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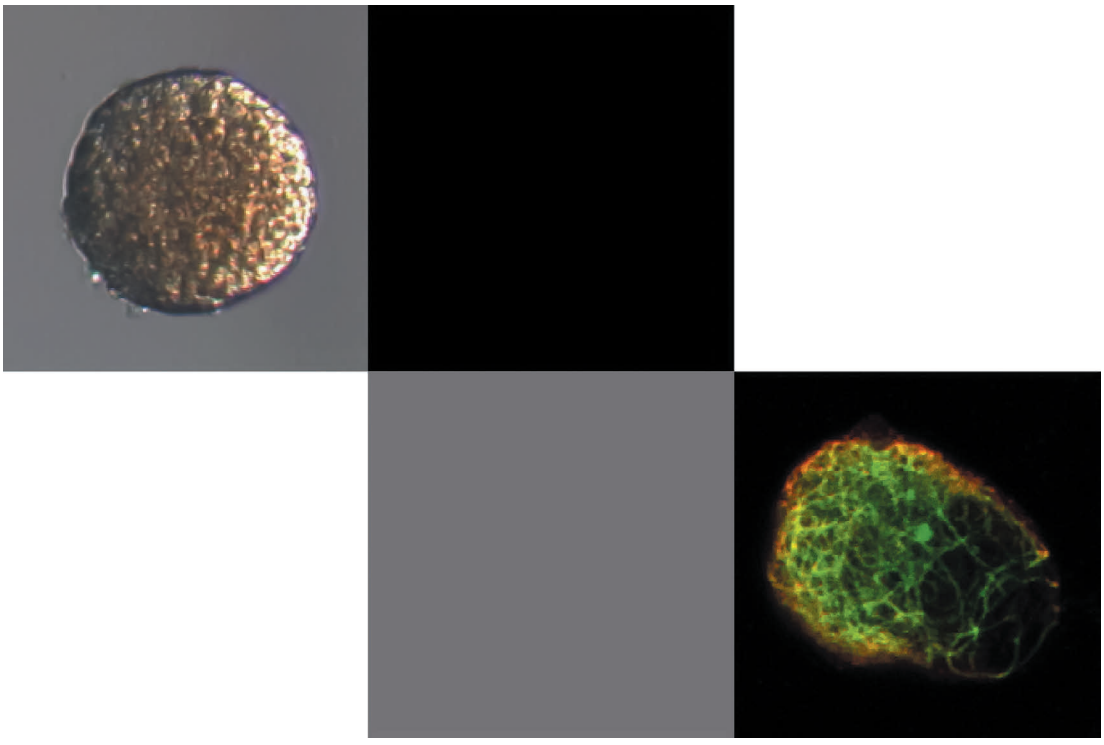
Keratins in the endocrine pancreas

- Novel regulators of cellular processes in β-cells



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To my wonderful family and friends.

"Never, never, never give up."

Winston Churchill

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ABSTRACT

Keratins (K) are the intermediate filament (IFs) proteins of epithelial cells and they are involved in multifaceted cellular functions, such as stress protection, organelle/protein-targeting, cell signaling and epithelial polarity. Keratin mutations have been linked to the cause of, or susceptibility to, several diseases, such as skin and liver diseases. Type 1 (T1D) and Type 2 (T2D) diabetes are dramatically increasing throughout the world. The mechanisms of T1D and T2D development are not fully understood, but include defective insulin production in β-cells. Still there is no cure for diabetes. However, T1D and T2D can be managed by insulin injections or insulin enhancing drugs. The roles of keratins in insulin-producing pancreatic β-cells and diabetes susceptibility have not been thoroughly investigated, and are the aim of the thesis. The hypothesis is that keratins are involved in the regulation of β-cell functions, such as insulin production.

In this thesis, the role of keratins in the endocrine pancreas and blood glucose regulation was investigated using biological model systems: Keratin 8 wild-type ($K8^{+/+}$), heterozygote ($K8^{+/-}$) and knockout ($K8^{-/-}$) mice. Streptozotocin was used to chemically induce T1D in $K8^{+/+}$, $K8^{+/-}$ and $K8^{-/-}$ mice, and a high-fat diet (HFD) was used as a model for T2D in $K8^{+/+}$ and $K8^{+/-}$ mice.

Characterization of keratin profiles in β-cells showed that K8 and K18 were the main keratin pair in $K8^{+/+}$ and $K8^{+/-}$ islets. Low levels of K7 were expressed in both $K8^{+/+}$ and $K8^{+/-}$ islets, while remnant K7 and K18 were found in $K8^{-/-}$ islets. $K8^{-/-}$ mice showed a decrease in fasting blood glucose levels, increased glucose tolerance and insulin sensitivity as well as impaired glucose-stimulated insulin secretion, compared to $K8^{+/+}$ mice. Complete K8 deficiency also led to fragmentation of the mitochondrial network in β-cells, which is connected to a decrease in both the mitochondrial fusion protein mitofusin 2 and the cytolinker trichoplein. Mitochondrial cytochrome c and electron transport chain complexes I and IV were decreased in $K8^{-/-}$ β-cells, which resulted in decreased mitochondrial membrane potential and ATP-production. Irregular and smaller insulin dense cores in $K8^{-/-}$ β-cells correlates with less insulin in $K8^{-/-}$ islets and β-cells. $K8^{-/-}$ mice showed an initial resistance to acute high-dose STZ-treatment, but not to chronic low-dose STZ-damage. The initial resistance could be due to mislocalization of the glucose transporter 2 (GLUT2) in $K8^{-/-}$ mice, since STZ uses GLUT2 to enter the cell. Interestingly, the partially K8 deficient $K8^{+/-}$ mice were more susceptible to low-dose STZ-induced T1D, compared to $K8^{+/+}$ mice. However, $K8^{+/-}$ mice showed no difference in blood glucose regulation, nor major difference in susceptibility to HFD, compared to $K8^{+/+}$ mice.

In conclusion, K8 and K18 constitute the main keratin pair in $K8^{+/+}$ and $K8^{+/-}$ islets. Keratins are maintaining normal insulin levels by involvement in β-cell mitochondrial ATP production and insulin vesicle morphology. On systemic level, keratins in β-cells regulate basal blood glucose levels, most likely in combination with insulin sensitive tissues, such as liver. In addition, keratins are crucial for β-cell stress protection against chemically induced T1D in mice. These novel findings on insulin production and cell stress protection in β-cells, shed light on the potential role of keratins in diabetes susceptibility and progression.

SWEDISH ABSTRACT/SVENSKT ABSTRAKT

Keratiner (K) är epitelcellernas intermediärfilament och de är involverade i många cellulära funktioner så som stresskydd, målstyrning av proteiner/organeller, cellsignalering och epitelcellernas polaritet. Keratinmutationer har förknippats med flera epitelsjukdomar, bland annat i huden och levern. Förekomsten av typ 1 och typ 2 diabetes (T1D; T2D) ökar kraftigt i värden, medan mekanismerna bakom diabetes, där β-cellernas insulinproduktion ofta är störd, inte är fullt kända. T1D och T2D är livslånga sjukdomar, men patienterna kan behandlas med insulininjektioner och insulinökande mediciner. Keratinernas roll i de insulinproducerande β-cellerna och för diabetesuppkomsten har inte tidigare studerats noggrant och är målsättningen för denna avhandling. Hypotesen är att keratiner är involverade i regleringen av β-cellernas funktioner, inklusive insulinproduktionen.

I den här avhandlingen undersöktes keratinernas roll i den endokrina bukspottkörteln och i regleringen av blodsockernivån med hjälp av biologiska modellsystem: K8-vildtyps- ($K8^{+/+}$), K8-heterozygot- ($K8^{+/-}$) och K8-knockout- ($K8^{-/-}$) transgena möss. Streptozotocin (STZ) användes för att kemiskt inducera T1D hos $K8^{+/+}$-, $K8^{+/-}$- och $K8^{-/-}$-möss, och en högfett-diet (eng. high-fat diet, HFD) användes som modell för T2D hos $K8^{+/+}$- och $K8^{+/-}$-möss.

Karakterisering av keratinprofiler visade att K8 och K18 är det främsta keratinparet i β-celler. Små mängder K7 förekom i både $K8^{+/+}$- och $K8^{+/-}$-öar, medan det fanns mycket små mängder K7 och K18 i $K8^{-/-}$-öar. $K8^{-/-}$-möss uppvisade lägre blodsockernivåer efter fasta, men hade ökad glukostolerans, insulinkänslighet och försämrad glukosstimulerad insulinutsöndring (GSIS), jämfört med $K8^{+/+}$-möss. Fullständig K8-brist ledde också till ett fragmenterat mitokondriellt nätverk i β-cellerna, samt till minskade proteinmängder av både det mitokondriella fusionsproteinet mitofusin 2 (MFN2) och det keratinbindande proteinet, trichoplein (TCHP). Mitokondriellt cytokrom c och elektrontransportkedjans komplex I och IV var minskade på proteinnivå i $K8^{-/-}$-β-celler, vilket resulterade i ett minskat mitokondriellt membranpotential och lägre produktion av ATP. Oregelbundna och mindre insulinfyllda insulinvesikelkärnor i $K8^{-/-}$-β-celler, ledde till mindre insulin i både $K8^{-/-}$-öar och β-celler. $K8^{-/-}$-möss uppvisade till en början ett svagt motstånd mot akut STZ-behandling hos $K8^{-/-}$-möss, men inte mot kronisk STZ-skada. Det initiala motståndet kan tänkas uppstå av misslokalisering av glukostransportör 2 (GLUT2) i $K8^{-/-}$-möss, eftersom STZ transporteras in i cellen via GLUT2. $K8^{+/-}$-möss var känsligare för kronisk STZ-inducerad T1D, jämfört med $K8^{+/+}$-möss. Dock uppvisade $K8^{+/-}$-möss ingen skillnad i blodglukosreglering, jämfört med $K8^{+/+}$-möss. $K8^{+/-}$-möss uppvisade inte heller någon kraftig förändring i känslighet för HFD jämfört med $K8^{+/+}$-möss.

Sammanfattningsvis är K8 och K18 det huvudsakliga keratinparet i $K8^{+/+}$- och $K8^{+/-}$-öar. Keratiner upprätthåller normal insulinnivå genom att reglera β-cellernas mitokondriella hälsa och ATP-produktion, samt insulinvesiklernas morfologi. På den systemiska nivån reglerar β-cellernas keratiner den basala blodglukosnivån, troligtvis tillsammans med insulinkänsliga vävnader, så som levern. Dessutom är keratiner viktiga för β-cellernas stresskydd mot kemiskt inducerad T1D hos möss. Dessa nya mekanismer för insulinproduktion och cellstresskydd i β-celler belyser en potentiell roll för keratiner vid uppkomsten och utvecklandet av diabetes.

LIST OF ORIGINAL PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following original publications and manuscript. The publications have been reprinted with permission from the copyright holders.

Study I. C.M. Alam, J.S.G. Silvander, E.N. Daniel, G.-Z. Tao, S.M. Kvarnström, P. Alam, M.B. Omary, A. Hänninen, D.M. Toivola (2013). Keratin 8 regulates β-cell stress responses and normoglycaemia. *Journal of Cell Science*, 126(24):5635-5644.

Study II. C.M. Alam, J.S.G. Silvander[#], T.O. Helenius[#], D.M. Toivola. Decreased levels of keratin 8 sensitize mice to streptozotocin-induced diabetes. Manuscript.

Study III. J.S.G. Silvander, S.M. Kvarnström, A. Kumari-Ilieva, A. Shrestha, C.M. Alam, D.M. Toivola (2017). Keratins regulate β-cell mitochondrial morphology, motility, and homeostasis. *FASEB Journal*, 31(10):4578-4587.

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PUBLICATION AND BOOK CHAPTER NOT INCLUDED IN THESIS

M.N. Asghar, J.S.G. Silvander, T.O. Helenius, I.A. Lähdeniemi, C. ALAM, L.E. Fortelius, R.O. Holmsten, D.M. Toivola (2015). The amount of keratins in colonic epithelial cells matter for stress protection. *PLoS One*. 10(5):e0127436.

P. Strnad, N. Guldiken, T.O. Helenius, J.O. Misiorek, J.H. Nyström, I.A.K. Lähdeniemi, J.S.G. Silvander, D. Kuscuoglu, D.M. Toivola (2015). Simple Epithelial Keratins, *Methods in Enzymology*, 568:351-388.

ABBREVIATIONS

ADT	Adenosin diphosphate
ATP	Adenosin triphosphate
EBS	Epidermolysis bullosa simplex
ER	Endoplasmic reticulum
GIP	Gastric inhibitory polypeptide
GLP	Glucagon-like peptide
GLUT	Glucose transporter
GSIS	Glucose stimulated insulin secretion
HCl	Hydrochloric acid
HFD	High-fat diet
HRP	Horseradish peroxidase
HSP	Heat shock protein
IAPP	Islet amyloid polypeptide
IF	Intermediate filament
IR	Insulin receptor
K	Keratin
$K8^{+/+}$	Keratin 8 wild type
$K8^{-/-}$	Keratin 8 heterozygote
$K8^{-/-}$	Keratin 8 knock out
MCU	Major calcium uniporter
MF	Microfilament
MFN	Mitofusin
MHC	Major histocompability complex
MIN6	Murine insulinoma cells
MSH	Mannitol-Sucrose-Hepes buffer

MT	Microtubules
Δψm	Mitochondrial membrane potential
mKRBH	Modified Krebs Ringer HEPES buffer
NF	Neurofilament
NOD	Non-obese diabetic
OPA1	Optic atrophy type 1
OXPHOS	Oxidative phosphorylation
PDX	Pancreatic and duodenal homeobox
PP	Pancreatic polypeptide
PTMs	Post-translational modifications
REG	Regenerative islet-derived
SEK	Simple epithelial keratins
SST	Somatostatin
STZ	Streptozotocin
TCHP	Trichoplein
T1D	Type 1diabetes
T2D	Type 2 diabetes
TEM	Transmission electron microscope
TLR	Toll-like receptor
TOM	Translocase of outer membrane
ULF	Unit-length filament
WB	Western blot

INTRODUCTION

The islets of Langerhans play an important role in maintaining human systemic blood glucose levels. Defective blood glucose regulation leads to severe complications, such as hypoglycemia and hyperglycemia that over the long run can be fatal for the patients. Over the last decades metabolic diseases, including diabetes, have spiked dramatically mostly due to sedentary lifestyle and over nutrition. Especially obesity is a known cause of metabolic diseases. The most common metabolic disease is diabetes, which is classified into type 1 (T1D) and type 2 (T2D). Both T1D and T2D involve faulty blood glucose regulation due to either loss of the insulin producing β-cells (T1D) or either insufficient insulin production or response (T2D). Although, there has been extensive research in the diabetes field, the full mechanisms of diabetes development are not known. Patients with diabetes can administer insulin or drugs that increase the effect of insulin in order to maintain normal blood glucose level. Accordingly, new knowledge about β-cell function and diabetes development is needed.

Keratin intermediate filaments are known to provide cellular stress protection and are involved in organelle management and cell signaling. Keratin mutations cause or predispose to several diseases, such as liver and skin diseases. Thus, keratins might be novel regulators of β-cell homeostasis and diabetes susceptibility. However, the roles of keratins in the endocrine pancreas have not been investigated. Thus, a better understanding of keratin biology could result in additional knowledge about β-cell functions and diabetes development.

REVIEW OF THE LITERATURE

1 Pancreas

The human pancreas consists of a head, body and tail region, and it is attached to the duodenum and spleen (Fig. 1)(In't Veld and Marichal, 2010) and located in close proximity to the digestive system. In contrast, the murine pancreas is more widely spread across the digestive system (In't Veld and Marichal, 2010). The pancreas consists of exocrine and endocrine cells, with the exocrine pancreas secreting essential digestive enzymes (Pandol, 2010), such as amylase, lipases and proteases. These enzymes are secreted by acinar cells and play a key role in digestion of the chyme (Case, 1978; Whitcomb and Lowe, 2007). Amylases digest starch and glycogen, lipases digest lipids, and proteases digest proteins (Whitcomb and Lowe, 2007). The acinar cells secrete their enzymes to the surrounding pancreatic ducts.

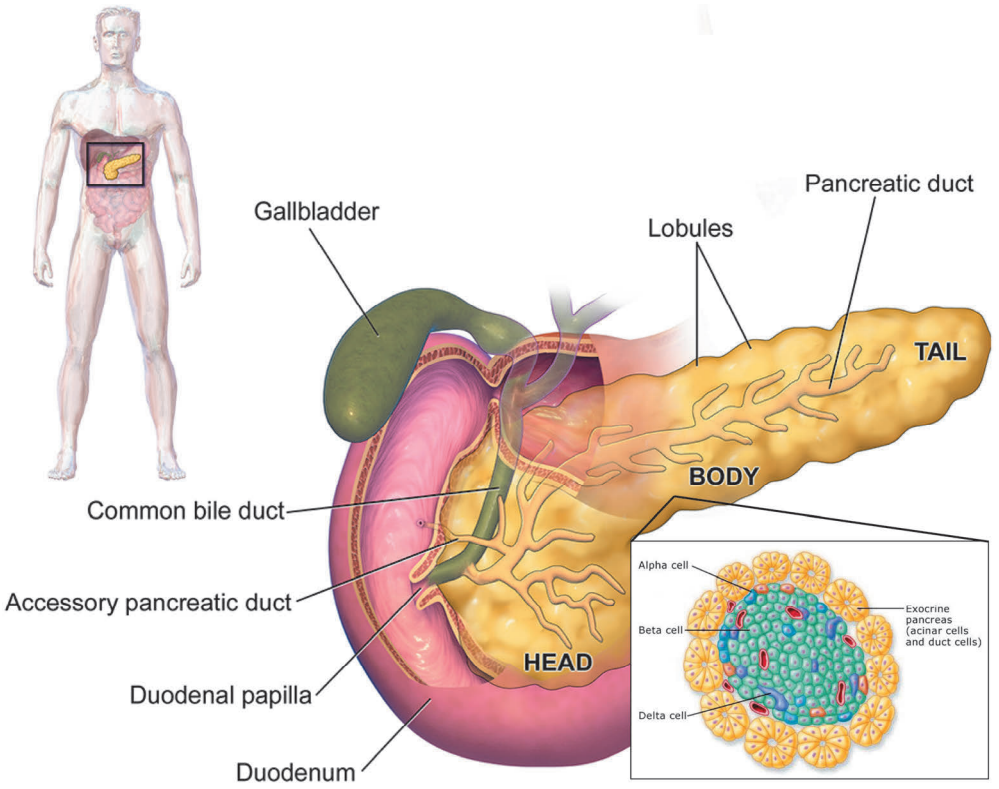

Figure 1. Anatomy and localization of the pancreas in the human body. *The pancreas is situated in close proximity to the digestive system and is divided into three regions: head, body and tail. Insert shows islet of Langerhans and the surrounding exocrine tissue [modified from blaussen.com (2014)].*

The endocrine part of the pancreas is embedded in the exocrine pancreas and constitutes only approximately 1-2% of the total pancreatic mass (In't Veld and Marichal, 2010). The endocrine pancreas is the focus of the thesis and is further discussed below.

1.1 Endocrine pancreas and the Islet of Langerhans

The islets of Langerhans are spherical clusters of endocrine secreting cells (Fig. 1). The primary function of these islets is to regulate blood glucose levels in order to maintain blood glucose homeostasis in the body. The islets of Langerhans were first described in 1869 by the German biologist Paul Langerhans. However, the name islet of Langerhans was introduced by the French histologist Edouard Laguesse in 1893 to honor Paul Langerhans (Navarro, 2014). In the following chapters, the properties and function of the islets of Langerhans will be discussed.

1.1.1 Cell types in the Islets of Langerhans

Islets of Langerhans consist of five types of endocrine secreting cells: α-cells, β-cells, δ-cells, pancreatic polypeptide (PP)-cells and ε-cells. ε-cells are the most recently discovered (Brissova et al., 2005; Wierup et al., 2002). The distribution of the endocrine secreting cells differs between human and mouse islets. Human islets have a randomized organization of cells, in contrast to mouse islets, which have a large core of β-cells surrounded with a mantle of the other cell types (Brissova et al., 2005; Wieczorek et al., 1998).

Table 1. Cell types in the islet of Langerhans and their main secreted hormones. *The Islet of Langerhans consists mainly of insulin-producing β-cells, but also α-cells, δ-cells, PP-cells and ε-cells (In't Veld and Marichal, 2010). The ratio of cell types can vary greatly between individual islets and islet location in the pancreas (Brissova et al., 2005).*

Cell type	Volume percent (%) of total islet mass	Main peptide hormone
α-cells	10-30	Glucagon
β-cells	70-80	Insulin
δ-cells	5-10	Somatostatin
PP-cells	1-2	Pancreatic polypeptide
ε-cells	1	Ghrelin

β-cells are the most prominent cell types in both human and mouse islets (up to 80%; Table 1)(In't Veld and Marichal, 2010). Insulin is the main secretory product produced, stored and secreted by β-cells. However, β-cells also secrete a small

amount of islet amyloid polypeptide (IAPP) (Westermark et al., 1990). The function of insulin is to stimulate glucose uptake by organs (e.g. liver, skeletal muscle and adipose tissue) after the postprandial rise of blood glucose levels. IAPP is co-secreted with insulin and involved in glycemic control. IAPP functions by stalling the gastric enzymes to avoid sudden spikes of high blood glucose levels (In't Veld and Marichal, 2010). Due to their central role in the endocrine pancreas, β-cells will be discussed in more detail in this thesis.

α-cells make up 10-30% of the total islet cells and the ratio of α-cells can vary across individual islets (In't Veld and Marichal, 2010). The primary function of α-cells is to produce and secrete glucagon. Glucagon is a polypeptide consisting of 29 amino acids and a product of proglucagon cleavage (Parker et al., 2002). Differential proteolytic cleavage of proglucagon results in glucagon-like peptides GLP-1 and GLP-2, which induce insulin secretion in response to elevated glucose levels. Glucagon is secreted during hypoglycemia in order to release glucose to the blood stream (Parker et al., 2002).

δ-cells are the third most common cell type in the islet of Langerhans and they produce the hormone somatostatin (SST) (DiGruccio et al., 2016). SST can inhibit both insulin and glucagon secretion (Hauge-Evans et al., 2009). As neuroendocrine and gastrointestinal cells also secrete SST, it has both a gastrointestinal suppressing effect and a growth hormone inhibiting effect. The regulation of δ-cells and the secretion of SST are not fully understood (DiGruccio et al., 2016). A link between diabetes and an increased number of δ-cells in severe T1D patients has been suggested, but the mechanism is still unknown (Brereton et al., 2015).

PP-cells are very few (2%) in the islet of Langerhans (Brereton et al., 2015). As their name implies, PP-cells secrete pancreatic polypeptide. Although, the function of the PP-cells is unclear, it is known that they can reduce gastric HCl secretion and bowel movement. PP-cells have also been linked to moderate inhibition of insulin and SST secretion (Arimura et al., 1979; Brereton et al., 2015). Interestingly, the PP hormone is also thought to have an anorexic effect; however, the underlying mechanism is still unknown (Batterham et al., 2003; Jesudason et al., 2007). Unlike other endocrine pancreas cells, PP-cells are not sensitive to glucose stimulation. Instead they are stimulated indirectly by the vagus nerve. Stimulation of the vagus nerve induces release of cholecystokinin, gastrin and secretin, which all promote the secretion of PP (Batterham et al., 2003).

The islets of Langerhans have been commonly considered to consist of four main cell types (Andralojc et al., 2009). However, ε-cells have recently been found in islets. These cells are immunohistochemically positive for the hormone ghrelin (Wierup et al., 2002). However, the function of ε-cells in islets is not known. Ghrelin is most

commonly secreted by gastrointestinal cells and is known to regulate appetite by binding to the ghrelin receptors in the brain (Andralojc et al., 2009).

A study showing a completely new islet cell type in humans was recently published (van der Meulen et al., 2017). These cells, named virgin β-cells, resemble mature β-cells, but lack their insulin secreting function. Therefore, these cells could be described as an immature type of β-cells. Whether they will be accepted as a new class of islet cells remains to be determined.

1.1.2 Glucose homeostasis

Regulation of blood glucose is a complex process, which involves several hormones (Table 2) aiming to maintenance of normal blood glucose level (4-6 mmol/L) (Roder et al., 2016). Insulin and glucagon are the main blood glucose-regulating hormones. β-cells secrete insulin when plasma blood glucose levels rise after a meal. This process in known as glucose-stimulated insulin secretion (GSIS), and will be discussed in detail in chapter 1.1.4. However, even though glucose stimulation is the main factor in insulin release, there are also other factors that impact the release of insulin (Roder et al., 2016). GLP-1 and gastric inhibitory polypeptide (GIP) are members of the incretins, which are secreted from α-cells and entero-endocrine L- and K-cells, respectively. Incretins have the ability to stimulate insulin secretion through potentiating Ca^{2+} influx or insulin release in β-cells (Parker et al., 2009; Reimann et al., 2008). Additionally, free long fatty acids can also stimulate insulin secretion through release of Ca^{2+} from intracellular storages (Itoh et al., 2003). The brain is also involved in regulating insulin secretion through insulin receptors (IR) in various brain cells and feedback through nerves (Roder et al., 2016). Circulating insulin molecules in the blood stream bind to IR in skeletal muscle, adipose and liver tissues (Khan and Pessin, 2002). This binding recruits glucose transporter 4 (GLUT4) to the plasma membrane of adipocytes and myocytes, thus promoting the import of glucose into these cells and lowering blood glucose (Saltiel and Kahn, 2001). Insulin also promotes effects on lipogenesis,synthesis of glycogen and proteins, and storage of the produced molecules (Roder et al., 2016).

Glucagon is an antagonist to insulin actions (Roder et al., 2016). If plasma blood glucose levels decrease, glucagon then promotes conversion of glycogen to glucose (glycogenolysis). Also this process occurs mainly in the liver. Glucagon also promotes glyconeogenesis, which further increases the plasma glucose levels (Freychet et al., 1988). Secretion of glucagon can also be increased by adrenaline secretion during stress situations, such as fight or flight response (Roder et al., 2016).

Table 2. Main hormones regulating blood glucose homeostasis. *Insulin and glucagon have antagonistic effects on glucose homeostasis and are the main hormones regulating this process (Roder et al., 2016; Saltiel and Kahn, 2001)*

Hormone	Secreted by	Function
Insulin	β-cells	1) Decreases blood glucose by increasing glucose uptake primarily in skeletal muscle and adipose tissue. 3) Anabolic effects: increases lipogenesis, and synthesis of glycogen and proteins. 4) Promotes storage of macromolecules in muscle, fat and liver tissue 5) Inhibits catabolic processes
Glucagon	α-cells	1) Increases blood glucose by: - Increases hepatic glycogenolysis - Promotes glyconeogenesis - Increases catabolic activity
GLP-1	L-cells	Increases insulin secretion
GIP	K-cells	Increases insulin secretion
Somatostatin	δ-cells	Inhibits insulin and glucagon secretion

1.1.3 Insulin production in β-cells

The *INS* gene encodes the preproinsulin protein, which is then converted into insulin (Fig. 2)(Fu et al., 2013). Preproinsulin has a signal peptide (leader region) in order for it to be imported into the endoplasmic reticulum, ER (Egea et al., 2005). Inside the ER, the leader region is removed by a signal peptidase and proinsulin is generated. The protein is further processed with protein folding and addition of disulfide bonds to the proinsulin peptide (Huang and Arvan, 1995). After protein processing the proinsulin is ready for transfer to the Golgi apparatus, where it is further processed and packed into immature insulin vesicles as proinsulin (Fu et al., 2013). The immature insulin vesicles are coated with clathrin. The peptide now consists of three parts: A-, B- and C-peptide (Fig. 2). The maturation process of insulin vesicles involves (i) acidification of the vesicle, (ii) cleavage and removal of the c-peptide (by PC1/2 and carboxypeptidase) and (iii) removal of the clathrin of the clathrin-coated vesicles (Orci et al., 1986; Orci et al., 1985). Inside the insulin vesicle, the insulin molecules are stored as hexamers with a Zn^{2+} in the center (Dunn, 2005). Because of the low solubility of the formed insulin hexamers, insulin vesicles have a dense core of crystalized insulin hexamers.

Insulin synthesis and secretion are regulated by a variety of factors; with glucose signaling sensed by β-cells being the most critical one (Poitout et al., 2006). Another important transcription factor in insulin regulation and secretion is pancreatic and

duodenal homeobox-1 (PDX-1), which is also necessary for β-cell development (Ohlsson et al., 1993).

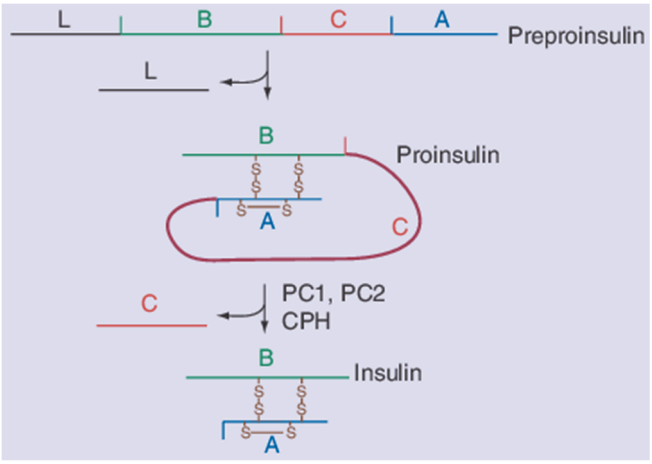

Figure 2. Preprocessing of the insulin molecule. *Insulin is translated as preproinsulin, which is cleaved to proinsulin inside the ER. Inside the Golgi network, vesicles containing proinsulin are considered as immature insulin vesicles. Removal of the C-peptide in proinsulin by PC1/2 and CPH results in insulin in mature insulin vesicles. L = leader region, A-, B-, C-peptides, S-S = disulfide bonds, PC = proprotein convertase, CPH = carboxypeptidase H.*

1.1.4 Glucose-stimulated insulin secretion (GSIS) in β-cells

GSIS is probably the most important function of β-cells (Orci et al., 1990). GSIS starts when glucose molecules in the blood stream trigger β-cells to produce and secrete insulin back to the blood stream. Glucose uptake by β-cells is mediated by GLUT2 (SLC2A2), a member of solute carrier family 2 (SLC2) glucose transporters (Zhao and Keating, 2007). GLUT2 is one of 14 glucose transporters in the GLUT family, all of which transport glucose through facilitated diffusion. GLUT proteins can be found in β-cells, hepatocytes, striated muscles, adipocytes, neurons, intestinal enteroendocrine and renal cells as the primary glucose transporter. Due to the high capacity and low affinity for glucose, GLUT2 is suitable as glucose sensor in the β-cells, since only high glucose concentrations will result in import (Thorens and Mueckler, 2010). Loss of GLUT2 is linked to decreased GSIS, resulting in hyperglycemia in mice (Orci et al., 1990)

After glucose molecules enter the β-cell facilitated by GLUT2, they are processed through glycolysis and oxidative phosphorylation (OXPHOS), thus, generating intracellular ATP molecules (Fig. 3)(Komatsu et al., 2013). This leads to a change in the cytoplasmic ATP/ADP ratio (Roder et al., 2016). ATP-sensitive K^+-channels close when ATP levels increase, resulting in depolarization of the β-cell membrane and opening of voltage-dependent Ca^{2+}-channels (Roder et al., 2016). Insulin vesicles

that are located in close proximity to the cell membrane will fuse with membrane and release the insulin molecules, due to the influx of Ca^{2+}. This is the rapid first phase (10 minutes) of the insulin secretion and the slower second phase will continue to release insulin for a longer period (2-3 h)(Olofsson et al., 2002). Thus, the insulin secretion process is biphasic and can rapidly react to hyperglycemic episodes. The first readily releasable pool of insulin vesicles, which is located near the membrane, is continuously recruited to the membrane in order to secure vesicles to be ready for secretion (Hao et al., 2005). Meanwhile, the second reserve pool of insulin vesicles needs to be transported to the membrane for release, thus, its slower secretion speed. The important molecules involved in the fusion of insulin vesicles to the cell membranes are SNARE complexes, including SNAP-25, syntaxin-1, Munc18, synaptobrevin 2 and synaptotagmin (Roder et al., 2016). The two forms of SNARE complexes are vesicle (v, synaptobrevin 2 and synaptotagmin) and target (t, SNAP-25 and syntaxin-1). Synaptobrevin 2 in t-SNARE initiates the process by binding to t-SNARE located on the cell membrane. The membrane fusion can be completed when synaptotagmin binds Ca^{2+} (Gut et al., 2001).

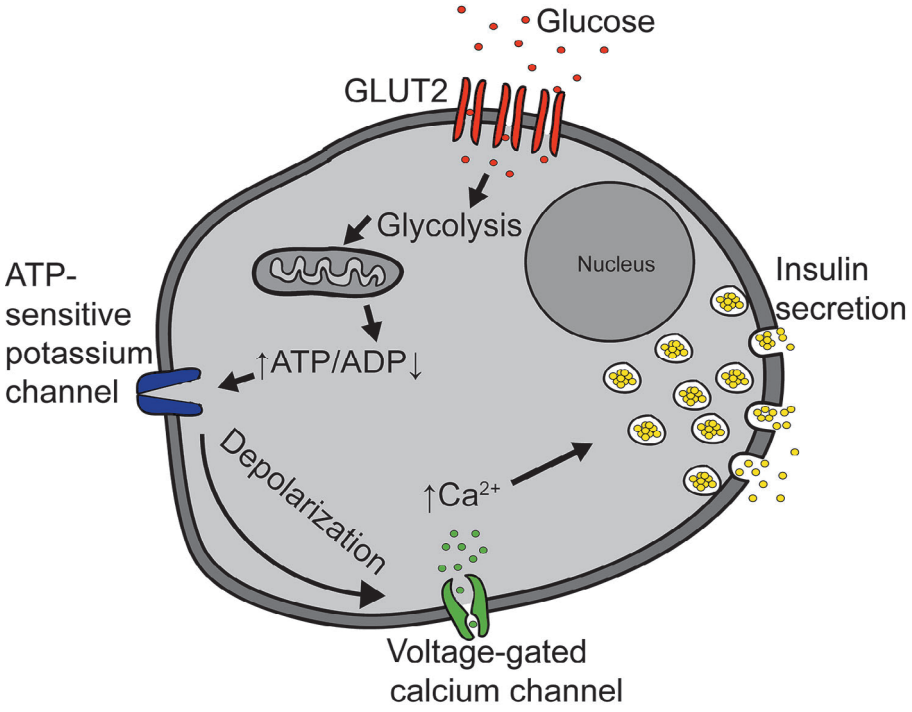

Figure 3. Schematic representation of GSIS. *Extracellular glucose is imported into the β-cell through the GLUT2 transporter. Glucose is processed through glycolysis and oxidative phosphorylation, OXPHOS, to generate an increase in the ATP/ADP ratio, which closes the ATP-sensitive K^+ channel. This causes depolarization of the cell membrane and influx of Ca^{2+} through the voltage-gated calcium channels. Ca^{2+} triggers the release of insulin vesicles.*

1.2 Diabetes

1.2.1 Type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disease that results in the destruction of β-cells (Atkinson et al., 2014). Although it was long thought to be a disease acquired at a young age, it is now known that people of all ages can develop T1D (Harjutsalo et al., 2008; Leslie, 2010). Finland and Sardinia have the highest incidence of T1D, while China, India and Venezuela have the lowest incidence (Patterson et al., 2009). Symptoms of T1D include increased thirst, appetite and urine secretion, along with hyperglycemia (fasting blood glucose value >7 mmol/L or basal blood glucose > 11 mmol/L) (Kilpatrick et al., 2009). Hyperglycemia can be fatal and lead to fatigue, blurred vision and coma (Kilpatrick et al., 2009).

T1D is divided into two subclasses, 1A and 1B (Dabelea et al., 2007). 1A has an autoimmune origin and 1B is classified as the remaining unspecific origins of the disease. The destruction of β-cells occurs when inflammatory cells infiltrate the islet of Langerhans, due to autoantibodies against β-cell associated proteins such as glutamic acid decarboxylase and insulin (Bingley, 2010). Loss of β-cell mass leads to insufficient insulin production, which results in hyperglycemia (Atkinson et al., 2014). The main cause of T1D is considered to be genetic, with the studied mutations in the HLA. However, viruses have also been implicated as cause of T1D. There is no cure for T1D, but the disease can be managed through daily insulin injections.

1.2.2 Type 2 diabetes

Type 2 diabetes (T2D) is a metabolic syndrome, which includes hyperglycemia, insulin resistance and decreased insulin secretion (Das and Elbein, 2006). The symptoms are similar to T1D, including polyuria, increased thirst and hunger. T2D is a major disease throughout the industrialized world (>150 million suffer from the disease) and the incidence is dramatically increasing (Lin and Sun, 2010). An important factor in developing T2D is obesity (as much as 80% of the cases), which leads to lipotoxicity and insufficient insulin secretion, due to loss in β-cell mass by apoptosis (Butler et al., 2003). In addition, insulin resistance is often the main cause of hyperglycemia, since the insulin responsive tissues, including muscles, fat and liver, do not act upon insulin stimulation due to defects in insulin receptor signaling (Bjornholm and Zierath, 2005). Under normal conditions insulin signaling activates intracellular PI3K/Akt/mTOR signaling pathways, however, free fatty acids can inhibit this particular pathway and results in insulin resistance. In turn, insulin resistance results in insufficient uptake of glucose by tissues and increased

circulating free glucose, which is toxic. Mutations, such as in the ABCC8 gene coding for the sulfonylurea receptor 1 in the K-ATP channel, have been linked to predisposition to developing T2D, especially in familial cases (Majithia and Florez, 2009). T2D is a severe disease that can results in long-term complications such as, hearth disease, stroke, blindness and kidney failure (Das and Elbein, 2006)

1.2.3 Models for the study of diabetes

Rodents (mainly mice and rats) have been the most widely used animal models to study diabetes. In this part, three of the most widely used diabetes models will be discussed.

1.2.3.1 Streptozotocin-induced diabetes

Streptozotocin (STZ) is a broad-spectrum antibiotic, produced by the soil bacteria *Streptomyces achromogenes* (Vavra et al., 1959). It is classified as a nitrosourea analogue (Fig. 4) with alkylating properties and was early on found to be toxic to β-cells, resulting in inhibition of insulin secretion (Lenzen, 2008; Rakieten et al., 1963). STZ enters β-cells facilitated by the GLUT2 transporter. Loss of GLUT2 leads to decreased STZ effect (Elsner et al., 2000; Tjalve et al., 1976). However, due to GLUT2-specific uptake of STZ, also other GLUT2 expressing cells such as hepatocytes and renal cells, are affected by STZ toxicity (Lenzen, 2008), which can cause unwanted side effects, such as renal failure and blood in the urine. Inside β-cells STZ acts as a DNA alkylating agent, primarily acting on the oxygen in guanine causing mutations. The cell's energy storages (NAD^+ and ATP) are depleted due to increased poly (ADP-ribose) polymerase (PARP) activity, as they try to repair the DNA damage (Sandler and Swenne, 1983), leading to β-cell death. STZ is also able to methylate proteins, which contributes to the toxicity (Lenzen, 2008).

Due to its toxic effects on β-cells STZ is used as a model for *in vivo* diabetes induction (Graham et al., 2011). Depending on the STZ dose, the disease-modeling effect can differ: Induction of high-dose STZ (single administration) leads to acute β-cell damage by DNA alkylation (Deeds et al., 2011). However, low-dose STZ, administered in multiple doses, leads to immune cell activation and islet infiltration by lymphocytes. STZ is also used in clinical medicine for treatment of islet carcinoma; however, it is not widely used due to severe side effects, such as urinal bleeding and hyperglycemia (Graham et al., 2011).

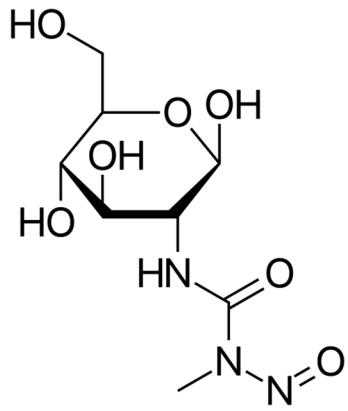

Figure 4. The molecular structure of STZ. *The STZ molecule is an antibiotic molecule with DNA alkylating effects in e.g. β-cells. It is used for inducing chemically induced diabetes in animal models.*

1.2.3.2 The non-obese diabetic mouse model

Non-obese diabetic (NOD) mice were bred for the first time in 1980 in Japan (Makino et al., 1980). These mice develop spontaneous diabetes of autoimmune origin, and polyuria, glycosuria and weight loss symptoms, similar to T1D in humans, (Leiter et al., 1990; Makino et al., 1980). The innate immune cell infiltration of the islet of Langerhans in NOD-mice starts after approximately three weeks, followed by CD4 and CD8 positive T-cells attack after five weeks (Jansen et al., 1994; Miyazaki et al., 1985). T1D is fully developed after 10 weeks (Wicker et al., 1994). Interestingly, it is predominantly female mice that develop the disease (Makino et al., 1980). As with the disease in humans, the main factor causing T1D in NOD-mice, is mutations in the major histo-compability complex (MHC) I/II (Pearson et al., 2016; Singal and Blajchman, 1973). Mutations in MHC I/II lead to faulty antigen presentation (Pearson et al., 2016). Due to the autoimmune properties of NOD-mice, these mice are the most investigated autoimmune animal models (Pearson et al., 2016).

1.2.3.3 High-fat diet

Western diets and unhealthy lifestyles are major causes of T2D and metabolic diseases (Lin and Sun, 2010). Experimental high-fat diets (HFD) involve treatment of animals with high-fat food (around 60% energy from fat), which results in the symptoms typical of T2D, such as weight gain, hyperglycemia, insulin resistance and increased inflammation (Heydemann, 2016). The development of T2D after HFD-treatment is strain dependent in mice (Kobayashi et al., 2014). The onset of HFD-induced T2D can vary from a few days to >10 weeks (Heydemann, 2016). Also, parameters such as weight increase and hyperglycemia tend to plateau after a

prolonged HFD-treatment. Old male mice (>8 months) seem to develop T2D faster on a HFD, compared to young mice (Heydemann, 2016).

2 Cytoskeleton and intermediate filaments

Cells have a rigid network of cyto-protective proteins named the cytoskeleton. It consists of microtubules (MTs), intermediate filaments (IFs) and microfilaments (MFs), which are grouped according to their subunits (Fletcher and Mullins, 2010). MTs are composed of α-, β- and γ-tubulin and are involved in dynamic processes such as intracellular transport and cell division (Howard and Hyman, 2003). MFs (most commonly known as actin filaments) consist of α-, β- and γ-actin subunits and are responsible for cellular movement (Chhabra and Higgs, 2007). IFs include a large family of proteins that have cell- and tissue-dependent expression and development (Herrmann et al., 2007). IFs are involved in stress protection (Toivola et al., 2010) and will be discussed below in detail. In addition to their composition, the members of the cytoskeleton also differ in the filament diameter, with MTs having the largest diameter and MFs the smallest (Table 3) (Fletcher and Mullins, 2010). As the name implies, IFs have an intermediate diameter (Herrmann et al., 2007).

Table 3. The members of the cytoskeleton. *Microtubules, intermediate filaments and microfilament are classified according to their protein subunits and differ in function and properties (Chhabra and Higgs, 2007; Herrmann et al., 2007; Howard and Hyman, 2003; Parry et al., 2007).*

Name	Subunit protein	Filament polarization	Filament diameter
Microtubules	Tubulin	Yes	25 nm
Intermediate filaments	Various	No	8-12 nm
Microfilament	Actin	Yes	6 nm

2.1 Intermediate filaments

IFs were described for the first time in 1968 in chicken embryo muscle cells (Ishikawa et al., 1968). The members of the IF family proteins are classified into six groups, depending on their properties and expression in different cell types (Herrmann et al., 2007). Keratins (K) are divided into type I and II and are the largest IF protein group (Moll et al., 2008). Keratins are the IFs of the epithelial cells. Type III IFs are found predominantly in mesenchymal and endothelial cells and include vimentin and desmin (Toivola et al., 2005). Neurofilaments (NF) are the IFs of neuronal cells and are classified as type IV IFs. Type V IFs, the lamins, are exclusively located in the nucleus, contrary to the other IFs, which are also and

largely found in the cytoplasm (Kim and Coulombe, 2007). Type VI is the newest class of IFs and consists of phakinin and filensin, which are located inside the eye lens (Szeverenyi et al., 2008).

2.1.1 Structure of intermediate filaments

Intermediate filaments are fibrous proteins, which form through the polymerization of monomeric protein subunits (Parry et al., 2007). The monomers are divided into three domains: head (N-terminal), rod and tail (C-terminal) (Fig. 5A). IFs are formed by head-to-head dimers of either hetero- or homologous monomer pairs (Fig. 5B). The connecting rod-domain of the dimer consists of two parallel coil α-helices. The uniform rod-domain is segregated into 1A, 1B, 2A and 2B segments, which are linked by linker-regions L1, L12 and L2 (Fig. 5A). Two dimers join to build a tetramer and two tetramers make an octamer, and so on. The unit-length filament (ULF) is assigned as the IF filament building block and consists of four IF octamers arranged in 3D space (Herrmann et al., 1999; Herrmann et al., 2002) (Fig. 4D). Since IFs lack polarity, many ULFs can be added at a time and from both ends of the filament (Kirmse et al., 2007). The filament formation is a spontaneous process and requires a free filament end and a ULF nearby (Windoffer and Leube, 2004; Woll et al., 2005). Most of the posttranslational modifications in IFs take place in the head (N-terminal)- and tail (C-terminal) regions.

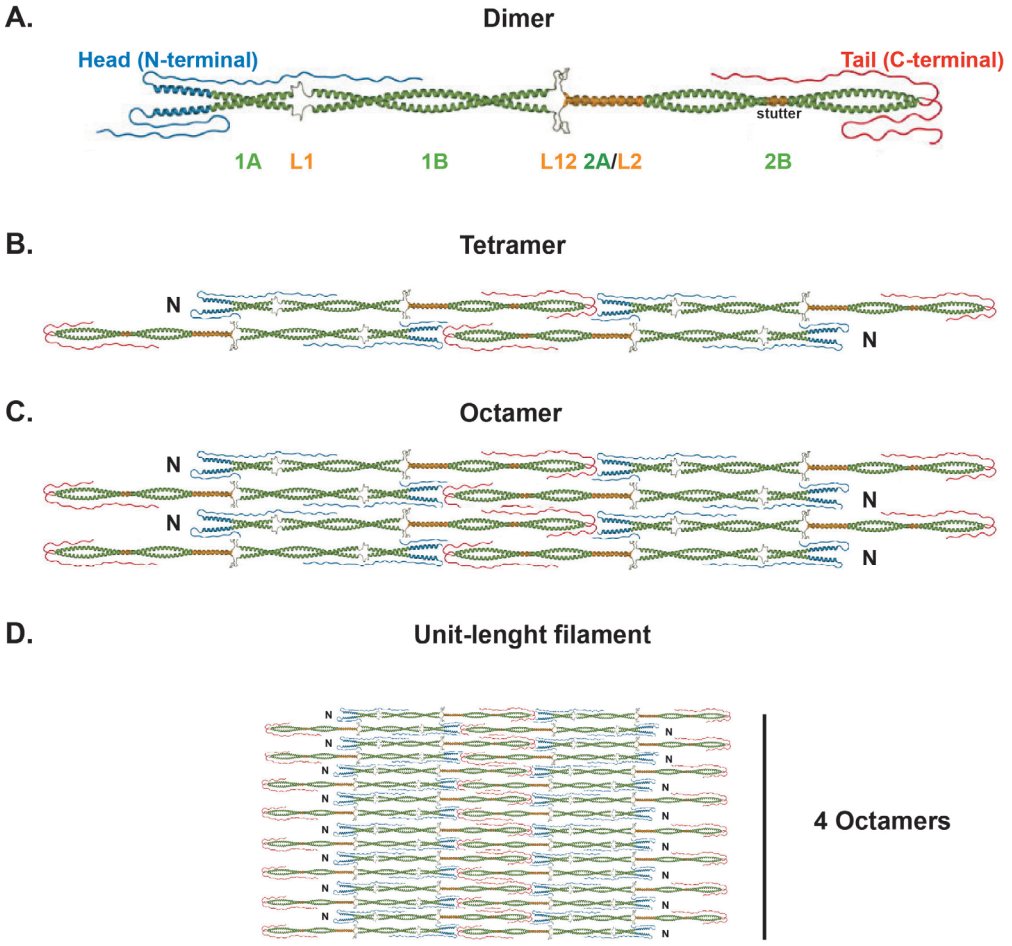

Figure 5. Structure of intermediate filaments illustrated with vimentin. *A. Two IF monomers bind to each other to form a dimer. B. Two dimers are attached in anti-parallel manner to form a tetramer. C. Two tetramers form an octamer. D. The functional IF unit is a unit-length filament, consisting of four octamers forming a rope-like 3D structure (modified from (Parry et al., 2007)).*

2.2 Simple epithelial keratins

The first keratin study was done on sheep wool (hair) keratins in 1952, and in the 1970's it was established that keratins are also present in epithelia (Crick, 1952; Franke et al., 1978). Knowledge of keratin biology and its importance has expanded over the past three decades (Oshima, 2007; Schwarz and Leube, 2016; Toivola et al., 2015).

Keratins are divided into type I and type II IFs and their classification depends on their biochemical properties (Moll et al., 2008). Type I keratins are all acidic proteins and are named K9 through K28 and K31 through K40. Type II keratins, K1 through

K8 and K71 through K86 have neutral or basic biochemical properties. Keratins are transcribed from 54 *KRT* genes located at 17q21.2 (type I keratins) and 12q13.13 (type II keratins) (Schweizer et al., 2006). However, *KRT18* localization is an exception, since it is located on chromosome 12q13.13 (Moll et al., 2008). Keratins are expressed in a tissue-specific manner and can roughly be grouped into hair/nail keratins, stratified keratins and simple epithelial keratins (Franke et al., 1981; Kim and Coulombe, 2007). Accordingly, keratins can be classified as "hard" or "soft" based on their properties (Coulombe and Omary, 2002). This thesis focuses on simple epithelial keratins (SEK), hereafter only named as keratins, which are present in single-layer epithelia including type I keratins K18-K20, K23; and type II keratins K7 and K8 (Omary et al., 2009). All keratins form obligate heterodimers of type I and type II keratins. Thus, single keratin monomers will be marked for degradation if no partner is present in the cytoplasm (Omary et al., 1998). Due to the cell- and tissue-specific expression pattern of keratins, all epithelial cells are considered to have a primary keratin pair and often a secondary pair is present (Moll et al., 2008). Hepatocytes express only K8/K18 and are more sensitive to keratin mutations (Omary et al., 2002; Toivola et al., 2015). In colonic crypt epithelial cells, however, K8/K19 is considered as the primary keratin pair, but also K7, K18, K20 are present at various levels in a cell type-dependent expression pattern (Ku et al., 1999; Zhou et al., 2003). It was recently shown that the newest SEK member, K23, is also present in the colon (Guldiken et al., 2016). Pancreatic ductal cells primarily express K8/K19, but they also express K7 and K18 (Ku et al., 1999). Overall, K7 and K19 have often been shown to be expressed in the duct lining epithelial cell (Moll et al., 2008).

2.2.1 Functions of simple epithelial keratins

Keratin network extends throughout the cytoplasm where it provides excellent cellular mechanical stress protection for cells (Toivola et al., 2010). The keratin network connects to adjacent cells by desmosomes, since keratins can directly bind to the desmosome component desmoplakin (Kouklis et al., 1994). The connection to basally located hemidesmosomes is made by keratin interaction with the cytolinker plectin (Andra et al., 2003). The ability of keratin filaments to link to both desmosomes and hemidesmosomes provides a rigid support for the epithelial cell barrier. Interestingly, absence of keratins in hepatocytes leads to unstable desmosomes, due to a decrease in desmoplakin levels (Loranger et al., 2006). Keratins have been shown to be upregulated during stress situations in both human and mouse, for example in liver, colon, pancreas and kidney (Djudjaj et al., 2016; Guldiken et al., 2015; Helenius et al., 2016; Zhong et al., 2004). Reduced amount of keratins is linked to improper protection of the colon during induced colitis in mice, emphasizing the importance of keratin filaments (Asghar et al., 2015). Keratin filament properties are modified by post-translational modifications (PTMs), such as

phosphorylation, glycosylation, acetylation, sumoylation and prenylation (Snider and Omary, 2014). Phosphorylation is the most common PTM of keratin and occurs at serine/threonine residues at the head and tail domain of keratins. Keratin phosphorylation is often linked to disruption of keratin filaments and increased keratin solubility (Snider and Omary, 2014). As a function of keratin stress protection, keratin phosphorylation increases during stress and keratins are suggested to act as "sponges" for phosphate groups (Ku and Omary, 2006; Toivola et al., 2004b; Toivola et al., 2010).

In addition to stress protection, keratins have many other important functions. For instance, they regulate mitochondrial morphology in hepatocytes, and are also involved in protein targeting of ion-transporters in colon epithelia (Asghar et al., 2016; Tao et al., 2009; Toivola et al., 2004a). Golgi becomes fragmented when the keratin mutant K8R90C is present in cells (Kumemura et al., 2004). In addition, keratins regulate polarity in epithelial cells, which is especially important in absorbing and secreting cells (Ameen et al., 2001; Oriolo et al., 2007). A recent study shows that K8 stabilizes notch1 and increases notch signaling in the colon (Lahdeniemi et al., 2017) leading to activation of notch1 downstream targets with impact on cell fate. Cell size and protein synthesis are linked to K17 and K8 regulation (Galarneau et al., 2007; Kim et al., 2006). This is likely due to the fact that keratins can act as scaffolds for signaling molecules, which is evidenced by the binding of signaling molecules, such as 14-3-3 proteins, Raf-1 kinase and PKC, to keratin filaments (Ku et al., 2004; Omary et al., 2006).

2.2.2 Keratins in human diseases

Keratins have been linked to a large number of diseases called keratinopathies (Szeverenyi et al., 2008; Toivola et al., 2015). The most common disease caused by a skin keratin mutation is Epidermolysis bullosa simplex (EBS; mutation in *KRT5* and *KRT14*) (Bonifas et al., 1991; Coulombe et al., 1991; Lane et al., 1992). Other skin diseases caused by keratin mutations include Epidermolytic hyperkeratosis (*KRT1, KRT10*), Dermatopia pigmentosa reticularis (*KRT14*) and Epidermolytic palmoplantar keratodema (*KRT16*) (Toivola et al., 2015).

Several keratin mutations predispose patients to certain diseases, which are triggered by other stresses (Toivola et al., 2015). The most common *KRT8* mutations, G62C and R341H (Strnad et al., 2010; Treiber et al., 2006), are associated with primary biliary cirrhosis and hepatitis, with G62C also being associated with liver cirrhosis (Ku et al., 2003; Ku et al., 2001; Szeverenyi et al., 2008). There is some conflicting evidence about the involvement of keratin mutations' (*KRT8* and *KRT19*) in the

development of inflammatory bowel diseases (Owens and Lane, 2004; Tao et al., 2007), therefore, further investigation is needed.

Keratins are often used as markers for characterizing tumors, especially those that have metastasized (Omary et al., 2009). The origin of metastasized tumors can be determined based on the specific keratin expression pattern (e.g. colorectal carcinomas express K20 and hepatic carcinoma express K8 and K18). Also circulating keratin fragments can be analyzed from blood samples of patients in order to detect and monitor specific cancers (Omary et al., 2009).

2.3 Cytoskeleton and β-cells in pancreas

Both MFs and MTs and their motor proteins are involved in the transport of insulin vesicles from the Golgi network to the cell membrane. Microtubule-based transport by kinesin-1 is primarily responsible for long-distance transport of insulin vesicles, while actin-based myosin V is involved in the short-distance transport (Park and Loh, 2008; Varadi et al., 2005). The mechanisms of myosin and kinesin transport are further discussed in the mitochondria dynamics chapter (3.2) below. There are two theories on how actin filaments impact the secretion of insulin vesicles. The traditional theory is that F-actin functions as a physical barrier and traps the vesicles in the filament meshwork (Porat-Shliom et al., 2013). Depolarization of F-actin would then lead to increased secretion. The other theory proposes that actin is in globular form during the first phase of insulin secretion, due to its binding to cofilin.(Uenishi et al., 2013). In the second phase actin is polymerized by the WASP-Arp2/3 complex, leading to F-actin network-mediated transport of insulin vesicles.

A few studies have linked keratins to a potential role in β-cells. For example, results from a small study showed that four out of ten patients with T1D, were found to carry keratin autoantibodies (Nakanishi et al., 1993). However, to my knowledge, there has been no follow up on this particular study. Mice bearing mutations (S30A, S31A or S49A) on the main K18 glycosylation sites, which leads to loss of K18 glycosylation are more susceptible to STZ-treatment compared to controls (Ku et al., 2010), implying a protective role of keratin in β-cells. Keratins have also been found upregulated after glucose stimulation *in vivo* (Ahmed and Bergsten, 2005), suggesting that keratins are glucose-responsive.

Both MFs and MTs have been associated to play crucial roles in β-cells, primarily in the transport and insulin vesicles and in insulin secretion (Park and Loh, 2008). However, the role of keratins in β-cells has not been investigated in detail.

3 Mitochondria

The name mitochondria means thread granules in Greek (Lehninger, 1964), since in the microscope this organelle appeared as small thread-like structures. These observations were based on studying microscopic dissections of eukaryotic cells. Mitochondria are unique organelles with their own DNA (mitochondrial DNA, mtDNA)(Anderson et al., 1981). Interestingly, the mtDNA is inherited from the maternal side of the offspring, which consequently reduces the risk of mtDNA mutation (Ingman et al., 2000; Mishra and Chan, 2014). The endosymbiotic theory proposes that mitochondria originate from bacteria that have been incorporated in cells in a symbiotic manner during evolution (Gray et al., 1999). In addition to having their own proteome, mitochondria also have a double membrane and a proton gradient-mediated energy production, similar to proteobacteria (Mishra and Chan, 2014). The mitochondria and their properties are discussed in further detail below.

3.1 Mitochondrial structure and function

The mitochondrion is a double-membrane bound organelle (Fig. 6), where its separating double-membrane plays an important role for mitochondrial function (Karbowski and Youle, 2011). The inter-membrane space, bound by the outer- and inner-membrane of the mitochondria, is crucial for the proton-gradient. The inner space of the mitochondria is called the matrix, where the mtDNA and mitochondrial ribosomes are located. Translation of mitochondrial proteins are executed in the matrix (Ingman et al., 2000). The mitochondrial inner-membrane is folded, and resulting protrusions are called cristae that significantly increases the surface area of the mitochondrial inner-membrane (Frey and Mannella, 2000).

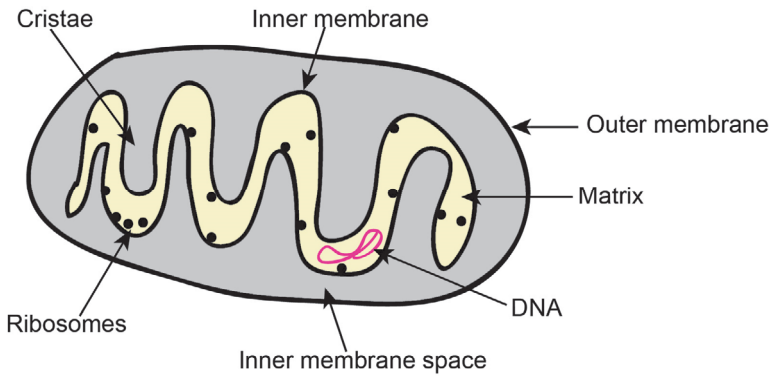

Figure 6. Schematic structure of the mitochondria. *Mitochondria are a double-membrane bound organelle. The inner membrane is folded into cristae, which envelopes the mitochondrial matrix. Mitochondrial ribosomes and DNA are located inside the matrix. Based on Kuhlbrandt, 2015.*

The main function of mitochondria is to support eukaryotic cells with energy in a usable form (ATP) by finalizing the oxidation steps of carbohydrates, lipids and proteins (Ryan and Hoogenraad, 2007). These molecules need to be first catabolized to acetyl-Coenzyme A (CoA) in order to be processed through the Krebs cycle inside the mitochondrial matrix. Carbohydrates are already metabolized in the cytosolic glycolysis, where pyruvate is produced and further converted to acetyl-CoA by pyruvate dehydrogenase. Lipids are converted to acetyl-CoA in the mitochondrial matrix through the β-oxidation reaction (Fromenty and Pessayre, 1995). Proteins can also be used as energy, but it is an energetically unfavorable process (Jois and Sleeman, 2017). First, the amino group (NH_2) from a protein is removed through deamination and keto acids are formed. These keto acids can be converted to acetyl-CoA through gluconeogenesis. The produced acetyl-CoA can then be used in the Krebs cycle as a substrate to produce free energy bound to nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) (Bartlett and Eaton, 2004). NADH is then transported to the electron transport chain (discussed in chapter 3.1.1).

Mitochondria are also important players regulating intracellular calcium levels, since Ca^{2+} can circulate between the cytosol and mitochondria (Baughman et al., 2011). Ca^{2+} import into mitochondria is mediated through the mitochondrial calcium uniporter (MCU) (Baughman et al., 2011). Local Ca^{2+} spikes promote mitochondrial Ca^{2+} influx, which increases Krebs cycle rate and electron transport chain production (Duchen, 1992). Mitochondria and ER function as calcium buffers and storage, since constant high levels of cytosolic Ca^{2+} are unhealthy for the cell (Duchen, 2000).

Cell death and apoptosis can be initiated by the mitochondria. Necrotic cell death involves opening of mitochondrial pores (Hunter and Haworth, 1979). Pore opening results in depletion of mitochondrial membrane potential and ATP synthesis, which cause rapid cell death (Baines et al., 2005). Programmed cell death or apoptosis has two pathways: the intrinsic and the extrinsic pathway (Li et al., 1997). In the intrinsic pathway, mitochondria are stimulated by an apoptotic signal leading to mitochondrial outer membrane pores and cytochrome c release. This stimulates the formation of apoptosome complex and caspase 3 activation. In the extrinsic pathway the cell is stimulated externally with apoptotic signals, leading to caspase 8 activation.

3.1.1 Oxidative phosphorylation

ATP production inside the mitochondria is a process of nutrient oxidation (Mitchell, 1961). This process is named oxidative phosphorylation (OXPHOS). This is a highly favorable process compared to glycolysis, due to its higher yield of ATP molecules. The electron transport chain consists of five large molecular complexes (I-V; table 4) embedded in the mitochondrial inner membrane (IMM).

Table 4. Mitochondrial OXPHOS complexes. *Mitochondria contain five complexes involved in the OXPHOS.*

Complex	Name
I	NADH: ubiquinone oxidoreductase
II	Succinate dehydrogenase
III	Cytochrome bc_1 complex
IV	Cytochrome c oxidase
V	ATP synthase

NADH from the Krebs cycle or glycolysis binds to complex I and donates two electrons (Fig. 7) (Efremov et al., 2010). These electrons are transferred to ubiquinone, which can move inside the IMM. This process generates energy for complex I to move four protons to the IMM, generating a proton gradient. Complex II reduces succinate to fumarate, generating electrons to the ubiquinone (Zhou et al., 2011). However, complex II does not transfer protons over the membrane. Two electrons bound to ubiquinone are released in complex III and transferred to two cytochrome c molecules, while simultaneously two protons are released from the ubiquinone (Crofts, 2004). This causes a reduction of ubiquinone to quinol, generating the transfer of four protons to the IMS. In complex IV cytochrome c releases electrons which, together with O_2, form H_2O molecules in the matrix (Zhu et al., 2010). This process generates transfer of protons from the matrix to the IMS. The built-up proton gradient in the IMS drives the ATP synthase, where ADP and inorganic phosphate produces ATP (Nakamoto et al., 2008). A defective OXPHOS pathway is associated with a decrease in ATP-production and ultimately cell death (Chandra and Singh, 2011).

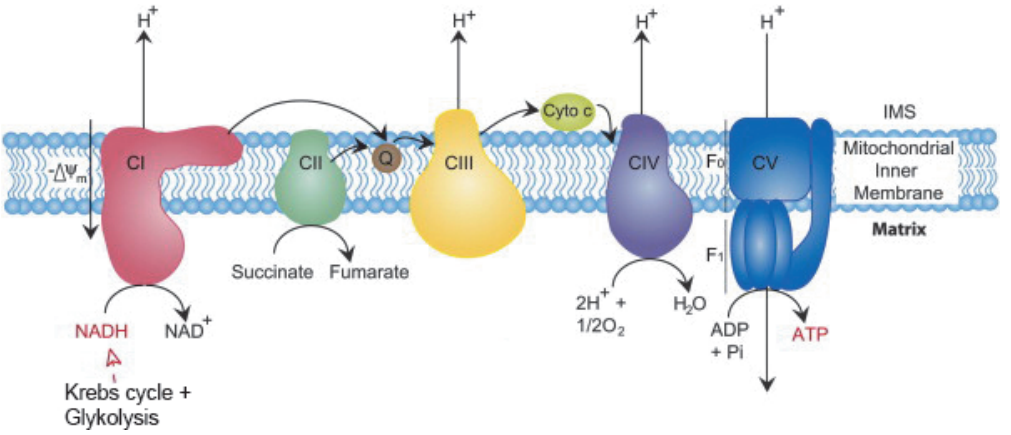

Figure 7. Schematic representation of the OXPHOS process. *OXPHOS reaction produces ATP by ATP synthase, which is driven by a proton gradient. The proton gradient is generated by OXPHOS complexes I-V. Modified from (Osellame et al., 2012).*

3.2 Mitochondrial dynamics

Most of the key mechanisms of mitochondrial dynamics were first discovered in yeast. However, in recent years, studies on mammalian cells have complemented this knowledge (Mishra and Chan, 2014). Mitochondria are highly dynamic organelles which undergo fusion and fission depending on cellular energy demands and to remove defective parts of the mitochondrial network, but also to distribute mitochondria during cell differentiation and division (Fig. 8) (Gomes et al., 2011; Liesa and Shirihai, 2013; Molina et al., 2009; Twig et al., 2008). Furthermore, dynamic fusion and fission processes enable mitochondria to communicate with each other (Mishra and Chan, 2014). Fusion of the mitochondrial network is promoted by low stress situations, increased OXPHOS, decreased availability of nutrients and DNA synthesis (Liesa and Shirihai, 2013; Mitra et al., 2009), whereas, fragmentation of the mitochondrial network occurs during apoptosis, severe cell stress mitosis, excessive nutrients and defective OXPHOS (Hackenbrock, 1966; Molina et al., 2009; Taguchi et al., 2007).

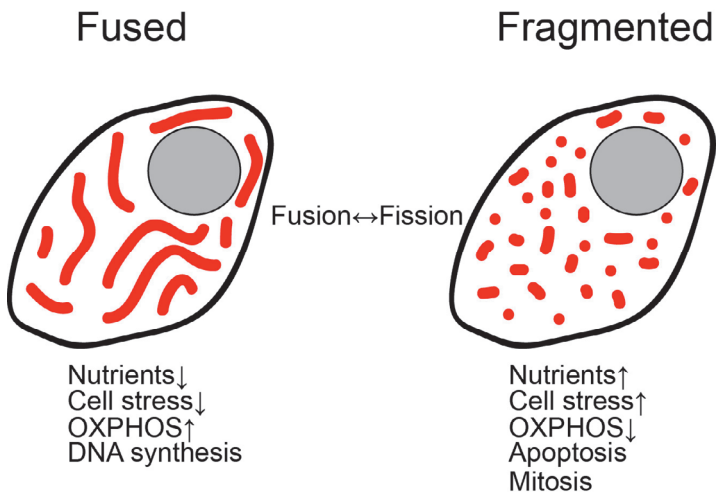

Figure 8. Balance between mitochondrial fusion and fission in a cell. *The dynamic state of mitochondria depends on the overall homeostasis of the mitochondria/cell. Mitochondria are illustrated in red color and nuclei in gray. Figure based on Wai and Langer, 2016.*

Mitochondrial dynamics does not only involve fusion and fission and their key proteins, discussed further in the next two paragraphs, but it is also regulated by the involvement of the cytoskeleton and the related proteins. The main proteins regulating mitochondrial motility and organelle transport are MTs and microtubule associated proteins (MAPs). MAPs are categorized into stabilizing and destabilizing proteins and motor proteins, which can move along the MT (Nogales, 2000). Kinesins and dynein

are the MT motor proteins (Vale, 2003). Motor protein transport in MT uses MT polarity to guide the direction of transport, with dynein moving towards the minus-end (retrograde transport) and kinesins toward the plus-end (anterograde transport). Kinesins are a large family of motor proteins and are divided into six families, where members of family 1 and 3 kinesins are involved in mitochondrial transport (Hollenbeck and Saxton, 2005). Kinesin-1 proteins bind mitochondria through the Milton receptors located on the outer mitochondrial membrane (OMM) (Stowers et al., 2002). Cytoplasmic dynein, present in animal cells, also plays roles in centrosome assembly. Axonemal dynein, the other known dynein type, is found in cilia and flagella, where it causes the sliding of MTs. Dynein binds mitochondria through the mitochondrial Rho GTPase 1 (MIRO1) (Morlino et al., 2014).

Mitochondria can also be transported across short distances on actin filaments using myosins as motor proteins. Myosin V transports mitochondria towards the actin plus-end and myosin VI towards the minus-end (Lister et al., 2004; Trybus, 2008). Also, IFs have been implied to play a role in mitochondrial motility (Schwarz and Leube, 2016), which will be discussed further in a later chapter 3.2.

3.2.1 Fusion of the mitochondrial network

Fusion of the mitochondria is a two-step process, which requires fusion of the double-membrane bound mitochondria, requiring three dynamin-related (GTPases) proteins: mitofusin 1 and 2 (MFN) and optic atrophy 1 (OPA1) (Chen et al., 2003; Cipolat et al., 2004). The role of these proteins is to produce a mechanical force to bring two OMMs and two IMMs of individual mitochondria in close proximity to allow membrane lipids to mix (Yoon et al., 2011). The fusion process starts by the binding of a MFN complex, (consisting of MFN isoforms 1 and 2) to another MFN complexes on an opposite mitochondrial OMM (Fig. 9) (Koshiba et al., 2004). The force generated by this process causes fusion of the OMMs. The fusion of the inner mitochondrial membrane is mediated by OPA1 (Cipolat et al., 2004). OPA1 exists in both short and long isoforms and in order for fusion to occur both isoforms need to be present (Song et al., 2007). As with fusion of the OMM, to achieve fusion of the IMM, OPA1 molecules attach to molecules on the opposite IMM (Fig. 9) (Song et al., 2009).

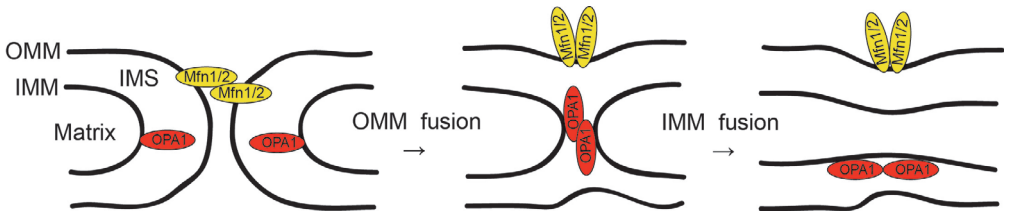

Figure 9. Mitochondrial fusion. *Fusion of the OMM is mediated by MFN1/2 (yellow), while the IMM is fused by OPA1 (red). Based on Mishra and Chan, 2014.*

Knockout of MFN1/2 causes fragmentation of the mitochondrial network and embryonic lethality in mice, highlighting the importance of the mitochondrial fusion process (Chen et al., 2003; Mishra and Chan, 2014). Interestingly, MFN2 mutations lead to the disease neuropathic Charcot-Marie-Tooth type 2A (Burte et al., 2015), whereas, knockout of OPA1 is linked to fragmented mitochondria, wide cristae, decreased insulin amount and dysfunctional GSIS in pancreatic β-cells (Zhang et al., 2011).

3.2.2 Fission of the mitochondrial network

The main actor in the fission process of the mitochondrial network is the dynamin-related protein 1 (Drp1; also known as DLP1) (Pitts et al., 1999). Drp1 is a GTPase that can form ring-like structures that can contract when GTP is present (Smirnova et al., 2001; Yoon et al., 2001). Drp1 proteins circulate between the cytosol and the OMM, although, their binding to OMM is very rapid (Smirnova et al., 2001). The recruitment of Drp1 to OMM is mediated by four proteins: FIS1, MFF, MiD49 and MiD59 (Labbe et al., 2014). When Drp1 proteins bind to these recruiting proteins, they form a contractile ring and separate the mitochondria into two parts in a GTP-dependent manner (Fig. 10) (Mears et al., 2011).

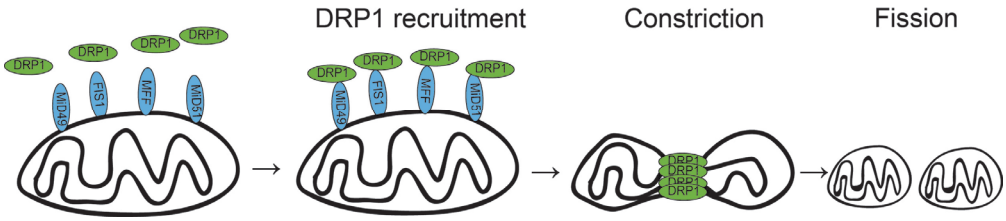

Figure 10. Mitochondrial fission. *Fission of mitochondria is mediated by DRP1 (green), which binds to OMM proteins (blue) FIS1, MFF, MiD49 and MiD59. After DRP1 binding to these proteins, constriction of mitochondria occurs, and fission is completed. Based on Mishra and Chan, 2014.*

Knockdown of Drp1 in various cell types causes tubular mitochondria (Waterham et al., 2007). Human Drp1 mutations are associated with fatal neonatal disorder (Waterham et al., 2007). Mouse studies have shown that knockout of Drp1 is embryonic lethal (Ishihara et al., 2009). Interestingly, liver-specific knockout of Drp1 causes resistance to high-fat induced obesity (Wang et al., 2015), suggesting that mitochondrial fission is important for systemic energy metabolism.

3.2 Mitochondria and intermediate filaments

The cytoskeleton is known to regulate mitochondrial function and properties. However, most studies have focused on actin and microfilaments (Glater et al., 2006; Morris and Hollenbeck, 1995). Interestingly, IFs are located in close proximity to

mitochondria (Toh et al., 1980), and over the last decade several studies have shown convincing evidence about the interaction between IFs and mitochondria (Gentil et al., 2012; Nekrasova et al., 2011; Winter et al., 2008). There are three proposed models for how IFs can regulate the mitochondrial function (Schwarz and Leube, 2016). One model proposes that IFs bind mitochondria directly or through a linker-protein. Another model puts forth the idea that IFs form a physical barrier that act as a hindrance for the mitochondria (Eckert, 1986; Nekrasova et al., 2011; Winter et al., 2008). The last model suggests that IFs are involved in cell signaling events that regulate mitochondria (Kumar et al., 2015).

Since the presence of mitochondria is necessary in cytoplasmic regions with high ATP demand, motility of mitochondria is an essential process (Morris and Hollenbeck, 1995). However, due to the calcium-buffering properties of mitochondria, immobile mitochondria are also required in certain situations (Chen et al., 2009). Mitochondria are typically transported using bidirectional, MT-based transport. In contrast, mitochondria use the actin cytoskeleton as anchors to become stationary (Glater et al., 2006; Pathak et al., 2010). Recent studies have shown that IFs are also involved in mitochondrial motility and anchoring. For example, knockout of NF-L in murine neurons leads to increased mitochondrial motility, while overexpression of NFs results in immobile mitochondria (Gentil et al., 2012; Straube-West et al., 1996). This is further supported by the increased mitochondria motility observed in fibroblasts where vimentin has been knocked out (Nekrasova et al., 2011). Mitochondrial motility is necessary for the organization of the mitochondrial network, which IFs have been shown to regulate. Knockout of desmin or K19 in muscle cells leads to disorganization of the mitochondrial network (Milner et al., 2000; Stone et al., 2007). Likewise, knockout of vimentin in cancer cells results in mitochondrial reorganization (Tang et al., 2008).

IFs have also been shown to alter mitochondria morphology. Neurons lacking NF-L and hepatocytes lacking K8 show smaller mitochondria (Gentil et al., 2012; Tao et al., 2009). Mutation of K18 (R90C) in hepatocytes is linked to fragmentation of the mitochondrial network (Kumemura et al., 2008b). Fragmentation of mitochondria has also been observed in cells lacking vimentin (Tang et al., 2008). $K8^{-/-}$ mitochondria in colonocytes show no major morphological difference, but have minor cristae alterations (Helenius et al., 2015).

Mitochondrial functions have also been shown to be regulated by IFs. Loss of K8 in colonocytes is known to cause a decrease in the mitochondrial protein HMGCS2, which suggests an involvement in the regulation of colonic ketogenic energy metabolism (Helenius et al., 2015). Knockout of vimentin, which binds mitochondria directly, results in decreased mitochondrial membrane potential (Chernoivanenko et al., 2015; Matveeva et al., 2015). $K8^{-/-}$ hepatocyte mitochondria

leak cytochrome c and show decreased ATP-production (Tao et al., 2009). Knockout of desmin in muscle cells leads to lower mitochondrial ATP-production (Milner et al., 2000). Loss of keratins in hepatocytes has been linked to increased mitochondrial hexokinase activity, which results in increased glycogen levels (Mathew et al., 2013). IFs can attach to mitochondria either by direct binding, as with vimentin, or through cytolinkers (Chernoivanenko et al., 2015; Schwarz and Leube, 2016). The plectin 1b isoform is a large cytolinker protein known to bind both mitochondria and IFs, and loss of plectin is associated with elongation of mitochondria (Rezniczek et al., 2003; Winter et al., 2008). Trichoplein (TCHP; also referred as mitostatin) is another cytolinker, which binds both K18 and K8 filaments and MFN2, and is anchoring mitochondria to the ER (Cerqua et al., 2010; Nishizawa et al., 2005). Interestingly, overexpression of TCHP is linked to altered mitochondrial morphology, including mitochondrial fragmentation in cervical cancer cells (Vecchione et al., 2009).

3.3 Mitochondria in β-cells and diabetes

Mitochondria play an especially important role in β-cells, where they elevate intracellular ATP concentration, which is crucial for proper GSIS (Zhang et al., 2001). It is important to distinguish between mitochondrial diabetes and mitochondrial dysfunction during diabetes. Mitochondrial diabetes refers to mitochondrial mutations resulting in diabetes, which can resemble both T1D and T2D (Maassen et al., 2004). However, these mutations are very rare. The A3243G mutation in the mitochondrial tRNA gene is the most common mitochondrial diabetes variant. Patients carrying this mutation develop diabetes at an average 40 years of age, and the disease is accompanied by hearing loss of high frequencies (Maassen et al., 2006b). Thus, the disease is referred to as maternally inherited diabetes and deafness (MIDD). The A3243G mutation results in defective mitochondrial protein synthesis and leads to decreased insulin secretion (Maassen et al., 2006a). This could be due to the increased amount of oxidative stress caused by the A3243G mutation, which leads to enhanced β-cell death (Kujoth et al., 2005).

Mitochondrial dysfunction has mostly been linked to T2D, where β-cell mitochondria tend to be swollen and fragmented (Anello et al., 2005; Higa et al., 1999; Lowell and Shulman, 2005). The amount of mitochondria is similar between T2D and non-diabetic β-cells; however, the total mitochondrial volume mass is increased in T2D β-cells (Anello et al., 2005). Also, the levels of mitochondrial proteins, including mitochondrial uncoupling protein 2 (UCP2), complex I and V are higher in T2D β-cells. It has also been shown that defective mitochondria and OXPHOS are involved in the development of T2D through oxidative stress-caused insulin resistance (Mootha et al., 2003; Robertson, 2006). Still the mechanism behind mitochondria and diabetes susceptibility is unknown

OUTLINE AND AIMS OF THE THESIS

Proper endocrine pancreas function is vital for maintaining homeostasis, such as blood glucose levels, in the human body. The dramatic increase in T1D and T2D incidence throughout the world calls for new insights into endocrine pancreas biology. Keratin filaments are important modulators and stress protectors in epithelial cells. Keratin deficiency and mutations have previously been linked to diseases in e.g. skin and liver. However, the roles of keratins in the insulin producing β-cells have not been thoroughly investigated. In this thesis, keratins have been studied to uncover their potential roles in vital cellular processes in pancreatic β-cells and endocrine pancreas. This includes search for new molecular mechanisms and players in the regulation of insulin secretion. Characterization of keratins present in β-cells has previously not been thoroughly investigated.

The thesis is divided into three connecting aims with focus on investigating the role of keratins in the endocrine pancreas:

Aim 1: Characterization of keratins and their functions in pancreatic islets homeostasis.

Aim 2: Investigation of the role of keratin deficiency in diabetes susceptibility and blood glucose regulation.

Aim 3: Investigate if keratins affect β-cell mitochondria function and morphology.

EXPERIMENTAL PROCEDURES AND MATERIAL

1 Methods

Detailed descriptions of methods used in the thesis can be found in the mentioned study (publication/manuscript).

Table 5. Methods used in the studies.

Method	Study
ATP luminescence assay	III
Confocal microscopy	I, II, III
ELISA assay	
-Insulin	I, II
Fasting blood glucose measurements	I, II
Glucose tolerance test	I, II
High-fat diet	II
High-salt extraction	I
Histology	
Immunofluorescence staining	I, II,III
Immunohistochemistry	I, II
Image analysis	
-GLUT2 cellular localization	I
-Mitochondrial morphology	III
-Tracking of mitochondria	III
Insulin tolerance test	I, II
Isolation and culture of islet of Langerhans	I, III
Isolation of mitochondria	III
Live-cell imaging	
-Mitochondria membrane potential	III
-Mitochondria motility	III
MIN6 cell culture and transfection	II, III
Mitochondrial membrane potential analysis	III
Mitochondrial motility analysis	III
SDS-PAGE and Western blot	I, II,III
Streptozotocin treatments	
-High-dose	I
-Low-dose	I, II
Subcellular fractionation	I
Transmission electron microscopy	I, III

2 Experimental animals

All animals were bred and raised at the Central Animal Laboratory at the University of Turku. The mice were bred and genotyped as described previously (Baribault et al., 1994; Zhong et al., 2007). The mice were sacrificed by CO_2 inhalation. Animal experiments were approved by the National Animal Experiment Board and conformed to the regulations set by the Finnish Act on Animal Experimentation.

Table 6. Experimental animals used in the studies.

Mice	Study
$K8^{+/+}$	I + II + III
$K8^{+/-}$	II
$K8^{-/-}$	I + III
Non-obese diabetic (NOD)	I

3 Antibodies

Antibodies were used in both western blotting and immunofluorescence staining analyses. Primary antibodies are listed in Table 7. Secondary antibodies are listed in Table 8 and were conjugated with either fluorophore or horseradish peroxidase (HRP).

Table 7. Primary antibodies and their applications in the studies. *Antibodies were used in western blotting (WB) and immunofluorescence staining (IF).*

Name (clone)	Manufacturer	Application	Study
Cytochrome c	Cell Signaling Technologies	WB	III
E-cadherin	BD Pharmingen	IF	I
Glucagon	BD Pharmingen	IF	I
GLUT2	Millipore	WB, IF	I + II
H2A.X	Invitrogen	IF	I
Hsc70	Stressgen	WB	I + II + III
Insulin	Santa Cruz Technologies	WB, IF	I + II + II
K7 (RCK 105)	Progen	WB, IF	I + II
K8 (Troma I)	Developmental Studies Hybridoma Bank	WB, IF	I + II + III
K18	Progen	WB, IF	I + II
K19 (Troma III)	Developmental Studies Hybridoma Bank	IF	I
K20	Progen	IF	I + II
MFN2	Sigma-Aldrich	WB, IF	II + III
MitoProfile Total OXPHOS	MitoScience	WB	III
OPA1	Cell Signaling Technologies	WB	III
TOM20	Santa Cruz Technologies	WB, IF	II + III

Table 8. Secondary antibodies and their applications in the studies. *Antibodies were used in western blotting (WB) and immunofluorescence staining (IF).*

Name	Manufacturer	Application	Study
Alexa Fluor 488	Invitrogen	IF	I + II + III
Alexa Fluor 546	Invitrogen	IF	I + II + III
Alexa Fluor 568	Invitrogen	IF	I + II + III
Anti-goat HRP	Cell Signaling Technologies	WB	I + II + III
Anti-mouse HRP	GE Healthcare	WB	I + II + III
Anti-rabbit HRP	Cell Signaling Technologies	WB	I + II + III
Anti-rat HRP	GE Healthcare	WB	I + II + III

RESULT AND DISCUSSION

1 K8 and K18 are the major IFs of β-cells, with low expression of K7 (Study I + II)

The knowledge that keratins are involved in diseases, and that the characterization of keratins in the endocrine pancreas had not been thoroughly investigated, led us to study the keratins in murine β-cells.

The only previous study investigating keratins in the endocrine pancreas showed that K8 and K18 are the main keratin pair (Bouwens, 1998). Immunofluorescence staining and western blotting for K8 and K18 on endocrine pancreas sections confirmed that K8 and K18 are the most common keratin pair in $K8^{+/+}$ β-cells (Study I, Fig. 1A, C; Fig. 11B). However, in $K8^{-/-}$ β-cells leftover K18 likely pairs with type II keratin K7 in a very sparse manner (Study I, Fig. 1B), indicating that K7 alone cannot compensate for the loss of K8. In order to determine if keratins are important in β-cell function, we were interested in identifying the keratins present in $K8^{+/-}$ β-cells. We found that in $K8^{+/-}$ β-cells, K8 and K18 still constitute the main keratin pair (Study II, Fig. 1A). However, the keratin levels are decreased >50% compared to $K8^{+/+}$ (Study II, Fig. 1B-D). The minor amount of K7 detected in $K8^{-/-}$ was not detected by western blotting of $K8^{+/-}$ islet lysates (not shown), but immunofluorescence staining for K7 in $K8^{+/+}$ and $K8^{+/-}$ pancreatic sections showed no difference (Study II, Suppl. Fig. 1), suggesting that K7 cannot compensate for the loss of one K8 allele. These results are similar to those of a study in colon, where partial loss of K8 did not lead to any major upregulation of other keratins, however, K7 is upregulated in the topmost cells of the crypts (Asghar et al., 2015). Interestingly, overall the expression of K8 is lower in the endocrine pancreas than in the exocrine pancreas (Study I, Fig. 1A). A potential explanation behind this difference is that the exocrine pancreas needs more stress protection, which can be provided by keratin filaments, since exocrine pancreatic cells produce and secrete several digestive enzymes. In conclusion, K8 and K18 are the main keratins in both $K8^{+/+}$ and $K8^{+/-}$ β-cells, but when K8 is absent, residues of K7 and K18 are still present.

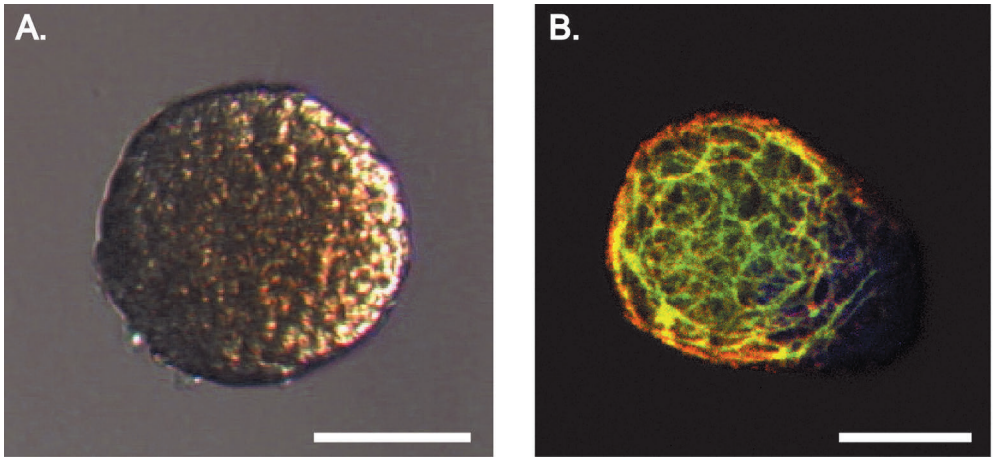

Figure 11. Isolated islet of Langerhans and β-cell. *(A) Isolated murine islet of Langerhans captured with phase-contrast light microscope. Scale bar = 100 um. (B) $K8^{+/+}$ β-cell immunofluorescence staining of K8 (green), K18 (red) and nuclei (blue). Scale bar = 5 um.*

2 Keratins in blood glucose regulation and diabetes susceptibility (Study I + II)

The roles of keratins in the endocrine pancreas and diabetes susceptibility have not been thoroughly investigated. Keratins have been linked to T1D in a small study (12 patients), where four out of ten patients with T1D had keratin autoantibodies in blood serum (Nakanishi et al., 1993). However, no other studies on the subject have been published. A study has shown that murine K18 glycosylation mutations (S30A, S31A or S49A) made the mutant mice more susceptible to STZ-treatment compared to controls, due to prevention of K18 glycosylation (Ku et al., 2010), thus, implicating a protecting role for keratin glycosylation in β-cells. Keratins have been shown to become upregulated in β-cells after glucose stimulation of C57Bl/6J mice *in vivo* (Ahmed and Bergsten, 2005). These previous studies led us to hypothesize that keratins are involved in diabetes susceptibility and the results of our investigation are presented in the following sub-chapters.

2.1 Keratins are involved in blood glucose regulation (Study I + II)

Since improper blood glucose regulation is a sign of diabetes (Vahidi et al., 2016), we investigated the effect of K8 expression level on blood glucose levels. Overnight fasting of $K8^{+/+}$ and $K8^{-/-}$ mice revealed that $K8^{-/-}$ mice have slightly lower fasting blood glucose levels than $K8^{+/+}$ (Study I, Fig. 2A). Also, glucose tolerance tests showed that $K8^{-/-}$ mice have significantly impaired glucose tolerance when

compared to $K8^{+/+}$ mice. For example, 30 minutes after glucose administration, $K8^{-/-}$ mice have average blood glucose levels at 13 mmol/L compared to 18 mmol/L for $K8^{+/+}$ mice (Study I, Fig. 2B). $K8^{-/-}$ mice also showed signs of increased insulin sensitivity during an insulin tolerance test (Study I, Fig. 2C), including increased tremor, fatigue, and delayed recovery from hypoglycemia, compared to $K8^{+/+}$. $K8^{+/+}$ and $K8^{-/-}$ mice both had similar fasting serum insulin levels; however, after glucose administration, $K8^{-/-}$ mice did not increase their serum insulin level, in contrast to $K8^{+/+}$ mice (Study I, Fig. 2D). These observations suggest that $K8^{-/-}$ mice have increased insulin sensitivity, but impaired GSIS. A possible explanation for these observations is that other organs involved in the regulation of blood glucose are partially responsible for the effect. It has been shown that loss of K8 and K18 in hepatocytes leads to increased hepatic glucose uptake and promotes glycogen production (Mathew et al., 2013). The glycogen production is further stimulated by insulin, which suggests that keratins in hepatocytes play an important role in regulating blood glucose levels. Another possibility could be that glucose regulation by skeletal muscles, which normally express small amounts of K8 and K19 (Stone et al., 2007), might be playing a role. Glucokinase (GK) is important in sensing intracellular glucose in β-cells by phosphorylating glucose to glucose-6-phosphate. It has been shown that GK amount and localization are regulated by keratins in K8 lacking hepatocytes (Mathew et al., 2013). Interestingly, there is no difference in GK localization or expression level between $K8^{+/+}$ and $K8^{-/-}$ β-cells (Fig. 12A,B), suggesting that keratins differentially regulate GK in β-cells and hepatocytes. This is further supported by the partial localization of GK in the nucleus in β-cells, but its exclusive cytoplasmic localization in hepatocytes. One of the GK isoforms is β-cell specific (Jetton et al., 1994), which could explain the differences between β-cells and hepatocytes mentioned above.

We also investigated if low K8 and K18 expression level in $K8^{+/-}$ mice (Study II, Fig. 1A-D) has an impact on blood glucose levels. Interestingly, there was no difference between $K8^{+/+}$ and $K8^{+/-}$ mice with respect to fasting blood glucose, glucose and insulin tolerance, or GSIS (Study II, Fig. 2A-D), which suggests that a ~50% loss of these keratins does not have an impact on blood glucose regulation. $K8^{+/-}$ mice on a HFD for 16 weeks neither showed abnormalities in GSIS (Study II, Fig. 6D) when administered 2g/kg glucose. Together, these data suggest that keratins are important for regulating blood glucose, and are able to maintain glucose homeostasis even when expressed at lower level. These results further emphasize the importance of hepatocytes in regulation of blood glucose.

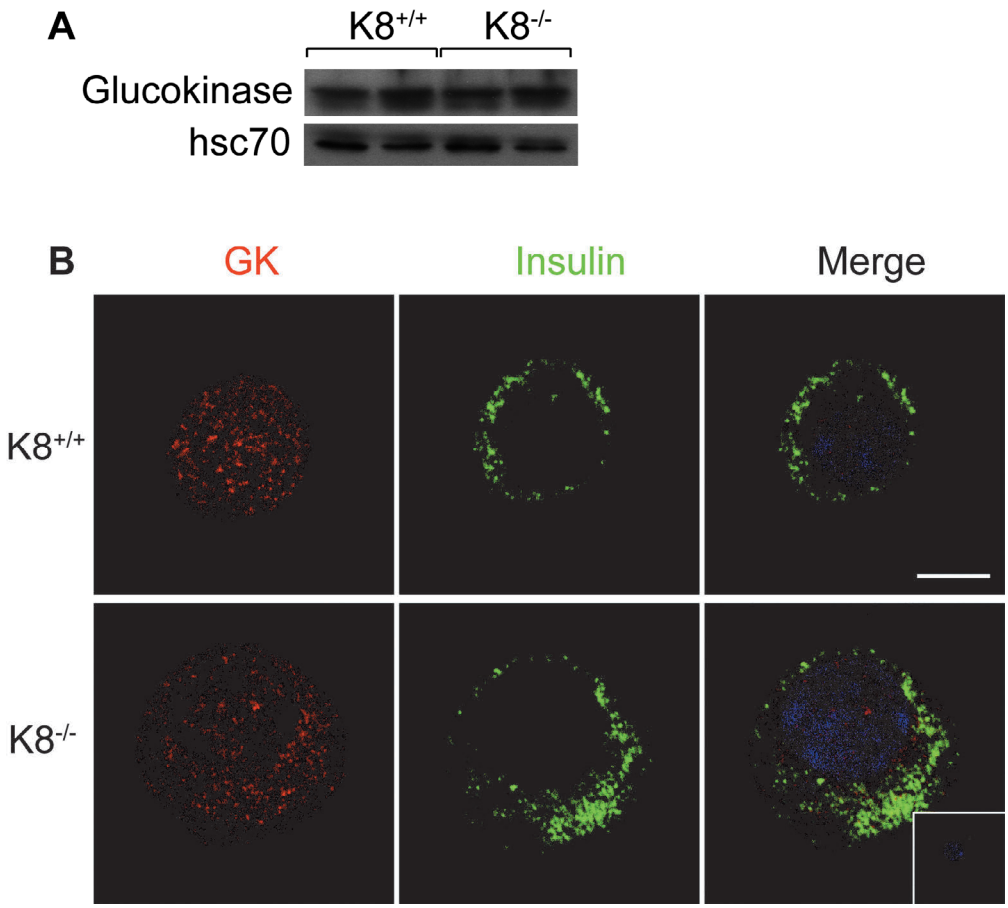

Figure 12. Keratins do not regulate GK protein amount or localization. *(A) Immunoblotting of isolated islet lysates from $K8^{+/+}$ and $K8^{-/-}$ mice for GK. n = 2 mice per sample of islets. (B) Immunofluorescence staining of cultured $K8^{+/+}$ and $K8^{-/-}$ β-cells for GK (red), insulin (green) and nuclei (blue). Insert in (B) shows secondary antibody control. Scale bar = 5 μm.*

2.2 Keratins participate in the regulation of insulin vesicle morphology and insulin content (Study I + II + III)

The impaired GSIS in $K8^{-/-}$ mice (Study I, Fig. 2C) led us to study the insulin vesicle morphology and insulin levels in relationship to the presence or absence of K8. Investigation of insulin vesicles by TEM shows smaller, paler and irregularly shaped dense cores when keratins are absent, compared to $K8^{+/+}$ insulin vesicles (Study I, Fig. 3A-C). There is also clustering and clumping of insulin vesicles in $K8^{-/-}$ β-cells (Study I, Fig. 3A) and the total amount of insulin, measured by ELISA, is decreased in $K8^{-/-}$ pancreas (Study I, Fig. 3D) compared to $K8^{+/+}$ pancreas. Reduced insulin expression in islet lysate was confirmed by western blotting (Study III, Fig. 4K, L).

The insulin amount in $K8^{+/-}$ islets and total pancreas, however, was unaltered compared to $K8^{+/+}$ pancreas (Study II, Fig. 2E-G). From these findings we show that keratins are involved in the regulation of insulin vesicle morphology and insulin production and/or release. The morphology of the insulin vesicles in $K8^{-/-}$ mice resembles the insulin vesicles in zinc transporter 8 knockout β-cells, which have an abnormal insulin/proinsulin ratio (Wijesekara et al., 2010). However, there is no difference in proinsulin ratio between $K8^{-/-}$ and $K8^{+/+}$ islets (not shown). Keratins have previously been suggested to regulate organelle morphology, targeting of proteins and cell size restricting of secretory goblet cells (Asghar et al., 2016; Tao et al., 2009; Toivola et al., 2004a). Thus, keratin filament targeting of insulin vesicles could be a potential mechanism underlying our observations. The insulin vesicle membrane's proteome consists of over 130 proteins (Brunner et al., 2007), which makes the search for possible keratin-binding partners a daunting task. Plectin, TCHP and 14-3-3 are all cyto-linkers known to bind keratins (Liffers et al., 2011; Nishizawa et al., 2005; Winter et al., 2008). Therefore, these cyto-linkers are candidate linkers between keratins and insulin vesicles. 14-3-3 proteins are especially interesting due to their GTPase-binding ability, since the insulin vesicles have several GTPases located on the cell membrane (Suckale and Solimena, 2010). Other potential proteins that might interact with keratin filaments are MFs and their actin-binding proteins, such as Rab27a, granuphilin, alpha-actinin and gelsolin (Wang and Thurmond, 2009). These proteins are all known to be involved in insulin vesicle release (Wang and Thurmond, 2009). In conclusion, keratins are involved in the regulation of insulin content and insulin vesicle morphology.

2.3 Delayed STZ-induced diabetes in $K8^{-/-}$ mice, but increased exocrine damage after low-dose STZ-treatment (Study I)

STZ is a common model used to chemically induce T1D in mice (Graham et al., 2011). We hypothesized that because of the loss of stress-protecting keratins, STZ-treatment of $K8^{-/-}$ mice would lead to an earlier induction of diabetes compared to $K8^{+/+}$ mice. Surprisingly, $K8^{-/-}$ mice showed resistance to acute high-dose STZ-induced stress (Study I, Fig. 4AB) by delaying hyperglycemia after a low-dose STZ-treatment (Study I, Fig. 5AB) compared to $K8^{+/+}$. However, after the low-dose STZ-treatment, $K8^{-/-}$ mice displayed more severe exocrine pancreas damage compared to $K8^{+/+}$. This observation is similar to a previous study where mice overexpressing mutations that disable K18 glycosylation show severe exocrine damage after STZ-treatment (Ku et al., 2010). It has previously been shown that the absence of K8 appears to enhance protection against stress in the exocrine pancreas (Toivola et al., 2000) due to the upregulation of regenerative islet-derived (Reg)-II (Zhong et al., 2007). In addition, overexpression of K8 in the exocrine pancreas causes pancreatitis

(Toivola et al., 2008). These observations reflect the impact of exocrine damage severity; chronic STZ-treatment is too severe for the protecting capacity of Reg-II.

2.4 $K8^{-/-}$-induced GLUT2 mislocalization in β-cells correlates with STZ resistance (Study I + II)

STZ is a nitrous urea analogue known to enter β-cells facilitated by GLUT2 (Elsner et al., 2000). GLUT1 and GLUT3 have been shown to be mislocalized in endothelial cells lacking type II keratins, leading to suppression of cellular biosynthesis (Vijayaraj et al., 2009). Therefore, we decided to investigate GLUT2 in $K8^{+/+}$ and $K8^{-/-}$ β-cells and immunofluorescence staining of GLUT2 in $K8^{-/-}$ islets showed mislocalization of the transporter to the β-cell cytoplasm (Study I, Fig. 4C-E), in contrast to the tightly plasma membrane bound GLUT2 in $K8^{+/+}$. This was confirmed by biochemical analysis that showed increased levels of GLUT2 in the cytoplasm of $K8^{-/-}$ β-cells. However, there was no difference in total GLUT2 protein levels between $K8^{-/-}$ and control cells (Study I, Fig. 4F). These data suggest that the mislocalization of GLUT2 protects the $K8^{-/-}$ β-cells from the effect of STZ by delaying its entry. This is supported by previous findings where ablation of GLUT2 led to decreased STZ sensitivity (Elsner et al., 2000).

Interestingly, the $K8^{+/-}$ model is also protected from STZ-induced damage, and low-dose STZ-treatment of $K8^{+/-}$ mice resulted in significantly earlier onset of diabetes, as measured by a blood glucose level higher than 14 mmol/L for two consecutive days (Study II, Fig. 3A-B). The $K8^{+/-}$ mice also had increased urine secretion (Study II, Fig. 3C), probably due to diabetes-induced kidney damage (Lenzen, 2008). As hypothesized, the localization of GLUT2 was primarily at the cell membrane in $K8^{+/-}$ mice (Study II, Fig. 5C). Therefore, the more severe low-dose STZ-induced damage is likely due to decreased keratin amount in $K8^{+/-}$ mice. Downregulation of K8 has been linked to negative regulation of toll-like receptor (TLR) signaling (Dong et al., 2016). A potential mechanism underlying the increased low-dose STZ-induced T1D in $K8^{+/-}$ mice could be attributed to increased TLR-mediated signaling, which might lead to increased inflammation in β-cells when induced by STZ-treatment (Dong et al., 2016). The evidence above highlights the duality of keratin functions, which depends on cell type, and which is further supported by the fact that $K8^{-/-}$ hepatocytes are fragile and not stress-protected (Toivola et al., 2001). In conclusion, the keratin expression levels correlate with diabetes susceptibility, as long as GLUT2 is plasma membrane-bound.

2.5 High-fat diet induces transiently higher blood glucose levels in $K8^{+/-}$ mice (Study II)

Given that $K8^{+/-}$ mice were highly sensitive to low-dose STZ-induced T1D, we tested whether these mice would also be sensitive to HFD. We hypothesized that HFD could accelerate the onset of T2D in $K8^{+/-}$ mice. It has been shown that HFD induces damage of hepatocytes in mice lacking K8 (Kucukoglu et al., 2014). $K8^{+/+}$ and $K8^{+/-}$ mice were fed HFD (60% energy from fat) for 16 weeks. $K8^{+/-}$ mice tested after exposure to the HFD showed no significant difference in blood glucose levels, weight or fat mass compared to $K8^{+/+}$ controls (Study II, Fig. 6A-C). However, there was a slight but non-significant increase in blood glucose levels of $K8^{+/-}$ mice during weeks 8-10 (week 8 $p = 0.06$; week 9 $p = 0.15$; week 19 $p = 0.06$). There was no observed islet damage in $K8^{+/+}$ and $K8^{+/-}$ islets when stained for histology (Study II, Suppl. Fig. 2). The mild keratin-related phenotype to a HFD is supported by that $K8^{+/-}$ gallbladders and livers have been shown to be resistant to lithogenic diet-induced damage (Tao et al., 2003), which is similar to our findings. Interestingly, it has been shown that HFD is not independently able to induce T2D and its effect is mouse strain-dependent (Kobayashi et al., 2014). In conclusion, exposure to a HFD induced dramatic weight and fat mass increase in both $K8^{+/+}$ and $K8^{+/-}$ mice, and transiently higher blood glucose levels in $K8^{+/-}$ mice compared to $K8^{+/+}$ mice. This may implicate that a full K8 deficiency in $K8^{-/-}$ mice would make the mice sensitive to a HFD, but it needs further investigation.

2.6 Upregulation of keratins after diabetes and chronic stress (Study I + II)

Keratins are well-known cellular stress protectors and are upregulated during stress situations in different tissues (Helenius et al., 2016; Toivola et al., 2010). Since diabetes is highly stressful and eventually lethal for β-cells, we examined what happens to keratins during two β-cell destruction models. We found that K8 and K18 was highly upregulated in β-cells and still present both in NOD islets and low-dose STZ-treated $K8^{+/+}$ islets (Study I, Fig. 6AB). However, the K8 partner K18 was not upregulated in $K8^{-/-}$ islets (Study I, Fig. 6C), suggesting that K8 is needed for proper stress protection by keratin upregulation.

$K8^{+/-}$ β-cells showed almost 50% more islet cell damage after two weeks of low-dose STZ-treatment and 20% more islet cell damage after five weeks, compared to $K8^{+/+}$ (Study II, Fig. 4A). This further supports the possibility of TLR-induced inflammation in $K8^{+/-}$ β-cells (Dong et al., 2016), where loss of keratins are linked with increased TLR signaling and inflammation. The $K8^{+/-}$ β-cells not destroyed by

STZ showed early stress protection two weeks after low-dose STZ-treatment (Study II, Fig. 4B), similar to $K8^{+/+}$ β-cells. However, five weeks after STZ-treatment, the K8 filaments were almost completely lost from the $K8^{+/-}$ islets, compared to the $K8^{+/+}$ islets where the recovery phase from STZ had started. This suggests that proper K8 levels are needed for complete recovery after chronic islet stress.

3 Keratins interact with mitochondria in β-cells which might regulate insulin production (Study III)

3.1 β-cell mitochondrial fusion is promoted by keratin-mediated stabilization of trichoplein-mitofusin complexes (Study III)

Dysfunctional mitochondria and mitochondrial mutations have been linked to diabetes and mitochondrial fragmentation (Jheng et al., 2012; Jitrapakdee et al., 2010). This, together with the crucial involvement of mitochondria in β-cell GSIS (Jitrapakdee et al., 2010; Supale et al., 2012) and evidence that K8 deficient pancreas has decreased amount of insulin (Study I, Fig. 3D), led us to investigate the role of keratins in β-cell mitochondria regulation. Ultra-resolution TEM imaging of $K8^{+/+}$ and $K8^{-/-}$ murine pancreas reveled that β-cells lacking K8 have an almost two-fold increase in the number of round and smaller mitochondria, compared with $K8^{+/+}$ (Study III, Fig. 1A-C,E). It is known that keratins and other IFs in several organs interact with mitochondria and can alter its morphology (Schwarz and Leube, 2016). Knockout of K8 or expression of the keratin filament disrupting mutation K18R90C in hepatocytes is associated with smaller mitochondria (Kumemura et al., 2008a; Tao et al., 2009). Also, knockout of NF-L in neurons leads to smaller mitochondria (Gentil et al., 2012). $K8^{-/-}$ β-cells had an increased amount of mitochondria with diffuse cristae (Study III, Fig. 1D), which might be related to the abnormal mitochondrial cristae described in $K8^{-/-}$ hepatocytes (Tao et al., 2009). These results show that keratins not only regulate mitochondrial size, but also the appearance of the cristae. Given that K17 has been found in the nucleus in epithelial tumor cells (Hobbs et al., 2016), a potential mechanism that may explain how keratins regulate mitochondrial cristae morphology may involve keratin dimers trapped inside the mitochondria.

The observed changes from long-shaped mitochondria in $K8^{+/+}$ β-cells to round-shaped in $K8^{-/-}$ cells led us to investigate the mitochondrial morphology more closely. Immunofluorescence staining of isolated $K8^{+/+}$ and $K8^{-/-}$ β-cells for the outer mitochondrial membrane protein TOM20 followed by 3D-rendering of confocal Z-stacks showed that $K8^{+/+}$ β-cells have a fused mitochondrial network. In contrast, cells lacking keratins had a partially fragmented mitochondrial network (Study III,

Fig. 2AB). This observation is similar to the effect of knocking out vimentin in 3T3 cells, which also leads to fragmentation of the mitochondrial network (Tang et al., 2008). This suggests that keratins in β-cells can regulate mitochondrial morphology and keratins are required to form a fused mitochondrial network.

MFN2, together with MFN1, are known to regulate the fusion of the mitochondrial outer membrane, while OPA1 is involved in the fusion of the inner membrane (Hoppins, 2014). Therefore, these fusion proteins became candidates in the search for a potential mechanism behind the fusion phenotype in $K8^{-/-}$ β-cell mitochondria. The cytolinker TCHP is known to bind both K8/K18 and MFN2 (Cerqua et al., 2010; Nishizawa et al., 2005). Interestingly, both MFN2 and TCHP protein levels were reduced in $K8^{-/-}$ β-cells, compared to $K8^{+/+}$ β-cells (Study III, Fig. 3A-C and E-G). Reduced levels of MFN2 have been associated with fragmentation of the mitochondrial network in both muscle cells and fibroblasts (Bach et al., 2003; de Brito and Scorrano, 2008). In contrast to the observation that overexpression of TCHP is linked to fragmentation of mitochondria in cancer cells (Vecchione et al., 2009), we noticed a reduction of TCHP in $K8^{-/-}$ β-cells, which suggests that the TCHP function might be cell type-specific. The OPA1 protein level was unchanged in $K8^{-/-}$ β-cells (Study III, Fig. 3A,D); however, knockout of OPA1 has previously been associated with mitochondrial fragmentation (Zhang et al., 2011). The unaltered OPA1 levels likely results in a milder mitochondrial fragmentation phenotype in $K8^{-/-}$ β-cells. The partial decrease in keratins was not enough to alter MFN2 protein levels, since there was no difference between $K8^{+/+}$ and $K8^{+/-}$ islets (Study II, Fig. 5A, B). In conclusion, keratins seem to stabilize the MFN2-TCHP complexes leading to a primarily fused mitochondrial network.

3.2 Keratins regulate mitochondrial complexes to maintain normal membrane potential, ATP levels and insulin levels in β-cells (Study III)

A fragmented mitochondrial network has been linked to disruption of mitochondrial homeostasis (Gomes et al., 2011; Jheng et al., 2012). This led us to investigate whether keratins can regulate mitochondrial functions in β-cells, such as OXPHOS and membrane potential. Knockout of MFN2 in cancer cells has previously been linked to defective mitochondrial complexes, and reduction in membrane potential and ATP-production (Bach et al., 2003; Chen et al., 2005; Mourier et al., 2015; Pich et al., 2005). Proper mitochondrial function is crucial in β-cells during basal conditions due to their involvement in GSIS through the maintenance of proper intracellular ATP/ADP-ratio and Ca^{2+} buffering (Jitrapakdee et al., 2010; Supale et al., 2012). As hypothesized, both of β-cells' mitochondrial

membrane potential and ATP levels decreased when K8 is absent (Study III, Fig. 4IJ), which corresponds to a downregulation of MFN2. This is supported by the fact that due to the increased IMM area, round and fragmented mitochondria produce less ATP than a fused mitochondrial network (Rossignol et al., 2004). Also, knockout of vimentin leads to a decrease in mitochondrial membrane potential in cultured fibroblasts (Chernoivanenko et al., 2015). Vimentin interacts with mitochondria by binding directly through a mitochondria-binding head region of the vimentin polypeptide. However, the sequences of the K8 and K18 corresponding region of do not align with the mitochondria-binding region of vimentin (data not shown), indicating that K8/K18 are not able to directly bind to mitochondria in the same way as vimentin. However, other potential undiscovered mitochondria-binding regions of the keratin filaments cannot be excluded.

Next, we investigated other pathways that could alter mitochondrial membrane potential and mitochondrial function, such as proteins involved in the OXPHOS pathway. Cytochrome c was selected as candidate due to its stabilizing properties of the OXPHOS complexes I and IV (Vempati et al., 2009). Knockout of cytochrome c in murine fibroblast leads to a decrease of those two complexes (Vempati et al., 2009). Correspondingly, we noticed over 50% decrease in both total and mitochondrial cytochrome c protein levels in $K8^{-/-}$ β-cells (Study III, Fig. 4A,G,H); and subsequently, a slight decrease in OXPHOS complex I and IV protein levels (Study III, Fig. 4A,B,E). However, the remaining OXPHOS complexes were unaltered (Study III, Fig. 4A,C,D,F). A possible mechanism underlying the reduction in cytochrome c in $K8^{-/-}$ β-cell mitochondria could implicate the import of the cytochrome precursor protein apocytochrome c. This and other nuclear encoded proteins are imported through the TOM and TIM complexes on the OMM/IMM respectively (Dudek et al., 2013). The measured TOM20 protein levels, which were used as a marker for mitochondria in study III, were unaltered in $K8^{-/-}$ β-cells (Study III, Suppl. Fig. 1B,C). This suggested that keratins do not alter TOM20 proteins directly. Heat shock proteins (HSPs) act as chaperones and guiding proteins ready for mitochondrial import. K8/K18 have previously been shown to bind to HSP70 (Liao et al., 1997). Therefore, a plausible hypothesis is that loss of keratins leads to destabilization of HSPs and a decrease in mitochondrial import. In conclusion, keratins maintain mitochondrial health, including OXPHOS complexes, mitochondrial membrane potential and intracellular ATP amount, which may contribute to proper insulin levels in β-cells (Study III, Fig. 4K, L).

3.3 Keratin-mitochondria-association stabilizes mitochondrial movements in β-cells (Study III)

The motility of mitochondria is highly depending on the cell type, as certain cell types e.g. neurons that need motile mitochondria (Chen et al., 2009). However, other mitochondrial properties, such as serving as calcium buffers, require stationary mitochondria (Chen et al., 2009; Morris and Hollenbeck, 1995). β-cells, where mitochondria are important as calcium buffers, favor stationary mitochondria (Chen et al., 2009). Given that loss of the IF protein vimentin in fibroblasts led to more motile mitochondria (Nekrasova et al., 2011), we were interested in investigating mitochondrial motility in isolated β-cells. Both $K8^{-/-}$ β-cells (Study III, Fig. 5A,B) and murine insulinoma, MIN6, cells (Study III, Suppl. Fig. 2AB) lacking keratins have 50% more motile mitochondria, compared with $K8^{+/+}$ β-cells and MIN6 transfected with K8/K18. This could mean that the keratin-cytolinker binding of mitochondria leads to a physical barrier of keratin filaments. In conclusion, keratins may regulate mitochondrial motility by physical scaffolding of mitochondria or mitochondrial-binding proteins.

CONCLUDING REMARKS

In this thesis, I have uncovered new insights about the role of keratins in the endocrine pancreas (summarized in Fig. 13). Characterization of keratin profiles in the endocrine β-cells shows that K8 and K18 constitute the most common keratin pair in both $K8^{+/+}$ and $K8^{+/-}$ β-cells while there are few keratin filaments of K7 and K18 in both $K8^{-/-}$ and $K8^{+/-}$ β-cells.

In β-cells, keratins modulate both insulin vesicles and mitochondrial morphology, contributing to proper insulin levels and mitochondrial health. Keratins are also involved in the targeting of GLUT2 to the plasma membrane in β-cells. The ability of keratins to maintain mitochondrial health in β-cells leads to a primarily fused mitochondrial network, in conjunction with stabilization of the TCHP-MFN2 complex that promotes fusion of mitochondria. Keratins are also able to regulate mitochondrial membrane potential and ATP levels, which are crucial for normal β-cell insulin production and secretion. Our findings also show that keratins are involved in blood glucose regulation by stabilizing fasting blood glucose, including both glucose and insulin tolerance. Mitochondria in keratin positive β-cells are primarily stationary, which may be due to physical scaffolding properties of the keratin network.

Proper keratin protein levels are important for islet stress protection in order to withstand acute or low-dose STZ-treatment of mice. $K8^{-/-}$ islets are initially able to withstand acute STZ-damage likely due to mislocalization of GLUT2 that also imports STZ, but are more sensitive to chronic STZ-induced exocrine damage. β-cells in diabetic mice (NOD mice) or mice with chronic stress (low-dose STZ-treatment) display an upregulation of K8 filaments. However, the reduced amount of keratin in $K8^{+/-}$ endocrine pancreas leads to higher diabetes susceptibility than in $K8^{+/+}$ mice probably due sufficient plasma membrane GLUT2 localization compared to $K8^{-/-}$ β-cells, highlighting the importance of the sufficient keratin amount in β-cells. However, partially decreased keratin amounts do not have an impact on systemic blood glucose regulation. HFD induces a transient, but non-significant, increase in blood glucose levels in $K8^{+/-}$ mice compared to $K8^{+/+}$ mice, suggesting that $K8^{+/-}$ β-cells contain sufficient amount of keratin filaments to withstand the HFD-induced β-cell stress.

In conclusion, keratins regulate insulin levels by regulating β-cell mitochondrial ATP production and insulin vesicle morphology. In addition, keratins may help target GLUT2 to the plasma membrane. Keratins are also involved in blood glucose regulation, most likely in cooperation with hepatocytes.

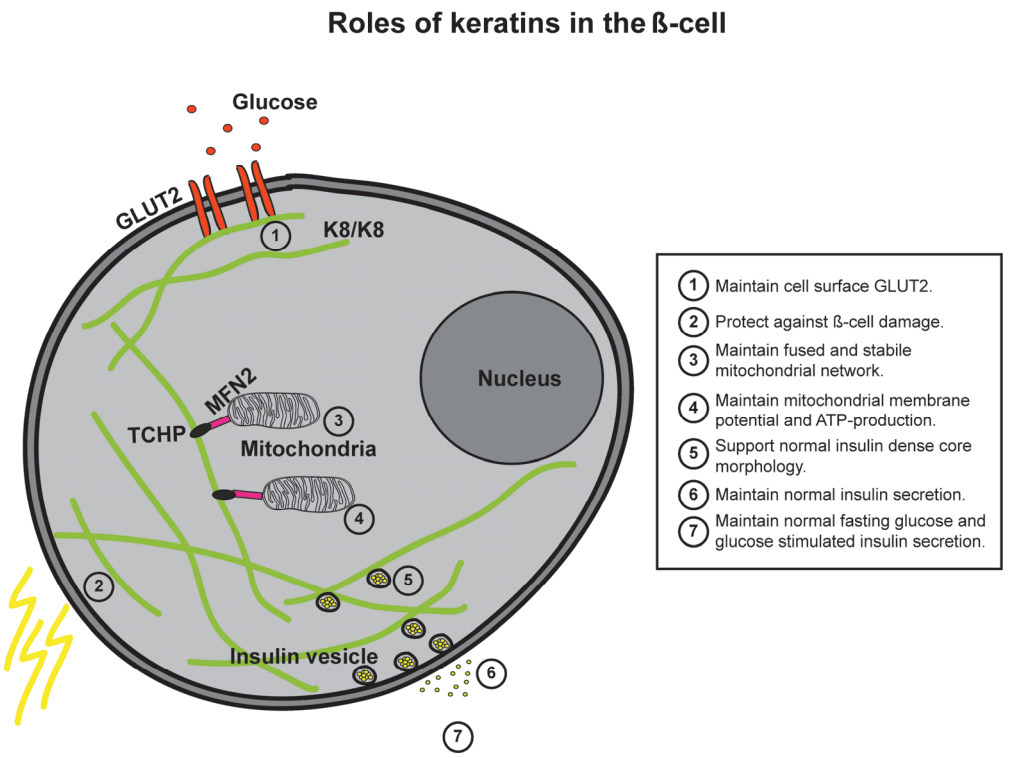

Figure 13. Roles of keratins in the β-cell. *Keratins maintain vital functions, such as mitochondrial health, insulin vesicle mitochondria and insulin level, in β-cells.*

FUTURE PROSPECTS

Whether and how keratin filaments can regulate insulin vesicle dense cores needs to be further investigated. Is there a direct keratin binding to these vesicles or does the interaction take place via cytolinkers, such as plectin or TCHP? MTs and MFs are involved in the transport and secretion of insulin vesicles; thus, it would be essential to investigate the relationship between keratins and insulin vesicles. Another key protein worth investigating is the scaffolding protein 14-3-3. The 14-3-3 proteins are known to bind to keratins and, due to their scaffolding properties, involved in several cell signaling pathways (Liffers et al., 2011). Answering these questions could greatly advance the knowledge about keratins and insulin secretion.

Another crucial question is whether the findings in this thesis also true in human β-cells. Therefore, a study regarding keratin impact on mitochondria and insulin vesicles in human β-cells can be of great interest. A potential connection between human patients with keratin mutations and diabetes susceptibility needs to be investigated. My study on this particular field is one of the first. One possible reason why the role of keratins in diabetes has been understudied is that keratins have been traditionally considered as static cellular stress protectors in epithelial cells, and as a result keratins are often omitted from biochemical analysis results due to poor knowledge about simple epithelial keratins. This underscores the importance of expanding the research of keratins in relation to diabetes. I believe that the novel knowledge about keratins in the endocrine pancreas in this thesis, constitute a good foundation for future research on the potential involvement of keratins in diabetes susceptibility and progression.

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Keratins in the endocrine pancreas

- Novel regulators of cellular processes in β-cells

This Ph.D. thesis describes the role of keratins in the endocrine pancreas. It shows that keratins are maintaining normal insulin levels by involvement in β-cell mitochondrial ATP production and insulin vesicle morphology. On systemic level, keratins in β-cells regulate basal blood glucose levels, most likely in combination with insulin sensitive tissues. In addition, keratins are crucial for β-cell stress protection against chemically induced T1D in mice. These novel findings on insulin production and cell stress protection in β-cells, shed light on the potential role of keratins in diabetes susceptibility and progression.

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