Brief Definitive Report

Promoter of the Mouse Gene Encoding Calcium-independent Nitric Oxide Synthase Confers Inducibility by Interferon γ and Bacterial Lipopolysaccharide

By Qiao-wen Xie, Richard Whisnant, and Carl Nathan

From the Beatrice and Samuel A. Seaver Laboratory, Department of Medicine, Cornell University Medical College, New York 10021

Summary

Inducible nitric oxide synthase (iNOS) can be expressed by many types of mammalian cells in response to diverse signals acting synergistically, including cytokines and microbial products. We previously showed that induction of iNOS in mouse macrophages by interferon γ (IFN-γ) and lipopolysaccharide (LPS) was at the transcriptional level. From a mouse genomic library, we now cloned a 1,749-bp fragment from the 5′-flanking region of the *iNOS* gene, and used S1 nuclease mapping and primer extension to identify the mRNA transcription start site within it. The mRNA initiation site is preceded by a TATA box and at least 22 oligonucleotide elements homologous to consensus sequences for the binding of transcription factors involved in the inducibility of other genes by cytokines or bacterial products. These include 10 copies of IFN-γ response element; 3 copies of γ-activated site; 2 copies each of nuclear factor-κB, IFN-α–stimulated response element, activating protein 1, and tumor necrosis factor response element; and one X box. Plasmids in which all or the downstream one half or one third of this region of *iNOS* were linked to a reporter gene encoding chloramphenicol acetyltransferase were transfected into cells of the RAW264.7 macrophage-like line. All these constructs conferred inducibility of the *iNOS* promoter by LPS, but only the construct containing all 1,749 bp conferred synergistic inducibility by IFN-γ plus LPS.

Nitric oxide (NO), a short-lived, radical gas, acts as an intercellular messenger in most or all mammalian organs, participating in vascular homeostasis, neurotransmission, and antimicrobial defense (1). Three genes encode NO synthases (NOS) of two biochemical types. Neuronal and endothelial NOS are constitutive but dormant until activated briefly by Ca^{2+} transients that sustain the binding of calmodulin. These have been termed cNOS (1). A third NOS is expressed only after transcriptional induction (2). Once synthesized, this NOS is active for prolonged periods, perhaps because it binds calmodulin without a requirement for elevation of Ca^{2+} above the levels in resting cells (3). Termed inducible NOS (iNOS), the latter form of the enzyme has been purified (4–6) and cloned (2, 7, 8) from mouse macrophages. iNOS mediates some of the cytotoxic action of activated macrophages, and when expressed in vascular smooth muscle, endothelium and/or liver, may play a key role in septic shock (9).

Three considerations have focused our interest on the *iNOS* promoter. First, immunologically inducible, high-output NO production is widely distributed, having been observed not only in macrophages, endothelium, vascular smooth muscle, and hepatocytes, but also in tumor cells, fibroblasts, mesangial cells, astrocytes, keratinocytes, and cardiac myocytes (1, 10–12). Second, in all these cells, maximal induction of iNOS depends on synergistic combinations of stimuli, the most effective ones varying with the cell type. For mouse macrophages, synergistic inductive combinations include IFN-γ, IFN-α or IFN-β plus LPS, or IFN-γ plus TNF-α or -β (13–16), whereas for rat and human hepatocytes, four signals are required: IFN-γ, TNF-α, IL-1β, and LPS (17). Finally, iNOS is subject to immunologic suppression, for example, by TGF-β (18), macrophage deactivation factor (18), and IL-4 (19). We have taken steps toward analyzing the molecular mechanisms by which iNOS is induced and suppressed, by cloning the promoter of the mouse *iNOS* gene, and distinguishing regions within it that are responsive to LPS alone or to the synergistic contribution of IFN-γ.

Materials and Methods

Materials. Recombinant murine IFN-γ (5×10^6 U/ml) was generously provided by Genentech (South San Francisco, CA). LPS was purchased from Sigma Chemical Co. (St. Louis, MO). Thioglycollate broth was from Difco Laboratories Inc. (Detroit,

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MI). Isotopes were obtained from Amersham Corp. (Arlington Heights, IL).

Cell Culture. Peritoneal macrophages were isolated from CD-1 mice (Charles River Laboratories, Wilmington, MA) 4 d after an intraperitoneal injection of thioglycollate broth (15). RAW 264.7, a mouse macrophage cell line, was obtained from the American Type Culture Collection, Rockville, MD. Cells were grown in RPMI 1640 (KC Biological, Lenexa, KS) with 10% fetal bovine serum and antibiotics (complete medium) at 37°C in 5% CO_2 (4). Nitrite accumulation in culture medium was measured as before (15).

Preparation of Probes. For DNA probes, template DNA fragments were reacted with Klenow enzyme, random primers, dATP, dGTP, dTTP, and [^{32}P]dCTP, according to the instructions provided with the Prime-a-Gene kit (Promega Corp., Madison, WI). For oligonucleotide probes, specific oligonucleotides were 5'-end-labeled with γ-[^{32}P]ATP by T4 polynucleotide kinase.

Cloning of 5'-Flanking Region from iNOS. The 5'-fragment of iNOS cDNA (nucleotides 1–845, see reference 2), comprised of 255 bp of 5'-untranslated region and 590 bp of NH_2-terminal coding region, was isolated from the clones described (2). This fragment served as a template to prepare a DNA probe for screening 20,000 cosmid colonies from a mouse genomic library provided by J. Mudgett (Merck Research Laboratories, Rahway, NJ). A positive clone was selected and its genomic DNA was digested by HincII. The products were subjected to Southern blot with a second DNA probe prepared from only the 5'-untranslated region of iNOS (nucleotides 1–200, see reference 2). A unique fragment of 1.7 kb hybridized and was subcloned into pBluescript SK+ at the HincII site to form pUP1 and pUP1r (in the two orientations). These plasmids were used to sequence the 1,749-bp insert (−1588 to +161) by the dideoxy method (20).

Constructs of iNOS Promoter Fused with Chloramphenicol Acetyltransferase (CAT) Gene. The 1,749-bp fragment of the 5'-flanking region of *iNOS* was excised from plasmid pUP1 and inserted, with the same orientation, into pCAT-Basic (Promega Corp.) upstream of the promoterless CAT gene to form the construct p1*iNOS-CAT*. Portions were removed from p1*iNOS-CAT* to form p2*iNOS-CAT*, p3*iNOS-CAT*, and p5*iNOS-CAT*, as specified below.

Initiation Site for mRNA. This was determined by S1 nuclease mapping with a single-stranded, end-labeled DNA probe (21) and by primer extension (22) with the following modifications: (*a*) the primer and RNA template were hybridized in buffer without formamide at 65°C for 2 h; and (*b*) avian myeloblastosis virus reverse transcriptase was used.

Transient Transfection of RAW 264.7 Cells and CAT Assays. RAW 264.7 cells were transfected by a modification of the DEAE-dextran procedure (23). After cells were washed twice with RPMI, 10 μg of plasmid DNA was added per 10^7 cells in 1 ml of RPMI without serum prewarmed to 37°C and containing DEAE-dextran (250 μg/ml) and 50 mM Tris (pH 7.4). The suspension was incubated at 37°C for 45–60 min followed by a 1-min shock with 10% DMSO at room temperature. The cells were washed, distributed to 100-mm plates, each with about 5×10^6 cells in 10 ml of complete medium, and incubated at 37°C in 5% CO_2. At least 24 h later, the medium was changed, and IFN-γ and/or LPS were added to some plates. About 14 h later, the cells were washed with ice-cold PBS, resuspended in 0.25 mM Tris (pH 7.8), and subjected to three cycles of freezing and thawing. Lysates were centrifuged (11,700 *g* for 10 min at 4°C). The supernatant was heated at 65°C for 10 min to inactivate CAT inhibitors and then centrifuged as above. The supernatant was assayed for CAT activity by TLC method (24). Protein content was determined as described by Bradford (25).

Results

Cloning and Analysis of a 1,749-bp 5'-Flanking Region of iNOS. A 1,749-bp HincII fragment at the 5'-flanking region of *iNOS* was cloned from a mouse genomic library and sequenced (Fig. 1 *A*). Both S1 nuclease mapping and primer extension (Fig. 2) identified the same initiation site (designated +1 in Fig. 1 *A*) 30 bp downstream of a TATA box. The TATA box is preceded by a multitude of oligonucleotide elements homologous to consensus sequences for binding of transcription factors involved in cytokine or LPS induction of other genes, including sequences specified as IFN-γ response element (γ-IRE) (10 copies) (26, 27); γ-activated site (GAS) (3 copies) (26, 28); nuclear factor-κB (NF-κB) (2 copies) (29); IFN-α–stimulated response element (ISRE) (2 copies) (28); activator protein 1 (AP-1) (2 copies) (30); TNF response element (TNF-RE) (2 copies) (31); and X box (1 copy) (26, 32).

S1 nuclease mapping and primer extension (Fig. 2) reinforced the previous conclusion (2) that no detectable mRNA for iNOS accumulates in RAW 264.7 macrophage-like cells or primary peritoneal macrophages without induction. Treatment with IFN-γ and LPS led to transcription of *iNOS* from the same initiation site in both cell populations.

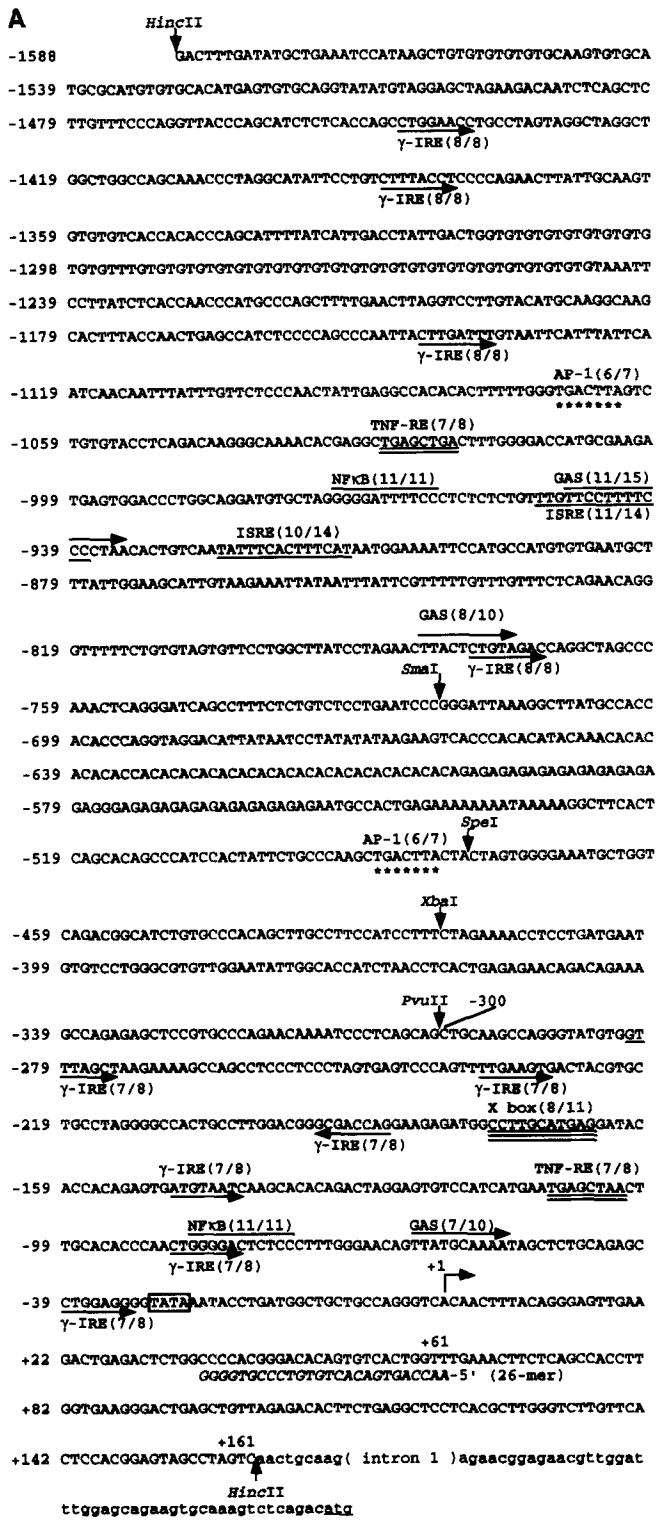
Promoter Activity of the Full-Length 1,749-bp Fragment and Its 5'-Truncated Constructs. The 1,749-bp 5'-flanking region of *iNOS* was fused to a promoterless *CAT* reporter gene in the vector pCAT-Basic to form the construct p1*iNOS-CAT*. This construct and the vector only were transfected into RAW 264.7 cells. As reflected by CAT activity, the 1,749-bp 5'-flanking region of *iNOS* conferred responsiveness to the combination of IFN-γ and LPS (Table 1 and Fig. 3). By itself, LPS induced promoter activity in a concentration-dependent manner. IFN-γ alone was ineffective. However, IFN-γ synergistically enhanced the response to LPS (Fig. 4 *A*).

Downstream portions of the 1,749-bp region were linked to the CAT gene to form the constructs p2*iNOS-CAT* and p3*iNOS-CAT* (Fig. 1 *B*). Both conferred inducibility by LPS in a concentration-dependent fashion. However, in contrast to p1*iNOS-CAT*, the constructs lacking the upstream half of the 1,749-bp fragment did not respond to the addition of IFN-γ (Fig. 4, *B* and *C*).

S1 nuclease mapping confirmed that the mRNA initiation site of the CAT transcript in the cells transfected with p1*iNOS-CAT* was at the same location as that for *iNOS* transcript itself in untransfected cells, and that no detectable CAT mRNA synthesis was initiated in transfected cells without induction by IFN-γ and LPS (data not shown).

Discussion

The 1,749-bp 5'-flanking region identified here as the promoter of the *iNOS* gene contains a remarkable multiplicity of consensus sequences for the binding of transcription factors involved in the induction of other genes by cytokines and bacterial products, such as IFN-γ, IFN-α, TNF, IL-2, and LPS. In addition, regions of GT- or AC-repeats considered



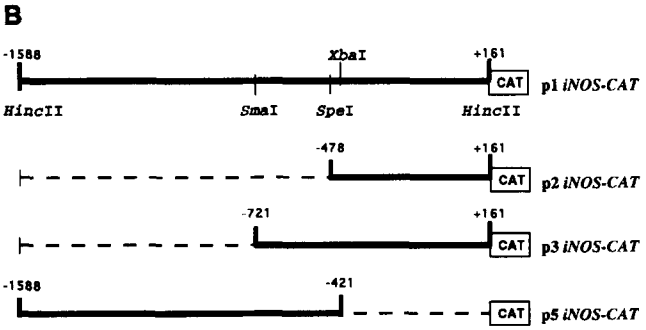


Figure 1. (*A*) The 5′-flanking region of *iNOS*. The nucleotide sequence (numbered *left*) of the 1,749-bp region (−1588 to +161) is shown (*upper case*). The sequence (*lower case*) following position +161 is from the cDNA of iNOS (2). Intron 1 is inserted at the parentheses (personal communication, Dr. J. Mudgett); the start codon for iNOS (atg) is in the second exon. A complementary sequence (*italics*) with 5′-end at +61 specifies the primer used for Fig. 2. (*Bent arrow*) The mRNA initiation site (*+1*); (*boxed*) TATA sequence. Elements are designated that are homologous to consensus sequences for binding of transcription factors involved in conferring responsiveness to cytokines or LPS in other genes, with the homology ratios in parentheses: (*horizontal underlined arrows*) γ-IRE; (*starred*) AP-1; (*double underlined*) TNF-RE; (*overlined*) NF-κB; (*underlined*) ISRE; (*overlined arrow*) GAS; (*triple underlined*) X box. (*Vertical arrows*) Restriction sites. (These sequence data are available from GenBank under accession number L09126). (*B*) CAT constructs. (*Dashed lines*) Deleted portions.

susceptible to formation of Z-DNA and sometimes conferring enhancer activity (33) occupied positions −1314 to −1245 and −645 to −600.

The whole 1,749-bp region, as in p1*iNOS-CAT*, conferred inducibility by LPS, along with synergistic responsiveness to the combination of IFN-γ plus LPS. The downstream one third, as in p2*iNOS-CAT*, contained a promoter responsive to LPS but unresponsive to the synergizing effect of IFN-γ.

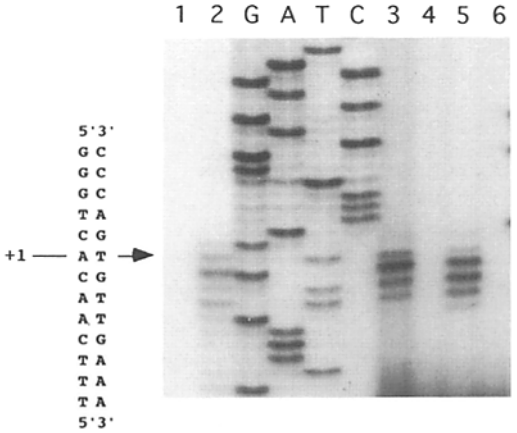


Figure 2. Determination of mRNA initiation site for *iNOS* transcript. The 26-mer indicated in Fig. 1 *A* was used for the sequence ladders (lanes *G*, *A*, *T*, and *C*). The same 26-mer was also used in primer extension after labeling the 5′-end. Single-stranded DNA complementary to nucleotides −300 to +61 was labeled at the same 5′-end and used in S1 nuclease mapping. (Lanes *1* and *2*) S1 mapping with total RNA from RAW 264.7 cells, without (lane *1*) or with (lane *2*) prior treatment of the cells for 8 h with IFN-γ (50 U/ml) and LPS (1 μg/ml). (Lanes *3–6*) Primer extension using RNA from RAW 264.7 cells (lanes *3* and *4*) or from primary macrophages (lanes *5* and *6*). The cells had been treated with (lanes *3* and *5*) or without (lanes *4* and *6*) IFN-γ and LPS (8 h for RAW 264.7 cells; 15 h for primary macrophages).

Table 1. *Inducibility of iNOS Promoter by LPS plus IFN-γ*

Plasmid	No. of Expts.	LPS plus IFN-γ	Acetylation	Fold induction
			%	
p1*iNOS-CAT*	8	– *	0.25 ± 0.03‡	
		+	10.94 ± 2.31	44
p*CAT-Basic*	5	–	0.19 ± 0.05	
		+	0.19 ± 0.03	1

* LPS (1 μg/ml) plus IFN-γ (50 U/ml).
‡ CAT activity in each transfectant is expressed as percent acetylation of chloramphenicol when 4 μg cell lysate protein was assayed at 37°C for 2 h (mean ± SEM).

The critical contribution of the upstream portion for responsiveness to IFN-γ may relate to its content of four γ-IRE, all of which match the consensus sequence perfectly (8/8). By contrast, the downstream half of the 1,749-bp fragment contains six γ-IRE, but each matches the consensus sequence less well (7/8). The upstream half also contains two ISRE lacking from the downstream half; two GAS (11/15, 8/10) versus one (7/10) in the downstream half; and one site each homologous to NF-κB, AP-1, and TNF-RE. Yet the upstream half tested alone (construct p5*iNOS-CAT*) was devoid of promoter activity, with or without LPS plus IFN-γ (data not shown).

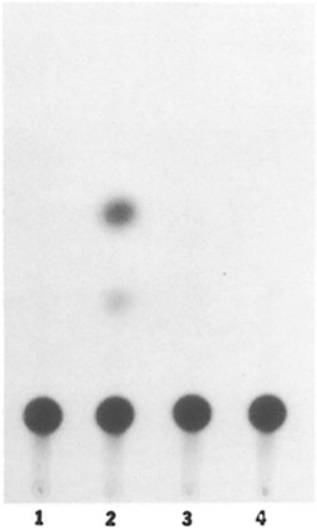


Figure 3. Demonstration of iNOS promoter activity. The construct p1*iNOS-CAT* (lanes *1* and *2*) or the vector itself, p*CAT-Basic* (lanes *3* and *4*), was transfected into RAW 264.7 cells. The cells were cultured in the absence (lanes *1* and *3*) or presence (lanes *2* and *4*) of IFN-γ (50 U/ml) and LPS (1 μg/ml). Cell lysates were then assayed for CAT and the ethyl acetate extract of the reaction mixture subjected to TLC followed by autoradiography.

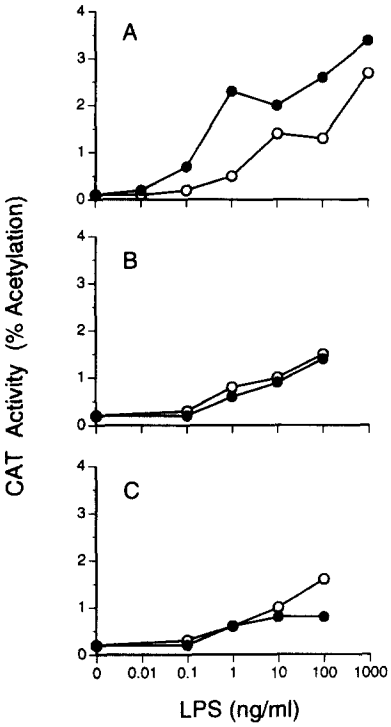


Figure 4. Concentration dependence of activation of *iNOS* promoter by LPS, and interaction with IFN-γ. CAT activity in RAW 264.7 cells transfected with (*A*) p1*iNOS-CAT*, (*B*) p2*iNOS-CAT*, or (*C*) p3*iNOS-CAT*. In each case, transfected cells were treated with the indicated concentrations of LPS with (●) or without (○) IFN-γ (50 U/ml). CAT activity was measured as described in Table 1. In each panel, all treatments were carried out with cells from the same transfected pool.

RAW 264.7 macrophage-like cells have been a useful tool for transfection with reporter constructs to demonstrate promoter activity in genes that are inducible in macrophages (34, 35). Both RAW 264.7 cells (2, 4, 13) and primary macrophages (14–16) produce iNOS when treated with combinations of cytokines and LPS. However, RAW 264.7 cells do not perfectly mirror the patterns of induction seen with primary mouse peritoneal macrophages. RAW 264.7 cells produce NO after exposure to LPS alone (13), whereas primary mouse peritoneal macrophages do so little or not at all (15). Conversely, primary mouse peritoneal macrophages respond to IFN-γ alone by producing NO (15), but early passage RAW 264.7 cells do not (our unpublished observations). Thus, a full analysis of synergistic induction and suppression of *iNOS* will require transfection of promoter-reporter constructs into additional cell types, including primary macrophages.

Perhaps the best understood example of transcriptional induction by IFN-γ involves a primary response gene coding for a guanylate binding protein of unknown function (28, 36, 37). The GAS in the guanylate binding protein promoter binds a 91-kD protein termed γ-activated factor (GAF). GAF has recently been cloned by virtue of its participation in the heterotetrameric transcription complex activated by IFN-α

(38). After treatment of human fibroblast FS2 cells with either IFN-γ or IFN-α, GAF is phosphorylated on tyrosine and translocates to the nucleus (39). Our preliminary experiments (data not shown) suggest that tyrosine phosphorylation may also participate in the induction of *iNOS*. GAF or a similar protein may undergo IFN-γ–induced tyrosine phosphorylation in the cytoplasm, translocate to the nucleus, and induce the expression of a transcription factor that binds the iNOS promoter. LPS may synergize with IFN-γ by augmenting this pathway, or by activating or inducing a distinct transcription factor(s) that may cooperate with proteins that bind the iNOS promoter in response to IFN-γ.

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Address correspondence to Dr. Qiao-wen Xie, Box 57, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

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References

1. Nathan, C. 1992. Nitric oxide as a secretory product of mammalian cells. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 6:3051.
2. Xie, Q.W., H.J. Cho, J. Calaycay, R.A. Mumford, K.M. Swiderek, T.D. Lee, A. Ding, T. Troso, and C. Nathan. 1992. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science (Wash. DC).* 256:225.
3. Cho, H.J., Q.W. Xie, J. Calaycay, R.A. Mumford, K.M. Swiderek, T.D. Lee, and C. Nathan. 1992. Calmodulin is a subunit of nitric oxide synthase from macrophages. *J. Exp. Med.* 176:599.
4. Stuehr, D.J., H.J. Cho, N.S. Kwon, M.F. Weise, and C.F. Nathan. 1991. Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. *Proc. Natl. Acad. Sci. USA.* 88:7773.
5. Yui, Y., R. Hattori, K. Kosuga, H. Eizawa, K. Hiki, and C. Kawai. 1991. Purification of nitric oxide synthase from rat macrophages. *J. Biol. Chem.* 266:12544.
6. Hevel, J.M., K.A. White, and M.A. Marletta. 1991. Purification of the inducible murine macrophage nitric oxide synthase. Identification as a flavoprotein. *J. Biol. Chem.* 266:22789.
7. Lyons, C.R., G.J. Orloff, and J.M. Cunningham. 1992. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J. Biol. Chem.* 267:6370.
8. Lowenstein, C.J., C.S. Glatt, D.S. Bredt, and S.H. Snyder. 1992. Cloned and expressed macrophage nitric oxide synthase contrasts with the brain enzyme. *Proc. Natl. Acad. Sci. USA.* 89: 6711.
9. Kilbourn, R.G., S.S. Gross, A. Jubran, J. Adams, O.W. Griffith, R. Levi, and R.F. Lodato. 1990. N^G-methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc. Natl. Acad. Sci. USA.* 87:3629.
10. Pfeilschifter, J., and K. Vosbeck. 1991. Transforming growth factor beta 2 inhibits interleukin 1 beta- and tumour necrosis factor alpha-induction of nitric oxide synthase in rat renal mesangial cells. *Biochem. Biophys. Res. Commun.* 175:372.
11. Heck, D.E., D.L. Laskin, C.R. Gardner, and J.D. Laskin. 1992. Epidermal growth factor suppresses nitric oxide and hydrogen peroxide production by keratinocytes. Potential role for nitric oxide in the regulation of wound healing. *J. Biol. Chem.* 267:21277.
12. Roberts, A.B., Y. Vodovotz, N.S. Roche, M.B. Sporn, and C. Nathan. 1992. Role of nitric oxide in antagonistic effects of TGF-β and IL-1β on the beating rate of cultured cardiac myocetes. *Mol. Endocrinol.* 11:1921.
13. Stuehr, D.J., and M.A. Marletta. 1987. Synthesis of nitrite and nitrate in murine macrophage cell lines. *Cancer Res.* 47:5590.
14. Iyengar, R., D.J. Stuehr, and M.A. Marletta. 1987. Macrophage synthesis of nitrite, nitrate, and N-nitrosamines: precursors and role of the respiratory burst. *Proc. Natl. Acad. Sci. USA.* 84:6369.
15. Ding, A.H., C.F. Nathan, and D.J. Stuehr. 1988. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J. Immunol.* 141:2407.
16. Drapier, J.C., J. Wietzerbin, and J.B. Hibbs, Jr. 1988. Interferon-gamma and tumor necrosis factor induce the L-arginine–dependent cytotoxic effector mechanism in murine macrophages. *Eur. J. Immunol.* 18:1587.
17. Nussler, A.K., S.M. Di, T.R. Billiar, R.A. Hoffman, D.A. Geller, R. Selby, J. Madariaga, and R.L. Simmons. 1992. Stimulation of the nitric oxide synthase pathway in human hepatocytes by cytokines and endotoxin. *J. Exp. Med.* 176:261.
18. Ding, A., C.F. Nathan, J. Graycar, R. Derynck, D.J. Stuehr, and S. Srimal. 1990. Macrophage deactivating factor and transforming growth factors-beta 1, -beta 2, and -beta 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. *J. Immunol.* 145:940.
19. Liew, F.Y., Y. Li, A. Severn, S. Millott, J. Schmidt, M. Salter, and S. Moncada. 1991. A possible novel pathway of regulation by murine T helper type-2 (Th2) cells of a Th1 cell activity via the modulation of the induction of nitric oxide synthase

on macrophages. *Eur. J. Immunol.* 21:2489.
20. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.
21. Greene, J.M., and K. Struhl. 1988. Analysis of RNA structure and synthesis. *In* Current Protocols in Molecular Biology. F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, editors. John Wiley & Sons. New York. 4.6.1–4.6.13.
22. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
23. Golub, E.I., H. Kim, and D.J. Volsky. 1989. Transfection of DNA into adherent cells by DEAE-dextran/DMSO method increases drastically if the cells are removed from surface and treated in suspension. *Nucleic Acids Res.* 17:4902.
24. Gorman, C.M., L.F. Moffat, and B.H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 2:1044.
25. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248.
26. Pearse, R.N., R. Feinman, and J.V. Ravetch. 1991. Characterization of the promoter of the human gene encoding the high-affinity IgG receptor: transcriptional induction by gamma-interferon is mediated through common DNA response elements. *Proc. Natl. Acad. Sci. USA.* 88:11305.
27. Yang, Z., M. Sugawara, P.D. Ponath, L. Wessendorf, J. Banerji, Y. Li, and J.L. Strominger. 1990. Interferon gamma response region in the promoter of the human DPA gene. *Proc. Natl. Acad. Sci. USA.* 87:9226.
28. Lew, D.J., T. Decker, I. Strehlow, and J.E. Darnell. 1991. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. *Mol. Cell. Biol.* 11:182.
29. Lenardo, M.J., and D. Baltimore. 1989. NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell.* 58:227.
30. Mackman, N., K. Brand, and T.S. Edgington. 1991. Lipopolysaccharide-mediated transcriptional activation of the human tissue factor gene in THP-1 monocytic cells requires both activator protein 1 and nuclear factor κB binding sites. *J. Exp. Med.* 174:1517.
31. Leitman, D.C., R.C. Ribeiro, E.R. Mackow, J.D. Baxter, and B.L. West. 1991. Identification of a tumor necrosis factor-responsive element in the tumor necrosis factor alpha gene. *J. Biol. Chem.* 266:9343.
32. Benoist, C., and D. Mathis. 1990. Regulation of major histocompatibility complex class–II genes: X, Y and other letters of the alphabet. *Annu. Rev. Immunol.* 8:681.
33. Hamada, H., and T. Kakunaga. 1982. Potential Z-DNA forming sequences are highly dispersed in the human genome. *Nature (Lond.).* 298:396.
34. Beutler, B., and T. Brown. 1991. A CAT reporter construct allows ultrasensitive estimation of TNF synthesis, and suggests that the TNF gene has been silenced in non-macrophage cell lines. *J. Clin. Invest.* 87:1336.
35. Benech, P.D., K. Sastry, R.R. Lyer, Q.G. Eichbaum, D.P. Raveh, and R.A. Ezekowitz. 1992. Definition of interferon γ-response elements in novel human Fcγ receptor gene (FcγRIb) and characterization of the gene structure. *J. Exp. Med.* 176:1115.
36. Decker, T., D.J. Lew, J. Mirkovitch, and J.J. Darnell. 1991. Cytoplasmic activation of GAF, an IFN-gamma–regulated DNA-binding factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:927.
37. Schindler, C., K. Shuai, V.R. Prezioso, and J.M. Darnell, Jr. 1992. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science (Wash. DC).* 257:809.
38. Schindler, C., X.-Y. Fu, T. Improta, R. Aebersold, and J.M. Darnell, Jr. 1992. Proteins of transcription factor ISGF-3: one gene encodes the 91- and 84-kDa ISGF-3 proteins that are activated by interferon α. *Proc. Natl. Acad. Sci. USA.* 89:7836.
39. Shuai, K., C. Schindler, V.R. Prezioso, and J.M. Darnell, Jr. 1992. Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kD DNA binding protein. *Science (Wash. DC).* 258:1808.