The Om(1E) Mutation in Drosophila ananassae Causes Compound Eye Overgrowth due to tom Retrotransposon-Driven Overexpression of a Novel Gene

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ABSTRACT

Optic morphology (Om) mutations in Drosophila ananassae are a group of retrotransposon (tom)-induced gain-of-function mutations that map to at least 22 independent loci and exclusively affect the compound eye morphology. In marked contrast to other Om mutations, which are characterized by fewer-than-normal and disorganized ommatidia, the Om(1E) mutation exhibits a peculiar phenotype as enlarged eyes with regularly arrayed normal ommatidia. To characterize the Om(1E) mutation, we have carried out molecular analyses. A putative Om(1E) locus cloned by tom tagging and chromosome walking contained two transcribed regions in the vicinity of tom insertion sites of the Om(1E) mutant alleles, and one of these regions was shown to be the Om(1E) gene by Pelement-mediated transformation experiments with D. melanogaster. The Om(1E) gene encodes a novel protein having potential transmembrane domain(s). In situ hybridization analyses demonstrated that the Om(1E) gene is expressed ubiquitously in embryonic cells, imaginal discs, and the cortex of the central nervous system of third instar larvae, and specifically in lamina precursor cells. Artificially induced ubiquitous overexpression of Om(1E) affected morphogenesis of wing imaginal disc derivatives or large bristle formation. These findings suggest that the Om(1E) gene is involved in a variety of developmental processes.

Many spontaneous mutations are caused by the insertion of transposons (for review see Shapiro 1983; Georgiev 1984). In the majority of cases, transposon-induced mutations result in complete or partial loss-of-function by disrupting gene structure or by suppressing gene expression. However, there exist several cases in which transposon insertions cause dominant gain-of-function mutations by activating expression of adjacent genes (e.g., Campuzano et al. 1986; Itoh et al. 1988; Ito et al. 1989; Hake 1992; Bradley et al. 1993). It is also included in this category that tumorigenesis in vertebrates is sometimes induced by proviral insertions of retroviruses that activate expression of protooncogenes by “promoter insertion mode” or “enhancer insertion mode” (for review see Nesse 1986). Such mutations often enable us to recognize hidden genes and their functions.

Optic morphology (Om) mutations in Drosophila ananassae are another example of gain-of-function mutations resulting from enhanced expression of relevant genes by transposon insertion; Om mutations are a group of semidominant mutations that map to at least 22 independent loci and exclusively display abnormalities of the compound eye morphology in locus-specific fashions (Hinton 1984; Moriwaki and Tobari 1993; for review see Tanda et al. 1993). Genetic and cytogenetic studies demonstrated that all Om mutations are associated with insertion of the tom retrotransposon (Shrimpton et al. 1986; Matsubayashi et al. 1992).

Three Om loci, Om(1A), Om(1D), and Om(2D), have so far been cloned and analyzed (Tanda et al. 1989; Matsubayashi et al. 1991a,b; Tanda and Corces 1991; Awasaki et al. 1994; Yoshida et al. 1994). In these mutants, tom elements are found nearby the relevant Om genes and activate their expressions in eye imaginal discs of third instar larvae, without affecting the structure of their products. These findings suggest that Om phenotypes may result from over- and/or ectopic-expression of the Om genes in eye imaginal discs and that a putative enhancer present within the tom element may be responsible for such changes (Tanda and Corces 1991; Awasaki et al. 1994; Yoshida et al. 1994).

The products of the Om genes have been characterized as follows: the Om(1D) and Om(1A) genes encode homeoproteins homologous to the D. melanogaster BarH1 and cut gene products, respectively (Kojima et al. 1991; Awasaki et al. 1994). BarH1, together with its sibling, BarH2, is required for the differentiation of R1/6 photoreceptors and primary pigment cells in the eye imaginal disc, as well as that of neurons and glial cells in the external sensory organ (HigashiJima et al. 1992a,b); the cut gene is known to play an important...
role in the differentiation of the sensory mother cell into the external sensory organ (Blochlinger et al. 1988, 1991). On the other hand, the Om(2D) gene encodes a novel protein containing the histidine/proline repeat (PRD repeat) motif (Yoshida et al. 1994). This motif has been found in several transcription factors of Drosophila and other species and hypothetically mediates the pH-dependent protein-protein interaction of eukaryotic transcription factors (Janknecht et al. 1991). Loss-of-function of the Om(2D) gene results in embryonic- or larval-lethality (Matsubayashi et al. 1991b). It is thus plausible that the Om(2D) gene also participates in some developmental pathways.

Based on the above findings, it is presumed that Om gene products may be capable of switching cell fates in given developmental pathways, and that Om phenotypes result from misleading of cell fates by excess Om gene products in differentiating eye imaginal discs. The possibility thus exists that studies of Om mutations may provide a way to investigate members of such “switching genes”. Om mutations may well have some merits to analyze: first, the transposon (i.e., tom)-tagging strategy facilitates cloning of Om genes; second, the gain-of-function nature of Om mutations allows the identification of genes difficult to recognize with loss-of-function mutations because of lethality or redundancy [e.g., Om(1D) = BarH1/H2].

Among the Om mutations, Om(1E) is quite unique because of its peculiar phenotype, exhibiting overgrowth of the compound eye without affecting the regular array of ommatidia and the ommatidial structure, while the other Om mutations result in fewer-than-normal and disorganized ommatidia. Three Om(1E) mutant alleles, Om(1E)53, Om(1E)59a, and Om(1E)109, have been isolated (Hinton 1984; Moriwaki and Tobari 1993) and cytologically mapped to 16D on the X chromosome (Shrimpton et al. 1986; Matsubayashi et al. 1992). In this report, we describe identification and characterization of the Om(1E) gene. We cloned a putative Om(1E) region and showed one of two transcribed regions therein to be the Om(1E) gene. The deduced Om(1E) gene product is a novel protein having presumptive transmembrane domain(s). The Om(1E) gene is expressed ubiquitously in embryonic cells, imaginal discs, and the cortex of the central nervous system of third instar larvae, and specifically in lamina precursor cells. Artificially induced ubiquitous overexpression of Om(1E) affected morphogenesis of wing imaginal disc derivatives or large bristle formation. These findings suggest that the Om(1E) gene is involved in various developmental processes.

**MATERIALS AND METHODS**

**Drosophila stocks:** D. ananassae stocks, Om(1E)53, Om(1E)59a, Om(1E)109, and their progenitor ca; px, which was used as wild-type in this study, were provided by C. W. Hinton. Revertants of Om(1E)53, Om(1E)53a, and Om(1E)53b were isolated from 8762 X chromosomes of Om(1E)53 males that had been irradiated with 30 Gy of γ-ray from a 60Co source. These revertants are viable and fertile, and maintained in males by crossing to C(1LR)f g/Y females (Y. N. Tobari, personal communication). The D38 strain was provided by Y. N. Tobari. The D. melanogaster w strain was obtained from the National Institute of Genetics, Mishima, Japan. All stocks were cultured at 25°C on standard medium containing yeast, cornmeal, malt, glucose, and agar.

**Scanning electron microscopy and immunocytochemistry:** Scanning electron microscopy and immunocytochemistry with MAB22C10 were made as described earlier (Awasaki et al. 1994). The ommatidial number per compound eye was counted on scanning electron micrographs of four female compound eyes.

**Molecular techniques:** Routine molecular techniques were according to Sambrook et al. (1989).

**Analyses of genomic DNA:** Preparation of genomic DNA was after Itoh et al. (1988). Genomic libraries were constructed with Sou3AI partially digested genomic DNAs and the λEMBL3 vector. The tom probe, proom1, is a 0.5-kb Sal fragment of A85-13 (Matsubayashi et al. 1991a) that contains all the tom sequences except for one of the two LTRs. Library screening and Southern hybridization were performed using digoxigenin-labeled probes and the DIG Luminescent Detection Kit (Boehringer Mannheim) according to the manufacturer’s instruction.

**In situ hybridization to polytene chromosomes:** was performed using digoxigenin-labeled probes and the DIG DNA Detection Kit (Boehringer Mannheim) following the method of Engels et al. (1986).

**RNA isolation and Northern blot analysis:** Total RNAs were extracted by the hot phenol method (Jowett 1986), and poly(A)+ RNAs were prepared using the mRNA purification kit (Pharmacia). Poly(A)+ RNAs (5 μg/lane) were separated on formaldehyde-denatured 1% agarose gels, blotted onto the Biodyne-A nylon membrane (Paul), and hybridized with 32Plabeled probes prepared with the Multiprime DNA labeling system (Amersham). As a control for the amount of RNA loaded in each lane, the blots were rehybridized with a D. melanogaster ras2 gene probe, pUC8-HB-1.2 kb (Bishop and Corces 1988), which was provided by V. G. Corces.

**Cloning and sequencing of cDNAs:** A cDNA library was constructed as described by Yoshida et al. (1994) with oligo-dT-primed cDNAs of wild-type third instar larvae and the Agt10 vector. The cDNA library was screened with the pEES4 probe for the t1 cDNA and with the pBS3.8 probe for the t2 cDNA (see Figure 3A) by the same method as for genomic library screening. The cDNA library was screened by the same method as for genomic library screening. The retrieved cDNAs were recloned in the pGEM7zf(−) plasmid vector (Promega). Nucleotide sequences were determined by the chain-terminating method (Sanger et al. 1977) using the BcaBest Dideoxy Sequencing Kit (Takara) and sequential deletion mutants (Henikoff 1984). The nucleotide sequence data in this study will appear in the GSDB, DDBJ, EMBL and National Center for Biotechnology Information nucleotide sequence databases with the following accession numbers: D37990 for t1 cDNA and D37989 for t2 cDNA.

**In situ hybridization to embryos and larval tissues:** Embryos were dechorionated, fixed, and devitellinized after Tautz and Pfeifle (1989). Hand-dissected larval tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) [130 mM NaCl in 10 mM phosphate buffer (pH 7.2)] on ice for 15 min, and then in 4% paraformaldehyde, 0.5% Triton-X 100 in PBS at room temperature for 15 min. Pretreatment, hybridization and signal-detection were made as described by Tautz and Pfeifle (1989) except that antisense RNA probes were used instead of double-stranded DNA probes. Digoxigenin-labeled RNA probes were synthesized from cDNAs.
cloned in the pGEM7zf(-) vector using the DIG RNA Labeling Kit (Boehringer Mannheim) according to the manufacturer’s instruction; the labeled RNAs were size-reduced to the average length of 50–100 bases by controlled alkaline hydrolysis to achieve the efficient hybridization (Cox et al. 1984). At the beginning and the end of the washing step after hybridization, the specimens were treated with 20 μg/ml RNase A, 0.5 M NaCl in 10 mM Tris buffer (pH 8.0) at 37°C for 30 min to remove the unhybridized probe. After signal detection, the specimens were washed in 0.1% Tween 20 in PBS and mounted in Aquatex (Merck).

**PElement-mediated transformation:** A 0.3-kb SalI-PstI fragment spanning −0.2 to +0.1 kb from the initiation site of the Hsp70 gene was excised from phsp70C4 (Tanda and Corces 1991) and cloned in the Xbal site of the CaSpeR vector (Pirrotta et al. 1985) so as to orient the Hsp70 promoter parallel to the w+ marker gene. This construct was named HspCaSpeR1. Furthermore, an EcoNI-TaqI fragment ranging nucleotides −13–434 of the 3′ tom LTR was excised from M8313 (Matsubayashi et al. 1991a) and cloned in the PstI site of the HspCaSpeR1 vector in the opposite direction to the Hsp70 promoter. This was named TLHspCaSpeR. The t1 and t2 cDNAs flanked by EcoRI linkers were cloned in each of HspCaSpeR1 (Hsp-t1/t2 constructs) and TLHspCaSpeR (TLHsp-t1/t2 constructs) at the EcoRI site downstream from the Hsp70 promoter (see Figure 5). Since the t1 cDNA lacked in 0.5 kb of the 3′ untranslated region containing the polyadenylation signal, its genomic counterpart was fused to the t1 cDNA before introducing into the vectors. The resulting constructs were cojected with pB25.7wC (Kareess and Rubin 1984) into embryos of the D. melanogaster w strain by the method of Rubin and Spradling (1982). Transformants were selected from the G1 flies and individually crossed to w flies. Homozygous transformant lines were established by single-paired sib-mating for further two or more generations.

**RESULTS**

**Phenotypes of Om(1E) mutant flies:** Three Om(1E) mutants, Om(1E)53, Om(1E)59a, and Om(1E)109 (Hinton 1984; Moriwaki and Tobari 1993) are all characterized by dorso-posteriorly enlarged compound eyes that look as if one extra eye is fused dorso-posteriorly to another (Figure 1). The average number of ommatidia per compound eye is 1353.3 ± 90.2 in Om(1E)53, 1058.3 ± 67.5 in Om(1E)59a, and 945.3 ± 71.8 in Om(1E)109, in contrast to 816.8 ± 34.9 in wild type. The array of ommatidia and the ommatidial structure are basically normal, and no other visible change was noted in the mutant (Tanda et al. 1993; our unpublished data).

Our histological study showed that the eye imaginal discs of Om(1E) mutants are normal, both in size and morphology, up to the early third larval instar stage; but during the middle and late third larval instar stages, the eye imaginal disc epithelium overgrows to form a bud that might later become a dorso-posteriorly protruded portion of the compound eye (Tanda et al. 1993; our unpublished data). This suggests that the Om(1E) phenotype may result from overproliferation of undifferentiated cells, but not from overproduction of photoreceptor precursor clusters at the expense of cells that are normally not involved in the cluster formation. This was tested in the present study by immu-

**Figure 1.** Scanning electron micrographs showing left compound eyes of wild-type (WT) and Om(1E)53 mutant female flies. Anterior is to the left.

nurstaining with neuron-specific antibody MAb22C10, which enables us to visualize photoreceptor precursors (Zipursky et al. 1984).

In the wild-type eye imaginal disc, single photoreceptor precursors (R8) are seen immediately posterior to the morphogenetic furrow, which sweeps from posterior to anterior across the eye imaginal disc, with clusters of three to five photoreceptor precursors in the farther posterior region, all being regularly arranged (Figure 2, A and C; for review see Wolff and Ready 1993). Such regular arrangement and density of the clusters does not seem to be affected by the Om(1E) mutation (Figure 2, B and D). This is in agreement with the above suggestion.

**Molecular cloning and characterization of the Om(1E) region:** The Om(1E) mutation is X-linked and associated with the tom insertion cytologically mapped to 16D (Matsubayashi et al. 1992; also designated 14C on Hinton’s map, Shrimpton et al. 1986). To clone the Om(1E) locus, we screened an Om(1E)53 genomic library with a tom probe, ptom1 (see MATERIALS AND METHODS). Since the Om(1E)53 genome has silent tom insertions in at least four other loci (Shrimpton et al. 1986; Matsubayashi et al. 1992; N. Juni, unpublished...
WT

Om(1E)53

FIGURE 2.—Whole mount eye imaginal discs from third instar larvae of wild type (left) and Om(1E)53 (right). (A and B) Eye imaginal discs stained with MAb22C10 highlighting photoreceptor precursor cells. (C and D) Higher magnifications of A and B, respectively, showing regions immediately posterior to the morphogenetic furrow. Note that the array and density of photoreceptor precursor clusters is normal in the mutant. Arrowheads mark positions of the morphogenetic furrow. Anterior is to the top.

data), retrieved clones were mapped by in situ hybridization to polytene chromosomes of the D38 strain that has a tom-free X chromosome (N. Juni, unpublished data). As a result, one of the retrieved clones, λOm53-49, hybridized uniquely to the 16D region (data not shown). Sequential chromosome walking on a wild-type (ca; px) genomic library was performed with this clone, and a putative Om(1E) region of 70 kb long was obtained (Figure 3A).

The genomic structure of Om(1E) alleles was analyzed by Southern hybridization using DNA probes derived from the cloned region (data not shown). As a result, 7- and 3-kb insertions were found in Om(1E)59a and Om(1E)109, respectively, ~25 kb downstream from the tom insertion site in Om(1E)53 (Figure 3A). To characterize these inserts, we cloned λOm59a-1 and λOm59a-2 from an Om(1E)59a genomic library, and λOm109-1 from an Om(1E)109 genomic library using the pHH3.8 probe that spans the insertion site (Figure 3A). Analyses of these clones by restriction mapping and Southern hybridization with the ptom1 probe revealed that the insertions are indeed tom elements, their direction of transcription being the same as that of the tom insertion in Om(1E)53 (data not shown). However, the tom element in Om(1E)109 is defective consisting of ~1 kb of 5’ and 2 kb of 3’ ends of the intact tom element.

To confirm the involvement of the cloned region in the Om(1E) mutation, we isolated two γ-ray-induced complete revertants of Om(1E)53, Om(1E)53R1 and Om(1E)53R2 and analyzed their genomic structures by Southern hybridization (data not shown). As a result, Om(1E)53R1 was found to have a breakpoint of a gross rearrangement within the cloned region (Figure 3A). In agreement with this, cytological observation of polytene chromosomes revealed that the rearrangement is T(1R;2L)16D;26C (data not shown). On the other hand, Om(1E)53R2 seems to have resulted from excision of the inserted tom element in toto (Figure 3A). Complete loss of inserted tom elements has often been seen in complete revertants of Om mutations (Tanda et al. 1989; Matsubayashi et al. 1991a; Awasaki et al. 1994). These results imply that the cloned region and the tom insertions therein may be responsible for the Om(1E) mutation.

Transcribed regions within the Om(1E) locus: We surveyed transcribed regions by Northern blot analyses using various DNA fragments spanning all over the cloned region as probes. As a result, two transcribed regions, t1 and t2, were identified between the tom insertion site of Om(1E)53 and that of Om(1E)59a or Om(1E)109 (Figure 3A). The t1 region expresses a 1.8-kb transcript throughout development except for the embryonic stage, whereas t2 encodes a 2.6-kb transcript that is expressed in embryos, late third instar larvae, pupae and adults (Figure 3B). No structural difference was noted between the t1 or t2 transcripts of wild type and Om(1E)53 judging from their sizes. In Om(1E)53R1, the t1 transcript is truncated to 1.3 kb (Figure 3B), although this seems not to affect any visible phenotype.

For further analyses, we screened a third larval instar cDNA library of wild type and obtained cDNA clones derived from t1 and t2. Approximate positions and orientations of the t1 and t2 transcribed regions, shown in Figure 3A, were determined from their cDNA sequences (see below) and partial genomic sequences (data not shown).

Localization of t1 and t2 RNAs in developing eye imaginal discs: The t1 and t2 transcripts were localized by in situ hybridization to whole mount eye imaginal discs using antisense RNA probes synthesized from the t1 and t2 cDNAs. In wild-type eye imaginal discs, t1 is expressed in a narrow region corresponding to the morphogenetic furrow at the late third larval instar stage (Figure 4A) but was not detected at the early third instar stage (data not shown), whereas t2 expression is almost at the background level throughout the third...
instar stage (Figure 4G). In eye imaginal discs of Om(1E)53, both t1 and t2 are overexpressed in a similar fashion: their expression is seen in the posterior region of the eye imaginal disc in early third instar larvae where no morphogenetic furrow is seen (data not shown), whereas in the late third instar stage, the region of expression is around the morphogenetic furrow then locating in the center of the eye imaginal disc (Figure 4, B and H). Such an activation of gene expression is not seen in Om(1E)53C1 and Om(1E)53E1 (Figure 4, E, F, K, and L). This indicates that the activated expression of t1 and t2 in Om(1E)53 is driven by the tom insertion.

Eye imaginal discs of Om(1E)59a and Om(1E)109 also display intense t2 expression as in Om(1E)53 (Figure 4, I and J), but they are not distinguishable from wild-type eye imaginal discs with respect to the t1 expression (Figure 4, C and D).

**Hsp70 promoter-t2 transformants mimic the Om(1E) phenotype:** To determine which region is the Om(1E) gene, *D. melanogaster* flies were transformed with either t1 or t2 cDNA, and their effects were studied. There are two ways for inducing the expression of the t1 and t2 cDNA: one is heat-induction using *Hsp70* promoter-cDNA fusion genes (Hsp-t1 and Hsp-t2 constructs, Figure
and the other is *tom* LTR-driven expression using constructs of *Hsp70* promoter-cDNA plus *tom* LTR that presumably carries a transcriptional enhancer. Our transformation experiments with a *tom* LTR-minimal promoter-*lacZ* reporter gene construct have shown that the *tom* LTR is a potential driver of gene expression in the eye imaginal disc (Awasaki et al. 1996). A DNA fragment containing nucleotides −13 to 434 of the 3′ *tom* LTR was therefore placed upstream of *Hsp-t1*/*t2* constructs in the opposite direction to prevent transcription starting from the *tom* promoter (TLHsp-t1 and TLHsp-t2 constructs, Figure 5). Using the *P* element-mediated transformation technique (Rubin and Spradling 1982), we established one *Hsp-t1*, six TLHsp-t1, one *Hsp-t2*, and five TLHsp-t2 transformant lines. Analyses of these transformants demonstrated that the t2 cDNA can evoke overgrowth of the compound eye (see below), while none of the *Hsp-t1* nor TLHsp-t1 transformants exhibit any morphological change (data not shown).

As compared with the *white* (*w*) strain (Figures 6A and 7), ~17% increase of ommatidial number was observed in Hsp-t2 transformants without heat-induction.

**Figure 4.**—*In situ* hybridization to t1 and t2 RNAs in whole mount eye imaginal discs. Expression of t1 (A–F) and t2 (G–L) transcripts in late third instar larval eye imaginal discs of wild-type (WT), *Om(1E)* mutants, and revertants. All eye imaginal discs are of female except for revertants. Positions of the furrow is marked by arrowheads.

**Figure 5.**—Schematic representation of gene constructs used for *P* element-mediated transformation.
Mutation in D. annaassae

FIGURE 6.—Phenocopy of Om(1E) exhibited by Hsp-t2 and TLHsp-t2 transformants. (A–D) Scanning electron micrographs showing female left compound eyes of white, and transformants carrying t2 cDNA constructs. Anterior is to the left. (A) white. (B) Hsp-t2 transformant received no heat induction. (C) Hsp-t2 transformant received heat induction during the early third instar stage. (D) An example of TLHsp-t2 transformants (line #4) received no heat induction. (E–G) Showing t2 expression in whole mount eye imaginal discs of white (E), Hsp-t2 transformant (F), and TLHsp-t2 transformant (line #2) (G). All eye imaginal discs were prepared from female late third instar larvae that received no heat induction. Arrowheads indicate positions of the morphogenetic furrow. Anterior is to the left.

(Figures 6B and 7). This seems to be due to leaky expression at a low level, since whole mount in situ hybridization shows that the t2 expression in eye imaginal discs of Hsp-t2 transformants without heat-induction is as weak as that of the w strain (Figure 6, E and F). On the other hand, ~40% increase of ommatidia was observed in Hsp-t2 transformants that had been subjected to three 1-hr heat-inductions at 37°C at 2-hr intervals during the early (feeding) third instar stage (Figures 6C and 7). When the transformants received the same treatment during the late (wandering) third larval instar stage, the increase of ommatidia was less prominent, being ~27% (Figure 7).

In addition to overgrowth of the compound eye, heat-induced ubiquitous overexpression of t2 during the third larval instar stage evoked other morphological changes. When the transformants received the heat-induction during the early third larval instar stage, the resulting flies exhibited deformation of the wing imaginal disc derivatives very often; many folds were formed around the wing hinge at the expense of the notum, the margin between the scutum and the scutellum became obscure, and the wing was held downward (Figure 8B). Such phenotypes were barely observed in the Hsp-t2 flies that had received the heat-induction during the late third larval instar to the early pupal stages. In this case, however, extra large bristles frequently appeared on the notum (Figure 8, C and D). The extra large bristles seemed to be ectopically formed independently of native bristles and had their own sockets.

It is noteworthy that the ommatidial numbers of the five independent TLHsp-t2 transformants (#1–#5) were always greater than those of the Hsp-t2 transformant when not treated with heat (27–55% increase, Figures 6D and 7). No other morphological change was observed. This suggests eye imaginal disc specific enhancement of gene expression by the tom LTR. Whole mount in situ hybridization confirmed that the t2 expression was intensely induced in eye imaginal discs of third instar larvae of TLHsp-t2 transformants, while no obvious t2 expression was seen in eye imaginal discs of w and Hsp-t2 larvae that received

FIGURE 7.—Average ommatidial numbers of female left eyes of white (w), Hsp-t2 and five independent TLHsp-t2 transformant lines (#1–#5). Hsp-t2 transformants were raised without heat induction (no) and with heat induction during the early third instar stage (@E3rd) or the late third instar stage (@L3rd). N = 4. Bars, SD.
no heat-treatment (Figure 6G). These results demonstrate that the transcribed region t2 is of the *Om(1E)* gene, and that the *tom* LTR may have a tissue-specific enhancer function.

**cDNA sequence and deduced protein of *Om(1E)*:** Figure 9A shows the nucleotide sequence of the *Om(1E)* cDNA and the deduced polypeptide sequence. The *Om(1E)* cDNA consists of 2597 bp in agreement with the mRNA length predicted from the Northern blot analysis. Potential polyadenylation signals (AATAAA) are at the nucleotide positions 2541 and 2553, followed by the poly(A) tail starting at the nucleotide position 2584. There are six repeats of RNA instability motif (ATTATA; Shaw and Kamene, 1986) within the putative 3' untranslated region, suggesting rapid turnover of this message. The cDNA contains a potential open reading frame (ORF) of 1590 nucleotides starting from the first methionine codon at the nucleotide position 311. The deduced protein is 330 residues long with the predicted molecular mass of 59 kDa. However, if started from the second methionine codon at the nucleotide position 509, the protein product may be 464 residues long with the predicted molecular mass of 53 kDa. Although these potential translation start sites do not match the typical translation start consensus of Drosophila (C/AAC/ AATG, Cavener 1987), the protein would start at one of these sites, because anti-*Om(1E)* antibodies detect ~55 kDa protein on a Western blot (N. Juni, unpublished data).

The overall sequence of the *Om(1E)* cDNA and the deduced protein have no significant homology with any other sequences registered in the DDBJ/GenBank/EMBL databases and the SWISS-PROT protein database. The deduced protein is rich in serine, arginine, and glutamine (~10% residues each), and has a poly-serine stretch (residues 125–151) following a threonine-rich stretch (residues 89–122) and a glutamine-rich stretch (residues 432–466). As shown by the GES-scaled hydrophobicity plot in Figure 9B (Engelman et al. 1986), the deduced protein is hydrophilic as a whole, having three large peaks of basic cluster (residues 154–162, 192–202, and 428–467). The last peak and the glutamine-rich stretch constitute a large hydrophilic domain in the carboxyl terminus (residues 428–467). In addition to these hydrophilic domains, there are two prominent hydrophobic domains (residues 62–82 and 117–137). The algorithm after Von Heijne (1992) predicts that these domains are of membrane-spanning potential. Thus, the *Om(1E)* protein is presumed to be a transmembrane protein having two transmembrane domains flanking a short extracellular loop, provided that the translation starts from the first methionine. On the other hand, given the second methionine codon as the translation start site, the first hydrophobic domain may be a signal...
Peptide. In this case, the Om(1E) protein may span the membrane at the second hydrophobic domain alone.

Spatial expression of the Om(1E) gene in embryogenesis and imaginal development: As a first step in inquiring into the normal function of the Om(1E) gene, its spatial expression was examined by in situ hybridization. Because the transcript of the Om(1E) gene appears to be expressed during embryogenesis and imaginal develop-
ment judging from Northern blot analysis, we were especially interested in examining its expression in normal embryos and imaginal primordia of third instar larvae.

The Om(1E) transcript is not present in embryos during the early cleavage stage (Figure 10A), but first appears around nuclei locating in the apical surface of the embryo at the syncytial blastoderm stage (Figure 10B), and then accumulates in all cells at the cellular blastoderm stage (Figure 10C). After this stage, ubiquitous distribution of the transcript was continuously observed throughout the embryonic stage (Figure 10, D–F), although a slightly higher level of the transcript is seen in the ventral furrow during the gastrulation stage (Figure 10D).

On the other hand, the Om(1E) transcript in imaginal discs of third instar larvae was not restricted within any particular areas, being weak and dispersed (data not shown). However, a characteristic distribution of the Om(1E) transcript was observed in the central nervous system of late third instar larvae; while a weak expression was seen all over the cortex of brain hemisphere and ventral ganglion, a prominent expression was seen around the entrance of the optic stalk in the brain of young third instar larvae (Figure 11, A and B), and the region of expression later formed a distinct C-shape (Figure 11, C and D). This region consists of an array of cells locating in the groove of the inner margin of the outer optic anlage and matches the distribution of lamina precursor cells (for review see MEINERTZHAGEN and HANSON 1993).

**Figure 10.** In situ hybridization to the Om(1E) transcript in whole mount embryos. (A) Early cleavage stage. (B) Syncytial blastoderm. The Om(1E) transcript is observed around nuclei (white spots). (C) Cellular blastoderm stage. (D) Ventral furrow formation stage. Note a higher level of expression in the ventral furrow (arrow). (E) Germ band extension stage. (F) Germ band shortening stage. Anterior is to the left. Bar, 100 μm.
**DISCUSSION**

**Involvement of the tom element in the Om(1E) and other Om mutations:** The hypothesis put forward by HINTON (1984) that the Om mutations are associated with the insertion of a transposable genetic element, tom, has been supported by the molecular analyses of the Om(1D), Om(2D), and Om(1A) loci (TANDA et al. 1989; MATSUBAYASHI et al. 1991a,b; TANDA and CORCES 1991; AWASAKI et al. 1994; YOSHIDA et al. 1994). The present study shows that this holds true for another Om mutation, Om(1E).

The Om(1E) mutation is similar to the other Om mutations in that the tom element inserted in the vicinity of the Om gene enhances the gene expression. In the Om(1A), Om(1D), and Om(2D) mutations, the tom inserts responsible for the mutant phenotypes reside in the regions up to 70 kb downstream of transcription initiation sites, and their directions are either parallel or reverse to those of Om genes. In this respect, Om(1E)59a and Om(1E)109 are similar to those Om mutants, but Om(1E)53 differs from the others in that the tom insert resides 15 kb upstream of the transcription initiation site of the Om(1E) gene.

The Om(1E)53 mutant also differs from the others in that the tom insert exerts a positive effect on expression of another nearby gene, tl, although overexpression of the gene appears to be limited only in eye imaginal discs and does not result in any abnormal phenotype. This is in contrast to the case of Om(2D) mutants; the tom insert responsible for the mutant phenotype has no detectable effects on the ornithine aminotransferase.

**FIGURE 11.—In situ hybridization to the Om(1E) transcript in third instar larval central nervous systems. Ventral view of whole mount preparations. Anterior is to the top. (A) Early third instar stage. (C) Late third instar stage. (B and D) Higher magnification of A and C, respectively, showing intense expression of the Om(1E) transcript in lamina precursor cells. Bars, 100 μm.**
(OAT) precursor gene present near the insertion site but affect the Om(2D) gene located farther distally (Yoshida et al. 1994).

It is an interesting question why tom elements cause Om mutations. Almost all mutations so far obtained in the Om mutability system cause defects in the morphology of adult compound eye (Hinton 1984; for review see Tanda et al. 1993), although the tom element is found in a number of loci in the genome (Shrimpton et al. 1986; Matsubayashi et al. 1992). This means that tom may have no preference of insertion sites, but it mutates Om genes when it is fortuitously inserted in their neighborhood. Taking into account all the findings so far obtained by molecular analyses of Om mutants, a most plausible explanation as to why the tom element induces Om mutations may be that the tom element carries an enhancer sequence or sequences that act on nearby genes with the help of an eye-disc specific regulatory factor, and that the Om genes might be a set of genes, the product of which exerts influence upon eye morphogenesis when present in excess. Similar to Om mutations are some retrotransposon-or retrovirus-induced gain-of-function mutations. In the Hairy-wing (Hu) mutation of D. melanogaster, the gypsy element inserts in the achaete (ac) gene result in truncation and overproduction of the transcript (Campuzano et al. 1986). The overexpression of ac is under the influence of the suppressor-of-Hairy-wing [su(Hu)] gene, of which product binds to the 5′ untranslated region of the gypsy element (Mazo et al. 1989). In the case of yeast ROAM (regulated overproducing alleles under mating signals) mutations, overexpression of various genes are induced by the MAT locus-dependent enhancer present in the 5′ leader sequence of Ty1 insertions (for review see Boeke 1989). Ty1 elements in ROAM mutations are located exclusively in the juxtaposition of target gene promoters in the opposite direction. Most analogous with the Om mutations are tumorigenic mutations in vertebrates that are induced by retroviral proviruses (for review see Nusse 1986). For example, the provirus of mouse mammary tumor virus (MMTV) activates the expression of several heterologous proto-oncogenes including int-1 (Wnt-1) and int-2 when inserted into loci either upstream or downstream of the target gene promoters, sometimes more than 10 kb apart (for review see Nusse 1988). The int genes are not expressed in normal mammary glands, but their expression is stimulated specifically in the organ under the influence of steroid hormones when the provirus is inserted in their vicinity. In this case, overexpression of the gene is brought about by a cooperative action of the hormone response element (Chandler et al. 1983; Scheideeriu et al. 1983) and the mammary gland-specific enhancer (Lefebvre et al. 1991; Mink et al. 1992; Mok et al. 1992) carried by the MMTV LTR.

Recently, Mozzer and Benzer (1994) have reported that the 17.6 transposable element of D. melanogaster, which has a considerable homology with the tom element (Tanda et al. 1988) and the avian leukemia-sarcoma viral genome (Kugimya et al. 1983), possesses lamina precursor cell-specific and eye imaginal disc-specific enhancers within its LTR. Thus, it is not rare that retrotransposons have tissue-specific enhancers in their LTRs that can act positively on relevant genes. To demonstrate the presence of tissue-specific enhancer in the tom LTR, we have ligated a tom LTR with lacZ and transformed D. melanogaster with this construct. As a result, it was found that the tom LTR is indeed effective in inducing lacZ expression in eye imaginal discs (Awasaki et al. 1996). Taking advantage of this, we have made constructs containing a tom LTR and cDNA sequences, and tested in the present study if transformants carrying these constructs mimic the Om(1E) phenotype. The results have clearly indicated that the tom LTR indeed possesses an eye imaginal disc-specific enhancer.

Possible function of the Om(1E) gene: The Om(1E) mutants exhibit overgrowth of the compound eye without affecting the regular array of ommatidia and the ommatidial structure. Our results indicate that the Om(1E) phenotype may result from overproliferation of undifferentiated cells induced by excess Om(1E) gene product in the eye imaginal disc.

The presumptive Om(1E) gene product is a novel transmembrane protein containing stretches of monoamino acids such as glutamine, serine, and threonine. The Om(1E) protein is likely to have either one or two membrane-spanning domains. In either case, the putative extracellular domain is highly rich in serine and threonine (~50%). Serine/threonine-rich extracellular domains are seen in several proteins that appear to be involved in cell interaction, such as α-agglutinin attachment subunit of yeast (Roy et al. 1991), cell surface antigen 114/A10 of mouse (Dougherty et al. 1989), and Zipper protein of Drosophila (Zhao et al. 1988), and are considered to be subject to O-glycosylation. Thus, the possibility may be envisaged that the Om(1E) gene product is a glycoprotein involving in intercellular communication that may be required for the growth control of imaginal discs.

Whole mount in situ hybridization studies have shown ubiquitous expression of the Om(1E) gene in embryonic cells, developing imaginal discs, and the cortex of larval central nervous system in normal individuals. Although these results suggest a possible role of the Om(1E) gene in those tissues, its actual function remains to be explored. Nevertheless, forced overexpression of the Om(1E) gene during the early third instar stage affects the morphogenesis of compound eye and notum in the transformants, suggesting the existence of a biological pathway affected by this gene in the development of these organs.

In addition, extra large bristles are induced by overexpression of the Om(1E) gene during the late larval or early pupal stage. This phenotype mimics that caused by mutations of genes involved in differentiation of the sensory organ precursor cell, such as gain-of-function
mutations of the *achaete* and/or *scaute* genes, or loss-of-function mutations of neurogenic genes (for review see GHISEN and DAMBY-CHAUDRIE 1989; SIMPSON 1990; CAMPUZANO and MODELL 1992). It is therefore probable that the *Om(1E)* gene might play a role in differentiation of the sensory organ precursor cell.

On the other hand, the *Om(1E)* gene is prominently expressed in lamina precursor cells. This suggests a possible *Om(1E)* function in an early phase of lamina cell differentiation. This is particularly of interest, because the lamina is currently one of the most interesting topics in Drosophila biology. A great number of morphological, developmental, and physiological studies on the lamina have been accumulated, and the lamina is the best characterized nervous system in Drosophila at present (for review see MEINERTZHAGEN and HANSON 1993). The lamina precursor differentiate to the lamina cortex through a wave of mitosis at the groove in response to progressive innervation of photoreceptor axons, implying cell-interaction-dependent differentiation (HOFBAUER and CAMPOS-ORTEGA 1990; SELLECK et al. 1992). This seems to be consistent with the above notion that the *Om(1E)* protein might be involved in cell interaction and cell proliferation. Recently, special attention has been paid to genes controlling development of the imaginal central nervous components, including the lamina (KRETZSCHMAR et al. 1991; TIX et al. 1991; DATTA et al. 1993). However, only a few genes have been characterized in this field of research, and little is known about the genetic basis of the lamina development. Thus, the *Om(1E)* gene might provide a breakthrough for this query. This merits further elaboration.

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