Lipopolysaccharide Induction of Tissue Factor in THP-1 Cells Involves Jun Protein Phosphorylation and Nuclear Factor κB Nuclear Translocation*

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Tissue factor (TF) expression is transiently induced in human monocytic THP-1 cells by lipopolysaccharide (LPS). We characterized the transcription factor complexes binding to the TF gene promoter LPS response element (LRE) (−220 to −172), which contains binding sites for nuclear factor κB (NFκB) and activator protein 1 (AP1) transcription factors, and examined the nature of the activation of these factors during a 24-h time course of LPS stimulation. We found proteolysis of the cytoplasmic inhibitory protein IκB and nuclear translocation of the NFκB/Rel family proteins p65 and c-Rel, corresponding to the transient binding of a p65/c-Rel heterodimer to the κB-like site of the LRE. AP1 binding to the LRE was found to be constitutive, with the majority of the AP1 complexes being JunD/Fra-2 heterodimers. A change in the activation state of the AP1 complexes was, however, found to be transient, as determined by JunD phosphorylation of AP1 bound to the proximal binding site. This directly correlates to the transient activation of Jun N-terminal kinase (SAPK/JNK). These data indicate that LPS induction of TF gene expression in monocytes THP-1 cells is regulated by both the transient phosphorylation of Jun-family proteins and the nuclear translocation and transient binding of NFκB/Rel proteins.

LPS-induced monocytes, TF mRNA levels increase as a result of transcriptional activation (7). In the monocytic cell line THP-1, TF gene expression is induced by LPS in a similar manner with, however, some increase in mRNA stability as well (9).

Functional studies in THP-1 cells identified an enhancer in the TF gene promoter that mediates LPS induction. This 56-base pair region (−227 to −172) is termed the LPS response element (LRE) (10). In addition, a second region (−85 to −52) containing Egr-1 binding sites has been identified that is also subject to inducible binding (11). Our study focused on the LRE that contains two AP-1 sites (a distal, low affinity site and a proximal, high affinity site) and an NFκB-like site. Mutation of any of these sites compromises LPS inducibility (10, 12), suggesting that all three are required for optimal LPS induction.

In this study we have analyzed the protein interactions with the LRE binding sites over a 24-h time course of LPS induction in THP-1 cells. We have determined that a number of regulatory mechanisms act to control TF gene transcription in response to LPS stimulation. These include the transient binding of a p65/c-Rel heterodimer from at least 30 min to 2 h, resulting from the proteolysis of IκB and nuclear translocation of p65 and c-Rel and transient phosphorylation of JunD in LRE-bound AP-1 complexes correlating to the activation of Jun N-terminal kinase (SAPK/JNK) from 10 min up to 1 h of LPS treatment. These data suggest multiple mechanisms acting co-operatively at the LRE enhancer element to direct a transient increase in TF mRNA levels in monocytic THP-1 cells.

EXPERIMENTAL PROCEDURES

Cell Lines—THP-1 cells (13) were grown in RPMI 1640 with l-glutamine and 25 mM HEPES buffer (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and 100 units/ml penicillin, 100 μg/ml streptomycin. Cells were routinely grown to a density of 1 × 10⁶ cells/ml and induced with 10 μg/ml LPS from Salmonella typhimurium (Sigma) for the times indicated in the figures.

TF Antigen Assay—THP-1 cells were recovered from suspension and washed in phosphate–buffered saline. Cell extracts were then prepared, and TF antigen levels were determined by enzyme-linked immunosorbent assay as described by Consomni and Bertina (14), using TF 4503 monoclonal antibody (American Diagnostica, Greenwich, CT) as the catching antibody and biotinylated TF 5 monoclonal antibody (Costar) as the tagging antibody. Recombinase P/Innolin (Baxter Diagnostica Inc., Deerfield, IL) was used as a standard after calibration against the standard of the Immunobind tissue factor enzyme-linked immunosorbent assay kit (American Diagnostica Inc., Greenwich, CT). The final results were expressed as ng TF/10⁶ cells.

RNA Isolation and Northern Blotting—Total RNA was prepared from THP-1 cells using the TRIzol reagent (Life Technologies, Inc.), a modification of the guanidinium isothiocyanate method (15). 15 μg of total RNA/time point were fractionated on 1% agarose, 0.22 M formaldehyde gels in 20 mM MOPS, 5 mM sodium acetate, pH 7.0, 1 mM EDTA and 0.22 M formaldehyde. After staining with ethidium bromide under UV light, RNA was transferred to Hybond-N filters (Amersham Pharmacia Biotech) by standard capillary transfer methods (16). The RNA was then immobilized by UV...
irradiation on a UV Stratagene 1800 (Stratagene, San Diego, CA). Filters were prehybridized for 3 h at 42 °C in 50% denatured formamide, 0.5% SDS, 5 × Denhardt’s solution, 5 × SSPE (0.9 M NaCl, 50 mM sodium phosphate, pH 7.7, 0.5 mM Na2EDTA), and 100 μg/ml sheared salmon sperm DNA. Filters were then hybridized for 18 h under the same conditions with a 32P-labeled glyceraldehyde-3-phosphate dehydrogenase cDNA probe (18).

**Electrophoretic Mobility Shift Assays**—Crude nuclear extracts were prepared for use in electrophoretic mobility shift assays (EMSA) essentially as described previously (11). Modifications include cell shearing by repeated aspiration through a 27-gauge needle rather than a Dounce homogenizer and the addition of phosphatase inhibitors (0.25 mM orthovanadate and 25 mM β-glycerophosphate) to all solutions. Protein concentrations of all extracts were determined using the Bio-Rad protein assay reagent.

The following oligonucleotides were radiolabeled using T4 polynucleotide kinase (Epigenic Technologies) and [γ-32P]ATP (Amersham Pharmacia Biotech) before being annealed to oligonucleotides of complementarity in 10% formamide, 50 mM NaCl, 1 mM dithiothreitol (binding buffer 1: 23 mM Hepes, pH 7.9, 113 mM NaCl, 9 mM MgCl2, 0.23 mM EDTA, 19% glycerol, 4.5 mM dithiothreitol, 19% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.75 μg/ml each leupeptin, aprotinin, antipain, pepstatin A) (19). Two binding reaction buffers were used; one optimized for AP1 binding (binding buffer 2: 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM dithiotreitol, 1 mM EDTA, 19% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.75 μg/ml each leupeptin, aprotinin, antipain, pepstatin A) (11) and a second optimized for NFκB binding (binding buffer 2: 20 mM Tris, pH 7.5, 50 mM NaCl, 1 mM dithiotreitol, 1 mM EDTA, 19% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.75 μg/ml each leupeptin, aprotinin, antipain, pepstatin A) (19). 20 μl of binding reactions contained 5 μg of nuclear extract, 0.5 ng of 32P-labeled double-stranded oligonucleotide, and 3 μg poly(dI-dC), unless otherwise stated. When looking specifically for phosphorylated proteins, the binding reactions also contained orthovanadate and β-glycerophosphate to allow examination of the relative contributions of the 32P-labeled and unphosphorylated protein standard used (New England Biolabs). Comparative blots of nuclear and cytoplasmic proteins were performed simultaneously and exposed to film for the same length of time.

**Antibodies**—The polyclonal antibodies used in the EMSA supershift assays and Western blot analyses were as follows: c-Jun/AP-1 (sc-44x); c-Jun/AP-1 (sc-45x); JunB (sc-46x); JunD (sc-74x); ATF-2 (sc-187x); c-Fos (sc-253x); c-Fos (sc-52x); FosB (sc-48x); Fra-1 (sc-605x); Fra-2 (sc-171x); NFκB p50 (sc-114x); NFκB p52 (sc-298x); NFκB p65 (sc-109x); RelB (sc-226x); c-Rel (C) (sc-71x); c-Rel (N) (sc-70x), all obtained from Santa Cruz Biotechnology; phospho-specific ATF-2 (Thr 71) (9164); phospho-specific SAPK/JNK (Thr-183/Tyr-185) (9251) were all obtained from New England Biolabs. The α-tubulin antibody used in the Western blot analysis was a kind gift from Dr. Ingrid Gaemers obtained from Dr. Pavel Draber (21, 22).

**RESULTS**

**LPS Stimulation of Monocytic THP-1 Cells Produces Transient Increases in the Levels of TF Antigen and mRNA**—To determine their response to LPS, monocytic THP-1 cells were incubated with 10 μg/ml LPS for various times up to 24 h. TF mRNA, analyzed by Northern blot hybridization (Fig. 1), increased by 30 min and reached a peak at 1 h. Levels dropped considerably by 2 h and had returned to preinduction levels at the following time points. Larger transcripts resulting from incomplete splicing (17) can be seen in addition to the mature 2.2-kilobase mRNA. A corresponding increase in the level of TF antigen was also observed, reaching a peak at 2 h then decreasing through to 24 h (data not shown).

**Binding of AP1 and NFκB Complexes**—The EMSA studies presented were predominantly carried out using the AP1α subunit (−213 to −172). This region of the TF LRE was chosen as AP1 consists of various dimers of Fos and Jun family proteins, and this oligonucleotide together with TPAP1distal allowed us to distinguish between AP1 complexes binding to the proximal and distal AP1 sites while maintaining possible effects on complex binding arising from the proximity of the adjacent high affinity proximal AP1 site and the κB-like site. We found that variation in the binding reaction conditions had a noticeable effect on the relative intensities of the DNA-protein complexes that were observed (Fig. 2). Under conditions that seemed to favor AP1 binding (binding buffer 1) two complexes became apparent whose intensity appears to be relatively constant throughout the time course (Fig. 2A). A very faint larger complex (I) and a smaller doublet complex (II), which constituted the majority of the binding to this oligonucleotide, are seen. Both complexes I and II could be competed with a 100-fold molar excess of AP1ακB and an AP1 consensus

**Fig. 1.** Time course of LPS induction of TF in THP-1 cells. THP-1 cells were cultured in the absence or presence of 10 μg/ml LPS for the times indicated. Total RNA was fractionated and transferred to a membrane; TF mRNA levels were determined using Northern hybridization with a TF cDNA probe. 15 μg of total RNA/time point were loaded. Equivalence of loading was examined by rehybridization of the stripped blot with a cDNA probe for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) house-keeping gene. kb, kilobases.
competitor but not with an NFκB consensus or a nonspecific competitor (Sp1), indicating that both complex I and II are AP1 complexes. Constitutive binding of an AP1 complex throughout the 24-h time course was also observed using an oligonucleotide containing the distal AP1 site (data not shown).

Under the conditions of binding buffer 2 (Fig. 2B), we see a much lower intensity of complex II, with complex I no longer visible, whereas complexes III and IV become more obvious. Complexes III and IV begin to appear at 30 min, with binding reaching a peak at 1–2 h. At 4 h and later, their presence is no longer detected. A 100-fold molar excess of AP1 PκB oligonucleotide both compete complexes III and IV. The AP1 consensus competes complex II (as seen in Fig. 2A), whereas the nonspecific competitor (Sp1) appears to compete only complex IV. These data demonstrate the transient binding of two NFκB complexes to the oligonucleotide between 30 min and 2 h, of which only complex III appears to have high affinity for the κB-like site.

Under conditions where the ratio of nuclear proteins to oligonucleotide is much higher (200-fold higher, binding buffer 1, Fig. 2C) we see the appearance of a new larger complex (V). This complex is present from 30 min to 2 h, similar to complexes III and IV binding in Fig. 2B. The AP1 PκB oligonucleotide and the NFκB consensus oligonucleotide compete complex V, as does the AP1 consensus competitor (in addition to complex II). A nonspecific competitor (Sp1) has no effect. These results indicate that complex V is a complex of the oligonucleotide AP1 PκB with both AP1 and NFκB binding simultaneously.

Characterization of the NFκB Complex—Supershift assays were carried out with the AP1 PκB oligonucleotide, the TFκB-like site oligonucleotide and the NFκB consensus oligonucleotide, and antibodies against specific NFκB/Rel proteins. With AP1 PκB (Fig. 3, lanes 1–10), supershifts of complex III were observed with an antibody against p65 and to a lesser extent, anti-c-Rel, but not with antibodies specific for other members of the NFκB/Rel family. The complex formed with the TFκB oligonucleotide (Fig. 3, lanes 11–20) had the same mobility and transient binding characteristics as complex III and was also supershifted by antibodies against p65 and c-Rel. The affinity of protein complexes for the TFκB site in isolation was much lower than with the adjacent AP1 proximal site present (lanes 11–20 of Fig. 3 were exposed four times longer than the rest of the assay). The complex formed with the NFκB consensus oligonucleotide (Fig. 3, lanes 21–30) was supershifted by antibodies against p50 and p65 but not by antibodies specific for other members of the NFκB/Rel family. These data show complex III to be a p65/c-Rel heterodimer.

LPS Induces Proteolysis of IκBa and Translocation of p65 and c-Rel from the Cytoplasm to the Nucleus—Nuclear and cytoplasmic extracts from THP-1 cells stimulated with LPS for the times indicated in the figure were examined by Western blot analyses (Fig. 4). Antibodies against p65 and c-Rel, the component proteins of the higher affinity complex III, and also the cytoplasmic inhibitory protein IκBa, were used. When nuclear extracts used in the EMSA studies were examined with an antibody against a cytoplasmic protein (α-tubulin) in Western blot analysis a certain proportion of cytoplasmic protein was observed. Therefore, to attain a greater degree of nuclear/cytoplasmic separation, an adapted technique of preparation (panel A); the second, in binding buffer 2 (panel B), both with the DNA probe present in excess; and a third, with binding buffer 1 but with the ratio of nuclear proteins to probe 200-fold higher (20 μg nuclear extract to 0.02 ng of oligonucleotide) (panel C). Under all conditions the effects of specific (AP1 PκB, AP1, and NFκB) and nonspecific (Sp1) competitors at 100-fold molar excess were analyzed.
was used (see “Experimental Procedures”). However, rather than single bands corresponding to the p65 and c-Rel proteins, we observe multiple bands after immunostaining of the Western blots, which we would suggest are because of partial degradation of the proteins during this preparation procedure. However, because we are mainly concerned with the localization of these proteins, the degradation was not considered to impede the interpretation of the data. IκBa and Fos/Jun family proteins show relatively little degradation on Western blots, indicating that p65 and c-Rel may be particularly susceptible to breakdown with this preparation method. We can see that very little p65 is evident in the nucleus in unstimulated cells. After 10 min of LPS induction, nuclear p65 begins to appear and peak at 1 h, declining again by 2 h. A concomitant decrease in cytoplasmic p65 corresponds to the observed increase in nuclear p65.

In the case of c-Rel, again very little protein is evident in the nuclei of unstimulated cells or at 10 min. However, by 30 min to 1 h, nuclear c-Rel has increased to a level that, unlike p65, appears to be maintained up to 24 h. The level of cytoplasmic c-Rel seems relatively constant with only a slight dip observed at 1 h, corresponding to the peak in nuclear c-Rel levels. The amount of IκBa seen in the cytoplasm drops sharply at 30 min, with levels beginning to increase again at 1 h, peaking at 2–4 h, and reaching preinduction levels by 24 h. This peak in IκBa expression corresponds to a peak in IκBa mRNA levels at 1 h (data not shown). Nuclear levels of IκBa in Western analysis were found to be low and constant, confirming breakdown and re-synthesis of cytoplasmic IκBa rather than translocation. These data are consistent with the proposal that IκBa is proteolyzed after induction of the NFκB system, releasing the NFκB proteins, which then translocate to the nucleus (10, 19, 23, 24). An NFκB element in the IκBa promoter (25) directs the increase in IκBa levels at later time points.

**Characterization of the AP1 Complexes**—Nuclear extracts prepared from THP-1 cells stimulated with LPS through a 24-h time course were used to analyze the identity of complex II in supershift assays (Fig. 5). In stimulated and unstimulated cells, complex II is completely supershifted by an antibody against JunD (Fig. 5A). An unidentified complex that seems to have a slightly higher mobility than complex II becomes evident when complex II is supershifted by anti-JunD. Antibodies specific for c-Jun and ATF-2 have no observable effect on complex II. The effect of an antibody against JunB was also investigated with negative results (data not shown). Further supershifts with antibodies against Fos proteins were also carried out. In the absence of LPS, complex II is completely supershifted by a broadly reactive Fos antibody. Anti-Fra-2, although not producing an observable supershift, does significantly decrease the intensity of complex II. Anti-c-
Fos has no effect. This situation remains the same following LPS stimulation of up to 24 h. The effects of antibodies against FosB and Fra-1 were also analyzed with negative results (data not shown).

Our data show complex II to be a JunD/Fra-2 heterodimer both before and after LPS stimulation of up to 24 h. The residual complex II observed in the presence of anti-Fra-2 may either be because of the antibody having a relatively low affinity for Fra-2 or because of the presence of an as yet unidentified Fos-related protein that forms a dimer with JunD. Supershift assays with the API distal site oligonucleotide demonstrated the binding of both JunD/Fra-2 and JunD/c-Fos heterodimers with no change in the components of the complex through the 24-h time course (data not shown). These data suggest slight differences in binding at the two adjacent API sites.

The Transient Phosphorylation of JunD—In the absence of a significant quantitative change in binding to the proximal API site through the 24-h time course (see Fig. 2A), we examined whether there was a change in the phosphorylation state of JunD. Supershift assays (Fig. 6) were carried out using anti-JunD alone or in combination with an antibody specific for

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**FIG. 5. Identification of AP1 complexes.** Nuclear extracts were prepared from THP-1 cells induced with LPS for the times indicated and subsequently used in supershift analyses with oligonucleotide AP1,αB and antibodies against Jun/ATF/CREB proteins (panel A, binding buffer 1) and Fos proteins (panel B, binding buffer 2).
JunD phosphorylated at Ser-100 (c-Jun phosphorylated at Ser-73, a conserved site, is also recognized). In the absence of LPS, complex II is supershifted with anti-JunD, with the addition of the phospho-specific Jun antibody (α-p-Jun) having little effect. A 30 min to 1 h of LPS stimulation, the anti-phospho-Jun antibody caused a further shift in the anti-JunD supershift (supershift I), producing a lower mobility complex (supershift II). The amount of observable “supershift II” is considerably reduced at periods of LPS incubation of 2 h and longer. These data indicate that the majority of the JunD bound to the AP1pκB oligonucleotide is not phosphorylated at its transactivation domain (Ser-100) in the absence of LPS, but with LPS induction of up to 1 h, transient phosphorylation occurs. The presence of phosphorylated JunD at 30 min to 1 h of LPS stimulation was confirmed by Western blot analyses using the phospho-specific Jun antibody (data not shown).

We also analyzed the activation state of SAPK/JNK, whose downstream targets include JunD. The data indicate no change in the levels of nuclear or cytoplasmic SAPK/JNK (Fig. 7, A and C) but clearly show activation by phosphorylation at the Thr-183/Tyr-185 residues beginning at 10 min and peaking at 30 min to 1 h (Fig. 7, B and D). The activation of SAPK/JNK directly reflects the appearance of phosphorylated JunD.

**DISCUSSION**

LPS activation of monocytes and monocytic THP-1 cells produces a transient increase in the levels of TF mRNA and TF antigen and activity (8, 9). Our data charting these levels in THP-1 cells through a 24-h time course show a correlation to previous reports, with a rapid induction of TF mRNA from 30 min to 1 h of LPS stimulation and TF antigen levels peaking at 2 h.

When we examined the TF gene promoter LRE in DNA binding studies, we found the transient binding of an NFκB complex and the constitutive binding of AP1 complexes. The NFκB complex was observed to bind the LRE site from at least 30 min up to 2 h of LPS stimulation and was found to contain p65 and c-Rel in a heterodimeric complex. The TF LRE NFκB-like sequence has been shown to be an optimal site for the binding of p65 and c-Rel but not p50 (26). A number of previous reports studying this NFκB complex identified its component proteins as p65 and c-Rel, in agreement with our data (26, 27). A different study, however, suggested that an Ets transcription factor is binding to the core sequence of the κB-like site upon LPS stimulation rather than an NFκB complex. We found no evidence of Ets proteins binding to this site using an antibody against Ets 1/2 in supershift assays (data not shown).

To confirm the predicted model of NFκB activation, we compared nuclear and cytoplasmic extracts for evidence of nuclear translocation. Our data show the proteolysis of the cytoplasmic inhibitory protein IκBα and the translocation of both p65 and c-Rel from the cytoplasm to the nucleus, corresponding to the appearance of the NFκB complex in gel shift studies. An earlier study charting the nuclear and cytoplasmic levels of the NFκB

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**FIG. 6.** Analysis of the phosphorylation state of JunD bound to the AP1pκB oligonucleotide. A supershift assay was carried out with nuclear extracts prepared from THP-1 cells treated with LPS over a 24-h time course. The supershifts resulting from a single antibody binding (anti-JunD) (supershift I) and dual antibody binding (anti-JunD and anti-phospho-Jun) (supershift II) are indicated.

**FIG. 7.** The phosphorylation state of SAPK/JNK during a 24-h time course of LPS induction. Nuclear extracts prepared from LPS-treated THP-1 cells were assessed for the levels of SAPK/JNK and phosphorylated SAPK/JNK. The two forms of SAPK/JNK, p54 and p46, are indicated. Nuclear extracts (panels A and B) and cytoplasmic extracts (panels C and D) were analyzed. A SAPK/JNK antibody (panels A and C) and a phospho-specific SAPK/JNK antibody recognizing phosphorylation at Thr-183/Tyr-185 were used. M, molecular mass markers.
proteins in THP-1 cells (19) found that p65 was present in the nucleus before LPS induction, with levels increasing on stimulation and continuing to be elevated for up to 24 h. This contrasts with our data, where p65 was not detected before stimulation and only translocated to the nucleus for a very short period (up to 2 h), corresponding to the appearance of NFκB complexes in the binding studies and the transiently elevated levels of TF gene transcription. This same study, in agreement with our data, observed the rapid nuclear translocation of c-Rel with elevated levels persisting for up to 24 h. The fact that c-Rel is present at these longer periods of LPS stimulation yet does not appear to bind the TF κB-like site suggests that either this site is specific for the binding of p65/c-Rel heterodimers and not c-Rel homodimers or that the DNA binding capacity of c-Rel is subject to regulation. A recent study demonstrated such a constitutive expression of c-Jun and c-Fos in human monocytes and their induction in response to LPS (35). A second study in THP-1 cells (11) used a larger oligonucleotide containing the entire LRE with an additional downstream Sp1 site and found LPS induction of c-Jun binding. c-Fos/JunD heterodimers were found preinduction, whereas following LPS stimulation, both c-Fos/JunD and c-Fos/c-Jun complexes were observed. Our data examining AP1 LRE binding through a 24-h time course of LPS induction shows a relatively constant degree of AP1 binding to AP1 κB throughout the 24-h time course, although we have consistently observed a decrease in the amount of complex II at 4 h and a subsequent increase at 24 h (Fig. 2A). Consistent with the study of Group and Donovan-Peluso (11), we find the majority of AP1 binding activity to contain JunD. In our study, JunD/Fra-2 bound at the proximal AP1 site (complex II), and both JunD/Fra-2 and JunD/c-Fos bound at the distal AP1 site, both at pre- and post-induction time points.

The Jun proteins may be regulated in their DNA binding and transactivation activities by means of changes in their phosphorylation state. Phosphorylation of c-Jun at the C terminus by casein kinase II or glycogen synthase kinase 3 prevents DNA binding (36), whereas phosphorylation at the N-terminal region (Ser-63/Ser-73) by the mitogen-activated protein kinase homologue SAPK/JNK enhances transcriptional activation. Ser-100 of JunD is a conserved phosphorylation site corresponding to Ser-73 of c-Jun and is also a target for SAPK/JNK (37, 38). Because our data showed no significant change in the degree of AP1 binding or in the composition of the complexes, we examined evidence for the activation states of the Jun proteins by assessing the phosphorylation of the amino acids that influence their transactivation domains. Our data clearly show the transient (30 min to 1 h) phosphorylation of JunD in the JunD/Fra-2 complex bound to the proximal AP1 site (Fig. 6, lanes 6 and 9). The activation of SAPK/JNK, for which JunD is a target, directly correlates to the appearance of phosphorylated JunD. Although only a small proportion of the cellular JunD is phosphorylated when examined by Western blot analysis (data not shown), gel mobility shift assays indicate that a high proportion of the proximal AP1 site-bound JunD is phosphorylated (Fig. 6), raising the question as to whether there may be some form of coordinated regulation of DNA binding and transactivation enhancement. Although we did not assess the phosphorylation state of JunD bound to the distal AP1 site, it seems probable that it, too, is phosphorylated at the transactivation domain upon LPS stimulation.
Our evaluation of transcription factor binding to the TF gene promoter LRE throughout a 24-h time course of LPS induction demonstrates a number of regulatory features (Fig. 8). These include the transient, inducible binding of a p65/c-Rel heterodimer to the TF κB-like site (30 min to 2 h) corresponding to the proteolysis of IκBα and the nuclear translocation of p65 and c-Rel as predicted by previously proposed models of NFκB activation (10, 19, 23) and the transient phosphorylation (30 min to 1 h) of JunD bound to the proximal AP1 site, reflecting the appearance of phospho-JunD in nuclear extracts. JunD is a target for SAPK/JNK, which we have demonstrated is activated by phosphorylation for this same period. The transient appearance of phospho-JunD in nuclear extracts. JunD is a target for SAPK/JNK and phosphorylation of target AP1 proteins directly correlate with the observed transient increases in TF mRNA in LPS-induced THP-1 cells through the 24-h time course.

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