Volume 289, number 2, 235–238 FEBS 10148 September 1991

ADONIS 001457939100859A

Identification of seven novel protein-tyrosine kinase genes of *Drosophila* by the polymerase chain reaction

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Received 14 June 1991

We used the polymerase chain reaction to identify 7 novel tyrosine-kinase genes (*dtk1* to −7) in *Drosophila melanogaster*. *dtk4* coded for a part of the kinase catalytic domain nearly identical in sequence to that of the human receptor for insulin-like growth factor 1, whereas sequences encoded by *dtk1* and *dtk2* were highly homologous to that of the chicken fibroblast growth factor receptor.

Protein-tyrosine kinase; Polymerase chain reaction; *Drosophila melanogaster*; Insulin-like growth factor 1 receptor; Fibroblast growth factor receptor

1. INTRODUCTION

Protein-tyrosine kinases (PTKs) are a large family of enzymes, many of which appear involved in various cellular processes via signal transduction [1]. Recent molecular and genetic analyses in *Drosophila* have shown some PTKs to function as determinants of cell fate [2–4]. *sevenless* (*sev*) [2] encodes a receptor-type PTK, and its abscence causes cells, normally destined to form the R7 photoreceptor, to differentiate into non-neuronal cells [2]. *torso* is essential for the differentiation of anterior and posterior terminal structures of embryo [3]; abnormal expression of a homologue of the vertebrate epidermal growth factor receptor (EGFR) [5] results in developmental defects in embryonic and ommatidial cells [4].

For further clarification of the development functions of the PTK family as a whole, identification and functional analysis of novel PTK genes would be important. In the present paper are presented the results of PCR analysis of the genomic DNA of *Drosophila* which identified 7 novel PTK genes including a putative homologue of the human insulin-like growth factor 1 receptor (IGF1R) gene [6].

2. MATERIALS AND METHODS

Fully degenerate oligonucleotides used as PCR primers are shown in Fig. 1. About 0.5 μg of *Drosophila* genomic DNA was used as a template in each PCR amplification, which was carried out for 30 cycles on a thermal cycler essentially according to the supplier's instructions (Perkins-Elmer/Cetus, USA). The primer concentration was 5 μM and the temperature cycles were as follows: 94°C for 1 min, 55°C for 2 min and 72°C for 2 min. Amplified products were treated with Klenow fragment, phosphorylated with T4 polynucleotide kinase and size-fractionated on 1.5% agarose. DNA fragments (200–220 bp) were isolated and cloned into the Bluescript vector at the *Sma*I site.

Colony hybridization probes A and B, respectively, were oligonucleotides corresponding to K(I,V)DFGLA (subdomain VII) and P(I,V)KWTAPE (subdomain VIII) (see Fig. 1). Hybridization (50°C, 12–16 h) was carried out in the solution containing 1 M NaCl, 10 mM EDTA, 50 mM Tris-HCl (pH 8.0), 0.2% sodium lauryl sulfate (SDS), 10× Denhardt's reagent [10], 0.2 mg/ml of heat-denatured herring sperm DNA and 1×10^6 cpm/ml of ^{32}P-labeled probe. After being washed in 0.9 M NaCl, 90 mM sodium citrate and 0.1% SDS at 25°C for 10 min, filters were incubated in the same solution at 50°C for 1 h.

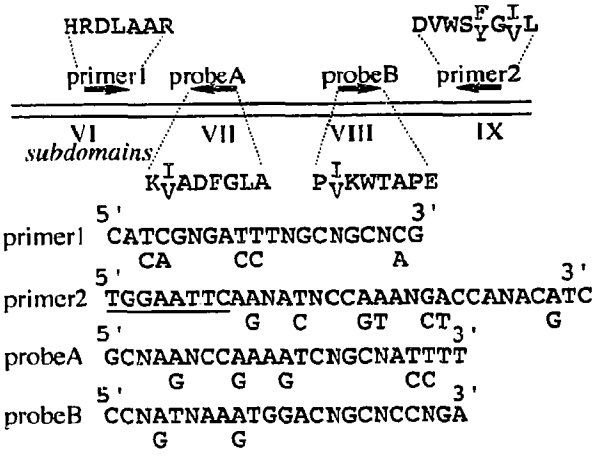

Fig. 1. PCR primers and hybridization probes. N denotes mixture of A, G, T and C. Underlined residues are additional bases connected for the *Eco*RI site.

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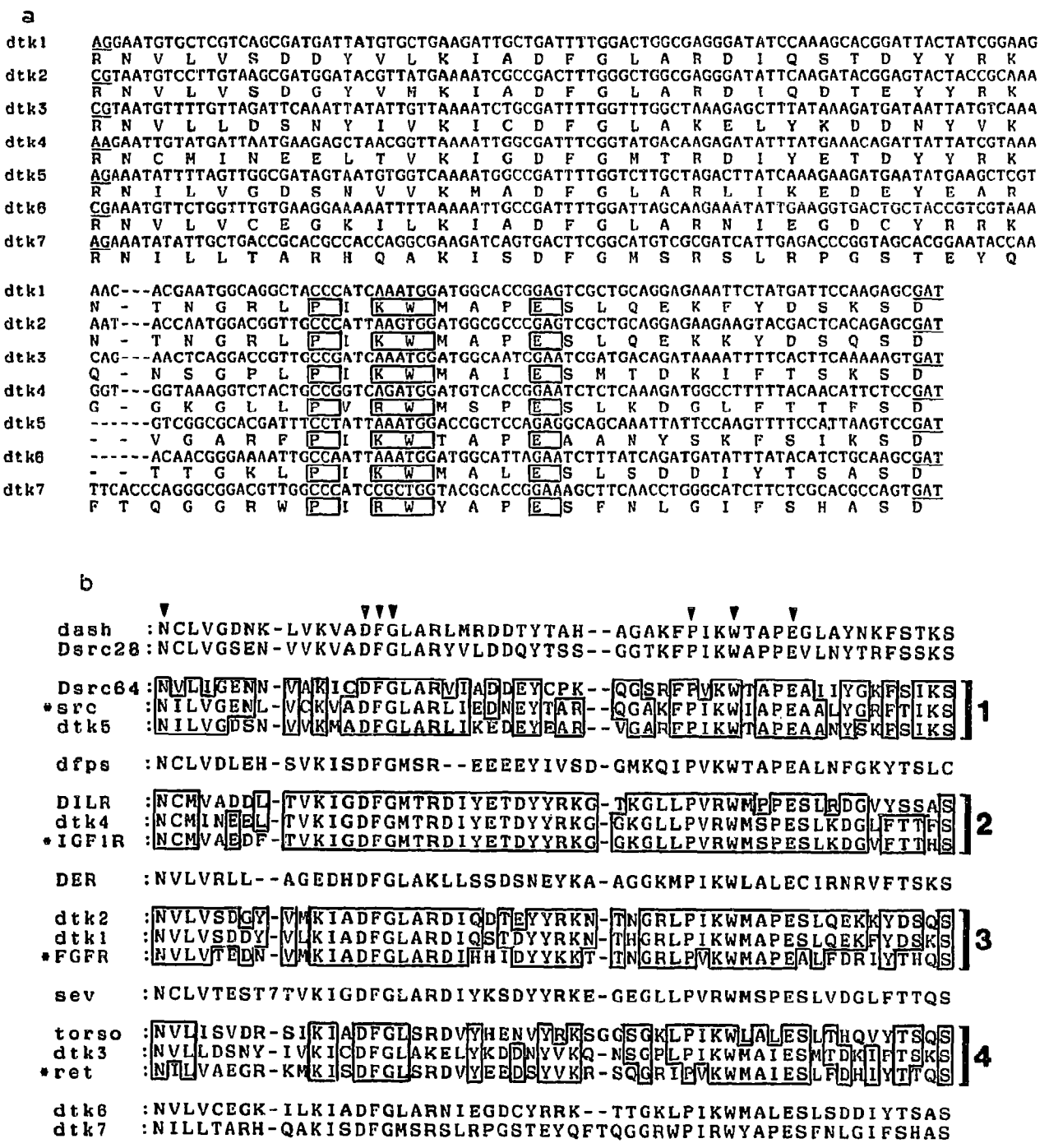

Fig. 2. (a) Nucleotide sequences of *dtk* genes and amino acid sequences of the putative translation products. Amino acids in P-(K/R)W····E are boxed. Underlined, partial primer sequences. (b) Amino acid sequence comparison of PTK kinase domains [1–3,5,6,9–15]. Homology to human *src* (region 1), *dtk4* (region 2), *dtk1* (region 3) and *dtk3* (region 4) are shown. Filled triangles, conserved amino acids in PTKs [1]; *, non-*Drosophila* sequences; 7, seven amino acid insert in *sev*.

3. RESULTS AND DISCUSSION

The catalytic domains of PTKs contain 11 subdomains conserved in sequence [1]. As in Fig. 1, the PCR primers used, correspond to the amino acid sequences, HRDLAAR and DVWS(F/Y)G(I/V)L, located in subdomains VI and IX, respectively. AAR, three consecutive, carboxyl-terminal amino acids of HRDLAAR are present in most of PTKs but absent in all serine/threonine kinases so far examined [1] (see Fig. 1). Thus, by using primer 1, the serine/threonine kinase genes could be eliminated efficiently. To further eliminate non-PTK sequences, size-fractionation and colony hybridization with probes A and B were carried out, since most exam-

Table I

Difference scores among vertebrate PTKs and *Drosophila* PTKs both known and newly-isolated

	src	fyn	abl	EGFR	fes	INSR	IGF1R	ros	FGFR	PDGFR	ret
Dsrc64	21.6	23.6	28.6	76.3	52.9	70.6	72.3	60.7	54.9	63.7	69.0
Dsrc28	35.0	35.4	23.8	83.6	41.1	61.0	63.2	54.8	64.4	76.6	80.8
dash	28.5	30.1	7.5	70.3	43.3	67.2	69.4	60.0	62.7	74.2	75.3
dfps	68.3	68.6	53.3	100.9	28.5	60.6	61.2	65.0	75.5	82.0	82.7
DER	85.7	87.4	81.4	40.5	119.3	94.6	99.4	88.0	83.5	94.2	79.5
DILR	73.7	74.7	69.3	92.1	57.7	7.8	9.3	23.9	38.5	66.3	47.9
sev	68.5	70.1	63.0	80.3	57.8	17.0	17.5	10.3	34.8	50.5	41.6
torso	80.3	80.6	71.9	54.2	73.9	39.5	42.0	37.7	36.4	54.9	29.9
dtk1	61.8	62.6	59.2	71.3	63.7	35.3	37.3	39.6	22.3	55.7	45.2
dtk2	58.4	55.9	57.2	69.7	59.3	37.0	38.6	41.4	21.0	56.0	40.3
dtk3	77.7	77.0	74.9	61.7	89.9	48.0	50.6	44.1	47.5	51.7	37.7
dtk4	74.8	77.4	71.7	97.1	60.9	7.7	4.2	23.5	45.4	60.5	53.3
dtk5	18.9	19.8	21.5	79.8	54.9	76.6	78.9	70.0	59.1	79.1	75.9
dtk6	65.4	63.4	57.2	67.1	67.1	58.2	59.7	53.4	42.5	54.1	48.4
dtk7	79.6	81.3	72.0	61.2	87.1	88.1	91.1	83.0	77.0	94.5	86.4

The scores are calculated by the method of Feng *et al.* with log-odds matrix [8].

ined PTK genes in *Drosophila* are not interrupted by introns in the relevant region (subdomains VI to IX) [2,3,5,9,10,14,15] and probes A and B partially represent conserved sequences of subdomains VII and VIII, respectively [1].

Examination was made of 155 independent clones, 39 of which were positive to probes A and/or B. Nucleotide sequence analysis showed 28 of the 39 positive clones to have inserts encoding PTK-related sequences and to represent 9 PTK genes, 7 of which (*dtk1* to *7*) had not been identified previously. The remaining two were *Dsrc64B* [9] and *Dsrc28C* [10]. No PTK-related sequence could be detected in 6 clones randomly selected from 116 negatives. Since, as expected, a motif diagnostic of PTK, P-(K/R)W---E, was found in all cases (Fig. 2a), all 7 *dtk* genes were concluded to encode novel PTKs in *Drosophila*.

By the difference score method of Fent et al. [8], the relevant kinase domains of *dtk* PTK sequences along with all 8 *Drosophila* PTKs so far identified were compared with those of several vertebrate PTKs (Table I). The regions to be examined were relatively short, but no essential discrepancy could be found between difference scores obtained in this study and reported phylogenetic relations [1], so far as known *Drosophila* and vertebrate PKTs were concerned. *dtk1* and *dtk2* were shown related to the FGF receptors [11], *dtk3* to *ret* [2], *dtk4* to the IGF1R [6], and *dtk5* to *src* [13]. Homology between *dtk4* and human IGF1R was particularly high. As shown in Fig. 2b, 48 of 54 (88%) positions were identical to each other, indicating the *dtk4* gene product to possibly be the counterpart of the human IGF1R in *Drosophila*. Difference scores in Table I along with amino acid sequence alignments in Fig. 2b also appear to indicate *dtk5* to be a member of non-receptor-type PTKs represented by *src* [13], and *dtk1* and *dtk2* to be cognate PTKs intimately related to the human FGF receptor [11]. *dtk3* also appears to be of the membrane-receptor type, in view of its marginal homologies to *torso* [3] and *ret* [12]. The remaining two genes, *dtk6* and *dtk7*, show high scores to any of the known PTKs, suggesting that these *dtk* genes might thus form novel, independent subfamilies of PTKs.

In summary, PCR was used to isolate 7 novel members of PTK genes, so that the total number of PTK genes cloned in *Drosophila* was doubled.

During the preparation of this manuscript, two papers appeared, one indicating serine/threonine kinase genes and the other PTK genes, respectively, to have been isolated from rice [16] and rat [17] cDNAs using PCR. Thus, these and the present results clearly demonstrate the effectiveness of PCR for systematic isolation of protein kinase genes from various sources.

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