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Independent control elements that determine yolk protein gene expression in alternative *Drosophila* tissues

(germ-line transformation/sex-, stage-, and tissue-specific gene expression/*cis*-acting elements)

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ABSTRACT **The adjacent and divergently transcribed *yp1* and *yp2* genes of *Drosophila* were separated at a site that is 342 base pairs upstream of *yp2* and 883 base pairs upstream of *yp1*. Each gene was separately used to transform *Drosophila* germ-line-producing flies with a single copy of the introduced gene. Transcripts from the introduced genes were found only in adult females. Thus, the introduced genes maintain the sex- and time-specific expression pattern of the endogenous *yp* genes. However, the pattern of tissue-specific expression differed among the two introduced genes and the endogenous genes. Transcripts from both of the endogenous genes are found in fat bodies and ovaries. In contrast, transcripts from the introduced *yp1* gene are found only in fat bodies, and those from the introduced *yp2* gene are found only in ovaries. Thus, each introduced DNA segment lacks at least one of the *cis*-acting elements required for the normal pattern of tissue-specific expression. These results indicate that the expression of a *yp* gene in different tissues is determined by different *cis*-acting elements.**

In *Drosophila melanogaster* each of the three egg yolk proteins is encoded by a different single-copy gene (1, 2). Transcripts of all three genes (*yp1*, *yp2*, and *yp3*) are abundant and occur with the same tissue-, time-, and sex-specific pattern. These transcripts are found only in adult females, where they are detected only in the ovarian follicle cells and the fat bodies of the head, thorax, and abdomen (refs. 2–4 and this paper). In addition to this tissue-, time-, and sex-specific control, there is either direct or indirect hormonal control of these genes (5, 6).

This paper describes an investigation of *cis*-acting control elements that determine the transcription pattern of two yolk protein genes, *yp1* and *yp2*. These two genes are divergently transcribed, and their 5′ ends are 1225 nucleotides apart (7–11). To locate the *cis*-acting elements and investigate their effects on transcription, we have introduced segments of the *yp1–yp2* gene region into the germ line of *Drosophila* by the *P* element-mediated transformation method (12). The sex-, time-, and tissue-specific occurrence of transcripts from the introduced genes indicates that there are two tissue-specifying elements acting on each gene. One is necessary for gene expression in fat bodies, and one, for expression in ovaries.

MATERIALS AND METHODS

Plasmid Constructions. The vector plasmid pRPL1 for *Drosophila* transformation was derived from pARP-23 (a gift from J. Posakony) by deleting an *Xba* I restriction fragment containing the *Drosophila* alcohol dehydrogenase gene (13). A 676-base-pair (bp) *Bgl* II/*Xmn* I restriction fragment from gene II of phage M13mp8 (14, 15) was inserted into the *yp1* and *yp2* genes. Plasmid pYP1-$M13_{676}$ was generated by inserting this M13 fragment into the unique (7) *Xho* I site of pYP1 by filling in the restriction enzyme-generated single-stranded ends (16) with avian myeloblastosis virus reverse transcriptase (Life Sciences) and then ligating the blunt ends. An identical M13 DNA fragment was inserted into *yp2* between the unique (8) *Stu* I and *Bgl* II sites of *yp2*, forming pYP2-$M13_{676}$. In each plasmid, the M13 fragment is in the same orientation relative to the direction of *yp* gene transcription.

The plasmid pRPL-YP1-$M13_{676}$ was formed (Fig. 1) by inserting a *Hin*dIII restriction fragment containing *yp1* and M13 DNA (from the plasmid pYP1-$M13_{676}$) into the *Xba* I site of pRPL1 by the partial-filling method of Hung and Wensink (17). The plasmid pRPL-YP2-$M13_{676}$ was formed by inserting a *Hin*dIII fragment containing the *yp2* and M13 DNA (from pYP2-$M13_{676}$) into the *Xba* I site of pRPL by the partial-filling method.

***Drosophila* Germ-Line Transformation.** Transformation experiments were carried out essentially according to Spradling and Rubin (12). Embryos from the ry^- host $ry^{\Delta 506}$ (a gift from W. Bender) were injected with 5 mM KCl/0.1 M sodium phosphate, pH 6.8, containing the intact helper *P* element clone, $p\pi 25.1$ (12) at 50 μg/ml and pRPL-YP1-$M13_{676}$ or pRPL-YP2-$M13_{676}$ at 300 μg/ml. Adults derived from injected embryos (the G_0 generation) were mated to $ry^{\Delta 506}$ partners and phenotypically wild-type (ry^+) progeny in the G_1 generation were selected. The ry^+ G_1 flies were mated individually to $ry^{\Delta 506}$ partners to produce the G_2 generation.

Nucleic Acid Manipulations. Total and poly(A)$^+$ RNA as well as DNA were prepared (1) from adult (3- to 5-day-old) flies heterozygous for the introduced gene. Restriction endonuclease (Boehringer Mannheim) digestions and T4 DNA ligase (New England Biolabs) reactions were performed as suggested by the supplier. Methods for DNA and RNA electrophoresis, blotting, hybridization, and autoradiography have been described (18). DNAs specifically complementary to *yp1* or *yp2* transcripts [pYP1-5′ or pYP2-5′ (2, 10)] and to the *Drosophila* α1 tubulin gene [pDmTα1-3′ (18)] were subcloned in M13mp8 and -mp9 (15), and single-stranded radiolabeled probes were prepared from the subclones (19). 3′-End labeling and nuclease S1-protection experiments were done as described (10).

RESULTS

Transformation of the *Drosophila* Germ Line with *yp1* and *yp2*. The adjacent *yp1* and *yp2* genes were separated by restriction enzyme digestion and then inserted into a vector DNA that facilitates the transformation experiments. These constructions generated two circular DNAs (Fig. 1): one

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Abbreviations: bp, base pair(s); kb, kilobase(s); *yp1*, yolk protein 1 gene; *yp2*, yolk protein 2 gene.
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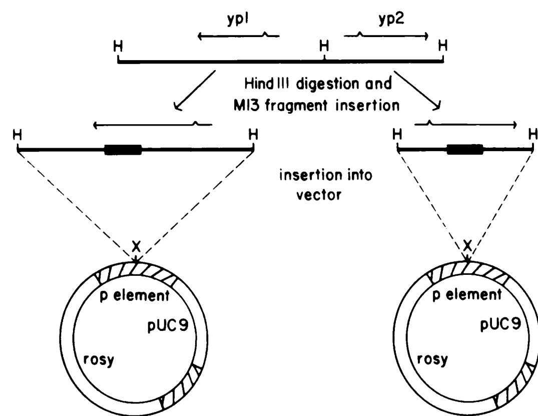

FIG. 1. Plasmid construction. The upper portion of this diagram represents cloned genomic DNA containing the *ypl* and *yp2* genes. The horizontal arrows indicate the transcribed portions of the genes and the direction of transcription. The caret symbols (Λ) on the arrows show intron locations. The separated *Hin*dIII (H) restriction fragments [4.8 and 2.8 kilobases (kb)] contain identical M13 DNA (thick portion of the line) inserted into a *Xho* I site in *ypl* or substituted for a 393-bp *Stu* I/*Bgl* II segment of *yp2*. These constructs were inserted into the *Xba* I (×) site of the transformation vector.

(ypl-M13) containing *ypl* and 883 bp of its upstream DNA and the other (yp2-M13) containing *yp2* and 342 bp of its upstream DNA. Together the two constructs contain all of the DNA between these divergently transcribed genes. As shown in Fig. 1, a DNA segment from bacteriophage M13 was inserted into these *yp* gene constructions to distinguish their transcripts from those of the endogenous *yp* genes. The vector DNA contains portions of the transposable *P* element of *Drosophila* (20) that permit transformation of the *Drosophila* germ line (12). It also contains the xanthine dehydrogenase (rosy) gene which serves as an eye color marker of successful transformation (21).

This paper describes progeny of one *yp2* transformant and three *ypl* transformants. The three *ypl*-transformed lines were selected on the basis of their fertility pattern and the chromosomal location of their introduced gene. One of the eight *ypl*-transformed lines obtained is female-sterile. We selected it as well as two female-fertile lines. One of the selected fertile lines has the *ypl-M13* gene on the X chromosome. The other two selected lines have this gene on autosomes. Since different orientations of *ypl* relative to the *P* element and rosy gene made no observable difference in the expression of the introduced gene, we will not distinguish between them.

Each Transformant Has a Single Copy of the Introduced *yp* Gene. To determine the number of gene copies that were stably integrated into the genome, Southern blots of restriction enzyme-digested genomic DNA were hybridized to radiolabeled *yp* genes. *Hin*dIII does not cut within the *yp2* gene. When this enzyme digests the nontransformed genome, it generates only one restriction fragment containing this single-copy gene (Fig. 2). In the transformed genome, the same enzyme generates two fragments that hybridize to the gene (Fig. 2). Since one of these has the same electrophoretic mobility as the endogenous gene, we conclude that the endogenous gene is undisturbed in the transformant. Since the two autoradiographic bands in the transformed genome are of very similar intensity, we conclude that there is a single copy of the *yp2-M13* gene. This conclusion is supported by the observation (Fig. 2) that the *Hin*dIII and the *Sac* I sites have only one arrangement in the vicinity of the introduced gene. As shown in this figure, there is a single

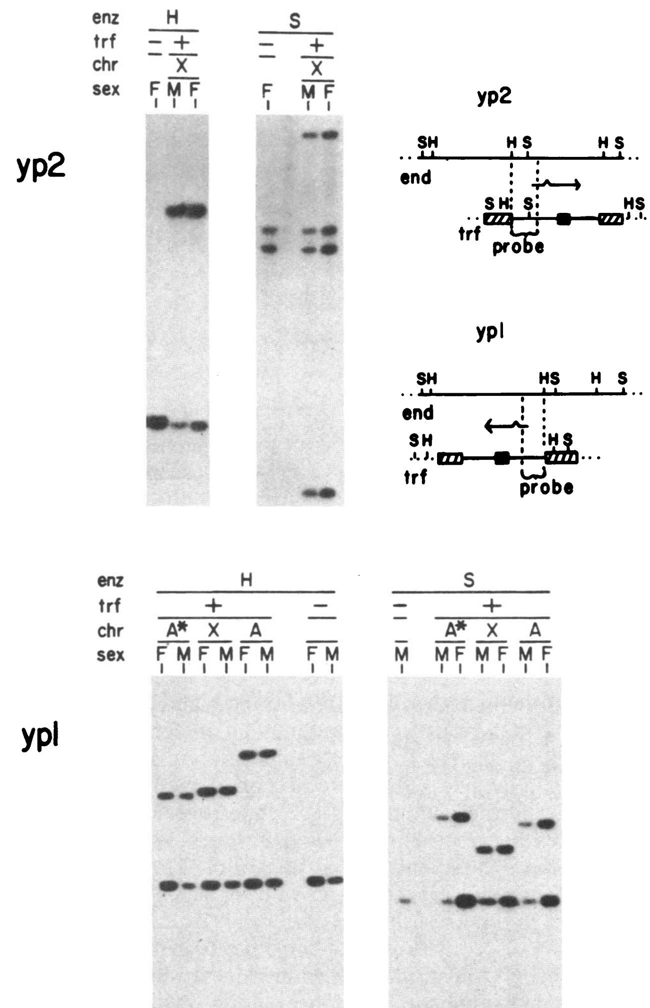

FIG. 2. Genomic copy number of the introduced genes. Genomic DNA from transformed (trf+) or nontransformed (trf−) adult males (M) or females (F) was digested with either restriction enzyme (enz) *Hin*dIII (H) or *Sac* I (S), electrophoresed on agarose gels, and blotted onto nitrocellulose paper. The transformed flies are heterozygous for the introduced gene. The *yp2* and *ypl* blots were hybridized to ^{32}P-labeled regions of *yp2* (pYP2-5′) and *ypl* (pYP1-5′), respectively, and then were autoradiographed. The introduced DNA is on either the X chromosome (chr X) or an autosome (A). In one case (*) the transformed line is female-sterile. The *Hin*dIII restriction fragments containing the endogenous *yp2* and *ypl* genes are 2.4 and 4.1 kb, respectively. The diagrams (not drawn to scale) show the arrangement of *Hin*dIII and *Sac* I restriction sites in and near the endogenous (end) and transforming (trf) *yp* genes. All symbols are as in Fig. 1.

copy of the *yp2-M13* gene in both the male and the female genome.

Results from similar experiments with *ypl* transformants (Fig. 2) indicate that they have single-copy inserts in both males and females. Another observation strengthens this conclusion. The endogenous *ypl* gene is on the X chromosome (1, 22). If the introduced gene were on an autosome, then in heterozygous males its copy number should equal that of the endogenous gene and in females it should be half that of the endogenous gene. Densitometry of the autoradiographs in this figure gave results that are in accord with this prediction (data not shown). In addition, these restriction enzyme digests indicate that the site of *ypl* insertion was different in each transformant. We conclude that each *ypl* transformant has a single copy of the gene inserted at a different chromosomal site.

Transcripts from the Introduced Genes Are Found in Fe-

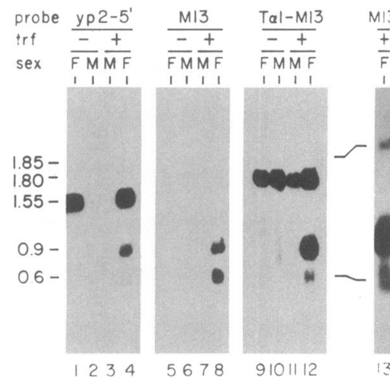

FIG. 3. Transcripts from the *yp2-M13* gene. Poly(A)$^+$ RNA (0.1 μg) from *yp2-M13*-transformed (trf+) or nontransformed (trf−) male (M) or female (F) adults were electrophoresed on formaldehyde/agarose gels and blotted onto nitrocellulose paper. Shown are three autoradiographs of one blot that had been hybridized to radiolabeled pYP2-5′ DNA (lanes 1–4), M13 DNA (lanes 5–8), or pDmTα1-M13 DNA (lanes 9–12). Between autoradiography and the next hybridization, radiolabeled DNA was eluted from the blot. Lane 13: an autoradiograph of another blot, exposed for a longer time.

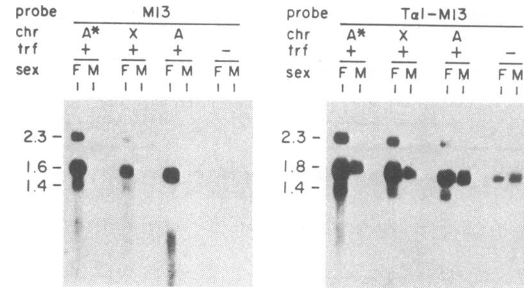

FIG. 4. Transcripts from the *yp1-M13* gene. Poly(A)$^+$ RNA (0.1 μg) from *yp1-M13*-transformed (trf+) or nontransformed (trf−) male (M) or female (F) adults was electrophoresed on formaldehyde/agarose gels and blotted onto nitrocellulose paper. Shown are two autoradiographs of one blot hybridized to either radiolabeled M13 or radiolabeled pDmTα1-M13 DNA. Between autoradiography and the next hybridization, radiolabeled DNA was eluted from the blot. All symbols are as in Figs. 2 and 3.

males but Not in Males. The RNAs transcribed from *yp2-M13* and the endogenous *yp2* are abundant in females but undetectable in males. In an RNA blot-hybridization experiment, radiolabeled DNA probe complementary to both *yp2-M13* and the endogenous *yp2* gene revealed transcripts in females but not in males (Fig. 3, lanes 1–4). Wild-type females have the expected *yp2* transcript. The transformed females have this transcript as well as at least two others which are products of the *yp2-M13* gene (lanes 4 and 8). The multiple transcripts from the introduced gene are due to processing or transcription termination at sites within the inserted M13 sequence. This observation was also made for transcripts of the *yp1-M13* gene (see below). To demonstrate that the results shown in Fig. 3 reveal female specificity of the transcripts rather than an absence of RNA in the male RNA preparations, the *yp2* probe was removed from the blot and the blot was then hybridized to a probe containing both the M13 sequence and the α1 tubulin gene of *Drosophila* (23). Transcripts from the latter gene are present at approximately equal concentrations in males and females (18) and thus serve as an internal standard for the amount of RNA present in each lane. An autoradiograph of this blot (Fig. 3, lanes 9–12) confirms the female-specific occurrence of transcripts from *yp2-M13*.

Similar experimental results showed that the *yp1* transformants had multiple *yp1-M13* transcripts that were detected only in females. Blot profiles of electrophoretically separated RNA from the *yp1* transformants (Fig. 4) show that M13-complementary RNAs are present only in females.

We conclude that both of the introduced genes, *yp1-M13* and *yp2-M13*, are transcribed. Furthermore, their transcripts, like those of the endogenous *yp* genes, are found only in females.

Most of the Transcripts from the Introduced Genes Terminate Within the M13 Sequence. The *yp2-M13* DNA includes an M13 segment that should increase the *yp2* transcript size from 1.55 to 1.85 kb (see Fig. 1). Thus its transcript should differ from the endogenous transcript in both length and complementarity to M13 DNA. Electrophoretically fractionated poly(A)$^+$ RNA from the *yp2* transformant had not one but at least two RNAs complementary to M13 DNA (Fig. 3, lane 8). Since poly(A)$^+$ RNA from nontransformed flies does not contain detectable complements to M13 DNA (lane 5), we conclude that these transcripts are from the *yp2-M13* gene. However, none of them is the length predicted, 1.85 kb. A much longer autoradiographic exposure from a similar experiment reveals that the predicted RNA is present, but at low abundance (lane 13).

Nuclease S1-protection experiments indicate that the short transcripts from the *yp2-M13* gene are due to either premature termination or cleavage at sites within the M13 sequence. This conclusion is diagrammed in Fig. 5. The results from these protection experiments show that the most abundant transcript from *yp2-M13* (labeled c in Fig. 5) has a 3′ terminus that maps to a site within the M13 DNA. The distance between this site and the normal 5′ end of *yp2* transcripts corresponds to the length (0.9 kb) of the most abundant transcript observed in blots of *yp2-M13* transcripts (Fig. 3). These experiments reveal other less abundant transcripts, including one (d in Fig. 5) whose 3′ end corresponds to the 3′ end of the *yp2-M13* gene and a cluster of several (b in Fig. 5) which correspond to the second-most abundant transcript (0.6 kb) observed in the blot. The 3′ termini of these short transcripts map approximately 25 bp upstream of M13 sequences that are either identical or similar to the eukaryotic RNA 3′-terminal-cleavage consensus sequence A-A-T-A-A-A (24, 25).

Nuclease S1/exonuclease VII-mapping of heteroduplexes between the *yp1-M13* construct and RNA from *yp1* transformants lead to a similar conclusion. The 5′ ends of *yp1-M13* RNA map to the same site as do transcripts from the endogenous *yp1* gene (data not shown). The 3′ ends map to the same M13 sites (Fig. 5, lanes 7–9) as did transcripts from *yp2-M13*.

Transcripts from the Introduced Genes Occur Only at the Developmental Time Expected for *yp* Genes. Endogenous *yp* gene transcripts occur in adult females but have not been detected at any other time in development. Total RNA from transformants at various developmental stages was tested for the presence of transcripts from the introduced *yp* genes. A blot-transfer of electrophoretically separated RNAs from the *yp2* transformant was hybridized to a probe containing both *yp2* and M13 sequences. An autoradiograph of this blot shows that transcripts from the endogenous *yp2* and the *yp2-M13* genes were detected only in adults (Fig. 6). A similar experiment in which an M13 probe was hybridized to blots containing RNAs from various developmental stages of the *yp1* transformants (Fig. 6) led to the same conclusion. Autoradiographs of both RNA blots after rehybridization to a probe containing both the α1 tubulin gene and M13 indicate that RNA was present in all RNA preparations (Fig. 6). Thus, the introduced genes are transcribed at the developmental times expected.

Transcripts from the *yp2-M13* Gene Are Detected Only in Ovaries, and Transcripts from the *yp1-M13* Gene Are De-

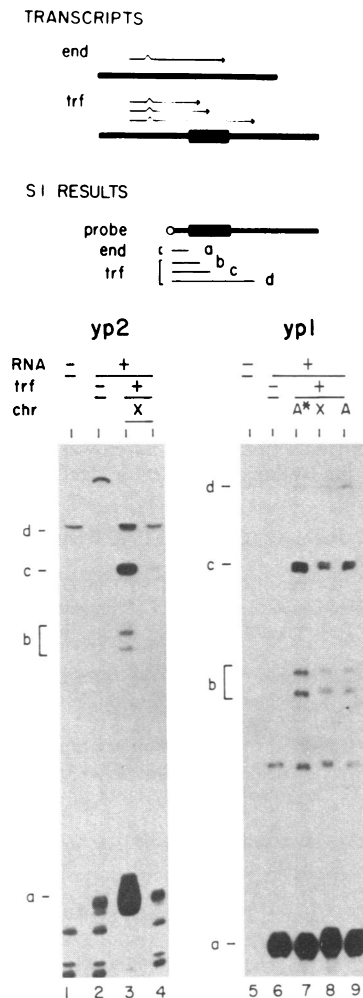

FIG. 5. RNA complementarity to the 3′ regions of *yp2-M13* and *yp1-M13* genes. (*Upper*) Transcript maps of the endogenous *yp2* and *yp1* genes and our interpretation of the nuclease S1-protection results. The 3′-end radiolabel of the probe DNA is represented by an open circle. The nuclease S1-resistant DNA fragments from the endogenous (end) and transforming (trf) genes are indicated by thin lines. (*Lower*) Autoradiographs of the electrophoresed products from nuclease S1-protection experiments. For *yp2* transcript mapping a 1.8-kb *Bst*EII/*Hin*dIII fragment from pYP2-M13$_{676}$ was 3′ labeled at the *Bst*EII end. For *yp1* transcript mapping, a 2.8-kb *Eco*RI/*Hin*dIII fragment from pYP1-M13$_{676}$ was 3′ labeled at the *Eco*RI end. The labeled DNA was denatured and hybridized without RNA (lanes 1 and 5), with RNA from nontransformed ry$^{\Delta 506}$ flies (lanes 2 and 6), or with RNA from flies transformed with *yp2-M13* (lanes 3 and 4) or *yp1-M13* (lanes 7–9). The hybridization reaction for lane 3 had 10-fold more RNA than reactions for lanes 2 and 4. Lower case letters mark fragments protected by the endogenous (a) and transformed (b–d) gene transcripts. Other symbols are as in Figs. 1 and 3.

tected Only in Fat Bodies. Transcripts from endogenous *yp* genes occur in two tissues, namely, fat bodies and ovaries. To determine the tissue specificity of transcripts from the introduced genes, we extracted RNA from hand-dissected female tissues and analyzed the various total RNA preparations by blot–hybridization. Transcripts from the endogenous *yp2* gene were present in the three tissue samples tested (Fig. 7): ovaries, abdominal fat bodies, and heads (which contain fat-body cells; see ref. 26). In the same samples, transcripts from the *yp2-M13* gene were present only in ovarian RNA (Fig. 7). In a similar experiment, we examined the tissue specificity of transcripts from the *yp1* transformants (Fig. 7). In each *yp1* transformant, transcripts from *yp1-M13* were detected in abdominal fat bodies and in the

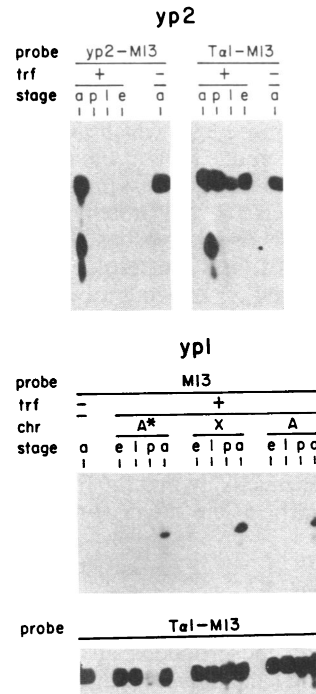

FIG. 6. Developmental pattern of transcripts from the introduced genes. Total RNA (5-μg sample) from embryos (e), larvae (l), pupae (p), or adults (a) was electrophoresed and blotted onto nitrocellulose paper. (*Upper*) Autoradiographs of a blot hybridized to radiolabeled pYP2-5′-M13 or pDmTα1-M13. (*Lower*) Autoradiographs of a blot hybridized to radiolabeled M13 DNA and then to pDmTα1-M13. Between autoradiography and the next hybridization, radiolabeled DNA was eluted from the blot. All symbols are as in Figs. 2 and 3.

head and thorax, two body parts that contain fat body cells. Transcripts from the *yp1-M13* gene were not detected in ovaries of the *yp1* transformants (Fig. 7).

These results with *yp1* and *yp2* transformants lead us to conclude that the *yp1* and *yp2* constructs are each missing DNA sequences that are necessary for transcripts to occur in one of the two tissues in which the endogenous genes are normally expressed. Since the patterns of expression are different, we conclude that a different tissue-specific expression element is missing from each construct. Furthermore, the sequences present in each construct are sufficient to produce approximately normal transcript concentrations in expressing tissues. This was indicated by densitometry of autoradiographs of the *yp2* ovarian RNA lane (Fig. 7) and of a nuclease S1-protection experiment that used thoracic RNA from *yp1* transformants. In tissues that express the introduced genes, transcripts from the heterozygous *yp2-M13* and

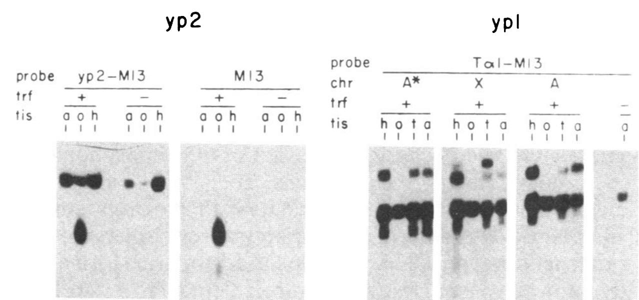

FIG. 7. Tissue specificity of RNAs from introduced genes. Total RNA (5 μg) from the ovary (o), head (h), thorax (t), or fat body of the abdomen (a) was electrophoresed and blotted onto nitrocellulose paper. (*Left*) Blot with RNA from the *yp2*-transformed flies was hybridized to radiolabeled pYP2-5′-M13 and then to M13 DNA. Between autoradiography and the next hybridization, radiolabeled DNA was eluted from the blot. (*Right*) Blot with RNA from *yp1*-transformed flies was hybridized to radiolabeled pDmTα1-M13. In this autoradiograph, the signal from nontransformed flies (trf–) is due to α1 tubulin RNA. We draw this conclusion because the same blot hybridized to radiolabeled M13 DNA gave no signal in this lane. An autoradiographic signal from this α1 tubulin transcript is present in all lanes of the blot. Transformed flies are heterozygous for the introduced gene. All symbols are as in Figs. 2 and 3.

yp1-M13 genes are present at 50% and 40%, respectively, of the levels of transcripts from the corresponding homozygous endogenous genes.

One Transformed Line Is Female-Sterile. The female-sterile *yp1-M13* line produces collapsed eggs which do not develop into adults. This sterility could be due either to insertion of the *yp1-M13* DNA into a region essential for fertility or to production of an aberrant yolk protein. The latter possibility seems unlikely for several reasons. First, seven of eight *yp1-M13*-transformed lines are fertile. Second, RNA blots (Figs. 6 and 7) and S1-protection results (Fig. 5) indicate that fertile and sterile lines have similar developmental and tissue-expression patterns for *yp1-M13* and no more than a 2-fold difference in transcript abundance. Furthermore, the yolk proteins from eggs of sterile and fertile flies are indistinguishable in abundance and electrophoretic mobility as determined by $NaDodSO_4$/10% PAGE (data not shown). Therefore, it seems likely that the female sterility is due to alteration of a gene at the site of *yp1-M13* insertion.

DISCUSSION

Germ-line transformation experiments have shown that several cloned genes are flanked by the *cis*-acting elements necessary for normal developmental and tissue-specific control (27–30). There is little information on the characteristics of such *cis*-acting elements. Elements controlling immunoglobulin genes are perhaps the best characterized. Experiments with cultured cells have shown that when particular elements are placed near the immunoglobulin genes, the elements dramatically increase transcript concentration (31–34). This effect appears to be tissue-specific because it occurs when the element-gene structure is introduced into cells derived from tissue that normally expresses immunoglobulin genes but not when the structure is placed in cells derived from tissue that does not express these genes.

The results described in this paper provide information about the *cis*-acting elements that control the tissue-specific pattern of yolk protein gene expression. The *yp* genes are transcribed in two different female tissues, the fat bodies and the ovaries. We find that *yp1* DNA segment used to transform the germ line of *Drosophila* contains the sequences necessary to produce near normal concentrations of *yp1* transcripts in female fat bodies but not all of the sequences necessary to produce detectable transcripts in the ovary. We conclude that this change from the normal expression pattern is due to the sequence of the introduced DNA and not to destruction of a *trans*-acting gene at the site of insertion. We draw this conclusion because the same *yp1*-containing segment inserted into different chromosomal sites gives the same results and because expression of the endogenous *yp1* gene is unaffected by the insertions. The simplest interpretation of the *yp1*-transformation results is that a DNA element that acts in *cis* to determine ovarian transcription is missing or damaged in the DNA segment used for transformation. We infer that two distinct control elements, a fat body element and an ovarian element, together determine the tissue specificity of *yp1* transcription. Results with *yp2-M13* lead to a similar inference, that for *yp2* there are two elements, one for fat body expression and one for ovarian expression. Thus, for each gene there are at least two *cis*-acting elements determining tissue-specific expression. Moreover, for each gene, each of the two elements identified determines expression in a different tissue.

These results indicate that different *cis*-acting elements can cause the same gene to be expressed in different tissues. Such elements have been predicted by models for the developmental and tissue-specific control of transcription. Results presented in this paper indicate that such elements exist. The developmental control model of Britten and Davidson (35) describes how these elements could allow a simple mechanism to coordinate the expression of many genes that are transcribed in different combinations in different tissues.

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