ical function of this enzyme during M phase.

REFERENCES AND NOTES

1. Y. Masui and H. J. Clarke, *Int. Rev. Cytol.* **57**, 185 (1979).
2. J. Gautier, C. Norbury, M. Lohka, P. Nurse, J. L. Maller, *Cell* **54**, 433 (1988); J. Gautier *et al.*, *ibid.* **60**, 487 (1990).
3. P. Nurse, *Nature* **344**, 503 (1990); A. Murray, *Curr. Biol.* **3**, 291 (1993).
4. J. C. Gerhart, M. Wu, M. W. Kirschner, *J. Cell Biol.* **98**, 185 (1984); L. M. Roy *et al.*, *ibid.* **113**, 507 (1991).
5. Y. Masui and C. L. Markert, *J. Exp. Zool.* **177**, 129 (1971).
6. N. Sagata, N. Watanabe, G. F. Vande Woude, Y. Ikawa, *Nature* **342**, 512 (1989); R. S. Freeman *et al.*, *J. Cell Biol.* **111**, 533 (1990).
7. N. Yew, M. L. Mellini, G. F. Vande Woude, *Nature* **355**, 649 (1992); I. Daar, R. S. Paules, G. Vande Woude, *J. Cell Biol.* **114**, 329 (1991).
8. N. Sagata, I. Daar, M. Oskarsson, S. D. Showalter, G. F. Vande Woude, *Science* **245**, 643 (1989).
9. N. Sagata, M. Oskarsson, T. Copeland, J. Brumbaugh, G. F. Vande Woude, *Nature* **335**, 519 (1988).
10. B. G. Gabrielli, L. M. Roy, J. Gautier, M. Philippe, J. L. Maller, *J. Biol. Chem.* **267**, 1969 (1992); B. G. Gabrielli, L. M. Roy, J. L. Maller, *Science* **259**, 1766 (1993).
11. Y. Gotoh *et al.*, *Nature* **349**, 251 (1991).
12. O. Haccard *et al.*, *Eur. J. Biochem.* **192**, 633 (1990).
13. C. B. Barrett, E. Erikson, J. L. Maller, *J. Biol. Chem.* **267**, 4408 (1992).
14. J. E. Ferrell, M. Wu, J. C. Gerhart, G. S. Martin, *Mol. Cell. Biol.* **11**, 1965 (1991).
15. Y. Gotoh *et al.*, *EMBO J.* **10**, 266 (1991).
16. N. G. Ahn *et al.*, *J. Biol. Chem.* **266**, 4220 (1991); C. M. Crews, A. Alessandrini, R. L. Erikson, *Science* **258**, 478 (1992); S. Matsuda *et al.*, *EMBO J.* **11**, 973 (1992); S. Nakielny, P. Cohen, J. Wu, T. Sturgill, *ibid.*, p. 2123.
17. S. Matsuda, Y. Gotoh, E. Nishida, *J. Biol. Chem.* **268**, 3277 (1993).
18. P. Dent *et al.*, *Science* **257**, 1404 (1992); L. R. Howe *et al.*, *Cell* **71**, 335 (1992); H. Kosako, E. Nishida, Y. Gotoh, *EMBO J.* **12**, 787 (1993); J. M. Kyriakis *et al.*, *Nature* **358**, 417 (1992); C. A. Lange-Carter, C. M. Pleiman, A. M. Gardner, K. J. Blumer, G. L. Johnson, *Science* **260**, 315 (1993); R. Seger *et al.*, *J. Biol. Chem.* **267**, 25628 (1992); C. F. Zheng and K. Guan, *ibid.* **268**, 11435 (1993).
19. J. Posada, N. Yew, N. G. Ahn, G. F. Vande Woude, J. A. Cooper, *Mol. Cell. Biol.* **13**, 2546 (1993); L. M. Roy *et al.*, unpublished results.
20. A. R. Nebreda and T. Hunt, *EMBO J.* **12**, 1979 (1993); E. K. Shibuya and J. V. Ruderman, *Mol. Biol. Cell* **4**, 781 (1993).
21. B. Sarcevic, E. Erikson, J. L. Maller, *J. Biol. Chem.*, in press.
22. N. K. Tonks, C. D. Diltz, E. H. Fischer, *ibid.* **263**, 6722 (1988).
23. J. Posada and J. A. Cooper, *Science* **255**, 212 (1992).
24. MAP kinase activity was assayed for 15 min at 30°C in 20 mM Hepes (pH 7.5), 2 mM EGTA, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), bovine serum albumin (0.1 mg/ml), 100 μM [γ-^{32}P]adenosine triphosphate (ATP) (1 to 5 cpm/fmol), with 200 μM EGF receptor peptide as substrate.
25. The pTZXA$^+$ plasmid (*7*) contained c-*mos*^{xe} cDNA in pTZ18R vector (Pharmacia). A 1.4-kilobase Eco RI–Hind III fragment containing the full-length c-*mos*^{xe} was subcloned into the Eco RI and Hind III sites of pMAL-cRI (New England Biolabs) and used to transform *E. coli* NM522 to produce the 77-kD maltose-binding protein fused to c-Mos^{xe} (MBP-Mos^{xe}).
26. I. Daar *et al.*, *Science* **253**, 74 (1991).
27. S. Hattori *et al.*, *J. Biol. Chem.* **267**, 20346 (1992); B. VanRenterghem, J. Gibbs, J. L. Maller, *ibid.* **268**, 19935 (1993).
28. C. B. Barrett, R. M. Schroetke, F. A. Van der Hoorn, S. K. Nordeen, J. L. Maller, *Mol. Cell. Biol.* **10**, 4408 (1990).
29. A. J. Muslin, A. MacNicol, L. T. Williams, *ibid.* **13**, 4197 (1993).
30. N. Shiina, T. Moriguchi, K. Ohta, Y. Gotoh, E. Nishida, *EMBO J.* **11**, 3977 (1992).
31. T. W. Sturgill, L. B. Ray, E. Erikson, J. L. Maller, *Nature* **334**, 715 (1988); D. Stokoe *et al.*, *EMBO J.* **11**, 3985 (1992).
32. J. Posada, J. Sanghera, S. Pelech, R. Aebersold, J. A. Cooper, *Mol. Cell. Biol.* **11**, 2517 (1991).
33. *Xenopus laevis* MAP kinase cDNA (*32*) was inserted into the pRSET vector (by L. E. Heasley and G. L. Johnson, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) and expressed in BL21(DE3) as described (*21*). The eluate was dialyzed against 20 mM Hepes (pH 7), 1 mM EGTA, 1 mM EDTA, 15 mM β-mercaptoethanol, 1 mM benzamidine, and 500 mM NaCl, then loaded onto a FPLC phenyl Superose HR 5/5 and eluted with a 24-ml gradient of 0 to 60% ethylene glycol and 500 to 0 mM NaCl.

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Requirement of Tyrosine Phosphorylation for Rapid Activation of a DNA Binding Factor by IL-4

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Interleukin-4 (IL-4) is an immunoregulatory cytokine produced by activated T lymphocytes to promote the growth and differentiation of cells that participate in immune defense. This study demonstrates the rapid activation of a specific DNA binding factor by IL-4. The IL-4 nuclear-activated factor (IL-4 NAF) appeared within minutes of IL-4 stimulation and recognized a specific DNA sequence found in the promoters of IL-4–responsive genes. Activation of this putative transcription factor required tyrosine phosphorylation, and antibodies specific for phosphotyrosine recognize the IL-4 NAF–DNA complex. Thus, IL-4 appears to transduce a signal to the nucleus through tyrosine phosphorylation of a latent DNA binding factor.

Interleukin-4 (IL-4) is a T cell lymphokine produced in response to mitogen or antigen stimulation (*1*). Initially discovered by its proliferative effect on B lymphocytes, it is now recognized to function in the survival, growth, and differentiation of T lymphocytes and other cells of the hematopoietic lineage and induces the expression of genes that function in immune recognition. In B lymphocytes, IL-4 activates transcription of the constant region of the immunoglobulin heavy chain genes (murine $C_\gamma1$ and C_ϵ and human $C_\gamma4$ and C_ϵ), promoting isotype class switching (*2*, *3*). IL-4 also induces transcription of the major histocompatibility class II (MHCII) genes (*4*) and a gene encoding a receptor for immunoglobulin E (IgE), FcεRII (*5*, *6*).

Interleukin-4 initiates these biological responses by binding to a specific cell surface receptor. The gene encoding the human IL-4 receptor has been isolated but does not appear to encode a receptor with intrinsic enzymatic activity (*7*). Although little is known of the IL-4 signal transduction pathway, studies have described the activation of phosphatidylinositol-3 kinase after IL-4 stimulation and provided evidence for the activation of both a tyrosine kinase and phosphatase (*8*, *9*). Here, we describe the tyrosine kinase–dependent activation of a putative transcription factor by IL-4.

Several of the biological effects of IL-4 are antagonized by another T cell lymphokine, interferon-γ (IFN-γ). Interferon-γ inhibits the immunoglobulin class switch to IgG1 and IgE induced by IL-4 (*10*) and also suppresses IL-4 induction of MHCII genes and the FcεRII gene in B cells (*11*). However, in macrophages both IFN-γ and IL-4 can induce the expression of MHCII genes (*12*). Therefore, IFN-γ and IL-4 can have either antagonistic or analogous effects on transcription. For this reason, we examined the ability of IL-4 to activate a DNA binding factor that could recognize an IFN-γ–stimulated response element.

To determine if IL-4 activates a transcription factor with DNA binding specificity for an IFN-γ response element, we analyzed electrophoretic mobility-shift assays (EMSAs) (Fig. 1). Nuclear extracts were prepared from human monocytic cells treated with IL-4, IFN-γ, or IL-4 and IFN-γ and were incubated with DNA corresponding to the IFN-γ response region of the FcγRI gene

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(*13*). A specific DNA binding factor was induced in cells that were stimulated with IFN-γ [identified as IFN-γ–activated factor (IFN-γ AF)]. Stimulation with IL-4 resulted in the appearance of a complex containing an IL-4 nuclear-activated factor (IL-4 NAF). The IL-4 NAF migrated with a slower mobility than that of IFN-γ AF and often appeared as a doublet in both monocytic cells and B cells (*14*). Because the effects of IFN-γ and IL-4 on specific gene expression can be antagonistic, we examined the ability of one cytokine to influence factor activation by the other cytokine. Pretreatment with the heterologous cytokine had no inhibitory effect on the binding of IFN-γ AF or IL-4 NAF. Because IL-4 and insulin initiate some common signaling events, we examined the effects of insulin (*15*). Treatment with insulin did not stimulate the appearance of IL-4 NAF.

If IL-4 NAF binding is necessary for the induction of gene expression by IL-4, the IL-4 NAF binding site should be present in responsive genes. An analysis of the promoter region of IL-4–stimulated genes revealed similar binding sites in many of the genes (Fig. 2A). Deletion of this site upstream of the $C_\gamma 1$ gene has been shown to inhibit transcriptional induction by IL-4 (*3*). The IL-4 NAF binding site has a GAA inverted repeat structure found in the IFN-γ response element of the FcγRI gene. The ability of IFN-γ and IL-4 to stimulate the transcriptional expression of a similar set of genes probably results from the activation of distinct DNA binding factors with similar DNA binding specificities. However, the mechanisms that mediate the antagonistic effects of IFN-γ and IL-4 on gene expression in B cells remain to be ascertained.

To ensure that IL-4 NAF can bind these similar sites in responsive genes, we used oligonucleotides that corresponded to the sites in the FcεRIIb promoter and the $C_\gamma 1$ promoter in an EMSA DNA competition experiment (Fig. 2B); these sites competed for binding of IL-4 NAF. Although these sites are not identical, they share the inverted repeat GAA structure (Fig. 2A). The IFN-γ activated site (GAS) of the guanylate-binding protein gene did not compete (*16*). Although induced by IFN-γ, the sequence of the GAS does not have an exact GAA inverted repeat consensus and may bind the factor weakly in this assay (Fig. 2B). The IFN-α–stimulated response element (ISRE) has tandem GAA repeats and did not compete for binding. An Xho I linker oligonucleotide served as a negative control.

The activation of latent IL-4 NAF is rapid, appearing within 5 min after IL-4 treatment, and is transient, disappearing by 2 hours (Fig. 3A). This activation indicates that IL-4 NAF functions in the IL-4 signal transduction pathway leading to the regulation of gene expression. The rapid activation of IL-4 NAF suggests that modification of a preexisting factor confers DNA binding activity. To determine the requirement for protein phosphorylation in the activation of IL-4 NAF, we evaluated the effect of protein kinase inhibitors (Fig. 3B). Before stimulation with IL-4, cells were treated with staurosporine, a broad spectrum kinase inhibitor with inhibitory activity for tyrosine kinases, or with genistein, a tyrosine kinase inhibitor (*17, 18*). Staurosporine completely blocked IL-4 NAF activation, and genistein inhibited activation by 95%. Therefore, tyrosine phosphorylation appears to be required for activation of IL-4 NAF.

To test whether IL-4 NAF itself was phosphorylated on tyrosine residues, we added phosphotyrosine monoclonal antibodies (anti-phosphotyrosine) to the DNA binding reaction before EMSA analysis (Fig. 4A). Increasing amounts of control monoclonal antibodies had no effect on the appearance or migration of IL-4 NAF. However, with the addition of anti-phosphotyrosine we observed a decrease in the amount of IL-4 NAF. This result suggests that IL-4 NAF is a target substrate for an IL-4–activated tyrosine kinase. In addition, tyrosine phosphorylation appears to be required for the DNA binding capability of IL-4 NAF (Fig. 4B). Treatment of nuclear extracts containing IL-4 NAF in vitro with a baculovirus-expressed protein tyrosine phosphatase inhibited the appearance of the IL-4 NAF–DNA complex (*19*). A tyrosine phosphatase has been reported to be induced by IL-4, and we

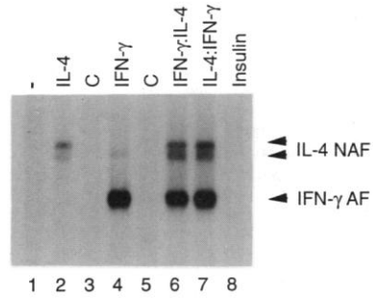

Fig. 1. Activation of a DNA binding factor by IL-4. Nuclear extracts were prepared from human THP-1 cells either untreated (lane 1) or treated with IL-4 (Upstate Biotechnology Inc., Lake Placid, New York) (20 ng/ml for 15 min) (lanes 2 and 3), IFN-γ (1000 U/ml for 15 min) (lanes 4 and 5), IFN-γ (15 min) and then IL-4 (15 min) (lane 6), IL-4 (15 min) and then IFN-γ (15 min) (lane 7), or insulin (Boehringer Mannheim) (10 μg/ml for 15 min) (lane 8) (*17, 18*). The EMSA was done with 10 μg of protein in a 15-μl reaction volume containing 12 mM Hepes (pH 7.9), 10% glycerol, 5 mM $MgCl_2$, 0.12 mM EDTA, 0.06 mM EGTA, 0.5 mM dithiothreitol, 2 μg of polydeoxyinosinic-polydeoxycytidylic acid, 0.12 μg of nonspecific plasmid DNA, and 40 ng of nonspecific single-stranded oligonucleotide. The reaction was incubated at room temperature for 10 min, and then 2 ng of end-labeled oligonucleotide corresponding to the IFN-γ response region of the FcγRI gene (nucleotides −33 to −14) was added (*13*). The mixture was incubated for 20 min before electrophoresis. Unlabeled oligonucleotide was included in the binding reactions at 50 times molar excess of probe to demonstrate specificity (lanes 3 and 5; C).

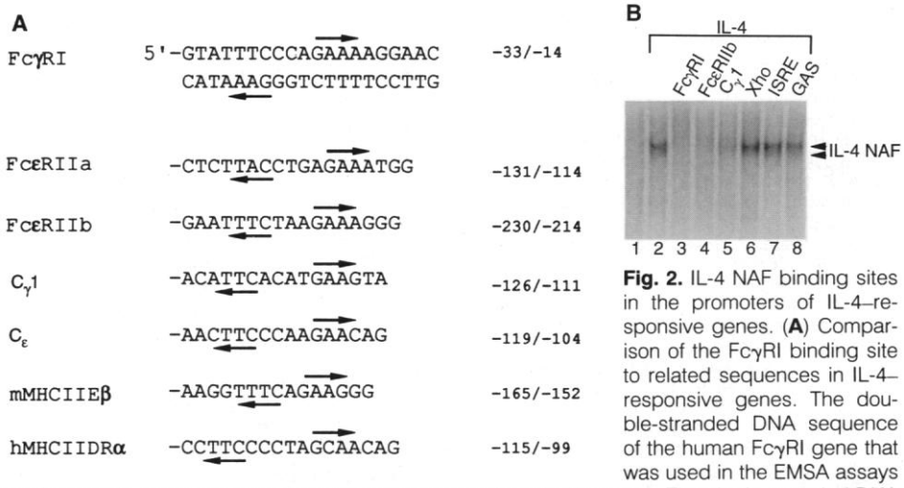

Fig. 2. IL-4 NAF binding sites in the promoters of IL-4–responsive genes. (**A**) Comparison of the FcγRI binding site to related sequences in IL-4–responsive genes. The double-stranded DNA sequence of the human FcγRI gene that was used in the EMSA assays is shown (*13*), and the inverted repeat structure is denoted with arrows. The single-stranded DNA sequences in the two promoters of the human FcεRII gene, FcεRIIa and FcεRIIb, are displayed (*6*). Similar sequences are shown in the promoters of the murine Ig heavy chain constant regions ($C_\gamma 1$ and C_ε), the murine MHCIIEβ, and the human MHCIIDRα (*2–4*). (**B**) IL-4 NAF binds to the FcεRIIb and $C_\gamma 1$ promoters. The EMSA was done with nuclear extracts from THP-1 cells either untreated (lane 1) or treated with IL-4 (20 ng/ml for 15 min) (lanes 2 through 8). The following double-stranded DNA oligonucleotides were added to the binding reactions at 150-fold molar excess of labeled probe: FcγRI (lane 3), FcεRIIb (lane 4) (nucleotides −229 to −215), $C_\gamma 1$ (lane 5) (nucleotides −128 to −109), Xho I linker (5′-CCCTCGAGGG-3′) (lane 6), ISRE (5′-GATCGGGAAAGGGAAAC-CGAAACTGAAGCC-3′) (lane 7), or GAS (5′-TCAGTTTCATATTACTCTAAATCCA-3′) (lane 8).

also found evidence for an endogenous tyrosine phosphatase (*9, 14*). To ensure that the loss of DNA binding was a result of the action of tyrosine phosphatase, we included sodium vanadate, a tyrosine phosphatase inhibitor, in the reactions, which specifically blocked this effect (Fig. 4B).

The signal transduction pathways of IFN-α and IFN-γ lead to the activation and tyrosine phosphorylation of latent transcription factors (*18, 20*). A 91-kD protein originally identified as a component of the IFN-α–stimulated transcription factor ISGF-3 is present in the IFN-γ–activated factors (*13, 20*). Because IL-4 NAF can bind to an IFN-γ response element, we tested for the presence of the 91-kD protein in IL-4 NAF. Nuclear extracts were prepared from cells that were treated with IL-4 or with IFN-γ. Specific polyclonal antisera that recognize the 91-kD protein were added to DNA binding reactions, and complex formation was measured by EMSA (Fig. 4C). The antisera to the 91-kD protein inhibited the appearance of the specific IFN-γ AF complex but did not inhibit the appearance of the IL-4 NAF complex. This immunological assay suggests that IL-4 NAF does not contain the 91-kD protein. Antibodies to the 113-kD and 48-kD components of ISGF-3 also did not recognize IL-4 NAF (*14*).

IL-4 NAF appears to function in the signal transduction pathway of IL-4. IL-4 NAF is activated within minutes of IL-4 stimulation and binds to a consensus DNA sequence in the promoters of IL-4–responsive genes. Previous studies of DNA binding proteins regulated by IL-4 have described factors that increase in appearance only after long periods of IL-4 treatment and therefore may represent secondary activation signals (*21*). This study describes the activation of a putative transcription factor by tyrosine phosphorylation. Signals activated by the IL-4–receptor complex at the cell surface may be transduced by IL-4 NAF to regulate gene expression in the nucleus.

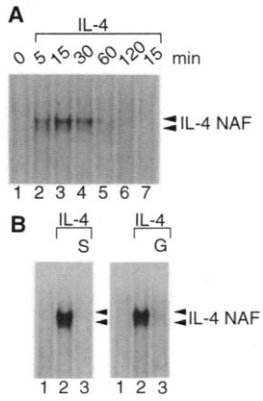

Fig. 3. Inhibition of IL-4 NAF by protein kinase inhibitors. (**A**) Time course of IL-4 NAF activation. Nuclear extracts were prepared from THP-1 cells either untreated (lane 1) or treated with IL-4 (20 ng/ml) for 5 to 120 min as indicated (lanes 2 to 6). The EMSA analysis was done with the FcγRI oligonucleotide. Unlabeled oligonucleotide at 50 times molar excess of labeled probe specifically inhibited IL-4 NAF–DNA binding (lane 7). (**B**) Inhibition of IL-4 NAF activation by staurosporine and genistein. Nuclear extracts were prepared from untreated THP-1 cells (lanes 1) or cells treated with IL-4 (20 ng/ml for 15 min) (lanes 2 and 3). Before IL-4 stimulation, cells were treated with staurosporine (S) (GIBCO, BRL) (1 μM for 60 min) or with genistein (G) (Kamiya Biomedical) (100 μg/ml for 12 hours) (lanes 3) (*17, 18*). The EMSA analysis was performed as described, and quantitation of autoradiographic signals was determined by densitometric scanning (Bio-Rad GS670).

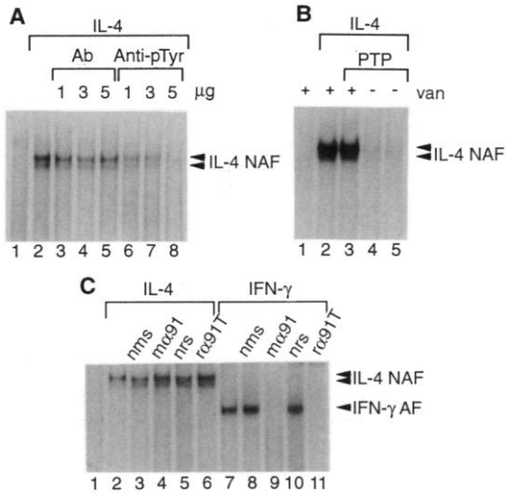

Fig. 4. Effect of specific antibodies on the IL-4 NAF–DNA complex. (**A**) EMSA was performed with nuclear extracts from untreated THP-1 cells (lane 1) or cells treated with IL-4 (20 ng/ml for 15 min) (lanes 2 through 8). Control monoclonal antibodies (Ab) (lanes 3 through 5) or anti-phosphotyrosine (anti-pTyr) (lanes 6 through 8) were added to the binding reactions in the quantities indicated for 1 hour before addition of the DNA. The anti-phosphotyrosine was a 1:1 mixture of 4G10 (Upstate Biotechnology, Inc., Lake Placid, New York) and PY20 (ICN Biomedical, Inc., Irvine, California). (**B**) Effect of protein tyrosine phosphatase (PTP) on the appearance of IL-4 NAF. THP-1 nuclear extracts from untreated cells (lane 1) or cells treated with IL-4 (lanes 2 through 5) (20 ng/ml for 15 min) were treated in vitro with 10 mM sodium vanadate (van; lanes 1 through 3) in the absence or presence of baculovirus-expressed PTP at 100 ng (lanes 3 and 4) or 20 ng (lane 5) per reaction (*19*). All extracts were incubated at 37°C for 30 min in 25 mM Hepes (pH 7.0), bovine serum albumin (1 mg/ml), and 0.2% β-mercaptoethanol followed by EMSA. (**C**) Antibodies specific for the 91-kD protein of ISGF-3 and IFN-γ AF do not recognize IL-4 NAF. THP-1 cells were untreated (lane 1), treated with IL-4 (20 ng/ml for 15 min) (lanes 2 through 6), or treated with IFN-γ (1000 U/ml for 15 min) (lanes 7 through 11). EMSA analysis was performed with nuclear extracts that had been reacted with 2 μl of polyclonal antisera for 2 hours at 4°C before DNA addition: normal murine serum (nms) (lanes 3 and 8); murine antiserum to the 91-kD protein (mα91) (lanes 4 and 9) (*22*); normal rabbit serum (nrs) (lanes 5 and 10); and rabbit 91-kD antiserum (rα91T) (lanes 6 and 11) (*20*).

REFERENCES AND NOTES

1. W. E. Paul, *Blood* **77**, 1859 (1991).
2. F. D. Finkelman *et al.*, *Annu. Rev. Immunol.* **8**, 303 (1990); J. Purkerson and P. Isakson, *FASEB J.* **6**, 3245 (1992); S. Gerondakis, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1581 (1990); P. Rothman *et al.*, *Mol. Cell. Biol.* **11**, 5551 (1991).
3. M. Xu and J. Stavenezer, *EMBO J.* **11**, 145 (1992).
4. C. Benoist and D. Mathis, *Annu. Rev. Immunol.* **8**, 681 (1990); L. H. Glimcher and C. J. Kara, *ibid.* **10**, 13 (1992).
5. H. Kikutani *et al.*, *Cell* **47**, 657 (1986); D. H. Conrad, *Annu. Rev. Immunol.* **8**, 623 (1990); M. Sarfati, S. Fournier, C. Y. Wu, G. Delespesse, *Immunol. Res.* **11**, 260 (1992).
6. A. Yokota *et al.*, *Cell* **55**, 611 (1988); U. Suter, G. Texido, H. Hofstetter, *J. Immunol.* **143**, 3087 (1989).
7. R. L. Idzerda *et al.*, *J. Exp. Med.* **171**, 861 (1990).
8. A. O. Morla, J. Schreurs, A. Miyajima, J. Y. J. Wang, *Mol. Cell. Biol.* **8**, 2214 (1988); G. M. McGarvie and W. Cushley, *Immunol. Lett.* **22**, 221 (1989); R. J. Isfort and J. N. Ihle, *Growth Factors* **2**, 213 (1990); L.-M. Wang *et al.*, *EMBO J.* **11**, 4899 (1992).
9. A. R. Mire-Sluis and R. Thorpe, *J. Biol. Chem.* **266**, 18113 (1991).
10. C. M. Snapper and W. E. Paul, *Science* **236**, 944 (1987).
11. J. J. Mond, J. Carman, C. Sarma, J. Ohara, F. D. Finkelman, *J. Immunol.* **137**, 353 (1986); T. DeFrance *et al.*, *J. Exp. Med.* **165**, 1459 (1987).
12. P. M. Stuart, A. Zlotnik, J. G. Woodward, *J. Immunol.* **140**, 1542 (1988); H. Cao, R. G. Wolff, M. S. Meltzer, R. M. Crawford, *ibid.* **143**, 3524 (1989).
13. R. N. Pearse *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4314 (1993); C. Perez, J. Wietzerbin, P. Benech, *Mol. Cell. Biol.* **13**, 2182 (1993); K.-I. Igarashi, M. David, A. Larner, D. S. Finbloom, *ibid.*, p. 3984; K. C. Gilmour and N. C. Reich, unpublished data.
14. H. Kotanides and N. C. Reich, unpublished data.
15. L.-M. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4032 (1993).
16. D. J. Lew, T. Decker, I. Strehlow, J. E. Darnell Jr., *Mol. Cell. Biol.* **11**, 182 (1991).
17. N. C. Reich and L. M. Pfeffer, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8761 (1990).
18. M. J. Gutch, C. Daly, N. C. Reich, *ibid.* **89**, 11411 (1992).
19. Q. Yang, D. Co, J. Sommercorn, N. K. Tonks, *J. Biol. Chem.* **268**, 6622 (1993).
20. C. Shindler, K. Shuai, V. R. Prezioso, J. E. Darnell Jr., *Science* **257**, 809 (1992); X.-Y. Fu, *Cell* **70**, 323 (1992); K. Shuai, C. Schindler, V. R. Prezioso, J. E. Darnell Jr., *Science* **258**, 1808 (1992).
21. M. Boothby, E. Gravallese, H.-C. Liou, L. H. Glimcher, *Science* **242**, 1559 (1988); H. Illges and A. Radbruch, *Mol. Immunol.* **29**, 1265 (1992).
22. M. J. Gutch and N. C. Reich, unpublished data. Murine antiserum to the 91-kD protein was generated by immunization with a pGEX bacterial fusion protein containing amino acids 601 to 727 of the 91-kD protein.

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