2021 multi-country
reviewed 9/2022

A Selective Look at Autophagy in Pancreatic β-Cells

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Diabetes 2021;70:1229–1241 | https://doi.org/10.2337/dbi20-0014

Insulin-producing pancreatic β-cells are central to glucose homeostasis, and their failure is a principal driver of diabetes development. To preserve optimal health β-cells must withstand both intrinsic and extrinsic stressors, ranging from inflammation to increased peripheral insulin demand, in addition to maintaining insulin biosynthesis and secretory machinery. Autophagy is increasingly being appreciated as a critical β-cell quality control system vital for glycemic control. Here we focus on the underappreciated, yet crucial, roles for selective and organelle-specific forms of autophagy as mediators of β-cell health. We examine the unique molecular players underlying each distinct form of autophagy in β-cells, including selective autophagy of mitochondria, insulin granules, lipid, intracellular amyloid aggregates, endoplasmic reticulum, and peroxisomes. We also describe how defects in selective autophagy pathways contribute to the development of diabetes. As all forms of autophagy are not the same, a refined view of β-cell selective autophagy may inform new approaches to defend against the various insults leading to β-cell failure in diabetes.

Pancreatic β-cell loss or dysfunction is central to diabetes development, as β-cells are responsible for producing, storing, and secreting insulin to maintain normoglycemia. Numerous stressors lead to β-cell failure in diabetes, including reactive oxygen species (ROS), mitochondrial damage, endoplasmic reticulum (ER) stress, inflammation, and toxic amyloid aggregates. Therefore, stress responses and quality control pathways that mitigate these stressors, including autophagy, are vital for maintaining β-cell homeostasis.

Autophagy is an evolutionarily conserved process that degrades and recycles cytoplasmic components via the lysosome, allowing for nutrient reallocation. Disruption of autophagy is associated with numerous human diseases, including diabetes (Table 1), and plays critical roles in metabolism, inflammation/inflammasome action (reviewed in 1,2), neurodegeneration, and tumor suppression (3,4). Autophagy is often viewed as a nonselective bulk degradation response activated during starvation to compensate for nutrient deficiency. However, we now have a broader view of autophagy. Autophagy is critical for basal cellular function in the presence of nutrients and is important for cellular metabolism, proliferation, and survival (5,6). While autophagy was initially characterized as a nonselective pathway, emerging evidence demonstrates that selective organellar autophagy governs the balance between normal cellular function and the development of disease (7,8). We therefore posit that defects in selective β-cell autophagy could potentiate β-cell failure in diabetes.

Recent studies suggest that several distinct forms of selective autophagy occur within β-cells (Fig. 1): mitochondria (mitophagy), secretory vesicle (crinophagy), lipid (lipophagy), aggregate (aggrephagy), ER (ER-phagy), and peroxisome (pexophagy). Each form of selective autophagy is tightly regulated and possesses its own unique autophagy receptors and machinery, thus distinguishing between forms of selective autophagy. In effect, autophagy is not a singular monolithic process, and all forms of autophagy are not the same. Our goal is to review the latest evidence supporting the underappreciated importance of selective autophagy to β-cell function, while underscoring the need for future work clarifying the significance of selective versus nonselective autophagy within β-cells.

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Received 29 October 2020 and accepted 22 March 2021

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Table 1—Human autophagy gene and diabetes associations

Nearby/mapped gene identified*	Disease association	Autophagy association	SNPs	Study accession no.
CDKN1B	T2D	Macroautophagy	rs2066827	GCST009379
BCL2	T2D	Macroautophagy	rs12454712	GCST004894, GCST009379, GCST010118, GCST007847
CAMKK2	T2D	Macroautophagy	rs3794205	GCST005414
MAP2K7	T2D	Macroautophagy	rs4804833	GCST009379
CALCOCO2	T2D	Selective autophagy (xenophagy)	rs10278	GCST007517
VEGFA	T2D	Macroautophagy	rs11967262	GCST009379
PTEN	T2D	Macroautophagy	rs1236816	GCST010118
CLN3	T1D	Macroautophagy	rs151234	GCST009875
CLEC16A	T1D	Macroautophagy, selective autophagy (mitophagy)	rs741172	GCST009875
CTSH	T1D	Macroautophagy	rs34593439	GCST009875

*This table was generated by cross-referencing mapped/nearby genes from published T1D and T2D GWAS polymorphisms with the human autophagy database. This table reflects the identified gene, the autophagy pathway associated with that gene, and the nearby SNP used to identify the gene.

SELECTIVE VERSUS NONSELECTIVE AUTOPHAGY

Nonselective/bulk macroautophagy (commonly referred to as autophagy) initiates sequestration of cellular contents following starvation by an expanding double-membraned phagophore, which engulfs cargo within an enclosed autophagosome. The autophagosome then fuses with the lysosome forming an autolysosome, which leads to degradation of autophagosome contents. It is clear, then, that lysosomal function is crucial for completion of the autophagic process, and any perturbations of lysosomal pH, function, or activity will impact autophagic flux. Decades of work have led to the elucidation of basic mechanisms and identification of the core autophagy machinery, also known as autophagy-related (ATG) genes. Autophagosome formation and cargo engulfment are regulated by ATG proteins, which are highly conserved from yeast to mammals (9). Interestingly, observations of selective autophagy actually predate the coining of the word autophagy. In the 1960s, autophagosomes were first described as double-membraned sequestration compartments delivering cytoplasmic cargo to the lysosome, whose cargo depended on both the cell-type and environmental conditions (10–12). Others types of autophagy in eukaryotic cells include microautophagy and chaperone-mediated autophagy, which bypass the need for a phagophore and instead directly deliver targets to the lysosome, and have previously been reviewed (13).

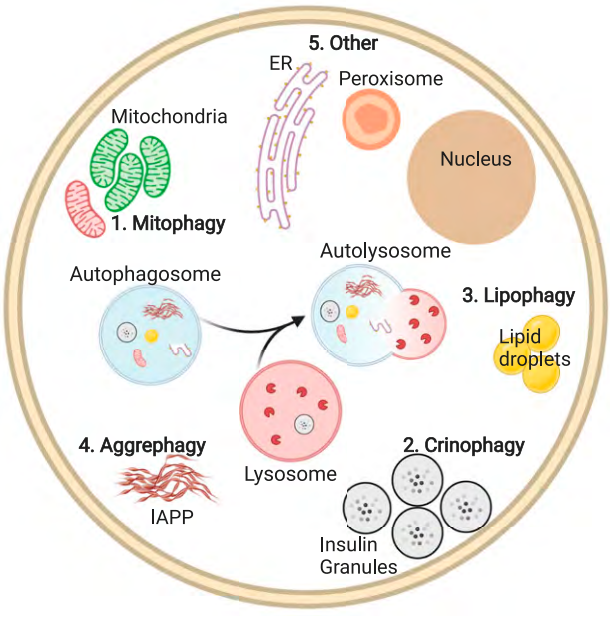

Figure 1—Overview of selective autophagy in β-cells. Selective autophagy relies on the formation of autophagosomes (blue circle) surrounding specific cellular cargo and subsequent fusion with lysosomes (red circle) for cargo degradation. In pancreatic β-cells, selective forms of autophagy that have been described to date include *1*) mitophagy, *2*) crinophagy, *3*) lipophagy, *4*) aggrephagy, and *5*) ER-phagy and pexophagy.

Selective macroautophagy requires the same core ATG proteins as nonselective/bulk autophagy and additionally uses a growing number of selective autophagy receptors (14). Selective autophagy receptors, including p62/SQSTM1, Nbr1, Optineurin, and Ndp52, bind proteins on specific target cargo and act as a scaffold between the cargo and the lipidated protein LC3 (LC3-II) on the expanding phagophore membrane. It is also important to note that specific individual proteins can be selectively targeted for degradation via these receptors, independently of entire organelles. While nonselective autophagy is principally a response to starvation, selective autophagy is important for both stress responses and cellular homeostasis, playing roles in the removal of damaged organelles, protein aggregates, target proteins, and pathogens. As such, it should not be surprising that

disruption of selective autophagy is associated with numerous human diseases, including diabetes (15,16).

Initial β-cell autophagy studies showed that loss of a key ATG protein, Atg7, in mouse β-cells led to impaired glucose tolerance and insulin secretion, decreased β-cell mass due to apoptosis, and an accumulation of dysfunctional mitochondria (17,18). Furthermore, β-cells from patients with type 2 diabetes (T2D) have increased autophagosomes, though it is unclear whether this represents a protective response to stress or a pathogenic dysregulation of autophagy (19). Since these initial observations, numerous studies focusing on loss of Atg7 (20–25) and other upstream autophagy regulators (26–28) have been performed in β-cells. Overall, autophagy is pivotal for β-cell health and function, yet variations in the duration and context of autophagy gene deletion can exert dramatically different effects on β-cell function (29). However, these models disrupt both bulk and selective autophagy, making it difficult to discern the relative importance of either in β-cells.

Mitophagy

Observations of mitochondrial cargo within autophagosomes date back to the 1960s (12), followed shortly thereafter by further reports of specific mitochondrial autophagy (30,31). In the late 1990s, certain nutrient deficiencies in hepatocytes and neurons were found to induce mitochondrial depolarization, followed by mitochondrial autophagy (32,33). In 2005, the term "mitophagy" was coined and proposed as a mechanism for eliminating aged and damaged mitochondria that would otherwise cause oxidative stress (34).

Selective mammalian mitophagy was first observed in the context of the PTEN-induced kinase-1 (PINK1)-Parkin pathway. PINK1 acts as a sensor for mitochondrial health and accumulates on the surface of damaged mitochondria to recruit and phosphorylate the cytoplasmic E3 ligase Parkin (Fig. 2). Following its mitochondrial recruitment and activation, Parkin ubiquitinates several outer mitochondrial membrane (OMM) proteins, which leads to the binding of selective autophagic adaptor proteins, including p62/SQSTM1, Nbr1, Optineurin, and Ndp52, via ubiquitin-interacting motifs (35,36). These adaptors directly conjugate ubiquitinated OMM proteins to LC3-II on the autophagophore membrane via LC3-interacting regions, allowing for autophagosome engulfment and subsequent mitochondrial degradation in the lysosome. While PINK1/Parkin-mediated mitophagy is the most well-studied form of mitophagy, Parkin-independent mitophagy has also been observed through recruitment of alternative E3 ligases to mitochondria (37), transmembrane-receptor mediated mitophagy (reviewed in 38), cardiolipin-mediated mitophagy (39), and activation of the novel E3 ubiquitin ligase Clec16a (40,41).

The ability of β-cells to secrete insulin in response to rising glucose levels depends on mitochondrial metabolism. Glucose metabolism increases mitochondrial ATP generation, altering the cytosolic ATP-to-ADP ratio and closing K_{ATP} channels, which is followed by cellular depolarization and insulin release. Thus, quality control mechanisms maintaining mitochondrial health and eliminating aged/damaged mitochondria are critical for β-cell function. Indeed, β-cells of human islet donors with T2D accumulate damaged and dysfunctional mitochondria (42). In the following sections, we will detail selective mitophagy pathways in β-cells, describing their role in cellular function and pathophysiology.

PINK1/Parkin-Mediated Mitophagy

The importance of the PINK1/Parkin pathway within β-cells to glucose homeostasis has been controversial. Hoshino et al. (43) observed that whole-body Parkin knockout mice have impairments in glucose tolerance and β-cell mitochondrial function and turnover and reduced glucose-stimulated insulin secretion (GSIS) following damage with the β-cell toxin streptozotocin. In contrast, using both pancreatic and β-cell–specific Parkin-deficient mouse models, we observed that Parkin was dispensable for glucose homeostasis, insulin secretion, β-cell mass, and islet architecture, both at baseline and in the context of diet-induced obesity (44) (summarized in Table 2). Of note, a similar inconsistency was observed when comparing the role of Parkin in adipocyte function in whole-body versus adipocyte-specific Parkin knockouts (44–46). These findings indicate that β-cell function may be increasingly dependent on Parkin when peripheral tissues concurrently lose their mitophagy machinery.

Another player in the PINK1/Parkin pathway is Miro1, an OMM GTPase that serves as a mitochondrial docking site for Parkin (47). Miro1 loss reduces Parkin translocation and impairs mitophagy (47). Miro1 expression is reduced in human T2D islets and *db/db* mouse islets, and β-cell–specific loss in mice impairs glucose homeostasis following diet-induced obesity (48). Miro1 deficiency disrupts β-cell mitophagy, causing impaired insulin secretion and impaired mitochondrial function (48).

As for the upstream mitophagy effector PINK1, global PINK1 loss in mice surprisingly improves glucose tolerance (49). PINK1-deficient islets showed increased basal and glucose-stimulated insulin release, as well as reduced residual insulin stores in the context of high glucose and decreased mitochondrial glucose responsiveness (49). Therefore, it appears PINK1 loss impairs the ability of β-cells to couple glucose uptake and metabolism with appropriate insulin release, causing dysregulated basal insulin secretion and hypersecretion at high glucose (49). This suggests a role for PINK1 in β-cell metabolism independent of mitophagy. Further, McWilliams et al. (50) determined that PINK1 was dispensable for basal β-cell

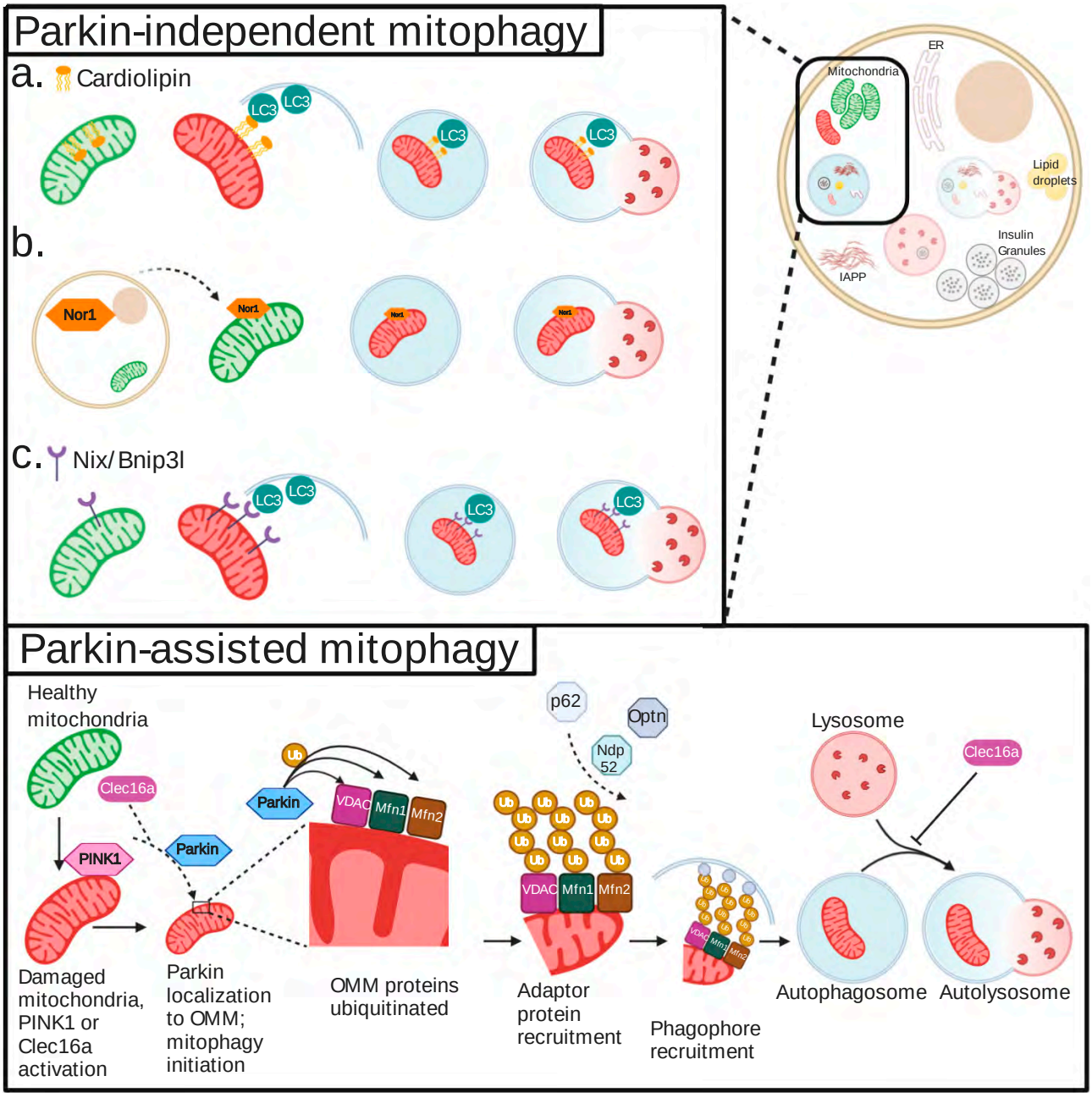

Figure 2— Mitophagy pathways in β-cells. Mitophagy can be initiated by a number of mechanisms, including those dependent (bottom) or independent (top) of the E3 ubiquitin ligase Parkin. During Parkin-mediated mitophagy, damaged mitochondria are recognized by activation of the Clec16a-Nrdp1-Usp8 complex, which leads Parkin to translocate to the OMM. Here, Parkin ubiquitinates a number of membrane proteins (including but not limited to VDAC, Mfn1, and Mfn2). The presence of ubiquitin (Ub) complexes signals to adaptor proteins capable of binding Ub and phagophore/autophagosome membrane resident proteins to traffic mitochondria to autophagosomes. Once the mitochondria are targeted to the phagophore, subsequent closure of the autophagosome and fusion with lysosomes result in selective mitochondrial clearance. These final steps are common to both Parkin-dependent and -independent mitophagy, as can be seen in *a*–*c* (top panel). Parkin-independent pathways of mitochondrial cargo recognition and clearance include the following (top panel): flipping of the phospholipid cardiolipin to the OMM, which can bind directly to LC3 on the expanding phagophore and is potentially targeted by the mitochondrial targeted tetrapeptide SS-31 (*a*); nuclear orphan receptor Nor1 translocation to the mitochondria (*b*); and increased expression of transmembrane receptor Nix/BNIP3L, which can again directly bind LC3 (*c*).

mitophagy in vivo. Interestingly, PINK1-deficient mice had increased expression of key β-cell transcription factors FoxA2, NeuroD, and Nkx6.1, which may explain their enhanced insulin secretion (49). Overall, there is no strong evidence to date that PINK1 controls β-cell function through mitophagy.

Table 2—Mouse models of selective autophagy

Gene	Model	Phenotype	Reference no.
Mitophagy			
Bnipl3 (Nix)	Whole-body knockout on *Pdx1*$^{+/-}$ background	Restored glucose homeostasis and insulin secretion compared with *Pdx1*$^{+/-}$ alone	65
Clec16a	Pancreatic and β-cell–specific knockouts	Impaired GTT, loss of GSIS and mitochondrial respiration in β-cells	40,41,53
Pink1	Whole-body knockout	Improved whole-body glucose tolerance and improved GSIS in isolated islets, reduced insulin islet content and mitochondrial respiration. Isolated islets have increased β-cell TFs	49
Prkn (Parkin)	Whole-body knockout	Impaired GTT and β-cell mitochondrial function, STZ treatment caused worse reduction in GSIS	43
Prkn (Parkin)	Pancreatic and β-cell–specific knockout	Normal GTT, insulin secretion, and β-cell mass on normal chow and high-fat diet	44
Rhot (Miro1)	β-cell–specific knockout	Impaired GTT after high-fat diet, reduced GSIS and mitochondrial function in β-cells	48
Tfb2m	β-cell–specific knockout	Progressive hyperglycemia and eventual diabetes due to impaired GSIS, reduced mitochondrial respiration, and loss of β-cell mass	60
Crinophagy			
Atp6ap2	β-cell–specific knockout	Impaired glucose tolerance, reduced GSIS and insulin content	87
Rab3a	Whole-body knockout	Glucose intolerance, reduced GSIS due to enhanced intracellular insulin degradation	83
Lipophagy			
Lipa (Lal)	Whole-body knockout	Lower fasting glucose, increased glucose-stimulated plasma insulin levels, improved glucose tolerance, increased GSIS in isolated islets	107 and unpublished observations*
Plin2	Whole-body knockout on *AkitaC96Y* background	Protected from diabetes compared with *Akita* alone mice, lowered ER stress and β-cell death	110
Aggrephagy			
hIAPP	β-cell–specific overexpression with *Atg7* knockout	Diabetes development with decreased β-cell mass due to apoptosis and oxidative damage	20,21,23
Uchl1	Whole-body *Uchl1*$^{+/-}$ nm3419 mutant with *hIAPP* overexpression	Accelerated diabetes onset compared with *hIAPP* overexpression alone, due to decreased β-cell mass via apoptosis	123

GTT, glucose tolerance test; TFs, transcription factors; STZ, streptozotocin. *G.L.P. and T.J.B., unpublished observations.

Clec16a-Mediated Mitophagy

Clec16a is an E3 ubiquitin ligase critical for regulating β-cell mitophagy (40,41,51). β-Cell–specific Clec16a loss impairs glucose homeostasis and insulin secretion and causes defects in mitochondrial turnover. Clec16a- deficiency results in increased Parkin levels and unrestrained Parkin activation, causing an accumulation of damaged mitochondria through overloaded mitophagy machinery (40). Clec16a forms a tripartite mitophagy complex with Nrdp1 and Usp8 to regulate mitophagic flux based on cellular demand. Specifically, Clec16a stabilizes the E3 ligase Nrdp1, which targets Parkin for proteasomal degradation to restrain Parkin activity during physiologically healthy states (41). Additionally, Usp8 contributes to the activation of Parkin following the induction of mitochondrial damage (41). Clec16a may also play a separate downstream role in mitophagy by promoting autophagosome-lysosome fusion (40) (Fig. 2).

Clec16a is implicated in the pathogenesis of type 1 diabetes (T1D), as it is a known T1D risk gene (52), and Clec16a deficiency sensitizes β-cells to inflammatory damage in both rodent and human islets (53). Further, Clec16a is transcriptionally regulated by the critical β-cell transcription factor and T2D susceptibility gene, *PDX1*, which may suggest a role for mitophagy in T2D pathogenesis (54). Indeed, the destabilization of the Clec16a-Nrdp1-Usp8 mitophagy complex in human islets by glucolipotoxicity further suggests that Clec16a-mediated mitophagy may be contributing to mitochondrial dysfunction in T2D (41).

Our recent studies of β-cell Clec16a deficiency suggest that excessive Parkin levels or activity could negatively impact β-cell function. In support of this concept, overexpression of Parkin reduced GSIS in Min6 insulinoma cells (55). Additionally, Kusminski et al. (56) determined that excess Parkin levels, caused by overexpression of the OMM protein mito-NEET, were detrimental to β-cell function. Mito-NEET overexpression in β-cells impaired mitochondrial oxidative capacity, leading to glucose intolerance and reduced GSIS. Mito-NEET induction increased β-cell

Parkin expression and aberrantly activated Parkin-mediated mitophagy (56). Deletion of Parkin in the context of mito-NEET overexpression rescued glucose homeostasis and insulin secretion, indicating that excess Parkin activity impaired β-cell function (56). Collectively, these studies point to the importance of fine-tuning and restraining aberrant Parkin activity to maintain β-cell health.

Cardiolipin-Mediated Mitophagy

The phospholipid cardiolipin is found in the mitochondrial inner membrane, and its translocation to the OMM via phospholipid scramblase-3 and mitochondrial nucleoside diphosphate kinase-D promotes mitophagy in neuronal cells (39,57). Externalized cardiolipin initiates mitophagy by binding cardiolipin-interacting sites on LC3 to mediate autophagosome engulfment of mitochondria (39) (Fig. 2*A*). There is limited knowledge on the role of cardiolipin-mediated mitophagy in β-cells. The mitochondrial-localized tetrapeptide, elamipretide (SS-31), which is thought to bind cardiolipin on the inner mitochondrial membrane to prevent fragmentation (58), promotes mitophagy following glucolipotoxic stress in the INS1 β-cell line (59). However, elamipretide did not restore insulin secretion defects in INS1 cells following glucolipotoxicity (59). Thus, the impact of cardiolipin-mediated mitophagy on β-cell function remains unclear.

Transcriptional Regulators of Mitophagy

Beyond the regulation of Clec16a-mediated mitophagy by Pdx1 (see *Clec16a-Mediated Mitophagy* above [54]), other transcriptional regulators also influence β-cell mitophagy. Transcription factor B2 (TFB2M) is an RNA methyltransferase and a component of the mitochondrial transcription initiation complex. TFB2M is necessary to maintain β-cell mitochondrial DNA content, mitochondrial respiration, and GSIS (60). TFB2M deficiency causes an accumulation of mitophagy intermediates, suggesting impaired mitophagy (60). Nor1/NR4A3, an orphan nuclear receptor that functions as a transcriptional activator and also has proapoptotic extranuclear roles, is upregulated in T2D islets. Nor1/NR4A3 translocates to the mitochondria following exposure of INS1 832/13 cells to inflammatory cytokines, which aberrantly enhances mitophagy, and reduces mitochondrial function and GSIS (61,62) (Fig. 2*B*).

Transmembrane Receptor–Mediated Mitophagy

Selective mitophagy can also be mediated through outer mitochondrial transmembrane proteins directly binding LC3 on the phagophore (Fig. 2*C*), such as the BCL2/adenovirus E1B 19 kDa protein–interacting protein 3-like (BNIP3L) (also known as Nix) (63). Nix and the closely related protein BNIP3 are upregulated during hypoxia and mediate mitophagy as an adaptive response to metabolic stress (reviewed in 38). Interestingly, *BNIP3* expression is upregulated in human islets following hypoxia, which may suggest the initiation of protective mitophagy (64). However, loss of Nix in mouse β-cells did not impair glucose homeostasis, insulin secretion, or autophagy, suggesting that Nix does not contribute to β-cell function through control of mitophagy (65). To our knowledge, the impact of BNIP3 in β-cell mitophagy is unexplored.

Mitochondrial Calcium and Mitophagy

The regulation of mitochondrial calcium has been connected to control of mitophagy in other metabolic tissues (66). In β-cells, mitochondrial calcium promotes respiration and ATP production by acting on calcium-sensitive enzymes (67,68). Calcium dysregulation disrupts mitochondrial membrane potential, increases ROS, and disrupts fission-fusion dynamics, all of which may potentiate mitophagy (68). Reducing mitochondrial calcium levels in primary mouse β-cells by silencing or genetic loss of the mitochondrial calcium uniporter (MCU) blunts glucose-stimulated ATP production and impairs ex vivo GSIS, indicating the importance of mitochondrial calcium dynamics to β-cell function (69,70). The direct impact of mitochondrial calcium dynamics on β-cell mitophagic flux, however, remains to be examined.

Mitochondrial-ER Contacts and Mitophagy

Mitochondrial-ER contact sites, which occur at mitochondria-associated membranes (MAMs), facilitate lipid and calcium exchange and are also proposed sites for autophagosome membrane formation (71). Following mitochondrial damage, PINK1/Parkin localize to MAMs and promote MAM formation, which is proceeded by autophagosome formation and mitophagy (72–74). The impact of MAMs on β-cell mitophagy is unclear. However, disruption of MAMs in primary mouse β-cells by selective EI24 loss impairs bulk autophagy (75). In INS1E β-cells, glucose stimulates MAM formation, and MAM disruption inhibits GSIS (76). Interestingly, chronic glucotoxicity also induces MAM formation in human islets (76). While a direct connection between MAMs and β-cell mitophagy is unclear, there is a significant role for MAMs in β-cell function, possibly through regulating β-cell mitophagy.

Selective mitochondrial turnover in β-cells is crucial to maintain β-cell health and function. Disruption of critical mitophagy regulators can precipitate apoptosis or hamper β-cell compensation for environmental stressors including insulin resistance or inflammation. It is also apparent that there are redundant β-cell mitophagy pathways (Fig. 2) that may compensate for each other to maintain β-cell mitochondrial health. While it is clear that mitochondrial structural and functional defects occur in human islets in T1D and T2D (42,53,77), future studies must resolve the importance of mitophagy in protection against the development of diabetes.

Crinophagy and Selective Insulin Granule Autophagy

Crinophagy refers to the selective degradation of secretory granules via direct fusion with lysosomes. It is therefore a specific form of microautophagy and thought to occur in the majority of secretory organ cells, including β-cells (78).

Insulin granule crinophagy was first observed in 1980 in rat islets (79). Due to the high demand for insulin production, it is not surprising that β-cells use insulin turnover mechanisms to ensure that a functional insulin pool is always ready for release. Indeed, the number of insulin granules inside lysosomes was found to be increased as extracellular glucose concentrations declined (80,81), indicating a potential mechanism for eliminating older insulin stores when they are not readily needed. Defects in crinophagy have also been observed recently in the islets of T1D donors (82). Additionally, insulin granule turnover by macroautophagy has been proposed. Thus, both forms of selective insulin granule turnover (crinophagy and granule autophagy) will be discussed below (83–87).

To investigate the mechanisms by which insulin granules reached the lysosome, Marsh et al. (83) impaired insulin granule exocytosis through deletion of the insulin granule trafficking protein Rab3A. In response to impaired insulin exocytosis, insulin degradation via both crinophagy and granule autophagy was upregulated to ensure optimal insulin storage levels (83). Similarly, loss of BAIAP3, a Munc13 protein involved in dense core vesicle priming and docking for exocytosis, enhanced lysosomal degradation of insulin granules (86).

Selective insulin degradation is also observed in vitro during nutrient depletion, when insulin release/requirements should be at a nadir (84). This form of crinophagy appears to be dependent on protein kinase D and proximity to Golgi apparatus (88) and was dubbed starvation-induced nascent granule degradation (SINGD) (84). SINGD may share commonalities with another insulin granule degradation pathway, Golgi membrane–associated degradation (GOMED), which is induced upon glucose deprivation in autophagy-deficient β-cells (89). Intriguingly, mTOR-mediated macroautophagy was inhibited by the lysosomal products released from SINGD (84), suggesting that selective insulin degradation can act independently and upstream of bulk macroautophagy. It will be interesting to determine whether SINGD contributes to the dampened secretion of insulin that is associated with fasting or starvation in vivo.

The method of insulin granule degradation depends not only on upstream stimuli but also on whether proinsulin, insulin, or misfolded insulin is the target cargo (85,90). In INS-1E cells, insulin is degraded primarily by crinophagy, whereas proinsulin is cleared via an autophagosome-mediated route (85). In a mouse β-cell line established by Nozaki et al. (91), inhibition of autophagosome formation after cycloheximide treatment protected proinsulin levels, providing additional evidence that proinsulin is preferentially degraded by macroautophagy (90). However, in Min6 insulinoma cells, knockdown of Atg7 decreased autophagosome formation and increased total cellular insulin levels (25), suggesting that autophagosome-mediated pathways also regulate mature insulin degradation. Conversely, inhibition of autophagosome formation with the selective PI3K inhibitor 3-methyladenine in mouse islets reduced islet insulin content, yet increased GSIS (25). The opposing results of autophagy inhibition on insulin content could be due to differences in model systems and methodologies (3-methyladenine vs. Atg7 deficiency) or may be due to differences in crinophagy, which were not assessed. Further work is necessary to reconcile these results.

The proinsulin mutant *Akita* C(A7)Y was resistant to clearance by macroautophagy due to sequestration within the ER in INS-1E cells (85). However, Akita mice were protected from diabetes development when autophagy was induced by the mTOR inhibitor rapamycin, reducing ER stress and preventing β-cell apoptosis (27). This may be due to protective activation of other selective forms of autophagy, such as ER-phagy, which has recently been shown to selectively clear mutant proinsulin from the ER (92).

The lysosomal V-ATPase accessory protein Atp6ap2 restricts lysosomal fusion events upstream of macroautophagy activation. Atp6ap2 was found to be pivotal for control of insulin granule degradation (87). While the modification of lysosome function can control insulin degradation, the specific effects of lysosome function on crinophagy or selective granule autophagy were not addressed.

Recent studies demonstrate that younger insulin granules are preferentially released by β-cells over aged granules, consistent with the need for clearance of older, less dynamic insulin granules (93,94). The exact method of insulin granule degradation, whether through macroautophagy or crinophagy, depends on the specific cargo and stimulus inducing degradation. β-Cells appear to prioritize lysosomal degradation of insulin granules and even preferentially select for insulin granule degradation over other proteins including toxic IAPP aggregates (90). However, the specific effectors of this pathway remain a mystery. Interestingly, the small GTPases Rab2 and Rab7, a SNAP complex (consisting of Vamp7, SNAP29, and Syntaxin13), and the homotypic fusion and protein sorting (HOPS) complex have been implicated in selective crinophagy of salivary gland glue granules in *Drosophila* (95). Investigating roles for these and other targets in crinophagy and selective granule autophagy will be critical to understand how β-cells maintain an optimal insulin pool for glucose homeostasis.

Lipophagy

Lipid oversupply is a commonly observed destructive force in metabolic syndrome and T2D. Lipotoxicity is detrimental to the function and survival of β-cells (96). Exogenous treatment of β-cells with fatty acids causes a number of cellular responses, including alteration in lipid composition and induction of ER stress (96), which in and of themselves can impact bulk autophagy. However, specific lipid autophagy, or lipophagy, was first observed

in 2009 when Singh et al. (97) found that lipid was a target of autophagic degradation in liver. Lipophagy has been extensively studied in the liver, hypothalamus, and fibroblasts (97–100), yet there has only been one article explicitly referencing β-cell lipophagy (25). However, electron microscopy images from murine and human islets after treatment with fatty acids frequently display lipid-containing autophagosomes (101), and many studies have explored the role of macroautophagy during lipid oversupply from high-fat feeding or ex vivo lipid exposure in β-cells (18,22,26,102,103). A recent proteomic screen identified a number of putative lipophagy-specific regulatory proteins (104), yet their role in β-cell function remains elusive. However, there are many inferences to the role of lipophagy in β-cell health and function to be found in the literature.

Some of the first insights into β-cell lipophagy arose from a study of high-fat diet feeding in β-cell–specific Atg7 knockout mice (18). High-fat diet increased autophagy in β-cells, and Atg7 deficiency led to apoptosis and impaired β-cell mass compensation to diet-induced obesity, suggesting that autophagy plays a critical protective role to lipid excess. The concept of lipid-induced β-cell lipophagy is further supported by the observations of lipid specifically within the autophagosomes of mouse and human islets after exogenous lipid exposure in isolated islets (101,105) and animal models (22,102). Indeed, in leptin-deficient *ob/ob* mice that develop extreme obesity, β-cell–specific Atg7 deficiency precipitated diabetes development due to β-cell apoptosis and loss of functional β-cell mass (22). β-cells appear to respond to lipid oversupply and obesity by increasing autophagy, while simultaneously decreasing lysosomal degradation of other cargo, including long-lived proteins (22), p62-conjugated aggregates (22,102), and mitochondria (26). These studies suggest that β-cells prioritize enhanced lipophagy at the expense of other degradative pathways, such as nonselective/bulk autophagy.

Lysosomal acid lipase (Lal), which is responsible for lipid turnover within the lysosome, has been observed to regulate β-cell function through lipophagy (25). Lipids can reach the lysosome in the form of cholesterol esters trafficked by direct endocytosis of LDL particles or cytosolic lipid droplets delivered by autophagosomes (106). Whole body loss of Lal caused reduced fasting glucose, and increased plasma insulin levels after a glucose bolus, along with a variety of lipid handling defects (107). In β-cells, inhibition of Lal promoted accumulation of cytosolic lipid droplets and enhanced GSIS, likely due to increased lipid intermediates amplifying insulin release (25). This study showed for the first time that impaired lipophagy caused lipid accumulation in pancreatic β-cells and positioned the lysosomal degradation of lipids as a key player in β-cell secretory function.

The maintenance and turnover of β-cell lipid droplets may also provide insights into lipophagy. Perilipin-2 (Plin2) is a membrane protein component of lipid droplets and recently has been implicated in regulating lipophagy in the liver and heart (108,109). Plin2 expression is increased following exogenous lipid exposure in β-cells (110), and Plin2 deficiency in Min6 insulinoma cells caused reciprocal upregulation of LC3-II with p62 downregulation following lysosomal inhibition with chloroquine, indicating increased autophagic flux (110). This study highlights that in the absence of a safe place to store exogenous lipid, i.e., lipid droplets, β-cells may upregulate autophagy/lipophagy to remove harmful lipid species.

Little is known regarding the function of lipophagy in human β-cells. Due to a higher frequency of lipid accumulation in human versus rodent islets, lipophagy could play a central role in human β-cell function. Indeed, initial observations of lipid droplets were made in human pancreatic tissue, demonstrating that human β-cells increase lipid storage with age (111). Later assessment of lipid storage in human β-cells using electron microscopy showed the presence of lipid/lysosomal structures, known as lipofuscin bodies (112). Lipofuscin bodies are waste storage organelles comprised of lipids with excessive peroxidation and cross-linking rendering them resistant to proteases and lipases within the lysosome. Lipofuscin bodies accumulated with age in human β-cells but were unchanged in T2D (112). Tong et al. (113) corroborated the increase in β-cell lipid droplets with age and also found lipid droplets increased substantially in T2D islets. While accumulation of indigestible lipofuscin bodies is a hallmark of aging β-cells, accumulation of potentially degradable lipid droplets in T2D islets may indicate changes in lipid metabolism and/or lipophagy. Indeed, a recent study demonstrated that glucose-stimulated lipolysis is impaired in human T2D islets (114), but the role of lipophagy in lipid handling in human β-cells and in T2D remains unexplored.

Aggrephagy of Toxic Islet Amyloid

Accumulation of protein aggregates is highly toxic and can disrupt cellular homeostasis. Many human diseases are associated with accumulation of intracellular aggregates, including neurodegenerative disorders and proteinopathies (reviewed in 115). While misfolded or damaged proteins can be degraded via the ubiquitin-proteasome system or by chaperone-mediated autophagy, large insoluble protein aggregates undergo selective aggregate autophagy, termed aggrephagy. Large protein aggregates are ubiquitinated and then associate with selective autophagy receptors (including p62/SQSTM1 or Nbr1), which recruit aggregates to the forming autophagosome (116). Both p62 and Nbr1 contain a C-terminal ubiquitin-associating domain that binds ubiquitinated aggregates, an LC3-interacting region that binds lipidated LC3 on the phagophore, and a Phox and Bem1 (PB1) domain, which assists in both phagophore recruitment and p62/Nbr1 self-interaction (116). While Nbr1 and p62 are implicated in

aggrephagy, they also have roles in other types of selective autophagy including xenophagy and pexophagy (117,118).

Aggrephagy may play a critical role in eliminating toxic human islet amyloid polypeptide (hIAPP) aggregates found in T2D (119). hIAPP is a 37-residue protein that is cosecreted with insulin in β-cells. Increased levels of hIAPP, present as both intracellular β-cell oligomers and extracellular deposits, are found in islets in T2D (119). As rodent IAPP does not oligomerize, several transgenic hIAPP overexpression models have been developed to study the effects of hIAPP in β-cells (120). Transgenic overexpression of hIAPP in rat islets increased autophagosomes, seen by accumulation of LC3-II and p62 (121). hIAPP may stimulate aggrephagy, thereby increasing p62, or alternatively could inhibit aggrephagy, leading to accumulation of p62 due to decreased clearance. Following hIAPP overexpression, genetic loss of p62 and lysosomal inhibition each sensitized β-cells to hIAPP-induced toxicity (121). Interestingly, p62 is dispensable for normal β-cell function (122), suggesting a specific importance of p62 in the handling of hIAPP aggregates. These results suggest that hIAPP induction of p62 is protective and likely indicates increased autophagy/aggrephagy.

Further addressing the importance of autophagy/aggrephagy in compensation for hIAPP toxicity, three independent groups concurrently reported mouse models with β-cell–specific hIAPP overexpression together with Atg7 deficiency to impair autophagy/aggrephagy. All groups determined that aggrephagy protected β-cells from hIAPP toxicity (20,21,23). Mice with hIAPP overexpression and Atg7 loss developed overt diabetes, decreased β-cell mass and proliferation, increased oxidative damage, and increased apoptosis compared with hIAPP-alone controls (20,21,23). In all models, β-cells responded to hIAPP toxicity by enhancing autophagy and sequestering hIAPP in inert p62-positive inclusions, which were absent in autophagy-deficient mice. Together, these studies suggest that activation of aggrephagy is a protective response to combat toxic intracellular hIAPP aggregates.

Aggrephagy of hIAPP in β-cells is also modulated by a novel protein, ubiquitin carboxyl-terminal hydrolase isozyme L1 (Uchl1), without formerly known roles in autophagy. Uchl1 is a de-ubiquitinase that promotes hIAPP degradation and is deficient in T2D β-cells (123). β-Cell Uchl1 deficiency exacerbated hIAPP toxicity and impaired autophagy in vivo (123). Following hIAPP overexpression, Uchl1 deficiency decreased β-cell mass, increased β-cell apoptosis, and accelerated diabetes onset (123). Uchl1 loss also enhanced the accumulation of cytoplasmic p62-positive hIAPP inclusions, which could represent overloading of the aggrephagy pathway (123). While Uchl1 is classically implicated in the ubiquitin/proteasome system, this observation suggests that Uchl1 also plays a critical role in β-cell aggrephagy (123).

Together, these studies suggest that hIAPP aggregates are removed by aggrephagy, which may be critical to maintain β-cell health and function in T2D. However, many of these studies use genetic loss of Atg7 to study aggrephagy responses to hIAPP aggregates in β-cells, which disrupt both nonselective and selective autophagy. Thus, a focus on known regulators of aggrephagy, including the scaffolding protein ALFY (124) and the sorting protein Ubiquilin-1 (125), is critical to refine the understanding of aggrephagy of hIAPP in β-cells. Moreover, new technologies to study aggrephagy, including an inducible multimerization module to force protein aggregation, combined with fluorescent reporters to quantify aggregate delivery to the lysosome (126), may provide further insights into β-cell aggrephagy.

Other Selective Autophagy

Other forms of selective autophagy, including ER-phagy and pexophagy, have both very recently been reported in β-cells. Selective ER-phagy in mammalian cells has been linked to a number of ER-resident receptors, including Fam134b, Sec62, Ccpg1, and Reticulon 3 (Rtn3) (127), among others identified in a recent genome-wide CRISPR screen (128). In β-cells, autophagic ER degradation is critical for clearance of insoluble ER-resident aggregates of misfolded Akita proinsulin and wild-type proinsulin, along with canonical ER-associated degradation pathways (92). The degradation of these ER-resident aggregates required Rtn3. Overexpression of Rtn3 promoted aggregate clearance in INS1 832/13 cells, likely due to enhanced selective ER-phagy (92). The role of other ER-phagy receptor mediators (128) in normal β-cell function, or in diabetogenic conditions, remains unknown.

Peroxisomes are genuinely mysterious organelles, whose functions are speculated to be in fatty acid or ROS metabolism (129). β-cell–specific loss of the key peroxisomal protein, Pex5, led to β-cell dysfunction and obvious vacuolation by electron microscopy, potentially indicative of impaired autophagy (129). Acute β-cell–specific autophagy deficiency impacted peroxisomal function, with increases in specific akyl- or alkenyl-phospholipids (plasmalogens) following loss of Atg7 (29). Intriguingly, these plasmalogen species also appear to regulate GSIS (130). Together, these data indicate that peroxisomal function, and specifically pexophagy, may be more important in β-cells than previously appreciated.

Pharmacologic Manipulation of Selective Autophagy

Given the diverse roles of selective autophagy within β-cells, the development of pharmacologic agents targeting selective autophagy may improve β-cell health and function in diabetes. Urolithin A, a metabolite produced by gut bacteria, induces mitophagy and improves mitochondrial health in humans (59). Two similar benzothiophenes also induce basal mitophagy and improve insulin secretion in INS1 cells and mouse islets (131).

Elamipretide (SS-31) also stimulates basal mitophagy and protects against mitochondrial fragmentation during nutrient excess in INS1 cells (59). Targeting mitochondrial calcium or mitochondrial ER contacts may be an additional mechanism to regulate mitophagy. Expanding the repertoire of drugs targeting other forms of selective autophagy, and understanding how these drugs impact human β-cell function, could provide a new avenue to treat diabetes.

CONCLUSIONS

Autophagy is often positioned as a bulk-degradative process activated in response to starvation. However, we are now are beginning to elucidate the nuanced roles autophagy plays in the calibrated and selective clearance of organelles/aggregates. The work described above lays the framework for recognizing the importance of selective autophagy in β-cells and moves beyond the cursory impression that all autophagy is the same. However, several studies on selective autophagy in β-cells still rely on manipulating machinery that also affects bulk autophagy. Thus, a coordinated focus on mechanisms underlying the selective turnover of cargo is needed to discern the roles of selective autophagy in β-cell health. More fully comprehending how, when, and why β-cells use selective autophagy pathways in response to distinct internal and external stressors will provide crucial insights to our understanding of β-cell failure in diabetes.

Acknowledgments. Figures were created with BioRender (biorender.com).

Funding. S.A.S. was supported by the JDRF (CDA-2016-189, SRA-2018-539, COE-2019-861); the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (R01 DK108921, U01 DK127747, and P30 DK020572); and the U.S. Department of Veterans Affairs (I01 BX004444). G.L.P. was supported by the American Diabetes Association (19-PDF-063). M.A.G. was supported by the National Institute of General Medical Sciences, NIH (T32 GM008322), and the NIDDK, NIH (F31 DK122761). The JDRF Career Development Award to S.A.S. is partly supported by the Danish Diabetes Academy and the Novo Nordisk Foundation.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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