Microtubules in Pancreatic β Cells: Convoluted Roadways Toward Precision

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Pancreatic islet β cells regulate glucose homeostasis *via* glucose-stimulated insulin secretion (GSIS). Cytoskeletal polymers microtubules (MTs) serve as tracks for the transport and positioning of secretory insulin granules. MT network in β cells has unique morphology with several distinct features, which support granule biogenesis (*via* Golgi-derived MT array), net non-directional transport (*via* interlocked MT mesh), and control availability of granules at secretion sites (*via* submembrane MT bundle). The submembrane MT array, which is parallel to the plasma membrane and serves to withdraw excessive granules from the secretion hot spots, is destabilized and fragmented downstream of high glucose stimulation, allowing for regulated secretion. The origin of such an unusual MT network, the features that define its functionality, and metabolic pathways that regulate it are still to a large extent elusive and are a matter of active investigation and debate. Besides the MT network itself, it is important to consider the interplay of molecular motors that drive and fine-tune insulin granule transport. Importantly, activity of kinesin-1, which is the major MT-dependent motor in β cells, transports insulin granules, and has a capacity to remodel MT network, is also regulated by glucose. We discuss yet unknown potential avenues toward understanding how MT network and motor proteins provide control for secretion in coordination with other GSIS-regulating mechanisms.

Keywords: microtubule, insulin, pancreatic beta cell, endocrine cell, kinesin, dynein, glucose-stimulated insulin secretion

INTRODUCTION

Pancreatic islet β cells regulate glucose homeostasis in vertebrates *via* glucose-stimulated insulin secretion (GSIS). This function is of critical importance to human health, because excessive GSIS causes hyperinsulinemic hypoglycemia that damages the brain and other tissues (Nessa et al., 2016), while insufficient secretion causes diabetes (Hudish et al., 2019; Alejandro et al., 2015). To avoid these pathological effects, the correct number of secretion-competent insulin granules (IGs) has to be prepared prior to a glucose stimulus. Moreover, these IGs must be positioned to the exocytosis sites at the plasma membrane at the right moment (Gao et al., 2007; Kasai et al., 2008; Verhage and

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Abbreviations: FIB-SEM, focused ion beam scanning electron microscopy; GDMT, Golgi-derived MT; GSIS, glucose-stimulated insulin secretion; IG, insulin granule; MT, microtubule; MAP, MT-associated protein.

Sorensen, 2008; Wang and Thurmond, 2009; Zhao et al., 2010; Gandasi et al., 2018). Several studies over the years have established that microtubules (MTs), which serve as tracks for IG transport, precisely regulate IG positioning over time (Heaslip et al., 2014; Hoboth et al., 2015; Zhu et al., 2015; Bracey et al., 2020; Ho et al., 2020; Trogden et al., 2021).

Our surprising findings are that the β-cell MTs serve two functions. In the long-term, they promote endocrine function *via* facilitating IG biosynthesis, which relies on the growth of new MTs (Trogden et al., 2019). In the short-term, they acutely attenuate GSIS by restricting the number of readily-releasable IGs at the secretion sites, depending on MT stabilization (Zhu et al., 2015; Bracey et al., 2020; Ho et al., 2020; Trogden et al., 2021). In this perspective, we will provide our current views on how MT networks in β cells are designed at the cellular and sub-cellular scales to precisely tune IG-transport and GSIS. Our current model is that β-cell MTs are built, modulated, and utilized by such intracellular factors as MT-associated proteins (MAPs) and molecular motors to regulate IG transport and secretion.

CHALLENGE OF INSULIN GRANULE TRANSPORT FOR CORRECT SECRETION LEVELS

The task of correctly positioning secretory vesicles for acute stimulated secretion is a complicated process. On one hand, IGs are produced from the trans-Golgi network in the inner cytoplasm (Hou et al., 2009). They must then be transported to underneath the plasma membrane for regulated release (Gao et al., 2007; Kasai et al., 2008; Verhage and Sorensen, 2008; Wang and Thurmond, 2009; Zhao et al., 2010; Gandasi et al., 2018). Since most of the IG transport in a β cell is MT-dependent (Varadi et al., 2002; Tabei et al., 2013; Heaslip et al., 2014; Zhu et al., 2015), overall IG distribution in the cytoplasm including the cell periphery is a result of such active transport.

On the other hand, insulin secretion occurs within minutes after high glucose treatment, while it takes hours for newly synthesized IGs to mature and reach the cell membrane (Hou et al., 2009). To provide a timely response, a large number of pre-processed secretion-competent IGs is awaiting the signal. In a resting β cell, several thousands of IGs fill the entire cytoplasm, and only ~4% of those are secreted at a given stimulus (Dean, 1973; Olofsson et al., 2002; Rorsman and Renstrom, 2003; Fu et al., 2013). This scenario dramatically differs from constitutive secretion when secretory vesicles are constantly produced and readily transported for immediate secretion.

Not surprisingly, numerous secretion-restricting mechanisms have evolved to prevent over-secretion *via* the occasional release of pre-stored IGs (Chatterjee Bhowmick et al., 2021). These mechanisms act in concert as a combination of locks on a door or filters in a pipe. Broadly defined, these "filters" include any cellular tool preventing uncontrollable secretion. For example, restricting calcium levels in the cytoplasm can be considered a filter, because calcium influx is needed for priming of docked IGs for secretion (Idevall-Hagren and

Tengholm, 2020; Omar-Hmeadi and Idevall-Hagren, 2021). Actin cytoskeleton provides another set of filters: actin remodeling and activity of myosins are thought to remove steric hindrance between IGs and the plasma membrane, drive short-distance IG transport to the secretion site, and provide mechanical force for the exocytic event itself (Varadi et al., 2005; Kalwat and Thurmond, 2013; Arous and Halban, 2015; Veluthakal and Thurmond, 2021). When we discuss MT-dependent positioning and transport of IGs in the cytoplasm, we must take the over-crowding of β-cell cytoplasm into account and consider that removal of IG from the secretion sites can act as one of those "filters". Our findings over the last few years support this model (Zhu et al., 2015; Bracey et al., 2020; Ho et al., 2020; Trogden et al., 2021). All the "filters" that prevent secretion at a steady-state (basal) conditions must be adjusted upon each stimulus to allow a proper number of IGs to be secreted (Idevall-Hagren and Tengholm, 2020; Omar-Hmeadi and Idevall-Hagren, 2021). Being one of the filters, the MT network and transport must be modified downstream of glucose in a precise and reversible manner. Indeed, emerging evidence indicates that both MT network itself and MT-dependent motor activity are regulated by glucose signaling in β cells (Donelan et al., 2002; McDonald et al., 2009; Heaslip et al., 2014; Zhu et al., 2015; Trogden et al., 2019; Ho et al., 2020; Trogden et al., 2021).

ORGANIZATION AND ORIGIN OF MICROTUBULE NETWORK IN β CELLS

Early work has assumed that MT networks in β cells resemble radial MT organization in other cells (Byers et al., 1980), and serve for directional delivery of IGs to the cell periphery (Lacy et al., 1972). Such a view emerged, in part, due to technical inability, at that time, to distinguish which MT networks belonged to functional β cells vs. other cell types in pancreatic primary cell cultures (Boyd et al., 1982). This view has been challenged by the demonstration of a complex MT network in Min6 cells (Varadi et al., 2003), followed by a series of data in primary functional β cells uncovering a dense non-radial interlocking mesh of MTs in mouse and human islet β cells (**Figure 1A**) (Zhu et al., 2015; Trogden et al., 2019; Bracey et al., 2020; Ho et al., 2020; Muller et al., 2021). Identification of the sites of MT origin (nucleation) has shown that the vast majority of β-cell MTs nucleates at the Golgi complex membrane (**Figure 1A**) (Zhu et al., 2015; Trogden et al., 2019). Such MTs, in contrast to those nucleated at the conventional MT-organizing centers (MTOCs), the centrosomes, are called Golgi-derived MTs, or GDMTs (Zhu and Kaverina, 2013; Sanders and Kaverina, 2015). More recently, a thorough analysis of three-dimensional confocal (Bracey et al., 2020) and electron microscopy (Muller et al., 2021) data has shown that in addition to inner meshwork, the islet β-cell MT network features a prominent array of peripheral MTs parallel to the plasma membrane (**Figure 1A**). We found that this sub-membrane MT bundle is locally stabilized by a MT-associated protein (MAP) tau (Ho et al., 2020).
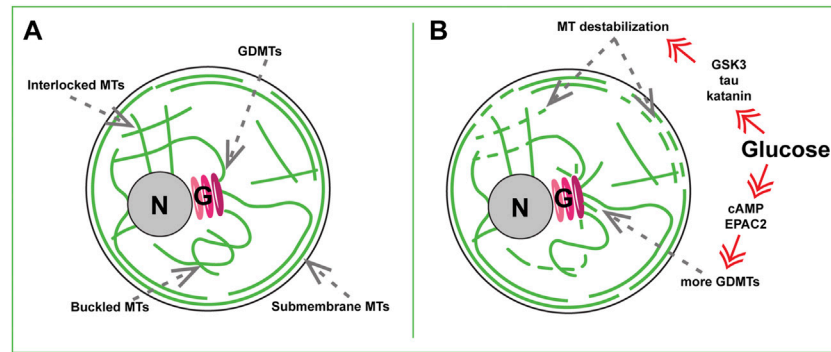
FIGURE 1 | MT sub-populations in a beta cell. **(A)** Steady-state basal glucose conditions. Newly nucleated GDMTs and the Golgi (G), Inner MT mesh formed by interlocked and buckled MTs, and submembrane MT array are shown. **(B)** High-glucose triggered MT remodeling *via* two signaling axes. Top: Kinase- (e.g. GSK3), tau phosphorylation-, and katanin severing-dependent MT destabilization. Bottom: cAMP and EPAC2- dependent enhancement of GDMT nucleation. Nucleus, N.

What could possibly produce a MT network so drastically different from the classic radial MT array? Switching MT nucleation to the Golgi can, in part, explain the non-radial MT pattern in the β-cell interior: because the Golgi in β cells is relatively large, GDMT minus ends are intrinsically distributed over a significant volume, contributing to the network complexity. In addition, irregular MT organization might result from their extended lifetimes: β-cell MTs are extraordinarily stable with a half-life of hours at basal glucose compared with minutes to tens of minutes in other cell types (Zhu et al., 2015; Ho et al., 2020). This increases their flexibility (Portran et al., 2017) and the probability of bending and buckling by intracellular forces over time (Odde et al., 1999; Straube et al., 2006; Bicek et al., 2009).

The origin of the sub-membrane MT bundle is, so far, a matter of speculation. GDMTs might extend from the Golgi to the cell periphery and bend to elongate along the plasma membrane (**Figure 1A**), however such long GDMTs have not yet been detected in β cells (Muller et al., 2021). Nevertheless, MTs do not nucleate anew at β-cell locations other than the Golgi, and, to a much lesser extent, the centrosome (Trogden et al., 2019), meaning that GDMTs must serve as precursors of most components of the MT network in a long run. In principle, MT polymer mass can increase without new nucleation, *via* using small fragments of existing MTs as seeds (Roll-Mecak and Vale, 2006). Interestingly, FIB-SEM studies found multiple small MT fragments at the β-cell periphery (Muller et al., 2021), which might serve as such seeds. These fragments were suggested to potentially arise from MT severing by katanin-family proteins directly in the sub-membrane area (Muller et al., 2021). Alternatively, these fragments could be short MTs nucleated elsewhere (most likely, at the Golgi) and transported to the cell periphery by a motor-dependent MT sliding, as described in other cell types (Jolly and Gelfand, 2010).

On an additional note, it is important to keep in mind that everything said above assumes that every β cell has a similar MT organization. This, however, is not the case. β cells are known to be extremely heterogeneous in their granularity, Ca^{2+} response, metabolic activity, GSIS level, and gene expression (Avrahami et al., 2017; Nasteska and Hodson, 2018; Miranda et al., 2021; Benninger and Kravets, 2022). Not an exception, MTs also vary significantly from one β cell to another, as obvious from the dramatic differences in the amount of long-lived MTs, detected by the detyrosinated tubulin immunostaining (Trogden et al., 2021). This means that certain β cells have stable, unchanging MT networks, while others have more labile, dynamic networks. Thus, some important subtypes of β-cell MT networks might potentially differ from the generalized picture described here. Moreover, specific local MT features within individual cells might have yet escaped averaged analyses (Bracey et al., 2020) or studies unavoidably restricted to a small sample number [e.g., FIB-SEM, (Muller et al., 2021)], and may be functionally very important. Thus, the heterogeneity of MTs within the β-cell population and their fine functional features remain an intriguing area of research.

β-CELL MICROTUBULE NETWORK REGULATION BY GLUCOSE

The critical features of β cells are to be able to respond to postprandial glucose stimulus properly and rapidly and to be able to revert to a steady-state condition after the glucose levels have been reduced. Like other important β-cellular systems, the MT network readily reacts to stimulation. Combined evidence (Heaslip et al., 2014; Zhu et al., 2015; Trogden et al., 2019; Ho et al., 2020; Muller et al., 2021) indicates that being very stable at basal, low-glucose, conditions, MTs undergo a significant turnover in high glucose: both destabilization/depolymerization of pre-existing MTs and simultaneous polymerization of new MTs (**Figure 1B**).

Live imaging assays indicate that MT depolymerization is triggered already 5 min after the high glucose application (Ho et al., 2020). This response relies on hyper-phosphorylation of MT stabilizer tau *via* glucose-responsive kinases GSK3, PKA, PKC, and CDK5 (Ho et al., 2020), which promotes tau detachment from MTs (Lindwall and Cole, 1984). While dynamics of the whole MT network is facilitated upon tau
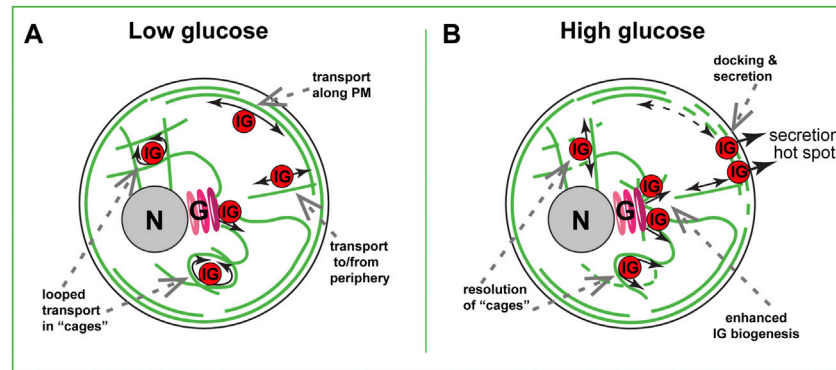
FIGURE 2 | MT-dependent IG transport in a beta cell. **(A)** Steady-state basal glucose conditions. Low number of nascent IGs are formed at the Golgi (G) in a GDMTs-dependent manner. In the inner cytoplasm, many IGs are trapped in MT cages and undergo looped transport. At the cell periphery, sub-membrane and randomly oriented MTs serve for bi-directional IG transport. **(B)** High-glucose triggered IG transport. Partial destabilization of inner meshwork allows for the resolution of "cages" and rare directional transport events. Local destabilization of submembrane MTs allows for interruption of IG withdrawal from the hot spots, leading to docking and secretion. Facilitated GDMT formation supports new IG biogenesis to replenish the IG population. Golgi, (G). Nucleus, N. Plasma membrane, PM.

removal, this effect is especially manifested at the submembrane MTs (**Figure 1B**), which initially contain a higher concentration of tau (Ho et al., 2020). This glucose-dependent MT destabilization coincides with a substantial fragmentation of MTs into small "seeds" (Muller et al., 2021), which may be potentially used to rebuild the submembrane bundle after glucose is cleared from the extracellular media. It is tempting to suggest that tau hyper-phosphorylation and detachment from MTs is a priming step for submembrane MT severing by katanin [as suggested in (Muller et al., 2021)], considering that tau is known to protect MTs from such severing in neurons or *in vitro* (Barbier et al., 2019).

In parallel with destabilization, glucose stimulation also leads to an increase in MT polymerization. This includes facilitated MT nucleation at the Golgi [**Figure 1B**, (Trogden et al., 2019)] and faster polymerization at peripheral MT plus ends (Heaslip et al., 2014). Such responses likely compensate for MT loss, so that the whole MT polymer mass is affected by glucose only to a slight (Zhu et al., 2015) or non-detectable level (Muller et al., 2021). At the same time, the amount of detyrosinated tubulin (Glu-tubulin) within MTs, a post-translational modification used as a readout for MT lifetime (Gundersen et al., 1987; Khawaja et al., 1988), is significantly decreased (Zhu et al., 2015; Ho et al., 2020; Trogden et al., 2021), which is a direct indication of high MT polymer turnover.

Intriguingly, signaling pathways downstream of glucose that trigger the processes of MT depolymerization versus repolymerization in β cells are distinct from one another. While MT destabilization is ATP-production and kinase-dependent (Ho et al., 2020), MT nucleation at the β-cell Golgi requires cAMP and cAMP effector EPAC2 (Trogden et al., 2019), another metabolic signaling axis involved in GSIS (Renstrom et al., 1997; Ramos et al., 2008). This suggests that the amount of MT polymer can be fine-tuned by relative inputs of those two pathways. In addition, MT subsets predominantly affected by those pathways are also distinct, meaning that while the MT polymer mass is mostly sustained,

the balance between different MT subsets is likely tilted at the time of secretion stimulation. We will next discuss how changing specific MT subsets and their relative representation within a cell affects β-cell function and fitness.

FUNCTIONS OF DISTINCT SUBPOPULATIONS OF β-CELL MICROTUBULE NETWORK

Complete depolymerization of MTs by nocodazole leads to enhanced GSIS (Zhu et al., 2015; Trogden et al., 2021), laying a base for our model that MTs serve as one of the "filters" for regulated, dosed secretion levels. What are the functions of distinct MT subsets and how do they affect secretion?

The net movement of insulin granules is non-directional and has characteristics of sub-diffusion, or random walk (Tabei et al., 2013; Zhu et al., 2015). This is, however, not true diffusion: the β-cell cytoplasm is too crowded for IG to move unless they are forcefully transported by molecular motors. Non-directional transport likely arises from a convoluted configuration of MT tracks in the β-cell interior (Varadi et al., 2003; Zhu et al., 2015), where IGs frequently switch tracks and/or follow buckled MT loops (**Figure 2A**). In addition, IGs likely often get restrained by the dense MT meshwork and other components of the crowded cytoplasm.

In the absence of other factors, sub-diffusive transport should move IGs down the concentration gradient: from the areas of high IG abundance to the areas of low IG abundance. Under conditions of IG depletion at the periphery after an extreme secretion wave (degranulation), this would deliver IGs from the cell interior to the cell periphery [positive MT regulation of secretion, as proposed in (Lacy et al., 1972; Boyd et al., 1982; Rorsman and Renstrom, 2003)]. Net IG transport in a healthy β cell does not enrich IG concentration at the cell periphery (Zhu et al., 2015), probably due to an ample IG abundance at that location (the lack of IG gradient). However, instances of direct IG

movement along MTs (**Figure 2A**) have been reported (Varadi et al., 2002; Heaslip et al., 2014; Hoboth et al., 2015). Such rare events deliver recently produced (young) insulin granules toward the periphery for secretion regardless of the gradient and have been proposed to serve as a positive MT regulation of secretion in functional β cells (Hoboth et al., 2015).

Instances of direct IG movement along MTs were also reported when observing IG transport by TIRF microscopy, which by design visualizes only peripheral, submembrane IGs (Varadi et al., 2003; Zhu et al., 2015). Such movements are consistent with utilizing peripheral MTs parallel to the membrane (**Figure 2A**, (Bracey et al., 2020). Those submembrane MTs get destabilized and fragmented upon glucose stimulation (Ho et al., 2020; Muller et al., 2021), prompting a hypothesis that this MT array must be interrupted for secretion to occur (**Figure 2B**). Indeed, preventing MT destabilization by taxol treatment inhibits GSIS (Howell et al., 1982; Zhu et al., 2015). Our computational model predicts that submembrane MTs, as long as they are connected with the inner MT network and serve as tracks for bi-directional IG transport, will promote the removal of IGs from the periphery, acting like a "sponge" (Bracey et al., 2020).

Interestingly, both computational (Bracey et al., 2020) and experimental (Zhu et al., 2015; Hu et al., 2021) data indicate that destabilization of submembrane MTs in the absence of glucose trigger does not strongly influence the amount of IGs at the periphery. Accordingly, at basal glucose levels, effects of MT destabilization on secretion are not detectable (Zhu et al., 2015; Trogden et al., 2021) unless the accumulated effects over several days are evaluated (Ho et al., 2020). However, destabilization under conditions of active IG retention [glucose-activated IG-plasma membrane association, or docking (Nagamatsu, 2006; Nagamatsu and Ohara-Imaizumi, 2008)], leads to a dramatic IG accumulation at the plasma membrane (Zhu et al., 2015; Hu et al., 2021). We speculate that MT destabilization promotes docking by eliminating fast IG movement and allowing for longer IG dwelling in the proximity of docking molecular machinery (**Figure 2B**). It is also possible that active transport physically breaks emerging protein interactions and rips some already docked IGs away from the secretion sites. Importantly, IG docking and secretion do not occur randomly across the plasma membrane. Rather, it is allowed only at so-called secretion "hot spots", cortical/plasma membrane locations where clustered exocytic machinery targets secretion into the bloodstream (Yuan et al., 2015b; Fu et al., 2017; Ohara-Imaizumi et al., 2019). MT-dependent mechanisms restrict functioning of those hot spots: MT destabilization by nocodazole increases the number of actively secreting hot spots per cell (Trogden et al., 2021). In part, the hot spots are activated in otherwise dormant, non-secreting β cell subpopulation. This suggests that the differences in MT stability observed over β cell population in an islet might be one of the mechanisms of functional β-cell heterogeneity, reviewed in (Miranda et al., 2021). MT presence also restricted the number of IGs secreted at each hot-spot location. This implies that if MTs act *via* removal of IGs from the docking sites, they remove all IGs from some secretion loci, and only a percent from others. Exact MT organization and

dynamics at secretion hot spots are unknown, and whether it is differential between hot spots, is yet to be understood. It also cannot be excluded that MTs regulate secretion hot-spot machinery through a different, non-IG-transport-dependent mechanism. For example, the turnover of hot-spot structural elements could be regulated by MTs similar to the integrin and membrane receptor recycling (Yoon et al., 2005; Pellinen et al., 2006; Balasubramanian et al., 2007). Along these lines, MTs were shown to promote the localization of clathrin pits to the vicinity of insulin secretion sites, which is necessary for compensatory endocytosis, and, potentially, molecular component turnover during secretion responses (Yuan et al., 2015a).

Considering MT roles in various trafficking processes in a β cell besides IG transport and positioning, it is important to consider functions of MT-dependent transport at earlier stages of insulin biogenesis. MTs are known to promote every stage of protein production and trafficking in the cytoplasm, including mRNA transport, ER shaping, ER-to-Golgi and Golgi-to-ER trafficking, and exit of secretory vesicles from the trans-Golgi network (TGN) (Palmer et al., 2005; Luini et al., 2008; Suter, 2018). It is plausible that all same steps are regulated in β cells and influence insulin production, as suggested by early studies (Malaisse-Lagae et al., 1979). It is indeed true for efficient budding on nascent IGs off the TGN (**Figure 2B**): under high glucose conditions when IG biogenesis must be intensified, without efficient MT nucleation at the Golgi IG biogenesis fails (Trogden et al., 2019). This means that GDMTs are critical in replenishing IG population after each secretion cycle and maintaining β-cell fitness. This function, likely similar to what was described for post-Golgi carrier formation during constitutive secretion (Polishchuk et al., 2003), indicates an important MT contribution to the positive regulation of insulin secretion at the IG biogenesis stage.

Thus, the MT network in β cells promotes IG availability in the long-term (*via* biogenesis and distribution in the cytoplasm) but restricts IG secretion in the short-term (by withdrawing IGs from secretion sites).

MICROTUBULE-DEPENDENT MOLECULAR MOTORS AND THEIR ROLE IN GLUCOSE-STIMULATED INSULIN SECRETION

As summarized above, we are starting to understand how MT networks are organized and metabolically tuned in β cells. Besides rearranging the MT geometry, IG transport can be tuned by activation of molecular motors or by changing the capacity of MTs to serve as tracks for specific motors (**Figures 3A,B**, (Yu et al., 2015; Monroy et al., 2018). MT-dependent motors are recognized by their capacity to move toward the plus- or minus- end of a MT. In a non-differentiated cell with a radial MT network plus-end directed motors drive center-to-periphery (anterograde) transport, while minus-end directed motors drive periphery-to-interior (retrograde) transport. At this point, we do not have a good understanding of MT plus
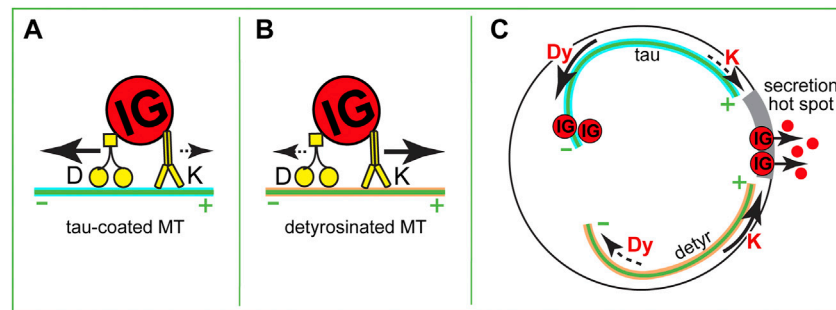
FIGURE 3 | Hypothetical roles of MT-dependent molecular motors in IG transport. **(A,B)** Variants of a tug-of-war between dynein and kinesin-1. **(A)** On a tau-coated MT, dynein overcomes kinesin-1 and transports an IG toward the minus end. **(B)** On a detyrosinated MT, kinesin-1 overcomes dynein and transports an IG toward the plus end. **(C)** A variant of IG delivery-withdrawn regulation. If in high glucose submembrane MTs are partially depolymerized so that the plus ends of remaining MTs are oriented toward a secretion hot spot (gray), different MT modifications could result in either withdrawal (dynein-dependent transport on a tau-coated MT) or delivery (kinesin-1-dependent transport on a detyrosinated MT) of IGs to the secretion sites.

and minus end distributions within complex β-cell MT networks. With largely non-centrosomal long-lived MTs, a significant MT population being parallel to the plasma membrane, and many short MT fragments, it is difficult to predict their polarity. Thus, it is elusive whether plus- or minus-end directed transport will be more efficient in taking IGs to or from the cell border and even less clear, to or from secretion hot spots. To understand specific motor functions, it is important to gather more knowledge on MT polarity and the regulation and function of specific motors in β cells.

The major molecular motor that is thought to drive insulin transport is conventional kinesin, or kinesin-1 (KHC, KIF5b) (Balczon et al., 1992; Varadi et al., 2002; Varadi et al., 2003). It is robustly present at isolated IGs (Balczon et al., 1992), and colocalized with insulin-containing vesicles in β-cell lines (MIN6 & INS-1) (Varadi et al., 2002). Inactivation of kinesin-1 in Min6 cells results in seized IG movement (Varadi et al., 2003). Moreover, a variety of kinesin-1 loss-of-function approaches in cell lines and in mice lead to decreased GSIS levels (Meng et al., 1997; Varadi et al., 2003).

The importance of kinesin-1 for GSIS is consistent with the glucose-dependent regulation of kinesin-1 activity. Interestingly, kinesin-1 heavy chain is heavily phosphorylated at basal (low glucose) conditions (Donelan et al., 2002). Kinesin-1 phosphorylation by a variety of kinases has been associated with inhibited motor activity, data accumulated mostly in vitro and in neurons (Morfini et al., 2016). Kinesin-1 becomes dephosphorylated upon high glucose stimulation (Donelan et al., 2002), which is correlated with faster insulin granule movement (McDonald et al., 2009; Zhu et al., 2015).

Collectively, these data strongly indicate that kinesin-1 positively regulated insulin secretion, either via promoting the steady-state distribution of IG in the cytoplasm, or by specifically targeting IGs to secretion hot spots. The first option would result from the already described non-directional sub-diffusive transport (Tabei et al., 2013; Zhu et al., 2015). The second option assumes the existence of a MT subset that has an accumulation of MT plus ends at secretion hot spots and favors kinesin-1 movement toward those spots (**Figure 3C**). Such a subset is yet to be detected in β cells, but it could be created via such mechanisms as detyrosination (**Figure 3B**), MAP7 accumulation, or other MAP/tubulin PTM variations (Barlan et al., 2013; Yu et al., 2015; Monroy et al., 2018).

Very little is known about the role of other MT-dependent motors in insulin transport. Retrograde transport of granules was implicated in the retrieval of granules away from the secretion sites during kiss-and-run exocytosis (Varadi et al., 2003), however, because of the lack of clarity in MT organization and polarity at those sites, it is yet unclear whether it is dynein or another motor that drives such retrieval. It is tempting to extrapolate the role of dynein as a "brake" that slows down plus-end directed movement [tug-of-war mechanism reported for other cell types (Bryantseva and Zhapparova, 2012)] to β cells (**Figures 3A,B**), however this hypothesis has not been tested yet. Interestingly, dynein activity is dramatically decreased on detyrosinated MTs (McKenney et al., 2016), which would lead to the release of a brake on kinesin-1 movement and make detyrosinated MTs strongly preferred tracks for plus end-directed IG transport (**Figure 1B**). Another extrapolation from the neuronal scenario calls for testing whether a local accumulation of specific MAPs [e.g. tau enrichment at the peripheral MT bundle: tau regulates the efficiency of several motors, restricting kinesin-1 but not dynein movement (Vershinin et al., 2007; Dixit et al., 2008; Chaudhary et al., 2018; Tan et al., 2019)] defines which motor takes advantage for the movement of IG in a certain direction or to a certain location (**Figure 3A**). Additionally, a variety of other MT-dependent motors that are expressed in β cells at lower levels might be important for fine-tuning IG transport.

Finally, it is important to consider the role of MTs and MT motors in the transport of other β-cell components, positioning of which can have indirect yet very important effects on insulin secretion. For example, the scaffolding of transcription factor SP1 at MTs by kinesin KIF12 promotes GSIS and glucose homeostasis in mice via regulating the oxidative stress (Yang et al., 2014). It is known from other cell models that kinesin-1 can reconfigure the MT network via transport and intracellular positioning of MT

fragments (Jolly and Gelfand, 2010). The main glucose-processing stations in cells, mitochondria, are positioned at the sites of energy consumption by MT motor-dependent transport [e.g., (Wang and Schwarz, 2009)]. An important process of insulin degradation/turnover could also be dependent on MT transport, since in other cell types, lysosome movement is mediated by both kinesin-1 (Hollenbeck and Swanson, 1990) and dynein motors (Harada et al., 1998; Jordens et al., 2001). MT-motor-dependent transport is also crucial for the organization of many other cellular features which could contribute to β-cell fitness.

CONCLUSION

To conclude, we are currently at an exciting nucleation point where increased understanding of MT organization and regulation will inform how GSIS is precisely tuned in endocrine islet β cells. Specifically, we now know that the β-cell MT network is built in a unique configuration. This configuration, surely the MT stability and possibly also the 3D precise arrangement, is varied within the cell population to contribute to β-cell functional heterogeneity. We also know that the MT network is remodeled downstream of glucose in such a way that both MT-dependent insulin biogenesis and secretion are allowed. Yet, mere MT presence serves as a negative regulator, adding to other "filter" mechanisms that prevent insulin over-secretion. Kinesin-1 is specifically activated by glucose to support GSIS, and there is still an intriguing possibility that other MT-dependent motors act in concert with kinesin-1 for the precision and restriction of the response. Thus, future studies to illustrate how MT-regulators and motor proteins interact are essential for a better understanding of β-cell function.

Another intriguing area of future research is dissecting the cooperation and hierarchy of the secretion-restricting "filters". To this end, depolymerization of the actin cytoskeleton, which strongly promotes GSIS (van Obberghen et al., 1973; Li et al., 1994; Thurmond et al., 2003), also leads to a partial MT network disruption and eliminates MT-dependent regulation of secretion (Zhu et al.,

2015). Furthermore, MT depolymerization affects neither glucose-induced calcium influx nor secretion enforced by membrane depolarization (Mourad et al., 2011; Trogden et al., 2021), suggesting that calcium-dependent component of GSIS is not controlled by MTs. At the same time, the intriguing question whether and how calcium-independent mechanisms downstream of glucose are affected by MT presence has received some mixed answers (Mourad et al., 2011; Trogden et al., 2021), indicating a potential for yet-unknown, condition-dependent, cooperation of those pathways. Studies on actin-MT cross-regulation in IG localization and Ca^{2+}-dependent vesicle-plasma membrane fusion should not only help with our understanding of diabetes, but also serve as a prototype in understanding how different cell types leverage the regulation and configuration of MTs to serve distinct physiological functions.

AUTHOR CONTRIBUTIONS

KMB reviewed the literature and wrote the initial review draft. GG reviewed the literature, contributed to the critical discussion and edited the manuscript. IK reviewed the literature, prepared the graphic and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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