Mammalian Exocyst Complex Is Required for the Docking Step of Insulin Vesicle Exocytosis*

Received for publication, February 14, 2005, and in revised form, April 28, 2005
Published, JBC Papers in Press, May 3, 2005, DOI 10.1074/jbc.M501674200

Takashi Tsuboi‡‡, Magalie A. Ravier‡, Hao Xie‡, Marie-Ann Ewart‡, Gwyn W. Gould***, Stephen A. Baldwin†††, and Guy A. Rutter‡‡‡

From the ‡‡Henry Wellcome Laboratories for Integrated Cell Signalling and Department of Biochemistry, School of Medical Sciences, University Walk, University of Bristol, Bristol BS8 1TD, United Kingdom, †Bradford Health Sciences, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom, and ‡‡School of Biochemistry and Microbiology, University of Leeds, Leeds LS2 9JT, United Kingdom

Glucose stimulates insulin secretion from pancreatic β cells by inducing the recruitment and fusion of insulin vesicles to the plasma membrane. However, little is currently known about the mechanism of the initial docking or tethering of insulin vesicles prior to fusion. Here, we examined the role of the SEC6-SEC8 (exocyst) complex, implicated in trafficking of secretory vesicles to fusion sites in the plasma membrane in yeast and in regulating glucose-stimulated insulin secretion from pancreatic MIN6 β cells. We show first that SEC6 is concentrated on insulin-positive vesicles, whereas SEC5 and SEC8 are largely confined to the cytoplasm and the plasma membrane, respectively. Overexpression of truncated, dominant-negative SEC8 or SEC10 mutants decreased the number of vesicles at the plasma membrane, whereas expression of truncated SEC6 or SEC8 inhibited overall insulin secretion. When single exocytotic events were imaged by total internal reflection fluorescence microscopy, the fluorescence of the insulin surrogate, neuropeptide Y-monomeric red fluorescent protein brightened, diffused, and then vanished with kinetics that were unaffected by overexpression of truncated SECs or SEC10. Together, these data suggest that the exocyst complex serves to selectively regulate the docking of insulin-containing vesicles at sites of release close to the plasma membrane.

Insulin is accumulated in large dense core vesicles within the pancreatic β cell and is released by exocytosis when glucose concentrations rise (1). Triggering of secretion appears to involve increases in intracellular ATP concentrations (or ATP/ADP ratio), closure of ATP-sensitive K+ channels, and Ca2+ influx through voltage-sensitive Ca2+ channels (1). Because secretion in response to nutrient secretagogues is usually biphasic, with a rapid early phase followed by a subsequent second or sustained phase (2), it has been proposed that functionally distinct groups of vesicles may exist and represent “reserve,” “readily releasable,” and “intermediate” pools (3, 4). At present, however, the mechanisms through which vesicles are recruited to the readily releasable pool, proposed to correspond to a subset of the “morphologically docked” (4) vesicles at the cell surface, are still incompletely understood (1).

Soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs)† are believed to be required for the trafficking of insulin and other secretory hormones from the endoplasmic reticulum to the Golgi apparatus, Golgi and trans-Golgi network to the plasma membrane, and finally exocytosis of mature vesicles (5, 6). Dense core vesicle trafficking to the plasma membrane can itself be divided into three stages: transport of insulin vesicles to the plasma membrane, their first interaction with the plasma membrane (docking or tethering), and then subsequent fusion at the plasma membrane (exocytosis). Although SNARE proteins are believed to regulate the final fusion step in β cells (7), the machinery involved in the initial docking or tethering steps and possibly in regulating the sustained phase of insulin secretion (1, 8) is undefined (9).

The budding yeast Saccharomyces cerevisiae has provided the genetic identification of proteins required for vesicle transport (10, 11). Six of the sec gene products, Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, and Sec15p, have been described in the vesicle transport complex, and mammalian homologues have been identified (12–14). Furthermore, two novel gene products, Exo70p and Exo84p, have been shown to be part of the complex (13, 15, 16), now known as the SEC6-SEC8 or “exocyst” complex.

The exocyst complex is concentrated at the sites of active vesicle exocytosis in the yeast bud and is essential for secretion (16, 17). Similarly, the mammalian exocyst is localized at the plasma membrane of nerve terminals (12) and, in differentiated PC12 cells, displays a punctate distribution at the termini of cell processes at or near sites of exocytosis (13) or on the secretory vesicle membrane (18). Such findings suggest that the exocyst complex may serve as part of vesicle-docking machinery at sites of regulated as well as constitu-

* This work was supported in part by grants (to G. A. R.) from the Wellcome Trust (Project 062321; Programme 067081/Z/02/Z), the Biotechnology and Biological Sciences Research Council, the Human Frontiers Science Program, the Juvenile Diabetes Research Foundation, and Diabetes UK. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be part by the payment of page charges. This article must therefore be

** Supported by Biotechnology and Biological Sciences Research Council Grant 17/C0221 and recipient of a Wellcome Trust for Research leave fellowship.

††† Supported by Biotechnology and Biological Sciences Research Council Grant 24/b17173.

‡‡‡ Recipient of a Wellcome Trust for Research leave fellowship. To whom correspondence should be addressed: Dept. of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, United Kingdom. Tel.: 44-117-954-6401; Fax: 44-117-928-8274; E-mail: g.a.rutter@bristol.ac.uk.

† The abbreviations used are: SNARE, soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor; EGFP, enhanced green fluorescent protein; mRFP, monomeric red fluorescent protein; NPY, neuropeptide Y; Tir, total internal reflection fluorescence.
Exocyst Complex in Insulin Vesicle Docking

The exocyst complex has been shown to be present in high concentrations in developing brain and is believed to play a role in synaptogenesis (12, 31). To examine the cellular localization of the complex in pancreatic β cells, formaldehyde-fixed pancreatic MIN6 β cells were probed with antibodies against the three exocyst complex subunits (SEC5, SEC6, and SEC8). Confocal images revealed that SEC5, SEC6, and SEC8 immunoreactivity was brightly displayed. Therefore, to probe subcellular localization (Fig. 1). SEC5 immunofluorescence was observed largely as diffuse, cytosolic fluorescence, although some punctate staining, partially colocalized with insulin, was apparent (Fig. 1, A–C). In contrast, SEC6 was found as punctate labeling, closely colocalized with insulin-positive vesicles (Fig. 1, D–F). SEC8 was mainly found on the plasma membrane of the cells (Fig. 1, G–I). These observations are similar to those made previously in PC12 cells (18) and suggest that the exocyst complex might regulate vesicle docking or fusion in pancreatic MIN6 β cells.

EXPERIMENTAL PROCEDURES

Expression Vectors—NPY-mRFP was constructed as described previously (21, 22). Plasmids encoding pIRES2-EGFP-tagged forms of N-terminally truncated sec8 (sec8-ΔN) and C-terminally truncated sec10 (sec10-ΔC) plasmids (23) were generously provided by Dr. S. C. Hsu (Rutgers University).

Generation of Truncated sec8 and sec8 Adenoviruses—cDNAs encoding C-terminal truncation mutants of rat sec8 (residues 1–333, Ad-sec8(ΔC)) or rat sec8 (residues 1–426, Ad-sec8(ΔN)) were amplified by PCR, subcloned into pcR2 (Invitrogen), and sequenced on both strands. These mutants corresponded to those identified by Novick and colleagues (17, 24, 25), which abrogate function (i.e. secretion) in yeast. We therefore postulated that the constructs would function as dominant negatives in mammalian cells. The cDNAs were subcloned into the pShuttle-CMV vector, linearized with PacI, and cotransformed with pADEasy into E. coli strain BL21(DE3) (Novagen). Recombinants were selected and amplified in E. coli DH5α. The chosen clones were linearized with PciI to expose the inverted terminal repeats and allow viral packaging when transfected in HEK293 cells. Large scale amplification and titer of viral stocks was performed as described previously (26).

MIN6 Cell Culture—MIN6 cells (27) were used between passages 20 and 35 and grown in Dulbecco’s modified Eagle’s medium containing 15% (v/v) heat-inactivated fetal calf serum (Invitrogen, Paisley, UK), 25 mM glucose, 5.4 mM KCl, 2 mM glutamine, 50 μM β-mercaptoethanol, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured in a humidified atmosphere at 37 °C with 5% CO2.

Imaging was performed in modified Krebs-Ringer buffer (KRB: 125 mM NaCl, 3.5 mM CaCl2, 0.5 mM NaH2PO4, 0.5 mM MgSO4, 3 mM glucose, 10 mM HEPES, 2 mM NaHCO3, pH 7.4, equilibrated with O2/CO2 (19:1)). Stimulation with KCl was achieved by perfusion of 50 mM glucose, 10 mM HEPES, 2 mM NaHCO3, pH 7.4, equilibrated with O2/CO2 (19:1). Stimulation with a long pass filter (515 nm for EGFP, 600 nm for mRFP).

To monitor exocytosis of NPY-mRFP constructs at the single vesicle level, we used a TIRF microscope similar to that described previously (26). The critical angle of our 1.45 numerical aperture objective lens is 67.5° and 2 the refractive indices of water (1.33) and glass (1.53). For n = 67.5° and λ = 488 nm, decay constant (d) becomes 81.1 nm, and effectively, a layer of 100–200 nm is the reach of the evanescent light.

Confocal Imaging—To assess the density of the vesicles in exocyst complex mutant-expressing cells, we employed a confocal microscope (Leica TCS-AOBS laser-scanning confocal microscope). Before monitoring vesicle density, the bottom of the cell was located by inspection in confocal mode. The focal plane was then moved up by 1 μm using a piezo motor, which drove the objective lens as controlled by the system software.

Generation and Purification of Polyclonal Anti-SEC5 Antibody—Antibodies were raised in sheep against residues 1–100 of the human SEC5 (GenBank™ accession number: NP_060773) sequence, encompassing the Rab-binding domain. The mouse SEC5 protein is identical in this region apart from residue 82, where arginine is replaced by lysine in the murine sequence. The relevant part of the coding region of SEC5 was inserted as a BamHI/EcoRI fragment into the vector pET28a. The resultant fusion protein, bearing an N-terminal hexahistidine tag, was expressed in E. coli and purified by chromatography on nickel-nitritotriacetic acid-agarose. Antibodies were purified from the anti-softmax by chromatography on a column of the fusion protein, immobilized on CNBr-activated Sepharose CL-4B.

Insulin Secretion Assay—MIN6 cells (5 × 105 cells/well) were seeded in 6-well microtiter plates and infected with null (control), truncated sec6, and truncated sec8 adenoviruses. Culture was continued for 24 h in Dulbecco’s modified Eagle’s medium containing 25 mM glucose and then at 3 mM glucose for another 16 h. Cells were then washed in phosphate-buffered saline and incubated in KRB medium and 3 mM glucose, 30 mM glucose, or 50 mM KCl. Incubations were performed for 30 min at 37 °C in a water bath. Total insulin was extracted in acidified ethanol (30). Total and secreted insulin were measured using radioimmunoassay by competition with 125I-labeled rat insulin (Linco Research, St. Charles, MO) according to the manufacturer’s instructions.

Data Analysis—Data are given as means ± S.E. Comparisons between means were performed using one-way analysis of variance with GraphPad Prism™ (GraphPad Software, San Diego, CA) software.

RESULTS

The Exocyst Complex Is Associated with Insulin-containing Vesicles—The exocyst complex has been shown to be present in high concentrations in developing brain and is believed to play a role in synaptogenesis (12, 31). To examine the cellular localization of the complex in pancreatic β cells, formaldehyde-fixed pancreatic MIN6 β cells were probed with antibodies against the three exocyst complex subunits (SEC5, SEC6, and SEC8). Confocal images revealed that SEC5, SEC6, and SEC8 immunoreactivity was brightly displayed. Therefore, to probe subcellular localization (Fig. 1). SEC5 immunofluorescence was observed largely as diffuse, cytosolic fluorescence, although some punctate staining, partially colocalized with insulin, was apparent (Fig. 1, A–C). In contrast, SEC6 was found as punctate labeling, closely colocalized with insulin-positive vesicles (Fig. 1, D–F). SEC8 was mainly found on the plasma membrane of the cells (Fig. 1, G–I). These observations are similar to those made previously in PC12 cells (18) and suggest that the exocyst complex might regulate vesicle docking or fusion in pancreatic MIN6 β cells.
Truncated Exocyst Component Decreases the Number of Docked Vesicles on the Plasma Membrane—To determine the significance of exocyst complex function in insulin vesicle recruitment or docking, MIN6 cells were cotransfected with NPY-mRFP and either pIRE2-EGFP-sec8-ΔN or pIRE2-EGFP-sec10-ΔC. The pIRE2-EGFP plasmid is a bicistronic vector that allows the expression of both EGFP and an exocyst subunit mutant as two separate proteins. NPY-mRFP was used here as a surrogate for insulin (22, 29) because the latter targets relatively poorly to the dense core vesicle after fusion here as a surrogate for insulin (22, 29) because the latter targets relatively poorly to the dense core vesicle after fusion.

The number of intracellular and plasma membrane-docked NPY-mRFP vesicles by TIRF and confocal microscopy, respectively (Fig. 2A–F). All of the cells examined that overexpressed truncated exocyst components showed a significant 40–50% decrease in the number of docked vesicles at the plasma membrane (control, 1.36 ± 2.2 vesicles/μm², n = 12; sec8-ΔN, 0.86 ± 2.8 vesicles/μm², n = 7; sec10-ΔC, 0.65 ± 1.1 vesicles/μm², n = 12) (Fig. 2G, open squares). In contrast, cells overexpressing sec8-ΔN or sec10-ΔC showed a non-significant ~10% decrease in the number of intracellular vesicles (Fig. 2G, filled squares).

We also performed adenoviral infection of Ad-sec6CT or Ad-sec8CT to observe the effect on the number of intracellular and plasma membrane-docked NPY-mRFP vesicles. Cells adenovirally infected with truncated exocyst components also displayed a significant 30% decrease in the number of docked vesicles at the plasma membrane (control, 1.43 ± 1.2 vesicles/μm², n = 6; Ad-sec6CT, 0.91 ± 1.8 vesicles/μm², n = 6; Ad-sec8CT, 0.88 ± 1.7 vesicles/μm², n = 6). However, there was no significant decrease in the number of intracellular vesicles in either Ad-sec6CT- or Ad-sec8CT-infected MIN6 cells (control, 1.54 ± 2.2 vesicles/μm², n = 6; Ad-sec6CT, 1.47 ± 2.1 vesicles/μm², n = 6; Ad-sec8CT, 1.51 ± 2.1 vesicles/μm², n = 6).

To further validate the above observations, we measured the number of docked vesicles in pancreatic MIN6 β cells. A–C, MIN6 cells were co-immunostained with sheep polyclonal anti-SEC6 and guinea pig polyclonal anti-insulin antibody and visualized with an Alexa 488 rabbit anti-sheep and an Alexa 568 goat anti-guinea pig secondary antibody. D–F, MIN6 cells were co-immunostained with mouse monoclonal anti-SEC6 and guinea pig polyclonal anti-insulin antibody and visualized with an Alexa 488 rabbit anti-mouse and an Alexa 568 goat anti-guinea pig secondary antibody. G–I, MIN6 cells were co-immunostained with mouse monoclonal anti-SEC8 and guinea pig polyclonal anti-insulin antibody and visualized with an Alexa 488 rabbit anti-mouse and an Alexa 568 goat anti-guinea pig secondary antibody. Scale bars = 5 μm.

Effect of Overexpression of Exocyst Complex on Vescicle Fusion—To determine whether the apparent inhibition of docking observed above might be due to the exocyst complex decreasing the rate at which individual vesicles fused, thus causing a potential "bottleneck" that prevented subsequent fusion events, the dynamics of single vesicle exocytosis were analyzed in single NPY-mRFP-expressing vesicles. Although as expected, exocytotic events were detected less frequently in sec8-ΔN- and sec10-ΔC-expressing cells than in cells transfected with empty vector control (Fig. 2E), the kinetics of individual fusion events were identical in each case (Fig. 3D). Thus, stimulation with 30 mM glucose caused NPY-mRFP-expressing vesicles to brighten and spread suddenly during the release of the fluorescent peptide (22, 29, 32) with an identical time course in control (Fig. 3A), sec8-ΔN-overexpressing cells (Fig. 3B), or sec10-ΔC-overexpressing cells (Fig. 3C). No difference...
was observed in the mean values for the rise time, half-widths, or decay time of fluorescence intensity between control and sec8-ΔN- or sec10-ΔC-overexpressing cells (data not shown). These data thus indicate that the exocyst complex does not alter the kinetics of individual insulin exocytosis events but rather regulates the docking step of insulin vesicles to the plasma membrane.

Effect of Overexpression of Exocyst Complex on Number of Vesicles on the Plasma Membrane—We next measured the dynamic changes in the number of insulin vesicles docked at the plasma membrane during stimulation with glucose. During glucose stimulation of control cells (Fig. 4, A and D, open circles), the total number of docked vesicles slightly increased by up to 110% of the initial value, consistent with previous observations (33). This apparent recruitment of new vesicles to docking sites might thus contribute to the refilling of the docked vesicle pool (possibly equivalent to a readily releasable pool) during the second phase of glucose-induced insulin secretion. In contrast, in either sec8-ΔN- or sec10-ΔC-overexpressing cells, the total number of docked vesicles during glucose stimulation remained low, with no appreciable appearance of newly recruited vesicles to the plasma membrane (Fig. 4, B and C). As a result, in cells expressing sec8-ΔN or sec10-ΔC, the total number of docked vesicles decreased by 10–40% of the initial number after 10 min of stimulation with high glucose concentrations (Fig. 4D, closed squares and triangles).

**Truncated Exocyst Component Does Not Affect Microtubule Dynamics**—To determine whether the effects of the dominant-negative sec constructs on vesicle distribution may be due to alterations in microtubule dynamics, the latter were assessed by monitoring the distribution of α-tubulin in null- or sec mutant-expressing cells. No differences were apparent in α-tubulin distribution in cells expressing any of the interfering sec constructs as assessed by confocal microscopy (Fig. 5). Thus, microtubule rearrangements would seem unlikely to explain the decreased vesicle recruitment to the cell surface.

**DISCUSSION**

We show here that the exocyst complex is present in significant concentrations in pancreatic MIN6 β cells. Unexpectedly, we observed very different patterns of immunofluorescence staining for intracellular SEC5, SEC6, and SEC8. Thus, SEC5 immunofluorescence was predominantly seen diffusely throughout the cytoplasm in MIN6 cells. In contrast, SEC6 and SEC8 immunofluorescence were predominantly localized to insulin vesicles and...
the plasma membrane, respectively (Fig. 1). Thus, it would appear that the exocyst complex does not exist chiefly as a preformed unit but that instead, formation of the holo-complex may accompany translocation of vesicles to docking (pre-exocytosis) sites at the plasma membrane. Several studies in epithelial cells have shown that a portion of the exocyst complex exists in a partially assembled state with some of the protein being found dissociated from SEC6 (39–41). We also analyzed here the docking and fusion of insulin vesicles in pancreatic MIN6 cells overexpressing truncated exocyst subunits. Our results showed that: 1) inhibition of the exocyst complex inhibited insulin secretion in response to cell depolarization or to glucose; 2) the kinetics of insulin secretion were identical in either control or truncated exocyst component-overexpressing cells; 3) the supply of vesicles and/or the ability to retain them on plasma membrane were impaired, resulting in a decrease in the number of docked insulin vesicles (to ~50% of that in normal MIN6 cells; Fig. 2D).

In conclusion, we present direct evidence that the exocyst complex contributes to the docking step of insulin vesicles on the plasma membrane in pancreatic β cells and may be involved in the recruitment of vesicles from a reserve to a readily releasable pool at the plasma membrane. Changes in the efficiency of the docking process may therefore play a role in the normal control of insulin release under conditions where secretion is strongly stimulated, such as during chronic hyperglycemia or during treatment with sulfonylureas. Because such alterations might conceivably contribute to insulin secretory deficiency in some form of type 2 diabetes, exocyst complex components may provide potential therapeutic targets in this disease.

Acknowledgments—We thank Katsuyuki Abe (Olympus Optical, Japan), Hitoshi Hatano (Olympus Optical, Europe GmbH), and Jean Ingram for generous technical support; Drs. Shu-Chan Hsu and Roger Y. Tsien for plasmids; and Rebecca Rowe for insulin secretion assays. We also thank Dr. Mark Jepson and Alan Leard of the Bristol MRC Cell Imaging Facility for technical support.

REFERENCES
1. Rutter, G. A. (2004) Diabetologia 47, 1861–1872
2. Burr, I. M., Balant, L., Stauffacher, W., Renold, A. E., and Grodsky, G. (1969) Lancet 2, 882–883
3. Bratanova-Tochkova, T. K., Cheng, H., Daniel, S., Gunawardana, S., Liu, Y. J., Mulvany-Musa, J., Schermerhorn, T., Straub, S. G., Yajima, H., and...
Sharp, G. W. (2002) *Diabetes* **51**, Suppl. 1, 83–90
4. Rorsman, P. (1997) *Diabetologia* **40**, 487–495
5. Rothman, J. E. (1994) *Nature* **372**, 55–63
6. Sudhof, T. C. (1995) *Nature* **375**, 645–653
7. Gat, A., Kiraly, C. E., Fukuda, M., Mikoshiba, K., Wollheim, C. B., and Lang, J. (2001) *J. Cell Sci.* **114**, 1709–1716
8. Straub, S. G., and Sharp, G. W. (2004) *Am. J. Physiol.* **287**, C565–C571
9. Rorsman, P., and Renstrom, E. (2003) *Diabetologia* **46**, 1029–1045
10. Rothman, J. E. (1994) *Nature* **372**, 55–63
11. Sudhof, T. C. (1995) *Nature* **375**, 645–653
12. Gut, A., Kiraly, C. E., Fukuda, M., Mikoshiba, K., Wollheim, C. B., and Lang, J. (2001) *J. Cell Sci.* **114**, 1709–1716
13. Straub, S. G., and Sharp, G. W. (2004) *Am. J. Physiol.* **287**, C565–C571
14. Rorsman, P., and Renstrom, E. (2003) *Diabetologia* **46**, 1029–1045
15. Novick, P., Feletti, D. L., and Scheller, R. H. (1999) *Trends Cell Biol.* **9**, 150–153
16. Hsu, S. C., Hazuka, C. D., Feletti, D. L., and Scheller, R. H. (1999) *Trends Cell Biol.* **9**, 150–153
17. TerBush, D. R., Maurice, T., Roth, D., and Novick, P. (1996) *EMBO J.* **15**, 6483–6494
18. TerBush, D. R., and Novick, P. (1995) *J. Cell Biol.* **130**, 299–312
19. Vik-Mo, E. O., Oltedal, L., Hoivik, E. A., Kleivdal, H., Eide, J., and Davanger, S. (2003) *Neuroscience* **119**, 73–85
20. Bajjalieh, S. M. (1999) *Curr. Opin. Neurobiol.* **9**, 321–328
21. Lin, R. C., and Scheller, R. H. (2000) *Annu. Rev. Cell Dev. Biol.* **16**, 19–49
22. Campbell, R. E., Tour, O., Palmer, A. E., Steinbach, P. A., Baird, G. S., Zacharias, D. A., and Tsien, R. Y. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 7877–7882
23. Tsuboi, T., da Silva Xavier, G., Leclerc, I., and Rutter, G. A. (2003) *J. Biol. Chem.* **278**, 52042–52051
24. Vega, I. E., and Hsu, S. C. (2003) *Neurosci. L.* **21**, 3839–3848
25. Bowser, R., Muller, H., Govindan, B., and Novick, P. (1992) *J. Cell Biol.* **118**, 1041–1056
26. Potenza, M., Bowser, R., Muller, H., and Novick, P. (1992) *Yeast* **8**, 549–558
27. Miyazaki, J., Araki, K., Yamate, E., Ikegami, H., Asano, T., Shibasaki, Y., Oka, Y., and Yamamura, K. (1990) *Endocrinology* **127**, 126–132
28. Pinton, P., Tsuboi, T., Ainscow, E. K., Pozzan, T., Rizzuto, R., and Rutter, G. A. (2003) *J. Biol. Chem.* **277**, 37702–37710
29. Tsuboi, T., McMahon, H. T., and Rutter, G. A. (2004) *J. Biol. Chem.* **279**, 47115–47124
30. Ainscow, E. K., Zhao, C., and Rutter, G. A. (2000) *Diabetes* **49**, 1149–1155
31. Hsu, S. C., Foletti, D. L., Hsu, S. C., Kee, Y., Hopf, F. W., and Scheller, R. H. (1999) *J. Neurosci.* **19**, 1324–1334
32. Ainscow, E. K., Zhao, C., and Rutter, G. A. (2000) *J. Neurosci.* **19**, 1324–1334
33. Hsu, S. C., Hopf, F. W., and Scheller, R. H. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 14438–14443
34. Ting, A. E., Hazuka, C. D., Hsu, S. C., Kirk, M. D., Bean, A. J., and Scheller, R. H. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 9613–9617
35. Grindstaff, K. K., Yeaman, C., Andandasabapathy, N., Hsu, S. C., Rodriguez-Boulan, E., Scheller, R. H., and Nelson, W. J. (1998) *Cell* **93**, 731–740
36. Boyd, C., Hughes, T., Pypaert, M., and Novick, P. (2004) *J. Cell Biol.* **167**, 889–901
37. Finger, P. F., Hughes, T. E., and Novick, P. (1998) *Cell* **92**, 559–571
38. Guo, W., Sacher, M., Barrowman, J., Ferro-Novick, S., and Novick, P. (2000) *Trends Cell Biol.* **10**, 251–255
39. Brymora, A., Valova, V. A., Larsen, M. R., Boufogalis, B. D., and Robinson, P. J. (2001) *J. Biol. Chem.* **276**, 29792–29797
40. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 2509–2514
41. Shin, D. M., Zhao, X. S., Zeng, W., Mozhayeva, M., and Muailem, S. (2000) *J. Biol. Chem.* **150**, 1101–1112
42. Moskalenko, S., Henry, D. O., Rosse, C., Mirey, G., Camonis, J. H., and White, M. A. (2002) *Nat. Cell Biol.* **4**, 66–72
43. Sugihara, K., Asano, S., Tanaka, K., Ishida, T., Ishida, H., and Nagamatsu, S. (2002) *J. Biol. Chem.* **277**, 3805–3808
44. Brymora, A., Valova, V. A., Larsen, M. R., Boufogalis, B. D., and Robinson, P. J. (2001) *J. Biol. Chem.* **276**, 29792–29797
45. Tsuboi, T., da Silva Xavier, G., Leclerc, I., and Rutter, G. A. (2003) *J. Biol. Chem.* **278**, 52042–52051
46. Tsuboi, T., and Rutter, G. A. (2000) *Curr. Biol.* **10**, 1101–1112
47. Wang, L., Li, G., and Sugita, S. (2004) *J. Biol. Chem.* **279**, 19875–19881
Mammalian Exocyst Complex Is Required for the Docking Step of Insulin Vesicle Exocytosis

Takashi Tsuboi, Magalie A. Ravier, Hao Xie, Marie-Ann Ewart, Gwyn W. Gould, Stephen A. Baldwin and Guy A. Rutter

J. Biol. Chem. 2005, 280:25565-25570.
doi: 10.1074/jbc.M501674200 originally published online May 3, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M501674200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 42 references, 18 of which can be accessed free at
http://www.jbc.org/content/280/27/25565.full.html#ref-list-1