Amino acid limitation regulates *CHOP* expression through a specific pathway independent of the unfolded protein response

Céline Jousse[a], Alain Bruhat[a], Heather P. Harding[b], Marc Ferrara[a], David Ron[b], Pierre Fafournoux[a,*]

[a] *UR 238 - Unité de Nutrition Cellulaire et Moléculaire, INRA de Theix, 63122 Saint Genès Champanelle, France*
[b] *Skirball Institute of Biomolecular Medicine, NYU Medical Center, 550 First Ave., New York, NY 10016, USA*

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Abstract The gene encoding CHOP (C/EBP-homologous protein) is transcriptionally activated by many stimuli and by amino acid deprivation. *CHOP* induction was considered to be due to an accumulation of unfolded protein into the ER (unfolded protein response (UPR)). We investigate the role of the UPR in the induction of *CHOP* by amino acid deprivation and show that this induction is not correlated with *BiP* expression (an UPR marker). Moreover, amino acid deprivation and UPR inducers regulate the *CHOP* promoter activity using distinct *cis* elements. We conclude that amino acid deprivation does not activate the UPR and regulates *CHOP* expression through a pathway that is independent of the UPR.

Key words: *CHOP*; Amino acid; Unfolded protein response; *BiP* (Grp78)

1. Introduction

All cells regulate gene expression in response to changes in the external environment such as nutrient availability. In mammals, plasma concentrations of nutrients are markedly affected by dietary or pathological conditions. The concentration of amino acids in the plasma is particularly sensitive to the nutritional state, with levels falling several-fold in cases of malnutrition. This may occur in response to a global limitation in protein intake or in response to specific limitations in essential amino acids [1,2].

The molecular mechanisms involved in the control of gene expression in response to amino acid deprivation (AAD) have been extensively studied in yeast [3]. In addition to specific controls of genes involved in the synthesis of individual amino acids, the yeast employs a general control process whereby a subset of genes are coordinately regulated by starvation of the cell for any single amino acid. In mammalian cells, such a global response has not been observed but specific examples of enzymes, transporters and mRNAs that are regulated by amino acid availability have been reported [4,5]. The current understanding of mechanisms involved in amino acid dependent control of gene expression is limited. Our ignorance extends both to the identity of the genes implicated in the response to amino acid deprivation and to the pathways that mediate it.

One of the genes strongly induced by an amino acid limitation is the *CHOP* gene (C/EBP-homologous protein, also known as *GADD153*) [6,7]. *CHOP* encodes a transcription factor that regulates certain aspects of the response of cells to stress [8,9]. The activation of *CHOP* transcription by stress has been characterized extensively at the molecular level. This analysis has led to the identification of a role for a signal, emanating from the endoplasmic reticulum (ER) in mediating the response of *CHOP* to multiple different stress inducers [10–12]. *CHOP* induction is linked to the activation of an ER stress response, one that is presumably mediated by the accumulation of malfolded proteins in the ER. This response, referred to as the unfolded protein response (UPR), occurs in a variety of stressful conditions and is associated with the transcriptional activation of the genes encoding ER chaperones such as the immunoglobulin binding protein BiP (also known as Grp78) [13,14]. Indeed *CHOP* and *BiP* are coordinately regulated during the response to different stress inducers [11,12]. Recent data suggest that this coordinate expression is mediated by the action of a common upstream signaling pathway [15]. However, the possible role of the ER unfolded protein response in mediating the effects of AAD has not been addressed to date. The results of such an analysis would be important to the restricted question of unity in the pathways involved in *CHOP* induction and to the more general question of the signaling pathway that links AAD to regulated gene expression. The major finding of this study indicates that amino acid starvation does not activate the UPR and therefore regulates *CHOP* expression through a pathway that is distinct from the ER stress signaling cascade.

2. Materials and methods

2.1. Cell culture and treatment conditions

HeLa and HepG2 cells, obtained from ATCC were cultured at 37°C under 95% air-5% CO_2, in Dulbecco's modified Eagle's medium F12 (DMEM/F12) (Sigma) containing 10% decomplemented fetal calf serum. CHO-K1 and CHO-tsH_1 cells (a gift of J. Pollard) were cultured at 34°C under similar conditions. For amino acid starvation experiments, sub-confluent cells were washed twice with phosphate-buffered saline (PBS) and refed with a medium containing the indicated concentrations of amino acids supplemented with 10% dialyzed fetal calf serum for the indicated time. DMEM/F12 medium devoid of leucine, glutamine, lysine and methionine (Sigma) was used to make media lacking leucine, glutamine or methionine. Media lacking other individual amino acids or glucose were made from DMEM/F12 medium free of amino acids and glucose (Gibco). The control medium was obtained by complementing the depleted medium with the corresponding amino acids. Each amino acid was added at the concentration of that present in DMEM/F12.

2.2. RNA isolation and Northern blot analysis

Total RNA was prepared as described previously [16]. Northern

*Corresponding author. Fax: +33 4 73 62 45 70.
E-mail: fpierre@clermont.inra.fr

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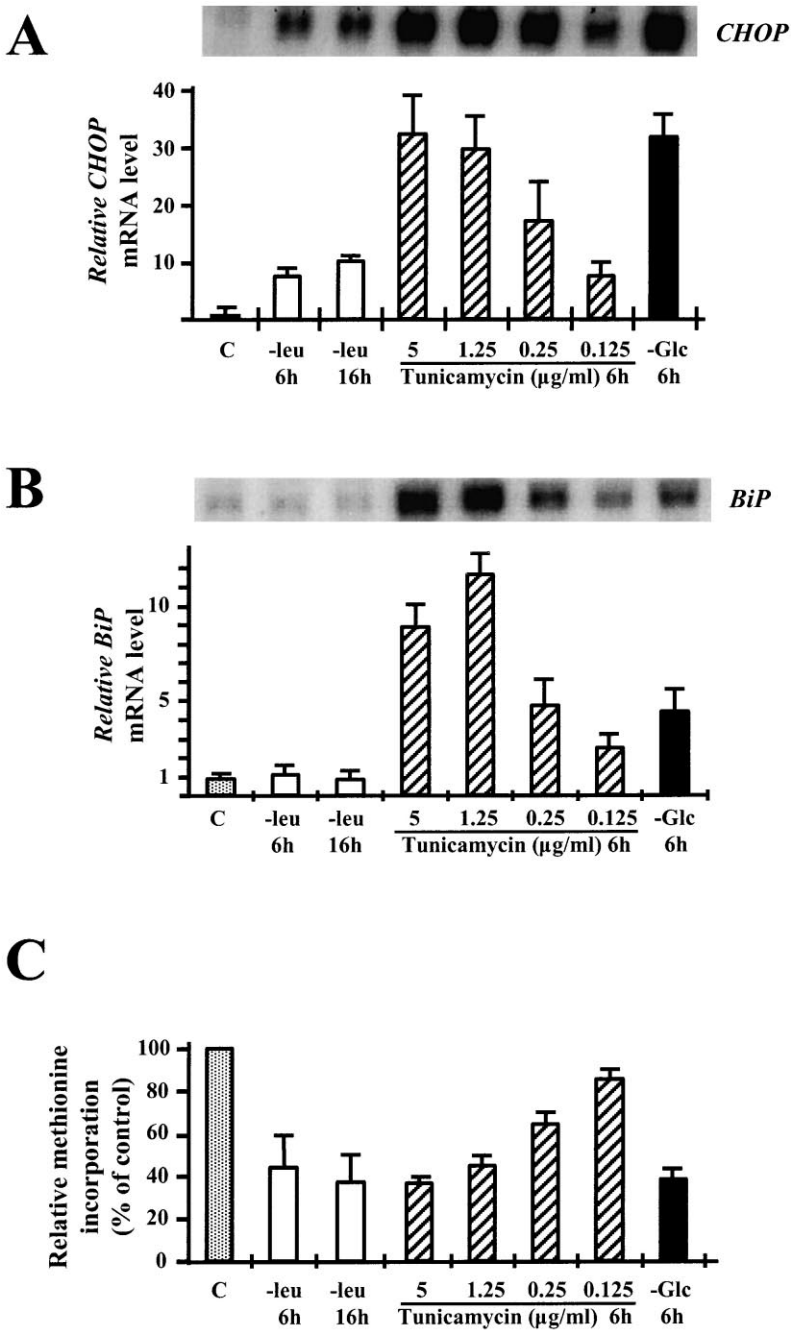

Fig. 1. Effect of leucine limitation, glucose starvation and tunicamycin treatment on the expression of *CHOP* and *BiP* mRNA. HeLa cells were incubated for 6 or 16 h in DMEM/F12 control medium (C), in medium lacking leucine (−leu) or glucose (−Glc), or containing different concentrations of tunicamycin (5, 1.25, 0.25, or 0.125 μg/ml) as indicated. Total RNA was extracted, and Northern blot analysis was performed. The blots were hybridized with a human probe corresponding to *CHOP* (A) or *BiP* (B). The blots were quantified as described in Section 2, then results are given as fold increase above the control value. The protein synthesis was measured by [^{35}S] methionine incorporation during the last 3 h of incubation (C).

blots were performed according to the procedure of Sambrook et al. [17]. RNA was cross-linked to the membrane by UV irradiation before hybridization was performed.

2.3. cDNA probe and hybridization

The human *CHOP* cDNA (BH1), generously provided by Dr. N.J. Holbrook [18] was used as a probe. BH1 plasmid was linearized by *Pst*I, and ^{32}P-riboprobes were synthesized [17] using T7 RNA polymerase (Promega). Prehybridization was carried out for 2 h at 55°C in 50% formamide, 6×SSC, 5×Denhardt's reagent, 0.5% SDS, 10 μg/ml yeast tRNA. Hybridization was carried out for 16 h at 55°C. The membranes were washed for 15 min at 55°C successively in 2×SSC containing 0.1% SDS, 0.5×SSC containing 0.1% SDS and 0.1×SSC containing 0.1% SDS. The human *BiP* cDNA and *IGFBP-I* cDNA (generously provided by Drs. Powell and Suwanichkul, Baylor College of Medicine, Houston, TX, USA) were used as a probe. The gel-purified *Pst*I fragments of both cDNAs were labeled by random priming with [α^{32}P]dCTP. Prehybridization was carried out for 2 h at 42°C in 50% formamide, 6×SSC, 5×Denhardt's reagent, 0.5% SDS. Hybridization was carried out for 16 h at 42°C. The membranes were washed for 15 min at 42°C successively in 2×SSC containing 0.1% SDS and 0.5×SSC containing 0.1% SDS. Hybridization signals were

visualized and quantified using a PhosphorImager (Molecular Dynamics) and the IMAGEQUANT software. To control for variation in either the amount of RNA in different samples or loading errors, all blots were rehybridized with a 18S cDNA probe (results not shown). Relative mRNA was determined as the ratio of mRNA to 18S RNA. The results are expressed as fold increase above the control value.

2.4. Plasmids used for transfection experiments

The plasmid p*CHOP*-LUC corresponds to the human *CHOP* promoter region from nucleotides −649 to +91 fused to the luciferase (LUC) gene in the pGL3 plasmid (Promega). The shorter promoter fragments were PCR generated using appropriate oligonucleotides containing *Hin*dIII and *Xho*I restriction sites at the 5′ end. The DNA fragment was then cloned in the pGL3 plasmid at the *Hin*dIII and *Xho*I site. The constructs were sequenced before utilization.

2.5. DNA transfection and luciferase assays

HeLa cells were plated in 12-well dishes and transfected by the calcium phosphate coprecipitation method as described previously [19]. Two μg of luciferase plasmid were transfected into the cells along with 0.1 μg of pCMV-βGal, a plasmid carrying the bacterial β-Galactosidase gene fused to the human cytomegalovirus immediate-early enhancer/promoter region, as an internal control. Cells were exposed to the precipitate for 16 h, washed twice in phosphate-buffered saline, and then incubated with DMEM/F12 containing 10% calf serum. Twenty-four h after transfection, cells were treated as indicated for 6 or 16 h. After treatment, cells were harvested in 150 μl of lysis buffer (Promega) and centrifuged at 13 000×*g* for 2 min. Twenty μl of the supernatant were assayed for luciferase activity (CD.LIFE, Anduze, France). β-Galactosidase activity was measured as described by Hall et al. [20]. Relative luciferase activity was given as the ratio of relative light unit/relative β-Gal unit. All values are the means calculated from the results of at least three independent experiments.

2.6. Protein synthesis determination

HeLa cells were incubated in the indicated medium. During the last 3 h of incubation, 0.5 μCi/ml ^{35}S L-methionine was added. The medium was then removed and the cells were incubated for 30 min in cold 5% trichloroacetic acid. The wells were washed three times with cold water. The radioactivity incorporation into trichloroacetic acid-precipitable material was measured by liquid scintillation counting after protein solubilization in 0.1 M NaOH plus 0.5% SDS. Results are given as a percentage of methionine incorporation into cells incubated in DMEM/F12 control medium.

3. Results

3.1. CHOP induction in response to leucine limitation is not correlated with BiP expression

To determine if the signal inducing *CHOP* expression in response to amino acid deprivation (AAD) emanates from the ER, we examined the correlation between *CHOP* and *BiP* expression in response to either leucine limitation or to agents and growth conditions that induce ER stress (tunicamycin, glucose starvation). For most of the experiments we used leucine starvation to induce amino acid limitation because (1) leucine is an essential amino acid that is poorly utilized by cells during a 16 h incubation period and (2) leucine, which is transported by system L, is rapidly equilibrated through the cell membrane [21,22].

Fig. 1A shows that *CHOP* is induced by a leucine limitation and by tunicamycin or glucose starvation. In the same experiment (Fig. 1B) *BiP* is strongly induced in response to glucose deprivation and tunicamycin treatment but is not affected by a 6 or 16 h leucine starvation. A more precise time course does not show any *BiP* induction in response to leucine starvation (results not shown). Fig. 1C demonstrates that all three conditions inhibit protein synthesis to comparable degrees. It is notable that a low tunicamycin concentration (0.125 μg/ml) that induces *CHOP* at the same magnitude as leucine limitation, does not significantly affect protein synthesis but induces *BiP* expression. These results demonstrate that *CHOP* induction in response to leucine limitation is not correlated with *BiP* expression.

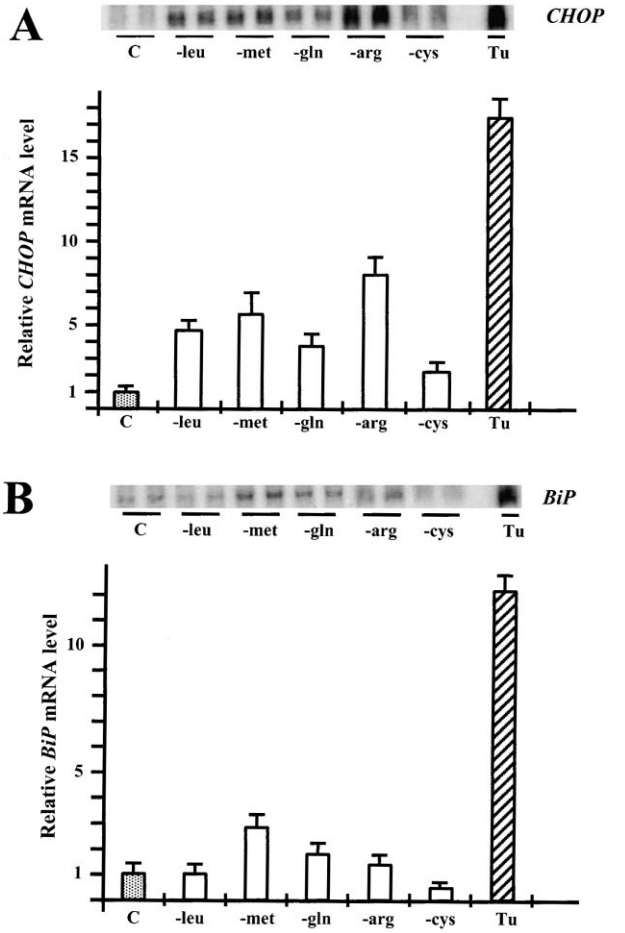

Fig. 2. Effect of limitation for methionine, glutamine, arginine or cystine on *CHOP* and *BiP* expression. HeLa cells were incubated for 16 h in DMEM/F12 control medium (C) or in medium lacking leucine (−leu), methionine (−met), glutamine (−gln), arginine (−arg), cystine (−cys). Tunicamycin treatment (1.25 μg/ml for 6 h) was used as a positive control for *CHOP* and *BiP* expression. Total RNA was extracted, and Northern blot analysis was performed. The blots were hybridized with a probe corresponding to *CHOP* (A) and *BiP* (B). After quantification, results are given as fold increase above the control value.

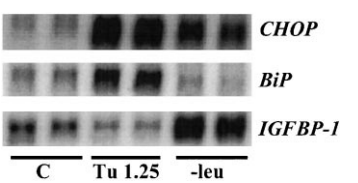

Fig. 3. Effect of leucine limitation and tunicamycin treatment on expression of *CHOP*, *BiP* and *IGFBP-1* mRNA in HepG2 cells. HepG2 cells were incubated for 6 h in DMEM/F12 control medium (C), in medium lacking leucine (−leu) or containing 1.25 μg/ml of tunicamycin. Total RNA was extracted, and Northern blot analysis was performed. The blots were hybridized with a probe corresponding to *CHOP*, *BiP* and *IGFBP-1*.

To better characterize the specificity of the amino acid dependent control of *CHOP* mRNA, cells were incubated for 16 h in a medium deficient in any one of several single amino acids and the *CHOP* and *BiP* mRNA content of the cells was measured. *CHOP* is induced in response to deprivation in any one of the amino acids tested here (Fig. 2) whereas *BiP* levels remained essentially unchanged. Methionine limitation gives rise to a weak induction of *BiP* compared to the induction observed in response to tunicamycin treatment. This may be attributed to a change in the reduced glutathione level and the redox potential in the cell [23] which can interfere with protein folding in the ER. These results show that *CHOP* is induced in response to starvation for different essential amino acids without affecting *BiP* expression.

3.2. *Tunicamycin does not affect the expression of IGFBP-1, an unrelated gene induced by amino acid deprivation in another cell line (HepG2)*

To determine if the lack of correlation between expression of AAD-regulated genes and *BiP* is not restricted to *CHOP*, we investigated the effect of both tunicamycin and leucine limitation on the expression of *IGFBP-1*. It has previously been demonstrated that *IGFBP-1* expression is induced upon leucine limitation in HepG2 cells [24,25].

Consistent with the results shown above, *CHOP* is induced both by leucine limitation and tunicamycin treatment in these cells, whereas *BiP* is induced only in response to tunicamycin (Fig. 3). *IGFBP-1* is strongly induced by leucine limitation but is not affected by tunicamycin treatment. These results indicate that in HepG2 cells, the induction of *IGFBP-1* expression in response to leucine starvation is not correlated with *BiP* expression.

3.3. *Uncharged tRNA accumulation induces CHOP but does not affect BiP expression*

In yeast it has been demonstrated that AAD leads to accumulation of uncharged tRNA species and it is hypothesized that such uncharged tRNAs participate in signaling the up-regulation of numerous genes [26]. In order to induce the accumulation of uncharged tRNA in mammalian cells independent of AAD, we used a mutant CHO cell line with temperature sensitive lesion in leucyl-tRNA synthetase (CHO-tsH_1 cells). When these cells are exposed to non-permissive conditions (39.5°C), the leucyl-tRNA synthetase is inhibited, consequently leucyl-tRNAs are not synthesized and translation is impaired [27–29]. Exposure of CHO-tsH_1 cells to non-permissive conditions results in a strong induction of *CHOP* whereas *BiP* expression is not affected (Fig. 4). When the same experiment is performed with the corresponding wild-type CHO cells (CHO-K1), *CHOP* and *BiP* expression are not affected by the temperature shift to 39.5°C. The magnitude of *CHOP* induction is similar to that observed in response to 6 h leucine starvation when the cells are incubated at 34°C. Both cell lines have the capacity to induce *BiP* expression in response to tunicamycin. It is notable that the basal *CHOP* signal in the mutant cells growing at 34°C is slightly higher than in the parental CHO-K1 cells. This presumably reflects a partial defect in leucyl-tRNA synthetase activity even at the permissive temperature (about 25% of the wild-type cells) [28]. These experiments suggest that a stress signal induced by the

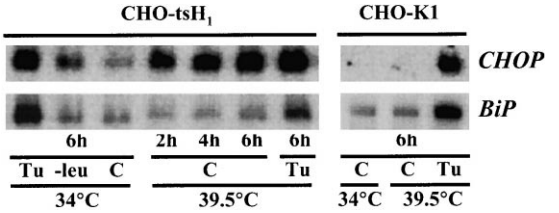

Fig. 4. Effect of leucyl-tRNA synthetase inhibition on the expression of *CHOP* and *BiP* mRNA. CHO-K1 or CHO-tsH_1 cells were incubated for 2, 4 or 6 h in DMEM/F12 control media (C), the same media lacking leucine (−leu), or containing 1.25 μg/ml of tunicamycin at the permissive temperature (34°C) or the non-permissive temperature (39.5°C). Total RNA was extracted, and Northern blot analysis was performed as described in Section 2. Each blot was hybridized with a probe corresponding to *CHOP* or *BiP*.

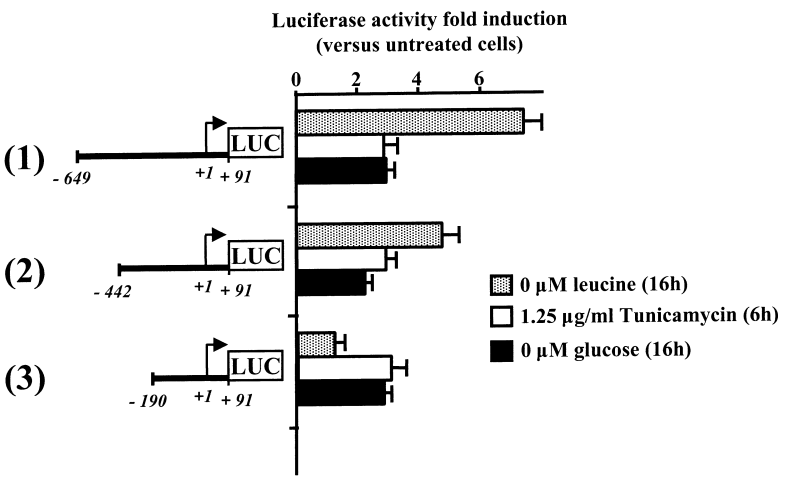

Fig. 5. Effect of deletion mutations on the activation of the *CHOP* promoter in response to leucine limitation, glucose starvation and tunicamycin treatment. The plasmids p*CHOP*-LUC (1), (2) and (3) correspond respectively to the human *CHOP* promoter region from nucleotide −649, −442, −190 to +91 fused to the luciferase (LUC) gene. HeLa cells were transiently transfected with these plasmids along with plasmid pCMV-βGal as described in Section 2. 24 h after transfection, cells were incubated for 6 or 16 h in DMEM/F12 control medium (untreated cells), lacking leucine (0 μM leucine), lacking glucose (0 μM glucose), or containing 1.25 μg/ml of tunicamycin. Cells were harvested for luciferase and β-Galactosidase assay. Results are given as the fold induction defined as the ratio of the relative LUC activity of treated cells to untreated cells.

presence of uncharged leucyl-tRNAs mediates *CHOP* induction without triggering a stress of the ER.

3.4. Amino acid starvation and agents inducing an ER stress regulate the CHOP promoter activity by using distinct cis elements

We have previously found that a promoter fragment, when linked to a reporter gene, is sufficient to mediate the regulation of *CHOP* expression by leucine starvation [7]. Serial deletions of the *CHOP* promoter were undertaken to highlight regions responsive to amino acid starvation. Luciferase activity driven by the longest *CHOP* promoter fragment tested is only minimally induced by agents that trigger the UPR whereas 16 h leucine starvation leads to a robust 8-fold increase in reporter gene activity (Fig. 5). It is noticeable that the expression of the endogenous *CHOP* gene is more induced in response to tunicamycin treatment or glucose deprivation than in response to AAD. The magnitude of promoter activation in response to leucine deprivation was markedly diminished by serial deletion whereas the response to tunicamycin treatment or glucose starvation was not affected by these deletions (Fig. 5). These results suggest that regulatory element(s) contained between −649 and −190 of the *CHOP* promoter are involved in mediating *CHOP* gene activation in response to leucine starvation but do not play an essential role in the induction of the gene by the UPR.

4. Discussion

Previous studies have emphasized the tight linkage between the activation of the *CHOP* gene and triggering of an ER stress response. *CHOP* expression had been shown to be coordinately regulated with the ER chaperone BiP and to be inducible by agents that lead to impairment in the folding environment of the ER. Recently, the link between *CHOP* expression and the UPR was furthered by the observation that a shared upstream signaling component, Ire1, participates in the co-induction of the *CHOP* and *BiP* genes in response to tunicamycin [15]. The major experimental observation presented here is that amino acid deprivation and unloading of tRNAs are stressful events in which *CHOP* induction is dissociated from the induction of *BiP*. Prior to this observation, it had been considered that amino acid limitation indirectly affects protein folding in the ER and activates *CHOP* through a pathway that is common to the inducers of the UPR [12]. Our findings suggest that *CHOP* induction can proceed by two different pathways, one that is ER dependent and the other ER independent. Our analysis of the *CHOP* promoter further suggests that both pathways feed into different *cis* acting DNA elements. It is likely that the *cis* element(s) involved in the response to ER stress are linked to Ire1 activation whereas the amino acid response element(s) are not dependent upon Ire1 activation. We have begun to define the elements responsible for promoter activation by AAD (Fig. 5) [30] but progress remains to make in identifying the regions responsive to ER stress. At this point we know that an 8 kb genomic fragment will recapitulate the marked induction of the gene by ER stress [15].

Our results also suggest that AAD does not have a significant effect on ER function. In mammalian cells, ER stress has been reported to activate at least two different pathways: one that culminates in the induction of ER chaperones such as BiP and another, referred to as the ER overload pathway and involves the activation of the transcription factor NF-kB [31]. Since NF-kB activity is not increased in response to leucine starvation (result not shown), it is likely that NF-kB does not participate to the amino acid regulation of *CHOP* expression.

The physiological significance of *CHOP* induction by an amino acid limitation is not known. Several studies have shown that severe protein malnutrition (kwashiorkor) leads to a drop of the blood amino acid concentration [2]. Under extreme nutritional conditions, cells might be subjected to limitation for essential amino acids at levels that might induce *CHOP*. We would speculate that CHOP participates in such downstream signaling in response to amino acid limitation, activating a subset of target genes [8]. Perhaps through its ability to inhibit the activity of C/EBP proteins [32], CHOP might participate in modulating these regulators of energy metabolism [33].

CHOP mRNA is regulated by a complex mechanism that is sensitive to the level of many different amino acids. The signaling pathways that recognize amino acid availability in mammalian cells have not been investigated extensively. In yeast, amino acid starvation gives rise to an accumulation of uncharged tRNA which stimulates the activity of the protein kinase GCN2. GCN2 turns on a signaling pathway that leads to the expression of numerous genes. In mammalian cells, previous observations suggested that tRNA charging is involved in the sensing of amino acid levels. Andrulis et al. [34] have demonstrated a correlation between asparagine starvation, amino acylation of $tRNA^{asn}$ and asparagine synthetase activity. Our results showing that inhibition of the leucyl-tRNA synthetase induces *CHOP* expression, are in agreement with this hypothesis. However, whether or not this signaling pathway is mechanistically analogous to the general control response of yeast is not presently known. Further work will be necessary to precisely determine the cascade of molecular events involved in the signaling pathway leading to activation of *CHOP* and similar genes by amino acid starvation.

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References

[1] Albanese, A.A. (1952) J. Clin. Nutr. 1, 44–51.
[2] Baertl, J.M., Placko, R.P. and Graham, G.G. (1974) Am. J. Clin. Nutr. 27, 733–742.
[3] Hinnebusch, A.G. (1988) Microbiol. Rev. 52, 248–273.
[4] Guerrini, L., Gong, S.S., Mangasarian, K. and Basilico, C. (1993) Mol. Cell. Biol. 13, 3202–3212.
[5] Kilberg, M.S., Hutson, R.G. and Laine, R.O. (1994) FASEB J. 8, 13–19.
[6] Marten, N.W., Burke, E.J., Hayden, J.M. and Straus, D.S. (1994) FASEB J. 8, 538–544.
[7] Bruhat, A., Jousse, C., Wang, X-Z., Ron, D., Ferrara, M. and Fafournoux, P. (1997) J. Biol. Chem. 272, 17588–17593.
[8] Wang, X.-Z., Kuroda, M., Sok, J., Batchvarova, N., Kimmel, R., Chung, P., Zinszner, H. and Ron, D. (1998) EMBO J. 17, 3619–3630.

[9] Zinszner, H., Kuroda, M., Wang, X.-Z., Batchvarova, N., Lightfoot, R.T., Remotti, H., Stevens, J.L. and Ron, D. (1998) Genes Dev. 12, 982–995.
[10] Halleck, M.M., Holbrook, N.J., Skinner, J., Liu, H. and Stevens, J.L. (1997) Cell Stress Chaperones 2, 31–40.
[11] Price, B.D. and Calderwood, S.K. (1992) Cancer Res. 52, 3814–3817.
[12] Wang, X.-Z. and Ron, D. (1996) Science 272, 1347–1349.
[13] Kozutsumi, Y., Segal, M., Normington, K., Gething, M.J. and Sambrook, J. (1988) Nature 332, 462–464.
[14] Lee, A.S. (1987) Trends Biochem. Sci. 12, 20–23.
[15] Wang, X.-Z., Harding, H.P., Zhang, Y., Jolicoeur, E.M., Kuroda, M. and Ron, D. (1998) EMBO J. 17, 5708–5717.
[16] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p. 7.46.
[18] Park, J.S., Luethy, J.D., Wang, M.G., Fargnoli, J., Fornace Jr, A.J., McBride, O.W. and Holbrook, N.J. (1992) Gene 116, 259–267.
[19] Davis, L.G., Dibner, M.D. and Battey, J. (1986) Basic Methods in Molecular Biology, Elsevier Science Publishing Co., Inc., New York.
[20] Hall, C.V., Jacob, P.E., Ringold, M. and Lee, F. (1983) J. Mol. Appl. Genet. 2, 101–109.
[21] Kilberg, M.S., Stevens, B.R. and Novak, D.A. (1993) Annu. Rev. Nutr. 13, 137–165.
[22] McGivan, J.D. and Pastor-Anglada, M. (1994) Biochem. J. 299, 321–334.
[23] Wang, S.T., Chen, H.W., Sheen, L.Y. and Lii, C.K. (1997) J. Nutr. 127, 2135–3141.
[24] Straus, D.S., Burke, E.J. and Marten, N.W. (1993) Endocrinology 132, 1090–1100.
[25] Jousse, C., Bruhat, A., Ferrara, M. and Fafournoux, P. (1998) Biochem. J. 334, 147–153.
[26] Hinnebusch, A.G. (1994) TIBS 19, 409–414.
[27] Clemens, M.J., Galpine, A., Austin, S.A., Panniers, R., Henshaw, E.C., Duncan, R., Hershey, J.W. and Pollard, J.W. (1987) J. Biol. Chem. 262, 767–771.
[28] Pollard, J.W., Galpine, A.R. and Clemens, M.J. (1989) Eur. J. Biochem. 182, 1–9.
[29] Thompson, L.H., Harkins, J.L. and Stanners, C.P. (1973) Proc. Natl. Acad. Sci. USA 70, 3094–3098.
[30] Bruhat, A., Jousse, C. and Fafournoux, P., submitted for publication.
[31] Pahl, H.L. and Baeuerle, P.A. (1995) EMBO J. 14, 2580–2588.
[32] Ron, D. and Habener, J.F. (1992) Genes Dev. 6, 439–453.
[33] McKnight, S., Lane, M.D. and Gluecksohn-Waelsch, S. (1989) Genes Dev. 3, 2021–2024.
[34] Andrulis, I.L., Hatfield, G.W. and Arfin, S.M. (1979) J. Biol. Chem. 254, 10629–10633.