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Monitoring and Manipulating Mammalian Unfolded Protein Response

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Abstract

The unfolded protein response (UPR) is a conserved, intracellular signaling pathway activated by endoplasmic reticulum (ER) stress. In mammalian cells, the UPR is controlled by three ER-resident transmembrane proteins: inositol-requiring enyzme-1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor-6 (ATF6), by which cytoprotective mechanisms are initiated to restore ER functions. However, if cellular homeostasis is not restored by the UPR's initial events, UPR signaling triggers apoptotic cell death, which correlates with the pathogenesis of a wide range of human diseases. The intrinsic function of the UPR in regulating cell survival and death suggests its importance as a mechanistic link between ER stress and disease pathogenesis. Understanding UPR regulatory molecules or signaling pathways involved in disease pathogenesis is critical to establishing therapeutic strategies. For this purpose, several experimental tools have been developed to evaluate individual UPR components. In this chapter, we present methods to monitor and quantify activation of individual UPR signaling pathways in mammalian cells and tissues, and we review strategies to artificially and selectively activate individual UPR signaling pathways using chemical–genetic approaches.

1. Introduction

The endoplasmic reticulum (ER) is the entrance site for newly synthesized polypeptides of membrane and secreted proteins. In the ER, residential chaperones and enzymes fold and assemble polypeptides before export to the rest of the secretory pathway. The ER's role in protein quality control is pivotal to maintaining cellular homeostasis. Pathologic and physiologic processes can disrupt ER protein folding and assembly, and under these circumstances, misfolded proteins accumulate in the ER lumen, leading to ER stress. ER stress is correlated with and causally linked to the pathogenesis of a wide range of human diseases, including neurodegenerative disease, metabolic and inflammatory disease, infection, and subtypes of cancer (He, 2006; Hotamisligil, 2010; Lin *et al.*, 2008; Moenner *et al.*, 2007). Why and how ER stress causes diseases and what molecular and cellular changes lead to diseases are questions that remain largely unanswered and require further investigation.

The unfolded protein response (UPR) is a conserved, intracellular signaling process activated by ER stress (Ron and Walter, 2007). In mammalian cells, the UPR comprises at least three parallel intracellular signaling pathways controlled by ER-resident

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transmembrane proteins: inositol-requiring enzyme-1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor-6 (ATF6). IRE1, PERK, and ATF6 detect disrupted ER homeostasis and then transmit this information to the rest of the cell by activating downstream signal transduction effectors. In experimentally induced conditions of acute ER stress, all three UPR branches are simultaneously activated and initiate cytoprotective events that suppress global protein translation to reduce the protein folding burden of the ER, upregulate the transcription of molecular chaperones and protein folding enzymes to increase folding capacity of the ER, and enhance the degradation of unfolded proteins via proteasomal machinery. However, if cellular homeostasis is not restored despite these actions, the UPR then triggers apoptosis.

The intrinsic function of the UPR in regulating cell survival and death in response to ER stress suggests its importance as a mechanistic link between ER stress and disease pathogenesis. However, the concomitant activation of multiple UPR signaling pathways by ER stress in mammalian cells has hindered precise assessments of the contribution of each specific UPR signaling pathway to disease pathogenesis. Identifying specific UPR regulatory molecules or signaling pathways involved in disease pathogenesis is critical to developing targeted therapies that prevent the development or progression of disease. Here, we review methods to monitor and quantify activation of individual UPR signaling pathways in mammalian cells and tissues, and we review strategies to artificially and selectively activate individual UPR signaling pathways using chemical–genetic approaches.

2. Monitoring Mammalian UPR

Here we present conventional protocols to detect and quantify the activity of IRE1, PERK, or ATF6 signaling in mammalian cell lines and tissues. Additionally, we review reporter systems developed for monitoring mammalian UPR activity.

2.1. Monitoring IRE1 activity

Upon induction of ER stress, IRE1α endonuclease (RNase) is activated and subsequently cleaves its substrate, X-box binding protein-1 (*Xbp-1*) messenger RNA (mRNA) (Calfon *et al.*, 2002; Yoshida *et al.*, 2001). *Xbp-1* mRNA has two conserved, overlapping open reading frames (ORFs). Activated IRE1α removes a 26-nucleotide (nt) intron from unspliced *Xbp-1* mRNA, leading to a translational frame shift to produce a protein encoded from the two ORFs. The product from spliced *Xbp-1* mRNA, XBP-1s, is an active transcription factor that upregulates the expression of ER associated degradation (ERAD) components and ER chaperones. Specificity of *Xbp-1* splicing by activated IRE1α is evidenced by mutation or knockout of IRE1α (Lee *et al.*, 2002). Therefore, monitoring *Xbp-1* splicing status is a specific molecular readout for IRE1α activity.

2.1.1. *Xbp-1* splicing assay for mammalian cells and tissues—Determination of *Xbp-1* splicing is performed by reverse transcriptase PCR (RT-PCR). Total RNA is extracted from cells or tissue lysate, and cDNA is synthesized by RT reaction with oligo-dT primer and Superscript III (Invitrogen, Carlsbad, CA). Sample cDNA is used as a template for PCR amplification with specific primers for *Xbp-1* (Table 11.1). PCR conditions are as follows: Step 1: 95 °C for 5 min; Step 2: 95 °C for 1 min; Step 3: 58 °C for 30 s; Step 4: 72 °C for 30 s Repeat steps 2–4 for 35 cycles Step 5: 72 °C for 5 min.

Differentiation of unspliced and spliced *Xbp-1* can be resolved on a 2.0–3.0% agarose/ 1×TAE gel. A minor hybrid amplicon species consisting of unspliced *Xbp-1* annealed to spliced *Xbp-1* is also typically observed and appears above the unspliced amplicon (Back *et al.*, 2005; Lin *et al.*, 2007).

2.2. Monitoring PERK activity

In response to ER stress, PERK forms dimers and undergoes autophosphorylation (Harding *et al.*, 1999). Consequently, PERK's kinase activity phosphorylates the alpha subunit of eukaryotic translation initiation factor-2 (eIF2α). Phosphorylation of eIF2α impairs ribosomal assembly on mRNA, thereby attenuating global protein translation (Harding *et al.*, 2000b). However, translation of activating transcription factor 4 (ATF4) is increased when eIF2α is phosphorylated because of the presence of multiple upstream ORFs in its cognate *Atf4* mRNA (Harding *et al.*, 2000a; Lu *et al.*, 2004a; Vattem and Wek, 2004). ATF4 subsequently induces expression of its target genes, including transcription factor C/EBP-homologous protein (CHOP). CHOP, ATF4, and phosphorylated eIF2α are therefore useful molecular markers for monitoring activity of PERK.

2.2.1. Quantitative PCR (qPCR) analysis of CHOP—Relative *Chop* mRNA expression is evaluated by qPCR. Total RNA extraction and RT reaction is performed to generate cDNA. *Rpl19*, a gene for ribosomal protein L19, whose transcription is not affected by ER stress, can be used as an internal control for normalization (Hollien and Weissman, 2006). Primer information for *Chop* and *Rpl19* is shown in Table 11.1 and qPCR conditions are as follows: Step 1: 95 °C for 5 min; Step 2: 95 °C for 30 s; Step 3: 58 °C for 30 s; Step 4: 72 °C for 30 s Repeat steps 2–4 for 40 cycles.

2.2.2. Western blotting of eIF2α and ATF4—Cells are homogenized by sonication in 1% NP-40 or 6 *M* urea, 20 m*M* HEPES pH = 8.0, containing protease and phosphatase inhibitors (Pierce, Rockford, IL). Twenty to thirty micrograms of total cell lysate are loaded onto SDS-PAGE mini gels and analyzed by Western blot. The following antibody dilutions are used: anti-eIF2α at 1:2000 (Cell Signaling, Natick, MA); anti-phospho-eIF2α at 1:500 (Cell Signaling); anti-ATF4 at 1:2000 (Santa Cruz Biotechnologies, Santa Cruz, CA). After overnight incubation with primary antibody, membranes are washed in PBS (or TBS) containing 0.1% Tween-20 and incubated in horseradish peroxidase (HRP)-coupled secondary antibody (Amersham, Piscataway, NJ) diluted 1:5000 in wash buffer. Immunoreactivity is detected using the enhanced chemiluminescence assay (Pierce). These conditions have been optimized for human protein samples and may require modification when applied to other mammalian cell types and tissues.

2.3. Monitoring ATF6 activity

The third stress sensor for ER stress in mammalian cells is ATF6 (Haze *et al.*, 1999). Upon induction of ER stress, ATF6 translocates from the ER to the Golgi apparatus where Site-1 Protease (S1P) and Site-2 Protease (S2P) cleave it (Ye *et al.*, 2000). After cleavage by S1P/S2P, the amino-terminus of ATF6, containing a basic leucine zipper (bZIP) transactivating domain, translocates to the nucleus as an active transcription factor. ATF6 increases ER protein folding capacity and assembly by transcriptional upregulation of molecular chaperones and/or enzymes. In particular, immunoglobulin heavy chain binding protein (*BiP/Grp78*) is robustly induced in response to ER stress and is a direct transcriptional target of ATF6 (Haze *et al.*, 1999). A simple and effective way to evaluate ATF6 activity is to measure *BiP/Grp78* mRNA or protein levels. In addition, Ron Prywes and colleagues established a method to monitor proteolytic cleavage using a FLAG-tagged ATF6 construct (Shen and Prywes, 2005). In this section, we briefly describe these methods of evaluating ATF6 activation.

2.3.1. qPCR analysis of BiP—Conditions for analysis of *BiP/Grp78* by qPCR are identical to *Chop*. Primer sequences for *BiP/Grp78* are shown in Table 11.1.

2.3.2. Western blotting of FLAG-ATF6—Cells are transfected with a p3×FLAG-ATF6 plasmid and protein lysate is prepared with lysis buffer as described above (Shen and Prywes, 2005). Detection is performed by Western blotting as described above using a 1:5000 dilution of primary antibody to FLAG (Sigma, St. Louis, MO).

2.4. Alternative approaches to UPR evaluation

Several reporter systems have been developed to monitor mammalian UPR activity. ER stress response elements (ERSE) (Yoshida *et al.*, 1998) and UPR elements (UPRE) (Yoshida *et al.*, 2000) have been identified within the promoter regions of UPR target genes, and UPR-dependent transcription factors bind to these regions to start their transcription. Constructs with conventional reporter proteins, such as luciferase and β-galactosidase, fused to UPRE and ERSE sequences can report ER stress, as evidenced by increased luciferase and β-galactosidase activities, in both cell culture and transgenic mice (Mao *et al.*, 2004; Wang *et al.*, 2000; Yoshida *et al.*, 1998). However, in these systems, the contribution of individual UPR pathways (IRE1 vs. PERK vs. ATF6) cannot be resolved.

Additionally, Iwawaki *et al.* (2004) established *in vitro* and *in vivo* green fluorescent protein (GFP) reporter systems to monitor the IRE1 pathway. They constructed a reporter plasmid containing a FLAG-tagged, partial sequence (410–633 nt) of hXBP-1 and a GFP variant (*Venus*) sequence under the control of the chicken β-actin promoter. This partial sequence of hXBP-1 includes a 26-nt intron that is spliced out by activated IRE1. Under normal condition, expression of Venus-fused *hXbp-1* mRNA contains the 26-nt intron, thereby halting translation via the stop codon located between the coding regions of hXBP-1 and Venus. Under ER stress conditions, the 26-nt intron is spliced out, inducing a frame shift and resulting in production of the hXBP-1-Venus fusion protein. Using this construct observation or quantification of Venus fluorescence allows monitoring of IRE1 activation. However, interpreting the on/off kinetics of UPR signaling might be difficult with this GFP-based approaches because the intrinsic stability of GFP may result in a prolonged fluorescence signal that remains elevated even after cessation of ER stress or the activation of a particular UPR pathway being monitored.

3. Chemical–Genetic Manipulation of Mammalian UPR

Common physiological and pharmacological inducers of ER stress (e.g., hypoxia, tunicamycin, and thapsigargin) concomitantly activate all UPR signaling pathways. To precisely identify the downstream effects of each signaling pathway, multiple chemical–genetic tools have been developed to selectively activate one UPR pathway at a time. Here we review chemical–genetic tools that enable selective activation of individual UPR signaling pathways in mammalian cells independent of ER stress.

The techniques described within take advantage of UPR initiation events by creating cell lines that mimic the activation of IRE1, PERK, or ATF6 proteins upon addition of cell-permeable, small molecules. Unlike simple gene deletion or constitutive overexpression, drug-induced activation acts quickly and reversibly. Furthermore, it avoids activating compensatory mechanisms in the cell arising from long-term UPR signaling. The establishment of stable, isogenic cell lines to study each pathway is recommended to (1) minimize ER stress arising from transfection or transduction protocols; (2) minimize epigenetic influences resulting from variable transgenic insertion; (3) permit longer analysis since the transgene is stably expressed; and (4) prevent overexpression bias by selecting stable clones with no elevation in basal ER stress compared to wild-type cells.

3.1. Chemical–genetic manipulation of IRE1

3.1.1. Activation of wild-type IRE1—IRE1α is a type I membrane protein consisting of an ER stress-sensor domain protruding into the ER lumen coupled to cytosolic kinase and RNase domains (Cox *et al.*, 1993). In response to ER stress, IRE1α oligomerizes, which induces *trans*-autophosphorylation of its cytosolic domains and activation of its RNase activity (Cox and Walter, 1996; Shamu and Walter, 1996; Sidrauski and Walter, 1997) (Fig. 11.1A). The activated RNase cleaves *Xbp-1* mRNA in the cytosol to generate spliced *Xbp-1* mRNA which encodes a potent bZIP-containing transcription factor that upregulates ERAD components and ER chaperones (Lee *et al.*, 2003b). In addition to catalyzing *Xbp-1* mRNA splicing, mammalian IRE1α also binds to a growing number of ER membrane and cytosolic proteins including TRAF2 (tumor necrosis factor receptor-association factor 2), BAX (Bcl-2-associated X protein), BAK (Bcl-2 homologous antagonist killer), BI-1 (BAX inhibitor-1), HSP90 (heat shock protein 90), and RACK1 (receptor for activated C-kinase 1) (Hetz *et al.*, 2006; Hetz and Glimcher, 2009; Lisbona *et al.*, 2009; Marcu *et al.*, 2002; Urano *et al.*, 2000). The IRE1–TRAF2 interaction leads to the activation of the c-Jun N-terminal kinase (JNK) signaling pathway (Urano *et al.*, 2000). The physiologic functions of other IRE1 protein–protein interactions are less well understood.

3.1.2. Activation of IRE1[I642G]—Several chemical–genetic approaches can selectively recapitulate IRE1's RNase activity in mammalian cells. ATP binding to IRE1's kinase domain is believed to activate IRE1's RNase function (Korennykh *et al.*, 2009). A generalized chemical–genetic strategy for sensitizing protein kinases to cell-permeable molecules, while not affecting wild-type kinases, was first applied in yeast (Bishop *et al.*, 2000; Papa *et al.*, 2003) and has since been adapted and demonstrated to be effective in regulating mammalian IRE1α (Han *et al.*, 2008; Hollien *et al.*, 2009; Lin *et al.*, 2007). A human IRE1α mutant bearing an isoleucine-to-glycine missense mutation at the gatekeeper position in the kinase domain, IRE1[I642G], enables ATP-competitive ligands to selectively bind its kinase domain thereby activating the RNase activity of this IRE1α allele (Fig. 11.1B).

1NM-PP1 (1-(tert-butyl)-3-(naphthalen-1-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine) is the most common ligand used to regulate IRE1 [I642G] and is commercially available (EMD Chemicals, Gibbstown, NJ). Addition of 1NM-PP1 to cells expressing IRE1[I642G] results in rapid *Xbp-1* mRNA splicing in multiple mammalian cell types including human embryonic kidney (HEK293), mouse embryonic fibroblast (MEF), and human prostate cancer (PC-3) (Han *et al.*, 2008; Hollien *et al.*, 2009; Lin *et al.*, 2007; Thorpe and Schwarze, 2010). This strategy robustly reproduces IRE1α signaling through *Xbp-1* mRNA splicing without perturbing other UPR pathways.

When using the IRE1[I642G] mutant, however, it is important to note its differences from WT IRE1α and what consequences it might have in evaluating the IRE1α signaling pathway as a whole. It is unclear whether the mutant IRE1α forms dimers and/or oligomers or whether this step is necessary for activation in this system whereas *trans*-autophosphorylation is absent and unnecessary for RNase activity in the mutant. Differences in activation mechanism may be critical, especially in light of any cross talk between UPR pathways. While typically thought of as cytoprotective, recently, Hollien *et al.* (2009) and Han *et al.* (2009) discovered that mammalian IRE1α promotes the cleavage of numerous additional mRNAs and that IRE1 may also promote apoptosis depending on the specific mRNAs degraded. These authors demonstrated that IRE1α with active phosphotransfer was able to reduce levels of ER-localized mRNA which preceded apoptosis—this activity was absent in kinase catalysis-compromised mutants such as IRE1[I642G]. This suggests that WT IRE1α can send proapoptotic signals independent of *Xbp-1* splicing and that

IRE1[I642G] uncouples these endonucleolytic outputs. This uncoupling may be therapeutically advantageous but may confound understanding of the endogenous role of IRE1 and the UPR.

Back *et al.* (2006) employed a different strategy to activate IRE1α via induced dimerization. By fusing the kinase and RNase domains of IRE1α to a modified FK506-binding domain (Fv1E), addition of a small organic molecule, AP20187, can serve as a dimerizer and recapitulate IRE1α's RNase activity. This strategy has also been adapted for inducible PERK activation and is described in more detail below. Additionally, others have successfully developed inducible spliced XBP-1 (XBP-1s) using the Tet-Off system (Lee *et al.*, 2003a); however, solely evaluating XBP-1s expression ignores additional targets of IRE1α RNase activity that may have physiological significance (Han *et al.*, 2009; Hollien *et al.*, 2009). Notably, neither system is suitable to study the physiologic functions of other IRE1 protein–protein interactions, as described above (Han *et al.*, 2009; Hollien *et al.*, 2009).

3.2. Chemical–genetic manipulation of PERK

3.2.1. Activation of wild-type PERK—PERK is an ER stress sensor protein that has an ER-luminal domain, bearing sequence and functional similarities to IRE1's luminal domain, and a cytosolic domain encoding a kinase. In response to ER stress, PERK oligomerizes, activating its kinase function (Fig. 11.1C). PERK kinase activity phosphorylates eIF2α which in turn attenuates protein translation by inhibiting ribosome assembly on mRNAs.

3.2.2. Activation of Fv2E–PERK—Chemical–genetic approaches have been developed that selectively recapitulate PERK's kinase activity in mammalian cells. Dimerization of PERK is a proximal step in its activation following ER stress. Fv2E–PERK comprises PERK's kinase domain fused to tandem-modified FK506-binding domains, Fv2E. Fv2E-PERK dimerization can be artificially induced by application of AP20187 (Fig. 11.1D). AP20187 is a cell permeable synthetic mimic of FK1012 (dimer of FK506) that can simultaneously bind two Fv2E domains and physically allow dimerization of Fv2E-fused proteins (Pollock and Rivera, 1999). Fv2E–PERK dimerization by AP20187 causes autophosphorylation of Fv2E–PERK, *trans*-phosphorylation of eIF2α, and downstream PERK signaling (Lu *et al.*, 2004a,b). This strategy robustly reproduces PERK signaling without necessitating ER stress or activating other UPR signaling pathways. AP20187-based activation of the PERK pathway has been demonstrated in Chinese hamster ovary (CHO), HEK293, MEF, and human mammary epithelial (MCF10A) cells, as well as in mice (Lin *et al.*, 2009; Lu *et al.*, 2004a,b; Oyadomari *et al.*, 2008; Sequeira *et al.*, 2007).

Fv2E–PERK fusion proteins can be created using standard cloning techniques and the ARGENT Regulated Homodimerization Kit (ARIAD Pharmaceuticals, Cambridge, MA). Fv2E is a modified FKBP domain with high affinity to AP20187, therefore commercially available anti-FKBP12 antibodies (Affinity Bioreagents, Rockford, IL) can be used to detect expression of the chimeric PERK protein by Western blot. AP20187 is commercially available from ARIAD Pharmaceuticals, can be diluted in DMF (diamethylformamide), and can be added directly to cell culture preparations.

3.3. Chemical–genetic activation of ATF6

3.3.1. Activation of wild-type ATF6—In contrast to IRE1 and PERK, ATF6 is a type II transmembrane protein with a carboxyl-terminus sensor domain facing the ER lumen coupled to a bZIP-containing transcription factor domain in its cytosolic amino terminus. In response to ER stress, ATF6 exits the ER and translocates to the Golgi (Shen *et al.*, 2002). ATF6 is sequentially cleaved into its active form by two proteases, S1P and S2P (Ye *et al.*,

2000) in the Golgi. Cleavage of ATF6 liberates the cytosolic fragment of ATF6 containing the bZIP transcription factor. The cleaved ATF6 fragment then translocates to the nucleus and upregulates transcription of target genes including ER protein-folding enzymes and ERAD proteins (Fig. 11.1E) (Haze *et al.*, 1999; Wu *et al.*, 2007; Yamamoto *et al.*, 2007).

3.3.2. Activation of TET-ATF6—Chemical–genetic approaches have been developed that selectively recapitulate ATF6's transcriptional factor activity in mammalian cells. Tetracycline-inducible induction of the cleaved ATF6 fragment encoding the cytosolic bZIP transactivational domain reproduces native ATF6 signaling in human cervical cancer cells (HeLa) and HEK293 cells (Okada *et al.*, 2002). In this system, the ATF6 fragment is placed under the control of a strong promoter containing multiple regulatory tet operator elements. In the absence of tetracycline or its derivative, doxycycline, tet repressors bind to the tet operators and inhibit transcription of ATF6. However, in the presence of doxycycline, the tet repressors bind the drug and undergo a conformational change that dissociates it from the tet operator. As a result, transcription of ATF6 is strongly induced and downstream transcriptional targets of ATF6 are upregulated (Fig. 11.1F). Other UPR signaling pathways are unaffected under these conditions.

There are several ATF6 isoforms; however, amino acid residues 1–373 of human ATF6α correspond to the active product of S1P/S2P-mediated proteolysis. This truncated ATF6α constitutively exists in the nucleus (Haze *et al.*, 1999). We recommend establishing stable Tet-On cell lines expressing this truncated form of ATF6α. For the establishment of a Tet-On system, there are several commercially available vectors and cell lines such as the T-REx system (Invitrogen, Carlsbad, CA) that makes creating of a stable ATF6α(373) cell line convenient and efficient.

Conditional regulation of ATF6 activity can also be achieved with the ERT2 (mutated estrogen receptor) system (Thuerauf *et al.*, 2007). Using this system, the functional domain of cleaved ATF6 is fused to ERT2. In the absence of ERT2 ligands (e.g., tamoxifen), ERT2 forms a complex with various cellular proteins, such as Hsp90, which mask ATF6. The addition of tamoxifen, however, blocks Hsp90 binding to ERT2, allowing ERT2–ATF6 to induce transcriptional activity of ATF6.

4. Concluding Remarks

UPR signaling pathways are likely to contribute to the pathogenesis and progression of numerous human diseases arising from protein misfolding or ER stress. Strategies to monitor and manipulate individual UPR signaling pathways can offer valuable tools to investigate the precise role of the UPR in disease pathogenesis and may offer new ways to combat diseases arising from ER stress.

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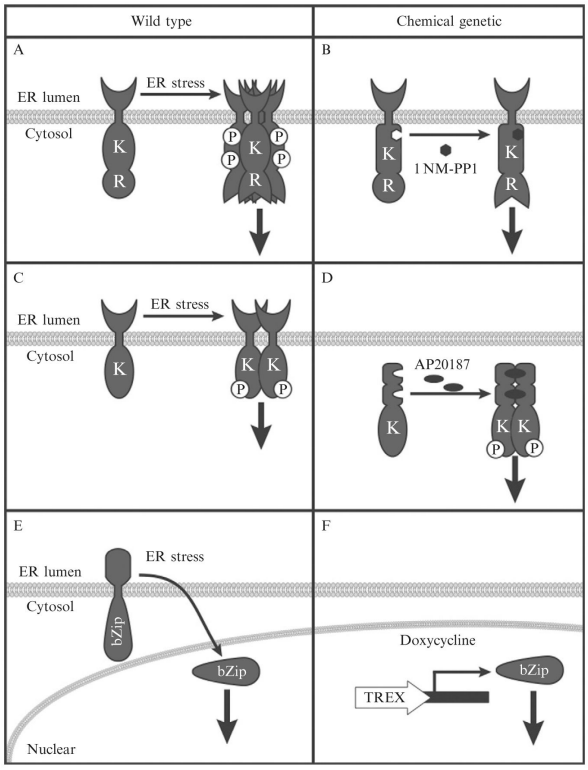

Figure 11.1.

Comparison of UPR activation by ER stress or chemical–genetics. (A) In response to ER stress, wild-type IRE1 oligomerizes, *trans*-autophosphorylates (*P*) its kinase domain (*K*), and activates its endoribonuclease (RNase) activity (*R*). (B) In response to 1NM-PP1, IRE1[I642G] activates its RNase activity. (C) In response to ER stress, wild-type PERK dimerizes, autophosphorylates, and activates its kinase. (D) In response to AP20187, Fv2E–PERK conditionally dimerizes, autophosphorylates, and activates its kinase function. (E) In response to ER stress, full-length of ATF6 is cleaved and its N-terminus containing a bZip DNA binding domain translocates to the nucleus to activate transcription of target genes. (F) In response to doxycycline, the cleaved form of ATF6 under the control of T-REx promoter is expressed and activates transcription of its target genes.

Table 11.1

PCR primer sets

		Sequence (5′–3′)		
Gene	**Species**	**Forward**	**Reverse**	**Product (bp)**
RPL19	Human	ATGTATCACAGCCTGTACCTG	TTCTTGGTCTCTTCCTCCTTG	233
	Mouse	ATGCCAACTCCCGTCAGCAG	TCATCCTTCTCATCCAGGTCACC	198
	Rat	TGGACCCCAATGAAACCAAC	TACCCTTCCTCTTCCCTATGCC	180
	Hamster	ATGCCAACTCCCGTCAGCAG	TCATCCTTCTCATCCAGGTCACC	198
BiP/Grp78	Human	CGGGCAAAGATGTCAGGAAAG	TTCTGGACGGGCTTCATAGTAGAC	211
	Mouse	CCTGCGTCGGTGTGTTCAAG	AAGGGTCATTCCAAGTGCG	201
	Rat	CCTGCGTCGGTGTATTCAAG	AAGGGTCATTCCAAGTGCG	201
	Hamster	TGGACCTGTTCCGATCTACC	GACAGCAGCACCGTATGCTA	206
Chop	Human	ACCAAGGGAGAACCAGGAAACG	TCACCATTCGGTCAATCAGAGC	201
	Mouse	ACGGAAACAGAGTGGTCAGTGC	CAGGAGGTGATGCCCACTGTTC	219
	Rat	ACGGACCACGAGTGGTCAGTGC	CAGGAGGTGATGCCAACAGTTC	219
	Hamster	AACGAGAGGAAAGTGGCTCA	TGTTCTCCCTACCCAGCATC	222
Xbp-1[a]	Human	TTACGAGAGAAAACTCATGGC	GGGTCCAAGTTGTCCAGAATGC	s-257, u-283
	Mouse	GAACCAGGAGTTAAGAACACG	AGGCAACAGTGTCAGAGTCC	s-179, u-205
	Rat	GAGTGGAGTAAGGCTGGTGG	TGGGTAGACCTCTGGGAGTTCC	s-223, u-249
	Hamster	TTGAGAGAGAAAACTCATGGC	GGGTCCAACTTGTCCAGAATGC	s-263, u-289

[a]Spliced form of *xbp-1* (s); unspliced form of *xbp-1* (u)