Intracellular ATP, cAMP, and Ca$^{2+}$ are major signals involved in the regulation of insulin secretion in the pancreatic β-cell. We recently found that the ATP-sensitive K$^+$ channel (K$\text{ATP}$ channel) as an ATP sensor, cAMP-GEFII as a cAMP sensor, Piccolo as a Ca$^{2+}$ sensor, and t-type voltage-dependent Ca$^{2+}$ channel (VDCC) can interact with each other. In the present study, we examined the effects of cAMP and ATP on the interaction of cAMP-GEFII and syntaxin-1, synaptotagmins, and Rab proteins (2,3). An increase in the intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$])$\text{_{i}}$ is the primary signal in most secretory cells. In neurons, the elevation of [Ca$^{2+}$]$\text{_{i}}$ results from Ca$^{2+}$ influx owing to opening of the voltage-dependent Ca$^{2+}$ channels (VDCCs) localized in the active zone, a specialized region where synaptic vesicles dock and fuse (4,5). CAZ (cytoskeletal matrix associated with the active zone) proteins have been suggested to organize the site of neurotransmitter release. These include Piccolo/Aconzin (6,7), Bassoon (8), Rim1 (9), Munc13-1 (10), and CAST/ERC (11,12). Piccolo/Aconzin is a 500-kDa protein with zinc fingers, a PDZ (PSD-95, Dlg, and ZO-1) domain, and two C$_2$ domains. Rim1, which is structurally related to Piccolo/Aconzin, is a 180-kDa protein that interacts with Rab3 (9). The pancreatic β-cell is a typical endocrine cell in which exocytosis of insulin-containing vesicles is regulated by a variety of intracellular signals. ATP, cAMP, Ca$^{2+}$, and diacylglycerol are the major intracellular signals involved in the regulation of insulin secretion (13).

We recently found that cAMP-GEFII/Epac2 (hereafter cAMP-GEFII) (14–16), acting as a cAMP sensor, interacts specifically with sulfonyleurea receptor-1 (SUR1) through nucleotide-binding fold (NBF)-1 of SUR1 (16). We also found that cAMP-GEFII mediates cAMP-dependent, protein kinase A (PKA)-independent insulin secretion, and that this requires interaction with both Rim2 and Piccolo (16–18). Piccolo forms a homodimer or a heterodimer with Rim2 in a Ca$^{2+}$-dependent manner, and Piccolo rather than Rim2 may function as the Ca$^{2+}$ sensor (18). In addition, Rim2 and Piccolo bind directly to α1,2-subunit of VDCCs (19). These findings show that the ATP, cAMP, and Ca$^{2+}$ sensors interact with each other.

In the present study, we investigated regulation of the interaction of the ATP-sensitive K$^+$ channel (K$\text{ATP}$ channel) and cAMP-GEFII. We also show direct interaction of SUR1, cAMP-GEFII, and Piccolo.

**RESEARCH DESIGN AND METHODS**

**Recombinant fusion proteins.** SUR1 (amino acid residues 508–1,003), SUR1 (508–702), Piccolo (4,505–4,708), Piccolo-C$_{\text{A}}$ (4,704–5,010), and Piccolo-C$_{\text{B}}$ (4,955–5,165) were expressed as a glutathione S-transferase (GST)-fused protein in BL21. The fusion proteins were purified by affinity chromatography with glutathione-resin (Amersham Biosciences). SUR1 (508–1,003) was also expressed as a maltose-binding protein (MBP)-fused protein in BL21. These fusion proteins were purified by affinity chromatography with amyllose-resin (New England BioLabs). Fragment containing α1,2-subunit (745–892) was subcloned in pGEMI7 vector (Clontech Laboratories, Palo Alto, CA) as a Myc-tagged protein.

**Pull-down assay.** Mouse insulin-secreting MIN6 cells were sonicated in binding buffer (20 mmol/l HEPES, pH 7.4, 150 mmol/l NaCl, 1 mmol/l EGTA, 5 mmol/l MgCl$_2$, and 0.5% NP-40). The cellular lysates were incubated with 0.5 μg GST-fused protein immobilized on glutathione-resin for 90 min at 4°C. The washed complexes were separated by SDS-PAGE and subjected to immunoblot analysis with anti-cAMP-GEFII antibody (16). COS-1 cells were transfected with pcMV-FLAG-mouse cAMP-GEFII using LipofectAmine PLUS (Invitrogen, Carlsbad, CA). The transfected cells were sonicated in binding buffer. The cellular lysates were incubated with MBP-SUR1 (508–1,003) immobilized on amyllose-resin for 90 min at 4°C. The washed complex was incubated with GST-Piccolo (4,505–4,758) for 2 h at 4°C. The sample was

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From the Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan. Address correspondence and reprint requests to S. Seino, Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017. E-mail: seino@med.kobe-u.ac.jp.

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The K_{ATP} channel in pancreatic β-cells plays a critical role in the regulation of glucose-induced and sulfonylurea-induced insulin secretion (20,21). The β-cell K_{ATP} channel is a hetero-octamer composed of pore-forming Kir6.2 subunits and regulatory SUR1 subunits (20,21). SUR1 has two intracellular nucleotide-binding folds (NBF-1 and NBF-2), each containing a Walker A and a Walker B motif. We recently established that the cAMP-binding protein cAMP-GEFII specifically interacts with NBF-1 of SUR1 (16,19). We have now found that the interaction requires both the Walker A and Walker B motifs. ATP has been shown to bind to NBF-1 of SUR1 (22), but the binding of ATP to NBF-1 did not affect interaction of SUR1 and cAMP-GEFII. Because cAMP-GEFII does not affect β-cell K_{ATP} channel activity under the conditions used (19), these findings suggest that SUR1 may function as a scaffold protein in addition to its role as the regulatory subunit of the K_{ATP} channel.

Interaction of cAMP-GEFII and SUR1 is decreased by a high cAMP concentration (19). Because binding of cAMP to cAMP-GEFII induces a conformational change of cAMP-GEFII (23), cAMP-GEFII might dissociate from the complex of SUR1 and cAMP-GEFII upon cAMP stimulation. Accordingly, rather than being activated, cAMP-GEFII may...
be anchored to SUR1 even when the ATP/ADP ratio is increased by the metabolism of glucose. This anchoring may be necessary to assemble the components in exocytosis, including the K\textsubscript{ATP} channel, VDCC, Rim2, and Piccolo.

Incretins such as glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) increase the cAMP concentration in pancreatic β-cells. We previously found that incretin-induced, PKA-independent insulin secretion is mediated by cAMP-GEFII (17). Lack of interaction between SUR1 and cAMP-GEFII could disrupt assembly of the exocytic components and lead to an impairment of cAMP-dependent, PKA-independent insulin secretion. This is supported by the finding that incretin-induced, PKA-independent insulin secretion is impaired in SUR1-deficient mice (24,25). We recently found that Piccolo in pancreatic β-cells interacts with cAMP-GEFII (18). Here we show that Piccolo interacts with SUR1 through cAMP-GEFII and with the α\textsubscript{1.2}-subunit of L-type VDCC in a Ca\textsuperscript{2+}-independent manner.

Based on these findings, we propose a model of the interactions of ATP, cAMP, and Ca\textsuperscript{2+} sensors in the exocytosis of insulin granules, as shown in Fig. 3. NBF-1 of SUR1 interacts with cAMP-GEFII. cAMP-GEFII and SUR1 form a complex with Piccolo. Piccolo forms heterodimer with Rim2 in a Ca\textsuperscript{2+}-dependent manner. Both Piccolo and Rim2 interact with L-type VDCC. An increase in cAMP concentration upon stimulation may control the dissociation of cAMP-GEFII from SUR1. Clarification of the temporal and spatial interaction of these molecules in exocytosis of insulin granules requires further investigation.

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