CCAAT Displacement Protein Competes with Multiple Transcriptional Activators for Binding to Four Sites in the Proximal gp91phox Promoter*

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CCAAT displacement protein (CDP) competes with transcriptional activating proteins for binding to each of four elements within the myeloid-specific gp91phox promoter. CDP exhibits the strongest affinity for a site centered at −110 base pairs (bp) of the promoter and progressively weaker affinities for three more distal binding sites. CDP binding to each site is down-regulated during terminal phagocytic differentiation, coincident with induction of gp91phox expression. Deletion of the high affinity CDP-binding site at −110 bp leads to inappropriate gp91phox promoter activity in HeLa, K562, and HEL cells. An overlapping binding site for the CCAAT box-binding factor CP1 is required for derepressed promoter activity in HeLa and K562 cells, but is dispensable in HEL cells, indicating that different cell types require distinct cis-elements for gp91phox promoter activity. Derepressed gp91phox promoter activity is further increased upon removal of a second CDP-binding site centered at −150 bp, revealing that CDP represses gp91phox expression via multiple cis-elements. We present a model in which restriction of gp91phox expression to mature myeloid cells involves competition between transcriptional activators and repressors for binding to multiple sites within the promoter.

The gp91phox gene encodes a component of the NADPH oxidase complex that is responsible for the generation of a respiratory burst in mature phagocytic blood cells (1). Absence of oxidase activity results in chronic granulomatous disease, an inherited disorder of phagocyte function, and may be due to disruption in the expression of any of several oxidase components, including gp91phox (1), p47phox (2), p67phox (3), or p22phox (4). The gp91phox gene is expressed nearly exclusively in terminally differentiating myelomonocytic cells (1), thereby providing a model system for studying the regulation of tissue- and stage-specific gene expression within the myelomonocytic lineage.

The proximal gp91phox promoter (−450 to +12 bp)% is sufficient, and cis-acting elements between −138 and −450 bp are required, to direct appropriate expression of linked reporter genes in a subset of monocytes/macrophages when introduced into transgenic mice (5). The proximal gp91phox promoter also appropriately directs reporter gene expression in stably transfected myeloid cells following stimulation by interferon-γ (IFN-γ) (6).

The binding of transcriptional activating factors to several sites in the gp91phox promoter is required for normal promoter function. Mutations that ablate a binding site for a factor denoted HAF-1 (hematopoietic associated factor) have been identified in the gp91phox promoter of chronic granulomatous disease patients who exhibit abnormal gp91phox transcription (7). Ablation of the HAF-1-binding site also prevents IFN-γ induction of gp91phox promoter activity in transfected myeloid cell lines (6). Additional transcriptional activating factors, denoted BIDs (binding increased during differentiation), bind to three sites in the gp91phox promoter and are also required for an IFN-γ response (8). The middle BID-binding site conforms to an IFN-stimulated response element and additionally serves as a binding site for IFN regulatory factor (IRF)-1 and -2 (9).

The gp91phox promoter is additionally under the control of transcriptional repression. Previously, we demonstrated that deletion of the region between −133 and −100 bp leads to inappropriate gp91phox promoter activity in transiently transfected cells that do not normally express the gp91phox gene (8). A ubiquitous CCAAT box-binding factor interacts with this promoter region when nuclear extracts derived from mature myeloid cells are analyzed. When using nuclear extracts isolated from nonexpressing cells, however, binding of the CCAAT box-binding factor is blocked by binding of the transcriptional repressor CCAAT displacement protein (CDP). The DNA binding activity of CDP is down-regulated during terminal differentiation of phagocytic cells (8). In addition, overexpression of cloned CDP in myeloid cell lines prevents induction of the gp91phox gene upon terminal phagocytic differentiation (9). Therefore, the stage-specific regulation of CDP is one important step in restricting gp91phox expression to mature phagocytic cells.

Molecular dissection of CDP revealed it to be a 180-kDa protein related to the Drosophila homeobox protein Cut (10–14). Cut is involved in determining cell fates in several Drosophila tissues including sensory organs and malpighian tubules (15–20). Cut expression is also detected in muscle, central nervous system, and ovarian follicle cells (21). However, the target genes and mechanism of action for Cut in Drosophila have not been reported.

We postulated that additional repressor elements reside up-

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The abbreviations used are: bp, base pairs; IFN, interferon; IRF, interferon regulatory factor; CDP, CCAAT displacement protein; EMSA, electrophoretic mobility shift assay.

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stream of the previously described CDP-binding site in the gp91phox promoter (8). This report describes the identification and functional characterization of three additional CDP-binding sites within the gp91phox promoter and illustrates the importance of transcriptional repression in the complex regulation of gp91phox expression.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—Plasmids containing the regions from -182 to +12 bp of wild-type sequence at their 5'-end and 40 bp of mutated sequence, including a CCAAT box derived from the α-globin promoter and 10 bp of unrelated sequence, and an XhoI site at their 3'-end were generated using the Sequence system (U.S. Biochemical Corp.). The resulting gp91phox promoter lacks the CDP-α site, but retains a CCAAT box in an unaltered position relative to the transcription initiation site. The entire nucleotide sequence of each mutagenized promoter fragment was determined using the Sequenase system (U.S. Biochemical Corp.). The -138 to +12 bp gp91phox promoter/luciferase constructs were generated by digesting the corresponding -450 to +12 bp constructs with HindIII, followed by intramolecular ligation.

Cell Culture and Transfections — The human cervical carcinoma cell line HeLa, the human monocyte/macrophage cell line HEL and in the undifferentiated myeloid cell line PLB985 (28) were synthesized on a 0.5 × Tris borateEDTA, 3% nonde- naturing polyacrylamide gel (except where otherwise indicated), and electrophoresis was carried out at 4°C and 25 mA for 90 min. Anti- serum raised against CDP (11), NF-Y (35), or preimmune serum was added to the binding reactions 10 min prior to the probe where indicated. NF-Y antisera was the generous gift of Diane Mathis.

RESULTS

Identification of Three Additional CDP-binding Sites within the Proximal gp91phox Promoter—Previously, we demonstrated that CDP binding to the gp91phox promoter (−132 to −86 bp) is necessary to repress promoter function in the erythroid cell line HEL and in the undifferentiated myeloid cell line PLB985 (8). We hypothesized that additional repressor sites were located upstream of −138 bp because a progressively greater degree of derepression was observed when constructs lacking the CDP-binding site were analyzed in the context of −1542 bp, −212 bp, or −138 bp of gp91phox promoter (8). Experiments were conducted to further characterize the gp91phox promoter region between −450 and −138 bp.

EMSA performed with nuclear extracts isolated from HeLa cells identifies three upstream gp91phox promoter fragments (CDP-β, −138 to −112 bp; CDP-γ, −241 to −192 bp; and CDP-δ, −382 to −313 bp) (Fig. 1A), each of which forms a complex that exhibits a mobility identical to that previously described for the CDP complex formed with the CDP-α probe (−137 to −76 bp) (Fig. 1B, −lanes). Similar complexes are also observed using nuclear extracts isolated from K562 and HEL cells (data not shown). Each of the four complexes is specifically disrupted by antisera raised against CDP (Fig. 1B, CDP-α lanes), but not by preimmune serum (preimmune lanes). Furthermore, each of these complexes is absent when EMSA is performed with nuclear extracts isolated from terminally differentiated PLB985 myeloid cells that lack CDP DNA binding activity (8) and express the endogenous gp91phox gene (Fig. 1C, Macrophage lanes). We conclude that four CDP-binding sites reside within the −382 to −76 bp region of the gp91phox promoter.

EMSA competition assays were performed to examine the sequence specificity and relative binding affinity of CDP for each of the four binding sites within the gp91phox promoter (Fig. 2). Each CDP complex is disrupted by the addition to the binding reaction of a molar excess of homologous double-stranded oligonucleotide competitor, but not by the addition of a heterologous oligonucleotide competitor (a CCAAT box element derived from the α-globin promoter). Cross-commission studies using the four CDP-binding sites as competitors reveal that CDP exhibits a range of affinities for the four binding sites within the gp91phox promoter. For example, the CDP-α site efficiently disrupts only the CDP-δ complex and...
partial disrupts the CDP-α complex at high competitor concentration. Overall, the affinity of CDP for each site correlates with the distance from the site of transcription initiation. CDP exhibits the greatest affinity for the CDP-α site and the weakest affinity for the CDP-δ site (Fig. 2).

The CDP-α and CDP-β oligonucleotides overlap by 25 bp. This region includes a portion of the DNase I footprint (−132 to −86 bp) produced by CDP binding to the CDP-α element (8). Additional studies were performed with oligonucleotides corresponding to truncated portions of CDP-β (Fig. 3A) to determine if CDP binding to the CDP-β oligonucleotide is due to the overlapping CDP-α sequence. An oligonucleotide competitor lacking the 3′-half of the overlapping region (Δ-CDP-β) exhibits a significantly reduced ability to disrupt the CDP complex formed with the full-length CDP-β-binding site probe (Fig. 3B, first triplet of lanes). However, a probe lacking the entire overlapping region (5′-CDP-β) and the Δ-CDP-β probe both produce CDP complexes in EMSA, although of weaker intensity compared with the full-length CDP-β-binding site probe (Fig. 3B, middle two triplets of lanes). Each of these CDP complexes is disrupted by competition with the full-length CDP-β oligonucleotide. However, a probe restricted to the overlapping region (3′-CDP-β) fails to form a CDP complex when used as a probe (Fig. 3B, last triplet of lanes). We conclude that the CDP-β complex is not determined.

![Fig. 1. CDP binds to four elements in the proximal gp91phox promoter.](image1)

A, shown is an illustration of the relative positions of oligonucleotide probes (CDP-α, -β, -γ, and -δ) derived from the gp91phox promoter and utilized in EMSA. B, CDP binds to each of four elements derived from the gp91phox promoter. EMSA was performed as described under “Experimental Procedures.” Probes were incubated with 3 μg of nuclear extract isolated from HeLa cells following preincubation with CDP antibody (CDP-ab) or preimmune serum as indicated. The CDP-γ probe used in this experiment corresponds to a dimer of the −241 to −192 bp gp91phox promoter, which explains the slightly higher position of the free probe and CDP complex. The position of the CDP complexes is indicated with an arrow. C, CDP binding to four gp91phox promoter elements is down-regulated during myeloid differentiation. Probes were incubated with 3 μg of nuclear extract isolated from PLB985 cells induced by phorbol 12-myristate 13-acetate treatment to differentiate along the macrophage lineage or nuclear extract isolated from HeLa cells. The position of the CDP complexes is indicated with an arrow.

![Fig. 2. Binding specificity and affinity of CDP for four binding sites within the gp91phox promoter.](image2)

EMSA was performed as described under “Experimental Procedures” using CDP-binding site probes, nuclear extract isolated from HeLa cells, and the indicated amount of nonradioactive oligonucleotides. 2 ng of competitor oligonucleotide correspond to an ~50-fold molar excess over the probe. The α-globin CCAAT box competition for the CDP-α probe is not shown since it has been previously reported not to affect the CDP complex (8). ND, not determined.
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![Diagram](image)

**Fig. 3.** Localization of the CDP-binding site within the CDP<sub>β</sub> oligonucleotide. A, shown is an illustration of oligonucleotides corresponding to regions of the gp91<sub>phox</sub> promoter that were used in EMSA: CDP<sub>α</sub>, −137 to −76 bp; CDP<sub>β</sub>, −182 to −112 bp; Δ-CDP<sub>β</sub>, truncated CDP<sub>β</sub> lacking one-half of the overlapping region between the CDP<sub>α</sub> and CDP<sub>β</sub> oligonucleotides (−182 to −124 bp); 5′-CDP<sub>β</sub>, truncated CDP<sub>β</sub> lacking the overlapping region between the CDP<sub>α</sub> and CDP<sub>β</sub> oligonucleotides (−182 to −137 bp); and 3′-CDP<sub>α</sub>, containing the region common to the CDP<sub>α</sub> and CDP<sub>β</sub> oligonucleotides (−138 to −112 bp). B, sequence upstream of CDP<sub>α</sub> is required for binding of CDP to the CDP<sub>β</sub> element. EMSA was performed as described under “Experimental Procedures” using 3 μg of nuclear extract isolated from HeLa cells. A competitor oligonucleotide containing the CP1-binding site of the α-globin promoter was used to identify the CP1 complex. The positions of the CDP and CP1 complexes are indicated by arrows. C, the CCAAT box-binding factor that binds to the CDP<sub>α</sub> probe is recognized by NF-Y (CP1) antiserum. EMSA was performed with the CDP<sub>α</sub> probe and nuclear extract isolated from 3T3 cells. 20 ng of α-globin competitor element contains an independent CDP-binding site, but sequence that overlaps with CDP<sub>α</sub> appears necessary for full binding affinity of CDP for the CDP<sub>β</sub> site.

The 3′-CDP<sub>β</sub> probe forms a complex with a CCAAT box-binding factor that is disrupted by competition with an oligonucleotide containing the α-globin promoter CCAAT box (Fig. 3B, last triplet of lanes). This is consistent with previous findings that a CCAAT box within the CDP<sub>α</sub> probe (−123 to −319 bp) is a binding site for a protein that exhibits a binding specificity similar to that of CP1 (8). Since the time of that report, antibodies directed against NF-Y, the murine homologue of CP1, have been described (35). An additional EMSA was performed with nuclear extracts isolated from murine 3T3 cells to determine if NF-Y antiserum affects the CCAAT box-binding factor complex produced with the CDP<sub>α</sub> probe. The CDP<sub>α</sub> probe generates a complex that is disrupted by the addition of a competitor derived from the α-globin CCAAT box region (Fig. 3C), consistent with the HeLa results presented in Fig. 3B. The addition of anti-NF-Y antiserum to the binding reaction alters the CCAAT box-binding complex and generates a supershifted band (Fig. 3C). This confirms that NF-Y, the murine homologue of CP1, binds to the CCAAT box at −123 to −119 bp of the gp91<sub>phox</sub> promoter. No effect is observed following the addition of normal serum to the reaction.

CDP Competes with Transcriptional Activating Factors for Binding to the gp91<sub>phox</sub> Promoter—CDP has been postulated to repress transcription by competing with transcriptional activating factors for overlapping promoter-binding sites (8, 36, 37). As illustrated in Fig. 4, we have previously demonstrated the presence of several binding sites for transcriptional activating proteins (CP1 and BID factors) that overlap the CDP-binding sites described in this report (8).<sup>2</sup> This suggests a model in which interaction of the activators with the gp91<sub>phox</sub> promoter is prevented by the binding of CDP in non-phagocytic cells. EMSA experiments were carried out under conditions of limiting probe to directly assess whether the binding of CDP is mutually exclusive with the binding of these activating factors.

When nuclear extracts isolated from HeLa cells are used in EMSA, the CDP complex predominates for the CDP<sub>α</sub>, β, γ, and δ gp91<sub>phox</sub> promoter probes (the CDP<sub>γ</sub> oligonucleotide is shifted 20 bp upstream relative to the CDP<sub>γ</sub> site)<sup>2</sup> (Fig. 5A). However, the CP1 and BID complexes become more intense following disruption of the CDP complex by the addition of a high affinity CDP-binding site competitor oligonucleotide (E 36) were added wherever indicated. NS, normal serum. The positions of the NF-Y (CP1) and supershifted (SS) complexes are indicated by arrows. Ab, antibody.
to the reaction (Fig. 5A, center lane for each probe), suggesting that CDP binds preferentially to each probe to the exclusion of BID and CP1. Similar results were obtained following preincubation of nuclear extract with anti-CDP antiserum (data not shown). An IRF-binding site is also found within the CDP-9 site, although BID and IRF-2 comigrate on a 3.5% gel. A similar experiment analyzed on a 6% gel revealed that both BID and IRF-2 binding are similarly increased following disruption of the CDP complex by the addition of the E36-binding site competitor (data not shown).

Ternary complexes containing probe and both CDP and a transcriptional activating factor should not form if CDP binds DNA in a mutually exclusive manner. Complexes trapped in the gel wells appear to contain CDP as they are disrupted by the addition of the E36 CDP-binding site oligonucleotide (Fig. 5, A and B). However, they are not affected by competition with oligonucleotides known to disrupt BID, IRF-2 (IRF-1 oligonucleotide), or CP1 (a-globin CCAAT box oligonucleotide) complexes or by an unrelated oligonucleotide (Mbo) (Fig. 5B). Hence, no ternary complex is detected that contains probe and both CDP and CP1, IRF, or BID.

**Functional Analysis of CDP-binding Sites**—A series of luciferase reporter gene constructs were generated that contain variable lengths of the gp91phox promoter, and hence different numbers of CDP-binding sites, to assess the contribution of the four CDP-binding sites to the transcriptional regulation of the
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![Functional analysis of CDP-binding sites on gp91\textsubscript{phox} promoter activity.](image)

The -450 to +12 bp gp91\textsubscript{phox} promoter/luciferase construct (wt450) produces a low level of reporter gene expression following transfection into cell lines that do not express the endogenous gp91\textsubscript{phox} gene, such as HEL, K562, and HeLa cells (Fig. 6). S'-Promoter truncations that remove one or two of the upstream CDP-binding sites (wt324 and wt212) have little effect on promoter activity. However, a truncation that removes the three upstream CDP-binding sites (wt138) results in a statistically significant increase in reporter gene expression as compared with the wt450 construct in both K562 and HEL cells (2.6-fold increase, p < 0.01; and 1.8-fold increase, p < 0.05, respectively). Deletion of four CDP-binding sites, leaving only the -100 to +12 bp region of the proximal gp91\textsubscript{phox} promoter, results in a further increase of promoter activity in K562 and HEL cells (5.4- and 19.5-fold increases, respectively; p < 0.01). None of the truncated gp91\textsubscript{phox} promoter fragments direct a significantly increased level of reporter gene expression in HeLa cells, however. These results suggest that CDP binding is required for repression of gp91\textsubscript{phox} gene expression in at least some non-phagocytic cells and confirm the functional significance of both the CDP-\(\alpha\) and CDP-\(\beta\)-binding sites in repressing gp91\textsubscript{phox} promoter activity in these cells.

Additional experiments were performed to analyze in more detail the functional significance of the CDP-\(\alpha\)-binding site. Specific deletion of the proximal CDP/CP1-binding site in the context of the -450 to +12 bp gp91\textsubscript{phox} promoter (del450) leads to derepression of luciferase expression in HEL cells (3.9-fold increase; p < 0.01), confirming the importance of the CDP-\(\alpha\)-binding site in the repression of the gp91\textsubscript{phox} promoter (Fig. 6). Similar results using a human growth hormone reporter gene were previously reported in both PLB985 and HEL cells for this deletion construct (8). Derepressed expression in HEL cells is also observed with this deletion in the context of -324 to +12 bp (del324) or -212 to +12 bp (del212) of gp91\textsubscript{phox} promoter (3.9- and 4.0-fold increases, respectively, relative to the corresponding wild-type constructs; p < 0.01) (Fig. 6).

However, these internal deletion constructs fail to exhibit a similar derepression in HeLa and K562 cells. Because the promoter deletion is not specific for the CDP-binding site, but rather also removes an overlapping binding site for CP1, we reasoned that in HeLa and K562 cells, the binding of CP1 may be necessary for gp91\textsubscript{phox} promoter activity in the absence of CDP binding. To test this hypothesis, a construct was created that lacks the CDP-\(\alpha\)-CDP-binding site, but retains a CP1-binding site (see "Experimental Procedures"). EMSA was performed with wild-type and mutated CDP-\(\alpha\)-site probes to determine the specificity of this mutation. An oligonucleotide competitor containing the CDP-specific mutation disrupts the CP1 complex, but not the CDP complex that forms with the wild-type probe (Fig. 7). When used as a probe, the oligonucleotide containing the CDP-specific mutation fails to generate a CDP complex, but forms a complex containing CP1 that is disrupted by a competitor oligonucleotide containing the CP1-binding site of the \(\alpha\)-globin promoter.

A luciferase reporter gene construct containing the CDP mutation within the CDP-\(\alpha\) element (mut450) produces significant derepressed expression following transfection into HeLa cells (4.8-fold increase; p < 0.01), K562 cells (7.7-fold increase; p < 0.01), and HEL cells (3.9-fold increase; p < 0.01) (Fig. 6). Similarly derepressed promoter activity is observed when the CDP-specific mutation is analyzed in the context of -324 to +12 bp (mut324) or -212 to +12 bp (mut212) of proximal gp91\textsubscript{phox} promoter. However, a significantly greater level of gp91\textsubscript{phox} promoter activity is observed when the CDP-specific mutation is analyzed in the absence of the CDP-\(\beta\) element (mut138) (HeLa cells, 7.1-fold increase; K562 cells, 16.4-fold increase; and HEL cells, 6.7-fold increase; p < 0.01). This confirms the importance of the CDP-\(\alpha\)-binding site in gp91\textsubscript{phox} regulation as well as the requirement of CP1 binding for full promoter activity in HeLa and K562 cells. In addition, the difference in all three cell lines between the mut212 and mut138 promoter activities provides additional functional evidence that the CDP-\(\beta\)-binding site also represses gp91\textsubscript{phox} expression.
DISCUSSION

CDP Is a Repressor of gp91<sub>phox</sub> Transcription—Transcriptional repressors play a crucial role in controlling gene expression during developmental processes (38–42). For example, neuron-specific silencer factor represses a wide range of neuron-specific genes and is down-regulated during neural differentiation (43). Hence, regulation of a repressor can act as a master regulatory switch, just as induction of transcriptional activators such as GATA-1 or MyoD has been postulated to serve as a global activator of specific developmental programs (44, 45).

This report examines four binding sites for the transcriptional repressor CDP within the proximal promoter region (−382 to −76 bp) of the myelomonocyte-specific gp91<sub>phox</sub> gene. Two of these CDP-binding sites repress gp91<sub>phox</sub> transcription in cells that do not express the endogenous gp91<sub>phox</sub> gene. Repression activity in transient transfection assays is not evident for the two distal CDP-binding sites. This may be because they appear to be the two weakest binding sites, and their effects may therefore be too subtle to detect in transient transfection assays. In addition, derepressed promoter activity following removal of upstream CDP-binding sites may require the retention of overlapping binding sites for transcriptional activators (Fig. 4), similar to the requirement for CP1 binding to the CDP-α site for expression in HeLa and K562 cells (Fig. 6). Efforts to specifically ablate the upstream CDP-binding sites have thus far been unsuccessful, but may be possible when the binding properties of the transcriptional activating protein(s) are fully characterized.

Significance of Multiple CDP-binding Sites in the gp91<sub>phox</sub> Promoter—The presence of four CDP-binding sites within the gp91<sub>phox</sub> promoter is the first example of multiple CDP-binding sites within a single promoter. However, a requirement for multiple binding sites has been reported for the activity of other transcriptional repressors, such as even-skipped, dorsal, and the Wilms’ tumor suppressor, WT1 (46–49). In addition, the binding of transcriptional repressors to multiple promoter sites can be cooperative. For example, binding of even-skipped to high affinity binding sites of the ultrabithorax gene promoter facilitates subsequent binding of even-skipped to low affinity sites that overlap the binding site for the transcriptional activator zeste (46). Exclusion of zeste binding appears to be at least one mechanism of transcriptional repression in this system. Similarly, dorsal binds cooperatively to multiple low affinity binding sites in the decapentaplegic gene (48), and WT1 binds to both high and low affinity sites within the insulin-like growth factor II promoter (49). Interaction between CDP molecules bound to the gp91<sub>phox</sub> promoter may similarly stabilize their binding and enhance the ability of CDP to exclude transcriptional activators. Alternatively, a single CDP molecule, which contains multiple DNA-binding domains (12, 31, 50), may simultaneously interact with more than one binding site, hence causing DNA looping and affecting the ability of a transcription initiation complex to form. The gp91<sub>phox</sub> promoter therefore provides an attractive system with which to further study the mechanism of CDP-mediated transcriptional repression.

Transcriptional Regulation of the gp91<sub>phox</sub> Gene Is Complex—EMSA competition experiments support the hypothesis that CDP binding to the gp91<sub>phox</sub> promoter is mutually exclusive with that of transcriptional activating factors (Figs. 4 and 5). The down-regulation of CDP thus appears to contribute to the apparent induction of BID complexes observed in EMSA when utilizing nuclear extracts isolated from differentiated myeloid cells. In addition, binding of HAF-1 to a more proximal promoter element is also necessary for normal gp91<sub>phox</sub> expression (6, 7). Hence, the regulation of the gp91<sub>phox</sub> promoter is complex and requires regulated interactions of both transcriptional repressors and activators with multiple promoter elements.

No derepression occurs in HeLa and K562 cells if the CDP-binding site within the CDP-α element is deleted, a mutation that also removes a CP1-binding site. However, reporter gene expression is enhanced 5–10-fold in these cell lines after the CP1-binding site is specifically restored. These results indicate that CP1 functions as a transcriptional activator of the gp91<sub>phox</sub> gene promoter and is necessary for the derepressed promoter activity evident in HeLa and K562 cells. This suggests that CDP represses the gp91<sub>phox</sub> promoter, at least in part, by preventing the binding of the ubiquitous CCAAT box-binding factor CP1.

In contrast, the derepressed gp91<sub>phox</sub> promoter activity observed in HEL and PLB985 cells does not require the CP1-binding site within the CDP-α element (Fig. 6 and Ref. 8). The molecular basis for variable CP1 requirements between cell types is currently under investigation, but is presumably due to differences between cell types in the complement of transcriptional activating factors that interact with other elements within the gp91<sub>phox</sub> promoter. The general phenomenon of different cell types requiring distinct cis-elements to direct transcription from the gp91<sub>phox</sub> promoter has been previously described. For example, promoter mutations in chronic granulomatous disease patients lead to the absence of gp91<sub>phox</sub> expression in the majority of phagocytes, while normal gp91<sub>phox</sub> expression persists in 5–10% of phagocytes (7). Another variant chronic granulomatous disease patient has been found to express gp91<sub>phox</sub> specifically in eosinophils, but not in other phagocytes (51). The defect responsible for this unusual
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Significant progress has recently been made in the identification of transcriptional activating factors controlling myeloid-specific gene expression. Functional binding sites for the transcriptional activators CCAAT/Enhancer-binding protein-β and PU.1 and the ubiquitous factor Sp1 have been identified in the promoters of many genes expressed in myeloid cells (52–60). However, these factors have not been detected binding to the gp91phox promoter. The model we present suggests an important role for the transcriptional repressor CDP in restricting expression of the gp91phox gene to mature myeloid cells. However, down-regulation of CDP DNA binding activity may not be sufficient to induce gp91phox expression, and the presence of additional lineage-restricted transcriptional activating factors may also be required for normal gp91phox promoter function. This might explain why the gp91phox gene is not induced in other cell types, such as myotubes, in which CDP has been reported to be down-regulated (10). The absence of activity for the wild-type –138 bp and –100 bp gp91phox promoter constructs in HeLa cells may reflect such a requirement for hematopoietic associated factors. Interestingly, HAF-1 binds at approximately –55 bp of the gp91phox promoter and is more abundant in K562 and HEL cells than in HeLa cells (6).

The –100 to +12 bp gp91phox promoter/luciferase construct directs significant decreased expression in HEL, K562, and PLB985 cells (Fig. 6 and Ref. 8), suggesting that cis-elements within this proximal region are capable of directing transcription in some non-phagocytic cells in the absence of CDP-mediated transcriptional repression. This assay system provides a means to dissect the cis-elements and cognate DNA-binding proteins required for derepressed promoter activity in the absence of CDP binding. This should provide additional insight into the mechanisms involved in CDP-mediated transcriptional repression as well as permit the identification of proximal promoter elements possibly involved in normal gp91phox transcription in mature phagocytes.

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