

# Origin of the Zebrafish Endocrine and Exocrine Pancreas

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Here, we report a detailed fate map of the zebrafish pancreas at the early gastrula stage of development (6 hours postfertilization; hpf). We show that, at this stage, both pancreas and liver progenitors are symmetrically localized in two broad domains relative to the dorsal organizer. We demonstrate that the dorsal and ventral pancreatic buds can derive from common progenitor pools at 6 hpf, but often derive from independent populations. Endocrine vs. exocrine pancreas show a similar pattern of progenitors, consistent with descriptions of the dorsal bud being strictly endocrine and the ventral bud primarily exocrine. In general, we find that endocrine/dorsal bud progenitors are located more dorsally than the exocrine pancreas/ventral bud progenitors. Later in gastrulation (10 hpf), pancreas progenitors have migrated to bilateral domains at the equator of the embryo. Our fate map will assist with design and interpretation of future experiments to understand early pancreas development. *Developmental Dynamics* 236:1558–1569, 2007. © 2007 Wiley-Liss, Inc.

**Key words:** zebrafish; pancreas; fate map; insulin; islet; ventral pancreatic bud; dorsal pancreatic bud

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## INTRODUCTION

The zebrafish is emerging as a useful model for the study of vertebrate organogenesis, combining powerful genetics with accessible, optically clear embryos that allow cell fates and movements to be traced. In recent years, several studies have revealed that the zebrafish pancreas shares many properties with the mammalian pancreas. Thus, the zebrafish pancreas consists of both an endocrine and an exocrine component (Slack, 1995; Biemar et al., 2001), and many molecular pathways acting during early pancreatic development are evolutionarily conserved (e.g., Biemar et al., 2001; Gu et al., 2004; Stafford et

al., 2004, 2006; Molotkov et al., 2005). However, to understand how pancreatic progenitors become separated from the previously undifferentiated cells of the endoderm germ layer, one must first determine when and where these cells originate. In this study, we have produced a fate map of the zebrafish endocrine and exocrine pancreas that will assist future studies of pancreas development.

As in other species, the zebrafish pancreas develops from distinct dorsal and ventral buds associated with the gut tube (Field et al., 2003; Wallace and Pack, 2003; Fig. 1). A transgenic line expressing green fluorescent protein in the postpharyngeal endoderm

(gut-GFP, Field et al., 2003) has allowed visualization of the morphogenesis of the two zebrafish pancreatic buds. The dorsal bud is located slightly posterior to the ventral bud, and we hereafter refer to them as the posterodorsal and anteroventral buds, respectively. The posterodorsal bud is the first to form, becoming morphologically apparent at 24 hours postfertilization (hpf); immunohistochemistry shows this bud is composed exclusively of endocrine pancreatic cell types. By 40 hpf, the anteroventral bud has formed on the ventral side of the gut tube; this second bud comprises primarily exocrine cells, although there are a few endocrine cells

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present. By 52 hpf, the two buds have merged (Field et al., 2003; schematized in Fig. 1) to form a single pancreatic islet of endocrine cells, surrounded by exocrine tissue, with the entire structure localized to the right side of the ventral midline.

Molecular markers reveal that pancreatic development is well under way at stages that precede bud morphogenesis. The earliest molecular marker of the zebrafish pancreas is the transcription factor Pdx1 (Biemar et al., 2001). Expression of Pdx1 in pancreatic progenitors is conserved in zebrafish, *Xenopus*, mouse, and chick; in all species Pdx1 labels both exocrine and endocrine pancreas progenitor cells, as well as part of the duodenum (Offield et al., 1996; reviewed in Edlund, 2002). The zebrafish *pdx1* gene is first expressed as early as the 10-somite stage (14 hpf), in bilateral patches of endoderm on either side of the midline (Biemar et al., 2001; schematized in Fig. 1A). Other pancreas cell type-specific markers begin to be expressed a short time later. The *islet-1* gene is first expressed in the endoderm by the 12-somite stage (15 hpf), specifically in cells of the endocrine pancreas (Biemar et al., 2001), and at later stages in the mesenchyme surrounding the pancreas and liver (Ahlgren et al., 1997). Expression of *insulin*, a marker of differentiated  $\beta$ -cells, can also be detected from 15 hpf (Biemar et al., 2001; Kim et al., 2005). Like *pdx1*, *insulin* expression is initially detected in cells located on both sides of the midline; however, by 24 hpf, the *insulin*-expressing  $\beta$ -cells are localized to the posterodorsal bud together with other endocrine pancreas-specific cell types (Biemar et al., 2001; Field et al., 2003). In contrast, markers of the exocrine pancreas progenitors, *mn2* and *ptf1a*, cannot be detected until 24 hpf and 32 hpf, respectively (Wendik et al., 2004; Zecchin et al., 2004; Lin et al., 2004). In summary, expression of cell type-specific molecular markers reveals a differentiation program in which a subset of endodermal cells become specified as pancreatic progenitors and go on to differentiate into distinct endocrine and exocrine pancreatic cell types.

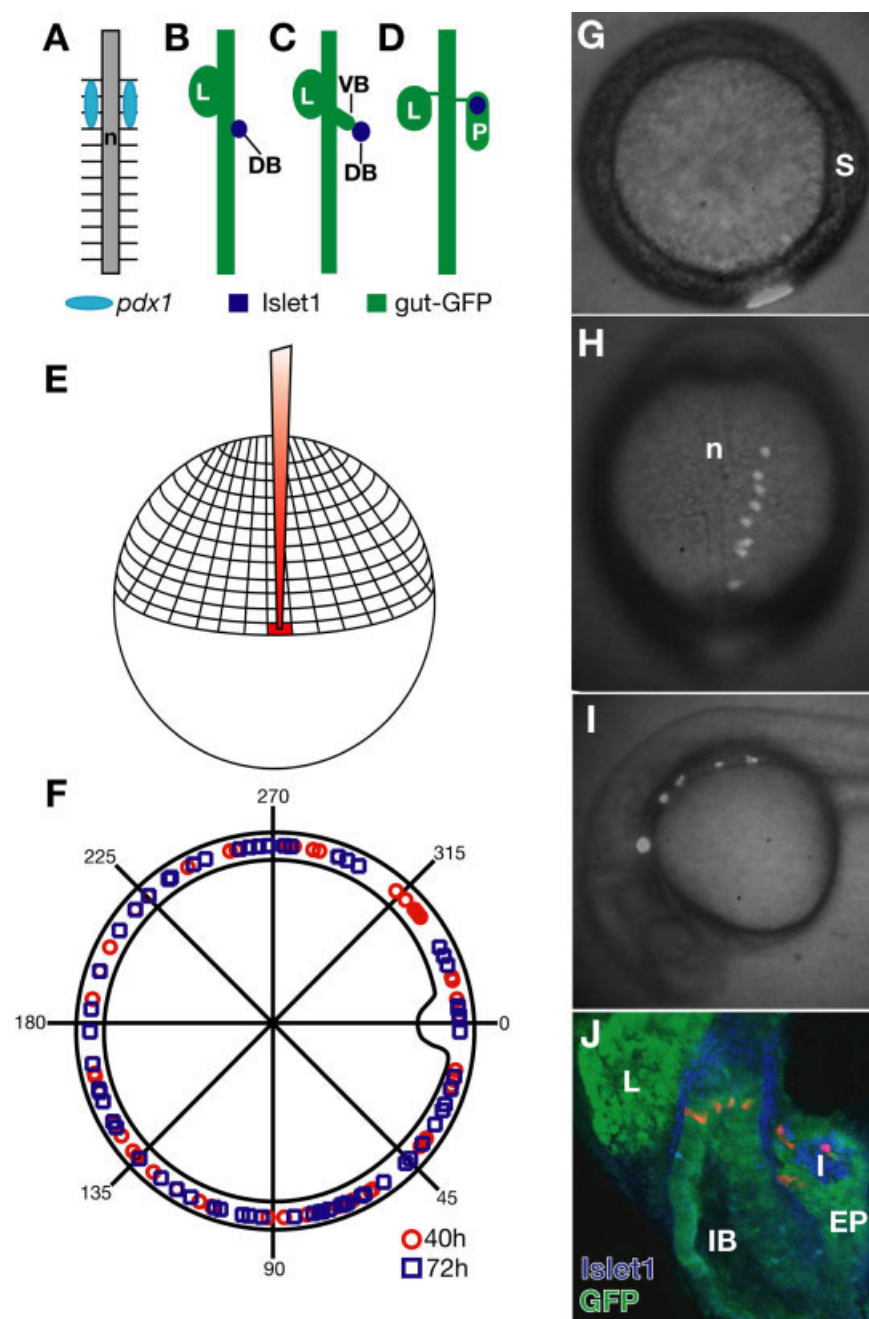
Fate maps can provide useful information about the location of progeni-

tor cells at developmental stages that precede expression of cell type-specific molecular markers. Previous work has shown that fate maps for all three germ layers of the zebrafish are established by early gastrulation (approximately 6 hpf; Kimmel et al., 1990; Woo and Fraser, 1995; Shih and Fraser, 1995; Melby et al., 1996; Warga and Nüsslein-Volhard, 1999; Vogeli et al., 2006). Fate maps are generated by labeling cells at early developmental stages and then determining what region or regions of the embryo the labeled cells contribute to at later developmental stages. Fate maps thus provide a probabilistic representation of where cells within a particular region are likely to be located at a later developmental stage, but do not provide any information on the state of commitment of the labeled cells. By contrast, tests of commitment require cell transplantation: cells can be termed committed if, when transplanted to an ectopic location, their differentiation program continues unperturbed. Experiments of this type have revealed that the majority of zebrafish endoderm cells are committed to an endodermal fate at 5 hpf, just as gastrulation begins (David and Rosa, 2001). These committed endoderm cells express the Sox17 transcription factor, a general endodermal marker, which is essential for endoderm development (David and Rosa, 2001; Reiter et al., 2001; Aoki et al., 2002). No experiments thus far have addressed when endodermal derivatives become committed to their specific fates.

Previously, a fate map of the zebrafish endoderm was generated by labeling cells in the blastula before gastrulation begins, establishing the location of their progeny in the late blastula at 5 hpf, before formation of germ layers, and again in the gastrula at 6 hpf, once the dorsal axis can be unambiguously identified, and then observing which endodermal organs these cells contributed to at 5 days post fertilization (Warga and Nüsslein-Volhard, 1999). This fate map showed that just before the involution movements of gastrulation begin, all the endodermal precursor cells are located within four cell diameters of the blastoderm margin. Once gastrulation commences, these endoderm progenitor cells are the earliest cell popula-

tion to internalize. The fate map further showed that the dorsoventral position of endoderm precursors in the early gastrula correlates approximately with their anteroposterior position in the 5 day old gut tube. For example, cells close to the dorsal organizer become pharyngeal endoderm, an anterior derivative, whereas cells from the ventral-most part of the gastrula become intestinal endoderm, a posterior derivative. This study also provided some limited evidence suggesting that progenitors of the asymmetric foregut organs, the pancreas and liver, may also be asymmetrically organized in the gastrula. Warga and Nüsslein-Volhard's (1999) data suggested that liver precursors derive from a dorsal region on the right side of the gastrula and a ventral region on the left, while the pancreas derives from dorsal regions on both sides, as well as a ventral region on the right side of the gastrula. These results raise the possibility that, already by the gastrula stage, some endodermal progenitors are asymmetrically localized.

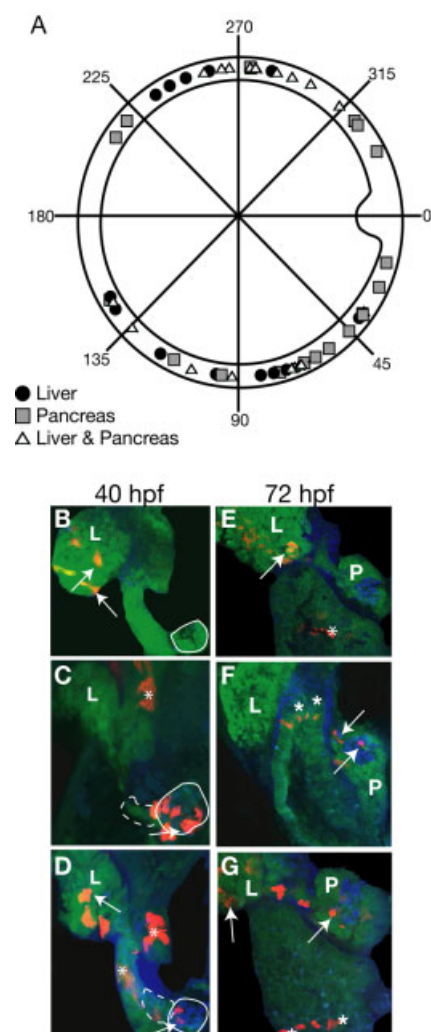
Most vertebrate fate maps that include endoderm have investigated broad general divisions of its derivatives from anterior to posterior along the gut (reviewed in Fukuda and Kikuchi, 2005). However, a fate map of the mouse liver and ventral foregut has been established (Tremblay and Zaret, 2005), and a fate map of the tadpole gut has revealed that the progenitors of *Xenopus* dorsal and ventral pancreatic buds are segregated as early as the neurula stage (stage 14; Chalmers and Slack, 2000). The former zebrafish fate map (Warga and Nüsslein-Volhard, 1999) did not supply extensive information on where zebrafish pancreatic progenitors originate within the undifferentiated early endoderm, nor did it address whether endocrine and exocrine pancreas cell types, or posterodorsal and anteroventral pancreatic buds, derive from the same or separate pools of endodermal progenitors. To address these questions, we have reinvestigated where progenitors of the pancreas originate from, and how these cells move, to produce the differentiated cell types. Only with this knowledge in hand can we hope to properly understand the inductive interactions critical to pancreas cell type specification.



**Fig. 1.** Development of the pancreas in zebrafish and general fate map methodology. **A:** *pdx1* is expressed bilaterally adjacent to the notochord of the anterior trunk at 14 hours postfertilization (hpf; based on Biemar et al., 2001). **B–D:** Morphogenesis of the pancreatic buds in zebrafish (based on Field et al., 2003). **B:** 24 hpf. The posterodorsal bud is visible. **C:** At 40 hpf. Both posterodorsal and anteroventral buds are visible, but have not fused. **D:** At 52 hpf. The buds have fused. **E:** One marginal blastomere was injected at the 512- to 2000-cell stage. **F:** Location of injected cells that gave rise to endoderm. Red circles were locations for the 40 hpf analysis, and blue squares were for the 72 hpf analysis. **G–J:** Embryos were imaged at various time points using brightfield and fluorescence, and photos were merged. **G:** At 6 hpf. **H:** At 10 hpf. **I:** At 24 hpf. **J:** Dorsal view of 72 hpf embryo showing rhodamine dextran-labeled cells in the exocrine and endocrine pancreas. Anti-*Islet1* (blue) labels the endocrine pancreas and anti-green fluorescent protein (GFP; green) labels the postpharyngeal endoderm in *gut-GFP* embryos. DB, posterodorsal pancreatic bud; EP, exocrine pancreas; I, pancreatic islet; IB, intestinal bulb; L, liver; N, notochord; P, pancreas; S, embryonic shield; VB, anteroventral pancreatic bud.

In this study, we establish a more complete early gastrula fate map of the zebrafish pancreas. We compare

the locations of pancreas and liver progenitors, of posterodorsal and anteroventral pancreatic bud progeni-



**Fig. 2.** Fate map of liver and pancreatic progenitors at 6 hours postfertilization (hpf). **A:** Polar plot showing location at 6 hpf of progenitors of the liver and pancreas. The embryos from the 40 hpf and 72 hpf analyses were combined. Gray squares are pancreatic progenitors, black circles are liver progenitors, and white triangles indicate a mixed liver and pancreas population. **B–G:** All confocal images are individual slices from a Z-stack. **B:** Embryo with labeled cells in the liver bud at 40 hpf. **C:** Embryo with labeled cells in the pancreatic bud. **D:** Embryo with labeled cells in the liver and pancreatic buds at 40 hpf. **E:** Example of embryo with labeled cells only in the liver at 72 hpf. **F:** Embryo with labeled cells in the pancreas at 72 hpf. **G:** Embryo with rhodamine dextran-labeled cells in the liver and the pancreas at 72 hpf. Arrows point to labeled cells that colocalize with the structure of interest. Asterisks (\*) point to rhodamine dextran-labeled cells that are not in the pancreas or liver. Dashed lines outline the anteroventral bud and solid lines outline the posterodorsal bud. L, liver; P, pancreas

tors, and of endocrine and exocrine pancreatic cell progenitors and find that liver and pancreas progenitors do



**TABLE 1. Average Dorsoventral Positions of Clones Contributing to Specific Endodermally Derived Tissues at 40 hpf<sup>a</sup>**

	Pharynx	Esophagus	Swim bladder	Posterodorsal bud	Anteroventral bud	Liver	Intestinal bulb <sup>b</sup>	Intestine
Ave	33	47	54	64	79	83	83	123
SD	30	22	18	33	31	31	42	47

<sup>a</sup>Average (Ave) dorsoventral positions (and standard deviation; SD) are indicated for all clones that gave rise to each endodermal structure. Location is indicated in degrees between the dorsal organizer (0°) and the ventral side (180°). Clones from both left and right hand sides have been combined. hpf, hours postfertilization.

<sup>b</sup>Note that others have referred to intestinal bulb as “stomach.”

not show strict asymmetric localization at 6 hpf as previously suggested. Our extended fate map also reveals that progenitors of the posterodorsal and anteroventral pancreatic buds are located in different yet overlapping domains. Endocrine and exocrine pancreatic progenitors are located in similar domains to the posterodorsal and anteroventral pancreatic buds, respectively, consistent with previous observations that the posterodorsal bud is exclusively endocrine and the anteroventral bud primarily exocrine. Our results suggest that endocrine progenitors derive from a population of gastrula stage cells that is, on average, located more dorsally than exocrine progenitors. We also determine the location of pancreatic progenitors in the late gastrula (10 hpf), as our previous studies demonstrated that this is an important developmental stage when retinoic acid signals are received by the endoderm to specify pancreas fate (Stafford and Prince, 2002; Stafford et al., 2006). We find that pancreas progenitors move significant distances during gastrulation to converge bilaterally on either side of the midline at the anterior of the trunk by 10 hpf. In summary, our studies indicate that, at the onset of gastrulation, there are already biases in the fates of pancreatic precursors, with respect to bud and associated histotype, dependent on where they originate in the gastrula. Moreover, by the end of gastrulation, pancreatic precursors are found to populate a unique equatorial latitude of the endodermal layer.

## RESULTS

This study expands on a previous fate map of the endoderm generated by

one of us (R.M.W.) and Nüsslein-Volhard (1999), by specifically addressing where progenitors of the pancreas originate in the gastrula of the zebrafish embryo. The earlier study showed that a cell labeled at the blastula margin of the 1,000-cell stage embryo has a high probability of giving rise to endoderm. Following a similar approach, we labeled single marginal blastomeres of gut-GFP embryos at the 512- to 2,000-cell stage with 70-kD lysinated rhodamine dextran (Fig. 1E) and localized the labeled progeny relative to the embryonic shield (dorsal organizer) of the early gastrula to determine the clone's position relative to dorsoventral (Fig. 1G). To simplify analysis and data presentation, we divided the embryo into quadrants: left-dorsal (1 degree–90 degrees), left-ventral (91 degrees–180 degrees), right-ventral (181 degrees–270 degrees), and right-dorsal (271 degrees–360 degrees). In each experimental embryo, we detected 2–16 rhodamine dextran-labeled cells at 6 hpf. The positions shown in the polar plots depicting our fate map data (e.g., Fig. 1F) indicate the mean location along the blastoderm margin of each clone of labeled cells.

Labeled cells were localized again at tail bud (10 hpf; Fig. 1H) and at 24 hpf (Fig. 1I), and their final locations and fate were determined either at 40 hpf, a stage immediately before fusion of posterodorsal and anteroventral pancreatic buds, or at 72 hpf, when marker analysis allows exocrine and endocrine pancreas fates to be distinguished (Fig. 1J). A total of 360 embryos were labeled, 125 of which had labeled cells in endodermal derivatives at later stages (40 hpf, 57 embryos; 72 hpf, 68 embryos; Fig. 1F).

Consistent with the previous report (Warga and Nüsslein-Volhard, 1999), not all of these 125 clones were germ layer restricted to the endoderm: some also contributed to mesodermal derivatives, and in almost all cases labeled blastomeres also contributed to the extraembryonic enveloping layer (data not shown). Statistical analyses confirmed that the locations of the endoderm progenitor populations were randomly distributed along the margin at 6 hpf (see Experimental Procedures section), again consistent with previous fate maps using this methodology (Warga and Nüsslein-Volhard, 1999; Kimmel et al., 1990). As predicted by the Warga and Nüsslein-Volhard endoderm fate map (1999), we found a positive correlation between a labeled clone's dorsoventral location at 6 hpf and its later anteroposterior position in the differentiating gut (Tables 1–4). Because the liver and pancreas are a relatively small portion of the gut, only 57 of the 125 endodermal clones contributed labeled cells to liver and/or pancreatic tissues (Tables 3, 4). These 57 clones form the basis for the remainder of our analysis.

## Fate Map of the Pancreas and Liver in Zebrafish

To determine the location of progenitors of the pancreas and the liver, we combined our analyses at 40 and 72 hpf (Fig. 2A). We found that progenitor cells contributing to either organ could derive from any of the four early gastrula stage quadrants (Fig. 2A). However, clones that contributed to the liver but not the pancreas (“liver-only” clones,  $n = 15$ ; Fig. 2B,E) were more likely to lie within the ventral half of the gastrula (Fig. 2A). By con-

**TABLE 2. Average Dorsoventral Positions of Clones Contributing to Specific Endodermally Derived Tissues at 72 hpf<sup>a</sup>**

	Pharynx	Esophagus	Endocrine pancreas	Swim bladder	Exocrine pancreas	Liver	Intestinal bulb*	Intestine
Ave	28	72	78	87	91	101	102	117
SD	28	36	39	48	35	29	33	43

<sup>a</sup>Average (Ave) dorsoventral positions (and standard deviation; SD) are indicated for all clones that gave rise to each endodermal structure. Location is indicated in degrees between the dorsal organizer (0°) and the ventral side (180°). Clones from both left- and right-hand sides have been combined. hpf, hours postfertilization.

<sup>b</sup>Note that others have referred to intestinal bulb as “stomach”.

**TABLE 3. Relationships of Cellular Contributions to Different Endodermal Tissues Based on the 40 hpf Analysis<sup>a</sup>**

	Pharynx	Esophagus	Swim bladder	Posterodorsal bud	Anteroventral bud	Intestinal bulb	Liver	Intestine
Pharynx	<b>13</b>	1	0	0	0	2	1	0
Esophagus	1	<b>4</b>	0	0	0	0	0	0
Swim bladder	0	0	<b>4</b>	3	4	2	3	1
Posterodorsal bud	1	0	3	<b>15</b>	6	4	8	2
Anteroventral bud	0	0	4	6	<b>11</b>	4	8	1
Intestinal bulb	0	0	2	4	4	<b>13</b>	4	1
Liver	1	0	3	8	8	4	<b>16</b>	1
Intestine	0	0	1	2	1	1	1	<b>15</b>

<sup>a</sup>The intersection between row and column gives the number of specimens that had cells localized in both tissues. Numbers in bold represent the total number of clones contributing to each tissue. Individual clones contributed to one, two, or more tissues. Tissues are listed in dorsoventral order from Table 1. hpf, hours postfertilization.

**TABLE 4. Relationships of Cellular Contributions to Different Endodermal Tissues Based on the 72 hpf Analysis<sup>a</sup>**

	Pharynx	Esophagus	Endocrine pancreas	Swim bladder	Exocrine pancreas	Liver	Intestinal bulb	Intestine
Pharynx	<b>15</b>	1	0	1	0	0	0	0
Esophagus	1	<b>12</b>	5	6	5	6	6	0
Endocrine pancreas	0	5	<b>16</b>	4	13	8	12	2
Swim bladder	1	6	4	<b>11</b>	5	3	5	0
Exocrine pancreas	0	5	13	5	<b>19</b>	11	13	2
Liver	0	6	8	3	11	<b>21</b>	15	4
Intestinal bulb	0	6	12	5	13	15	<b>27</b>	2
Intestine	0	0	2	0	2	4	2	<b>17</b>

<sup>a</sup>The intersection between row and column gives the number of specimens that had cells localized in both tissues. Numbers in bold represent the total number of clones contributing to each tissue. Individual clones contributed to one, two, or more tissues. Tissues are listed in dorsoventral order from Table 1. hpf, hours postfertilization.

trast, clones that contributed to the pancreas but not the liver (“pancreas-only” clones,  $n = 19$ ; Fig. 2C,F) tended to lie within the dorsal half of the gastrula (Fig. 2A). These liver-only progenitors were located significantly further from the dorsal organizer than the pancreas-only progenitors (Mann–

Whitney  $U$  Test,  $P = 0.01$ ). Consistent with these data the average location of all clones that contributed to the liver was more ventral than the average location of all clones that contributed to the pancreas (Tables 1, 2). Clones that contributed to both pancreas and liver ( $n = 23$ ; Fig. 2D,G) tended to be

located in more intermediate positions (Fig. 2A). We found a slight bias for both pancreas and liver clones to come from the left-dorsal quadrant, although this is largely explained by the increased number of endodermal clones labeled in that quadrant (Fig. 1F): 54% of the clones that gave rise to

endoderm in the left-dorsal quadrant gave rise to pancreatic or liver tissues as compared to 39% for the left-ventral, 42% for the right-ventral, and 45% for the right-dorsal. These data do not support the hypothesis of a robust left/right asymmetrical distribution of progenitors. In summary, while a single labeled blastomere can contribute to both liver and pancreas, there is nevertheless bias for more dorsally located endoderm cells in the early gastrula to contribute to the pancreas, and more ventrally located endoderm cells to contribute to the liver.

### Fate Map of the Posterodorsal and Anteroventral Pancreatic Buds

To determine whether progenitor cells for the posterodorsal and anteroventral buds derive from specific locations in the gastrula, we analyzed our labeled clones at a stage in development when both pancreatic buds are visible but not fused (40 hpf). We found that individual clones of cells contributed to the posterodorsal bud, anteroventral bud, or frequently both (Fig. 3B–D; Table 3). Table 3 also indicates that while many of the clones contributed to two or more tissue types, these contributions tended to be limited to tissues that are closely juxtaposed. In general, the majority of clones that contributed to the posterodorsal but not anteroventral bud originated from both dorsal quadrants (9/10 clones, Fig. 3A). By contrast, clones that contributed to the anteroventral but not posterodorsal bud ( $n = 5$ ) tended to originate from more ventral locations. Clones that contributed only to the posterodorsal bud were significantly closer to the dorsal organizer than clones that contributed only to the anteroventral bud (Mann-Whitney  $U$  test,  $P = 0.029$ ). Clones of cells that contributed to both buds ( $n = 5$ ) tended to be located in intermediate positions between these domains (Fig. 3A). We conclude that, although a single labeled clone can contribute to both pancreatic buds, there is nevertheless some bias by early gastrula stage for more dorsally located endoderm cells to give rise to posterodorsal bud derivatives, and more ventrally located endoderm cells to

give rise to anteroventral bud derivatives.

### Fate Map of the Exocrine and Endocrine Pancreas

The pancreas is a composite organ consisting of both exocrine and endocrine cells. We tested whether progenitors of endocrine vs. exocrine cells derive from the same respective regions of the 6 hpf fate map as posterodorsal vs. anteroventral bud cells. For this study, we raised labeled embryos to 72 hpf, when the pancreatic buds have merged and enlarged. At this stage, we could easily distinguish endocrine cells from exocrine by monitoring endocrine-specific expression of *islet1* (Fig. 4C–E). Data from 72 hpf, shown in Figure 2A, was re-analyzed by excluding clones that contributed to liver but not pancreas and asking what proportion of the remaining clones contributed to endocrine, exocrine, or combined endocrine and exocrine pancreatic cell types (Fig. 4A; Table 4).

Consistent with the 40 hpf fate map data, we found that clones that gave rise to endocrine and/or exocrine pancreas derived from all four quadrants of the gastrula ( $n = 22$ ; Fig. 4). The clones that contributed to the endocrine but not exocrine pancreas (endocrine-only;  $n = 3$ ) were located in the dorsal half of the embryo at 6 hpf, while the majority of clones that contributed to the exocrine but not endocrine pancreas (exocrine-only clones; 5/6) were located in the ventral half of the embryo (Fig. 4A,B). The more dorsal location of endocrine-only clones relative to exocrine-only clones was statistically significant as determined by the Mann-Whitney  $U$  test ( $P = 0.024$ ). Notably, we found no bias toward right or left side. We also found that clones that contributed to both exocrine and endocrine pancreas (mixed clones;  $n = 13$ ) were scattered randomly around the margin (Rayleigh test for uniform circularity,  $P > 0.10$ ; Fig. 4A). However, further examination revealed that mixed clones that gave rise to a low percentage of exocrine cells were located more dorsally, whereas mixed clones that gave rise to a high percentage of exocrine cells were located more ventrally (Fig. 4B). We conclude that, like the bias at

6 hpf for posterodorsal vs. anteroventral pancreatic bud, there is a correlated bias for endocrine vs. exocrine pancreatic cell types. In general, cells located more dorsally within the early gastrula have a higher probability of giving rise to endocrine cell types of the posterodorsal bud, whereas cells located more ventrally have a higher probability of giving rise to exocrine cell types of the anteroventral bud (compare Fig. 3A with 4A).

### Location of Pancreatic Progenitors at Tail Bud

In addition to determining the location of the pancreas progenitors at 6 hpf, we asked where they are located at 10 hpf, when retinoic acid is required to specify their fate (Stafford and Prince, 2002). At this developmental stage, the embryo completely encompasses the yolk cell, and expression of *sox17* (Alexander and Stainier, 1999) reveals that the endodermal cells are distributed extensively over the yolk cell in broad bilateral swaths extending dorsolaterally from the midline (Fig. 5A,B). Examining embryos in dorsal view at 10 hpf (tail bud stage), and then again at 40 hpf, allowed us to determine the likely position of the pancreatic progenitors at 10 hpf. We did this by superimposing all the individual cells from multiple clones that gave rise to pancreas (Fig. 5C; 20 embryos). For each clone, the labeled cells were pseudocolored with a unique color, revealing a region that included cells of every color (gray square in Fig. 5C). This region should represent the common domain where pancreatic progenitors are located at 10 hpf.

To test the hypothesis that the equatorial domain indicated in Figure 5C does indeed include pancreatic progenitors, we performed time-lapse video microscopy of labeled clones in *pdx1*-GFP transgenic fish (Huang et al., 2001). Labeled endoderm cells were followed from 10 hpf until approximately 16 hpf, after onset of *pdx1*-GFP expression (Biemar et al., 2001) in the pancreatic domain. By tracing backward in time, we were able to determine that cells which contributed to the Pdx-GFP-labeled pancreas were located at approximately the level of the first somite when it

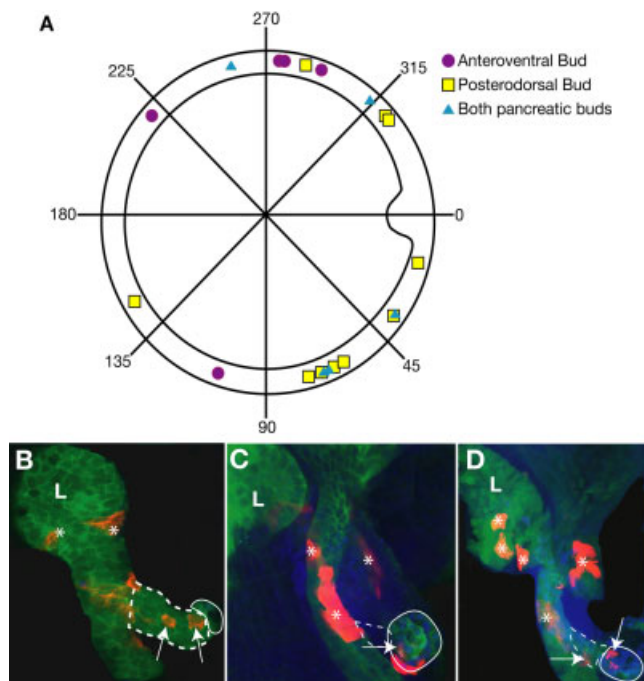


Fig. 3.

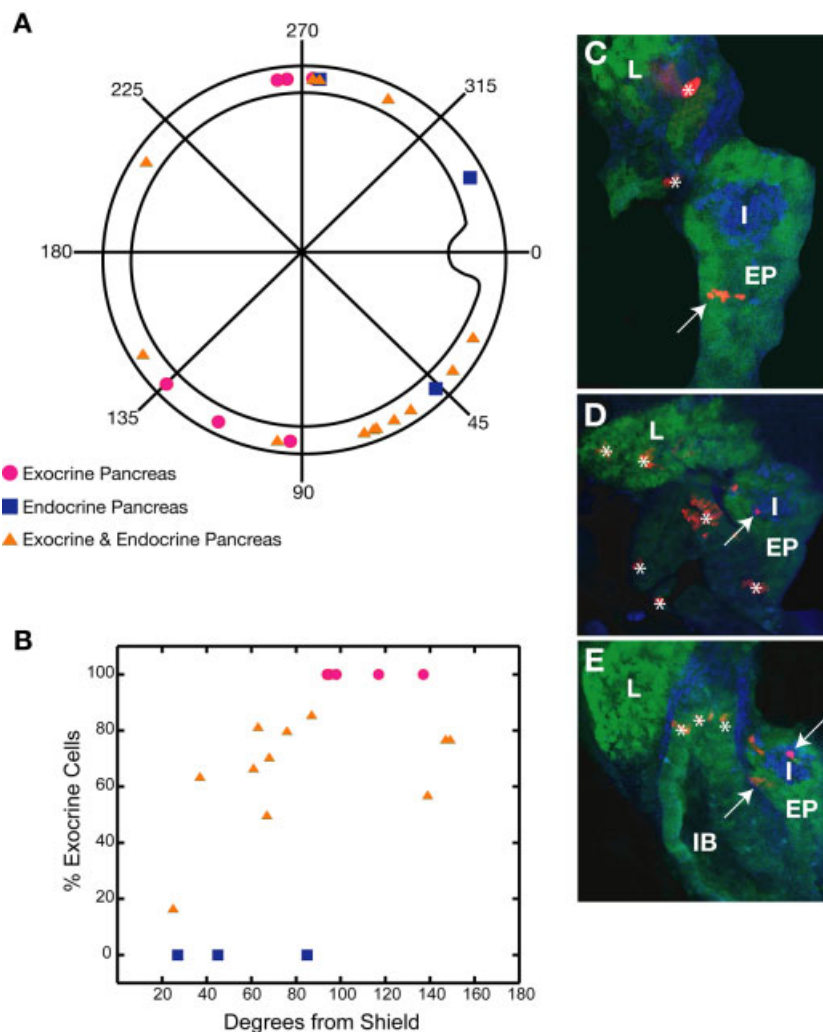


Fig. 4.

formed (Fig. 5D–G). These cells then moved medially toward the notochord, and subsequently moved posteriorly to the level of the second somite (Fig. 5D–J). Later they could be seen in the pancreas (Fig. 5K). Thus, cells contributing to the pancreas are located in the endodermal layer at approximately the equatorial region of the 10 hpf embryo.

## DISCUSSION

In this study, we have established a more complete gastrula fate map of the zebrafish pancreas, including the origin of the posterodorsal and anteroventral pancreatic buds, and the endocrine and exocrine precursors. Although a relationship between different cell lineages of the endocrine pancreas has been established in the mouse (reviewed by Herrera, 2000), this is the first study of any vertebrate where progenitors of the endocrine vs.

**Fig. 3.** Fate map of the posterodorsal and anteroventral pancreatic buds. **A:** Polar plot of the locations of the posterodorsal and anteroventral pancreatic bud progenitors at 6 hours postfertilization (hpf). These points are the pancreas positive locations seen in Figure 2A. **B–D:** Confocal slices of representative 40 hpf embryos. **B:** Example of embryo with labeled cells in the anteroventral bud (purple circles in A). **C:** Embryo with rhodamine dextran-labeled cells in the posterodorsal bud (yellow squares in A). **D:** Embryo with labeled cells in both buds (blue triangles in A). Arrows point to labeled cells that colocalize with the structure of interest. Asterisks (\*) point to rhodamine dextran-labeled cells that are not in the pancreas or liver. The dashed line encircles the location of the ventral bud, and the solid line encircles the location of the dorsal bud.

**Fig. 4.** Fate map of the endocrine and exocrine pancreas in zebrafish at 6 hours postfertilization (hpf). **A:** Polar plot of the locations of endocrine and exocrine pancreatic progenitors at mid-gastrulation. **B:** Graph of percentage exocrine cells at 72 hpf vs. dorsoventral location of the clone at 6 hpf. The numbers of exocrine and endocrine cells were counted, and the percentage exocrine is the number of exocrine cells divided by the total number of cells. **C–E:** Confocal slices of representative 72 hpf embryos. **C:** Embryo with rhodamine dextran-labeled cells in the exocrine pancreas. **D:** Embryo with labeled cells in the endocrine pancreas. **E:** Embryo with labeled cells in both the endocrine and exocrine pancreas. Blue cells are islet1 positive. Arrows point to labeled cells that colocalize with the structure of interest. Asterisks (\*) point to rhodamine dextran-labeled cells that are not in the pancreas or liver. EP, exocrine pancreas; I, pancreatic islet; IB, intestinal bulb; L, liver.



exocrine pancreas have been mapped. Our data confirm the findings of the previous report that blastula stage cells are still multipotent at 3–4 hpf and that clones established at this stage can contribute to multiple endodermal (and often mesodermal) derivatives (Warga and Nüsslein-Volhard, 1999). Moreover, our fate map agrees with this previous report that dorsoventral position of endoderm progenitors at 6 hpf correlates with later anteroposterior location along the gut. Significantly, although we found that some individual clones contributed to both the posterodorsal and anteroventral pancreatic buds, there is a trend for posterodorsal bud progenitors to be located more dorsally than anteroventral bud progenitors at 6 hpf. Likewise, and consistent with previous reports that the posterodorsal bud gives rise to mainly endocrine and the anteroventral bud gives rise to mainly exocrine cell types (Field et al., 2003), we found that endocrine progenitors tend to be located more dorsally and exocrine progenitors more ventrally. We also established the position of the pancreatic progenitors at the end of gastrulation (10 hpf), interestingly, by this developmental stage they are mainly clustered in two foci on either side of the dorsal midline at the equator of the embryo.

### Right/Left Asymmetry of the Liver and Pancreas

Previously, one of us (R.M.W.) and Nüsslein-Volhard (1999) mapped all endoderm derivatives and suggested that pancreas and liver progenitors are located asymmetrically in the zebrafish gastrula. Our new results do not support this conclusion. Rather, we find that pancreas and liver progenitors derive from both right and left sides, with no significant asymmetry. This apparent difference between the previous and current fate map probably reflects the increased number of pancreas and liver clones analyzed in this study. Endoderm fate maps of *Xenopus* and mouse similarly indicate no initial right/left asymmetry of pancreas or liver progenitors (Chalmers and Slack, 2000; Tremblay and Zaret, 2005). Nevertheless, eventually the liver and pancreas are asymmetrically located in vertebrate

embryos, the pancreas on the left and the liver on the right. Thus, at some point during normal organogenesis progenitors must cross the midline. We conclude that establishment of foregut organ cell fate is primarily influenced not by right/left position, but by dorsoventral location in the gastrula.

### Dorsoventral Asymmetry of the Pancreas Fate Map

As previously stated, this study confirms the previous finding (Warga and Nüsslein-Volhard, 1999) that, in general, endoderm progenitors show a positive correlation between dorsoventral position in the early gastrula and anteroposterior location of their derivatives along the gut. This predicts that the more dorsally derived posterodorsal bud precursors should eventually be located anterior to the ventrally derived anteroventral bud precursors. This prediction is not borne out, although it should be noted that there is only a very minor anteroposterior separation of the two buds in zebrafish. A fate map of the early neurula stage *Xenopus* embryo (Chalmers and Slack, 2000) indicates that dorsal and ventral bud progenitors are segregated by stage 14 and that dorsal pancreatic bud progenitors derive from a more posterior location than the ventral bud progenitors. This apparent discrepancy between the zebrafish and *Xenopus* fate maps may well be a consequence of the later developmental stage at which the *Xenopus* fate map was generated.

In our fate map, already by 6 hpf we observe a partial separation of the progenitor domains for the two pancreatic buds. This finding suggests that the pools of progenitor cells that will later contribute to the individual buds of the pancreas may already be acquiring differences by gastrulation stages. However, our data reveal only a broad trend in progenitor locations: individual cells do not always follow this trend. We, therefore, cannot conclude that pancreas progenitor cells have already responded to molecular signals and made a decision regarding their future fate at this early stage. Rather, we suggest that pools of progenitor cells are located in regions of the gastrula that receive distinct molecular signals and that their pattern-

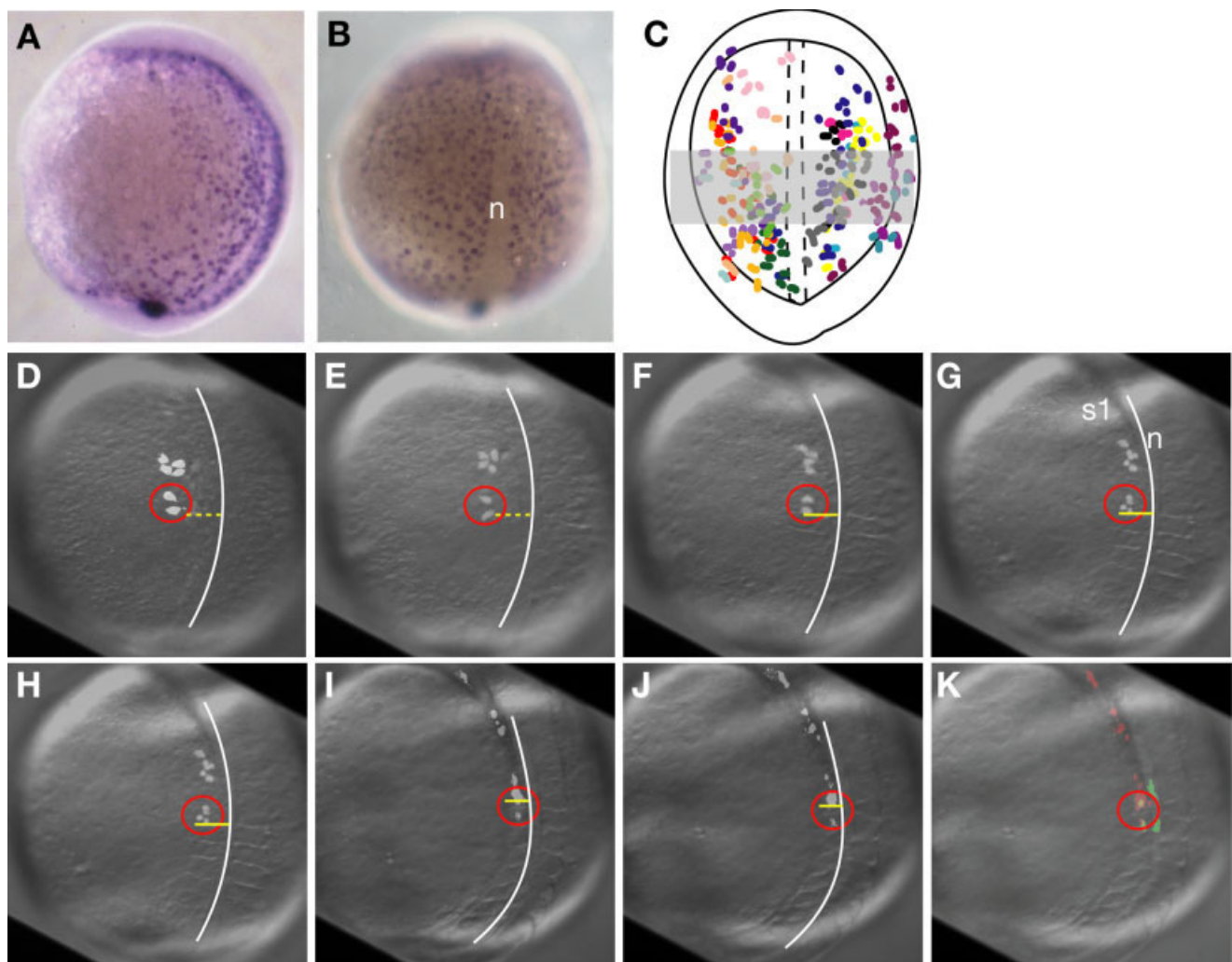
ing is further influenced by the environments through which they move on route to their final destinations; this model is schematized in Figure 6.

A similar model has been proposed to explain how fast and slow muscle precursors acquire their different fates (Hirsinger et al., 2004). These authors reported that the fast and slow muscle progenitor pools derive from separate yet overlapping regions of the 6 hpf embryo (similar to the progenitor pools contributing to individual pancreatic buds). Their cell transplantation studies revealed that muscle progenitors are not committed to fast vs. slow fate at 6 hpf, but become committed by the three-somite stage (11–11.5 hpf). This commitment is proposed to be a consequence of the signals the cells received during gastrulation movements.

Evidence already exists that molecular signals critical for normal pancreas development are acting during gastrulation. For example, in the mouse, it has been demonstrated that instructive signals from the mesoderm and ectoderm act to regionalize the late gastrula-stage endoderm. Endoderm not receiving this anteroposterior information is incapable of responding to subsequent inductive signals that impart organ identity (Wells and Melton, 2000). Secreted signals that function during gastrulation stages include Bmps and their antagonists, and mutant analyses indeed indicate a role for Bmp signaling in early zebrafish pancreas development (Tiso et al., 2002). In *swirl/bmp2b* mutants, the number of endocrine pancreas progenitors is decreased. By contrast, in *dino/chordin* mutants (a Bmp inhibitor) the number of endocrine pancreas progenitors is increased. We suggest that additional molecular pathways will also prove to be influencing the earliest stages of pancreas cell type specification.

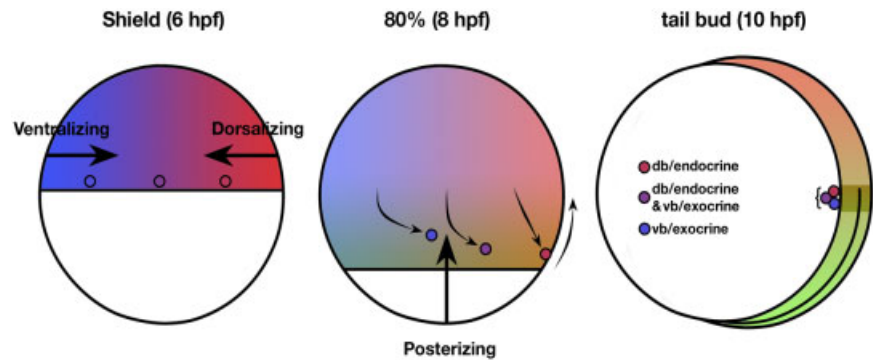
Cell transplantation is a useful technique in the zebrafish that allows tests of cell or tissue autonomy of gene function, as well as of the timing of cell commitment (Ho and Kane, 1990; Ho and Kimmel, 1993). Recently, we developed an approach to target transplanted cells specifically to the endoderm (Stafford et al., 2006). However, this technique was not able to effectively target cells to the endocrine





**Fig. 5.** Location of cells at tail bud that potentially give rise to the pancreas. **A:** Lateral view of *sox17* expression at tail bud. **B:** Dorsal view of *sox17* expression at tail bud. n: notochord. **C:** Locations of all cells (excluding cells in the extreme anterior and posterior) from 20 different embryos that had one or more labeled cells in one or both of the pancreatic buds. Each color represents a different embryo. Embryos were mounted in dorsal view with the equator of the embryo (approximately the level of the first somite) closest to the viewer. The gray square marks an area that includes labeled cells from all embryos and marks the likely location of the pancreatic progenitors at the end of gastrulation (tail bud stage, 10 hours postfertilization [hpf]). The outline of the notochord is indicated by dashed lines. **D–J:** Representative confocal projections from time-lapse experiment between 95% epiboly and 14 somites (approximately 9.5 hpf–16 hpf). Images are approximately equally spaced in time. Red circle contains cells that later colocalize with *pdx1*–green fluorescent protein (GFP) positive cells. **K:** Location of rhodamine dextran-labeled cells (red) at 16 hpf with cells that are positive for GFP (green). The dashed yellow line is the future division between somites 1 and 2. The solid yellow line marks the boundary between somites 1 and 2. The solid white line marks the notochord.

pancreas of every specimen. Based on our new fate map, we suggest that such transplants should be targeted to the dorsal quadrants of the early gastrula (Fig. 6B) to maximize the probability of donor cells contributing to the pancreatic islet. Finally, although our fate maps reveal the location of pancreas progenitors at two developmental stages, our experiments do not address when these cells become committed to distinct fates. Future studies, transplanting pancreas progenitors to other locations at specific time



**Fig. 6.**

points, should establish when in development their commitment occurs.

### Fate Map of the Pancreas at Tail Bud Stage

Our fate map at the end of gastrulation reveals that pancreas progenitors lie bilaterally at the equator of the embryo, at approximately the level of the future first somite. Following *Pdx1*-GFP transgenic embryos by time-lapse video microscopy confirms this equatorial location of progenitors at 10 hpf (tail bud stage) and also reveals that pancreas progenitor cells move posteriorly once they have converged at the midline, consistent with a previous report (Kim et al., 2005). Comparison of our 6 hpf and 10 hpf fate maps shows that pancreas progenitors move significant distances during gastrulation and, despite a wide range of locations at 6 hpf, migrate to a distinct anteroposterior domain of the endoderm by 10 hpf. We

suggest that, although endocrine and exocrine progenitors will become closely juxtaposed as early as 10 hpf, the locations of their progenitors at 6 hpf require these cells to move along different trajectories, thus, as we propose above, these movements would allow them to be influenced by distinct sets of molecular signals (Fig. 6). Of interest, it is at approximately 10 hpf that retinoic acid (RA) signals function to specify pancreas (Stafford and Prince, 2002). Embryonic RA is synthesized by the action of the *RALDH2* enzyme, and at tail bud stage the *raldh2* gene is expressed in anterior paraxial mesoderm (Begemann et al., 2001). We have previously established not only that anterior paraxial mesoderm is the source of the RA signals that specify pancreas, but also that these RA signals are received directly in the endoderm (Stafford et al., 2006). Our 10 hpf fate map now confirms that the pancreatic progenitors lie immediately beneath this source of RA.

Despite intensive study of the pancreas, little is known about the molecular pathways controlling its earliest specification. For example, we do not know what target genes are activated by RA at tail bud stage to specify pancreas. Although *vhnf1* has been shown to function downstream of RA in pancreas specification, endodermal expression of this gene has not been reported at stages before *insulin* expression (Song et al., 2007). Currently, there are no specific molecular markers for zebrafish pancreatic progenitors before the onset of *pdx1* expression at 14 hpf. In this study, we have revealed the locations of the pancreatic progenitors at 6 and 10 hpf. In the future, our fate map should prove useful in determining whether candidate genes are likely expressed in pancreas progenitors at stages that precede the onset of *pdx1* expression. Notably, one possible candidate is *hlxb9*, which is expressed in a subset of endodermal cells from late gastrulation (Wendik et al., 2004). It will be interesting to compare the expression patterns of such early endodermal genes to our fate map, helping to establish earlier markers and possible cell fate restrictions to the pancreas.

## EXPERIMENTAL PROCEDURES

### Fish

Cell fates were determined in the gut-GFP line (Field et al., 2003). Time-lapse experiments were conducted in the *pdx1*-GFP line (Huang et al., 2001) following similar procedures to those described by Warga and Kane (2003). Embryos were staged according to Kimmel et al. (1995).

### Injections/Fate Map

Injections and fate determination were performed as described (Warga and Nüsslein-Volhard, 1999). The 70-kD lysinated rhodamine dextran (Molecular Probes) was injected into a single marginal blastomeres at the calculated 512 and 2,000-cell stage (approximately 2.75–3.25 hpf). Because we were primarily interested in the dorsoventral location of pancreatic progenitors, we did not analyze clones before involution. At 6 hpf, when the dorsal side is morphologically visible, embryos were mounted animal pole up so that the location of a clone could be determined relative to the embryonic shield (dorsal); in general there were 2–16 labeled cells including EVL cells at 6 hpf (Fig. 1G). In a portion of these clones, cells were also localized at tail bud stage (10 hpf) and at 24 hpf using the embryonic axis as a reference (Fig. 1H,I). Gastrula stage, embryos were mounted in 3% methylcellulose, for older stages embryos were also anesthetized with MS-222. Brightfield and fluorescent pictures were taken at each time point (e.g., Fig. 1G–J). All the embryos were subsequently fixed at either 40 hpf or 72 hpf in 4% paraformaldehyde and then processed for various markers.

For experiments to follow cell movements from 10 hpf to the onset of *pdx1* expression. We labeled cells as described above in *pdx1*-GFP transgenic zebrafish (Huang et al., 2001). After localizing cells at 6 hpf, candidate embryos were mounted at late gastrulation in low percentage agarose (0.15%). Brightfield and fluorescent pictures were taken every 10 minutes from 95% epiboly (approximately 9.5 hpf) to the 14 somite stage (approximately 16 hpf) using a Zeiss Spinning Disk confocal microscope, images

**Fig. 6.** Model indicating how endocrine and exocrine pancreatic progenitors may be patterned as a consequence of exposure to different molecular influences during gastrulation. At shield stage (6 hours postfertilization [hpf]) marginal cells are already committed to the endodermal lineage and are subject to the global patterning signals that establish the dorsoventral axis. At this stage, there is a broad trend for more dorsally located endoderm cells to later contribute to the dorsal bud/endocrine lineage and for more ventral cells to contribute to the ventral bud/exocrine lineage. However, this model should not be taken to suggest that cells have made decisions at this stage regarding pancreas cell fate vs. other endodermal fates. As gastrulation proceeds (80% epiboly; 8 hpf) marginal cells have internalized and are moving toward their appropriate location on the anteroposterior axis. During these epiboly stages cells are subject to posteriorizing influences. The different trajectories followed by dorsally derived vs. ventrally derived endoderm cell populations may allow these cell populations to be exposed to different molecular influences, thus further refining their tendency to contribute to dorsal bud/endocrine lineage vs. ventral bud/exocrine lineage. By tail bud (10 hpf) pancreatic progenitors have reached an equatorial zone (bracket). While all pancreas progenitors are now localized in the same region, cells derived from the ventral gastrula are more likely to become exocrine, whereas cells derived from the dorsal gastrula are more likely to become endocrine. db, posterodorsal bud; vb, anteroventral bud.

were processed using MetaMorph 6.3 software. Labeled cells that colocalized with GFP-positive cells at 16 hpf were subsequently traced backward to their location at 10 hpf. Two separate embryos had rhodamine dextran-labeled cells colocalizing with GFP.

### Immunohistochemistry

Embryos were stained with anti-GFP 488 (Molecular Probes) and anti-islet1 (Hybridoma Bank) using an anti-mouse 633 secondary antibody (Molecular Probes). Embryos were imaged using a Zeiss LSM 510 confocal microscope with a 40 $\times$  objective for 40 hpf embryos and a 25 $\times$  objective for 72 hpf embryos. All images shown are individual slices from Z-stacks. Only embryos with labeled cells in the endoderm were used for the analysis. Additionally, embryos were specifically scored for cells in the liver, endocrine and/or exocrine pancreas at 72 hpf or in the liver, posterodorsal bud and/or anteroventral bud of the pancreas at 40 hpf. In situ hybridization was performed as previously described (Stafford and Prince, 2002) for the *sox17* riboprobe (Alexander and Stainier, 1999).

### Data Analysis

At 6 hpf, the angle from dorsal was drawn from the middle of the embryonic shield (dorsal organizer) to each end of the clone to calculate the range using ImageJ (<http://rsb.info.nih.gov/ij/>). The average angle from shield for each clone was determined by drawing an angle through the middle of the shield and middle of the clone and was graphed using Kaleidograph 4.01 (Synergy Software). Angles were graphed using a polar plot with 0 degrees defined as dorsal. Quadrants were defined as left-dorsal (1 degree–90 degrees), left-ventral (91 degrees–180 degrees), right-ventral (181 degrees–270 degrees), and right-dorsal (271 degrees–360 degrees).

For this study, a total of 360 embryos were injected, 125 of which contributed to endodermal tissues: 68 embryos were included in the 72 hpf analysis and 57 embryos were included in the 40 hpf analysis. Cellular locations at 6 hpf were randomly distributed at shield stage in both 40 hpf

and 72 hpf analyses (Rayleigh test for circular uniformity: 40 hpf,  $P > 0.50$ ; 72 hpf,  $P > 0.10$ ; Zar, 1996). For each plot (Figs. 2A, 3A, 4A), we considered whether mixed populations of cells (i.e., liver and pancreas [Fig. 2A], posterodorsal and anteroventral pancreatic buds (Fig. 3A), and endocrine and exocrine pancreatic cells [Fig. 4A]) derived only from large size clones. We did not find that larger clones were more likely to produce a mixed population of cells. To determine whether the locations of clones differed between the pancreas and liver, posterodorsal and anteroventral pancreatic buds, and endocrine and exocrine pancreas, a Mann–Whitney test was used (Zar, 1996).

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### REFERENCES

Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H. 1997. Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. *Nature* 385: 257–260.  
 Alexander J, Stainier DYR. 1999. A molecular pathway leading to endoderm formation in zebrafish. *Curr Biol* 9:1147–1157.  
 Aoki TO, David NB, Minchiotti G, Saint-Etienne L, Dickmeis T, Persico GM,

Strahle U, Mourrain P, Rosa FM. 2002. Molecular integration of casanova in the Nodal signalling pathway controlling endoderm formation. *Development* 129: 275–286.  
 Begemann G, Schilling TF, Rauch G, Geisler R, Ingham PW. 2001. The zebrafish neckless mutation reveals a requirement for *raldh2* in mesodermal signals that pattern the hindbrain. *Development* 128: 3081–3094.  
 Biemar F, Argenton F, Schmidtke R, Epperlein S, Peers B, Driever W. 2001. Pancreas development in zebrafish: early dispersed appearance of endocrine hormone expressing cells and their convergence to form the definitive islet. *Dev Biol* 230:189–203.  
 Chalmers AD, Slack JMW. 2000. The *Xenopus* tadpole gut: fate maps and morphogenetic movements. *Development* 127: 381–392.  
 David NB, Rosa FM. 2001. Cell autonomous commitment to an endodermal fate and behaviour by activation of Nodal signalling. *Development* 128:3937–3947.  
 Edlund H. 2002. Pancreatic organogenesis - developmental mechanisms and implications for therapy. *Nat Rev Genet* 3:524–532.  
 Field HA, Dong PDS, Beis D, Stainier DYR. 2003. Formation of the digestive system in zebrafish. II. Pancreas morphogenesis. *Dev Biol* 261:197–208.  
 Fukuda K, Kikuchi Y. 2005. Endoderm development in vertebrates: fate mapping, induction and regional specification. *Dev Growth Differ* 47:343–355.  
 Gu GQ, Wells JM, Dombkowski D, Preffer F, Aronow B, Melton DA. 2004. Global expression analysis of gene regulatory pathways during endocrine pancreatic development. *Development* 131:165–179.  
 Herrera PL. 2000. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127:2317–2322.  
 Hirsinger E, Stellabotte F, Devoto SH, Westerfield M. 2004. Hedgehog signaling is required for commitment but not initial induction of slow muscle precursors. *Dev Biol* 275:143–157.  
 Ho RK, Kane DA. 1990. Cell-autonomous action of zebrafish *Spt-1* mutation in specific mesodermal precursors. *Nature* 348: 728–730.  
 Ho RK, Kimmel CB. 1993. Commitment of cell fate in the early zebrafish embryo. *Science* 261:109–111.  
 Huang HG, Vogel SS, Liu NG, Melton DA, Lin S. 2001. Analysis of pancreatic development in living transgenic zebrafish embryos. *Mol Cell Endocrinol* 177:117–124.  
 Kim HJ, Schleifarth JR, Jessurun J, Sumanas S, Petryk A, Lin S, Ekker SC. 2005. *Wnt5* signaling in vertebrate pancreas development. *BMC Biol* 3:23.  
 Kimmel CB, Warga RM, Schilling TF. 1990. Origin and organization of the zebrafish fate map. *Development* 108:581–594.



- Kimmel CB, Ballard WW, Kimmel SR, Uhlmann B, Schilling TF. 1995. Stages of embryonic-development of the zebrafish. *Dev Dyn* 203:253–310.
- Lin JW, Biankin AV, Horb ME, Ghosh B, Prasad NB, Yee NS, Pack MA, Leach SD. 2004. Differential requirement for *ptf1a* in endocrine and exocrine lineages of developing zebrafish pancreas. *Dev Biol* 274: 490–503.
- Melby AE, Warga RM, Kimmel CB. 1996. Specification of cell fates at the dorsal margin of the zebrafish gastrula. *Development* 122:2225–2237.
- Molotkov A, Molotkova N, Duester G. 2005. Retinoic acid generated by *Raldh2* in mesoderm is required for mouse dorsal endodermal pancreas development. *Dev Dyn* 232:950–957.
- Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BLM, Wright CVE. 1996. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 122:983–995.
- Reiter JF, Kikuchi Y, Stainier DYR. 2001. Multiple roles for *Gata5* in zebrafish endoderm formation. *Development* 128: 125–135.
- Shih J, Fraser SE. 1995. Distribution of tissue progenitors within the shield region of the zebrafish gastrula. *Development* 121:2755–2765.
- Slack JMW. 1995. Developmental biology of the pancreas. *Development* 121:1569–1580.
- Song J, Kim HJ, Gong Z, Liu NA, Lin S. 2007. *Vhnf1* acts downstream of *Bmp*, *Fgf*, and *RA* signals to regulate endocrine beta cell development in zebrafish. *Dev Biol* 303:561–575.
- Stafford D, Prince VE. 2002. Retinoic acid signaling is required for a critical early step in zebrafish pancreatic development. *Cur Biol* 12:1215–1220.
- Stafford D, Hornbruch A, Mueller PR, Prince VE. 2004. A conserved role for retinoid signaling in vertebrate pancreas development. *Dev Genes Evol* 214:432–441.
- Stafford D, White RJ, Kinkel MD, Linville A, Schilling TF, Prince VE. 2006. Retinoids signal directly to zebrafish endoderm to specify insulin-expressing beta-cells. *Development* 133:949–956.
- Tiso N, Filippi A, Pauls S, Bortolussi M, Argenton F. 2002. BMP signalling regulates anteroposterior endoderm patterning in zebrafish. *Mech Dev* 118:29–37.
- Tremblay KD, Zaret KS. 2005. Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. *Dev Biol* 280:87–99.
- Vogeli KM, Jin SW, Martin GR, Stainier DYR. 2006. A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. *Nature* 443: 337–339.
- Wallace KN, Pack M. 2003. Unique and conserved aspects of gut development in zebrafish. *Dev Biol* 255:12–29.
- Warga RM, Kane DA. 2003. One-eyed pinhead regulates cell motility independent of *Squint/Cyclops* signaling. *Dev Biol* 261: 391–411.
- Warga RM, Nüsslein-Volhard C. 1999. Origin and development of the zebrafish endoderm. *Development* 126:827–838.
- Wells JM, Melton DA. 2000. Early mouse endoderm is patterned by soluble factors from adjacent germ layers. *Development* 127:1563–1572.
- Wendik B, Maier E, Meyer D. 2004. Zebrafish *mnx* genes in endocrine and exocrine pancreas formation. *Dev Biol* 268: 372–383.
- Woo K, Fraser SE. 1995. Order and coherence in the fate map of the zebrafish nervous system. *Development* 121:2595–2609.
- Zar JH. 1996. *Biostatistical analysis*. 3rd Ed. Upper Saddle River, NJ: Prentice Hall.
- Zecchin E, Mavropoulos A, Devos N, Filippi A, Tiso N, Meyer D, Peers B, Bortolussi M, Argenton F. 2004. Evolutionary conserved role of *ptf1a* in the specification of exocrine pancreatic fates. *Dev Biol* 268: 174–84.