Activation of β-major globin gene transcription is associated with recruitment of NF-E2 to the β-globin LCR and gene promoter

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The mouse β-globin gene locus control region (LCR), located upstream of the β-globin gene cluster, is essential for the activated transcription of genes in the cluster. The LCR contains multiple binding sites for transactivators, including Maf-recognition elements (MAREs). However, little is known about the specific proteins that bind to these sites or the time at which they bind during erythroid differentiation. We have performed chromatin immunoprecipitation experiments to determine the recruitment of the erythroid-specific transactivator p45 NF-E2/MafK (p18 NF-E2) heterodimer and small Maf proteins to various regions in the globin gene locus before and after the induction of murine erythroleukemia (MEL) cell differentiation. We report that, before induction, the LCR is occupied by small Maf proteins, and, on erythroid maturation, the NF-E2 complex is recruited to the LCR and the active globin promoters, even though the promoters do not contain MAREs. This differentiation-coupled recruitment of NF-E2 complex correlates with a greater than 100-fold increase in β-major globin transcription, but is not associated with a significant change in locus-wide histone H3 acetylation. These findings suggest that the β-globin gene locus exists in a constitutively open chromatin conformation before terminal differentiation, and we speculate that recruitment of NF-E2 complex to the LCR and active promoters may be a rate-limiting step in the activation of β-globin gene expression.

The mammalian β-globin gene loci are models for studying the activation of gene expression during development. The mouse β-globin gene locus contains two embryonic and two adult β-genes ordered as they are expressed during development (Fig. 1*A*). Expression of genes in the locus is regulated by the locus control region (LCR), which extends from 30 to 60 kb upstream of the adult β-globin genes. The murine LCR consists of six DNase I hypersensitive sites (HS1–6; Fig. 1*A*). An additional HS doublet located far upstream of the LCR (−62.5 kb 5′ of Ey) and of unknown function has been described recently (1). Targeted deletions of HS1–6 in both cultured cell lines and mice have revealed that the LCR is not required to initiate or maintain open chromatin and basal transcription, suggesting that the main function of the LCR in the endogenous locus is to enhance transcription (2, 3). Each hypersensitive site contains Ap-1-, Sp-1-, and/or GATA-like sites. The AP-1-like sites in HS2 and HS3 are closely related to the Maf-recognition elements [MAREs; TGCTGAC(T/GT)TCAGCA]. The MAREs in the murine β-globin LCR can be recognized by small Maf family members, including MafK (p18 NF-E2), erythroid specific p45 NF-E2, ubiquitously expressed Nrf1/LCR-F1/TCF11, the Bach factor family, or Nrf2/ECH (4).

The erythroid-specific transactivator NF-E2 was initially purified from murine erythroleukemia (MEL) cells and was shown to be a heterodimer of p45 NF-E2 and MafK (5–8). p45 NF-E2, the larger subunit of NF-E2, is a member of basic leucine zipper (bZIP) family and resembles the cap 'n' collar family of proteins in *Drosophila*. Although p45 NF-E2 does not bind DNA directly, it has been shown to function as a transactivator of globin gene expression. *p45 NF-E2*-deficient MEL cells fail to express globin genes (9), and globin gene expression can be restored by the induction of a tethered NF-E2 complex (10). However, targeted disruption of *p45 NF-E2* has little effect on erythroid maturation in mice (11, 12), presumably because of functional redundancy between p45 NF-E2 and other bZIP family proteins. Recently, chromatin immunoprecipitation (ChIP) studies have revealed that p45 NF-E2 is recruited to HS2 in the murine β-globin gene LCR (13) and to HS1 to HS4 in the human β-globin gene LCR (14). Interestingly, p45 NF-E2 can also interact with a subunit of TFIID ($TAF_{II}130$; ref. 15) and with CBP, a coactivator with histone acetyltransferase activity (16, 17). These findings raise the possibility that p45 NF-E2 may communicate with the basal transcription machinery and mediate gene activation.

The smaller subunit of NF-E2 (5, 6), MafK, is closely related to other small Maf family members, MafF and MafG (18–20). MafK contains a basic-leucine zipper domain required for dimerization and DNA binding, but lacks a transactivation domain (6, 18, 21). Overexpression of antisense MafK in MEL cells decreases globin gene expression (22), and overexpression of MafK in MEL cell causes terminal differentiation (22, 23), suggesting that MafK plays an important role in erythroid differentiation. However, both genetic and biochemical studies suggest that MafK is not the only partner of p45 NF-E2 *in vivo* (21, 24). All small Maf family members can form a complex with p45 NF-E2 and transactivate MARE-linked reporter genes (21, 25, 26), and both MafG and MafK are widely expressed in hematopoietic cells (19). In addition, MafK mutant mice are indistinguishable from wild-type mice (24, 27), suggesting that other small Maf family members can compliment MafK activity *in vivo*. Thus, currently, it is not clear which small Maf family members actually interact with the β-globin gene locus *in vivo*.

We have used viral-transformed MEL cells as a model system to study the nuclear events during erythroid terminal differentiation and maturation. MEL cells are blocked in the early stage of erythropoiesis, and morphologically MEL cells resemble proerythroblasts. MEL cells proliferate in culture until induced to undergo erythroid maturation (28). One MEL cell subclone, 745A, can be efficiently induced into terminal differentiation in the presence of DMSO, resulting in a strong activation of adult β-globin gene expression (22, 28).

Abbreviations: LCR, locus control region; MARE, Maf-recognition element; MEL, murine erythroleukemia; HS, hypersensitive site; ChIP, chromatin immunoprecipitation; RT, reverse transcription.

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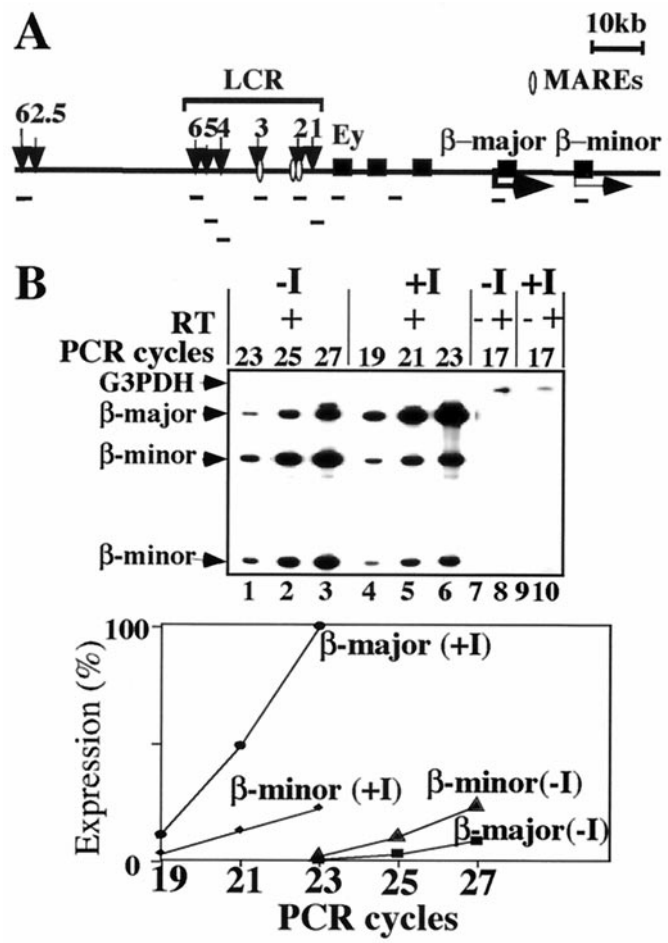

Fig. 1. Structure of the mouse β-globin gene locus, and mouse β-globin gene expression in MEL cells. (*A*) PCR primer pairs for ChIP analyses were designed to amplify the LCR hypersensitive sites, inactive Ey globin promoter, intergenic region, and active β-major and β-minor globin promoters. Each primer sequence is published as supplemental data. (*B*) RT-PCR analysis of two adult β-globin genes (β-minor and β-major) was performed with a primer pair that coamplifies β-minor and β-major globin cDNAs followed by restriction enzyme digestion of the β-minor globin product to distinguish β-minor and β-major globin products. PCR products of cDNA from uninduced 745A MEL cells after 23, 25, and 27 cycles (lanes 1–3) and induced 745A MEL cells after 19, 21, and 23 cycles (lanes 4–6) were digested and loaded on a 6% polyacrylamide gel. Quantitation of digested PCR products is shown (*Lower*). Each product was quantified and then normalized to the length of the product. Values were then standardized by dividing by control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal (lanes 8 and 9).

Using the ChIP assay, we have mapped the regions recognized by p45 NF-E2, MafK, and small Maf members in the β-globin gene locus in uninduced and induced 745A MEL cells. We show that in uninduced cells small Maf family proteins occupy the LCR. On MEL cell induction, the NF-E2 complex is recruited to the LCR and to the active gene promoters. This differentiation-coupled recruitment of NF-E2 is not associated with any significant change in locus-wide histone H3 acetylation of the β-globin gene domain. We speculate on the significance of these findings for the regulation of β-globin gene activation.

Materials and Methods

Cell Lines, Culture Conditions, and Antibodies. 745A MEL cell line (obtained from J. Robert-Lézénès, Hôspital Paul-Brousse, Villejuif, France) was grown in RPMI medium 1640 containing 10% FCS. Over 90% of the cells were hemoglobinized after 5 days in 2% DMSO, as estimated by benzidine staining. Anti-p45 NF-E2 (sc-291) and anti-MafK (anti-p18 NF-E2:sc-477) polyclonal antibodies were obtained from Santa Cruz Biotechnology. The anti-p18 NF-E2 antibody reacts only with MafK, whereas the anti-small Maf antiserum used in these experiments reacts with both MafK and MafG (29). The affinity of the small Maf antiserum for MafK is approximately twice that for MafG (29). An antibody against-H3 histone acetylated at Lysine 9 and 14 was obtained from Upstate Biotechnology. Anti-GFP antibody (Living Colors A.v. peptide antibody from CLONTECH) was used as a control IgG in ChIP experiments.

Reverse Transcription (RT)-PCR Analysis. Expression analysis by RT-PCR was performed by using primer pairs for adult globin genes (HBG1 + 2) as described (30): Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control Amplimer Set (CLONTECH) was used as a control primer for RT-PCR experiments.

ChIP and Quantitative PCR. Chromatin fixation and purification procedures were as described (31). Confluent cells (1 to 2×10^8) were fixed in 60 ml RPMI medium 1640 with 1% formaldehyde for 5 min at room temperature. Immunoprecipitations with anti-p45 NF-E2 and MafK antibodies (31) and with anti-small Maf and anti-acetylated H3 antibodies (32) were performed as described. PCR reactions were performed as described (31, 33) with minor modifications. Duplex PCR reactions were performed in 1× buffer II (Applied Biosystems), 1.25 mM $MgCl_2$, 0.2 mM dNTP, 5 pmol of each globin gene primer set, 5 pmol of myoD1 gene primer set, 1 μCi (1 Ci = 37 GBq) [α-^{32}P]dCTP, and 0.05 units of AmpliTaq Gold. The PCR protocol was 95°C for 10 min, followed by 28 cycles of 96°C for 30 sec, 54°C for 30 sec, and 72°C for 30 sec. The PCR primers are published as supplemental data on the PNAS web site, www.pnas.org.

Results

***β*-Major and *β*-Minor Globin Gene Expression and Histone H3 Acetylation.** Previous studies suggested that histone acetylation precedes activated β-globin gene transcription (refs. 33 and 34; D. Schübeler, M.G., and M. A. Bender, unpublished data). To investigate the relationship between histone acetylation and β-globin gene activation during 745A MEL cell differentiation, we determined β-globin gene expression and the status of H3 acetylation before and after induction.

As determined by RT-PCR (Fig. 1*B*), before induction, both β-major and β-minor globin gene transcripts are detected after 23 cycles of PCR, with a β-minor globin/major globin ratio of 3.4. After induction, both transcripts are detected after only 17 cycles of PCR (data not shown), reflecting increases of 159-fold in β-major globin and 10-fold in β-minor globin transcripts. Thus, after MEL cell induction, the ratio of β-minor globin to β-major globin is inverted (0.27), as has been reported (35).

To determine the level of histone acetylation before and after induction, we performed ChIP experiments with cross-linked chromatin from MEL cells and an antibody that recognizes acetylated histone H3. A set of ten PCR primer pairs was designed to amplify various regions of the β-globin gene locus: the main DNase I hypersensitive sites in the LCR, the inactive Ey globin promoter, the active β-major and β-minor globin promoters, and the intergenic region located between the β-major globin gene and Ey globin genes (Fig. 1*A*). The myoD1 gene served a negative control; MyoD is expressed only in skeletal muscle and its precursors (36) and, like the β-globin gene locus, is located on murine chromosome 7. The enrichment of β-globin gene signals relative to myoD1 was determined by duplex-PCR under conditions of linear amplification (Fig. 2*A*). The ratio of the two PCR products was determined for the antibody-bound fraction and normalized to the ratio of the input

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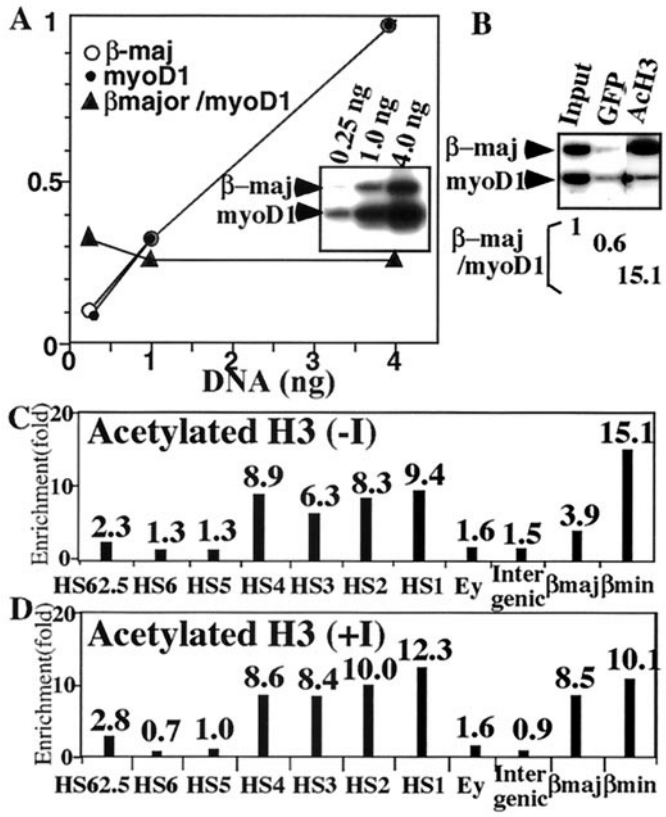

Fig. 2. Histone H3 acetylation profile of the β-globin gene locus before and after MEL cell differentiation. (*A*) Duplex PCR assays. Chromatin was purified from induced 745A MEL cells. PCR reactions were performed by using a primer pair to amplify the β-major globin gene promoter and a primer pair that amplifies the myoD1 gene. To confirm linear PCR amplification, a serial dilution from 0.25 ng to 4 ng DNA was done. Both a linear PCR amplification and a constant ratio between β-major globin/myoD1 were observed. (*B*) ChIP experiment using control rabbit IgG and anti-acetylated H3 antibodies. Chromatin from uninduced 745A MEL cells was immunoprecipitated by using control rabbit IgG (anti-GFP antibody). The ratio of products obtained with β-globin gene locus and myoD1 gene primers was measured for the input and antibody-bound sample. The globin/myoD1 ratio from the bound fractions was normalized by dividing by the globin/myoD1 ratio from the input material to determine enrichment for β-globin gene sequences during immunoprecipitation. (*C* and *D*) Histone H3 acetylation before and after induction. Anti-acetylated H3 antibody was used for ChIP analyses. Duplex PCR was performed by using the cross-linked chromatin from uninduced (*C*) and induced 745A MEL cells (*D*). ChIP and PCR reactions were repeated at least twice with consistent results. Quantification of duplex PCR results is shown. The ratio of products obtained with β-globin gene and myoD1 gene primers was measured for the input (I) and antibody-bound sample (Ab). The β-globin/myoD1 ratio from the bound fractions was normalized by dividing by the β-globin/myoD1 ratio from input material to determine enrichment for β-globin gene sequences during immunoprecipitation.

materials. A control IgG showed no enrichment of the β-globin gene locus in ChIP assay (Fig. 2*B*).

In uninduced MEL cells, histone H3 acetylation of HS1, 2, 3, and 4 is enriched 9.4-, 8.3-, 6.3-, and 8.9-fold, respectively (Fig. 2*C*). Whereas neither the Ey globin promoter nor the intergenic region was enriched, the β-minor globin promoter was enriched 15.1-fold, and the β-major globin gene promoter was enriched only 3.9-fold relative to myoD1. Thus, histones are highly acetylated over the β-globin gene locus before MEL cell differentiation, whereas β-globin gene expression levels are 10- to ≈150-fold lower than those after induction. This suggests that H3 hyperacetylation at both the LCR and promoters is not sufficient for high levels of β-globin gene expression. In induced MEL cells, HS1, 2, 3, and 4 were enriched 12.3-, 10-, 8.4-, and 8.6-fold relative to myoD1, respectively (Fig. 2*D*); this level of acetylation is not significantly different from that observed in uninduced cells. These results are consistent with a recent study in which a different MEL subclone was examined (34). Whereas neither the Ey globin promoter nor the intergenic region was enriched after induction, acetylation at the β-major globin promoter increased from 3.9- to 8.5-fold during induction. This approximate 2-fold increase in H3 acetylation of the β-major globin promoter correlates with an approximate 150-fold increase in β-major globin transcripts.

Recruitment of the NF-E2 Complex to the β-Globin Gene Locus During Induction. To examine the recruitment of NF-E2 complex to the β-globin gene locus in uninduced and differentiated MEL cells, we performed ChIP experiments with anti-p45 NF-E2 and anti-MafK antibodies and duplex-PCR analyses with the same primer pairs used in the analysis of histone H3 acetylation. By sequence analysis, three MAREs capable of binding NF-E2 *in vitro* are present in the murine β-globin gene locus: two in HS2, one in HS3 (37).

In uninduced MEL cells, p45 NF-E2 binding to HS2 is enriched 3.3-fold, relative to myoD1 (Fig. 3*A*, lanes 11 and 12). No significant enrichment is observed in other regions of the locus. After induction, the enrichment at HS2 increased to 33-fold, and HS1, HS3, and HS4 were enriched 6.8-, 4.3-, and 4-fold (Fig. 3*B*, lanes 7–14), respectively. In these differentiated cells, no significant enrichment was detected in the inactive Ey-globin promoter or the intergenic region (Fig. 3*B*, lanes 15–18); however, the β-major and the β-minor globin promoters were enriched 3.9- and 2.2-fold (Fig. 3*B*, lanes 19–22), respectively. These results suggest that p45 NF-E2 interacts with both HS1–4 and active gene promoters and that the interaction occurs primarily after cells are induced to differentiate.

The results obtained with the anti-MafK antibody (Fig. 3 *C* and *D*) are quite similar to those obtained with the anti-p45 NF-E2 antibody. HS2 was enriched 2.9-fold in uninduced MEL cells, relative to myoD1 (Fig. 3*C*, lanes 11 and 12). After induction, this enrichment increased to 30-fold (Fig. 3*D*, lanes 11 and 12), and HS1, 3, 4, and 5 were enriched 5.5-, 5.3-, 5.8-, and 4.6-fold, respectively (Fig. 3*D*, lanes 5–14). Although neither the Ey globin promoter nor the intergenic region were enriched (Fig. 3*D*, lanes 15–18), the β-major and β-minor globin promoters were enriched 6.0- and 2.9-fold, respectively (Fig. 3*D*, lanes 19–22), after induction. Consistent with the requirement of MafK for p45 NF-E2 recruitment to DNA, our data show that both MafK and p45 NF-E2 interact with HS1–4 and the active gene promoters after differentiation of MEL cells, suggesting that NF-E2 complexes are recruited to the globin gene locus on erythroid differentiation.

Small Maf Occupancy in the β-Globin Gene Locus in Uninduced MEL Cells. Our ChIP analyses revealed a 10-fold increase in binding of p45 NF-E2 and MafK to the globin gene locus after MEL cell induction. However, *in vivo* footprinting data have indicated that, even before induction, the MAREs in HS2 are occupied (38). MAREs can be bound by small Maf family members. Both MafK and MafG are expressed in MEL cells, whereas no MafF transcripts are detected in uninduced or differentiated MEL cells (data not shown). Therefore, we performed the ChIP assay, using an anti-small Maf antiserum that recognizes both MafG and MafK (29).

In uninduced MEL cells, HS2 and HS3 were enriched 9.4- and 5.2-fold by the small Maf antiserum, respectively (Fig. 4*A*, lanes 9–14), whereas no significant enrichment was detected when using anti-MafK antibody (Fig. 3*C*, lanes 11 and 12). With the anti-small Maf antiserum, the enrichment of HS2 increased from 9.4- to 22.1-fold relative to myoD1 during induction (Fig. 4*A* and

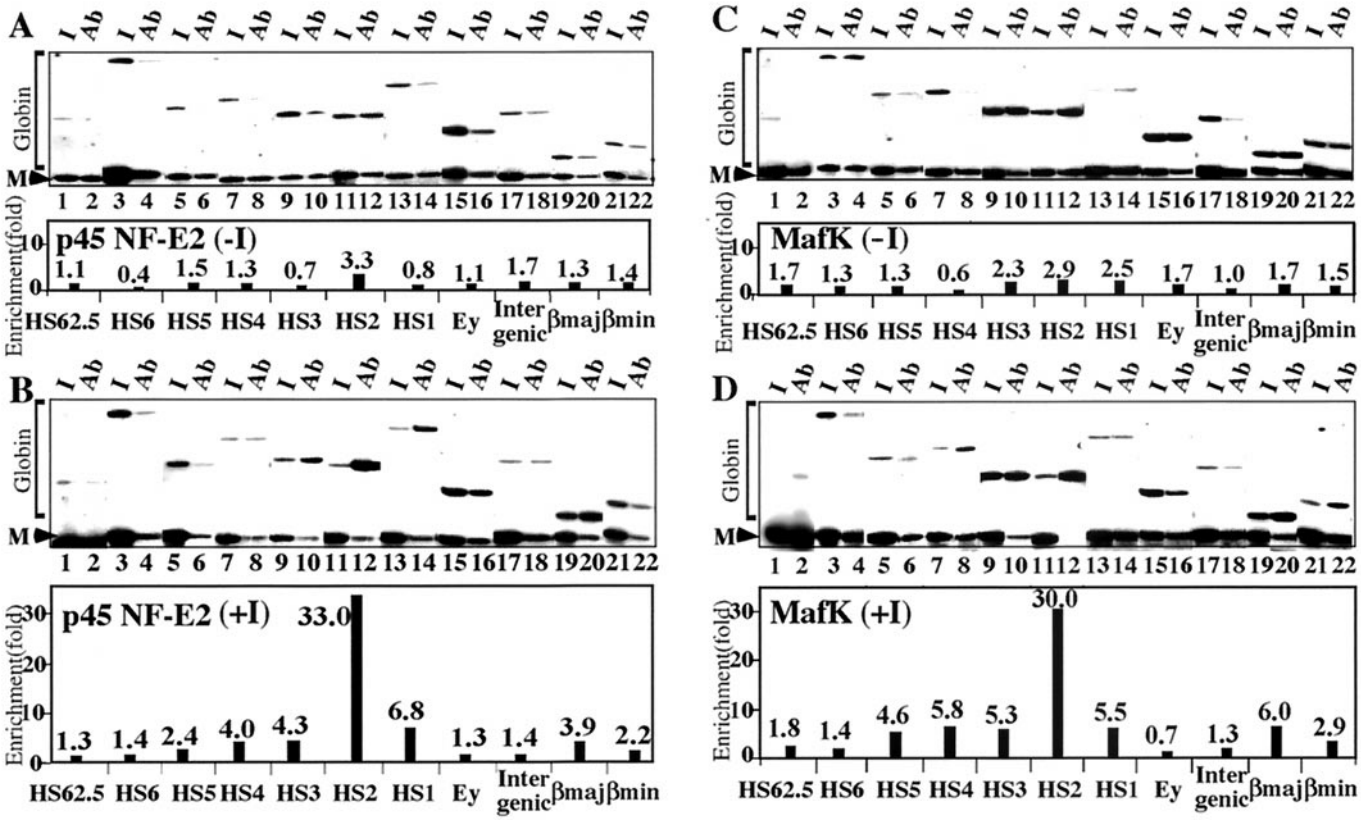

Fig. 3. p45 NF-E2 and MafK recruitment during induction. Anti-p45 NF-E2 antibody (*A* and *B*) and anti-MafK antibody (*C* and *D*) were used for ChIP experiments. Duplex PCR was performed by using the cross-linked chromatin from uninduced (*A* and *C*) and induced 745A MEL cells (*B* and *D*). ChIP and PCR reactions were repeated at least twice to verify results. Quantification of duplex PCR results is shown. The ratio of products obtained with β-globin gene locus (Globin) and myoD1 gene (M) primers was measured as described in Fig. 2.

B, lanes 11 and 12), suggesting that the anti-small Maf antiserum efficiently recognizes a factor binding to the globin gene locus, both in uninduced and induced MEL cells. Because the antibody specific for MafK showed only 2.9-fold enrichment of the globin gene locus relative to myoD in uninduced MEL cells, and only MafG and MafK are expressed in MEL cells, the major factor interacting with the LCR before induction is likely to be MafG.

Discussion

Histone H3 Acetylation Is Not Sufficient for Activated β-Globin Gene Transcription. In uninduced MEL cells, the murine β-globin gene locus is in a DNase I sensitive conformation (39, 40); moreover, LCR HSs are formed in uninduced 745A MEL cells (M. Bulger and M.G., unpublished observation). Here we report that histone H3 is highly acetylated in the globin gene locus both before and after activated transcription of the locus in MEL cells. Thus, other than minor changes in H3 acetylation at the promoters of the active genes, the uninduced and differentiated states of the β-globin gene locus are indistinguishable at the levels of H3 acetylation and nuclease sensitivity. These results are consistent with our recent analysis of mice homozygous for a targeted deletion of the LCR. The level of adult β-globin transcripts in the LCR deletion mice is less than 5% that of wild-type mice; however, the LCR deletion and wild-type mice display similarly high levels of H3 and H4 hyperacetylation at the active gene promoters (D. Schübeler, M.G., and M. A. Bender, unpublished data). Thus, our analyses of the murine β-globin gene locus in both MEL cells and in LCR deletion mice suggest that histone hyperacetylation at the β-globin promoter is not sufficient to achieve activated β-globin transcription. Clearly, there may be other chromatin modifications, such as histone H3 methylation, phosphorylation, and/or acetylation of specific lysine residues that distinguish the β-globin gene loci in committed and differentiated cells, as well as in wild-type and LCR deletion mice.

Recently, Forsberg *et al.* reported moderate levels of histone acetylation in HS2 and the β-major globin gene in the uninduced state of another MEL cell line, and a 2-fold increase in this acetylation after DMSO treatment (34). Although we did not observe a significant increase in acetylation at the LCR, we did observe an approximate 2-fold increase in acetylation of the β-major globin promoter. These minor differences in results could be due to differences in the MEL cell lines examined or different technical approaches, such as different procedures for quantification of PCR products.

NF-E2 Complex Recruitment to the β-Globin Gene Locus Correlates with Activated Transcription of the β-Globin Gene. Previously, p45 NF-E2, the larger subunit of NF-E2, was shown to associate with HS2 both in induced MEL cells and in mouse fetal liver cells (13), and with HS1–4 in the human β-globin gene locus in K562 cells (14). These interactions were demonstrated in cells producing significant levels of globin gene transcripts and were limited to only part of the globin gene locus. Here, we examined the recruitment of p45 NF-E2, MafK, and small Maf proteins to the upstream hypersensitive sites, intergenic region, and gene promoters in both induced and uninduced MEL cells.

Our ChIP experiments revealed that small Maf family members are bound to the LCR in uninduced MEL cells. The anti-small Maf antiserum used in these experiments recognizes the two small Mafs expressed in MEL cells, MafG and MafK.

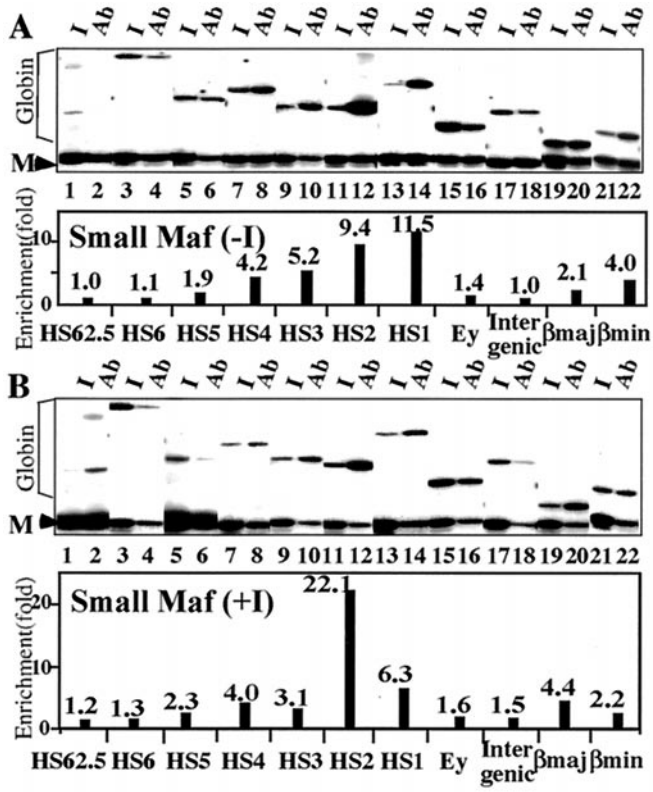

Fig. 4. Small Maf occupancy of the β-globin gene locus. Anti-small Maf antiserum, which recognizes both MafG and MafK, was used for ChIP analyses. Duplex PCR was performed by using the cross-linked chromatin from uninduced (*A*) and induced 745A MEL cells (*B*). ChIP and PCR reactions were repeated at least twice to verify results. Quantification of duplex PCR results is shown. The ratio of products obtained with the β-globin gene locus (Globin) and myoD1 gene (M) primers was measured as described in Fig. 2.

Because the anti-MafK-specific antibody detected 10-fold less binding of MafK to the globin gene locus in uninduced cells compared with induced cells, we suggest that in uninduced cells MafG is the predominant Maf binding to the β-globin gene locus. Whether or not MafG binding also occurs after induction remains to be determined.

Similar to MafK, p45 NF-E2 enrichment at the LCR in uninduced MEL cells is 10 times less than that in induced MEL cells. After induction, high-affinity binding of p45 NF-E2 and MafK is observed at HS1–4 (Fig. 3 *B* and *D*). Thus, we conclude that although the LCR MAREs are occupied before and after MEL cell induction (38), different protein complexes occupy these sites in the two cellular states. In the uniduced state, such complexes could include small Maf homodimers, as well as MafG/MafK heterodimers or small Maf/Bach1 heterodimers (41); in the induced state, the predominant complex is NF-E2 (Fig. 5).

The low level NF-E2 complex enrichment detected at the LCR in uninduced MEL cells could be due to stable occupancy in a small subpopulation of cells that differentiate spontaneously or to uniform, but transient NF-E2 binding in uninduced MEL cells. However, in either case, the induction-associated increase in NF-E2 complex binding to the β-globin gene locus correlates with a greater than 100-fold increase in β-major globin transcription.

We also observed NF-E2 interaction with promoter regions in which no MAREs are present. Thus, the NF-E2 interaction with the gene promoters may be mediated by protein–protein interaction, for example through CBP (16, 17) or $TAF_{II}130$ (15). There are at least two mechanistic possibilities for such interactions (Fig. 5). First, NF-E2 bound to the LCR might be directly recruited to the active gene promoter by means of either looping (42, 43) or linking (44) of DNA sequences. Another possibility is that NF-E2 might interact with both the LCR and gene promoters independently. In this case, two different NF-E2-containing complexes, one at the LCR and one at the promoter, would exist. These possibilities can be tested by determining the status of NF-E2 recruitment to the promoters in β-globin gene loci from which the LCR has been deleted.

Activation of β-Globin Gene Transcription. Significant levels of p45 NF-E2 and MafK transcripts are present in uninduced 745A MEL cells (22), raising the question of why NF-E2 is not efficiently recruited to the globin gene locus before differentiation. Recently, we observed that, in uninduced 745A MEL cells,

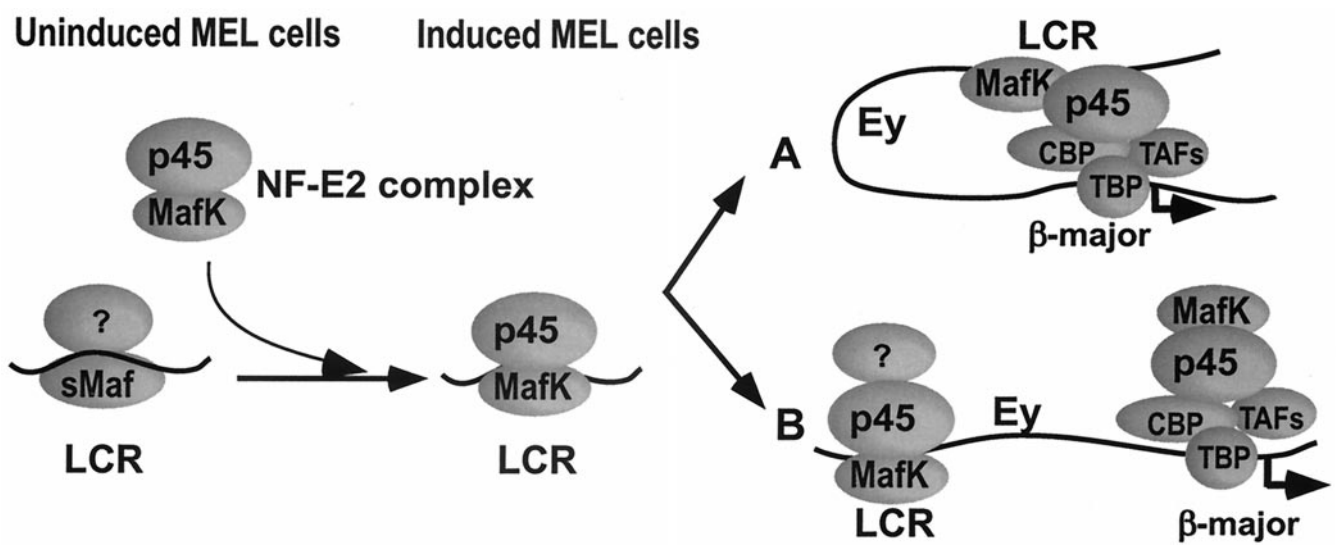

Fig. 5. Models of NF-E2 recruitment to the β-globin gene locus during terminal differentiation. NF-E2 is recruited to the β-globin gene locus on MEL cell induction. (*A*) NF-E2 at the LCR may communicate with basic transcription machinery through protein–protein interaction with coactivators or TAFs. (*B*) NF-E2 may associate with two independent protein complexes that bind to the LCR and the active promoters.

MafK and p45 NF-E2 are located in different nuclear compartments: MafK is located in heterochromatin in both mitotic and interphase nuclei, whereas p45 NF-E2 is located in euchromatin compartments. On MEL cell induction, MafK relocates away from heterochromatin (C. Francastel, W. Magis, and M.G., unpublished data). This suggests that the sequestration of these NF-E2 subunits in different nuclear compartments in uninduced MEL cells may be a rate-limiting step in NF-E2 complex formation and binding to the β-globin gene locus.

On induction-associated recruitment of NF-E2 to the β-globin gene locus, activated transcription occurs. However, the mechanism(s) underlying NF-E2-associated activation of β-globin transcription is not clear. It is possible that NF-E2 recruitment to the globin gene locus may be essential for chromatin modifications that were not detected in our analyses and that mediate recruitment of factors important for the initiation of transcription.

Alternatively NF-E2 may act downstream of transcription initiation. Promoter-proximal pausing of RNA polymerase II is a rate-limiting step after transcription initiation, and transcriptional activators have been shown to increase elongation efficiency (45, 46). Thus, it is possible that NF-E2 or factors recruited by it may modify chromatin and/or RNA polymerase II itself into a form that is more competent for elongation. Determination of complexes bound to the promoter before and after MEL induction will be important in understanding how basal transcription of the β-globin genes is converted to activated transcription.

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