Mechanisms controlling the expression of the components of the exocytotic apparatus under physiological and pathological conditions

A. Abderrahmani*, V. Plaisance†, P. Lovis† and R. Regazzi†[1]

*Department of Internal Medicine, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland, and †Department of Cell Biology and Morphology, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland

Abstract

The last decade has witnessed spectacular progress in the identification of the protein apparatus required for exocytosis of neurotransmitters, peptide hormones and other bioactive products. In striking contrast, our knowledge of the mechanisms determining the expression of the components of the secretory machinery has remained rudimentary. Since modifications in secretory functions are associated with several physiological processes and contribute to the development of human pathologies, a better knowledge of the control of the expression of the genes involved in exocytosis is urgently needed. Recent studies have led to the identification of transcription factors and other regulatory molecules such as microRNAs that modulate the cellular level of key controllers of the exocytotic process. These findings furnish a new perspective for understanding how secretory functions can adapt to normal physiological conditions and shed light on the mechanisms involved in the development of important human diseases such as diabetes mellitus characterized by defective release of bioactive compounds.

The molecular machinery underlying the process of exocytosis

Convergence of data obtained in different model systems including yeast, *Caenorhabditis elegans*, *Drosophila* and mammals led to the identification of numerous components of the protein apparatus governing exocytosis. These components include members of the SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor) and Rab families. The SNAREs constitute a group of evolutionarily conserved proteins characterized by the presence of amino acid sequences, referred to as SNARE motifs, which allow them to form thermodynamically favourable complexes. There is now compelling evidence that the assembly of these complexes represents one of the key events driving fusion of secretory vesicles with their target compartments [1]. The function of the vesicular-associated SNARE protein VAMP2 (vesicle-associated membrane protein 2) and of the plasma membrane-associated SNAREs syntaxin-1 and SNAP25 has been investigated in detail. It is now universally accepted that the assembly of a complex between these three proteins is essential for hormone and neurotransmitter release [1]. In cells with specialized secretory functions that do not express these SNAREs, other closely related homologues fulfil analogous roles, emphasizing the requirement for an appropriate set of SNAREs to carry out exocytosis.

Rabs are monomeric GTPases sharing structural homologies with the Ras oncogene product. The human genome comprises approx. 60 genes encoding Rab GTPases. The members of this large protein family have specific tissue distribution patterns and display characteristic intracellular locations overlapping those of organelles participating in the secretory or endocytic pathways. Members of the Rab3 (Rab3A–D) and Rab27 (A–B) subfamilies are associated with secretory vesicles destined for fusion with the plasma membrane and are major regulators of exocytosis in a variety of cell systems, including not only neurons and secretory cells from endocrine and exocrine glands but also lymphocytes, platelets and melanocytes [2]. Rab3 and Rab27 isoforms act by recruiting a group of effector molecules on the surface of secretory organelles. Some of the Rab effectors, such as melanophilin and MyRIP (myosin VIIa- and Rab-interacting protein), link the secretory vesicles to the cell cytoskeleton and regulate organelle movement. Others, including rabphilin and granuphilin, are able to interact with proteins located at the target membranes and are thought to direct vesicle docking [3].

Mechanisms controlling the expression of the components of the secretory apparatus

Despite major progress in understanding the molecular events governing exocytosis, the mechanisms controlling the

Key words: diabetes mellitus, exocytotic apparatus, inducible cAMP early repressor (ICER), microRNA (miRNA), Rab, transcription factor.

Abbreviations used: CRE, cAMP-response element; CREM, CRE modulator; ICER, inducible cAMP early repressor; miRNA, microRNA; MyRIP, myosin VIIa- and Rab-interacting protein; NEFA, non-esterified fatty acid; NRSE, neuron restrictive silencer element; RE-1, repressor element 1; REST, RE-1 silencing transcription factor; SNAP25, 25 kDa synaptosome-associated protein; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; VAMP, vesicle-associated membrane protein.

[1]To whom correspondence should be addressed (email Romano.Regazzi@unil.ch).

expression of the main players in this process have been poorly explored. Variations in the level of key components of the secretory apparatus have been associated with various physiological and pathological conditions, including learning, ischaemia, schizophrenia and diabetes mellitus [4–8]. However, in most cases, the molecular determinants responsible for the observed changes have not been identified. Indeed, only very few studies investigated the promoter activities of the genes encoding the components of the exocytotic machinery, and the transcription factors driving the expression of these genes remain largely unknown. In view of the potential impact that modifications in the secretory activities may have on a variety of fundamental biological processes, a better knowledge of the mechanisms that regulate the expression of the genes involved in exocytosis is urgently needed.

The pancreatic β-cell is one of the cell systems in which alterations in the secretory function can have major consequences for human health. In fact, insulin release from pancreatic β-cells plays a pivotal role in the achievement of blood glucose homoeostasis and inappropriate tuning of this process can lead to diabetes mellitus. Type 2 (non-insulin-dependent) diabetes mellitus, the most frequent form of the disease, is characterized by impaired insulin sensitivity of target tissues and variable degrees of β-cell dysfunction. Under normal conditions, a feedback loop exists between insulin sensitivity and insulin secretion such that decreases in sensitivity of peripheral tissues are balanced by increases in secretion. In Type 2 diabetic patients, the amount of insulin secreted by pancreatic β-cells is insufficient to compensate for the degree of insulin resistance [9].

Chronic exposure of pancreatic β-cells to adverse environmental conditions such as hyperglycaemia, elevated concentrations of NEFAs (non-esterified fatty acids) or low-density lipoproteins are associated with alterations in the secretory properties and favour the progression towards diabetes. Defects in insulin exocytosis observed under these conditions include abnormally elevated hormone release in the absence of stimuli and impaired secretion evoked by physiological secretagogues [10]. Secretion of insufficient amounts of insulin appears to be due both to reduction in the number of exocytotic events and to a premature termination of the fusion process resulting in an increase in the number of events in which the release of the granule content is incomplete [11]. These alterations in the secretory mechanism are at least in part related to derangements in metabolism that affect the complex signalling cascades normally triggering exocytosis in β-cell. However, accumulating evidence indicates that defective insulin release in diabetic patients may also result from differences in the level of critical components of the secretory machinery of pancreatic β-cells. In fact, the expression of SNAREs and SNARE-interacting proteins in pancreatic islets were found to be decreased both in diabetic patients and in rat Type 2 diabetes models [4,5]. The molecular mechanisms responsible for the defective expression of these proteins in disease states have not been yet elucidated.

Transcription factors regulating the expression of Rab GTPases and SNAREs

Prolonged exposure to elevated glucose concentrations such as those encountered in pre-diabetic and diabetic stages has a profound impact on β-cell gene expression possibly linked to diminished activity of important transcription factors and/or to the induction of transcriptional repressors. Indeed, the transcriptional repressor ICER (inducible cAMP early repressor) has been shown to be up-regulated after long-term exposure to high glucose or NEFA [12,13] and in a rat model for Type 2 diabetes [14]. Transgenic mice with β-cell-directed overexpression of ICER-Iγ suffer from severe diabetes [15]. This is due to a combination of adverse effects elicited by the presence of inappropriate levels of the transcriptional repressor. Indeed, ICER inhibits proliferation and promotes apoptosis of pancreatic β-cells [16]. Moreover, sustained up-regulation of ICER leads to suppression of insulin gene expression and to defective insulin secretion in response to secretagogues [15]. Interestingly, a number of promoters of the genes coding for key components of the machinery of exocytosis contain potential CREs (cAMP-response elements) and might be regulated by transcription factors from the CREB (CRE-binding protein) and CREM (CRE modulator)/ICER families [17]. We demonstrated that members of the CREM/ICER family do indeed bind to the CRE identified in the promoters of Rab3A, Rab27A and of two of their effectors, granuphilin and Noc2 [17]. Consistent with this observation, we found that overexpression of ICER-Iγ in insulin-secreting cells inhibits the expression of these important regulators of β-cell exocytosis and leads to a defect in insulin secretion [17]. Chronic exposure to high glucose diminishes the expression of Rab3A, Rab27A and their effectors [17]. Transfection with an ICER antisense construct or silencing of the gene by RNA interference was sufficient to restore the expression of the components of the exocytotic machinery of insulin-secreting cells kept under hyperglycaemic conditions [17]. These observations point to an important role for the transcriptional repressors of the CREM/ICER family in the control of genes of the exocytotic apparatus (Figure 1). Interestingly, ICER is induced in a variety of physiological and pathological conditions not only in pancreatic β-cells and in other endocrine tissues but also in neurons [18,19]. Taken together, these findings suggest a more general role for ICER in the control of the secretory functions under different physiological and pathological conditions, including long-term synaptic plasticity and brain insult.

Many other transcription factors are likely to control the expression of proteins necessary for insulin exocytosis but currently we have only very few clues about their possible identity. Some hints about the transcription factors governing the expression of components of the exocytotic machinery are available in neurons, another cell system endowed with highly specialized secretory functions. In neurons, the expression of SNAP25 (25 kDa synaptosome-associated protein) is controlled by transcription factors of the POU (Pit-Oct-Unc) family. Thus Brn-3a and Brn-3c enhance the expression

Figure 1 | Effect of ICER on the expression of Rab GTPases and of their targets

Prolonged exposure of pancreatic β-cells to supraphysiological levels of glucose or NEFA induces the expression of the transcriptional repressor ICER [12,13,17]. This leads to a strong decrease in the transcription of Rab3A, Rab27A and of their effectors granuphilin and NOC2 and, in turn, to defects in insulin exocytosis [17].

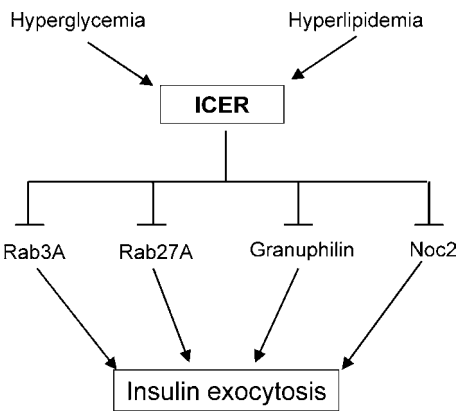
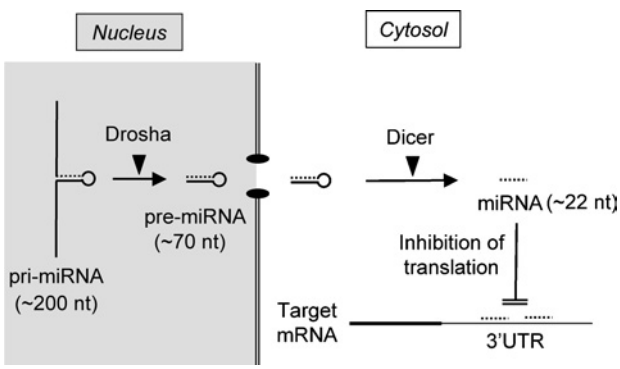


Figure 2 | Biosynthesis and mode of action of miRNAs

miRNAs are encoded in the genome as inverted repeats and are transcribed as long primary transcripts (pri-miRNAs). They are then processed to stem–loop precursors of approx. 70 nt (pre-miRNAs) by the nuclear RNase III-like enzyme Drosha. Pre-miRNAs are transported from the nucleus to the cytoplasm where mature miRNAs are excised from pre-miRNAs by Dicer, another RNase III-like enzyme. Mature miRNAs inhibit gene expression by partially pairing to sequences in the 3′-untranslated region (3′-UTR) of their target mRNAs.



of SNAP25, while Brn-3b represses both basal and Brn-3a-induced transcriptional activities [20]. At present, it is not known whether these transcription factors are present in pancreatic β-cells and whether their level is altered under conditions in which SNAP25 expression is changed such as hyperlipidaemia [12] or diabetes [5]. Recently, a combination of bioinformatic and biochemical approaches revealed that many genes encoding proteins involved in vesicular trafficking contain a functional *cis* repressor element of 21 bp termed RE-1 (repressor element 1) or NRSE (neuron restrictive silencer element). These genes include SNAP25, synapsin I and complexin I [21,22]. The NRSE binds the REST (RE-1 silencing transcription factor), a Krüppel-like zinc finger protein, also named neuron-restrictive silencer factor. REST silences the expression of its target genes in all cells except neurons and β-cells where the predominant isoform of this repressor (REST1) is absent or present at very low levels [23–25]. This mechanism allows expression of a selected group of exocytotic genes only in neuronal and endocrine cells. Evidence for the involvement of REST in the control of the secretory functions of pancreatic β-cell is already available. Indeed, re-introduction of exogenous REST in β-cells suppresses expression of its target genes and has a deleterious impact on insulin secretion in response to secretagogues [26]. A possible implication of REST in the dysfunction of the secretory machinery in neurological disorders has been suggested. Thus, in neurons, REST levels are abnormally elevated following ischaemic and epileptic insults [27]. Interestingly, the decrease in acetylcholine release following ischaemia is associated with a reduction in the expression of SNAP25 and of other REST target genes including synapsin I [6,28,29]. These changes could be caused by the inappropriate induction of REST. At present, alterations in REST expression in mature β-cells have not been reported.

miRNAs (microRNAs) as new potential regulators of the secretory functions

Recently, a new paradigm of RNA-directed regulation of gene expression has emerged. Eukaryotic cells were found to express hundreds of small (~22 nt) non-coding RNAs called miRNAs that act as specificity determinants to direct translational repression of a number of mRNA targets [30]. These small non-coding RNAs inhibit gene expression by partially pairing to one or more sequences in the 3′-untranslated region of their target mRNAs (Figure 2). Sophisticated computational approaches led to the identification of thousands putative target genes for miRNAs [31,32]. The immense potential of miRNAs as controllers of gene networks is just beginning to unfold but there is already substantial evidence indicating that they are involved in the control of a variety of physiological processes including development, cell proliferation, apoptosis and tissue differentiation [30]. Moreover, several human diseases have already surfaced in which miRNAs or their machinery might be implicated [33]. Recent data have demonstrated that miRNAs are also key regulators of specialized pancreatic β-cell functions. Indeed, expression of appropriate levels of miR-375 is required for glucose-induced insulin secretion [34]. miR-375 appears to modulate the expression of myotrophin, a protein potentially involved in cytoskeleton dynamics [34]. This discovery is likely to represent only the very beginning of the connection between miRNAs and the machinery of exocytosis. In fact, a number of miRNAs are predicted using bioinformatic tools to target several key components of the machinery of exocytosis (Table 1). These computational predictions need to be experimentally verified but, since false positive rates estimates are relatively low [31,32], at least a subset of miRNAs is likely to contribute to the control of the expression of the protein

Table 1 | **Several genes coding for proteins of the secretory machinery are putative miRNA targets**

miRNAs (miR) predicted to control the expression of key components of the apparatus required for hormone and neurotransmitter release [32].

VAMP2	SNAP25	Syntaxin-1	Rab3A	Rab27A	Noc2	Granuphilin	MyRIP
miR-34	miR-27	miR-138	None	miR-124	None	miR-136	miR-34
miR-338	miR-128	miR-217					miR-101
miR-150	miR-23	miR-133					miR-182
miR-206	miR-200	miR-200					miR-7
miR-1	miR-153	miR-107					miR-96
miR-30	miR-222	miR-15					miR-141
miR-133	miR-221	miR-103					miR-200
miR-32	miR-206	miR-195					miR-124
miR-153	miR-1	miR-16					let-7
	miR-301	miR-29					miR-98
	miR-130						miR-302
	miR-142						

apparatus necessary to sustain hormone and neurotransmitter release. In view of the present knowledge of the role of miRNAs in cell differentiation and development, it can be argued that given combinations of these small non-coding RNAs are required to achieve and maintain an appropriate secretory phenotype. Should this assumption turn out to be correct, then adaptations of the machinery of exocytosis to different physiological and pathological conditions may be associated with alterations in miRNA expression patterns. Methodologies allowing global determination of miRNA expression patterns and rapid assessment of the function of selected miRNAs are exponentially growing. Thanks to these new techniques, the possible contribution of miRNAs to adaptive and pathological processes involving changes in secretory functions is likely to be soon unveiled.

Conclusion and perspectives

After years of intense investigations, we have a good knowledge of the key components of the machinery governing exocytosis. Detailed analysis of the expression of the main players in exocytosis revealed that the level of some of them is subjected to variations during physiological and pathological processes in which cellular secretory functions are modified. The future challenge will be to unravel the network of transcription factors and miRNAs susceptible to regulate the activity of the secretory machinery. The acquisition of this essential information will help in defining the molecular events that drive normal adaptation processes and participate in the development of human diseases such as diabetes mellitus. In view of its potential impact in a variety of research fields, we have no doubt that this topic will be at the centre of future studies. Major progresses are therefore expected in a relatively short period of time.

References

1 Jahn, R. (2004) Ann. N.Y. Acad. Sci. **1014**, 170–178
2 Seabra, M.C., Mules, E.H. and Hume, A.N. (2002) Trends Mol. Med. **8**, 23–30
3 Fukuda, M. (2005) J. Biochem. (Tokyo) **137**, 9–16
4 Gaisano, H.Y., Ostenson, C.G., Sheu, L., Wheeler, M.B. and Efendic, S. (2002) Endocrinology **143**, 4218–4226
5 Ostenson, C.G., Gaisano, H., Sheu, L., Tibell, A. and Bartfai, T. (2006) Diabetes **55**, 435–440
6 Raghavendra Rao, V.L., Bowen, K.K., Dhodda, V.K., Song, G., Franklin, J.L., Gavva, N.R. and Dempsey, R.J. (2002) J. Neurochem. **83**, 1072–1086
7 Davis, S., Rodger, J., Stephan, A., Hicks, A., Mallet, J. and Laroche, S. (1998) Learn. Mem. **5**, 375–390
8 Honer, W.G., Falkai, P., Bayer, T.A., Xie, J., Hu, L., Li, H.Y., Arango, V., Mann, J.J., Dwork, A.J. and Trimble, W.S. (2002) Cereb. Cortex **12**, 349–356
9 Weir, G.C. and Bonner-Weir, S. (2004) Diabetes **53** (Suppl. 3), S16–S21
10 Ling, Z., Kiekens, R., Mahler, T., Schuit, F.C., Pipeleers-Marichal, M., Sener, A., Kloppel, G., Malaisse, W.J. and Pipeleers, D.G. (1996) Diabetes **45**, 1774–1782
11 Tsuboi, T., Ravier, M.A., Parton, L.E. and Rutter, G.A. (2006) Diabetes **55**, 1057–1065
12 Busch, A.K., Cordery, D., Denyer, G.S. and Biden, T.J. (2002) Diabetes **51**, 977–987
13 Zhou, Y.P., Marlen, K., Palma, J.F., Schweitzer, A., Reilly, L., Gregoire, F.M., Xu, G.G., Blume, J.E. and Johnson, J.D. (2003) J. Biol. Chem. **278**, 51316–51323
14 Inada, A., Yamada, Y., Someya, Y., Kubota, A., Yasuda, K., Ihara, Y., Kagimoto, S., Kuroe, A., Tsuda, K. and Seino, Y. (1998) Biochem. Biophys. Res. Commun. **253**, 712–718
15 Inada, A., Hamamoto, Y., Tsuura, Y., Miyazaki, J., Toyokuni, S., Ihara, Y., Nagai, K., Yamada, Y., Bonner-Weir, S. and Seino, Y. (2004) Mol. Cell. Biol. **24**, 2831–2841
16 Inada, A., Weir, G.C. and Bonner-Weir, S. (2005) Biochem. Biophys. Res. Commun. **329**, 925–929
17 Abderrahmani, A., Cheviet, S., Ferdaoussi, M., Coppola, T., Waeber, G. and Regazzi, R. (2006) EMBO J. **25**, 977–986
18 Mioduszewska, B., Jaworski, J. and Kaczmarek, L. (2003) J. Neurochem. **87**, 1313–1320
19 Della Fazia, M.A., Servillo, G., Foulkes, N.S. and Sassone-Corsi, P. (1998) FEBS Lett. **434**, 33–36
20 Morris, P.J., Dawson, S.J., Wilson, M.C. and Latchman, D.S. (1997) Neuroreport **8**, 2041–2045
21 Abderrahmani, A., Niederhauser, G., Plaisance, V., Roehrich, M.E., Lenain, V., Coppola, T., Regazzi, R. and Waeber, G. (2004) J. Cell Sci. **117**, 2239–2247
22 Bruce, A.W., Donaldson, I.J., Wood, I.C., Yerbury, S.A., Sadowski, M.I., Chapman, M., Gottgens, B. and Buckley, N.J. (2004) Proc. Natl. Acad. Sci. U.S.A. **101**, 10458–10463
23 Atouf, F., Czernichow, P. and Scharfmann, R. (1997) J. Biol. Chem. **272**, 1929–1934
24 Chong, J.A., Tapia-Ramirez, J., Kim, S., Toledo-Aral, J.J., Zheng, Y., Boutros, M.C., Altshuller, Y.M., Frohman, M.A., Kraner, S.D. and Mandel, G. (1995) Cell **80**, 949–957

25 Abderrahmani, A., Steinmann, M., Plaisance, V., Niederhauser, G., Haefliger, J.A., Mooser, V., Bonny, C., Nicod, P. and Waeber, G. (2001) Mol. Cell. Biol. **21**, 7256–7267
26 Abderrahmani, A., Niederhauser, G., Plaisance, V., Haefliger, J.A., Regazzi, R. and Waeber, G. (2004) FEBS Lett. **565**, 133–138
27 Calderone, A., Jover, T., Noh, K.M., Tanaka, H., Yokota, H., Lin, Y., Grooms, S.Y., Regis, R., Bennett, M.V. and Zukin, R.S. (2003) J. Neurosci. **23**, 2112–2121
28 Ishimaru, H., Takahashi, A., Ikarashi, Y. and Maruyama, Y. (1994) Brain Res. **639**, 66–72
29 Ishimaru, H., Takahashi, A., Ikarashi, Y. and Maruyama, Y. (1994) Neuroreport **5**, 601–604
30 Ambros, V. (2004) Nature **431**, 350–355
31 Lewis, B.P., Burge, C.B. and Bartel, D.P. (2005) Cell **120**, 15–20
32 Krek, A., Grun, D., Poy, M.N., Wolf, R., Rosenberg, L., Epstein, E.J., MacMenamin, P., da Piedade, I., Gunsalus, K.C., Stoffel, M. and Rajewsky, N. (2005) Nat. Genet. **37**, 495–500
33 Alvarez-Garcia, I. and Miska, E.A. (2005) Development **132**, 4653–4662
34 Poy, M.N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P.E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P. and Stoffel, M. (2004) Nature **432**, 226–230

Received 16 June 2006