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Regulation of β-cell function through the primary cilium

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Abstract
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Primary cilia are rod-like sensory organelles extending from the surface of most mammalian cells, and recent studies indicate they play critical roles in islet cell function and hormone secretion. Their sensory ability is achieved by specific receptors that initiate signal transduction within the cilium, enabling localized signaling events that can propagate into the cell and modulate its function. Using intact mouse and human islets, we show that GABA and somatostatin, two major paracrine factors in the islet microenvironment, exert their effects through ciliary receptors. We found that $GABA_{B1}$ receptors were enriched at the ciliary base, but mobilized distally upon GABA binding and selectively induced ciliary Ca^{2+} influx via activation of voltage-dependent Ca^{2+} channels. At the same time, cytosolic Ca^{2+} increases were prevented from propagating into the cilium due to enhanced Ca^{2+} extrusion at the cilia base, thus isolating the cilium from cytosolic Ca^{2+} signals. Somatostatin, secreted from islet δ-cells, directly activated ciliary SSTR3 receptors on neighboring β-cells as a consequence of their close proximity within the islet microenvironment. This localized signaling resulted in a rapid reduction of cAMP specifically within the cilium, promoting sustained nuclear entry of the cilia-dependent transcription factor GLI2. This mechanism operated in parallel with the canonical Hedgehog pathway and was critically dependent on ciliary Ca^{2+} signaling. We further showed that somatostatin was released directly onto β-cell cilia in intact islets, establishing primary cilia as a key site for paracrine regulation of β-cell function. Islets isolated from patients with type-2 diabetes were found to contain cells with reduced cilia length, which in turn led to reduced proximity between β-cell cilia and islet δ-cells. Additionally, cGMP was identified as another important ciliary second messenger. Both GLP-1 and atrial natriuretic peptide stimulated cGMP formation in β-cells, and the nucleotide freely diffused into the cilium, where it triggered increases in Ca^{2+} at least in part through activation of cyclic nucleotide-gated channels. Moreover, cGMP increases, similar to cAMP reductions, induced stable nuclear translocation of GLI2, indicating intricate interdependence of cAMP and cGMP signaling that may converge on ciliary Ca^{2+}. These findings highlight the primary cilium as a specialized and unique signaling compartment for integrating and interpreting paracrine, endocrine and intracellular cues, with important implications for islet cell function.

Keywords: cAMP, Ca2+, cGMP, primary cilia, β-cell, δ-cell, Hedgehog

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To mom and dad

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Sanchez GM, **Incedal TC**, Prada J, O'Callaghan P, Dyachok O, Echeverry S, Dumral Ö, Nguyen PM, Xie B, Barg S, Kreuger J, Dandekar T, Idevall-Hagren O. (2022) The β-cell primary cilium is an autonomous Ca^{2+} compartment for paracrine GABA signaling. *J Cell Biol,* 2;222(1): e202108101

II. **Incedal Nilsson C**, Dumral Ö, Sanchez G, Xie B, Müller A, Solimena M, Ren H, Idevall-Hagren O. (2025) Locally released somatostatin triggers cAMP and Ca^{2+} signaling in primary cilia to modulate pancreatic β-cell function. *EMBO J.*

III. **Incedal Nilsson C**, Dumral Ö, Idevall-Hagren O. cGMP signaling in the primary cilium of islet cells. (manuscript)

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Contents

Abbreviations

AC	Adenylyl Cyclase
ANP	Atrial Natriuretic Peptide
ATP	Adenosine Triphosphate
BBS	Bardet-Biedl Syndrome
bPAC	Bacterial Photoactivated Adenylyl cyclase
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CNG	Cyclic Nucleotide-Gated (channels)
FFAR4	Free Fatty Acid Receptor 4
GABA	γ-Aminobutyric acid
GABBR1	$GABA_{B1}$ receptor
GABBR2	$GABA_{B2}$ receptor
GLP-1	Glucagon-Like Peptide-1
Hh	Hedgehog
IBMX	3-Isobutyl-1-methylxanthine
IFT	Intraflagellar Transport
NCX	Na^{+}-Ca^{2+} Exchanger
NPRA	Natriuretic Peptide Receptor A
PDE	Phosphodiesterase
pGC	Particulate Guanylyl Cyclase
PKA	Protein Kinase A
PKG	Protein Kinase G
RhGC	Rhodopsin-Guanylyl Cyclase
Shh	Sonic hedgehog
SMO	Smoothened
SSTR	Somatostatin Receptor
T2D	Type 2 Diabetes
VGCC	Voltage-Gated Calcium Channel

Introduction

The pancreas is a vital organ responsible for regulating glucose homeostasis through the secretion of key hormones from endocrine cells situated within the islets of Langerhans. β-cells secrete insulin, which lowers blood glucose by promoting uptake and storage, while α-cells release glucagon, which increases glucose production during fasting states. δ-cells secrete somatostatin, a potent inhibitor of both insulin and glucagon release that maintain islet hormone balance. Disruptions in this tightly regulated system contribute to metabolic disorders, particularly type 2 diabetes (T2D), which is characterized by insulin resistance, β-cell dysfunction, and impaired intra-islet communication.

Recent findings suggest that the primary cilium, a microtubule-based sensory organelle present on most mammalian cells, plays key roles in islet cell function. Cilia act as signaling hubs, integrating external cues to regulate intracellular pathways. In pancreatic β-cells, ciliary signaling influences hormone secretion through modulation of second messengers such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and Ca^{2+}. Notably, ciliary dysfunction in pancreatic islet cells has been implicated in impaired glucose homeostasis, contributing to β-cell dysfunction, and insulin resistance. This suggests that defective ciliary signaling may be a previously underappreciated factor in the pathogenesis of T2D. Understanding how ciliary signaling shapes islet function could provide new insights into diabetes pathophysiology and identify novel therapeutic targets.

Background

Primary cilia: sensory organelles of mammalian cells

Primary cilia are solitary, microtubule-based organelles extending from most mammalian cells' surface, acting as specialized sensory hubs for the extracellular environment (1, 2). They are present in various tissues, including pancreatic islets, and play crucial roles in cellular communication and metabolic regulation (3, 4). Cilia assembly is tightly regulated by the cell cycle, forming during the G0/G1 phase as cells exit mitosis and disassembling before mitosis (G2-M phase) (5, 6). Structurally, the cilium is anchored by the basal body, derived from the mother centriole and docks at the membrane. The basal body consists of nine microtubule triplets (A-, B-, C- tubules), where the C-tubule ends at the distal end, and the remaining A- and B- tubules extend to form the transition zone, which is a ciliary gate that regulate protein entry into and exit from the cilia (7). Surrounding the ciliary base, the ciliary pocket engages in endocytic trafficking and protein transport to shape the ciliary membrane (7). Beyond the transition zone, microtubules doublets extend to form the axoneme, initially following 9+0 architecture. However, in β-cell cilia, the axonemal microtubules exhibit structural alteration, indicating 9+0 architecture is not consistently maintained along the cilia (8, 9). Islets cilia are found projecting toward the apical side of the cells (10), positioning them to sense the islet-derived factors and contribute to paracrine signal transduction (3, 11). Islet cilia have been shown to interact with neighbouring islet cells and their cilia, as well as with intra-islet nerve fibers (8). It has been reported that primary cilia form synapses with neurons in the brain (12, 13), and a similar organization in the islet suggests functional connectivity between islet cilia and their surrounding environment. Disruptions in ciliary structure and function contribute to a group of diseases known as ciliopathies, most of which are associated with obesity. There is growing evidence linking ciliary dysfunction to metabolic disorders such as obesity and T2D (14, 15). Similarities between loss of cilia phenotype and β-cell dysfunction in T2D suggest a potential role for cilia in disease development, as observed in mouse models (15). Understanding ciliary signaling and its role in islet function may provide new insights into metabolic disorders, including diabetes.

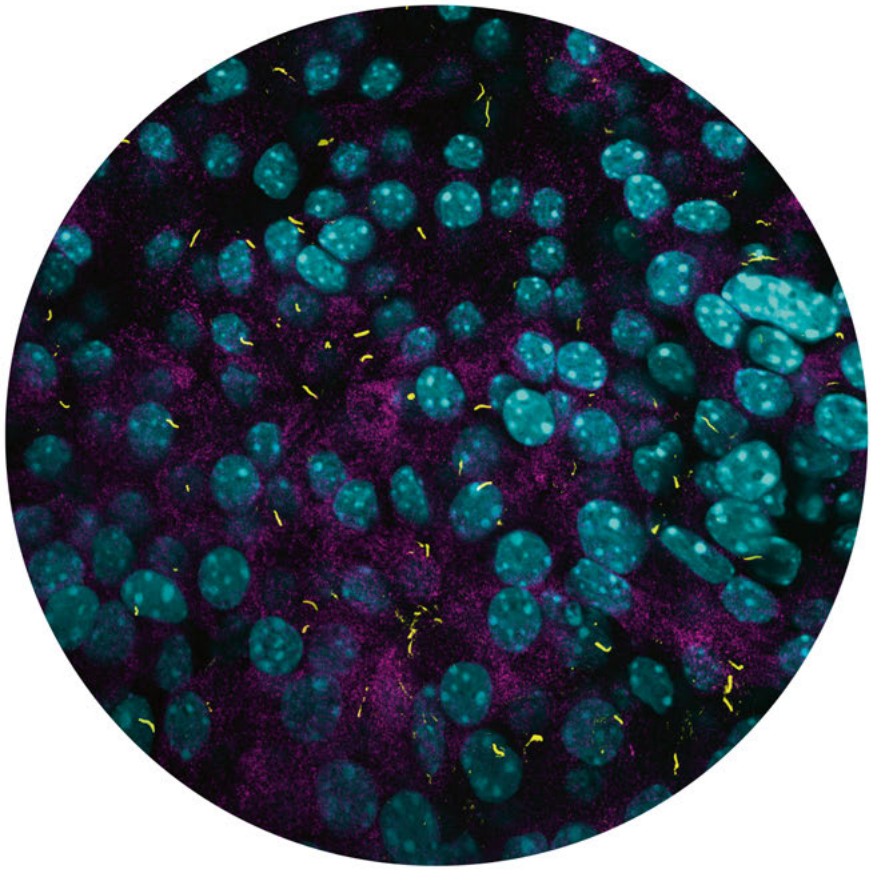

Figure 1. Islets of Langerhans are equipped with primary cilia. Mouse islets were immunostained against acetylated tubulin to visualize cilia (yellow) and against insulin to visualize β-cells (magenta). Nuclei are shown in cyan.

The ciliary membrane

The ciliary membrane originates from the plasma membrane, yet it differs from it and has a distinct protein and lipid composition. The primary cilium, densely packed with receptors and ion channels, has an exceptionally high surface-to-volume ratio, approximately 10-15 fold higher than the cell body (16, 17), which enhances signal detection and transduction efficiency. Recent advancements in proteomics, especially cilia-targeted proximity-labeling techniques have expanded our understanding of ciliary membrane composition, revealing a wide array of ciliary-localized receptors, ion channels, and downstream effectors that dynamically change in response to signaling

molecules and ciliopathy-associated mutations (18, 19). Some examples of receptors identified in cilia include transforming growth factorβ receptors, insulin receptors, dopamine receptors, serotonin receptors, melanocortin receptors, free fatty acid receptors, and somatostatin receptors (20-23).

The lipid composition of the ciliary membrane regulates signalling processes. The ciliary membrane is highly enriched in phosphatidylinositol 4-phosphate (PtdIns4P) but, in contrast to the plasma membrane, contains very little 4,5-bisphosphate (PtdIns(4,5)P_2) (24). The transition zone membrane predominantly contains phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and PtdIns(4,5)P_2 (25). This compartmentalized localization of phospholipids is tightly regulated by INPP5E, a phosphatase that maintains ciliary lipid homeostasis by dephosphorylating the 5'-position of the inositol ring of PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 (26). The localization of distinct lipids in the ciliary membrane is critical for the localization of GPCRs. For example, PtdIns(4,5)P_2 at the transition zone binds to the adaptor protein TULP3 through its tubby domain and and help to recruit GPCRs to the cilia base for subsequent IFT-mediated transport into the cilium (27). The ciliary lipid composition is not static but is subject to regulation through interactions with the intraflagellar transport (IFT) machinery (28). Disruptions in IFT-associated cargo adaptors can alter ciliary lipid composition, leading to defects in signaling pathways and contributing to ciliopathies (28). Besides phosphoinositides, the ciliary membrane is highly enriched in sterols that contribute to the formation of lipid rafts and microdomains that serve as platforms for signaling pathways (25). A prime example of this functional compartmentalization is Hedgehog signaling, which is directly modulated by cholesterol accessibility (29). Through this unique lipid and protein composition, the primary cilium functions as a dynamic cellular antenna, finely tuned to receive various extracellular signals.

Ciliary trafficking

The proper function and ciliary membrane plasticity require the transport of proteins and receptors from the cytoplasm. Receptor transport from the trans-Golgi network to the ciliary pocket is facilitated by a complex network of small GTPases and adaptor proteins, which coordinate vesicle trafficking to the periciliary membrane. Once at the ciliary base, receptor-containing vesicles dock near the transition zone, where their transport shifts from vesicular trafficking to IFT-dependent transport (14). IFT is a highly regulated, bidirectional transport system critical for shuttling proteins, receptors, and other cargo into and out of cilia (30). IFT involves two main protein complexes,

IFT-A and IFT-B, which mediate retrograde and anterograde transport, respectively. IFT-A is involved in retrograde protein transport and ciliary protein import, functioning together with the heterodimeric motor protein dynein-2 (31, 32). IFT-B is responsible for anterograde transport, moving cargo from the ciliary base toward the tip via the kinesin-II motor, and at the ciliary tip, IFT trains undergo remodeling, activating dynein-2 for retrograde transport back to the base (14). Both IFT-A and IFT-B are always present in IFT trains, but dynein-2 remains autoinhibited during anterograde transport to prevent interference with kinesin-II (33). Another crucial player in ciliary trafficking is the Bardet-Biedl syndrome (BBS) protein complex, also known as the BBSome. The BBSome is crucial in trafficking GPCRs into and out of the cilium by associating with IFT-A/B complexes (34). It is recruited to ciliary membranes via the small GTPase ARL6, which, in its GTP-bound form, interacts with BBS1 (23, 31). Retrieval of receptors from the cilium also relies on the BBSome and is, at least for some receptors, driven by ubiquitination (35). The precise control of receptor and signaling protein localization within cilia is essential for their ability to dynamically respond to extracellular changes, and mutations in IFT-A/B complexes and BBSome proteins disrupt this finely tuned trafficking system, leading to ciliopathies.

Cilia-dependent signaling

The hedgehog pathway is oriented around primary cilia (Figure 2.), serving as an evolutionarily conserved signaling mechanism with crucial roles during development. For example, it has been shown to regulate cell differentiation during pancreas organogenesis (36). The pathway operates through ciliary cAMP modulation, influencing the balance between repressor and activator forms of GLI transcription factors. When a Hedgehog ligand is present, it binds to the Patched receptor, releasing its inhibition on the atypical GPCR Smoothened (SMO), which then traffics into the cilium. Once inside, SMO inhibits protein kinase A (PKA) activity by lowering cAMP levels, allowing the conversion of GLI transcription factors from repressors to activators. This processing occurs primarily in the cilia tip, and following activation GLI proteins are transported to the cilia base where they exit into the cytosol and translocate into the nucleus, thus promoting Hedgehog signaling. In contrast, in the absence of Hedgehog, Patched locks SMO on the plasma membrane, preventing it from entering the cilium (37). Instead, the orphan receptor GPR161 accumulates inside cilia (38) and maintains constitutive PKA activity by promoting cAMP synthesis, which in turn keeps GLI in its repressor state and suppresses Hedgehog target gene expression. Hedgehog functions as

a morphogen, with its effects being dose- and time-dependent, determining the ratio of activator to repressor forms and ultimately shaping the transcriptional response (39). In the context of β-cells, disruptions of this pathway by triggering GLI2 activation in the absence of primary cilia disturb glucose-stimulated insulin secretion and induce dedifferentiation of β-cells by negatively regulating the expression of β-cell identity genes (40). The ciliary control of Hedgehog signaling emerges as a critical factor in preserving β-cells' function and a shift in the Hedgehog dynamics highlighting the importance of precise control.

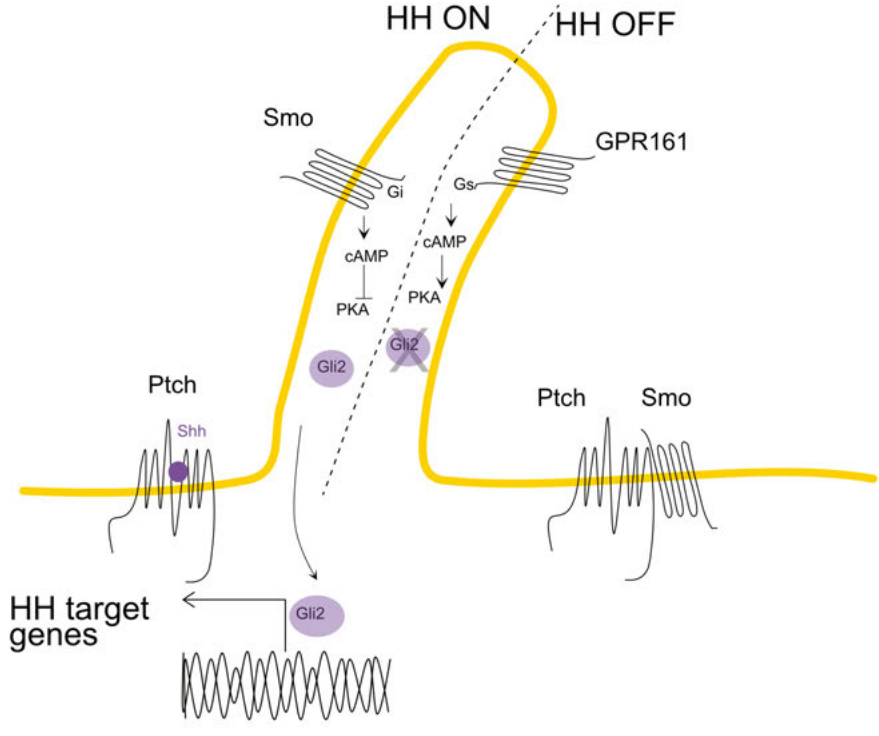

Figure 2. The Hedgehog pathway is oriented around the primary cilium: Hedgehog binding to Patched determines the GLI-mediated gene transcription through control of SMO localization and ciliary cAMP levels.

Compartmentalization of cAMP signaling in cilia

Since the volume of the cytoplasm is roughly 5,000 times greater than that of the cilium, the diffusion of small molecules from the cilioplasm into the cytoplasm has a negligible impact on overall cytoplasmic concentrations. Instead,

ciliary Ca^{2+} and cAMP are more likely to exert specific effects by activating effector proteins localized within or at the base of the primary cilium. This selective compartmentalization allows cilia to function as specialized signaling hubs where GPCRs interact with distinct signaling partners, fine tuning cellular responses. The melanocortin-4 receptor and the neuropeptide Y2 receptor both regulate food intake and energy homeostasis, and these effects require the receptors to be located to the ciliary membrane (41). Melanocortin 4 receptor, a Gαs-coupled receptor, increases ciliary cAMP levels, leading to activation of PKA and anorexigenic signaling (42). Conversely, neuropeptide Y2 receptor-coupling to Gαi, reduces ciliary cAMP levels and promotes orexigenic signaling (41). The coexistence of these receptors within the same cilium suggests that local cAMP regulation is finely tuned to balance metabolic homeostasis. Another aspect of signal compartmentalization is that the same receptor, when localizing to the cilia membrane, exhibits downstream coupling that is different from that of the same receptor localizing to the plasma membrane, as has been shown for the bile acid receptor, TGR5. This receptor signals through Gαs at the plasma membrane but switches to Gαi when localized to the cilium, altering cAMP dynamics and eventually reducing cell proliferation via ERK activation (43). Although receptor activation shapes ciliary cAMP signals via control of adenylate cyclase activity, this regulation also depends on the local action of phosphodiesterases (PDE). Several PDEs are either enriched, or exclusively found, in primary cilia. For example, ciliary PDE4 restricts ciliary cAMP production and supresses CREB pathway activation through an inhibitory effect on PKA activity (44). The unique spatial regulation of cAMP within the cilium controls precise signaling dynamics, allowing cells to differentiate the signals derived from cilia or cytosol and relay them onto global cell function. Parallel to cAMP, cGMP is another nucleotide second messenger with important roles in certain types of specialized cilia. In olfactory cilia, cGMP binds cyclic nucleotide gated (CNG) channels to induce Ca^{2+} transients (45) while in ciliary photoreceptors, it controls light-dependent ion channel gating (46). Like cAMP, cGMP serves as a critical regulator of ciliary signaling, supporting the compartmentalized nature of ciliary signal transduction.

Compartmentalization of Ca^{2+} signaling in cilia

Ca^{2+} is another bona fide signaling molecule in the cilia that regulates cell type-specific functions such as vasodilation (47), osteogenesis (48), olfaction (49), and left-right symmetry establishment (50). Although the ciliary membrane is continuous with the plasma membrane, it maintains a more positive

membrane potential (51) and is decorated with various Ca^{2+} channels, including TRPV4, TRPC1, and PKD2, whose expression is highly cell-type specific (44, 51, 52). In non-excitable cells, constitutively open ciliary heteromeric PKD1-L1/PKD2-L1 channels have been shown to maintain a Ca^{2+} gradient between the cilium and cytoplasm (51). However, whether such a gradient is present in other cell types, especially excitable cells that experience frequent fluctuations in the intracellular Ca^{2+} concentration, remains to be determined. This Ca^{2+} gradient appears to be essential for Hedgehog signaling by regulating the trafficking of GLI transcription factors (51). In neurons, ciliary Ca^{2+} signaling is actively modulated by Shh with TRPC3 channels residing in the primary cilium acting as key mediators of local Ca^{2+} influx in response to Shh stimulation (52).

While several studies have shown that cytosolic Ca^{2+} can enter the cilium via diffusion, its functional role remains closely tied to Hedgehog pathway regulation. Ca^{2+}-sensitive adenylyl cyclases (53) (AC3, AC5, and AC6) are localized to the cilium (54, 55), integrating Ca^{2+} signals to regulate cAMP levels. AC5 and AC6 negatively regulate the Hedgehog pathway by increasing cAMP production and PKA activation (56), whereas Ca^{2+}-activation of AC3 represses Hedgehog signaling during neural tube patterning (52). This relationship shows how changes in ciliary Ca^{2+} can modulate ciliary cAMP levels, ultimately influencing global cellular function.

Although Ca^{2+} is a ubiquitous secondary messenger in intracellular homeostasis, its precise spatial and temporal regulation within the cilium remains an active research area. In β-cells, the Ca^{2+} concentration undergoes regular fluctuations due to influx through voltage-gated calcium channels (VGCCs) on the plasma membrane and release from intracellular stores, but whether these changes propagate into the cilium and influence cilia signaling is not known. It is likely that ciliary Ca^{2+} homeostasis is maintained through efficient buffering systems or active extrusion processes, though the exact mechanisms remain unclear. Nevertheless, the tight regulation of ciliary Ca^{2+} is likely a prerequisite for further downstream signaling.

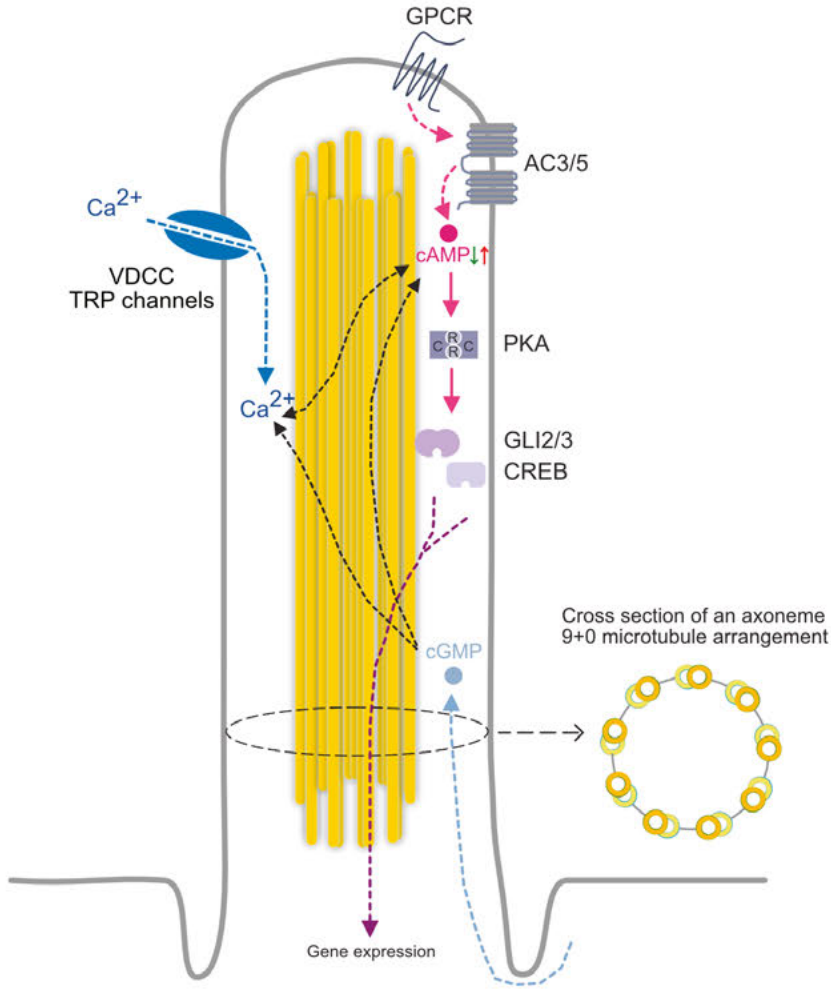

Figure 3. Primary cilia as a signaling hub. Many GPCRs localize to primary cilia and couple to adenylyl cycle activity, stimulating or inhibiting cAMP production: Ca^{2+}, another important secondary messenger, enters cilia via VGCC or TRP channels.

Islet cilia in β-cell function

The primary cilium functions as a dynamic sensor within the islet microenvironment, detecting local changes and modulating hormone secretion in response to immediate physiological changes. This is accomplished through activation of cilia-localized GPCRs, including free fatty acid receptor 4 (FFAR4), somatostatin receptor 3 (SSTR3), and the insulin receptor, each

facilitating distinct signaling pathways that modulate β-cell function (Figure 4.) (11, 21, 57). Deletion of β-cell cilia results in defective glucose handling, increased fasting blood glucose levels, and impaired somatostatin-mediated insulin suppression, highlighting the role of cilia in islet paracrine regulation (3, 57). While plasma membrane-localized SSTR2 modulates β-cell activity in response to basal somatostatin levels, ciliary SSTR3 regulates insulin secretion at elevated glucose concentrations (11). This suggests a parallel signaling mechanism where receptors at the plasma membrane and within the cilium function in distinct but complementary ways. Rather than requiring specific receptor subtypes, being localized to the cilium may enhance receptor sensitivity to ligands. Studies show that ciliary receptors can exhibit unique coupling properties or lower activation thresholds compared to their plasma membrane counterparts. For instance, in adipocytes, ciliary insulin receptor substrate 1 is phosphorylated at nanomolar insulin concentrations, whereas plasma membrane IRS1 requires significantly higher levels (58). The increased sensitivity may be a result of the unique composition of the cilium or ciliary membrane, and perhaps also facilitated by localized ligand delivery. In islets, the tight physical proximity of α-, β-, and δ-cells suggests that primary cilia facilitate direct interactions within specialized domains inaccessible to other cellular regions. This structural arrangement likely stabilizes juxtacrine signaling (59) and enhances contact-dependent communication (60). Similar interactions have been observed in hippocampal neurons, where serotonin release is directed toward primary cilia in a synapse-like manner (12), reinforcing the idea that cilia function as specialized signaling compartments. Evolutionarily, the cilium has been linked to the origins of neuronal post-synapses, with early ciliated cells exhibiting post-synaptic-like proteins before the emergence of the nervous system (61). The primary cilium acts as an evolutionarily conserved hub for localized signaling, integrating metabolic cues and coordinating intercellular communication to fine-tune hormone secretion and maintain islet homeostasis.

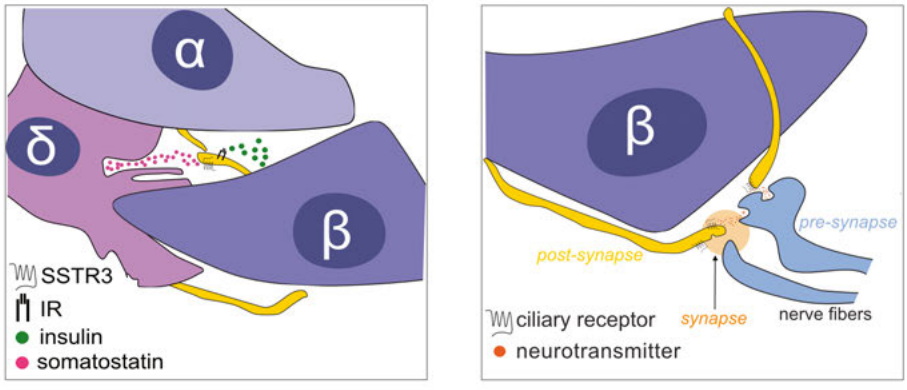

Figure 4. Primary cilia are sensors of the islet environment. The primary cilia detect islet-derived factors like insulin and somatostatin. The primary cilia may also detect neurotransmitters released from the islet nerve fibers.

The islet microenvironment

The islets of Langerhans are specialized endocrine clusters within the pancreas that play a crucial role in maintaining glucose homeostasis. Despite comprising only 1–2% of the pancreas, islets receive up to 20% of the pancreatic blood supply (62), enabling efficient nutrient sensing and distribution of secreted hormones to target tissues. This efficiency arises from their dense glomerular-like capillary network (63), which facilitates the rapid exchange of nutrients, oxygen, and signaling molecules between the islet cells and the circulation. Due to their unique vascularization, islet endocrine cells are directly exposed to systemic glucose concentrations, and function as the glucose sensors of the body (63, 64). The islets contain several endocrine cell types, of which insulin secreting β-cells make up 50% of islet cells in humans, whereas in mice, they consist of 75%, indicating species differences in islet composition and organization. In contrast, α-cells, which secrete glucagon to elevate blood glucose levels in response to fasting or in between meals, account for 35–40% in human islets and 15–20% in mice. δ-cells, which release somatostatin, make up 10–15% in humans and ~5% in mice, while pancreatic polypeptide-secreting γ-cells are found in smaller numbers (65, 66). The structural arrangement of the islet cells is designed to facilitate efficient hormone secretion and communication, which is facilitated by electrical coupling and auto- and paracrine effects (64). Beyond vascularization and cellular organization, pancreatic islets are innervated by cholinergic, adrenergic, and peptidergic nerves, which contribute to hormonal regulation (67, 68) as observed in human and mouse islets (69, 70).

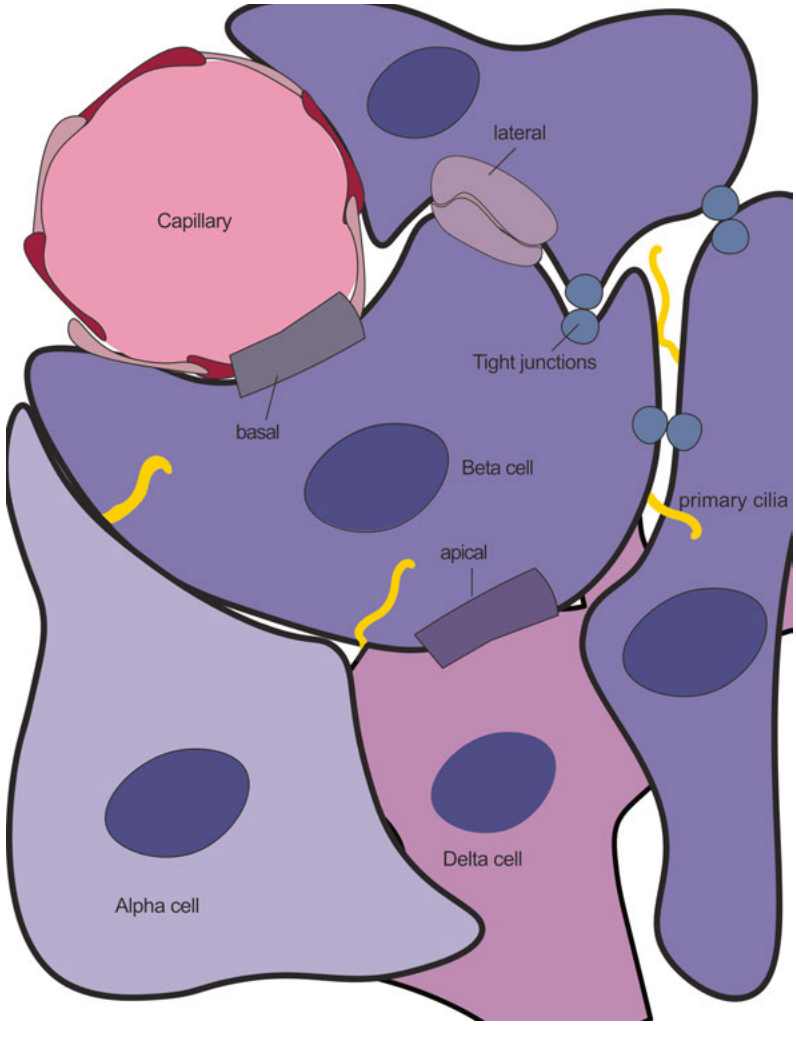

Figure 5. Islet cells are polarized and primary cilia project towards the apical side, facing away from the blood vessels.

Insulin secretion: maintaining glucose balance

The β-cell is the predominant cell type in the islets of Langerhans and is polarized with an apical domain from which the primary cilium extends into the extracellular space (Figure 5.). The β-cell edges face the lateral domain where glucose transporters and the Ca^{2+} sensing machinery are situated, and secretion preferentially occurs towards the vessel-adjacent basal domain (10). β-cells act as glucose sensors, and when glucose levels rise, they take up glucose in a facilitative manner that differs between species. In mice, glucose transport is mediated by GLUT2, a low-affinity transporter, allowing rapid equilibration

of intracellular and extracellular glucose, with the ensuing glucose uptake not being rate limiting in downstream metabolic processes (71). Human β-cells primarily express GLUT1, which has a higher glucose affinity (72), thereby enabling insulin secretion at lower glucose levels. Once glucose is inside the β-cell, glucose metabolism is initiated by glucokinase, the rate limiting enzyme in glucose metabolism, leading to glucose phosphorylation and subsequent further processing through glycolysis and oxidative metabolism. This metabolic cascade leads to the generation of ATP, increasing the ATP/ADP ratio, which serves as a critical regulatory signal for insulin secretion. A rise in the ATP/ADP ratio leads to closure of ATP-sensitive K^+ (K_{ATP}) channels in the β-cell plasma membrane, reducing K^+ efflux and triggering membrane depolarization. The increase in membrane potential activates VGCCs, leading to Ca^{2+} influx and a rise in intracellular Ca^{2+} concentration, which is the primary trigger for insulin granule exocytosis.

Paracrine regulation of the β-cell

Insulin secretion is tightly regulated by signals from surrounding cells that are part of a local communication network within the islet. One well-known modulator of insulin secretion is somatostatin, which inhibits insulin and glucagon secretion. Somatostatin secretion is positively regulated by glucose, and released somatostatin acts through activation of somatostatin receptors (SSTRs) on β- and α- cells, coupling to $G_{i/o}$ proteins that inhibit adenylate cyclases, thereby lowering cAMP levels and reducing hormone exocytosis (73). Somatostatin release is further enhanced by ghrelin, a peptide hormone mainly produced in the stomach (74), but also secreted by rare ε-cells in the islet (75). Ghrelin signals via Gαq-coupled receptors on δ-cells (76, 77), increasing somatostatin secretion and inhibiting insulin secretion probably as a result of paracrine inhibition (78).

Another important modulator of insulin secretion is glucagon-like peptide-1 (GLP-1), which is secreted both as a paracrine factor from islet α-cells and as a systemic signal from glucose-sensing cells in the intestine (79). GLP-1R activation on β-cells activates AC, increases cAMP production, and amplifies insulin release by enhancing Ca^{2+} influx and insulin vesicle exocytosis (80).

γ-Aminobutyric acid (GABA) is secreted from β-cells and exhibits glucose-dependent effects on insulin secretion. At low glucose levels, $GABA_A$ receptors on the β-cell can promote insulin secretion (81). However, at high glucose levels, GABA instead suppresses insulin secretion by reducing cAMP via G_i signaling in β-cells (82) and by elevating somatostatin secretion via δ-cell $GABA_A$ (81) receptors. Together, these paracrine mechanisms ensure

precise regulation of insulin secretion, balancing glucose homeostasis through coordinated islet signaling.

Type 2 diabetes

About 537 million adults have diabetes, and T2D accounts for nearly 90% of these cases (83). Although T2D is primarily defined by elevated blood glucose levels, it is a chronic and complex disease driven by metabolic and homeostatic disturbances that progresses over time. The prevalence of the disease correlates with lifestyle choices but is also influenced by genetic and epigenetic changes (84, 85). The pathophysiology of T2D is characterized by tissue insulin resistance and an inadequate compensatory insulin secretory response due to β-cell dysfunction. This results in reduced glucose uptake in peripheral tissues and increased hepatic glucose production, contributing to chronic hyperglycemia. β-cell dysfunction plays a critical role in disease progression, leading to insufficient insulin secretion. Impaired insulin signaling, mitochondrial dysfunction, and increased endoplasmic reticulum stress all contribute to β-cell dysfunction and ultimately lead to β-cell death and a reduction in β-cell mass (86). Recent studies have also revealed that primary cilia play a crucial role in glucose homeostasis, with ciliary dysfunction being linked to reduced glucose-stimulated insulin secretion and diabetes progression (3, 57, 87). Furthermore, dysregulation of cilia-associated genes has been associated with an increased risk of T2D (15).

cAMP and cGMP production

cAMP is generated from ATP through the activity of ACs (AC1-9), which are membrane-bound proteins that are activated downstream of GPCRs. Ca^{2+} activates AC1, AC3, and AC8, while AC5 and AC6 are inhibited by Ca^{2+} and by PKA mediated phosphorylation (88). Their distinct localization further influences their function, with AC3 and AC5 reported to localize to primary cilia (55, 89), and AC8 being mostly found in raft-like domains in the plasma membrane, where it is required for GLP-1-mediated Ca^{2+} flux (90). Rodent islets predominantly express AC3, AC6, and AC8 (91, 92), whereas human islets express AC3, AC6, and AC8 (92).

Parallel to cAMP signaling, cGMP is synthesized from guanosine triphosphate by particulate guanylyl cyclase (pGCs) (93). These transmembrane receptors contain an extracellular ligand-binding domain, a single transmembrane region, and an intracellular guanylyl cyclase domain, which catalyzes cGMP

production upon ligand binding (94, 95). There are at least seven pGC isoforms (GC-A to GC-G), but only three of them have a ligand (96). Atrial natriuretic peptide (ANP) and B-type natriuretic peptide activate natriuretic peptide receptor-A (NPR-A) and C-type natriuretic peptide activates natriuretic peptide receptor-B (97). The NPR-A receptor is expressed in islets and its activation triggers the cGMP/PKG pathway and inhibits PDE3B activity (98, 99), exemplifying the intricate cross-talk between cAMP and cGMP.

cAMP and cGMP dynamics

The balance of cAMP and cGMP levels is maintained through their production and degradation by PDEs. PDE1, PDE3 and PDE4 are generally considered the most important for cAMP regulation in islet cells. PDE1 activity is Ca^{2+} dependent with PDE1C as the main isoform, capable of degrading both cAMP and cGMP (100). PDE3 has a dual specificity, but exhibits a unique kinetic property where cGMP acts as a competitive inhibitor of cAMP degradation (101), meaning that higher cGMP levels enhance cAMP signaling via PDE3 inhibition. Unlike PDE3, PDE4, particularly the PDE4C isoform, exclusively hydrolyzes cAMP and does not act on cGMP (102, 103). PDE4 likely contributes to local cAMP compartmentalization, affecting pulsatile insulin secretion dynamics rather than global cAMP degradation (104). Additionally, PDE2 also degrades both cAMP and cGMP, but cGMP binding to PDE2 enhances cAMP degradation, leading to a negative cGMP-to-cAMP crosstalk (105), in contrast to PDE3-mediated positive cGMP-to-cAMP crosstalk. PDE5 is known to selectively hydrolyze cGMP, playing a major role in regulating intracellular cGMP levels (106); however, PDE2 and PDE5 expression in islet cells remains to be elucidated.

Signal compartmentalization

cAMP formation is counteracted by PDE-mediated degradation, and the tight interplay between synthesis and degradation can give rise to cAMP oscillations (104, 107). A complex network of regulatory mechanisms orchestrates the temporal and spatial spread of cAMP, thus leading to formation of signalling nanodomains. During the basal state, cAMP is mainly bound to intracellular binding sites (108) and free diffusion of cAMP only occurs when its concentration significantly surpasses the number of available binding sites (109). A key player in cAMP compartmentalization is the Type I regulatory subunit of PKA, RIα , which undergoes liquid-liquid phase separation, leading to the

formation of RIα bodies, which act as concentrated hubs of cAMP and PKA activity. Formation of these signaling domains effectively limits the free diffusion of cAMP and confines its effects to specific cellular domains (110, 111). This dynamic sequestration is crucial for maintaining the localized nature of cAMP signaling within cells. Given the localized and compartmentalized nature of cAMP signaling, it is expected that organelles frequently serve as the locations where cAMP signalosomes/nanodomains are observed. One such organelle is the primary cilium, but whether it maintains an independent cAMP pool or is in equilibrium with that in the cytosol remains debated. Some studies report equal cAMP concentrations in cilia and cytosol, suggesting free diffusion (17, 112), whereas others indicate that ciliary cAMP levels can be up to five fold higher (~4 μM), indicating localized production and restricted diffusion (54). Given the role of cilia as a signaling hub, understanding how cells distinguish ciliary cAMP signals from cytosolic cAMP is important. One possible explanation is that ciliary PKA is selectively regulated by ciliary cAMP, allowing it to drive specific cellular responses, such as modulating Hh signaling. Due to the small volume of cilia, the production of even a small number of cAMP molecules leads to a significant increase in cAMP concentration within the ciliary space. As a result, even in the absence of a physical diffusion barrier or differences in PKA distribution, ciliary PKA remains highly sensitive to local cAMP changes. This compartmentalized regulation enables ciliary and extraciliary cAMP to transmit distinct signals, with ciliary PKA activity specifically shaping Hh pathway responses (17, 44, 54, 112, 113) through the regulation of GLI transcription factors that shuttle between the cilium and nucleus. Ultimately, this demonstrates how cells employ spatially confined cAMP-PKA signaling to regulate broader cellular responses with high specificity.

Aims

The overall aim of this study was to investigate the role of primary cilia in the islets of Langerhans. To achieve this, we developed tools for direct measurements of ciliary signaling within intact islets and, by combining these with high-resolution live cell imaging, explored how these structures regulate islet cell function. The specific aims were to:

1. Develop methodology to study signaling in islet β-cell primary cilia (I, II, III)

2. Identify cilia-dependent signaling pathways in islet β-cells (I, II, III)

3. Determine second messenger dynamics in islet β-cell cilia (I, II, III)

4. Determine if β-cell primary cilia structure or function is altered in type-2 diabetes (II)

Methodology

β-cell models

This thesis primarily utilized three distinct models of pancreatic β-cells: MIN6 cells grown as monolayers, MIN6 cells assembled into pseudoislets and intact mouse islets of Langerhans. Each model serves a unique purpose yet remains interconnected. In particular, since our focus is on primary cilia, these models provide complementary advantages in studying ciliary signaling. Monolayer cultures allow for detailed biochemical and genetic analyses, pseudoislets re-establish key cell–cell interactions, and intact mouse islets preserve the complete micro-organ context with paracrine communication that closely mimics in vivo conditions.

Clonal MIN6 β-cells of passages (17-30) (114) were maintained in Dulbecco´s modified Eagle´s medium (DMEM) containing 25 mM glucose and supplemented with 2 mM GlutaMAX, 50 μM β-mercaptoethanol, 100 U/mL penicillin, 100 μg/ml streptomycin, and 15% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2. The MIN6 cell line shows glucose uptake and metabolism and glucose-induced insulin secretion properties similar to those of primary islets but without the paracrine influence of other cell types, such as delta cells**.** Monolayer MIN6 cells provide a simplified and homogenous system that facilitates molecular analyses such as protein expression via transient transfection. However, the monolayer format lacks the three-dimensional cell-cell interactions present in vivo, and because primary cilia are uniquely positioned on the apical surface, this model limits our ability to employ TIRF microscopy imaging effectively, as the cilia project away from the coverslip.

When MIN6 cells are allowed to aggregate into pseudoislets, they establish intercellular contacts and demonstrate enhanced functional responses with improved glucose-stimulated insulin secretion (115). In our second study, we focused on the effect of somatostatin on the primary cilium of β-cells; thus, using MIN6 pseudo-islets provided us with a β-cell-like islet. Exogenous somatostatin was added to mimic the interaction of the β-cell cilia with delta cells. Another advantage of using MIN6 pseudo-islets is that gene transcription could be easily manipulated. We optimized the transfection protocol to

achieve 60% gene silencing with various siRNAs. In the case of ciliary receptors, we experienced during this study that relying only on pharmacology is not the best option because the coupling of the receptor in the cilium may be different from the receptor present on the plasma membrane, or different receptor interactors or modulators may not be present in cilia (116). For example, although the GABBR1 forms obligate heterodimers with GABBR2 in the plasma membrane, we only detect the GABBR1 subunit in the cilium of islet cells; consequently, the absence of GABBR2 receptor renders the GABBR1 receptor insensitive to its antagonist. To address this, we instead relied on siRNA-mediated silencing. To silence specific genes, we transiently transfected MIN6 cells using a lipid reagent (Lipofectamine 2000) and 5 hours later we supplemented the cell-siRNA mixture with more siRNA and a different lipid reagent (Lipofectamine RNAiMAX), plated the mixture as a small drop in a non-stick plate and incubated it as a drop overnight. The following day, cells were fed with a complete medium, and gene knockdown was assessed using qPCR or immunofluorescence. It is important to note that overnight transfection was performed in the presence of 10% FBS, which prevents the cells from sticking to the plate and helps them form pseudo-islets.

Intact mouse islets represent the gold standard for studying β-cell function within a physiologically intact micro-organ. Mouse islets maintain the complex cytoarchitecture of different endocrine cell types, with β-cells predominantly located in the core and alpha cells in the outer mantle. This organization supports intricate paracrine interactions and preserves the long-term differentiated state of the cells, including stable primary cilia (117). The use of primary islets gives us the advantage of studying the function of the primary cilium in an intact micro-organ, as well as investigating the signaling of the primary cilium in a primary tissue that preserves both tissue architecture and function, unlike most other studies of the primary cilium, which has been performed in immortalized cell lines (54, 118). Moreover, since islet cells have very low, if any, proliferative capacity (119), their cilia are maintained over long periods, enabling them to develop a highly specialized phenotype. In Study II, the use of mouse islets was indispensable, as we established the spatial localization of primary cilia relative to somatostatin-secreting delta cells, thereby demonstrating how primary cilia sense delta cell derived somatostatin.

Human islets represent the most relevant model for this work, providing a valuable source for our study. We had the privilege of accessing human islets isolated at the Academic Hospital in Uppsala by the Nordic Network for Clinical Islet Transplantation. Human islets exhibit high variability in cilia number and length, likely due to handling and isolation procedures. Additionally, the composition of endocrine cells varies considerably from donor to donor, with

reported differences in the number of δ-cells (120). This inherent variability and the relatively small sample size pose challenges for studying human islets. Furthermore, we also obtained islets from donors with type 2 diabetes, allowing us to explore potential disease-related alterations in ciliary signaling.

Our multi-model approach from monolayers to intact human islets provides a comprehensive analysis of islet ciliary signaling and function. This strategy enables us to understand the role and involvement of this unique organelle across a spectrum of models, from simplified cellular systems to the intricate microenvironment of human islets.

Visualization of primary cilia

We primarily used acetylated α-tubulin to visualize primary cilia in mouse, human, and MIN6 pseudo-islets. While cilia staining in mouse and MIN6 pseudo-islets was clear and well-defined, acetylated α-tubulin, also present in neuronal fibers, produced dense signals at the islet periphery, complicating visualization. Due to the increased background signal, human islets posed additional challenges. Given their inherent heterogeneity, we speculate that the complex extracellular matrix and diverse cell composition may contribute to nonspecific antibody binding. Additionally, the isolation procedures for human islets could alter protein structure and accessibility, potentially affecting antibody reactivity. We also used ARL13B and polyglutamylated tubulin as complementary markers to label primary cilia. Furthermore, fluorescently tagged proteins such as Smoothened-Cherry, ARL13B-Halo, and SSTR3-GFP were successfully employed in the study, providing an alternative approach to visualize primary cilia.

Measurements of ciliary Ca^{2+}

There are numerous sensors available for Ca^{2+} imaging, yet many of them do not perform optimally in the small organelle of the primary cilium. Most live-cell imaging studies have been successfully conducted in the cytosol or submembrane regions, but targeting this sensor to cilia did not yield the desired performance (121).

Full-length 5HT6 receptor is a cilia-localized Gs-coupled receptor known to increase cAMP upon activation. This receptor was fused to the green Ca^{2+} sensor GGECO1 to generate a cilia-localized Ca^{2+} indicator (121). However, as previous studies had shown that 5HT6 receptors can exhibit intrinsic activity, we generated another Ca^{2+} sensor by fusing the Ca^{2+} sensor GCaMP5G

with the Smoothened receptor (23). The Smoothened receptor can also possess intrinsic activity, and its activation leads to a decrease in cAMP levels (122). Both sensors, 5HT6-GGECO1 and Smo-GCaMP5G, were packed in E5 serotype adeno-virus vectors under the control of cytomegalovirus promoter. Intact mouse islets were infected with 2.5 µl high titration virus (>10^{12}-10^{13} viral particle/ml) in 200 µl of culture medium for three hours and subsequently allowed to express the indicator for 24 h. Cilia Ca^{2+} imaging was performed using total internal reflection fluorescence microscopy. Both sensors exhibited similar distribution and performance, with no major effects of the overexpressed receptors on cilia function, including changes in ciliary Ca^{2+} levels. However, a higher-affinity sensor could have captured more events, as the current sensors, with Kd of approximately 749 nM (5HT6-G-GECO1) and 542 nM (Smo-GCaMP5G) (123), may have limited sensitivity to Ca^{2+} fluctuations in the low-nanomolar range. Expanding the detection range could have provided better resolution of subtle ciliary Ca^{2+} dynamics. Both indicators are green-shifted, making them difficult to combine with optogenetic tools activated by blue light or with FRET-based sensors, which require spectral separation for optimal performance. Despite indicators being targeted to cilia, detecting the indicator in the plasma membrane was still possible, allowing us to record from both compartments simultaneously.

Measurements of ciliary cAMP and cGMP

Development of suitable ciliary cAMP sensor took considerable effort. Initially, efforts were made to develop a red cAMP sensor to combine with the green Ca^{2+} sensors. RflincA (124) and Pink Flamindo (125), two red-shifted cAMP sensors, were fused separately with Smoothened, 5HT6, and mArl13b. Their cilia localization was confirmed. However, these sensors exhibited low brightness under basal conditions, which complicated the experiments. As an alternative, a FRET (Förster resonance energy transfer) sensor with low cAMP affinity (K_D 9 µM), referred to as Epac-S^{H188}, was used (126). The FRET sensor outperformed the red cAMP sensors in terms of dynamic response range and brightness. Through the fusion of Epac-S^{H188} with Smo, 5HT6, or mArl13b, our approach successfully targeted the probe within the cilia of islet cells. This targeting allowed for ciliary cAMP recordings; importantly, all the sensors demonstrated consistent kinetics while reporting similar resting cAMP levels. Subsequently, the experiments proceeded with the use of cilioplasmic mArl13b- Epac-S^{H188} as the preferred configuration, since the overexpression of both 5HT6 and Smo, which are coupled to cAMP-synthesizing ACs, may influence cAMP dynamics in the cilium.

cAMP is structurally similar to cGMP, and previous cGMP sensors have shown only moderate selectivity for cGMP over cAMP (127). Therefore, choosing an appropriate cGMP sensor is important, mainly because in β-cells, cAMP functions as a key amplifier of insulin secretion and has been the primary focus of many studies, emphasizing its importance and regulation, while the real-time live imaging of cGMP in intact islets remains unreported. We used the recently developed FRET based biosensor, ScGI, which shows strong specificity for cGMP over cAMP (EC_{50} ~ 125 μM for cAMP, EC_{50} ~ 0.2 μM (128)). ScGI was expressed in intact human islets by adenovirus-mediated transduction, and TIRF microscopy imaging revealed homogenous distribution without enrichment in any intracellular compartments. Further ScGI was also targeted to cilia by fusion with mArl13b and its ciliary localization confirmed by confocal microscopy.

Measurements of the sub-plasma membrane cAMP concentration

To visualize the dynamics of the catalytic Cα subunit of PKA, we expressed a plasma membrane-localized translocation biosensor based on non-fluorescent PKA regulatory subunit anchored to the plasma membrane by a CaaX-motif (ΔRIIβ-CaaX) and mCherry-tagged catalytic Cα (mCherry-PKA-Cα) (129). At low cAMP concentrations, Cα associates with PKAIIβ at the plasma membrane, and when cAMP levels increase, it dissociates from the plasma membrane into the cytosol and becomes available for participation in enzymatic reactions.

Optogenetic tools

We examined how second messengers, including cAMP and cGMP, regulate each other and affect the signaling in islet cell cilia. One optogenetic tool we used was bPAC (bacterial photoactivated adenylyl cyclase), a light-inducible AC enzyme derived from Beggiatoa (130). It is a genetically engineered protein that responds to blue light by catalyzing the conversion of ATP into cAMP. Using bPAC provided a great advantage because it allows for non-invasive and reversible control of cAMP signaling using blue light, without the need for ligands or drugs. However, a limitation of bPAC was its activation light spectrum, which overlapped with the excitation wavelengths of our genetically encoded Ca^{2+} indicator and cAMP FRET sensor. This required careful experimental design to ensure proper activation of bPAC while minimizing

spectral interference with the Ca^{2+} and cAMP sensors. We employed bPAC in conjunction with ciliary Ca^{2+} recordings to investigate the impact of cytosolic cAMP elevation on ciliary Ca^{2+} signaling. To address this, we included a separate group of islets without bPAC expression to validate our results and isolate the effects of cAMP elevation. We extended our investigations by combining bPAC with a red PKA translocation sensor. By utilizing blue light activation of bPAC, we were able to monitor the trafficking dynamics of the liberated catalytic Cα subunit of PKA to the islet cilia. This approach provided valuable insights into the translocation of PKA and its association with cAMP signaling in the ciliary compartment.

We used Rhodopsin-Guanylyl Cyclase (RhGC) as an optogenetic tool to modulate cytosolic cGMP levels with high temporal precision. RhGC is a plasma membrane-localized enzyme that can convert guanosine triphosphate into cGMP upon activation by green light illumination (131). By utilizing RhGC, we could specifically elevate cytosolic cGMP levels in a controlled manner.

Fluorescence microscopy

Fluorescence microscopy enables us to visualize structures invisible to the naked eye. First discovered in 1845 by Frederick Herschel, fluorescence was later applied in the early 1900s to visualize tissues and pathogens. This approach eventually evolved into fluorescence microscopy, pioneered by Carl Zeiss. Today, we can observe cells, their organelles, and even individual proteins with lateral resolutions down to 50 nm. In this study, we primarily employed spinning disk confocal microscopy and total internal reflection fluorescence (TIRF) microscopy to visualize primary cilia in the intact islets of Langerhans. Spinning disk confocal microscopy allowed us to obtain images throughout the islet volume, while TIRF microscopy provided high-resolution imaging of cilia from cells that were localized close to the surface of the islet. The choice of microscopy technique depended on specific questions. Mainly, spinning disc confocal microscopy, which combines the speed of a widefield illumination with optical sectioning of a confocal microscopy, was used to acquire high-resolution z-stack images of cilia extending deep into the islet (for determination of ciliary length, number, and localization within the islet). On the other hand, the TIRF microscope restricts imaging to a small volume about 200 nm above the glass coverslip surface, which gives us a distinct advantage during live cell cilia recordings by enabling high-resolution imaging of cilia close to the cell membrane with a high signal-to-noise ratio. Trapping cilia under the weight of the islet minimizes motion artifacts, facilitating more

stable recordings. The potential drawbacks with this methodology are that we are recording from a specific subset of cilia (those that protrude out of the islet) and that the cilia are immobilized between the islet and the coverslip, which may alter the biophysical properties of the cilia. All cilia Ca^{2+}, cAMP and cGMP measurements were performed using TIRF microscopy, and given that the cilia diameter is 200 nm, we were able to record the entire cilia volume within the evanescent field, with minimal signal contamination from deeper parts of the cell. For long experiments, TIRF illumination is less phototoxic for cells, exhibits less photobleaching and better axial resolution than confocal microscopy.

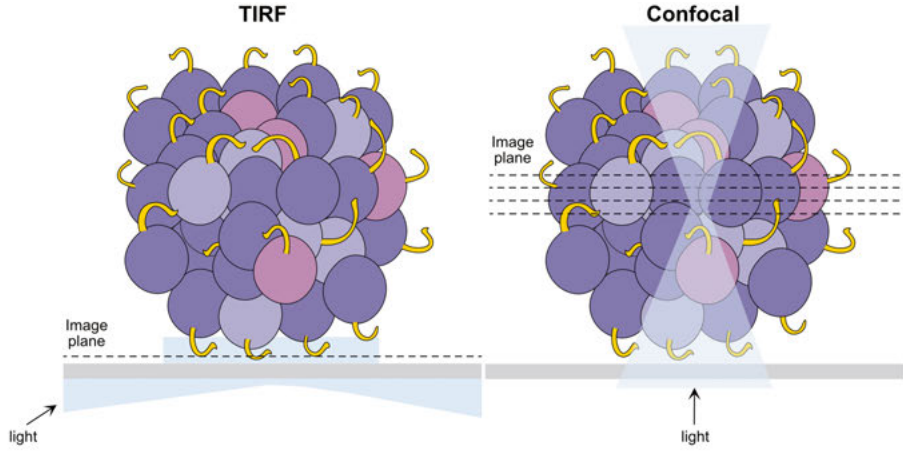

Figure 6. Comparison of imaging techniques: TIRF vs Confocal microscopy

TIRF selectively illuminates a very thin region near the coverslip, providing high resolution imaging of submembrane events. Confocal microscopy provides high speed optical sectioning as pinholes scan across the sample in rows to build up an image.

Image analysis

Image analysis was performed using MetaFluor and the Fiji version of ImageJ (132). The concentrations of cAMP and cGMP were expressed as the ratio of CFP to YFP fluorescence emission, and the fluorescence changes are shown relative to the initial basal fluorescence. The magnitude of the responses was often calculated by time-averaging the signal over the stimulation period. For ciliary recordings, changes in fluorescence intensity over time were determined from the segmented region covering the cilia and a part of the cytoplasm, and values were normalized to pre-stimulatory levels. Ciliary Ca^{2+} activity appeared as spontaneous flashes. To analyze these events, regions of interest covering the cilium were drawn, and the mean pixel intensities were

extracted throughout the experiment. These intensities were then converted into binary values using a threshold set at 20% above basal levels—any event exceeding this threshold was counted and used for subsequent ciliary Ca^{2+} analysis.

Results and Discussion

The primary cilium of islet β-cells (I, II)

The primary cilium has recently been recognized as a critical organelle regulating β-cell function. In my studies, I first characterized primary cilia in intact mouse and human islets by immunostaining and fluorescence microscopy. In mouse islets, staining with an antibody against acetylated tubulin revealed that most islet cells are equipped with a single primary cilium, averaging approximately 8 μm in length. Notably, cilia were not randomly distributed; instead, they tended to cluster in certain regions, where occasional cilia–cilia contact zones were observed. In some cases, individual cells even displayed two primary cilia, a finding later confirmed by ultrastructural examinations of pancreatic sections (8).

Primary cilia were detected in human islets, with an average length of around 5 μm. Furthermore, human islet cilia displayed a characteristic bulging tip, possibly indicating cilia with active signaling, as also observed in mouse islets. In mechanosensitive cilia, these swellings are critical for facilitating membrane budding in response to fluid stress (133) and releasing signaling molecules for effective cell-to-cell communication, as established in neuronal primary cilia (134). Moreover, dilated tips have been associated with the accumulation of activated GPCRs, which are cleared through signal-dependent ectocytosis - a vital process for proper Hedgehog signaling (135). These observations suggest that the bulging tips in islet cilia may similarly serve as specialized hubs for receptor clearance and signal modulation. Importantly, islets obtained from patients with T2D demonstrated a reduction in cilia length by approximately 25%. A similar decrease in cilia length was also observed in vitro when islets from non-diabetic donors were exposed to 0.5 mM palmitic acid for 7 days to mimic a diabetogenic environment. These observations show that primary cilia undergo dynamic length regulation under both physiological and diabetic conditions.

The primary cilium as a signaling hub; compartment-specific localization of GABBR1 receptors (I)

The sensory capacity of primary cilia relies on the spatiotemporal localization of specific receptors and the transduction of external cues to ensure proper regulation of cilia-tuned cell functions. The expression of a particular set of ciliary GPCRs and their activation patterns are key determinants of cellular responses. In this study, we investigated the expression and functional dynamics of two GPCRs, the GABBR1 and SSTR3, within the primary cilia of islet cells.

Immunostainings of GABA receptors revealed distinct localization patterns in islet cells. While GABRA receptors and GABBR2 were primarily found in cytosolic vesicles, GABBR1 was distinctly localized to the primary cilia. Using stimulated emission depletion microscopy, we observed that GABBR1 was concentrated at the ciliary base, occupying a segment of approximately 2 μm in length. This localization was further confirmed by co-staining with γ-tubulin, a marker of the ciliary base, and was lost following GABBR1 knockdown, verifying its specificity. Additionally, the use of two different antibodies, one targeting an intracellular epitope and the other an extracellular epitope, further validated the localization of the receptor within the cilia. The compartmentalized distribution of GABBR1 has been observed in neurons, particularly in hippocampal pyramidal cells, where it is restricted to the post-synaptic part of the plasma membrane, dendritic shafts and spines (136-139). Given that dendritic spines serve as specialized compartments for neurotransmitter signaling, it is tempting to speculate that primary cilia may function similarly, acting as specialized sensory domains where synapse-like signaling events could occur. This raises the possibility that ciliary GABA signaling contributes to intercellular communication within the islet microenvironment, potentially influencing β-cell function.

Upon GABA stimulation, GABBR1 exhibited a dynamic behavior, moving from the ciliary base toward more distal regions. This receptor mobility suggests that GABBR1 participates in cilia-specific signaling events, potentially modulating β-cell function in response to locally produced GABA, which is known to influence insulin secretion (140). One possible explanation could be that base-localized GABBR1 receptors might represent a state in which receptors exit the organelle following activation induced by the ligand. This would be consistent with recent studies showing that almost all ciliary receptors, including SSTR3, TGFβ- receptors, and the Patched receptor, exit the cilia via signal-dependent retrieval into the cell (141-143). This retrieval is mainly mediated by the BBSome through motif recognition of activated GPCRs by the β-arrestin 2 and BBSome components, allowing them to transit through the

transition zone and exit the cilia (144). It is worth noting that $GABA_B$ receptors might lack this recognition motif by β-arrestin 2 as it was previously shown that baclofen-induced activation of $GABA_B$ receptors failed to internalize due to lack of β-arrestin 2 recognition (145).

While GABBR1 in intact mouse islets displayed dynamic redistribution upon activation, this phenomenon was not observed in MIN6 pseudo-islets, where GABBR1 was present throughout the cilium membrane, highlighting differences between primary and clonal cells. The difference in GABBR1 localization between different models may stem from variations in GABA availability or paracrine signaling, as GABA inhibits glucagon secretion, enhances somatostatin release, and exerts an autocrine effect by promoting insulin secretion (140, 146-148). In intact islets, native GAD isoforms ensure proper GABA compartmentalization, and contribute to the regulation of GABA secretion. In contrast, the lack of expression of GAD 65 in MIN6 pseudo-islets and altered GABA synthesis may disrupt this regulation (149). The uniform ciliary distribution of GABBR1 in MIN6 cells suggests that a paracrine cue in intact islets could be essential for spatially controlled receptor dynamics.

The primary cilium as a somatostatin target; SSTR3 localization to islet cell cilia (II)

SSTR3 is a well-established ciliary receptor previously identified in the cilia of MIN6 cells and mouse and human islet cells, (4, 118). Its trafficking to the cilium is mediated by the IC3 loop, which contains a ciliary localization signal that enables receptor trafficking through an IFT-A-dependent mechanism (38, 150). SSTR3 localization has been reported to be dynamic with somatostatin binding inducing receptor internalization from both the ciliary and plasma membranes (143, 151). This ligand-induced internalization has been observed across various cell types, including neurons and fibroblasts, and results in ciliary exit of SSTR3 (143). In cases where the recetor retrieval pathway is impaired, such as through mutations in the BBSome complex, SSTR3 can instead leave the cilium via ectocytosis at the ciliary tip (135).

In contrast to previous studies, our findings revealed a distinct SSTR3 phenotype in islet cilia, where the receptors remained stably localized within the cilia even after prolonged somatostatin stimulation (up to 18 hours). This stability is likely due to the use of endogenous SSTRs in our models, where cells are continuously exposed to somatostatin. In contrast, earlier studies primarily relied on overexpressed receptors in cell lines, which may not accurately reflect native receptor dynamics (135). While earlier studies focused on tracking receptor exit dynamics, our approach instead used immunostaining to

visualize endogenous SSTR3 localization within the cilium. This technique shows the receptor localization within the overall cilioplasm and ciliary membrane but may not be sensitive enough to detect subtle receptor exit events. It is also important to note that we have checked the functional activity of these receptors after 18h of stimulation with somatostatin. The receptors remained responsive to exogenous somatostatin, indicating an active pool of SSTR3 still present in the cilia after prolonged exposure to its ligand. A stable pool of SSTR3 in the cilia could be well-suited for detecting a tonic somatostatin signal, similar to ciliary receptors in the nervous system, which are proposed to sense synaptic spillover (152).

Spontaneous cilia Ca^{2+} flashes in islets of Langerhans (I, II, III)

Signal transduction pathways in β-cells often converge on key secondary messengers such as cAMP and Ca^{2+}, which propagate signals to control key physiological processes, including insulin secretion. To visualize Ca^{2+} dynamics specifically within islet cell cilia, we used genetically encoded, cilia-localized Ca^{2+} sensors: Smo-GCaMP5G-mCherry and 5HT6-GGECO1. While previous studies have shown that Ca^{2+} is a critical second messenger in primary cilia, its exact function remains debated. Earlier works showed that cilia maintain higher Ca^{2+} concentrations than the cytoplasm, primarily through the action of heteromeric TRP channel complex PKD1L1–PKD2L1 (51). However, our TIRF microscopy recordings revealed that the GCaMP5G to mCherry fluorescence ratio was either similar to, or lower, in the cilia compared to the surrounding cytoplasm. During our experiments, islets were kept in 3mM glucose and we observed spontaneous Ca^{2+} flashes in the cilia of intact mouse islets. Similar spontaneous ciliary Ca^{2+} transients were observed in human and MIN6 pseudo islets. These flashes have rapid onset and offset transitions and lasts approximately one minute. Removal of extracellular Ca^{2+} abolished all ciliary Ca^{2+} flashes indicating that these events are driven by Ca^{2+} influx from the extracellular environment. However, the precise mechanism underlying these spontaneous flashes remains unknown, and whether they are triggered by an extracellular ligand or an intracellular signaling requires further investigation.

Ca^{2+} microdomains in islet cilia (I)

The high spatial and temporal resolution of our TIRF microscopy imaging allowed us to pinpoint the origin of the spontaneous ciliary Ca^{2+} flashes and

to track the propagation of Ca^{2+} along the cilia. Most of the flashes originate from the distal part of the cilium, with about 30% of the events beginning at the ciliary tip before diffusing along the ciliary shaft. Interestingly, in some instances, we observed the formation of stable Ca^{2+} microdomains within the cilia. These stable regions may indicate the localized presence of specific ion channels that facilitate or regulate Ca^{2+} influx. Similar to other cells, Ca^{2+} entry in islet cilia could be driven by PC-2 channels, which are known to regulate ciliary Ca^{2+} dynamics in various systems (51). However, our experimental data also suggest that alternative pathways contribute to these events. Specifically, we found that CNG channels and VGCCs are present within islet cilia, likely contributing to ciliary Ca^{2+} dynamics. In sensory neurons, NPHP-2 (part of inversin) anchors CNG channels within the proximal ciliary region (153), suggesting a similar compartmentalized expression of ion channels may exist in islet primary cilia. We detected CNGA3 expression along the ciliary shaft, but the precise distribution of other channels remains unclear. Given that GABBR1 is exclusively expressed in the proximal region, it is plausible that other receptors and channels also exhibit distinct localization patterns. The spatial distribution of these unknown channels could be responsible for the formation of Ca^{2+} microdomains and may display functional blocks that restrict or direct Ca^{2+} flow within specific ciliary compartments. These Ca^{2+} microdomains in islet cilia resemble compartmentalized Ca^{2+} signaling observed in dendritic spines and cardiac cells (154). In dendritic spines, actin-rich structures create microdomains that localize and regulate synaptic Ca^{2+} signals, enabling precise control of synaptic plasticity (155). Similarly, in cardiac muscle cells, the T-tubule system forms defined Ca^{2+} microdomains that orchestrate excitation–contraction coupling (156). Although primary cilia are built on tubulin-based microtubule blocks rather than an actin framework, recent work suggests that actin filaments can integrate into cilia and influence ciliary structure and function, potentially contributing to the spatial regulation of Ca^{2+} microdomains (157, 158).

Primary cilia as an autonomous Ca^{2+} organelle (I)

In β-cells, cytosolic Ca^{2+} is critical for insulin secretion regulation and undergoes oscillatory concentration changes in response to glucose stimulations. The cilioplasm is continuous with the cytosol, so the question arises whether cytosolic Ca^{2+} can enter the cilium. To answer this question, we elevated the cytoplasmic Ca^{2+} concentration by depolarization. This caused a pronounced increase in cytosolic Ca^{2+}, but the ion did not spread throughout the cilia. Next, we subdivided each cilium into smaller segments and observed a slight

increase in the segment closest to the base of cilia, but this rise of Ca^{2+} did not propagate to the more distal part of the organelle. Removal of extracellular Na^{+} slowed down the Ca^{2+} extrusion rate and allowed cytosolic Ca^{2+} to freely enter the cilium, suggesting the involvement of the Na^{+}/Ca^{2+} exchanger (NCX). Considering that NCX relies on the Na^{+} gradient and has been reported in cilia of other cell types, it is a strong candidate for mediating ciliary Ca^{2+} extrusion in islet cells (159).

Next, we determined whether Ca^{2+} released from intracellular stores could diffuse into primary cilia. Addition of charbachol, which triggers IP_3-mediated Ca^{2+} release from the ER, resulted in a sharp, transient increase in the cytosolic Ca^{2+} concentration that was accompanied by a localized ciliary Ca^{2+} increase that was confined to a ~3 μm region at the cilia base, similar to observation made with depolarization-induced Ca^{2+} increases. Interestingly, the Ca^{2+} increase at the cilia base was more pronounced than in the adjacent cytosol, indicating that Ca^{2+} may be directly funneled from the ER into the cilium. In neuronal cells, IP_3 receptors were essential for Shh-induced ciliary Ca^{2+} activity (52), raising the possibility that a similar mechanism could regulate ciliary Ca^{2+} dynamics in islet cells. The primary cilium has a more positive membrane potential than the bulk plasma membrane (51), which indicates that it is insulated from changes in general membrane potential. Consistent with this, direct depolarization caused Ca^{2+} influx in the soma, but not in the primary cilia, of islet cells. In fact, depolarization even suppressed spontaneous Ca^{2+} flashes in the cilium, possibly by enhancing Ca^{2+} extrusion. Such isolation could be particularly important in excitable cells like β-cells, which are exposed to fluctuations in cytosolic Ca^{2+} as part of their primary function. Isolation against cytosolic Ca^{2+} changes would therefore facilitate the generation of intrinsic Ca^{2+} signals and allow cilia to function as autonomous Ca^{2+} organelles.

Cyclic nucleotide dynamics in islet cell primary cilia (I, II, III)

cAMP is a major second messenger in islet cells and is also implicated in ciliary signaling in many other cell types. We therefore investigated whether ciliary cAMP dynamics are directly influenced by changes in the cytosolic cAMP concentration. Using both low- and intermediate-affinity Epac-based cAMP indicators fused to ciliary-targeting proteins such as 5HT6, Smoothened, and mArl13b, we measured resting cAMP levels in the cytosol and primary cilia. Consistently across all indicators, we observed no significant difference between cytosolic and ciliary cAMP under basal conditions. These findings align with previous studies that reported no basal gradient between

the cytosol and cilia (113). However, they contrast with another study using the pH-resistant CADDIS sensor, which suggested that ciliary cAMP levels are approximately five times higher than in the cytosol (54). One important consideration is that our FRET-based sensor includes YFP, whose fluorescence is enhanced in alkaline environments. Since we have reported that primary cilia in islet cells maintain a more alkaline pH (160), this could have artificially increased the FRET signal, leading to an overestimation of cAMP levels in cilia. If ciliary cAMP were indeed much higher in islet cells, as suggested (54), we should have detected a difference. The fact that we did not observe any difference suggests that ciliary cAMP is comparable to cytosolic cAMP or lower, but the pH-dependent YFP enhancement could be masking the difference. The regulation of ciliary cAMP dynamics in the cilium remains a topic of ongoing debate. Our findings suggest that the primary cilium of islet cells lacks a cAMP barrier (17, 112, 113), as stimulation of β-cells with a non-ciliary GLP-1 receptor agonist resulted in an initial increase in cytosolic cAMP, followed by an elevation of cAMP in the cilia approximately 20 seconds later, supporting the idea of free diffusion. This contrasts with findings that cAMP within the cilium is compartmentalized and regulated by $PI(3,4,5)P_3$ signaling (54). Interestingly, the addition of IBMX, which is a broad PDE inhibitor, caused cAMP levels to increase first in the cilium before increasing in the cytosol. This suggests that the cilium has higher basal PDE activity, allowing cAMP to accumulate more rapidly when degradation is inhibited (44).

cGMP is another second messenger that is closely related to, and cross-talks with, cAMP, but much less is known regarding both dynamics and downstream signaling. In human islets, we found that ANP and GLP-1 stimulate cGMP production in the cytosol, and that depolarization also triggers cGMP formation in a Ca^{2+}-dependent manner. By combining optogenetic tools and pharmacological approaches, we further demonstrated that cGMP can freely diffuse from the cytosol into the cilium, suggesting that ciliary cGMP levels are not independently regulated but rather mirror cytosolic changes. This contrasts Ca^{2+} signaling, which appears to be more compartmentalized within the cilium, potentially enabling localized regulation independent of cytosolic fluctuations.

cAMP reduction, not elevation, establishes cilia as an isolated cAMP compartment (I, II)

While previous data showed that ciliary cAMP levels are largely influenced by cytosolic changes, a key question remains: how can cilia generate localized

signals in such an interconnected environment? It is important to note that cilia have a 10-15-fold greater surface-to-volume ratio than the soma (161) and that their surface is densely packed with receptors and ion channels. By its very nature, the cilium should be able to generate its own distinct signals to operate effectively. To explore this, we shifted focus from cAMP elevation to its reduction. SSTR3 is a well-established ciliary receptor that inhibits AC activity upon activation, which in turn leads to PDE-mediated lowering of cAMP. To test whether the cilium can isolate itself in terms of cAMP reduction, we stimulated cells with somatostatin in the presence of low concentrations of the AC activator, forskolin. cAMP levels decreased in both cilium and cytosol; however, only the ciliary effect was dependent on SSTR3. In simpler terms, knockdown of SSTR3 disrupted the ciliary cAMP response, rendering it distinct from the cytosolic cAMP dynamics. Then we wondered if cAMP reductions would affect ciliary Ca^{2+} levels, given the close interplay between cAMP and Ca^{2+} signaling, e.g. through Ca^{2+} inhibition of AC5/6 activity (49) and Ca^{2+} activation of PDE1 (162). Upon somatostatin treatment, we observed that the reduction in cAMP levels initiated ciliary Ca^{2+} signaling after a delay of 5–10 minutes without affecting cytosolic Ca^{2+} levels. This Ca^{2+} activity was inhibited by simultaneous elevation of the cAMP concentration using PACAP, forskolin, an FFAR4 agonist or bPac activation, as well as by siRNA-mediated knockdown of SSTR3. Direct inhibition of PKA with a general blocker also induced Ca^{2+} signaling in primary cilia of both mouse islet cells and MIN6 pseudoislets. Earlier studies suggested that Gi receptor activation was directly linked to Ca^{2+} mobilization and entry (163, 164). However, more recent findings indicate that Gi-mediated Ca^{2+} influx depends entirely on active Gαq (165). In cilia, TIRF microscopy revealed that Ca^{2+} influx primarily originated from the extracellular space rather than intracellular stores, making Gq involvement unlikely. Instead, Gi proteins have been shown to activate TRPC4 currents (166, 167), which may contribute to the observed ciliary Ca^{2+} events. Additionally, cAMP reduction may lead to a compensatory increase in cGMP through effects on PDE activity, which could activate CNG channels and promote Ca^{2+} influx. These findings suggest that cAMP lowering is a critical regulatory mechanism for ciliary Ca^{2+} entry, a concept we aim to investigate further.

In pancreatic β-cells, where cAMP elevation is essential for insulin secretion, these findings show that the cilium function as a signaling checkpoint. By responding to inhibitory signals like somatostatin, cilia can locally degrade cAMP, providing a mechanism to counterbalance cytosolic cAMP increases. This finely tuned regulation allows the cilium to modulate cellular responses independently, despite lacking a cAMP barrier against the cytoplasm.

cGMP elevation triggers ciliary Ca^{2+} signaling (I, III)

cGMP is one of the second messenger, which has not been studied as much as cAMP, yet their dynamics are linked through shared regulation by PDEs. However, this interaction is not solely dependent on competition for phosphodiesterases. In olfactory neurons, cGMP activates PKG, which inhibits AC activity, effectively restricting cAMP to localized microdomains. This level of regulation highlights how cGMP can directly influence cAMP signaling beyond PDE competition (168).

In islet cilia we found that lowering cAMP levels with somatostatin triggered ciliary Ca^{2+} influx. Surprisingly, elevating cGMP levels, either through activation of transmembrane guanyl cyclases with ANP, through inhibition of PDE5, or via optogenetic activation of RhGC, also initiated ciliary Ca^{2+} activity. This effect was mediated via CNGA3 channels, as knockdown of CNGA3 abolished ANP-induced ciliary Ca^{2+} influx. To assess the extent of cAMP and cGMP pathway integration, we measured ciliary Ca^{2+} activity in the presence of agents that elevate cGMP (ANP) and decrease cAMP (somatostatin), allowing us to observe their combined effects on ciliary signaling dynamics. In cells exhibiting somatostatin-induced ciliary Ca^{2+} signaling, ANP potently suppressed this response, while it had the opposite effect in the rest of the cilia. These results indicate complex cross-talk between cAMP and cGMP pathways that converge on ciliary Ca^{2+} signaling, but that differs across cilia populations. Such opposing dynamics between cyclic nucleotides have been observed in other systems where local and distal interactions between cAMP and cGMP create subcellular compartments that define neuronal polarity (169, 170). These findings emphasize the role of localized cyclic nucleotide signaling in shaping the plasticity of the cellular environment, and also indicate that cytosolically generated cGMP may have the ability to modulate cilia-intrinsic signaling pathways that depend on local cAMP lowering, such as the Hh pathway (17).

Primary cilia as an active somatostatin hub; receiving the signal (II)

After showing that islet cilia are equipped with SSTR3 (and to some extent SSTR5) and that these are functionally active through coupling to cAMP lowering and ciliary Ca^{2+} influx, it was next crucial to determine whether primary cilia can sense endogenous somatostatin released by islet δ-cells. 3D segmentation of FIB-SEM images of mouse pancreas showed that β-cell primary cilia are in close contact with δ-cells, suggesting direct communication. The

irregular morphology of δ-cells (171) may contribute to this proximity, resulting in more frequent interactions between cilia and δ-cells. Immunofluorescence microscopy of mouse and human islets showed that each δ-cell contacted an average of 3.2 cilia, compared to just 1.8 cilia for non-δ cells. Next, we checked if endogenous somatostatin is released onto primary cilia using a recently developed somatostatin sensor (SST1.0), based on circularly permuted GFP fused to SSTR5 (172), which localizes to both the plasma membrane and primary cilia. Mouse islets expressing SST1.0 were stimulated with 10 mM glucose, leading to an increase in SST1.0 fluorescence at both the plasma membrane and primary cilia with similar amplitudes. Notably, the frequency of SST1.0 responses was 1.8-fold higher in primary cilia, suggesting that somatostatin secretion is at least partially directed towards the cilia. Next, we wanted to know if endogenous somatostatin could activate ciliary SSTRs and initiate ciliary signaling. Mouse islets expressing a ciliary Ca^{2+} indicator were stimulated with ghrelin, a hormone known to trigger Ca^{2+}-dependent somatostatin release (76, 77). Ghrelin stimulation elicited ciliary Ca^{2+} responses in approximately 40% of the cilia. These responses were confined to cilia located in specific islet regions, likely near δ-cells. Somatostatin released from δ cells within intact mouse islet can therefore initiate ciliary Ca^{2+} signaling, indicating a paracrine communication between δ cell and β-cell cilia. Examination of human islets from non-diabetic and type-2 diabetic organ donors revealed disruptions in this cilia-δ-cell communication in type 2 diabetes. Immunofluorescence staining revealed both reduced δ-cell numbers and shortened cilia in islets from type 2 diabetes donors, resulting in an increased distance between δ-cells and primary cilia. This structural change may impair the paracrine signaling seen in healthy islets and contribute to altered somatostatin signaling and β-cell dysfunction in type 2 diabetes.

Primary cilia as an active somatostatin hub; transmitting the signal (II)

Somatostatin is a major paracrine hormone that inhibits insulin secretion in β-cells primarily through plasma membrane-localized SSTR2, which lowers cytosolic cAMP concentrations (173). Beyond this well-established mechanism, islet cilia have been implicated in hormone secretion dynamics, including somatostatin signaling (3). While β-cell-specific cilia knockout abolishes the inhibitory effect of exogenous somatostatins on insulin secretion, direct SSTR3 inhibition or SSTR3 knockdown does not enhance glucose-stimulated insulin secretion (3, 11), suggesting that SSTR3 may play roles beyond acute control of hormone release.

We demonstrated that somatostatin exposure immediately lowers ciliary cAMP and triggers ciliary Ca^{2+} influx in an SSTR3-dependent manner. This finding aligns with the Hedgehog signaling pathway, which operates through the primary cilium and relies on reduced ciliary cAMP levels to activate transcriptional regulators like GLI2. Given the close proximity of β-cell cilia to δ-cells, allowing direct somatostatin access, we hypothesize that cilia act as integrative hubs for extracellular cues, mediating long-term transcriptional changes in response to sustained somatostatin exposure. In the islet microenvironment, where cilia are continuously exposed to somatostatin, even at low glucose levels (120), this positioning may allow cilia to fine-tune β-cell function over time through constant receptor activation and downstream signaling. Supporting this, MIN6 cells and mouse islets exposed to somatostatin for 18 hours showed GLI2 exit from the cilium and its subsequent nuclear entry in an SSTR3-dependent manner. Somatostatin-induced GLI2 entry was abolished using a cilia specific Ca^{2+} chelator, highlighting the complex interplay between secondary messengers in regulating ciliary output. The effect of somatostatin on GLI2 nuclear entry mirrored the response observed with the Hedgehog pathway activator SAG, with somatostatin inducing a comparable magnitude of GLI2 translocation. This suggests that somatostatin may exert some of its effects through cilia-dependent transcriptional control. How this regulation relates to its already established role as a regulator of the transcription of its own receptors in β-cells remains to be determined (174). Long term effects of somatostatin were further observed in MIN6 pseudo islets expressing cytosolic cAMP indicator ($EpacS^{H187}$). Both somatostatin and SAG treatments attenuated GLP-1-induced cAMP elevation, and SSTR3 knockdown showed that these somatostatin-mediated changes were SSTR3-dependent. Together, these results show that somatostatin can act as a cilia-dependent transcriptional regulator, operating in parallel with its acute suppression of insulin secretion to help maintain functional β-cells.

Conclusions

1. The primary cilium acts as a distinct Ca^{2+} compartment in islet cells, maintaining an isolated Ca^{2+} microenvironment through Na^{+}-dependent Ca^{2+} extrusion at the ciliary base. In contrast, the cyclic nucleotides cAMP and cGMP can freely diffuse into primary cilia from the cytosol.

2. cAMP and cGMP concentration changes are closely connected to ciliary Ca^{2+} signaling, with both cAMP reductions and cGMP increases causing increased ciliary Ca^{2+} influx.

3. In human islets, activation of the cell surface receptors for ANP and GLP-1 or voltage-dependent Ca^{2+} influx stimulates cytosolic cGMP production.

4. GABBR1 receptors localized to the base of β-cell primary cilia, and GABA binding to the receptor leads to receptor mobilization to more distal parts of the cilium and is accompanied by ciliary Ca^{2+} influx.

5. SSTR3 selectively localizes to the primary cilium, where its activation leads to both acute lowering of ciliary cAMP and increase of ciliary Ca^{2+} and to more sustained activation of GLI2 and its nuclear translocation.

6. Islet cilia function as an active somatostatin hub by facilitating direct interactions with nearby somatostatin-secreting δ-cells, where somatostatin secretion is directed towards, and detected by, ciliary receptors on β-cells, enabling targeted paracrine regulation.

7. Islet cell cilia length is reduced in T2D, resulting in increased distance between β-cell cilia and δ-cells that may lead to loss of connectivity.

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"It´s so easy to laugh, it´s so easy to hate. It takes strength to be gentle and kind."
The Smiths. (1986). *I know it´s over.*

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