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The Mouse *mafB* 5′-Upstream Fragment Directs Gene Expression in Myelomonocytic Cells, Differentiated Macrophages and the Ventral Spinal Cord in Transgenic Mice

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The b-Zip transcription factor MafB is an essential determinant of neural development and an inducer of monocytic differentiation. The MafB protein is expressed in a variety of tissues including the developing spinal cord, retina, myelomonocytic lineages of hematopoietic cells, and peritoneal macrophages. However, the tissue-specific regulatory mechanism of *mafB* gene expression and its biological relevance have not been examined in detail. Here, we report, for the first time, analysis of the regulatory mechanism and tissue-specific expression of the *mafB* gene *in vivo* using transgenic mice. A transgene, containing the 8.2-kb sequence flanking the 5′ end of the *mafB* exon, directed the expression of a GFP reporter gene specifically in the retina, myelomonocytic lineages of hematopoietic cells, peritoneal macrophages and the ventral spinal cord. *In situ* hybridization analysis showed that the reporter gene expression specifically recapitulates the endogenous expression profile of *mafB* in the retina and spinal cord. FACS analysis revealed that the Gr-1, Mac-1 and F4/80 antigens were present on most of the GFP-positive hematopoietic cells from transgenic adult bone marrow and spleen. On the other hand, B220 CD4, 8, and Ter119 cells were almost absent from among the GFP-positive cells examined. These observations suggest that gene regulatory regions located in the 8.2-kb upstream region of *mafB* are responsible for directing *mafB* expression in the retina, myelomonocytic lineages, peritoneal macrophages and the ventral spinal cord.

Key words: *mafB/kr*, macrophage, retina, spinal cord, transgenic mouse.

Abbreviations: GFP, green fluorescence protein; FACS, fluorescence activated cell sorter; RT-PCR, reverse transcription polymerase chain reaction; dpc, days post coitus.

The *maf* oncogene was initially identified in an acutely oncogenic avian retrovirus, AS42, which induces musculoaponeurotic fibrosarcomas *in vivo* and the transformation of chick embryo fibroblasts (CEFs) *in vitro* (*1*). The product of the *maf* proto-oncogene and its relatives (the Maf family proteins) share a conserved basic region and an amphipathic helix (bZip) motif that mediate dimer formation and DNA binding to the Maf Recognition Element (MARE) (*1*). Large Maf proteins, c-Maf, MafB, L-Maf/MafA/SMaf and NRL, have an acidic domain that enables transcriptional activation (*1–5*). On the other hand, small Maf proteins lack this transcriptional activation domain and function as either transcriptional activators or repressors according to the dimeric partner molecules, such as CNC, Bach or the small Maf itself (*6*, *7*).

Mutations in MafB, a Large Maf protein, have been reported to be responsible for the phenotypic abnormalities observed in the *Kreisler* mutant mouse (*8*). The original *Kreisler* mutant mouse was identified on X-ray mutagenesis screening due to its hyperactive behavior, characterized by head tossing and running in circles (*9*). This phenotype is caused by dis-segmentation of the rhombomere, indicating that MafB is indispensable for rhombomere segmentation. Chemical mutagenesis, involving ethylnitrosourea (ENU), has generated mice homozygous for mutant alleles of kr^{ENU}. Analysis of these mice showed that *mafB* is specifically required for the differentiation of glomerular visceral epithelial cells (podocytes) (*10*, *11*).

The importance of MafB in monocyte and macrophage function is suggested by its selective expression in these lineages but not in other hematopoietic cells, which is in contrast to the cases of other myeloid transcription factors (*12*). During differentiation, MafB is not found in multipotent progenitors, but expressed at moderate levels in myeloblasts, and strongly upregulated in monocytes and macrophages (*13*). MafB is known to interact with and inhibit the transactivation activity of Ets-1, a transcription factor containing a helix-turn-helix DNA binding domain, thereby interrupting erythroid differentiation (*13*).

Analysis of *Kreisler* mutant mice has also provided information regarding the transcriptional regulation of MafB. The mutation consists of a chromosomal inversion, with a minimal physical length of 1-Mb, occurring within

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the *mafB* genetic locus (*10*). Further experiments suggested that one of the inversion break points is located 30-kb 5′ upstream of the *mafB/kr* gene (*10*). Although *mafB* expression in the embryonic caudal hindbrain is dramatically abolished in the *Kreisler* mutant mouse, in various other organs including the intestine, liver, spleen and bone marrow, *mafB* expression remains unchanged, indicating that the *Kreisler* mutation generates hindbrain specific gene inactivation (*12*).

These observations caused us to hypothesize that the *mafB* gene regulatory regions that direct expression in tissues other than the hindbrain rhombomere could be located downstream of the 30-kb 5′ flanking region of the *mafB* gene. To investigate this hypothesis, we prepared a reporter transgene construct by fusing the 5′ upstream region of the *mafB* gene to a GFP reporter. To analyze the gene regulatory mechanism under more physiological conditions, we generated several transgenic mice lines, and then analyzed the GFP expression in these mice, since reporter analysis involving cell lines and transient transfection provides little information about tissue specific gene regulatory function. This analysis demonstrated that an 8.2-kb fragment, 5′ to the *mafB* gene, could direct reporter gene expression selectively in myelomonocytic cells and macrophages, but not in other hematopoietic lineages including erythroid and lymphoid cells. Various other endogenous *mafB*-expressing tissues, including the embryonic ventral spinal cord and retina, also showed reporter gene expression. These results indicate that the specific expression of *mafB* in myelomonocytic lineages, peritoneal macrophages, the embryonic spinal cord and the retina is controlled by the 8.2-kb upstream region of the *mafB* gene. This promoter fragment could prove useful for the analysis of myelomonocytic differentiation and development of the neural system, and elucidation of the function of MafB in the cells of these tissues.

MATERIALS AND METHODS

Transgene Vector and Generation of Transgenic Mice—The *mafB* genomic clone was obtained from a 129SV/J mouse library (Stratagene, La Jolla, CA) using mouse *mafB* cDNA as the probe. An 8.2-kb genomic fragment, spanning from the *Sac*I site at –7.8-kb to +364-bp relative to the transcription initiation site (*14*), was ligated to the green fluorescent protein (GFP) cDNA in the pCMX-SAH/Y145F plasmid (a gift from Dr Kazuhiko Umesono, Kyoto University). This plasmid, designated as p8.2*mafB*GFP, was linearized and purified with a Qiagen Gel Extraction kit (Qiagen GmbH, Germany), adjusted to a concentration of 5ng/μl, and then injected into fertilized murine eggs. Transgene integration was confirmed by PCR of genomic DNA from mouse tails. The primers used were: sense, AAACGGCCACAAGTTCAG; antisense, GAAGTTCACCTTGATGCC.

On Southern blot hybridization analysis, genomic DNA was digested with *Bam*HI and *Eco*RI, electrophoretically separated, and then transferred to a nylon membrane. A 1.5-kb genomic fragment, spanning 5′ *Bam*HI to *Apa*I site, was used as the probe.

GFP Immunohistochemistry and mafB In Situ Hybridization Analysis—Eye balls or 12.5–18.5 dpc mouse embryos were directly fixed for 2 h in 4% paraformaldehyde in PBS after dissection, followed by overnight incubation in 30% sucrose at 4°C. On the following day, the tissues were embedded in Tissue-Tek OCT compound (Sakura, Tokyo) and frozen in liquid nitrogen. Frozen sections (7–10 μm thick) were cut at –20°C with a LEICA cryostat (Leica Instruments, Germany). The tissue sections were mounted in DAKO fluorescent mounting medium (DAKO Corporation, CA), and then incubated in 0.6% hydrogen peroxide for 35 min to eliminate endogenous peroxidase activity. After rinsing in a phosphate buffer, the sections were incubated in Tris-buffered saline (TBS) containing 3% normal goat serum and 0.25% Triton X-100 for 30 min. The sections were then incubated with rabbit polyclonal anti-GFP antibodies (Molecular Probes, Eugene, OR) diluted 1:1,000 in TBS containing 1% normal goat serum and 0.25% Triton X-100. GFP immunoreactivity was visualized using an avidin-biotin-peroxidase system (Vector Laboratories, CA) with 0.01 M imidazole acetate buffer containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide.

For *in situ* hybridization, the embryo sections were treated with Proteinase K for at least ten minutes. After postfixation, the embryos were prehybridized and then hybridized with a *mafB* RNA probe created from a 1-kb fragment of the *mafB* exon (*15*). The embryos were subsequently incubated with alkaline phosphatase-conjugated anti-digoxigenin antibodies (Roche Molecular Biochemicals). Hybridization signals were visualized using BM Purple AP Substrate (Roche Molecular Biochemicals).

FACS Analysis of Peritoneal Macrophages and Bone Marrow Cells—Peritoneal macrophages were isolated from peritoneal cavities after injection of 2 ml of 5% thioglycollate broth, followed by peritoneal lavage with PBS 3–4 days later (*16*). The isolated peritoneal macrophages were directly subjected to FACS analyses after being washed and resuspended in 1XPBS. Bone marrow cells were isolated from the femurs of adult mice and most erythrocytes were removed by the specific gravity centrifugation method with HISTPPAQUE-1083 (Sigma, St, Louis, MO). Then, the cells were washed once in ice-cold 1XPBS. Non-specific binding was blocked by incubation of the cells in 5% mouse serum for 15 min, followed by incubation with antibodies for 10 min. We used phycoerythrin (PE)-conjugated anti-Mac-1, anti-Gr-1, anti-B220, anti-CD4, 8, anti-Ter119 (Pharmingen, San Diego, CA), and anti-F4/80 (Serotec, Oxford, UK) antibodies.

Examination of GFP+ cells among peritoneal macrophages and bone marrow cells by Flow Cytometry was performed with a FACS Calibur and Vantage flow cytometer (Becton Dickinson, Mountain View, CA), and the data were analyzed with CellQuest (Becton Dickinson Immunocytometry Systems, San Jose, CA) or Flowjo (Tree Star Inc., San Carlos, CA) software.

RT-PCR Analysis—On RT-PCR analysis, 1 μg of the total RNA extracted from peritoneal macrophages was used for cDNA synthesis using Super Script II™ reverse transcriptase (Invitrogen Corp., Carlsbad, CA) and random hexamer primers. The GFP primers used were: sense, AAACGGCCACAAGTTCAG; antisense, GAAGT-

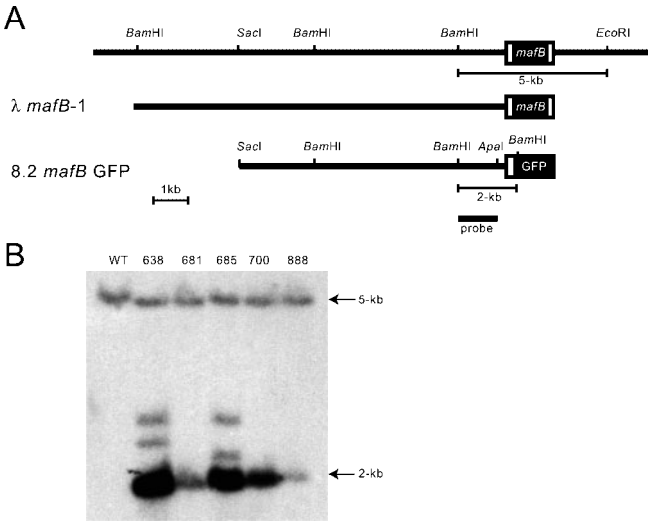

Fig. 1. **Generation of 8.2*mafB*GFP transgenic mice.** (A) Schematic representation of the 10-kb *mafB* genomic λ-phage clone and the transgene construct used in the transgenic assay. 1.1-kb GFP cDNA was ligated to the 8.2-kb *mafB* promoter fragment (see "MATERIALS AND METHODS"). (B) Southern-blot analysis of the founder (F0) transgenic mice. Genomic DNA prepared from five independent transgenic lines was digested with both *Eco*RI and *Bam*HI at 37°C overnight. The double digestion generated 5.0-kb and 2.0-kb bands for the wild-type and transgenic allele respectively, with the probe indicated in (A).

TCACCTTGATGCC. The *mafB* primers used were: sense, GAGGGATCCCATGGAGTACGTCAAGCACT; antisense, GAGGAATTCCCTCGAGATGGGTCTTCGGT.

RESULTS

Generation of mafB8.2-GFP Transgenic Mice—To determine whether or not the *mafB* upstream sequence has the ability to direct gene expression in cell-lineage specific and developmental stage-specific manners, we fused the 5′ flanking region to a GFP reporter gene (Fig. 1A). We first isolated phage clones, λ*mafB*-1, containing the 10-kb upstream region and the exon of mouse *mafB*. The 8.2*mafB*GFP construct comprised 8.2-kb of the *mafB* upstream genomic fragment, spanning from the 5′ *Sac*I site to +364-bp relative to the transcription initiation site, ligated to a GFP reporter gene, and included a small t-antigen intron and a polyadenylation site derived from the SV40 gene. Transgenic mice were generated using this construct. Our aim was to identify the regulatory regions that mediate GFP reporter gene expression in a manner reflecting the expression of endogenous *mafB* in tissues including peritoneal macrophages, myelomonocytic lineages, eyes and neural tissues. Blot hybridization analysis of tail DNA revealed five independent transgenic lines (Fig. 1B) that passed transgenes on to their offspring. These lines were selected for further analysis.

The 8.2-kb mafB 5′ Upstream Region Directs GFP Expression to the Inner Segment, and Inner and Outer Granular Layers of the Retina—To ensure the reproducibility of the expression profile, we examined five independent established lines of transgenic mice. In some instances, we also analyzed transgenic founder embryos directly. Analysis of adult mice demonstrated high levels of GFP transgene expression in the retinas of two independent transgenic lines (Fig. 2, A–D, and Table 1). Transverse sections of the eyeballs revealed transgene expression in three distinct layers of the retina (Fig. 2B). HE staining of the flanking section showed histologically that these layers correspond to the inner and outer granular layers, and the inner segments (Fig. 2D). To permit direct comparison of the expression patterns of the transgenes and endogenous *mafB* expression, *in situ* hybridization was performed on wild-type adult mice using a *mafB* antisense oligonucleotide probe. As expected,

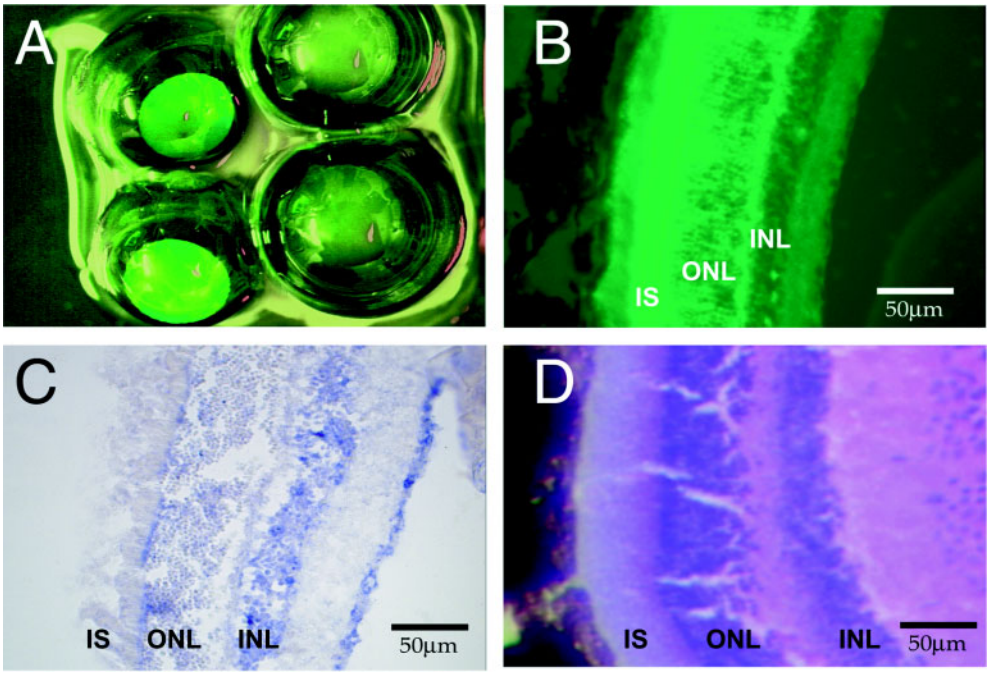

Fig. 2. **GFP expression in the retinas of 8.2*mafB*GFP transgenic mice.** (A) The eye balls of an adult transgenic mouse (left two) and a wild-type mouse (right two). Note the expression of GFP observed in the retina through the lens. (B) The expression of GFP in a section of an adult retina of line 685. Note the expression of GFP specifically in IS, inner segments; ONL, outer nuclear layer; and INL, inner nuclear layer. (C) The pattern of *mafB* mRNA expression in the adult retina. *In situ* hybridization with the *mafB* antisense riboprobe revealed *mafB* mRNA expression in IS, ONL and INL. Sections incubated with the sense *mafB* probe do not show any hybridization (data not shown). (D) A representative bright-field photomicrograph of an HE-stained adult retina illustrating the cellular organization.

Table 1. **Transgene expression sites in adult transgenic mouse lines.**

Transgenic line	Copy number	Brain	Retina	Bone marrow	Peritoneal macrophage
685	7	+	+	–	–
700	4	+	+	+	+
681	2	+	–	–	–
638	9	+	–	+	+
888	1	+	–	–	–

GFP expression was examined by fluorescence imaging and immunohistochemistry for brain and retina, and RT-PCR and FACS analysis for bone marrow cells and peritoneal macrophages. +, positive; –, negative.

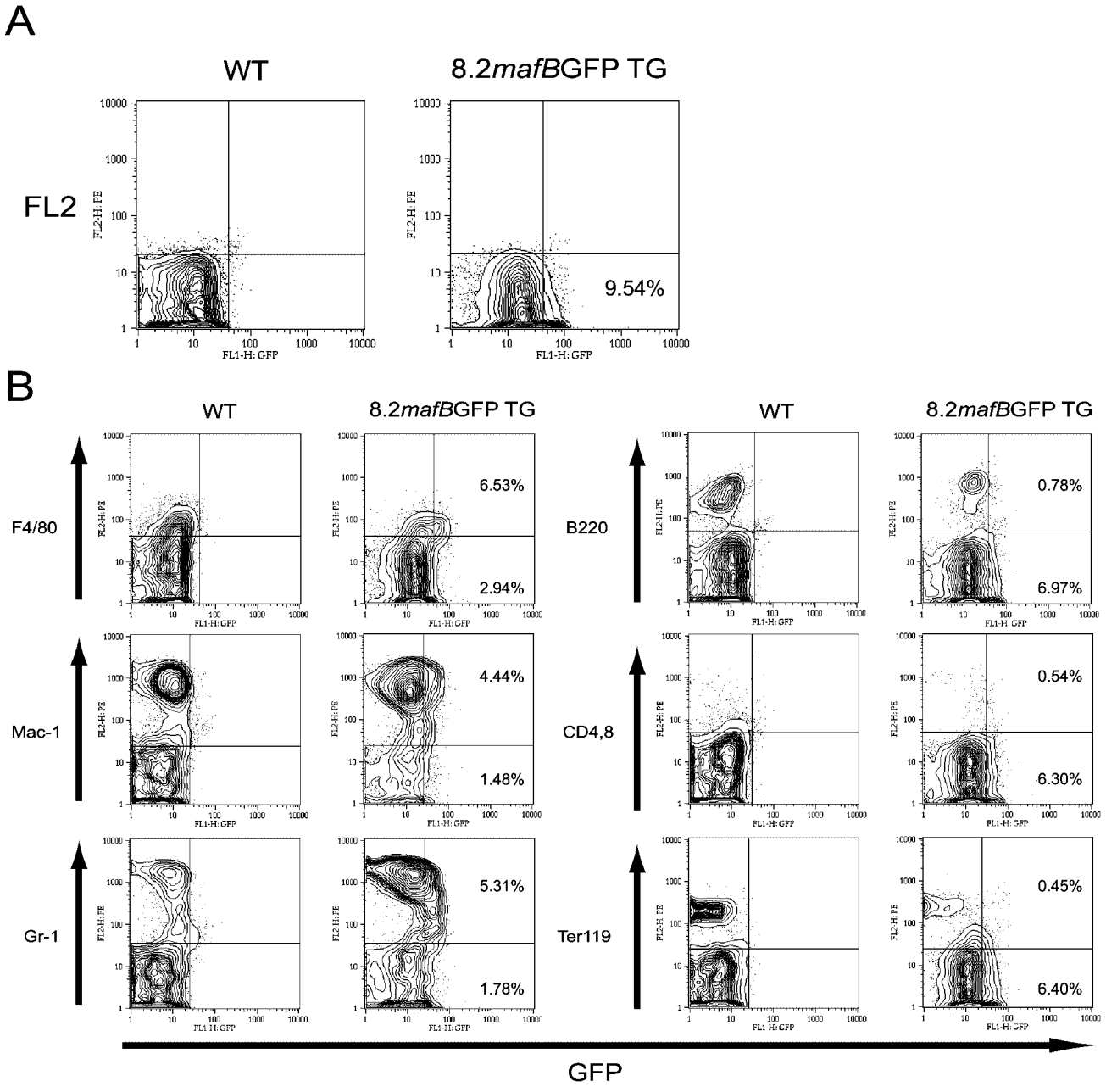

Fig. 3. **GFP-positive cells in the bone marrow of adult 8.2*mafB* transgenic mice (line 700) are associated with cell surface markers characteristic of the myelo-monocytic lineage.** Bone marrow cells of transgenic mice were aspirated out, and single-cell suspensions were analyzed by FACS after staining with monoclonal antibodies. PE-conjugated anti-F4/80, anti-Mac-1, anti-Gr-1, anti-B220, anti-CD4, 8, and anti-Ter119 were used. (A) GFP-positive cells were found to comprise 9.54% of the adult bone marrow cells. (B) Most of the GFP-positive cells were stained by anti-F4/80, anti-Mac-1 and anti-Gr-1, while B220 and CD4, 8 were largely undetectable in the GFP-positive fraction.

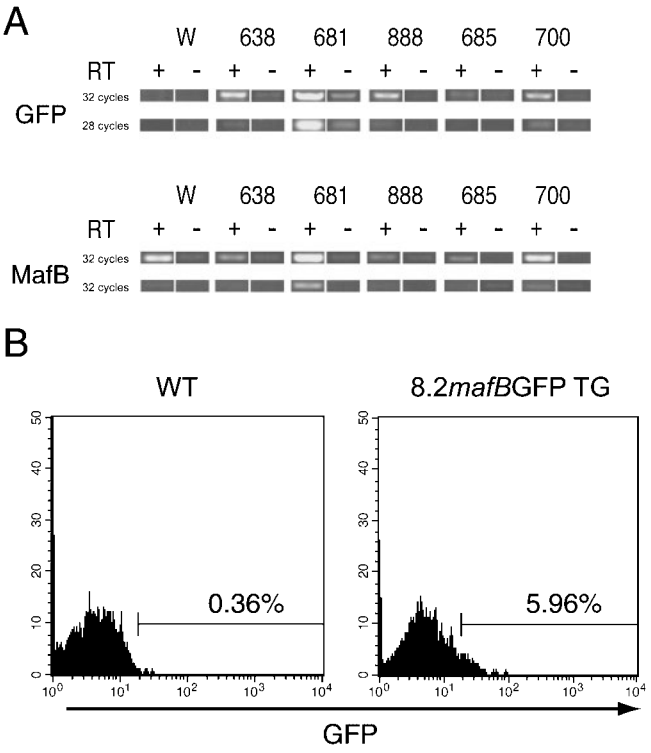

Fig. 4. **Peritoneal macrophages express GFP mRNA and fluorescence.** (A) Results of RT-PCR. RNA was prepared from peritoneal macrophages isolated from peritoneal cavities after injection of 2 ml of 5% thioglycollate broth, followed by peritoneal lavage with PBS 3–4 days later. The RT reaction was conducted in either the presence (+) or absence (–) of reverse transcriptase. A band representing the transcript derived from the introduced GFP cDNA and the endogenous *mafB* gene were observed for several transgenic lines. (B) FACS analysis of peritoneal macrophages. GFP-positive cells were specifically observed in the 8.2*mafB*GFP transgenic mice (line 700).

endogenous transcripts of the *mafB* gene were expressed in the outer and inner granular layers, and inner segments of the retina (Fig. 2C).

However, GFP reporter gene expression was only faintly detected in the epithelial cells of 14.5 dpc fetal lenses (data not shown), which are known to be representative of cells expressing MafB in the lens (*15*, *17*). These results suggested that the 8.2-kb upstream region contained regulatory elements capable of directing expression to the inner and outer granular layers, and the inner segments of the retina of adult mice, but lacked elements necessary for high level expression in the epithelial cells of 14.5 dpc fetal lenses.

The 8.2-kb mafB 5′-Upstream Region Directs GFP Reporter Gene Expression in Monocyte-Macrophage Lineages of Adult Bone Marrow Cells—To examine the expression of GFP in hematopoietic lineages, we determined the frequency of GFP-positive cells in the bone marrow of adult 8.2*mafB*GFP transgenic mice. Single-cell suspensions of adult bone marrow cells were analyzed by FACS. GFP-positive cells were found to comprise 9.54% of the adult bone marrow cells (line 700) (Fig. 3A). We next stained the adult bone marrow cells with antibodies recognizing specific cell surface markers and then performed FACS analysis. Most of the GFP-positive cells in adult bone marrow were stained with anti-Gr-1, anti-Mac-1, or anti-F4/80 antibodies, but were negative for CD4, CD8, B220, and Ter119, which indicates that GFP was expressed specifically in myelomonocytes and differentiated macrophages (Fig. 3B, compare upper right boxes of 8.2*mafB*GFP TG with WT). These data thus indicated that cells positive for GFP, whose expression is directed by the 8.2-kb *mafB* gene regulatory region, were primarily granulo-monocytic cells and differentiated macrophages of adult bone marrow.

Peritoneal Macrophage-Specific Expression of the mafB Gene Is Regulated by the 8.2-kb 5′-Upstream Region—Since it has been reported that MafB was strongly expressed in peritoneal macrophages (*13*), we analyzed the GFP reporter expression in peritoneal macrophages of 8.2*mafB*GFP transgenic mice. Adult 8.2*mafB*GFP transgenic mice and wild-type littermates were injected intraperitoneally with thioglycollate to harvest peritoneal cells. RT-PCR analysis clearly demonstrated the expression of reporter GFP and the endogenous *mafB* gene in peritoneal macrophages (Fig. 4A). We then performed FACS analysis to examine the GFP fluorescence in peritoneal macrophages of the transgenic mice. Two independent transgenic lines (lines 638 and 700) showed GFP fluorescence in peritoneal macrophages, indicating that GFP expression recapitulated endogenous MafB expression in peritoneal macrophages of 8.2*mafB*GFP transgenic mice (Fig. 4B and Table. 1).

Transgene Expression in the Embryonic Ventral Spinal Cord—To determine transgene expression during neural development, embryos from at least two 8.2*mafB*GFP transgenic lines were analyzed at different times from 8.5 to 14.5 dpc. The expression of GFP was first detected in the developing spinal cord at 10.5 dpc. GFP immunohistochemical analysis of axial sections of transgenic embryos demonstrated strong expression of the transgene in the ventral region of the spinal cord at 12.5 dpc (Fig. 5, A–C). *In situ* hybridization analysis revealed that endogenous *mafB* mRNA was expressed in the ventral spinal cord of 12.5 dpc embryos (Fig. 5D). Therefore, the pattern of GFP expression in transgenic embryos recapitulated the spatial pattern of endogenous *mafB* expression in the developing spinal cord. Expression of the transgene in the 5~6th segments of the rhombomere in 8.5–9.5 dpc embryos was scarcely detectable (data not shown), a site representative of *mafB* gene expression and abolished in the *Kreisler* mutant (*12*). These observations suggested that the 8.2-kb *mafB* upstream region was able to direct expression of the transgene in the *mafB*-expressing ventral spinal cord in 12.5 dpc embryos but lacked elements necessary for expression in the rhombomeres of 8.5–9.5 dpc embryos. These results are consistent with previous reports that suggested the hindbrain-specific regulatory region is located far 5′ from the *Kreisler* break point, *i.e.* 30-kb upstream of the *mafB* exon (*10*).

DISCUSSION

We have investigated the promoter activity that directs tissue-specific *mafB* gene expression, utilizing transgenic mice harboring a GFP reporter gene under the control of

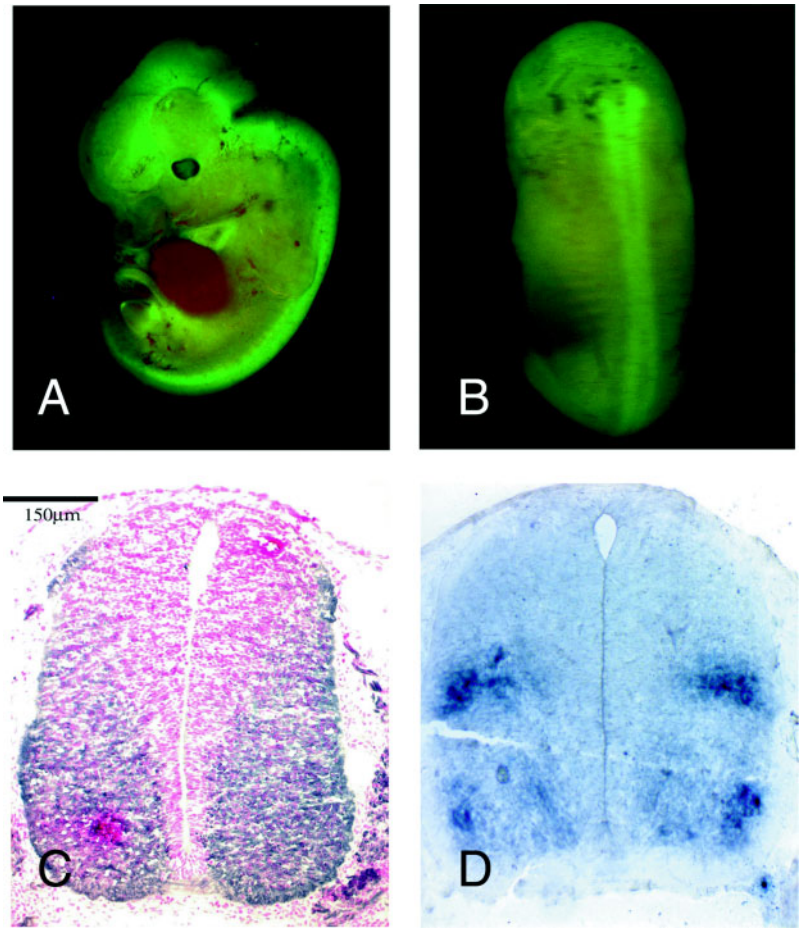

Fig. 5. **Embryonic neural tissue-specific expression of GFP in 8.2*mafB*GFP transgenic mice.** (A and B) Whole-mount analysis of GFP expression in 12.5 dpc transgenic mice. (C) GFP immunohistochemical analysis of transverse sections of embryonic neural tissues demonstrated expression of the transgene in the ventral region of the spinal cord at 12.5 dpc. (D) The pattern of *in situ* hybridization with the *mafB* antisense riboprobe in a 12.5 dpc wild-type embryo. The sense probe did not show any hybridization (data not shown).

the 5′-flanking sequence of the mouse *mafB* gene. Our analysis of five transgenic mice lines demonstrated that the 8.2-kb 5′-flanking region of the mouse *mafB* gene encodes elements that are necessary and sufficient for the tissue-specific gene expression in the retina, monocytes-macrophages, and the developing spinal cord.

We observed that endogenous *mafB* mRNA was expressed in the inner and outer granular layers of the retina. The 8.2-kb *mafB* upstream region directed GFP expression in the same layers. Therefore, the 8.2-kb *mafB* upstream region recapitulates the spatial pattern of endogenous *mafB* expression in the retina. Nrl, a large Maf family transcription factor, is expressed specifically in the rod photoreceptor layer of photoreceptor cells, and the inner and outer granular layers and is required for rod photoreceptor development (*18*). In Nrl knock-out mice, functional switching of photoreceptors from a rod to an S-cone phenotype in photoreceptor cells has been observed, demonstrating that Nrl plays a pivotal role in determination of the photoreceptor phenotype (*19*). Within the layer of photoreceptor cells, the Nrl expression pattern is homologous to that of *mafB*, as we observed here. Overall, it is plausible that Nrl and MafB may be partners in a common regulatory network in the retina.

Although it has been reported that MafB is expressed in lens epithelial cells (*15*, *17*), we did not detect significant expression of the GFP reporter. Given that in *Kreisler* mutant mice MafB expression in lens epithelial cells is conserved (Kawauchi *et al*., unpublished observation), we suspect the regulatory region necessary for MafB expression in these cells is located within the 30-kb to 8.2-kb 5′ upstream region of the MafB exon.

In this study we showed, by means of FACS analysis of adult bone marrow cells and peritoneal macrophages, that the specific expression of MafB in monocyte-macrophage lineages was recapitulated in 8.2*mafB*GFP transgenic mice. Most of the GFP-positive hematopoietic cells in 8.2*mafBGFP* transgenic mice were stained for anti-F4/80, Mac-1, and Gr-1, but were negative for CD4, CD8, B220, and Ter119. This suggests that the regulatory regions that direct the expression of MafB specifically in myelomonocytes and macrophages are located in the 8.2-kb fragment 5′ upstream of the mouse *mafB* gene.

Upon inspection of the 8.2-kb nucleotides sequence, we found a consensus ets-binding site at 7.8-kb upstream and a consensus C/EBP-binding site at 86-bp upstream of the transcription initiation site of the *mafB* gene. PU.1, an ets family transcription factor, is known to be required for the differentiation of both mature granulocytes and macrophages (*20*, *21*). Although PU.1 can induce myeloid lineage commitment efficiently in multipotent progenitors, it cannot drive monocyte/macrophage terminal differentiation in myeloblasts (*22*). MafB is able to induce macrophage differentiation directly from myeloblasts *in vitro* (*23*), suggesting that PU.1 may induce myeloid line-

Table 2. **Transgene expression sites in embryos.**

Transgenic line	Brain	Retina	Spinal cord	Pancreas	Heart	Kidneys	Limbs
685	$+^{3}$	$+^{3}$	–	–	–	–	$+^{3}$
700	$+^{1,2,3,4}$	$+^{1,2,3,4}$	$+^{1,2,3,4}$	$+_{4}$	$+^{1,2,3,4}$	$+^{4}$	$+^{1,2,3,4}$
681	–	–	–	–	–	–	–
638	$+_{3}$	–	–	$+_{4}$	–	–	$+_{3}$
888	$+_{3}$	–	$+_{3}$	–	–	–	$+_{3}$
F0-1	$+_{2}$	–	$+_{2}$	–	–	–	$+_{2}$
F0-2	$+_{2}$	–	$+_{2}$	–	–	–	$+_{2}$
F0-3	$+_{2}$	–	$+_{2}$	–	–	–	$+_{2}$

GFP expression was examined by fluorescence imaging and immunohistochemistry of these tissues. The embryos examined were: 1, 10.5 dpc; 2, 12.5 dpc; 3, 14.5 dpc; 4, 18.5 dpc. +, positive; –, negative.

age commitment and subsequently trigger MafB expression during the terminal differentiation stage of macrophages. CCAAT/enhancer-binding protein beta (C/EBPβ) regulates the transcription of myelomonocytic genes. Targeted inactivation of the *C/EBP*β gene in the mouse results in macrophage dysfunction, impaired tumor cell killing, and a lymphoproliferative disorder (*24–26*). Macrophages from C/EBPε knock-out mice are defective in phagocytosis, and the production of IL-10 and IL-12 induced through the LPS stimulation pathway. These observations indicate that C/EBPβ or ε is required later than PU.1 during myelomonocytic differentiation. Therefore, the CCAAT site located in the upstream region of *mafB* could have an indispensable function by facilitating the interaction between C/EBPs and MafB on the differentiation of macrophages. Further studies on the molecular basis of macrophage differentiation should reveal any functional or regulatory interaction(s) between PU.1, C/EBPs and Maf family proteins.

Previous studies have shown that *mafB*/*maf-1* mRNA is expressed specifically in the spinal cord of murine embryos (*12*, *27*). In accordance with this observation, GFP expression was observed in the ventral spinal cord of 12.5 dpc transgenic embryos. This GFP expression profile recapitulates endogenous *mafB* mRNA expression, as revealed by *in situ* hybridization analysis. We found that the 8.2-kb upstream sequence contains four consensus GATA-binding sites (data not shown). It has been reported that GATA-2 and -3 are expressed in the embryonic ventral spinal cord and regulate developing V2 interneurons (*28*). It is possible that MafB might contribute to the differentiation of V2 interneurons under the control of the GATA transcription factors.

Recently, it was reported that MafB was specifically expressed in glomerular epithelial cells and required for foot process formation by podocytes (*11*, *29*). So, we examined GFP expression in the kidneys of 18.5 dpc transgenic embryos. Although one of the five established 8.2*mafB*GFP transgenic lines showed GFP expression in the kidneys, GFP-positive cells were observed in the renal tubules and mesenchyme as well as glomerular epithelial cells (data not shown and Table 2). Therefore, these observations suggest that the 8.2-kb *mafB* upstream region was insufficient for regulation of the gene expression specifically in podocytes, *i.e.* not in other renal cells.

In summary, we have shown here that the *mafB* gene expression in monocytes, macrophages, the retina and the ventral spinal cord is regulated by the 8.2-kb 5′-upstream region of the *mafB* gene. Further analysis to identify a regulatory module should reveal the molecular mechanism of cell differentiation and enable targeted expression of various factors in distinct tissues. At the same time, this promoter fragment should be a powerful tool for elucidation of the MafB function through various experiments, such as transgenic overexpression or rescue of MafB deficiency *in vivo*.

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