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Short Communication

Sox9, a novel pancreatic marker in *Xenopus*

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ABSTRACT The pancreas is a mixed exocrine and endocrine gland involved in the control of many homeostatic functions. During embryogenesis, the pancreas arises from dorsal and ventral evaginations of the foregut that will subsequently fuse into a single organ. The characterization of early genes expressed in the developing pancreas is critical to understand its specification and differentiation. Here we report the expression pattern of *Sox9*, a member of the Sox family of transcription factors, during development of the *Xenopus* pancreas and compare its expression to that of a well characterized pancreatic marker, *Pdx1*. By whole-mount *in situ* hybridization, *Sox9* was first detected at stage 25 in the pancreatic anlagen - dorsally in the prospective foregut and ventrally on each side of the liver diverticulum. As development proceeds, *Sox9* expression can be used to trace the development of the dorsal and ventral pancreatic buds and their repositioning associated with the dynamic movements of the gastrointestinal tract. *Sox9* expression in the pancreatic rudiment was identical to that of *Pdx1*. However, while *Pdx1* is expressed in both the pancreatic buds and the duodenum, *Sox9* was restricted to ventral and dorsal pancreatic buds. *Sox9* and *Pdx1* are thus two of the earliest genes expressed in the presumptive pancreatic tissue.

KEY WORDS: *Xenopus, gut, pancreas, Sox9, Pdx1*

Morphologically and functionally the pancreas has distinct exocrine and endocrine components. The exocrine cells produce enzymes (elastase, amylase, trypsinogen) released into the digestive tract via a system of ducts, and the endocrine cells secrete hormones (insulin, glucagon, somatostatin) directly released into the bloodstream. Abnormal pancreatic function can lead to severe pathologies including pancreatitis and diabetes mellitus. The development of the pancreas depends on reciprocal interactions between the mesoderm and the endoderm mediated by secreted proteins of the FGF, TGF-β and hedgehog families (Reviewed in Kim and Hebrok, 2001). The pancreas arises from a dorsal and a ventral evagination of the foregut that will subsequently coalesce to form the mature pancreas (Slack, 1995). In the recent years, a number of transcription factors that show restricted expression in the developing pancreas have been identified, and mutations in some of these genes have provided important information on the processes involved in the specification and development of this organ (Reviewed in Edlund, 2002). Here we describe the expression pattern of *Sox9* (Spokony *et al.*, 2002), a member of the Sox family of transcription factors, during development of the *Xenopus* pancreas.

By whole-mount *in situ* hybridization *Sox9* is first detected at stage 25 in two discrete domains in the undifferentiated endoderm, dorsally in the prospective foregut and ventrally on each side of the future liver diverticulum (Fig 1A, stage 25). At stage 35/36 the morphologically recognizable dorsal pancreatic bud is first apparent. Ventral pancreatic rudiments become evident a few hours later at stage 37/38, on each side of the hepatic cavity (Nieuwkoop and Faber, 1967; Kelly and Melton, 2000). *Sox9* is strongly expressed in both dorsal and ventral pancreatic rudiments at these stages (Fig 1A-B, stage 35/36; and Fig 1A, stage 37/38). As development proceeds, *Sox9* expression persists in the dorsal and ventral buds now located on the left side due to the rotation of the developing gut (Fig. 1A, stage 40).

By stage 42 the dorsal and ventral rudiments of the pancreas have fused into a single organ and movements of the gastrointestinal tract have brought the pancreas into a ventral position (Fig 2, stage 42). At stage 45 the coiling of the intestine has displaced the pancreas towards the right side in a dorsal-lateral position, where it will remain during further development (Kelly and Melton, 2000). *Sox9* is strongly expressed in the pancreas and the main pancreatic duct at this stage (Fig 2, stage 45). Later in embryogenesis (stage 50, the latest stage examined in this study) *Sox9* is detected throughout the entire pancreatic tissue without any obvious restriction to specific cell types (not shown).

Abbreviations used in this paper: FGF, fibroblast growth factor: TGF-β, transforming growth factor beta.

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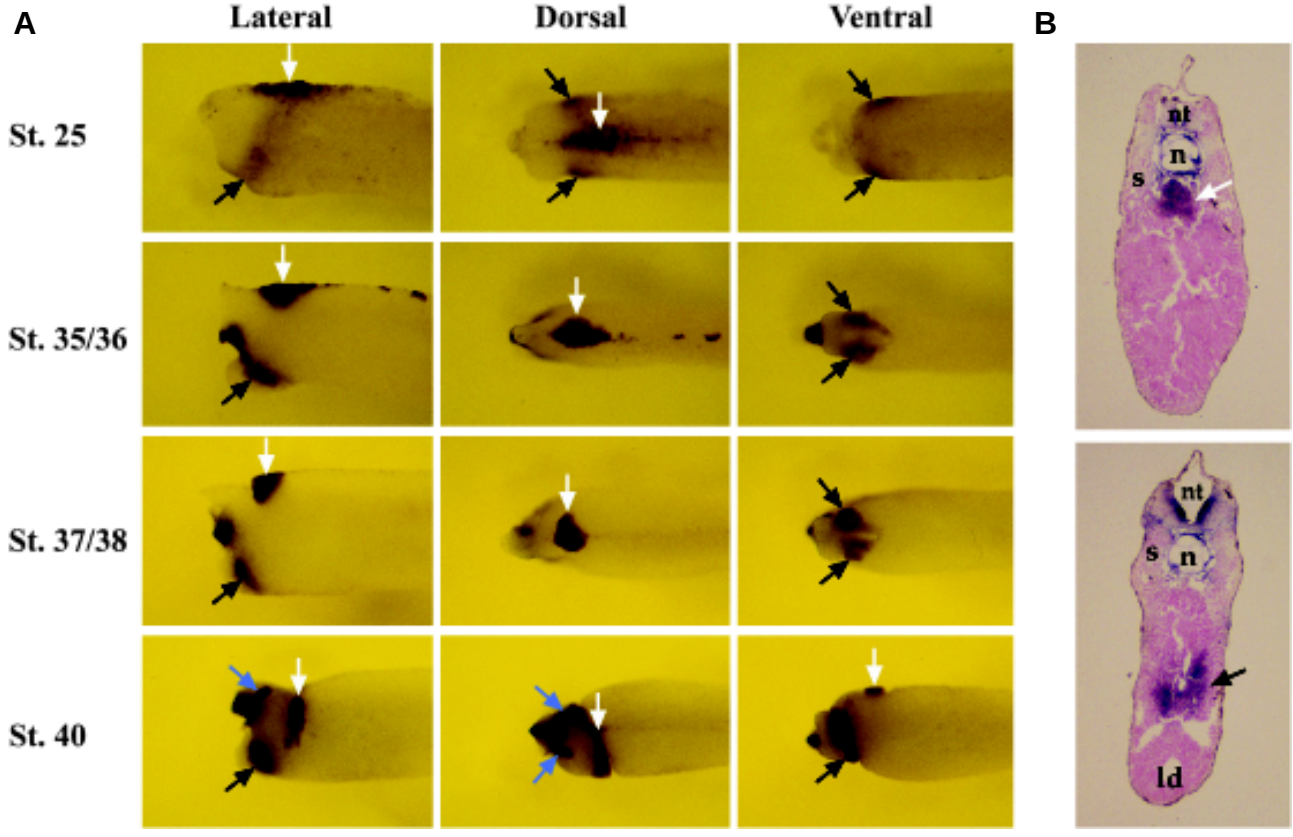


Fig. 1. Pancreatic expression of *Sox9* at stages 25-40 in whole guts and tissue sections. (A) *Whole-mount* in situ *hybridization of* Sox9 *on embryonic guts at different stages. Dissected whole embryonic guts are viewed from the lateral, dorsal or ventral sides.* Sox9 *is detected in the dorsal (white arrows) and ventral (black arrows) pancreatic rudiments/buds from stage 25 through stage 40.* Sox9 *is also expressed in the lung buds (blue arrows) at stage 40.* **(B)** *Transverse sections of a stage 35/36 embryo.* Sox9 *is expressed in the dorsal (white arrow, upper panel) and ventral (black arrow, lower panel) pancreatic rudiments. At this stage* Sox9 *is also expressed in the notochord and the neural tube. ld, liver diverticulum; nt, neural tube; n, notochord; s, somite.*

The homeodomain protein *Pdx1* is one of the earliest genes expressed in the mammalian pancreas, and mutation in *Pdx1* gene resulted in complete pancreas agenesis (Ahlgren *et al.*, 1996; Offield *et al.*, 1996). In *Xenopus*, *Pdx1* (a.k.a. *XlHbox8*) is expressed within the pancreatic rudiments and the duodenum prior to stage 35/36 (Wright *et al.*, 1988). *Pdx1* expression in the prospective pancreatic tissue is initiated as early as *Sox9*, around stage 25 (not shown). A comparison of *Sox9* and *Pdx1* expression at stage 31 and stage 40 indicate that these transcription factors have identical expression domains within the pancreatic rudiments (Fig 3). However, while *Pdx1* is expressed in both the pancreatic buds and the duodenum, *Sox9* appears to be exclusively restricted to ventral and dorsal pancreatic buds (Fig 3). At stage 40, *Sox9* is also detected in another endoderm derivative, the lung buds (Fig 3, stage 40).

The developmental expression of a number of pancreatic markers has been reported in *Xenopus* (Wright *et al.*, 1988; Kelly and Melton, 2000; Kim *et al.*, 2001; Horb and Slack, 2002). These include nuclear factors (Pax6, NeuroD, Islet1, Pdx-1 and XpabpII), hormones (insulin, glucagon and somatostatin) and digestive enzymes (amylase, elastase, trypsinogen and carboxypeptidase A). As summarized in Fig 4, with the exception of *Sox9* and *Pdx1*, first expressed around stage 25 in the prospective pancreatic rudiments, most of these markers are not detected until the pancreatic buds become discernable

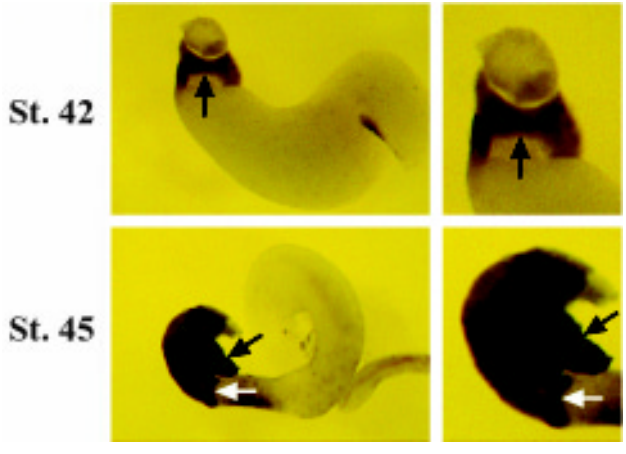


Fig. 2. Whole-mount *in situ* hybridization of *Sox9* on dissected and uncoiled embryonic guts at stages 42 and 45. Sox9 *expression persists in the pancreas at stage 42 as the dorsal and ventral rudiments fuses into a single organ located ventrally (black arrow). At stage 45,* Sox9 *is detected in the pancreas (black arrow) and the main pancreatic duct (white arrow). Right panels are higher magnification views of the panels on the left.*

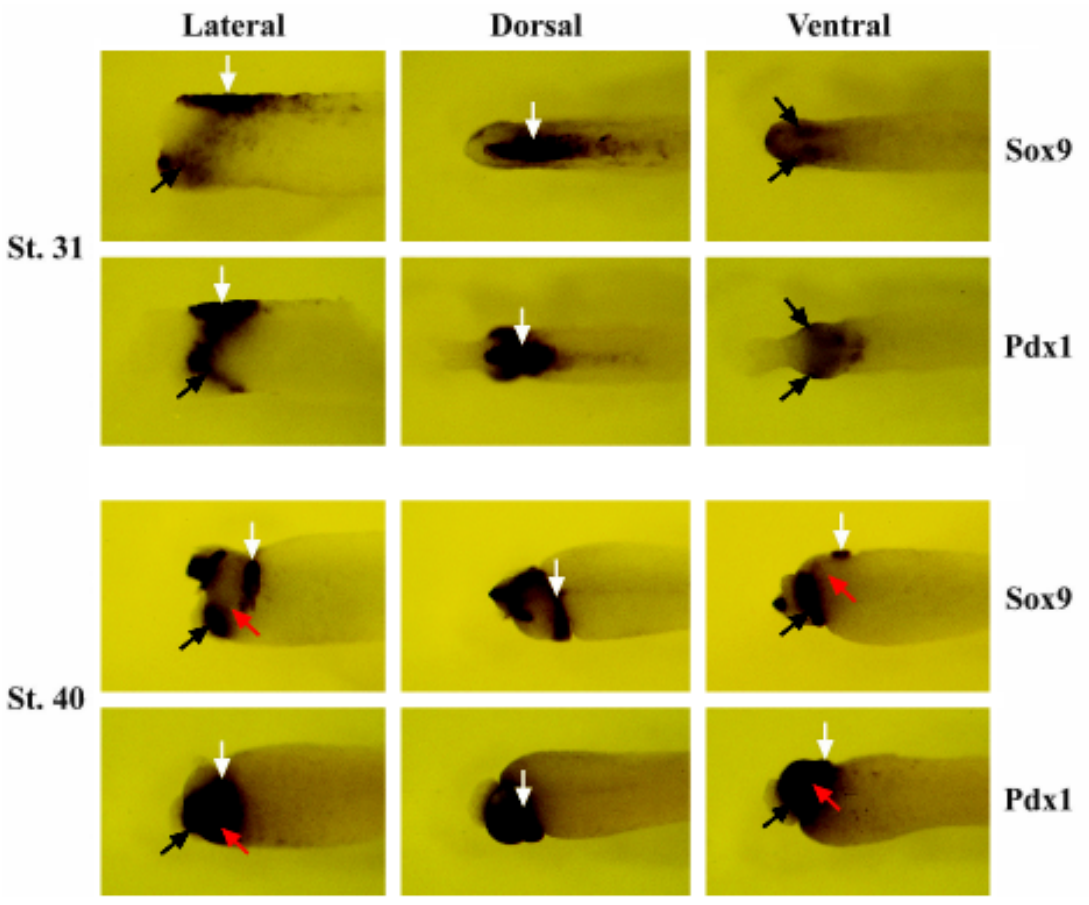
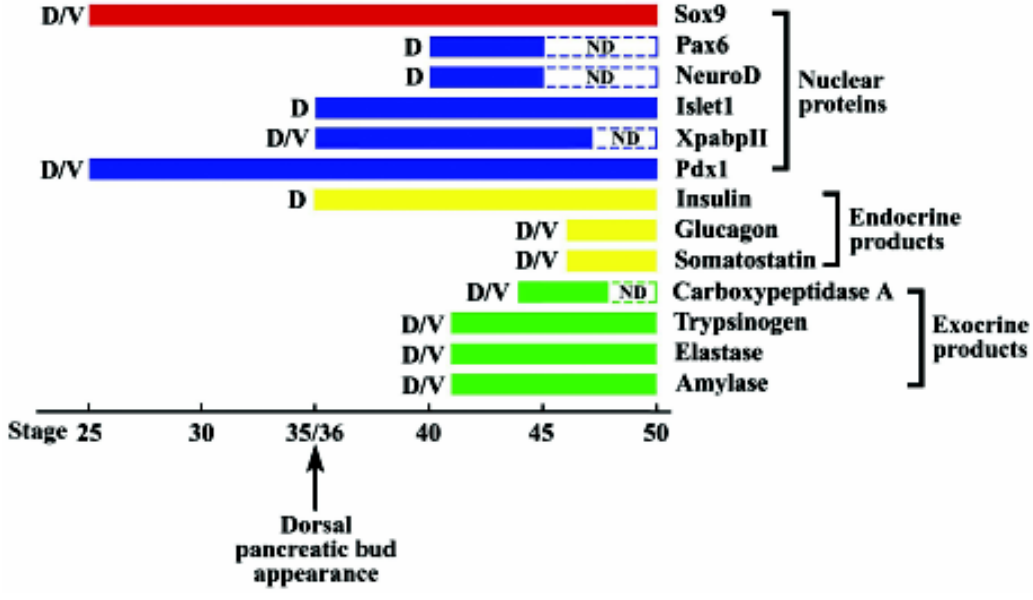


Fig. 3 (Above). Comparison of *Sox9* and *Pdx1* expression at stage 31 and stage 40. *Dissected whole embryonic guts are viewed from the lateral, dorsal or ventral sides. The position of the dorsal pancreatic bud (white arrows), ventral pancreatic bud (black arrows) and duodenum (red arrows) is indicated.* Pdx1 *is expressed in the pancreatic buds and the duodenum.* Sox9 *is restricted to ventral and dorsal pancreatic rudiments. In all panels, anterior is to the left.*

Fig. 4 (Right). Summary of the developmental expression of thirteen pancreatic markers in *Xenopus*. *The timing of expression of these markers was based on this work (* Sox9 *and* Pdx1*) and other published studies, as follow:* Pdx1 *(Wright* et al.*, 1988),* Pax6, NeuroD, Islet1, *insulin, glucagon, somatostatin and carboxypeptidase A (Kelly and Melton 2000);* XpabpII *(Kim* et al.*, 2001);* amylase, elastase *and* trypsinogen *(Horb and Slack, 2002). ND, expression not determined at these stages; D, expression limited to the dorsal pancreatic bud; D/V, expression in both dorsal and ventral pancreatic buds. This diagram does not take into account quantitative differences in the levels of expression of these markers at various stages.*



(Wright *et al.*, 1988; Kelly and Melton, 2000; Kim *et al.*, 2001; Horb and Slack, 2002). Therefore, *Sox9* is one of the earliest genes expressed in the developing pancreatic tissue and has the potential to be a valuable tool to analyze the specification and development of the pancreas.

We have previously shown that Sox9 is required for neural crest development in *Xenopus* using a morpholino-mediated "knockdown" of Sox9 protein (Spokony *et al.*, 2002). In these studies Sox9 morpholino was primarily targeted to the ectoderm layer; work is currently in progress to address the function of Sox9 during formation of the pancreas by targeting this morpholino to blastomeres at the 32-cell stage that contribute to the pancreatic rudiments. Heterozygous mutations in Sox9 result in Campomelic Dysplasia (CD), a lethal human disorder characterized by autosomal XY sex reversal and severe skeletal malformations (Foster *et al.*, 1994; Wagner *et al.*, 1994). Recent studies indicate that *Sox9* is also expressed in human and mouse pancreas (Piper *et al.*, 2002; Lioubinski *et al.*, 2003), and in human this expression has been proposed to correlate with pancreas abnormalities observed in patients affected by Campomelic Dysplasia (Piper *et al.*, 2002), suggesting an important function for Sox9 in pancreas development.

Experimental Procedures

Isolation of Pdx1

Xenopus Pdx1 was amplified by PCR using specific primers (forward, TTA CAA AGA GCC CTG TGC GTT C and reverse, TCC CTT CCC CTA ATA ACC CGT C) based on the published sequence of *Xenopus Pdx1*| *XlHbox 8* (Wright *et al.*, 1988). The 1143 bp PCR product was ligated into pGEMT-easy (Promega) and sequenced.

In situ *hybridization*

Embryos were staged according to Nieuwkoop and Faber (1967). After removal of the epidermis, whole embryonic gut was dissected from embryos at different stages, fixed in MEMFA and processed for whole-mount *in situ* hybridization as previously described (Harland, 1991). Antisense DIG-labeled probes (Genius kit, Roche) were synthesized using *Sox9* (Spokony *et al.*, 2002) and *Pdx1* cDNA as template. Hybridization signal was detected with an alkaline-phosphatase coupled anti-digoxigenin antibody (Roche) and visualized using BM purple as a substrate (Roche). For *in situ* hybridization on section, embryos at stage 35 and 50 were fixed in MEMFA for 1 hour, embedded in Paraplast and 12µm sections hybridized with a *Sox9* probe following the procedure described by Henry *et al.* (1996). Sections were briefly counter stained with eosin.

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