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Detection and localization on *Drosophila melanogaster* polytene chromosomes of sequences homologous to oncogene *yes*

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Abstract. Sequences homologous to oncogene *yes* (Y73/Esh/sarcoma viral oncogene cDNA) in the *Drosophila melanogaster* Oregon genome were detected by *in situ* hybridization on salivary gland chromosomes. Three separate sites, 8D/X, 57BC/2R and 95CD/3R, were identified. Presence of sequences highly homologous to *yes* in the genomic DNA was confirmed by dot blot hybridization under high stringency conditions.

Keywords. Oncogene *yes*; *Drosophila melanogaster*; *in situ* hybridization.

1. Introduction

Oncogenes have been shown to be conserved across widely separated phylogenetic lines in normal cell genomes (Toyoshima *et al.* 1987). The cellular counterparts (proto-oncogenes) of viral oncogenes have been assumed to have a role to play in the normal growth of cells and organisms. A number of oncogenes have been cloned and their primary sequences have been determined, revealing relationships of oncogene products to molecules that function in normal cells, such as growth factors, growth factor receptors or hormone receptors, protein kinases and regulatory proteins (Bishop 1983, 1985; Heldin and Westermark 1984; Adamson 1987; Ohlsson and Pfeifer-Ohlsson 1987). Vertebrate oncogenes have been shown to have homologues with conserved sequences in organisms as distantly related as yeast and *Drosophila*, implying the vital role of oncogene products in cellular growth and differentiation (Shilo and Weinberg 1981). Oncogenes have been assigned roles in tissue-specific and developmental stage-specific processes (Adamson 1987; Ohlsson and Pfeifer-Ohlsson 1987; Tsonis 1988) in the vertebrates. In *Drosophila* the classical, biochemical and molecular-genetic approaches can be easily combined to study oncogenes. Proto-oncogenes that represent all the major families of vertebrate oncogenes (*src*, *ras* and *myc*/*myb*) have been found in *Drosophila* (Shilo 1987). At least five homologues of vertebrate oncogenes have been demonstrated to be developmentally regulated in *Drosophila* (Lev *et al.* 1984, 1985a, b; Katzen *et al.* 1985; Madhavan *et al.* 1985; Mozer *et al.* 1985; Simon *et al.* 1985; Telford *et al.* 1985; Petruzzelli *et al.* 1986; Kammermeyer and Wadsworth 1987). Expression of the *src* homologue has been shown to be tissue-specific and developmentally regulated (Lev *et al.* 1984; Simon *et al.* 1985). The *Drosophila* homologue of oncogene *int*-1 has been found to be identical with the segment polarity gene *wingless* (Rijsewijk *et al.* 1987; Cabrera *et al.* 1987), while the oncogene *myc* was found to have similarity with two of the genes of the achaete–scute (AS–C)

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gene complex of *Drosophila* (Villares and Cabrera 1987). At the protein level, the product of a maternal-effect gene, *dorsal*, of *Drosophila melanogaster* and the avian oncogene v-*rel* protein seem to have 50% amino acid sequence homology (Steward 1987), while another homoeotic gene of *Drosophila*, *sevenless*, has been found to encode a putative transmembrane receptor with a domain homologous to the tyrosine protein kinase specific to *src* and *ras* oncogenes (Hafen *et al.* 1987).

Oncogene *yes* (v-onc from avian sarcoma virus Y73) encodes a tyrosine protein kinase and has been included in the *src* family (Kawai *et al.* 1980; Kitamura *et al.* 1982). It has been shown to be associated with fibrosarcoma in chick (Kitamura *et al.* 1982) and is specifically expressed in chicken kidney (Shibuya *et al.* 1982). The viral (Y73) *yes* protein and the *src* product have 356 amino acids common between them (Kitamura *et al.* 1982) but, there is no hybridization at the nucleic acid level between the two gene sequences under optimum stringency conditions (Kawai *et al.* 1980; Yoshida *et al.* 1980). It was therefore of interest to search for a *yes*-homologous sequence in the *D. melanogaster* genome. Here we report the presence of DNA sequences homologous to v-*yes*, and localization of these sequences on *D. melanogaster* polytene chromosomes.

2. Materials and methods

2.1 *In situ hybridization*

In situ hybridization was carried out as per Neel *et al.* (1982).

Polytene chromosome spreads were prepared from third instar larval salivary glands of *D. melanogaster* Oregon (Atherton and Gall 1972). Clean glands were squashed in 45% acetic acid. Chromosome spreads were observed under a phase contrast microscope and good preparations were frozen either in liquid nitrogen or at −70°C to remove the siliconized coverslip off the slide. Squashes were then dehydrated by passing them through grades of ethanol and stored at 4°C until used.

Plasmid pYS (a gift from T. Yamamoto, Institute for Medical Sciences, University of Tokyo, Japan), which was used as probe, has a 1·5-kbp *Sin*1–*Sin*1 cDNA fragment of avian sarcoma virus Y73 corresponding to v-*yes* oncogene in the *Sal*I, site of pBR322 (Kitamura *et al.* 1982). Plasmid DNA (500 ng) was nick-translated using 20 μM [^{3}H]dGTP (37 Ci/mmol, Amersham, England) in a 25-μl reaction mixture containing 60 μM each of dATP, dCTP and dTTP, and 2·5 μl of DNA Pol I/DNase I enzyme mix (Bethesda Research Labs., USA) at 14°C for 2 h. Unpolymerized nucleoside triphosphates were removed from the labelled probe by gel filtration through a spun column of Sephadex G-50. Since only one labelled nucleoside triphosphate was used, the probe had specific activity of $7{\cdot}8 \times 10^5$ cpm/μg.

Chromosome preparations were treated with RNase A (100 μg/ml) in 2× saline sodium citrate (SSC) pH 7·0 at 37°C for 2 h, rinsed in 2× SSC at room temperature, and then dehydrated. Chromosomal DNA was denatured by treating the spreads with 70% formamide in 2× SSC at 70°C for 3–4 min followed by a wash in chilled 70% ethanol and dehydration. Hybridization mixture (50% formamide, 0·2% polyvinylpyrrolidone, 0·2% Ficoll, 0·2% BSA, 250 μg/ml denatured calf thymus DNA, 5% dextran sulphate and 500 ng/ml labelled probe) was kept at 75°C for 10 min and transferred quickly to ice to denature the probe. A 30 μl aliquot of this solution was applied to each slide and hybridization was carried out at 37°C for 20 h in a moist chamber saturated with 50% formamide/2× SSC. Post-

hybridization washes were in 50% formamide/2× SSC (v/v) twice at 37°C for 10 min each, followed by two washes in 2× SSC at 39°C for 10 min each and 4 to 5 washes in 2× SSC at room temperature for 5 min each. Preparations were then dehydrated, air-dried, and coated with Ilford L4 emulsion diluted 1:1 with 2% glycerol, 0·02% Triton X-100, and exposed for 1 to 2 months at 4°C in a light-proof box. Exposed slides were developed in D19 developer for 3 min at 13°C and fixed. Chromosomal preparations were stained with 1·5% lactoaceto-orcein. Spreads showing silver grains were photographed and the bands were identified by comparison with a cytogenetic map of *D. melanogaster* Oregon (Ashburner and Novitski 1976).

2.2 *DNA preparation and dot blot hybridization*

Plasmid pYS DNA was extracted by the alkaline lysis method of Birnboim and Doly (1979). Chromosomal DNA of *D. melanogaster* Oregon was extracted from first instar larvae hatched from eggs laid on agar smeared with 2% acetic acid. This eliminated contamination by yeast DNA. The DNA extraction method used was a modified form of the one described by Blin and Stafford (1976). DNAs were blotted onto Biodyne A membrane (Pall Ultrafine Filtration Corp., USA) in varying concentrations, denatured using 0·5 N NaOH/1·5 M NaCl for 5 min, and neutralized. Blots were exposed to UV light (310 nm) for 3 min on either side. Mouse DNA was used as a positive control. Plasmid pYS was nick-translated according to the protocol of Rigby *et al.* (1977) to a specific activity of 2×10^7 cpm/μg using α-[^{32}P]dCTP. Two different hybridization conditions were used: (i) 5× Denhardt's solution, 5× SSC, 50 mM sodium phosphate (pH 7·0), 0·1% SDS and 50% formamide at 42°C, and (ii) 5× Denhardt's solution, 5× SSC, 10% dextran sulphate, 1% BSA, 5 mM EDTA, 65 mM sodium phosphate (pH 7·0), 0·1% SDS and 35% formamide at 40°C. Hybridization was carried out for 24 h. Blots were washed using 2× SSC, 25 mM sodium phosphate (pH 7·0), 1 mM EDTA, 0·1% SDS at 68°C for 90 min or 0·2× SSC, 0·1% SDS for 30 min at 55°C. Blots were then exposed to X-ray film (Kodak X-OMAT) with intensifying screens for 1 day at −20°C.

3. Results and discussion

Avian virus Y73-specific v-*yes* DNA sequence hybridized at three locations on salivary gland polytene chromosomes of *D. melanogaster*. These sites were located on three different chromosomes: 8D on X, 57B/C on 2R and 95C/D on 3R (figure 1). Previously 8D has been identified as the 'lozenge' locus (Merriam 1984) while 95D and 57C have been shown to be associated with the heat-shock protein hsp 68 (Holmgren *et al.* 1979) and head-specific RNA (Levy *et al.* 1982) respectively. Analysis of DNA from human × mouse hybrid cells has shown that there are multiple copies of v-*yes*-related genes in the human genome: c-*yes*-1, the prototype of *yes*, has been assigned to chromosome 18, and c-*yes*-2, presumably a pseudogene, to chromosome 6 (Semba *et al.* 1985). The human proto-*yes* gene (c-*yes*-1) has been mapped at 18q21 by *in situ* hybridization (Yoshida *et al.* 1985).

The transforming gene product of v-*yes* has a protein kinase activity that phosphorylates tyrosine on target proteins (Kawai *et al.* 1980; Kornbluth *et al.* 1987)—a common feature of the products of all *src*-group oncogenes. This protein

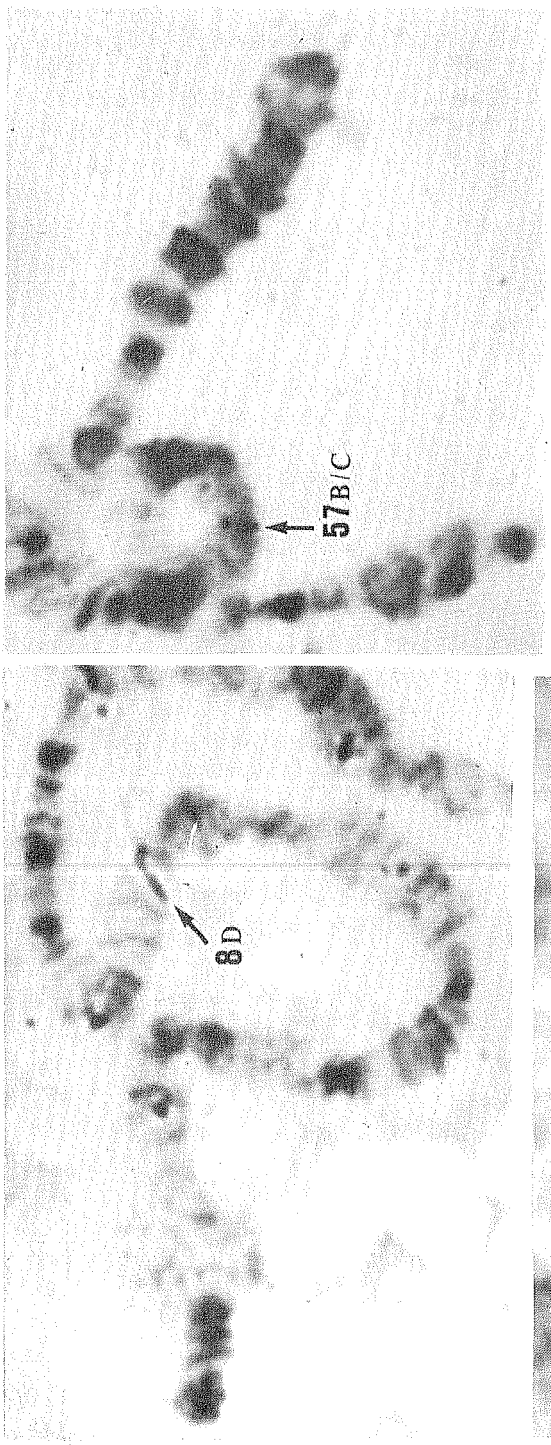

Figure 1. *In situ* localization of *v-yes* on salivary gland polytene chromosomes of *D. melanogaster.*

is associated with the inner surface of the plasma membrane in chick embryo fibroblasts (Sefton 1986). It is not yet known why there should be different genes coding for proteins that have similarity in structure and function and are conserved and expressed in normal cells. For instance, cellular homologues of v-*src* are found in salmon, chicken, and mammals like mouse, calf and man, suggesting some important function for this gene, as for other oncogenes (Spector *et al.* 1978). However, whether different substrates exist in specific tissues for the enzymes coded by these genes, and hence the multiplicity of such genes, is not yet clear. There is a possibility that these genes are regulated differentially in various tissues for multiple, changing functions. The c-*fps* and c-*abl* genes are expressed predominantly in lymphoid cells (Shibuya *et al.* 1982) and in the testes (Muller *et al.* 1982) respectively, while expression of both c-*src* and c-*abl* is carefully regulated during *Drosophila* embryogenesis (Lev *et al.* 1984). It is also possible that divergence in the tyrosine kinase gene family gave rise to enzymes with similar substrate specificities but the activities of these enzymes are regulated by different mechanisms. The cytoplasmic catalytic domain of the epidermal growth factor (EGF) receptor shows protein kinase activity only after binding of EGF to the extracellular amino-terminal domain, whereas $p60^{c\text{-}src}$ exhibits apparently constitutive tyrosine kinase activity and possesses no identified regulatory domain (Sefton 1986). The search for *Drosophila* homologues of these oncogenes is important because the possible roles of these genes in *Drosophila* development can be studied. In *Drosophila* it has been observed that proto-oncogenes like D-*src* are expressed in actively dividing cells like those of imaginal discs as well as in non-dividing, terminally differentiated cells like those of nervous tissue (Lev *et al.* 1984; Simon *et al.* 1985; Shilo 1987). It is possible that proto-oncogenes play an important role not only in cell proliferation but also in differentiation. The gene c-*yes* is known to be expressed in most avian tissues at a level significantly higher than that of c-*src* (Shibuya *et al.* 1982). Transcripts of c-*yes* are unusually abundant in chicken kidney. It will be interesting to see tissue-specific expression, if any, of *yes*-homologous sequences in *Drosophila* with reference to the Malpighian tubules. Transcripts of c-*yes*-1, the human *yes* gene, have been detected in various human malignant cells. Of these, epidermoid carcinoma A431 cells, which expressed a high number of receptors for EGF, showed a high level of transcription of c-*yes*-1 (Semba *et al.* 1985). Another member of the *src* gene family, v-*erb B*, is known to be related to the EGF receptor gene (Ullrich *et al.* 1984). It has been also suggested that the *yes* gene product might contribute to receptor-associated cell proliferation (Yoshida *et al.* 1985).

Significant hybridization was observed between v-*yes* and *D. melanogaster* genomic DNA sequences at 35% as well as 50% formamide concentrations and was found to be quite stable even after subsequent washing at higher stringency conditions (55°C, 0·2× SSC, 0·1% SDS) (figure 2). When Shilo and Weinberg (1981) detected *Drosophila* DNA homologues to vertebrate oncogenes for the first time, they had used 35% formamide to favour formation and stabilization of the less stable hybrids. Also the washing conditions used in their protocol were less stringent (2× SSC at 68°C) compared to conditions described here. At the DNA level, the *yes* transforming gene is distinct from the *src* gene, the longest homologous stretch being of only 17 nucleotides, but at the protein level the homology is 82% (Kitamura *et al.* 1982). Most of the codons are different at the third position and therefore suggest regulation of expression partly also by relative

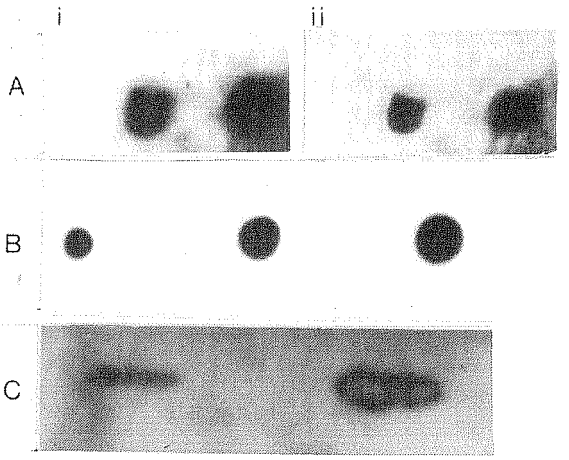

Figure 2. DNA dot blot hybridizations with plasmid pYS. **A,** 300 and 600 ng of *D. melanogaster* larval DNA were hybridized in 35% formamide and blots were washed for 30 min at (i) lower stringency in 2× SSC, 25 mM sodium phosphate (pH 7·0), 1 mM EDTA, 0·1% SDS at 68°C, and (ii) at higher stringency in 0·2× SSC, 0·1% SDS at 55°C. **B,** Mouse liver DNA; hybridization in 50% formamide and blot was washed at 42°C in 0·2× SSC, 0·1% SDS for 30 min. **C,** *D. melanogaster* larval DNA; hybridization was in 50% formamide and blot was washed at 55°C in 0·2× SSC, 0·1% SDS for 30 min.

abundance of specific tRNA isoacceptors. The hybridization detected on *Drosophila* chromosomes using the v-*yes* probe is thus not due to *src*-homologous sequences. Further, D-*src* in *D. melanogaster* has been located at 29A/2L and 64B/3L (Hoffman–Falk *et al.* 1983; Simon *et al.* 1983), which are different from the locations reported for *yes* here. It will therefore be of interest to clone *yes*-homologous DNA sequences from *D. melanogaster* to study their structural and regulatory features, as well as tissue-specific and developmental stage-specific expression.

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References

Adamson E. D. 1987 Oncogenes in development. *Development* 99: 449–471

Ashburner M. and Novitski E. 1976 *The genetics and biology of Drosophila* (London and New York: Academic Press) vol. 1a

Atherton D. and Gall J. G. 1972 Salivary gland squashes for *in situ* nucleic acid hybridization studies. *Drosoph. Inf. Serv.* 49: 131–133

Birnboim H. C. and Doly J. 1979 A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7: 1513–1523

Bishop J. M. 1983 Cellular oncogenes and retroviruses. *Annu. Rev. Biochem.* 52: 301–354

Bishop J. M. 1985 Viral oncogenes. *Cell* 42: 23–38

Blin N. and Stafford D. W. 1976 A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res.* 3: 2303–2308

Cabrera C. V., Alonso M. C., Jonston P., Phillips R. G. and Lawrence P. A. 1987 Phenocopies induced with antisense RNA identify the *wingless* gene. *Cell* 50: 659–663

Hafen E., Besler K., Edstroem J.-E. and Rubin G. M. 1987 *sevenless*, A cell-specific homeotic gene of *Drosophila*, encodes a putative transmembrane receptor with a tyrosine kinase domain. *Science* 236: 55–63

Heldin C. H. and Westermark B. 1984 Growth factors: mechanism of action and relation to oncogenes. *Cell* 37: 9–20

Hoffman–Falk H., Einat P., Shilo B.-Z. and Hoffman F. M. 1983 *Drosophila melanogaster* DNA clones homologous to vertebrate oncogenes: Evidence for a common ancestor to the *src* and *abl* cellular genes. *Cell* 32: 589–598

Holmgren R., Livak K., Morimoto R., Freund R. and Meselson M. 1979 Studies of cloned sequences from four *Drosophila* heat shock loci. *Cell* 18: 1359–1370

Kammermeyer K. L. and Wadsworth S. C. 1987 Expression of *Drosophila* epidermal growth factor receptor homologue in mitotic cell populations. *Development* 100: 201–210

Katzen A. L., Kornberg T. B. and Bishop J. M. 1985 Isolation of the proto-oncogene c-*myb* from *Drosophila melanogaster*. *Cell* 41: 449–456

Kawai S., Yoshida M., Segawa K., Sugiyama H., Ishizaki R. and Toyoshima K. 1980 Characterization of Y73, an avian sarcoma virus: a unique transforming gene and its product, a phosphopolyprotein with protein kinase activity. *Proc. Natl. Acad. Sci. USA* 77: 6199–6203

Kitamura N., Kitamura A., Toyoshima K., Hirayama Y. and Yoshida M. 1982 Avian sarcoma virus Y73 genome sequence and structural similarity of its transforming gene product to that of Rous sarcoma virus. *Nature* (*London*) 297: 205–208

Kornbluth S., Sudol M. and Hanafusa H. 1987 Association of the polyoma virus middle-T antigen with c-*yes* protein. *Nature* (*London*) 325: 171–173

Lev Z., Kimchie Z., Hessel R. and Segev O. 1985a Expression of *ras* cellular oncogenes during development of *Drosophila melanogaster*. *Mol. Cell. Biol.* 5: 1540–1542

Lev Z., Leibovitz N., Segev O. and Shilo B.-Z. 1984 Expression of the *src* and *abl* cellular oncogenes during development of *Drosophila melanogaster*. *Mol. Cell. Biol.* 4: 982–984

Lev Z., Shilo B.-Z. and Kimchie Z. 1985b Developmental changes in expression of the *Drosophila melanogaster* epidermal growth factor receptor gene. *Dev. Biol.* 110: 499–502

Levy L. S., Ganguly R., Ganguly N. and Manning J. E. 1982 The selection, expression and organization of a set of head-specific genes in *Drosophila*. *Dev. Biol.* 94: 451–464

Madhavan K., Bilodeau-Wentworth D. and Wadsworth S. C. 1985 A family of developmentally regulated, maternally expressed *Drosophila* RNA species detected by a v-*myc* probe. *Mol. Cell. Biol.* 5: 7–16

Merriam J. 1984 *Drosophila* genetic map. In *Genetic maps 1984* (ed.) S. J. O'Brien (Cold Spring Harbor: Cold Spring Harbor Laboratory) vol. 3, pp. 304–308

Mozer B., Marlor R., Parkhurst S. and Corces V. 1985 Characterization and developmental expression of a *Drosophila ras* oncogene. *Mol. Cell. Biol.* 5: 885–889

Muller R., Slamon D. J., Tremblay J. M., Cline M. J. and Verma I. M. 1982 Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. *Nature* (*London*) 299: 640–644

Neel B. G., Jhanwar S. C., Chaganti R. S. K. and Hayward W. S. 1982 Two human c-*onc* genes are located on the long arm of chromosome 8. *Proc. Natl. Acad. Sci. USA* 79: 7842–7846

Ohlsson R. I. and Pfeifer-Ohlsson S. B. 1987 Cancer genes, proto-oncogenes and development. *Exp. Cell Res.* 173: 1–16

Petruzzelli L., Herrera R., Arenas-Garcia R., Fernandez R., Birnbaum M. J. and Rosen O. M. 1986 Isolation of a *Drosophila* genomic sequence homologous to the kinase domain of the human insulin receptor and detection of the phosphorylated *Drosophila* receptor with an anti-peptide antibody. *Proc. Natl. Acad. Sci. USA* 83: 4710–4714

Rigby P. W. J., Dieckmann M., Rhodes C. and Berg P. 1977 Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113: 237–258

Rijsewijk F., Schuermann M., Wagenaar E., Parren P., Weigel D. and Nusse R. 1987 The *Drosophila* homologue of the mouse mammary oncogene *int*-1 is identical to the segment polarity gene *wingless*. *Cell* 50: 649–657

Sefton B. M. 1986 The viral tyrosine protein kinases. In *Retroviruses 4* (eds) P. K. Vogt and H. Koprowski, *Current Topics in Microbiology and Immunology* (Berlin: Springer-Verlag) vol. 123, pp. 39–72

Semba K., Yamanashi Y., Nishizawa M., Sukegawa J., Yoshida M., Sasaki M., Yamamoto T. and Toyoshima K. 1985 Location of the c-*yes* gene on the human chromosome and its expression in various tissues. *Science* 227: 1038–1040

Shibuya M., Hanafusa H. and Balduzzi P. C. 1982 Cellular sequences related to three new *onc* genes of avian sarcoma virus (*fps*, *yes* and *ros*) and their expression in normal and transformed cells. *J. Virol.* 42: 143–152

Shilo B.-Z. 1987 Proto-oncogenes in *Drosophila melanogaster*. *Trends Genet.* 3: 69–72

Shilo B.-Z. and Weinberg R. A. 1981 DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 78: 6789–6792

Simon M. A., Dress B., Kornberg T. B. and Bishop J. M., 1985 The nucleotide sequence and the tissue-specific expression of *Drosophila* c-*src*. *Cell* 42: 831–840

Simon M. A., Kornberg T. B. and Bishop J. M. 1983 Three loci related to the *src* oncogene and tyrosine-specific protein kinase activity in *Drosophila*. *Nature* (*London*) 302: 837–839

Spector D. H., Varmus H. E. and Bishop J. M. 1978 Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates. *Proc. Natl. Acad. Sci. USA* 75: 4102–4106

Steward R. 1987 *Dorsal*, an embryonic polarity gene in *Drosophila*, is homologous to the vertebrate proto-oncogene c-*rel*. *Science* 238: 692–694

Telford J., Burckhardt J., Butler B. and Pirrotta V. 1985 Alternative processing and developmental control of the transcripts of the *Drosophila abl* oncogene homologue. *EMBO J.* 4: 2609–2615

Toyoshima K., Yamamoto T., Kawai S. and Yoshida M. 1987 Viral oncogenes v-*yes* and v-*erb B* and their cellular counterparts. *Adv. Virus Res.* 32: 97–127

Tsonis P. A. 1988 Oncogenes take a place in pattern formation. *Trends. Biochem. Sci.* 13: 4–5

Ullrich A., Coussens L., Hayflick J. S., Dull T. J., Gray A., Tam A. W., Lee J., Yarden Y., Libermann T. A., Schlessinger J., Downward J., Mayes E. L. V., Whittle N., Waterfield M. D. and Seeburg P. H. 1984 Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermal carcinoma cells. *Nature* (*London*) 309: 418–425

Villares R. and Cabrera C. V. 1987 The *achaete–scute* gene complex of *Drosophila melanogaster*: conserved domains in a subset of genes required for neurogenesis and their homology to *myc*. *Cell* 50: 415–424

Yoshida M., Kawai S. and Toyoshima K. 1980 Uninfected avian cells contain structurally unrelated progenitors of viral sarcoma genes. *Nature* (*London*) 287: 653–654

Yoshida M. C., Sasaki M., Mise K., Semba K., Nishizawa M., Yamamoto T. and Toyoshima K. 1985 Regional mapping of the human proto-oncogene c-*yes*-1 to chromosome 18 at band q21·3. *Jpn. J. Cancer Res.* (*GANN*) 76: 559–562