ANTIOXIDANTS & REDOX SIGNALING
Volume 6, Number 5, 2004

News & Views

Regulation of Heme Oxygenase-1 Gene Transcription: Recent Advances and Highlights from the International Conference (Uppsala, 2003) on Heme Oxygenase

JAWED ALAM,[1,2] KAZUHIKO IGARASHI,[3] STEPHAN IMMENSCHUH,[4] SHIGEKI SHIBAHARA,[5] and REX M. TYRRELL[6]

ABSTRACT

Recent investigations into the regulation of heme oxygenase-1 gene (*hmox-1*) transcription have exposed mechanisms of increasing diversity and complexity worthy of a gene whose expression is modulated by a seemingly endless array of physiological, pathophysiological, and nonphysiological agents and conditions. For instance, contrary to initial and prevalent assumptions that inducer-dependent gene stimulation is mediated principally by the positive action of transcription activators, it now appears that such induction may occur secondarily to deactivation of the repressor protein, Bach1. As a further complication, heme and cadmium, two potent inducers of the *hmox-1* gene, inhibit Bach1 function by different mechanisms—by inhibition of DNA binding or promotion of nuclear export, respectively. Bach1 also plays a role in signal-dependent *hmox-1* gene repression, an increasingly appreciated phenomenon that is manifested in a species- and cell-specific manner. Although extreme concentrations of the heme oxygenase-1 protein resulting from the opposing phenomena of gene activation and repression have physiological consequences, even minor modulation in the level of this enzyme, as elicited by variations in the length of a dinucleotide repeat region within the human *hmox-1* promoter, may be of clinical relevance. Finally, mechanistic diversity is also apparent in the type and combination of protein kinase-dependent, signal transduction pathways used during *hmox-1* gene activation.
Antioxid. Redox Signal. 6, 924–933.

INTRODUCTION

HEME OXYGENASE (HO) ENZYMES regulate cellular heme and iron levels by catalyzing the rate-limiting reaction in heme catabolism: the oxidative cleavage of b-type heme molecules to yield equimolar quantities of biliverdin IXα, carbon monoxide (CO), and iron. In a non– rate-limiting reaction, biliverdin is subsequently converted to bilirubin by the action of biliverdin reductase. One mammalian isoform, HO-1, is widely recognized as a ubiquitous protective enzyme under various conditions of cellular stress. Although the cytoprotective function of HO-1 has been largely attributed to its ability to eliminate the prooxidant heme molecule and to generate catalytic products with antioxidant, anti-inflammatory, antiapoptotic, and cell-signaling properties, manifestation of this cytoprotection is critically dependent on the stimulation of HO-1 expression in response to the stress environment.

[1]Department of Molecular Genetics, Ochsner Clinic Foundation, New Orleans, LA 70121.
[2]Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, New Orleans, LA 70112.
[3]Department of Biochemistry, Hiroshima University School of Medicine, Kasumi 1–2-3, Hiroshima 734–8551, Japan.
[4]Institut für Klinische Chemie und Pathobiochemie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany.
[5]Department of Molecular Biology and Applied Physiology, Tohoku University School of Medicine, Aoba-ku, Sendai, Miyagi 980–8575, Japan.
[6]Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY U.K.

A variety of physiological and nonphysiological stressors, including the substrate heme, endotoxin, inflammatory cytokines, long-wavelength (320–380 nm) ultraviolet A radiation (UVA), hyperthermia, and heavy metals, stimulate HO-1 expression and activity, primarily via activation of the *hmox*-1 gene (2). Studies on the gene activation process have revealed multiple and varied mechanisms of gene regulation, a complexity that was readily evident in the presentations at the *International Conference on Heme Oxygenase: Regulation, Function and Clinical Applications*. In this report, we summarize the more compelling data on *hmox-1* gene regulation presented at the conference and organize this information into three specific topics that represent the more recent advances in the field: (a) a mechanism of gene activation based on derepression, (b) atypical regulatory features of the human *hmox-1* gene; and (c) the diversity of protein kinase-dependent signaling cascades used during *hmox-1* gene activation.

INDUCTION OF THE MURINE *HMOX-1* GENE BY DEREPRESSION

Analysis of the mouse *hmox-1* locus, the best characterized among the various *hmox-1* genes, has identified a 10-bp DNA sequence motif, termed the stress response element (StRE), that is present in multiple copies within two upstream enhancer regions and that mediates transcription activation in response to almost all HO-1 inducers thus far tested. Among these inducers are agents or conditions as diverse as heme, lipopolysaccharide, diesel exhaust particles, nitric oxide (NO), and various quinones and electrophiles. The StREs[1] are targets of multiple dimeric proteins generated by intrafamily homodimerization or intra- and interfamily heterodimerization of individual members of the Jun, Fos, CREB, ATF, Maf, and Cap'N'Collar–basic-leucine zipper (CNC-bZIP) subclasses of the basic-leucine zipper superfamily of transcription factors. Accumulating evidence argues for a particularly important role of CNC-bZIP factors in inducer-dependent *hmox-1* gene regulation (2).

Mammalian CNC-bZIP proteins, characterized by a sequence domain (*i.e*., the CNC domain) homologous to a region within the fruit fly homeotic selector protein encoded by the *cap'n'collar* gene, include p45, nuclear factor–erythroid 2 (NF-E2)-related factor 1 (Nrf1), Nrf2, Nrf3, Bach1, and Bach2. CNC-bZIP proteins do not form intrafamily dimers and heterodimerize most prominently with "small" Maf proteins, including MafF, MafG, and MafK (37). For example, the p45·MafK heterodimer constitutes the erythroid-specific transcription factor NF-E2, a protein that binds to the locus control regions of the β-globin gene. Small Maf proteins do not contain transcription activation domains and, thus, the transcription activity of NF-E2-type factors is derived from the CNC-bZIP subunit. One CNC-bZIP factor in particular, Nrf2, contains a potent transcription activation domain and has been implicated in *hmox*-1 gene induction in response to multiple agents, including heme, heavy metals, arsenite, curcumin, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, and various electrophiles (2).

Unlike Nrf2, Bach proteins do not harbor canonical transcription activation domains, and therefore Bach·small Maf dimers would be expected to function as repressors, rather than activators, when bound to target sequences. Recent studies by Igarashi and colleagues (40, 42, 53, 54) have confirmed this expectation and also revealed that Bach1-mediated repression plays a critical role in the regulation of HO-1 expression.

Regulation by heme

Initial experiments demonstrated that Bach1·MafK dimers bind to the *hmox-1* StREs and that this binding leads to repression of a linked reporter gene. This repressive activity of Bach1 was even more convincingly substantiated by gene-targeting experiments in mice that revealed that loss of Bach1 function was sufficient to uncouple *hmox-1* from stress-responsive control, resulting in constitutive expression of HO-1. Importantly, Bach1 repressor activity is dominant over the activator function of other StRE-binding proteins, such as Nrf2, effectively maintaining *hmox-1* activity at low levels under normal conditions (53).

Aside from its ability to bind to the StREs, another feature of Bach1 that is both critical and relevant to *hmox-1* gene regulation is that, *in vitro*, it binds heme—the prototypical HO-1 inducer—with high affinity (K_D = 140 n*M* for recombinant Bach1). Importantly, heme binding inhibits the DNA-binding activity of Bach1, suggesting a simple model for induction of HO-1 expression: inactivation of Bach1-mediated *hmox-1* gene repression by increased cellular levels of heme (40). Evidence in support of this model was presented by Kazuhiko Igarashi. Analysis of the *hmox-1* locus in NIH3T3 fibroblasts indicated that this gene is not repressed by hypoacetylation of the chromatin microenvironment, but rather exists in a preactivated state as the H3 histones at both the StRE-containing enhancer and the proximal promoter regions were hyperacetylated irrespective of gene activity. In contrast, *de novo* hyperacetylation and hypermethylation of H3 was induced in the transcribed region upon exposure of cells to heme. Basal level repression of transcription, therefore, is mediated primarily by Bach1. Using chromatin immunoprecipitation (CHIP) assays, Igarashi and colleagues demonstrated that, as observed *in vitro*, heme also promotes displacement of Bach1 from the *hmox-1* enhancers *in vivo*, resulting in gene derepression.

Given the preactivated status of the chromatin structure even under unstimulated conditions, it is not unreasonable to ask if removal of Bach1 from the *hmox-1* enhancers is sufficient to promote optimal *hmox-1* gene activity. The CHIP assays noted above revealed that displacement of Bach1 from the *hmox-1* StREs by heme was followed by binding of Nrf2 (presumably as Nrf2·small Maf dimers) to these elements, suggesting that high level gene transcription also requires the subsequent action of an StRE-binding protein with activator function. This idea is consistent with the results of Alam *et al*. (4), who

[1]The StRE is structurally and functionally similar to the Maf response element (MARE) and the antioxidant response element (ARE) and, to varying degrees, all three terms have been used interchangeably to describe the inducer-responsive *cis*-acting sequences of the *hmox-1* gene. For historical reasons and to avoid confusion, the StRE nomenclature has been used exclusively in this report.

demonstrated that Nrf2 is necessary for *hmox-1* gene induction by heme in rat kidney proximal tubular cells.

Regulation by cadmium

If Bach1-mediated repression of the *hmox-1* gene is a general and ubiquitous phenomenon, then one obvious question is how nonheme HO-1 inducers inactivate Bach1 protein. Given the multiplicity and the varied nature and structure of HO-1 inducers known to act via the StRE, it is unlikely that all such agents bind to Bach1 to inhibit its activity. One appealing possibility is that these inducers act indirectly by increasing the intracellular concentration of inhibitory heme molecules, by either promoting release of heme moieties from hemoproteins or mobilizing a putative "free" heme pool. This type of regulation is reminiscent of a general hypothesis proposed almost 30 years ago that induction of HO activity by nonheme agents occurs secondarily to the release of heme from cytochrome P-450. This proposition was challenged early on and, as noted by Mahin Maines (34), is "encumbered by considerable exceptions."

Data presented by K. Igarashi at the conference, and now published (54), also do not support the heme-dependent mechanism noted above at least in the case of cadmium, a potent HO-1 inducer. Rather these data argue that cadmium sponsors *hmox-1* derepression by promoting export of Bach1 from its site of action, the nucleus. Interestingly, cadmium-induced export of Bach1 is mediated *in trans* by the C-terminal region that is conserved between Bach1 and Bach2. Not surprisingly, cadmium also promotes nuclear export of Bach2, suggesting that this agent can alleviate any potential repression of the *hmox-1* gene mediated by Bach2. As cadmium, like heme, stimulates Nrf2 expression by inhibiting its degradation via the ubiquitin–proteasomal pathway (52), the net effect of these processes will be a relatively greater nuclear abundance of StRE activators (*e.g.*, Nrf2·small Maf dimers) compared with StRE repressors (*e.g.*, Bach·small Maf dimers), resulting in *hmox-1* gene activation.

A general model for hmox-1 *gene activation*

Based on the studies with heme and cadmium, we propose a simple but general model for inducer-dependent activation of the murine *hmox-1* gene (Fig. 1). As noted earlier, even in nonstimulated cells, the *hmox-1* promoter and enhancers already exist in a transcriptionally competent state as judged by the acetylation status of the H3 histone. Presumably, in this "open" conformation, the promoter can accommodate RNA polymerase II and the basic transcription machinery, which may even mediate a low basal rate of gene activity. Then, in the simplest model, the eventual rate of transcription in both nonstimulated and stimulated cells will be largely dependent on the transcription competency of the protein(s) bound at the StREs. Transcription activators, such as Nrf2, by interacting directly with the basic transcription machinery or indirectly via recruitment of coactivators, would sponsor high rates of transcription. On the other hand, transcriptionally inert proteins, such as Bach and small Maf isoforms, would be unable

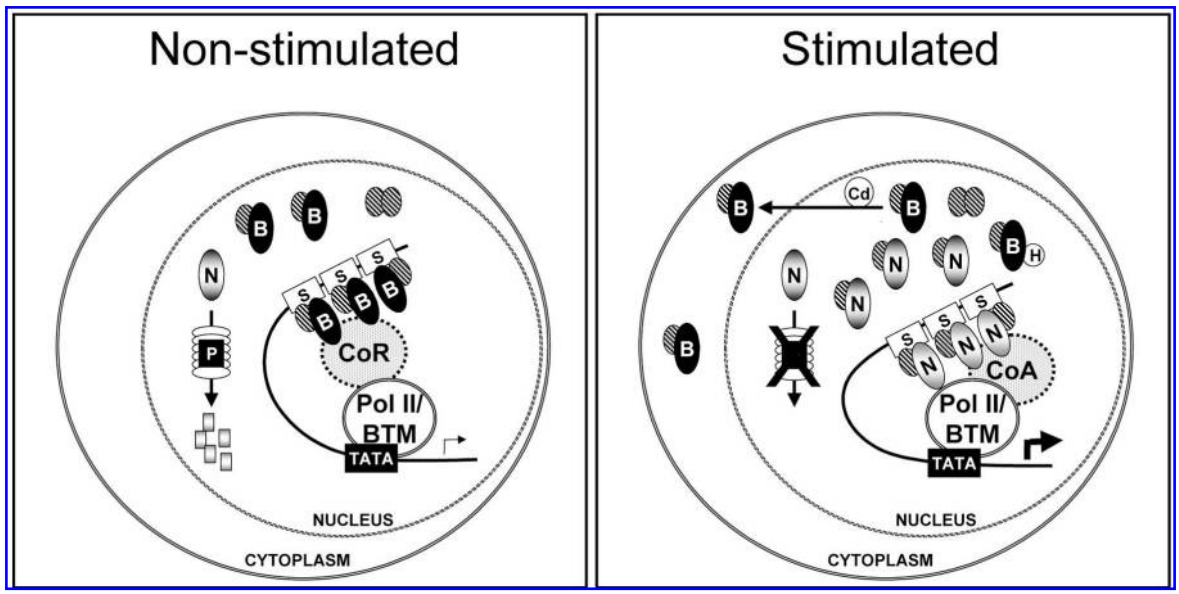

FIG. 1. A general model for *hmox-1* gene activation. In nonstimulated cells, the chromatin status of the gene permits access by RNA polymerase II and the basal transcription machinery (Pol II/BTM), which may promote low levels of transcription. Nrf2 (N) is rapidly degraded by the 26S proteasome (P), and the relatively more abundant Bach1 (B) binds to the StREs (S) as Bach1·Small Maf (hatched ovals) dimers. Bach1 cannot stimulate, and may even further repress through recruitment of corepressors (CoR) (9), the rate of transcription. Upon cellular stimulation, heme (H)-type inducers bind to Bach1 and either prevent its interaction with, or displace it from, the StREs. Furthermore, cadmium (Cd)-type inducers promote nuclear export of Bach1. Simultaneously, these stimuli inhibit Nrf2 degradation, permitting greater nuclear abundance of this activator. Nrf2, as Nrf2·Maf dimers, binds to the StREs and increases the rate of transcription by interacting with the BTM or recruitment of coactivators (CoA) (26) or both. TATA, TATA box.

to stimulate the basal rate of transcription and may even further depress this default level of gene activity by active repression mechanisms. Clearly, in this model, the competition between various StRE-binding proteins for StRE occupancy is the primary determinant of the level of gene activity. In nonstimulated cells, we suggest that this competition favors occupancy by factors such as Bach1·Maf or Bach2·Maf dimers for at least two general reasons. Firstly, CNC-bZIP·small Maf factors are relatively more stable dimers and/or bind to StREs with higher affinities than other factors, such as Maf·Maf or Jun·Jun dimers. Secondly, transcription factors with potent activation domains in general (36), and some StRE binding activators specifically (*e.g.*, Nrf2, c-Jun, c-Fos) (4, 18, 52), are highly unstable proteins that are maintained at exceedingly low cellular concentrations under normal conditions. Conversely, DNA-binding proteins lacking such domains (*e.g.*, Bach1 and small Maf proteins) would be expected to accumulate to relatively greater abundancies.

We further suggest that cellular stimulation by HO-1 inducers alters the prevailing equilibrium to one that favors StRE occupancy by activators such as Nrf2·Maf dimers. This alteration is achieved by the action of a two-pronged mechanism. On the one hand, HO-1 inducers increase the relative abundancy of Nrf2, presumably by inhibiting its proteasomal degradation. Simultaneously, these agents limit Bach1 occupancy, either by inhibiting its DNA-binding activity or by promoting nuclear export. Of course, other as of yet uncharacterized mechanisms may also contribute to inducer-mediated stimulation of Nrf2 activity and diminution of Bach1 activity. The net effect of these concerted operations will be the exchange of factors at the StREs and an increase in the rate of *hmox-1* transcription. Eventually, lessening of the stress environment either by termination of exposure or by sequestration, cellular extrusion, or metabolic inactivation of the stress agent will cause a reversal of these processes and a return to the default state observed in nonstimulated cells.

DISTINCTIVE CHARACTERISTICS IN THE REGULATION OF THE HUMAN *hmox-1* GENE

The number, location, and sequence of the StREs are essentially conserved between the mouse and human *hmox-1* genes, suggesting that the StRE pathway also plays a dominant role in induction of human HO-1 expression. Support for this contention is provided by recent reports implicating the StRE sequences and Nrf2 in activation of the human *hmox-1* gene by shear stress (10) and gold compounds (25). Overall, however, analysis of the human StRE pathway is limited, and further experimentation will be required to determine the importance of this regulatory system. Indeed, as was evident at the conference, recent studies on the human *hmox-1* gene have focused more on the potential mechanistic differences in the regulation of this gene compared with that of its rodent counterparts. For instance, Anupam Agarwal and colleagues provided evidence for the existence of previously uncharacterized regulatory loci, including an intragenic enhancer and an oxidized low-density lipoprotein-responsive region (19, 20). Although it is presently unclear if these are regulatory sequences specific to the human *hmox-1* gene, they are certainly distinct from the StRE-containing enhancers. Two additional characteristics of the human *hmox-1* gene were highlighted by the presentation of Shigeki Shibahara and colleagues: (a) cell-specific repression of human HO-1 expression by agents, such as hypoxia, interferon-γ, and hyperthermia, that typically promote induction of HO-1 expression in rodent cells; and (b) fine-tuning of human *hmox-1* gene transcription by a highly polymorphic dinucleotide repeat sequence.

Regulated hmox-1 *gene repression*

Mechanism of human hmox-1 *gene repression by hypoxia.* Expression of human HO-1 is inducible or repressible, depending on cell type or cellular microenvironment (47). For example, expression of HO-1 is reduced by hypoxia in several types of cultured human cells (29, 39), induced in human dermal fibroblasts (43), and unaffected in explants of normal human chorionic villi from term placentas (5). These results expose underlying intertissue differences in the regulation of HO-1 expression by hypoxia. Interestingly, Bach1 is consistently induced in cultured human cells in which HO-1 expression is repressed by hypoxia, suggesting that Bach1 may be involved in hypoxia-mediated *hmox-1* down-regulation. Support for this hypothesis was recently provided by Kitamuro *et al*. (29), who showed that Bach1 and one of the conserved StREs noted above mediate this down-regulation. Notably, the mechanism of hypoxia-mediated *hmox-1* repression appears to represent a specific variation of the general model of gene activation depicted in Fig. 1 insofar as the repression is achieved by altering the relative abundancy or availability of the Bach1 repressor.

In contrast to that observed in human cells, hypoxia coordinately induced expression of both HO-1 and Bach1 mRNAs in cultured rat and monkey cells, demonstrating that increased expression of Bach1 does not automatically result in inhibition of *hmox-1* transcription (29). Given that the Bach1 binding sites (*i.e.*, StREs) are essentially conserved between rodent and primate *hmox-1* genes, the species-specific variation in the hypoxia response suggests other factors—for instance, inhibitory proteins in rodent and monkey cells or corepressors in human cells—influence Bach1 activity and ultimately control the response of the *hmox-1* gene in certain circumstances.

Similar to hypoxia, HO-1 expression is repressed in cultured human cells in response to interferon-γ (29, 55) or an iron chelator, desferrioxamine (29, 39), each of which induces Bach1 expression. Whether Bach1 is involved in the repression of HO-1 expression by these agents, however, remains to be investigated.

Human hmox-1 *gene repression by hyperthermia.* The proximal promoter region of the rat *hmox-1* gene contains a functional heat shock element (HSE), and HO-1 mRNA expression and activity are increased in rat cells by heat shock (48). Moreover, hyperthermia induces the expression of HO-1 mRNA and protein in the rat brain (13). Thus, rat HO-1 is considered a *bona fide* heat shock protein. In contrast, HO-1

mRNA expression and activity are not inducible by heat shock in many types of human cells despite the fact that the human *hmox-1* promoter contains a potential HSE (41, 49, 61). For example, in human alveolar macrophages isolated from lavage fluid, heat shock increased the expression of HSP70, but instead reduced the expression of HO-1 protein (41). Moreover, HO activity was not induced by heat shock in cultured human cells (57, 61).

The lack of HO-1 induction by hyperthermia in human cells is intriguing as fever is an evolutionary conserved response in host defense. Okinaga *et al*. (41) have suggested that the sequences flanking the human *hmox-1* HSE have evolved to prevent the hyperthermia-mediated induction of HO-1 expression, possibly to limit the accumulation of heme degradation products, an abundance of which may be toxic under certain circumstances. One such condition may be cerebral malaria, where overproduction of the heme degradation products during the associated fever may be toxic to the brain (47, 50).

Physiological significance of hmox-1 *gene repression.* Expression levels of HO-1 may affect the intracellular heme pool, which in turn influences the availability of heme for the synthesis of various hemoproteins, such as soluble guanylate cyclase and cyclooxygenase (1, 16). In addition, the repression of HO-1 expression may transiently increase the intracellular heme levels, which may facilitate binding of heme to Bach1, thereby derepressing transcription of the target genes of Bach1 (40). Thus, the suppression of HO-1 production is responsible for the feedback regulation of heme catabolism mediated by intracellular heme (47). In addition to the secondary effects of heme availability, there are two direct consequences of the inhibition of HO-1 expression. This repression reduces energy expenditure consumed during oxidative catabolism of heme and also prevents the local accumulation of CO, iron, and bilirubin beyond acceptable threshold levels (47). In the latter scenario, because HO-1 is responsible for the turnover of iron that is essential for cell proliferation, repression of this enzyme could restrict iron supply to cancer cells or certain pathogens, such as bacteria and protozoa, harbored by a host.

Fine-tuning of human hmox-1 *gene transcription*

To date, two distinct polymorphisms have been identified within the human *hmox-1* gene promoter: (a) variability in the length of a GT dinucleotide repeat region and (b) a single nucleotide polymorphism ($-413A \rightarrow T$) (47). Whereas the functional consequence of the single nucleotide polymorphism is currently unknown, the $(GT)_n$ polymorphism may be of significant clinical relevance. The GT repeat lengths have been classified into three separate alleles, *i.e.,* S (small) (<27 repeats), M (medium) (27–32), and L (long) (≥33), and initial studies by Shibahara and colleagues revealed an association between the L alleles and susceptibility to pulmonary emphysema (59). Subsequent reports, including data provided by Oswald Wagner and colleagues at the conference, have documented an association between the $(GT)_n$ polymorphism and various diseases, including restonosis after balloon angioplasty and abdominal aortic aneurysm (14, 45, 46). In general, adverse outcomes are associated with the L/L allele, whereas beneficial outcomes are associated with the S/S allele.

The simplest explanation for the above correlations is that the L/L genotype leads to reduced production of the cytoprotective HO-1 enzyme, an idea based on the experiments of Yamada *et al*. (59), who showed that the "small" *hmox-1* promoter conferred greater basal activity and hydrogen peroxide responsiveness to a linked reporter gene than the "long" promoter. Given the limitations of promoter/reporter gene transfection assays, the recent report by Hirai *et al*. (21) demonstrating a comparable behavior of the endogenous *hmox-1* L/L and S/S alleles is reassuring. In this study, lymphoblastoid cell lines were established from human subjects possessing either the S/S or L/L alleles. Although no statistical difference was observed between the S/S and L/L alleles with respect to basal HO-1 mRNA production or enzyme activity, the S/S allele was associated with greater expression of HO-1 in response to hydrogen peroxide exposure and protection of the lymphoblastoid cells from apoptosis during such exposure. These data provide more convincing proof for the notion that the short $(GT)_n$ alleles are associated with relatively higher levels of HO-1 expression and presumably greater production of the cytoprotective mediators biliverdin, bilirubin, and CO. Interestingly, the long $(GT)_n$ alleles may provide a beneficial role under certain circumstances, such as cerebral malaria (47), where reduced expression of HO-1 may be an important defense mechanism. Of note, the mouse and rat *hmox-1* promoters do not harbor dinucleotide repeats at positions comparable to the human repeats. The murine gene does contain a pentanucleotide TCTCT repeat in this region, but no particular function has yet been ascribed to these sequences (3).

SIGNALING PATHWAYS UTILIZED FOR *hmox-1* GENE ACTIVATION

Although heme may function as a ligand and interact directly with a sequence-specific DNA binding protein (*i.e.*, Bach1) to activate the *hmox-1* gene, this mode of action is not, nor can be, utilized by the majority of HO-1 inducers. Rather, as accumulating evidence indicates, most inducers elicit increased HO-1 expression by activating one or more signaling cascades that eventually converge on transcription activators (or repressors) that regulate the *hmox-1* gene. Aside from such transcription factors and their cognate binding sites, other components of the signal transduction pathways that have been identified include various receptors, second messenger molecules, protein kinases, and phosphatases. Given the multiplicity and complexity of the signaling cascades used, the present discussion will, of necessity, focus on select pathways and kinases involved in *hmox-1* gene regulation in liver-derived cells and those involved in the response to UVA irradiation, as highlighted in the presentations by S. Immenschuh and R. Tyrrell.

Protein kinases that mediate hepatic HO-1 induction by cyclic nucleotides

The second messengers cyclic AMP (cAMP) and cyclic GMP (cGMP) play a major role in the intracellular signal transduction events of various hormones and extracellular stimuli. Elevation of these cyclic nucleotides activates the downstream kinases cAMP-dependent protein kinase A

(PKA) and cGMP-dependent protein kinase G (PKG), respectively. Bakken *et al*. (6) initially showed that treatment with glucagon, as well as dibutyryl cAMP, induces hepatic HO enzyme activity in rats. More recently, Immenschuh and colleagues have observed that HO-1 expression is also induced by cAMP in primary cultures of rat hepatocytes, an effect that is mediated by a cAMP response element/activator protein-1 site within the rat *hmox-1* proximal promoter and occurs via activation of the PKA signaling pathway (22, 24). This response and mechanism are not limited to hepatocytes as PKA-dependent, cAMP-mediated *hmox-1* gene activation is also observed in cultured vascular smooth muscle cells (11). Furthermore, the PKA signaling pathway not only is involved in cAMP-dependent induction, but also participates in *hmox-1* gene activation by oxidized phospholipids in human umbilical vein endothelial cells (31).

The signaling gas, NO, can induce HO-1 expression in multiple tissue and cell types, including the intact liver (38) and isolated hepatocytes (28). At least in certain cellular context, this induction is controlled at the level of gene transcription (17). As NO potently stimulates soluble guanylate cyclase activity, resulting in high levels of cGMP production, it is not unreasonable to expect that cGMP serves as a second messenger for NO-mediated *hmox-1* gene activation. Consistent with such a mechanism, cGMP treatment itself increases *hmox-1* gene transcription, via activation of PKG, not only in hepatocyte cultures (23), but also in endothelial cells (44). It should be noted, however, that NO can induce HO-1 expression independent of cGMP in some experimental settings (17, 28). Nonetheless, it is apparent that both PKA and PKG signaling pathways are involved in the induction of HO-1 expression in hepatic cells.

Role of mitogen-activated protein kinases (MAPKs) in stress-dependent hmox-1 *gene activation in hepatic cells*

An important role for stress-mediated cellular responses has been ascribed to MAPKs, a family of serine-threonine protein kinases that couple extracellular signals to intracellular events, such as gene expression, cell proliferation, and apoptosis. Three major subfamilies of MAPKs—extracellular regulated signal–kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 kinases—have been identified and extensively studied. MAPKs are activated via phosphorylation of their threonyl and tyrosyl residues by MAPK kinases (MKKs), which, in turn, are phosphorylated and activated by the upstream MKK kinases (MKKKs) (32).

As to the regulatory role of MAPKs in HO-1 expression, divergent results have been reported for stress-dependent stimulation of *hmox-1* gene activity in hepatic cells from different species. For instance, Kong and co-workers have implicated several MKKKs, including MEKK1 (MAPK/ERK kinase 1), TAK1 (transforming growth factor-β-activated kinase 1), and ASK1 (apoptosis signal-regulated kinase 1), in arsenite-mediated, Nrf2-dependent HO-1 up-regulation in human HepG2 hepatoma cells (62). Furthermore, this response is, in part, dependent on the activity of JNK1 and its associated upstream kinase MKK4, but apparently does not require p38α or its associated upstream kinase MKK3. On the other hand, stimulation of HO-1 expression by arsenite in rat hepatocytes requires signaling through both the JNK and MKK3/p38 pathways (27). Whereas JNK1 apparently mediates this induction via activation of c-Jun, the role of the p38 kinase pathway is less straightforward. Whereas the p38γ isoform stimulates rat *hmox-1* promoter activity, the p38α, β, and δ isoforms function as negative regulators. Interestingly, a conserved E-box sequence that binds to the transcription regulator Max in rat hepatocyte nuclear extracts is required for both p38-dependent stimulation and inhibition of promoter activity. Presumably, the Max dimerization partner ultimately determines the regulatory outcome of p38 signaling. Finally, yet another combination of MAPKs—ERK and JNK family members—has been implicated in the activation of the *hmox-1* gene by arsenite in chicken hepatocytes (12). These studies clearly point to species-specific differences in the utilization of MAPK signaling cascades and their target transcription factors during stress-dependent induction of HO-1 expression in the hepatic environment.

Activation of signaling cascades by UVA radiation

UVA (320–380 nm) radiation is an oxidizing carcinogen (56), and most of its biological effects are dependent on oxygen. Singlet oxygen is a crucial intermediate in UVA-induced cell killing (58), and several lines of evidence, although indirect, strongly indicate that it is an early intermediate in UVA induction of the *hmox-1* gene (7). The next steps in the process have not been firmly established, but appear to involve lipid oxidation products and metabolites (8). Oxidized lipids such as hydroxynonenal, as well as those found in oxidized low-density lipoprotein (*e.g.,* 13-hydroperoxyoctadecadienoic acid) (20) and oxidized membrane phospholipids (*e.g.,* oxidized 1-palmitoyl-2-arachidonyl-*sn*- glycero-3-phosphorylcholine) (31), are potent inducers of HO-1 expression, but there is evidence that enzymatic processing of UVA-damaged lipids to prostaglandins via the phospholipase A2/cyclooxygenase pathway may also be critical to UVA activation (8). Prostaglandins are strong inducers of HO-1 (15, 33). Finally, ceramides may also be involved because they are generated by UVA (60) and have been implicated in UVA activation of the ICAM-1 gene (15). The predominant pathways undoubtedly vary with cell type, and the underlying factors controlling this remain to be elucidated.

Given that UVA can generate, via both enzymatic and nonenzymatic mechanisms, a plethora of lipid intermediates with potential involvement in signaling pathways, it is not surprising that reports on UVA-induced signaling are complex and often discordant. Under the appropriate conditions, UVA can clearly lead to activation of each of the three major classes of MAPKs in both fibroblasts and epidermal cells (30, 33, 51). Although there are reports that UVA radiation activates both JNK2 and p38, but not ERKs, in human fibroblasts (30), others report the selective activation of only p38 kinases (33). In other studies (63, 64) in which phosphorylation of specific amino acids in the autoinhibitory domains of specific kinases were studied, UVA-activated ERKs, JNKs, p38, and phosphatidylinositol 3-kinases were all involved in phosphorylation and activation of the S6 ribosomal protein kinase, $p70^{S6K}$, whereas only activated ERKs and JNKs, but not p38 kinase, were involved in phosphorylation and activation of the $p90^{RSK}$ kinase. Because UVA responses appear to

be mediated primarily by singlet oxygen, additional insight should be provided by looking at the effects of singlet oxygen alone on the signaling pathways. Again p38 kinase and JNKs (30), as well as ERKs, appear to be activated by pure singlet oxygen in fibroblasts.

The exact pathway(s) by which *hmox*-1 is activated by UVA in human fibroblasts is not clear, although various kinases are probably involved. UVA activates protein kinase C (35), which in turn has an antiapoptotic influence possibly linked to the antiapoptotic properties of HO-1. Interestingly, studies with kinase inhibitors (Tanew and Tyrrell, unpublished observations) have implicated certain protein kinase C isoforms in the UVA-mediated up-regulation of HO-1. The precise balance of pro- and antiapoptotic events following UVA radiation will clearly depend on cell type and dose. Elucidating how this balance is controlled is a key to understanding the carcinogenic properties of UVA.

SUMMARY AND FUTURE PROSPECTS

The discovery of Bach1 as a basal and signal-induced repressor of the *hmox-1* gene and of derepression as a component of the transcription activation mechanism has unfolded a new chapter in the field of *hmox1* gene regulation accompanied by, as is often the case with new concepts, a long list of unanswered questions. For instance, is Bach1 a dominant and ubiquitous repressor of the *hmox-1* gene? If so, how do HO-1 inducers other than heme and cadmium inactivate this repression? Some stimuli such as UVA radiation, which are known to promote heme release from intracellular proteins, may act indirectly though the liberated heme. Whether there are sufficient amounts of such "releasable" heme in all cell types, however, is a fair question. If the mechanism by which cadmium inhibits Bach1 activity is any indication, nuclear extrusion may be a more common mechanism utilized by other HO-1 inducers to promote gene derepression. There is no indication that such a mechanism requires cadmium-Bach protein interaction, but rather is dependent on the ERK signaling cascade (54). Of course, one cannot exclude the possibility of other mechanisms for Bach1 inactivation, such as inhibition of interaction with corepressor molecules. Heme and cadmium induce the *hmox-1* gene not only by inhibiting Bach1 repressor activity, but also by promoting activator (*e.g.*, Nrf2) function. Whether such duality is a common feature of HO-1 inducers remains to be determined. In principle, the inducer-responsive activator in such a concerted mechanism need not be Nrf2 or even an StRE-binding protein and could act through a distinct *cis*-element as appears to be the case for oxidized low-density lipoprotein. The identification of Bach1 as signal-induced repressor raises additional questions about its function. The most immediate of these is whether Bach1 also mediates repression of the human *hmox-1* gene in response to interferon-γ and desferrioxamine.

The signal transduction pathways activated by HO-1 inducers that culminate in gene induction are now being elucidated. So far, only a limited number of stimuli and cell types have been subjected to this sort of analysis. If results from these limited studies are any indication, however, the majority of HO-1 inducers are likely to use a complex network of protein kinases (and phosphatases)—the exact combination of which will vary depending on the nature of the inducing agent and the type and species of the cells under investigation—to promote *hmox-1* gene activation. Finally, although the prevailing assumption in this field has been that the signaling cascades stimulated by HO-1 inducers ultimately impinge on the function of various transcription activators, it is now abundantly clear that one must also examine the effects of these pathways on repressor activity.

ABBREVIATIONS

cAMP, cyclic AMP; cGMP, cyclic GMP; CHIP; chromatin immunoprecipitation; CNC-bZIP, Cap'N'Collar–basic-leucine zipper; CO, carbon monoxide; ERK, extracellular signal-regulated kinase; *hmox*-1, heme oxygenase-1 gene; HO, heme oxygenase; HSE, heat shock element; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKK, MAPK kinase; MKKK, MAPK kinase kinase; NF-E2, nuclear factor–erythroid 2; NO, nitric oxide; Nrf, NF-E2-related factor; PKA, protein kinase A; PKG, protein kinase G; StRE, stress response element; UVA, ultraviolet A.

ACKNOWLEDGMENTS

The work of R.M.T. is supported by the International Association for Cancer Research (U.K.).

REFERENCES

1. Abraham NG, Quan S, Mieyal PA, Yang L, Burke-Wolin T, Mingone CJ, Goodman AI, Nasjletti A, and Wolin MS. Modulation of cGMP by human HO-1 retrovirus gene transfer in pulmonary microvessel endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 283: L1117–L1124, 2002.
2. Alam J and Cook JL. Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway. *Curr Pharm Des* 9: 2499–2511, 2003.
3. Alam J, Cai J, and Smith A. Isolation and characterization of the mouse heme oxygenase-1 gene. Distal 5′ sequences are required for induction by heme or heavy metals. *J Biol Chem* 269: 1001–1009, 1994.
4. Alam J, Killeen E, Gong P, Naquin R, Hu B, Stewart D, Ingelfinger JR, and Nath KA. Heme activates the heme oxygenase-1 gene in renal epithelial cells by stabilizing Nrf2. *Am J Physiol Renal Physiol* 284: F743–F752, 2003.
5. Appleton SD, Marks GS, Nakatsu K, Brien JF, Smith GN, Graham CH, and Lash GE. Effects of hypoxia on heme oxygenase expression in human chorionic villi explants and immortalized trophoblast cells. *Am J Physiol Heart Circ Physiol* 284: H853–H858, 2003.
6. Bakken AF, Thaler MM, and Schmid R. Metabolic regulation of heme catabolism and bilirubin production. I. Hormonal control of hepatic heme oxygenase activity. *J Clin Invest* 51: 530–536, 1972.

7. Basu-Modak S and Tyrrell RM. Singlet oxygen: a primary effector in the ultraviolet A/near-visible light induction of the human heme oxygenase gene. *Cancer Res* 53: 4505–4510, 1993.
8. Basu-Modak S, Luscher P, and Tyrrell RM. Lipid metabolite involvement in the activation of the human heme oxygenase-1 gene. *Free Radic Biol Med* 20: 887–897, 1996.
9. Brand M, Ranish JA, Kummer NT, Hamilton J, Igarashi K, Francastel C, Chi TH, Crabtree GR, Aebersold R, and Groudine M. Dynamic changes in transcription factor complexes during erythroid differentiation revealed by quantitative proteomics. *Nat Struct Mol Biol* 11: 73–80, 2004.
10. Chen XL, Varner SE, Rao AS, Grey JY, Thomas S, Cook CK, Wasserman MA, Medford RM, Jaiswal AK, and Kunsch C. Laminar flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. *J Biol Chem* 278: 703–711, 2003.
11. Durante W, Christodoulides N, Cheng K, Peyton KJ, Sunahara RK, and Schafer AI. cAMP induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle. *Am J Physiol* 273: H317–H323, 1997.
12. Elbirt KK, Whitmarsh AJ, Davis RJ, and Bonkovsky HL. Mechanism of sodium arsenite-mediated induction of heme oxygenase-1 in hepatoma cells. Role of mitogen-activated protein kinases. *J Biol Chem* 273: 8922–8931, 1998.
13. Ewing JF and Maines MD. Rapid induction of heme oxygenase 1 mRNA and protein by hyperthermia in rat brain: heme oxygenase 2 is not a heat shock protein. *Proc Natl Acad Sci U S A* 88: 5364–5368, 1991.
14. Exner M, Schillinger M, Minar E, Mlekusch W, Schlerka G, Haumer M, Mannhalter C, and Wagner O. Heme oxygenase-1 gene promoter microsatellite polymorphism is associated with restenosis after percutaneous transluminal angioplasty. *J Endovasc Ther* 8: 433–440, 2001.
15. Grether-Beck S, Bonizzi G, Schmitt-Brenden H, Felsner I, Timmer A, Sies H, Johnson JP, Piette J, and Krutmann J. Non-enzymatic triggering of the ceramide signalling cascade by solar UVA radiation. *EMBO J* 19: 5793–5800, 2000.
16. Haider A, Olszanecki R, Gryglewski R, Schwartzman ML, Lianos E, Kappas A, Nasjletti A, and Abraham NG. Regulation of cyclooxygenase by the heme–heme oxygenase system in microvessel endothelial cells. *J Pharmacol Exp Ther* 300: 188–194, 2002.
17. Hartsfield CL, Alam J, Cook JL, and Choi AM. Regulation of heme oxygenase-1 gene expression in vascular smooth muscle cells by nitric oxide. *Am J Physiol* 273: L980–L988, 1997.
18. Hershko A and Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 67: 425–479, 1998.
19. Hill-Kapturczak N, Sikorski E, Voakes C, Garcia J, Nick HS, and Agarwal A. An internal enhancer regulates heme- and cadmium-mediated induction of human heme oxygenase-1. *Am J Physiol Renal Physiol* 285: F515–F523, 2003.
20. Hill-Kapturczak N, Voakes C, Garcia J, Visner G, Nick HS, and Agarwal A. A cis-acting region regulates oxidized lipid-mediated induction of the human heme oxygenase-1 gene in endothelial cells. *Arterioscler Thromb Vasc Biol* 23: 1416–F1422, 2003.
21. Hirai H, Kubo H, Yamaya M, Nakayama K, Numasaki M, Kobayashi S, Suzuki S, Shibahara S, and Sasaki H. Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. *Blood* 102: 1619–1621, 2003.
22. Immenschuh S and Ramadori G. Gene regulation of heme oxygenase-1 as a therapeutic target. *Biochem Pharmacol* 60: 1121–1128, 2000.
23. Immenschuh S, Hinke V, Ohlmann A, Gifhorn-Katz S, Katz N, Jungermann K, and Kietzmann T. Transcriptional activation of the haem oxygenase-1 gene by cGMP via a cAMP response element/activator protein-1 element in primary cultures of rat hepatocytes. *Biochem J* 334: 141–146, 1998.
24. Immenschuh S, Kietzmann T, Hinke V, Wiederhold M, Katz N, and Muller-Eberhard U. The rat heme oxygenase-1 gene is transcriptionally induced via the protein kinase A signaling pathway in rat hepatocyte cultures. *Mol Pharmacol* 53: 483–491, 1998.
25. Kataoka K, Handa H, and Nishizawa M. Induction of cellular antioxidative stress genes through heterodimeric transcription factor Nrf2/small Maf by antirheumatic gold(I) compounds. *J Biol Chem* 276: 34074–34081, 2001.
26. Katoh Y, Itoh K, Yoshida E, Miyagishi M, Fukamizu A, and Yamamoto M. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. *Genes Cells* 6: 857–868, 2001.
27. Kietzmann T, Samoylenko A, and Immenschuh S. Transcriptional regulation of heme oxygenase-1 gene expression by MAP kinases of the JNK and p38 pathways in primary cultures of rat hepatocytes. *J Biol Chem* 278: 17927–17936, 2003.
28. Kim YM, Bergonia HA, Muller C, Pitt BR, Watkins WD, and Lancaster JR Jr. Loss and degradation of enzyme-bound heme induced by cellular nitric oxide synthesis. *J Biol Chem* 270: 5710–5713, 1995.
29. Kitamuro T, Takahashi K, Ogawa K, Udono-Fujimori R, Takeda K, Furuyama K, Nakayama M, Sun J, Fujita H, Hida W, Hattori T, Shirato K, Igarashi K, and Shibahara S. Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. *J Biol Chem* 278: 9125–9133, 2003.
30. Klotz LO, Pellieux C, Briviba K, Pierlot C, Aubry JM, and Sies H. Mitogen-activated protein kinase (p38-, JNK-, ERK-) activation pattern induced by extracellular and intracellular singlet oxygen and UVA. *Eur J Biochem* 260: 917–922, 1999.
31. Kronke G, Bochkov VN, Huber J, Gruber F, Bluml S, Furnkranz A, Kadl A, Binder BR, and Leitinger N. Oxidized phospholipids induce expression of human heme oxygenase-1 involving activation of cAMP-responsive element-binding protein. *J Biol Chem* 278: 51006–51014, 2003.
32. Kyriakis JM and Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 81: 807–869, 2001.

33. Le Panse R, Dubertret L, and Coulomb B. p38 mitogen-activated protein kinase activation by ultraviolet A radiation in human dermal fibroblasts. *Photochem Photobiol* 78: 168–174, 2003.
34. Maines MD. *Heme Oxygenase: Clinical Applications and Functions*. Boca Raton, FL: CRC Press, Inc., 1992, pp. 163–165.
35. Matsui MS and DeLeo VA. Induction of protein kinase C activity by ultraviolet radiation. *Carcinogenesis* 11: 229–234, 1990.
36. Molinari E, Gilman M, and Natesan S. Proteasome-mediated degradation of transcriptional activators correlates with activation domain potency in vivo. *EMBO J* 18: 6439–6447, 1999.
37. Motohashi H, Shavit JA, Igarashi K, Yamamoto M, and Engel JD. The world according to Maf. *Nucleic Acids Res* 25: 2953–2959, 1997.
38. Motterlini R, Hidalgo A, Sammut I, Shah KA, Mohammed S, Srai K, and Green CJ. A precursor of the nitric oxide donor SIN-1 modulates the stress protein heme oxygenase-1 in rat liver. *Biochem Biophys Res Commun* 225: 167–172, 1996.
39. Nakayama M, Takahashi K, Kitamuro T, Yasumoto K, Katayose D, Shirato K, Fujii-Kuriyama, Y and Shibahara S. Repression of heme oxygenase-1 by hypoxia in vascular endothelial cells. *Biochem Biophys Res Commun* 271: 665–671, 2000.
40. Ogawa K, Sun J, Taketani S, Nakajima O, Nishitani C, Sassa S, Hayashi N, Yamamoto M, Shibahara S, Fujita H, and Igarashi K. Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *EMBO J* 20: 2835–2843, 2001.
41. Okinaga S, Takahashi K, Takeda K, Yoshizawa M, Fujita H, Sasaki H, and Shibahara S. Regulation of human heme oxygenase-1 gene expression under thermal stress. *Blood* 87: 5074–5084, 1996.
42. Oyake T, Itoh K, Motohashi H, Hayashi N, Hoshino H, Nishizawa M, Yamamoto M, and Igarashi K. Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. *Mol Cell Biol* 16: 6083–6095, 1996.
43. Panchenko MV, Farber HW, and Korn JH. Induction of heme oxygenase-1 by hypoxia and free radicals in human dermal fibroblasts. *Am J Physiol Cell Physiol* 278: C92–C101, 2000.
44. Polte T, Abate A, Dennery PA, and Schroder H. Heme oxygenase-1 is a cGMP-inducible endothelial protein and mediates the cytoprotective action of nitric oxide. *Arterioscler Thromb Vasc Biol* 20: 1209–1215, 2000.
45. Schillinger M, Exner M, Mlekusch W, Ahmadi R, Rumpold H, Mannhalter C, Wagner O, and Minar E. Heme oxygenase-1 genotype is a vascular anti-inflammatory factor following balloon angioplasty. *J Endovasc Ther* 9: 385–394, 2002.
46. Schillinger M, Exner M, Mlekusch W, Domanovits H, Huber K, Mannhalter C, Wagner O, and Minar E. Heme oxygenase-1 gene promoter polymorphism is associated with abdominal aortic aneurysm. *Thromb Res* 106: 131–136, 2002.
47. Shibahara S. The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism. *Tohoku J Exp Med* 200: 167–186, 2003.
48. Shibahara S, Muller RM, and Taguchi H. Transcriptional control of rat heme oxygenase by heat shock. *J Biol Chem* 262: 12889–12892, 1987.
49. Shibahara S, Sato M, Muller RM, and Yoshida T. Structural organization of the human heme oxygenase gene and the function of its promoter. *Eur J Biochem* 179: 557–563, 1989.
50. Shibahara S, Kitamuro T, and Takahashi K. Heme degradation and human disease: diversity is the soul of life. *Antioxid Redox Signal* 4: 593–602, 2002.
51. Silvers AL, Bachelor MA, and Bowden GT. The role of JNK and p38 MAPK activities in UVA-induced signaling pathways leading to AP-1 activation and c-Fos expression. *Neoplasia* 5: 319–329, 2003.
52. Stewart D, Killeen E, Naquin R, Alam S, and Alam J. Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. *J Biol Chem* 278: 2396–2402, 2003.
53. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, Tashiro S, Takahashi S, Shibahara S, Alam J, Taketo MM, Yamamoto M, and Igarashi K. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *EMBO J* 21: 5216–5224, 2002.
54. Suzuki H, Tashiro S, Sun J, Doi H, Satomi S, and Igarashi K. Cadmium induces nuclear export of Bach1, a transcriptional repressor of heme oxygenase-1 gene. *J Biol Chem* 278: 49246–49253, 2003.
55. Takahashi K, Nakayama M, Takeda K, Fujia H, and Shibahara S. Suppression of heme oxygenase-1 mRNA expression by interferon-γ in human glioblastoma cells. *J Neurochem* 72: 2356–2361, 1999.
56. Tyrrell RM. UVA (320–380 nm) radiation as an oxidative stress. In: *Oxidative Stress: Oxidants and Antioxidants*, edited by Sies H. London: Academic Press, 1991, pp. 57–83.
57. Tyrrell RM. Solar ultraviolet A radiation: an oxidizing skin carcinogen that activates heme oxygenase-1. *Antioxid Redox Signal* 6: 834–840, 2004.
58. Tyrrell RM and Pidoux M. Singlet oxygen involvement in the inactivation of cultured human fibroblasts by UVA (334 nm, 365 nm) and near visible radiations. *Photochem Photobiol* 49: 407–412, 1989.
59. Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, and Sasaki H. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 66: 187–195, 2000.
60. Yang Y, Sharma A, Sharma R, Patrick B, Singhal SS, Zimniak P, Awasthi S, and Awasthi YC. Cells preconditioned with mild, transient UVA irradiation acquire resistance to oxidative stress and UVA-induced apoptosis: role of 4-hydroxynonenal in UVA-mediated signaling for apoptosis. *J Biol Chem* 278: 41380–41388, 2003.
61. Yoshida T, Biro P, Cohen T, Muller RM, and Shibahara S. Human heme oxygenase cDNA and induction of its mRNA by hemin. *Eur J Biochem* 171: 457–461, 1988.
62. Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, Tan TH, and Kong AN. Activation of mitogen-activated protein kinase

pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. *J Biol Chem* 275: 39907–39913, 2000.

63. Zhang Y, Dong Z, Nomura M, Zhong S, Chen N, and Bode AM. Signal transduction pathways involved in phosphorylation and activation of p70S6K following exposure to UVA irradiation. *J Biol Chem* 276: 20913–20923, 2001.
64. Zhang Y, Zhong S, Dong Z, Chen N, Bode AM, and Ma W. UVA induces Ser381 phosphorylation of p90RSK/MAPKAP-K1 via ERK and JNK pathways. *J Biol Chem* 276: 14572–14580, 2001.

Address reprint requests to:
Dr. Jawed Alam
Department of Molecular Genetics
Ochsner Clinic Foundation
1516 Jefferson Highway
New Orleans, LA 70121

E-mail: jalam@ochsner.org

Received for publication May 20, 2004; accepted June 13, 2004.

This article has been cited by:

1. Hsi-Lung Hsieh , Hui-Hsin Wang , Cheng-Ying Wu , Chuen-Mao Yang . 2010. Reactive Oxygen Species-Dependent c-Fos/Activator Protein 1 Induction Upregulates Heme Oxygenase-1 Expression by Bradykinin in Brain Astrocytes. *Antioxidants & Redox Signaling* **13**:12, 1829-1844. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Shigeki Shibahara , Feng Han , Bin Li , Kazuhisa Takeda . 2007. Hypoxia and Heme Oxygenases: Oxygen Sensing and Regulation of Expression. *Antioxidants & Redox Signaling* **9**:12, 2209-2226. [Abstract] [Full Text PDF] [Full Text PDF with Links]
3. Stefan W. Ryter , Hong Pyo Kim , Kiichi Nakahira , Brian S. Zuckerbraun , Danielle Morse , Augustine M.K. Choi . 2007. Protective Functions of Heme Oxygenase-1 and Carbon Monoxide in the Respiratory System. *Antioxidants & Redox Signaling* **9**:12, 2157-2174. [Abstract] [Full Text PDF] [Full Text PDF with Links]
4. Prof. Mahin D. Maines . 2005. The Heme Oxygenase System: Update 2005. *Antioxidants & Redox Signaling* **7**:11-12, 1761-1766. [Citation] [Full Text PDF] [Full Text PDF with Links]