Published in final edited form as:

Mol Cell Endocrinol. 2010 July 8; 323(1): 70–75. doi:10.1016/j.mce.2009.12.009.

Gut Endocrine Cell Development

Catherine Lee May^{1,2,4} and Klaus H. Kaestner^{3,4}

¹ Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA

² Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA

³ Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA

⁴ Institute for Diabetes, Obesity and Metabolism, Philadelphia, Pennsylvania 19104, USA

INTRODUCTION

Endocrine cells are scattered throughout the gastrointestinal mucosa from the stomach to the colon and constitute one of the largest endocrine systems in the body. Enteroendocrine cells labeled by chromogranin A comprise about 1% of all epithelial cells in the gastrointestinal tract and are surrounded by mucus, chief, and parietal cells in the stomach, and enterocyte, goblet and Paneth cells in the intestine (Figure 1). The enteroendocrine system consists of at least 15 different cell types that can be classified based on their main hormonal products and on the ultrastructure of their secretory granules [1]. A given enteroendocrine cell secretes one or more hormone or hormone-like substance, which is released directly into the lamina propria and diffuses into the capillaries. These hormones include gastrin, histamine, serotonin, cholecystokinin (CCK), somatostatin and glucagon-like peptides (GLP1 and 2) [1]. Although enteroendocrine cells are very scarce, they are essential regulators of digestion, gut motility, appetite, and metabolism.

The development of enteroendocrine cells is a fascinating biological problem: how is the relative proportion of the individual subtypes maintained? How are so many different cell types specified from a common precursor? And how is the endocrine compartment maintained in the gastrointestinal epithelium with its rapid and life-long turnover? However, the study of the mechanisms of enteroendocrine cell differentiation is not only an “academic exercise” but of relevance to human health and disease on multiple levels. First, deficiencies in enteroendocrine cell specification or survival contribute to human diseases, as exemplified by congenital malabsorptive diarrhea, discussed in detail below, which is due solely to deficient enteroendocrine cells. Second, several enteroendocrine cells play a role the control of glucose homeostasis and thus diabetes, and there relatedness to pancreatic beta-cells makes transdifferentiation of enteroendocrine cells an interesting possibility. Therefore, lessons learned from the study of normal gut endocrine cell development promise to be instructive to

Correspondence to: KHK, kaestner@mail.med.upenn.edu, Address: 415 Curie Blvd. CRB 752B Philadelphia, PA, 19104 USA Fax: 215-573-5892; Phone: 215-898-8759. CLM, catheril@mail.med.upenn.edu, Address: 3615 Civic Center Boulevard, ARC 516E Philadelphia, PA, 19104 USA Fax: 215-590-3709; Phone: 267-426-0116.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Embryonic Origin of Enteroendocrine Cells

The gastrointestinal tract is lined with a monolayer of cells that undergo continuous and rapid renewal, in contrast to the epithelia of the other endoderm-derived organs such as liver and lung that differentiate early in life and turn over slowly in adulthood [2–6]. Enteroendocrine cells found in the gastrointestinal tract express several hormones, and even transcriptional regulators, originally associated with the nervous system. Therefore, the ontology of enteroendocrine cells has long been controversial, with some scientists suggesting an endodermal, and others a neural crest origin. However, lineage tracing experiments have convincingly demonstrated that all epithelial cell types of the intestinal mucosa, including enteroendocrine cells, are differentiated from pluripotent stem cells in the crypts (or the neck region of gastric glands), and are therefore endoderm-derived [7–14]. Thus, although neurons and enteroendocrine cells express a common set of genes, they are of divergent embryological origin.

Each intestinal crypt contains 4–6 stem cells; however, the exact identity of these cells was revealed only recently. Wnt signaling had been shown to play an important role in the proliferative activity of the normal intestinal crypt [15–17]. Based on this knowledge, Clevers and colleagues identified a Wnt target gene, *Lgr5*/GPR49, as restricted in expression to the stem cell compartment, which is located at the very base of the crypt in the small intestine (Figure 3) [9]. Most importantly, genetic lineage tracing studies using a *Lgr5*-CreER^{T2} transgene and Rosa26R reporter mice demonstrated conclusively that all epithelial lineages, including enteroendocrine cells, are derived from *Lgr5*-expressing stem cells in the intestine [9].

Notch signaling in enteroendocrine cell differentiation

Notch is a transmembrane receptor that mediates local cell-cell communication and coordinates a signaling cascade schematized in Figure 2 [18]. Unlike other receptors, Notch functions both at the cell surface to receive signals and in the nucleus to regulate gene expression [18]. The key players of the Notch pathway in mammals are the ligands (Delta and Jagged), the receptor (Notch 1 through 4) and a transcription factor (RBP-J κ) that translates the signaling process into target gene activation. Ligand binding activates proteolytic cleavage and post-translational modification of Notch, releasing the Notch intracellular domain (Notch IC) from the cell surface ([19]; Figure 2). The Notch IC then translocates into the nucleus and associates with RBP-J κ , a DNA binding protein [21], to form a complex that binds to and activates the promoters of the hairy/enhancer of split (HES) gene family [20]. Hes1 or its relatives then repress proendocrine bHLH transcription factors such as Neurogenin 3 (Ngn3) by binding to their promoters or enhancers [21–23].

One of the major functions of Notch signaling is to mediate lateral inhibition between adjacent cells, preventing neighboring cells from adopting the same fate [24,25]. Involvement of the Notch signaling pathway in the differentiation of enteroendocrine cells was first suggested by the observation that there are never two enteroendocrine cells adjacent to each other in the gastrointestinal epithelium. This was confirmed by studies in which loss of Notch function resulted in excess enteroendocrine cells [21,26]. Notch signaling controls the bHLH transcription factor-driven differentiation program to select individual endocrine cells from expanding precursor cells [26–28]. In addition to its role in the initial decisions of epithelial lineage determination, Notch signaling is also important during later stages of enteroendocrine cell differentiation to fine-tune the number of the various enteroendocrine cell types [21,29–32].

bHLH transcription factors in enteroendocrine cell differentiation

Three proendocrine bHLH transcription factors, all targets of Notch signaling, have been shown to contribute to enteroendocrine cell differentiation through loss-of-functions studies in mice [29–33]. *Math1*, *Neurogenin 3* (*Ngn3*) and *NeuroD* are all related to the *Drosophila atonal* gene, which is critical in neural differentiation in the fly [34–36]. This family of transcription factors functions in sequential order, where one factor activates another to control the initial specification of the enteroendocrine cells as well as their final differentiation.

Math1 is activated first in the immature crypts and villi of the intestinal epithelium during fetal development, although it is not detected in the stomach [32]. *Math1* is required for the initial specification of all three secretory lineages of the intestine: goblet, Paneth and enteroendocrine cells [32]. Furthermore, it has been shown through lineage tracing studies that all secretory lineages in the intestine are derived from *Math1*-expressing precursor cells [32]. Later expression of lineage-specific transcription factors such as *Sox9* for Paneth cells, *Klf4* for goblet cells and *Ngn3/NeuroD* for enteroendocrine cells is required for the proper differentiation of these secretory cells (Figure 3) [29–31,37,38]. Recently, analysis using an intestine-specific deletion of *Math1* in adult mice has revealed the importance of *Math1* in the balance between enterocytes and enteroendocrine cells, as intestinal precursors preferentially differentiate into enterocytes at the expense of secretory lineages in the *Math1*-deficient intestine (Figure 3) [33].

Ngn3 is first activated in the intestine as early as embryonic day 12.5 (E12.5), similar to what is observed in the developing pancreas [39]. This expression persists into adulthood where expression is found in the intestine and glandular stomach [29]. *Ngn3* acts downstream of the transcription factor *Math1*, because *Ngn3*-deficient animals still express *Math1* (Figure 3) [29,30].

The contribution of *Ngn3* to enteroendocrine cell differentiation varies between intestine and stomach. In both mice and humans, all intestinal enteroendocrine cells are *Ngn3*-dependent, with no enteroendocrine cells left in the intestinal epithelium of mice homozygous for a null mutation in *Ngn3*, and few were found in humans homozygous for a *NGN3* point mutation [29,40,41]. In the humans, *NGN3* mutation has been reported as the cause of congenital malabsorptive diarrhea [41]. Interestingly, the role of *Ngn3* in the development of enteroendocrine cells in the stomach is more limited. Thus, while the number of glucagon-, somatostatin-, and gastrin-secreting cells is reduced in *Ngn3*-null mice, serotonin-, histamine-, and ghrelin-expressing cells are still present [29,30].

While loss-of-function studies suggest that normal enteroendocrine differentiation requires *Ngn3* [29,30], it is less clear if *Ngn3*⁺ precursor cells contribute only to the enteroendocrine population. Several groups have developed transgenic mouse models to address this issue. In the first model, the *Ngn3* promoter and 6.9 kilobases of upstream sequence were used to direct expression of the bacterial *LacZ* gene. In these mice, some, but not all enteroendocrine cells were marked by the transgene. A potential limitation of this model is the short *Ngn3* promoter fragment employed for the transgene construction, leaving the possibility that both exclusion of essential *Ngn3* enhancer elements and transgene position effects might have affected the outcome of this lineage tracing experiment.

To overcome these limitations, Andrew Leiter's group derived mice in which the Cre-recombinase encoding cDNA was placed under the control of a *Ngn3* BAC (bacterial artificial chromosome) containing 183 kilobases of the *Ngn3* locus [42]. Using this model, Leiter and colleagues demonstrated that all intestinal endocrine cells arise from *Ngn3*-Cre⁺ cells. This study, however, produced a new puzzle, as a few goblet and Paneth cells were also marked by the *Ngn3*-Cre transgene [42]. These observations raise a crucial issue with genetic lineage

tracing using the Cre/loxP system that is often overlooked. Because of the extraordinary specificity and activity of the site-specific DNA recombinase Cre, very low levels of expression are sufficient to effect excision of loxP-flanked targets. Thus, cells in which the Ngn3-Cre transgene is only minimally activated will be permanently marked by β -galactosidase from the recombined Rosa26R (or any other reporter) locus, even though there is yet no Ngn3 protein detectable in the same cell. In other words, the observed activation of β -galactosidase in a small percentage of goblet and Paneth cells (and the occasional entire crypt) may be due to stochastic low-level activation of the Ngn3-Cre transgene.

To better characterize the progenitors leading from the stem cells to the Ngn3⁺ enteroendocrine lineage, Bjerknes and Chen [43] have analyzed the rate of cell flux through different stages of enteroendocrine cell differentiation, finding that most if not all of the Ngn3⁺ cells are in the enteroendocrine lineage and Ngn3⁺ cells do not contribute significantly to non-endocrine lineages [43]. The importance of Ngn3 in the enteroendocrine lineage was also demonstrated by gain-of-function experiments in which Ngn3 was over-expressed throughout the developing intestinal epithelium, resulting in increased numbers of enteroendocrine cells at the expense of goblet cells [44].

Genetic analysis of the third *atonal*-related bHLH gene, *NeuroD*, was the first to link Notch signaling to enteroendocrine cell differentiation [31]. Unlike Ngn3, expression of *NeuroD* is restricted to a subset of enteroendocrine cells [45,46]. *NeuroD* has been shown to control terminal differentiation of secretin-producing cells by coordinating transcription of the secretin gene with cell cycle arrest [31,47,48]. In addition, *NeuroD*-deficient mice lack secretin- and cholecystokinin (CCK)-producing cells in the intestine, thus defining a subset of the enteroendocrine cell lineage [31]. It has been suggested that *NeuroD* acts downstream of Ngn3 and is regulated by Ngn3, which was confirmed by the absence of *NeuroD* expression in the *Ngn3*-deficient mice (Figure 3) [29,49].

Hes1, which is transcriptionally controlled by Notch signaling, encodes a bHLH transcriptional repressor [22,50] and antagonizes the activity of the aforementioned bHLH transcriptional activators [51–54]. *Hes1* is critical for maintaining endocrine precursor pools in the undifferentiated state. In *Hes1*-deficient mice several bHLH factors are up-regulated, resulting in precocious differentiation of enteroendocrine subtypes in the stomach and intestine [21]. In these mice, excess numbers of glucagon-, CCK/gastrin-, somatostatin- and GIP- cells are found in the gastric epithelium during development [21]. In addition, increased numbers of cells positive for serotonin, CCK, proglucagon, somatostatin, and GIP are found in the intestine [21]. There more goblet cells and fewer enterocytes in *Hes1*-deficient mice, suggesting that the secretory lineage is augmented at the expense of enterocytes in the intestine (Figure 3) [21].

Transcription factors other than bHLH factors have also been shown to play critical roles during enteroendocrine cell differentiation. As mentioned above, concomitant with the premature differentiation of enteroendocrine cells in the *Hes1*-deficient animals [21], the *Pax4*, *Pax6*, *Nkx2.2*, *Isl-1* genes are activated in the gut [21]. Instead of controlling the global differentiation of enteroendocrine cells like the bHLH genes, these factors are likely to play important roles in fine-tuning cell specification decisions within the enteroendocrine population.

Zinc-finger transcription factors in enteroendocrine cell differentiation

The zinc-finger containing transcription factor Gfi1 is expressed in scattered cells throughout the developing intestine epithelium. Once the intestine matures, Gfi1 is found mainly in the crypts but also in some villi in the small intestine [55]. Double immunostaining analysis showed that Gfi1 expression is restricted to enteroendocrine lineages [55]. In addition, Gfi1 is expressed within the crypt in Math1-dependent progenitors, and Gfi1 expression is affected in *Math1*-

deficient embryos, suggesting that Gfi1 acts downstream of Math1 in the differentiation program (Figure 3B) [55].

Another zinc-finger protein, Insm1 (also termed IA-1), is found in scattered cells of the intestinal epithelium, many of which also co-express NeuroD1 and chromogranin A [56]. In *Insm1* null mice, substance P or neurotensin expressing endocrine cells are absent and serotonin, CCK or PYY expressing cells are present at a reduced number, while enteroendocrine precursors cells are generated normally [56]. This genetic analysis reveals that Insm1 controls differentiation of specific enteroendocrine lineages in the intestine and acts downstream from Notch and Ngn3 after the initial specification of enteroendocrine precursors.

Homeodomain transcription factors in enteroendocrine cell differentiation

As mentioned briefly above, many homeobox genes including *Isl-1*, *Pdx1*, *Nkx6.1* and *Nkx2.2* have been shown to be involved in enteroendocrine cell differentiation. *Isl-1* transactivates somatostatin expression in islet tumor cells *in vitro* [57] and its expression is restricted to G/D precursors (gastrin/somatostatin co-expressing cells) and a subpopulation of somatostatin cells in the stomach [58]. This implies that *Isl-1* could be involved in the asymmetric division of G/D precursors [58]. Unfortunately, thus far examination of the requirement of *Isl-1* in enteroendocrine differentiation has not been possible due to the early embryonic lethality of the *Isl-1* null mice [59].

Pdx1 is expressed in the pancreatic buds at the onset of pancreatic organogenesis [60]. Deletion of *Pdx1* leads to an arrest of pancreatic differentiation and altered duodenal morphology [61–64]. Most relevant here is the fact that the *Pdx1*-deficient duodenum is also devoid of gastrin cells [65]. Interestingly, *Pdx1*-deficient mice have an increased number of serotonin cells in the distal stomach [65]. *Pdx1* is clearly essential for normal gastrin cell development in the gastric epithelium; however, other factors must also contribute to this lineage since not all gastrin cells are *Pdx1* positive and a few gastrin cells still remain in the *Pdx1*-deficient mice [65].

Paired and homeodomain transcription factors in enteroendocrine cell differentiation

Two paired box (Pax) genes, *Pax4* and *Pax6*, are expressed throughout the gastrointestinal tract during development. Mice deficient for either *Pax4* or *Pax6* fail to properly elaborate pancreatic endocrine cells [66,67]. Similar to their role in the pancreas, the two genes also control important stages of enteroendocrine cell differentiation in the gut.

Deletion of *Pax4* affects enteroendocrine cell development only in the rostral gastrointestinal tract. In the stomach of *Pax4* deficient mice, the number of somatostatin and serotonin-expressing cells is significantly reduced, while gastrin-expressing cells are not affected. In the duodenum, serotonin-, secretin-, CCK-, GIP-, and PYY- expressing cell differentiation is severely impaired [68]. Interestingly, there are no significant differences in enteroendocrine cell numbers in the ileum and colon between the *Pax4* deficient mice and the controls [68].

Pax6 expression is also detected in the developing gastrointestinal epithelium. In *Pax6* deficient mice, there is a severely diminished elaboration of somatostatin- and gastrin-expressing cells in the distal stomach; however, serotonin-expressing cells are not affected. In addition, the number of GIP-expressing cells is reduced in the duodenum of *Pax6* deficient mice [68].

NK class and homeodomain transcription factors in enteroendocrine cell differentiation

Nkx6.1 expression is detected in the gastric mucosa, but not in the duodenum [69]. The majority of cells expressing Nkx6.1 are serotonin-expressing cells, with a smaller population expressing gastrin [70]. In *Pdx1*-deficient mice, Nkx6.1 expression is absent, indicating Pdx1 acts upstream of Nkx6.1 in the enteroendocrine cell program [70].

Nkx2.2 expression is first detected in the proximal forestomach and then in scattered cells throughout the small intestine, and expression persists through birth [71]. Many intestinal hormone-producing enteroendocrine populations are reduced in *Nkx2.2*-deficient mice, including serotonin-, CCK-, GIP-, and gastrin-expressing cells [71]. Interestingly, the number of ghrelin-expressing cells is significantly increased in the *Nkx2.2* deficient mouse [71]. Further analysis suggested that ghrelin-expressing cells are specified at the expense of other endocrine cell lineages in the intestine while the total number of enteroendocrine cells remains unchanged [71].

Nkx6.3 expression is detected exclusively within the epithelium of the antrum in developing, newborn and adult mice [72,73]. Phenotypic analysis using *Nkx6.3* null mice showed a selective and severe reduction in gastrin-secreting G cells and an increase in the number of somatostatin-expressing D cells. Interestingly, *Nkx6.3* null mice express normal levels of transcription factors (Pdx1, Pax6 and Ngn3) that are required for the development of gastrin-secreting G cells [29,30,65,68,73]. Conversely, normal expression of *Nkx6.3* was detected in the *Ngn3* null animals [73]. These data suggest that Nkx6.3 functions independently of other transcription factors in G cell differentiation.

Summary and future directions

Many unanswered questions remain regarding how enteroendocrine cells are specified in the gastrointestinal tract, and if it might be possible to control their relative contribution with therapeutic benefit. Unlike endocrine cells in the pancreas, which cluster together to form islets, enteroendocrine cells are scattered as individual cells throughout the gastrointestinal mucosa. Very little is known about what directs the region-specific expression of hormones in the gut. Although the functions of several key transcription factors during enteroendocrine cell differentiation have been uncovered using genetic means, exactly how these factors direct and control the gene expression in the multitude of endocrine cell types remains unresolved. It is also not known whether and to what extent these transcription factors control hormone expression in the adult. However, this limitation will be overcome in the near future through the use of inducible gene ablation with the Cre/LoxP system, which permits gene deletion at all stages of ontogeny. Because of the continuous renewal of the gastrointestinal epithelium throughout adult life, it might be possible one day to manipulate the differentiation of these cells for the treatment of metabolic and gastrointestinal diseases.

Acknowledgments

The authors thank Dr. Joshua R. Friedman for critical reading of the manuscript and Johanna Murray for providing the immunostaining images. Related work in KHK's laboratory was supported by NIH grants R01-DK053839 and R01-DK055342, and in CLM's laboratory by NIH grant R01-DK078606.

References

1. Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E. The "normal" endocrine cell of the gut: changing concepts and new evidences. *Ann N Y Acad Sci* 2004;1014:1–12. [PubMed: 15153415]

2. Cardoso WV, Lu J. Regulation of early lung morphogenesis: questions, facts and controversies. *Development* 2006;133:1611–24. [PubMed: 16613830]
3. Darlington GJ. Molecular mechanisms of liver development and differentiation. *Curr Opin Cell Biol* 1999;11:678–82. [PubMed: 10600708]
4. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006;43:S45–53. [PubMed: 16447274]
5. Rawlins EL, Hogan BL. Ciliated epithelial cell lifespan in the mouse trachea and lung. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L231–4. [PubMed: 18487354]
6. Zaret KS. Regulatory phases of early liver development: paradigms of organogenesis. *Nat Rev Genet* 2002;3:499–512. [PubMed: 12094228]
7. Barker N, Clevers H. Tracking down the stem cells of the intestine: strategies to identify adult stem cells. *Gastroenterology* 2007;133:1755–60. [PubMed: 18054544]
8. Barker N, van de Wetering M, Clevers H. The intestinal stem cell. *Genes Dev* 2008;22:1856–64. [PubMed: 18628392]
9. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 2007;449:1003–7. [PubMed: 17934449]
10. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian Theory of the origin of the four epithelial cell types. *Am J Anat* 1974;141:537–61. [PubMed: 4440635]
11. Fontaine J, Le Lievre C, NMLD. What is the developmental fate of the neural crest cells which migrate into the pancreas in the avian embryos? *Gen Comp Endocrinology* 1977;33:394–404.
12. Le Douarin NM. On the origin of pancreatic endocrine cells. *Cell* 1988;53:169–171. [PubMed: 3282672]
13. Le Douarin NM, Teillet MA. The migration of neural crest cells to the wall of the digestive tract in avian embryo. *J Embryol Exp Morphol* 1973;30:31–48. [PubMed: 4729950]
14. Rawdon BB, Andrew A. Origin and differentiation of gut endocrine cells. *Histol Histopathol* 1993;8:567–80. [PubMed: 8358166]
15. Hoffman J, Kuhnert F, Davis CR, Kuo CJ. Wnts as essential growth factors for the adult small intestine and colon. *Cell Cycle* 2004;3:554–7. [PubMed: 15044853]
16. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, Clevers H. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking *Tcf-4*. *Nat Genet* 1998;19:379–83. [PubMed: 9697701]
17. Pinto D, Gregorieff A, Begthel H, Clevers H. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev* 2003;17:1709–13. [PubMed: 12865297]
18. Lai EC. Notch signaling: control of cell communication and cell fate. *Development* 2004;131:965–73. [PubMed: 14973298]
19. Ray WJ, Yao M, Mumm J, Schroeter EH, Saftig P, Wolfe M, Selkoe DJ, Kopan R, Goate AM. Cell surface presenilin-1 participates in the gamma-secretase-like proteolysis of Notch. *J Biol Chem* 1999;274:36801–7. [PubMed: 10593990]
20. Jarriault S, Le Bail O, Hirsinger E, Pourquie O, Logeat F, Strong CF, Brou C, Seidah NG, Isral A. Delta-1 activation of notch-1 signaling results in HES-1 transactivation. *Mol Cell Biol* 1998;18:7423–31. [PubMed: 9819428]
21. Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P, Madsen OD. Control of endodermal endocrine development by *Hes-1*. *Nat Genet* 2000;24:36–44. [PubMed: 10615124]
22. Sasai Y, Kageyama R, Tagawa Y, Shigemoto R, Nakanishi S. Two mammalian helix-loop-helix factors structurally related to *Drosophila* hairy and Enhancer of split. *Genes Dev* 1992;6:2620–34. [PubMed: 1340473]
23. van Den Brink GR, de Santa Barbara P, Roberts DJ. Development. Epithelial cell differentiation--a Mather of choice. *Science* 2001;294:2115–6. [PubMed: 11739944]
24. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science* 1999;284:770–6. [PubMed: 10221902]

25. Gaiano N, Nye JS, Fishell G. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. *Neuron* 2000;26:395–404. [PubMed: 10839358]
26. Apelqvist A, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, Hrabe de Angelis M, Lendahl U, Edlund H. Notch signalling controls pancreatic cell differentiation. *Nature* 1999;400:877–81. [PubMed: 10476967]
27. Chitnis A, Henrique D, Lewis J, Ish-Horowicz D, Kintner C. Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene Delta. *Nature* 1995;375:761–6. [PubMed: 7596407]
28. de la Pompa JL, Wakeham A, Correia KM, Samper E, Brown S, JAR, Nakano T, Honjo T, Mak TW, Rossant J, Conlon RA. Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development* 1997;124:1139–1148. [PubMed: 9102301]
29. Jenny M, Uhl C, Roche C, Duluc I, Guillermin V, Guillemot F, Jensen J, Kedinger M, Gradwohl G. Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *Embo J* 2002;21:6338–47. [PubMed: 12456641]
30. Lee CS, Perreault N, Brestelli JE, Kaestner KH. Neurogenin 3 is essential for the proper specification of gastric enteroendocrine cells and the maintenance of gastric epithelial cell identity. *Genes Dev* 2002;16:1488–97. [PubMed: 12080087]
31. Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ. Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. *Genes Dev* 1997;11:2323–34. [PubMed: 9308961]
32. Yang Q, Bermingham NA, Finegold MJ, Zoghbi HY. Requirement of Math1 for Secretory Cell Lineage Commitment in the Mouse Intestine. *Science* 2001;294:2155–2158. [PubMed: 11739954]
33. Shroyer NF, Helmuth MA, Wang VY, Antalffy B, Henning SJ, Zoghbi HY. Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology* 2007;132:2478–88. [PubMed: 17570220]
34. Ben-Arie N, Bellen HJ, Armstrong DL, McCall AE, Gordadze PR, Guo Q, Matzuk MM, Zoghbi HY. Math1 is essential for genesis of cerebellar granule neurons. *Nature* 1997;390:169–72. [PubMed: 9367153]
35. Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N, Weintraub H. Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. *Science* 1995;268:836–44. [PubMed: 7754368]
36. Sommer L, Ma Q, Anderson DJ. neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. *Mol Cell Neurosci* 1996;8:221–41. [PubMed: 9000438]
37. Katz JP, Perreault N, Goldstein BG, Lee CS, Labosky PA, Yang VW, Kaestner KH. The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. *Development* 2002;129:2619–28. [PubMed: 12015290]
38. Mori-Akiyama Y, van den Born M, van Es JH, Hamilton SR, Adams HP, Zhang J, Clevers H, de Crombrughe B. SOX9 is required for the differentiation of paneth cells in the intestinal epithelium. *Gastroenterology* 2007;133:539–46. [PubMed: 17681175]
39. Schwitzgebel VM, Scheel DW, Connors JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development* 2000;127:3533–42. [PubMed: 10903178]
40. Heller RS, Jenny M, Collombat P, Mansouri A, Tomasetto C, Madsen OD, Mellitzer G, Gradwohl G, Serup P. Genetic determinants of pancreatic epsilon-cell development. *Dev Biol* 2005;286:217–24. [PubMed: 16122727]
41. Wang J, Cortina G, Wu SV, Tran R, Cho JH, Tsai MJ, Bailey TJ, Jamrich M, Ament ME, Treem WR, Hill ID, Vargas JH, Gershman G, Farmer DG, Reyen L, Martin MG. Mutant neurogenin-3 in congenital malabsorptive diarrhea. *N Engl J Med* 2006;355:270–80. [PubMed: 16855267]
42. Schonhoff SE, Giel-Moloney M, Leiter AB. Neurogenin 3-expressing progenitor cells in the gastrointestinal tract differentiate into both endocrine and non-endocrine cell types. *Dev Biol* 2004;270:443–54. [PubMed: 15183725]

43. Bjerknes M, Cheng H. Neurogenin 3 and the enteroendocrine cell lineage in the adult mouse small intestinal epithelium. *Dev Biol* 2006;300:722–35. [PubMed: 17007831]
44. Lopez-Diaz L, Jain RN, Keeley TM, VanDussen KL, Brunkan CS, Gumucio DL, Samuelson LC. Intestinal Neurogenin 3 directs differentiation of a bipotential secretory progenitor to endocrine cell rather than goblet cell fate. *Dev Biol* 2007;309:298–305. [PubMed: 17706959]
45. Mutoh H, Fung BP, Naya FJ, Tsai MJ, Nishitani J, Leiter AB. The basic helix-loop-helix transcription factor BETA2/NeuroD is expressed in mammalian enteroendocrine cells and activates secretin gene expression. *Proc Natl Acad Sci U S A* 1997;94:3560–4. [PubMed: 9108015]
46. Naya FJ, Stellrecht CM, Tsai MJ. Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. *Genes Dev* 1995;9:1009–19. [PubMed: 7774807]
47. Mutoh H, Naya FJ, Tsai MJ, Leiter AB. The basic helix-loop-helix protein BETA2 interacts with p300 to coordinate differentiation of secretin-expressing enteroendocrine cells. *Genes Dev* 1998;12:820–30. [PubMed: 9512516]
48. Ratineau C, Petry MW, Mutoh H, Leiter AB. Cyclin D1 represses the basic helix-loop-helix transcription factor, BETA2/NeuroD. *J Biol Chem* 2002;277:8847–53. [PubMed: 11788592]
49. Huang HP, Liu M, El-Hodiri HM, Chu K, Jamrich M, Tsai MJ. Regulation of the pancreatic islet-specific gene BETA2 (neuroD) by neurogenin 3. *Mol Cell Biol* 2000;20:3292–307. [PubMed: 10757813]
50. Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. Signalling downstream of activated mammalian Notch. *Nature* 1995;377:355–8. [PubMed: 7566092]
51. Chen H, Thiagalingam A, Chopra H, Borges MW, Feder JN, Nelkin BD, Baylin SB, Ball DW. Conservation of the Drosophila lateral inhibition pathway in human lung cancer: a hairy-related protein (HES-1) directly represses achaete-scute homolog-1 expression. *Proc Natl Acad Sci U S A* 1997;94:5355–60. [PubMed: 9144241]
52. Ishibashi M, Ang SL, Shiota K, Nakanishi S, Kageyama R, Guillemot F. Targeted disruption of mammalian hairy and Enhancer of split homolog-1 (HES-1) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. *Genes Dev* 1995;9:3136–48. [PubMed: 8543157]
53. Oellers N, Dehio M, Knust E. bHLH proteins encoded by the Enhancer of split complex of Drosophila negatively interfere with transcriptional activation mediated by proneural genes. *Mol Gen Genet* 1994;244:465–73. [PubMed: 8078474]
54. Ohsako S, Hyer J, Panganiban G, Oliver I, Caudy M. Hairy function as a DNA-binding helix-loop-helix repressor of Drosophila sensory organ formation. *Genes Dev* 1994;8:2743–55. [PubMed: 7958930]
55. Shroyer NF, Wallis D, Venken KJ, Bellen HJ, Zoghbi HY. Gfi1 functions downstream of Math1 to control intestinal secretory cell subtype allocation and differentiation. *Genes Dev* 2005;19:2412–7. [PubMed: 16230531]
56. Gierl MS, Karoulias N, Wende H, Strehle M, Birchmeier C. The zinc-finger factor Insm1 (IA-1) is essential for the development of pancreatic beta cells and intestinal endocrine cells. *Genes Dev* 2006;20:2465–78. [PubMed: 16951258]
57. Leonard J, Serup P, Gonzalez G, Edlund T, Montminy M. The LIM family transcription factor Isl-1 requires cAMP response element binding protein to promote somatostatin expression in pancreatic islet cells. *Proc Natl Acad Sci U S A* 1992;89:6247–51. [PubMed: 1352885]
58. Larsson LI, Tingstedt JE, Madsen OD, Serup P, Hougaard DM. The LIM-homeodomain protein Isl-1 segregates with somatostatin but not with gastrin expression during differentiation of somatostatin/gastrin precursor cells. *Endocrine* 1995;3:519–524.
59. Pfaff SL, Mendelsohn M, Stewart CL, Edlund T, Jessell TM. Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* 1996;84:309–20. [PubMed: 8565076]
60. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. *Embo J* 1993;12:4251–9. [PubMed: 7901001]
61. Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CV, Teitelman G. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal

- epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* 1995;121:11–8. [PubMed: 7867492]
62. Jonsson J, Ahlgren U, Edlund T, Edlund H. IPF1, a homeodomain protein with a dual function in pancreas development. *Int J Dev Biol* 1995;39:789–98. [PubMed: 8645563]
63. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 1994;371:606–9. [PubMed: 7935793]
64. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996;122:983–95. [PubMed: 8631275]
65. Larsson LI, Madsen OD, Serup P, Jonsson J, Edlund H. Pancreatic-duodenal homeobox 1 -role in gastric endocrine patterning. *Mech Dev* 1996;60:175–84. [PubMed: 9025070]
66. Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. *Nature* 1997;386:399–402. [PubMed: 9121556]
67. St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P. Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. *Nature* 1997;387:406–9. [PubMed: 9163426]
68. Larsson LI, St-Onge L, Hougaard DM, Sosa-Pineda B, Gruss P. Pax 4 and 6 regulate gastrointestinal endocrine cell development. *Mech Dev* 1998;79:153–9. [PubMed: 10349628]
69. Jensen J, Serup P, Karlsen C, Nielsen TF, Madsen OD. mRNA profiling of rat islet tumors reveals nkx 6.1 as a beta-cell-specific homeodomain transcription factor. *J Biol Chem* 1996;271:18749–58. [PubMed: 8702531]
70. Oster A, Jensen J, Edlund H, Larsson LI. Homeobox gene product Nkx 6.1 immunoreactivity in nuclei of endocrine cells of rat and mouse stomach. *J Histochem Cytochem* 1998;46:717–21. [PubMed: 9603782]
71. Desai S, Loomis Z, Pugh-Bernard A, Schunk J, Doyle MJ, Minic A, McCoy E, Sussel L. Nkx2.2 regulates cell fate choice in the enteroendocrine cell lineages of the intestine. *Dev Biol* 2008;313:58–66. [PubMed: 18022152]
72. Alanentalo T, Chatonnet F, Karlen M, Sulniute R, Ericson J, Andersson E, Ahlgren U. Cloning and analysis of Nkx6.3 during CNS and gastrointestinal development. *Gene Expr Patterns* 2006;6:162–70. [PubMed: 16326147]
73. Choi MY, Romer AI, Wang Y, Wu MP, Ito S, Leiter AB, Shivdasani RA. Requirement of the tissue-restricted homeodomain transcription factor Nkx6.3 in differentiation of gastrin-producing G cells in the stomach antrum. *Mol Cell Biol* 2008;28:3208–18. [PubMed: 18347062]

STOMACH

- Mucus cell
- Chief cell
- Endocrine cell
- Parietal cell

Chromogranin A

INTESTINE

- Enterocyte
- Goblet cell
- Endocrine cell
- Stem cell
- Paneth cell

Chromogranin A

Figure 1.

Illustration of the major cell types found in the gastrointestinal epithelium. Gastric epithelium contains mucus, chief, Parietal, and endocrine cells, whereas enterocyte, goblet, endocrine, and Paneth cells are found in the intestinal epithelium. Intestinal stem cells are depicted in red in the diagram. Chromogranin A staining marks all enteroendocrine cells in the gastrointestinal epithelium.

Endocrine cell

Non-Endocrine cell

Figure 2.

Involvement of the Notch signaling pathway during endocrine cell differentiation. The Notch ligand Delta is first up-regulated in differentiating endocrine cells. It binds to Notch on neighboring non-endocrine cells, which results in release of the Notch intracellular domain (Notch IC). Notch IC translocates into the nucleus and interacts with RBP-Jk to activate target genes such as Hes1. Hes1 then inhibits proendocrine bHLH transcription factors such as Ngn3.

Figure 3.

Overview of enteroendocrine cell differentiation in the intestinal epithelium. Stem cells marked by *Lgr5* are located in the crypt and give rise to all four cell types found in the intestinal epithelium. Expression of *Math1/Hes1* restricts secretory lineages (Paneth, goblet and endocrine cells) from the absorptive lineage (enterocyte). Once this initial specification is established, lineage-specific transcription factors such as *Sox9* for Paneth cells, *Klf4* for goblet cells and *Gfi1/Ngn3/NeuroD* for enteroendocrine cells are required for the proper differentiation of their respective secretory lineages.