Emerging roles for the ER stress sensor IRE1α in metabolic regulation and disease

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Inositol-requiring enzyme 1 (IRE1) is an endoplasmic reticulum (ER)-resident transmembrane protein that senses ER stress and is evolutionarily conserved from yeast to humans. IRE1 possesses both Ser/Thr protein kinase and endoribonuclease (RNase) activities within its cytoplasmic domain and is activated through autophosphorylation and dimerization/oligomerization. It mediates a critical arm of the unfolded protein response to manage ER stress provoked by lumenal overload of unfolded/misfolded proteins. Emerging lines of evidence have revealed that in mammals, IRE1α functions as a multifunctional signal transducer that responds to metabolic cues and nutrient stress conditions, exerting profound and broad effects on metabolic homeostasis. In this review, we cover recent advances in our understanding of how IRE1α integrates a variety of metabolic and stress signals and highlights its tissue-specific or context-dependent metabolic activities. We also discuss how dysregulation of this metabolic stress sensor during handling of excessive nutrients in cells contributes to the progression of obesity and metabolic disorders.

In eukaryotic cells, the endoplasmic reticulum (ER) is the largest membrane-bound organelle that forms a contiguous network with interconnected sheets and tubules (1). Spreading throughout the cell, it is typically classified into two types, smooth ER and rough ER. The ER performs a diversity of essential cellular functions, including calcium (Ca^{2+}) storage and release, protein synthesis and processing, lipid biosynthesis and trafficking, and membrane biogenesis. Dynamically interacting with many other organelles (e.g. mitochondria, endosomes, Golgi, lysosomes, and lipid droplets) through membrane contact sites (2–4), the ER is generally thought to be implicated not only in exchanging ions, metabolites, lipids, and proteins, but also in conveying crucial cellular signals. Thus, adaptive regulatory mechanisms for maintaining the dynamic ER integrity are vitally important for cell homeostasis and survival in response to changes in nutritional states and a variety of other environmental challenges.

The ER is the primary site for the synthesis, folding, modification, and trafficking of nearly one-third of the cellular proteome, particularly transmembrane or secreted proteins. An overload of unfolded proteins or excess accumulation of misfolded proteins within the ER lumen instigates a state of the so-called “ER stress,” leading to activation of a highly-conserved adaptive response referred to as the unfolded protein response (UPR) (5–10). The notion of UPR was initially proposed over 30 years ago by Kozutsumi et al. (11) based on the observation that misfolded protein-elicited signals from the ER could trigger the induction of two glucose-regulated proteins, which are later known to be the ER protein chaperones. This was followed by elaborate characterizations, through yeast genetics approaches, of the delicate intracellular UPR signaling pathways (6, 12, 13). The ER-resident transmembrane proteins, PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1), are the essential signal transducers in mediating the three classical arms of the UPR. Coordinated activation of these UPR sensors serves as the quality control mechanism to govern ER proteostasis and cope with ER stress. This is achieved through shutting down the cellular protein translation machinery, while transcriptionally activating gene expression programs to enhance the ER’s protein-folding capacity and to promote the elimination of terminally unfolded/misfolded proteins by ER-associated degradation (ERAD) (14). Under prolonged or severe ER stress conditions, however, homeostasis cannot be restored at the ER, and overactivation of the UPR leads to activation of cell death programs (15). A plethora of studies over the last 30 years or so have uncovered the central roles of the UPR signaling arms in the control of many aspects of cell physiology, particularly cell fate decisions (16, 17), as well as their implications in a wide
range of pathologies, including cancer, neurodegeneration, and metabolic diseases (18-25).

IRE1, encoded by the Ern1 (Endoplasmic reticulum to nucleus signaling 1) gene, is the most ancient and evolutionarily-conserved UPR sensor that was originally identified in yeast Saccharomyces cerevisiae (26, 27). As an ER-localized type I transmembrane protein (i.e. a membrane-spanning protein with the extracellular/lumenal portion at its N terminus), IRE1 harbors an N-terminal luminal domain (LD) that can sense the protein-folding status within the ER, and a C-terminal cytoplasmic effector domain that possesses both protein serine/threonine kinase and endoribonuclease (RNase) activities (Fig. 1) (5, 6, 28). Two IRE1 isoforms, designated IRE1α and IRE1β, are encoded by the mammalian genome, with IRE1α found to be the most abundantly and ubiquitously expressed (29). Upon ER stress, IRE1α is activated through dimerization/oligomerization and trans-autophosphorylation, resulting in allosteric activation of its C-terminal RNase domain (30, 31). Activation of IRE1α’s RNase activity in turn catalyzes the unconventional splicing of the mRNA encoding X-box–binding protein 1 (XBP1), removing an intron (26-nucleotide in mouse and human) that contains a double hairpin structure (6, 7). Subsequent ligation by an RNA ligase RtcB-mediated ligation yields the spliced form of Xbp1 (Xbp1s) mRNA and generates a transcriptionally active transcription factor XBP1s to initiate a key UPR gene expression program to cope with ER stress and maintain the homeostatic control of cell proliferation and metabolism. IRE1α can also degrade select sets of mRNAs or pre-miRNAs through a process termed RIDD. In addition, IRE1α can interact with an increasing number of protein partners (indicated by X, Y, and Z) and presumably serves as a signaling platform composed of regulatory factors as well as signaling effectors. Under metabolic stress conditions, IRE1α may act as a multitasked protein machinery that is involved in regulating many aspects of metabolism.

Figure 1. Activation of IRE1α as a metabolic stress sensor. In addition to protein folding overload within the ER lumen, the mammalian ER-localized transmembrane stress sensor IRE1α can also sense the indicated nutrient or metabolic signals that activate its downstream effector activities. As a UPR signal transducer with an N-terminal LD for detecting unfolded/misfolded proteins, IRE1α possesses a cytoplasmic domain with dual kinase/RNase activities. Upon typical ER stress, IRE1α activation involves dimerization/oligomerization and autophosphorylation. Activated IRE1α RNase can catalyze the unconventional splicing of Xbp1 mRNA, removing a 26-nucleotide intron from the unspliced Xbp1 (Xbp1u) mRNA. Subsequently, RNA ligase RtcB-mediated ligation yields the spliced form of Xbp1 (Xbp1s) mRNA and generates a transcriptionally active transcription factor XBP1s to initiate a key UPR gene expression program to cope with ER stress and maintain the homeostatic control of cell proliferation and metabolism. IRE1α can also degrade select sets of mRNAs or pre-miRNAs through a process termed RIDD. In addition, IRE1α can interact with an increasing number of protein partners (indicated by X, Y, and Z) and presumably serves as a signaling platform composed of regulatory factors as well as signaling effectors. Under metabolic stress conditions, IRE1α may act as a multitasked protein machinery that is involved in regulating many aspects of metabolism.
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(33), which was initially thought to eliminate select ER-localized mRNA species (34). Moreover, IRE1 is known to form high-order clusters at the ER membrane (35, 36), and IRE1α can interact with many functionally important protein partners such as the proapoptotic BCL-2 family members BAX and BAK, the scaffold proteins TRAF2, TRAF6, and RACK1, and the transcription factor STAT3, potentially acting as a signaling platform to regulate apoptosis, inflammation, and proliferation (37–44). Thus, IRE1 is a genuine multitasked stress-sensing machinery that performs a myriad of cellular functions via a complex signaling network.

Numerous studies have implicated IRE1α signaling, through the three aforementioned mechanisms, in a diverse range of biological processes in mammals, including cell survival/death determination, immunity, and metabolism, which have been previously reviewed (16, 22, 45–47). In particular, ER stress is highly associated with obesity and insulin resistance (22, 48, 49), which arises from chronic imbalance of food intake and energy expenditure. As indicated by the implication of the IRE1α–XBP1 pathway in obesity-related metabolic disorders (50), molecular elucidation of the complex mechanisms linking the IRE1α signaling network during handling of an excessive load of nutrients to chronic inflammation and metabolic dysfunctions has been a very active and vigorous research field. Currently, it is increasingly recognized how important it is to dissect the metabolic roles of IRE1α as well as its mechanisms of action in the context of different cell types and various nutrient stress conditions. Therefore, we intend to summarize herein the recent progress with regard to the physiological roles of IRE1α in metabolic organs based on studies of various animal models, with a focus upon its tissue- or cell type-specific regulatory actions in energy balance and glucose/lipid metabolism. We will also discuss how dysregulation of IRE1α under overnutrition-elicited metabolic ER stress may mediate the pathological progression of obesity and related metabolic diseases, including type 2 diabetes, nonalcoholic fatty liver disease (NAFLD), and atherosclerosis.

IRE1α integrates nutrient, hormonal, and metabolic stress signals

Given the unique ER structure spreading throughout the cell to handle both the protein folding task and the intracellular flow of ions, nutrients, and metabolites, it is naturally not surprising that the ER undergoes dynamic remodeling in response to nutritional changes and metabolic challenges. As the key signal transducer in the regulatory machinery that governs proteostasis and ER’s functional integrity, IRE1α can respond not only to an increased load of protein folding within the ER lumen, but also to other intracellular signals derived from metabolite fluxes such as lipid composition alterations that lead to disruption of ER Ca2+ storage (51). It has been well-documented how the luminal domain of IRE1α senses unfolded/misfolded proteins during ER stress, either through directly binding to unfolded proteins or via its dissociation from the ER chaperone protein BiP/GRP78 (binding-immunoglobulin protein/glucose-regulated protein 78) (8). It remains largely unclear, however, whether there exist alternative modes of IRE1α-activating mechanisms that do not stem from within the ER lumen.

Animal model studies, particularly in the setting of obesity or other metabolic stress conditions, have led to an increasing recognition that IRE1α in metabolic organs can respond to changes in the body’s nutritional and metabolic states (Fig. 1). The activation of IRE1α signaling, which can be typically evaluated by measuring its phosphorylation state or Xbp1 mRNA splicing activity, has been frequently observed in multiple tissues and cell types from dietary and genetic obesity mouse models. This state of so-called “metabolic ER stress” is presumably provoked by a multitude of stimuli arising from both systemic and tissue microenvironmental changes in the face of energy surplus. From studies of metabolically-active organs or cell types in response to metabolic stress-inducing factors, emerging lines of evidence suggest that IRE1α can virtually integrate three major types of signals: nutrients, hormones, and immunological stimuli. Although an altered load of protein folding, i.e. increased accumulation of luminal unfolded/misfolded proteins, can be viewed as a major stressor for activating IRE1α signaling under most circumstances, additional factors are also very likely involved in triggering its activation, through either cytosolic signaling cross-talk or perturbations in the ER’s structural integrity, e.g. lipid composition changes that cause ER membrane remodeling (51).

Glucose-sensing of IRE1α

The nutrient-sensing property of IRE1α can be best-exemplified by its ability to respond to high-glucose stimulation in professional secretory pancreatic islet β-cells (42, 52, 53). Several groups have reported that high glucose is able to stimulate the phosphorylation of IRE1α at Ser724 within its kinase activation domain in cultured insulinoma β-cell lines as well as in primary pancreatic islets, resulting in elevated Xbp1 mRNA splicing (53) without inducing the activation of the PERK–eIF2α branch of the UPR (42). Although the molecular signaling mechanism by which glucose stimulates the selective activation of the IRE1α arm is largely obscure, this glucose-responsive phosphorylation of IRE1α, mostly likely reflecting its activation, is coupled to insulin biosynthesis (42, 52). Furthermore, research from our group revealed that glucose-stimulated IRE1α phosphorylation can be negatively regulated through glucose-induced IRE1α association with the adaptor protein receptor for activated C kinase 1 (RACK1), which in turn recruits protein phosphatase PP2A for its dephosphorylation (42). Interestingly, prolonged high-glucose exposure was found to cause dissociation of PP2A from RACK1 along with a higher degree of IRE1α phosphorylation, mimicking that under the chemically-induced ER stress state (42). This suggests that IRE1α’s glucose-sensing activation is dynamically controlled.

Lipid-sensing of IRE1α

Lipid species can also stimulate the activation of the IRE1α-signaling pathway in different types of cells such as β-cells, macrophages, and endothelial cells. Free fatty acid (FFA) has been documented as a typical ER stressor, which is thought to have a key role in lipotoxicity-induced β-cell death (54). In macrophages, it was demonstrated that saturated fatty acid
(SFA) can activate IRE1α in a manner that does not rely on its unfolded protein-sensing ability. This, in turn, promotes the activation of the NOD-like receptor family, pyrin domain-containing 3 (NLPR3) inflammasome in the “metabolically-activated” macrophages that drive high-fat diet (HFD)-induced tissue inflammation (55). Interestingly, the ability of SFAs to stimulate IRE1α activation is thought to be associated with its flux into phosphatidylcholine (PC), which may contribute to the perturbation of ER lipid composition. In addition, cholesterol trafficking to the ER membrane has been proposed to cause depletion of ER Ca2+ storage, activating the UPR, including the IRE1α-XBP1 pathway (56). However, it remains unknown whether IRE1α directly mediates cholesterol-induced cytotoxicity or exerts cytoprotective actions during ER stress-associated apoptosis of cholesterol-loaded macrophages, which has a key role in the progression of atherosclerosis (56). In addition, oxidized low-density lipoproteins, as well as the lipid oxidation products 7-ketocholesterol and 4-hydroxynonenal, were also shown to evoke the activation of the IRE1α–XBP1 pathway in human endothelial cells (57). How is IRE1α sensing these lipid molecules? Recent studies have shown that IRE1α can be activated by SFA through an unexpected unique mechanism without involving its luminal sensing of protein folding load (58). The transmembrane (TM) domain of IRE1α was found to contain intrinsic structural elements for detecting ER membrane lipid saturation, allowing the IRE1α’s TM domain to dimerize for its activation in response to membrane aberrancy (59–61). It will be interesting to interrogate further whether such lipid sensing of IRE1α occurs in localized regions/subdomains of the ER membrane undergoing lipid bilayer stress and through a similar mechanism as suggested for yeast Ire1 protein (62).

**IRE1α activation by metabolic hormones**

Investigations of how IRE1α is metabolically regulated, mostly carried out in the liver, have revealed that IRE1α can be activated by both anabolic and catabolic hormones. Due to the onset of insulin resistance in mice following HFD feeding, hyperinsulinemia was found to accompany elevations in phosphorylation at Ser724 of hepatic IRE1α and Xbp1 mRNA splicing (63). Furthermore, prolonged insulin exposure could activate the IRE1α–XBP1 pathway in nonobese diabetic livers as well as in primary hepatocytes, which was linked to up-regulation of the de novo lipogenic program through XBP1s-mediated control of steroid regulatory element-binding protein 1c (SREBP1c) (63). Notably, this could be suppressed by inhibition of the mammalian target of rapamycin–dependent protein synthesis. Acute activation of the IRE1α–XBP1 pathway was also documented in the liver of mice upon refeeding, presumably attributable, at least in part, to the increased postprandial blood insulin level (64). Insulin is an anabolic hormone known to stimulate both protein synthesis and lipogenesis in the liver, and insulin stimulation can lead to an increased load of protein folding within the ER lumen while altering the lipid composition of the ER membrane as well. Paradoxically, animal studies from our group uncovered that, upon short-term starvation, phosphorylation at Ser724 of IRE1α was stimulated without significant activation of its Xbp1 mRNA–splicing activity in the liver when compared with that under the ad libitum–fed state; furthermore, glucagon secreted from pancreatic islet α-cells during fasting was able to induce IRE1α phosphorylation in primary hepatocytes (65). More surprisingly, we found that glucagon or epinephrine signaling-activated PKA could directly phosphorylate IRE1α at Ser724 in a fashion independent of its autophosphorylation ability (65). This catabolic activation of IRE1α was implicated in promoting glucagon stimulation of the hepatic gluconeogenic program (65), but the precise mechanism by which IRE1α regulates hepatic glucose production remains unclear. It is tempting to speculate the possibility that hepatic IRE1α phosphorylation at Ser724 in response to the fasting/feeding cycle is coupled to the circadian control of its activation (66), and IRE1α is likely to sense the circadian input and participate in the rhythmic control of hepatic lipid metabolism (66) as well as gluconeogenesis (67).

**IRE1α activation by inflammatory stimuli**

Early studies have indicated that IRE1α is connected to JNK and IκBK–NF-κB inflammatory pathways through interacting with TNF-receptor–associated factor 2 (TRAF2) (41, 68). More recent immunometabolic research has pointed to the critical involvement of IRE1α in regulating or mediating immune responses that can profoundly influence metabolic homeostasis. In this context, it has been shown that IRE1α in primary macrophages could be selectively activated by Toll-like receptor agonists such as lipopolysaccharide (LPS), which did not appear to elicit typical ER stress response; and this, in turn, could drive M1-polarized activation of macrophages (also termed classically activated macrophages) and the inflammasome gene expression program (69–71). Moreover, our research on IRE1α signaling in adipose tissue macrophages (ATMs) in relation to obesity-associated metabolic inflammation showed that IL-4, a cytokine known to promote M2-polarized activation of macrophages (also termed alternatively-activated macrophages) (72, 73), was also able to induce IRE1α phosphorylation and activate its Xbp1 mRNA-splicing activity (70). Interestingly, two innate immune sensors, nucleotide-binding oligomerization domain 1 (NOD1) and NOD2, were found to be associated with the IRE1α–TRAF2 axis, and pharmacological inhibition analyses suggested that IRE1α might serve as a key component in sensing cytosolic danger signals to trigger the pro-inflammatory responses (e.g. IL-6 production) during tissue damage or microbial infection (74). These findings suggest a crucial role of IRE1α in coupling ER stress to inflammation, which may have particular relevance in obesity-associated metabolic inflammation.

Taken together, it is most likely that under metabolic stress, IRE1α may respond to a cluster of metabolic conditions, including hyperglycemia, hyperinsulinemia, hyperlipidemia, abnormal levels of blood glucagon, and SFAs, as well as various immune response stimuli (e.g. higher LPS levels as a result of increased intestinal permeability during diet-induced obesity) or pro-inflammatory cytokines. It is also worth noting that the IRE1α–XBP1 pathway can be activated in the liver of mice following long-term starvation or when fed a ketogenic (75) or high-fructose diet (76), as well as in cancer cells when mice are fed a low-protein isoaloric diet but not a low-carbohydrate
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**Regulatory actions of IRE1α in metabolic tissues**

Thus far, we have summarized recent results revealing IRE1α as a nutrient stress sensor that can be activated in response to changes in metabolic cues. Now, we will focus on its regulatory functions in metabolic homeostasis. As mentioned previously, a great number of animal model investigations, especially in the settings of obesity or other stress conditions, have demonstrated critical roles of IRE1α signaling in various metabolic tissues, including hypothalamus, pancreatic islets, liver, and adipose tissue (Fig. 2). In particular, the IRE1α–XBP1 pathway has been most extensively interrogated using loss-of-function and gain-of-function strategies (23, 50). These studies have revealed the tissue- or cell type-specific, and in most cases context-dependent, features of IRE1α regulation of metabolism, unveiling the complex network of IRE1α signaling that interconnects with metabolic and inflammatory pathways.

**IRE1α regulation of energy balance in hypothalamus**

The hypothalamus of the central nervous system serves as the headquarters of energy balance control via a complex neuroendocrine circuitry to regulate food intake and energy expenditure. Located in the arcuate nucleus of hypothalamus, orexigenic agouti-related peptide neurons, and anorexigenic pro-opiomelanocortin (POMC) neurons are the best-characterized leptin-responsive neurons with opposing regulatory functions, which act to integrate the nutritional states of the body and orchestrate the central control of energy balance (80). Documented studies in mouse models have shown that ER stress is implicated in hypothalamic inflammation-associated insulin and leptin resistance during diet-induced obesity (81, 82). Interestingly, Williams et al. (85) reported a mouse model with inducible expression of XBP1s specifically in POMC neurons, which exhibited protection against HFD-induced obesity with improved leptin and hepatic insulin sensitivity. XBP1s expression in POMC neurons also led to an up-regulated thermogenic program in adipose tissues, along with higher Xbp1 mRNA splicing in the liver that is thought to represent a cell-nonautonomous UPR signaling mechanism between the brain and liver (84). In addition, Yao et al. (85) reported another mouse model in which exons 16 and 17 encoding a portion of the kinase domain within the Xbp1s gene were deleted in POMC neurons, and in this model, POMC neuron-specific IRE1α deficiency resulted in marginal acceleration of HFD-induced obesity with considerable impairments in food intake and energy expenditure. Loss of IRE1α in POMC neurons was thought to aggravate ER stress-induced leptin and insulin resistance (85). In contrast, Xiao et al. (86) reported a different IRE1α-deficient mouse model in which exon 2 of the Xbp1s gene was specifically deleted in POMC neurons, which exhibited significant resistance to HFD-induced obesity and improvement of insulin resistance. It was also shown that POMC neuron-specific ablation of IRE1α led to increased energy expenditure and leptin sensitivity with higher production of α-melanocyte-stimulating hormone in the hypothalamus (86). This apparent discrepancy in the phenotypes of these two mouse models likely resulted from the difference in the regions of IRE1α protein that were targeted for genetic deletion. Whereas removal of exon 2 was shown to result in a complete abrogation of IRE1α protein expression (75), deletion of exons 16 and 17 could lead to generation of a mutant IRE1 protein with deletion within its kinase domain, namely a 115-kDa ΔIRE1α protein as recently reported in B-cells (87). Although it remains unclear whether such a mutant IRE1α protein could act in POMC neurons (e.g., through forming signaling clusters with its interacting partners) to affect leptin sensitivity, it is reasonable to speculate that the findings in these animal models largely reflect the multifaceted functions of IRE1α protein.

**IRE1α modulation of pancreatic islet cells**

Studies of several conditional IRE1α or XBP1 knockout mouse models have established that IRE1α can regulate the...
entire process of insulin production from pancreatic islet \( \beta \)-cells, ranging from insulin biosynthesis, processing, and secretion through both its \( Xbp1 \) mRNA splicing and RIDD activities. Despite that sustained production of XBP1s was shown to induce dysfunction and apoptosis in primary \( \beta \)-cells (88), genetic ablation of XBP1 in mice resulted in the loss of \( \beta \)-cells, decreased insulin content, and impaired insulin maturation, largely ascribable to hyperactivation of IRE1\( \alpha \) whose increased RIDD activity could act to degrade mRNAs encoding proinsulin-processing enzymes (89). Furthermore, in two mouse models with genetic deletion of IRE1\( \alpha \) in pancreatic \( \beta \)-cells, the IRE1\( \alpha \)--XBP1 pathway was shown to not only regulate oxidative proinsulin folding, insulin processing, glucose-stimulated secretion of insulin, and protection against oxidative stress (53, 90), but also to promote pancreatic islet growth and \( \beta \)-cell proliferation in the face of obesity and insulin resistance (91). Therefore, properly-balanced control of IRE1\( \alpha \)'s \( Xbp1 \) mRNA splicing versus its RIDD activity is essential for the maintenance of \( \beta \)-cell homeostasis and insulin-producing capacity. Notably, a mouse model with pancreatic islet \( \alpha \)-cell--specific \( Xbp1 \) knockout was reported to display glucose intolerance, with an inability to suppress glucagon secretion upon glucose stimulation (92). Similar to \( \beta \)-cells, loss of XBP1 also caused hyperactivation of IRE1\( \alpha \), leading to increased JNK activation and impaired insulin signaling in \( \alpha \)-cells. It remains unknown, however, whether IRE1\( \alpha \)'s RNase activity is directly involved in regulating glucagon secretion in \( \alpha \)-cells.

**IRE1\( \alpha \) regulation of liver glucose and lipid metabolism**

The liver is an essential metabolic organ in which various metabolic pathways in glucose and lipid metabolism are interconnected and converged. A series of animal model studies have demonstrated that IRE1\( \alpha \) serves as a key component of the ER's nutrient-sensing network (22), acting to regulate many aspects of hepatic metabolism. There exists an intricate network connecting the IRE1\( \alpha \)--XBP1 branch to metabolic and inflammatory pathways (21). Conceivably, the regulatory roles of IRE1\( \alpha \) signaling revealed by these investigations largely rely upon the feeding conditions and dietary compositions, unveiling the dynamic nature of IRE1\( \alpha \) signaling actions in response to different types of metabolic stress.

The IRE1\( \alpha \)--XBP1 pathway in the liver can be activated upon refeeding and after insulin stimulation (63, 64), indicating that it can mediate the anabolic actions of insulin. This notion is supported by XBP1s promotion of hepatic lipogenesis directly through XBP1s-dependent up-regulation of SREBP1c and fatty-acid synthase (63). Accordingly, studies of mice with liver-specific XBP1 knockouts also revealed its role in regulating the lipogenic program when fed high-carbohydrate diets (93). Moreover, following feeding of high-fructose chow, mice with liver-specific XBP1 deficiency showed reduced hepatic steatosis and improved hepatic insulin sensitivity in parallel with reduced hepatic diacylglycerol (DAG) content and protein kinase \( \varepsilon \) (PK\( \varepsilon \)) activity, despite increased ER stress and IRE1\( \alpha \) activation-associated JNK (76). Remarkably, the nuclear translocation of XBP1s protein has been shown to be facilitated by associating with the regulatory subunits of phosphoinositide 3-kinase, p85\( \alpha \), and p85\( \beta \), the critical components of the insulin-signaling network (94, 95). Moreover, this nuclear translocation process was reported to be enhanced by interacting with bromodomain-containing protein 7 (BRD7), which could be further promoted by insulin but was found to be suppressed in the state of obesity and insulin resistance (96).

Additional evidence further suggests that XBP1s may serve as an effector in contributing to the metabolic actions of insulin. XBP1s was demonstrated to interact with FoxO1, the transcription factor that is negatively controlled by insulin signaling, acting to direct its proteosomal degradation and suppress hepatic gluconeogenesis (97). Given that XBP1s protein was also known to interact with FoxO1 for its proteosomal degradation (98), it is of particular interest to unveil the molecular details regarding how endogenous XBP1s proteins can cooperate in the control of FoxO1 protein turnover during metabolic stress. Moreover, both the stress-activated protein kinase p38 mitogen-activated protein kinase (MAPK) and I\( \kappa \)B kinase \( \beta \) (IKK\( \beta \)) of the inflammatory pathway were shown to phosphorylate XBP1s (99, 100), thereby enhancing its nuclear translocation (99) or protein stability (100). Depending upon the genetic or HFD-induced obesity mouse models, nuclear XBP1s localization was arguably found to be impaired (50) along with abnormal hepatic p38 MAPK and I\( \kappa \)K\( \beta \) activity (99, 100), and restoration of nuclear XBP1s expression in the liver could ameliorate hyperglycemia. It remains enigmatic, however, whether phosphorylation of endogenous XBP1s by p38 MAPK or I\( \kappa \)K\( \beta \) in vivo can influence its selection of targeted gene sets for transcriptional activation or its ability and selectivity for interacting with other protein factors, such as the orphan nuclear receptor small heterodimer partner or the Cullin3-SPOP (speckle-type POZ protein) E3 ligase complex (101), under different metabolic stress conditions. Highlighting again the relevance of differing animal models and experimental contexts, adenovirus-mediated XBP1s expression in the liver exerted an unexpected anti-lipogenic effect in both HFD-induced and genetically-obese mice with insulin resistance, leading to suppressed lipogenesis and alleviated hepatic steatosis with lower PK\( \varepsilon \)c activity and enhanced macrolipolysis (102).

Research from our group has uncovered that hepatic IRE1\( \alpha \) can also be activated under catabolic states. We found in mice that glucagon-activated PKA kinase could stimulate IRE1\( \alpha \) phosphorylation at Ser\( ^{724} \) without activating its \( Xbp1 \) mRNA splicing in the liver following a short-term fast, and IRE1\( \alpha \) phosphorylation at Ser\( ^{724} \) was markedly elevated owing to higher hepatic PKA activation in mice with genetic obesity (65). Moreover, knockdown of hepatic IRE1\( \alpha \) expression reduced the gluconeogenic program and normalized glucose intolerance in the face of obesity-associated metabolic stress (65). However, we found that prolonged starvation or feeding of a ketogenic diet resulted in activation of the IRE1\( \alpha \)--XBP1 pathway in the liver (75), presumably arising from higher levels of metabolic stress signals (e.g. glucagon as well as FFAs). Further interrogations using hepatocyte-specific IRE1\( \alpha \) knockout mice revealed that IRE1\( \alpha \) could promote fatty acid oxidation and ketogenesis as well as lipid secretion in the liver, and it exerted these catabolic effects largely through XBP1s-dependent transcriptional up-regulation of peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)), the master starvation regulator (75). Interestingly, the IRE1\( \alpha \)–
XBP1 pathway was also documented to directly activate the transcriptional expression of starvation hormones fibroblast growth factor 21 (FGF21) (103) and growth differentiation factor 15 (GDF15) (104), which might also contribute to promoting hepatic fatty acid β-oxidation and ketogenesis, thereby counteracting ER stress-related liver steatosis.

In line with its role in hepatic lipid homeostasis, Wang et al. (105) employed hepatocyte-specific IRE1α knockout mice and showed that the IRE1α→XBP1 pathway could regulate the assembly and secretion of triglyceride (TG)-rich very-low-density lipoproteins (VLDLs); and loss of IRE1α in the liver exerted a hypolipidemic effect, which could be partially attributed to XBP1s up-regulation of protein-disulfide isomerase, a key enzyme in the control of VLDL assembly by affecting microsomal triglyceride transfer protein activity (105). Interestingly, So et al. (106) took advantage of the fact that XBP1 deficiency in mice led to a feedback hyperactivation of IRE1α and identified the RIDD activity of such highly-activated IRE1αs in down-regulating a number of mRNAs encoding proteins involved in lipid and lipoprotein biogenesis such as angiopoietin-like protein 3, which is also an XBP1s target gene. As anticipated, hepatic XBP1 ablation showed a plasma lipid-lowering effect in dyslipidemic ApoE-deficient mice (106). Further supporting its regulatory role in hepatic lipid secretion, the IRE1α→XBP1 pathway was recently found to orchestrate the transcriptional up-regulation of the COPII vesicle secretory program in another mouse model with hepatocyte-specific IRE1α abrogation (107), thus affecting ApoB-associated lipid secretion in response to nutritional changes. Putting these findings together, it can be postulated that bidirectional modulating strategies of targeting IRE1α RNase for inhibiting its Xbp1 mRNA splicing while boosting its RIDD activity would bring about beneficial effects upon lowering atherogenic blood lipids.

From these extensive studies, it can be appreciated that the IRE1α→XBP1 pathway actively participates in the control of a wide range of metabolic pathways under either anabolic or catabolic conditions in the liver. It is worth further dissecting the molecular modifications of IRE1α or XBP1s under these opposing metabolic states, which may govern its functional outputs. Nonetheless, the findings reported thus far have illustrated the highly-complex mechanisms by which hepatic IRE1α responds to metabolic cues and operates to regulate the metabolic switches and pathways in fuel metabolism. It should be reiterated that, most likely due to differences in genetic manipulations or experimental conditions, different phenotypic effects have been observed. For instance, XBP1 was shown to have a lipogenic role in hepatocyte-specific XBP1 knockout mice when fed a fructose chow diet (76), whereas an anti-lipogenic effect of adeno viral XBP1s expression was reported in obese mouse models with elevated plasma triglyceride levels observed (102). In the former model, reduced lipid secretion due to hyperactivation of IRE1α’s RIDD activity might also contribute to the decreased plasma TG levels, while the abolished XBP1s-dependent lipogenesis showed its prominent effect when fed a high-fructose diet (76); whereas in the latter, XBP1s promotion of VLDL secretion could be a predominant factor for alleviating hepatic steatosis under the reported experimental settings. For another example, mice with liver-specific knockouts of IRE1α (through deletion of exons 16 and 17 within the kinase domain that presumably could result in production of the 115-kDa ΔIRE1α protein (87)) showed an elevated degree of hepatic steatosis upon chemical ER stress or following HFD (45% fat) feeding (108, 109). However, we observed that when fed a HFD (60% fat), mice with hepatocyte ablation of IRE1α (through removal of exon 2 of the Ern1 gene) exhibited a slight alleviation of hepatic steatosis in the obesity-accelerated, diethylnitrosamine-induced hepatocellular carcinoma (HCC) model (110). These results point to potential previously unrecognized functional outputs of IRE1α that may be independent of its kinase or RNase activities.

**IRE1α regulation of adipose tissue function**

Adipose tissue plays a pivotal role in the control of whole-body energy balance and glucose metabolism, functioning not only as the energy storage depot but also as an active endocrine organ that secretes many adipokines such as leptin, adiponectin, and inflammatory cytokines (111, 112). Three types of adipocytes have been characterized, including unilocular, energy-storing white adipocytes, and energy-dissipating, mitochondrial uncoupling protein 1 (UCP1)-expressing brown adipocytes and beige/brite adipocytes with thermogenic capacity (113). Adipose tissue is known to regulate many physiological aspects of metabolic homeostasis, ranging from glucose uptake, lipid storage, to energy expenditure. Adipose tissue inflammation is a hallmark of obesity (114), and adipose-resident immune cells, particularly polarized activation of macrophages, are believed to exert profound effects upon adipocyte function and insulin sensitivity (71–73, 115). Increased ER stress signaling in white adipose tissue has been described in both animal models and human patients with obesity and diabetes (22, 48, 116), and emerging evidence from studies of XBP1 in adipocytes and IRE1α in ATMs suggests that IRE1α signaling is critically involved in the regulation of adipose tissue inflammation, metabolism, and remodeling.

The IRE1α→XBP1 pathway has been documented to be required for adipogenesis, with defective adipocyte differentiation observed in XBP1-deficient mouse embryonic fibroblasts and 3T3-L1 cells with knockdown of XBP1 or IRE1α (117). Because the adipogenic defect in XBP1-deficient cells could be rescued by restored XBP1s expression, IRE1α-mediated Xbp1 mRNA splicing was thought to be a crucial mechanism in adipogenic control (117). Interestingly, PKA-dependent activation of the IRE1α→XBP1 pathway was shown to up-regulate the expression of UCP1 in primary brown adipocytes (118), the essential mitochondrial protein that dissipates chemical energy as heat during thermogenic respiration. However, a mouse model study showed that deletion of adipocyte XBP1 had no effect on adipocyte formation or on systemic metabolism when fed a regular or high-fat diet (119). By contrast, inducible XBP1s overexpression in adipocytes resulted in reduced adiposity in genetic or diet-induced obese mouse models (120), and this anti-obesity effect of adipocyte XBP1s was thought to result from stimulated uridine biosynthesis in adipocytes, which has been linked to maintaining whole-body metabolic balance, including thermogenesis (121). Moreover, XBP1s overexpression in adipocytes was also reported in lean and obese mouse
models that exhibited improvement in insulin resistance and glucose intolerance (122), largely because of the enhancement of ER chaperone-mediated maturation of high-molecular-weight adiponectin, the multifunctional adipokine that exerts a broad range of beneficial effects upon metabolic health (123). These studies thus suggest that adipocyte IRE1α plays regulatory roles in adipose tissue metabolism through an XBP1-dependent mechanism under certain circumstances, although its XBP1-independent actions have yet to be explored.

In addition, given that macrophages are the primary effector cells in orchestrating tissue-immune and inflammatory responses (124), we studied metabolic ER stress and IRE1α’s function in ATMs in response to nutrient overload. Utilizing mice in which IRE1α was specifically ablated, by removal of exon 2 within the Ern1 gene, in myeloid cells, we found that IRE1α deficiency led to balanced M1/M2 polarization of ATMs, in parallel with marked reduction of adipose tissue inflammation, prominent enhancement of brown adipose tissue activation, and white adipose tissue browning (70). Mice with myeloid IRE1α deletions were completely protected against HFD-induced obesity, insulin resistance, hyperglycemia, dyslipidemia, and hepatic steatosis (70), indicating a powerful role for IRE1α in governing the activation states of ATMs to promote energy expenditure through boosting the thermogenic ability of brown and beige adipocytes. These findings suggest that macrophage IRE1α can sense nutrient overload and promote pro-inflammatory M1 polarization while suppressing M2 polarization, thus linking metabolic ER stress to adipose inflammation, energy imbalance, and metabolic disorders. However, the following remains to be further deciphered: the molecular nature of the signals derived from nutrient overload that IRE1α senses, and how IRE1α-deficient macrophages communicate with adipocytes to affect their metabolic properties (125).

**Mechanisms of IRE1α dysregulation linking ER stress to metabolic dysfunction**

Now that we have summarized the broad metabolic roles of IRE1α signaling in different metabolic tissues, we will discuss the potential mechanisms that may lead to its dysregulation during metabolic ER stress. Overnutrition, *i.e.* chronic energy surplus, is a major factor driving the pathogenic progression of obesity, which is highly associated with a chronic, subacute state of inflammation along with dramatic structural remodeling of the gut microbiome (126–128). Since the early reports that implicated ER stress in obesity, insulin resistance, and type 2 diabetes (129, 130), a great deal has been learned about the potential mechanisms by which the IRE1α signaling branch of the UPR links metabolic ER stress to obesity-associated tissue inflammation and metabolic disorders, including type 2 diabetes, NAFLD, and atherosclerosis. IRE1α is believed to be subjected to multiple layers of regulatory surveillance (50), and very complex mechanisms have been indicated to cause its dysregulation under various stress conditions. It can be hypothesized that under chronic metabolic stress, nutrient overload may provoke a number of intrinsic alterations to IRE1α, such as phosphorylation status and other post-translational modifications, which may underlie its conversion from an adaptive activation state in physiological settings to a maladaptive, aberrant activation state under pathological conditions (Fig. 3). Such conversion is supposed to profoundly affect IRE1α’s functional effector outputs, leading to abnormal enzyme activities (*e.g.* Xbp1 mRNA splicing versus RIDD), substrate specificities, or signaling efficiencies. Mechanistically, IRE1α dysregulation may stem from altered sensing of metabolic stress signals, disrupted actions of its regulatory partners, and/or aberrant assembly of the multiprotein signaling platform.

**Aberrant IRE1α phosphorylation**

Hyperactivation of IRE1α has been extensively observed and documented in the state of obesity based upon its increased phosphorylation level, mostly as determined by phosphorylation at Ser724 within the activation loop of the kinase domain, usually in parallel with elevated Xbp1 mRNA splicing. As a typical ER stress sensor, activation of IRE1α involves its dimerization/oligomerization and auto-phosphorylation at multiple sites (35, 131), but it is unclear whether IRE1α contains preferentially phosphorylated residues upon activation in response to different stress signals. Surprisingly, we found that hepatic IRE1α could be phosphorylated by glucagon-activated PKA kinase at Ser724, and it had much fewer phosphorylation sites in the livers of genetically-obese mice when compared with that induced by experimental ER stress or upon its overexpression (65). It has yet to be determined whether this PKA-dependent metabolic activation of IRE1α occurs in other metabolic tissues, or how it may affect IRE1α’s autophosphorylation profile and regulate its downstream effector actions in metabolic control. Furthermore, IRE1α phosphorylation at particular residues may occur only selectively in response to certain types of stimuli, as exemplified by the bacterial subtilase cytotoxin-induced phosphorylation at Ser725 of IRE1α, which is also located within the activation loop of the kinase domain, in B-cells (87).
fore, it requires more in-depth investigations to dissect whether and how the extent of IRE1α phosphorylation at critical sites within different functional domains can govern its activation state and dictate its RNase enzyme activity or specificity, e.g. its Xbp1 mRNA splicing versus RIDD actions. During the conversion from an adaptive to a maladaptive activation state, abnormal IRE1α phosphorylation states may ensue as a result of disruption of mechanisms that control its phosphorylation. For example, it is currently unclear whether disruption of the RACK1–PP2A feedback regulatory loop identified in pancreatic β-cells (42) occurs in the liver during obesity-induced metabolic ER stress, thus causing IRE1α hyperphosphorylation at Ser724 and subsequently the dysregulated gluconeogenic program and hyperglycemia (65). Interestingly, it was shown that the RACK1–PP2A regulatory loop was suppressed by TRAF6 association with IRE1α, resulting in LPS-induced hyperactivation of IRE1α RNase activity and enhancement of the inflammatory gene expression program in macrophages (44). It is worth further dissecting whether this mechanism is also involved in promoting M1 polarization of ATMs during HFD-induced adipose inflammation (70).

**Post-translational IRE1α modifications**

With respect to the possible alterations in IRE1α protein that may disturb its functional outputs, many other types of post-translational modifications, in addition to phosphorylation, of IRE1α are also likely to play a role. Remarkably, Yang et al. (132) reported that in the face of obesity, inflammation-associated increase of inducible nitric-oxide synthase (iNOS) activity resulted in S-nitrosylation of hepatic IRE1α within its RNase domain, leading to a progressive decline in its Xbp1 mRNA splicing activity in both genetic ob/ob and HFD-induced obesity mouse models. Moreover, restored expression of a nitrosylation-resistant version of IRE1α in obese mice with liver-specific IRE1α deficiency normalized Xbp1 mRNA splicing and improved glucose homeostasis. This suggests that inflammation-induced S-nitrosylation impairs IRE1α activity, which may represent one molecular type of obesity-associated IRE1α dysregulation (132). Interestingly, this iNOS-directed dysregulation of the IRE1α–XBP1 pathway was found to be a crucial mechanism in cardiomyocyte dysfunction in a mouse model of heart failure with preserved ejection fraction (133). Moreover, such S-nitrosylation was also shown to impair hepatic IRE1α’s RIDD activity, resulting in derepression of miR-200 and miR-34, two miRNAs that target the 3’UTRs of PPARo and SIRT1 mRNAs to attenuate their translation (109). This in turn was thought to promote hepatic steatosis in mice following HFD feeding (109). In addition, it has to be pointed out that IRE1α protein can be subjected to additional types of modification that may affect its functional outputs. For instance, in response to oxidative stress, cytoplasmic reactive oxygen species (ROS) was shown to induce sulfenylation at a conserved cysteine within the kinase activation loop of IRE1α, inhibiting its phosphorylation and Xbp1 mRNA–splicing activity while initiating an antioxidant response (134). Given the important role of mitochondria in ROS generation and cellular metabolism, it is of particular interest to elucidate whether such sulfenylation can link mitochondrial dysfunction to metabolic ER stress in obesity and metabolic diseases. Moreover, although the Sel1L–Hrd1 ERAD complex was shown to regulate IRE1α protein stability (135), several E3 ubiquitin ligases have also been documented to interact with and catalyze IRE1α ubiquitination, including synoviolin (136), TRAF6 (44), C terminus of HSC70-interacting protein (CHIP) (137), and mitochondrial ubiquitin ligase (MITOL/MARCH5) (138). Although synoviolin-mediated ubiquitination was shown to promote its degradation, TRAF6- and CHIP-dependent ubiquitination of IRE1α was found to affect, respectively, its phosphorylation by interfering with the RACK1–PP2A regulatory loop or its activation of the TRAF2–JNK pathway. Intriguingly, MITOL-directed Lys-63–linked chain ubiquitination of IRE1α at lysine 481 was reported to occur at the mitochondria-associated ER membrane (MAM), preventing IRE1α hyper-oligomerization and reducing its RIDD activity. Therefore, it warrants more extensive, in-depth investigations in order to pinpoint the distinct types of modification of IRE1α in the context of how they may bring about maladaptive effector functions of the IRE1α signaling platform in metabolic control.

**Alterations in regulatory proteins**

Given the increasing number of proteins that have been identified as IRE1α interactors (38), it is conceivable that IRE1α most likely operates within a multiprotein complex machinery containing both regulatory partners and signaling effectors. Many regulatory proteins have been recently reported to either suppress or enhance IRE1α signaling, which include Bax inhibitor-1 (Bcl-I), an evolutionarily conserved ER-resident protein that interacts through its C-terminal cytosolic domain with IRE1α and negatively controls IRE1α’s RNase activity (140–142); ERdj4/DNAJB9, an ER luminal co-chaperone acting as a selective repressor of IRE1α via promoting a complex between BiP and IRE1α (143); heat-shock protein 72 (Hsp72), a stress-inducible cytosolic molecular chaperone that forms a stable complex with the cytosolic domain of IRE1α to enhance its activation (144); protein kinase C substrate 80K-H (PKCSh/heatocystin), an ER luminal noncatalytic subunit of β-subunit of glucosidase II that interacts with and boosts the autophosphorylation and oligomerization of IRE1α upon ER stress (145); and Hsp47, an evolutionarily conserved ER luminal chaperone that directly binds to IRE1α with high affinity and displaces its negative regulator BiP to facilitate IRE1α oligomerization (146). Although the interplays between these potential IRE1α regulators under metabolic stress conditions remain to be determined, it will be intriguing to decipher how changes in their regulatory modes may contribute to the dysregulation of IRE1α during chronic metabolic ER stress, consequently affecting the homeostatic control of metabolism. Indeed, hepatic Bi-1 protein has been found to be down-regulated in the state of obesity (147), along with hyperactivation of the IRE1α–XBP1 pathway (103). Adenoviral restoration of Bi-1 protein expression suppressed hepatic IRE1α activation to decrease XBP1s protein production and gluconeogenesis, protecting mice from obesity-associated insulin resistance and glucose intolerance but resulting in higher hepatic steatosis with hypolipidemia (147). This again reflects the multifaceted effector functions of IRE1α signaling that could lead to bidirectional outputs, i.e. reduced
hepatic lipid secretion due to deficient XBP1s production and improved insulin sensitivity and suppressed gluconeogenic programming due to inhibition of the activation degree of IRE1α. Supporting this notion, genetic ablation of BI-1 in mice was found to markedly aggravate ER stress–induced or chronic HFD feeding–induced nonalcoholic steatohepatitis (NASH), which was accompanied by unrestrained hepatic IRE1α signaling as indicated by higher XBP1s protein level and NLRP3 inflammasome activation (148). Curiously, pharmacological inhibition showed that the metabolic effects of this BI-1 deficiency-evoked dysregulation of IRE1α could be largely attributable to its elevated RNAse activity (148), which in a certain extent contradicted the findings reported for the liver-specific IRE1α knockout mouse model (109). For yet another example, despite that metabolic ER stress may not arise solely from protein folding overload, alterations in ER luminal chaperone proteins may affect the capacity of IRE1α for sensing or detecting abnormal protein folding, and this can also contribute to IRE1α dysregulation. In this regard, adenoviral expression of the ER chaperone Bip/glucose-regulated protein 78 (GRP78) in the livers of obese ob/ob mice reduced liver XBP1s protein and inhibited SREBP-1c production, leading to prominent alleviation of hepatic steatosis (149). Thus, these findings suggest the important roles of the regulatory molecules in provoking IRE1α dysregulation during chronic ER stress, but the predominant downstream effectors linking aberrant IRE1α signaling to its metabolic disrupting actions remain to be further defined.

**Maladaptive outputs of IRE1α effector actions**

As a multiprotein signaling platform, it has yet to be clearly illustrated how maladaptive activation of IRE1α through differing states of phosphorylation and/or modification affects its downstream effector outputs, whether by altering its kinase/RNAse activities or disrupting its signaling cascades. Most of IRE1α’s cellular functions are believed to be mainly performed through activation of its RNAse activities. Whereas alterations in both Xbp1 mRNA splicing and RIDD activities of IRE1α have been frequently reported in animal models with obesity and metabolic dysfunctions, it remains obscure whether IRE1α can be selectively activated to dictate its RNAse output in terms of Xbp1 mRNA splicing versus RIDD of select mRNA substrates.

In the state of obesity and metabolic ER stress, hyperactivation of IRE1α leads to elevated Xbp1 mRNA splicing, and XBP1s protein produced from this unconventional splicing may exert a broad range of metabolic effects in different metabolic tissues as mentioned previously. In most cases, XBP1s can be viewed as an adaptive effector in the homeostatic control of metabolism, and its defective nuclear translocation or stability has been linked to disruption of glucose metabolism (23, 50, 97, 99, 100). However, maladaptive consequences of higher Xbp1 mRNA splicing were also reported. In hepatocytes during palmitate-induced lipotoxic ER stress, XBP1s could promote the release of pro-inflammatory extracellular vesicles for recruiting macrophages to the liver (150), which is considered to be a potential NASH–promoting factor (151). In obese mouse models, elevated plasma LPS concentration due to increased intestinal permeability is thought to promote insulin resistance, and LPS-activated production of XBP1s was shown to induce the acetyltransferase P300, which in turn could acetylate insulin receptor substrate 1/2 (IR51/2) to diminish insulin signaling (152). Therefore, these findings suggest the context-dependent metabolic impacts of IRE1α’s Xbp1 mRNA splicing in response to different stress signals.

It has been increasingly recognized that hyperactivation of IRE1α’s RIDD activity can also mediate its maladaptive, pathological effects during obesity-induced metabolic stress. For instance, overactivation of IRE1α in ATMs was found to down-regulate Irf4 and Klf4 mRNA levels through its RIDD activity, thereby suppressing M2-polarized macrophage activation and connecting metabolic ER stress to adipose inflammation and insulin resistance during HFD-induced obesity (70). In pancreatic β-cells, severe experimental ER stress or IRE1α overexpression was shown to cause RIDD of insulin mRNA as well as β-cell apoptosis (89, 153, 154), although it is unclear whether stress-induced disruption of the RACK1–PP2A control of IRE1α activity can contribute to the hyperactivation of its RIDD activity under chronic metabolic ER stress. Interestingly, hyperactivated IRE1α was also reported to increase the stability of mRNA encoding thioredoxin-interacting protein (TXNIP), likely by its RIDD control of a TXNIP destabilizing microRNA, miR17, which in turn couples ER stress to activation of the NLRP3 inflammasome and pancreatic β-cell death (155, 156). Notably, mammalian IRE1α may also employ distinct catalytic mechanisms for Xbp1 mRNA splicing and RIDD as found in yeast (157), and opposing Xbp1 mRNA splicing and RIDD outputs have been documented in glioblastoma aggressiveness (158). In this sense, it requires more exquisite dissections to determine which of the RNAse outputs may play predominant roles in metabolic dysfunctions in a given tissue or cell type under a certain metabolic stress condition with clinical relevance.

Aberrant IRE1α signaling represents another maladaptive mechanism stemming from chronic activation of IRE1α during metabolic stress. Mice with liver XBP1 haploinsufficiency were shown to exhibit insulin resistance with increased JNK activation, which might be largely attributable to IRE1α hyperactivation in the context of XBP1 deficiency (129). Similarly, hepatocyte-specific Xbp1-deficient mice, when chronically fed a high-fat/sugar diet, developed enhanced liver injury and fibrosis but less steatosis in parallel with higher JNK activation and inflammatory signatures (159), which might also result from the IRE1α–TRAF2 signaling axis. Moreover, we found that in the face of HFD-induced obesity, hepatic IRE1α could promote activation of the IKKβ–NF-κB cascade, likely through its interaction with TRAF2, thereby enhancing, in a feed-forward fashion, the sustained activation of the IRE1α–STAT3 pathway during obesity acceleration of HCC progression (110). It can be anticipated that more signaling molecules from the IRE1α platform will be uncovered in mediating metabolic ER stress–related derangement of metabolic control.

All these studies highlight a critical role for IRE1α in linking metabolic ER stress to obesity and metabolic diseases, and it is essential to further decipher the molecular and cellular features of maladaptive dysregulation of IRE1α in different tissues or cell types in response to a diversity of factors from the tissue microenvironment at differing stages during metabolic disease
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progression (160). In addition to its phosphorylation, modification, enzyme activity, and downstream signaling effector, it should also be borne in mind that most likely IRE1α does not operate alone at the ER membrane in vivo as a homodimeric or homooligomeric machinery, as indicated by its in vitro structures (161), to perform its regulatory functions or exert its deteriorating effects upon metabolic homeostasis. Given IRE1α’s clustering property in cells under ER stress or when overexpressed, it has yet to be pinpointed how the maladaptive dysregulation of IRE1α involves its interacting protein partners. In this regard, it is worth noting that cystolic ABL kinases were recently documented to localize to the ER membrane and promote the clustering and hyperactivation of IRE1α, thereby driving ER stress-associated pancreatic β-cell death (162). Moreover, imatinib, the anti-cancer tyrosine kinase inhibitor that could disrupt the ABL–IRE1α interaction, was shown to blunt IRE1α hyperactivity and reduce β-cell apoptosis, resulting in the reversal of autoimmune diabetes in the mouse model (162). This underscores the translational importance of elucidating the mechanistic details of IRE1α dysregulation that may underpin the pathogenesis of metabolic disorders.

Concluding remarks

Over the last decade, remarkable progress has been made toward our molecular and physiological understanding of IRE1α’s metabolic actions as well as its mechanistic connections to obesity-associated metabolic inflammation and metabolic disorders. However, it is only proper to conclude that what we have learned thus far can be largely regarded as the “tip of the iceberg” owing to the complex, multifaceted mechanisms by which IRE1α operates as a multitasked protein machinery in adaptively coping with metabolic stress, and how IRE1α regulation may go awry during the pathological progression of metabolic disorders. A plethora of outstanding and fascinating questions remain to be carefully addressed before we can translate our discoveries into developing IRE1α-targeting therapeutics to combat the devastating epidemic of metabolic diseases. For instance, functioning as a bona fide ER-localized “signalome,” what are the molecular components and cellular signatures of IRE1α machinery during the sensing of different metabolic stress signals? How is this multiprotein machinery temporally and spatially regulated in cells, or does it perform context-dependent functions in compartmentalized locations, i.e. at particular ER subdomains making contact sites with other organelles? Within the assembly of the IRE1α-signaling platform, are the interacting protein partners serving as regulatory auxiliary factors that are subjected to metabolic surveillance control, and how do they govern IRE1α’s distinct modes of action or effector outputs? As recently reported, IRE1α might serve as a scaffold to regulate the distribution of inositol 1,4,5-triphosphate receptors at MAMs, thus facilitating the Ca2+ transfer from the ER to mitochondria regardless of the ER stress state (163). It is likely that IRE1α localization at certain ER subdomains or membrane contact sites can respond to local metabolic changes or stress signals from within other organelles in the cell. Investigations aiming to answer these questions with respect to the subcellular context-dependent functions of IRE1α will provide new knowledge on the molecular determinants of IRE1α dysregulation that can be rather dynamic.

Among all the potential mechanisms that may give rise to maladaptive outputs from dysregulated IRE1α signaling, it is imperative to pinpoint the precise and distinct features in the control of IRE1α’s dynamic clustering, RNase activity and substrate selectivity, and signaling effectors. This will allow us to gain more insight into how IRE1α dysregulation in metabolic tissues underlies its crippling actions on metabolic control. Toward this end, it is particularly worth exploring the key factors that drive the conversion of IRE1α from an adaptive to maladaptive activation state, leading to its aberrant effector outputs. Thus far, we have witnessed encouraging progress with therapeutic translation based on molecular dissection of the connection between macrophage IRE1α hyperactivation and metabolic inflammation in obesity, insulin resistance, and atherosclerosis. In this scenario, IRE1α represents a highly-attractive therapeutic target among the three UPR pathways (164). For instance, given that IRE1α was found to be highly activated in lipid-laden macrophages infiltrating the atherosclerotic lesions during metabolic ER stress (165), small molecule inhibitors of IRE1α RNase have shown promising efficacy in counteracting the progression of atherosclerosis in animal models (166). Employing novel single-cell methodologies as in the case for unraveling the pathogenesis of NASH (139), future research will lead to more thorough understanding of the complex cell type- and context-dependent mechanisms by which the IRE1α machinery operates in metabolic control, as well as the pathomechanisms by which its dysregulation promotes the pathological progression of metabolic disorders. This will pave the way for selective targeting with precision of IRE1α’s distinct maladaptive outputs, offering new avenues for development of therapeutic agents against obesity and metabolic diseases.

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