Erythroid Gene Suppression by NF-κB*

Received for publication, December 3, 2002, and in revised form, March 14, 2003
Published, JBC Papers in Press, March 17, 2003, DOI 10.1074/jbc.M212278200

Jan-Jan Liu‡§§, Shin-Chen Hou‡¶, and C.-K. James Shen‡‡§§

From the ‡Institute of Molecular Biology, Academia Sinica, Nankang, the §Institute of Molecular Medicine, National Taiwan University, and the ¶College of Life Sciences, National Defense University, Taipei 115, Taiwan, Republic of China

NF-κB/Rel transcription factors play essential roles to mediate the immune response and apoptosis, and they have also been implicated in cellular differentiation such as erythropoiesis. To elucidate the possible role(s) of NF-κB in erythroid gene regulation and erythropoiesis, we have carried out transient transfection studies of the human embryonic/fetal erythroid cell line K562 and mouse adult erythroid MEL cells. It is shown that tumor necrosis factor-α represses the transcription activity directly by either α or ζ globin promoter in a dose-dependent manner. Furthermore, different NF-κB family members could effectively repress the transfected α-like globin promoters in K562 as well as in MEL cells. The involvement of NF-κB pathway is supported by the ability of a NF-κB-specific, dominant negative mutant to block the tumor necrosis factor-α or p65-mediated suppression of the α-like globin promoter activities. The suppression appears to be mediated through cis-linked HS-40 enhancer. Finally, stably transfected K562 cells overexpressing p65 contain reduced amounts of the p45/NF-E2 RNA and functional NF-E2 proteins. Our studies have identified a new set of targets of NF-κB. We suggest that the relatively high activity of the NF-κB pathway in early erythroid progenitors is involved in the suppression of erythroid-specific genes. Later in differentiation, together with other changes, the decline of the amounts of the NF-κB family of factors leads to derepression and consequent increase of NF-E2, which in turn would activate a subset of erythroid-specific genes.

The mammalian α-like (embryonic ζ, adult α2, α1, and α) and β-like (embryonic e, fetal γG and γA, adult δ and β) globin gene clusters together have provided a paradigm for the analysis of coordinated and differential gene expression. Understanding the molecular basis of human globin gene switch during development would also provide essential knowledge and alternative therapeutic strategies for the treatment of severe hemoglobinopathies including sickle cell anemia and thalassemias (1, 2).

It has become clear that the developmental switch on and off of different globin gene transcription is controlled by the synergistic interactions between different globin promoters and their upstream regulatory elements (3–7). These elements, namely, the locus control region of the β-like globin gene cluster (4, 6, 7) and the HS-40 enhancer of the α-like globin gene cluster (5), and the promoters each consists of specific sequence motifs that are bound with nuclear factors in an erythroid cell- and developmental stage-specific manner (Refs. 8 and 9 and references therein). Among the DNA-binding proteins known to function in globin gene regulation are the erythroid-enriched transcription factors NF-E2 (10), GATA-1 (11), and EKLF (12). There are also cofactors that physically interact with the above DNA-binding transcription factors, for example, FOG (13) and chromatin remodeling complexes (14).

When compared with other gene systems, relatively little is known about the signal transduction pathways regulating the globin gene transcription. One study in human primary erythroid progenitors and the human embryonic/fetal erythroid cell line K562 showed that IL-6 down-regulated the γ globin mRNA level. However, the molecular basis of this repression remains unknown (15). Another transgenic mice study has shown that a NF-κB-binding motif at the 3′ side of the human ζ globin gene is required for its complete silencing in the adult mice (16). Finally, it has been implicated that serine-threonine phosphorylation might regulate the activity of NF-E2 (17, 18).

NF-κB/Rel is a family of potent inducible regulatory factors consisting of p65/RelA, p50(p105), p52(p100), RelB, and c-Rel (Refs. 19 and 20 and references therein). The NF-κB pathway could be activated by a variety of extracellular stimuli to regulate the expression of genes required for immune and inflammatory responses, for cell growth and differentiation, as well as for suppressing apoptosis (21–23). In general, upon stimuli such as exposure to the various cytokines, the IkB inhibitory proteins are phosphorylated and degraded. The liberated NF-κB is then translocated to the nucleus, binds to specific κB elements (GGGRNNYYCC), and modulates by gene transcription. More recently, several lines of evidence have suggested that NF-κB also plays a crucial role in erythropoiesis (24). RelB knockout mice develop normally but exhibit abnormalities in hematopoiesis and multiorgan inflammation (25). Rel/RelA-deficient mice displayed multiple hematopoietic cell defects and erythropoiesis impairment (26). Also, NF-κB subunits p65, p50, and p52 are all expressed during early normal erythroid proliferation (day 7–10 erythroblasts), and their levels decline during differentiation (24). It was also postulated (24) that NF-κB factors modulate the erythropoiesis via its downstream target genes, c-myc and c-myc, the expression of which are required for the erythroid development (27–29). We show below that activation of the NF-κB pathway in two different types of erythroid cells represses the expression of the α-like globin genes. We have also carried out experiments to investigate the molecular basis of this NF-κB-mediated repression.

* The work was supported by the National Health Research Institute, The National Science Council, and the Academia Sinica (Taipei, Taiwan, R.O.C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Present address: Dept. of Anesthesiology-0818, UCSD, 9500 Gilman Dr., La Jolla, CA 92037.
‡ To whom correspondence should be addressed. Tel.: 11-886-2-27821436; Fax: 11-886-2-27884177; E-mail: csksher@ccvax.sinica.edu.tw.

1 The abbreviations used are: IL, interleukin; GFP, green fluorescent protein; HA, hemagglutinin; CMV, cytomegalovirus; GH, growth hormone; TNF, tumor necrosis factor; EMSA, electrophoretic mobility shift assay; Luc, luciferase.
Experimental Procedures

Plasmids—The reporter constructs pHS40-597GH, pHS40-a590GH, and their mutated versions have been described previously (8, 9). The constructions of pHS40-a590 and pHS40-597GH were made in the following way. First, a SacI/XbaI fragment encompassing the HS-40 enhancer from pBS-HS40-a590GH was ligated with SacI/XhelI-cut pGh3 (Promega) to create pGh3-HS40. Then a blunt FstI-NcoI fragment containing the a1 globin promoter sequence from –574 to +41 was ligated with HindIII/I-colt pGh3-HS40 after the HindIII end was generated by generating pHS40 and pHS40-597Gh. To construct pHS40-597Luc, a SacI/BamHI fragment containing the HS-40 enhancer and a globin promoter from pHS40-597GH was cloned into the SacI/BgII sites of pGh3. p65 cDNA, amplified by RT-PCR, was inserted between the XbaI and KpnI sites of pGh3 to get pGh3/Hap65 encoding a Hap65 fusion protein. The HindIII/KpnI fragment from pGh3/Hap65 was inserted in-frame into pEGFP-C1 (Clontech), resulting in pEGFP-Hap65 directing the synthesis of GFP-HA-tagged p65 fusion under the control of CMV promoter. pGh3–at87/+41Luc was constructed by ligating a Smal/I-colt fragment encompassing the a1 globin promoter from –87 to +41 with Smal/I-colt pGh3. pGh3–(NA)–at87/+41Luc contains two tandem NF-E2/AP1-binding sites cloned into the Smal site of pGh3–at87/+41Luc. pSV40 and pRSV-p84 expression plasmids were constructed by cloning fragments flank the HindIII sites and containing the full coding regions of p45 and p65, respectively, into pRSV/Sp (Invitrogen). pCMV/p65, p32H, and pCMV-Ix/Bs/322/363A, also termed pCMV-IxBoxA2BA (see Fig. 1), were kindly provided by Dr. Greene (30), Dr. Chernoff (31), and Dr. Peters (32), respectively.

Cell Linedine DNA Transfection—Human erythroid K562 (33) and mouse erythroid MEL (34) cells were cultured under 5% CO2 at 37 °C. MEL cells were kindly provided by Dr. Greene (30), Dr. Chernoff (31), and Dr. Peters (32), respectively.

Cytoplasmic RNA Extraction and Northern Blot Analysis—Total RNA was extracted from cells with the use of RNAzol B (CinnaGene, Inc.) according to the manufacturer's instructions. Northern blot analysis, total RNAs were extracted by means of a commercial Trizole reagent (Invitrogen). 10 μg of each RNA sample were separated on a 1.0% SSC, 0.1% SDS once and 0.2 SSC, 0.1% SDS at 55 °C. The blots were subjected to autoradiography or direct quantitation with a PhosphorImager.

EMS—The nuclear extracts were prepared essentially as described by Dignam et al. (36). All of the DNA binding reactions were performed using a volume of nuclear extract equivalent to 0.5 μg of nuclear protein. For Northern and Western blot analysis of the p45/NF-EL expression, serum stimulation of the cells was for 2–3 and 6–12 h, respectively. The blot hybridization procedures essentially followed Sambrook et al. (35). For Western blot analysis, the extracts were prepared by cell lysis in 25 mm HEPES, pH 7.5, 0.3 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5% Triton X-100, 3 mm diethiolether, 50 mM β-glycerol phosphate, 1 μg/ml of 1 mM Na2VO4, 10 μg/ml of each Klenow enzyme with [32P]dATP for random priming. After overnight hybridization at 42 °C with standard buffer containing 50% formalde, the blot was washed successfully in 2× SSC, 0.1% SDS once and 0.2× SSC, 0.1% SDS at 55 °C. The blots were subjected to autoradiography or direct quantitation with a PhosphorImager.

EMSA—The nuclear extracts were prepared as described by Dignam et al. (36). All of the DNA binding reactions were performed using a volume of nuclear extract equivalent to 0.5 μg of nuclear protein. These oligonucleotides were used as the probe. The probes were labeled with [32P]dATP by random priming method. The binding reaction for the IgB site was carried out in a buffer containing 20 mm HEPES, pH 7.9, 60 mm KCl, 1 μg of poly(dI-dC), 10% glycerol, 0.2 mm EDTA, 0.5 mm phenylmethylsulfonyl fluoride, and 0.5 mm diethiolether. 3.2 mm MgCl2 was included in the reaction mixtures for NF-E2 or NF-Y binding. For Sp1 binding, the reaction mixture contained 10 μg of each RNA sample were separated on a 1.0% agarose, 6% formaldehyde gel, transferred to a nylon membrane, and hybridized with DNA fragments amplified by PCR and containing α globin, β globin, and β-actin cDNA, respectively. A 1.9-kb HindIII fragment containing human p45 cDNA was used as the probe. The probes were labeled with [32P]dATP by random priming method. After overnight hybridization at 42 °C with standard buffer containing 50% formalde, the blot was washed successfully in 2× SSC, 0.1% SDS once and 0.2× SSC, 0.1% SDS at 55 °C. The blots were subjected to autoradiography or direct quantitation with a PhosphorImager.
RESULTS

Activation of the NF-κB Pathway Represses Human α and ζ Globin Promoters—Transient transfection was used to test whether the NF-κB pathway might be involved in the regulation of the α-like globin gene expression. The human erythroleukemia cell line K562 was transfected with the reporter plasmids pHS40-α590Luc or pHS40-ζ597Luc. At 5–6 h posttransfection, the cells were treated with TNF-α or IL-6, respectively, for 24 h prior to the reporter assay. As shown in Fig. 1, treatment with TNF-α, but not IL-6, reduced the luciferase activity of either ζ (Fig. 1A) or α (Fig. 1B) globin promoter. To further examine whether TNF-α exerted this effect through the NF-κB pathway, the cells were cotransfected with pCMV-IκBoA(S32A/S36A). This plasmid, termed pCMV-IκBoAα32A36 in the following, expresses IκBoSA32A36, also known as IκBoAA or IκB, which inhibits TNF-α- or IL-1-induced NF-κB activation (37). Indeed, TNF-α-mediated repression of the human ζ and α globin promoters was relieved by cotransfection of pCMV-IκBoAα32A36 (Fig. 1). These data together suggested that activation of the NF-κB pathway by TNF-α could suppress the transcriptional activities of transfected human α-like globin promoters in K562 cells.

The functional roles of NF-κB subunits in suppressing the human α-like globin promoters were further studied by the cotransfection assay. Consistent with Fig. 1, a dose-dependent suppressive effect by p65 in K562 cells was observed when either growth hormone (Fig. 2A) or luciferase (Fig. 2B) was used as the reporter. In particular, cotransfection with 10 μg of pCMV-p65 decreased the ζ and α promoter activities by 90 and 80%, respectively (Fig. 2A, middle bars). Although IκBoAA by itself had no effect, coexpression of this subunit reversed the p65-mediated suppression of either globin promoter (Fig. 2A, top two bars). These data indicate that p65 could down-regulate the transcriptional activities of the human ζ and α globin promoters through the NF-κB pathway. Cotransfection of MEL cells with pHS40-α590 GH and pCMV-65 gave similar results (data not shown).

Members of the NF-κB Family Other Than p65 Are Also Capable of Repressing the α-Like Promoters—Similar to p65, several other NF-κB members including p50, p65-p50, and c-Rel significantly repressed the ζ globin promoter in transfected K562 cells (Fig. 2B, left panel). Coexpression of p50-p50 also repressed transfected human α globin promoter in MEL cells (Fig. 2B, right panel). In addition, the repression by these factors correlated well with the expression of NF-κB activity in the transfected cells, as measured by a NF-κB-dependent reporter assay (data not shown). Furthermore, coinexpression of a dominant negative mutant of p50, p50ΔSp, had no suppression effect on the transfected globin promoters (Fig. 2B, top bars of both panels). The data of Fig. 2B indicated that the HS-40-mediated activities of the human α-like globin promoters could be repressed by activation of the NF-κB pathway through the functioning of different members of the NF-κB family.

Target Sites of Suppression of the α-Like Globin Promoters by NF-κB—To determine the sites of action by the NF-κB factors to suppress the α-like globin promoters, we compared the promoter activities in transfected K562 cells in the presence or absence of the cis-linked HS-40 enhancer. Interestingly, the suppression phenomena were not seen for the enhancerless plasmid pα590GH (Fig. 3) or pζ597GH (data not shown). This result suggests that suppression by p65 or other NF-κB factors is mediated mainly through the HS-40 enhancer region instead of the promoter(s) per se.

The HS-40 enhancer consists of at least five functional motifs that are bound with factors in vivo in an erythroid lineage- and developmental stage-specific manner (8, 33, 38, 39). These motifs include a GT motif, two GATA-1-binding sites, and two NF-E2/AP1-binding sites, 5’-NA and 3’-NA (8). In vitro, the NA motif(s) could be recognized by a number of different factors including the erythroid-enriched NF-E2 and more ubiquitously expressed AP1, small maf (avian musculoaponeurotic fibrosarcoma virus) homodimers, and Bah1, Bah2, Bah3, etc. (Refs. 38, 40, and 41 and references therein). To examine whether the NA motifs are involved in the suppression of the HS-40 enhancer function by p65, transient transfection experiments were carried out with a reporter plasmid, pGL3-(NA)2 (−87/+41)Luc, containing two tandem copies of the NF-E2/AP1-binding sites, or NA, in front of an α globin minimal promoter. The tandem NA sites confer red activation of the reporter by coexpression of p45 and p18, i.e. NF-E2 (Fig. 4, compare top and bottom bars of left panel). Coexpression of p65 in K562 cells, on the other hand, resulted in a marked decrease of the reporter activity (Fig. 4, left panel, second bar from bottom). Similar to the experiments with pHS40-α590GH or pHS40-ζ597GH, cotransfection with IκBoAA reversed the p65-mediated suppression. In contrast, the expression of p65 and/or IκBoAA had little effect on the control reporter construct, pGL3-α(−87/+41)Luc, without the two NA sites (Fig. 4, right panel). The data of Fig. 4 together with Fig. 3 suggest that presence of cis-linked NA sites is both necessary and sufficient for p65-mediated repression of the α-like globin promoters.

Repression of Endogenous α-Like Globin Messages by Exogenous p65—To study the effects of p65 on the expression of endogenous α-like globins, stably transfected K562 cells over-
expressing p65 were generated. This was accomplished by the transfection of K562 cells with pEGFP-HAp65 encoding GFP as well as a HA-p65 fusion protein. As a control, K562 cells were also transfected with the vector pEGFP-C1 encoding only the GFP. After selection with G418, two pools with integrated pEGFP-HAp65, K(p65) and K(p65)/H11032, and one pool integrated with the vector, K(V), were isolated by cell sorting. Expression of recombinant p65 in these stable clones was then determined by Western blot analysis. As exemplified by K(p65), both the HA and p65 epitopes could be detected in K562 overexpressing p65 (Fig. 5A, left two panels, lanes 3) but not in the parental K562 or K(V) cells (Fig. 5A, left two panels, lanes 1 and 2). Consistent with previous reports that p65 positively regulates Iκα (42, 43), the K(p65) cells (Fig. 5A, right top panel, lane 3) and K(p65)/ cells (see Fig. 7B) also have elevated levels of IκB.

Next, we performed electrophoretic mobility shift and transient transfection assays to test the DNA binding abilities and transactivation potential of the recombinant p65 in the K(p65)
cells (Fig. 5B, top panel). Two specific protein-DNA complexes could be observed when K(p65) nuclear extract was incubated with a radiolabeled oligonucleotide, IgκB, the κB site from the immunoglobulin κ light chain enhancer (Fig. 5B, lane 2). In contrast, there was no obvious formation of these complexes in the control K(V) extract (Fig. 5B, lane 1). The cold wild-type competitor oligonucleotide, but not a mutant one, efficiently inhibited the complex formation (Fig. 5B, compare lanes 3 and 4). In addition, anti-p65 or anti-p50 (Fig. 5B, lanes 5 and 6), but not unrelated anti-IκB and anti-ɛ-Jun antibodies (data not shown), supershifted these complexes. Thus, these complexes most likely resulted from binding of p65 homodimer (Fig. 5B, upper band) or p65/p50 heterodimer (Fig. 5B, lower band) to the IgκB probe. There was no difference in the formation of the NF-Y complex in K(V) and K(p65) extracts (Fig. 5B, bottom panel).

To further explore whether the recombinant HA-p65 in K(p65) cells could play a role in transcriptional regulation, a functional assay was performed. A reporter plasmid pxB-Luc was transfected into proliferating K(p65) and K(V) cells. As shown in Fig. 5C, the luciferase activity in transfected K(p65) cells was 35-fold higher than that of the K(V) cells, suggesting that HA-p65 indeed could induce gene expression through binding to the κB enhancer in the plasmid. Taken together, the above immunoblot analysis, DNA binding study, and pxB-Luc reporter assay suggested that recombinant HA-p65 is functional in K562 cells.

Whether the endogenous α-like globin gene transcription in K562 cells was affected by expression of exogenous p65 was examined by Northern blot analysis (Fig. 5D). Remarkably, the steady-state levels of α and ζ globin transcripts were greatly reduced, by at least 70 and 50%, respectively, in K(p65) cells (Fig. 5D, compare lane 1 with lane 2). Similar to previous studies of K562 cells, hemin also induced the levels of the globin transcripts in both K(V) and K(p65) cells with or without prior treatment with hemin. The levels of the α and ζ mRNAs are indicated as the percentages relative to the respective RNAs of the uninduced K(V) cells, after calibration against the level of the actin mRNA.

Formation of NF-E2/DNA Complex Is Reduced in K(p65) Cells—As already shown in Figs. 3 and 4, the suppression of the α-like globin promoters by p65, and probably by other NF-κB components as well, is most likely mediated through protein-DNA complex(es) formed at the NA motifs of the HS-40 enhancer. It is thus logical to examine whether p65 exerts this suppression effect by interference with NF-E2-DNA complex formation at the NA motifs. To accomplish this, we used EMSA to assay nuclear extracts prepared from K(V) and K(p65) cells (Fig. 6). Similar to the previous studies (for example, see Ref. 44), several prominent protein-DNA complexes, including the major AP-1 and a minor NF-E2, were observed with the control K(V) extract (Fig. 6A, lane 2). These complexes could be com-
peted out with the cold NA oligonucleotide (Fig. 6A, lane 3) but not with the IκB oligonucleotide (Fig. 6A, lane 4). The addition of anti-p45 and anti-c-Jun antibodies supershifted the NF-E2 and AP1 complexes, respectively (Fig. 6A, lanes 5 and 6). Use of preimmune serum had no obvious effect (Fig. 6A, lane 7). Interestingly, however, the NF-E2 complex could barely be seen with the nuclear extract from K(p65) cells (Fig. 6A, compare lanes 1 and 2). These data indicated that the presence of p65 in K(p65) reduced or inhibited the formation of NF-E2/DNA complex. No significant variations in the intensities of Sp1 and NF-Y complexes in the two nuclear extracts were detected (Fig. 6B), which argued against a general inhibition of protein-DNA complex formation by p65.

Expression of p45 Is Repressed in p65 Overexpressing Cells—Among the possible reasons for the above reduction of NF-E2/DNA complex formation is the reduced level of NF-E2 as caused by the presence of p65. To address this possibility, we investigated the expression level of the erythroid-enriched sub-

DISCUSSION

We have investigated the regulation of the α-like globin promoter activities as affected by the activation of the NF-κB pathway. It appears, from DNA transfection analysis, that different NF-κB factors could suppress the α-like globin promoters in erythroid cells derived from either embryonic/fetal or adult lineage. The suppression required the presence in cis of the HS-40 enhancer and was mediated at least in part through the NF-E2/AP-1-binding sites, or NA motif, in the enhancer. Indeed, activation of the NF-κB pathway repressed the expression of the erythroid-specific subunit, p45, of NF-E2, thus leading to the reduction of NF-E2/DNA complex formation with the NA motif(s).

Although in most cases NF-κB acts as an activator for gene expression, inhibition by NF-κB is not without precedents. For example, studies on Dorsal, a Drosophila homologue of the members of the mammalian NF-κB/Rel family, suggested that NF-κB negatively regulated the expression of maternal effect genes controlling the dorsal-ventral pattern formation (45). Also, TNF-α reduced extracellular matrix deposition through inhibition of the expression of the structural matrix components such as the type I collagen (46). Finally, as already mentioned, two studies have linked globin gene silencing with the NF-κB signaling (Refs. 15 and 16; see further discussion below). In contrast to the positive regulation of gene expression by NF-κB in which the factor(s) binds to κB motif(s) and interacts with the basic transcription complex or coactivators such as CBP/p300 (47), the molecular mechanisms through which the NF-κB factors exert their negative regulatory effects are not well defined (46, 48).

In the case of the human α and ζ globin genes, we showed that their expression in erythroid cells could be negatively regulated by activation of the NF-κB pathway. This could be accomplished either by treatment of the cells with TNF-α (Fig. 1) or by ectopic expression of the NF-κB family of factors such as p65, p50, and c-Rel (Fig. 2). Further analysis of the amounts of endogenous α-like globin messages and p45 in K562 cells either stably expressing exogenous p65 (Figs. 5D, 6, and 7) or...
NF-κB and Erythropoiesis

treated with TNF-α (data not shown) suggested that negative regulation of the α-like globin promoters by NF-κB may result in part from repression of the p45 gene. As shown previously by different groups, p45 is a subunit of NF-E2, a DNA-binding transcription activator (10) stably bound in vivo in the HS-40 enhancer and the locus control region of the β-like globin gene cluster (8, 49) and is required for transcriptional activation of the globin genes in erythroid cells (Refs. 8, 50, and 51 and references therein). The negative regulation of expression of either the α-like globins or p45 gene by NF-κB could be regulated at the transcriptional level either directly by NF-κB factor(s) or indirectly by its downstream targets, such as c-myb (28, 29) and the CCAAT/enhancer-binding protein factor (46) through binding to negative regulatory DNA motifs. Alternatively, the suppression of p45 expression by NF-κB could occur post-translationally. In fact, comparison of the Northern and Western blot data of Fig. 7 strongly suggested that suppression of the p45/NF-E2 expression by NF-κB also occurred at the translational or post-translational level. Finally, it should be noted that the suppression of the α-like globin gene expression by NF-κB might not entirely be mediated through p45 suppression.

In combination with several previous studies, we propose a model for the functional relationship between NF-κB and NF-E2 in erythroid gene regulation and erythropoiesis. It is known that NF-E2 not only transactivates erythroid-specific genes such as the globins (50), but it is also involved in erythropoiesis (52, 53). On the other hand, the NF-κB factors are highly expressed in early burst-forming units-erythroblast-derived precursors, but their levels decline during erythropoiesis (24). This is in contrast to the increase of NF-E2 during maturation of the erythroid cells (10). Our demonstration of the suppression of p45/NF-E2 by NF-κB thus provides a partial explanation for the reciprocal changes of the amounts of NF-κB and NF-E2 during erythropoiesis. To expand further, through repression of p45, the NF-κB factor could be involved in the silencing of not only the globins but also other NF-E2-activated genes in hematopoietic progenitors and early stage erythroid cells. Although the model needs to be refined, it strongly suggests that inactivation of the NF-κB pathway is one requirement for the activation of a subset of erythroid-specific genes including the globins, as well as for the normal progression of erythropoiesis.

Acknowledgments—We thank Dr. H.-F. Yang Yen for useful discussions and Dr. S.-H. Wen for help on the manuscript preparation. We also thank Drs. Greene, Chernoff, Peters, T. Maniatis, and Ming-Zong Lai for the generous gifts of the plasmids.

REFERENCES

1. Stamatosyanopoulos, G., and Nienhuis, A. W. (1987) The Molecular Basis of Blood Diseases, pp. 66–105, Saunders, Philadelphia, PA.
2. Blau C. A., and Stamatosyanopoulos G. (1994) Curr. Opin. Hematol. 1, 136–142.
3. Orkin, S. H. (1995) Eur. J. Biochem. 231, 271–281.
4. Grosveld, F. (1999) Curr. Opin. Genet. Dev. 9, 152–157.
5. Higgs, D. R. et al. (1989) Blood 73, 1081–1104.
6. Engel, J. D., and Tanimoto, K. (2000) Cell 100, 499–502.
7. Bulger, M., and Groudine, M. (1999) Genes Dev. 13, 2465–2477.
8. Wen, S. C., Roder, K., Hu, K. Y., Rombel, I., Gavva, N. R., Daftari, P., Kuo, Y. Y., Wang, C., and Shen, C.-K. K. (2000) Mol. Cell. Biol. 20, 1903–2003.
9. Rombel, I., Hu, K. Y., Zhang, Q., Papayannopoulou, T., Stamatosyanopoulos, G., and Shen, C.-K. K. (1999) Proc. Natl. Acad. Sci. U. S. A. 92, 6454–6458.
10. Andrews, N. C., Erdjument-Bromage, H., Davidson, M. B., Tempst, P., and Orkin, S. H. (1998) Nature 396, 725–728.
11. Tsai, S. F., Martin, P. I. K., Zon, L. I., D’Andrea, A. D., Wong, G. G., and Orkin, S. H. (1989) Nature 338, 446–451.
12. Miller, J. J., and Bieker, J. J. (1993) Mol. Cell. Biol. 13, 2776–2786.
13. Tsang, A. P., Visvader, J. E., Turner, C. A., Fujikawa, Y., Yu, C., Weiss, M. J., Crossley, M., and Orkin, S. H. (1997) Cell 90, 109–119.
14. Armstrong, J. A., and Emerson, B. M. (1996) Mol. Cell. Biol. 16, 5634–5644.
15. Perry, A. E., Baliga, S. B., Monteiro, C., and Pace, B. S. (1997) J. Biol. Chem. 272, 20030–20037.
16. Wang, Z., and Liebhaber, S. A. (1999) EMBO J. 18, 2218–2228.
17. Garingo, A. D., Subhasis, M., Andrews, N. C., and Pitz, R. B. (1995) J. Biol. Chem. 270, 9169–9177.
18. Nagai, T., Igarashi, K., Akasaka, J., Furuyama, K., Fujita, H., Hayashi, N., Yamamoto, M., and Sassa, S. (1998) J. Biol. Chem. 273, 5388–5393.
19. Karin, M., and Yen, B.-N. (2000) Annu. Rev. Immunol. 18, 621–663.
20. Perkins, N. D. (2000) Trends Biochem. Sci. 25, 434–440.
21. Thanso, D., and Maniatis, T. (1995) Cell 80, 529–532.
22. Foo, S. Y., and Nolan, G. P. (1999) Trends Genet. 15, 229–235.
23. Hatada, E. N., Krauppman, D., and Scheiderent, C. (2000) Curr. Opin. Immunol. 12, 52–58.
Erythroid Gene Suppression by NF-κB
Jan-Jan Liu, Shin-Chen Hou and C.-K. James Shen

J. Biol. Chem. 2003, 278:19534-19540.
doi: 10.1074/jbc.M212278200 originally published online March 17, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212278200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 30 of which can be accessed free at
http://www.jbc.org/content/278/21/19534.full.html#ref-list-1