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A Small Region Surrounding the Distal Promoter of the *hunchback* Gene Directs Maternal Expression

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While maternally provided factors play essential roles in the earliest processes of embryogenesis, little is known about the control of female germline-specific gene expression. Primer extension analysis and *in situ* hybridization experiments demonstrate that in adult *Drosophila* females, transcripts of the gap gene *hunchback* (*hb*) are produced only by the distal (P1) promoter and that this expression is largely restricted to the ovarian nurse cells. A deletion analysis of the *hb* promoter using *lacZ* reporter constructs defines a 1.2-kb genomic DNA fragment surrounding the P1 promoter that is sufficient to reproduce the wild-type pattern of *hb* ovarian transcript accumulation. By contrast, the subfragments of this region we have tested fail to direct specific ovarian expression.

INTRODUCTION

Despite the importance of maternally contributed mRNAs and proteins for the early development of the embryos of many metazoan organisms, relatively little is known about the control of maternal gene expression (Davidson, 1986). In insects that undergo meroistic oogenesis, such as *Drosophila*, the cellular machinery of the oocyte and many of the maternal factors controlling the earliest events of embryonic pattern formation are produced by the germ line-derived nurse cells. By analyzing specific genes, it should be possible to address the question of whether a single or multiple mechanisms are responsible for the nurse cell transcription of various maternally contributed regulatory factors. Additionally, the definition of a relatively small DNA fragment capable of directing specific gene expression in nurse cells would offer a useful experimental tool for studies of oogenesis and early embryogenesis.

Toward these ends, we have studied the *cis* regulation of ovarian expression of the segmentation gene *hunchback* (*hb*), a member of the gap gene class. The gap genes constitute the first zygotic level of the genetic hierarchy controlling segmentation in the *Drosophila* embryo, and their expression responds directly to maternal positional information (see Hülskamp and Tautz, 1991, for review). *hb* is unique within this group in exhibiting both maternal and zygotic expression. The *hb* gene has two promoters, each generating transcripts with a distinct first exon that splices to a common second exon containing the protein coding region (see Fig. 3; Tautz *et al.*, 1987; Bender *et al.*, 1988; our unpublished results). The distal (P1) promoter is responsible for all maternal expression (Tautz *et al.*, 1987; Bender *et al.*, 1988; this paper). Maternal *hb* mRNA is not translated in the ovary (Tautz and Pfeifle, 1989), and in the early embryo its translation is subject to inhibition by the posterior determinant *nanos* (*nos*). In the absence of *nos* activity, hb protein translated from maternal transcripts acts to repress expression of two other gap genes, *knirps* and *giant*, in the posterior half of the embryo, leading to a loss of abdominal segmentation (reviewed by St. Johnston and Nüsslein-Volhard, 1992). Thus, the negative regulation of *hb* maternal mRNA function is critical to establishing a normal anteroposterior body plan in the *Drosophila* embryo. However, *hb* maternal expression itself is apparently dispensable under laboratory conditions, since embryos lacking any maternal *hb* contribution can be fully rescued by one copy of the *hb* gene provided paternally (Lehmann and Nüsslein-Volhard, 1987). Nevertheless, the phenotype of *hb* null embryos is more severely mutant if they are derived from a *hb* mutant female germline (Lehmann and Nüsslein-Volhard, 1987). In addition, maternal *hb* expression is conserved in the housefly *Musca*, estimated to have diverged from *Drosophila* more than 100 million years ago (Sommer and Tautz, 1991), suggesting that this expression may serve some as yet undetected function.

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In this paper we demonstrate that *hb* transcripts accumulate to high levels in the nurse cells of the adult ovary, analyze in detail the normal spatial pattern of *hb* ovarian expression by *in situ* hybridization, and define a minimal genomic DNA fragment of 1.2 kb that is sufficient to confer this pattern of expression on a reporter gene.

MATERIALS AND METHODS

General Molecular Biology Methods

General molecular biological techniques were as described in Ausubel *et al.* (1987) and Sambrook *et al.* (1989).

Primer Extension Assays

Primer extension reactions (Ausubel *et al.*, 1987) contained 50 μg of total RNA and a mixture of two ^{32}P end-labeled oligonucleotides (Operon Technologies), each complementary to one of the *hb* promoter-specific first exons. Distal promoter probe: 5′-GCTGCCTGTCAATGCTGGCGACTTTC-3′. Proximal promoter probe: 5′-CGTAAGGATTTGCAAGTATATTATTGGTGC-3′. The general integrity of the RNA preparations was evaluated by ethidium bromide staining following agarose gel electrophoresis.

β-Galactosidase Activity Staining

All operations were carried out at room temperature. Ovaries were dissected in PBTr [1× PBS (phosphate-buffered saline), 0.1% Triton X-100] from mated, well-fed flies 3–4 days after eclosion and fixed in a 1:1 mixture of heptane and 0.5% glutaraldehyde in 1× PBS for 10 min. The tissue was then washed extensively in PBTr, dissected into ovarioles, and incubated for 30–60 min in Fe/NaP buffer (Ashburner, 1989) containing 0.2% X-gal. After staining, tissue was washed in PBTr, dehydrated through graded ethanol washes and two acetone washes, and then transferred to Epon to make permanent mounts. Ovaries were further dissected after transfer to the microscope slide.

In Situ Hybridization

In situ hybridizations were performed with the Boehringer-Mannheim Genius system (Tautz and Pfeifle, 1989) as adapted for ovaries by Suter and Steward (1991), with the following additional changes: Proteinase K concentration was reduced to 5 μg/ml, anti-sense RNA was used as the probe, and the hybridization and washing steps were carried out at 55°C. A full-length cDNA clone (J3) of a *hb* transcript deriving from the distal (P1) promoter was used as the template for synthesis of a *hb* antisense RNA probe. The J3 clone was isolated in our laboratory from a cDNA library representing poly(A)$^+$ RNA of 2–4-hr embryos (Brown and Kafatos, 1988). The *hb* riboprobe was transcribed from the T7 promoter of *Hin*dIII-linearized J3 plasmid DNA. The *lacZ* antisense RNA probe was transcribed from the T3 promoter of plasmid pBluescript II KS + *lacZ* (J. Treisman and C. Desplan, personal communication) linearized with *Pst*I. Following development of the phosphatase reaction, tissue was dehydrated and mounted as described above.

Plasmid Constructions

The P element transformation vectors used in this study were constructed as follows (maps and further details are available upon request):

CaSpeRlacZ. The CaSpeRlacZ vector permits analysis of the expression pattern of promoter-containing DNA fragments (see Van Doren *et al.*, 1992). A similar vector has been described by Thummel *et al.* (1988). The *lacZ* reporter gene (including a translation leader from the *Drosophila Adh* gene) was derived from pC4AUGβgal (Thummel *et al.*, 1988) as follows. First, the *Xba*I site of pC4AUGβgal was ablated by cleaving with *Xba*I, blunting the termini, and religating. Then, a *Bam*HI linker (New England Biolabs No. 1021) was inserted in the *Sma*I site and the *Bam*HI–*Eco*RI fragment containing the *Adh* leader and *lacZ* gene was cloned into the polylinker of the CaSpeR P element vector (Pirrotta, 1988).

HZCaSpeR. The HZCaSpeR vector is useful in assaying the capacity of *cis*-regulatory DNA fragments to confer expression on a heterologous promoter. The 3.8-kb *Xba*I–*Eco*RI fragment comprising the *Hsp*70 basal promoter/*lacZ* gene fusion from HZ50PL (Hiromi and Gehring, 1987) was cloned into CaSpeR (Pirrotta, 1988).

The fusion gene constructions described below are diagrammed in Fig. 3:

Lac 12, Lac 8.0, Lac 6.6, Lac 5.3. The 12-, 8.0-, 6.6-, and 5.3-kb *hb* promoter fragments (all of which include both the P1 and the P2 promoters) were all cloned into the *Xba*I site of CaSpeRlacZ (see above) using at the 5′ termini a genomic *Xba*I site (8.0) or an *Xba*I linker (12, 6.6, 5.3; New England Biolabs No. 1081) and at the common 3′ terminus a genomic *Xba*I site in the second exon of *hb*. This latter site lies 4 nt downstream of the splice acceptor, but 10 nt upstream of the presumptive start of translation. The resulting constructs should produce transcripts that initiate at the appropriate promoter, contain the promoter-specific untranslated leader exon, splice into the shared second exon, and continue directly into the *Adh* translation leader and *lacZ* reporter gene sequence of CaSpeRlacZ.

Lac Δ1.6. An internal 1.6-kb *Sal*I fragment was deleted from the intron sequence of Lac 5.3.

Lac Δ2.3. An internal 2.3-kb *Sal*I to *Hin*dIII fragment was deleted from the intron sequence of Lac 5.3. The *Hin*dIII terminus was blunted and a *Sal*I linker (New England Biolabs No. 1027) added for religation.

cgf-B. The 1.9-kb *Eco*RI-*Bam*HI genomic DNA fragment surrounding the *hb* P1 transcription start was fused to an *Eco*RI-*Xba*I fragment from the 5′ untranslated leader of *hb* cDNA clone J3 (see above). The latter fragment includes the exon 1-2 splice junction of a P1 mRNA, so the construct will produce transcripts with a mature P1 5′ leader. *Xba*I linkers were added to the blunted *Bam*HI terminus, and the fusion was cloned into the *Xba*I site of CaSpeRlacZ.

cgf-H. The 0.9-kb *Eco*RI-*Hin*dIII genomic DNA fragment surrounding the *hb* P1 transcription start was fused to the same *Eco*RI-*Xba*I cDNA fragment as for cgf-B. *Pst*I linkers (New England Biolabs No. 1024) were added to the blunted *Hin*dIII terminus, and the fusion was cloned into CaSpeRlacZ as an *Xba*I-*Pst*I fragment.

Lac 1.9, Lac 0.9. The 1.9-kb *Eco*RI-*Bam*HI and 0.9-kb *Eco*RI-*Hin*dIII genomic DNA fragments, respectively, surrounding the *hb* P1 transcription start were each blunt-end cloned into the blunted *Xba*I site of CaSpeRlacZ.

HZ 0.36. The 0.36-kb *Eco*RI-*Hin*dIII genomic DNA fragment just downstream of the P1 transcription start was subcloned into the corresponding sites of pBluescript II KS+ (Stratagene), excised at the flanking *Xba*I and *Kpn*I vector sites, and cloned into HZCaSpeR with the *Hin*dIII site proximal to the promoter (thus, the fragment lies in the same orientation relative to the basal *Hsp70* promoter as it does relative to P1 in the genome).

Germline Transformation

The recipient strain for P element-mediated germline transformation (Rubin and Spradling, 1982) was w^{1118}. For each construct, homozygous individuals from at least three independent transformant lines were analyzed for expression of the transgene.

RESULTS AND DISCUSSION

hb Transcripts in the Ovary Initiate at the Distal (P1) Promoter

Previous studies have used Northern blot hybridization analysis of RNAs from adult females and early embryos (prior to the onset of zygotic transcription) to infer that *hb* maternal transcripts derive from the distal (P1) promoter (Tautz *et al.*, 1987; Bender *et al.*, 1988). We have confirmed and extended these results using the more sensitive technique of primer extension to detect transcripts and determine their 5′ termini. Each of the *hb* promoters generates a transcript with a unique first exon (see Fig. 3; Tautz *et al.*, 1987; Bender *et al.*, 1988; our unpublished results), permitting the design of primers specific for each type of *hb* mRNA. In addition, we chose primer sequences located different distances from the expected transcription initiation site of the corresponding promoter, so that the P1- and P2-specific extension products would be clearly distinguishable by size. This allowed a direct comparison of transcript accumulation from the two promoters in a single primer extension reaction.

Total RNA from whole adult males and from ovariectomized females contains a very low level of transcript deriving from P1 and no detectable transcript from P2 (Fig. 1A, lanes 1 and 2). By contrast, P1 transcripts are relatively abundant in RNA from adult ovaries (Fig. 1A, lane 3). Again, no P2 transcripts are detected. As would be expected if the ovarian *hb* transcripts are provided to the mature oocyte, this same pattern of extension products is observed in embryos 0–2 hr after egg-laying (Fig. 1A, lane 4), prior to the onset of zygotic transcription (Edgar and Schubiger, 1986). These results demonstrate that *hb* transcripts in adult females are strongly enriched in the ovary, and that stable ovarian *hb* RNAs are initiated exclusively from the P1 promoter.

We also used the primer extension assay to compare ovarian RNA with RNA from a staged series of embryos. The maternal *hb* transcripts present in adult ovaries and in 0–2-hr embryos (Fig. 1B, lanes 1,2) appear to utilize the same transcription start site as the P1-derived transcripts present throughout most of embryogenesis (Fig. 1B, lanes 3–8). Transcripts initiated at the P2 promoter accumulate only between 2 and 4 hr (Fig. 1B, lane 3), the temporal window surrounding the cellular blastoderm stage, in agreement with previous studies (Tautz *et al.*, 1987; Bender *et al.*, 1988).

Localization of hb Transcripts in the Ovary

We examined the spatial distribution of *hb* transcript in the ovary by *in situ* hybridization with a *hb* antisense RNA probe (Tautz and Pfeifle, 1989; see Sommer and Tautz, 1991). As shown in Figs. 2A,2B, ovarian *hb* RNA is produced only by the germline-derived nurse cells; there is no apparent accumulation of *hb* transcript from the oocyte nucleus or from the somatic follicle cells. *hb* RNA is first detectable in nurse cells at stage 7 and then dramatically increases in abundance at stage 8,9 (staging by the criteria of Mahowald and Kambysellis, 1980). The elevated signal in the later stage egg chambers most likely reflects both accumulation of transcript and the general rise in transcriptional activity that accompa-

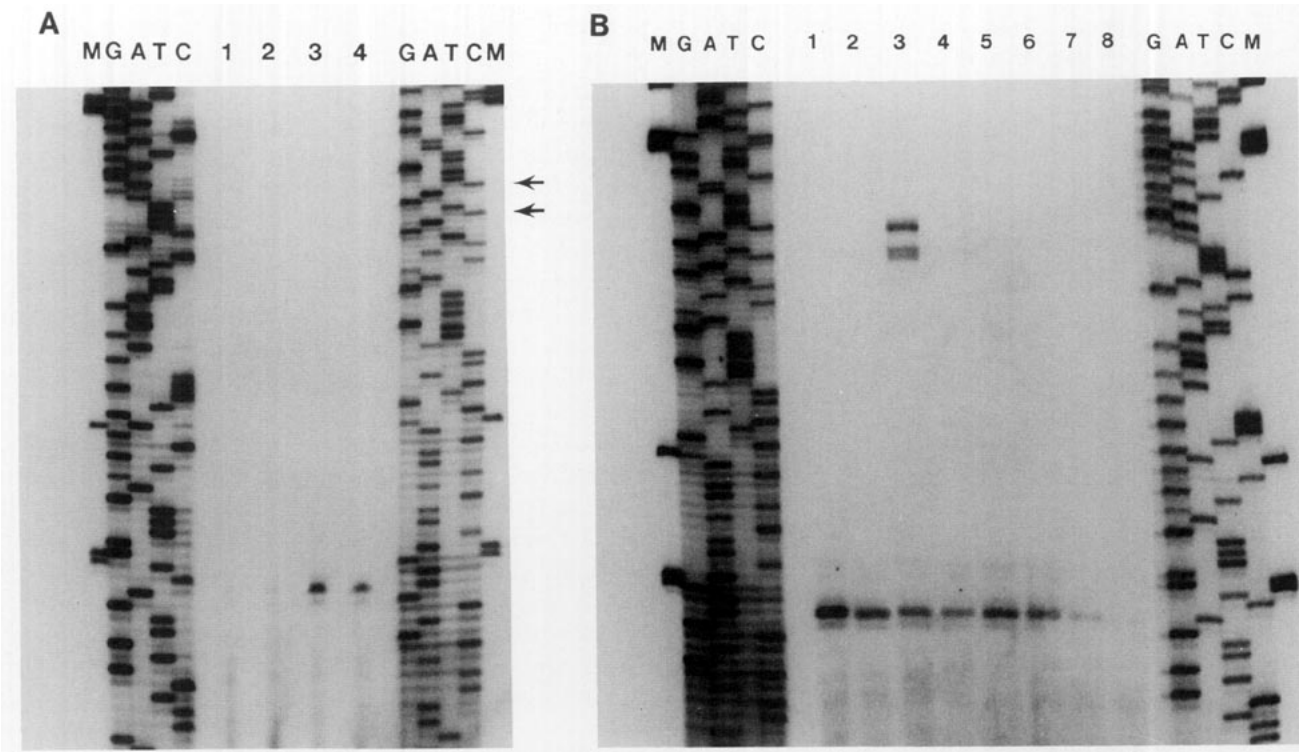

FIG. 1. Primer extension analysis of *hb* transcripts. Primer extension reactions (see Materials and Methods) contained two end-labeled antisense oligonucleotide primers, one with its 5′ end corresponding to a position 49 nt downstream of the predicted distal (P1) transcription initiation site and the other with its 5′ end corresponding to a position 70 and 74 nt downstream of the predicted proximal (P2) transcription start sites. Sequencing ladders (GATC) were generated using the identical labeled primers, as appropriate, in dideoxy sequencing reactions (Sanger *et al.*, 1977) on cloned genomic DNA from either the distal (A, left; B, right) or proximal (A, right; B, left) promoter region of *hb*. Size marker (M) is an *Hpa*II digest of pSP65 DNA (Promega), fill-in labeled with [^{32}P]dCTP using Klenow polymerase. (A) Numbered lanes display primer extension products of 50 μg of total RNA from adult males (1), adult females minus ovaries (2), ovaries (3), and 0–2-hr embryos (4). All extension products detected derive from distal (P1) promoter transcripts; arrows at upper right indicate the expected positions of extension products from transcripts initiated at the proximal (P2) promoter (see B). (B) Numbered lanes display primer extension products of 50 μg of total RNA from (1) adult ovaries, (2) 0–2-hr embryos, (3) 2–4-hr embryos, (4) 4–6-hr embryos, (5) 6–8-hr embryos, (6) 8–12-hr embryos, (7) 12–16-hr embryos, (8) 16–22-hr embryos. The sizes of the extension products observed in these reactions confirm that the transcription initiation sites predicted for *hb* (Tautz *et al.*, 1987) are used *in vivo*.

nies the increase in ploidy of the nurse cells. By stage 12, *hb* transcript is no longer detectable in the nurse cells (data not shown).

Promoter Analysis

To define the specific *cis*-regulatory sequences necessary to drive the *hb* nurse cell expression pattern, we used P element-mediated germline transformation (Rubin and Spradling, 1982) to establish stable transgenic lines of flies bearing different *hb* promoter–*lacZ* fusions and tested them for their ability to recapitulate this expression. We initially tested four constructs containing uninterrupted *hb* promoter fragments of 12, 8.0, 6.6, and 5.3 kb, all having the same 3′ terminus inside the second exon before the coding region (P1 + P2 fusions: Lac 12, Lac 8.0, Lac 6.6, and Lac 5.3; Fig. 3). Since all four fragments contain both *hb* promoters and the sequences necessary for proper splicing, the structure of the fusion gene transcripts should be indistinguishable from normal *hb* transcripts up to the *lacZ* coding sequences.

Although maternal *hb* transcript is not translated in the ovary (Tautz and Pfeifle, 1989), all four of these constructs yield an identical pattern of ovarian β-galactosidase activity (shown in Fig. 2D) that largely mimics the transcript distribution of the normal *hb* gene (Figs. 2A,2B). The activity of the reporter enzyme is restricted to the germline and is first detectable earlier than *hb* mRNA, in egg chambers of stage 5,6. *In situ* hybridization with a *lacZ* RNA probe reveals that the reporter transcript, like the normal hb transcript, is first barely detectable at stage 7 (compare Fig. 2F with Figs. 2A,2B). Older egg chambers show slightly more β-galactosidase activity, but a striking increase in staining intensity appears in stage 10 (Fig. 2D). At this stage also, the ooplasm first begins to show some weak β-galactosidase

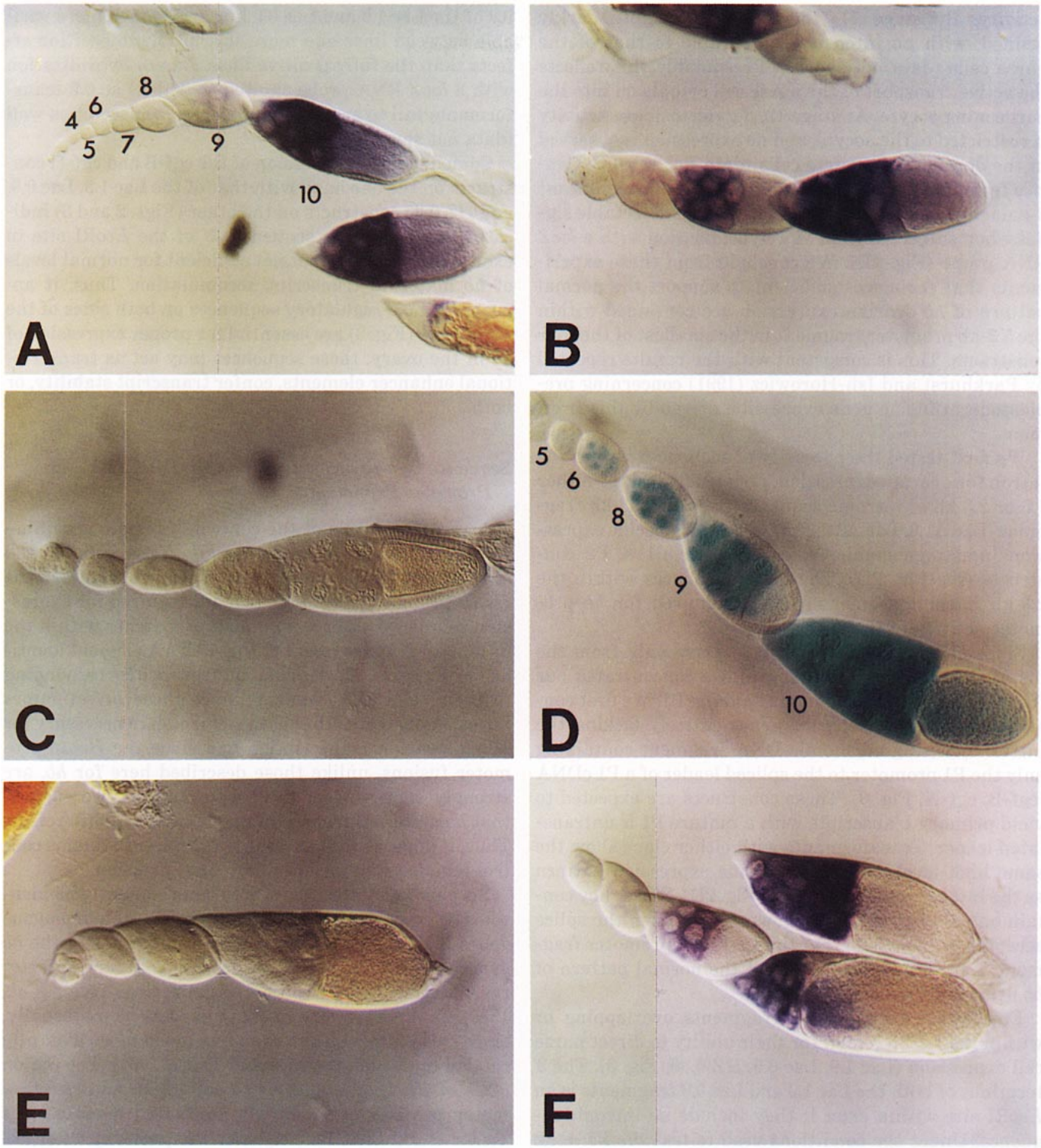

FIG. 2. Expression of the endogenous *hb* gene and of a *hb–lacZ* promoter–reporter fusion gene in the ovary. (A,B) Distribution of endogenous *hb* transcripts in the ovaries of w^{1118} flies, revealed by *in situ* hybridization with an antisense RNA probe. (C,D) Ovaries stained for β-galactosidase activity. Shown are the transformation recipient strain w^{1118} (C) and a transformant line carrying the cgf-H promoter–*lacZ* fusion construct (D). (E,F) Distribution of *lacZ* reporter transcript in ovaries, revealed by *in situ* hybridization with an antisense RNA probe. Shown are w^{1118} (E) and cgf-H (F). The detection of apparently strong β-galactosidase activity (D) earlier in oogenesis than *lacZ* RNA is detected (F) may be an artifact of the concentration of X-gal reaction product in nuclei or may result from a greater sensitivity of β-galactosidase versus alkaline phosphatase activity detection (the latter is used for the *in situ* hybridization). The expression patterns of the P1 + P2 fusions (see Fig. 3) are indistinguishable from those shown for the minimal cgf-H construct (D,F). Egg chamber stages (Mahowald and Kambysellis, 1980) are numbered in A and D.

activity. By stage 11, the ooplasm is quite darkly stained, with an intensity comparable to that of the nurse cells (data not shown). Presumably this reflects the active transport of the nurse cell cytoplasm into the burgeoning oocyte. At stage 12, β-galactosidase activity is restricted to the oocyte, and no expression is observed in the degenerating nurse cells (data not shown). Ovaries from flies of the recipient strain show no significant β-galactosidase activity (Fig. 2C) and no detectable signal when subjected to *in situ* hybridization with a *lacZ* RNA probe (Fig. 2E). We conclude from these experiments that sequences sufficient to support the normal pattern of *hb* ovarian expression are contained within the 5.3-kb promoter fragment in the smallest of the four constructs. This is consistent with the results reported by Parkhurst and Ish-Horowicz (1991) concerning preblastoderm fusion gene expression driven by this fragment.

We next tested the necessity of sequences within the intron for ovarian expression. Constructs lacking either 1.6 or 2.3 kb of intronic sequence from the 5.3-kb fragment (Lac Δ1.6, Lac Δ2.3; Fig. 3) showed specific expression indistinguishable from the other P1 + P2 constructs (see Fig. 2D). Thus, the sequences within the 2.3-kb intronic segment are not required for *hb* promoter activity in the ovary.

Since *hb* maternal transcripts derive only from the distal (P1) promoter (see above), we concentrated our further analysis on fragments flanking P1. We first constructed a pair of reporter genes entirely lacking the intron by fusing a genomic DNA fragment containing only the P1 promoter to the spliced leader of a P1 cDNA (cgf-B, cgf-H; Fig. 3). These constructs are expected to yield primary transcripts with a mature P1 5′ untranslated leader. Transformants with either clone show the same high-level, nurse cell-specific expression pattern as the intact promoter fusions (Fig. 2D). Since they contain only 6 bp of sequence downstream from the splice acceptor, we conclude that the 1.2-kb P1 promoter fragment is fully sufficient to direct the normal pattern of *hb* ovarian expression.

Finally, we tested three fragments overlapping or within the 1.2-kb region for their ability to direct nurse cell expression (Lac 1.9, Lac 0.9, HZ 0.36; Fig. 3). The 3′ terminus of both the Lac 1.9 and Lac 0.9 fragments is an *Eco*RI site within exon 1; they include no intronic sequence. Under the conditions used to test the P1 + P2 constructs (30–60 min incubation time for β-galactosidase activity staining), all three constructs showed no or barely detectable activity. When the incubation was extended to 21 hr, a low level of nurse cell-specific staining was observed in the Lac 1.9 and Lac 0.9 lines; in parallel reactions, the P1 + P2 lines became grossly overstained (data not shown). We also observed that the weak activity of the Lac 1.9 and Lac 0.9 lines was much more variable between lines and more susceptible to position effects than the fully positive lines. *In situ* hybridization with a *lacZ* RNA probe showed that the Lac 0.9 transformants fail to accumulate reporter transcript as well (data not shown).

Comparing the expression of the cgf-B and cgf-H constructs on the one hand with that of the Lac 1.9, Lac 0.9, and HZ 0.36 constructs on the other (Figs. 2 and 3) indicates that P1 leader sequences 3′ of the *Eco*RI site in exon 1 are necessary but not sufficient for normal levels of *hb* maternal transcript accumulation. Thus, it appears that *cis*-regulatory sequences on both sides of the *Eco*RI site (Fig. 3) are essential for proper expression of *hb* in the ovary; these sequences may act as transcriptional enhancer elements, confer transcript stability, or both.

Sequence Comparisons with the Minimal 1.2-kb hb Promoter Fragment

A recent analysis of the control of female germline-specific expression of the *Drosophila Hsp26* gene (Frank *et al.*, 1992) identified binding sites for two factors in the *Hsp26* promoter, both of which are required for expression. While we observe a number of matches within the minimal 1.2-kb fragment to the AATAA element identified by Frank *et al.*, we found no sequence corresponding to their CAACAA element. Since these investigators have demonstrated that both elements are necessary for *Hsp26* ovarian transcription and since the *Hsp26* promoter fusions, unlike those described here for *hb*, are strongly expressed in the oocyte nucleus, we suggest that *hb* maternal transcription is controlled differently. Thus, it appears that at least two different systems control female germline-specific gene expression.

Specific regulatory sequences can frequently be identified by comparing the promoter regions of homologous genes in different species. Such a comparison of the *hb* genes of *Drosophila virilis* and *Drosophila melanogaster* reveals their nearly identical organization (Treier *et al.*, 1989). *D. virilis* is very likely to express *hb* maternally, since this pattern is observed in a much more distantly related dipteran (Sommer and Tautz, 1991). The region of *D. virilis hb* homologous to our 1.2-kb minimal promoter exhibits seven highly conserved domains [see Treier *et al.*, 1989, for sequence comparison]. Of these, one corresponds to the basal promoter, including the TATA and CAAT boxes, and a second to the splice donor site. The remaining five are possible candidates for sites of action of nurse cell-specific transcriptional regulators. Interestingly, some of these regions contain multiple repeats of the consensus binding site for the GAGA factor (C/AGAGAGAG; Biggin and Tjian, 1989). GAGA

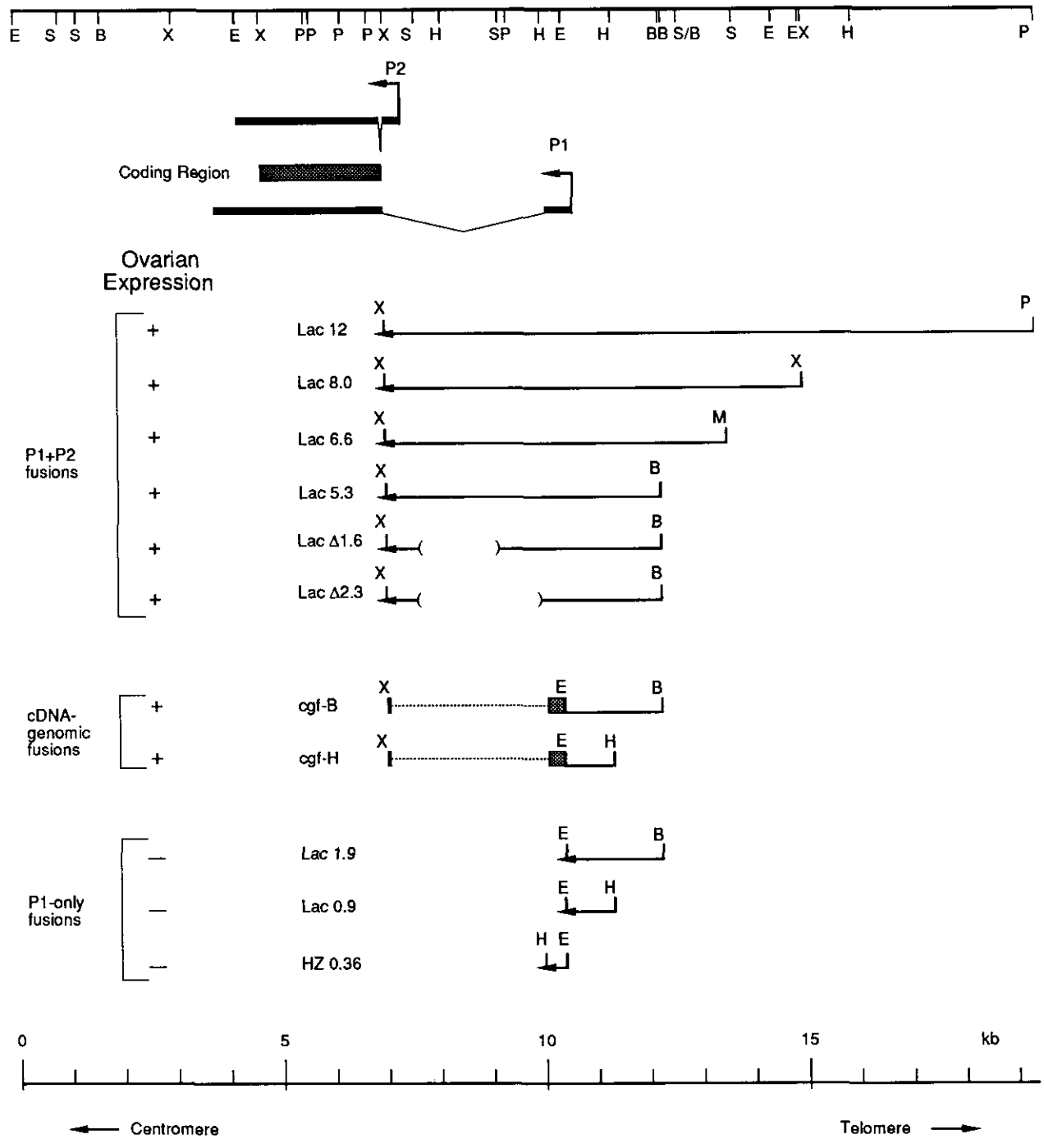

FIG. 3. Diagram of the *hb* promoter fragments analyzed in this study. At the bottom is a scale in kilobases (kb), with the origin set at the leftmost *Eco*RI site (E). The map is shown in standard *Drosophila* cytological orientation for the right arm of chromosome 3; centromere/telomere directions are indicated. At the top is a restriction map of genomic DNA in the vicinity of the *hb* gene. The *hb* transcription unit is diagrammed below the restriction map. Transcription start sites of the two promoters (P1 or distal, and P2 or proximal) are indicated by arrows. Exon sequences in mature *hb* mRNAs are indicated by filled bars; thin lines denote intron sequences. The *hb* protein coding sequence is shown as a hatched bar. Genomic DNA sequences used in fusion gene constructs (see Materials and Methods) are indicated by solid lines. Dotted line joining the two segments of the cgf fusions indicates the absence of the intron in the cDNA portion of these constructs. Ability or inability of each construct to direct ovarian expression in transgenic flies is indicated at left (+ or −). Restriction enzyme code: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; M, *Sma*I; P, *Pst*I; S, *Sal*I; and X, *Xba*I.

factor has been shown to be a transcriptional anti-repressor *in vitro* (Kerrigan *et al.*, 1991).

Although *hb* mRNA is not translated in the ovary, our promoter-reporter fusions are translated. Thus, sequences that normally inhibit this translation in the wild-type *hb* gene are apparently located 3′ of the *Xba*I site used as the 3′ terminus of the promoter fragments tested here. The maternal posterior gene system acts through *nos* to locally inhibit the embryonic translation of *hb* maternal mRNA through sequences in the latter's 3′ untranslated region (Wharton and Struhl, 1991). Although nos is maternally transcribed (Wang and Lehmann, 1991), it is unknown whether it is maternally translated and thus whether it could control *hb* maternal translation. If *nos* mRNA is maternally translated, a mechanism must exist to prevent the uniform distribution of maternal nos protein in the oocyte, as this would block *bicoid* and *hb* translation (Wharton and Struhl, 1991) throughout the embryo.

Our analysis of *hb* maternal expression has defined a 1.2-kb genomic DNA fragment surrounding P1 promoter that is sufficient to drive ovarian nurse cell-specific transcription. A fine structure analysis of the necessity and sufficiency of sequences within the 1.2-kb region will define more precisely the *cis*-regulatory elements responsible for this activity.

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Note added in proof. Lukowitz *et al.* [*Mech. Dev.* **45**, 105–115 (1994)] have independently demonstrated the role of P1 leader sequences in controlling *hb* expression in the ovary.

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