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Reparative inflammation takes charge of tissue regeneration

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Abstract

Inflammation underlies many chronic and degenerative diseases, but it also mitigates infections, clears damaged cells and initiates tissue repair. Many of the mechanisms that link inflammation to damage repair and regeneration in mammals are conserved in lower organisms, indicating that it is an evolutionarily important process. Recent insights have shed light on the cellular and molecular processes through which conventional inflammatory cytokines and Wnt factors control mammalian tissue repair and regeneration. This is particularly important for regeneration in the gastrointestinal system, especially for intestine and liver tissues in which aberrant and deregulated repair results in severe pathologies.

The intestinal epithelium is the most rapidly self-renewing tissue in the mammalian body — cells have a life cycle of 3–4 days. Multiple studies have demonstrated that the intestinal stem cells (ISCs) in intestinal crypt compartments are dependent on Wnt homeostatic signals[1]. ISCs are marked by the expression of the transmembrane receptor Lgr5 and reside at the bottom of the crypts, where they are intermingled with Paneth cells, one of their daughter cells. Paneth cells produce bactericidal proteins (for example, lysozyme) and peptides (defensins and cryptdins), thus participating in gut innate immunity (Fig. 1a). Paneth cells also produce a range of niche signals that support ISCs, including epidermal growth factor (EGF), Wnt3 and Notch ligands[2]. Although Wnt3 is redundant with other Wnts *in vivo*[3], it is essential for the expansion of ISC-generated organoids *in vitro*[1].

The healthy liver contains very few proliferative cells. Increased Wnt activity mainly occurs around the liver's central veins, resulting in a signalling gradient that governs 'zonation' of the liver lobule — the differential distribution of liver enzymes and metabolic functions

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Note added in proof: A paper recently appeared online while the current Review was in press that underscored the regenerative role of IL-22 by demonstrating that it directly promotes proliferation of isolated ISCs in culture (C. A. Lindemans *et al.* Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration *Nature* http://dx.doi.org/10.1038/nature16460; 2015).

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along the portal-tract-to-central-vein axis[4]. A pool of pericentral diploid hepatocytes has been described using lineage-tracing based on the Wnt-driven adult stem cell (ASC) gene *Axin2*. This cell population mediates homeostatic hepatocyte renewal and is controlled by Wnts produced by endothelial cells adjacent to the central vein[5]. However, the liver has two different and powerful regenerative responses when damaged[6]. Partial hepatectomy (when up to 65% of the organ is removed) activates a unique response, during which the remaining, fully differentiated, healthy diploid hepatocytes enter the cell cycle and, within a matter of weeks, restore the liver mass to its original size[7]. A number of the inflammatory signals described in this Review play a major part in this type of liver regeneration by acting on mature hepatocytes, but Wnt signals are also thought to have a role[8,9]. A very different pattern of regeneration, often given the pathological description oval-cell response or ductular reaction[10,11], is elicited by insults that debilitate all hepatocytes, such as exposure to liver toxins, viral infections or immune attack. Oval cells are postulated to be bipotent stem cells (they have the capacity to generate hepatocytes and biliary cells) that are derived from the biliary tract system (the canals of Hering; Fig. 1b). The paucity of unique markers for oval cells and their apparent absence in the healthy liver has complicated mechanistic studies of these elusive stem cells, and their relevance remains a subject of intense debate (see later).

Inflammation, and normal and abnormal damage repair

People often react to inflammation and its five signs: *dolor* (pain), *calor* (heat), *rubor* (redness), *tumour* (swelling) and *functio laesa* (loss of function) by taking an anti-inflammatory medication. But inflammation is an important protective response that, along with the elimination of its primary triggers (foreign organisms, dead cells or physical irritants), plays a crucial part in the regeneration of injured tissues. Too little inflammation can result in tissue destruction by harmful triggers, especially bacteria, whereas chronic unresolved inflammation culminates in a host of pathologies, including cancer and fibrosis. The link between inflammation and cancer is reviewed extensively elsewhere[12,13], as is the link between inflammation and fibrosis[14]. Both of these pathologies can be viewed as attempts at tissue repair that have gone awry. In this Review, we argue that self-limiting acute inflammation is essential for a proper restorative response, and we focus on this topic. Wound healing and injury repair facilitate the resolution of inflammation by restoring barrier function. Self-resolving inflammation is the first stage of wound repair and is followed by tissue formation and eventual remodelling[15]. Although not as extensively studied as its innate immune or antimicrobial functions, the regenerative function of inflammation is evolutionarily conserved and has been amply documented in the fruit fly *Drosophila*, in which genetic analysis has highlighted its role in regeneration of adult tissues such as the injured midgut[16] and in closure of larval wounds[17].

Evolutionarily conserved repair pathways in the fly gut

The fly innate immune system is activated on engagement of pattern recognition receptors (PRRs) by pathogen-associated molecular patterns (PAMPs), setting in motion highly conserved signalling cascades that impinge on NF-κB, AP-1 and STAT transcription factors, which are also the main regulators of the mammalian inflammatory response[18]. In innate

immunity, these pathways are important for the induction of antimicrobial peptides in haemocytes, the fat body and the midgut — the fly equivalents of myeloid cells, the liver and the mammalian intestine, respectively. The same pathways also control regeneration and wound healing by stimulating proliferation of ISCs and ASCs, and modulating their differentiation[16]. Reactive oxygen species (ROS) produced during tissue injury and infection are an important cue that couples inflammation to ISC proliferation through activation of Jun N-terminal kinases (JNKs) and the antioxidant transcription factor and NRF2 homologue CncC[19]. JNK stimulates ISC proliferation by activating Fos (AP-1), which is also activated by growth factors of the EGF family. In addition, JNK contributes to the induction of Upd (a homologue of inter-leukin (IL)-6) family members that activate JAK–STAT signalling in both ISCs and visceral muscle cells, in which it also induces expression of growth factors (EGF family members) that directly stimulate ISC proliferation[19]. JNK activation also has a crucial role in epithelial sheet movement and cell migration, which are the first steps in wound closure. Completion of the regenerative and wound-healing response depends on compensatory proliferation of activated ASCs, normal differentiated cells (NDCs) or dedifferentiated cells that assume a cellular identity associated with an increased proliferative potential. The same general mechanisms control epithelial regeneration in mammals (Fig. 2 and Table 1), although in this process a much larger orchestra of cell types and regulatory cytokines and growth factors is involved (Table 2), and these require more intricate conducting.

In addition to these classic inflammatory signalling pathways, the Wnt pathway — well known for its role in ASC homeostasis in mammals[20] — is emerging as an additional player in inflammatory tissue regeneration. Although it is less prominent than mammalian Wnt, Wingless signalling in flies drives self-renewal and cooperates with JAK–STAT to regulate homeostatic ISC proliferation and maintenance[21]. Strikingly, when flies sustain intestinal damage through ingestion of the bacterium *Pseudomonas entomophila* or the intestinal irritant dextran sulfate sodium (DSS), Wingless expression is upregu-lated in enteroblasts — ISC daughter cells. These enteroblast-secreted, inflammatory Wnt signals activate downstream components that lead to enhanced ISC proliferation[22]. Similarly, inflammation-induced regeneration in mammals is guided by crosstalk between numerous cell types (such as the innate immune Paneth cells and ISCs that flank each other in crypts; Fig. 1a); the cytokines and growth factors that they produce; and ASCs (Fig. 2).

Notably, the constant contact that the gastrointestinal system has with microbes has allowed it to acquire important immune functions, including prevention of bacterial invasion and maintenance of tolerance. In addition, liver parenchymal cells, and to a lesser extent intestinal epithelial cells (IECs), metabolize and detoxify foodborne, waterborne and microbiota-generated toxic compounds. These protective functions are associated with a certain degree of collateral damage, which causes cell loss through physical attrition, chemical injury and immune destruction — processes that are especially pronounced in the mucosal lining of the gut. To prevent tissue loss and dysfunction and maintain homeostasis, the mammalian gastrointestinal system manifests strong regenerative capacity throughout its life.

Microbiota and regeneration

The human gut contains up to 100 trillion bacterial cells that belong to as many as a thousand different species, and a similar complexity is found in the murine gastrointestinal microbiota[23]. The gut microbiota mainly consists of commensal microbes that exhibit symbiotic relationships with their host. In addition to modulating nutrient metabolism and absorption, the gut microbiota influences intestinal development and function[24], and shapes the gastrointestinal immune landscape[25]. Mucosal erosion or injury allows commensal microbes and/or microbial macromolecules to penetrate and thereby activate macrophages, dendritic cells and T lymphocytes in the lamina propria. Activated immune cells produce numerous inflammatory cytokines, including tumour necrosis factor (TNF), IL-6, IL-10 and IL-17 family members. In addition to the propagation of intestinal inflammation, these cytokines control the regenerative response, which depends on ISC proliferation. Once the mucosa regenerates, microbial translocation and further inflammation are prevented. However, substantial disruption of the healthy microbiota caused by extensive and prolonged antibiotic use, especially in neonates and children, can result in a life-threatening pathology called necrotizing enterocolitis, in which mucosal injury results in cell death without regeneration[26]. A similar condition can be elicited in mice by giving them broad-spectrum antibiotics, and can be prevented with oral administration of microbial products — such as lipopolysaccharide (LPS) — that induce the production of inflammatory cytokines through Toll-like receptor (TLR)4 activation[27]. One such cytokine is IL-6, which prevents IEC death[28]. However, TLR4 and IL-6 have also been implicated as pathogenic factors in necrotizing enterocolitis[29]. Interestingly, although the intestinal microbiota of *Drosophila* is much simpler (featuring fewer than 20 microbial species), it also has an important role in intestinal regeneration. In the fly, symbiotic bacteria promote normal tissue growth, whereas potential pathogens produce uracil, which stimulates ROS production by activating a G-protein-coupled receptor (GPCR) that leads to p38 and JNK activation, induction of dual oxidase and ISC proliferation[30]. Although limited ROS production stimulates ISC proliferation, extensive ROS generation can lead to the loss of epithelial homeostasis, which may underlie age-associated tissue dysfunction[19]. It remains to be determined whether a similar mechanism operates in the mammalian gut, in which the much more complex microbiome controls the amplitude of cytokine gene expression. More specific effects cannot be ruled out, and certain symbiotic microbes may even attenuate damaging inflammation.

The intestinal microbiota also influences liver regeneration[31]. The liver detoxifies LPS and other compounds derived from the gut microbiota that reach it through portal circulation. Thus, partial hepatectomy or extensive liver injury induced by carbon tetrachloride increases the local concentration of LPS, which promotes liver regeneration through TLR4 activation and induction of inflammatory cytokines[31, 32]. Consistent with this hypothesis, germ-free rats have defective liver regeneration[31], and administration of synbiotics (a mixture of probiotics and prebiotics) restores liver regeneration in these animals and enhances liver function in patients who have undergone hepatectomy[33]. The gut microbiota also stimulates expression of reparative cytokines, partly through complement system activation[34–36].

Sterile inflammation and its sensors

In the absence of microbes, tissue damage and cell death still evoke sterile inflammation[37]. As already mentioned, the microbiota mainly dictates the amplitude of the inflammatory response and its output, whereas damage-associated molecular patterns (DAMPs) initiate the response[37]. Cell death, especially necrotic death, and tissue damage cause the release of DAMPs, which include extracellular nucleic acids and chromatin components, ATP and other nucleotides, uric acid, cytoskeletal fragments, heat-shock proteins and oxidized mitochondrial DNA (mtDNA), all of which are sensed by PRRs. In addition to TLRs, many DAMPs affect membrane permeability to potassium and calcium, resulting in mitochondrial damage that culminates in leakage of mtDNA and mitochondrial ROS that cause inflammasome activation and IL-1β and IL-18 secretion[38]. The most important inflammasome for DAMP sensing is NLRP3, but its role in gastrointestinal regeneration and tissue repair is controversial. Some studies suggest that NLRP3 activation promotes epithelial integrity and regeneration, at least indirectly, through IL-1 and IL-18 production[39,40], but others have shown that IL-18 damages the intestinal mucosa, disrupts its barrier function[41] and inhibits goblet-cell maturation during colitis[42].

The response to sterile inflammatory triggers is evolutionarily conserved and has been studied in *Drosophila*, in which it is also involved in wound repair and regeneration[17]. In addition to PRR activation, sterile inflammation in flies and mammals results in activation of matrix metalloproteases (MMPs)[43–45], which are involved in the processing and release of cytokines and growth factors. The MMP ADAM17 controls regeneration of the injured colonic mucosa by shedding EGF receptor (EGFR) ligands and TNF[44]. ADAM17 is also involved in Notch cleavage, providing a pathway through which inflammatory stimuli activate Notch signalling[45]. In summary, TLR activation is responsible for the initial surge in cytokine gene transcription, inflammasome priming and MMP induction, whereas inflammasome activation controls IL-1β and IL-18 secretion. MMP activation results in the release of cell-anchored cytokines and growth factors, such as TNF and EGF family members (Table 1).

Cytokines and tissue repair

Inflammation controls regenerative processes through several cytokines and growth factors. One of the first cytokines implicated in tissue repair was IL-6, which promotes liver regeneration after partial hepatectomy or CCl_4-induced injury[34,35]. Shortly after, the primary inflammatory cytokine TNF was also found to control liver regeneration[46]. Initially, these results were counter-intuitive because these cytokines, especially TNF, were thought to mediate tissue damage. But concurrent work revealed that TNF inhibits apoptotic cell death by activating NF-κB[47,48]. NF-κB activation also inhibits necrosis[49], and a site-specific complement inhibitor — CR2–CD59, which blocks a membrane attack complex and increases hepatic TNF and IL-6 expression — strongly stimulates liver regeneration, even after 90% hepatectomy[50]. IL-6 and TNF also promote regeneration of the injured intestinal mucosa[28,51–54], acting directly on epithelial cells by engaging IL-6 receptor (IL-6R):gp130 heterotetramers and TNF receptor 1 (TNFR1), respectively. TNF signalling also stimulates IL-6 expression, and TNF blockade in rats inhibits IL-6 production along with liver

regeneration[55]. TNFR1 engagement is required for stimulation of hepatocyte proliferation not only after partial hepatectomy[56] but also in hepatocellular carcinoma progenitors of mice with nonalcoholic steatohepatitis[57]. TNF also promotes epithelial regeneration through the Notch pathway[58], the activation of which may depend on ADAM17 and EGFR expression and engagement[44,59]. Another TNF family member, lymphotoxin (LT), contributes to liver regeneration by binding to the LTβ receptor[60]. In *Drosophila*, TNF family members are used to control intestinal immunity and regeneration through the immune deficiency pathway[19], further underscoring the ancient origin of inflammatory control of tissue repair.

IL-6 is the prototypical member of a large cytokine family, which also includes the mammalian proteins IL-11, IL-27, IL-31, leukaemia inhibitory factor (LIF), oncostatin M (OSM), leptin, ciliary neurotrophic factor (CNTF) and cardiotrophin-1, all of which are capable of stimulating cell proliferation and survival[61]. Most of these cytokines signal through heterodimeric or heterotetrameric receptors that use the gp130 signal transducing subunit[62] (Fig. 3). IL-6-related proteins, known as Upd proteins, are present in *Drosophila*, in which they stimulate intestinal repair and regeneration either through direct effects on ISCs or by inducing expression of EGFR ligands in underlying muscle cells[21]. Other IL-6 family members, especially IL-11, LIF and OSM, may also contribute to gastrointestinal epithelial regeneration. In mammals, these cytokines can have indirect effects on epithelial regeneration by inducing the expression of EGFR ligands, the release of which is ADAM17-dependent[44,63,64]. For instance, IL-6 induces amphiregulin (Areg) expression in the mammalian intestine[65].

Another important regenerative cytokine is IL-22, a member of the IL-10 family[66]. IL-22 is produced by lymphocytes, especially T helper 17 (T_H17) cells and innate lymphoid cells (iLCs), and by certain myeloid subsets, but unlike most cytokines, it does not target other leukocytes. Instead, IL-22 acts on epithelial cells and fibroblasts to stimulate proliferation, inhibit death and delay terminal differentiation. IL-22 receptor engagement results in JAK–STAT3 activation, as well as mitogen-activated protein kinase (MAPK) activation, including extracellular signal-regulated kinase (ERK), p38 and JNK activation[66]. Thus, IL-22 action is mainly dedicated to immune control of tissue repair. Fittingly, infection of IL-22-deficient mice with *Citrobacter rodentium* results in increased mucosal damage[67], and exogenous IL-22 ameliorates inflammation in a DSS-colitis model[68]. IL-22 also prevents concanavalin-A-induced hepatocyte death[69,70], provides protection against acute pancreatitis by inducing Reg family members[66] and can directly stimulate proliferation of epithelial cells, including ISCs[68]. Like T_H17 cells, iLCs also produce IL-17 cytokines, which include the six members A to F. These cytokines are important regulators of epithelial barrier integrity[71]. Curiously, the primordial member of this family is the *Drosophila* protein Spätzle, the ligand of Toll (or Toll-1, the prototypical TLR)[72]. IL-17 cytokines bind heterodimeric receptors that use IL-17RA as a common subunit[73], the engagement of which causes activation of NF-κB and MAPKs, which in turn induce antimicrobial immunity and tissue remodelling. IL-17-induced genes encode antimicrobial peptides, such as β-defensins and Reg3γ, and inflammatory cytokines, including IL-6 (refs 71, 74). Like IL-22 deficient mice, IL-17RE-deficient mice exhibit enhanced mucosal damage after *C. rodentium* infection[75]. Although this phenotype may be due to the antimicrobial effects of IL-17 signalling, IL-17RA engagement can directly stimulate IEC proliferation[76]. Curiously, IL-17 cytokines are not as

important in liver regeneration as IL-22 (ref. 69); the stronger regenerative effect of IL-22 could be due to its ability to directly activate STAT3. IL-17 cytokines, however, can lead to indirect STAT3 activation in epithelial cells by stimulating immune cells to produce IL-6 (ref. 76). See Table 2 for a summary of key regeneration-stimulating cytokines.

Signalling pathways in inflammation-led regeneration

Several evolutionarily conserved signalling pathways connect inflammatory inputs to the regenerative response (Fig. 2). First and foremost is the MAPK–AP-1 pathway, which also contributes to inflammation-driven regeneration and repair in *Drosophila*[16,18]. Even in *Drosophila*, JNK and p38 have additional targets, including Foxo, which controls stress resistance and antioxidant gene expression, and the AP-1-related factor ATF2, which controls dual-oxidase expression[16]. In mammals, the AP-1 component c-Jun controls liver regeneration, partly by suppressing p53 and p38 MAPK activities[77] and inducing cyclin D1 expression on JNK1 activation[78]. JNK–AP-1 signalling, however, can have opposing and complex effects on hepatocyte proliferation and survival, partly through the inhibition of pro-survival NF-κB activity[79–81], the induction of Nos2-generated pro-survival signals[82], the stimulation of cell-cycle progression[78] and the modulation of mitochondria-dependent apoptosis[83]. JNK-dependent AP-1 and p38 MAPK also act in non-parenchymal liver cells to induce TNF and IL-6, which, as already described, control liver regeneration[83]. EGFR ligands regulate epithelial homeostasis through MAPKs in the *Drosophila* midgut[84] and the mammalian intestine, in which ERK MAPKs control ISC proliferation and migration of their progeny along the crypt–villus axis. Src-mediated p38 activation stimulates the migration, but not the proliferation, of an IEC-derived cell line[85], but this remains to be demonstrated *in vivo*. p38 and other MAPKs also exert regenerative effects through ADAM17 activation[45].

Another evolutionarily conserved inflammatory signalling pathway that controls epithelial tissue integrity and survival is the IKK–NF-κB pathway[86]. NF-κB activation is needed to protect hepatocytes from TNF-induced apoptosis and allow them to respond to proliferative signals generated by TNFR1 engagement[87]. Some of NF-κB's protective effects are mediated through GADD45β, which inhibits prolonged JNK activation. Others, however, depend on inducible anti-apoptotic proteins such as c-Flip[79,80]. NF-κB activation in immune cells contributes to liver regeneration and hepatocyte proliferation by promoting synthesis of IL-6, TNF, LT and other cytokines[88]. IKKβ-dependent NF-κB also protects the intestinal epithelium from injury induced by DSS, ionizing radiation or ischaemia–reperfusion[51,89–91]. Epithelial IKKβ, however, has no protective role in chronic colitis caused by IL-10 deficiency[92]. NF-κB may further enhance proliferation of differentiated IECs by potentiating β-catenin signalling[93], and like AP-1 it contributes to the induction of IL-6 and other cytokines in lamina propria immune cells[89]. These cytokines promote IEC survival and ISC proliferation by activating STAT3 (ref. 52) (Fig. 3); the *Drosophila* counterpart DStat responds to Upd proteins, which control ISC proliferation[94]. STAT3 is activated by the tyrosine kinases JAK1 and JAK2 in response to gp130 or IL-22R signalling and is subject to feedback inhibition by SOCS3 (ref. 95). All of these regulators positively or negatively modulate intestinal homeostasis[95] and are present in *Drosophila*, in which they are known as Hop (DJAK) and Socs (SOCS3). STAT3 also contributes to expression of antimicrobial

peptides in Paneth cells[96,97], which provide a niche for mammalian ISCs. Like NF-κB, with which it collaborates[98], STAT3 is not needed for the development or maintenance of the uninjured intestinal epithelium, possibly because its loss may be compensated for by STAT1 (ref. 99). Notably, human STAT3 and JAK2 genes have been identified as susceptibility loci for inflammatory bowel disease[100]. STAT3 is activated by IL-6 and related cytokines, and is required for the stimulation of liver regeneration after partial hepatectomy, toxic damage or inflammation-induced injury[101] because it induces genes with products that maintain cell survival and promote proliferation[102].

Two other functionally linked and evolutionarily conserved signalling pathways with key roles in regeneration and gastrointestinal homeostasis are the Hippo–YAP (Fig. 3) and Notch signalling pathways[94,103,104]. YAP and its orthologue TAZ are transcriptional co-activators of TEAD transcription factors, which control a gene-expression program that stimulates cell proliferation, suppresses cell death and induces other receptors and ligands[103,105–107]. In non-stimulated epithelial cells, YAP and TAZ remain in the cytoplasm and undergo proteasomal degradation, as a result of phosphorylation of inhibitory serine residues by Warts (in *Drosophila*) or LATS1/2 kinases (in mammals)[107]. Genetic loss of these protein kinases or their activators (Hippo or MST1/2) results in YAP/TAZ dephosphorylation, stabilization, nuclear translocation and activation of TEAD and other transcription factors, with which these co-activators interact. Loss of cell–cell contact, cell adhesion or cytoskeletal integrity leads to transient Hippo/MST inhibition and consequent YAP/TAZ activation[107]. In both *Drosophila* and mammals, the Hippo pathway restricts uncontrolled ISC proliferation, whereas activation of YAP and its fly homologue Yorkie is required for intestinal epithelial regeneration after injury[108–114]. More refined genetic analysis in *Drosophila* demonstrated that Hippo–YAP signalling in differentiated IECs controls ISC proliferation, such that Yorkie/YAP activation after epithelial injury results in the production of signals by differentiated cells that act on neighbouring ISCs. Given the dependence of inhibitory Hippo signalling on cell–cell and cell–matrix contact, or cytoskeletal integrity, it was assumed that disruption of the gut epithelial lining results in Yorkie/YAP activation owing to inhibition of Hippo/MST kinase activity. However, an entirely different mechanism has recently been identified, at least in mammals (Fig. 3). Injury of the intestinal mucosa results in penetration of commensal microbes or their products (for example, LPS), leading to induction of IL-6, IL-11, IL-22 and related cytokines by lamina propria macrophages and dendritic cells. IL-6 and IL-11 activate gp130 signalling and induce the association of tyrosine-phosphorylated gp130 with the Src family kinases (SFKs) Src and Yes, thereby stimulating their tyrosine kinase activity[65]. Activated SFKs interact with YAP and phosphorylate it to induce its stabilization and nuclear translocation[65]. YAP target genes induced through this pathway include those that encode Notch receptors and ligands, and Areg, which acts on ISCs to enhance their proliferation. These findings demonstrate that YAP is also subject to positive regulation and can be rapidly activated in response to inflammatory signals, which, as well as IL-6 family members, may also include IL-22. Accordingly, interference with SFK-dependent YAP activation attenuates inflammation-induced intestinal regeneration even though it does not affect the parallel JAK– STAT3 pathway[65].

YAP activation in IECs induces several Notch receptors and ligands, including Notch1, Notch3 and DLL3 (ref. 65). This results in activation of Notch, which maintains ISCs and transit-amplifying cells in the crypt compartment in a highly proliferative and undifferentiated state[115,116]. Persistent Notch activation inhibits the generation of secretory cell types (goblet, enteroendocrine and Paneth cells) and slows the differentiation of absorptive enterocytes[115,117]. A similar phenotype has been observed with persistent YAP activation owing to either MST1/2 ablation[112,113] or intestinal-specific expression of a constitutively active gp130 variant[65]. γ-Secretase inhibition in mice with activated Notch or gp130 signalling in IECs led to the restoration of epithelial differentiation and homeostasis[65,115]. Conversely, ectopic expression of activated Notch in the *Drosophila* gut results in rapid differentiation of ISCs into enterocytes and inhibition of secretory-cell formation, along with ISC depletion[118]. Of note, *Drosophila* Src is also involved in intestinal regeneration, although its downstream targets have not been identified[119].

The Wnt signalling cascade is arguably the most prominent regulator of ASCs in mammalian epithelia. Its role was first revealed in the maintenance of small intestinal crypt stem cells[120]. It has since been extended to other perpetually self-renewing tissues, including hair follicles, the colon and stomach epithelium[20]. Most other organs have very little homeostatic proliferation under physiological conditions, but respond with a burst of regenerative proliferation when damaged. Although not extensively investigated yet, it seems likely that Wnts have a central role in regenerative stem-cell activity. The Wnt target gene products Lgr5 and Axin2 have emerged as common markers of constitutive, as well as damage-activated, types of Wnt-driven ASCs[20] (Fig. 3).

Despite intense ISC baseline activity, intestinal crypts become even more active (hyperplastic) during episodes of damage and inflammation, further increasing ISC and Paneth cell numbers and cellular output. Indeed, mice carrying a loss-of-function allele of the Wnt-agonistic receptor Lgr4 have a considerably higher susceptibility to DSS-induced colitis, concomitant with greatly reduced ISC numbers[121]. During these temporary hyperplastic phases, Wnt signalling activity increases markedly[122]. In the colon, regeneration of lost epithelium and the subsequent correct patterning of new crypts involves non-canonical Wnt5a activity[123]. Increases in size and number of Paneth cells are typically seen during an inflammatory or damage response, and consequent crypt hyperplasia can be viewed as a special form of inflammation that is not mediated by bone-marrow-derived immune cells, but by an endodermal epithelial cell with a prominent role in host defence and regeneration — the Paneth cell[124]. Paneth cells that are normally restricted to the small bowel appear in the human colon alongside chronic inflammation, a phenomenon that is well known to pathologists and called Paneth cell metaplasia. But other regenerative signalling molecules, such as Notch and YAP, inhibit Paneth-cell formation[113,115]. Thus, the size of the ISC niche is probably kept in check by the balance between the different regeneration-promoting pathways reviewed above. Since the original description of label-retaining ISCs, or +4 cells, near the crypt bottom[125], these cells — which serve as a reserve for the ISC population — have been the focus of intense research. The most notable marker for the +4 cell has been Bmi1 (ref. 126). Although +4 cell markers such as Bmi1 seem to be shared between $Lgr5^{+}$ and +4 ISCs[127,128], a pool of transient, non-dividing Paneth cell precursors near the crypt base may actually serve as the reserve stem-cell pool during

periods of damage[128,129]. The signals that control these quiescent cells have not been revealed, but it seems safe to assume that Wnt signals have a central role in this process.

Wnt signals are also emerging as crucial regulators of liver repair. A study in the zebrafish *Danio rerio* showed that biliary epithelial cells (BECs) are a crucial stem-cell source for injury repair when hepatocyte proliferation is compromised. Massive hepatocyte loss results in the dedifferentiation of BECs into hepatoblast-like cells and the subsequent formation of highly proliferative hepatocytes to restore liver mass. This process is strongly dependent on an intact *Wnt2b* gene, implying that Wnt signals are involved in this oval-cell-like response[130]. Nonetheless, lineage-tracing studies in mice have failed to detect any contribution from oval cells or other bile-duct-derived cells to hepatocyte regeneration after chemical injury[131]. Furthermore, a population of periportal hepatocytes that do not express the metabolic functions that characterize fully differentiated hepatocytes have been identified in the normal liver, surrounding the central vein (Fig. 1b). These cells — termed hybrid hepatocytes because they express *Sox9* and other bile-duct genes — do not metabolize CCl_4 (and therefore escape its toxic effect), undergo several rounds of proliferation and repair the damage[132]. When transplanted into a diseased liver, hybrid hepatocytes have higher regenerative capacity than normal hepatocytes or oval cells.

Although Wnts may control the weak proliferative activity of diploid pericentral hepatocytes, Wnt signalling seems to be particularly important for ‘waking up’ quiescent cells in the biliary tree after generalized liver damage[133–135]. The Wnt-dependent stem-cell marker Lgr5 is not expressed in healthy liver, but it is induced in small cells that carry biliary markers when mice are given hepatocyte toxins, such as CCl_4 (ref. 136). Indeed, CCl_4 treatment leads to a massive induction of Wnt proteins and Wnt-supporting R-spondins in the damaged liver. Lineage tracing has revealed that these induced $Lgr5^+$ cells generate large numbers of hepatocytes and bile-duct cells in the damaged areas, which is indicative of their bipotency. Such $Lgr5^+$ cells can be grown over long periods of time in a Wnt-based three-dimensional culture system as epithelial organoids that contain hepatocyte-like cells and BECs. Similarly, a human BEC generates ever-growing organoids that consist of hepatocyte-like cells as well as BECs. When transplanted into mice, BECs yield mature hepatocytes[137]. The source of Wnts and R-spondins during CCl_4-induced damage remains to be determined, but it has been reported that the resident liver macrophages (Kupffer cells) are a major source of Wnt after partial hepatectomy[4]. Similarly, in a model of chronic liver damage, macrophage engulfment of sterile hepatocyte debris induced Wnt3a expression, and when these macrophages were removed, no new hepatocytes were formed[138]. Thus, although more studies are required, it seems that Wnts produced by inflammatory cells in the damaged liver and Wnt-pathway activity are essential components of the signalling toolbox that the liver exploits for its regeneration. The relationship between hybrid hepatocytes and the $Lgr5^+$ progenitors previously discussed also needs to be investigated. Finally, an alternative mode of Wnt signalling has been described that involves the non-canonical Wnt receptor–ligand pair ROR1/2 and Wnt5A, which directly activates YAP signalling[139] (Fig. 3). This provides a further example of the close interconnection between regenerative signalling pathways.

Harnessing inflammatory regeneration for therapy

Balancing the positive and negative effects of the inflammatory reaction has been key for the design of clinical treatments for a multitude of diseases. The advantages of removing harmful foreign agents and infected or damaged cells need to be weighed against the disadvantages of chronic or uncontrolled inflammation in which friendly inflammatory fire causes more harm than good. In this Review, we have discussed an aspect of inflammation that has received much less attention. The inflammatory reaction not only deals with what has to be removed but also supports the rebuilding of what has been lost. In other words, it is an important driver of the regenerative response, an ancient function that has been evolutionarily conserved between flies and mammals. Orchestrating the action of the numerous cellular components involved in sensing and executing inflammation, and balancing the ensuing regenerative response are key to optimal recovery. Indeed, regeneration and mucosal healing have been suggested as key treatment goals that predict sustained remission and resection-free survival for inflammatory bowel disease[28]. But the key question is this: which of the known regenerative cytokines or signalling molecules can be harnessed to achieve this therapeutic goal? Answering this question is fundamental, because when regeneration falls short, there will be insufficient tissue for the affected organ to function, and when regeneration goes awry, the wrong tissue might be produced, resulting in scar formation or fibrosis. Although myofibroblasts are essential components of both inflammatory and regenerative responses, their extensive proliferation without subsequent removal causes aberrant regeneration and excessive collagen deposition, all of which fall under the fibrosis umbrella[14]. Worse still, the signalling pathways that are activated during inflammation to support regeneration are, in one way or another, all drivers of cancer — a known complication of chronic inflammation[13]. Furthermore, regenerative cytokines such as IL-17 or IL-22 may be responsible for the development of resistance to cancer drugs[140]. However, the neutralization of these regenerative responses creates another serious problem. The major dose-limiting factor in chemotherapy or radiotherapy for cancer is mucositis — severe mucosal inflammation. Blockade of cancer-promoting inflammatory signals should not, therefore, be combined with mucositis-inducing therapies. Likewise, maintenance of liver function is essential for proper detoxification of chemotherapeutic drugs, and the presence of liver fibrosis prevents the use of such drugs in the treatment of liver cancer. Obviously, much remains to be learned about the role of inflammation in functional and practical tissue restoration. Undoubtedly, such knowledge will be crucial to support optimal tissue repair — the right kind of tissue produced in the right amount and at the right location — during an ongoing inflammatory response.

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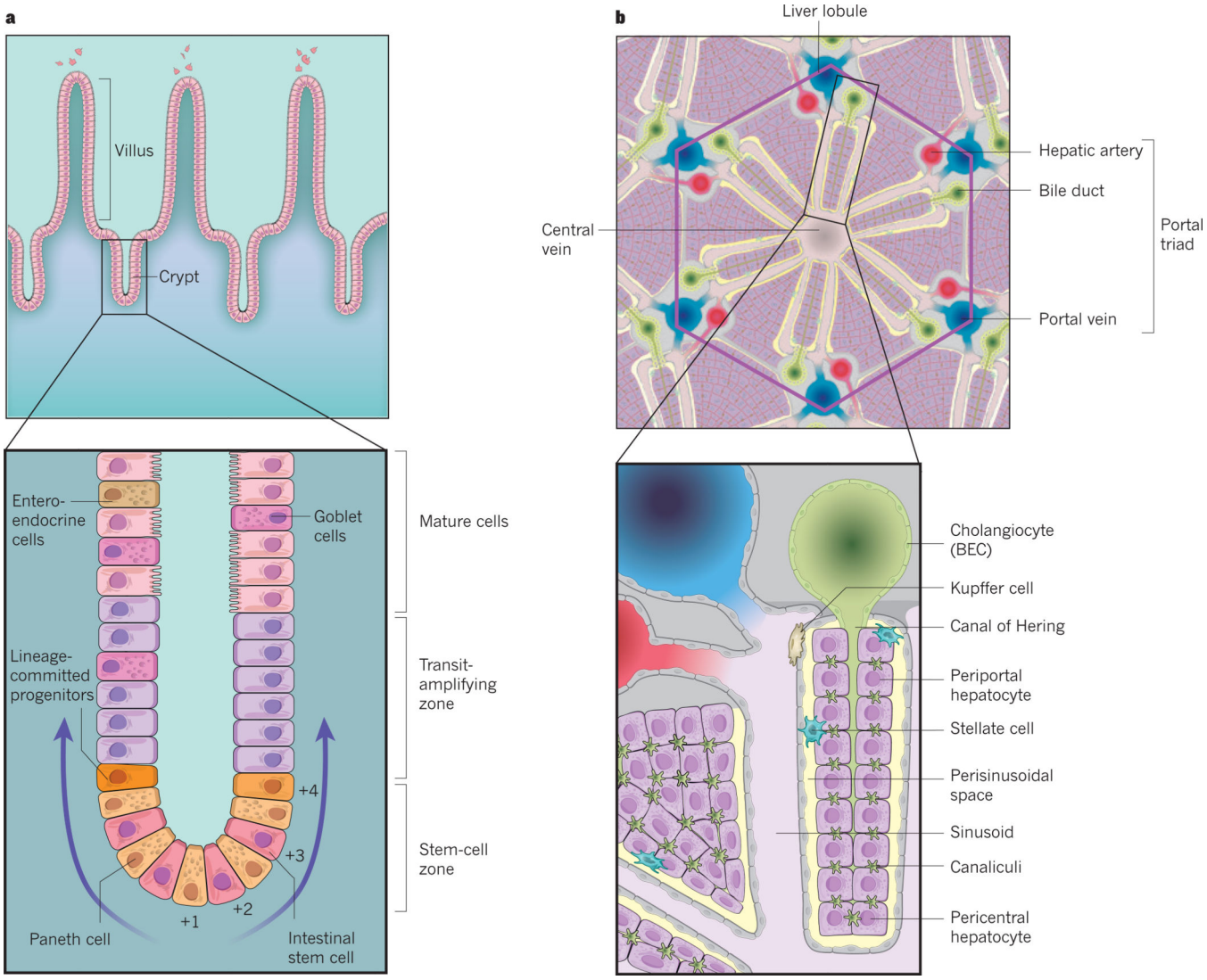

Figure 1. The microanatomy of the cellular compartments responsible for intestinal and liver regeneration

a, Intestinal stem cells (ISCs) are located at the bottom of crypts in the stem-cell zone. They sit wedged between Paneth cells, which provide niche factors. The stem-cell positions are denoted by +1 to +3 from the bottom of the crypt. The label-retaining cells at the +4 position can act as reserve stem cells when crypt damage occurs. The transit-amplifying zone above the stem-cell zone contains rapidly proliferating lineage-committed progenitors that mature as they move up the crypt–villus axis. On injury, ISCs expand and repair the mucosa to restore the gut permeability barrier. **b**, The liver is made up of roughly hexagonal functional units called lobules. Within these, cords of hepatocytes stretch from the central vein to the portal triad. Hepatocytes differ in function, depending on their position (pericentral to periportal). On the apical side, hepatocytes enclose canaliculi, bile channels that lead outwards to intrahepatic bile ducts. Along the way, bile passes through the canal of Hering, which is lined by hepatocytes and cholangiocytes. Basolaterally, hepatocytes face the perisinusoidal space. This transitional compartment between hepatocyte and sinusoid is

delimited by discontinuous, fenestrated endothelial cells and contains hepatic stellate cells. Kupffer cells, a specialized population of liver macrophages, reside at the interface between the perisinusoidal space and sinusoid. Blood from the portal vein and the hepatic artery mixes in sinusoids and flows towards the central vein, transporting both oxygen from the lungs and nutrients from the digestive tract to the liver. Although liver stem cells have not been identified, pericentral diploid hepatocytes were suggested to be responsible for homeostatic hepatocyte renewal. Chronic liver injury, however, leads to expansion of periportal hybrid hepatocytes, which repair the injured liver parenchyma.

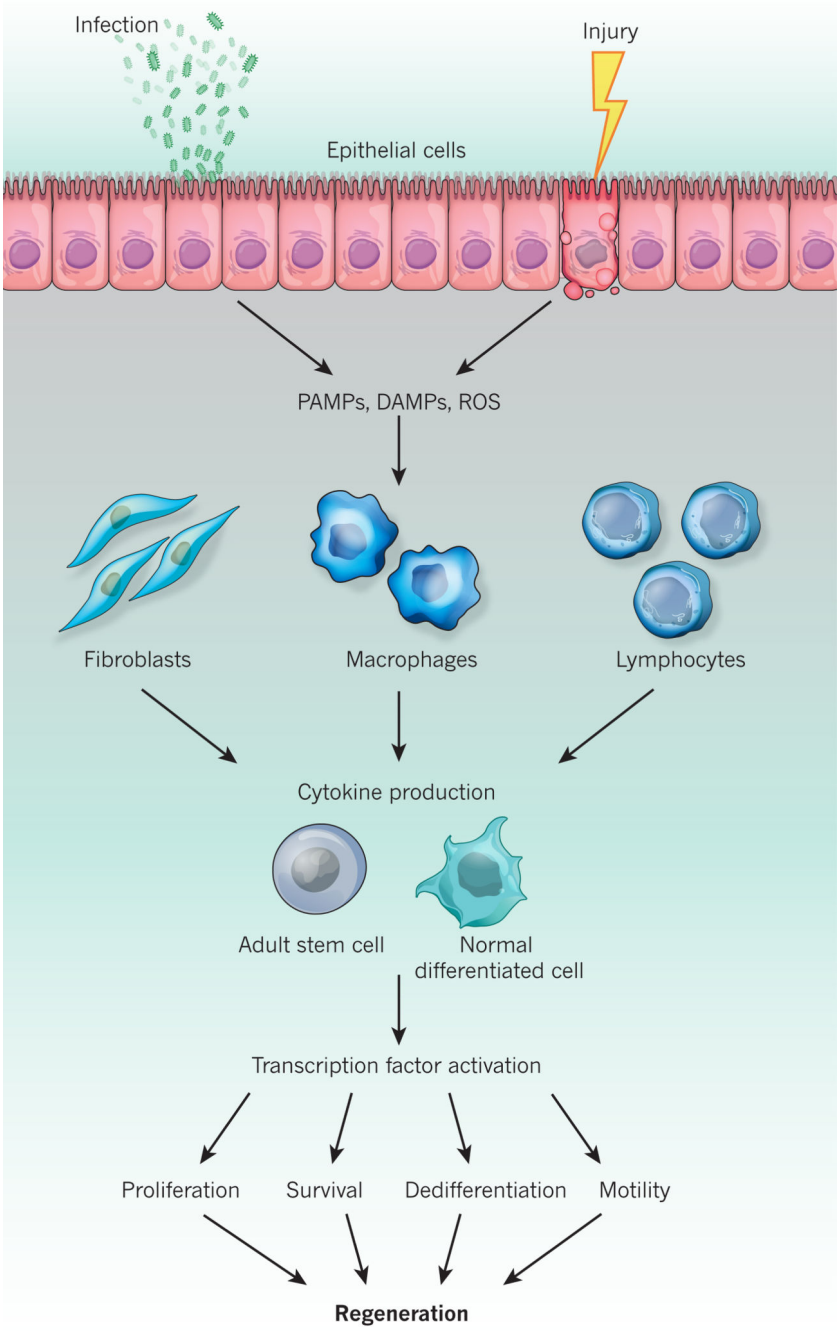

Figure 2. Mechanisms through which infection and injury induce a regenerative inflammatory response

Injury or infection of epithelial tissues leads to the generation of pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and reactive oxygen species (ROS). These induce the production of cytokines (tumour necrosis factor, interleukin (IL)-6, IL-11, IL-17 or IL-22) through tissue constituents such as immune cells. Some of the most common inflammatory cytokines trigger signalling pathways in adult stem cells or normal differentiated cells that culminate in the activation of transcription factors (AP-1, NF-κB, STAT3, YAP or Notch intracellular domain). These then mount a

regenerative response by inducing genes that encode growth factors, stimulate cell-cycle progression, prevent cell death, promote dedifferentiation and the acquisition of ‘stemness’, and enhance cell motility and migration.

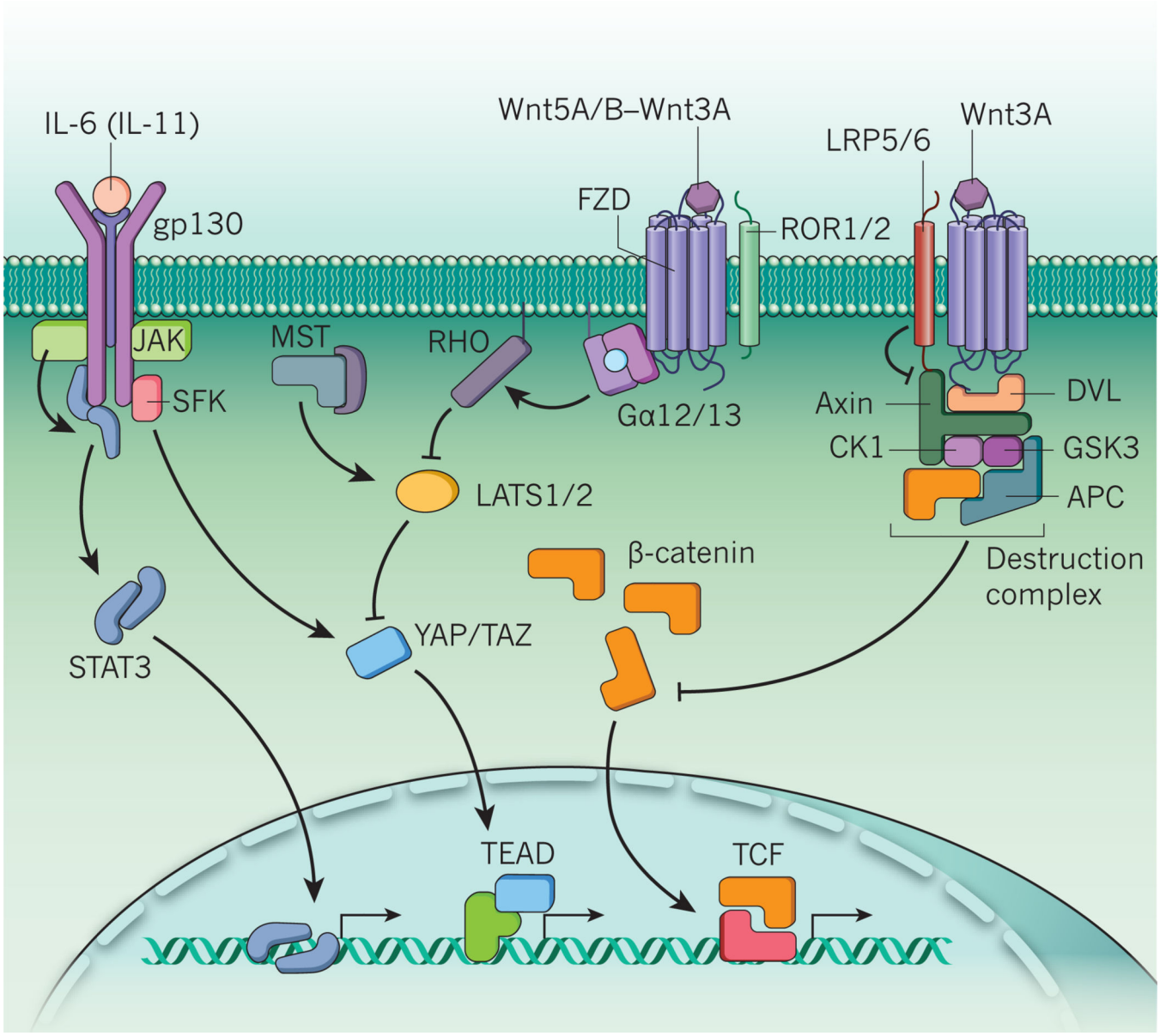

Figure 3. Signalling pathways in inflammation-driven regeneration

Cytokine signalling, the Wnt pathway (canonical and non-canonical) and Hippo-independent Src family kinase (SFK)-YAP signalling connects inflammatory signals to the regenerative response. Interleukin (IL)-6, and other members of the cytokine family, signal through receptors that use the gp130 subunit. STAT3 is activated by the tyrosine kinases JAK1 and JAK2 in response to gp130 or IL-22R signalling. In addition, cytokine signalling recruits SFK to gp130, which leads to phosphorylation, stabilization and nuclear translocation of YAP and its orthologue TAZ. Nuclear YAP and TAZ act as transcriptional co-activators of TEAD transcription factors. In non-stimulated epithelial cells, YAP and TAZ remain in the cytoplasm and undergo proteasomal degradation as a result of phosphorylation of inhibitory serine residues by LATS1/2 kinases. LATS1/2 is activated by Hippo/MST and inhibited by non-canonical Wnt signalling (FZD-ROR1/2 complex) owing to Gα12/13-

dependent Rho activation. Canonical Wnt signalling (FZD-LPR5/6 complex), however, stabilizes β-catenin by inhibiting its continuous degradation by the destruction complex. Stabilized β-catenin can then translocate to the nucleus and stimulate TCF-mediated gene transcription.

Table 1

Sensors and activators of regenerative inflammation

Molecular class	Activators	Products
TLR	PAMPs and DAMPs	*Tnf, Il1β, Nlrp3* and *Mmp* mRNAs, IL-23, IL-6 and L-22
NLR (NLRP3)	ATP, uric acid, mtDNA and mtROS	L-1β and IL-18
MMP/ADAM	TLR, TNF and IL-1 through AP-1 and NF-κB	TNF, EGF, Areg and Ereg

Molecular class refers to the major sensors and mediators of regenerative inflammation. Areg, amphiregulin; DAMPs, damage-associated molecular patterns; EGF, epidermal growth factor; Ereg, epiregulin; IL, interleukin; MMP, matrix metalloprotease; mRNA, messenger RNA; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; NLR, NOD-like receptor; PAMPs, pathogen-associated molecular patterns; TLR, Toll-like receptor; TNF, tumour necrosis factor.

Table 2

Regeneration-inducing cytokines and their properties

Cytokines	Source	Direct effectors
TNF	T lymphocytes, macrophages and epithelial cells	NF-κB, MAPKs and AP-1
IL-6	Lymphocytes, myeloid cells, fibroblasts and epithelial cells	JAK–STAT3, MAPKs, SFKs, YAP and Notch
IL-22	T_H17 cells, iLCs and some myeloid cells	JAK–STAT3, MAPKs, SFKs, YAP and Notch
IL-17	T_H17 cells, γδT cells and iLCs	NF-κB and MAPKs

IL, interleukin; iLCs, innate lymphoid cells; MAPKs, mitogen-activated protein kinases; SFKs, Src family kinases; T_H17, T helper 17; TNF, tumour necrosis factor.