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Molecular analysis of the *asense* gene, a member of the *achaete-scute* complex of *Drosophila melanogaster*, and its novel role in optic lobe development

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The *achaete-scute* complex (AS-C) comprises five genetic regions: *achaete*, *scute* (*sc*)α, *lethal of sc*, *scβ* and *scγ*. Each region promotes the determination and positional specification of different, but partially overlapping, subsets of neural elements of *Drosophila*. In this work, we report a molecular characterization of the *scγ* region. It comprises 22 kb of DNA and contains two transcription units, only one of which, named *asense* (*ase*), seems involved in neurogenesis. *ase* encodes a protein that shares with other three AS-C proteins a domain containing a helix-loop-helix motif characteristic of a group of DNA-binding proteins. In the embryo, *ase* is expressed in neural precursor cells, a pattern consistent with the known requirement of *scγ* for the development of the larval nervous system. In late third-instar larvae, the gene is expressed in developing structures of the central nervous system (CNS), namely the anlagen of the optic lobes and in many cells, including neuroblasts, of the central brain and ventral ganglia. Its removal leads to anatomical defects in the adult optic lobes. This is the first demonstration of a role for the AS-C in the development of the adult CNS.

Key words: achaete-scute complex/*asense*/neurogenesis/optic lobe

Introduction

The *achaete-scute* complex (AS-C) is involved in the development of sensory organs (SOs) and central nervous system (CNS) of *Drosophila* (reviewed by Ghysen and Dambly-Chaudière, 1988). Muller (1955) and García-Bellido (1979) proposed the subdivision of the complex in four genetic regions, namely: *achaete* (*ac*), *scute* (*sc*)α, *lethal of sc* (*l'sc*) and *scβ* (from distal to proximal). Subsequently, Dambly-Chaudière and Ghysen (1987) and Jiménez and Campos-Ortega (1987) described a fifth region, *scγ*. The deletion of either *ac* or *scγ* removes specific subsets of larval SOs, and that of *ac*, *scα*, *scβ* or *scγ* suppresses different subsets of adult SOs. The *l'sc* region is required for the development of the embryonic CNS. In its absence, most neuroblasts segregate normally but a large part of their progeny cells degenerate and die (Jiménez and Campos-Ortega, 1979, 1987). This damage is greatly enhanced by the simultaneous deletion of *l'sc* and other AS-C regions (Jiménez and Campos-Ortega, 1987). This suggests that *ac*, *scα* and *scγ* also play a role, albeit less important than that of *l'sc*, in embryonic CNS development.

The molecular analysis of the complex has shown that the AS-C comprises ~90 kb of DNA and contains several transcription units separated by long, non-transcribed regions (Campuzano *et al.*, 1985) (Figure 1). Four transcription units give rise to structurally related proteins (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; F.González and J.Modolell, unpublished). Their longest conserved domain contains a putative helix-loop-helix motif which is shared by the *Drosophila daughterless* (Caudy *et al.*, 1988), *twist* (Thisse *et al.*, 1988), *Enhancer of split* (Klämbt *et al.*, 1989) and *extramachrochaetae* proteins (J.Garrell and J.Modolell, in preparation; H.M.Ellis, D.R.Spann and J.W.Posakony, personal communication), and the mammalian *myc* (Villares and Cabrera, 1987; Alonso and Cabrera, 1988), MyoD1 (Davis *et al.*, 1987), myogenin (Wright *et al.*, 1989), Myf-5 (Braun *et al.*, 1989) and immunoglobulin enhancer-binding proteins (Murre *et al.*, 1989). This domain confers on these latter proteins the ability to bind as dimers to specific DNA enhancer sites. This suggests that all the aforementioned proteins are transcriptional regulators (Murre *et al.*, 1989).

Each of these four AS-C genes is located within one of the genetically defined regions. The genes were formerly named T5, T4, T3 and T1a or T8. However, as proposed elsewhere (Ghysen and Dambly-Chaudière, 1988; Romani *et al.*, 1989), they will be called *ac*, *sc*, *l'sc* and *asense* (*ase*), their corresponding genetic regions being *ac*, *scα*, *l'sc* and *scγ* (Figure 1). A considerable body of evidence indicates that these transcription units are responsible for the neurogenic functions of the complex (Alonso and Cabrera, 1988; Balcells *et al.*, 1988; reviewed in Ghysen and Dambly-Chaudière, 1988). Within the long, non-transcribed regions separating these units, there seem to be enhancer-like elements that control the spatial pattern of expression of at least the *sc* gene (Ruiz-Gómez and Modolell, 1987; Ghysen and Dambly-Ghaudière, 1988; Romani *et al.*, 1989).

The *scγ* region was genetically defined by the breakpoints of the *Df(1)sc*^{19} and *Df(1)260-1* (Figure 1) (Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987). Although part of the DNA of *scγ* had been cloned and its transcription was partially characterized (Campuzano *et al.*, 1985; Alonso and Cabrera, 1988), the molecular analysis of this region was still far from complete. Consequently, in this work, we have determined the molecular extent of *scγ*, re-examined its transcription, sequenced the *ase* gene, determined the *ase* spatial expression in both the embryo and third instar larva and analyzed the phenotype of the *ase* deletion in adult flies. The results indicate that *ase* is responsible for previously known *scγ* functions, and reveal a role for this gene in the development of the adult optic lobes.

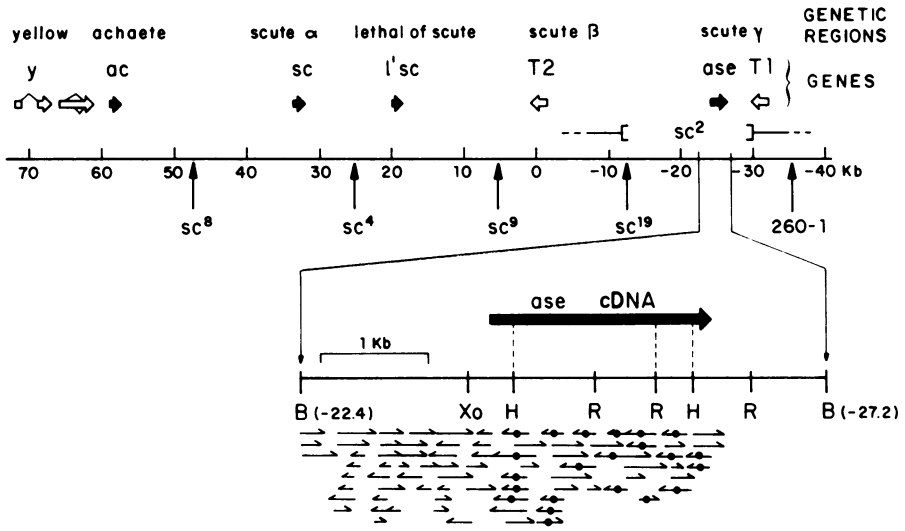


Fig. 1. Simplified physical map of the AS-C and detailed location of the *ase* gene, with indication of the strategy used for sequencing it. On top, the genetic subdivision of the region (García-Bellido, 1979; Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987) is indicated. Below, thick horizontal arrows show the transcribed regions with the corresponding names of the genes (Campuzano *et al.*, 1985; Chia *et al.*, 1986). Filled arrows indicate genes whose products share conserved domains (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; F.González and J.Modolell, unpublished results). The location of the sc^2 deletion is indicated. Thin vertical arrows indicate position of breakpoints of $In(1)sc^8$, $In(1)sc^4$, $In(1)sc^9$, $T(1;2)sc^{19}$ and *Df(1)260-1*. These breakpoints have been used to define the genetic regions. The position of the *Df(1)260-1* breakpoint has been derived from the positive *in situ* hybridization of a genomic clone, extending proximally from the end of our previous walk-through the AS-C (Campuzano *et al.*, 1985), to the *Df(1)260-1* chromosome and from new fragments appearing in Southern blot analyses containing *Df(1)260-1*/FM6 genomic DNA. All data are consistent with the breakpoint being located within a 2.2 kb region (coordinates −33.7 to −35.9). The region containing the *ase* gene is shown at 15× larger scale. Restriction sites are: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; Xo, *Xho*I. Negative numbers in parentheses indicate coordinates of the indicated *Bam*HI sites in the physical map of the AS-C (Campuzano *et al.*, 1985). The extent of the *ase* cDNA used in this work is indicated by a large, thick arrow. At the bottom, the strategy of sequencing is indicated. Below the DNA line, arrows indicate the number of times and the direction in which the sequence of any stretch of DNA was read in different gels. Dotted arrows, cDNA clones; non-dotted arrows, genomic clones.

Results

Molecular extent of the scγ region

The *scγ* region extends between the breakpoints of $Df(1)sc^{19}$ and *Df(1)260-1* (Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987). The first breakpoint is found at coordinate −12.7 of the molecular map of the AS-C (Figure 1) (Campuzano *et al.*, 1985). The second one has now been found within the 2.2 kb of DNA adjacent to the end of our previous chromosomal walk (see legend to Figure 1). Therefore, *scγ* spans ~22 kb.

Transcriptional analysis of the scγ region

The proximal part of the *scγ* region contains the T1 gene, expressed most abundantly in late third-instar larvae, pupae and adults (Campuzano *et al.*, 1985). In late third-instar larvae, T1 is exclusively and very abundantly expressed in trachea (not shown). This pattern of expression makes a role of T1 unlikely in the development of the CNS or adult sensory organs.

The *ase* gene was found in a search for genes encoding a domain containing the putative helix−loop−helix motif present in AS-C proteins (S.Campuzano, unpublished; Beamonte and Modolell, 1989). A 214 bp *Xmn*I fragment of the *l'sc* gene (encoding most of the conserved domain) cross-hybridized with a 1.2 kb *Xho*I−*Eco*RI fragment (coordinates −23.9 to −25.1), which in RNA blots detects a 2.8 kb transcript present at low abundance in 0−12 h embryos, late third-instar larvae and 0−1 day old pupae (Figure 2). *ase* corresponds to the T8 gene recently described by Alonso and Cabrera (1988) (see Figure 3), although our estimate of the transcript size is larger (2.8 versus 1.6 kb)

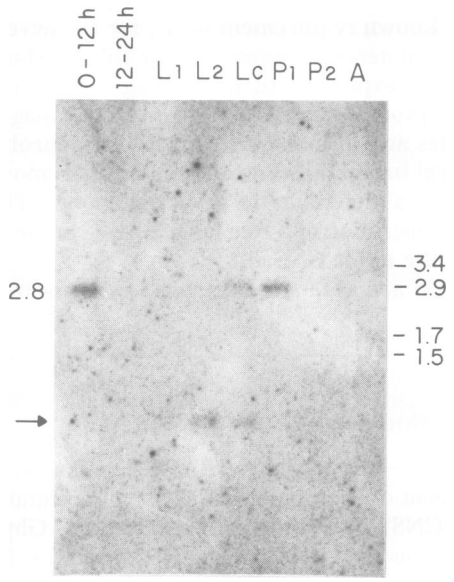


Fig. 2. Developmental profile of the *ase* RNA. A blot containing in each lane 5 μg of wild-type Oregon R poly(A)$^+$ RNA of the indicated stage was hybridized with a single-stranded RNA probe (7.5×10^6 c.p.m.) synthesized on a 1.2 kb *Eco*RI−*Xho*I template which is homologous to the 5′ half of the *ase* RNA (Figure 1). After washing in 0.1 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate at 65°C, the blot was exposed with a Du Pont Quanta III intensifying screen for 21 days. RNA standards (rRNA from *Saccharomyces cerevisiae* and *Escherichia coli*) were run in parallel in the same gel. A smaller transcript present in third-instar larvae usually lit up with this probe. This RNA was not transcribed from the *scγ* region since it was still present in RNA prepared from sc^2 larvae, which are deficient for the DNA used to synthesize the probe (Figure 1).

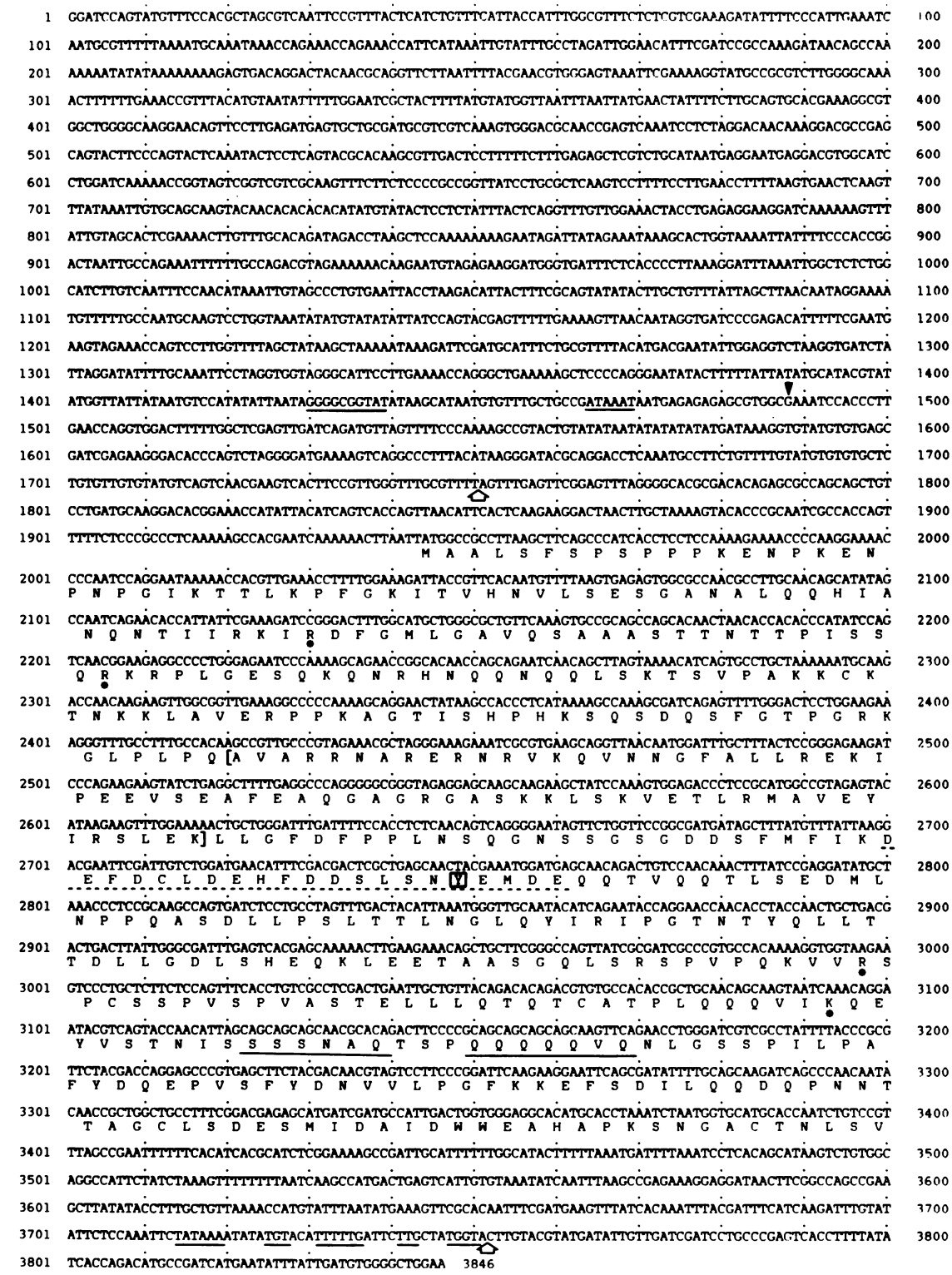


Fig. 3. Sequence of the *ase* gene and its putative protein product. The sequence shown corresponds to the genomic DNA. Vertical arrows following T_{1750} and A_{3751} show start and end of the colinearity region between genomic and cDNA clones. The only difference between the two sequences is T_{3698}, which is a G in the cDNA. At the 5′ end, the cDNA clone has a stretch of 19 Cs followed by 14 As and a piece of DNA 115 bp long, extremely A/T rich (77%) and unrelated to the rest of the cDNA or genomic sequence. This short piece of DNA seems a cloning artefact. At positions 287 and 321 nt upstream from the colinearity region, putative TATA and GC boxes are found (underlined). Primer extension experiments (Materials and methods) suggest that G_{1488} (arrowhead) is the start of transcription. At the 3′ end, the cDNA clone has a poly(dA) tail. This most likely marks the site of transcription termination (T_{3750} or A_{3751}). A putative polyadenylation signal followed by a TG region (Birnstiel *et al.*, 1985) are immediately upstream (underlined). In the protein sequence, asterisks point to basic amino acids flanking the PEST regions. The domain containing the putative helix−loop−helix motif is between brackets. For an alignment of the domain of the four AS-C proteins see Alonso and Cabrera (1988), and for the amino acids probably involved in forming the helices see Murre *et al.* (1989). The acidic region with a tyrosine (boxed) that may correspond to a phosphorylation domain is underlined with dashes. Oligoglutamine and oligoserine stretches corresponding to *opa* sequences are also underlined. Comparison of this nucleotide sequence with the partial sequence of *ase* of Alonso and Cabrera (1988) shows that the following bases are missing in the previously published sequence: C_{2084}, C_{3315}, G_{3379}, G_{3518} and G_{3591}.

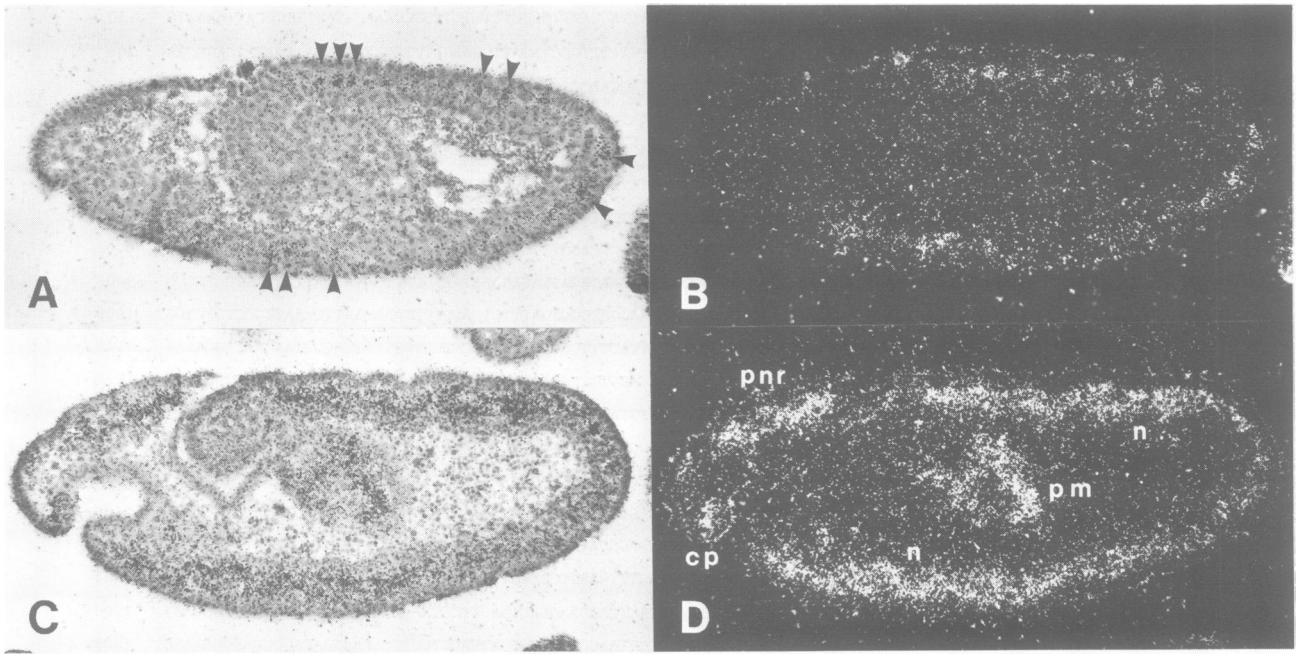


Fig. 4. Expression of the *ase* gene in embryos. The figure shows sagittal sections of embryos with the cephalic region towards the left. (**B**) and (**D**) are dark field images of the sections shown in (**A**) and (**C**) respectively. The earliest detection of *ase* expression was localized in internal cells of the neural primordium, presumably neuroblasts (**A**, arrowheads; **B**). At later stages (**C** and **D**), *ase* became more abundantly expressed. Transcripts were detected in many cells of the primordium of the CNS (n) and in the procephalic neurogenic region (pnr), clypeolabrum (cp) and the posterior midgut rudiment (pm). These last two regions are known to have neural precursors. This RNA pattern indicates that *ase* is also expressed in the neuroblast progeny.

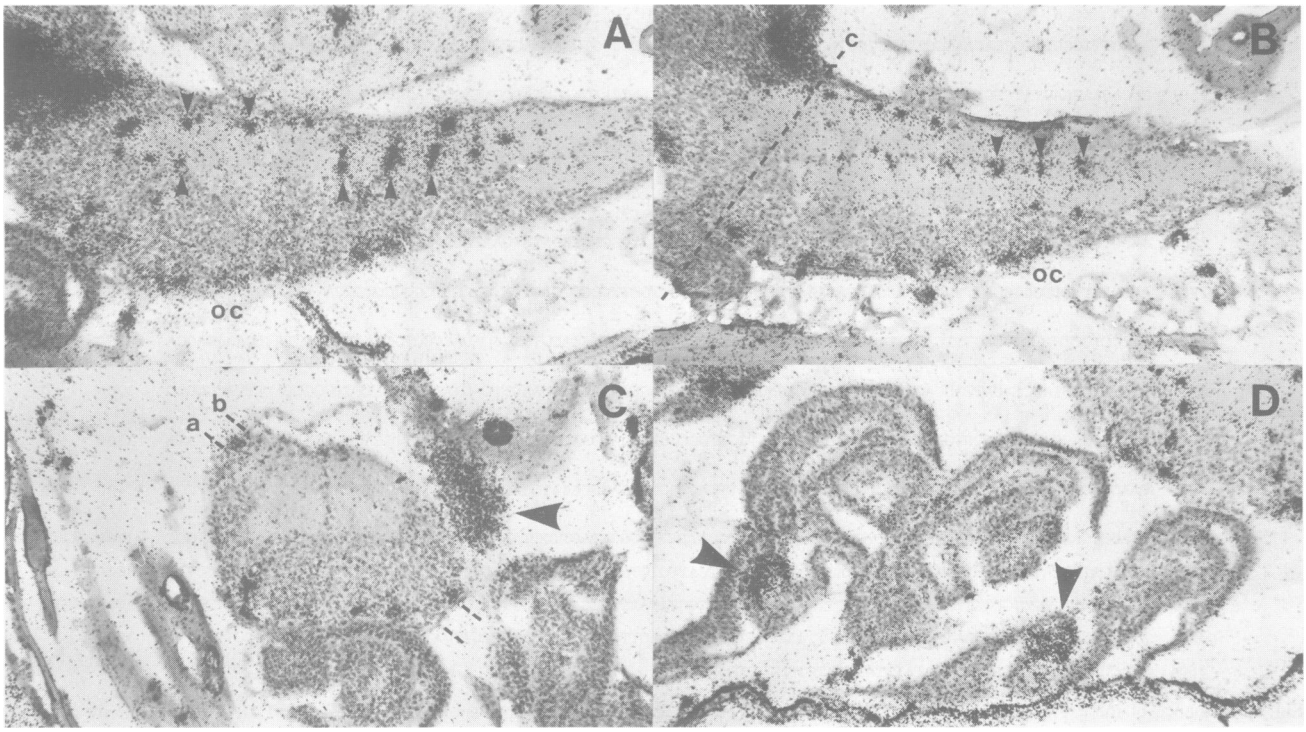


Fig. 5. Expression of *ase* in the ventral ganglion (**A**–**C**) and leg imaginal discs (**D**) of late third-instar larvae. (**A**) and (**B**) are two sections of the same ventral ganglion separated by two intervening sections (12 μm). They are roughly parallel to the antero-posterior axis and tilted with respect to the dorso-ventral axis as indicated in (**C**) (a,b). Anterior is to the left and dorsal towards the top. In the ventral region, the label appears in the form of small but relatively open clusters (oc). These signals may correspond to large neuroblasts present in this region (Truman and Bate, 1988) or neuroblasts and a few neighboring cells, possibly their immediate progeny. More compact signals are found on cells surrounding the neuropile (**A**, arrowheads) or in the midline layer of cell bodies that longitudinally bisect the neuropile (**B**, arrowheads). Neuroblasts are also found in these positions (Truman and Bate, 1988). Note the metameric arrangement of labeled cells surrounding part of the neuropile and in the medial layer. (**C**) Transverse section of the ventral ganglion at approximately the position and the tilt indicated in **B** (c). This section belongs to the same brain as those shown in Figure 6(A)–(D). Note the clusters of label probably corresponding to neuroblasts in the periphery of the ganglion. The large area of label (arrowhead) corresponds to a piece of brain lobe. (**D**) Section of leg discs showing relatively large areas of label (arrowheads). Labeling of larval cuticle (bottom of figure) is a common artefact observed with many ^{35}S-labeled probes.

and its developmental profile of expression is more complex than those reported by these authors.

Our research for additional transcription units within the *scγ* region has been negative. We have been unable, either with nick-translated probes or RNA probes, to detect the RNA of a transcribed region (T9) reported by Alonso and Cabrera (1988).

Characterization of the ase gene

We have isolated and sequenced a cDNA clone of the *ase* transcript. We have also sequenced 3.8 kb of genomic DNA which includes the whole transcribed region. Both sequences are identical, except for 1 nt, along 2001 nt (Figure 3) and contain a 1596 nt long ORF. Its first methionine codon matches the *Drosophila* consensus for translation start signals: A at positions −3 and −4, G at position +1 from the ATG codon (Cavener, 1987). The following coding sequence is in agreement with the *Drosophila* codon usage (not shown). The deduced *ase* protein has 486 amino acids (Figure 3). In addition to the domain with the helix−loop−helix motif (see Introduction), it has other interesting features. Near the N terminus, the sequence is rich in proline residues (7 out of 15 amino acids). Near the center, it contains an acidic region (10 acidic residues out of 21) with a tyrosine. The *ac, sc* and *l'sc* proteins have similar regions but their amino acid sequences are different and they are located at the C terminus (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; F.González and J.Modolell, unpublished). Like these proteins, the *ase* product has PEST sequences, i.e. regions rich in proline, glutamic acid, serine and threonine flanked by basic amino acids that promote protein turnover (Rogers *et al.*, 1986). Similarly to many *Drosophila* genes, *ase* contains CAX repeats coding for either glutamine or serine (*opa* repeats, Wharton *et al.*, 1985).

The *ase* gene sequence of Figure 3 differs from the previously reported 1.6 kb sequence of part of the gene (Alonso and Cabrera, 1988) in five places (legend to Figure 3). Three of the discrepancies are located within the ORF shown in Figure 3 and cause the resulting putative protein to have 396 instead of 486 amino acids.

Spatial distribution of the ase mRNA

Our analysis of the spatial distribution of *ase* RNA in embryos agrees with that reported by Alonso and Cabrera (1988). Thus, we shall summarize our findings. Expression becomes first detectable in internal cells of the neural primordium, presumably neuroblasts and their progeny (Figure 4A and B). Expression peaks in embryos of stages 10−11, as defined by Campos-Ortega and Hartenstein (1985). RNA is then detected in most cells of the CNS primordium and also in the labrum, optic lobe rudiment, procephalic neurogenic region and posterior midgut rudiment (Figure 4C and D). Expression also occurs in regions where peripheral nervous system (PNS) precursor cells are located. Expression lasts at least until germ band retraction is well advanced (not shown). Thus, *ase* transcription starts later in development and lasts longer in the neuroblast progeny than that of the other proneural genes of the AS-C (Cabrera *et al.*, 1987; Romani *et al.*, 1987).

ase is also expressed in late third-instar larvae (Figures 2, 5 and 6). The RNA is very scarce in imaginal discs, except for one relatively large cluster of cells in leg discs (Figure 5D). Extremely weak expression is occasionally detected in the presumptive wing margin and dorsal radius of the wing disc (not shown). In contrast, strong *ase* expression occurs in the CNS (Figures 5 and 6), most prominently in cells distributed as a cap on each optic lobe. These cells form the outer anlage and proliferate actively to give rise to ganglion mother cells of the lamina and medulla. [For descriptions of the *Drosophila* brain and its development see White and Kankel (1978) and Kankel *et al.* (1980).] Expression also ocurs in the inner anlage of the optic lobes, which gives rise to cells of the medulla and lobula complex.

In the central brain and ventral ganglion, expression takes place in small clusters of cells or single cells. Their distribution resembles that of neuroblasts and their immediate progeny (Truman and Bate, 1988). Thus, they are found in the periphery of the ganglia (Figures 5A−C and 6A,F) and in layers bordering the neuropiles (Figures 5A, B and 6C, D). We conclude that throughout the CNS *ase* is expressed in many or most of its actively proliferating cells.

The ase deficiency affects the optic lobes

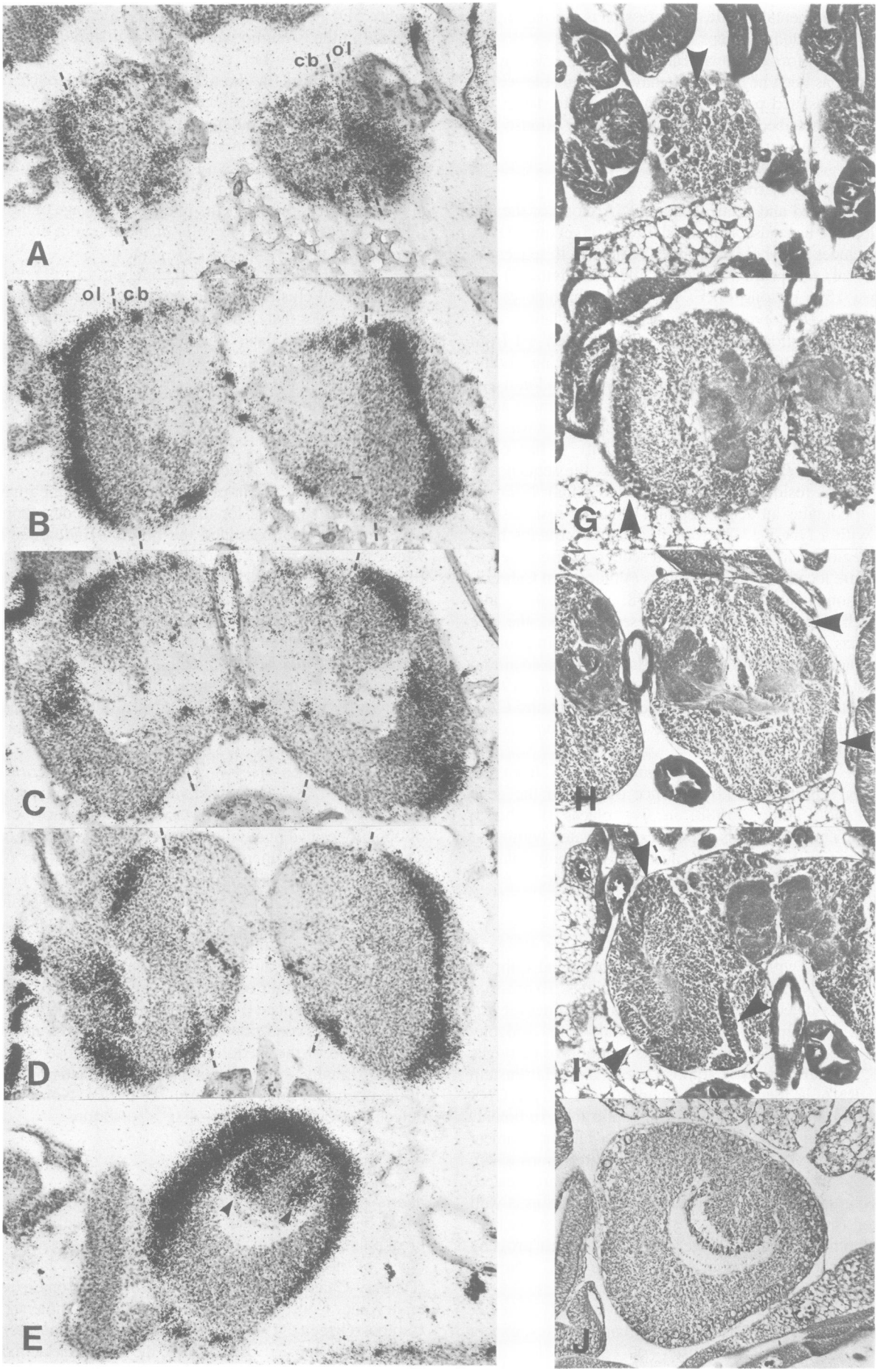
The expression of *ase* in proliferative cells of the imaginal CNS, suggested a role for this gene in CNS development. Consequently, we examined the brains of adult sc^2 flies, which lack *ase* and 17 kb of flanking regions (Figure 1). In >75% of the brains, we detected morphological anomalies in one or both optic lobes. Most often, the defects consisted in ectopic fiber bundles that crossed the lobula plate neuropile at variable locations and distorted its configuration (Figure 7B). Much less frequently, we observed distortions of the medulla and lobula neuropiles (not shown).

To verify that these anomalies were due to the absence of *ase* and not to uncharacterized recessive mutations present in the sc^2 chromosome, we examined the brains of sc^2/sc^{B57} and $sc^{B57}/+$ flies. [sc^{B57} is an interstitial deletion that removes the AS-C and inactivates the adjacent proximal complementation group (EC4).] In sc^2/sc^{B57} flies, >90% of optic lobes had anomalies. Some were like those seen in sc^2 mutants, but others were more extreme. One of the most common was the presence of misrouted fiber bundles that presumably originate in the posterior part of the lamina, avoid the outer chiasma, run along the posterior and medial border of the medulla neuropile and finally cross this neuropile to end at the anterior part of the medulla (Figure 7D,F). Other ectopic fiber bundles crossed both the lobula and lobula plate (Figure 7C) or the medulla neuropiles without obvious connection with the lamina. Gross alterations of the shape of the medulla neuropile were also seen (not shown). Finally, some brains had altered relative positions of the optic lobe ganglia (Figure 7D and E). None of these defects were found in control $sc^{B57}/+$ brains.

Discussion

ase is responsible for part of the scγ functions

It has been reported that the *scγ* deletion, defined by the breakpoints of $Df(1)sc^{19}$ and *Df(1)260-1*, suppresses several types of neural elements, like a subset of larval SOs (Dambly-Chaudière and Ghysen, 1987), neurons from the embryonic CNS (in the absence of *l'sc*; Jiménez and Campos-Ortega, 1987), adult abdominal chaetae (sc^2 mutant; Lindsley and Grell, 1968), the extra chaetae induced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by the *Tuft* mutation (A.García-Bellido, personal communication), and photoreceptor, as well as pigmentary cells, of the retina (Jiménez and Campos-Ortega, 1987). Within $sc\gamma$ we have found only the *ase* transcription unit as a candidate for neurogenic function. However, all the phenotypic effects of the $sc\gamma$ deletion cannot in principle be adscribed to the lack of this gene because *Df(1)260-1* uncovers both the AS−C and the immediately adjacent *1(1)EC4* complementation group (K.White, cited in Jiménez and Campos-Ortega, 1987). A transcription unit that lies immediately to the right of the *Df(1)260-1* breakpoint most likely corresponds to the *1(1)EC4* locus, since molecular lesions associated with two *1(1)EC4* alleles have been found within it (F.González, unpublished). *Df(1)260-1* should then affect the expression of this transcription unit. Still, the available data indicate that the suppression of larval SOs and adult chaetae, as well as the optic lobe damage described here (see below), are due to the absence of *ase*, for they occur in both $sc\gamma^-$ and sc^2 flies (C.Dambly-Chaudière, personal communication) and the sc^2 mutation is not an allele of *1(1)EC4* (García-Bellido, 1979). In contrast, *ase* removal does not affect the retina (compare Figure 7F with Figure 5 of Jiménez and Campos-Ortega, 1987), suggesting that its damage is due to loss of *1(1)EC4* function. Finally, the lesions of the embryonic CNS (in the absence of *l'sc*) may be caused by the lack of either or both genes.

The putative ase protein

The *ase* protein shares with the *ac, sc* and *l'sc* proteins a 66 amino acid long domain containing a putative helix−loop−helix motif that may be the structural characteristic of a new family of transcriptional regulators (see Introduction). *ac, sc* and *l'sc* proteins also share an acidic domain, located at their carboxyl ends, which bears a tyrosine residue that may be a substrate for a tyrosine kinase (Villares and Cabrera, 1987). The *ase* protein also has an acidic domain with a tyrosine, even though it has a different composition and it is internally located. The presence of such a domain in the four AS-C proteins suggests that it may have a function. Whether this is the activation of transcription, as has been suggested for other transcription factors with both basic and acidic domains (reviewed by Guarente, 1988), or the modulation of their activity by tyrosine kinases, is unknown. It should be stressed, however, that in at least two cases this domain is not essential for neurogenic activity. Hw^J and Hw^{Ua} mutants have truncated versions of the *ac* and *sc* proteins respectively, which lack the acidic domain (Villares, 1986). These proteins still promote SO development (Campuzano *et al.*, 1986), although their activity has not been characterized in detail.

The embryonic ase expression

In the embryo, *ase* seems to be expressed in neuroblast and/or in many or most of their progeny cells. Its pattern of expression contrasts with those of the other AS-C genes. They start to be expressed earlier, in the blastoderm; later, their RNAs are spatially restricted to different but partially overlapping subsets of neuroblasts and their progeny (Cabrera *et al.*, 1987; Romani *et al.*, 1987); and finally their expression terminates earlier than that of *ase*. The widespread expression of *ase* contrasts with the phenotypic effect of its deletion, which only removes a subset of larval SOs. As previously suggested (Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987; Romani *et al.*, 1987), this may be due to *ase* expression being dispensable in many cells and/or to an almost complete replacement of *ase* function by the other AS-C genes.

A role of ase in adult optic lobe development

In late third-instar larvae, *ase* is expressed almost exclusively in the CNS. Expression occurs in the outer and inner anlagen of the optic lobes, and in many individual cells or small groups of cells of the central brain and ventral ganglion. In the latter case, the distribution of label bears a strong resemblance to the distribution of neuroblasts (Truman and Bate, 1988). Thus, we tentatively conclude that expression occurs in many, if not all, actively dividing precursors of the adult nervous system. Expression may also occur in some of the immediate neuroblast progeny.

Consistent with this pattern of expression, the removal of *ase* causes abnormalities in the adult optic lobes. The most prominent consist in the appearance of ectopic fiber bundles crossing the optic lobe neuropiles and in misrouted axonal efferents from the posterior lamina, which avoid the outer chiasma. It should be stressed that, due to the experimental approach used, additional subtle abnormalities may have escaped detection. Moreover, we have not investigated the presence of defects in other parts of the CNS.

In general, the deletion of *ase* or other AS-C genes causes the removal of subsets of neural cells (see Introduction). Thus, in ase^- flies loss of neural cells may also cause the damage to the optic lobes. Since cellular recognition and adhesion play a major role in axon guidance and, therefore, in establishing the configuration of the nervous system

Fig. 6. Expression of *ase* in the optic lobes and central brain of late third instar larvae. **(A)**−**(D)** are different parallel sections from the same brain. Sections are approximately perpendicular to the antero-posterior axis, but tilted towards the posterior end of the larva as indicated in Figure 5B (c). Anterior is towards the top of the figures. **(F)**−**(I)** are non-hybridized sections of a different brain at approximately the same level and orientation as those in **(A)**−**(D)** respectively; they are shown to facilitate interpretation of the pattern of label. In many sections, the separation between optic lobe (ol) and central brain (cb) can be clearly seen and is pointed at by dashes. **(A)** and **(F)** are sections very close to the brain surface. Note the strong expression in the optic lobes (outer anlage) and the clusters of expression in the central brain **(A)** which most likely correspond to individual large neuroblasts present in its outer layer **(F**, one is pointed at by an arrowhead). **(B)** This section is ~18 μm deeper into the brain than that in **(A)**. Strong expression is seen in two bands near the surface of the optic lobes. These correspond to the outer anlage, a region of enlarged, actively proliferating cells **(G**, arrowheads). We cannot rule out that expression may also occur in the progeny cells immediately adjacent to the anlage. Expression is also seen in individual neuroblasts near the central brain surface (small clusters). **(C)** In this section, 42 μm below the previous section, three regions of labeling can be see in the right optical lobe. The anterior and posterior ones correspond to the outer anlage, here split into two parts **(H**, arrowheads). The middle, more internal label, also seen in the contralateral lobe and in **(D)** corresponds to the inner anlage. Cells of the inner anlage are more loosely distributed than those of the outer anlage. Labeling of this inner region is seen in nine consecutive sections out of the 22 that span the complete right cerebral lobe. Note the small, intense spots of label in the border of the central brain neuropile which may correspond to internal neuroblasts. **(D)** In this section, 18 μm below the previous one, a continuous band of label is again seen in the right optical lobe, while in the left the label occurs in four regions. Two of them correspond to the outer anlage and the other two to different parts of the inner anlage. Three of these regions with distinct cellular morphology are arrowed in **(I)**. **(E)** and **(J)** show sections roughly parallel to the anterior-posterior axis and tilted approximately as shown in Figure 5(C). The outer anlage forms a cap of cells on the optical lobe **(J)** and *ase* is expressed in it **(E)**. Arrowheads in **(E)** point to label in the inner anlage.

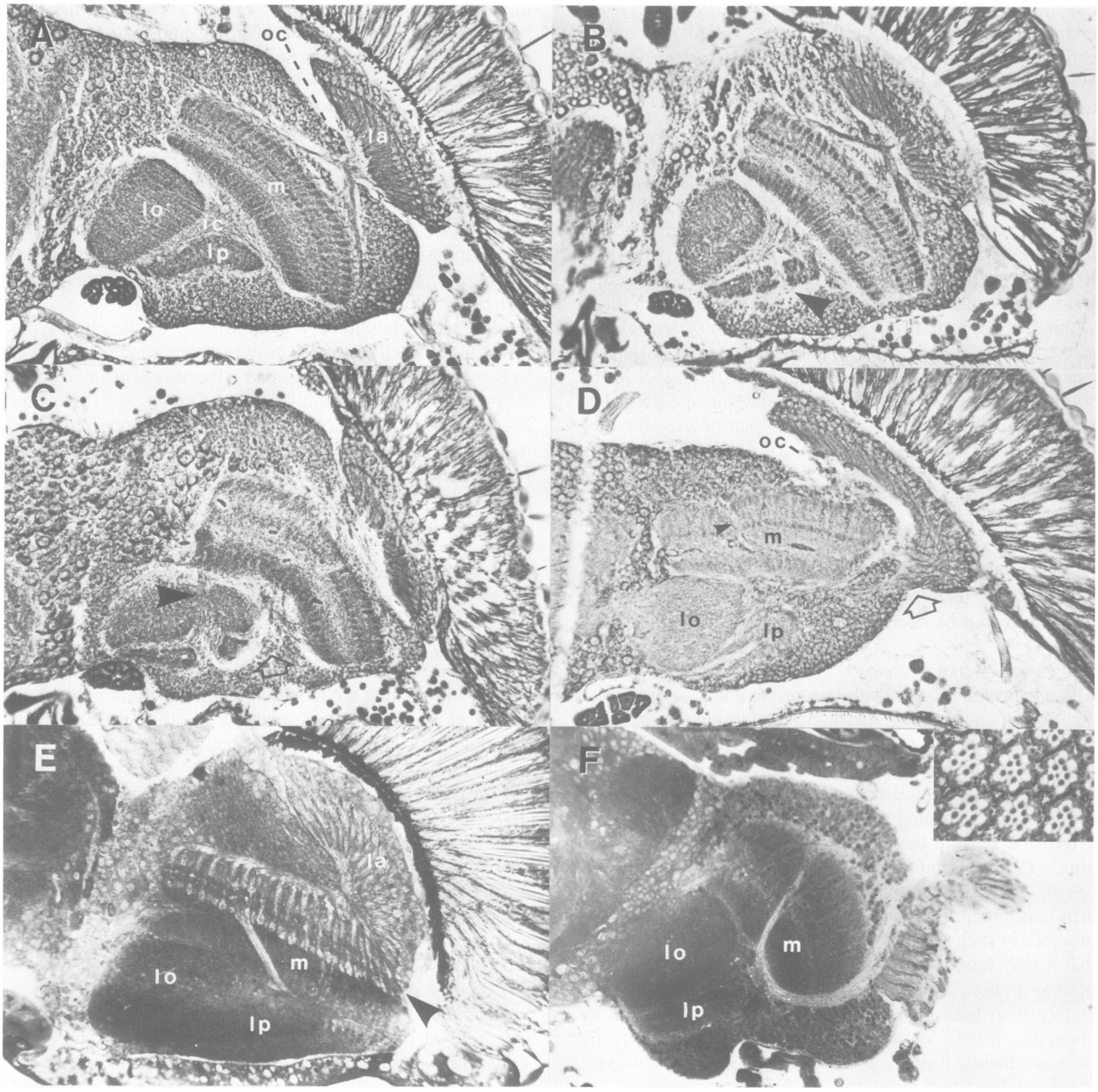

Fig. 7. Horizontal sections through brains of wild type (**A**), sc^2 (**B**) and sc^2/sc^{B57} (**C**–**F**) flies showing one of their optic lobes. Sections (**A**)–(**D**) correspond to pharate pupae and were stained by the Holmes–Blest silver impregnation method. Sections (**E**) and (**F**) are from adult flies and were stained with toluidine blue–methylene blue. All sections are shown at the same scale; anterior is towards the top. (**A**) Wild-type lobe showing the disposition of the lamina (la), medulla (m), lobula (lo) and lubula plate (lp) neuropiles. The sole connection between lamina and medulla consists in the intersecting axonal fibers that form the outer chiasma (oc). ic, inner chiasma. (**B**) sc^2 lobe showing an ectopic fiber bundle (arrowhead) crossing the lobula plate neuropile and abutting into the inner chiasma. (**C**) sc^2/sc^{B57} lobe showing an ectopic bundle (arrowhead) crossing the lobula, inner chiasma and lobula plate. The distal part of the lobula plate neuropile is absent and replaced by cell bodies (arrow). (**D**) A mutant optic lobe showing an abnormal position of the medulla neuropile, and the misrouting of posterior lamina efferents that avoid the outer chiasm (oc) and penetrate the medulla in an ectopic position (arrow). This nerve bundle runs along the posterior-medial border of the medulla neuropile, penetrates this neuropile and ends up near or at the anterior-distal border of the neuropile. The course of a similar misrouted bundle is more clearly seen in (**F**). The small arrowhead in (**D**) points at a neuropile distortion created by the path of the misrouted bundle. Note that posterior lamina efferents normally innervate the anterior medulla (**A**). Thus, in the mutant lobes (**D**) and (**F**), the misrouted efferents seem to project onto their normal region, but following an abnormal path. The optic lobe in (**D**) has, in addition, a reduced number of cells in its cortex (compare with **A**). (**E**) shows that medullar cortex is absent in the posterior part of this ganglion (arrowhead). An ectopic bundle is also clearly seen. Inset in (**F**) shows a section of retina from a sc^2/sc^{B57} head; it is undistinguishable from a wild-type retina. Its magnification is 2.5 times that of the other sections. Retina and most of the lamina in (F) was removed to allow good penetration of fixative.

(Goodman *et al.*, 1984; Doe *et al.*, 1985; Palka, 1987), it seems plausible that even moderate cell loss may lead to misrouting of fiber bundles. In *rough* mutants, the loss or improper differentiation of photoreceptor cells induces gross misrouting of fibers emerging from the lamina (Meyerowitz and Kankel, 1978; Rubin, 1989). In *small optic lobes* (*sol*) mutants, certain cell types of the medulla are missing and neurons from the lobula complex establish abnormal

connections with the medulla (Fischbach and Heisenberg, 1981). Evidently, a more detailed analysis of the ase$^-$ phenotype is required to characterize the primary lesion of the *ase* deficiency. It is of interest that mutations in four X-linked genes (*irre A–D*) induce misrouting of fiber bundles similar to those found in the *ase* deficiency (Boschert *et al.*, 1989).

The *ase* product, like other AS-C proteins, is most likely a transcription factor that helps regulate the activity of 'downstream' genes involved in the development of neural cells. This factor is present in many proliferating precursors of the CNS, but it seems unnecessary for at least some of their progeny to differentiate normally. This is a recurring theme found for several genes presumably implicated in transcriptional control within the NS (the four AS-C genes, *fushi-tarazu* and *even-skipped*): their deletions seem to affect fewer cells than those in which they are expressed (Romani *et al.*, 1987, 1989; Doe *et al.*, 1988a,b). Thus, it is tempting to suggest that the control of 'downstream' genes may be accomplished by different combinations of transcriptional factors, some factors not being essential in many cells because others can substitute for them.

Materials and methods

Drosophila stocks

Df(1)sc^{B57}/FM6 was obtained from M.Brand. We have determined that it carries an interstitial deficiency with the distal breakpoint located between *y* and *ac* and the proximal one to the right of *Df(1)260-1* (F.González, unpublished). It is deficient for the whole AS-C and mutant for *1(1)EC4*. *Df(1)260-1*/FM6 and *sc^2 pn*/CDX were obtained from the collection of A.García-Bellido. *Df(1)260-1* is a spontaneous, apparently terminal deficiency of the X chromosome. It is deficient for the whole AS-C. The *sc^2* mutation was X-ray induced and is deficient for most of the *scγ* region (Figure 1). See Lindsley and Grell (1968) for further description of these mutants.

Molecular cloning of scγ region

Five genomic equivalents of a wild-type Canton S library (Maniatis *et al.*, 1978) were screened (Maniatis *et al.*, 1982) with a 3.9 kb *Eco*RI fragment of phage λsc53. This fragment corresponds to the proximal end of our previous chromosomal walk (Campuzano *et al.*, 1985). Three different phages were isolated. The one extending most proximally was further analyzed and used to determine the *Df(1)260-1* breakpoint.

Drosophila head sectioning and staining

Whole heads, from pharate adults dissected out of the pupal case, were fixed and embedded in paraffin as described by Jäger and Fischbach (1987). Sections (7 μm) were stained with the Holmes–Blest silver–gold procedure (Blest, 1961). Alternatively, adult heads were collected from etherized flies, one retina was cut out to allow better penetration of fixative and the heads fixed in glutaraldehyde and osmium tetroxide (Franke *et al.*, 1969). Heads were embedded in plastic (Epon), and sections (2 μm) were stained at 55°C with toluidine blue–methylene blue.

cDNA clones

A 3–12 h embryo cDNA library constructed by L.Kauvar and T.Kornberg was screened (3×10^5 clones) with the 1.2 kb *Xho*I–*Eco*RI fragment (coordinates 23.9–25.1; Figure 1) which hybridized with the *ase* RNA. Five clones containing a 2.2 kb insert with two internal *Eco*RI sites were isolated.

DNA sequencing

Appropriate subclones of cDNA and genomic DNA in pEMBL18(+) (Dente *et al.*, 1983) were sequenced by the dideoxynucleotide termination procedure (Sanger *et al.*, 1977) as modified by Tabor and Richardson (1987) to use chemically modified T7 DNA polymerase. Synthetic oligonucleotides of defined sequence (15 nt long) were used to extend sequences and fill in gaps. Consensus sequences were constructed with the help of Staden's (1984) computer programs. Sequences were analyzed using the University of Wisconsin GCG software package (Devereux *et al.*, 1984).

Primer-extension analysis

Primer-extension experiments were performed according to Bensi *et al.* (1985). A 21 nt long oligonucleotide complementary to the sequence extending from C_{1521} to G_{1541} was synthesized, labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (3000 Ci/mmol) and hybridized in 50 mM Tris, pH 7.7, 75 mM KCl, 10 mM dithiothreitol and 15 mM $MgCl_2$ with 35 μg of poly(A)$^+$ RNA from 0–12 h embryos. Incubations were at 65°C for 15 min followed by 30 min at 55°C. The excess primer was removed by precipitating the RNA in ethanol. The RNA was redissolved in the above buffer and the primer was extended with AMV-reverse transcriptase and deoxynucleotide triphosphates, by incubation at 42°C for 1 h. The length of the DNA was determined in a 10% acrylamide sequencing gel. Extension was 33 nt long, suggesting that transcription starts at G_{1488}.

Other procedures

Phage and *Drosophila* DNA preparations, RNA preparations, Southern and RNA blot analyses and single-stranded RNA probe preparations were performed as described (Maniatis *et al.*, 1982; Modolell *et al.*, 1983; Melton *et al.*, 1984; Campuzano *et al.*, 1985). *In situ* hybridizations of ^{35}S-labeled RNA probes to embryo and larval sections were performed by the method of Ingham (1985) and Ingham *et al.* (1985) as modified by Romani *et al.* (1987, 1989).

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