Review

Pancreatic β-cell signaling: toward better understanding of diabetes and its treatment

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Abstract: Pancreatic β-cells play a central role in the maintenance glucose homeostasis by secreting insulin, a key hormone that regulates blood glucose levels. Dysfunction of the β-cells and/or a decrease in the β-cell mass are associated closely with the pathogenesis and pathophysiology of diabetes mellitus, a major metabolic disease that is rapidly increasing worldwide. Clarification of the mechanisms of insulin secretion and β-cell fate provides a basis for the understanding of diabetes and its better treatment. In this review, we discuss cell signaling critical for the insulin secretory function based on our recent studies.

Keywords: cell signaling, insulin, cAMP, Epac2, pancreatic acinar cell, transdifferentiation

Introduction

The pancreatic β-cell plays a central role in the maintenance of glucose homeostasis by secreting insulin, a key metabolic hormone that regulates blood glucose levels. Dysfunction of the β-cell and/or a decrease in β-cell mass are associated closely with the pathogenesis and pathophysiology of diabetes mellitus, a metabolic disease that has come to afflict more than 250 million people in the world at the present time and is still increasing in prevalence globally.[1)] Studies of pancreatic β-cells provide a basis for understanding diabetes mellitus and its better treatment as well as the regulation of blood glucose levels. Current trends of pancreatic β-cell research include the mechanisms of the regulation of insulin secretion by cell signaling, the mechanisms of β-cell fate, the roles of the β-cell in the pathogenesis and pathophysiology of diabetes mellitus, and the development of novel therapies targeting the β-cell. In the past two decades, we have focused on the following issues: 1) identification of novel signaling pathways (molecules) in the regulation of insulin secretion, 2) the manner in which the intracellular signals that regulate insulin secretion are spatially and temporally integrated in β-cells, 3) the mechanism of acquisition of insulin secretory function in the process of β-cell differentiation, and 4) the relationship between abnormalities in β-cell signaling and the development of diabetes. We are also endeavoring to translate the information obtained by our basic research into clinical practice. In this review, the cell signaling critical for insulin secretory function will be discussed by describing our recent studies on the potentiation of insulin secretion by cAMP signaling and the induction of insulin secreting-cells from exocrine pancreas *in vitro*.

1. Potentiation of insulin secretion by cAMP signaling

Stimulus–secretion coupling is a fundamental biological event in the β-cells.[2),3)] Ca^{2+}, ATP, cAMP, and phospholipid-derived signals such as inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) are the major intracellular signals in insulin secretion (Fig. 1). Glucose-stimulated insulin secretion (GSIS) is the principal mechanism of insulin secretion.

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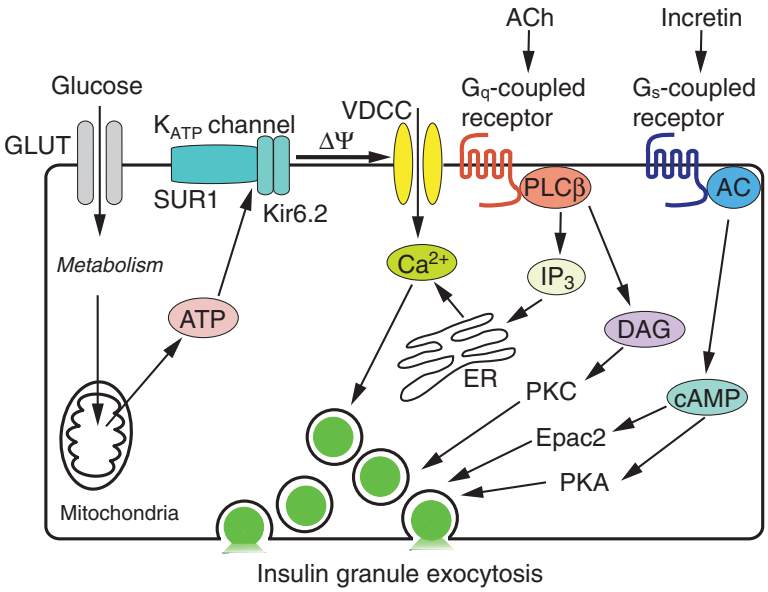

Fig. 1. GSIS and its potentiation. Glucose is transported into the pancreatic β-cell by the glucose transporter (GLUT). Metabolism of glucose increases ATP production, closing the K_{ATP} channels, which results in membrane depolarization ($\Delta\psi$), thus opening of voltage-dependent calcium channels (VDCC) and allowing Ca^{2+} influx. The resultant rise in $[Ca^{2+}]_i$ triggers insulin secretion. Insulin secretion is also modulated by hormones and neurotransmitters. Incretins such as GLP-1 and GIP bind to Gs-coupled receptors and activate adenylyl cyclase (AC), which increases intracellular levels of cyclic AMP. cAMP activates both PKA and Epac2 to potentiate insulin secretion. Achetylcholine (ACh), a major parasympathetic neurotransmitter, binds to Gq-coupled receptors and activates phospholipase Cβ (PLCβ). PLCβ activation generates phospholipid-derived messengers. Among these, DAG activates PKC and IP3 mobilizes Ca^{2+} from intracellular storage sites.

According to the generally accepted model of GSIS, an increase in the ATP concentration (or ATP/ADP ratio) due to increased glucose metabolism closes the ATP-sensitive K^+ (K_{ATP}) channels, depolarizing the β-cell membrane and opening the VDCCs, which allows Ca^{2+} influx. The resultant rise in $[Ca^{2+}]_i$ in the β-cell triggers exocytosis of insulin granules. Thus, K_{ATP} channels couple metabolic changes to membrane potential.[4] In addition to GSIS, signals involved in the potentiation of GSIS are also important for normal regulation of insulin secretion. These include cAMP, DAG, and IP3, among which cAMP is particularly important for amplifying GSIS. cAMP is now known to have diverse actions in insulin secretion. We have recently found that the cAMP sensor cAMP-GEFII (Epac2) (referred to as Epac2 hereafter)-mediated mechanism is required for the action of sulfonylureas, widely used drugs in the treatment of diabetes, as well as for the potentiation of GSIS by cAMP in a protein kinase A (PKA)-independent manner.

Modes of insulin granule exocytosis. Insulin granule exocytosis includes several steps including recruitment, docking, priming, and fusion to the plasma membrane. Recently, investigation of insulin granule exocytosis has been refined by the use of the total internal reflection fluorescence microscopy (TIRFM) system.[5)–7)] We monitored the dynamics of insulin granule exocytosis from primary cultured pancreatic β-cells infected with adenovirus carrying insulin fused with yellow fluorescent protein (Venus).[6)] Analyses by TIRFM in detail revealed that the exocytotic process can be classified into three modes based on the dynamics of the granules: mode 1, in which predocked granules are immediately fused to the plasma membrane by stimulation (named *old face*); mode 2, in which granules are newly recruited by stimulation and immediately fused (a docking state can hardly be detected by TIRFM) to the plasma membrane (named *restless newcomer*); and mode 3, in which granules are newly recruited by stimulation, but are first paused or docked and then fused to the plasma membrane (named *resting newcomer*)[6)] (Fig. 2A). We investigated to determine whether the different modes of insulin granule exocytosis might be caused by different stimuli. Fusion events induced by potassium stimulation occur immediately upon stimulation with potassium, and are transient. The majority of these granules are *old face*. In contrast, fusion events

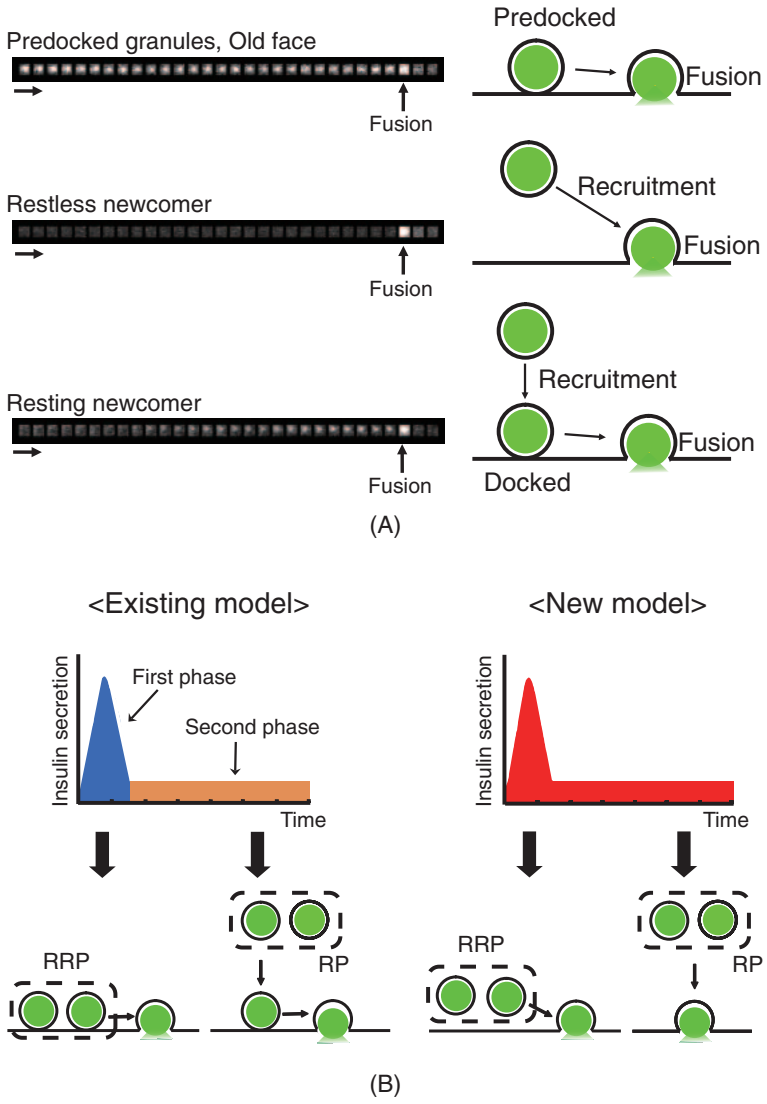

Fig. 2. A. Modes of insulin granule exocytosis. There are three modes of insulin granule exocytosis, based on the dynamics of the granules. *Old face*: predocked granules that are fused to the plasma membrane by stimulation. *Restless newcomer*: granules that are newly recruited by stimulation and immediately fused to the plasma membrane. *Resting newcomer*: granules that are newly recruited by stimulation, docked, and fused to the plasma membrane by stimulation. Sequential images (1 μm × 1 μm) acquired every 300 msec show these different dynamics; the flash indicates the fusion events. B. Models of GSIS. In the existing model of GSIS (Ref. 8), the first phase results from a readily releasable pool (RRP) comprising docked insulin granules ("*old face*" as defined); the second phase results from a reserve pool (RP) comprising granules located farther away ("*resting newcomer*" as defined). In the new model, a RRP responsible for the first phase is located more than 50 nm from the plasma membrane but is yet immediately releasable, and both the first and second phases of GSIS comprise *restless newcomer*. Blue, orange, and red indicates insulin granule exocytosis comprising *old face*, *resting newcomer*, and *restless newcomer*, respectively.

induced by glucose stimulation are biphasic: a first phase of acute increase and a second phase of sustained increase. Interestingly, both phases of fusion events induced by glucose stimulation involve mostly *restless newcomer*. The existing model of GSIS indicates that the first phase of insulin secretion results from the fusion of insulin granules from a readily releasable pool (RRP) comprising predocked granules (*old face*)[8]; the second phase results from fusion of insulin granules from a reserve pool (RP) comprising granules located farther away.[9] Our data suggest a new model of exocytosis in which both phases are caused by newly recruited granules (*restless newcomer*), some of which are from a

distance away from the plasma membrane yet are readily releasable (Fig. 2B). In fact, we found that the granule pool providing *restless newcomer* is likely to be located more than 50 nm from the plasma membrane.[10)]

To ascertain which granule pools are involved in glucose and potassium stimulation, β-cells were sequentially treated with the secretagogues under two different protocols.[10)] In one protocol, in which the cells were stimulated with high potassium (60 mM) after glucose stimulation (16.7 mM), the fusion events occurred immediately and transiently, and were caused mainly by *old face*, as was found in the case of potassium stimulation alone without initial glucose stimulation. In another protocol, in which the cells were stimulated with glucose after potassium stimulation, both the first and second phases of fusion events induced by glucose stimulation were caused mostly by *restless newcomer*, similar to the case in which the cells were stimulated with glucose alone without initial potassium stimulation. These findings indicate that potassium stimulation and glucose stimulation induce fusion of granules from distinct pools.

Importance of cAMP signaling in insulin secretion. It is well known that oral administration of glucose induces a greater insulin secretion than a comparable glucose challenge given intravenously.[11),12)] This is the so-called incretin effect, which refers to the potentiating action of gastrointestinal hormones on insulin secretion, including GLP-1 (Glucagon-like peptide 1) and GIP (glucose-dependent insulinotropic polypeptide, originally called gastric inhibitory polypeptide).[13),14)] GLP-1 and GIP are released in response to the ingestion of nutrients from gastrointestinal endocrine L-cells and K-cells, respectively.[15)] Both of these hormones potentiate GSIS[16)] by activation of cAMP signaling in pancreatic β-cells.[17)–19)] cAMP acts on insulin secretion at various steps.[20)–22)] In normal pancreatic islets, *in vitro* concentration-dependency of GSIS displays a sigmoidal curve[23)] in which a glucose concentration exceeding 6 mmol/l is required for triggering insulin secretion. On the other hand, a study in man showed that GLP-1 infusion induced a significant increase in insulin secretion even at fasting glucose levels of around 5.1 mmol/l.[24)] In addition, it has been reported that GLP-1 endows glucose-insensitive β-cells with glucose-competency, probably by modulating K_{ATP} channel activity.[25)] These findings suggest a mechanism by which cAMP may induce glucose responsiveness of pancreatic β-cells. We recently found that treatment with GLP-1 induced glucose responsiveness in K_{ATP} channel-deficient (Kir6.2 null) mice in which there was almost no insulin secretion in response to glucose,[22)] indicating that activation of cAMP signaling is important for induction of glucose responsiveness in K_{ATP} channel-deficient β-cells and that GLP-1 induces GSIS by the K_{ATP} channel-independent mechanism.

GSIS is usually assessed by the insulin response to a rapid and large increase in glucose concentration, *e.g.*, from 2.8 mM to 16. 7 mM *in vitro*. However, such drastic change in glucose concentration is unlikely to occur *in vivo*, in which blood glucose levels gradually increase within a narrow range when glucose is taken orally. To confirm the importance of cAMP signaling in GSIS under physiological conditions, we increased the glucose concentration in a stepwise manner in the presence or absence of cAMP in perfused pancreas. Interestingly, these small and stepwise increases in glucose concentration from 2.8 mM to 11 mM failed to trigger insulin secretion in the absence of 8-Bromo-cAMP or GLP-1, but dramatically evoked insulin secretion in its presence. Such GSIS was almost completely abolished by treatment with niflumic acid. Considering that the blood glucose concentrations increase gradually after meal ingestion from 4–5 mmol/l to less than 8.0 mmol/l[26)] with concomitant rises in incretin levels, pancreatic β-cell cAMP signaling activated by incretins may well play an important role in maintaining the blood glucose level within the physiological range. Thus, niflumic-sensitive channels seem to be critically involved in the mechanism of induction of GSIS by cAMP in the physiological state.[22)]

Role of Epac2 (cAMP-GEFII)/Rap1 signaling in insulin secretion. cAMP is a universal intracellular second messenger involved in regulation of various cellular functions in many cell types. It has long been thought that cAMP regulates the potentiation of insulin secretion through PKA phosphorylation of various proteins associated with the insulin secretory process.[2),27)–29)] However, it is now known that cAMP potentiates insulin granule exocytosis by PKA-independent as well as PKA-dependent mechanisms, the former involving the cAMP-binding protein Epac2.[30)–32)] Epacs, Epac1 (also known as cAMP-GEFI) and Epac2 (cAMP-GEFII), have so far been identified.[33)–35)] Recently, a novel variant Epac2 lacking the N-terminal cAMP-binding domain of the known Epac2 was found in mouse adrenal glands and designated Epac2B (*i.e.* adrenal type); Epac2 is now called Epac2A (brain/β-cell type).[36)] A previously

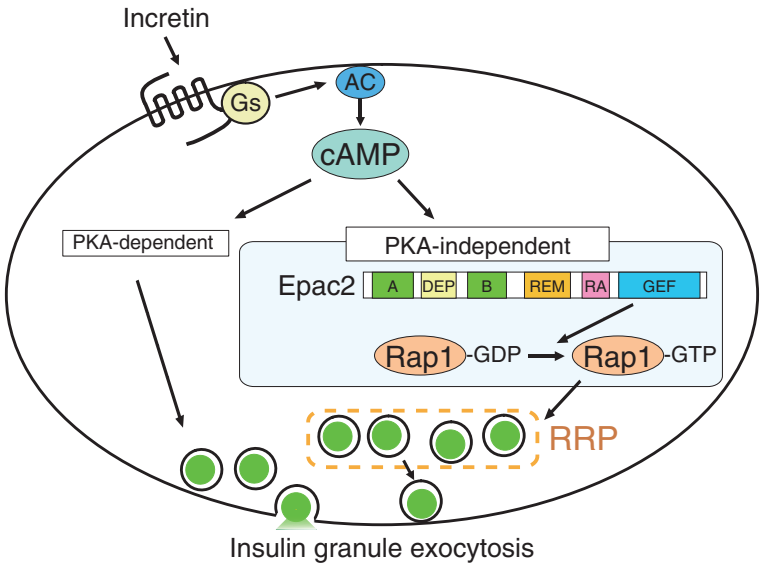

Fig. 3. Potentiation of insulin secretion by Epac2/Rap1 signaling. Activation of Epac2/Rap1 signaling promotes cAMP-induced, PKA-independent insulin granule exocytosis by increasing the size of a readily releasable pool (RPP) near the plasma membrane. Epac2 includes cAMP-binding domain A and cAMP-binding domain B, DEP domain, REM, RA domain, and GEF.

identified variant, which is specifically expressed in liver, is referred to as Epac2C (liver type).[37] Both Epac1 and Epac2 proteins possess guanine nucleotide exchange activity towards the small G-proteins Rap1 and Rap2 in a cAMP-dependent manner.[33)–35)] Structurally, both Epac proteins have common features: a cAMP-binding domain and DEP (Dishevelled, Egl-10, and Pleckstrin), REM (Ras exchange motif), and GEF (guanine nucleotide exchange factor) domains. Epac1 possesses one cAMP-binding domain while Epac2 possesses two cAMP-binding domains. Binding of cAMP to Epac2 is thought to cause conformational changes that elicit GEF activity toward Rap proteins.[38)]

We previously showed that Epac2 mediates the potentiation of cAMP-dependent, PKA-independent insulin secretion.[27),31),39)] To clarify the role of Epac2 in insulin granule exocytosis directly, we examined exocytosis in pancreatic β-cells isolated from Epac2-deficient (Epac2 null) mice using the TIRFM system.[6)] Although there was no difference in glucose-induced exocytosis of insulin granules, we found that potentiation by cAMP of the first phase of glucose-induced exocytosis was significantly impaired in Epac2 null mice.[6)] In addition, Rap1, which is activated by cAMP specifically through Epac2 in pancreatic β-cells, was found to be required for PKA-independent cAMP-potentiated insulin secretion. Thus, Epac2/Rap1 signaling is important for the potentiation by cAMP of the first phase of GSIS. We therefore propose that Epac2/Rap1 signaling regulates cAMP-induced insulin granule exocytosis by controlling the size of the RRP, most likely through regulation of granule density[6),10)] (Fig. 3).

Epac2 as a novel target of antidiabetic sulfonylurea drugs. K^+ channels regulated by intracellular ATP concentrations were first reported in guinea pig and rabbit cardiac cell membranes[40)] and were later found in many cell types, including pancreatic islet cells.[4)] K_{ATP} channels are critical in the regulation of GSIS by coupling ATP, produced by the metabolism of glucose, to electrical activity of the β-cell membrane, leading to calcium influx through opening of VDCCs. It was found that tolbutamide, an antidiabetic drug, inhibits K_{ATP} channels in pancreatic β-cells, which suggested that the channels are the target of sulfonylureas (SUs).[41)] In 1995, the sulfonylurea receptor (now called SUR1) from pancreatic β-cell cDNA libraries was cloned by Aguilar-Bryan *et al.*[42)] SUR1 is a member of the ATP-binding cassette (ABC) protein superfamily. We cloned Kir6.2,[43)] a member of the inwardly rectifying K^+ channel family, and established that the β-cell K_{ATP} channel is composed of Kir6.2 and SUR1,[43)] which provided a basis of understanding of K_{ATP} channels. Kir6.1 was cloned prior to Kir6.2.[44)] The K_{ATP} channel is a hetero-octameric complex comprising two subunits: a pore-forming subunit Kir6.x (Kir6.1 or Kir6.2) and a regulatory subunit SURx (SUR1, SUR2A, or SUR2B).[45),46)] Different

combinations of Kir6.1 or Kir6.2 and SUR1 or SUR2 variant (mix and match) form K_{ATP} channels with differing nucleotide and SU sensitivities that play distinct physiological and pathophysiological roles in different tissues.[47] While Kir6.2 plus SUR1 constitutes pancreatic β-cell K_{ATP} channels, Kir6.2 plus SUR2A constitutes cardiac and skeletal muscle K_{ATP} channels. Kir6.2 plus SUR2B constitutes smooth muscle K_{ATP} channels and Kir6.1 plus SUR2B constitutes vascular smooth muscle K_{ATP} channels, both of which are somewhat ATP-insensitive, nucleotide diphosphate-activated, and glibenclamide-sensitive K^+ channels. It is now known that mutations of K_{ATP} channels cause persistent hyperinsulinemic hypoglycemia of infancy and neonatal diabetes, depending on loss-of-function mutation or gain-of-function mutation.[48] K_{ATP} channels, as metabolic sensors, play critical roles in various functions such as insulin secretion, glucagon secretion,[49] and protection against hypoxia-induced seizure[50] and ischemic heart.[51]

Mice lacking K_{ATP} channels (Kir6.2 null mice and SUR1 null mice) were generated.[52),53] Neither glucose nor tolbutamide stimulation elicited any change in $[Ca^{2+}]_i$ in Kir6.2 null β-cells.[52] Importantly, neither glucose nor tolbutamide stimulation caused a significant insulin secretion in Kir6.2 null mice. The study of SUR1 null mice confirmed also that both glucose-induced and sulfonylurea-induced insulin secretion depend critically on the activity of β-cell K_{ATP} channels.[53] Based on these findings, it is generally accepted that the primary target of SUs is SUR1 and that action of SUs is mediated by closure of the K_{ATP} channels through binding to SUR1.

In the course of our study of the Epac2-mediated mechanisms of insulin secretion, we developed a fluorescence resonance energy transfer (FRET)-based Epac2 sensor (termed C-Epac2-Y) in which the full-length Epac2 is fused amino-terminally to enhanced cyan fluorescent protein (ECFP) and carboxyl-terminally to enhanced yellow fluorescent protein (EYFP).[54] Epac2 is a closed form in the inactive state,[32] so that ECFP and EYFP are located very closely to each other (within 10 nm), which causes FRET. Upon cAMP binding to Epac2, Epac2 changes its conformation to an open form. As a result, ECFP and EYFP separate away, so that FRET does not occur (active state). Utilizing this principle, we are able to monitor the activation status of Epac2. In the search for agents that activate Epac2 using this FRET sensor, we found that tolbutamide, glibenclamide, chlorpropamide, acetohexamide, and glipizide significantly decrease the FRET response in COS-1 cells transfected with Epac2 FRET sensor, in different degrees and varying kinetics, suggesting strongly that these SUs activate Epac2. However, gliclazide, another SU, does not decrease the FRET response. These findings indicate that the core structure of sulfonylurea is required for binding to Epac2. The fact that gliclazide, a sulfonylurea having a side chain on the urea group larger than that of other sulfonylureas, does not decrease FRET suggests that the structure or size (or both) of the side chain on the urea group determines the capability of interaction between sulfonylureas and Epac2. Direct binding of SU to Epac2 was confirmed by specific binding of radiolabeled glibenclamide to Epac2 expressed in COS-1 cells. Binding of [^{3}H]glibenclamide to Epac2 was inhibited by unlabeled tolbutamide or unlabeled glibenclamide in a concentration-dependent manner, indicating that tolbutamide binds specifically to Epac2 at the same binding site as glibenclamide does. Specific binding of glibenclamide to Epac2 was not significantly inhibited in the presence of 8-Bromo-cAMP, suggesting that the sulfonylurea-binding site is distinct from the cAMP-binding sites.

We also found that tolbutamide and glibenclamide activate Rap1 in clonal pancreatic β-cells (MIN6 cells), but gliclazide does not. Interestingly, although there was no difference in GSIS between wild-type mice and Epac2 null mice, tolbutamide-induced insulin secretion and glibenclamide-induced insulin secretion from isolated pancreatic islets of Epac2 null mice were significantly reduced, compared to those of wild-type mice. However, there was no significant difference in insulin secretion in response to gliclazide between wild-type mice and Epac2 null mice. Furthermore, the insulin response to oral administration of tolbutamide alone or concomitant administration of glucose and tolbutamide in Epac2 null mice was significantly reduced, compared to that in wild-type mice, and the glucose lowering effect of tolbutamide in Epac2 null mice was significantly less than that in wild-type mice.

As described above, it is well established that SUs stimulate insulin secretion by eliciting a series of ionic events including closure of K_{ATP} channels, opening of VDCCs, and Ca^{2+} influx into the β-cells. Although closure of the K_{ATP} channels is prerequisite for SUs to stimulate insulin secretion, activation of Epac2/Rap1 signaling is required for SUs to exert their full effects in insulin secretion. Considering the role of Epac2/Rap1 signaling in insulin granule

exocytosis described above, SUs may well increase the size of a RRP of insulin granules near the plasma membrane (Fig. 4).

2. Induction of insulin secreting-cells from pancreatic exocrine cells *in vitro*

Although it has been reported that embryonic stem (ES) cells can be manipulated to produce insulin,[55)–58)] there are several obstacles to their clinical use. Transplantation of ES cell derivatives into human recipients can result in formation of ES cell-derived tumors.[59)] Ethical problems also arise in the acquisition of human ES cells.[60)] In addition, it has been suggested that both the production and the release of insulin in such manipulated ES cells may be abnormal.[61),62)] On the other hand, recent studies have shown that insulin-secreting cells can be generated *in vitro* from adult non-β-cells including mouse and human pancreatic duct cells,[62)–65)] rat hepatic oval cells,[66)] and mouse bone marrow cells.[67)] Although it was thought that terminally differentiated cells do not change their phenotype, accumulating evidence suggests that phenotypic plasticity is retained in differentiated cells. The most intriguing example is induced-pluripotent stem (iPS) cells, which have ES-cell like properties, generated from somatic cells by introducing defined factors.[68)] Insulin-secreting cells have been generated from such iPS cells.[69)] However, the use of iPS cells retains problems similar to those involved in the use of ES cells except for the ethical issues. On the other hand, it has been shown that adult cells occasionally change their phenotype without reprogramming into ES-like pluripotent cells in chronic damage and tissue regeneration, a phenomenon often called transdifferenation.[70)] The pancreas is an organ in which metaplasia, a pathological state involving transdifferentiation,[71),72)] frequently occurs. For example, hepatocyte-like cells appear in human pancreatic cancer in some cases,[73)] and experimental conditions such as copper depletion can lead to the development of pancreatic hepatocytes in rodents.[74),75)] Since a large number of pancreatic exocrine cells can be obtained as a byproduct of islet transplantation, the exocrine pancreas is a potential source for the generation of transplantable surrogate β-cells. *In vitro* generation of insulin-positive cells from pancreatic exocrine cells has been reported by several groups.[76),77)] However, neither direct evidence of the origin of these cells nor their precise insulin secretory properties was shown in those studies.

Generation of insulin-positive cells from pancreatic exocrine cells. We have established a method for the generation of insulin-secreting cells from pancreatic exocrine cells in mice.[78)] The pancreatic exocrine cell-enriched fraction was prepared by Ficoll density gradient centrifugation. By this method, pancreatic exocrine cells are recovered as a pellet, and are then stained with dithizone, a zinc-chelating agent, to remove contaminated pre-existing pancreatic β-cells. The resulting fraction contains >90% of amylase-positive cells, approximately 5% of cytokeratin-positive cells, and less than 0.01% of insulin-positive cells. The exocrine cell-enriched fractions were then cultured in RPMI-1640 medium supplemented with 0.5% fetal calf serum (FCS) and 20 ng/ml of EGF. Under these conditions, the cells readily formed aggregates and became smooth spheroids within a few days (Fig. 5). We found that a subset of the cells in these colonies expressed insulin. Most insulin-positive cells were detected at the peripheral of small colonies. When the isolated pancreatic exocrine cells were cultured in suspension using dishes treated with 2-methacryloyloxyethyl phosphorylcholine (MPC), which interferes with cell attachment, insulin production was increased compared to that in monolayer culture. The frequency of insulin-positive cells was increased to ~5% of total cells on day 4. The insulin-positive cells also expressed C-peptide, indicating *de novo* biosynthesis of insulin in these cells. Both amylase and elastase, which are acinar cell-specific enzymes, were strongly detected before culture (day 0) at both mRNA (RT-PCR) and protein (immunoblotting) levels. Immunocytochemistry showed that both amylase- and elastase-positive cells comprised more than 90% of total cells in the initial preparation (day 0), but less than 5% 4 days after culture. While CK (ductal marker)-positive cells were rare at day 0, CK-positive ductal structures were found frequently at day 4 and day 6. Although insulin-positive cells were generally negative for amylase, a few cells clearly expressed both insulin and amylase, suggesting that the insulin-producing cells in the culture are derived from amylase/elastase-expressing mature pancreatic acinar cells.[78)]

Origin of the newly made insulin-positive cells. To identify the origin of the newly-made insulin-producing cells, we utilized the method of cell lineage tracing. In this method, ROSA26-loxP-stop-loxP-ECFP reporter mouse (R26R-ECFP)-expressing ECFP that can be activated through the action of Cre recombinase[79)] and adenoviruses in which either amylase-2 (Ad-pAmy-Cre) or elastase-1 (Ad-pEla-

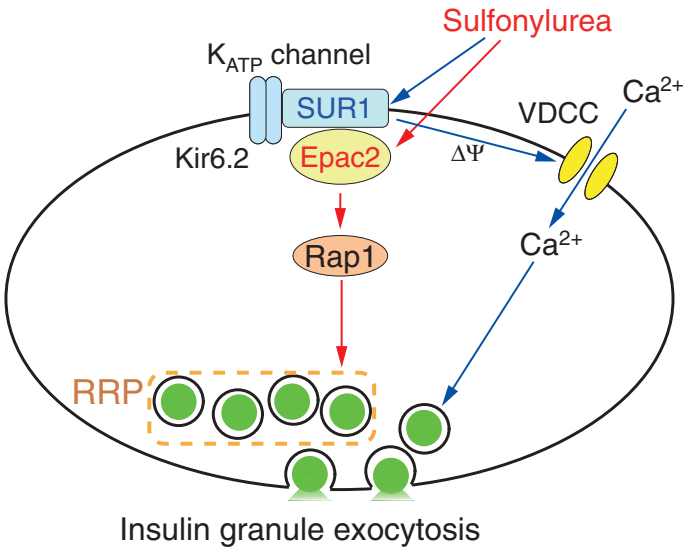

Fig. 4. Model of sulfonylurea stimulation of insulin secretion. SUs close K_{ATP} channels by binding to SUR1, which leads to calcium influx by opening of VDCCs. SUs also bind Epac2 to activate Rap1 signaling, which increases the size of a RRP of insulin granules near the plasma membrane.

Cre) promoter that drives the expression of Cre recombinase are used. Pancreatic exocrine cells from R26R-ECFP mice were infected with these adenoviruses to label pancreatic acinar cells, and cultured as described. After the culture, ECFP-expressing insulin-positive cells were frequently found, demonstrating that the insulin-positive cells originate from amylase-expressing pancreatic acinar cells.[78] We also found that glucagon-positive cells, somatostatin-positive cells, and a subset of CK-positive cells in the culture also originate from the acinar cells.[80] Accordingly, pancreatic acinar cells possess plasticity to convert into both endocrine and ductal cells.

Properties of the newly made cells. Glucose transporters, glucokinase, K_{ATP} channels, VDCCs, molecules associated with the exocytotic machinery (SNAREs), and prohormone convertases (PC1/3 and PC2) are required for proper GSIS. The expressions of all of these molecules were induced or up-regulated in pancreatic acinar cells after the culture. The expression profiles of the exocrine pancreas-derived insulin-producing cells became similar to those of pancreatic islets. In addition, formation of insulin-containing granules was confirmed by immunoelectron microscopy, indicating that these cells can secrete insulin in a regulated manner. Indeed, KCl at a high concentration stimulated insulin secretion from the pancreatic acinar-derived cells, indicating the occurrence of Ca^{2+}-triggered exocytosis. Insulin secretion also was

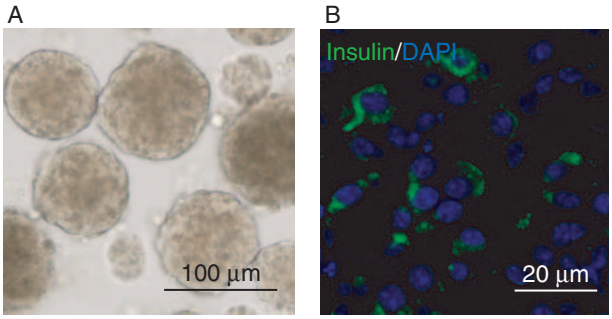

Fig. 5. Spheroids formed by pancreatic acinar cell culture. A. The pancreatic acinar cells form spherical cell clusters within a few days by culture with EGF. B. Insulin-positive cells are shown in culture.

increased by glibenclamide, a sulfonylurea commonly used in treatment of diabetes, indicating that functional K_{ATP} channels are expressed. Importantly, GSIS from exocrine pancreas-derived cells was detected in a concentration-dependent manner, indicating that the cells are glucose responsive. In addition, GLP-1 (7-36 amide), an incretin, potentiated insulin secretion in the presence of relatively high concentrations of glucose, indicating that the cAMP-mediated potentiation system also is present in the cells (Fig. 6). These results show that pancreatic acinar-derived cells have qualitatively similar insulin secretory properties to those of native pancreatic β-cells. However, insulin content in these

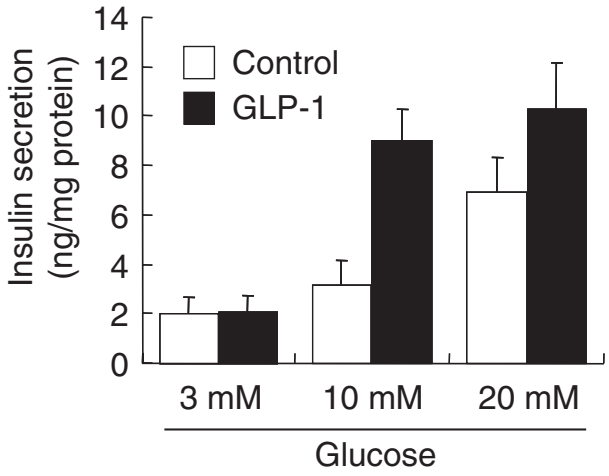

Fig. 6. Insulin secretion from transdifferentiated pancreatic acinar cells. Insulin secretion is stimulated by glucose and enhanced by glucagon-like peptide-1 (GLP-1) (7–36 amide) (100 nM).

pancreatic acinar-derived cells (including both insulin-positive and insulin-negative cells) is only 1/400 that of native pancreatic islets. Since the frequency of insulin-positive cells is approximately 5% of total cells in the culture, the insulin content of the newly made insulin-positive cells is about 1/20 that of a native β-cell[78] on a cell to cell basis.

Dedifferentiation and redifferentiation processes in induction of the insulin-secreting cells. During development of the pancreas, several transcription factors are known to play critical roles in β-cell differentiation. Pdx1, a transcription factor seen early in the developing pancreas and restricted to β-cells in adults, was induced in pancreatic acinar-derived cells by the culture at both mRNA and protein levels. Other transcription factors characteristic of endocrine pancreas (NeuroD1, Isl1, Pax6, Nkx2.2, Nkx6.1, Hlxb9, HNF1a, and Foxa1) also were induced. Foxa2 is expressed in early endoderm[81] and all pancreatic cell types including endocrine and exocrine cells in adult.[82] Although we could not detect Foxa2 expression in isolated acinar cells, probably due to the low expression level, Foxa2 was induced at both mRNA and protein levels after the culture. HNF6, which is required for the expression of Foxa2,[83] also was induced. Ptf1a expression was increased by the culture. Although Ptf1a was thought to be an exocrine-specific transcription factor,[84] a recent study has shown that this factor also is expressed in the pancreatic bud and is required for differentiation into all three cell types (endocrine, exocrine, and duct) of the pancreas.[85] These results suggest that pancreatic acinar cells dedifferentiate into cells having the properties of endocrine progenitors.

Cell signaling required for induction of insulin-secreting cells from pancreatic acinar cells *in vitro*. We found that enzymatic dissociation of the pancreas leads to activation of the EGF receptor and its downstream signaling. The EGF receptor was not activated before dissociation but was activated (tyrosine phosphrylated) by enzymatic dissociation. When an inhibitor of EGF receptor kinase (AG1478) was applied, transdifferentiation of isolated pancreatic acinar cells into insulin-secreting cells was inhibited,[78] demonstrating that activation of the EGF receptor is essential for the transdifferentiation. In embryonic pancreas, EGF increases the number of undifferentiated endocrine precursor cells, and, upon removal of EGF, a large number of β-cells are differentiated,[86] suggesting that EGF may be important for the proliferation of endocrine precursors and/or endow the cells with commitment to endocrine lineage. We have found that activation of the PI3-kinase pathway is critical for the transdifferentiation of pancreatic acinar cells into insulin-secreting cells and have shown that isolated pancreatic acinar cells form spheroids (3-D structures) when cultured in the presence of EGF.[87] In the presence of a PI3-kinase inhibitor, LY294002, the formation of spheroids was blocked and induction of the genes characteristic of pancreatic β-cells was significantly suppressed, demonstrating that PI3-kinase is essential for the transdifferentiation of pancreatic acinar cells into insulin-secreting cells. These results suggest that cell–cell interaction is involved in the process. Although cadherin-mediated cell–cell adhesions were disrupted by enzymatic dissociation of pancreatic acinar cells, they were recovered by culture with EGF. Since cell-to-cell contact is known to play important roles in fate determination of many cell types, the impacts of inhibition of cell-to-cell contact on such transdifferentiation were investigated using a neutralizing antibody against E-cadherin (ECCD-1). When spheroid formation in isolated acinar cells was inhibited by addition of the antibody, inductions of genes specific for pancreatic β-cells were significantly suppressed, while transcription factors seen early in pancreas development such as HNF6 and Foxa2 were still up-regulated[87] (Fig. 7). Activation of PI3-kinase resulted in up-regulation of both E-cadeherin and β-catenin, major components of cadherin-mediated cell–cell adhesion, and blocked degradation of these proteins, which leads to the recovery of the adhesion.

It has been reported that acinar-to-β cell transdifferentiation is not found *in vivo*.[88] This is probably because loss of cell adhesion of pancreatic acini rarely occurs *in vivo*.

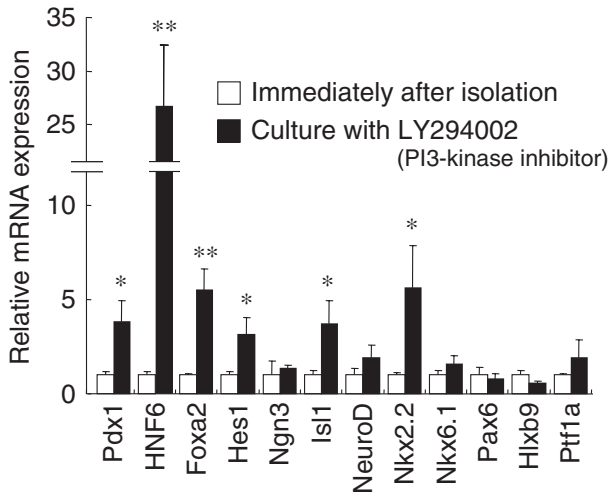

Fig. 7. Dedifferentiated state in pancreatic acinar cells. Gene expression profiling of isolated pancreatic acinar cells cultured in the presence of LY294002 and nicotinamide was performed. Quantification of changes in gene expression is shown. The mRNA expression level of each gene on day 0 was considered as 1. $^{*}P < 0.05$; $^{**}P < 0.01$, unpaired t-test.

Model of the mechanism of induction of insulin-secreting cells from pancreatic acinar cells *in vitro*. A model of pancreatic acinar-to-β cell transdifferentiation is depicted in Fig. 8: enzymatic dissociation of exocrine pancreas disrupts the epithelial structures of acini, including cadherin-mediated cell–cell adhesion, which causes dedifferentiation of the acinar cells. Spontaneous activation of the EGF receptor and its downstream signals occurs after isolation of the cells, and the addition of EGF enhances these signals. Remodeling of cell–cell interaction by restored cadherin-mediated cell–cell adhesion, which is promoted by activation of the PI3-kinase pathway, induces redifferentiation of the dedifferentiated pancreatic acinar cells into insulin-secreting cells. Thus, loss and restoration of PI3-kinase-dependent cadherin-mediated cell–cell adhesion is essential for induction of insulin-secreting cells from pancreatic acinar cells *in vitro*.

Coda

Although the discovery of insulin in 1921 by Banting and Best brought the first and foremost good news to patients with diabetes mellitus, how the pancreatic β-cell produces and secretes insulin and how the β-cell is generated and maintained are still puzzling questions. Normal pancreatic β-cells are

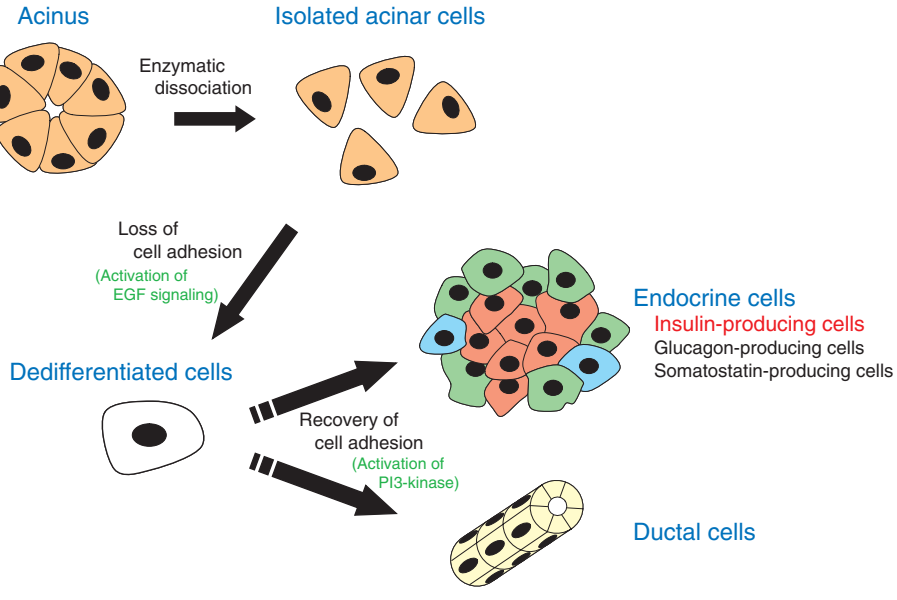

Fig. 8. Model for pancreatic acinar-to-β cell transdifferentiation. Enzymatic dissociation disrupts epithelial structures of acini, resulting in loss of cadherin-mediated cell–cell adhesion, which causes dedifferentiation of the acinar cells. Meanwhile, EGF receptors are activated, which is followed by activation of the PI3-kinase pathway. Within a few days of culture, cadherin-mediated cell–cell adhesion is recovered by the enhanced expression of E-cadherin, which is essential for redifferentiation of the dedifferentiated cells into insulin-secreting cells.

equipped with a very precise apparatus that allows adjustment of insulin secretion in response to moment-to-moment changes in blood glucose levels, and thus precisely maintain blood glucose levels within the narrow physiological range that protects the various organs from the development of the many serious diabetic complications. In addition to insulin, various antidiabetic drugs targeting β-cells have been developed to treat diabetes. Understanding the signaling mechanisms of insulin secretion and those of the generation of β-cells should provide clues for development of novel therapeutic strategies as well as understanding the signaling mechanisms of insulin secretion and those of the generation of β-cells should provide clues for development of novel therapeutic strategies as well as clarification of the pathogenesis and pathophysiology of diabetes.

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References

1) International Diabetes Atlas, http://www.diabetesatlas.org/
2) Prentki, M. and Matschinsky, F.M. (1987) Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol. Rev. **67**, 1185–1248.
3) Wollheim, C.B. and Sharp, G.W. (1981) Regulation of insulin release by calcium. Physiol. Rev. **61**, 914–973.
4) Ashcroft, F.M. (1988) Adenosine 5′-triphosphate-sensitive potassium channels. Annu. Rev. Neurosci. **11**, 97–118.
5) Ohara-Imaizumi, M., Nakamichi, Y., Tanaka, T., Ishida, H. and Nagamatsu, S. (2002) Imaging exocytosis of single insulin secretory granules with evanescent wave microscopy: distinct behavior of granule motion in biphasic insulin release. J. Biol. Chem. **277**, 3805–3808.
6) Shibasaki, T., Takahashi, H., Miki, T., Sunaga, Y., Matsumura, K., Yamanaka, M. *et al.* (2007) Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. Proc. Natl. Acad. Sci. USA **104**, 19333–19338.
7) Kasai, K., Fujita, T., Gomi, H. and Izumi, T. (2008) Docking is not a prerequisite but a temporal constraint for fusion of secretory granules. Traffic **9**, 1191–1203.
8) Rorsman, P. and Renstrom, E. (2003) Insulin granule dynamics in pancreatic β cells. Diabetologia **46**, 1029–1045.
9) Eliasson, L., Abdulkader, F., Braun, M., Galvanovskis, J., Hoppa, M.B. and Rorsman, P. (2008) Novel aspects of the molecular mechanisms controlling insulin secretion. J. Physiol. **586**, 3313–3324.
10) Seino, S., Takahashi, H., Fujimoto, W. and Shibasaki, T. (2009) Roles of cAMP signalling in insulin granule exocytosis. Diabetes Obes. Metab. **11**, 180–188.
11) McIntyre, N., Holdsworth, C.D. and Turner, D.S. (1964) New interpretation of oral glucose tolerance. Lancet **2**, 20–21.
12) Elrick, H., Stimmler, L., Hlad, C.J. Jr. and Arai, Y. (1964) Plasma insulin response to oral and intravenous glucose administration. J. Clin. Endocrinol. Metab. **24**, 1076–1082.
13) Bell, G.I., Santerre, R.F. and Mullenbach, G.T. (1983) Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature **302**, 716–718.
14) Brown, J.C., Mutt, V. and Pederson, R.A. (1970) Further purification of a polypeptide demonstrating enterogastrone activity. J. Physiol. **209**, 57–64.
15) Elliott, R.M., Morgan, L.M., Tredger, J.A., Deacon, S., Wright, J. and Marks, V. (1993) Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J. Endocrinol. **138**, 159–166.
16) Dupre, J., Ross, S.A., Watson, D. and Brown, J.C. (1973) Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab. **37**, 826–828.
17) Thorens, B. (1992) Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc. Natl. Acad. Sci. USA **89**, 8641–8645.
18) Usdin, T.B., Mezey, E., Button, D.C., Brownstein, M.J. and Bonner, T.I. (1993) Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology **133**, 2861–2870.
19) Yasuda, K., Inagaki, N., Yamada, Y., Kubota, A., Seino, S. and Seino, Y. (1994) Hamster gastric inhibitory polypeptide receptor expressed in pancreatic islets and clonal insulin-secreting cells: its structure and functional properties. Biochem. Biophys. Res. Commun. **205**, 1556–1562.
20) Holst, J.J. and Gromada, J. (2004) Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am. J. Physiol. Endocrinol. Metab. **287**, E199–E206.
21) Miki, T., Minami, K., Shinozaki, H., Matsumura, K., Saraya, A., Ikeda, H. *et al.* (2005) Distinct effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 on insulin secretion

and gut motility. Diabetes **54**, 1056–1063.
22) Fujimoto, W., Miki, T., Ogura, T., Zhang, M., Seino, Y., Satin, L.S. *et al.* (2009) Niflumic acid-sensitive ion channels play an important role in the induction of glucose-stimulated insulin secretion by cyclic AMP in mice. Diabetologia **52**, 863–872.
23) Gembal, M., Detimary, P., Gilon, P., Gao, Z.Y. and Henquin, J.C. (1993) Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K^+ channels in mouse B cells. J. Clin. Invest. **91**, 871–880.
24) Vilsboll, T., Krarup, T., Madsbad, S. and Holst, J.J. (2003) Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul. Pept. **114**, 115–121.
25) Holz, G.G.t., Kuhtreiber, W.M. and Habener, J.F. (1993) Pancreatic β-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). Nature **361**, 362–365.
26) Suzuki, H., Fukushima, M., Okamoto, S., Takahashi, O., Shimbo, T., Kurose, T. *et al.* (2005) Effects of thorough mastication on postprandial plasma glucose concentrations in nonobese Japanese subjects. Metabolism **54**, 1593–1599.
27) Seino, S. and Shibasaki, T. (2005) PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol. Rev. **85**, 1303–1342.
28) Malaisse, W.J. and Malaisse-Lagae, F. (1984) The role of cyclic AMP in insulin release. Experientia **40**, 1068–1074.
29) Jones, P.M. and Persaud, S.J. (1998) Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic β-cells. Endocr. Rev. **19**, 429–461.
30) Eliasson, L., Ma, X., Renstrom, E., Barg, S., Berggren, P.O., Galvanovskis, J. *et al.* (2003) SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. J. Gen. Physiol. **121**, 181–197.
31) Ozaki, N., Shibasaki, T., Kashima, Y., Miki, T., Takahashi, K., Ueno, H. *et al.* (2000) cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat. Cell Biol. **2**, 805–811.
32) Bos, J.L. (2006) Epac proteins: multi-purpose cAMP targets. Trends Biochem. Sci. **31**, 680–686.
33) de Rooij, J., Zwartkruis, F.J., Verheijen, M.H., Cool, R.H., Nijman, S.M., Wittinghofer, A. *et al.* (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature **396**, 474–477.
34) Kawasaki, H., Springett, G.M., Mochizuki, N., Toki, S., Nakaya, M., Matsuda, M. *et al.* (1998) A family of cAMP-binding proteins that directly activate Rap1. Science **282**, 2275–2279.
35) de Rooij, J., Rehmann, H., van Triest, M., Cool, R.H., Wittinghofer, A. and Bos, J.L. (2000) Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. J. Biol. Chem. **275**, 20829–20836.
36) Niimura, M., Miki, T., Shibasaki, T., Fujimoto, W., Iwanaga, T. and Seino, S. (2009) Critical role of the N-terminal cyclic AMP-binding domain of Epac2 in its subcellular localization and function. J. Cell. Physiol. **219**, 652–658.
37) Ueno, H., Shibasaki, T., Iwanaga, T., Takahashi, K., Yokoyama, Y., Liu, L.M. *et al.* (2001) Characterization of the gene EPAC2: structure, chromosomal localization, tissue expression, and identification of the liver-specific isoform. Genomics **78**, 91–98.
38) Rehmann, H., Das, J., Knipscheer, P., Wittinghofer, A. and Bos, J.L. (2006) Structure of the cyclic-AMP-responsive exchange factor Epac2 in its auto-inhibited state. Nature **439**, 625–628.
39) Kashima, Y., Miki, T., Shibasaki, T., Ozaki, N., Miyazaki, M., Yano, H. *et al.* (2001) Critical role of cAMP-GEFII—Rim2 complex in incretin-potentiated insulin secretion. J. Biol. Chem. **276**, 46046–46053.
40) Noma, A. (1983) ATP-regulated K^+ channels in cardiac muscle. Nature **305**, 147–148.
41) Sturgess, N.C., Ashford, M.L., Cook, D.L. and Hales, C.N. (1985) The sulphonylurea receptor may be an ATP-sensitive potassium channel. Lancet **2**, 474–475.
42) Aguilar-Bryan, L., Nichols, C.G., Wechsler, S.W., Clement, J.P.t., Boyd, A.E. 3rd, Gonzalez, G. *et al.* (1995) Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science **268**, 423–426.
43) Inagaki, N., Gonoi, T., Clement, J.P.t., Namba, N., Inazawa, J., Gonzalez, G. *et al.* (1995) Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science **270**, 1166–1170.
44) Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M. *et al.* (1995) Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. J. Biol. Chem. **270**, 5691–5694.
45) Aguilar-Bryan, L. and Bryan, J. (1999) Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr. Rev. **20**, 101–135.
46) Seino, S. (1999) ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. Annu. Rev. Physiol. **61**, 337–362.
47) Seino, S. and Miki, T. (2003) Physiological and pathophysiological roles of ATP-sensitive K^+ channels. Prog. Biophys. Mol. Biol. **81**, 133–176.
48) Ashcroft, F.M. (2005) ATP-sensitive potassium channelopathies: focus on insulin secretion. J. Clin. Invest. **115**, 2047–2058.
49) Miki, T., Liss, B., Minami, K., Shiuchi, T., Saraya, A., Kashima, Y. *et al.* (2001) ATP-sensitive K^+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. Nat. Neurosci. **4**, 507–512.
50) Yamada, K., Ji, J.J., Yuan, H., Miki, T., Sato, S., Horimoto, N. *et al.* (2001) Protective role of ATP-sensitive potassium channels in hypoxia-induced

generalized seizure. Science **292**, 1543–1546.
51) Suzuki, M., Sasaki, N., Miki, T., Sakamoto, N., Ohmoto-Sekine, Y., Tamagawa, M. *et al.* (2002) Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. J. Clin. Invest. **109**, 509–516.
52) Miki, T., Nagashima, K., Tashiro, F., Kotake, K., Yoshitomi, H., Tamamoto, A. *et al.* (1998) Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. Proc. Natl. Acad. Sci. USA **95**, 10402–10406.
53) Seghers, V., Nakazaki, M., DeMayo, F., Aguilar-Bryan, L. and Bryan, J. (2000) Sur1 knockout mice. A model for K_{ATP} channel-independent regulation of insulin secretion. J. Biol. Chem. **275**, 9270–9277.
54) Zhang, C.L., Katoh, M., Shibasaki, T., Minami, K., Sunaga, Y., Takahashi, H. *et al.* (2009) The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. Science **325**, 607–610.
55) Soria, B., Roche, E., Berná, G., León-Quinto, T., Reig, J.A. and Martín, F. (2000) Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes **49**, 157–162.
56) Lumelsky, N., Blondel, O., Laeng, P., Velasco, I., Ravin, R. and McKay, R. (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science **292**, 1389–1394.
57) Assady, S., Maor, G., Amit, M., Itskovitz-Eldor, J., Skorecki, K.L. and Tzukerman, M. (2001) Insulin production by human embryonic stem cells. Diabetes **50**, 1691–1697.
58) D'Amour, K.A., Bang, A.G., Eliazer, S., Kelly, O.G., Agulnick, A.D., Smart, N.G. *et al.* (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat. Biotechnol. **24**, 1392–1401.
59) Odorico, J.S., Kaufman, D.S. and Thomson, J.A. (2001) Multilineage differentiation from human embryonic stem cell lines. Stem Cells **19**, 193–204.
60) McLaren, A. (2001) Ethical and social considerations of stem cell research. Nature **414**, 129–131.
61) Rajagopal, J., Anderson, W.J., Kume, S., Martinez, O.I. and Melton, D.A. (2003) Insulin staining of ES cell progeny from insulin uptake. Science **299**, 363.
62) Hansson, M., Tonning, A., Frandsen, U., Petri, A., Rajagopal, J., Englund, M.C. *et al.* (2004) Artifactual insulin release from differentiated embryonic stem cells. Diabetes **53**, 2603–2609.
63) Ramiya, V.K., Maraist, M., Arfors, K.E., Schatz, D.A., Peck, A.B. and Cornelius, J.G. (2000) Reversal of insulin-dependent diabetes using islets generated *in vitro* from pancreatic stem cells. Nat. Med. **6**, 278–282.
64) Bonner-Weir, S., Taneja, M., Weir, G.C., Tatarkiewicz, K., Song, K.H., Sharma, A. *et al.* (2000) *In vitro* cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. USA **97**, 7999–8004.
65) Yang, L., Li, S., Hatch, H., Ahrens, K., Cornelius, J.G., Petersen, B.E. *et al.* (2002) *In vitro* transdifferentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc. Natl. Acad. Sci. USA **99**, 8078–8083.
66) Gao, R., Ustinov, J., Pulkkinen, M.A., Lundin, K., Korsgren, O. and Otonkoski, T. (2003) Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. Diabetes **52**, 2007–2015.
67) Tang, D.Q., Cao, L.Z., Burkhardt, B.R., Xia, C.Q., Litherland, S.A., Atkinson, M.A. *et al.* (2004) *In vivo* and *in vitro* characterization of insulin-producing cells obtained from murine bone marrow. Diabetes **53**, 1721–1732.
68) Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell **126**, 663–676.
69) Tateishi, K., He, J., Taranova, O., Liang, G., D'Alessio, A.C. and Zhang, Y. (2008) Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J. Biol. Chem. **283**, 31601–31607.
70) Shen, C.N., Slack, J.M. and Tosh, D. (2000) Molecular basis of transdifferentiation of pancreas to liver. Nat. Cell Biol. **2**, 879–887.
71) Slack, J.M. and Tosh, D. (2001) Transdifferentiation and metaplasia—switching cell types. Curr. Opin. Genet. Dev. **11**, 581–586.
72) Tosh, D. and Slack, J.M. (2002) How cells change their phenotype. Nat. Rev. Mol. Cell Biol. **3**, 187–194.
73) Yeung, R.S., Weese, J.L., Hoffman, J.P., Solin, L.J., Paul, A.R., Engstrom, P.F. *et al.* (1993) Neoadjuvant chemoradiation in pancreatic and duodenal carcinoma. A phase II study. Cancer **72**, 2124–2133.
74) Rao, M.S., Dwivedi, R.S., Subbarao, V., Usman, M.I., Scarpelli, D.G., Nemali, M.R. *et al.* (1988) Almost total conversion of pancreas to liver in the adult rat: a reliable model to study transdifferentiation. Biochem. Biophys. Res. Commun. **156**, 131–136.
75) Dabeva, M.D., Hwang, S.G., Vasa, S.R., Hurston, E., Novikoff, P.M., Hixson, D.C. *et al.* (1997) Differentiation of pancreatic epithelial progenitor cells into hepatocytes following transplantation into rat liver. Proc. Natl. Acad. Sci. USA **94**, 7356–7361.
76) Song, K.H., Ko, S.H., Ahn, Y.B., Yoo, S.J., Chin, H.M., Kaneto, H. *et al.* (2004) *In vitro* transdifferentiation of adult pancreatic acinar cells into insulin-expressing cells. Biochem. Biophys. Res. Commun. **316**, 1094–1100.
77) Baeyens, L., De Breuck, S., Lardon, J., Mfopou, J.K., Rooman, I. and Bouwens, L. (2005) *In vitro* generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia **48**, 49–57.
78) Minami, K., Okuno, M., Miyawaki, K., Okumachi, A., Ishizaki, K., Oyama, K. *et al.* (2005) Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc. Natl. Acad. Sci. USA **102**, 15116–15121.

79) Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., Tanabe, Y., Jessell, T.M. *et al.* (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev. Biol. **1**, 4.
80) Okuno, M., Minami, K., Okumachi, A., Miyawaki, K., Yokoi, N., Toyokuni, S. *et al.* (2007) Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. Am. J. Physiol. Endocrinol. Metab. **292**, E158–E165.
81) Edlund, H. (2002) Pancreatic organogenesis—developmental mechanisms and implications for therapy. Nat. Rev. Genet. **3**, 524–532.
82) Sund, N.J., Vatamaniuk, M.Z., Casey, M., Ang, S.L., Magnuson, M.A., Stoffers, D.A. *et al.* (2001) Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. Genes Dev. **15**, 1706–1715.
83) Rausa, F., Samadani, U., Ye, H., Lim, L., Fletcher, C.F., Jenkins, N.A. *et al.* (1997) The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target gene HNF-3 beta in the developing murine liver and pancreas. Dev. Biol. **192**, 228–246.
84) Krapp, A., Knöfler, M., Ledermann, B., Bürki, K., Berney, C., Zoerkler, N. *et al.* (1998) The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes Dev. **12**, 3752–3763.
85) Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R.J. and Wright, C.V. (2002) The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat. Genet. **32**, 128–134.
86) Cras-Méneur, C., Elghazi, L., Czernichow, P. and Scharfmann, R. (2001) Epidermal growth factor increases undifferentiated pancreatic embryonic cells *in vitro*: a balance between proliferation and differentiation. Diabetes **50**, 1571–1579.
87) Minami, K., Okano, H., Okumachi, A. and Seino, S. (2008) Role of cadherin-mediated cell-cell adhesion in pancreatic exocrine-to-endocrine transdifferentiation. J. Biol. Chem. **283**, 13753–13761.
88) Desai, B.M., Oliver-Krasinski, J., De Leon, D.D., Farzad, C., Hong, N., Leach, S.D. *et al.* (2007) Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. J. Clin. Invest. **117**, 971–977.

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Profile

Susumu Seino graduated from Kobe University School of Medicine in 1974. After spending years of clinical practice in hospitals, he moved to Department of Internal Medicine, Kyoto University School of Medicine in 1978, where he started his research career with studies on pancreatic hormones and received D. M. Sci. (equivalent to Ph.D.) under mentorship of Professor Hiroo Imura. He, then, moved to the USA and worked at University of Michigan in Ann Arbor, University of California in San Francisco, and University of Chicago. During almost 10 years of period in the USA, he and his colleagues succeeded in cloning of many genes related to glucose metabolism, including various primate insulins, guinea pig glucagon, human insulin receptor and its mutant in insulin-resistant diabetes, various glucose transporter isoforms, and somatostatine receptors. After returning to Japan, as Professor of Chiba University, his research centered around on pancreatic β-cells, including molecular mechanisms of insulin secretion, roles of β-cells in diabetes, and regenerative medicine of β-cells. In 2003, he returned to his alma mater, Kobe University Graduate of School of Medicine and currently appointed as Professor and Head of the Division of Diabetes and Endocrinology and Professor and Head of the Division of Cellular and Molecular Medicine. His research has revealed how insulin secretion from the pancreatic β-cells is regulated by glucose and cAMP signaling. Seino's ground-breaking and fundamental discoveries on the structure of the ATP-sensitiven K^+ channel have led to a new understanding of the ion channel in not only pancreatic β cells but also in heard and many other tissues and cells. In addition, his research on the ATP-sensitive K^+ channel laid the groundwork for new approaches for treating patients with permanent neonatal diabetes. His recent discovery of the protein Epac2 (cAMP-GEF II) as a target of anti-diabetic drugs may lead to new treatments for diabetes. His group also discovered that pancreatic exocrine cells can convert into insulin-secreting cells *in vitro*, suggesting that they are a potential source of β-cells replacement therapy in diabetes. He is actively developing his research at Kobe University Graduate School of Medicine. For these achievements he received awards such as the Asia & Oceania Medal from The Society for Endocrinology, UK, Hargedorn Award from The Japan Diabetes Society, Academic Award of the Mochida Memorial Foundation from The Mochida Memorial Foundation, and The Naito Foundation Research Prize from The Naito Foundation, as well as the honor of The Kroc Lectureships at the University of Chicago and the University of Washington in Seattle.

Profile

Tadao Shibasaki graduated from Meiji University School of Agriculture in 1992. He received his master's degree in medical science at Tsukuba University Graduate School of Medicine in 1994, and then, Ph.D in medical science in 1998 at Chiba University School of Medicine. Shibasaki started his research activities in Professor Susumu Seino's laboratory at Chiba University School of Medicine. His research theme has been centered around insulin granule exocytosis in pancreatic β-cells. Shibasaki and his colleagues found that cAMP potentiates insulin granule exocytosis by PKA-independent as well as PKA-dependent mechanisms, the former involving the cAMP-binging protein cAMP-GEFII (Epac2).

In 2003, Shibasaki moved his foothold of his research work to Kobe University Graduate School of Medicine as an Assistant Professor to Professor Susumu Seino. He is breaking new ground to look into the regulation of insulin granule dynamics using bioimaging techniques. Currently he has been engaging himself in the projects on molecular mechanism of insulin secretion, computer simulation model of insulin secretion, and metabolome analysis.

Profile

Kohtaro Minami graduated from Nagoya University School of Agriculture in 1988. Upon receiving his master's degree at Nagoya University Graduate School of Agriculture in 1990, he joined Mitsui Pharmaceuticals, where he worked as a scientist on drug discovery in the field of thrombosis and diabetes until 2000. In 2001, he joined Chiba University School of Medicine as a Research Associate to Professor Susumu Seino. Minami's research at Chiba University was focused on mechanism of normal regulation and dysfunction of insulin secretion, mainly using newly cloned pancreatic β-cell lines, obtained his Ph.D in medical science. He, then, moved to Translational Research Center, affiliated to Kyoto University Hospital, as Associate Professor and was involved in the β-cell regeneration project, headed by Professor Seino. Minami and his colleagues succeeded in generating insulin-secreting cells from differentiated pancreatic acinar cells for the first time and elucidated a part of the mechanism of *in vitro* transdifferentiation, which represents a basis of regenerative medicine of diabetes. In 2007, he joined Professor Seino's laboratory again at Kobe University Graduate School of Medicine, where he has been devoting himself in the projects on β-cell regeneration, molecular mechanism of insulin secretion, β-cell specific gene manipulated animals, and metabolome analysis.