Emerging roles for the ubiquitin-proteasome system and autophagy in pancreatic β-cells

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1Division of Cell and Molecular Biology, Toronto General Research Institute, University Health Network, Toronto; Departments of 2Biochemistry, 3Molecular Genetics, 4Institute of Medical Science, and 5Physiology, University of Toronto; and 6Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada

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Hartley T, Brumell J, Volchuk A. Emerging roles for the ubiquitin-proteasome system and autophagy in pancreatic β-cells. Am J Physiol Endocrinol Metab 296: E1–E10, 2009. First published September 23, 2008; doi:10.1152/ajpendo.90538.2008.—Protein degradation in eukaryotic cells is mediated primarily by the ubiquitin-proteasome system and autophagy. Turnover of protein aggregates and other cytoplasmic components, including organelles, is another function attributed to autophagy. The ubiquitin-proteasome system and autophagy are essential for normal cell function but under certain pathological conditions can be overwhelmed, which can lead to adverse effects in cells. In this review we will focus primarily on the insulin-producing pancreatic β-cell. Pancreatic β-cells respond to glucose levels by both producing and secreting insulin. The inability of β-cells to secrete sufficient insulin is a major contributory factor in the development of type 2 diabetes. The aim of this review is to examine some of the crucial roles of the ubiquitin-proteasome system and autophagy in normal pancreatic β-cell function and how these pathways may become dysfunctional under pathological conditions associated with metabolic syndromes.

autophagy; insulin secretion; ubiquitin proteasome; endoplasmic reticulum stress

THE REGULATION OF BLOOD GLUCOSE is accomplished by several organs that maintain appropriate levels during fasting as well as in postprandial states. In the latter case, the pancreas, specifically the pancreatic β-cells found in the islets of Langhans, is essential for blood glucose disposal. The β-cell is the only source of circulating insulin, which is essential for both stimulating peripheral tissue glucose uptake and inhibiting liver glucose production, among other metabolic effects (112). Glucose is the main metabolite responsible for regulating insulin secretion from the β-cell (3), although other metabolites such as free fatty acids can also stimulate secretion and the process can be modulated by incretin hormones such as glucagon-like peptide 1 (24, 88). In addition to stimulating insulin secretion, glucose also stimulates insulin production, both by increasing insulin gene transcription and by increasing insulin translation (Fig. 1) (79, 116, 121, 122). Pancreatic β-cells are central to blood glucose regulation, and if they are destroyed by the immune system or are either dysfunctional or significantly depleted then type 1 or type 2 diabetes, respectively, can result. As a secretary cell that must secrete large amounts of insulin this particular cell type is particularly susceptible to certain stresses.

Even under fasting glucose levels an estimated 40–50% of total cellular protein produced by purified rat β-cells is insulin and stimulatory glucose levels acutely increase insulin biosynthesis (107). To accommodate this large demand the β-cell has evolved an extensive endoplasmic reticulum (ER) (25). It is therefore not unexpected that the β-cell is very sensitive to conditions in which the ER protein folding capacity is not sufficient to meet protein folding demands. Such a state is referred to as ER stress and the cellular response to such conditions is referred to as the unfolded protein response (UPR). The UPR is involved in sensing the protein folding capacity of the ER and eliciting cellular effects that reduce ER stress by transiently attenuating translation, increasing chaperone expression, and increasing degradation of misfolded proteins (101). In mammalian cells this is mediated primarily by three ER stress sensors (PERK, IRE-1, and ATF6) that sense the level of misfolded protein in the ER and transmit downstream signals. The PERK pathway via eIF2-α phosphorylation transiently attenuates translation and induces gene expression changes via upregulation of the transcription factor ATF4. IRE-1 signals by splicing the XBP-1 mRNA that results in enhanced XBP-1 translation, whereas ATF6 is activated by regulated proteolysis once it is trafficked to the Golgi. XBP-1 and ATF6 regulate the induction of the majority of UPR response genes. The intricacies of ER stress sensing and the complement of responses mediated by these pathways is reviewed in Ref. 101. It has recently been appreciated that as a consequence of its high secretory protein production the pancreatic β-cell must rely on an efficient UPR system to maintain ER homeostasis (25, 29). Chronic or persistent ER stress can result in the induction of apoptosis (92). Thus the β-cell may be particularly susceptible to programmed cell death in conditions such as obesity and diabetes, where insulin demands are high.
In addition to susceptibility to ER stress, the pancreatic \(\alpha\)-cell is also known to be sensitive to oxidative stress. This is likely due to the fact that the \(\alpha\)-cell expresses relatively low levels of major antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase that function to remove reactive oxygen species (34, 68, 119). Pancreatic \(\alpha\)-cells are sensitive to conditions such as chronically elevated glucose and free fatty acid levels that increase the amount of reactive oxygen species within cells (99, 100). Such chronic conditions are known to cause adverse effects such as reduced insulin expression and increased cellular damage and cell death.

Both ER stress and increased oxidative stress can also lead to the accumulation of misfolded and/or aggregated proteins in the cell (101, 108). Such material needs to be removed to prevent protein aggregates from accumulating to toxic levels. Two principal systems, the ubiquitin-proteasome system and autophagy, function to maintain cellular homeostasis by recycling macromolecules and in the latter case entire organelles by intracellular degradation. This review will examine the role of both systems in pancreatic \(\alpha\)-cell physiology and the potential consequences that may result when they are overwhelmed or do not function efficiently.

**The Ubiquitin-Proteasome System in Pancreatic \(\alpha\)-Cells**

One of the main mechanisms for the degradation of cellular proteins is the ubiquitin-proteasome system. Proteins targeted...
for degradation are marked by ubiquitination and are degraded by the 26S proteasome. Three distinct enzymes are required to link ubiquitin onto proteins: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-carrier or conjugating proteins), and E3 (ubiquitin-protein ligase), which recognizes a specific protein substrate and catalyzes the transfer of activated ubiquitin to the client protein. Because the ubiquitin-proteasome system can control protein abundance, the cellular functions are varied and diverse and include general protein degradation, regulation of gene transcription, maintenance of protein quality control, immune system regulation, and regulation of amino acid levels, among many other roles (19, 41, 83, 118). The mechanistic details of ubiquitin-proteasome-mediated protein degradation are described in the above reviews and will not be discussed further here. The specificity for protein targeting for ubiquitin-proteasome degradation is mediated by the E3 enzymes, which comprise a large family of proteins (28, 39, 85, 105). Although the protein targets of the E3 enzymes for the most part still need to be identified, several of the E3s have been implicated in human diseases such as cancer, cardiovascular, autoimmunity, and other conditions (28, 33, 85, 105). Some E3 enzymes appear to be expressed at relatively high levels in islets and β-cells on the basis of analysis of microarray expression data available on internet databases (see Supplemental Table S1). However, biochemical analysis of the function and targets of the various E3 enzymes expressed in β-cells is lacking.

The role of the ubiquitin-proteasome system in pancreatic β-cells is turning out to be highly complex. In this section we will discuss some recent studies examining the function of the ubiquitin-proteasome system in maintaining insulin biosynthesis and secretion, in ER-associated degradation (ERAD), and in the degradation of cytosolic protein aggregates and how proteasome dysfunction may contribute to pathogenesis in pancreatic β-cells.

Ubiquitin-proteasome system and insulin biosynthesis, secretion, and signaling. Several recent studies have demonstrated an important role for the ubiquitin-proteasome system in β-cell physiology including maintaining proper insulin levels and facilitating normal secretion (summarized in Fig. 2). Although it is clear that the ubiquitin-proteasome system is essential for maintaining insulin production and secretion, exactly how it is involved has yet to be worked out. Most of the studies have used proteasome inhibitors and have monitored insulin biosynthesis and secretion. Proteasome inhibition with

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Summary of some of the roles of ubiquitin-proteasome system and autophagy in pancreatic β-cells. In addition to the general function of autophagy observed in other tissues described in the text, autophagy in β-cells serves to dispose of aggregated proteins that accumulate in aggresome-like structures (ALIS) that result from chronic conditions such as elevated hyperglycemia, free fatty acids (FFA) and reactive oxygen species (ROS) (A), retire old insulin granules (B), and potentially relieve ER stress that can result from the accumulation of misfolded proteins (C). The ubiquitin-proteasome system has been shown to regulate the activation of membrane channels such as the VDC channel and the KATP channel (I), as well as play a role in the regulation of proinsulin transcription (II), but how this occurs or what role this plays remains unclear. In addition, the ubiquitin-proteasome system is also the degradative machinery of the ER-associated degradation system (ERAD) (III), which functions to eliminate misfolded proteins retrotranslocated from the ER lumen. The ubiquitin-proteasome system may also serve to degrade misfolded cytosolic and ER proteins that accumulate in pancreatic β-cells as a result of islet amyloid polypeptide (IAPP) deposition (IV) that is commonly found in islets of type 2 diabetic individuals. See text for details.
lactacystin has been reported to reduce proinsulin synthesis in mouse islets, which seems counterintuitive given that proteasome inhibition might be expected to result in increased insulin levels (58). This study, however, also reported that proteasome inhibition induces ER stress, which may attenuate translation and indirectly reduce insulin biosynthesis.

Proteasome inhibition has also been shown to affect glucose-stimulated insulin secretion in β-cells, although different results have been reported. Proteasome inhibition in mouse islets (58) and MIN6 cells (55) inhibits acute glucose-stimulated insulin secretion, whereas lactacystin enhanced acute glucose-stimulated insulin secretion in rat islets (71). The role of the ubiquitin-proteasome system in the insulin secretion pathway is likely to be complex since recent studies have shown that the surface expression of molecules involved in regulated insulin secretion such as the ATP-sensitive potassium (K<sub>ATP</sub>) channel (124) and the activity of the voltage-dependent Ca<sup>2+</sup> channel (55) are dependent on the ubiquitin-proteasome system. The latter study has shown that the α-subunit of the voltage-dependent Ca<sup>2+</sup> channel undergoes ubiquitination and that proteasome inhibition impairs channel activity. Thus the proteasome may control the abundance or activity of multiple proteins that regulate the complex process of glucose-stimulated insulin secretion (Fig. 1).

In addition, the ubiquitin-proteasome system may regulate the function of other important pathways in the β-cell. For instance, insulin has been shown to promote ubiquitin-proteasome system mediated degradation of insulin receptor substrate 2 (IRS2) (15, 127). Thus the latter study has shown that IRS2 is critical for pancreatic β-cell function and cell survival (8). It remains to be determined whether the levels of IRS2 are controlled by the ubiquitin-proteasome system in β-cells, but the possibility exists that β-cell survival is dependent on the proteasomes’ ability to correctly regulate IRS2 levels under various external conditions.

Chronic cytokine exposure associated with chronic inflammation in obesity and diabetes has been implicated in causing dysfunction in several tissues including β-cells (26, 43). Some studies have identified that cytokine signaling in pancreatic β-cells involves proteasomal degradation of key signaling components. One study has shown that cytokines can increase cytosolic calcium levels, which leads to ubiquitination and degradation of islet-brain 1/JNK interacting protein 1 (1). IB1/JIP1 normally prevents JNK activation, a signaling pathway implicated in induction of apoptosis in response to cytokines in β-cells (16). The ubiquitin-proteasome system is also implicated in cytokine-induced activation of the NF-κB transcription factor, which also plays a role in the induction of apoptosis. Cytokine-induced degradation of IκBα and activation of NF-κB in islets and pancreatic β-cell lines can be inhibited by proteasome inhibitors (64). Although inhibiting the proteasome itself is unlikely to be a useful therapeutic strategy for reducing the cytotoxic effects of cytokines, targeting the E3 ligases specifically responsible for ubiquitination of IκBα or IκBα may be.

The ubiquitin-proteasome also plays a role in cell proliferation. The Skp2 ubiquitin ligase was recently shown to control cellular p27 levels in β-cells, an important molecule involved in β-cell proliferation (128). The identification of ubiquitin-proteasome targets in pancreatic β-cells is only just beginning and it is likely that the activity or abundance of many key signaling proteins will be shown to be intimately dependent on this system.

Several genes involved in the ubiquitin-proteasome system are downregulated in a mouse pancreatic β-cell line with impaired insulin secretion, suggesting that defects in this system may lead to impaired secretion (81). Clearly more work is required to delineate the role of the ubiquitin-proteasome system in maintaining normal insulin biosynthesis and secretion in pancreatic β-cells as well as in regulating cellular signaling pathways in this cell type.

Ubiquitin-proteasome system and ER stress. In addition to controlling the abundance of cytosolic proteins the ubiquitin-proteasome system is also required for degrading terminally misfolded ER proteins via the ERAD system. As mentioned above, owing to the requirement of β-cells to maintain insulin production this cell type is particularly sensitive to ER stress. In addition, conditions associated with obesity and the development of diabetes, such as chronically elevated cytokines, free fatty acids, and glucose, have been shown to cause ER stress in both in vivo and in vitro models (11, 54, 56, 66, 117). Induction of the UPR during times of ER stress results in a transient decrease in the overall amount of translation within the β-cell and an increase in the levels of ER chaperones (15, 127). Interestingly, although ER stress leads to PERK-mediated translational inhibition, recovery from translational repression seems to be critical for β-cell function and survival (15, 127). Thus the β-cell must also likely upregulate ERAD components during ER stress. The ERAD system degrades terminally misfolded proteins by retrotranslocating them out of the ER into the cytosol where they are degraded by the ubiquitin-proteasome system (57, 80). This may be important for maintaining normal insulin biosynthesis since proteasome inhibition reduces proinsulin biosynthesis (58). Whether misfolded insulin in normal β-cells or in obese or diabetic conditions is actually retrotranslocated to the cytosol for ubiquitin-dependent proteasome degradation requires experimental verification. However, this appears likely since misfolded insulin in the Akita diabetic mouse induces expression of ERAD components, which are required for intracellular degradation of the misfolded mutant insulin (2).

Ubiquitin-proteasome system and islet amyloid. Another situation in which the ubiquitin-proteasome system may be important to β-cell dysfunction in diabetes involves islet amyloid deposition. A pathophysiological feature of type 2 diabetes is islet amyloidosis in the pancreatic islet (46). The main component of islet amyloid is islet amyloid polypeptide (IAPP), also known as amylin (14, 51, 73, 120). IAPP is a normal product of the pancreatic islet β-cell and is stored along with insulin in secretory granules. Islet amyloid localizes to areas of cell degeneration and the process of amyloidosis has been associated with progressive loss of pancreatic β-cell mass by apoptosis (9, 46, 50, 72). The reason why islet amyloid deposits form is not completely understood, although it may involve impaired processing of the IAPP precursor molecule that triggers the process. Pancreatic β-cell toxicity is likely due to oligomerizing propensity of IAPP intermediates, rather than the mature fibrils (20, 49, 77).

The mechanism by which amyloid deposits cause cell death is not entirely understood, although some recent work has been
suggestive of several potential effects. A property of IAPP oligomers closely associated with cytotoxicity is the formation of nonselective membrane channels (82). In addition, both extracellular and intracellular amyloidosis have been shown to cause ER stress and an associated increase in ubiquitinated proteins (12, 45). Overexpression of human IAPP (hIAPP) that has a propensity to aggregate in mice caused ER stress activation including increased expression of spliced Xbp-1 (45). Human islets treated with hIAPP showed increased ER stress and decreased proteasome activity, resulting in the accumulation of ubiquitinated proteins (12). These effects may contribute to apoptotic cell death if as a result of ER stress the UPR is unable to restore ER function and the accumulation of unfolded proteins overwhelms the proteasome, leading to decreased proteasome activity (4, 76). Thus chronic ER stress combined with insufficient degradation by the ubiquitin-proteasome may trigger apototic pathways leading to cell death (92).

Ubiquitin-proteasome system and cellular protein aggregates. Several cellular stresses including heat shock and agents that increase mitochondrial and oxidative stress can cause protein misfolding and the accumulation of cytosolic protein aggregates (62, 108). If the accumulation of cytosolic protein aggregates exceeds disposal the aggregates can be stored in cytosolic structures called aggresomes (62). Aggresomes form from small protein aggregates that are targeted along microtubules to the microtubule organizing center, where they coalesce to form a larger structure. Material targeted to aggresomes is degraded by the ubiquitin-proteasome system, but also by autophagy (discussed below). In addition to aggresomes, other structures referred to as aggresome-like structures (ALIS) have been discovered that result from accumulation of misfolded proteins or aborted translation products (67, 110). The clearance of aggregated material is dependent on both the ubiquitin-proteasome system and autophagy (18, 30, 110). Such structures have recently been observed to accumulate in chronic conditions associated with diabetes in pancreatic β-cells, but they are likely degraded mainly via the autophagy system (discussed below) (53).

Autophagy in Pancreatic β-Cells

In addition to the ubiquitin-proteasome system, cells also rely on autophagy for the degradation of cellular components. Autophagy is a degradative system that is executed by two ubiquitin-like protein conjugation reactions: Atg5 is conjugated to Atg12, which then forms a complex with Atg16L1. This complex acts as an E3 ligase to promote the conjugation of LC3 (a homologue of yeast Atg8) to the phospholipid phosphatidylethanolamine (36, 47, 87). Lipid-conjugated LC3 is thought to promote membrane interactions (tethering and hemifusion), promoting the completion of autophagosome formation and/or their fusion with lysosomes (84, 109). The Atg5-12-16L1 complex is thought to localize LC3 conjugation to sites where autophagy occurs (32), and this targeting may be regulated by members of the Rab GTPase family (48). Because LC3 is covalently attached to membranes involved in autophagy, it serves as a powerful marker to visualize the autophagy process (52). Upstream regulators of autophagy in mammalian cells include class III PI3-kinase VPS34, which is part of a complex with the tumor suppressor Beclin-1 (10), and the kinases ULK-1/2 (38). The mechanism of autophagosome formation and the source of membrane required for autophagy are not clear, possibly complicated by the fact that autophagy is a heterogeneous process (96). Indeed, there are different variations of autophagy depending on the organelle or substrates being degraded. In all cases autophagy terminates with the delivery of cytoplasmic cargo into the lysosome for degradation and recycling of macromolecular components. Several recent reviews deal with the molecular details of these complex pathways (59, 69).

It should also be mentioned that in addition to degradation of long-lived proteins and elimination of cellular organelles, autophagy is used to produce amino acids under nutrient-depleted conditions. Insulin signaling, which enhances protein synthesis, also inhibits autophagy in insulin target tissues (17, 78). Thus there is the potential for alterations in this control in insulin-resistant states such as diabetes (78). In this review we will focus on the current understanding of what is known about the role of autophagy in pancreatic β-cells. We will highlight recent findings on the function of autophagy in the ER stress response, in potential degradation of aggregated proteins, and in the degradation of insulin granules.

Autophagy and the degradation of cytosolic proteins. Although the ubiquitin-proteasome system is involved in the degradation of numerous cytosolic proteins and those retro-translocated from the ER, some studies have shown that certain proteins are not degraded by this system (37, 60, 94, 95). Inhibiting the proteasome has been shown to activate autophagy, suggesting that the two are functionally coupled and can act in a compensatory fashion to one another or autophagy may serve as a backup system for proteasomal degradation (21). In addition, autophagy does not seem to be limited to bulk degradation. Increasing evidence supports the idea that autophagy can act in a selective manner. Indeed, autophagy can be the preferred mechanism for the degradation of specific proteins (90). Ding and Yin (22) have recently reviewed the current understanding on how misfolded protein degradation is divided between autophagy and the ubiquitin-proteasome system.

As mentioned previously, several cellular stresses can cause the accumulation of cytosolic protein aggregates. The clearance of some of this aggregated material has been shown to be dependent, at least in part, on the autophagy pathway (30). In diabetes chronic hyperglycemia induces elevated reactive oxygen species production (99, 100). Recently, we have discovered that chronic hyperglycemia induces the appearance of ubiquitinated-protein aggregates in a pancreatic β-cell line (INS-1 832/13) (53). Such ubiquitinated protein aggregates are also present in β-cells (and other cell types) in the Zucker diabetic fatty rat during the development of diabetes (53). Treatment of INS1 832/13 pancreatic β-cells with 3-MA (an inhibitor of autophagy), but not epoxomicin (an inhibitor of the proteasome) induced the appearance of ubiquitinated aggregates. Thus, at least in this context, the disposal of misfolded or aggregated protein appears to be mediated by autophagy. Why such ubiquitinated protein aggregates accumulate during hyperglycemia is not known, although it is possible that these structures are storage sites for nonfunctional proteins during times of stress. These are then removed via the autophagy pathway once cellular conditions improve. In the β-cell the proteins targeted to the ubiquitinated protein aggregates still
need to be identified. It is possible that insulin is retrotranslocated to the cytosol or that autophagy is responsible for degrading areas of the ER that have accumulated and sequestered such aggregates.

How ubiquitinated protein aggregates are targeted for autophagy-mediated degradation has yet to be established. In this context it would be interesting to determine whether p62, which is targeted to cytoplasmic ubiquitin-containing protein aggregates in some cells (7), is present in these structures in β-cells. p62 interacts with the autophagosome marker protein LC3 and with proteins containing K63-linked polyubiquitin and may facilitate autophagy-mediated clearance of cellular inclusions (61, 111). p62 has recently been shown to be expressed in β-cells (65), but its intracellular distribution and function in the β-cell was not examined.

In addition to chronic hyperglycemia, diabetes associated with obesity is characterized by elevated circulating free fatty acids. Free fatty acids are known to induce apoptosis in pancreatic β-cells in vitro (16). In one study with isolated human islets, chronic exposure to high levels of free fatty acids showed clear examples of autophagy in electron microscopic images (74). Whether or not the upregulated autophagy is contributing to promoting survival in this case or is itself a causative factor in promoting apoptosis remains to be determined.

Autophagy, ER stress, and degradation of ER components. Misfolded protein accumulation in the ER has traditionally been thought to be removed primarily by the ERAD system that is induced by the UPR (101). Recently, however, the importance of autophagy in the ER stress response has been established (6, 44). In yeast the process may involve a novel form of autophagy specifically induced by ER stress that results in the formation of autophagosomes that include ER membranes (5, 6). Interestingly, these structures appear to selectively sequester portions of the ER but do not seem to be involved in degrading the sequestered material. It will be interesting to determine whether similar structures occur in mammalian cells and whether they differ from more traditional autophagosomes involved in degrading macromolecules.

There are several examples of mutant versions of membrane or secretory proteins that aggregate in the ER and appear to require the autophagy system for degradation. Expression of a mutant version of a protein called dysferin present in muscle cells induced autophagy, which was required to degrade dysferin aggregates (31). This shows that in some cases aggregated proteins in the ER can be degraded by autophagy, which may act in conjunction with the ERAD system. Another pathological example where autophagy may play a prominent role is familial neurohypophyseal diabetes insipidus (13). In a cell culture model expression of a mutant version of the secretory protein vasopressin causes an accumulation of the protein in the ER and induces markers of autophagy (13). Inhibition of autophagy results in increased cell death, suggesting that autophagy activation acts as a prosurvival mechanism. Another example is α1-antitrypsin deficiency, which results from a mutant form of this protein that tends to aggregate in the ER of liver cells, preventing its normal secretion (93). A series of studies have determined that autophagy is a critical system that is used for the degradation and removal of mutant α1-antitrypsin (93). In all of these cases it is aggregated protein that appears to require autophagy for degradation, whereas soluble misfolded protein is likely disposed of by the ERAD pathway.

Although not all forms of aggregated protein accumulation that cause ER stress necessarily activate the UPR (42), there is a link between the UPR signaling pathways and autophagy induction. In mammalian cells some studies have shown that induction of autophagy requires the JNK kinase (70, 126). JNK can be activated by the IRE-1 pathway of the UPR (115). Recent studies have shown that IRE-1 is required for autophagy induction in ER-stressed mammalian cells (21, 89). The accumulation of LC3-positive vesicles can be triggered by the ER stress-inducing compounds tunicamycin or thapsigargin, which is dependent on IRE1 but not PERK or ATF6 (89). In addition, studies in yeast have shown that the IRE1 pathway can regulate autophagy induction in that cell (125). However, the precise involvement of the UPR pathways in autophagy induction remains to be sorted out as some recent studies have concluded that the PERK–eIF2α pathway also has an important role in inducing autophagy (31, 63). ER stress has also been shown to lead to the release of calcium from the ER into the cytosol, and calcium can in turn activate various kinases and proteases potentially involved in autophagy (44).

eIF2α has also been implicated as an autophagy mediator in several other models unrelated to ER stress per se (104), but to the integrated stress response (101). Viral infection, amino acid starvation, and heme depletion are all thought to induce autophagy through other kinases that phosphorylate eIF2α such as protein kinase R, general control nonderepressible-2 (GCN2), and heme-regulated inhibitor (HRI).

The role of autophagy in the ER stress response in pancreatic β-cells has not yet been reported. Although acute ER stress response may not require autophagy, it is possible that during times of chronic ER stress activated by chronic conditions (elevated glucose, cytokines, and free fatty acids), the autophagy system may be required to deal with an overwhelming accumulation of misfolded or aggregated proteins.

Autophagy and the degradation of insulin granules. In response to elevated glucose insulin is secreted by the exocytosis of granules at the cell surface and enhanced insulin translation is mainly responsible for replenishing the lost insulin (114). Interestingly, insulin synthesis is maintained even under nonstimulatory glucose concentrations, indicating that the cell has a natural bias toward continual replenishment of insulin stores (3, 98, 107). However, β-cells contain a relatively constant number of secretory granules whose half-life is ~3–5 days (35, 102). Thus insulin granules must undergo degradation to maintain granule numbers relatively constant. Early studies determined that granule degradation in β-cells occurs by a process called crinophagy, the fusion of insulin granules with lysosomes (35, 91, 106). More recent studies have suggested that autophagy is also responsible for the degradation of insulin granules (114). Studies in Rab3A-deficient mice, which have defective insulin secretion, have shed some insight into the role of autophagy in maintaining granule number (75). Despite the secretion defect, insulin biosynthesis remains normal in these animals and hence a net overproduction occurs. However, total insulin content remains relatively normal in Rab3A(-/-) mouse islets owing to a marked upregulation in autophagic degradation of insulin granules. This suggests that autophagy can play a protective role in β-cells during signaling disconnection between insulin synthe-
sis and secretion (75). Interestingly, these authors also found that there was a marked decrease in the expression of the lysosomal protein LAMP-2 in the islets of these mice. LAMP-2, which is known to play a significant role in autophagy (27, 86), may be a potential regulator of granule degradation in β-cells.

The role of autophagy in normal β-cell physiology and the regulation of this process to maintain β-cell granule number requires further investigation. Similarly, it is possible that defects in the regulation of autophagy occur in diabetes. This may contribute to imbalanced insulin content and as a result reduced secretion capacity. In addition, a recent study has shown that autophagy is important in the degradation of mitochondria in β-cells and this process may be dysfunctional in diabetes (113).

Conclusion and Future Directions

Although both the ubiquitin-proteasome system and autophagy have already been shown to mediate important functions in the pancreatic β-cell, the repertoire of cellular pathways these systems control is only beginning to be uncovered. These are essential systems for controlling protein and organelle abundance and are likely to be central in modulating many aspects of β-cell function. Future studies using β-cell-specific knock-out models of important genes involved in the ubiquitin-proteasome system and autophagy pathways should provide more detail into the major cell pathways (signaling, secretion, cell apoptosis, etc.) that these pathways control and the mechanisms involved.

In addition the study of these systems in the context of diabetes is also just beginning. There are likely to be defects in the ubiquitin-proteasome system and/or autophagy in diabetes that may adversely affect β-cell function. Such defects may lead to the inappropriate accumulation of dysfunctional mitochondria, insulin granules, or activation of apoptotic pathways. Furthermore, it is becoming apparent that cellular stress of various forms can induce dysfunctional protein folding, which can cause the accumulation of misfolded and aggregated proteins in cells (40, 123). The inability of the ubiquitin-proteasome system and autophagy pathways to clear protein aggregates may be intimately related to cell death in diseases such as diabetes. The future study of these systems in the context of diabetes should uncover the defects that arise and potential methods to improve the function of these systems to potentially counteract the detrimental effects of diabetes on pancreatic β-cell function.

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