

Glycogen synthase kinase3 beta phosphorylates serine 33 of p53 and activates p53's transcriptional activity

ABSTRACT

Phosphorylation of p53 serine 33 by GSK3 can regulate transcriptional activity. However, GSK3 β is not believed to be involved in the underlying mechanism of DNA damage response. Instead, it may serve as the link between a specific form of protein KOH and an activating non-DNA damage mechanism.

INTRODUCTION

The NFAT family, which includes genes like innate, adaptive, and autoimmune cells, is a group of transcriptional activators that promote the expression or expression of specific genes. NFATp, NFATc, and NFAT4 are involved in the activation of T and B cells. Similarly, NFATc is essential for the proper functioning of cardiac muscle-cell differentiation, while NFAT3 plays a role in cardiac hypertrophy. Additionally, several studies have implicated NFAT4/x in development of immature thymocytes.

Meanwhile, only NFAT5 plays transcriptional regulation of osmotic stress response genes. NFAT proteins are conserved in the cytoplasm of resting cells as phosphoproteins, with the exception of NFAT5, and their nuclear localization is controlled by a specific phosphatase and corresponding multiple kinases. Antigen stimulation in T cells triggers a calcium-dependent signaling pathway that activates the activating factor calcineurin, directly dephosphorylating 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, within the nucleus. Through sequencing analysis, it was discovered that the DNA-binding domain and a region known as the NFAT homology region (NHR) share similar sequences with other regions of the same NFAT protein(s) (Figure 1A). All NFAT members share the same DNA-binding domains, which enable them to bind DNA with sequence specificity as monomers. Moreover, all NFAT proteins exhibit NHRs located N-terminal to the DNA binding domain and regulate nuclear localization in cells. The NHR has a high phosphorylation level for NFAT proteins, and the phosphatase calcineurin binds directly to sequences in the same region. Regions beyond the NHR and DNA binding domain are believed to contain transcriptional activation domains. Murine NFATp's N- and C-terminal regions (amino acids 1-171 and 727-927), which function as activation domains when fused to a heterologous DNA binding domain in Jurkat cells, are used in transient transfection assays. The sequence of amino acids that make up the C-terminus of NFAT proteins may be unique and cause functional differences among NFAT family members. Several activators can interact with NFAT to promote synergistic activation of transcription on specialized promoters that respond to the molecule. These promoter elements are typically composed of several NFAT-specific sites, such as the interleukin-2 (IL-2) promoter, which has five NFAT binding sites in a region spanning 250 bp upstream of the TATA box. NFAT and AP-1 sites are found in four IL-2 regulated NFAT enzymatic sites, which form composite elements that control cooperative binding of NFAT proteins and other supplementary cytochromosomal protein components (AP-1) and synthetic adenosine triphosphate (ATP) into phages. In biochemical experiments, the DNA-binding domains of all NFAT proteins (excluding NFAT5) and Fos/Jun heterodimers are sufficient for binding cooperative DNA through interacting with RNA. The NFAT/cJun/CFos/DNA complex's structural analysis suggests that the DNA binding domain of NFAT and the

basic leucine zipper regions of TCF serve as additional pathways for NFAT to interact with DNA. To this day, biochemical analysis of these proteins has been limited to portions. While isolating active forms of full-length NFAT proteins is a more challenging task, it is possible to recover smaller, soluble, and truncated forms. Domain-based investigations have been a valuable tool for studying DNA-binding domains related to NFAT, but full-length proteins were necessary for biochemical analysis of transcriptional activation and regulated nuclear association. The reason for this is mainly because the domains responsible for nuclear association and those governing transactivation are situated in areas adjacent to the central DNA binding domain. Therefore, we developed methods for expressing and purifying full-length recombinant human (NFATp) using a reasymmeted transcription system, and found that despite the fact that activation requires regions of NFATp outside the DNA binding domain, innate transcriptional activator expression can still be reached. Furthermore, we have shown that human NFATp synthesized in insect cells by a recombinant baculovirus is actually an in vitro phosphoprotein displaying calcineurin-regulated association with nuclei.

CONCLUSION

In vivo experiments have revealed an osmotic stress-dependent serine phosphorylation of the eukaryotic histidine kinase homologue DokA. The phosphorylation is not dependent on the conserved histidine residue, which is crucial for two-component systems and is unlikely to occur through autophosphorylation. This supports the notion that eukaryotic homologues of bacterial signal transduction systems could be involved in serine/threonine kinases-related signaling pathways.