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Molecular analysis of the locus *elav* in *Drosophila melanogaster*: a gene whose embryonic expression is neural specific

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The *embryonic lethal abnormal visual system* (*elav*) locus in *Drosophila melanogaster*, a vital gene mapping within the 1B5-1B9 region of the X-chromosome has been cloned and analysed. Previous developmental analyses have shown that in addition to the embryonic requirement there is a post-embryonic requirement for *elav* function in the cells of the visual system. A DNA segment containing *elav*$^+$ function was defined through germ line transformation experiments. This region encodes three embryonic poly(A)$^+$ RNAs and two adult transcripts which are preferentially expressed in the head. *In situ* hybridization experiments clearly demonstrate that the embryonic expression of *elav* is restricted to the nervous system.

Key words: developmental expression/*elav* locus/germ line transformation/neurogenetics/RNA localization

Introduction

The potential of molecular genetics as a tool to study the mechanisms underlying neuronal development, structure and function is just beginning to be appreciated. In the fruit fly, *Drosophila melanogaster*, a large number of loci have been genetically identified as being required for normal function or development of the nervous system (reviewed in Hall, 1982). Further understanding of the mechanisms by which these gene products act will depend on their detailed molecular characterization. Thus far, molecular analyses have been initiated for only a handful of these genes. Examples include: *Notch*, a locus that affects neurogenesis (Artavanis-Tsakonas *et al.*, 1983; Kidd *et al.*, 1983); *period*, a locus that affects biological rhythms (Reddy *et al.*, 1984; Bargiello and Young, 1984); and *nina E*, a locus that encodes opsin (O'Tousa *et al.*, 1985; Zuker *et al.*, 1985).

In this paper the initial molecular characterization of the gene *embryonic lethal abnormal visual system* (*elav*), a locus whose function is required in at least some cells of the nervous system and may be neural specific (Campos *et al.*, 1985; Homyk *et al.*, 1985), is described. Our previous developmental study of mutant alleles at this locus and analysis of genetic mosaics led to the following conclusions: (i) *elav* maps within the 1B5-9 interval of the X chromosome, a region that had been previously identified as being essential for the proper formation of the embryonic nervous system (Jimenez and Campos-Ortega, 1979; White, 1980); (ii) mutations at the locus cause embryonic lethality; (iii) the *elav* function is autonomously essential in the eye; (iv) the gene function is autonomously essential for the normal development of the optic lobes; and (v) the *elav* function is not required for the viability of all cell types. Therefore, there is a role for *elav* function at least during embryogenesis and, post-embryonically, in the cells of the developing visual system.

Molecular analysis of *elav* was initiated using a previously cloned piece of genomic DNA from the 1A6-1B1 region of the X chromosome (R.J.Fleming, personal communication), and a chromosomal inversion to jump into the *elav* region. This inversion is associated with a lethal allele of the locus, the *elav*^{G3} chromosomal aberration, which has breakpoints near 1A6 and 1B5-9. Approaches used to define the limits of the putative *elav* encoding DNA included molecularly defining the breakpoints of a set of terminally deficient chromosomes that either delete or retain *elav* gene function, and analyzing RNAs transcribed from the vicinity of the proximal *elav*^{G3} breakpoint. The transcriptional pattern of relevant genomic sequences was also studied by developmental Northern analysis. DNA sequences that provide the embryonic function were further defined using P-element-mediated transformation and subsequent rescue of the *elav* lethal alleles by this transduced segment of DNA. To obtain information on the tissue distribution of the *elav* transcripts, we probed serial sections of staged embryos with anti-sense *elav* RNA.

Results

Molecular cloning of the elav region DNA

The gene *elav* is located within the salivary segment 1B5-9 of the X chromosome of *D. melanogaster* (Lefevre, 1981; Campos *et al.*, 1985). To clone the DNA corresponding to the gene *elav*, advantage was taken of a mutation *elav*^{G3} which is associated with a small chromosomal inversion with breakpoints near 1A6-1B1 and 1B5-9. Because this inversion is associated with a mutant allele of *elav*, it was reasoned that the proximal breakpoint in the vicinity of 1B5-9 was likely to be very near or within this gene (Figure 1). The distal breakpoint in the vicinity

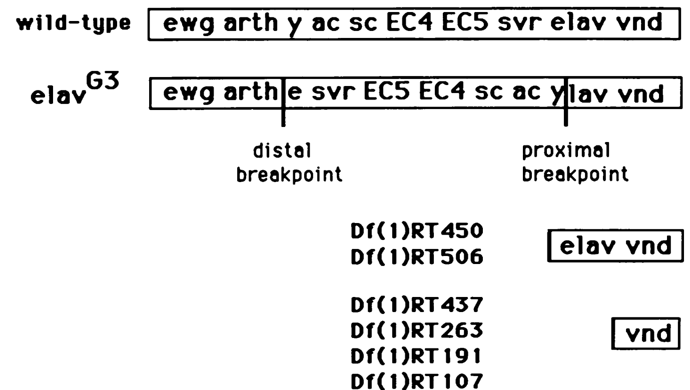

Fig. 1. Chromosomal aberrations used to define the *elav* gene. Top box shows the complementation groups that map to the *ewg*–*vnd* region of the X-chromosome. Below the vertical bars indicate the inversion breakpoints of the *elav*^{G3} allele used to jump into the *elav* region. *Df(1)RT*$^{\#}$ denotes the terminal deletions used to define the *elav* gene DNA, the deleted complementation groups are not drawn in.

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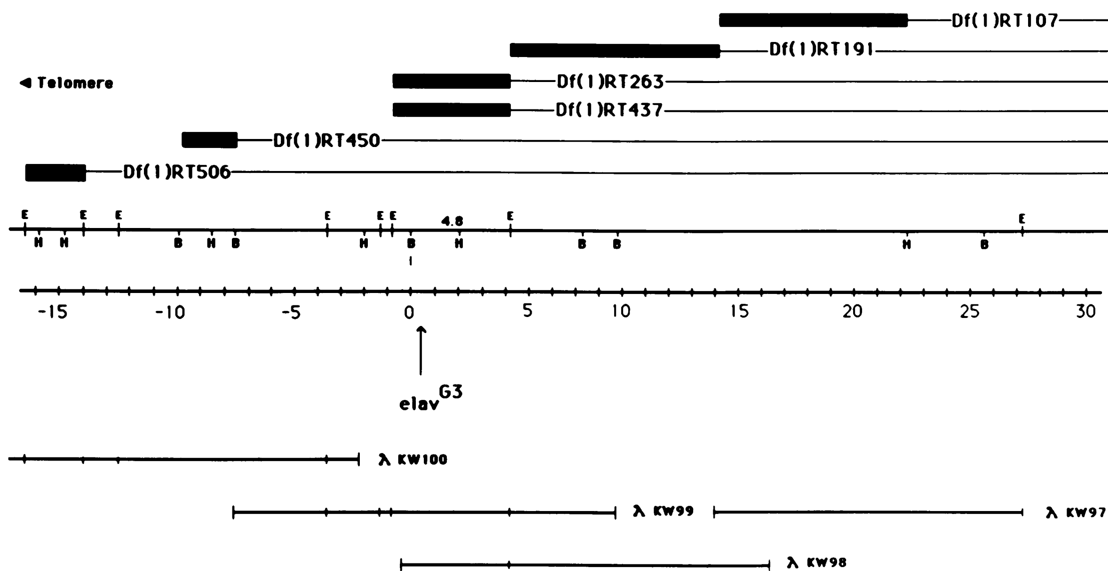

Fig. 2. Mapping of recombinant phage and chromosome breakpoints in the *elav* region. The *Drosophila* DNA contained in four overlapping recombinant phage (KW100, KW99, KW98, KW97) is shown at the bottom of the figure. '0' on the scale corresponds to the *Bam*HI site next to the $elav^{G3}$ breakpoint u 1 to jump in this region. In the restriction map R = *Eco*RI sites, B = *Bam*HI and H = *Hin*dIII. On the top, the breakpoints of terminal deletions that either retain [*Df(1)RT450*, *Df(1)RT506*] or abolish [*Df(1)RT263*, *Df(1)RT437*, *Df(1)RT107* and *Df(1)RT191*] *elav* function are shown. Each breakpoint has been localized to a region denoted by bold bars. The empty region preceding these bars designates the deleted DNA.

of 1A6-1B1 was likely to be immediately distal to the gene *yellow* which maps in the 1B1 interval and which has been previously cloned (Biessmann, 1985; Parkhurst and Corces, 1986). Approximately 90 kb of DNA distal to the coding region of *yellow* has been cloned by R.Fleming in our laboratory in a chromosomal walk from the *yellow* to the *erect wing* locus (Fleming, personal communication). Genomic Southern blots of DNA from flies heterozygous for the mutation $elav^{G3}$ were probed with recombinant phage from the *yellow–erect wing* DNA walk. We were able to define molecularly the distal inversion breakpoint of $elav^{G3}$ to a 2.0-kb genomic *Eco*RI fragment ~35 kb from the coding sequences for the gene *yellow* (data not shown).

The strategy to jump into the proximal 1B5-9 region of the $elav^{G3}$ inversion, using the sequences spanning the distal inversion breakpoints was similar to that used by Bender *et al.* (1983). In brief, a genomic DNA library constructed of DNA from flies heterozygous for the $elav^{G3}$ inversion was probed with a wild-type DNA clone which spanned the distal inversion breakpoint. From this library, recombinant phage that contained the 'fusion fragment' DNA sequences from the proximal breakpoint of the inversion were obtained. The fusion fragment DNA was then used to isolate wild-type DNA in the vicinity of the proximal breakpoint of the $elav^{G3}$ by probing wild-type genomic libraries. Subsequently, by standard chromosome walking techniques (Bender *et al.*, 1983) ~70 kb of DNA has been isolated in overlapping recombinant phage from the vicinity of the $elav^{G3}$ proximal breakpoint. Figure 2 shows the restriction map of 30 kb of DNA surrounding this breakpoint. The *Bam*HI restriction site closest to the $elav^{G3}$ breakpoint in question has been arbitrarily chosen as position '0'.

Delimiting the gene

The locus *elav* is flanked by two vital genes, distally by *silver* (*svr*) and proximally by *ventral nervous system condensation defective* (*vnd*) (Campos *et al.*, 1985). A series of terminally deficient X chromosomes provided the means to limit the *elav* gene. These deficiencies were generated by Dr J.Mason using an X-ray dependent mutator gene *mu-2* (Mason *et al.*, 1986). The genetic breakpoint of each of the terminal deletions was determined by performing complementation tests with a representative allele of the lethal complementation groups *svr*, *elav* and *vnd* as in Campos *et al.* (1985). In this way a set of deficiency chromosomes that have retained the *elav* gene was defined. Similarly, a set of deficiency chromosomes with breakpoints in or proximal to the *elav* gene was defined on the basis of retaining *vnd* but not *elav* gene function (Mason *et al.*, 1986). Figure 1 depicts the subset of deficiencies that flank *elav* and are relevant to the molecular analysis presented in this paper.

Genomic Southern blots of DNA isolated from flies carrying a chromosome deleted of segments flanking the *elav* gene were probed with subcloned DNA from the −30 to +20 kb region. We thus mapped the breakpoints for two of the eight terminal deficiencies with their genetic breakpoints distal to *elav* region. The deletion that complements *elav* mutations with its breakpoint closest to the $elav^{G3}$ breakpoint is *Df(1)RT450* (Figure 2). This lesion maps to a restriction fragment between −8 and −10 kb. This small region is therefore the distal limit of the *elav* locus. The deletion with the second closest breakpoint, *Df(1)RT506*, has a breakpoint within a restriction fragment between −14 and −17 kb (Figure 2).

Terminal deletions that fail to complement *elav* lethal alleles may be either completely deficient for the gene or break within the gene; therefore their breakpoints are expected to be proximal to the *Df(1)RT450* breakpoint. Approximately 40 terminal deletions that are vnd^+ but abolish *elav* functions have been reported (Mason *et al.*, 1986). An arbitrarily chosen set of nine deficiency stocks was analyzed by Southern blotting. The breakpoints for all of these deletions have been defined to within ~50 kb of DNA from the *Df(1)RT450* breakpoint. As expected, these breakpoints were all proximal to the *Df(1)RT450* breakpoint. The deficiency chromosomes with breakpoints closest to the *Df(1)RT450* lesion are *Df(1)RT437* and *Df(1)RT263* (Figure 2). These breakpoints are located within the 4.8-kb *Eco*RI restriction fragment (Figure 2), the same fragment to which the $elav^{G3}$

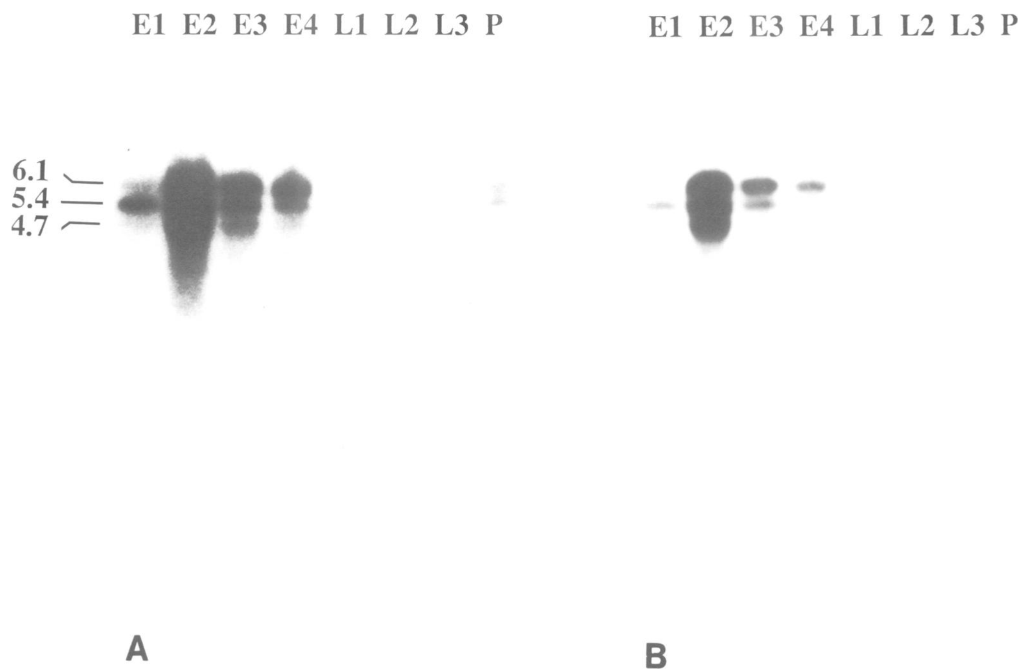

Fig. 3. Developmental Northern blot analysis of the poly(A)$^+$ RNAs encoded in the vicinity of the *elav*^{G3} breakpoint. The 4.8-kb *Eco*RI fragment that spans the breakpoint of the *elav*^{G3} inversion was nick-translated and hybridized to a Northern blot of poly(A)$^+$ RNA extracted from various stages as follows: embryos; from 0 to 6, 6 to 12, 12 to 18 and 18 to 24 h are labeled E1–E4 respectively; first instar larvae, L1; second instar, L2; climbing third instar, L3 and 1-day pupae, P. Each lane contained ~4 μg of poly(A)$^+$ RNA. The blot was subsequently hybridized to a ribosomal protein probe to control for loading of the samples (*rp49*, James *et al.* 1986). The **A** and **B** panels represent 24 h and 17 h exposure of the same blot. In **B**, note the presence of three bands in the E2 lane, lane E3 was underloaded in relation to all other lanes. Note the signal in lane P. The molecular size standards used were *Hin*dIII fragments of a λ phage.

breakpoint maps. The breakpoint for *Df(1)RT191* was localized to a 10 kb region (+4 to +14 kb) and that for *Df(1)RT107* was determined to be within an 8 kb region (+15 to +23 kb, Figure 2).

Genomic Southern blots of DNA from five different strains carrying *elav* alleles were also probed with DNA from −15 to +10 kb. No abnormalities were observed with the exception of *elav*^{G3} as previously described.

The elav^{G3} *chromosome breaks within a transcribed DNA region*

Previous developmental–genetic analyses have demonstrated a requirement for *elav* function during embryogenesis and in the adult visual system (Campos *et al.*, 1985; Homyk *et al.*, 1985). The embryonic function is disrupted by the *elav*^{G3} mutation, as mutant *elav*^{G3} embryos do not hatch (Campos *et al.*, 1985). We have also observed an eye defect associated with hemizygous *elav*^{G3} clones in gynandromorphic mosaics (data not shown). These data indicate that the *elav*^{G3} inversion behaves genetically as a mutant allele of the *elav* locus. Therefore we decided to probe for RNA transcripts in the vicinity of the *elav*^{G3} proximal breakpoint.

The 4.8-kb *Eco*RI subclone of the recombinant phage KW99 (Figure 2), which spans the *elav*^{G3} proximal breakpoint, was initially used to probe Northern blots of poly(A)$^+$ RNA obtained from 6- to 12-h embryos (Figure 3). Three transcripts, ~6.1, 5.4 and 4.7 kb in length (respectively A^E, B^E and C^E), were detected. That the *elav*^{G3} inversion breakpoint maps within this genomic region suggests that this transcriptional unit corresponds to the *elav* gene.

Developmental analysis of elav transcripts

Northern blots of poly(A)$^+$ RNA, isolated from embryonic and postembryonic stages, were probed with the 4.8-kb *Eco*RI fragment (Figure 3). The 5.4 kb transcript (B^E) is detected in the RNA samples from 0–6 h embryos and is present throughout embryonic development. The 6.1-kb and 4.7-kb transcripts (A^E and C^E respectively) are present mainly from 6 to 18 h of embryonic development. During larval development these transcripts are undetectable by our Northern analysis. Two transcripts A^P and B^P are detected during pupal stages, though at a much lower level than seen during embryogenesis (Figure 3). In adult flies the same 4.8-kb *Eco*RI fragment used to probe the developmental Northern in Figure 3, hybridizes to two transcripts, A^A and B^A of ~6.1 and 5.4 kb respectively (Figure 4).

The transcripts A^A and B^A observed in the adult are head-enriched based on Northern blots where equal amounts of poly(A)$^+$ RNA from isolated heads and whole adults were loaded in adjacent lanes (Figure 4).

Characterization of the embryonic elav transcripts

To characterize the three embryonic transcripts, Northern blots of poly(A)$^+$ RNA extracted from 6- to 12-h-old embryos were probed with contiguous subclones from the genomic DNA region −10 to +7 (Figure 5). The subclones c, d, e, f and g hybridize to each of the three embryonic transcripts. Furthermore the λ phage KW 97 (see Figure 2) that contains 14 kb of DNA proximal to KW 99 was also used to probe Northern blots. It detected one transcript in 6- to 12-h embryos and whole adults of much smaller size than the *elav* transcripts. These data taken together suggest that transcripts B^E and C^E, 5.4 kb and 4.7 kb, respectively, share extensive homology as they are encoded within a 7.8-kb genomic region. The longest transcript, A^E, is also detected by the distal subclones a and b which span ~6 kb of genomic DNA and fail to detect the smaller transcripts B^E and C^E.

Fig. 4. Head versus whole adult Northern blots. 4 μg of poly(A)$^+$ RNA extracts from fly heads was loaded on an agarose gel next to an approximately equal amount of poly(A)$^+$ RNA obtained from intact adult flies (males and females). The Northern blot was hybridized to the 4.8-kb *Eco*RI fragment that spans the *elav*^{G3} breakpoint. Subsequently the same blot was hybridized to an *rp49* probe to demonstrate the equal loading of the samples (lower panel).

To determine the direction of transcription, subclones b and f were cloned into the plasmid pGEM-1 to obtain single-stranded RNA probes. Positive Northern blot signals from single-stranded probes indicate that the three transcripts are all transcribed in a proximal to distal direction as shown in Figure 5.

A cDNA, ~2.3 kb in length was isolated by probing an embryonic cDNA library with the 4.8-kb *Eco*RI fragment. The cDNA shared homology with the genomic subclones e, f and g (Figure 5) and with the three embryonic transcripts. In addition, Southern analysis showed that subclone h has a very small homology with the cDNA indicating that the 5′ end of the cDNA is within this fragment. This same subclone (h) does not detect any transcripts in our Northern blots indicating that the transcribed segment is indeed very small.

In summary, the genomic sequences homologous to the putative *elav* transcriptional unit are spread over a 16-kb genomic region. The transcriptional unit is composed of at least three RNAs distinguishable on the basis of size. DNA segments that specifically hybridize to either of the two short embryonic transcripts B^E or C^E have not yet been identified. The distal subclones a and b contain sequences specific to the longest transcript A^E.

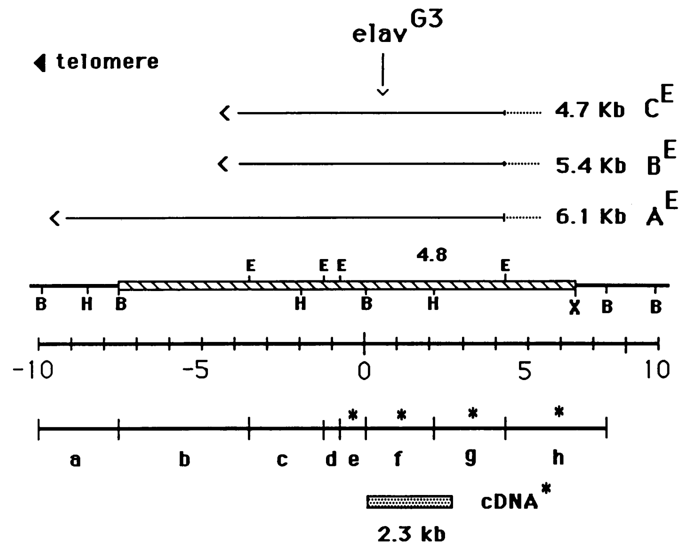

Fig. 5. Characterization of the embryonic *elav* transcripts. The RNAs transcribed in the vicinity of the *elav*^{G3} inversion breakpoint are indicated above the DNA restriction map. The restriction map and the scale are as in Figure 1. The individual fragments used to probe Northern blots are indicated below the restriction map (subclones a to h). These fragments were subcloned into pEMBL 9$^+$, nick-translated and hybridized to Northern blots of 6- to 12-h poly(A)$^+$ RNA. The subclones c–g hybridize to transcripts A^E, B^E and C^E, while subclones a and b hybridize only to transcript C^E. The b and f subclones were subsequently cloned into pGEM-1 and single-stranded RNA probes were used to determine the direction of transcription. The direction of transcription is denoted by the arrowheads. The hatched area of the restriction map corresponds to the DNA fragment inserted in the *Drosophila* genome through P-mediated transformation. The asterisks indicate the restriction fragments that have homology to the embryonic cDNA used in the *in situ* hybridization experiments.

Transformation assay

The 13.5-kb *Bam*HI–*Xba*I DNA fragment shown in Figure 5 was introduced into the germ line of *D. melanogaster* through P-element-mediated transformation (Rubin and Spradling, 1982). This fragment probably includes the entirety of transcripts B^E and C^E but is missing the 3′ end of transcript A^E. Two independent transformed fly lines were established.

Each line was tested for the ability of the insert to restore the *elav* gene function, as assayed by rescue of lethality caused by *elav* mutant alleles. As shown in Table I, both transformed lines were able to rescue the lethality of all alleles of the *elav* gene tested but were not able to rescue the lethality caused by mutations in the adjacent vital loci *vnd* and *svr*.

Embryonic elav expression is limited to the central nervous system

In order to determine whether the embryonic requirement for the *elav* gene function is neural in nature, the tissue distribution of *elav* transcripts in the embryo has been studied. The single-stranded RNA probe was transcribed from the 2.3-kb embryonic cDNA described earlier and hybridized to tissue sections prepared from 6- to 12-h-old embryos.

Figure 6A and B show an autoradiogram of a sagittal section of an embryo in bright and dark field respectively. As seen by the localization of silver grains, the *elav* transcripts appear to be present exclusively and with relatively uniform distribution in the brain and ventral nervous system. This pattern of localization was observed in all embryos of this stage analyzed (144/144). No localization was observed when the sense strand was used as a probe. In preliminary studies with embryos in earlier and

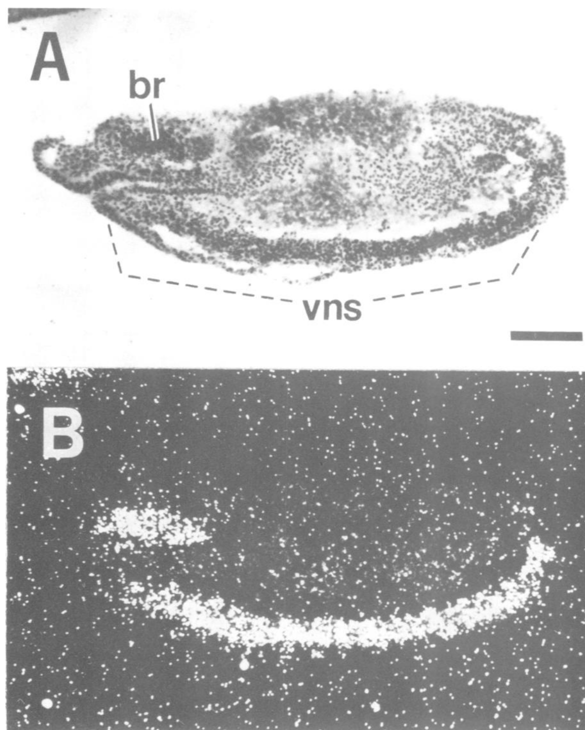

Fig. 6. Distribution of *elav* transcripts during embryogenesis. Tissue section of 9:20–10:20 h embryos (Stage 13; Campos-Ortega and Hartenstein, 1985) hybridized with anti-sense RNA probe of the 2.3-kb cDNA. Autoradiographs exposed for 19 days. Anterior is to the left and ventral is down. (**A**) Parasaggital section, bright field. (**B**) Corresponding dark-field photomicrograph. b, brain; vns, ventral nervous system. The horizontal bar represents 50 μm.

later stages of development, we have observed that the *elav* transcripts are not detected in the nuclear blastoderm and that they appear to remain neural specific in the later half of embryogenesis.

Discussion

Approximately 70 kb of genomic sequence in the 1B5-9 region of the X chromosome has been cloned. The *elav* gene transcriptional unit spans ~16 kb of genomic DNA. The assignment of the distal border of the gene is based on the breakpoint of *Df(1) RT450* which retains the *elav* gene function but is deleted for all genes distal to *elav* (Figures 1 and 2). The breakpoint of *Df(1)RT450*, and the 3′ end of the *elav* transcriptional unit have been localized to a 2.2-kb genomic *Bam*HI fragment (subclone a in Figure 5). In Northern analysis, the distal subclones (a and b) detect only the long embryonic transcript A^E (Figure 5). The assignment of *Df(1)RT450* as the left (distal) end is also consistent with the finding that breakpoints for chromosomal lesions that disrupt the *elav* function [*Df(1)RT263, 437, 191* and *107*] were all localized proximal to the *Bam*HI fragment (subclone a, Figure 5). The 5′ end of the *elav* transcriptional unit has been tentatively localized to a genomic *Eco*RI–*Xba*I fragment (Figure 5). The strongest data supporting this fragment as the proximal border (coordinate +7 in Figure 5) are based on the rescue of embryonic lethality of mutant *elav* alleles by the transduced DNA segment (Table I).

The 13.5-kb DNA fragment used for transformation does not contain the DNA sequence corresponding to the 3′ end of transcript A^E (Figure 5). Nevertheless it contains DNA sequences necessary for *elav* embryonic function as it allows mutant *elav* embryos to bypass the embryonic lethal phase (Table

Table I. Survival index of hemizygous *elav* mutant flies carrying transduced *elav* DNA

Transformed strain	$elav^{1}$	$elav^{G3}$	$elav^{M3}$	$elav^{ts1}$	svr^{J0}	vnd^{6}
Tf(2) *elav*						
13.5 × A	0.47	0.80	0.80	0.47	0	0
Tf(2) *elav*						
13.5 × B	0.97	1.0	0.87	1.0	0	0

Tf stands for transformant, the number in parentheses indicates the chromosome where the insert is located, *elav* 13.5 indicates the *Bam*HI–*Xba*I fragment used, and X indicates that the construct was in a transformation vector containing the *Xanthine dehydrogenase* gene, A and B indicate different lines. Males of the genotype T(2)*elav* X13.5/+;*ry/ry* were crossed to females of a given mutant allele/X-balancer. The X-balancer chromosome carries a lethal mutation unrelated to *elav*. All the mutant alleles used were recessive lethals (Campos *et al.*, 1985). The *elav* alleles used were $elav^{1}$, $elav^{M3}$, $elav^{G3}$, $elav^{ts1}$. Alleles svr^{J0} and vnd^{6} representing the flanking lethal complementation groups were used as controls. Among the progeny of this cross all males are expected to die, unless the *elav* X13.5-transduced segment rescues the mutants. Since only half the males are expected to carry the *elav* X13.5 insert, the survival index was defined as no. of *elav* mutant males × 2/no. of wild-type females. According to the different alleles used, the rescued males will carry different markers. The $elav^{1}$, $elav^{ts1}$ alleles and the vnd^{6} allele are marked with *yellow* and *chocolate; $elav^{M3}$* with y^2, *white ivory, cut* and *forked*, svr^{J0} with *yellow* and *white*.

I). It can be concluded that part of the 3′ end is not essential for embryonic viability. The rescue of the embryonic lethality for the alleles tested is not complete for at least one of the transformed strains (Table I). This could be due to the phenomenon of position effect (Goldberg *et al.*, 1983; Spradling and Rubin, 1983; Levis *et al.*, 1985; Daniels *et al.*, 1986).

Genetic mosaic analysis had placed the lethal focus in the ventral area of the blastoderm. The major tissue derivatives of this region are the central nervous system, the mesoderm and the alimentary tissue (Poulson, 1950; Technau and Campos-Ortega, 1985). Our earlier suggestion that the embryonic requirement for *elav* may be neural was based on the post-embryonic neural requirement for the gene (Campos *et al.*, 1985).

As predicted, the *in situ* hybridization data demonstrate that the expression of the *elav* transcripts is specific to the central nervous system of the embryo. The embryonic cDNA from which the anti-sense RNA strand was synthesized as a probe for tissue *in situ* hybridization has homology to all three embryonic transcripts, and thus the autoradiograms represent the localization of all transcripts. The grains appeared to be uniformly distributed over the cortical areas of the brain lobes and ventral ganglion (Figure 6). However, whether or not the *elav* transcripts are present in every neural cell is not known. The period when the highest levels of transcript are observed, 6–18 h after egg laying, coincides with extensive cell division and differentiation in the nervous system (Campos-Ortega and Hartenstein, 1985).

The requirement of *elav* function in the formation of a normal visual system (Campos *et al.*, 1985; Homyk *et al.*, 1985) predicts the presence of *elav* transcripts in adults. In fact, the *elav* transcripts were found in adults, preferentially expressed in the head suggesting that *elav* expression continues to be nervous system-specific at this stage. Detailed understanding of the spatial expression of *elav* within the embryonic and adult nervous systems will require further study. The construction of probes specific to each of the three different transcripts will allow further investigation into the details of the temporal and spatial expression of this locus.

Few genes known to be expressed exclusively in the nervous system have been cloned and analysed regarding the spatial

distribution of transcripts. Genes for which transcript localization is known, are mostly involved in specialized neural functions and, as expected, have their transcripts limited to specific cell types (O'Tousa *et al.*, 1985; Zuker *et al.*, 1985; Montell *et al.*, 1985). The embryonic transcription of the *period* (*per*) locus in *Drosophila* is limited to the nervous system (James *et al.*, 1986). In contrast to the *elav* transcripts, the *per* transcripts show an apparent spatial localization within each ganglion of the ventral cord (James *et al.*, 1986).

Developmentally regulated multiple transcripts have been reported for several eukaryotic genes (Rosenfeld *et al.*, 1984; Breibart *et al.*, 1985; Falkenthal *et al.*, 1985; Rosek and Davidson, 1986). Tissue- or development-specific variation in the various steps of mRNA processing can be responsible for RNA heterogeneity. Multiple transcripts can be generated by alternate utilization of promoters (Benyajati *et al.*, 1983), splicing patterns (Nevins *et al.*, 1980) or poly(A)$^+$ sites (Setzer *et al.*, 1980). The significance of each of the three *elav* embryonic transcripts as well as their relationship to each other has not yet been established. Multiple *elav* transcripts may reflect protein heterogeneity as reported for other *Drosophila* genes (e.g. Falkenthal *et al.*, 1985; Karlik and Fryberg, 1986; Berstein *et al.*, 1986). It is also conceivable that in the embryo, *elav* transcriptional heterogeneity is a reflection of cell type-specific processing of the primary transcript.

Thus far the molecular analyses of the *elav* locus have corroborated the prediction of the earlier developmental genetic analysis (Campos *et al.*, 1985). The expression of *elav* in the embryo is unique, in that the transcripts are limited to the nervous system, appear to be uniformly distributed, and are present as early as 6 h after egg laying, a time at which important early developmental events are taking place. This pattern suggests that the *elav* gene may encode a general function required in the development and/or maintenance of the nervous system. The molecular characterization of the *elav* gene will facilitate a more thorough understanding of the processes disrupted by the *elav* mutations.

Materials and methods

Stocks

D. melanogaster were raised on cornmeal/agar/molasses/yeast medium at 25°C, 60% relative humidity. A Canton-S strain was used as source of genomic DNA, poly(A)$^+$ RNA and tissue sections. The genetic analysis of the *elav* mutations reported in this paper has been described elsewhere (Campos *et al.*, 1985). The terminal deletions used to define the *elav* gene DNA were kindly provided by J.Mason (Mason *et al.*, 1986).

Construction and screening of Drosophila genomic DNA and cDNA libraries

Flies heterozygous for the $elav^{G3}$ inversion were used to construct a genomic DNA library essentially as described by Frischauf *et al.* (1983). Briefly, a partial *Sau*3A digest of adult DNA was fractionated in a 5–25% sucrose gradient and the fragments ~20 kb in length were pooled and precipitated with ethanol. DNA from the λ vector EMBL4 was fully digested with *Bam*HI, sedimented in a 5–25% sucrose gradient and the fractions containing the vector arms pooled and precipitated wtih ethanol. The ligation of *Drosophila* DNA and EMBL4 arms was done as described by Maniatis *et al.* (1982), the recombinant DNA was packaged and infected into *Escherichia coli* Q359 cells. This library was screened with the 2.0-kb genomic *Eco*RI fragment which spans the distal breakpoint of the $elav^{G3}$ inversion. Three positive clones were isolated and identified as fusion derivatives of the distal inversion breakpoint. A 2.4-kb *Eco*RI fragment contiguous to the breakpoint fusion fragment was then used to isolate positive clones from a wild-type library generated by Maniatis *et al.* (1978). The retrieval of *elav* region DNA in both directions was continued by the technique of chromosome walking as described by Bender *et al.* (1983). For this purpose we used either the Maniatis library or another library generated by D.Curtis.

The cDNA library was prepared by L.Kauvar. Briefly, poly(A)$^+$ RNA from 3- to 12 h Canton-S embryos was used as template for reverse transcriptase. The cDNA was tailed with *Eco*RI linkers and ligated to *Eco*RI arms of λ gt10 DNA. The recombinant phage were packaged and infected into C600 cells. The genomic DNA and cDNA libraries were screened by hybridizing [^{32}P]DNA probes labelled by nick translation to nitrocellulose transfers of phage plaques (Maniatis *et al.*, 1982).

DNA and RNA extractions

DNA was prepared from adult flies as described by Pirrota *et al.* (1983). Total RNA from embryos, larvae and pupae aged as indicated in Figure 4, was obtained according to Vincent *et al.* (1984). Total RNA from adult flies, heads and bodies was extracted as described by Barnett *et al.* (1980). Poly(A)$^+$ RNA was prepared by passing total RNA through an oligo(dT) cellulose column (Aviv and Leder, 1972).

Plasmid constructions

The *E. coli* strain Q359 and the plasmid pGEM-1 and pGEM-2 containing the SP6 and T7 RNA polymerase promoters were a gift from A.Bedard. The pEMBL vector 9$^+$ as well as the JM 101 cells were given by M.Gray. The P-element transformation vector cp 20.1 was a gift from J.Lingappa. Restriction fragments isolated from KW 100, 99, 98 and 97 were ligated to linearized pEMBL 9$^+$ DNA. The ligated DNA was transformed into JM 101 (amp^s) cells and assayed on selective plates (cf. antibiotic resistance and β-galactosidase expression). DNA obtained from ampicillin-resistant, white colonies was analyzed in order to identify clones containing *Drosophila* DNA inserts. The λgt10 phage containing the cDNA insert and selected pEMBL 9$^+$ genomic subclones were subcloned into the pGEM-1 or pGEM-2 plasmids. The P-element transformation vector was constructed by inserting *elav* DNA into cp 20.1 double digested with *Sal*I and *Xba*I. Correct plasmid constructions were size selected and verified by restriction mapping and Southern analysis.

Preparation of radiolabelled probes for hybridization DNA probes

Restriction fragments were purified by the method of band interception in agarose gels using Schleicher and Schuell NA-45 DEAE membrane (procedure as provided by manufacturer). Isolated restriction fragments, phage or plasmid DNA were radiolabelled by [^{32}P]dCTP (800 Ci/mmol, from New England Nuclear) by nick translation (Golden *et al.*, 1980).

Riboprobes

The pGEM-1 and pGEM-2 vector systems (Promega Biotec) were used to generate single-stranded RNA probes of both sense and anti-sense strand of any given sequence as described by the manufacturer. The RNA was labelled with either ^{32}P or ^{3}H by incorporation of labelled ribonucleotides. The tritiated riboprobes used for *in situ* hybridizations were labelled with [^{3}H]UTP (NEN NET-520) to a specific activity of $\sim 2 \times 10^7$ c.p.m./μg.

Preparation and hybridization of Southern and Northern blots

Ten micrograms of genomic DNA were electrophoresed on 0.8% agarose gels, blotted and hybridized according to procedures described in Maniatis *et al.* (1982). For Northern blots, ~4 μg of poly(A)$^+$ RNA was fractionated on a 1% agarose gel containing 2.2 M of formaldehyde (Maniatis *et al.*, 1982). The RNA gel was blotted onto GeneScreen Plus, according to procedures provided by NEN Research Products. Hybridizations with labelled probes were carried out as described (Colot and Rosbash, 1982). Southern blots were washed in 2 × SSC/0.1% sodium dodecyl sulfate (SDS) for 15 min at room temperature followed by a 2-h wash in 0.1 × SSC–0.5% SDS at 60 or 68°C. Northern blots were washed in 1 × SSC–0.1% SDS at room temperature for 30 min and in 0.1 × SSC–0.1% SDS at 55°C for 30 min. In the case of hybridization with single-stranded RNA probes the Northern blots were washed at 68°C instead of 55°C. RNA and DNA sizes were estimated by comparison to DNA standards.

Generation and manipulation of transformants

Germ line transformants were produced by microinjection of cloned DNA (Spradling and Rubin, 1982; Rubin and Spradling, 1982). We used the cp 20.1 P-element transformation vector (Simon *et al.*, 1985) in which a fragment of the *elav* region, 13.5 kb in size, has been inserted in only one orientation (Figure 5). This vector contains the *rosy* gene which serves as an easily scored eye color marker. The transformation cocktail also contained the helper P-element plasmid pHST which is able to provide transposase function but is unable to insert into the *D. melanogaster* genome. The *elav* containing transformation vector was at a final concentration of 400 μg/ml and the helper plasmid at 50 μg/ml in the microinjection cocktail.

Homozygous ry^{506} embryos were injected using the techniques described by Rubin and Spradling (1982). The strain ry^{506} has a deletion of a segment of the xanthine dehydrogenase coding sequence and therefore can not revert (Cote *et al.*, 1985). The G0 adults that emerged from the injected embryos were mated to homozygous ry^{506} flies and the G1 flies exhibiting wild-type eye color were considered successful transformants that were maintained by selection for ry^+ eye color. Males heterozygous for the *elav* X13.5 insert were obtained by crossing the ry^+ from the transformant brood to ry^{506}. The chromosomal location of the transduced DNA segment was established through standard genetic procedures.

In situ hybridizations

The entire process from embryo preparation through hybridization was performed essentially as described by Ingham *et al.* (1985). Canton-S embryos were prefixed for 15–40 min by the phase partition of Zalokar and Erk (1977). Synthesis of the tritiated probes has already been described. These probes were alkaline hydrolyzed as described in Cox *et al.* (1984) to generate fragments ~150 bp in length. Slides were coated with poly-L-lysine instead of poly-D-lysine. Slides were coated with Kodak NTB-2 emulsion (diluted 1:1 with 600 mM ammonium acetate) and developed with Kodak Dektol (1:1 with water for 2 min at 17°C), and fixed with Kodak Fixer (17°C, 5 min). Post-development, tissue sections were stained in Mayer Hematoxylin for 30 min as described by Humason (1972).

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Note added in proof

Recent *in situ* hybridization experiments in our laboratory have demonstrated the presence of *elav* transcripts in the larval CNS and in the ventral ganglion of adults (S.N.R. unpublished results). Therefore, the head enrichment of *elav* transcripts, as well as the absence of signal in the larval stages, as reported in this paper, may represent the dilution of nervous system RNA by total organism RNA.