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IMP-L2: an essential secreted immunoglobulin family member implicated in neural and ectodermal development in *Drosophila*

James C. Garbe, Eric Yang and James W. Fristrom*

Department of Molecular and Cell Biology, Genetics Division, University of California, Berkeley, Berkeley, CA 94720 USA

*Author for correspondence

SUMMARY

The *Drosophila IMP-L2* gene was identified as a 20-hydroxyecdysone-induced gene encoding a membrane-bound polysomal transcript. IMP-L2 is an apparent secreted member of the immunoglobulin superfamily. We have used deficiencies that remove the *IMP-L2* gene to demonstrate that *IMP-L2* is essential in *Drosophila*. The viability of *IMP-L2* null zygotes is influenced by maternal *IMP-L2*. *IMP-L2* null progeny from *IMP-L2*$^+$ mothers exhibit a semilethal phenotype. *IMP-L2* null progeny from *IMP-L2* null mothers are 100% lethal. An *IMP-L2* transgene completely suppresses the zygotic lethal phenotype and partially suppresses the lethality of *IMP-L2* null progeny from *IMP-L2* null mothers. In embryos, IMP-L2 mRNA is first expressed at the cellular blastoderm stage and continues to be expressed through subsequent development. IMP-L2 mRNA is detected in several sites including the ventral neuroectoderm, the tracheal pits, the pharynx and esophagus, and specific neuronal cell bodies. Staining of whole-mount embryos with anti-IMP-L2 antibodies shows that IMP-L2 protein is localized to specific neuronal structures late in embryogenesis. Expression of IMP-L2 protein in neuronal cells suggests a role in the normal development of the nervous system but no severe morphological abnormalities have been detected in *IMP-L2* null embryos.

Key words: *Drosophila*, immunoglobulin, maternal effect

INTRODUCTION

Molecular recognition and intercellular adhesion are major aspects of morphogenetic processes in multicellular organisms. In particular, the development of the nervous system depends on cellular interactions mediated by adhesion molecules, signalling molecules and cell surface receptors. One aspect of nervous system development that is extensively studied at the molecular level is the outgrowth and fasciculation of axons (reviewed in Bixby and Harris, 1991). In vertebrates, a number of putative guidance molecules have been identified based on in vitro and in vivo studies using purified protein substrates (Bixby and Harris, 1991; Tomaselli et al., 1986) or antibody perturbation (Fraseret al., 1988; Landmesseret al., 1988; Silver and Rutihauser, 1984). These studies have provided some understanding of the molecular mechanisms involved in nervous system development, but the experimental approaches used do not necessarily identify the function of specific molecules. The identification of proteins in *Drosophila* that are homologous to vertebrate molecules (Fessler and Fessler, 1989; Grenninglogh and Goodman, 1992; Grenninglogh et al., 1991a; Hortsch and Goodman, 1991) makes possible experimental approaches that combine cellular and molecular characterization with classical genetic analysis.

Among the molecules believed to be functionally important during nervous system development are members of the immunoglobulin (Ig) superfamily. Four transmembrane Ig superfamily members, fasciclin II (Grenninglogh et al., 1991a), fasciclin III (Patel et al., 1987), neuroglian (Bieber et al., 1989) and a neurotrophin receptor homolog, Dtrk (Pulido et al., 1992), have been identified as neural cell adhesion molecules in *Drosophila*. In addition, at least five other Ig family proteins have been characterized in *Drosophila*.

In this report, we present results of our characterization of *Drosophila IMP-L2* (Osterbur et al., 1988), a member of the Ig superfamily. The genomic clone containing IMP-L2 sequences was isolated in a differential screen designed to isolate genes encoding 20-hydroxyecdysone (20HE)-induced secreted or membrane-bound proteins (Natzle et al., 1986). The *IMP-L2* transcription unit was identified as encoding a 20HE-induced, membrane-bound polysomal RNA. In situ hybridization using antisense RNA as probe to detect IMP-L2 transcripts in frozen tissue sections of imaginal discs or metamorphosing animals indicated that the major sites of *IMP-L2* expression were the peripodial epithelium of imaginal discs in prepupae and the imaginal abdominal histoblasts of early pupae (Osterbur et al., 1988). Both tissues undergo a process of hormonally induced spreading followed by fusion with adjacent imaginal tissues to form the continuous adult epithelium. These data impli-

cated the *IMP-L2* gene product in the process of epithelial spreading and fusion. We show here that IMP-L2 is a member of the Ig superfamily and is apparently a secreted protein. Like most characterized Ig genes in *Drosophila*, *IMP-L2* is expressed in neuronal cells suggesting a role in the normal development of the nervous system. Also like other characterized Ig genes in *Drosophila*, no severe morphological abnormalities are detected in *IMP-L2* null embryos. Genetic analysis demonstrates that *IMP-L2* is an essential gene in *Drosophila*. Absence of zygotic *IMP-L2* results in semilethality of null progeny. *IMP-L2* null progeny from *IMP-L2* null mothers are 100% lethal. The zygotic lethality is completely suppressed in the presence of an IMP-L2 transgene. The lethal phenotype of *IMP-L2* null progeny from *IMP-L2* null mothers is partially suppressed by an IMP-L2 transgene.

MATERIALS AND METHODS

Drosophila stocks

Drosophila deficiency stocks used in this analysis were *Df(3L)ems*^{13} (64B1,2-64E), *Df(3L)GN19* (63F9,11-64B1,2) and *Df(3L)A466* (63D1,2-64B1,2). The Oregon-R stock used in this study has been continuously maintained in our laboratory since 1965. Balancer stocks and *w*^{1118}; *ry*^{506} P[ry^{+} Δ2-3]99B are described in Lindsley and Zimm (Lindsley and Zimm, 1992).

Isolation of genomic and cDNA clones

Genomic clones were isolated from a *D. melanogaster* genomic library made from randomly sheared embryonic DNA (Maniatis et al., 1978). The library was screened using the ^{32}P-labeled 3.0 kb fragment from λ64B1 carrying the 3′ portion of the *IMP-L2* gene (p3.0 in Osterbur et al., 1988). The same 3.0 kb fragment was used as the probe to isolate the cDNA clone from a cDNA library derived from hormone-induced imaginal discs (Osterbur, 1986).

Hybridizations

Nucleic acid blotting and hybridizations were performed according to standard procedures (Sambrook et al., 1989). For Southern blots, genomic DNA was digested with the restiction enzyme *Eco*RI. The resulting products were electrophoretically separated on 0.7% agarose gels and transferred to Nytran membranes (Schleicher and Schuell). RNA samples were denatured with glyoxal, electrophoretically separated on 1.0% agarose gels and transferred to Nytran membranes. Hybridizations were performed at 65°C in 5× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0) with 0.5% SDS, 10× Denhardt's solution, and 100 μg/ml sheared denatured salmon sperm DNA. Filters were washed at 65°C in three changes of 0.2× SSC and exposed to X-ray film.

DNA sequencing

To sequence the IMP-L2 genomic and cDNA clones, restriction fragments were cloned into the single-strand M13 phage vectors mp18 and mp19 (Norrander et al., 1983). Sequencing reactions were performed by dideoxy chain termination (Sangeret al., 1977) using Sequenase version 2.0 (US Biochemicals). Reaction products were resolved on 6% polyacrylamide/8M urea gels. Sequences were analyzed with the MacVector DNA sequence analysis program (International Biotechnologies, Inc.) Database searches were done using FASTDB (Brutlag et al., 1990) from Intelligenetics, Inc. and FASTA and TFASTA (Pearson and Lipman, 1988).

Generation of anti-IMP-L2 polyclonal antibodies

The coding region of IMP-L2 representing amino acids 22 to 263 was used to generate anti-IMP-L2 antibodies. The cDNA-containing plasmid, pL2C302, was digested with *Sac* II and blunt-ended with the Klenow fragment of DNA polymerase I. The DNA was purified by phenol extraction and ethanol precipitated. The DNA was resuspended and digested with *Eco* RI. The digestion products were electrophoretically separated on a low melting point agarose gel and the *Sac* II-*Eco* RI insert fragment isolated. The insert was ligated into the *Sma* I and *Eco* RI sites of plasmid pGEX-3X (Pharmacia-LKB) encoding glutathione-S tranferase (GST; Smith and Johnson, 1988). Recombinant plasmids were transformed into *E. coli* and ampicillin-resistant colonies selected. Bacteria expressing the GST/IMP-L2 fusion protein (M_r=54×10^3) were used for subsequent isolation of the fusion protein. The GST/IMP-L2 fusion protein was purified from induced bacterial cultures by lysing the bacteria with lysozyme and Triton X-100 followed by low speed (5000 ***g***) centrifugation. The insoluble pellet containing the GST/IMP-L2 fusion protein was resuspended and washed several times in phosphate-buffered saline (PBS) plus 0.1% Triton X-100. The pellet was resuspended in PBS and solubilized in 0.1% SDS followed by extensive dialysis against PBS. The solubilized fusion protein (100 μg/ml in PBS) was used to immunize female Fisher 344 rats. For the initial injection antigen was emulsified in Freund's complete adjuvant. Antigen emulsified in Freund's incomplete adjuvant was used for booster injections. Anti-GST/IMP-L2 sera were collected and stored frozen in aliquots at −20°C. Anti-GST/IMP-L2 antibodies were affinity purified by binding to GST/IMP-L2 protein immobilized on an Immunopure column (Pierce Biochemicals).

Western blotting

Protein samples dissolved in Laemmli buffer (Laemmli, 1970) were separated on 12.5% polyacrylamide/SDS gels and electrophoretically transferred to ECL nitrocellulose membranes (Amersham). Membranes were blocked in Tris-buffered saline (TBS; 150mM NaCl, 20mM Tris pH 8.0) with 3% powdered milk, 1% BSA and 0.5% Tween 20. Affinity-purified GST/IMP-L2 antibody was bound in the same blocking solution. Filters were washed 3× 15 minutes in 50 ml TBS with 0.5% Tween 20 to remove excess antibody. Filters were blocked a second time for 1 hour and horse radish peroxidase (HRP)-conjugated goat anti-rat antibody (1:1000, Cappel) was bound in blocking solution. Excess secondary antibody was removed by washing as above. HRP-catalyzed ECL reactions were done according to manufacturer's instructions and the filters exposed to X-ray film.

Collection and fixation of embryos

Embryos were collected from flies kept in 1/2 pint bottles maintained at 22°C. Embryos were dechorionated en masse with 50% commercial bleach and fixed in a two-phase mixture of 7.4% formaldehyde in PEM buffer (100 mM Pipes, 1 mM $MgCl_2$, 2 mM EGTA, pH 6.9), and heptane (Karr and Alberts, 1986). Fixed embryos were devitellinized with methanol and rinsed with 100% methanol to remove residual heptane. Embryos that were not used immediately were stored in 100% methanol at −20°C.

Antibody staining of embryos

Embryos in 100% methanol were rehydrated stepwise in PBS with 0.1% TWEEN 20 and 0.1% Triton X-100. Embryos were blocked in PBS with 0.1% Tween 20, 0.1% Triton X-100, 5% normal goat serum and 1 mg/ml BSA. Affinity-purified anti-GST/IMP-L2 antibody was used at an equivalent dilution of 1:500. Whole antisera were used at a dilution of 1:500-1:1000. FITC-conjugated goat anti-rat antibody (Cappel) was used at a dilution of 1:500. To visualize the embryonic nervous system, embryos were stained with rabbit anti-horseradish peroxidase (Jackson Immunoresearch Laboratories) followed by Cy3-conjugated goat anti-rabbit

antibody (Jackson Immunoresearch Laboratories). Fluorescently stained embryos were examined with a Zeiss microscope.

In situ hybridization of embryos

Embryos in 100% methanol were rehydrated and processed for in situ hybridization according to the procedures of Tautz and Pfeifle (Tautz and Pfeifle, 1989). Randomly primed, digoxigenin-labeled probe was prepared with the Genius DNA labeling and detection kit (Boehringer Mannheim) according to the manufacturer's instructions. Digoxigenin-labeled sense and antisense DNA probes were synthesized as described by Sturzl et al., (1992). Color development of the alkaline phosphatase reaction product was monitored under a dissecting microscope. The reaction was terminated by washing the embryos extensivley in PBS. Embryos were mounted in 70% glycerol/1× PBS.

Embryo transformations

The plasmid used for P-element mediated rescue of *IMP-L2*-deficient flies included *IMP-L2* genomic sequences extending from an *Eco* RV site at −1000 to a *Sal* I site at +4200. The IMP-L2 DNA was cloned into the P-element transformation vector CaSpeR-4 (Pirrotta, 1988). Plasmid DNA at a concentration of 500 μg/ml was injected into embryos of the genotype *w*; ry^{506} P[ry^{+} Δ2-3]99B. Surviving adults were crossed to *w*; *TM3 Sb* /*TM6B* to identify transformants.

RESULTS

Molecular analysis

The isolation and initial characterization of the *IMP-L2*-containing genomic clones λ64B1 and λ64B2 have been described previously (Natzle et al., 1986; Osterbur et al., 1988). Isolation of additional clones provided a total of 32 kb of genomic sequence representing 12 kb of upstream (centromere proximal) DNA, the *IMP-L2* gene and 15 kb of downstream (centromere distal) DNA (Fig. 1A). The 3.3 kb IMP-L2 transcription unit comprises three exons and two introns (Fig. 1B) producing a single mRNA species of 2.4 kb (revised from 3.0 kb in Osterbur et al., 1988). We have found no evidence from RNA blot hybridizations for alternative RNA processing. In addition to the *IMP-L2* gene, four other transcription units are represented in the cloned genomic sequences (Osterbur et al., 1988). These transcription units have not been fully characterized but only the three transcription units closest to *IMP-L2* (indicated in Fig. 1A) are relevant to the genetic analysis discussed below.

The sequence of the *IMP-L2* gene and conceptual translation product is presented in Fig. 2. The transcription start site (+1) was determined by the method of Hu and Davidson (1986; data not shown). A closely spaced TATA box begins 17 nucleotides upstream from the transcription start site. A canonical polyadenylation signal (nt +3319-3324) is present 13 nucleotides upstream from the polyadenylation site. A large open reading frame (ORF) is present in the sequence beginning at position +1684 from the transcription start site (+740 on the mRNA). Three small ORFs are located in the leader region (discussed below). The large ORF encodes a conceptual protein of 263 amino acids. At the amino terminus are 23 hydrophobic amino acids with properties characteristic of a signal sequence (von Heijne, 1983). Removal of the signal sequence would produce a protein of 240 amino acids with a calculated relative molecular mass of 27×10^{3}. No consensus N-X-$^{S}/_{T}$ sites for N-linked glyco-

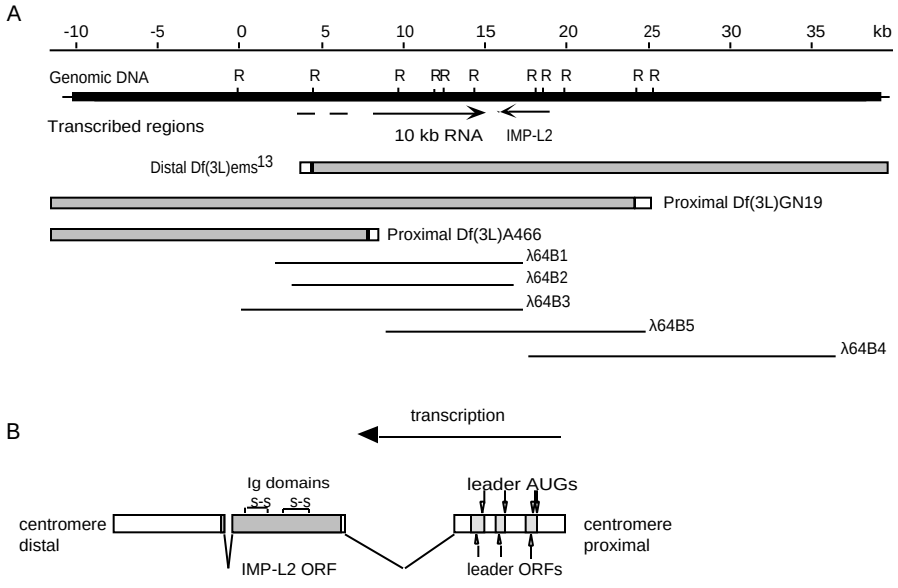

Fig. 1. (A) Molecular map of the *IMP-L2* gene region. The *IMP-L2* gene is located in band 64B1-2 on the left arm of chromosome three. *Df(3L)ems*^{13} breaks 12 kb distal to *IMP-L2*. *Df(3L)GN19* breaks 5 kb proximal to *IMP-L2*. *Df(3L)A466* breaks 8 kb distal to *IMP-L2*. The hatched bars indicate the extent of the three deficiencies identified by analysis of genomic Southern blots. R identifies *Eco* RI restriction endonuclease sites. A 10 kb transcript broken by Df(3L)A466 is indicated as are two distal transcripts deleted by all deficiencies. Further characterization of IMP-L2 genomic and cDNA clones has indicated errors in the original mapping of clones as reported in Osterbur et al. (1988). Specifically, the λ64B2 clone is wholly within the limits of λ64B1 and neither clone includes sequences from the first exon of *IMP-L2*. The genomic DNA represented by the additional genomic clones that have been isolated are indicated. The entire *IMP-L2* gene is present in clone λ64B5. The size of the IMP-L2 transcript has been revised from 3.0 kb to 2.4 kb. A further correction concerns the sequences located immediately centromere distal to *IMP-L2*. The 1.7 kb transcript indicated in Osterbur et al. (1988) has not been detected using probes derived from these genomic sequences (M. Prout and J. W. Fristrom, unpublished data). (B) Molecular map of the *IMP-L2* gene. The mature IMP-L2 mRNA is derived from three exons and two introns as indicated. The direction of transcription is centromere proximal to distal. The long open reading frame encoding the IMP-L2 protein begins in the second exon. The conceptual protein product is 263 amino acids long with a 23 amino acid signal sequence. Four AUG codons and three short open reading frames are found in the mRNA leader (discussed in text).

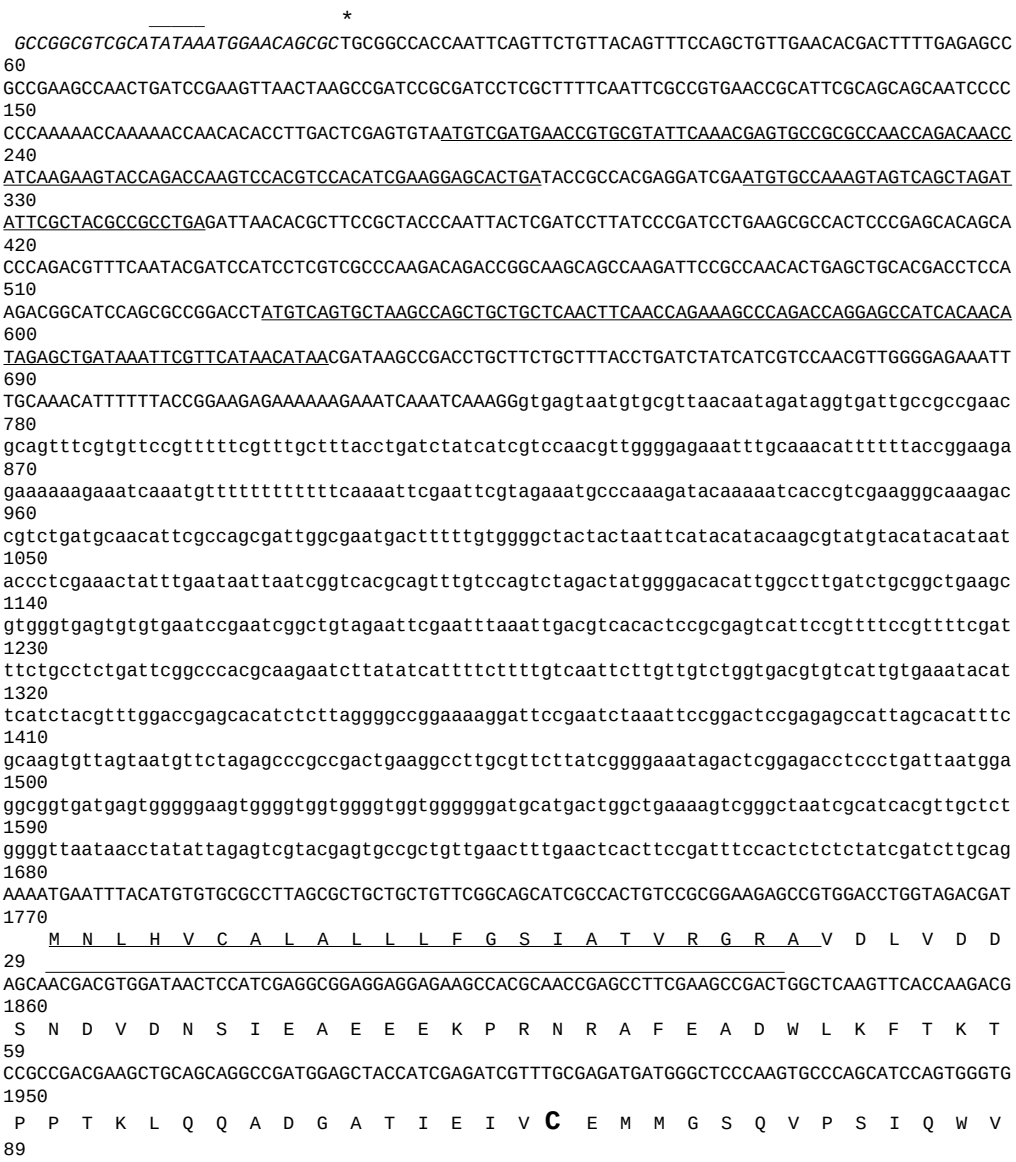

Fig. 2. IMP-L2 DNA and conceptual protein sequence. The DNA sequence shown extends from a *Nae*I site at −29 from the transcription start site to the *Sca* I site at +3350. The TATA box (−17 to −13) is overlined and the transcription start site (+1) is indicated with an asterisk and right arrow. Three short open reading frames in the mRNA leader (discussed in text) are underlined. Intron sequences are in lowercase, the polyadenylation signal (+3329-3333) is underlined and the polyadenylation site is indicated with an up arrow. Genomic sequences before the start site and after the polyadenylation site (+3347) are italicized. The first nucleotide of the cDNA clone corresponds to nucleotide 129 of the genomic sequence. The probable signal sequence of the IMP-L2 protein is double underlined and the four cysteines of the Ig domains are indicated in boldface. There are thirteen nucleotide differences between the genomic DNA and the cDNA clone: 647:T→C; 1893:T→C; 1960:T→C; 2190:G→A; 2196:C→A; 2200:G→A; 2298:C→T; 2319:T→C; 2689→2691:CAA→ATC; and 2702→2703: AA deleted. The G→A transition at 2200 results in a conservative amino acid substitution of isoleucine for valine 173. This sequence has been submitted to the GenBank database under the accession number L23066.

sylation are present. The absence of a transmembrane domain in the IMP-L2 conceptual protein and the absence of hydrophobic sequences indicative of phosphoinositol linkage (Ferguson and Williams, 1988) suggest that the IMP-L2 protein product is secreted. Thirteen nucleotide differences were detected in the sequences of the genomic and cDNA for IMP-L2 (see legend, Fig. 2). Of these differences, seven are in the protein-coding region but only one results in a change of the protein sequence; a conservative substitution of isoleucine for valine at amino acid 173. We believe these differences reflect the differences between the two *D. melanogaster* stocks used in the construction of the genomic and cDNA libraries from which clones were isolated.

Similarity searches of protein and nucleic acid databases revealed significant similarity between IMP-L2 and proteins in the immunoglobulin (Ig) superfamily. Further analysis showed that two immunoglobulin domains constitute the bulk of the IMP-L2 protein. Consistent with this interpreta-

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IMP-L2  DLMGSNIQLP CRVHARPRAE ITWLNNENKE .IVQGHRHRV LANGD..... ....LLISEI KWEDMGNYKC IARNVVGKDT
Nrg     TVDGRNVTIK CRVNGSPKPL VKWLRASNW. ..LTGGRYNV QANGD..... ....LEIQDV TFSDAGKYTC YAQNKFGEIQ
Ama     QMVSHSAELE CSVQGYPAPT VVWHKNG..V .PLQSSRHHE VANTASSSGT TTSVLRIDSV GEEDFGDYYC NATNKLGHAD
Lach    QALQYDMDLE CHIEAYPPPA IVWTKDD..I .QLANNQHYS ISHFATADEY TDSTLRVITV EKRQYGDYVC KATNRFGEAE
FasII   SWEQRKANLS CLAMGIPNAT IEWHWNGRKI KDLYDTNLKI VGTGPRSD.. ....LIVHPV TRQYYSGYKC IATNIHGTAE
                * ★ * * *      *              *      *          * *  *    * * *  ★ * *  *
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Fig. 3. Alignment of the second Ig domain of IMP-L2 with similar Ig domains of *Drosophila* Neuroglian (Nrg; Ig-5), Amalgam (Ama; Ig-3), Lachesin (Lach; Ig-3), and fasciclin II (FasII; Ig-5). Residues that are identical in three of the five sequences are indicated with an asterisk. The strongest similarity is found in the regions around the conserved cysteine residues (shown in boldface). The aligned sequences begin 10 residues before the first cysteine and end 10 residues after the second cysteine in each Ig domain. The alignment was performed with the PILEUP program from the University of Wisconsin Genetic Computer Group using the algorithm of Needleman and Wunsch [Needleman, 1970 no. 341].

tion, the amino acid residues that characterize Ig domains are conserved in IMP-L2 and the highest level of similarity between IMP-L2 and other Ig family proteins is around the conserved cysteines. Comparison of the IMP-L2 sequence to that of other *Drosophila* Ig domain-containing proteins showed most similarity to Neuroglian (Nrg; Bieber et al., 1989). IMP-L2 was also found to be highly similar to the vertebrate Ig proteins mouse L1 (Moos et al., 1988) and chicken contactin/F11 (Ranscht, 1988; Brümmendorf et al., 1989). These proteins all have a similar structure and are believed to be evolutionarily related (Grenninglogh et al., 1991a). These proteins also have been identified as neural cell adhesion molecules. Less sequence similarity was found between IMP-L2 and other *Drosophila* Ig proteins such as Fasciclin II (Grenninglogh et al., 1991b) and the two secreted Ig molecules Amalgam (Seeger et al., 1988) and Lachesin (Karlstrom et al., 1993). The greatest degree of similarity to other Ig family members was consistently observed with the second Ig domain of IMP-L2 (Fig. 3). The identification of IMP-L2 as a member of the Ig superfamily thus suggests that IMP-L2 may be involved in some aspect of cell adhesion. However, the small size and apparent secreted character of IMP-L2 also indicate a role different from that of transmembrane cell adhesion molecules.

Although three additional start codons are present in the mRNA, several lines of evidence indicate that the large ORF encodes the IMP-L2 protein. First, this ORF displays codon usage consistent with that of other *Drosophila* genes (Ashburner, 1989) and has the proposed initiator AUG in a favorable context for translational initiation (Kozak, 1986; Cavener, 1987; Cavener and Ray, 1991;). Second, the conceptual translation product from this ORF possesses two Ig domains with sequence similarity to other Ig domain-containing proteins suggesting evolutionary conservation that would not be expected in noncoding DNA. Third, this is the only ORF that encodes a protein with a signal sequence. The presence of a signal sequence would be expected based on the isolation scheme and the association of the IMP-L2 mRNA with membrane-bound polysomes. Fourth, antisera made against a glutathione S-transferase (GST)/IMP-L2 fusion protein translated in the reading frame of this ORF recognize a M_r 32×10^3 protein in wild-type flies that is absent from *IMP-L2*-deficient flies (see below). Although the first small ORF encountered on the mRNA also has a favorable translation initiation context and appropriate codon usage for *Drosophila*, the sequence similarity and immunological detection of the large ORF product indicate that it is the major though not necessarily the only translation product from the *IMP-L2* gene (see discussion).

Genetic analysis

Chromosomal deficiencies extending into the 64B region were characterized by Southern analysis to determine if they removed the *IMP-L2* locus. Three of these, *Df(3L)ems^{13}* (64B1,2-64E), *Df(3L)GN19* (63F-64B1,2) and *Df(3L)A466* (63D1,2- 64B1,2), hereafter referred to as *ems^{13}*, *GN19* and *A466*, were found to have breakpoints within 15 kb of the *IMP-L2* gene as illustrated in Fig.1A. The *ems^{13}-GN19* overlap removes 25 kb of DNA and deletes the *IMP-L2* gene (see Fig. 4). Three additional transcription units are deleted in *ems^{13}/GN19* heterozygotes, two encoding 1.2 kb mRNAs and one encoding a 10 kb mRNA. The *ems^{13}-A466* overlap removes 14 kb of DNA distal to the *IMP-L2* gene but does not remove *IMP-L2* which is still expressed in an apparently normal manner (M. Prout and J. W. Fristrom, unpublished). The two smaller transcription units are deleted in *ems^{13}/A466* heterozygotes while the 10 kb transcript is broken at its 5′ end and produces a shorter transcript of 8 kb probably from an adventitious promoter (M. Prout and J. W. Fristrom, unpublished data).

Unexpectedly, the GN19 deficiency chromosome was semiviable when heterozygous with the ems^{13} deficiency. However, the viability of *ems^{13}/GN19* F1 heterozygous progeny lacking the IMP-L2 gene was variably reduced (ranging from 0 to 60%) and was frequently less than 10% of Mendelian expectations (Table 1). The direction of the cross had no obvious effect on the viability of *IMP-L2* deficient progeny (data not shown). In contrast, *ems^{13}/A466* heterozygotes were recovered at near Mendelian expectations (Table 2) suggesting that *IMP-L2* is the only gene deleted in *ems^{13}/GN19* heterozygotes that is essential for full viability. The recovery of *ems^{13}/A466* heterozygotes further suggests that deficiencies in the region do not reduce F1 zygotic viability nonspecifically.

Examination of the *ems^{13}/GN19* heterozygote larvae and adults revealed no gross morphological defects in epidermal structures that would be expected if *IMP-L2* were essential for epithelial morphogenesis. Specifically, all imaginal disc-derived and abdominal histoblast-derived structures were present in adults and were seen to have spread and fused normally with adjacent tissues. Furthermore, all adult cuticular structures were present and appeared in their

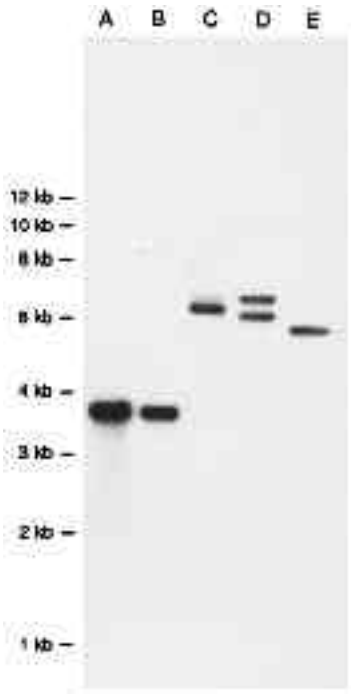

Fig. 4. Autoradiogram showing that the *IMP-L2* gene is absent in *ems^{13}/GN19* heterozygotes. Southern blots were done as described in Materials and Methods. (Lanes A,B) DNA from *IMP-L2*$^{+}$ adults, *ems^{13}/TM6B* (Lane A) and *GN19/TM6B* (Lane B). A single 3.7 kb band from the endogenous *IMP-L2* gene is detected. (Lanes C,-E) DNA from P[*w*$^{+}$*IMP-L2*$^{+}$]; *ems^{13}/GN19* adults (lane C, line 337.221; lane D, line 337.481; lane E, line 337.551). Note absence of the 3.7 kb band and the presence of new bands derived from the transformed *IMP-L2* gene.

normal positions. Similarly, examination of *ems^{13}/GN19* heterozygote embryos revealed no gross defects in development of the nervous system, the epidermis or the digestive system. More detailed morphological examinations are in progress to identify possible abnormalities in embryos that are associated with the absence of the *IMP-L2* gene.

Table 1. Viability of *IMP-L2* null heterozygotes

Cross: *GN19/TM3 Sb* ♀♀ × *ems^{13}/TM6B* ♂♂

Cross no.	*ems^{13}/GN19*	*ems^{13}/TM3 Sb*	*GN19/TM6B*	*TM6B/TM3 Sb*
1	1 (1%)	0	80	83
2	1 (1%)	0	119	106
3	25 (31%)	0	82	80
4	10 (12%)	0	85	80
Totals	37 (10.4%)	0	365	349

ems^{13}/GN19 heterozygotes are *IMP-L2* null and exhibit a zygotic semilethal phenotype. Data from four parallel crosses are presented. Viable *ems^{13}/GN19* heterozygotes were recovered at an average of about 10% of Mendelian expectations (range from <1% to 31% in these four crosses). Numbers in parentheses reflect the *ems^{13}/GN19* progeny that were recovered as a percentage of the number expected assuming a 1:1:1 ratio among the viable progeny of the cross. The *ems^{13}* deficiency uncovers a lethal locus on the *TM3 Sb* chromosome, thus, *ems^{13}/TM3 Sb* heterozygotes were not recovered in these crosses. Four other chromosomal deficiencies that uncover the region 64B3 to 64B15,17 were also lethal with *TM3 Sb*.

Viable *ems^{13}/GN19* heterozygote males were sterile indicating that at least one of the deleted loci is required for male fertility. The A466 deficiency does not delete the *IMP-L2* gene but *ems^{13}/A466* males were also sterile, thereby excluding *IMP-L2* as a single cause of the male sterility.

Viable *ems^{13}/GN19* heterozygote females exhibit maternal effect embryonic lethality. This was initially recognized when *ems^{13}/GN19* heterozygote females were mated to *IMP-L2* deficiency/balancer males (e.g. *ems^{13}/GN19* × *GN19/TM6B*). In these crosses, only progeny carrying the paternal *TM6B* chromosome were recovered (Table 3). A paternally supplied *A466* chromosome (*IMP-L2*$^{+}$) rescued the maternal effect lethality as efficiently as an *IMP-L2*$^{+}$ balancer chromosome (Table 3).

To determine the lethal phase of the maternal effect, embryos from the cross *ems^{13}/GN19* ♀♀ × *GN19/TM3 Sb Ser* ♂♂ were examined. Data tabulated from several collections showed that only 66% of the embryos that hatched survived to eclose as adults. All eclosed adults carried the *IMP-L2*$^{+}$ balancer chromosome. Examination of the collection plates revealed that many first instar larvae died shortly after hatching. A few additional embryos appeared to develop through stage 17 but did not hatch. In comparison, in the cross *ems^{13}/GN19* ♀♀ × Oregon-R ♂♂, 86% of hatched eggs eclosed as adults. We conclude that the major lethal phase for the *ems^{13}/GN19* progeny of *ems^{13}/GN19* mothers is during early first instar.

To verify that the observed zygotic and maternal effect embryonic lethality was due to loss of *IMP-L2*, cloned genomic *IMP-L2* sequences were introduced into *Drosophila* via P-element-mediated germ-line transformation. The transformation construct included the *IMP-L2* gene, 1.0 kb of upstream sequence and 1.0 kb of downstream sequence in the transformation vector CaSpeR-4 (Pirrotta, 1988). Nine independent transformants were recovered with insertion sites on the X and second chromosomes. Presence of the transposed *IMP-L2* gene was confirmed by Southern blotting. The crosses indicated in Table 4 (five independent transformant lines) permitted us to determine the rescuing ability of IMP-L2 with respect to the viability of *ems^{13}/GN19* heterozygotes.

In F_1 *ems^{13}/GN19* heterozygotes (Table 4A), the presence of the IMP-L2 transgene resulted in full rescue of viability as determined by the number of P[*w*$^{+}$; *IMP-L2*$^{+}$] *ems^{13}/GN19* adults compared to P[w+; *IMP-L2*$^{+}$] *GN19/TM3*. As an internal control for viability, the number of *w; ems^{13}/GN19* adults was <10% of Mendelian expectations compared to *w; GN19/TM3.* DNA from the P[*w*$^{+}$; *IMP-L2*$^{+}$] *ems^{13}/GN19* progeny was analyzed by Southern blotting and confirmed the presence of both deficiency chromosomes and the P[*w*$^{+}$, *IMP-L2*$^{+}$] insert (Fig. 4).

To determine the rescuing ability of *IMP-L2* on the maternal effect lethality, *w*; P[*w*$^{+}$, *IMP-L2*$^{+}$]; *ems^{13}/GN19* females were crossed to *w*; P[*w*$^{+}$, *IMP-L2*$^{+}$]; *GN19/TM3 Sb Ser* males (Table 4B). Rescue of lethality was indicated by recovery of non *TM3 Sb Ser* G2 progeny. No *w; ems^{13}/GN19* G2 progeny were recovered. Rescue of the maternal effect embryonic lethality by the transgene was only 10-20% efficient compared to a wild-type *IMP-L2* gene. Whether this low efficiency was due to insufficient regulatory sequences for normal maternal and zygotic expression or a

Table 2. Viability of *IMP-L2*$^+$ heterozygotes

cross	*ems^{13}/A466*	*ems^{13}/TM6B*	*A466/TM3*	*TM6B/TM3*
ems^{13}/TM3 Sb Ser ♀♀ × *A466/TM6B* ♂♂	54 (26%)	43 (21%)	74 (36%)	36 (17%)
A466/TM6B ♀♀ × *ems^{13}/TM3 Sb Ser* ♂♂	64 (22%)	99 (34%)	76 (26%)	54 (18%)

ems^{13}/A466 heterozygotes are *IMP-L2*$^+$ and do not exhibit a zygotic semilethal phenotype. Data from reciprocal crosses are shown. Numbers in parentheses represent the progeny recovered in each class as a percentage of the total number of progeny for the cross. Viable *ems^{13}/A466* deficiency transheterozygotes are recovered in numbers consistent with Mendelian expectations (26% and 22%). Note that the lethal locus on *TM3 Sb* uncovered by *ems^{13}* is not present on the *TM3 Sb Ser* chromosome. Thus, *ems^{13}/TM3 Sb Ser* heterozygotes are viable.

nonspecific effect of the mothers being heterozygous for the deficiencies is not known.

Four lines were also tested for paternal rescue of the maternal effect lethality. As shown in Table 4C, *ems^{13}/GN19* progeny from *ems^{13}/GN19* mothers can be rescued by a paternally supplied *IMP-L2* transgene. The similar results in crosses where the *IMP-L2* transgene was derived from both the maternal and paternal parent or only the paternal parent suggest that zygotic expression of *IMP-L2* provides the rescuing function in the crosses described. *IMP-L2* transgenic animals transformed with a construct containing 2.5 kb of upstream sequence also showed zygotic rescue of lethality but at a lower efficiency than the transgene with 1.0 kb of upstream sequence (data not shown) suggesting the presence of negative regulatory elements in the additional upstream sequences.

To summarize, genetic analysis demonstrates that *IMP-L2* is an essential gene in *Drosophila* and that *IMP-L2*-deficienct embryos exhibit a lethal phenotype that is influenced by maternal *IMP-L2*. Rescue experiments with an *IMP-L2* transgene conclusively show that the lethal phenotype is due to the loss of *IMP-L2*.

Expression of IMP-L2

The genetic analyses of *IMP-L2* indicated that partial *IMP-L2* function for the developing embryo can be obtained from maternal components supplied during oogenesis. To address this point further, total RNA was isolated from embryos during the first 8 hours of development at 23°C and probed to identify IMP-L2 transcripts (Fig. 5A). RNA from embryos aged 0-2 hours had no detectable IMP-L2 mRNA and embryos aged 2-4 hours had only a barely detectable amount although the blots were intentionally overexposed to detect low levels of transcript. In contrast, embryos aged 4-6 hours and 6-8 hours had very high levels of IMP-L2 mRNA. This result indicated that the *IMP-L2* function supplied to the embryo during oogenesis is not provided by IMP-L2 mRNA.

To characterize the distribution of IMP-L2 mRNA during embryonic development, in situ hybridizations were done on embryos ranging in age from 0 to 18 hours (i.e. stages 1-17; Fig. 6A-F). Identical results were obtained using strand-specific DNA probes (data not shown). IMP-L2 mRNA was first detected in cellular blastoderm embryos in a series of seven distinct stripes spanning 20% to 70% egg length (Fig. 6A) in a pattern similar to pair rule genes. The IMP-L2 stripes extended dorsoventrally through the neurogenic and dorsal epidermal regions. The stripes did not extend into the region of the presumptive mesoderm (Fig. 6B) and were also absent from the cells that form the amnioserosa. During germ-band elongation, the stripe pattern was modified. At stage 8, 14 broad bands of staining were localized to the neurogenic ectoderm (Fig. 6C). In stage 9 embryos, strong signal was detected surrounding the tracheal pits (Fig. 6D). During stages 15-16, IMP-L2 mRNA was detected in the pharynx, frontal sac, the esophagus, surrounding the posterior spiracles (Fig. 6E) and the lateral bipolar dendrite neurons [lbd; Bodmer and Jan, 1987 no. 250; Bodmer et al., 1989 no. 251]. By stage 17, IMP-L2 mRNA was limited to the lbd neurons (part of the transverse nerve) and other structures that show accumulation of the protein (Fig. 6F, and see below).

Antisera against the GST/IMP-L2 fusion protein were raised as described in Materials and methods. Production of antibodies against the IMP-L2 portion of the fusion protein was verified by cross-reactivity of the antisera with a lacZ/IMP-L2 fusion protein. On western blots (see Materials and methods), affinity-purified anti-GST/IMP-L2 antibodies recognized a protein from Oregon-R white prepupae with an apparent M_r=32×10^3 (Fig. 5B). Preimmune serum did not recognize this protein and the protein was absent in white

Table 3. Viability of G2 progeny from *IMP-L2* null mothers

cross	*ems^{13}/GN19* (*IMP-L2*$^-$)	*ems^{13}/TM3 Sb Ser* & *GN19/TM3 Sb Ser*
ems^{13}/GN19 ♀♀ × *GN19/TM3 Sb Ser* ♂♂	0	922
	ems^{13}/A466 (*IMP-L2*$^+$)	*ems^{13}/TM3 Sb Ser* & *GN19/TM3 Sb Ser*
ems^{13}/GN19 ♀♀ × *A466/TM3 Sb Ser* ♂♂	476	818

ems^{13}/GN19 heterozygote females are *IMP-L2* null. Viable progeny recovered from the cross *ems^{13}/GN19* ♀♀ × *GN19/TM3 Sb Ser* ♂♂ were only from eggs fertilized by *TM3 Sb Ser* sperm. *IMP-L2* null progeny were not recovered. Viable progeny recovered from the cross *ems^{13}/GN19* ♀♀ × *A466/TM3 Sb Ser* ♂♂ included both *ems^{13}/A466* and the *ems^{13}/TM3 Sb Ser* and *GN19/TM3 Sb Ser* progeny. The A466 deficiency does not delete the *IMP-L2* gene but does delete or break other identified transcription units in the region, indicating that the maternal effect lethality of *IMP-L2*-deficient embryos is due to loss of maternal and zygotic *IMP-L2* function.

Table 4. (A) Rescue of F1 zygotic semilethality by an *IMP-L2* transgene

Cross: P[w^+, *IMP-L2*$^+$]; *GN19/TM3 Sb* ♀♀ × w^{1118}; *ems*^{13}/*TM3Sb Ser* ♂♂

Line	P[w^+,*IMP-L2*$^+$]; *ems13/GN19*	*ems*^{13}/*GN19*	P[w^+,*IMP-L2*$^+$]; *GN19/TM3SbSer*	*GN19/TM3SbSer*
337.011	152	0	148	108
337.092	158	0	171	127
337.221	129	7	201	191
337.481	189	5	239	97
337.551	124	13	137	141
TOTALS	752	25	896	664

An *IMP-L2*$^+$ transgene rescued the zygotic semilethal phenotype of *ems*^{13}/*GN19* heterozygotes. Five independent *IMP-L2*$^+$ transformed lines were tested. Progeny carrying the *IMP-L2*$^+$ transgene were identified as w$^+$ and confirmed by Southern hybridization. For all lines tested, the number of *ems*^{13}/*GN19* heterozygote progeny carrying the transgene approached Mendelian expectations. In contrast, *ems*^{13}/*GN19* heterozygote progeny lacking the transgene were recovered at less than 5% of Mendelian expectations.

Table 4. (B) Rescue of lethality in G2 progeny of *IMP-L2*$^+$, *ems*^{13}/*GN19* females

Cross: *w*; P[w^+, *IMP-L2*$^+$]; *ems*^{13}/*GN19* ♀♀ × *w*; P[w^+, *IMP-L2*$^+$]; *GN19/TM3 Sb Ser* ♂♂

Line	*w*; P[w^+,*IMP-L2*$^+$]; *ems*^{13}/*GN19*	*w*; *ems*^{13}/*GN19*	*w*; P[w^+,*IMP-L2*$^+$]; Def/*TM3 Sb Ser*	*w*; Def/*TM3 Sb Ser*
337.011	4 (17%)	0	48	7
337.091	26 (34%)	0	154	69
337.221	12 (34%)	0	71	21
337.481	16 (15%)	0	219	59
337.551	12 (25%)	0	98	63
TOTALS	70 (24%)	0	590	219

An *IMP-L2*$^+$ transgene partially rescues the maternal effect lethality of *ems*^{13}/*GN19* females. Five independent transformed lines were tested. In the cross, *w*; P[w^+,*IMP-L2*$^+$]; *ems*^{13}/*GN19* ♀♀ × *w*; P[w^+, *IMP-L2*$^+$]; *GN19/TM3 Sb Ser* ♂♂, viable *IMP-L2*$^+$ *ems*^{13}/*GN19* G2 progeny were recovered at frequencies ranging from 14% to 32% of Mendelian expectations (mean=24%) compared to transgenic deficiency/*TM3, Sb, Ser* siblings. No *IMP-L2* null deficiency transheterozygotes were recovered indicating that maternal expression of the *IMP-L2* transgene alone is insufficient to rescue the lethal phenotype. The Def/*TM3 Sb Ser* progeny class includes both *ems*^{13}/*TM3 Sb Ser* and *GN19/TM3 Sb Ser*.

Table 4. (C) Rescue of lethality in G2 progeny of *IMP-L2*$^-$, *ems*^{13}/*GN19* females by a paternal *IMP-L2*$^+$ transgene

Cross: ems13/GN19 ♀♀ × P[w$^+$, IMP-L2$^+$]; GN19/TM3 Sb (TM6B) ♂♂

Line	*ems13/GN19*	*GN19/TM3 Sb*	*ems*^{13}/*TM6B* & *GN19/TM6B*
337.011	40 (8%)	492	
337.221	48 (21%)		459
337.481	8 (14%)		117
337.551	9 (13%)		138

A paternally supplied *IMP-L2*$^+$ transgene partially rescues the maternal effect lethality of *IMP-L2* null *ems*^{13}/*GN19* females. In the cross *ems*^{13}/*GN19* ♀♀ × *w*; P[w^+, *IMP-L2*$^+$]; *GN19/TM6B* ♂♂ viable *IMP-L2*$^+$ *ems*^{13}/*GN19* G2 progeny were recovered at frequencies ranging from 16% to 42% of Mendelian expectations (mean=25%) compared to deficiency/*TM3 Sb Ser* siblings. *IMP-L2*$^+$ transgenic progeny were identified as Tb$^+$ or Sb$^+$. The Df/*TM6B* progeny class includes transgenic and non-transgenic progeny of genotypes *ems*^{13}/*TM6B* and *GN19*/*TM6B*. *ems*^{13}/*TM3 Sb* progeny were not recovered (see legend, Table 1).

prepupae of *ems*^{13}/*GN19* heterozygotes that lacked the *IMP-L2* gene (Fig. 5B). The size of the presumptive IMP-L2 product as detected by western blotting of *Drosophila* proteins (M_r=32×10^3) is larger than the predicted size of the conceptual translation product (M_r=27×10^3). Although no sites for N-linked glycosylation are present in the conceptual IMP-L2 protein sequence, the difference between the predicted and observed size may be due to O-linked glycosylation, other post-translational modifications, or aberrant migration.

The genetic analysis and northern blot results suggested that IMP-L2 protein should be present in embryos as a maternally supplied product. A faint IMP-L2 protein signal was detected on western blots for embryos aged 2-4 hours and a stronger signal was seen for embryos aged 4-6 and 6-8 hours (data not shown) in agreement with the observed changes in IMP-L2 mRNA levels. However, several attempts to detect IMP-L2 protein in extracts from 0-2 hour embryos were inconclusive. IMP-L2 protein may be present in such low abundance as to be undetectable above background using the antibodies available. Alternatively, the antigenic epitopes may be blocked by modifications specific for this developmental stage.

Affinity-purified anti-GST/IMP-L2 antibody was also used to identify the sites of IMP-L2 protein accumulation during embryonic development. Although we expected the IMP-L2 antigen to be present in embryos after 4 hours of development, no localized IMP-L2 could be detected until

approximately stage 16, after dorsal closure. At this stage, several sites of IMP-L2 staining appear on the lateral region of embryos in a segmentally repeating pattern (Fig. 5G, H). This staining pattern is detectable through stage 17. The lateral staining structures in the embryo abdominal region were identified as the lbd neurons (Bodmer et al., 1989; Bodmer and Jan, 1987) when embryos were double stained with anti-GST/IMP-L2 and anti-HRP, which stains all neurons (Jan et al., 1985). The earliest stage of embryonic development at which IMP-L2 staining of the lbd neurons has been detected is when the anterior fascicle has formed and the differentiation of the sensory organs is progressing but not complete.

In the thoracic segments, similarly positioned neuronal cell bodies and their axons were also stained with the IMP-L2 antibodies. The identity of these neurons is not known but they most likely correspond to multiple dendritic neurons described by Bodmer and Jan (1987). Also stained were two neurons in the anterior region of the embryo positioned adjacent to the pharynx. IMP-L2 protein was also detected in two closely positioned cells or cell clusters on the dorsolateral surface of the embryonic optic lobes (data not shown). A final staining structure was also located in the cephalic region of the embryo. This bilobed structure was positioned between the lobes of the supraesophageal ganglion and posterior to the supraesophageal commissure. This structure did not appear to stain with anti-HRP antibody but may represent specialized cells that will constitute part of the ring gland or lymph glands.

In summary, staining of whole-mount embryos with anti-IMP-L2 antibodies showed that IMP-L2 protein was localized to specific neuronal structures late in embryogenesis. All sites of IMP-L2 protein localization in stage 16-17 embryos have been identified as sites of IMP-L2 mRNA expression. This was in contrast to earlier stages of embryogenesis when IMP-L2 mRNA was abundant and IMP-L2 protein was detectable by western blotting but no protein could be localized in whole-mount embryos.

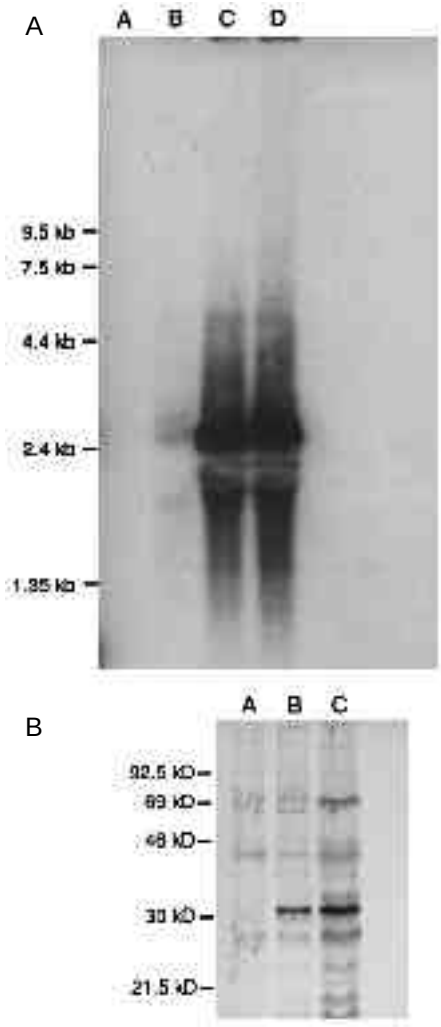

Fig. 5. (A) Expression of IMP-L2 mRNA during early embryogenesis. Autoradiogram of northern blots done as described in Materials and Methods. Total RNA was isolated from embryos aged 0-2 hours (lane A), 2-4 hours (lane B), 4-6 hours (lane C) and 6-8 hours (lane D). No signal was detected in 0-2 hour embryo RNA and only weak signal was detected in 2-4 hour RNA. Strong signal was detected beginning in 4-6 hour and 6-8 hour RNA. The film was intentionally overexposed to detect faint signal in the 0-2 hour and 2-4 hour RNA samples.
(B) Autoradiogram of western blot of proteins from *IMP-L2*$^{+}$ and *IMP-L2*$^{-}$ prepupae. Prepupae of genotype *ems*^{13}*/GN19* (lane A) and Oregon-R (lanes B and C) were crushed in SDS sample buffer and the proteins separated by SDS-PAGE. Proteins were transferred to a nitrocellulose filter and probed with affinity-purified anti-IMP-L2 antibody. HRP-conjugated secondary antibody was used in the detection reaction with the Amersham ECL detection system. Note the strong signal in the Oregon-R samples for IMP-L2 (M_r=32×10^3) that is absent in the *ems*^{13}*/GN19* sample. The film was intentionally overexposed to show the absence of IMP-L2 protein in the *ems*^{13}*/GN19* sample.

DISCUSSION

Our analysis of *IMP-L2* has identified it as a new member of the immunoglobulin superfamily in *Drosophila* with an essential developmental role during embryogenesis. *IMP-L2*$^{-}$ embryos from *IMP-L2*$^{+}$ mothers show a partial lethal phenotype (average viability reduced to less than 10% of expected). *IMP-L2*$^{-}$ embryos from *IMP-L2*$^{-}$ mothers were 100% lethal during late embryogenesis or as early first instar larvae. Genetic analysis suggests that IMP-L2 product may be supplied to the oocyte during oogenesis. Although we have been unable to detect IMP-L2 mRNA or protein in 0-2 hour embryos, we suggest that IMP-L2 protein is present below the level of detection and is sufficient to permit viability of *IMP-L2* null zygotes. In the absence of maternal IMP-L2, zygotically produced IMP-L2 product, synthesized after cellular blastoderm formation, rescues the lethal phenotype. An *IMP-L2* transgene provided zygotic rescue at 10-20% of the efficiency of an endogenous *IMP-L2* gene. The *IMP-L2* transgene does not appear to provide sufficient IMP-L2 function to rescue *ems*^{13}*/GN19* heterozygous progeny of *ems*^{13}*/GN19* heterozygous mothers.

We have not identified the cause of lethality in *IMP-L2* null *Drosophila*. The lbd neurons that accumulate IMP-L2 protein are present in both F_1 and G_2 *IMP-L2* null progeny indicating that *IMP-L2* is not required for the formation of

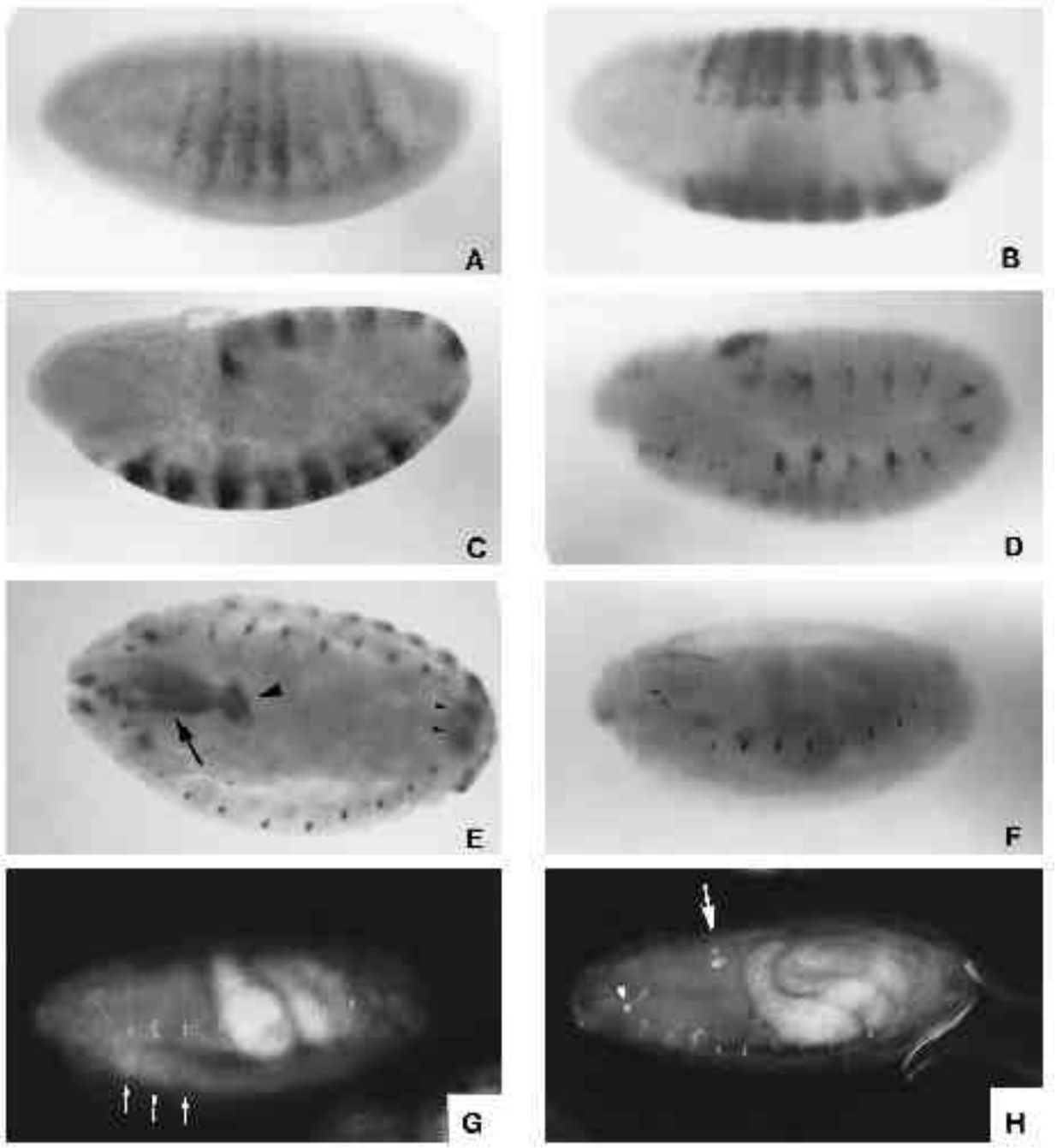

Fig. 6. Photomicrographs of embryos showing expression of IMP-L2 mRNA and protein during embryogenesis. Embryos were prepared for in situ hybridization (Fig. 6A-F) as described in Materials and Methods. The digoxigenin-labeled probe was detected using anti-digoxigenin antibody coupled to alkaline phosphatase. To localize IMP-L2 protein (Fig. 6G,H), embryos were stained with affinity-purified anti-GST/IMP-L2 primary antibodies and FITC-conjugated goat anti-rat secondary antibodies. All embryos are oriented with anterior to the left. Lateral view of embryo at the cellular blastoderm stage (A) shows IMP-L2 mRNA in seven stripes. Ventrolateral view of embryo (B) at the cellular blastoderm stage shows IMP-L2 mRNA is not detected in the presumptive mesoderm. Lateral view of embryo after germ band extension (C). IMP-L2 mRNA is present in fourteen segmentally repeating regions of the ventral neuroectoderm. Lateral view of embryo prior to germ band retraction (D) showing IMP-L2 mRNA expression in the neuroectoderm is reduced and is replaced by expression surrounding the tracheal pits. Dorsal view of stage16 embryo (E) shows major sites of IMP-L2 mRNA expression including the pharynx (large arrow), esophagus (large arrowhead) and posterior spiracles (small arrowheads) in addition to the lateral bipolar dendrite (lbd) neurons and several other cell bodies. Lateral view of stage 17 embryo (F). IMP-L2 mRNA expression is limited to the lbd neurons in the abdomen and a pair of neurons adjacent to the pharynx. IMP-L2 protein is detected in whole-mount embryos beginning at stage 16. Lateral view of stage 16 embryo showing IMP-L2 protein on the lbd neurons in the abdomen (G, small arrowheads). Dorsolateral view of stage 16 embryo (H) shows staining of the lbd neurons, the anterior neurons adjacent to the pharynx (small arrow), and a structure near the supraesophageal ganglion (large arrow).

these neurons. Whether the other cells and tissues that accumulate IMP-L2 protein are also unaffected is currently being investigated. It is also possible that the lethal defect will be found at sites where IMP-L2 mRNA is expressed although the protein is not detected.

The results of our analysis raise three major issues regarding the role of *IMP-L2* during development. First, the essential embryonic requirement for *IMP-L2* can be partially met if the mother is *IMP-L2*$^+$. This suggests that *IMP-L2* is a maternal effect locus. Second, although IMP-L2 mRNA is abundant in many sites after cellular blastoderm formation, IMP-L2 protein is only localized late in development close to the stage when *IMP-L2* null zygotes die. Third, like other *Drosophila* Ig molecules, *IMP-L2* is primarily expressed in neural cells. This suggests a role for *IMP-L2* in the proper development or function of the nervous system.

Zygotic and maternal effect lethality

The developmental processes occurring during the early stages of embryogenesis are mediated by maternal products supplied during oogenesis. These maternally supplied gene products direct subsequent development by modulating the hierarchical expression of zygotic genes involved in embryonic pattern formation. Maternal effect embryonic lethal mutations are defined as those that have no obvious effect on the morphology or viability of homozygous mutant females but result in lethality of their embryos (Schüpach and Wieschaus, 1986, 1989).

Another group of maternal effect mutations are those where the gene products required for embryonic development can be supplied either by the maternal genome during oogenesis or the zygotic genome during embryogenesis. Among these mutations are *maroonlike* (Chovnick et al., 1969), *fused* (Fausto-Sterling, 1971), *cinnamon* (Baker, 1973), *rudimentary* (Fausto-Sterling, 1971), *deep orange* (Garen and Gehring, 1972), *abnormal oocyte* (Mange and Sandler, 1973) *almondex* (Lehmann et al., 1983), *pecanex* (LaBonne and Mahowald, 1985) and *l(1)pole hole* (Perrimon et al., 1985). Common to all of these, mutant females lay eggs that do not undergo normal embryogenesis unless the zygote receives a wild-type allele, paternally. In the case of *l(1)pole hole*, the severity of the lethal phenotype is substantially reduced but not eliminated by the paternally supplied allele. The zygotic and maternal effect lethal phenotype of *IMP-L2* null embryos suggests that *IMP-L2* is a member of this class of maternal effect loci.

Post-transcriptional regulation

The second point of interest in our analysis is the apparent discrepancy between where IMP-L2 mRNA and IMP-L2 protein were expressed. As described, IMP-L2 transcripts are first observed during the cellular blastoderm stage. Subsequently, high levels of IMP-L2 mRNA accumulate in the region of the ventral neuroectoderm and around the tracheal pits. Ultimately, transcripts are detected only in the lateral bipolar neurons. In contrast, we have detected no localized IMP-L2 protein accumulation during the early stages of embryogenesis. Although it is detected in western blots, IMP-L2 protein localization is first seen only in stage 16-17 embryos. There are three possible reasons for our inability to localize IMP-L2 protein in early embryonic stages. First, the IMP-L2 protein may be secreted from the cell and rapidly diffuse into the surrounding tissues. A consequence of the IMP-L2 protein being secreted is that the phenotypic effects leading to lethality may occur in regions that show no evidence of IMP-L2 mRNA or protein synthesis. Second, turnover of the IMP-L2 protein after it is secreted may be sufficiently rapid that the protein does not accumulate to detectable levels for immunolocalization. Third, and most intriguing, IMP-L2 mRNA may be subject to some form of post-transcriptional or translational regulation mediated by sequences in the leader.

Based on the scanning model of translational initiation, Kozak has argued that most RNAs with small ORFs in the leader represent incompletely processed transcripts, not the mRNA species that are actually translated (Kozak, 1989, 1991). Indeed, for some characterized transcripts, alternatively processed mRNAs have been identified that do not have ORFs in the leader (see Kozak, 1989, 1991). Thus, it is possible that the IMP-L2 transcript represented by the 2.4 kb cDNA is an incompletely processed RNA that is not translated. This raises the possibility that during embryogenesis, a minor IMP-L2 mRNA species would be synthesized that lacks ORFs in the leader and is translated in the cells where IMP-L2 protein is detected. Although our analysis has not indicated the presence of alternatively processed IMP-L2 mRNAs, this possibility cannot be excluded.

Kozak's arguments also raise a significant point regarding the function of *IMP-L2* in *Drosophila.* In vertebrates, most transcripts with ORFs in the leader encode proteins that function in growth regulation or signal transduction including proto-oncogenes and transcription factors (Table II in Kozak, 1991). Overexpression of these proteins would be detrimental and incomplete RNA processing would be one mechanism to down-regulate gene expression. By analogy, IMP-L2 protein may be required in small amounts or only in specific tissues and overexpression of *IMP-L2* may be detrimental or lethal. In vitro mutagenesis of the IMP-L2 leader or overexpression of the IMP-L2 protein would address this issue. At present, we do not know whether the IMP-L2 cDNA represents an incompletely processed transcript that is not translated or the normal IMP-L2 mRNA with ORFs in the leader. To distinguish conclusively between these and other possibilities will require the identification of a second IMP-L2 mRNA with an ORF-free leader. However, in either situation, the predicted result would be the same as our experimental results; the amount of IMP-L2 protein detected would be lower than expected based on the amount of IMP-L2 transcript.

Functions of immunoglobulin domain-containing molecules in *Drosophila*

The third major point raised by our results derives from our analysis showing *IMP-L2* is a member of the Ig superfamily. To speculate on the possible role of *IMP-L2*, it is useful to consider the apparent function of other Ig domain-containing proteins in *Drosophila* and the data derived from studies on Ig superfamily proteins from vertebrates. Although several Ig domain-containing proteins have been identified in vertebrates, functional analysis has been limited to experiments involving purified proteins as substrates or antibody perturbation. The *Drosophila* homologs of some of

the vertebrate proteins have been identified, making them ideal candidates for combined molecular and genetical analysis.

At least nine Ig superfamily members have been identified and characterized in *Drosophila* (Hortsch and Goodman, 1991). Two of these, Neuroglian, (Bieber et al., 1989) and Fasciclin II, (Grenninglogh et al., 1991b), have been characterized as cell adhesion molecules, having extracellular regions consisting of Ig and fibronectin type III domains. The cell adhesion molecule Fasciclin III (Snow et al., 1989) has three extracellular Ig domains that are highly divergent. The *Drosophila* fibroblast growth factor receptor (D-FGFR; Glazer and Shilo, 1991; Klämbt et al., 1992) and neurotrophin receptor (Dtrk; Pulido et al., 1992) have cytoplasmic kinase domains. *Drosophila* homologs of the phosphotyrosine phosphatases DLAR and DPTP, (Streuli et al., 1989; Tian et al., 1991) have cytoplasmic phosphatase domains. These kinase and phosphatase domains indicate roles in intercellular signalling through familiar signal transduction pathways.

The *Drosophila* secreted Ig family members are Amalgam, Lachesin and IMP-L2. The *amalgam* (*ama*) gene was identified as a transcription unit within the *Antennapedia* complex (Seeger et al., 1988). The conceptual product is a secreted protein with three Ig domains that accumulates on various mesodermal and neural cells during embryogenesis. No *ama*-specific null mutations are known. Analysis of deficiencies that delete *ama* and the adjacent *zen* and *bicoid* loci indicate no obvious neurological defects that can be attributed to the loss of *ama* (Seeger et al., 1988). The sequence of the Ama protein is most similar to N-CAM (Barthels et al., 1987; Hemperly et al., 1986) and Fasciclin II (Harrelson and Goodman, 1988). Lachesin was identified immunologically and is most similar to Amalgam (Karlstrom et al., 1993). No mutations in the *lachesin* gene have been described. *IMP-L2* was identified as an ecdysone-responsive gene in imaginal discs. *IMP-L2*, however, is essential for development. Embryos with IMP-L2 protein-null genotypes die primarily in the 1st instar, and like mutants of *fasciclin II* and *neuroglian*, have no severe abnormalities. Our data clearly indicate an essential role for *IMP-L2*. We predict, however, that the loss of *IMP-L2* function results in subtle morphological abnormalities.

Three conclusions emerge from the characterization of Ig family members in *Drosophila*. The first is that, among their various sites of expression, most *Drosophila* Ig family members are expressed in neurons. Thus, Ig family members are strongly implicated in various aspects of neural development.

The second conclusion is that loss of an Ig protein product may not result in major disruptions of the embryonic nervous system but may produce subtle defects. Loss of neuroglian results in a general loss of intercellular adhesiveness that is most evident in the PNS. Fasciclin II null embryos lack specific axons in an otherwise normal appearing CNS. These results are consistent with the possibility that these gene products have very specific roles in development, e.g. affecting particular axons but not the entire CNS or PNS. These results are also consistent with the lethal phenotypes of some of the mutants. For example, failure to hatch from the egg or death during the early first instar could easily result from the miswiring of the CNS and resultant motor defects. Finally, these results are also consistent with the re-emerging view of functional redundancy, in which the same function may be mediated by different molecules, such that loss of one of these molecules will have little or no obvious effect. The data for *nrg* (Bieber et al., 1989) could thus be interpreted to show that, although Nrg is involved in intercellular adhesion of neural cells, other molecules (e.g., integrins; Hynes, 1992) also promote adhesion in those cells. Likewise, the function of an independent protein could compensate for the loss of *ama*.

The third conclusion is that secreted *Drosophila* Ig family members have unknown functions. This is true even though most *Drosophila* Ig family genes have been identified as homologs of mammalian or, in the case of the fasciclins, grasshopper genes. Both *ama* and *IMP-L2* encode secreted proteins. Mammalian counterparts of these genes with known functions apparently have not been described. A secreted form of N-CAM has been found, but its function is unknown (Gower et al., 1988; Pizzey et al., 1989). Axonin-1, a possible chicken homolog of TAG-1 and F3, is expressed by axons and contains fibronectin as well as Ig domains. In its PI anchored form, axonin-1 functions as a substrate adhesion molecule (Zuellig et al., 1992). It has been proposed (Stoekli et al., 1991) that the secreted form of axonin-1 is an anti-adhesion molecule. Mouse F3, which also contains both Ig and fibronectin domains, may stimulate neurite outgrowth in its secreted form by binding to cell surface receptors to elicit a signal transduction response and not by acting mechanically as an adhesion molecule (Durbec et al., 1992; Saffell et al., 1992). It is not known whether the activities of axonin-1 and F3 are mediated by the fibronectin domains or the Ig domains. None the less, secreted Ig family members, by binding to cell surfaces or matrices may act at a distance from their sites of synthesis as adhesion molecules, as ligands for signal transduction, as tropic molecules (Durbec et al., 1992) or as anti-adhesion molecules. We infer that IMP-L2 protein may also bind to specific cell adhesion molecules. Because IMP-L2 is essential for development, it provides an unusual opportunity to study the developmental function of a secreted Ig family member.

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