Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 7214–7219, June 1999
Biochemistry

NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun

CRISTINA LÓPEZ-RODRÍGUEZ, JOSÉ ARAMBURU, ANDREW S. RAKEMAN, AND ANJANA RAO*

Department of Pathology, Harvard Medical School, The Center for Blood Research, Boston, MA 02115

Communicated by Stephen C. Harrison, Harvard University, Cambridge, MA, April 29, 1999 (received for review March 24, 1999)

ABSTRACT **NFAT transcription factors are related to NF-κB/Rel proteins and form cooperative complexes with Fos and Jun on DNA. We have identified an NFAT-related protein, NFAT5, which differs from the conventional NFAT proteins NFAT1–4 in its structure, DNA binding, and regulation. NFAT5 contains a NFAT-like Rel homology domain, conserves the DNA contact residues of NFAT1–4, and binds DNA sequences similar to those found in the regulatory regions of well-characterized NFAT-dependent genes. However, it lacks the majority of Fos/Jun contact residues and does not bind cooperatively with Fos and Jun to DNA. Unlike NFAT1–4, whose nuclear import is tightly regulated by calcineurin-mediated dephosphorylation, NFAT5 is a constitutively nuclear phosphoprotein regardless of calcineurin activation. These features suggest that unlike the conventional NFAT proteins, NFAT1–4, which activate gene transcription by integrating inputs from calcium/calcineurin and protein kinase C/mitogen-activated protein kinase signaling pathways, NFAT5 participates in as-yet-unidentified signaling pathways in diverse immune and nonimmune cells.**

The NFAT family of transcription factors contains four members, NFAT1 (NFATp), NFAT2 (NFATc), NFAT3, and NFAT4 (NFATx), each expressed as several isoforms related by alternative splicing (1–6) (reviewed in ref. 7). Genes encoding NFAT proteins are transcribed in almost every tissue tested, although the expression of individual proteins is fairly tissue restricted (5, 7). NFAT1, NFAT2, and NFAT4 are expressed in immune cells, and their roles in the immune response have been well characterized (8–15). Additionally, NFAT2 has been implicated in cardiac valve development (16, 17) and NFAT3 in cardiac hypertrophy (18).

The transcriptional function of NFAT proteins is regulated at several levels. When activated by a rise in intracellular calcium, the calmodulin-dependent phosphatase calcineurin dephosphorylates NFAT proteins in the cytoplasm, exposing their nuclear localization sequences and inducing their translocation into the nucleus (19–22). Calcineurin binds to NFAT proteins by recognizing a conserved targeting motif (PxIxIT) present near the N terminus of the NFAT regulatory domain (19). The regulatory domain also contains a nuclear localization sequence (NLS), a serine-rich region ([SP]SPxSS[S]xSSx-SxxS[D/E]), and three ''SP motifs'' (SPxxSPxxSPxx-[SPxx]xxx[D/E][D/E]) (reviewed in refs. 7 and 23). Once NFAT proteins are in the nucleus, their central Rel-like domain dictates their binding specificity for DNA (7). A major level of regulation is conferred by the ability of NFAT proteins to form stable, cooperative DNA-binding complexes with dimers of the AP-1 (Fos/Jun) family at composite NFAT:AP-1 DNA elements that have been identified in multiple NFAT-regulated genes (7, 24, 25). This combinatorial mechanism results in integration of the calcium/calcineurin and protein kinase C/mitogen-activated protein kinase signaling pathways that are activated by stimulation of the T cell receptor as well as other immune and nonimmune pathways (7, 26).

The calcineurin dependence of NFAT proteins makes them targets of the immunosuppressive drugs cyclosporin A (CsA) and FK506 (27, 28). These powerful agents not only have contributed in a major way to the success of transplant surgery over the last 15 years (27, 28), but also may prove important in the treatment of cardiac hypertrophy (18).

We have cloned an additional member of the NFAT family, NFAT5. The NFAT5 gene is widely transcribed and encodes a protein of 1,455 aa. In contrast to the conventional NFAT proteins, NFAT1–4, which show high and moderate sequence identity in their DNA-binding and N-terminal regulatory domains, respectively, NFAT5 exhibits a clear relation to NFAT proteins only in its Rel-like DNA-binding domain. The DNA-binding specificity of NFAT5 is similar to that of NFAT1, but the NFAT5 DNA-binding domain differs from the DNA-binding domains of NFAT1–4 in that it does not cooperate with Fos/Jun at NFAT:AP-1 composite sites. A striking feature of NFAT5 is its constitutive nuclear localization that is not modified on cellular activation. Taken together, our data indicate that NFAT5 is a target of signaling pathways distinct from those that regulate NFAT1–4, and that it is likely to modulate cellular processes in a wide variety of cells.

MATERIALS AND METHODS

cDNA Cloning. A search of the expressed sequence tag database, conducted by using the conserved nucleotide sequences encoding the DNA-binding domains of NFAT1–4, yielded the clone W27762, a 743-bp sequence that when translated showed partial homology with the last 60 residues of the NFAT DNA-binding domain. By using primers from this conserved region we amplified a region by reverse transcription–PCR and expanded the original sequence by 5′ and 3′ rapid amplification of cDNA ends (RACE) using human brain RNA. A probe based on the 5′ RACE product was used to screen a λgt10 human brain cDNA library (CLONTECH). For each stretch of NFAT5 sequence at least two independent cDNA clones were sequenced on both strands. Protein motifs were identified by using PROFILESCAN and PROSITE programs.

Northern Analysis. Northern blots (CLONTECH) bearing poly(A)$^+$ mRNA from different human tissues were hybridized with a 285-base antisense cDNA probe (bp 1020–1305 of NFAT5) obtained by primer extension.

PCR-Based Selection and Amplification of DNA-Binding Sites. The DNA-binding domains of NFAT1 (amino acids 398–694) (29) and NFAT5 (residues 175 to 471) were expressed as hexahistidine-tagged proteins in *Escherichia coli* and used to identify optimal DNA-binding sites for NFAT1

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

Abbreviation: CsA, cyclosporin A.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF134870).

*To whom reprint requests should be addressed at: The Center for Blood Research, 200 Longwood Avenue, Boston, MA 02115. e-mail: arao@cbr.med.harvard.edu.

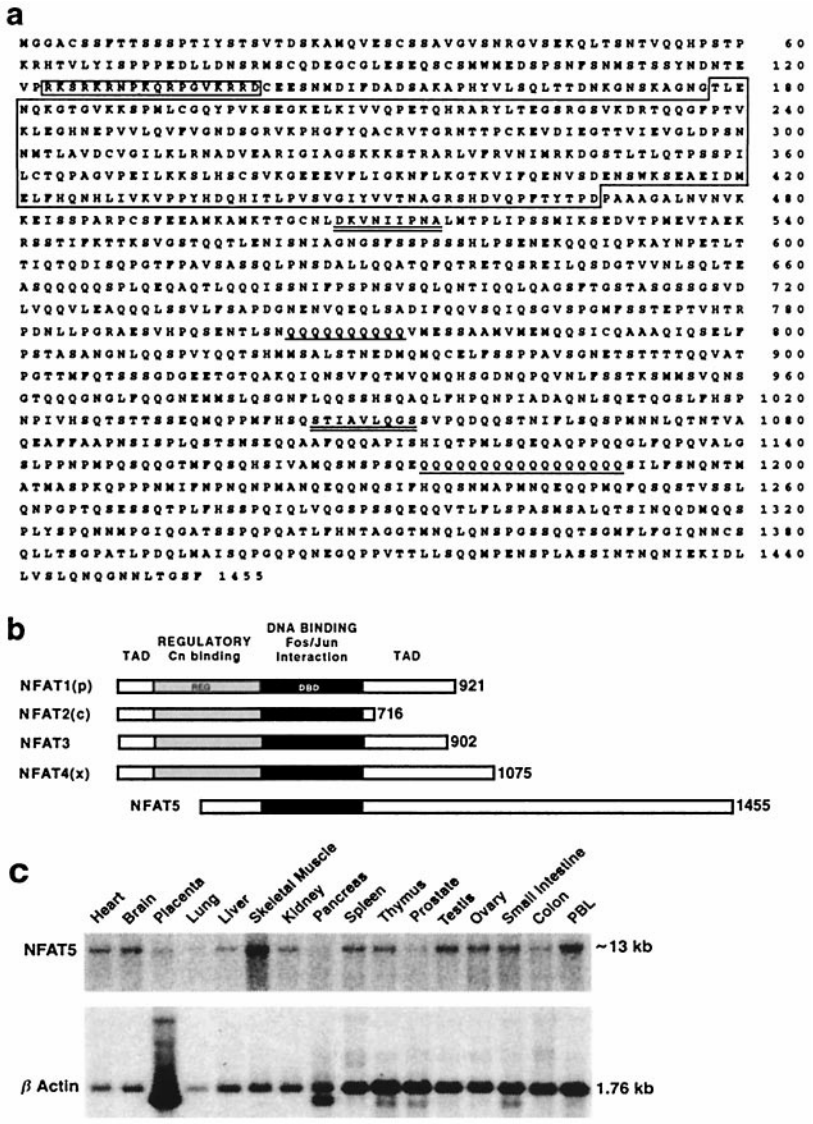

FIG. 1. Sequence and expression pattern of NFAT5. (*a*) Predicted amino acid sequence of human NFAT5. The Rel homology region and a bipartite nuclear localization sequence are indicated by large and small boxes, respectively. Two polyglutamine stretches and a putative protein interaction motif (41) in the C-terminal region are singly and doubly underlined. (*b*) Schematic comparison of the five NFAT family members. Selected splice variants of NFAT1–4 are shown. The DNA-binding domain (DBD) conserved in NFAT1–5 and the regulatory domain (REG) represented in NFAT1–4 are indicated. TAD, transactivation domain. (c) Widespread expression of NFAT5 mRNA. The single NFAT5 transcript (≈13 kb) is expressed at highest levels in skeletal muscle, brain, heart, and peripheral blood leukocytes (PBL), and at moderate to detectable levels in other tissues. The blots were rehybridized with a human β-actin cDNA probe as a control for RNA loading.

and NFAT5 (31). A double-stranded DNA library with 18 randomized bases was used in the binding site selection experiments (30). Four rounds of selection were used in each case: 20 nM recombinant protein was used in the first round of selection and 100 nM in the three subsequent rounds. Binding conditions were 10% glycerol, 10 mM Hepes, 120 mM NaCl, 0.5% NP-40, 0.8 mg/ml BSA, 0.25 mM DTT, and 20 μg/ml poly(dI):poly(dC).

Electrophoretic Mobility-Shift Assay. Binding conditions were the same as for the PCR selection experiments, with the radio-labeled probe used at 500 pM. NFAT1 and NFAT5 DNA-binding domains were used at 5 nM and 200 nM, respectively, to obtain comparable intensities of DNA-protein complexes in the absence of Fos and Jun. Recombinant c-Fos and c-Jun [full-length for Fig. 3*b*, c-Fos (118–211) and c-Jun (186–334) for Fig. 3*c*] (29) were tested over a 2–200 nM range and used at 40 nM in the experiments of Fig. 3. Neither NFAT1 nor NFAT5 bound cooperatively to the ARRE-2 element in the presence of several other basic leucine zipper proteins tested, including C/EBPα, C/EBPβ, C/EBPδ, ATF2, or FosB (proteins kindly provided by C. Vinson, National Cancer Institute, Bethesda, MD) (31). Oligonucleotide sequences were: murine IL-2 promoter ARRE-2 site, 5′-GCCCAAAG-AGGAAAATTTGTTTCATACAG-3′; and ARRE-2 (CONS AP-1) site, 5′-CCCCAAAGAGGAAAATTTGAC-TCATACAG-3′.

Expression Plasmids. The NFAT5 plasmid used for mammalian expression was tagged with six copies of the Myc epitope tag at the N terminus and green fluorescent protein (GFP) at the C terminus. The presence of GFP did not affect the behavior of NFAT5. The truncated, constitutively active form of the calcineurin A chain (ΔCn) (32) was coexpressed with calcineurin B chain.

Cell Lines and Transfections. The Cl.7W2 murine v-fos-transformed T cell clone has been described (33). C2C12 myoblast cells were kindly provided by Andrew Lassar, Harvard Medical School, Boston. Jurkat T cells were transfected by electroporation in serum-free medium (250 V, 960 μF).

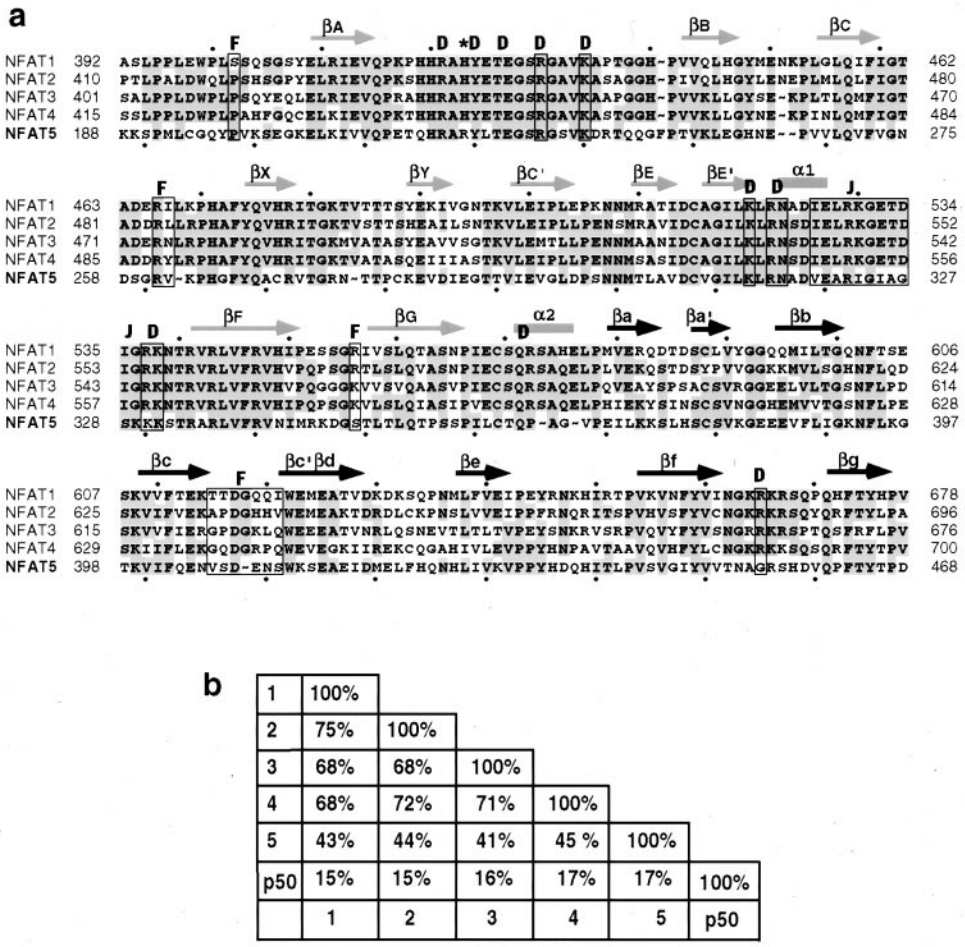

FIG. 2. Comparison of the DNA-binding domains of NFAT1–5. (*a*) Alignment of the Rel homology regions of human NFAT1–5. Residues that are identical in at least three of the five proteins are shown in gray. The arrows and bars above the sequence indicate the secondary structure elements deduced from the crystal structure of the NFAT1:Fos:Jun:DNA complex (25). Light and dark arrows are used for the N- and C-terminal portions of the Rel homology domain. The residues implicated in DNA (D), Fos (F), and Jun (J) contacts are boxed. The asterisk indicates alteration of the invariant His residue in the DNA-recognition loops of NFAT1–4 to Arg in NFAT5. (*b*) Pairwise sequence comparison of the Rel homology regions of p50 and NFAT1–5. Numbers indicate the percentage of identical residues.

HEK293 cells were transfected by calcium phosphate precipitation. For reporter assays, Jurkat cells were stimulated 24 hr after transfection with 2 μM ionomycin and 10 nM phorbol 12-myristate 13-acetate, and cell extracts were assayed for reporter activity 6–10 hr later (32). Results (mean ± SE) were analyzed by using the Student's *t* test.

Metabolic Labeling. Metabolic labeling with ^{32}P-orthophosphate and immunoprecipitations were done as described (34). Cells were lysed in 50 mM Hepes (pH 7.4), 10 mM sodium pyrophosphate (NaPPi), 110 mM NaCl, 5 mM iodoacetamide, 2.5 mM EGTA, 1% Triton X-100, 0.5 mM DTT, 10 units/ml DNase I, 2 mM PMSF, 50 μg/ml aprotinin, and 25 μM leupeptin. Immunoprecipitates were boiled in Laemmli buffer supplemented with 10 mM NaPPi.

Immunocytochemistry. Immunocytochemistry was performed as described (34), using rabbit polyclonal antisera generated against the N-terminal region (amino acids 2–177) or the DNA-binding domain (amino acids 175–471) of human NFAT5, or against the anti-67.1 peptide of murine NFAT1 (34). The specificity of staining was confirmed in all cases by using preimmune sera, as well as immune sera preabsorbed with the appropriate recombinant proteins.

RESULTS AND DISCUSSION

Molecular Cloning and Expression Pattern of NFAT5. cDNA clones encoding NFAT5 were isolated from a λgt10 human brain cDNA library. The longest sequence compiled from overlapping clones had an ORF of 4,365 bp and encoded a predicted protein of 1,455 aa (Fig. 1 *a* and *b*). NFAT5 mRNA (≈13 kb) was detected in all tissues examined, with higher expression in heart, brain, skeletal muscle, and peripheral blood lymphocytes (Fig. 1*c*).

The Rel homology region of NFAT5 showed 41–45% identity with NFAT1–4 and 15–17% identity with p50 NF-κB in pairwise comparisons (Fig. 2 *a* and *b*), indicating that NFAT5 is an outlying member of the NFAT family with a more distant relationship to the Rel family. However, NFAT5 lacks the moderately conserved regulatory domain located N-terminal to the DNA-binding domains of NFAT1–4 (7, 20, 21, 32, 35, 36). The C-terminal region of NFAT5, which is long (987 aa) and glutamine-rich (19.3%), shows no sequence similarity to the C-terminal regions of NFAT1–4 (Fig. 1*a*), consistent with the fact that there is little or no sequence conservation among the C-terminal regions of NFAT proteins (7).

Restricted DNA-Binding Specificity of NFAT5. The Rel homology region of NFAT5 is identical with that of NFAT1–4 at 11 of the 14 DNA contact residues identified in the crystal structure of the NFAT1:Fos:Jun:DNA complex (25) (Fig. 2*a*), and both NFAT1 and NFAT5 selected the extended sequence TGGAAA from a randomized oligonucleotide pool (30). However, NFAT1 showed an absolute preference only for the

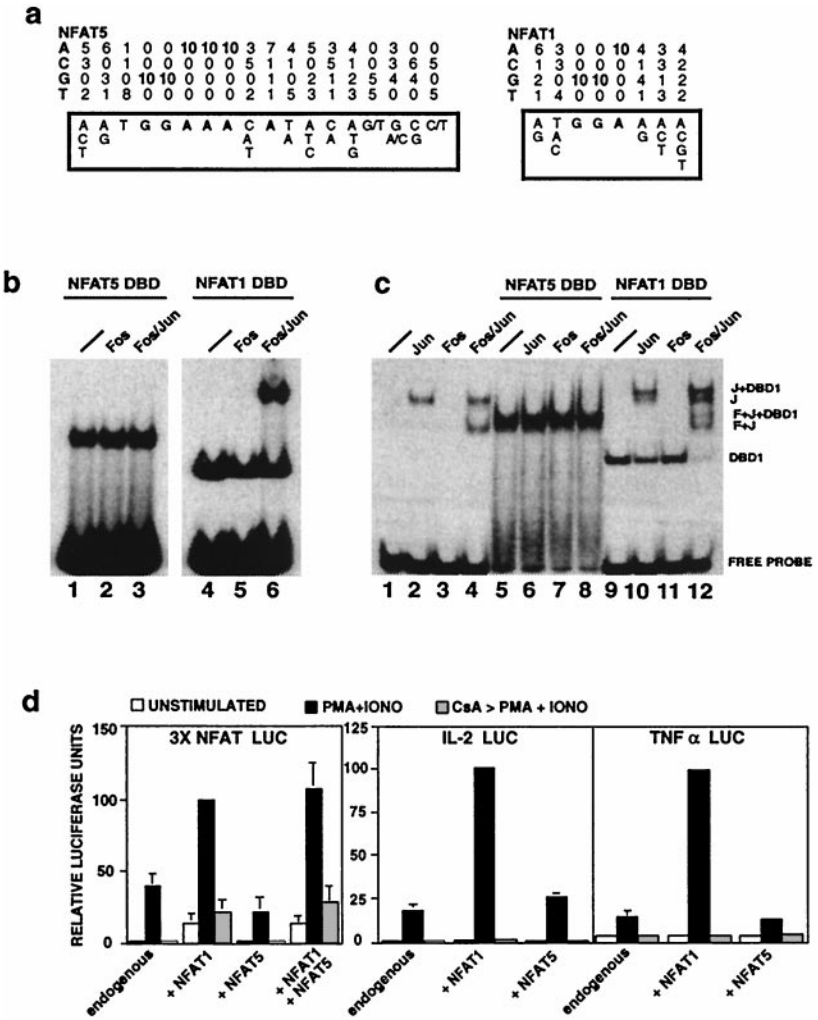

FIG. 3. DNA binding, Fos-Jun interaction, and transactivation by NFAT5. (*a*) Identification of the optimal binding site for NFAT5. The boxed sequences summarize the results of aligning 45 NFAT5-selected and 21 NFAT1-selected PCR clones, obtained from four independent selections. At each position, the probability of occurrence of each of the four nucleotides is normalized to an overall value of 10, with the numbers rounded off to the nearest integer. Bases showing a greater than 60% probability of occurrence are indicated in bold. (*b*) NFAT5 does not bind cooperatively with Fos and Jun to the murine IL-2 promoter ARRE-2 element. DBD, DNA-binding domain. (*c*) NFAT5 does not bind cooperatively with Fos and Jun to the ARRE-2 element modified to contain a strong consensus AP-1 site. (*d*) NFAT5 lacks the ability to activate luciferase (LUC) expression driven by three copies of the ARRE-2 site, the human IL-2 promoter, or the human tumor necrosis factor α (TNF-α) promoter. PMA, phorbol 12-myristate 13-acetate; IONO, ionomycin.

GGA core whereas NFAT5 showed a clear preference for the longer sequence PuTGGAAAnA (Fig. 3*a*).

The DNA recognition loop of NFAT5 (RARYLTEG) contains an arginine rather than a histidine at position 3, and thus is intermediate between Rel (RFRYxCEG) and NFAT1–4 (RAHYxTEG). This arginine residue may participate in DNA contact by NFAT5, as demonstrated for the corresponding substitution in NFAT2 (37). In the α2 linker region, the residue corresponding to Gln-571 of NFAT1 is conserved in NFAT5. This glutamine residue makes contact with adenine-3 of the core GGA binding sequence in NFAT1 (25), and its conservation in NFAT5 most likely accounts for the fact that NFAT5 selectes the same core GGA-binding sequence as NFAT1 (Fig. 3*a*). However NFAT5 diverges from NFAT1–4 in other key features of the α2 linker region. In particular, Arg-572, a residue known to make a major DNA contact in NFAT1, is substituted with proline in NFAT5 (Fig. 2*a*). This alteration, together with the overall loss of two residues in the α2 linker region (QRSAHE > QPAG), the introduction of the helix-breaking residue Gly-367, and the charge difference imposed by substitution of the invariant arginine (Arg-572) and two invariant glutamates (Glu-568 and Glu-576) with uncharged residues, may combine to force a different orientation of the N- and C-terminal domains of the NFAT5 DNA-binding domain relative to the orientations expected for NFAT1–4 (25).

NFAT5 Does Not Bind Cooperatively with Fos and Jun to DNA. In contrast to the conservation of DNA contact residues, the residues participating in Jun interaction are poorly conserved in NFAT5: only three of the 11 invariant Jun-contact residues of NFAT1–4 remain identical in NFAT5 (25) (E'F loop, Fig. 2*a*). Five of the six nonconservative changes (K>I, E>I, T>A, D>G and I>S) are likely to alter substantially the overall conformation of this loop; in particular, the Thr-533 to Ala change abrogates Jun interaction by NFAT2 (38). However, the invariance of Glu-320 and the conservation of the hydrophobic patch centered on Phe-473 are compatible with conservation of DNA interactions and loop structure in the region nearest the DNA (25). Consistent with these features, NFAT5 bound to the murine IL-2 promoter ARRE-2 sequence, which fits the consensus for a strong NFAT5-binding site (Fig. 3 *a* and *b*), but did not form cooperative complexes with Fos and Jun, even when the ARRE-2 element was modified so that the nonconsensus AP-1 site adjacent to the

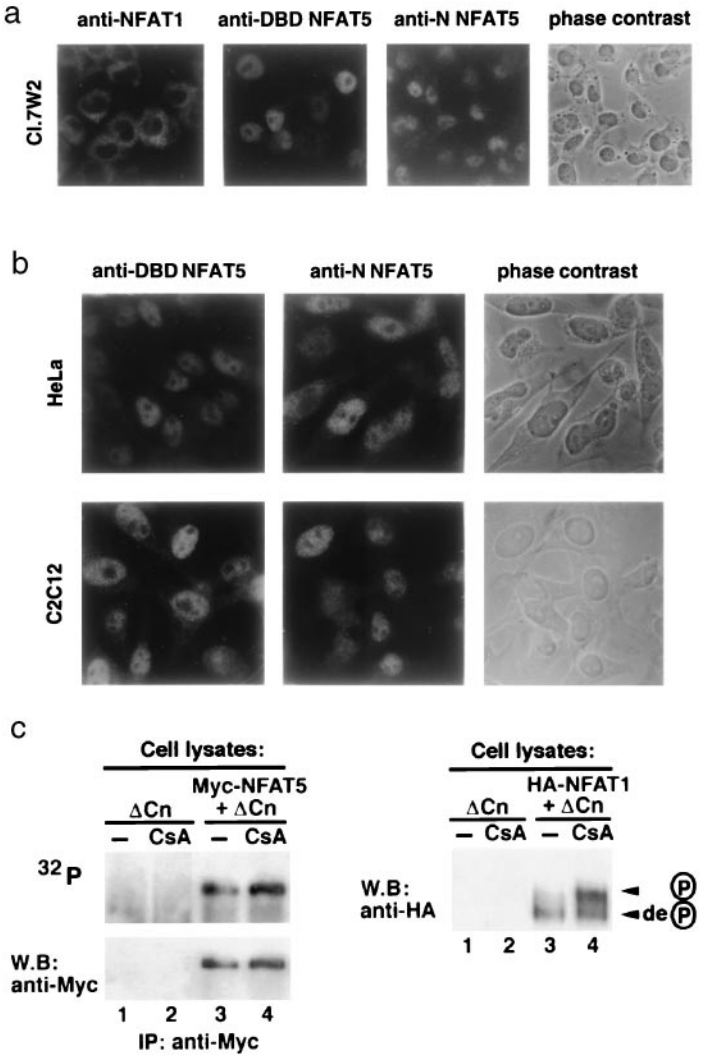

FIG. 4. Nuclear localization and phosphorylation status of NFAT5. (*a*) Nuclear localization of endogenous NFAT5 in a resting murine T cell clone. The first three panels show immunocytochemical staining of Cl.7W2 murine T cells with anti-NFAT1, or antisera against the N-terminal region (anti-N) or DNA-binding domain (anti-DBD) of NFAT5. (*Right*) Phase-contrast photomicrograph of the field stained with anti-N of NFAT5. (*b*) Nuclear localization of endogenous NFAT5 in resting HeLa (fibroblast, *Upper*) and C2C12 (myoblast, *Lower*) cells. (*Left* and *Middle)* Antisera against the NFAT5 DNA-binding domain (anti-DBD) or N-terminal region (anti-N) of NFAT5. (*Right*) Phase-contrast photomicrograph of the same field stained with anti-N. (*c*) Analysis of NFAT5 phosphorylation. (Upper) 293 cells expressing ΔCn or Myc-NFAT5-GFP and ΔCn were metabolically labeled with ^{32}P-orthophosphate in the absence or presence of 1 μM CsA. NFAT5 was immunoprecipitated (IP) with anti-Myc and detected by autoradiography (^{32}P) and Western blotting (W.B.). (*Lower*) In a parallel experiment, activity of ΔCn and inhibition by CsA were monitored by expressing hemagglutinin (HA)-tagged NFAT1 in 293 cells and evaluating its phosphorylation status in SDS-lysates by Western blotting with anti-HA. Arrows indicate the dephosphorylated and phosphorylated forms of NFAT1.

NFAT site was altered to a strong consensus AP-1 site (39) (Fig. 3 *b* and *c*).

NFAT5 Does Not Transactivate Conventional NFAT-Dependent Reporters. Despite its ability to bind DNA elements resembling those recognized by NFAT1–4, NFAT5 did not activate several NFAT-dependent reporter plasmids in Jurkat T cells, nor suppress the activity of endogenous NFAT (Fig. 3*d*). We confirmed that NFAT5 was expressed as a full-length protein and was localized to the nucleus under these conditions (data not shown). Similar results also were obtained with the 3xNFAT-Luc reporter in a nonlymphoid cell line, the human embryonic kidney cell line HEK293. The inability of NFAT5 to activate transcription in these cell types under these conditions may be explained in several (not mutually exclusive) ways. NFAT5 function may require specific conditions of stimulation or may occur only in specific cell types, for instance NFAT5 itself, or a partner protein needed for NFAT5 function needs to be activated via specific signaling. Thus a simultaneous scanning for appropriate promoters/enhancers, cell types, and stimulation conditions may be required to define optimal conditions for NFAT5-mediated transactivation and to identify any partner proteins that might be involved.

NFAT5 Is a Nuclear Phosphoprotein. We investigated the subcellular localization and phosphorylation status of NFAT5, focusing on its potential regulation by calcineurin. Endogenous NFAT5 is constitutively present in the nucleus of a resting T cell clone (Fig. 4 *a* and *b*), in striking contrast with the cytoplasmic localization of NFAT1 in the same cells (Fig. 4*a*). This nuclear localization is consistent with the presence of a bipartite nuclear localization sequence in the N-terminal region of NFAT5 (40) (Fig. 1*a*) and was unaffected by treatment with phorbol 12-myristate 13-acetate, ionomycin, and CsA, alone or in combination (5–60 min; data not shown). NFAT5 also is expressed and localized to the nucleus in HeLa cells and C2C12 myoblast cells (Fig. 4*b*). NFAT5 is phosphorylated in transfected 293 cells, and its overall level of phosphorylation (unlike that of NFAT1) was not affected by coexpressing a truncated, constitutively active form of calcineurin (ΔCn) (32) or by inhibiting calcineurin activity with CsA (Fig. 4*c*).

In summary, NFAT5 shares DNA-binding specificity with the conventional NFAT proteins NFAT1–4 but differs strikingly in two other features considered characteristic of the NFAT family of proteins. Unlike NFAT1–4, NFAT5 does not cooperate with Fos and Jun in the DNA, nor is its subcellular location regulated by calcineurin-mediated dephosphorylation. The distinct structural and functional properties of NFAT5 indicate that it is unlikely to be a target of the same signaling pathways that regulate NFAT1–4 and mediate their cooperation with Fos and Jun. The existence of NFAT5 raises the possibility of new regulatory mechanisms for NFAT-modulated genes.

We thank Patrick Hogan, Ed Lamperti, Keith Blackwell, Lin Chen, Andrew Lassar, and the members of the laboratory for reagents, valuable discussions, and advice. This work was supported by National Institutes of Health Grants CA-42471 and AI-40127 (to A.R.). C.L.-R was supported by a Postdoctoral Fellowship from Ministerio de Educacion y Ciencia, Spain. J.A. is a recipient of an Arthritis Foundation Postdoctoral Fellowship.

1. McCaffrey, P. G., Luo, C., Kerppola, T. K., Jain, J., Badalian, T. M., Ho, A. M., Burgeon, E., Lane, W. S., Lambert, J. N., Curran, T., *et al*. (1993) *Science* **262,** 750–754.
2. Northrop, J. P., Ho, S. N., Chen, L., Thomas, D. J., Timmerman, L. A., Nolan, G. P., Admon, A. & Crabtree, G. R. (1994) *Nature (London)* **369,** 497–502.
3. Park, J., Takeuchi, A. & Sharma, S. (1996) *J. Biol. Chem.* **271,** 20914–20921.
4. Chuvpilo, C., Zimmer, M., Kerstan, A., Glockner, J., Avots, A., Escher, C., Fischer, C., Jankevikcs, E., Berberich-Siebelt, F., Schmitt, E. & Serfling, E. (1999) *Immunity* **10,** 261–269.
5. Hoey, T., Sun, Y. L., Williamson, K. & Xu, X. (1995) *Immunity* **2,** 461–472.
6. Masuda, E. S., Naito, Y., Tokumitsu, H., Campbell, D., Saito, F., Hannum, C., Arai, K. & Arai, N. (1995) *Mol. Cell. Biol.* **15,** 2697–2706.
7. Rao, A., Luo, C. & Hogan, P. G. (1997) *Annu. Rev. Immunol.* **15,** 707–747.
8. Xanthoudakis, S., Viola, J. P., Shaw, K. T., Luo, C., Wallace, J. D., Bozza, P. T., Luk, D. C., Curran, T. & Rao, A. (1996) *Science* **272,** 892–895.
9. Kiani, A., Viola, J. P., Lichtman, A. H. & Rao, A. (1997) *Immunity* **7,** 849–860.
10. Viola, J. P., Kiani, A., Bozza, P. T. & Rao, A. (1998) *Blood* **91,** 2223–2230.
11. Ranger, A. M., Hodge, M. R., Gravallese, E. M., Oukka, M., Davidson, L., Alt, F. W., de la Brousse, F. C., Hoey, T., Grusby, M. & Glimcher, L. M. (1998) *Immunity* **8,** 125–134.
12. Yoshida, H., Nishina, H., Takimoto, H., Marengere, L. E., Wakeham, A. C., Bouchard, D., Kong, Y. Y., Shahinian, A., Bachmann, M., Ohashi, P. S., *et al*. (1998) *Immunity* **8,** 115–124.
13. Oukka, M., Ho, I. C., de la Brousse, F. C., Hoey, T., Grusby, M. J. & Glimcher, L. H. (1998) *Immunity* **9,** 295–304.
14. Ranger, A. M., Oukka, M., Rengarajan, J. & Glimcher, L. H. (1998) *Immunity* **9,** 627–635.
15. Hodge, M. R., Ranger, A. M., Charles de la Brousse, F., Hoey, Y., Grusby, M. J. & Glimcher, L. M. (1996) *Immunity* **4,** 397–405.
16. Ranger, A. M., Grusby, M. J., Hodge, M. R., Gravallese, E. M., de la Brousse, F. C., Hoey, T., Mickanin, C., Baldwin, H. S. & Glimcher, L. H. (1998) *Nature (London)* **392,** 186–190.
17. de la Pompa, J. L., Timmerman, L. A., Takimoto, H., Yoshida, H., Elia, A. J., Samper, E., Potter, J., Wakeham, A., Marengere, L., Langille, B. L., *et al*. (1998) *Nature (London)* **392,** 182–186.
18. Molkentin, J., Lu, J.-R., Antos, C. L., Markham, B., Richardson, J., Robbins, J., Grant, S. R. & Olson, E. N. (1998) *Cell* **93,** 215–228.
19. Aramburu, J., Garcia-Cozar, F., Raghavan, A., Okamura, H., Rao, A. & Hogan, P. G. (1998) *Mol. Cell* **1,** 627–637.
20. Luo, C., Shaw, K. T., Raghavan, A., Aramburu, J., Garcia-Cozar, F., Perrino, B. A., Hogan, P. G. & Rao, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8907–8912.
21. Beals, C. R., Clipstone, N. A., Ho, S. N. & Crabtree, G. R. (1997) *Genes Dev.* **11,** 824–834.
22. Zhu, J., Shibasaki, F., Price, R., Guillemot, J. C., Yano, T., Dotsch, V., Wagner, G., Ferrar, P. & Mckeon, F. (1998) *Cell* **93,** 851–861.
23. Crabtree, G. (1999) *Cell* **96,** 611–614.
24. Jain, J., McCaffrey, P. G., Valge-Archer, V. E. & Rao, A. (1992) *Nature (London)* **356,** 801–804.
25. Chen, L., Glover, J. N. M., Hogan, P. G., Rao, A. & Harrison, S. C. (1998) *Nature (London)* **392,** 42–48.
26. Crabtree, G. R. & Clipstone, N. A. (1994) *Annu. Rev. Biochem.* **63,** 1045–1083.
27. Schreiber, S. L. & Crabtree, G. R. (1992) *Immunol. Today* **13,** 136–142.
28. Sigal, N. H. & Dumont, F. J. (1992) *Annu. Rev. Immunol.* **19,** 519–560.
29. Jain, J., Burgeon, E., Badalian, T. M., Hogan, P. G. & Rao, A. (1995) *J. Biol. Chem.* **270,** 4138–4145.
30. Blackwell, T. K. (1995) *Methods Enzymol.* **254,** 604–618.
31. Ahn, S., Olive, M., Aggarwal, S., Krylov, D., Ginty, D. D. & Vinson, C. (1998) *Mol. Cell. Biol.* **18,** 967–977.
32. Luo, C., Burgeon, E., Carew, J. A., McCaffrey, P. G., Badalian, T. M., Lane, W. S., Hogan, P. G. & Rao, A. (1996) *Mol. Cell. Biol.* **16,** 3955–3966.
33. Valge-Archer, V. E., de Villiers, J., Sinskey, A. J. & Rao, A. (1990) *J. Immunol.* **145,** 4355–4364.
34. Shaw, K. T., Ho, A. M., Raghavan, A., Kim, J., Jain, J., Park, J., Sharma, S., Rao, A. & Hogan, P. G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11205–11209.
35. Masuda, E. S., Liu, J., Imamura, R., Imai, S. I., Arai, K. I. & Arai, N. (1997) *Mol. Cell. Biol.* **17,** 2066–2075.
36. Shibasaki, F., Price, E. R., Milan, D. & McKeon, F. (1996) *Nature (London)* **382,** 370–373.
37. Zhou, P., Sun, L. J., Dotsch, V., Wagner, G. & Verdine, G. L. (1998) *Cell* **92,** 687–696.
38. Sun, L. J., Peterson, B. R. & Verdine, G. L. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4919–4924.
39. Chen, L., Oakley, M. G., Glover, J. N., Jain, J., Dervan, P. B., Hogan, P. G., Rao, A. & Verdine, G. L. (1995) *Curr. Biol.* **5,** 882–889.
40. Robbins, J., Dilworth, S. M., Laskey, R. A. & Dingwall, C. (1991) *Cell* **64,** 615–623.
41. Murre, C., McCaw, P. S. & Baltimore, D. (1989) *Cell* **56,** 777–783.