

RECOGNITION OF DNA BY THE NK-2 HOMEODOMAIN

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The NK-2 homeobox gene (Kim, Y., and Nirenberg, M. (1989) Proc. Natl. Acad. Sci. USA, **86**, 7716-7720) is expressed initially by nuclei in late stage 4 or early stage 5 *Drosophila* embryos that comprise the ventral half of the ventral neurogenic anlage and by some nuclei in the procephalic region. These nuclei are precursors of neuroectodermal cells that give rise to subsets of neuroblasts, ganglion mother cells, and neurons in the subesophageal ganglion, ventral nerve cord, stomatogastric nervous system, and some cephalic ganglia. NK-2 also is expressed in the anterior and posterior midgut primordia. (Nakayama, K., Nakayama, N. Kim, Y., Webber, K., Lad, R., and Nirenberg, M., in preparation). The NK-2 homeodomain and flanking regions (77 amino acid residues, termed NK-2H) was synthesized in *E. coli* and purified. Nucleotide sequences of DNA binding sites for NK-2H were identified by purifying oligonucleotides with central random sequences by repetitive NK-2H-Sepharose affinity column chromatography. Purified oligonucleotides were amplified by PCR and cloned. The consensus nucleotide sequence of the NK-2H DNA binding site, obtained by sequencing 77 clones, is T(T/C)AAGTG(G/C). The K_D of NK-2H for the consensus sequence is 2×10^{-10} M. Twenty putative high-affinity and 13 lower affinity NK-2 binding sites were found in 2.2 kb of the 5'-flanking sequence of the NK-2 gene, which suggests that NK-2 protein may be required to maintain NK-2 gene expression. Many NK-2 binding sites for DNA were found with overlapping or adjacent putative sites for other proteins, such as E(spl)m8, dorsal, snail, or other homeodomain proteins, which suggests that NK-2 protein may compete with some proteins that regulate gene expression for occupancy of NK-2 binding sites in DNA.

Circular dichroism measurements and 1D NMR spectra showed that the t_m for denaturation of NK-2H is approximately 25°C at pH 4.4 and that denaturation is fully reversible. NK-2H has considerably less α -helical content and a less stable conformation than does the *Antp* homeodomain (Otting, G. et al. (1989) EMBO J. **7**, 4305-4309). NK-2H uniformly enriched with ¹⁵N was examined by 2D and 3D NMR. The results show that NK-2H has a novel homeodomain conformation.