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The Promoter of Mouse Transcription Repressor *bach1* Is Regulated by Sp1 and *Trans*-Activated by Bach1[1]

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The Maf recognition element (MARE) is regulated by both activators and repressors. Bach1 and Bach2 repress MARE-dependent transcription by forming heterodimers with Maf-related oncoproteins. In order to gain an understanding of the regulation of *bach1* gene expression, we analyzed the structure of the mouse *bach1* gene. Comparison of the exon-intron structure of the *bach1* gene with those of other NF-E2–related genes indicated that *bach1* and *bach2* constitute an evolutionarily distinct subfamily among bZip factors. The *bach1* promoter region contains two GC boxes that are important for its basal activity and are bound by Sp1 in K562 cell extracts. In addition, we found an evolutionarily conserved MARE-like element located downstream of the transcription initiation site. Deletion of this element resulted in a higher promoter activity in K562 cells. Bach1 *trans*-activated its own promoter depending on the presence of the MARE-like element in co-transfection assays. However, Bach1 did not bind to the MARE-like element in electrophoretic mobility shift assays (EMSA). These results suggest that Bach1 activates its own promoter indirectly by inhibiting the putative repressor. Such a positive feedback regulation by the repressor Bach1 may play an important role in maintaining the expression of Bach1 while consolidating repression of other genes with MARE.

Key words: Maf, oncogene, promoter, transcription factor, transcription repression.

In higher eukaryotes, it is not unusual that a single *cis*-DNA element is targeted by both activators and repressors. The presence of repressors may be important for setting thresholds and/or maximal levels of gene expression, or for terminating gene expression after induction. As such, it is critical to understand how the levels and functions of repressors are regulated. The Maf recognition element (MARE) is found in regulatory regions of genes involved in hematopoiesis and oxidative stress response (*1–5*). MARE is bound by various sets of heterodimers of transcription factors generated between Maf family proteins and NF-E2–related factors (*6*). Among NF-E2–related factors, Bach1 and Bach2 function as transcription repressors (*3*, *7–9*), whereas others, namely NF-E2 p45, Nrf1, Nrf2, and Nrf3, function as activators (*10–13*). Such a functional distinction among Maf partner molecules appears to reflect their structural features. Bach1 and Bach2 possess the BTB/POZ domain in their N-terminal regions (*7*). The BTB/POZ domain is found in various nuclear and cytoplasmic proteins and is known to mediate protein-protein interaction and transcriptional repression (*8*, *14–18*). In contrast, other NF-E2–related factors are conventional transcription activators possessing activation domains at their N-termini (*13*, *19*).

Maf proteins play important roles in hematopoietic and neural cell differentiation (*6*, *20*). For example, MafG and MafK regulate platelet formation (*21–23*), whereas MafB regulates monocytic differentiation (*24*). c-Maf induces monocytic differentiation and apoptosis in bipotent myeloid progenitors (*25*). Among the partner molecules for the Maf family, Nrf2 is known to regulate oxidative stress–inducible genes (*4*). Furthermore, de-regulation of the Maf/NF-E2 system appears to lead to tumorigenesis (*26*). To understand how the activities of these factors are regulated in a diverse set of systems and how their de-regulation leads to cell transformation, it may be helpful to focus on the repressor class of molecules. Along this line, we showed previously that the DNA binding activity of the Bach1/small Maf oligomer is negatively regulated by heme through its direct binding to Bach1 (*27*). Thus, Bach1 may constitute a novel regulatory pathway that couples metabolism and gene expression. In an attempt to gain further understanding its regulation of expression, we have now analyzed the structure of the mouse *bach1* gene and identified its promoter

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Abbreviations: MARE, Maf-recognition element; EMSA, electrophoretic mobility shif assay; NF-E2, nuclear factor–erythroid 2; BAC, bacterial artificial chromosome.

region. A comparison of the *bach1* and *bach2* genes indicate that they constitute an evolutionarily distinct subfamily of NF-E2–related factors. The regulation of the *bach1* gene involves a unique positive-feedback loop. Bach1 transactivates its own promoter depending on the presence of an evolutionarily conserved *cis*-element. However, Bach1 fails to bind to this element *in vitro*. The results suggest that Bach1 may function as an indirect activator of its own promoter as well as a direct repressor of genes with MARE.

MATERIALS AND METHODS

Phage and Bacterial Artificial Chromosome (BAC) Clones—Genomic lambda phage clones were isolated from a 129sv genomic library (Stratagene) using mouse *bach1* or *bach2* cDNA fragments as probes. Using a *bach1* probe, two partially overlapping clones were isolated and their restriction enzyme sites were determined. Using a *bach2* probe, more than six phage clones were isolated. Exons were identified by hybridization experiments and their sequences were determined using an ABI 377 sequencer. To isolate the entire mouse *bach1* gene, a genomic BAC library (Genomesystems) was screened by PCR. Primers were 5′-CGGATAATTTCGCTCTCACG-3′ and 5′-TTCCGACTCATGGTTCTGGA-3′, designed to amplify the basic region of *bach1*.

5′ Rapid Amplification of cDNA End (RACE)—mRNA was isolated from 10 day mouse whole embryos by the acid guanidinium thiocyanate-phenol-chloroform extraction method (*28*) and using oligo(dT)-latex beads (Takara). Nested RACE reactions to amplify the 5′ ends of the bach1 cDNA were carried out using a RACE cDNA amplification kit (Clontech). Primers for the first and second PCR were 5′-CCTTCTTCCGCTGGTCATTG-3′ and 5′-ATACCGCACTCTCACTCACAGACATCCT-3′, respectively. Both primers are located within the second exon of *bach1*. Amplified cDNAs were cloned into pGEM-T vector (Promega) after gel purification of the bands and their sequences were determined.

Construction of Reporter Plasmids—bach1 promoter-luciferase plasmids were constructed using the pGL3 basic vector (Promega). The longest promoter fragment was isolated as a 3.2 kb *Eco*RI–*Pst*I restriction fragment. Nested deletions from the 5′ end were constructed using the exonuclease III and mung bean nuclease system (Stratagene). Deletion junctions were determined by sequencing each clone.

Transfection Assay—Human erythroid K562 cells were grown in RPMI1640 medium (Nissui Japan) containing 10% fetal calf serum. Transfection was carried out using FuGENE6 reagent (Boehringer Mannheim) and 1 μg of reporter plasmids and 0.2 μg of control plasmid (sea pansy luciferase gene driven by elongation factor gene promoter, Ref. *3*). At 24 h post-transfection, cell lysates were prepared using the Luciferase Assay System (Promega) following the supplier's protocol. Luciferase activities were measured with a Biolumat Luminometer (Berthold). Fire-fly luciferase activity was normalized for transfection efficiency as determined by the control sea pansy luciferase activity.

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts of K562 cells were prepared from 10^7 cells as described previously (*8*). A probe DNA containing the GC box of the *bach1* promoter was prepared by annealing oligonucleotides (5′-AGCTACGGGGCGGGGCCCGGGG-3′ and 5′-CCCCGGGCCCCGCCCCGTAGCT-3′). A probe DNA containing the MARE-like sequence of *bach1* exon 1 was prepared from oligonucleotides (5′-AGAGGGAGTGAGTCACCTGACC-3′ and 5′-GGTCAGGTGACTCACTCCCTCT-3′). The double-stranded oligonucleotide was labeled with [γ-^{32}P]ATP and T4 Polynucleotide Kinase (TOYOBO). Incubation of K562 cell extracts with the DNA probes was carried out at room temperature for 20 min under the conditions described previously (*8*). Recombinant proteins of Bach1 and MafK were described previously (*8*). Where indicated, antibodies were added to the binding reaction at 1/10 or 1/5 dilutions, with incubation for 30 min before the addition of DNA. The reaction products were electrophoresed on 4% polyacrylamide gels. The anti-Sp1 antibody (SC-59) was obtained from Santa Cruz.

RESULTS

Genome Structure of bach1 and bach2—The murine *bach1* locus was cloned from a λ phage library of 129/SvJ mouse genomic DNA using bach1 cDNA as a probe. Two recombinant phage clones were identified and found to contain some of coding exons for Bach1. To obtain clones covering entire exons, a bacterial artificial chromosome (BAC) library of 129/SvJ genomic DNA was screened by PCR, resulting in two recombinants. One of the BAC clones was analyzed further by restriction enzyme site mapping. The positions of the exons were determined by subcloning of restriction enzyme DNA fragments and sequence analyses. This BAC clone (184K21) was found to contain all of the Bach1 cDNA sequence. As shown in Fig. 1, the mouse *bach1* gene comprises five exons. The junction sequences of the exon-intron boundaries conform to the GT/AG rule (Table I). Bach1 contains at least two functional domains (Fig. 1). At the N-terminal region, it carries a BTB/POZ domain that mediates protein interactions. Toward the C-terminus, it carries a basic region-leucine zipper (bZip) domain that mediates dimer formation with Maf-related proteins and DNA binding. Exon 2 of *bach1* gene is the first

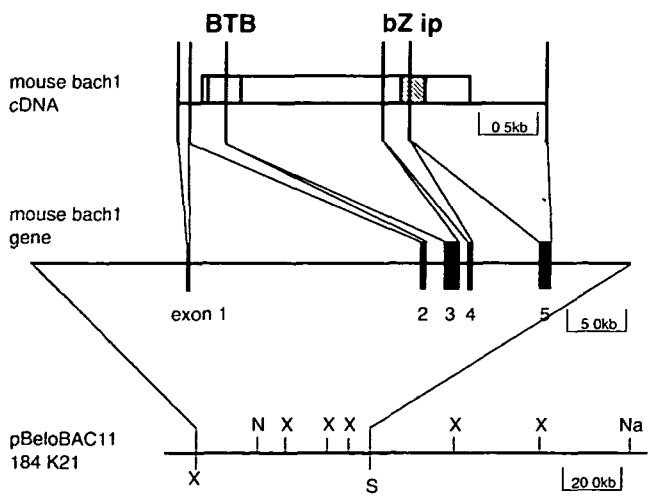

Fig. 1. **Structure of the mouse *bach1* gene.** The cDNA and genome structures are depicted on the first and third lines, respectively. Exon-intron structures are depicted on the second line. X, *Xho*I; N, *Not*I; S, *Sal*I; Na, *Nar*I.

coding exon, and encodes most of the BTB/POZ domain. Exon 3 encodes a portion of the BTB domain and the intervening region between the BTB/POZ and bZip domains. An interesting feature of the *bach1* gene is that the bZip domain is encoded by two separate exons (exons 4 and 5). The entire sequence of human chromosome 21 was reported recently, and revealed the presence of human BACH1 on this chromosome (*29*). Exon-intron structures are well conserved between human and mouse (data not shown).

Since there have been no reports describing the structure of the *bach2* gene, we examined the structure of the mouse *bach2* gene to elucidate further the evolutional relationships. By analyzing phage and BAC clones, the *bach2* gene was found to be very large. The BAC clones 62G11 and 71C7, each covering overlapping 120 kb stretches of genomic DNA, were found to contain both exon 1 and exon 2 but not other exons. We could not cover the entire region as a contig. However, analysis of the individual exons on the BAC clones, as well as λ phage clones, revealed that the *bach2* gene possesses exon structures very similar to those of *bach1*. It is composed of at least 7 exons (Fig. 2), at least three exons encoding the 5′ UTR. Most of its BTB domain is encoded by a single exon (tentatively assigned as exon 4), the basic-leucine zipper by two separate exons (exons 6 and 7), and the intervening region by a single exon (exon 5).

In clear contrast to *bach1* and *bach2*, the bZip domains of other NF-E2–related factors are encoded by single exons (*12*, *30–32*). Together with the results of phylogenetic tree analysis of the sequences of the basic region (*13*), and the fact that only Bach1 and Bach2 possess the BTB/POZ domain, the results described here establish that Bach1 and Bach2 constitute a separate group among NF-E2–related transcription factors in terms of evolution.

Identification of the Promoter Region of bach1—To determine the transcriptional initiation site of the Bach1 mRNA, 5′-RACE analysis was performed using mRNA isolated from 10 day mouse whole embryos. After cloning amplified DNA fragments into a plasmid, we determined the sequences of 14 randomly picked clones. As shown in Fig. 3, the results indicated the presence of two major 5′ ends juxtaposing the end of the cDNA sequence. We therefore assigned these two positions as the major transcription start sites for the mouse *bach1* gene.

To examine the function of the putative promoter region, a set of reporter gene plasmids carrying varying lengths of the upstream region were constructed (Fig. 4). These reporter plasmids were introduced into K562 cells, which express high levels of bach1 (E. Ito, unpublished observation), and the reporter gene activities were compared. The results can be summarized as follows. First, because the longest construct (pBPPst) directed high levels of expression, the region preceding the transcription initiation sites indeed contains a functional promoter. Second, there is a negative regulatory element(s) between the −3.1 and −1.6 kb region and a positive regulatory element(s) between −1.6 and −0.7 kbp. Third, because further deletions toward −88 bp from the transcription start site had no significant effects, a minimal promoter region should be confined within the −88 bp region. The presence of negative regulatory element(s) in the upstream region was also supported by com-

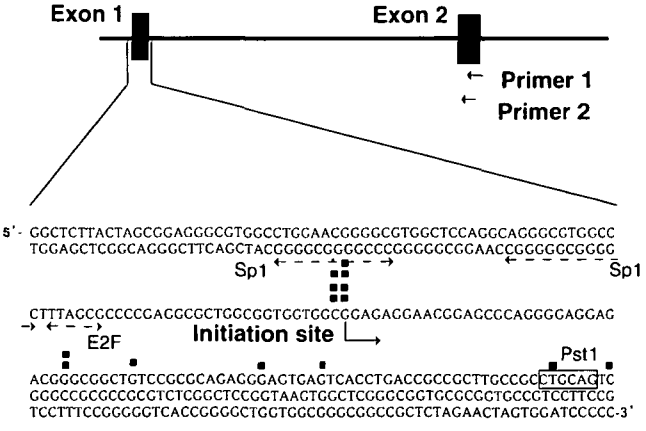

Fig. 3. **Determination of transcription start sites.** The 5′ ends of the Bach1 mRNA were determined by RACE. Positions and numbers of the ends of the cDNA clones are indicated by squares above the genome sequence. Potential transcription factor binding sites are indicated below the DNA sequence.

TABLE I. **Exon/intron boundary sequences of the mouse *bach1* gene.**

Exon	Size (bp)	5′ splice donor	3′ splice acceptor	Size (bp)	Intron
1	113	CTCGGCTCCG ***gt***aagtggct	gtgtttgc***ag*** GTCGATGACA	~20,000	1
2	295	GCCTGAAGAG ***gt***gaggggcc	gtgtttgc***ag*** GTAACGGTTA	~3,750	2
3	1,345	GGACTGTGAG ***gt***aagtggac	gtgtttgc***ag*** GTGAAGCTGC	~1,500	3
4	203	CGAGAAGCTG ***gt***aagtgtgt	ctctgcac***ag*** CAAAGTGAAA	~6,500	4
5	1,267	GTAATCTGTT			

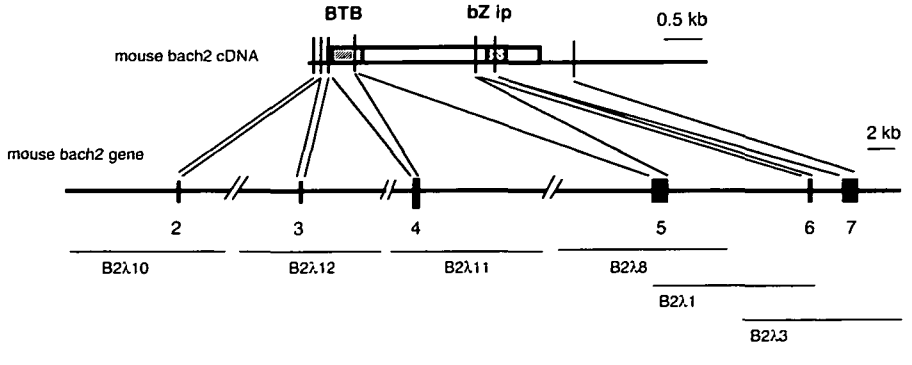

Fig. 2. **Structure of the mouse *bach2* gene.** The cDNA and genome structures are depicted on the first and second lines, respectively. The corresponding λ phage clones are indicated below the second line.

paring the activities of pBPSac and pBPSacdel reporter plasmids. Even though the −88 bp region contains a minimal promoter region, this activity was efficiently repressed by fusing the −3.1 to −2.1 kb region. These results indicate that, as defined in transient transfection assays, the bach1 promoter is composed of at least three regions: an upstream negative regulatory region, a minimal core promoter region, and a stimulatory region that resides between the first two regions. In addition, since pBPP1, which contains as few as 13 bp upstream of the transcription start sites (see Fig. 5), showed considerable expression as compared with the empty vector (pGL3basic), the region around the transcription initiation site appears to possess an initiator activity.

Binding of Sp1 to the bach1 Promoter—A homology search using the mouse *bach1* promoter region indicated a significant similarity to a region preceding the putative first exon of human *bach1* (Fig. 5), corroborating that this region is involved in gene regulation. Furthermore, a comparison of the mouse and human sequences revealed several features of the mouse bach1 promoter. There are two well-conserved, potential Sp1 binding sites within the mini-

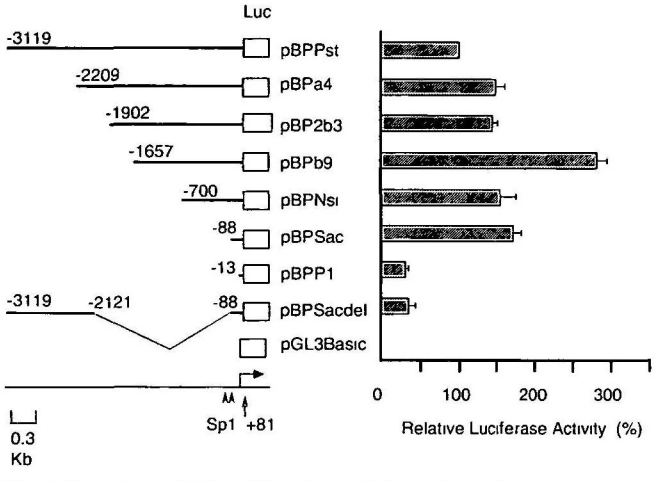

Fig. 4. **Functional identification of the minimal promoter region of *bach1*.** Luciferase reporter plasmids carrying various regions upstream of the transcription start sites were transfected into K562 cells and luciferase activities were determined. Results of at least three independent experiments each carried out in duplicate are shown.

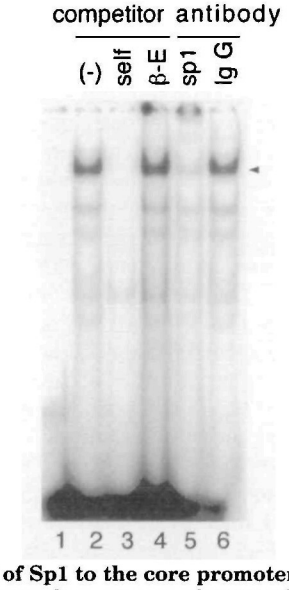

Fig. 6. **Binding of Sp1 to the core promoter region.** EMSA was carried out using nuclear extracts of K562 cells and a probe DNA from the core promoter region of *bach1*. Competition for binding was done with excess unlabelled probe DNA (lane 3) or MARE oligonucleotide from the chicken β-globin enhancer (lane 4). Effects of anti-Sp1 (lane 5) or anti-E2A (lane 6) antibodies were compared. The arrowhead indicates the specific binding complex.

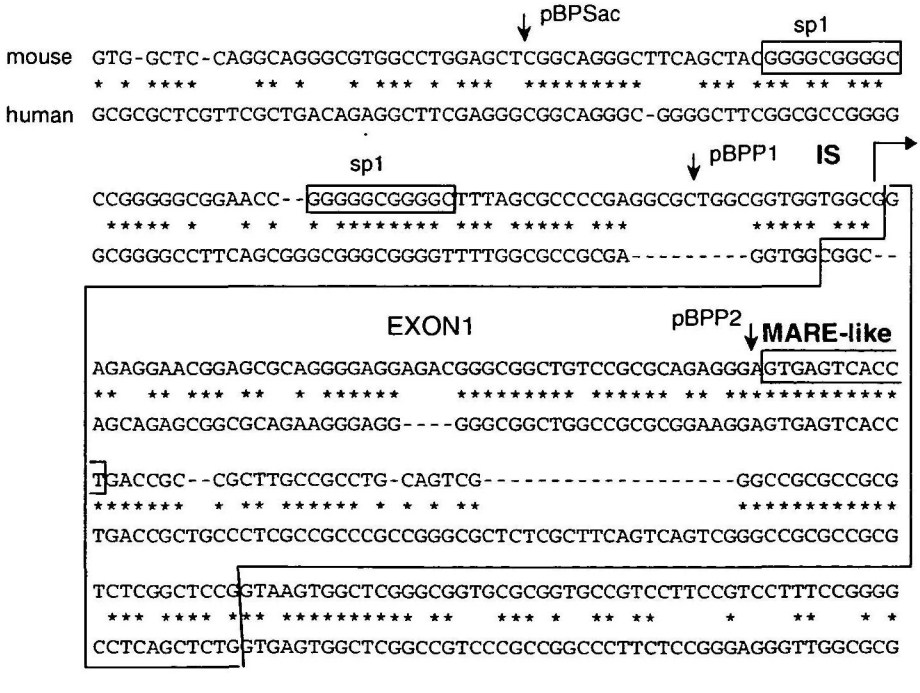

Fig. 5. **Comparison of the mouse *bach1* core promoter and exon 1 sequence with the corresponding region of human *BACH1*.** The main transcription initiation site of mouse *bach1* is indicated by a horizontal arrow. The 1st exon is boxed. The junctions of three reporter gene constructs are indicated by vertical arrows. Two potential Sp1 sites and the MARE-like sequence are boxed.

mal promoter region. Since deletion of this region (pBPP1) substantially decreased promoter activity as shown above, these two potential Sp1 sites may be important for the basal activity of the promoter. To determine the nature of binding factors, EMSA analysis was carried out using one of the potential Sp1 binding sites as a probe and K562 nuclear extracts (Fig. 6). The probe generated a single prominent complex. Formation of the complex was inhibited by excess unlabeled probe DNA but not by an oligonucleotide containing the MARE of the β-globin enhancer (lanes 3 and 4), indicating that the inhibition was due to a sequence-specific binding activity. An antibody against Sp1 efficiently inhibited the binding activity while a control IgG showed no effect (lanes 5 and 6). These results strongly suggest that the basal activity of the *bach1* promoter is regulated mainly by Sp1.

Transactivation of the bach1 Promoter by Bach1—Comparison of the mouse and human sequences also revealed that the 1st exons, which do not possess any coding sequence, are well conserved. Interestingly there is a conserved MARE-like sequence within the 1st exon (Figs. 5 and 7A). The presence of a MARE-like sequence raises the possibility that *bach1* promoter activity is regulated by itself or by other MARE-binding factors. Since self- or cross-regulation may be important for regulation of a repressor gene, we investigated whether the MARE-like sequence plays any functional role. To examine whether this MARE-like sequence is important for the promoter activity, it was deleted from the promoter reporter plasmid pBPSac (Fig. 7B), and this deletion plasmid was compared with its parent plasmid by transfecting them into K562 cells. As shown in Fig. 7C, the deletion of the MARE-like sequence resulted in a roughly twofold higher promoter activity (compare columns 1 and 3). Thus, the MARE-like sequence is involved in the negative regulation of the *bach1* promoter.

The possibility that Bach1 represses its own promoter activity was tested by co-transfection assay. For this purpose, we utilized the Bach1-MafK tethered molecule described previously (*33*). We used the fusion molecule of Bach1 and MafK first because Bach1 does not bind to DNA in the absence of a small Maf protein (*7*), and second, because if we were to transfect individual expression plasmids, it would be difficult to understand the molecular mechanisms of their effects since Bach1 and MafK may interact individually with some endogenous bZip proteins. Tethering Bach1 and MafK provides control over the specificity of multiple bZip partner interactions (*33*). As shown in Fig. 7C, co-expression of the Bach1-MafK fused molecule stimulated the promoter activity twofold (compare columns

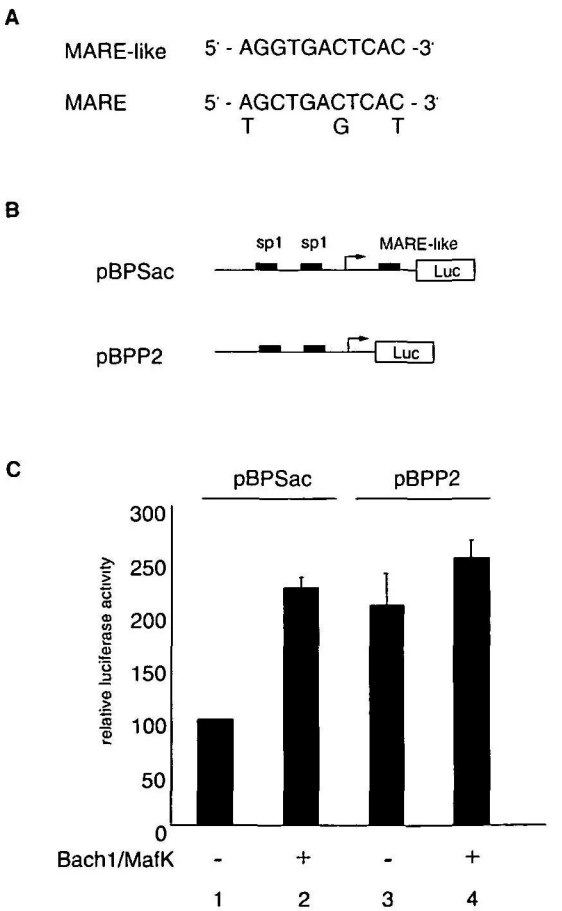

Fig. 7. **Activation of the *bach1* core promoter by Bach1.** A: The MARE-like sequence located downstream of the transcription initiation site is compared with the NF-E2-type MARE. B: Schematic representation of two reporter plasmids with and without the MARE-like sequence. C: Indicated reporter plasmids were introduced into K562 cells with or without the expression vector for the Bach1-MafK tethered molecule, and relative luciferase activities were determined. The results are the means of three independent experiments each carried out in duplicate.

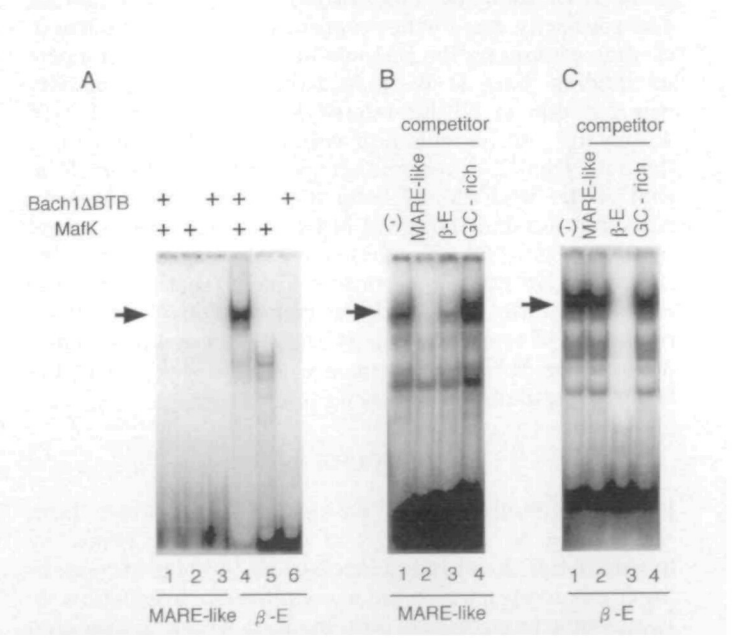

Fig. 8. **Specific binding activity to the MARE-like sequence in K562 cells.** A: Binding of recombinant Bach1ΔBTB and MafK to oligonucleotide probes containing the MARE-like sequence (lanes 1–3) or the MARE from the β-globin enhancer (lanes 4–6) was analyzed in EMSA. The arrow indicates the Bach1ΔBTB/MafK heterodimer binding complex. B: Nuclear extracts of K562 cells were incubated with probe DNA containing the MARE-like sequence to examine binding activities in EMSA. Competitors were the MARE-like (lane 2), the chicken β-globin MARE (lane 3), or the bach1 SP1 site (lane 4) oligonucleotides. The arrow indicates the specific binding complex. C: Recombinant Bach1ΔBTB and MafK were incubated with the probe DNA containing the chicken β-globin MARE. Binding of the heterodimer (indicated by the arrow) to various sequences was examined by competition assay as in panel B.

1 and 2). This mild effect was judged to be significant since the stimulatory effect was dependent on the presence of the MARE-like sequence: pBPP2 showed no significant response to Bach1-MafK (columns 3 and 4). Thus, even though Bach1 is known to function as a repressor through MARE, it activates its own promoter activity depending on the presence of the MARE-like sequence.

To examine whether Bach1 binds to the MARE-like sequence, we carried out EMSA analysis using the site as a probe. Oligonucleotide probes containing either the *bach1* MARE-like sequence or the MARE from the chicken β-globin enhancer were incubated with Bach1 and/or MafK expressed in *Escherichia coli*. We used recombinant a Bach1 protein lacking the BTB domain (Bach1ΔBTB) to avoid a complex shift pattern due to protein–protein interactions through the BTB domain (*8*). Very surprisingly, Bach1 failed to bind to the *bach1* MARE-like sequence while it bound to the MARE within the β-globin enhancer as a heterodimer with MafK (Fig. 8A). These results indicate that the effect of Bach1 through the MARE-like sequence in transfection assays can not be explained by direct Bach1/small Maf binding. If Bach1 regulates its promoter indirectly, then there may be a factor that binds to the MARE-like sequence of the *bach1* promoter. To explore this possilibity, we carried out EMSA analysis of K562 cell extracts using the MARE-like sequence of the *bach1* promoter as a probe. The results indeed showed the presence of a specific binding activity with which excess unlabelled probe DNA competed efficiently (Fig. 8B, lanes 1 and 2). The specificity was further confirmed since excess oligonucleotide containing the Sp1 site of *bach1* failed to compete for binding (lane 4). We judged that the binding activity was not due to NF-E2–related factors since the MARE within the β-globin enhancer was only weakly competitive (lane 3). This DNA recognition specificity is different from that of the Bach1/MafK heterodimer (Fig. 8C). These results indicate that the *bach1* MARE-like sequence is bound by a factor in K562 cells whose recognition specificity is distinct from NF-E2–related factors. Taken together with the results that the MARE-like sequence is involved in the repression of promoter activity, the specific complex generated on the MARE-like sequence may be involved in the negative regulation of the *bach1* promoter.

DISCUSSION

Functional studies of NF-E2–related factors have been complicated by the scarcity of defined target genes, by apparent functional redundancies, and by their absence in lower eukaryotes, which are more amenable to genetic analyses. Some progress has been made, however, to implicate p45, Nrf1, Nrf2, and Nrf3 in gene activation and Bach1 and Bach2 in gene repression. In this work, we corroborated their functional and evolutional relationships by analyzing the *bach1* and *bach2* gene structures. An ancestor gene for NF-E2–related factors most likely underwent gene duplication, generating two gene classes, one of which led to four activator genes (p45, nrf1, nrf2, and nrf3), and the other to two repressor genes (*bach1* and *bach2*) after subsequent gene duplication. Interestingly, each of the activator class genes is closely associated with each of the four Hox gene clusters HoxA, -B, -C, and -D (*13*, *34*). In contrast, *bach1* and *bach2* are not associated with any HOX cluster: human *bach1* and *bach2* are located on chromosomes 21 and 6, respectively, which do not carry HOX clusters. Thus, the duplication that generated the two classes appears to have occurred before the duplication of the Hox gene clusters (*i.e.*, before the origin of vertebrates, predating the divergence of amphioxus and vertebrates). After gene duplication, one of the duplicated genes gave rise to p45, Nrf1, Nrf2, and Nrf3 during genome expansion accompanying vertebrate radiation. The other duplicated gene was retained and diverged to a new specificity with repressor function, resulting in Bach1 and Bach2. This conjecture is supported by the fact that, while *Drosophila* transcription factor Cap'n'color (CNC) is related to the NF-E2 family, it is more closely related to the activator class and distinct from Bach1 and Bach2. Furthermore, we could not find any counterpart for *bach1* and *bach2* in the *Drosophila* genome sequence database. Thus, the presumptive ancestor gene for *bach1* and *bach2* may have been recruited into the system after the evolution of insects. It should be noted that the BTB domain is one of the most prevalent expansions in human protein domains, being found in more than 100 proteins in the human proteome (*35*). Among BTB domain proteins, Bach1 and Bach2 are unique in that they possess the bZip structure as a DNA binding domain (*35*). Other BTB domain proteins possess Zn finger DNA binding domains or Kelch-like actin-binding domains. These observations, together with their exon-intron structures, suggest that Bach1 and Bach2 were generated by the shuffling of protein domains into a new combination, and that they represent a relatively new protein subfamily among higher eukaryotes.

Transcription regulation through MARE in higher eukaryotes involves activation and repression by its binding factors (*2*, *19*, *23*, *36*, *37*). Thus, the regulation of the intracellular levels of these factors must be critical for proper gene expression. Our results indicate that the basal level of *bach1* gene expression is regulated by Sp1, which binds to the promoter region. The presence of a MARE-like sequence within the promoter region of the mouse and human *bach1* genes is intriguing. We found that Bach1 functions as an activator of its own promoter depending on the presence of the MARE-like sequence. Based on several observations, however, this activator function of Bach1 reflects its indirect activity. The most simple interpretation of our results is that Bach1 negatively regulates the activ-

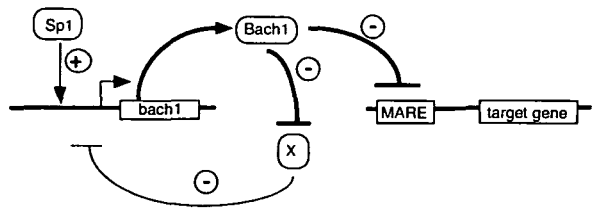

Fig. 9. **A model for stable but dynamic gene regulation by Bach1.** The basal level of *bach1* expression is regulated by the GC boxes in the promoter region and Sp1. Bach1 directly or indirectly inhibits a repressor molecule (designated X here), further maintaining its expression as well as consolidating the repression of other genes with MARE. Increased levels of heme inhibit Bach1 DNA binding activity, thus relieving the target genes from repression by Bach1 followed by activation by p45-related factors. Strong regulatory interactions are indicated by thick lines.

ity of the MARE-like element–binding protein present within K562 cells. Since the MARE-like sequence is involved in the negative regulation of the *bach1* promoter, the net effect of Bach1 expression is the activation of the *bach1* promoter. This hypothesis postulates a relatively simple feedback mechanism to maintain the expression levels of repressor molecules (Fig. 9). With such a regulatory circuit to maintain the expression of the repressor by itself, a cell may be able to fix a certain type of gene expression program among a set of programs available to the cell. The fact that the DNA binding activity of Bach1 is directly regulated by heme (27) suggests that the stability of the gene expression program regulated by Bach1 is influenced by intracellular heme concentrations. Thus, the expression of MARE-containing genes can be stably repressed by Bach1, or enhanced by de-repression through the inactivation of Bach1 and subsequent activation due to the binding of p45-related factors (Fig. 9). This hypothesis should be tested in a more physiological context and such experiments are now underway in our laboratories.

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