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Chimeric mice reveal clonal development of pancreatic acini, but not islets

E. Scott Swenson¹, Julie Xanthopoulos¹, Timothy Nottoli², James McGrath², Neil D. Theise³, and Diane S. Krause⁴

¹ Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT

² Animal Genomics Services, Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT

³ Department of Pathology, Beth Israel Hospital Medical Center, New York, NY

⁴ Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT

Abstract

Intestinal crypt stem cells establish clonal descendants. To determine whether the pancreas is patterned by a similar process, we used embryonic stem (ES) cell chimeric mice, in which male ES cells were injected into female blastocysts. Fluorescence in situ hybridization for the Y chromosome (Y-FISH) revealed clonal patterning of ES-derived cells in the adult mouse small intestine and pancreas. Intestinal crypts were entirely male or entirely female. Villi contained columns of male or female epithelial cells, consistent with upward migration of cells from the crypts which surround them. Within the exocrine pancreas, acini were entirely male or entirely female, consistent with patterning from a single stem/progenitor cell. Pancreatic islets contained a mixture of male and female cells, consistent with patterning from multiple progenitors. Male-female chimeric mice demonstrate that the adult mouse exocrine pancreatic acinus is patterned from a single stem/progenitor cell, while the endocrine pancreas arises from multiple progenitors.

Keywords

Mouse pancreas development; embryonic stem cell chimera; pancreatic acinus; pancreatic islet

Introduction

The rapid cell turnover in the mammalian intestine requires continual production of new cells from tissue-committed stem cells in the crypt. Stem and progenitor hierarchies in the small intestine may be inferred from lineage tracing studies in which neighboring cells differ in a phenotypic marker. Early examples of this approach relied on mosaic expression of X-linked

Corresponding author: E. Scott Swenson MD, PhD, Section of Digestive Diseases, Yale University School of Medicine, New Haven, CT 06520-8019, Email: scott.swenson@yale.edu, Phone: 203-785-2441, Fax: 203-785-7273.

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No conflicts of interest exist.

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genes in female mice[1;2], in which allelic differences could be distinguished immunohistochemically. These studies demonstrated that crypts were clonal, while villi were oligoclonal, arising from the crypts surrounding them. These findings were confirmed using mouse embryo aggregation chimeras varying in lectin binding[3] or Major Histocompatibility Complex H2 alleles[4]. Clonal crypts and oligoclonal villi were also seen by X-gal staining in the intestine of mice generated by injecting nontransgenic ES cells into LacZ-expressing blastocysts[5]. Together, these findings strongly support the now familiar model of intestinal development whereby a single stem cell populates each crypt. Homeostasis in the adult small intestine is maintained by a limited number of progenitors which give rise to Paneth cells, enterocytes, goblet cells and enteroendocrine cells. As the maturing enterocytes and goblet cells emerge from the crypt onto the villus and migrate upward, they eventually undergo programmed cell death and are shed into the intestinal lumen.

A similar approach has been taken with blood[6], adrenal gland [7;8] and pancreas[9]. To study the pancreas, aggregation chimeras were prepared from nontransgenic mice and mice expressing a human insulin transgene. The embryonic origin of individual cells in adult mice was distinguished using an antibody specific for the human insulin C-peptide.

Insulin-producing populations of cells in the Islets of Langerhans contained cells both positive and negative for the human C-peptide, indicating that islets arise from more than one progenitor cell[9]. The possibility that glucagon and somatostatin-producing cells arose from clonal precursors was not excluded, and the clonality of pancreatic acinar cells was not investigated. Subsequent investigations using Cre-Lox lineage tracing[10] or long-term labeling with analogs of BrdU[11] in mice indicated that insulin-producing beta cells are replaced by division of mature beta cells rather than endocrine precursors.

The mouse pancreas develops from budding of the dorsal and ventral foregut endoderm beginning at embryonic day 9.5. Cells in the dorsal and ventral pancreatic buds undergo commitment to exocrine or endocrine differentiation. The functional exocrine unit, the acinus, arises by proliferation of exocrine precursors and extensive arborization, resulting in clusters of acini connected to ducts which deliver digestive secretions to the duodenum[12;13;14]. Pancreatic endocrine cells also arise from the dorsal and ventral buds, initially appearing as scattered cells, and assembling to form islets between embryonic day 14 and 18[15].

There is, as yet, no general agreement on the fundamental question of whether the adult exocrine pancreas contains stem or progenitor cells. Understanding these basic processes of tissue development and maintenance may improve our ability to limit acute pancreatic injury, improve the success of therapeutic regeneration and cell therapy, and inhibit neoplastic transformation.

By using the Y chromosome to distinguish between ES-derived and blastocyst-derived cells, we found that each pancreatic acinus overwhelmingly contained either male cells or female cells, but not both. In contrast, pancreatic islets consistently contained both male and female cells. These results demonstrate that pancreatic acini arise from a single stem/progenitor cell, and confirm that pancreatic islets arise from multiple progenitors.

Materials and Methods

Experimental animals

C57Bl/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Male embryonic stem cells were kindly provided by Dr. Frank Costantini (Columbia University, New York, NY). Production of ES chimeric mice was performed as described [16]. Only mice found to be male/female chimeras were informative. We considered using green fluorescent protein

(GFP) as a marker of ES cell origin and in vivo clonality, but learned in control experiments that GFP is not uniformly expressed in every cell of the “ubiquitous” beta-actin-GFP mouse [17]. This would have led to the inevitable and potentially erroneous interpretation that cells of the pancreatic acinus or islet were polyclonal in origin. We also considered using LacZ transgenic ES cells but encountered difficulty staining pancreatic tissue uniformly in control animals (not shown).

Animals were housed at the Yale University School of Medicine, in a temperature and humidity-controlled 12 hour light/dark cycle environment with free access to rodent chow and water. All animal use protocols were approved by the Yale University School of Medicine Institutional Animal Care and Use Committee.

Tissue sampling, fixation and processing

Tissues from 3 month old chimeric mice were fixed in phosphate buffered formalin (Fisher Scientific, Morris Plains, NJ), embedded in paraffin and cut in 3 micron sections.

Y chromosome fluorescence in situ hybridization (Y-FISH) and immunofluorescence

Slides were heated to 60°C for two minutes, deparaffinized in Citrisolv (Fisher Scientific, Fairlawn, NJ), and rehydrated through graded alcohols to phosphate buffered saline (PBS). Antigen retrieval was performed using sodium citrate buffer in steam for 15 minutes. Digoxigenin-labeled mouse Y chromosome probe was applied as described [18], and detected using a Rhodamine-conjugated secondary antibody to digoxigenin (Roche Diagnostics, Mannheim, Germany). Y chromosome-positive acinar nuclei were counted in male/female chimeric pancreas and male controls and compared by t-test. Pancreatic exocrine cells were selected for counting based on their characteristic size, shape, location, and cytoplasmic autofluorescence. Potential clonality of pancreatic ducts was examined by costaining Y-FISH and pancytokeratin. Following detection of the Y chromosome probe, rabbit anti-cow cytokeratin polyclonal antibody (1:100 dilution, Z0622, DAKO, Carpinteria, CA) was applied and subsequently detected with goat anti-rabbit FITC (1:100 dilution, F2765, Invitrogen Molecular Probes, Carlsbad, CA).

The potential clonality of alpha, beta and delta cells in chimeric islets was investigated using colocalization of Y chromosome with immunofluorescence for insulin, glucagon, or somatostatin. Following Y-FISH, slides were incubated with rabbit anti-insulin (1:100 dilution, sc-9168, Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-glucagon (1:200 dilution, Glu-001 mAb, Novo Nordisk, Bagsvaerd, Denmark) or rabbit anti somatostatin (1:50 dilution, A0566, DAKO USA, Carpinteria, CA). Primary antibodies were detected with goat anti-rabbit FITC (1:100 dilution, F2765, Invitrogen Molecular Probes, Carlsbad, CA) or goat anti-mouse FITC (1:100 dilution, F2761, Invitrogen Molecular Probes). Fluorescence mounting media with DAPI (4',6-diamidino-2-phenylindole, Vector Labs, Burlingame, CA) was applied. The number of Y-positive, hormone-positive cells was quantitated in chimeric mice and compared with male controls by t-test.

Image acquisition and processing

Tissue sections were visualized and photographed using an Olympus BX-51 microscope. Y-FISH was colocalized with cytokeratin, insulin, glucagon, or somatostatin using a dual-wavelength channel filter set designed for simultaneous visualization of FITC and Rhodamine (Chroma Technology, Rockingham, VT). Photomicrographs were taken using a digital camera and IPLab imaging software (BD Biosciences, Rockville, MD). Images were obtained using excitation and emission filter sets for DAPI, rhodamine (Y-chromosome), and FITC (cytokeratin, insulin, glucagon, or somatostatin).

Results

Twenty eight mice exhibited coat color chimerism, qualitatively estimated from 5–50%. Five of these mice were subsequently determined to be male-female chimeras with a relatively low percentage of male chimerism in the pancreas (visually estimated at 10% or less). In these 5 mice, clusters of male cells were easily distinguished from the majority of female, Y chromosome-negative cells.

Analysis of chimeric adult small intestine by Y-FISH demonstrated that crypts were populated exclusively by male or female epithelial cells, but not both (Figure 1A). Villus enterocytes appeared as columns of male or female cells, arising from nearby crypts. Since the blood was also chimeric, lamina propria cells contained, as expected, a mixture of male and female cells and were not considered in the analysis of epithelial cell clonality. Analysis of chimeric adrenal cortex revealed linear arrays of Y chromosome-positive cells (Figure 1B), consistent with previous investigations of embryonic patterning in the adrenal gland [7; 8]. The clonality of small intestinal crypts and patches of adrenal cortex is consistent with previous findings using other methods, validating our approach to in vivo clonality using Y-FISH in male/female chimeric mice.

Analysis of the chimeric pancreas by Y-FISH demonstrated patches of Y-positive cells. In some sections, the Y-positive patches appeared to involve whole lobules, but the presence of smaller clusters of Y-positive acinar cells in directly adjacent lobules (Figure 2C) argues against clonal patterning at the level of the pancreatic lobule and suggests patterning at the level of the acinus. This finding also underscores the importance of studying chimeras with a low percentage of male ES contribution, so that merged patches of Y-positive cells are not interpreted as a single patch. Careful examination revealed several Y-positive male acini, clearly surrounded by Y-negative female acini (Figure 2C–E). These images are highly reminiscent of the all-or-none pattern in the small intestinal crypt, and suggest that acini arise from a single progenitor cell. Pancreatic acini from male controls and male-female chimeras were quantitated by Y-FISH. Two considerations were critical in this analysis. Partial nuclear sampling due to 3 micron sectioning inevitably underestimates the proportion of Y-positive acinar cells, in both male controls and experimental chimeras. Therefore, we compared the percentage of Y-positive nuclei in chimeric mice with male controls. Second, pancreatic acini may be diploid or tetraploid [19] (Figure 2A, 2C–E), so that individual acinar cell nuclei may contain one or two Y chromosomes. To account for this, we counted the number of acinar nuclei which contained at least one Y chromosome, rather than the total number of Y signals.

Seventy-two control male acini, each containing 3–12 nuclei were counted; 338 acinar nuclei contained at least one Y chromosome (Figure 3A), out of a total of 427 nuclei counted (79%). Therefore, we hypothesized that if pancreatic acini arise from a single progenitor, the efficiency of Y-FISH labeling for male acini in a male-female chimeric mouse would also be 79%. If acini arise from multiple progenitors, then the proportion of acinar cells labeled by Y-FISH in chimeric mice would be diluted by female cells, to less than 79%. Among acini containing at least one Y-positive acinar cell, 684 out of 880 (78%, $p > 0.05$ vs male control) acinar nuclei were Y-positive. Therefore, pancreatic acini are clonal and appear to represent the descendants of a single progenitor cell.

We also attempted to investigate the clonality of pancreatic ducts in relation to the acini they serve using this approach. Large and intermediate ducts contained a mixture of Y-positive and Y-negative cells (Figure 2E), likely reflecting their confluence with smaller ducts more proximal to the acinus. Among ducts lined by only 3 or 4 cells, 1 or 2 cells were always Y-negative. Due to the small size and low number of duct cells available for counting Y chromosome signals in any given cross section of small duct, we could not confidently apply

the same quantitative comparison of chimeric vs male control to determine the level at which clonality is seen in the ducts.

In eight male control islets, containing a total of 481 nuclei, 369 (77%) contained a Y chromosome. In contrast, the proportion of male cells in the pancreatic islets of chimeric mice was always less than male controls (Figure 3C), suggesting that islets are not clonal. We further investigated whether subsets of islet cells expressing insulin, glucagon, or somatostatin represented clonal populations. Among insulin-positive cells, 303 out of 400 (76%) were Y positive in male controls, while only 325 out of 675 (48%, $p < 0.05$) were Y-positive in chimeric mice (Figure 3D). Among glucagon-expressing cells, 244/345 (71%) were Y-positive in male controls, while only 154/307 (50%, $p < 0.05$) were Y-positive in chimeric mice (Figure 3E). Among somatostatin-expressing cells, 114/183 (62%) were Y-positive in male controls, while 74/220 (34%, $p < 0.01$) were Y-positive in chimeric mice (Figure 3F). These results indicate that hormone-secreting cells in the pancreatic islet are not clonal populations.

Discussion

In contrast to the small intestine, cell turnover in the normal adult exocrine and endocrine pancreas is relatively slow. However, long-term homeostasis demands eventual replacement of lost cells from either remaining mature cells or a progenitor pool. Here, we present evidence that pancreatic acini are clonal in origin, consistent with a model in which a single progenitor cell gives rise to all the exocrine cells of one acinus. A limitation of this approach is that our data reflect embryonic patterning events giving rise to clonal populations, but do not directly demonstrate the progenitor cell(s) which maintain homeostasis in the adult organ. Whether pancreatic acinar cells in the adult are replaced by division of remaining mature acinar cells, or from a multipotent stem cell during normal aging is not known. Similar questions regarding the identity and location of intestinal stem cells remain unresolved [20]. In the small intestine there is general agreement that adult crypts are clonal, but the identity of the intestinal “stem cell” has been variously ascribed to rapidly cycling populations of crypt base columnar cells expressing *Lgr5* [21] or slowly cycling cells located just above Paneth cells in the +4 position, marked by expression of *Bmi1* [22] or *DCamKL1* [23].

Also unknown is whether regeneration of the exocrine pancreas after acute or chronic injury occurs through proliferation of mature acinar cells or a local progenitor. While there are no known unique markers of pancreatic stem cells, it is possible to identify slowly cycling progenitors in the pancreas using the label-retention cell (LRC) technique used to identify stem cell niches in the bone marrow[24], skin[25], liver[26], intestine[27], kidney[28], and prostate [29]. This pulse-chase approach requires a transient labeling phase during which a nucleotide such as BrdU is incorporated, followed by a washout phase during which dividing cells gradually dilute out the label, leaving only the most slowly cycling cells with detectable BrdU. This approach has been applied to the mouse pancreas[30] during normal development and after injury. BrdU-positive LRC were located within and around islets, and surrounding acini and ducts. In most cases, the LRC did not stain for markers of pancreatic endocrine or exocrine differentiation, suggesting that they reflect a population of uncommitted phenotype. In addition, up to 30% of LRC expressed c-Met, a potential marker of pancreatic progenitor cells. After 90% pancreatectomy or streptozotocin treatment, BrdU-positive cells could be found in ducts or insulin-expressing cells, respectively, indicating at least partial regeneration of exocrine and endocrine pancreas from local precursors.

If the adult pancreas contains progenitor cells, then one would predict that early loss of these progenitors would be compensated by proliferation of residual progenitors. In a mouse model of transient inducible ablation of *pdx1*-expressing precursors with Diphtheria toxin during development up to embryonic day 10.5, a pancreatic remnant of about 40% of normal weight

was found, containing nearly normal proportions of duct, exocrine and endocrine tissues. These mice survived, but the pancreas never achieved normal total mass, even in adult mice[31]. This finding suggests that residual progenitors were not able to compensate for early losses and supports a model whereby mature cells in the pancreas arise only from preexisting mature cells with limited proliferative potential, rather than stem cell precursors. To independently confirm this, ES-complementation studies were performed, in which normal ES cells were injected into *pdx1*-null blastocysts. The pancreata that developed in these mice contained duct, exocrine and endocrine cells and were always entirely ES-derived. However, the total pancreatic mass was reduced below normal, approximately in proportion to the overall contribution of ES cells to the chimeric mouse. The failure of normal ES-derived cells to compensate for the loss of pancreatic mass during development is consistent with the Diphtheria toxin-mediated pancreatic ablation studies. Both findings indicate that cell-autonomous programs limit proliferative expansion of endodermal progenitors arising from the gut tube[31].

In conclusion, our data demonstrate that the adult exocrine pancreas is patterned from single progenitor cells at the level of pancreatic acini. This suggests that there may be a stem cell niche in the pancreatic acinus which should be explored through other lineage tracing approaches. The mixed clonality of endocrine cells in the pancreatic islet does not exclude the possibility that endocrine cells arise from progenitor cells. Endocrine cells in the islets may arise from more than one progenitor, or from existing mature endocrine cells.

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References

1. Griffiths DF, Davies SJ, Williams D, Williams GT, Williams ED. Demonstration of somatic mutation and colonic crypt clonality by X-linked enzyme histochemistry. *Nature* 1988;333:461–3. [PubMed: 3374587]
2. Ponder BA, Schmidt GH, Wilkinson MM, Wood MJ, Monk M, Reid A. Derivation of mouse intestinal crypts from single progenitor cells. *Nature* 1985;313:689–91. [PubMed: 3974703]
3. Schmidt GH, Garbutt DJ, Wilkinson MM, Ponder BA. Clonal analysis of intestinal crypt populations in mouse aggregation chimaeras. *J Embryol Exp Morphol* 1985;85:121–30. [PubMed: 3989447]
4. Ponder BA, Schmidt GH, Wilkinson MM. Immunohistochemistry in the analysis of mouse aggregation chimaeras. *Histochem J* 1986;18:217–27. [PubMed: 3528064]
5. Wice BM, Gordon JI. Forced expression of Id-1 in the adult mouse small intestinal epithelium is associated with development of adenomas. *J Biol Chem* 1998;273:25310–9. [PubMed: 9737997]
6. Ueno H, Weissman IL. Clonal analysis of mouse development reveals a polyclonal origin for yolk sac blood islands. *Dev Cell* 2006;11:519–33. [PubMed: 17011491]
7. Iannaccone P, Morley S, Skimina T, Mullins J, Landini G. Cord-like mosaic patches in the adrenal cortex are fractal: implications for growth and development. *Faseb J* 2003;17:41–3. [PubMed: 12475906]
8. Morley SD, O'Donohoe EA, Hughes KE, Irving C, Willis SM, Heasman S, West JD. Mosaic patch patterns in chimeric and transgenic mice suggest that directional growth in the adrenal cortex begins in the perinatal period. *Endocr Res* 2002;28:657–62. [PubMed: 12530679]
9. Deltour L, Leduque P, Paldi A, Ripoché MA, Dubois P, Jami J. Polyclonal origin of pancreatic islets in aggregation mouse chimaeras. *Development* 1991;112:1115–21. [PubMed: 1682130]

10. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004;429:41–6. [PubMed: 15129273]
11. Teta M, Rankin MM, Long SY, Stein GM, Kushner JA. Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell* 2007;12:817–26. [PubMed: 17488631]
12. Means AL, Leach SD. Lineage commitment and cellular differentiation in exocrine pancreas. *Pancreatology* 2001;1:587–96. [PubMed: 12120241]
13. Edlund H. Pancreas: how to get there from the gut? *Curr Opin Cell Biol* 1999;11:663–8. [PubMed: 10600706]
14. Slack JM. Developmental biology of the pancreas. *Development* 1995;121:1569–80. [PubMed: 7600975]
15. Edlund H. Developmental biology of the pancreas. *Diabetes* 2001;50(Suppl 1):S5–9. [PubMed: 11272202]
16. Hogan B.; Beddington, R.; Costantini, F.; Lacy, E. Manipulating the mouse embryo: A laboratory manual. Cold Spring Harbor Press; Cold Spring Harbor: 1994.
17. Swenson ES, Price JG, Brazelton T, Krause DS. Limitations of green fluorescent protein as a cell lineage marker. *Stem Cells* 2007;25:2593–600. [PubMed: 17615263]
18. Donnelly DS, Zeltermann D, Sharkis S, Krause DS. Functional activity of murine CD34+ and CD34 – hematopoietic stem cell populations. *Exp Hematol* 1999;27:788–96. [PubMed: 10340393]
19. Oates PS, Morgan RG. Changes in pancreatic acinar cell nuclear number and DNA content during aging in the rat. *Am J Anat* 1986;177:547–54. [PubMed: 2433936]
20. Barker N, van de Wetering M, Clevers H. The intestinal stem cell. *Genes Dev* 2008;22:1856–64. [PubMed: 18628392]
21. Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgard R. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet* 2008;40:1291–9. [PubMed: 18849992]
22. Sangiorgi E, Capecchi MR. Bmi1 is expressed in vivo in intestinal stem cells. *Nat Genet* 2008;40:915–20. [PubMed: 18536716]
23. May R, Riehl TE, Hunt C, Sureban SM, Anant S, Houchen CW. Identification of a novel putative gastrointestinal stem cell and adenoma stem cell marker, doublecortin and CaM kinase-like-1, following radiation injury and in adenomatous polyposis coli/multiple intestinal neoplasia mice. *Stem Cells* 2008;26:630–7. [PubMed: 18055444]
24. Bradford GB, Williams B, Rossi R, Bertoncello I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp Hematol* 1997;25:445–53. [PubMed: 9168066]
25. Morris RJ, Potten CS. Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells in vitro. *Cell Prolif* 1994;27:279–89. [PubMed: 10465012]
26. Kuwahara R, Kofman AV, Landis CS, Swenson ES, Barendswaard E, Theise ND. The hepatic stem cell niche: Identification by label-retaining cell assay. *Hepatology*. 2008
27. Potten CS, Morris RJ. Epithelial stem cells in vivo. *J Cell Sci Suppl* 1988;10:45–62. [PubMed: 3077942]
28. Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q. The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 2004;114:795–804. [PubMed: 15372103]
29. Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D, Shapiro E, Lepor H, Sun TT, Wilson EL. Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *J Cell Biol* 2002;157:1257–65. [PubMed: 12082083]
30. Teng C, Guo Y, Zhang H, Zhang H, Ding M, Deng H. Identification and characterization of label-retaining cells in mouse pancreas. *Differentiation* 2007;75:702–12. [PubMed: 17381540]
31. Stanger BZ, Tanaka AJ, Melton DA. Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* 2007;445:886–91. [PubMed: 17259975]

Figure 1.

Fluorescence in situ hybridization for the Y chromosome (Y-FISH) in male/female chimeric jejunum and adrenal gland. Nuclei are counterstained with DAPI (blue). The Y chromosome signal appears as a pink nuclear dot. A. Small intestinal crypts are entirely male (arrowheads) or entirely female. Villi are formed by the cells which emerge from the crypts surrounding them. B. Nearly linear arrays of cells of the adrenal cortex are either male or female, consistent with patterning from a progenitor at the base of the array. Magnification 200x.

Figure 2.

Analysis of clonality in the exocrine pancreas. Male cells are stained by Y-FISH (pink). Nuclei are counterstained with DAPI (blue). The green and orange colors are autofluorescence of acinar cell cytoplasm and zymogen granules, respectively. A: Male control - note that some nuclei contain two Y chromosomes, consistent with normal tetraploidy in some acinar cells. In acinar cells where 2 or more Y chromosomes were detected in a single nucleus, only one was counted in our analysis of clonality. 79% of male acinar nuclei were Y-positive, while 21% of acinar nuclei did not appear to have a Y chromosome due to sectioning artifact. B: Female control demonstrates the absence of background staining. C, D: Male-female chimeric mouse pancreas in which male acini (arrowheads) are surrounded by female acini. E: Large and intermediate ducts (stained green with cytokeratin antibody, arrowheads) contained both male and female cells. Magnification 200x.

Figure 3.

Analysis of clonality in the endocrine pancreas. Male cells are stained by Y-FISH (pink). Insulin, glucagon, and somatostatin are stained in green (D–F, respectively). Nuclei are counterstained with DAPI (blue). A: Male control islet stained by Y-FISH. 77% of islet nuclei were Y-positive, while the remaining 23% of nuclei did not appear to have a Y chromosome due to sectioning artifact. B: Female control shows absence of staining. C: Chimeric islet. The percentage of Y-positive islet cells was always less than in male controls. D–F: Colocalization of Y chromosome (pink) and insulin, glucagon or somatostatin (green) in chimeric islet. The percentage of Y-positive cells among each population of hormone-expressing cells was always less than in male controls (see text). Magnification 200x (A–D) or 100x (E, F).