Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9037–9041, November 1990
Neurobiology

Molecular cloning of an olfactory gene from *Drosophila melanogaster*

(*olfE*/cytogenetic mapping/germ-line transformation/transcript analysis)

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Communicated by Allan Spradling, August 22, 1990 (received for review February 16, 1990)

ABSTRACT **An olfactory gene *olfE*, which affects response to benzaldehyde in larvae and adults of *Drosophila melanogaster*, has been mapped between two breakpoints on the X chromosome. The breakpoints have been shown to lie at a distance no greater than 25 kilobases (kb). A 14-kb genomic fragment from this region has been used for germ-line transformation of *olfE* mutant flies, and in one of three transformant lines obtained, rescue of the olfE phenotype is observed by two separate behavioral assays. Transcript analysis of the region that rescues the olfE phenotype has shown one major transcript at 5.4 kb and a minor one at 1.7 kb. Both of these transcripts are probably alternatively spliced products of the *olfE* gene. A developmental and tissue-specific profile of the 5.4-kb olfE message has shown that it is present at all developmental stages, suggesting that the gene may be multifunctional.**

Despite the widespread occurrence of the sense of smell among higher organisms, the molecular basis of olfaction is poorly understood. Mainly two approaches, biochemical and genetic, have been taken in an attempt to identify molecules involved in olfaction. The genetic approach has helped identify a number of X chromosome-linked olfactory genes in *Drosophila melanogaster*. Flies mutant for these genes exhibit altered olfactory behavior toward particular odors at both larval and adult stages (1, 2). Specifically, mutations in the genes *olfA*, *olfB*, *olfE*, *olfF* (3), and pentagon (2), change olfactory behavior toward the odorant benzaldehyde. Sensing of all other chemicals is normal, suggesting that the wild-type genes encode olfactory receptors for benzaldehyde. Alternatively, because benzaldehyde is the only repellant known for adult *Drosophila* and response to it is mediated through a specialized neural circuit (4), mutations in genes that alter features of this circuit would also specifically affect the olfactory response to benzaldehyde. Neither olfactory receptors nor the molecules that make up the elements of such an olfactory neural circuit have, thus far, been identified in any organism. The molecular nature of the products of these olfactory genes are, therefore, of considerable interest. Here I describe the molecular cloning of the gene *olfE*. This gene has been mapped between two closely placed breakpoints, and the region between these breakpoints has been used for germ-line transformation and phenotypic rescue of olfE mutant flies. Analysis of transcripts encoded by the region used for germ-line transformation has shown the presence of one major RNA at 5.4 kilobases (kb). This transcript is ubiquitously present at all developmental stages and in both heads and bodies of adult flies, suggesting that an olfactory gene may, in fact, be multifunctional.

MATERIALS AND METHODS

***Drosophila* Stocks and Strains.** The wild-type strain used in all cases was Canton-Special (Canton-S). The olfE mutant line was isolated from the Canton-S strain after ethyl methanesulfonate mutagenesis by C. Ayyub in this laboratory. Its phenotype and genetic mapping have been described (3). Most other strains of *Drosophila* used were obtained from the California Institute of Technology. The $Dp(1;3)sn^{13al}/Ki$ strain was obtained from the stock center at Umea, Sweden. The strain used for supplying *P* element transposase in excising the Car20–E2 insert from transformed line 5m2, was $CyO/Sp;ry^{506}$ *Sb* $P[ry^{+}\Delta 2\text{–}3](99B)/TM6,Ubx$ obtained from W. R. Engel's laboratory (University of Wisconsin, Madison) (5). The third chromosome balancer stock used for making the excised chromosome homozygous was $T(2;3)Ata/CyO$; *TM3*, *ry Sb*.

Olfactory Behavior Tests. (*i*) The Y maze. This is an olfactometer based on the principle of a choice test (1). For each reading ≈50 flies were given the choice between a tube with odor and a tube with odorless air. The response index (RI) for each individual reading was calculated by subtracting number of flies on the smell side (S) from those on the control side (C) and dividing by the total number of flies that participated in the test [RI = (S − C)/(S + C)]. A detailed construction of the adult Y maze used has been published (3). (*ii*) The olfactory jump test. This test has been described in detail (2, 6). In this case a slightly modified version described in ref. 3 was used.

Analysis of DNA. Genomic DNA from adult *Drosophila* was prepared by standard methods. Approximately 2–5 μg of DNA was used for each restriction endonuclease digestion. These digestions were performed according to the manufacturer's specifications. Restriction endonucleases were generally obtained from New England Biolabs. Gel electrophoresis of the DNA and its subsequent transfer to nylon membranes (Hybond-N from Amersham) was according to published procedures (7). Hybridization of the membranes to radiolabeled probes was done following the procedure given in ref. 8. Radiolabeling of the gel-purified DNA fragments was by random-primed labeling (9). [α-^{32}P]dATP obtained from Amersham was used for making the probe.

Analysis of RNA. Total RNA was prepared and separated on agarose gels according to standard procedures. Each lane of 1.2% agarose gel was loaded with 10–15 μg of RNA. Conditions for running RNA gels and transfer of RNA to nylon membranes have been described (10). Hybridization of the membranes was as described above for DNA samples.

Germ-Line Transformation. Embryos obtained from flies of the genotype *olfE ry* were injected with the recombinant plasmid Car20–E2. This plasmid was constructed by subcloning a 14-kb genomic fragment in the *Sal* I site of plasmid Carnegie 20. The genomic fragment was obtained from a phage isolated from a Canton-S/EMBL3 library. It is flanked by *Sal* I sites, one of which is in the genomic insert and the other of which is from the polylinker in vector EMBL3; the extent of this genomic fragment is shown in Fig. 1*A*. Standard

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Abbreviations: RI, response index; Canton-S, Canton-Special.

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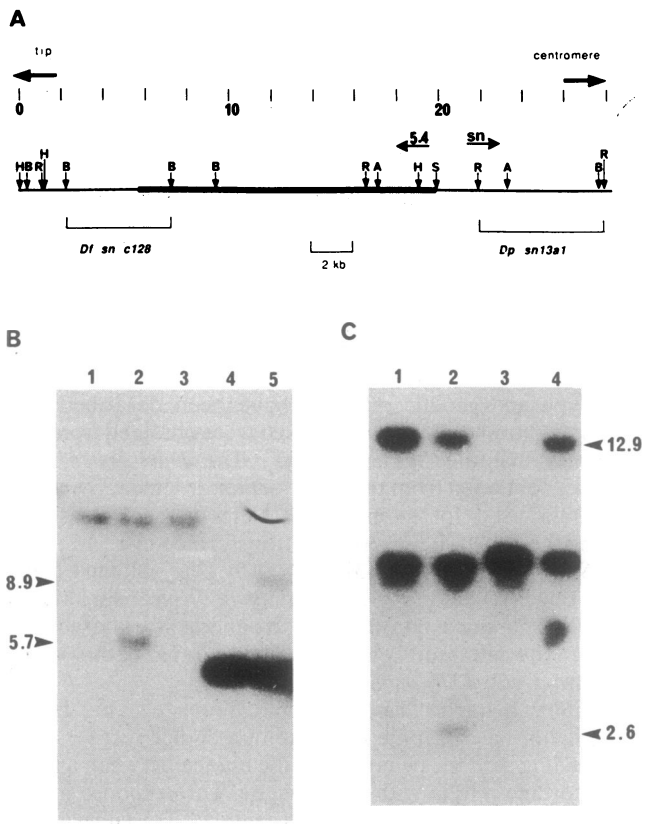


FIG. 1. Molecular mapping of the breakpoints that define the limits of the *olfE* gene. (*A*) Restriction map of the *olfE* region. Restriction endonuclease sites have been denoted: A, *Ava* I; B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; S, *Sal* I. Each scale bar denotes 2 kb, and 0 kb is at the left. $Df(1)sn^{c128}$ extends in the direction of the centromere, whereas $Dp(1;3)sn^{13al}$ extends in the opposite direction toward the chromosome tip. Direction of transcription for the 5.4-kb and *sn* transcripts (data from personal communication with J. Paterson and K. O'Hare) is indicated above the map. (*B*) Mapping of the breakpoint for $Df(1)sn^{c128}$. Genomic DNA from flies of the Canton-S strain (lanes 1, 3, and 4) and $Df(1)sn^{c128}$/Canton-S strain (lanes 2 and 5) was digested with *Eco*RI (lanes 1, 2, and 3) and *Bam*HI (lanes 4 and 5). After electrophoresis and transfer of the DNA to a nylon membrane, as described, the membrane was hybridized to the fragment marked *Df(1)c128* in Fig. 1*A*. (*C*) Mapping of the breakpoint for $Dp(1;3)sn^{13al}$. Genomic DNA from Canton-S flies (lanes 1 and 3) and from flies of the genotype Canton-S/$Y;Dp(1;3)sn^{13al}$/+ (lanes 2 and 4) was digested with the restriction endonuclease *Ava* I (lanes 1 and 2) and with *Eco*RI (lanes 3 and 4). As in Fig. 1*B*, the DNA was electrophoresed and transferred to a nylon membrane. In this case the membrane was hybridized to the fragment marked *Dp* sn^{13al} in Fig. 1*A*. Numbers at sides indicate kilobase pairs.

published procedures were followed for microinjection and selection of transformants (11). Three independent ry^+ transformant lines were obtained. Excision of the recombinant plasmid Car20–E2 insert from the line 5m2 was done by mating virgin females from the homozygous 5m2/5m2 stock to males of the strain containing the *P* transposase.

RESULTS AND DISCUSSION

Cytogenetic Mapping of *olfE*. To clone the *olfE* gene it was first necessary to obtain a precise genetic map position of this gene. This had, in part, been accomplished, and the gene mapped between the markers crossveinless and vermilion on the X chromosome. Within this interval it was found to map under a duplication of polytene bands 7A8–8A5 [*Dp(1;2)-FN107*] and a deficiency of bands 7D1–D5 [$Df(1)sn^{c128}$] (3). Overlapping this region, in part, lies another chromosomal translocation $Dp(1;3)sn^{13al}$, in which the polytene bands 6C5–7C9 from the X chromosome have been duplicated on the third chromosome (12). As seen from the results in Table 1, $Dp(1;3)sn^{13al}$ also covers the *olfE* gene. When *olfE* virgin females are crossed with males carrying $Dp(1;3)sn^{13al}$ all males in the next generation carry *olfE* mutant X chromosomes, whereas only half carry $Dp(1;3)sn^{13al}$. These can be easily distinguished from males not carrying $Dp(1;3)sn^{13al}$ by the presence of a dominant marker *Ki* on the sister chromosome. RIs of both classes of males to benzaldehyde were obtained in the Y-maze test paradigm (Table 1). The RI of *olfE*/*Y*;$Dp(1;3)sn^{13al}$ flies is not significantly different from that of wild-type flies, indicating that the *olfE* gene is covered by the duplicated segment 6C5–7C9. This result is probably not from the effect of a different genetic background of the duplication flies because the expected mutant RI is obtained for *olfE*/*Y*;+/*Ki* flies. In addition, progeny from a control cross, in which wild-type females (Canton-S) were mated to males of the strain carrying $Dp(1;3)sn^{13al}$, have also been tested and were found to give normal responses as expected.

Molecular Mapping. Mapping of *olfE* under $Dp(1;3)sn^{13al}$ agrees with earlier genetic data placing it under *Dp(1;2)-FN107* because the two duplications are known to overlap from bands 7A8 to 7C9 (12). There is, however, a discrepancy in placing it under $Df(1)sn^{c128}$ because no overlap was known to exist between $Dp(1;3)sn^{13al}$ and $Df(1)sn^{c128}$. It was, therefore, important to determine precisely the two breakpoints of $Dp(1;3)sn^{13al}$ at 7C9 and $Df(1)sn^{c128}$ at 7D1. To do this, genomic DNA from flies carrying either $Df(1)sn^{c128}$ or $Dp(1;3)sn^{13al}$ chromosomes was analyzed. λ clone probes from the singed region, obtained in the course of a chromosomal walk initiated from the singed locus (13, 14), were used as probes on DNA blots for determining the breakpoints. A restriction map of the region in which the two breakpoints map is given in Fig. 1*A*. It is derived from the data in refs. 13 and 14. Various cloned fragments from the region shown in the figure and neighboring regions were used as probes for determining the breakpoints. As seen from the results shown in Fig. 1*B*, the breakpoint of $Df(1)sn^{c128}$ lies within a 5.3-kb *Bam*HI fragment (2.5–7.8 in the restriction map). When genomic DNA isolated from either Canton-S or $Df(1)sn^{c128}$/Canton-S flies is digested with restriction enzymes and probed with this 5.3-kb fragment, the following results are obtained: a common band is observed at 14.6 kb (Fig. 1*B* *Eco*RI digests, lanes 1–3) and 5.3 kb (*Bam*HI digests, lanes 4 and 5). In addition, there is another band observed in the $Df(1)sn^{c128}$/Canton-S lanes at 5.7 kb in the *Eco*RI digest (Fig. 1*B*, lane 2) and at 8.9 kb in the *Bam*HI digest (Fig. 1*B*, lane 5). Because the additional band is seen with the same probe in restriction digests by two different enzymes, it is probably not caused by restriction-site polymorphisms in the

Table 1. Cytogenetic mapping of *olfE*

Genotype	RI	SD	*n*	*P*
+/+ (Canton-S)	−0.34	0.03	10	
$olfE^{x26}$	−0.14	0.07	19	<<0.0005
+/*Y*;$Dp(1;3)sn^{13al}$	−0.34	0.03	3	>>0.005
+/*Y*;+/*Ki*	−0.33	0.02	3	>>0.005
olfE/*Y*;$Dp(1;3)sn^{13al}$	−0.29	0.03	9	>>0.005
olfE/*Y*;+/*Ki*	−0.15	0.03	7	<<0.0005
+/$Df(1)sn^{c128}$	−0.37	0.08	5	>>0.005
olfE/$Df(1)sn^{c128}$	−0.12	0.01	6	<<0.0005

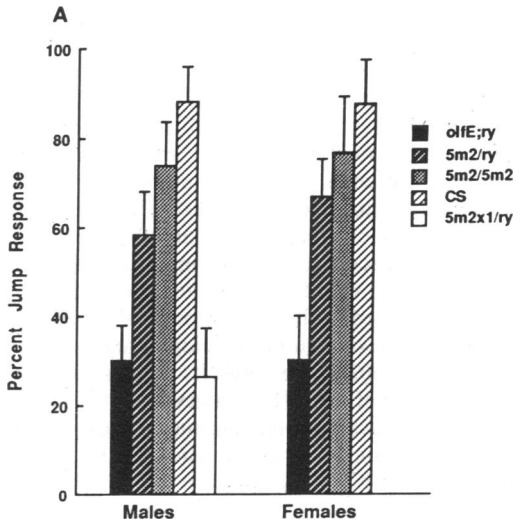
Mean values of RI values of adult *Drosophila* to a 1:10,000 dilution of benzaldehyde are given with SD, as obtained by testing in the Y maze. Negative values for the RI indicate that benzaldehyde is a repellant. *n* denotes number of readings taken. *P* values were determined by comparing each mean RI with wild-type (Canton-S) value using Student's *t* test. Values for strains carrying $Df(1)sn^{c128}$ are from Ayyub *et al.* (3).

Df(1)sn^{c128} chromosome. A similar analysis of DNA from *Dp(1;3)sn^{13a1}* flies (Fig. 1*C*) places that breakpoint in a 5.9-kb *Eco*RI fragment that lies from 22 to 27.8 kb in the restriction map in Fig. 1*A*. The two breakpoints were independently confirmed by hybridization of a 2.0-kb *Eco*RI–*Sal* I fragment that lies from 18 to 20 kb in Fig. 1*A* to polytene chromosome squashes of both the deficiency and the duplication. As expected, this fragment hybridizes to the X chromosome and the third chromosome of *Dp(1;3)sn^{13a1}* males, but it does not hybridize to X chromosomes from males with the genotype *Df(1)sn^{c128}/Y;Dp(1;2)FN107/bwD* (14).

Germ-Line Transformation. Mapping of breakpoints for *Df(1)sn^{c128}* and *Dp(1;3)sn^{13a1}* in the 7C9–7D1 region clearly demonstrates an overlap of ≈25 kb between them. Because *olfE* is uncovered by *Df(1)sn^{c128}* and covered by *Dp(1;3)-sn^{13a1}* (Table 1), it must lie within this 25-kb interval. A transcript analysis of this region has been independently done by J. Paterson and K. O'Hare (personal communication). This analysis had shown that the 5′ end of the singed transcript maps near the *Eco*RI site at 21.8 kb on the restriction map in Fig. 1*A*, and the direction of singed transcription is toward the centromere. Another transcript in this region was found to initiate at ≈19.7 kb in Fig. 1*A* (near the *Sal* I site). This transcript is 5.4 kb in length, and from its map position it appears likely to be the *olfE* transcript. To confirm this, I have used some of this region for *P* element-mediated germ-line transformation of *olfE* flies (11). The precise region used for germ-line transformation is marked with a bold line in Fig. 1*A*. This region was subcloned from the original λ phage clone into the *Sal* I site of plasmid Carnegie 20 (15). Due to the necessity of performing group tests for looking at olfactory behavior, injections of the recombinant plasmid (Car20–E2) were in *olfE;ry^{506}* embryos. Surviving adults were mated with *olfE;ry^{506}*, and the progeny were screened for germ-line transformants. Because both *olfE$^+$* and *ry$^+$* genes are integrated as a unit, germ-line transformants were identified by first looking for flies with *ry$^+$* eye color. Three independent *ry$^+$* transformants were obtained in this way. These transformants were mated with *olfE;ry* flies, and both *ry* and *ry$^+$* progeny obtained were independently tested for olfaction. Position of the Car20–E2 insert in two of the three lines was established by *in situ* hybridization to polytene chromosomes (data not shown). The insert is at band position 94E/F in line 5m2 and at band position 43E in line 15f2. These positions were further confirmed by observing segregation of the *ry$^+$* phenotype from appropriately marked second and third chromosomes. In line 4f2 the insert is on the second chromosome, but its position on the polytene chromosomes has not been determined. The presence of a single insert in each case was established by looking at Southern blots of genomic DNA from each of the transformant lines by using appropriate restriction digests and probes (data not shown).

Olfactory Testing of Germ-Line Transformants. Two separate olfactory behavior tests have been used for testing progeny of the transformant lines. Testing by the olfactory jump assay showed that the percentage response of one transformant line (5m2) was consistently higher than that of *olfE;ry* flies (Fig. 2*A*). The 14-kb genomic fragment cloned in Car20–E2 can thus partially rescue the olfE mutant phenotype, indicating that the *olfE* gene is, indeed, encoded within this fragment. However, the percentage jump response obtained with transformant line 5m2 (≈60%), although significantly higher than that of *olfE;ry* flies (≈30%), is still not equivalent to the wild-type response of Canton-S flies, which is >85%. This partial rescue is probably from inadequate expression of *olfE*, possibly caused by lack of the complete 5′ sequences needed for its expression (see below). The partial rescue may also be the result of the effect of chromosomal position on expression of integrated genes, which has been reported (16–18). In either of these two cases it should be possible to improve expression and, hence, the response, by introducing a second copy of the insert. This was done by interbreeding single pairs of *olfE*;5m2/*ry* flies to obtain lines that were *olfE*;5m2/5m2. As shown in Fig. 2*A*, introduction of a second copy of the 5m2 insert in the *olfE;ry* background has a statistically significant effect; the percentage jump response of 5m2/5m2 flies increases to 75%, suggesting that increasing copy number of what may be an underactive copy of the wild-type gene could lead to a cumulative effect on behavior. Enhanced expression of an inserted gene due to multiple copies has been reported with the gene for xanthine dehydrogenase (16).

Somewhat different results were obtained when the 5m2 line was tested in the Y maze. Flies with single copies of the insert gave variable responses that could not be properly quantified (data not shown). However when flies with two copies of the insert (5m2/5m2) were tested in the Y maze their responses were normal (Fig. 2*B*). Two explanations can be offered for not seeing a measurable effect of one copy of the 5m2 insert in the Y maze. The jump test and the Y maze measure different responses, the neural circuits for which



B

Genotype	RI	SD	*n*	*P*
Canton-S	-0.34	0.03	10	
olfE^{x26};ry^{506}	-0.18	0.02	10	<<0.0005
5M2/5M2 (two copies)	-0.29	0.04	10	>>0.005

FIG. 2. Germ-line transformation and phenotypic rescue of *olfE*. (*A*) Behavioral testing of transformant line 5m2 by the jump assay. Percentage jump values for each of the strains indicated were obtained from 20 readings, and each reading was calculated by scoring 20 individual fly jumps measured in response to a stream of air that was sucked through a reservoir of undiluted benzaldehyde. Error bars denote SD. Male and female flies were tested separately to be certain of the absence of sexual dimorphism in the test. CS, Canton-S. (*B*) Behavioral testing of transformant line 5m2 by the Y maze. For the Y maze, flies were tested as described in the legend for Table 1.

may have some common and some different elements. Thus, while level of expression of a single 5m2 insert may be enough to activate the jump circuit, it may not be adequate for establishing a choice response required in the maze. Alternatively, our inability to observe a difference in the maze may simply be from the fact that this test is less sensitive. Thus, although the difference between mutant and wild type is considerable in the jump test (30% and 85%), their difference in the Y maze is much smaller (0.15 and 0.30), so that in the latter test it is difficult to reliably measure values that lie between mutant and wild type. A better understanding of the neural pathways used in the two responses is needed before any further hypothesis can be developed and tested.

Excision of the *olfE* Insert Results in Loss of Wild-Type Phenotype. Rescue of the olfE phenotype by the insert in line 5m2 supports the earlier molecular mapping of *olfE*. While it is possible to explain why rescue was seen in one transformant line of three, this fact does raise the possibility that the one line showing rescue of the olfE phenotype (5m2) may contain a modifier of *olfE*, also on the third chromosome. It was, therefore, important to show that it is, in fact, the Car20–E2 insert on the third chromosome that causes rescue of the olfE phenotype. To do this, the insert, which is flanked by *P* element ends derived from the Carnegie 20 vector, was excised by mating flies homozygous for the insert to a strain that makes *P* element transposase (5). Excision of the Car20–E2 insert was monitored by looking for *ry* flies, and the excised chromosome was followed by mating flies carrying it with a dominantly marked third chromosome balancer stock. Two homozygous lines, each bearing an independent excision event, were constructed (5m2x1 and 5m2x2). Males from these lines were mated to *olfE;ry* virgin females, and males from the F_1 progeny were tested by the jump assay. In both excised lines tested no rescue of the olfE phenotype was observed. Results for line 5m2x1 are given in Fig. 2*A*.

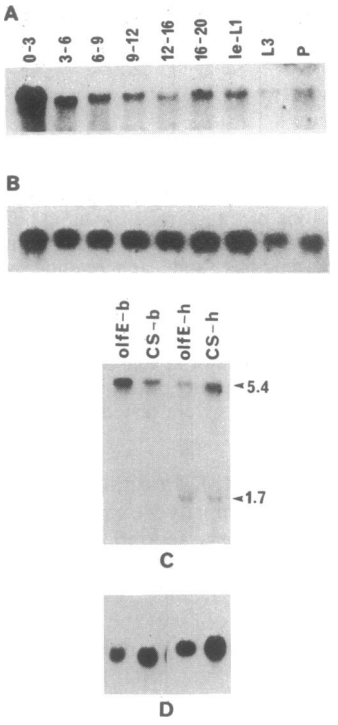


FIG. 3. Developmental and tissue-specific profile of *olfE* transcripts. (*A* and *B*) Total RNA was isolated from differentially staged embryos (obtained after the time in hours indicated above each lane), late embryos and first instar larvae (Ie-L1), third instar larvae (L3), and pupae (P). (*C* and *D*) Total RNA was isolated from heads (h) and bodies (b) of adult Canton-S (CS) and *olfE* flies. The RNA was electrophoresed and subsequently transferred to nylon membranes. Membranes were hybridized to a cloned genomic fragment from *olfE* (5.7–16.2 kb, Fig. 1*A*) in *A* and *C*. *B* and *D* show the same membrane as in *A* and *C*, respectively, after washing off the *olfE* probe and hybridizing to a plasmid encoding a gene for a *Drosophila* ribosomal protein; this procedure was done to check for the amount of RNA in each lane.

Transcript Analysis of the *olfE* Gene. From the data so far, it is clear that *olfE* is encoded within a 14-kb genomic fragment that has been used for germ-line transformation of *olfE* mutants. This region has been analyzed for transcripts at all developmental stages and also in heads and bodies of adult flies (Fig. 3). The probes used were genomic fragments obtained from λ phage clones of the region (14). A single major transcript of 5.4 kb is detected by the two genomic probes a and b shown in Fig. 4*A*. Data for probe b are shown in Fig. 3. This probe, in addition, detects a smaller and rarer transcript of 1.7 kb in adults, which appears enriched in RNA from heads. To study the relationship of these two transcripts a number of cDNA clones from both embryonic and head cDNA libraries have been isolated by using probe b. One

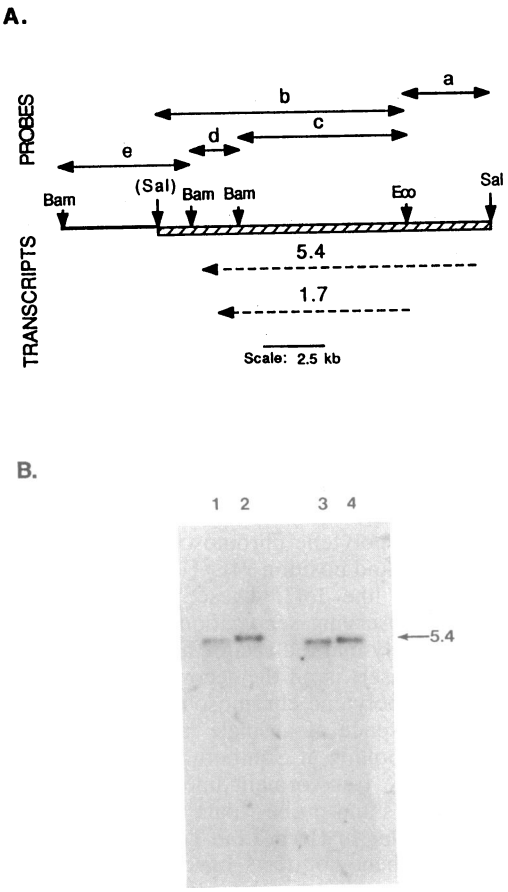


FIG. 4. Mapping of transcripts encoded by the Car20–E2 insert (hatched line). (*A*) Restriction endonuclease sites are as follows: Bam, *Bam*HI; Eco, *Eco*RI; Sal, *Sal* I. Only sites used for generation of probes are marked. The *Sal* I site in parenthesis is derived from the polylinker in EMBL3 vector and does not exist in the genome of Canton-S flies. Extent of region encoding the 5.4-kb and 1.7-kb transcripts, as derived by hybridization of cDNA clones and genomic fragment probes a–e, is shown below the restriction map. (*B*) Hybridization of a 5′ cDNA clone insert (lanes 1 and 2) and λE7-1 insert (lanes 3 and 4) to RNA blots. Approximately 10 μg of total RNA extracted from adult bodies (lanes 1 and 3) and heads (lanes 2 and 4) was electrophoresed and transferred to nylon membranes. Hybridization of the filters to gel-purified insert fragments was as described. The 5′ cDNA clone of the 5.4-kb transcript was provided by J. Paterson and K. O'Hare.

such embryonic cDNA clone (λE7-1) has a 3-kb insert that hybridizes to a single band of 5.4 kb on RNA blots from heads and bodies of adult flies, indicating that it corresponds to the 5.4-kb transcript (Fig. 4*B*, lanes 3 and 4). Preliminary sequence analysis of this clone has shown the presence of a poly(A) tail at one end. The end of this clone maps within a 1.8-kb *Bam*HI fragment, depicted as probe d in Fig. 4*A*, thus placing the 3′ end of the 5.4-kb transcript in this fragment. The 5′ end of this transcript had earlier been mapped close to the *Sal* I site of probe a, by partial sequencing of genomic and 5′ cDNA clones (see Fig. 1*A*, data from personal communication with J. Paterson and K. O'Hare). Hybridization of one such 5′ cDNA clone to an RNA blot, similar to the one used for hybridization of the 3′ cDNA clone λE7-1, is shown in Fig. 4*B*, lanes 1 and 2. As for the 3′ cDNA clone hybridization is to a single band at 5.4 kb. The gene encoding the 5.4-kb transcript thus extends almost the complete length of the Car20–E2 insert (hatched line in Fig. 4), indicating that this is, in fact, the *olfE* gene. The rare 1.7-kb transcript seen in adult heads is, thus, probably an alternatively spliced product of the same gene for the following reasons. A probe lying within the ends of the 5.4-kb transcript (probe c in Fig. 4*A*) also detects the 1.7-kb transcript at a similar intensity to that seen with probe b in Fig. 3*B* (data not shown). Furthermore, it is not detected by the neighboring 5.3-kb *Bam*HI fragment (probe e, Fig. 4*A*). This analysis places the coding regions for both the 5.4-kb and 1.7-kb transcript in overlapping segments of DNA (Fig. 4*A*) and suggests that the two transcripts may be alternately spliced products of the same gene. This idea has recently been supported by partial sequence analysis of λE7-1 and a 300-base-pair (bp) head cDNA clone (λheadE-1), which has shown that the two clones share ≈160 bp of sequence but diverge at both 5′ and 3′ ends (data not shown). λheadE-1 is likely to be a cDNA clone of the 1.7-kb transcript because no other transcript corresponding to this region has been detected in the head. However, confirmation of this by hybridization of the λheadE-1 insert to an RNA blot has not been possible—perhaps due to the small insert size and rarity of the 1.7-kb message. A complete sequence analysis of full-length cDNA clones for both the 5.4- and 1.7-kb transcripts is required to unambiguously show the relationship of the two transcripts.

The *olfE* gene, thus, has at least two transcripts, one or both of which could be involved in its olfactory function. Because the mutant phenotype of *olfE* is seen at both larval and adult stages, one would expect the relevant transcript also to be present in at least these two stages. Absence of the 1.7-kb transcript from the larval stages suggests that this transcript may not be directly involved in olfactory function, leaving the 5.4-kb transcript as the likely candidate. Fig. 3*A* shows that this transcript is present at all stages of development, although it is considerably enhanced in very early embryos of 0–3 hr. In adults it is present in both heads and bodies and shows no change in size or quantity in the existing *olfE* allele (*x26*). Differences in amounts between the lanes in Fig. 3*C* are not reproducible. The ubiquitous nature of the 5.4-kb transcript suggests that the *olfE*-encoded product may also have a function other than in olfaction, and from the presence of this transcript in embryos, it may play a role in development of the nervous system. The idea that some olfactory genes may be multifunctional is supported by another olfactory behavior gene pentagon, which in addition to its olfactory phenotype also has a morphological phenotype (2). From the results to date, definition of the mechanism by which *olfE* affects olfactory behavior is not possible. Further analysis of the cloned gene coupled with a study of other alleles should help elucidate the function(s) of *olfE* more precisely.

I thank my colleague Veronica Rodrigues for her help at many different stages of this work and Jayashree Paranjpe for help with the behavioral testing. I am also grateful to Jamie Paterson and Kevin O'Hare at the Imperial College, London, for sending me cDNA clones of the 5.4-kb transcript as well as sharing their data on the 5.4-kb transcript and the singed locus with me before publication.

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