Lipopolysaccharide Induction of the Tumor Necrosis Factor-α Promoter in Human Monocytic Cells

REGULATION BY Egr-1, c-Jun, AND NF-κB TRANSCRIPTION FACTORS

(Jin Yao, Nigel Mackman, Thomas S. Edgington, and Sao-Tah Fan)†

From the Department of Immunology, The Scripps Research Institute, La Jolla, California 92037

Biosynthesis of tumor necrosis factor-α (TNF-α) is predominantly by cells of the monocytic lineage. This study examined the role of various cis-acting regulatory elements in the lipopolysaccharide (LPS) induction of the human TNF-α promoter in cells of monocytic lineage. Functional analysis of monocytic THP-1 cells transfected with plasmids containing various lengths of TNF-α promoter localized enhancer elements in a region (−182 to −37 base pairs (bp)) that were required for optimal transcription of the TNF-α gene in response to LPS. Two regions were identified: region I (−182 to −162 bp) contained an overlapping Sp1/Egr-1 site, and region II (−119 to −88) contained CRE and NF-κB (designated κB3) sites. In unstimulated THP-1, CRE-binding protein and, to a lesser extent, c-Jun complexes were found to bind to the CRE site. LPS stimulation increased the binding of c-Jun-containing complexes. In addition, LPS stimulation induced the binding of cognate nuclear factors to the Egr-1 and κB3 sites, which were identified as Egr-1 and p50/p65, respectively. The CRE and κB3 sites in region II together conferred strong LPS responsiveness to a heterologous promoter, whereas individually they failed to provide transcriptional activation. Furthermore, increasing the spacing between the CRE and the κB3 sites completely abolished LPS induction, suggesting a cooperative interaction between c-Jun complexes and p50/p65. These studies indicate that maximal LPS induction of the TNF-α promoter is mediated by concerted participation of at least two separate cis-acting regulatory elements.

† The abbreviations used are: TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide; bp, base pair(s); PMA, phorbol 12-myristate 13-acetate; EMSA, electrophoretic mobility shift assay; CRE, cAMP-responsive element; CREB, CRE-binding protein.

This paper is available on line at http://www.jbc.org

Vol. 272, No. 28, Issue of July 11, pp. 17795–17801, 1997
Printed in U.S.A.

Revised manuscript received May 7, 1997.

This work was supported by National Institutes of Health Grants HL-16411 and HL-48872. 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.

† To whom correspondence should be addressed: Dept. of Immunology, IMM 17, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: 619-784-8226; Fax: 619-784-8480; E-mail: sfan@scripps.edu.
the human TNF-α gene. Using LPS stimulation as a paradigm, we find that a mechanism involving several transcription factors is required for maximal TNF-α promoter activity in human monocytes.

**EXPERIMENTAL PROCEDURES**

**Cell and Reagents**

THP-1 monocytic cells were maintained in medium RPMI 1640 with 8% fetal calf serum. Human peripheral blood monocytes were isolated by gradient centrifugation as described (18). Antibodies used in electrophoretic mobility shift assay (EMSA): anti-Egr-1, anti-CREB, anti-ATF-2, anti-c-Jun, anti-JunB, anti-JunD, anti-c-Fos, anti-Fos B, anti-p50, anti-p65, anti-c-Rel, and anti-Ets1/2 were form Santa Cruz Biotechnology (Santa Cruz, CA).

**Plasmids**

5' Deletion Series—pTNF(-1311)Luc, pTNF(-1185)Luc, pTNF(-615)Luc, pTNF(-479)Luc, pTNF(-295)Luc, pTNF(-162)Luc, pTNF(-120)Luc, pTNF(-95)Luc, and pTNF(-36)Luc (19) were generously provided by Dr. J. Economou (UCLA, Los Angeles, CA). pTNF(-295)Luc, 17,636; pTNF(-182)Luc, 13,288; pTNF(-161)Luc, 13,335; pTNF(-120)Luc, 11,813; pTNF(-95)Luc, 9457; pTNF(-36)Luc, 1659; the background is 425 light units. Similar results were observed in two independent experiments.

**Heterologous Promoter Series**—Multiple copies of oligonucleotides containing sequences from the human TNF-α promoter were cloned upstream of the minimal SV40 promoter driving expression of the luciferase reporter gene in pGL2-promoter (Promega Corp.). Sequences of oligonucleotides used for producing these constructs are listed in Table I. All plasmids were verified by DNA sequencing.

**RESULTS**

**Fig. 1. LPS induction of the TNF-α promoter from a 5' deletion series.** The 5' boundaries of plasmids containing various truncations of the TNF-α promoter are shown. The average fold induction of luciferase activity expressed by each plasmid in transiently transfected THP-1 cells in response to LPS (10 μg/ml) is shown with S.D. Results of a representative experiment are shown. The basal luciferase activity (light units) levels per 10⁶ cells are as follows: pTNF(-1311)Luc, 13,546; pTNF(-1185)Luc, 12,606; pTNF(-615)Luc, 19,319; pTNF(-479)Luc, 12,311; pTNF(-295)Luc, 17,636; pTNF(-182)Luc, 13,288; pTNF(-161)Luc, 13,335; pTNF(-120)Luc, 11,813; pTNF(-95)Luc, 9457; pTNF(-36)Luc, 1659; the background is 425 light units. Similar results were observed in two independent experiments.

**Fig. 2. LPS induction of TNF-α promoter from mutant series.** The position of mutations in corresponding regulatory motifs are marked. Oligonucleotides used for producing these plasmids are listed in Table I. The sB3 mutant was generated using the sB3 m2 oligonucleotide (Table I). Fold induction of luciferase activity expressed by each plasmid in transiently transfected THP-1 cells in response to LPS of duplicate samples with S.D. are shown. Results are representative of four independent experiments.
DNA Transfection

A DEAE-dextran transfection procedure (18, 20) was used. Briefly, 3 × 10^7 THP-1 cells were resuspended in 1 ml of Tris-buffered saline and incubated for 10 to 20 min at 37 °C with 5 mg of plasmid DNA and 80 μg of DEAE-dextran (Pharmacia, Uppsala, Sweden). During incubation, cells were monitored closely for permeability to trypan blue. Transfection was stopped by adding large volumes of Tris-buffered saline, usually after 10 min, when 20–30% of cells are permeable to trypan blue. After washing with Tris-buffered saline, cells were cultivated in media for 48 h. Cells were stimulated with 5 μg/ml LPS (Escherichia coli O111:B4 purchased from Calbiochem, La Jolla, CA) in a 96-well plate at 1 × 10^6 cells/well. After 7 h of incubation at 37 °C, cells were harvested and luciferase activity was determined using an assay kit (Promega) and the Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA).

Nuclear Extracts and EMSA

Nuclear extracts were prepared from 5 × 10^6 THP-1 cells or peripheral blood monocytes stimulated under various conditions as described (18, 20). Protein concentrations in nuclear extracts were determined by BCA protein assay (Pierce). Oligonucleotide probes were radiolabeled using [α-32P]dCTP (Amersham). Nuclear extracts (1–5 μg) were incubated with radiolabeled oligonucleotide probes (0.2–1 × 10^6 cpm) in 2 × binding buffer (100 mM KCl, 1 mM EDTA, 10% glycerol, 2 mM dithiothreitol, 2 mg/ml bovine serum albumin, 0.2% Nonidet P-40, and 40 mM Heps) for 20 min at room temperature. Samples were subjected to electrophoresis through 6% non-denaturing acrylamide gels (Novex, San Diego, CA) in 0.5 × Tris borate-EDTA buffer. For antibody supershift experiments, nuclear extracts were precubated 20 min with 2 μg of antibody before the addition of the labeled probe.

RESULTS

Localization of DNA Elements Involved in the Transcriptional Activation of the TNF-α Gene in Response to LPS—To define the 5’ boundary of LPS responsive elements, THP-1 cells were transiently transfected with a series of plasmids containing progressive truncations of the 5’ promoter sequence between −1311 bp and −36 bp. Deletion of sequences upstream of −182 had no significant effect on LPS-inducibility of the TNF-α promoter activity (Fig. 1). Removal of a region from −182 to −162, which contains an Sp1/Egr-1 overlapping site, reduced LPS inducibility by 50% (from 13.3-fold to 7.8-fold). Removal of a region between −161 and −95, which removes Ets, CRE, and kB (kB3) sites, further reduced LPS inducibility (Fig. 1). Finally, deletion to −36 removed a region containing a Sp1 site and abolished basal promoter activity. These results provide new and substantial evidence that LPS induction of the TNF-α gene in monocytes involves at least two regulatory elements; region I (−182 to −162) and region II (−120 to −96).

Determination of the Roles of Various Binding Sites in the Human TNF-α Promoter by Functional Analysis of Mutant Plasmids—To determine the functional role of nuclear binding motifs in the region identified by 5’-truncation analysis, plasmids with specific site-directed mutations were examined. Mutation in the Egr-1 site (−169 bp), the CRE site (−106 bp), as
Functional Analysis of the CRE and xB3 Sites in Heterologous Promoter Plasmids—To further explore the potential of these nuclear factor binding motifs to function as enhancer elements, we examined their ability to confer inducibility to heterologous promoter. Neither tandem copies of the xB3 nor two copies of the CRE sites alone conferred LPS inducibility to a SV40 minimal promoter (Fig. 3). However, when two or three copies of a DNA fragment spanning both the CRE and the xB sites were cloned upstream of the SV40 promoter strong LPS inducibility was observed (Fig. 3, A and B). Mutation of either the CRE site (−110 to −86 m1) or the xB3 site (−110 to −86 m2) completely abolished LPS inducibility (Fig. 3B). These results suggest that the LPS induction of human TNF-α transcription requires cooperative interaction between proteins bound to the CRE and xB sites. There is only 1 base pair separating the CRE and xB3 sites. Whether the close proximity of the CRE site and the xB3 site is required for the optimal transactivation of TNF-α was investigated for these experiments, 5, 10, or 15 additional base pairs were added between CRE and xB3 sites and oligonucleotides were cloned into pGL2-promoter. These insertions created 1/2, 1, or 1 1/2 extra turns of the DNA helix between these two sites. Fig. 3C shows that insertion of DNA between these two sites abolished LPS inducibility.

Identification of Transcription Factors That Bind to the Egr-1, CRE, and xB3 Sites—EMSA were performed to determine which transcription factors bind to sites in regions I and II of the human TNF-α promoter.

Region I: Egr-1 and Sp1—An oligonucleotide containing only the Egr-1 site bound an LPS and phorbol ester-inducible complex (Fig. 4A). This complex was not observed when an oligonucleotide containing a mutant Egr-1 site was used as the probe (Fig. 4A, lanes 4–6). In addition, this LPS-inducible complex was supershifted by anti-Egr-1 antibody (Fig. 4A, lane 9), demonstrating that Egr-1 bound to this site.

A prominent complex was observed when nuclear extracts from unstimulated cells were incubated with an oligonucleotide spanning the overlapping Sp1/Egr-1 sites (−182 to −157 bp) (Fig. 4B, lane 1). This complex was not induced by LPS (lane 2) but was supershifted using an anti-Sp1 antibody (lane 3). In addition, this complex was not observed using an oligonucleotide containing a mutated Sp1 site (Fig. 4B, lanes 5–8). Taken together, these results demonstrated that Sp1 bound to this site. In addition to the constitutively expressed Sp1 complex, LPS stimulation of cells resulted in the formation of an Egr-1 complex that bound to the oligonucleotide containing overlapping Sp1/Egr-1 sites (Fig. 4B, lane 2). Similarly, this LPS inducible Egr-1 complex was observed using the Sp1mut/Egr-1 oligonucleotide as a probe (Fig. 4B, lane 6). The Egr-1 complex

![Figure 4](image)

**FIG. 4.** LPS induction of Egr-1 nuclear factor. A, nuclear extract from unstimulated THP-1 or THP-1 stimulated with LPS (10 μg/ml) or PMA (10 nM) for 36 h were probed with labeled oligonucleotide probes of either wild type sequence or mutant sequence of Egr-1 site (Table I). B, probes used were oligonucleotide spanning overlapping Sp1/Egr-1 site (−182 to −157) or oligonucleotide containing base substitutions in Sp1 site, Sp1mut/Egr-1 (−182 to −157 m1, Table I). For antibody supershift experiment, nuclear extracts were incubated with antibody (2 μg) for 20 min before addition of probes.

| Table I | Oligonucleotides used in this study |
|---------|-----------------------------------|
| Name    | Position | Sequence (5’–3’) |
| Egr-1   | −168 to −145 | CCCCCCCCCGCGATGGAGAGAA |
| Egr-1m  | −168 to −145 | CCCCCCCCCGCGATGGAGAGAA |
| CRE     | −112 to −92  | TCCAGATGAGCTCAATGAGTTTC |
| CREm1   | −112 to −92  | TCCAGATGAGCTCAATGAGTTTC |
| CREm2   | −112 to −92  | TCCAGATGAGCTCAATGAGTTTC |
| xB1     | −594 to −577 | AAGCCTGGGACAGCCCCG |
| xB2     | −217 to −200 | TGTGAGGGGTATCTTGTGA |
| xB3     | −103 to −86  | GCTCATGAGTTTCCTCAC |
| xB3m1   | −103 to −86  | GCTCATGAGTTTCCTCAC |
| xB3m2   | −103 to −82  | CATGAGCTCATGAGTTTCCTCAC |
| −182 to −157 | −182 to −157 | CTTCATGGATTCCTCACCAAG |
| −182 to −157m1 | −182 to −157 | CTTCATGGATTCCTCACCAAG |
| −110 to −86 | −110 to −86 | CAGATGAGCTCATGAGTTTCCTCAC |
| −110 to −86ml | −110 to −86 | CAGATGAGCTCATGAGTTTCCTCAC |
| −110 to −86n2 | −110 to −86 | CAGATGAGCTCATGAGTTTCCTCAC |
| −110 to −86+5 | −110 to −86 | CAGATGAGCTCATGAGTTTCCTCAC |
| −110 to −86+10 | −110 to −86 | CAGATGAGCTCATGAGTTTCCTCAC |
| −110 to −86+15 | −110 to −86 | CAGATGAGCTCATGAGTTTCCTCAC |

Respective binding sequences are in bold type. Base substitutions or insertions are underlined.
was not affected by the addition of an anti-Sp1 antibody (Fig. 4B, lanes 3 and 7), but was supershifted with an anti-Egr-1 antibody (Fig. 4B, lanes 4 and 8).

Region II: κB3 and CRE—EMSA were performed with oligonucleotides spanning three putative κB sites: κB1 (−594 to −577), κB2 (−216 to −199), and κB3 (−104 to −87) (Table I). As depicted in Fig. 5A, LPS stimulation of cells resulted in the formation of nuclear protein-DNA complexes with κB1 (lane 2) and κB3 (lane 6), but not with κB2 (lane 4). More protein bound to κB1 than κB3. Similar results were found using nuclear extracts from human peripheral blood monocytes (Fig. 5B). Monospecific anti-p50 and anti-p65 antibodies were used in supershift experiments to identify the protein composition of the complexes formed with the κB1 and κB3 sites. As shown in Fig. 5C, the LPS induced complex binding either to the κB1 or to the κB3 oligonucleotides was supershifted by anti-p50 and also by anti-p65 antibodies (Fig. 5C), indicating that they were composed of p50/p65 heterodimers. Furthermore, mutation of the κB3 site (κB3 m2, Table I) abolished binding of p50/p65 (data not shown). Importantly, the same mutation significantly reduced LPS inducibility of the TNF-α promoter (Fig. 2).

Using a prototypic CRE site as a probe, we demonstrated that CREB was constitutively expressed in unstimulated THP-1 cells and that CREB binding was not induced by LPS (Fig. 6A). In contrast, LPS stimulation increased the amount of protein binding to the non-consensus CRE site from the TNF-α promoter (Fig. 6B, compare lanes 1 and 4). Antibody supershift experiments were performed to determine the proteins that bound to the CRE site (Fig. 6B). In unstimulated cells, the majority of the complex was supershifted using an anti-CREB antibody, whereas only a minor supershift was observed using an anti-c-Jun antibody. In LPS-stimulated cells, the anti-c-Jun antibody supershifted the majority of the complex, whereas the anti-CREB antibody formed a minor supershift band. These results suggest that LPS does not increase the binding of CREB, consistent with our results using a prototype CRE site (Fig. 6A), and that LPS increases binding of c-Jun-containing complexes to the CRE site from the TNF-α promoter.

Using an oligonucleotide containing a mutated CRE site (CREm2, Table I), we observed a reduction in the total amount of protein binding (Fig. 6C, lane 2). In addition, LPS stimulation did not increase complex formation (lane 3). This complex was supershifted by an anti-CREB antibody (lane 4) but was not recognized by an anti-c-Jun antibody (lane 5), suggesting that small amounts of CREB still bound to the mutated CRE site. Since these same base substitutions completely abolished the functional activity of the CRE site in transfected cells (Fig. 3B), these results provide additional evidence that c-Jun-containing complexes, rather than CREB, play a crucial role in LPS induction of the TNF-α promoter.

**DISCUSSION**

In this report, we have defined two *cis*-acting regulatory elements in the human TNF-α promoter that mediated maximal LPS induction of TNF-α gene expression in cells of monocytic lineage. Region I contained an overlapping Sp1/Egr-1 site (−182 to −162), whereas region II (−120 to −96) contained CRE and κB sites.

Functional studies demonstrated that Egr-1 binding to region I was required for LPS induction of the TNF-α promoter in monocytes. Egr-1 protein expression was induced by LPS stimulation (21). The Egr-1 site at −169 bp is part of the Sp1/Egr-1 overlapping sequence motif. In unstimulated monocytes, Sp1

---

**Fig. 5.** LPS induction of NF-κB (p50/p65). Nuclear extracts from THP-1 (A) or peripheral blood monocytes (B) were probed with oligonucleotide spanning κB1, κB2, or κB3 site (Table I). C, nuclear extracts were preincubated with antibodies (2 μg) for 20 min before adding probes.

**Fig. 6.** LPS induction of nuclear factors binding to CRE site. Nuclear extracts from unstimulated or LPS-stimulated THP-1 cells were probed with oligonucleotide probe spanning the prototypic CRE sequence (A), TNF-α promoter wild type CRE sequence (B), or mutant CRE sequence (CREm2, C). Sequences of these oligonucleotides are shown in Table I. For antibody supershift experiments, nuclear extracts were incubated with various antibodies (2 μg) for 20 min before adding probes.
binds to this site, whereas upon LPS stimulation it is likely that Egr-1 displaces Sp1 to mediate induction of TNF-α promoter activity. Previously, we have shown that Egr-1 can displace Sp1 from a similar overlapping Sp1/Egr-1 site (22). The role of Sp1 bound to this upstream Sp1 site at -172 is unknown, although our results using a plasmid containing a mutation in the Sp1 site suggest that it does not mediate basal expression. In contrast, mutation of the Sp1 site at -56 bp dramatically reduces basal expression by 65%.2

Further functional studies showed that the CRE and κB3 sites in region II were required for LPS induction of the TNF-α promoter. We demonstrated that κB3 (-97) bound p50/p65 heterodimers. In contrast, we found no role for κB1 (-588) despite its ability to bind p50/p65. A recent study by Trede et al. (11) also showed a role for κB3 in LPS induction of the TNF-α promoter in THP-1 cells. However, these investigators did not identify other regulatory regions, possibly due to the low level of induction (about 4-fold) of the TNF-α promoter (11). In addition, our studies are in agreement with an earlier report (16), showing that p50/p65 binds with much less avidity to κB3 than κB1 (Fig. 5). Therefore, it is possible that protein-protein interaction of p50/p65 with c-Jun proteins bound to the adjacent CRE site is required to stabilize the formation of a transcriptional complex that mediates induction of the TNF-α promoter.

In monocytic cells, the CRE site in the TNF-α promoter constitutively bound CREB (Fig. 6). Upon LPS stimulation, the amount of CREB binding remains unchanged, but there was a marked increase in c-Jun binding. LPS induces c-jun expression (23), suggesting that the increases in c-Jun-containing complexes observed in this study were due to de novo protein synthesis. Furthermore, the transactivating activity of these c-Jun complexes may be increased due to post-translational phosphorylation (24). As reported recently, LPS stimulation of THP-1 cells resulted in rapid activation of JNK (25). The phosphorylation of c-Jun by JNK significantly enhances the transactivation potential of these factors (26). The non-consensus CRE sequence of TNF-α promoter TGAGCCTCA was shown to have a lower binding affinity for c-Jun/ATF-2 complex than a consensus CRE sequence (27). These variations of sequences and the resulting variation in binding of these bZIP proteins could play a role in the modulation of TNF-α expression in response to various signals. Importantly, base substitution in the CRE site that reduced CREB binding but abolished c-Jun binding abrogated the functional activity of this site, suggesting that c-Jun-containing complexes bound to region II are required for LPS induction of the TNF-α promoter. The molecular mechanisms by which binding of the nuclear proteins at this CRE site regulate the activation of TNF-α gene expression await further elucidation.

Parallel to our findings for monocytic cells, it was demonstrated recently that both the κB3 site and the adjacent upstream CRE site are required for the calcium-stimulated TNF-α transcription in human T cells (4). However, in contrast to our data for monocytes, these investigators reported that the κB3 site bound NFATp and not p50/p65 (28). Moreover, contrary to our findings in monocytes, no constitutive or induced CREB protein binding to the CRE site was observed in T cells (4). Instead, the CRE-binding complex was shown to consist almost exclusively of c-Jun/ATF-2 heterodimers. Furthermore, both the CRE site and adjacent upstream Ets site were essential for both basal promoter activity in T cells and responsiveness to PMA (5). It appears that the Ets site did not play a significant role in basal TNF-α promoter activity in monocytes, since it was not affected by mutation of the Ets site.2 Together, these results demonstrate the marked difference in the regulation of the TNF-α promoter in human T cells and monocytes.

In this study, we showed that LPS induction of a heterologous promoter by region II required both the CRE and κB3 sites, suggesting a functional cooperation between the transcription factors bound to these sites. Similar cooperation has also been demonstrated in the induction of another cytokine gene, IFN-β (29), as well as in the expression of E-selectin (30). In both cases, ATF-2/Jun proteins were shown to interact with p50/p65 proteins. In addition, p50/p65 has been shown to physically interact with ATF-2 (29), and c-Jun (31). These direct associations are considered an important mechanism by which transcriptional factors cooperate to induce gene expression.

The novel findings presented here support the notion that concerted participation of proteins bound to the Egr-1, CRE, and κB3 sites mediates the induction of the TNF-α promoter in human monocytic THP-1 cells in response to LPS. Future studies will determine if similar regulatory pathways control TNF-α gene expression in monocytes and macrophages. Further elucidation of the cooperative interactions of transcription factors bound to these cis-acting regulatory elements are essential to our understanding of the transcriptional regulation of the TNF-α gene in human monocytes. Understanding of the molecular mechanisms by which these nuclear factors regulate TNF-α gene expression should lead to design of specific inhibitors that will counteract the pathological effects of TNF-α in various diseases.

Acknowledgments—We thank Dr. James S. Economou for the generous gift of plasmids, and Dr. Craig Dickinson and Paul Oeth for advice.

REFERENCES

1. Tracey, K. J., and Cerami, A. (1994) Annu. Rev. Med. 45, 491–503
2. Han, J., Brown, T., and Beutler, B. (1990) J. Exp. Med. 171, 465–475
3. Han, J., Huez, G., and Beutler, B. (1991) J. Immunol. 146, 1843–1848
4. Tsai, R. Y., Jain, J., Pesavento, P. A., Rao, A., and Golffeld, A. E. (1996) Mol. Cell. Biol. 16, 459–467
5. Kramer, B., Weigmann, K., and Kronke, M. (1995) J. Biol. Chem. 270, 6577–6583
6. Rhodes, K. L., Golub, S. H., and Economou, J. S. (1992) J. Biol. Chem. 267, 22102–22107
7. Kramer, B., Meichle, A., Hensel, G., Charnay, P., and Kronke, M. (1994) Biochim. Biophys. Acta 1219, 413–421
8. Lettman, D. C., Ribeiro, R. C. J., Mackow, R. E., Baxter, J. D., and West, B. L. (1991) J. Biol. Chem. 266, 9343–9348
9. Tsytsykova, A. V., Chatila, T., Goldfeld, A. E., and Geha, R. S. (1995) J. Immunol. 155, 902–908
10. Adamson, E. D., and Mackman, N. (1996) J. Exp. Med. 171, 35–44
11. Trede, N. S., Tsytyskova, A. V., Chatila, T., Goldfeld, A. E., and Geha, R. S. (1995) J. Immunol. 155, 902–908
12. Leitman, D. C., Mackow, R. E., Williams, T., Baxter, J. D., and West, B. L. (1992) Mol. Cell. Biol. 12, 1352–1356
13. Shakhov, A. N., Collot, M. A., Vassalli, P., Nedosposav, S. A., and Jongeneelen, W. J. (1990) J. Exp. Med. 171, 9498–9506
14. Goldfeld, A. E., Doyle, C., and Mandis, T. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 9769–9773
15. Ziegler-Heitbrock, L. H. W., Sternsdorf, T., Liese, J., Belohradsky, B., Weber, C., Wodell, A., Schreck, R., Bauerle, P., and Strobel, M. (1993) J. Immunol. 151, 6086–6093
16. Oeth, P., and Mackman, N. (1995) Blood 86, 4144–4152
17. Oeth, P., and Mackman, N. (1995) Blood 86, 4144–4152
18. Fan, S.-T., Mackman, N., Cui, M.-J., and Edgington, T. S. (1995) J. Immunol. 154, 3268–3274
19. Economou, J. S., Rhodes, K., Essner, R., McBride, W. H., Gasson, J. C., and Morton, D. L. (1989) J. Exp. Med. 170, 321–326
20. Coleman, D. L., Bartiss, A. H., Sukhatme, V. P., Liu, J., and Rupprecht, H. D. (1992) J. Immunol. 149, 3405–3405
21. Cui, M.-Z., Parry, G. C. N., Oeth, P., Larson, H., Smith, M., Huang, R.-P., Goldfeld, A. E., and Mackman, N. (1996) J. Biol. Chem. 271, 2731–2739
22. Okada, W. H., Esselinck, M. T., Halle, M. R., and Vellenga, E. (1993) Blood 103, 337–343
23. Fan, S.-T., Mackman, N., Brand, K., and Edgington, T. S. (1995) J. Exp. Med. 174, 1517–1526
24. Coleman, D. L., Bartiss, A. H., Sukhatme, V. P., Liu, J., and Rupprecht, H. D. (1992) J. Immunol. 149, 3405–3405
25. Adamson, E. D., and Mackman, N. (1996) J. Biol. Chem. 271, 16483–16486
26. Hambleton, J., Weinstein, S. L., Lem, L., and DeFranco, A. L. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 2774–2777
27. Smeal, T., Binetruy, B., Mercado, D., Groover-Bardwick, E., Heidecker, G., Rapp, U. R., and Karin, M. (1992) Mol. Cell. Biol. 12, 3507–3513
28. Yao, J., and S.-T. Fan, submitted for publication.
27. Newell, C. L., Deisseroth, A. B., and Lopez-Berestein, G. (1994) *J. Leukocyte Biol.* **56**, 27–35
28. McCaffrey, P. G., Goldfeld, A. E., and Rao, A (1994) *J. Biol. Chem.* **269**, 30445–30450
29. Du, W., Thanos, D., and Maniatis, T. (1993) *Cell* **74**, 887–898
30. Whitley, M. J., Thanos, D., Read, M. A., Maniatis, T., and Collins, T. (1994) *Mol. Cell. Biol.* **14**, 6464–6475
31. Kaszubska, W., Hoof van Huijsduijnen, R., Ghersa, P., DeRaemy-Schenk, A. M., Chen, B. P. C., Hei, T., DeLamarter, J. F., and Whelan, J. (1993) *Mol. Cell. Biol.* **13**, 7180–7190