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Identification and characterization of oncogene *yes*-homologous genomic clones from *Drosophila melanogaster*

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Abstract. The proto-oncogene *yes* encodes a protein tyrosine kinase described as a member of the non-receptor protein tyrosine kinases family (NRPTK). We have identified two clones from the genomic library of *Drosophila melanogaster* which hybridized to the *v-yes* oncogene (encoded by the Y73/Esh Avian Sarcoma Virus). These clones represent two different genomic sequences from *Drosophila*. By RNA slot blot hybridization, we have demonstrated the presence of *yes* homologous sequences in the RNA of larvae (the third instar), pupae and an embryonic cell line of *Drosophila*. On northern analysis of the cell line RNA, three transcripts (3·8, 2·0 and 1·0 kb) hybridized with the *yes* sequence. Oncogene *yes* is thus well conserved across widely separated phylogenetic lines like human, chicken and *Drosophila* indicating involvement in essential biological functions.

Keywords. Oncogene homologue; protein tyrosine kinases; *Drosophila melanogaster*; oncogene *yes*.

1. Introduction

Protein tyrosine kinases play an important role in many cellular pathways, though their precise physiological roles remain enigmatic. In *Drosophila*, seven genes encoding protein tyrosine kinases have been identified – four of these encode cell surface receptors – torso, sevenless, insulin receptor and DER, while three of them are proto-oncogene homologues – *src*, *abl*, *fes/fps* (Marshall 1989; Katzen *et al.* 1991).

Proto-oncogene *yes* encodes a non-receptor protein tyrosine kinase related to the *src* group of oncogene protein kinases (Sudol 1991). All these proteins share extensive amino acid homology particularly in the tyrosine kinase domain (Kitamura *et al.* 1982). However, at high stringency conditions there is no hybridization between the DNAs of these genes due to differences in codon usage (Kawai *et al.* 1980; Yoshida *et al.* 1980). The nucleotide sequence of chicken *yes* cDNA is available (Sudol *et al.*1988). Proto-oncogene *yes* encodes a 62 kd protein which is myristoylated and attached to the cell membrane (Sudol 1991). It has been seen to localize at cell adhesion plaques (Marshall 1989). Levels of *c-yes* mRNA have been shown to be developmentally regulated (Lev *et al.* 1984; Zhao *et al.*

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1990). Expression of *yes* is highest in cerebellum, in particular in the Purkinje cells and mRNAs have also been detected in kidney and liver (Zhao *et al.* 1990).

In *Drosophila*, molecular genetic and biochemical approaches can be easily combined to unravel the involvement of oncogenes in normal cellular processes. Oncogene *yes* homologous sequences were localized by *in situ* hybridization on salivary gland polytene chromosomes of *Drosophila melanogaster* at three separate sites 8D/X, 57 BC/2R and 95 CD/3R (Samant *et al.* 1989). We have screened a genomic library of *Drosophila melanogaster* embryonic DNA in lambda EMBL4 by hybridization with the *v-yes* sequence. Several clones were identified as presumptive positives and two clones (D-yes 41 and D-yes 42) which hybridized at high stringency were characterized further. Southern hybridization of the restriction enzyme digested DNA of the two clones confirmed the presence of *yes* homologous sequences. Dot blot hybridization of poly A^+ mRNA from H-33 cell line confirmed its expression in *Drosophila*. Northern analysis revealed three major *yes* homologous transcripts of sizes 3·8, 2·0 and 1·0 kb in *Drosophila melanogaster*.

2. Materials and methods

Bacterial strain *E. coli* K802 was used as host for growing phage EMBL4. Genotype of K802: *hsd* R^+, *hsd* M^+, gal^-, met^-, supE.

Cell line: Drosophila embryonic cell line H-33 was used to isolate RNA. The cell line was grown in Schneider's medium and maintained at 28°C.

Developmental stages third instar and pupae of *Drosophila melanogaster* Oregon were used for isolation of RNA.

2.1 *Isolation of DNA from phage and Southern blotting*

DNA was isolated from genomic clones as described by Miller (1987). DNA from the two clones (D-yes 41 and D-yes 42) was digested with *BamHI*, electrophoresed on 1% agarose gel and transferred onto Hybond-N membrane (Amersham) under denaturing conditions (1·5 M NaCl, 0·5 N NaOH). The 6·1 kb fragment of D-yes 41 was electroeluted, labelled by primer extension using α^{32} P dCTP (Feinberg and Vogelstein 1983) and used as a probe. The blot was prehybridized for 1 h at 42°C. Hybridization was carried out in 5 × SSC, 5 × Denhardt's solution, 50 mM phosphate buffer, pH 7·0, 0·1% SDS, 35% formamide and 0·1 mg/ml bovine serum albumin for 16–18 hours. The blot was washed first in 2 × SSC, 0·1% SDS at 42°C for 45 minutes and at 55°C for 45 minutes. It was then washed at 0·5 × SSC, 0·1% SDS, 42°C for 45 minutes and at 55°C for 45 minutes and autoradiographed. This blot was washed subsequently at a stringency of 0·1% SSC, 0·1% SDS, 52°C.

2.2 *Isolation of cellular RNA and dot/slot blot*

Total cellular RNA was isolated from H-33 cell line and from various stages of *Drosophila* (Perbal 1988). Cellular RNA from H-33 cell line, third instar larvae and pupae of *Drosophila* were spotted on Hybond-N membrane in five different concentrations (0·12, 0·25, 0·5, 1·0 and 2·0 μg) and challenged with 6·1 kb fragment

of D-yes 41. Hybridization was carried out at 42°C for 16 h in 35% formamide. The blot was washed at 2 × SSC, 0·1% SDS and then at 0·2 × SSC, 0·1% SDS, 42°C.

RNA from H-33 cell line was fractionated on oligo dT column. Poly A^+ mRNA was spotted (1 μg and 5 μg) onto Hybond-N and probed with p YS plasmid labelled by nick translation (Rigby *et al.* 1977), using α^{32}P dCTP (specific activity: 2×10^7 cpm/μg DNA). The blot was washed as described earlier.

2.3 *Northern blot analysis*

Total cellular RNA was extracted from H-33 cell line and the third instar larval stage of *Drosophila*. Total RNA (10 μg) was treated with formamide/formaldehyde at 65°C for 5 min before loading on the gel. RNAs were electrophoresed on 1.4% agarose gel containing formaldehyde (Lebrach *et al.* 1977; Thomas 1980). RNA was transferred onto Hybond-N membrane in 10 × SSPE.

Northern blot was challenged with p YS plasmid labelled by nick-translation (Rigby *et al.* 1977) using α^{32} P dCTP. Blots were washed finally in 0·2 × SSC, 0·1% SDS at 42°C.

Northern blot containing H-33 RNA was challenged with 6·1 kbp fragment of D-yes 41, washed at 0·5 × SSC, 0·1% SDS at 42°C and autoradiographed.

2.4 *Preparation of probe*

i) Plasmid DNA was isolated by the alkaline lysis method (Birnboim and Doly 1979). Plasmid p YS has a 1·5 kbp *SinI-SinI* cDNA fragment of Avian Sarcoma Virus Y73 corresponding to *v-yes* oncogene in the *SalI* site of pBR322 (Kitamura *et al.* 1982). Plasmid DNA (500 ng) was nick translated (Rigby *et al.* 1977) using 50 μCi of α^{32} P dCTP, specific activity of 3×10^7 cpm/μg of DNA was achieved.

ii) The 6·1 kb fragment of D-yes 41 was electroeluted and labelled by primer extension (Feinberg and Vogelstein 1983).

3. Results and discussion

The DNAs from clones D-yes 41 and D-yes 42 were digested with *BamHI* and electrophoresed on 1% agarose gel. The restriction enzyme digestion pattern of the two clones is shown in figure 1a. On Southern hybridization with *v-yes* insert, the 6·1 kb band of D-yes 41 and the 7·1 kb band of D-yes 42 showed strong hybridization signals (data not shown). This suggested that these fragments could harbor sequences homologous to the tyrosine kinase domain of *v-yes*. The other fragments which do not hybridize to *v-yes* may represent non-tyrosine kinase domains of the proteins, flanking sequences and/or introns. We then used the 6·1 kb fragment, isolated from D-yes 41, as a hybridization probe. Two fragments, namely, 7·1 kb and 2·0 kb from D-yes 42 hybridized to the 6·1 kb fragment (figure 1b). This indicated that the 6·1 kb fragment has homology with the two fragments (7.1 and 2·0 kb) from D-yes 42. However, at higher stringency (figure 1c) there was no hybridization to 7·1 and 2·0 kb fragments from D-yes 42 indicating that these two clones harbored related but non-identical regions. We have thus identified two distinct *yes* homologous clones from the genomic library of *D. melanogaster*.

In chickens and humans, presence of multiple oncogene *yes* homologous genomic sequences has been suggested (Toyoshima *et al.* 1987) In the human, *C-yes-1* gene localizes to chromosome 6, spans 30 kb and has 12 exons (Yoshida *et al.* 1985). *C-yes* 2 is a pseudogene (1·8 kb) present on chromosome 18 (Semba *et al.* 1985). The number, size and arrangement of introns/exons of *Drosophila src* gene are similar to the vertebrate gene while the organization of *fes/fps* and other related genes in *Drosophila* is significantly different from that in vertebrates (Katzen *et al.* 1991).

Cytoplasmic RNAs were isolated from the third instar larvae, pupae and *Drosophila* cell line, spotted onto Hybond-N and hybridized with 6.1 kb insert from D-yes 41 (figure 2A). RNAs from the third instar larvae, pupae and *Drosophila* H-33 cell line all showed a positive signal indicating presence of oncogene *yes* homologous transcripts. The concentration of *yes* homologous RNA was much higher in the H-33 embryonic cell line indicating enhanced transcription (high level of steady state RNA). Poly $(A)^+$ mRNA purified from total RNA of the embryonic cell line by oligo dT cellulose fractionation hybridized extensively with the oncogene *yes* (figure 2B). Thus, the *yes* homologous RNA is represented in poly $(A)^+$ RNA in the H-33 cell line. The RNA from the third instar larvae and H-33 cell line were electrophoresed, northern blotted and hybridized with *v-yes*. Three distinct RNAs hybridized with *yes* – namely 3.8 kb, 2.0 kb and 1.0 kb (figure 3). Although the RNA from the cell line and the third instar larvae showed the same size classes their relative abundance was variable. The 2.0 kb band was present in much higher proportion in the third instar larvae. In another set of experiments, the 6.1 kb fragment from D-yes 41 was used as a hybridization probe to challenge the northern blot of RNA from the cell line. The same three RNA species 3.8, 2.0 and 1.0 kb hybridized to the D-yes 41 fragment. These transcripts may arise from the *D-yes* sequences present at different loci or they may be products of alternate and differential splicing. The sizes of the chicken *yes* mRNA are 3.7 and 3.9 kb (Sudol *et al.* 1988), while the size of the human *yes* mRNA is 4.3 kb (Sudol 1989). We have screened a *Drosophila* cDNA library in lambda gt 10 and have obtained a cDNA clone which has a 3·8 kb insert (data not shown). It would be interesting to compare the insect and vertebrate genes in order to examine the intron–exon organization and changes in nucleotide and amino-acid sequence.

Thus *yes* oncogene is developmentally regulated in chickens and humans and the *src* and *yes* sequences seem to be differentially expressed (Sudol *et al.* 1988, Sudol 1991; Zheng *et al.* 1989; Zhao *et al.* 1990). Our studies in *Drosophila* demonstrate the presence of *yes* homologous transcripts in an embryonic cell line, the third instar larvae and pupae. The developmental regulation and stage specificity of expression of *D-yes* sequences in *Drosophila* would give important insight into the expression of this proto-oncogene in normal cells. *D-src*, a related gene in *Drosophila* has been localized to 29A/2L, and 64B/3L in the polytene chromosomes of salivary glands (Hoffman-Falk *et al.* 1983; Simon *et al.* 1983). Transcripts homologous to *D-src* have been detected in actively dividing cells like those of imaginal discs and terminally differentiated non-dividing cells like the nervous tissue (Lev *et al.* 1984; Shilo 1987). It has been suggested that oncogene *yes* is involved in receptor associated cell proliferation and signal transduction (Sudol 1991; Bishop 1991). The complex pattern of expression of *yes* indicates its involvement in many cellular pathways. *abl*, also a member of NRPTK, has nuclear localization. Three of the protein tyrosine kinases have been implicated in retinal development in *Drosophila* namely sevenless (encodes a transmembrane receptor), *src* and *ras* (Katzen *et al.*

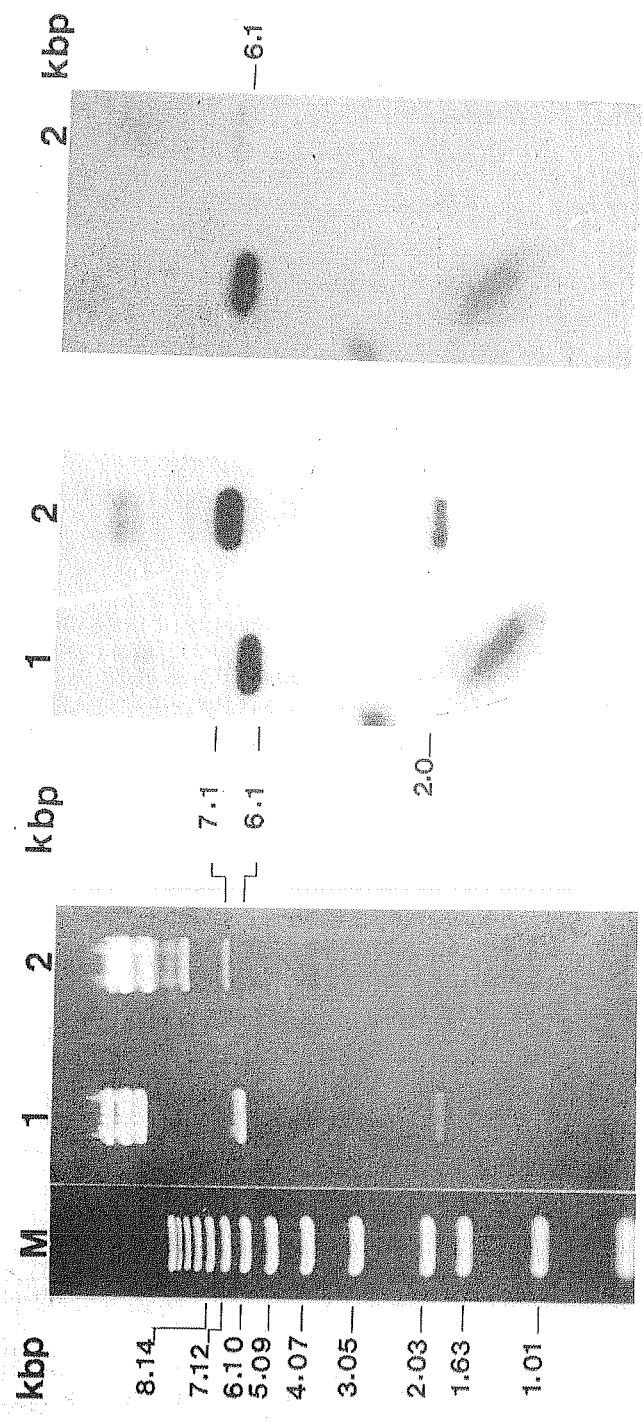

Figure 1. (**a**) *BamHI* digestion pattern of D-yes 41 and D-yes 42. Lane 1: Molecular weight marker, Lane 2: D-yes 41 and Lane 3: D-yes 42. (**b**) Southern blot of *BamHI* digested DNAs from D-yes 41 and D-yes 42, probed with 6·1 kb fragment of D-yes 41, washed in 0·5 × SSC at 42°C and autoradiographed. (**c**) Blot washed at high stringency: 0·1 × SSC at 60°C and autoradiographed.

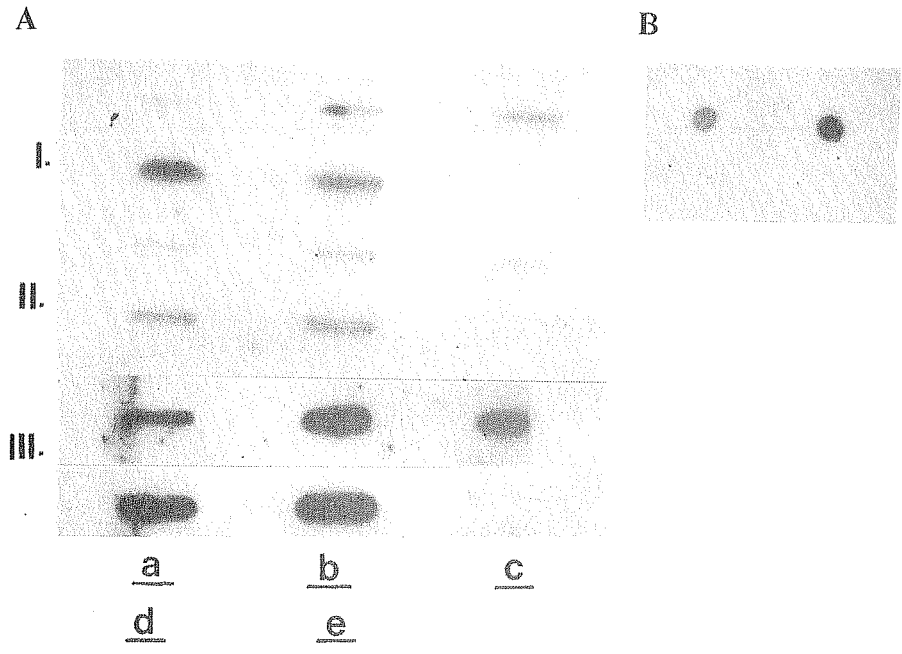

Figure 2. (A) Slot blot of total cellular RNA from (I) third instar larvae (II) Pupae and (III) H-33 cell line, spotted in five different concentrations a) 0·12 μg, b) 0·25 μg, c) 0·5 μg, d) 1·0 μg and e) 2·0 μg and probed with 6·1 kb fragment of D-yes 41. (**B**) Poly A^+ RNA from H-33 cell line spotted in two different concentrations (1·0 μg and 5·0 μg) and probed with pYS.

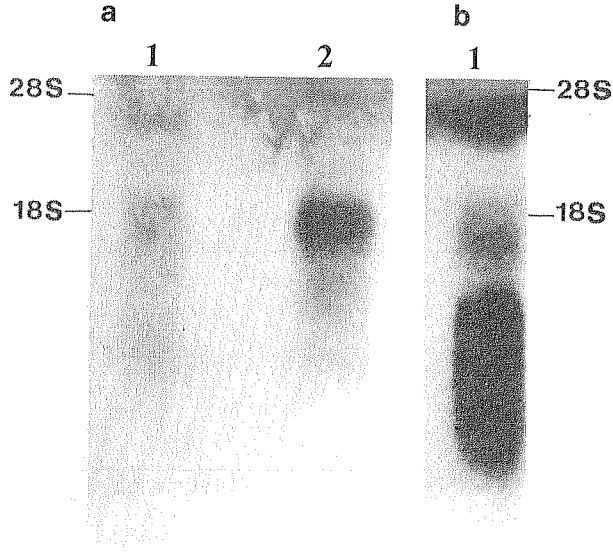

Figure 3. (**a**) Total RNA (10 μg) from H-33 cell line (lane 1) and the third instar stage (lane 2) of *Drosophila* electrophoresed on 1·4% agarose/formaldehyde gel and probed with pYS. (**b**) Total RNA (lane 1) from H-33 cell line electrophoresed on 1·4% agarose/formaldehyde gel and probed with 6·1 kb fragment of D-yes 41.

1991). The *Drosophila* homologue of the oncogene *int 1* is a segment polarity gene *Wingless*, while homologue of *int 2* is similar to the fibroblast growth factor. The *achaetescute* complex is homologous to the *myc* gene and the maternal effect gene *dorsal* shows 50% amino acid homology with oncogene *v-rel* protein (Tsonis 1988; Sudol *et al.* 1988). The oncogene homologues are thus closely related to genes involved in pattern formation, stress related responses and in normal development and differentiation (Shilo 1987; Marshall 1989; Bishop 1991).

In *Drosophila*, we have thus identified two distinct *yes* homologous genes, and three transcripts. *v-yes* DNA sequence has been shown to hybridize at three different locations in *Drosophila* salivary gland polytene chromosomes, 8D on X, 57B/C on 2R and 95-C/D on 3R (Samant *et al.* 1989). Previously 8D has been identified as a 'Lozenge' locus (Merriam 1984) while 95D and 57C have been shown to be associated with heat-shock protein hsp68 (Holmgren *et al.* 1979) and head specific RNA (Levy *et al.* 1982) respectively. Detailed analysis of the *yes* homologues in *D. melanogaster* would give fundamental information on structure and functional regulation of *yes* and other related genes.

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