Phenformin Activates the Unfolded Protein Response in an AMP-activated Protein Kinase (AMPK)-dependent Manner*

Received for publication, February 19, 2013, and in revised form, March 30, 2013 Published, JBC Papers in Press, April 2, 2013, DOI 10.1074/jbc.M113.462762

Liu Yan1, Haibo Sha5, Robin L. Davisson9, and Ling Qi6,5,2

From the 1Graduate Program in Biochemistry, Molecular and Cell Biology, 5Division of Nutritional Sciences, and 6Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Background: The cross-talk between UPR activation and metabolic stress remains largely unclear.

Results: Phenformin treatment activates the IRE1α and PERK pathways in an AMPK-dependent manner.

Conclusion: AMPK is required for phenformin-mediated IRE1α and PERK activation.

Significance: Our findings demonstrate the cross-talk between UPR and metabolic signals.

Activation of the unfolded protein response (UPR) is associated with the disruption of endoplasmic reticulum (ER) homeostasis and has been implicated in the pathogenesis of many human metabolic diseases, including obesity and type 2 diabetes. However, the nature of the signals activating UPR under these conditions remains largely unknown. Using a method that we recently optimized to directly measure UPR sensor activation, we screened the effect of various metabolic drugs on UPR activation and show that the anti-diabetic drug phenformin activates UPR sensors IRE1α and pancreatic endoplasmic reticulum kinase (PERK) in both an ER-dependent and ER-independent manner. Mechanistically, AMP-activated protein kinase (AMPK) activation is required but not sufficient to initiate phenformin-mediated IRE1α and PERK activation, suggesting the involvement of additional factor(s). Interestingly, activation of the IRE1α (but not PERK) pathway is partially responsible for the cytotoxic effect of phenformin. Together, our data show the existence of a non-canonical UPR whose activation requires the cytosolic kinase AMPK, adding another layer of complexity to UPR activation upon metabolic stress.

Alteration of endoplasmic reticulum (ER)3 homeostasis initiates signaling pathways, which allow communication between the ER and other cellular organelles, collectively termed the unfolded protein response (UPR). Activation of UPR has been associated, causally in some instances, with various physiological and pathological conditions such as cancer, obesity, type 2 diabetes, and cardiovascular diseases (1, 2). The expansion of ER folding capacity and the reduction of nascent peptide load by UPR assist the recovery and reset of ER homeostasis. However, UPR can act as a double-edged sword, as prolonged UPR activation may induce apoptosis (3).

Upon accumulation of misfolded proteins in the ER lumen, canonical UPR in mammals is initiated by activation of three major ER transmembrane sensors: IRE1α, pancreatic endoplasmic reticulum kinase (PERK), and ATF6 (3). Under ER-stressed conditions, the binding of misfolded proteins and/or the dissociation of chaperone GRP78 results in IRE1α dimerization, trans-autophosphorylation, oligomerization with the help of the actin cytoskeleton (4), and activation of its RNase activity (5, 6). Activated IRE1α splices 26 nucleotides from the Xbp1 mRNA to generate the Xbp1s (spliced) transcript, which encodes the transcription factor XBP1s, responsible for the induction of various ER chaperones (7, 8). The activation mechanism for PERK may be similar to that for IRE1α because their luminal domains are interchangeable (9). However, unlike IRE1α, PERK has only a kinase domain, which trans-autophosphorylates itself and phosphorylates eIF2α upon activation (10). Phosphorylation of eIF2α attenuates global translation while paradoxically increasing the expression of ATF4 and CHOP (c/EBP homology protein) (11). Unlike IRE1α and PERK, ATF6 translocates to the Golgi and undergoes proteolysis to release its N-terminal domain, which encodes an active transcription factor. N-terminal ATF6 may be responsible for the up-regulation of genes involved in protein folding, trafficking, and degradation (12).

Many of the mechanistic insights into UPR activation were garnered from the use of pharmacological drugs such as DTT, which blocks disulfide bond formation (13); thapsigargin (Tg); which inhibits the ER calcium pump (14); and tunicamycin (Tm), which impairs ER protein glycosylation (15). Physiologically, UPR has been detected in various metabolic state changes such as in obese liver and adipose tissues (16) and in refeeding pancreas (17). Interestingly, glucagon-induced IRE1α activation is dependent on PKA, supporting the possibility of direct regulation of UPR by cytosolic metabolic pathways (18). However, how metabolic stress activates UPR is poorly understood.

To address whether and how metabolic signals cross-talk with UPR pathways and facilitate UPR activation, we took advantage of a recently developed Phos-tag-based method. This method allows for visual assessment and quantitative
detection of UPR activation at the sensor level (17, 19, 20). We applied several metabolic drug treatments that mimic physiological energy deprivation, and we tested their effects on UPR activation. To our surprise, among all of the tested drugs, only the hypoglycemic agent phenformin potently activated the IRE1α and PERK pathways. In addition, overexpression of the chaperone GRP78 attenuated phenformin-mediated UPR activation, whereas translation inhibition had no effect. Mechanistically, phenformin-induced UPR required the kinase activities of both UPR sensors as well as AMP-activated protein kinase (AMPK), but not LKB1 (liver kinase B1). Interestingly, the AMPK agonist 5-amino-1-β-d-ribofuranosylimidazole-4-carboxamide (AICAR) was not sufficient to activate UPR, suggesting the involvement of additional factor(s) in phenformin-mediated UPR activation. Thus, our study implicates a requirement for AMPK in phenformin-mediated UPR activation and provides a framework for further studies to understand the cross-talk between metabolic signals and UPR activation.

EXPERIMENTAL PROCEDURES

Cell Lines—HepG2, 266-6, 3T3-L1, mouse embryonic fibroblast (MEF), and Phoenix cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. PERK null (PERK−/−) and WT control MEFs were gifts from Dr. Douglas Cavender (Pennsylvania State University). AMPKα1Δα2−/−, AMPKα1+/+, LKB1−/−, and LKB1+/− MEFs (gifts from Dr. Reuben Shaw, Salk Institute) were described previously (21). IRE1α−/− MEFs stably expressing WT and mutant IRE1α were generated as described previously (22). XBP1 shRNA- or luciferase shRNA (control shRNA)-expressing 3T3-L1 cells were described previously (20).

Reagents—Tm and Tg (Calbiochem) were dissolved in Me2SO and used at 5 μg/ml and ~75−300 μM, respectively. AICAR (Toronto Research Center, Toronto, Ontario, Canada) was used at 2 mM. Phenformin, 2-deoxy-d-glucose, rotenone, and rapamycin (Sigma-Aldrich) were used at 50 μM, 2 mM, 1 μM, and 80 μM, respectively. Cycloheximide (CHX) dissolved in ethanol (Calbiochem) was used at 50 μg/ml. The selection drugs puromycin and G418 were purchased from Sigma and Calbiochem, respectively. Phos-tag was purchased from the NARD Institute, Ltd. (Amagasaki, Japan).

Drug Treatment—106 cells were plated in 6-well plates in culture medium and incubated overnight. Before experiments, the medium was changed to serum-free DMEM supplemented with 0.05% BSA and 1% penicillin/streptomycin. 0.5 h later, drugs were added for the indicated times. For CHX treatment, cells were pretreated with 50 μg/ml CHX for 30 min and throughout the drug treatment. At the end of experiments, cells were snap-frozen in liquid nitrogen before analysis.

Plasmids—Mouse WT and K618A PERK cDNAs (gifts from Dr. David Ron, University of Cambridge) (10) were subcloned into the pBabe-puro vector. Human WT IRE1α cDNA in the pMSCV retroviral vector was provided by Dr. Claudio Hetz (University of Chile) (23). pMSCV-K599A IRE1α was generated by mutagenesis (22).

Stable Cell Line Generation—PERK−/− MEFs stably expressing pBabe-WT PERK and pBabe-mutant PERK and 3T3-L1 fibroblasts stably expressing PERK shRNA and its control were generated using retroviral transduction as described previously (20). pSUPER-neo vectors (provided by Dr. Lee Kraus, University of Texas Southwestern Medical Center) encoding control shRNA (gatagggctgaatacaca) or PERK shRNA (aggggaagctgaggata) were used for retroviral transduction and stable cell line generation.

Adenoviral Infection—HepG2 cells in 12-well plates were infected with adenoviruses (5 μl of 1012 particles/ml encoding LacZ or GRP78 (24). After overnight incubation, cells were incubated in DMEM with 0.05% BSA and 1% penicillin/streptomycin for 0.5 h and then mock-treated or treated with Tg, Tm, or phenformin.

Western Blotting—Cell nuclear fractionation, whole cell lysate preparation, and Western blot analysis were performed as described (20). Phosphatase treatment of whole cell lysates and Phos-tag gel was performed as described (17, 19). Quantification of Western blots was done using the ChemiDoc XRS+ system with ImageLab software (Bio-Rad) or ImageJ.

Antibodies—Goat anti-GRP78 (1:1000), rabbit anti-ATF4 (1:1500), mouse anti-CHOP10 (1:500), rabbit anti-XBP1 (1:1000), and rabbit anti-HSP90 (1:5000) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-AMPK (phospho-Thr-172; 1:1000), rabbit anti-acetyl-CoA carboxylase (phospho-Ser-79; 1:1000), rabbit anti-elf2α (phospho-Ser-51; 1:1000), rabbit anti-IRE1α (1:1000), rabbit anti-PERK (phospho-Thr-980; 1:1000), rabbit anti-S6 (phospho-Ser-240/Ser-244; 1:1000), rabbit anti-AMPK, rabbit anti-acetyl-CoA carboxylase, rabbit anti-elf2α, rabbit anti-PERK, and mouse anti-S6 antibodies were purchased from Cell Signaling (Beverly, MA). Rabbit anti-cAMP-responsive element-binding protein (CREB) antibody (1:6000) was from Dr. Marc Montminy (Salk Institute). The secondary antibodies used were HRP-conjugated goat anti-rabbit and anti-mouse IgG and HRP-conjugated donkey anti-goat IgG (1:10,000; Bio-Rad).

Quantitative PCR and RT-PCR—Total mRNA extractions were carried out using a TRIzol-based protocol (Molecular Research Center, Inc.) and reverse-transcribed using the Super-Script III kit (Invitrogen). Quantitative PCR was performed with the primers described previously (17). RT-PCR analysis of spliced and unspliced Xbp1 was performed as described (20). RT-PCR products were separated by electrophoresis on a 2% agarose gel (Invitrogen) containing ethidium bromide and visualized using the ChemiDoc XRS+ system.

Survival Assay—2−5 × 104 3T3-L1 fibroblasts stably expressing control, XBP1, or PERK shRNA were plated in each well of a 24-well plate; incubated overnight; and treated with 5 mM phenformin for the indicated times. Cells were snap-frozen in liquid nitrogen and thawed at room temperature (25°C). DNA content as an indication of relative cell numbers was measured using the CyQUANT kit (Invitrogen) following the supplier’s protocol.

Statistical Analysis—All experiments were repeated at least twice. Results are expressed as the mean ± S.E. Comparisons between groups were made using Student’s unpaired two-tailed
AMPK-dependent UPR Activation

RESULTS

Phenformin Induces IRE1α and PERK Phosphorylation—To study the link between metabolic pathways and UPR, we tested the effect of various metabolic drugs on UPR sensor activation in the hepatoma cell line HepG2. Several drugs with the capacity to affect cellular metabolic state were selected, including phenformin (25) and rotenone (26), inhibitors of mitochondrial electron transport; a non-metabolizable glucose analog that blocks glycolysis, 2-deoxy-D-glucose (27); and an inhibitor of mitochondria, mitochondrial electron transport, as rotenone, another mitochondrial respiration inhibitor, had the opposite effect on IRE1α phosphorylation (Fig. 1, C and D).

To further characterize the nature of phenformin-mediated activation of IRE1α and PERK, we performed the following two experiments to attenuate ER stress. First, cells were pretreated with the protein translation inhibitor CHX. As expected, inhibition of protein translation greatly reduced both basal and Tg-induced IRE1α and PERK phosphorylation (Fig. 1, C and D). By contrast, phenformin-mediated IRE1α and PERK activation remained unaffected by CHX (Fig. 1, C and D). Second, we infected HepG2 cells with an adenovirus encoding the ER chaperone GRP78, which is known to increase ER folding capacity and thereby alleviate UPR activation (24, 29). Overexpression of GRP78 significantly attenuated phenformin-mediated IRE1α and PERK phosphorylation compared with the controls (Fig. 1, E and F). The effect of GRP78 on Tg-induced ER stress in HepG2 cells was modest, likely due to the expression level of GRP78, as more a dramatic effect of GRP78 was observed in HEK293T cells, where viral infection was more efficient (Fig. 1G). Thus, we conclude that the accumulation of unfolded/misfolded proteins is responsible, at least in part, for phenformin-mediated UPR activation.

As expected, most metabolic drugs affected cellular metabolic state within the 2-h treatment as reflected by the decreased phosphorylation of ribosomal protein S6 (Fig. 1B). However, only phenformin, a derivative of biguanides, significantly elevated IRE1α and PERK phosphorylation to a level comparable to that of classical ER stressors such as Tg and Tm (Fig. 1, C and D, and data not shown). The effect of phenformin on UPR sensors was not due merely to the inhibition of mitochondrial electron transport, as rotenone, another mitochondrial respiration inhibitor, had the opposite effect on IRE1α phosphorylation (Fig. 1, C and D).

Phenformin treatment activates UPR sensors IRE1α and PERK. A, Phos-tag (PT) analysis of IRE1α and Western blot analysis of PERK in HepG2 cells treated with 75 nM Tg for 2 h. Whole cell lysates were treated with or without λ-phosphatase (PP). HSP90 was used as a loading control. B, Western blot analysis of phospho-S6 in HepG2 cells treated with Tm (5 μg/ml), phenformin (Ph; 5 mM), 2-deoxy-D-glucose (2DG; 2 mM), rotenone (Rot; 1 μM), or rapamycin (Rap; 80 μM) for 2 h. C, Western blot analysis of IRE1α and PERK in HepG2 cells treated with various drugs as described for A and B. In the CHX group, HepG2 cells were pretreated with 50 μg/ml CHX for 30 min and throughout the drug treatments. D and F, quantitation of the percentage of phosphorylated IRE1α in total IRE1α in C and E. E and G, Western blot analysis of IRE1α and PERK in HepG2 (E) and HEK293T (G) cells infected with adenoviruses (Ad) overexpressing LacZ or GRP78, followed by drug treatment. Data are representative of at least two independent experiments. Values are the mean ± S.E. with three repeats. Statistical analysis was done by Student’s t test. *p < 0.05. p, hyperphosphorylated; 0, non-phosphorylated.
AMPK-dependent UPR Activation

Phenformin-mediated IRE1α and PERK Activation Is a General Phenomenon—To determine whether this is a general phenomenon, we examined in detail the effect of phenformin on UPR in various cell types. In MEFs, phenformin treatment increased AMPK phosphorylation and decreased S6 phosphorylation, as expected (Fig. 2A). Consistent with the data in HepG2 cells shown in Fig. 1, phenformin induced IRE1α phosphorylation (Fig. 2A) and Xbp1 mRNA splicing (Fig. 2B). Similarly, phenformin increased PERK and eIF2α phosphorylation and CHOP protein levels (Fig. 2B). Moreover, activation of the IRE1α and PERK pathways was supported by the increased transcript levels of UPR target genes, including Grp78, Erdj4, Chop, and Gadd34 (Fig. 2D). Similar observations were obtained in pancreatic acinar 266-6 cells (Fig. 2E), the β-cell line INS-1, and the macrophage cell line RAW (data not shown). Thus, our data show that phenformin-induced UPR activation is a general phenomenon regardless of cell type.

AMPK Is Required for Phenformin-mediated IRE1α and PERK Activation—The phenformin effect is partially mediated by AMPK (30). We next investigated whether AMPK is required for phenformin-mediated UPR activation. To this end, we examined UPR activation in AMPK<sup>−/−</sup> MEFs lacking both AMPKα1 and AMPKα2 catalytic domains. Indeed, activation of the IRE1α pathway, including IRE1α phosphorylation and Xbp1 mRNA splicing, was completely abolished by the loss of AMPK (Fig. 3, A and B). This was not due to intrinsic defects of UPR activation in AMPK<sup>−/−</sup> cells, as Tg-induced IRE1α activation was not blocked (Fig. 3, A and B). At the mRNA level, AMPK deficiency blocked phenformin-induced (but not Tg-induced) Erdj4 expression (Fig. 3C). Similarly, activation of the PERK pathway by phenformin (but not Tg) was significantly reduced in AMPK<sup>−/−</sup> MEFs as shown by Thr-980 PERK phosphorylation (Fig. 3D) and induction of CHOP at both the mRNA and protein levels (Fig. 3, C and E). Of note, lack of phosphorylation of acetyl-CoA carboxylase, a direct AMPK target, in AMPK<sup>−/−</sup> MEFs in response to phenformin confirmed the complete loss of AMPK activity (Fig. 3D). Thus, our data suggest that AMPK is required for phenformin-mediated IRE1α and PERK activation.

LKB1 Is Dispensable for the Phenformin Effect—LKB1 is an upstream kinase of AMPK in some settings (31, 32). We next investigated whether LKB1 is required in phenformin-mediated UPR activation. Comparable levels of IRE1α and PERK hyperphosphorylation were observed in LKB1<sup>−/−</sup> and WT cells treated with phenformin (Fig. 4A). This was further supported by the similar induction of CHOP protein (Fig. 4A). Thus, LKB1 is dispensable for phenformin-mediated UPR activation. Indeed, phenformin-induced acetyl-CoA carboxylase phosphorylation did not require LKB1 (Fig. 4B), suggesting the presence of other AMPK upstream kinase(s).

AMPK Activation Is Not Sufficient to Activate IRE1α and PERK—We next investigated whether AMPK is sufficient to induce UPR using another AMPK agonist, AICAR (33). Treatment of AICAR increased AMPK phosphorylation at Thr-172 and decreased S6 phosphorylation, as expected (Fig. 5A). However, no increase in IRE1α and PERK phosphorylation was observed even with prolonged treatment (Fig. 5B), arguing that AMPK activation is not sufficient to promote IRE1α and PERK activation. Rather, we noticed that IRE1α phosphorylation was decreased with time, suggesting that AMPK activation alone
may down-regulate ER stress, likely through inhibition of protein translation (reduced phospho-S6). This observation is consistent with previous reports showing that AMPK activation alone alleviates ER stress in various cell types (34–39).

**Kinase Activities of Both IRE1α and PERK Are Indispensable for Their Activation by Phenformin**—In canonical UPR, the kinase activities of IRE1α and PERK are required for trans-autophosphorylation and activation. To test the involvement of the kinase activities of IRE1α and PERK in phenformin-mediated activation, we generated PERK knock-out (PERK−/−) MEFs stably expressing the empty vector pBABE, WT PERK, or kinase-dead K618A PERK (40) and measured phenformin-induced phosphorylation. The kinase-dead K599A IRE1α was responsive to phenformin treatment (Fig. 6B). In conclusion, intact IRE1α and PERK kinase domains are required for the phenformin effect, suggesting that the phenformin effect is mediated through the kinase activities of IRE1α and PERK.

**Activation of the IRE1α-XBP1 Pathway Partially Mediates the Cytotoxicity of Phenformin**—Cytotoxicity is a potential side effect of prolonged phenformin treatment (41). As UPR is a key...
AMPK-dependent UPR Activation

Figure 6. Kinase activities of IRE1α and PERK are required for the phenformin effect. A, Western blot analysis of the PERK pathway in PERK−/− MEFs stably expressing empty vector, WT PERK, or kinase-dead K618A PERK and treated with 300 nM Tg and 5 mM phenformin (Ph) for 2 h. MEFs were used as a control. TAM, PPA, and CHOP were detected using nuclear extracts of the same cells. B, Western blot analysis of IRE1α phosphorylation in IRE1α−/− MEFs stably expressing WT or K599A IRE1α and treated with phenformin (5 mm) for 6 h. CREB was used as a loading control for the nuclear fraction. Data are representative of at least two independent experiments. PT, Phos-tag, p, hyperphosphorylated; 0, non-phosphorylated.

Discussion

Our study demonstrates phenformin-induced UPR activation in an AMPK-dependent manner. The downstream outcomes of this phenformin-induced UPR strongly resemble those of the UPR triggered by traditional ER stress inducers, including activation of downstream canonical events of the IRE1α and PERK pathways such as the requirement of kinase activities of IRE1α and PERK, Xbp1 splicing, eIF2α phosphorylation, and induction of ER chaperones. However, the activation mechanism appears to be distinct: phenformin-mediated UPR activation is resistant to cycloheximide treatment and requires the cytosolic energy sensor AMPK. On the basis of these findings, we conclude that activation of UPR sensors can be facilitated by metabolic signals from outside of the ER lumen upon metabolic challenges.

In addition to the activation mechanism, our data show that phenformin-mediated UPR activation may be responsible for the cytotoxic effect of phenformin. It has been shown that the IRE1α and PERK pathways differentially regulate cell survival in response to ER stress (2). Whereas the PERK pathway promotes cell death in part via CHOP-mediated induction of pro-apoptotic genes such as Bim (42), the IRE1α-XBP1 pathway may promote cell survival via induction of the ER folding capacity (43–45). In contrast, several recent reports showed that high levels of XBP1s can also be pro-apoptotic (46, 47). Here, our data showed an improved cell survival rate in XBP1-deficient cells treated with phenformin but no difference in PERK-deficient cells, suggesting that the IRE1α-XBP1 pathway is pro-apoptotic in this context. In line with our study, a previous report showed that another biguanide derivative, metformin, which selectively up-regulates the PERK pathway, resulted in no cell apoptosis (48). Thus, the role of UPR branches in the cell fate decision is likely to be cell type- and context-specific.

Phenformin has been removed from the anti-diabetic drug market due to its liver toxicity and potency for inducing lactic acidosis, whereas its analog metformin remains on the market (49). Phenformin has recently attracted interest because of its greater antitumor efficacy compared with metformin in mouse models (50). The enhanced cell cycle inhibitor p21 has been suggested as one of the mechanisms underlying the inhibition of cell proliferation and induction of apoptosis in phenformin-treated cancer cells (51). However, the detailed mechanisms of its cytotoxicity are still not fully understood. Our data suggest...
UPR as a potential mechanism for the cell fate determination of phenformin. Whether the liver toxicity or the anticancer effect of phenformin is mediated by the IRE1α-XBP1 pathway requires further investigations using in vivo models.

Several recent studies have shed light on the emerging cross-talks between UPR and metabolic signaling pathways such as the AMPK or mTOR pathways (52). For example, a recent study showed that the RNase activity of IRE1α may be required for nitric oxide-mediated activation of AMPK (53), whereas UPR activation down-regulates the mTOR pathway to enhance autophagy, presumably to provide amino acids by degradation of unnecessary proteins (54). Conversely, AMPK activation reduces translation and attenuates ER stress and UPR (34–39), similar to our observation. Nonetheless, our data show that phenformin activates UPR in an AMPK-dependent manner and that AMPK deficiency completely abolishes phenformin-induced UPR while having no effect on Tg-induced UPR. Thus, these new data suggest that AMPK is required but not sufficient to activate UPR. We speculate that additional phenformin-responsive factor(s) may be required in this process (Fig. 7C). We speculate that signals from inside the ER may act as the additional factor and exert a synergistic effect with AMPK to activate UPR sensors upon phenformin treatment, as overexpression of GRP78 attenuates the phenformin effect on UPR.

Possible mechanisms include the interruption of folding, maturation of GRP78 attenuates the phenformin effect on UPR. We speculate that additional phenformin-responsive factor(s) may be required in this process (Fig. 7C). We speculate that signals from inside the ER may act as the additional factor and exert a synergistic effect with AMPK to activate UPR sensors upon phenformin treatment, as overexpression of GRP78 attenuates the phenformin effect on UPR. Possible mechanisms include the interruption of folding, maturation, and secretion of ER proteins.

Our study reveals the complexity of mammalian UPR activation, which seems to be tightly regulated by multiple signaling pathways in addition to stress in the ER. We recently showed the involvement of non-muscle myosin II and the actin cytoskeleton in activation of IRE1α in response to ER stress (4). Thus, we postulate that UPR sensors not only sense the ER environment but also are able to integrate cytosolic signals such as the actin cytoskeleton and metabolic state.

The challenge that lies ahead is to determine the nature of physiological signals triggering ER stress and activating UPR as well as to quantitate the amount of ER stress in a physiological setting. We recently demonstrated the importance and efficacy of a Phos-tag-based approach in quantitating and visualizing ER stress (17, 19, 20). This approach takes advantage of the fact that IRE1α is separated into two bands on a Phos-tag gel, with the slower migrating band representing the phosphorylated form, which tightly correlates with IRE1α and UPR activation. Many laboratories have successfully used this approach to detect IRE1α activation under more physiological conditions in various cell and tissue types. In this study, with the aid of this method, we have reported a case of UPR activation facilitated by metabolic signals in the cytosol. Understanding the alternate modes of UPR activation will be pivotal and instrumental for the development of therapeutic strategies that modulate ER stress and homeostasis in human health and diseases.

Acknowledgments—We thank Drs. Douglas Cavener, Claudio Hetz, Lee Kraus, Marc Montminy, David Ron, and Reuben Shaw for reagents. We also thank Dr. Stipanuk (Cornell University) and the Qi laboratory members for helpful discussions.

REFERENCES

structural determinant located at the interdomain region of mammalian inositol-requiring enzyme 1a. J. Biol. Chem. 286, 30859–30866.


