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Tertiary Structure and DNA Binding Specificity of the vnd/NK-2 Homeodomain : Transgenic Alteration of Embryonic Neurogenesis

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The importance in embryonic neurogenesis of tertiary structure and DNA binding specificity of the protein encoded by the vnd/NK-2 homeobox gene is analyzed. The in vivo ectopic expression patterns of the wild type and four mutant vnd/NK-2 genes are analyzed together with the expression of two down-stream target genes, ind and msh, that are down-regulated by vnd/NK-2. Three of these mutants are conserved region segmental deletions (i.e., tinman, NK-2 box and acidic box) and the fourth, Y54M vnd/NK-2, corresponds to a single amino acid residue replacement in the homeodomain. The Y54M replacement was chosen because tyrosine in position 54 of the vnd/NK-2 homeodomain and other members of the NK-2 class of homeodomain transcription factors is the most important amino acid residue in sequence specific binding to an unusual DNA that contains 5 CAAGTG 3 as it core. Of the four ectopically expressed mutant genes examined, only the Y54M alteration acts as a knockout, where ability to down-regulate ind and msh was suppressed completely. The acidic domain deletion mutant showed limited down-regulation capability. By contrast, both the tinman and the NK-2 box deletion mutants behaved fully as functional vnd/NK-2 genes in their ability to repress ind and msh. The NMR determined tertiary structures of the Y54M vnd/NK-2 homeodomain in vitro both free and bound to DNA that contains 5 CAAGTG 3 or 5 CAATGG 3 in its core are compared with the wild type analog. The only structural differ- ence observed for the mutant homeodomain in the complex with the consensus DNA involved a closer interaction of the methionine-54 with A2 rather than C3 of the β strand of the DNA. This subtle change in the homeodomain-DNA complex resulted in modifications of bin- ding affinities to DNA. These changes resulting from a single point mutation constitute the mole- cular basis for the phenotypic alterations observed upon ectopic expression of the Y54M vnd/NK-2 gene during embryogenesis. A critical relationship between the regulatory role of the NK-2 class of homeobox genes in development and highly explicit homeo- domain-DNA interactions is thereby established. The corresponding rescue experiment where the 5 CAAGTG 3 containing sites are replaced with 5 CAATGG 3 become of considerable interest.