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Research paper

Activation of the antioxidant response in methionine deprived human cells results in an HSF1-independent increase in HSPA1A mRNA levels

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ABSTRACT

In cells starved for leucine, lysine or glutamine heat shock factor 1 (HSF1) is inactivated and the level of the transcripts of the HSF1 target genes HSPA1A (Hsp70) and DNAJB1 (Hsp40) drops. We show here that in HEK293 cells deprived of methionine HSF1 was similarly inactivated but that the level of HSPA1A and DNAJB1 mRNA increased. This increase was also seen in cells expressing a dominant negative HSF1 mutant (HSF379 or HSF1-K80Q), confirming that the increase is HSF1 independent. The antioxidant N-acetylcysteine completely inhibited the increase in HSPA1A and DNAJB1 mRNA levels upon methionine starvation, indicating that this increase is a response to oxidative stress resulting from a lack of methionine. Cells starved for methionine contained higher levels of c-Fos and FosB mRNA, but knockdown of these transcription factors had no effect on the HSPA1A or DNAJB1 mRNA level. Knockdown of NRF2 mRNA resulted in the inhibition of the increase in the HSPA1A mRNA, but not the DNAJB1 mRNA, level in methionine starved cells. We conclude that methionine deprivation results in both the amino acid deprivation response and an antioxidant response mediated at least in part by NRF2. This antioxidant response includes an HSF1 independent increase in the levels of HSPA1A and DNAJB1 mRNA.

1. Introduction

Amino acids are the building blocks of proteins and can also serve as intermediates in metabolism. The amino acid availability is closely monitored [1]. When cells sense a lack of one or more amino acids the amino acid response is mounted. Upon accumulation of uncharged tRNAs the general control non-derepressible 2 (GCN2) kinase is activated [2] and subsequently eukaryotic translation initiation factor 2α (eIF2α) is phosphorylated. eIF2α phosphorylation then leads to the inhibition of the global protein synthesis and the selective translation of some mRNAs, e.g. ATF4 mRNA (reviewed in Ref. [3]). ATF4 is an important player in the amino acid response: most amino acid responsive genes are ATF4 targets of which asparagine synthetase (ASNS) is the most widely studied one [4,5]. Petti et al. [6] showed that in yeast methionine starvation differs from starvation for other amino acids in that survival was substantially higher compared to for example leucine starvation. Furthermore, methionine has been shown to have a unique effect on fecundity in *Drosophila* upon dietary restriction, a restriction in food intake that does not lead to malnutrition. Grandison et al. [7] described a decrease in fecundity upon reduced food intake, and adding back methionine alone was sufficient to increase fecundity to the same level as did full feeding. Adding back other amino acids did not show this effect. These data thus suggest that starvation for methionine does not equal starvation for other amino acids.

Next to its importance in protein synthesis, the essential amino acid methionine is also involved in the transsulfuration pathway, a pathway in which methionine is converted via the formation of S-adenosylmethionine (SAM) into homocysteine and subsequently cysteine [8]. Cysteine availability is important for the synthesis of glutathione, a molecule that has been shown to have a strong antioxidative effect. A lack of methionine might thus have an effect on the formation of glutathione and can thereby affect the oxidative status of the cell. In the literature conflicting results about methionine deprivation and the antioxidant response are described. Erdmann et al. [9] showed that addition of L-methionine reduced free radical formation in endothelial cells through the

Abbreviations: GCN2, general non-derepressible 2; ATF, activating transcription factor; ASNS, asparagine synthetase; SAM, S-adenosylmethionine; HMOX1, heme oxygenase 1; GSTP, glutathione S-transferase P; HSF1, heat shock factor 1; HSE, heat shock element; NAC, N-acetylcysteine; NRF2, nuclear factor (erythroid-derived 2)-related factor 2; ARE, antioxidant response element; TPA, 12-*O*-tetradecanoyl phorbol13-acetate; TRE, TPA-responsive element; KEAP1, Kelch-like ECH associated protein 1; EpRE, electrophile-responsive element.

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induction of heme oxygenase 1 (HMOX1). On the other hand it was described that methionine supplementation increases mitochondrial ROS production and mitochondrial DNA oxidative damage in rat liver mitochondria [10] and vice versa that methionine restriction decreases mitochondrial ROS generation and oxidative damage to mitochondrial DNA and proteins, indicating the activation of the antioxidant response [11,12]. Recently, Lin et al. [13] demonstrated that the increased synthesis of glutathione S-transferase P (GSTP) in methionine restricted rat hepatocytes is due to activation of the transcription factor NRF2, a factor involved in the antioxidant response.

We have previously shown that upon starvation for leucine, lysine or glutamine the transcription factor heat shock factor 1 (HSF1), which regulates the proteotoxic stress response, is inactivated and that the mRNA levels of the HSF1 target genes HSPA1A, DNAJB1 and HSP90AA1 levels are strongly decreased [14]. The complex cellular response to methionine starvation made us wonder whether HSF1 is also inactivated in methionine starved cells. We show here that HSF1 indeed also loses its DNA binding affinity in methionine starved cells, but that, unexpectedly and in contrast to what is found during starvation for other amino acids, HSPA1A and DNAJB1 mRNA levels do increase. This increase was not dependent on HSF1 but, at least for HSPA1A mRNA, on NRF2. These data show that in methionine starved cells an antioxidant response is superimposed on the amino acid response.

2. Materials and methods

2.1. Tissue culture

T-REx HEK293-pcDNA5, HEK-HSF379 (dnHSF1) and HEK-HSF1 K80Q cell lines were generated as described before [14,15]. The cells were cultured at 37 °C/5% CO_2 in high glucose DMEM medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Blasticidin (1.65 μg/ml; Invitrogen) and 100 μg/ml hygromycin were also added to the culture medium during maintenance of the cell lines, but were omitted during experiments. For amino acid starvation experiments cells were washed with PBS and subsequently DMEM/F12 medium (Sigma) with or without methionine or leucine, supplemented with 10% dialyzed fetal calf serum, was added for the indicated times.

2.2. RNA isolation and reverse transcription

Total RNA was isolated using Trizol (Invitrogen). 1 μg of RNA was treated with DNaseI (Amplification grade; RNase-free; Invitrogen). Subsequently, 5 mM $MgCl_2$, RT-buffer, 1 mM dNTPs, 18.75 units AMV reverse transcriptase, 20 units RNase inhibitors and 1.25 μM oligo(dT) were added to a total volume of 20 μl. Reverse transcription was performed for 10 min at 25 °C, 60 min at 42 °C and 5 min at 95 °C. For QPCR analysis, cDNA was 10-fold diluted.

2.3. Chromatin immunoprecipitation

T-REx HEK293-pcDNA5 cells were cultured for 24 h in the presence or absence of methionine. Chromatin immunoprecipitation was performed as described in Ref. [16], except that cells were crosslinked for 15 min with 1% formaldehyde. After quenching with 125 mM glycine, cells were washed twice with ice cold PBS and resuspended in ice cold lysis buffer (50 mM HEPES·KOH pH 7.6, 140 mM NaCl, 1 mM EDTA pH 8.0, 1% (v/v) Triton X-100, 0.1% NaDOC and 1× protease inhibitor cocktail). Antibodies used for ChIP were rabbit polyclonal ATF4 antibody (sc-200; Santa Cruz) and rabbit polyclonal HSF1 antibody (SPA-901; Stressgen). ChIP samples were analyzed by QPCR with the primer sets listed in Supplementary data Table S1.

2.4. Electrophoretic mobility shift assay

HEK293 cells were cultured for 24 h in the presence or absence of methionine. Cells were immediately harvested and nuclear extracts were prepared using NE-per nuclear and cytoplasmic reagents (Pierce). Extracts were aliquoted and stored at −80 °C. Oligonucleotide probes were end-labeled with ^{32}P. The sequences of the oligonucleotides used in EMSA are listed in Supplementary data Table S1. The EMSA protocol was adapted from Refs. [17,18]. A mixture containing 5 μg nuclear extract and 3 μg poly dIdC in binding buffer [20 mM HEPES pH 7.9, 100 mM KCl, 1 mM EDTA, 1 mM DTT, 4% (v/v) Ficoll, 1× PhosSTOP (Roche)] was incubated for 20 min on ice. 0.01 pmol radiolabeled oligonucleotide was added and the samples were incubated for 20 min at room temperature. DNA–protein complexes were separated on a pre-run 4% polyacrylamide gel in 0.25× TBE with recirculation of the buffer. The gel was dried and signals were visualized using a PhosphorImager.

2.5. RNA interference

The control siRNA against luciferase (5′-CGUACGCGGAAUACUUCGAdTdT-3′), NRF2 siRNA (5′-CAGCAUGCUACGUGAUGAAdT dT-3′) and NRF2 siRNA#2 (5′-CCAGUGGAUCUGCCAACUAdT dT-3′) were purchased from Eurogentec. C-Fos (sc-29221) and FosB (sc-35403) siRNAs were purchased from Santa Cruz Biotechnology. HEK293 cells were cultured in 6-well plates and transfected with 50 nM siRNA using oligofectamine transfection reagent (Invitrogen) according to the manufacturer's instructions. 48 h after transfection cells were re-transfected as described above. Medium was changed to DME/F12 with or without methionine 24 h after re-transfection and cells were harvested 24 h later.

2.6. Quantitative real-time PCR

Quantitative real-time PCR was performed using the StepOnePlus™ Real-Time PCR System with *Power* SYBR® Green PCR Master mix (Applied Biosystems) using the following amplification protocol: 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Per reaction 3 μl of diluted cDNA or ChIP material was used and the DNA was amplified using primers for the sequences of interest, listed in Supplementary data Table S1. Two-tailed Student's *t* tests were performed to calculate the significance of the data.

3. Results and discussion

3.1. Increased HSPA1A and DNAJB1 mRNA levels in methionine deprived HEK293 cells

In a previous study we described that leucine, lysine or glutamine deprivation results in the inactivation of HSF1 and a concomitant decrease in heat shock protein mRNA levels [14]. To determine whether methionine deprivation has a similar effect, we measured HSPA1A and DNAJB1 mRNA levels in methionine starved HEK293 cells and found these, in contrast with the effect of leucine, lysine and glutamine starvation, to be increased (Fig. 1A). Already within 4 h of methionine starvation an increase in HSPA1A mRNA was seen; the maximal level was reached within 8 h (Fig. 1B). The mRNA level of DNAJB1 increased only later (Fig. 1C). Note that, although the increase in HSPA1A mRNA level upon methionine deprivation was consistently found, the extent of change in the HSPA1A mRNA levels varied between 3- and 30-fold between

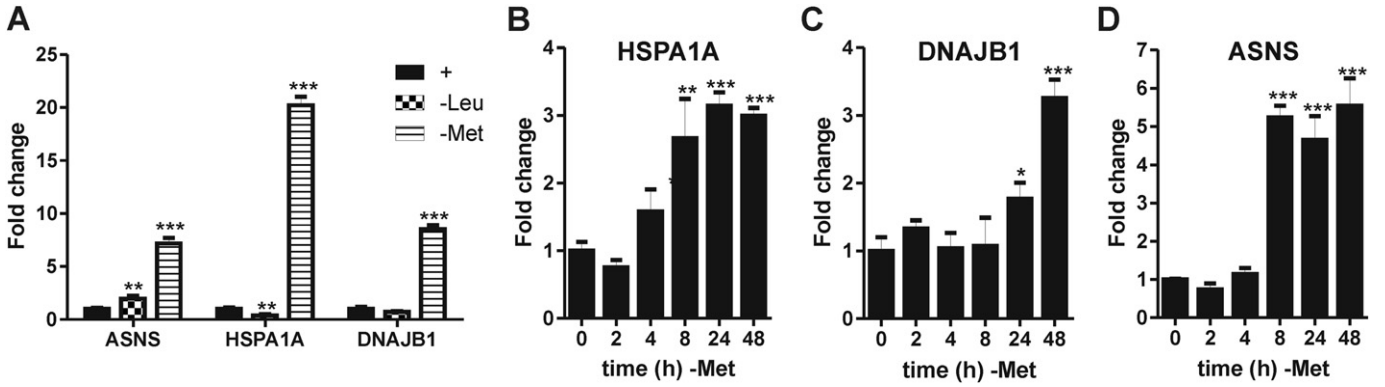

Fig. 1. Methionine starvation increases HSPA1A and DNAJB1 mRNA levels. (A) HEK293 cells were deprived of leucine or methionine for 24 h. (B–D) HEK293 cells were deprived of methionine and harvested at the indicated time points. mRNA levels were determined by QPCR analysis and are shown relative to GAPDH mRNA levels. Error bars represent SD; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, relative to fed cells (+ or 0).

different experiments (compare Fig. 1A and B) for unknown reasons and in spite of our efforts to keep culture conditions identical between experiments. Note also that compared to the induction levels of HSPA1A mRNA upon heat stress, the effect of methionine deprivation is relatively small. The protein levels of HSPA1A and DNAJB1 did not noticeably increase in methionine starved cells (Fig. S1); presumably the rate of de novo protein synthesis in methionine starved cells is low. ASNS mRNA levels were 5-fold increased upon starvation for methionine (Fig. 1D), indicating that the amino acid response was activated. The increase in HSPA1A and DNAJB1 mRNA suggests that HSF1 is active when cells are starved for methionine. To determine whether HSF1 is indeed activated upon methionine deprivation, we performed an electrophoretic mobility shift assay with nuclear extracts of methionine deprived cells and examined binding of HSF1 to the heat shock element (HSE). Unexpectedly, this showed that HSF1 binding to the HSE decreases in nuclear extracts of methionine starved cells compared to fed cells (Fig. 2A), just as it does when cells are starved for other amino acids [14]. The HSF1 protein level or cellular location was not affected by methionine deprivation (Fig. S2). As a control we measured binding to the NSRU sequence of the ASNS promoter, which showed increased binding in nuclear extracts of

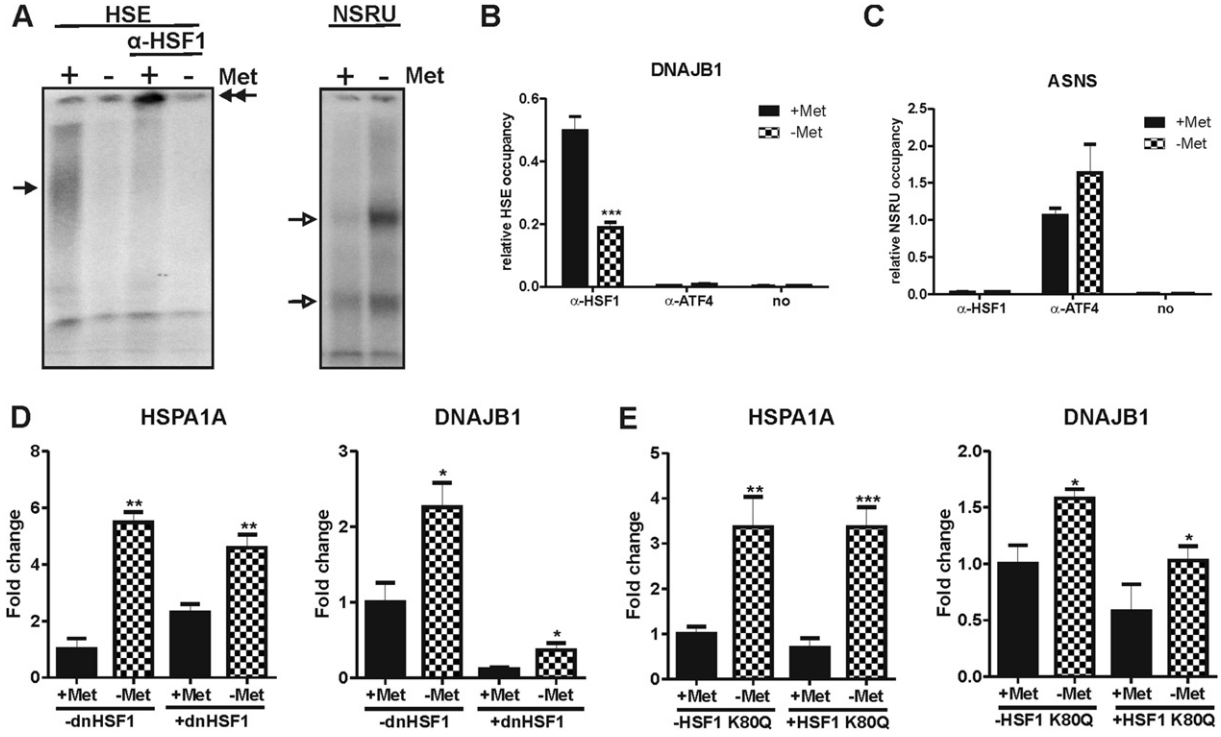

Fig. 2. The increase in HSPA1A and DNAJB1 mRNA levels upon methionine starvation is HSF1 independent. (A) HEK293 cells were deprived of methionine for 24 h. Nuclear extracts were used in an electrophoretic mobility shift assay with a double-stranded oligonucleotide with the NSRU or HSE sequence. Supershifts were induced with an anti-HSF1 antibody. Single closed arrows indicate the primary HSE complexes formed; double closed arrows indicate the supershifted HSE complexes. Open arrows indicate the NSRU complexes formed. (B and C) HEK293 cells were starved for methionine for 24 h. Chromatin immunoprecipitation was performed using an anti-HSF1 or an anti-ATF4 antibody. Bound chromatin was analyzed by QPCR using a primer set surrounding the HSE of the DNAJB1 promoter or the NSRU of the ASNS promoter. As a control the ChIP was performed without an antibody. (D and E) HEK293-dnHSF1 or HEK293-HSF1 K80Q cells were deprived of methionine in the presence or absence of doxycycline and harvested after 24 h. mRNA levels were determined by QPCR analysis and are shown relative to GAPDH mRNA levels. Error bars represent SD; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, relative to +Met.

methionine deprived cells compared to fed cells. The loss in HSF1 binding was confirmed by chromatin immunoprecipitation with an anti-HSF1 antibody: less HSF1 was bound to the heat shock element of the DNAJB1 promoter in methionine starved cells (Fig. 2B). At the same time an increase in ATF4 binding to the ASNS promoter was detected (Fig. 2C). Thus, even though HSPA1A and DNJAB1 mRNA levels increase upon starvation for methionine, HSF1 does lose its DNA binding affinity. These results imply that the increase in these HSP mRNA levels upon methionine deprivation is independent of HSF1. To confirm these findings, we used two cell lines stably expressing an HSF1 mutant, either a dominant negative HSF1 (dnHSF1) [15], which lacks the activation domain, or HSF1 K80Q [19], which contains a point mutation in the DNA binding domain and is therefore unable to bind to the DNA. We measured the HSPA1A and DNAJB1 mRNA levels upon methionine starvation in the presence of either of these HSF1 mutants. In the absence of mutant HSF1 expression, both cell lines showed an increase in HSPA1A and DNAJB1 mRNA levels upon deprivation of methionine (Fig. 2D and E). When doxycycline was added, i.e. when the HSF1 mutants were expressed, HSPA1A and DNAJB1 mRNA levels were still increased relative to methionine fed cells. Note that DNAJB1 mRNA levels are strongly decreased upon expression of dnHSF1 in either fed or starved cells (Fig. 2D); this is in agreement with our previous observations [15], where DNAJB1 mRNA levels were 4-fold decreased in the presence of dnHSF1. All together, these data indicate that the increase in HSP mRNA levels is indeed independent of HSF1.

3.2. Activation of the antioxidant response in methionine starved HEK293 cells

As mentioned above, a lack of methionine could have an effect on the oxidative status of the cell, which could signal the increase in the heat shock protein mRNA levels. If so, addition of an antioxidant would counteract these changes. When we deprived cells of methionine and added the antioxidant N-acetylcysteine (NAC) both HSPA1A and DNAJB1 mRNA levels were strongly decreased compared to those in cells that were deprived of methionine without the addition of NAC (Fig. 3A and B). The addition of NAC appears to abolish the amino acid response in that the ASNS mRNA level no longer increased when methionine was withdrawn (Fig. 3C). However it has been shown before that addition of NAC induces ER stress in HeLa cells [20], which in turn activates transcription of the ASNS gene [21]. This would obscure the response of the ASNS promoter to amino acid deprivation. Overall, the fact that addition of the antioxidant NAC completely inhibited the increase in HSPA1A and DNAJB1 mRNA levels in methionine starved cells indicates that this increase is due to oxidative stress caused by the lack of methionine. Indeed we do find an increase in HMOX1 mRNA levels upon methionine deprivation (Fig. 4A), indicating activation of the antioxidant response.

It has been shown previously that oxidizing agents can induce the transcription of heat shock protein genes via HSF1 activation [22–24]. The results described above, however, demonstrated an HSF1 independent increase in HSPA1A and DNAJB1 mRNA levels, which is most likely mediated by the activation of another transcription factor. An important player in the antioxidant response is NRF2 (nuclear factor (erythroid-derived 2)-related factor 2), a transcription factor that controls the expression of antioxidant response element (ARE)-regulated antioxidant and cytoprotective genes [25,26], among which is HMOX1. Recently, it has been shown that in rat primary hepatocytes NRF2 is indeed activated upon methionine starvation [13]. Another candidate is the transcription factor AP1, as glutathione depletion has been shown to result in increased expression of c-Fos and FosB, both AP1 family members [27]. AP1 binds to the TRE (TGACTCA; 12-*O*-tetradecanoyl phorbol13-acetate (TPA)-responsive element) of which the sequence is identical to that of the core of the ARE, the NRF2 consensus binding sequence (ATGACTCAGCA). When we examined binding to the ARE in nuclear extracts from methionine starved cells in an EMSA, we found increased complex formation compared to fed cells (Fig. 4B). The signal of the complex binding to the ARE probe was decreased when we competed with a 10-fold molar excess of cold ARE oligo (Fig. 4B). These data show that indeed the activity of NRF2 and/or AP1 is increased in methionine deprived cells.

To look further into possible roles for AP1 and NRF2 in the increased HSP mRNA levels in methionine deprived cells, we knocked down c-Fos, FosB, or NRF2 mRNA and examined the effect on HSPA1A and DNAJB1 mRNA levels. Methionine deprivation indeed led to a 10- to 15-fold increase in c-Fos and FosB mRNA levels (Fig. S3A, B). Curiously, knocking down c-Fos mRNA resulted in an even larger increase in the FosB mRNA level in methionine deprived cells (Fig. S3B). Conversely, knocking down FosB mRNA enhanced the level of c-Fos mRNA (Fig. S3A). HSPA1A and DNAJB1 transcript levels remained unaffected when either c-Fos or FosB mRNA was knocked down (Fig. S3C and D). These data suggest that c-Fos and FosB are not responsible for the increase in HSPA1A or DNAJB1 mRNA levels upon methionine deprivation, although we

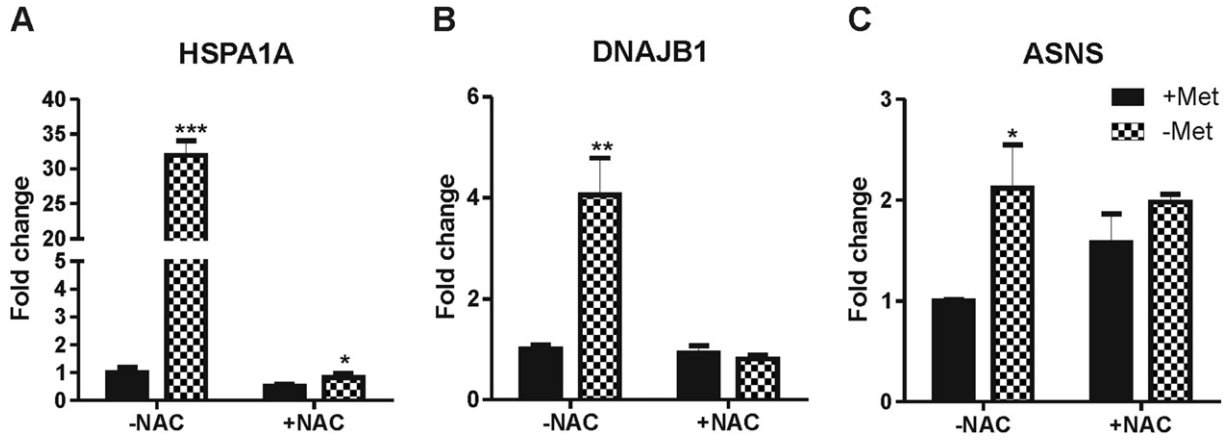

Fig. 3. N-acetylcysteine inhibits the increase in HSPA1A and DNAJB1 mRNA levels upon methionine starvation. (A–C) HEK293 cells were deprived of methionine for 24 h in the presence or absence of 10 mM N-acetylcysteine (NAC). mRNA levels were determined by QPCR analysis and are shown relative to GAPDH mRNA levels. Error bars represent SD; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, relative to +Met.

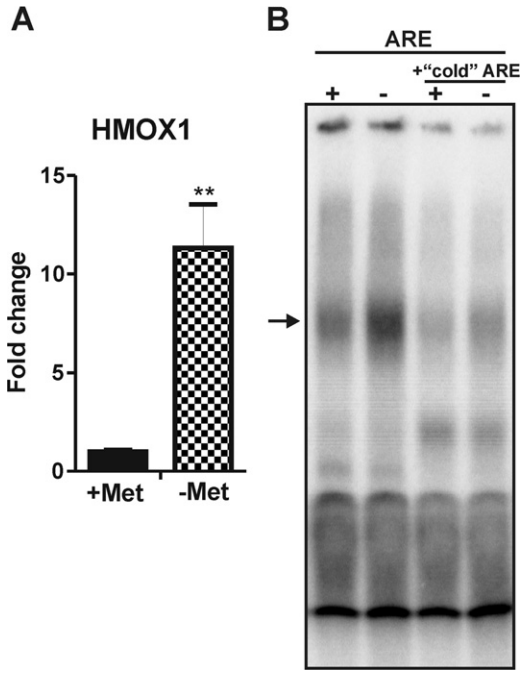

Fig. 4. Increased DNA binding to the ARE sequence in methionine starved cells. (A) HEK293 cells were deprived of methionine for 24 h. HMOX1 mRNA levels were determined by QPCR analysis and are shown relative to GAPDH mRNA levels. Error bars represent SD; $^{**}P < 0.01$, relative to +Met. (B) HEK293 cells were deprived of methionine for 24 h. Nuclear extracts were used in an electrophoretic mobility shift assay with a double-stranded oligonucleotide with the ARE sequence. A 10-fold molar excess of unlabeled ARE probe ("cold") was added to determine the specificity of the signal. The arrow indicates the ARE complexes formed.

cannot rigorously exclude that the lack of c-Fos is compensated for by enhanced synthesis of FosB or vice versa. The level of NRF2 mRNA increased only slightly in methionine deprived cells (Fig. S4). The activity of NRF2 is regulated post-translationally by the cysteine rich protein KEAP1 (*K*elch-like ECH associated protein 1), which acts as a sensor for oxidative stress. Under non-stress conditions KEAP1 retains NRF2 in the cytoplasm [28] and maintains it at a low level through KEAP1-dependent ubiquitination and proteasomal degradation [29–33]. Upon oxidative stress, cysteines in the KEAP1 protein are oxidatively modified, resulting in a conformational change and release of NRF2 [30]. When we depleted NRF2 mRNA by siRNA the increase in mRNA levels of its target gene HMOX1 upon methionine starvation was inhibited by about 50% (Fig. 5A). The increase in HSPA1A mRNA levels was also inhibited by about 50% upon siRNA treatment for NRF2 (Fig. 5B), whereas the increase in DNAJB1 mRNA levels (Fig. 5C) and ASNS mRNA levels (Fig. 5D) was not affected. These results were confirmed using a second NRF2 siRNA (Fig. S5). We did not detect a change in HSPA1A mRNA stability in methionine deprived cells either in the presence or absence of NRF2 mRNA (data not shown), unlike in leucine or glutamine starved cells where HSPA1A mRNA is destabilized [14,34]. These data thus suggest that the increase in HSPA1A mRNA level is due to NRF2 directed enhanced transcription. Almeida et al. [35] described the presence of an electrophile-responsive element (EpRE), to which NRF2 can bind, in the promoter of the Hsp70 gene in zebrafish and this element was conserved between mouse and zebrafish. We were unable to detect an involvement of NRF2 in the increase in DNAJB1 mRNA levels in methionine deprived cells, even though the murine Dnajb1 gene has been reported to be a target of Nrf2 [36,37]. The delay in the increase in DNAJB1 mRNA suggests that this increase may be a secondary effect. Apparently, in methionine restricted cells there is a difference between the regulation of transcription of the HSPA1A and DNAJB1 promoters. In contrast to HSPA1A, DNAJB1 is

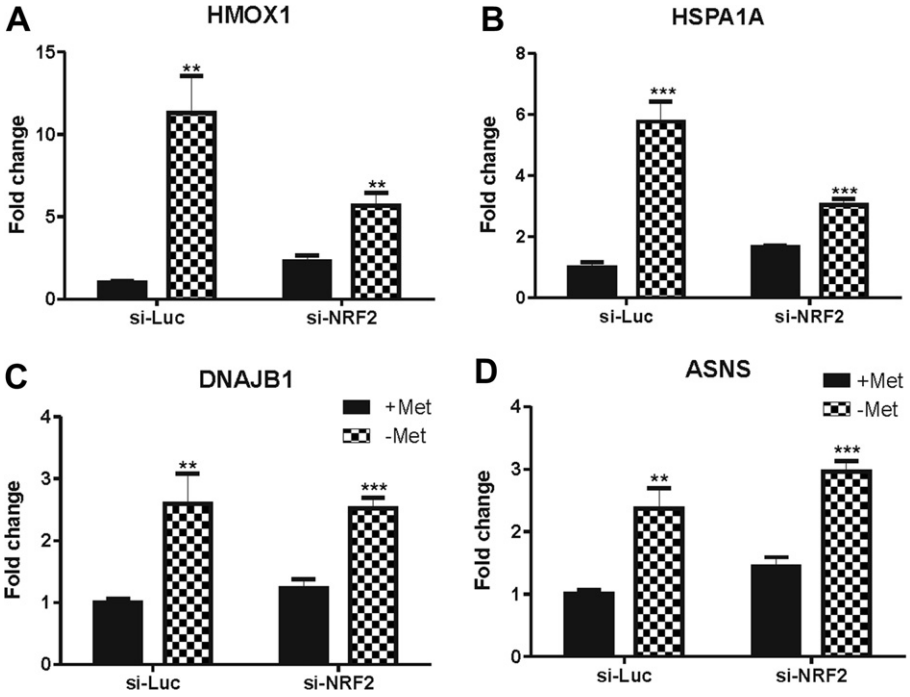

Fig. 5. Transcript levels in NRF2 siRNA treated, methionine deprived cells. (A–D) HEK293 cells were transfected for 96 h with siRNA against NRF2 or luciferase as a control with a re-transfection at 48 h. 24 h after re-transfection cells were deprived of methionine for 24 h. mRNA levels were determined by QPCR analysis and are shown relative to GAPDH mRNA levels. Error bars represent SD; $^{**}P < 0.01$; $^{***}P < 0.001$, relative to +Met.

present in high levels in non-stressed cells and not much is known about the regulatory elements of the promoter of the DNAJB1 gene.

Our results show that methionine deprivation results in increased HSPA1A and DNAJB1 mRNA levels, in spite of the inactivation of HSF1, probably due to the activation of the antioxidant response simultaneously with the amino acid deprivation response. The increase in HSP mRNA levels is relatively low compared to that in heat stressed cells and it is not clear whether the increase in the level of the resulting gene products, the heat shock proteins, is sufficient to protect the cell from proteotoxic stress resulting from the change in cellular oxidative status. It could well be that the products of other NRF2 target genes are of more importance in dealing with the damage resulting from the oxidative insult. As described previously [14], leucine starvation results in the inactivation of HSF1 and a subsequent decrease in HSP mRNA levels, which would lead to a loss in chaperoning capacity and enhanced sensitivity to proteotoxic stress, i.e. cellular frailty. Starvation for methionine does also result in the inactivation of HSF1, but heat shock mRNA levels do not decrease, suggesting that methionine deprived cells remain robust.

Our studies were performed using methionine starved tissue culture cells and our results may not apply directly to methionine restriction, which has been shown to increase lifespan in rodent studies [38–41]. The beneficial effects of methionine restriction are likely to be mediated in part by tissue-specific effects on the transcriptome, as in the case of caloric restriction [42], and in part by systemic factors, such as a lower IGF-1 level [40,43]. Our results do suggest that in methionine restricted cells the antioxidant response is primed and can be quickly called into action to prevent deleterious effects if the methionine level drops too low. However, the loss of HSF1 activity in methionine deprived cells would be predicted to affect longevity adversely [44], but this is perhaps compensated by the increase in the level of at least some heat shock proteins. At least in *C. elegans*, the loss of HSF1 can be compensated in part by exogenous expression of small heat shock proteins [45]. Intriguingly, methionine restriction also delays tumor growth (for review, see Ref. [46]). Malignant cells are dependent upon an HSF1 directed transcriptional program that is distinct from the HSF1 directed heat shock response [47]. It would be of interest to determine whether loss of HSF1 activity in methionine deprived tumor cells plays a role in the inhibitory effect of methionine restriction on tumor growth.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.biochi.2013.01.017.

References

[1] G. Wu, Amino acids: metabolism, functions, and nutrition, Amino Acids 37 (2009) 1–17.
[2] T. Krishnamoorthy, G.D. Pavitt, F. Zhang, T.E. Dever, A.G. Hinnebusch, Tight binding of the phosphorylated alpha subunit of initiation factor 2 (eIF2alpha) to the regulatory subunits of guanine nucleotide exchange factor eIF2B is required for inhibition of translation initiation, Mol. Cell. Biol. 21 (2001) 5018–5030.
[3] R.C. Wek, H.Y. Jiang, T.G. Anthony, Coping with stress: eIF2 kinases and translational control, Biochem. Soc. Trans. 34 (2006) 7–11.
[4] C. Zhong, C. Chen, M.S. Kilberg, Characterization of the nutrient-sensing response unit in the human asparagine synthetase promoter, Biochem. J. 372 (2003) 603–609.
[5] H. Chen, Y.X. Pan, E.E. Dudenhausen, M.S. Kilberg, Amino acid deprivation induces the transcription rate of the human asparagine synthetase gene through a timed program of expression and promoter binding of nutrient-responsive basic region/leucine zipper transcription factors as well as localized histone acetylation, J. Biol. Chem. 279 (2004) 50829–50839.
[6] A.A. Petti, C.A. Crutchfield, J.D. Rabinowitz, D. Botstein, Survival of starving yeast is correlated with oxidative stress response and nonrespiratory mitochondrial function, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) E1089–E1098.
[7] R.C. Grandison, M.D. Piper, L. Partridge, Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*, Nature 462 (2009) 1061–1064.
[8] G.J. McBean, The transsulfuration pathway: a source of cysteine for glutathione in astrocytes, Amino Acids 42 (2012) 199–205.
[9] K. Erdmann, N. Grosser, H. Schroder, L-Methionine reduces oxidant stress in endothelial cells: role of heme oxygenase-1, ferritin, and nitric oxide, AAPS J. 7 (2005) E195–E200.
[10] J. Gomez, P. Caro, I. Sanchez, A. Naudi, M. Jove, M. Portero-Otin, M. Lopez-Torres, R. Pamplona, G. Barja, Effect of methionine dietary supplementation on mitochondrial oxygen radical generation and oxidative DNA damage in rat liver and heart, J. Bioenerg. Biomembr. 41 (2009) 309–321.
[11] A. Sanz, P. Caro, V. Ayala, M. Portero-Otin, R. Pamplona, G. Barja, Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins, FASEB J. 20 (2006) 1064–1073.
[12] I. Sanchez-Roman, A. Gomez, J. Gomez, H. Suarez, C. Sanchez, A. Naudi, V. Ayala, M. Portero-Otin, M. Lopez-Torres, R. Pamplona, G. Barja, Forty percent methionine restriction lowers DNA methylation, complex I ROS generation, and oxidative damage to mtDNA and mitochondrial proteins in rat heart, J. Bioenerg. Biomembr. 43 (2011) 699–708.
[13] A.H. Lin, H.W. Chen, C.T. Liu, C.W. Tsai, C.K. Lii, Activation of Nrf2 is required for up-regulation of the pi class of glutathione S-transferase in rat primary hepatocytes with l-methionine starvation, J. Agric. Food Chem. 60 (2012) 6537–6545.
[14] S.M. Hensen, L. Heldens, C.M. van Enckevort, S.T. van Genesen, G.J. Pruijn, N.H. Lubsen, Heat shock factor 1 is inactivated by amino acid deprivation, Cell Stress Chaperones (Jan 16 2013) (Epub ahead of print).
[15] L. Heldens, R.P. Dirks, S.M. Hensen, C. Onnekink, S.T. van Genesen, F. Rustenburg, N.H. Lubsen, Co-chaperones are limiting in a depleted chaperone network, Cell. Mol. Life Sci. 67 (2010) 4035–4048.
[16] S. Denissov, M. van Driel, R. Voit, M. Hekkelman, T. Hulsen, N. Hernandez, I. Grummt, R. Wehrens, H. Stunnenberg, Identification of novel functional TBP-binding sites and general factor repertoires, EMBO J. 26 (2007) 944–954.
[17] E.J. Klok, S.T. van Genesen, A. Civil, J.G. Schoenmakers, N.H. Lubsen, Regulation of expression within a gene family. The case of the rat gamma B- and gamma D-crystallin promoters, J. Biol. Chem. 273 (1998) 17206–17215.
[18] M.M. Thiaville, E.E. Dudenhausen, C. Zhong, Y.X. Pan, M.S. Kilberg, Deprivation of protein or amino acid induces C/EBPbeta synthesis and binding to amino acid response elements, but its action is not an absolute requirement for enhanced transcription, Biochem. J. 410 (2008) 473–484.
[19] S.D. Westerheide, J. Anckar, S.M. Stevens Jr., L. Sistonen, R.I. Morimoto, Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1, Science 323 (2009) 1063–1066.
[20] D. Guan, Y. Xu, M. Yang, H. Wang, X. Wang, Z. Shen, N-acetyl cysteine and penicillamine induce apoptosis via the ER stress response-signaling pathway, Mol. Carcinog. 49 (2010) 68–74.
[21] A. Gjymishka, N. Su, M.S. Kilberg, Transcriptional induction of the human asparagine synthetase gene during the unfolded protein response does not require the ATF6 and IRE1/XBP1 arms of the pathway, Biochem. J. 417 (2009) 695–703.
[22] M.R. Jacquier-Sarlin, B.S. Polla, Dual regulation of heat-shock transcription factor (HSF) activation and DNA-binding activity by H_2O_2: role of thioredoxin, Biochem. J. 318 (Pt 1) (1996) 187–193.
[23] H. Liu, R. Lightfoot, J.L. Stevens, Activation of heat shock factor by alkylating agents is triggered by glutathione depletion and oxidation of protein thiols, J. Biol. Chem. 271 (1996) 4805–4812.
[24] Y. Zhang, Y.H. Ahn, I.J. Benjamin, T. Honda, R.J. Hicks, V. Calabrese, P.A. Cole, A.T. Dinkova-Kostova, HSF1-dependent upregulation of Hsp70 by sulfhydryl-reactive inducers of the KEAP1/NRF2/ARE pathway, Chem. Biol. 18 (2011) 1355–1361.
[25] K. Itoh, T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Hatayama, M. Yamamoto, Y. Nabeshima, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, Biochem. Biophys. Res. Commun. 236 (1997) 313–322.
[26] T.H. Rushmore, M.R. Morton, C.B. Pickett, The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity, J. Biol. Chem. 266 (1991) 11632–11639.
[27] M. Fratelli, L.O. Goodwin, U.A. Orom, S. Lombardi, R. Tonelli, M. Mengozzi, P. Ghezzi, Gene expression profiling reveals a signaling role of glutathione in redox regulation, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 13998–14003.
[28] K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J.D. Engel, M. Yamamoto, Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain, Genes Dev. 13 (1999) 76–86.

[29] S.B. Cullinan, J.D. Gordan, J. Jin, J.W. Harper, J.A. Diehl, The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase, Mol. Cell. Biol. 24 (2004) 8477–8486.
[30] K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, T. O'Connor, M. Yamamoto, Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles, Genes Cells 8 (2003) 379–391.
[31] M. McMahon, K. Itoh, M. Yamamoto, J.D. Hayes, Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression, J. Biol. Chem. 278 (2003) 21592–21600.
[32] D.D. Zhang, M. Hannink, Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress, Mol. Cell. Biol. 23 (2003) 8137–8151.
[33] D.D. Zhang, S.C. Lo, J.V. Cross, D.J. Templeton, M. Hannink, Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex, Mol. Cell. Biol. 24 (2004) 10941–10953.
[34] M.M. Eliasen, M. Brabec, C. Gerner, J. Pollheimer, H. Auer, M. Zellner, G. Weingartmann, F. Garo, E. Roth, R. Oehler, Reduced stress tolerance of glutamine-deprived human monocytic cells is associated with selective down-regulation of Hsp70 by decreased mRNA stability, J. Mol. Med. 84 (2006) 147–158.
[35] D.V. Almeida, B.F. Nornberg, L.A. Geracitano, D.M. Barros, J.M. Monserrat, L.F. Marins, Induction of phase II enzymes and hsp70 genes by copper sulfate through the electrophile-responsive element (EpRE): insights obtained from a transgenic zebrafish model carrying an orthologous EpRE sequence of mammalian origin, Fish Physiol. Biochem. 36 (2010) 347–353.
[36] R. Hu, C. Xu, G. Shen, M.R. Jain, T.O. Khor, A. Gopalkrishnan, W. Lin, B. Reddy, J.Y. Chan, A.N. Kong, Identification of Nrf2-regulated genes induced by chemopreventive isothiocyanate PEITC by oligonucleotide microarray, Life Sci. 79 (2006) 1944–1955.
[37] R. Hu, C. Xu, G. Shen, M.R. Jain, T.O. Khor, A. Gopalkrishnan, W. Lin, B. Reddy, J.Y. Chan, A.N. Kong, Gene expression profiles induced by cancer chemopreventive isothiocyanate sulforaphane in the liver of C57BL/6J mice and C57BL/6J/Nrf2 (−/−) mice, Cancer Lett. 243 (2006) 170–192.
[38] N. Orentreich, J.R. Matias, A. DeFelice, J.A. Zimmerman, Low methionine ingestion by rats extends life span, J. Nutr. 123 (1993) 269–274.
[39] J.P. Richie Jr., D. Komninou, Y. Leutzinger, W. Kleinman, N. Orentreich, V. Malloy, J.A. Zimmerman, Tissue glutathione and cysteine levels in methionine-restricted rats, Nutrition 20 (2004) 800–805.
[40] R.A. Miller, G. Buehner, Y. Chang, J.M. Harper, R. Sigler, M. Smith-Wheelock, Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance, Aging Cell 4 (2005) 119–125.
[41] L. Sun, A.A. Sadighi Akha, R.A. Miller, J.M. Harper, Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age, J. Gerontol. A Biol. Sci. Med. Sci. 64 (2009) 711–722.
[42] N.J. Linford, R.P. Beyer, K. Gollahon, R.A. Krajcik, V.L. Malloy, V. Demas, G.C. Burmer, P.S. Rabinovitch, Transcriptional response to aging and caloric restriction in heart and adipose tissue, Aging Cell 6 (2007) 673–688.
[43] V.L. Malloy, R.A. Krajcik, S.J. Bailey, G. Hristopoulos, J.D. Plummer, N. Orentreich, Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction, Aging Cell 5 (2006) 305–314.
[44] S.K. Calderwood, A. Murshid, T. Prince, The shock of aging: molecular chaperones and the heat shock response in longevity and aging – a mini-review, Gerontology 55 (2009) 550–558.
[45] A.L. Hsu, C.T. Murphy, C. Kenyon, Regulation of aging and age-related disease by DAF-16 and heat-shock factor, Science 300 (2003) 1142–1145.
[46] P. Cavuoto, M.F. Fenech, A review of methionine dependency and the role of methionine restriction in cancer growth control and life-span extension, Cancer Treat. Rev. 38 (2012) 726–736.
[47] M.L. Mendillo, S. Santagata, M. Koeva, G.W. Bell, R. Hu, R.M. Tamimi, E. Fraenkel, T.A. Ince, L. Whitesell, S. Lindquist, HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers, Cell 150 (2012) 549–562.