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Adult gastric stem cells and their niches

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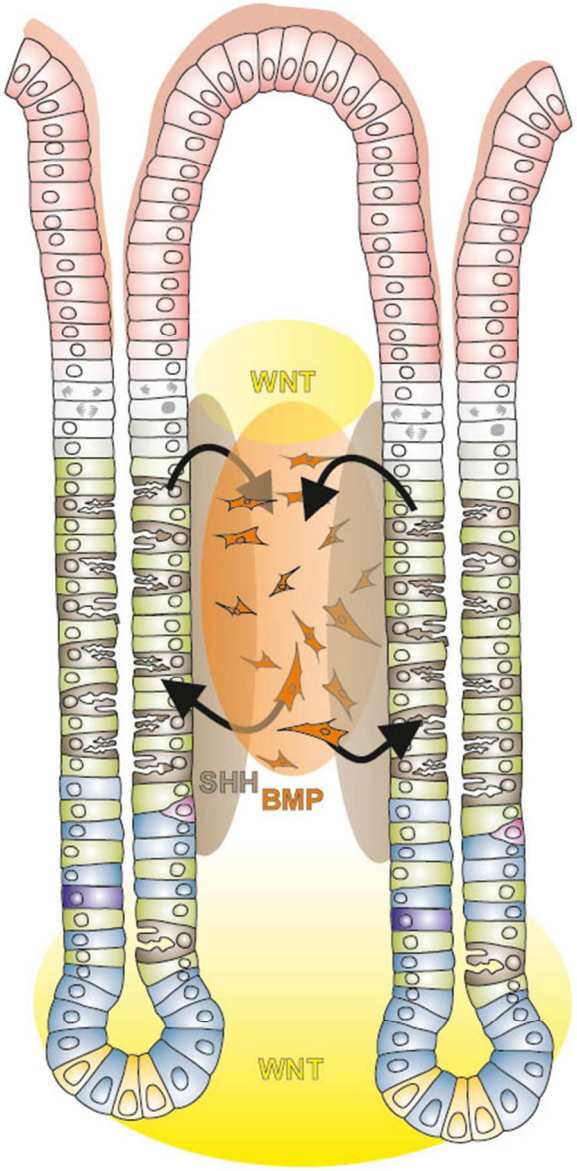

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Abstract

[Adult gastric stem cells replenish the gastric epithelium throughout life. Recent studies have identified diverse populations of stem cells, progenitor cells and even differentiated cells that can regain stem cell capacity, so highlighting an unexpected plasticity within the gastric epithelium, both in the corpus and antrum. Two niches seem to co-exist in the gastric unit: one in the isthmus region and the other at the base of the gland, although the precise features of the cell populations and the two niches ~~is~~ are currently under debate. A variety of gastric organoid models have been established, providing new insights into niche factors required by the gastric stem cell populations. Here we review our current knowledge of gastric stem cell populations, their markers and interactions, important niche factors, and different gastric organoid systems.]

[The gastric architecture]

[The stomach is a part of the gastrointestinal tract. While proximal esophagus and distal intestine have the form of a muscular tube, the stomach has a characteristic bean shape, designed to be loaded with the incoming food for primary digestion and disinfection [1]. The acidic gastric fluid kills incoming pathogens, denatures proteins and aids in the absorption of metals. In the corpus region, the amylases from saliva, as well as peptidases secreted from the glands of the corpus digest the

carbohydrates and proteins. Muscular movements disperse the food and coat it thoroughly in mucus, especially in the distal pyloric antrum, before it leaves the stomach through the pyloric sphincter into the intestinal duodenum [1,2].

The stomach architecture varies between species. In the human stomach, the squamous epithelium of the esophagus is followed by the glandular epithelium of the proximal cardia (Fig 1A). The murine stomach lacks the cardia, but has a large proximal region called the forestomach, which is lined by a ~~thin,~~ keratinized, squamous epithelium with a few cardiac type glands at the junction (Fig 1B). The mucosa of all other parts of the stomach is lined by a simple columnar epithelium with many tubular invaginations into the lamina propria that are termed gastric units. A murine corpus unit contains about 200 cells [3], while the much shorter antral unit contains about 45 cells [4]. The units are divided into four anatomical parts. From the opening, they are named as: (I) the pit (or foveola), populated by mucus-producing cells, (II) the isthmus, a fairly small region containing immature, proliferating progenitor cells (III) the neck and (IV) the base (Fig 1C). While the pit and the isthmus are relatively similar in all glandular gastric units throughout the stomach, the cellular composition of the neck and the base varies between the regions of the stomach, reflecting the specific functions of these regions [5]. Six differentiated cell types have been described: (1) pepsinogen-secreting zymogenic cells or chief cells (hereafter called chief cells), (2) acid-producing oxyntic or parietal cells (hereafter called parietal cells), (3) surface mucus cells, foveolar mucus cells or pit mucus cells (hereafter called pit mucus cells), (4) mucus neck cells, (5) enteroendocrine cells and (6) rare fibrillovesicular, brush, caveolated, or tuft cells (Fig 1D).

The cellular composition of a gland defines its function. In the mouse antrum, glands can either contain only mucus cells (pure mucus glands) or they can contain mucus cells and some parietal cells (mixed mucus oxyntic glands). The latter are mostly located onwards from the intermediate zone and then throughout the corpus [5]. In contrast, in humans some antral glands contain a few parietal cells~~parietal cells are distributed throughout the antrum~~, and there are also glands that contain chief cells in the antrum [6]. In the mouse and human corpus, typical oxyntic glands contain parietal cells, mucus cells and chief cells [5,6]. Enteroendocrine cells are dispersed throughout all glands, but the type of enteroendocrine cell varies, e.g. with gastrin-positive cells being highly abundant in the antrum [6].

Using pulse-chasing with radioactive or modified nucleoside labeling, early studies detected rapid proliferation in the isthmus in adult mice. Labeled cells appeared to migrate bidirectionally up to the pit and down to the gland. The pit region has a rapid turnover of about 3 days [7], while chief cells at the gland exhibit impressive lifespans of at least 6 months [8]. These studies thus demonstrated that the gastric epithelium constantly renews itself in adulthood. The adult stem cells that fuel this constant need for new cells were thought to reside in the tissue itself. In this review, we will discuss our current understanding of gastric stem cells, their markers and niches. There are excellent recent reviews about gastric development [9] and tumorigenesis [10–12], so these topics will not be discussed in detail here.]

[Gastric stem cells]

[Prior to the identification of gastric stem cell markers, early studies using electron microscopy that aimed to identify candidate stem cells focused on characteristics of cells that were known from

embryonic stem cells, such as abundant free ribosomes, scant organelles and a large reticulated nucleolus [13]. These studies identified cells in the isthmus of gastric glands that were particularly undifferentiated and it was hypothesized that these may be the stem cells. Less differentiated cells were also identified by electron microscopy at the base of the glands, and it was deduced from their characteristics that these may be progenitors of the chief cell lineage [13,14]. Analysis of specific transgenes, mutations in mitochondrial DNA or strain-specific genes in chimeric mice showed that almost all gastric glands are monoclonal, meaning that they originate from a single stem cell that won the competition of functional stem cells within the gland [15–17].

With the development of genetic lineage tracing methods, clonal analysis of single stem cells became possible. In general, lineage tracing by the activation of a reporter gene for colorimetric or fluorescent signal tracks all the progeny of a given cell up to the end of the mouse's life span (reviewed by Kretzschmar and Watt [18]). The first lineage tracing studies in the mouse stomach used transgenic beta-galactosidase (*LacZ*) under the ubiquitously expressed Rosa26 promoter for visualization together with random chemical mutagenesis. When mutagenesis randomly silenced *LacZ* expression, unstained clones appeared within otherwise uniformly LacZ-stained epithelium. Histological analysis of these clones together with marker genes of different gastric lineages showed that clonal glands contain all markers of the four main lineages of the stomach, demonstrating that a common progenitor or stem cell exists [19].]

[Markers of gastric stem cells]

[Following studies then used genetic lineage tracing from specific cell types to attempt to pinpoint the gastric stem cell. For this, an inducible Cre recombinase (e.g. *CreERT*) gene is brought under the control of the promoter of a putative stem cell marker in order to activate the Cre recombinase only in the putative stem cell population. When crossed with the *Rosa26-STOP-LacZ* reporter mouse, where the expression of LacZ is inhibited by a genetic roadblock flanked by LoxP sites (a floxed "STOP" cassette), the LacZ reporter will lose the STOP cassette only in the target cell type upon induction of Cre activity (e.g. with administration of tamoxifen). Importantly, after labeling, this irreversible genetic trait is inherited by all daughter cells, allowing fate mapping of the progeny of a targeted single cell (reviewed by Kretzschmar and Watt [18]). While this technique generally has a low impact on the normal function of most tissues, strong Cre activity can lead to parietal cell loss and activity therefore needs to be well controlled [20], especially as parietal cells play a specific role in the organization of the gastric architecture (see below).

This technical development has induced a spurt in gastric stem cell marker discovery over the past decade. Two schools of thought have emerged, with one discovering more and more markers for stem cells in the base or neck of the gland and the other following up on the isthmus stem cell hypothesis. This debate is reminiscent of the recent scientific controversy about the "+4 stem cell" and the "crypt base columnar stem cell" in the intestine, before the field reached agreement on a unifying theory (reviewed by Clevers [21]).

There is a set of markers which label populations of stem cells at the base or neck region of the glands. The gene leucine-rich-repeat-containing G-protein-coupled receptor 5 (*Lgr5*) marks continuously cycling stem cells at the base of intestinal crypts as well as antral glands [22,23]. Lineage tracing from *Lgr5-Egfp-IRES-CreERT2* mice showed that these cells rapidly replenish antral

glands in homeostasis and that monoclonal glands are maintained throughout the life of the mouse [23]. Single molecule fluorescence *in situ* hybridization (FISH) confirmed expression of *Lgr5* in all glands of the antrum [24]. Contrary to the belief that stem cells divide asymmetrically to preserve one (pre-determined) stem cell and one amplifying daughter cell, destined to differentiate, multicolor lineage tracing and mathematical model fitting showed that *Lgr5*-positive cells divide symmetrically to produce two daughter cells which are phenotypically equal. In fact it is the neutral competition for niche space which permits only those daughter cells that stay within the niche to remain as stem cells [24,25].

Interestingly, *Lgr5* lineage tracing was not observed in the adult corpus. However, this may be due to a poorly understood technical constraint of the specific mouse model used, because endogenous *Lgr5* expression could still be detected when using a second mouse model which expresses a fusion of the diphtheria toxin receptor (*DTR*) and enhanced green fluorescent protein (*Egfp*) under the control of the *Lgr5* locus [26]. An additional stem cell marker which was identified because it is co-expressed with *Lgr5* in the intestine, the gene tumor necrosis factor receptor superfamily member 19 (*Tnfrsf19*) which encodes the protein TROY, is highly expressed at the base of corpus glands. *Tnfrsf19*-positive cells in the corpus express *Lgr5* based on microarray and qRT-PCR analyses [27]. Tracing from *Tnfrsf19*-positive cells produced the monoclonal glands typical of tracing from stem cells and co-stainings demonstrated that traced cells contain all cell types of the corpus epithelium, indicating that TROY-marked cells at the base of the glands may be stem cells. Surprisingly, post-FACS expression analysis and immunohistochemistry demonstrated that *Tnfrsf19*/TROY marks differentiated chief cells at the bottom of the glands. Tracing from another chief cell marker, the transcription factor basic helix-loop-helix family member a15, termed *Bhlha15* or *Mist1*, also produced traced monoclonal glands. This indicates that *Tnfrsf19*/TROY marks differentiated chief cells at the bottom of the glands that can act as stem cells, making this a prime example of the remarkable plasticity of the system. Additional experiments suggested this slowly proliferating cell type to be a reserve stem cell population that aids the rapid repair of the epithelium after damage [27].

Another population of reserve stem cells was marked with the gene villin (*Vil1*) at the base and neck of antral glands. In comparison to *Lgr5*-positive cells, *Vil1*-positive cells are rare and cycle only slowly. When mice were treated with the pro-inflammatory cytokine interferon-gamma (IFNγ), tracing showed monoclonal glands. The authors therefore concluded that *Vil1* marks rare quiescent stem cells that most likely do not contribute to homeostasis but may act as a reservoir of stem cells for repair after injury [28].

Just above the *Lgr5*-expressing zone, two other populations of stem cells that contribute to normal homeostasis were identified using lineage tracing. The gastrin receptor cholecystokinin B receptor (*Cckbr*) marks a population that has some overlap with the *Lgr5*-positive population. Ectopic expression of progastrin increased the overlap between the populations, again indicating cell plasticity within the system [29]. Sex determining region Y (SRY)-box 2 (*Sox2*) marks rare cycling cells just above but mutually exclusive from the *Lgr5*-expressing zone, with the relationship of this population to the *Cckbr*-labeled population currently unknown [30].

Recently, Hayakama and colleagues revived the hunt for the elusive isthmus stem cell [31]. They used tracing from the *Mist1* locus, the abovementioned gene that marks chief cells and is necessary

for their development [32,33]. In this new study, *Mist1* was found to mark not only chief cells at the base of the gland but also other cells in the isthmus. Clonal expansion assessed by multicolor tracing was predominantly observed in the isthmus region. Depletion of dividing cells by 5-FU markedly reduced the number of traced cells, whilst ablation of Lgr5-positive cells using *Lgr5-DTR:EGFP* mice [26] did not. The authors thus concluded that *Mist1* also marks an unidentified isthmus stem cell population as well as chief cells. This new result unveiled the complicated nature of gastric corpus gland organization. Further analysis will be needed to define the true identity of the hidden isthmus stem cell population as well as the progenitor role of Troy- and/or *Lgr5*-expressing chief cells, also bearing in mind the co-existence of two stem cell populations (slow and fast cycling stem cells) in the gut epithelium and other organs (reviewed by Li and Clevers [34]). Gastric glands may be another example of this, possessing two different stem cell populations with varying levels of plasticity, longevity and cycling rate.]

[The gastric stem cell niche]

[An inspiring study recently investigated how sonic hedgehog (*Shh*) and bone morphogenetic protein (*Bmp*) shape the gut [35]. In the intestine, *Shh* is expressed uniformly by the epithelium. The intestinal architecture with repeating invaginations creates areas at the tip of the villi where SHH is highly concentrated in the mesenchyme and consequently induces Bmp. Thus, the mere shape of the tissue alone leads to the formation of a BMP gradient, with high BMP activity at the tip of the villi. As the BMP signal becomes high at the villus cluster, it restricts the localization of the intestinal stem cells to the base of the invaginations, away from BMP activity. [35].

In the stomach, however, the expression pattern of *Bmp* differs markedly from that of the gut, because here SHH is not secreted by all cells but only by parietal cells. Thus, *Bmp4* is expressed in the parietal cell-rich area, which is the neck region of the gland [36] (Fig. 2). This is of central importance because SHH is a major regulator of gastric epithelial proliferation and differentiation. Pharmacological inhibition of SHH induced proliferation in the epithelium [36], whilst the deletion of parietal cells inhibits chief cell differentiation [37,38] and is the first step on the road to gastric cancer (reviewed by Mills and Sansom[10]). Taken together, this suggests that SHH signaling by parietal cells forms the negative mold for the stem cell zones, which can be established only outside of the parietal cell/SHH/BMP4 zones, i.e. in the isthmus and the base of the gland.

In gastric development, hedgehog (*Hh*) signaling also counteracts the activity of Notch [39], but whether or not this mechanism plays a role in adulthood is unclear. Notch signaling is known to be important in intestinal stem cell maintenance and acts as a molecular switch during cellular differentiation to determine whether a differentiating cell enters the adsorptive lineage (Notch on) or the secretory lineage (Notch off). In the stomach, however, Notch signaling affects all cells, most likely due to the lack of absorptive cells. Judging from the expression of the Notch target gene hairy and enhancer of split-1 (*Hes1*), Notch is active specifically in the isthmus region [40]. The Samuelson group recently showed that Notch is also active at the base of antral glands by using a previously reported but very elegant genetic model [41]. In this system, *CreERT2* is fused to the Notch receptor and so only able to enter the nucleus after Notch activation and cleavage. Notch activation by ectopic expression of Notch1 intracellular domain1 (*Nicd1*) in antral *Lgr5*-positive stem cells induces proliferation that leads to antral polyps [41]. Kim and Shivdasani showed that activation of Notch by the expression of *Nicd1* under the promoter of the H+/K+ ATPase of parietal cells, *Atp4b*, reprograms

parietal cells or committed progenitor cells into functional stem cells [40]. Administration of the Notch antagonist dibenzazepine (DBZ) abolished proliferation in antral and corpus glands and led to an increase of mucus cells [40]. Thus, active Notch is important for stem cell maintenance and proliferation.

The third pathway regulated by *Hh* is fibroblast growth factor 10 (*Fgf10*) signaling. During lung and pituitary gland development, *Hh* restricts *Fgf10* expression [42,43]. In the developing stomach, *Fgf10* is expressed in the mesenchyme and knockout of either *Fgf10* or its receptor *Fgfr2* results in defects in gland formation [44,45], but its role in adulthood is unclear. In summary, hedgehog signaling is a major node in gastric development and stem cell maintenance due to its regulation of niche factors such as BMP, Notch, and FGF10.

While Wnt family members are of central importance in the intestine, their role in the stomach is not fully understood. During stomach development, the level of Wnt reporter expression is clearly reduced in the stomach region, an effect which is controlled by underlying BarH-like homeobox 1 (*Barx1*)-positive mesenchymal cells [46]. This down-regulation of Wnt activity is suggested to be essential for defining stomach fate during early gut patterning. On the other hand, transcriptome analysis of laser-captured isthmus cells following parietal ablation suggested that *Wnt* is expressed in isthmus stem cells [47]. Some Wnt target genes, including the stem cell markers *Lgr5* and *Troy*, are expressed at the base of antral as well as corpus glands [23,27]. Experimental activation of the Wnt pathway in gastric stem cells leads to gastric cancer in animal models [23,31,48] and genetic mutations in the Wnt pathway are also detected in human gastric cancers, although to a much lower extent than in colon cancers [49,50]. Taken together, there is a wide range of evidence pointing to the importance of Wnt signaling in the stomach (Fig. 2) and it would therefore be very interesting to see the expression pattern of Wnt ligands in the stomach by an advanced new technology such as single molecule FISH [51].

Epidermal growth factor (EGF) signaling also plays a major role in gastric cell proliferation. Treatment of rats with EGF stimulated epithelial growth throughout the gastrointestinal tract [52]. In Ménétier's disease, excessive EGF signaling (especially via EGF family member transforming growth factor alpha, TGFa) induces foveolar hyperplasia [53].

Gastrin (Gast) is secreted by specialized enteroendocrine cells in the antrum, the G-cells. Progastrin is the incompletely cleaved precursor of gastrin. Both bind to the CCK2 receptor (encoded by the *Cckbr* gene), which is predominantly expressed on parietal cells and enterochromaffin-like (ECL) cells in the corpus. However, gastrin receptors are also expressed in neck progenitors or stem cells in the gland base [29,54]. Ectopic expression of gastrin induces proliferation in the gastric mucosa [55], and the addition of gastrin to gastric cell lines also induces proliferation [54]. Interestingly, progastrin or gastrin treatment increased the overlap between the *Cckbr*-positive population and the *Lgr5*-positive population of cells, suggesting that it may stimulate an interconversion [29].

While the past decade has provided essential information about gastric stem cell niche factors, we are still searching for the exact identity of cells providing the niche, although there seems to be a tightly regulated interplay between the mesenchyme and the epithelium. Parietal cells in the epithelium act as a linchpin as they secrete SHH to instruct the mesenchyme. This has obvious clinical implications, as tissue damage that leads to loss of parietal cells (such as *Helicobacter pylori [H. pylori]* infection or experimental chemical damage) causes tissue rearrangement characterized by

the expansion of a metaplastic lineage co-expressing mucus neck and chief cell markers (reviewed by Mills and Sansom [10]). Recent evidence has suggested that endothelial cells and gastric innate lymphoid cells secrete Wnt ligands and may form a perivascular niche. Infection with *H. felis* increased the expression of *Wnt5a* in the stroma. Depletion of innate lymphoid cells reversed this effect and also reduced the metaplastic foci caused by *H. felis* infection [31]. Depletion of macrophages reduces metaplasia after parietal cell loss [56], and the SHH-induced myeloid population is also required for metaplasia progression [57]. Thus, immune cells may provide an additional environment for gastric de-differentiation and progenitor cell metaplasia.

Here we have reviewed important niche factors for the gastric epithelium in development, adult tissue homeostasis and disease. Despite their importance, the identity of the cells providing these niche factors remains elusive. Finding the exact localization of gastric stem cells will help to unveil the required cellular niche for the gastric stem cell. The importance of the niche factors Wnt, EGF, FGF-10, GAST, and inhibition of BMP is further highlighted by the *in vitro* reconstitution of the stem cell niche, which has led to the development of the long-term adult gastric stem cell organoid culture.]

[Creating the gastric stem cell niche *in vitro* - growing organoids]

[While the pluripotent stem cell field had surged forward following the discovery of the Yamanaka factors, most adult stem cells were still widely believed to be refractive to long-term culture until a seminal study by Sato and colleagues demonstrated the opposite. In this study, authors seeded single, sorted *Lgr5*-positive intestinal stem cells into extracellular matrix and added the essential niche factors such as the BMP inhibitor noggin (NOG), EGF and the LGR5-ligand and Wnt agonist R-spondin (RSPO). This matrix and growth factor combination allowed the stem cells to divide and grow into 3-dimensional (3D) multicellular structures [58]. As they contained all differentiated cell types of the intestinal epithelium, as well as stem cells and progenitors, this new *in vitro* structure was termed an “organoid” to emphasize its striking resemblance to the *in vivo* counterpart tissue. Intestinal organoids are long-lived and can be expanded in culture for more than a year [58].

Based on this technique, culture conditions for murine gastric organoids were established using previous knowledge regarding niche factors (Fig 3). By mimicking the natural stem cell niche, researchers enabled the gastric stem cell to grow and expand *in vitro*. The murine gastric organoid depends on EGF, NOG and RSPO, as with the intestinal organoid, plus the additional growth factors WNT3a, FGF-10 and GAST [23]. Human gastric organoids additionally require inhibition of the TGFβ-pathway [59]. Other studies have used Nicotinamide and/or p38 inhibitor for human gastric organoids [60,61], as these were previously used for human intestinal cultures [62].

Both mouse and human gastric organoids can be maintained and expanded long-term while retaining the capacity to generate differentiated cell types, thus demonstrating the longevity and multipotency of the cultured gastric stem cells. Progenitors and differentiated cells cooperate to autonomously create the domains of the gland and the pit – a phenomenon called self-organization. [58,59]. This is quite surprising, given the fact that the niche factors are presented uniformly in culture. It is surmised that epithelial organization must generate an intrinsic local gradient of important signals in order to properly pattern the structure under this uniform environment. Cultures from antral as well as corpus glands differentiate into mucus pit cells (especially after

WNT3a-withdrawal), mucus neck cells, chief cells and rare enteroendocrine cells [23,59,61]. Parietal cells are present in the corpus organoids directly after isolation [61,63] but lost during passaging [59,63]. This is likely due to the fact that the growth factor cocktail (containing WNT3a and NOG) does not support or prevents differentiation to parietal cells. This can also be inferred from observations in mice, where experimental activation of the Wnt pathway as well as expression of noggin in parietal cells leads to parietal cell loss [48,64]. Gastric organoids differentiate to almost pure pit cell cultures under Wnt-withdrawal, supporting the hypothesis that there may be a Wnt gradient in the gastric unit similar to the one in the intestine [23,27,59,60].

A second generation of organoid cultures derived from pluripotent stem cells (PSCs) has been particularly useful to study gastric development (Fig. 3). McCracken and colleagues used either induced PSCs (iPSCs) or embryonic stem cells (ESCs) and generated human antral organoids following a precisely orchestrated modulation of signaling pathways. First, they differentiated PSCs towards definitive endoderm by activating nodal using activin and BMP4. Then, posterior foregut fate was induced by addition of Wnt, FGF-4, NOG and retinoic acid (RA). To direct the cells towards antral specification, spheres were placed into extracellular matrix and RA, NOG and EGF were added, as these factors induce growth, differentiation and glandular folding of the gastric antral epithelium. When levels of EGF were reduced, enteroendocrine cells became more abundant, suggesting that EGF may play a specific role in enteroendocrine development [65]. A second PSC-derived model used a less defined route of stepwise differentiation but arrived at impressively functional parietal cells. They first grew embryoid bodies from ESCs. In a floating dish, these cultures usually differentiate into definitive endoderm. In a second step, they then mixed the cultures with mesenchyme, plated them into adherent culture conditions, activated SHH and inhibited Wnt signaling. This yielded stomach primordium-like spheres. In the third step, spheres were placed into extracellular matrix and supplemented with FGF10, Wnt, NOG and RSPO. The resultant stomach organoids then formed fully mature chief and parietal cells. Acid secretion can be stimulated in these organoids using histamine [66].]

[Organoids as a surrogate stem cell assay and models for gastric disease]

[Organoids hold much promise for basic research as well as for clinical applications. For example, since the organoids allow observation of the progeny of single sorted cells *in vitro*, organoid formation now serves as a new surrogate stem cell assay (Fig. 4). Indeed, murine cells positive for the aforementioned stem cell markers, i.e. *Lgr5*, *Tnfrsf19*, *Cckbr* and *Mist1*, were all sorted respectively and were able to form organoids and differentiate into the cells of the stomach *in vitro* [23,27,29,31]. Also, human (unmarked) sorted single cells could form gastric organoids *in vitro*, providing experimental evidence for the existence of human gastric stem cells [59].

Organoids are also used to study human diseases *in vitro*. For example, *H. pylori* can be microinjected into gastric organoids and induces an inflammatory response. Interestingly, this response depends on the cell types present in the organoids [59,67]. A bacterial virulence factor, cytotoxicity associated gene A (CagA), is translocated into epithelial cells in organoids or derived from organoids [60], where it interacts with the met proto-oncogene (c-MET) [61] and induces proliferation [61,65].

Perhaps the current most relevant clinical application so far is the generation of organoids from a range of patients, so building "living biobanks" [68,69], as organoids can be grown from healthy human tissue as well as from metaplastic or tumor tissues [59,62,68]. In contrast to previous efforts to generate lines from patient tumors, tumor organoids can be generated from patients with a much higher success rate. Just like organoids from the healthy epithelium, tumor organoids can be expanded, are long-lived, can be frozen and thawed and are amenable to a wide range of standard laboratory techniques. In a colon cancer organoid study, genotypes known from previous large scale genome analysis are well represented within the colon cancer organoid biobank. Large scale drug screening in organoids confirmed known associations of genotypes and reactions to drugs [68], allowing the hope that organoids can in the future be used to bridge the gaps between drug discovery and patient trials, and to establish methods for personalized treatment.]

[Conclusion and outlook]

[Using lineage tracing and additional evidence from organoid technology, researchers have identified several marker genes for gastric stem cells. Probably more markers are yet to be uncovered, especially a specific marker for the isthmus stem cell. The considerable controversy regarding the interrelation of different populations awaits a unifying theory. Technical advances, such as intravital imaging, single molecule FISH, single cell sequencing and the development of a novel Cre line that marks all cells or a specific cell type in the stomach will be useful to advance our knowledge of gastric stem cells, their plasticity and the role of specific niche factors. The establishment of gastric organoids opens up a new window into *in vitro* disease modeling and cancer biology. Gastric cancer affects a huge population, with one million new cases every year, and it is one of the leading causes of cancer death. Also, gastric ulcers and chronic gastritis significantly affect quality of life and can pose a serious health threat. Thus, a detailed understanding of this relatively understudied organ is of the utmost importance. The recent advances in the stomach field, represented by the establishment of gastric organoid cultures and the identification of novel stem cell populations, provides a firm foundation to investigate the pathophysiology of the stomach. Current research focuses on the complete hierarchy and organization of gastric epithelium, the identification of cancer-initiating cells, epithelial-pathogen interactions and immune-epithelial communications.]

Figures and Captions

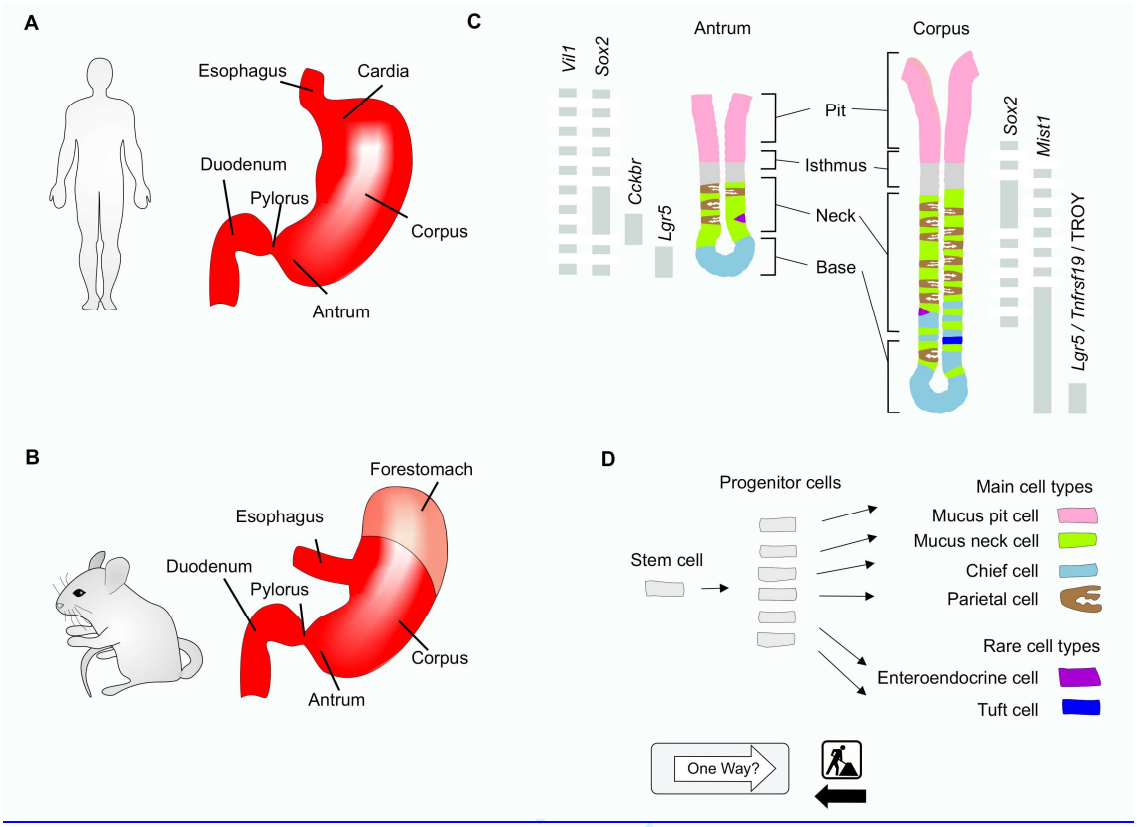

Figure 1: Gastric architecture, cell types and stem cell markers. A and B: The general architecture of the human (A) or murine (B) stomach and the different gland types present. C: Organization of antral and corpus glands and associated stem cell markers. Grey bars mark the regions of expression of the respective marker. D: Flow chart detailing differentiation from stem cells via (committed) progenitor cells to differentiated cells. Main cell lines in the stomach are mucus pit cell (pink), mucus neck cell (green), chief cell (blue) and parietal cells (brown). Rare cell lines are enteroendocrine cells (purple) and tuft cells (dark blue). The general dogma postulates that differentiation is a one way street from stem cells towards terminally differentiated cells. However, recent studies have shown a remarkable plasticity, due to which differentiated cells could re-gain stem cell capacity displayed especially in cases of damage and repair.

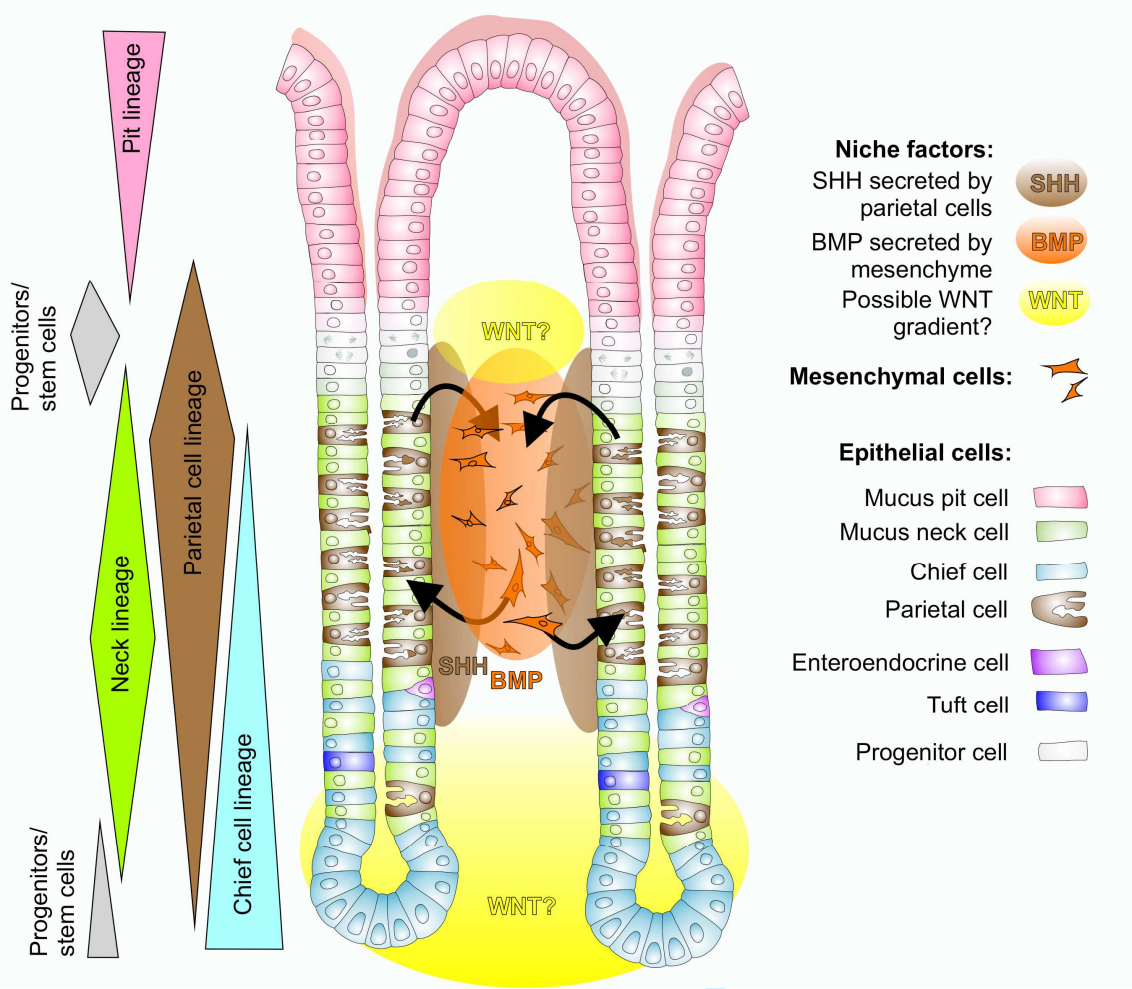

Figure 2: Distribution of cell types and niche factors in the gland. Bars on the left side depict the abundance of the cell types. Circles between the two corpus glands depict proposed gradients of niche factors. The abundance of SHH-secreting parietal cells in the neck region leads to the formation of a gradient of SHH. ~~This~~ SHH then regulates mesenchymal BMP expression. There is only indirect data for the proposed WNT gradients depicted here. It's possible source ~~The source of other niche factors, such as WNT,~~ is not clear.

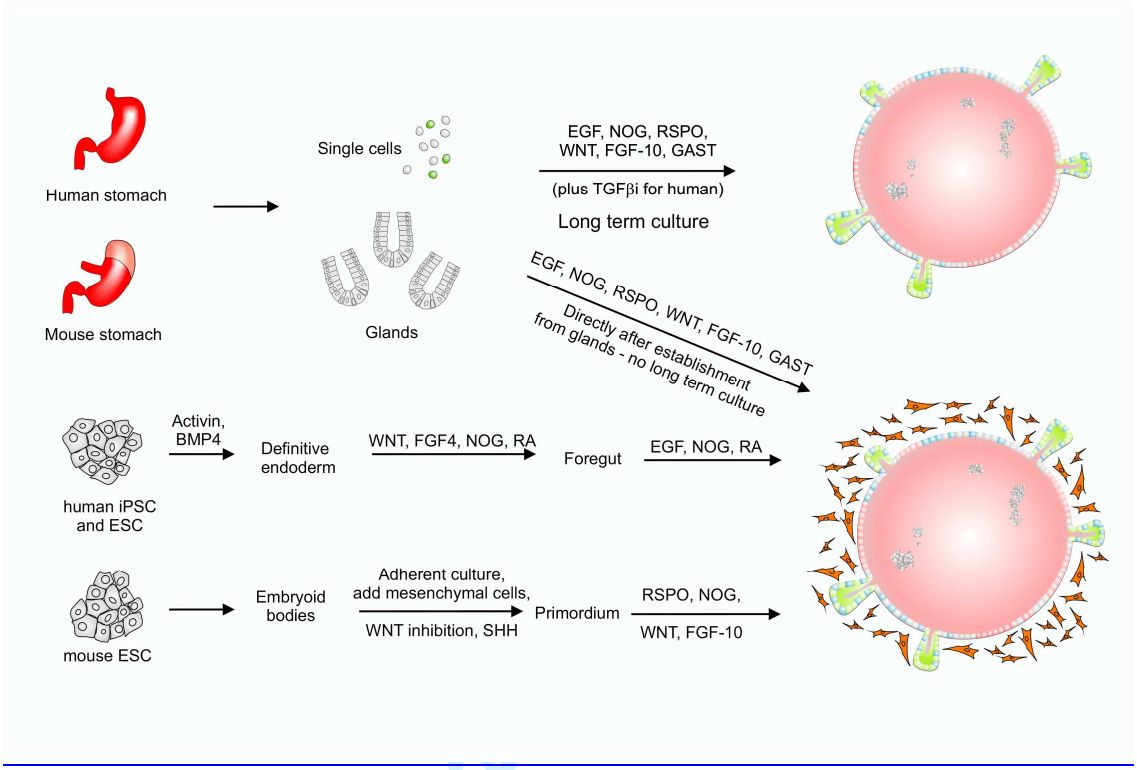

Figure 3: Overview of organoid culture systems. Tissue resident adult stem cells (ASC)-derived organoids from mouse and human can be maintained long-term in culture. Over time, they become purely epithelial. PSC-derived organoids recapitulate the developmental steps that lead to the formation of gastric tissue and contain both mesenchyme as well as epithelium, but cannot be expanded long-term

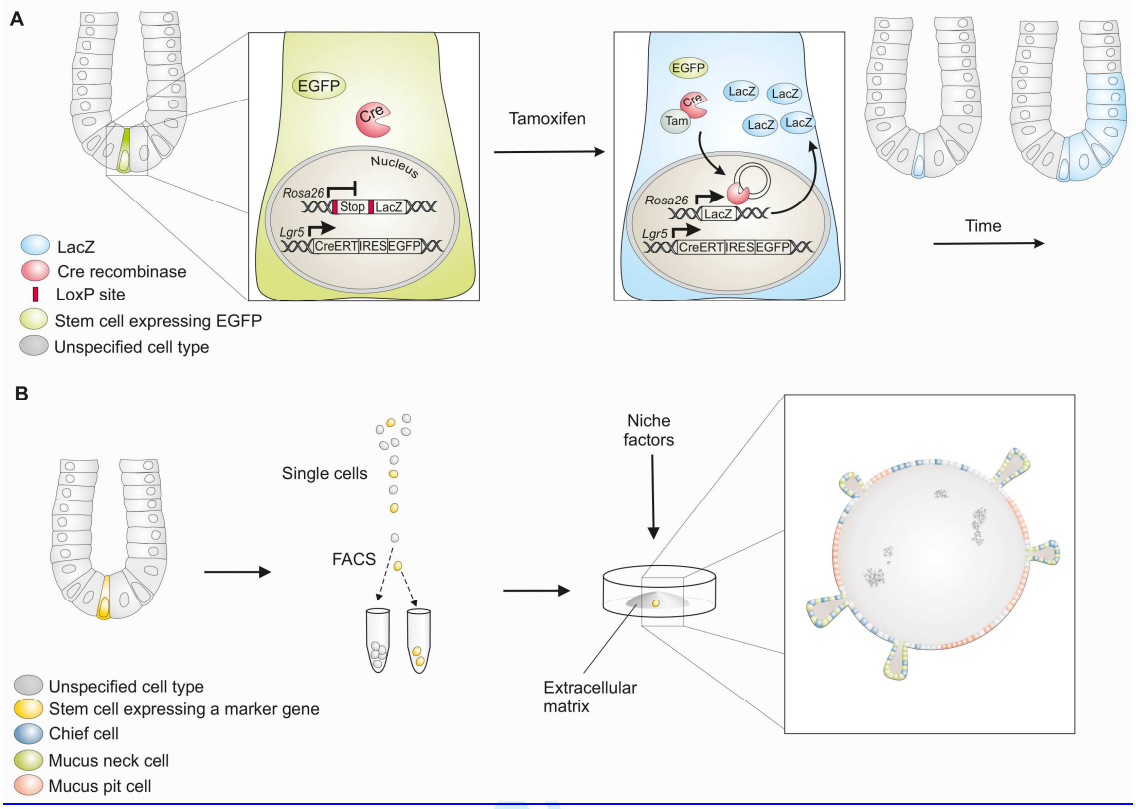

Figure 4: Techniques to assay stem cell capacity. A: Lineage tracing enables *in vivo* fate mapping of specific cells. Lineage tracing from stem cells results in characteristic ribbons of clonally expanded cells. B: Organoids can generate the lineages of a specific tissue epithelium *in vitro* and thus serve as an *in vitro* surrogate model to test stem cell capacity.

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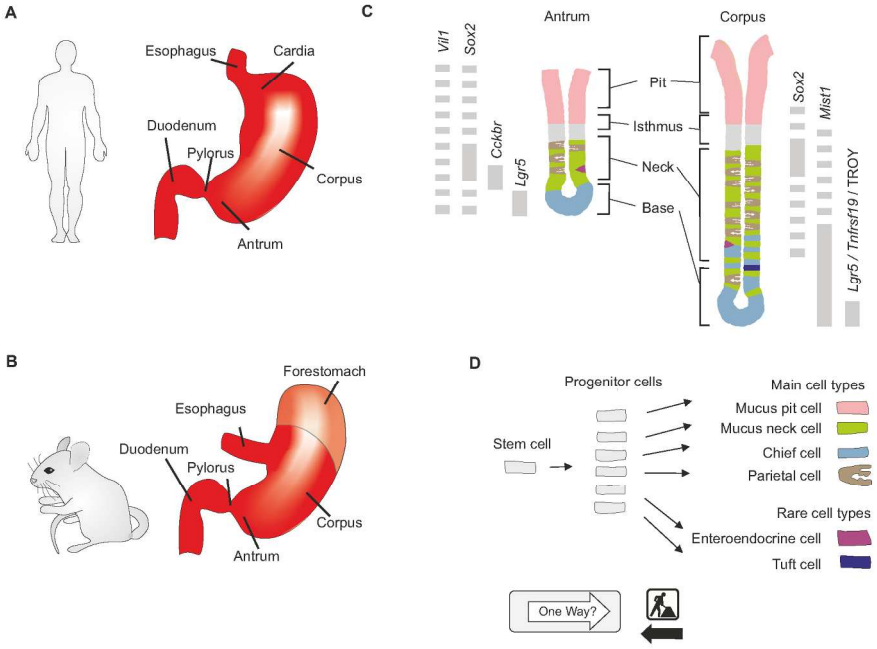

Figure 1

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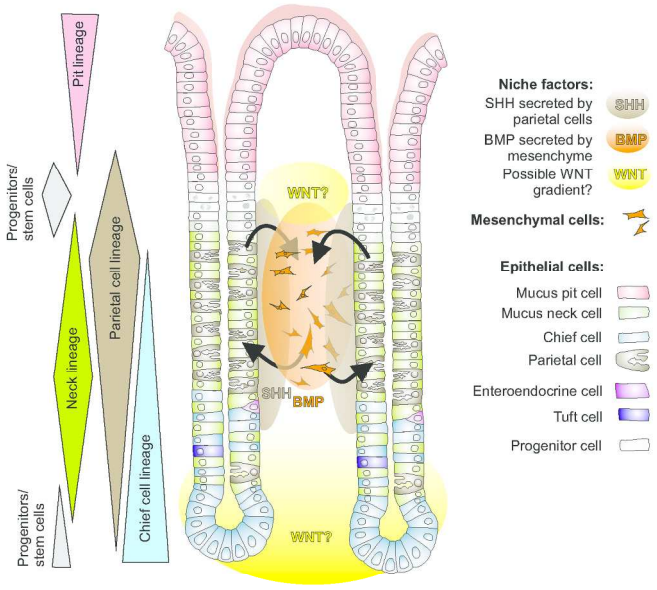

Figure 2

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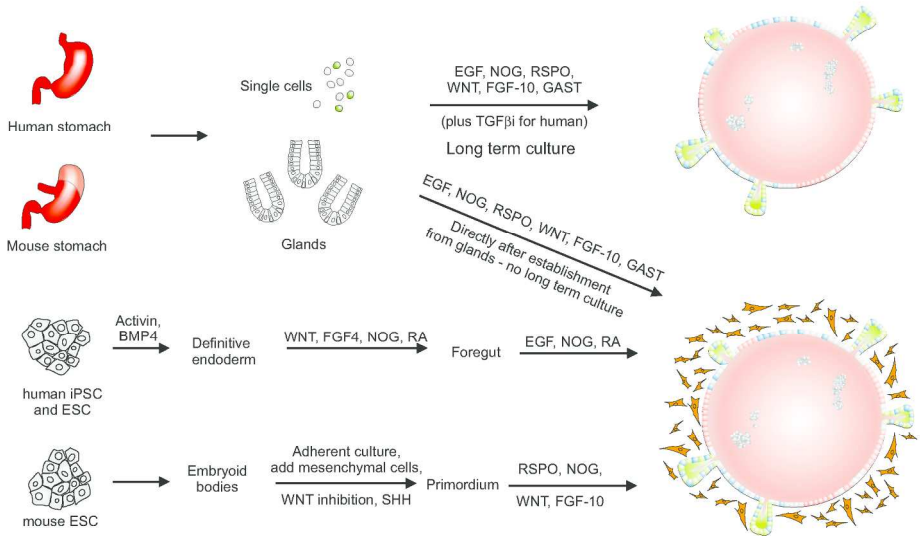

Figure 3

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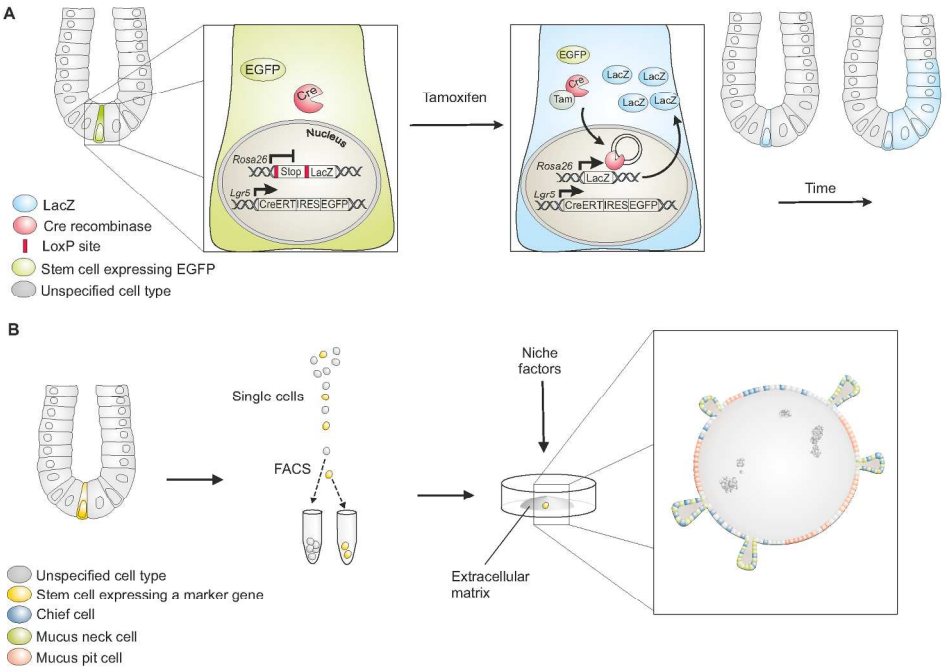

Figure 4

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