Stress-Induced Translational Regulation Mediated by RNA Binding Proteins: Key Links to β-Cell Failure in Diabetes

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In type 2 diabetes, β-cells endure various forms of cellular stress, including oxidative stress and endoplasmic reticulum stress, secondary to increased demand for insulin production and extracellular perturbations, including hyperglycemia. Chronic exposure to stress causes impaired insulin secretion, apoptosis, and loss of cell identity, and a combination of these processes leads to β-cell failure and severe hyperglycemia. Therefore, a better understanding of the molecular mechanisms underlying stress responses in β-cells promises to reveal new therapeutic opportunities for type 2 diabetes. In this perspective, we discuss posttranscriptional control of gene expression as a critical, but underappreciated, layer of regulation with broad importance during stress responses. Specifically, regulation of mRNA translation occurs pervasively during stress to activate gene expression programs; however, the convenience of RNA sequencing has caused translational regulation to be overlooked compared with transcriptional controls. We highlight the role of RNA binding proteins in shaping selective translational regulation during stress and the mechanisms underlying this level of regulation. A growing body of evidence indicates that RNA binding proteins control an array of processes in β-cells, including the synthesis and secretion of insulin. Therefore, systematic evaluations of translational regulation and the upstream factors shaping this level of regulation are critical areas of investigation to expand our understanding of β-cell failure in type 2 diabetes.

Paradigms of β-Cell Demise in Type 2 Diabetes

During the progression of type 2 diabetes (T2D), insulin resistance places a growing burden on pancreatic β-cells to produce and secrete greater quantities of insulin. Eventually, the demand for insulin surpasses the functional capacity of β-cells, leading to hyperglycemia. Furthermore, this discrepancy worsens as the disease advances due to a decline in the number and in the functioning of β-cells. This outcome, known as β-cell failure, leads to severe hyperglycemia and diabetic complications (1–3). There are several proposed mechanisms to explain the development of β-cell failure in T2D, including impaired insulin secretion, β-cell apoptosis, and loss of β-cell identity, termed "dedifferentiation" (Fig. 1) (4). However, the relative contribution of these processes toward T2D pathogenesis is unclear and may be dependent on the severity of the disease and the genetic makeup of the individual (3,5).

In response to insulin resistance in obese individuals, β-cells can augment insulin secretion as much as five times that seen in healthy control subjects despite only a 50% increase in β-cell mass (6,7). This indicates that the insulin secretory capacity of a single β-cell is dynamic and can be expanded to accommodate increased demand. Thus, the ability of β-cells to compensate for insulin resistance is dependent not just on the number of β-cells present in the pancreas but also on the functional capacity of those cells. Indeed, it has been suggested that the initial cause of hyperglycemia in T2D is primarily a consequence of defects in insulin secretion, not a reduction in the number of β-cells (2,3).

On the other hand, it is clear that long-standing cases of T2D and severe hyperglycemia are associated with a significant decline in the number of β-cells (8). One cause of reduced β-cell mass in T2D is an increase in β-cell apoptosis (2,3). Examination of pancreatic tissue from autopsies indicated that individuals with T2D had reduced β-cell mass that was associated with increased rates of β-cell apoptosis (9). It has recently come into question, however,

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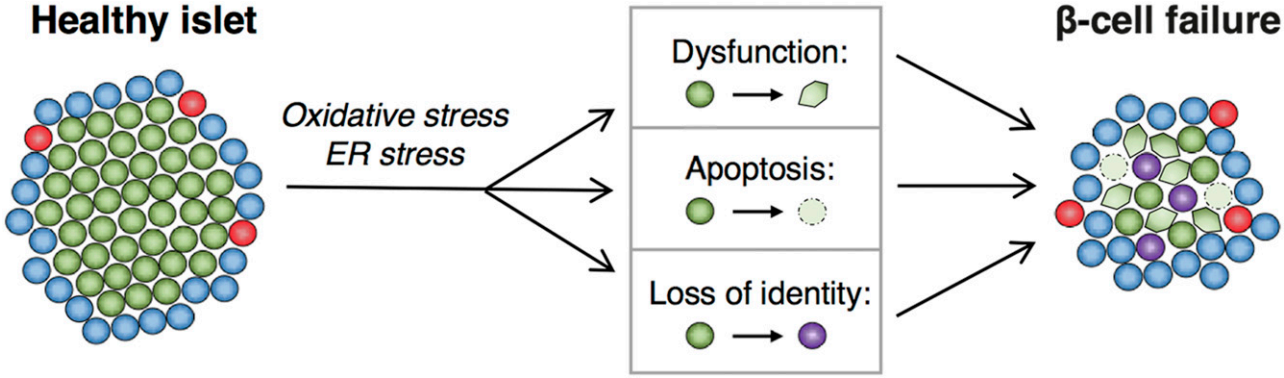

Figure 1—Paradigms of pancreatic β-cell demise in T2D. Increased demand for insulin secretion and rising glucose levels lead to oxidative and ER stress in β-cells. Prolonged stress leads to dysfunction, apoptosis, and/or dedifferentiation of β-cells. Collectively, these processes contribute to β-cell failure that underlies T2D. Depicted are endocrine cells of the pancreas including β-cells (green), α-cells (blue), and δ-cells (red). Dysfunction, apoptosis, and loss of identity are depicted as labeled in the figure.

whether cell death can fully explain the decrease in β-cell number in T2D. An alternative explanation has been proposed in which β-cells lose their cell identity during the development of T2D, which entails the acquisition of features normally restricted to progenitor cells or other endocrine cell types (10,11). This model has been considered plausible partly because there is known to be a high degree of cellular plasticity among endocrine cells of the pancreas (12). Evidence to support this concept includes the acquisition of progenitor markers in islets seen in several mouse models of diabetes, reductions in key β-cell transcription factors in islets from organ donors with diabetes, and reexpression of the progenitor marker ALDH1A3 in islets from individuals with T2D (13–15). While the precise definition of β-cell dedifferentiation in T2D will require further refinement and experimental validation, the idea that reduced β-cell mass in T2D is not solely caused by apoptosis raises the exciting possibility that this loss may be reversible (7).

β-Cell Stress in T2D

In order to develop new treatment strategies to prevent or reverse β-cell failure in T2D, the molecular mechanisms underlying these deleterious processes need to be elucidated. For example, identification of signaling pathways or regulatory factors that govern β-cell identity and apoptosis under disease conditions could provide new targets for therapeutic intervention. A common theme underlying these processes is that β-cells endure various forms of cellular stress in the pathogenesis of T2D. In this context, the term "stress" refers to a significant deviation from a homeostatic set point that causes cellular dysfunction and/or damage. In particular, an imbalance in redox homeostasis, or oxidative stress, and an imbalance in protein folding homeostasis, or endoplasmic reticulum (ER) stress, have been implicated in β-cell demise in diabetes.

Oxidative Stress

Reactive oxygen species (ROS) are normal byproducts of cellular respiration, and the major endogenously produced forms of ROS include hydrogen peroxide, superoxide, and hydroxyl radical (16,17). When generated at low levels, ROS can function as critical signaling molecules. For example, treatment of islets with low levels of hydrogen peroxide has been found to increase insulin secretion (18). When present at high levels, however, ROS can be very detrimental to the cell by damaging lipids, proteins, and DNA, and the onset of ROS-mediated injury is referred to as oxidative stress (19). Oxidative stress is proposed to be a central contributor to β-cell failure in T2D. Islets from patients with T2D show increased levels of 8-hydroxy-2′-deoxyguanosine, a marker of oxidative damage to DNA (20). Animal models of T2D, such as chronic high-fat diet feeding in mice, also display signs of oxidative damage in β-cells (21).

The development and resolution of oxidative stress depends on the balance between the generation of ROS and the levels of antioxidant enzymes that detoxify these molecules. Antioxidant genes are broadly expressed across tissues due to their essential role in preventing oxidative stress, but their relative expression levels can vary to meet the needs of a particular cell type. In fact, β-cells are thought to be particularly sensitive to oxidative stress due to low expression levels of antioxidant genes (22). The effect of ROS production on β-cell homeostasis is complex because it is dependent on both the type and subcellular location of the oxidant (23–25). Thus, a more precise understanding of the molecular mechanisms underlying oxidative stress in β-cells during conditions associated with T2D, including the pertinent factors promoting and ameliorating ROS levels, may provide new therapeutic opportunities for T2D (17).

ER Stress

Another instance of homeostasis that is critical for cell viability is the balance between the abundance of nascent peptides and the protein folding capacity of the ER. If protein synthesis exceeds the folding capacity of the ER, there will be an accumulation of unfolded proteins, termed ER stress. Prolonged ER stress can lead to protein aggregation, cellular dysfunction, and apoptosis (26). Since a

critical function of β-cells is to synthesize and process proinsulin polypeptides, the balance between protein synthesis and folding is particularly important in these cells. In T2D, ER homeostasis is disrupted when insulin resistance causes the demand for insulin synthesis to overwhelm the folding capacity of β-cells (27).

The response to ER stress consists of a highly conserved set of processes known as the unfolded protein response (UPR). The presence of unfolded proteins in the ER activates three distinct branches of the UPR: IRE1α/XBP1, PERK, and ATF6. Together, these factors activate a cellular program that initially aims to restore protein folding homeostasis by increasing expression of ER chaperones and degrading unfolded proteins. Chronic activation of the UPR, however, promotes cell death via proapoptotic factors, such as CHOP (28).

Pancreata from T2D patients show evidence of ER stress in β-cells, including induction of CHOP and dysregulation of the UPR mediators ATF6 and XBP1 (29,30). Mutations in the gene *EIF2AK3*, which encodes PERK, leads to permanent neonatal diabetes due to a reduction in the number of β-cells (31). On the other hand, genetic deletion of CHOP rescues β-cell survival and function in several mouse models of T2D (32). Furthermore, recent findings show that misfolded proinsulin complexes accumulate in the ER of β-cells during prediabetic conditions, indicating that disruption of ER homeostasis is an early event in the development of T2D (33). Thus, a better understanding of the cellular factors influencing the onset of and recovery from ER stress may uncover new therapeutic targets in T2D.

Translational Regulation and Cellular Stress

Changes in the extracellular environment that cause cellular stress lead to reprogramming of gene expression, which, in the acute setting, generally promotes the restoration of homeostasis. Chronic activation of these stress-induced gene networks, however, may lead to cellular dysfunction or death. Therefore, thorough characterization of stress-induced gene programs in β-cells and the factors controlling these changes is an essential step in better understanding T2D pathogenesis. Importantly, these coordinated shifts in gene expression can occur not only at the transcriptional level but also by posttranscriptional mechanisms. Indeed, a critical and prominent part of stress responses across cell types is the regulation of mRNA translation, which allows for rapid changes in gene expression to restore homeostasis. For example, one component of the UPR is increased translation of the mRNA encoding ATF4, a transcription factor that upregulates genes involved in protein folding (34,35).

Since translational regulation may not be accompanied by changes in mRNA abundance, common gene expression analyses such as RNA-seq will not detect this layer of gene regulation. As such, translational regulation represents an essential yet understudied mechanism of cellular adaptation during stress. Several approaches, including ribosome profiling, translating ribosome affinity purification (TRAP), RiboTag, and polysome profiling, address this gap in knowledge by determining ribosome occupancy, a proxy for translational efficiency, transcriptome-wide. For example, these methods have demonstrated a striking reprogramming of translation for hundreds of transcripts during ER stress and heat shock (36–38). Therefore, it will be critical to integrate these analyses into studies of β-cell failure to more comprehensively define the compendium of genes dysregulated under disease conditions as well as the upstream factors controlling these changes.

Overview of Translation Initiation

Translation initiation is a highly regulated, multistep process that is generally considered to be the rate-limiting step of translation (39). The process of translation initiation requires recruitment of the 43S preinitiation complex (PIC) to the 5′ end of the mRNA, scanning of the 43S PIC to a start codon, and formation of a competent ribosomal complex for elongation (Fig. 2*A*). For most mRNAs, this process is dependent on the presence of two particular RNA modifications that are added posttranscriptionally: a 7-methylguanylate cap at the 5′ end (5′ cap) and a stretch of adenosine monophosphates at the 3′ end (poly(A) tail). Upon export to the cytoplasm, mRNA is bound at the 5′ cap by the eukaryotic translation initiation factor 4E (eIF4E), which is part of a heterotrimeric complex called eIF4F that also includes a scaffold protein eIF4G and an RNA helicase eIF4A. Separately, the small ribosomal subunit (40S) is decorated by many additional translation initiation factors, including eIF2, the initiator tRNA, and the multisubunit factor eIF3, and this large complex is collectively called the 43S PIC. Interaction between eIF4G and eIF3 facilitates the recruitment of the 43S PIC to the mRNA 5′ cap, after which the 43S PIC scans along the mRNA until a start codon is encountered. This is followed by the assembly of an 80S ribosomal complex, which requires GTP hydrolysis by eIF2 and recruitment of the 60S ribosomal subunit (40).

In addition to the assembly of translation machinery at the 5′ end of mRNAs, efficient translation also depends upon factors binding to the 3′ end, most importantly the poly(A)-binding protein (PABP). This factor binds to the poly(A) tail and eIF4G to facilitate RNA circularization (Fig. 2*A*). These interactions are thought to increase the efficiency of translation by enhancing eIF4F binding to the 5′ cap and facilitating the reinitiation of ribosomal subunits at start codons after termination, termed ribosome recycling (41).

While these steps encompass translation initiation for most transcripts, certain properties of mRNAs can promote alternative mechanisms of translation initiation. For example, some mRNAs contain a highly structured 5′ untranslated region (UTR) that inhibits scanning of the 43S PIC (Fig. 2*B*). In this case, efficient translation initiation requires supplementary factors, such as additional RNA helicases, to unwind the RNA secondary structures and promote scanning (42). Besides providing structural blockades for translation, RNA secondary structure in

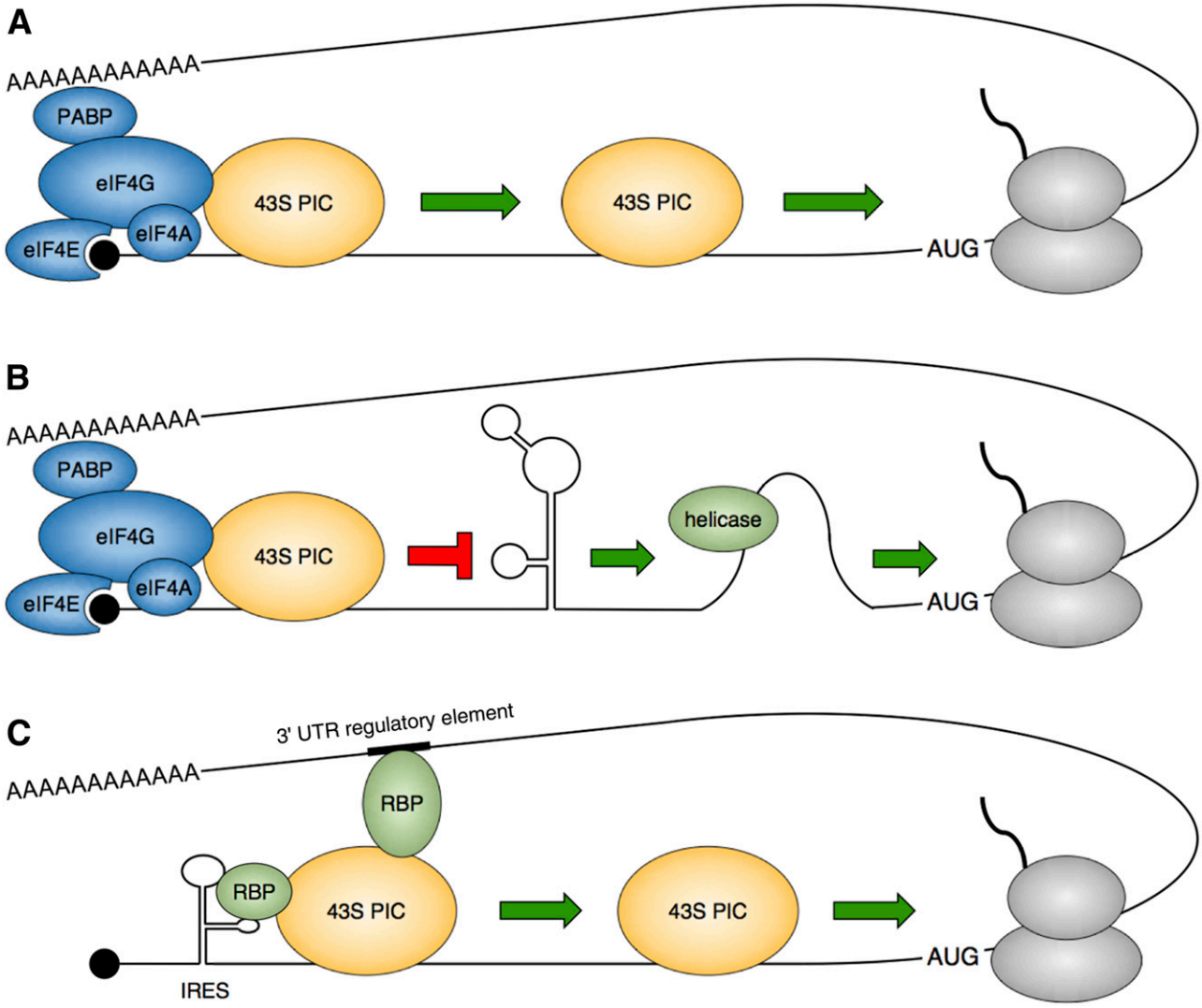

Figure 2—Mechanisms of RBP-mediated regulation of mRNA translation. *A*: Canonical cap-dependent translation initiation requires recruitment of the 43S PIC to the 5′ end of the mRNA by binding to the eIF4F complex (eIF4E-eIF4G-eIF4A), followed by 43S PIC scanning to a start codon and assembly of an 80S ribosomal complex (shown in gray). Interaction between eIF4G and PABP facilitates RNA circularization. *B*: RNA secondary structures may confer selective translational regulation via inhibition of 43S PIC scanning. RNA helicase activity relieves this inhibition via unwinding secondary structures and facilitating scanning. *C*: Cap-independent recruitment of the 43S PIC can be mediated by RBP binding to 5′ or 3′ UTR regulatory elements, including IRESs. Note: the various factors illustrated in the models are drawn for ease of visualization and do not depict actual size ratios.

5′ UTRs can also influence the function of initiation factors. For example, eIF3 generally promotes translation initiation via recruitment of the 43S PIC, however, certain mRNAs contain 5′ UTR structural elements that cause eIF3 to act as a translational repressor (43).

A useful paradigm for studying atypical modes of translation initiation is the translation of viral mRNAs. Viruses are completely dependent on the host translation machinery for their replication and propagation. Counterintuitively, however, many viruses incapacitate the translation machinery of infected cells to shut off translation of host mRNAs. This is achieved by a broad range of mechanisms, including expression of a viral protease that cleaves eIF4G (poliovirus), reducing eIF4E expression levels (enterovirus 71), sequestration of eIF3 via viral proteins (measles virus), and cleavage of PABP by viral proteases (retroviruses) (44). While this impairment of translation cripples the host's defense mechanisms, it also presents the conundrum of how to translate viral mRNAs. For this reason, viruses have evolved various mechanisms to initiate translation using host machinery in a cap-independent manner. One prominent example of this phenomenon is the presence of an internal ribosomal entry site (IRES) near the 5′ end of viral mRNAs. IRESs are highly structured RNA elements that recruit the 40S ribosomal subunit to mRNA in a cap-independent manner. There are several different classes of IRESs, each of which require a distinct subset of trans-acting factors, including translation initiation factors, RNA binding proteins (RBPs), and viral proteins, for efficient translation (45). For example, the IRES in

poliovirus mRNA requires eIF4F and eIF3 for recruitment of the 40S ribosomal subunit, whereas hepatitis C virus mRNA requires eIF3 but not eIF4F and cricket paralysis virus mRNA does not require either of these factors (46).

Importantly, many of these mechanisms for alternative initiation are thought to be pirated from their hosts, meaning that certain host mRNAs also employ these mechanisms under certain conditions. For example, the mRNA encoding c-MYC contains an IRES element, which confers cap-independent translation that is stimulated by the RNA binding protein hnRNPK (47). The broad range of mechanisms employed by viruses for translation initiation raises the intriguing possibility that we have only just begun to understand the usage of these processes for translational regulation of endogenous mRNAs. Indeed, the multifaceted process of translation initiation is regulated at various steps during stress conditions, allowing for the tight control of both global translation rates and selective translation of key mRNAs.

Regulation of Global Translation Rates

Global translation rates must be tightly controlled to optimize cellular efficiency and avoid protein misfolding. For example, mTOR signaling increases protein synthesis in part via inactivation of the inhibitory factor 4E-BP1 to promote cellular growth and proliferation (48). However, prolonged mTOR signaling in β-cells during lipotoxicity leads to excess translation rates that eventually cause ER stress (49). Since protein synthesis consumes a significant amount of cellular energy, dampening global translation rates during stress conserves cellular resources and promotes cell survival (27). For example, the UPR influences global translation rates by several mechanisms. First, activated PERK phosphorylates eIF2α, which reduces its function and suppresses translation initiation (26). This regulation is critical for β-cell adaptation to stress in vivo, as mutation of the phosphorylated site of eIF2α in mice led to the accumulation of unfolded proteins and defective insulin secretion during high-fat feeding (50). Second, 4E-BP1, which reduces global translation rates by sequestering eIF4E, is upregulated by ATF4 and ATF5 during ER stress (51–53). Loss of 4E-BP1 in β-cells leads to increased apoptosis during conditions of ER stress due to unchecked translation rates (54). Third, ER stress impacts global translation rates in β-cells via inhibition of the mTOR signaling pathway as seen in the Akita mouse model and insulin mutations in patient-derived induced pluripotent stem cells (55,56). Thus, dampening translation is critical for the adaptation of β-cells to ER stress because it reduces the protein folding burden and conserves cellular resources.

Selective Regulation of Translation

In addition to the regulation of global translation rates, the selective translation of mRNAs impacts cellular homeostasis during stress indirectly by altering the gene expression program of the cell. This mechanism for altering gene expression may be advantageous over transcriptional regulation because it occurs downstream of transcription and thus can very quickly alter protein levels (37). In β-cells, the importance of translational regulation is epitomized by proinsulin biosynthesis. The acute adaptation of β-cells to high levels of glucose includes increased translation of the mRNA encoding proinsulin, and this upregulation of proinsulin biosynthesis significantly exceeds any change in global translation, indicating a selective translation mechanism (57). Similarly, the GLP-1 analog exendin-4 enhances proinsulin biosynthesis via increased mRNA translation (58).

The directive for selective translation is encoded in the mRNA and can be achieved by several mechanisms. First, selective translation can be governed by regulatory sequences in the mRNA that are bound by sequence-specific factors that regulate translation, such as RBPs or microRNAs. Second, mRNAs can be translationally regulated based on the presence of RNA secondary structures rather than primary sequence elements. Lastly, upstream open reading frames (uORFs) in 5′ UTRs can affect the reassembly of ribosome complexes on downstream canonical initiation codons (40). The translational induction of genes containing uORFs, such as ATF4, ATF5, and CHOP, during ER stress has been well characterized to be a consequence of eIF2α phosphorylation by PERK, leading to increased translation reinitiation at the downstream coding sequences (59). However, the mechanisms controlling translational regulation during stress via primary sequence or secondary structural elements are less understood.

Importantly, these regulatory elements may be present in a cohort of genes that are functionally related, allowing for the coordinated reshaping of gene expression toward a specific purpose. This notion forms the basis of the "RNA-operon" theory, which postulates that trans-acting factors, such as RBPs, may integrate environmental signals with the posttranscriptional regulation of specific genes that share a common function (60). This model is especially intriguing in the context of β-cell adaptation to pathophysiologically relevant conditions; however, the role of RBPs in shaping the β-cell gene expression program in response to external cues is largely unknown.

RBPs regulate all aspects of posttranscriptional RNA processing and handling, including splicing, stability, localization, and translation. In fact, mRNAs are continuously bound by a range of RBPs, indicating that these factors are ideally situated to impart rapid changes in gene expression (61). The target specificity of an RBP is directed by its RNA binding domain, which interacts with RNA in a sequence- and structure-dependent manner (62).

A key mechanism by which RBPs impart translational regulation during stress conditions is to substitute for inactivated translation initiation factors on select mRNA targets. For example, RBPs can bind to 5′ UTR IRES elements to promote assembly of translation machinery in a cap-independent manner during various stress conditions associated with inactivation of eIF4E (Fig. 2*C*) (63).

Alternatively, RBPs can promote translation initiation during stress independent of IRES elements. For example, the RBP RBM4 binds to 3′ UTR sequence elements to promote cap-independent translation of select mRNAs during hypoxia (64). Therefore, RBPs help to shape the cellular response to environmental cues by activating stress-dependent gene programs.

RBP Functions in β-Cells

In order to fully understand the mechanisms shaping gene expression patterns in β-cells, particularly during disease-relevant stress conditions, the repertoire of RBPs that impact β-cell differentiation, viability, and function will need to be elucidated. Given the central role of proinsulin production, processing, and secretion, much of our early understanding of RBP function in β-cells has come from the study of proinsulin biosynthesis. For example, the RBP polypyrimidine tract-binding protein (PTB) enhances the stability of proinsulin mRNA via binding to its 3′ UTR (65). Subsequent studies expanded the role of PTB in β-cells to include controlling the stability of mRNAs encoding secretory granule proteins, indicating that this factor coordinates posttranscriptional gene expression to modulate the secretory capacity of the cell (66,67). Interestingly, PTB also binds to 5′ UTR IRES elements to promote cap-independent translation of mRNAs encoding proinsulin and secretory granule proteins during glucose stimulation (68), highlighting the multifunctionality of RBPs to control various steps of posttranscriptional gene expression.

The RBP HuD, initially reported to be neuron-specific, is expressed in β-cells and binds to the 5′ UTR of the proinsulin mRNA to represses its translation in a glucose-dependent manner (69). Furthermore, HuD levels are decreased in islets from diabetic *db/db* mice (70), suggesting a link to pathophysiologically relevant conditions. Functional studies also linked HuD to triglyceride accumulation and autophagosome formation in β-cells, which could at least in part be attributed to the translational regulation of the mRNAs encoding INSIG1 and ATG5, respectively (70,71). Another target of HuD, mitofusin 2, has also linked this RBP to mitochondrial dysfunction in β-cells during diabetic conditions (72). Lastly, depletion of HuD, also known as ELAVL4, in β-cells reduces apoptosis during cytokine stress; however, the targets underlying this phenotype are unknown (73).

Beyond the regulation of proinsulin biosynthesis, RBPs also establish secretory function of β-cells via regulation of genes involved in various components of the insulin secretion pathway. For example, the RBP CUGBP1 stabilizes the mRNA encoding PDE3B, a phosphodiesterase that mediates cAMP hydrolysis, resulting in reduced intracellular cAMP levels and impaired glucose-stimulated insulin secretion (GSIS) (74). Further, CUGBP1 levels are increased in islets from diabetic mice, including *db/db* and high-fat diet models (74), suggesting this factor may contribute to defects in insulin secretion in T2D-associated conditions. Similarly, the RBP DDX1 impacts insulin secretion by at least two mechanisms. First, DDX1 regulates hundreds of alternative splicing events in β-cells, and depletion of DDX1 impairs glucose-dependent calcium influx at least in part via altered splicing of several voltage-gated calcium channels (75). Second, DDX1 is an RNA helicase that binds to and enhances the translation of the proinsulin mRNA. Under conditions of high free fatty acid levels, DDX1 is phosphorylated and dissociates from the proinsulin mRNA, likely contributing to reduced proinsulin biosynthesis during lipotoxicity (76). Another RBP, RBFOX1, influences GSIS in β-cells via splicing of Gelsolin, a calcium-activated actin-binding protein that enhances insulin secretion (73).

The RBP RBM4 has been implicated in glucose homeostasis based on the observation that *Rbm4*-deficient mice have impaired glucose tolerance and insulin secretion in vivo. This correlated with a reduction in β-cell mass in adult mice and altered isoform expression of key β-cell genes, including *Isl1*, *Pax4*, *Pax6*, and *Glut2*, in embryonic pancreata (77). This suggests that RBM4 is a critical regulator of alternative splicing in β-cells; however, this RBP is a multifunctional protein that regulates various steps of posttranscriptional gene expression, including stress-dependent translational controls (64,78). Therefore, further investigation of this RBP in β-cells under stress conditions may uncover novel mechanisms of stress adaptation.

In addition to effects on insulin biosynthesis and secretion, RBPs have also been linked to β-cell apoptosis. For example, the RBP Musashi-1 is expressed in β-cells, and overexpression of this factor reduces apoptosis under cytokine and ER stress conditions (79). Given its role as a translational repressor in neurons (80), identification of the target mRNAs being regulated by Musashi-1 in β-cells may reveal new pathways implicated in stress-dependent apoptosis. Another RBP, AUF1, promotes cytokine-mediated β-cell apoptosis, although the targets responsible for this effect are unknown (81). Depletion of the splicing factors NOVA1 or NOVA2 increases cytokine-mediated apoptosis, indicating a prosurvival role for these factors (73,82). Finally, an hnRNPK/DDX3X complex regulates the translation of the mRNA encoding JUND, a pro-oxidant and proapoptotic factor in β-cells, during metabolic stress in an ERK-dependent manner (83,84).

Concluding Remarks and Future Directions

While several studies have begun to uncover RBPs that regulate critical aspects of β-cell biology, including proinsulin biosynthesis, insulin secretion, and cell viability (Table 1), we are just beginning to understand the scope of their influence over β-cell adaptation or dysfunction during stress conditions. To better understand the mechanisms underlying these effects, future studies should focus on comprehensively defining the downstream targets of these factors in β-cells. This can be achieved by integrating genomic approaches to assess RBP binding sites, such as CLIP-seq, with changes in gene expression after RBP depletion or overexpression.

Table 1—Role of RBPs in pancreatic β-cells

RBP	Role in β-cells	Reference
PTB	Increases stability of mRNAs encoding proinsulin and secretory granule proteins Promotes cap-independent translation of proinsulin mRNA during glucose stimulation	(65,66) (68)
HuD	Inhibits translation of mRNA encoding proinsulin Regulates autophagosome formation, triglyceride accumulation, and mitochondrial fusion Promotes apoptosis during cytokine stress	(69) (70–72) (73)
CUGBP1	Inhibits GSIS via stabilization of mRNA encoding PDE3B	(74)
DDX1	Regulates alternative splicing, including of voltage-gated calcium channel genes Enhances the translation of proinsulin mRNA	(75) (76)
RBFOX1	Regulates actin remodeling via alternative splicing of Gelsolin	(73)
RBM4	Regulates alternative splicing of *Isl1*, *Pax4*, *Pax6*, and *Glut2* in embryonic pancreata	(77)
MSI1	Reduces apoptosis caused by cytokine and ER stress	(79)
AUF1	Increases apotosis caused by cytokine stress	(81)
NOVA1/2	Reduces apoptosis caused by cytokine stress	(73,82)
hnRNPK	Regulates translation of the pro-oxidant factor JUND during metabolic stress	(83,84)

Importantly, technologies that assess ribosome binding density, such as ribosome profiling, will be especially useful to uncover targets undergoing translational regulation. These data sets promise to uncover new genes impacting β-cell biology and to advance our understanding of gene networks organized by posttranscriptional mechanisms.

In addition to their downstream targets, the importance of RBPs for disease states should be investigated by assessing the cellular conditions that alter their expression level or posttranslational modifications. For example, islets from diabetic *db/db* mice display decreased levels of HuD, increased levels of CUGBP1, and increased phosphorylation of hnRNPK (70,74,84), indicating these RBPs may participate in altering gene expression programs during disease states. Importantly, stress-induced posttranslational modifications may change the cohort of genes regulated by an RBP by altering its RNA binding activity, protein-protein interactions, or subcellular localization. Thus, identifying cellular stresses and/or signaling pathways that regulate RBP function is critical to fully delineate the gene expression programs regulated by these factors in β-cells.

Another important area of investigation will be expansion of the repertoire of RBPs that regulate posttranscriptional gene expression in β-cells during cellular stress. A candidate-based approach can be used to identify potential RBPs based on expression patterns or known function in other cell types. For example, the RBP RBM4 controls selective translation of mRNAs during hypoxic stress in a glioblastoma cell line (64) and during arsenite stress in HeLa cells (78); however, whether this RBP impacts stress-dependent translational regulation in β-cells is unknown. Candidate RBPs may also be chosen based on tissue-specific expression patterns. Indeed, an analysis of neuron-enriched RBP expression patterns in β-cells led to the identification of novel roles for HuD, NOVA2, and RBFOX1/2 in insulin secretion or apoptosis (73). With a constantly growing body of genomic and proteomic data sets in β-cells, analyses to search for RBPs associated with disease states or stress conditions promise to uncover new candidates for further investigation.

In contrast to a "top-down" strategy in which compelling candidates are chosen prior to investigation of downstream mRNA targets and cellular processes, a "bottom-up" strategy can also be employed in which cohorts of genes with stress-dependent expression patterns are first identified. Common features of these genes, such as sequence elements, can then be used to infer the upstream RBPs coordinating their expression. This approach is particularly relevant for genome-wide assessments of ribosome binding density, a proxy for translational efficiency, as transcriptionally regulated genes will not confound the enrichment analysis. For example, we previously found enrichment of a cytosine-rich sequence element in 3′ UTRs of genes with altered ribosome occupancy during PDX1 deficiency, leading us to investigate the poly(C)-binding protein hnRNPK in β-cells (84). Further, inferring which RBPs may be regulating a cohort of genes can be strengthened by overlap with genome-wide RNA-protein interaction data sets, such as CLIP-seq. Fortunately, binding sites have already been mapped for over 100 RBPs in K562 and HepG2 cell lines (85,86), allowing for an unbiased comparison against a broad array of potential regulatory factors.

While mRNA translation is clearly a highly regulated process, the mechanisms shaping this posttranscriptional control over gene expression during stress are incompletely understood. Translational regulation impacts critical processes relevant for T2D progression, including proinsulin biosynthesis and β-cell viability. Therefore, a better understanding of the mechanisms controlling translation during disease-relevant conditions may uncover new therapeutic opportunities. Further, stress causes widespread changes in translation, such as a shift from cap-dependent to cap-independent initiation, and prolonged alterations in translation initiation may lead to disruption of gene networks

critical for β-cell identity. As such, stress conditions may present a reprogramming window that can result in loss of β-cell identity. To investigate this possibility, it will be particularly important to study mechanisms controlling the stress-dependent translation of key β-cell transcription factors, which are required to establish and maintain β-cell gene expression networks. Finally, regulation of mRNA translation can also include the production of truncated or extended polypeptides via translation initiation at noncanonical start codons, especially during stress conditions (87). Intriguingly, defects in translation initiation of the insulin mRNA have recently been linked to the generation of an immunogenic peptide from an alternative open reading frame (88). Further investigation into mechanisms controlling translation initiation in β-cells during stress may therefore shed light on the development of autoimmunity in type 1 diabetes.

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