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The islet hormone exocytosis machinery in type-2 diabetes

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Abstract

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Type-2 diabetes (T2D) is characterized by progressive β-cell dysfunction and impaired insulin secretion, yet the molecular mechanisms remain incompletely understood. In pancreatic β-cells, insulin secretion occurs through exocytosis, a process whereby insulin-containing secretory granules dock at the plasma membrane, recruit proteins that make up the SNARE-dependent exocytosis machinery (priming), and ultimately fuse with the membrane to release their content to the extracellular space. This thesis investigates the molecular machinery governing insulin granule dynamics and exocytosis in both healthy and diabetic conditions. Through analysis of secretory machinery components, we demonstrate distinct roles for SNARE binding protein Munc18 isoforms. Both Munc18 isoforms support granule docking, but Munc18-1 is strikingly required for exocytosis. On a molecular level, both isoforms bind to syntaxin, but are not recruited to the granule release site to the same extent. Our investigation of the v-SNARE protein VAMP8 reveals its predominant localization to endosomal compartments rather than insulin granules. Furthermore, we identify VAMP8 as a negative regulator of insulin secretion, likely by competing with VAMP2 at the release site, suggesting a new regulatory mechanism in β-cell function. We further examine phosphatidylinositol transfer protein alpha (PITPNA) as a critical regulator of insulin granule maturation. Modulation of PITPNA levels in human islets directly impacts insulin granule exocytosis, its silencing impairs secretion while overexpression enhances it. Importantly, restoring PITPNA expression in T2D islets reverses diabetes-related secretory defects, suggesting its loss may contribute to β-cell failure. Finally, using a novel ATP biosensor, we demonstrate that insulin granules can undergo either complete fusion, releasing both peptides and small molecules, or partial fusion that selectively releases only small transmitter molecules. This differential cargo release is regulated through cellular polarity and becomes dysregulated in T2D. Collectively, these findings provide insight into the molecular mechanisms controlling insulin granule trafficking and release, revealing multiple points of dysregulation in T2D.

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To my family
献给我最爱的家人♥

List of Papers

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

I. **Liangwen Liu**, Muhmmad Omar-Hmeadi, Santiago Echeverry Alzate, Irina Česnokova, Per-Eric Lund, Jan Saras, and Sebastian Barg. "Functional differences of Munc18 isoforms during insulin granule exocytosis reflect their affinities for the release site." ***Journal of Biological Chemistry*** (2024): *under revision*.

II. **Liangwen Liu**, Misty Marshall, Jan Saras, and Sebastian Barg. "VAMP8 is an endosomal v-SNARE that supports GLP1-receptor recycling and inhibits insulin granule exocytosis in pancreatic β-cells." ***Diabetes*** (2024): *under revision*.

III. YT Yeh, C Sona, X Yan, Y Li, A Pathak, MI McDermott, Z Xie, **L Liu**, ... & Poy, M. N. (2023). "Restoration of PITPNA in Type 2 diabetic human islets reverses pancreatic beta-cell dysfunction." ***Nature Communications***, 14(1), 4250.

IV. **Liangwen Liu**, Jan Saras, Sebastian Barg. "Altered fusion pore behavior in human type-2 diabetes favors nucleotide- over peptide-release from insulin granules." *Manuscript*.

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Additional publications not included in the thesis:

I. Marques C, Friedrich F, **Liu L**, et al. Global and Spatial Metabolomics of Individual Cells Using a Tapered Pneumatically Assisted nano-DESI Probe[J]. ***Journal of the American Society for Mass Spectrometry***, 2023, 34(11): 2518-2524.

II. Omar-Hmeadi M, **Liu L**, Echeverry S, et al. Quantification of Secretory Granule Exocytosis by TIRF Imaging and Capacitance Measurements[M]//Chromaffin Cells: Methods and Protocols. New York, NY: ***Springer*** US, 2022: 179-186.

III. Marques C, **Liu L**, Duncan K D, et al. A direct infusion probe for rapid metabolomics of low-volume samples[J]. ***Analytical Chemistry***, 2022, 94(37): 12875-12883.

IV. Kamranvar, S. A., Gupta, D. K., Wasberg, A., **Liu, L.**, Roig, J., & Johansson, S. (2022). Integrin-mediated adhesion promotes centrosome separation in early mitosis. ***Cells***, 11(8), 1360.

V. Gupta, D. K., Kamranvar, S. A., Du, J., **Liu, L.**, and Johansson, S. (2019). Septin and Ras regulate cytokinetic abscission in detached cells. ***Cell Division***, 14(1), 8.

Contents

Abbreviations

ATP	Adenosine Triphosphate
cAMP	Cyclic Adenosine Monophosphate
CAPS	Ca^{2+}-dependent activator protein in secretion
EGFP	Enhanced Green Fluorescent Protein
ELISA	Enzyme-Linked ImmunoSorbent Assay
EM	Electron microscopy
FACS	Flow Cytometry
GABA	γ-aminobutyric acid
GIP	Glucose-dependent Insulinotropic Polypeptide
GLP-1	Glucagon-Like Peptide-1
GLUT	Glucose Transporter
GSIS	Glucose Stimulated Insulin Secretion
K_{ATP}	ATP-sensitive K^+ channel
LDCV	Large Dense Core Vesicles
Munc13	Mammalian uncoordinated 13
Munc18	Mammalian uncoordinated 18
NSF	N-ethylmaleimide-Sensitive Factor
PI (4,5) P2	Phosphatidylinositol- 4,5-Bisphosphate
PM	Plasma Membrane
RP	Reserve Pool of Granules
RRP	Readily Releasable Pool of Granules
SLMV	GABA-containing Synaptic-Like Micro Vesicle
SNAP25	25kDa, Synaptosome Associated Protein
SNARE	Soluble N-Ethylmaleimide–Sensitive Factor Attachment Protein Receptor
sptPALM	single-molecule tracking photoactivated localization microcopy
T1D	Type-1 Diabetes
T2D	Type-2 Diabetes
TIRF	Total Internal Reflection
VAMP	Vesicle Associated Membrane Protein
VDCCs	Voltage-Dependent Ca^{2+} Channels
α-SNAP	α-Soluble NSF-Attachment Protein
PITPNA	phosphatidylinositol transfer protein alpha

Introduction

Diabetes

Insulin is the only blood glucose-lowering hormone to maintain glucose homeostasis in human physiology, and its unique role lead to an unparalleled central position in the diabetes research and treatment. Inadequate circulating levels of insulin impair glucose uptake from the bloodstream and its storage in liver, muscle, and adipose tissue, leading to chronic hyperglycemia, which is the hallmark of diabetes[1,2]. Diabetes manifests primarily in two forms: type-1 diabetes (T1DM, 5-10%) and type-2 diabetes (T2DM, 90-95%). T1DM is an autoimmune disorder characterized by the destruction of pancreatic β-cells through autoimmune attack, though the specific trigger remains unclear. T2DM is characterized by insulin resistance and glucose intolerance, ultimately progressing to insufficient insulin secretion from β-cells. However, the molecular mechanisms underlying impaired insulin secretion in diabetic patients remain incompletely understood. Therefore, investigating the molecular mechanisms that regulate both normal and pathological insulin release is crucial for a better understanding of diabetes pathogenesis.

Pancreatic islet microenvironment

Pancreatic islets, also known as islets of Langerhans, are organized structures that control endocrine function. The islets contain three main hormone-producing cell types: α cells producing glucagon, β-cells secreting insulin, and δ cells releasing somatostatin[3–6]: Each cell type possesses mechanisms for sensing glucose and other nutrients, while simultaneously integrating neural and paracrine signals within a microenvironment that includes blood vessels, immune cells, and neural projections. The regulation of hormonal output relies on paracrine signaling between adjacent islet cells. This local signaling is further enriched by the co-secretion of neurotransmitter-like molecules (GABA, ATP, glutamate) with insulin and glucagon[7–9]. These signaling molecules bind to specific receptors on islet cells, which triggers cellular responses. The spatial architecture of islets facilitates two modes of signaling: local paracrine communication and systemic hormone distribution. While paracrine signals operate within the immediate cellular environment, insulin and glucagon must reach the bloodstream to achieve their systemic effects. For instance, β-cells

are in direct contact with islet capillaries and exhibit polarized secretion preferentially directed toward the vascular interface[10]. However, the subcellular mechanisms enabling this specialized spatial organization of hormone release remain to be fully characterized.

Glucose-stimulated insulin secretion (GSIS)

In pancreatic β-cells, insulin undergoes processing to form Zn^{2+}-stabilized hexamers, which are packaged into large dense-core vesicles (LDCVs, also known as secretory granules). Other small molecules (ATP, GABA, glutamate) are pumped into these granules for storage[11]. The exocytosis secretion of insulin-containing granules is primarily triggered by increased blood glucose concentration[12] (Figure 1). Following food intake, elevated blood glucose levels enter β-cells through facilitated diffusion via glucose transporter type 2 (GLUT2)[13]. Once internalized, glucose is metabolized through glycolysis, starting with its phosphorylation to glucose-6-phosphate, and subsequently proceeds the Krebs cycle[14,15]. This generates ATP, leading to an elevated intracellular ATP/ADP ratio[16,17]. The increased ATP/ADP ratio closes ATP-sensitive potassium channels (K_{ATP}) in the membrane, resulting in membrane depolarization and action potential firing. These electrical signals active voltage-gated calcium channels (VGCCs), allowing Ca^{2+} influx into the β cell. The subsequent elevation in cytoplasmic Ca^{2+} concentration triggers exocytosis of insulin-containing granules[18–20]. Insulin is co-released with other granule cargo molecules, including islet amyloid polypeptide (IAPP, or amylin)[21], ATP[7], zinc[22], the neurotransmitters GABA[8] and glutamate[9], which contribute to local signaling within the islet.

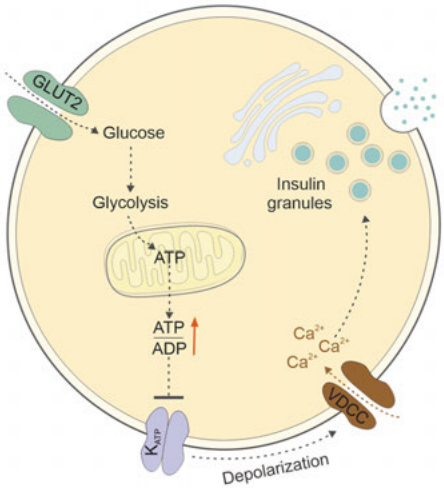

Figure 1. Glucose-stimulated insulin secretion pathway in pancreatic β-cells. Schematic representation of the mechanisms underlying insulin secretion. Following glucose uptake via GLUT2 transporters, cellular metabolism through glycolysis and the Krebs cycle elevates the ATP/ADP ratio. This triggers closure of K_{ATP} channels, leading to membrane depolarization and subsequent activation of voltage-gated calcium channels (VGCCs). The resulting Ca^{2+} influx stimulates exocytosis of insulin-containing granules.

Biphasic insulin secretion reflects functional granule pools

The insulin secretory response to glucose exhibits a characteristic biphasic pattern, with an initial rapid phase with high release rates lasting several minutes, followed by a slowly developing sustained release phase[23]. This typical biphasic pattern has been consistently observed in the human body, isolated islets, and single β-cells when exposed to elevated glucose concentrations[24–27]. The biphasic nature of insulin secretion reflects that not all granules are immediately released, and the release capacity depends on their location on the plasma membrane (PM). Single-cell studies have led to the concept of two distinct functional pools: the readily releasable pool (RRP) and the reserve pool (RP). Each β cell contains ~10^4 secretory granules, but only ~1% of these are released immediately upon stimulation. This group of granules is classified as RRP and thought to be primed at the PM[28–30]. Following the rapid depletion of RRP, granules from the RP undergo a sequential process to maintain sustained insulin secretion[29,31]. This process begins with granule translocation and docking at the PM, followed by priming reactions through which granules acquire release competence. Finally, these primed granules (corresponding to RRP) fuse with PM upon Ca^{2+} signals and release insulin (Figure 2). Continuous mobilization of granules from the RP to replenish the RRP underlies the second, slower phase of sustained insulin secretion. T2DM is distinguished by the near complete loss of first-phase insulin secretion in response to glucose challenge, accompanied by diminished second-phase insulin secretion[32,33].

Regulatory factors of insulin secretion

Insulin secretion is regulated by multiple physiological factors beyond glucose, including both stimulatory and inhibitory signals. Key metabolic stimulators include free fatty acids[18] and amino acids[34]. the most potent enhancers are incretin hormones, primarily glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)[35–37], which are released by intestinal cells upon food intake. These incretins bind to their respective G protein-coupled receptors (GLP-1R and GIPR) on β-cells, activating adenylyl cyclase to increase intracellular cAMP levels, thereby amplifying glucose-stimulated insulin secretion (GSIS)[38–40]. The increased cAMP levels orchestrate insulin secretion through two parallel pathways: PKA (protein kinase A)[41] and Epac2 (exchange protein directly activated by cAMP)[42,43]. PKA enhances insulin secretion by phosphorylating both K_{ATP} channels[44,45] and Ca^{2+} channels[46], which increases the number of highly Ca^{2+} sensitive insulin granules[47]. Epac2 triggers calcium release via ryanodine receptors, facilitating granule docking through Rim2α complex formation, and expanding the

readily releasable granule pool (RRP)[48,49]. In contrast, several factors serve as physiological brakes on insulin secretion, including epinephrine[50], leptin[51], zinc ions (Zn^{2+})[52], and somatostatin released by neighboring islet δ-cells[53].

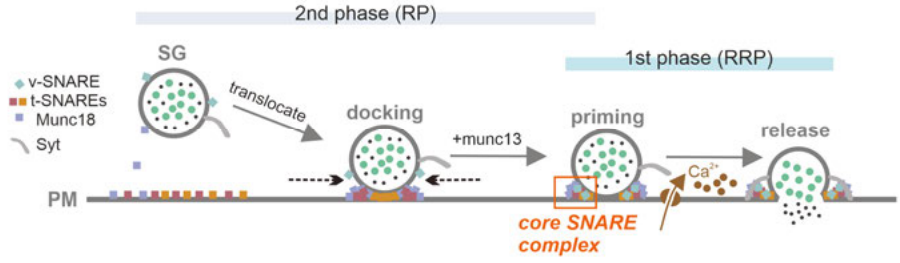

Figure 2. Sequential steps of insulin granule exocytosis and molecular machinery involved in each step. Cartoon illustrates the progressive stages of insulin secretory granule (SG) trafficking and fusion at the plasma membrane (PM). The process occurs in two phases: the readily releasable pool (RRP, 1st phase) and reserve pool (RP, 2nd phase). Key molecular components are depicted: v-SNAREs (diamond) on vesicles, t-SNAREs (rectangles) on the PM, Munc18 (purple squares), and Synaptotagmin (Syt, curved lines). The sequential steps include: a) Initial SG translocation to the PM; b) Docking: establishment of initial molecular contacts at the PM; c) Priming: SNARE complex assembly facilitated by Munc13; d) Final Ca^{2+}-triggered fusion and release, where the core SNARE complex (highlighted in orange box) mediates membrane fusion.

Exocytosis machinery

In pancreatic β-cells, proinsulin synthesis initiates in the endoplasmic reticulum (ER) and undergoes maturation steps in the Golgi apparatus. The processed insulin molecules are packaged into mature secretory granules that gradually acidify. These mature insulin granules are distributed throughout the cytosol and eventually translocated to and docked at the PM. The ultimate fusion of these insulin granules with the PM is regulated by protein-protein and protein-lipid interactions. The molecular machinery governing membrane fusion is highly conserved from yeast to humans[54,55]. Extensive molecular and physiological studies have established a unified model for membrane fusion mechanisms. Many of the proteins that are involved in the regulation of neurotransmitter release have been identified also in the pancreatic β cell, and they participate in insulin secretion. Biochemical analyses across various secretory systems have revealed a ternary core complex that includes SNARE proteins in vesicles (v-SNAREs: synaptobrevin/VAMP) and target membranes (t-SNAREs: syntaxin and SNAP25) that have a characteristic conserved coiled-coil SNARE motif domain exposed in the cytosol. A v-SNARE and two t-SNAREs converge into a tight four-helix bundle through their SNARE motif, forming a core SNARE complex that pulls the two membranes

together, leading to membrane fusion (Figure 3). Specific isoforms of SNARE proteins serve different membrane fusion pathways. For example, in β-cells, VAMP2/syntaxin-1/SNAP-25 is involved in insulin granule exocytosis, whereas VAMP8/syntaxin3/SNAP-25 mediates unconventional exocytosis of insulin granules, where "newcomer" granules undergo exocytosis immediately after arrival at the PM[56–58]. Moreover, membrane fusion of lysosome and autophagosome requires VAMP8/syntaxin-17/SNAP-29, and recycling endosome fusion is facilitated by VAMP8/syntaxin-4/SNAP-23. In addition, several accessory proteins facilitate and regulate SNARE dependent fusion, in particular N-ethylmaleimide-sensitive (NSF) and α-soluble NSF-attachment protein (α-SNAP), the Ca^{2+}-sensor synaptotagmin (Syt), the syntaxin binding protein Munc18, and the priming factor Munc13.

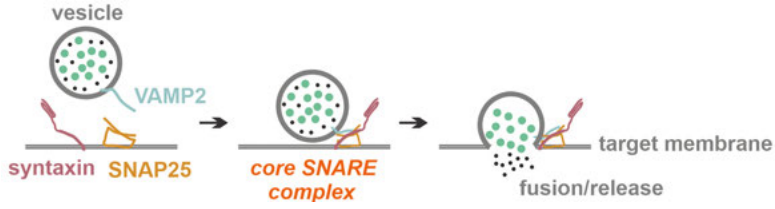

Figure 3. Core SNARE complex assembly in membrane fusion. The ternary SNARE complex forms between vesicle-associated v-SNAREs (synaptobrevin/VAMP) and plasma membrane-localized t-SNAREs (syntaxin and SNAP25). These proteins interact through their conserved cytosolic coiled-coil SNARE motifs to form a tight four-helix bundle, providing the force necessary for membrane fusion.

The release site harbors many copies of exocytosis proteins

The molecular machinery orchestrating insulin granule docking and exocytosis is characterized by organized protein clusters at specialized release sites. These dynamic assemblies form rapidly after granule arrival, rather than relying on pre-existing docking platforms. Upon granule arrival at the release site, syntaxin-1 is sequentially recruited and self-assembles into clusters of 50-70 copies within 50-100nm domains at the PM[59–61].This clustering process, promoted by Munc18, establishes stabilization at the release site[62]. This rapid clustering is followed by sequential recruitment of SNAP25 and Munc13 after a 1-2 minutes delay[61]. At each primed granule site, more than 100 SNARE proteins are clustered into a highly structured molecular complex spanning 100~110nm in diameter. While this cluster contains numerous proteins, membrane fusion requires only 3-4 SNARE complexes to generate sufficient force through their zippering mechanism to overcome the fusion barrier[63,64]. The clusters are beneath docked granules, that they stabilize docking, and that the

excess SNAREs may act as reservoir to supply trimeric snare complex formation.

Molecules in docking

Granule docking requires precise molecular interactions to be established in specific sites at the PM. Two GTP-binding proteins, Rab3 and Rab27, serve as key regulators in the docking process[65–67]. Rab3 coordinates granule targeting to appropriate PM docking sites through interactions with its binding partner RIM, while Rab27 limits granule mobility via interaction with its effector protein Granuphilin[6869]. Following these initial interactions, clustering of SNARE proteins syntaxin and its chaperone Munc18 are recruited to the docking site[61]. At the docking site, Munc18 forms a high-affinity complex with syntaxin (binding affinity 2-10 nM) in a 1:1 stoichiometry[70,71 72]. This interaction maintains syntaxin in an autoinhibitory closed conformation, where Munc18 arches around a four-helix bundle comprising syntaxin's SNARE domain and N-terminal H_{abc} domain helices. The Munc18-syntaxin complex serves multiple functions: it prevents the early recruitment of other SNARE proteins[60,73–75], facilitates syntaxin trafficking from the ER to the PM, and protects syntaxin from degradation[62,76]. These molecular chaperons (Munc18 and syntaxin) stabilize the docking state, as evidenced by decreased protein levels when either component is knocked down[77,78].

Molecules in priming

Priming is a critical maturation step that prepares near-membrane granules for release. This process involves conformational changes in SNARE proteins and the formation of partially zippered SNARE complexes[79,80]. Two key proteins, Munc13 and Munc18, orchestrate SNARE complex assembly[81–83]. Munc13 facilitates proper complex formation by catalyzing syntaxin-1's transition from a closed to an open state, enabling its interaction with SNAP25 and VAMP2[84]. Munc18 enhances SNARE assembly and acts as a template for syntaxin-1 and VAMP2 interaction, effectively bringing opposing membranes into close proximity[85–88]. Recent findings demonstrate that Munc18-1 forms a ternary template complex with VAMP2 and syntaxin-1, significantly enhancing SNARE assembly efficiency[82,89,9091]. SNAP-25 rapidly incorporates into this template complex, leading to partially zippered SNARE complex formation. During this process, Munc18-1 dissociates from the four-helix bundle while maintaining its interaction with syntaxin's N-terminal domains[91]. This conserved complex template (Munc18-1:syntaxin-1:VAMP2) ensures both speed and accuracy in SNARE assembly. Moreover, the interaction between SNAREs and Munc18-1 in the template complex contributes to the

proofreading of SNARE pairings, thus preventing promiscuous SNARE pairing. Any SNAREs cannot enter the SNARE complex assembly without prior binding to Munc18-1 in the template complex[92–94 89].

Molecules in fusion

The final stages of priming involve Syt and complexin (Cpx) binding to the partially zippered SNARE complex, forming a super-complex ready for Ca^{2+}-triggered membrane fusion[95]. The transition of the partially zippered SNARE complex to the fully zippered state requires the Ca^{2+} signals to facilitate fusion pore opening and membrane fusion. Upon Ca^{2+} influx, binding to Syt triggers Cpx release from the syt-SNARE-Cpx-syt complex, enabling complete SNARE complex zippering[96–98]. Doc2b is another high-affinity Ca^{2+} sensor that mediates vesicle fusion[99,100]. Still, it exhibits an inhibitory role during the prolonged calcium elevation by protecting unprimed vesicles from premature fusion to ensure proper priming[101]. Coupling to the Ca^{2+} sensors promotes the full zippering of SNARE assembly and fusion of the vesicles with the PM. After fusion, the SNARE complex undergoes ATP-dependent disassembly mediated by NSF and α-SNAP, recycling of SNARE proteins for subsequent fusion events[102].

Fusion pore dynamics

The initial step of fusion begins with the formation of a narrow fusion pore ($\leqslant$3 nm)[103], establishing the first aqueous connection between the secretory granule lumen and extracellular space. These narrow pores comprising both lipids and proteins[104,105], exhibits dynamic behavior similar to ion channels, flickering between open and closed states. This behavior suggests the presence of mechanisms that stabilize the pore and restrict its expansion. The restricted pore diameter creates a size-dependent sequence of cargo release: small molecules such as ATP and other nucleotides are released rapidly (within 100-200 milliseconds post-fusion, detected by purinoreceptor 2 (P2X2) current spikes)[106–109],followed by observable changes in granule pH (monitored via pH-sensitive fluorescent proteins), and finally, the release of larger peptide cargo including insulin, which requires further pore expansion[108,110–112]. Electrophysiological studies in INS1 cells have revealed that this temporal sequence of release is tightly controlled by pore dynamics[106,107].

The fusion pore can undergo two distinct fates: irreversible expansion (termed full fusion)[106,107,113] or transient opening followed by closure (termed kiss-and-run or cavicapture)[106,108,114–116]. During full fusion, complete mixing of secretory granule and plasma membranes occurs, enabling the release of

large molecular weight cargo. In contrast, kiss-and-run events involve temporary pore formation followed by resealing, allowing intact retrieval of the secretory granule into the cytoplasm. In β-cells, kiss-and-run events constitute approximately 20-50% of exocytotic events, while not resulting in insulin release[106,108]. However, these events are expected to contribute to local signaling within the islet, as smaller granule constituents (nucleotides, glutamate, or GABA) are released even without full pore expansion. For instance, co-released ATP from insulin granules synchronizes β-cells, stimulates insulin secretion[117], suppresses glucagon release[118], and activates macrophages[119]. Recent studies have shown key regulatory pathways controlling fusion pore expansion in β-cells, including GLP1/cAMP signaling cascades (modulated by exendin and DPP4 inhibitors)[120] and by local PIP2 generation[121], which recruits BAR domain/endocytosis proteins to release sites. Despite these insights, the physiological significance of fusion pore dynamics in modulating β-cell secretion remains to be fully elucidated.

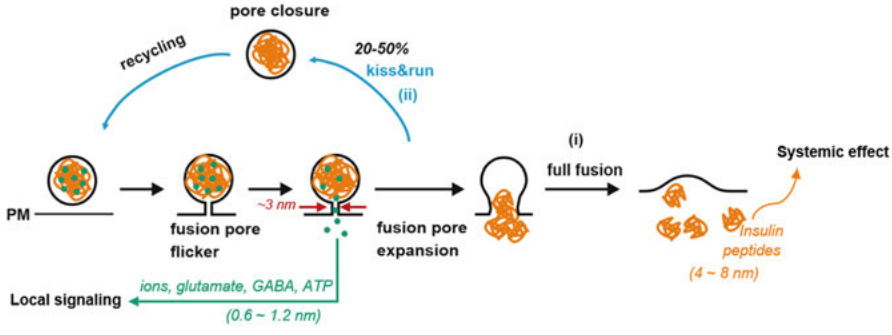

Figure 4. Sequential stages of insulin granule fusion pore dynamics and differential cargo release. The diagram illustrates the temporal progression of insulin granule fusion and differential cargo release. Initial dense-core vesicles (containing hormone peptides [orange] and small molecules like nucleotides and neurotransmitters [green dots]) dock at the plasma membrane (PM), followed by fusion pore formation (⩽3 nm diameter). Small molecule release (ATP, GABA, glutamate, and ions [0.6-1.2 nm]) occurs rapidly through the initial fusion pore, enabling local signaling within the islet environment. Fusion pore expansion facilitates proton exchange with the extracellular space, resulting in a transient fluorescence increase. The expanded pore enables insulin peptide release before eventually collapse, with released hormone peptides entering the bloodstream to mediate systemic metabolic effects. Secretory granules undergo one of two distinct fates during fusion pore expansion: (i) complete fusion with full membrane integration and total content release, or (ii) "kiss-and-run" exocytosis (20-50% of events) where the granule maintains integrity for recycling. This model synthesizes evidence from capacitance measurements, fluorescence imaging, and ATP release studies.

β-cell polarity

Pancreatic β-cells exhibit distinct structural polarity, characterized by three specialized domains: basal membrane facing capillaries, lateral membrane maintaining cell-cell contacts, and apical membrane oriented away from capillaries[122]. This polarized architecture is crucial for normal β-cell function, particularly in directing hormone secretion[10]. The basal membrane, which interfaces with capillaries, is enriched with presynaptic scaffold proteins including RIM, Bassoon, Piccolo, CAST, and ELKS - proteins originally characterized in neuronal synapses[123 10,124–126]. These proteins create specialized active zones by recruiting and organizing essential exocytotic machinery, including voltage-dependent calcium channels (VDCCs), cytoskeletal proteins, and SNARE proteins[127–129]. This molecular scaffold establishes an efficient environment optimized for insulin secretion directly into the bloodstream. The functional significance of this polarity is demonstrated by preferential insulin granule fusion at basement membrane contacts through FAK signaling, and higher insulin concentration at the vascular face[130]. Notably, β-cells maintain and even elongate to preserve direct capillary contact[130], suggesting evolutionary optimization for efficient hormone delivery. While some of these proteins have studied in the regulation of insulin secretion. However, the organization and interaction of these active zone proteins in β-cells have not been fully understood.

Methods for studying exocytosis and intracellular granule trafficking

The observation and quantification of vesicle exocytosis and intracellular trafficking have evolved significantly over the past three decades through various methodological approaches. Each technique offers distinct advantages and limitations in studying these cellular processes. Enzyme-linked immunosorbent assay (ELISA), introduced for insulin detection in the 1980s, represents a fundamental biochemical approach that quantifies secreted substances in the supernatant through enzymatic reactions[131,132]. Nowadays, this technique demonstrates enhanced sensitivity in measuring not only insulin secretion but also the intracellular content of proinsulin and C-peptide[133,134]. However, ELISA measurements require large amounts of cells and offer little temporal resolution. Cell capacitance measurement is the electrophysiological technique to monitor exocytosis and intracellular granule dynamics, exploiting the proportional relationship between cell capacitance and surface area[135,136]. When secretory granules fuse with the plasma membrane, the resulting changes in cell surface area can be detected with exceptional temporal resolution (~1 ms) at the single cell level, enabling the detection of individual

granule fusion events[137]. While this technique offers remarkable sensitivity, it measures the net membrane dynamics resulting from both exocytosis and endocytosis. A notable limitation is that it cannot distinguish secretion events between insulin-containing secretory granules and other vesicle types, such as GABA-containing synaptic-like microvesicles (SLMVs)[138–140]. Electron microscopy (EM) uses a beam of electrons to generate magnified images that provide information on vesicles from the fixed cells, for example, to quantify the near membrane granule numbers in beta cells at the time of fixation[141]. It cannot be used to view living cells since the electrons destroy them. Therefore, there are limitations in detecting granule movement and granule release. A combination of cell capacitance measurement and EM indicated that the initial secretion comes from the granules in the vicinity of the PM[28].

Imaging exocytosis and release in single living cells

While traditional methodologies provide valuable insights into cellular secretion, live-cell fluorescence microscopy represents a significant advance by enabling direct observation of individual secretory granule dynamics in living cells. This approach primarily utilizes fluorescent protein-tagged peptides that are normally sorted into granules, to be used as secretory granule markers. This labeling strategy permits visualization and tracking of individual vesicles throughout the secretory process, including pre-exocytotic, exocytotic, and post-exocytotic phases. Two principal fluorescence microscopy techniques have emerged as powerful tools for investigating granule trafficking and exocytosis: confocal microscopy and total internal reflection fluorescence (TIRF) microscopy.

Confocal microscopy generates thin optical sections (~500 nm) through selective elimination of out-of-focus light via a pinhole mechanism[142]. This capability enables three-dimensional reconstruction through z-stack imaging, providing more information on the distribution of cellular structure and proteins within the overall cell.

TIRF microscopy employs evanescent wave excitation to achieve superior resolution through the generation of an extremely thin optical section (~100 nm) at the coverslip interface [143]. This selective illumination specifically captures vesicle dynamics near the PM while excluding signals from deeper cellular regions. Moreover, TIRF microscopy significantly reduces photobleaching and phototoxicity, allowing relatively longer observation times with less damage to the sample. Given that insulin secretory granules typically range from 50-140 nm in diameter, TIRF microscopy provides an ideal methodology for investigating secretory events that occur near the PM in live single cells[144], and it is the primary imaging technique employed throughout this thesis work.

Methods

Experimental pancreatic β cell models

This thesis work employed two in vitro models to study insulin secretion dynamics: primary human islets and the INS1 pancreatic β-cell line. Human pancreatic islets, obtained from organ donors, represent the most physiologically relevant experimental system. Following enzymatic dispersion with trypsin-containing buffer and culture on poly-L-lysine-coated coverslips, these primary cells were used for single-cell imaging after transfection or/and infection with viral vectors to express the fluorescent reporter constructs.

The INS1 cell line, derived from rat insulinoma, served as the β-cell line model due to their unlimited proliferation capacity and relatively easy handling. INS1 cells exhibit efficient transfection using liposome-based reagents and adenoviral infection. Importantly, INS1 cells maintain stable glucose responsiveness, demonstrating a physiologically relevant ~6-fold enhancement in insulin secretion when glucose concentrations are elevated from 3 mM to 15 mM[145]. These properties make INS1 cells particularly suitable for single β-cell secretion kinetic studies.

Insulin granule exocytosis measurements in vitro

Two methods were employed to assess insulin exocytosis *in vitro*:

1) TIRF Microscopy (Figure 5A). This technique enabled the visualization of insulin secretion at the single β-cell level using fluorescent labeled secretory granules. Insulin granules were labeled with fluorescent cargo peptides (e.g., neuropeptide Y [NPY]) or granule-associated proteins (e.g., Rab3, vesicle-associated membrane protein 2 [VAMP2], etc.) and exocytotic events were identified by the rapid disappearance of tracked granules in time-lapse recordings (Figure 5B-C).

2) Capacitance measurements via Patch-Clamp technique. This method exploits the proportional relationship between granule exocytosis and increased cell surface area, manifesting as changes in cell capacitance. We mainly used patch-clamp technique as an alternative method to assess the secretion trend

of a whole cell and compare it with the cell footprint exocytosis events from TIRF data.

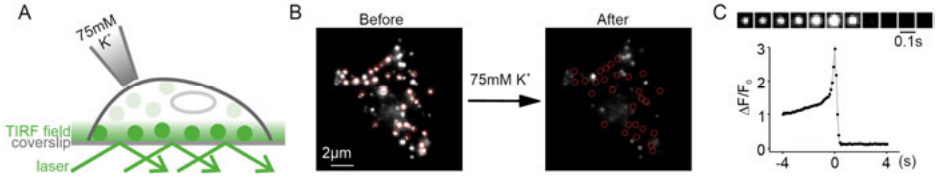

Figure 5. Visualization of single insulin granule exocytosis using TIRF microscopy. A: Principle of TIRF microscopy in live single cells, illustrating selective illumination of fluorophores within the evanescent field (TIRF field, ~100 nm from the plasma membrane interface). **B:** Representative TIRF micrograph of an INS1 cell expressing NPY-EGFP. Discrete fluorescent puncta correspond to individual insulin-containing secretory granules. Granules undergo exocytosis (indicated by red circles) upon membrane depolarization with 75 mM K^+ solution. **C:** Individual exocytotic events are identified by the rapid loss of fluorescence signal.

Image analysis of granule-associated protein dynamics

To investigate protein dynamics during granule trafficking and exocytosis, we expressed the fluorescence-labeled proteins together with the labeled granules, to enable the quantification of protein dynamics corresponding to granule states (Figure 6). The binding of fluorescently labeled proteins to granules was analyzed using MetaMorph software. The analysis defines three regions: a centered area (c, 3×3 pixels) on a randomly picked granule, a surrounding annular region (a, 5×5 pixels), and a background area (bg) outside the cell footprint. Protein binding affinity to granules was quantified as ΔF (c-a), representing "on-granule" fluorescence, while S (a-bg) indicates "off-granule" fluorescence in the surrounding region. The binding affinity of proteins to granules is expressed as the ratio ΔF/S, calculated either as cell averages for still images or each frame for time-lapse recordings.

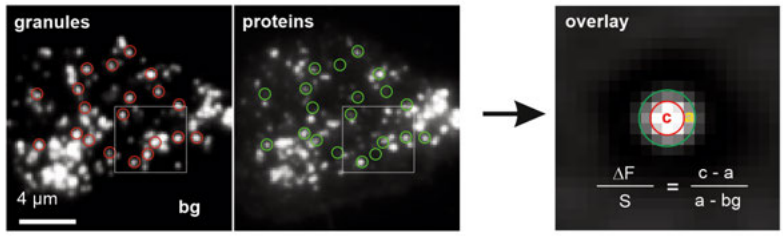

Figure 6. Image analysis for protein binding affinity to granules. An example of INS1 cell co-expressing the granule marker NPY-mCherry and t-SNARE protein syntaxin-1-EGFP under TIRF microscopy. The distribution of fluorescent labeled syntaxin-1 clusters are circled in green. The binding affinity (ΔF/S) of syntaxin-1 to secretory granules was analyzed using MetaMorph software as shown.

Single molecule tracking and analysis

To perform single molecule tracking, we need to express the protein of interest fused to a fluorescent protein (e.g., EGFP) using a truncated promoter (ΔCMV) to achieve low expression levels suitable for single molecule detection. The samples were imaged using a TIRF microscope equipped with a high NA objective ($\geqslant$1.45), sensitive EMCCD/sCMOS camera, and appropriate laser illumination. Data was acquired at 20 fps with 20-50ms exposure time, maintaining cell temperature at 32°C. 500-1000 frames per sequence were recorded, ensuring molecule density was $<1/\mu m^2$ and including cell-free regions for background correction.

For analysis, background was subtracted and corrected for drift if necessary. We used ImageJ plugins (Particle Tracker or QuickPALM) to detect and track single molecules, setting appropriate thresholds for intensity and particle size. Trajectories were analyzed by calculating mean square displacement and fitting displacement histograms with a two-component diffusion model to determine diffusion coefficients. For residence time analysis, we measured how long molecules remained within defined regions of interest and fitted dwell time distributions with double-exponential functions. Afterwards, we validated single molecule behavior through photobleaching step analysis and diffraction-limited spot size verification. Key measurements to be reported include diffusion coefficients, residence times, mobile/immobile fractions, and localization precision. Success depends on achieving appropriate expression levels, optimizing imaging conditions for good signal-to-noise ratio, and including proper controls throughout the analysis.

Objectives

This thesis aims to investigate key proteins involved in exocytosis and to explore alterations in cellular function during diabetic disorders, deepening our understanding of islet physiology and pathology. This research includes three main objectives:

1. Characterizing the roles of crucial proteins (Paper I & II) in regulated exocytosis and insulin secretion, with a focus on understanding how these proteins contribute to the control of insulin release.

2. Investigating the role of the phosphatidylinositol transfer protein alpha (PITPNA) in T2D, and exploring a novel molecular pathway that may be crucial for maintaining β-cell health and function (Paper III).

3. Investigating fusion pore behavior and co-release of peptide and small-molecule molecules from insulin granules, with focus on changes in human T2D that may contribute to impaired insulin secretion (Paper IV).

Results and Discussion

Paper I

Munc18-1 orchestrates both docking and priming of secretory granules for exocytosis[77,146–149]. In β-cells, Munc18-1 co-clusters with syntaxin-1 at granule sites, and docking of granules depends on syntaxin-1 clustering[61,150]. However, how Munc18-1 is involved in clustering for granule docking remains unknown, and relatively little work has been done with Munc18-2. In this work, we created CRISPR/Cas9-mediated single and double knockouts of Munc18-1 and -2 in INS1 cells to investigate their respective roles during insulin exocytosis. Using TIRF microscopy, we investigated granule docking, priming, and syntaxin clustering through knockout studies (Munc18-1 KO, Munc18-2 KO, and Munc18-1/2 DKO) and subsequent rescue experiments examining Munc18 isoforms recruitment to granule release sites.

Munc18 isoforms in granule docking and priming

We used three different approaches to study granule docking: 1) quantification of near-membrane granules from snapshot images; 2) measurement of newly arriving granules residence time at the PM; 3) analysis of granule lateral mobility through trajectory tracking (CD: cage diameter). While the density of near-membrane granules remained unchanged across all genotypes, newly arriving granules in DKO cells exhibited significantly reduced residence times (~30 s) compared to control. Analysis of CD revealed increased granule mobility in Munc18-2 KO and DKO cells, whereas Munc18-1 KO cells showed no significant alterations. These findings suggest that Munc18-2 plays a more prominent role in granule docking than Munc18-1, and the absence of both isoforms leads to increased granule undocking events.

Assessment of granule priming through high K^+-stimulated insulin exocytosis revealed a strong reduction (~90%) in secretory responses in both Munc18-1 KO and DKO cells, while Munc18-2 KO cells maintained control levels of secretion. These findings were verified by single-cell capacitance measurements, indicating a critical role for Munc18-1 in insulin granule exocytosis, which is consistent with the function of Munc18-1 in regulating exocytosis in other secretory cell types.

Munc18 isoforms in syntaxin clustering

Analysis of syntaxin clustering revealed distinct isoform-specific interactions. Syntaxin-1 clustering was markedly impaired in all knockout variants, whereas syntaxin-3 clustering deficits were restricted to Munc18-2 KO and DKO cells. The differential efficacy of Munc18 isoforms in granule priming may reflect either disparate endogenous expression levels or preferential syntaxin chaperon binding, with Munc18-1/syntaxin-1 potentially constituting a more efficient priming complex compared to Munc18-2/syntaxin-3. Collectively, these data suggest a separate role of the isoform effect, where Munc18-2/syntaxin-3 predominantly mediates granule docking, while Munc18-1/syntaxin-1 facilitates granule priming.

Rescue experiments in knockout cells

Rescue experiments in Munc18-1 KO cells utilizing point mutations that disrupt specific binding modes: Munc18-1_{EK} prevent clasping around the 4-helix bundle of closed syntax-in, and Munc18-1_{EA} prevents binding to syntaxin's N-peptide[72,151–153]. Munc18-1_{EK} and Munc18-1_{EA} demonstrated only partial restoration of insulin exocytosis, whereas wild-type Munc18-1 achieved the complete rescue. This reduced efficacy correlates with diminished clustering at granule docking sites. Expression of Munc18-2 in Munc18-1 KO cells yielded partial secretory recovery, suggesting that the relative inefficiency of Munc18-2 in supporting exocytosis is independent of expression levels and may instead reflect intrinsic properties of Munc18-2/syntaxin-3 chaperoning. The inferiority of Munc18-2 to Munc18-1 in exocytosis seems to be independent of the expression level, which may be due to the lower efficiency of chaperones of Munc18-2 and syntaxin-3 in regulating exocytosis. Similar to observations in Munc18-1 null chromaffin cells, the substitution of Munc18-1 with Munc18-2 supported vesicle docking to the same extent as Munc18-1, but only partially promoting vesicle exocytosis[154].

In DKO cells, both wild-type isoforms fully restored secretory function, and surprisingly, even the mutants Munc18-1_{EK} and Munc18-1_{EA} supported substantial exocytosis despite their weaker association with docked granules and syntaxin-1. The difference in rescue outcomes between single and double knockout may suggest that Munc18-2 is involved in, or competes with the priming function of Munc18-1.

Single molecule dynamics of Munc18/syntaxin interactions

Single molecule imaging via TIRF microscopy revealed the membrane-based interactions between Munc18-1/2 and syntaxin-1. Both proteins showed diffusion across the plasma membrane with similar track durations. Direct observation confirmed co-diffusion of Munc18s with syntaxin-1. Analysis of

survival curves using double exponential decay functions showed comparable interaction times for both Munc18-1 and Munc18-2 with syntaxin-1, with no significant differences in fast or slow components. These observations support a model where unclustered, freely diffusing syntaxin-1 molecules remain bound to Munc18, consistent with their proposed chaperoning function (Figure 7).

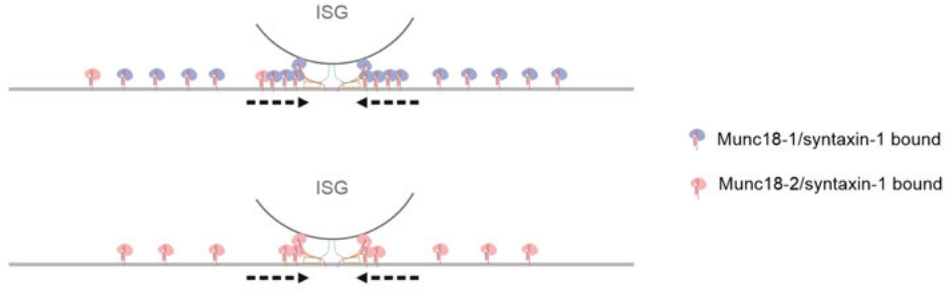

Figure 7. Munc18 behavior at the release site during docking and priming. Single-molecule imaging reveals that Munc18 associates with both clustered syntaxin beneath granules and individual diffusing syntaxin molecules in the plasma membrane. Syntaxin is recruited to clusters in its closed (Munc18-bound) conformation prior to granule docking. At granule docking sites, syntaxin clusters serve to stabilize granule-membrane physical interactions and provide a local reservoir of syntaxin molecules to facilitate SNARE complex formation. ***Paper I***

Paper II

In multiple secretory cell types, v-SNAREs play a key role in shuttling vesicles to various endosomal compartments to influence the distribution of membrane proteins. Reduced expression of v-SNAREs leads to defects in the regulated secretion. For example, VAMP2 is well described to mediate conventional exocytosis of dense core granules and synaptic vesicles. Previous studies have shown that VAMP8 mediates the exocytosis of "newcomers" insulin secretory granules, which fuse with the plasma membrane immediately upon arrival with minimal or no docking time. However, conflicting evidence showed that VAMP8-null mice exhibited increased total insulin secretion and improved glucose homeostasis. Therefore, we investigated the role of VAMP8 in pancreatic β-cells.

VAMP8-positive vesicles are endosomes

We first aimed to determine the localization of VAMP8 in β-cells, and to test its association with different fluorescence-labelled vesicles: NPY- or Rab3-positive insulin granules, Rab5-positive early endosomes, Rab7-positive late

endosomes, Rab11-positive recycling endosomes. After TIRF imaging analysis, we found that VAMP8 was not localized to insulin granules, but was associated with endosomal compartments and had much higher binding affinity to recycling endosomes. K^+-stimulated exocytosis revealed that recycling endosomes also delivered VAMP8 to the PM. Previous work have pointed out that VAMP8-dependent recycling endosomes deposit the required t-SNARE at immune synapses, facilitating the fusion of cytotoxic granules [155], which provided us with an idea of which proteins important for β-cell function are carried by VAMP8-positive recycling endosomes. We tested several important membrane receptors and found that VAMP8-positive vesicles delivered GLP-1R and GLUT2 to the PM (Figure 8).

Depending on the characteristics of the "newcomers", they stay at the PM for a short time and diffused rapidly. We studied the behavior of VAMP8-positive vesicles by measuring the residence time and exocytosis time course. We found that VAMP8-positive vesicles exhibited a linear monophasic secretion pattern with a very short residence time of ∼4 seconds, in contrast to insulin granules. The latter stay immobile beneath the PM for several minutes and exhibit a biphasic secretion manner as described previously[61]. Finally, we used tetanus neurotoxin (TeNT) studies to differentiate the signaling pathway of VAMP2 (TeNT-sensitive) and VAMP8 (TeNT-insensitive) mediated exocytosis. We found that TeNT blocked insulin secretion only, with no effect on VAMP8 vesicle secretion. Taken together, our results conflict with the previous declaration of VAMP8 "newcomers", and we state that VAMP8 is not associated with insulin granules, but is involved in the endosomal pathway that recycles important membrane proteins back to the PM.

VAMP8 may inhibit insulin secretion by competing with VAMP2

According to a previous study, overexpression of VAMP8 inhibits GSIS in mouse insulinoma cells[56]. To study the role of VAMP8 in insulin secretion, we overexpressed VAMP8 in β-cells, K^+-stimulated insulin exocytosis was inhibited in both human islet cells and INS1 cells. Gene expression analysis of VAMP8 showed a significant anti-correlation with insulin secretion from isolated human islets[156]. These data reveal an inhibitory function of VAMP8 in mediating insulin secretion. To study how VAMP8 inhibits exocytosis, we deleted the transmembrane domain of VAMP8 (V8-ΔTMD), preventing its anchoring to vesicles. We found that soluble VAMP8 was still able to inhibit insulin exocytosis. One possibility is that overexpressed soluble VAMP8 interacts with other SNAREs to form VAMP8/syntaxin-1/SNAP25 complex that occupies their binding to VAMP2-localized insulin granules, thereby inhibiting insulin secretion in an indirect way (Figure 8).

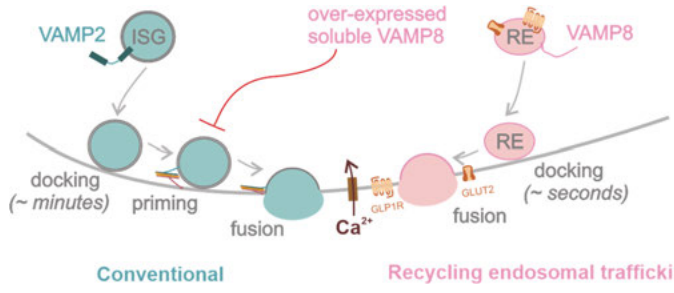

Figure 8. VAMP8 in endosomal trafficking and insulin secretion. VAMP8 localizes to endosomal compartments and mediates Ca^{2+}-dependent exocytosis of Rab11-positive recycling endosomes. Through endosomal recycling, VAMP8-positive vesicles facilitate the trafficking of essential membrane proteins, including GLP1R and GLUT2, back to the plasma membrane. VAMP8 overexpression inhibits VAMP2-mediated insulin granule exocytosis. ***Paper II***

Paper III

PITPNA is a member of the phosphatidylinositol transfer protein family, known for facilitating phospholipid transport between cellular membranes and stimulating phosphatidylinositol 4-kinase activity. While PITPNA's role has been documented in various cell types, its specific function in pancreatic β-cells has remained largely unexplored. This study aims to provide a comprehensive understanding of PITPNA's role in β-cell physiology and its relevance to diabetes. By employing a multifaceted approach that combines studies in mouse models with analyses of human islets, we seek to unravel the intricate relationship between PITPNA and β-cell function.

PITPNA regulates insulin secretion and β-cell function

The researchers found that PITPNA plays a crucial role in regulating insulin secretion and β-cell function. They demonstrated that PITPNA is required for efficient insulin granule maturation, docking, and secretion in pancreatic β-cells. Conditional deletion of PITPNA in mouse β-cells led to impaired glucose-stimulated insulin secretion, reduced β-cell mass, and hyper-glycemia. In human islets, knockdown of PITPNA resulted in decreased insulin secretion and granule docking, while overexpression enhanced these processes.

PITPNA mediates PtdIns-4-P synthesis and ER stress responses

The study showed that PITPNA is essential for maintaining proper levels of phosphatidylinositol-4-phosphate (PtdIns-4-P) in the trans-Golgi network of β-cells. Inhibition of PITPNA in human islets disrupted PtdIns-4-P localization and synthesis. Loss of PITPNA also induced ER stress, increased proinsulin accumulation, and altered

mitochondrial morphology and function in β-cells. These defects were associated with increased expression of ER stress markers like CHOP and BiP/GRP78.

Restoring PITPNA expression rescues β-cell function in T2D

Importantly, the researchers found that PITPNA expression was significantly reduced in islets from human subjects with T2D compared to non-diabetic controls. Remarkably, restoring PITPNA expression in isolated islets from type 2 diabetic donors rescued glucose-stimulated insulin secretion, insulin granule maturation and docking, and alleviated ER stress. This suggests that enhancing PITPNA activity could potentially reverse β-cell dysfunction in T2D.

Paper IV

Our study reveals fundamental alterations in insulin granule exocytosis dynamics in T2D, particularly concerning the relationship between ATP and peptide release. By implementing the GRAB_ATP biosensor[157], especially the ATP1.0-L variant with enhanced temporal resolution, we have investigated into the complex fusion dynamics of β-cell secretory function.

Temporal sequence of cargo release

Using high-resolution TIRF microscopy combined with the ATP1.0-L biosensor and NPY-tdmOrange2 as fusion pore indicators, we established the temporal sequence of cargo release during single exocytotic events. ATP release consistently preceded detectable pH changes in the granule lumen, occurring within milliseconds of fusion pore opening, followed by peptide release. This sequential release pattern suggests a regulated mechanism where small signaling molecules (ATP) are released before larger hormone cargo (insulin), potentially allowing for local signaling within the islet microenvironment before systemic hormone release. The balance of release phenotype between ATP and insulin release from non-diabetic β-cells show predominant co-release events (both ATP and NPY released, 59%), around 5 times more than partial release events (ATP release with NPY remained trapped, 13%), ensuring efficient hormone delivery.

Domain-specific exocytosis patterns

Our investigation of extracellular matrix effects revealed that surface composition fundamentally influences β-cell secretory function. Culture on substrate coated with ECM components enhanced exocytosis, with fibronectin coating

showing particularly robust effects. Notably, we observed twice the number of exocytotic events on fibronectin-coated versus E-cadherin surfaces in healthy donors, indicating clear domain preferences for secretion. Fusion pore behaviors differed between domains: E-cadherin surfaces showed shorter fusion pore durations (E-cad: 0.97 ± 0.05 seconds) compared to fibronectin surfaces (FN: 1.31 ± 0.06 seconds). This domain-specific pattern of exocytosis suggests a complex structural basis for directed hormone release, potentially optimizing the balance between local and systemic signaling within the islet microenvironment (Figure 9).

Pathological fusion pore dynamics change in diabetes

Our findings demonstrate significant alterations in the exocytotic release patterns of pre-diabetic and type 2 diabetic β-cells compared to healthy controls. Specifically, we observed a shift in release frequency, where diseased β-cells show reduced co-release but increased partial release events, indicating compromised full fusion capabilities. This impairment is further evidenced by significantly reduced fusion pore open durations in both pre-diabetic and T2D β-cells. Importantly, we found that healthy β-cells exhibit domain-specific granule exocytosis, with higher frequency of exocytotic events on fibronectin-coated substrates compared to E-cadherin-coated surfaces. However, this spatial preference is abolished in both pre-diabetic and T2D conditions, suggesting a change of the spatial organization of exocytosis in the disease state.

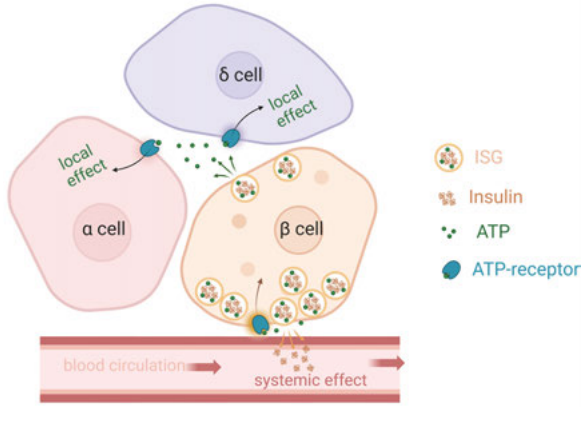

Figure 9. Domain-specific secretory mechanisms in pancreatic β-cells. Illustration of the hypothesis that β-cells exhibit polarized exocytosis based on subcellular location: hormones (e.g., insulin) are preferentially released towards capillaries for systemic circulation, while paracrine signaling molecules (e.g., ATP) are released towards neighbouring cells for local intra-islet communication. This spatial organization of release mechanisms ensures efficient hormone distribution systemically while maintaining local cell-cell signaling within the islet. ***Paper IV*** *(Created in https://BioRender.com)*

Conclusion

Our studies reveal that the molecular machinery governing insulin secretion operates through regulatory pathways involving Munc18 isoforms, PITPNA, VAMP8, and fusion pore dynamics. These components demonstrate remarkable functional specificity in controlling the secretory process. The Munc18 isoforms show distinct but overlapping functions, with Munc18-1 being critical for granule priming while both Munc18-1 and -2 support docking through their interaction with syntax-in. We established VAMP8 as an endosomal SNARE in β-cells. Notably, we also found that it acts as an inhibitory regulator of insulin granule exocytosis through competition with VAMP2 for SNARE complex formation, contrary to a previous report about its role in newcomer exocytosis. Further, the studies reveal dysregulation of the secretory machinery in T2D. The reduction in PITPNA expression coincides with altered fusion pore dynamics, suggesting a coordinated disruption of the secretory machinery. Particularly significant is the shift toward partial fusion events that release only small transmitter molecules. This suggests that T2D pathology involves not just reduced insulin secretion but substantial changes in the mode of exocytosis. The loss of domain-specific exocytosis patterns in diabetic β-cells points to cell polarity as an important factor for insulin granule release. Some of these findings may have therapeutic implications. For example, expression of PITPNA restored insulin secretion in T2D islets, suggesting the protein as a promising therapeutic target. Additionally, the observation that fusion pore dynamics and content release are modulated by cAMP signaling may have implications for the clinical use and development of GLP1-receptor agonists. Collectively, these studies in this thesis advance our understanding of β-cell dysfunction in T2D by revealing how disruption of multiple components of the secretory machinery contributes to disease progression, while also identifying promising new therapeutic targets.

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论文摘要

2 型糖尿病（T2D）的主要病理特征是胰岛 β 细胞功能进行性衰退和胰岛素分泌障碍，然而其具体分子机制仍未完全阐明。在胰腺 β 细胞中，胰岛素通过精密的胞吐作用机制分泌：含胰岛素的分泌颗粒首先与质膜对接，继而招募 SNARE 依赖性胞吐机制相关蛋白进行启动，最终通过膜融合将胰岛素释放至细胞外空间。本研究旨在深入探讨在生理和病理状态下调控胰岛素颗粒动态转运和胞吐作用的分子机制。

通过系统分析分泌机制的关键组分，我们揭示了 SNARE 结合蛋白 Munc18 不同亚型在胞吐过程中的差异性功能。研究发现，尽管 Munc18 的两种亚型均能促进颗粒对接，但 Munc18-1 在胞吐过程中发挥着不可替代的作用。在分子水平上，这两种亚型虽然都能与 syntaxin 结合，但它们被募集到颗粒释放位点的效率存在显著差异。针对 v-SNARE 家族成员 VAMP8 的深入研究表明，该蛋白主要定位于内体腔室而非胰岛素颗粒中，并作为胰岛素分泌的负调节因子发挥作用。我们推测，其抑制效应可能通过与 VAMP2 在释放位点的竞争机制实现，这一发现为理解 β 细胞功能调控提供了新的视角。

在此基础上，我们进一步识别出磷脂酰肌醇转运蛋白 α （PITPNA）是调控胰岛素颗粒成熟的关键分子。研究证实，在人源胰岛中调节 PITPNA 表达水平能够直接影响胰岛素的胞吐过程：降低 PITPNA 表达抑制胰岛素分泌，而提高其表达则增强分泌功能。值得注意的是，在 T2D 患者胰岛中恢复 PITPNA 表达能够逆转糖尿病相关的分泌功能障碍，这一发现提示 PITPNA 表达下调可能是导致 β 细胞功能衰竭的重要因素之一。

最后，借助新型 ATP 生物传感器，我们首次观察到胰岛素颗粒存在两种不同的融合模式：完全融合（同时释放多肽和小分子物质）和部分融合（选择性释放神经递质）。研究发现，这种差异性释放过程受细胞极性调控，且在 T2D 病程中出现显著紊乱。综上所述，本研究深化了对胰岛素颗粒转运和释放分子机制的认知，揭示了 T2D 发病过程中多个潜在的调控靶点。

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