Molecular Cloning and Analysis of *l(1)ogre*, a Locus of *Drosophila melanogaster* With Prominent Effects on the Postembryonic Development of the Central Nervous System

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ABSTRACT

Previous genetic studies have shown that wild-type function of the *l(1)ogre* (*lethal (1) optic ganglion reduced*) locus is essential for the generation and/or maintenance of the postembryonic neuroblasts including those from which the optic lobe is descended. In the present study molecular isolation and characterization of the *l(1)ogre* locus was carried out to study the structure and expression of this gene in order to gain information about the nature of *l(1)ogre* function and its relevance to the development of the central nervous system. About 70 kilobases (kb) of genomic DNA were isolated that spanned the region where *l(1)ogre* was known to reside. Southern analysis of a *l(1)ogre* mutation and subsequent *P* element-mediated DNA transformation mapped the *l(1)ogre*$^+$ function within a genomic fragment of 12.5 kb. Northern analyses showed that a 2.9-kb message transcribed from this 12.5-kb region represented *l(1)ogre*. A 2.15-kb portion of a corresponding cDNA clone was sequenced. An open reading frame (ORF) of 1,086 base pairs was found, and a protein sequence of 362 amino acids with one highly hydrophobic segment was deduced from conceptual translation of this ORF.

THE optic lobe of the fruit fly *Drosophila melanogaster* consists of four interconnected ganglia (lamina, medulla, lobula and lobula plate) which process and integrate visual information from the retina and transmit it to the central brain (reviewed by KANKEL *et al.* 1980; FISCHBACH and DITTRICH 1989). The anatomy of these structures is relatively simple and has been comparatively well characterized both in terms of the detailed morphology of individual neuronal elements as well as the overall structure of tissues (FISCHBACH 1983; GAREN and KANKEL 1983; FISCHBACH and DITTRICH 1989). Studies also exist which define many of the aspects of the ontogeny of this subset of the central nervous system (*e.g.* see WHITE and KANKEL 1978; CAMPOS-ORTEGA and HARTENSTEIN 1985; HOFBAUER and CAMPOS-ORTEGA 1990). From the standpoint of a genetic analysis, the optic lobe, indeed the entire visual system, is completely dispensable for organismal viability (at least within the environment defined by the standard conditions of laboratory culture); this has allowed the isolation of mutations which cause a near elimination of the optic lobe or its precursors or give rise to an obvious perturbation of its structure (*e.g.* see MEYEROWITZ and KANKEL 1978; CAMPOS, GROSSMAN and WHITE 1985; LIPSHITZ and KANKEL 1985; review by FISCHBACH *et al.* 1989).

The *l(1)ogre* locus (lethal (1) optic ganglion reduced), which plays an essential role in the normal development of the optic lobe, was originally identified via a mutation which impaired visual pattern recognition (LIPSHITZ and KANKEL 1985). To date one viable and three lethal alleles have been isolated at this locus (NICKLAS and CLINE 1983; LIPSHITZ and KANKEL 1985). Histological observations at the light microscopic level on adults carrying the viable allele detected extensive morphological abnormalities in the optic lobe but not elsewhere. Similar analyses of rare adult escapers carrying lethal alleles demonstrated structural aberrations in many other areas of the CNS, as well as the optic lobes, but not outside of the CNS proper. Previous genetic mosaic studies (LIPSHITZ and KANKEL 1985) have suggested that gene action within or in the vicinity of the optic lobe primordia is responsible for the following phenotypes: defective vision, defective flight and abnormalities in the optic lobe structure.

Examination of mutant larvae at the light microscopic level showed no detectable abnormalities except for a significant reduction of the postembryonic neuroblasts and their offspring. Those neuroblasts include the aggregates of neuroblasts called the optic formation centers which give rise to the adult optic lobe (LIPSHITZ and KANKEL 1985) and the giant neuroblasts distributed over the periphery of the CNS (SINGH, SINGH and KANKEL 1989). Electron microscopic studies of mutant organisms revealed extensive cell degeneration in the late-larval CNS (SINGH, SINGH and KANKEL 1989). Thus it seems that the phenotypes in the CNS are caused by a combination of reduced

postembryonic neurogenic activity and cell degeneration. The EM examination revealed more subtle structural abnormalities in the imaginal discs, their derivatives and muscles, but it is not yet known whether these defects are the primary effects of the mutations or the consequences of higher order interactions.

These genetic and developmental studies have generated a hypothesis that the wild-type function of the *l(1)ogre* gene is crucial for and specific to postembryonic neurogenesis. An attempt was made to clone this gene so as to test this hypothesis by studying (1) the specificity of its expression and (2) the biochemical function(s) of its product(s). In this article, we report the molecular cloning of this gene and the amino acid sequence of its hypothetical protein product.

MATERIALS AND METHODS

Purification of plasmid and lambda phage DNA: Plasmid and phage DNA were isolated according to MANIATIS, FRITSCH and SAMBROOK (1982). In some mini-preparations of lambda phage DNA, DNA could not be well digested with restriction enzymes; in those cases, DNA was further purified with Elutip (Schleicher & Schuell).

Purification of nucleic acids from *D. melanogaster*: Genomic DNA and poly(A$^+$) RNA were isolated according to MANIATIS, FRITSCH and SAMBROOK (1982) and Artavanis-Tsakonas, Muskavitch and YEDBOVNICK (1983), respectively.

Radioisotopic labeling of double-stranded DNA templates: Double-stranded DNA templates were radioisotopically labeled either by nick-translation or random primed labeling. Nick-translation was carried out according to MANIATIS, FRITSCH and SAMBROOK (1982) or with a nick-translation kit from BRL (Bethesda Research Laboratories). Random primed labeling was done according to FEINBERG and VOGELSTEIN (1984).

Southern and Northern hybridization analysis: Double-stranded DNA fragments were separated by electrophoresis, denatured and neutralized according to MANIATIS, FRITSCH and SAMBROOK (1982). RNA was denatured and run in agarose gels containing formaldehyde according to MANIATIS, FRITSCH and SAMBROOK (1982). Nucleic acids were transferred onto either nitrocellulose filters (Schleicher & Schuell) or GeneScreen (NEN, Du Pont NEN Research Products) according to MANIATIS, FRITSCH and SAMBROOK (1982) or instructions from NEN, respectively. After blotting, filters were baked at 80° for 2 hr (Southern filters) or 4 hr (Northern filters) under vacuum. Hybridization was carried out at 42° in hybridization buffer (50% formamide, 10 × Denhardt's solution, 50 mM Tris-Cl (pH 7.5), 0.1% sodium pyrophosphate, 1.0% SDS, 1 M NaCl, 10% dextran sulfate and 100 μg/ml sheared salmon sperm DNA boiled and added right before use). Radioisotopically labeled probes were added to an activity of 1×10^6 dpm (Southern analysis) or 5×10^6 dpm (Northern analysis) per 1 ml of the hybridization buffer.

***In situ* hybridization to polytene chromosomes:** DNA templates were nick-translated with bio-16-dUTP (ENZO Biochem, Inc.) and a trace amount of deoxy [1′,2′,5-^{3}H] cytidine 5′-triphosphate (to measure the incorporation of bio-16-dUTP). Preparation of polytene chromosome squashes and hybridization were done according to ZUKER, COWMAN and RUBIN (1985) except that hybridization was done at 37° in hybridization buffer (40% formamide, 4 × SSC, 50 mM each of Na_2HPO_4 and NaH_2PO_4 and 200 μg/ml tRNA). The biotin signal was detected with DETEK I-*hrp* signal generating system (ENZO) according to instructions from the manufacturer.

***P* element-mediated DNA transformation:** The pπ25.7wc helper plasmid was amplified in HB101 *Escherichia coli* cells in L broth with 200 mg/liter ampicillin. Microinjection into ry^{506} embryos was carried out according to SPRADLING (1986). The concentrations of 4K12.5Xh/Carnegie 20 (see below) and pπ25.7wc in the injection buffer (5 mM KCl and 0.1 mM $NaPO_4$ (pH 7.8)) were about 1 mg/ml and 0.1 mg/ml, respectively.

Nucleotide sequencing: A 2.15-kb cDNA fragment to be sequenced was subcloned into the M13mp18 vector. Nested deletions were generated using the CYCLONE kit (IBI). Sequencing reactions were done with a Sequenase DNA sequencing kit (USB). All the sequencing reactions were done with dITP (in place of dGTP) as well as with dGTP. Alternatively restriction fragments from a cDNA clone were subcloned into the pGEM-3Zf(−) vector (Promega) and sequenced as double-stranded templates using EP6 or T7 Promotor Sequencing Primer (Promega).

Reduced silver staining procedure: This was carried out according to HARTE and KANKEL (1983).

RESULTS

Chromosome walking: Cytogenetic studies localized the *l(1)ogre* locus between the breakpoints of deficiencies *Df(1)Sxl^{bt}* (in salivary region 6E1/2) and *Df(1)HA32* (in 6E4/5) on the × chromosome (Figure 1a; NICKLAS and CLINE 1983; LIPSHITZ and KANKEL 1985). J. K. LIM kindly provided us with a genomic DNA clone, Oregon R :: C11, from the 6E4/5 region along with information about its orientation. By an *in situ* hybridization to the wild-type and *Df(1)HA32* polytene chromosomes, it was found that this clone resided proximal to the *Df(1)HA32* breakpoint in 6E4/5, as it hybridized to the wild-type chromosome but not to the deficient chromosome (data not shown). Since *l(1)ogre* resides distal to this breakpoint (Figure 1a), a distal chromosome walk was initiated from Oregon R :: C11 using a Canton S genomic library in the Charon 4A lambda phage vector (MANIATIS *et al.* 1978) and a Canton S genomic library in the EMBL3 vector (a generous gift from HENRY SUN). The *Df(1)HA32* breakpoint was mapped by Southern analysis (Figure 2a). The *Df(1)Sxl^{bt}* breakpoint in 6E1/2 was approximately localized between the distal ends of clones 8E and 9D, as the former did not hybridize to the deficient chromosome while the latter did (Figure 2b). Thus, the genomic DNA (about 70 kb) between the two breakpoints was isolated (Figure 1b). At least part of the *l(1)ogre* locus should reside in this region. Hereafter this region bracketed by the two breakpoints will be referred to as the *l(1)ogre* region.

Mapping of transcripts in the *l(1)ogre* region: Next we searched, by Northern analysis, for poly(A$^+$) messages transcribed from the *l(1)ogre* region. We examined embryonic, early larval and late larval pop-

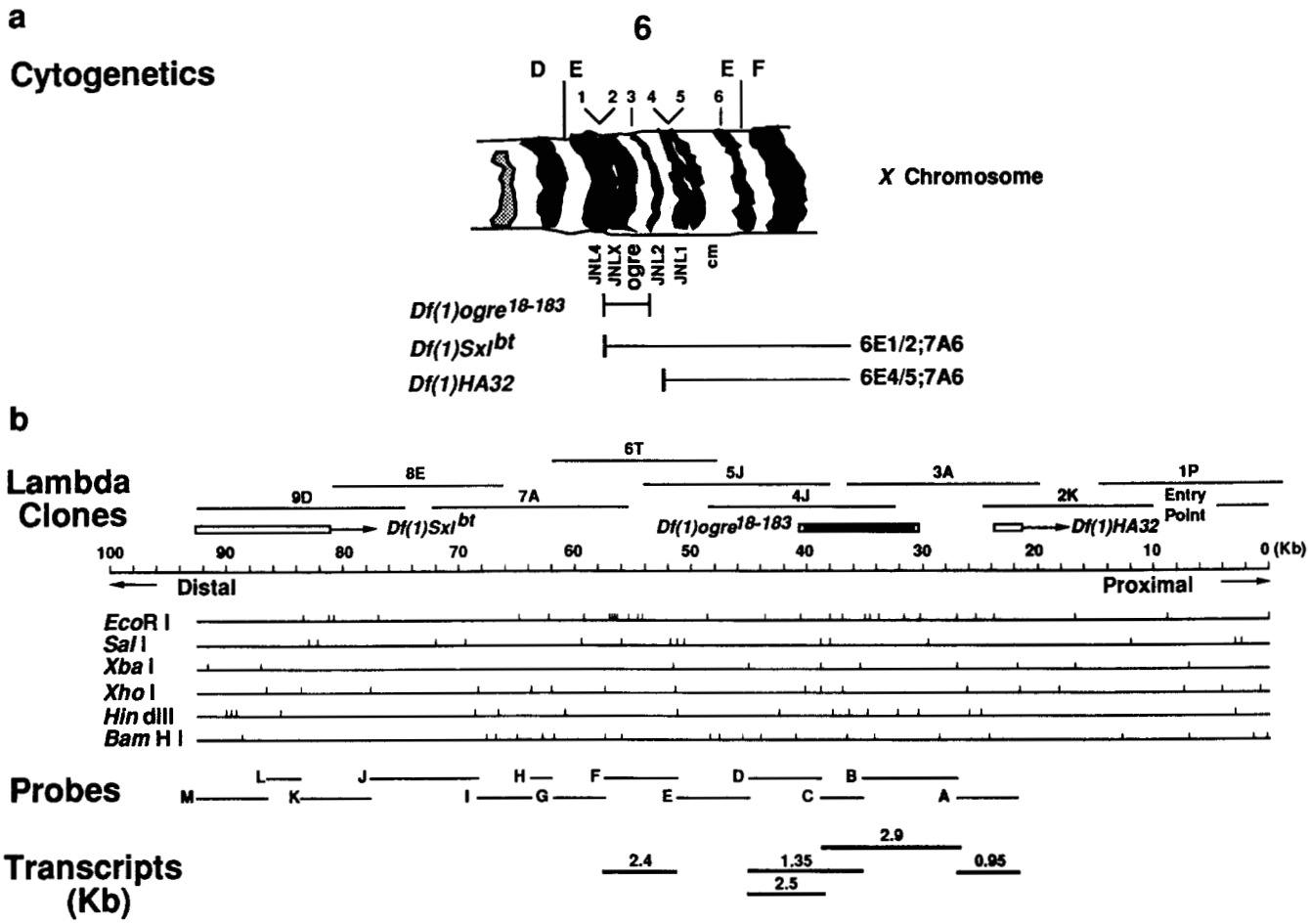

FIGURE 1.—Cytogenetic location of the *l(1)ogre* locus and mapping of recombinant phages, the *Df(1)HA32*, *Df(1)Sxl*^{bt} and *Df(1)ogre*$^{18\text{-}183}$ breakpoints and transcripts in the *l(1)ogre* region. (**a**) Cytogenetic map of salivary region 6E of the × chromosome. *l(1)ogre* was mapped between the breakpoints of *Df(1)HA32* in 6E4/5 and *Df(1)Sxl*^{bt} in 6E1/2 together with the *JNL2* and *JNLX* loci (NICKLAS and CLINE 1983; LIPSHITZ and KANKEL 1985). The spontaneous mutation *18–183* (SCHALET 1986) was found to be a small deletion and to uncover *l(1)ogre* (see the text and Figure 4b); with A. P. SCHALET's permission, this mutation was renamed as *Df(1)ogre*$^{18\text{-}183}$. Complementation tests showed this deletion to be *JNL2+* but *JNLX-* (WATANABE 1989). (**b**) A chromosomal walk to isolate genomic DNA in the *l(1)ogre* region and the location of the *Df(1)HA32* breakpoint in 6E4/5, the *Df(1)Sxl*^{bt} breakpoint in 6E1/2 (see Figure 2), the *Df(1)ogre*$^{18\text{-}183}$ breakpoint (see Figure 4) and transcripts in the *l(1)ogre* region. The scale is in kb, based on a zero point at the proximal end of the entry point to the walk. The lambda phage clones are shown at the top, and the location of the breakpoint, determined as shown in Figure 2, is immediately above the coordinate scale. The location of the *Df(1)Sxl*^{bt} breakpoint is approximate (see text). See Figure 4 for the localization of the *Df(1)ogre*$^{18\text{-}183}$ breakpoint. A restriction map using six enzymes is immediately below the scale. Thirteen probes (A–M) were made for Northern analyses by electrophoretically purifying the respective restriction fragments. For each Northern hybridization, 5 μg of poly(A$^+$) RNA from each of the following three stages was used: (1) embryos (0–24 hr after egg laying (AEL) at 25°); (2) A 1:1 mixture of first instar larvae (33 ± 2 hr AEL) and second instar larvae (60 ± 2 hr AEL); (3) A 1:1 mixture of early third instar (90 ± 2 hr AEL) and late third instar (120 ± 2 hr AEL) larvae. A map of detected transcripts is shown at the bottom.

ulations of the Oregon R wild-type strain for transcripts, as previous genetic studies had suggested that the *l(1)ogre* gene should be expressed in the embryonic and/or larval stages (LIPSHITZ and KANKEL 1985). Five transcripts were found clustering proximally in this region (Figure 1b); all were expressed in all three stages examined. With the hope of identifying transcript(s) from the *l(1)ogre* locus, we compared these transcripts from wild-type third instar larvae with the ones from mutant individuals carrying the *ljnl3* allele, the strongest allele available at that time (LIPSHITZ and KANKEL 1985). The only noticeable difference in the transcription profile between the wild type and *ljnl3*-bearing individuals involved the 2.9-kb transcript whose expression was somewhat reduced in the mutants (Figure 3A), suggesting the possibility that this was a *l(1)ogre* transcript. The amount of the 1.35- and 0.95-kb transcripts in the mutants was comparable to that in the wild-type (Figure 3, A and B). The 2.5- and 2.4-kb transcripts (Figure 1b) were observed in the *ljnl3*-bearing mutants. However, because of their low abundance, comparison could not be successfully made between the wild type and the mutants (data not shown).

A Southern analysis of genomic DNA of the *18-183* mutation: After the transcript mapping had been completed, a spontaneous lethal mutation, *18-183* (SCHALET 1986), was found to be *l(1)ogre*$^-$ (K. A.

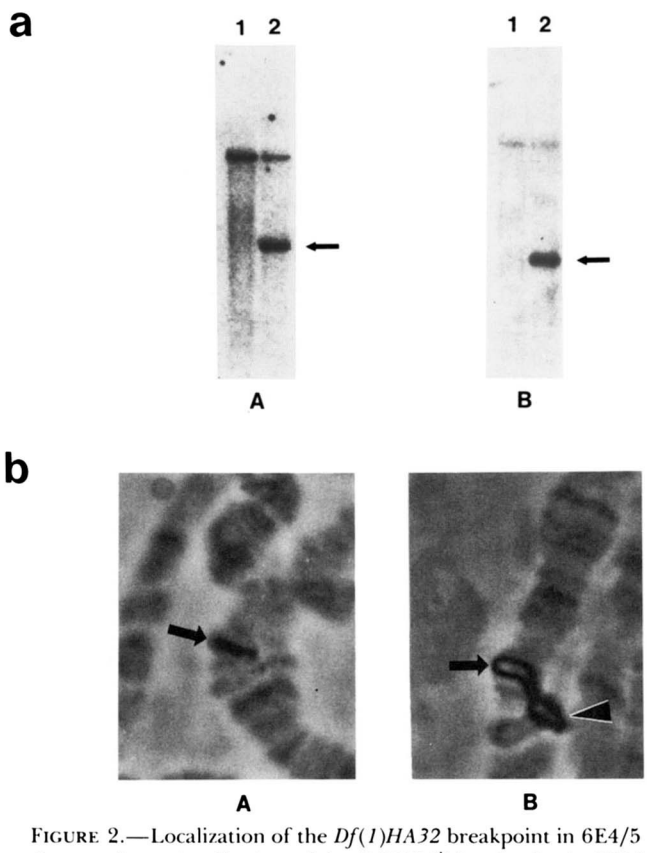

FIGURE 2.—Localization of the *Df(1)HA32* breakpoint in 6E4/5 and approximate localization of the *Df(1)Sxl^{bt}* breakpoint in 6E1/2. (**a**) Southern analysis to localize the *Df(1)HA32* breakpoint. Genomic DNA was extracted from adult females of the following two genotypes: lane 1, wild-type M56i (Amherst); lane 2, *Df(1)HA32*/+ (+ represents the wild-type *X* chromosome from M56i; *Df(1)HA32* was generated on the M56i *X* chromosome). One microgram of genomic DNA from those two materials was cleaved with *Eco*RI (panel A) or *Hin*dIII (panel B), separated by electrophoresis, blotted and hybridized with a ^{32}P-labeled *Eco*RI fragment (16.7–23.8 according to the coordinate system of Figure 1). Anomalous fragments (3.9 kb in panel A and 3.4 kb in panel B) are found (arrows) that are presumed to contain the deficiency. In panel A a fragment of about 15 kb was also detected; we presume that the *Eco*RI site at 16.7 in Figure 1b (the restriction map is based on the Canton S genomic DNA) is absent in the M56i chromosome and the absence results in the formation of a 15.1-kb fragment (8.7–23.8). In panel B a 22-kb *Hin*dIII fragment (3.0–25.0) was also detected. (**b**) *In situ* hybridization of the following two lambda clones to polytene chromosomes from *Df(1)Sxl^{bt}*/+ females (+ represents the wild-type *X* chromosome from Oregon R): panel A, lambda clone 8E; panel B, 9D (Figure 1). 8E is seen to hybridize to the wild-type *X* chromosome at 6E1/2 (the arrow in A) but not to the *Df(1)Sxl^{bt}* chromosome, while 9D hybridizes both to the wild type (the arrow in B) and *Df(1)Sxl^{bt}* (the arrowhead) *X* chromosomes at 6E1/2.

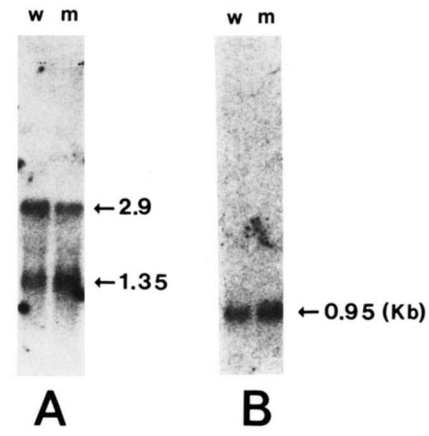

FIGURE 3.—Comparison of the wild-type and *l(1)ogre^{ljnl3}* mutant larvae in transcription of the 2.9-, 1.35- and 0.95-kb transcripts. A sample of 10 μg of poly(A$^+$) RNA from wild-type (Oregon R) third instar larvae ("w" lanes) and mutant third instar larvae (*l(1)ogre^{ljnl3}* males, "m" lanes) was separated by electrophoresis, blotted onto nitrocellulose filters and hybridized to the following two probes (see Figure 1 for the locations of the probes): panel A, probe C; and panel B, probe A. The transcription pattern of the 1.35- and 0.95-kb messages in the mutant larvae is comparable to the wild-type pattern, while the amount of the 2.9-kb transcript is reduced in the mutants (panel A).

STARK, unpublished observation). As spontaneous mutations are often associated with transposon insertions, genomic DNA of the *18-183* mutation was examined by Southern analysis for molecular lesions which might indicate the location of the *l(1)ogre* locus. The *ljnl3* allele (the most severe among the chemically generated alleles, LIPSHITZ and KANKEL 1985) was also analyzed. No abnormalities were detected in the *ljnl3* genomic DNA in the *l(1)ogre* region (data not shown), but *18-183* was found to be a small deficiency of about 10 kb (Figure 4a). With A. P. SCHALET's agreement, *18-183* was renamed as *Df(1)ogre$^{18\text{-}183}$*. Subsequent complementation tests against two lethal mutations in the *l(1)ogre* region, *JNL2* and *JNLX*, have found that *Df(1)ogre$^{18\text{-}183}$* is *JNL2$^+$* but *JNLX$^-$* (WATANABE 1989).

Transcripts in the vicinity of the *Df(1)ogre$^{18\text{-}183}$* deletion: This Southern analysis suggested that the *Df(1)ogre$^{18\text{-}183}$* deficiency uncovered at least a portion of the *l(1)ogre* locus. If this was true, the structural region of the *l(1)ogre* locus should be within or in the vicinity of the deletion. The transcripts around the deficiency were mapped by using smaller probes (Figure 4b) than in Figure 1. As shown in Figure 4b, the deficiency should delete, at least partly, the transcribed regions for the 2.9-kb, 1.35-kb and possibly 2.5-kb transcripts.

***P* element-mediated DNA transformation and identification of the *l(1)ogre* transcript:** The above results suggested that the 2.9-kb transcript might be the *l(1)ogre* message since this transcript was less abundant in mutant larvae carrying the *ljnl3* allele than in the wild-type, and at least part of the transcribed region of the genomic DNA for this transcript was deleted by the *Df(1)ogre$^{18\text{-}183}$* deficiency. To test that possibility, a *P* element-mediated DNA transformation (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982) was carried out. A genomic fragment 4K12.5Xh (Figure 4b) which spanned the entire transcribed region of the 2.9-kb message was subcloned into the ry$^+$ Carnegie 20 vector (RUBIN and SPRADLING 1983) and introduced into ry$^-$ embryos by

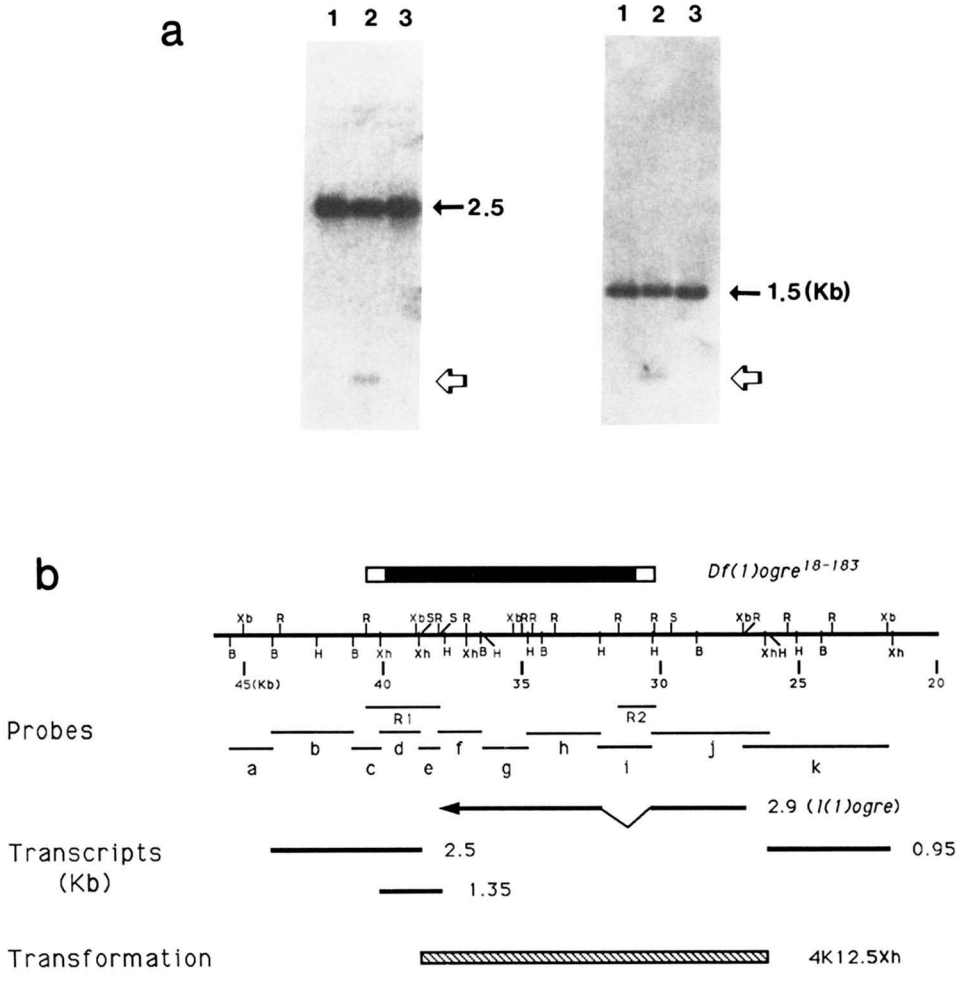

FIGURE 4.—Molecular characterization of the *18-183* mutation. (**a**) Southern analysis of the *18-183* mutation. Genomic DNA was isolated from adult females of the following three genotypes: lane 1, wild-type Canton S; lane 2, *18-183*/+ (+ represents a wild-type *X* chromosome from Canton S); lane 3, *18-37*/+. Both *18-183* and *18-37* were derived from the wild-type M56i (Amherst) strain (SCHALET 1986); *18-37* is an *X*-linked lethal mutation genetically mapped outside of the *l(1)ogre* region (K. A. STARK, personal communication). A sample of 1 μg of *Eco*RI-cleaved genomic DNA from the above mentioned three materials was Southern-blotted and hybridized with two probes, R1 (panel A) and R2 (panel B). R1 and R2 are *Eco*R I genomic fragments of 2.5 and 1.5 kb in size, respectively (see **b**). Both probes detected apparently the same anomalous fragment, 0.65 kb, in lanes 2 (open arrows), indicating that about 10 kb of genomic DNA between the *Eco*RI sites at +30.1 and +40.6 (according to the coordinate system of **b**) is deleted in the *18-183* *X* chromosome leaving 0.65 kb between the two sites. (**b**) A molecular map around the *Df(1)ogre*$^{18\text{-}183}$ (renamed from *18-183*, see text) deletion. The coordinate system is the same as in Figure 1. The location of the *Df(1)ogre*$^{18\text{-}183}$ deletion is shown at the top: the open boxes and the filled box indicate the breakpoints and the deleted portion, respectively. Immediately above the coordinate scale is a restriction map (a part of the map in Figure 1) employing the following six enzymes: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; S, *Sal*I; Xb, *Xba*I; and Xh, *Xho*I. Transcripts from embryonic, early larval and late larval stages (see the legend for Figure 1) are more finely mapped with eleven probes (a–k). The arrow representing the 2.9-kb message indicates the direction of transcription (5′ to 3′). Genomic fragment 4K12.5Xh shown at the bottom successfully rescued *l(1)ogre* phenotypes in a *P* element-mediated DNA transformation (Figure 6).

microinjection. About two thousand embryos were injected and at least seventeen ry$^+$ lines were obtained. G_2 males from five of these ry$^+$ transformant lines were crossed to *l(1)ogre* mutants according to the scheme in Figure 5 in order to test whether the 4K12.5Xh genomic DNA could rescue *l(1)ogre* phenotypes. In crosses with each of the five lines the lethalities of the *ll523*, *lj555* and *ljnl3* alleles were rescued (Table 1, the 6-1 line was not tested against the *ll523* allele). Three of these lines (6-1, 9-3 and 12-2) were further tested for the rescue of the mutant optic lobe phenotype. All three lines restored the wild-type optic lobe structure; see Figure 6 for an illustration involving the 9-3 line. Thus, we concluded that the functional part of the *l(1)ogre* gene resided in the 4K12.5Xh DNA. Four transformant lines (9-3, 12-2,

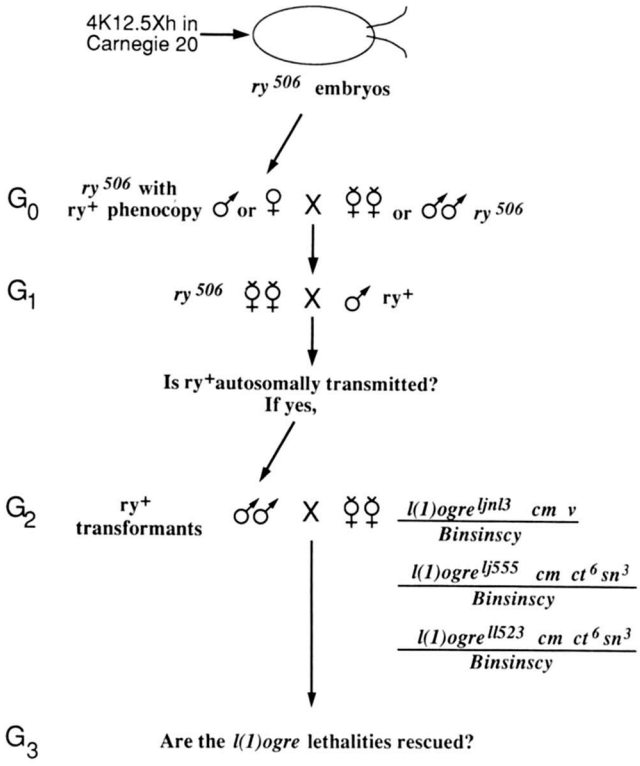

FIGURE 5.—Scheme for *P* element-mediated transformation to show that the 4K12.5Xh fragment contains the functional part of the *l(1)ogre* locus. About 2000 ry^{506} embryos were microinjected with a mixture of the following two plasmids: (1) the 4K12.5Xh fragment subcloned into the Carnegie 20 vector and (2) the pπ25.7wc helper plasmid. Among the injected organisms which grew into adulthood (G_0), 120 expressed ry^+ phenocopy from the ry^+ marker on Carnegie 20; these ry^+ adults were individually mated to ry^{506} partners. A ry^+ phenotype was observed among G_1 offspring in 17 of these pair-mated lines: the ry^+ G_1 offspring in those lines should have had 4K12.5Xh and the ry^+ locus from Carnegie 20 integrated into their genome. ry^+ G_1 males were mated to ry^{506} females to establish transformant stocks; by analyzing the G_2 offspring it could be determined whether 4K12.5Xh integration was into the *X* chromosome or one of the autosomes. G_2 males from five transformant lines with autosomal integration were mated to *l(1)ogre^{ljnl3} cm v/Binsinscy*, *l(1)ogre^{lj555} cm ct^6 sn^3/Binsinscy* and *l(1)ogre^{ll523} cm ct^6 sn^3/Binsinscy* females to see the rescue of *l(1)ogre* lethalities by the 4K12.5Xh fragment. All five of those transformant fathers gave rise to cm v or cm ct sn offspring, showing that 4K12.5Xh contained the *l(1)ogre* locus (Table 1). In addition to this rescue of the lethalities, histological examination of the cm v offspring showed that the optic lobe phenotype was also rescued by 4K12.5Xh (Figure 6a). *ry*, *cm* and *v* are all eye-color markers (LINDSLEY and GRELL 1968), and *Binsinscy* is an *X* chromosome balancer (LINDSLEY and ZIMM 1987).

24-1 and 27-1) failed to rescue the lethalities of two other genetic loci in the *l(1)ogre* region, *JNLX* and *JNL2* (WATANABE 1989) or the lethality of *Df(1)ogre$^{18\text{-}183}$*, presumably because this deficiency deleted at least one other vital gene (data not shown).

As seen in Figure 4b, the 4K12.5Xh fragment apparently included the entire transcribed region for the 2.9-kb message and a part of the transcriptional unit for the 1.35-kb message. To find out which of these messages was encoded by the *l(1)ogre* locus, the

TABLE 1

Male offspring from crosses (see Figure 5) of G_2 transformant males to females carrying the *ljn13,lj555* or *ll523* allele of *l(1)ogre* over the *Binsinscy* balancer

Transformant line	*l(1)ogre^{ljn13} cm v/Binsinscy* No. of male offspring		*l(1)ogre^{lj555} cm dt^6 sn^3/Binsinscy* No. of male offspring		*l(1)ogre^{ll523} cm ct^6 sn^3/Binsinscy* No. of male offspring	
	cm v	*Binsinscy*	*cm ct v*	*Binsinscy*	*cm ct v*	*Binsinscy*
6-1	35	58	15	42		
9-3	32	41	21	27	23	36
12-2	44	54	14	57	26	41
24-1	15	24	11	30	12	29
27-1	25	27	26	17	30	32
Control[a]	3[b]	190	1	23	0	45

[a] ry^{506} males were used as fathers.

[b] All three males showed optic lobe phenotypes characteristic to *l(1)ogre*.

G_2 transformant male parents have either chromosome 2 or 3 heterozygous for the transformant chromosome over a wild-type second chromosome or a ry^{506} third chromosome, respectively.

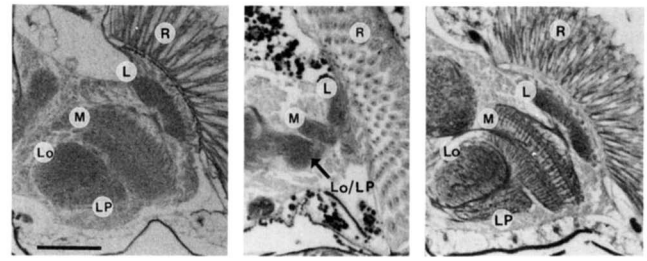

FIGURE 6.—Restoration of the wild-type optic lobe structure by the 4K12.5Xh genomic fragment. Horizontal sections through the adult head of the following three genotypes: panel A, a wild-type adult male (Oregon R); panel B, a mutant, male, pharate-adult pupa (*l(1)ogre^{ljnl3} cm v/Y*); and panel C, a G_3 (Figure 5) transformant adult male (*l(1)ogre^{ljnl3} cm v/Y*; $Tr^{9\text{-}3}/+_2$. $Tr^{9\text{-}3}$ and $+_2$ designate the transformant second chromosome from the 9-3 line and a wild-type second chromosome, respectively). Anterior is up and lateral is to the right. The wild-type optic lobe is composed of lamina (L), medulla (M), lobula (Lo), and lobula plate (LP), and located medial to the retina (R). In the mutant, the volume of those four ganglia is reduced, the medulla is facing anteriorly, and the division of lobula and lobula plate is not seen. The optic lobe of the transformant is indistinguishable from the wild-type optic lobe. Bar = 50 μm (in A).

presence/absence of these messages was examined in *Df(1)ogre$^{18\text{-}183}$* mutant larvae and in *Df(1)ogre$^{18\text{-}183}$* individuals carrying the ectopically integrated 4K12.5Xh DNA from the 9-3 transformant line. As shown in Figure 7, both messages were absent in the mutants while the 4K12.5Xh DNA restored the transcription of the entire 2.9-kb message but only part of the 1.35-kb one. This observation and results of *in situ* hybridization studies (WATANABE 1989; described below in DISCUSSION) led us to conclude that the 2.9-kb message is the *l(1)ogre* transcript. The absence of the 2.9-kb message in *Df(1)ogre$^{18\text{-}183}$* mutants was confirmed by *in situ* hybridization analysis (WATANABE 1989).

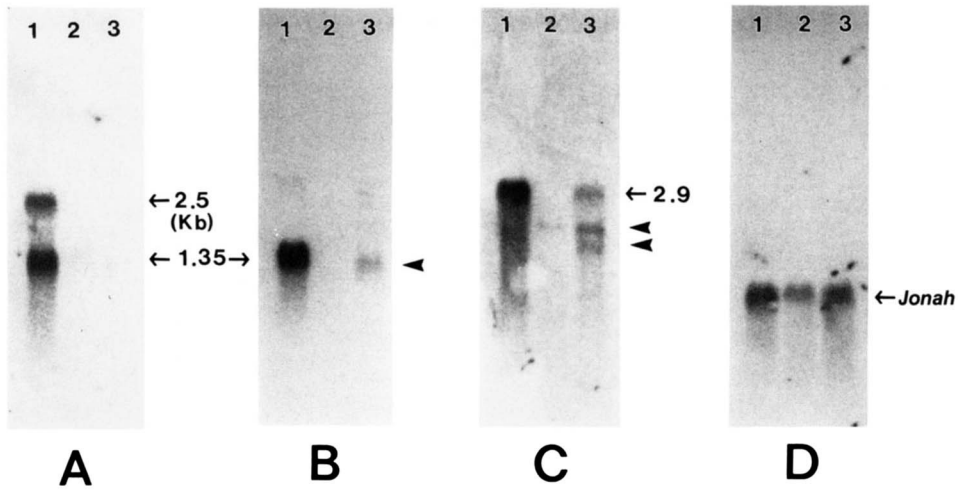

FIGURE 7.—Transcription of the 2.9-, 1.35- and 2.5-kb messages in the following three materials: lane 1, wild-type (Oregon R) male third instar larvae; lane 2, mutant male ($Df(1)ogre^{18\text{-}183}/Y$) third instar larvae; and lane 3, a mixture of mutant ($Df(1)ogre^{18\text{-}183}/Y$) and transformant ($Df(1)ogre^{18\text{-}183}/Y$; $Tr^{9\text{-}3}/+_2$) male third instar larvae. Approximately 5 μg of poly(A$^+$) RNA from each of these materials was separated by electrophoresis and hybridized with the following probes: Panel A, genomic fragment **d** (Figure 4b); B, genomic fragment **e**; C, a 2.15-kb *Xho*I fragment of the LK-1 cDNA clone (see text); D, the aDm2353 clone for the 0.91-kb *Jonah* transcript (CARLSON and HOGNESS 1985). As seen in panel D, less amount of RNA was apparently loaded on lane 2 than the other two lanes. In the mutant larvae the 2.5-, 1.35- and 2.9-kb transcripts were not detected with the above-mentioned probes (lane 2 of panels A, B and C). In the transformant larvae transcription of the 2.9-kb message was detected (lane 3 of panel C). Two shorter transcripts (the arrowheads in panel C), 2.0 and 1.5 kb in size, are also seen in the transformants. The 2.0-kb transcript, but not the 1.5-kb one, was detected in the mutant larvae (lane 2 of panel C). See DISCUSSION for arguments on these transcripts. A 1.3-kb message was detected with genomic probe **e** (the arrowhead in panel B). This message was not detected with genomic probe **d**, indicating that part of this message came from outside of the 1.35-kb transcription unit, presumably the region next to the insertion site of the 4K12.5Xh DNA.

Isolation of *l(1)ogre* cDNA clones: The following three embryonic libraries were screened for cDNA clones of the 2.9-kb mRNA with two separate genomic probes to select against partial cDNA clones:

About 1.5 × 10^5 plaques of a 3–12-hr AEL (after egg lay) Oregon R embryonic library in λgt10 (POOLE *et al.* 1985) were screened with two genomic probes **g** and **j** (Figure 4b) which detected about 200 and 40 positive plaques, respectively. Twenty-three plaques were positive to both probes; among these, eight clones were randomly selected and analyzed by restriction mapping. The cDNA LK-1 had the longest insert, 2.45 kb.

About 7.5 × 10^4 plaques of a 12–24-hr AEL Oregon R embryonic library (POOLE *et al.* 1985) were screened with probes **g** and **j** (Figure 4b) which detected 9 and 0 positive plaques, respectively. None of these were further analyzed.

About 1.5 × 10^5 colonies of an 8–12-hr AEL embryonic library in the plasmid vector pNB40 (BROWN and KAFATOS 1988) were screened with the probes **f** and **j** (Figure 4B) which detected 162 and 105 positive colonies, respectively. Among those which were positive to both probes, seven clones were isolated, all of which had inserts of the same size. Restriction analysis of two clones showed that both had the same insert which was a part of LK-1 (data not shown).

Nucleotide sequence of the LK-1 cDNA clone and the physicochemical properties of the putative *l(1)ogre* protein: The LK-1 cDNA clone was selected for sequence analysis, as it contained the largest insert (2.45 kb) obtained. A 2.15-kb *Xho*I fragment of LK-1 was subcloned into the *Sal*I site of the M13mp18 vector in two different orientations. Deletions of various sizes were produced in the inserts; these deletions allowed sequencing of different portions of the insert. Alternatively subfragments from the *Xho*I fragment was subcloned into the pGEM-3Zf(--) vector and sequenced. Thus both strands of the 2.15-kb *Xho*I fragment were completely sequenced.

The subcloned cDNA fragment turned out to be 2155 bp long, and a single long open reading frame (ORF) of 1086 bp (362 amino acids) was identified (Figure 8). This open reading frame is likely to encode a protein for the following three reasons: (1) The frequencies of the codons used within this frame, but not outside, were in good agreement with the codon usage bias of *D. melanogaster* (data not shown). (2) The sequence of four nucleotides immediately upstream from the start codon (nucleotide positions 908–910 in Figure 8) was in fair agreement with the consensus sequence flanking translation start sites in *D. melanogaster*; the four-nucleotide sequence in the current instance is C A A G while the consensus sequence is C/A A A A/C (CAVENER 1987). (3) Antisera raised against the 16-amino acid segment at the C terminus (CEFAKQVEPSKHDRAK) of this hypothetical protein detected antigen in postembryonic

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   1  TCGAGATTTG GCGAACTGTA GATGGAGTGG CAGTTGGTGC AGCGATCAGG TGGCCAGATC CAGTGGAGTA
  71  TGGCAATGAG AATGGCGGTT AGACTTGATA TTCGGGTTTG TTTGTACTTT TTTAAAGGCA CTTGACCAGA
 141  GGAAATTGAG CCCGTACTGC GATTGTGCAA ATGATGATTA TTGGCGGCAA TGTTACGAGC ATCCGTTATC
 211  CTGCAAGCTA CCATGTTCCC CCCCCCCGGT CGACGGCGAC GGCGACAGCG TGCTACAAAA GCCATTGCGA
 281  GCGTCTACGC GGCGCTCGAA AACACAGGAA AATTATAAGA AAATTTCGAA ACACAGCGAA AATCGTGAAA
 351  ACCCCTTGTA CACGACAAAC GGAATACGAC CGAATTGCTT AAAGAAACTA AAGTATACAT TTTTTTTTGG
 421  AAATTTCCAT TTTGCGTTGG TATTTTTTGC AGTGAGTGTG TGTGCGCGGT TTTTTCTTTT GACAGTCATA
 491  TTTCGAAAAT ATATTGTTTG AGGCCCTGTG GGCTGCATTC GAGAACCTGT TTCATGCAAA TTCTACGGCC
 561  ACCTGGCAGC TGATTCAAAA AATAAAACAA AAAAATTTAA AGAAGAGGAA GCAGTCCCAA AACAAACACA
 631  CACACACGCC ATGCTATGAC AAAAAATCAA AAATATAAAG AAGAGCAGCA AAACAAAAAG CCAGAAGAAG
 701  AGCCTGACAA GACAACGGAA TTCTGCTTGA TATGCATCGA AAGCTGAAGG CGAAGAGAAT TCGAAACAAG
 771  ACAAGAACCG GCAAGAGAAA GTTAAAGTCG CGTCTAGCAT AACAACGTTT CCGTTTGGGA AGATCAAACA
 841  CACTGCCCGG AGGAGAAGCT CCCGAAAGAT CCTGATCCTT GCCCACTGAG ATCCAAACTG TGCCAAG

 908  ATG TAT AAG TTG CTG GGT AGC CTG AAG AGC TAC CTC AAG TGG CAG GAC ATC CAG ACG GAC
   1  MET Tyr Lys Leu Leu Gly Ser Leu Lys Ser Tyr Leu Lys Trp Gln Asp Ile Gln Thr Asp

 968  AAC GCC GTC TTC CGG CTG CAC AAC TCC TTT ACC ACG GTG CTC CTG CTA ACC TGC AGC CTG
  21  Asn Ala Val Phe Arg Leu His Asn Ser Phe Thr Thr Val Leu Leu Leu Thr Cys Ser Leu

1028  ATC ATC ACC GCC ACC CAG TAC GTG GGC CAG CCG ATT AGC TGC ATC GTC AAT GGC GTA CCG
  41  Ile Ile Thr Ala Thr Gln Tyr Val Gly Gln Pro Ile Ser Cys Ile Val Asn Gly Val Pro

1088  CCG CAC GTG GTC AAC ACG TTC TGC TGG ATC CAC AGC ACT TTC ACC ATG CCG GAC GCT TTT
  61  Pro His Val Val Asn Thr Phe Cys Trp Ile His Ser Thr Phe Thr MET Pro Asp Ala Phe

1148  CGC AGA CAG GTT GGC CGA GAG GTG GCT CAT CCC GGT GTG GCC AAT GAT TTT GGC GAC GAG
  81  Arg Arg Gln Val Gly Arg Glu Val Ala His Pro Gly Val Ala Asn Asp Phe Gly Asp Glu

1208  GAT GCC AAG AAG TAC TAC ACC TAC TAC CAG TGG GTG TGC TTC GTG CTT TTC TTC CAG GCC
 101  Asp Ala Lys Lys Tyr Tyr Thr Tyr Tyr Gln Trp Val Cys Phe Val Leu Phe Phe Gln Ala

1268  ATG GCC TGT TAT ACG CCC AAA TTC CTG TGG AAT AAA TTC GAG GGC GGA CTG ATG CGC ATG
 121  MET Ala Cys Tyr Thr Pro Lys Phe Leu Trp Asn Lys Phe Glu Gly Gly Leu MET Arg MET

1328  ATT GTG ATG GGT CTG AAT ATC ACG ATC TGC ACC CGC GAG GAG AAG GAG GCC AAA CGC GAT
 141  Ile Val MET Gly Leu Asn*Ile Thr Ile Cys Thr Arg Glu Glu Lys Glu Ala Lys Arg Asp

1388  GCC CTG CTG GAC TAT CTG ATC AAG CAC GTG AAG CGC CAC AAG CTG TAC GCC ATT CGG TAC
 161  Ala Leu Leu Asp Tyr Leu Ile Lys His Val Lys Arg His Lys Leu Tyr Ala Ile Arg Tyr

1448  TGG GCC TGC GAA TTT CTC TGC TGC ATC AAC ATT ATC GTG CAG ATG TAT CTG ATG AAT CGC
 181  Trp Ala Cys Glu Phe Leu Cys Cys Ile Asn Ile Ile Val Gln MET Tyr Leu MET Asn Arg

1508  TTC TTC GAT GGC GAG TTC CTC TCG TAC GGC ACC AAT ATC ATG AAG CTT TCG GAT GTG CCG
 201  Phe Phe Asp Gly Glu Phe Leu Ser Tyr Gly Thr Asn Ile MET Lys Leu Ser Asp Val Pro

1568  CAG GAG CAA AGG GTG GAT CCC ATG GTC TAT GTG TTC CCC CGG GTG ACC AAG TGC ACC TTC
 221  Gln Glu Gln Arg Val Asp Pro MET Val Tyr Val Phe Pro Arg Val Thr Lys Cys Thr Phe

1628  CAC AAG TAT GGT CCC TCT GGT TCG CTG CAG AAG CAC GAC TCA CTC TGC ATC CTG CCG CTG
 241  His Lys Tyr Gly Pro Ser Gly Ser Leu Gln Lys His Asp Ser Leu Cys Ile Leu Pro Leu

1688  AAC ATT GTG AAC GAG AAG ACG TAC GTG TTC ATC TGG TTC TGG TTC TGG ATC CTG CTC GTC
 261  Asn Ile Val Asn Glu Lys Thr Tyr Val Phe Ile Trp Phe Trp Phe Trp Ile Leu Leu Val

1748  CTG CTC ATC GGA CTG ATA GTG TTC CGT GGC TGC ATT ATC TTT ATG CCG AAA TTC CGA CCC
 281  Leu Leu Ile Gly Leu Ile Val Phe Arg Gly Cys Ile Ile Phe MET Pro Lys Phe Arg Pro

1808  CGC CTC CTG AAC GCC AGC AAT CGC ATG ATT CCG ATG GAG ATC TGT CGC TCG CTG TCC CGC
 301  Arg Leu Leu Asn Ala Ser Asn Arg MET Ile Pro MET Glu Ile Cys Arg Ser Leu Ser Arg

1868  AAA CTG GAC ATC GGT GAC TGG TGG CTA ATC TAT ATG CTG GGT CGC AAT CTT GAT CCG GTC
 321  Lys Leu Asp Ile Gly Asp Trp Trp Leu Ile Tyr MET Leu Gly Arg Asn Leu Asp Pro Val

1928  ATC TAC AAG GAC GTG ATG AGC GAG TTT GCC AAG CAG GTG GAG CCC TCC AAG CAC GAC CGT
 341  Ile Tyr Lys Asp Val MET Ser Glu Phe Ala Lys Gln Val Glu Pro Ser Lys His Asp Arg

1988  GCC AAG TAGACCAGAT GTACGAGATG ATGCCCCCCG ATATGCCGTT CGATATATCA ATCACCCTGC
 361  Ala Lys

2054  CTATTAGTCC CCATCATTTT GTCCGAAACC CGAAATTCCG AATTCTGTTT TGGATTTTTT ATTTTTATTT
2124  AGCACAAATC ATACTACTTA GTTGAAACTC GA
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FIGURE 8.—Nucleotide sequence of the 2.15-kb *Xho*I fragment of the LK-1 cDNA clone and conceptual translation of an open reading frame of 1086 nucleotides. The fragment turned out to be 2155 bp long. Over an open reading frame (nucleotide position 908–1993), amino acid sequence is shown in the three-letter representation below the respective codons. The asterisk at amino acid position 146 shows the location of a potential glycosylation site. The underlined segment (amino acid position 268–288) represents the potential transmembrane region (see text).

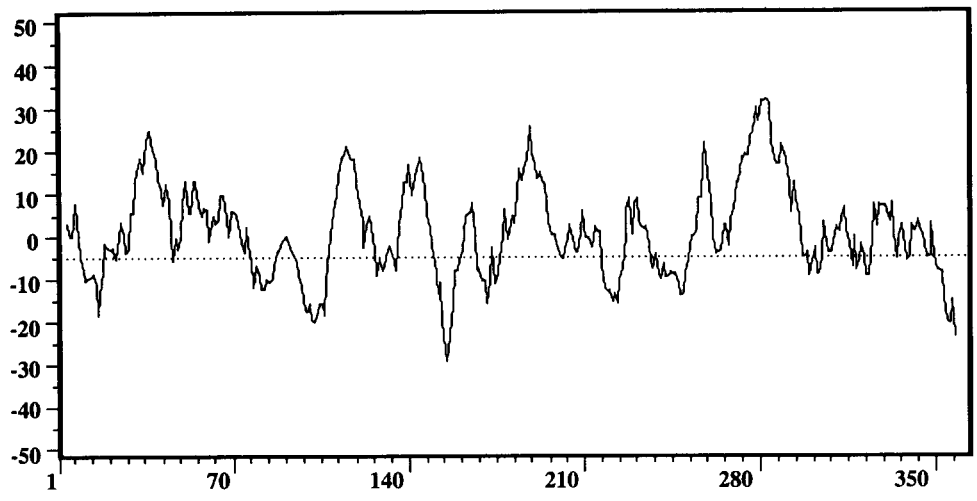

FIGURE 9.—Hydropathy profile (KYTE and DOOLITTLE 1982) of the hypothetical *l(1)ogre* protein produced by the SOAP program (PC-GENE program package from Intelligenetics). The computation was done using an interval of nine amino acids.

neuroblasts in the larval CNS (T. WATANABE and D. R. KANKEL, unpublished results).

The molecular weight and isoelectric point of the protein deduced from conceptual translation of the ORF were 42,582 and 9.04, respectively (assuming that there is no signal cleavage site; see below).

Hydropathy analysis (KYTE and DOOLITTLE 1982) (Figure 9) detected a few hydrophobic domains in the hypothetical *l(1)ogre* protein. The HELIXMEM program (part of the PC-GENE program package from Intelligenetics; EISENBERG *et al.* 1984), which classified hydrophobic segments as transmembrane, surface, or globular based on their hydrophobicities and hydrophobicity moments, predicted that the helices 29–49 (the position of amino acids from the N terminus), 51–79 and 111–131 were globular; 178–198 surface; and 268–288 monomeric transmembrane. According to the hydrophobicity indices of KYTE and DOOLITTLE (1982), the 268–288 segment has an average hydrophobicity index of 2.49; this argues for the interpretation that this segment is transmembrane because segments which have an average hydrophobicity above 1.6 have a high probability of being transmembrane domains (KYTE and DOOLITTLE 1982). The other four segments have average hydrophobicity indices below 1.6 (29–49, 1.42; 51–71, 0.81; 111–131, 1.01; and 178–198, 1.30). Furthermore, the 268–288 segment is followed, on the C terminus side, by a cluster of basic amino acids (VON HEIJNE 1985a): arginine at 289 and 299 and lysine at 297 and 301. These analyses suggested that the hypothetical *l(1)ogre* protein was a transmembrane protein with extracellular (1–267, see below for a potential signal peptide), transmembrane (268–288), and intracellular (289–362) domains. There is one potential glycosylation site at 146 (Figure 8).

A computer analysis found no typical signal peptide in the deduced *l(1)ogre* protein; the PSIGNAL program from PC-GENE (VON HEIJNE 1986) predicted (based on the amino acid composition from -12 to +2 with respect to a specific, potential cleavage site) the likeliest cleavage site to be between +45 and +46 from the N terminus. However the segment 1–45 may be too long to be a signal peptide, given that eukaryotic signal peptides are usually about 20 amino acids long with the longest extreme around 35 amino acids (VON HEIJNE 1985b).

Searches for proteins with homologous primary structures: Proteins with sequences similar to that of the hypothetical *l(1)ogre* protein were sought with the FASTA program (PEARSON and LIPMAN 1988) in the NBRF/PIR (release 24) and SWISS-PROT (release 10.0) protein databanks and with the TFASTA program (PEARSON and LIPMAN 1988) in an "on-the-fly" translation of the GenBank nucleic acid sequence library (release 59). R. F. DOOLITTLE was kind enough to search his own personal protein databank using the sequence from the hypothetical *l(1)ogre* protein. Several protein sequences were found with optimized scores above 90; the statistical significance of these similarities were evaluated with the RDF2 program (PEARSON and LIPMAN 1988). None of these similarities seemed to be significant, as the *z*-values (LIPMAN and PEARSON 1985) were not significantly above 6. Thus, no proteins have been found that have primary structures significantly homologous to that of the *l(1)ogre* protein.

DISCUSSION

The identification of the *l(1)ogre* transcript: *P* element-mediated DNA transformation has shown that the functional part of the *l(1)ogre* locus resides within the 4K12.5Xh genomic DNA. This genomic fragment contains the entire transcribed region of the 2.9-kb message (Figure 4b). The 2.9-kb message is transcribed from the ectopically inserted 4K12.5Xh DNA of the 9-3 transformant line (Figure 7C, lane 3). The probe (the 2.15-kb *Xho*I fragment from the LK-1 cDNA clone) also detected two shorter transcripts (2.0 kb and 1.5 kb) in the transformant larvae (Figure

7C, lane 3). In this figure it is hard to detect these shorter transcripts in the wild-type larvae (Figure 7C, lane 1), because they would be shadowed by the 2.9-kb message. In a previous Northern analysis using genomic DNA probes **g** and **h** (Figure 4b) a very low level of the 2.0-kb transcript was detected in the wild-type individuals (data not shown). Because the 2.0-kb message was detected also in *Df(1)ogre*$^{18\text{-}183}$ larvae (Figure 7C, lane 2), it follows that the 2.0-kb message is transcribed from a separate gene and cross-hybridizes to the probe. It is not clear whether the 1.5-kb message is transcribed in the wild-type individuals. It was once detected with the **h** genomic probe; however this observation was not reproducible. This transcript may, as the 2.0-kb transcript, be produced by a separate gene. But it is also possible that it is an alternative product of the gene encoding the 2.9-kb transcript *via* alternative splicing or use of a cryptic promotor. Given that a 1.5-kb transcript was not detected in *Df(1)ogre*$^{18\text{-}183}$ larvae (Figure 7C, lane 2), the latter possibility is more likely.

The 4K12.5Xh transformant DNA covers part (0.7 kb, at most) of the transcribed region of the 1.35-kb message (Figure 4b). A low level of a 1.3-kb transcript was detected with probe **e** in the transformant larvae (Figure 7B, lane 3). This novel transcript was not detected with probe **d** (Figure 7A, lane 3), indicating that it was a chimeric transcript consisting of sequences from the 1.35-kb transcription unit and also from the region next to the insertion site of the 4K12.5Xh DNA.

Two lines of Northern analysis data indicate that the 2.9-kb transcription unit, rather than the 1.35-kb unit, represents the *l(1)ogre* locus: (1) As has been just discussed, transcription of the 2.9-kb message was restored by the 4K12.5Xh DNA, while only part of the 1.35-kb message was transcribed from 4K12.5Xh. (2) The transcription of the 2.9-kb message, but not the 1.35-kb one, was reduced in the *l(1)ogre*^{ljnl3} larvae compared to the wild type (Figure 3).

In situ hybridization analyses have supported the above indication. A previous genetic study found that *l(1)ogre* mutations did not have maternal phenotypes (LIPSHITZ and KANKEL 1985). Thus it would be expected that *l(1)ogre* transcripts were not accumulated in oocytes in the adult ovary. Accumulation of the 1.35-kb transcript was detected in nurse cells and oocytes in the adult ovary (T. WATANABE and D. R. KANKEL, unpublished results), suggesting that this transcript is not the *l(1)ogre* transcript. The 2.9-kb message, on the other hand, was not detected in oocytes (WATANABE 1989). In the larval brain accumulation of the 2.9-kb message was detected in primordia of the optic lobe (WATANABE 1989), as expected for *l(1)ogre* transcript(s) from previous genetic studies (LIPSHITZ and KANKEL 1985).

Thus we conclude that the 2.9-kb transcription unit represents the *l(1)ogre* locus. As discussed above, a possibility remains that the 1.5-kb message (Figure 7d, lane 3) is also from the *l(1)ogre* gene. However, given that this transcript is barely detectable in the wild type, it is unlikely that this transcript represents the *l(1)ogre*$^{+}$ function.

The molecular-genetic map in the *l(1)ogre* region: A previous study by NICKLAS and CLINE (1983) mapped two additional genes, *JNL2* and *JNLX*, in the *l(1)ogre* region. *JNL2* is an embryonic lethal mapped proximal to *l(1)ogre* by a distance of about 0.1 cM (NICKLAS and CLINE 1983). The 0.95-kb message is the only embryonic pA^{+} message detected proximal to the 2.9-kb *l(1)ogre* message and consequently may be a *JNL2* transcript. *JNLX* is a semilethal gene with its lethal phase in the pupal or adult stage (NICKLAS and CLINE 1983). Because it is leaky, its meiotic map position is imprecise (NICKLAS and CLINE 1983). A complementation test has shown that *Df(1)ogre*$^{18\text{-}183}$ is *JNLX*$^{-}$. *Df(1)ogre*$^{18\text{-}183}$ uncovers the 1.35-kb and 2.5-kb messages (Figure 7), suggesting the possibility that one of these messages is a *JNLX* transcript.

The structure of the *l(1)ogre* transcript: The nucleotide sequence of the 2.15-kb fragment of an embryonic cDNA clone (LK-1) revealed an open reading frame of 1086 bp. This ORF is likely to be translated (into a protein of 362 amino acids), as judged from the codon usage within it and by the presence of the translation start consensus sequence of four nucleotides immediately upstream from its putative translation-initiation site. In a recent study with polyclonal antibodies raised against the 16 amino acid C terminus portion of this hypothetical protein, *l(1)ogre* protein was detected in the optic formation centers and giant neuroblasts in the third instar larval CNS (T. WATANABE and D. R. KANKEL, unpublished results)

It is equivocal whether the *l(1)ogre* protein is transmembrane or not: On one hand, the hydropathy analyses (Figure 9) strongly suggest that the segment from amino acid positions 268–288 is membrane-spanning. On the other hand, the putative signal sequence is much longer than average (45 bp *vs.* about 20 bp; VON HEIJNE 1985b). In the above-mentioned study with polyclonal antibodies, *l(1)ogre* protein was (at the light microscope level) detected throughout the cytoplasm without significant localization. At this level of resolution we could not tell whether *l(1)ogre* proteins were in the cytosol or on organellar membranes.

On the function of *l(1)ogre*: The function of a cloned gene may be readily studied by two molecular approaches. The first approach utilizes the sequence of the protein product of the gene. If a protein with a known biochemical function is found that has primary structure homologous to that of the gene prod-

uct, it suggests a possibility that the gene product performs a comparable function. In the present study searches failed to find a protein sequence which were homologous to that of the *l(1)ogre* product.

In the second approach, tissue/stage specificity of expression of the gene is investigated with nucleotide or antibody probes in search for a correlation between gene expression and a biological process. Temporal/spatial specificity of *l(1)ogre* expression has been studied by an *in situ* analysis (WATANABE 1989) and immunofluorescence with the above-mentioned polyclonal antibodies (T. WATANABE and D. R. KANKEL, unpublished results). As expected from previous genetic studies, expression was seen in the optic formation centers, the primordia of the optic lobe, and also in giant neuroblasts. These observations are compatible with previous genetic studies which showed requirement of *l(1)ogre* in postembryonic neurogenesis (LIPSHITZ and KANKEL 1985; SINGH, SINGH and KANKEL 1989). However, the hypothesized specificity of *l(1)ogre* expression to postembryonic neurogenesis was not confirmed, as expression was also detected in tissues outside of the CNS (*e.g.*, imaginal discs in larvae). The relevance of *l(1)ogre*$^+$ function in these tissues remains unknown.

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