Cloning of full coding chicken cDNAs for the homeobox-containing gene *Hoxd-13*

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Homeobox-containing genes, which are characterized by a highly conserved 183 bp sequence known as a homeobox, encode sequence-specific DNA binding transcription factors. They have been shown to be involved in the specification of positional identity, segmentation, and body pattern during *Drosophila* development (1). On the basis of their regulated temporal and spatial expression during embryogenesis, homeobox-containing genes of the 5' end of the *Hoxd* cluster (formerly named the Hox 4 cluster) (2), are thought to be involved in the regulation of pattern formation and specification of positional information during vertebrate limb development (3, 4, 5). In order to isolate cDNA clones corresponding to chicken *Hoxd-13* (formerly called CHox-4.8 or CHox-4g) (2), we have screened a cDNA library prepared from RNA isolated from 4–6 days (Hamburger-Hamilton stage 22–30) (6) embryonic chick limb buds with a polymerase chain reaction generated probe. The probe was prepared by amplification of an aliquot of the same library using amplification primers (5'-primer, 5'-CGAGATGTGCGT-CTACC-3'; 3'-primer, 5' CTGTCCGCAAGGAATCG-3') designed from the available chicken *Hoxd-13* sequence (4). Ten positive clones were isolated and sequenced. The map of the *Hoxd-13* mRNA derived from the cDNA clones is shown in figure 1. Based on comparisons of the nucleotide and deduced amino acid sequences with the published genomic sequence for the 2nd exon of the chicken (4, 5, 7), mouse (7) and human (8) genes, the clones were confirmed to correspond to chicken *Hoxd-13*. The deduced amino sequence is identical to the published amino acid sequences for human (8) and mouse *Hoxd-13* (7) homeodomains, but differs from the homeodomains of human *HOX A13* and *HOX C13* (the paralogs of *Hoxd-13* in *Hox A* and *C* cluster) (9) by 9 amino acids. Two out of ten clones contain sequence at their 5' ends, which is different from the sequence in the other clones. Based on identity with the genomic clone (4, 7), the position of the intron in other Hox genes, and sequence with homology to the 3' splice acceptor consensus sequence, this sequence appears to correspond to a retained intron. Clones which appeared to be primed at 2 different polyadenylation sites were obtained. Four were primed at the 3' site, and 3 at the more 5' polyadenylation site. A probe 5' to the more proximal polyadenylation site hybridized to mRNAs of approximated 1.6 and 2.5 kb whereas a probe 3' to the proximal polyadenylation site hybridized only to the 2.5 kb RNA (figure B 2). Three clones appeared to be primed on internal A-rich regions.

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Figure 1. Map of *Hoxd-13* mRNA derived from cDNA clones. The positions of the translation initiation codon (AUG), termination codon (TERM), and the alternative polyadenylation site [(A)_n] are indicated. The homeobox is indicated by the shaded rectangle and the probes used for Northern blots as probe 1 and probe 2. Intron indicates the site at which the intron sequences present in 2 of the clones are located.

Figure 2. The chicken *Hoxd-13* gene uses two polyadenylation sites. Northern blots of total RNA from Hamburger-Hamilton stages 25 and 28 (6) chick limbs hybridized with probe 1 (A) and probe 2 (B).

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