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Screen to identify the novel pancreatic gene Synaptotagmin 13 (Syt13)

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Bibliographic information published by the German National Library:

The German National Library lists this publication in the National Bibliography; detailed bibliographic data are available on the Internet at http://dnb.dnb.de .

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Imprint:

Copyright © 2016 GRIN Verlag
ISBN: 9783668296107

This book at GRIN:

https://www.grin.com/document/338115

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TECHNISCHE UNIVERSITÄT MÜNCHEN

Screen to identify the novel pancreatic gene *Synaptotagmin 13* (*Syt13*)

Stefanie Julia Willmann

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktor der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ. Prof. Dr. E. Grill

Prüfer der Dissertation:

1. Univ. Prof. Dr. M. Hrabe de Angelis
2. Univ. Prof. Dr. H. Lickert

Die Dissertation wurde am …….. bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am …….. angenommen.

Danksagung

Ich möchte mich auf dieser Seite bei allen bedanken die mich auf diesem Weg begleitet haben. Insbesondere will ich hier einige beim Namen nennen.

Insbesondere will ich mich bei Herrn Professor Dr. Heiko Lickert bedanken, für die Möglichkeit meine Promotion in seiner Arbeitsgruppe anzufertigen. Die Begeisterung für die Wissenschaft und dein Wissen hat mich stark beeindruckt. Ausserdem will ich mich natürlich noch bei Professor Dr. Martin Hrabe de Angelis bedanken, für die Unterstützung und fachliches Wissen.

Bei Herrn Professor Dr. Grill bedanke ich mich herzlich für den Vorsitz in meiner Prüfungskommission.

Bei dem Team der Arbeitsgruppe Lickert will ich mich für die langjährige Unterstützung bedanken. Besonders Dr. Ingo Burtscher für das offene Ohr bei allen Fragen, die Einführung in konfokale Mikroskopie, Immunhistochemische Färbungen und ES Zellkultur. Bei Dr. Aurelia Raducanu für die Unterstützung in dem nicht immer leichten Screening Projekt und die anschliessende Mausarbeit. Zusätzlich noch bei Dr. Mostafa Bahkti für die Unterstützung und Übernahme im Syt13 Projekt. Ausserdem will ich Aimee Bastidas-Ponce viel Erfolg bei der Weiterführung wünschen – es ist ein vielversprechendes Projekt.

Insbesondere gilt mein Dank an Dr. Bomi Jung, Heide Oller und Dr. Alexander Korostylev für die Unterstützung und hilfreichen Tipps. Auch geht ein herzlicher Dank an Dr. Silvia Engert für die Unterstützung in manchmal schwierigen Situationen. Donna Thompson – du bist eine Perle und dir gebührt extra Dank für deinen besonderen Einsatz in der Arbeitsgruppe. Anne, Wenke, Bianca und Kerstin haben wundervoll geholfen bei alltäglichen Problemen und Arbeiten wie PCRs, Maushausbesuche, Bestellungen und andere kleine Dinge im Laboralltag. Ausserdem noch zu erwähnen sind Dr. Nikola Müller, Steffen Sass, Dr. Martin Irmler und Dr. Harald Staiger für die gute Zusammenarbeit und Austausch in den verschiedenen Projekten.

Ausserdem will ich mich noch bei dem IDR Team bedanken für die Unterstützung und Informationen die das Projekt vorangetrieben haben. Hier geht mein besonderer Dank an Daniela Padula, Martin Preusse, Esra Karaköse, Anika Böttcher, Noah Mozurri, Lisann Heyner und Felizitas Schmid und natürlich nicht zu vergessen Erik Bader.

Aber der größte Dank geht an meine Familie für die Unterstützung in der Zeit – Danke das ihr für mich da wart und immer ein offenes Ohr hattet. Danke auch an Alex, Alex, Manu, Dani, Alex, Elke und nicht zu vergessen Rainer!

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1 Abstract

Screen to identify the novel pancreatic gene *Synaptotagmin 13* (*Syt13*)

The multipotent pancreatic progenitors segregate in the secondary transition (E12.5-15.5) into the pancreatic lineages endocrine, ductal and acinar in mouse development. Different signaling pathways as WNT, FGF, Notch, BMP, SHH and RA define anterior-posterior axis and the lineage decision of the organ. The precise mechanism of the formation of the endocrine lineage will allow generation of *in vitro* β-cells for cell-replacement therapy and identify potential disease genes. Thus, we utilized the *Foxa2-Venus fusion* (*FVF*) knock-in reporter mouse to separate the pancreatic endodermal epithelium (FVF^+) from the surrounding non-endodermal tissue (FVF^-) in the secondary transition. Subsequent global gene expression analyses identified 886 temporal significantly regulated genes in both tissue compartments. By using the public database Genepaint, spatial expression of the genes in the embryo and in the pancreatic region was analyzed in classifying the *in situ mRNA* expression pattern at E14.5 into tip/acinar, epithelium and mesenchyme. In a next step, GO term analyses classified the majority of the genes according to their predicted protein function. The selection of pancreatic genes, yet functionally not described are under the criteria of temporal regulated expression in the secondary transition, lineage prognosis by spatial pancreatic *mRNA* hybridization pattern and predicted protein function. In a next step, we generated Knock in/knock out mice lines to analyze the function and mechanism of these pancreatic candidate genes. Taken together, we successfully accomplished a global gene expression profile during secondary transition of the pancreas, inspected WNT, FGF, Notch, BMP, SHH pathway components and pancreatic lineage determinants. The pancreatic gene candidate, *Synaptotagmin 13 (Syt13)* is expressed in the secondary transition, the trunk *mRNA* hybridization pattern similar to endocrine TF factor *Ngn3* and the gene functionally not described. *Syt13* is a member of the large family of *Synaptotagmins* that are known to regulate vesicle docking and fusion for neurotransmitter and secretory granule release. Expression analysis using the *Syt13 lacZ* reporter gene revealed endocrine-specific expression in pancreas organogenesis and in the adult Islets of Langerhans. In addition, we observe in *Syt13* deficient pancreas delamination defects of endocrine progenitors during secondary transition. Also, we quantified a decline of endocrine differentiated cells characterized through Insulin. In collaboration with Harry Staiger and Hans-Ullrich Häring from the University in Tübingen we identified a single nucleotide polymorphisms (SNP; rs11038374) in *SYT13* that correlates with insulin secretion defects in a cohort of 2100 individuals with increased risk for diabetes. Thus, *Syt13* is expressed and regulates the endocrine lineage in mouse and may present a promising target for diabetes therapy.

Screening und Identifizierung des neuen Pankreas Gen *Synaptotagmin 13* (*Syt13*)

In der Pankreasentwicklung differenzieren aus einem Pool von Vorläuferzellen in der sekundären Transition (E12.5-15.5) die endokrine, exokrine und duktale Zelllinien. In dem Prozess selbst sind Gewebeinteraktionen und komplexe Signalwege involviert. Wir haben ein Transkriptionsprofil erstellt um die zeitliche und räumliche Expression regulatorischer und funktioneller Gene zu charakterisieren. Dabei haben wir spezifisch als Zeitpunkt die sekundäre Transition gewählt in der sich die verschiedenen pankreatischen Ziellinien abspalten.

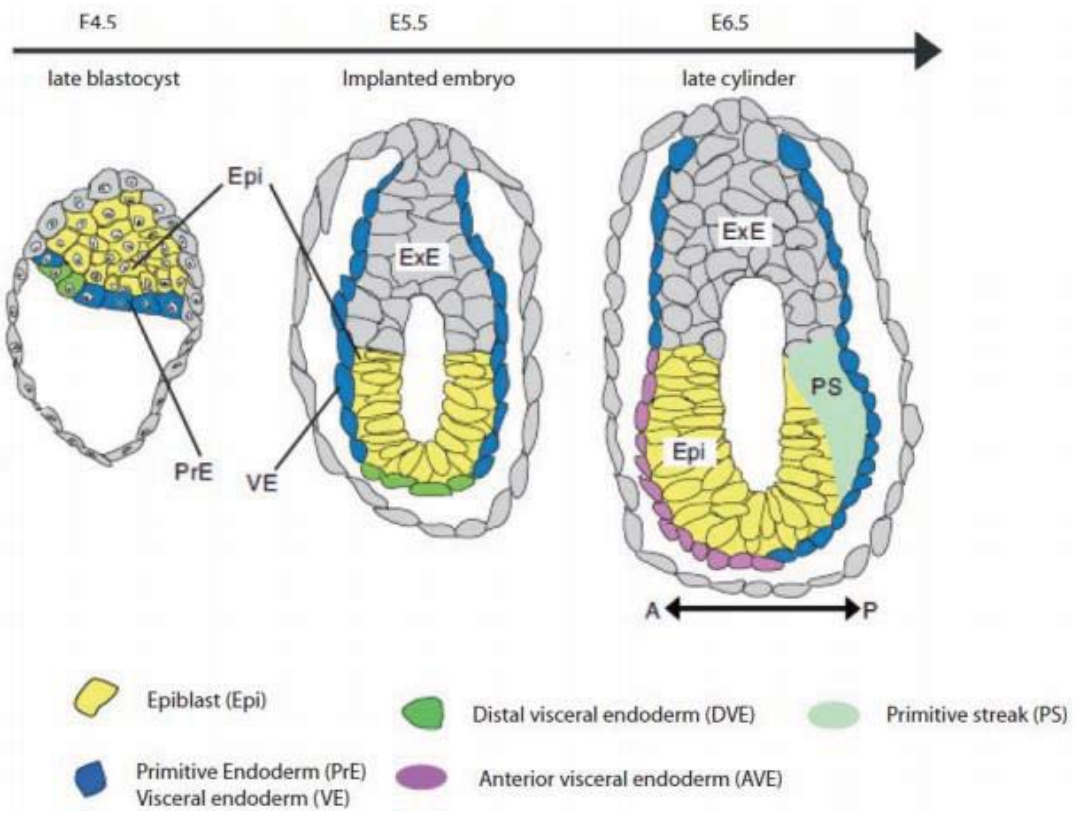
Mit der Forkhead Transkriptionsfaktor *Foxa2*^{Venus} (*FVF*) Mauslinie konnten wir pankreatisches Epithelium (*FVF*$^{+}$) von umgebenden Mesenchym (*FVF*$^{-}$) trennen. In dem anschließenden globalen Transkriptionsprofil identifizierten wir 886 signifikant regulierte Gene in beiden Kompartimenten, was auf essentielle Gewebeinteraktionen in der Pankreasentwicklung hinweist. Die Expression aus unserem Profil konnten wir dann mit der öffentlich zugänglichen Datenbank Genepaint für bekannte und unbekannte Gene bestätigen. Mit diesem Ansatz haben wir zudem die unterschiedlichen pankreatischen Linien nach bestimmten Mustern klassifiziert und zwar in Exokrin, Endokrin und Mesenchym. Für bekannte und unbekannte pankreatische Gene konnten wir somit die zeitlich und räumlich begrenzte Expression bei der Abspaltung in unterschiedlichen pankreatische Linien und die entsprechende Linie aufzeigen. In einem nächsten Schritt generierten wir verschiedene transgene Mauslinien um Funktion und Mechanismus der unbekannten Gene näher zu untersuchen. Zusammengefasst zeigt unser Transkriptionsprofil, das bekannte und unbekannte Gene in Gewebeinteraktionen und Stoffwechselwegen notwendig sind für die Entwicklung des Pankreas.

Eines der unbekannten identifizierten Gene ist *Synaptotagmin 13* (*Syt13*), mit räumlich und zeitlich begrenzter Expression in der sekundären Transition. Erste *LacZ* Reportergen Expressionsdaten weisen auf *Syt13* in endokrinen Zellen und adulten Langerhans'schen Inseln hin. Wir zeigen, das *Syt13* im Pankreas notwendig ist für die Abspaltung in die endokrine Linie und für die Bildung der endokrinen Zellmasse. In Kollaboration mit Harry Staiger (Universität Tübingen) konnten wir zudem *Syt13* direkt auf Diabetes beziehen, Genome-wide association studies (GWAS) Studien zeigen auf single nucleotide polymorphism (SNP; rs11038374) in *SYT13* die Defekte in der Insulinsekretion aufweisen. Unsere Daten schlagen vor, das *Syt13* in der endokrinen Zellentstehung eine Rolle spielt. Die weiteren Aussichten für *Syt13* sind dabei therapeutische Ansätze um endokrine Linien zu expandieren oder um diese zu induzieren.

2 Introduction

2.1 The early embryonic development

The development of the embryo starts with the formation of the zygote out of the fertilized egg. Thus, the fertilized zygote is capable of producing all the different lineages responsible for the embryo proper. Subsequent cell divisions onwards lead from the zygote to the 8-cell stage, termed as morula (Gardner 2001; Piotrowska and Zernicka-Goetz 2001; Piotrowska-Nitsche and Zernicka-Goetz 2005). These 5 cell cycles are commonly referred to as cleavage decisions, which occur in the absence of cellular growth or in the increase of total cell mass – leading to the blastocyst. At embryonic stage (E) 3.5, a cavity will form in the early blastocyst and will separate the embryo into two cell populations: the outer lining extraembryonic trophectoderm (TE) and the inner cell mass (ICM), oriented at one side of the cavity. This lineage segregation process is determined as the first cell fate decision. Further differentiation occurs within the ICM, specifying in the second cell fate decision the primitive Endoderm (PrE) and the Epiblast (Epi) (Figure 2.1 – late blastocyst). The PrE does not contribute to the embryo itself, presumably later as extra-embryonic (ExE) tissue to the yolk sac, whereas the ICM gives rise to the embryo proper (Cockburn and Rossant 2010; Johnson et al. 1986). Prior implantation into the maternal uterus at E4.5, the embryo elongates to an egg-shaped structure. The PrE covers the Epi in the cavity, so that the embryonic lineage will be enclosed by the PrE on one side and by the TE on the other side. In the next step, the PrE expands and lines the luminal surface of the TE and the distal visceral endoderm (DVE) (Figure 2.1 – implanted embryo). Thus, the DVE mediates activity of wingless type MMTV integration family (WNT), fibroblast growth factor (FGF), Bone morphogenetic protein (BMP) and Nodal for the differentiation and patterning of the Epi (Lewis and Tam 2006; Sumi et al. 2013; Sumi et al. 2008; de Sousa Lopes et al., 2004; Beddington and Robertson 1999). In a next step, the Anterior-Posterior (AP) patterning of the body axis is initiated in a dynamic process by variations in the endodermal structure, regionalized gene expression domains and morphogenetic signaling (Tam and Loebel, 2007). At E6.5, the cells of the DVE migrate into the prospective anterior region of the embryo and become the anterior visceral endoderm (AVE) of the late cylinder (Figure 2.1 – late cylinder). In the AVE activity of Wnt and Nodal inhibitors restrict the action of this signaling pathways to the posterior side of the embryo. Thus, formation of the primitive streak (PS) is initiated posterior of the AVE. With the formation of the PS at E6.5, the gastrulation and establishment of the three principal germ layers takes place (Beddington and Robertson 1999; Burtscher and Lickert 2009; Lawson et al. 1986).



modified from Arias et al., 2013

Figure 2.1: Early stages of mouse embryo development

In the blastocyst cells get orientated into Epi, distal visceral endoderm (DVE) and primitive endoderm (PrE). The Extraembryonic tissue (ExE) does not contribute to the embryo itself. After implantation Epi cells are immigrating into the primitive streak, egress and displace the AVE. Prior to gastrulation, proximal-distal (PD) and AP are established through morphogenetic signals.

Abbreviations: ExE = Extraembryonic; VE = Visceral endoderm; PrE = Primitive endoderm; Epi = Epiblast; AP = Anterior-Posterior; DVE = Distal visceral endoderm; AVE = Anterior visceral endoderm; PS = Primitive streak; PrE = primitive endoderm.

2.2 Development of endodermal derived organs

The gastrulation is initiated through formation of the PS on the opposite side of the AVE. In the steps of gastrulation, Epi cells migrate into the PS and segregate into distinct cell populations. The clonal analysis of Epi fate highlighted that temporal and spatial ingression of the Epi in the PS defines the three principal germ layers: endoderm, mesoderm and ectoderm.

With this in mind, Epi cells remaining in the blastocyst are still competent of the different embryonic lineages (Morani et al., 2013). As gastrulation is initiated, the Epi cells migrate through the posterior region in the PS and transform into the Exe mesoderm (Kinder et al., 1999). The center and most likely anterior part of the PS contributes to the mesodermal derived cells as cardiac, cranial, paraxial and

axial mesoderm (Kinder et al., 2001). Contrary, the most anterior end of the PS forms the definitive endoderm (DE) (Wells and Melton 1999; Engert et al. 2013). In further steps migrates the DE to the anterior region of the embryo via different routes and displaces the AVE. At the end of gastrulation, the DE covers as a cell-layer sheet the embryo with approximately 500 cells. (Tam and Loebel 2007; Lawson and Pedersen 1987). The DE will form the primitive gut tube through folding of this epithelial sheet. The folding appears at the anterior and posterior ends. Thus, anterior endoderm forms the anterior intestinal portal (AIP) defining the foregut and equally, the posterior endoderm folds to establish the caudal intestine portal (CIP) defining the hindgut region. At the end of folding of the DE, turning of the whole embryo completes internalization as a tube. The derivatives of the gut regions are regionalized in foregut, midgut and the hindgut. The foregut gives rise to the largest number of structures; it forms the pharynx and its derivative as the respiratory part, esophagus, stomach, duodenum and the liver with the pancreas. The midgut is the supplier of the gastrointestinal part of the embryo, with the exception of the upper duodenum. Contrary, the hindgut contributes to the urogenital tract (The atlas of mouse development).

In the ventral foregut endoderm development, organ domains are established through transcription factors and signals from the mesenchyme. The hepatic endoderm is induced by FGF signaling from the mesenchyme and is marked by *Hematopoietically expressed Homeobox* (*Hhex*) expression, establishing the liver (Zorn and Wells 2010; Zorn and Wells 2009) The first obvious indication of pancreas morphogenesis takes place approximately at embryonic stage E8.0. It is noteworthy that all pancreatic epithelial cells derive from a common pool of pancreatic progenitors that evaginate from the dorsal and ventral section of the foregut/midgut junction. A signaling cascade mainly contributed by mesodermal-derived FGF, BMP, WNT, Activin A, retinoic acid (RA) and repression of sonic hedgehog (SHH) leads to the outgrowth of the pancreatic protrusions (Raducanu and Lickert 2012; Pan and Wright 2011). Upon a gut roation between E11 and E12, the ventral pancreas switches dorsally and the proximity to the dorsal pancreatic bud leads to the fusion of the two lobes. This period involves tubulogenesis in the ongoing organogenesis which includes micro lumen fusion and the rearrangement of the epithelial sheet into a stratified epithelium (Kesavan et al. 2009). Along with plexus remodeling and proliferation, the pancreatic epithelium (PE) transforms into a branched single luminal ductal system. Simultaneously, in the so called secondary transition, the PE differentiates into the pancreatic lineages Subsequently, the formerly multipotent pancreatic progenitors (MPP) will be specified by lineage commitment through a temporal and spatial restricted transcription factor program. Lineage tracing experiments and Gain/Loss-of function analyses in genetically modified mice further gained insights into the morphogenetic and transcriptionally mechanism which facilitate maturation of the pancreatic anlagen (Mehta and Gittes 2005; Li et al. 2004; Gittes et al. 1996).

The earliest transcription factors that characterize the pancreatic buds are *Pancreatic and duodenal factor 1* (*Pdx1*), *pancreas transcription factor 1 alpha* (*Ptf1α*) and *Homeobox Protein HB9* (*Hlxb9*). In addition, combined ectopic expression of *Pdx1* and *Ptf1α* converts posterior endoderm into endocrine and exocrine pancreatic tissue, highlighting the importance of both factors for pancreas initiation (Afelik et al. 2006; Sherwood et al. 2009) Although, *wingled helix/forkhead box 1/2* (*Foxa1/2)*, *NK homeobox x 6-1* (*Nkx6-1*) and *Nkx2-2* are co-expressed in the pancreatic initiation stage, but their expression is broadly prevalent in the foregut endoderm. These multipotent cells give later on rise to all the different lineages of the adult pancreas, consisting of exocrine, ductal and endocrine cell. A landmark article by Golosow and Grobstein demonstrated that cytodifferentiation of the PE is promoted by the surrounding non-endodermal tissue. Experiments either stripping of the mesenchyme of the endodermal-derived, as well as pancreatic explants separated by a porous barrier of the non-endodermal part in trans filter experiments, could define the necessity of tissue interactions. In both projections the PE failed to proliferate. Thus, highlighting the tissue interaction in pancreas organogenesis (Golosow et al., 1962; Willmann et al., 2016).

2.3 Development of the pancreas

The adult pancreas is a compound gland which is important for the nutrient metabolism and composes of an exocrine compartment with acinar cells and ductal cells and an endocrine compartment composed of α, β ε, δ and pancreatic polypeptide (PP) cells organized in structures called the Islets of Langerhans. The acinar cells secrete digestive enzymes which are transported through the ductal system into the duodenum where they catalyze the digestion of nutrients. The endocrine cells secrete hormones such as glucagon, insulin, ghrelin, somatostatin and PP into the blood stream to maintain a fine balanced hormone homeostasis. Understanding the developmental process that leads to the formation of the functional organ is essential to generate potential therapeutic strategies for diabetes (Zorn and Wells 2010; Zorn and Wells 2009).

Pancreas formation appears at around E8.0 likely in the last steps of gastrulation with the separation of the primitive gut tube into foregut and hindgut. In this stage, inductive mesodermal signals including FGFs, BMPs, Activin and RA induce an area in the lateral endoderm and a dorsal domain in the midline endoderm to become pancreatic tissue as shown by expression of the transcription factor (TF) *Pdx1*, a key regulator of pancreas development (Stanger et al. 2007; Mehta and Gittes 2005; Gittes et

al. 1996; Gittes et al., 2009). Continuous signals of Wnt and FGF-signaling from the surrounding tissues activate expansion of the two buds into the surrounding mesenchyme. The stratified epithelium becomes polarized and microlumens are generated throughout the bud which later fuse together to form a luminal epithelial plexus (Figure 2.2). The stratified epithelial bud contains MPP and few differentiated endocrine cells which are mainly glucagon-positive (Herrera et al., 2000). This first differentiation event takes place between E9.0 and E11.5 is called the primary transition. At around E11.5, gut tube rotation brings the two pancreatic buds in close proximity to one another and this event leads to their fusion into a single pancreatic primordium, which shapes the future organ. Massive plexus remodeling leads to the organization of a tubular structure from which branches continue to form through an epithelial branching morphogenesis process. The epithelial cells are still multipotent and capable of differentiation into the three pancreatic lineages: duct, acinar and endocrine. From E12.5 until E15.5, the three pancreatic lineage decisions are subsequently being made in the process of the secondary transition. The segregation of the epithelium initiates patterning into a distal tip region and a proximal trunk. Thereby, the cells representing the tip region will be assigned for the exocrine lineage, which are characterized through expression of *Carboxypeptidase 1* (*Cpa1*), which expresses cells and co-expression of *Ptf1α*. The pattern of the trunk region is commonly represented by ductal/endocrine factors illustrated through *Sry related Homeobox 9* (*Sox9*) and *Neurogenin 3* (*Ngn3*). Thus, the ductal cells are bi-potent for either the ductal or endocrine lineage and restricted in the MPP PE. In the secondary transition at E12.5 – 15.5 in the ductal compartment the progenitor pool of the endocrine progenitors marked by *Ngn3*$^{+}$- cells delaminate out of the ductal cord through a process which is likely regarded as epithelial-mesenchymal transition (EMT) (Gouzi et al. 2011). Shortly after delamination, the endocrine precursors migrate into the PE and aggregate into the precursor Islets of Langerhans. From E18.5 onwards and in the first days after birth, the Islet of Langerhans morphogenesis takes place, which are indicated through the α-, δ-, ε-,PP- and β-cells that form clusters, invade the PE and acquire the typical architecture of the adult Islet of Langerhans (Zorn & Wells 2009; Gittes 2009).

In pancreas organogenesis, tubulogenesis is tightly spatio- and temporal regulated, as the lineages including branching morphogenesis develop in the proliferating pancreas (Willmann et al. 2016). The expansion of the pancreas alters compared to branching morphogenesis of different tubular organs as lung, kidney and ureteric bud (Karihaloo et al. 2005). The classical branching morphogenesis suggests that peripheral growth accompanied with epithelial expansion, contrary to the pancreatic branching, implicates plexus formation, proliferation and remodeling and further plexus formation. Postnatal (P), the PE is transformed into a branched single luminal ductal system that is necessary to transporting digestive enzymes. Currently, tubulogenesis remains widely unknown as the polarity establishment in the different lineages of the pancreas especially affecting the evolving β-cells. This might be an

interesting point of research, especially regarding the polarity within the adult Islets of Langerhans, respective β-cells (Kone et al. 2014; Granot et al. 2009; Lo et al. 2012; Martin-Belmonte and Perez-Moreno 2011).

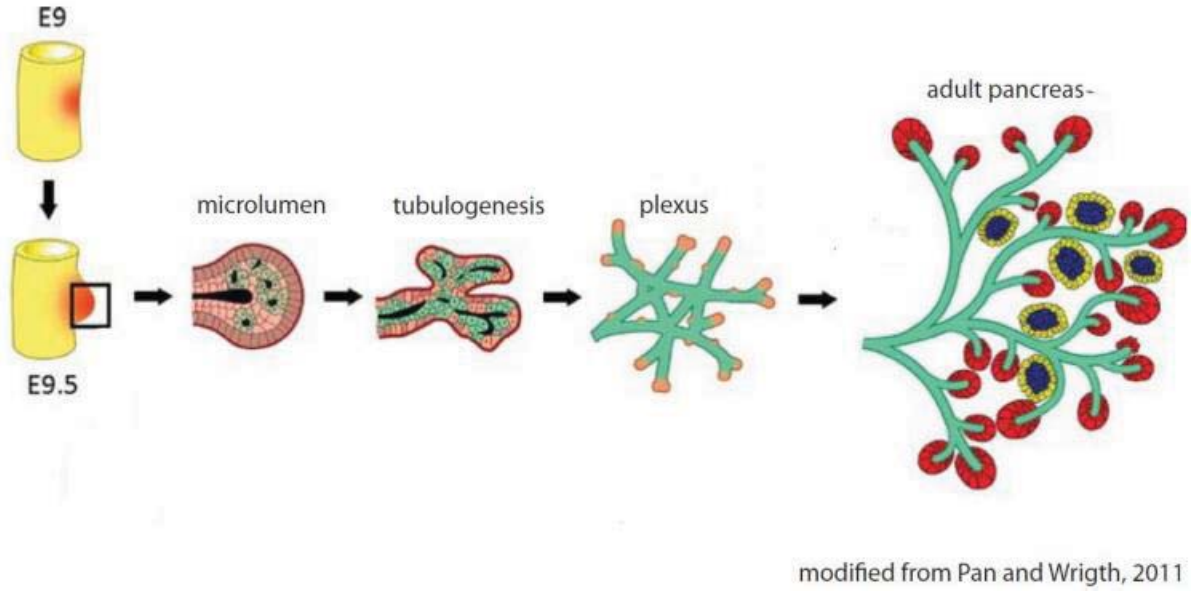


Figure 2.2: Tubulogenesis of the embryonic pancreas

The pancreas buds out of the anterior endoderm and at E11, micro lumen within the PE appear. Through a process called tubulogenesis at the beginning of the secondary transition, patterning into the different lineages determines specific regions. Epithelial remodeling (plexus) leads to the adult pancreas including defined compartments as the exocrine lineage (adult pancreas – red ball) and the endocrine Islets of Langerhans (adult pancreas – blue balls with β-cells; outer layer in yellow α-, δ-, ε- and pp-cells)

Abbreviations: E = embryonic stage; PP = pancreatic polypeptide; PE = pancreatic epithelium

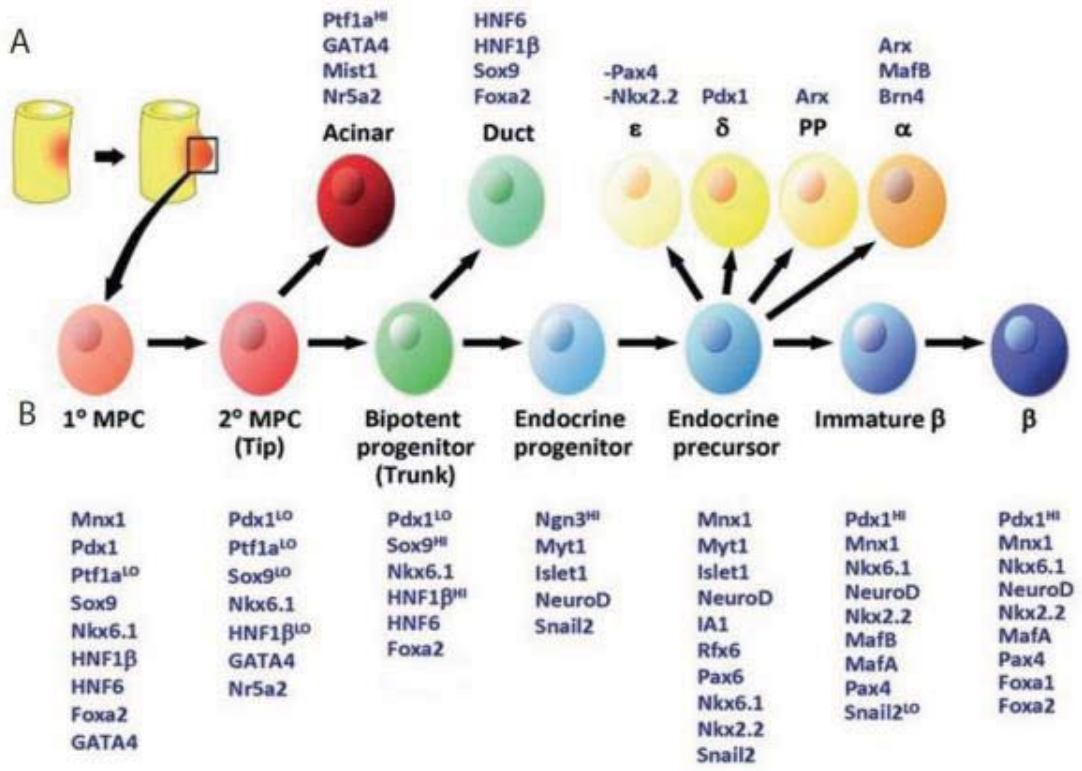
2.4 Regulatory networks of pancreas development

In pancreas organogenesis, a transcription factor hierarchy orchestrates the segregation into the different cell types. The entry point of pancreas initiation is well characterized by the TF *Pdx1*, as in the MPP after pancreas initiation Pdx1 serves as a marker for the PE (Gao et al. 2008).

Contrary, the adult pancreas consists of different cell types represented in the different lineages: acinar and ductal cells and the endocrine compartment comprising of the Islets of Langerhans which include α-, δ-, ε- and PP-cells and the β-cells. As the pancreas evolves, the PE is characterized by *Pdx1* expression, thereby reflecting subpopulations as $Pdx1^{low}$ and $Pdx1^{high}$. The $Pdx1^{low}$ population is specifically required for the exocrine lineage, whereas the lineage determining factor for the endocrine

compartment is represented by the subpopulation Pdx1^{high} (Holland et al. 2002). A transcription factor hierarchy is guiding the pancreatic outgrowth, consisting of Pdx1^{low} expressing PE within acinar cells represented by Ptf1α^{High} expression, as suppression of *Ptf1α* induces acinar-to-endocrine formation (Hesselson et al. 2011). In addition, members of the *GATA family* such as *GATA binding protein 4* (*Gata4)* and *basic helix-loop-helix 1 (Mist1)* regulate acinar cell proliferation (Pan and Wright 2011; Jia et al. 2008). Thus, *Mist1* is scattered on the foregut wall at stage E10.5 and after E13.5 and will be restricted to the acinar progenitors and mature exocrine cells. *Gata4* expression is restricted to the acinar fate in the E, in the adult pancreas Gata4 expression localizes specifically in the α - and β-cells of the Islets of Langerhans (Xuan et al. 2012). The factor *nuclear receptor subfamily 5 group a2* (*Nr5a2*) controls the early MPP the progenitor cells in the PE and after separation into tip and trunk pattern the acinar differentiation. It acts directly or through regulatory interactions with the acinar determining factors as *Gata4*, *Ptf1α* and the *recombining binding protein suppressor of hairless* (*Rbpjl*) (Hale et al. 2014).

In addition, the *SRY related gene 9* (*Sox9*) determines the ductal lineage independently of *Foxa2* and *Hnf6* (Dubois et al. 2011). In the adult pancreas, the ductal lineage is responsible for the transportation of digestive enzymes from the surrounding exocrine tissue into the duodenum. Intercalating blood vessels in the Islets of Langerhans transport the hormones into the blood stream for maintaining the precise blood glucose level in the adult pancreas. Through the use of a gain-of-function, in mice constantly misexpressing *Aristaless related homeobox* (*Arx*), β-cells were converted into pancreatic PP and α-cells (Courtney et al. 2013). Besides decreasing β-cell mass, mice developed hyperglycemia and died (Figure 2.3 A). There are several more regulatory genes and combinations, thus pointing to a fine-tuned spatio- and temporal network of TF in the secondary transition in pancreas organogenesis.



modified from Pan and Wright et al., 2011

Figure 2.3: Lineage hierarchy of the pancreatic lineages with cell specific genes for the adult pancreas (A) and the embryonic pancreas from multipotent state at E8.0 to the mature β cell (B).

(A) The adult pancreas consist of acinar cells (Ptf1α ^{high}, GATA4, Mist1 and Nr5a2), ductal lineage (Hnf6, Hnf1β, Sox9 and Foxa2) and the Islets of Langerhans (α-, δ-, ε- and PP-cells and β-cell).

(B) Multipotent progenitors get restricted to the β cell by different lineage commitments.
The transcription factor cascade starts with multipotent pancreatic cells (MPC) (Mnx1, Pdx1, Ptf1α ^{low}, Sox9, Nkx6-1, Hnf1β, Hnf6, Foxa2, GATA4), the second multipotent state is illustrated in a classical tip pattern (Pdx1^{low}, Ptf1α ^{low}, Sox9^{low}, Nkx6-1, Hnf1β^{high}, Hnf6 and Foxa2). Bi-potent progenitors are although characterizing the trunk pattern (Pdx1^{low}, Sox9^{high}, Nkx6-1, Hnf1β^{high}, Hnf6, Foxa2). In the next step, endocrine progenitors segregate in the PE (Ngn3^{high}, Myt1, Isl1, NeuroD, Snail2). Endocrine progenitors will led to endocrine precursors (Mnx1, Myt1, Isl1, NeuroD, Rfx6, Pax6, Nkx6-1, Nkx2-2, Snail2) and to immature β cells (Pdx1^{high}, Mnx1, Nkx6-1, NeuroD, Nkx2-2, MafB, MafA, Pax4, Snail2^{low}) to β cells (Pdx1^{high}, Mnx1, Nkx6-1, NeuroD, Nkx2-2, MafB, Pax4, Foxa1 and Foxa2)

Abbreviations: Ptf1α = pancreas specific transcription factor 1 α; Gata4 = Gata binding protein 4; Mist1 = basic helix-loop-helix protein; Nr5a2 = nuclear receptor subfamily 5 group a; Hnf6 = Hnf homeobox 6; Hnf1ß = Hnf homeobox 1ß; Sox9 = Sry related homeobox 9; Foxa2 = wingled helix/forkhead box 2; Foxa1 = wingled helix/forkhead box 1; PP = pancreatic polypeptide; MPC = multipotent pancreatic cells; Mnx1 = Moto neuron and pancreas homeobox 1; Pdx1 = pancreatic and duodenal homeobox 1; Nkx6-1 = Nk homeobox 6-1; Nkx2-2 = Nk homeobox 2-2; Ngn3 = Neurogenin 3; Myt1 = Myelin transcription factor 1; Isl1 = Isl lim homeobox 1; NeuroD = Neuronal Differentiation 1; Snail2 = Snail family Zinc Finger 2; Rfx6 = regulatory factor x 6; MafA = V-maf musculoaponeurotic fibrosarcoma oncogene homolog A; MafB = V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; Pax4 = paired homeobox x 4.

In the development of the pancreas, in conjunction with *Ptf1α* and *Foxa2*, *Pdx1* facilitates the expansion of the PE (Stanger et al. 2007). Thereby in later stages, it acts as an glucose-responsive regulator of insulin gene expression, likely for *glucagon-like peptide-1* (*GLP-1*) (Hay et al. 2005). *Ptf1α* and *Foxa2* act upstream of *Pdx1* with the combined expression in the MPP of the PE as early as the pancreatic formation is initiated at E8.0. In the later steps of pancreas organogenesis and finally in the adult pancreas, the total pancreatic cell mass depends strikingly on these marker onset (Stanger et al., 2007). The first lineage segregation appears as early as E9.5 which does not affect the adult pancreas. After the first lineage segregation, a second lineage segregation arises with a typical expression pattern that is described as a tip and trunk. The typical tip pattern represents the MPP at E14.5. The marker onset illustrating the typical bi-potent trunk pattern for the ductal and endocrine lineage in the PE at E14.5 is illustrated by the TF Pdx1^{low}, Ptf1α ^{low}, Sox9^{low}, HNF1β^{low}, NK homeobox, family 6- 1(Nkx6-1), Gata4 and Nr5a2 (Zhou et al., 2007). The compartmentalization of the ductal and endocrine lineage leads to a subset of Neurogenin3^{high} (Ngn3^{high}), Myelin transcription factor 1 (Myt1), ISL LIM homeobox 1 (Isl1), neuronal differentiation 1 (NeuroD) and Snail Family Zinc Finger 2 (Snail2) expressing cells (Figure 2.3 B). Thus, the endocrine progenitors still maintain the MPP state of the endocrine lineage, meaning that all the different subcells in the mature Islets of Langerhans derive out of this progenitor pool. In a next step in pancreas organogenesis, the immature β-cells will develop in the precursor Islets of Langerhans to the final mature β-cells. Factors that determine this specific lineage commitment include Pdx1^{high}, motor neuron and pancreas homeobox 1 (Mnx1), Nkx6-1, NeuroD, Nkx-.2, V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog B (MafB), MafA, Paired Box 4 (Pax4) and Snail2^{low} (Figure 2.3 B). As Snail factors determine the process of EMT, in the mature Islets of Langerhans *Snail2* is no longer expressed (Schaffer et al. 2013; Seymour et al. 2012; Herrera 2000).

2.5 The model of endocrine formation

In line with pancreas development, the endocrine lineage at approximately E13.5 will be restricted. Specific factors that drive the endocrine lineage are described as trunk pattern in the PE and is reflected by an epithelial cord pattern, which is illustrated by *Sox9* expression. Within this cord-like structure, the expression of the *Ngn3*-transient population represents the endocrine progenitors (Gradwohl et al. 2000; Gu et al. 2002; Schwitzgebel et al. 2000; Jensen 2004; Zhou et al. 2007). Mainly, lineage tracing experiments implicated that *Ngn3* as a basic helix-loop-helix transcription factor is necessary for the establishment of all endocrine cells as glucagon, Insulin, somatostatin and PP which first assemble into the Islets of Langerhans at E18.5 (Gradwohl et al. 2000; Herrera 2000). The latest attempts showed that *Sox9* deficient mice illustrate a severe reduction in endocrine progenitors marked by *Ngn3*, which

implicates that *Sox9* acts upstream of the endocrine lineage (Seymour et al. 2012). Furthermore, Lynn and Seymour proposed a cell-autonomous role for *Sox9* in *Ngn3* induction, which suggests an negative feedback-loop for co-related expression of *Sox9* and *Ngn3* (Shih et al. 2012; Seymour et al. 2012). These results further determine the importance of *Ngn3* as a key TF for the endocrine progenitor. In addition, upstream TF as *Sox9* and *Pdx1*, specifically in the duct, regulate expression of endocrine precursor *Ngn3*. The signal cascade for the regulation of *Ngn3* is still controversial, as there might be extrinsic and intrinsic signals that affect the proliferation of the endocrine lineage. Interestingly, signals from mesenchyme might not play a role in endocrine formation - as close proximity of epithelium to the mesenchyme accelerates the exocrine fate, whereas missing contact of epithelium to the mesenchyme leads to the endocrine lineage (Li et al. 2004).

In the 1970s, Pictet and Rutter already postulated that these endocrine progenitors delaminate out of the epithelial sheet and cluster to precursor Islets of Langerhans. This delamination process accompanies with alteration in contact to other cells. In the neuron crest system, delamination is well studied and described as transcriptionally controlled by the *Snail* family of TF (Sanitarias et al. 2002; Pictet et al. 1972).

In the process itself, *Snail family Zinc finger* TF are involved and members of the *Rho subfamily of GTPases*. Recently published by Rukstalis, *Snail2/Slug* is co-expressed with endocrine progenitor *Ngn3* and still maintained in a subset of differentiated endocrine cells (Rukstalis et al. 2006; Rukstalis and Habener 2007). These results link to epithelial and to EMT of endocrine progenitors as they leave the ductal cord. In development, cancer cells and metastases the mechanism of EMT is well studied. In the mechanism of EMT the epithelial cells remodel their polarity as they change from epithelial state to mesenchyme. The characteristic of the epithelium is described as apical-basal (AB) polarity including a polarized actin cytoskeleton. As cells change into a mesenchymal state, polarity changes from AB to front-rear and cell junction remodel as the cell moves out of the epithelial sheet. Main TF of EMT are *Snail2, Zinc Finger E-Box Binding Homeobox* (*Zeb*) and *Twist family BHLH transcription factor* (*Twist*). Another hallmark of EMT is described as the switch of *E-cadherin* (*Ecad*) to *N-cadherin*. The loosening of the epithelial character includes the down regulation of *E-cad*. Graphin-Botton had already published that *Ngn3* overexpression leads to endocrine differentiation. Further work by Gouzi showed that *Ecad* is transcriptionally down regulated in endocrine precursors with *Snail2* under the control of *Ngn3*. These results suggest that endocrine progenitors undergo delamination with at least partial EMT. Which mechanism regulates endocrine formation remains elusive and is in focus in the field of diabetes research, as it will help trigger the endocrine commitment *in vivo* (Johansson et al. 2007; Gouzi et al. 2011).