Cell Stem Cell
Minireview

Intestinal Stem Cells in Mammals and *Drosophila*

Andreu Casali[1,2] and Eduard Batlle[1,3,*]
[1]Institute for Research in Biomedicine (IRB Barcelona), Baldiri Reixac 10, 08028 Barcelona, Spain
[2]Institut de Biologia Molecular de Barcelona (CSIC), Baldiri Reixac 10, 08028 Barcelona, Spain
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain
*Correspondence: eduard.batlle@irbbarcelona.org
DOI 10.1016/j.stem.2009.01.009

In the last few years, our knowledge of intestinal stem cell biology has flourished. Here we review recent developments in this exciting field, paying special attention to the characterization of *Drosophila* and mammalian intestinal stem cells.

Mammalian and *Drosophila* Intestinal Stem Cells

In mammals, the inner lining of the intestinal tube is a monostratified epithelium folded into millions of invaginations known as crypts (Figure 1A). The small intestine of an adult mouse contains roughly a million crypts, each producing around 300 cells per day, every day of the mouse's life. This remarkable yield is ultimately sustained by a small population of stem cells (four to six cells) that reside at the base of each crypt. The progeny of mammalian intestinal stem cells (ISCs) does not differentiate immediately, but rather it is amplified by cell division during a process of continuous upward migration along the crypt axis. Around 150 undifferentiated cycling progenitor cells (or transient amplifying [TA] cells) occupy the crypt length. Progenitor cells divide with fast kinetics (about 1 division every 12 hr). Cell-cycle arrest and functional differentiation occur as migrating TA cells reach the upper part of the crypt. Three differentiated cell types populate the intestinal tract: mucosecreting, enteroendocrine, and absorptive cells. The small intestine contains an additional secretory cell type, Paneth cells, which localizes at the base of the crypts intermingled with the ISCs (Barker et al., 2008b; Van der Flier and Clevers, 2008).

The location and the precise identity of mammalian ISCs have been controversial issues due to the lack of specific marker genes and assays to study their properties (Barker et al., 2008b). In a decisive work, Hans Clevers and colleagues have recently identified bona fide gastrointestinal stem cells at the bottommost positions of the stomach, small intestine, and colon crypts (Barker et al., 2007). ISCs in the intestinal tract can be specifically recognized by the expression of *Lgr5*, a Wnt target gene that codifies for an orphan G protein coupled receptor of unknown function. Lgr5+ ISCs are multipotent, divide approximately once every day, and are capable of regenerating the intestinal epithelium for long periods (>12 months). The recent finding that Lgr5 also labels hair follicle epithelial stem cells (Jaks et al., 2008) may suggest that this gene is a general marker for Wnt-activated stem cells. More recently, *Bmi1*, a member of the polycomb family of chromatin remodelers, has been proposed as another ISC marker gene in the proximal small intestine (Sangiorgi and Capecchi, 2008). Bmi1+ ISCs also exhibit long-term regeneration potential but are located just above Paneth cells, around position +4 from the crypt base. This distinct localization may suggest that Lgr5 and Bmi1 identify different ISCs in the crypts, although a proper comparison of the features of both populations has not been performed yet.

The presence of regenerative cells in the intestine of some arthropods was reported more than century ago, but it has not been until recently that *Drosophila* intestinal stem cells were revealed. In a series of seminal articles, the Spradling and Perrimon labs described the nature of adult *Drosophila* midgut ISCs (mgISCs) (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006), while the Hartenstein group identified ISCs in the hindgut (hgISCs) (Takashima et al., 2008). The lining of the fruit fly midgut (i.e., the equivalent of the mammalian small intestine) is a pseudostratified epithelium composed mostly of large polyploid cells of absorptive function (enterocytes or EC) intermingled with two less abundant populations of diploid cells, enteroendocrine (EE) cells and midgut stem cells (mgISCs) (Figure 1B). mgISCs sit in basal position relative to the rest of epithelial cell types and show a wedge-like morphology that to some extent resembles that of mouse Lgr5+ cells (Ohlstein and Spradling, 2006, 2007). Unlike in mammals, mgISCs are the only known cell type in the posterior midgut that proliferates, as their progeny is not further amplified. Upon cell division, the descendants of mgISCs regenerate the stem cell pool and/or become quiescent progenitor cells (known as enteroblasts or EB cells), which ultimately differentiate to EC or EE cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). A detailed analysis of midgut structure is only available for the posterior region. Thus, some regional variation in numbers and properties of intestinal cells may be present along the anterior-posterior axis of the intestine, as it occurs in mammals.

The *Drosophila* midgut arises from the endoderm, like the mammalian intestinal tract. However, the hindgut, which is the anatomical equivalent of the mammalian large intestine, develops from ectodermal imaginal discs. In adult flies, ectodermal-derived tissues such as those of external structures (cuticle, wings, eyes, etc.) remain postmitotic, yet the epithelium of the hindgut retains regeneration capacity. Unlike in mammalian crypts or in the fly midgut, renewal in the hindgut epithelium does not occur from the basal cell layer toward the lumen but rather along the anterior-posterior axis (Takashima et al., 2008) (Figure 1C). Hindgut intestinal stem cells (hgISCs) can be specifically labeled by a GFP reporter of the Jak/Stat pathway. They reside in a narrow compartment around the hindgut-midgut boundary (the spindle cell zone [SCZ]), whereas their progeny migrates toward the posterior end (Takashima et al., 2008) (Figure 1C). In a fashion that resembles the transient amplifying compartment of the mammalian crypts, hgISC descendants

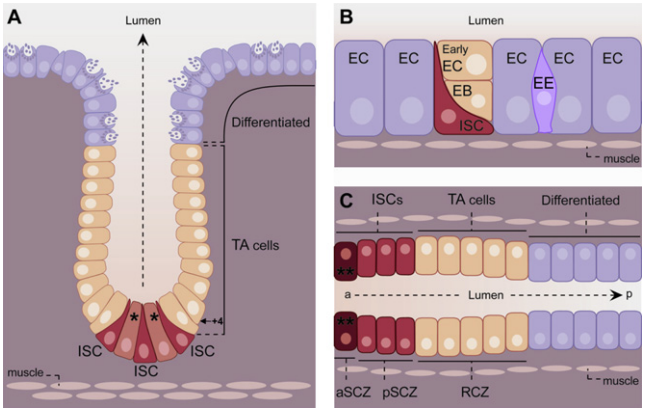

Figure 1. Similarities between Mammalian and *Drosophila* Intestinal Epithelium

Organization of a mammalian colon crypt (A) and the *Drosophila* midgut (B) and hindgut (C) epithelium. In each panel, ISCs are depicted in red, progenitor cells in light orange, and differentiated cells in blue. Cells in (A) labeled with asterisks represent Paneth cells in the small intestine or other secretory types in the colon. In (C), ISCs marked with two asterisks are Wg-secreting cells that localize in the anterior region of the spindle zone. Arrow indicates the direction of migration and cell renewal, i.e., from the base toward the lumen in crypts, or in an anterior (a)-posterior (p) fashion in the hindgut. ISC, intestinal stem cells; TA, transient amplifying; EB, enteroblast; EC, enterocyte; EE, enteroendocrine cells; aSCZ and pSCZ, anterior or posterior spindle cell zone, respectively; RCZ, round cell zone.

continue to proliferate vigorously while traveling through the round cell zone (RCZ). Apparently, transient amplifying hindgut cells proliferate with faster ratios than hgISCs, yet the precise kinetics of division remains unknown. The onset of cell-cycle arrest and functional differentiation occurs as transient amplifying cells reach the most posterior region of the hindgut.

Maintaining ISCs in Mammals and Flies: The Wnt/Wg Connection

A wealth of data have put forward an essential role for Wnt signaling in the maintenance of mammalian ISCs (Van der Flier and Clevers, 2008). Mice engineered to lack Wnt signaling in the intestinal epithelium lose the crypt progenitor compartment. Conversely, constitutive activation of the Wnt pathway results in a massive expansion of crypt progenitor/stem cell numbers and the onset of intestinal tumorigenesis. The majority of human colorectal cancers (CRCs) are initiated by mutations in the tumor suppressor gene *Apc*, which switches on the Wnt pathway in a constitutive fashion. Remarkably, most Wnt target genes induced by APC mutations in intestinal tumors are physiologically expressed in crypt ISCs and/or in transient amplifying progenitor cells (Van der Flier et al., 2007). A prime example is *Lgr5*, which was originally identified as a Wnt signaling-induced gene in CRC cells (Barker et al., 2007). Recently, the tumor-initiating potential of different crypt cell populations has been assessed (Zhu et al., 2008; Barker et al., 2008a). These studies have revealed that deletion of the *Apc* gene in mouse ISCs triggers tumor formation with high efficiency, whereas transient amplifying progenitor cells or differentiated cells are relatively resistant to Wnt-driven transformation. Thus, it appears that some specific features of ISCs are required to initiate CRC. Overall, these data have led to the notion that Wnt signaling sustains the expression of the crypt ISC gene program, which upon mutational activation of Wnt pathway is constitutively imposed on tumor-initiating cells (i.e., on stem cells and perhaps also on early progenitor cells) (van de Wetering et al., 2002; Barker et al., 2007, 2008a; Van der Flier and Clevers, 2008).

The picture that emerges from genetic analysis of *Drosophila* ISCs is similar to that of mammalian ISCs. mgISCs also receive Wg, which is apparently secreted by muscle cells immediately underneath the epithelium (Lin et al., 2008). Blockage of Wg secretion results in a small but consistent decrease of mgISC numbers and proliferation. Indeed, inhibition of Wg downstream signaling in mgISCs results in partial loss of stem cells due to reduced proliferation and premature differentiation. Authors observed two outcomes from the activation of Wg signaling in mgISCs depending on the experimental setting: mgISCs null clones for GSK3β/shaggy, a negative regulator of the Wg pathway, increase proliferation of mgISCs without disturbing their differentiation. On the other hand, overexpression of Wg or of constitutive active β-catenin/armadillo resulted in accumulation of stem and progenitor-like cells. Thus, physiological Wg signaling contributes to mgISC self-renewal, yet further analysis is required to dissociate its specific effects on proliferation and stem cell specification. In the *Drosophila* hindgut, hgISCs receive paracrine Wg secreted from specialized stem cells that reside in the most anterior compartment of the SCZ (aSCZ) (Takashima et al., 2008). Interestingly, genetic manipulation of Wg levels suggests that, while Wg promotes overall proliferation and survival of stem and progenitor cells, high Wg levels may be specifically required to promote stemness in hgISCs. Thus, the current model proposes that cells residing near the aSCZ (the Wg source) retain ISC features that would be gradually lost as they migrate away from this niche (Takashima et al., 2008).

The discoveries made in the *Drosophila* intestine may provide insight to help solve several long-lasting questions about the role of Wnt signaling in mammalian crypts. First, it is not well understood if physiological Wnt signaling is only activated in ISCs or also in transient amplifying progenitor cells. Whereas the highest accumulation of nuclear β-catenin is observed in cells at the bottommost positions of the crypts, lower levels of β-catenin can also be detected in the nucleus of cells located above ISC positions (van de Wetering et al., 2002). Furthermore, direct β-catenin/Tcf target genes show different expression patterns along the crypt axis, including those that are ISC restricted (e.g., *Lgr5*) or present in ISCs plus early progenitors (e.g., *Myc*), Paneth cell restricted (e.g.. Cryptdins), or expressed in a decreasing gradient from the crypt base throughout the transient amplifying compartment (e.g., *Ephb2*) (Van der Flier et al., 2007). Second, despite many efforts, the location of the source of Wnt ligands required for mammalian ISC maintenance remains unknown. Several Wnt family members are expressed in crypt epithelial cells, which may suggest autocrine or paracrine signaling (Van der Flier and Clevers, 2008), but no clear candidate to mediate effects on self-renewal has been revealed to date. This missing information is essential to understand how the position of ISCs is defined and what mechanisms operate to create the stem cell niche. Third, it is largely unknown what specific stem cell properties are codified within the β-catenin/Tcf target gene program. Wnt signaling promotes the

proliferation of CRC cells by inducing the expression of c-Myc and other mediators of cell division (van de Wetering et al., 2002). This function is also essential for the perpetuation of ISCs and transient amplifying progenitor cells. Conditional deletion of *Myc* in the crypts results in reduction of the number and size of progenitor cells (Muncan et al., 2006). Likewise, *Apc* mutant cells lacking *Myc* are not capable of initiating intestinal tumors in mice (Sansom et al., 2007). However, it is unclear if Wnt signaling plays additional roles in promoting stemness and long-term regeneration potential besides acting as a promitogenic stimulus for ISCs.

Diversifying ISC Progeny via Notch Signaling

As it often occurs in other developmental systems, Notch acts in concert with Wnt signaling to control the fate of ISCs and their descendants. In the mammalian intestine, progenitor cells become committed toward one of the differentiated cell types as they migrate along the crypt axis. This process implies at least one binary decision, i.e., to become an absorptive cell (enterocyte) or to differentiate toward a secretory phenotype (goblet, enteroendocrine, or Paneth cells). This decision depends critically on Notch signaling. Mice with a conditional deletion of RBP-J, the transcription factor that partners with Notch intracellular domain, showed a massive conversion of all intestinal epithelial cell types to secretory cells (mainly to goblet cells) (van Es et al., 2005). Conversely, expression of a constitutively active form of Notch receptor in the intestine blocks the generation of mucosecreting and enteroendocrine cells (Fre et al., 2005). In addition, modulation of Notch signaling has profound effects on the proliferation capacity of intestinal cells. In the absence of RBP-J function, all crypt proliferative cells (presumably including ISCs) became cell cycle arrested differentiated goblet cells (van Es et al., 2005), whereas enforced Notch activity expands the proliferative compartment (Fre et al., 2005). Accordingly, it has been shown recently that Notch1 and Notch2 repress the levels of the cell-cycle inhibitors $p27^{kip1}$ and $p57^{kip2}$ in the crypts (Riccio et al., 2008). The defects induced by *Rbp-j* loss-of-function are phenocopied by treatment of mice with drugs that inhibit the activity of γ-secretase, a protease required for Notch receptor activation (van Es et al., 2005). Remarkably, γ-secretase inhibitors are capable of transforming APC mutant tumor cells into cell-cycle-arrested differentiated mucosecreting cells despite constitutive activation of the Wnt pathway (van Es et al., 2005). This finding has encouraged many pharmaceutical companies to explore the possibilities of pharmacological inhibition of Notch signaling for treatment of CRC. Overall, these results indicate that Notch signaling is required to sustain self-renewal in crypt and cancer cells. Of note, inhibition of Wnt signaling in mice not only results in loss of progenitor cells but also halts differentiation toward the goblet and enteroendocrine lineages (Van der Flier and Clevers, 2008). Thus, it appears that a combination of Notch and Wnt signaling specifies different cell types in the intestinal epithelium of mice: Wnt+ Notch− crypt cells are forced toward goblet or enteroendocrine differentiation, Wnt− Notch+ cells are converted to enterocytes, whereas Wnt+ Notch+ cells maintain an undifferentiated phenotype. Downstream of Notch and Wnt, a cascade of transcription factors further diversify the progeny of stem cells and control their differentiation (reviewed in Van der Flier and Clevers, 2008). It is not well understood, though, how differential Notch and Wnt signaling is regulated at the spatial and temporal levels in crypt cells to ensure the right proportions of each cell type. Similarly, whether subsequent commitment in the lineage hierarchy (e.g., mucosecreting versus endocrine differentiation) may also be a Notch/Wnt-driven process remains unclear.

In *Drosophila* intestinal cells, Notch signaling is used in a fashion similar to that of their mammalian counterparts, albeit with some notable differences. Midgut ISCs, EBs, and early-differentiated cells all express Notch receptor, but Delta, a ligand for Notch, is only present in mgISCs (Ohlstein and Spradling, 2006, 2007). Lack of Delta in the mgISCs produces tumor-like expansion of cells that resemble either ISCs or EE cells. On the other hand, constitutive Notch activation in mgISC results in reduced proliferation and premature differentiation toward an EC fate (Ohlstein and Spradling, 2007; Micchelli and Perrimon, 2006). These observations suggest that Delta expression in mgISC induces Notch signaling in daughter cells, which in turn triggers the differentiation process. Therefore, lineage selection and differentiation do not seem to rely on supporting cells or surrounding tissues. Instead, mgISC themselves play an active role in supplying signals and instructing daughter cells. Selection between absorptive or endocrine lineage depends on differential Notch signaling. mgISCs containing high levels of cytoplasmatic Delta-rich vesicles induce high levels of Notch activity in daughter cells, which will in turn promote EC differentiation. In contrast, mgISCs that express low levels of Delta will specify their progeny to become EE cells (Ohlstein and Spradling, 2007). How the amount of Delta-rich vesicles in mgISCs is regulated is not yet clear, and neither is what controls the relative numbers of enteroendocrine versus adsorptive cells. Further work is also required to understand the hierarchy between Notch and Wg/WNT signaling pathways. Finally, it's important to point out that Notch signaling seems to have opposite outputs regarding self-renewal in the mammalian and *Drosophila* intestinal epitheliums. Genetic or pharmacological blockade of Notch activity in mice causes the depletion of the progenitor cell compartment by promoting differentiation (van Es et al., 2005). On the contrary, reduction of Notch signaling in *Drosophila* intestine induces overgrowth of mgISCs due to impaired differentiation (Ohlstein and Spradling, 2006, 2007; Micchelli and Perrimon, 2006). Concordantly, excess of Notch signaling amplifies the number of progenitor cells in mammalian crypts (Fre et al., 2005), whereas it induces the differentiation of *Drosophila* mgISC (Ohlstein and Spradling, 2006, 2007; Micchelli and Perrimon, 2006).

Drosophila Gut as a Model for Understanding the Relationship between Age, Stress, Cancer, and Stem Cells

In adult organisms, tissue homeostasis is maintained by the balance between removal of dead cells and production of new cells by adult stem cells. As organisms age and/or agents such as oxidative stress induce tissue damage, the tight control on proliferation and differentiation on the stem cell population is lost, favoring the conditions for age-related diseases, such as cancer. Indeed, it has been hypothesized that cancer and aging may be considered stem cell diseases, cancer having been proposed to be the result of growth-promoting mutations within a given stem cell and aging representing the natural exhaustion

and depletion of the stem and progenitor pools. How adult stem cells respond to age or injury is still not clear, but new data recently obtained in *Drosophila* have shed some light on the effect of age or injury in the stem cell population of the fly gut (Biteau et al., 2008; Choi et al., 2008; Amcheslavsky et al., 2009).

Age or treatment with tissue-damaging agents disrupts the basal membrane organization of the intestine and induces accumulation of clusters of abnormally differentiated cells (Choi et al., 2008; Biteau et al., 2008). In aged flies, a marked increase in JNK activity induces overproliferation of mgISCs, perhaps as a response to a damaged basement membrane or to compensate for the loss of differentiated cells. Concomitantly, JNK activity also disturbs Delta/Notch expression resulting in cells that retain Delta but at the same time show activity of the Notch signaling reporter Su(H)-lacZ (Biteau et al., 2008). Apparently, aberrant Delta/Notch signaling impedes the differentiation of EBs toward ECs, leading to the accumulation of polyploid EC-like cells that do not express EC markers. Interestingly, only one cell within the aberrantly differentiated cluster expresses Delta but not Su(H)-lacZ, thus retaining normal ISC identity (Biteau et al., 2008). Several issues need further investigation: first, it is unclear how the aberrant expression of Delta in ISC descendants affects the differentiation toward the EC lineage or why the number of EE cells remains mainly unaffected in aged flies. Second, more in-depth analysis is needed to unveil the signals that promote increased ISC division upon damage. In this regard, the Ip laboratory has recently provided evidence that systemic levels of insulin regulate mgISC division both in normal conditions and in flies treated with tissue-damaging agents (Amcheslavsky et al., 2009). Unfortunately, the authors did not investigate whether insulin signaling is modulated upon damage or if the damaged intestine may somehow promote the release of insulin-like peptides (DILPs) by brain cells. Third, upon injury with chemical agents such as DSS, the mammalian intestinal mucosa is repaired very efficiently. In *Drosophila* it is not clear whether the observed response will also help to regenerate the damaged intestine or, on the contrary, if we are simply witnessing an aberrant process of tissue degeneration with no consequences for repair.

The cancer aging model proposes that tumor suppressor mechanisms carrying an anticancer function may inadvertently contribute to aging by causing stem cell attrition. In the adult intestine of *Drosophila*, widespread activation of Notch signaling activity seems to be important to restrict ISC proliferation induced by JNK. But this restriction comes at a price, as the resulting abnormal Delta/Notch signaling is responsible for defects in the differentiation of EB cells and thus for the decline in intestinal function (Biteau et al., 2008). In mammals, c-*jun* and TCF4/β-catenin seem to interact in vivo in a JNK-dependent manner, and this interaction regulates intestinal tumorigenesis by integrating JNK and APC/β-catenin pathways (Nateri et al., 2005). In *Drosophila* JNK appears to be involved in aging and stress damage responses. Open questions are whether JNK plays a role in mammalian intestinal homeostasis and more importantly whether a putative crosstalk between JNK and Wg could mediate the relationship between aging and cancer. These intriguing and relevant issues could now be addressed in *Drosophila* and mouse, taking advantage of the new tools reviewed here.

ACKNOWLEDGMENTS

We thank Xavier Franch-Marro and Elena Sancho for proofreading and critical discussion. We apologize for omitting many important contributions due to lack of space and strict limits in the number of references.

REFERENCES

Amcheslavsky, A., Jiang, J., and Ip, Y.T. (2009). Cell Stem Cell *4*, 49–61.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., and Clevers, H. (2007). Nature *449*, 1003–1007.

Barker, N., Ridgway, R.A., van Es, J.H., van de Wetering, M., Begthel, H., van den Born, M., Danenberg, E., Clarke, A.R., Sansom, O.J., and Clevers, H. (2008a). Nature. Published online December 17, 2008. 10.1038/nature07602.

Barker, N., van de Wetering, M., and Clevers, H. (2008b). Genes Dev. *22*, 1856–1864.

Biteau, B., Hochmuth, C.E., and Jasper, H. (2008). Cell Stem Cell *3*, 442–455.

Choi, N.H., Kim, J.G., Yang, D.J., Kim, Y.S., and Yoo, M.A. (2008). Aging Cell *7*, 318–334.

Fre, S., Huyghe, M., Mourikis, P., Robine, S., Louvard, D., and Artavanis-Tsakonas, S. (2005). Nature *435*, 964–968.

Jaks, V., Barker, N., Kasper, M., van Es, J.H., Snippert, H.J., Clevers, H., and Toftgard, R. (2008). Nat. Genet. *40*, 1291–1299.

Lin, G., Xu, N., and Xi, R. (2008). Nature *455*, 1119–1123.

Micchelli, C.A., and Perrimon, N. (2006). Nature *439*, 475–479.

Muncan, V., Sansom, O.J., Tertoolen, L., Phesse, T.J., Begthel, H., Sancho, E., Cole, A.M., Gregorieff, A., de Alboran, I., Clevers, H., and Clarke, A.R. (2006). Mol. Cell. Biol. *26*, 8418–8426.

Nateri, A.S., Spencer-Dene, B., and Behrens, A. (2005). Nature *437*, 281–285.

Ohlstein, B., and Spradling, A. (2006). Nature *439*, 470–474.

Ohlstein, B., and Spradling, A. (2007). Science *315*, 988–992.

Riccio, O., van Gijn, M.E., Bezdek, A.C., Pellegrinet, L., van Es, J.H., Zimber-Strobl, U., Strobl, L.J., Honjo, T., Clevers, H., and Radtke, F. (2008). EMBO Rep. *9*, 377–383.

Sangiorgi, E., and Capecchi, M.R. (2008). Nat. Genet. *40*, 915–920.

Sansom, O.J., Meniel, V.S., Muncan, V., Phesse, T.J., Wilkins, J.A., Reed, K.R., Vass, J.K., Athineos, D., Clevers, H., and Clarke, A.R. (2007). Nature *446*, 676–679.

Takashima, S., Mkrtchyan, M., Younossi-Hartenstein, A., Merriam, J.R., and Hartenstein, V. (2008). Nature *454*, 651–655.

Van der Flier, L.G., and Clevers, H. (2008). Annu. Rev. Physiol. Published online September 22, 2008. 10.1146/annurev.physiol.010908.163145.

Van der Flier, L.G., Sabates-Bellver, J., Oving, I., Haegebarth, A., de Palo, M., Anti, M., van Gijn, M.E., Suijkerbuijk, S., van de Wetering, M., Marra, G., and Clevers, H. (2007). Gastroenterology *132*, 628–632.

van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Batlle, E., Coudreuse, D., Haramis, A.P., et al. (2002). Cell *111*, 241–250.

van Es, J.H., van Gijn, M.E., Riccio, O., van den Born, M., Vooijs, M., Begthel, H., Cozijnsen, M., Robine, S., Winton, D.J., Radtke, F., and Clevers, H. (2005). Nature *435*, 959–963.

Zhu, L., Gibson, P., Currle, D.S., Tong, Y., Richardson, R.J., Bayazitov, I.T., Poppleton, H., Zakharenko, S., Ellison, D.W., and Gilbertson, R.J. (2008). Nature. Published online December 17, 2008. 10.1038/nature07589.