Identification of ERSE-II, a New cis-Acting Element Responsible for the ATF6-dependent Mammalian Unfolded Protein Response*

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Herp is a 54-kDa membrane protein in the endoplasmic reticulum (ER). The mRNA expression level of Herp is increased by the accumulation of unfolded proteins in the ER. Transcriptional changes designed to deal with this type of ER stress is called the unfolded protein response (UPR). Most mammalian UPR-target genes encode ER-resident molecular chaperones: GRP78, GRP94, and calreticulin. The promoter regions of these genes contain a cis-acting ER stress response element, ERSE, with the consensus sequence of CCAAT-N_9-CCACG. Under conditions of ER stress, p50ATF6 (the active form of the transcription factor, ATF6) binds to CCACG when CCAAT is bound by the general transcription factor, NF-Y/CBF. Here, we report the genomic structure of human Herp and the presence of a new ER stress response element, ERSE-II, in its promoter region. The gene for Herp consists of eight exons, localized to chromosome 16q12.2–13. The promoter region contains a single ERSE-like sequence. In reporter gene assays, disruption of the cis-element resulted in a partial reduction of the transcriptional response to ER stress, suggesting that the element is functional for the UPR. These results also suggest the involvement of additional elements in the UPR. Further analysis, using an optimized plasmid containing an mRNA-stabilizing sequence, revealed ERSE-II (ATTGG-N-CCACG) as the second ER stress response element. Interestingly, ERSE-II was also dependent on p50ATF6, in a manner similar to that of ERSE, despite the disparate structure. The strong induction of Herp mRNA by ER stress would be achieved by the cooperation of ERSE and ERSE-II.

The expression of Herp, a 54-kDa endoplasmic reticulum (ER)-resident protein, is induced by the unfolded protein response (UPR) (1). The UPR is a transcriptional response to remedy the accumulation of unfolded proteins in the ER. Most previously identified UPR-target genes encode ER-resident molecular chaperones and folding enzymes, such as GRP78/BiP, GRP94, protein-disulfide isomerase, and calreticulin. In contrast, this ER luminal proteins, Herp is an integral membrane protein, both N and C termini of which face the cytoplasmic side of the ER. This membrane topology makes it unlikely that Herp acts as a molecular chaperone for proteins in the ER. Herp plays an unknown role in the cellular survival response to the accumulation of unfolded proteins in the ER.

The ER provides an optimal environment for the synthesis, folding, and assembly of membrane and secreted proteins. The accumulation of unfolded or misfolded proteins in the ER under conditions of “ER stress” threatens the normal functioning of eukaryotic cells. Although the physiological conditions inducing ER stress are not fully understood, the cellular response to the stress is essential for homeostasis (comprehensively reviewed by Kaufman (Ref. 2)). The ER-stress responses are currently categorized to three mechanisms: transcriptional induction, translational attenuation, and degradation (reviewed by Mori (Ref. 3)). In addition, ER stress activates c-Jun N-terminal kinases (4) and induces caspase-12-mediated apoptosis (5).

The molecular mechanism of the UPR is extensively defined in the yeast, Saccharomyces cerevisiae. The ER luminal domain of Ire1p, an ER-resident type I transmembrane protein, senses the accumulation of unfolded proteins in the ER, activating its cytoplasmic endoribonuclease domain through homo-oligomerization and trans-autophosphorylation (6, 7). Activated Ire1p triggers the production of a transcription factor, Hac1p, through an unusual mRNA splicing mechanism (8–10). Hac1p binds to the UPR-dependent cis-acting element, UPRE, in the promoter regions of UPR-target genes to activate their transcription (8, 11, 12). In mammals, two Ire1p orthologs, IRE1α and IRE1β, have been identified (13, 14), although the substrates of their endoribonuclease activities are unknown. ATF6 has been identified as the transcription factor responsible for the UPR (15). The quiescent form of ATF6 (p90ATF6), a type II-transmembrane protein, is embedded in the ER membrane and proteolyzed in an ER stress-dependent manner (16). The liberated N-terminal fragment (p50ATF6) translocates to the nucleus, binding to the mammalian UPR-dependent cis-acting element, designated the ER stress response element (ERSE) (15, 17).

ERSE, possessing a consensus sequence of CCAAT-N_9-CCACG, is necessary and sufficient for the induction of at least three major ER chaperones (GRP78, GRP94, and calreticulin) (15). This sequence is present in the proximal promoter regions of previously identified UPR-target genes. It is also found in a new element, ERSE-II (ATTGG-N-CCACG), which contributes to the transcriptional response to ER stress. These findings suggest a role for Herp in the cellular survival response to ER stress.

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The nucleotide sequence reported in this paper has been submitted to the GenBank™/EMBL/DDBJ Data Bank with accession number AB034990.

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§ The abbreviations used are: ER, endoplasmic reticulum; UPR, unfolded protein response; ERSE, ER stress response element; FISH, fluorescence in situ hybridization; HUVEC, human umbilical vein endothe-elial cell; UTR, untranslated region; GM-CSF, granulocyte-mono-cyte colony-stimulating factor; MMS, methyl methanesulfonate; bp, base pair(s); PCR, polymerase chain reaction; PBS, phosphate-buffered saline.

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of many ER stress-responsive proteins (15, 18). The general transcription factor, NF-Y/CBF, binds to the CCAAT motif of ERSE (17, 19, 20). Under conditions of ER stress, p50ATF6 binds to the CCACG motif of ERSE, resulting in the transcriptional induction of ER chaperones (17). Multiple ER stress-responsive genes, however, possess proximal promoter regions without an ERSE sequence, such as FKB13 (15), asparagine synthetase (21), ATF3 (22), and RTP/NDRG1.2

In the present paper, we demonstrate the existence of a new ER stress response element, ERSE-II, found in the Herp promoter region. In a manner similar to ERSE, ERSE-II mediates the ATF6-dependent UPR.

**EXPERIMENTAL PROCEDURES**

**Cloning and Sequencing of the Human Herp Gene—**A human whole blood-encoded Herp (Stratagene) was screened to obtain genomic clones encoding Herp using the PCR-based screening method described previously (23). Positive phages were cloned utilizing the Escherichia coli XL1-Blue MRA strain as host. PCR was performed using 5'-TGGTTTTCTCCGGTTACAC-3' and 5'-AGAGAACCAGGATCTC-3' as primers with the plate lysates as templates. Two positive phages were cloned by limiting serial dilution. The insert DNAs isolated from these clones (~17 kilobases each) were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PerkinElmer Life Sciences) cloned by limiting serial dilution. The insert DNAs isolated from these clones (~17 kilobases each) were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PerkinElmer Life Sciences).

**Fluorescence in Situ Hybridization (FISH) Analysis—**The P1-derived artificial chromosome clone containing the Herp gene was isolated from a human PAC DNA library (GenomeSystems) using Herp cDNA as a probe. DNA from the clone, labeled by nick translation with digoxigenin (Roche Molecular Biochemicals), was transfected HUVECs with 0.5 ng/ml anti-Herp rabbit polyclonal antibody (1) and 10 μg/ml anti-FLAG M2 mouse monoclonal antibody (Eastman Kodak Co.). Cells were then incubated with Oregon Green 514-conjugated goat anti-rabbit IgG (Molecular Probes) and rhodamine Red-X-conjugated goat anti-mouse IgG (Molecular Probes) for 1 h. After washing with PBS, fluorescence was visualized using a confocal laser-scanning microscope with FLUOVIEW (Olympus).

**RESULTS**

**Genomic Structure of Human Herp—**We obtained a complete sequence of the human Herp gene (GenBank® accession no. AB034990) by comparison of two partially overlapping clones, isolated from the human genomic DNA library, to the Herp cDNA sequence (GenBank® accession no. AB034989). The gene was officially designated HERPUD1 by the HUGO Gene Nomenclature Committee. The gene structure, sequences across the exon-intron junctions, and the sizes of exons and introns are shown in Fig. 1 (A and B). The Herp gene contains eight exons and spans 11,738 bp in length. The identified exon-intron junctions agreed with the intron 5'-GT and 3'-AG consensus sequences. The 5'-terminal transcription start site had been previously determined by cap-site hunting (1). Exon 1 encoded the 5'-UTR and the first 49 N-terminal residues including the initial Met codon. The stop codon and the 3'-UTR were encoded by exon 8.

To localize the Herp gene on human chromosomes, we performed FISH analysis utilizing DNA from the P1-derived artificial chromosome clone containing the Herp gene. Labeled Herp DNA was hybridized to chromosomes derived from peripheral blood lymphocytes. Eighty metaphase cells were analyzed; 73 exhibited specific labeling of the 16q12.2-13 region (Fig. 1C).

**Sequence of the 5’-Flanking Promoter Region—**We sequenced the ~6-kilobase pair 5’-flanking region of the Herp gene. Computer analysis by TFSEARCH using the TRANSFAC data base (24) revealed many potential transcription factor-binding sites within the sequence. The proximal 200-bp sequence upstream of the transcriptional start site, including several putative cis-acting regulatory elements, is shown in Fig. 1D. The canonical TATA box, specifying the transcriptional start site, is found in close proximity to exon 1. Two CAAT boxes were also identified. The 5’-flanking region contained several GC boxes (GGGCG), suggesting multiple Sp1-binding sites.

The Herp promoter region contains one ERSE-like sequence, 5'-CCAAATGGCGGGGAGGCACA-3' located upstream of the TATA box (Fig. 1D). ERSE is a cis-acting regulatory element identified in the promoters of mammalian UPR target genes (15). ERSE, with a consensus of CCAAT-N9-CACG, is necessary and sufficient for the induction of the ER-resident molecular chaperones, GRP78, GRP94, and calreticulin. Although 2 K. Kokame, H. Kato, and T. Miyata, unpublished data.
Fig. 1. Human Herp gene. A, genomic organization. Exons are indicated by black boxes and numbered. The initial Met and stop codons appear in exons 1 and 8, respectively. B, exon-intron boundaries. The exon sequences, deduced from comparison to the Herp cDNA, are given in uppercase letters, whereas the intron sequences are in lowercase letters. All introns start with GT and end with AG. The potential polyadenylation signal is underlined, and the polyadenylation site at the 3' end of exon 8 is indicated by an arrowhead. The complete sequence data have been submitted to the GenBank/EMBL/DDBJ databases under accession number AB004390. The gene has been officially designated HERPUD1 by the HUGO Gene Nomenclature Committee. C, FISH analysis. Labeled DNA containing the Herp gene was hybridized to metaphase chromosomes. The hybridization signals on chromosome 16 are indicated by arrows. D, sequence of 5'-flanking region. Exon 1 is shaded. Negative numbers indicate the distance of the nucleotide from the transcription start site. Putative regulatory motifs are labeled with lines. The motif corresponding to ERSE is shown in black-boxed white letters.

the G nucleotide at the 3' end of the consensus sequence is replaced by an A in the Herp ERSE, we predict this sequence functions in the UPR-dependent induction of Herp expression at the transcriptional level.

Functional Mapping of the Herp Promoter—A series of reporter plasmids containing sense fragments of the Herp 5'-flanking region (from nucleotide -5000 to -200) upstream of the firefly luciferase gene were transfected into HUVECs. The firefly luciferase activity in each assay was normalized to a cotransfected Renilla luciferase plasmid, pRL-SV40, to compensate for a varied efficiency of transfection.

The basal luciferase activity of plasmid containing the longest 5'-flanking sequence (-5000/+98) exhibited approximately half the activity of an SV40 promoter control (Fig. 2). Thapsigargin, an inhibitor of ER-resident Ca\(^{2+}\)-ATPase, is used experimentally to activate the UPR. Following a 6-h treatment with 1 \(\mu\)M thapsigargin, luciferase activity increased significantly (-4.3-fold) over basal activity, consistent with previous results demonstrating the induction of Herp mRNA by thapsigargin (1). The SV40 promoter vector did not respond to thapsigargin treatment. Removal of the -5000 to -1800 region of Herp resulted in an increase of basal activity, suggesting the existence of silencing element in the region; little effect, however, was observed in the response to thapsigargin. Both the basal and thapsigargin-treated activities of plasmids containing -1000/+98, -800/+98, -600/+98, and -400/+98 were similar in magnitude to -1800/+98. Although removal of the -400 to -200 region resulted in a reduction of basal activity, the strong induction of luciferase activity in response to thapsigargin remained intact. We, therefore, concluded that the cis-elements responsible for the response to thapsigargin treatment would lie within the region 200 bp upstream of the transcription start site. In the following experiments, we used a plasmid containing the -200/+98 region to analyze this hypothesis in detail.

Disruption of ERSE in the Herp Promoter—One ERSE-like sequence, \(3'\)CCAATggcggccACCA\(-70\), is contained within the Herp 5'-flanking region. As the A nucleotide at the 3' end was different from a G in the ERSE consensus, CCAT-N\(_{4}\)-CCACG, we examined the transcriptional effect of this nucleotide difference. Basal and thapsigargin-treated activities of the plasmid containing CCACg (Fig. 3A, line 2) were similar to those of the original plasmid (line 1), suggesting that the A nucleotide functions similarly to a G nucleotide in the response to thapsigargin. We performed site-directed mutagenesis on two motifs of ERSE and examined the effects on the transcriptional induction following thapsigargin treatment. Throughout this paper, the term "mutation" is defined as the substitution of A, C, G, and T for C, A, T, and G, respectively. Disruptive mutation of either of the two motifs, CCAT or CCAAC, resulted in a partial reduction of the thapsigargin-dependent induction of luciferase activity (Fig. 3A, lines 3 and 4), indicating their involvement in the induction. Mutation of both motifs, however, did not completely abrogate the response to thapsigargin (line 5). These results suggest that other cis-elements are involved in thapsigargin-dependent transcriptional induc-
tion. Under these experimental conditions, however, the observed inducibilities were not high enough to define the elements. We, therefore, modified the plasmid DNAs to effectively monitor the difference in activity with or without thapsigargin treatment.

Optimization of the Reporter Plasmid to Monitor the Induction Effectively—Observation of the effects of stimulants on transcriptional induction in reporter gene assays is contingent on a faster turnover of mRNA produced from the test plasmid DNA. We, therefore, introduced an AT-rich sequence into the 3'-UTR of the firefly luciferase plasmids. The 51-nucleotide stretch (TAATATTTATATATTTATATTTTTAAAATATTTATTATTTATTTATTTAA), known to selectively destabilize mRNA, was identified from the 3'-UTR of GM-CSF cDNA (25).

Insertion of this sequence into the luciferase 3'-UTR of the plasmid containing the Herp promoter region resulted in dramatic reduction of the basal activity; the activity in the presence of thapsigargin was relatively unchanged (compare lines 1 and 2 in Fig. 3B). As a result, the induction rate of thapsigargin treatment increased from 2.8 to 7.7 in this assay. Insertion of the AT-rich sequence into the control pGL3-Control plasmid, containing the SV40 promoter, had little effect on the ratio of basal to thapsigargin-treated activities (1.1 to 1.4, lines 3 and 4), although the luciferase activities were reduced in both cases. We utilized this optimized plasmid to identify additional transcriptional control elements in the Herp promoter region.

Identification of ERSE-II—To identify additional cis-elements involved in thapsigargin induction, we made a series of mutant plasmids that also contained the disrupted ERSE. First, we searched the region from nucleotide -2196 to -89, making 11 sets of consecutive 10-bp mutations. After measuring the resulting luciferase activities (Fig. 4A), we found that basal activities were reduced when two regions, -2186GCGGGT TGCA and -2176TCAGCCCGTG, were mutated, although the induction by thapsigargin treatment remained intact (lines 4 and 5). Mutation of -126GCCGATTGGG or -116CCACCGTGGGG, however, resulted in a significant decrease in basal activity without a significant change in the induction rate by thapsigargin treatment (lines 6 and 7).
crease of luciferase activity upon thapsigargin treatment, despite little effect on basal activity (lines 10 and 11). To identify the nucleotides involved in the thapsigargin response, we assessed the effects of 14 nucleotide mutations crossing these two regions on luciferase activity (Fig. 4B). Mutations at -122, -121, -120, -119, -118, -116, -115, -114, -113, and -112 demonstrated inhibitory effects on the thapsigargin-induced response of luciferase activity (lines 3-13 except line 8). These results indicate that the 11-bp stretch, 122ATTGGgC-132, in the Herp promoter region is responsible for the transcriptional response to thapsigargin. This 11-bp sequence contains two motifs forming the ERSE consensus, CCAAT (complementary to ATTGG) and CCACG, although the orientation of the first sequence is inverted. We termed this cis-element, ERSE-II.

Functional Contribution of ERSE and ERSE-II to the UPR—To compare the activity of ERSE and ERSE-II, we measured the luciferase activity of plasmids containing combination of mutations in these two cis-elements. We utilized not only thapsigargin but also tunicamycin (light gray bars), 10 μg/ml thapsigargin (closed bars), 10 μg/ml tunicamycin (light gray bars), or 10 mM mercaptoethanol (dark gray bars). Tg, thapsigargin; Tm, tunicamycin; Me, mercaptoethanol.

Effect of ATF6 Overexpression on the Herp Promoter Activity—The general transcription factor, NF-Y, constitutively binds the CCAAT motif of ERSE (17, 18). The transcription factor, ATF6, on the ER membrane is activated by proteolysis in response to ER stress; the resultant N-terminal soluble form (p50ATF6) moves into nuclei to bind directly to the CCACG motif (16, 17). We, therefore, examined the effect of p50ATF6 overexpression on the induction of Herp expression. As the cleavage site involved in conversion from p90ATF6 to p50ATF6 is unknown, we utilized ATF6(366), an N-terminal soluble fragment containing the entire basic region and majority of the leucine zipper region of ATF6. ATF6(366) translocates to the nucleus to enhance the levels of GRP78 mRNA (16). Upon transfection of the expression plasmid encoding FLAG-tagged ATF6(366) into HUVECs, a fraction of transfected cells possessed nuclei recognized by an anti-FLAG-tag antibody, indicating that the expressed ATF6(366) was present in nuclei (Fig. 6A, red signal). Cells with immunonegative nuclei were also observed, likely due to a failure of transfection. Following staining of cells with an anti-Herp antibody, immunopositive signals of the ER in ATF6(366)-expressing cells were stronger than those in cells without ATF6(366) (Fig. 6A, green signal). This suggests that overexpressed ATF6(366) functions in vivo to induce the expression of Herp in the ER.

To demonstrate that p50ATF6 induces the transcriptional activity of the Herp promoter, the plasmid containing the -200/+98 region of Herp was cotransfected into HUVECs in conjunction with the ATF6(366)-expression plasmid. As expected, coexpression of ATF6(366) resulted in an enhancement of luciferase activity (Fig. 6B, line 1). The induction was partially reduced when the two motifs, CCAAT and CCAC, of ERSE were disrupted (line 2), indicating that both the effect of ATF6(366) is dependent on the cis-element and that other elements are involved in this induction. Disruption of both motifs, ATF6G (complementary to CCAAT) and CCACA, of ERSE-II also demonstrated a partial reduction in induction (line 3). Disruption of both elements, ERSE and ERSE-II, resulted in a complete loss of the ATF6 effect (line 4). These data suggest that both ERSE and ERSE-II are involved in the ATF6-dependent UPR.

p50ATF6 binds directly to the CCACG portion of ERSE to exert its ability as a trans-factor (17). Mutation of CCACA/G motifs of both ERSE and ERSE-II in the Herp promoter abrogated the inducible effect of ATF6(366) (Fig. 6B, line 5).
gesting that the enhancer activity of p50ATF6 requires the CCACG sequences of both ERSE and ERSE-II. p50ATF6 binds to CCACG only when CCAAT is bound by NF-Y, exactly 9 bp upstream of CCACG (17). Mutation of the CCAAT motifs of both ERSE and ERSE-II abrogated the ATF6 effect as well (line 6). The indispensability of NF-Y binding is also applicable to ERSE-II as well as ERSE, despite the differences in both the direction and interval of CCAAT and CCACG in ERSE-II from those in ERSE.

DISCUSSION

We identified two cis-acting elements responsible for the UPR-dependent transcriptional induction in the proximal promoter region of the Herp gene. CCAATgggcggcgcgCCACA is almost identical to the 19-nucleotide consensus sequence of ERSE, CCAAT-Np-CCACG (15). The other, ATTTGG-N-CCACG, is a new element, termed ERSE-II. ERSE-II also contains two motifs, CCAAT (complementary to ATTTGG) and CCACG, although the orientation and the interval between them are different from ERSE. Moreover, ERSE-II functions as an ATP6-dependent fashion, in the same manner as the original ERSE.

The A nucleotide at position 19 in ERSE of the Herp promoter differs from a G of the ERSE consensus. Our data, however, could not demonstrate a significant functional difference in response to thapsigargin treatment between A and G at this position (Fig. 3A). Yoshida et al. (15) demonstrated that substitution of the nucleotide G to T was a crucial mutation, impairing the UPR; they did not, however, examine the effect of substitution to A. Furthermore, ERSE-like sequences also appear in the human ER stress-responsive genes, GRP58 (15) and SERCA2 (26), with a sequence of CCAAT-Np-CCACA. Therefore, the ERSE consensus should be described as containing the sequence: CCAAT-Np-CCACG/G.

It has been reported that the transcription factors, NF-Y and ATF6, simultaneously bind to the CCAAT and CCACG portions of ERSE, respectively (17). The former is considered to bind in a constitutive manner, independent of the UPR. The latter binds only when it is converted from the ER membrane-embedded p90ATF6 to the soluble p50ATF6 by processing induced by ER stress (16, 17). Binding of ATF6 to CCACG requires the binding of NF-Y to the CCAAT sequence at a position exactly 9 bp upstream of CCAAT (17). This 9-bp distance is critical; neither 8 nor 10 bp is acceptable (17). The unidirectional necessity, however, of CCAAT and CCACG was not investigated. Our data indicate that the role of ERSE-II as a cis-acting element was exerted by ATF6 as was the case with that of ERSE (Fig. 6). Both the CCACG and the ATTTGG (complementary to CCAAT) sequences of ERSE-II were critical for ATF6-mediated transcription. Although direct evidence is not available, it is likely that both NF-Y and p50ATF6 bind to ERSE-II to enhance transcription (Fig. 7). We observed specific binding of NF-Y to the CCACG sequence of ERSE-II in vitro (data not shown). If our model is correct, the inverse direction of two motifs may be necessary when the distance between them is 1 bp, not 9. A study of the steric structure of protein-DNA interaction will help determine the validity of this argument. By analogy to ERSE, other transcription factors, such as CREB-RP (15) and XBP-1 (17), may bind to the CCACG sequence of ERSE-II.

Most UPR-target genes, such as GRP78, GRP94, and the gene for calreticulin, are fully activated by multiple copies of ERSE (15, 18). Despite a single ERSE, however, Herp mRNA induction by the UPR is very strong as compared with other ER chaperones (1). ERSE-II may cooperate with ERSE to facilitate the strong induction of Herp in response to ER stress. We searched for ERSE-II in other ER stress-responsive genes to demonstrate the function of this sequence in the response to cellular stress. ORP150 is an ER-resident protein whose expression is induced by hypoxia; three distinct mRNA species are produced by alternative promoters (27). One of them was preferentially induced by hypoxia and ER stress, in a manner dependent on a single ERSE-like sequence (−93CACATgggcggcgcgCCACG−75) in the promoter region (27). We found two ERSE-II-like sequences, −267ATTGGaCCACG−277 and −160ATTGGaCCACG−170, upstream of the ERSE. They also might be involved in the UPR.

Recently, van Laar et al. (28) identified a human methyl methanesulfonate (MMS)-inducible gene, Mif1, identical to Herp. The mRNA is also induced by tunicamycin, osmotic shock, and UV irradiation. Although they demonstrated that one cis-element, ERSE, was involved in the response to tunicamycin, ERSE-II was not mentioned. The induction of Mif1 by MMS was mediated by neither ERSE nor ERSE-II but by a 122-bp fragment (−257 to −136). As MMS also induces the mRNA expression of GRP78 (28, 29), GRP94 (28), and CHOP (29), known UPR-target genes, these genes and Herp may share an additional cis-acting MMS response element.

The function of Herp is still unknown. It was believed that all proteins encoded by UPR-target genes functioned as molecular chaperones and folding enzymes to relieve the disturbance of the ER. As the majority of the molecule is exposed to the cytoplasm, Herp may play a role independent of molecular chaperones and folding enzymes to relieve the disturbance of the ER. As the majority of the molecule is exposed to the cytoplasm, Herp may play a role independent of molecular chaperones and folding enzymes to relieve the disturbance of the ER.
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2. Kaufman, R. J. (1999) Genes Dev. 13, 1211–1233
3. Mori, K. (2000) Cell 101, 451–454
4. Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., and Ron, D. (2000) Science 287, 664–666
5. Nakagawa, T., Zhu, H., Morishima, N., Li, E., Xu, J., Yankner, B. A., and Yuan, J. (2000) Nature 403, 98–103
6. Cox, J. S., Shamu, C. E., and Walter, P. (1993) Cell 73, 1197–1206
7. Mori, K., Ma, W., Gething, M. J., and Sambrook, J. (1993) Cell 74, 743–756
8. Cox, J. S., and Walter, P. (1996) Cell 87, 391–404
9. Kawahara, T., Yanagi, H., Yura, T., and Mori, K. (1997) Mol. Biol. Cell 8, 1845–1862
10. Mori, K., Ogawa, N., Kawahara, T., Yanagi, H., and Yura, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4660–4665
11. Mori, K., Kawahara, T., Yoshida, H., Yanagi, H., and Yura, T. (1996) Genes Cells 1, 803–817
12. Mori, K., Ogawa, N., Kawahara, T., Yanagi, H., and Yura, T. (1998) J. Biol. Chem. 273, 9912–9920
13. Tirasophon, W., Welihinda, A. A., and Kaufman, R. J. (1998) Genes Dev. 12, 1812–1824
14. Wang, X. Z., Harding, H. P., Zhang, Y., Jolicoeur, E. M., Kuroda, M., and Ron, D. (1998) EMBO J. 17, 5728–5737
15. Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998) J. Biol. Chem. 273, 33741–33749
16. Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999) Mol. Biol. Cell 10, 3787–3799
17. Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., and Mori, K. (2000) Mol. Cell. Biol. 20, 6755–6767
18. Roy, B., and Lee, A. S. (1999) Nucleic Acids Res. 27, 1437–1443
19. Roy, B., and Lee, A. S. (1995) Mol. Cell. Biol. 15, 2263–2274
20. Roy, B., Li, W. W., and Lee, A. S. (1996) J. Biol. Chem. 271, 28995–29002
21. Babu-Tesmann, I. P., Chen, C., Zheng, C., Siu, F., Schuster, S. M., Nick, H. S., and Kilberg, M. S. (2000) J. Biol. Chem. 275, 26976–26985
22. Cai, Y., Zhang, C., Nawa, T., Aso, T., Tanaka, M., Oshiro, S., Ichijo, H., and Kitajima, S. (2000) Blood 96, 2140–2148
23. Kokame, K., Kato, H., and Miyata, T. (1996) J. Biol. Chem. 271, 29659–29665
24. Heinemeyer, T., Wingender, E., Reuter, I., Hermjakob, H., Kel, A. E., Kel, O. V., Ignatieva, E. V., Ananko, E. A., Podkolodnaya, O. A., Kolpakov, F. A., Podkolodny, N. L., and Kolchanov, N. A. (1996) Nucleic Acids Res. 24, 672–676
25. Shaw, G., and Kamen, R. (1986) Cell 46, 659–667
26. Caspersen, C., Pedersen, P. S., and Treiman, M. (2000) J. Biol. Chem. 275, 22363–22372
27. Kaneda, S., Yura, T., and Yanagi, H. (2000) J. Biochem. (Tokyo) 128, 529–538
28. van Laar, T., Schouten, T., Hoogervorst, E., van Eck, M., van der Eb, A. J., and Terleth, C. (2000) FEBS Lett. 480, 123–131
29. Wang, X. Z., Lawson, B., Brewer, J. W., Zinszner, H., Sanjay, A., Mi, L. J., Boorstein, R., Kreibich, G., Hendershot, L. M., and Ron, D. (1996) Mol. Cell. Biol. 16, 4273–4280
30. Travers, K. J., Patil, C. K., Wodicka, L., Lockhart, D. J., Weissman, J. S., and Walter, P. (2000) Cell 101, 249–258