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Cis-acting sequences sufficient for correct tissue and temporal specificity of larval serum protein 1 genes of *Drosophila*

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We have constructed hybrid genes in which the coding region of the bacterial gene chloramphenicol acetyl transferase (CAT) has been linked to varying lengths of upstream sequences of *Drosophila* genes for larval serum sequence 1 (LSP1). These have been inserted into a P-element transformation vector and subsequently transferred into the germ-line of recipient flies. Transformants carrying the CAT gene linked to 1650 bp, 570 bp or 377 bp of upstream LSP1α sequences, or 745 bp or 471 bp of upstream β sequences express CAT with the same developmental and tissue specificity as the endogenous LSP1 genes. Constructs having only 66 bp of upstream LSP1β sequences, however, show extremely low levels of CAT expression in tissues and at developmental stages in which LSP1 is not expressed. We discuss the significance of short regions of homology between the DNA upstream of the α and β genes, which lie within the regions identified by the transformation experiments as being required for the *cis*-regulation of LSP1 synthesis.

Key words: Drosophila/LSP1 genes/transformation/CAT/developmental specificity

Introduction

Larval serum protein 1 of *Drosophila melanogaster* is comprised of three polypeptide chains, α, β and γ, encoded by single copy genes at 11A7-B9 on the X-chromosome, 21D2-22A1 on the second chromosome and 61A1-6 on the third chromosome (Roberts and Evans-Roberts, 1979; Smith *et al.*, 1981). The three genes which are co-ordinately expressed in the fat body of third instar larvae encode polypeptides that associate as a heterohexamer (Wolfe *et al.*, 1977) which comprises 8% of total protein at puparium formation. It is exported into the haemolymph and subsequently re-absorbed into the fat body. The peptides have been shown to be closely related by immunological studies and peptide mapping (Wolfe *et al.*, 1977; Brock and Roberts, 1980). The genes are similarly organized (McClelland *et al.*, 1981) and form stable heteroduplexes over most of their length that can be visualized in the electron microscope and have a thermostability indicative of between 85 and 90% sequence homology (Smith *et al.*, 1981). DNA sequencing experiments around the 5′ ends of the three genes indicate strong sequence conservation in the region encoding the untranslated 5′ end of the mRNA and the long open reading frame of the first intron. There is little homology between the genes within the intron and in the region upstream of the TATA box (Delaney *et al.*, 1986).

Jowett (1985) has introduced a wild-type allele of the LSP1α gene into the germ line of a strain of flies carrying an electrophoretic variant of the endogenous α gene. This transforming DNA has ~1.5 kb of upstream flanking sequences, and the protein it encodes can be readily distinguished from that of the endogenous gene with which it is co-ordinately expressed. In order to examine transcription, we have found it necessary to adopt a different strategy, since although there are γ^0 alleles which show no transcription, the α^0 and β^0 alleles are transcribed at a low level (D.Roberts, T.Jowett, D.Smith and D.Glover, unpublished data), and the sequences are cross-homologous. We therefore linked 1.65 kb of upstream α gene sequences and 2.25 kb of upstream β gene sequences to the bacterial gene, chloramphenicol acetyl transferase (CAT), and showed that this gene could be expressed with the developmental specificity of LSP1 when these constructs were introduced into the *Drosophila* germ line (Davies *et al.*, 1986). The *cis*-acting DNA sequences responsible for the developmental regulation of LSP1 gene transcription must therefore lie in the region upstream of the protein coding sequences within which there is little sequence homology at the three chromosomal sites.

In this paper we describe experiments in which we have delimited the *cis*-acting regulatory sequence. We have constructed a further series of hybrid genes with varying lengths of upstream sequences from the LSP1α and β genes driving the CAT gene. We show that sequences responsible for developmental regulation lie within 377 and 471 nucleotides upstream of the α and β genes respectively, within which are two short regions of sequence homology that we have previously described (Delaney *et al.*, 1986).

Results

LSP1–CAT hybrid genes

We wished to study the *cis*-acting sequences that regulate LSP1 transcription by re-introducing genes containing all or part of these sequences back into flies by P-element mediated germ-line transformation. Initial transformation experiments indicated that sequences 1.65 kb and 2.25 kb upstream of the α and β genes respectively would drive the expression of the bacterial gene CAT with the same temporal and tissue specificity as the endogenous LSP1 genes (Davies *et al.*, 1986). In order to further delimit the *cis*-acting sequences necessary for correct temporal and tissue specific expression, we have constructed a further series of hybrid genes. These have varying lengths of upstream sequences from the LSP1 α and β genes linked at position +22 (relative to their initiation site for transcription) to the CAT gene which has 42 nucleotides of bacterial DNA upstream of its initiation codon for translation. Downstream of the CAT gene, the constructs contain a segment of SV40 DNA that has the splice sites and polyadenylation site of the t-antigen gene. The hybrid LSP1–CAT gene together with the *rosy* transformation marker are flanked by the terminal regions of a P-element. The construction of these recombinant DNA molecules is described in detail in Materials and methods together with complete molecular maps (Figure 6). The

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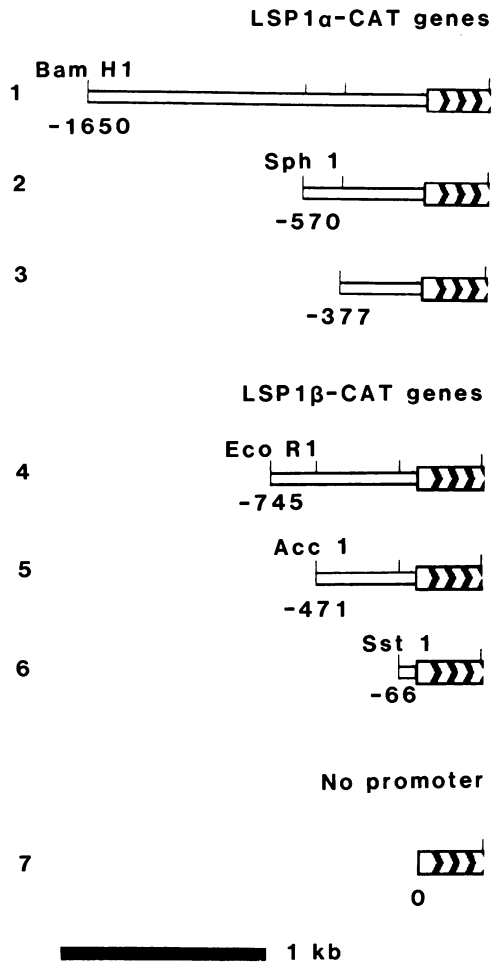

Fig. 1. LSP1 upstream sequences present in the hybrid genes. The LSP1 upstream sequences are indicated by the open narrow boxes. The length of DNA in bp remaining upstream of the start site of transcription and the restriction sites that delimit this DNA are indicated at the 5′ end of the LSP1 sequence. The CAT gene is shown as a wide box shaded with chevrons. These hybrid genes are contained within the plasmids **1**, pP[(ry)LSP1α-CAT]; **2**, pP[(ry)α-CAT 0.57]; **3**, pP[(ry)α-CAT 0.38]; **4**, pP[(ry)β-CAT 0.75]; **5**, pP[(ry)β-CAT 0.47]; **6**, pP[(ry)β-CAT 0.07] and **7**, pP[(ry)CAT 0].

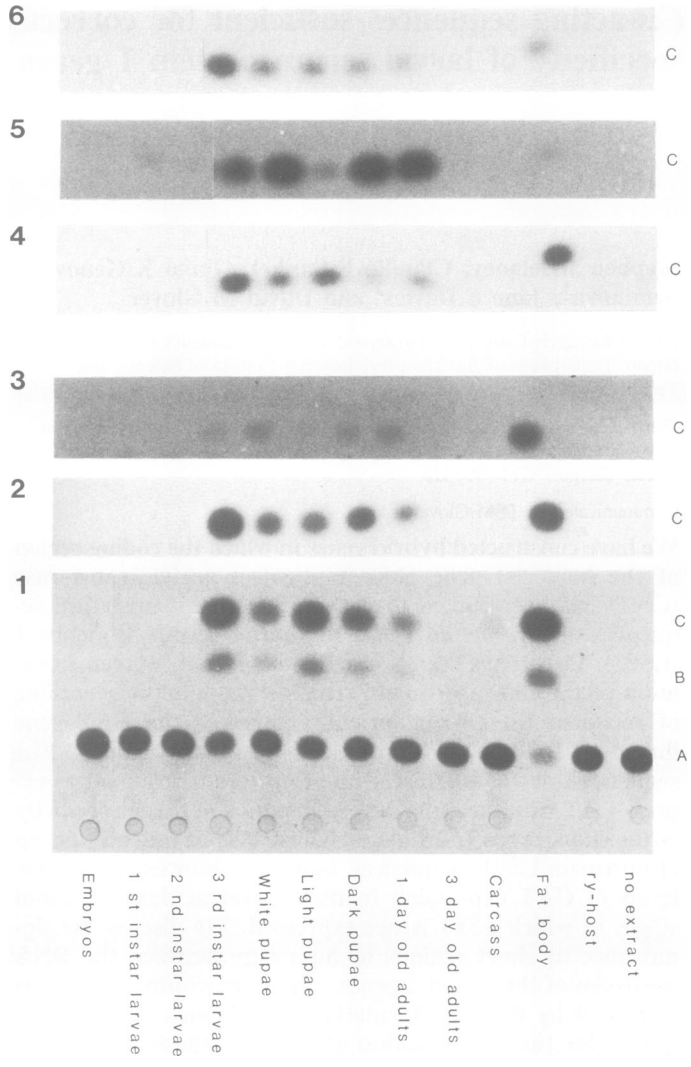

Fig. 2. CAT assays of transformant lines. Whole animals of various stages and tissues dissected from third instar larvae were assayed for CAT activity using the standard protocol. The amount of protein extract used for the assays on extracts of whole animals was 12.5 μg. This is approximately equivalent to the extract from 1/5 of a third instar larva. The exception was the experiment in row 5 for which 20 μg was assayed. The volumes of tissue extract which were assayed were also equivalent to that obtained from 1/5 of a larva, except for the experiments in rows 1 and 3 where an amount equivalent to 1/2 of a larva was used. The early larval instars were staged by the appearance of the mouth-hooks and spiracles, whereas the third instar larvae were harvested during the wandering stage. 'White pupae' corresponds to stage P1 of Bainbridge and Bownes (1981), 'light pupae' to stages P2 and P11, and 'dark pupae' between stages P12 and P15. Adults were aged in vials following eclosion. 'ry-host' is protein extract prepared from ry^{506} wandering third instar larvae. The experiments were carried out on the following transformed lines: **row 1**, αT2/CyO; **row 2**, P[α 0.57]/P[α 0.57]; **row 3**, P[α 0.38]/P[α 0.38]; **row 4**, P[β 0.75] T4; **row 5**, P[β 0.47] S1/TM3; **row 6**, P[β 0.47] S18/CyO.

essential features of the hybrid LSP1−CAT genes are depicted in Figure 1. The α gene constructs contain 1650 bp, 570 bp and 377 bp of upstream LSP1α sequence, and the β gene constructs 745 bp, 471 bp and 66 bp of upstream LSP1β sequences. We introduced these plasmids into ry^{506} embryos together with the non-mobile helper P-element, pπ25.7wc (Karess and Rubin, 1984), which provides transposase activity. Details of these transformed lines are given in Materials and methods. With two exceptions which are indicated later in the text, the lines used in this work contained single inserts as demonstrated by Southern blotting analysis of transformant DNA (Delaney, 1987).

Specificity of expression of CAT in transformant lines

To test whether the expression of the CAT gene in the transformed lines followed the same developmental and tissue specificity as that shown by the LSP1 genes, we selected lines of transformants that had hybrid LSP1−CAT genes inserted at single chromosomal sites, prepared protein extracts from eight developmental stages ranging from embryos to 3-day-old adults, and assayed these for CAT activity. In addition, assays were performed on extracts made from fat body dissected from wandering third instar larvae, and from the residual carcass. A complete autoradiogram of a TLC plate showing the conversion of ^{14}C-labelled chloramphenicol (A) to its two mono-acetyl derivatives (B and C) is shown in Figure 2 for extracts of transformants carrying the construct with 1650 bp of upstream α sequences (row 1). Only that section of the autoradiograms showing the major acetylated product of the CAT assay is shown for the other transformants. These correspond to assays carried out upon extracts prepared from flies transformed with constructs containing 570 bp (row 2), or 377 bp (row 3) of upstream sequences from the

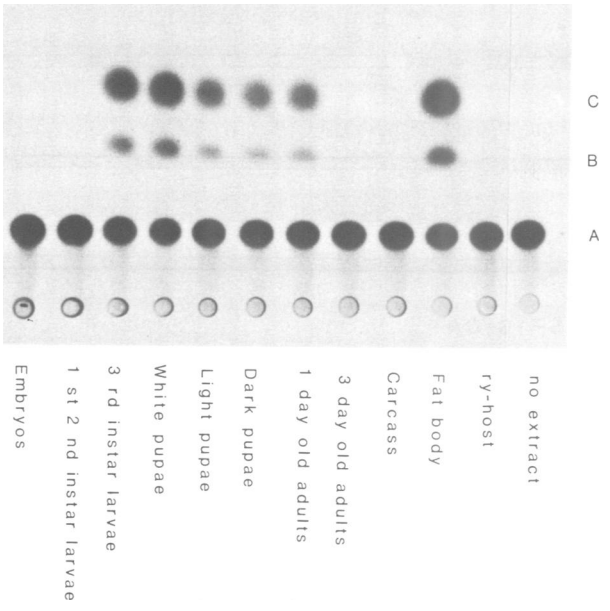

Fig. 3. CAT activity detected using the regenerative assay of S.A.Mitsialis and F.A.Kafatos (personal communication) upon whole animals of various stages and tissue dissected from third instar larvae of the P[α 0.38]/P[α 0.38] transformed line. The animals were staged in the same manner as for Figure 2 except that first and second instar larvae were combined. The amount of whole animal protein extract assayed was 12.5 μg, and the amount of dissected tissue was equivalent to ½ a third instar larvae larva. The increase in sensitivity over a conventional assay was ~5-fold.

Table I. Quantitation of CAT activity in wandering third instar larvae or white pupae of transformed lines expressed as the percentage conversion of chloramphenicol into its acetylated derivatives by 12.5 μg of tissue (equivalent to 1/5 of a third instar larvae)

	Third instar larvae	White pupae
α series		
αT2/CyO	71	80
P[α 0.57]/P[α 0.57]	62	58
P[α 0.38]/P[α 0.38]	2	3.5
β series		
P[β 0.75] T1	37	24
P[β 0.47] S1/P[β 0.47] S1	50	41
P[β 0.47] S18/CyO	30	22
P[β 0.07] S1	0.1	nd
P[β 0.07] S23	0.05	nd
Promoterless		
P[CAT 0]/CyO	0	nd

Assays were performed within the linear response range of either the conventional or the regenerative CAT assay. The data were normalized by comparing the results obtained with extracts giving a response within the linear range of both types of assay.
nd: not determined.

LSP1α gene, and 745 (row 4) or 471 (rows 5 and 6) of upstream sequences from the LSP1β gene. The lines of flies used were either homozygous for the chromosome carrying the insertion (rows 2 and 3), heterozygous for the chromosome carrying the insertion and a balancer chromosome (rows 1, 5 and 6), or mixtures of homozygotes and heterozygotes in a G2 line (row 4—see Materials and methods).

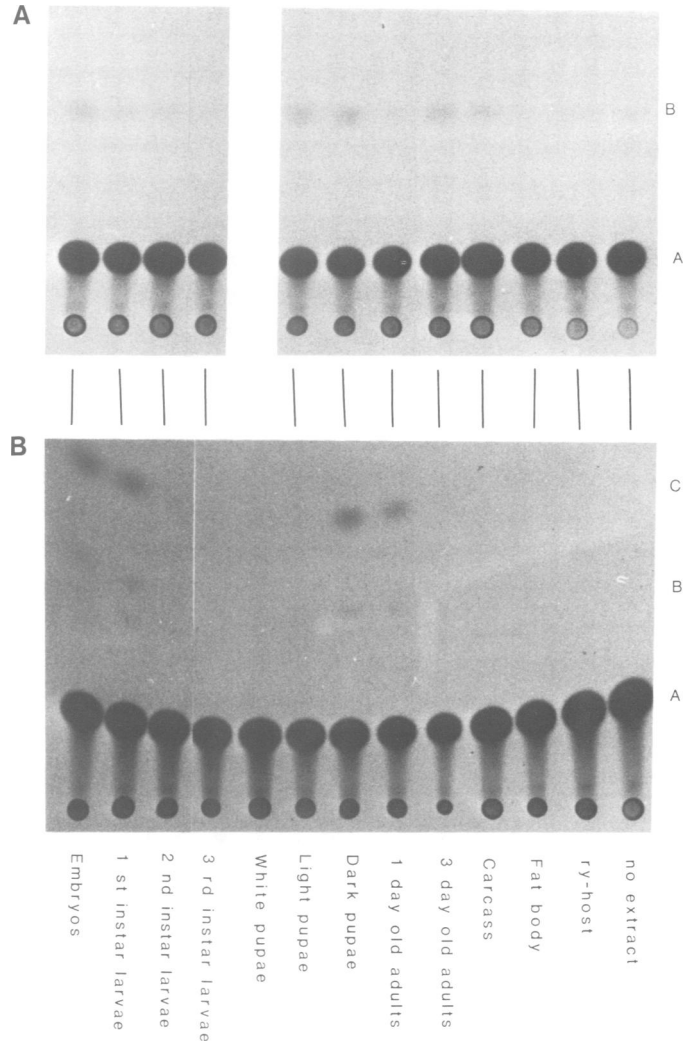

Fig. 4. Regenerative CAT assays performed upon extracts of whole animals of various stages and tissues dissected from third instar larvae of the transformant lines P[β 0.07] S1 (**panel A**) and P[β 0.07] S31 (**panel B**). The amount of protein extract from whole animals assayed was 20 μg. Volumes of tissue extract equivalent to that obtained from one larva (**panel A**) or to ⅓ of a larva (**panel B**) were assayed.

The temporal pattern of expression shown by all these transformants follows that normally shown by the LSP1 polypeptides. CAT is only expressed in third instar larvae, pupal stages and in 1-day-old adults. Expression is not detectable in embryos, in the first two larval instars and in 3-day-old adults, even after overexposure of the autoradiograms. In all of these transformants, CAT expression also shows the tissue specificity of the LSP1 genes, predominating in the fat body. In some cases the extract of the residual carcass also showed some CAT activity, at a level approximately 1/15 to 1/30 of that in fat body extracts. It seems most likely that this activity is the result of contamination of the carcass samples with fat body cells as the fat body is both delicate and distributed throughout the larva and therefore difficult to dissect from the animal.

The levels of CAT activity during development are roughly comparable for the assays shown in Figure 2, the exception being the construct with 377 bp of upstream LSP1α sequences. The line carrying this construct, which is homozygous for a single insertion on chromosome 3, shows levels of CAT expression much lower than the other transformants. Low levels of expression were also

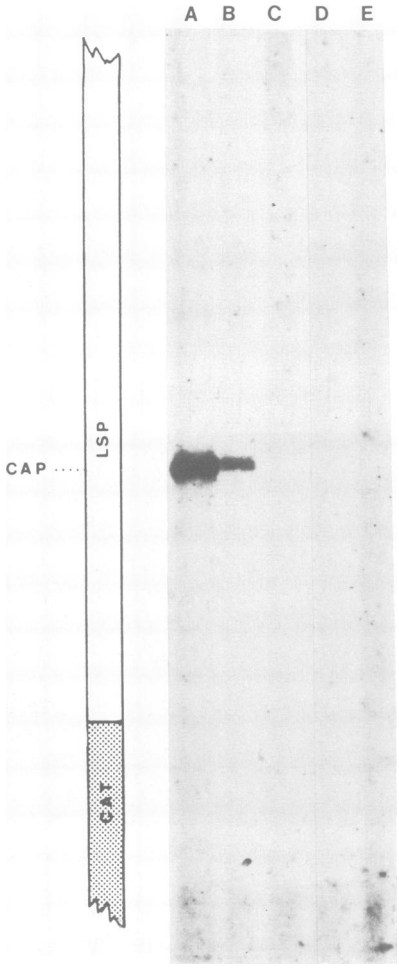

Fig. 5. Primer extension analysis of RNA using a ^{32}P-labelled oligonucleotide complementary to the CAT transcript. Each annealing reaction was carried out with 30 μg of total RNA extracted from third instar larvae of: **A**, αT2/CyO; **B**, P[α 0.57]/P[α 0.57] and **C**, P[α 0.38]/P[α 0.38], which have various upstream LSP1α sequences; and **D**, P[CAT 0]/Cy which contains no LSP1 sequences; and **E**, the host strain for transformation. Products were resolved on a 7% polyacrylamide gel. The marker was a dideoxy sequencing reacting using the same CAT oligonucleotide as primer so that the position of the transcription start or CAP site could be read directly from the sequence ladder. The position of the LSP1 CAP site is indicated on the left of the figure as well as the start of the CAT sequences.

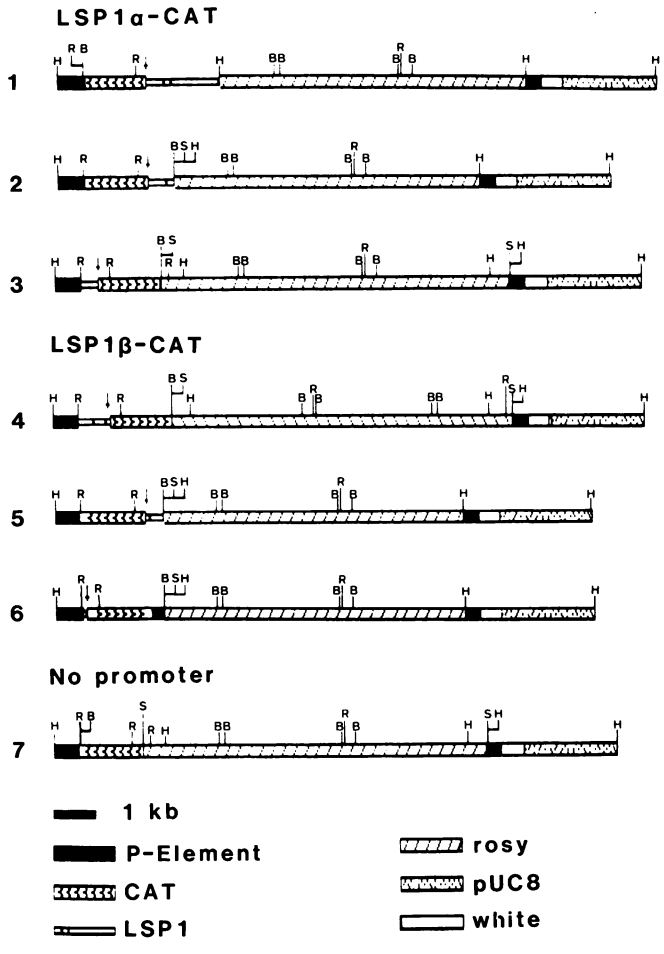

Fig. 6. Molecular maps of the LSP−CAT P-element transposons. Sites for the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII and *Sal*I are indicated by the letters B, R, H and S respectively. The lines within the narrow boxes representing the LSP1 sequences indicate the positions to which the upstream sequences have been removed. The arrows above the boxes indicate the position of the LSP1 CAP sites. The transposons are named according to the nomenclature suggested by Spradling and Rubin (1983). The transposons are as follows: **1**, pP[(ry)LSP1α-CAT]; **2**, pP[(ry)α-CAT 0.57]; **3**, pP[(ry)α-CAT 0.38]; **4**, pP[(ry)β-CAT 0.75]; **5**, pP[(ry)β-CAT 0.47]; **6**, pP[(ry)β-CAT 0.07] and **7**, pP[(ry)CAT 0]. The orientation of the genes within these transposons is indicated by the direction of shading in the CAT and *rosy* genes which are both transcribed from right to left in plasmid 1, while the direction of transcription of the P-element is from left to right in each case.

seen with the G2 parental line from which it was derived, which contains an additional site of insertion (data not shown). In both cases, however, the specificity of expression was similar to that shown by the other transformants. This was confirmed by repeating the assays using a protocol which achieves a greater degree of sensitivity using phosphotransacetylase and acetyl phosphate to regenerate acetyl CoA, which normally becomes limiting in the standard reaction due to self hydrolysis (S.Mitsialis and F. Kafatos, personal communication). When the CAT assays were repeated on these lines using the regenerative protocol the tissue and temporal specificity clearly resembled that of the other α transformants (Figure 3). CAT activity is restricted to those stages and tissues in which LSP1 polypeptides can be detected. Quantitation of CAT activity in third larval instar and pupal extracts indicates that the level of CAT expression in flies carrying this construct is almost two orders of magnitude lower than any of the other transformants in this group (Table I).

In contrast to the above results, an abnormal temporal and tissue-specific pattern of expression is seen in the transformants containing only 66 bp of LSP1β upstream sequences. Of 11 G2 parental transformant lines obtained for this construct, eight were assayed for CAT activity. Five lines were found to express CAT in either their third larval instar or pupal stages, and of these three were selected for further study. Figure 4 shows a developmental time course of CAT expression on two of these lines P[β 0.07] S1 and P[β 0.07] S31 using the sensitive CAT assay. CAT activity could be detected at varying levels throughout development including in the extracts prepared from embryos, first and second instar larvae and 3-day-old adults. In addition, CAT assays performed on tissues dissected from larvae of the line P[β 0.07] S1 showed that activity predominated in the carcass and not the fat body. The levels of CAT activity in third instar larvae were reduced by approximately three orders of magnitude compared with the construct having 745 bp of upstream β sequences (Table

I). Similar observations were made with tissue dissected from larvae of line P[β 0.07] S1/CyO which bears a genetically isolated second chromosome, carrying a subset of the seven insertions present in P[β 0.07] S1. CAT activity was also just detectable in the larvae of P[β 0.07] S23 which contains a single insertion. That this aberrant expression is dependent on the residual 66 bp of upstream LSP1β sequences is shown by the results obtained in transformants carrying the construct of the CAT gene with no upstream LSP1 sequences, from which we were unable to detect any expression at most of these stages, although in one such transformant it was just possible to detect CAT activity in 3-day-old adults.

We can conclude from these experiments that *cis*-acting elements sufficient for correct tissue and temporal specificity lie within 377 bp upstream of the LSP1α gene and between 66 and 471 upstream of the LSP1β gene.

Analysis of CAT transcripts in transformed flies

In order to confirm that the levels of CAT reflected the levels of transcripts, and to determine if transcription was initiating correctly at the LSP1 CAT site, we carried out primer extension experiments on RNA prepared from third instar larvae of the transformed lines. We annealed the RNA to a synthetic oligonucleotide complementary to a sequence close to the 5′ end of the CAT gene (Walker *et al.*, 1983) and incubated this with reverse transcriptase and deoxynucleoside trisphosphates in order to extend the primer to the 5′ end of the mRNA. The intensity of the signal given by the extended primer is in proportion to the abundance of the template and reflects the results of the CAT assays and Northern blot analyses of the RNA (data not shown). The results with the α series of transformants are shown in Figure 5. We were able to detect extended primer of the correct length in larvae transformed with constructs having 1650 bp and 570 bp of upstream LSP1α sequences. The abundance of transcripts in the transformants having 377 bp of upstream LSP1α sequences, which was expected to be low from the CAT assays, was in fact insufficient for us to be able to detect extended primer. This correlation of RNA levels with the level of CAT activity was borne out in primer extension assays upon RNA from the β series of transformants which also showed that transcription of the hybrid genes was initiating at the correct nucleotide (data not shown). Extended transcripts could not be detected in control experiments with RNA from transformants in which there were no LSP1 sequences upstream of the CAT gene, or in RNA from the recipient strain used in the transformation experiments.

Discussion

In this paper we show that the bacterial gene for CAT can be expressed in *D.melanogaster* with temporal and tissue specificity directed by the upstream regions of two of the LSP1 genes. In these transformants CAT expression is maximal in the fat body and in the third larval instar and pupal stages of development. The hybrid genes that retain this specificity contain 1650, 590 or 377 bp of upstream LSP1α sequences; or 745, or 471 bp of upstream LSP1β sequences. Transformants in which the CAT gene is linked to only 66 nucleotides of upstream LSP1β sequences, on the other hand, showed extremely low levels of CAT expression with incorrect developmental and tissue specificity. Furthermore, constructs having no LSP1 sequences upstream of CAT show no activity in third larval instar, although with one such construct we were just able to detect CAT in 3-day-old adults. These data indicate that the sequences that mediate the temporal and tissue specificity must reside within a surprisingly short sequence, namely between +22 and −377 bp of the LSP1α gene and between 66 and 471 bp upstream of the LSP1β gene.

We observed two instances in which the levels of CAT expression is significantly reduced. Although the 377 nucleotides upstream of the α gene can direct the correct specificity of expression, they may be insufficient to control the level of expression. Thus CAT activity in larvae carrying this hybrid gene is two orders of magnitude lower than with constructs having longer upstream sequences and it is difficult to detect the transcript in primer extension experiments. In order to draw definitive conclusions regarding the effect of sequences upstream of position −377 upon the level of transcription it would be necessary to obtain more lines of flies transformed with this construct. Nevertheless, we note that a parental line having an additional site of insertion of the hybrid gene gave comparably low levels of expression, suggesting that the result is probably not simply a consequence of insertion of the hybrid gene at a chromosomal location unfavourable for its expression. Furthermore, Jowett and co-workers have noted reduced levels of expression in flies transformed with an electrophoretic variant of the α gene driven by a comparable segment of upstream sequences (personal communication). The second case of low expression, some three orders of magnitude lower than in any of the other transformants, was with a construct containing only 66 bp of LSP1β upstream sequence. Moreover, these 66 bp were insufficient to confer the LSP1 developmental specificity on the CAT gene and low levels of activity were detected in inappropriate tissues and at inappropriate times. These were in the residual carcass following dissection of the fat body, in embryos, early larval stages and 3-day-old adults. It is possible that we have fortuitously created a *cis*-acting controlling element *de novo* by placing the 66 nucleotides of β sequences adjacent to P-element sequences, and that it is this new element which is directing low expression with novel specificity. A more likely explanation is that we are seeing the influence of a neighbouring promoter. This could either be the P-element promoter or that of the *rosy* gene. The construct contains its own TATA box and it may be sufficient for the chromatin of the flanking genes to become 'opened up' for transcription, thereby permitting a low level of transcription of the CAT gene. As the level of transcription of the CAT gene from the 66 bp of upstream β sequences is so low, it is impossible to confirm by primer extension which promoter is being used, even though it is possible to detect CAT activity. It is also possible that the transcriptional influence of a chromosomal gene adjacent to the transforming sequence is creating a region of active chromatin, but this seems unlikely since we observed the result in a number of independent transformants.

The control construct which has no LSP1 sequences showed no expression in third instar larvae, although we have been able to observe extremely low levels of CAT expression in some adult transformants bearing the pP[CAT 0] transposon, but not at any other developmental stages. This may result from the proximity of the *rosy* gene, since the levels of xanthine dehydrogenase activity are highest in the adult (Covington *et al.*, 1984). We are unable to map these transcripts and even the CAT activity is barely detectable.

The LSP1 α, β and γ genes have retained very strong homologies despite being distributed to the X-, second and third chromosomes of *D. melanogaster* during evolution. We recently examined the sequence homologies around the 5′ ends of the three genes (Delaney *et al.*, 1986). This revealed strong DNA sequence conservation in the 5′ untranslated regions of the mRNA and within the first exon's long open reading frame. Little of the 5′

untranslated region is present within our constructs which have their junction with the CAT gene at nucleotide +22. The three genes also show strong sequence conservation around their TATA boxes and transcription initiation sites extending from nucleotide position −36 to +24. However, it appears that the sequences responsible for tissue and developmental specificity lie further upstream. What very little homology there is upstream of the genes occurs in two regions. The first, which we have termed region 'A', is found beginning at nucleotides −200, −216 and −377 upstream of the α, β and γ genes respectively as follows:

```
α −200 A A T G C A A A T C T G G A C T C G A T A T G   −178
       * * *     * *   * * * * *     *   * * * * *

β −216 A A T A G A A G T C T G G C T T T G A T A A G   −194
       * * *     * *     * * *         * * * * * *

γ −377 A A T T A A A C   C T G A A   C T G A T A T G   −357
```

Another homology, region 'B', occurs between the sequences beginning 111, 130 and 192 nucleotides upstream of the α, β and γ genes respectively. These homologous sequences may be aligned as follows:

```
α −111 A G C A C C T G A G A T A C A C C C   − 94
       * * * * * * * * * * * * * * * * * *

β −130 A G C A C C T G A G A T A C A C C C   −113
       *   * * * * * * * *     *         *

γ −182 A C C A C C T G A A T T G A G G C A   −165
```

These sequence homologies occur within segments of DNA upstream of the α and β genes that we have shown in this paper to be essential for developmental specificity. They must therefore represent strong candidates for sequences that are recognized by *trans*-acting factors to co-ordinate the expression of the genes. Our results suggest additional experiments to test the functional significance of these sequences, either by specific mutagenesis or by specifically placing them upstream of other marker genes for further germ-line transformation studies.

Materials and methods

DNA constructions

Construction of transformation plasmids took one of two routes: the first involved a three step cloning in which the LSP1 sequences were cloned directly into the transformation vector, Carnegie 4 (Rubin and Spradling, 1983), which does not contain the *rosy* gene. A 1.64 kb *BglII*−*Bam*HI fragment derived from pSV0CAT-*BglII* (Gorman *et al.*, 1982), having the CAT gene upstream of a segment of SV40 DNA carrying a polyadenylation site, was cloned into the unique *Bam*HI site of this construct. Subsequently, an 8.1 kb *SalI* fragment carrying the *rosy* gene was introduced into the unique *SalI* site in the Carnegie 4 polylinker of constructs in which the CAT gene was in the appropriate orientation relative to the LSP1 promoter sequences. The fragment LSP1 DNA introduced into pP[(ry)α-CAT 0.38] in this way was a fragment terminating −377 bp upstream and at the *Bam*HI +22 bp downstream of the transcription start site. This fragment was generated during 'shotgun sequencing' (Delaney *et al.*, 1986) and was cleaved from sequencing vectors in an *Eco*RI−*Bam*HI digest. The fragment introduced into pP[(ry)β-CAT 0.75] was a 0.77 kb *Eco*RI−*Bam*HI fragment from pR^{b}LSP1β:1 (Smith *et al.*, 1981) extending between −745 and +22 bp relative to the transcription start site. The same method was used to construct a transformation vector which did not contain any LSP1 upstream sequences except that the first step was omitted. The alternative approach was to fuse the LSP1 sequences to the CAT gene in an appropriate plasmid and excise the hybrid gene using sites at the end of the CAT gene and at suitable positions in the LSP1 upstream sequences. Following end-repair the fragment was then introduced by blunt end ligation into the *HpaI* site of Carnegie 20. The segments of LSP1 genes introduced into the constructs in this way were: in pP[(ry)α-CAT 0.57], an end-repaired *SphI*−*Bam*HI fragment derived from pαCAT4 (Delaney, 1987) which has the α gene sequences between −570 and +22 bp relative to the transcription start site; in pP[(ry)β-CAT 0.47], an end-repaired *AccI*−*Bam*HI fragment derived from p229 (C.Sunkel, unpublished data) which effectively has the LSP1β sequence between −471 and +22 bp; and in pP[(ry)β-CAT 0.07], an end-repaired *SstI*−*XhoI* fragment derived from pPC112 (Davies *et al.*, 1986) which extends from −69 to +22 bp of the transcription start site. The construction of pP[(ry)LSP1α-CAT] is described in Davies *et al.* (1986). The resulting plasmids are shown in Figure 6.

Germ line transformation

Transformation of homozygous ry^{506} was carried out essentially as described by Spradling and Rubin (1982) except that the helper P-element was the transposition defective pπ25.7wc (Karess and Rubin, 1984). Surviving adults derived from injected (G0) embryos were mated individually to the recipient ry^{506} strain and ry^{+} G1 flies were selected. These were mated individually to the host strain and resulting ry^{+} G2 progeny self-crossed to establish 'G2 lines'.

Transformants were named according to the transforming sequences present; for example transformants obtained from micro-injection of the plasmid pP[(ry)α-CAT 0.57] are referred to as P[α 0.57] together with an additional letter and number to indicate individual G2 lines. With the exception of the lines P[β 0.75] T1 and P[β 0.07] S23, transposition appeared to give rise to multiple insertions. However, individual chromosomes bearing transforming sequences were isolated using standard crosses permitting the establishment of lines containing single transforming genes and the identification of the chromosome carrying the introduced genes. The lines obtained were made homozygous where possible or otherwise maintained against CyO or TM3 balancer chromosomes (Lindsley and Grell, 1968). With the exception of P[β 0.75] and P[α 0.38] this gave rise to sublines which had different chromosomal locations of transposons that did not differ significantly in the qualitative pattern of CAT expression during development.

CAT assays

The standard CAT assay was performed as described in Davies *et al.* (1986). The regenerative assay was carried out following the protocols of S.A.Mitsialis and F.A.Kafatos (personal communication) by adding 1 unit of phosphotransacetylase derived from *Leuconostoc mesenteroides* (Sigma) and adding 0.3 μCi of [^{14}C]chloramphenicol. The reaction was allowed to proceed overnight at 37°C. [^{14}C]Chloramphenicol and its acetylated derivatives were extracted and analysed in the same way as for the standard assay. Protein determinations were made using the dye binding procedures of Bradford (1976) using a commercially available dye reagent (Bio-rad). Calibration curves were constructed using BSA protein standards.

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References

Bainbridge,S.P. and Bownes,M. (1981) *J. Embryol. Exp. Morphol.*, **66**, 57−80.
Bradford,M.M. (1976) *Anal. Biochem.*, **72**, 248−254.
Brock,H.W. and Roberts,D.R. (1980) *Eur. J. Biochem.*, **106**, 129−135.
Covington,M., Fleenor,D. and Devlin,R.B. (1984) *Nucleic Acids Res.*, **12**, 4559−4573.
Davies,J.A., Addison,C.F., Delaney,S.J., Sunkel,C. and Glover,D.M. (1986) *J. Mol. Biol.*, **189**, 13−24.
Delaney,S.J. (1987) Ph.D. Thesis, University of London.
Delaney,S.J., Smith,D.F., McClelland,A., Sunkel,C. and Glover,D.M. (1986) *J. Mol. Biol.*, **189**, 1−11.
Gorman,C., Moffat,L.F. and Howard,B.H. (1982) *Mol. Cell. Biol.*, **2**, 1044−1051.
Jowett,T. (1984) *EMBO J.*, **4**, 3789−3795.
Karess,R.E. and Rubin,G.M. (1984) *Cell*, **38**, 135−142.
Lindsley,D. and Grell,E. (1968) *Carnegie Institute of Washington*, Publication no. 627.
McClelland,A., Smith,D.F. and Glover,D.M. (1981) *J. Mol. Biol.*, **153**, 257−272.
Roberts,D.B. and Evans-Roberts,S. (1979) *Genetics*, **93**, 663−679.
Rubin,G.M. and Spradling,A.C. (1983) *Nucleic Acids Res.*, **11**, 6341−6351.
Smith,D.F., McClelland,A., White,B.N., Addison,C.F. and Glover,D.M. (1981) *Cell*, **23**, 441−449.
Spradling,A.C. and Rubin,G.M. (1982) *Science*, **218**, 341−347.
Spradling,A.C. and Rubin,G.M. (1983) *Cell*, **34**, 47−57.
Walker,M.D., Edlund,T., Boulet,A. and Rutter,W. (1983) *Nature*, **306**, 557−561.
Wolfe,J., Akam,M.E. and Roberts,D.R. (1977) *Eur. J. Biochem.*, **79**, 47−53.

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