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Proliferation of both somatic and germ cells is affected in the *Drosophila* mutants of *raf* proto-oncogene

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The genomic and cDNA fragments of *Drosophila melanogaster*, homologous to human c-*raf-1*, were cloned. The nucleotide sequence predicted the primary structure of a polypeptide of 666 amino acid residues with a highly conserved Ser-Thr kinase domain on its carboxy terminal half. *Draf-1* was mapped to the 2F region of the X chromosome. Two newly induced recessive lethals belonging to a complementation group in this region were identified to be defective in *Draf-1* by P element-mediated rescue experiments. The mutants die at larval/pupal stages. The mutant larvae are apparently normal, but they harbor serious defects in the organs containing proliferating cells of both somatic and germ line origins. Maternal effects on embryogenesis indicated that *Draf-1* is also required in early larval development.

Key words: raf oncogene/*Drosophila* mutant/Ser-Thr kinase/proliferation/recessive lethal

Introduction

An increasing number of oncogenes have been identified with their dominant characters. However, to understand the normal functions of these proto-oncogenes, it is important to analyze their recessive characters. For this *Drosophila melanogaster* is one of the most useful organisms. A number of genes homologous to the vertebrate oncogenes have been identified in this species (Shilo, 1987).

Cell cycle-dependent phosphorylations of many regulatory and structural components of cell division have been observed in many vertebrate cells (e.g. Inglis *et al.*, 1976; Gerace and Blobel, 1980; Halleck *et al.*, 1984). These observations suggest an importance of the phosphorylation reactions in cell proliferation. To elucidate the roles of protein kinases in the regulation of proliferation, we have started a genetic analysis on the *raf* proto-oncogene in *Drosophila*.

The *raf* oncogene was first identified in the murine sarcoma virus 3611 (3611-MSV) (Rapp *et al.*, 1983) and its avian homolog, *mil*, has been found in the avian carcinoma virus MH2 (Jansen *et al.*, 1983). Activated human *raf* genes have also been found in a stomach cancer (Shimizu *et al.*, 1985) and in a glioblastoma line (Fukui *et al.*, 1985). The viral oncogenes encode *gag-raf* and *gag-mil* fusion proteins which carry protein kinase activities specific to serine and threonine residues (Moelling *et al.*, 1984). Structures of the activated *raf/mil* oncogenes suggest an involvement of the kinase activity in tumor formation (Mölders *et al.*, 1985; Bonner *et al.*, 1986; Nakatsu *et al.*, 1986; Dozier *et al.*, 1987; Fukui *et al.*, 1987). However, little is known about the function of the *raf* proto-oncogene.

Recently, cloning of two *Drosophila* genes homologous to the human c-*raf-1* gene has been reported (Mark *et al.*, 1987). However, their results including the partial nucleotide sequence of one of them, *Draf-1*, are far from our understanding of the structure and function of the genes. Here we describe the complete nucleotide sequence of *Draf-1* and isolation of the mutants defective in this gene. The mutant phenotypes revealed an involvement of the gene in proliferation.

Results

Cloning and nucleotide sequencing of Draf-1

A 1.7 kb insert of pTC1171, a cDNA clone containing the activated human *raf* oncogene (Nakatsu *et al.*, 1986), detected single bands on the Southern blots of *Drosophila* DNA. The fragment was used to isolate its *Drosophila* homolog, *Draf-1*. A genomic clone, Ch28-*Draf-1*, with an insert of ~17 kb long was obtained and its 0.9 kb *Pst*I fragment (fragment b in Figure 1) hybridized to the human probe. This fragment was employed to screen a cDNA library. The restriction maps of the genomic and cDNA fragments are summarized in Figure 1. The cDNA fragment from the

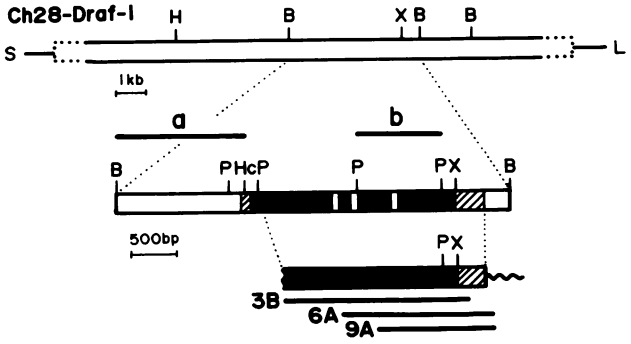

Fig. 1. Restriction maps of the genomic and cDNA fragments of *Draf-1*. The restriction map of the genomic clones, CH28-*Draf-1*, is shown at the top. S and L indicate the short and long arms, respectively, of the λ phage vector. A portion of the genomic fragment is enlarged in the middle and the fragments used for hybridization were indicated with bars, a and b. Three of the overlapping cDNA clones are indicated below the restriction map of the cDNA fragment. Coding sequences are filled and the introns are indicated by open boxes. The 5′ and 3′ untranslated regions are hatched. The direction of transcription is from left to right. Restriction sites: B, *Bam*HI; H, *Hin*dIII; Hc, *Hin*cII; P, *Pst*I; X, *Xba*I.

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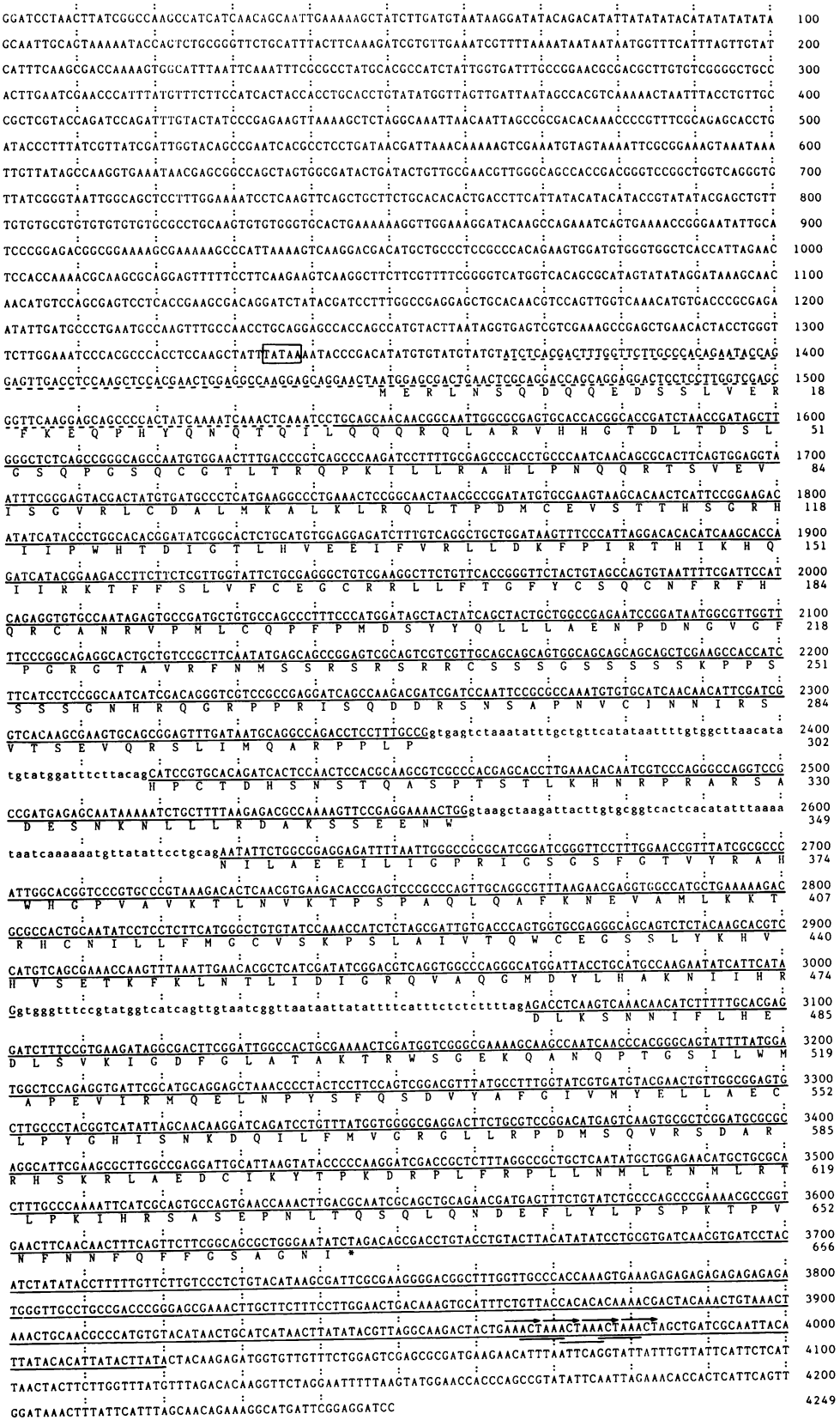

Fig. 2. The nucleotide and amino acid sequences of *Draf-1*. The nucleotide sequences were determined by the chain termination method (Sanger *et al.*, 1977). The complete nucleotide sequence of the genomic 4.3 kb *Bam*HI fragment is shown. The sequences also seen in the cDNA fragments are underlined. The intron sequences are indicated by lower case letters. The putative TATA box sequence is boxed and the presumptive 5′ region of the transcript is underlined with broken lines. Arrows indicate the tandem repeats of a pentamer and the possible poly(A) addition signals are underlined. The amino acid sequence predicted from the nucleotide sequence is shown by one-letter code below the nucleotide sequence.

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MERLNSQDQQ EDSSLVERFK EQPH---YQN QTQILQQQRQ LARVHHGTDL TD-SLGSQPG SQCGTLTRQP KILLRAHLPN QQRTSVEVIS GVRLCDALMK  96
MEH--IQGAW KTISNGFGFK DAVFDGSSCI SPTIVQQFGY QRRASDDGKL TDPSKTSN-- ---------- --TIRVFLPN KQRTVVNVRN GMSLHDCLMK  84

ALKLRQLTPD MCEVSTTHSG ----RHIIPW HTDIGTLHVE EIFVRLLDKF PIRTHIKHQI IRKTFFSLVF CEGCRRLLFT GFYCSQCNFR FHQRCANRVP 192
ALKVRGLQPE CCAVFRLLHE HKGKKARLDW NTDAASLIGE ELQVDFLEHV PL---TTHNF ARKTFLKLAF CEICQKFLLN GFRCQTCGYK FHEHCSTKVP 181

MLCQPFPMDS YYQLLLAENP DNGVGFPGRG TAVRFNMSSR SRSRRCSSSG SSSSSKPPSS SSGNHRQGRP PRISQDDRSN SAPNVCINNI RSVTSEVQRS 292
TMCVDWSNIR --QLLLFPNS TIGDSGVPAL PSLTMRRMRE SVSRMPVSSQ HRYSTPHAFT FNTSSPSSEG S-LSQRQRST STPNVHMVST TLPVDSRMIE 278

LIMQARPPLP HPCTDHSNST QASPTSTLKH ------NRPR AR-SA-DESN KNLLLRDAKS SEENWNILAE EILIGPRIGS GSFGTVYRAH WHGPVAVKTL 384
DAIRSHSESA SPSALSSSPN NLSPTGWSQP KTPVPAQRER APVSGTQEKN KIRPRGQRDS SYY-WEIEAS EVMLSTRIGS GSFGTVYKGK WHGDVAVKIL 377

NVKTPSPAQL QAFKNEVAML KKTRHCNILL FMGCVSKPSL AIVTQWCEGS SLYKHVHVSE TKFKLNTLID IGRQVAQGMD YLHAKNIIHR DLKSNNIFLH 484
KVVDPTPEQF QAFRNEVAVL RKTRHVNILL FMGYMTKDNL AIVTQWCEGS SLYKHLHVQE TKFQMFQLID IARQTAQGMD YLHAKNIIHR DMKSNNIFLH 477

EDLSVKIGDF GLATAKTRWS GEKQANQPTG SILWMAPEVI RMQELNPYSF QSDVYAFGIV MYELLAECLP YGHISNKDQI LFMVGRGLLR PDMSQVRSDA 584
EGLTVKIGDF GLATVKSRWS GSQQVEQPTG SVLWMAPEVI RMQDNNPFSF QSDVYSYGIV LYELMTGELP YSHINNRDQI IFMVGRGYAS PDLSKLYKNC 577

RRHSKRLAED CIKYTPKDRP LFRPLLNMLE NMLRTLPKIH RSASEPNLTQ SQLQNDEFLY -L-PSPKTPV NFNNFQFFGS AGNI.....Draf          666
PKAMKRLVAD CVKKVKEERP LFPQILSSIE LLQHSLPKIN RTASEPSLHR AAHTEDINAC TLTTSPRLPV -F.................h-c-raf-1    648
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Fig. 3. Comparison of *Draf-1* with h-c-*raf-1*. The deduced amino acid sequence of *Draf-1* is compared to that of h-c-*raf-1* (Bonner *et al.*, 1986). The upper and lower sequences represent *Draf-1* and h-c-*raf-1*, respectively. A minimum number of insertions (indicated by dashes) were introduced to optimize the similarity. Identical amino acids are indicated by asterisks. The highly conserved protein kinase domains start at positions indicated with arrowheads. The conserved cysteine and histidine residues in the cysteine-rich region are indicated with dots.

clone 3B showed a single strongly hybridizing band and several very faint bands on the Southern blot under low stringency hybridization conditions. The result indicates that *Draf-1* is unique in the *Drosophila* genome and suggests the presence of several distantly related genes. *Draf-2* described by Mark *et al.* (1987) may be one of them.

The complete nucleotide sequences of the genomic 4.3 kb *Bam*HI fragment and the cDNA fragments were determined (Figure 2). Comparison of the sequences revealed the presence of three short introns of 64, 68 and 65 bp. The nucleotide sequences at the splicing junctions agree well with the consensus sequence for splicing sites (Mount, 1982). The poly(A) tail starts at nucleotide 4020 or 4021. Around 55 bp upstream to this point, four tandem repeats of a pentamer, AAACT, were found and they produce three overlapping repeats of a hexamer, ACTAAA which would be the signal for poly(A) addition. A TATAA sequence was found at nucleotide 1334 in the genomic sequence, and we have tentatively identified the transcription start site around nucleotide 1365, that is 25 bp downstream from the putative TATA box. The length of the putative transcription unit after removal of introns is ~2.6 kb, and it agrees well with the actual mRNA size of 2.9 kb (see below) if we assume that a poly(A) tail of ~300 bp is added.

Based on this assumption, the first ATG codon is located at nucleotide 1449 followed by a long open reading frame specifying 666 amino acid residues. The sequence predicted a primary structure of polypeptide with a mol. wt of 75 513. The entire *Draf-1* gene would be ~4 kb long or less and has only three short introns. These features are in contrast to those of h-c-*raf-1* which is composed of 17 exons encompassing >50 kb (Bonner *et al.*, 1986).

We concluded that the 4.3 kb *Bam*HI fragment does not contain other transcription units, since the 1.4 kb *Bam*HI–*Hin*cII fragment (fragment a in Figure 1) did not show any significant hybridization by our Northern blot analysis. The sequence does not contain any possible genes for the small RNA species such as tRNAs and snRNAs. The results indicate that the fragment contains only *Draf-1*.

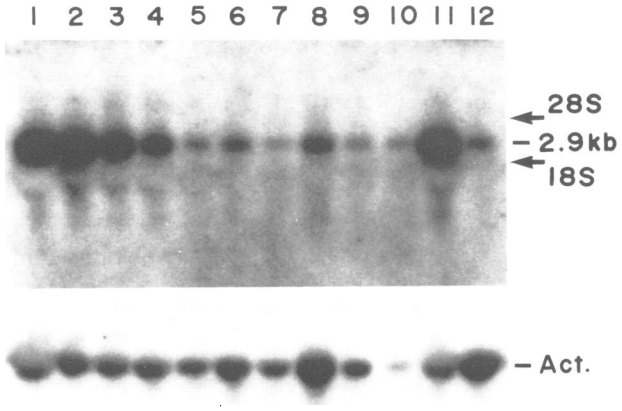

Fig. 4. Expression of *Draf-1* during *Drosophila* development. Poly(A)$^+$ RNAs were prepared from the materials at various developmental stages. 5 μg of poly(A)$^+$ RNAs were applied in each lane except lane 12, in which 15 μg was applied. The human placental RNA was used as a size marker. The 4.3 kb *Bam*HI fragment was used as a probe. The filter was rehybridized with a cytoplasmic actin probe, Act42A (Fyrberg *et al.*, 1983), to ascertain that no significant degradation of RNA has occurred. Each lane contains poly(A)$^+$ RNA extracted from: (1) unfertilized eggs, (2) 0- to 4-h-old embryos, (3) 4- to 10-h-old embryos, (4) 10- to 22-h-old embryos, (5) 1st instar larvae, (6) 2nd instar larvae, (7) 3rd instar larvae, (8) 2- to 3-day-old pupae, (9) adult males, (10) adult females, (11) fully grown ovaries, and (12) 3rd instar larvae hemizygous or homozygous for *l(1)raf*^{1}.

Comparison to human c-raf-1

The predicted amino acid sequence was compared to that of h-c-*raf-1* (Bonner *et al.*, 1986). As shown in Figure 3, a considerable similarity was found over the entire region (45% positional identity in the alignment). The highest similarity (65% positional identity) was found in the carboxy-terminal halves (from residue 361 to the end) which correspond to the protein kinase domain. This implies that *Draf-1* carries a protein kinase activity specific to serine and threonine residues.

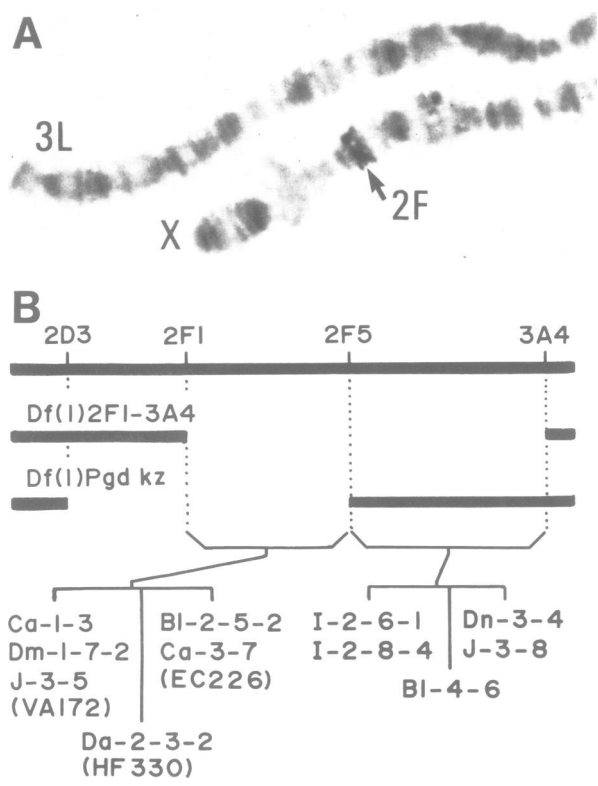

Fig. 5. Mapping of *Draf-1* and genetic analysis of the 2F region. (**A**) The biotinylated plasmid DNA containing the insert of the cDNA clone 3B (Figure 1) was hybridized to the squash of salivary gland chromosomes. Hybridization was visualized by staining with alkaline phosphatase. The arrow indicates the hybridizing band in the 2F region. (**B**) The chromosomal segment around the 2F region is schematically represented. The positions and the ranges of deficiencies in *Df(1)2F1-3A4* and *Df(1)Pgd kz* are indicated. Out of 213 recessive lethals, 11 were mapped between 2F and 3A4 by testing the lethality of *lethal/Df(1)2F1-3A4* heterozygotes. They were classified into six complementation groups. Three of the complementation groups were identified between 2F5 and 3A4 by testing the viability of *lethal/Df(1)Pgd kz*. Each of the remaining three complementation groups mapped between 2F1 and 2F5 was found to be allelic to VA172, HF330 or EC226 (Lefevre, 1981; Perrimon *et al.*, 1984).

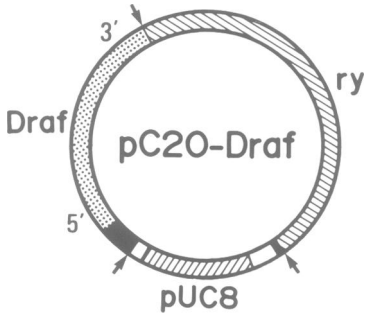

Fig. 6. The construct of pC20-Draf. The genomic 4.3 kb *Bam*HI fragment was cloned into the *Sal*I site of pCarnegie 20 after blunt end formation. The arrows indicate the *Hin*dII sites and the cleavage at these sites produces three fragments containing *Draf-1* (dotted), *ry* (hatched) and pUC8 (hatched) sequences. The split P element sequences are indicated by the filled boxes.

Similarity is rather lower in the amino-terminal halves (29% positional identity), but significant similarities were found at several blocks: residues 71–110, 150–195, 266–277 and 323–355. These sequences may represent functionally important domains. The characteristic cysteine-rich sequence conserved in the protein kinase C, c-*raf-1* and *A-raf* (Knopf *et al.*, 1986; Parker *et al.*, 1986; Ishikawa *et al.*, 1986) is also conserved in *Draf-1*. The results indicate that h-c-*raf-1* and *Draf-1* have originated from a common ancestral gene and their basic structural features are conserved.

Table I. Suppression of the lethal mutations by the transfected P[*Draf*$^+$; *ry*$^+$]

	+/Y; P[*Draf*$^+$; *ry*$^+$]/– (wild-type eye color)				+/Y; –/– (*ry* eye color)			
	l/+	*FM7c*/+	*l*/Y	*FM7c*/Y	*l*/+	*FM7c*/+	*l*/Y	*FM7c*/Y
I-2-6-1/*FM7c*	337	273	119	163	389	326	3[a]	207
I-2-8-4/*FM7c*	356	357	127	222	252	238	1[a]	189
Bl-4-6/*FM7c*	356	344	1[a]	181	318	279	1[a]	262
J-3-8/*FM7c*	402	369	2[a]	278	323	289	1[a]	178
Dn-3-4/*FM7c*	111	105	0	54		(ND)[b]		

[a]Sterile. They are probably X/O males due to non-disjunction.
[b]Not done.

Draf-1 gene expression

The 4.3 kb *Bam*HI fragment detected a single band of 2.9 kb on the Northern blots of RNAs from various developmental stages (Figure 4). The amount of the mRNA is relatively high during the first 4 h of embryonic development, and lower levels of expression were observed all through the remaining developmental stages. Abundance of the *Draf-1* gene transcripts in the adult ovary suggests transfer of the maternal mRNA into the ooplasm. In fact, the transcripts are accumulated in the unfertilized eggs laid by virgin females. Thus, the transcripts in the early embryonic stages are mainly contributed by the maternal gene expression.

Mapping of the Draf-1 gene

In situ hybridization on the salivary gland chromosomes located the *Draf-1* gene in the 2F region, near the tip of the X chromosome (Figure 5A). A Southern hybridization analysis revealed that *Draf-1* is deleted in *Df(1)2F1-3A4* but not in *Df(1)Pgd kz* (the locations and the ranges of the deletions are shown in Figure 5B) (data not shown). Therefore, *Draf-1* lies between 2F5 and 3A4.

Screening Draf-1 mutants as recessive lethals

As *Draf-1* is unique and highly conserved, we expected that its defect might be lethal. A series of X-linked recessive lethal mutations have been induced with methylnitrosourea (MNU) or ethylnitrosourea (ENU) (Ayaki and Yoshikawa, 1986). Out of 213 stocks examined, 11 mutations classified into six complementation groups were mapped to the region between 2F1 and 3A4 (Figure 5B). Three of the complementation groups were further identified in the region between 2F5 and 3A4, where *Draf-1* has been mapped.

Identification of the mutants by P element-mediated gene transfer

The 4.3 kb *Bam*HI fragment was cloned into pCarnegie 20 (Rubin and Spradling, 1983) to make a construct of pC20-*Draf* (Figure 6). The plasmid DNA was injected into the *ry*^{506} embryos together with pπ25.7wc as a helper (Rubin and Spradling, 1983), and the transformants were

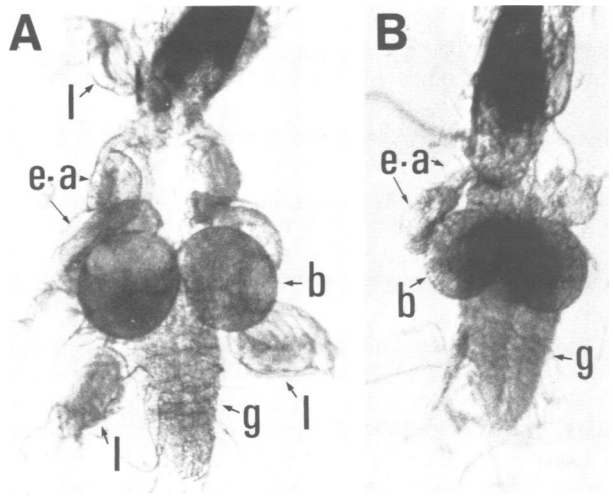

Fig. 7. Imaginal disc abnormalities in the mutant. Imaginal disc-ganglion complexes are dissected out of normal (**A**) and mutant (**B**) larvae. In the mutant larvae, under-developed eye-antenna discs were observed but other major imaginal discs could not be detected. Abbreviations: b, brain lobe; e.a, eye-antenna disc; g, ventral ganglion; l, leg disc.

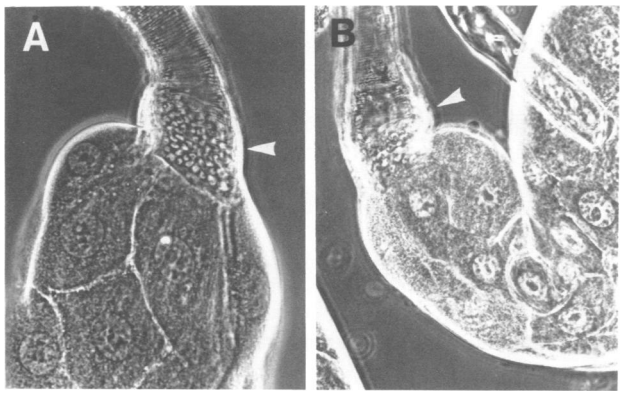

Fig. 8. Imaginal salivary gland cells in the mutant. Salivary glands from normal (**A**) and mutant (**B**) mature larvae were dissected and visualized by phase-contrast optics. Focusing at various depths revealed that imaginal rings (arrowheads) in normal larvae are filled with imaginal salivary gland cells, while in the mutants, they are populated by a smaller number of cells.

selected by using the ry^+ marker. Two transformants, B-12 and B-13, were obtained among 151 embryos injected. The transformants were mated to ry^{506} and approximately the same number of flies with the *ry* eye color and the wild-type eye color were obtained. Southern analysis of DNAs of these flies, and the pattern of transmission of P[*Draf*$^+$; ry^+] indicated a single autosomal integration in B-13 and a single X chromosomal integration in B-12. We therefore used B-13 for further studies.

The mutations mapped near *Draf-1* (Figure 5B) were tested for the defect in *Draf-1* by crossing to B-13. The heterozygous *lethal/FM7c* females were mated to the wild-type eye colored males of B-13 which are heterozygous for the autosomal transgene. In these crosses, males of two types of sex chromosome composition would be generated: *lethal*/Y and *FM7c*/Y. If the mutation is within *Draf-1*, half of the males of the former genotype will be rescued by the exogenous *Draf-1*. As shown in Table I. Two mutants, I-2-6-1 and I-2-8-4, belonging to the same complementation group did produce a number of non-*FM7c*/Y males but other mutants did not. A small number of non-*FM7c*/Y males obtained in these crosses were all sterile, suggesting that they are X/O males due to non-disjunction in the female germ cells. Suppression could not be seen when the *ry* eye colored brothers of B-13, the males transformed with pCarnegie 20, or the original ry^{506} males were used in the crosses. These results indicate that the rescue effect is due to the exogenous *Draf-1* sequence. As the fragment used for transformation contains only *Draf-1*, the mutants, I-2-6-1 and I-2-8-4, were identified to be defective in *Draf-1* and they were named as *l(1)raf*^{1} and *l(1)raf*^{2}, respectively.

As inferred from the number of *FM7c*/+ females as internal standards (the low viability of *FM7c*/Y males is taken into account), only 70% of the *l(1)raf*/Y; P[*Draf*$^+$; ry^+]/− males were rescued. This may be due to the incomplete expression of the transgenic *Draf-1* gene depending on the chromosomal site of integration or to the absence of some sequence necessary for the full expression of *Draf-1* in the 4.3 kb *Bam*HI fragment.

Phenotypes of the mutants

The lethal phase of both *l(1)raf*^{1} and *l(1)raf*^{2} is at larval/pupal stage. To identify the mutant larvae, the *yellow (y)* mutation was introduced into the *l(1)raf*^{1} chromosome by genetic recombination and the recombinant chromosome was balanced over *Binsc* which is wild-type for *y*. The *y* mutation makes the larval mouth hook brownish rather than black in the wild-type. The *y l(1)raf*^{1}*/Binsc* females were crossed to either *y l(1)raf*^{1}*/B*^{s}*y* $^+w^+$ Y or +/Y males. The *y* larvae in the former cross are the females homozygous for *l(1)raf*^{1} and those in the latter cross are hemizygous males. In both crosses >95% of the eggs hatched and ~25% of the larvae were *y*, indicating that the mutation has no apparent zygotic lethal effect on embryogenesis. About 60% of the larvae pupated while the rest died as fully grown larvae.

The larval body is composed of three kinds of cells: larval cells, imaginal cells and germ cells. The latter two continue to proliferate during larval development while larval cells have lost their ability to divide. The larva grows by increasing cell size through endomitosis. Analysis of the mutant larvae revealed defects in the cells which need continuous proliferation. Upon dissection of the mutant larvae, imaginal discs could not be detected except underdeveloped eye-antenna discs (Figure 7). The number of imaginal salivary gland cells is also significantly reduced (Figure 8). Reduction of the sizes was also observed in other organs containing proliferating cells, such as brain lobes and lymph glands (not shown). Furthermore, both testes and ovaries are much smaller than those in the normal larvae (Figure 9). These phenotypes indicate that proliferating cells of both somatic and germ line origins are severely affected in the mutant and suggest that *Draf-1* is required for the normal rate of proliferation. Although *l(1)raf*^{2} has not been studied extensively, essentially the same abnormalities were observed.

The mutation in *l(1)raf*^{1} was induced in the male germ cell with MNU which induces mostly point mutations (Vogel and Natarajan, 1979). Poly(A)$^+$ RNA from the mutant third instar larvae is shown to contain apparently normal-

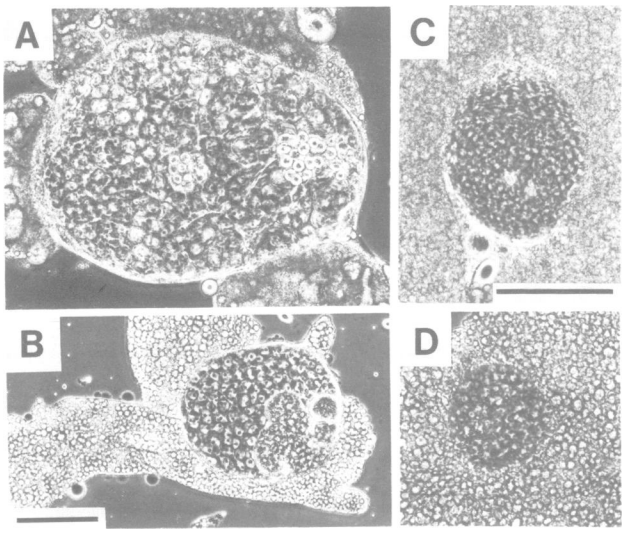

Fig. 9. Gonads of mature mutant larvae. Gonads from mature larvae of normal male (**A**), mutant male (**B**), normal female (**C**), and mutant female (**D**) were dissected out and were visualized by phase-contrast optics. (A) and (B), and (C) and (D), respectively, are at the same magnifications. Gonads are surrounded by adipose cells. Bars represent 50 μm.

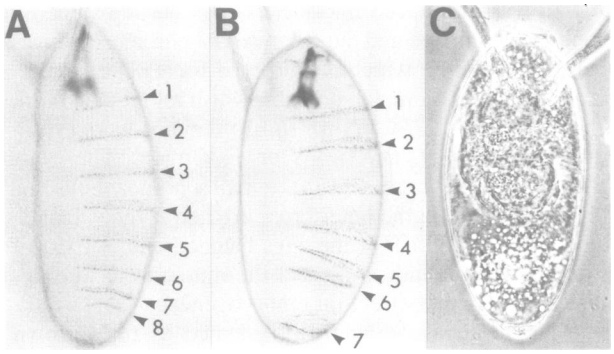

Fig. 10. Maternal effects of *Draf-1* on the embryonic development. The germ mosaics were induced in *l(1)raf*^{1}/*Fs(1)K1237* by X-irradiation at late embryonic stages. The resulting female flies were mated to Oregon-R males and were allowed to lay eggs. Eggs were collected at 24 h intervals and kept 12 h at 25°C before subjecting to a cuticle pattern examination (Van der Meer, 1977). (**A**) A control egg laied by an Oregon-R female; (**B**) a mutant embryo showing the late abnormalities, and (**C**) a mutant showing the early abnormalities. Embryos were visualized by bright field optics in (A) and (B), and by phase-contrast optics in (C). Abdominal segments are numbered.

sized transcripts (Figure 4), suggesting no gross structural alteration had taken place, at least in the coding sequence.

Maternal effects

The apparently normal larval viability of the mutants may be due to the maternal *Draf-1* gene activity. The accumulation of the maternal *Draf-1* transcripts in the ooplasm (Figure 4) supports this idea. This point was further confirmed by the germ-line mosaic analysis (Perrimon and Gans, 1983). The dominant female-sterile mutation, *Fs(1)K1237*, prevents the formation of full-grown oocytes. The *l(1)raf*^{1} chromosome was made heterozygous with *Fs(1)K1237* and the heterozygous females were X-irradiated at the late embryonic stages. Mitotic recombination induced by X-rays would produce the germ-line clones, which are homozygous for *l(1)raf*^{1} but are devoid of *Fs(1)K1237*. If *Draf-1* is not required for ovarian development, we could expect that such germ cells will develop into mature oocytes without maternal *Draf-1* gene activity. Dissection of the adult flies showed that ~5% of the females had unilaterally developed ovaries with fully grown oocytes. The results indicate that *Draf-1* is not essential for the differentiation of the female germ cells. Such females crossed to the wild-type males actually laid eggs but the eggs never hatched, indicating that the maternal *Draf-1* gene activity is necessary for embryonic development.

Analysis of the mutant embryos showed two types of abnormalities. About half of the embryos were nearly full grown but they showed abnormalities at the posterior portions. The cuticle preparations of such embryos revealed defective segmentation at the posterior end (Figure 10B). The remaining embryos showed only a mass of disorganized cells (Figure 10C). Such a dimorphism of the lethal phenotypes could be explained by the paternal contribution of the *Draf-1* gene activity, and the late and early arrested embryos may represent female and male embryos, respectively. In fact, when the mosaic females were mated to $+/B^{s}y^{+}w^{+}$Y males which carry the wild-type *Draf-1* gene on both sex chromosomes, >90% of the embryos showed the late abnormalities. As the expression of zygotic genes takes place mainly after cellular blastoderm formation (Edgar and Schubiger, 1986), the significant rescue of the embryonic development by the paternal gene suggests that *Draf-1* is not essential for the preceding nuclear cleavage divisions.

These mutant phenotypes resemble those of *l(1)pole hole (l(1)ph)* (Perrimon *et al.*, 1984, 1985). The allelism remains to be examined.

Discussion

In *Drosophila*, three types of cell growth take place during development: nuclear cleavage divisions during the early embryonic stage, divisions of undifferentiated diploid cells and endomitosis in differentiated cells. The phenotypes of the *Draf-1* mutants indicate that the gene is an important component for the division of diploid cells of both somatic and germ lines but is not essential for two other specialized processes. These unique features of *Drosophila* growth and the maternal effect of the gene made the mutants viable during larval development. A number of late lethal mutants with disc-less or small disc phenotypes have been reported (Shearn *et al.*, 1971; Shearn and Garen, 1974; Kiss *et al.*, 1976). Some of them might be defective in the genes homologous to other vertebrate oncogenes and/or the genes necessary for the division of diploid cells. Thus, *Drosophila* would provide us an excellent system for studying genes essential for cell proliferation.

v-*mil* of MH2 has been reported to induce a production of chicken myelomonocytic growth factor (cMGF) in avian macrophages (Graf *et al.*, 1986). If *Draf-1* is responsible for such an endocrine stimulation of growth, its defect would be non-cell-autonomous. A preliminary experiment on the mosaics produced by using the unstable ring X chromosome (Hotta and Benzer, 1972) showed that this is not the case. Although the possibility that only the neighboring cells receive the growth stimuli could not be rigorously excluded, the cell-autonomy of *Draf-1* may be compatible with the idea that it is involved in the transduction of signals within the cells.

The *cdc2, wee1* and *nim1* genes of the fission yeast encode protein kinases organizing a cascade of phosphorylation that regulates the cell cycle (Russell and Nurse, 1987a,b). An importance of phosphorylation reactions in the regulation of proliferation has been observed in many vertebrate cells (e.g. Inglis *et al.*, 1976; Gerace and Blobel, 1980; Halleck *et al.*, 1984). *Draf-1* may be involved in such a regulatory network of proliferation. The mutants obtained in this study may be useful as a starting point for the identification of the genes involved in such networks.

Materials and methods

Fly stocks

Flies were reared on yeast–corn meal–agar medium at 25 ± 0.5°C. A series of X-linked recessive lethals was induced in Canton-S stock by methylnitroso-urea (MNU) or ethylnitrosourea (ENU) (Ayaki and Yoshikawa, 1986) and have been kept by balancing over *FM7c*. VA172, HF330 and EC226 (Lefevre, 1981; Perrimon *et al.*, 1984), and *Fs(1)K1237* v^{24}*/C(1)DX, y f/B*^{S}Y (Perrimon and Gans, 1983) were from A.P.Mahowald. *Df(1)2F1-3A4/FM6/B*$^{s}y^{+}w^{+}$Y and *Df(1)Pgd kz/FM7c* were supplied by T.Tanimura. The *B*$^{s}y^{+}w^{+}$Y chromosome carries the distal portion of X chromosome including the 2F region, and it was used for the complementation tests and for the construction of the homozygous mutant females.

Molecular procedures

DNA was extracted from adult flies as described (McGinnis *et al.*, 1983). A genomic library was constructed by *Sau*3AI partial digestion of Oregon-R DNA and ligation into the *Bam*HI site of Charon 28. A λgt10 library constructed from mRNA of 3- to 12-h old embyros was provided by L.M.Kauvar (Poole *et al.*, 1985). Low stringency hybridization conditions used for screening the genomic library were as described (Shilo and Weinberg, 1981). For DNA sequencing, fragments were cloned into pGEM3 (Promega Biotec) and a series of deletion mutants were generated by using the Kilo-sequencing Kit (Takara Shuzo Co., Ltd., Japan). DNA sequences were determined by the chain termination method (Sanger *et al.*, 1977) using synthetic primers.

RNA was extracted by the guanidium–CsCl gradient method (Chirgwin *et al.*, 1979) and poly(A)$^{+}$ RNA was isolated by oligo-dT cellulose column chromatography. RNA was blotted onto Transa-Bind papers (Schleicher and Schuell) after separation in the agarose gels (Thomas, 1983).

In situ hybridization on the squashes of polytene chromosomes was performed as described (Langer-Safer *et al.*, 1982; Leary *et al.*, 1983). Microinjection of DNA into embyros was made according to Karess (1985).

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