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DOC2b is a SNARE regulator of glucose-stimulated delayed insulin secretion

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ABSTRACT

Insulin secretion is precisely regulated by blood glucose with unique biphasic pattern. The regulatory mechanism of the second-phase insulin release is unclear. In this study, we report that DOC2b (double C2 domain protein isoform b), a SNARE related protein, was associated with insulin vesicles and translocated to plasma membrane within several minutes upon high-glucose stimulation followed by an interaction with syntaxin4, but not syntaxin1. This binding specificity and the time course of DOC2b translocation were suitable for the regulation of second-phase insulin release. Increased DOC2b expression enhanced glucose-stimulated insulin secretion. In contrast, silencing DOC2b inhibited delayed release of insulin, without affecting rapid (~7 min) phase secretion. Interestingly, DOC2b had no effects on KCl-triggered insulin release. These data suggest that DOC2b may be a regulator for delayed (second-phase) insulin secretion in MIN6 cells.

Introduction

Appropriate secretion of insulin from pancreatic β-cells is critically important to the energy homeostasis. The secretion of this hormone is precisely regulated by blood glucose with unique biphasic pattern [1,2]. The first-phase occurs just after exposure to glucose, followed by prolonged second-phase release. Fundamental mechanisms of glucose-stimulated first-phase release have been vigorously studied for a few decades. It involves the following sequential steps: rise in the ATP/ADP ratio by oxidative glycolysis, closure of ATP-sensitive potassium (K_{ATP}) channels, depolarization of the plasma membrane, opening of voltage-dependent calcium channels, increase in intracellular calcium concentration ($[Ca^{2+}]_i$), and activation of membrane fusion machinery [3–5]. Whereas, the second-phase of glucose-induced insulin secretion is regulated mainly by K_{ATP} channel-independent pathway [6,7]. Despite intensive investigations, however, the mechanisms of second-phase release are still largely unknown.

In the view of insulin granule dynamics in β-cells, insulin secretion is primarily achieved by membrane fusion processes of insulin granules to plasma membrane. These processes are mediated by a set of highly conserved membrane proteins known as SNARE machinery (i.e. syntaxins, VAMPs, SNAPs), and its regulatory proteins [8–16]. However, little is known about the precise mechanisms how glucose regulates SNARE machinery. Notably, no SNARE regulator has been identified for the second-phase of insulin exocytosis. In neurons, calcium sensor proteins such as synaptotagmins have critical roles in vesicle fusion process through the binding to SNARE proteins [17,18]. Although some isoforms of synaptotagmins have been identified in pancreatic β-cell [19–21], there are no definite evidences that synaptotagmins regulate insulin secretion to date.

The universal role of Ca^{2+} as a trigger for regulated exocytosis predicts the existence of conserved proteins capable of activating the fusion machinery upon binding Ca^{2+} in pancreatic β-cells. Although many proteins have been suggested to play such a role in many type of cells, tandem C2 domain proteins have attracted the most attentions as the putative calcium sensors. DOC2 (double C2 domain) protein family have identified as a novel protein having tandem C2 domains that targeted to membrane phospholipids in $[Ca^{2+}]_i$ dependent manner [22,23]. Many proteins have been identified to be involved in the insulin-vesicle fusion in pancreatic β-cells [19,20,24,25]; however, there are no candidates of Ca^{2+} sensor proteins suitable for relatively slow second-phase release. Furthermore, the connection between glucose and calcium signals in the second-phase insulin secretion is also obscure.

Previously, we have investigated the functional role of DOC2b (one of the isoforms of DOC2 family proteins) on exocytosis in adipocytes and found that it regulates the relatively slow-time scale (several minutes order) step of vesicle fusion [26,27]. Herein, we showed that DOC2b was translocated from intracellular compartment to the plasma membrane upon glucose stimulation, and bound syntaxin4, but not syntaxin1, in pancreatic β-cells. This binding was $[Ca^{2+}]_i$ dependent. DOC2b expression enhanced and its silencing inhibited delayed insulin secretion. Our data

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suggested that DOC2b may be a positive regulator of membrane fusion and have a role on second-phase secretion of insulin.

Materials and methods

Reagents and antibodies. Mouse DOC2 cDNA constructs (DOC2a, DOC2b) were kindly provided by Dr. Rory Duncan (University of Edinburgh, UK). The polyclonal antibody against to DOC2a and DOC2b were generated against the peptide sequence CYLKELEQAEQGPGL and CGARDDDEDVDQL, respectively. Monoclonal anti-myc antibody (Clone 9E10) was from Covance (NJ, USA). The other antibodies to DOC2b, syntaxin4, syntaxin1A were products of Synaptic Systems GmbH (Goettingen, Germany). The siRNA duplex and control oligonucleotides were synthesized by Invitrogen (CA, USA).

Cell culture. MIN6 cells (a gift from Dr. Jun-ichi Miyazaki, Osaka University [28]) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose supplemented with 15% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 5 μl/L β-mercaptoethanol at 37 °C in a humidified atmosphere (5% CO_2). Cells were passaged every 4–5 days at 70–80% confluence. For retrovirus packaging, Plat-E cells were maintained in DMEM containing 10% fetal bovine serum, 1 μg/ml puromycin (Sigma, MO, USA), and 10 μg/ml blasticidin S (Funakoshi, Tokyo, Japan).

RT-PCR. Total RNA was extracted from MIN6 cells using ISOGEN (NIPPON GENE, Tokyo, Japan). Purified RNA was converted to cDNA by SuperScriptII reverse transcriptase (Invitrogen). RT-PCR was performed using the following primers: DOC2a forward; 5′-TCGCATGACCATCAACATCC-3′, DOC2a reverse; 5′-CTTCAGGTAACAGGATATGC-3′, DOC2b forward; 5′-AAAGGATCCAAGGCAGAGGACAAGTCTCTGG-3′, DOC2b reverse; 5′-AAACTCGAGTCAGTCGCTCACTACAGCCC-3′.

Preparation of adenoviruses and transfection. Adenovirus producing myc-tagged DOC2b and eGFP were prepared by AdEasy Adenoviral Vector System (Stratagene, CA, USA) according to the manufacturer's instructions. All amplified viruses were purified by the cesium chloride centrifugation method and stored at −80 °C. MIN6 cells were infected by these adenoviruses with the m.o.i. of ~30.

Immunoprecipitation and immunoblotting. Cells were lysed in lysis buffer [20 mM Hepes (pH 7.2), 100 mM NaCl, 1 mM EDTA, 25 mM NaF, 1 mM sodium vanadate, 1 mM benzamidine, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT] and the protein concentration was measured with BCA protein assay reagent (Pierce, IL, USA). For immunoprecipitation, the cell lysate was preincubated with protein-G Sepharose at 4 °C for 1 h to remove nonspecific bindings. Then, samples were incubated with primary antibody at 4 °C for 8–12 h followed by incubation with protein-G Sepharose. Lysates and immunoprecipitates were resolved by SDS–PAGE and transferred to polyvinylidene difluoride membranes (GE Healthcare, UK). The membranes were incubated with primary antibodies for 8–12 h. Protein signals were visualized using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence substrate kit (GE Healthcare, UK).

DOC2b-shRNA construct, retrovirus preparation and generation of stable cell line. Short hairpin-RNA was designed to have a 5′-AAGCCAGATGTAGACAAGAAATC-3′ sequence. Synthetic complementary single-stranded DNA of the target sequence was annealed, and the double-stranded DNA was inserted into RNAi-Ready pSIREN-RetroQ vector (Clontech, CA, USA). This plasmid vector was transfected into Plat-E to obtain the viruses using Lipofectamine 2000 transfection reagent (Invitrogen). Forty-eight hours after the transfection, supernatants containing retroviruses were harvested and purified by centrifugation and filtration. MIN6 cells were infected with these retroviruses and kept in culture containing 1 μg/ml puromycin for at least 1 week to obtain stable cell-lines lacking DOC2b.

Measurement of insulin secretion. MIN6 cells were seeded and grown in 24-well plates for 3–4 days. The cells were preincubated in KRH buffer containing 3 mM glucose for 30 min at 37 °C. Then the cells were treated with 0, 3, 12 or 25 mM glucose with or without 30 mM KCl for 60 or 7 min. At the end of incubation, KRH buffer (supernatant) were stored for insulin determination. The cell was lysed in 0.5% NP-40 and used for the determination of protein concentration. Insulin concentrations were measured using Rat insulin ELISA kit (Morinaga, Yokohama, Japan). The results were normalized by cellular protein content.

Immunofluorescence and immunoelectron microscopy. Immunostaining and sample preparation for fluorescence and electron microscopy were performed by the methods described previously [27,29]. See detailed in Supplementary methods.

Results

Expression profile of DOC2 proteins in MIN6 cells and mouse islets

DOC2 family protein was identified as a group of type C tandem C2 domain proteins in neuron and was reported to regulate docking and fusion of synaptic vesicles in $[Ca^{2+}]_i$ dependent manner [22,23]. This protein family consists of three isoforms, DOC2a, -b and -γ. DOC2a have been reported to be expressed in neuronal cells, whereas DOC2b is more widely expressed. DOC2γ is localized to the nucleus and has no Ca^{2+}-binding activity because of amino acid substitutions at the Ca^{2+} binding site [30]. Therefore, to clarify the involvement of DOC2 proteins in insulin secretion, we first investigated the presence of DOC2a and -b mRNA in the insulin-secreting cells MIN6. As shown in Fig. 1A, both DOC2 mRNAs were expressed in MIN6 cells. We next determined the protein expression of DOC2a and b in insulin-secreting cells by Western blotting. As shown in Fig. 1B, DOC2b isoform is predominantly expressed in pancreatic β-cells. Therefore, we focused on DOC2b protein as a candidate of Ca^{2+} sensor for insulin secretion.

DOC2b translocates to plasma membrane in response to glucose

Since DOC2b was reported to localized in the cytosol under the basal condition and translocated to plasma membrane upon stimulation in neuronal cells [31,32], we determine the subcellular localization of DOC2b in MIN6 cells. We performed immunofluorescent microscopy using polyclonal anti-DOC2b antibody or the cells expressing myc-tagged DOC2b. The confocal images showed that endogenous DOC2b, as well as expressed myc-DOC2b, was distributed throughout the cells in the basal state of 3 mM glucose. In contrast, when the cells were treated with high glucose (12 or 25 mM), DOC2b was translocated to the plasma membrane as shown in Fig. 2A. Interestingly, cell-permeable calcium chelating agent BAPTA-AM inhibited DOC2b translocation. Moreover, we

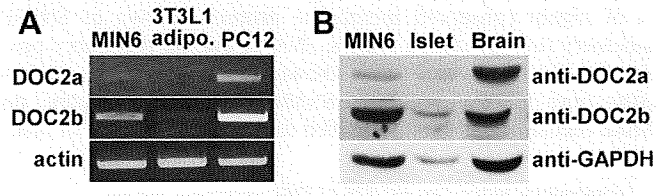

Fig. 1. Expression profiles and distributions of DOC2 proteins. (A) The expression of DOC2a and DOC2b in MIN6 cells, 3T3-L1 adipocytes, and PC12 were analyzed by RT-PCR. (B) Endogenous DOC2 proteins in MIN6 cells, mouse islet and mouse brain were determined by Western blot using anti-DOC2b and DOC2a antibodies.

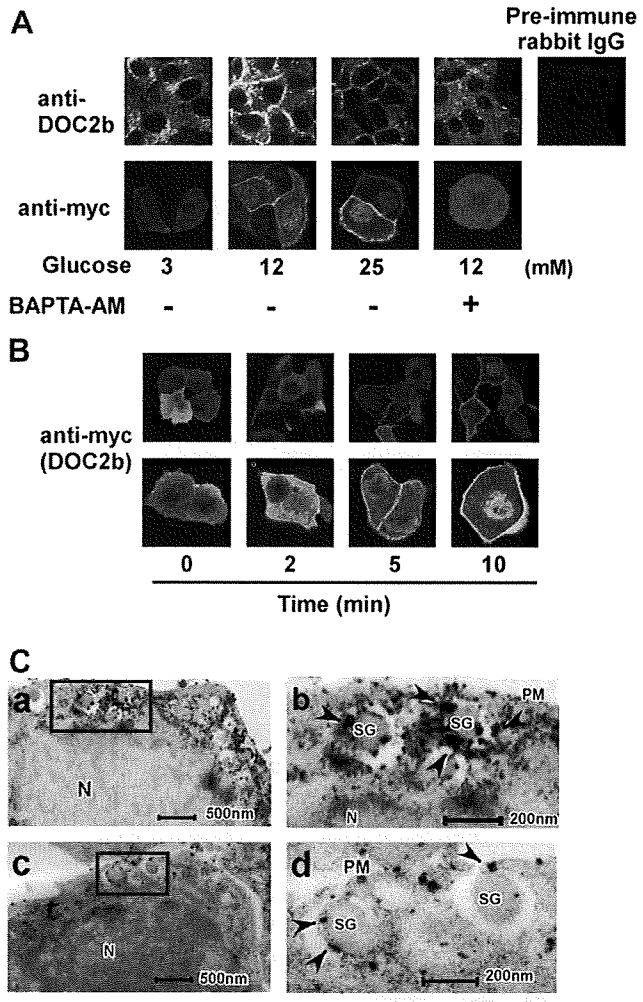

Fig. 2. Intracellular localization of DOC2b in MIN6 cells. (A,B) MIN6 cells were expressed or left untreated with adenovirus containing myc-tagged DOC2b 48 h prior to experiments. After the preincubation in KRH buffer for 0.5 h, cells were treated with 3, 12 or 25 mM glucose for 10 min (A) or the time indicated (B) and fixed followed by immunostaining with anti-DOC2b (for determination of endogenous expression) or anti-myc antibody followed by FITC-labeled secondary antibody. Stained cells were observed by confocal microscopy. In order to determine the role of $[Ca^{2+}]_i$, some cells were pre-treated with 30 mM BAPTA-AM for 30 min. The cells staining with normal rabbit IgG were used for negative control. C: Ultrathin-section of MIN6 cells were immunolabeled with anti-DOC2b antibody using avidin–biotin complex method and observed under a Hitachi H-7500 electron microscope without uranyl acetate or lead staining. Allow heads show the staining detected by anti-DOC2b antibody. Panel b and d are enlarged images of a and c, respectively. SG, secretary granule; N, nucleus; PM, plasma membrane.

determined the time scale of DOC2b translocation in MIN6 cells. As shown in Fig. 2B, DOC2b was accumulated at plasma membrane about 5–10 min after the glucose stimulation. This relatively slow-time scale of DOC2b translocation is not suitable for the first-phase secretion of insulin.

DOC2b is localized at insulin vesicles

Since DOC2b has double C2 domains, it can be targeted to the membrane phosphatidyl inositoles [33]. To confirm the precise membrane localization of endogenous DOC2b in pancreatic β-cells, we examined ultrathin-sections of MIN6 cells by immunoelectron microscopy using anti-DOC2b antibody. Interestingly, DOC2b-immunoreactive density was mostly found on the periphery of large dense core granules (insulin vesicles) near the plasma membrane (Fig. 2C).

DOC2b binds to syntaxin4 upon glucose stimulation

Recently we found a novel mechanism that DOC2b regulates membrane fusion through binding to syntaxin4 in adipocytes [27]. To investigate the role of DOC2b on insulin secretion, we first determined the DOC2b-binding partner in MIN6 cells. As shown in Fig. 3A and B, DOC2b–syntaxin4 binding was increased upon glucose stimulation and pre-treatment with BAPTA-AM decreased this interaction, suggesting that high glucose triggers DOC2b–syntaxin4 interaction in the presence of calcium ions. Since recent studies suggest that syntaxin1 was a t-SNARE for first-phase insulin secretion in pancreatic β-cells [12], we next assessed the interaction between DOC2b and syntaxin1 by immunoprecipitation experiments. As shown in Fig. 3A and C, DOC2b–syntaxin1 interaction was under the detectable level. These results, taken together with the data shown in Fig. 2, suggest the possibility that DOC2b regulates the second-phase insulin secretion through binding with syntaxin4.

DOC2b positively regulates glucose-stimulated insulin secretion

We next focused on the role of DOC2b in glucose-stimulated insulin secretion in MIN6 cells. As shown in Fig. 4A, adenoviral overexpression of myc-DOC2b in MIN6 cells caused significant increase in insulin secretion compared with control cells ($p < 0.05$, $n = 3$) at high glucose concentration. Next, we introduced short hairpin-RNA ($shRNA_{DOC2b}$) into MIN6 cells by retroviral system to induce specific degradation of the DOC2b mRNA. Under these conditions, DOC2b protein expression was decreased to 10–20% of the control level (Fig. 4B). Using this stable cell-line lacking DOC2b, we measured rapid and prolonged (delayed) insulin secretion in response to glucose. As shown in Fig. 4C, insulin secretion for the first 60 min period was decreased by 20–54% in DOC2b silenced cells, compared with the control cells. However, during the first 7 min after glucose stimulation, we could not find any differences between the cells. These results raise the possibility that DOC2b may regulate second-phase secretion of insulin in MIN6 cells. In order to better assess this phase-dependency of DOC2b on insulin secretion, we conducted additional experiment using depolarization dependent, first-phase specific secretagogue KCl. As expected, KCl-stimulated insulin secretion did not differ both in DOC2b overexpressing and silencing cells compared to the respective control cells. These results support the aforementioned hypothesis that DOC2b may involve in the second-phase secretion of insulin.

Discussion

Insulin secretion is fundamentally important for glucose homeostasis and strictly regulated by blood glucose. However, its precise regulatory mechanism is not fully understood. In general, vesicular exocytosis occurs when appropriate stimulus (i.e. an increase in $[Ca^{2+}]_i$) arrives to trigger the fusion of secretory vesicles with the plasma membrane. These membrane fusion processes are initiated with the formation of core complex consisting of SNARE proteins [18]. However, a number of additional factors are required to bring membrane fusion *in vivo*. These factors are called SNARE regulators. In pancreatic β-cells, a lot of SNARE regulators such as synaptotagmins I, II, III, V, and VII, were initially reported to be involved in insulin-granule exocytosis [19–21], but there remains uncertainty about their specificity [5]. Since we recently identified DOC2b as a positive SNARE regulator for the fusion step of vesicles containing glucose transporter 4 (GLUT4) in adipocytes [27], we speculatively investigated the role of DOC2b on insulin secretion in MIN6 cells.

In this report, we first confirmed the expression of DOC2b in islets and MIN6 cells (Fig. 1). Then, we revealed that DOC2b was localized around the insulin vesicles at cell periphery (Fig. 2C),

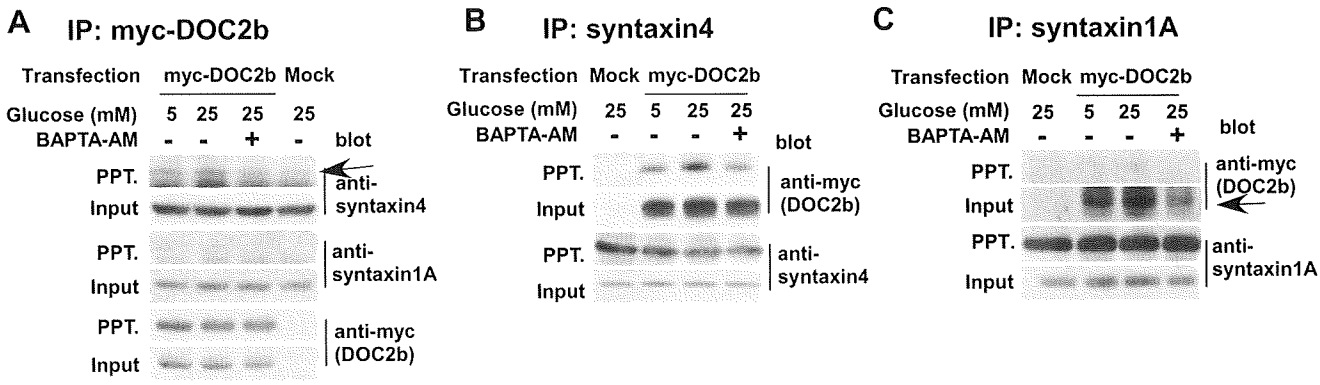

Fig. 3. DOC2b interacts with syntaxin4 in glucose and Ca^{2+} dependent manner. Myc-tagged DOC2b and eGFP control (Mock) were expressed in MIN6 cells by adenovirus vectors. After the preincubation for 30 min in KRH buffer containing 5 mM glucose, the cells were treated with 5 or 25 mM glucose for 15 min in the presence or absence of 30 mM BAPTA-AM. Immunoprecipitation was performed by monoclonal anti-myc (A), polyclonal anti-syntaxin4 (B), or monoclonal anti-syntaxin1A (C) antibodies. Precipitates were separated by SDS–PAGE and blotted with anti-myc, anti-syntaxin4, and anti-syntaxin1A antibodies.

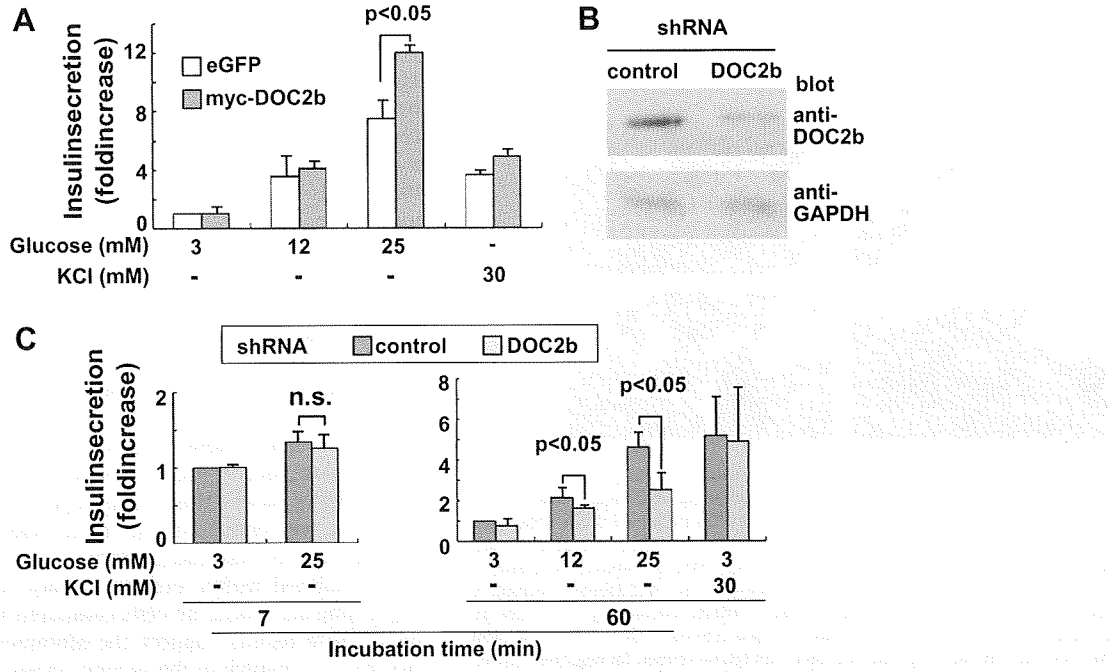

Fig. 4. Role of DOC2b on glucose-stimulated insulin secretion in MIN6 cells. MIN6 cells expressing DOC2b (A) or $shRNA_{DOC2b}$ (B,C) were treated with 3, 12, 25 mM glucose or 30 mM KCl for 60 min (A) or the time indicated (C). At the end of glucose stimulation, secreted insulin concentrations were measured by ELISA and normalized by cellular protein content. Knock-down efficacy was determined by Western blot using anti-DOC2b antibody (B). Values are mean ± SD from three independent experiments.

and translocated to the plasma membrane in response to high glucose (Fig. 2A and B). Interestingly, this translocation was intracellular calcium dependent manner. Glucose induced interaction between DOC2b and syntaxin4, but not syntaxin1 (Fig. 3). Finally, we showed that overexpression of DOC2b increased and silencing of DOC2b decreased glucose-induced insulin secretion (Fig. 4), suggesting the regulatory role of DOC2b on insulin secretion. These data were consistent with the aforementioned hypothesis that DOC2b positively regulates insulin secretion in β-cells.

One of the key findings of this study is the time scale of the regulation by DOC2b. As shown in Fig. 4C, DOC2b silencing did not affect the insulin secretion during the first 7 min after exposure to glucose. In contrast, DOC2b silencing apparently decreased the delayed (~60 min) insulin secretion. These data were consistent with the slow time course of DOC2b translocation to plasma membrane, suggesting the role of second-phase specific insulin secretion. To date, there are no reports on SNARE regulators for the second-phase insulin secretion. These results were supported by the observation that DOC2b has no effects on KCl-stimulated insulin secretion (Fig. 4A and C), the first-phase specific secretagogue. Furthermore, in agreement with the report that syntaxin1 mediates first-phase specific insulin secretion [12], our observation that DOC2b specifically binds to syntaxin4, but not syntaxin1 (Fig. 3A and C), is consistent with its role on the second-phase release of insulin.

Another interesting observation in this study is the essential role of $[Ca^{2+}]_i$ in glucose induced DOC2b translocation (Fig. 2A). DOC2b binding to syntaxin4 is also $[Ca^{2+}]_i$ dependent (also in [27]). Ke et al. reported that DOC2b did not interact with syntaxin4 in pancreatic β-cells [34]. This apparent discrepancy must be attributable to the different experiment conditions. They performed their experiments using a buffer without Ca^{2+}. DOC2b has tandem C2 domains and several Ca^{2+} binding sites, and is structurally similar to the well-known calcium sensor synaptotagmins,

suggesting that $[Ca^{2+}]_i$ might be necessary for proper function of DOC2b.

In conclusion, our results allow us to draw the following two conclusions. First, DOC2b was translocated to plasma membrane and interacted with syntaxin4 upon high-glucose stimulation in $[Ca^{2+}]_i$ dependent manner. Second, DOC2b regulates glucose induced delayed insulin secretion in MIN6 cells. In summary, our data suggest that DOC2b may be a SNARE regulator for the second-phase secretion of insulin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.04.133.

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WFS1 遺伝子と糖尿病

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*WFS1*遺伝子とWolfram症候群

WFS1 遺伝子は Wolfram 症候群の原因遺伝子として同定された[1]．Wolfram 症候群は常染色体性に劣性遺伝し，若年発症のインスリン依存性糖尿病と視神経萎縮を主徴とする．他に，感音性難聴，中枢性尿崩症，尿路異常，精神神経障害などを合併し，主要な 4 徴候(Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness)の頭文字をとって DIDMOAD 症候群ともよばれる．典型例では 3〜8 歳で発症するインスリン分泌不全による糖尿病が初発症状となり，遅れて視力障害，尿崩症，聴力障害や多彩な神経症状をきたす[2]．

Wolfram 症候群患者の剖検例では膵 Langerhans 島の萎縮とβ細胞の選択的な脱落が認められることから，その原因遺伝子はβ細胞の“生存”に必須である[3]．Wolfram 症候群の原因遺伝子としては，ほかに *CISD2* (*WFS2*)遺伝子が知られ，少数の患者で変異が同定されている．

*WFS1*遺伝子の構造と機能

ヒト *WFS1* 遺伝子は 8 つのエクソンにより構成され，Wolfram 症候群での変異の大多数は最大エクソンである第 8 エクソンに存在する．変異の種類は患者によりさまざまで，hot spot や founder effect を示唆する共通の変異は存在しない．

WFS1 蛋白は 890 アミノ酸よりなる分子量約 100 kDa の，小胞体に存在する 9 回膜貫通型の膜蛋白である．ほぼすべての臓器，組織で発現されるが，組織内での発現には特異性がみられ，膵では Langerhans 島のβ細胞におもに発現され，α細胞や外分泌組織には発現されない．また，中枢神経組織では海馬(CA1)，嗅結節，扁桃体および梨状皮質に強い発現が認められる[4,5]．

WFS1 蛋白の機能は十分には解明されていないが，その発現は小胞体ストレスで誘導され，WFS1 蛋白の欠損自体が小胞体ストレスを惹起すること，また，*WFS1* 欠損細胞は小胞体ストレスに対して脆弱でアポトーシスをきたしやすいことから，小胞体ストレスと深くかかわることが示唆されている．*WFfs1* 遺伝子欠損マウスが作製されているが，このマウスではβ細胞の減少とインスリン分泌障害が認められる．ββ細胞死はインスリン抵抗性の増強によるβ細胞への小胞体ストレス亢進により著明に加速される[6]．WFS1 蛋白を欠損するβ細胞では，ブドウ糖やカルバコール刺激によるインスリン分泌が障害されている．また，これらによる刺激時の細胞内カルシウム上昇も障害されており，小胞体へのカルシウムの再取込みの障害によると推察されている[7]．これらの観察は，WFS1 蛋白が小胞体膜の(カルシウム)イオンチャネルの活性を調節する可能性を示唆し，その欠損によるカルシウムホメオスタシスの異常が小胞体ストレスを惹起し，また小胞体ストレス亢進によるアポトーシスの，すくなくとも一部を説明しうる．WFS1 蛋白はカルシウム依存性にカルモデュリンと結合しうることが示され，WFS1 蛋白の機能がカルシウム-カルモデュリンにより調節を受ける可能性も示唆されている[8]．また，ごく最近，WFS1 蛋白が小胞体ストレスシグナルを伝達する重要な転写因子である ATF6 を抑制的に制御している可能性も示唆された．著者らは，β細胞においては WFS1 蛋白は小胞体のみならず，インスリン分泌顆粒に多く存在し，分泌顆粒内の pH を制御しうることを見出している(Hatanaka et al. manuscript in preparation)．このように，WFS1 蛋白の機能については現在活発に研究が展開されているが，いぜん不明な点も多い．

2型糖尿病と*WFS1*遺伝子

2 型糖尿病においてもβ細胞量の減少が，その発症や進展の重要な要因である．*WFS1* 遺伝子の軽度の発現低下や WFS1 蛋白の機能異常は，2 型糖尿病の発症素因になりうる．事実，*WFS1* 遺伝子の比較的頻度の高いバリアントである 611 番目のヒスチジンのアルギニンへの変化(H611R)が 2 型糖尿病と関連することがイギリス人の集団で示された．続いて，このバリアントを含む 4 つの SNP が 2 型糖尿病と強く関連することがイギリス人や Ashkenazi のユダヤ人で示され，さらに白人の他の集団で確認されている[9](図 1)．同様の傾向は Diabetes Prevention Program 参加者の

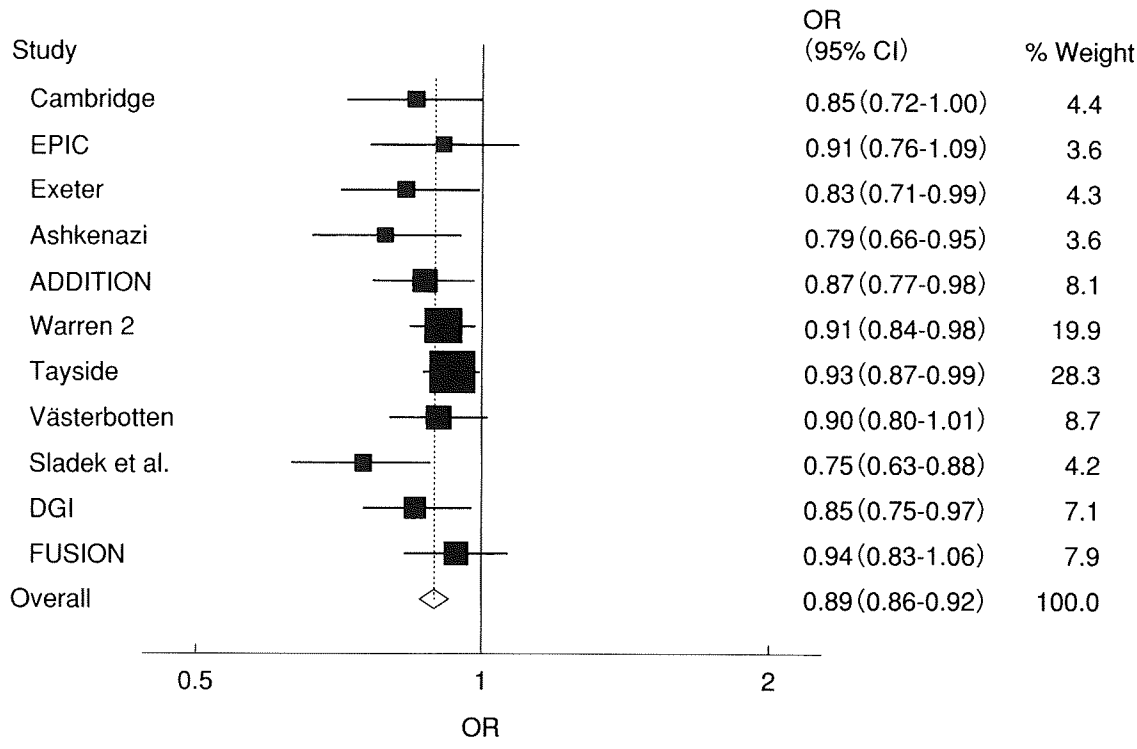

図 1　*WFS1* 遺伝子のSNPと2型糖尿病の関連解析についてのメタ解析[10]

現在までに発表されたデータ，および未発表のデータを含めてメタ解析を行った．SNP rs10010131は2型糖尿病と有意に関連した($p=5.4\times10^{-11}$)．

集団でも認められ，*WFS1* 遺伝子の SNP はβ細胞機能を規定する可能性が示唆されている．このように，*WFS1* 遺伝子は2型糖尿病遺伝子のリストに加えられるに至っている[10]．日本人では *WFS1* 遺伝子の SNP と2型糖尿病に弱い相関がみられているが，大規模な解析は行われておらず，*WFS1* 遺伝子と2型糖尿病の関連は明確には示されていない[11]．白人で2型糖尿病と強く関連する SNP の MAF(minor allele frequency)が2%程度であるため，十分な解析力を得るためには相当大規模スケールでの相関解析が必要と思われる．ほとんどの日本人は，欧米人での解析で2型糖尿病と強く相関した SNP の risk allele を有している点は興味深い．

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