Nuclear Factor of Activated T Cells (NFAT)-dependent Transactivation Regulated by the Coactivators p300/CREB-binding Protein (CBP)

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Summary

p300 and cAMP response element-binding protein (CREB)-binding protein (CBP) are members of a family of coactivators involved in the regulation of transcription and chromatin. We show that transcription factors of the nuclear factor of activated T cells (NFAT) family bind p300/CBP and recruit histone acetyltransferase activity from T cell nuclear extracts. The NH$_2$-terminal transactivation domain of NFAT1 and the phospho-CREB- and E1A-binding sites of p300/CBP are involved in the interaction. The viral oncoprotein E1A inhibits NFAT-dependent transactivation in a p300-dependent manner. Recruitment of the coactivators p300/CBP by the transactivation domains of NFAT proteins is likely to play a critical role in NFAT-dependent gene expression during the immune response.

Key words: transcriptional regulation • histone acetyltransferases • T cells • nuclear factor of activated T cells • cytokine gene expression

Histone acetyltransferases (HATs)$^1$ and histone deacetylases have been closely implicated in the mechanism of transcriptional activation and repression of multiple genes (for a review, see references 1 and 2). Acetylation of the NH$_2$-terminal regions of histones is considered to be crucial for the accessibility of transcription factors to nucleosomal templates. All core histones can be variably acetylated by a number of proteins designated HAT (1, 3), which include p300/CAMP response element-binding protein (CREB)-binding protein (CBP) (4, 5), the p300/CBP-associated factor P/CAF (6), and the nuclear receptor coactivator ACTR (7). A diverse and increasing number of transcription factors and some elements of the basal transcription machinery are able to form stable physical complexes with and respond to the coactivating properties of p300/CBP (for a review, see references 8 and 9). However, the precise mechanisms by which transcriptional activators stimulate the transcriptional machinery through p300/CBP remain unclear.

p300/CBP coactivators serve also as multifunctional adaptors that coordinate cell cycle progression with transcriptional regulation (10). These coactivators are recruited in signaling pathways dependent on p53, a tumor suppressor and a common target for genetic alteration in human cancers (11). Interestingly, there is accumulating evidence for the role of CBP and p300 in tumorigenesis (9). Specifically, certain cases of acute myeloid leukemia have been linked to recurrent chromosomal translocations that result in in frame fusions of CBP or p300 to the monocytic leukemia zinc finger protein (12) and myeloid/lymphoid leukemia (13, 14) gene products. Moreover, the viral oncoprotein E1A inhibits host gene transcription, by itself binding and presumably using the HAT activities of p300/CBP (9).

The nuclear factor of activated T cells (NFAT) regulates the inducible transcription of cytokine genes and other genes critical for the immune response (for a review, see reference 15), thereby indirectly regulating immune cell proliferation. The most NH$_2$-terminal region (≈100 amino acids [aa]) of NFAT proteins contains a strong acidic transactivation domain, whose function in resting cells is partially masked by the adjacent regulatory domain (16). We show here that the NH$_2$-terminal transactivation domains of NFAT proteins are capable of recruiting HATs. Immunoprecipitates of NFAT from T cell nuclear extracts contain an associated HAT activity that is accounted for at least partially by p300/CBP. NFAT-dependent transactivation is inhibited by the viral oncoprotein E1A in a p300-dependent manner. The p300/CBP proteins, acting as chromatin-remodeling factors and/or interacting with the basal

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$^1$Abbreviations used in this paper: aa, amino acid(s); AP-1, activating protein-1; CBP, CREB-binding protein; CREB, cAMP response element-binding protein; DBD, DNA-binding domain; GST, glutathione S-transferase; HA, hemagglutinin; HAT, histone acetyltransferase; hGH, human growth hormone; Luc, luciferase; NFAT, nuclear factor of activated T cells.
transcription machinery, may play an important role in the NFAT-dependent expression of inducible genes.

Materials and Methods

Antibodies. Antihemagglutinin (anti-HA; 12CA5 murine mAb against the influenza HA peptide; Boehringer Mannheim Biochemicals, Indianapolis, IN) was used to immunoprecipitate HA-tagged p300. Rabbit polyclonal antibodies against an NH$_2$-terminal peptide (67.1), the DNA-binding domain (DBD), and a COOH-terminal peptide (anti-NFAT1-C) of NFAT1 were used as described (17, 18). TAT antibody, used as a negative control for immunoprecipitation, was provided by Dr. B. Cullen (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD [19]).

Plasmids. pLGP3-mNFAT1(A) (Δ146–398) was made by subcloning a SacI-HindIII fragment from pBSTΔ5 into the expression plasmid pLGP3, between SacI and HindIII in the multicloning site. pBSTΔ5 was made by removing a fragment SfiI-XhoI from pBS-mNFAT1-C and religating blunt ends. 6xHis-hNFAT1(1–415), GAL4-Chatsworth, CA) that had been filled in with Klenow. The construction of pEFBOS-mNFAT1-C, pLGP3-mNFAT1-C, GAL4-hNFAT1(1–415), GAL4-ΔSP2, and GAL4-hNFAT2(1–418) has been described previously (16, 20). The NFAT luciferase reporter plasmid (NFAT3x-Luc) containing three copies of the distal NFAT site of the murine IL-2 promoter (21), was provided by Dr. D. McKean (Mayo Clinic, Rochester, MN). The GAL4-luciferase reporter plasmid (GAL4-Luc) (22), the cDNA plasmids pCMV-b-HA-p300, pCMV-b-E1A (23), the E1A mutant deficient in binding to p300 (pE1A.15–35 [24]), and plasmids encoding the glutathione S-transferase (GST)-CBP fusion proteins, CBP1 (aa 1–117), CBP2 (aa 117–737), CBP3 (aa 737–1626), and CBP4 (aa 1680–1891) (25), were provided by Dr. M. Montminy (Joslin Diabetes Center, Boston, MA). pSV-human growth hormone (hGH) was used to normalize transfection levels.

Transfection. Plasmid DNAs were introduced into Jurkat human T cells by electroporation (0.25 V, 960 μF) using a gene pulser (Bio-Rad Laboratories, Hercules, CA) and 0.4-cm gap cuvettes (26). Transfection of 293T cells (human embryonic kidney fibroblasts), originally referred to as 293Tα1690neo (27), was performed using the calcium phosphate coprecipitation technique. For each lane of the immunoprecipitation experiments, five 10-cm dishes with 0.5 x 10$^6$ cells each were transfected with 4 μg plasmid DNA. 36 h after transfection, cells were stimulated for 25 min at 37°C with 1 μM ionomycin and 10 nM PMA. Cell extracts were assayed for luciferase activity (Luciferase Assay System; Promega Corp., Madison, WI) as described previously (26). Transfection efficiencies were determined by measuring the hGH concentration in the culture media with a radiomimunometric assay kit (Nichols Institute, San Juan Capistrano, CA).

Results and Discussion

NFAT Associates with HATs in T Cells. Nuclear extracts were prepared with the activated cells, and NFAT1 was immunoprecipitated using several antibodies. After extensive washing, the immune complexes were tested for HAT activity with 1 μM ionomycin and 10 nM PMA. Cell extracts were assayed for luciferase activity (Luciferase Assay System; Promega Corp., Madison, WI) as described previously (26). Transfection efficiencies were determined by measuring the hGH concentration in the culture media with a radiomimunometric assay kit (Nichols Institute, San Juan Capistrano, CA).

NFAT Interacts with p300 in Cells. Nuclear extracts were prepared with the activated cells, and NFAT1 was immunoprecipitated using several antibodies. After extensive washing, the immune complexes were tested for HAT activity with 1 μM ionomycin and 10 nM PMA. Cell extracts were assayed for luciferase activity (Luciferase Assay System; Promega Corp., Madison, WI) as described previously (26). Transfection efficiencies were determined by measuring the hGH concentration in the culture media with a radiomimunometric assay kit (Nichols Institute, San Juan Capistrano, CA).

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noprecipitated from whole-cell lysates, and the immunoprecipitates were tested for the presence of coprecipitating HA-p300. Antibodies against the DBD and COOH-terminal regions of NFAT1 coimmunoprecipitated HA-p300, as judged by Western blotting of the immunoprecipitations with anti-HA (Fig. 2 A, lanes 3 and 4). Antibody against the NH2-terminal region of NFAT1 gave very weak coimmunoprecipitation of HA-p300 (Fig. 2 A, lane 2), which was only detected by overexposure (data not shown), again suggesting that the NH2-terminal region of NFAT1 was involved in the interaction with HA-p300. All antibodies immunoprecipitated equivalent amounts of NFAT1 (Fig. 2 A, bottom). The antibody control showed no immunoreactive band corresponding to HA-p300 (Fig. 2 A, lane 1), indicating the specificity of the interaction. Conversely, anti-HA coimmunoprecipitated a fraction of nuclear NFAT1 as shown by Western blotting with anti-NFAT1 (Fig. 2 B, lane 2). The coimmunoprecipitated fraction of NFAT1 migrated with higher apparent molecular weight than the bulk of the NFAT1 protein (compare lanes 1 and 2), suggesting that some posttranslational modification of NFAT1 (e.g., phosphorylation) might be required for that optimal interaction with p300. These results are the first demonstration of an interaction of NFAT transcription factors with the coactivators and HATs p300 and CBP.

The NH2-terminal region of NFAT1 Interacts with CBP In Vitro. To identify the region of NFAT1 and p300/CBP involved in the NFAT1–p300/CBP interaction, we performed binding experiments using bacterially expressed recombinant proteins. GST fusion proteins containing different regions of CBP were incubated with hexahistidine-tagged NFAT1(1–415), and the level of NFAT1 binding was assessed by Western analysis. These experiments showed that NFAT1 interacted directly with regions CBP2 (aa 117–737) and CBP4 (aa 1680–1891), where CBP2 includes the phospho-CR3E–binding site and CBP4 includes the E1A-binding site (Fig. 3 A). Equivalent amounts of each GST fusion protein were used in the binding assay as seen by Ponceau red staining (data not shown). Binding between CBP and NFAT was specific because no immunoreactive band was seen with GST alone or with other portions of CBP. The interaction seen in these in vitro experiments was weak, and may reflect either the lack of some posttranslational modification of NFAT1 required for optimal interaction or the lack of an accessory protein present in nuclear extracts (see Fig. 2 B). The interaction of p300/CBP with signal transducer and activator of transcription Stat1 also involves the participation of two widely separated regions of p300/CBP (31), although most of the transcription factors described to date interact only with a single region (9).

An Intact Regulatory Domain Is Not Required for Interaction with p300. The NH2-terminal region of NFAT1 comprises a strong acidic transactivation domain and an adjacent regulatory domain, the NFAT homology region (13). To test the importance of the regulatory domain, we...
E1A Inhibits NFAT-dependent Transactivation in a p300-dependent manner. (A) The NFAT3x-Luc reporter plasmid and expression plasmids encoding full-length NFAT1 (NFAT1-FL) or NFAT1ΔReg were cotransfected into Jurkat cells along with expression plasmids encoding wild-type E1A (WT) or the E1A mutant defective for p300 binding (Mut). (B) The GAL4-Luc reporter and expression plasmids encoding GAL4-NFAT1(1–415), GAL4-NFAT2(1–418), or GAL4-NFAT1ΔSP2 were cotransfected into Jurkat cells along with expression plasmids encoding wild-type E1A (WT) or the E1A mutant defective in p300 binding (Mut). After 36 h of transfection, cells were left unstimulated or stimulated overnight with ionomycin and PMA and assayed for luciferase assay. Results are representative of several experiments. Transfection efficiencies were normalized by measuring hGH expression from a cotransfected RSV-hGH plasmid.
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