HSF1-TPR Interaction Facilitates Export of Stress-induced HSP70 mRNA* S

Received for publication, May 16, 2007, and in revised form, September 25, 2007. Published, JBC Papers in Press, September 25, 2007. DOI 10.1074/jbc.M704054200

Hollie S. Skaggs 1, Hongyan Xing, Donald C. Wilkerson, Lynea A. Murphy, Yiling Hong 2, Christopher N. Mayhew 3, and Kevin D. Sarge 4

From the Department of Molecular and Cellular Biochemistry, Chandler Medical Center, University of Kentucky, Lexington, Kentucky 40536-0084

Stress conditions inhibit mRNA export, but mRNAs encoding heat shock proteins continue to be efficiently exported from the nucleus during stress. How HSP mRNAs bypass this stress-associated export inhibition was not known. Here, we show that HSF1, the transcription factor that binds HSP promoters after stress to induce their transcription, interacts with the nuclear pore-associating TPR protein in a stress-responsive manner. TPR is brought into proximity of the HSP70 promoter after stress and preferentially associates with mRNAs transcribed from this promoter. Disruption of the HSF1-TPR interaction inhibits the export of mRNAs expressed from the HSP70 promoter, both endogenous HSP70 mRNA and a luciferase reporter mRNA. These results suggest that HSP mRNA export escapes stress inhibition via HSF1-mediated recruitment of the nuclear pore-associating protein TPR to HSP genes, thereby functionally connecting the first and last nuclear steps of the gene expression pathway, transcription and mRNA export.

The up-regulation of heat shock proteins such as HSP70 that occurs in response to exposure to elevated temperature and many other stress conditions is vital for the ability of cells to survive these stresses. Because of their crucial cytoprotective function, it is very important that up-regulation of HSP expression after stress occur as rapidly and as efficiently as possible. An intriguing finding of past studies is that stress conditions inhibit the export of many mRNAs from the nucleus, but mRNAs encoding heat shock proteins continue to be efficiently exported during stress (1–7). However, the mechanism by which HSP mRNAs bypass this stress-associated export inhibition was not known.

HSF1 is the transcription factor responsible for up-regulating transcription of HSP70 and other genes in response to elevated temperature and other stress conditions. HSF1 performs this function by undergoing stress-induced trimerization to the DNA-binding form and then interacting with heat shock elements in the promoters of these genes to increase their transcription (8, 9). TPR is a 270-kDa polypeptide that is associated with the nuclear basket on the nucleoplasmic face of the nuclear pore complex (10–17). Previous data suggest that TPR is involved in the export of both mRNAs and proteins from the nucleus (13, 16, 18–20). During the course of yeast two-hybrid experiments in our laboratory, we identified the existence of an interaction between HSF1 and the TPR protein. The results presented here suggest that the HSF1-TPR interaction could be important for the export of HSP mRNAs during stress conditions.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Assay—The interactions between pGBD-HSF1 and pVP16-TPR-(14–117) and pVP16-TPR-(1218–1320) were characterized by streaking yeast (strain p694A) containing these constructs or pGBD-HSF1 bait and empty pVP16 plasmid (as a negative control) on plates lacking tryptophan and leucine; tryptophan, leucine, and histidine; or tryptophan, leucine, and alanine.

In Vitro Binding Assay—In vitro translated 35S-labeled TPR-(14–117) or TPR-(1218–1320) was incubated with GST-S-HSF1 and GST bound to glutathione-agarose. After washing, bound proteins were analyzed by SDS-PAGE and autoradiography to detect the 35S-labeled TPR proteins. The amounts of GST-HSF1 and GST proteins bound to the beads were determined by SDS-PAGE followed by Western blotting using goat anti-GST polyclonal antibody (Amersham Biosciences).

Immunoprecipitation Analysis—HeLa cells (American Type Culture Collection) were heat-shocked at 42 °C for 1 h, and extracts of these cells were then subjected to immunoprecipitation using rabbit anti-HSF1 polyclonal antibody (21) or rabbit nonspecific IgG followed by Western blotting using mouse anti-TPR monoclonal antibody (Oncogene Research Products).

Chromatin Immunoprecipitation Assay—ChIP assays were performed with Jurkat cells as described previously (22) using...
Cloning junctions were checked by sequencing. One site was generated using PCR-based strategy to amplify the relevant regions of the stress-inducible HSP70i gene and histone H4 gene, as follows: HSP70i, 5'-ctcagggtcctggc-3' and 5'-tgacccatacgcagc-3'; and histone H4, 5'-gagaggcggggagaatg-3' and 5'-ttggaggtgcggttagt-3'. PCR products were then separated on polyacrylamide gels and stained with ethidium bromide. ChIP samples were also analyzed by quantitative real-time PCR as described below.

**HSP mRNA Export Analysis**—pEGFP-TPR-(14–117) and pEGFP-TPR-(1218–1320) were generated using a PCR-based strategy to amplify the relevant regions while adding restriction sites to each end to allow ligation into the pEGFP-C2 vector. PCR products were then separated on polyacrylamide gels with ethidium bromide staining to ensure that they were of the expected size. For quantitative PCR analysis of the L32 ribosomal protein mRNA, the probe for L32 was described previously (24).

**RNA Immunoprecipitation Analysis**—HeLa cells (1.5 × 10⁶) were transfected with 2 μg of either the HSP70i-luciferase or RSV-luciferase plasmid using Effectene following the manufacturer's instructions. Cells were then heat-shocked for 1 h at 42 °C, washed once with ice-cold phosphate-buffered saline, and cross-linked with 2% paraformaldehyde for 12 min while rotating. Cross-linking was quenched with 125 mM glycine for 5 min; and cells were washed twice with ice-cold phosphate-buffered saline, harvested by scraping, and snap-frozen in liquid nitrogen. Cells were resuspended in 2 ml of low stringency radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl (pH 7.5), 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.05% SDS, 1 mM EDTA, 150 mM NaCl, 1 × Complete protease inhibitor mixture (Roche Applied Science), and 80 units of RNaseOUT (Invitrogen)). Pipetted 20 times, and incubated on ice for 10 min. Cells were then sonicated three times (80–90% output) for 20 s and centrifuged at 16,000 × g for 10 min at 4 °C. The supernatant was preclarified with 20 μl of protein G-Sepharose (GE Healthcare) and washed with low stringency RIPA buffer and 100 μg/ml yeast tRNA (Ambion, Inc.) for 2 h at 4 °C. During the preclarification, the low stringency RIPA buffer-washed protein G-Sepharose beads were coated with 5 μg of either anti-TPR antibody or mouse IgG (Sigma) in low stringency RIPA buffer. The preclarified supernatant was then incubated with the antibody-coated beads for 90 min at 4 °C with rotation. Complexes were washed five times for 10 min each at room temperature with 1 ml of high stringency RIPA buffer (50 mM Tris-HCl (pH 7.5), 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 1 M NaCl, 1 mM urea, and 0.2 M phenylmethylsulfonyl fluoride). Beads were then resuspended in 100 μl of 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 10 mM dithiothreitol, and 1% SDS, and cross-links were reversed for 1 h at 70 °C. mRNA was isolated as described above, and samples were analyzed by quantitative PCR as described below.

**Quantitative Real-time PCR**—This was performed with the Mx4000 system (Stratagene) using Brilliant SYBR Green QPCR Master Mix (Stratagene). Samples were checked for specific amplification using dissociation curve analysis included with the software. PCR products were also assayed on polyacrylamide gels with ethidium bromide staining to ensure that they were of the expected size. For quantitative PCR analysis of the TPR-HSP70 promoter ChIP assay, the primers used were as follows: HSP70, 5'-caacaccctccacgagcct-3' and 5'-tctgtaaggggagaatgc-3'; and histone H4, 5'-gagaggcgggagaattg-3' and 5'-gtgtagctgctgsggaa-3'. The C_{threshold} values were normalized to input DNA (DNA before the immunoprecipitation step) and IgG controls. Data are represented as fold differences above mouse control IgG relative to input DNA using the formula 2^{ΔΔC_{threshold}} = 2^{(C_{threshold} - C_{input})}. For the experiments analyzing luciferase cDNA, primers used for quantitative PCR analysis were 5'-gagaggcggggagaa-3' and 5'-gagaggcgggagaatg-3'. The C_{threshold} values were normalized to input cDNA (cDNA made from genomic contamination. cDNA was prepared from samples using ImProm-II reverse transcriptase (Promega Corp.) and oligo(dT)₁₆ primers. cDNA samples were assayed by quantitative real-time PCR as described below.

**HSF1-TPR Interaction Facilitates Export**
HSF1-TPR Interaction Facilitates Export

total RNA before the immunoprecipitation step) and IgG controls, which were set as 1 unit. Data are represented as fold differences relative to these two values using the formula $2^{\frac{(C_{t_{Input}} - C_{t_{Input(IgG)}}) - (C_{t_{Input(TPR)} - C_{t_{Input(IgG)}}})}{C_{t_{Input(TPR)} - C_{t_{Input(IgG)}}}}$.

For experiments analyzing endogenous HSP70 mRNA levels, the following primers were used: human HSP70, 5'-caccctgcctgttgga-3' and 5'-ttctcgcgacctagc-3'; and human L32, 5'-catctctctcctcacta-3' and 5'-aaccctgtagctc-3'. The data presented represent two independent experiments in which values obtained utilizing the formula $2^{\Delta \Delta C_{t}}$ were averaged. The results are graphed as relative differences between cytoplasmic and nuclear HSP70 mRNA levels compared with the control samples (GFP alone), which were set to a value of 1.

RESULTS

To further the understanding of the regulation and function of HSF1, we performed a yeast two-hybrid screen using the HSF1 protein as a bait. Two of the interacting clones that were obtained from this screen represented two different regions of the TPR protein. One of the HSF1-interacting regions of TPR identified by the yeast two-hybrid screen comprises a sequence near the N terminus (amino acids 14–117), whereas the other is located close to the middle of the protein (amino acids 1218–1320) (Fig. 1, A and B). As an independent test of the interaction between HSF1 and these two regions of TPR and to determine whether the interaction is direct, in vitro binding experiments were performed in which in vitro translated 35S-labeled TPR-(14–117) and TPR-(1218–1320) were incubated with GST-HSF1 or GST bound to glutathione-agarose beads. The results confirmed the ability of both regions of TPR to interact with HSF1 (Fig. 1C).

To determine whether endogenous HSF1 and TPR proteins interact and, if so, whether the interaction between these proteins is regulated in a stress-dependent manner, immunoprecipitation analysis was performed using extracts of cells kept at 37 °C or cells subjected to heat stress at 42 °C for 1 h. Fig. 2 shows that endogenous HSF1 and TPR did associate and that more HSF1-TPR complex was observed in extracts of stressed cells compared with non-stressed cells.

In multicellular eukaryotes, HSF1 binds to heat shock gene promoters in response to stress conditions. Therefore, the data presented in Fig. 2 indicating that HSF1 interacts with TPR in a stress-induced manner prompted the question of whether TPR might also associate with the promoter of the stress-inducible HSP70 gene when cells are exposed to stress. We tested this hypothesis using the ChIP assay. The results demonstrate that a low level of TPR association was detected within cross-linking distance of the HSP70 promoter in cells kept at 37 °C and that a higher level of TPR was associated with the HSP70 promoter in cells that were subjected to stress treatment at 42 °C for 1 h (Fig. 3A, upper panels). TPR was not found to associate with the promoter region of the histone H4 gene, indicating the specificity of its HSP70 promoter association (Fig. 3A, lower panels). As a complementary approach, we repeated this experiment and used quantitative real-time PCR for the analysis. The results (Fig. 3B) are consistent with the finding of increased association between TPR and the HSP70 promoter in response to exposure to stress conditions.

On the basis of previous results indicating a role for TPR in mRNA export (13, 18, 19), including the finding that the yeast TPR ortholog Mlp1p interacts with the mRNA export heterogeneous nuclear ribonucleoprotein Nab2p (19), we hypothesized that the recruitment of TPR to the HSP70 promoter might function as a way to specifically promote association between...
TPR and the stress-induced transcripts that arise from this gene. We tested this hypothesis using an RNA immunoprecipitation approach. In this experiment, HeLa cells were transfected with expression constructs in which the luciferase gene is transcribed from either the stress-inducible human HSP70 gene promoter or the RSV promoter. The transfected cells were subjected to a heat shock treatment (42 °C), after which they were incubated with the chemical cross-linking agent paraformaldehyde, and extracts of these cells were then immunoprecipitated using anti-TPR antibody. RNA isolated from the TPR-containing complexes was reverse-transcribed into cDNAs, which were then analyzed by quantitative real-time PCR using a luciferase primer pair. The results of this experiment (Fig. 4) indicate that significantly more luciferase mRNA transcripts generated from the HSP70 promoter were associated with the TPR protein compared with luciferase mRNAs transcribed from the RSV promoter.

The results described above indicate that stress conditions result in increased interaction between HSF1 and TPR, increased association of TPR with the HSP70 promoter, and the preferential association of TPR with mRNAs arising from transcription from the HSP70 promoter. In light of the data suggesting a role for TPR in mRNA export (13, 18, 19), we hypothesized that these events could be part of a mechanism for specifically enhancing the export of mRNAs transcribed from heat shock gene promoters. To test this hypothesis, we sought to deter-

FIGURE 3. TPR associates with the HSP70 promoter in response to stress. A, ChIP assay was performed on non-stressed (37 °C) or stressed (42 °C, 60 min) Jurkat cells using anti-TPR antibody or control IgG and PCR primers specific to the promoter region of the stress-inducible HSP70i gene (upper panels) or the histone H4 gene (lower panel). PCR products were separated on a polyacrylamide gel and stained with ethidium bromide. B, ChIP assay was performed as described for A, but analysis was done by quantitative real-time PCR. Data are from triplicate experiments. Data are shown as means ± S.E.

FIGURE 4. TPR interacts with mRNAs transcribed from the HSP70 promoter. RNA immunoprecipitation analysis was performed to measure the amounts of luciferase (Luc) mRNA transcribed from the HSP70 promoter versus the RSV promoter that are associated with the TPR protein. The results were normalized to the levels of each mRNA and to control IgG samples (which were set to a value of 1). Data are from triplicate experiments. Data are shown as means ± S.E.
HSF1-TPR Interaction Facilitates Export

A

GFP-Tpr (14–117)
GFP-Tpr (1218–1320)

-GFP-Tpr segment
-GFP

β-actin

B

GFP
GFP-Tpr (14–117)
GFP-Tpr (1218–1320)

C

GFP
GFP-Tpr (14–117)
GFP-Tpr (1218–1320)

Probe

Luc
L32

hsp70-Luc

RSV-Luc

D

Relative hsp70 mRNA (Relative to control = 1)

GFP-Tpr (14–117)
GFP-Tpr (1218–1320)

C
N

FIGURE 5. Expression of HSF1-interacting regions of TPR inhibits export of endogenous HSP70 mRNA and a reporter transcript expressed from the HSP70 promoter. A, HeLa cells were transfected with GFP-TPR-(14–117), GFP-TPR-(1218–1320), or GFP alone and then subjected to Western blot analysis using anti-GFP antibody. B, HeLa cells transfected with GFP-TPR-(14–117), GFP-TPR-(1218–1320), or GFP alone were subjected to heat treatment at 42 °C for 60 min, and HSF1 immunoprecipitates from extracts of these cells were then subjected to anti-TPR Western blotting. C, HeLa cells were cotransfected with the GFP-TPR-(14–117), GFP-TPR-(1218–1320), or GFP alone construct along with either a HSP70 promoter-driven (upper panels) or RSV promoter-driven (lower panels) reporter plasmid and subjected to heat shock treatment at 42 °C for 60 min, and mRNA from the cytoplasmic (C) and nuclear (N) fractions of these transfected cells was then analyzed by RNase protection assay using a probe that detects luciferase mRNA or mRNA encoding the L32 ribosomal protein (as a control). The results indicate that cells transfected with GFP-TPR-(14–117) or GFP-TPR-(1218–1320) exhibited a decrease in the cytoplasmic levels of luciferase mRNA expressed from the heat shock element-containing HSP70 promoter compared with cells transfected with GFP (Fig. 5C, upper panels). Transfection of GFP-TPR-(14–117) or GFP-TPR-(1218–1320) did not change the nuclear versus cytoplasmic levels of luciferase mRNA expressed from the non-heat shock element-containing RSV promoter compared with transfection of GFP alone, indicating the HSP70 promoter selectivity of the effect (Fig. 5C, lower panels).

Finally, to test whether expression of these two regions of the TPR protein is able to inhibit export of endogenous HSP70i mRNAs, we performed an experiment similar to the one shown in Fig. 5D, except that the cells were transfected with only the GFP-TPR-(14–117), GFP-TPR-(1218–1320), or GFP expression construct (no luciferase reporter constructs). After subjecting the transfected cells to heat shock treatment at 42 °C for 60 min, mRNA from the cytoplasmic and nuclear fractions of these cells was reverse-transcribed and then analyzed by quantitative real-time PCR using primers that amplify the HSP70i sequence or the L32 sequence (normalizing control). The results of this experiment (Fig. 5D) are presented graphically as
the relative levels of endogenous HSP70 mRNA in the nucleus or cytoplasm, normalized for L32 mRNA levels, compared with the results for the control cells (transfected with GFP alone), which were set to a value of 1. The results indicate that the export of endogenous HSP70i mRNA, like that of the heat shock element-driven luciferase mRNAs above, was decreased in cells transfected with GFP-TPR-(14–117) or GFP-TPR-(1218–1320) compared with cells transfected with GFP (Fig. 5D).

DISCUSSION

The results presented in this work indicate that, in response to stress, the TPR protein interacts with the stress gene transcriptional regulator HSF1, is recruited to the HSP70 promoter region, and preferentially associates with mRNAs transcribed from this promoter compared with those expressed from a non-stress-induced promoter and that the HSF1-TPR interaction is required for efficient export of HSP mRNAs from the nucleus during stress. The association of TPR with these mRNAs may be assisted by its interaction with mRNA-binding heterogeneous nuclear ribonucleoproteins such as Nab2p (19). The TPR protein is known to be able to form homodimers (25). Thus, once it is complexed with HSP mRNAs, TPR could facilitate their export by docking with TPR found at the nucleoplasmic face of nuclear pore complexes (10–17).

These results reveal the existence of a direct functional connection between the first and last nuclear steps in the gene expression pathway, transcription and export of mRNAs from the nucleus. The HSF1-TPR interaction and its downstream events could serve as a mechanism for bypassing the inhibition of mRNA export that occurs in response to stress and/or to increase the kinetics of export of HSP mRNAs so that cells can express these crucial cytoprotective proteins as soon as possible. One intriguing question for future studies is whether the TPR protein is recruited to other genes to aid in the export of their mRNAs from the nucleus.

Acknowledgments—We are grateful to Dan Noonan and Brian Finlin for providing plasmid constructs and to other members of the laboratory for insightful discussions.

REFERENCES

1. Sadis, S., Hickey, E., and Weber, L. A. (1988) J. Cell. Physiol. 135, 377–386
2. Galbouzi, J. E., Brennan, C. M., Stenberg, M. G., Swanston, M. S., Eversole, A., Maizels, N., and Steitz, J. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3073–3078
3. Saavedra, C., Tung, K. S., Amberg, D. C., Hopper, A. K., and Cole, C. N. (1996) Genes Dev. 10, 1608–1620
4. Tani, T., Derby, R. J., Hiraoka, Y., and Spector, D. L. (1996) Mol. Biol. Cell 7, 173–192
5. Liu, Y., Liang, S., and Tartakoff, A. M. (1996) EMBO J. 15, 6750–6757
6. Krebber, H., Taura, T., Lee, M. S., and Silver, P. A. (1999) Genes Dev. 13, 1994–2004
7. Bond, U. (2006) FEBS Lett. 580, 266–272
8. Voellmy, R. (2003) Cell Stress Chaperones 9, 122–133
9. Holmberg, C. I., Tran, S. E., Eriksson, J. E., and Sistonen, L. (2002) Trends Biochem. Sci. 27, 619–627
10. Cordes, V. C., Reidenbach, S., Rackwitz, H. R., and Franke, W. W. (1997) J. Cell Biol. 136, 515–529
11. Zimowska, G., Aris, J. P., and Paddy, M. R. (1997) J. Cell Sci. 110, 927–944
12. Shah, S., Tugendreich, S., and Forbes, D. (1998) J. Cell Biol. 141, 31–49
13. Bangs, P., Burke, B., Powers, C., Craig, R., Purohit, A., and Doxsey, S. (1998) J. Cell Biol. 143, 1801–1812
14. Strambio-de-Castillia, C., Blobel, G., and Rout, M. P. (1999) J. Cell Biol. 144, 839–855
15. Fontoura, B. M., Daies, S., Blobel, G., and Zhong, H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3208–3213
16. Frostell, P., Guan, T., Subauste, C., Hahn, K., and Gerace, L. (2002) J. Cell Biol. 156, 617–630
17. Krull, S., Thyberg, J., Bjorkroth, B., Rackwitz, H. R., and Cordes, V. C. (2004) Mol. Biol. Cell 15, 4261–4277
18. Vinciguerra, P., Iglesias, N., Camblong, J., Zenklusen, D., and Stutz, F. (2005) EMBO J. 24, 813–823
19. Green, D. M., Johnson, C. P., Hagan, H., and Corbett, A. H. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1010–1015
20. Cornett J., Cao, F., Wang, C. E., Bates, G. P., Li, S. H., and Li, X. J. (2005) Nat. Genet. 37, 198–204
21. Sarge, K. D., Murphy, S. P., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 1392–1407
22. Xing, H., Wilkerson, D. C., Mayhew, C. N., Lubert, E. J., Skaggs, H. S., Goodson, M. L., Hong, Y., Park-Sarge, O. K., and Sarge K. D. (2005) Science 307, 421–423
23. Giguere, V., Hollenberg, S. M., Rosenfeld, M. G., and Evans, R. M. (1986) Cell 46, 645–652
24. Hobbs, M. V., Weigle, W. O., Noonan, D. I., Torbett, B. E., McEvilly, R. L., Koch, R. J., Cardenas, G. J., and Ernst, D. N. (1993) J. Immunol. 150, 3602–3614
25. Hase, M. E., Kuznetsov, N. V., and Cordes, V. C. (2001) Mol. Biol. Cell 12, 2433–2452