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Bach1 Functions as a Hypoxia-inducible Repressor for the Heme Oxygenase-1 Gene in Human Cells*

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Heme oxygenase 1 (HO-1) catalyzes heme breakdown, eventually releasing iron, carbon monoxide, and bilirubin IXα. HO-1 is induced by its substrate heme and various environmental factors, which represents a protective response against oxidative stresses. Here we show that hypoxia represses HO-1 expression in three human cell types but induces it in rat, bovine, and monkey cells, indicating the inter-species difference in the hypoxic regulation of HO-1 expression. The hypoxia-mediated repression of HO-1 expression is consistently associated with the induction of Bach1, a heme-regulated transcriptional repressor, in human cells. Bach1 is a basic leucine zipper protein, forming a heterodimer with a small Maf protein. Expression of HO-1 was also reduced in human cells when exposed to interferon-γ or an iron chelator desferrioxamine, each of which induced Bach1 expression. In contrast, induction of HO-1 expression by $CoCl_2$ is associated with reduced expression of Bach1 mRNA. Thus, expression of HO-1 and Bach1 is inversely regulated. We have identified a Maf recognition element in the human *HO-1* gene that is required for repression of a reporter gene by hypoxia and targeted by Bach1. Therefore, Bach1 functions as a hypoxia-inducible repressor for the *HO-1* gene, thereby contributing to fine-tuning of oxygen homeostasis in human cells.

Heme oxygenase (EC 1.14.99.3) is the rate-limiting enzyme in heme catabolism that cleaves heme at the α-methene bridge to form biliverdin IXα, carbon monoxide, and iron (1, 2). Biliverdin IXα is immediately converted by biliverdin reductase to bilirubin IXα that is transported to the liver for conjugation and excretion into bile (3). There are two isozymes of heme oxygenase, heme oxygenase-1 (HO-1)[1] and heme oxygenase-2 (HO-2) (4, 5). HO-1 is inducible whereas HO-2 is constitutively expressed in human cells (6). Expression of HO-1 mRNA is highly increased in human cells by the substrate heme (7), heavy metals (8, 9), UV irradiation (10), and nitric oxide donors (11–14). Because bilirubin IXα functions as a natural radical scavenger (15, 16), induction of HO-1 probably represents a protective response against oxidative stress. The physiological importance of HO-1 has been confirmed by the phenotypic consequences of the HO-1-deficient mice (17) and a patient with HO-1 deficiency (18).

Induction of HO-1 has been extensively studied for the last few decades by many investigators. In contrast, repression of HO-1 expression has been largely ignored, despite its physiological importance (3). We have shown that HO-1 is not induced or rather reduced by heat shock in human cells (19), whereas rat HO-1 is a heat shock protein (20, 21). The expression levels of HO-1 are also decreased in human glioblastoma cells by the treatment with interferon-γ (22). In addition, hypoxia represses HO-1 mRNA expression in primary cultures of human umbilical vein endothelial cells (HUVECs), human astrocytes, and human coronary arterial endothelial cells (23). On the other hand, hypoxia increased HO-1 expression in rat liver (24) and heart (25) and in various cultured animal cells, including Chinese hamster ovary cells (26), rat ventricular smooth muscle cells (27, 28), and rat myocytes (29). These results suggest the inter-species difference in the regulation of HO-1 gene expression by hypoxia between human and animal cells.

The inter-species variations in the hypoxic response are of clinical significance because hypoxia is involved in the pathophysiology of various disorders, including ischemic heart disease, cerebrovascular disease, cancer, sleep apnea syndrome, and chronic obstructive pulmonary disease, which account for common causes of death and disability in the developed world. Mammalian cells respond to hypoxia in part by increased expression of several genes coding for erythropoietin (30), vascular endothelial growth factor (31), adrenomedullin (32, 33), and glycolytic enzymes (34, 35), all of which cooperate to protect cells and tissues against the hypoxic state. Hypoxia-inducible

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This paper is dedicated to the late emeritus Prof. Tamotsu Takishima who was instrumental in initiating the collaborative work on hypoxic response.

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[1] The abbreviations used are: HO, heme oxygenase; BBMVEC, bovine brain microvascular endothelial cell; HUVECs, human umbilical vein endothelial cells; HIF, hypoxia-inducible factor; MARE, Maf recognition element; FBS, fetal bovine serum; EMSA, electrophoretic mobility shift assay; NF-E2, nuclear factor erythroid 2.

factor-1 (HIF-1) serves as a key regulator that induces the expression of most of these genes (36).

Transcription repressor Bach1 is a basic leucine zipper (bZip) protein and forms heterodimers with one of the small Maf proteins (*i.e.* MafK, MafF, and MafG) that bind to the Maf recognition element (MARE) (37, 38), which may repress transcription of certain target genes. Recently, Sun *et al.* (39) have shown that ho-1 is constitutively expressed at higher levels in many tissues of *bach1*-deficient mice, indicating that Bach1 acts as a negative regulator of transcription of the mouse *ho-1* gene. In fact, the Bach1-MafK heterodimer binds to the MAREs of the *ho-1* gene enhancers, thereby repressing transcription (39). Importantly, heme abrogated the repressor function of Bach1 by inhibiting its binding to the MAREs of the *ho-1* gene, which is consistent with the notion that the *in vitro* DNA-binding activity of Bach1 is negatively regulated by direct Bach1-heme interaction (40). Furthermore, Bach1 is widely expressed in human tissues that have been analyzed (41).

This study was aimed to clarify the mechanism by which hypoxia represses the HO-1 expression in human cells. Here we show that hypoxia induces the expression of Bach1, which is consistently associated with the repression of HO-1 expression in human cells. Furthermore, we have identified a MARE in the human *HO-1* gene enhancer that is responsible for the repression by hypoxia and is targeted by Bach1. Thus, Bach1 is a newly recognized hypoxic regulator and functions as an inducible repressor for the *HO-1* gene in several human cell types.

EXPERIMENTAL PROCEDURES

Cell Cultures—A human glioblastoma cell line, T98G, was obtained from the American Type Culture Collection and was cultivated in minimum essential medium supplemented with 5% fetal bovine serum (FBS) at 37 °C under 5% CO_2/95% room air. To examine the effect of hypoxia on the expression of heme oxygenase-1, T98G glioblastoma cells were placed in a chamber filled with 5% CO_2/94% N_2/1% O_2, as previously reported (33). The cells were cultivated for 6–48 h and were harvested for RNA extraction. In parallel, T98G cells were cultivated under 5% CO_2/95% room air for the same periods and used as normoxia control. In another series of experiments, T98G cells were exposed to 260 μM desferrioxamine or 150 μM $CoCl_2$ under normoxia for 6, 12, 18, and 24 h, and were harvested for total RNA extraction. A549 human lung cancer cells were obtained from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan), and were cultured in RPMI 1640 medium containing 10% FBS. HUVECs were obtained from Kurabo and cultured in EGM-2 medium (Takara) containing 2% FBS. C6 rat glioma cells were obtained from Human Science Research Resource Bank and cultured in Ham's F10 medium containing 15% horse serum and 2.5% FBS. Bovine brain microvascular endothelial cells (BBMVECs), which are primary endothelial cells derived from microvessels of the bovine brain, were obtained from Cell Applications, Inc. (San Diego, CA). BBMVECs were cultivated in the BBMVEC growth medium (Cell Applications, Inc.) following the manufacturer's protocol. COS7 monkey kidney cells were cultured in Dulbecco's modified Eagle's medium, containing 10% FBS, 2 mM L-glutamine, and 4500 mg/liter glucose. These cell cultures were maintained for 6–48 h at 37 °C under 5% CO_2/95% room air (normoxia) or in a chamber with 5% CO_2/94% N_2/1% O_2. The number of viable cells was determined with Cell Counting Kit-8 according to the manufacturer's protocol (Dojindo).

Northern Blot Analysis—Total RNA was extracted from cultured cells by the guanidinium thiocyanate-cesium chloride method and subjected to Northern blot analysis (42). The northern probe used for heme oxygenase-1 mRNA was the *Xho*I/*Xba*I fragment (−64/923) derived from the human heme oxygenase-1 cDNA, pHHO1 (7). The northern probe used for human Bach1 mRNA was the *Pst*I fragment of human Bach1 cDNA (41). The rat Bach1 cDNA segment was prepared from C6 glioma RNA by reverse-transcription and polymerase chain reaction (RT-PCR) using a forward primer (5′-ACAAGGACGGAGCCCTGGCACTGCC-3′) and a reverse primer (5′-GAGTCCGTGCTGCAAATGTCACTCC-3′). The primer set was designed from the rat genome data base (clone AC095903), and the region covered by this primer set shows 89% identity to the mouse Bach1 cDNA sequence (37). The rat Bach1 cDNA fragment of 598 bp was cloned into pCR-BluntII-TOPO (Invitrogen), yielding pCR-rBach1. The nucleotide sequence of the cDNA insert is identical to that of the rat genome data base (AC095903). The expression of β-actin mRNA was examined as an internal control. The probe for β-actin mRNA was the *Sma*I/*Sca*I fragment (nucleotides 124–1050) of a human β-actin cDNA provided by Dr. T. Yamamoto (Tohoku University). These DNA fragments were labeled with [α-^{32}P]dCTP (Amersham Biosciences) by the random priming method and were used as hybridization probes. Total RNA (15 μg per sample) was electrophoresed on 1.0% agarose gels containing 2 M formaldehyde, transferred to nylon membranes filter (Zeta-probe membrane; Bio-Rad), and fixed with a UV-linker (Stratalinker 1800; Stratagene). The RNA blot was hybridized with each ^{32}P-labeled probe, as detailed previously (33). Radioactive signals were detected by exposing the filters to x-ray films (X-AR5; Kodak) or with a Bioimage Analyser (BAS 1500; Fuji Film Co. Ltd.). The exposure time to x-ray films varied depending of the experiments: about 12 h for the induction of HO-1 and at least 48 h for the repression of HO-1 expression. The intensity of hybridization signals was determined by photo-stimulated luminescence with a Bioimage Analyzer.

Western Blot Analysis—T98G human glioblastoma cells or C6 rat glioma cells were lysed in triple detergent lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 100 μg/liter phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml Nonidet P40, and 0.5% sodium deoxycholate. The cell lysates were centrifuged at 15,000 × *g* for 10 min, and the supernatant (10 μg of protein) was analyzed on a SDS-polyacrylamide gel (10%). Nuclear extracts (5 μg of protein) of T98G cells were also used for Western blot analysis to confirm the expression of Bach1 protein. The proteins in the gel were treated with 20% methanol buffer containing 48 mM Tris, 39 mM glycine, and 0.037% SDS and electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore Corporation), which was pretreated with the same buffer. Expression of HO-1 and Bach1 was determined with anti-HO-1 antibody (6) and with anti-Bach1 antibody (37), respectively. The specific immunocomplexes were detected with a Western blot kit (ECL Plus, Amersham Biosciences).

HO-1 mRNA Stability Assays—T98G cells and C6 rat glioma cells were incubated for 12 h in fresh medium under hypoxia or normoxia in the presence or absence of $CoCl_2$, followed by addition of actinomycin D (1 μg/ml). The cells were further incubated for 2, 4, and 6 h after the addition of actinomycin D and harvested at each time point for RNA extraction.

Transient Transfection Assays—A549 lung cancer cells were seeded in a 6-cm diameter dish 24 h before DNA transfection. Cells were transfected by the calcium phosphate method with modifications, as described previously (43). Reporter plasmids used were pHHOL constructs containing the promoter region of the human *HO-1* gene upstream from the Firefly luciferase gene (43). Two constructs, pHHOL15 and pHHOL14, contain the $(GT)_{15}AT(GT)_{14}$ repeat in the proximal promoter region (44, 45). The internally deleted construct pHHOL20 and its derivatives were described previously (43). Base changes were introduced into the MARE of pHHOL20 by the method based on polymerase chain reaction (46), yielding pHHOLΔ20 (see Fig. 7*B*). A reporter plasmid, pSV40 promoter-Epo HRE-Luc, contains four copies of hypoxia-response element (5′-GATCGCCCTACGTGCTGTCTCA-3′; the core sequence underlined) and was used as a positive control for hypoxic induction (47). A549 cells were incubated for 24 h with 8.0 μg of reporter plasmid DNA and 0.5 μg of internal control DNA (pRL-TK), then re-fed with fresh medium and incubated for 12 h. The pRL-TK contains the thymidine kinase promoter region upstream of Renilla luciferase (Promega). Following the 12-hour incubation, cells were treated for 12 h under hypoxia or normoxia. Soluble extracts were prepared from the transfected cells and assayed for luciferase activity by Dual-Luciferase reporter assay system (Promega). The luciferase activity was measured with Lumat LB9507 (Berthold).

Electrophoretic Mobility Shift Assay (EMSA)—T98G glioblastoma cells were incubated under hypoxia or normoxia for 3–24 h, and their nuclear extracts were prepared as described previously (43). A probe for EMSA was a synthetic double-stranded hHO-1 MARE (5′-GATTTTGCTGAGTCACCAGTGCCTCCTCAG-3′), end-labeled with [γ-^{32}P]ATP (ICN Moravek). Nuclear extracts (1.0 μg of protein) were incubated with the labeled probe at 37 °C for 10 min in the 40-μl reaction mixture containing 20 mM HEPES (pH 7.9), 0.1 μg/μl poly(dI-dC), 0.1 μg/μl bovine serum albumin, 0.5 mM dithiothreitol, 9 mM $MgCl_2$, 1 mM EDTA, 20 mM KCl, and 4% glycerol (40). The binding reaction was also performed with the nuclear extracts prepared from untreated T98G cells in the presence of unlabeled competitors, the probe (hHO-1 MARE), or hHO-1-MARE mut (5′-GATTTTTCTTAGTTACCAGTGCC-

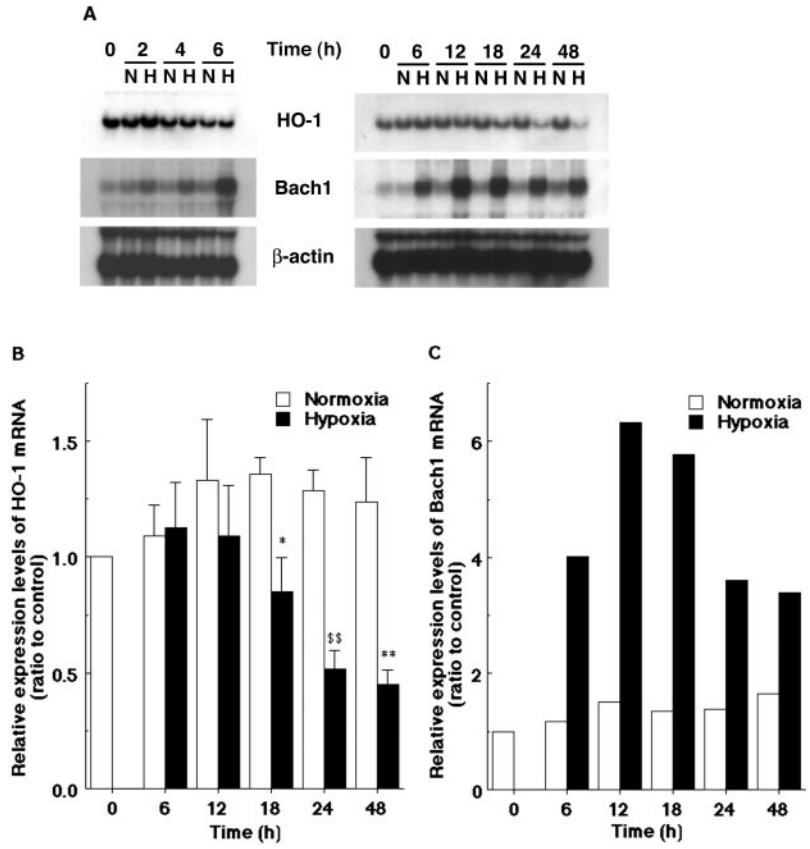

FIG. 1. **Effects of hypoxia on expression of HO-1 and Bach1 mRNAs in T98G glioblastoma cells.** *A*, Northern blot analysis. T98G cells were cultivated under normoxia (20% oxygen; *N*) or hypoxia (1% oxygen; *H*) for the indicated hours and then harvested for RNA preparation. The *lane* labeled with *0* contained RNA prepared from the untreated cells. *Top panel*, HO-1 mRNA; *middle panel*, Bach1 mRNA; *bottom panel*, β-actin mRNA as an internal control. The data shown are from one of four independent experiments with similar results. *B*, relative expression levels of HO-1 mRNA (means ± S.E., $n = 4$). The intensity of hybridization signals in the *right panel* of *A* was quantified with a Bioimage analyzer, and the intensity representing HO-1 mRNA was normalized with respect to the intensity for β-actin mRNA in each experiment. The ratio of each normalized value to that of the control (indicated with *0*) is shown as the relative expression levels of HO-1 mRNA. *, $p < 0.05$; **, $p < 0.01$; $$, $p < 0.001$. *C*, relative expression levels of Bach1 mRNA (means of two experiments with similar results). The intensity of hybridization signals of Bach1 mRNA was quantified as described in *B*.

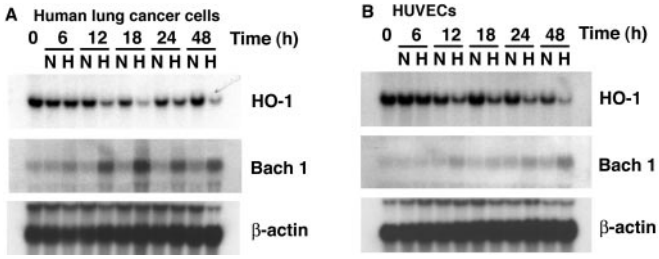

FIG. 2. **Effects of hypoxia on expression of HO-1 and Bach1 mRNAs in other human cells.** A549 human lung cancer cells (*A*) and HUVECs (*B*) were cultivated under normoxia (*N*) or hypoxia (*H*) for the indicated hours. Shown are the Northern blot analyses, as described in the legend to Fig. 1.

TCCTCAG-3′) that is carried by pHHOLΔ20. The resulting mixture was separated on a 4% polyacrylamide gel in 0.5× Tris-borate-EDTA buffer (89 mM Tris, 89 mM borate, and 2 mM EDTA) buffer at 200 V for 2 h at 4 °C.

EMSA was also performed with a recombinant Bach1 protein, BA1G174–739, carrying amino acid positions 174–739 of mouse Bach1, fused to glutathione *S*-transferase (40). In this series of EMSA, the hHO-1 MARE was labeled with digoxigenin using the DIG Oligonucleotide 3′-End Labeling kit (Roche Molecular Biochemicals). The competitors used were chicken *β-globin* enhancer (CβE) (5′-TCGACCCGAAAGGAGCTGACTCATGCTAGC-3′) (48) and control MARE (5′-GTGGTGCTGAGTCATAGGAGAAG-3′) (49). BA1G174–739 (180 ng) and FLAG-tagged MafK (20 ng) were left on ice for 20 min in 40 μl of the reaction buffer (40). The digoxigenin-labeled hHO-1 MARE was added to the above solution with or without an unlabeled competitor, and the reaction mixture was incubated at 37 °C for 10 min. The protein·DNA complexes, separated on a 4% polyacrylamide gel, were transferred onto nylon membranes and were detected using the DIG Luminescent Detection kit (Roche Molecular Biochemicals).

Statistics—Data are shown as mean ± S.E., unless otherwise stated. The statistical analysis was performed by one-way analysis of variance and Fisher's protected least significant difference multiple comparison test.

RESULTS

Repression of Heme Oxygenase-1 by Hypoxia Preceded by Induction of Bach1 in Human Cells—We analyzed the effect of hypoxia on the expression of HO-1 and Bach1 mRNAs in T98G human glioblastoma cells by Northern blot analysis, because T98G cells are able to respond to hypoxia by inducing adrenomedullin and express mRNAs for HIF-1α and HIF-β that constitute HIF-1 (33). Hypoxia decreased expression levels of HO-1 mRNA and conversely increased the expression of Bach1 mRNA (Fig. 1*A*). In contrast, hypoxia had no noticeable effect on the expression levels of β-actin mRNA. Under the hypoxic conditions employed, the growth or survival rate of T98G cells remained unchanged by 48 h (data not shown). The levels of HO-1 mRNA were decreased by 12 h after exposure to hypoxia and reached the lowest level by 48 h (about 36% of the parallel 48-h control) (Fig. 1*B*). The hypoxia-mediated reduction of HO-1 mRNA expression is associated with the reduction of HO-1 protein levels (data not shown), as observed in HUVECs (23). Induction of Bach1 mRNA expression was detectable at 2 h after exposure to hypoxia (Fig. 1*A*) and reached the maximum by 12 h (Fig. 1*C*). The expression levels of Bach1 mRNA remained at the increased levels by 48 h.

To explore whether the repression of HO-1 expression and induction of Bach1 expression by hypoxia seen in T98G glioblastoma cells represents a general response, we repeated similar experiments in A549 human lung cancer cells and primary culture of HUVECs. It is noteworthy that HIF-1 is functionally activated in HUVECs under our hypoxic conditions (23). In both cell types, the reduction of HO-1 mRNA levels was detectable at 12 h after exposure to hypoxia, when the levels of Bach1 mRNA were higher than those under normoxia (Fig. 2, *A* and *B*). The expression levels of HO-1 mRNA were reduced to the lowest level by 48 h after exposure to hypoxia, whereas the expression levels of Bach1 mRNA were maintained at higher levels under hypoxia. The effect of hypoxia on expression pro-

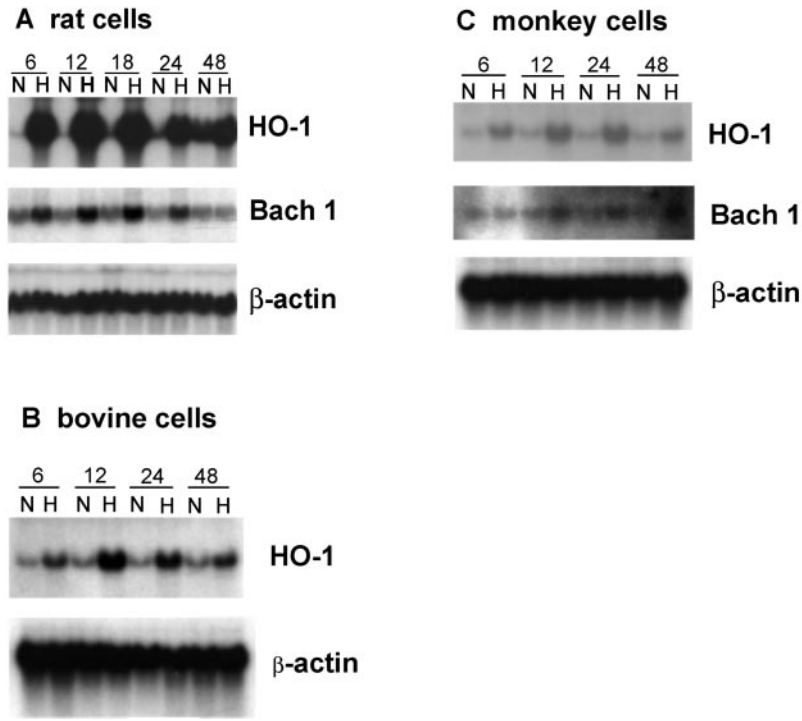

FIG. 3. **Induction of HO-1 mRNA expression by hypoxia in animal cells.** C6 rat glioma cells (*A*), bovine primary culture of BBMVECs (*B*), and COS7 monkey kidney cells (*C*) were cultivated under normoxia (*N*) or hypoxia (*H*) for the indicated hours. Shown are the Northern blot analyses.

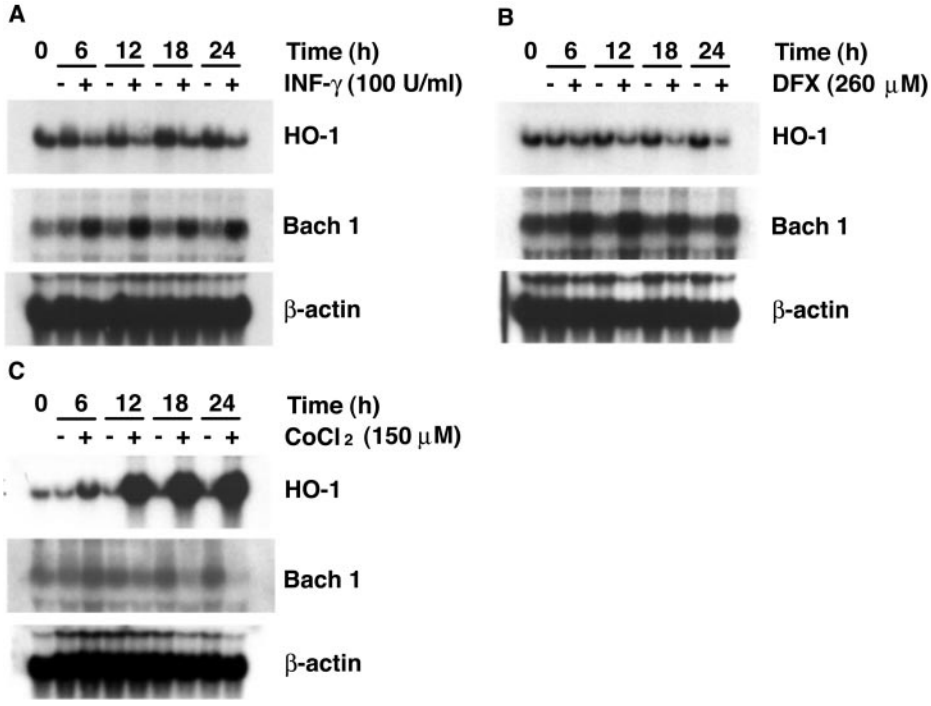

FIG. 4. **Effects of interferon-γ, desferrioxamine, and cobalt chloride on HO-1 and Bach1 mRNA expression.** T98G human glioblastoma cells were treated with interferon-γ (*INF*-γ, 100 units/ml) in *A*, desferrioxamine (*DFX*, 260 μM) in *B* or cobalt chloride ($CoCl_2$, 150 μM) in *C* for the indicated hours. Shown are the Northern blots of HO-1 mRNA (*upper panel*), Bach1 mRNA (*middle panel*), and β-actin mRNA (*lower panel*). The data represent one of two experiments with similar results.

files of HO-1 and levels of Bach1 mRNA were similar to the results observed in T98G cells although less marked. Thus, hypoxia induces expression of Bach1 mRNA in three types of human cells, which is consistently associated with the hypoxia-mediated repression of HO-1 mRNA expression.

Hypoxic Induction of HO-1 mRNA Expression in Animal Cells—We then analyzed the effects of hypoxia on the expression of HO-1 mRNA in C6 rat glioma cells, BBMVECs, and COS7 monkey kidney cells (Fig. 3) as well as in NIH3T3 mouse fibroblasts (data not shown). Hypoxia consistently induced the HO-1 mRNA expression in these cells, suggesting the species difference in the regulation of HO-1 expression by hypoxia. Expression levels of HO-1 mRNA in C6 rat cells were noticeably increased by 48 h of incubation even under normoxia, which may be due to a certain type of metabolic stress generated during the long-term culture. In fact, rat HO-1 is a heat shock protein (20). Bach1 mRNA expression was induced by hypoxia in C6 rat cells and COS7 monkey kidney cells (Fig. 3, *A* and *C*). Bach1 mRNA expression was undetectable in BBMVECs with a human cDNA probe.

Induction of Bach1 Expression by Other Repressors for HO-1 Expression—We next examined the effects of interferon-γ and an iron chelator, desferrioxamine, on Bach1 mRNA expression in T98G glioblastoma cells, as both reagents repress the HO-1 expression in human cells (22, 23). We also included $CoCl_2$ as a positive control for the induction of HO-1. Hypoxia and $CoCl_2$ exerted the opposing effects on the HO-1 mRNA expression in HUVECs, despite that both activated the function of HIF-1

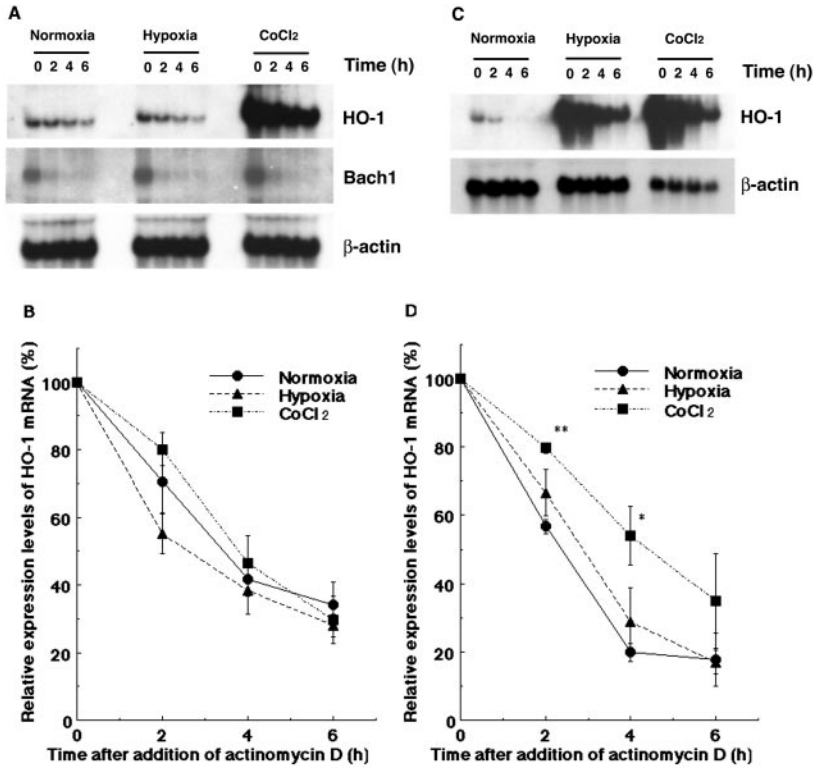

FIG. 5. **Effects of hypoxia and $CoCl_2$ on the stability of HO-1 mRNA.** T98G human glioblastoma cells (*A* and *B*) or C6 rat glioma cells (*C* and *D*) were incubated for 12 h under hypoxia or under normoxia in the absence or presence of 150 μM $CoCl_2$. Cells were further incubated for 2, 4, or 6 h after addition of actinomycin D (1 μg/ml) and harvested for RNA preparation. Other conditions are the same as in Fig. 1. *A* and *C*, Northern blot analysis (*left panel*, normoxia; *center*, hypoxia; *right*, $CoCl_2$). The data shown are from one of three independent experiments with similar results. *B* and *D*, relative expression levels of HO-1 mRNA. The intensities representing HO-1 mRNA at the time of addition of actinomycin D under each condition were considered to be 100%. The data shown are mean ± S.E. ($n = 3$). In *D*, *, $p < 0.05$; **, $p < 0.01$ compared with normoxia.

(23). HO-1 mRNA expression levels were decreased time-dependently by the treatment with interferon-γ (Fig. 4*A*) or desferrioxamine (Fig. 4*B*), but remarkably increased by the treatment with $CoCl_2$ (C). The repression of HO-1 mRNA expression by interferon-γ or desferrioxamine was consistently associated with the induction of Bach1 mRNA. Thus, interferon-γ and desferrioxamine exhibited the effects similar to those of hypoxia on the expression of HO-1 and Bach1 mRNAs (see Fig. 1). In contrast, Bach1 mRNA levels were reduced time-dependently during the treatment with $CoCl_2$ and reached the lowest levels at 24 h. Thus, the inducion of HO-1 mRNA expression by $CoCl_2$ was associated with the repression of Bach1 mRNA expression. Taken together, these results indicate that the expression levels of HO-1 and Bach1 mRNA are inversely regulated.

Stability of HO-1 mRNA under Hypoxia—We then analyzed the effects of hypoxia on the stability of HO-1 mRNA in T98G human glioblastoma cells and that in C6 rat glioma cells (Fig. 5), because the hybridization signal representing human *HO-1* gene transcripts is too low under the basal conditions to detect its reduction by nuclear run-on assays (43, 50). In T98G cells, the half-life of HO-1 mRNA was about 3 h under normoxia, which is similar to the value determined in other human cells (12, 23). Hypoxia appeared to reduce the half-life of HO-1 mRNA, and $CoCl_2$ prolonged the half-life of HO-1 mRNA, although these effects were not statistically significant (Fig. 5*B*). Thus, the observed repressive effect of hypoxia on HO-1 mRNA expression is mainly due to the decreased transcription of the *HO-1* gene. It is noteworthy that the half-life of Bach1 mRNA is much shorter than 2 h.

In C6 rat glioma cells, the half-life of HO-1 mRNA was about 2.5 h under normoxia. $CoCl_2$ induced HO-1 mRNA expression in C6 cells, as did hypoxia (Fig. 5*C*). There was no significant change in the half-life of HO-1 mRNA under hypoxia, but $CoCl_2$ prolonged the half-life of HO-1 mRNA to ~4.5 h. Thus, the induction of HO-1 mRNA expression in C6 cells by hypoxia is mainly due to the increased transcription, and the induction by $CoCl_2$ is in part due to the reduced degradation rate.

Bach1 as a Repressor for the Human HO-1 Gene—We next studied the role of Bach1 in the promoter activity of the human *HO-1* gene by transient co-transfection assays in A549 human lung cancer cells. Co-expression of Bach1 caused significant reduction of the expression of a reporter plasmid pHHOL15 carrying the 4.5-kb upstream region (Fig. 6*A*). Deletion studies have localized the *cis*-acting region (positions −4.5 to −4 kb) that is required for the basal promoter activity and for the Bach1-mediated repression. Incidentally, this *cis*-acting region contains the composite enhancer (14) that consists of the cadmium-responsive element (43, 51) and a putative MARE. To localize a *cis*-acting element that is responsible for the Bach1-mediated repression we used the internally deleted construct pHHOL20 that contains the upstream *cis*-acting region (−4.5 to −4 kb) at the position −283 of the human *HO-1* gene (Fig. 6*B*). Bach1 reduced the expression levels of pHHOL20 and its derivative pHHOL23 carrying the MARE, but not a construct pHHOL22, lacking the MARE. Together with the fact that Bach1 represses the mouse *ho-1* gene transcription through MAREs (39), these results suggest that the identified MARE may be involved in the repression of the human *HO-1* gene by Bach1. It is noteworthy that the basal expression level of pHHOL23, lacking the 5′ portion of the upstream region, is lower than that of pHHOL20, suggesting that the entire upstream region is required for the efficient transcription of the *HO-1* gene.

Repression of the Human HO-1 Gene Promoter Activity by Hypoxia—We then studied the effect of hypoxia on the promoter activity of the human *HO-1* gene by transient expression assays. Hypoxia significantly reduced the expression of pHHOL15 but not pHHOL14 (Fig. 7*A*), whereas hypoxia consistently induced expression of a construct containing four copies of hypoxia-response element by more than 3-fold (data not shown). Thus, the *cis*-acting region (positions −4.5 to −4 kb) is required for the reduction of reporter expression by hypoxia. Moreover, expression of the internally deleted constructs, pHHOL20 and pHHOL23, carrying the MARE was reduced under hypoxia, but not pHHOL22 (Fig. 7*B*). Importantly, the

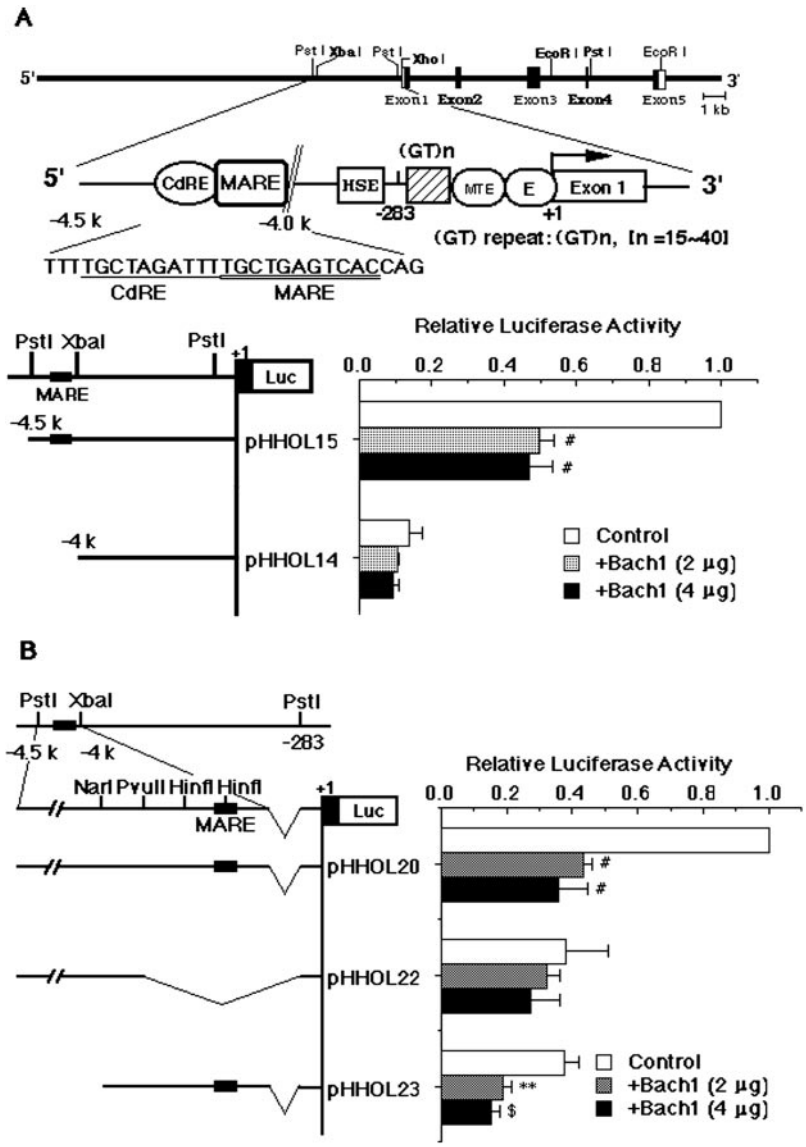

FIG. 6. **Role of Bach1 in the promoter activity of the human *HO-1* gene.** The human *HO-1* gene promoter is schematically shown at *top*. The composite enhancer constitutes the cadmium-responsive element (*CdRE*) and MARE, and the proximal promoter region contains the potential heat shock element (*HSE*) (19), the E box motifs (*MTE* and *E*) (9, 50), and the *(GT)n repeat* (44, 45). Note that the internally deleted constructs contain the (GT)n repeat. A549 lung carcinoma cells were transfected with each reporter construct together with Bach1 expression plasmid or vector plasmid. Relative luciferase activity is shown as the ratio to the normalized luciferase activity obtained with a mock plasmid and pHHOL15 (*A*) or pHHOL20 (*B*). The data are means ± S.D. of five independent experiments.

hypoxia-mediated repression was not detectable with a construct, pHHOLΔ20, carrying a mutated MARE, suggesting that the MARE located at −4 kb may be required for the hypoxic repression of *HO-1* gene expression. It is also noteworthy that the basal promoter activity of pHHOLΔ20 is lower than that of pHHOL20. Thus, the MARE is also required for the efficient transcription of the *HO-1* gene under basal conditions.

Induction of the MARE-binding Activity by Hypoxia—Hypoxia increased the amounts of immunoreactive Bach1 protein in nuclear extracts of T98G glioblastoma cells in a time-dependent manner (Fig. 8*A*). Unexpectedly, Bach1 protein was also increased after a 12–24-h incubation even under normoxia, which may reflect a preferential nuclear translocation of Bach1 under a certain metabolic stress generated during the long-term culture of T98G cells. Bach1 binds to a MARE as heterodimers with small Maf proteins (37) that are ubiquitously expressed. EMSA showed that a MARE of the human *HO-1* gene was bound *in vitro* by nuclear proteins prepared from the T98G cells exposed to hypoxia (Fig. 8*B*), indicating that the nuclear extracts also contain a member of Maf proteins. Importantly, the MARE-binding activities increased in parallel with the hypoxic induction of Bach1 protein. In addition, the MARE-binding activities increased in nuclear extracts prepared from cells after the 12-h incubation under normoxia, which appears in parallel with the induction of Bach1 protein. Attempts to demonstrate the supershifted complex of the detected band with the anti-Bach1 antibody were unsuccessful, probably due to the properties of the antibody or inaccessibility of the antibody to the Bach1·Maf complex on the DNA under the conditions employed. Consequently, we used recombinant Bach1 and MafK proteins to confirm the involvement of Bach1 in the binding to the MARE of the human *HO-1* gene, because a heterodimer of Bach1 and MafK binds to a MARE consensus *in vitro* (37, 40). As shown in Fig. 8*C*, the MARE was specifically bound by the complex of recombinant Bach1 and MafK.

DISCUSSION

The expression levels of HO-1 mRNA are decreased in human cells by the treatment with hypoxia, desferrioxamine, or interferon-γ, each of which consistently induces Bach1 mRNA expression. In addition, expression levels of Bach1 mRNA are decreased by the treatment with $CoCl_2$ that remarkably induces HO-1 expression. Thus, there is an inverse relationship in the expression levels between HO-1 and Bach1. These results suggest that Bach1 may function as a metabolic sensor. Importantly, the present study identifies Bach1 as a hypoxia-inducible regulator that represses the transcription of the *HO-1* gene in human cells; Bach1 represents a component of the hypoxia-inducible repressor because Bach1 functions as heterodimers with one of small Maf proteins (37–40). Under

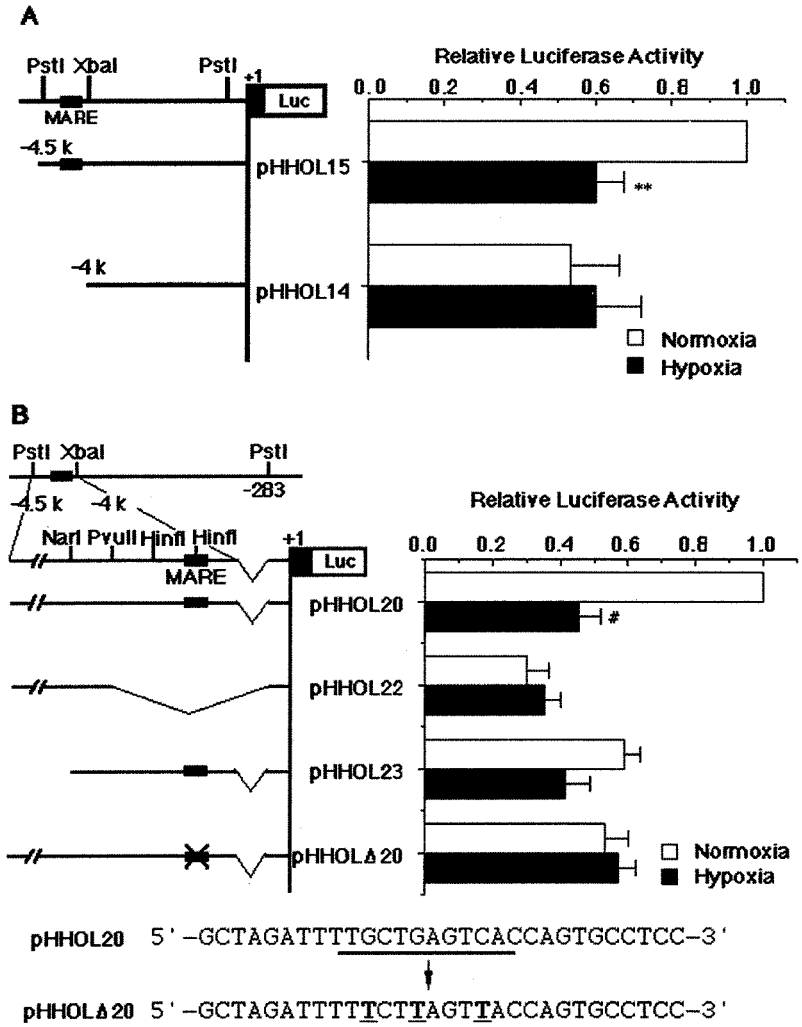

FIG. 7. **Hypoxia-mediated repression of the human *HO-1* gene promoter function.** A549 lung carcinoma cells were transfected with each reporter construct and incubated under normoxia or hypoxia. Relative luciferase activity is shown as the ratio to the normalized luciferase activity obtained with pHHOL15 (*A*) or pHHOL20 (*B*) under normoxia. The base changes introduced into the MARE are shown in *bold*. The data are means ± S.D. of five independent experiments.

our hypoxic conditions, HIF-1, a key regulator in hypoxic response, is functionally activated in A549 human lung cancer cells as well as in T98G glioblastoma cells (33) and HUVECs (23). Human cells therefore fine-tune oxygen homeostasis by inducing Bach1 and HIF-1α in response to hypoxia.

The MARE located at −4 kb is required for the hypoxic repression of the human *HO-1* gene expression and is bound by Bach1. Importantly, MARE is also bound by nuclear factor erythroid 2 (NF-E2) that is a heterodimer of an erythroid-specific subunit (p45) and the small Maf proteins (48, 52–54). Furthermore, heterodimers of NF-E2-related factor 2 and one of the small Maf proteins activate transcription of the mouse *ho-1* gene by binding to the MAREs (55–57). Taken together with the overexpression of ho-1 in many tissues of the *bach1*-deficient mouse (39), these results suggest that transcription of the *ho-1* gene may vary depending on the availability of transcriptional activators, such as NF-E2-related factor 2, and the repressor Bach1. In fact, expression of HO-1 mRNA is induced by hypoxia in C6 rat glioma cells (Fig. 3*A*) and monkey kidney cells (Fig. 3*C*), in which Bach1 mRNA is also induced by hypoxia.

An important question that remains to be answered is the physiological implication for the hypoxia-mediated repression of HO-1 expression that is usually maintained at relatively low levels. We provide three explanations that are not mutually exclusive. First, the repression of HO-1 expression reduces energy expenditure consumed for heme catabolism because the reaction catalyzed by HO-1 requires at least 3 mol of oxygen and 4 mol of NADPH to cleave 1 mole of heme (1, 2). Second, it prevents the local accumulation of carbon monoxide, iron, and bilirubin IXα beyond certain threshold levels in the HO-1-expressing cells and their surroundings, thereby preventing the tissue damage, as the heme breakdown products are potentially toxic to cells (58). For example, bilirubin IXα causes bilirubin encephalopathy in certain newborns (3). The repression of HO-1 expression could also restrict iron supply to cancer cells or certain pathogens that might be carried by a host because HO-1 is important in the turnover of iron that is an essential requirement for cell proliferation. This possibility is plausible for the repression of HO-1 expression mediated by interferon-γ and desferrioxamine. Third, it represents a mechanism by which the intra-cellular heme level is maintained at the narrow range by the balance between HO-1 and Bach1. Importantly, Bach1 functions as a heme-regulated transcriptional repressor and loses its DNA-binding activity when bound by heme (39, 40); namely, heme inhibits the DNA-binding activity of Bach1 (40), thereby leading to de-repression of the downstream target genes of Bach1. Consistent with this unique property of Bach1, heme oxygenase activity is inducible by hemin in all the cultured cells examined, including human, monkey, mouse, pig, and rat cells (59, 60). Identification of the target genes of Bach1 other than *HO-1* will give further insights into the host defense mechanism against metabolic stress.

The inter-species variations in the hypoxic regulation of *HO-1* gene expression are of particular significance, even

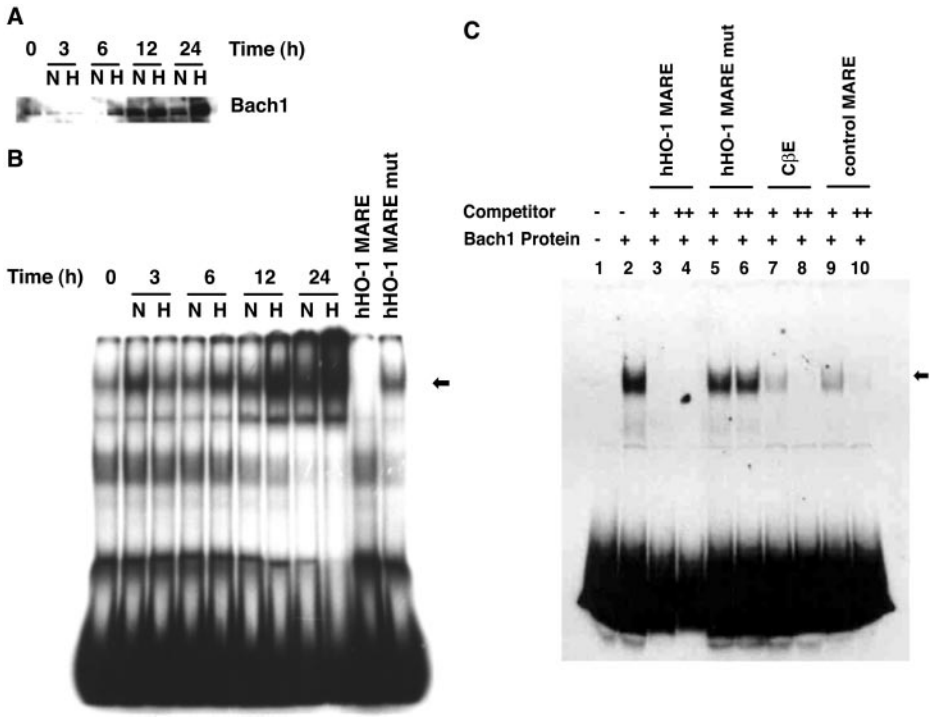

FIG. 8. **Induction of the MARE-binding activity by hypoxia.** *A*, Western blot analysis of the Bach1 protein. Each *lane* contained nuclear extracts prepared from T98G glioblastoma cells exposed to normoxia or hypoxia for the indicated hours. *B*, EMSA with nuclear extracts. Nuclear extracts of untreated T98G cells were incubated with the ^{32}P-end labeled probe in the absence or presence of an indicated competitor. The competitors used are the probe itself (hHO-1 MARE) and a mutated hHO-1 MARE. The *first lane* shows the MARE-binding activity detected in untreated T98G cells (0 h). An *arrow* indicates the specific protein·DNA complex. *C*, EMSA with recombinant Bach1 and MafK. *Lane 1* represents a buffer control lacking proteins. The digoxigenin-labeled hHO-1 MARE was incubated with recombinant Bach1 and MafK in the absence (*lane 2*) or the presence of indicated competitors (*lanes 3–10*). The competitors used were the probe itself (*lanes 3* and *4*), the mutated hHO-1 MARE (*lanes 5* and *6*), chicken *β-globin* enhancer (C*β*E) (*lanes 7* and *8*), and a control MARE (*lanes 9* and *10*). The protein·DNA complex is indicated with an *arrow*.

though we cannot generalize experimental findings in cultured cells or animal models to the human condition. A simple question has been provoked why hypoxia induces HO-1 expression in animal cells, if the repression of HO-1 is so important in the host defense, as discussed for human cells. Many studies have established that hypoxia induces HO-1 expression in animal cells (see Introduction). Here we also show the hypoxic induction of HO-1 in rat, bovine, and monkey cells (Fig. 3). Likewise, hypoxia induces HO-1 mRNA expression in NIH3T3 mouse fibroblasts (data not shown). Thus, the differential regulation of HO-1 expression between human and these animal cells may reflect the differences in the availability of certain transcription factors for the *HO-1* gene. The inter-species variation might reflect the defense strategy uniquely developed in humans (19), and its implications have been discussed in the relevance to the pathogenesis of cerebral malaria (3).

Importantly, there is at least one report that shows the induction of HO-1 expression by hypoxia in cultured human dermal fibroblasts (61). In this case, dermal fibroblasts were exposed to hypoxia in the culture medium containing 0.5% FBS instead of the maintenance culture medium that contains high glucose and 10% FBS, suggesting that these cells might be exposed to the combined stresses of nutrients deprivation and hypoxia. Moreover, the stabilization of HO-1 mRNA appears to be responsible for the hypoxic induction of HO-1 in these human dermal fibroblasts. It is therefore conceivable that expression of HO-1 mRNA is differentially regulated in human cells by hypoxia depending on cell types.

In summary, hypoxic repression of *HO-1* gene expression is consistently associated with the induction of Bach1 expression in several human cell types. Induction or down-regulation of HO-1 in human cells by pharmacological means will be a promising strategy for the treatment of various disorders. Bach1 is an alternate target to modulate the expression of HO-1. The present study suggests that Bach1 serves as a component of hypoxia-inducible repressor in human cells. Future studies will be aimed at analyzing whether expression of the Bach1 gene is under the regulation of HIF-1.

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REFERENCES

1. Tenhunen, R., Marver, H. S., and Schmid, R. (1968) *Proc. Natl. Acad. Sci. U. S. A.* **61,** 748–755
2. Yoshida, T., and Kikuchi, G. (1978) *J. Biol. Chem.* **253,** 4230–4236
3. Shibahara, S., Kitamuro, T., and Takahashi, K. (2002) *Antiox. Redox Signal.* **4,** 593–602
4. Shibahara, S., Muller, R., Taguchi, H., and Yoshida, T. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 7865–7869
5. Cruse, I., and Maines, M. D. (1988) *J. Biol. Chem.* **263,** 3348–3353
6. Shibahara, S., Yoshizawa, M., Suzuki, H., Takeda, K., Meguro, K., and Endo, K. (1993) *J. Biochem.* (*Tokyo*) **113,** 214–218
7. Yoshida, T., Biro, P., Cohen, T., Muller, R. M., and Shibahara, S. (1988) *Eur. J. Biochem.* **171,** 457–461
8. Taketani, S., Kohno, H., Yoshinaga, T., and Tokunaga, R. (1989) *FEBS Lett.* **245,** 173–176
9. Sato, M., Ishizawa, S., Yoshida, T., and Shibahara, S. (1990) *Eur. J. Biochem.* **188,** 231–237
10. Keyse, S. M., and Tyrrell, R. M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 99–103
11. Takahashi, K., Hara, E., Suzuki, H., and Shibahara, S. (1996) *J. Neurochem.* **67,** 482–489
12. Takahashi, K., Hara, E., Ogawa, K., Kimura, D., Fujita, H., and Shibahara, S. (1997) *J. Biochem.* (*Tokyo*) **121,** 1162–1168
13. Hara, E., Takahashi, K., Tominaga, T., Kumabe, T., Kayama, T., Suzuki, H., Fujita, H., Yoshimoto, T., Shirato, K., and Shibahara, S. (1996) *Biochem. Biophys. Res. Commun.* **224,** 153–158
14. Hara, E., Takahashi, K., Takeda, K., Nakayama, M., Yoshizawa, M., Fujita, H., Shirato, K., and Shibahara, S. (1999) *Biochem. Pharmacol.* **58,** 227–236
15. Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N., and Ames, B. N. (1987) *Science* **235,** 1043–1046

16. Yamaguchi, T., Shioji, I., Sugimoto, A., Komoda, Y., and Nakajima, H. (1994) *J. Biochem. (Tokyo)* **116,** 298–303
17. Poss, K. D., and Tonegawa, S. (1997) *Proc. Natl. Acad. Sci. U. S. A.,* **94,** 10919–10924
18. Yachie, A., Niida, Y., Wada, T., Igarashi, N., Kaneda, H., Toma, T., Ohta, K., Kasahara, Y., and Koizumi, S. (1999) *J. Clin. Invest.* **103,** 129–135
19. Okinaga, S., Takahashi, K., Takeda, K., Yoshizawa, M., Fujita, H., Sasaki, H., and Shibahara, S. (1996) *Blood* **87,** 5074–5084
20. Shibahara, S., Muller, R. M., and Taguchi, H. (1987) *J. Biol. Chem.* **262,** 12889–12892
21. Sato, M., Fukushi, Y., Ishizawa, S., Okinaga, S., Muller, R. M., and Shibahara, S. (1989) *J. Biol. Chem.* **264,** 10251–10260
22. Takahashi, K., Nakayama, M., Takeda, K., Fujita, H., and Shibahara, S. (1999) *J. Neurochem.* **72,** 2356–2361
23. Nakayama, M., Takahashi, K., Kitamuro, T., Yasumoto, K., Katayose, D., Shirato, K., Fujii-Kuriyama, Y., and Shibahara, S. (2000) *Biochem. Biophys. Res. Commun.* **271,** 665–671
24. Bonkovsky, H. L., Lincoln, B., Healey, J. F., Ou, L. C., Sinclair, P. R., and Muller-Eberhard, U. (1986) *Am. J. Physiol.* **251,** G467-G474
25. Katayose, D., Isoyama, S., Fujita, H., and Shibahara, S. (1993) *Biochem. Biophys. Res. Commun.* **191,** 587–594
26. Murphy, B. J., Laderoute, K. R., Short, S. M., and Sutherland, R. M. (1991) *Br. J. Cancer* **64,** 69–73
27. Lee, P. J., Jiang, B. H., Chin, B. Y., Iyer, N. V., Alam, J., Semenza, G. L., and Choi, A. M. K. (1997) *J. Biol. Chem.* **272,** 5375–5381
28. Morita, T., Perrella, M. A., Lee, M. E., and Kourembanas, S. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 1475–1479
29. Eyssen-Hernandez, R., Ladoux, A., and Frelin, C. (1996) *FEBS Lett.* **382,** 229–233
30. Semenza, G. L., and Wang, G. L. (1992) *Mol. Cell. Biol.* **12,** 5447–5454
31. Shweiki, D., Itin, A., Soffer, D., and Keshet, E. (1992) *Nature* **359,** 843–845
32. Nakayama, M., Takahashi, K., Murakami, O., Shirato, K., and Shibahara, S. (1998) *Biochem. Biophys. Res. Commun.* **243,** 514–517
33. Kitamuro, T., Takahashi, K., Nakayama, M., Murakami, O., Hida, W., Shirato, K., and Shibahara, S. (2000) *J. Neurochem.* **75,** 1826–1833
34. Firth, J. D., Ebert, B. L., Pugh, C. W., and Ratcliffe, P. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 6496–6500
35. Semenza, G. L., Jiang, B. H., Leung, S. W., Passantio, R., Concordet, J. P., Maire, P., and Giallongo, A. (1996) *J. Biol. Chem.* **271,** 32529–32537
36. Wang, G. L. Jiang, B.-H., Rue, E. A., and Semenza, G. L. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 5510–5514
37. Oyake, T., Itoh, K., Motohashi, H., Hayashi, N., Hoshino, H., Nishizawa, M., Yamamoto, M., and Igarashi, K. (1996) *Mol. Cell. Biol.* **16,** 6083–6095
38. Igarashi, K., Hoshino, H., Muto, A., Suwabe, N., Nishikawa, S., Nakauchi, H., and Yamamoto, M. (1998) *J. Biol. Chem.* **273,** 11783–11790
39. Sun, J., Hoshino, H., Takaku, K., Nakajima, O., Muto, A., Suzuki, H., Tashiro, S., Takahashi, S., Shibahara, S., Alam, J., Taketo, M. M., Yamamoto, M., and Igarashi, K. (2002) *EMBO J.* **21,** 5216–5224
40. Ogawa, K., Sun, J., Taketani, S., Nakajima, O., Nishitani, C., Sassa, S., Hayashi, N., Yamamoto, M., Shibahara, S., Fujita, H., and Igarashi, K. (2001) *EMBO J.* **20,** 2835–2843
41. Kanezaki, R., Toki, T., Yokoyama, M., Yomogida, K., Sugiyama, K., Yamamoto, M., Igarashi, K., and Ito, E. (2001) *J. Biol. Chem.* **276,** 7278–7284
42. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual,* 2nd, pp. 7.39–7.52, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
43. Takeda, K., Ishizawa, S, Sato, M., Yoshida, T., and Shibahara, S. (1994) *J. Biol. Chem.* **269,** 22858–22867
44. Shibahara, S., Sato, M., Muller, R. M., and Yoshida, T. (1989) *Eur. J. Biochem.* **179,** 557–563
45. Kimpara, T., Takeda, A., Watanabe, K., Itoyama, Y., Ikawa, S., Watanabe, M., Arai, H., Sasaki, H., Higuchi, S., Okita, N., Takase, S., Saito, H., Takahashi, K., and Shibahara, S. (1997) *Hum. Genet.* **100,** 145–147
46. Higuchi, R., Krummel, B., and Saiki, R. K. (1988) *Nucleic Acids Res.*, **16,** 7351–7367
47. Ema, M., Taya, S., Yokotani, N., Sogawa, K., Matsuda, Y., and Fujii-Kuriyama, Y. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 4273–4278
48. Igarashi, K., Kataoka, K., Itoh, K., Hayashi, N., Nishizawa, M., and Yamamoto, M. (1994) *Nature* **367,** 568–572
49. Nagai, T., Igarashi, K., Akasaka, J., Furuyama, K., Fujita, H., Hayashi, N., Yamamoto, M., and Sassa, S. (1998) *J. Biol. Chem.* **273,** 5358–5365
50. Muraosa, Y., and Shibahara, S. (1993) *Mol. Cell. Biol.* **13,** 7881–7891
51. Takeda, K., Fujita, H., and Shibahara, S. (1995) *Biochem. Biophys. Res. Commun.* **207,** 160–167
52. Andrews, N. C., Kotkow, K. J., Ney, P. A., Erdjument-Bromage, H., Tempst, P., and Orkin, S. H. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 11488–11492
53. Igarashi, K., Itoh, K., Motohashi, H., Hayashi, N., Matuzaki, Y., Nakauchi, H., Nishizawa, M., and Yamamoto, M. (1995) *J. Biol. Chem.* **270,** 7615–7624
54. Kataoka, K., Igarashi, K., Itoh, K., Fujiwara, K. T., Noda, M., Yamamoto, M., and Nishizawa, M. (1995) *Mol. Cell. Biol.* **15,** 2180–2190
55. Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M., and Nabeshima, Y. (1997) *Biochem. Biophys. Res. Commun.* **236,** 313–322
56. Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M. (2000) *J. Biol. Chem.* **275,** 16023–16029
57. Alam, J., Stewart, D., Touchard, C., Boinapally, S., Choi, A. M., and Cook, J. L. (2000) *J. Biol. Chem.* **274,** 26071–26078
58. Shibahara, S. (1994) in *Regulation of Heme Protein Synthesis* (Fujita, H., ed), pp. 103–116, Alpha Med Press, Dayton, Ohio
59. Shibahara, S., Yoshida, T., and Kikuchi, G. (1978) *Arch. Biochem. Biophys.* **188,** 243–250
60. Shibahara, S. (1988) *Sem. Hem.* **25,** 370–376
61. Panchenko, M. V., Farber, H. W., and Korn, J. H. (2000) *Am. J. Physiol.* **278,** C92-C101

GENES: STRUCTURE AND REGULATION:

Bach1 Functions as a Hypoxia-inducible Repressor for the Heme Oxygenase-1 Gene in Human Cells

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