Characterization of DNA binding, transcriptional activation, and regulated nuclear association of recombinant human NFATp

ABSTRACT

We conclude that NFATp is a bona fide transcriptional activator. Moreover, the reagents and methods that we developed will facilitate future studies on the mechanisms of transcriptional activation and nuclear accumulation by NFATp, a member of an important family of transcriptional regulatory proteins.

INTRODUCTION

Background NFAT (Nuclear Factor of Activated T cells) is a family of transcriptional activators that stimulate the expression of genes including those encoding immunomodulatory cytokines. The transcriptional effects of individual NFAT family members, including NFATp, NFATc, NFAT3, NFAT4/x, and NFAT5, are beginning to emerge. For example, NFATp, NFATc, and NFAT4 participate in the activation of T and B cells. NFATc appears to be critical for proper cardiac muscle-cell differentiation, and NFAT3 functions in cardiac hypertrophy. NFAT4/x has been implicated in development of immature thymocytes. NFAT5 is involved in the transcriptional regulation of osmotic stress response genes. All NFAT proteins except NFAT5 exist as phosphoproteins and are maintained in the cytoplasm of resting cells. NFAT nuclear localization is regulated by the action of a specific phosphatase and a number of kinases. In the case of T cells, antigen stimulation elicits a calcium-dependent signaling pathway that results in the activation of calcineurin, which directly dephosphorylates NFATp in preparation for nuclear import. Once in the nucleus, NFAT can bind DNA elements in target promoters, often in association with other resident and co-induced nuclear proteins. Sequence comparison of NFAT proteins revealed that the DNA-binding domain and a region referred to as the NFAT homology region (NHR) share sequence similarity, while other regions of the NFAT proteins share little or no sequence similarity (a schematic of NFATp is shown in Figure 1A). The DNA-binding domains are similar among all NFAT members and allow NFAT proteins to bind DNA with sequence specificity as monomers. NFAT proteins (except NFAT5) contain NHRs located N-terminal to the DNA binding domain that function to regulate nuclear localization in cells. NFAT proteins are highly phosphorylated in the NHR, and the phosphatase calcineurin binds directly to sequences in the NHR. Regions outside of the NHR and DNA binding domain are thought to contain transcriptional activation domains. For example, the N- and C-terminal regions (amino acids 1-171 and 727-927, respectively) of murine NFATp function as activation domains when fused to a heterologous DNA binding domain in transient transfection assays in Jurkat cells. The C-terminal regions of NFAT proteins are unique in sequence and may be responsible for functional differences among NFAT family members. A number of activators can interact with NFAT to potentiate synergistic activation of transcription on NFAT-responsive promoters that characteristically consist of multiple NFAT-specific sites. One example is the interleukin-2 (IL-2) promoter, which contains five NFAT binding sites in a region spanning 250 bp upstream of the TATA box. Four of the IL-2 NFAT sites are part of composite elements consisting of NFAT and AP-1 sites that direct the cooperative binding of NFAT proteins and AP-1 components. In biochemical experiments the DNA-binding domains of NFAT proteins (with the exception of NFAT5) are sufficient for cooperative DNA-binding in association with Fos/Jun heterodimers. As supported by structural analysis of the NFAT/cJun/cFos/DNA complex,

physical interactions between the DNA binding domain of NFAT and the basic leucine zipper regions of cJun and cFos enhance their interaction with DNA. To date, biochemical analysis of NFATp and other NFAT members has been conducted using only portions and not the full-length versions of these proteins. Recovery of smaller, soluble, truncated forms of NFAT proteins is feasible relative to the more difficult task of isolating active forms of full-length NFAT proteins. Although such domain-based investigations have been constructive in studying the activity and the properties of the NFAT DNA-binding domains, a biochemical analysis of transcriptional activation and regulated nuclear association of NFAT necessitated the characterization of full-length proteins. This is mainly because the domains exhibiting the transactivation properties and those controlling nuclear association are located in regions flanking the central DNA binding domain. We therefore established protocols for expressing and purifying full-length recombinant human NFATp. Utilizing a reconstituted transcription system, we found that NFATp is a bona fide transcriptional activator and that activation requires regions of NFATp outside the DNA binding domain. In addition, we discovered that human NFATp produced in insect cells using a recombinant baculovirus is a phosphoprotein that demonstrates calcineurin-regulated association with nuclei in vitro.

CONCLUSION

Conclusions We have found that human NFATp is a bona fide transcriptional activator and that regions outside of the central DNA binding domain are required for NFATp to activate transcription. Our experiments in a reconstituted transcription system lacking contaminating AP-1 proteins and using DNA templates with high affinity NFAT sites demonstrate that NFATp can function as a transcriptional activator on its own. We have also shown that recombinant NFATp purified from insect cells is a phosphoprotein that exhibits association with nuclei in vitro only after dephosphorylation by calcineurin. The recombinant full length and deletion mutants of human NFATp used in the studies described here as well as the methods that we have developed will be valuable for future biochemical studies of the function of NFATp in DNA binding, transcriptional activation, phosphorylation/dephosphorylation, and nuclear association.