J Mol Hist (2005) 36:15–24

Epithelial lineages of the small intestine have unique patterns of GATA expression

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Received 2 July 2004 and in revised form 2 September 2004

Summary

The ability of the GATA family of factors to interact with numerous other factors, co-factors, and repressors suggests that they may play key roles in tissues and cells where they are expressed. Adult mouse small intestine has been shown to express GATA-4, GATA-5, and GATA-6, where they have been implicated in the activation of a number of intestinal genes. Determination of which GATA factor(s) are involved in a specific function in tissues expressing multiple family members has proven difficult. The immunohistochemical analysis presented here demonstrate that within the mouse small intestine GATA-4/-5/-6 are found to be uniquely distributed among the various differentiated lineages of the intestinal epithelium. Among differentiated cells GATA-4 is found only in the villous enterocytes. GATA-5 is absent from enterocytes, but was found in the remaining lineages: goblet, Paneth and enteroendocrine. Additionally, high levels of GATA-6 are found in only one of these differentiated cell types, the enteroendocrine lineage. The observed distribution suggests that the GATA factors may have distinct roles in lineage allocation, lineage maintenance, and/or terminal differentiation events in small intestine.

Introduction

The luminal surface of the small intestine is folded into finger-like projections called villi and recessed pits known as crypts. The epithelium covering this surface is a deceptively simple structure, yet one that exhibits defined patterning along various axes as well as a huge regenerative capacity that maintains that pattern throughout the life of the organism. Stem cells anchored near the base of the crypt give rise to a population of transit amplifying cells, also located in the crypt (Moog 1981, Madara & Trier 1987, Gordon 1989, Potten *et al.* 1997). These cells give rise to the four types of differentiated epithelial cells. The majority, ~80–90%, of the cells generated are destined to become enterocytes that are responsible for the absorption and metabolism of various molecules. About 5–10% of the cells become goblet cells that synthesize and secrete components of the mucous barrier that coat the intestine. Enteroendocrine cells are a heterogeneous group of cells that represent <1% of the epithelium. These cells collectively secrete a variety of hormone molecules. These three types of cells are generally found on the villus. The fourth type, the Paneth cell, is found at the base of the crypt. Lineage restricted differentiation occurs as cells migrate bi-directionally away from the stem cells. For cells migrating toward the villus, differentiation is complete by the time the cells reach the crypt-villus juncture and emerge onto the villus proper. Over the next three to five days, cells migrate up the villus structure until they are sloughed or undergo apoptosis at the villus tip. Differentiated Paneth cells migrate to the crypt base and they occupy this position for about three weeks before they are replaced. Allocation of cells into these four lineages is known to change in response to disease states, environmental or surgical insult, or position along the small intestine (Dvorak & Dickersin 1980, Ehrmann *et al.* 1990, Kamal *et al.* 2001, Couto *et al.* 2002, Porter *et al.* 2002, Wasserberg *et al.* 2003, Wong, *et al.* 2004). However, the complex set of cues that control this process are unknown. The current study shows that the GATA proteins are uniquely distributed to play a potential role in this process.

The GATA family are zinc finger transcription factors that recognize and bind to DNA with the sequence WGATAR. The six members are traditionally divided into two groups: those involved in hematopoietic development and differentiation (GATA-1/-2/-3) and those involved in mesendodermal development (GATA-4/-5/-6). GATA-4/-5/-6 all have a wide-ranging expression pattern throughout many mouse organs (Molkentin 2000). Northern analysis of GATA-4/-5/-6 has shown that these factors are expressed in adult small intestine (Arceci *et al.* 1993, Morrisey *et al.* 1996, Morrisey *et al.* 1997, Dusing *et al.* 2003) where they have been implicated in the regulation of numerous intestinal genes (Fitzgerald

et al. 1998, Boudreau *et al.* 2002, Divine *et al.* 2003, Dusing *et al.* 2003, Kiela *et al.* 2003, Divine *et al.* 2004). Yet, a precise role for the GATA factors in intestinal development and differentiation has not been established. Both GATA-4 and GATA-6 null embryos die before birth, e8-9 and e5.5-7.5 respectively, well before intestinal development (Molkentin *et al.* 1997, Koutsourakis *et al.* 1999). GATA-5 null mice survive with no gross gastrointestinal defect, although a detailed analysis of the intestinal epithelium was not described (Molkentin *et al.* 2000). The studies described here elucidate a pattern for GATA-4/-5/-6 expression within the mouse small intestine. Moreover, the segregation of these factors into cells of the different lineages suggests the intriguing possibility that they play a role in intestinal epithelial lineage commitment and/or maintenance analogous to that played by GATA-1/-2/-3 in hematopoietic development.

Materials and methods

Mice

All mice were sacrificed upon arrival from the supplier according to approved NIH and CCHMC protocols (CCHMC/IACUC protocol # 3D05039).

Immunohistochemistry

Rabbit IgG and antibodies generated against GATA-4, GATA-5, GATA-6, and chromogranin A were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Each experiment was performed on multiple mice. Tissues of interest were harvested from 10–12-week old FVB/N mice from Taconic Farms (Germantown, NY). A 2 cm segment of mouse small intestine adjacent to the pyloric sphincter was harvested as duodenum. A 2 cm jejunum sample was harvested from the middle of the length of the small intestine. Ileum was a 2 cm segment harvested adjacent to the cecum. Intestinal segments were flushed with warm 1XPBS and fixed for 8 h in 4% paraformaldehyde/1XPBS at 4 °C. Tissues were rinsed twice in 70% ethanol/1XPBS and stored in 70% ethanol. Tissue embedding, sectioning and periodic acid Schiff-Alcian Blue histochemical stain were performed by the CCHMC Department of Pathology. 6 μm sections were heated 15 min to 65 °C, cooled to room temperature, deparaffinized and rehydrated. Antigen retrieval was performed by heating slides for 10 min at 95 °C in 10 mM sodium citrate pH 6.0 and then cooling them covered at room temperature until they reached 60–65 °C. Slides were washed 3 times for 2 min in water, 1 time for 10 minutes in 0.5% hydrogen peroxide, and 2 times for 5 min in 1XPBS. Slides were then incubated 1 h in a humidified chamber at room temperature covered with a blocking solution containing 1XPBS/1.5% goat serum. Blocking serum was removed and a solution containing the primary antibody at 1 μg/ml (anti-GATA-4/-5/-6) or 0.5 μg/ml (anti-chromogranin A) in the blocking serum was added. Control slides received rabbit IgG at 1 μg/ml in lieu of a specific primary antibody. Slides were allowed to incubate overnight at 4 °C in a humidified chamber. Detection of primary antibody binding was done using the rabbit ABC Staining Kit (Santa Cruz Biotechnology) as per the manufacturer's instructions. Sections were counterstained briefly in hematoxylin and then dehydrated. Sections were visualized on an Axioplan 2 microscope with AxioVision 4 software by Zeiss (Thornwood, NY). Photos comparing intestinal segments are from the same mouse and were processed simultaneously.

Fluorescent immunohistochemistry

Goat antibody directed against chromogranin A was from Santa Cruz Biotechnology. FITC-conjugated donkey anti-goat and Texas Red-conjugated donkey anti-rabbit fluorescent secondary antibodies were from Jackson ImmunoResearch Laboratories, Inc (West Grove, PA). Slides were treated as indicated in immunohistochemistry through the overnight binding of the primary antibody except that the hydrogen peroxide wash step was omitted. Slides were washed 3 times in 1XPBS. All subsequent steps were performed in the dark. Sections were covered with blocking serum containing 1XPBS/1.5%donkey serum/1.5 μg/ml FITC donkey anti-goat antibody/1.5 μg/ml Texas Red donkey anti-rabbit antibody and incubated for 1 h at room temperature in a humidified chamber. Slides were washed 3 times with 1XPBS, twice with 0.1 M sodium phosphate, and twice with 0.05 M sodium phosphate. Sections were stored in the dark at 4 °C and photographed within 72 h of completion.

Results

GATA-4/5/6 are localized within the epithelial layer of the small intestine

Previous Northern analysis of GATA-4/-5/-6 showed that the mRNA for each of these factors is expressed in mouse small intestine and that for GATA-4 and GATA-5 levels vary along the length of the small intestine (Dusing *et al.* 2003). Immunohistochemical analysis using antibodies specific for each of the GATA factors was performed to localize the proteins within the small intestine along the anterior/posterior (A/P) axis. These studies revealed that all three proteins could be found within the intestinal epithelium (Figure 1–3) where GATA protein is indicated by the deposition of a brown

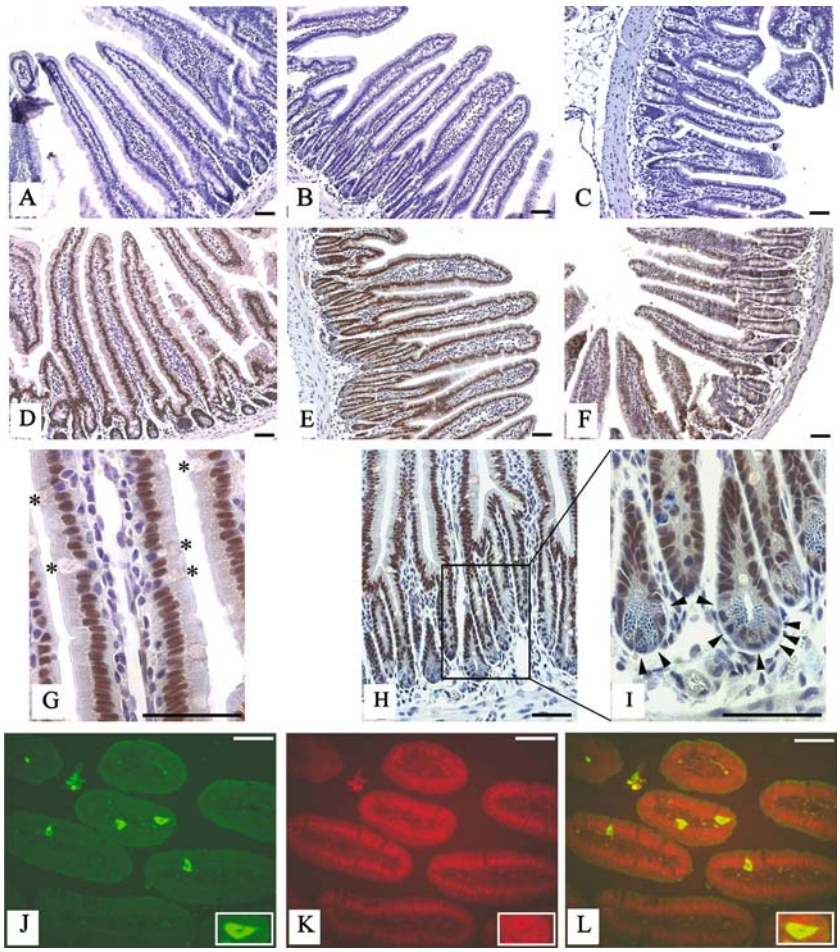

Figure 1. GATA-4 is only observed in intestinal enterocytes. Immunohistochemical analysis of adult mouse duodenum (A, D), jejunum (B, E, G–L) and ileum (C, F) using either a non-specific antibody (A–C) or an antibody directed against GATA-4 (D–I) are shown. Goblet cells in G are marked with an asterisk. Paneth cells in I are indicated with arrowheads. Fluorescent immunohistochemistry of mouse jejunum using the pan-enteroendocrine marker anti-chromogranin A (J) and anti-GATA-4 (K) antibodies do not label the same cells (Merged image L). Scale bar in each panel is 50 μm. Insets in panels J–L are 2.5X original.

product. Each protein was observed to have a unique, but consistent distribution within the cell types of the intestinal epithelium (described below), regardless of their relative position along the length of the small intestine. Fluorescent immunohistochemical studies also confirmed that the GATA factors are differentially distributed among the cell lineages that comprise the epithelial layer.

GATA-4 is expressed only in the enterocytic lineage

Representative studies of mouse intestine are shown in Figure 1, where GATA-4 protein can be seen distributed widely throughout the nuclei of the intestinal epithelial cells (Figure 1D–F). In contrast, only the blue hematoxylin counterstain was seen in control samples (Figure 1A–C). Immunohistochemical analysis showed that the majority (85–90%) of the villous epithelial cells are positive for GATA-4. These GATA-4 positive cells correspond to those of the enterocyte lineage (Figure 1G). 10–15% of the villous cells, however, did not express GATA-4. Based on cell morphology, a significant number of these GATA-4 negative cells appear to be those of the goblet lineage and are marked by an asterisk (Figure 1G). The remaining GATA-4 negative cells found on the villus represent the enteroendocrine lineage. Fluorescent immunohistochemical studies were used to confirm that these cells are indeed enteroendocrine cells. Antibodies directed against the pan-enteroendocrine marker chromogranin A (Figure 1J) and GATA-4 (Figure 1K) each labeled a non-overlapping population of cells (Figure 1L) indicating that GATA-4 is not expressed in this lineage. Sections incubated with a control antibody showed no fluorescence (data not shown). The region at the base of the crypts throughout the intestine did not show any evidence of GATA-4 expression (Figure 1H–I). The GATA-4 negative cells located at the base of the crypt (arrowheads in Figure 1I) possess apical secretory granules that are characteristic of the Paneth cell lineage (Madara and Trier 1987).

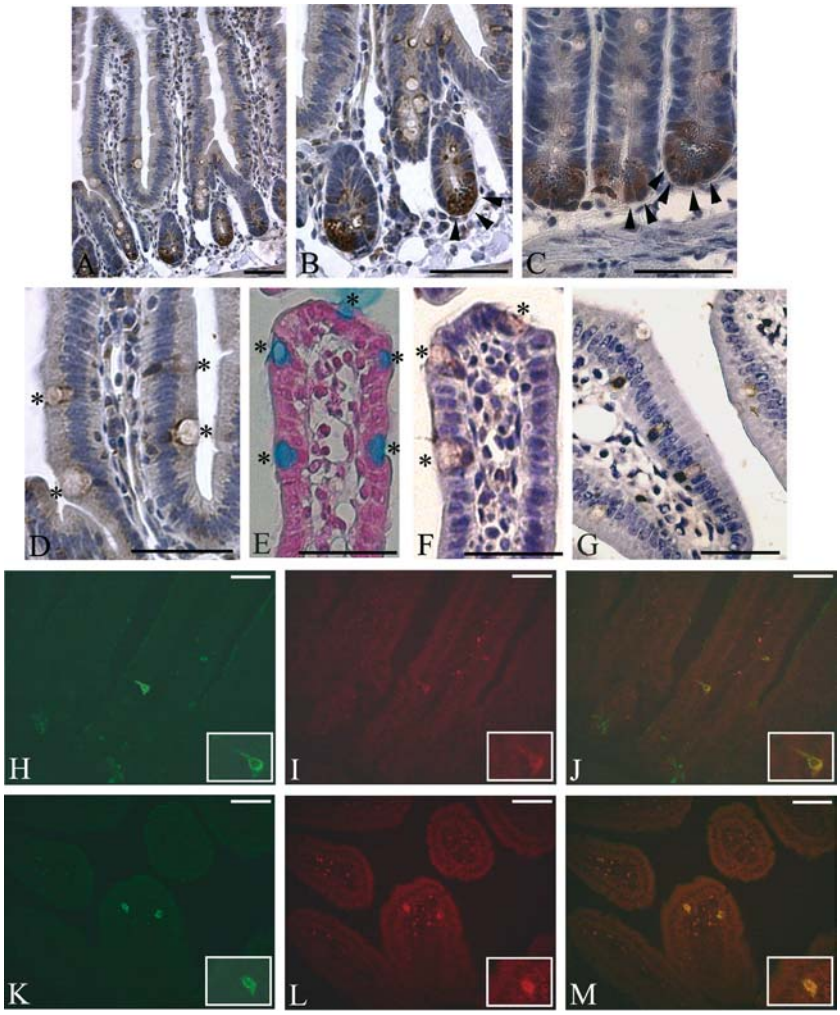

Figure 2. GATA-5 is expressed in Paneth, goblet and enteroendocrine cells. Immunohistochemical analysis of mouse jejunum using GATA-5 specific antibody is shown in A–D, F, and G. E and F represent serial sections stained with either the goblet cell stain Alcian Blue (E) or the GATA-5 antibody (F). Asterisks in D–F indicate goblet cells. Arrowheads indicate some of the Paneth cells in B and C. GATA-5 positive cells that lack goblet morphology are shown in G. Fluorescent immunohistochemistry of mouse jejunum using anti-chromogranin A (H, K) and anti-GATA-5 (I, L) antibodies label the same cells (Merged images J, M). Scale bar in each panel is 50 μm. Insets in panels H–M are 2.5X original.

The transit amplifying and stem cells are positioned above the Paneth cells within the crypt. Interestingly, many of the cells in this region, but not all, stain positive for GATA-4 (Figure 1H–I).

Reduced levels of GATA-4 immunostaining were consistently observed in the ileal epithelium (Figure 1F) compared to the epithelium of either duodenum (Figure 1D) or jejunum (Figure 1E). In studies utilizing less antibody, GATA-4 staining in the ileum and the transit-amplifying region of the crypt was observed to be much less intense and in some sections not evident (data not shown). A similar cellular distribution profile for GATA-4 in mouse small intestine has been observed previously (Boudreau *et al.* 2002, Divine *et al.* 2004). This pattern of GATA-4 protein along the length of the intestine correlates with the pattern previously observed for GATA-4 mRNA. GATA-4 is the predominant GATA mRNA throughout the proximal 2/3 of the small intestine, exhibiting a sharp decrease as the intestine transitions from jejunum to ileum (Dusing *et al.* 2003).

GATA-5 is found in cells of the secretory lineages

GATA-5 protein is also readily observed in all intestinal segments localized to the epithelial layer of the small intestine (Figure 2A), where in some cells it is observed to be distributed throughout the nucleus and cytoplasm of the cell. Overall, the cellular distribution of GATA-5 protein within the epithelium is very different from that seen for GATA-4. In all intestinal segments, high level GATA-5 staining is observed within the crypt region, concentrated within cells located at the base of the crypt containing apical secretory granules (Figure 2B–C). These GATA-5 positive cells correspond to the lineage previously discussed

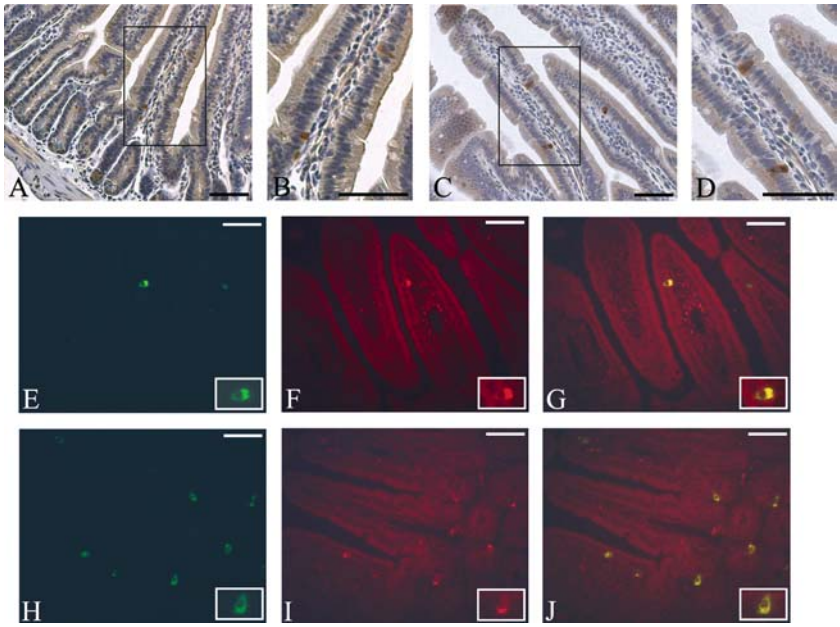

Figure 3. GATA-6 is expressed primarily in enteroendocrine cells. Immunohistochemical analysis of mouse jejunum with anti-GATA-6 antibody (A–D) stains a very small number of epithelial cells. Fluorescent IHC of mouse jejunum using anti-chromogranin A (E, H) and anti-GATA-6 (F, I) antibodies label the same cells (Merged images G, J). Magnification of A, C, and E–J are at 40X. Scale bar in each panel is 50 μm. Insets in E–J are 2.5X original.

and identified as Paneth cells. Along the villous epithelium, the majority of cells corresponding to the enterocytic lineage do not express detectable GATA-5. However, a subset of epithelial cells is clearly GATA-5 positive (Figure 2D,F,G). As demonstrated below, these GATA-5 expressing cells are those of the enteroendocrine and goblet lineages.

Examination of alternate serial sections with either Alcian Blue, a histochemical stain specific for goblet cells (Figure 2E), or the GATA-5 antibody (Figure 2F) demonstrate that there is very good correlation of the Alcian Blue positive cells with the GATA-5 positive ones confirming that these cells (marked by asterisk) are goblet cells. Two of the goblet cells observed in Figure 2E are not represented in the section on Figure 2F and therefore, there is no GATA-5 staining.

Not all the GATA-5 positive cells located on the villus could be identified as goblet cells (Figure 2G). The remaining GATA-5 positive cells were suspected to be cells of the enteroendocrine lineage. Fluorescent immunohistochemical analysis like that used in Figure 1 was used to test for co-localization of GATA-5 (Figure 2I,L) and the enteroendocrine marker chromogranin A (Figure 2H,K). Both markers are observed in a small number of cells that are generally triangular in shape (see insets for each photo). The merged image (Figure 2J,M) shows co-localization of these markers confirming that enteroendocrine cells are GATA-5 positive. Co-localization of these two markers was not always visible as the fluorescent staining observed with the GATA-5 antibody in these cells was generally quite low. So, it remains possible that enteroendocrine cells, known to be a heterogeneous population, are a mixture of GATA-5 positive and GATA-5 negative sub-lineages. Sections receiving the non-specific antibody showed no fluorescence (data not shown).

GATA-5 was found at significant levels in cells of all three of the secretory lineages. Two of these secretory lineages, Paneth and goblet, increase in number by about two-fold in ileum compared to jejunum (Moe 1955, Cheng 1974, Elmes 1976, Bry *et al.* 1994). This increase in number of the cells observed to express GATA-5 protein correlates with the two to three-fold increase in GATA-5 mRNA observed previously in ileum versus proximal intestine (Dusing *et al.* 2003).

GATA-6 is expressed primarily in the enteroendocrine lineage only. GATA-6 was found to be extremely low or absent from the great majority of the epithelial cells of both the villus and the crypt (Figure 3A,C). A very small number of discrete cells stain strongly positive for GATA-6 within the cytoplasm (Figure 3B,D). These cells are very few in number and have the characteristic triangular shape of enteroendocrine cells. Fluorescent immunohistochemical studies demonstrated co-localization of GATA-6 (Figure 3F,I) and chromogranin A (Figure 3E,H) within the same cells (Figure 3G,J), thereby, confirming their identity as enteroendocrine cells. In fact, all observed GATA-6 positive cells were also found to be chromogranin A positive and vice versa (not shown) indicating that all the sub-types of cells within this variant lineage express GATA-6.

Enteroendocrine cells make up only a small portion of the epithelium and are found peppered throughout its

length. Both the observed pattern of GATA-6 protein and the GATA-6 mRNA levels, which were observed to be low throughout the length of the mouse small intestine (Dusing *et al*. 2003), are consistent with expression in this diverse population of cells. The remaining lineages: enterocytes, goblet and Paneth cells were not observed to express detectable levels of GATA-6 under these conditions. Recently a more widespread distribution of GATA-6 has been described in mouse intestinal epithelium (Divine *et al*. 2004). Using similar conditions to the ones described in that work (i.e. higher antibody concentrations), the high level staining of the enteroendocrine population was still readily apparent. Additionally, low level GATA-6 staining could be observed throughout the epithelium similar to that described (data not shown). It is possible that the enterocyte lineage expresses low levels of GATA-6 in addition to high levels of GATA-4. Regardless, it is clear that high-level GATA-6 expression is confined to the enteroendocrine cells of the epithelium.

Therefore, in addition to a defined pattern of expression along the length of the small intestine (confirmed here), GATA-4, GATA-5, and GATA-6 each show a discrete lineage-specific distribution in the intestinal epithelium as well. High-level expression is confined to enterocytes for GATA-4, and to enteroendocrine cells for GATA-6, while GATA-5 is highly expressed in the Paneth, goblet and enteroendocrine cell populations.

Discussion

GATA-4, GATA-5, and GATA-6 are found localized to the mouse intestine throughout its development in increasingly restricted patterns within the epithelium (Nemer & Nemer 2003). All three are found within the anterior intestinal portal prior to fusion on e9. Overlapping expression persists through e11.5 in the pseudostratified epithelium lining the primitive gut tube where the immature villous epithelium is a mixture of GATA-4/-5/-6 positive cells. As the columnar epithelium forms and begins to differentiate late in rodent gestation, e15–e19 (Calvert & Pothier 1990), markers for differentiated enterocytes, goblet and endocrine cells can be detected. Developing villi at e17.5 have already been described as exhibiting GATA-4 and GATA-6 in a complementary pattern, a pattern that we find persists into adulthood (enterocyte *versus* enteroendocrine). This distinction is not surprising as markers of enteroendocrine differentiation are often developmentally the first to be observed (Ratineau *et al*. 2003). At this same stage the GATA-4 and GATA-5 expression patterns are overlapping. The complementary GATA-4 and GATA-5 expression observed in adult (enterocyte *versus* enteroendocrine/goblet) is apparently not fully established. The robust GATA-5 expression in the crypt associated with the Paneth cells was not observed in these studies. Paneth cells are not evident in mice until two weeks after birth when the intervillus space is remodeled and crypts are generated. At this time morphologically recognizable Paneth cells can be detected in the newly formed crypts (Bry *et al*. 1994) and presumably high-levels of GATA-5 would then first be observed. The results from the experiments presented here show that the pattern of GATA-4/-5/-6 expression in adult mouse epithelium is similar to that described through e17.5 (Nemer & Nemer 2003) and suggests that the GATA factors become segregated into the different cell types as the epithelium completes the process of differentiation. In the adult mouse intestinal epithelium, high-levels of GATA-4 are found within the enterocytes of the proximal 2/3 of the small intestine and at somewhat lower levels in both ileal enterocytes and most immature cells populating the crypt. GATA-5 is found in cells of all three of the secretory lineages. While, GATA-6 is observed at high-levels only in enteroendocrine cells (Figure 4).

Previous studies observed GATA-5 and GATA-6 distributed widely throughout the mouse intestinal epithelium in late gestation (e17.5) (Nemer & Nemer 2003). However, expression of GATA-5 protein in adult intestinal epithelium had not been previously observed (Divine *et al*. 2004). The studies described here show that GATA-5 protein has a widespread distribution throughout the adult intestinal epithelium more consistent with the pattern observed during embryogenesis. This distribution is also supported by the 2–3 fold increase in GATA-5 mRNA in ileum where the number of Paneth and goblet cells is higher

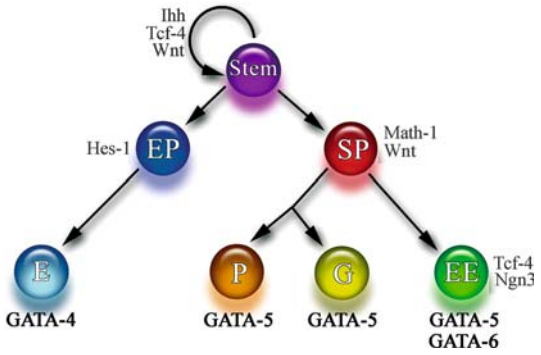

Figure 4. Lineage commitment in intestine. Four lineages of epithelial cells: enterocyte (E – light blue), Paneth (P – orange), goblet (G – yellow) and enteroendocrine (EE – green) are derived from a common progenitor cell (stem-purple). Enterocytes are believed to arise from a separate branch that may or may not utilize an enterocyte precursor cell (EP – dark blue). There is strong evidence that the secretory lineages (EE, P, and G) arise along a common branch that is separate from the enterocytic branch and that a secretory precursor cell may be utilized (SP – red). Proteins known to be vital to epithelial differentiation are shown in gray. Expression of the GATA factors among the differentiated cell populations suggests a role for them in lineage allocation.

(Dusing *et al.* 2003). The apparent discrepancies may be related to the wide variety of GATA antibodies available, since this study and previous studies (1 embryonic, 1 adult) were performed utilizing different antibodies (Nemer and Nemer 2003, Divine *et al.* 2004). Previously, low level of GATA-6 protein was observed uniformly distributed throughout the epithelium (Divine *et al.* 2004). While uniform low-level expression was observed in some of our experiments, the major site of GATA-6 protein was observed to be the enteroendocrine lineage of epithelial cells. These cells make up a very small proportion of the epithelial cells (< 1%, sometimes as few as 2–3/cross-section) and could easily be overlooked, especially in sections exhibiting the low-level epithelial staining. Both studies are consistent with the low uniform levels of GATA-6 mRNA observed throughout the intestine, the result of either low-level uniform expression throughout intestinal epithelium or high-level expression in a few cells uniformly distributed throughout the epithelium. However, the latter distribution of GATA-6 into enteroendocrine cells is consistent with the embryonic distribution that is described as complementary to GATA-4 (enterocyte). The presence of GATA-6 in cells of the enteroendocrine lineage is also supported by the presence of GATA-6 protein in STC-1 an enteroendocrine-like cell line derived from intestine (Dimaline *et al.* 1997).

Immunohistochemical staining with GATA-5 and GATA-6 antibodies revealed an apparent localization for these proteins throughout the nucleus and cytoplasm of the cell. The cytoplasmic staining observed does not appear to be the result of non-specific antibody adherence as no other antibody tested, including controls, resulted in such a staining pattern. While this observation is unusual for a GATA protein, there are other examples of cytoplasmic localization for mammalian GATA factors (Siltanen *et al.* 2003, Nemer & Nemer 2003, Gillio-Meina *et al.* 2003, Caramori *et al.* 2001, Divine *et al.* 2004). One very elegant study in ovarian epithelial cells, utilizing the same GATA-6 antibody as the studies described in this paper, has shown that GATA-6 can be found localized exclusively to the cytoplasm. In this study, both nuclear and cytoplasmic GATA-6 antibody-reactive protein was specifically lost in cells treated with GATA-6 siRNA (Capo-chichi *et al.* 2003) compelling evidence for the antibody's specificity and the observation that GATA-6 can be observed within the cytoplasm. A yeast GATA protein, Gln3p, has also been observed to translocate to the cytoplasm as a direct result of phosphorylation or other modification. In this case, the factor has been shown to re-enter the nucleus under the direction of certain cell signals (Bertram *et al.* 2000, Carvalho *et al.* 2001, Cooper 2002). Modification of some mammalian GATA factors has been observed (Partington & Patient 1999, Morimoto *et al.* 2000, Liang *et al.* 2001, Tremblay & Viger 2003). In one instance, phosphorylation resulted in export to the cytoplasm (Morisco *et al.* 2001). However, that cytoplasmic GATA factor was rapidly degraded and transport in the reverse direction has not as yet been described in a system other than yeast. Regardless, a generally cytoplasmic localization of GATA-5 and GATA-6 would explain why various laboratories investigating gene regulation in intestine have been able to readily identify GATA-4 but not GATA-5 or GATA-6 protein from mouse intestinal nuclear extracts (Boudreau *et al.* 2002, Dusing *et al.* 2003). The lack of GATA-5 and GATA-6 in these extracts has always been puzzling, given that GATA-5 and GATA-6 mRNA are present in ample amounts to generate detectable levels of these proteins. The results presented here suggest that this may be due to a depletion or absence of these proteins from the nuclei of the cells that are the source of the extract. As yet, we have no explanation for the observed localization of GATA-5 and GATA-6 and more studies will have to be done to determine the function and significance of cytoplasmic localization of these GATA factors in the intestinal epithelium.

The GATA proteins seem uniquely positioned to play a pivotal role in lineage allocation in the intestinal epithelium. Very little is understood about the mechanisms governing this process (Clatworthy & Subramanian 2001, de Santa Barbara *et al.* 2003). Some of the key molecules believed to be involved are shown in Figure 4. Maintenance, but not establishment, of the adult stem cell population has been shown to require molecules like Indian hedgehog (Ramalho-Santos *et al.* 2000), Tcf-4 (Korinek *et al.* 1998) and the Wnt pathway (Pinto *et al.* 2003). Development of the secretory lineages requires the bHLH protein Math1. Absence of Math1 results in a complete lack of goblet, endocrine and Paneth cells with no apparent affect on the enterocytes (Yang *et al.* 2001). This protein is believed to utilize the Delta/Notch signaling pathway to promote the separation of the secretory lineages from the enterocytic lineage. Many components of this signaling pathway are expressed in adult mouse intestine, most within the crypt region (Schroder & Gossler 2002). The involvement of this mechanism is supported by the phenotype observed in mice with a targeted Hes-1 gene (Jensen *et al.* 2000). Notch signaling activates transcription of Hes1, a repressor of bHLH (Math1) transcription. Loss of Hes1 results in derepression of Math1 expression and an increase in allocation of cells to the secretory lineages at the expense of the enterocyte lineage. Derivation of intestinal endocrine cells is known to require the bHLH protein, neurogenin3, as loss of neourogenin3 results in a specific loss of all enteroendocrine cells within the small intestine (Jenny *et al.* 2002). The HMG domain protein Tcf4 is also required (Korinek *et al.* 1998) for differentiated endocrine cells.

GATA proteins have been shown to be key regulators of stem cell maintenance, lineage allocation and terminal gene expression profiles during hematopoiesis. A number of intriguing similarities exist between hematopoietic cell differentiation and intestinal epithelial cell differentiation. Each of these tissues in the adult animal must maintain a specific high-volume pattern of cell replication and differentiation. Portions of hematopoiesis have also been shown to rely on members of the hedgehog, Tcf/LEF/Wnt, Delta/Notch/HES, and GATA families of transcription factors (Orkin 1995, Staal *et al.* 2001, Hirasawa *et al.* 2002) for the correct cell-type specification. Interestingly, in hematopoietic differentiation the GATA proteins have been shown to be an integral part of the Notch signaling pathway (Kumano *et al.* 2001, Staal *et al.* 2001, Anderson *et al.* 2002). In some cases, decisions regarding stem cell maintenance versus differentiation are related to increasing expression levels of one GATA family member with respect to another (Grass *et al.* 2003). Studies also suggest that the relative dosage of an individual GATA factor may be involved in differentiation decisions (Kulessa *et al.* 1995, McDevitt *et al.* 1997).

The GATA proteins have been observed to play a role in regulating a number of genes expressed in terminally differentiated cells of the intestinal epithelium. The results presented here suggest that these genes may be activated and/or repressed by GATA-4/-5/-6 in a lineage-specific manner. In addition, given the observed distribution of these proteins within the intestinal epithelium, it seems possible that GATA-4/-5/-6 utilize mechanisms similar to those utilized by GATA-1/-2/-3 during hematopoietic development in order to facilitate intestinal epithelial differentiation. GATA-4 has been observed in the less differentiated cells of the crypt where the process of differentiation commences and the differential distribution of GATA-4/-5/-6 proteins among the lineages of the intestinal epithelium suggests that these proteins may be involved in the processes of lineage-specification or lineage maintenance. Additional studies will be required to determine if the GATA factors are involved in earlier stages of epithelial differentiation or whether their role is limited to the events of terminal differentiation.

Acknowledgements

Thank you to Elizabeth Maier and Brad Bemiss for their tireless efforts in daily laboratory operations. Special thanks to Jim Wells, Jennifer Kordich, and Gregg Sabla for their impetus to this project and for their technical assistance. This work was funded by NIH grant R01 DK52343 (to D.A.W). Tissue processing and sectioning was performed in collaboration with Cincinnati Children's Hospital Medical Center Department of Pathology and was supported by DDRDC grant R24 DK064403.

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