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Developmental- and Eye-Specific Transcriptional Control Elements in an Intronic Region of a Ca^{2+}-Activated K^+ Channel Gene

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The range of electrical properties that a neuron or muscle cell can manifest is determined by which ion channel genes it expresses and in what amounts. The *Drosophila slowpoke* Ca^{2+}-activated K^+ channel gene has four distinct promoters. Here we assess the role that a downstream intronic region, called the C2/C3 region, plays in modulating Promoter C1 and Promoter C2 activity. Promoter C1 and Promoter C2 appear to be responsible for all neuronal and muscle expression, respectively. Transgenic flies were used to determine the expression pattern from each promoter in the presence and absence of the C2/C3 region. Deletion of this region silences Promoter C1 in adult but not larval CNS and causes a substantial reduction in Promoter C2 activity in adult but not larval muscle. The C2/C3 region also activates Promoter C1 in the animal's eye. By placing the C2/C3 region adjacent to a basal HSP70 promoter we have demonstrated that it contains elements that can specifically activate a heterologous promoter in the eye and in adult but not larval muscle. These results demonstrate that the C2/C3 region has a important role in regulating *slowpoke* developmental expression in the CNS and musculature and in regulating eye expression.

INTRODUCTION

Nerve cells and muscle fibers transmit information in the form of electrical impulses. These impulses are produced by the activity of and the interplay between different ion channels in the cell membrane. In many systems, electrophysiological and molecular techniques have been used to catalog the channel types expressed in different cells. Different Na^+ channel subtypes are expressed in the adult and embryonic CNS and in skeletal and cardiac muscle. In turn, a brain can be subdivided into overlapping patterns of K^+ channel gene expression (Perney and Kaczmarek, 1993). From these analyses, it is clear that ion channel gene expression is both temporally and spatially regulated in species ranging from *Drosophila* to man (Mandel, 1992; Broadie and Bate, 1993; Perney and Kaczmarek, 1993; Hong and Ganetzky, 1994; Sheng *et al.*, 1994; Becker *et al.*, 1995). Furthermore, the transcription of K^+ channels can be altered by electrical stimulation, growth factors, hormones, and seizures, leading to the supposition that alterations in channel expression could function in modulating the synaptic plasticity of the mature nervous system (Perney and Kaczmarek, 1993).

Since the transcriptional control of channel genes ultimately determines the range of electrical properties that a cell can adopt, we would like to understand more about how channel genes are transcriptionally regulated. Toward this end, we are studying the transcriptional control of the *Drosophila slowpoke* (*slo*) gene. This gene encodes a Ca^{2+}-activated K^+ (CAK) channel that is molecularly and functionally homologous to the vertebrate BK or Maxi-K CAK channels (Atkinson *et al.*, 1991; Butler *et al.*, 1993). CAK channels respond to a change in internal Ca^{2+} by opening a transmembrane K^+-specific ion pore. Once activated by Ca^{2+}, the channel's activity can be modulated by changing membrane potential.

The complete expression pattern of the gene has been documented using anti-Slo antibodies, *in situ* hybridization and reporter gene constructs (Becker *et al.*, 1995). The *slo* gene is widely expressed in the CNS, PNS, musculature, and tracheal cells and in a limited fashion in the eye and midgut. We would like to understand how the *slo* transcriptional control region is organized. Previously, we used 5′ RACE, RNase protection, and cDNA clones to map four transcription start sites. These four promoters are distributed over a 5-kb region of genomic DNA and are called Promoters C1, C1b, C1c, and C2. During RNA processing the unique 5′ exon produced by each promoter is spliced to a common exon called C3.

Deletion mapping of the transcription control region

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identified the position of sequences required for expression in the CNS, musculature, trachea, and midgut. None of the deletions advanced further 3′ than Promoter C2 (the most 3′ promoter to be mapped). Downstream of this promoter is exon C2, an intron, and exon C3. In this work we demonstrate that this downstream, promoterless region contains elements required for CNS expression, modulates expression in a developmental stage-specific manner, and directs expression to the animal's eye.

METHODS

The construction of *slo* promoter gene constructs P1, P3, and P6 has been previously described (Becker *et al.*, 1995; Brenner *et al.*, 1996). All deletion constructs were carried in the vector pCaSperβ-gal (Thummel *et al.*, 1988) and were transformed into embryos by co-injecting the transformation constructs (1 μg/μl) and a helper plasmid pπ25.7wc (200 ng/μl) into w^{1118} embryos (Spradling, 1986). Transformed chromosomes were maintained in either the homozygous state or by using the balancer chromosomes CyO and TM6 (Lindsley and Zimm, 1992). β-Galactosidase staining of sectioned adult tissues and dissected larval tissues was performed as described in Becker *et al.* (1995). The adult pattern was assayed in at least three independent transformants except for the P6 construct. Only two independent transformants were used to determine the P6 adult pattern. In adults, at least 5 different animals were stained for each transformation construct. The larval expression pattern was established by examining a minimum of four independent transformants. At least 10 different animals were examined for each construct.

Construction of slowpoke Transformation Constructs

Restriction maps helpful in understanding this section are found in Fig. 1. The P1 transformation construct was described in Becker *et al.* (1995) and the P3 and P6 constructs were described in Brenner *et al.* (1996). For convenience they will be briefly described here. To build P1, cosmid S10 (Atkinson *et al.*, 1991) was digested with *Xho*I and *Apa*I. An 11-kb *Xho*I–*Apa*I genomic fragment containing the slowpoke promoters was isolated and subcloned into a modified pCaSperβgal (Thummel *et al.*, 1988). This produced a translational fusion between *slo* exon C3 and the lacZ gene such that transcription from any of the *slo* promoters will cause the production of β-galactosidase. P3 was built by digesting P1 with *Bam*HI and *Bgl*II. Cohesive end ligation joined the *Bam*HI site in exon C1 to the *Bgl*II site in exon C2, resulting in the loss of material between the sites. P3 contains Promoter C1 but not Promoters C1b, C1c, or C2. P6 was built by digesting P1 with *Xho*I and *Pst*I, converting the ends to blunt ends, and ligating the plasmid shut. This results in the loss of all material 5′ of the *Pst*I site and the removal of Promoters C1, C1b, and C1c.

To build P8, *Bgl*II was used to digest P1. This DNA was then subjected to partial *Bam*HI digestion and a 4-kb DNA fragment was gel purified. The 5′ end of this fragment is the *Bam*HI site within exon C1 and its 3′ end is the *Bgl*II site within exon C2. The fragment was subcloned into the *Bam*HI site of the transformation vector pCasperAUGβgal (Thummel *et al.*, 1988). P8 includes Promoters C1b, C1c, and C2 but not Promoter C1. Expression from the *slo* promoters will cause the expression of β-galactosidase.

To build P9, the *Xho*I–*Apa*I fragment that contains the entire transcriptional control region was subcloned into the *Xho*I–*Apa*I sites of pBluescript(SK^+) to produce plasmid pBSXA. pBSXA was digested with *Bam*HI. The enzyme cuts in the vector and at the *Bam*HI site in exon C1. This releases a 5-kb DNA fragment that includes all sequences 5′ to the *Bam*HI site of exon C1. This fragment was subcloned into the *Bam*HI of pCasperAUGβgal to produce plasmid P9. P9 contains the CNS1 box and Promoter C1, but not Promoters C1b, C1c, or C2.

In construct p10a a minimal HSP70 promoter was placed adjacent to the C2/C3 intronic region. PCR was used to specifically amplify the −57 to +85 region of the HSP70 promoter (Ingolia *et al.*, 1980). The 5′ primer introduced a *Xho*I and a *Pst*I site into the sequence. The 3′ primer was downstream of a *Pst*I and a *Bam*HI site. The amplification product was digested with *Xho*I and *Bam*HI. The P1 transformation construct (Becker *et al.*, 1995) was also digested with *Xho*I and *Bgl*II. In P1, *Xho*I is the most 5′ restriction site in the P1 genomic DNA and *Bgl*II cuts within exon C2. This released from the vector a large fragment that includes Promoters C1, C1b, C1c, and C2. This fragment was replaced with the HSP70 promoter-bearing *Xho*I/*Bam*HI fragment to produce P10a. The remaining *slo* genomic DNA in p10a begins at the *Bgl*II site within exon C2 and ends at the *Apa*I site in exon C3. Therefore, it contains 213 n of exon C2, the 629-n C2/C3 intron, and 204 n of exon C3. This DNA does not contain any previously identified *slo* promoters. P10b was built by digesting P10a with *Pst*I and then ligating closed all free ends. P10b was the ligation product which had lost the HSP70 promoter containing *Pst*I fragment.

Anti-HRP Staining of Frozen Adult Sections

Following β-galactosidase activity staining the coverslips were floated off in PBS. The sections were washed once for 5 min in PBS and then incubated in a PBS, 0.1% Tween 20 solution (PBT) containing a 1/25 dilution of goat fluorescein-conjugated anti-HRP antibody (Cappell Research) for 1 hr at room temperature. The sections were then washed three times in PBT for 15 min. Anti-HRP staining was viewed using fluorescence microscopy (Zeiss 450-490/FT510/LP520 filter set).

RESULTS

In previous work we mapped the location of four *slo* promoters and DNA elements that are required for expression in specific tissue types (Brenner *et al.*, 1996). The four *slo* promoters are called Promoters C1, C1b, C1c, and C2 and are named after the unique 5′ exon whose synthesis they specify. Deletion analysis had identified two regions, called CNS box 1 and CNS box 2, required for expression in the CNS, one region required for midgut expression, and a region required for expression in tracheal cells and musculature. The position of each of these regions and the four promoters is summarized in Fig. 1A for convenience. In this work we test the function of the C2/C3 intron-bearing region in the expression of the *slo* gene. This intron is located between exon C2 and exon C3.

To determine the role played by the intron in gene regula-

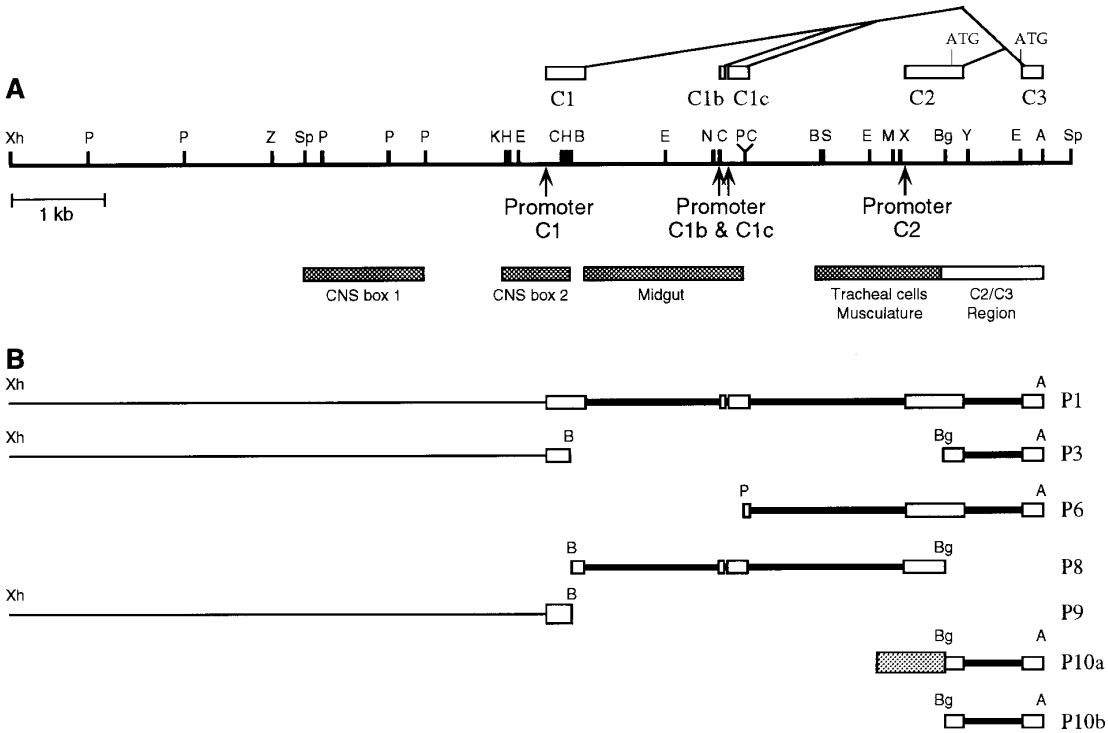

FIG. 1. (A) Transcriptional control region of *slo* gene. The horizontal line represents a restriction map of genomic DNA in the vicinity of the *slo* promoters. Position of all mapped promoters is identified. Above the map is shown the splicing pattern of the first four exons of the gene. Open boxes represent exons and lines denote their splicing pattern. ATG represents putative translation start sites (Brenner *et al.*, 1996). Labeled gray boxes below the genomic map represent regions that when deleted cause loss of expression in a particular tissue (Brenner *et al.*, 1996). The single open box below the genomic map identifies the position of the C2/C3 intron. (B) *slo* reporter gene constructs. This map is aligned with the map in A. A thin line represents DNA not part of a transcription unit, open boxes represent exons, and thick lines represent introns. Refer to A for position of promoters and exon names. For each construct a lacZ gene is inserted at the 3′ end of the insert (rightmost position in the map above). The terminal restriction sites of all DNA fragments are identified by the abbreviations Xh, B, Bg, P, or A. The large gray box in P10a is a HSP70 minimal promoter element which can respond to enhancer elements in the C2/C3 intronic region. Abbreviations: CNS, central nervous system; A, *Apa*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; M, *Mun*I; N, *Nhe*I; P, *Pst*I; S, *Sma*I; Sp, *Spe*I; X, *Xba*I; Xh, *Xho*I; Y, *Xmn*I; Z, *Sph*I.

tion we compared Promoter C1 and C2 activity in the presence and absence of the intron. As an additional test for regulatory elements the intron was placed adjacent to a minimal HSP70 promoter and tested for its ability to cause this promoter to be expressed in a tissue-specific manner. To delete the intron we relied on conveniently located restriction sites. Consequently, when the intron was removed portions of both exons C2 and C3 were also removed. Similarly, when the intron was tested for enhancer activity (as in P10a) or promoter activity (as in P10b), we were actually testing the intron and flanking portions of the C2 and C3 exons. Therefore, our analysis really maps control elements to the C2/C3 region. However, the most likely residence for these elements is in the intron itself. The C2/C3 region is identified by the open box in Fig. 1A. All expression constructs include a lacZ reporter gene. Each construct was transformed into *Drosophila* embryos and transformed stocks of animals were produced. Then the tissue- and the developmental-specific expression patterns of the constructs were determined by staining sectioned adults, dissected larval brains, and body wall muscles for β-galactosidase activity. The P1 construct includes the full-length *slo* transcriptional control region. It has been previously described and shown to be capable of reproducing the entire *slo* expression pattern in all developmental stages (Becker *et al.*, 1995). Figure 1B shows maps of the P1 construct and the various deletions used to determine the function of the C2/C3 region.

The C2/C3 Region Does Not Contain a Promoter Element

It is important to note that the C2/C3 region does not itself contain a promoter. This is most convincingly demonstrated by the P10b construct. In three independent chromosome insertions, P10b showed no expression in muscle fi-

bers or neurons in the adult or in larvae (Figs. 2 and 3). Furthermore, previous promoter mapping studies using 5′ RACE did not identify a transcription start site within the C2/C3 intron (Becker *et al.,* 1995). If this region does not contain a promoter then any affect it has on expression must result from the presence of control elements that modify the expression of upstream promoters.

The C2/C3 Region is Important for Adult but Not Larval Muscle Expression

The involvement of the C2/C3 region in muscle expression is shown by comparison of the P1, P6, P8, and P10a transformants. P6 is expressed in adult and larval muscle at the same level as the full-length P1 construct (Fig. 2). Of the four promoters, P6 contains only Promoter C2. P8 contains all sequences found in P6 (and additional 5′ sequence) but is missing the C2/C3 region (Fig. 1B). In larval muscles the levels of expression of P1, P6, and P8 are comparable. However, in adult muscle, P8 expresses at much lower levels than P1. Therefore, the C2/C3 region must contain information important for expression in adult muscle but not larval muscle.

This was further tested by examining the expression of P10a in both larval and adult muscles. In P10a, the *Bgl*II/*Apa*I fragment that contains all of the C2/C3 region is adjacent to a HSP70 minimal heat shock promoter (Fig. 1B). By itself, this promoter is essentially silent in flies. However, when an enhancer element is placed adjacent to it, β-galactosidase is expressed in the tissue-specific pattern dictated by the enhancer. Others have used this promoter as a tool for detecting enhancers and documenting their tissue-specificity (Hiromi and Gehring, 1987; Bowtell *et al.,* 1989). We have used it to characterize the transcription elements contained in the C2/C3 region. P10a is not active in larval muscle; however, it is expressed in the adult musculature (Fig. 2). Therefore, region C2/C3 appears to contain transcription elements that activate the minimal HSP70 promoter within the adult but not larval musculature.

Region C2/C3 Is Required for Adult Brain Expression

The P3 construct is expressed in neuronal tissue but not in muscles, trachea, or midgut. In the CNS, P3 is expressed in essentially the same pattern and at the same abundance as the full-length P1 construct (Brenner *et al.,* 1996). P3 is expressed in the larval central brain, mushroom bodies, and ventral nerve cord but not in the immature larval optic lobes. In adults, P1 and P3 are expressed in the central brain, including mushroom bodies, optic lobes, and thoracic ganglion (Figs. 3 and 4). P3 is a modified version of P1 in which all the material between exons C1 and C2 has been deleted. P3 contains Promoter C1, CNS box 1, and CNS box 2, regions previously shown to be required for CNS expression (Brenner *et al.,* 1996). It does not contain any of the other promoters.

To determine how the C2/C3 region modulates expression from Promoter C1 we built a construct which was identical to P3 except that it was missing the C2/C3 region. This construct is called P9. Comparison of the expression patterns of P3 and P9 demonstrates that loss of the C2/C3 region causes a dramatic change in Promoter C1 activity in both the adult and larval brain. In the larval brain P9 is expressed at much lower levels than P3 (Fig. 3). P9 expression is weak in the ventral nerve cord and almost undetectable in the central brain itself. Both regions of the brain require extended staining periods to visualize. Mushroom body expression in the central brain is typically visible as shown in Fig. 3. In adults P9 expression is undetectable in the adult central brain, mushroom bodies, optic lobes, and in the thoracic ganglion (Fig. 3).

The P10a construct demonstrates that the C2/C3 elements are, by themselves, not sufficient to activate the HSP70 promoter in the larval or adult brain, ventral nerve cord, or thoracic ganglion. P10a is not expressed in any of these tissues. Therefore, transcription elements within the C2/C3 region, while necessary for proper expression in the CNS, are not sufficient in the absence of other *slo*-associated elements. P10a, however, is transcriptionally active in other tissues (discussed below).

Eye Expression

Both the endogenous *slo* gene and the P1 construct have been shown to be expressed in the *Drosophila* eye (Becker *et al.,* 1995). As demonstrated in the P9 construct, removal of this region causes complete loss of expression in the eye (Fig. 3). This indicates that the C2/C3 region contains elements that activate Promoter C1 in the eye. Interestingly, in P3, in which the C2/C3 region is much closer to Promoter C1, eye expression is more robust than with the full-length P1 construct.

Eye expression is also observed in the P10a construct. This indicates that elements within the C2/C3 region are sufficient to activate a minimal HSP70 promoter in the eye. Conversely, the P6 construct is not expressed in the eye, even though it contains both a promoter, Promoter C2, and the C2/C3 intron. Apparently, the region's eye-specific elements are incapable of causing Promoter C2 to be active in the eye. P1, P3, and P10a are expressed to different levels in the eye. P1 is very weakly expressed, P3 is much more abundant, and P10a is strongly expressed. In the eye, expression is seen at the level of photoreceptors R1 through R6, R7, and R8.

DISCUSSION

The *slo* Ca^{2+}-activated K^+ channel gene has a complex transcriptional control region. It contains at least four promoters and has been shown to be transcriptionally active in neurons, skeletal and smooth muscle, tracheal cells, and cells of the midgut. Previously, deletion analysis was used

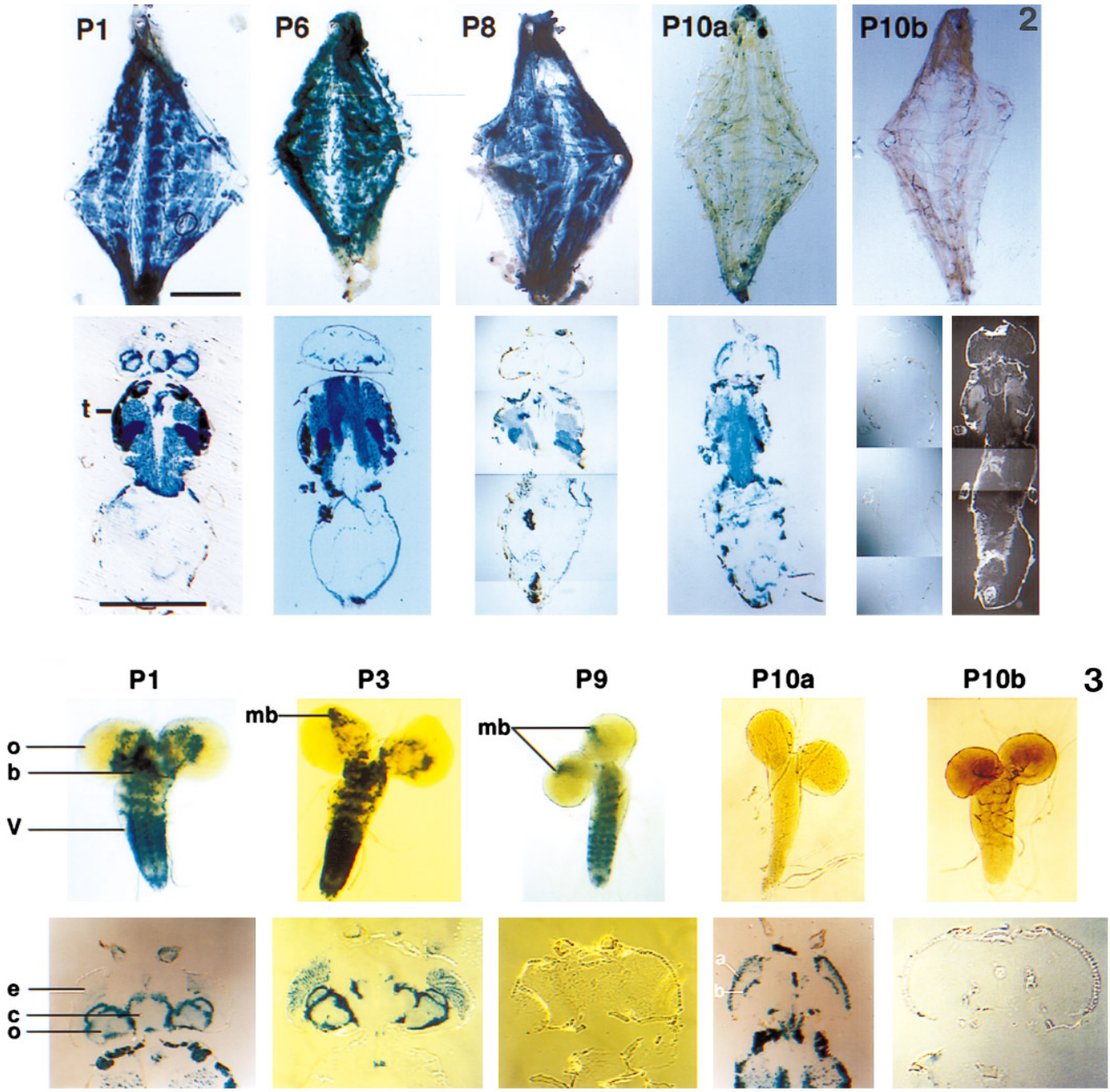

FIG. 2. Expression of reporter genes in larval body wall muscles and adult muscle. Shown below the name of each transformant is a larval body wall followed by a section through an adult animal. P1, P6, and P8 show uniform expression in the muscle fibers of the body wall. At this resolution body wall staining represents expression in muscle fibers. In the adult muscle expression can be most easily seen in the flight muscles located in the thorax (t). The adult p10b animal is represented by a light-field photograph to show the lack of reporter expression (in the complete absence of β-galactosidase expression contrast is poor) and an exposure using autofluorescence to show the presence of tissue. Bar, 1 mm.

FIG. 3. The C2/C3 intronic region is required for proper CNS expression. Each reporter gene construct is represented by an intact larval brain (top) and a section through the head of an adult (bottom). P1 shows the expression pattern of the intact transcriptional control region. In the P1 larval brain an optic lobe (o), a brain hemisphere (b), and the ventral nerve cord (V) have been labeled. The P1 adult brain is labeled to identify an eye (e), the central brain (c), and the optic lobes (o) which consist of the lamina, medulla, lobula, and lobula plate. For all brain structures, the β-galactosidase activity is most abundant in the cortical regions which contain the neuronal cell bodies. In the P3 and P9 larval brains, the expression in the mushroom bodies is easily seen (mb). In P10a expression is limited to the eye at the positions of photoreceptors R1 through R6 (labeled as a) and photoreceptor R8 (labeled as b). No expression of P10a occurs in the adult brain. Positively staining material near the brain represents muscle fibers. Bars, 0.5 mm.

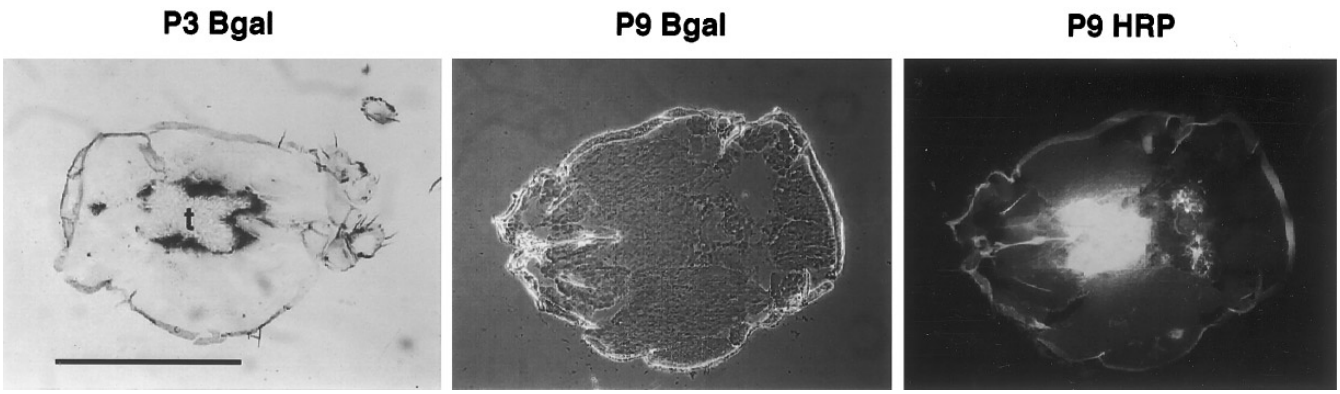

FIG. 4. Expression of reporter genes in the thoracic ganglion. Transverse sections through the thoraces of P3 and P9 transformants. The thoracic ganglion (t) is labeled. Both P3 and P9 were stained for β-galactosidase activity. Only P3 expresses β-galactosidase in the thoracic ganglion. P9 was also stained with anti-HRP antibody to identify the position of the β-galactosidase negative thoracic ganglion. Bar, 0.5 mm.

to map tissue-specific control regions for each of these tissue types (Brenner *et al.*, 1996). However, each and every one of the deletion constructs contained the intron-bearing C2/C3 region. Therefore, the possibility existed that we had overlooked control elements within the intron. This region does not seem to contain a promoter since previous mapping of transcription start sites did not identify a promoter (Brenner *et al.*, 1996) and furthermore, the C2/C3 region did not function as a promoter in transformed *Drosophila* tissue. This work focuses on two of the four *slo* promoters, Promoters C1 and C2, and their interaction with elements within the C2/C3 region. To do this we compared the expression pattern of a lacZ reporter gene in transgenic flies in the presence and absence of the C2/C3 region. The P3 reporter gene demonstrates that in conjunction with the appropriate control elements Promoter C1 can produce the entire *slo* CNS expression pattern. Similarly, the P6 reporter shows that when paired with the appropriate elements Promoter C2 is able to reproduce the muscle expression pattern. Both constructs include the C2/C3 region.

The P9 reporter is basically identical to P3 except that it is missing the C2/C3 intron (and portions of the flanking exons). Therefore, comparison of the P9 and P3 expression patterns tells us how the C2/C3 region influences the expression pattern of Promoter C1. Deletion of the C2/C3 region does not obviously alter the pattern of Promoter C1 expression in the larval brain but seems to merely reduce its intensity. That is, P9 (without the C2/C3 region) and P3 (with the C2/C3 region) are expressed in the same regions of the brain; however, P9 expression is much weaker than P3 expression. Of course, there may be some cell to cell variation in the pattern between P3 and P9 that remain to be discovered. Therefore, this leads to the conclusion that in the larval brain, the C2/C3 region does not impart tissue-specificity per se, upon Promoter C1, but acts to augment its expression in the tissues specified by other upstream elements. It appears that neuronal tissue-specificity is imparted by elements within CNS box 1 and 2. It has been shown that deletion of these sequences causes a steep reduction in the number of cells in which promoter C1 is active but does not seem to reduce the level of expression in these cells (Brenner *et al.*, 1996).

In adult animals the C2/C3 deletion causes an absolute loss of expression in all parts of the CNS (optic lobes, central brain, and thoracic ganglion). This is the second region that has been shown to be required for Promoter C1 activity in the adult CNS expression, the first being CNS box 1 (Brenner *et al.*, 1996). Because the C2/C3 deletion eliminates all activity we cannot determine if it acts by augmenting Promoter C1 activity in the cells as specified by other control elements or if it also plays a fundamental role in identifying the cell type. The most parsimonious hypothesis is the former since in it the C2/C3 region affects Promoter C1 activity in the same manner in both larval and adult CNS.

Using the P6, P8, and P10a constructs we addressed the role that the C2/C3 region plays in Promoter C2 expression. P6 contains only Promoter C2 and flanking sequences but expresses β-galactosidase in muscles in the same pattern as the complete transcription control region. P8 also contains Promoter C2 and additional upstream sequences but not the C2/C3 region. P8 is expressed in the same pattern as P6 in both larvae and adults with the exception that in the adult P8 expression is greatly reduced. The level of larval muscle expression, however, is comparable between the P6 and P8. Based solely on this result it would appear that the C2/C3 region acts to enhance Promoter expression in the tissues specified by other elements. However, we cannot exonerate the C2/C3 region from any influence upon tissue-specific expression because the P10a construct is active in adult flight muscle. This is not a nonspecific activation of the HSP70 promoter since it is not active in the CNS. Notably, the C2/C3 region causes the basal HSP70 promoter to be active in adult but not larval musculature. Therefore a muscle-specific control element(s) must exist in the C2/C3

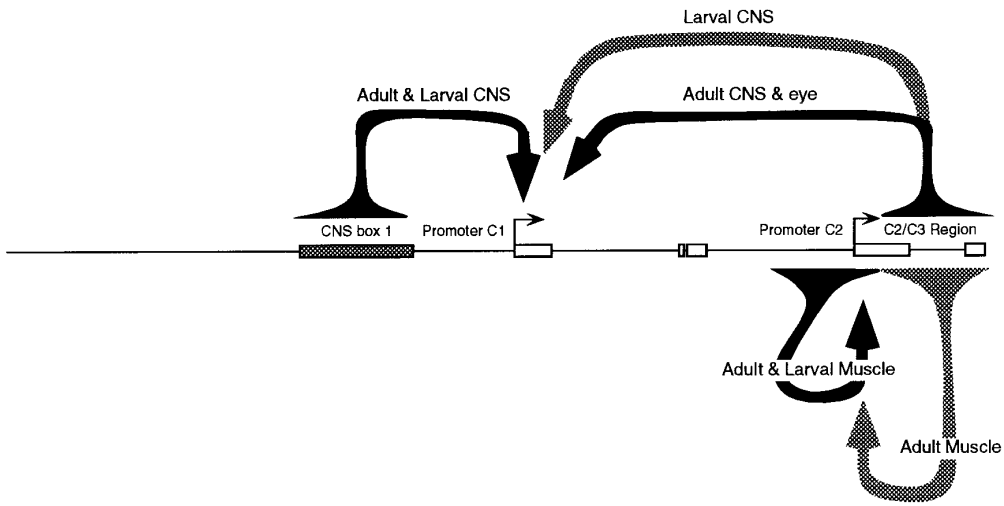

FIG. 5. Summary of regulatory interactions essential for proper developmental expression from Promoter C1 and Promoter C2. Each arrow represents a positive regulatory interaction. The base of the arrow identifies DNA sequence that contains tissue- or developmental-specific regulatory elements. These elements modulate the promoter identified by the arrowhead and the labels identify a tissue or developmental stage in which the elements act. Black arrows indicate that the control elements are absolutely required for promoter activity, while gray arrows indicate that some expression still occurs in the absence of the control elements. The C2/C3 interactions are described in this paper; all other interactions were originally described in Brenner *et al.* (1996).

region. Clearly elements in the C2/C3 region also respond strongly to the developmental stage since expression is not seen in larval muscle.

The effect of the C2/C3 region on Promoter C1 activity in the CNS and Promoter C2 expression in the muscles is similar. In both tissues the loss of the C2/C3 region affects expression in the adult to a greater extent that in the larvae. With respect to the CNS, larval expression is reduced but adult expression is completely eliminated. With respect to the musculature larval expression is not affected or is affected very little while in the adult expression is dramatically reduced. The presence within the C2/C3 region of an element that responds to the animal's developmental stage but is not itself involved in specifying cell type-specific expression could account for these results.

The C2/C3 region very clearly causes P10a to be active in the eye. It has been shown that the complete *slo* transcriptional control region (P1) is also expressed in the eye. Three copies of a photoreceptor-conserved element I exist within the C2/C3 intron (Brenner *et al.*, 1996). This element is found associated with genes expressed in retinal cells in *Drosophila*, cows, mice, and chickens (Kikuchi *et al.*, 1993). It appears that P10a expresses in photoreceptor cells and perhaps other cell types in the eye. The expression pattern in both P10a and the P1 constructs appears to be the same; however, the level of P10a expression is far greater. Interestingly, P3 also shows enhanced eye expression. The increased eye expression from P3 can be accounted for either because of the loss of a negatively acting element which reduced expression in the eye or because the ability of the C2/C3 region to influence Promoter C1 is dependent upon the distance between the two regions.

The C2/C3 region, by itself, is able to direct the HSP70 basal promoter to be active in the eye. Therefore, it is of some note that the C2/C3 region is unable to force Promoter C2 to be active in the eye even though it is adjacent to it. Sequences proximal to Promoter C2 could actively prevent it from being active in the eye. For vertebrate genes, it is increasingly common to find that their tissue-specificity also includes a negative component which prevents expression in inappropriate cell types (Li *et al.*, 1993; Chong *et al.*, 1995; Gherzi *et al.*, 1995; Hoeben *et al.*, 1995; Muller, 1995). An alternative explanation is that Promoter C2 is merely incompatible with the elements in the C2/C3 region while Promoter C1 and the HSP70 promoter are compatible with these elements. Promoter C2 is a TATA-less promoter while both Promoter C1 and the HSP70 promoter have easy to recognize TATA boxes. This alone may serve to insulate Promoter C2 from the eye specific elements in the C2/C3 region. Examples of the tuning of enhancers to specific TATA boxes certainly exist in Drosophila (Li and Noll, 1994).

The *slo* gene has a complex transcriptional control region in which expression in fundamentally different cell types is driven by distinct promoters. A summary of the regulatory circuitry controlling Promoter C1 and Promoter C2 is presented in Fig. 5. The C2/C3 intronic region has a role in regulating both promoters during development. In concert with the CNS Box, the intronic region activates transcription from Promoter C1 in the adult and larval CNS. How-

ever, the degree of activation is greater in the adult than larval CNS. In muscles, the C2/C3 region enhances Promoter C2 activity in adults but does not seem to play any role in larval muscle expression.

Potassium channels are highly regulated, both in development and in response to plastic changes in the cell (Broadie *et al.*, 1993; Partiseti *et al.*, 1993; Perney *et al.*, 1993). Controlling the correct number of ion channels is critical for maintaining the electrical properties of a cell. In *Drosophila*, altering the number of Ca^{2+}-activated K^+ channels has been shown to alter synaptic efficacy and the shape and duration of muscle action potentials (Gho and Ganetzky, 1992; M. Stern, personal communication). Moreover, altering potassium channel expression may perturb the neuronal development or affect cell morphology (Jones and Ribera, 1994; Budnik *et al.*, 1990). This work shows that the C2/C3 intronic region plays an important role in regulating slowpoke developmental and tissue-specific expression and provides a foundation for understanding how the electrical properties of a cell are regulated.

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