Transformation Mapping of the Regulatory Elements of the 
Ecdysone-Inducible P1 Gene of Drosophila melanogaster

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The transcription of the P1 gene is induced by 20-hydroxyecdysone in fat bodies of third-instar larvae. Germ line transformation showed that sequences between −138 to +276 contain elements required for a qualitatively correct developmental and hormonal regulation of P1 transcription. Sequences from −138 to −68 are essential for this expression.

Expression of the P1 gene is restricted spatially to the larval fat body and temporally to the end of the third instar (1, 5, 9, 10, 11, 18), when the ecdysteroid titer rises considerably before the onset of pupariation (19). P1 mRNA transcription is inducible by 20-hydroxyecdysone in ecdysteroid-deficient larvae homozygous for the I(3) ecdysone 1st (ecd1) mutation (10, 17, 29). Cycloheximide does not prevent reporter 20-hydroxyecdysone (17).

We had used germ line transfer of a hybrid gene containing an Escherichia coli gpt (EcoGpt)-simian virus 40 (SV40) reporter gene to demonstrate that control elements essential for the hormonal and developmental specificities of transcription of the P1 gene lie within positions −1300 to +276 relative to its transcription start (15). We have achieved a more precise mapping of these elements by analyzing the expression of deletion derivatives of this initial P1-EcoGpt-SV40 construct.

**P1-EcoGpt-SV40 hybrid constructs and establishment of transformed lines.** The starting point for the constructions was plasmid pDmHX21 (15) containing a 1.5-kbp HindIII-Sau3A P1 fragment (positions −1300 to +276 relative to the P1 mRNA start site) linked to the EcoGpt-SV40 sequences (16, 20, 33) inserted into the BamHI site of the polylinker of the Carnegie 2 transformation vector (25). The P1 sequences were shortened by deleting sequences between the EcoRI site of the polylinker and restriction sites at positions −775, −256, −138, −68, and +80 (14) (Fig. 1). A 4-kbp HindIII

![Diagram](https://example.com/diagram.png)

**FIG. 1.** P1-EcoGpt-SV40 constructs and developmental pattern of expression in transgenic lines. Hybrid constructs are designated according to the length of the 5' P1 upstream sequences retained in the construct. The P1 sequences including the mRNA start site, the 196-bp first exon, the 59-bp intron, and 21 bp of the second exon to position +276 (thick lines and filled boxes) were fused at position +276 to the EcoGpt coding sequence (stippled boxes) linked to the SV40 t-antigen intron and polyadenylation sequences (hatched boxes) as described previously (15) and in the text. All transformed lines contained one insert except lines B13, B14, Hp4, S8, S12, which contained two inserts as determined by Southern blot analysis (data not shown). Hybrid transcripts were detected (+) or not detected (−) by Northern blot analysis of RNA from fat bodies of late third-instar larvae (Fig. 2). For 20-hydroxyecdysone (20-OHecdysone) inducibility, + indicates that induction of the hybrid transcripts was detected by feeding 20-hydroxyecdysone to ecdysteroid-deficient larvae as described in the legend to Fig. 5. NT, Hormonal induction was not tested because no transcription of the hybrid transcripts was taking place in these lines.

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fragment (-1300 to -5300) was inserted upstream to the p-1300 construct to obtain the p-5300 transposon. Transgenic lines containing one copy of the recombinant transposons inserted into the genome of a recipient stock (2cb2) homozygous for the rosy<sup>206</sup>, scarlet, and ecodl mutations were obtained by using standard germ line transformation techniques (24, 31).

**Analysis of the steady-state level of transcription of the hybrid constructs.** Hybrid transcripts were detected in all lines harboring copies of the p-5300, p-1300, p-775, p-256, and p-138 constructs (Fig. 2). In contrast, no trace of hybrid transcripts could be detected in the transformed lines for the p-68 and p+80 constructs (data not shown).

A similar pattern of one major 1.7-kb and two minor 1.9- and 1.3-kb transcripts was observed in all cases (Fig. 2). The 5' end of each of these transcripts was mapped by a primer extension assay (Fig. 3). A major 239-bp product corresponds to the expected length for a transcript initiated at the natural P1 mRNA start site and normally spliced. A 298-bp fragment indicated the presence of a minor fraction of unspliced transcripts. 5' nuclease protection mapping of the 3' end of the hybrid transcripts indicated that termination of transcription of the hybrid gene occurred at three different sites within the SV40 sequences (Fig. 3B): a minor site at the polyadenylation site of the SV40 t-antigen gene, a major site approximately 200 bp upstream of this site, and an additional minor site localized at the 5' border of the t-antigen intron. The small t-antigen intron was spliced in the two longer hybrid transcripts (Fig. 3). A unique transcription start site and alternate use of three different transcription termination sites within the SV40 sequences account thus for the uniform pattern of three hybrid transcripts.

In all transformed lines, the level of accumulation of the hybrid transcripts was found to be much lower than that of the endogenous P1 mRNA. A 100- to 50-fold-lower level was determined in the case of the H1 line by densitometry scanning of the hybridization signals obtained on the same filter with a P1-specific probe (Fig. 4). In the other transformed lines, a similar or lower level was found (Fig. 2). The fluctuations noticed between different lines for the same construct (Fig. 2) probably result from an effect of genomic sequences flanking the different insertion sites (8, 26, 31, 32). From these data, no major influence of the length of the P1 flanking sequences on the level of expression of the reporter gene could be detected within the interval from -256 to -5300.

The low level of accumulation of the P1-Ecogpt-SV40 transcripts may reflect their reduced stability. The level of transcription of a P1-Adh hybrid gene in which the coding sequence of the alcohol dehydrogenase gene of *Drosophila*
melanogaster had been brought under the control of the −1300 to +80 P1 sequences has been found to be very similar to that of the endogenous P1 gene (5, 8a).

Developmental and hormonal regulation of transcription of the hybrid constructs. We had shown previously that the tissue and stage specificities of transcription of the p-1300 construct were identical to those of the endogenous P1 gene (15). The same specificities were found in different transformed lines for the p-5300, p-775, p-256, and p-138 hybrid constructs. While Northern (RNA) blot analysis showed expression in fat bodies from late third-instar larvae (Fig. 2), no hybrid transcripts were detectable, even after a prolonged exposure of the autoradiograms, in RNA samples prepared from other tissues (including salivary glands, gut, epidermis, brain, and imaginal discs) pooled from the same larvae (data not shown). This finding demonstrates that the hybrid gene was transcribed in all cases with the same spatial and temporal pattern as that of the P1 endogenous gene, i.e., in the fat body at the end of the third larval instar.

Because the recipient 2cb2 stock contained the ecd118 mutation (7, 30), the ecdysteroid dependence of the transcription of the hybrid constructs was tested by a shift of third-instar larvae of the transgenic lines to the restrictive temperature (29°C). At this temperature no transcription was observed in the case of the p-5300, p-1300, p-775, p-256, and p-138 constructs, indicating that their expression remained dependent on an elevated ecdysteroid titer (Fig. 1).

This conclusion was further confirmed in the case of the p-775 and p-256 constructs. First, a hormone feeding experiment (15) carried out on the B2 and Hpl1 lines showed that 20-hydroxyecdysone induces the transcription of the hybrid transcripts in ecdysteroid-deficient larvae (Fig. 5A). This finding was confirmed in an independent hormone induction test performed on ecdysteroid-deficient larvae hemizygous for the dop118 mutation (2) and one copy of the p-256 or the p-775 construct (Fig. 5B).

The major conclusion drawn from the deletion analysis described above is that one or several cis-acting elements involved in the developmentally regulated transcription of the P1 gene lie close to its mRNA start site, as already described for other Drosophila genes (13, 23, 28, 35). Sequences localized downstream of +276 thus appear completely dispensable for qualitatively correct hormonal and
developmental specificities. Although only one transgenic line has been tested with the p-138 construct, sequences upstream of this position also appear dispensable.

To date, the regulatory elements of only a few ecdysone-inducible genes have been analyzed in detail (see references 12, 27, and 34 and references therein). Riddihough and Pelham (21) identified a 23-bp hyphenated dyad element in the hsp27 promoter as containing a putative binding site for an ecdysone receptor (EcRE) (Table 1). Cherbas et al. (4) recently proposed an EcRE consensus sequence from binding studies with the ecdysone-inducible Eip28/29 gene (Table 1). Attempts to detect similar EcRE elements in the P1 gene identified an 11-bp sequence between positions -316 and -306 (Table 1). This element, however, which is removed in the p-256 and p-138 constructs, appears dispensable for 20-hydroxyecdysone induction. Another sequence matching the hsp27 element was found between -90 and -102 on the noncoding strand (Table 1). The functional significance of this sequence, which is part of an imperfect palindrome between positions -139 and -90, remains to be assessed. Removal of sequences localized between positions -68 and -138 is correlated with a loss of expression of the p-68 construct, indicating that essential sequences for the expression of the P1 gene are located in this region. Recent results to be reported elsewhere demonstrate the presence of a transcriptional enhancer element in this region (8a).

Our results indicate that spatial, temporal, and 20-hydroxyecdysone regulation of the P1 gene is mediated by tightly linked regulatory elements. Further work will be directed at elucidating how much of a physical and a functional overlap exists between them.

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REFERENCES


**TABLE 1. Sequence comparison**

<table>
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<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Estrogen</td>
<td>G G T C A N N N T G A/T C C</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>G A C A N N N T G T T/C C</td>
</tr>
<tr>
<td>Thyroid</td>
<td>G G T C A T - - - G A C C</td>
</tr>
<tr>
<td>Eip28/29</td>
<td>G G T C A N T G A A/C C</td>
</tr>
<tr>
<td>hsp27</td>
<td>G G T T C A A T G C A C</td>
</tr>
<tr>
<td>P1 (-316/306)</td>
<td>G A T C A A T G A A C</td>
</tr>
<tr>
<td>P1 (-90/102)</td>
<td>G G G T T G A A T G A A T</td>
</tr>
</tbody>
</table>

*a* Shown are sequence similarities of two sequence elements of the proximal promoter of the P1 gene (14) and the putative EcRE of the Eip28/29 (4) and hsp27 (21) genes. Vertebrate hormone response elements (1) are shown for comparison.