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Short Sequence-Paper

DR-78, a novel *Drosophila melanogaster* genomic DNA fragment highly homologous to the DNA-binding domain of thyroid hormone-retinoic acid-vitamin D receptor subfamily

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Degenerate oligodeoxyribonucleotides were designed for both ends of the DNA-binding domain of members of the nuclear receptor superfamily. PCR amplified *Drosophila melanogaster* DNA was purified and cloned (DR plasmids). Genomic λDASH clones were identified at high stringency with an amplified DR-78 plasmid DNA and isolated. The partial sequence shows a very probable open reading frame which would encode a peptide highly homologous to members of the thyroid hormone-retinoic acid-vitamin D receptor subfamily. The fragment corresponds to a single copy gene and was mapped at position 78D of chromosome three by in situ hybridization.

Different members of the nuclear receptor superfamily have been described in *Drosophila melanogaster*, see, e.g., Refs. 1–4. Ecdysone receptor, *Ultraspiracle* (*Usp*), a gene coding for an homologous of vertebrate RXR genes, and E75 protein seem to be involved in ecdysone response during metamorphosis. FTZ-F1, *Knirps* and *Knirps-related* are probably participating in segmentation processes.

To search for new members of the nuclear receptor superfamily, we designed two degenerate primers corresponding to the two zinc fingers of the DNA binding domain of vertebrate receptors [5] (Fig. 1). After PCR amplification of genomic *Drosophila* DNA and posterior cloning, we isolated and sequenced several clones with partial homology to different receptors. For one of them, DR-78, we screened a λDASH genomic *Drosophila* library (kindly provided by J.N. Jan Laboratory, UCSF) and isolated several λ clones.

Southern blot of restriction enzyme-digested genomic DNA from *Drosophila melanogaster* was probed with the DR-78 clone. Hybridization under high-stringency conditions gave only a single band indicating that the DR-78 fragment derives from a single copy sequence (data not shown).

Physical maps of genomic clones were aligned and the PCR probe was localized by hybridization to a 3.6 kb *Eco*RI fragment. DR-78 nucleotide sequence and its conceptual translation product bear strong resemblance to the nuclear receptor zinc fingers domain (C region) [6], with the characteristic structure C-X_2-C-X_{13}-C-X_2-C and C-X_5-C-X_{12}-C-X_4-C (Fig. 2). In each of the genes of the nuclear receptor superfamily that have been examined, an intron had been found in between the two zinc fingers. This intron occurs either one codon after [7] or within the tenth codon after [8] the final cysteine residue of the first finger. DR-78 encodes a possible intron, of 57 bp, one codon after the cysteine, which presents good putative donor and acceptor splicing sites (Fig. 2A).

DR-78 is highly homologous to members of the thyroid hormone-retinoic acid-vitamin D receptor subfamily [9] and would code for Glu, Gly and Gly at the discriminatory positions at the base of the first finger

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The DR-78 sequence data reported in this paper have been submitted to the EMBL/GenBank Data Libraries under the accession number X73045.

Abbreviations: PCR, polymerase chain reaction; hRAR, human retinoic acid receptor; RXR, retinoid X receptor; v-*erbA*, viral oncogene *erbA*; rGR, rat glucocorticoid receptor; hGR, human glucocorticoid receptor; cER, chicken estrogen receptor; hER, human estrogen receptor; hPR, human progesterone receptor.

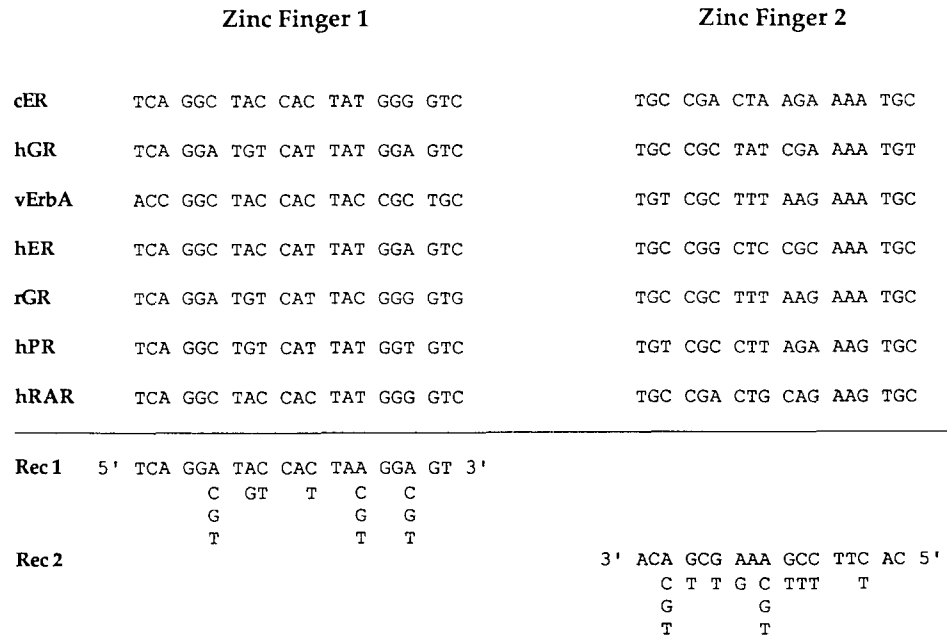

Fig. 1. Conserved sequences for zinc fingers 1 and 2 of hRAR, (human retinoic acid receptor), hPR (human progesterone receptor), v-*erbA* (viral oncogene *erbA*), rGR (rat glucocorticoid receptor), hGR (human glucocorticoid receptor), cER (chicken estrogen receptor) and hER (human estrogen receptor). Rec 1 and Rec 2 are the degenerate primers designed for both zinc fingers.

(P box) as these receptors do. It is well known that DNA binding specificity is mediated, at least in part, by these three variable residues. Highest homology is reached with retinoic acid receptors (hRARγ) [5] (71.5% at DNA; 68% identity at protein level (C domain)) (Fig. 2B).

DR-78 nucleotide and aminoacid homology to nuclear receptors extends outside of the PCR-amplified sequence. This confirms that DR-78 clone corresponds to a bona fide receptor genomic sequence and not to a PCR artifact. Nevertheless, homology dissapears outside the C domain (Fig. 2B).

By 'in situ' hybridization to polytene chromosomes, using a biotinylated DR-78 225 bp probe, we were able to detect a single signal at position 78D (data not shown).

78D is a major early-late puffing site [10] inducible by β-ecdysone. We suggest that a putative nuclear receptor encoded at this position could have a role in the ecdysone-induced cascade of regulatory events during *Drosophila* metamorphosis [11].

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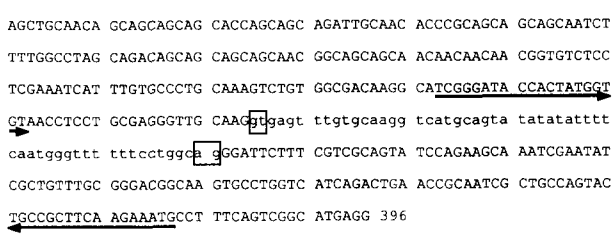
A



B

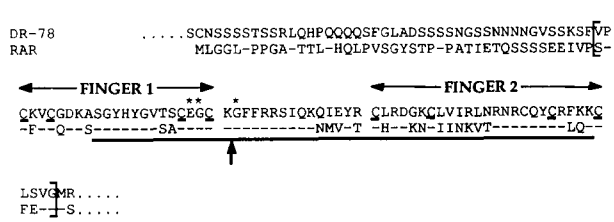


Fig. 2. Homology between the predicted sequences of DR-78 and nuclear receptors. (A) partial genomic sequence of a λ clone spanning PCR-amplified fragment DR-78. Sequences corresponding to primers Rec 1 and Rec 2 are underlined. Putative intron sequence is shown in lowercase characters, tentative donor and acceptor sites are boxed. (B) Predicted aminoacid sequence of DR-78, aligned with the corresponding sequence of hRARγ [5]. Zinc fingers 1 and 2 are shown. Cysteines are underlined. * symbols indicate residues characteristics of the P box in the thyroid hormone-retinoic acid-vitamin D receptors subfamily. Intron position is marked with an arrow. Brackets represent the beginning and the end of the C Domain. PCR-amplified sequence is underlined.

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