Cdc42 and Rac1 in Pancreatic Tubulogenesis and Islet Formation

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Cdc42 and Rac1 in Pancreatic Tubulogenesis and Islet Formation

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Faculty of Medicine
Lund University
2009

with permission from the Faculty of Medicine at Lund University, this thesis will be publicly defended on November 27 at 13.00 in Segerfalksalen, BMC, Sölvegatan 19, Lund

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**Abstract**

Tubes form the basic structure of several organs like lungs, kidneys, glandular organs like the pancreas, mammary and salivary glands. Tube formation involves a series of dynamic and interdependent cellular processes, including cytoskeletal reorganization, assembly of intercellular junctional complexes, and cell polarization. Understanding how cells polarize and coordinate tubulogenesis during organ formation is a central question in biology. Tubulogenesis often coincides with cell lineage specification during organ development. Hence, an elementary question is whether these two processes are independently controlled, or whether proper cell specification depends on formation of tubes.

To address these fundamental questions, we have studied the functional role of Cdc42 in pancreatic tubulogenesis. We present evidence that Cdc42 is essential for tube formation, specifically for initiating microlumen formation and later for maintaining apical cell polarity. Finally, we show that Cdc42 controls cell specification non-cell-autonomously by providing the correct microenvironment for proper control of cell fate choices of multipotent progenitors.

To understand the process and importance of endocrine cell delamination and migration from the ductal epithelium, we used transgenic mouse models expressing the dominant active Cdc42 (Cdc42 DA) or the dominant negative Rac1 (Rac1 DN) in the insulin cells. Active Cdc42 enhanced E-cadherin and F-actin at the cell-cell contacts and blocked the delamination of newly formed insulin cells. The beta cell numbers were severely reduced and resulted in hyperglycemia. Expression of dominant negative Rac1 did not affect the delamination but impaired the subsequent migration and organization of islet cells. E-cadherin was enhanced in the cell-cell contacts of the transgenic islet cells expressing Rac1 DN.

Our results describe the process of tube formation in glandular organs like pancreas and identified key regulators in the process. In addition, we show tube formation plays a crucial role in establishing correct microenvironment for proper cell fate specification. Both Cdc42 and Rac1 is regulated during endocrine cell delamination and migration from the ductal epithelium.

**Key words:** Cdc42, Rac1, microlumen, tubulogenesis, cell polarity, morphogenesis, delamination, migration and Pancreas.
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About the Cover Image:

An illustration representing the terminal ends of a branching pancreatic epithelium (yellow). The mesenchymal cells (red) and the extra cellular matrix proteins (turquoise) influence cell fate specification along the tubular epithelium.
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III. Rac1 regulates pancreatic islet morphogenesis.

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<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPKC</td>
<td>atypical protein kinase c</td>
</tr>
<tr>
<td>Arp</td>
<td>actin related protein</td>
</tr>
<tr>
<td>Cdc</td>
<td>cell division cycle</td>
</tr>
<tr>
<td>C.elegans</td>
<td>Caenorhabditis elegans</td>
</tr>
<tr>
<td>Cre</td>
<td>cyclization recombination</td>
</tr>
<tr>
<td>Dlg</td>
<td>disc large</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>epithelial cadherin</td>
</tr>
<tr>
<td>ECM</td>
<td>extra cellular matrix</td>
</tr>
<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
</tr>
<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
</tr>
<tr>
<td>GAP</td>
<td>GTPase activating protein</td>
</tr>
<tr>
<td>GEF</td>
<td>guanine nucleotide exchange factor</td>
</tr>
<tr>
<td>Hnf</td>
<td>hepatocyte nuclear factor</td>
</tr>
<tr>
<td>HUVEC</td>
<td>human umbilical vein endothelial cell</td>
</tr>
<tr>
<td>IA</td>
<td>insulin associated</td>
</tr>
<tr>
<td>Lgl</td>
<td>lethal giant larvae</td>
</tr>
<tr>
<td>MAGUK</td>
<td>membrane- associated guanylate kinase</td>
</tr>
<tr>
<td>MDCK</td>
<td>Madin Darby canine kidney cells</td>
</tr>
<tr>
<td>Maf</td>
<td>v-maf musculoaponeurotic fibrosarcoma oncogene homolog B</td>
</tr>
<tr>
<td>Ngn</td>
<td>neurogenin</td>
</tr>
<tr>
<td>Nkx</td>
<td>NK homeodomain protein</td>
</tr>
<tr>
<td>Par</td>
<td>partitioning defective</td>
</tr>
<tr>
<td>Pals</td>
<td>protein linked to protein associated with Lin-7</td>
</tr>
<tr>
<td>Pax</td>
<td>paired homeobox</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Patj</td>
<td>Pals- associated tight junction protein</td>
</tr>
<tr>
<td>Pdx1</td>
<td>pancreatic and duodenal homeobox 1</td>
</tr>
<tr>
<td>PDZ</td>
<td>PSD-95, disc large, ZO-1</td>
</tr>
<tr>
<td>Ptf</td>
<td>pancreas specific transcription factor</td>
</tr>
<tr>
<td>Sox</td>
<td>SRY-box containing protein</td>
</tr>
<tr>
<td>TGN</td>
<td>Trans Golgi network</td>
</tr>
<tr>
<td>WASP</td>
<td>Wiskott-Aldrich syndrome protein</td>
</tr>
<tr>
<td>WAVE</td>
<td>WASP family verprolin- homologous protein</td>
</tr>
<tr>
<td>ZO</td>
<td>Zonula occludens</td>
</tr>
</tbody>
</table>
Introduction

Tube formation

Tubes form the basic structure of several organs like lungs, kidneys, glandular organs including pancreas, mammary and salivary glands, the vascular system, gastrointestinal and urinary tracts. Biological tubes provide the transport ways for vital fluids and gases from one site to another. For example, vascular tubes allow the transport of $O_2$ and nutrients throughout the body. The ductal tubes in the pancreas allow the secretion of digestive enzymes into the duodenum (Lubarsky and Krasnow, 2003).

Tubes are generally made of a single layer of polarized cells with a distinct apical surface facing the lumen, lateral surface in contact with the neighboring cell and a basal surface contacting the extra cellular matrix. An absolute requirement of a biological tube is that a lumen must form and the lumen must be enclosed and unobstructed. Even though there is tremendous structural diversity in tube size, shape and connecting patterns, the basic strategy of tube formation share common developmental principles (Hogan and Kolodziej, 2002).

Defining how tubes form is an important question in developmental biology and it helps us to gain knowledge on the etiology of diseases and potentially their treatment through organ regeneration. Also how tube formation controls cell fate specification is a fundamental question to understand.

Models for studying tubulogenesis

There are several model systems available to study tubulogenesis and each of these models has their advantages and limitations over other models. Some reagents and techniques work best in specific systems. For example the simple cell culture models like MDCK epithelial cells is well suited to understand the mechanistic insights of tube formation, while it has limitations to incorporate complexities of organ architecture (Martin-Belmonte et al., 2007). The advantages and limitations of both in vivo and in vitro model systems are listed in the table (Table 1).

It is possible to dissect and culture in vitro entire epithelial organs, such as the salivary gland, pancreas, kidney and lung, from mice (Andrew and Ewald, 2009). This provides an easy way to screen several molecular targets controlling tubulogenesis. In addition, explant cultures provide a suitable system to follow the morphogenetic processes during tube formation by time lapse in vivo imaging.

Different strategies to make tubes

The different strategies of tube formation can be broadly divided into two general
<table>
<thead>
<tr>
<th>Model Systems</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCK- Mammalian epithelial cell line derived from adult canine kidney (Zegers et al., 2003).</td>
<td>In vitro model. Simple to use, suitable for genetic manipulation and biochemical assays.</td>
<td>Does not represent any developmental stages during kidney development.</td>
</tr>
<tr>
<td>HUVEC- Endothelial cell line derived from human umbilical vein (Davis et al., 2007).</td>
<td>In vitro model to study angiogenesis. Suitable for genetic and biochemical manipulations.</td>
<td>HUVEC are derived from a unique extra embryonic tissue and this creates limitations in generalizing the results.</td>
</tr>
<tr>
<td>C.elegans- Excretory system (Buechner, 2002)</td>
<td>The single cell kidney in C.elegans is the simplest in vivo model to understand tubulogenesis.</td>
<td>General limitations associated with simple organisms in extrapolating the results to mammalian systems.</td>
</tr>
<tr>
<td>Drosophila- Trachea and Salivary gland (Baer et al., 2009)</td>
<td>Excellent genetic model. Wide ranges of mutants are available. Suitable to study tube formation and cell fate specification. It is a suitable model for live cell imaging techniques.</td>
<td>Characteristic interspecies difference in epithelial organization. The barrier junctional complex differs in location and organization compared to mammals.</td>
</tr>
<tr>
<td>Zebra fish- Gut and vascular system (Bagnat et al., 2007; Kamei et al., 2006)</td>
<td>Vertebrate model to follow tube formation by live imaging. Wide ranges of mutants are available to dissect the molecular pathways in tubulogenesis.</td>
<td>Technical limitations to carry out biochemical assays. Not suitable to study tubulogenesis in specialized organs like mammary gland.</td>
</tr>
<tr>
<td>Mouse- Lung, kidneys and glandular organs (Andrew and Ewald, 2009)</td>
<td>Mammalian in vivo model. Suitable for genetic manipulations.</td>
<td>Fewer mutant models and genetic tools are available compared to Drosophila and zebra fish model systems.</td>
</tr>
</tbody>
</table>

**Table 1:** In vitro models like MDCK and HUVEC are extensively used to study tubulogenesis in combination with in vivo genetic models like Drosophila, Zebra fish and Mouse.
classes with respect to the state of tissue polarity at the onset of tubulogenesis.

1. Formation of tubes from pre-polarized epithelia

2. Formation of tubes from unpolarized epithelia

**Formation of tubes from pre-polarized epithelia**

The formation of tubes from pre-polarized epithelia occurs through two related mechanisms- wrapping and budding; requires only a change in cell shape/organization (Figure 1).

**a. Wrapping**

Wrapping occurs when cells within an epithelial sheet undergo coordinated apical constriction, which causes an inward curving or bending of the sheet of cells. This continues until the edges of the sheet meet and seal to form a tubular structure that lies parallel to the plane of the epithelium. The best examples of wrapping are neurulation in vertebrates and mesoderm invagination during gastrulation of *D.melanogaster*. Cells that form tubes by wrapping first adopt a columnar morphology giving rise to an epithelial placode of cells that are elongated along the apico-basal axis. Subsequent actinomyosin driven contraction of the apical cell surface in a subset of these cells changes their shape from columnar to pyramidal. This drives the bending and internalization of the placode (Lubarsky and Krasnow, 2003)

Proteins associated with apical constrictions are proposed to be involved in wrapping. In Drosophila, DRhoGEF2 has been shown to regulate the cell shape change during segmental groove formation (Mulinari et al., 2008). Also recently Shroom 3 (a PDZ protein) was shown to interact with ROCK kinases and play a critical role in chicken neural tube closure (Haigo et al., 2003; Nishimura and Takeichi, 2008).
**Figure 1:** Tube formation from pre-polarized epithelia occurs through wrapping and budding.

**b. Budding**

Budding occurs when cell within an epithelium invaginate through apical constriction and subsequently extend in a direction that is orthogonal to the plane of original epithelium. Orthogonal extension of the tube can occur through additional cell recruitment, cell division and/or cell elongation. Tube formation during branching morphogenesis of many organs, including the mammalian lung and Drosophila tracheal system occurs through budding. New branches bud from the walls of an existing branch, and the lumen of the new branch is a direct extension of the lumen of the parental branch (Baer et al., 2009).

Fgf10 is expressed in a dynamic fashion in the mesenchyme surrounding the epithelial branch tips, and is intimately linked to the branching process in the lung (Cardoso and Lu, 2006).

**Formation of tubes from unpolarized epithelia**

The formation of tubes from an unpolarized group of cells which occurs during the development of mammary gland, kidney, salivary gland and pancreas, first requires the establishment of a polarized epithelium with a distinct apical and basolateral cellular domains. As the cells acquire polarity, they form tubes either by cavitation, cord hollowing or cell hollowing (Figure 2).

**a. Cavitation**

Cavitation occurs by hollowing out a tube through the elimination of cells located in the centre of a cylinder of cells. Cavitation has been reported during mammary gland development, mammalian salivary gland formation and proamniotic cavity formation. The clearance process may occur through apoptosis, but the process is poorly understood.

Bim is a member of the proapoptotic BCL2 family gene and loss of Bim during mammary development in mice delays lumen formation. Other processes like autophagy have been suggested to occur during cavitation (Mailleux et al., 2007).

**b. Cord hollowing**

In cord hollowing, epithelial cells migrate and aggregate to form a solid cord like structure in which the lumen is subsequently made by the formation of an open space between the cells facing the future luminal side. Cord hollowing is observed in vasculogenesis, during gut development and in neurulation. Generation of apical- basal polarity is the key process in cord hollowing and how this is achieved in different tissues varies considerably. For example the process of cord hollowing during the Zebrafish gut development and neurulation differs.
During lumen formation in Zebra fish intestine, apical polarization occurs at multiple sites within the cord by the establishment of apical junctions involving tight junctional proteins like Zonula occludens (ZO-1). Next, microlumens form at the sites of polarization. Finally, these lumens expand upon influx of ions and water and coalesce into a single lumen. Mutations in atypical protein kinase Cλ (aPKC-λ) generate multiple lumens in the gut and mutations in Claudin 15 impair lumen coalescence (Bagnat et al., 2007; Horne-Badovinac et al., 2001).

During the zebra fish neurulation, cells on both sides of the midline of the neural rod divide to produce daughter cells with a mirror image polarity. The mirror symmetric cell divisions establish the distribution of apical proteins along the midline. To form only a single apical surface leading to a single lumen opening, mirror- symmetric divisions have to be properly positioned within the tissue. This might be regulated by non-canonical Wnt/planar polarity pathway. Mutations in Van Gogh- like2 (Vangl2) generate two lumens on either side of the midline. Although the primary polarity establishment varies between the gut and neural tissues, the process of lumen expansion is similar in both systems (Ciruna et al., 2006). In MDCK cyst model both cord hollowing and cavitation is involved in lumen formation (Martin-Belmonte and Mostov, 2008).

**Figure 2:** Tube formations in unpolarized epithelia can occur through cavitation, cord hollowing or cell hollowing.

### c. Cell hollowing

Lumen formation occurs within single cells, resulting in seamless structures
in which the lumen is not lined by AJ complexes. Cell hollowing occurs in the excretory cell of *C. elegans* and in the tracheal system of *Drosophila melanogaster*.

During *C. elegans* embryogenesis, the excretory cell extends branched processes, ultimately forming two canals on each side of the body. The extensions are tunneled by a single lumen with blind endings. At the onset of lumen formation, a large vacuole is generated by pinocytosis. Subsequently, this vacuole extends tubular arms that eventually coalesce and remodel to form a mature lumen (Berry et al., 2003). Several mutants affecting tube formation within the excretory cell have been identified and referred as exc mutants.

**Novel modes of tube formation**

Recently some novel modes of tube formation like cell wrapping/cell fusion and cell assembly has been reported (Figure 3).

**a. Cell wrapping/cell fusion**

During *C. elegans* digestive tract development, a novel way to form doughnut shaped cells with lumen inside and lacking an adherens junction seal has been reported. The pharynx and intestine are multicellular tubes. Between the two is the gut tube formed by seamless single cells known as pmi and vpi1. These two cells move across the midline of the forming tube, wrap itself around the axis of the future lumen and finally fuse with it self (Rasmussen et al., 2008).

**b. Cell assembly**

![Figure 3: Tube formation by cell assembly. Slit-Robo signaling (green and blue).](image-url)
During *D. melanogaster* heart tube development, two rows of myoendothelial cells line up along the midline and extend membrane processes towards the mirroring cell on the other side of the midline. In epithelial cells this would result in adhesion along the lateral contact. However, in these myoendothelial cells junctions occur only at the dorsal and ventral most regions resulting in the formation of an enclosed luminal tube between the two rows of cells. Slit-Robo signaling has been reported to be involved in repelling adhesion along the lateral contact and thereby establishing a lumen (Medioni et al., 2008).

**Apical polarization and de novo lumen formation**

Tube formation in unpolarized epithelia begins with the establishment of a de novo apical domain. Establishment of apical domain requires the coordinated targeting of apical proteins to the surface and the formation of junctional structures with the neighboring cells. Regulation of cell polarity occurs at three major levels.

A. Intrinsic mechanisms include the sorting and fusion of proteins from the Golgi to the target membrane.

B. Protein complexes of Par/Crumbs and Scribble family control the identities of the plasma membrane domains.

C. Extrinsic cues mediated by cell adhesion and ECM controls the orientation of cell polarity.

It is highly possible that all the three mechanisms regulate the overall polarity of the cells/tissue (Mellman and Nelson, 2008).

**Cell polarization by vesicular targeting**

After synthesis in the endoplasmic reticulum (ER), the newly synthesized membrane proteins are sorted by the coatmer protein complex- II (COPII) machinery and delivered to the Golgi complex through SNARE proteins. Intra-Golgi transport and retrograde transport from the Golgi to the ER are regulated by the COPII machinery. At the trans-Golgi network (TGN), the proteins are sorted into vesicles by intrinsic sorting motifs and cytoplasmic adapter complexes and are transported along cytoskeletal elements to the plasma membrane (Figure 4). Vesicle- tethering and proteins of SNARE family mediate protein delivery to the plasma membrane. Some proteins are internalized through another set of adaptors and delivered to an endosome (E), from which they are recycled back to the plasma membrane (Figure 4) (Lee et al., 2004).

The apical sorting motifs are localized in the extracellular or trans-membrane domains of proteins. Extracellular domain motifs contain N- and O- linked oligosaccharide chains. Apically sorted proteins like Occludin contains N-linked glycosylation motif where as sucrase isomaltase contains O-linked glycosylation
Glycosyl phosphoinositol (GPI) anchored proteins are sorted into the apical pathway in the Golgi complex. This occurs by the GPI-anchored protein oligomerization in lipid rafts that are rich in glycosphingolipids, spingomyelin and cholesterol. Proteins like heamagglutinin and neuraminidase are sorted through the lipid rafts (Simons and Ikonen, 1997). Another mechanism of sorting proteins to apical surface occurs through transcytosis, where membrane proteins from the basolateral surface are endocytosed, delivered to endosomes, resorted and transported to the apical surface (Casanova et al., 1990).

The fusion of vesicles with the correct membrane domain is a crucial step in polarity establishment. Fusion of vesicles at the target plasma membrane is mediated by SNARE complex. The SNARE complex includes vesicle (v)-SNARE (such as Vesicle-associated membrane protein (VAMP)) and target (t)-SNAREs (syntaxins). In polarized epithelial cells, different v-SNAREs and t-SNAREs regulate the apical and basolateral transport.

Mesh works of short actin filaments are localized underneath the plasma membrane and provide the scaffold to transport membrane proteins. The actin cytoskeleton controls cell polarity and protein trafficking at several levels.

**Figure 4**: Process of vesicle generation and the transport between the ER and Golgi. Apical polarization by vesicle targeting occurs through different processes.
Cell polarization by intracellular protein complexes

Epithelial cells establish two types of polarity, apical-basolateral (A-BL) polarity and planar polarity. The development of apical-basolateral polarity is crucial for establishing permeability barriers and to maintain ionic homeostasis along apical-basal axis. Three interacting protein complexes mediate A-BL polarization,

a. Par/aPKC complex, consisting of Par3, Par6, Cdc42 and atypical protein kinaseC (aPKC).

b. Crumbs complex, consisting of Crumbs, Pals1, PATJ.

c. Scribble complex, consisting of scribble, Disc large (Dlg) and lethal giant larvae (Lgl).

These three protein complexes interact and by a process of mutual exclusion define A-BL axis in epithelial cells (Assemat et al., 2008) (Figure 5).

Figure 5: Three polarity complexes and interactions.

a. Par polarity complex

The Par complex of proteins was first indentified in a C.elegans mutation screen for zygoti-axis specification. Par1 and Par4 (also known as LKB1) encode protein kinases, Par3 and Par6 contain PDZ domains, Par5 is a 14-3-3 protein and Par2 a zinc finger protein (Macara, 2004). The Par proteins are evolutionarily conserved.

Activation of LKB1 (Par4) in single epithelial cells lacking cell-cell adhesion, induce apical- basal polarization. Induction of STRAD activates LKB1, upon LKB1 activation single cells rapidly remodel their cytoskeleton to establish apical brush border. Tight junctional proteins like ZO-1 redistribute around these apical brush borders and the apical and basolateral markers sort to their respective
membrane domains. This finding contradicts the previous views and strongly suggests that a single cell can fully polarize even in the absence of junctional cell-cell contacts and LKB1 induces cell polarization in a cell-autonomous fashion (Baas et al., 2004).

The two PDZ domain containing Par members Par3 and Par6 along with the Ser/Thr kinase, atypical protein kinase C (aPKC) and the RHO GTPases protein Cdc42 constitute the Par polarity complex. The Par complex was first described as essential proteins for asymmetric cell divisions in the C.elegans zygote (Watts et al., 1996). Three Par6 proteins encoded by three different genes have been identified in mammals, Par6A/C, Par6B and Par6D/G. The Par6 protein contains three domains

1. PB1 (Phox/Bem) domain, in the N-terminus that binds to other PB1 domain containing proteins like aPKC.

2. CRIB (Cdc42 Rac interaction binding) domain, in the middle that binds to activated Cdc42 or Rac.

3. PDZ (PSD-95, disc large, ZO-1) domain, in the C-terminus that binds to proteins like Par3 and Crb3.

Although the three Par6 proteins are structurally similar, they have different patterns of tissue and sub cellular distribution. Our current knowledge on the specific function of Par6 in different tissues and the importance of the differences in their spatiotemporal expression is largely unclear (Assemat et al., 2008).

Two Par3 genes, Par3A and Par3B have been identified in mammals. The PDZ domain of Par3A interacts with Par6 and JAM (junctional adhesion molecule). Par3A also contains an aPKC-binding domain, which is phosphorylated by aPKC. Par3A is widely expressed in heart, kidney and brain. Par3B is strongly expressed in the kidney, lung and skeletal muscle. Par3A and Par3B are partially located in the cell-cell contacts regions and co-localized with ZO-1 in tight junctions (Lin et al., 2000).

Two aPKC genes have been identified in mammals, aPKC λ/ι and aPKCζ. Unlike conventional PKC, aPKC contains a PB1 domain in the N-terminus that interacts with Par6. The aPKCs are not activated by Ca$^{2+}$ and diacyl-glycerol as classical PKCs. The catalytic domain is conserved among all PKCs (including aPKCs). The catalytic domain phosphorylates Par3 and Lgl. In addition, Yamanaka et al have demonstrated that both Lgl and Par3 can form independent complexes with aPKC/Par6 and regulate epithelial cell polarity (Yamanaka et al., 2003). In the MDCK epithelial cells, aPKCs localize with the Par proteins at tight junctions. Over-expression of Kinase dead aPKC mutants blocks tight junction formation and mis-localize Par6 and Par3 in MDCK epithelial cells (Suzuki et al., 2001).
**b. Crumbs polarity complex**

The crumbs (Crb) polarity complex was first identified in Drosophila and subsequently in vertebrates. Mammalian Crb are trans-membrane proteins and the other members of this polarity complex like Pals1 and Patj are cytoplasmic scaffolding proteins.

In mammals three Crb genes have been identified namely Crb 1, 2 and 3. Crb1 and 2 are mainly expressed in the retina and brain and crb2 is also expressed in the kidney (den Hollander et al., 2001; van den Hurk et al., 2005). Crb3 is ubiquitously expressed in all epithelial tissues. The importance of tissue specific expression and their functional importance are largely unknown. The extracellular domain of Crb1 and 2 contains three laminin A/G domains and 19 and 14 EGF like domains respectively. On the contrary Crb3 has very short extracellular domain. The cytoplasmic tail consists of two domains, a FERM (band 4.1- ezin-radixin-moesin) domain and a PDZ domain. Mutations in Crb1 have been reported in retinal diseases like Leber congenital amaurosis (LCA) and retinis pigmentosa (RP). Mutations in Crb1 impair cell adhesion properties and or apical surface formation in MDCK cells.

Pals1 is the mammalian homologue of the Drosophila stardust (Std) protein and it is an adapter protein interacts with Crb and Patj. Pals1 is highly expressed in the placenta, kidney and at moderate levels in the brain, heart and skeletal muscle. Pals1 has multiple protein-protein interactions domains and it belongs to the MAGUK (membrane-associated guanylate kinase) family. It has two L27 domains, a PDZ domain, a SH3 domain, a hook domain and a GUK domain. Loss of Pals1 in MDCK cells leads to tight junction and polarity defects. Furthermore, loss of Pals1 resulted in concomitant loss of Patj but apparently no effect on the Crb expression or distribution. The underlying mechanism of Pals1s effect on Patj expression is unknown.

Patj (Pals- associated tight junction protein) and Mupp1 (multiple PDZ domain protein) are the two mammalian homologues of Drosophila Dpatj. The expression of Patj is restricted mainly to epithelial tissues like bladder, testis, ovary, small intestine, pancreas, kidney and lung. The interaction between Pals1 and Patj occurs through the L27 domains. The N-terminus of Patj contains the L27 domain followed by ten PDZ domains. The 6th PDZ domain interacts with ZO3 and the 8th PDZ domain interacts with claudin1 and thereby communicates with the tight junctional components. Over expression of patj disrupts the tight junctional localization of ZO1, ZO3 and Occludin, suggests that Patj may be involved in tight junction stabilization. Down regulation of Patj affects Crb3 and Pals1 localization to the apical membrane and at tight junctions. These data strongly suggest that Patj provides the link between the lateral (Occluding and ZO3) and apical (Pals1 and Crb3) components of tight junctions and stabilizes the Crb polarity complex (Assemat et al., 2008).
c. Scribble Polarity complex

The mammalian Scribble polarity complex includes three proteins namely Scrib, Dlg and Lgl and it is localized in the basolateral domain of epithelial cells. The Scrib protein is a member of the LAP family proteins and consists of 16 repeats of LRR motif at the N-terminal domain, followed by 2 LAP- specific domain and 4 PDZ domains at the C-terminus. Scrib can directly bind to tight junction proteins ZO2 via the PDZ domain. Suppression of Scrib in MDCK cells delay tight junction assembly and affects epithelial morphology.

There are five mammalian DLG proteins and it contains 3 PDZ domains, a SH3 domain, a hook domain and a GUK domain. Mice expressing truncated Dlg-1 showed delayed growth and die perinatally. The mutant mice showed defects in urino-genital organs but no effect was seen in cell-cell junctional complexes.

Four mammalian Lgl proteins have been identified, Lgl1-4 and they all contain WD40 domain. Lgl 1 and 2 are homologues to the Drosophila Lgl and expressed in the brain, kidney and testis. The Lgl proteins are localized in the lateral membrane, below the adherens junction. Loss of Lgl1 in mice impaired the asymmetric localization of Numb and resulted in severe hydrocephalus (Klezovitch et al., 2004).

Cdc42- the centre of polarity

Cdc42 was first identified as a polarity regulator in Saccharomyces cerevisiae (yeast). Mutations in Cdc42 impaired the budding process and altered the actin cytoskeleton. The general function of Cdc42 in regulating cell polarity is well conserved from yeast to mammals (Etienne-Manneville, 2004). Yeast- two hybrid screens identified Par6 to be key downstream effectors of Cdc42 in establishing cell polarity (Lin et al., 2000).

Cdc42 belongs to the family of RHO GTPases. Cdc42, RhoA and Rac1 have been extensively studied in different model systems. Cdc42 cycles between an active GTP bound or inactive GDP bound state. The activity of Cdc42 is controlled by GAPs (Rho GTPases activating protein) and GEFs (Rho guanine nucleotide exchange factor). Our current knowledge of Rho GTPases function is mainly derived from studies expressing the dominant active and negative versions of the proteins. There are general limitations regarding the specificity of the dominant active/negative versions on a specific GTPase.

Recent advances in gene targeting have generated several mouse models where, specific Rho GTPases can be knocked out in a cell/tissue specific manner (Heasman and Ridley, 2008).

Role of Cdc42 in establishing cell polarity has been extensively studied in various model organisms/systems. A recent study from Belmonte et al, demonstrated the requirement of Cdc42 in localizing Par-aPKC complex to the apical domain.
and in apical domain formation using a three dimensional (3D) MDCK cyst model (Martin-Belmonte et al., 2007).

Global knockout of Cdc42 in mice is embryonic lethal and the embryos die before E7.5 (Chen et al., 2000). Cdc42 is preferentially localized in the neural progenitor cells at the apical ventricular zone (VZ) and not in the basal subventricular zone (SVZ). Cdc42 is required for localizing adherens junction and Par proteins in the neural progenitors at the apical VZ. Cdc42 was knocked out in the neural progenitors during mouse embryogenesis. In the Cdc42 KO mice, the loss of Par complex localization is associated with the gradual conversion of apical progenitors to basal progenitors. As a consequence more neurons were generated (Cappello et al., 2006). In addition, apical localization of Par6 and aPKC was abolished following Cdc42 loss in the telencephalic neuroepithelium of the brain (Chen et al., 2006). Loss of Cdc42 in the skin caused a failure to stabilize beta catenin and consequently the fate determination of progenitors in hair follicle was altered (Wu et al., 2006). During early embryonic development in mice, loss of Cdc42 in embryoid bodies caused defects in cell polarity and cell-cell contacts. The phosphorylation levels of aPKC were reduced in the Cdc42 KO cells (Wu et al., 2007).

Cell polarization by extrinsic cues

Single epithelial cell grown in suspension- in the absence of cell-cell and cell-Extra cellular matrix (ECM) adhesion does not develop polarity. These cells die by programmed cell death (anoikis). When a single epithelial cell is grown on a laminin-coated surface, they develop A-BL polarity. The cell-ECM adhesion not only influences vesicular transport but also regulate the transcription of genes to establish a polarized architecture. The cell-cell adhesion molecule E-cadherin is critical for localizing the exocyst complex and to ensure that basolateral vesicles are delivered to their target domains. The communication of polarization cues could involve ECM interactions and it can occur through various receptors, e.g. integrins, dystroglycan and proteoglycan molecules (Deng et al., 2003; Yu et al., 2005). Alternatively, communication between cells could involve adhesion receptors, such as cadherins, or sensing of diffusible factors, such as morphogens, chemo attractants, and chemo repellants (Gumbiner, 2005; Gurdon and Bourillot, 2001).

Cell polarity defects in diseases

Various diseases can occur due to impaired protein trafficking or defective polarity complex proteins and cell-cell adhesion or cell-ECM adhesion proteins. Mutations in protein sorting machinery occur in several epithelial organs like kidney, lung and intestine. Missorting of proteins to wrong membrane domains can affect their functional properties. Some of the most common disease occurring due to the cell polarity defects is listed below (Table 2).
Pancreas development

Pancreas organogenesis begins with the evagination of the dorsal and ventral anlagen from the foregut endoderm. In mice, this event starts at embryonic day (E) 8.5. The initial pancreatic bud contains two major cell types, the pancreatic epithelium and the surrounding mesenchymal cells which are crucial for epithelial growth and differentiation (Gittes, 2008). All multipotent pancreatic progenitors in the early pancreatic epithelium express the pancreatic and duodenal homeobox gene 1(Pdx1). All mature cell types of the adult pancreas, including the exocrine, endocrine and duct cells, arise from these Pdx1+ progenitors. The specification of endoderm to a pancreatic fate does not require Pdx1, but it is crucial for the

<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein</th>
<th>Defect</th>
</tr>
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<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>CFTR (ΔF508)</td>
<td>Exit from endoplasm reticulum.</td>
</tr>
<tr>
<td></td>
<td>CFTR (C-terimal PDZ)</td>
<td>Exit from Golgi apparatus.</td>
</tr>
<tr>
<td>Polycystic Kidney defect</td>
<td>Polycystin-1 and 2</td>
<td>Protein mislocalization of Na+/K+ ATPase, EGFR and growth or mitotic defects.</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>Kinesin-2</td>
<td>Rhodopsin transport Missorting of photoreceptor apical membrane</td>
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<td>Rhodopsin</td>
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<td>Situs inversus</td>
<td>Left-right dynein</td>
<td>Cilium motility</td>
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<td>Oncogenesis</td>
<td>Scribble and Lgl</td>
<td>Decreased expression levels found in colorectal and malignant melanoma</td>
</tr>
<tr>
<td>Microvillus inclusion disease</td>
<td>Myo5b and Rab8</td>
<td>Dysfuctional apical domain and vesicular accumulation</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>Lkb1 (par4)</td>
<td>Overgrowth of cells in the gastrointestinal tract</td>
</tr>
<tr>
<td>CRASH syndrome</td>
<td>L1 cell adhesion molecule</td>
<td>Loss of axon and dendrite localization. Corpus callosum hypoplasia, retardation and aphasia</td>
</tr>
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Table 2: Cell polarity in diseases.
expansion of the pancreatic progenitors. Loss of Pdx1 in mice arrests the growth of pancreatic primordia at a very early stage and eventually results in pancreatic agenesis (Offield et al., 1996). Another important transcription factor, pancreas specific transcription factor 1a (Ptf1a) is also expressed along with Pdx1 in the multipotent progenitors (Figure 6) (Yoshitomi and Zaret, 2004).

The multipotent pancreatic progenitors expand without any lineage commitment until the secondary transition, which occurs between E13- E14 in mouse. At this point, the progenitors commit into three different lineages, the exocrine, the endocrine and the duct. Several transcriptional networks play a crucial role in cell fate commitment towards different lineages (Jorgensen et al., 2007).

a. Exocrine cells

The exocrine cells are the major cell types in the adult pancreas; it constitutes about 90% of the total pancreatic mass. The exocrine or acinar cells secrete digestive enzymes like proteases, lipases and nucleases into the duodenum through the ductal network. The signaling factors that segregate the commitment of endocrine and exocrine compartments are of great interest (Gittes, 2008). The pancreatic mesenchyme plays a crucial role in guiding the pancreatic progenitors to exocrine fate. By culturing the pancreatic epithelium devoid of mesenchyme, the progenitors were committed to an endocrine fate and the exocrine commitment was completely retarded (Duvillie et al., 2006).

Expression of exocrine markers like amylase is observed around E14.5 in the branching tips/acini of the mouse pancreatic epithelium. Ptf1a, which is expressed in the early multipotent progenitors also play a critical role in exocrine commitment. The expression of Ptf1a is detectable in acinar cells from E14.5 onwards. In Ptf1a null mice, the acinar and duct cells were completely absent while the endocrine were formed but migrated into the spleen (Gittes, 2008). The transcription factor Mist1 is expressed in acinar cells and in Mist1 null mice the exocrine tissue is extensively disorganized and the acinar cells acquire a ductal fate. These data suggest that Mist1 is crucial for acinar cell function and stability (Pin et al., 2001).

b. Duct cells

The ductal cells of the pancreas constitute about 10% in number and 4% in volume. The duct cells deliver the digestive enzymes secreted by the acinar cells into the duodenum. Interlobular ducts arise from the main pancreatic duct and join the different lobes of the pancreas. Within the lobes, the intercalated ducts drain the acini. Our current knowledge on the transcriptional regulation of duct cell lineage is limited (Grapin-Botton, 2005).

Recently the hepatocyte nuclear factor-6 (Hnf-6) has been shown to control the differentiation of inter and intralobular ducts with in the pancreas. The Hnf-6
null mice show severe defects in ducts with enlarged lumina and multiple cysts. Also the formation of primary cilia was impaired and the adherens junction proteins like beta catenin were mislocalized (Pierreux et al., 2006). Also a recent study showed the expression of Sox9 in centroacinar and ductal cells of the adult pancreas. Pancreas specific Sox9 KO showed cystic duct structures and reduced exocrine differentiation (Seymour et al., 2007).

c. Endocrine cells

Endocrine cells of the pancreas constitute about 5% of the total volume. The endocrine cells of the pancreas delaminate and migrate out of the ductal epithelium to form the islet of Langerhans. Ngn3 is a master regulator of endocrine differentiation and Ngn3 null mice completely lack all endocrine cells in the pancreas. Moreover, forced expression of Ngn3 is sufficient for endocrine differentiation and majority of them turn into glucagon expressing α cells (Gittes, 2008).

Pax6 is one of the major downstream targets of Ngn3. Pax6 null mice showed a severe reduction in α and β cells. Several other downstream targets like NeuroD and Insulin associated (IA1) is important for endocrine development. The family of NK homeodomain members likes Nkx2.2, Nkx6.1 and Nkx6.2 is also crucial for endocrine differentiation (Jorgensen et al., 2007).

![Figure 6: Differentiation of Pdx1+ multipotent progenitors into all pancreatic lineages.](image)

**Morphogenesis and cell differentiation**

During pancreas organogenesis, the expansion of multipotent progenitors occurs in parallel with lumen formation and extensive branching morphogenesis of the
pancreatic epithelium. The pancreatic epithelium forms finger like protrusion called the “branching tips” and invade the surrounding mesenchyme (Zhou et al., 2007). The exocrine cells secreting the digestive enzymes are found in the branching tips, while the ductal cells form the trunk of the branching tree. A subset of cells in the truck region expresses the endocrine progenitor marker Ngn3. These Ngn3+ cells differentiate into endocrine cells and delaminate from the ductal epithelium into the mesenchyme. The delaminated endocrine cells aggregate and form the islets (Gittes, 2008). Thus islet formation involves several morphogenetic processes like delamination and migration, which requires extensive cytoskeletal remodeling (Figure 7).

**Figure 7**: Delamination and migration of endocrine cells from the ductal epithelium. The delaminated endocrine cells cluster to form the islets of Langerhans.
The family of RHO GTPases has been shown to control several morphogenetic processes like directional cell migration, cell-cell adhesion, cell polarity and cytoskeletal remodeling. Three members of the family have been extensively studied—Rac1, Cdc42 and RhoA. Rac1 controls actin polymerization and filopodia formation via the WAVE (WASP family verprolin-homologous protein) and Arp2/3 complex. Dominant negative Rac1 inhibits lamellipodium extension, membrane ruffling and migration in epithelial cells, fibroblast and macrophages. Ablation of Rac1 during Drosophila tracheal development increased the cell-cell adhesion protein E-cadherin and impaired the remodeling processes. These results strongly support the importance of Rac1 in several key cell biological processes controlling morphogenesis (Heasman and Ridley, 2008).

Dominant active and negative forms of Cdc42 impair filopodia formation and show defects in actin polymerization. Cdc42 controls actin polymerization via the WASP (Wiskott-Aldrich syndrome protein) and Arp2/3 complex proteins. Over expression of Rho isoforms induce stress fiber formation in fibroblast. Both RhoB and RhoC knockout mice are viable with no major developmental defects. RhoA plays a key role in regulating the actomyosin contractility through the Rho Kinase (ROCK) and stimulates actin polymerization via the diaphanous related formins (Dia) (Heasman and Ridley, 2008).

**Mesenchyme in epithelial branching and differentiation**

The instructive function from mesenchyme to the epithelium was established based on the classic transplantation experiments. When combined with embryonic mammary mesenchyme, skin epithelium from mouse or even chicken can be respecified to form mammary branches and milk producing alveoli. Although, the salivary mesenchyme combined with mammary epithelium forms branches in the salivary gland pattern but it retains the ability to form milk-producing alveoli. These results suggest that cell differentiation and branch pattern are differentially controlled by both intrinsic and extrinsic factors. The stroma, which regulates epithelial differentiation and branching, plays an indispensable role in stem cells maintenance and regeneration of the mammary gland (Lu and Werb, 2008).

The small intestine offers an interesting example of regeneration and establishment of a stem cell niche. The small intestine can be divided into a relatively undifferentiated crypt at the base and an organized villi consisting of differentiated cells. From the ultrastructural studies it is clear that the multipotent stem cells are interspersed in the crypt along with the terminally differentiated paneth cells. This region is a “stem cell zone” more typically referred as a niche. This niche is established during early morphogenesis of the intestinal development. The signals emanating from the mesenchyme plays a crucial role in establishing and maintaining the niche (Fuchs, 2009).
Diabetes

Diabetes is solely defined by elevated blood glucose due to impaired insulin production or function or both. According to world health organization, 170 million people in the world are already suffering from diabetes and the number is expected to double by 2030. Diabetes can be broadly classified as Type I and Type II diabetes. Type I diabetes is an autoimmune disorder, where the insulin producing beta cells are targeted by the immune cells. Type II diabetes is characterized by insulin resistance and impaired glucose tolerance. Conventionally the diabetic patients are treated with multiple insulin injections.

The strategies to cure type I diabetes include preventing the loss of beta cells and or replacing the loss with new cells. Recently, type I patients transplanted with beta cells from islet donors restore blood glucose to normal levels. This opens up an opportunity to generate beta cells from various stem/progenitor cells, which can be transplanted into patients (Madsen, 2005).

Currently, various invitro differentiation protocols have been tried to expand and differentiate insulin producing beta cells. The human embryonic stem cells (hESCs) and the recent discovery of induced pluripotent stem cells (IPS) cells are potential source for cell replacement therapy. We need better understanding of the basic developmental program guiding the formation and maturation of insulin cells during normal embryonic development to be able to recapitulate them invitro.

Methods

Tissue specific knockouts and transgenic mouse models

The roles of different RHO GTPases have been extensively studied in different mammalian cell culture models using dominant active and dominant negative approaches. With recent advances in gene targeting and transgenic mouse models, new information on the specific role of these each GTPases in cytoskeletal organization and organ developments are revealed. Constitutive knock out (KO) of Cdc42 and Rac1 die very early in gestation and limits the opportunity to study their role in organ development. However, generation of floxed mice for Cdc42 and Rac1 provide an opportunity to study their specific role in tissues/cell types of our interest using Cre/lox recombination (Heasman and Ridley, 2008).

The coding exon in the gene of our interest is flanked with two loxp sequences (flocking). By expressing cre recombinase using a tissue/cell type specific promoter, the gene of interest will be loxed out (removed) creating a tissue/cell type specific KO. All the pancreatic cell types are derived from the Pdx1+ multipotent progenitors. Thus, the expression of cre recombinase under the control of Pdx1 promoter will generate a pancreas specific KO. Expression of cre recombinase can also be controlled with certain drugs like tamoxifen. By
injecting tamoxifen at different gestation periods, the gene of our interest can be knocked out at a specific time point. Also using tissue specific promoters, genes can be over expressed in the tissue of our interest (Gu et al., 2002).

**Explants culture**

It is possible to culture the entire epithelial organs as explants cultures and this technique has been extensively used in pancreas, salivary gland, kidney etc. The pancreatic explants can be cultured in 3D at the air-liquid interface on a filter (Edsbagge et al., 2005). Pancreatic explants can also be cultured on fibronectin coated cover slips and they grow two dimensionally (2D) (Percival and Slack, 1999). The 2D system is an easy way to evaluate branching processes during pancreas organogenesis. Both the 3D and 2D system is suitable for screening signaling molecules of interest in a fast way. It also provides a platform to follow the morphogenetic processes by live cell imaging.

**Microscopy**

Recent advancement in microscopy and computer programming has made it possible to generate 3D reconstructions of tissue samples. By analyzing the whole-mount immunofluorescence with confocal microscopy followed by 3D reconstructions generates a better understanding of the organ as a whole (Jorgensen et al., 2007). By combining the latest advanced fluorescence analysis with classical tools like electron microscopy strengthen our understanding on the cell biological aspects of organ development.

**Present investigation**

**Aim:**

1. To describe the process of tube formation during organogenesis.
2. To identify key regulators in tube formation.
3. To address the role of morphogenesis in cell fate specification.
4. To describe the process of endocrine cell delamination and migration during pancreas organogenesis and to identify the molecular regulators.

**Hypothesis:**

Key morphogenetic processes control tubulogenesis and endocrine development during pancreas development. Members of the Rho GTPases family have been shown to control cytoskeletal dynamics and regulate cell biological processes like cell-cell adhesion, cell polarity and cell migration. We hypothesized that RHO GTPases are involved in regulating branching morphogenesis, polarity establishment, endocrine cell delamination and migration during pancreas
Results

Cell polarization drives tubulogenesis in pancreas (paper I)

To understand the process of tube formation, we characterized the cell polarity status in the pancreatic epithelium throughout gestation. Using whole mount immunofluorescence and transmission electron microscopy, we describe key morphogenetic processes.

1. The early pancreatic tissue at E10.5 is multilayered and lack apical-basal cell polarity. The epithelium lacks luminal structures, except for the opening into the duodenum.

2. Lumen formation starts with the stochastic appearance of small openings called “microlumen” in the pancreatic epithelium at E11.5. The microlumen represents the de novo apical domain.

3. The de novo apical domain formation starts with the cell polarization initiation in a single cell. The apical surface expands from a single cell to the neighboring cells.

4. Formation of a microlumen establishes the common apical surface shared between several neighboring cells.

5. The microlumen expands and coalesces to establish a continuous luminal network at E12.5.

6. The first sign of a fully established tube is observed at E13.5. As the tubes are formed, the pancreatic epithelium undergoes extensive remodeling. The establishment of a fully polarized monolayered epithelium is observed at E15.5.

Cdc42 is required for tube formation (paper I)

Cdc42 was ablated in the developing pancreas by crossing Cdc42 floxed mice with Pdx1 cre mice. The heterozygotes for Cdc42 were indistinguishable from wildtype (WT) littermates.

1. Ablation of Cdc42 in the developing pancreas blocks tube formation and the KO pancreas completely lack tubes.

2. Microlumen formation and establishment of a common apical surface is impaired in the Cdc42 KO.

3. Impaired microlumen formation blocks subsequent steps in tube formation.
4. Results from the mosaic deletion of Cdc42 using Pdx1 Cre ER suggest that Cdc42 is required in a cell autonomous manner to establish and maintain a bona fide apical surface. Loss of Cdc42 favors delamination from the epithelium.

5. Failure to form tubes impairs epithelial morphogenesis and results in fragmentation of the epithelium.

6. Regulation of aPKC and Rho Kinase (ROCK) is important for tube formation.

**Impaired tube formation alter cell specification (paper I)**

1. Ablation of Cdc42 increased acinar cell differentiation at the expense of endocrine and duct lineage commitment.

2. The decrease in endocrine and duct lineage commitment is non-cell autonomously caused by cdc42 ablation.

3. The non-cell autonomous effects are due to the altered tissue architecture and the epithelial- ECM interactions in the Cdc42 KO.

4. Laminin promotes exocrine lineage commitment and functional blocking of laminin-1 in the Cdc42 KO restores acinar differentiation to normal levels.

**Cdc42 and Rac1 regulates endocrine delamination and migration (paper II and III)**

To understand the process of endocrine cell delamination and migration, we generated transgenic mouse models expressing the dominant active Cdc42 (Cdc42 DA) or the dominant negative Rac1 (Rac1 DN) in insulin cells.

1. Expression of Cdc42 DA in insulin cells impairs delamination from the ductal epithelium.

2. The transgenic insulin+ cells that failed to delaminate express high levels of polarized F-actin and cell-cell adhesion molecule E-cadherin.

3. Severe reduction in beta cell number, impaired islet morphogenesis and hyperglycemia is observed in the Cdc42 DA transgenic mice.

4. Expression of Rac1 DN in insulin cell impairs migration and alters islet shape and morphology.

5. The transgenic islets expressing Rac1 DN, show increased accumulation of E-cadherin in cell-cell contacts.
6. EGF-R ligand betacellulin acts upstream of Rac1 and regulates islet migration.

Discussion

Establishment of a common apical surface is crucial for tube formation (Paper I)

Tube formation in multilayered epithelia can occur through different mechanisms like cavitation and cord hollowing. Based on our results, it is clear that the process of cord hollowing guides tube formation in the pancreas. Acquisition of apical polarity in single epithelial cells is the initial trigger, which spreads cross the immediate neighbors and form microlumen. Several neighboring cells share this common apical surface and it is the crucial event to initiate tube formation. Recent studies in Zebrafish gut development describe similar formation of stochastic lumens within the unpolarized epithelium and also complete polarization of single epithelial cells has been reported in intestinal cell culture studies (Baas et al., 2004; Bagnat et al., 2007; Horne-Badovinac et al., 2001).

Cdc42 is crucial for the establishment of common apical surface shared between the neighbors and the failure to achieve this blocks tube formation. Cdc42 KO can still generate an apical domain, but it fail to spread to neighboring cells. The failure to initiate apical polarization in neighboring cells could be due to several reasons. First, Cdc42 is required to signal the neighbors to polarize; this communication failure can be due to the impaired tight junctional coupling with neighbors. Second, Cdc42 is required for the neighbors to receive the polarization signals. Third, it can be a combination of both the above-mentioned reasons.

Results from the aPKC ablation suggest that Cdc42 acts as a master regulator of tube formation and the signaling through aPKC is not the only mechanism of regulation. Also Cdc42 controls Rho Kinase activity during lumen formation and currently there is no in vivo data on the Cdc42-ROCK cross talks. Further understanding the signaling between Cdc42 and the ROCK will provide more insight into the molecular mechanisms of lumen formation in vivo.

Tubes establish the microenvironment required for proper cell fate specification (Paper I)

Understanding the relationship between morphogenesis and cell differentiation is the most important aspect of organ development. It is fundamental to understand how multipotent progenitors make lineage decisions and how morphogenesis establishes different microenvironments to guide proper lineage commitment. The in vitro explants studies clearly suggest that non-cell autonomous effects drive the cell fate alteration in the Cdc42 KO. The alteration in the epithelial-mesenchymal interaction and the consequent effects on the ECM- epithelial interaction increases acinar differentiation. Both the duct and the endocrine
specification are compromised due to the impaired morphogenesis. Consistently, blocking aPKC alters morphogenesis and endocrine cell commitment. On the contrary, mosaic deletion of Cdc42 does not change the tissue asymmetry and the cell fate specification is unaltered. In conclusion, these results provide an explanation for how a direct role of Cdc42 in tissue architecture secondarily specifies microenvironments permissive for specification of multipotent Pdx1+ pancreatic progenitors.

**Role of microenvironment during in vitro differentiation protocols to generate insulin cells (Paper I)**

There is great need for generating insulin producing beta cells for cell replacement therapies and various attempts has been made to generate them from different stem/progenitor pools. Our results strongly emphasize the importance of tube formation and morphogenesis in cell fate specification. Currently, the in vitro differentiation protocols have largely ignored the importance of microenvironments to guide cells into specific lineage commitments. Understanding the molecular mechanisms in establishing specific microenvironments will improve the efforts to generate insulin cells in vitro.

**Delamination of beta cells is important for its expansion and maintenance (Paper II)**

The newly formed insulin cells need to detach itself from the neighboring cells and delaminate from the ductal epithelium. The ductal epithelium is fully polarized with clear apical-basal polarity and the endocrine cells delaminate through the basal surface. The delaminated endocrine cells cluster and form islets. Basement membrane proteins surround the islet structures, but they do not have any clear apical-basal polarity like the ductal epithelium.

Both cell-cell adhesion and cell polarity must be regulated in order for the insulin+ cells to delaminate. Enhanced cell-cell adhesion proteins like E-cadherin and cytoskeletal components like F-action by active Cdc42 in insulin cells impair delamination. Consequently, the insulin+ cells fail to maintain their differentiated status by staying within the ductal epithelium. As a result, the beta cell numbers were severely reduced resulting in hyperglycemia. These results strongly emphasize that delamination is required for the beta cells to maintain their differentiated status. The microenvironment with in the ductal epithelium is not favorable for beta cells to maintain and expand.

**Rac1 regulates islet cell migration (Paper III)**

Dynamic regulation of actin cytoskeleton is required for the endocrine cells to migrate away from the ductal epithelium. Rac1 is important for the cytoskeletal rearrangement and expression of dominant negative Rac1 impair islet migration. Rac1 is also required for the subsequent steps to establish proper
islet morphology. Also dynamic regulation of cell-cell adhesion is required to establish proper islet architecture and the enhanced E-cadherin alter the normal morphogenetic events. This finding proposes a model; where the EGF-R ligand betacellulin induced Rac1 regulate cell-cell adhesion and cytoskeleton.

Conclusions

1. Apical polarization drives tube formation in multilayered unpolarized epithelia like pancreas.

2. Cdc42 is required for establishing common apical surface involving several cells, which is a prerequisite for tube formation.

3. Cdc42 plays a cell autonomous role in establishment and maintenance of a bona fide apical surface.

3. Tube formation establishes tissue asymmetry and microenvironments required for proper tissue commitment.

4. Delamination of beta cells creates the microenvironment favorable for its maintenance and expansion.

5. Rac1 modulates cytoskeleton and cell-cell adhesion during islet migration and morphogenesis.

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Populärvetenskaplig sammanfattning

Utvecklingen av levande organismer och dess funktion involverar ett antal biologiska processer. Att förstå basal principer och mekanismer under utvecklingen leder till ökad förståelse som är viktig för att förstå sjukdomars uppkomst.

Diabetes är en av dessa sjukdomar, där de insulinproducerande cellerna är oförmöga att kontrollera glukosnivåerna i blodet. I min doktorsavhandling har jag fokuserat på bildandet av tuber i bukspottkörteln samt hur olika celltyper bildas. Resultatet från min studie visar att bildandet av dessa tuber i bukspottkörteln är viktiga för insulinproducerande cellers utveckling. Denna kunskap kan användas för att utveckla terapier för att behandla diabetes.

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