

## LINEAGE COMMITMENT AND MATURATION OF EPITHELIAL CELLS IN THE GUT

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### 1. ABSTRACT

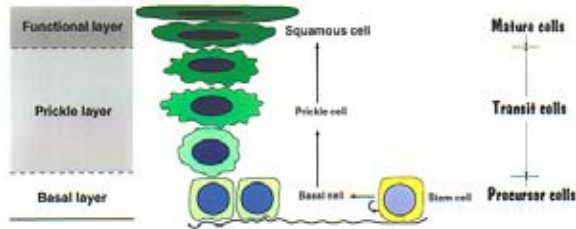
The dynamic concepts of gut epithelial cell populations which heralded the era of modern gut cell biology have been generally substantiated by recent studies and are still being correlated with functional properties. Multipotent stem cells are anchored in specific locations along the gut epithelium where decisions concerning proliferation and differentiation/migration pathways are made. Stem cells give rise to lineage precursors which transform into transit cells and sequentially express lineage specific features during their differentiation program. Morphologically and functionally mature cells along the gut epithelium are dynamically heterogeneous. 1) The squamous lineage of the esophagus forms a stratified epithelium which has an average turnover time of about 7.5 days. 2) In the stomach, the oxyntic pit-gland unit includes pit, zymogenic and parietal cells which respectively migrate outwards, inwards, and in both directions; their turnover times average 3, 194 and 54 days, respectively. 3) The mucous units of the pyloric antrum are populated by pit cells which migrate outwards and gland cells which migrate inwards; their turnover times are about 3 and 1-60

days, respectively. 4) In the crypt-villus units of the small intestine, while both absorptive and goblet cells migrate outwards and for each the turnover time is about 3 days, Paneth cells migrate inwards and their turnover time is about 15 days. 5) In the crypts of the descending colon, both vacuolated-columnar and goblet cells migrate outwards and for each the turnover time is about 5 days. The ascending colon has an additional cell type called deep crypt secretory cells which migrate inwards and their turnover time is about 14-21 days. Finally, while the factors maintaining the gut epithelium in a steady state remain to be elucidated, this epithelium represents a remarkable system for studying the biological features of stem cells and their hierarchies.

### 2. INTRODUCTION

The lining epithelium of the gut has fascinated many cell biologists due to its dynamism. It was Bizzozero (1) and Bensley (2) who first noted the phenomena of mitotic activity in the gut epithelium. To early workers in

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**Figure 1.** Diagram of cell differentiation in the esophageal epithelium. The proliferative stem cells are located in the basal layer where they undergo differentiation-associated migration to become prickle cells which transform into squamous cells.

gut cell biology the advent of markers of cell dynamics and thus of the time dimension *in vivo* came as an exciting breakthrough. Particularly important was the application of DNA-labeling by radio-thymidine and the radioautographic technique to detect the marker in single cells (3). Pioneering experiments demonstrated that the cells of gut epithelium could be distinguished from one another by their proliferative capability, age and renewing rate, one of the first hints of gut cell dynamics. Thus, the gut epithelium was seen to be a cellular renewal system in a steady state (4). These early steps in unraveling the dynamics of gut epithelium marked the beginning of modern gut cell biology. Concepts of gut cell dynamics have since become fundamental to understanding the organization of the gut epithelium and its capacity for protection, secretion, absorption and adaptation.

The foregoing principles are exemplified by work on rodent gut cell dynamics. The mouse, in particular, has become the model system for chimeric, transgenic, chimeric-transgenic, knock-out, and knock-in technology (5-7). Several promoters of cell-specific genes have become available to deliver biologically interesting foreign gene products to different cell lineages at various positions along the gut. In addition to these gain-of-function experiments, the promoters can also be used for designing loss-of-function experiments in each cell lineage along the gut (8). These experiments would help answering many questions of cellular dynamics including factors regulating gut cell production and maintaining epithelial organization of the gut.

This article will start with a brief description of the general organization of the gut epithelium, then new insights into stem cells and cellular hierarchies of the gut epithelium will be summarized, and finally the main morpho-dynamic features of the various epithelial cell lineages along the gut will be presented.

### 3. GENERAL ORGANIZATION OF THE GUT EPITHELIUM

In mice, the gut epithelium is first identifiable at embryonic day 7 as a single layer of proliferative endodermal cells (9). Then, within a few days the endoderm forms pseudostratified epithelium. Eventually, elongation of the gut tube occurs with remarkable changes in the epithelial features which are associated with

compartmentalization of the gut. While the esophagus is lined by several layers of epithelial cells, the lining cells of the stomach and intestines form a single layer which increases its surface area by forming numerous evaginating and/or invaginating epithelial units.

The principle cells of the esophageal epithelium form three successive layers or strata (figure 1): basal layer (stratum basalis or germinativum), prickle or spinous or intermediate layer (stratum spinosum), and functional or superficial layer (stratum corneum). In rodents, where keratinization is the main feature of the epithelium, an additional distinct granular layer (stratum granulosum) is found between the functional and prickle layers. A few enteroendocrine cells and melanocytes are scattered along the basal layer. In addition, antigen-presenting Langerhans cells are occasionally present in the prickle cell layer. In humans, some cells from the basal and prickle layers extend into the underlying lamina propria, and thus connective tissue papillae are formed (10).

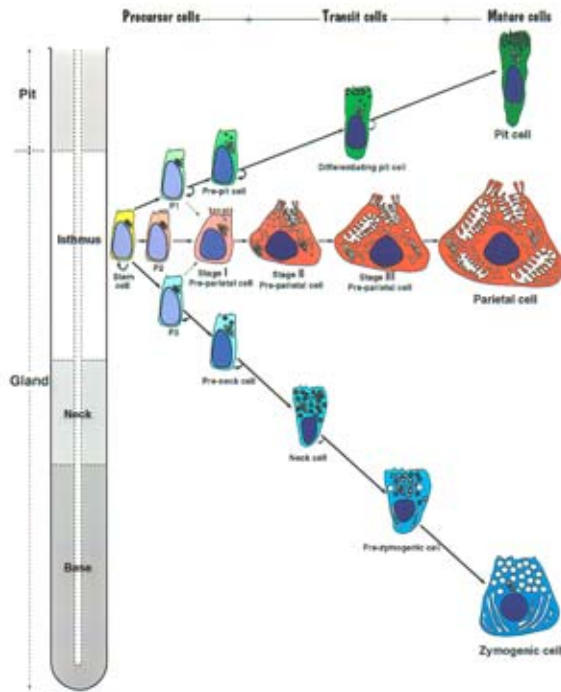
In the stomach, the oxyntic epithelium invaginates to form numerous short pits continuous with long tubular glands (11-13). Each gland can be divided into isthmus, neck and base regions (figure 2). In the mouse, the pit-gland unit is lined by a monolayer of about 200 cells (13). These cells include several types: mucus-secreting pit cells in the pit, a diverse group of precursor cells in the isthmus, mucus-secreting neck cells in the neck, and pepsinogen-secreting pre-zymogenic and zymogenic cells in the base. The acid-secreting parietal (oxyntic) cells, as well as enteroendocrine and caveolated cells, are scattered throughout the unit.

In the pyloric antrum, the epithelium invaginates to form long pits continuous with short glands made of three regions: isthmus, neck and base (figure 3). In the mouse, the pyloric antral pit-gland unit is lined by a monolayer of about 250 cells. The pit is lined by mucus-secreting pit cells; the isthmus, by a diverse of precursor cells; and the neck/base by gland mucous cells (14). In addition, enteroendocrine and caveolated cells are scattered throughout the pit-gland unit.

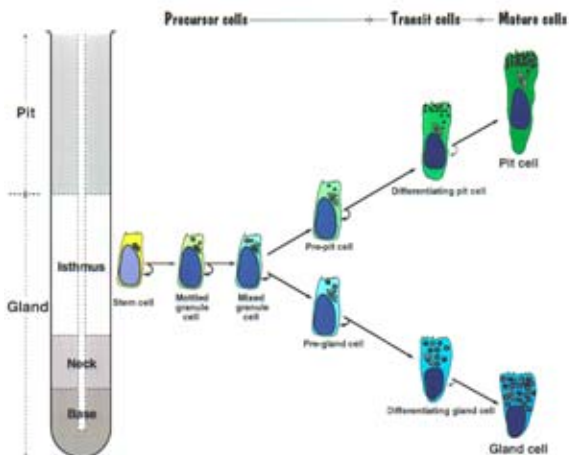
The small intestinal epithelium invaginates to form small crypts continuous with large evaginating villi (figure 4). Although the crypts are much more smaller than the villi, they are much more numerous (15). An adult mouse has a total of about 1.1 million crypts; each crypt contains about 250 cells (16). In addition to the proliferative precursor cells, the crypts contain lysozyme- and cryptidine-producing Paneth cells at the bottom. The absorptive, goblet (mucous), enteroendocrine and caveolated cells are all scattered along the crypt-villus unit (17).

The epithelial lining of the mouse colon invaginates to form numerous crypts (figure 5). There are about 700,000 crypts containing a total of about 700 million epithelial cells in the mouse colon (18). The size of crypts and their lining cell types vary in the ascending and descending portions of the colon. In the ascending colon,

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**Figure 2.** Diagram of cell differentiation in the oxyntic pit-gland unit of the stomach. The stem cells are located in the isthmus region which we enlarged here to accommodate all precursor cells seen at the right. The stem cells give rise to three precursors: pre-pit cell precursor (P1), pre-parietal cell precursor (P2) and pre-neck cell precursor (P3) which respectively evolve into pit, parietal and zymogenic cell lineages. The straight arrows for pit and zymogenic cell lineages indicate the migration pathways, and curved arrows indicate cells capable of mitosis. After their differentiation in the isthmus, parietal cells migrate in both directions.



**Figure 3.** Diagram of cell differentiation in the mucous pit-gland unit of the pyloric antrum. The gland includes three equal regions: isthmus (enlarged here to accommodate all precursor cells), neck and base. Stem cells are located in the isthmus and give rise to mottled granule cells which undergo several rounds of cell division and produce mixed granule cells. The latter give rise to pre-pit and pre-neck cells which evolve to form pit and gland cell lineages, respectively.

crypts are shorter (about 20 vs. 30 cell positions) and are populated by a smaller number of cells (about 300 vs. 700 cells/crypt) than in the descending colon. Cell types in the descending colon include vacuolated-columnar, goblet, enteroendocrine and cavuolated cells. These cells are all scattered throughout the crypt wall, contrary to the proliferative precursor cells which are located in the crypt base (19). In the ascending colon, an additional morphologically distinct cell type, deep crypt secretory cell, is located in the crypt base. Here the vacuolated-columnar and goblet cells are found in the crypt top, and the precursor cells, in the mid crypt (20, 21).

## 4. STEM CELLS OF THE GUT EPITHELIUM

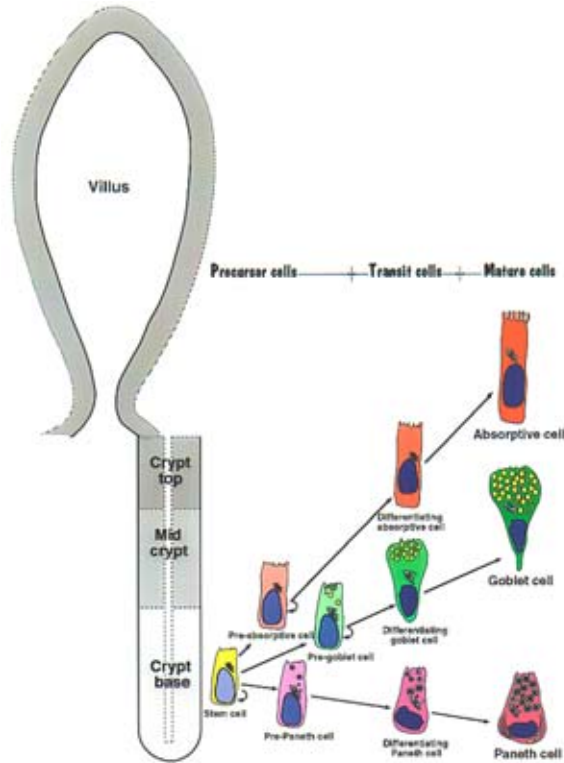
Even though specific biomarkers for stem cells of normal gut epithelium are not yet available, these cells can be defined by two major criteria. First, morphologically, they are undifferentiated and exhibit embryonic cell-like features: high nucleus-to-cytoplasm ratio, a nucleus with much diffuse chromatin and large reticulated nucleoli, and cytoplasm containing a few small organelles but many free ribosomes (22, 23). Second, functionally, they have a high capacity to proliferate so as to ensure their own renewal while producing lineage precursors which differentiate to form transit cells committed to become mature cells (24-26). Therefore, potential stem cells in a population can be tentatively identified by electron microscopy combined with 3H-thymidine radioautography; stem cells would be the least differentiated and most proliferative cells (23).

The stem cells of the gut are stationary anchored in specific locations: (i) the basal layer of the esophageal epithelium (27, 28), (ii) the isthmus region of the oxyntic gland where their turnover time is about 2.5 days (29), (iii) the isthmus region of the pyloric antral gland where their turnover time is about 1 day (30), (iv) the crypt base of the small intestine where they are arranged in an annulus (26, 31), (v) the mid crypt of the ascending colon (20, 21, 32), and (vi) the crypt base of the descending colon (19).

Because of their proliferative capabilities, the stages of the cell cycle are re-defined in the stem cells of the pyloric antrum (33, 34) and duodenum (35). It appears that the duration of their mitosis is about 8.4 hr including 4.8 hr for prophase and respectively, 0.2, 0.06 and 3.3 hr for metaphase, anaphase and telophase. Thus, the duration of prophase is quite longer than hitherto believed; i.e. chromatin condensation of the prophase begins during DNA-synthesizing (S) stage which starts during interphase and lasts for about 5.8 hr (35).

New insights into the small intestinal stem cells are introduced by cytotoxic radiation damage where the stem cells are defined by their regenerative capabilities. The location of stem cells has been confirmed and precisely defined in the fourth cell stratum from the crypt bottom (17). More insights have been introduced by the powerful technology of mouse aggregation chimeras and transgene expression regarding the clonality and number of the multipotent stem cells in each epithelial unit and their

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**Figure 4.** Diagram of cell differentiation in the crypt villus unit of the small intestine. The crypt include three equal regions: crypt top, mid crypt and crypt base (the crypt base has been enlarged here to fit the precursor and Paneth cells). The stem cells are located in the crypt base and give rise to three main precursors: pre-absorptive, pre-goblet and pre-Paneth cells which respectively evolve into absorptive, goblet and Paneth cell lineages.

capacity to encode spatial memory or retain a positional address along the cephalocaudal axis of the gut (reviewed in 25).

## 5. CELLULAR HIERARCHIES OF THE GUT EPITHELIUM

In the gut epithelium, cells go through five main stages. They are initially present as stem cells which divide and produce new stem cells as well as uncommitted and/or committed precursor cells which represent the second stage in gut epithelial cell life. The uncommitted precursor cells exhibit dual lineage features and eventually become committed precursor cells with features of one lineage. These precursor cells are usually capable of undergoing equivalent mitosis and thus amplifying the population before entering the next stage (29, 30). Transit cells represent the third stage during which cellular specification gradually occurs by synthesizing new gene products. This may be encountered morphologically by gradual changes in cell structure, immuno- or lectin-cytochemically by expression of new proteins or sugar residues, and biochemically by changes in enzymatic activities, protein composition and messenger RNA expression. The fourth stage is that of the mature cells which have completed their

differentiation and thus becoming actively functional. In the last stage of terminal cells there is a gradual deterioration and eventually cells undergo death and elimination (36).

## 6. SQUAMOUS CELL LINEAGE OF THE ESOPHAGUS

Cells of squamous lineage are arranged in three layers made of three morphologically distinct cell types (figure 1).

### 6.1. Basal cells

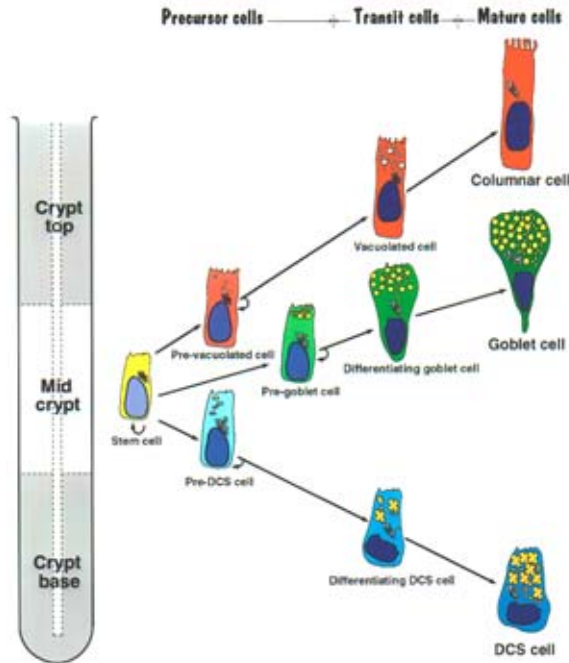
Basal cells appear cuboidal or low columnar and, in humans, form about 15% of the whole epithelium (28), whereas in rats they form about 62% of all nucleated cells (27). Basal cells rest on a basement membrane and their basal plasmalemmae have numerous extensions projecting into the underlying connective tissue forming hemidesmosomes (10). In addition to the presence of a few bundles of tonofilaments, basal cells exhibit signs of immaturity: a high nucleus-to-cytoplasm ratio, nucleus rich in euchromatin, large irregular nucleolus, abundant free ribosomes, rudimentary Golgi apparatus, few cisternae of rough endoplasmic reticulum (ER), and few small mitochondria. In mice, dividing stem cells are randomly distributed along the basal layer (37, 38), whereas in humans, they predominate in the inter-papillary region of the basal layer (38). The division of basal stem cells occurs in a combination of symmetrical (or equivalent) and asymmetrical (or differential) mitoses (37, 39). When a basal stem cell divides, it may produce two basal cells (equivalent basal mitosis), or one basal and one prickle cells (differential mitosis), or two prickle cells (equivalent outgoing mitosis). After a single 3H-thymidine injection, the decrease and eventual loss of labeled basal cells within a few days indicates that they turn over quite rapidly. Within 12 hr, basal cells start their migration with 3% of them reaching the prickle layer. This small fraction may represent the cells which are formed by differential mitosis. By 24 hr, many basal cells become prickle cells and the average transfer rate is about 1% per hr. The average turnover time of basal cells is about 4.5 days (27).

### 6.2. Prickle cells

As the name indicates, these cells are located in the intermediate prickle cell layer. In rats, they represent about 30% of all nucleated epithelial cells. They arise by differentiation and upward migration of basal cells. They appear polyhedral and are characterized by numerous cytoplasmic finger-like extensions projecting into the intercellular spaces and forming desmosomes with those of neighbor cells. Gap and tight junctions are also found between these cells (40). The presence of these interconnected extensions gives this cell layer the prickle or spinous appearance. Tonofilaments of desmosomes extend into the cytoplasm to form numerous bundles. During their migration towards the functional layer, prickle cells show signs of differentiation which vary in different species. In human, the cells gradually flatten, tonofilaments become more and more numerous, rosettes of glycogen particles as well as membrane-coating granules appear in the cytoplasm



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**Figure 5.** Diagram of cell differentiation in the crypt of the ascending colon. The stem cells are anchored in the mid crypt and give rise to three main precursors: pre-vacuolated, pre-goblet and pre-deep crypt secretory (pre-DCS) cells which respectively differentiate into columnar, goblet and DCS cell lineages.

and gradually increase in amount (41). The granules are electron dense and only occasionally appear lamellated as those of the epidermis. They are numerous near the superficial surface of the cells and because they contain some acid phosphatases, they are believed to be part of the lysosomal system, and thus play a role in breaking down desmosomes in the superficial layer before desquamation. In rabbits, prickly cells produce small granules which after exocytosis their glycoprotein contents act as barriers to paracellular permeability (42). In rats, prickly cells produce keratohyaline granules, and thus transform into granular cells which form about 8% of all nucleated cells. It takes about 48 hr for basal cells to migrate through the prickly layer and reach the granular layer (27).

### 6.3. Squamous cells

Squamous cells are markedly flattened and primarily composed of tonofilaments. The superficial cell membrane forms numerous microplicae. In rats and mice, there is a conspicuous gradual disappearance of the nuclei and organelles towards the most superficial layer (27). In humans, the flattened cells of the superficial layer contain basophilic granular structures (clumped organelles), membrane-coating granules and occasionally a small number of parakeratotic granules, but do not undergo true cornification. However, a few scattered areas of slight cornification are normally seen. In rodents, the superficial epithelial cells undergo soft keratinization. The average turnover time of the whole squamous epithelial cell lineage is about 7.5 days (27).

## 7. CELL LINEAGES IN THE BODY OF THE STOMACH

All cells lining the pit-gland unit of the oxyntic epithelium originate from the isthmal stem cells and then migrate in an outward and/or inward directions. They constitute three main lineages (figure 2).

### 7.1. Pit cell lineage

#### 7.1.1. Pre-pit cell precursors

In the isthmus, about 67% of the progeny of the stem cells produced daily become pre-pit cell precursors (P1 in figure 2). They are characterized by a small Golgi apparatus that produces prosecretory vesicles at the trans-face. These vesicles vary in density but contain uniformly fine particulate material. These cells are partially committed and have two different progenies. The majority (99%) become pre-pit cells and only 1% become pre-parietal cells with pre-pit cell-like secretory granules. The development of a pre-pit cell precursor into a pre-pit cell is manifested by the maturation of the trans-Golgi vesicles into dense secretory granules. In the case of the pre-parietal cell, there is also an elongation of the apical microvilli (29).

#### 7.1.2. Pre-pit cells

Pre-pit cells are located in the upper portion of the isthmus and are characterized by a few 200 nm-wide, dense secretory granules. An average of 10 pre-pit cells are present in each isthmus. Radioautography has revealed that they have two sources of origin. About 57% come from the differentiation of pre-pit cell precursors, the remaining 43%, from their own mitosis. After a pulse of 3H-thymidine, 25% of pre-pit cells become labeled. With time, label increases to reach 33% at 6 hr, then gradually decreases to 1% at 4 days, and completely disappears thereafter. In continuous 3H-thymidine labeling experiments, almost all pre-pit cells become labeled by 2 days. Both single injection and continuous labeling experiments confirm the short turnover time of pre-pit cells (2.5 days, 29). The fate of pre-pit cells is to become pit cells. This occurs as the activity of the cell increases and an increasing number of larger and larger secretory granules are produced and accumulate at the apex before exocytosis (29).

#### 7.1.3. Pit cells

These cells are located in the pit region, and are characterized by a dense apical group of mucous granules. The pit cells migrate outward along the pit wall to reach the gastric luminal surface in a few days (43). During pit cell migration, the apical group of granules enlarges due to increase in number and size of newly produced secretory granules, from 250 to 400 nm (43). In addition, cells gradually elongate with tapering of their basal cytoplasm, nucleoli become condensed, the amount of ribosomes diminishes, and the mitochondria decrease in size.

Pit cells close to the pit-isthmus border retain some ability to divide. Thus, pit cells are not only developed from maturation of pre-pit cells, but some are also produced by their own mitosis. Even though a pit

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region may include a few large parietal cells, the migration of pit cells along the pit wall occurs in a fairly regular pipe-line manner. It takes about 60 hr for a pit cell to reach the surface. At the luminal surface, the transit time is only 12 hr. The overall turnover time of pit cells averages 3 days (43).

### 7.2. Zymogenic cell lineage

#### 7.2.1. Pre-neck cell precursors

About 24% of the stem cells produced daily become pre-neck cell precursors (P3 in figure 2). These precursors are characterized by prosecretory vesicles at the trans-face of their Golgi apparatus containing dense irregular material with light periphery. They are partially committed precursors and the fate of 98% of them is to become pre-neck cells; the remaining 2% become pre-parietal cells with cored secretory granules similar to those of pre-neck cells (29).

#### 7.2.2. Pre-neck cells

Pre-neck cells are located in the lower portion of the isthmus; they average 1.8 cells per isthmus. They are characterized by a few 400 nm-wide secretory granules which appear dense with a light core. They are mitotically active (11% become labeled after a radio-thymidine pulse) and their division yields new pre-neck cells and cells committed to develop into neck cells. The turnover time of pre-neck cells is about 3 days (29).

#### 7.2.3. Neck cells

These cells, also called "mucous neck cells", are located in the neck region and are characterized by many dense mucous granules which usually contain a light core made of pepsinogen (44). Neck cells close to the isthmus have a fewer and smaller granules (430 nm-wide) than those close to the base (700 nm). After their production in the isthmus from transformation of pre-neck cells or in the high neck segment from their own mitosis, neck cells migrate inward while completing their differentiation toward the mucous phenotype. Neck cells are not end cells; i.e. their fate is not to degenerate and die. They spend from 7 to 14 days in the neck region. Then, at the neck-base border, their phenotype gradually changes from mucous to serous (45).

#### 7.2.4. Pre-zymogenic cells

In the upper segment of the base region of the pit-gland unit of the mouse, there is a group of cells producing secretory granules which appear intermediate between those of neck cells and those of zymogenic cells. These granules contain two different components: electron dense mucus, and light pepsinogen. Cells with similar criteria are also described in guinea pigs (44) and rats (46). In the mouse, these cells are classified into subtypes I, II and III, according to whether the dense mucous component is, respectively, more abundant than, about equal to, or less abundant than the light pepsinogenic component (45). Moreover, prosecretory vesicles at the Golgi trans-face of each of these subtypes exhibit differences parallel to those occurring in the granules. In the basal cytoplasm, rough ER cisternae are more abundant in subtype III than in subtypes I and II. The existence of further intermediates

between these subtypes indicate that they transform into one another (I → II → III), and thus gradually change their phenotype to become more and more pepsinogenic. The gradual decrease in their mucus production have led to the production of granules which are entirely pepsinogenic (45).

### 7.2.5. Zymogenic cells

These pepsinogen-secreting cells are characterized by spherical zymogen granules with homogeneously light pepsinogenic content. As zymogenic cells migrate inward, their phenotype specificity increases, as suggested by the measurement of zymogen granules, 780-nm-wide in the high base vs. 1070-nm-wide in the low base. The production of larger and larger granules is in line with the increase in the amount of rough ER cisternae and also with the enlargement of the nucleolus. Zymogenic cells are end cells which eventually acquire signs of degeneration and finally die at the gland bottom after a long turnover time of ~194 days (45).

### 7.3. Parietal cell lineage

#### 7.3.1. Pre-parietal cell precursors

Little is known about these precursors (P2 in figure 2). They are defined in developing transgenic animal model in which the precursors of acid-secreting cell lineage have been amplified. They are characterized by embryonic cell-like features, in addition to having numerous apical microvilli with little glycocalyx (47, 48).

#### 7.3.2. Pre-parietal cells

Pre-parietal cells are characterized by having long apical microvilli and incipient canaliculi. Pre-parietal cells do not undergo mitosis at any stage of their development (49). Based on the presence or absence of some secretory granules, pre-parietal cells are divided into three variants: (i) pre-parietal cells with no secretory granules which directly develop from pre-parietal cell precursors, (ii) pre-parietal cells with a few small dense granules similar to the granules of pre-pit cells which develop from pre-pit cell precursors, and (iii) pre-parietal cells carrying a few cored granules similar to those in pre-neck cells which develop from pre-neck cell precursors.

Development of pre-parietal cells into parietal cells occurs in three stages. First, an increase in the surface area of the apical plasma membrane forms long numerous microvilli. Second, a few small H<sub>2</sub>K-ATPase-containing tubules and vesicles appear in the cytoplasm, and the apical membrane invaginates to form an incipient canaliculus at one side of the nucleus. Third, an additional canaliculus appears on the other side of the nucleus and the number and size of mitochondria gradually increase. Finally, expansion of the canaliculi and overall increase in cell size lead to the formation of a fully mature parietal cell (48-50). The formation of a pre-parietal cell takes about one day, and their maturation into a parietal cell requires at least two more days (49).

#### 7.3.3. Parietal cells

Parietal cells are produced in the isthmus and migrate bi-directionally along the pit-gland axis. This

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migration pathway has been visualized by radioautography. Radio-labeled cells are first seen in the isthmus. With time, they appear in the pit in an outward direction and also in the neck in an inward direction until they reach the blind end of the unit. In situ hybridization studies and biochemical analysis have demonstrated that the synthetic/secretory activity of parietal cells vary along the pit-gland axis (36). Young parietal cells in the isthmus and neck are more active than old parietal cells in the pit and base regions. The estimated turnover time of parietal cells is about 54 days (49).

Ablation of parietal cells in genetically manipulated animal models (47, 51-55) has been associated with a block in the terminal differentiation of zymogenic cells. Thus, in addition to the fact that parietal and zymogenic cells have a common source of origin, it seems that the former produces some regulatory factors necessary for the terminal differentiation of the latter.

### 8. CELL LINEAGES IN THE PYLORIC ANTRUM OF THE STOMACH

In the isthmus region of the mucous units of the pyloric antrum, the immediate descendants of the stem cells are called "mottled-granule cells" (56). They are characterized by embryonic cell-like features, but in addition they carry a few small (235 nm) mottled granules in their apical cytoplasm (figure 3). These uncommitted precursor cells represent 39% of the isthmal cells; they undergo clonal expansion and divide four times before giving rise to "mixed-granule cells" characterized by a mixture of small dense granules and large cored granules. Mixed-granule cells divide and give rise to dense granule cells (here called pre-pit cells) and core granule cells (here called pre-gland cells). The turnover time for each of the mottled- and mixed-granule cells is about 1 day (56).

#### 8.1. Pit cell lineage

##### 8.1.1. Pre-pit cells

Pre-pit cells represent about 17% of all isthmal cells, and are usually located near the pit border. Both the morphological features and dynamic behavior of these cells are quite similar to those of the pre-pit cells in the isthmus region of the oxyntic pit-gland units (29, 57).

##### 8.1.2. Pit cells

These cells are located in the pit region, and represent about 180 cells per pit-gland unit. The mode of migration and structural features of these cells are similar to those in the oxyntic epithelium (29). They also have the same turnover time (3 days, 57).

#### 8.2. Gland cell lineage

##### 8.2.1. Pre-gland cells

These poorly differentiated cells are characterized by having a few small (280 nm) cored granules. The pre-gland cells represent about 28% of the isthmal cells and predominate near the neck border. These cells duplicate before their differentiation-associated migration to cross the neck border and become gland cells (58).

##### 8.2.2. Gland cells

Gland cells are located in the neck and base regions of the mucous pit-gland unit and represent about 37 cells per unit. Along the neck-base axis, gland cells exhibit more and larger cored granules toward the base. The granule size varies from 380 nm in the neck to 580 nm in the base. In addition, with the inward migration of gland cells, the amount of ribosomes diminishes, the rough ER cisternae become numerous, and the Golgi apparatus increases in size (58).

The isthmus of each mucous unit produces about 12 gland cells per day by differentiation of pre-gland cells. Gland cells also retain some mitotic activity which gradually diminishes toward the gland bottom. Thus, a total of 29 gland cells are added daily to the gland cell population. Gland cells migrate inward to the gland bottom in a gradually decreasing rate. The average time spent by a gland cell in the neck region is about 10 hr and in the base region, about 200 hr. This pattern of gland cell renewal is known as "cascade" pattern of cell renewal. Therefore, the overall turnover time of gland cells is highly variable, from 1 to 60 days (58).

### 9. CELL LINEAGES IN THE SMALL INTESTINE

The renewal capacity of the small intestinal epithelium is remarkable. In mice, about two thirds of the crypt cells pass through the cell cycle every 12 hr, and every crypt produces about 13-16 new cells/hr which undergo differentiation-associated migration and form several cell lineages (figure 4) that supply 2-3 neighboring villi (17).

#### 9.1. Absorptive cell lineage

In mice, there is an overwhelming majority of members of this lineage along the small intestinal crypt-villus unit (>80% of all epithelial cells).

##### 9.1.1. Pre-absorptive cells

Pre-absorptive cells are located in the crypt base. They exhibit stem cell-like features and relatively long microvilli. These cells retain some ability to divide and thus, are produced by their own mitosis as well as by differentiation of stem cells. Pre-absorptive cells migrate outward along the crypt base to reach the mid crypt where they gradually differentiate; the microvilli elongate to form brush border. When these cells reach the crypt top, the differentiation is complete as the absorptive cells are formed (31).

##### 9.1.2. Absorptive cells

These cells are located along the crypt top and the whole villus. They are characterized by the absence of secretory granules and the presence of a prominent apical brush border. The absorptive cells migrate outward along the crypt wall and reach the villus surface in a few days (31). During migration of absorptive cells, they show signs of differentiation: the cell gradually elongates, apical microvilli become long and numerous, lateral membranes interdigitate with neighbor cells, the nucleus gradually become heterochromatic and closer to the cell

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center, and the nucleoli become condensed and small. In the cytoplasm, the amount of free ribosomes diminishes, the Golgi apparatus becomes prominent, mitochondria become abundant, and rough ER cisternae increase in amount. The migration of members of the absorptive cell lineage is slow in the crypt, and gradually increases to become approximately constant in the crypt top and along the villus. The overall turnover time of absorptive cells averages 3 days (31).

It has been recently shown that extracellular matrix (59) and some adhesion molecules, such as N-cadherin and beta-catenin play an important role in maintaining the differentiation program of the absorptive cells and homeostasis of the epithelium (60, 61).

Along the crypt-villus axis, the functional activity of the absorptive cells follows a pattern parallel to their morphological changes. <sup>3</sup>H-fucose radioautography has revealed that the turnover rate of the glycoprotein (brush border enzyme) in the absorptive cells is very rapid. Within 5 min the fucose reaches the Golgi region and by 90 min most of the fucose is on the microvilli. However, the amount of incorporated fucose is variable along the crypt-villus axis. It is low in the crypt, rises towards the villus base, remain high along the mid-villus, then sharply declines at the villus tip (22).

### 9.2. Goblet cell lineage

The number of goblet cells increases from the duodenum to the jejunum to the ileum; they respectively constitute 4, 6 and 12% of all epithelial cells (62).

#### 9.2.1. Pre-goblet cells

Pre-goblet cells, also called oligomucous cells, are located in the crypt base compartment of the small intestine where they exhibit stem cell-features, and also show, as a sign of early differentiation, a few small mucous granules. These granules are usually homogeneously pale (common oligomucous cells), but sometimes they include a dense core (granular oligomucous cells). Pre-goblet cells retain some capacity for mitosis and so, they originate by their own mitosis as well as by differentiation of the stem cells. Then, they migrate outward and reach the mid crypt within 12-24 hours. As they migrate, more granules are produced and accumulate in the supranuclear cytoplasm. Thus, they are transformed into goblet cells (62).

#### 9.2.2. Goblet cells

These cells are scattered along the mid crypt up to the villus tip. They are characterized by a large group of mucous granules in the supranuclear cytoplasm. From the mid crypt, the goblet cells migrate outward to reach the villus surface in one day (62). With their migration, goblet cells producing cored mucous granules (granular mucous cells) gradually change their phenotype and produce homogeneously pale mucous granules. The overall turnover time of goblet cells averages 3 days (62).

### 9.3. Paneth cell lineage

The presence, morphology and number of Paneth cells vary in different species. They are absent in cats and

predominate in the crypts of ant-eating Brazilian bears (63). In mice, they are located in the lower third of the crypts and represent 3.3% of the duodenal crypt cells and, respectively, 7.5 and 6.6% in the jejunum and ileum (31).

#### 9.3.1. Pre-Paneth cells

These cells are located in the crypt base next to their ancestor stem cells. In addition to their stem cell-like features, they carry a few small granules with central dense cores and light halos. These cells migrate toward the crypt bottom while maturing into Paneth cells (64).

#### 9.3.2. Paneth cells

Paneth cells are characterized by apical secretory granules which exhibit an electron dense core and a light halo. The basal cytoplasm contains abundant cisternae of rough ER. Paneth cells exhibit more and larger haloed granules toward the crypt bottom, where they are more numerous. The granule size varies from about 500 nm close from the mid crypt border to about 3000 nm in the bottom of the crypt (64). This is due to production of a gradually larger granules. In addition, with the inward migration of Paneth cells, the rough ER cisternae become numerous, and the Golgi increases in size.

Paneth cells along the crypt base can not divide by mitosis. Thus, they are only developed from maturation of the stem cells (64, 65). With time, Paneth cells migrate inward to the crypt bottom. The overall turnover time of Paneth cells is about 15 days (64).

The location and relatively long residency time of Paneth cells, and the nature of their secreted gene products, suggest that they may influence the structure and/or function of the stem cell niche. However, it has been recently found that ablation of Paneth cells in transgenic mice has no detectable effect on the proliferation/differentiation program in the crypts or on host-microbial interactions (66).

## 10. CELL LINEAGES IN THE COLON

In the mouse colon, each crypt produces 14-21 cells/hr (67), whereas, in the rat, the rate of cell production is slightly lower, 7-11 cells/crypt/hr (68). Dynamics of the colonic stem cells vary in the ascending and descending portions of the colon (69, 70). While stem cells are located in the mid crypt of the ascending colon and there is a bi-directional mode of migration (figure 5), they are located in the crypt base of the descending colon and cellular migration occurs in an outward direction (32). Moreover, in the ascending colon, the cell cycle time is longer (19 vs. 15 hr), and proliferating cells are less numerous (90 vs. 190 cells/crypt) in comparison to the descending one. The latter explains the higher risk of colon cancer in the descending colon.

### 10.1. Vacuolated-columnar cell lineage

Cells of this lineage form about 80% of the crypt cell population in the descending colon and include three main members (figure 5).



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### 10.1.1. Pre-vacuolated cells

These poorly differentiated cells are located in the mid crypt of the ascending portion of the mouse colon or the crypt base of its descending portion. They exhibit a few small periodic acid-Schiff negative vacuole-like granules in their apical cytoplasm. Pre-vacuolated cells retain some ability to divide. Thus, they are developed from maturation of stem cells, and also by their own mitosis. With time, pre-vacuolated cells accumulate more and larger granules and become vacuolated cells (19).

### 10.1.2. Vacuolated cells

These cells are located in the upper two thirds of the crypts of the ascending portion of the colon and the lower two-thirds of the crypts of the descending colon. They are characterized by the presence of pale vacuole-like granules in the apical cytoplasm. As the cells migrate upwards, the granules become large and numerous. The nuclei of vacuolated cells are basal and darkly stained; mitochondria are scanty and free ribosomes are numerous. A few microvilli may project into the crypt lumen (19). Vacuolated cells migrate outward along the lower two thirds of the descending colonic crypts in a constant speed. 1-2 days after their production, they acquire a striated border characteristic of absorptive cells. Thus, they gradually differentiate toward the absorptive columnar phenotype. This transformation process is completed in about 3 days (69).

### 10.1.3. Columnar cells

Columnar cells are found in the crypt top of ascending and descending colons. They are characterized by an apical striated border composed of packed microvilli which are less prominent than those of the small intestinal absorptive cells. Columnar cells maintain the same migration speed as vacuolated cells. In the descending colon, the overall turnover time of vacuolated-columnar cells averages 4.6 days (19).

## 10.2. Goblet cell lineage

In the mouse descending colon, cells of this lineage form about 16% of the whole crypt cell population (19). They are evenly distributed throughout the crypt except at the crypt bottom and at the surface where they are very few. They are characterized by various number of mucous granules.

### 10.2.1. Pre-goblet cells

Pre-goblet cells are also called oligomucous cells and are located in the mid-crypt of ascending colon and the crypt base of descending colon. These cells are characterized by a few mucous granules. In some cells, the granules are grouped into a narrow theca. These cells are capable of mitosis, and thus they are developed from maturation of stem cells, and also by their own mitosis (19). Following their production, pre-goblet cells migrate upward along the crypt wall and show signs of gradual maturation. Within 1-2 days, they accumulate more and more mucous granules and the theca gradually enlarges and, thus, they become mature goblet cells. This transformation process is completed in about 2 days (69).

### 10.2.2. Goblet cells

Goblet cells are located in the upper third (or two thirds) of the ascending (or descending) colon. The

mucous granules are large and numerous and thus, the theca is swollen to give a typical goblet appearance. The dark nuclei are squeezed at the base of the goblet. In the descending colon, it takes about 1 day for a goblet cell to migrate along one third of the crypt and reach the surface. The overall turnover time of goblet cells is similar to that of the vacuolated-columnar cells; it averages 4.6 days (19).

## 10.3. Deep crypt secretory cell lineage

Members of this lineage are found in several species including mice, rats as well as humans. They differ from goblet cells in location, histochemistry and ultrastructure (20). They lack the goblet-shaped theca, but exhibit many mucous granules which appear large and light in mice and rats, but small and dark in humans (21). These cells are produced from stem cells located in the mid crypt as pre-deep crypt secretory cells and undergo migration-associated differentiation. They reach the crypt bottom as mature deep crypt secretory cells. In mice, they have an average turnover time of about 14-21 days (21).

## 11. ENTEROENDOCRINE CELL LINEAGES

The peptide- or polypeptide-producing enteroendocrine cell lineages are scattered throughout the gut epithelium. Members of these lineages usually occur singly along the gut epithelium. In mice, they represent about 7% of all cells in the stomach body (13) and 3% in the pyloric antrum (57). In the small intestine, they are more abundant in the crypts than in the villi and generally form about 0.5% of all crypt-villus cells (31). They form about 0.4% of cells in the colon (71). The identification of enteroendocrine cells of different lineages depends on the size, shape, electron density and immunocytochemical specificity of their secretory granules (72). After a long debate about the neuronal vs. epithelial origin of these cells, it has been established that they share a common stem cell with other epithelial lineages in the gut (73-77). Thus, enteroendocrine cells represent several cell lineages which originate from the epithelial stem cells of the gut.

### 11.1. Pre-enteroendocrine cells

These are immature cells producing a few small endocrine-like secretory granules. They have been described in the isthmus region of the oxyntic glands of the stomach (74, 75), in the crypt base of the small intestinal epithelium (76) and in the crypt base of the descending colon (71).

Pre-enteroendocrine cells are occasionally seen undergoing mitosis in the stomach (74) and intestine (76). Thus, they originate mainly by differentiation of the stem cells as well as by their own mitoses. Radioautographic labeling of these immature forms of enteroendocrine cells has revealed that in the stomach body, they mature in the isthmus and then migrate bi-directionally to reach the pit and base regions after about 16 days. In the small intestine they migrate outwards and reach the crypt top by 1-2 days where they produce more and more granules. After 1-2 more days, they reach the villi where they are transformed into mature enteroendocrine cells (76). In the colon, it takes at least 1 day for a pre-enteroendocrine cell to be

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formed; it differentiates into enteroendocrine cell and reaches the mid crypt by 7 days. Pre-enteroendocrine cells are, thus, left behind by the more rapidly migrating pre-vacuolated and pre-goblet cells (71).

### 11.2. Enteroendocrine cells

These are the mature forms of the endocrine cells which are located throughout the gastric pit-gland units, the small intestinal villi and the colonic crypts (71, 74, 76). They are characterized by a large group of granules in the infranuclear cytoplasm and may have bundles of cytoplasmic filaments which appear relatively few due to increase in cell size. With time, enteroendocrine cells migrate in an inward or outward directions. The overall turnover time of enteroendocrine cells is estimated at about 60 days in the stomach corpus (estimated from 74), 4 days in the small intestine (76, 77), and 23 days in the descending colon (71).

## 12. M CELL LINEAGE

The antigen-sampling M cells are also called membranous or microfold cells, and are found overlying lymphoid follicles in the small as well as large intestines of rodents and humans. They do not show signs of lipid absorption as do their neighbor absorptive cells, but are able to internalize cationized ferritin (78). In rabbits, alpha 1-2-linked fucose- and N-acetylgalactosamine-specific lectins can be used as markers for M cells (79).

### 12.1. Pre-M cells

In rabbits, pre-M cells are also found in the intestinal crypts around lymphoid follicles and express little alpha 1-2-linked fucose and N-acetylgalactosamine (79). They are characterized by many free ribosomes and numerous microvilli at the cell apex (78). These cells are not capable of mitosis, they originate from the crypt-base stem cells. It takes at least one day for pre-M cells to be generated. Then, they migrate outward to cover the dome-shaped lymphoid follicles while differentiating into mature functional M cells (79).

### 12.2. M cells

M cells are flattened due to accumulation of lymphoid cells in the underlying connective tissue. They are characterized by central cytoplasmic "pocket" containing lymphoid cells (80). In M cells, both free ribosomes and apical microvilli are few. These cells are actively capable of internalizing luminal molecules, particles or microorganisms and transporting them to the intercellular spaces. In rabbits, they express much alpha 1-2-linked fucose and N-acetylgalactosamine (80).

## 13. CAVEOLATED CELL LINEAGE

Mature members of this lineage are described throughout the gastric and intestinal epithelial linings by Nabeyama and Leblond (81). In the gastric epithelial units, they are most numerous in the pit, isthmus and neck regions (13).

### 13.1. Pre-caveolated cells

These immature cells are described in the isthmus regions of the gastric units (74, 75), and the crypt base

regions of the descending colon (71). They are very rare and appear plump with narrow apices and few caveoli.

Pre-caveolated cells originate by differentiation of the gut stem cells (71, 74). Their maturation is followed by bi-directional migration in the gastric units, or by their outward migration in the descending colonic crypts.

### 13.2. Caveolated cells

These mature cells are characterized by a plump body with narrow apex projecting microvilli into the luminal surface. The cytoplasm exhibits prominent lysosomes and numerous caveoli separated by bundles of filaments extending from the core of the microvilli deep to the sides of the nucleus (74). The long axis of the ovoid nucleus tends to be parallel to the basement membrane. In the mid crypt regions of the descending colon, caveolated cells exhibit a basal cytoplasmic process which becomes longer in the crypt top, but short at the luminal surface (71).

In the stomach, the little data available have shown that caveolated cells follow a bi-directional mode of migration similar to that of enteroendocrine and parietal cells (74). In the descending colon, it takes about 1 day for a caveolated cell to be produced; they migrate outward and spend about 4 days in the crypt base, and 0.5 day in each of the middle and upper thirds of the crypt. The overall turnover time of caveolated cells is about 8 days in the colon (71).

## 14. CELL DEATH IN THE GUT EPITHELIUM

Cell death is a feature of renewing tissues including gut epithelium (22). To maintain homeostasis of the gut epithelium, the addition of new cells by proliferation and differentiation of stem cells is balanced by programmed death and eventual loss of mature cells. Programmed cell death has three morphologically distinct types: 1) apoptosis, which is characterized by cell shrinkage, condensation and detachment from neighbor cells, 2) autophagic (necrosis-like) cell death characterized by vacuoles, and 3) death by fragmentation without pyknotic nuclear changes or vacuoles (82). Elimination of dead cells occurs by either extrusion into the gut lumen or by phagocytosis by neighboring cells.

In the esophagus cell loss occurs by the shedding of squamous cells at the surface; they eventually lose attachment with underlying cells and desquamate. Also, apoptosis occurs but very rapidly and, thus, difficult to encounter (40).

At the luminal surface of the stomach, pit and parietal cells lose their functional activities and undergo autophagic (necrosis-like) or apoptotic cell death. While necrotic cells are directly extruded into the gastric lumen (a kind of holocrine secretion), apoptotic cells are phagocytosed by healthier neighbors which eventually undergo extrusion (43, 49, 57). In the bottom of the gastric glands, both zymogenic and parietal cells undergo degeneration and are directly extruded into the gland lumen or are phagocytosed by healthier neighbors. Connective

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tissue macrophages may invade the gastric gland and participate in the elimination of dead cells (49). The pyloric gland cell degeneration and loss occurs in both the neck and base regions. It is estimated that about 18 cells are daily lost from the neck, 10 at the neck-base border and 1 from the base. Dead cell elimination occurs by extrusion into the gland lumen or by phagocytosis (58).

At the villus tip of the small intestine, the cells degenerate and drop into the gut lumen (31, 62). In the crypt bottom, apoptotic Paneth cells are rarely encountered. They are rapidly extruded into the crypt lumen or phagocytosed by neighboring immature cells (64).

In the colon, columnar and goblet cells undergo programmed death at the luminal surface (19) and deep crypt secretory cells, at the crypt bottom (21). Degeneration and loss of enteroendocrine, caveolated and M cells also occur in the gut epithelium.

## 15. PERSPECTIVES

In this article we have summarized the present concepts of cellular dynamics throughout the gut epithelium. Over the last few years, striking advances have been made using molecular techniques to perturb the biological features of the gut epithelium and hence revealing new insights into the stem cells and their hierarchies. However, many aspects regarding gut cell dynamics still remain unknown, such as 1) the factors keeping balance between cell proliferation and cell loss, and 2) the mechanisms controlling the differentiation pathway of the stem cell, and coordinating balance between proliferation and differentiation of stem cells and their immediate descendants. Answers to these questions may lead to better understanding to the pathogenesis of various gut diseases such as tumors and ulcers. Finally, we believe that gut cell dynamics will remain a topic of active research and should be viewed in an integrated form because of the similarities observed in the origin, morphology and behavior of the stem cells.

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