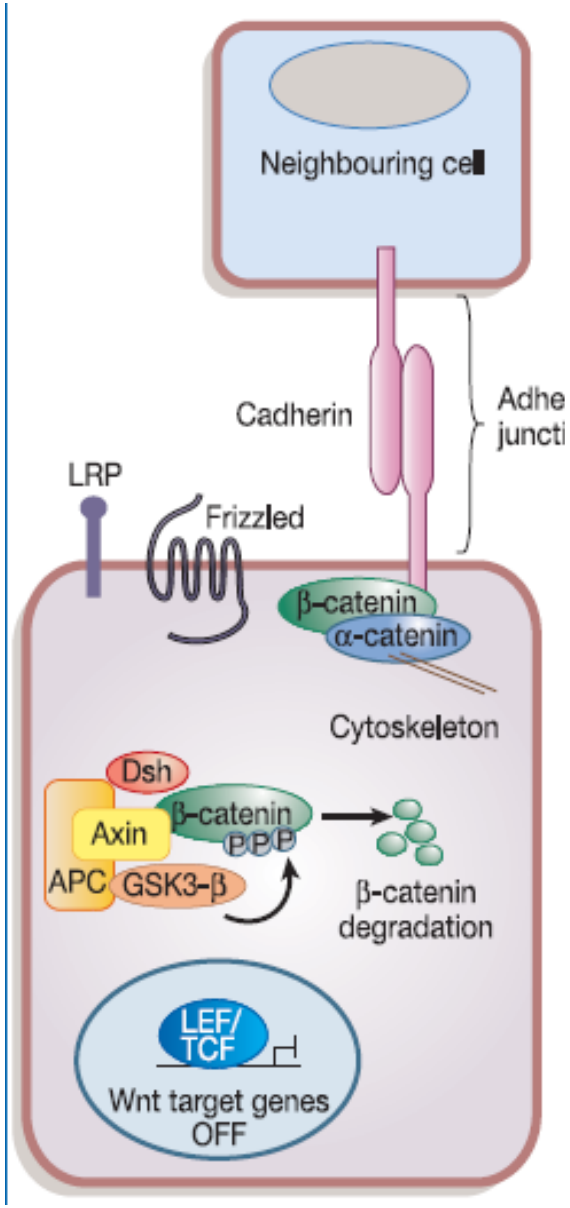
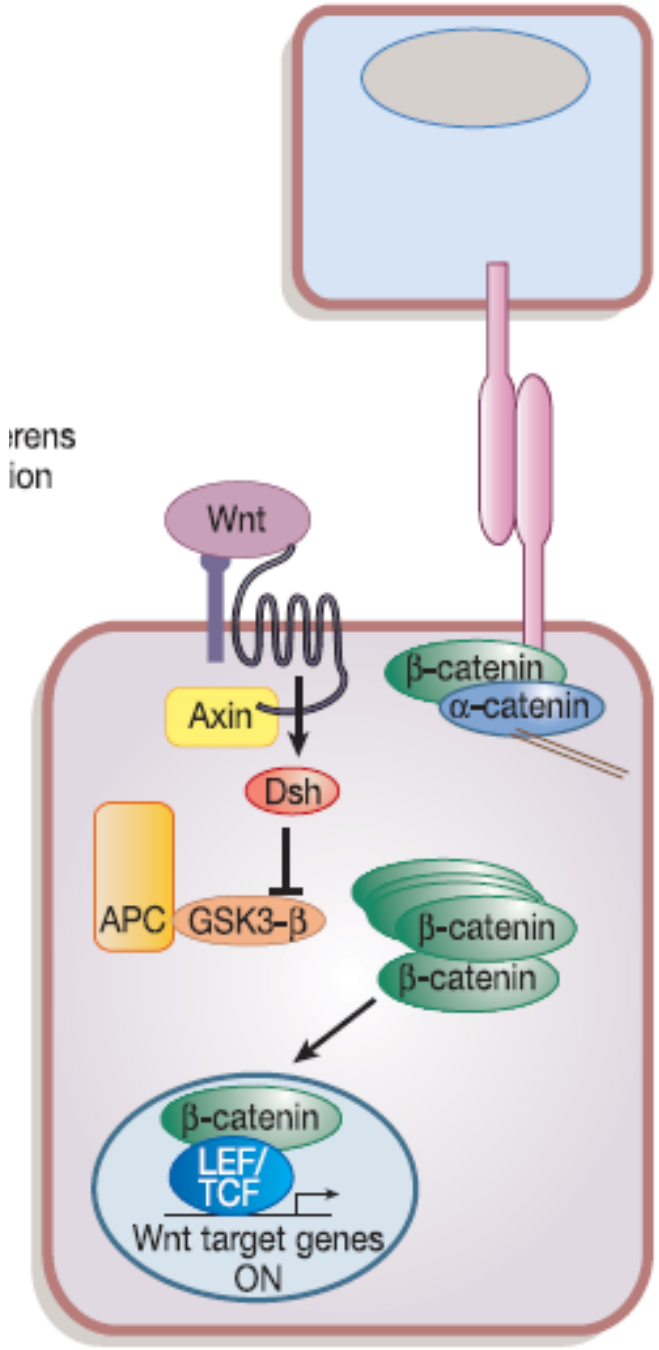
The Wnt/β-catenin signalling pathway

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

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

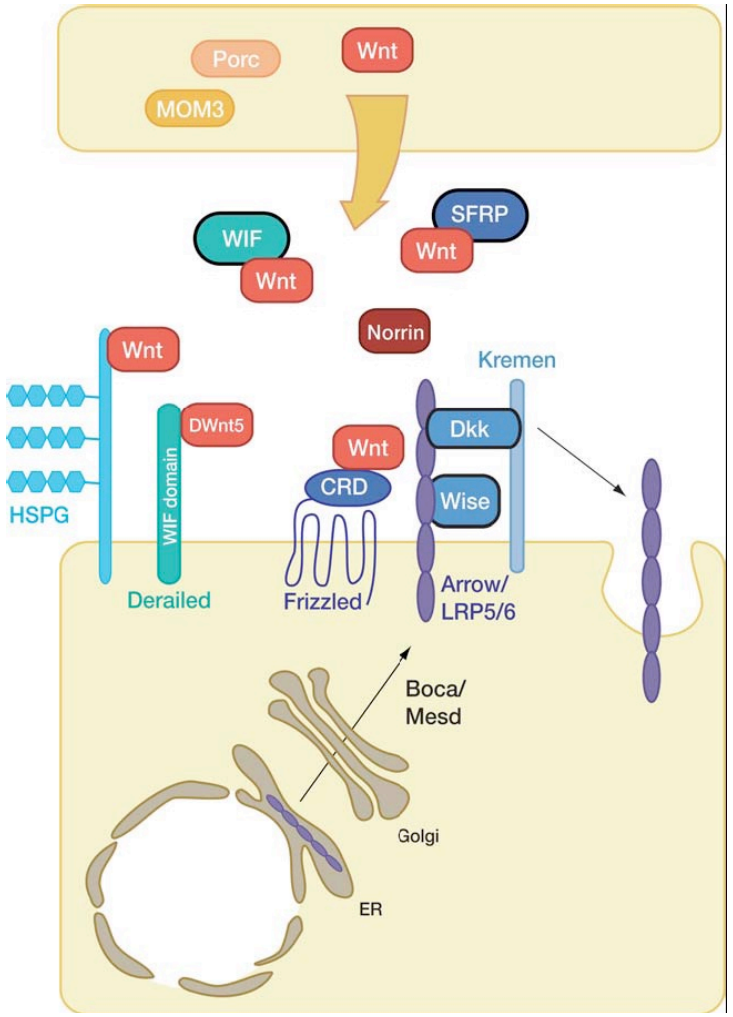




In the presence of Wnt ligand, Axin is recruited to the plasma membrane. b-catenin is then released from the multiprotein degradation complex and accumulates in the cytoplasm in a stabilized non-phosphorylated form.

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

- In vertebrates, Wnt proteins are inhibited by direct binding to either secreted frizzled-related protein (SFRP) or Wnt inhibitory factor (WIF).
- SFRP is similar in sequence to the cysteine-rich domain (CRD) of Frizzled, one of the Wnt receptors.



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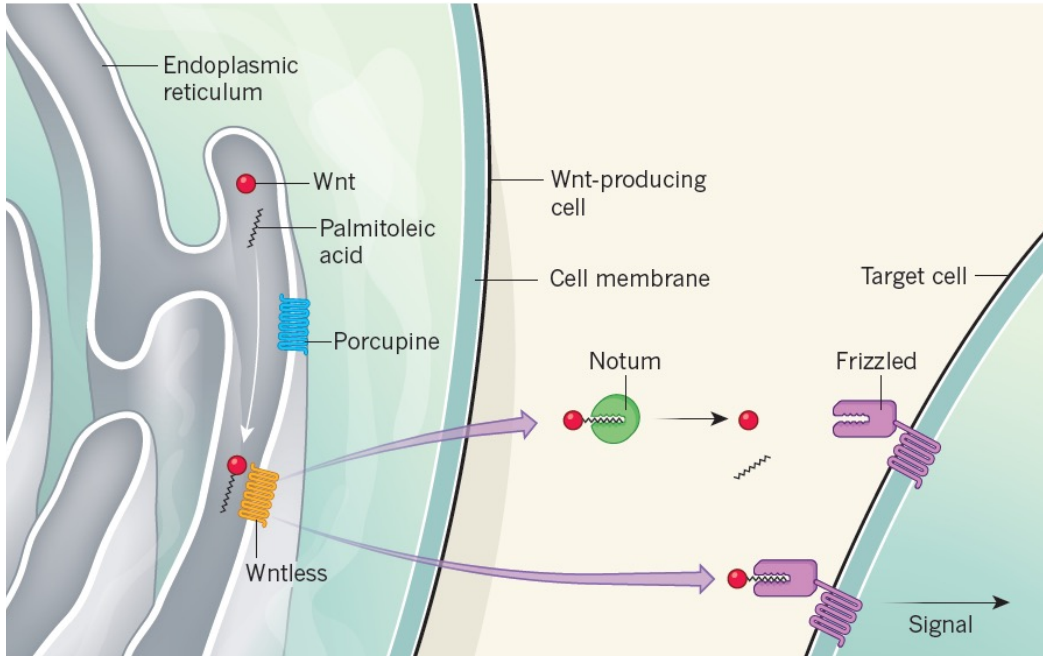
Notum deacylates Wnt proteins to suppress signalling activity

Satoshi Kakugawa[1]*, Paul F. Langton[1]*, Matthias Zebisch[2]†*, Steven A. Howell[1], Tao-Hsin Chang[2], Yan Liu[3], Ten Feizi[3], Ganka Bineva[4], Nicola O'Reilly[4], Ambrosius P. Snijders[5], E. Yvonne Jones[2] & Jean-Paul Vincent[1]



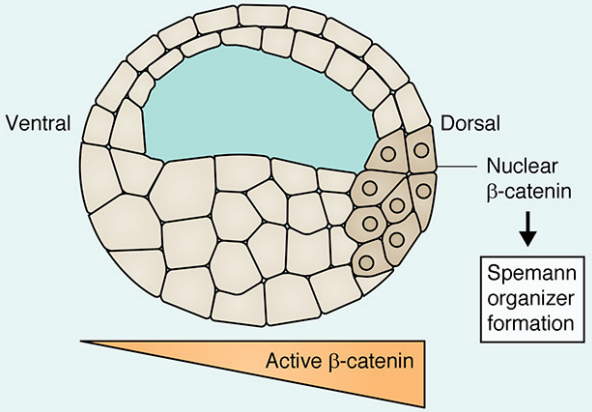
Notum shoots the messenger in Wnt signalling.
In Wnt-producing cells, the Wnt protein is made in the endoplasmic reticulum. There, an acyl group is added to Wnt by the membrane-spanning enzyme Porcupine. Secreted Wnt binds to its receptor Frizzled on target cells. This binding depends on the acyl group 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.

Wnt signalling is essential in early embryonic development

Wnt signaling in embryonic development

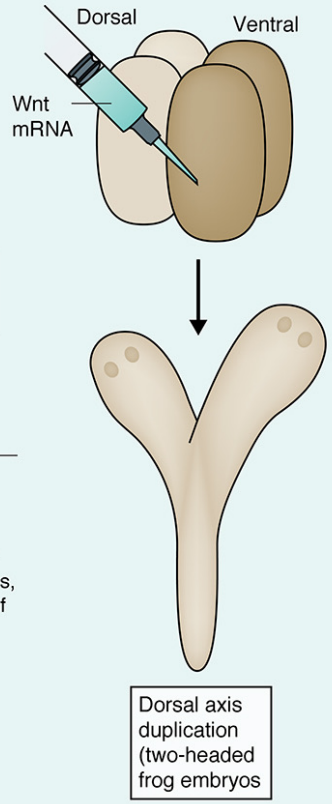
Blastula

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



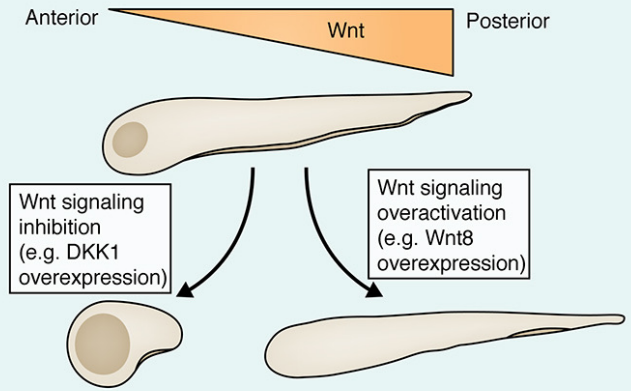
Four-cell embryo

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



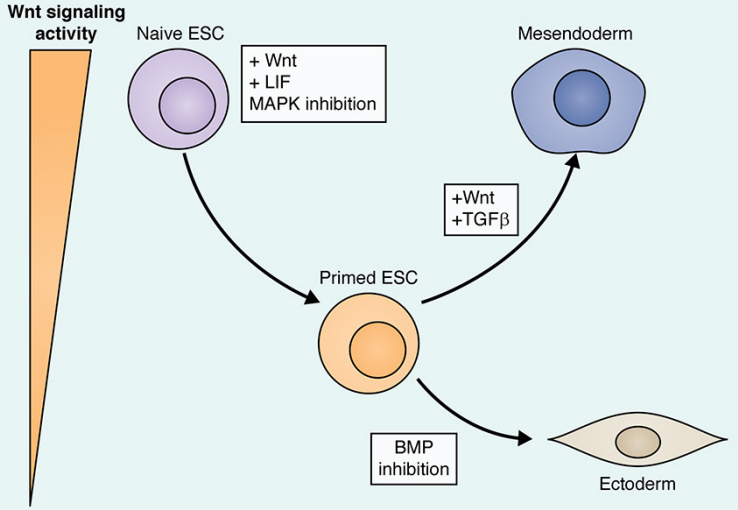
Post-gastrulation

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



Wnt signaling in pluripotent cells

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



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

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

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

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

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

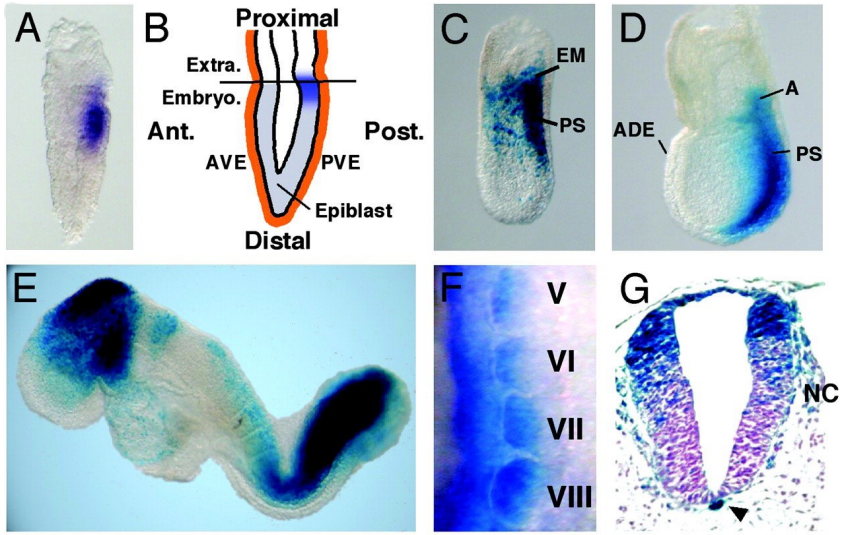
Wnt7b Placental development defects
Respiratory failure, defects in early mesenchymal proliferation leading to lung hypoplasia

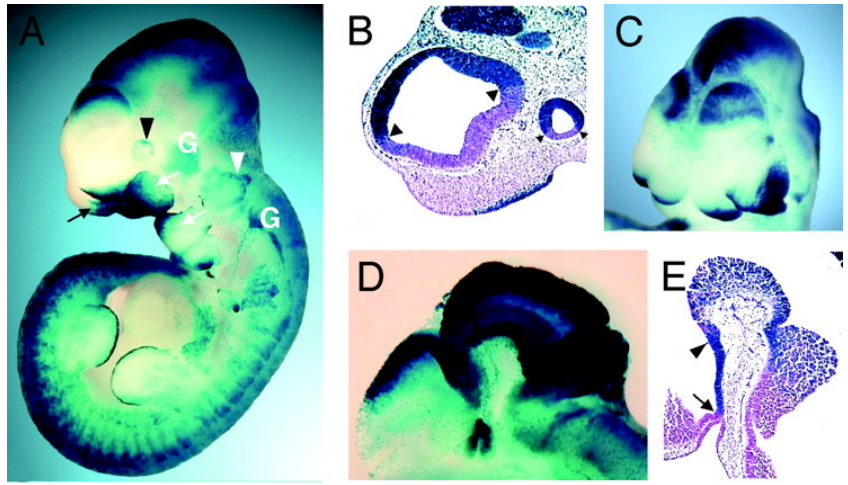
Wnt11 Ureteric branching defects and kidney hypoplasia

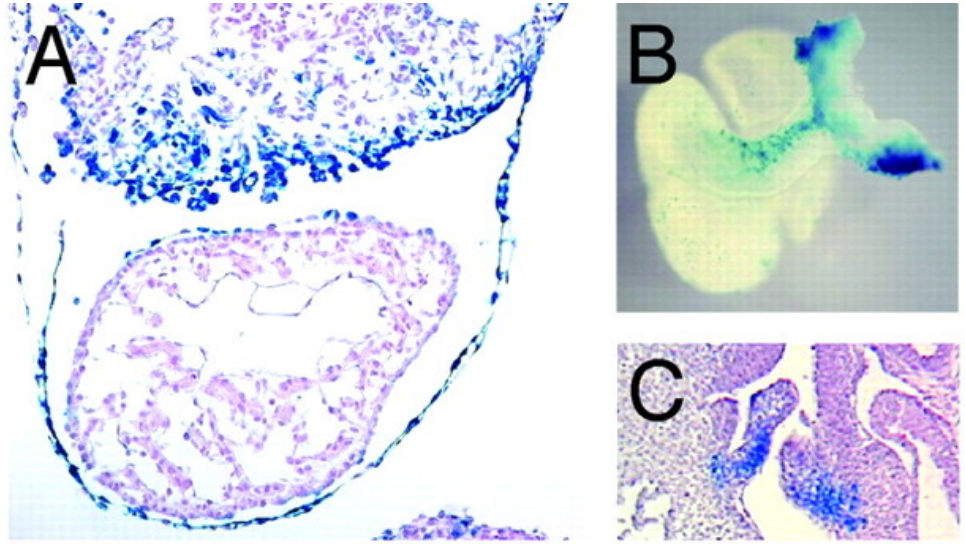
Mapping Wnt/β-catenin signaling during mouse development and in colorectal tumors

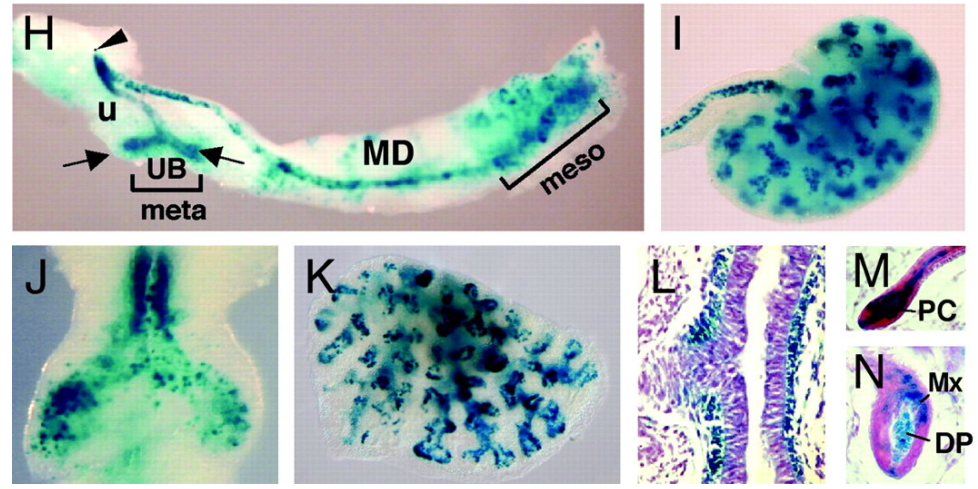
Silvia Maretto*, Michelangelo Cordenonsi*, Sirio Dupont*, Paola Braghetta*, Vania Broccoli†, A. Bassim Hassan‡, Dino Volpin*, Giorgio M. Bressan*, and Stefano Piccolo*§

*Histology and Embryology Section, Department of Histology, Microbiology, and Medical Biotechnology, University of Padua, 35131 Padua, Italy; †Stem Cell Research Institute, H. S. Raffaele, 20132 Milan, Italy; and ‡Cell and Development Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom

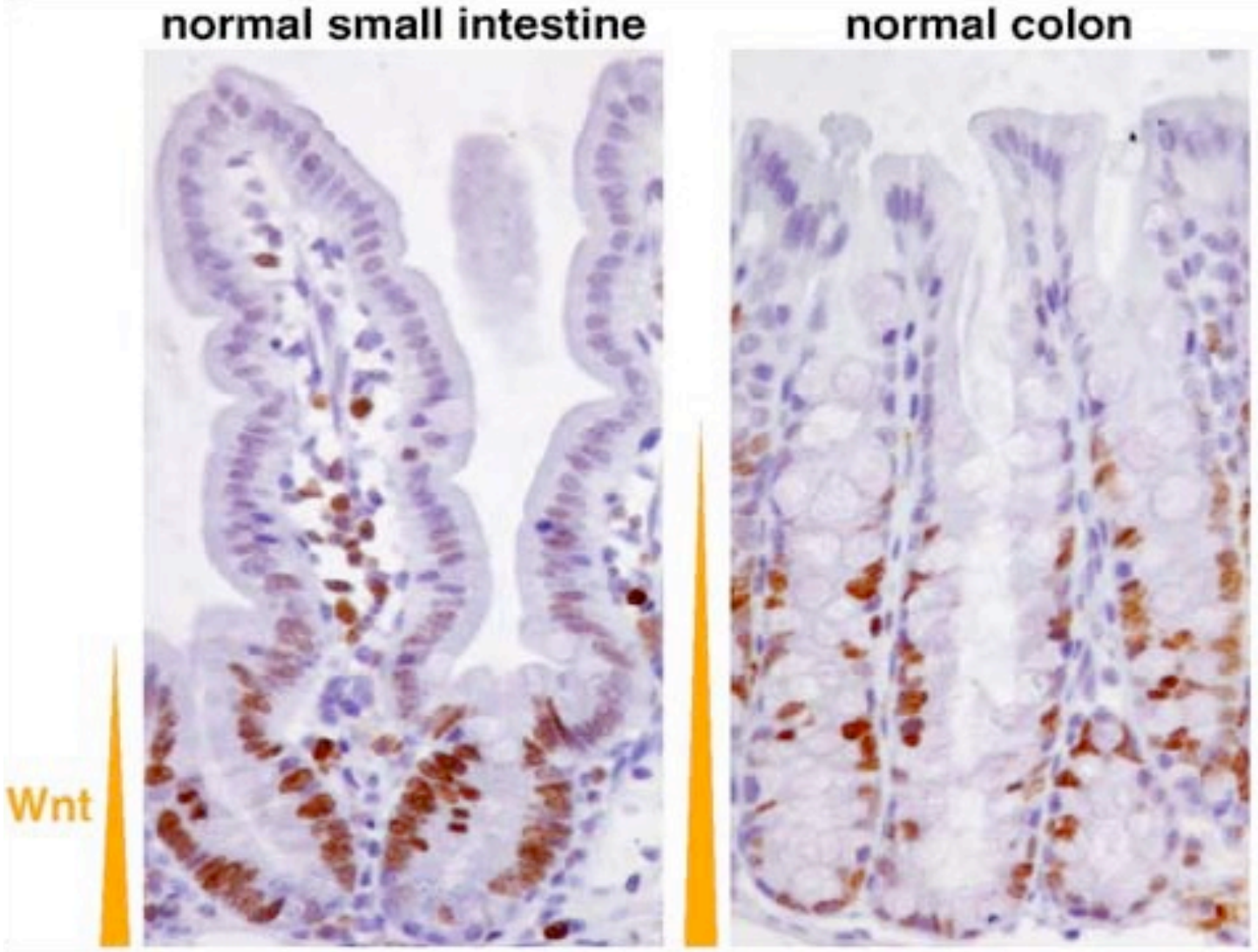




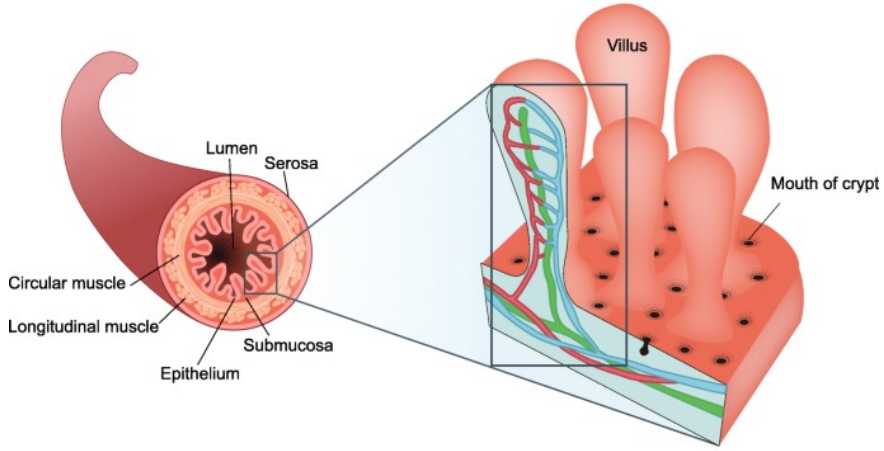


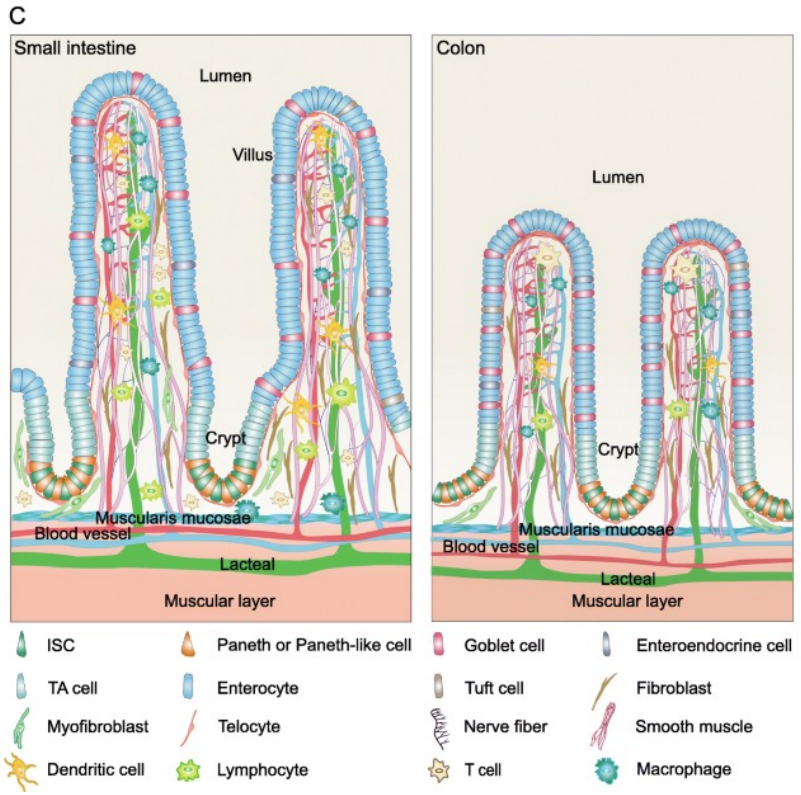


The Wnt cascade is the dominant force in controlling cell fate along the crypt-villus axis.



The intestinal epithelium: a dynamic tissue

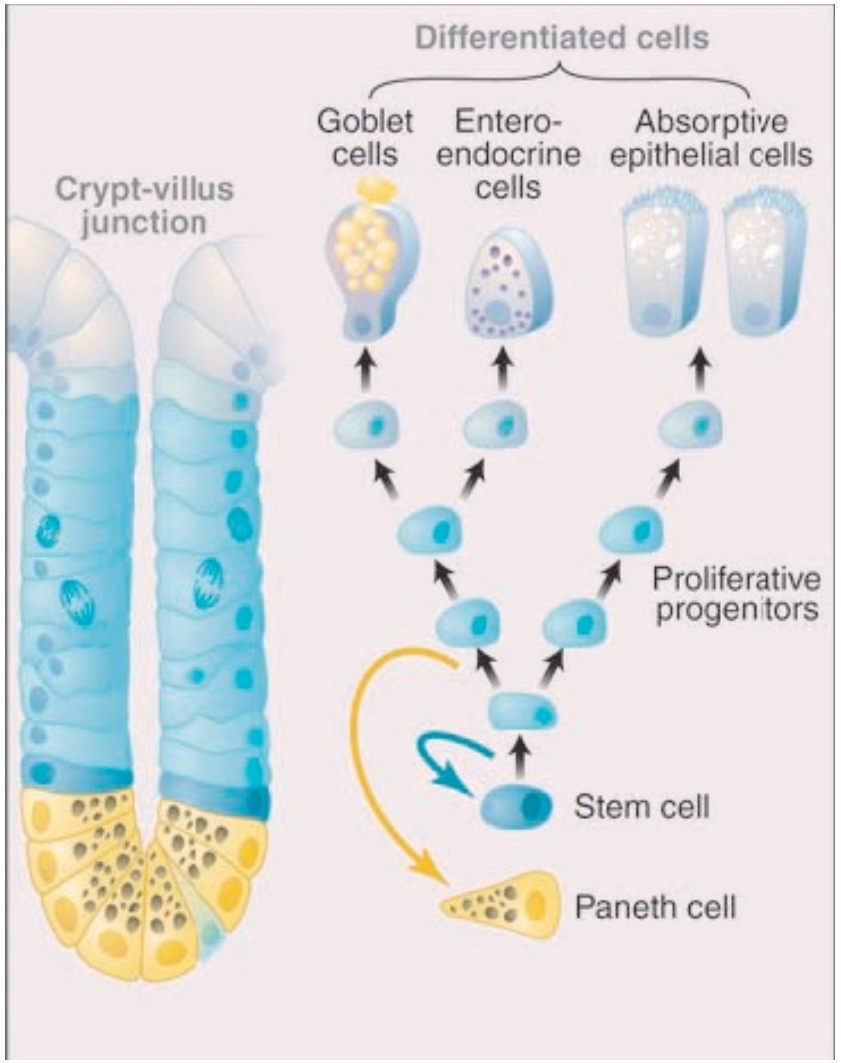




The absorptive epithelium of the small intestine is ordered into submucosal invaginations, the crypts of Lieberkuhn, and luminal protrusions termed villi.

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.



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

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

These daughter cells gradually differentiate into 4 epithelial lineages:

1- absorptive cells or enterocytes;

2- mucus-producing goblet cells;

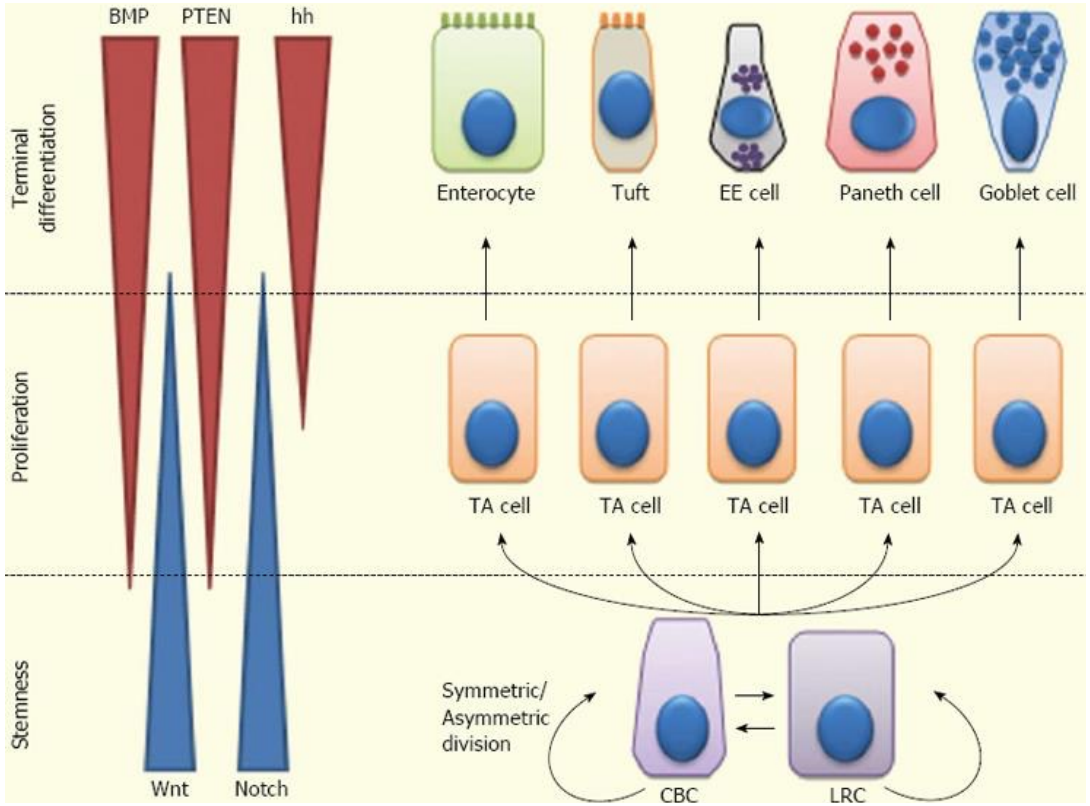
3- enteroendocrine cells, secreting hormones such as serotonin or secretin;

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

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

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

Lineage specification of intestinal stem cells



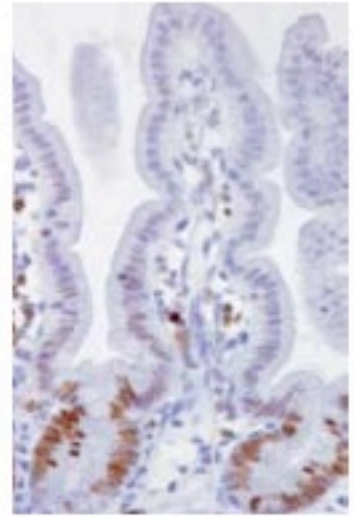
Intestinal stem cells divide asymmetrically or symmetrically to maintain the stem cell compartment. ISCs give rise to Transit Amplifying (TA) cells which actively proliferate and can further differentiate into enterocytes, tuft cells, enteroendocrine (EE) cells or goblet cells. Wnt signaling maintains the stem-like phenotype of ISCs, while Notch signaling maintains the proliferation of progenitor cells.

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

Normal Wnt signalling

Stem cells

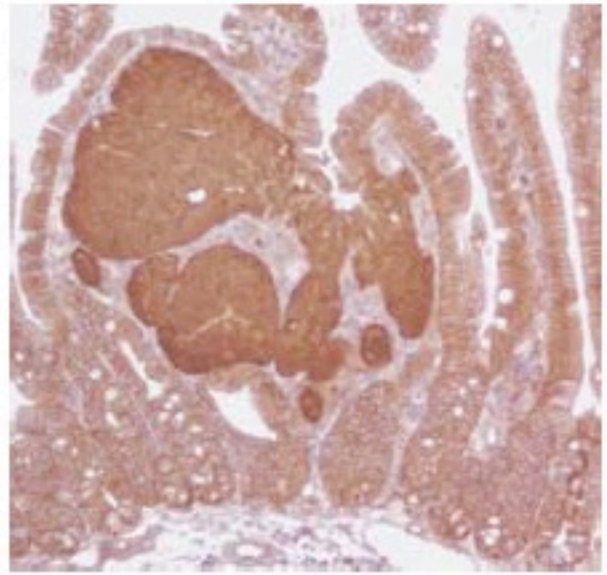
Progenitors



Normal tissue

Dysregulated Wnt signalling in stem cells

Dysregulated Wnt signalling in progenitor cells



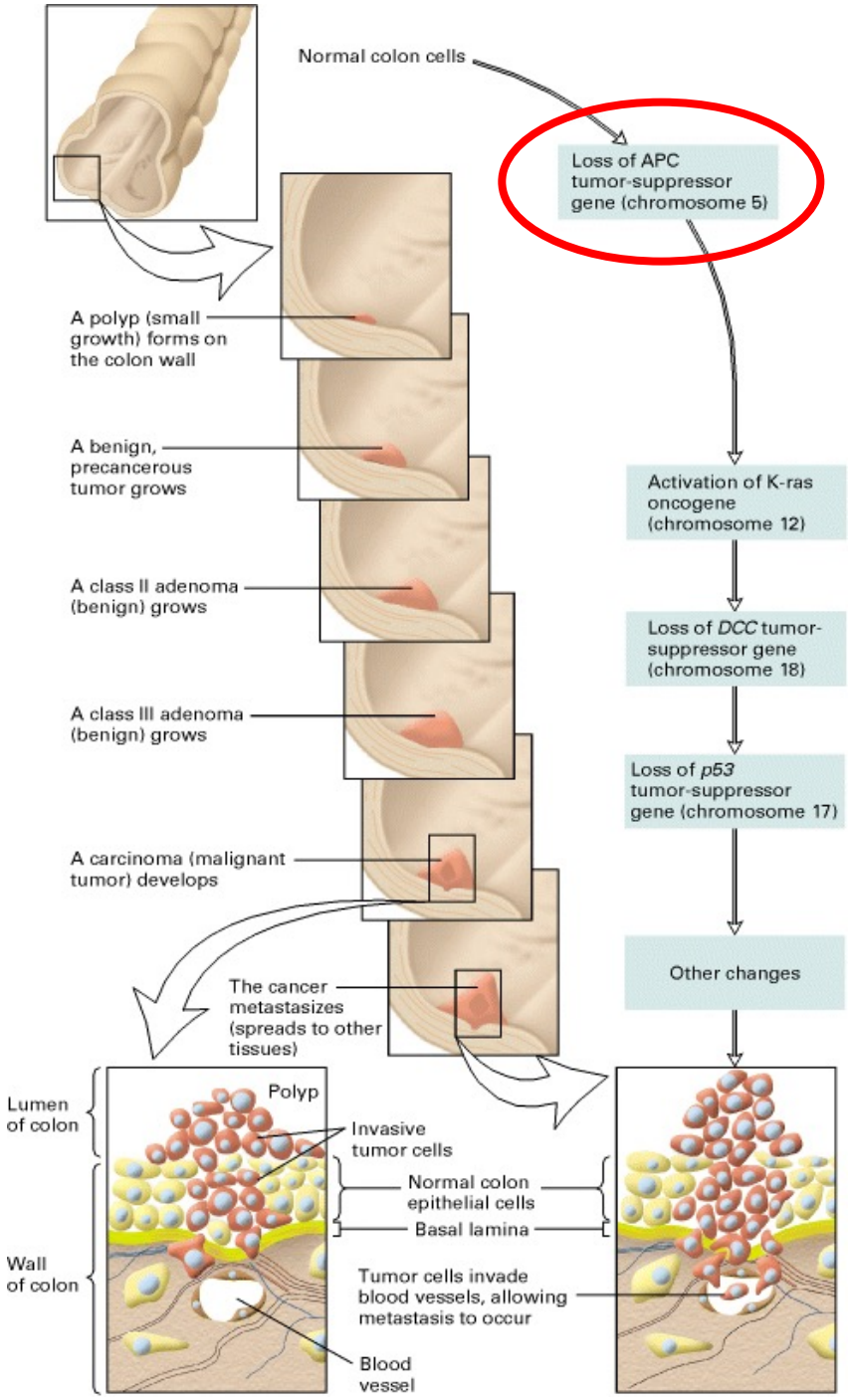
Cancer

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

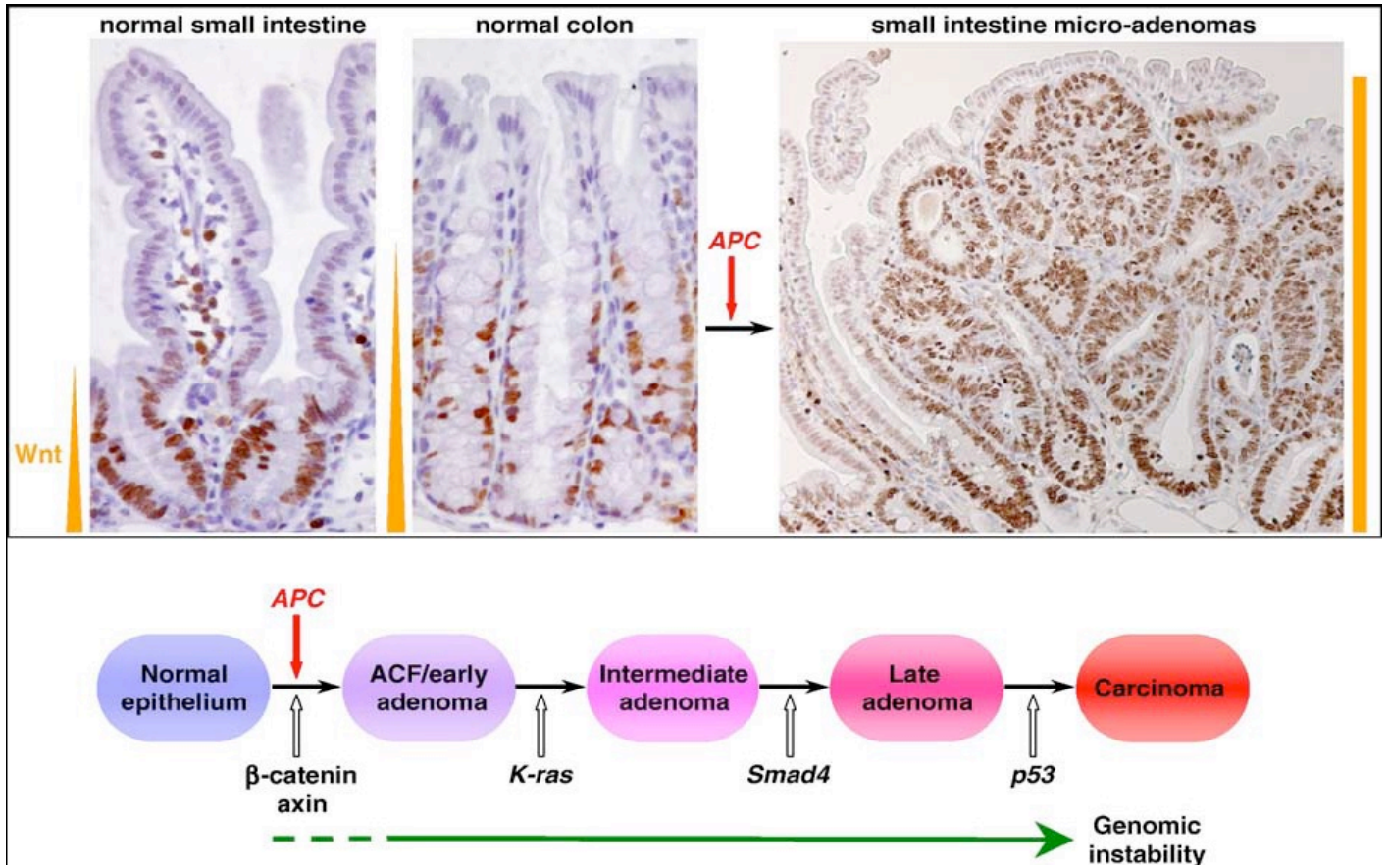
(i) colorectal tumors result from mutational activation of oncogenes combined with the inactivation of tumor-suppressor genes,

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

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



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

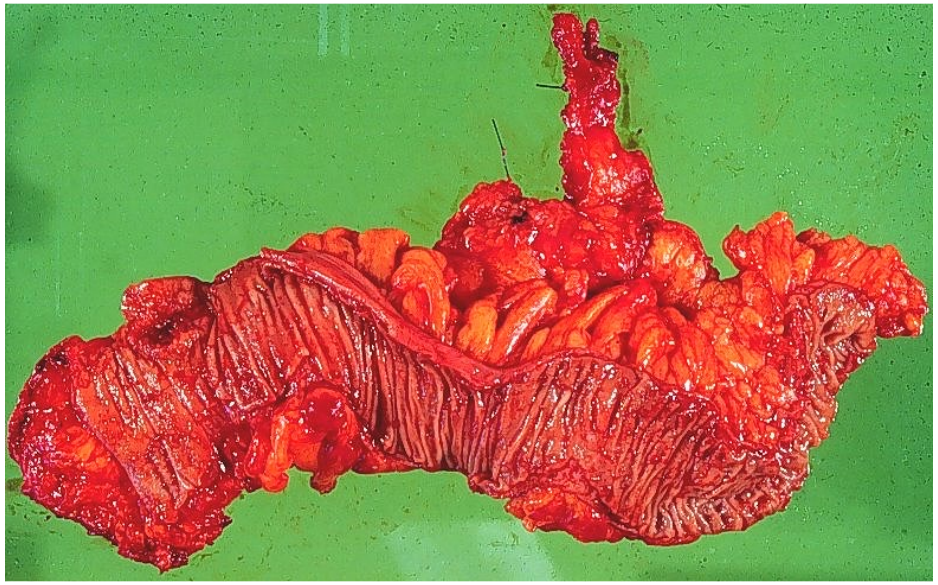


•Inactivating mutations in the APC gene (that selectively disable binding to b-catenin) or activating mutations in b-catenin (that remove the regulatory phosphorylation sites) lead to nuclear accumulation of b-catenin .

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

The Wnt pathway in colon cancer

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



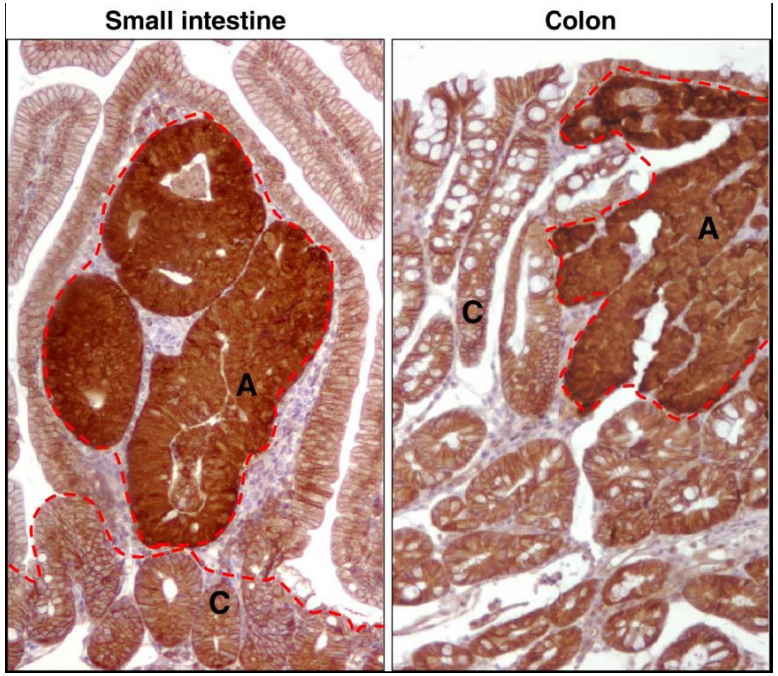
In FAP, as in most sporadic CRCs, tumorigenesis occurs incrementally. The earliest lesions in the colon or the rectum are “aberrant crypt foci” which progress to benign tumors termed adenomas or adenomatous polyps. Colorectal polyps can eventually develop into malignant tumor stages termed adenocarcinomas.

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

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

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

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



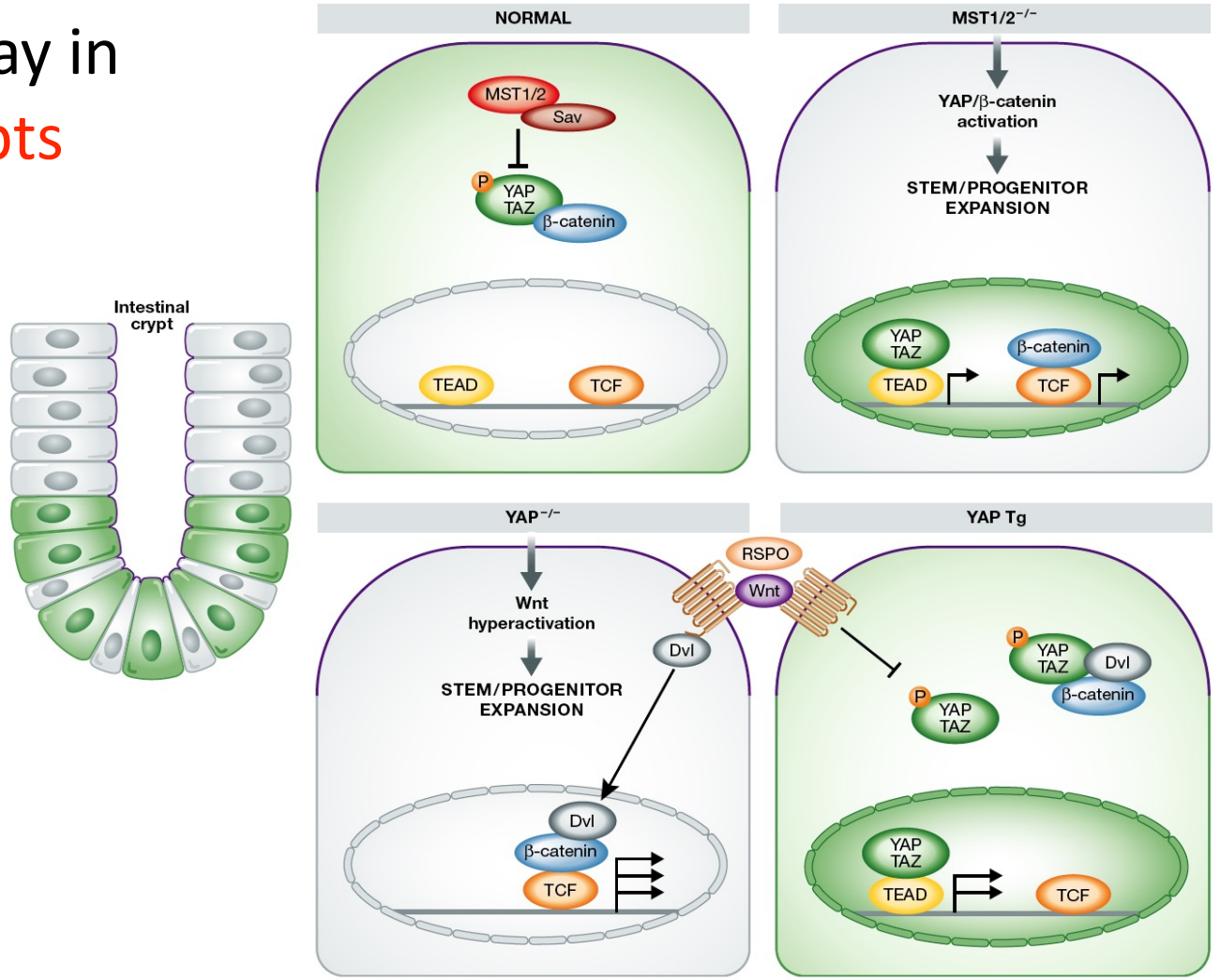
•Nuclear β-catenin accumulates in the crypt stem cell/progenitor compartments in small intestine and colon;

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

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

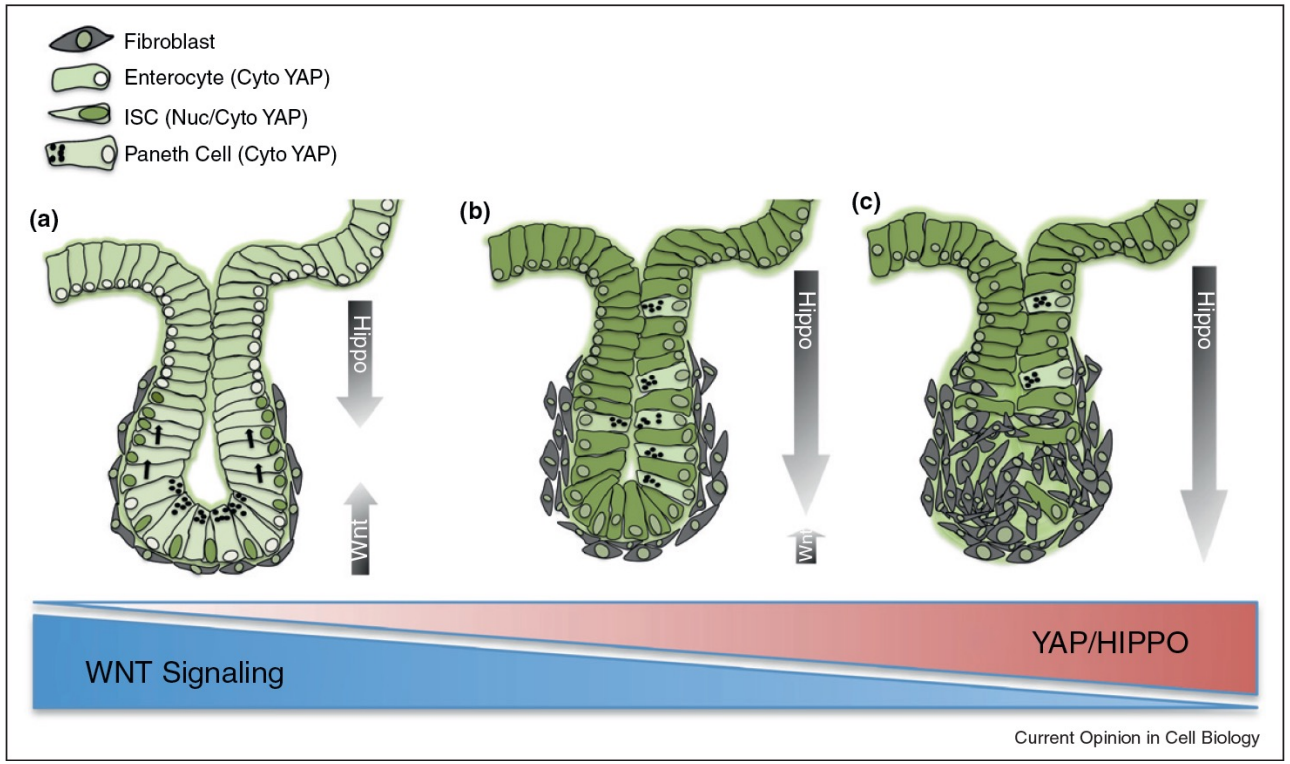
Wnt signaling is absolutely required for driving and maintaining crypt stem cell/progenitor compartments, and, thus, is essential for homeostasis of the intestinal epithelium.

The Hippo pathway in the intestinal crypts



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

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.

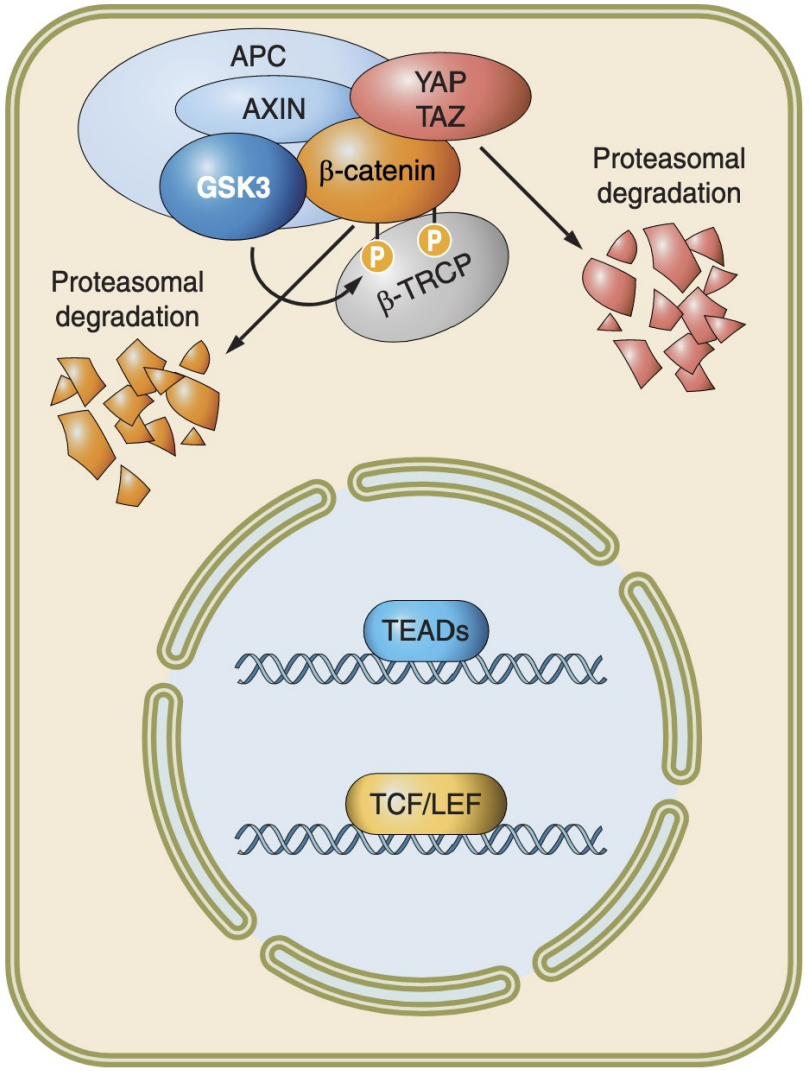
(b) When YAP is overabundant in the cytoplasm, Wnt signaling is repressed and the ISC niche is disrupted, causing aberrant upward migration of Paneth cells and loss of ISCs.

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

YAP/TAZ orchestrate the Wnt response

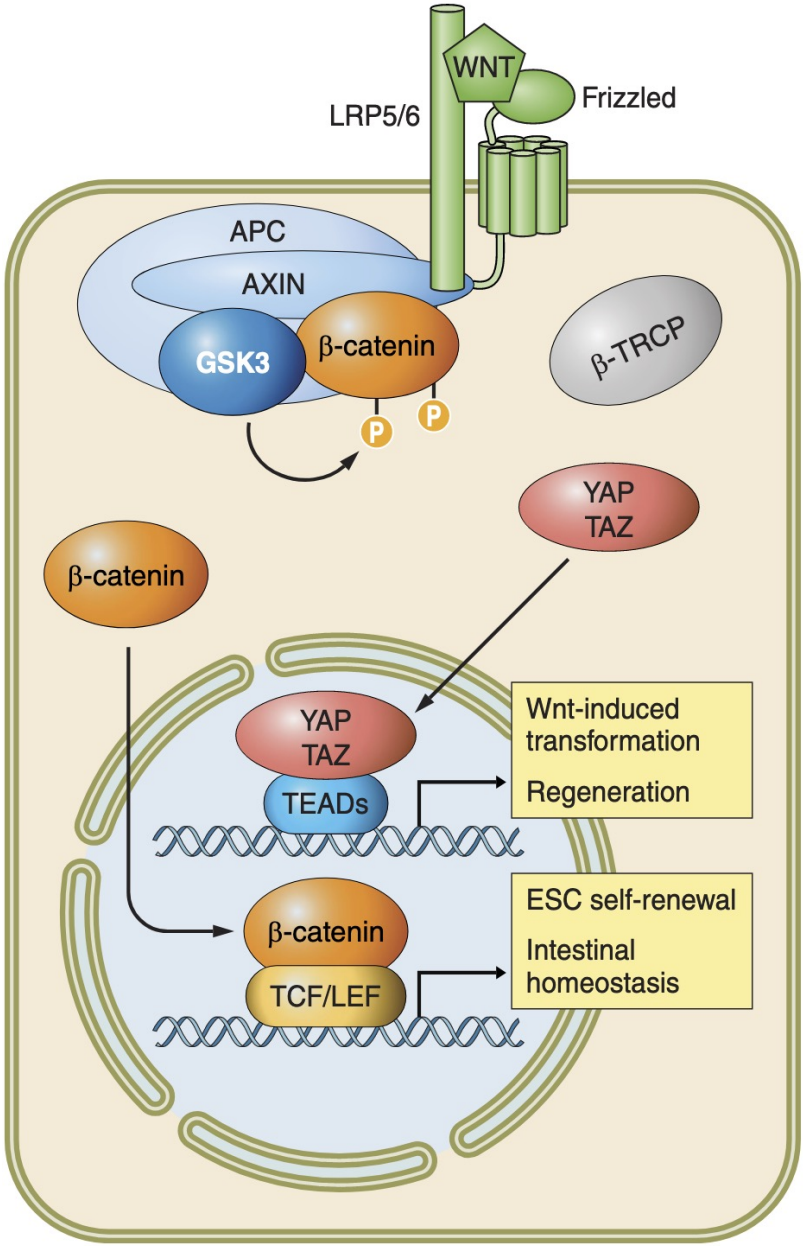
YAP/TAZ are not only messengers of the cell's structural features, but also of Wnts, a leading family of growth factors involved in cell proliferation, stem cell expansion, regeneration, and tumorigenesis (36, 154). Recent work highlighted a deep integration of YAP/TAZ in the Wnt pathway that mechanistically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

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

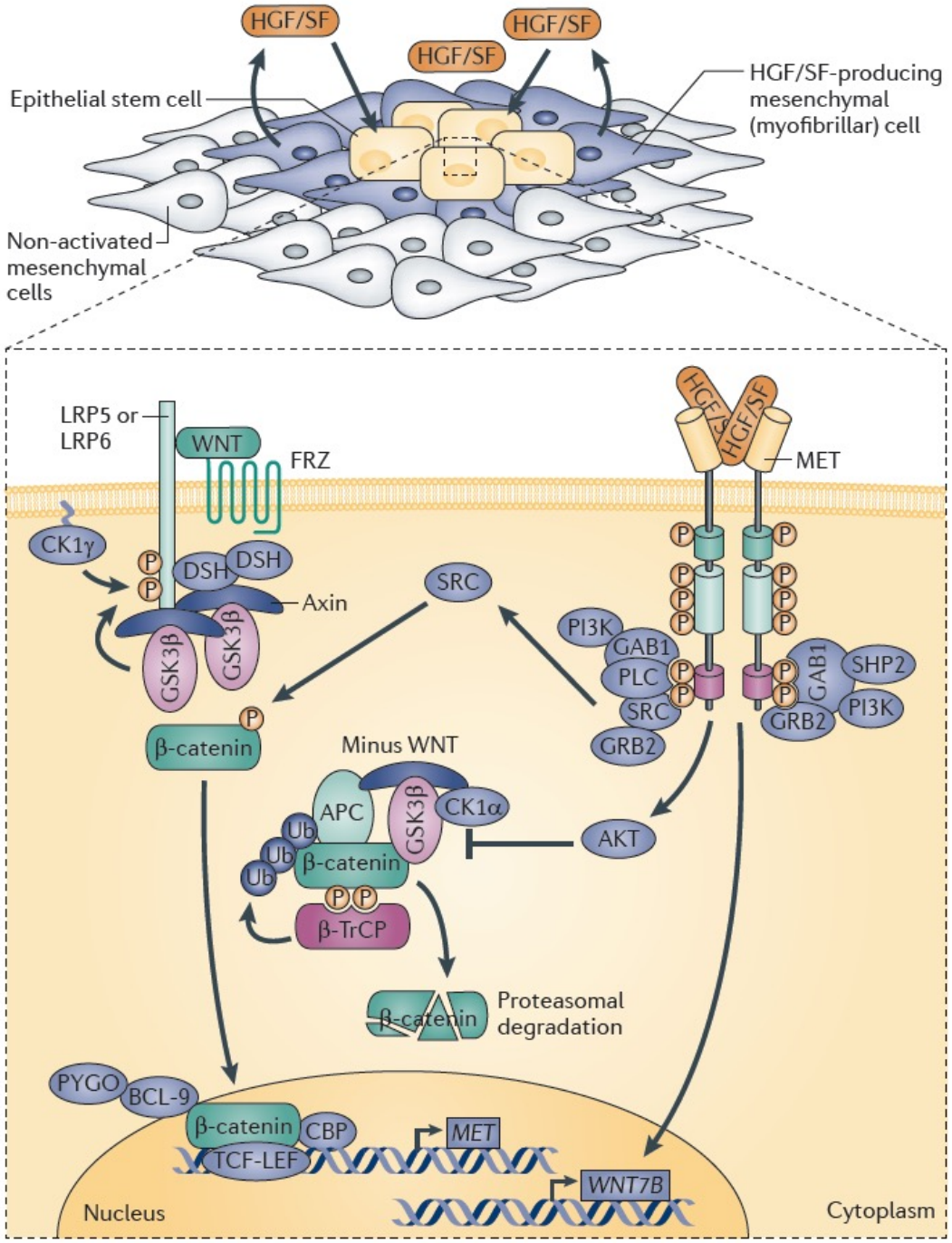


generation (12). The arrival of a Wnt ligand triggers the association between the Wnt receptor LRP6 and Axin with concomitant release of YAP/TAZ from the destruction complex (8). The consequence of such release is again two-fold: *1*) without YAP/TAZ, the destruction complex is now "invisible" to β-TrCP, favoring β-catenin accumulation; and 2) YAP/TAZ can now accumulate in the nucleus leading to the activation of Wnt-induced, YAP/TAZ-dependent transcriptional responses

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



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).

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]

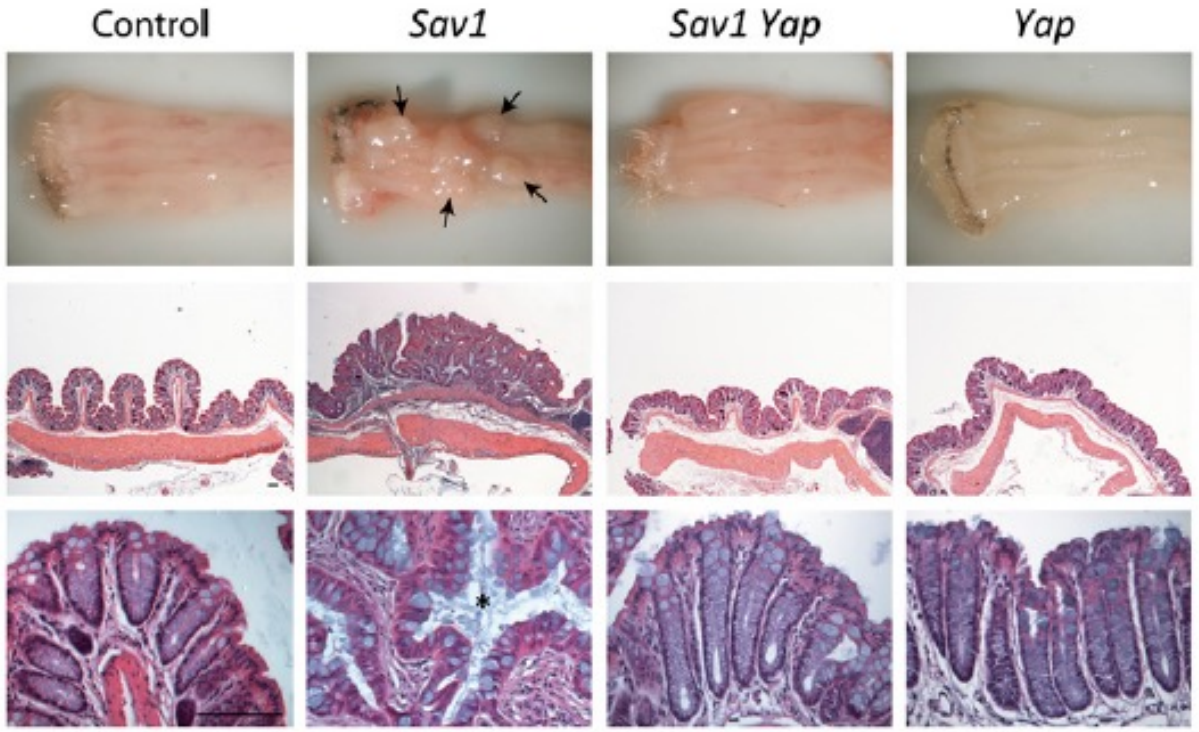


Figure 5. DSS-induced regeneration accelerated polyp development in *Sav1*-deficient colons in a *Yap*-dependent manner. (*A*) Distal colon of 12-wk-old wild-type, *Sav1*, *Yap*, or *Sav1 Yap* double-mutant mice treated with 2.5% DSS for 4 d, followed by normal drinking water for 3 mo. Note the presence 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