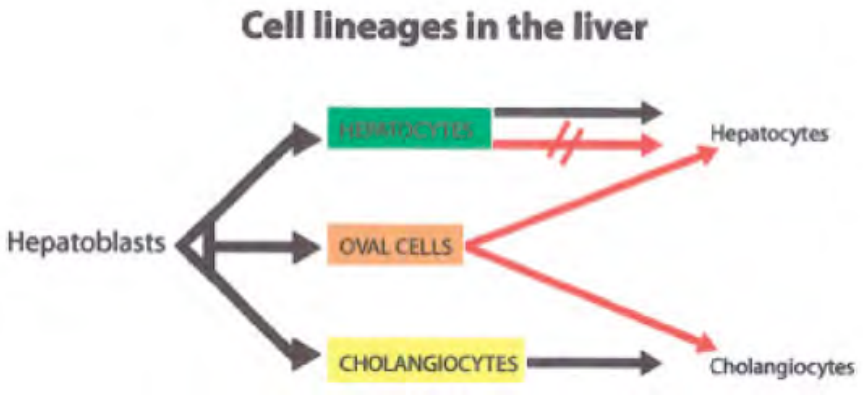


Table 1. Marker genes commonly used to identify oval cells in adult liver

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

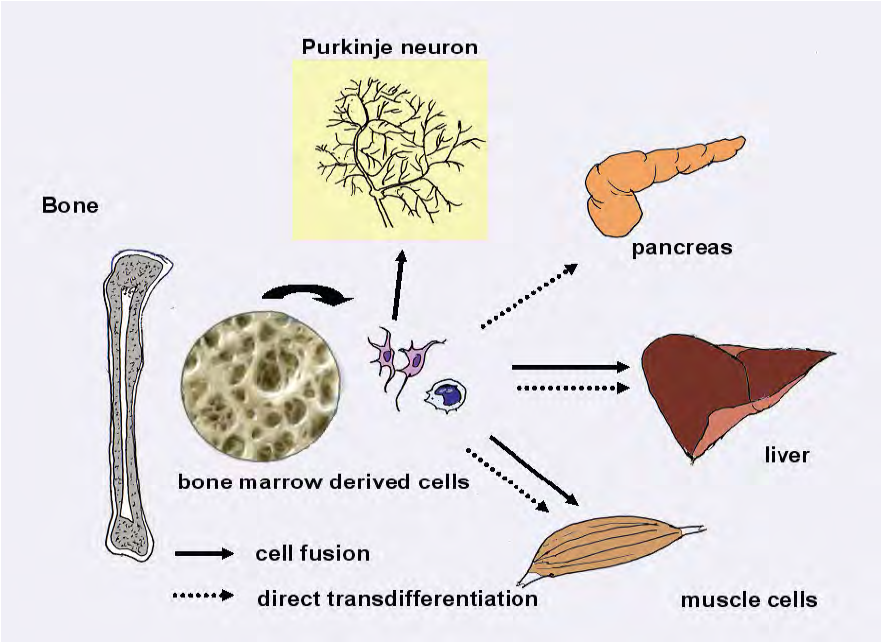
During liver regeneration, oval cells give rise to both hepatocytes and bile duct cells. This pathway can be viewed as a 'second line of defence' following liver injury, when hepatocyte-mediated repair cannot be accomplished.

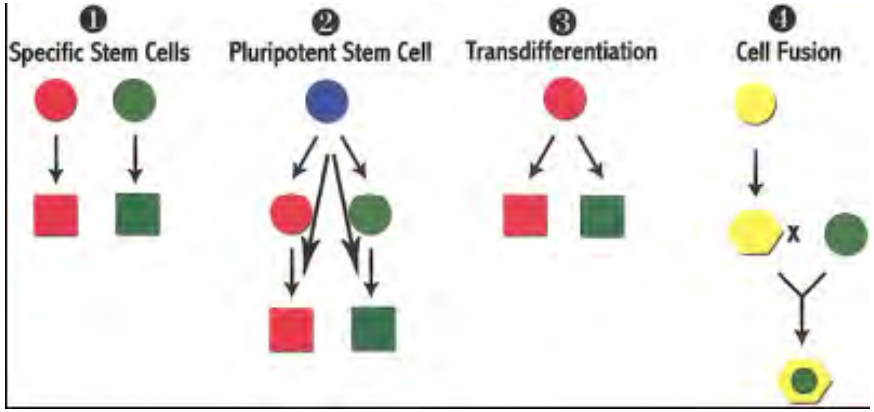
Mr Hyde

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.

Bone Marrow Cells and Hepatocyte Production: Differentiation, Transdifferentiation and Cell Fusion

Hematopoietic stem cells (HSC) and bone marrow mesenchymal stem cells are able to generate many different types of tissue cells (a property known as *transdifferentiation*) and can choose multiple differentiation pathways (a property called *differentiation plasticity*).





The generation of hepatocytes from bone marrow cells is a **very rare event** in liver transplantation and repopulation after injury and such hepatocytes are produced by cell fusion rather than by a transdifferentiation mechanism.

LETTER

https://doi.org/10.1038/s41586-018-0075-5

De novo formation of the biliary system by TGFβ–mediated hepatocyte transdifferentiation

Johanna R. Schaub[1,12], Kari A. Huppert[2,12], Simone N. T. Kurial[1,3,12], Bernadette Y. Hsu[1,3], Ashley E. Cast[2], Bryan Donnelly[4], Rebekah A. Karns[2], Feng Chen[1], Milad Rezvani[1], Hubert Y. Luu[5], Aras N. Mattis[6,7], Anne-Laure Rougemont[8], Philip Rosenthal[7,9], Stacey S. Huppert[2,10,13]* & Holger Willenbring[1,7,11,13]*

studies in mice with a fully developed biliary system[3–6]. In contrast to bile duct development[7–9], we show that de novo bile duct formation by hepatocyte transdifferentiation is independent of NOTCH signalling. We identify TGFβ signalling as the driver of this compensatory mechanism and show that it is active in some patients with ALGS. Furthermore, we show that TGFβ signalling can be targeted to enhance the formation of the biliary system from hepatocytes, and that the transdifferentiation-inducing signals and remodelling capacity of the bile-duct-deficient liver can be harnessed with transplanted hepatocytes. Our results define the

In more than 90 percent of cases, mutations in the JAG1 gene cause Alagille syndrome. A few people with Alagille syndrome have mutations in NOTCH2. Changes in either the JAG1 gene or NOTCH2 gene disrupt the Notch signaling pathway. As a result, errors may occur during development, especially affecting the bile ducts, heart, spinal column, and certain facial features.

Alagille syndrome is a genetic disorder that can affect the liver, heart, and other parts of the body. One of the major features of Alagille syndrome is liver damage caused by abnormalities in the bile ducts which are narrow, malformed, and reduced in number (bile duct paucity). As a result, bile builds up in the liver and causes scarring that prevents the liver from working properly to eliminate wastes from the bloodstream. Alagille syndrome is also associated with several heart problems, including impaired blood flow from the heart into the lungs (pulmonic stenosis). Pulmonic stenosis may occur along with a hole between the two lower chambers of the heart (ventricular septal defect) and other heart abnormalities. This combination of heart defects is called tetralogy of Fallot.

SELECTED REFERENCES: LIVER

LIVER REGENERATION: FROM MYTH TO MECHANISM

*Rebecca Taub**‡

NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 5 | OCTOBER 2004 |

Review *TRENDS in Cell Biology* Vol.15 No.10 October 2005

ELSEVIER

Thinking outside the cell: proteases regulate hepatocyte division

Fazilat F. Mohammed and Rama Khokha

Liver Regeneration and Repair: Hepatocytes, Progenitor Cells, and Stem Cells

Nelson Fausto[1]

HEPATOLOGY, Vol. 39, No. 6, 2004

Unanswered questions:

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

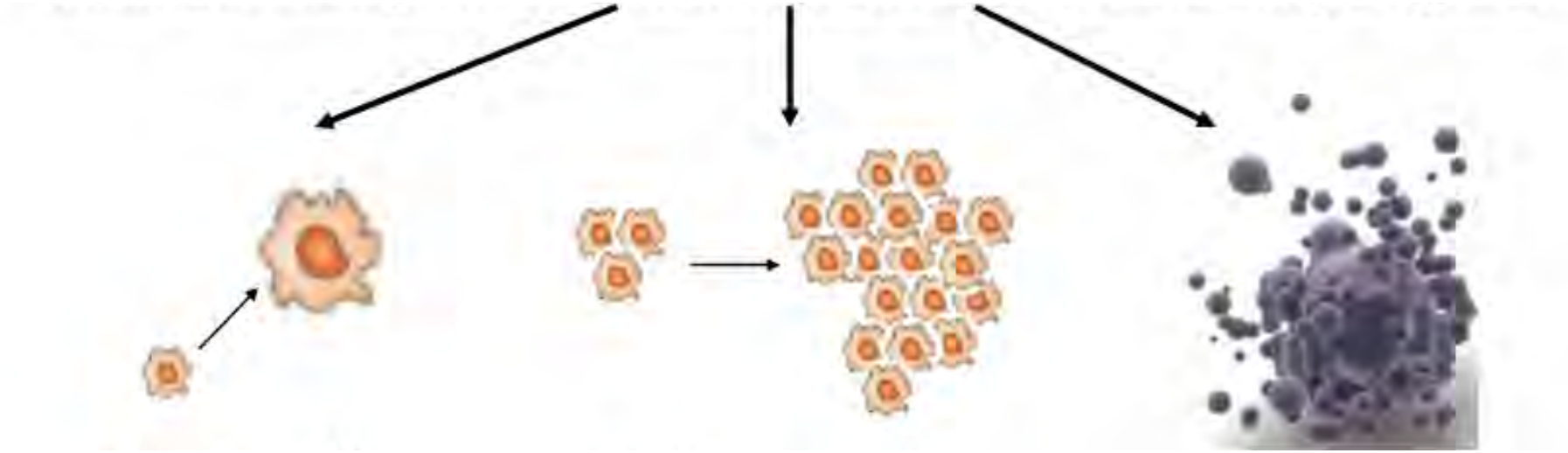
- how do these mechanisms relate to normal regulation of organ size during development?

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

<u>How is the organ size regulated?</u>



Hypertrophic growth

Proliferation

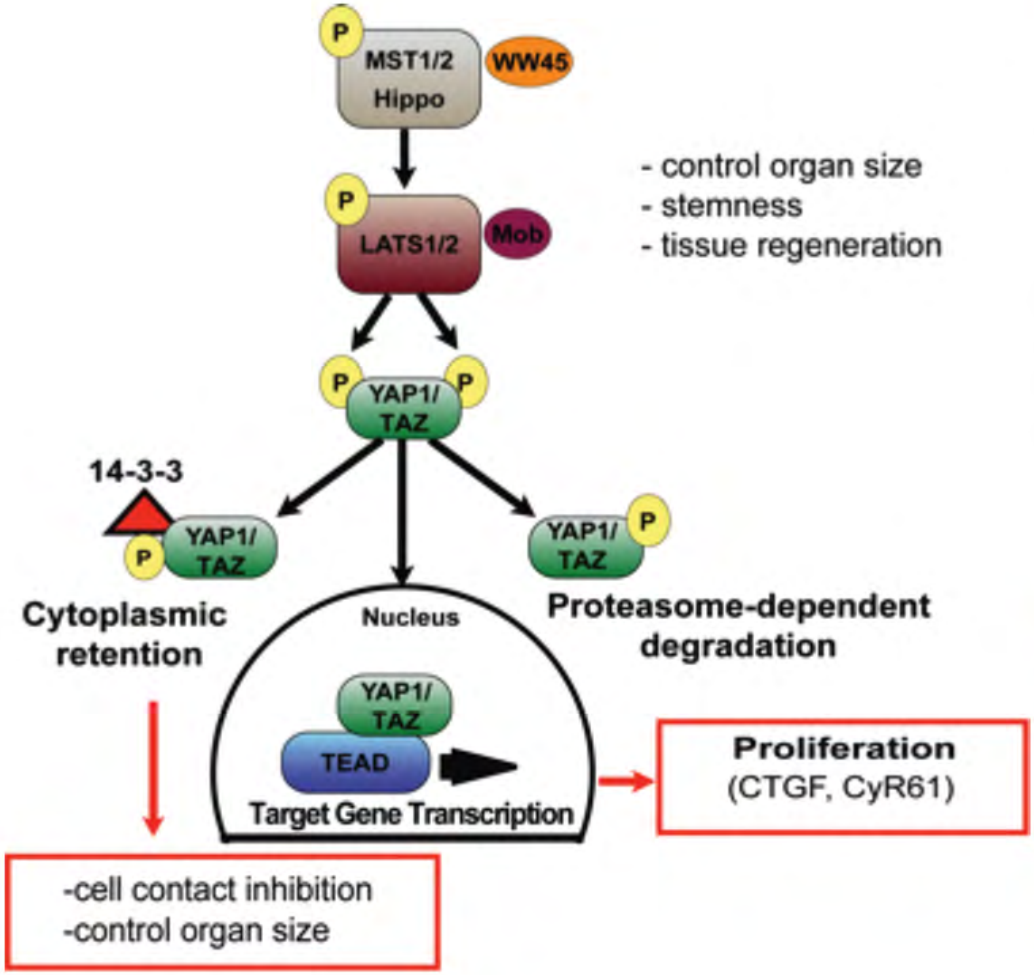
Apoptosis

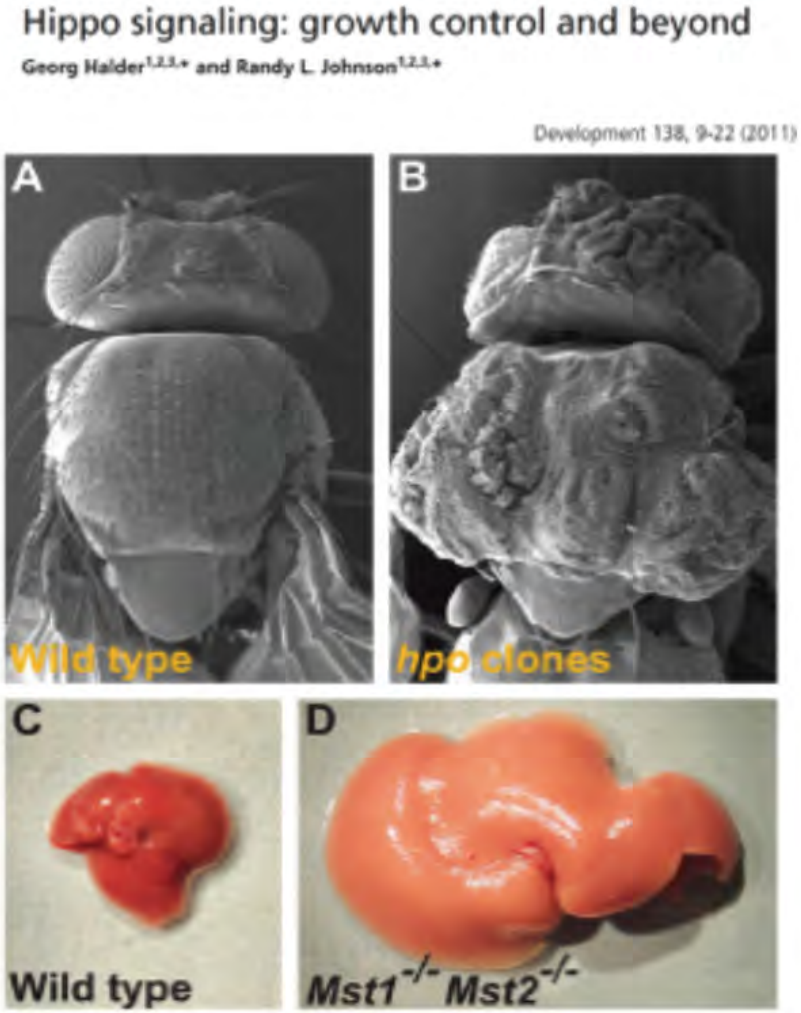
Proliferation

(Hyperplastic growth)

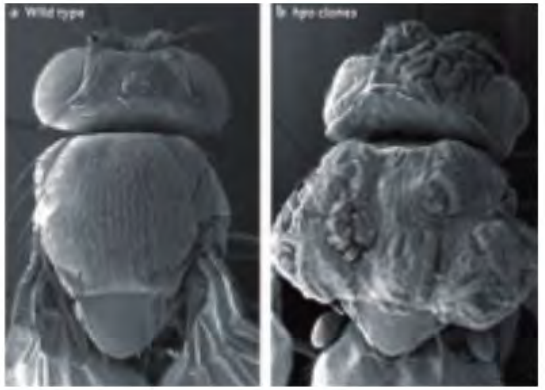
Hippo Pathway

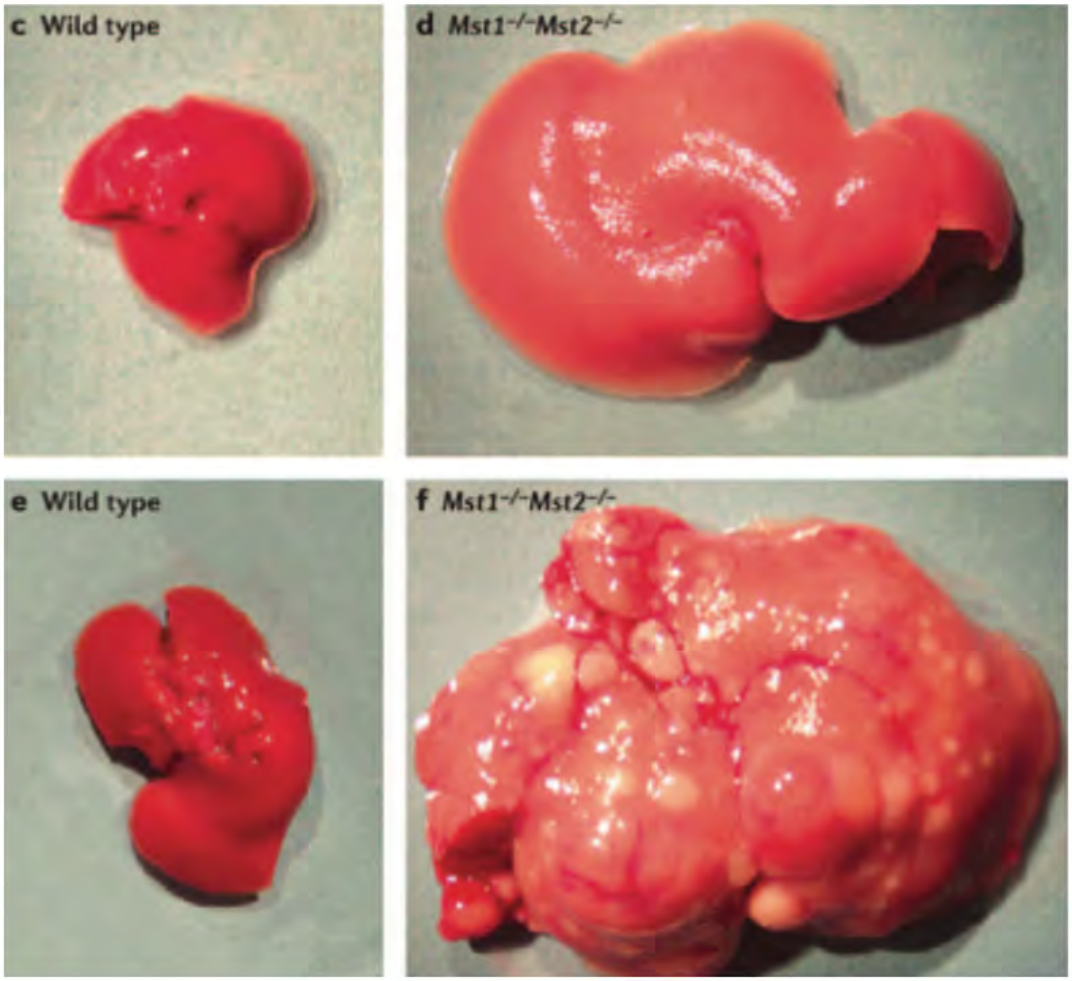
Hippo/YAP Signaling Pathway





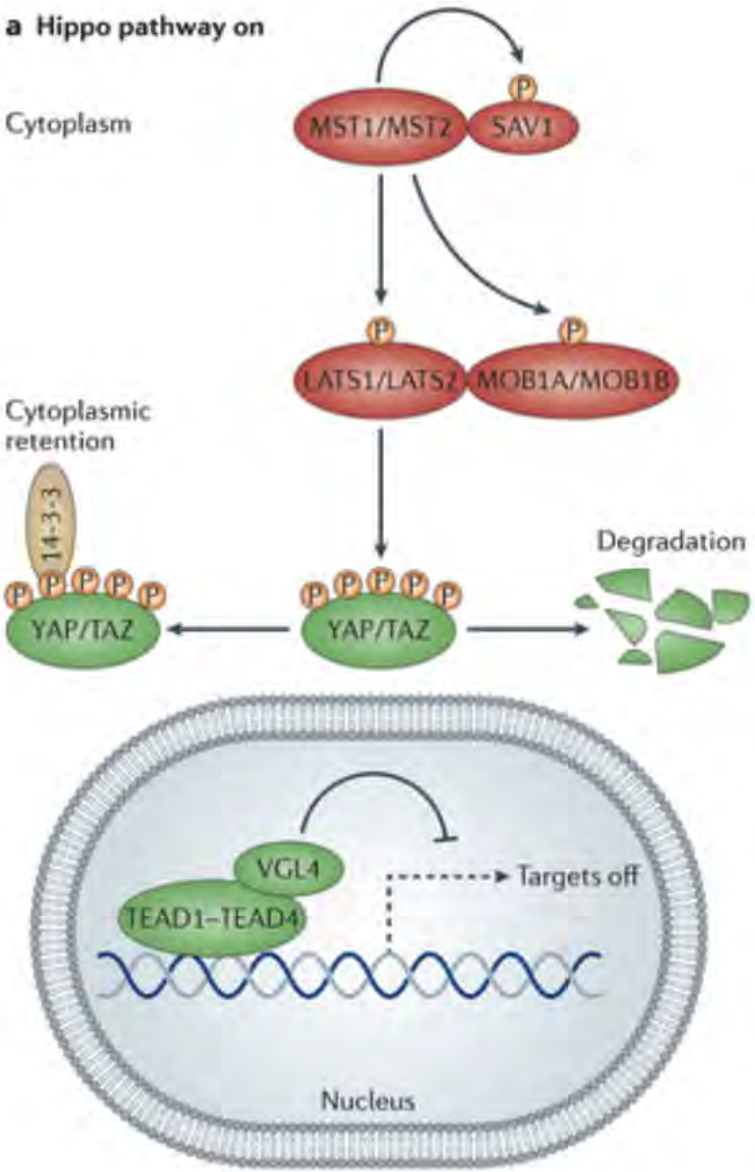
Hippo signaling pathway





- 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)



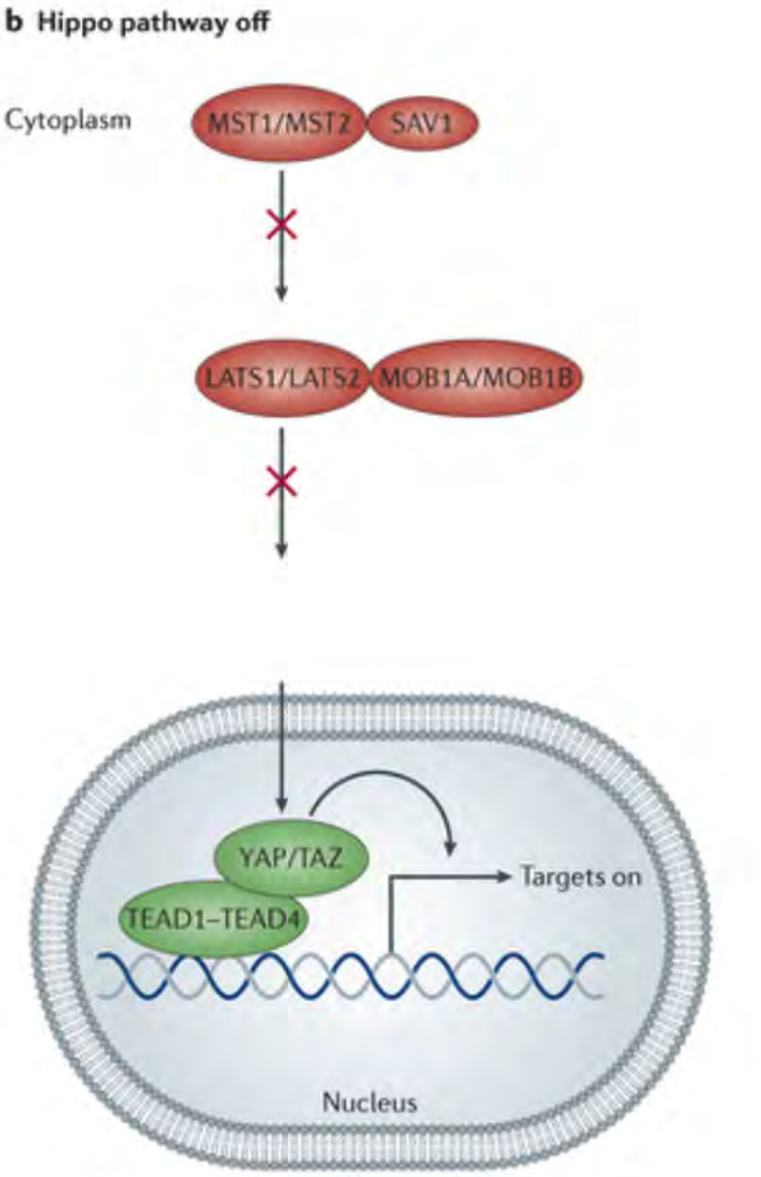
Johnson et al., 2013.

Hippo signaling pathway

When the Hippo pathway is **ON**, the mammalian STE20-like protein kinase1 (MST1) or MST2 phosphorylate Salvador homolog 1 (SAV1) and together they phosphorylate and activate MOB kinase activator 1A, MOB1B, the large tumor suppressor homolog 1 (LATS1) and LATS2 kinases, which then phosphorylate the Yes-associated protein (YAP) and the transcriptional coativator with PDZ-binding motif (TAZ).

Phosporylated YAP and TAZ are sequestered in the cytoplasm by the 14-3-3 protein and shunted for proteasomal degradation.

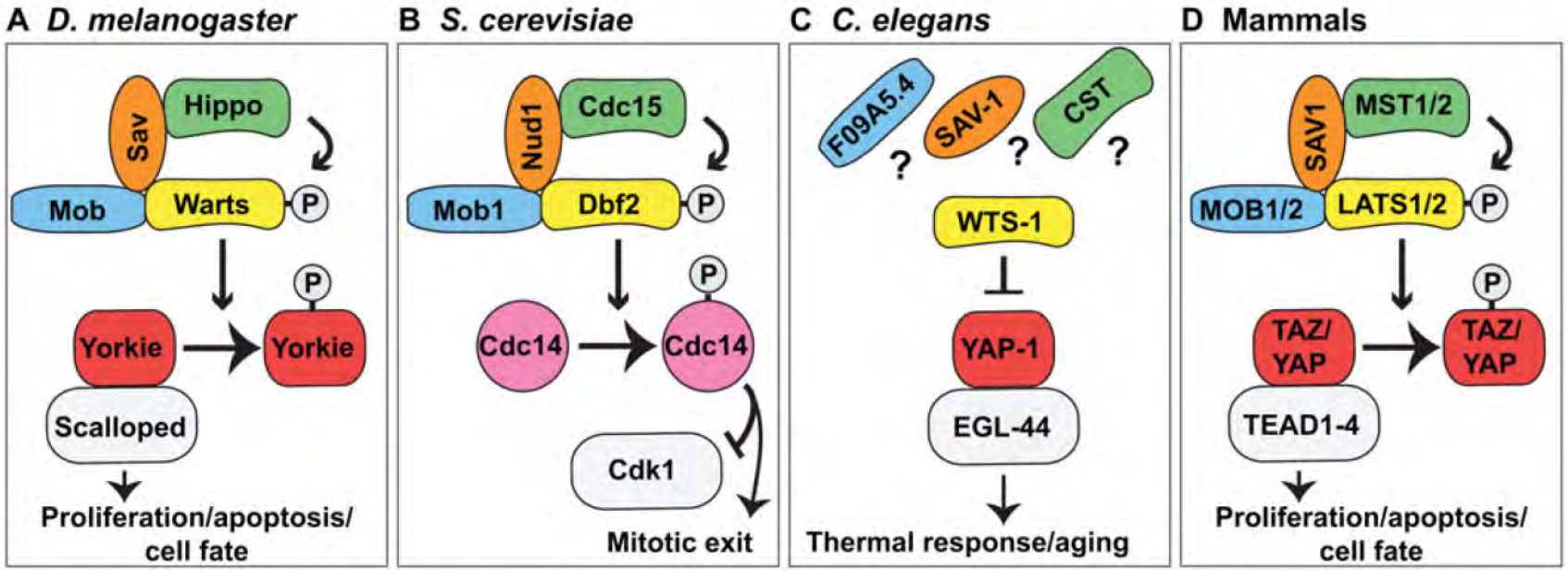
As a results, the TEA domain containing sequence-specific transcription factors (TEADs) associate with the transcription cofactor vestigial-like protein4 (VGL4) and suppress target gene expression (pro-proliferative and anti-apoptotic genes).



Hippo signaling pathway

When the Hippo pathway is **OFF**, the kinases MST1, MST2, LATS1 and LATS2 are inactive, so YAP and TAZ are not phosphorylated and accumulate in the nucleus where they displace VGL4 and form a complex with TEADs, which promote the expression of target genes.

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.