Heme regulates the dynamic exchange of Bach1 and NF-E2-related factors in the Maf transcription factor network

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Contributed by Mark Groudine, December 5, 2003

Small Maf proteins serve as dual-function transcription factors through an exchange of their heterodimerization partners. For example, as heterodimers with hematopoietic cell-specific p45 NF-E2 or NF-E2-related factors (Nrf), they activate the β-globin or antioxidative stress enzyme heme oxygenase 1 (HO-1) genes, respectively. In contrast, together with Bach1, they repress these same genes. However, the signals leading to this partner exchange are not known. Using chromatin immunoprecipitation assays in NIH 3T3 cells, we show that heme, an inducer of *ho-1*, promotes displacement of Bach1 from the MafK-occupied *ho-1* enhancers, which is followed by Nrf2 binding to these elements. Whereas histone H3 at the *ho-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 transcribed region. These data indicate that, under normal conditions, the chromatin structure of *ho-1* is in a preactivation state, but transcription is repressed by Bach1. Heme induces switching of Maf dimers, resulting in *ho-1* expression. Heme also promotes displacement of Bach1 from the β-globin locus control region without affecting MafK binding in murine erythroleukemia cells. Thus, heme functions as a signaling molecule for gene expression in higher eukaryotes.

The basic region leucine zipper (bZip) transcription factors, including Jun, Fos, and Maf families, form an extensive heterodimeric network (1) and regulate proliferation, apoptosis, differentiation, and stress responses. The multiple protein interactions in the network may generate redundancy in target gene activation and/or provide a more complex regulatory network. We addressed these possibilities by using the heme oxygenase 1 (HO-1) and β-globin genes, critical target genes of the Maf oncoprotein family, as model systems. HO-1 is the rate-limiting enzyme involved in heme degradation, generating ferrous iron, carbon monoxide, and biliverdin, which is rapidly reduced to bilirubin (2). HO-1 is essential for higher eukaryotes to cope with various aspects of cellular stress and to regulate cellular iron homeostasis (3, 4). The transcription of *ho-1* is induced by the substrate heme (5). Thus, heme functions not only as a substrate but also as an inducer of the cytoprotective HO-1 system. Additionally, the transcription of *ho-1* is robustly induced in mammalian cells by oxidative stress and various proinflammatory stimulants (reviewed in ref. 6). The induction of HO-1 by diverse stress conditions provides a model for addressing the mechanistic basis for such multifaceted responses.

The E1 and E2 *ho-1* enhancers contain multiple stress-responsive elements (7) that also conform generally to the Maf-recognition element (MARE, ref. 8). The heterodimers of the small Maf proteins and Nrf2 activate *ho-1* through binding to MAREs (9–12). In contrast, heterodimers of small Maf and Bach1 or Bach2 repress transcription (13–15). Bach1 repressor activity is dominant over the activity of *ho-1* activators, such as Nrf2, effectively inhibiting *ho-1* expression under normal conditions (15). These results indicate that *ho-1* regulation involves a competition between the activator Nrf2 and the Bach1 repressor for interactions with the small Maf proteins.

A similar competitive regulation by Maf heterodimers operates on the β-globin gene. MAREs, originally referred to as NF-E2 sites, play a critical role in the enhancer function of the β-globin locus control region (LCR) (16, 17). Biochemical and chromatin immunoprecipitation (ChIP) studies have shown that the small Maf/Bach1 heterodimer binds to MAREs within the β-globin LCR in murine erythroid leukemia (MEL) cells before terminal differentiation (14, 18). After DMSO-induced terminal differentiation of MEL cells, NF-E2, a heterodimer of p45 and the small Maf proteins (19–21), occupies MAREs to activate transcription (18, 22).

Currently, little is known regarding factor exchange at the *ho-1* enhancers upon transcriptional activation or the alterations in chromatin structure that accompany the switch between repression and activation. We hypothesize that heme might be a key molecule in this switch, because the binding of heme to Bach1 inhibits its DNA-binding activity (23). In addition, heme has been implicated in transcriptional regulation of the β-globin gene (24). Here, we use ChIP assays to examine enhancer occupancy and histone modifications associated with the repressed and activated *ho-1* states. Our results suggest that heme triggers dynamic changes in the composition of enhancer-binding proteins and histone modifications of the transcribed region.

Materials and Methods

Antibody Generation and Immunoblotting Analysis. Anti-Bach1 polyclonal rabbit antisera were generated by immunizing rabbits with a GST fusion protein of mouse Bach1 (amino acid residues 174–415). Sera from two rabbits (A1-4 and A1-5) were useful in ChIP assays. The A1-5 serum was used in experiments reported here; however, essentially similar results were obtained with A1-4. Immunoblotting was performed by using whole-cell extracts prepared from cells and various tissues as described (14).

RT-PCR Analysis. Preparation of RNA and cDNA from NIH 3T3 cells was performed as described (25). Primers to amplify HO-1 cDNA were 5′-ACATCGACAGCCCCACCAAGTTCAA-3′ and 5′-CTGACGAAGTGACGCCATCTGTGAG-3′. β-Actin primers were 5′-TGCCCATCTATGAGGGTTACG-3′ and 5′-TAGAAGCATTTGCGGTGCACG-3′. β-Major and GAPDH primers were as described (22). Quantification of PCR products was performed with Bioanalyzer 2100 and the DNA 1000 LabChip kit (Agilent, Palo Alto, CA) or real-time PCR (Applied

Abbreviations: ChIP, chromatin immunoprecipitation; MARE, Maf recognition element; MEL, murine erythroid leukemia; LCR, locus control region; HO-1, heme oxygenase 1; Nrf, NF-E2-related factors; HS2, hypersensitive site 2; pol II, RNA polymerase II.

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Biosystems PRISM 7700). Serial 3-fold dilutions of cDNA were used to ensure linearity of amplification.

ChIP. Chromatin fixation and purification procedures were as described (22). In brief, NIH 3T3 cells were fixed by adding formaldehyde to 1% final concentration for 10 min at room temperature. Cells were then sonicated to prepare chromatin suspensions of ≈300- to 1,000-bp DNA in length. Immunoprecipitations were carried out as described (22). Antibodies used were anti-MafK (26), anti-p18 (sc-477; Santa Cruz Biotechnology), anti-Bach1 (A1-4 and A1-5; described above); anti-Nrf2 (sc-13032; Santa Cruz Biotechnology), and anti-RNA polymerase II (anti-pol II) (sc-899; Santa Cruz Biotechnology). Histone antibodies antiacetylated H4 (06-866), anti-acetylated histone H3 (06-599), antiacetylated histone H3K18 (07-354), K23 (07-355), K27 (07-360), anti-dimethyl H3K4 (07-030), and anti-dimethyl-histone H3K9 (07-212) were purchased from Upstate Biotechnology. Anti-trimethyl H3K4 antibody (ab8580) was purchased from Abcam (Cambridge, UK). Normal rabbit serum was used in negative control experiments. PCR reactions were carried out by using *Ex Taq* DNA polymerase (Takara Shuzo, Kyoto). PCR products were quantified by using Bioanalyzer 2100 and the DNA 1000 LabChip kit (Agilent) or the real-time PCR LightCycler (Roche Diagnostics). Relative enrichment was calculated as the difference between specific antibody and normal IgG signals that were normalized to the respective input signals. Primers (5′ to 3′) were as follows: P1, GGAGCTTCCCTAGACGGG and TCATCTTGGGAACCAGGC; P2, GAACATGAAGACCTGGTTTTGG and ATTCCCAGCCCTGTGCTAATT; P3, GAGGACAGGACCTGTTGGCTAA and TCTGCCACTGAGCTACACCAT; P4, AGGTAAAAAAAGCCAGGCCCT and CCAGCCACACAGCACTATGTAA; P5, AGGATAAGGGACACCGGTCAC and CAGAGTTTCCGCATACAACCA; P6, TTGTAACAGACTTGCCAGAG and CACTCACTGGTTGTATGCG; P7, GGGTGCTGCACTCCATGAG and GCTTGGTTTGCTGACACTTGC; P8 (E1), TGAAGTTAAAGCCGTTCCGG and AGCGGCTGGAATGCTGAGT; P9, GGCTGAAGGTCTAGTGTTTGCAT and TGGTTATTTGTTGGGATCTCG; P10 (E2), GGGCTAGCATGCGAAGTGAG and AGACTCCGCCCTAAGGGTTC; P11, AGATTGAGGATATCACCCCATC and TTTGCGAATGCTTTACAGTGTAC; P12, TCTCCTGAAACTCCAGAAGTGAG and TCGAGTTTCCAGCATCTTATCTT; and P13, CTGGAGTGAGGATTTGATTTTTG and AAGGGAGGTCAGAGGACAGTTT. Primers and ChZP conditions for the mouse β-globin locus were as described (18).

Results

Generation of Anti-Bach1 Antibodies. To study the dynamics of Bach1 *in vivo*, we first generated antibodies against Bach1 by immunizing rabbits with recombinant GST-Bach1. To examine their utility, we performed an immunoblotting analysis with cell lysates from thymus and spleen of wild-type and *bach1*-deficient mice (Fig. 1*A*). The antibodies (A1-5) detected an antigen (indicated by the arrow) with a mobility expected for Bach1 in the extracts only from wild-type cells (the identity of the antigen indicated by the filled circle is unknown). We further analyzed NIH 3T3 cells transfected with a control plasmid or Bach1 expression plasmid. The anti-Bach1 antibodies identified an antigen in both lysates (Fig. 1*B*, lanes 1 and 2). As expected, the signal was more intense in the overexpressing cell extract (lane 2). When the antibodies were preabsorbed with recombinant GST-Bach1, the reactivity was lost (lanes 3 and 4). These results demonstrate the utility of these Bach1 antibodies for detecting endogenous Bach1.

Binding of Bach1 to *ho-1* Enhancers *in Vivo*. Expression of HO-1 was strongly induced in mouse NIH 3T3 cells by adding hemin [ferric protoporphyrin IX that is reduced to ferroprotoporphyrin IX (heme) within cells] to the culture medium. Induction of HO-1 mRNA was evident within 2 h after hemin treatment and reached a maximum level by 4 h (Fig. 1*C* and data not shown). We investigated binding of Bach1 to the enhancers of *ho-1* (see Fig. 3) before and after hemin treatment by using ChIP assays with the anti-Bach1 antibodies (Fig. 1*D*). Bach1 binding to the E1 and E2 enhancers of the *ho-1* gene was enriched in control NIH 3T3 cells cultured under normal conditions. In contrast, little Bach1 binding was detected when cells were treated with hemin for as much as 5 h (Fig. 1*D*) or as short as 1 h (Fig. 2*C*). As controls for the specificity of enrichment, we examined the promoter regions of *rag2* and *mcm5* (*mcm5* is located 9 kb downstream of *ho-1*). No enrichment of the *rag2* or *mcm5* promoter regions was observed in the control or hemin-treated cells (Fig. 1*D* and data not shown). These results indicate that Bach1 occupies the enhancers when *ho-1* is repressed, whereas it is displaced from the *ho-1* enhancers by increased intracellular levels of heme. We next examined binding of the small Maf proteins to the enhancers by using anti-MafK antibodies that also detect MafG and MafF. Binding of small Maf proteins is clearly enriched in the enhancer regions in both the control and hemin-treated cells (Fig. 1*E*). These results confirm that MafK or other small Maf proteins occupy the enhancers irrespective of the *ho-1* transcription state. Similar dynamics of factor binding

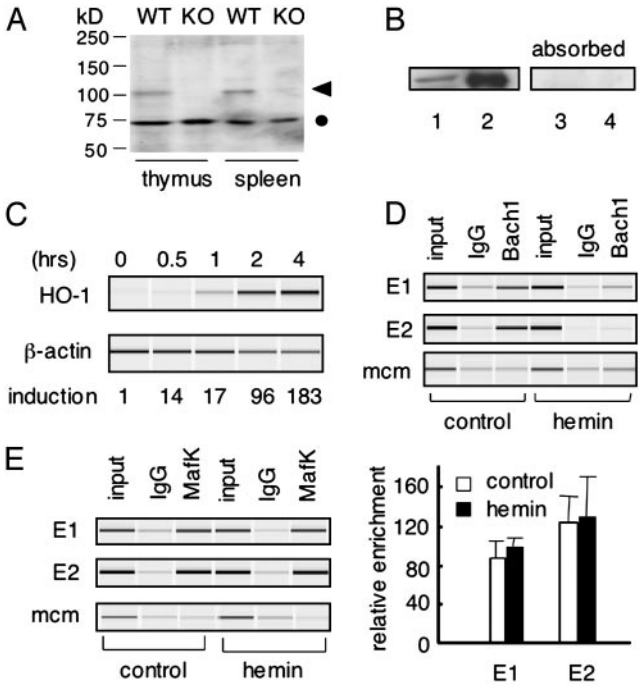

Fig. 1. Hemin induces the departure of Bach1 from *ho-1* enhancers. (*A*) Immunoblotting analysis of thymus and spleen extracts of wild-type or *bach1*-deficient mice was performed with anti-Bach1 antibodies. The arrowhead indicates a band with the expected mobility for Bach1. The filled circle indicates an antigen whose identity is unknown. (*B*) Immunoblotting analysis of whole-cell extracts of NIH 3T3 cells transfected with control plasmid (lanes 1 and 3) or Bach1 expression plasmid (lanes 2 and 4). Bach1 antibodies were preabsorbed with GST-Bach1 (lanes 3 and 4). (*C*) NIH 3T3 cells were treated with 10 μM hemin for the indicated periods. Expression of HO-1 mRNA was determined by RT-PCR. Relative levels of HO-1 mRNA corrected for β-actin mRNA levels are shown below the lanes. (*D* and *E*) ChIP assays were carried out with Bach1 (*D*) or MafK (*E*) antibodies or control rabbit IgG. NIH 3T3 cells were treated with or without hemin for 5 h. Gel images of PCR products of E1, E2, and *mcm5* promoter (mcm) using input and precipitated chromatin as template are shown. Relative levels of enrichment of each genomic DNA region are shown (*E Right*). These results represent three independent experiments.

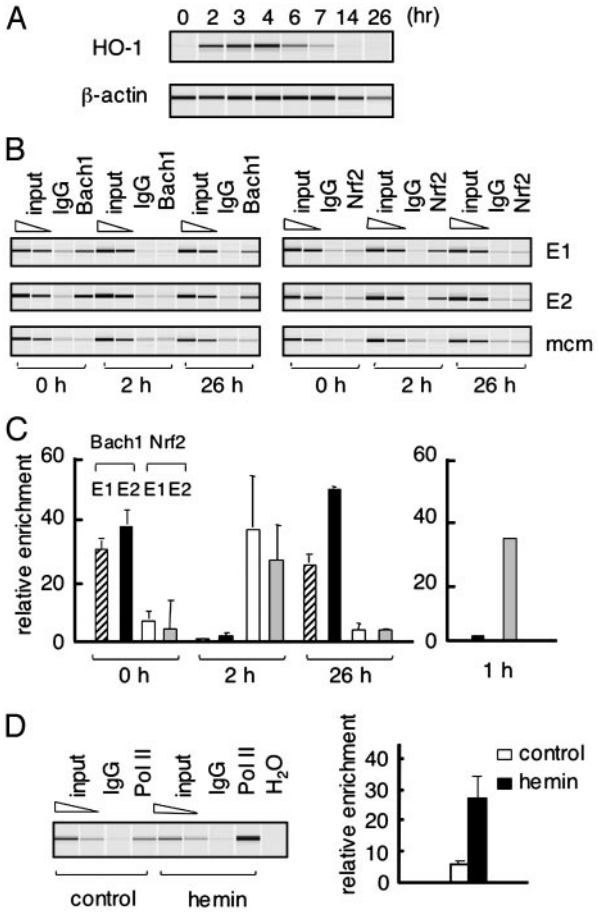

Fig. 2. Competitive binding of Bach1 and Nrf2 regulates RNA polymerase recruitment and promoter clearance. (*A*) NIH 3T3 cells were treated with 10 μM hemin for 2 h, washed, and cultured further in hemin-free medium. Expression of HO-1 mRNA was determined by RT-PCR. The number of hours after hemin treatment is indicated above the lane. (*B*) Results of ChIP assays using anti-Bach1 antibodies (*Left*) or anti-Nrf2 antibody (*Right*) at indicated time points. To ensure linearity of PCR reactions, 3-fold serial dilutions of input were used. (*C*) Levels of relative enrichment of E1 and E2 genomic DNA fragments by anti-Bach1 or anti-Nrf2 antibodies. These results represent at least three independent experiments (*Left*). Binding of Bach1 and Nrf2 to E2 after 1 h of hemin treatment is shown (*Right*). (*D*) ChIP assays using anti-pol II antibody. Gel image shows PCR products of *ho-1* promoter. Relative levels of enrichment obtained in three independent experiments are shown (*Right*).

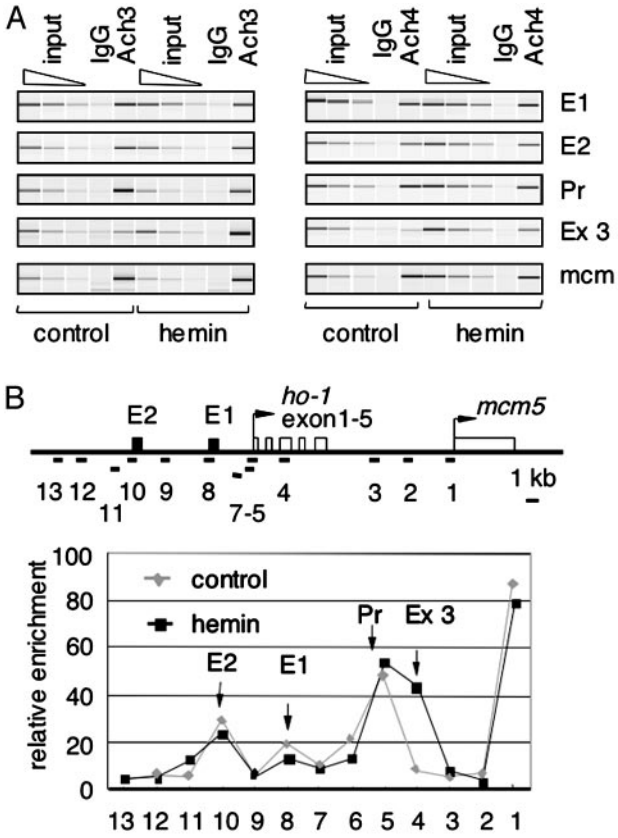

Fig. 3. Localized histone acetylation at the *ho-1* locus before and after induction. (*A*) ChIP assays using antidiacetylated histone H3 (K9 and K14) (*Left*) or antiacetylated histone H4 (K5, K8, K12, and K16) (*Right*) with or without hemin treatment. Gel images show PCR products of E1, E2, promoter (Pr), and exon 3 (Ex3) regions. The *mcm5* promoter was used as a control. (*B*) Schematic representation of mouse *ho-1* and *mcm5* locus (*Upper*). Numbers show locations of primers (p1–p13) used to amplify various genomic regions. ChIP assays were performed by using anti-diacetylated histone H3 (K9 and K14) antibodies. Means of relative enrichment from three experiments are shown below.

were observed by using anti-Bach1 and anti-MafK antibodies when cells were treated for 5 h with 10 μM cadmium, another strong inducer of *ho-1* (data not shown).

Competitive Binding of Bach1 and Nrf2 Regulates Polymerase Recruitment and Clearance. Having established that Bach1 binds the HO-1 enhancers when HO-1 is repressed, we next investigated two critical issues regarding *ho-1* regulation. First, because both Bach1 and Nrf2 compete for the small Maf proteins, they may also compete for enhancer occupancy. Second, because heme is degraded by HO-1, the inhibitory effect of heme on Bach1 may be transient, conferring a reversibility to this regulatory system. To test these hypotheses, we treated NIH 3T3 cells with hemin for 2 h, then washed hemin from the medium and cultured the cells in the absence of hemin. These procedures allow a transient increase in intracellular levels of heme followed by degradation of heme by HO-1. HO-1 mRNA reached a maximum level by 4 h and then declined to the basal level within 14 h (Fig. 2*A*). These data suggest that HO-1 was repressed again in response to degradation of heme. Using this same experimental protocol, we next examined the dynamics of binding of Bach1 and Nrf2 to the enhancers. Using anti-Bach1 antibodies in ChIP assays, we observed that binding of Bach1 disappeared within 2 h, whereas Bach1 was recruited again to the enhancers by 26 h (Fig. 2*B Left*). The pattern of binding of Nrf2 to the enhancers was opposite to that of Bach1 (Fig. 2*B Right*), demonstrating a clear, transient binding to the enhancers after hemin treatment. Quantification of these data clearly indicates reciprocal Bach1 and Nrf2 binding to the enhancers (Fig. 2*C*) taking place within 1 h (Fig. 2*C Right*). Neither Bach1 nor Nrf2 was enriched at the promoter regions of the *mcm5* or *rag2* genes (Fig. 2*B* and data not shown), confirming the specificity of these ChIP assays. We also detected no binding of Bach1, MafK, or Nrf2 to other regions on the *ho-1* locus detected by primer sets 3, 9, and 12 (Fig. 3*B*). These results suggest that heme regulates expression of *ho-1* through affecting a mutually exclusive binding of Bach1 and Nrf2 to the enhancers.

Gene expression can be regulated by recruitment of RNA polymerase II (27) and by events downstream of preinitiation complex formation, such as clearance of RNA polymerase from the promoter (28). To gain insight into the pathway of *ho-1* gene activation, we performed ChIP assays with an anti-pol II antibody. Whereas some recruitment of RNA polymerase II in the promoter of the *ho-1* gene was observed before hemin treatment,

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it was further stimulated by hemin treatment (Fig. 2*D*). Similar results were obtained when cells were treated with cadmium (data not shown). These results suggest that Bach1 represses the *ho-1* gene by reducing the efficiency of pol II recruitment. The finding of some pol II recruitment to the *ho-1* promoter before hemin/cadmium treatment is also consistent with at least some *ho-1* templates being in a poised state before induction.

Histone Acetylation at the *ho-1* Locus. The level of histone H3 and H4 acetylation has been shown to correlate directly with the transcription status of many genes; transcriptionally active regions are associated with hyperacetylated histones, whereas transcriptionally silent regions are associated with hypoacetylated histones (29). Thus, to better understand the role of Bach1 in the regulation of *ho-1*, we investigated histone modifications at the *ho-1* locus by using ChIP assays with anti-acetylated histone H3 (K9 and K14) and H4 (K5, K8, K12, and K16) antibodies. Surprisingly, we found that histones H3 and H4 at the *ho-1* enhancers and promoter were already hyperacetylated in NIH 3T3 cells under normal conditions, when the gene is repressed by Bach1 (Fig. 3*A*). The levels of acetylation did not change significantly after treatment with hemin (Fig. 3*A*) or cadmium (data not shown). To examine the distribution of histone acetylation at the locus, we used a battery of primer sets, covering 5.5 kb upstream of E2 to 4.7 kb downstream of the last *ho-1* exon (Fig. 3*B*). In regions other than the enhancers and promoter, such as the region between E1 and E2 and the region between E1 and the promoter, histone H3 was not hyperacetylated under normal culture conditions or after hemin treatment (Fig. 3*B Lower*). In contrast, histones in the exon 3 region became hyperacetylated on hemin treatment.

To examine acetylation at additional sites of histone H3, we performed ChIP assays with antibodies specific to single acetylated K18, K23, or K27 of histone H3. We observed high levels of acetylation at histone H3 K18 and K27 in E1, E2, and promoter regions of *ho-1* before and after hemin treatment (Fig. 4*A*). In contrast, only low levels of acetylation at K23 were detected (Fig. 4*A*). Thus, hyperacetylation at K9 and/or K14, and K18 and K27 of histone H3 marks *ho-1* regulatory domains before and after induction.

Histone Methylation at the *ho-1* Locus. Using anti-dimethyl and trimethyl-histone H3 K4 antibodies in ChIP assays, we found that histone H3 K4 at the enhancers and promoter of *ho-1* were hypermethylated under normal culture conditions (Fig. 4*B*). It has been suggested that methylation at K4 of histone H3 is related to gene activation, whereas methylation at H3 K9 is related to silencing of genes (30–33). We did not detect any enrichment of methylated H3 K9 at the enhancers or promoter (data not shown). These results suggest that the chromatin structure of the *ho-1* gene is already in an activated state when the gene is repressed.

Surprisingly, dimethylation and trimethylation of H3 K4 in the enhancer and promoter regions decreased ≈40% after hemin treatment. Concomitantly, the exon 3 region became hypermethylated at K4 of H3 (Fig. 4*B*). In contrast, no obvious difference in methylation at K4 of H3 was detected at the promoter region of *mcm5* before or after induction (Fig. 4*B*). Furthermore, on immunoblotting analysis with the same antibodies, the global levels of trimethylation at H3 K4 did not differ before and after hemin treatment (data not shown). Thus, it seems that histones on the *ho-1* regulatory domains undergo a specific reduction in the levels of H3 K4 methylation, although the mechanistic basis for this change is not clear. The induction of acetylation and methylation in the transcribed region may reflect chromatin remodeling by pol II-associated modifying enzymes (34).

Taken together, these results indicate that, under normal culture

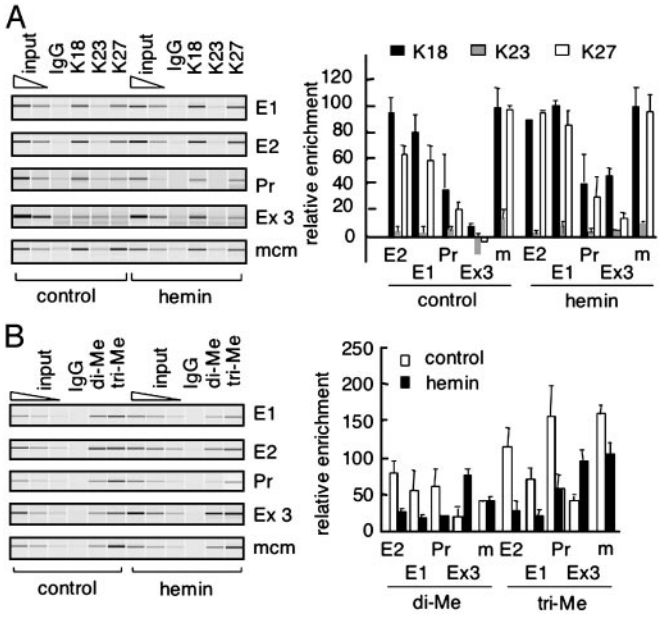

Fig. 4. Site-specific modifications of histone H3 lysines at the *ho-1* locus. (*A*) Anti-acetylated histone H3 K18, K23, or K27 antibody was used in ChIP analyses. Relative levels of enrichment are shown (*Right*). (*B*) Methylation of histone H3 K4 in *ho-1* locus. Anti-dimethylated or trimethylated histone H3 K4 antibody was used for ChIP experiments. Results of two experiments are shown (*Right*).

conditions, the *ho-1* chromatin domain is characterized by histone modifications that are compatible with gene expression rather than silencing. Thus, the inactive *ho-1* chromatin domain repressed by Bach1 may be in an open or preactivated state rather than in a condensed, heterochromatin-like structure. Consistent with this finding, we observed similar levels of DNase I hypersensitivity in the promoter region in thymocytes before and after *ho-1* expression (J.S. and K.I., unpublished observation).

Heme Induces the Departure of Bach1 from the β-Globin LCR. MafK/p18 NF-E2 serves as a dual-function molecule on the β-globin LCR. MafK shifts from a repressive to an activating mode during erythroid differentiation by exchanging its partner molecule from Bach1 to p45 NF-E2 (14, 18). Because heme is synthesized at high levels during erythroid differentiation, we investigated whether heme induces displacement of Bach1 from the β-globin LCR. We treated MEL cells with hemin and examined binding of Bach1 to the MAREs within the hypersensitive site 2 (HS2) enhancer of the β-globin LCR. As shown in Fig. 5*A*, Bach1 is bound to HS2 in uninduced MEL cells. Within 2 h after hemin treatment, Bach1 binding to HS2 is no longer detected. We observed a weak reassociation after 4 h of hemin treatment (Fig. 5*A*), but the significance of this observation is not clear. We observed similar but less dramatic binding dynamics of Bach1 to the β-globin promoter (Fig. 5*A*). In contrast to Bach1, MafK remained bound to HS2 irrespective of hemin treatment (Fig. 5*B*). Thus, heme induced the selective departure of Bach1 from the β-globin MAREs without affecting the binding of MafK. Surprisingly, however, p45 was not significantly recruited to MAREs after hemin treatment (data not shown). Consistent with this observation, β-globin mRNA levels did not change much after the hemin-induced departure of Bach1 (Fig. 5*C*). On treating cells with 2% DMSO, which induces erythroid differentiation of MEL cells, the levels of β-globin mRNA remained low for the first 24 h and gradually increased up to 100-fold by 4 days (Fig. 5*C* and data not shown). In addition, as

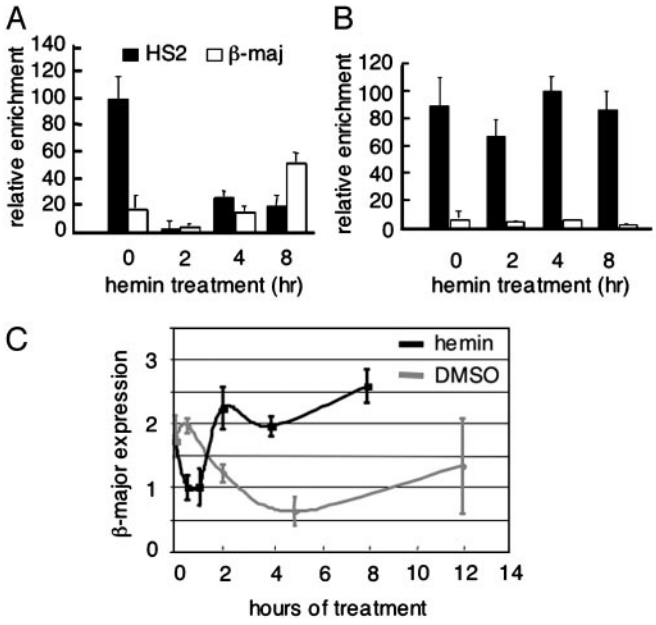

Fig. 5. Hemin induces departure of Bach1 from the β-globin LCR. (*A* and *B*) ChIP experiments were performed with anti-Bach1 antibodies (*A*) or anti-p18 (*B*) after treating MEL cells with hemin for the indicated periods. The anti-p18 antibodies recognize MafK and do not crossreact with MafG (18). Enrichment at HS2 (filled bars) and β-major globin gene promoter (open bars) regions was examined. (*C*) Levels of β-globin mRNA were determined by real-time PCR after 10 μM hemin or 2% DMSO treatment.

shown (18, 22, 28), p45 recruitment to the LCR and β-globin promoter is not detectable in uninduced cells and peaks 4–5 days after DMSO treatment. These results indicate that the heme-mediated departure of Bach1 from the LCR is not sufficient for the activation of β-globin expression or the induction of MEL cell differentiation.

Discussion

The maintenance of genes in a stable off state by DNA sequence-specific repressor proteins is often associated with the generation of repressive epigenetic chromatin structures through histone modifications, such as methylation at K9 of H3 (35). In addition, some genes that are silent, but have the potential to be active in a given cell type, are associated with hyperacetylated histones before their activation (36). However, little is known regarding the chromatin state associated with genes that are repressed conditionally, without losing the potential for expression. We show here that localized histone acetylation and methylation of the enhancers and promoter of *ho-1* marked both the activated and the repressed *ho-1* states. Inducers such as heme triggered subunit exchange of the enhancer-binding transcription factors, RNA polymerase recruitment and clearance, and acetylation and methylation of histone tails within the transcribed domain. The patterns of the transcription factor dynamics and the histone modifications observed in this study suggest that Bach1 actively maintains a quiescent expression state of the *ho-1* gene. Thus, the repression of *ho-1* under normal conditions is not a default pathway mediated by a repressive chromatin structure or by a pancellular lack of activator function (e.g., Nrf2).

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 may be able to establish and/or maintain a "poised" state that allows a gene to undergo subsequent rapid induction (37). Our results indicate that the small Maf proteins function as both stand-in factors, a function originally suggested by Enver and Greaves (38), and activators by exchanging their heterodimerization partners. The localized histone H3 acetylation at K9 and K14 and methylation at K4 in the enhancer and promoter regions before *ho-1* gene activation suggest that the Bach1/small Maf complex allows chromatin activation but simultaneously inhibits transcription, a function that is predicted for a stand-in factor. Such stand-in factors may provide a mechanism that permits poising of a locus for expression before the environmental or cellular cues required for the final steps in transcriptional activation. Because repressors are significant components of several mammalian heterodimeric transcription factor networks, including Myc/Max and E2F (39–41), they, like the Bach1/small Maf complex, may also play a role as stand-in, preactivation factors.

Our results suggest that intracellular levels of heme regulate the exchange of small Maf protein partners in not only the stress-responsive control of HO-1 but also the differentiation-associated control of β-globin expression. Based on the analyses of p18 (MafK) protein complexes before and after MEL cell differentiation, we proposed a model that may explain how the enhancer function of the β-globin LCR is regulated (18). Before differentiation, MafK/Bach1 recruits corepressor complexes, including NuRD, which is associated with repression of β-globin transcription. Upon erythroid differentiation induced by DMSO, an exchange of MafK-binding partners from Bach1 to p45 NF-E2 is associated with the formation of the NF-E2 activator complex (MafK/p45 heterodimer) on the LCR and promoter, resulting in β-globin gene expression. Because we showed in this study that an exogenous hemin treatment of MEL cells displaced Bach1 from the LCR, the replacement of Bach1 by the activator p45 may be initiated by heme that is synthesized at high levels during erythroid differentiation. However, the regulation of *ho-1* and β-globin gene transcription differ in that NF-E2 was not recruited to the LCR, and β-globin mRNA remained low shortly after hemin treatment. Therefore, in contrast to the *ho-1* gene, other signals are clearly required to induce activator recruitment and β-globin gene transcription after the departure of Bach1. Such signals may trigger alterations in the subnuclear localization of the β-globin locus and/or subunits of NF-E2 (42). Clearly, the identification of such signals may provide insights into the regulation of β-globin gene during erythroid differentiation. Because heme is involved in various metabolic processes, the Bach1/small Maf dimers may regulate other genes as well to transduce metabolic activity into changes in gene expression.

We thank Dr. Tomoyuki Sawado for advice on ChIP assays and Drs. Robert Eisenman and Gerd Blobel for critical and helpful comments on the manuscript. J.S. is supported by a fellowship from the Japanese Society for the Promotion of Science. M.B. is supported by a long-term fellowship from the Human Frontier Science Program Organization. This work was supported by grants-in-aid from the Ministry of Education, Science, Sport, and Culture of Japan (to K.I.) and by National Institutes of Health Grants DK44746 and HL57620 (to M.G.).

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