Identification of cis-Regulatory Elements Required for Larval Expression of the Drosophila melanogaster Alcohol Dehydrogenase Gene

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**ABSTRACT**

The Alcohol dehydrogenase (Adh) genes of two distantly related species, Drosophila melanogaster and Drosophila mulleri, display similar, but not identical, patterns of tissue-specific expression in larvae and adults. The regulatory DNA sequences necessary for wild-type Adh expression in D. mulleri larvae were previously reported. In this paper we present an analysis of the DNA sequences necessary for wild-type Adh expression in D. melanogaster larvae. We show that transcription from the proximal promoter of the melanogaster Adh gene is regulated by a far upstream enhancer and two or more elements near the transcription start site. The enhancer is tissue specific and stimulates transcription to high levels in fat body and to lower levels in midgut and malpighian tubules whether linked to the proximal promoter or to a heterologous promoter. The enhancer activity localized to at least two discrete regions dispersed over more than 1.7 kb of DNA. Deletion of any one of these subregions reduces Adh transcription in all three larval tissues. Similarly, two regions immediately upstream of the proximal promoter start site are necessary for wild-type transcription levels in all three tissues. Thus, each of the identified regulatory elements is sufficient for low levels of Adh gene expression in all three larval tissues, but maximal levels of expression requires the entire set.

The Adh genes of Drosophila provide an attractive system for studying the mechanisms that control cell type-specific transcription during eukaryotic development. Adh is transcribed in the larval and adult fat bodies of every Drosophila species thus far examined. It is also transcribed in other tissues which vary from species to species (Dickinson 1980a; Batterham et al. 1983; Rabinow and Dickinson 1986; Rowan and Dickinson 1986). Interspecific crosses and germline transformation experiments have shown that species-specific differences in Adh expression are determined by cis-acting elements closely linked to the Adh genes rather than by differences in the trans-acting factors (Dickinson and Carson 1979; Dickinson 1980b; Rabinow and Dickinson 1986; Fischer and Maniatis 1986; Brennan and Dickinson 1988).

To study the mechanisms that control tissue-specific transcription of Adh, we have compared the cis-acting regulatory elements necessary for Adh transcription in the larval tissues of two distantly related species, Drosophila mulleri and Drosophila melanogaster. The D. mulleri locus contains three closely linked genes: a pseudogene; an adult-specific gene, Adh-2; and a larval-specific gene, Adh-1 (Fischer and Maniatis 1985). In contrast, D. melanogaster has a single Adh gene transcribed from two tandem promoters (Benyajati et al. 1983; see Figure 1). The proximal promoter is active from mid-embryogenesis through the first part of the third larval instar, while the distal promoter is active in mid-embryogenesis, at the end of the third larval instar, and in adults. Adh-1 and Adh-2 appear to be functionally analogous to the proximal and distal promoters, respectively, of the melanogaster Adh gene, since the mulleri genes retain their normal pattern of expression when introduced into the melanogaster genome (Fischer and Maniatis 1986).

The proximal promoter of the D. melanogaster Adh gene and the Adh-1 gene of mulleri are expressed in essentially the same larval tissues, that is, in fat body, midgut and malpighian tubules (Fischer and Maniatis 1985, 1986). Detailed analysis of the sequences involved in the tissue-specific expression of Adh-1 showed that it is controlled by two fat body-specific enhancers and an upstream promoter element (Fischer and Maniatis 1988; see Maniatis, Goodbourn and Fischer 1987, for a review of upstream promoter elements, UPEs). Synergistic interactions between the enhancers and the UPE lead to high levels of transcription in midgut and malpighian tubules, as well as in fat body (Fischer and Maniatis 1988).

Here we present an analysis of the sequences necessary for correct tissue-specific transcription from the proximal promoter of the melanogaster Adh gene. We
show that the proximal promoter is regulated by an enhancer (called the larval enhancer) and two or more elements near the transcription start site. The enhancer is composed of at least two subregions which act synergistically to stimulate transcription in the larval fat body, midgut and malpighian tubules. Two elements immediately upstream of the transcription start site are also required for wild-type levels of transcription in these three larval tissues. Thus, Adh regulation in *D. melanogaster* and *D. mulleri* larvae is similar, in that each Adh regulatory element stimulates transcription in multiple tissues in its natural context. However, the individual regulatory elements in the two systems are not strictly analogous, as discussed below.

**MATERIALS AND METHODS**

**Construction of transformed Drosophila lines:** P element vectors containing modified Adh genes (see Figure 2) and the selectable marker ry+ were injected, along with the transposase plasmid (KARES and RUBIN 1984) into ry506 or Adh(6R)cn;ry506 embryos as described (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982; GOLDBERG, POSAKONY and MANIATIS 1983). Genes introduced into the rySo6 genome were cloned in the 5’ to 3’ fragment (-5000 to -660) was inserted in the 5’ to 3’ flanking DNA (GOLDBERG 1980). Plasmids were constructed as described in this paper were cloned in the AdhF element vector. The endpoints of the Adh genes designed to localize the larval enhancer were kept over the balancer chromosome TM2 (LINDSLEY and GRELL 1968).

**Transformation vectors:** All Adh fragments originated from plasmid sAF2, which contains the Adh+ allele and flanking DNA (GOLDBERG 1980). Plasmids were constructed using standard methods (MANIATIS, FRTSCH and SAMBROOK 1982). The basic structure of the modified Adh genes is shown in Figure 2. D-5000, D-660, ALE/hs/ADH, hs/ADH are described elsewhere (CORBIN and MANIATIS 1989a, b; FISCHER and MANIATIS 1988). Chromosomal linkages were assigned and homozygous lines were selected as described (GOLDBERG, POSAKONY and MANIATIS 1983; FISCHER and MANIATIS 1988). Most lines discussed in the paper are homozygous. Insertions which were homozygous lethal were kept over the balancer chromosome TM2 (LINDSLEY and GRELL 1968).

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**Plasmid pP-386** 

The plasmid pP-386 was made by inserting an XbaI linker into the HpaI site (at -386 of the proximal promoter) of D-660, cutting with XbaI, and ligating under dilute conditions to delete Adh sequences 5′ to -386. A 4.4-kb XbaI fragment containing the larval enhancer (including sequences from -660 to -386) was inserted into the XbaI site of plp-386 to make ALE/P-386.

**Transcription vectors:** All Adh fragments originated from plasmid sAF2, which contains the Adh+ allele and flanking DNA (GOLDBERG 1980). Plasmids were constructed using standard methods (MANIATIS, FRTSCH and SAMBROOK 1982). The basic structure of the modified Adh genes is shown in Figure 2. D-5000, D-660, ALE/hs/ADH, hs/ADH are described elsewhere (CORBIN and MANIATIS 1989a, b; FISCHER and MANIATIS 1988). Chromosomal linkages were assigned and homozygous lines were selected as described (GOLDBERG, POSAKONY and MANIATIS 1983; FISCHER and MANIATIS 1988). Most lines discussed in the paper are homozygous. Insertions which were homozygous lethal were kept over the balancer chromosome TM2 (LINDSLEY and GRELL 1968).

**RNase protection:** Total nucleic acid was prepared from Drosophila larvae as described (FISCHER and MANIATIS 1986). Most RNA preparations were isolated from third instar larvae within four hours of the second-to-third instar molt. However, in our initial attempts to localize the larval enhancer, we analyzed RNA from populations of -50 larvae collected as embryos over a 12-h period and aged for 72 h (78 ± 6 hr). In our culture conditions most of the populations were predominantly early third instar larvae, but transformants with low levels of Adh activity sometimes developed more slowly than those with high levels. Differences in the developmental stage of the samples could potentially skew Adh RNA measurements, since the activity of the proximal promoter peaks in a narrow developmental window in early third instar larvae (SAVAKIS, ASHURNER and WILLIS 1986). Therefore, data from the 12-h collections is included only for one line each of the D-5000, D-3345 and D-660 transformants, for three lines each of D-2830 and D-2830/-660 transformants and for both D-2190 transformants.

**Larval tissues** were dissected from eight to ten third instar larvae in ice cold Drosophila Ringer’s solution and immediately homogenized in RNA buffer (FISCHER and MANIATIS 1986).

**RNA analysis:** Northern blots were prepared essentially as described (MANIATIS, FRTSCH and SAMBROOK 1982). RNA samples were fractionated through 1.2–1.6% agarose gels containing 2.2 μg/ml formaldehyde, blotted onto GeneScreen nylon membranes (New England Nuclear), and cross-linked to the membrane by irradiating 5 min with 600 μW/cm² of UV light. The membrane was then baked at 80°C under vacuum for 1 hr (MELTON et al. 1984) and hybridized at 65°C overnight as described (THOMAS 1980) using uniformly labeled, single-stranded RNA probes. Probes were prepared as described (MELTON et al. 1984) except that BSA was added to a concentration of 60 μg/ml. Unhybridized probe was removed by washing in 0.2 × SSC, 0.5% SDS, and 0.02% sodium pyrophosphate at 65°C. Blots were stripped of probe by boiling 10 min in H₂O.

**Quantitative RNase mapping experiments** were carried out as described (ZINN, D’AVID and MANIATIS 1985), except that hybridizations were performed at 37°C without prior incubation at 85°C, and RNase digestions were at 25°C for 30 min.

**Hybridization probes:** The plasmid pSP6MEL was provided by J. FISCHER (FISCHER and MANIATIS 1986). The plasmid pSP6αTUB was made by inserting an approxi-
Regulation of Adh Gene Expression

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RESULTS

The proximal promoter of the D. melanogaster Adh gene is stimulated by the Adh larval enhancer (ALE, Figure 1; POSAKONY, FISCHER and MANIATIS, 1985; CORBIN and MANIATIS, 1989b). To determine whether the larval enhancer stimulates transcription in one or more tissues, we measured Adh transcripts from isolated tissues of larvae transformed with either a full-length Adh gene, which contains the larval enhancer, or a gene that lacks the enhancer (Figure 2A). The full-length gene was much more active in fat body than in malpighian tubules, and was more active in malpighian tubules than in midgut (Figure 2A). Note that lanes 5 and 6 were overloaded relative to the other lanes, as indicated by the α-tubulin control). Long exposures of the autoradiogram shown in Figure 2A show that the truncated Adh gene was expressed at very low levels in fat body, malpighian tubules, and midgut. Thus, the larval enhancer markedly stimulates Adh transcription in all three tissues, but has the largest effect in fat body.

The larval enhancer is primarily active in fat body: To determine the activity of the larval enhancer in the absence of potential regulatory elements near the Adh promoters, we linked the larval enhancer to a truncated hsp70 promoter, which in turn was linked to the Adh coding sequence (Figure 2B, the ALE/hs/ADH fusion gene). The truncated hsp70 promoter lacks inherent tissue- and temporal-specific control elements (Lis, Simon and Sutton 1983; Hiromi and Gehring, 1987; Garabedian, Shepherd and Wensink 1986; Fischer and Maniatis 1988). However, the Adh coding sequences could contain tissue-specific regulatory elements. To control for this possibility, we compared transcripts from the ALE/hs/ADH fusion gene to transcripts from a control gene, which lacks the larval enhancer (Figure 2B, the hs/ADH gene. Like the full-length Adh gene, the ALE/hs/ADH fusion gene was transcribed to the highest levels in fat body) (Figure 2B, lane 2), to lower levels in malpighian
Figure 3.—Diagram of proximal promoter deletions. All of the genes shown have a common 3’ endpoint 640 bp downstream of the polyadenylation signal. In the genes ALE/P-386, ALE/P-242, ALE/P-187, ALE/P-110, and ALE/P-80, the larval enhancer (−5000 to −660) was placed immediately adjacent to −386, −242, −187, −110, and −80 of the proximal promoter, respectively. The D-5000 gene (described in Figure 2A) is shown for comparison.

...tubules (lane 6), and to even lower levels in midgut (lane 4). In contrast, the control gene was expressed at very low or undetectable levels in these three tissues (Figure 2B, lanes 1, 3 and 5). The larval enhancer thus appears to be both necessary and sufficient for transcription in all three larval tissues, although it is most active in the fat body.

Localization of regulatory elements near the proximal promoter: The larval enhancer and sequences downstream of −386 of the proximal promoter are sufficient for wild-type levels of Adh transcription in early third instar larvae (CORBIN and MANIATIS 1989a, b). As visualized by a histochemical stain specific for ADH enzyme activity, larvae transformed with a gene containing only these sequences (called ALE/P-386 lines, see Figure 3) express high levels of Adh in a pattern indistinguishable from wild-type larvae (Table 1).

To further localize important regulatory elements near the transcription start site, we fused the larval enhancer to 5’ truncations of the proximal promoter (Figure 3). Transcription levels decreased ~3-fold when sequences upstream of −242 were deleted (Table 2, line 3). On average, transcription levels remained the same when sequences upstream of −187 and −110 were deleted, but decreased another 4-fold when sequences upstream of −80 were deleted (Table 2, lines 4–6). These data indicate that two regions near the promoter are important for Adh transcription, one between −382 and −242 and one between −110 and −80. Note, however, that genes with 110 bp of 5’ promoter sequence produced markedly different levels of Adh transcripts in different transformed lines (as reflected in the particularly large standard deviation (sD), Table 2, line 5). Since three of these lines displayed transcription levels as low as those of the −80 lines (data not shown), the −187 to −110 region could also contain all or part of a regulatory element.

We used the histochemical stain to determine whether the elements near the proximal promoter affect Adh expression in one or all Adh-expressing larval tissues. When sequences upstream of −242 were deleted, long incubations were required to visualize ADH activity in the fat body and midgut, and no activity was detected in malpighian tubules (Table 2). As additional sequences were deleted, fat body and midgut staining became weaker and more variable and, as expected, malpighian tubules still did not stain (Table 2). These results suggest that each deletion that lowers the overall levels of Adh transcription, does so in fat body and midgut. Although malpighian tubules did not stain when sequences upstream of −242 were deleted, RNA analysis from the three tissues indicates that transcription is reduced to approximately the same extent in all three tissues (data

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**TABLE 1**

Localisation of ADH activity in transformed lines by histochemical staining

<table>
<thead>
<tr>
<th>Genotype</th>
<th>FB</th>
<th>MG</th>
<th>MPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ry^SN</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>D-5000</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>ALE/P-386</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>ALE/P-242</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>ALE/P-187</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>ALE/P-110</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ALE/P-80</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Adh^M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Third instar larvae of the indicated genotypes were dissected, fixed, and stained for ADH enzyme activity as described (URSPRUNG, SOFER and BURROUGHS 1970; GOLDBERG, POSAKONY and MANIATIS 1983). Staining treatments were for 15–60 min. The number of + signs roughly represents the rapidity and intensity of the staining observed in different tissues. A − sign indicates that no staining above background was observed even with treatments of up to 60 min. The data represent the staining patterns observed in several (three or more) dissections of each transformed line of the indicated genotypes (see Table 2 for number of independent transformed lines). Larvae with the wild-type Adh^k gene (ry^SN larvae) show intense ADH activity in the fat body (FB), midgut (MG; this includes the gastric cecae, anterior and posterior midgut), and malpighian tubules (MPT). No staining is observed in the hindgut or proventriculus. Larvae from the recipient line, Adh^k cn; ry^M show no ADH activity even after staining for more than 60 min. Larvae transformed with D-5000 or ALE/P-386 show intense staining in the same tissues as ry^SN larvae, although malpighian tubule staining is more variable than for ry^SN larvae. Larvae transformed with ALE/P-242, ALE/P-187, ALE/P-110 or ALE/P-80 show no ADH activity in malpighian tubules even after staining incubations of over 1 hr, and reduced activity in fat body and midgut. The staining observed in the fat body and midgut of ALE/P-110 and ALE/P-80 larvae is generally more variable and splotchy than that seen in the lines with more active Adh genes (data not shown). The lack of staining in malpighian tubules probably reflects the limitations of the assay, rather than the loss of a malpighian tubule specific element because, even with the wild-type gene, histochemical staining in malpighian tubules is generally weaker and more variable than in other tissues (MARONI and STAMEY 1983; URSPRUNG, SOFER and BURROUGHS 1970; our unpublished results).
The 5' end of the larval enhancer lies between -5000 and -3550: The activity of the larval enhancer was abolished when sequences upstream of -3550 were deleted (Figure 5, lanes 10-12; Figure 5, compare D-3550 with D-660). Genes with fewer 5' sequences produced the same low levels of Adh transcripts (Figure 5, lanes 13-17; Figure 4). Thus, the sequences between -5000 and -3550 are essential for even minimal activity of the larval enhancer.

The combined data of the 5' and 3' deletion series indicate that the individual larval enhancer elements cannot stimulate Adh transcription independently. Neither element III nor the hypothetical element I is sufficient to stimulate transcription above basal levels (Figure 4). Element II has not been tested individually, but it is unlikely to be active, since elements I plus II do not augment Adh transcription (Figure 4). Only when elements II plus III are present do transcription levels rise above base line controls. Thus, both elements II and III, and possibly element I, must act synergistically to achieve maximal levels of Adh transcription.

**DISCUSSION**

The D. mulleri Adh-1 gene and the proximal promoter of the D. melanogaster Adh gene are expressed at similar times and in similar tissues during larval development. In this study we show that tissue-specific transcription from the proximal promoter of the melanogaster gene is controlled by a far upstream enhancer (the larval enhancer), and two or more elements near the transcription start site. The D. mulleri
**FIGURE 4.** Localization of larval enhancer activity. The *Adh* gene constructs used to map the larval enhancer were named according to their 5' endpoint or the endpoints of the internally deleted sequences. The 3' endpoint for all of the genes is 640 bp beyond the polyadenylation signal. The 5' endpoint for the internally deleted genes D-1845/-660, D-2395/-660, D-2830/-660 is 5000 bp upstream of the distal promoter start site. To the right of the diagram, is the number of lines examined and the mean transcription level ± the standard deviation (sD). The standard deviation was defined (SKOOG and WEST 1982). In the lower part of the figure, the limits of the three larval enhancer elements are shown. The existence of element I is purely hypothetical (as denoted by the question mark) because the differences observed between the two D-1845/-660 lines and the D-5000 lines are not statistically significant.

*Adh-1* gene is also regulated by a distant enhancer, the 3' element, and two elements near the transcription start site, box A (–91 to –60) and box B (–269 to –181; FISCHER and MANIATIS 1988). In both genes, each regulatory element stimulates *Adh* transcription in three different larval tissues. However, the regulatory elements of the *melanogaster* gene do not appear to be strictly analogous with those of the *mulleri* gene (see below).

**The Adh enhancers:** Both the *mulleri* and *melanogaster* genes are stimulated by distantly located enhancers, the 3' element and the larval enhancer, respectively. The larval enhancer is located at least 1300 bp upstream of the start site of the proximal *Adh* promoter (Figure 4), whereas the *mulleri* *Adh-1* 3' element is located downstream of the polyadenylation signal (FISCHER and MANIATIS 1988). The activity of the larval enhancer localizes to two, and possibly three, separate sub-elements dispersed over 1.7 kb to 4.4 kb (Figure 4). A family of repeated sequences is distributed throughout the larval enhancer region, not simply within elements I, II and III (Table 3), suggesting that the sequences between the sub-regions may also play a role in stimulating *Adh* transcription levels. Curiously, sequences related to these repeats are also found within the *D. melanogaster* adult enhancer (D. FALB and T. MANIATIS, unpublished results). Although no single enhancer subelement is sufficient to stimulate transcription, together they stimulate *Adh* transcription ~8-fold. This kind of enhancer organization is not unprecedented. For example, the SV40 enhancer and the Drosophila Sgs4 gene enhancer are also composed of multiple elements, which cannot stimulate transcription independently (HERR and GLUZMAN 1985; HERR and CLARKE 1986; ZENKE et al. 1986; JONGENS et al. 1988). Similar analyses have not been carried out for 3' element of the *mulleri Adh-1* gene.
The larval enhancer, and the *mulleri* 3′ element and box B are all tissue-specific enhancers. In their normal contexts, each of these enhancers stimulate transcription in three larval tissues. In isolation from their respective Adh promoters, however, their activities appear to differ. The larval enhancer stimulates transcription from the *hsp70* promoter predominantly in fat body, but also in malpighian tubules and midgut. In contrast, the 3′ element and Box B stimulate transcription of the same promoter exclusively in fat body (FISCHER and MANIATIS 1988). These differences support the suggestion that Adh function is necessary in
TABLE 3

Sequence repeats within the larval enhancer

<table>
<thead>
<tr>
<th>Sequence motif</th>
<th>Location of first nucleotide</th>
<th>Match</th>
</tr>
</thead>
<tbody>
<tr>
<td>TATGCAAATTAAGCCGAA</td>
<td>-566</td>
<td>N/A</td>
</tr>
<tr>
<td>TATACATTTT</td>
<td>-966</td>
<td>8/10</td>
</tr>
<tr>
<td>TATGCAAA</td>
<td>-1108</td>
<td>9/9</td>
</tr>
<tr>
<td>TATGCAATT</td>
<td>-1166</td>
<td>10/10</td>
</tr>
<tr>
<td>TAACAAATT</td>
<td>-1776</td>
<td>8/10</td>
</tr>
<tr>
<td>ATATAAATTA</td>
<td>-1898</td>
<td>8/10</td>
</tr>
<tr>
<td>TGCAAGTAA</td>
<td>-1955</td>
<td>9/10</td>
</tr>
<tr>
<td>GCCAATTAAGCTCAA</td>
<td>-2934</td>
<td>1/5</td>
</tr>
<tr>
<td>TATGCAATT</td>
<td>-2957</td>
<td>10/10</td>
</tr>
<tr>
<td>GCAAATTTA</td>
<td>-3916</td>
<td>8/9</td>
</tr>
<tr>
<td>TATGTAATTAA</td>
<td>-4098</td>
<td>11/12</td>
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<td>GCACCAACA</td>
<td>-553</td>
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<tr>
<td>GCAGTTAAGAA</td>
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<tr>
<td>AGCAACACA</td>
<td>-2541</td>
<td>8/8</td>
</tr>
<tr>
<td>CAGCAGCA</td>
<td>-2706</td>
<td>8/9</td>
</tr>
</tbody>
</table>

The larval enhancer contains two families of repeated motifs, as indicated. For each family of repeats, the first repeat shown is within the adult enhancer (D. FALK and T. MANIATIS, unpublished results), rather than the larval enhancer. The first basepair of each repeat is given relative to the distal promoter. N/A, not applicable.

Although both the larval enhancer and 3′ element are tissue-specific, low levels of transcription are observed in the appropriate tissues in the absence of these elements (FISCHER and MANIATIS 1988; Table 1). These observations suggest that the Adh promoter regions and the enhancers contain redundant tissue-specificity elements. In the melanogaster proximal promoter, tissue-specific transcription is apparently regulated by the elements located between -386 and -242 and between -187 and -80 of the proximal promoter (Figure 6). Similarly, box A and box B are sufficient for low levels of tissue-specific transcription from the mulleri gene (Figure 6; FISCHER and MANIATIS 1988). Several other Drosophila genes also contain enhancers that affect the level, rather than the pattern, of transcription, for example, the Sgs3, actin, and P3 genes (BOURROUS and RICHARDS 1985; GLANGRANDE, METTLING and RICHARDS 1987; MEYEROWITZ et al. 1987; GEYER and FRYBERG 1986; HIROMI, KUROIWA and GEHRING 1985; HIROMI and GEHRING 1987).

Elements near the Adh promoters: It is likely that at least some of the same trans-acting factors regulate both Adh-I and the melanogaster proximal promoter, since Adh-I is regulated in its normal pattern when introduced into the melanogaster genome. We therefore compared the DNA sequences within the regulatory elements of the mulleri and melanogaster Adh genes with each other and with known protein binding sites.

The -386 to -242 region of the melanogaster proximal promoter is required for wild-type levels of transcription. A 50-bp sequence from within this region is necessary for high levels of Adh expression in somatic transformation assays (SOFER and MARTIN 1987). This 50-bp sequence contains the binding site for a protein identified in nuclear extracts from Drosophila Kc cells (designated P3; U. HEBERLEIN and R. TJIAN, personal communication), and a similar sequence motif is found in the mulleri box B region, CCG(TG/AA)CAGAGC. Less extensive sequence similarities between box B and the melanogaster proximal promoter are detected downstream of -242. Although observations suggest...
that the melanogaster -386/ -242 region might be functionally equivalent to the mulleri box B, one or more copies of box B are sufficient to stimulate transcription from a heterologous promoter (FISCHER and MANIATIS 1988), whereas two copies of the melanogaster -386 to -214 region are not (V. CORBIN and T. MANIATIS, unpublished data). Possibly the melanogaster -386 to -214 region contains only a subset of the box B activities.

A second protein, P2, binds downstream of P3, between -270 and -230 (HEBERLEIN, ENGLAND and TJIAN 1985). However, its binding site is not conserved between the mulleri and melanogaster genes, or among different melanogaster strains. Moreover, the P2 binding site can be deleted without effecting expression from the proximal promoter in somatic transformation assays (SOFER and MARTIN 1987). From these data we argue that the P2 site is not critical for Adh expression in larvae, and that the drop in transcription observed when the -386 to -242 sequence was deleted was caused by the deletion of sequences other than the P2 binding site.

The region between -187 and -80 of the melanogaster proximal promoter is also necessary for correct levels of Adh transcription. This region contains several small regions of homology to sequences in box A and box B of the mulleri gene. The most dramatic of these homologies, a 17/24-bp match, appears between -133 to -110 of the melanogaster gene and box A of the mulleri gene. This region contains a binding site for the nuclear factor, Adf-1 (-150 to -102; HEBERLEIN, ENGLAND and TJIAN 1985). However, the Adf-1 binding site itself (-150 to -104) is not important for transcription from the proximal promoter in in vitro transcription systems or in the somatic transformation assays (HEBERLEIN, ENGLAND and TJIAN 1985; SOFER and MARTIN 1987). Similarly, in our experiments we found that deletion of sequences between -187 and -110 did not, on average, reduce Adh transcription levels.

The region between -110 and -80 also contains a small region of homology with mulleri box B, TCGCGTA. This conserved region overlaps the binding site for another nuclear factor, P0 (-98 to -76). The region corresponding to the P0 binding site is important for high levels of Adh transcription in the somatic transformation assay (SOFER and MARTIN 1987), in germline transformants (this paper), and in an in vitro transcription system (HEBERLEIN, ENGLAND and TJIAN 1985). Taken together, these observations suggest that P0 and P3 may be two of the elements necessary for proper regulation of the proximal promoter. However, Kc cells do not appear to express their endogenous Adh genes, and express transfected Adh genes only at very low levels (SOFER and MARTIN 1987; BENYAJATI and DRAY 1984). Thus, the P0 and P3 sites may be important, but they are not sufficient for high levels of Adh expression. Finally, the conservation of short, but not long, stretches of DNA sequence in the regulatory regions of the mulleri Adh-1 gene and the melanogaster Adh proximal promoter suggests that, although the same transcription factors may regulate Adh transcription in both species, the precise arrangement of these factors may not be critical.

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LITERATURE CITED


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