

Evidence for a new family of evolutionarily conserved homeobox genes

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The homeodomain is a DNA binding domain about 60 amino acids in length that occurs in many developmental regulatory proteins. Based on their degree of relatedness, homeodomain sequences have been grouped into 10 different families plus some unclassified sequences (1). Using a set of degenerate oligonucleotides (hom3 and en5) designed to anneal to homeoboxes, we employed the polymerase chain reaction (PCR) (2), to amplify a portion of a homeobox from genomic DNA of the leech, *Helobdella triserialis* (*Htr*). The amplified homeobox fragment was used as a probe to screen an *Htr* genomic library, from which positive recombinants were plaque purified. From one of these, *Htr-A2*, the homeobox cross-hybridizing region was sequenced. The corresponding homeodomain is unlike those of any of the previously described 10 families but is quite similar to the X1-HBox8 homeodomain (3) from the frog, *Xenopus laevis* (*Xl*) (shown below). Underlined portions of the nucleic acid sequence are homologous to the oligonucleotide primers used in the PCR amplification. Asterisks signify amino acids in the frog sequence that are identical to those in the leech sequence. In the region compared, 37 of 42 amino acids are identical. In addition, X1-HBox8 and *Htr-A2* homeodomains have in common a histidine (H) residue (amino acid 44) in the putative recognition helix region (4, 5), which is not present in any other homeodomain. For the region compared, this level of sequence identity surpasses that observed between chordates and annelids

for the *engrailed* gene family (unpublished results), one that is considered well conserved throughout many phyla. This high degree of homology identifies a new family of highly conserved homeobox genes.

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AAC AAA AGA ACT CGC ACA GCT TAC AGC AGA TCA CAA CTT TTC GAA TTA GAG AAA GAG
TTT CAT TTC GAT AAA TAT ATT TCG AGA CCA AGA AGA GTG GAA CTG GCC TCC TCT CTC
AAT TTG ACT GAA CGA CAT ATT AAG ATC TGG TTT CAA AAC CGT CGA ATG AAA TGG AAA
AAA ATG GAA
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10 20 30 40 50 60

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Htr NKRTRTAYSRSQ LLELEKEFHFDKYISRPRRVELASSLNLTERHIKIWFQNR RMKWKME
X.l. **L*N*****VM*****E*
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hom3 GAATTCGA (AG) (TC) TCGA (AG) AA (AG) GA (AG) TT (TC) CAT
en5 TT (AG) TT (TC) TG (AG) AACCAGAT (TC) TT (AGT) AT
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