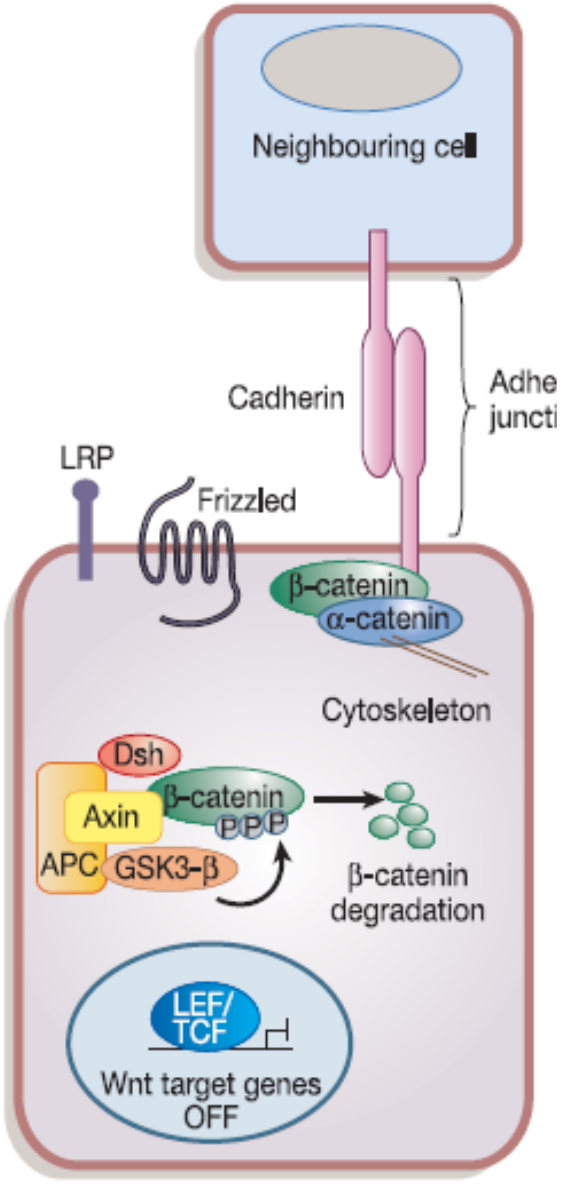
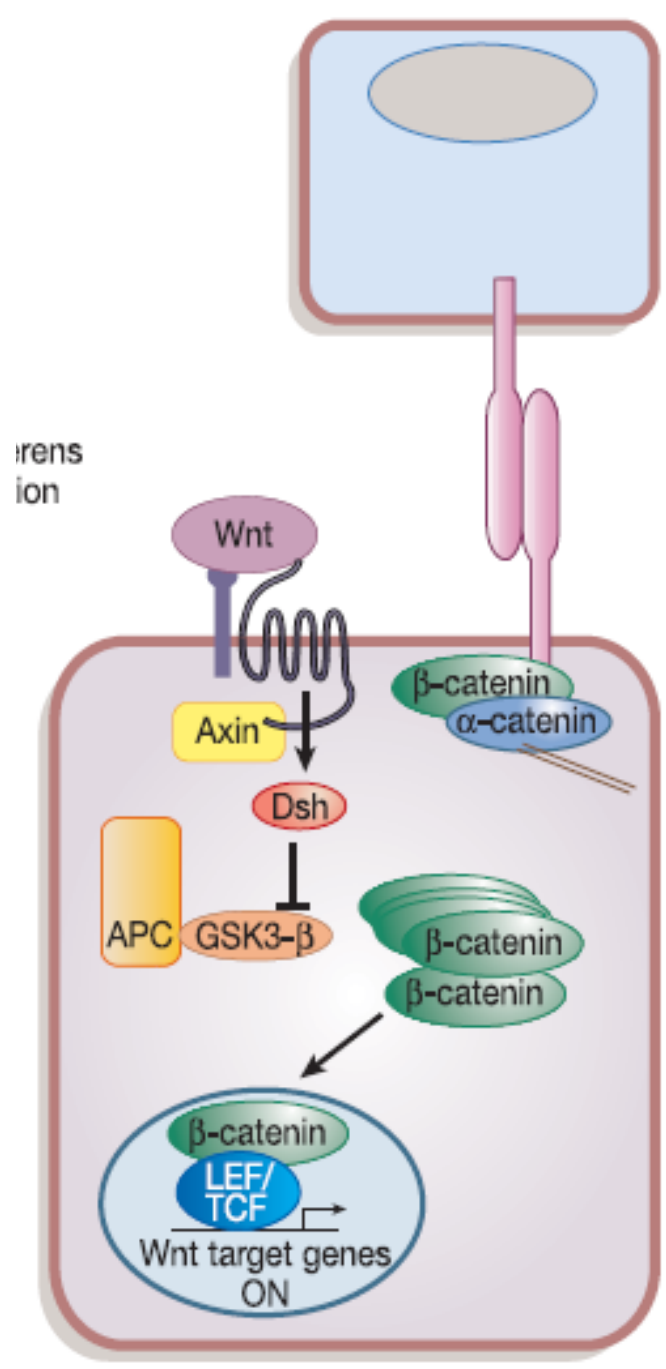
The Wnt/β-catenin signalling pathway

In the **absence of Wnt ligand**, β-catenin is sequestered in a *multiprotein degradation complex* containing the scaffold protein Axin, APC, casein kinase I (CKI) and glycogen synthase kinase 3β (GSK3β).

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

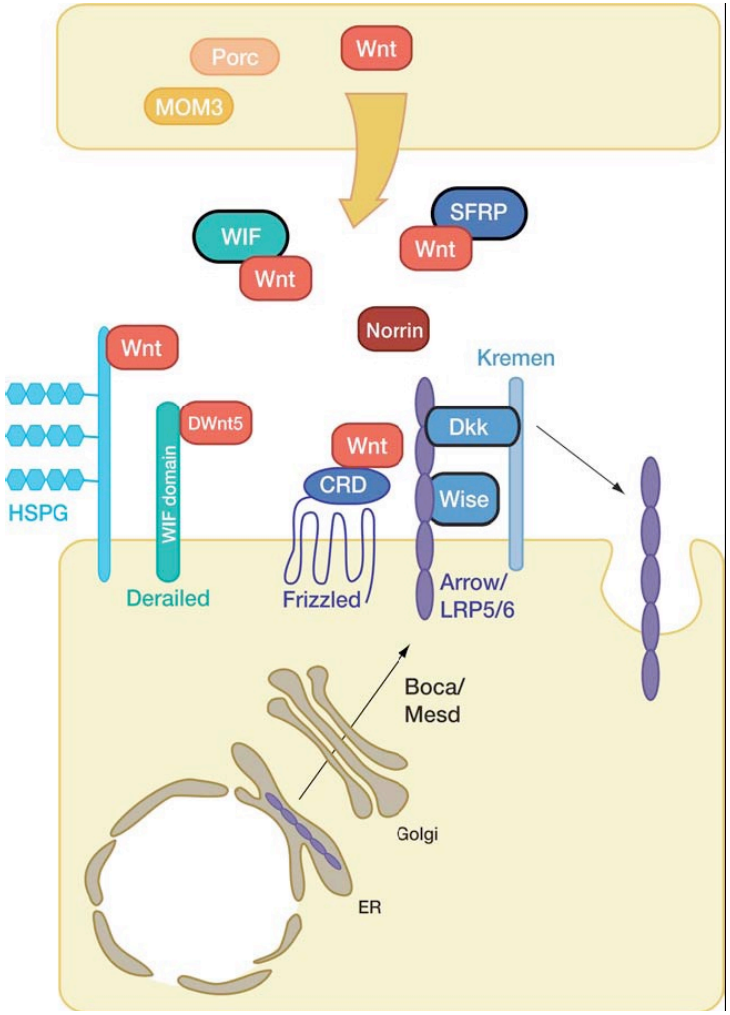




In the **presence of Wnt ligand**, Axin is recruited to the plasma membrane. β-catenin is then released from the multiprotein degradation complex and accumulates in the cytoplasm in a stabilized non-phosphorylated form.

As a consequence, β-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.



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Notum deacylates Wnt proteins to suppress signalling activity

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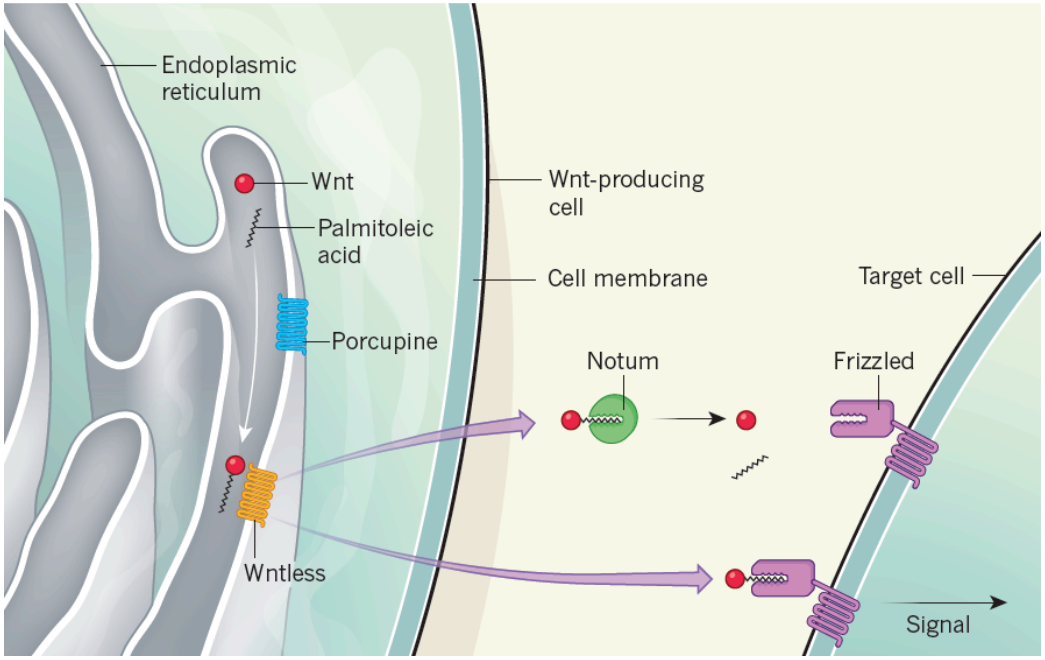


Notum shoots the messenger in Wnt signalling.
In Wnt-producing cells, the Wnt protein is made in the endoplasmic reticulum. There, an acyl group is added to Wnt by the membrane-spanning enzyme Porcupine. Secreted Wnt binds to its receptor Frizzled on target cells. This binding depends on the acyl group in Wnt.
Kakugawa *et al*. report that the Wnt–Frizzled interaction is inhibited by the extracellular enzyme Notum, which specifically removes the acyl group from Wnt.

TABLE 1 Wnt mutant phenotypes in the mouse

Gene	Knockout (KO) phenotypes or other functions	Redundancies/ similarities with other KO	References
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)	Redundant with Wnt3a and Wnt4; similar to TCF1	(Ikeya et al. 1997, Mulroy et al. 2002)
Wnt3	Early gastrulation defect, perturbations in establishment and maintenance of the apical ectodermal ridge (AER) in the limb	In limbs, similar to loss of β-catenin	(Barrow et al. 2003, P. Liu et al. 1999)
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	Redundant with Wnt1, similar to LEF1/TCF1	(Aulehla et al. 2003; Galceran et al. 1999, 2000; Ikeya et al. 1997; Lee et al. 2000; Yoshikawa et al. 1997)
Wnt4	Defects in female development; absence of Mullerian duct, defects in adrenal gland development Decrease in thymocyte number (with Wnt1 KO)	Wnt1	(Heikkila et al. 2002, Mulroy et al. 2002, Vainio et al. 1999)
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		(Li et al. 2002, Liang et al. 2003, Yamaguchi et al. 1999, Y. Yang et al. 2003)
Wnt7a	Female infertility; in males, Mullerian duct regression fails Delayed maturation of synapses in cerebellum		(Hall et al. 2000, Parr & McMahon 1998)
Wnt7b	Placental development defects Respiratory failure, defects in early mesenchymal proliferation leading to lung hypoplasia		(Parr et al. 2001, Shu et al. 2002)
Wnt11	Ureteric branching defects and kidney hypoplasia		(Majumdar et al. 2003)

TABLE 4 Frizzled phenotypes in mammals

Gene	Knockout (KO) phenotypes or other functions	References
Fz3 (*Mfz3*)	Defect in fiber tracts in the rostral CNS Perturbed anterior-posterior guidance of commissural axons	(Lyuksyutova et al. 2003, Wang et al. 2002)
Fz4 (*Mfz4*)	Cerebellar, auditory, and esophageal defects In humans, retinal angiogenesis in familial exudative vitreoretinopathy (FEVR)	(Wang et al. 2001, Robitaille et al. 2002, Xu et al. 2004, Toomes et al. 2004)
Fz5 (*Mfz5*)	Essential for yolk sac and placental angiogenesis	(Ishikawa et al. 2001)
Fz6 (*Mfz6*)	Hair patterning defects	(Guo et al. 2004)

WNT SIGNALING IN CANCER AND HUMAN DISEASE

- In adults, mis-regulation of the Wnt pathway also leads to a variety of abnormalities and degenerative diseases

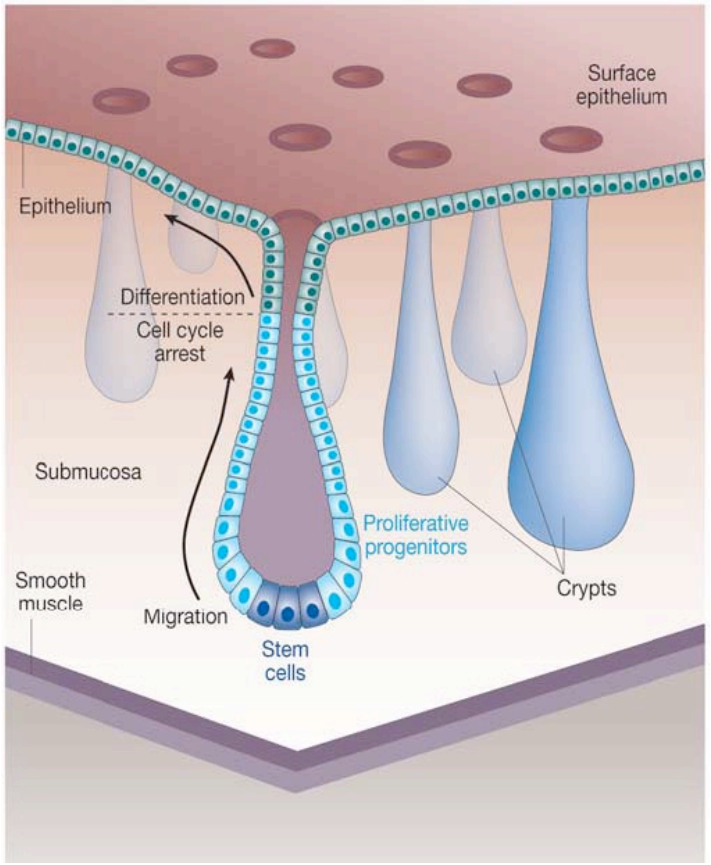
TABLE 5 Human genetic diseases and Wnt signaling components

Gene	Disease	References
WNT3	Tetra-amelia	(Niemann et al. 2004)
LRP5	Bone density defects Vascular defects in the eye (osteoperosis-pseudoglioma syndrome, OPPG; familial exudative vitreoretinopathy, FEVR)	(Boyden et al. 2002, Gong et al. 2001, Little et al. 2002, Toomes et al. 2004)
FZD4	Retinal angiogenesis defects (familial exudative vitreoretinopathy, FEVR)	(Robitaille et al. 2002 Xu et al. 2004, Toomes et al. 2004)
Axin2	Tooth agenesis Predisposition to colorectal cancer	(Lammi et al. 2004)
APC	Polyposis coli, colon cancer	(Kinzler et al. 1991, Nishisho et al. 1991)

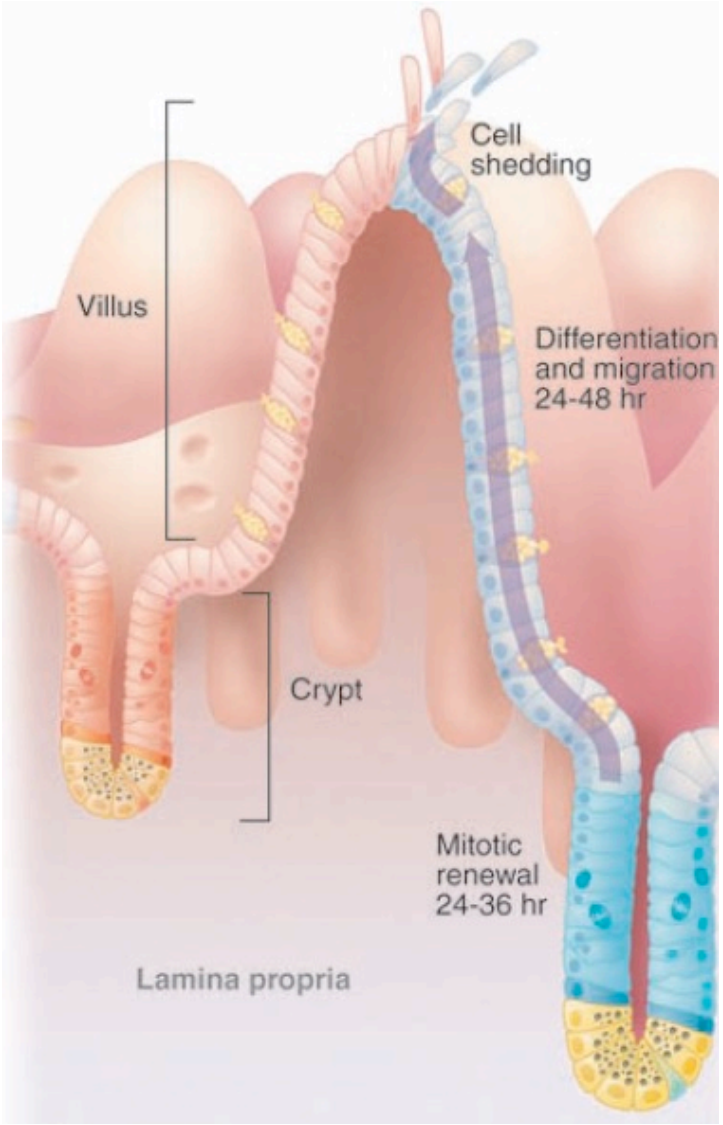
From crypt physiology to colon cancer

- Current evidence indicates that the Wnt cascade is the single most dominant force in controlling cell fate along the crypt-villus axis.

- In Tcf4 -/- neonatal mice, the villus epithelial compartment appears unaffected but the crypt progenitor compartment is entirely absent, implying that physiological Wnt signalling is required for maintenance of the crypt progenitor phenotype.



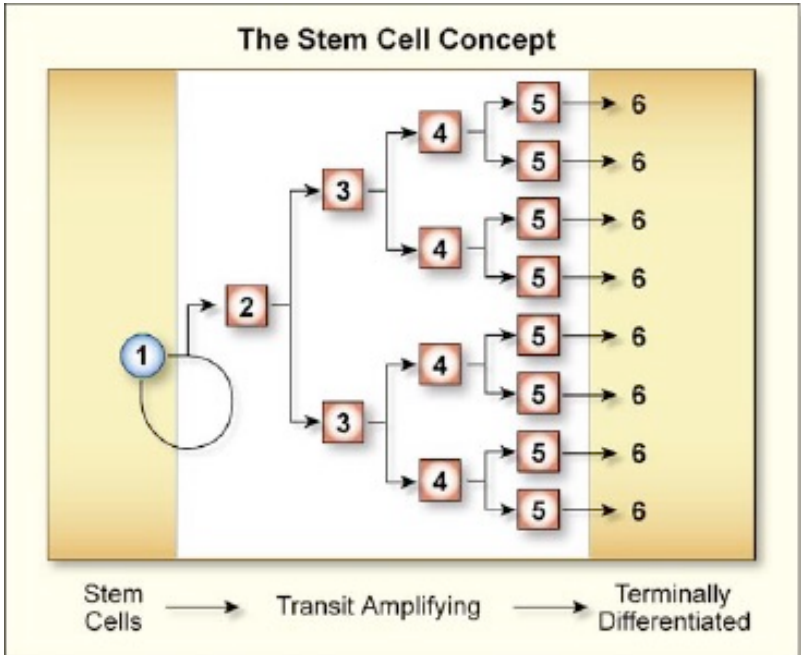
The intestinal epithelium: a dynamic tissue



The absorptive epithelium of the **small intestine** is ordered into submucosal invaginations, the **crypts of Lieberkuhn**, and luminal protrusions termed **villi**.

1- The **crypt** is mainly a *proliferative* compartment, *monoclonal* and is maintained by multipotent stem cells.

2- The **villus** represents the *differentiated* compartment, it is *polyclonal* as it receives cells from multiple crypts.



Stem cells in intestinal crypts are characterized by distinctive features:

(1) Monoclonal origin,

(2) Retention of an undifferentiated phenotype,

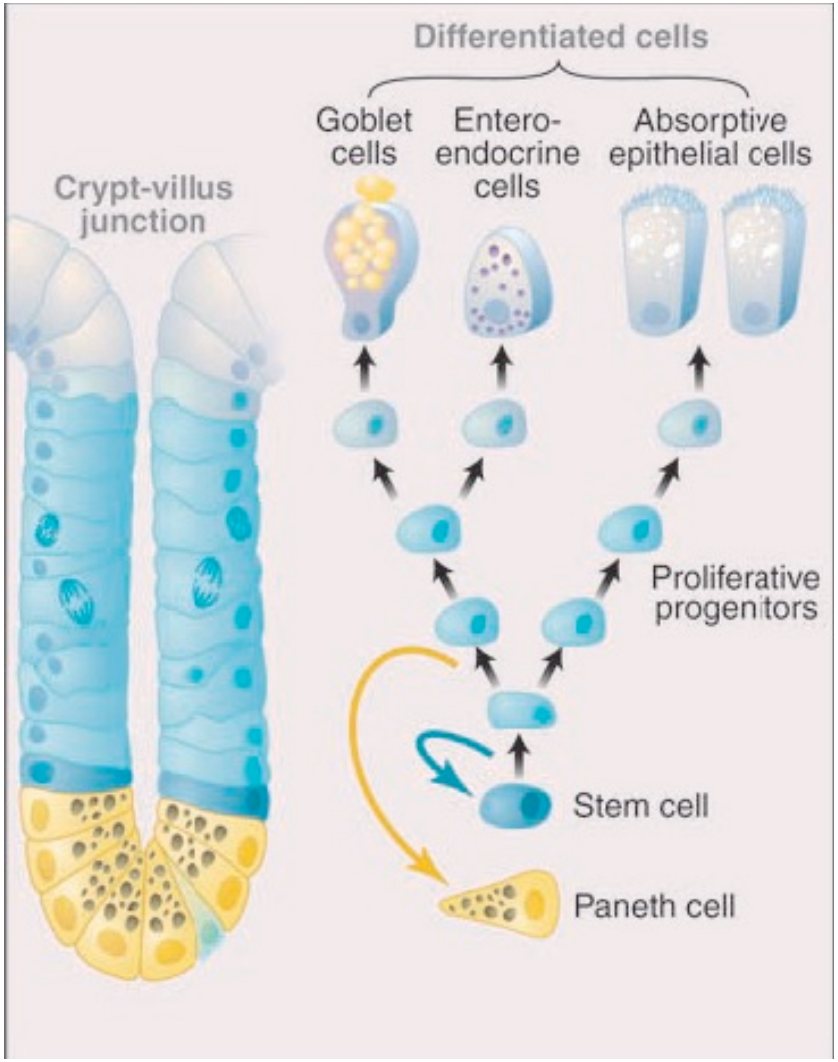
(3) Ability to divide asymmetrically,

(4) Multipotency,

(5) High proliferative potential throughout life,

(6) Ability to repopulate entire crypts upon injury,

(7) Anchorage at the crypt base, in the stem cell niche.



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**;

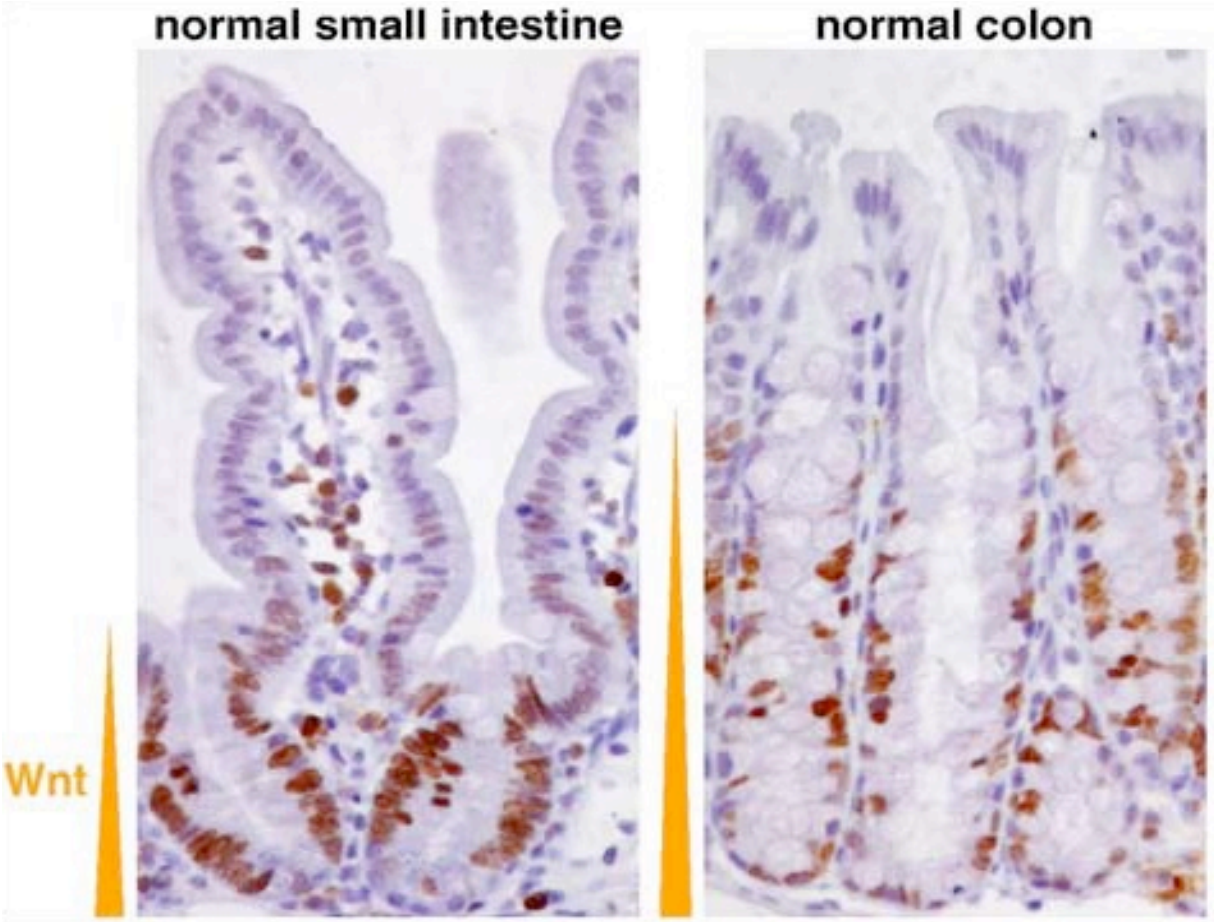
3- **enteroendocrine cells**, secreting hormones such as serotonin or secretin;

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.

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



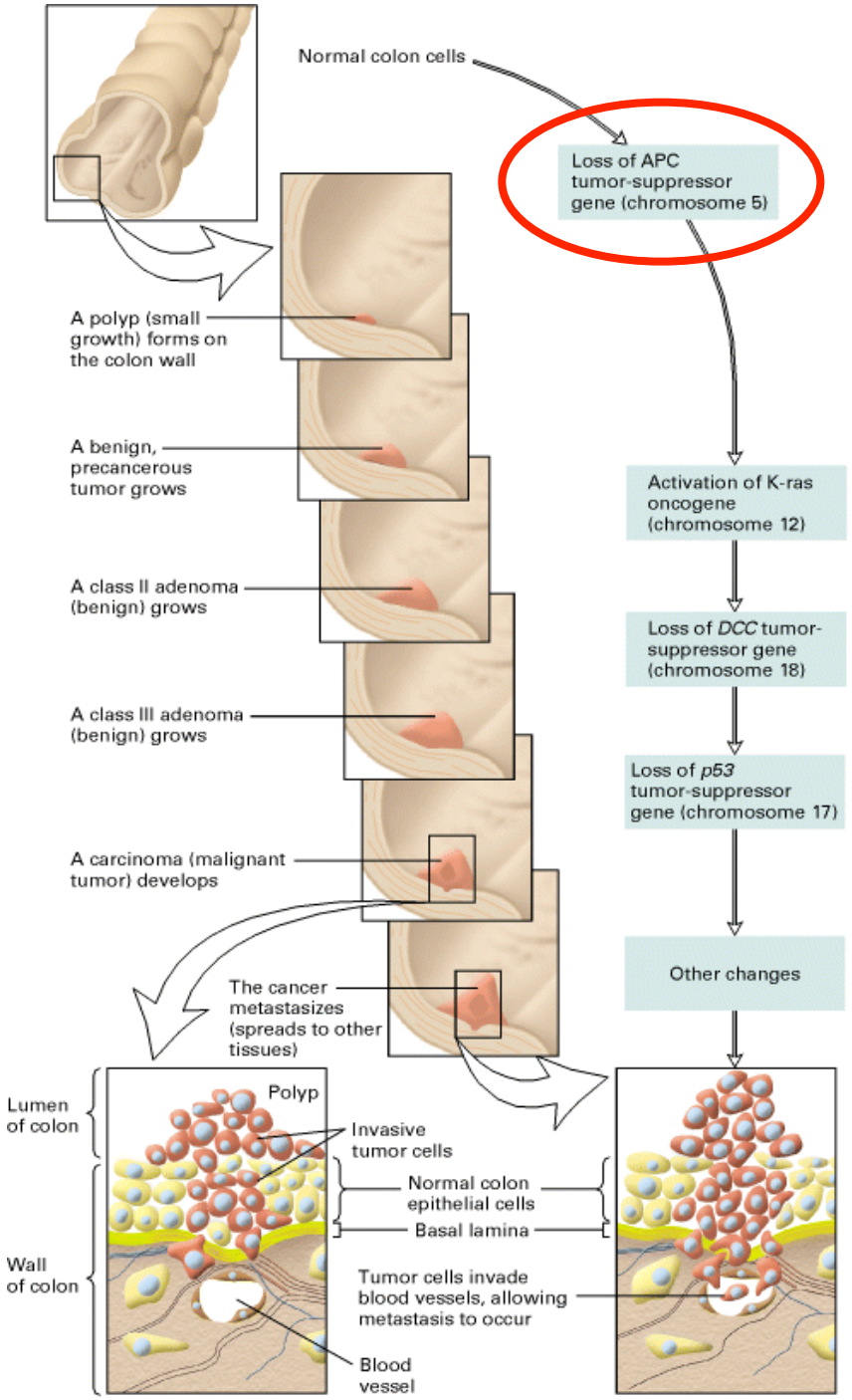
The multistep evolution of cancer

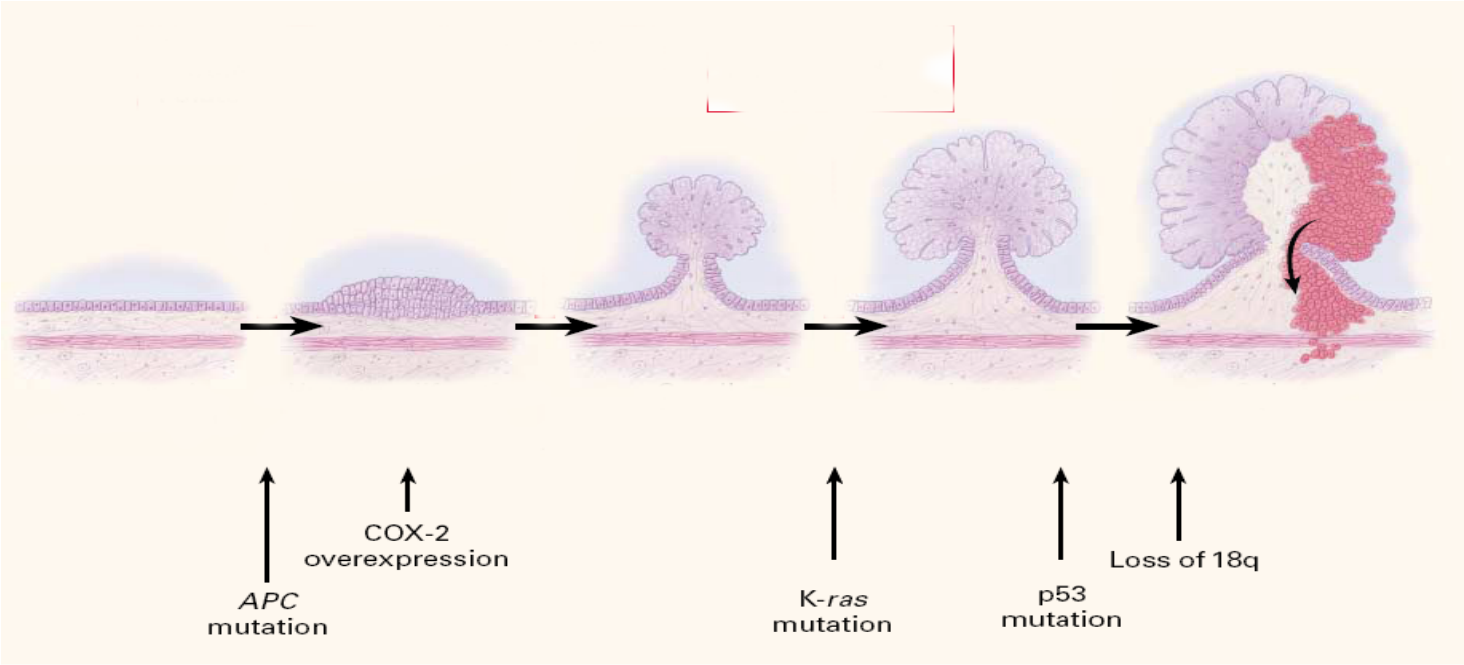
(Fearon & Vogelstein, 1990)

(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.





•In the **adenoma-carcinoma sequence**, the earliest identifiable lesion is a **small dysplastic lesion** in the colonic epithelium.

•Aberrant crypt foci expand over time to form macroscopically visible **adenomatous polyps**.

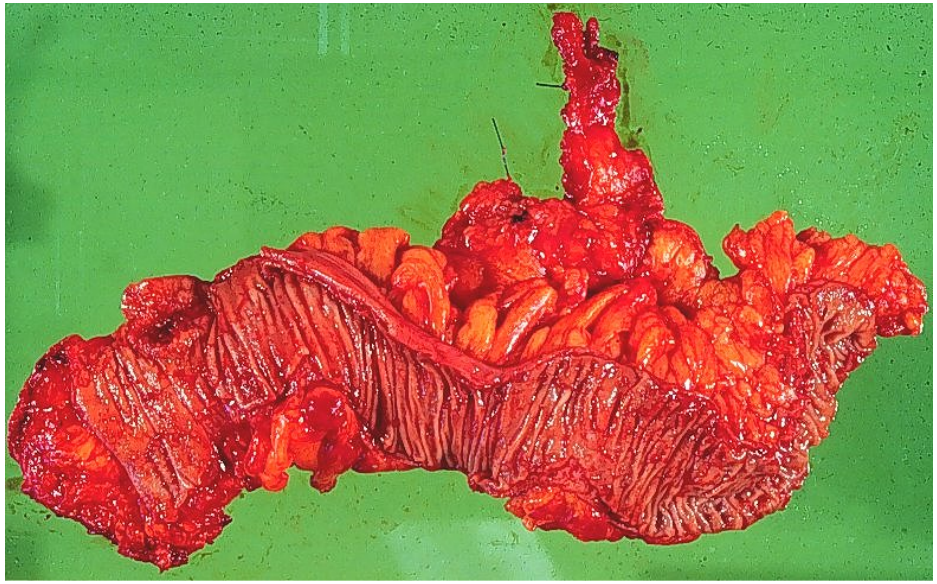
•Adenomas first advance to the **carcinoma in situ** stage.

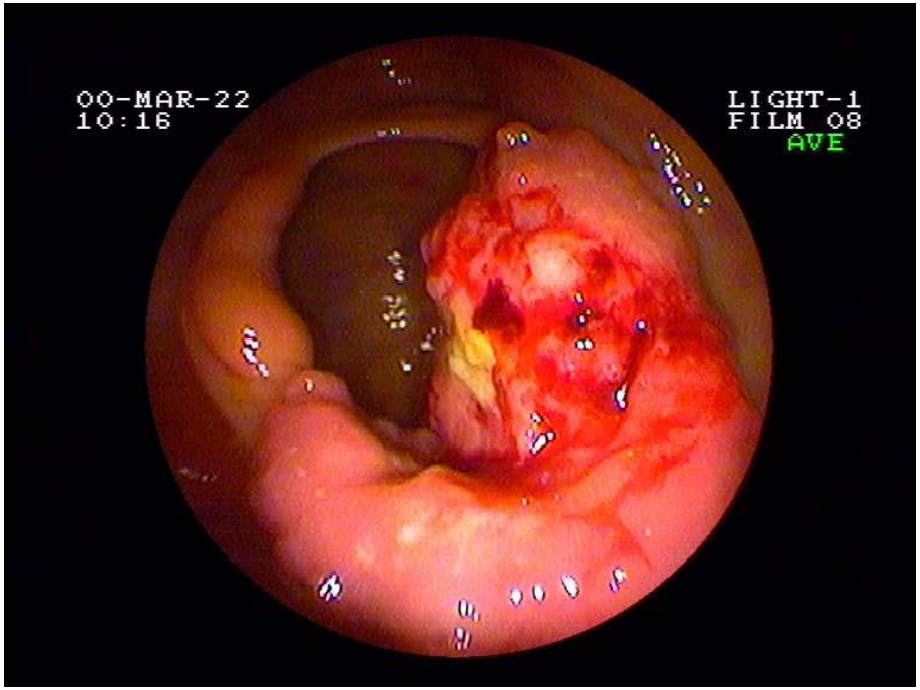
•Overtly **invasive carcinomas** often represent the first clinical presentation of colorectal tumors.

•Little is known about the genetic alterations and precise mechanisms driving progression from early stage in situ carcinomas through the successive clinically defined stages of regional invasion and distant **metastases**.

Most colon cancers (85%) are sporadic.
Familial cases (15%) fall into two categories:

- Hereditary non-polyposis colon cancer (HNPCC)
- 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.

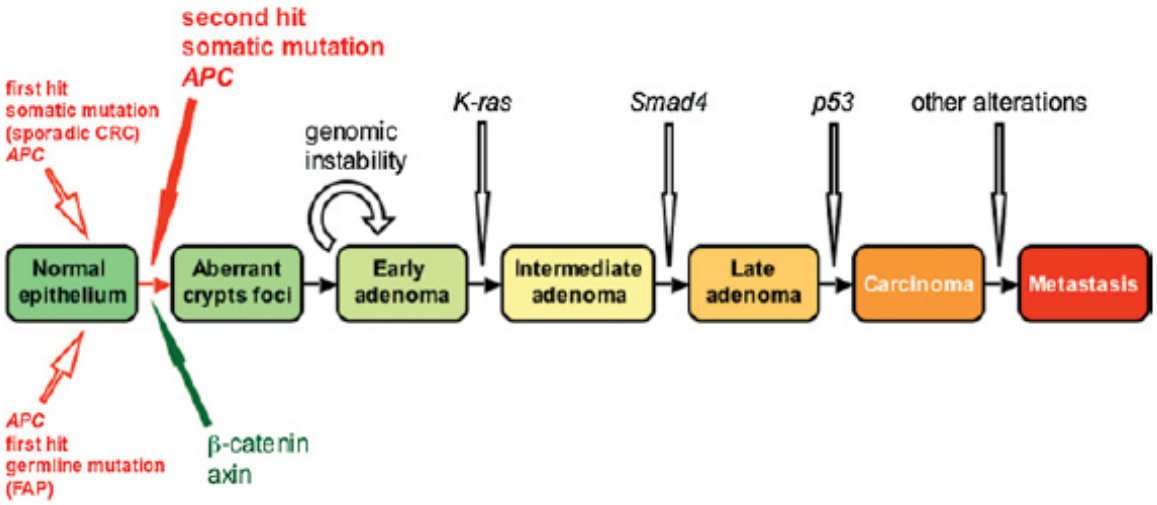
FAP patients develop hundreds to thousands of adenomatous polyps in the colon and rectum at an early age, of which a subset inevitably progresses to carcinomas if not surgically removed.

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

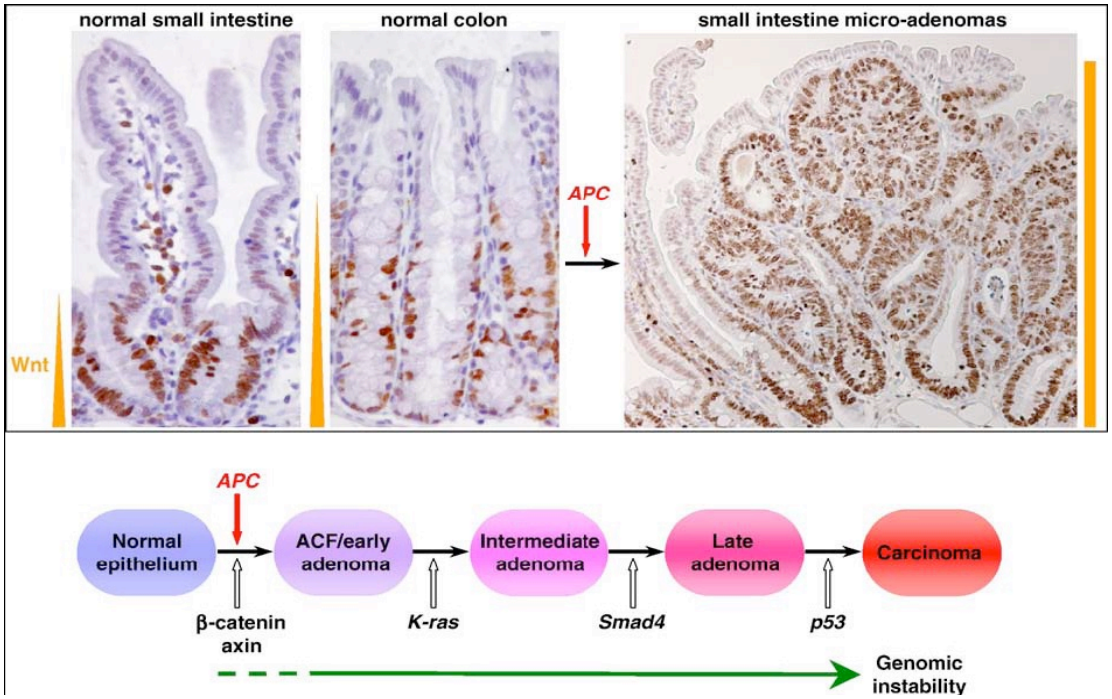
The Wnt pathway in colon cancer

- The APC gene was originally discovered to be the culprit in a hereditary cancer syndrome termed familial adenomatous polyposis (FAP).
- FAP patients, inheriting one defective APC allele, develop large numbers of colon polyps, or adenomas, early in life.
- Individual polyps are clonal outgrowths of epithelial cells in which the second APC allele is inactivated.

- 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.



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



•In crypts, Wnt signaling maintains a proliferative phenotype in multipotent stem cells.

•The absence of Wnt signaling in the villus compartment results in rapid cell cycle arrest and differentiation.

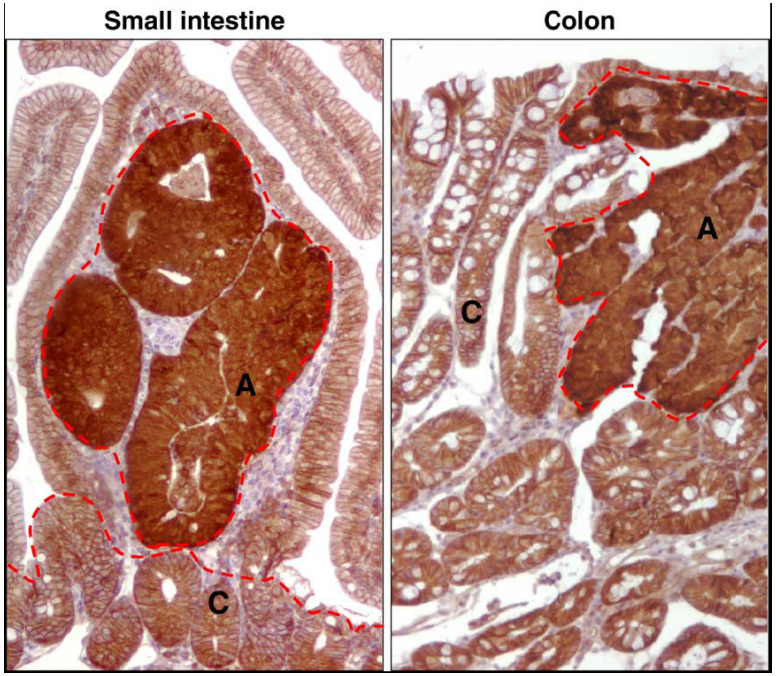
•Inactivating mutations in the APC gene (that selectively disable binding to β-catenin) or activating mutations in β-catenin (that remove the regulatory phosphorylation sites) lead to nuclear accumulation of β-catenin .

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

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.

What about the intestine?

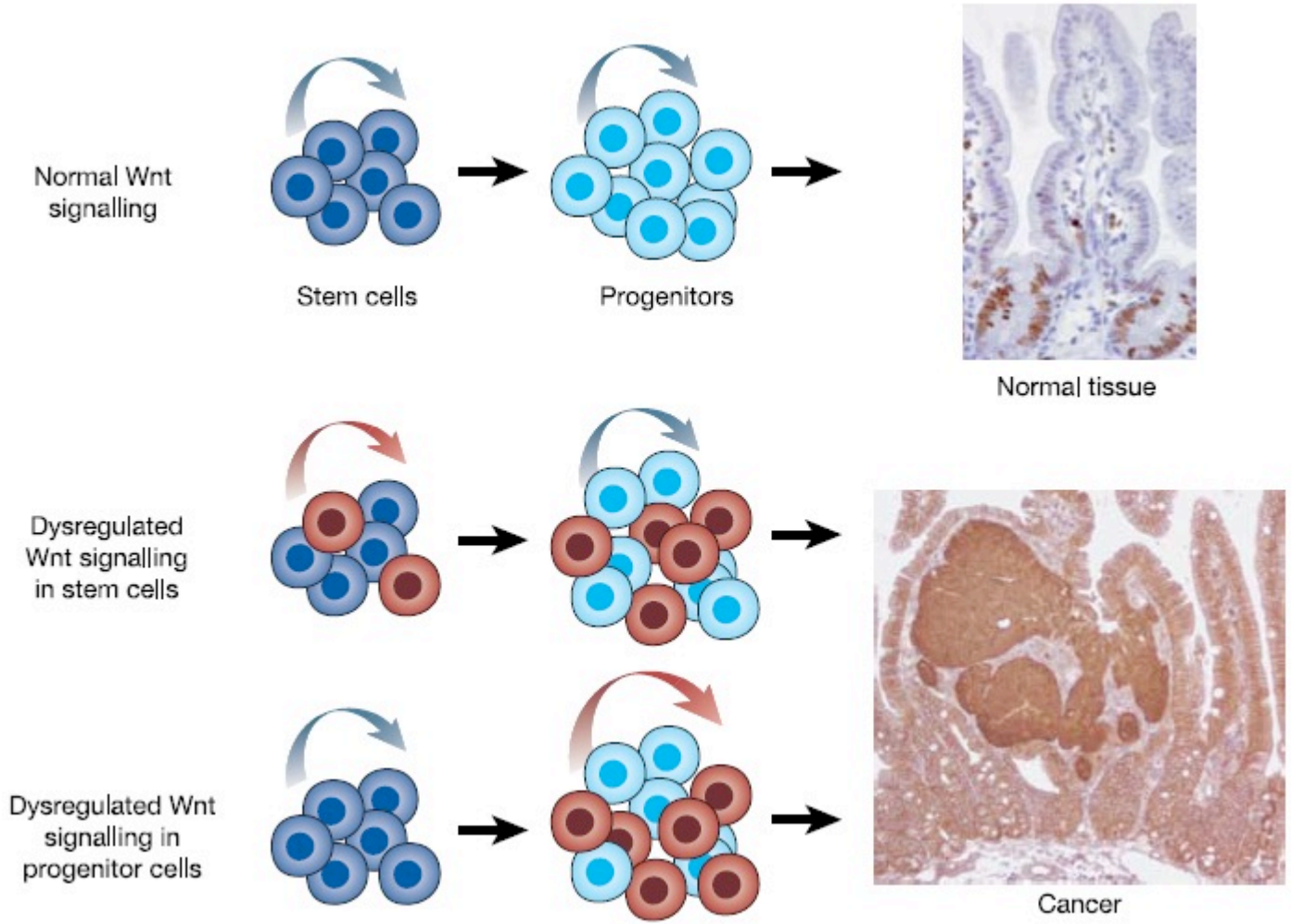


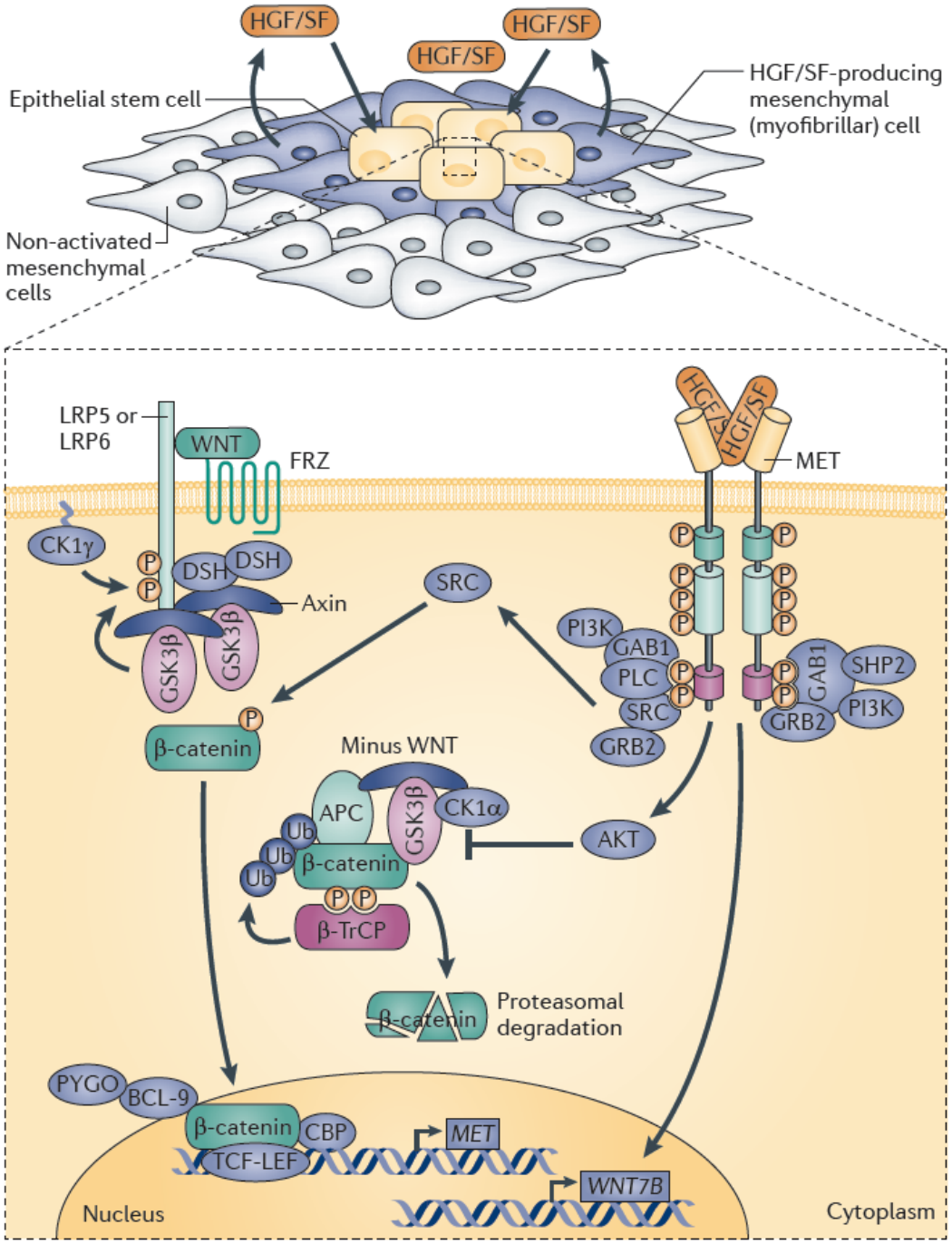
•Nuclear β-catenin accumulates in the crypt stem cell/ progenitor compartments in small intestine and colon;

•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.


Normal Wnt signalling
Stem cells
Progenitors
Normal tissue
Dysregulated Wnt signalling in stem cells
Dysregulated Wnt signalling in progenitor cells
Cancer



Cooperation between the HGF and WNT–β-catenin pathways.

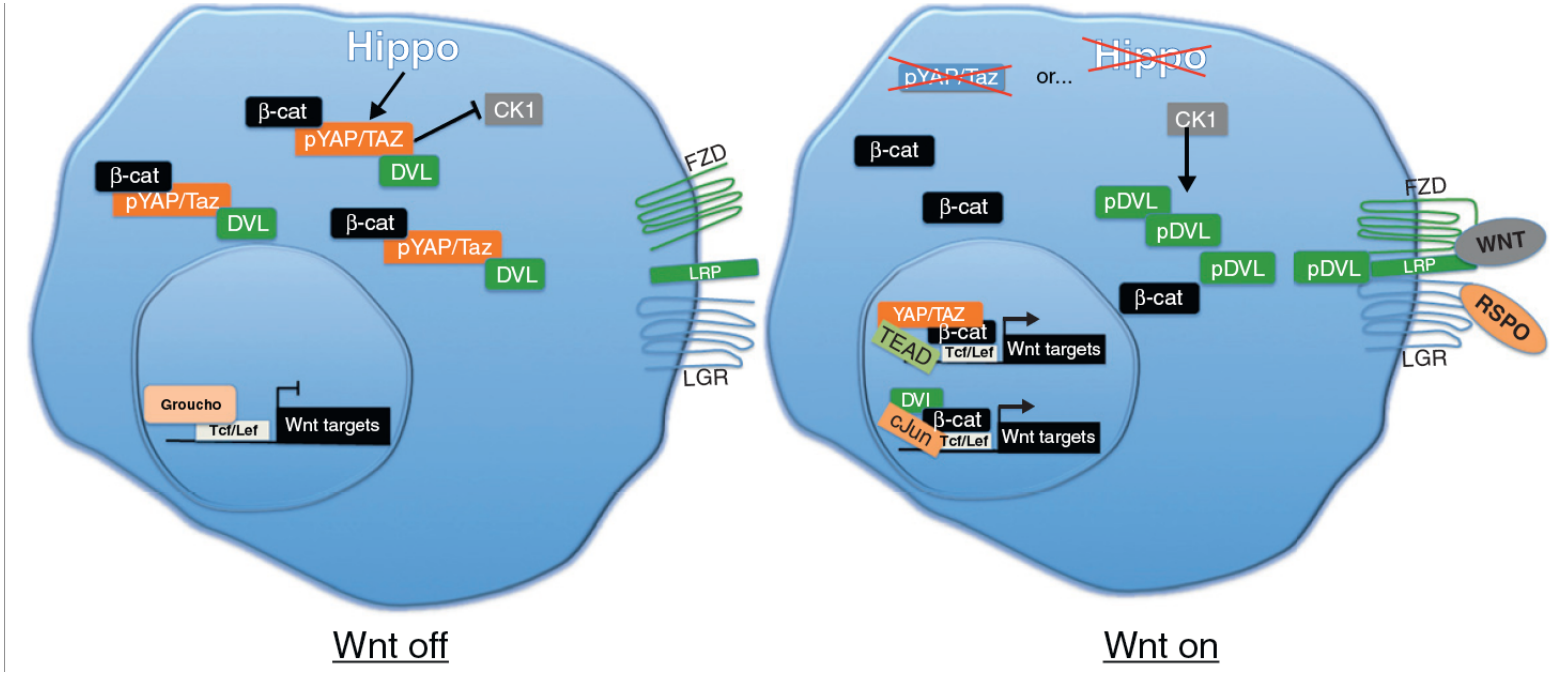
A recent report has shown that interaction of stroma-derived hepatocyte growth factor/ scatter factor (HGF/SF) controls the maintenance of stem cell-like properties of colon cancer cells, which is a function of WNT–β-catenin signalling.

A stem cell niche (top of the figure) contains epithelial (cancer) stem cells (shown in yellow) that are surrounded by mesenchymal (myofibrillar) niche cells (shown in blue), which secrete HGF/SF. Multiple mechanisms have been reported to allow interactions between MET and WNT–β-catenin signalling in epithelial cells, of which a few are shown here.

MET can contribute to the transcriptional activation of WNT ligands, such as *WNT7B*.

MET can also induce the activation of β-catenin–TCF–LEF-target genes; for example, through direct or indirect (SRC) tyrosine phosphorylation (P) and nuclear targeting of β-catenin, or by inhibition of the β-catenin degradation complex by AKT phosphorylation of glycogen synthase kinase-3β (GSK3β).

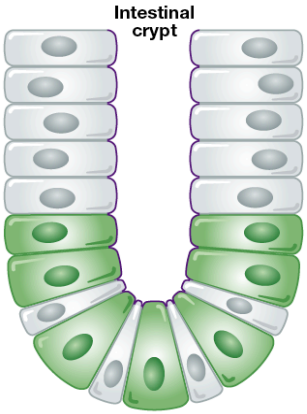
Regulation of Wnt signaling by the Hippo pathway

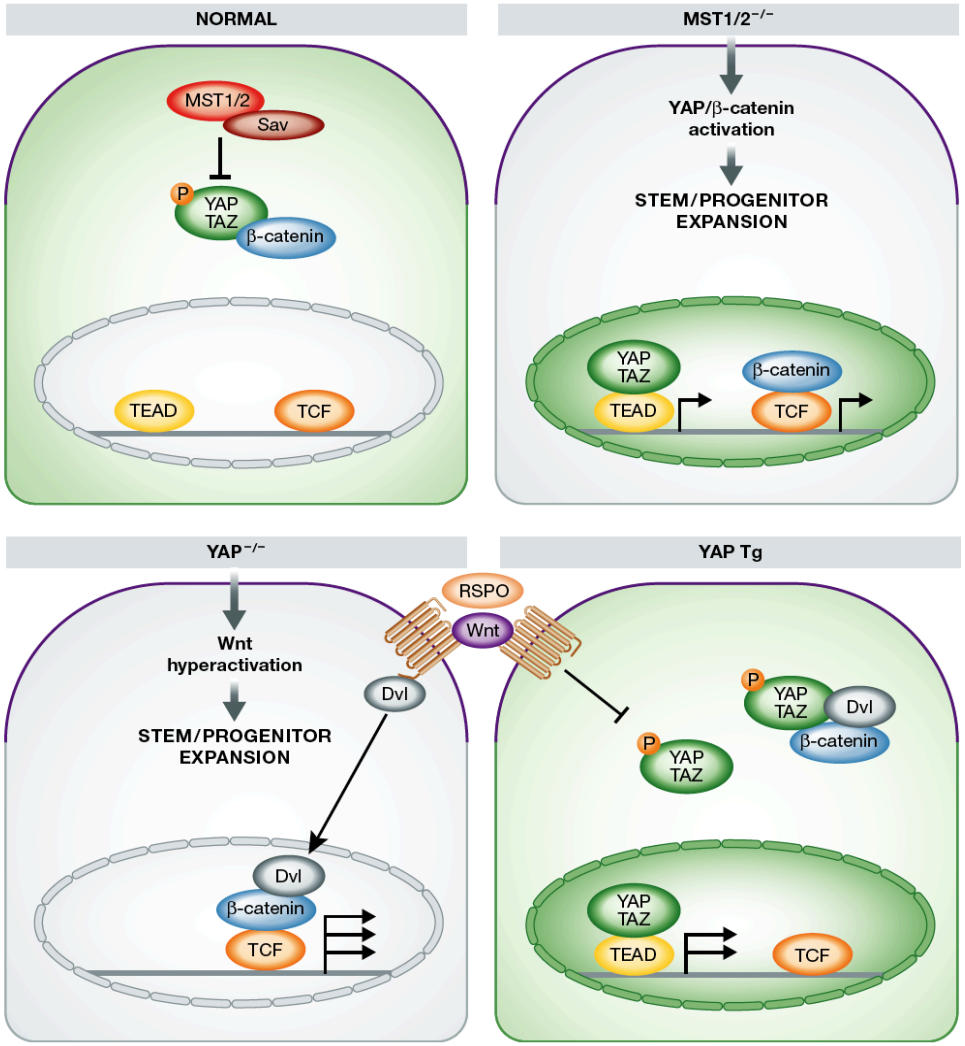


In the Wnt off state, YAP and TAZ are phosphorylated and sequestered in the cytoplasm. When located in the cytoplasm, YAP/TAZ interact with DVL and β-catenin. Cytoplasmic YAP inhibits the nuclear translocation of DVL, whereas TAZ inhibits the activity of CK1, blocking DVL phosphorylation. Some evidence also suggests that cytoplasmic YAP hinders β-catenin translocation to the nucleus.

In the Wnt on state, R-spondins can synergize with Wnt ligand and activate the β-catenin transcriptional program. Under these conditions, if YAP/TAZ is lost, or Hippo signaling is ablated, cells experience hyperactive Wnt owing to increased DVL phosphorylation and/or nuclear accumulation as well as additional nuclear β-catenin. Once in the nucleus, DVL acts as a transcriptional co-factor to induce β-catenin target genes in conjunction with cJUN. In addition, there is evidence that YAP interacts with β-catenin and TEAD in the nucleus to activate a pro-growth gene expression program.

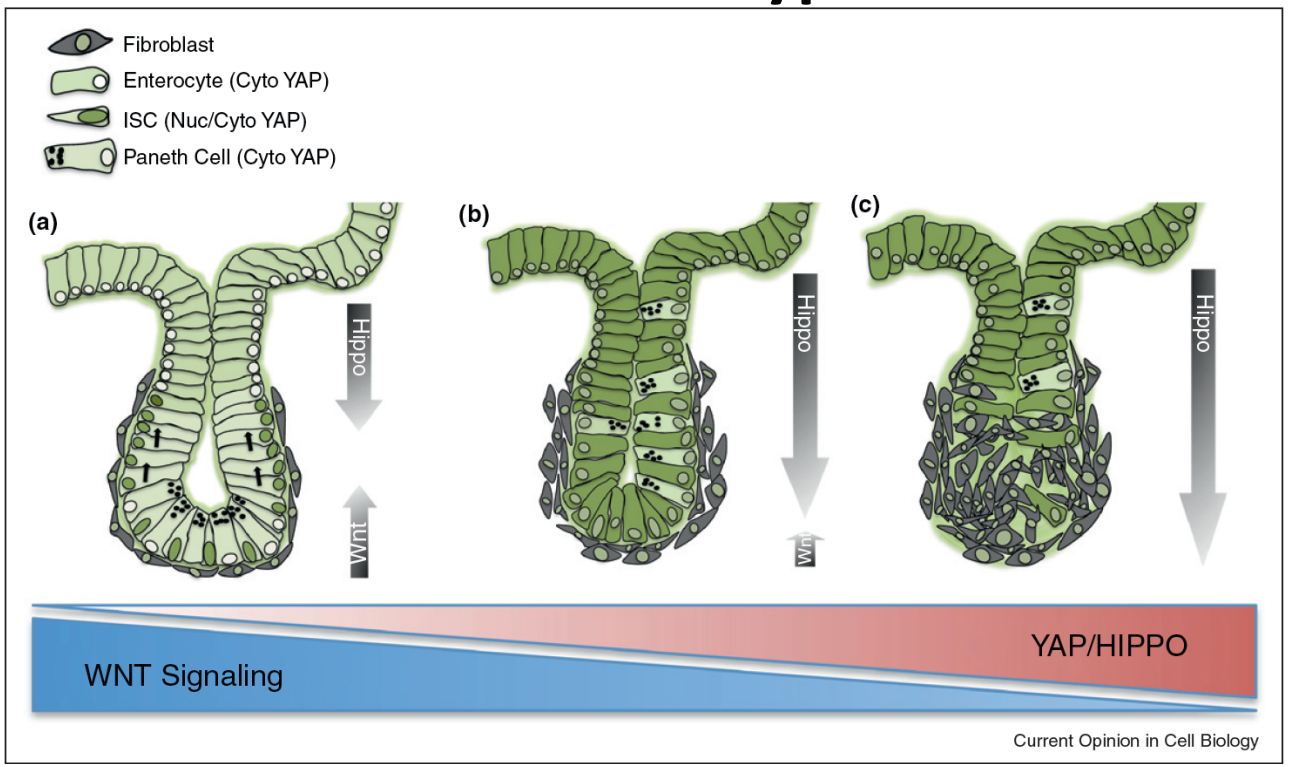
The Hippo pathway in the intestinal crypts





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).

Potential mechanism for YAP repression of Wnt in intestinal crypts



(a) Under normal conditions, YAP levels and Wnt signaling are balanced. Wnt signaling is received by ISCs in intestinal crypts from Paneth cells as well as other sources. ISCs divide and cells progress upward out of the crypt and begin to differentiate. YAP is found in the nucleus of ISCs and some other crypt cells, but is primarily cytoplasmic in the upper crypt and villi, where it is likely that Hippo targets YAP for phosphorylation. It is currently unclear if Hippo activity is strictly found in the villi as compared the crypts. Although immunohistochemical analysis would suggest that Hippo is primarily active in villi, this deserves more analysis.

(b) When YAP is overabundant in the cytoplasm, Wnt signaling is repressed and the ISC niche is disrupted, causing aberrant upward migration of Paneth cells and loss of ISCs.

(c) Because of ISC loss, the intestinal epithelium degenerates.

The Hippo pathway in colon cancer

The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program

Jing Cai,[1] Nailing Zhang,[1] Yonggang Zheng,[1] Roeland F. de Wilde,[2] Anirban Maitra,[2] and Duojia Pan[1,3]

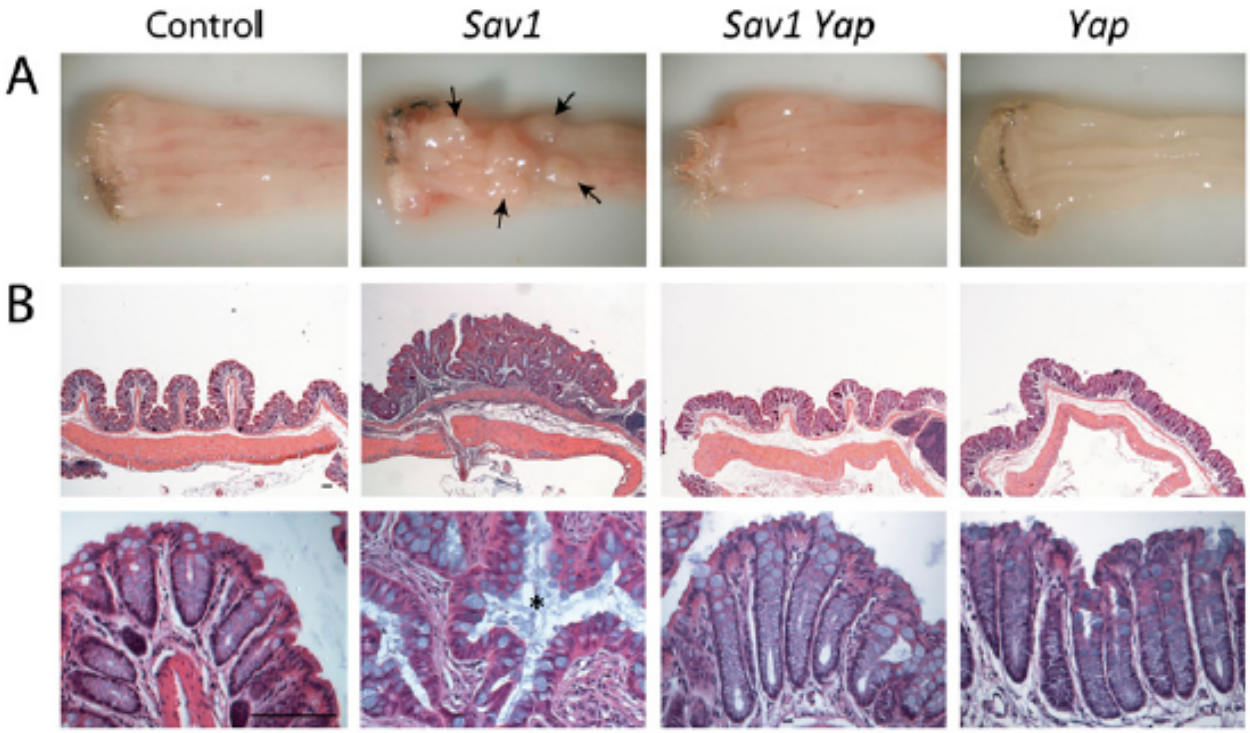


Figure 5. DSS-induced regeneration accelerated polyp development in *Sav1*-deficient colons in a *Yap*-dependent manner. (*A*) Distal colon of 12-wk-old wild-type, *Sav1*, *Yap*, or *Sav1 Yap* double-mutant mice treated with 2.5% DSS for 4 d, followed by normal drinking water for 3 mo. Note the presence of multiple large colonic polyps in the *Sav1*-deficient colon (arrows). (*B*) H&E staining of colonic sections from animals in *A*. The *top* and *bottom* panels show the corresponding low- and high-magnification images, respectively. Note the presence of serrated crypt epithelium in *Sav1*-deficient polyps (asterisk). (*C*) Ki67 staining of colon

YAP/TAZ as oncogenes and tumour suppressors

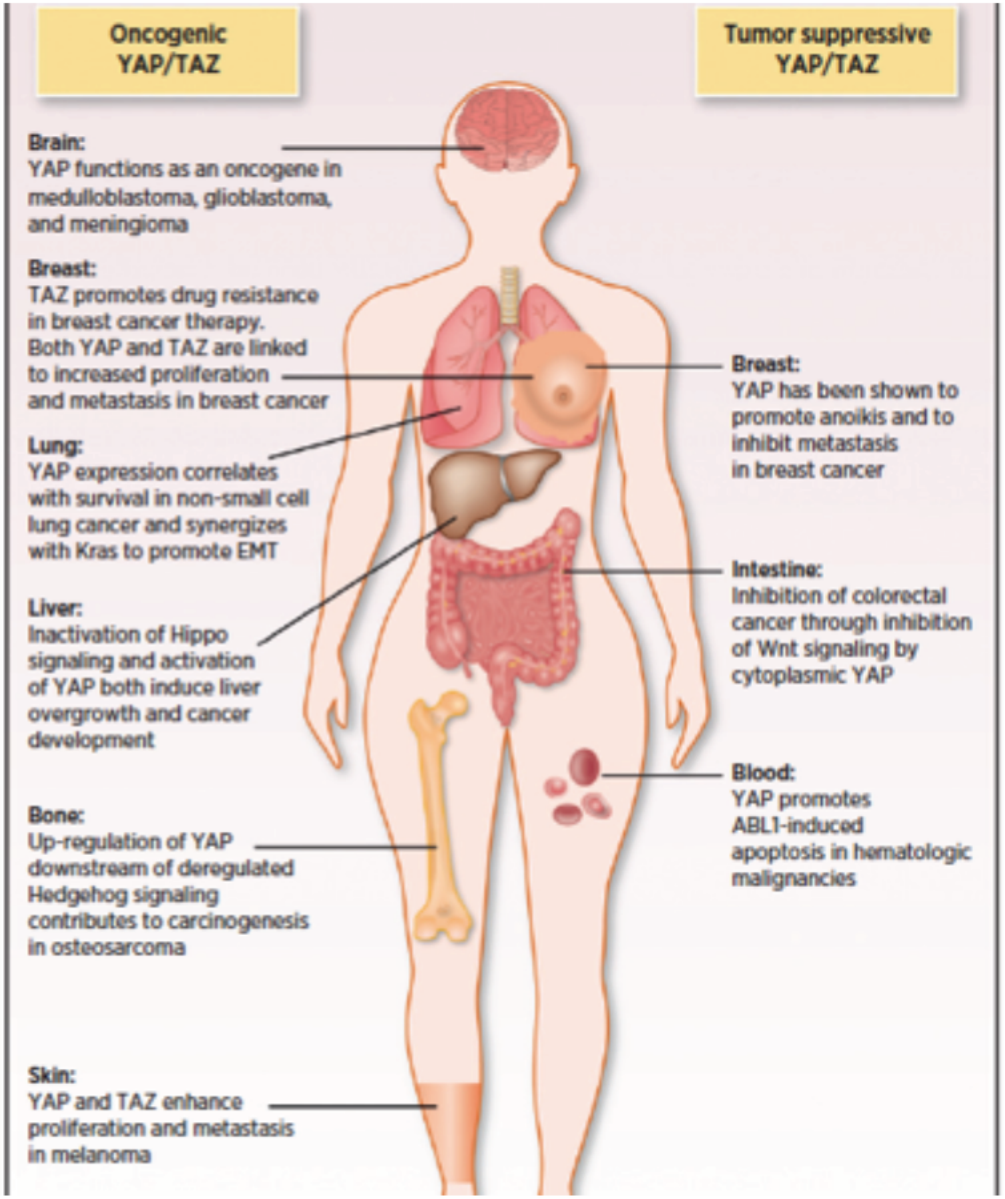


Figure 2.
Oncogenic and tumor-suppressive functions of YAP and TAZ in human cancer (86, 151, 162). See text for details.

YAP/TAZ as sensors of ECM in cardiac progenitors

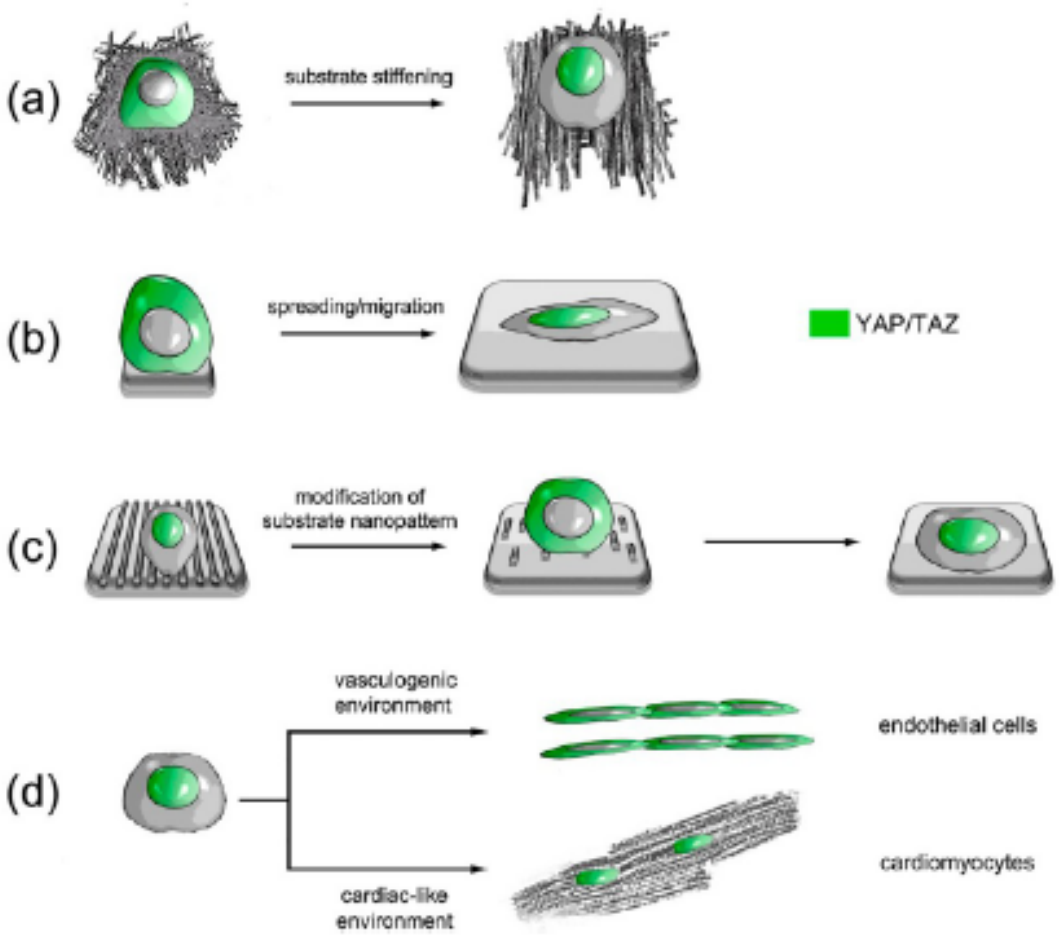
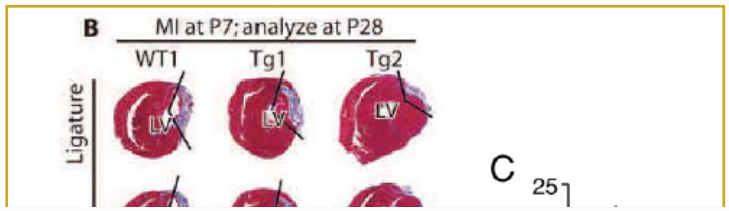


Figure 7. YAP/TAZ control cardiac progenitor cell fate by acting as sensors of extracellular matrix composition. YAP/TAZ activity as transcriptional coactivators is regulated *via* their phosphorylation in the cytoplasm. Phosphorylated YAP/TAZ are thought to be inactive when retained in the cytoplasm. Nuclear shuttling is triggered in cardiac progenitor cells by substrate stiffening (a), cell spreading or migration (b), and modifications in substrate nanopattern (c). More importantly, the regulation of YAP/TAZ intracellular localization is required for cardiac progenitor cell fate decision (d). When a vasculogenic environment (like Matrigel) is provided, YAP/TAZ are retained in the cytoplasm, while they shuttle to the nucleus when cardiac progenitors are cultured in cardiogenic conditions (E = 10 kPa).

The Hippo pathway in the heart

- **Costitutive expression of YAP in the heart after MI**

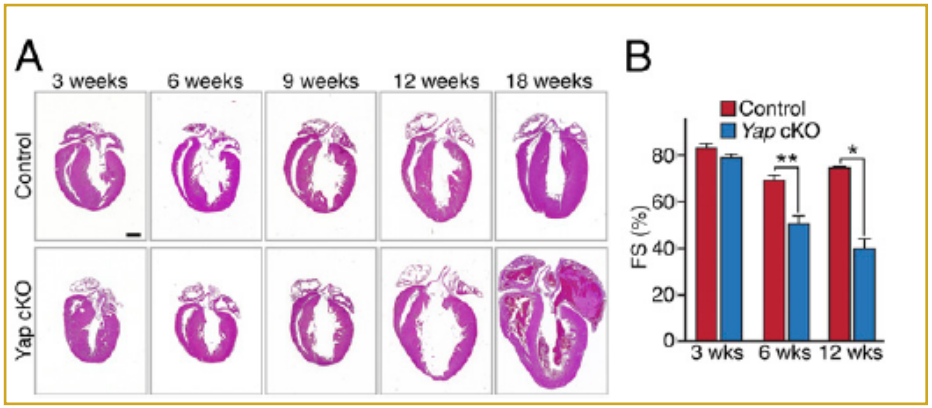


YAP S112A transgenic mice

Hippo pathway effector Yap promotes cardiac regeneration

Mei Xin[a], Yuri Kim[a], Lillian B. Sutherland[a], Masao Murakami[a], Xiaoxia Qi[a], John McAnally[a], Enzo R. Porrello[a], Ahmed I. Mahmoud[b], Wei Tan[a], John M. Shelton[b], James A. Richardson[a,c], Hesham A. Sadek[b], Rhonda Bassel-Duby[a], and Eric N. Olson[a,1]

- **YAP deletion in the heart**



→ Lethal cardiomyopathy

J Neurosci. 2017 Mar 29;37(13):3465-3477. doi: 10.1523/JNEUROSCI.2934-16.2017. Epub 2017 Feb 17.

Muscle Yap Is a Regulator of Neuromuscular Junction Formation and Regeneration.

Zhao K[1], Shen C[1,2], Lu Y[1,3,4], Huang Z[1,5], Li L[1], Rand CD[6], Pan J[1,7,8], Sun XD[1], Tan Z[1], Wang H[1], Xing G[1], Cao Y[1], Hu G[9], Zhou J[9], Xiong WC[1,7,8], Mei L[10,7,8].

⊕ Author information

Erratum in

Correction: Zhao et al., "Muscle Yap Is a Regulator of Neuromuscular Junction Formation and Regeneration". [J Neurosci. 2017]

Abstract

Yes-associated protein (Yap) is a major effector of the Hippo pathway that regulates cell proliferation and differentiation during development and restricts tissue growth in adult animals. However, its role in synapse formation remains poorly understood. In this study, we characterized Yap's role in the formation of the neuromuscular junction (NMJ). In *HSA-Yap*$^{-/-}$ mice where Yap was mutated specifically in muscle cells, AChR clusters were smaller and were distributed in a broader region in the middle of muscle fibers, suggesting that muscle Yap is necessary for the size and location of AChR clusters. In addition, *HSA-Yap*$^{-/-}$ mice also exhibited remarkable presynaptic deficits. Many AChR clusters were not or less covered by nerve terminals; miniature endplate potential frequency was reduced, which was associated with an increase in paired-pulse facilitation, indicating structural and functional defects. In addition, muscle Yap mutation prevented reinnervation of denervated muscle fibers. Together, these observations indicate a role of muscle Yap in NMJ formation and regeneration. We found that β-catenin was reduced in the cytoplasm and nucleus of mutant muscles, suggesting compromised β-catenin signaling. Both NMJ formation and regeneration deficits of *HSA-Yap*$^{-/-}$ mice were ameliorated by inhibiting β-catenin degradation, further corroborating a role of β-catenin or Wnt-dependent signaling downstream of Yap to regulate NMJ formation and regeneration.**SIGNIFICANCE STATEMENT** This paper explored the role of Yes-associated protein (Yap) in neuromuscular junction (NMJ) formation and regeneration. Yap is a major effector of the Hippo pathway that regulates cell proliferation and differentiation during development and restricts tissue growth in adult animals. However, its role in synapse formation remains poorly understood. We provide evidence that muscle Yap mutation impairs both postsynaptic and presynaptic differentiation and function and inhibits NMJ regeneration after nerve injury, indicating a role of muscle Yap in these events. Further studies suggest compromised β-catenin signaling as a potential mechanism. Both NMJ formation and regeneration deficits of *HSA-Yap*$^{-/-}$ mice were ameliorated by inhibiting β-catenin degradation, corroborating a role of β-catenin or Wnt-dependent signaling downstream of Yap to regulate NMJ formation and regeneration.

KEYWORDS: YAP; neuromuscular junction; regeneration; β-catenin

- **Modelli animali di YAP KO presentano difetti nella formazione delle giunzioni neromuscolari pre- e postsinaptiche con deficit sia nelle dimensioni che nella loro distribuzione**

Cell Rep. 2016 Mar 8;14(9):2224-2237. doi: 10.1016/j.celrep.2016.02.021. Epub 2016 Feb 25.

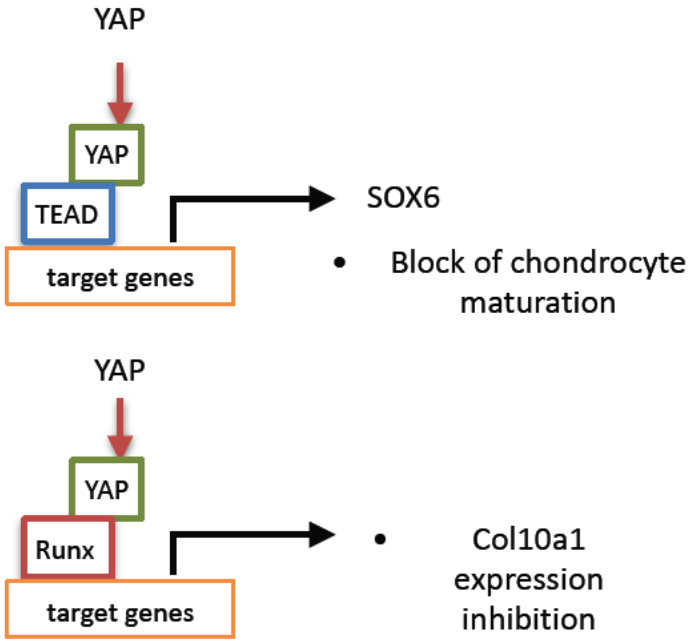
Yap1 Regulates Multiple Steps of Chondrocyte Differentiation during Skeletal Development and Bone Repair.

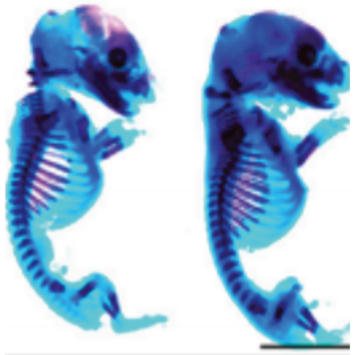
Deng Y[1], Wu A[2], Li P[3], Li G[4], Qin L[5], Song H[2], Mak KK[6].

Author information

Abstract

Hippo signaling controls organ size and tissue regeneration in many organs, but its roles in chondrocyte differentiation and bone repair remain elusive. Here, we demonstrate that Yap1, an effector of Hippo pathway inhibits skeletal development, postnatal growth, and bone repair. We show that Yap1 regulates chondrocyte differentiation at multiple steps in which it promotes early chondrocyte proliferation but inhibits subsequent chondrocyte maturation both in vitro and in vivo. Mechanistically, we find that Yap1 requires Teads binding for direct regulation of Sox6 expression to promote chondrocyte proliferation. In contrast, Yap1 inhibits chondrocyte maturation by suppression of Col10a1 expression through interaction with Runx2. In addition, Yap1 also governs the initiation of fracture repair by inhibition of cartilaginous callus tissue formation. Taken together, our work provides insights into the mechanism by which Yap1 regulates endochondral ossification, which may help the development of therapeutic treatment for bone regeneration.





Deng et al 2016

Role of TAZ in the Epithelial-Mesenchymal Transition

TAZ Promotes Cell Proliferation and Epithelial-Mesenchymal Transition and Is Inhibited by the Hippo Pathway[▽]

Qun-Ying Lei,[1,2]* Heng Zhang,[2] Bin Zhao,[4] Zheng-Yu Zha,[2] Feng Bai,[5] Xin-Hai Pei,[5] Shimin Zhao,[2,3] Yue Xiong,[2,5]* and Kun-Liang Guan[2,4]

Summary

Box 2. YAP and TAZ regulation in response to cellular stress

Cells are constantly subjected to external and internal stresses that endanger their integrity. YAP and TAZ are regulated in response to a range of these cellular stresses (Figure I). Cytokinesis failure and extra centrosomes alter small G protein signaling and thereby activate LATS2 kinase. LATS2 in turn inhibits YAP and TAZ and stabilizes p53, thus arresting cells at G1 phase to prevent tumorigenesis [143]. Treatment of cancer cells with antitubulin drugs exerts a mitotic block, which activates cyclin-dependent kinase 1 (CDK1) [194]. CDK1 directly phosphorylates and inhibits YAP function to induce apoptosis. However, the role of CDK1-mediated YAP phosphorylation is controversial as mitotic phosphorylation of YAP by CDK1 promotes mitotic defects and potentiates oncogenic functions of YAP [195]. DNA damage activates the tyrosine kinase ABL through ATM–JNK signaling, which in turn phosphorylates YAP to stimulate proapoptotic functions of YAP in complex with p73 [196]. Endoplasmic reticulum (ER) stress has also recently been implicated in YAP regulation. The ER stress transducer PKR-like ER kinase (PERK) phosphorylates eukaryotic initiation factor 2α (eIF2α) during the adaptive stage of the unfolded protein response (UPR), which suppresses general protein synthesis and specifically induces the translation of ATF4, which regulates YAP transcription [197]. ER stress thus induces YAP expression through the PERK–eIF2α–ATF4 axis to prevent cell death during the adaptive stage of the UPR, while prolonged ER stress activates Hippo signaling to inhibit YAP and promote apoptosis [197].

YAP and TAZ likewise respond to external stresses evoked by these cellular microenvironments. Hyperosmotic stress induces tyrosine phosphorylation of TAZ by the ABL kinase, which facilitates the interaction between TAZ and nuclear factor of activated T cells 5 (NFAT5) to inhibit NFAT5 function in osmoregulatory transcription [198]. YAP/TAZ are activated by interstitial flow-driven shear stress and promote osteogenic differentiation of mesenchymal stem cells [147,199]. Rho GTPase appears to be involved in this regulation, although the precise mechanism remains unknown [148]. Oxidative stress evoked by ischemia and reperfusion in the mouse heart activates the Hippo pathway to antagonize a functional YAP–FOXO1 complex, leading to enhanced oxidative stress-induced cell death [200]. By contrast, hypoxia deactivates the Hippo pathway by destabilizing LATS2 through SIAH2 ubiquitin ligase-induced degradation [33]. Energy stress, such as inhibition of glucose metabolism and ATP production, induces AMP-activated protein kinase (AMPK)-mediated phosphorylation of Angiomotin-like 1 (AMOTL1) to stabilize and increase AMOTL1, which in turn stimulates LATS [35]. Furthermore, LATS also senses and is activated by glucose starvation in an AMPK-independent manner [201]. In addition, AMPK inhibits YAP by directly phosphorylating it at least two distinct sites [201,202]; therefore, energy stress inhibits YAP nuclear activity by several mechanisms [35,201,202].

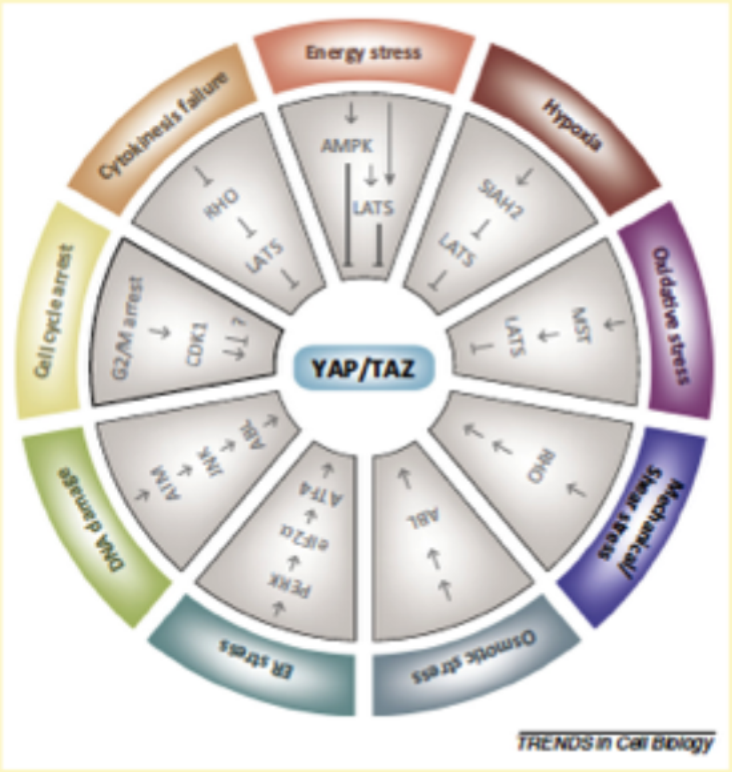
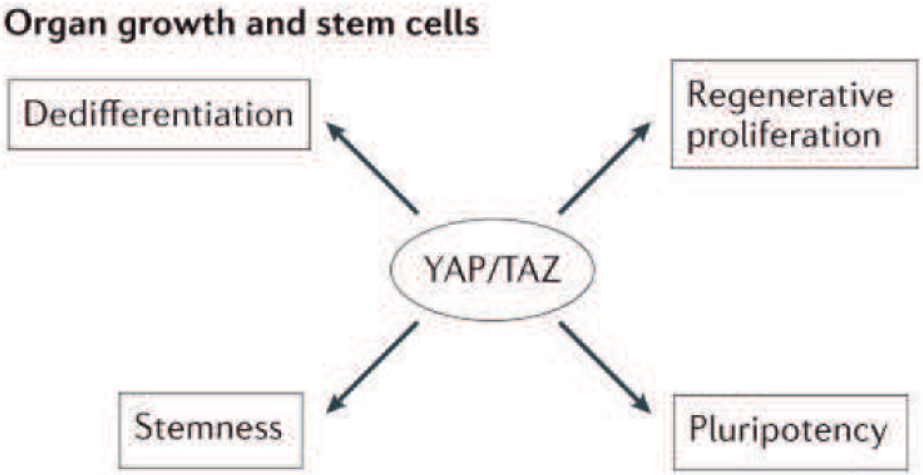
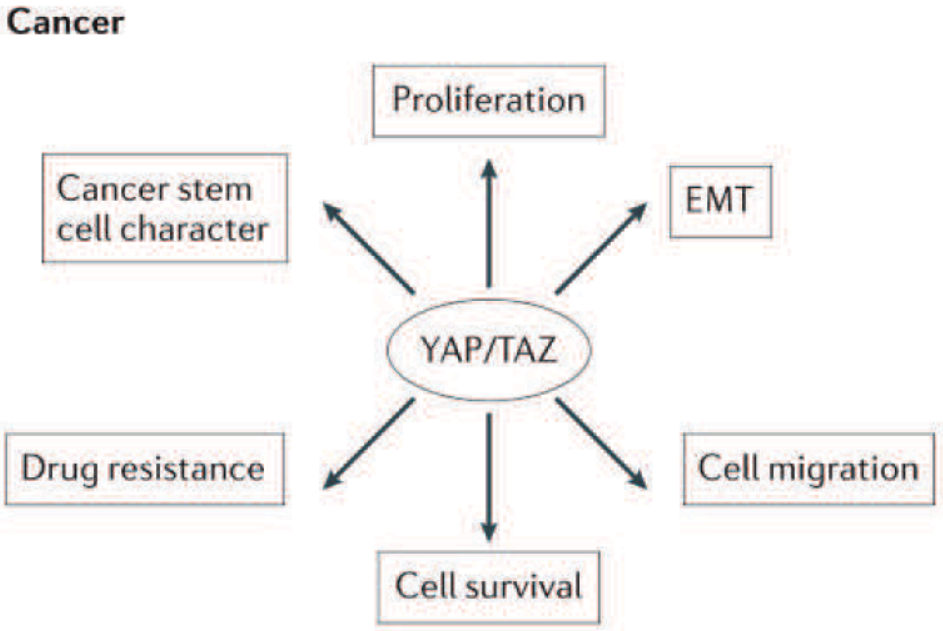


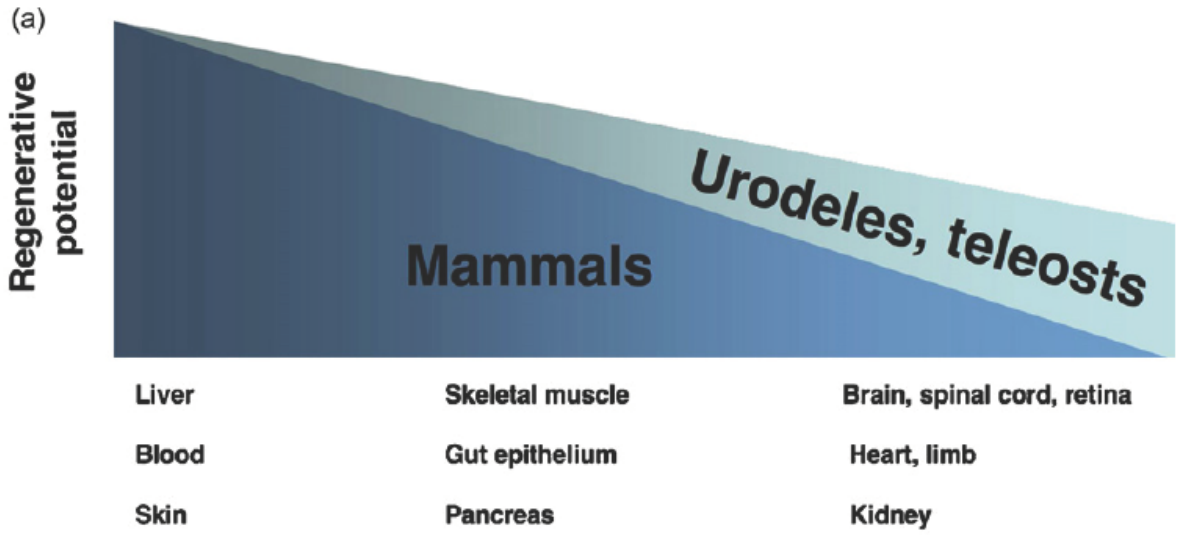
Figure I. Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ) regulation in response to cellular stress.

Summary


Organ growth and stem cells
Dedifferentiation
Regenerative proliferation
YAP/TAZ
Stemness
Pluripotency

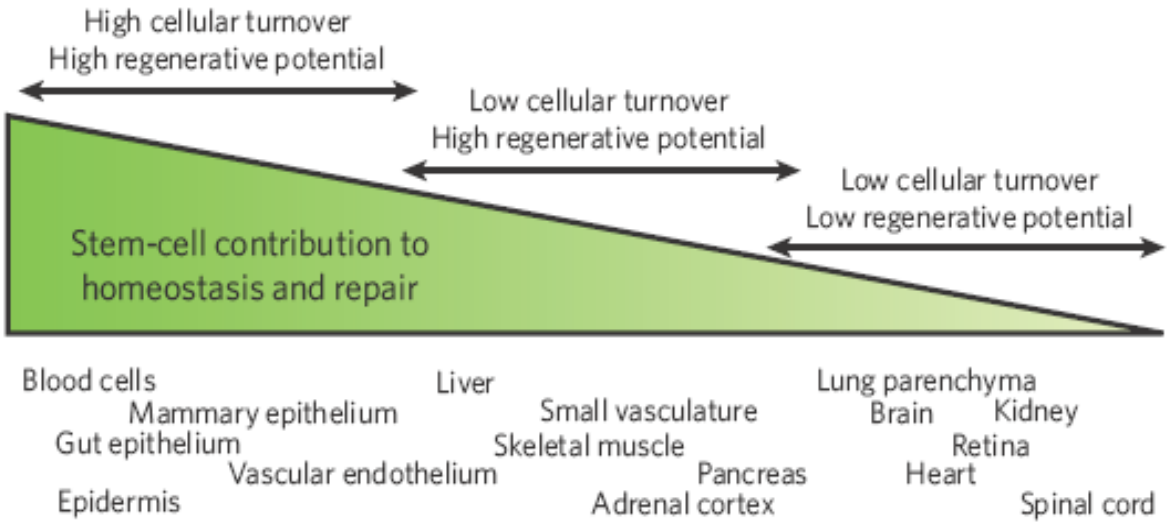

Cancer
Proliferation
Cancer stem cell character
EMT
YAP/TAZ
Drug resistance
Cell migration
Cell survival

How do species other than mammals regenerate organs?



Species or group	Regenerative capabilities	Microarray	Transgenesis	Knockout/knock down	Genome sequenced
Invertebrates					
Hydra	All tissues and organs	No	Yes	RNAi	No
Planarians	All tissues (neurons, muscles, epithelia) and organs (brain, sensory organs, digestive system, musculature)	Yes	No	RNAi	Yes
Ascidians	All tissues and organs	Yes	Yes	Morpholinos	Yes
Vertebrates					
Newts	Limbs, tail, heart, lens, spinal cord, brain, jaw, retina, hair cells of the inner ear	Yes	Yes	Morpholinos	No
Axolotls	Limbs, tails, heart, spinal cord, brain	Yes	Yes	Morpholinos	No
Frogs	Pre-metamorphic limbs, tail, retina, lens, hair cells of the inner ear	Yes	Yes	Morpholinos	Yes
Zebrafish	Fins, tail, heart, liver, spinal cord, hair cells of inner ear, lateral line	Yes	Yes	Mutagenesis, morpholinos	Yes
Chicks	Hair cell of the inner ear	Yes	Yes	Morpholinos	Yes
Mice	Liver, digit tips	Yes	Yes	Mutagenesis, homologous recombination	Yes

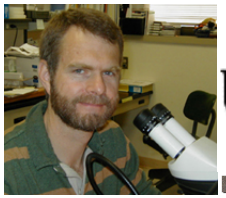
Tissue heterogeneity and stem-cell functionality for homeostasis and repair



The extent to which the effects of ageing on the resident stem cells determine the phenotype of an aged tissue is likely to correlate with the extent to which stem cells are responsible for normal tissue homeostasis and repair. Along this spectrum, tissues generally fall into one of three categories.

1. Tissues with high turnover (such as blood, skin and gut) have a prominent stem-cell compartment and, by definition, have high regenerative capacity.
2. Tissues with low turnover but high regenerative potential might use different strategies to ensure effective repair in the setting of acute injury.
3. Tissues with low turnover and low regenerative potential might have stem cells that mediate only limited tissue repair. Although there has been much interest in harnessing the potential of stem cells in the brain and heart for therapeutic purposes, for example, there is limited endogenous repair capacity of these tissues following acute injuries.

In Urodeles Amphibians:



THE UNIVERSITY OF UTAH
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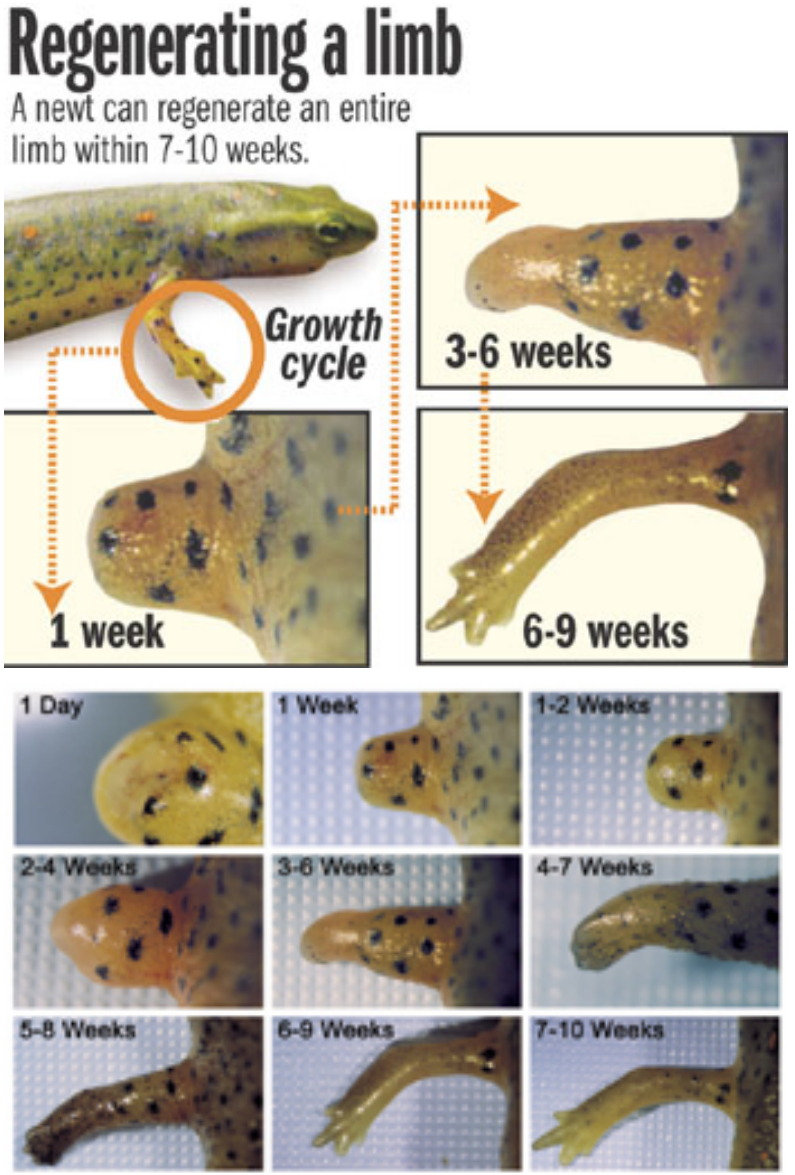
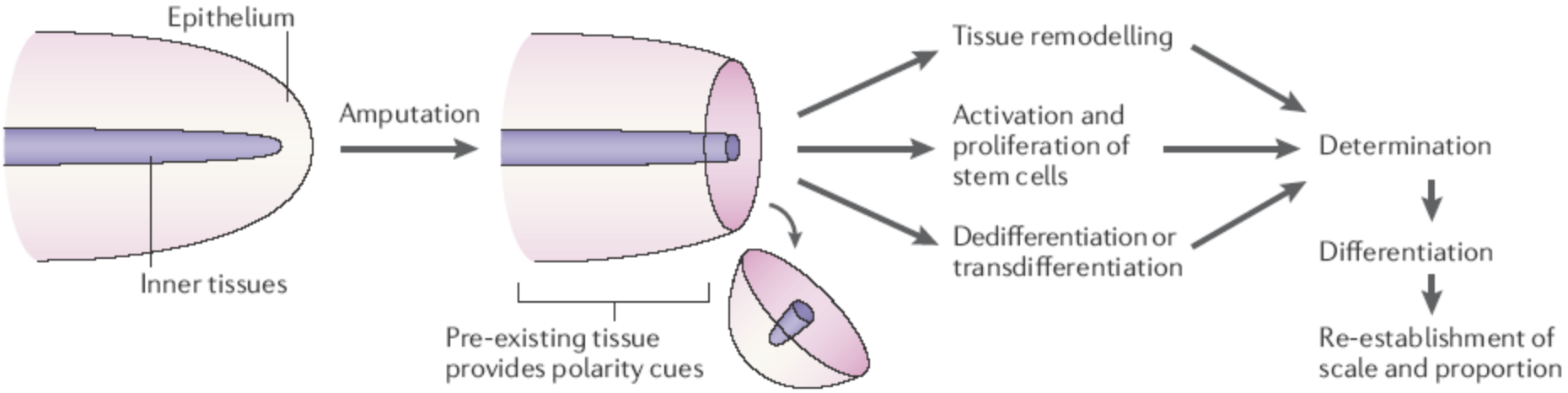


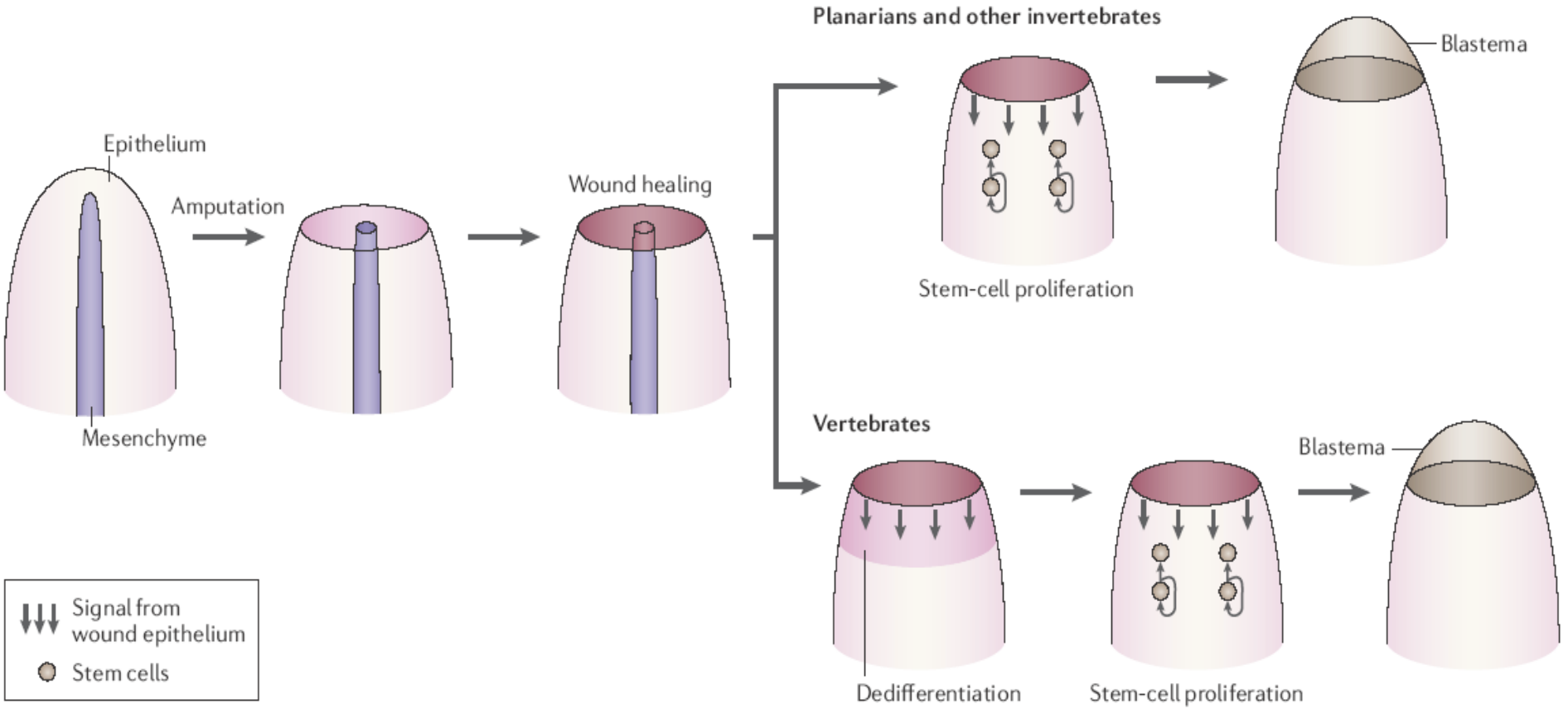
Basic mechanisms of regeneration.



After amputation, wound healing occurs. After wound healing three processes can be activated, either independently or together.

- **Hydra** undergo remodelling of pre-existing tissues to regenerate amputated parts.
- **Planarians** undergo both tissue remodelling and proliferation of resident adult somatic stem cells
- In **Vertebrates**, both stem-cell proliferation and the dedifferentiation of the cells that lie adjacent to the plane of amputation take place. The cells that respond to the stimulus of amputation eventually undergo determination and differentiation, resulting in new tissues that must then functionally integrate with and scale to the size of the pre-existing tissues.

Basic steps in the formation of blastema in vertebrates and invertebrates.



In **Vertebrates**, there is evidence that both stem cells and cell-dedifferentiation processes have a role in blastema-mediated regeneration.

In **Invertebrates** such as planarians, stem-cell proliferation seems to have a pivotal role.

Regeneration in the newt limb: blastema formation

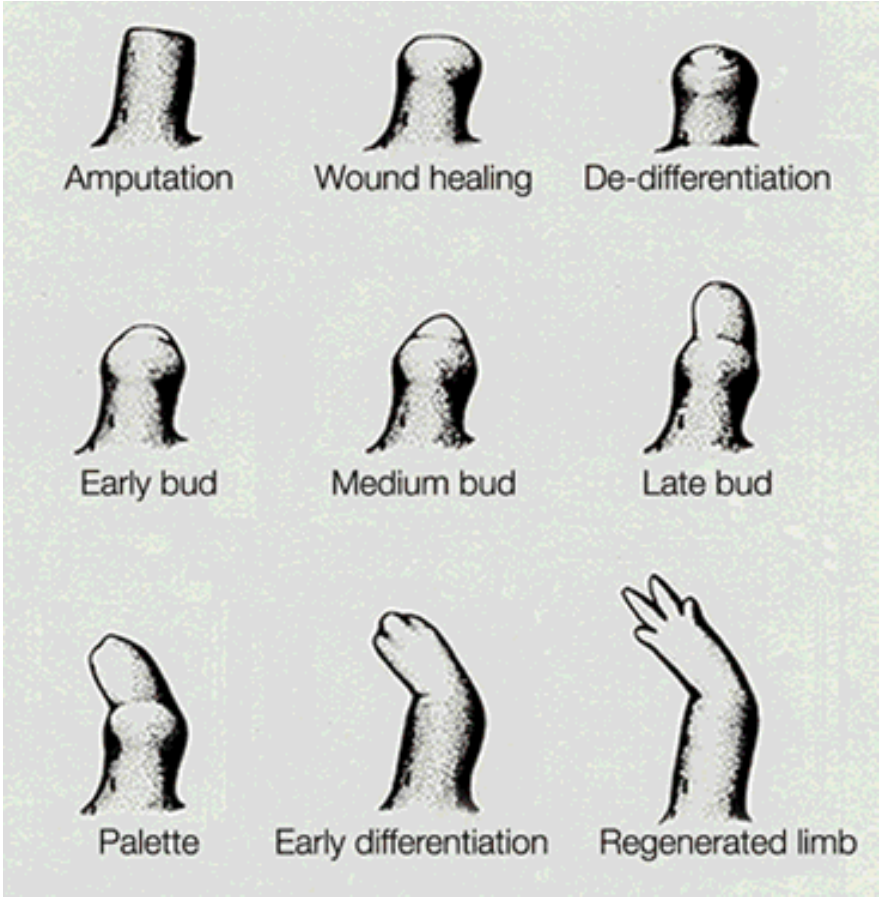
After amputation, cartilage, connective tissue and muscle cells loose their differentiated characteristics and form a **blastema.**

Blastema: a mesenchymal growth zone that undergo proliferation, differentiation and morphogenesis to regenerate the limb

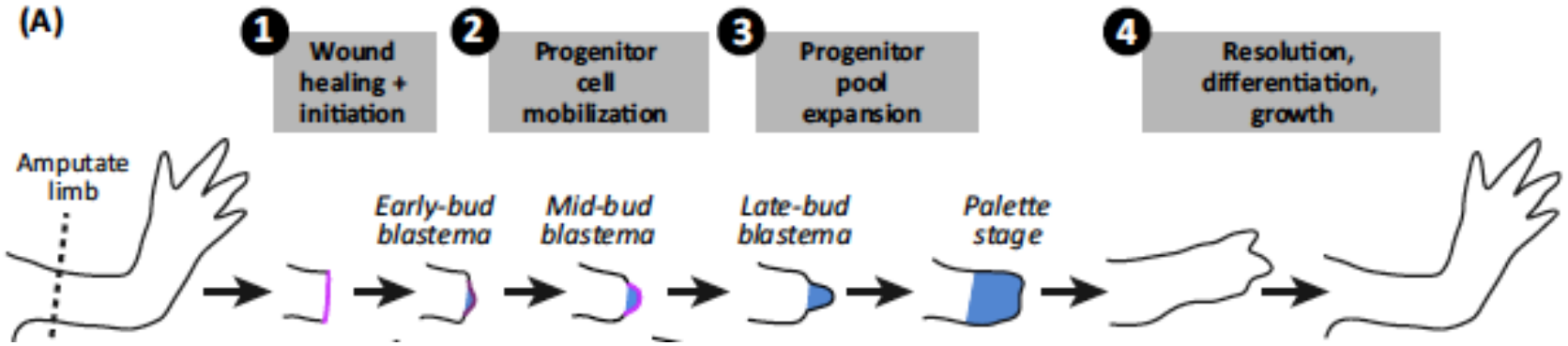
Does blastema formation involve **cellular dedifferentiation** or **activation of quiescent stem cells?**

Cellular dedifferentiation does appear to occur during newt limb blastema formation together with stem cells proliferation.

Transcription factors *msxb* and *msxc* are induced during blastema formation. These data are intriguing, as they are candidates for inducing dedifferentiation in mammalian cells and/or maintaining cells in undifferentiated state.



Outline of Cellular Events During Limb Regeneration



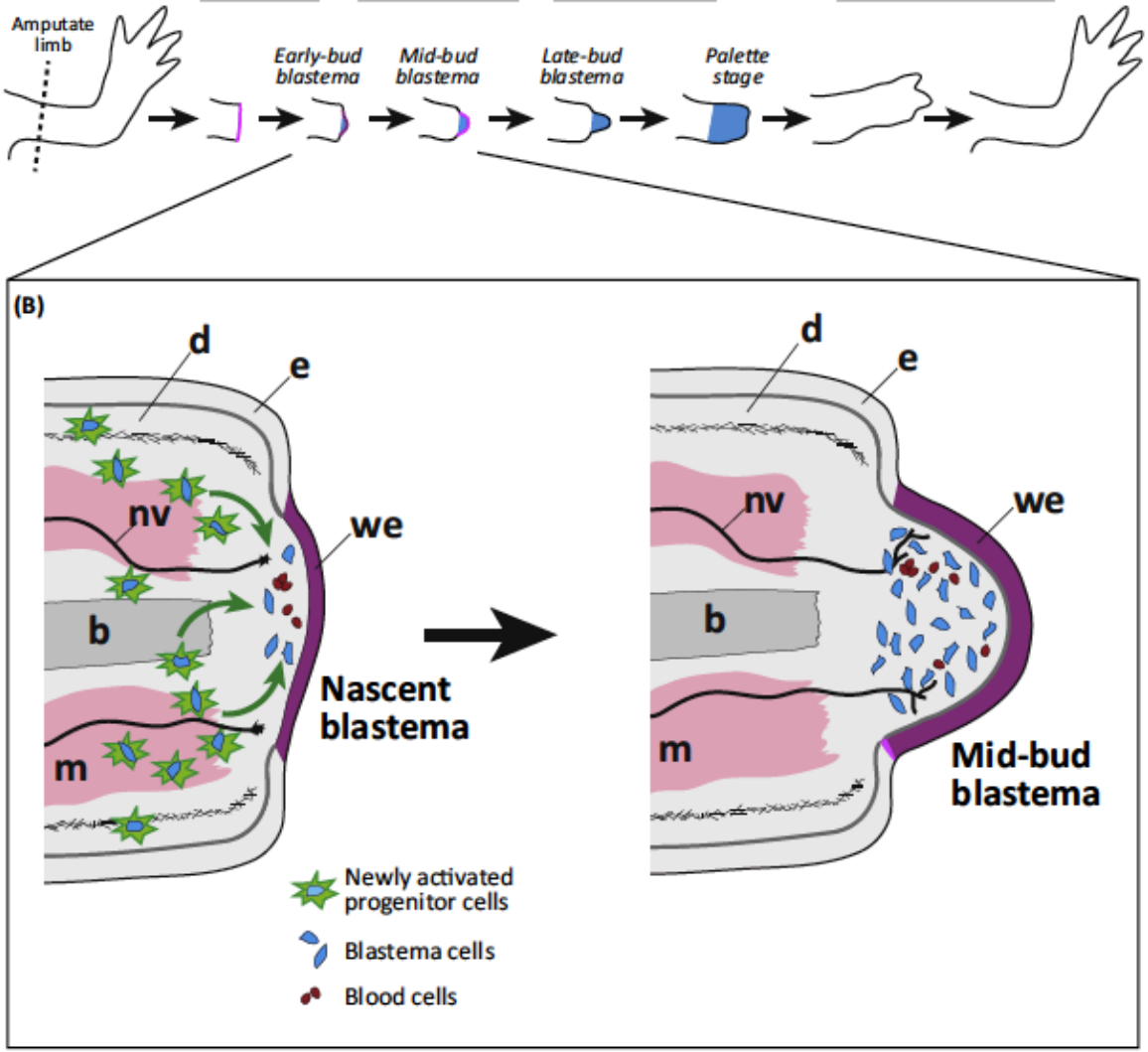
General progression from unamputated to fully regenerated.

(1) Immediately following amputation (within 24 h), a thin wound epidermis (magenta) forms across the cut stump via migration of stump epidermal cells. The wound epidermis thickens as cells within it proliferate.

(2) A visible bump, termed a blastema (blue), forms beneath the wound epidermis. Blastema cells are derived from activated progenitor cells within various stump tissues that migrate to the tip.

(3) Blastema cells proliferate to expand the progenitor pool.

(4) The initial regeneration response resolves, cells begin to undergo differentiation, and the limb continues to grow to the appropriate size



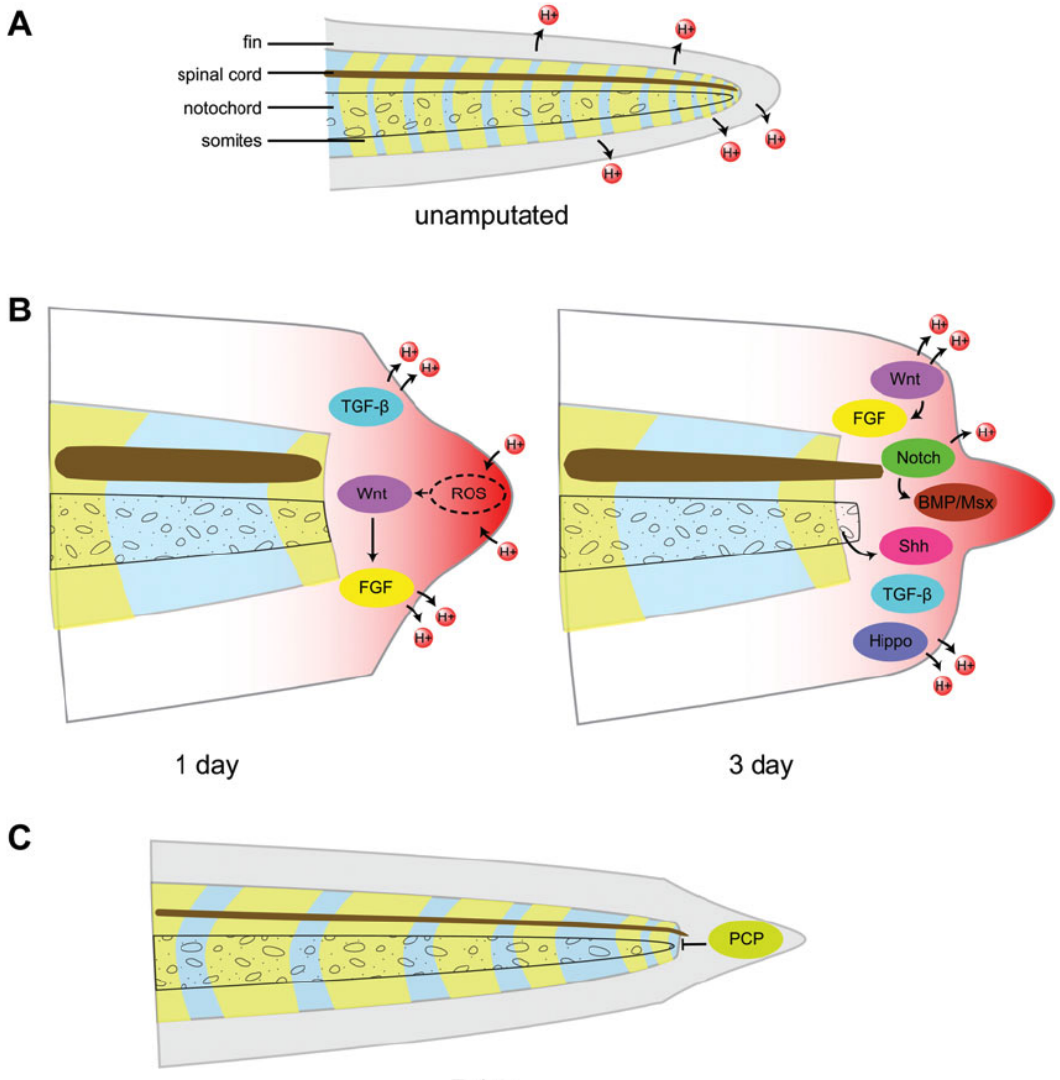
(B) Architectures of tissues such as bone and muscle are locally deconstructed near the amputation plane and are therefore shown as jagged. Newly activated progenitor cells, which give rise to future blastema cells, are cued to re-enter the cell cycle and some fraction of them presumably migrate to the space immediately below the wound epidermis. Blood cells, both red and white, are intermingled with blastema cells. A ‘nascent blastema’ is equivalent to very early bud stage blastema in other literature.

Received: 22 August 2016 | Revised: 9 September 2016 | Accepted: 12 September 2016
DOI: 10.1002/reg2.69

REVIEW

WILEY Regeneration Open Access

The cellular and molecular mechanisms of tissue repair and regeneration as revealed by studies in *Xenopus*



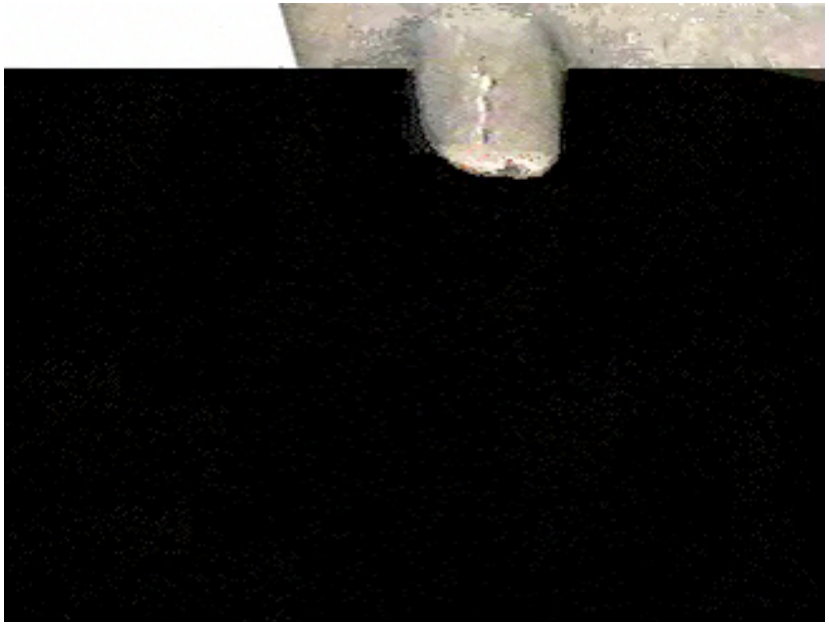
Stages of tadpole tail regeneration.

A *Xenopus* tadpole tail is composed of a number of axial structures including the spinal cord, notochord, and somites.

An unamputated tail is in a polarized state, sustained by V-ATPase pumps in the skin.
After amputation, wounded tail is depolarized and simultaneously reactive oxygen species (ROS) are produced at the amputation site.
Downstream targets of the ROS include Wnt, FGF, Shh, TGF-β, BMP, Notch, and Hippo pathways. V-ATPases are also upregulated at this stage to repolarize the skin.
A fully functional tail is regenerated 7 days after amputation.

Regenerative potential of *Ambystoma mexicanum* (Axolotl)

Regenerative potential of *Ambystoma mexicanum* (Axolotl)



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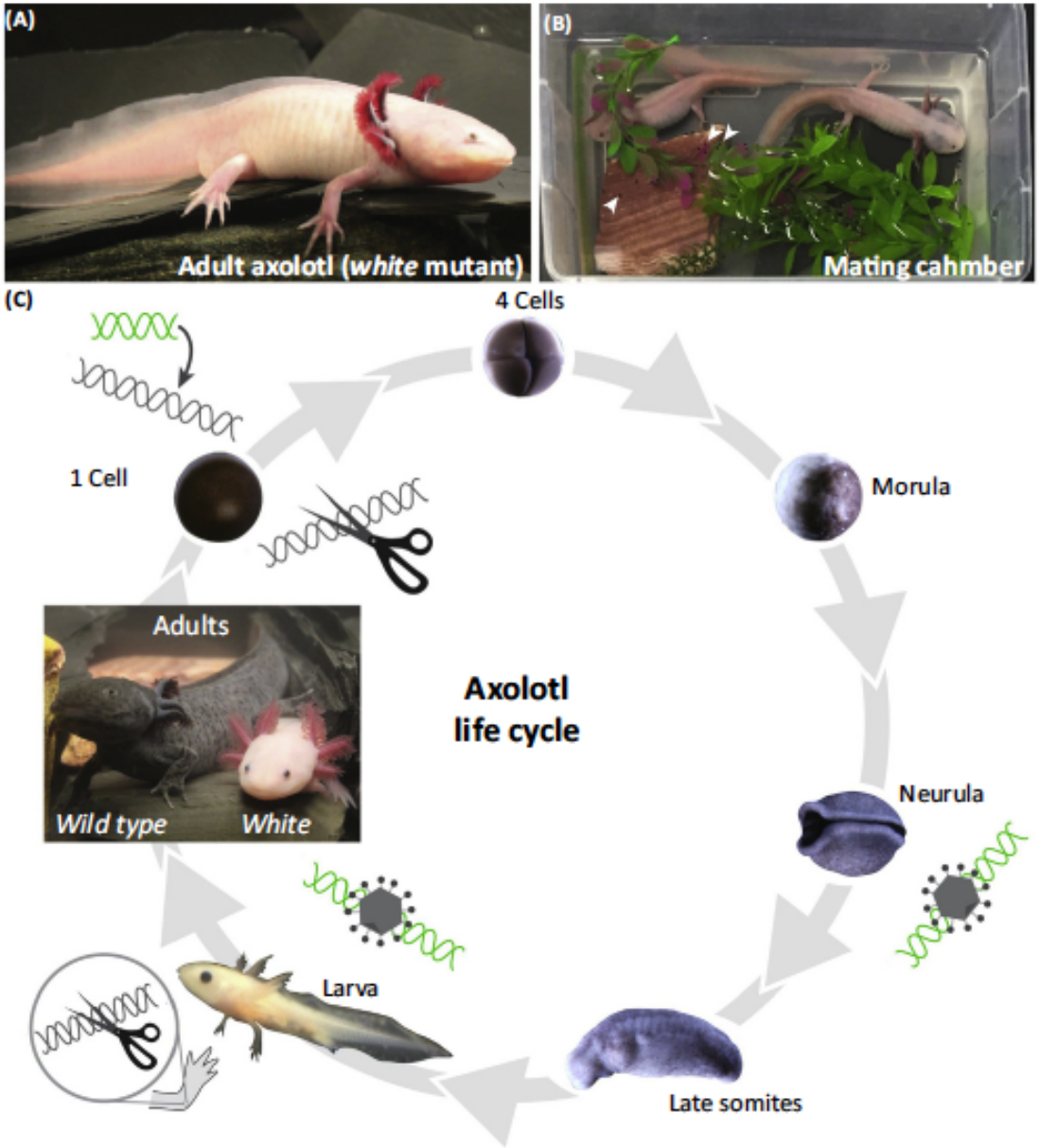
Trends in Genetics, August 2017, Vol. 33, No. 8

Review

CellPress

Advances in Decoding Axolotl Limb Regeneration

Brian J. Haas[1,*] and Jessica L. Whited[2,*]



Developmental Cell
Article

Developmental Cell *39*, 411–423, November 21, 2016 © 2016

CellPress

Live Imaging of Axolotl Digit Regeneration Reveals Spatiotemporal Choreography of Diverse Connective Tissue Progenitor Pools

Joshua D. Currie,[1,2,*] Akane Kawaguchi,[1] Ricardo Moreno Traspas,[1] Maritta Schuez,[1] Osvaldo Chara,[3,4] and Elly M. Tanaka[1,2,5,6,*]

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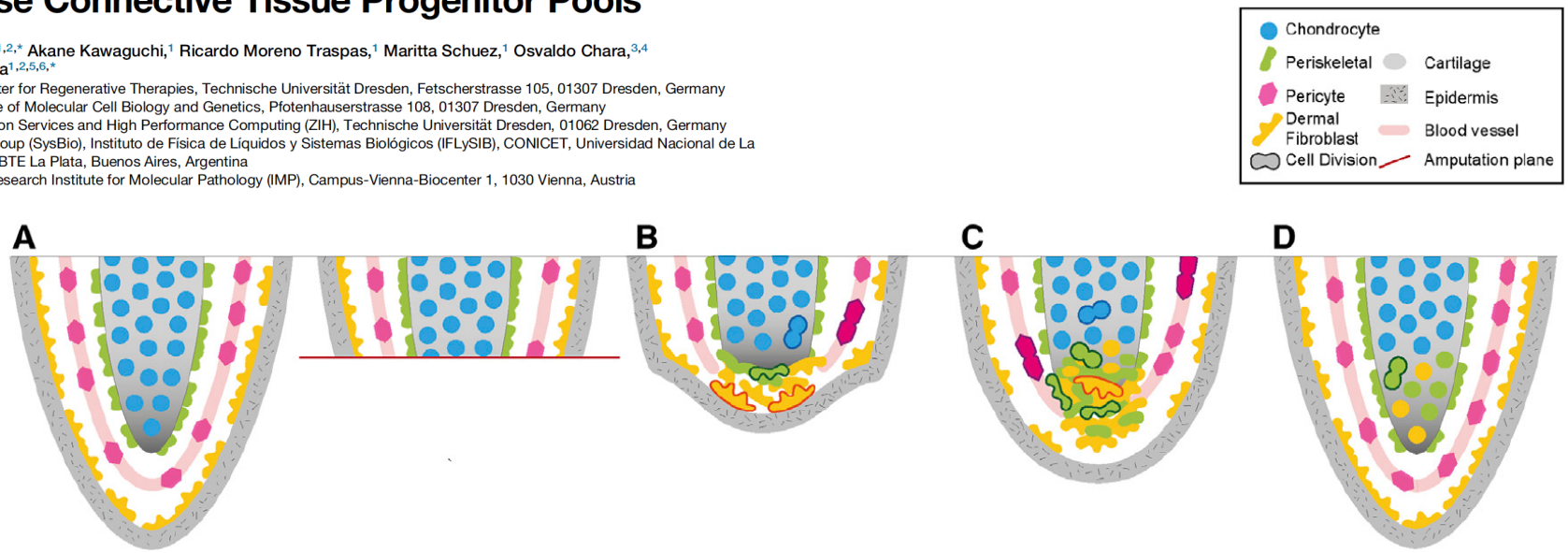
[4]Systems Biology Group (SysBio), Instituto de Física de Líquidos y Sistemas Biológicos (IFLySIB), CONICET, Universidad Nacional de La Plata (UNLP), B1900BTE La Plata, Buenos Aires, Argentina
[5]Present address: Research Institute for Molecular Pathology (IMP), Campus-Vienna-Biocenter 1, 1030 Vienna, Austria
[6]Lead Contact



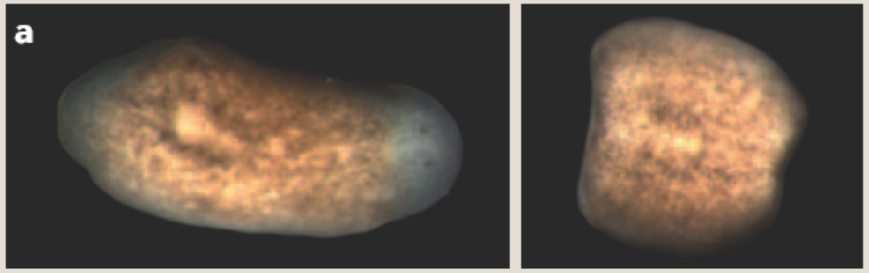
(A) The digit starts as an intact tissue with various connective tissue subcompartments. Chondrocytes, blue; pericytes, pink; periskeletal cells, green; dermal fibroblasts, orange.
(B) At the early stage after amputation, chondrocytes proliferate in situ and remain in place. Pericytes also divide behind the amputation plane. Periskeletal cells migrate without cell division across the surface of the skeleton, while dermal cells migrate underneath the wound epidermis (gray).
(C) In the mid-phase of regeneration, pericyte cells migrate along blood vessels into the blastema but retain their identity. Early-migrating periskeletal cells and dermal cells start proliferating, often already within the newly forming cartilage core. Migration of other periskeletal and dermal cells from behind the amputation plane continues.
(D) In the late phase of migration, tissue boundaries are already visible and late-migrating dermal cells contribute to lateral soft connective tissue.

Whereas a wound in humans gets covered with skin tissue, axolotls transform nearby cells into stem cells and recruit others from farther away to gather near the injury. There, the cells begin forming bones, skin and veins in almost the same way as when the animal was developing inside the egg. Each tissue contributes its own stem cells to the effort.

Transforming growth factor-β is THE key signalling molecule in axolotl regeneration

Common signaling pathways inducing regeneration

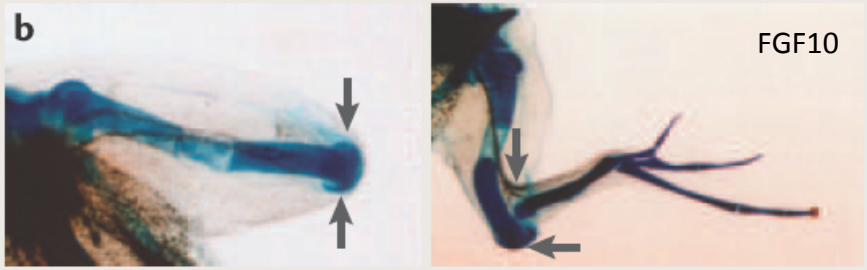
Planaria

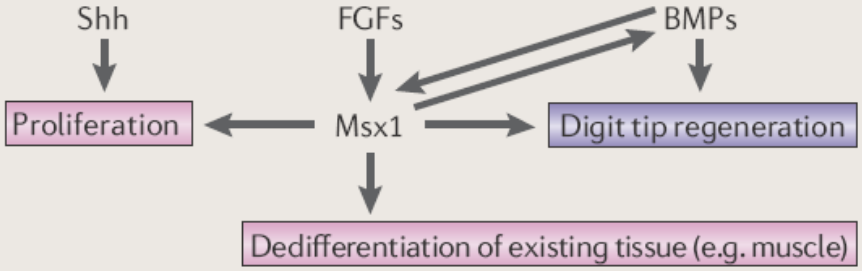


TGFB → TGFBR (?) → SMAD4 → Blastema formation

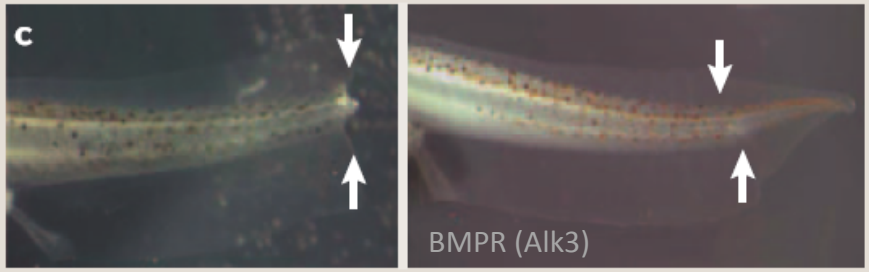
Cephalic and caudal regeneration in planarians is inhibited by the abrogation of the TGF-β pathway (silencing the expression of smad4)

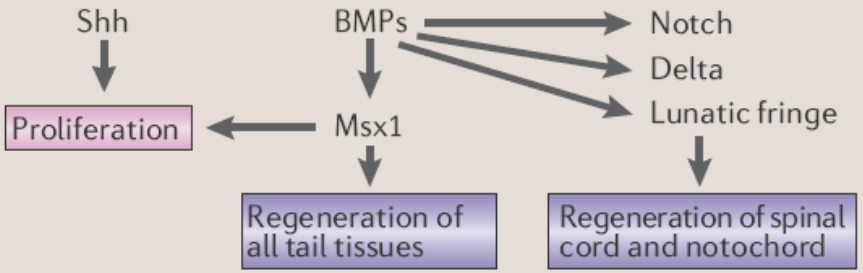
Newt





Zebrafish





Major signaling pathways involved in cellular differentiation are extensively conserved!

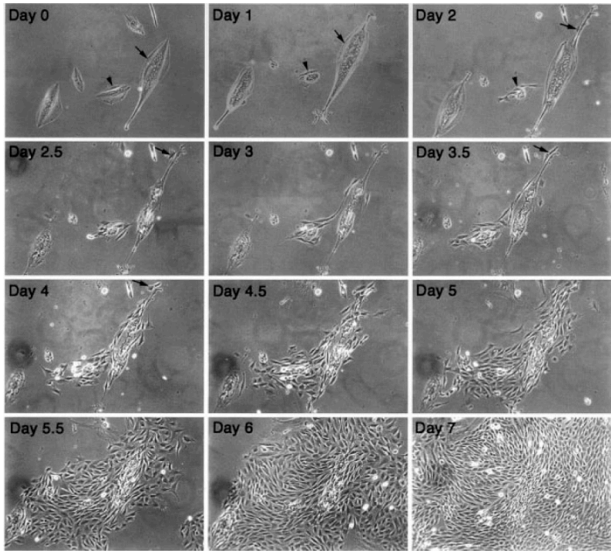
Signalling pathway	Species or group		
	Hydra magnipapillata	*Schmidtea mediterranea*	Vertebrates
TGFB	Yes	Yes	Yes
Notch	Yes	Yes	Yes
Wingless	Yes	Yes	Yes
Hedgehog	Yes	Yes	Yes
JAK/STAT	Unknown	Yes	Yes
EGF receptor	Yes	Yes	Yes
FGF receptor	Yes	Yes	Yes
Toll/NFκB	Unknown	Yes	Yes

Mammalian cells might maintain the pathways required to respond to the proper “pro-regeneration” signals

Cell, Vol. 103, 1099–1109, December 22, 2000, Copyright ©2000 by Cell Press

Dedifferentiation of Mammalian Myotubes Induced by msx1

Shannon J. Odelberg,*§‖ Angela Kollhoff,† and Mark T. Keating*†‡‖#
*Division of Cardiology, Department of Internal Medicine



Mononucleated cells from dedifferentiated myotubes exhibit signs of pluripotency (subjected to chondrogenic, osteogenic, adipogenic, and myogenic inducing signals)