J Physiol 594.17 (2016) pp 4791–4803

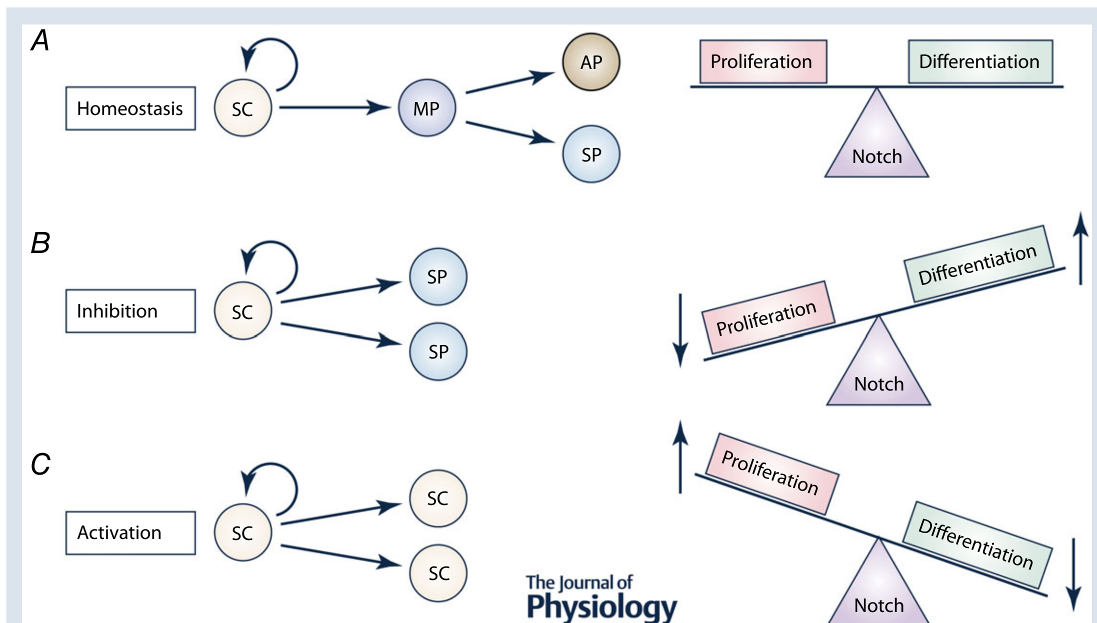
TOPICAL REVIEW

Notch regulation of gastrointestinal stem cells

Elise S. Demitrack[1] and Linda C. Samuelson[1,2]

[1] *Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA*
[2] *Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA*

The Journal of Physiology



Abstract The gastrointestinal (GI) tract epithelium is continuously replenished by actively cycling stem and progenitor cells. These cell compartments are regulated to balance proliferation and stem cell renewal with differentiation into the various mature cell types to maintain tissue homeostasis. In this topical review we focus on the role of the Notch signalling pathway to regulate GI stem cell function in adult small intestine and stomach. We first present the current view of stem and progenitor cell populations in these tissues and then summarize the studies that have established the Notch pathway as a key regulator of gastric and intestinal stem cell function. Notch signalling has been shown to be a niche factor required for maintenance of GI stem cells in both tissues. In addition, Notch has been described to regulate epithelial cell differentiation. Recent studies have revealed key similarities and differences in how Notch regulates stem cell function in the stomach

Elise Demitrack is a Research Investigator in the Samuelson laboratory at the University of Michigan. Her current research is focused on signalling pathways that regulate gastric stem cells during homeostasis and disease. **Linda Samuelson** is the John A. Williams Professor of Gastrointestinal Physiology at the University of Michigan. She holds a joint appointment in the Department of Internal Medicine, and is also the Associate Director for the Center for Organogenesis at the University of Michigan. Her laboratory focuses on the development and function of gastrointestinal tissues, with an emphasis on Notch pathway regulation of gastric and intestinal stem cells.

This review was presented at the 2015 FASEB Summer Research Conference entitled 'Gastrointestinal Tract XVI: GI homeostasis, the microbiome and the barrier, development and disease' August 2–7, 2015, Steamboat Springs, Colorado, USA.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

DOI: 10.1113/JP271667

compared to intestine. We summarize the literature regarding Notch regulation of GI stem cell proliferation and differentiation, highlighting tissue-specific functions to compare and contrast Notch in the stomach and intestine.

(Received 8 October 2015; accepted after revision 19 January 2016; first published online 5 February 2016)
Corresponding author L. C. Samuelson: 109 Zina Pitcher Pl., 2041 BSRB, Ann Arbor, MI 48109, USA.
Email: lcsam@umich.edu

Abstract figure legend Notch regulation of intestinal epithelial cell homeostasis. *A*, during homeostatic conditions, Notch maintains the intestinal stem cell (SC) pool and promotes proliferation of a multi-potential (MP) progenitor cell that will further differentiate into an absorptive progenitor (AP) or secretory progenitor (SP) cell in the intestine. Therefore, Notch is required to maintain the balance of stem cell proliferation and differentiation. *B*, during Notch inhibition, stem cell proliferation is reduced and progenitor cells are fated towards the secretory lineage. This results in a net reduction in proliferation and increased secretory cell differentiation. *C*, in contrast, Notch activation leads to expansion of the stem cell compartment, resulting in increased stem cell proliferation and an overall reduction in epithelial cell differentiation.

Abbreviations ADAM10, a disintegrin and metalloproteinase 10; bHLH, basic helix–loop–helix; CBC, crypt base columnar; GFP, green fluorescent protein; GI, gastrointestinal; ISC, intestinal stem cell; NICD, Notch intracellular domain; QSC, quiescent stem cell; TA cells, transit-amplifying cells.

Introduction

Notch regulates key cellular processes such as proliferation and differentiation via communication between adjacent cells. The Notch pathway is unique in that Notch signals are transmitted between adjacent cells, such that Notch activity in one cell can induce distinct function in a neighbouring cell. This process, termed lateral inhibition, is used in many different contexts in developing and adult tissues to establish cell boundaries, to pattern cellular differentiation and regulate stem cell function (Koch *et al.* 2013).

In mammals, there are four Notch receptors (NOTCH 1–4) and five Notch ligands (Delta-like (DLL) 1, 3, 4 and Jagged (JAG) 1, 2), which are all transmembrane proteins (Kopan & Ilagan, 2009). Notch signalling involves the engagement of ligand-expressing (signal-sending) and receptor-expressing (signal-receiving) cells to initiate proteolytic cleavage events to release an intracellular signalling fragment of the receptor: NICD (Notch intracellular domain) (Fig. 1). In intestine, the initial cleavage event excising the extracellular receptor domain occurs via the cell surface sheddase a disintegrin and metalloproteinase 10 (ADAM10) (Tsai *et al.* 2014); whether ADAM10 also performs this function in the stomach is unknown. Notch signals are subsequently transmitted to the nucleus after an intramembrane cleavage event orchestrated by the γ-secretase complex to release NICD. Once in the nucleus, NICD interacts with the DNA-binding protein RBPJ, converting a transcriptional repressor complex into an activator complex via recruitment of co-activators such as Mastermind. This complex can then activate transcription of Notch target genes, such as those in the hairy and enhancer of split (Hes) family. Notch responses are highly tissue specific; currently only limited information is known about Notch target genes and their function in GI tissues.

In both intestine (Fre *et al.* 2011; Pellegrinet *et al.* 2011) and stomach (Demitrack *et al.* 2015; Gifford *et al.* 2016), lineage tracing experiments in mouse genetic models have revealed active NOTCH receptor signalling in adult stem cells, suggesting an important function for this signalling pathway in stem cell homeostasis in both tissues. Indeed, as detailed in this review, pathway inhibition or activation disrupts proliferation and differentiation, with Notch signalling required for stem cell maintenance. Progress to understand Notch regulation of GI stem cells has primarily emerged from analysis of mouse genetic and pharmacological models. In addition, recent utilization of intestinal and gastric organoid cultures has allowed focus on epithelial cell effects without the confounding issues related to potential Notch function in other cell types (immune, neuronal, mesenchymal, vascular, etc.). The ability to generate organoids from human GI tissue samples presents a promising model system to study Notch regulation of human stem cells.

Overexpression of Notch pathway components has been demonstrated in gastric and intestinal cancers (Reedijk *et al.* 2008; Piazzi *et al.* 2011; Hsu *et al.* 2012; Bauer *et al.* 2015), which underscores the importance of this signalling pathway to promote cell proliferation. Outside of the GI tract, Notch pathway dysregulation is associated with several human cancers and thus this pathway is a prime target of therapeutic interest (Takebe *et al.* 2015). However, the use of pan-Notch inhibitors is associated with GI toxicity due to the importance of this pathway for GI stem cell maintenance (van Es *et al.* 2005*b*). Thus it is crucial to define pathway components and mechanisms of

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Notch function in the gut to design strategies that target components of this pathway relevant for human disease without disrupting normal GI stem cell function.

Notch regulation of intestinal epithelial cell homeostasis

Intestinal stem cells. The small intestinal epithelium is rapidly renewed by actively cycling crypt base columnar (CBC) stem cells. Each crypt contains several CBCs positioned between the Paneth cells at the crypt base (Fig. 2*A*). One imaging study of green fluorescent protein (GFP)-marked CBCs suggested approximately 14 stem cells reside in each intestinal crypt (Snippert *et al.* 2010), while another study using a functional, clonal labelling technique estimated 5–7 CBCs per crypt (Kozar *et al.* 2013). Regardless, the field of intestinal stem cell biology has largely advanced through the use of gene expression profiling and lineage tracing techniques, which first established the R-spondin receptor and Wnt target gene *Lgr5* as a marker for the active CBC stem cell population (Fig. 2*A*) (Barker *et al.* 2007). Since then, other markers of these stem cells have emerged, including *Olfm4* (van der Flier *et al.* 2009), *Ascl2* (van der Flier *et al.* 2009), *Sox9* (Formeister *et al.* 2009; Furuyama *et al.* 2011) and others (Munoz *et al.* 2012).

In addition to the active CBC stem cell, another stem cell population identified as the '+4', 'reserve', or 'quiescent' stem cell (QSC), has been described. Markers for these QSCs include *Bmi1* (Sangiorgi & Capecchi, 2008), *mTert* (Montgomery *et al.* 2011), *Hopx* (Takeda *et al.* 2011) and

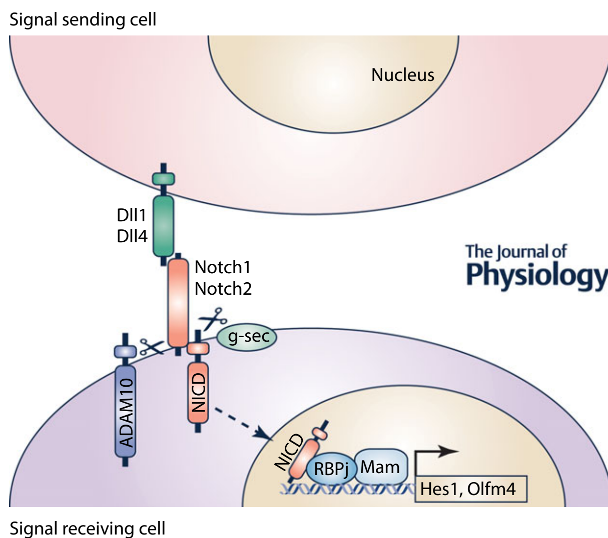


Figure 1. Notch signalling in the intestinal epithelium
Transmission of a Notch signal involves engagement of two cells: a signal-sending cell (top) that expresses Notch ligand (DLL1 and DLL4 in intestine), and a signal-receiving cell (bottom) that expresses Notch receptor (NOTCH1 and NOTCH2 in intestine and stomach). After ligand/receptor engagement, the Notch receptor is proteolytically cleaved: first by ADAM10, which releases the extracellular domain, and then by the γ-secretase complex, which cleaves the receptor at the cell membrane to release the Notch intracellular domain (NICD). NICD translocates to the nucleus where it recruits a transcriptional co-activator complex that activates downstream transcription of Notch target genes, such as *Hes1* and *Olfm4*. RBPJ, Recombining binding protein suppressor of hairless; Mam, Mastermind (Both proteins are part of the transcriptional activation complex that interacts with NICD).

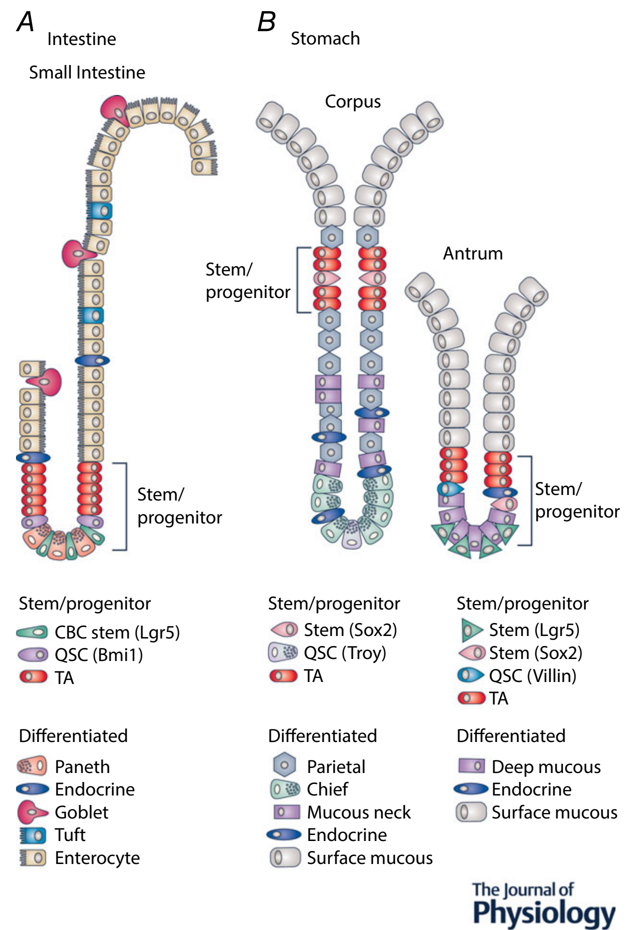


Figure 2. Stem and differentiated cells of the small intestine and stomach
Schematic diagram of the cellular composition of small intestinal crypt/villus units and gastric corpus and antral gland units, highlighting selective markers that are proposed to define distinct stem cell populations. In both stomach and intestine, stem cells proliferate to form transit-amplifying (TA) progenitors which differentiate into the mature cell types of each tissue, as indicated. The mature cell types are replaced every several days (intestine and antrum) to several months (corpus). *A*, in the intestine, active Lgr5-expressing CBC stem cells are located at the crypt base, intercalated between Paneth cells. A quiescent Bmi1-marked stem cell (QSC) population is located approximately 4 cell positions from the crypt base. *B*, in the stomach, stem and progenitor cells are located in the upper mid-region in corpus and at the gland base in antrum. LGR5 marks active stem cells in the antrum, but not corpus. Other potential gastric stem cell markers are discussed in the text.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Lrig1 (Powell *et al.* 2012). This stem cell population differs from CBCs in that they divide infrequently and are not normally responsible for epithelial cell maintenance, but can be activated in response to loss of LGR5^{+} CBCs to support epithelial cell homeostasis (Tian *et al.* 2011; Yan *et al.* 2012; Buczacki *et al.* 2013). However, it remains unclear if there is a single, dedicated population of QSCs or if multiple different cell populations are capable of occupying open intestinal stem cell (ISC) niche spaces left absent through loss of the active CBCs. Recent gene expression profiling of the LGR5^{+} CBC population showed that these cells also express QSC markers, further questioning whether distinct stem cell populations exist (Munoz *et al.* 2012). Furthermore the observation that crypt cells co-expressing endocrine and stem cell markers can de-differentiate to occupy open ISC niche spaces during CBC loss (Schonhoff *et al.* 2004; Sei *et al.* 2011; van Es *et al.* 2012; Van Landeghem *et al.* 2012; Buczacki *et al.* 2013) suggests substantial cellular plasticity exists for cells in the crypt to allow remodelling or repair to maintain homeostasis (Mills & Sansom, 2015).

The field has relied heavily on genetic mouse models to both identify and study stem cells in the intestine. This has enabled remarkable progress over the past few years as discovery of stem cell markers has allowed genetic approaches to label and manipulate stem cell populations. However, over-reliance on marker expression to define stem cells can be a limitation as outcomes may report marker gene regulation instead of stem cell function. Moreover, engineered gene alleles do not always faithfully reflect endogenous gene expression, due to patchy expression patterns or a different protein half-life for Cre or GFP. This is perhaps best illustrated by the expression pattern of the *LGR5-EGFP-CreERT2* allele that is widely used in the field, with incomplete expression in all LGR5^{+} CBCs and perdurance of GFP into transit-amplifying (TA) cells (Barker *et al.* 2007). Thus researchers need to be cognizant of the specific limitations of the transgene alleles employed in their study. Moreover functional and morphological criteria in addition to marker expression should be included to support conclusions regarding specific stem cell populations.

Small intestinal CBCs give rise to TA progenitor cells, which undergo several rounds of cell division before differentiating into the mature intestinal epithelial cell types. In intestine there are two distinct categories of differentiated cell lineages: absorptive (enterocytes) and secretory (goblet, enteroendocrine, Paneth and tuft cells) (Fig. 2*A*). Several studies have demonstrated that the Notch pathway is key to directing progenitor cell differentiation to absorptive *versus* secretory cell fate (see review by Noah & Shroyer, 2013).

Notch regulation of ISCs. Notch receptor and ligand mRNAs have been detected in both epithelial and mesenchymal cells of the developing and adult rodent intestine (Schröder & Gossler, 2002; Sander & Powell, 2004; Shimizu *et al.* 2014), and functional studies have confirmed active Notch signalling in ISCs. Analysis of mouse strains with Notch receptor-CreERT fusion genes demonstrated active NOTCH1 and NOTCH2 receptor signalling in CBCs by development of long-lived lineage tracing events after tamoxifen treatment (Fre *et al.* 2011; Pellegrinet *et al.* 2011). In addition, gene expression studies showed Notch target gene expression in intestinal crypts, with localization of *Hes1* and *Olfm4* to CBC cells (Fre *et al.* 2011; Pellegrinet *et al.* 2011; VanDussen *et al.* 2012). Finally, gene expression profiling showed that Paneth cells, which are directly adjacent to CBC stem cells at the crypt base, express the Notch ligand *Dll4* (Sato *et al.* 2011). The finding that Paneth cells express genes known to support ISC function (*Dll4*, *Wnt3*, *EGF*) suggests that these cells might function as ISC niche cells (Sato *et al.* 2011). However, this idea is controversial because other studies have shown that Paneth cells are not required for intestinal epithelial cell homeostasis (Garabedian *et al.* 1997; Shroyer *et al.* 2007; Durand *et al.* 2012; Kim *et al.* 2012). Thus, it is unlikely that Paneth cells are the sole source of Notch ligand.

Lineage marking cells with a Notch ligand *Dll1-CreERT* transgene showed expression in a subset of secretory progenitor cells, suggesting that committed TA cells may also be a source of Notch ligand for the GI stem cell (van Es *et al.* 2012). Furthermore, the colon, which is devoid of Paneth cells, requires Notch signalling for stem/progenitor cell proliferation and differentiation, suggesting that Paneth cells are not the sole source of Notch ligand, although a Paneth-like goblet cell has been postulated to serve this function in the colon (Rothenberg *et al.* 2012). Notch regulation of colonic stem cells is presumed to be similar to small intestine. However, because most studies have not included colonic stem cells in their analysis this issue has not been investigated in depth.

Many studies have suggested that Notch signalling is crucial for CBC maintenance, with Notch pathway inhibition resulting in reduced stem cell proliferation. Global Notch inhibition via pharmacological blockade of the γ-secretase complex led to an overall reduction in intestinal proliferation, and more specifically, reduced proliferation and fewer LGR5-expressing CBCs (van Es *et al.* 2005*b*; Pellegrinet *et al.* 2011; VanDussen *et al.* 2012). Consistent with the observed lineage tracing from CBCs expressing the NOTCH1 and NOTCH2 receptors (Fre *et al.* 2011; Pellegrinet *et al.* 2011), inhibition of both NOTCH1 and NOTCH2 receptor signalling via treatment with neutralizing antibodies or by genetic deletion resulted in reduced progenitor cell proliferation (Riccio *et al.* 2008; Wu *et al.* 2010; Tran *et al.* 2013; Carulli *et al.* 2015) and reduced LGR5^{+} CBC number (Carulli *et al.* 2015). Furthermore these studies suggested that NOTCH1 is

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

the dominant Notch receptor regulating CBCs (Carulli *et al.* 2015). Moreover, genetic inhibition of other Notch pathway components, such as the DLL1 and DLL4 ligands (Pellegrinet *et al.* 2011), the Notch receptor protease ADAM10 (Tsai *et al.* 2014) or the NICD binding partner RBPJ (van Es *et al.* 2005*b*) have been shown to reduce progenitor cell proliferation or loss of CBC stem cell function. Taken together, these data point to an essential role for Notch signalling to support intestinal CBCs.

Although much work has been devoted to understanding Notch regulation of CBC stem cells, much less is known about whether Notch plays a role in regulating QSCs. It was recently shown that global deletion of the QSC marker *Bmi1* results in modest reductions in intestinal length, progenitor cell proliferation and *Lgr5* expression, as well as a mild goblet cell hyperplasia, suggesting that BMI1 may interact with Notch signalling and that intestinal QSCs may contribute to intestinal epithelial cell homeostasis in the absence of injury (Lopez-Arribillaga *et al.* 2015). Moreover, this study showed that treatment of intestinal organoid cultures with the pan-Notch inhibitor DAPT resulted in reduced BMI1 expression, suggesting that Notch may regulate the BMI1$^+$ QSC population. Furthermore, recent studies investigating the consequences of individual deletion of the *Notch1* and *Notch2* receptor genes in the intestinal epithelium showed impaired crypt regeneration post-irradiation injury, suggesting that Notch signalling may be required for recruitment of QSCs to active stem cells, or for functional restoration of the CBC stem cell compartment after QSC activation (Carulli *et al.* 2015). These data highlight a potential role for Notch in regulating intestinal QSCs; however, further studies are needed to define Notch pathway function for maintenance or activation of this stem cell population.

Notch regulation of intestinal cell differentiation. In the intestine, Notch signalling is thought to direct the differentiation of multipotential progenitors into absorptive enterocytes by inhibiting the programme of secretory cell differentiation. Notch effects on cell fate have been primarily studied in mouse models where pathway disruption by pharmacological inhibition or genetic deletion has been shown to induce a profound secretory cell hyperplasia (Fig. 3*A*) (Jensen *et al.* 2000; van Es *et al.* 2005*b*; Riccio *et al.* 2008; Pellegrinet *et al.* 2011). Conversely, studies of pathway activation in transgenic mice expressing a constitutively active form of NICD showed loss of secretory cell types (Fre *et al.* 2005; Stanger *et al.* 2005).

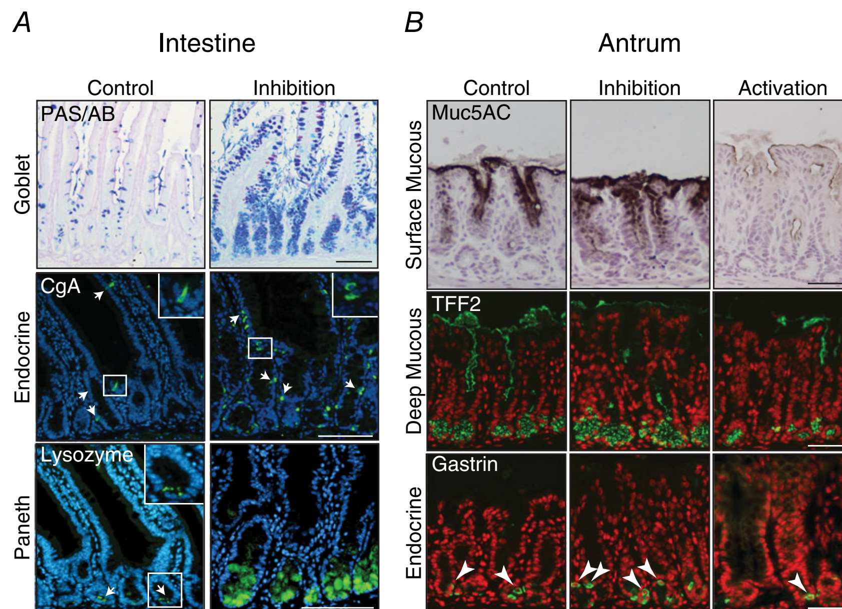


Figure 3. Notch regulates gastointestinal epithelial cell differentiation
A, inhibition of Notch with the γ-secretase inhibitor dibenzazepine (DBZ) induced expansion of all intestinal secretory cell lineages. Analysis of intestinal differentiated lineages by histological analysis, including goblet cells (PAS/AB), endocrine cells (chromogranin A; CgA) and Paneth cells (lysozyme) in control or Notch-inhibited mouse duodenum. Arrows indicate CgA$^+$ endocrine and lysozyme$^+$ Paneth cells. Insets are magnified from lower-powered boxed regions. *B*, analysis of gastric antral epithelial cells, including surface mucous cells (Muc5AC), deep mucous cells (TFF2) and endocrine cells (gastrin) in control, Notch-inhibited and Notch-activated (*Lgr5-CreER^{T2}; ROSA^{NICD}*) mice. Notch inhibition induced differentiation of all three epithelial cell types, while Notch activation induced a generalized reduction in differentiation. Arrowheads indicate gastrin$^+$ endocrine cells. Scale bars: 50 μm (*B*) or 100 μm (*A*). Figure modified from VanDussen *et al.* (2012) and Demitrack *et al.* (2015).

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Regulation of secretory cell fate occurs via Notch pathway inhibition of the basic helix–loop–helix (bHLH) transcription factor atonal homolog 1 (ATOH1, also called MATH1 in mouse) (reviewed in Noah & Shroyer, 2013). ATOH1 is expressed in secretory progenitors as well as differentiated secretory cell types and genetic deletion and activation studies in mouse models have demonstrated that ATOH1 is the key transcriptional activator inducing the full programme of secretory cell differentiation to generate goblet, Paneth and endocrine cells (Yang *et al.* 2001; Shroyer *et al.* 2007; VanDussen & Samuelson, 2010). The key Notch effector regulating *Atoh1* expression is thought to be the Notch target gene *Hes1*. Genetic deletion of *Hes1* in mouse results in excessive differentiation of secretory cell types, consistent with a Notch inhibition phenotype (Jensen *et al.* 2000; Ueo *et al.* 2012). Furthermore, Notch disruption results in decreased expression of *Hes1* and increased expression of *Atoh1*. The *Atoh1* promoter has been described to contain HES binding sites (Zheng *et al.* 2011) and it is thought that Notch-induced HES1 acts as a transcriptional repressor to silence *Atoh1* transcription and suppress secretory cell fate.

Other than suppressing secretory cell fate through regulation of *Atoh1*, Notch signalling does not appear to play an active role in enterocyte differentiation. This was demonstrated by analysis of mice with both intestine-specific deletion of *Atoh1* and disruption of Notch signalling. The intestinal epithelium of these mice was filled with enterocytes, suggesting that Notch is not required to promote enterocyte differentiation (Kazanjian *et al.* 2010; Kim & Shivdasani, 2011*a*).

Notch signalling can induce differential cell fate of adjoining cells. This process of Notch-induced lateral inhibition is thought to establish the homeostatic pattern of absorptive and secretory cell types in the intestine (reviewed in Sancho *et al.* 2015). ATOH1-expressing progenitor cells express Notch ligand, which would activate Notch signalling in surrounding cells to turn off *Atoh1* and promote absorptive cell fate (Fre *et al.* 2005; Kim & Shivdasani, 2011*a*). Thereby, secretory cell types in the small intestine are surrounded by absorptive enterocytes.

Several transcription factors have been identified to function downstream of ATOH1 to regulate specification of the mature secretory cell types (Fig. 4). Mouse loss-of-function and overexpression studies have demonstrated that the bHLH transcription factor Neurogenin 3 (NEUROG3) directs enteroendocrine cell differentiation (Jenny *et al.* 2002; Lee *et al.* 2002; Lopez-Diaz *et al.* 2007). GFI1, a zinc-finger protein family member, has been shown to be a direct ATOH1 target gene that directs secretory progenitor cells to form goblet or Paneth cells (Shroyer *et al.* 2005). Differentiation of goblet/Paneth progenitor cells into mature goblet cells also involves the transcription factor SPDEF (Noah *et al.* 2010). It is currently unknown how progenitor cells are fated towards goblet or Paneth cells, but this process probably involves SPDEF, KLF4 (Zheng *et al.* 2009), SOX9 (Bastide *et al.* 2007; Mori-Akiyama *et al.* 2007), FGF10 (Al Alam *et al.* 2015) and Wnt (van Es *et al.* 2005*a*).

A progenitor cell population expressing high levels of the Notch ligand DLL1 has been identified (van Es *et al.* 2012; Buczacki *et al.* 2013). These cells express low levels of stem cell markers and high *Atoh1*, characteristics of committed secretory progenitor cells. Lineage tracing studies showed that these $DLL1^+$ cells labelled short-lived clones containing all four secretory cell types suggesting that they are multipotential secretory cell precursors (van Es *et al.* 2012). Additionally, during radiation-induced loss of CBCs these committed progenitor cells exhibited plasticity by regaining stemness to regenerate the epithelium, thereby also functioning as QSCs (van Es *et al.* 2012; Buczacki *et al.* 2013). This finding supports the notion that TA progenitors can fill open niche spots left vacant when CBC stem cells are lost.

Notch regulation of gastric epithelial cell homeostasis

Gastric stem cells. While a large volume of literature exists regarding Notch pathway regulation of intestinal stem cells, much less is known about Notch regulation of gastric stem cells, largely due to the paucity of molecular markers for stem cells in this tissue. The glandular stomach is divided into two regions: the corpus, which secretes gastric acid and digestive enzymes into the gastric lumen, and the more distal antrum, which secretes the hormone gastrin into the blood. Gastric stem cells exist in both regions to generate the various differentiated gastric lineages throughout adult life (Fig. 2*B*). However, there are distinct differences in stem/progenitor cell location as well as marker expression in these regions. In the corpus, proliferating cells (and presumably stem cells) are located in the middle of the corpus glands, while in the antrum, stem and TA progenitor cells are located at the gland base, in a pattern similar to that observed in the intestinal crypt (Fig. 2). In line with the similar patterning, the CBC marker LGR5 has been identified as a marker of antral stem cells, based on lineage tracing analysis and initiation of organoid cultures from FACS-sorted LGR5-GFP stem cells (Barker *et al.* 2010). Approximately 8 $LGR5^+$ stem cells are present in each antral gland (Leushacke *et al.* 2013).

In contrast to the antrum, corpus stem cell markers are less well established. Although LGR5 marks a precursor to the corpus stem cell, adult corpus stem cells do not express this marker (Barker *et al.* 2010). SOX2 has been shown to be expressed in both corpus and antral stem cells (Fig. 2*B*), with long-lived lineage stripes observed in both compartments after activation of a reporter gene by

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

the Sox2-CreER^{T2} driver (Arnold *et al.* 2011). However, this gene is also expressed in differentiated gastric cell types in addition to stem cells. Importantly, in contrast to LGR5, SOX2 does not label ISCs, thus allowing genetic manipulation of gastric epithelial cells without affecting the intestine. Markers described for facultative gastric stem cells include *Villin*, which marks a rare quiescent antral stem cell (Qiao *et al.* 2007), and *Troy*, which is expressed in a small population of differentiated chief cells (Stange *et al.* 2013). Both of these markers are expressed in non-proliferating gastric cells that are capable of expanding and replenishing entire gastric glands after injury, similar in function to QSCs in the intestine (Fig. 2). Additionally, *Lrig1*, a reported intestinal QSC marker, also marks a population of gastric stem cells in both corpus and antrum, although this marker also appears to label differentiated cells in both regions (Powell *et al.* 2012). Clearly more work is needed to identify specific markers of gastric stem cells that are not expressed in differentiated gastric cell types or in intestinal stem cells.

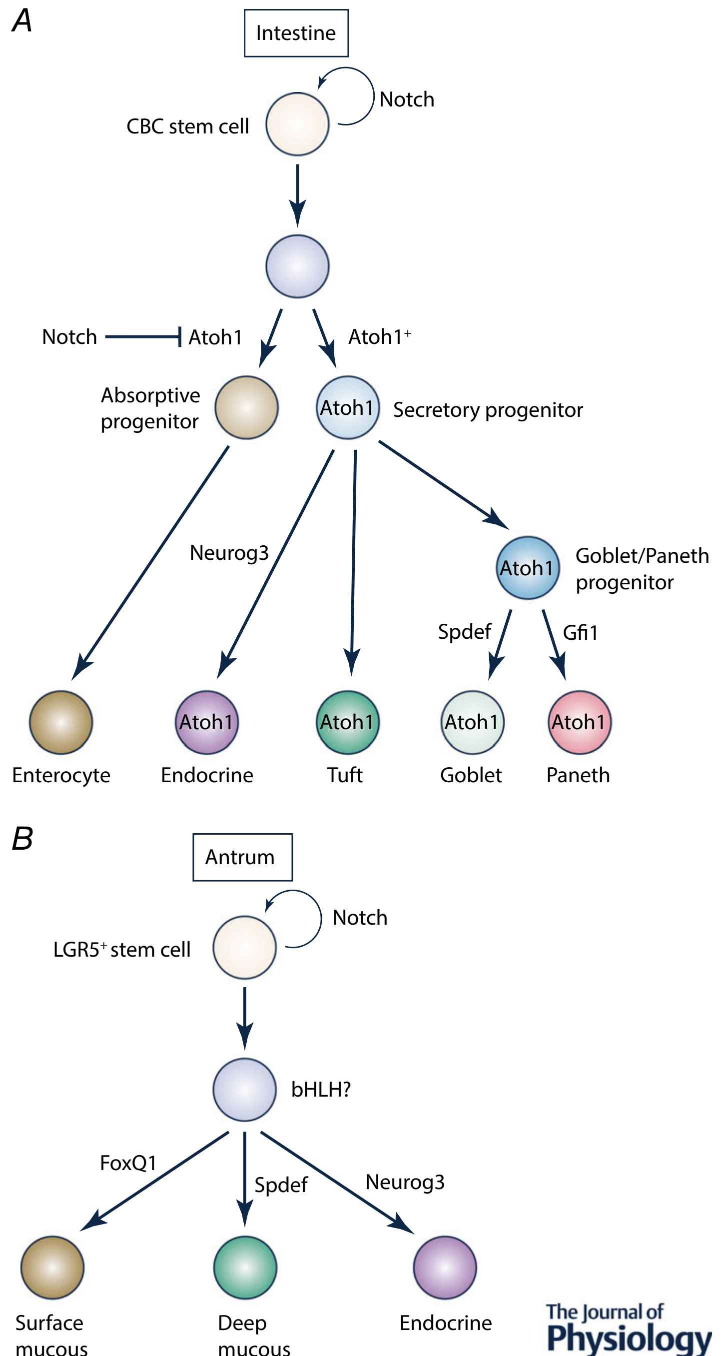


Figure 4. Model of Notch regulation of intestinal and gastric epithelial cell differentiation
Notch is required for renewal of both intestinal (*A*) and gastric antral (*B*) stem cells. In the intestine, Notch signalling represses the bHLH transcriptional regulator ATOH1 to form absorptive enterocytes. ATOH1$^+$ secretory progenitor cells are fated towards 1 of 4 mature secretory cell types: endocrine, tuft, goblet or Paneth. Note that SPDEF and/or GFI1 regulate goblet and Paneth cell differentiation while NEUROG3 induces endocrine cell differentiation. A master transcriptional regulator, possibly a member of the bHLH family, has not been identified for the gastric antrum, although transcription factors that promote differentiation of each mature lineage are known. In the stomach, recent studies suggest that Notch functions to regulate gastric stem cell proliferation *versus* differentiation, and not choice between different differentiated cell types.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Notch regulation of gastric stem cell proliferation. Notch regulation of adult gastric stem cells has only recently been established. Lineage tracing from cells with active NOTCH1 signalling has been demonstrated in antral stem cells (Demitrack *et al.* 2015; Gifford *et al.* 2016). Functional studies have shown that similar to its effect in the intestine, Notch signalling promotes gastric epithelial cell proliferation. This was demonstrated by the reduction in proliferation observed after pharmacological blockade of the γ-secretase complex in both corpus (Kim & Shivdasani, 2011*b*) and antrum (Kim & Shivdasani, 2011*b*; Demitrack *et al.* 2015; Gifford *et al.* 2016). Importantly, examination of the LGR5-expressing antral stem cells after Notch manipulation showed alterations in stem cell function. Notch inhibition was found to reduce stem cell proliferation, while Notch activation by genetic expression of NICD caused increases in proliferation and stem cell function (Demitrack *et al.* 2015). This study also showed that gastric organoids were similarly affected by Notch, with decreased efficiency of establishment and organoid growth with Notch pathway inhibition, and increased efficiency and growth with Notch activation. Over time, chronic NICD expression in parietal progenitor cells of the corpus (Kim & Shivdasani, 2011*b*) or $LGR5^+$ antral stem cells (Demitrack *et al.* 2015) resulted in the formation of hyperplastic polyps, further establishing Notch as a crucial pathway regulating proliferation.

Similar to intestine, NOTCH1 and NOTCH2 appear to be the key receptors mediating Notch effects in the antrum. Specific blockage of these two receptors by treatment with inhibitory antibodies led to reduced proliferation and alteration of differentiation similar to global Notch inhibition with a γ-secretase inhibitor (Gifford *et al.* 2016). Furthermore, NOTCH1 appears to be the dominant receptor regulating gastric stem cells, similar to what has been shown in intestine (Carulli *et al.* 2015; Gifford *et al.* 2016). Analysis of gastric organoids established from human antral tissue confirmed that Notch signalling is essential to maintain human gastric stem cells through NOTCH1 and NOTCH2 signalling (Gifford *et al.* 2016). Thus human gastric stem cells appear to be regulated similarly to the mouse, validating the mouse as an effective model to study Notch regulation.

Notch regulation of gastric stem cell differentiation. The Notch pathway has also been shown to regulate gastric epithelial cell differentiation. However, in contrast to intestine, where Notch is key to cell fate determination (Fig. 3*A*), in the stomach Notch appears to similarly affect all gastric lineages. These studies largely focused on the antrum, where cellular turnover is relatively rapid, occurring within a week, rather than the corpus where cell turnover ranges from a few days (surface mucous cells) to 6 months (chief cells). Notch inhibition increased antral cellular differentiation, including surface mucous, deep mucous and endocrine cells while Notch activation resulted in decreased numbers of all lineages (Fig. 3*B*) (Demitrack *et al.* 2015; Gifford *et al.* 2016). These findings were demonstrated in both *in vivo* mouse models and *ex vivo* gastric organoid cultures.

Although a master transcriptional regulator of cellular differentiation in the stomach has not been identified, transcription factors that regulate differentiation of mature antral lineages are known. As such, Notch signalling affects the expression of these factors in line with the cell lineage changes, including *FoxQ1* (surface mucous cells), *Spdef* (deep mucous cells) and *Neurog3* (endocrine cells), although whether this is direct or indirect is not known (Fig. 4) (Demitrack *et al.* 2015). In sum, these data suggest that active Notch signalling promotes proliferation and represses cellular differentiation in the antrum.

Comparison to Notch effects in the intestine. Notch regulation of gastric and intestinal epithelial cell homeostasis has many parallels, yet also distinct differences. Notch signalling promotes stem cell proliferation in both stomach and intestine, serving as an essential niche factor for the active $LGR5^+$ stem cell. In contrast, regulation of cell fate is fundamentally different in these two tissues. In the gastric antrum, Notch appears to affect choice between stem cell proliferation or differentiation to maintain homeostasis, and does not promote the differentiation of one lineage at the expense of another as has been established in intestine via regulation of ATOH1. More work is needed to determine whether a Notch-dependent master transcriptional regulator, such as another bHLH family member, exists for differentiation of gastric epithelial lineages. However, it is important to note that in contrast to the intestine the adult stomach only contains secretory cell types.

Notch and antral tissue expansion. In the gastric antrum, constitutive Notch activation has been shown to induce gland fission, a tissue growth phenomenon thought to be associated with increased stem cell number (Demitrack *et al.* 2015). Gland fission is a process by which a single gland bifurcates, or splits, into two separate glands (Lee, 1985). It is an essential part of postnatal stomach growth during the time that gastric glands are undergoing cellular maturation (Nomura *et al.* 1998; Keeley & Samuelson, 2010). Fission is rarely observed in the adult stomach. Recent studies have shown that NICD activation in $LGR5^+$ antral stem cells induces antral gland fission and eventual formation of hyperproliferative undifferentiated polyps (Demitrack *et al.* 2015). In the human stomach, cancer-initiating mutations in gastric stem cells have been shown to spread via gland fission (McDonald *et al.* 2008); however, whether Notch plays a role in this process in human is not known.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Notch and gastrointestinal disease

Dysregulation of Notch signalling has been implicated in tumorigenesis of a variety of tissues, particularly in the haematopoietic system where activating mutations in the NOTCH1 receptor lead to T cell acute lymphoblastic leukaemia (Demarest *et al.* 2008). Recent studies also point to a link between overexpression of Notch pathway components and colorectal cancer (Reedijk *et al.* 2008; Fre *et al.* 2009; Chu *et al.* 2010; Kazanjian & Shroyer, 2011; Guilmeau, 2012); these studies have been nicely summarized in a recent review (Noah & Shroyer, 2013).

Much less is known about the role of Notch in gastric cancer, although correlative studies between high NOTCH1 and NOTCH2 expression and gastric cancer morbidity have been published (Yeh *et al.* 2009; Hsu *et al.* 2012; Bauer *et al.* 2015). The observation of NICD-induced polyps in mouse genetic models suggests that Notch pathway activation induces aberrant stem and progenitor cell proliferation, which could then initiate and/or sustain gastric tumours (Kim & Shivdasani, 2011*b*; Demitrack *et al.* 2015). It has also been postulated that Notch may be involved in a pre-cancerous, metaplastic condition in the stomach known as spasmolytic polypeptide-expressing metaplasia (SPEM) (Mutoh *et al.* 2006), which typically results from loss of parietal cells and chronic inflammation. ATOH1, which is normally absent from the normal mouse and human stomach, has been shown to be upregulated in a mouse model of intestinal metaplasia as well as in the metaplastic human stomach (Mutoh *et al.* 2006). Whether ectopic activation of ATOH1 is due to the intestinalization of the stomach in these situations or includes a role for Notch in promoting these metaplastic conditions remains to be elucidated.

Conclusions and future directions

The Notch pathway is crucial for gastrointestinal epithelial cell homeostasis, regulating stem cells in both the stomach and intestine. Notch is required for stem cell maintenance and manipulation of Notch signalling affects stem cell function and number. Notch inhibition is associated with *reduced* stem cell proliferation and activity while Notch activation is associated with *increased* stem cell proliferation and activity. Together the findings suggest that Notch is a key niche factor regulating stem cell self-renewal in gastrointestinal tissues.

Our understanding of Notch regulation of gastrointestinal stem cells has primarily been developed through extensive studies of mouse pharmacological and genetic models. With the recent development of methods to grow epithelial organoids from primary human tissue there will be future opportunities to directly study human gastrointestinal stem cells. Notch pathway regulation of intestinal cell homeostasis is highly conserved, with parallel functions reported in other organisms, such as *Drosophila* (Micchelli & Perrimon, 2006) and zebrafish (Yang *et al.* 2009), suggesting that this pathway will also be a crucial regulator in man. Indeed, recent analysis of human gastric organoids demonstrated that Notch regulates human antral stem cells similar to what has been shown in the mouse (Gifford *et al.* 2016). Describing the key Notch pathway components and molecular mechanisms driving human gastrointestinal stem cell function will be important to further our understanding of human gastrointestinal epithelial cell maintenance and repair.

Some timely unanswered questions that need to be addressed to further our understanding of Notch regulation of mouse and/or human stem cells include:

- How does Notch regulate gastrointestinal stem cell number?
- Does Notch regulate the 'quiescent' stem cell population?
- Which cells present Notch ligand to regulate stem cell function? Are there niche cells in the stomach equivalent to Paneth cells in the intestine?
- Which Notch ligands function in the stomach?
- Does Notch regulate differentiation of cells in the gastric corpus?
- What are the mechanisms of Notch regulation of human gastrointestinal stem cells?

References

Al Alam D, Danopoulos S, Schall K, Sala FG, Almohazey D, Fernandez GE, Georgia S, Frey MR, Ford HR, Grikscheit T & Bellusci S (2015). Fibroblast growth factor 10 alters the balance between goblet and Paneth cells in the adult mouse small intestine. *Am J Physiol Gastrointest Liver Physiol* **308**, G678–G690.

Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, Sengupta S, Seandel M, Geijsen N & Hochedlinger K (2011). Sox2^{+} adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell* **9**, 317–329.

Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M, Danenberg E, van den Brink S, Korving J, Abo A, Peters PJ, Wright N, Poulsom R & Clevers H (2010). Lgr5^{+ve} stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* **6**, 25–36.

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ & Clevers H (2007). Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* **449**, 1003–1007.

Bastide P, Darido C, Pannequin J, Kist R, Robine S, Marty-Double C, Bibeau F, Scherer G, Joubert D, Hollande F, Blache P & Jay P (2007). Sox9 regulates cell proliferation and is required for Paneth cell differentiation in the intestinal epithelium. *J Cell Biol* **178**, 635–648.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Bauer L, Takacs A, Slotta-Huspenina J, Langer R, Becker K, Novotny A, Ott K, Walch A, Hapfelmeier A & Keller G (2015). Clinical significance of NOTCH1 and NOTCH2 expression in gastric carcinomas: an immunohistochemical study. *Front Oncol* **5**, 94.

Buczacki SJ, Zecchini HI, Nicholson AM, Russell R, Vermeulen L, Kemp R & Winton DJ (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* **495**, 65–69.

Carulli AJ, Keeley TM, Demitrack ES, Chung J, Maillard I & Samuelson LC (2015). Notch receptor regulation of intestinal stem cell homeostasis and crypt regeneration. *Dev Biol* **402**, 98–108.

Chu D, Li Y, Wang W, Zhao Q, Li J, Lu Y, Li M, Dong G, Zhang H, Xie H & Ji G (2010). High level of Notch1 protein is associated with poor overall survival in colorectal cancer. *Ann Surg Oncol* **17**, 1337–1342.

Demarest RM, Ratti F & Capobianco AJ (2008). It's T-ALL about Notch. *Oncogene* **27**, 5082–5091.

Demitrack ES, Gifford GB, Keeley TM, Carulli AJ, VanDussen KL, Thomas D, Giordano TJ, Liu Z, Kopan R & Samuelson LC (2015). Notch signaling regulates gastric antral LGR5 stem cell function. *EMBO J* **34**, 2522–2536.

Durand A, Donahue B, Peignon G, Letourneur F, Cagnard N, Slomianny C, Perret C, Shroyer NF & Romagnolo B (2012). Functional intestinal stem cells after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1). *Proc Natl Acad Sci USA* **109**, 8965–8970.

Formeister EJ, Sionas AL, Lorance DK, Barkley CL, Lee GH & Magness ST (2009). Distinct SOX9 levels differentially mark stem/progenitor populations and enteroendocrine cells of the small intestine epithelium. *Am J Physiol Gastrointest Liver Physiol* **296**, G1108–G1118.

Fre S, Hannezo E, Sale S, Huyghe M, Lafkas D, Kissel H, Louvi A, Greve J, Louvard D & Artavanis-Tsakonas S (2011). Notch lineages and activity in intestinal stem cells determined by a new set of knock-in mice. *PLoS One* **6**, e25785.

Fre S, Huyghe M, Mourikis P, Robine S, Louvard D & Artavanis-Tsakonas S (2005). Notch signals control the fate of immature progenitor cells in the intestine. *Nature* **435**, 964–968.

Fre S, Pallavi SK, Huyghe M, Lae M, Janssen KP, Robine S, Artavanis-Tsakonas S & Louvard D (2009). Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. *Proc Natl Acad Sci USA* **106**, 6309–6314.

Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, Hosokawa S, Elbahrawy A, Soeda T, Koizumi M, Masui T, Kawaguchi M, Takaori K, Doi R, Nishi E, Kakinoki R, Deng JM, Behringer RR, Nakamura T & Uemoto S (2011). Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet* **43**, 34–41.

Garabedian EM, Roberts LJ, McNevin MS & Gordon JI (1997). Examining the role of Paneth cells in the small intestine by lineage ablation in transgenic mice. *J Biol Chem* **272**, 23729–23740.

Gifford GB, Demitrack ES, Keeley TM, Tam A, La Cunza N, Dedhia PH, Spence JR, Simeone DM, Louvi A, Siebel CW & Samuelson LC (2016). Notch1 and Notch2 receptors regulate mouse and human gastric antral epithelial cell homeostasis. *Gut* (in press), doi: 10.1136/gutjnl-2015-310811.

Guilmeau S (2012). Notch signaling and intestinal cancer. *Adv Exp Med Biol* **727**, 272–288.

Hsu KW, Hsieh RH, Huang KH, Fen-Yau Li A, Chi CW, Wang TY, Tseng MJ, Wu KJ & Yeh TS (2012). Activation of the Notch1/STAT3/Twist signaling axis promotes gastric cancer progression. *Carcinogenesis* **33**, 1459–1467.

Jenny M, Uhl C, Roche C, Duluc I, Guillermin V, Guillemot F, Jensen J, Kedinger M & Gradwohl G (2002). Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *EMBO J* **21**, 6338–6347.

Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P & Madsen OD (2000). Control of endodermal endocrine development by Hes-1. *Nat Genet* **24**, 36–44.

Kazanjian A, Noah T, Brown D, Burkart J & Shroyer NF (2010). Atonal homolog 1 is required for growth and differentiation effects of notch/gamma-secretase inhibitors on normal and cancerous intestinal epithelial cells. *Gastroenterology* **139**, 918–928.

Kazanjian A & Shroyer NF (2011). NOTCH signaling and ATOH1 in colorectal cancers. *Curr Colorectal Cancer Rep* **7**, 121–127.

Keeley TM & Samuelson LC (2010). Cytodifferentiation of the postnatal mouse stomach in normal and Huntingtin-interacting protein 1-related-deficient mice. *Am J Physiol Gastrointest Liver Physiol* **299**, G1241–G1251.

Kim TH, Escudero S & Shivdasani RA (2012). Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. *Proc Natl Acad Sci USA* **109**, 3932–3937.

Kim TH & Shivdasani RA (2011*a*). Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression. *J Biol Chem* **286**, 11427–11433.

Kim TH & Shivdasani RA (2011*b*). Notch signaling in stomach epithelial stem cell homeostasis. *J Exp Med* **208**, 677–688.

Koch U, Lehal R & Radtke F (2013). Stem cells living with a Notch. *Development* **140**, 689–704.

Kopan R & Ilagan MX (2009). The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* **137**, 216–233.

Kozar S, Morrissey E, Nicholson AM, van der Heijden M, Zecchini HI, Kemp R, Tavare S, Vermeulen L & Winton DJ (2013). Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. *Cell Stem Cell* **13**, 626–633.

Lee CS, Perreault N, Brestelli JE & Kaestner KH (2002). Neurogenin 3 is essential for the proper specification of gastric enteroendocrine cells and the maintenance of gastric epithelial cell identity. *Genes Dev* **16**, 1488–1497.

Lee ER (1985). Dynamic histology of the antral epithelium in the mouse stomach: I. Architecture of antral units. *Am J Anat* **172**, 187–204.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Leushacke M, Ng A, Galle J, Loeffler M & Barker N (2013). Lgr5^{+} gastric stem cells divide symmetrically to effect epithelial homeostasis in the pylorus. *Cell Rep* **5**, 349–356.

Lopez-Arribillaga E, Rodilla V, Pellegrinet L, Guiu J, Iglesias M, Roman AC, Gutarra S, Gonzalez S, Munoz-Canoves P, Fernandez-Salguero P, Radtke F, Bigas A & Espinosa L (2015). Bmi1 regulates murine intestinal stem cell proliferation and self-renewal downstream of Notch. *Development* **142**, 41–50.

Lopez-Diaz L, Jain RN, Keeley TM, VanDussen KL, Brunkan CS, Gumucio DL & Samuelson LC (2007). Intestinal Neurogenin 3 directs differentiation of a bipotential secretory progenitor to endocrine cell rather than goblet cell fate. *Dev Biol* **309**, 298–305.

McDonald SA, Greaves LC, Gutierrez-Gonzalez L, Rodriguez-Justo M, Deheragoda M, Leedham SJ, Taylor RW, Lee CY, Preston SL, Lovell M, Hunt T, Elia G, Oukrif D, Harrison R, Novelli MR, Mitchell I, Stoker DL, Turnbull DM, Jankowski JA & Wright NA (2008). Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. *Gastroenterology* **134**, 500–510.

Micchelli CA & Perrimon N (2006). Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature* **439**, 475–479.

Mills JC & Sansom OJ (2015). Reserve stem cells: Differentiated cells reprogram to fuel repair, metaplasia, and neoplasia in the adult gastrointestinal tract. *Sci Signal* **8**, re8.

Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra S & Breault DT (2011). Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci USA* **108**, 179–184.

Mori-Akiyama Y, van den Born M, van Es JH, Hamilton SR, Adams HP, Zhang J, Clevers H & de Crombrugghe B (2007). SOX9 is required for the differentiation of Paneth cells in the intestinal epithelium. *Gastroenterology* **133**, 539–546.

Munoz J, Stange DE, Schepers AG, van de Wetering M, Koo BK, Itzkovitz S, Volckmann R, Kung KS, Koster J, Radulescu S, Myant K, Versteeg R, Sansom OJ, van Es JH, Barker N, van Oudenaarden A, Mohammed S, Heck AJ & Clevers H (2012). The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent '+4' cell markers. *EMBO J* **31**, 3079–3091.

Mutoh H, Sakamoto H, Hayakawa H, Arao Y, Satoh K, Nokubi M & Sugano K (2006). The intestine-specific homeobox gene Cdx2 induces expression of the basic helix–loop–helix transcription factor Math1. *Differentiation* **74**, 313–321.

Noah TK, Kazanjian A, Whitsett J & Shroyer NF (2010). SAM pointed domain ETS factor (SPDEF) regulates terminal differentiation and maturation of intestinal goblet cells. *Exp Cell Res* **316**, 452–465.

Noah TK & Shroyer NF (2013). Notch in the intestine: regulation of homeostasis and pathogenesis. *Annu Rev Physiol* **75**, 263–288.

Nomura S, Esumi H, Job C & Tan SS (1998). Lineage and clonal development of gastric glands. *Dev Biol* **204**, 124–135.

Pellegrinet L, Rodilla V, Liu Z, Chen S, Koch U, Espinosa L, Kaestner KH, Kopan R, Lewis J & Radtke F (2011). Dll1- and Dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. *Gastroenterology* **140**, 1230–1240.

Piazzi G, Fini L, Selgrad M, Garcia M, Daoud Y, Wex T, Malfertheiner P, Gasbarrini A, Romano M, Meyer RL, Genta RM, Fox JG, Boland CR, Bazzoli F & Ricciardiello L (2011). Epigenetic regulation of Delta-Like1 controls Notch1 activation in gastric cancer. *Oncotarget* **2**, 1291–1301.

Powell AE, Wang Y, Li Y, Poulin EJ, Means AL, Washington MK, Higginbotham JN, Juchheim A, Prasad N, Levy SE, Guo Y, Shyr Y, Aronow BJ, Haigis KM, Franklin JL & Coffey RJ (2012). The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell* **149**, 146–158.

Qiao XT, Ziel JW, McKimpson W, Madison BB, Todisco A, Merchant JL, Samuelson LC & Gumucio DL (2007). Prospective identification of a multilineage progenitor in murine stomach epithelium. *Gastroenterology* **133**, 1989–1998.

Reedijk M, Odorcic S, Zhang H, Chetty R, Tennert C, Dickson BC, Lockwood G, Gallinger S & Egan SE (2008). Activation of Notch signaling in human colon adenocarcinoma. *Int J Oncol* **33**, 1223–1229.

Riccio O, van Gijn ME, Bezdek AC, Pellegrinet L, van Es JH, Zimber-Strobl U, Strobl LJ, Honjo T, Clevers H & Radtke F (2008). Loss of intestinal crypt progenitor cells owing to inactivation of both Notch1 and Notch2 is accompanied by derepression of CDK inhibitors p27Kip1 and p57Kip2. *EMBO Rep* **9**, 377–383.

Rothenberg ME, Nusse Y, Kalisky T, Lee JJ, Dalerba P, Scheeren F, Lobo N, Kulkarni S, Sim S, Qian D, Beachy PA, Pasricha PJ, Quake SR & Clarke MF (2012). Identification of a cKit^{+} colonic crypt base secretory cell that supports Lgr5^{+} stem cells in mice. *Gastroenterology* **142**, 1195–1205 e1196.

Sancho R, Cremona CA & Behrens A (2015). Stem cell and progenitor fate in the mammalian intestine: Notch and lateral inhibition in homeostasis and disease. *EMBO Rep* **16**, 571–581.

Sander GR & Powell BC (2004). Expression of notch receptors and ligands in the adult gut. *J Histochem Cytochem* **52**, 509–516.

Sangiorgi E & Capecchi MR (2008). *Bmi1* is expressed *in vivo* in intestinal stem cells. *Nat Genet* **40**, 915–920.

Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M & Clevers H (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415–418.

Schonhoff SE, Giel-Moloney M & Leiter AB (2004). Neurogenin 3-expressing progenitor cells in the gastrointestinal tract differentiate into both endocrine and non-endocrine cell types. *Dev Biol* **270**, 443–454.

Schröder N & Gossler A (2002). Expression of Notch pathway components in fetal and adult mouse small intestine. *Gene Expr Patterns* **2**, 247–250.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Sei Y, Lu X, Liou A, Zhao X & Wank SA (2011). A stem cell marker-expressing subset of enteroendocrine cells resides at the crypt base in the small intestine. *Am J Physiol Gastrointest Liver Physiol* **300**, G345–G356.

Shimizu H, Okamoto R, Ito G, Fujii S, Nakata T, Suzuki K, Murano T, Mizutani T, Tsuchiya K, Nakamura T, Hozumi K & Watanabe M (2014). Distinct expression patterns of Notch ligands, Dll1 and Dll4, in normal and inflamed mice intestine. *PeerJ* **2**, e370.

Shroyer NF, Helmrath MA, Wang VY, Antalffy B, Henning SJ & Zoghbi HY (2007). Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology* **132**, 2478–2488.

Shroyer NF, Wallis D, Venken KJ, Bellen HJ & Zoghbi HY (2005). Gfi1 functions downstream of Math1 to control intestinal secretory cell subtype allocation and differentiation. *Genes Dev* **19**, 2412–2417.

Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD & Clevers H (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134–144.

Stange DE, Koo BK, Huch M, Sibbel G, Basak O, Lyubimova A, Kujala P, Bartfeld S, Koster J, Geahlen JH, Peters PJ, van Es JH, van de Wetering M, Mills JC & Clevers H (2013). Differentiated *Troy*$^{+}$ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* **155**, 357–368.

Stanger BZ, Datar R, Murtaugh LC & Melton DA (2005). Direct regulation of intestinal fate by Notch. *Proc Natl Acad Sci USA* **102**, 12443–12448.

Takebe N, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, Yang SX & Ivy SP (2015). Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat Rev Clin Oncol* **12**, 445–464.

Takeda N, Jain R, LeBoeuf MR, Wang Q, Lu MM & Epstein JA (2011). Interconversion between intestinal stem cell populations in distinct niches. *Science* **334**, 1420–1424.

Tian H, Biehs B, Warming S, Leong KG, Rangell L, Klein OD & de Sauvage FJ (2011). A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* **478**, 255–259.

Tran IT, Sandy AR, Carulli AJ, Ebens C, Chung J, Shan GT, Radojcic V, Friedman A, Gridley T, Shelton A, Reddy P, Samuelson LC, Yan M, Siebel CW & Maillard I (2013). Blockade of individual Notch ligands and receptors controls graft-versus-host disease. *J Clin Invest* **123**, 1590–1604.

Tsai YH, VanDussen KL, Sawey ET, Wade AW, Kasper C, Rakshit S, Bhatt RG, Stoeck A, Maillard I, Crawford HC, Samuelson LC & Dempsey PJ (2014). ADAM10 regulates Notch function in intestinal stem cells of mice. *Gastroenterology* **147**, 822–834.

Ueo T, Imayoshi I, Kobayashi T, Ohtsuka T, Seno H, Nakase H, Chiba T & Kageyama R (2012). The role of Hes genes in intestinal development, homeostasis and tumor formation. *Development* **139**, 1071–1082.

van der Flier LG, Haegebarth A, Stange DE, van de Wetering M & Clevers H (2009). OLFM4 is a robust marker for stem cells in human intestine and marks a subset of colorectal cancer cells. *Gastroenterology* **137**, 15–17.

VanDussen KL, Carulli AJ, Keeley TM, Patel SR, Puthoff BJ, Magness ST, Tran IT, Maillard I, Siebel C, Kolterud A, Grosse AS, Gumucio DL, Ernst SA, Tsai YH, Dempsey PJ & Samuelson LC (2012). Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. *Development* **139**, 488–497.

VanDussen KL & Samuelson LC (2010). Mouse atonal homolog 1 directs intestinal progenitors to secretory cell rather than absorptive cell fate. *Dev Biol* **346**, 215–223.

van Es JH, Jay P, Gregorieff A, van Gijn ME, Jonkheer S, Hatzis P, Thiele A, van den Born M, Begthel H, Brabletz T, Taketo MM & Clevers H (2005*a*). Wnt signalling induces maturation of Paneth cells in intestinal crypts. *Nat Cell Biol* **7**, 381–386.

van Es JH, Sato T, van de Wetering M, Lyubimova A, Nee AN, Gregorieff A, Sasaki N, Zeinstra L, van den Born M, Korving J, Martens AC, Barker N, van Oudenaarden A & Clevers H (2012). Dll1^{+} secretory progenitor cells revert to stem cells upon crypt damage. *Nat Cell Biol* **14**, 1099–1104.

van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, Begthel H, Cozijnsen M, Robine S, Winton DJ, Radtke F & Clevers H (2005*b*). Notch/γ-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* **435**, 959–963.

Van Landeghem L, Santoro MA, Krebs AE, Mah AT, Dehmer JJ, Gracz AD, Scull BP, McNaughton K, Magness ST & Lund PK (2012). Activation of two distinct Sox9-EGFP-expressing intestinal stem cell populations during crypt regeneration after irradiation. *Am J Physiol Gastrointest Liver Physiol* **302**, G1111–G1132.

Wu Y, Cain-Hom C, Choy L, Hagenbeek TJ, de Leon GP, Chen Y, Finkle D, Venook R, Wu X, Ridgway J, Schahin-Reed D, Dow GJ, Shelton A, Stawicki S, Watts RJ, Zhang J, Choy R, Howard P, Kadyk L, Yan M, Zha J, Callahan CA, Hymowitz SG & Siebel CW (2010). Therapeutic antibody targeting of individual Notch receptors. *Nature* **464**, 1052–1057.

Yan KS, Chia LA, Li X, Ootani A, Su J, Lee JY, Su N, Luo Y, Heilshorn SC, Amieva MR, Sangiorgi E, Capecchi MR & Kuo CJ (2012). The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *Proc Natl Acad Sci USA* **109**, 466–471.

Yang J, Chan CY, Jiang B, Yu X, Zhu GZ, Chen Y, Barnard J & Mei W (2009). hnRNP I inhibits Notch signaling and regulates intestinal epithelial homeostasis in the zebrafish. *PLoS Genet* **5**, e1000363.

Yang Q, Bermingham NA, Finegold MJ & Zoghbi HY (2001). Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science* **294**, 2155–2158.

Yeh TS, Wu CW, Hsu KW, Liao WJ, Yang MC, Li AF, Wang AM, Kuo ML & Chi CW (2009). The activated Notch1 signal pathway is associated with gastric cancer progression through cyclooxygenase-2. *Cancer Res* **69**, 5039–5048.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society

Zheng H, Pritchard DM, Yang X, Bennett E, Liu G, Liu C & Ai W (2009). KLF4 gene expression is inhibited by the Notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* **296**, G490–G498.

Zheng X, Tsuchiya K, Okamoto R, Iwasaki M, Kano Y, Sakamoto N, Nakamura T & Watanabe M (2011). Suppression of hath1 gene expression directly regulated by hes1 via Notch signaling is associated with goblet cell depletion in ulcerative colitis. *Inflamm Bowel Dis* **17**, 2251–2260.

Additional information

Competing interests

None declared.

Funding

E.S.D. was supported by NIH F32-DK093340, MICHR PTSP CTSA UL1TR000433 and NIH T32-HD007505. L.C.S. has research support through NIH P01-DK062041, NIH 5R01-DK096972-03, NCI P50-CA130810 and the American Gastroenterological Association (AGA) Research Foundation.

© 2016 The Authors. The Journal of Physiology © 2016 The Physiological Society