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Novel aspects on pancreatic beta-cell signal-transduction

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ABSTRACT

Pancreatic β-cells release insulin in appropriate amounts in order to keep blood glucose levels within physiological limits. Failure to do so leads to the most common metabolic disorder in man, diabetes mellitus. The glucose-stimulus/insulin-secretion coupling represents a sophisticated interplay between glucose and a variety of modulatory factors. These factors are provided by the blood supply (such as nutrients, vitamins, incretins etc.), the nerval innervations, cell-cell contacts as well as by paracrine and autocrine feedback loops within the pancreatic islet of Langerhans. However, the underlying mechanisms of their action remain poorly understood.

In the present mini-review we discuss novel aspects of selective insulin signaling in the β-cell and novel insights into the role of higher inositol phosphates in insulin secretion. Finally we present a newly developed experimental platform that allows non-invasive and longitudinal *in vivo* imaging of pancreatic islet/β-cell biology at single-cell resolution.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a heterogenous, complex metabolic disorder with a dramatic increase in incidence worldwide. It is characterized by defects in both insulin secretion by the pancreatic β-cell and insulin action in peripheral target tissues, mainly muscle, liver, fat, and brain. Defects in insulin action in the peripheral organs, commonly referred to as insulin resistance, is often associated with obesity. Currently it is assumed that T2DM develops, when pancreatic β-cells fail to respond to the increasing demand in insulin created by the peripheral insulin resistance. Interestingly, results from genome-wide association studies in large study samples performed over the past two years have revealed an increasing number of target genes with potentially associated functions in the pancreatic islet or even pancreatic β-cell [1]. This pinpoints the importance of an integrated signal-transduction in the β-cell for proper function and survival. There is a growing body of evidence suggesting that for an appropriate function, the β-cell relies on multiple signals provided by nutrients, incretins, the nervous system as well as by the architecture of the pancreatic islet of Langerhans.

The main signal-transduction cascades that allow the β-cell to respond to an increase in blood glucose with the secretion of appropriate amounts of insulin in order to keep blood glucose within narrow limits have been dissected. In this stimulus-secretion coupling glucose is taken up by the β-cell via glucose transporters and is converted into glucose-6-phosphate by the neuroendocrine isoform of glucokinase, which is believed to serve as the β-cell glucose sensor [2]. Glucose-6-phosphate is then metabolized in glycolysis and the Krebs cycle, resulting in an increase in the ATP/ADP-ratio. This leads to the closure of ATP-sensitive K^+-channels, which in turn results in depolarization of the plasma membrane and subsequent opening of voltage-gated L-type Ca^{2+}-channels. Influx of Ca^{2+} leads to an increase in cytoplasmic-free Ca^{2+} concentration, which finally triggers exocytosis of insulin [3].

Although the importance of the above mentioned factors, e.g. nutrients, incretins, vitamins, humoral and nervous stimuli, autocrine and paracrine feedback loops, are appreciated to modulate signal-transduction cascades that regulate β-cell function and survival, the underlying mechanisms remain poorly understood. Moreover, in order to understand the dynamics of β-cell function and survival under normal conditions and the development of β-cell dysfunction in diabetes, these processes must be studied in the β-cell in the context of the intact islet of Langerhans *in vivo* thereby providing an intact capillary network and adequate innervation. In the present mini-review we will discuss novel aspects of β-cell signal-transduction as well as present a newly developed experimental platform that allows non-invasive and longitudinal *in vivo* imaging of pancreatic islet/β-cell biology at single-cell resolution.

2. New insights into the mechanisms underlying the selective insulin signal-transduction in the β-cell

Research over the past 13 years has clearly demonstrated that the pancreatic β-cell is a target for positive insulin feedback action.

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Since we and others reported in 1998 that insulin positively affects the regulation of its own biosynthesis [4,5], data from several laboratories have shown that this autocrine feedback loop is important for proper β-cell function and survival by regulating gene expression, ion flux, insulin secretion, β-cell size and proliferation as well as β-cell survival (reviewed in [6]). However, little is known about how selectivity is achieved in this plethora of insulin actions. We have addressed this by analyzing how insulin by signaling through the two isoforms of the insulin receptor, i.e. the A-type (IR-A) and the B-type (IR-B), can regulate the transcription of three candidate/model genes, namely the insulin gene, the pancreatic β-cell transcription unit of the glucokinase gene (βGK) and the c-fos gene [4,7–11]. Insulin activates all three model genes simultaneously in the same cell by involving three different signal-transduction cascades (Fig. 1). While insulin up-regulates the transcription of its own gene by signaling via IR-A and IRS-2/PI3K class Ia/mTORC1/p70 s6 k [4], it requires signaling via IR-B to activate transcription of βGK and c-fos genes in the same cell [8,10]. Selectivity in IR-B-mediated signaling, again, is achieved by the involvement of different signaling cascades. Insulin activates βGK transcription by signaling from plasma membrane-standing receptor complexes, while IR-B-mediated activation of the c-fos gene needs clathrin-dependent endocytosis of the receptor and signaling from the early endosome pool [10]. Since IR-B is poorly recycled and mainly believed to undergo lysosomal degradation, signal-transduction from internalized IR-B was highly unexpected. In the light of these findings, our data do not only lend support to the suggestion that endocytic organelles can play a role in insulin signal propagation, but shows this for a receptor type that is thought to be preferentially degraded following endocytosis. Moreover, we identified two different sites in the IR-B β-subunit being involved in the selective activation of the two different signaling cascades. While activation of βGK requires the integrity of the juxtamembrane NPEY-motif of IR-B and signaling via class II PI3K-C2α/PDK1/PKBα, up-regulation of the c-fos promoter needs the intact C-terminal YTHM-motif of IR-B and signal-transduction through PI3K Ia/p52Shc/MEK1/ERK [10,11]. Although both IR-A-mediated activation of the insulin gene and IR-B-mediated activation of βGK is initiated from plasma membrane-standing receptor complexes, the different signaling cascades originate from IRs in different plasma membrane micro-domains [9]. Selectivity in signal-transduction is here achieved by involving different classes of PI3K. While IR-A via its juxtamembrane NPEY-motif and IRS-2 recruits class Ia PI3K, IR-B via the same NPEY-motif preferentially associates with the class II PI3K member PI3K-C2α [11]. In contrast to L6 myotubes, where insulin-stimulated PI3K-C2α generates PtdIns(3)*P* [12,13], this kinase generates PtdIns(3,4)P_2 in the pancreatic β-cell, which allows the selective activation of PKBα/Akt1 and subsequent up-regulation of βGK biosynthesis. Consequently, knockdown of PI3K-C2α or PKBα in β-cells impairs glucose-stimulated insulin release [11].

In conclusion, our data demonstrate that selectivity in signal-transduction via the two IR isoforms can be achieved by the spatio-temporal segregation of the signaling events within the same cell. Future experiments will have to show the additional involvement in selective insulin signaling of other potential insulin target receptors, such as IGF-1R and its hybrid receptors with either IR-A or IR-B.

3. New insights into the roles of higher inositol polyphosphates in the β-cell

Besides phosphorylated inositol lipids, as illustrated by the role of class Ia PI3K and class II PI3K-C2α in insulin feedback action (see

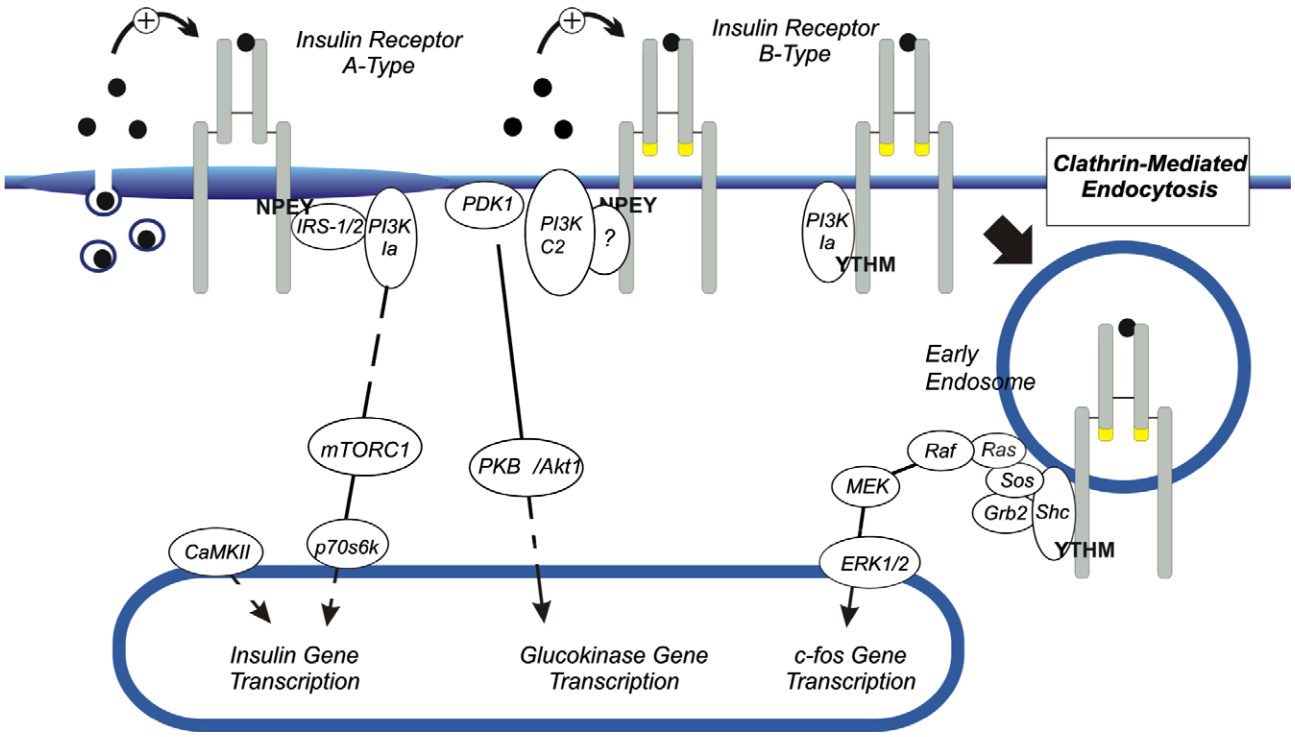

Fig. 1. Schematic illustration of selective insulin signaling via the two insulin receptor isoforms, A-type and B-type, in the pancreatic β-cell using the transcriptional regulation of the insulin gene, the β-cell transcription unit of the glucokinase gene and the c-fos gene as biological endpoints. While insulin up-regulates the transcription of its own gene by signaling via IR-A/IRS-2/PI3K class Ia/mTORC1/p70s6k, it requires signaling via IR-B/class II PI3K-C2α/PDK1/PKBα to activate transcription of the glucokinase gene. Whereas these signaling pathways are initiated from IR complexes that reside in different micro-domains of the plasma membrane, IR-B-meditated activation of the c-fos gene requires clathrin-dependent endocytosis of the IR and signaling via p52Shc/MEK1/ERK from the early endosome pool.

above), the roles of higher inositol polyphosphates in pancreatic β-cell function has become more and more evident in recent years [14,15] (the different inositol phosphate pools in the β-cell are illustrated in Fig. 2). For example, the impact of inositolhexakisphosphate (InsP_6) on β-cell function is complex. InsP_6 has been shown to directly stimulate both exocytosis and endocytosis [16,17]. The latter effect is mediated by dynamin and is dependent on the activation of calcineurin and PKC as well as on the inhibition of synaptojanin [17]. While the effect of InsP_6 on exocytosis can be mimicked by inositol(1,3,4,5,6)pentakisphosphate (InsP_5), the positive effect on Ca^{2+}-influx via L-type Ca^{2+}-channels is more InsP_6-specific [18]. Expression of a cytosolic version of the multiple inositol polyphosphate phosphatase, i.e. Cyt-MIPP, in the β-cell line HIT M2.2.2 leads to a decrease in the levels of InsP_5 and InsP_6 and an increase in InsP_3 [19]. The latter causes an increase in basal cytoplasmic-free Ca^{2+} concentration in these cells, i.e. to levels found in normal β-cells, which results in improved secretagouge-stimulated insulin release.

Besides being metabolically degraded, InsP_6 also serves as a substrate for inositolhexakisphosphate kinases, such as IP6K1–3, to become further phosphorylated to diphosphoinositol pentakisphosphate (InsP_7). Pancreatic β-cells, which express IP6K1 and IP6K2, have in contrast to other cells high basal levels of InsP_7 [20]. While overexpression of either IP6K1 or IP6K2 increases InsP_7 levels and stimulates insulin secretion from the readily releasable pool to a similar extent as exogenously applied InsP_7, only siRNA-mediated knockdown of IP6K1, but not IP6K2, impairs insulin exocytosis [20]. These data show that maintenance of high InsP_7 levels in the β-cell ensures its full exocytotic capacity and suggest a critical role of IP6K1.

Future work will reveal the underlying molecular mechanisms mediating the stimulatory effects of InsP_7. Potential target sites for the InsP_7-action are (i) the recruitment process of insulin granules from the reserve pool to the plasma membrane, (ii) the priming of docked insulin granules, and (iii) the molecular mechanisms directly involved in the fusion event of the insulin granule. Conceivable mechanisms of InsP_7-action are (i) to serve as a scaffold molecule to allow multi-molecular interactions, (ii) to serve as an energy donor in pyrophosphorylation reactions, or (iii) to serve as a substrate for the generation of higher inositol pyrophosphates, such as InsP_8 [21].

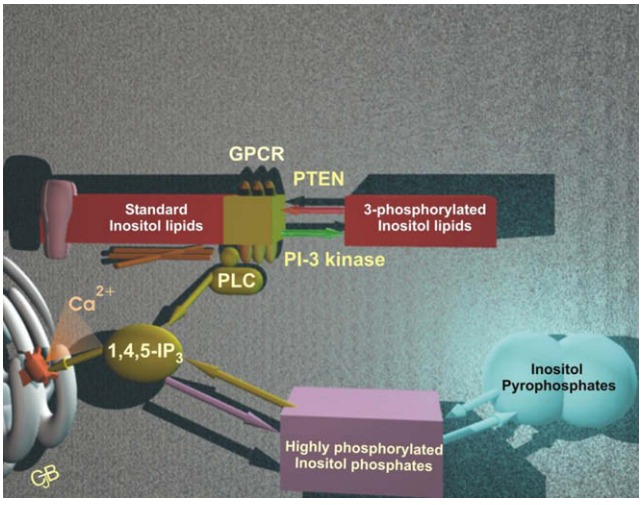

Fig. 2. Schematic illustration of the different inositol phosphate pools in the pancreatic β-cell. While receptor-activated PI3Kinases contribute to the generation of 3-phosphorylated inositol lipids, PLC-generated Ins(1,4,5)P_3 not only regulates the release of Ca^{2+} from intracellular stores but also feeds into the pool of highly phosphorylated inositol phosphates, such as InsP_5 and InsP_6, as well as of inositol pyrophosphates, such as InsP_7 that have important functions in β-cell physiology.

4. Non-invasive and longitudinal *in vivo* imaging of pancreatic β-cell function

A major challenge for medical research is to find a way to study complex biological processes like signal-transduction at the cellular level under physiological and pathophysiological conditions non-invasively *in vivo*. Accordingly, *in vitro* β-cell signal-transduction has been studied in detail but little is known about islet behavior *in situ*, either in their native environment or after transplantation. Here we discuss a new experimental platform that we have recently developed, which allows *in vivo* imaging of pancreatic β-cell mass and function non-invasively and longitudinally in islets at single-cell resolution [22,23]. Our approach is to transplant pancreatic islets into the anterior chamber of the eye of mice and rats for functional microscopic imaging (Fig. 3). Due to its optical and structural properties, the eye is optimally suited as a body-window for non-invasive imaging. The cornea is transparent and the grafts are easily vascularized and innervated because of the rich blood and nerve supply of the iris that forms the bed of the anterior chamber of the eye. After transplantation into the anterior chamber of the eye via injection through the cornea, the islets engraft on the iris and are readily observed and imaged through the cornea. Immunohistochemical analysis of the engrafted islets shows that the islet composition in terms of α-cell/β-cell ratio does not change over time and that fine structures, such as the fenestrae in the microcapillaries, are maintained. That the engrafted islets are functional is verified by the fact that they maintain normal glucose homeostasis of recipient mice that have been rendered diabetic by killing their endogenous β-cells chemically with streptozotocin or alloxan [22].

To visualize blood vessels in the islet grafts, we injected fluorescently labeled dextran into the tail vein of the recipient mouse. The vascularization pattern of the graft becomes more complex with time. Longitudinal imaging of the same islet grafts shows that after 4 weeks the vascularization process reaches a plateau, showing a vasculature-density for the islets equivalent to that found in the pancreas [22]. Hence, this imaging platform will enable researchers to investigate angiogenesis at high resolution in real-time in a mammalian model. Moreover, this platform will allow studying the microcirculation within the pancreatic islet under normal and diabetic conditions. Similarly, it is possible to study the dynamics of islet innervation by using mice as recipients that express for example GFP specifically in their neurons. With retrograde tracing from the eye we will be able to investigate to what brain areas the nerves associated with the various endocrine cells project.

A further potential of this imaging platform is to monitor non-invasively changes in β-cell mass *in vivo* over time under pathophysiological conditions and to test the efficacy of therapeutical interventions. Initial experiments demonstrate that engrafted islets in the anterior chamber of the eye can be used as visible representatives of their natural counterparts in the pancreas. This allows monitoring longitudinally the gain in β-cell mass in the ob/ob mouse as well as the loss of β-cells in response to autoimmune destruction in the BB-rat, i.e. an animal model of T1DM. The latter observation together with the fact that allograft rejection can be monitored in real-time illustrates that this experimental platform can be used to study the fundamental molecular aspects of the immunological reaction [24]. In other words, the immune-privilege that is transiently provided by this transplantation site is finally broken following vascularization of the graft.

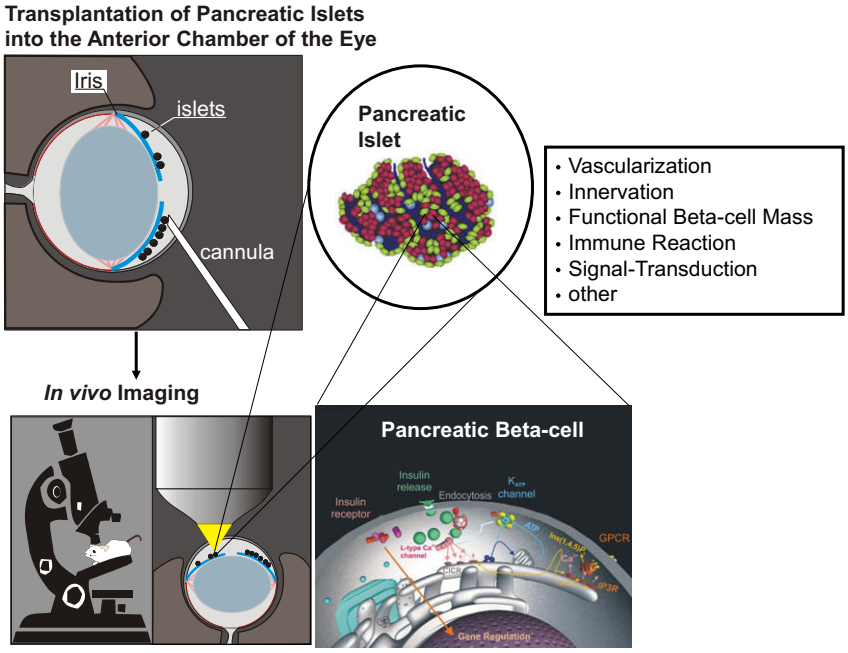

Fig. 3. Schematic illustration of the *in vivo* imaging platform to study pancreatic β-cell physiology and pathology. Following their isolation, pancreatic islets of Langerhans are transplanted into the anterior chamber of the eye, where they engraft on the iris, become vascularised and innervated. Since the cornea represents a natural body-window, this transplantation site allows non-invasive, longitudinal *in vivo* imaging of the islet with high resolution, i.e. at the single-cell level, and to study various aspects of islet/β-cell function under normal as well as diabetic conditions.

Another potential of this transplantation site is the possibility to study the details of signal-transduction in the pancreatic β-cell *in vivo*. This can be achieved by using genetically engineered biosensors that reflect key-events in β-cell biology, e.g. steps in the stimulus-secretion coupling, gene expression, β-cell profileration, and apoptosis. Biosensors to be used in these experiments are genetically engineered proteins that carry fluorescent probes which allow imaging of cellular signaling events by measuring with spatio-temporal resolution (i) fluorescence resonance energy transfer (FRET) between suitably labeled interacting partners as well as (ii) the fluorescence intensities of signaling components *per se*. Biosensors can be specifically expressed in β-cells by employing the insulin promoter. A potential strategy will be to use virus-based expression vectors for transduction of islets *in vitro* prior to their transplantation into the eye. Similarly, expressing these biosensors under the control of the glucagon- or somatostatin-promoter will allow non-invasive real-time imaging of signal-transduction in pancreatic α- and δ-cells, respectively.

Last, but not least, the anterior chamber of the eye offers the potential to study human pancreatic islet cell biology under physiological as well as pathological conditions. It is clear from our own studies and studies by others that the human pancreatic islet is different from rodent islets in terms of both structure and function [25,26]. Therefore it is of great importance to clarify signal-transduction processes in human islets under normal and pathological conditions. For this purpose we transplant human islets into immunodeficient nude mice, that have been subjected to streptozotocin-treatment to kill endogenous β-cells and/or pancreatectomy to eliminate residual islet function in the host. Our initial studies show that, similar to rodent islets, the dynamics of human islet vascularisation can be monitored longitudinally. Moreover, our data demonstrate that transplantation of human islets reverses streptozotocin-induced diabetes in nude mice proving functionality of the engrafted islets [27].

In conclusion, this novel imaging platform will allow us to move *in vitro* islet cell research into *in vivo* analysis of the complex molecular mechanisms regulating hormone release from the endocrine pancreas in health and disease.

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References

[1] I. Prokopenko, M.I. McCarthy, C.M. Lindgren, Type 2 diabetes: new genes, new understanding, Trends Genet. 24 (2008) 613–621.
[2] F.M. Matschinsky, A lesson in metabolic regulation inspired by the glucokinase sensor paradigm, Diabetes 45 (1996) 223–241.
[3] P.O. Berggren, O. Larsson, Ca2+ and pancreatic B-cell function, Biochem. Soc. Trans. 22 (1994) 12–18.
[4] I.B. Leibiger, B. Leibiger, T. Moede, P.O. Berggren, Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways, Mol. Cell 1 (1998) 933–938.
[5] G.G. Xu, P.L. Rothenberg, Insulin receptor signaling in the β-cell influences insulin gene expression and insulin content. Evidence for autocrine β-cell regulation, Diabetes 47 (1998) 1243–1252.
[6] I.B. Leibiger, B. Leibiger, P.O. Berggren, Insulin signaling in the pancreatic beta cell, Annu. Rev. Nutr. 28 (2008) 233–251.
[7] B. Leibiger, K. Wåhlander, P.O. Berggren, I.B. Leibiger, Glucose-stimulated insulin biosynthesis depends on insulin-stimulated insulin gene transcription, J. Biol. Chem. 275 (2000) 30153–30156.
[8] B. Leibiger, I.B. Leibiger, T. Moede, S. Kemper, R.N. Kulkarni, C.R. Kahn, L.M. de Vargas, P.O. Berggren, Selective signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic β cells, Mol. Cell 7 (2001) 559–570.
[9] S. Uhles, T. Moede, B. Leibiger, P.O. Berggren, I.B. Leibiger, Isoform-specific insulin receptor signaling involves different plasma membrane domains, J. Cell Biol. 163 (2003) 1327–1337.
[10] S. Uhles, T. Moede, B. Leibiger, P.O. Berggren, I.B. Leibiger, Selective gene activation by spatial segregation of insulin receptor B signaling, FASEB J. 21 (2007) 1609–1621.

[11] B. Leibiger, T. Moede, S. Uhles, C.J. Barker, M. Creveaux, J. Domin, P.O. Berggren, I.B. Leibiger, Insulin-feedback via PI3K-C2α activated PKBα/Akt1 is required for glucose-stimulated insulin secretion, FASEB J. 26 (2010), doi:10.1096/fj.09-148072.
[12] T. Maffucci, A. Brancaccio, E. Piccolo, R.C. Stein, M. Falasca, Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation, EMBO J. 22 (2003) 4178–4189.
[13] M. Falasca, W.E. Hughes, V. Dominguez, G. Sala, F. Fostira, M.Q. Fang, R. Cazzolli, P.R. Shepherd, D.E. James, T. Maffucci, The role of phosphoinositide 3-kinase C2α in insulin signaling, J. Biol. Chem. 282 (2007) 28226–28236.
[14] C.J. Barker, I.B. Leibiger, B. Leibiger, P.O. Berggren, Phosphorylated inositol compounds in β-cell stimulus-response coupling, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E1113–E1122.
[15] P.O. Berggren, C.J. Barker, A key role for phosphorylated inositol compounds in pancreatic beta-cell stimulus-secretion coupling, Adv. Enzyme Regul. 48 (2008) 276–294.
[16] A.M. Efanov, S.V. Zaitsev, P.O. Berggren, Inositol hexakisphosphate stimulates non-Ca^{2+}-mediated and primes Ca^{2+}-mediated exocytosis of insulin by activation of protein kinase C, Proc. Natl. Acad. Sci. USA 94 (1997) 4435–4439.
[17] M. Hoy, A.M. Efanov, A.M. Bertorello, S.V. Zaitsev, H.L. Olsen, K. Bokvist, B. Leibiger, I.B. Leibiger, J. Zwiller, P.O. Berggren, J. Gromada, Inositol hexakisphosphate promotes dynamin I- mediated endocytosis, Proc. Natl. Acad. Sci. USA 99 (2002) 6773–6777.
[18] O. Larsson, C.J. Barker, A. Sjoholm, H. Carlqvist, R.H. Michell, A. Bertorello, T. Nilsson, R.E. Honkanen, G.W. Mayr, J. Zwiller, P.O. Berggren, Inhibition of phosphatases and increased Ca^{2+} channel activity by inositol hexakisphosphate, Science 278 (1997) 471–474.
[19] J. Yu, B. Leibiger, S.N. Yang, J.J. Caffery, S.B. Shears, I.B. Leibiger, C.J. Barker, P.O. Berggren, Cytosolic multiple inositol polyphosphate phosphatase in the regulation of cytoplasmic free Ca^{2+} concentration, J. Biol. Chem. 278 (2003) 46210–46218.
[20] C. Illies, J. Gromada, R. Fiume, B. Leibiger, J. Yu, K. Juhl, S.N. Yang, D.K. Barma, J.R. Falck, A. Saiardi, C.J. Barker, P.O. Berggren, Requirement of inositol pyrophosphates for full exocytotic capacity in pancreatic beta cells, Science 318 (2007) 1299–1302.
[21] C.J. Barker, C. Illies, R. Fiume, G.C. Gaboardi, J. Yu, P.O. Berggren, Diphosphoinositol pentakisphosphate as a novel mediator of insulin exocytosis, Adv. Enzyme Regul. 49 (2009) 168–173.
[22] S. Speier, D. Nyqvist, O. Cabrera, J. Yu, R.D. Molano, A. Pileggi, T. Moede, M. Kohler, J. Wilbertz, B. Leibiger, C. Ricordi, I.B. Leibiger, A. Caicedo, P.O. Berggren, Noninvasive *in vivo* imaging of pancreatic islet cell biology, Nat. Med. 14 (2008) 574–578.
[23] S. Speier, D. Nyqvist, M. Kohler, A. Caicedo, I.B. Leibiger, P.O. Berggren, Noninvasive high-resolution *in vivo* imaging of cell biology in the anterior chamber of the mouse eye, Nat. Protoc. 3 (2008) 1278–1286.
[24] M. Abdulreda, R.D. Molano, G. Faleo, A.L. Bayer, J. Molina, P.O. Berggren, C. Ricordi, A. Caicedo, A. Pileggi, The anterior chamber of the eye allows performing noninvasive, repeated live imaging of islet allograft rejection, Xenotransplantation 16 (2009) 415.
[25] O. Cabrera, D.M. Berman, N.S. Kenyon, C. Ricordi, P.O. Berggren, A. Caicedo, The unique cytoarchitecture of human pancreatic islets has implications for islet cell function, Proc. Natl. Acad. Sci. USA 103 (2006) 2334–2339.
[26] M. Brissova, M.J. Fowler, W.E. Nicholson, A. Chu, D.M. Harlan, A.C. Powers, Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy, J. Histochem. Cytochem. 53 (2005) 1087–1097.
[27] A. Caicedo, R. Rodriguez, R.D. Molano, C. Ricordi, P.O. Berggren, A. Pileggi, Noninvasive, live imaging studies of human islet cell biology in the anterior chamber of the eye, Xenotransplantation 16 (2009) 415–416.