

FUNCTIONAL ANALYSIS OF *FOXP3*

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Mutations in *FOXP3* have been identified in individuals with the fatal autoimmune disease known as Immune Dysregulation Polyendocrinopathy Enteropathy X-linked syndrome (IPEX). In mice, the *Foxp3* gene has recently been shown to be necessary and sufficient for the presence of CD4⁺ CD25⁺ regulatory T cells. A naturally-occurring mutation has been identified in *Foxp3* in *Scurfy* mice; males hemizygous for this mutation are characterized by having enlarged spleens, lymph nodes and livers and the presence of activated lymphocytes in the skin and liver, resulting in death at about 4 weeks of age. *Foxp3* is a member of the winged-helix/forkhead family of transcription factors and a member of the *Foxp* subfamily of transcriptional repressors. FoxP3 contains a zinc finger, a leucine zipper and a C-terminal forkhead (FKH) DNA-binding domain. Other members of the *Foxp* subfamily, FoxP1, FoxP2 and FoxP4 have all been shown to repress transcription of their respective targets. FoxP1/2 repress transcription by associating with the corepressor protein C-terminal binding protein (CtBP-1), and the association domain is localized to the amino terminus of the respective proteins. Our laboratory has previously shown that FoxP3 functions as a transcriptional repressor. FoxP3 represses transcription of a luciferase reporter containing the -280 NF-AT binding site from the murine IL-2 promoter, which also contains a consensus binding site for the FKH domain of FoxP3. Also, this repression is dependent on the presence of the FKH domain. Using a luciferase reporter containing binding sites for the yeast GAL4 DNA-binding domain (DBD), we have determined that the leucine zipper and zinc finger domains of FoxP3 (when fused to GAL4-DBD) are neither sufficient nor required for its transcriptional repressor function. The mechanism by which FoxP3 represses transcription of the IL-2 locus is the aim of the present study. We show by expression of cDNA encoding for overlapping regions of the amino terminus of FoxP3 (fused to GAL4-DBD), that amino acids 67-132 of FoxP3 are required to repress transcription of a luciferase reporter containing binding sites for the yeast Gal4 DBD and separate binding sites for NF-AT. This suggests that FoxP3 functions by recruiting components of transcriptional machinery and not by competing with transcriptional activators (e.g. NF-AT) for binding to DNA.