Brazilian Journal of Medical and Biological Research (1998) 31: 271-279
ISSN 0100-879X

Cell lineage relationship in the stomach of normal and genetically manipulated mice

S.M. Karam

Department of Anatomy, Faculty of Medicine, Kuwait University, Safat, Kuwait

Abstract

The oxyntic mucosa of the mouse stomach is lined with a heterogeneous population of cells that form numerous short pits continuous with long tubular glands. Tritiated thymidine radioautography has made it possible to pinpoint the origin of all cell types and to follow the differentiation/migration of different cell lineages along the pit-gland unit. The proliferating multipotent stem cells functionally anchored in the upper glandular region, the isthmus, give rise to three main lineage precursors: 1) pre-pit cells, which migrate upward to the pit while differentiating into mucus-producing pit cells; 2) pre-neck cells, which migrate downward to the glandular neck while differentiating into mucus-producing neck cells that, by approaching the glandular base, gradually change their phenotype into pepsinogen- and intrinsic factor-producing zymogenic cells; 3) pre-parietal cells, which differentiate into acid-producing parietal cells in the isthmus and then undergo bipolar migration towards the pit and the glandular base. Thus, parietal cells are the only cells that complete their differentiation in the isthmus and then migrate to be scattered throughout the pit-gland unit. To determine whether parietal cells play a role in controlling decisions about cell fate within the pit-gland unit, the gastric epithelium has been examined in transgenic mice expressing the H,K-ATPase ß-subunit$^{-1035\ to\ +24}$/simian virus 40 large T antigen fusion gene. The blockade in parietal cell differentiation in these mice produces an amplification of lineage precursors, a marked depletion of zymogenic cells and an increase in pit cell census. Ablation of parietal cells in another transgenic mouse model expressing the H,K-ATPase ß-subunit$^{-1035\ to\ +24}$/diphtheria toxin fragment A fusion gene also produces amplification of lineage precursors, and similar effects on zymogenic and pit cell census. These findings strongly suggest that parietal cells produce regulatory signals that control the cellular differentiation program of both pit and zymogenic cell lineages, and would hopefully improve our ability to identify the cellular pathways leading to malignant transformation.

Correspondence
S.M. Karam
Department of Anatomy
Faculty of Medicine
Kuwait University
P.O. Box 24923
Safat 13110
Kuwait
Fax: 00-965-531-8454
E-mail: karam@hsc.kuniv.edu.kw

Presented at the 5th International Symposium on Radioautography, São Paulo, SP, Brasil, August 24-26, 1997.

Research supported by the Kuwait Foundation for Advancement of Sciences (No. KFAS 95-07-02).

Received August 4, 1997
Accepted August 20, 1997

Key words
- Radioautography
- Gastric epithelium
- Stem cells
- Cell proliferation/ differentiation programs
- Lineage progenitors
- Transgenic mice

Introduction

The mouse stomach consists of three main regions: the fundus which is lined with stratified squamous keratinized epithelium, the pyloric antrum which is continuous with the duodenum and lined with one layer of cells that invaginates to form numerous long pits continuous with short mucous glands, and the body (or corpus) which forms the main central region of the stomach and is lined with one layer of cells that forms numerous short pits continuous with long tubular glands. In this review, we will concentrate only on the epithelial cells of the body region of the mouse stomach.

The pit-gland units found in the corpus region of the mouse stomach are not static structures. Cellular proliferation, commitment, migration-associated differentiation, and death programs occur perpetually along the pit-gland axis of these units. The extensive dynamism of this epithelium is further indicated by its rapid regeneration after damage. In addition, interactions amongst the different gastric epithelial cell populations play a major role in determining the structure and function of the pit-gland unit. The main objective of this review is to summarize all of these fundamental features of the pit-gland units in the body region of the mouse stomach from studies in which tritiated thymidine radioautography, electron microscopy, multilabeled immunocytochemistry, and genetic manipulation techniques have been utilized.

Normal mice

Morphological identification of the main cell types along the pit-gland unit. Since the 1830s, it has been recognized that the mammalian gastric mucosa contains numerous glandular structures comprising mucus-producing glands in the pylorus and pepsinogen-producing glands in the corpus (1). In the mouse, the glands of the corpus open into the luminal surface via short pits. These pit-gland units, also called "zymogenic units" (2), consist of a structurally and functionally heterogeneous population of cells that include several cell types. 1) The mucus-secreting pit cells, or surface mucous cells, are found in the pit region and on the luminal surface and are characterized by a group of dense mucous granules (3-5). The granules are packed in an organelle-free apical area called ectoplasm (2,6). In the mouse, there are 37 pit cells per unit; their Golgi apparatus produces a uniformly fine particulate content packed in prosecretory vesicles which eventually form secretory granules. The diameter of the granules varies around 350 nm. The granule contents are homogeneously dense except on the free surface where they may acquire a core (2,6). Two lectins, *Ulex europaeus* type 1 agglutinin and cholera toxin B subunit, can be used as markers for pit cells in adult and developing mice (7,8). 2) The acid-secreting parietal cells have been extensively investigated by Forte et al. (9), Helander (4) and Ito (5). In the mouse, there are 26 parietal cells per unit; they are scattered along the pit and all three glandular regions and are characterized by an intracellular canalicular system, cytoplasmic tubulovesicular elements, long numerous microvilli lining canalicular/apical membranes and large numerous mitochondria. Antibodies against the α- and ß-subunits of the H,K-ATPase (10-12), the cytoskeletal protein ezrin (13) and the Lewis^x blood group antigen, Galb1,4(Fuca1,3)GlcNacb1 (8,14), as well as the lectin *Dolicus biflorus* agglutinin (7,8), are all molecular markers for parietal cells in developing and adult mice. 3) The mucus/pepsinogen-secreting neck cells have been well characterized as an entity separate from zymogenic and pit cells by Wattel and Geuze (15). The mouse stomach contains 13 neck cells per unit; they are located in the neck region and their Golgi apparatus produces dense irregular material packed in the center of prosecretory vesicles and light material

packed at the periphery of the same vesicles. These vesicles form cored granules scattered throughout the cytoplasm in comparison to the apical granules of pit cells. The diameter of neck cell granules varies around 570 nm. The *Grifforia simplifolica* II lectin can be used as a marker for neck cells (7,8) in both adult and developing mice. 4) The pepsinogen-secreting zymogenic cells have been extensively studied by Samloff (16) and Hersey (17). The mouse stomach contains 67 zymogenic cells per unit; they are typical serous cells characterized by a basal stack of rough ER cisternae and apical zymogen granules with a homogeneously pale content (2). Antibodies against pepsinogen and intrinsic factor are utilized as markers specific for mouse zymogenic cells (8,18,19). During morphogenesis, while pit, neck and parietal cells appear as early as embryonic day 18, typical zymogenic cells only appear at postnatal day 21 (8). 5) The peptide-secreting entero-endocrine cells have been characterized and extensively studied by Solcia et al. (20). The subtypes of these cells vary based on the shape of the secretory granules and their peptide content. In the mouse, there are 13 entero-endocrine cells per unit comprising several subtypes. They are scattered along the four unit regions but are mainly found in the base (2). 6) The villin-rich caveolated cells have been discovered by Hammond and LaDeur (21) and Nabeyama and Leblond (22); they are characterized by a microvillous tuft protruding into the glandular lumen, and long narrow convoluted caveoli that open between the microvilli. In the mouse, they are very few (1 cell per 2-3 units) but may be found in any of the four gland regions (2). 7) In the upper segment of the base region, there are pre-zymogenic cells (2) which are characterized by a Golgi apparatus producing prosecretory vesicles and secretory granules whose contents appear to be intermediate between those of neck cells and zymogenic cells. In the mouse stomach, there are 5 pre-zymogenic cells per unit. These cells can be identified by neck cell-specific lectins and pepsinogen-specific antibodies, markers for both neck and zymogenic cells (8,19).

Identification of the gastric epithelial precursor cells in the isthmus region. While parietal, entero-endocrine and caveolated cells are scattered in the four unit regions, pit cells are localized to pit, neck cells to the neck and zymogenic cells to the base. The cells found in the isthmus region appear to be small and devoid of prominent signs of differentiation. Stevens and Leblond (3) first suggested a role for these isthmal cells, i.e., continuous replacement of cells lining the pit and gastric lumen. With the advent of tritiated thymidine radioautography, the frequent mitosis of isthmal cells and their outward migration along the pit wall have been visualized (23). The presence of few "undifferentiated" cells among the isthmal cells has been reported in the stomach of the rat (24) and mouse (25). Our serial sections of the isthmus region of the mouse gastric gland confirmed the presence of these undifferentiated cells, and revealed seven different additional early committed precursor cells (2,26). 1) Undifferentiated granule-free cells exhibit an embryonic cell-like feature, i.e., scanty cytoplasm loaded with free ribosomes, much diffuse nuclear chromatin, and large reticulated nucleoli. The mitochondria and rough ER cisternae are scanty, and the Golgi apparatus is primitive. Because of the absence of secretory granules in the cytoplasm of these undifferentiated cells, they are called "granule-free" cells (2,26). All seven cell types cited below have features similar to those of the undifferentiated granule-free cells, but in addition they have a feature indicating early commitment. 2) Pre-pit cell precursors are characterized by a Golgi apparatus producing prosecretory vesicles similar to those of the pit cells. 3) Pre-neck cell precursors have Golgi apparatus producing prosecretory vesicles similar to those of the neck cells. No specific markers are available

for the granule-free cells or the precursors of both pre-pit and pre-neck cells. 4) Pre-pit cells are characterized by a Golgi apparatus producing prosecretory vesicles similar to those of pre-pit cell precursors and pit cells. They also have dense secretory granules similar to those of pit cells, but are fewer and smaller; their diameter varies around 200 nm. 5) Pre-neck cells are characterized by a Golgi apparatus producing prosecretory vesicles similar to those of pre-neck cell precursors and neck cells. They also have cored secretory granules similar to those of neck cells, but are fewer and smaller; their diameter varies around 398 nm. 6) Pre-parietal cells are characterized by parietal cell-like features, i.e., long apical microvilli and an incipient intracellular canaliculus; they include three subtypes: one carrying a few secretory granules similar to those of pre-pit cells, the second with cored granules similar to those of pre-neck cells, and the third devoid of any granules. 7) Pre-entero-endocrine cells are characterized by a few endocrine-type secretory granules. 8) Pre-caveolated cells are characterized by few caveoli and microvilli similar to those of caveolated cells.

The stem cell of the mouse gastric epithelium. In renewing epithelia, stem cells are defined by their high proliferative capacity to ensure their own persistence while producing committed cells (27) and their primitive embryonic cell-like features (28). Tritiated thymidine radioautography combined with electron microscopy revealed that the undifferentiated granule-free cells are the most proliferative cell type (30 min labeling index = 32%) and the most primitive among other isthmal cells, and are therefore considered to be the stem cells of the gastric epithelium (26).

Proliferation, differentiation and migration pathways along the pit-gland unit as revealed by tritiated thymidine radioautography. Tritiated thymidine has been utilized to label the proliferating cells of the pit-gland unit and to follow their differentiation/migration pathways with time. Radioautographs thus represent the source of valuable data on the labeling indices of various cell types at different time intervals. Cell proliferation is restricted to the isthmus region where granule-free cells are the most proliferative; pre-pit and pre-neck cells and their precursors also have some potential for mitosis, whereas pre-parietal cells do not divide. The shift in tritiated thymidine labeling that occurs with time from pre-pit to pit cells has confirmed morphological findings and indicated that they constitute one lineage (6) that migrates upwards to the free surface (Figure 1). The shift in the labeling from pre-neck to neck cells has confirmed morphological studies and indicated that they belong to one lineage. The morphological features of pre-zymogenic cells and the fact that they acquire thymidine labeling after neck cells and before the zymogenic cells indicate that they represent a transition during the transformation of neck cells into zymogenic cells (29). Thus, pre-neck, neck, prezymogenic and zymogenic cells all constitute one lineage that migrates towards the bottom of the gland (Figure 1). Also, the shift in labeling from pre-parietal to parietal cells indicates that they constitute a third lineage, but with a bipolar mode of migration towards either the pit orifice or gland bottom (30). Similar to parietal cells, the entero-endocrine and caveolated cells develop in the isthmus and undergo bipolar migration (31; Figure 1). The turnover time of the different gastric epithelial cell types is determined by continuous infusion of a low dose of tritiated thymidine into mice, which are then sacrificed at different time intervals. From the cumulative increase in the labeling indices of each cell type, the rate of cellular turnover and the turnover time can be estimated (Figure 1).

Little is known about the factors that control cellular proliferation and differentiation programs of the gastric epithelium. Since

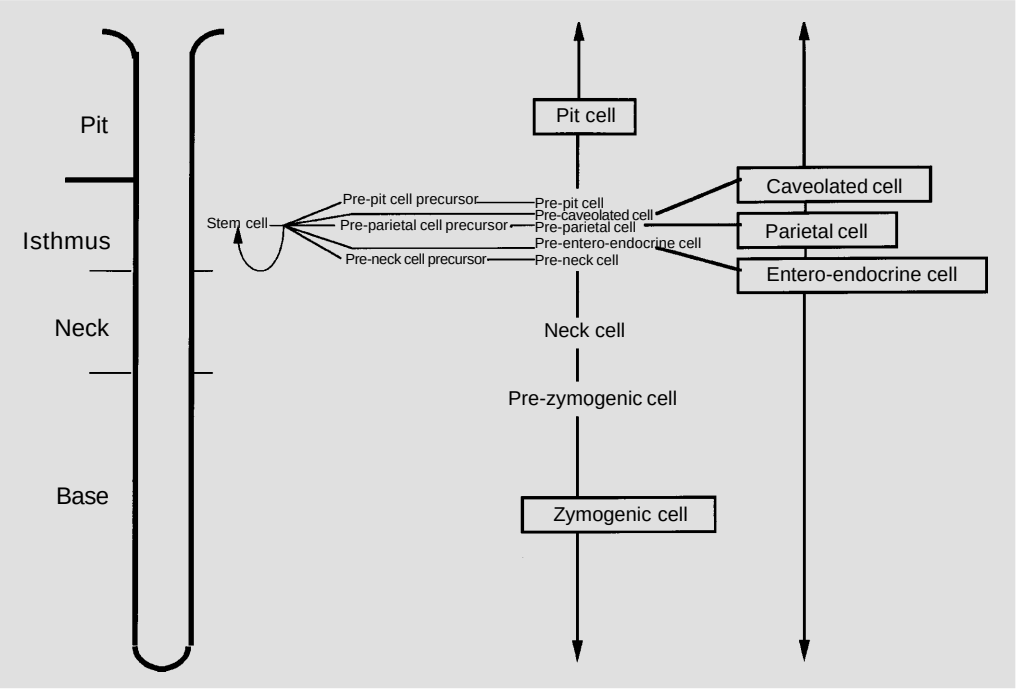

Figure 1 - Cell lineage relationships in the pit-gland unit of the corpus region of the mouse stomach. In the isthmus region of the gastric gland, the undifferentiated granule-free cell is the multipotent stem cell that divides to replace itself and to produce five lineage precursors. 1) Pre-pit cell precursors give rise to pre-pit cells that migrate upward to the pit while differentiating into pit cells. 2) Pre-parietal cell precursors give rise to pre-parietal cells which complete their differentiation into parietal cells in the isthmus and then undergo bipolar migration to the pit as well as to the neck/base regions. 3) Pre-neck cell precursors give rise to pre-neck cells that migrate downward into the neck while differentiating into neck cells which then transform into zymogenic cells in the base via a pre-zymogenic stage. 4) Pre-caveolated cells transform into caveolated cells in the isthmus and then undergo bipolar migration. 5) Pre-entero-endocrine cells differentiate into entero-endocrine cells in the isthmus and then undergo bipolar migration.

parietal cells appear to be strategically located among the precursor cells of the isthmus, and also scattered throughout the unit, they seem to have a role in cellular proliferation and commitment that perpetually occur in the isthmus. Immunohistochemical and *in situ* hybridization experiments have shown that both the α- and ß-subunits of H,K-ATPase are expressed in the pre-parietal and parietal cells (12,32). However, the synthetic activity of parietal cells is higher in the isthmus and neck regions than in the base. In addition, functional assays have revealed that parietal cells in the isthmus and neck are more active in acid secretion than basal parietal cells. Thus, a possible role for parietal cells other than acid secretion has been proposed. Covalent blockade of H,K-ATPase, the major protein of parietal cells, with omeprazole enhances the physiological degeneration of parietal cells. Furthermore, alteration in isthmal cell proliferation and commitment programs occurs, as indicated by the increase in the census of both the proliferating precursor cells and the non-proliferating committed pre-parietal cells (12).

To date there is no cell culture system that can reproduce cell lineages of the gastric epithelium for identifying factors that modulate cellular proliferation and differentiation. An alternative approach is to design a gain-of-function experiment by introducing a biologically interesting foreign gene into the mouse genome and generating transgenic mice.

Genetically manipulated mice

Transgenic mice overexpressing transforming growth factor α (TGFα). The epidermal growth factor (EGF) family of peptides includes TGFα which seems to play an important role in the regulation of cellular proliferation and differentiation programs in various tissues. The activity of TGFα is mediated by the EGF receptor, a tyrosine kinase that phosphorylates both itself and additional substrates upon ligand-specific activation/dimerization (33). Both TGFα and EGF receptors have been localized to the gastric epithelial cells by using immunohistochemistry and Northern blot analysis (34,35). It

has been shown that TGFα stimulates gastric mucosal growth (36) and also inhibits gastric acid secretion (37). Merlino and coworkers (38) have used the mouse metallothioneine I promoter to generate transgenic mice overexpressing human TGFα in several tissues. In these mice, TGFα disrupts the normal program of gastric epithelial cell differentiation. While pit cells and isthmal precursor cells are greatly expanded, parietal and zymogenic cells are depleted. These results suggest that TGFα plays an important role in the regulation of gastric epithelial cell differentiation.

A more powerful approach to the study of gastric epithelial biology is to utilize cis-acting regulatory elements that control transcription of a gastric cell lineage-specific gene in expressing any foreign gene in that cell lineage. Lorenz and Gordon (18) first found that nucleotides -1035 to +24 of the H,K-ATPase ß-subunit gene include transcriptional regulatory elements able to direct expression of any foreign gene in the parietal cell lineage.

Transgenic mice expressing the H,K-ATPase ß-subunit$^{-1035\ to\ +24}$/simian virus 40 large T antigen fusion gene. Simian virus 40 large T antigen (SV40 TAg) is an oncoprotein that binds p53 and induces cell proliferation. To test whether re-entry of the committed non-proliferating pre-parietal cells into the cell cycle would affect the cellular differentiation program in the gastric epithelium, Li et al. (19) used the regulatory elements of the H,K-ATPase ß-subunit gene to generate transgenic mice expressing SV40 TAg in their parietal cell lineage. In these mice, the proliferation-associated amplification of pre-parietal cells and the blockade of their differentiation into parietal cells starting from embryonic day 18 was not unexpected, whereas the accompanying loss of zymogenic cells was surprising. The latter finding indicates that members of the parietal cell lineage regulate the differentiation of neck cells to zymogenic cells (8,19). Furthermore, electron microscopic examination of the amplified population of pre-parietal cells in these transgenic mice at embryonic day 18 and postnatal day 1 revealed signs of their early commitment and transformation of undifferentiated (stem) cells into pre-parietal cells. Thus, the first member of the parietal cell lineage was identified, i.e., the pre-parietal cell precursor (Figure 1), which is characterized by loss of apical membrane glycocalyx and gradual elongation of microvilli (8). The amplification of pre-parietal cells and their precursors in these transgenic mice provides an excellent system to study the molecular and biochemical features of the early committed precursors of the acid-secreting parietal cell. With age these transgenic mice develop gastric adenocarcinoma (Karam SM, Li Q and Gordon JI, unpublished results).

Transgenic mice expressing the H,K-ATPase ß-subunit$^{-1035\ to\ +24}$/diphtheria toxin A subunit fusion gene. It is known that when diphtheria toxin fragment A is introduced into cells it inhibits protein synthesis and induces cell death. Further insights into the role of parietal cells are obtained by using the transcriptional regulatory elements of the H,K-ATPase ß-subunit gene to direct expression of diphtheria toxin gene in the parietal cell lineage (39). In these mice, dead parietal cells are either extruded into the gland lumen or phagocytosed by neighboring cells. Loss of parietal cells is accompanied by 1) a 7-fold increase in the number of pre-parietal cells, 2) a 5-fold increase in the number of undifferentiated granule-free (stem) cells, and early precursors of pit and zymogenic cell lineages, 3) a block in the differentiation program of the zymogenic cell lineage with accumulation of pre-neck cells and depletion of their zymogenic cell descendants, and 4) an enhancement in the differentiation program of pit cell lineage with a 2-fold amplification of both pre-pit and pit cells. Thus, mature parietal cells influence the commitment program among gastric epithelial precursors and modulate

the migration-associated terminal differentiation of both pit and zymogenic cell lineages. With age, the diphtheria toxin transgenic mice develop gastric adenocarcinoma in which the predominant cells are the normally proliferating isthmal cells, granule-free cells and their pre-neck and pre-pit descendants (Li Q, Karam SM and Gordon JI, unpublished results).

Transgenic mice expressing the H,K-ATPase ß-subunit promoter/herpes simplex virus 1 thymidine kinase fusion gene. Further progress in the field of genetic manipulation techniques to study gastric epithelial cell biology has been provided by the possibility of controlling the expression of a foreign gene in a specific cell lineage. Canfield et al. (40) have used the thymidine kinase ablation technique which is based on the selective toxicity of the antiherpetic drug ganciclovir to cells expressing a herpes virus thymidine kinase suicide gene. The generated transgenic animals provide a powerful system in which parietal cells are normally produced, but upon treatment with the antiherpetic drug ganciclovir, they are completely ablated. Discontinuation of the drug was associated with reemergence of parietal cells and restoration of the glandular architecture. In support of the findings of Gordon and coworkers (8,19,39), parietal cell ablation in these mice is found to be associated with a block in the terminal differentiation program of zymogenic cells (40). Thus, parietal cells appear to be the source of trophic factors that are essential for maintaining the differentiation of zymogenic cells.

Inhibin knockout mice. Activins A and B are members of the transforming growth factor ß superfamily which is known to regulate cellular proliferation and differentiation programs in several tissues (41). Matzuk and co-workers (42) have generated mice homozygous for a null allele of the inhibin α subunit. These inhibin knockout mice develop gonadal tumor and overexpress activins. The stomachs of these mice have no parietal cells. Removal of the gonads prior to development of tumors prevents the ablation of parietal cells. Detailed immunohistochemical and electron microscopic analysis of inhibin knockout mice has revealed that overexpression of activins produces a block in the differentiation of pre-parietal to parietal cells, a block in the transformation of neck into zymogenic cells, and a marked increase in pre-pit and pit cells as well as pre-caveolated and caveolated cells (Li Q, Karam SM, Coerver KA, Matzuk MM and Gordon JI, unpublished results). Thus, 1) activins play an important role in modulating the differentiation program of the gastric epithelial cell lineages, 2) several lines of evidence strongly suggest that members of the parietal cell lineage are essential for maintaining the normal programs of gastric epithelial cell proliferation and differentiation, and 3) it seems that altering parietal cells by using H2-receptor antagonists or proton pump inhibitors during treatment of peptic ulcer disease may elucidate the dynamics of the whole epithelium.

Tritiated thymidine radioautography is a powerful tool that can be used to characterize normal and abnormal programs of epithelial cell proliferation, differentiation and migration. Inferring the biological features of an epithelium by either gain- or loss-of-function experiments in genetically manipulated mice provides an opportunity to study factors that maintain homeostasis and interactions between different cells within the epithelium. Results of both radioautographic and genetic manipulation experiments should then lead to progress in our understanding of the gastric epithelial cell biology.

References

1. Bensley RR (1932). The gastric glands. In: Cowdry EV (Editor), *Special Cytology*. PB Hoeber, New York.
2. Karam SM & Leblond CP (1992). Identifying and counting all epithelial cell types present in the "corpus" of the mouse stomach. *Anatomical Record*, 232: 231-246.
3. Stevens CE & Leblond CP (1953). Renewal of the mucous cells in the gastric mucosa of the rat. *Anatomical Record*, 115: 231-245.
4. Helander HF (1981). The cells of the gastric mucosa. *International Review of Cytology*, 70: 217-289.
5. Ito S (1987). Functional gastric morphology. In: Johnson LR (Editor), *Physiology of the Gastrointestinal Tract*. 2nd edn. Raven Press, New York.
6. Karam SM & Leblond CP (1993). Dynamics of epithelial cells in the "corpus" of the mouse stomach. II. Outward migration of pit cells. *Anatomical Record*, 236: 280-296.
7. Falk PG, Roth KA & Gordon JI (1994). Lectins are sensitive tools for defining differentiation programs of mouse gut epithelial cell lineages. *American Journal of Physiology*, 266 (*Gastrointestinal and Liver Physiology*, 29): G987-G1003.
8. Karam SM, Li Q & Gordon JI (1997). Gastric epithelial morphogenesis in normal and transgenic mice. *American Journal of Physiology*, 272 (*Gastrointestinal and Liver Physiology*, 35): G1209-G1220.
9. Forte JG, Hanzel DK & Urushidani T (1989). Mechanisms of parietal cell function. In: Garner A & Whittle BJR (Editors), *Advances in Drug Therapy of Gastrointestinal Ulceration*. John Wiley & Sons, Sussex.
10. Smolka A, Helander H & Sachs G (1983). Monoclonal antibodies against H^+-K^+-ATPase. *American Journal of Physiology*, 245 (*Gastrointestinal and Liver Physiology*, 8): G589-G596.
11. Crothers Jr JM, Chow DC & Forte JG (1993). Omeprazole decreases H^+-K^+-ATPase protein and increases permeability of oxyntic secretory membranes in the rabbit. *American Journal of Physiology*, 256 (*Gastrointestinal and Liver Physiology*, 19): G231-G241.
12. Karam SM & Forte JG (1994). Inhibiting gastric H^+-K^+-ATPase activity by omeprazole promotes degeneration and production of parietal cells. *American Journal of Physiology*, 266 (*Gastrointestinal and Liver Physiology*, 29): G745-G758.
13. Hanzel DK, Urushidani T, Usinger WR, Smolka A & Forte JG (1989). Immunological localization of an 80-kD phosphoprotein to the apical membrane of gastric parietal cells. *American Journal of Physiology*, 256 (*Gastrointestinal and Liver Physiology*, 19): G1082-G1089.
14. Bry LP, Falk PG & Gordon JI (1996). Genetic engineering of carbohydrate biosynthetic pathways in transgenic mice demonstrates cell cycle-associated regulation of glycoconjugate production in small intestinal epithelial cells. *Proceedings of the National Academy of Sciences, USA*, 93: 1161-1166.
15. Wattel W & Geuze JJ (1978). The cells of the rat gastric groove and cardia. *Cell and Tissue Research*, 186: 375-391.
16. Samloff IM (1971). Pepsinogens, pepsin and pepsin inhibitors. *Gastroenterology*, 60: 586-604.
17. Hersey S (1994). Gastric secretion of pepsins. In: Johnson LR (Editor), *Physiology of the Gastrointestinal Tract*. 3rd edn. Raven Press, New York.
18. Lorenz RG & Gordon JI (1993). Use of transgenic mice to study regulation of gene expression in the parietal cell lineage of gastric units. *Journal of Biological Chemistry*, 268: 26559-26570.
19. Li Q, Karam SM & Gordon JI (1995). Simian virus 40 T antigen-induced amplification of pre-parietal cells in transgenic mice. Effects on other gastric epithelial cell lineages and evidence for a p53-independent apoptotic mechanism that operates in a committed progenitor. *Journal of Biological Chemistry*, 270: 15777-15788.
20. Solcia E, Capella C, Buffa R, Usillini L, Fiocca R & Sessa F (1987). Endocrine cells of the digestive system. In: Johnson LR (Editor), *Physiology of the Gastrointestinal Tract*. 2nd edn. Raven Press, New York.
21. Hammond JB & LaDeur L (1969). Fibrillovesicular cells in the fundic glands of the canine stomach. Evidence for a new cell type. *Anatomical Record*, 161: 393-412.
22. Nabeyama A & Leblond CP (1974). "Caveolated cells" characterized by deep surface invaginations and abundant filaments in mouse gastro-intestinal epithelia. *American Journal of Anatomy*, 140: 147-166.
23. Messier B & Leblond CP (1960). Cell proliferation and migration as revealed by radioautography after injection of thymidine-H^3 into male rats and mice. *American Journal of Anatomy*, 105: 247-285.
24. Corpron RE (1966). The ultrastructure of the gastric mucosa in normal and hypophysectomized rats. *American Journal of Anatomy*, 118: 53-90.
25. Kataoka K & Sakano Y (1984). Panoramic observation of the mouse gastric mucosa by superwide-field electron microscopy. *Archivum Histologicum Japonicum*, 47: 209-221.
26. Karam SM & Leblond CP (1993). Dynamics of epithelial cells in the "corpus" of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. *Anatomical Record*, 236: 259-279.
27. Hall PA (1989). What are stem cells and how are they controlled? *Journal of Pathology*, 158: 275-277.
28. Leblond CP (1981). The life history of cells in renewing systems. *American Journal of Anatomy*, 160: 113-158.
29. Karam SM & Leblond CP (1993). Dynamics of epithelial cells in the "corpus" of the mouse stomach. III. Inward migration of neck cells followed by progressive transformation into zymogenic cells. *Anatomical Record*, 236: 297-313.
30. Karam SM (1993). Dynamics of epithelial cells in the "corpus" of the mouse stomach. IV. Bidirectional migration of parietal cells ending in their gradual degeneration and loss. *Anatomical Record*, 236: 314-322.
31. Karam SM & Leblond CP (1993). Dynamics of epithelial cells in the "corpus" of the mouse stomach. V. Behavior of entero-endocrine and caveolated cells. General conclusions on cell kinetics in the oxyntic epithelium. *Anatomical Record*, 236: 333-340.
32. Karam SM, Yao X & Forte JG (1997). Functional heterogeneity of parietal cells along the pit-gland axis. *American Journal of Physiology*, 272 (*Gastrointestinal and Liver Physiology*, 35): G161-G171.
33. Carpenter G (1987). Receptors for epidermal growth factor and other polypeptide mitogens. *Annual Review of Biochemistry*, 56: 881-914.
34. Beauchamp RD, Barnard JA, McCutchen CM, Charner JA & Coffey Jr RJ (1989). Localization of transforming growth factor α and its receptor in gastric mucosal cells. *Journal of Clinical Investigation*, 84: 1017-1023.

35. Yasui W, Ji ZQ, Kuniyasu H, Ayhan A, Yokozaki H, Ito H & Tahara E (1992). Expression of transforming growth factor alpha in human tissues: immunohistochemical and Northern blot analysis. *Virchows Archiv A Pathological Anatomy and Histopathology*, 421: 513-519.
36. Chen MC, Lee AT & Soll AH (1991). Mitogenic response of canine fundic epithelial cells in short-term culture to transforming growth factor α and insulin-like growth factor I. *Journal of Clinical Investigation*, 87: 1716-1723.
37. Guglietta A, Lesch CA, Romano M, McClure RW & Coffey RJ (1994). Effect of transforming growth factor-α on gastric acid secretion in rats and monkeys. *Digestive Diseases and Sciences*, 39: 177-182.
38. Sharp R, Babyatsky MW, Takagi H, Tagerud S, Wang TC, Bockman DE, Brand SJ & Merlino G (1995). Transforming growth factor α disrupts the normal program of cellular differentiation in the gastric mucosa of transgenic mice. *Development*, 121: 149-161.
39. Li Q, Karam SM & Gordon JI (1996). Diphtheria toxin-mediated ablation of parietal cells in the stomach of transgenic mice. *Journal of Biological Chemistry*, 271: 3671-3676.
40. Canfield V, West AB, Goldenring JR & Levenson R (1996). Genetic ablation of parietal cells in transgenic mice: A new model for analyzing cell lineage relationships in the gastric mucosa. *Proceedings of the National Academy of Sciences, USA*, 93: 2431-2435.
41. Jenkin G, McFarlane J & de Kretser DM (1995). Inhibin and activin in embryonic and fetal development in ruminants. *Journal of Reproduction and Fertility*, 49 (Suppl): 177-186.
42. Matzuk MM, Finegold MJ, Su JGJ, Hsueh AJW & Bradley A (1992). α-Inhibin is a tumour-suppressor gene with gonadal specificity in mice. *Nature*, 360: 313-319.