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ScienceDirect
Developmental Biology 298 (2006) 415–429
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Gata6 is an important regulator of mouse pancreas development

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Received for publication 13 February 2006; revised 2 June 2006; accepted 27 June 2006
Available online 4 July 2006

Abstract

Gata4, Gata5, and Gata6 represent a subfamily of zinc-finger transcriptional regulators that are important in the development and differentiation of numerous tissues, including many endodermally-derived organs. We demonstrate that Gata4 and Gata6 have overlapping expression patterns in the early pancreatic epithelium. Later, Gata4 becomes restricted to exocrine tissue and Gata6 becomes restricted to a subset of endocrine cells. In addition, we show Gata6, but not Gata4, physically interacts with Nkx2.2, an essential islet transcription factor. To begin determining the roles that Gata4 and Gata6 play during pancreatic development, we expressed Gata4-Engrailed and Gata6-Engrailed dominant repressor fusion proteins in the pancreatic epithelium and in the islet. At e17.5, transgenic Gata6-Engrailed embryos exhibit two distinct phenotypes: a complete absence of pancreas or a reduction in pancreatic tissue. In the embryos that do form pancreas, there is a significant reduction of all pancreatic cell types, with the few differentiated endocrine cells clustered within, or in close proximity to, enlarged ductal structures. Conversely, the majority of transgenic Gata4-Engrailed embryos do not have a pancreatic phenotype. This study suggests that Gata6 is an important regulator of pancreas specification.

Keywords: Gata4; Gata6; Pancreas development; Islet

Introduction

The pancreas regulates glucose homeostasis in two important ways: the endocrine pancreas produces and secretes hormones, while the exocrine pancreas produces and secretes digestive enzymes. The endocrine cells cluster into islets, comprised of α, β, δ, PP, and ε cells that produce glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin, respectively. In the mouse, development of the pancreas begins with patterning of the foregut endoderm around embryonic day (e) 8.5. Cells are committed to a pancreatic fate by virtue of their unique position within the endoderm and their proximity to adjacent tissues (Bort et al., 2004; Kim et al., 1997; Kim and Melton, 1998). In response to signals from surrounding tissues, evagination of the endoderm leads to formation of the dorsal and ventral pancreatic buds. From e9.5 to birth, the cells of the pancreatic epithelium execute a complex program of proliferation, branching, and differentiation that results in the formation of three primary structures: the endocrine pancreas, the exocrine pancreas, and the ductal network (Pictet and Rutter, 1972; Slack, 1995).

Numerous transcription factors have been implicated in the sophisticated regulatory cascade that occurs during pancreas development. Before specification of the pancreatic epithelium, Pdx1 and Ptf1a can be detected in endodermal regions that will form the dorsal and ventral buds. Pdx1 and Ptf1a null mice do not develop a mature pancreas, demonstrating that both transcription factors are required for pancreas development (Jonsson et al., 1994; Krapp et al., 1998; Offield et al., 1996). Early Pdx1 expression is necessary for regionalization of the primitive gut endoderm and specification of the early pancreatic epithelium, while a second wave of expression is necessary for maturation of β cells and insulin regulation (Guz et al., 1995; Wilding and Gannon, 2004) as well as formation of the exocrine pancreas

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doi:10.1016/j.ydbio.2006.06.046

(Hale et al., 2005). The basic helix–loop–helix (bHLH) factor Ptf1a is initially required for specification of all pancreatic cell types, and has been shown to regulate exocrine differentiation and enzyme expression during later developmental stages (Kawaguchi et al., 2002; Rose et al., 2001).

Other transcription factors are also required for differentiation of the endocrine pancreas. The bHLH transcription factor Ngn3 is transiently expressed in all cells of the endocrine lineage and is recognized as a marker of endocrine progenitor cells (Gu et al., 2002). Nkx6.1 is required for proper differentiation of β cells in the developing pancreas (Sander et al., 2000). Pax6 is required for normal development of the islet cell types and has been shown to function as an activator of endocrine hormone gene transcription (Sander et al., 1997). The homeodomain-containing transcription factor Nkx2.2 is also required for proper differentiation of endocrine cell types (Sussel et al., 1998). Recent studies in our lab have centered on identifying transcription factors that work in concert with Nkx2.2 to regulate gene expression during pancreas development. Potential candidates include members of the Gata transcription factor family because they are known to interact with Nkx2 family members in other organs. For example, Gata6 and Nkx2.1 interact during lung epithelial development (Bruno et al., 2000; Liu et al., 2002a; Weidenfeld et al., 2002) and Nkx2.5 interacts with Gata4 and Gata5 during heart development (Stennard et al., 2003).

In the mouse, the Gata transcription factor family has six members and these zinc finger DNA binding proteins recognize the consensus sequence A/T-GATA-A/G. Gata1, Gata2, and Gata3 are known to be important for the differentiation of various cell types in the hematopoietic system while Gata4, Gata5, and Gata6 have been implicated in the development of organs derived from endoderm and mesoderm (Molkentin, 2000; Patient and McGhee, 2002). Gata4 is expressed in the primitive endoderm, heart, liver, small intestine, and gonads of the embryonic mouse (Arceci et al., 1993). Characterized as an important activator of many cardiac genes, Gata4 is required for cardiomyocyte maturation and formation of the heart tube. In addition, Gata4 is essential for ventral morphogenesis as Gata4 null embryos die between e7.5 and e9.5 due to defects in development of the visceral endoderm (Kuo et al., 1997; Molkentin et al., 1997; Narita et al., 1997). Expression of Gata6 during embryonic development is observed in the primitive streak, allantois, muscle, heart, lung, and gut (Morrisey et al., 1996). Gata6 plays an important role in lung development, specifically in branching morphogenesis and later stage differentiation of the lung epithelium (Keijzer et al., 2001; Koutsourakis et al., 2001; Liu et al., 2002b; Yang et al., 2002). Mice deficient for Gata6 die between e5.5 and e7.5, due to a defect in the formation of extraembryonic tissues in the developing blastocyst (Koutsourakis et al., 1999; Morrisey et al., 1998).

Recently, there have been two conflicting reports on Gata expression in the pancreas. Ritz-Lazer et al. (Ritz-Laser et al., 2005) used RT-PCR to demonstrate that Gata4 and Gata6 are both present in the islet and in endocrine cell lines, and suggest that Gata4 activates glucagon expression in vitro. However, Ketola et al. (Ketola et al., 2004) reported that Gata6 is expressed in the endocrine pancreas, while Gata4 expression is restricted to the exocrine pancreas. Further, they suggest that Gata4 and Gata6 are not expressed in the early pancreatic epithelium. The experiments described in our report attempt to resolve this incongruence and extend the analysis to attempt to determine the in vivo function of these Gata factors during embryonic pancreas development.

In this study, we demonstrate that both Gata4 and Gata6 are expressed at the onset of embryonic mouse pancreas development. Further, comprehensive expression analysis confirms that Gata4 and Gata6 are expressed in mutually exclusive pancreatic domains at later developmental stages. Since both Gata4 and Gata6 null mice die prior to formation of the pancreatic buds, we utilized dominant repressor Gata transgenic mice as an alternative in vivo approach to investigate the role of Gata factors in pancreas development. This strategy has been used successfully to study Gata6 during various stages of embryonic lung development (Koutsourakis et al., 2001; Liu et al., 2002b; Yang et al., 2002) and the role of ETS domain transcription factors in mouse development (Liu et al., 2003). We generated transient transgenic mice that express the repression domain of the *Drosophila* Engrailed protein fused to either full-length Gata4 or Gata6 or the Gata6 DNA binding domain. Expression of each fusion protein is driven by the pancreas-specific Pdx1 promoter element (Apelqvist et al., 1997; Li and Edlund, 2001; Stoffers et al., 1999). From this work, we conclude that Gata6 plays an important role in pancreas specification as well as the development of endocrine cell types.

Materials and methods

Construction of the Gata transgenes and generation of transgenic embryos

Three Gata transgenic constructs were created as follows: The repression domain of the *Drosophila* Engrailed (EnR) gene (encoding amino acids 1–298) was PCR-amplified from the pCS2:EnR plasmid (K. Artinger, UCHSC). Primers included 5′ *Bam*HI and 3′ *Xho*I sites for cloning. A FLAG tag was added, in frame, by cloning the EnR sequence into the *Bam*HI and *Xho*I sites of the pCMV-Tag4B vector (Stratagene) to produce pCMV-Tag4B-EnR. Full-length Gata6 cDNA, full-length Gata4 cDNA, and the Gata6 DNA binding domain (encoding amino acids 232–354) were PCR-amplified from cDNA and cloned into the pCR®-Blunt II-TOPO® vector (Invitrogen). The Gata cDNA inserts were released by digestion and cloned into the *Bam*HI site of pCMV-Tag4B-EnR to produce pCMV-Tag4B-G6FLEnR, pCMV-Tag4B-G4FLEnR, and pCMV-Tag4B-G6DBDEnR. Finally, the fusion constructs were PCR-amplified from their respective pCMV-Tag4B vectors and blunt-end cloned into the *Eco*RI site of the pPDX1-*Eco*RI vector (J. Jensen, UCHSC) to produce Pdx1:G6FLEnR, Pdx1:G4FLEnR, and Pdx1:G6DBDEnR. A control transgenic construct was created as follows: The repression domain of the *Drosophila* Engrailed gene (EnR) was PCR-amplified from the pCS2:EnR plasmid using primers that added a 5′ *Eco*RI site and nuclear localization sequence (encoding PKKKRKV) as well as a 3′ *Xho*I site. The PCR product was cloned into the pCR®-Blunt II-TOPO® vector to produce TOPO:NLSEnR. A FLAG tag was added, in frame, by cloning the NLSEnR sequence into the *Eco*RI and *Xho*I sites of pCMV-Tag4B to produce pCMV-Tag4b-NLSEnR. The NLSEnR construct was PCR-amplified from pCMV-Tag4b-NLSEnR and blunt-end cloned into the *Eco*RI site of the pPDX1-*Eco*RI vector to produce Pdx1:NLSEnR. Pdx1:G6FLEnR, Pdx1:G6DBDEnR, and Pdx1:NLSEnR were linearized with *Dra*III, while Pdx1:G4FLEnR was linearized with *Sap*1. The transgenic constructs were subsequently purified using the QIAquick Gel