A Novel Transcription Factor Regulates Expression of the Vacuolar H\(^+\)-ATPase B2 Subunit through AP-2 Sites during Monocytic Differentiation* 

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During monocytic differentiation, the cellular content of vacuolar H\(^+\)-ATPase (V-ATPase) increases more than 4-fold. We have shown previously that amplified expression of the B2 subunit of the V-ATPase occurs solely by increased transcription, and that the 5'-untranslated region of the B2 gene, containing multiple consensus binding sites for the transcription factors AP-2 and Sp1, is required for this expression. The present study demonstrates that AP-2 binding sequences are essential for increased transcription from the B2 promoter during monococyte-macrophage differentiation, and that AP-2, expressed exogenously in THP-1 and other cells, activates transcription from the B2 promoter. In mobility shift assays, a nuclear factor from THP-1 and U-937 cells was identified that binds to several AP-2 response elements within the B2 promoter, but does not react with AP-2 antibodies, and has a DNA sequence binding affinity profile that differs from AP-2. These findings suggest that a novel AP-2-like transcription factor is responsible for V-ATPase B subunit amplification during monocye differentiation.

The mammalian vacuolar H\(^+\)-ATPase, or V-ATPase,\(^1\) is a multisubunit complex that transports protons electrogenically across the membranes of intracellular endocytic and secretory compartments in all eukaryotic cells and the plasma membrane in certain specialized cell types. Its role in acidifying organelles is required for protein transport, processing, and degradation. In the plasma membrane, the V-ATPase participates in transcellular transport of H\(^+\) and other ions and in defending cytosolic pH.

The V-ATPase is similar in structure to the F\(_{0}\)F\(_{1}\)-ATPases (1, 2) and is composed of two distinct macrodomains. The transmembrane domain (V\(_{0}\)) is composed of proteolipids and other integral membrane proteins and transmits protons through the lipid bilayer (3–7). The cytosolic domain of the enzyme (V\(_{1}\)) is composed of three copies each of the “A” subunit (70 kDa) and the “B” subunit (56–58 kDa), as well as a single copy of the “C” subunit (42 kDa), “D” (33 kDa), “E” (31 kDa), and “F” (14 kDa) subunits (reviewed in Ref. 8). The A subunit contains the site of catalytic ATP-binding site (9, 10), although the B subunit likely participates in catalysis (11) and may have a regulatory role (12, 13).

Two isoforms of the B subunit have been identified in mammals (14–16). They are encoded by different genes and differ in amino acid sequence at the amino and carboxyl termini, although no functional differences have yet been identified. Expression of the B1 isoform is restricted to only a few tissues; it is found at highest levels in kidney and placenta. The B2 isoform is ubiquitously expressed and is most abundant in kidney and brain.\(^2\) Cells of the monocytic lineage, including monocytes, macrophages, and osteoclasts, express V-ATPase with a B2 subunit both in intracellular compartments and on their plasma membranes (18). Macrophages express the V-ATPase on their plasma membranes to aid in intracellular pH regulation when exposed to acidic environments such as abscission tissue (19, 20); osteoclasts employ the V-ATPase in the ruffled membrane to generate an acidic microenvironment required for bone resorption (21).

In prior studies, we showed that during monocytic-to-macrophage differentiation the cellular content of V-ATPase subunits increases by as much as 5-fold in native monocytes and by 3–4-fold in the monocytic cell line THP-1 (18). Both transcriptional and post-transcriptional mechanisms of amplification were observed among the different V-ATPase subunits examined (18). The increased expression of the B2 subunit (approximately 3.5-fold) occurred solely by transcriptional activation. We isolated and characterized the proximal promoter region of the B2 gene and found that the DNA in the 5'-untranslated region of the gene was required for transcriptional activation during monocyte differentiation and that this region contained multiple binding sites for transcription factors Sp1 and AP-2.

AP-2 was first identified as a \(M_{r} = 52,000\) transcription factor (22, 23) that had unique patterns of expression in different tissues during embryonic development (24). Isoforms of AP-2 were later identified that were products of alternative splicing of RNA from a single gene (25, 26). Subsequently, Moser et al. (27) isolated murine genomic and cDNA clones for a homologue of AP-2, designated AP-2\(\beta\), encoded by a second gene. cDNAs from the two genes share an overall identity of 76% at the amino acid level, with the strongest homology in the carboxyl-terminal DNA binding/dimerization domain (85% identity, 92% similarity). Both AP-2\(\beta\) and the original AP-2 isoform (now designated AP-2\(\alpha\)) bind to the DNA consensus sequence 5'-GCCNNNGGC-3', although many variants of this sequence have been identified (28). The functional significance of the isoforms is unclear; both AP-2\(\beta\) and AP-2\(\alpha\) activate transcription to an equal extent, and the patterns of expression...
of both genes are highly similar.

In this report, we show that AP-2 binding sites in the promoter of the V-ATPase B2 gene are functional in vivo and are required for regulation of B2 expression during monocyte-to-macrophage differentiation. We identify a nuclear factor from human monocytic cell lines that binds to AP-2 consensus binding sites but is distinct from AP-2α and AP-2β as determined by DNA binding affinity and immunological reactivity.

**EXPERIMENTAL PROCEDURES**

**Materials**—Unless specified otherwise, all reagents were obtained from Sigma and were reagent grade.

**Isolation and Culture of Cells**—THP-1 and U-937 cells were obtained from the American Type Culture Collection, Rockville, MD, and were cultured in RPMI 1640 with 10% fetal bovine serum (HyClone; Logan, UT), 2 mM L-glutamine, and 50 μg/ml gentamicin in a 5% CO2 incubator. THP-1 cells were induced to differentiate by addition of 160 nM tetradecanoylphorbol-13-acetate (TPA) as described previously (18, 29).

**DNase I Footprinting**—DNase I footprinting was performed essentially as described (18), with the exception that the noncoding strand of probe −338 to +84 was radiolabeled.

**Promoter Constructs**—The isolation and sequence of the B2 gene proximal promoter was described previously (18). B2 promoter fragments were inserted at the 5′ end of the luciferase reporter gene in the vector pGL3 (Promega), with the exception of B2 fragment −317 to −52, which was subcloned into the vector pGL2 (Promega). The −317 to −124 site 1 mutant was created by using restriction enzymes to excise a portion of the wild-type promoter containing binding site 1 and then ligating in synthetic oligonucleotides containing the mutant site. In this manner, the core AP-2 binding sequence of site 1 was changed from 5′-GGTCTGGCC-3′ to 5′-AATATAAAA-3′. Recombinant human AP-2α was expressed from an SV40-derived promoter in the vector pSAP2 (a kind gift of M. Tainsky, University of Texas M.D. Anderson Cancer Center).

**Transfection of Cells**—Transfections of THP-1 cells were performed as described previously (18, 30). For AP-2α co-transfection experiments, 5 μg of B2 promoter-luciferase reporter plasmid plus 2 μg of pRSVcat, a vector for expression of chloramphenicol acetyltransferase (CAT) used to normalize luciferase activity for transfection efficiency, were co-transfected with 0−2 μg of pSAP2. Cloning vector pGEM-5Z was added to the DNA mixtures as needed to equalize the DNA concentration among transfections. Forty-eight hours after transfection, cells were harvested and prepared for luciferase and CAT assays. The experiments using transfection and subsequent differentiation of THP-1 cells were performed as described previously (18). Luciferase and CAT assays were performed as described previously (18, 32).

**Isolation of AP-2α cDNAs from THP-1 Cells**—THP-1 cells were induced with TPA for 24 h as described above. mRNA was isolated using the RNasey Amphone kit (Invitrogen, San Diego, CA), following the manufacturer's instructions. cDNA synthesis and subcloning into the λ vector Uni-ZAP were performed using a commercially available cDNA synthesis kit (Stratagene; La Jolla, CA). For screening of this library, 1 × 108 plaques on nitrocellulose filters (Schleicher & Schuell) were probed with a 32P-labeled 361-base pair Psrl cDNA fragment corresponding to a region within the DNA-binding domain of human AP-2α (spanning amino acid residues 278−399; EMBL/GenBank accession number Y00029). Following hybridization of the probe, filters were washed under final conditions of 42°C in 2× SSPE and 0.1% SDS. Plaques were purified through multiple rounds of screening, and pBluescript plasmids containing the inserts of interest were excised from the Uni-ZAP vector using ExAssist interference-resistant helper phage (Stratagene).

**Nuclear Extracts and Gel Mobility Shift Assays**—Nuclear extracts from dividing or differentiated THP-1 cells were prepared by the method of Dignam et al. (33), except nuclear proteins were further purified by precipitation with 55% saturated ammonium sulfate prior to dialysis. Nuclear extracts from other cell types were prepared by a modification of the method of Lee et al. (34) for small cell numbers.

**Expression of Exogenous AP-2 Increases Expression from B2 Gene Promoters**—To determine whether AP-2 can bind to the B2 promoter in vivo and mediate induction of gene expression, we co-transfected into undifferentiated THP-1 cells up to 2 μg of the plasmid pSAP2, for exogenous expression of human AP-2α (35), with a plasmid containing one of several B2 promoter-luciferase reporter constructs. Five promoter constructs were used. 1) A fragment spanning from −317 to +30 containing AP-2 binding sites 1–8; 2) fragment −317 to −52 containing AP-2 binding sites 1–5; 3) fragment −317 to −124 containing site 1 only; 4) −317 to −199, containing no AP-2 binding sites except the sequence at the transcriptional start site; and 5) −317 to −124mut, containing a mutated AP-2 binding site 1.

**RESULTS**

The 5′-Untranslated Region of the B2 Promoter Contains Multiple AP-2 Binding Sites—In previous DNase I footprinting studies in which the coding strand of the B2 promoter was labeled (18), we found that the 5′-untranslated region and proximal coding region of the B2 promoter contained five sequences capable of binding purified AP-2α in vitro. In the present study, we performed footprinting experiments in which the non-coding strand was labeled. We identified three additional potential AP-2 binding sites (Figs. 1 and 2). The five AP-2 binding sites noted previously are designated sites 2, 5, 6, 7, and 8. Of the three newly identified AP-2 binding sequences, two (sites 3 and 4), composed of the sequence 5′-GGTCTGGCC-3′ (where R indicates purine), were found embedded within Sp1 binding sites. The most proximal site (site 1) contained a near-perfect palindromic sequence 5′-GGTCTGGCC-3′. Purified AP-2α also bound at the G + C-rich region of transcriptional initiation, although this site would not be expected to be active in vivo due to interference from the basal transcription complex. Promoter deletion studies (Fig. 3, discussed below) show that this site does not mediate AP-2-enhanced transcription in vivo.
began to decrease (not shown), most likely due to self-interference of AP-2 transcriptional activation as described by Kannan et al. (35). Expression of exogenous AP-2α in the cell lines LLC-PK1 (a porcine kidney proximal tubule line), 293 (a human embryonic kidney fibroblast line), and HepG2 (a human hepatoma line) also resulted in increased activity of the B2 promoter, but higher concentrations of pSAP-2 plasmid were required for promoter activation in 293 and HepG2 cells (data not shown). These results demonstrate that AP-2α is capable of activating transcription from the V-ATPase B2 promoter in multiple cell lines.

**FIG. 1.** DNase I footprint analysis of the 5′-untranslated region of the B2 gene. A DNA probe spanning the entire 5′-untranslated region (−207 to −1) was incubated with purified Sp1 (lane 1) or AP-2α (lane 3), digested with DNase I, and separated by electrophoresis as described under “Experimental Procedures.” A total of eight AP-2 binding sites and two Sp1 binding sites were identified.

**FIG. 2.** AP-2 and Sp1 binding sites in the first exon of the V-ATPase B2 gene. The AP-2 binding sites are underlined and numbered in accordance with Fig. 1. Sp1 binding sites (GC boxes) are in bold type. The nucleotide sequence was reported previously (18).

**FIG. 3.** Activation of transcription from the B2 promoter by AP-2α. Fragments of the B2 promoter containing indicated numbers of AP-2 binding sites were ligated to the 5′ end of a luciferase reporter cDNA. The constructs were co-transfected into THP-1 monocytes with varying amounts of the AP-2α expression vector pSAP2 as shown, and with the plasmid pRSVcat as described under “Experimental Procedures.” Activity is expressed as the ratio of luciferase activity to CAT activity. At least four independent experiments were performed for each construct.

**FIG. 4.** Deletion analysis of promoter activity in the 5′-untranslated region of the B2 gene. Luciferase reporter constructs containing the indicated fragments of the B2 promoter were transfected into THP-1 monocytes. After 2 days, the transfected cells were divided and incubated an additional 5 h with and without 160 nM TPA. Bars (mean ± S.E.) represent the luciferase activity in TPA-treated cells expressed as a percent of activity in the control undifferentiated cells. Data from the top four constructs and from promoter fragment −274 to −199 were reported previously (18) and are shown for comparison. *, p < 0.002. The number of independent trials for each construct is shown in parentheses.
those mediated by longer constructs, a result similar to that obtained by coexpression of exogenous AP-2 with this promoter fragment (see Fig. 3). These results suggest that site 1, or surrounding sequences, accounts for at least part of the transcriptional response to a differentiation stimulus. To determine whether deletion of site 1 abolished transcriptional activation during monocyte-to-macrophage differentiation, we examined the ability of construct −317 to −124mut, containing a mutation in site 1 (see Fig. 3 and “Experimental Procedures”), to enhance transcription during monocyte-macrophage differentiation. In THP-1 monocytes transfected with this construct, no increase in luciferase activity was observed following phorbol ester treatment (Fig. 4). This indicates that AP-2 binding sites have an essential role in induction of B2 expression during monocyte-to-macrophage differentiation.

A Monocytic Nuclear Protein Binds to AP-2 Consensus Sequences—To identify monocytic nuclear proteins capable of binding AP-2 binding sites, we performed gel mobility shift assays using nuclear extracts from undifferentiated or differentiated THP-1 cells. Nuclear extracts from both THP-1 monocytes and THP-1 macrophages contained a protein that bound to radiolabeled oligonucleotides containing AP-2 binding site 1 from the B2 promoter (Fig. 5A). Binding specificity was demonstrated by addition of either unlabeled probe or an unlabeled oligonucleotide containing a different AP-2 binding site (from the human metallothionein IIa basal level element (28)), which abolished binding of the nuclear proteins to the labeled probe; addition of an irrelevant oligonucleotide, however (in this case, the binding sequence for Sp1, another G+C-rich sequence), did not abolish binding of the proteins to the labeled probe. The amount of site 1 oligonucleotide bound by nuclear extract was increased in THP-1 macrophages compared with THP-1 monocytes (Fig. 5A).

To determine if the apparent increase in the AP-2-like factor in THP-1 macrophages was due to a more efficient extract preparation, we assayed the same extracts for Sp1 binding proteins. Studies in U-937 cells, another human monocytic leukemia cell line, have shown that the Sp1 content of monocytes does not change significantly during TPA-induced differentiation into macrophages (36). We found that the Sp1 content of THP-1 cell nuclear extract preparations exhibited a minor decrease during differentiation to macrophages (Fig. 5B), suggesting that greater recovery of transcription factors was not the reason for the increase in AP-2-like factor binding. The basis for the slight decrease in the Sp1 content of THP-1 cell nuclear extract is unclear but may have caused underestimation of the increase in AP-2-like factor binding.

We next incubated THP-1 nuclear extracts, or purified AP-2α, with oligonucleotides containing different AP-2 binding sequences to determine whether any differences in binding affinity existed among several sequences of DNA capable of binding AP-2α (Fig. 6). The oligonucleotide probes included the following: 1) a commercially available AP-2 binding sequence (Promega) from the human metallothionein IIa distal basal level element (hMtIIa BLE; (28)), which abolished binding of the nuclear proteins to the labeled probe; addition of an irrelevant oligonucleotide, however (in this case, the binding sequence for Sp1, another G+C-rich sequence), did not abolish binding of the proteins to the labeled probe. The amount of site 1 oligonucleotide bound by nuclear extract was increased in THP-1 macrophages compared with THP-1 monocytes (Fig. 5A).

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\[ LANE \]

A

Extracts

THP-1 monocyte:

THP-1 macrophage:

Competitive Oligonucleotides

Site 1:

HMtIIa:

Sp1:

- + + + - - -

- - - + - - +

- - - + - - +

- - - + - - +

B

LANE

THP-1 monocyte ext.:

THP-1 macrophage ext.:

Recombinant Sp1:

Sp1 oligo competition:

- + + - - -

- - - + + +

- - - + - - +

- - - + - - +

Fig. 5. Mobility shift analysis of THP-1 nuclear extracts probed with B2 promoter AP-2 site 1 oligonucleotide. A, nuclear extracts from control THP-1 cells (monocytes, lanes 2–5) or TPA-treated cells (macrophages, lanes 6–9) analyzed for protein-DNA complex formation using labeled double-stranded oligonucleotides corresponding to AP-2 binding site 1 of the B2 promoter. Unlabeled competitor oligonucleotides (50-fold excess over labeled probe) were added to mixtures to determine specificity of binding: lanes 3 and 7, competition with unlabeled site 1 probe; lanes 4 and 8 competition with the AP-2 binding site of the human metallothionein IIa basal level element; lanes 5 and 9, competition with an Sp1 binding site probe (GC box), demonstrating lack of competitive binding to the THP-1 cell nuclear factor. B, nuclear extracts from THP-1 monocytes (lanes 2 and 3) and macrophages (lanes 4 and 5) were analyzed for binding to a double-stranded oligonucleotide containing an Sp1 binding site. Lanes 3 and 5, competition with unlabeled probe; lane 6, binding of probe to recombinant Sp1. The nuclear extracts (lanes 2–5) produced protein-DNA complexes with different mobilities, most likely due to different post-translation modifications of Sp1 as described previously (17).
AP-2α exhibited different relative affinities for the probes tested. The hMtIIa BLE site oligonucleotide bound strongly to recombinant AP-2α, forming a single protein-DNA complex. When incubated with THP-1 extracts, the hMtIIa BLE site oligonucleotide formed multiple protein-DNA complexes. In contrast, the B2 site 1 and 5 oligonucleotide probes formed a single predominant protein-DNA complex in THP-1 nuclear extracts and bound to recombinant AP-2α weakly in comparison to the hMtIIa probe. Quantitation of the THP-1 factor (see Fig. 7, below) showed the concentration of the THP-1 factor using the gel mobility shift assay. A constant amount of THP-1 monocyte extract was incubated with increasing amounts of radiolabeled site 5 probe, and the amount of protein-DNA complex formed was assayed, allowing the number of moles of probe bound to be determined when all of the available THP-1 factor was complexed (Fig. 7A). The intensity of the factor-DNA complex at saturation was compared with a standard curve of free site 5 probe at a known specific activity (Fig. 7B). Assuming a 1:1 molar ratio of factor to DNA in the complex, we estimated the concentration of THP-1 monocyte factor in our standard DNA-binding reactions (i.e. Fig. 6) to be 0.12 nM. In contrast, the concentration of recombinant AP-2α in the reactions shown in Fig. 6 was 40 nM. Thus, even at a concentration 330-fold greater than the THP-1 factor, AP-2α bound to the site 1 and 5 probes only weakly. Both the difference in the affinity of AP-2α and the THP-1 factor for different probes, and the differences in mobility of the protein-DNA complexes formed, strongly suggest that the two proteins are distinct.

As an additional and independent method for determining whether the THP-1 nuclear factor was different from AP-2α, we analyzed the protein-DNA complexes using the antibody “supershift” method (37) to determine whether the factor is immunologically similar to AP-2 (Fig. 8). Antibody to the carboxyl terminus of human AP-2α (Santa Cruz Biotechnology), incubated with nuclear proteins prior to probe addition and electrophoresis, did not produce a mobility supershift of protein-DNA complexes from THP-1 cells (from either the monocytic or macrophage-like forms), but the antibody shifted the mobility of the entire AP-2α-oligonucleotide complex. Although the antibody was not tested directly in supershift assays for binding to AP-2β, it does detect AP-2β on immunoblots and should have caused a mobility supershift if the THP-1 factor were AP-2β (27).

To determine whether other cells of the monocytic lineage have an AP-2 site-binding protein with properties similar to
the factor in THP-1 cells, we performed mobility shift and supershift assays with nuclear extracts from the promonocytic cell line U-937 (Fig. 8). Like THP-1 cells, U-937 nuclear extracts contained a protein that formed complexes with AP-2 consensus binding sequences, but showed a higher mobility than AP-2α in gel mobility shift assays, and did not exhibit a mobility supershift with the anti-AP-2α antibody. The protein-DNA complexes from the THP-1 and U-937 nuclear extracts failed to show any mobility supershift with addition of as much as 5 μg of anti-AP-2 antibody (not shown).

Because AP-2-like factors have not been well studied in monocytic cell lines, we examined the possibility that THP-1 cells express novel proteins with homology to AP-2 DNA-binding domains. A THP-1 macrophage library was created and screened at low stringency using a cDNA probe corresponding to the DNA-binding domain of AP-2. A THP-1 macrophage library was created and screened at low stringency using a cDNA probe corresponding to the DNA-binding domain of AP-2. Three plaques were isolated from approximately 1 × 10^6 screened plaques that exhibited a positive signal through three rounds of screening. All three recombinant phage plaques contained the same insert, which encoded portions of the DNA binding domain and 3′-untranslated region of AP-2α (nucleotides 1184–2103 relative to the start of transcription). Thus, THP-1 cells express AP-2α, even though the predominant DNA-binding protein in the mobility shift assays appeared to be a distinct factor.

To address the possibility that the monocytic factor was indeed AP-2α, but was exhibiting altered mobility and immunoreactivity due to effects of the nuclear extract, we performed experiments in which recombinant AP-2α was added to THP-1 nuclear extracts, and the mixture was used to perform mobility shift and supershift assays (Fig. 9). When incubated with THP-1 nuclear extracts, AP-2α retained its original mobility and ability to react with the anti-AP-2α antibody, whereas the monocytic factor was unaffected (Fig. 9). These data, along with those shown in Figs. 6–8, indicate that the predominant factor in THP-1 nuclei that binds to AP-2 binding sites in the B2 promoter is distinct from AP-2.

DISCUSSION

Leukemic cell lines of the myeloid lineage, such as THP-1, have served as excellent tools in studies of the terminal differentiation processes of hematopoietic cells (38). In this report, we have demonstrated that AP-2 binding sites are essential for amplification of V-ATPase B2 subunit transcription during macrophage differentiation. We have identified a nuclear factor in THP-1 cells that binds to these sites and whose binding activity increases following differentiation of THP-1 cells into macrophages. Our results therefore suggest that a factor related to the AP-2 family of factors is involved in gene regulation during macrophage differentiation. The predominant protein from THP-1 cell nuclear extracts forming protein-DNA complexes with AP-2 binding sites, however, is a novel factor distinct from AP-2.

Recent studies have implicated transcription factors from multiple gene families as critical for terminal differentiation of macrophages. These factors include members of the STAT (39) and C/EBP (40) families; Pu.1, a member of the ets family (41); and Egr-1, a zinc finger transcription factor (42), among others. Although the role of transcription factor AP-2 in terminal differentiation has been elucidated in other cell types, including neuroectodermal and epidermal cells (43, 44), the role of AP-2...
in monocytic differentiation and gene expression has been addressed in only a few studies that have used the U-937 cell model. AP-2 binding sites were found to be required for tumor necrosis factor α expression induced by granulocyte-macrophage colony-stimulating factor but not by phorbol ester (45). AP-2 binding sequences were also found not to have any enhancer activity for transcription of downstream reporters during phorbol ester-induced differentiation of U-937 cells (46). Although these findings appear to conflict with our results, we have shown that THP-1 cells closely mimic primary blood monocytes in their ability to express V-ATPase during differentiation, whereas U-937 cells do not (18). The mechanisms that cause increased transcription of the B2 subunit in primary blood monocytes and phorbol ester-induced THP-1 cells therefore may not be functional in phorbol ester-treated U-937 cells. In support of this possibility, we found that the AP-2 site-binding activity was lower in nuclear extracts from phorbol ester-treated U-937 cells than from control U-937 promonocytes (data not shown).

The promoter of the V-ATPase B2 subunit contains eight functional AP-2 binding sites, and at least one of these sites is necessary for increased expression from the B2 promoter during macrophage differentiation. We have not determined if site 1 is the only active site in the B2 promoter nor if other downstream sequences are active in vivo. Promoters containing site 1 alone (−317 to −124) showed transcriptional activity somewhat less than those containing additional AP-2 binding sites and an Sp1 site (−317 to −96; Figs. 3 and 4), but these differences did not reach statistical significance. Four of the AP-2 binding sites (sites 2–5) are in such close proximity to Sp1-binding sites that interference, due to binding of Sp1, is likely to occur. We have tested this by performing gel mobility shift assays with an oligonucleotide probe that contains AP-2 binding sites 4 and 5 and the overlapping Sp1-binding site. When incubated with THP-1 monocyte or macrophage nuclear extracts, the only detectable protein-DNA complexes formed were with Sp1 (data not shown). These results suggest that Sp1 in the nuclear extracts interferes with the binding of the THP-1 factor to AP-2 sites 4 and 5. Competition by transcription factors for adjacent and overlapping binding sites has been noted previously for several promoters and is a mechanism by which the action of factors can be regulated (47–49). DNase I footprinting of promoter using the nuclear extracts will be required, however, for a direct assessment of protein-DNA interactions in the B2 subunit promoter.

The identity of the factor from THP-1 and U-937 cell nuclei identified here is unknown. A probe containing the conserved DNA binding domain of AP-2α that was used to screen a THP-1 cell cDNA library at low stringency hybridized only to a single gene product, AP-2α itself. Since AP-2α is expressed in these cells, it is unclear why it was not detected in the mobility shift assays. The most likely explanation is that the THP-1 factor binds AP-2 consensus sequences in the B2 promoter with a higher affinity than AP-2α, as shown in Fig. 6. When mobility shift assays were performed on THP-1 nuclear extracts using an oligonucleotide probe that binds strongly to purified AP-2α (the hMIIα BLE site), several protein-DNA complexes were formed, one of which was nearly identical in size to the complex formed with recombinant AP-2α. In experiments using the site 1 or site 5 probes, a barely detectable protein-DNA complex was occasionally present with the same mobility as the AP-2α-hMIIα BLE complex, but it was not observed reproducibly enough to allow determination of its identity.

Although these studies have not excluded the possibility that AP-2α has a role in B2 transcription in vivo, our experiments suggest that the THP-1 factor identified here is the predominant activator of the B2 promoter in cells of the monocytic lineage. Preliminary studies indicate that a factor similar to the one described here is present in differentiated bone marrow and spleen cell cultures enriched for osteoclasts (data not shown). Efforts to purify this factor and determine its identity are in progress.

The B2 isoform of the vacuolar H+-ATPase appears to be a “housekeeping” gene product, residing in the V-ATPases that acidify the intracellular membranes of vertebrate cells (8, 16, 50). Many housekeeping genes contain G+C-rich TATA-less promoters, similar to the B2 subunit promoter. In contrast to most housekeeping genes, however, the V-ATPase B2 subunit is highly expressed in a small population of cells that are specialized for proton secretion, such as macrophages, osteoclasts, and the renal proximal tubule, in cells that maintain high rates of endocytosis and exocytosis, including macrophages and proximal tubule cells, and in cells that require V-ATPase activity for solute transport, such as neurons. AP-2-like proteins may provide a mechanism by which amplified expression of the V-ATPase may occur in specific cell types. It is perhaps not coincidental that the kidney and brain, which express high levels of B2 mRNA, also express very high levels of AP-2 proteins during development and in adulthood. Our studies may have identified a unique transcription factor, binding to AP-2 sites, that confers the capacity for cells to maintain high levels of B2 expression.

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3 B. S. Lee and S. L. Gluck, unpublished results.
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