Multipotential stem cells in adult mouse gastric epithelium

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Bjerknes, Matthew, and Hazel Cheng. Multipotential stem cells in adult mouse gastric epithelium. Am J Physiol Gastrointest Liver Physiol 283: G767–G777, 2002.—Previous studies of chimeric animals demonstrate that multipotential stem cells play a role in the development of the gastric epithelium; however, despite much effort, it is not clear whether they persist into adulthood. Here, chemical mutagenesis was used to label random epithelial cells by loss of transgene function in adult hemizygous ROSA26 mice, a mouse strain expressing the transgene lacZ in all tissues. Many clones derived from such cells contained all the major epithelial cell types, thereby demonstrating existence of functional multipotential stem cells in adult mouse gastric epithelium. We also observed clones containing only a single mature cell type, indicating the presence of long-lived committed progenitors in the gastric epithelium. Similar results were obtained in duodenum and colon, showing that this mouse model is suitable for lineage tracing in all regions of the gastrointestinal tract and likely useful for cell lineage studies in other adult renewing tissues.

parietal cells; zymogen cells; mucous cells; committed progenitors; cell lineage tracing; small intestine; colon; ROSA26 mice

THE GASTRIC EPITHELIUM is organized into numerous gastric units. Each unit is composed of three main structural elements: a planar surface epithelium, tubular invaginations of the surface epithelium called pits, and tubular extensions of the pits called glands. The simple epithelium forming gastric units is continuously renewed. Most cell production occurs near the junction between the pit and its associated gland, in a region called the isthmus. The epithelium is composed of several cell types derived, it is proposed, from a multipotential stem cell population found in the isthmus, possibly among the immature-looking, granule-free cells of the isthmus (15, 17, 18, 22, 25, 29). Different cell types follow different migratory paths from the isthmus. In the corpus of the stomach, the mucus-secreting pit cells migrate up to the pit and surface, whereas cells in the zymogenic lineage migrate down into the glands. Parietal, enteroendocrine, and caveolated cells migrate in both directions (15, 17, 20, 22–25, 29). In the antrum, units are similarly organized but consist mostly of mucous cells, although parietal and enteroendocrine cells are also found in some antrum units (18, 27, 30).

A lineage model has been proposed both for corpus and for antrum units based on circumstantial morphological and cell kinetic evidence. Some of the granule-free cells located in the isthmus are proposed to act as multipotential stem cells giving rise to prepit, preparietal, and preneck cells, which then act as progenitors committed to their respective cell lineages (22, 25, 30). However, there is no direct evidence in the adult stomach for multipotential stem cells. Nor is it known whether any of the committed progenitors are long lived, as is the case in the small intestinal epithelium (6).

Studies of chimeric mammals (chimeric either as a result of random X-chromosome inactivation during development or as a result of mixing blastomeres from different mouse strains) show that most gastric units are monoclonal by adulthood, although they need not have started out that way (33, 35, 36, 44, 45). Thus, at some stage during development, there are single cells capable of generating all epithelial lineages. However, we cannot conclude from such results that multipotential stem cells exist in the adult gastric epithelium, because it remains plausible, for example, that the multipotential cells are lost during development but leave behind a series of committed progenitors to maintain the adult epithelium. Similarly, although transgenic mouse studies of the effects of parietal cell ablation (10, 31, 41) or of signal-transduction pathways (7, 26, 32) indicate regulation of gastric progenitor populations, they provide only suggestive evidence for adult multipotential stem cells and various committed progenitor populations. A direct demonstration of the persistence of a common stem cell population into adulthood is still lacking.

In the adult small intestine and colon, direct evidence for the existence of multipotential stem cells has been obtained by inducing somatic mutations in one of a variety of endogenous genes and then examining the clones of cells derived from such mutated cells (6, 14, 19).
The db-1 locus works well for small intestine (6, 48) but suffers from normally variegated expression in stomach and parts of the colon (unpublished observations; see also Ref. 45), making it unsuitable for lineage tracing in these tissues. The enzyme glucose-6-phosphatase has been used for similar studies both in colon and in small intestine (14, 38), but these mice are not commercially available and have not been applied to the stomach. Here, we introduced the use of a well-characterized strain of transgenic mice for lineage tracing and used it to establish the presence of multipotential stem cells and committed long-lived progenitors in the gastric epithelium.

We used chemical mutagenesis of a transgene to label a cell and its progeny. Transgenic mice expressing the bacterial gene for β-galactosidase (lacZ) are available from Jackson Laboratories as the ROSA26 strain. They were originally constructed by infecting embryonic stem cells with a retrovirus promotor trap construct (13). One of the resulting lines has one copy of lacZ inserted into the genome (13, 49). The associated mouse promotor drives β-galactosidase expression in all adult tissues tested, including the gastrointestinal tract. These mice were originally generated as a byproduct of a program to look for genes with interesting expression patterns but have since been used principally in transplantation or chimeric mouse experiments as markers for tissue of specific origin. To apply these mice to lineage tracing, we took advantage of the fact that mice hemizygous for lacZ contain only one transgene allele. Thus cells incurring inactivating mutations in the single lacZ allele (for example, a point mutation introducing a stop codon) will lose β-galactosidase activity and will not stain for the enzyme. If the mutated cell is a progenitor, all of its progeny will inherit the nonfunctional allele and hence will also be unstained and thus distinguished from neighboring stained cells. This allows clones of related cells to be identified.

MATERIALS AND METHODS

Isolated epithelium from stomach, duodenum, and descending colon obtained from male C57BL/6J mice was stained overnight at 37°C for β-galactosidase (40) to determine the background level of intrinsic galactosidase activity in these tissues. The background level of intrinsic galactosidase activity was negligible using this procedure (data not shown).

N-ethyl-N-nitrosourea dosing and animals. N-ethyl-N-nitrosourea (NEU) was used as the chemical mutagen in this study. NEU (250 mg/kg) was administered intraperitoneally (6) to 7-wk-old male hemizygous ROSA26 mice. Groups of two animals each were killed 5, 10, 15 (3 animals), 20, 30, and 48 wk after NEU administration. Control animals (injected with vehicle only) were killed 10, 15, 25, and 42 wk after injection. Under anesthesia, the animals were perfused with 30 mM EDTA in PBS, and the epithelium from duode-
num, descending colon, and stomach was isolated by vibration (5) into 0.25% glutaraldehyde in PBS and fixed for 10 min. The isolated epithelium was stained overnight at 37°C for β-galactosidase (40).

We refer to unstained cells as mutant cells, clones of mutant cells as mutant clones, and gastric units containing a mutant clone as mutant units. Similarly, small intestine and colon crypts containing a clone of unstained cells are referred to as mutant crypts. To determine the fraction of mutant units in the stomach, an average of ~11,500 randomly selected gastric units from each animal was screened for mutant clones. Additional mutant units were then screened to allow more data to study the characteristics of mutant clones (an average of ~30 mutant units per animal were studied). To determine the fraction of mutant crypts in duodenum and colon, an average of ~6,500 and 8,900 randomly selected crypts from each animal were screened, respectively.

Identification of the four main mature gastric cell types with differential interference contrast microscopy. All mutant units were microdissected and mounted on slides to determine the clone composition and distribution using differential interference contrast (DIC) microscopy. Mature parietal and zymogen cells were easily recognized with DIC microscopy. Recognition of mucous and enteroendocrine cells required experience. Our ability to recognize these cells with DIC microscopy was initially confirmed using specific staining. Enteroendocrine cells were stained with antibodies specific for chromogranin A (DAKO Diagnostics Canada, Mississauga, Canada), whereas mucous cells were stained with Kasten’s fluorescent periodic acid Schiff (PAS) method (12).

Cell identification using specific gastric epithelial cell markers. Multiple labeling with specific gastric epithelial cell markers was used to confirm the presence of the four main mature gastric epithelial cell types in homogeneous units.

Fig. 2. Differential interference contrast photomicrographs showing the characteristic morphology of the four mature gastric cell types. A and B: parietal cells are readily recognized by their characteristic circumnuclear canaliculi (arrows). Also evident are abundant mitochondria in the cytoplasm. C and D: zymogen cells have basal nuclei (arrows) and many supranuclear secretory granules. E-H: mucous cells. E: mucous cells with basal nuclei (arrows) and aggregates of mucous granules (arrowheads) in the luminal aspect of the cells. F: mucinous nature of the luminal aggregate was confirmed by Kasten’s fluorescent periodic acid Schiff (PAS) method to stain mucin. G and H: low magnification images to show the location (arrow) of mucous cells within gastric units. Remnants of 2 gastric units are attached to the top of the unit. I-L: enteroendocrine cells. J and K: enteroendocrine cells are characterized by their prominent nuclei (arrows) and small granules. J and L: identity of enteroendocrine cells was confirmed by staining with antibodies specific for chromogranin A. L: 3 other enteroendocrine cells located in other focal planes are also visible. G and H are at same magnification; bar in H is 100 μm; remaining panels at same magnification as A, whose bar represents 10 μm.
(units in which all cells are unstained and hence represent a clone derived from a single stem cell, see RESULTS). Rabbit primary antibodies specific for chromogranin A, intrinsic factor, and H⁺,K⁺-ATPase α-subunit were used sequentially to label enteroendocrine, zymogen, and parietal cells, respectively. Peroxidase-conjugated donkey anti-rabbit secondary antibodies were used with a different enzyme substrate after each primary to differentiate between the three cell types.
(the deposit formed by the reaction product masks the antibody complexes, thereby minimizing interaction with subsequent antibodies). Mucous cells were demonstrated with alcian blue staining.

Briefly, homogeneous units were first treated with rabbit primary antibodies specific for human chromogranin A (1:400, DAKO Diagnostics) followed by peroxidase-conjugated donkey anti-rabbit antibodies (1:2,000, Jackson ImmunoResearch, West Grove, PA). A 3,3′-diaminobenzidine substrate kit (Vector Laboratories, Burlingame, CA) was used to yield a brown reaction product. The units were then processed with rabbit primary antibodies specific for human intrinsin factor (1:400, a gift from Dr. D. H. Alpers; see also Ref. 42) followed by a Vector SG substrate kit (Vector Laboratories) to yield a black reaction product. Next, the units were processed with rabbit antibodies specific for porcine H^+K^+-ATPase α-subunit (1:100, Calbiochem, San Diego, CA) followed by an 3-amino-9-ethylcarbazole substrate kit (Vector Laboratories) to yield a red reaction product. Finally, the units were treated for 30 min with 0.5% alcian blue in 2.5% acetic acid (pH 2.6) to stain the mucin in mucous cells blue (6).

RESULTS

lacZ expression in gastric units from ROSA26 mice. lacZ transgene product staining was observed in all cells in the vast majority of corpus and antrum units (Fig. 1). The staining density appeared to decrease slightly with cell maturity, due perhaps to increased numbers of intracellular organelles in the mature cell types (Fig. 1). Also contributing to a nonuniform appearance is the fact that units have a cylindrical geometry. Thus there is a double layer of cytoplasm in the more central portions of the unit, making it appear more densely stained. This effect is enhanced by the protrusion of parietal cells from the lateral surface of the unit (Fig. 1, E-G). There was little variation in staining intensity between units in a given animal; however, we did notice variation in staining intensity between animals (perhaps due to slight variation in duration of fixation). Clones of unstained cells are readily seen against this background of stained cells (see below).

Identification of the four main mature gastric cell types with DIC microscopy. Mature parietal and zymogen cells are easily recognized with DIC microscopy. Parietal cells are characterized by the presence of the distinctive circumnuclear canaliculi and the numerous surrounding mitochondria (21). Both organelles are evident under DIC microscopy (Fig. 2, A and B). Zymogen cells contain many characteristic supranuclear secretory granules that can easily be seen (Fig. 2, C and D) (21). Morphological recognition of mucous cells with DIC microscopy is more subtle, because individual mucous granules are not discernible; however, collectively, the mucous granules appear as a distinc-

Fig. 3. Differential interference contrast photomicrographs showing mutant units from mouse antrum (A, B), corpus (C-P), duodenum (Q), and colon (R). Mutant cells (i.e., cells that have lost function of the lacZ transgene) are unstained, and normal cells are stained blue. The gastric units shown were microdissected from neighboring units in epithelial sheets to improve photographic clarity. In mixed units, there is often the misleading impression of substantial variation in the staining intensity of the normal cells, compared with the range of staining intensity observed in units from control animals (Fig. 1), due primarily to the fact that there can be a double layer of stained cells in some portions of the mixed unit, whereas in other areas, there may be unstained cells underlying or overlying the stained cells making them appear more lightly stained than their neighbors. A: 2 mixed units containing a spanning clone [15 wk after N-ethyl-N-nitrosourea (NEU)]. The unit on the right is branching. B: homogeneously mutant unit (left) next to a mixed unit with a spanning clone (48 wk after NEU). C-E: mixed units showing partial clones extending progressively further down the units 5 (C, D) and 15 wk (E) after NEU. Partial clones contained >1 cell type; for example, in C, mucous cells (arrow) and parietal cells (arrowheads) are visible. F: branching mixed unit with a spanning clone (20 wk after NEU). G: mixed unit with a spanning clone next to a homogeneously unit (50 wk after NEU). They are probably daughters of a mixed unit that branched. H: mixed unit with a restricted clone containing only mucous cells (15 wk after NEU). I: mixed unit with a restricted clone containing only parietal cells (20 wk after NEU). J: mixed unit with a restricted clone containing only zymogen cells (30 wk after NEU). K: mixed unit containing a spanning clone and 2 residual stained zymogen cells (30 wk after NEU). L: mixed unit with residual stained zymogen cells in its base to the right of a normal unit (48 wk after NEU). M: branching mixed unit that likely will give rise to 2 mixed units (10 wk after NEU). N: branching mixed unit that will likely give rise to 1 homogeneous and 1 mixed unit (30 wk after NEU). O: homogeneously unit (the patch of stained cells near its top belongs to a neighboring normal unit) next to a branching mixed unit (15 wk after NEU). The 2 units are likely daughters of a mixed unit. P: branching mixed unit that will likely give rise to a homogeneous and a normal unit (5 wk after NEU). Q: homogeneously mutant duodenal crypt feeding mutant cells onto the villus (48 wk after NEU). R: homogeneously mutant and a normal crypt from colon (15 wk after NEU).
tive aggregate visible in the luminal aspect of the cells, consistent with description based on electron microscopy (21). This means of identification was initially confirmed with Kasten’s fluorescent PAS method to stain mucin (Fig. 2, E-H). Enteroendocrine cells are recognized by their prominent nucleus and basal collection of small granules (21; Fig. 2, I and K), as initially confirmed by staining with antibodies specific for chromogranin A (Fig. 2, J and L).

Classification of mutant clones in gastric units. Most mutant units contained a mixture of stained and unstained cells and are referred to as mixed units (Fig. 3A). Some mutant units contained only mutant cells and are referred to as homogenous units (Fig. 3B). Clones could be variously categorized. The following scheme proved useful for both corpus and antrum: 1) spanning clone; mutant cells distributed throughout the length of a unit, although not necessarily occupying the entire unit; thus all homogenous units and many mixed units contained spanning clones (Fig. 3, A, B, F, G, K, M, O); 2) partial clone; mutant cells distributed in the top one-third of a unit, and possibly lower, but not throughout the full length of a unit (Fig. 3, C-E); 3) restricted clone; mutant cell distribution does not include the top one-third of the unit or clones containing only a single mature cell type (i.e., mucous, parietal, or zymogen cells; Fig. 3, H-J).

Frequency of mutant units. In the gastric epithelium, NEU induced a significant increase in the frequency of mutant units compared with the background control frequency (Fig. 4). The average frequency of mutant units in control mice was 0.000217, whereas the average frequency in NEU-treated mice was 0.00136. There was no significant variation in clone frequency with time. The fraction of mutant units in control animals that were mixed was 0.89, whereas the remaining 0.11 were homogenous. About 0.56 of mutant units in controls contained spanning clones, whereas 0.22 contained partial and 0.22 contained restricted clones. There was no statistical evidence of change during the experimental period (from 10 to 42 wk of age) in the relative distribution of the types of clones in control animals, in contrast to the significant changes observed in the NEU-treated animals we now describe.

Dynamics of spanning and partial clones in NEU-treated animals. Most mutant units in the corpus contained either a spanning or a partial clone (Fig. 5A). The proportion of mutant units with spanning clones increased significantly with time (1.32% per week; P = 0.0000232), whereas the proportion of units containing partial clones decreased (−0.867% per week; P = 0.0009). The proportion of mutant units containing restricted clones seemed to slowly decrease with time (Fig. 5A), but the slope was not significantly different from 0 (−0.0045% per week; P = 0.06). Clonal expansion occurred more rapidly in the antrum than in the corpus. From 20 wk onward, only spanning clones were observed in antrum (Fig. 5B) in contrast to the corpus, where partial clones were still observed at 48 wk. Restricted antrum clones were occasionally observed, but their numbers were so sparse that we refrain from making detailed comments on them.

Dynamics of mixed and homogenous units in NEU-treated animals. With time the proportion of mixed units decreased while that of homogenous units increased significantly (0.7% per week; P = 0.001; Fig. 5C), although even at 48 wk, mixed units made up the majority of mutant units. It is important to note, especially at the later time points, that in a substantial fraction of mixed units, the only stained cells were mature cells (usually parietal or zymogen cells; Fig. 3, K and L). This suggests that in such units, all cells were clonally related with the exception of residual long-lived mature normal cells, which were not derived from the mutant stem cell and hence were stained. Figure 5E shows the result of combining into a single “homogenous” category the homogenous units and the mixed units whose stained cells were all mature cells. Note that by 48 wk, the majority of mutant units are of the homogenous type. A similar pattern was observed in the antrum (Fig. 5, D and F).

Homogenous units contain the four main epithelial cell lineages. Homogenous units containing the four main cell lineages (parietal, zymogen, enteroendocrine, and mucous cells) were observed using DIC, indicating that all four lineages were derived from a single common stem cell in the adult. The presence of the four lineages in homogenous units was subsequently confirmed by simultaneous lineage-specific staining (parietal, zymogen, enteroendocrine, and mucous cells were demonstrated by presence of H+-, K+-ATPase α-subunit, intrinsic factor, chromogranin A, and mucin, respectively; Fig. 6).

Branching mutant units. Branching mutant units (Fig. 3A, E, F, and M-P) were observed at all time points both in corpus and in antrum (Fig. 5, G and H). The branching rate of mutant units we observed is similar to the branching rate reported by others in normal mouse gastric units (35), indicating that mutation of the transgene does not affect the behavior of the
population. The proportion of branching mutant units appeared to decrease with time in the corpus (−0.2% per week; \( P = 0.048 \)), but the decrease in the antrum was not significant (−0.4% per week; \( P = 0.096 \)). The decrease is probably due to aging, because decrease in the rate of branching with age has been reported in normal mouse gastric units (35).

**Mutant crypts in the intestine.** Mutant crypts were readily detected in duodenum and colon of NEU-treated mice (Fig. 3, Q and R). The process of clonal purification is much more rapid in duodenum and colon than in stomach, so a majority of mutant crypts (an average of 94% of mutant duodenum and 95% of mutant colon crypts) was homogeneously mutant. As
a result, little of interest occurred in colon and duodenum during the course of our observations. Our purpose in reporting the results is to establish the applicability of this mouse model to the entire gastrointestinal tract. In duodenum, the average fraction of crypts containing a mutant clone was $0.006 \pm 0.0006$ in NEU-treated vs. $0.00017 \pm 0.00012$ in control mice. In colon, the average fraction of crypts containing a mutant clone was $0.004 \pm 0.00036$ in NEU-treated mice vs. $0.00014 \pm 0.00012$ in control mice.

**DISCUSSION**

We show that ROSA26 (13, 49) is a simple and broadly applicable model for lineage tracing in all regions of the adult mouse gastrointestinal tract. We have used it specifically to establish the presence of both multipotential stem cells and committed long-lived progenitors in the adult mouse gastric epithelium. 

*Adult gastric epithelium has multipotential stem cells.* We report the generation in adult mice of long-lived mutant clones containing the four main epithelial...
cell types (parietal, zymogen, enteroendocrine, and mucus; Figs. 3 and 6). These clones frequently expand to occupy entire gastric units. These observations directly demonstrate the existence of multipotential stem cells in the adult gastric epithelium. It might be argued that such units could have resulted from simultaneous mutations in two or more long-lived committed progenitor cells in the same unit and hence, that we have not demonstrated a common stem cell. However, given that transgene inactivation by mutagenesis in a given cell is random and independent of events in neighboring cells, the probability that the transgene is inactivated in two or more long-lived progenitor cells in the same unit should be very small compared with the probability of mutation of a single progenitor in the unit. This makes simultaneous mutation untenable as an explanation for our observations, because we observed far more long-lived clones containing multiple cell types (spanning or partial clones) than clones containing a single cell type (restricted clones; Fig. 5A).

With time, partial clones seem to convert into spanning clones. This conclusion is derived from the similar composition of the two clone types and the fact that their frequencies follow inverse time courses (units containing partial clones decrease in frequency with a corresponding increase in frequency of spanning clones; Fig. 5A). Thus it is likely that partial clones gradually expand to become spanning clones, probably through the slow downward migration of cells in the parietal and zymogen lineages. This suggests that the multipotential stem cells are located in the upper portions of the unit, a conclusion consistent with previous morphological observations localizing the putative stem cells, the granule-free cells, to the isthmus (22, 29).

Adult gastric epithelium contains long-lived committed progenitors. Multipotential stem cells are not the only long-lived progenitor population in the stomach. We observed clones containing only parietal, zymogen, or mucous cells (Fig. 3, H-J) as late as 48 wk after NEU administration, suggesting the presence of long-lived committed progenitors for each of these lineages in the stomach. Because mature gastric mucous cells normally live for only a few days (22, 23, 28, 29), our finding of persistent mucous-only clones indicates the existence of long-lived mucous progenitors. The situation regarding zymogen and parietal cells is less clear, however, because mature zymogen and parietal cells may live for hundreds of days (20, 24) making it difficult to specify bounds on the lifespan of the committed progenitor. However, the large number of cells contained in many of these clones, their extensive distribution along the axis of the unit, and the slow migration rate of these cell types (20, 22) suggest the existence of committed progenitors with prolonged proliferative capacity. There is precedence for committed long-lived progenitors in the gastrointestinal tract. In the small intestine, long-lived committed columnar and mucous cell progenitors have been documented (6).

Multistep accumulation of mutations by clones of long-lived progenitor cells is thought to be a mechanism leading to many cancers (4, 34, 37, 46). It is usually assumed that the multipotential stem cells are the only long-lived progenitor in the gastrointestinal epithelium and hence are the only population at significant risk of cancer. However, the long-lived committed progenitors we have observed could also be at risk of accumulating the mutations leading to cancer. Therefore, a characterization of the various long-lived committed progenitors might aid our understanding of specific forms of gastrointestinal cancer (1–3, 9, 11, 19, 39, 43, 47). Committed long-lived progenitors might also be targets, in addition to stem cells, for long-term gastrointestinal gene therapy (8). Finally, development of better means to protect the gut from the damaging effects of radiation and chemotherapy may benefit from an understanding of the subtleties of the various progenitor populations and their regulation.

Clonal segregation in adult units. The majority of mutant units contained a mixture of stained and unstained cells at all time points, but with time, the proportion of mixed units decreased, whereas that of homogeneous units increased (Fig. 5, C and D), suggesting a transformation from mixed to homogeneous units. The lacZ-positive cells in a substantial number of the mixed units, especially at the later time points, were mature parietal or zymogen cells (Fig. 3, K and L). This suggests that in such units, the stem cell population is clonally homogeneous but residual long-lived mature normal cells persist. A plot of the proportion of mutant units containing either no stained cells or only mature stained cells indicates the impact of the long-lived mature cell populations on the homogenization process (compare Fig. 5, C and E).

The processes leading to homogenization of mixed units are important but poorly understood. Clonal purification may result from clonal partitioning after unit branching, from clonal extinction, or from a combination of both processes. New units are thought to result from a process of unit branching (16, 27) that presumably is accompanied by a distribution of the progenitor population among the resulting units. Nomura et al. (35) report that “monoclonal units do not emerge immediately following splitting of a polyclonal gland.” However, we observed numerous examples of mutant units, which, on completion of the branching process, would likely yield at least one homogeneous unit from a mixed parental unit (Fig. 3, N and P). Also, clusters of neighboring units were observed in which some units were mixed, whereas neighbors were homogeneously mutant, suggestive of branching events that resulted in one mixed and one homogeneous unit (Fig. 3, B, G, and O). Clonal extinction is also clearly important for the homogenization process. This includes the extinction of stem cells, committed progenitors, and all of their extant mature progeny. It is important to realize that the long life of some of the mature cell populations in gastric units slows down the homogenization of a mutant unit. For example, it may take hundreds of days for all of the original population of stained zymogen and parietal cells to die off. Furthermore, the presence of long-lived committed progenitors and any potential competition among various stem
cells inhabiting units undoubtedly add subtleties to the process.

Adult colon and small intestine have multipotential stem cells. Although not entirely novel, it is worth reporting our observation of homogeneously mutant crypts both in the small intestine and in colon using this model system. First, it confirms the applicability of the hemizygous ROSA26 mice to lineage tracing in these regions of the gastrointestinal tract and confirms the presence of multipotential stem cells. Second, it is important to note that our use of a transgene marker serves to complement previous studies using mutation of physiological genes (dlb-1 or glucose-6-phosphatase; see also Refs. 6, 14, 38, and 48). It was plausible that loss of endogenous gene function altered cellular behavior in some way that either gave the mutant cells a competitive advantage or changed adhesive characteristics causing the cells to segregate. Transgene mutation is more likely to be neutral. Therefore, we have confirmed that clonal purification is a normal process in small intestine and colon, not an artifact resulting from loss of function of an endogenous gene.

Potential issue with the use of a transgene marker, such as the bacterial gene lacZ, is the generation of clones by epigenetic mechanisms such as suppression of gene expression by methylation. A comparison of the frequency of lacZ-negative clones in small intestine from control mice reported here with that of clones from control mice marked by an endogenous gene and scored similarly in isolated epithelium (6) indicates from control mice marked by an endogenous gene and that clones from control mice reported here with that of clones by epigenetic mechanisms such as suppression of an endogenous gene.

In conclusion, we present evidence directly demonstrating the existence of a common stem cell population in the adult mouse gastric epithelium. Furthermore, we have shown that this ubiquitously expressed transgene model is applicable for lineage tracing in the entire gastrointestinal tract and is likely applicable to other renewing tissues. The specific transgene used, lacZ, could be replaced with other convenient transgenes; for example, green fluorescent protein.

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