Nuclear Factor of Activated T Cells 2 Transactivation in Mast Cells

A NOVEL ISOFORM-SPECIFIC TRANSACTIVATION DOMAIN CONFFERS UNIQUE FceRI RESPONSIVENESS*

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Murine nuclear factor of activated T cells (NFAT)2.a/β differ by 42 and 28 unique amino-terminal amino acids and are differentially expressed. Both isoforms share conserved domains that regulate DNA-binding and subcellular localization. A genetic “one-hybrid” assay was used to define two distinct transactivation (TA) domains: in addition to a conserved TAD present in both isoforms, a second, novel TAD exists within the β-specific amino terminus. Pharmacologic inhibitors Gö6976 and rottlerin demonstrate that both conventional and novel protein kinase C (PKC) family members regulate endogenous mast cell NFAT activity, and NFAT2 TA. Overexpression of dominant active PKCθ (which has been implicated in immune receptor signaling) induces NFAT2.a/β TA. Mutations within the smallest PKCθ-responsive transactivation domain demonstrate that the PKCθ effect is at least partially indirect. Significantly, the β-specific domain confers greater ability to TA in response to treatment with phorbol 12-myristate 13-acetate/ionomycin or lipopolysaccharide, and unique sensitivity to FceRI signaling. Accordingly, overexpression of NFAT2.β results in significantly greater NFAT- and interleukin-4 reporter activity than NFAT2.a. These results suggest that whereas NFAT2 isoforms may share redundant DNA-binding preferences, there are specialized functional consequences of their isoform-specific domains.

Nuclear factor of activated T cells (NFAT)1 transcription factors regulate critical events in vertebrate cell development and differentiation (for review, see Ref. 1). NFAT was first characterized as an essential component of inducible IL-2 transcription in T cells (2), but is now known to denote a family of transcription factors that include four Ca2+-responsive NFAT family members (NFAT1–4) (1, 3). NFAT family members have specialized functions. For example, deletion of the NFAT1 gene results in dysregulated lymphoproliferation and a Th2-dominant cytokine response (15). In contrast, NFAT2−/− lymphocytes are defective in proliferation and production of Th2 cytokines (16, 17). Such phenotypes suggest that NFAT2 activity drives Th2 cytokine production.

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‡ The abbreviations used are: NFAT, nuclear factor of activated T cells; PKC, protein kinase C; TA, transactivation; DBD, DNA-binding domain; PMA, phorbol 12-myristate 13-acetate; Cn, calcineurin; IL, interleukin; LPS, lipopolysaccharide; CAT, chloramphenicol acetyltransferase; TA, transactivate; AAD, acidic activation domains.
PKCs regulate this activity. In addition, a novel acidic activa-
sion to PMA and ionomycin. Both conventional and novel
act independently to confer transcriptional activation in re-
imparts NFAT2.

The existence of multiple isoforms of NFAT1, -2, and -4 suggests that there is even more complexity in the regulation and function of NFAT family members. Strategies used to
target NFAT genes for deletion result in the loss of expression of all isoforms derived from the targeted locus. Thus, the con-
tribution of individual NFAT isoforms to unique patterns of gene expression has not yet been evaluated in vivo. We previ-
ously cloned two isoforms of NFAT2 from a murine mast cell cDNA library that differ only at their amino termini (5).

Because the sequences that regulate DNA-binding and nu-
clear transport are identical in murine NFAT2.a and NFAT2.b, we hypothesized that the isoform-specific regions confer dis-
tinct abilities to transactivate transcription, perhaps allowing for cell- and signal-specific NFAT activities. In this study we used a genetic one-hybrid assay to identify transactivation domains (TADs) within NFAT2.a and NFAT2.b. Two regions act independently to confer transcriptional activation in re-
sponse to PMA and ionomycin. Both conventional and novel
PKCs regulate this activity. In addition, a novel acidic activa-
tion domain (Glu21Asp39) within the β-specific amino terminus imparts NFAT2.b with greater ability to transactivate in re-
sponse to a broad range of PMA and ionomycin concentrations. Significantly, it also provides NFAT2.b with the unique ability to respond to FcεRI cross-linking. These data demonstrate that there is functional specialization among NFAT2 isoforms that is regulated at the level of transactivation in response to spe-
cific activation signals.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures and Reagents—**CFTL-15 mast cells were cultured as previously described (5) except 3.3 ng/ml recombinant murine IL-3 (BIOSOURCE Intl., Camarillo, CA) was substituted for WEHI3B su-
permatant. MCs (obtained from American Type Culture Collection) were cultured in RPMI 1640 supplemented with 15% feta-
 bovine serum and 30% WEHI3B supernatant. COS-1 cells (American Type Culture Collection) were maintained in Dulbecco's modified Eagle's medium (4.5 g/liter glucose and 1.5 g/liter sodium bicarbonate) supplemented with 10% fetal bovine serum (Invitrogen). Cells were stimu-
lated with PMA (Sigma), ionomycin (Calbiochem), or LPS (Sig-
ma). PMA and ionomycin concentrations (20 ng/ml and 1 µg/ml, re-
spectively) were uniform with the exception of the PMA/ionomycin dose-
response shown in Fig. 3B, which utilized five 10-fold dilutions from 0.2 µg/ml PMA and 10 µg/ml ionomycin. FcεRI cross-linkage was achieved through IgG1-α-IgE treatment, briefly. CFTL-15 mast cells were preincu-
bated for 2 days in 0.5 µg/ml purified mouse IgE (BD Biosciences); 24 h after transfection, cells were resuspended in 200 µl of complete
media with 5.0 µg/ml IgE at 4 °C for 30 min; cells were washed twice in
serum-free RPMI to remove unbound IgE, and resuspended in 200 µl of complete media with 5.0 µg/ml rat α-mouse IgE (Southern Biotech,
Birmingham, AL) at 37 °C for 45 min. Cells were transfected to 12-well plates in complete media until harvest (48 h post-transfection). PKC
inhibitors Go6976 and rottlerin (Calbiochem) were dissolved in Me2SO, and added to cells 30 min prior to PMA/ionomycin treatment.

**Transfections—**5.0 × 10^4 CFTL-15 cells were electroporated in 0.5 ml of serum-free RPMI at 400 V and 425 µF in 0.4-cm gap cuvettes with a Gene-Pulser II (Bio-Rad). All CFTL-15 shocks contained 50 µg of DNA (Invitrogen). Samples were incubated for 10 h with DNA at room temperature prior to electroporation, and were allowed to recover on ice for 10 min post-electroporation.

**One-hybrid Constructs and CAT Assays—**PCR-generated fragments of murine NFAT2.a and NFAT2.b were cloned in-frame into the Gal4 DBD expression construct, pM (Clontech). The isoform-specific regions were generated by α1−44 and α2−30. Single amino acid differences were constructed with the QuikChange™ mutagenesis kit (Stratagene, La Jolla, CA). CFTL-15 assays were performed with 0.001–10 µg of effector
construct and 5 µg of GCAT reporter construct (Clontech). Within an experiment, equal molar quantities of effector constructs were used. PKCα expression constructs were the kind gift of A. Altman (La Jolla Institute for Allergy and Immunology).

For overexpression analysis, NFAT2.a and NFAT2.b were cloned into the expression vector pcDNA3 (Invitrogen); 20 µg of the expression constructs were transiently transfected into CFTL-15 cells with 20 µg of either NFAT- or IL-4-CAT reporter constructs. Twenty-four hours post-
electroporation, CFTL-15 cells were split into two to three samples and treated with PMA/ionomycin, or IgE/α-IgE. Forty-eight hours post-
transfection, CAT extracts were harvested by using EDTA/NaCl (TEN)/Trition X-100 method (22). CAT activity was measured in a scin-
tillation/diffusion assay as previously described (23). All CAT results
are representative of at least three independent experiments. The NFAT reporter contains 3 tandem repeats of the IL-2 NFAT site upsteam of the IL-2 promoter (+72 to +251) (24). The NFAT sites each contain the composite NFAT-AP-1 "NFAT-responsive element" (Fig. 4A). The NFAT reporter was the kind gift of T. J. Murphy (Emory University). The IL-4 reporter construct contains sequences corre-
sponding to −797 to +5 base pairs of the murine IL-4 promoter and has been previously described (23).

**Cell Extracts and Immunodetection—**Extracts from COS-1 and CFTL-15 cells were prepared by collecting and washing approximately 1.0 × 10^6 cells in cold phosphate-buffered saline. Cells were resus-
pered in 200 µl of 1.2× Laemmlı sample buffer (25) on ice. Extracts were heated to 95 °C for 5 min, then either sonicated or passed through a 22-gauge needle to disrupt chromatin. Extracts were then centrifuged at 10,000 × g for 10 min. After gel electrophoresis, proteins were electroblotted to nitrocellulose for 20 min at 15 V in transfer buffer (48 µg Tris, 39 µg glycine, 20% methanol). Blots were blocked in 5% dry milk/TBST (0.05% Tween 20) for 1 h at room temperature. Primary antibodies specific for either Gal4 DBD (clone RR5C1, Santa Cruz Biotechnology, Santa Cruz, CA) or PKCα (clone E-7, Santa Cruz Bio-
technology) were added to the same solution for an additional hour. Blots were washed in TBST (5 min each), blocked in 5% dry milk/TBST (5 min) and incubated with secondary antibodies conjugated to horseradish peroxidase in 5% dry milk/TBST (0.05% Tween 20) for 1 h at room temperature.

**Reverse Transcription-PCR Analysis—**RNA was harvested from 1.0 × 10^6 cells with RNA STAT-60 reagent (Tel-Test Inc., Friendswood, TX). 1.0 µg of DNase I-treated RNA was used as a template in a Superscript II™ (Invitrogen) first-strand synthesis reaction. PCR prim-
ers to detect PKCα transcripts were: PKCα forward, 5'-CTCCTCACA-
GAGTATGTCGAATCA-3' and PKCα reverse, 5'-AATTCATCGATCCTTGTGTCACTCA-3'.

**RESULTS**

**Two Distinct NFAT2 Transactivation Domains Function in Mast Cells—**To examine the ability of murine NFAT2 isoforms to transactivate transcription, one-hybrid effector constructs (Fig. 1A) were used. These constructs express the Gal4 DBD fused to discrete regions of NFAT2, but exclude the NFAT Rel-homology domain. The Gal4 DBD (amino acids 1−147) con-
tains a nuclear localization signal that targets one-hybrid pro-
teins to the nucleus. As a result, these molecules localize exclu-
sively to reporter cis-elements. Furthermore, transactiva-
tion measured in the assay is not a result of association with AP-1 family members (which make contacts within the NFAT Rel-homology domain). Effector constructs were co-transfected into mast cells with a reporter whose expression is regulated by
five Gal4 cis-elements and a minimal E1b promoter. These experiments were performed in the non-transformed, IL-3-dependent mast cell line, CFTL-15. These cells express functional FcR\(\gamma \)280, and release a number of mast cell mediators when activated through this receptor including histamine, IL-4, IL-13, tumor necrosis factor (TNF)251, and IL-6 (26). They also express MMCP-4, a serine protease expressed by mature connective tissue mast cells (27).

As shown in Fig. 1B, the “full-length” amino-terminal constructs, \(\alpha 1-429\) and \(\beta 1-415\), as well as \(31-415\) (which excludes the isoform-specific domains) were active in this assay. \(\beta 1-30\) and \(91-415\) represent the smallest independent constructs that can transactivate. All active constructs possess both basal and stimulation responsive (20 ng/ml PMA and 1 \(\mu\)g/ml ionomycin) activity. The common region encompassing amino acids \(91-415\) includes the previously defined human NFAT2 transactivation domain (TAD-A, amino acids 113–205), whereas \(\beta 1-30\) represents a novel TA domain. Amino acids 30–90 and the carboxyl terminus (amino acids 550–704), regions shared by both NFAT2.\(\alpha \) and NFAT2.\(\beta \), as well as the isoform-specific region of NFAT2.\(\alpha \) (amino acids \(\alpha 1-44\)), have no independent transactivation ability. \(\alpha 1-44\) is inactive over a broad range of effector concentrations, conditions under which \(\beta 1-30\) demonstrates a concentration-dependent ability to transactivate (Fig. 1C).

Regions that have no independent ability to transactivate can contribute to the activity of adjacent domains. For example, the basal and inducible activity of \(\beta 1-90\) is greater than that of \(\beta 1-30\), yet constructs containing amino acids \(30-90\) alone are inactive. Deletion of \(30-90\) from the \(31-415\) construct (represented by \(91-415\)) has only a minimal affect on activity, indicating that this modulating influence is exerted primarily on the \(\beta 1-30\) transactivation domain.

These differences in transactivation are not the result of significant variations in the inherent stability of the hybrid effector molecules or in their ability to be expressed. Whole cell lysates were isolated from COS-1 cells transiently transfected with one-hybrid effector constructs and subjected to Western blot analysis. Immunodetection of Gal4 fusion proteins was performed with \(\alpha\)-Gal41-147 clone RK5C1.

\[\text{NFAT2 Isoform-specific Transactivation Activity}\]

\[\text{FIG. 1. Analysis of NFAT2 transactivation in mast cells. A, upper, schematic representation of structural differences between NFAT2.}\(\alpha/\beta\) isoforms and location of common regulatory elements including: SPRIEIT sequence (black box), SP boxes (gray boxes), and the Rel-homology domain. A, lower, structure of NFAT2-Gal4 fusion one-hybrid effector constructs. B, CFTL-15 mast cells co-transfected with 2.5 \(\mu\)g of the indicated effector construct and a Gal4 reporter gene were stimulated with PMA and ionomycin 24 h post-transfection. Data are normalized to transactivation activity of the Gal4 DBD alone (±S.D.) and is representative of three independent experiments. C, \(\beta 1-30\) dose responsive transactivation. The one-hybrid assay was performed with the indicated quantities of isoform-specific effector constructs. Data are representative of three independent experiments. B, demonstration of one-hybrid effector molecule expression. Whole cell lysates from COS-1 cells transfected with the indicated one-hybrid effector constructs were subjected to Western blot analysis. Immunodetection of Gal4 fusion proteins was performed with \(\alpha\)-Gal41-147 clone RK5C1.}\]
exception of β1–30 and β1–90. Whereas differences in NFAT2 isoform stability have not been reported, the acidic β-specific amino terminus may confer a shorter protein half-life on these constructs. This result also indicates that the levels of transactivation for these two constructs (Fig. 1B) may be underestimated by this assay. Therefore, we are unable to determine whether the overall ability of NFAT2.β to transactivate represents additive or synergistic contributions from the two distinct transactivation domains (91–415 and the β-specific domain).

**β1–30 Contains a Novel Acidic Activation Domain: Glu5-Asp19**—The β-specific amino terminus is highly charged (pI = 2.9) and contains 7 acidic residues (Asp/Glu) interspersed by a number of hydrophobic residues including phenylalanine, a pattern conserved in many acidic activation domains (AAD) (28). An alignment of the amino termini of human NFAT2.C and murine NFAT2.β (Fig. 2B) reveals a conserved unit of acidic/hydrophobic residues. We tested whether the acidic “core” of this domain, Glu5-Asp19 is able to transactivate transcription in mast cells. The results shown in Fig. 2A demonstrate that the activity of Glu5-Asp19 fully complements the ability of β1–30 to drive transcription, and respond to PMA/ionomycin. Thus, a novel 15-amino acid acidic activation domain exists within the β-specific amino terminus of NFAT2.
mycin treatment alone to induce transactivation of NFAT2.α and NFAT2.β. As shown in Fig. 3A, PMA treatment alone results in a modest increase in transactivation, suggesting that PMA-induced signals do contribute to transactivation. However, signals induced by ionomycin or PMA/ionomycin have the most significant affects on inducible activity. The addition of cyclosporin A inhibits PMA/ionomycin-induced transactivation (Fig. 3A). Because one-hybrid effector proteins are targeted to the nucleus via the Gal4 nuclear localization signal, this finding suggests that calcineurin participates in the regulation of both NFAT2 subcellular localization and transactivation.

At the fixed doses of PMA and ionomycin used in these assays, β1–415 consistently transactivates better than α1–429 (Figs. 1B and 3A). It is possible that the isoform-specific regions confer differential ability to activate transcription in response to varying activation signal strength. For example, NFAT2.α may respond equivalently to NFAT2.β only at high signal strengths. To test this possibility, transfected cells were treated with increasing concentrations of PMA and ionomycin. As shown in Fig. 3B, the absolute level of transactivation differs between the two isoforms over the entire activator concentration range. However, both isoforms demonstrate inducible transactivation at the same PMA/ionomycin concentrations, indicating that there is no qualitative transactivation difference in response to signal strength.

Endogenous Mast Cell NFAT Activity Is Influenced by Both Novel and Conventional PKCs—Several Ser/Thr kinases have been demonstrated to regulate NFAT transactivation, including: CaMK IV, Cot-1, PIM-1, and PKCζ (29–32). The role of PKC family members in the transduction of immunoreceptor signals is clearly established (for review see Ref. 33). In T cells, T cell receptor-proximal kinases activate PLCγ, leading to the generation of diacylglycerol and inositol triphosphate. Both of these signaling intermediates are necessary to activate conventional PKCs (including α, β, and γ isoforms). Only diacylglycerol-responsive signals (mimicked by PMA treatment) are required for the activation of novel PKCs (δ, ε, μ, and θ). To further characterize the molecular mechanisms that regulate NFAT activity, we used pharmacologic inhibitors Go6976 (specific for conventional PKCs) (34) and rottlerin (specific for novel PKCs δ and θ) (35). We first examined the effect of these inhibitors on endogenous NFAT activity using an NFAT reporter construct (Fig. 4A). Both Go6976 and rottlerin inhibit NFAT reporter activity induced in response to PMA/ionomycin treatment and IgE receptor cross-linking, indicating that both conventional and novel PKCs regulate physiologic mast cell NFAT activity (Fig. 4B).

The novel PKC, PKCθ, has been implicated in T cell receptor signaling pathways. In activated T cells, PKCθ is rapidly recruited to the T cell receptor (36). PKCθ also associates with the actin cytoskeleton (37). Both PKCθ mRNA and protein are detectable in CFTL-15 mast cells (Fig. 4C). In addition, overexpression of dominant active PKCθ (A148E) increases basal and ionomycin-induced NFAT reporter activity (Fig. 4D).

PKCθ Induces NFAT2 Transactivation—Because endoge-
Fig. 7. Differential sensitivity of NFAT2 α/β to physiological stimuli. A, transactivation in response to high affinity IgE receptor stimulation is mediated by β1–30 TAD. CFTL-15 cells transfected with the indicated one-hybrid effector constructs (0.02 μg) were stimulated with either PMA/ionomycin, or via IgE/IgE treatment to cross-link FcεRI. B, deletion of β1–30 ablates FcεRI-responsive transactivation. CFTL-15 cells transfected with the indicated one-hybrid effector constructs (0.02 μg) were stimulated with increasing doses of IgE/IgE as described under “Experimental Procedures.” C, β1–30 confers FcεRI-responsive transactivation in MC9 mast cells. MC9 cells transfected by DEAE-dextran (58) with 8 μg of G5CAT reporter and 2 μg of the indicated one-hybrid effector constructs were stimulated with increasing doses of IgE/IgE as described under “Experimental Procedures.” D, LPS induces NFAT2 α/β transactivation. Transactivation of α1–429 and β1–415 (0.02 μg) in CFTL-15 cells were treated with 0.001–100 μg/ml LPS. Data are representative of three independent experiments.

The β-Specific Transactivation Domain Confers Unique Responsiveness to IgE Receptor Cross-linking—Mast cells are activated by a variety of agonists including neuropeptides, proteases, bacterial products, and specific antigen (38, 39). Antigen cross-linkage of the high affinity IgE receptor (FcεRI) expressed on mast cells results in the activation of signaling cascades mediated by Ca2+ and diacylglycerol and is perhaps the best characterized mode of mast cell activation (40). The use of PMA and ionomycin to activate mast cells can also provide these intracellular signals and bypasses the need for cell surface receptor engagement. However, these agents cannot faithfully mimic early receptor-proximal events that may affect signal strength and outcome of the response. Thus, we considered the possibility that physiological activators (that deliver quantitatively or qualitatively different signals) would lead to differences in the ability of NFAT2 isoforms to transactivate.

Cross-linking of FcεRI by treatment of transfected cells with IgEα/IgE results in inducible transactivation of β1–30 and β1–415 but not α1–44, α1–429, or 31–415 (Fig. 7A). Even under conditions where cells were treated with a range of IgEα/IgE concentrations, α1–429 and 31–415 are unable to respond (Fig. 7B). We repeated this experiment in a second IgE-responsive, non-transformed mast cell line, MC9. Fig. 7C
IL-4 reporter activity.

A and H9262 data demonstrating that NFAT2 promoter is also more active in cells overexpressing NFAT2 reporter construct containing 797 bp of the proximal IL-4 promoter. IL-4 reporter activity in CFTL-15 cells transfected with 20 μg of the indicated construct. Cells were stimulated via IgE/α-IgE treatment to cross-link FcεRI. IL-4 promoter (~797 bp) activity in CFTL-15 cells transfected with 20 μg of the indicated construct. Cells were stimulated via IgE/α-IgE treatment to cross-link FcεRI. Data are representative of three independent experiments (±S.D.).

Fig. 8. NFAT2,β overexpression induces greater NFAT and IL-4 reporter activity. A, NFAT reporter activity in CFTL-15 cells transfected with 20 μg of the indicated NFAT2 expression construct. Cells were stimulated via IgE/α-IgE treatment to cross-link FcεRI. B, IL-4 promoter (~797 bp) activity in CFTL-15 cells transfected with 20 μg of the indicated construct. Cells were stimulated via IgE/α-IgE treatment to cross-link FcεRI. Data are representative of three independent experiments (±S.D.).

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molecules that regulate NFAT subcellular localization (calcineurin and glycogen synthase kinase for example), resulting in an increased ability of endogenous NFAT2,β to enter the nucleus and activate transcription.

DISCUSSION

NFAT2,α and NFAT2,β differ by 42 and 28 unique aminoterminal amino acids but share common sequences that regulate nuclear import and export as well as DNA binding activity. In this study we provide evidence that there are differences in the regulation of these two isoforms at the level of transcriptional activation ability. Two distinct domains contribute to NFAT2 transactivation. One is located within the conserved NFAT homology region present in both isoforms. A second domain is contained within the β isoform-specific region. The activity of both domains appears to be regulated by novel and conventional PKCs, including PKCδ. The presence of the β-specific sequence is associated with both quantitative and qualitative differences in transactivation ability: NFAT2,β demonstrates a greater ability to transactivate in response to both PMA/ionomycin and LPS over a range of signal strengths when compared with NFAT2,α. In addition, NFAT2,β but not NFAT2,α can mediate high levels of transcriptional activation in response to FcεRI cross-linking.

We demonstrate that the β-specific domain is necessary for optimal responsiveness to all these signals. Deletion of amino acids 1–30 from β1–415 (represented by the 31–415 construct) ablates FcεRI responsiveness (Fig. 7, B and C) and significantly reduces PMA/ionomycin inducible activity (Fig. 1B). A 15-amino acid sequence, Glu5–Asp19, located within β1–30 is responsible for this activity and has the hallmarks of an AAD. AADs are comprised of acidic amino acids interspersed with hydrophobic residues (Phe in particular) and are among the most potent transcriptional activators (28). It has been shown that AADs of p53 and VP16 make contacts with the RNA polymerase II holoenzyme through the co-activator, hTAF1,β1 (43, 44). These protein-protein contacts depend on a motif: F-X-X-P, that occurs in two orientations within Glu5–Asp19 (forward, amino acids 12–16, and reverse, amino acids 14–18). Studies to determine whether the β-specific TAD also mediates its transactivation function through this co-activator are underway.

Proteins containing acidic activation domains such as VP16 and p53 are also characterized by a short half-life (45, 46). Molinari et al. (47) have used multimers of acidic activation domains to show that protein half-life is inversely correlated with transcriptional activity; they and others speculate that this is one mechanism used to regulate the activity of strong transactivators. The observation that β1–30 has the lowest steady-state protein level among our effector constructs may be a reflection of this phenomenon.

The varied abilities of NFAT2 isoforms to transactivate transcription in response to cell type-specific signals likely contributes to unique patterns of gene expression. How might this be accomplished? At the level of signal transduction, distinct signals can elicit differential co-activator activation and recruitment and lead to varying levels of transcriptional activation. We speculate that Igε receptor cross-linking results in the activation of one or more co-activators recruited by the β-specific, but not the common (91–415) transactivation domain, leading to selective transactivation. Signals downstream of the Igε receptor on mast cells may also facilitate increased association of co-factors such as CBP/p300 (which can contribute enzymatic activities (histone acetyltransferase activity in this case) (48)) with NFAT2,β (via the β1–30 and 91–415 domains). These co-activators could act to increase local acetylation and enhance transcriptional activation relative to NFAT2,α by reducing the “chromosomal barrier” to transcription.

At least two possible mechanisms may reconcile these findings: 1) NFAT2,α can still associate with AP-1 family members, and thus recruit the AP-1-dependent transactivation ability to the promoter, and/or 2) overexpression of NFAT2,α may titrate out
In contrast to our findings, Monticelli and Rao (49) recently reported that overexpression of "constitutively active" NFAT1 and NFAT2 (including isoforms corresponding to α and β) did not result in differential IL-4 expression in response to PMA/ ionomycin treatment in primary T cells. These apparently contradictory observations may reflect either signal- or cell-specific differences in the ability of NFAT2 isoforms to induce transcription. Alternatively, the IL-4 gene may be representative of a subset of NFAT-target genes that have low energetic barriers to transcription (because of their chromosomal context), and thus may not be differentially regulated by NFAT2 isoforms of distinct transactivation capabilities.

The presence of consensus PKCα sites (ST-X-K/R) throughout the 31–415 "common region," and within α1–44 led us to examine the role of PKC family members in NFAT2 transactivation. The novel PKC, PKCδ, has received particular attention for its role in transduction of T cell receptor signals upstream of calcineurin in other models of PKC activation. We and others have shown that NFAT transactivates calcineurin to induce a co-activator required for NFAT2 transactivation in response to LPS. Our finding may represent a flux necessary to drive NFAT nuclear localization. These data and NFAT2 isoforms of distinct transactivation capabilities.

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