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Forum Review

The Heme-Bach1 Pathway in the Regulation of Oxidative Stress Response and Erythroid Differentiation

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ABSTRACT

Heme—as a prosthetic group of proteins required for oxygen transport and storage, respiration, and biosynthetic pathways—is essential for practically all forms of life. Additionally, the degradation products of heme (i.e., carbon monoxide, biliverdin, and bilirubin) produced by the enzymatic actions of heme oxygenase (HO) and biliverdin reductase, possess various biological activities *in vivo*. In mammalian cells, heme also functions as an intracellular regulator of gene expression by virtue of its ability to bind to Bach1, a transcription factor that functions in association with small Maf proteins. Normally, such complexes function as repressors by binding to specific target sequences, the Maf recognition element (MARE), within enhancers of genes encoding proteins such as HO-1 and β-globin. By binding to Bach1, heme induces selective removal of the repressor from the gene enhancers permitting subsequent occupancy of the MAREs by activators that, interestingly, also contain small Maf proteins. Thus small Maf proteins play dual functions in gene expression: complexes with Bach1 repress MARE-dependent gene expression, whereas heterodimers with NF-E2 p45 or related factors (Nrf1, Nrf2, and Nrf3) activate MARE-driven genes. By modulating the equilibrium of the small Maf heterodimer network, heme regulates expression of the cytoprotective enzyme HO-1 during the stress response and of β-globin during erythroid differentiation. Implications of such heme-regulated gene expression in human diseases including atherosclerosis are discussed. *Antioxid. Redox Signal.* 8, 107–118.

HEME COUPLES METABOLIC AND GENE ACTIVITIES

THE COUPLING OF metabolic activity and gene expression is fundamental in maintaining cellular homeostasis. In virtually all organisms, heme functions as an essential prosthetic group of many proteins. In this capacity, heme is indispensable for the transport and storage of oxygen, the generation of energy by respiration, many biosynthetic reactions, and the detoxification of xenobiotics. Additionally, in lower eukaryotes and prokaryotes, heme binds to several transcription factors that regulate genes involved in oxygen utilization (83, 114, 149).

In the yeast *Saccharomyces cervisiae*, heme directly mediates the effects of oxygen upon gene transcription through the heme activator protein Hap1 (25, 40, 47, 108, 150). In response to heme, Hap1 stimulates expression of gene products involved in respiration, in controlling oxidative damage, and in repression of genes required for growth under anaerobic conditions. At low heme concentrations, Hap1 exists in an inactive, higher order complex in association with heat shock protein 90 (Hsp90) and other Hsps. Heme binds directly to HAP1 to convert the inactive complex to a functional activator (74). Because the rate of heme synthesis is directly related to the prevailing oxygen levels in the yeast, this regulatory pathway allows for oxygen-responsive gene expression via monitoring of intracellular heme levels.

Hap1 contains seven heme regulatory motifs (HRMs) consisting of the cysteine-proline (CP) dipeptide in which the

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cysteine residue serves as a critical ligand for the fifth coordination of heme. In Hap1, HRM7 is the key site for the effect of heme on transcriptional activity (75). In addition to Hap1, HRMs are found in diverse heme-binding proteins such as δ-aminolevulinate synthase (73), heme lyase (127), heme-regulated inhibitor kinase HRI (21, 20, 26, 116), bacterial iron response regulator Irr (114), mammalian iron regulatory protein 2 (IRP2) (60), and Bach1 (96). The recurrence of HRMs in various proteins involved in heme and/or iron metabolism suggests that heme directly regulates heme- and iron-related functions through diverse target proteins. In this review, we will focus on the mechanisms and roles of the heme-Bach1 pathway in two different biological contexts, the oxidative stress response and erythroid cell differentiation.

BACH1 AS A TARGET OF HEME ACTION IN HIGHER EUKARYOTES

Studies on nuclear factor-erythroid 2 (NF-E2) eventually led to the discovery of Bach1. NF-E2 was first identified as an erythroid- and megakaryocyte-restricted factor that binds to the erythroid-specific promoter region of the gene encoding porphobilinogen deaminase (PBGD) (79), one of the enzymes in the heme synthetic pathway. NF-E2 is a heterodimer formed between the basic region-leucine zipper (bZip) proteins p45 and p18 (7, 50). p45 is expressed in hematopoietic cells of the erythroid, megakaryocytic, and mast cell lineages (6). p18 is one of the small Maf family proteins (MafK, MafF, or MafG) (39, 52, 64, 65, 66, 94) and their expression is not restricted to hematopoietic cells (39, 52, 99), indicating that the hematopoietic-specific function of NF-E2 is specified by p45.

NF-E2 p45, together with the *Drosophila* Cap'n-Collar (CNC) protein (81), defines a bZip subfamily. While there is only a single CNC-related gene in nematode and fruitfly, there are four members in higher eukaryotes (17, 57, 69, 82). These CNC-related factors form heterodimers with the small Maf proteins and function as activators. Among the mammalian CNC-related factors, Nrf2 plays critical roles in the induction of the phase II detoxifying enzymes and oxidative stress responsive genes (18, 55, 58, 59). In *nrf2*-deficient mice, induction of genes encoding glutathione S-transferase (a phase II enzyme), HO-1, and peroxiredoxin 1 is severely diminished. Nrf1 also participates in the oxidative stress response (72, 90). In chimeric mice, loss of Nrf1 function results in liver cell apoptosis in late-gestation fetuses that is accompanied with increased oxidative stress and impaired expression of antioxidant genes (22). Combined deficiency in *nrf1* and *nrf2* causes early embryonic lethality with marked oxidative stress in cells (76). Thus, both Nrf2 and Nrf1 play overlapping, critical roles in the oxidative stress response. The function for Nrf3 remains elusive (27). Interestingly, gene loci for p45, Nrf1, Nrf2, and Nrf3 are each linked to one or the other of the four homoeotic box (*Hox*) gene clusters in the mice and human genomes, indicating that these genes have evolved during the genome-wide duplication of *Hox* gene clusters.

Bach1 and Bach2 possess bZip domains that are more distantly related to the CNC factors but also form heterodimers with the small Maf proteins (104). In transfection assays Bach1 and Bach2 function primarily as repressors. The presence of a BTB/POZ domain, which is implicated in transcription repression and chromatin structure remodeling, distinguishes Bach1 and Bach2 from many of the other bZip proteins (104). Bach2 is expressed in developing neuronal cells and B cells (49, 87) and in the latter is required for the execution of antibody class switching and somatic hypermutation, critical genetic events during the antibody response (89). Because overexpression of Bach2 induces apoptosis in response to oxidative stress, Bach2 may counteract the cytoprotective roles of Nrf2 and/or Nrf1 (88, 134). In contrast to p45 or other activator genes, neither *Bach1* nor *Bach2* loci are linked to the *Hox* gene clusters, suggesting a historical divergence of the activator and repressor genes.

Bach1 is a heme-binding protein (96), a characteristic initially alluded to by the brownish pigmentation of the soluble, recombinant Bach1 purified from *Escherichia coli*. Bach1 possesses six CP motifs and deletion studies have demonstrated that the heme-binding region is confined within the C-terminal portion that possesses four of the CP motifs (Fig. 1). Mutations in all four of these CP motifs abolish heme interaction. The effects of heme upon Bach1 are two-fold. First, heme markedly inhibits the DNA binding activity of Bach1 (96). Although CP motifs are definitely involved in this regulation, the identity of the specific motif (or motifs) required for this effect remains elusive. Second, heme induces nuclear export of Bach1 (131). Inhibition of heme synthesis enhances nuclear accumulation of Bach1, whereas treatment of cells with hemin results in nuclear exclusion of Bach1. A region containing CP3 and CP4 is critical for heme-induced nuclear exclusion. This region binds heme *in vitro* and functions as a heme-regulated, Crm1-dependent nuclear export signal; the heme-binding activity is critical for the Crm1-mediated export. Bach1 thus extends the regulatory roles for heme in protein sorting: heme is known to inhibit mitochondrial transport of δ-aminolevulinate synthase by binding to its mitochondrial targeting signal sequence (73, 86).

In addition to the heme-regulated nuclear export signal, Bach1 possesses another nuclear export signal at its C-terminus. This signal, originally discovered on Bach2 as a cytoplasmic localization signal (CLS) (48), is activated by cadmium in an ERK-dependent manner (130). While both nuclear export signals are Crm1-dependent, detailed mechanisms such as whether these signals bind to Crm1 directly remain obscure. Thus, Bach1 responds to heme and cadmium, two potent inducers of HO-1, by distinct mechanisms. In addition to the

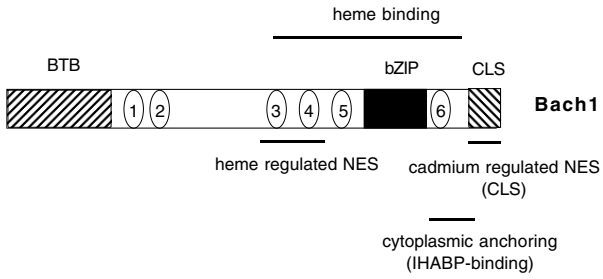

FIG. 1. Schematic representation of Bach1 structure. *Numbered circles* represent CP motifs. Details are described in the main text.

regulation at the level of nuclear export, subcellular localization of Bach1 is further regulated by its interaction with the cytoplasmic, microtubule-associated, intracellular hyaluronic acid binding protein IHABP (147). IHABP binds to Bach1 between the leucine zipper and the CLS. Human cells express an alternative spliced variant of Bach1, Bach1t, that lacks the IHABP-binding region. As expected, Bach1t is constitutively nuclear and, when co-expressed, exhibits the ability to translocate Bach1 to the nucleus (63). This region is essential for achieving cytoplasmic accumulation of Bach1. In summary, the subcellular compartmentalization of Bach1 is regulated through the combination of a nuclear localization signal, two distinct nuclear export signals, and cytoplasmic trapping by IHABP. Bach1 may integrate various signals to regulate target gene expression.

REGULATION OF THE HEME OXYGENASE-1 GENE BY BACH1

Heme oxygenase-1 (HO-1) is the inducible isozyme of HO that degrades heme, generating ferrous iron, carbon monoxide (CO), and biliverdin (that is rapidly reduced to bilirubin). These molecules are not mere waste products. Iron is re-utilized for the synthesis of new heme proteins and biliverdin and bilirubin possess antioxidant and anti-inflammatory activities *in vivo* (10, 102). Carbon monoxide, like nitric oxide, functions as an important neural second messenger and also suppresses apoptosis of endothelial cells and proliferation of smooth muscle cells (13, 14, 84, 107, 125). Reduced proliferation of smooth muscle cell appears to result from CO-mediated hypophosphorylation and activation of the tumor suppressor protein Rb (107). CO also inhibits activation of NF-κB to ameliorate inflammation. The precise mechanisms by which CO causes these effects, however, are still unclear. It should be noted that heme oxygenases are the only enzymes that generate CO within a cell. The cytoprotective roles of bilirubin and CO suggest a role for HO-1 as an antioxidant and anti-inflammatory defense enzyme that converts heme, which is a strong prooxidant when not bound to proteins, into antioxidants and a second messenger. Indeed, whereas its role in lower eukaryotes is not clear, HO-1 is essential for higher eukaryotes in order to cope with various aspects of cellular stress and to regulate cellular iron metabolism (110, 143). In clinical conditions, HO-1 expression has been associated with increased resistance to tissue injuries such as ischemic reperfusion injury (30, 45, 95, 126, 143). Importantly, the expression of HO-1 is induced by oxidative stress and the substrate heme (1, 122, 135). Thus, heme functions as both an inducer and a substrate of the cytoprotective HO-1 system. As HO-1 plays a crucial role in cell survival under oxidative stress, it is important to understand how the expression of *hmox-1* is quickly and efficiently activated upon oxidative stress. While a gene therapy approach employing HO-1 has been investigated, a more ideal approach would be to manipulate endogenous HO-1 expression with small molecules. Such a strategy necessitates an in depth understanding of *hmox-1* regulation.

The induction of mouse *hmox-1* by heme and other oxidants is regulated principally by two upstream enhancers, E1 and E2, which function in a cooperative manner (1, 2, 3, 5). Both enhancer regions contain multiple copies of the so-called stress response element (StRE, ref. 54). E1 also contains a cadmium-responsive element (CdRE) that juxtaposes one of the StREs (132). At present, it remains unclear whether the StREs and CdRE cooperate to regulate HO-1 in response to cadmium. Work on the v-Maf oncoprotein by Nishizawa and colleagues (65) revealed that v-Maf and Maf-related cellular factors are DNA binding proteins that recognize specific DNA sequence termed the Maf recognition element (MARE). Two types of MAREs are defined: T-MARE embeds a TPA-response element (TRE) while C-MARE embeds a cAMP-response element (CRE). This observation inspired the idea that the StRE and MARE are structurally and functionally related to each other. Interestingly, the antioxidant responsive element (ARE) is also related to MARE (58). (Given these similarities, the term MARE will be used hereafter to identify these sequences). The MAREs within the E2 enhancer mediate hemin-inducible expression of reporter genes. E2 possesses the ability to protect a reporter gene from chromatin position effect and this action is dependent on the presence of intact MAREs (29). Thus, these enhancers appear to regulate transcription in part by affecting the chromatin structure.

Among the MARE-binding factors, Nrf2 and Bach1 play critical roles in the regulation of *hmox-1* (4, 128). Gene targeting experiments in mice revealed that, in the absence of Bach1, *hmox-1* is expressed constitutively at high levels in various tissues under normal physiological conditions (128). On the other hand, Nrf2-deficient cells show reduced expression of HO-1 when stimulated with various inducers (55, 56, 71). In *bach1/nrf2* compound deficient mice, expression of *hmox-1* is reduced in tissues like liver, lung, and heart, demonstrating antagonistic activities of Bach1 and Nrf2 in the regulation of *hmox-1* in these tissues (128).

The genetic relation of these two factors has been confirmed biochemically. Chromatin immunoprecipitation (ChIP) assays in NIH3T3 cells and thymocytes revealed that, under resting conditions, Bach1•small Maf heterodimers occupy the E1 and E2 enhancers of *hmox-1* (128, 129) (Fig. 2). Heme and cadmium promote displacement of Bach1 from the enhancers, which is followed by Nrf2 binding to these elements. Thus, regulation of *hmox-1* involves a direct sensing of heme levels by Bach1 (by analogy to *lac* repressor sensitivity to lactose), generating a simple feedback loop whereby the substrate affects repressor-activator antagonism (128, 129).

β-GLOBIN, ANOTHER TARGET OF REGULATORY HEME

Heme also regulates expression of the β-globin genes. It is well established that heme regulates the translation initiation of globin mRNAs in erythroid cells (44, 139). In this system, heme controls protein synthesis by directly regulating the activity of the heme-regulated inhibitor kinase, HRI. HRI, which is expressed predominantly in erythroid cells, regulates the synthesis of both α- and β-globins in erythroid cells by inhibiting the general translation initiation factor eIF2. This inhibition occurs when the intracellular concentration of heme is low, thereby preventing the synthesis of globin polypeptides

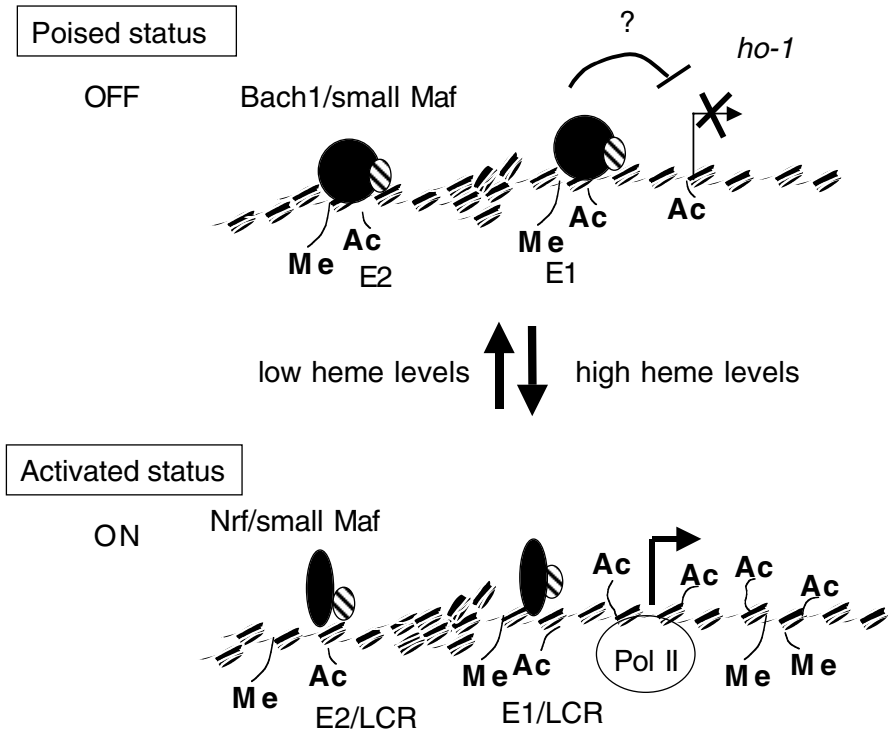

FIG. 2. On and OFF states of heme oxygenase-1 gene. When the heme levels are low in a cell, Bach1•small Maf heterodimer binds to the *hmox-1* enhancers to maintain the holding status. Chromatin structure is maintained active as judged from histone modification patterns in the enhancer and promoter regions. Increased levels of heme inhibit DNA binding activity of Bach1 and induce its nuclear export. Nrf2 or other CNC proteins bind to the enhancers to stimulate transcription. A similar switching of transcription factors is observed on the β-globin genes. How Bach1 maintains the quiescent state of target genes is unknown.

in excess of heme. When the heme concentration is high, heme binds to HRI through its HRM to inhibit the kinase activity. In addition to translational regulation, heme induces transcription of the β-globin gene (19). The couplings of synthesis of heme and globin at two levels are presumably necessary for avoiding oxidative damages due to accumulation of free heme molecules.

The human and mouse β-globin gene loci have provided a long-standing paradigm for cell type-specific gene regulation. The β-globin locus control region (LCR), located far upstream of the globin genes, is composed of five DNase I-hypersensitive sites (HS1-HS5) and mediates high level expression of all the β-like globin genes in erythroid cells (33, 34, 42, 138). These HS elements cooperate to generate a functional unit, the LCR-holocomplex or chromatin hub, which then interacts in a stage-specific manner to stimulate transcription of individual globin genes (15, 16, 31, 38, 80, 106). HS1 to HS5 contain a variety of *cis*-regulatory elements, including the GATA element and MAREs (originally termed NF-E2 binding site). MAREs are critical for both erythroid-specific and heme-dependent enhancer activities of the β-globin LCR.

In a way similar to *hmox-1* regulation, Bach1•MafK heterodimers bind to the MAREs within the β-globin LCR in MEL cells prior to terminal differentiation (12, 53) (Fig. 2). Bach1•MafK recruits co-repressor complexes including NuRD (12), which may be involved in repression of β-globin transcription. Upon erythroid differentiation induced by DMSO, Bach1 departs from the locus and is replaced by NF-E2, the heterodimer of p45 and the small Maf proteins (12, 118). This in turn leads to displacement of the co-repressors and recruitment of co-activators, resulting in globin gene expression. Thus, the small Maf proteins play dual functions in the regulation of β-globin genes depending on their partners. In addition to this partner switching, MafK proteins relocate within the nucleus upon differentiation (37). Before induction of differentiation, MafK colocalizes with centromeric heterochromatin; terminal differentiation of MEL cells is accompanied by the relocation of MafK to the euchromatic regions and formation of MafK•p45 heterodimers. Upon differentiation, the β-globin gene loci relocate away from heterochromatin compartments to euchromatin. Thus, small Maf heterodimers appear to regulate gene expression by affecting subnuclear positioning of target genes. The centromeric targeting of MafK requires dimerization, but not with an erythroid-specific partner, and the transactivation domain of p45 may be necessary and sufficient to prevent its localization in centromeric heterochromatin. Whether Bach1 is also involved in the centromeric targeting of MafK has not been examined.

Involvement of NF-E2 in erythroid cell differentiation was suggested by its role as an enhancer-binding protein for expression of the β-globin gene, by the lack of β-globin mRNA expression in a mouse erythroleukemia (MEL) cell line (CB3)

with no NF-E2 protein as a result of integration of the Friend viral genome within the p45 gene locus (77), and by the restoration of globin mRNA in these cells by transduction of p45 cDNA (70, 77). Consistent with the critical role of NF-E2 in globin gene expression, the DNA binding activity of NF-E2 increases during erythroid differentiation of MEL cells (78, 79, 92, 118, 133, 142). Furthermore, overexpression of MafK/p18 in MEL cells stimulates transcription of β-globin and α-globin genes (36, 51). Unexpectedly, however, targeted disruption of the p45 gene in mice showed severely reduced platelet formation but only a mild defect in erythropoiesis (123, 124). The reported small Maf-deficient mice also show no apparent defect in β-globin gene expression (100, 121). It is conceivable that, under these conditions, other bZip factors related to p45- and/or small Mafs compensate for the deficiency in NF-E2 activity. Alternatively, other *cis*-elements within the LCR may play roles similar to the MAREs and these redundant functions may be utilized particularly during conditions of NF-E2 deficiency or limitation. Of course, these possibilities are not mutually exclusive.

SWITCHING OF STAND-IN FACTORS AND ACTIVATORS BY HEME

As summarized above, induction of *hmox-1* and β-globin genes involves a switch of small Maf from a repressing to an activating state in a process that is initiated by departure of Bach1 from the small Maf-occupied enhancers. Why is it necessary to repress these genes with MARE-binding repressors? What is the advantage to utilize small Maf proteins in both repression and activation? Results of experiments that examine the chromatin structure of these genes may partly answer these questions.

The maintenance of genes in a stable off state is often associated with the generation of repressive epigenetic chromatin structures through histone modifications, such as methylation at residue lysine (K)9 of histone H3 (68, 93, 120). However, little is known regarding the chromatin state associated with genes that are repressed conditionally, without losing the potential for expression. Considering the multiple steps in transcription activation, it has been postulated that many genes may be preset for activation in a cell. Specialized transcription factors often called "stand-in factors", a function originally suggested by Enver and Greaves (32), may be able to establish and/or maintain a "poised" or "quiescent" state that allows a gene to undergo subsequent rapid induction (101).

The results of ChIP analyses strongly suggest that the small Maf proteins function as both stand-in factor and activators by exchanging their heterodimerization partners (12, 128, 129). While histone H3 at the *hmox-1* enhancers and promoter is hyperacetylated irrespective of gene activity, exposure of cells to heme results in *de novo* hyperacetylation and hypermethylation of histone H3 in the downstream transcribed region (129) (see Fig. 2). Thus, under normal conditions, the chromatin structure of *hmox-1* is in a preactivation state, but transcription is repressed by Bach1. Heme and cadmium induce switching of small Maf-containing heterodimers, resulting in RNA polymerase recruitment and clearance, acetylation and methylation of histone tails within the transcribed domain, and *hmox-1* transcription (129). Importantly, the localized histone H3 acetylation at K9 and K14, as well as methylation at K4, are observed in the enhancer and promoter regions prior to *hmox-1* gene activation (129). In mouse erythroid cells, hyperacetylation of histone H3 and H4 and dimethylation of K4 of H3 are restricted to the LCR and the β-major and β-minor promoters within the β-globin locus (67). In MEL cells, there is no significant change in the pattern and levels of histone H3 acetylation before or after induction of differentiation (118, 119). Another report indicates that the acetylation levels of both H3 and H4 increase two-fold at the HS2 and β-major and β-minor promoter regions upon differentiation (35). In any case, the β-globin locus goes through a preactivation state in erythroid cells prior to actual gene expression.

The patterns of the transcription factor dynamics and the histone modifications on *hmox-1* and globin loci suggest that the Bach1•small Maf complex allows chromatin activation but simultaneously inhibits transcription, a function that is predicted for a stand-in factor. Bach1 actively maintains a quiescent expression state of these genes. Small Maf•Bach1 may provide a mechanism that permits "poising" of a locus prior to the environmental or cellular cues required for the final steps in transcriptional activation. The observation that HS2 of the β-globin LCR is the first site to be opened along erythroid differentiation (61) supports a maintenance role for the MAREs. Analysis of proteins that associate with Bach1 and their functions will be critical in understanding the molecular nature of "poising". Since repressors are significant components of several mammalian heterodimeric transcription factor networks, including Myc•Max and E2F (11, 137), they, like the Bach1•small Maf complex, may also play a role as stand-in, preactivation factors.

Another salient feature of Bach1 is its activity as an architectural transcription factor (53, 148). This seems very important when we consider the fact that both *hmox-1* enhancers and β-globin LCR possess clustered MAREs. Current models for the β-globin LCR suggest that regulatory elements, separated over long chromosomal distances, may communicate with each other by direct protein:protein interactions (23). In this regard, the presence of the BTB/POZ domain in Bach1 (and Bach2) is of particular interest. Large looped DNA structures between MAREs located in different regulatory sites within the human β-globin LCR are formed *in vitro* in the presence of Bach1•MafK heterodimers and formation of these DNA loops require the Bach1 BTB/POZ domain (148). Interestingly, the Bach1 BTB/POZ domain mediates formation of homo-oligomers (53). Furthermore, repression of the enhancer activity of micro-LCR by Bach1 is partially dependent on the BTB/POZ domain (148). We surmise that such a higher order protein-DNA structure may be involved in the "poising" of the *hmox*-1 and β-globin genes. Such a function may be generic to other BTB/POZ transcription factors many of which are now categorized as repressors. This idea is reinforced by the fact that the BTB/POZ domain was originally discovered in *Drosophila* proteins that are implicated in the regulation of chromatin structure (97).

What is the reason for utilization of small Maf proteins in both conditional repression and activation? A similar strategy

is utilized in the regulation by the ATF/CREB-related bZip factor Sko1/Acr1 in yeast (91, 140). Sko1 represses expression of genes that are inducible upon hyperosmotic stress (105, 111) by recruiting Cyc8-Tup1 co-repressor to inhibit transcription (41, 111). Upon hyperosomotic stress, the MAP kinase Hog1 associates with target promoters, phosphorylates Sko1, and converts Sko1-Cyc8-Tup1 repressor complex into an activator complex that then recruits SAGA and SWI/SNF complexes (112, 113). This feature (i.e., employment of the same factors in repression and activation) provides a mechanism to achieve a rapid on/off switch of transcriptional activity, including during the post-stress period when it is necessary to rapidly turn off gene activity. Whereas the β-globin gene regulation is irreversible (i.e., uni-directional), the dual functionality of small Maf proteins in both repression and activation may allow efficient switching from off to on states of other genes.

AN EVOLUTIONAL VIEW OF THE NF-E2 SYSTEM: HOMODIMER VERSUS HETERODIMER

Having overviewed the NF-E2 system, we come back to the question regarding the grand design of the system. Whereas the CNC- and Maf-related activators are found in lower eukaryotes such as Skn1 in *Caenorhabditis elegans*, Bach1 and Bach2 are present only in higher eukaryotes. In *Ciona intestinalis*, there is only one gene each for Bach, Nrf, large Maf, and small Maf proteins (145). At present, there is no Bach-like gene found in lower eukaryotes. Thus, ascidians represent the most primitive organisms that utilize the complete core set of these factors including Bach. From an evolutionary point of view, Bach is a relatively new gene in the Maf system, suggesting that it is required for functions specific to higher eukaryotes and/or fine-tuning of the bZip system. The former idea is supported by the involvement of Bach2 in the regulation of class switch DNA recombination and somatic hypermutation of antibody genes in B lymphoid cells (89). B cells and plasma cells that utilize such genetic events are restricted to vertebrates. Overexpression of Bach1 in transgenic mice causes bone marrow fibrosis, suggesting that Bach1 may be involved in the regulation of bone marrow microenvironment, which is also an organ specific to vertebrates (136).

The function of Bach1 (and likely Bach2) as a stand-in factor is obviously important for fine-tuning of gene expression. It is possible that, in lower eukaryotes, this function of Bach1 is fulfilled by the small Maf homodimers that also function as repressors. For instance, MARE-driven genes can be repressed by increasing the level of small Maf protein in transfected cells or in transgenic mice (52, 85). Under such circumstances (i.e., excess small Maf over activator subunits), however, it may be difficult to turn on target genes because inactivation of homodimer function could inevitably lead to inhibition of the activating function as heterodimers. Furthermore, homodimers and heterodimers of small Maf show distinct DNA sequence specificities. For example, Bach1•small Maf and p45•small Maf heterodimers can target the same DNA sites that may not be good binding sites for small Maf homodimers. By evolving a dedicated repressive partner such as Bach1, the NF-E2 system may have become able to repress gene expression without losing the potentiality of activation.

REGULATION OF GENE ACTIVITY BY HEME, AN EXPANDING ARENA OF RESEARCH

Recent studies summarized here have established that Bach1 activity is regulated by heme *in vivo*, thus identifying heme as a signaling molecule in gene expression in higher eukaryotes. Under normal conditions, a "free", "exchangeable", or "regulatory" heme pool is often invoked to explain the regulatory effects of heme on cellular processes (117). In the case of the bacterial iron response regulator, heme is transferred to the regulator from its last synthetic enzyme, ferrochelatase, to exert a regulatory role (115). Such a direct transfer of heme to target proteins may be important to avoid oxidative damages due to free heme which is a potent prooxidant. It is possible that mammalian cells also contain such a carrier protein that shuttles "regulatory" heme to Bach1 in the nucleus. Because such a carrier protein for heme would be expected to play important roles in erythroid differentiation and stress response, identification of this putative protein will be crucial to understanding the entire picture of heme-mediated gene regulation. Along this line, another interesting question is the molecular mechanism that coordinates induction of HO-1 and sequestration of iron that is released by HO-1. Considering the fact that free iron catalyzes generation of oxygen radicals, its sequesteration by proteins such as ferritin is essential. Expression of ferritin is regulated at the level of gene transcription by factors such as Nrf2 as well as at the translational level by iron regulatory proteins (IRP1 and IRP2 in mammalian cells) (60, 109). While heme regulates degradation of IRP2 via promoting its ubiquitination by the E3 ligase HOIL-1 (146), there may be additional mechanisms that coordinate the heme degradation and iron sequestration.

The "regulatory" heme pool may fluctuate for two general reasons. First, heme levels reflect activities of the heme synthetic and degradation pathways. Considering the critical role of heme as a prosthetic group to multiple proteins in diverse metabolic pathways, Bach1 may regulate other genes as well to transduce the activity of heme metabolism into changes in gene expression. Second, when cells are injured, they release their contents, resulting in a local accumulation of heme (141). Heme released in this manner has been suggested to play a signaling role in the inflammatory process. Released heme may be taken up by surrounding cells and then bind to target molecules such as Bach1. This scenario postulates a new function for heme as a stress-signaling molecule.

In higher eukaryotes, heme is known to bind to another mammalian transcription factor NPAS2 (28). The fact that Bach1, NPAS2, and Hap1 have no apparent evolutionary or functional relationship suggests the involvement of heme in a diverse range of gene expression pathways and its key role as a signaling molecule in coupling metabolic activity and gene activity. In an analogy to the Hap1 system, Bach1 and/or NPAS2

may be involved in an oxygen-regulated gene expression. Alternatively, gasses such as CO may regulate their activities through heme-mediated binding. Indeed, NPAS2 is regulated by CO (28). However, the putative connection with O_2 or CO has not been addressed for Bach1.

Thus far, *hmox-1* and the β-globin genes are the only known targets of Bach1. MARE or MARE-related elements are found in diverse sets of genes encoding proteins with distinct functions such as thromboxane synthase (144), γ-crystalline (24), p53 (43), insulin (62), and IL-4 (46). Although Bach1 could be a generic repressor of MARE-driven genes, it is more likely to regulate a specific subset of such genes. Identification of additional direct target genes is obviously necessary to understand the role of heme as a gene regulatory molecule. The heme-Bach1 regulatory pathway also deserves further investigation because of the potential clinical significance of heme metabolism. For example, deregulation of heme metabolism has been implicated in mitochondrial and neural decay (8). Additionally, HO-1 plays a critical cytoprotective role in diverse clinical settings such as ischemic reperfusion injury and transplantation (9, 30, 45, 103, 126, 151). Gene responses under such conditions may also involve the heme-Bach1 pathways. Recently, we have found that progression of atherosclerosis is significantly reduced in *bach1*-deficient mice when assayed with the cuff injury model (98). Taken together with the fact that HO-1 has anti-inflammatory and antiatherosclerotic properties, Bach1 may represent a novel molecular target in anti-inflammatory and antiatherosclerotic therapy. The Bach1-deficient mice will provide excellent models to investigate these issues.

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ABBREVIATIONS

Bach1, BTB and CNC homology 1; bZip, basic region-leucine zipper; BTB, Broad complex/Tramtrack/Bric-a-brac; CdRE, cadmium-responsive element; ChIP, chromatin immunoprecipitation; CLS, cytoplasmic localization signal; CNC, Cap'n-Collar; CO, carbon monoxide; CP, cysteine-proline; CRE, cAMP-response element; HO, heme oxygenase; HRMs, heme regulatory motifs; Hsp, heat shock protein; HS, DNase I-hypersensitive site; IHABP, intracellular hyaluronic acid binding protein; IRP, iron regulatory protein; LCR, locus control region; MARE, Maf recognition element; MEL, mouse erythroleukemia; NF-E2, nuclear factor-erythroid 2; Nrf, NF-E2-related factor; POZ, Poxvirus and zinc finger; StRE, stress response element; TRE, TPA-response element.

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