Nuclear relocation of a transactivator subunit precedes target gene activation

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Murine erythroleukemia (MEL) cells are a model system to study reorganization of the eukaryotic nucleus during terminal differentiation. Upon chemical induction, MEL cells undergo erythroid differentiation, leading to activation of globin gene expression. Both processes strongly depend on the transcriptional activator NF-E2. Before induction of differentiation, both subunits of the NF-E2 heterodimer are present, but little DNA-binding activity is detectable. Using immunofluorescence microscopy, we show that the two NF-E2 subunits occupy distinct nuclear compartments in uninduced MEL cells; the smaller subunit NF-E2p18 is found primarily in the centromeric heterochromatin compartment, whereas the larger subunit NF-E2p45 occupies the euchromatin compartment. Concomitant with the commitment period of differentiation that precedes globin gene activation, NF-E2p18, along with other transcriptional repressors, relocates to the euchromatin compartment. Thus, relocation of NF-E2 p18 may be a rate-limiting step in formation of an active NF-E2 complex. To understand the mechanisms of NF-E2 localization, we show that centromeric targeting of NF-E2p18 requires dimerization, but not with an erythroid-specific partner, and that the transactivation domain of NF-E2p45 may be necessary and sufficient to prevent its localization in centromeric heterochromatin. Finally, using fluorescence *in situ* hybridization, we show that, upon differentiation, the β-globin gene loci relocate away from heterochromatin compartments to euchromatin. This relocation correlates with both transcriptional activation of the globin locus and relocation of NF-E2p18 away from heterochromatin, suggesting that these processes are linked.

Cellular differentiation in eukaryotes is accompanied by the coordinate activation and silencing of specific subsets of genes. Most studies of tissue-specific gene regulation have focused on the identification of cell type-specific transcription factors involved in the acquisition of a specific cell fate. However, recent studies have revealed that the nuclear compartmentalization of transcription factors and regions of the genome may play an important role in regulating gene expression (reviewed in refs. 1 and 2). Changes in nuclear organization often accompany changes in cell fate, suggesting that cell type-specific nuclear organization may participate in establishing specific patterns of gene expression characteristic of terminally differentiated cells (reviewed in ref. 3).

The murine erythroleukemia (MEL) cell line provides a model system to study the molecular and nuclear events associated with terminal differentiation of erythroid cells. MEL cells are blocked at an early stage of erythroid maturation; however, they can be induced to undergo most subsequent steps of normal erythroid differentiation, including hemoglobin synthesis and cell-cycle exit, by exposure to DMSO or other chemical agents (ref. 4; reviewed in ref. 5).

NF-E2 is a member of the leucine-zipper family of transcriptional activators and recognizes the consensus sequence TGCTGA(G/C)TCA located in the regulatory sequences of a number of erythroid-specific genes, including the locus control regions (LCRs) of both α- and β-globin genes (reviewed in ref. 6). The murine NF-E2 complex was first identified in MEL cells and consists of an hematopoietic-specific subunit NF-E2p45 (7), associated with a ubiquitous small Maf-protein subunit NF-E2p18 (also known as MafK) (8). NF-E2p18, which does not contain a transactivation domain, can bind DNA as a homodimer or heterodimer with other basic leucine zipper proteins (9, 10). Consistent with its lack of transactivation domain, NF-E2p18 homodimers can act as transcriptional repressors through NF-E2 DNA-binding sites (11, 12). NF-E2p45 binds DNA as an obligate heterodimer with p18 (8) and contains a transactivation domain essential for target gene activation.

Several studies have demonstrated that the activity of the globin LCRs depends on the integrity of their NF-E2 DNA-binding sites (13–17). Moreover, globin gene expression is not detectable in an NF-E2p45-null MEL cell subline (18). However, it can be restored by overexpression of NF-E2p45, demonstrating the dependence of globin gene expression on the NF-E2 complex (19).

The levels of NF-E2p18 and NF-E2p45 proteins do not change significantly after DMSO-induced differentiation of MEL cells. However, NF-E2 DNA-binding activity and transcriptional activity increase markedly upon induction (14, 20). NF-E2p18 is expressed at significantly lower levels than NF-E2p45, and overexpression of NF-E2p18 in uninduced MEL cells leads to an increase in NF-E2 DNA binding activity, increased globin gene expression, and accelerated erythroid differentiation (20, 21). Conversely, antisense inhibition of NF-E2p18 prevents globin gene expression and erythroid differentiation (20).

By analogy to other transcription factors, the ability of NF-E2 to activate globin gene expression is likely to depend on discrete domains that mediate DNA binding and transcriptional activation. Although the basic leucine zipper domain is required for dimerization and DNA-binding (22), and the N-terminal transactivation domain of NF-E2p45 interacts directly with several coactivators and components of the transcriptional machinery (23–27), the mechanism by which NF-E2 activates globin gene expression is not yet clear. Significant levels of both NF-E2 subunits are present before induction of MEL cell differentiation; however, only low-level NF-E2 DNA-binding activity and globin gene expression are detected in uninduced cells. Thus, the NF-E2-mediated activation of globin gene expression in induced cells must be mediated by posttranslational modification and/or protein–protein interactions that do not occur in uninduced cells.

Here, we show that, upon commitment of MEL cells to terminal differentiation, NF-E2p18 relocates from heterochromatic nuclear compartments to euchromatic compartments in which NF-E2p45 is located. Thus, NF-E2 activity may be regu-

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Abbreviations: LCR, locus control region; MEL, murine erythroleukemia; DAPI, 4′,6-diamidino-2-phenylindole.

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lated by the nuclear compartmentalization of its subunits. We also show that, like NF-E2p18, the β-globin loci relocate from heterochromatic nuclear compartments after induction of MEL cell differentiation, suggesting that these processes may be linked.

Materials and Methods

Cell Culture. MEL cells, strain 745A, were maintained in culture and induced to differentiate in 2% DMSO as described (28). Synchronization of MEL cells was achieved by flow cytometric sorting of live cells in the G_1 phase of the cell cycle, after vital staining of DNA with Hoechst 33342 (29). After sorting, cells were cultured in the presence or absence of DMSO for 4, 8, 12, or 20 h and subjected to immunostaining as described below. In experiments using inhibitors of differentiation, dexamethasone was added at a final concentration of 10 μM and imidazole at a concentration of 7.3 mM.

Constructs and Transient Transfections. Wild-type or mutant cDNAs, as described in the text, were amplified by PCR. NF-E2p18 cDNA (28) was cloned into the mammalian expression vector pCS2-MT in-frame with six Myc epitope tags in the N terminus (30) and NF-E2p45 cDNA (28) into the pME18 expression vector in-frame with one Flag epitope in the N terminus (31). MEL cells were electroporated as described (28); 2–3 days after transfection, tagged proteins were detected by immunofluorescence as described below.

Immunofluorescence. MEL cells were attached to coated slides (VWR Scientific, superfrost plus), rinsed briefly in PBS, and incubated with 4% paraformaldehyde in 20 mM KH_2PO_4, 130 mM NaCl, 20 mM KCl, 10 mM EGTA, 2 mM $MgCl_2$, and 0.1% Triton-X100 for 20 min at room temperature (RT). After fixation/permeabilization, slides were rinsed three times in PBS, incubated 10 min at RT in PBS/100 mM glycine, and blocked for 1 h in PBS containing 5–10% donkey normal serum (Jackson ImmunoResearch). For immunostainings, the following antibodies were used: rabbit polyclonal anti-NF-E2p18 (C-16; Santa Cruz Biotechnology); rabbit polyclonal anti-NF-E2p45 (C-19; Santa Cruz Biotechnology); rat monoclonal anti-GATA-1 (N6; Santa Cruz Biotechnology); mouse monoclonal anti-polymerase II (H14; Babco , Richmond, CA); goat polyclonal anti-HDAC1 (Santa Cruz Biotechnology); rabbit polyclonal anti-MeCP2 (Upstate Biotechnology, Lake Placid, NY); tetramethylrhodamine B isothiocyanate-conjugated mouse monoclonal anti-Flag (M2, Sigma); FITC-conjugated mouse monoclonal anti-Myc epitope (9E10; Santa Cruz Biotechnology). Fixed cells were incubated overnight at 4°C with primary antibody diluted in PBS/donkey serum at 1/200 for anti-NF-E2p18, NF-E2p45, and GATA-1 antibodies, 1/1,000 for the anti-HDAC1, Flag, and Myc-Tag antibodies, and 1/500 for the anti-MeCP2 antibody. Slides then were washed three times in PBS and incubated in PBS/serum containing directly conjugated secondary antibodies (1/500). Secondary antibodies were FITC-conjugated donkey anti-rabbit IgG, Texas red-conjugated donkey anti-rat IgG, Texas red-conjugated donkey anti-mouse IgM (Jackson ImmunoResearch). After labeling, slides were washed three times in PBS and mounted in antifade media (Vectashield, Vector laboratories) containing 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). At least 40 (range of 40 to 200) cells were examined in each experiment except when two plasmids were transfected, in which case at least 20 cells were examined. Representative results are shown in the figures.

Fluorescence *in Situ* Hybridization. Fluorescence *in situ* hybridization was performed as described (32), using a 15-kb probe encompassing the β-globin genes. Scoring of loci associated or dissociated from dense foci of DAPI was performed as described by Brown *et al.* (33).

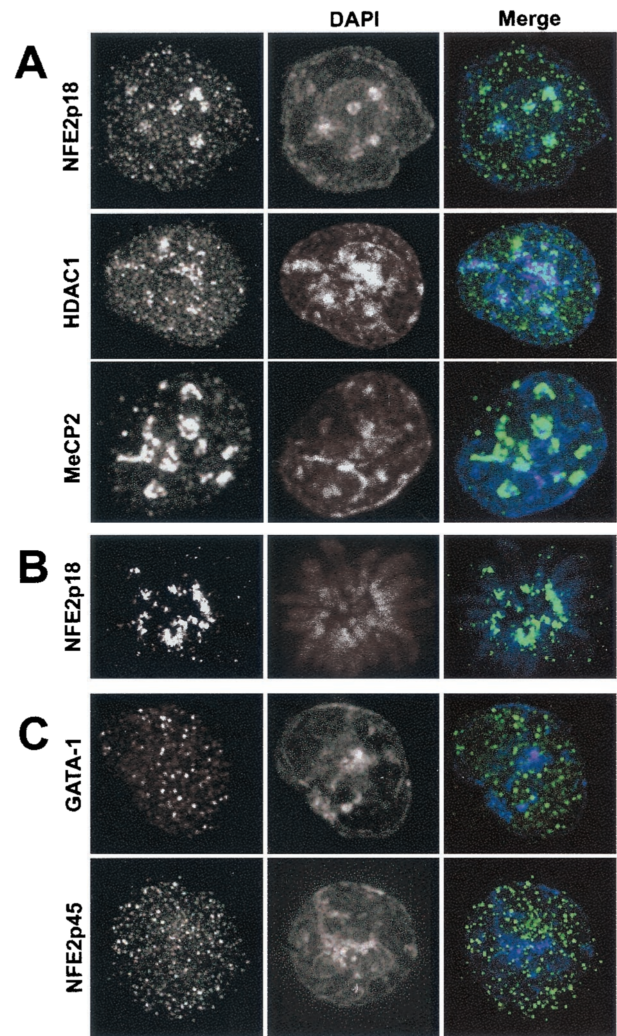

Fig. 1. Targeting of NF-E2p18 but not NF-E2p45 to centromeric heterochromatin. Cells were stained with antibodies against proteins indicated on the left followed by FITC-conjugated secondary antibodies (*Left*) and DAPI (*Center*). (*Right*) The merge of both images. (*A* and *C*) Staining of undifferentiated MEL interphase cells. (*B*) Example of metaphase chromosomes from MEL cells. (Magnifications: ×100.)

Results

NF-E2 p45 and NF-E2p18 Occupy Distinct Compartments in the Nucleus of Undifferentiated MEL Cells. We used immunofluorescence microscopy to examine the localization of NF-E2p45 and NF-E2p18 in uninduced MEL cell nuclei. Staining with an NF-E2p18-specific antibody revealed overlapping fluorescence with regions of dense staining DAPI, which interacts strongly with murine γ-satellite DNA, a component of centromeric heterochromatin (Fig. 1*A Top*). In addition, NF-E2p18 staining is observed in the pericentromeric regions and the nuclear periphery, which is also enriched in heterochromatin proteins, and a subset of NF-E2p18 is located in nonheterochromatic compartments. Thus, before MEL cell differentiation, the majority of NF-E2p18 associates with heterochromatic compartments. In metaphase chromosomes, NF-E2p18 is also targeted to pericentromeric regions (Fig. 1*B*). These results demonstrate that NF-E2p18 associates with the centromeric compartment throughout the cell cycle and during mitosis. In clear contrast,

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the erythroid-specific subunit NF-E2p45 is concentrated in distinct foci dispersed throughout the nucleus of undifferentiated MEL cells and excluded from centromeric heterochromatin (Fig. 1*C Bottom*). Thus, although a small amount of the two subunits of the NF-E2 complex may colocalize in uninduced MEL cells, the majority of NF-E2p18 and NF-E2p45 occupy distinct nuclear compartments before MEL cell differentiation.

Because *in vitro* experiments suggested that, in the absence of its transactivation partners, NF-E2p18 might function as a repressor (10), we investigated whether other general transcriptional repressors occupy the same nuclear compartment as NF-E2p18 in undifferentiated MEL cells. Interestingly, HDAC1 and MeCP2 also associate with pericentromeric heterochromatin (MeCP2 almost exclusively) in the undifferentiated cells (Fig. 1*A*). In contrast, the erythroid-specific positive regulator GATA-1 shows a punctate pattern similar to that of NF-E2p45 (Fig. 1*C*). These results suggest that the nucleus of undifferentiated MEL cells may be divided into distinct compartments enriched for complexes containing either transcriptional repressors or activators.

Nuclear Relocation of NF-E2p18 upon Commitment of MEL Cells to Terminal Differentiation. The results described above suggest that the relocation of the small subunit of the NF-E2 complex away from centromeric heterochromatin may be a rate-limiting step in formation of the NF-E2p18/p45 complex. Thus, we investigated the subnuclear location of NF-E2p18 and NF-E2p45 after induction of MEL cell differentiation.

In terminally differentiated MEL cells, NF-E2p18 adopts a punctate nuclear pattern, excluded from centromeric heterochromatin and similar to the pattern observed with the NF-E2p45 antibody in uninduced cells (Fig. 2*A*, compare with Fig. 1*C*). Moreover, HDAC1 and MeCP2 also relocate away from centromeres upon erythroid differentiation (compare Figs. 1*A* and 2*A*).

The relocation of NF-E2p18 from centromeric to noncentromeric nuclear compartments could be a consequence of terminal erythroid differentiation. Alternatively, this relocation may be an early event associated with the commitment to differentiation. To investigate these possibilities, we analyzed the nuclear location of NF-E2p18 in synchronized MEL cells at different times after DMSO addition and in the presence and absence of inhibitors of erythroid differentiation.

Relocation of NF-E2p18 was observed as soon as after 8–12 h of DMSO treatment. By 20 h, which is before globin gene activation (ref. 34 and references therein), NF-E2p18 is excluded from centromeres in most cells. Treatment of cells with DMSO and dexamethasone, an inhibitor of the DMS0-induced commitment to erythroid differentiation, prevents the relocation of NF-E2p18 away from centromeric heterochromatin. In contrast, in cells treated with DMSO and imidazole, which inhibits aspects of erythroid differentiation including the DMSO-induced increase in globin mRNA level, but permits the DMSO-induced commitment to differentiation (35), NF-E2p18 is relocated away from centromeric heterochromatin within 20 h (Fig. 2*B*). Together, these experiments suggest that the dissociation of NF-E2p18 from centromeric heterochromatin occurs when MEL cells are irreversibly committed to erythroid differentiation, but before globin gene expression is activated.

As the anti-NF-E2p18 and p45 antibodies both were generated in rabbits, it is technically difficult to use them simultaneously to ascertain whether NF-E2p18 and p45 colocalize in subnuclear compartments in differentiated MEL cells. As another approach to determine the localization of the NF-E2 subunits, we transiently expressed Flag-tagged NF-E2p45 and Myc-tagged NF-E2p18 proteins in MEL cells (Fig. 3). In uninduced cells, the tagged NF-E2p18 is efficiently targeted to centromeric foci whereas the tagged NF-E2p45 is located away from centromeres.

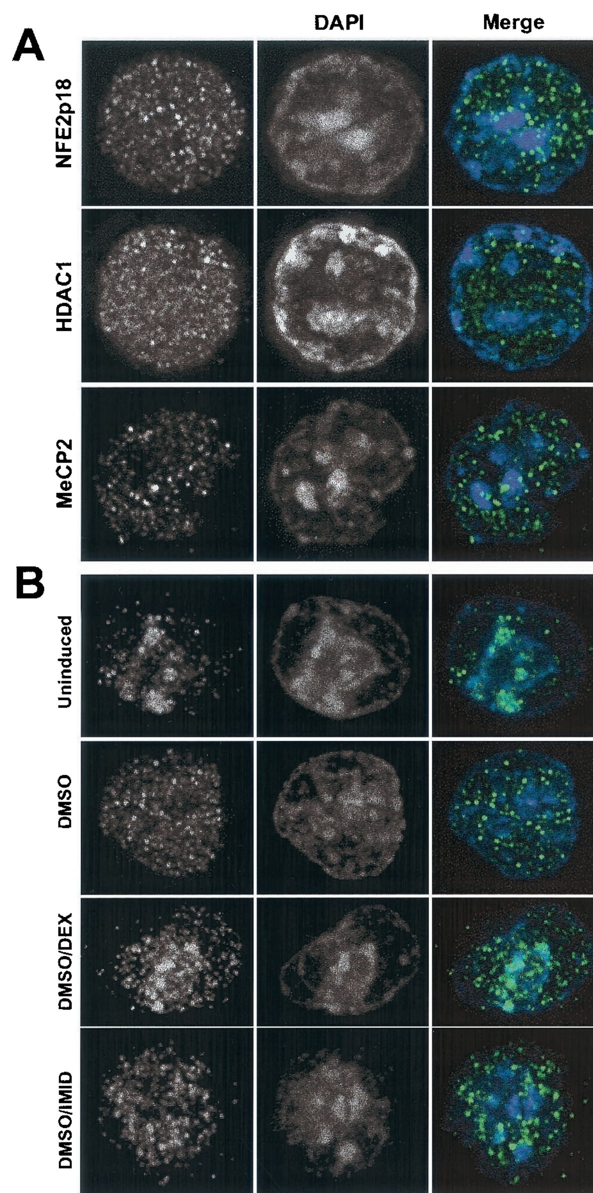

Fig. 2. Relocation of NF-E2p18 after MEL cell commitment to differentiation. (*A*) Differentiated MEL cells after 4 days of DMSO treatment. Cells were stained as in Fig. 1. (*B*) Staining with antibodies against NF-E2p18 of uninduced MEL cells or DMSO-treated, alone or in association with inhibitors of erythroid differentiation, for 12 h. DEX, Dexamethasone; IMID, imidazole. (Magnifications: ×100.)

However, upon DMSO treatment for 24 h, NF-E2p18 relocates away from centromeres, and consequently a significant overlap of the two subunits is observed only after differentiation.

Centromeric Targeting of NF-E2p18 Requires Dimerization But Not with an Erythroid-Specific Partner. Because NF-E2p18 (MafK) is expressed in many cell types, we investigated whether the pericentromeric location of NF-E2p18 observed in uninduced erythroid cells also occurred in other murine cell lines. Immunofluorescence microscopy revealed that NF-E2p18 localizes to pericentromeric heterochromatin in the nuclei of NIH/3T3 murine fibroblasts (Fig. 4*A*). This finding suggests that the centromeric targeting of NF-E2p18 does not require an erythroid-specific nuclear architecture or erythroid-specific partners.

As a preliminary approach to understanding the mechanism

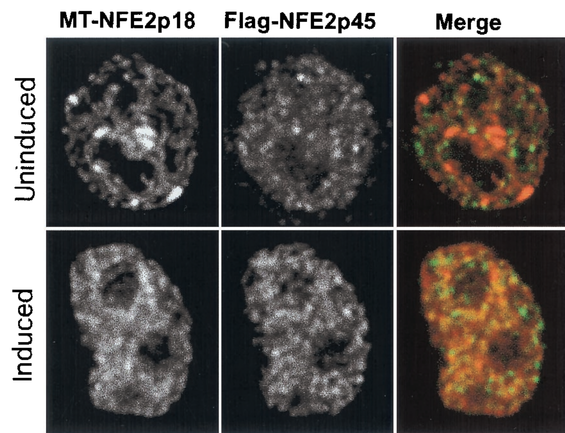

Fig. 3. Transcriptional activators and repressors occupy distinct compartments in the nucleus of MEL cells. MEL cells were transiently transfected with expression vectors encoding tagged versions of the wild-type NF-E2 subunits, p18 (red) and p45 (green). The yellow color results from the overlap between red and green fluorescence. Overlap between the two subunits increases with treatment of the cells with DMSO for 24 h. (Magnifications: ×100.)

by which NF-E2p18 is targeted to centromeric heterochromatin, we transiently expressed mutant NF-E2p18 proteins in both MEL cells (Fig. 4*B*) and NIH/3T3 cells (data not shown) and analyzed their nuclear localization. Although the full-length NF-E2p18 is efficiently targeted to centromeric foci, deletion of the leucine-zipper motif results in the delocalization of NF-E2p18 away from centromeres. This result suggests that homodimerization or heterodimerization with another member of the basic leucine zipper family is required for the centromeric targeting of NF-E2p18. However, as dimer formation is a prerequisite for NF-E2p18 DNA-binding activity, we cannot exclude the possibility that the failure of the deletion mutant to localize to centromeres results from its inability to bind DNA.

The NF-E2p45 Transactivation Domain Is Necessary and Sufficient for Localization Away from Centromeric Heterochromatin. The transactivation domain of NF-E2p45 is required for the activation of erythroid-specific gene expression. To further investigate the relationship between transcriptional activation and nuclear location, we examined whether the NF-E2p45 transactivation domain is required for location of NF-E2p45 to discrete nuclear foci. We transiently transfected MEL cells with Flag-tagged full-length NF-E2p45 or a Flag-tagged mutants (NFE2p45 ΔTA) containing deletions of the first 80 (ΔN80) or 206 (ΔN206) amino acids of the transactivation domain. The wild-type Flag-NF-E2p45 protein exhibits a punctate pattern similar to that revealed by the anti-NF-E2p45 antibody against the native protein, whereas the NFE2p45 ΔTA mutants are not found in discrete foci, but are distributed more diffusely throughout the nucleus (Fig. 4*C*). Interestingly, and in contrast to the full-length protein, the NFE2p45 ΔTA mutants are not excluded from centromeres. When coexpressed with NF-E2p18, the majority of NF-E2p45ΔTA is now targeted to centromeric heterochromatin (Fig. 5), whereas when coexpressed with the dimerization-deficient NF-E2p18, both show a diffuse pattern away from centromeric heterochromatin. These results suggest that NF-E2p45 lacking a transactivation domain can be targeted to centromeric heterochromatin through its dimerization with NF-E2p18.

To determine whether the transactivation domain of NF-E2p45 is sufficient to exclude centromeric localization, we fused the first 80 aa of this domain to the N terminus of NF-E2p18, in-frame with both the Myc tag and NF-E2p18. As shown in Fig.

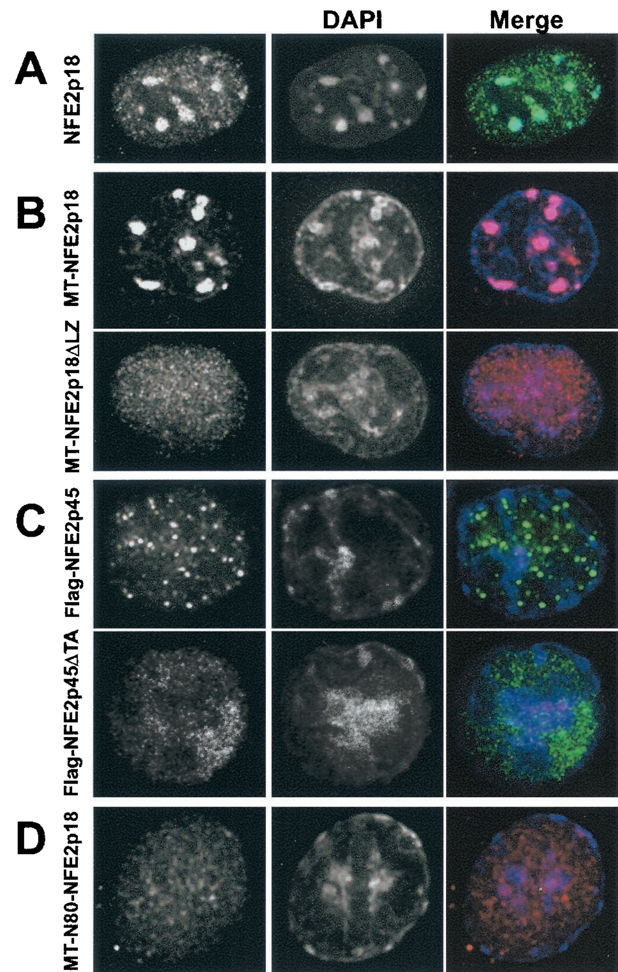

Fig. 4. Identification of protein domains involved in targeting of NF-E2. (*A*) Staining of NIH/3T3 cells with antibodies against NF-E2p18. (*B*) Expression of a Myc-tagged NF-E2p18; full-length protein (*Upper*) and deletion of the C-terminal dimerization domain (NF-E2p18DLZ) (*Lower*). (*C*) Expression of a Flag-tagged NF-E2p45; full-length protein (*Upper*) and deletion of the N-terminal transactivation domain (*Lower*). (*D*) Localization of NF-E2p18 containing 80 aa of the NF-E2p45 transactivation domain at its N terminus. (Magnifications: ×100.)

4*D*, addition of this part of the NF-E2p45 transactivation domain results in exclusion NF-E2p18 from centromeric heterochromatin.

Together, these results suggest that the NF-E2p45 transactivation domain, presumably through interaction with other activators, may prevent association of NF-E2p45 with centromeric heterochromatin, or alternatively, recruit NF-E2p45 to discrete foci in "euchromatin."

The β-Globin Loci Are Relocalized After MEL Cell Differentiation. We recently reported that NF-E2p18 is bound to the LCR of the β-globin locus before and after induction of differentiation (36). Here, we show that NF-E2p18 relocates away from centromeric heterochromatin upon induction of erythroid differentiation. Thus, we investigated the nuclear location of the globin loci during differentiation. Using the same fixation conditions as in the immunofluorescence studies, we performed fluorescence *in situ* hybridization analyses to determine the position of the globin loci relative to centromeric heterochromatin. Scoring of "associated" and "nonassociated" loci was as described by Brown *et al.* (33). As shown in Fig. 6, before induction of differentiation, one allele of the globin locus is associated with the heterochro-

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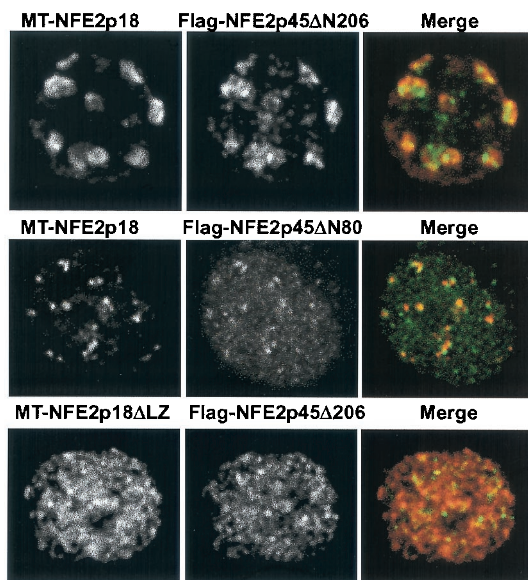


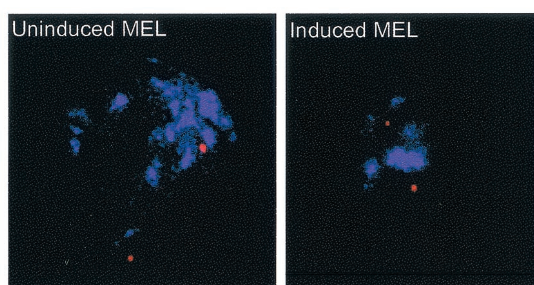
Fig. 5. Centromeric targeting of transactivation domain-deficient NF-E2p45 mutants Myc-tagged NF-E2p18, full length or mutant (NF-E2p18DLZ), was cotransfected with deletion mutants of NF-E2p45 lacking the first 80 (NF-E2p45DN80) or 206 (NF-E2p45DN206) N-terminal amino acids of the transactivation domain. (Magnifications: ×100.)

matin-enriched nuclear periphery, whereas the other allele is associated with a centromeric heterochromatin. Alternatively, both alleles are associated with centromeric heterochromatin (not shown). However, after induction of differentiation, both alleles become excluded from heterochromatin.

Thus, upon MEL cell differentiation, the globin loci relocate away from subnuclear compartments enriched in heterochromatin. This relocation correlates with both activation of transcription of the globin genes and relocation of NF-E2p18.

Discussion

The data presented here suggest that the activity of the transcription factor NF-E2, which is required for MEL cell differentiation and globin gene expression, may be regulated through the nuclear compartmentalization of its subunits. We also find that NF-E2 may be involved in positioning of the globin locus away from heterochromatin to a nuclear compartment permissive for transcription. These results complement our previous observation that the NF-E2 site in an erythroid-specific enhancer is essential for the maintenance of expression and nuclear location of a transgene away from heterochromatin in erythroid cells (32).



	2 associated	1 associated 1 periphery	2 non-associated
Uninduced MEL (n=50)	16	28	6
Induced MEL (n=42)	2	4	36

Fig. 6. Relocation of the globin loci upon induction of differentiation. (*Upper*) Examples of fluorescence *in situ* hybridization detection of the β-globin loci in interphase MEL cells. The β-globin loci are detected with a Texas red-conjugated probe (red); murine centromeres are revealed by their bright DAPI staining (blue). (Magnifications: ×100.) (*Lower*) Before induction of differentiation, the β-globin loci preferentially associate with centromeric heterochromatin or the nuclear periphery. Upon induction of differentiation, both loci are found dissociated from these heterochromatin compartments.

Nuclear Compartmentalization of NF-E2 Subunits. Using indirect fluorescence and transient expression of tagged proteins, we have shown that, before induction of erythroid differentiation, NF-E2p18 and p45 occupy largely separate nuclear compartments. Whereas a significant portion of NF-E2p18 protein is associated with pericentromeric heterochromatin, both during interphase and mitosis, NF-E2p45 is found in discrete domains scattered throughout the nucleus, but excluded from heterochromatin. Although low levels of NF-E2p18/p45 complex may form in the euchromatic compartments of uninduced cells, we propose that the sequestration of these subunits in distinct nuclear compartments may be a rate-limiting step in the formation of active complex. Previous reports have described cytoplasmic sequestration as a mechanism to prevent the activation of transcription factors. However, this study demonstrates inactivation of a transcription factor by sequestration of its subunits in distinct nuclear compartments.

Upon the induction of MEL cell differentiation, NF-E2p18, together with the two transcriptional repressors studied, MeCP2 and HDAC1, are located away from centromeric heterochromatin. The consequences of this redistribution are at least 2-fold. First, NF-E2p18 and p45 may colocalize in the same nuclear compartment. Because of the nature of the existing NF-E2p18 and p45 antibodies, we have not been able to demonstrate colocalization of these subunits by indirect fluorescence. However, the tagged versions of these proteins overlap significantly in the euchromatic compartment 24 h after induction of transiently transfected MEL cells. Second, the increased concentration of transcriptional repressors in the euchromatin compartment correlates with the increased silencing of nonerythroid genes that accompanies cellular differentiation. The composition of facultative heterochromatin that forms during cellular differentiation is not well characterized (reviewed in ref. 3). However, we propose that these delocalized transcriptional repressors, HDAC1 and MeCP2, may help form facultative heterochromatin and establish silent compartments outside of the constitutive centromeric heterochromatin.

The biological significance of centromeric targeting of NF-E2p18 is not clear. The movement of NF-E2p18 away from centromeres occurs within the first cell cycle after induction. Thus, centromeres may represent storage sites from which NF-E2p18 can be released rapidly to form an active complex with NF-E2p45. Alternatively, NF-E2p18 may be involved in recruiting transcriptional silencers, such as HDAC1 and MeCP2, to centromeric heterochromatin, thereby mediating the silencing of MARE-containing target genes.

Localization of Transcription Factors to Centromeres. Pericentromeric localization and associated gene silencing activity have been reported previously for a number of factors. For example, Ikaros, a transcriptional regulator required for normal lymphocyte development, may act as a transcriptional repressor when associated with Mi-2 and HDAC in centromeric heterochromatin (37). Krüppel-associated box-containing zinc finger proteins (KRAB-ZFPs) repress transcription via interaction with the corepressor KRAB-associated protein-1 (KRAB-1), and both interact with foci of heterochromatin containing HP1 (38). Targeting of KAP-1 to centromeric heterochromatin may be a

consequence of its direct interaction with the heterochromatin protein HP1. The targeting of Ikaros (39) and NF-E2p18 (this study) to foci of centromeric heterochromatin is abolished by deletion of the carboxyl-terminal domains required for DNA binding and dimerization. Thus, and in apparent contrast to KAP-1, centromeric targeting of Ikaros and NF-E2p18 may be mediated through direct DNA binding and/or the formation of homodimers or heterodimers.

The efficient pericentromeric targeting of overexpressed Ikaros or NF-E2p18 suggests that targeting to this compartment is an intrinsic property of these proteins and represents the default site where they localize in the nucleus. Thus, the relocation of NF-E2p18 to the euchromatic compartment upon the terminal differentiation of erythroid cells, as well as the location of Ikaros in euchromatin in resting B cells, may require post-transcriptional modifications or protein–protein interactions. In this regard, we have demonstrated that the transactivation domain of NF-E2p45 is both necessary to maintain NF-E2p45 away from centromeric heterochromatin and sufficient to target NF-E2p18 away from centromeric heterochromatin. The 80 N-terminal amino acids of this transactivation domain are also necessary and sufficient for interaction with TAFII130 (23), suggesting that this domain may prevent centromeric targeting via interaction with coactivators.

Silencing and Activation of Globin Gene Expression. Gene silencing in mammalian cells may be mediated by positioning of a gene in proximity to the repressive heterochromatic compartment in interphase nuclei (32, 33, 40, 41), and it has been proposed that certain factors may mediate gene silencing by recruiting target genes to centromeric heterochromatin compartments (32). Recently, we reported that the low basal expression of the globin locus in undifferentiated cells correlates with occupancy of the LCR by NF-E2p18 and the related factor MafG (36). We now show that it also correlates with positioning of NF-E2p18 (and MafG, not shown) to foci of centromeric heterochromatin. Thus, we propose that NF-E2p18 and related small Maf family members, when targeted to centromeric heterochromatin, may prevent activation of β-globin gene transcription.

Interestingly, LCR DNaseI hypersensitivity and general histone H3 acetylation of the β-globin loci are indistinguishable in uninduced and differentiated MEL cells (36, 42). In addition, basal transcription of the adult β-globin genes occurs in undifferentiated cells (36). Thus, as reported previously (32), proximity to heterochromatin compartments *per se* is not sufficient to suppress these aspects of chromatin structure or basal transcription. Upon the induction of differentiation, NF-E2 DNA-binding activity becomes detectable, and NF-E2p45 occupancy of the globin locus increases more than 30-fold (36). At the same time, the globin loci and NF-E2p18 relocate away from heterochromatin compartments and adult β-globin transcription increases $\approx$150-fold (36). Additional studies will be required to determine the ordering of these events and the mechanisms underlying them.

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