Histone H3 methylated at lysine 4 (H3-meK4) co-localizes with hyperacetylated histones H3 and H4 in transcriptionally active chromatin, but mechanisms that establish H3-meK4 are poorly understood. Previously, we showed that the hematopoietic-specific activator NF-E2, which is required for β-globin transcription in erythroleukemia cells, induces histone H3 hyperacetylation and H3-meK4 at the adult β-globin genes (βmajor and βminor). Chromatin immunoprecipitation analysis indicated that NF-E2 occupies hypersensitive site two (HS2) of the β-globin locus control region. The mechanism of NF-E2-mediated chromatin modification was investigated by complementation analysis in NF-E2-null CB3 erythroleukemia cells. The activation domain of the hematopoietic-specific subunit of NF-E2 (p45/NF-E2) contains two WW domain-binding motifs (PXY-1 and PXY-2). PXY-1 is required for activation of β-globin transcription. Here, we determined which step in NF-E2-dependent transactivation is PXY-1-dependent. A p45/NF-E2 mutant lacking 42 amino acids of the activation domain, including both PXY motifs, and a mutant lacking only PXY-1 were impaired in inducing histone H3 hyperacetylation, H3-meK4, and RNA polymerase II recruitment. The PXY motifs were not required for transactivation in the context of a GAL4 DNA-binding domain fusion to p45/NF-E2 in transient transfection assays. As the PXY-1 mutant occupied HS2 normally, the chromatin modification defect occurred post-DNA binding. PXY-1 was not required for recruitment of the histone acetyltransferases p300 and p300 to HS2. These results indicate that PXY-1 confers chromatin-specific transcriptional activation via interaction with a co-regulator distinct from CBP/p300 or by regulating CBP/p300 function.

Mechanisms that dynamically regulate chromatin structure at localized sites and over broad chromosomal regions are crucial for controlling nuclear processes such as transcription. A common mode of regulating chromatin structure is the post-translational modification of core histones, of which histone acetylation is the most extensively studied (1, 2). Hyperacetylated histones are often enriched in transcriptionally active chromatin (3, 4), although reductions in acetylation can occur upon transcriptional activation (5). Acetylation impacts transcription in part by increasing DNA accessibility within the nucleosome (6) and by perturbation of higher order chromatin folding (7, 8).

Analogous to acetylation, methylation of lysine 4 of histone H3 (H3-meK4) marks transcriptionally active chromatin (9–13). By contrast, histone H3 methylated at lysine 9 is associated with transcriptional repression (14–16). At least one mechanism by which H3-meK4 functions is by inhibiting binding of the nucleosome remodelling deacetylase repressor complex to the amino-terminal tail of histone H3, thereby favoring the transcriptionally active state (17). Acetylated and methylated lysines within histones can also function as ligands to bind bromodomain- and chromodomains-containing co-regulators, respectively (18–20).

ChIP analysis enables the measurement of histone modification patterns in living cells (2, 21). Histone modification patterns of the chicken (9, 22) and murine (23, 24) β-globin loci containing the erythroid-specific and developmentally regulated β-globin genes have been defined, and progress has been made in identifying factors/signals that establish and maintain these patterns (23, 24). Activation of β-globin transcription is mediated by a LCR, which consists of four erythroid-specific DNase I-hypersensitive sites (HS 1–4) and a constitutive site (HS5) 10–50 kb upstream of the β-globin genes (see Fig. 1A) (25). Two additional HS6, HS6 and HS7, are located upstream of HS5 (27). Each HS contains multiple ubiquitous and erythroid-specific transcription factor-binding sites. HS2 contains tandem Maf response elements (28), which mediate binding by the hematopoietic-specific transcription factor NF-E2 (29–33). Mutation of the Maf response elements abrogates HS2 enhancer activity in transfection assays and in transgenic mice (28, 34–37), whereas mutations of other binding sites partially inhibit enhancer activity (36, 38, 39).

NF-E2 is a heterodimeric activator consisting of a hematopoietic-specific basic leucine zipper subunit, p45/NF-E2 (29, 31), and a broadly expressed subunit, p18, a member of the small Maf family of basic leucine zipper proteins (30). Targeted disruption of the p45/NF-E2 gene in mice revealed that NF-E2 is required for megakaryopoiesis (40), whereas studies in p45/NF-E2-null CB3 erythroleukemia cells revealed an important

The abbreviations used are: H3-meK4, histone H3 methylated at lysine 4; βmajor Pro, βmajor promoter; βmaj 3’, βmajor exon 3; CBP, cAMP-responsive element-binding protein-binding protein; ChIP, chromatin immunoprecipitation; HS, DNase-hypersensitive site; LCR, locus control region; MEL, mouse erythroleukemia cell line; NF-E2, nuclear factor erythroid 2; Pol II, RNA polymerase II; RT, reverse transcriptase.
role in activating β-globin transcription (32, 33, 41, 42). We used complementation analysis in CB3 cells to show that NF-E2 establishes a component of the histone modification pattern of the murine β-globin locus. In adult erythroid cells, the murine β-globin locus is hyperacetylated at the LCR and at the adult β-globin genes (βmajor and βminor) (11, 43, 44) (see Fig. 1). By contrast, the embryonic/fetal β-globin genes (Eγ and βF1), located centrally within the locus, reside within a ~30-kb subdomain of reduced acetylation. The patterns of histone acetylation and H3-meK4 are nearly indistinguishable (11). NF-E2 establishes H3 hyperacetylation and H3-meK4 (11, 45) at the adult β-globin genes, whereas histone modifications at the LCR are largely NF-E2-independent.

Although NF-E2-dependent transcriptional activation is associated with chromatin modification (11, 45, 46), the mechanism by which NF-E2 functions is not understood. Amino acid sequences required for transactivation activity of p45/NF-E2 are located near its amino terminus, and this region is not homologous to other proteins (47, 48). Deletion of amino acids 42–83 of murine p45/NF-E2 (M1 mutant) (see Fig. 2A) strongly inhibits p45/NF-E2-mediated activation of endogenous β-globin transcription in CB3 cells (47, 48). As p45/NF-E2 binds the transcriptional co-activators and histone acetyltransferases CBP and p300 in vitro (49, 50), CBP/p300 might mediate NF-E2-dependent histone acetylation. The activation domain of p45/NF-E2 also interacts with WW domains (48, 51), which consist of 38–40 amino acids that bind PPXY motifs (52). Numerous transcription factors have PPXY motifs within their activation domains, suggesting that this motif confers an important transcriptional function. It is unknown whether a specific WW domain target for p45/NF-E2 exists in vivo. The activation domain of murine p45/NF-E2 contains one PPXY motif (PXY-1) at amino acids 79–83 of murine p45/NF-E2 (PPPPY), which is conserved in human p45/NF-E2 (PPPPY). A second PPXY motif (PXY-2) at amino acids 61–65 (PPPTY) of murine p45/NF-E2, is incompletely conserved in human p45/NF-E2 (PPTTY). PXY-1, but not PXY-2, is required for p45/NF-E2-mediated activation of endogenous β-globin transcription in CB3 cells (48). Analysis of the specificity of p45/NF-E2-WW domain interactions revealed that p45/NF-E2 differentially binds multiple WW domains in vitro, with the highest affinity binding being to a WW domain from the ubiquitin ligase, WWP1 (48). Given the unique sequence of the p45/NF-E2 activation domain, the lack of information on how PPXY motifs function in transcription, and the crucial role of NF-E2 in controlling megakaryopoiesis, we conducted an analysis to determine which step in NF-E2-mediated transactivation is PXY-1-dependent.

EXPERIMENTAL PROCEDURES

Cell Culture and Stable Transfections—Mouse erythroleukemia (MEL) (53) and p45/NF-E2-null CB3 (41) cells were maintained in Dulbecco’s modified Eagle’s medium (Biofluids) containing 5% fetal bovine serum, 5% calf serum, and 1% antibiotic/antimycotic (all components from Invitrogen). The human erythroleukemia cell line K562 (54) was maintained in Iscove’s modified Eagle’s medium (Biofluids) containing 10% fetal bovine serum and 1% antibiotic/antimycotic. Expression vectors encoding wild-type p45/NF-E2, the M1 deletion mutant (Δ42–83), and the PXY-1 alanine substitution mutant (AA substituted for PTY) were described previously (47, 48). Stably transfected clones of CB3 cells expressing wild-type p45/NF-E2 and the M1 and PXY-1 mutants were selected and maintained in 1 mg/ml G418 Sulfate (Calbiochem).

Quantitative ChIP Assay—ChIP analysis was performed as described (23, 45) except that the analysis of CBP binding used 1% formaldehyde for cross-linking and 1% (v/v) anti-CPB antibody. The cells were incubated for 4 days with 1.5% Me2SO (Sigma) to induce erythroid maturation. Immunoprecipitated DNA was analyzed by real time PCR (Applied Biosystems Prism 7000). The primers were designed by Primer Express 1.0 software (PE Applied Biosystems) to amplify 50–150-bp subregions within the endpoints of the predefined standard PCR primers (43). Primers were based on Hbbβ haplotype sequences (GenBank accession numbers Z13985, X14061, AF128269, and AF133300). Samples from at least three independent immunoprecipitations were analyzed. Primer pairs generated single products based upon dissociation curves post-amplification and agarose gel analysis of PCR products. The product was measured by SYBR Green fluorescence in 25-µl reactions. The amount of product was determined relative to a standard curve generated from serial dilutions of input DNA samples for each primer set/sample set combination. Values for the unknowns were determined relative to these standard curves.

Forward and Reverse Primers for Quantitative ChIP Assay—(5′–3′): HbA59AGCATTCTCAGTTCTCAGCGCTTGTA; βmajor (βmajor Pro), CAGGGGAAAATATGCTTGTCATCA and GTGACGAGATTTGCCCTTTCC; βmajor exon 3 (βmajor 3′), GCCCTGCTCGAATGTTACCA and TTTCAAGGGAAAGGAGAAGA; interventing region 16, TGGCCATTTTACTAGTATATTCG and TAGACTTCTGATTTGATAGGTOG; and RPII215 promoter (RPII215), GGGACTCTATAGAGGGATCTAGCT and TCGGCCCTCTTCGAGAAG.

Antibodies—Anti-pol-I (n-20, sc-899) was obtained from Santa Cruz Biotechnology. Anti-diacylated histone H3 (H3-599), anti-triacetylated H4 (H4-866), and anti-H3-meK4 (07-030) antibodies were obtained from Upstate Biotechnology, Inc. The anti-p45/NF-E2 polyclonal antibody generated against purified recombinant hexahistidine-tagged p45/NF-E2 was described previously (48). The anti-CBP polyclonal antibody (CBP-3) was generated in rabbits against a fragment of murine CBP (amino acids 737–1626) fused to glutathione S-transferase, which was purified by adsorption to glutathione-Sepharose resin. The p52KI-mCBP3 plasmid used to express GST-CBP(737–1626) was a kind gift from Dr. Marc Montminy (Salk Institute). Similar results were obtained in two experiments with another anti-CBP antibody (SC-369; Santa Cruz Biotechnology), which does not cross-react with the homologous protein p300. Preimmune serum was used as a control for ChIP.

RNA Isolation and RT-PCR—Total RNA was prepared with Trizol (Invitrogen) from the same cell cultures used for ChIP. cDNA was prepared by reverse transcribing RNA (1 µg) with 250 ng of a 5.3 mixture of random and oligo(dT) primers. This was followed by incubation with reverse transcriptase (Superscript II; Invitrogen), 10 mU dithiothreitol, RNasin (Promega), and 0.5 µM dNTPs at 42 °C for 1 h. The reaction mixture was diluted to a final volume of 150 µl and heat-inactivated at 95–99 °C for 5 min. RT-PCR reactions (25 µl) contained 2.5 µl of cDNA, 12.5 µl of SYBR Green (Applied Biosystems), and the appropriate primers. Product accumulation was monitored by SYBR Green fluorescence. Dissociation curves post-amplification and agarose gel analysis of PCR products showed that primer pairs generated single products. Relative expression levels were determined from a standard curve of serial dilutions of MEL cDNA samples. Forward and reverse primers for real-time RT-PCR (5′–3′): β-globin, GAGCTGGCCACTCGT-CACGT and GATCATACTGCCCCAGGACC; and glyceraldehyde-3-phosphate dehydrogenase, TGCCCCATGTGGTGATG and TGGTGC-TGATGCCCCTC.

Protein Analysis—Protein samples were prepared from the same cell cultures used for ChIP. Total protein was prepared by boiling cells for 5 min in SDS sample buffer (50 mM Tris, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromphenol blue, 10% glycerol). Extracts from 1 × 10⁶ cells were resolved on SDS-polyacrylamide gels, transferred to Immobilon P membranes (Millipore), and analyzed by Western blotting with anti-p45/NF-E2 antibody and protein A-peroxidase (Sigma) (48). The bands were detected with ECL Plus (Amer sham Biosciences).

Luciferase Assays and Luciferase Assays—Luciferase assays were performed on MEL cells at 6–8 days after transfection with 3 µg of total DNA using Superfect (Qiagen). The DNA mixture contained 1 µg of pG5Tiluc reporter, increasing amounts of either pGal4-p45 (1–90) or pGal4-p45(1–90)/PXY-1/2 for the expression of wild-type or mutated p45/NF-E2, respectively, and pUC19 to normalize the DNA concentration. MEL cells at 10⁶ were transfected with 5 µg of total DNA using 1 µl of DIMEIRE-C (Invitrogen), and some constructs were used as for the K562 cells transfection. Transfected cells were harvested after 40 h, and lysates were assayed for luciferase activity with a Promega luciferase assay kit. The luciferase values were normalized to the protein content of the lysates using a Bradford assay with γ-globin as a standard. pGal4Tiluc contains five Gal4-binding sites, a TATA box from the adenovirus major late promoter, and the RPII215 promoter driving expression of β-globin, GAGCTGGCCACTCGT-CACGT and GATCATACTGCCCCAGGACC; and glyceraldehyde-3-phosphate dehydrogenase, TGCCCCATGTGGTGATG and TGGTGC-TGATGCCCCTC.
Fig. 1. P45/NF-E2-mediated activation of endogenous \( \beta \)-globin transcription in CB3 cells. A, the diagram shows the organization of the murine Hbb\( \alpha \) \( \beta \)-globin locus and the positions of the embryonic (Ey and \( \beta H1 \)) and adult (\( \beta \text{major} \) and \( \beta \text{minor} \)) \( \beta \)-globin genes and the upstream HSs, which comprise the LCR. The locations of real-time PCR amplicons are depicted below the locus. B, domain organization of murine p45/NF-E2. The sequence below p45/NF-E2 depicts amino acids 42–83, which were deleted to generate the M1 mutant. The shaded PTY and PSY sequences represent PTY-1 and PTY-2 motifs, respectively. PTY of PXY-1 was substituted with AAA to generate the PXY-1 mutant. PXY-20 and PXY-70 expressed mutants in which PXY-1 and PXY-2 motifs were substituted with AAA (GALA-p45/1–90PXY/1–2)). Luciferase activity was assayed in cell lysates, and the numbers of relative light units (RLU) were normalized to the protein content of the lysates (means \( \pm \) S.E., three independent experiments). C, Murine Hbb\( ^{\alpha} \)/\( \beta \)-globin locus. Real-time PCR primers: 1. 900; 2. EY; 3. EYH1; 4. EYH1R; 5. HBB; 6. HBBR; 7. βH1; 8. βH1R; 9. LCRF; 10. LCRF. D, quantitative real-time RT-PCR analysis of \( \beta \)-globin expression in the cell lines of B. The relative levels of \( \beta \)-globin expression were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (means \( \pm \) S.E., three independent experiments).

RESULTS AND DISCUSSION

The PXY-1 Motif of p45/NF-E2, Which Mediates Transcriptional Activation from Chromatin, Is Not Required in Transient Transfection Assays—To investigate the mechanism of NF-E2-mediated transactivation, we generated stably transfected clonal lines of CB3 cells, which express either wild-type p45/NF-E2 or the M1 or PXY mutants of p45/NF-E2 (Fig. 1B). Two independently derived clonal cell lines were analyzed for each mutant, and each line was grouped with MEL and CB3 controls and referred to as group 1 or group 2. D, quantitative real-time RT-PCR analysis of \( \beta \)-global expression in the cell lines of B. The relative levels of \( \beta \)-globin expression were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (means \( \pm \) S.E., three independent experiments).

48). PXY-20 and PXY-70 expressed mutants in which PXY-1 was substituted with alanines, which also strongly reduces transactivation (48). Clones stably expressed wild-type p45/NF-E2 and the M1 and PXY-1 mutants at similar levels and, importantly, at levels similar to endogenous p45/NF-E2 in MEL cells (Fig. 1C). p45/NF-E2 exists as two isoforms resulting from alternative translational start site usage, although no functional distinctions have been ascribed to the isoforms (29, 31). Quantitative real-time RT-PCR analysis was used to measure endogenous \( \beta \text{major} \) transcription in the cell lines. As expected (48), p45/NF-E2 expression in CB3 cells reactivated \( \beta \text{major} \) transcription (Fig. 1D) (41, 42). By contrast, the M1 and PXY-1 mutants were almost completely defective in activating \( \beta \)-globin transcription.

The analysis of Fig. 1 demonstrated the crucial requirement of amino acids 42–83 and PXY-1 of p45/NF-E2 for activation of endogenous \( \beta \text{major} \) transcription in CB3 cells. To investigate the mechanism of the PXY-1 requirement, we tested whether PXY-1 is necessary for transactivation in transient transfection assays in mouse MEL (Fig. 2A) and human K562 (Fig. 2B) erythroleukemia cells. MEL cells represent a later developmental stage than K562 cells, expressing mainly adult \( \beta \)-globin (53) versus the predominantly fetal \( \gamma \)-globin expression of K562 cells (54). The cells were transiently co-transfected with a luciferase reporter gene containing five GAL4-binding sites and increasing amounts of vector expressing the GAL4 DNA-binding domain alone (GALA) or GAL4 fused to amino acids 1–90 of p45/NF-E2 (GALA-p45 (1–90)) or to amino acids 1–90 of p45/NF-E2, in which PXY-1 and PXY-2 motifs were substituted with AAA (GALA-p45/1–90PXY/1–2)). Luciferase activity was assayed in cell lysates, and the numbers of relative light units (RLU) were normalized to the protein content of the lysates (means \( \pm \) S.E., three independent experiments). B, K562 cells were co-transfected as described for A (means, two independent experiments).
expression vector concentration, GAL4-p45 (1–90) and GAL-
p45(1–90PXY-1/2) equally activated luciferase activity, whereas at higher concentrations, GAL4-p45(1–90PXY-1/2) was 20–40% less active than GAL4-p45 (1–90) (Fig. 2A). Despite the small decrease in activity relative to GAL4-p45 (1–90) in MEL cells, GAL4-p45(1–90PXY-1/2) conferred high level transactivation. GAL4-p45(1–90PXY-1/2) strongly activated luciferase activity, identical to GAL4-p45 (1–90) in K562 cells (Fig. 2B). The GAL4 DNA-binding domain alone did not affect luciferase activity in either cell line. Thus, in both MEL and K562 cells, PXY-1/2 was not necessary for strong transactivation, in contrast to the crucial PXY-1 requirement for activation of endogenous βmajor transcription. These results suggest that PXY-1 is preferentially required for transcriptional activation from endogenous chromatin and therefore might mediate essential chromatin remodeling events.

Establishment of an Overlapping Pattern of Histone Acetylation and H3-meK4 at the Endogenous β-Globin Locus Is PXY-1-dependent—Based on the role of PXY-1 for activation of endogenous βmajor transcription but not for transactivation in transient assays, we tested whether PXY-1 mediates chromatin modification. A quantitative real time PCR-based ChIP assay was conducted to measure the relative levels of acetylated H3 and H4 and H3-meK4 at the βmajor promoter (βmaj Pro) (Fig. 3B), at βmajor exon 3 (βmaj 3') (Fig. 3C), and at the constitutively active promoter of the RPII215 gene (Fig. 3D), which encodes the large subunit of Pol II. As described previously (11, 56, 57), SYBR Green fluorescence reflected the expected homogenous PCR product, and the assays were conducted under conditions in which product accumulated linearly with respect to input DNA (Fig. 3A).

Acetylated H3 and H3-meK4 levels at βmaj Pro and at βmaj 3' were low in CB3 cells and were induced upon expression of wild-type p45/NF-E2 (Fig. 3, B and C). By contrast, the M1 and PXY-1 mutants of p45/NF-E2 were impaired in their activities to induce H3 hyperacetylation (group 1, 82 and 88% decrease, respectively, relative to wild type; group 2, 93 and 100% decrease, respectively, relative to wild type) and H3-meK4 (group 1, 68 and 69% decrease, respectively, relative to wild type; group 2, 56 and 73% decrease, respectively, relative to wild type). Acetylated H4 levels varied only slightly in the cell lines, consistent with previous results showing that p45/NF-E2 preferentially induces H3 versus H4 hyperacetylation (11, 45). Also consistent with previous results was the finding that the p45/ NF-E2-dependent induction of H3 hyperacetylation and H3-
meK4 was greater at βmaj 3' versus βmaj Pro (group 1, 10-fold versus 3-fold, respectively; group 2, 6-fold versus 2-fold, respectively). The activity of p45/NF-E2 to induce H3 hyperacetylation and H3-meK4 at βmajor was specific, because the levels of these histone modifications at RPII215 were similar in the cell lines (Fig. 3D). Thus, PXY-1 is important for establishment of a component of the histone modification pattern of the endogenous β-globin locus. Furthermore, the lack of a major PXY-1 requirement for transactivation in transient assays (Fig. 2) is consistent with a role of PXY-1 in mediating chromatin modification, which would not be expected to profoundly influence the activity of nonreplicating plasmid templates.

GATA-1 and NF-E2 cooperatively recruit Pol II to the βmaj Pro (58). The impaired induction of H3 hyperacetylation and H3-meK4 at βmaj Pro (Fig. 3B) by the M1 and PXY-1 mutants of p45/NF-E2 might result in a less accessible promoter, thereby creating an obstacle to Pol II recruitment. To test this possibility, quantitative ChIP analysis was conducted to measure Pol II occupancy at the βmajor and RPII215 promoters. Although wild-type p45/NF-E2 strongly recruited Pol II to βmaj Pro, the M1 and PXY-1 mutants failed to recruit Pol II

![Fig. 3. PXY-1 is important for p45/NF-E2-mediated establishment of H3 hyperacetylation and H3-meK4 at the endogenous β-globin locus. The cells were incubated for 4 days with 1.5% Me2SO. Quantitative ChIP analysis was used to measure the relative levels of acetylated H3 (acH3), acetylated H4 (acH4), and H3-meK4 at the βmaj Pro (B), βmaj 3' (C), and at RPII215 (D) in CB3 cells lacking or expressing wild-type p45/NF-E2 or the M1 or PXY-1 mutants. A shows plots of relative SYBR Green fluorescence versus input DNA, demonstrating the linearity of the real time PCR-based ChIP assay. The numbers of relative units were derived as described under “Experimental Procedures.” Two independently derived clonal cell lines were analyzed for each mutant, and each line was grouped with a CB3 control and referred to as group 1 or group 2 (mean ± S.E., three independent experiments). PI, preimmune.](http://www.jbc.org/)

![Fig. 4A.](http://www.jbc.org/) Pol II occupancy was high at the RPII215 promoter, and expression of wild-type p45/NF-E2 and the M1 and PXY-1 mutants did not affect Pol II recruitment (Fig. 4B). Thus, amino acids 42–83, which define the activation domain, and PXY-1 are necessary to recruit Pol II to βmaj Pro, consistent with the role of these sequences in inducing the histone modifications associated with increased chromatin accessibility.

Despite the Impaired Chromatin Modification and Pol II Recruitment, p45/NF-E2 M1 and PXY-1 Mutants Bind HS2—Given the impaired induction of H3 hyperacetylation, H3-meK4, and Pol II recruitment, we tested whether the p45/NF-E2 mutants are competent to occupy the LCR. It is conceivable that PXY-1 is required for subnuclear localization of p45/NF-E2, which might be important for NF-E2 to gain access to the LCR. A role for the developmental control of p18 subnuclear localization in MEL
cells has been proposed (59). Quantitative ChIP analysis revealed that wild-type p45/NF-E2 and the M1 and PXY-1 mutants occupied HS2 similarly (Fig. 5), strongly suggesting that the PXY-1-dependent step in chromatin modification and $\beta$-globin transcription occurs post-DNA binding.

An obvious step in transactivation in which the PXY-1 mutant might be compromised is CBP/p300 recruitment, because CBP/p300 mediates NF-E2-dependent transactivation in transient transfection assays (49, 50). CBP/p300 is also a mediator of GATA-1-dependent transactivation (60). Furthermore, GATA-1 induces H3 hyperacetylation at the $\beta$-maj Pro (11, 61) and both H3 and H4 hyperacetylation at the LCR (61). CBP/p300 has been implicated as a mediator of these acetylation events (61).

We used quantitative ChIP to test whether CBP/p300 is recruited to HS2. CBP/p300 occupied HS2 similarly in CB3 cells and in CB3 clonal lines expressing wild-type p45/NF-E2 or the M1 or PXY-1 mutants (Fig. 6A). No CBP/p300 occupancy was detected at intervening region 16 (Fig. 6B), which resides several kb downstream of $\beta$-minor and has no known functional sequences (Fig. 1A). The association of CBP/p300 with HS2 in CB3 cells indicates that another factor, potentially GATA-1, recruits CBP/p300 to HS2 in this system. CBP/p300 occupancy of HS2 increases upon GATA-1 expression in GATA-1-null G1E cells (62). Nevertheless, CBP/p300 recruitment might be insufficient to convey an activation signal to the promoter. NF-E2 might be required to activate the histone acetyltransferase activity of CBP/p300. Of relevance to this possibility is the report that NF-E2 activates histone acetyltransferase activity of CBP/p300 in vitro (62).

A Nuclear WW Domain Mediator of NF-E2-dependent Transactivation from Chromatin?—Analysis of chromatin modifications at $\beta$-major in CB3 cells expressing either wild-type p45/NF-E2 or the M1 or PXY-1 mutants demonstrated that the activation domain and PXY-1 are important for establishing H3 hyperacetylation and H3-meK4. Importantly, mutagenesis of PXY-1 yielded a defect identical to deletion of the activation domain (M1 mutant), indicating that the activity of the activation domain to induce H3 hyperacetylation and H3-meK4 can be attributed to PXY-1. The PXY-1 activity to induce chromatin modification might involve a direct mechanism in which PXY-1 facilitates binding of p45/NF-E2 to a histone acetyltransferase and/or histone methyltransferase or an indirect mechanism in which PXY-1 facilitates binding of an intermediate protein, which recruits these enzymes. Regardless, PXY-interacting WW domain proteins and other co-regulators have not been co-purified with endogenous NF-E2.
Endogenous p45 NF-E2 co-purified with p18, but not with other polypeptide components (30, 31). Because the purification was conducted under high stringency conditions, it is not surprising that only the high affinity leucine zipper-mediated p45/NF-E2-p18 interaction was preserved. Even though CBP/p300 interacts with p45/NF-E2 with moderate affinity and high specificity in vitro, and the CBP/p300 inhibitor E1A abrogates NF-E2 nuclear transactivation (50), endogenous p45/NF-E2 has not been co-purified with endogenous CBP/p300. A simple explanation for these results is that the high salt used to extract NF-E2 from nuclei dissociates NF-E2-co-regulator interactions. Expression cloning and yeast two-hybrid screening have identified p45/NF-E2-interacting WW-domain-containing proteins, the ubiquitin ligase Itch (63), and NAP2P (64), which might regulate NF-E2 function. Other candidate interactors, which are nuclear WW domain proteins but have not been shown to interact with p45/NF-E2, include Yes-associated protein (YAP) (65–67) and the related TAZ protein (68), which have co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity. Intriguingly, the yeast histone methyltransferase Set2, which methylates Lys36 of histone H3, has co-activator activity.
A WW Domain-binding Motif within the Activation Domain of the Hematopoietic Transcription Factor NF-E2 Is Essential for Establishment of a Tissue-specific Histone Modification Pattern

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