MOLECULAR AND CELLULAR BIOLOGY, Sept. 1990, p. 4967–4969
0270-7306/90/094967-03$02.00/0

Vol. 10, No. 9

Oxidant Stress Leads to Transcriptional Activation of the Human Heme Oxygenase Gene in Cultured Skin Fibroblasts

STEPHEN M. KEYSE,† LEE ANN APPLEGATE, YVONNE TROMVOUKIS, AND REX M. TYRRELL*

Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland

Received 9 March 1990/Accepted 13 June 1990

Treatment of cultured human skin fibroblasts with near-UV radiation, hydrogen peroxide, and sodium arsenite induces accumulation of heme oxygenase mRNA and protein. In this study, these treatments led to a dramatic increase in the rate of RNA transcription from the heme oxygenase gene but had no effect on mRNA stability. Transcriptional activation, therefore, appears to be the major mechanism of stimulation of expression of this gene by either oxidative stress or sulfydryl reagents.

We have reported specific induction of a 32-kilodalton stress protein in human skin fibroblasts by both near-UV radiation (UV-A [320 to 380 nm]) and the oxidizing agent hydrogen peroxide (H_2O_2) (8). Recently, we have identified this protein as heme oxygenase (9), the rate-limiting enzyme in the degradation of cellular heme. Induction of heme oxygenase leads to a reduction in the cellular pool of heme and heme-containing proteins, thereby removing potential pro-oxidant catalysts (5, 6). Furthermore, bilirubin, the end product of the heme degradation pathway, is a molecule with intrinsic antioxidant properties (17). Induction of this enzyme in human cells may therefore constitute an inducible protective mechanism against oxidative damage (9).

Treatment of human skin fibroblasts with UV-A, H_2O_2, or the sulfydryl reagent sodium arsenite leads to a dramatic increase in steady-state levels of heme oxygenase mRNA (9), consistent with the possibility that induction of the gene is regulated primarily at the mRNA level. To characterize the regulation of this stress response in human cells further, we compared changes in the rates of nuclear RNA synthesis and mRNA stability after different inducing treatments.

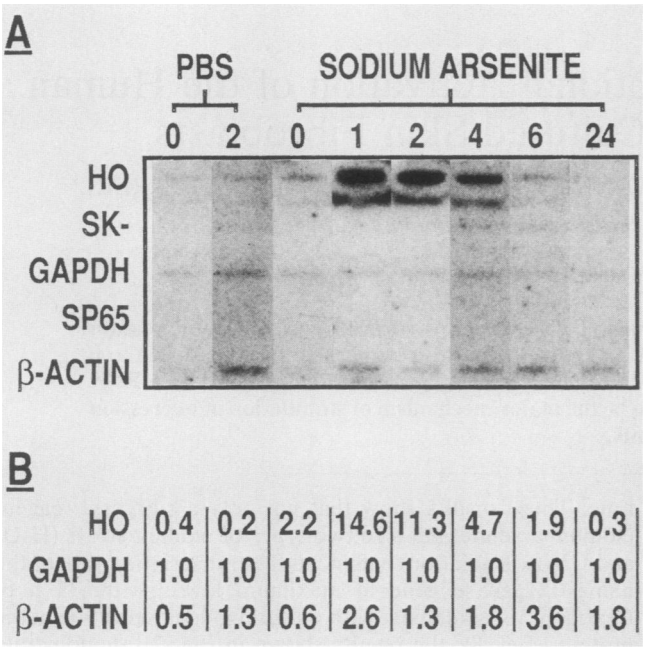
Rates of heme oxygenase RNA transcription. FEK4 fibroblasts (18) were treated with UV-A and chemicals as previously described (8, 9) and incubated for various times before preparation of nuclei. Transcription assays (runoff) were performed as described by Groudine et al. (4). The result of a representative experiment in which cells were treated with 50 μM sodium arsenite for 30 min and then assayed for levels of heme oxygenase transcription at various times up to 24 h after treatment is shown in Fig. 1A and B. The rate of heme oxygenase transcription was maximally elevated 1 h after sodium arsenite treatment. The transcription rate then declined steadily, reaching background levels by about 6 h. This increase was specific to the heme oxygenase gene, as neither the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene nor the β-actin gene showed a consistent pattern of increased transcription rate. Hybridization signals were quantitated by densitometry (Fig. 1B), and the results of this and additional transcription assays performed on cells following treatment with either H_2O_2 or UV-A are summarized in Fig. 2A as a plot of the fold change in the transcription rate (relative to untreated cells) versus the incubation time. These results show that not only a sulfydryl reagent (sodium arsenite) but also two types of oxidant stress (H_2O_2 and UV-A irradiation) enhanced the transcription rate of the heme oxygenase gene to maximum levels within 1 h of treatment. In agreement with our previous observation at the protein level (8), the results shown in Fig. 2B demonstrate that the relative increases in heme oxygenase transcription rates and steady-state mRNA levels were strictly dependent on the UV-A fluence applied to the cell population.

To investigate further the relationship between the transcription rate and steady-state levels of heme oxygenase mRNA, cytoplasmic RNA was extracted from the same cell samples used to prepare nuclei for the transcription assays (Fig. 2A) and analyzed by Northern (RNA) blotting as previously described (9). Treatment with all three inducers led to an increase in steady-state levels of heme oxygenase mRNA, which reached maximum levels approximately 2 to 4 h following treatment, i.e., approximately 2 h later than the peaks of transcriptional activity (Fig. 3).

Stability of heme oxygenase mRNA. We measured the half-life of heme oxygenase mRNA in cells exposed to either sodium arsenite or oxidative stress (H_2O_2) and compared these values with the mRNA half-life in untreated cells. Cells were incubated for 1 h after treatment to allow induction of heme oxygenase mRNA before addition of the transcriptional inhibitor dactinomycin. Heme oxygenase and GAPDH mRNA levels were then determined by Northern blot analysis at various times up to 8 h after addition of the inhibitor. The results of an experiment in which cells were treated with phosphate-buffered saline (control) or 50 μM sodium arsenite (induced) are shown in Fig. 4A and B, respectively. Heme oxygenase mRNA decayed over the time course of the experiment in both treated and untreated cells. In contrast, GAPDH mRNA was not degraded significantly, even 8 h after addition of dactinomycin. To determine mRNA half-lives, the blots shown in Fig. 4A and B, together with autoradiograms obtained in parallel experiments in which cells were treated with 200 μM H_2O_2, were quantitated by densitometry. Heme oxygenase mRNA signals were then normalized to 1.0 at $t = 2$ h, and the data are presented in Fig. 4C as an exponential plot of the heme oxygenase mRNA level versus time. The slopes of these curves indicated that the half-life of heme oxygenase mRNA was approximately 3.5 h both in untreated cells and in cells treated with sodium arsenite. The slopes of the three curves were not significantly different (t test; $P = 0.05$).

Our results directly demonstrate that both oxidant stress

* Corresponding author.
† Present address: Clare Hall Laboratories, Imperial Cancer Research Fund, South Mimms, Hertfordshire EN6 3LD, England.



B

HO	0.4	0.2	2.2	14.6	11.3	4.7	1.9	0.3
GAPDH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
β-ACTIN	0.5	1.3	0.6	2.6	1.3	1.8	3.6	1.8

FIG. 1. Autoradiogram showing runoff transcription of the heme oxygenase (HO), GAPDH, and β-actin genes. Nuclei were isolated from human skin fibroblasts at various times (0 to 24 h) following treatment with either phosphate-buffered saline (PBS) or sodium arsenite (50 μM, 30 min), and transcription reactions were performed as described by Groudine et al. (4). Nitrocellulose filters containing bound cDNAs and appropriate vector controls were then hybridized with the labeled transcripts, and the resulting signals were visualized by autoradiography. Bound DNAs are labeled to the left while times in hours before isolation of nuclei are indicated across the top. SK− and SP65 are vector controls. (B) Quantitation of hybridization signals in panel A by scanning densitometry. The values given for each time point were first corrected for the vector background and then normalized relative to the signal for GAPDH.

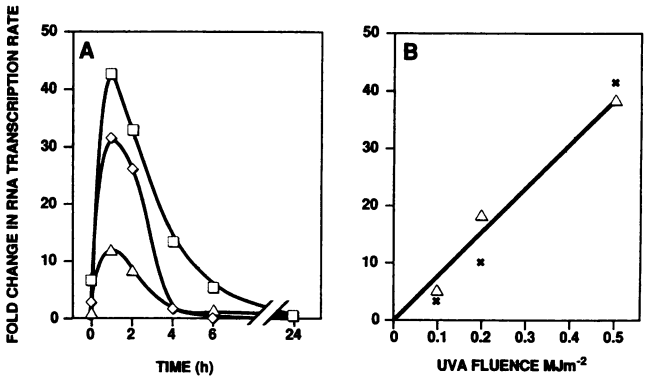


FIG. 2. (A) Graph showing the fold increase in the transcription rate of the heme oxygenase gene (relative to controls treated with phosphate-buffered saline) versus time in nuclei isolated from cells treated with 50 μM sodium arsenite for 30 min (□) or 100 μM H_2O_2 for 30 min (◇) or irradiated with UV-A (△) at 0.2 MJ m^{-2}. Data were calculated from the densitometric analysis of the runoff experiment shown in Fig. 1B (sodium arsenite) and additional experiments. (B) Graph of the fold increase in the transcription rate (relative to that in unirradiated cells) in nuclei isolated from cells 2 h after irradiation with increasing fluences of UV-A (△). Also shown is the increase in the cytoplasmic level of heme oxygenase mRNA extracted from the same cells and assayed by Northern blotting (×). Data for the runoff assay were calculated on the basis of densitometric scanning of the corresponding autoradiogram exactly as for Fig. 1B. Cytoplasmic mRNA levels were determined by scanning an autoradiogram of a Northern blot hybridization and normalization of the heme oxygenase signals relative to GAPDH.

and sulfydryl reagents increase the rate at which the human heme oxygenase gene is transcribed. We conclude that this stimulation of transcription is the major mechanism by which the steady-state level of mRNA is increased. This is supported by the observation that neither of these two classes of inducers caused a significant change in the half-life of heme oxygenase mRNA in treated cells.

Heme oxygenase activity is increased in the livers of rats by its substrate, heme, and by heavy metals (7, 11, 13). Recently, it has been shown that the rate of transcription of the rat gene is increased in response to heat shock (15) and that the transcription rate of the mouse gene is enhanced by treatment with cadmium and heme (1). However, the dramatic enhancement of transcription that we now report to result from oxidant stress is unlikely to share a common mechanism with these effects for several reasons. (i) The human heme oxygenase gene has been shown to be essentially unresponsive to heat shock (9, 19). (ii) It is unlikely that the oxidant stress response is mediated by a rapid stimulation of endogenous heme levels, particularly since the heme biosynthetic enzyme, aminolevulinate synthase, is normally down regulated under conditions which stimulate heme oxygenase synthesis (12). (iii) The transcriptional effects of both heat shock and cadmium on the rat heme oxygenase gene are consistent with a recent DNA sequence analysis of its 5′-flanking region which revealed the presence of a consensus sequence for the heat shock element and two copies of core sequences found in the metal-regulatory elements of the metallothionein genes (14). Although a genomic clone containing the human heme oxygenase gene has recently been isolated and the sequence of its 5′-flanking region has been determined (16), there is no functional information available concerning the sequences responsible for any of the inducible responses observed.

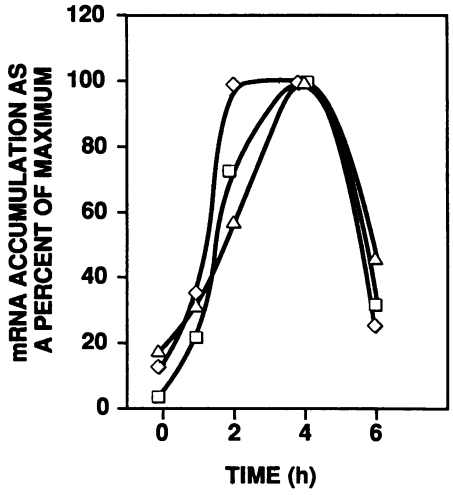


FIG. 3. Graph showing steady-state levels of heme oxygenase mRNA versus time in cells treated with sodium arsenite (□), H_2O_2 (◇), and UV-A (△). Data were calculated on the basis of densitometric scanning of Northern blots of cytoplasmic RNA isolated from the same populations of cells used in the corresponding runoff transcription experiments and probed with heme oxygenase and GAPDH. Signals were normalized to GAPDH to correct for loading errors.

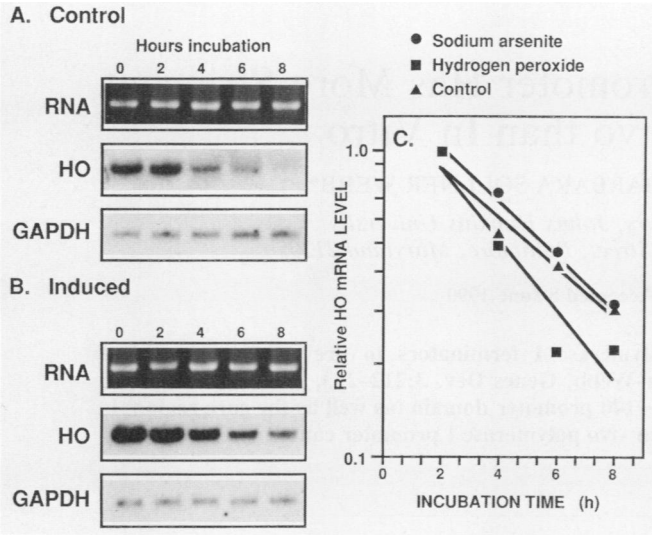


FIG. 4. Northern blot analysis of heme oxygenase (HO) and GAPDH mRNA levels in human skin fibroblasts at various times (0 to 8 h) following addition of the transcriptional inhibitor dactinomycin (5 μg/ml). Control cells (A) were treated with phosphate-buffered saline. Induced cells (B) were treated with 50 μM sodium arsenite for 30 min. Dactinomycin was added 1 h posttreatment in both cases. The autoradiogram shown in panel A represents a much longer exposure of the film than that shown in panel B. The upper panels show ethidium bromide staining of equalized RNA samples. (C) Quantitative analysis. The Northern blots obtained in this and additional experiments in which cells were treated with 200 μM H_2O_2 were subjected to scanning densitometry to determine relative levels of heme oxygenase mRNA. Data for each set of experimental samples were normalized to 1 at t = 2 h and plotted on an exponential scale. The lines of best fit were determined by the least-squares method.

The enhancement of expression of various bacterial genes by oxidative stress appears to be a protective response (2, 3). Although functional proof of a protective role for heme oxygenase is lacking, we speculate that induction of transcriptional activity of the gene by oxidative stress is an analogous response that occurs in mammalian cells. At least for UV-A irradiation and H_2O_2, generation of active oxygen intermediates appears to be the trigger for induction (10). Current studies aimed at identification of the DNA sequence elements involved in the transcriptional activation should provide information on the signal transduction pathways involved in the regulation of human genes by oxidative stress.

This research was supported by grants from the Swiss National Science Foundation (3.186.088) and the Swiss League Against Cancer and was undertaken during the tenure of a research training fellowship awarded to L.A.A. by the International Agency for Research on Cancer.

We thank R. D. Wood for critical reading of the manuscript.

LITERATURE CITED

1. **Alam, J., S. Shibahara, and A. Smith.** 1989. Transcriptional activation of the heme oxygenase gene by heme and cadmium in mouse hepatoma cells. J. Biol. Chem. **264**:6371–6375.
2. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defences against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
3. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature (London) **304**:466–468.
4. **Groudine, M., M. Peretz, and H. Weintraub.** 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. Mol. Cell. Biol. **1**:281–288.
5. **Gutteridge, J. M. C.** 1987. The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. Biochem. Biophys. Res. Commun. **917**:219–223.
6. **Gutteridge, J. M. C., and A. Smith.** 1988. Antioxidant protection by haemopexin of haem-stimulated lipid peroxidation. Biochem. J. **256**:861–865.
7. **Ishizawa, S., T. Yoshida, and G. Kikuchi.** 1983. Induction of heme oxygenase in rat liver. J. Biol. Chem. **258**:4220–4225.
8. **Keyse, S. M., and R. M. Tyrrell.** 1987. Both near ultraviolet radiation and the oxidising agent hydrogen peroxide induce a 32-kDa stress protein in normal human skin fibroblasts. J. Biol. Chem. **262**:14821–14825.
9. **Keyse, S. M., and R. M. Tyrrell.** 1989. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA-radiation, hydrogen peroxide and sodium arsenite. Proc. Natl. Acad. Sci. USA **85**:99–103.
10. **Keyse, S. M., and R. M. Tyrrell.** 1990. Induction of the heme oxygenase gene in human skin fibroblasts by hydrogen peroxide and UVA (365nm) radiation: evidence for the involvement of the hydroxyl radical. Carcinogenesis **11**:787–791.
11. **Kikuchi, G., and T. Yoshida.** 1983. Function and induction of the microsomal heme oxygenase. Mol. Cell. Biochem. **53/54**: 163–183.
12. **Maines, M. D.** 1989. New developments in the regulation of heme metabolism and their implications. Crit. Rev. Toxicol. **12**:241–314.
13. **Maines, M. D., and A. Kappas.** 1976. Studies on the mechanism of induction of heme oxygenase by cobalt and other metal ions. Biochem. J. **154**:125–131.
14. **Muller, R. M., H. Taguchi, and S. Shibahara.** 1987. Nucleotide sequence and organisation of the rat heme oxygenase gene. J. Biol. Chem. **262**:6795–6802.
15. **Shibahara, S., R. M. Muller, and H. Taguchi.** 1987. Transcriptional control of the rat heme oxygenase by heat shock. J. Biol. Chem. **262**:12889–12892.
16. **Shibahara, S., M. Sato, R. M. Muller, and T. Yoshida.** 1989. Structural organisation of the human heme oxygenase gene and the function of its promoter. Eur. J. Biochem. **179**:557–563.
17. **Stocker, R., Y. Yamamoto, A. F. McDonagh, A. N. Glazer, and B. N. Ames.** 1987. Bilirubin is an antioxidant of possible physiological importance. Science **235**:1043–1046.
18. **Tyrrell, R. M., and M. Pidoux.** 1986. Quantitative differences in host cell reactivation of ultraviolet damaged virus in human skin fibroblasts and epidermal keratinocytes cultured from the same foreskin biopsy. Cancer Res. **46**:2665–2669.
19. **Yoshida, T., P. Biro, T. Cohen, R. M. Muller, and S. Shibahara.** 1988. Human heme oxygenase cDNA and induction of its mRNA by heme. Eur. J. Biochem. **171**:457–461.