

## Chapter 12

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# The role of the cytoskeleton in transport and release of insulin-containing granules by pancreatic beta-cells

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Key words: **Actin; insulin; kinesin; microtubules; myosin Va; rab-GTPases**

**Abstract** Insulin secretion by beta-cells is stimulated by a rise in blood glucose level and occurs in two phases: a first-phase of short duration leading to the release of a small number of insulin-containing granules and a second-phase lasting up to several hours. During the first phase primed insulin-granules constituting the ready releasable pool (RRP) are exocytosed, whereas during the second phase this RRP is constantly replenished by granules from the reserve pool. During replenishment insulin-granules have to be transported from more intracellular locations towards the exit-sites on the plasma membrane. Microtubulues and the motor protein kinesin perform the long distance transport of insulin-granules, subsequently the motor protein myosin Va accomplishes their transfer along short F-actin filaments to the docking sites at the plasma membrane, where the granules are tethered by the formation of a ternary complex of Rab27a and granuphilin residing on the granular membrane and syntaxin 1a on the plasma membrane, leading to membrane fusion and insulin secretion.

keywords: ###, ###, ###, ###, ###, ### **Actin; insulin; kinesin; microtubules; myosin Va; rab-GTPases**

## Introduction

The pancreatic islets are clusters of endocrine cells that secrete a number of different peptide hormones. The islets were first discovered in 1869 by Paul Langerhans (Berlin; Germany) and are named in his honour “Langerhans islets”. It was

20 years later that Oskar Minkowski and Joseph von Mering (Straßburg, then Germany) induced Diabetes mellitus in dogs by removing the pancreas. Early attempts to treat diabetic patients with pancreatic extracts failed (Zülzer, Berlin, Germany). In 1916 Paul Paulescu (Bucharest, Romania) for the first time prepared a pancreatic extract enriched in insulin that he used successfully to treat diabetic dogs. Further work in his laboratory was held up by the 1<sup>st</sup> World War, and it was not until August 1921 that his results were published. In 1921 Banting and Best (Toronto, Canada) essentially repeated the results of Paulescu, and in 1922 they treated diabetic patients for the first time successfully using their highly insulin-enriched pancreatic extract. Among their first patients was a 5-year old diabetic boy who, under continuous insulin treatment, lived till the age of 76 (see also Alberti, 2001).

The human pancreas contains about one million dispersed Langerhans islets composed of five different cell types (A, B, D, E, or  $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\epsilon$ , and PP), each specialised for the synthesis of a particular peptide hormone. With 65-80% the insulin secreting B- or beta ( $\beta$ -) cells are the most abundant islet cell type (Fig. 1A,B). Insulin is the sole hormone that lowers the concentration of blood glucose. Therefore defects in its release will invariably lead to the metabolic disease diabetes mellitus. Insulin secretion by  $\beta$ -cells is stimulated by an increase in blood glucose and usually occurs with a biphasic time course, i.e. rapid initial and a slow but sustained second phase. Because diabetes type 2 is characterised by the absence of the first phase and a reduction of insulin release during the second phase, understanding the cellular mechanism of the biphasic insulin secretion and its disturbance is of paramount importance.

## **Models to study insulin secretion**

Insulin secretion by  $\beta$ -cells has been studied in whole rodent pancreas preparations, isolated rat or mouse islets or primary  $\beta$ -cells. The response of these organotypical preparations or primary cell culture systems to glucose and other secretagogues may correspond most closely to their *in vivo* behaviour. However, after isolation they survive only for a short period of time. Therefore attempts have been made to obtain and to recapitulate the data obtained from animal models with clonal or established  $\beta$ -cell lines, because they offer the advantage of propagation in cell culture and ease of handling. These cells can be stimulated by glucose to release insulin, however, their response is in most cases not clearly biphasic, although short and sustained insulin release responses can be evoked by modulating the external glucose concentration.

## Metabolic effects of glucose in $\beta$ -cells

After nutrient uptake, the blood glucose increases. Beta-cells take up glucose by facilitated diffusion catalysed by the GLUT2 transporter. Intracellularly, glucose is metabolised by glycolysis, leading to an increase of the ATP/ADP concentration ratio. Increased ATP inhibits the ATP-sensitive  $K^+$ -channel resulting in an intracellular  $K^+$ -increase and membrane depolarisation that subsequently opens voltage-gated  $Ca^{2+}$ -channels. The increase in cytosolic  $Ca^{2+}$ -ions is supposed to be the main trigger for exocytosis of the insulin-containing granules. A number of other secretagogues like KCl, cAMP and IBMX (isobutyl-methylxanthin, an inhibitor of PDEs) induce only a short response. They do not elicit the metabolic effects of glucose, in particular the ATP-increase necessary for sustained insulin release (for a review see also Rorsman and Renström, 2003).

## The response of the $\beta$ -cell

As an exported protein, pre-proinsulin is synthesized by ribosomes attached to the rough endoplasmic reticulum (rER) of the  $\beta$ -cells as a single polypeptide chain (pre-proinsulin) of 110 residues including the N-terminal signal sequence, which is removed within the rER generating proinsulin of 84 residues. Enclosed in small vesicles, proinsulin is transported from the rER to the Golgi-complex. Within the trans-Golgi-network (TGN) proinsulin is processed to mature insulin by excision of the connecting peptide to generate the A-chain of 21 and the B-chain of 30 residues. The chains are connected by two disulfide bridges. During package in secretory granules by the TGN mature insulin is complexed with  $Zn^{2+}$ -ions, inducing inactivation and aggregation (of  $Zn^{2+}$ -containing insulin-hexamers) to the crystalline dense cores within the insulin-granules visible in EM-images (Fig. 1C).

The cytoplasm of each  $\beta$ -cell is filled with more than 10,000 insulin-containing vesicles or dense-core granules (Fig. 1D), which are released by regulated exocytosis (Fig. 1E,F). Under resting conditions (fasting) the  $\beta$ -cell has to block the release of insulin-containing granules in order to secure a low blood insulin-level. After stimulation only a small fraction of these granules release their content by exocytosis into the extracellular space around the  $\beta$ -cells, from where the released insulin rapidly diffuses into adjacent capillaries. Nutrient uptake, especially the increase in blood glucose level stimulates  $\beta$ -cells to exocytose insulin-containing granules. During exocytosis the granular membrane fuses with the plasma membrane finally leading to fusion and subsequent fission of both membranes and release of the granular content into the extracellular space.

The elevation of blood glucose induces a biphasic insulin release: a rapid initial and transient phase lasting only a few minutes and a second sustained release up to

several hours depending on the duration of the blood glucose elevation. The rapid first phase is characterized by the release of a relatively small amount of insulin from granules, which are docked in close apposition to the plasma membrane (see Fig. 1E) and already “primed” for discharge (see also Fig. 1F). Subsequently the insulin secretion returns almost to the resting level. However, after nutrient uptake the blood glucose level usually remains elevated for longer periods of time and initiates the second sustained phase leading to the release of a larger amount of insulin.

It has been estimated that during the first phase only about 50 “primed” insulin-granules are exocytosed. These are only a fraction of the granules visualised by morphological methods to be “tethered” to the plasma membrane. It has been suggested that the granules’ “primed” state is due to direct complexation to the voltage-gated  $\text{Ca}^{2+}$ -channel protein (Barg et al. 2002). In contrast, during the second phase about 1,000 to 2,000 granules are transported from more distant regions of the cytoplasm towards the plasma membrane and discharged. Thus, stimulated  $\beta$ -cells are able to mobilize two different pools of insulin-containing granules: a readily releasable pool (RRP) of about a fifty immediately releasable primed granules and a few thousand (about up to 2,000) granules of the so-called reserve pool that have to be either primed or translocated from more central cytoplasmic regions to the plasma membrane.

## **The intracellular cytoskeleton**

The intracellular cytoskeleton appears to be responsible for both the inhibition of exocytosis during the resting phase and the active transport of insulin-granules necessary for sufficient insulin release after stimulation. Principally the cytoskeleton is composed of three more or less independent filamentous systems: (i) the actin containing microfilaments (MF) and its associated proteins, (ii) the microtubules (MT) composed of the  $\alpha,\beta$ -tubulin heterodimer, and (iii) the intermediate filaments, which almost exclusively fulfil mechanically stabilising functions and will not be further described in this review. Specific motor proteins interact with either microfilaments or microtubules and are involved in the various forms of cellular motility. Motor proteins of the myosin family interact with microfilaments to generate force and kinesins and cytoplasmic dyneins perform transport processes of intracellular vesicles along microtubules.

## **Some basic properties of microtubules**

Cytoplasmic microtubules are long straight filamentous structures with a diameter of 23 to 25 nm (Fig. 1G). They are composed of  $\alpha,\beta$ -tubulin heterodimers

(molecular mass:  $2 \times 55$  kDa), which associate head-to-tail to long protofilaments. Thirteen protofilaments associate laterally to a closed tube, the microtubule. Microtubules (MT) are polarized, possessing two different ends with different affinities for the tubulin heterodimer (Fig. 1G). Tubulin molecules preferentially associate to the so-called plus-ends. The closely related  $\alpha$ - and  $\beta$ -tubulin subunits have a molecular mass of about 55 kDa and firmly bind one molecule of GTP. The GTP bound to  $\beta$ -tubulin is exchangeable and hydrolyzed to GDP during the polymerisation process, whereas the GTP bound to  $\alpha$ -tubulin is not hydrolysed and exchanged. Addition of tubulin heterodimers to the plus-end generates a so-called GTP-cap, since GTP-hydrolysis by the  $\beta$ -tubulin molecules occurs with a time lag. MTs with a plus-end GTP-cap are stable, but once this GTP is hydrolysed to GDP rapid shrinkage of the microtubule from this end occurs (see also Desai and Mitchison 1997; Otto, 1987; van der Vaart et al. 2009).

Intracellularly, the MTs originate from the centrosome, also termed microtubule organisation centre (MTOC), which is located close to the cell nucleus, and extend to the cell periphery. Their minus-ends are tagged into the MTOC probably by association with a specific tubulin molecule ( $\gamma$ -tubulin). Their plus-ends are located peripherally where their tendency to shrink is reduced or blocked by binding of capping-proteins to the minus-ends (see also Fig. 2).

Single cytoplasmic MTs extend from the cell centre to its periphery and form the tracks along which vesicles can be transported over long distances in both directions. Specific motor proteins attached to vesicles or intracellular organelles crawl along the surface of MTs and thereby translocate vesicular structures either centripetally or -fugally. The main representatives of MT associated motor proteins are members of the kinesin and dynein families. These motor proteins associate with the vesicular membrane and move these in a processive manner along MTs. Most kinesins (Fig. 1G) translocate their cargo (vesicles) from the minus-towards the plus-end (centrifugally), whereas the cytoplasmic dyneins transport in the opposite direction (Hirokawa et al. 1998; Kamal and Goldstein, 2000).

### **Conventional kinesin transports insulin-granules during second-phase secretion**

The second phase of glucose-stimulated insulin secretion can last for several hours. It has been estimated that 5 to 40 insulin-containing granules are released per cell and minute during this phase (Barg et al., 2002). Therefore insulin-granules from the so-called storage or reserve pool located more centrally within the cell have to be translocated to the peripheral release sites.

It has been demonstrated that highly specific MT disrupting drugs like colchicine or nocodazole block the second phase without affecting the initial fast phase of insulin secretion (Farshori and Goode, 1994). Given the direction of their

movement, it appears plausible that members of the kinesin family transport the insulin-granules along MTs by (Varadi et al. 2002).

Kinesins are elongated heterotetrameric proteins composed of two heavy and two light chains. The heavy chains form the three main structural domains: the two N-terminal globular heads followed by an  $\alpha$ -helical coiled-coil and finally two tail regions with the attached light chains (Fig. 1G). The head regions form the highly conserved motor domains, each containing an ATPase centre and MT-binding site. ATP-hydrolysis drives the force generating power stroke when attached to a MT. The tail regions function as cargo-binding domains whose interaction with vesicular membranes is mediated by specific adaptor proteins located on the cytoplasmic face of the cargo vesicles.

Kinesins spend a large fraction of the ATPase cycle attached to the MTs and are processive motor proteins. Processivity is further supported by the fact that a head once bound to a MT does not dissociate before the second head has attached to the next binding region towards the plus-end of the MT. Thus the two heads of a kinesin molecule can perform a large number (around 100) of alternate ATPase and translocation cycles leading to migration of the whole kinesin along the MT in a hand-over hand fashion, thereby transporting attached vesicles over long distances.

The kinesins are a large family of motor proteins and most eukaryotic cells express several kinesin variants, which fulfil specific transport functions determined by their cargo-binding sites. It has been shown that the so-called conventional kinesin heavy chain or kinesin I can be detected on isolated insulin granules of established  $\beta$ -cell lines (Varadi et al., 2002; 2003). Furthermore, transfection of  $\beta$ -cells of established lines with a dominant negative mutated kinesin or kinesin I specific siRNA results in clear reduction of the intracellular movement of insulin-containing granules and second phase insulin secretion after sustained glucose-stimulation (Varadi et al. 2002; 2003). These data indicate that the kinesin-dependent insulin-granule transport functions to replenish the readily releasable pool.

Kinesin activity depends on the intracellular ATP-concentration, which is increased after glucose stimulation of  $\beta$ -cells. The higher the external glucose concentration the higher is the elevation of the intracellular ATP-concentration. Indeed, using permeabilised clonal  $\beta$ -cells demonstrated that the speed of insulin-granule movement correlates with the externally added ATP-concentration. Thus, the speed of replenishment of the readily releasable pool is modulated by the external glucose concentration (Varadi et al. 2002).

### **Some basic properties of F-actin filaments**

The basic building blocks of the actin cytoskeleton are the microfilaments composed of actin subunits (Fig. 1H). The cytoskeleton and also the microfila-

ment system is a highly dynamic system, it is constantly remodelled according to the cellular needs. A high fraction of the intracellular actin is maintained in monomeric form, this reserve pool of G-actin is used for the constantly occurring reorganisation of the actin cytoskeleton.

Monomeric globular (G-)actin has a molecular mass of 42 kDa and contains firmly bound one molecule ATP, which is hydrolysed into ADP and inorganic phosphate (Pi) after polymerization and incorporation into an actin filament (F-actin). Whereas the Pi is rapidly released the ADP remains firmly attached to F-actin subunits generating two different filament ends: the fast growing plus or barbed end containing exposing ATP-actin subunits and the slow-growing minus or pointed end with ADP-actin subunits. The different filament ends are the basis for the polarized addition and dissociation of subunits to F-actin filaments: ATP-actin subunits attach to the barbed ends and ADP-actins dissociate from the pointed ends. After dissociation the actin bound ADP is exchanged for ATP leading and able to re-associate at the barbed end to the so-called treadmilling which describes the fact that under steady state conditions a single actin subunit after association to the barbed end travels through the whole filament before it dissociates from the minus or pointed end (Wegner, 1976). In motile cells a thin veil-like extension of the plasma membrane (the lamellipodium) actively protrudes in the direction of cell migration. The lamellipodial forward movement is achieved by the continuous addition of new actin molecules to a branched F-actin network, whose barbed ends are oriented to cell periphery (Lai et al. 2008).

In many tissue cells, including  $\beta$ -cells, an F-actin network is often concentrated immediately underneath the plasma membrane forming the so-called cortical web (Fig. 1H). This network of short F-actin filaments is attached to the plasma membrane at multiple points by proteins of the so-called ERM (ezrin/radixin/moesin) family, which link it to particular cell adhesion molecules or extracellular matrix receptors like the integrins (Tsukita and Yonemura, 1999). As in motile cells the barbed end of the F-actin network of  $\beta$ -cells are supposed to be oriented towards the cell periphery.

### **The role of the actin cytoskeleton during exocytosis**

Although considerable knowledge about the function and regulation of the microfilament system has been accumulated in recent years, its exact role in exocytotic and especially in insulin secreting  $\beta$ -cells is still not completely understood due to the fact that it appears to simultaneously fulfil a number of diverse functions. Staining of  $\beta$ -cells with TRITC-phalloidin demonstrated the existence of the above mentioned dense network of F-actin filaments underneath the plasma membrane. It has been demonstrated that glucose entry into  $\beta$ -cells induces an immediate reorganisation of the cortical F-actin net into shorter filaments (Wang and Thurmond 2009).

It was suggested that this cortical web physically blocks the access of insulin-containing granules to the plasma membrane in resting  $\beta$ -cells and furthermore might also impede their discharge after stimulation unless disassembled or reorganised. This assumption gained support from data showing that F-actin disrupting drugs, especially the highly specific latrunculins, induce an increased insulin discharge during both phases after glucose stimulation (Tomas et al. 2006). However, latrunculin exposure did not induce insulin release of unstimulated cells (Nevins and Thurmond, 2003; Tomas et al. 2006).

Therefore proteins with F-actin fragmenting activity were suspected to aid insulin exocytosis after stimulation. These include gelsolin and the closely related scinderin (Bruun et al. 2000), which is, however, expressed only in very low amounts in  $\beta$ -cells. Gelsolin fragments (severs) F-actin filaments after  $\text{Ca}^{2+}$ -ion activation (Yin, 1988) and its possible role in insulin secretion was repeatedly assessed by gelsolin siRNA-knock-down or comparing clonal  $\beta$ -cells differing in its expression (Tomas et al., 2006). Gelsolin “minus”-cells exhibited longer F-actin filaments, which in contrast to gelsolin-normal cells were not depolymerised after glucose stimulation, indicating a crucial role for gelsolin in the normal stimulus-secretion pathway. Consequently, these  $\beta$ -cell variants exhibited a reduced insulin release after stimulation that was, however, considerably increased after latrunculin exposure (Tomas et al. 2006). In summary, these authors concluded that efficient insulin release by stimulated  $\beta$ -cells necessitated depolymerisation of the cortical F-actin in order to accomplish fast and efficient replenishment of the RRP from the reserve pool (Tomas et al. 2006).

This account cannot represent the whole story, since it was observed that latrunculin or other F-actin fragmenting manipulations (like transfection with *Clostridium botulinum* C2-toxin) in particular in clonal  $\beta$ -cells containing only a low number of insulin-granules induce an inhibition of insulin release (Li et al. 1994). Therefore additional functions appear to be performed by the microfilament system.

### **Myosin Va and F-actin are necessary for the final delivery of insulin-granules to the plasma membrane**

Kinesin transports insulin-granules towards the plasma membrane, however, the final step of their transport to the release sites is performed by the interplay between F-actin filaments and the motor protein myosin Va. Proposals suggesting the involvement of myosins in secretory vesicle transport were made long before the discovery of MT associated motor proteins (Li et al. 1994). In contrast to conventional myosins II as supposed in these early suggestions, the analysis of the myosin V-deficient mouse model “dilute” clearly demonstrated the general involvement of the un-conventional myosin Va in intracellular vesicle transport. Diluted mice have a postnatal life expectancy of only a few weeks due to a variety of neurological defects; their most obvious phenotype is the impaired transport of

melanosomes and delivery to keratinocytes and hairs leading to reduced (diluted) fur pigmentation (Futaki et al. 2000).

Myosin Va molecules are composed of two identical heavy chains ( $M_r = 215$  kDa), which dimerise in their coiled-coil domain (Fig. 1H'). The N-terminal motor domains are followed by long  $\alpha$ -helical shafts (lever arms), which are stabilised by six light chains per shaft four of which are  $Ca^{2+}$ -ion binding calmodulins. Subsequently the  $\alpha$ -helical shafts unite to the coiled-coil domain, which finally forms two separate globular cargo-binding (vesicle) domains (Chenney et al. 1993; see Fig. 1H'). A number of particular properties make myosin Va motors ideally suited for vesicular transport: (i) they bind to both MTs and F-actin, therefore secretory vesicle can be equipped simultaneously with both kinesin and myosin Va; (ii) in contrast to conventional myosins they spend a large fraction of the ATPase cycle attached to F-actin ("high duty rate"); and (iii) they are processive motors performing multiple large steps (36 nm each) due to their long lever arms towards the barbed end of F-actin (Sakamoto et al. 2008). Recent data demonstrated that myosin Va is a component of the insulin-granule membrane (Varadi et al. 2005) indicating that during second-phase secretion the final step of granule transport from the reserve pool to the release sites on the plasma membrane also necessitates F-actin.

## Control of granule docking

In contrast to neurons, there are no defined release sites for secretory granules in endocrine cells and the slow (rate-limiting) process of insulin-granule docking has been made responsible for the release of low amounts of insulin after stimulation. Selective intracellular docking and fusion of vesicles is controlled by a particular group of small GTPase proteins, the Rab-proteins (ras-like proteins from brain). There are more than 60 Rab-proteins in human cells involved in specific vesicle-membrane targeting. The Rab-proteins also indirectly control the association of myosin Va with insulin-granula as well as melanosomes and other secretory vesicles. In melanocytes, this is quite well understood, and involves the interaction of Rab27 with melanophilin, which in turns interacts with myosin Va. Interestingly, this interaction only occurs in the dendritic region, with earlier transport from the perinuclear region probably occurring via interaction of Rab proteins with kinesin and motion on MTs after initial concentration near the MTOCs by the interaction of Rab7 with the dynein motor system.

Rab-proteins also direct the process of tethering, i.e. the initial contact of for instance a vesicle with a target membrane, and are supported by specific tethering factors, which are either coiled-coil or multimeric protein complexes. A mutation leading to functional deficiency of Rab27a in mice (ashen-mice) leads to pigmentation disorder and to decreased insulin-release after  $\beta$ -cell stimulation (Waselle et al. 2003). In  $\beta$ -cells, Rab27a is attached to the cytoplasmic face of the insulin-

granules and associated with Slac2-c and additionally with granuphilin, a molecule related to melanophilin. The Rab27a-granuphilin complex mediates the tethering of the granules to the plasma membrane by binding to Munc18-1 and syntaxin 1a (Torii et al., 2004). Thus, membrane-specific tethering is mediated by Rab27a, which forms a ternary complex with granuphilin and syntaxin 1a. However, the thus tethered insulin-granules are not yet primed and supposed to be even release-incompetent awaiting the activation of the fusion machinery (Gomi et al. 2005). A further Rab 27 effector (i.e. protein which binds to the GTP form of Rab 27) found on secretory granules is MyRip, which also interacts with myosin Va. Several other Rab proteins have also been implicated in insulin secretion (Izumi et al., 2003; Desnos et al., 2003 and 2007).

### **Control of granule and plasma membrane fusion by F-actin**

Exocytosis requires the fusion of two separate unit membranes: the vesicle and plasma membrane, and the subsequent fission to allow the discharge of the vesicular content (see Fig. 1F). These processes are catalysed by the interaction of two sets of membrane proteins – the SNARE proteins (= soluble N-ethylmaleimide-sensitive factor attachment protein receptor). In mammalian cells there are more than 20 SNARE proteins, which convey specificity to membrane fusion events. In addition, the Rab-proteins bound to the vesicular membrane further control the specificity of the membrane interactions. The largely  $\alpha$ -helical cytoplasmic domains of both vesicular v-SNARE and target membrane t-SNARE interact with each other under the formation of highly stable coiled-coils in order to bring the two separate membranes in close apposition (docking), fusion and finally fission. The molecular details of these processes are not yet completely understood, but it is known that the energy for this process is delivered by the final dissociation and reactivation of the entangled SNAREs, which is delivered by the ATP-consuming soluble chaperon-like NSF-protein. The insulin-granule v-SNARE has been identified as the vesicle associated membrane protein 2 (VAMP2) and the plasma membrane t-SNARE as syntaxin-4, which appears to be involved at least in the second-phase insulin release.

t-SNAREs are often blocked by binding of inhibitory proteins in order to avoid indiscriminate fusion events. Surprisingly it was found that the inhibitory protein for syntaxin-4 is F-actin that specifically interacts with two of its  $\alpha$ -helical domains (Jewell et al. 2008). This interaction is disrupted after glucose entry into the  $\beta$ -cell probably being part of the then initiated F-actin reorganisation events that not only relieve the apparent physical barrier of the F-actin cortical web to the transfer of granules from the reserve pool to the plasma membrane, but more specifically allow the protein-protein interactions necessary for granule discharge.

Very little is known so far about the signalling pathways, which after glucose entry lead to microfilament reorganisation. It has been shown that the Rho-family

GTPase proteins Cdc42 and Rac1 are transiently activated immediately after glucose entry. It will be interesting to identify their effector proteins responsible for the reorganisation of the F-actin cortical web in insulin-secreting  $\beta$ -cells.

## Summary

Insulin-granule transport is a dual process depending on microtubules for long distance transport and F-actin for the final delivery to the release sites at the plasma membrane. In addition, F-actin appears to fulfil multiple functions before and after  $\beta$ -cells stimulation that necessitate tightly controlled reorganisation events of its supramolecular organisation, which are far from being fully apprehended.

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**Fig. 1.** Images of pancreatic islets and  $\beta$ -cells. Pancreatic islet visualised by the classical hematoxylin-eosin staining (A) and (B) immunostaining with anti-insulin antibody (B). Electron microscopical image (transmission electron microscopy) of two adjacent  $\beta$ -cells (C) and after electron tomography (D) giving in green the mitochondria and in blue the secretory granules (according to Marsh et al. 2001). (E) Single insulin-granule docked to the plasma membrane and (F) during discharge. (G) Part of a  $\beta$ -cell showing MTs (green) and insulin-granules (blue) in close proximity. (G') Gives a schematic representation of a MT with protofilaments, which are built by the linear aggregation of tubulin heterodimers and an attached kinesin molecule (HC = heavy and LC = light chain). (H) Gives an electron tomography images of the cortical web of a fibroblastic cell (according to Baumeister) with attached small secretory granules. (H') Schematic representation of a F-actin filament with an attached myosin Va molecule (for details see text).