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Germ Line and Embryonic Expression of Fex, a Member of the *Drosophila* F-Element Retrotransposon Family, Is Mediated by an Internal *cis*-Regulatory Control Region

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The F elements of *Drosophila melanogaster* belong to the superfamily of long interspersed nucleotide element retrotransposons. To date, F-element transcription has not been detected in flies. Here we describe the isolation of a member of the F-element family, termed Fex, which is transcribed in specific cells of the female and male germ lines and in various tissues during embryogenesis of *D. melanogaster*. Sequence analysis revealed that this element contains two complete open reading frames coding for a putative nucleic acid-binding protein and a putative reverse transcriptase. Functional analysis of the 5′ region, using germ line transformation of Fex-*lacZ* reporter gene constructs, demonstrates that major aspects of tissue-specific Fex expression are controlled by internal *cis*-acting elements that lie in the putative coding region of open reading frame 1. These sequences mediate dynamic gene expression in eight expression domains during embryonic and germ line development. The capacity of the *cis*-regulatory region of the Fex element to mediate such complex expression patterns is unique among members of the long interspersed nucleotide element superfamily of retrotransposons and is reminiscent of regulatory regions of developmental control genes.

Retrotransposons are a large class of mobile elements that have been studied extensively as factors of genome instability. They can be grouped in two large subclasses, LTR (long terminal repeat) and non-LTR retrotransposons (2, 15). Non-LTR retrotransposons, which are also called long interspersed nucleotide elements (LINEs), lack terminal repeats which are a characteristic of LTR retrotransposons. Mammals carry a single major family of LINEs, known as L1 elements (14). Primates alone harbor between 10,000 and 100,000 L1 members in their genomes. It has been shown that insertions of these elements can cause mutations in genes: a de novo insertion of an L1 element into the factor VIII gene can cause hemophilia A in humans (24). Similarly, an insertion into the *myc* locus was shown to cause breast carcinoma (35). *Drosophila* LINEs are a more heterogeneous set of sequences that do not occur at such a high copy number as do those in mammals. Nevertheless, they constitute an abundant class of mobile elements with several hundred copies and are very highly represented in the heterochromatin (7, 12, 13, 22, 41, 45). Mobilization of LINEs occurs most likely via reverse transcription of an RNA intermediate, a process called retrotransposition, which often leads to the truncation of the 5′ ends of the elements. The mobilization process is precisely regulated, and the biochemistry of this process is still under investigation (6, 9, 42). The role of LINEs in genome evolution and potentially also in genome organization is only poorly understood. Some of the LINEs have been shown to be active in a tissue-specific manner during mammalian and *Drosophila* development (28, 32, 47). However, apart from the knowledge about their expression domains, not much is known about whether the gene products of these elements also have a function in developmental processes.

The *Drosophila* LINEs can be subdivided into several families, among them the family of F elements. This family has been estimated to consist of approximately 60 to 80 members which are located in the chromocenter and at euchromatic sites in the genome (12). Like other LINEs, full-size F elements are thought to code for two open reading frames (ORFs); ORF 1 most likely codes for a nucleic acid-binding protein, and ORF 2 is believed to code for a polypeptide with sequence homology to viral reverse transcriptases (2, 15). It has not been shown whether these putative proteins are actually translated and how they coordinate the transposition event in vivo. The study of these processes is hampered by the fact that F-element transcription has not been detected in flies.

Here we present the isolation and characterization of a previously uncharacterized member of the *D. melanogaster* F-element family which we named Fex. This element was found to be integrated into the intronic regions of the developmental control gene *pointed* at chromosomal location 94F. Fex is a full-size element of 4,690 bp and codes for two complete ORFs. We show that Fex is transcribed in follicle and nurse cells during oogenesis and in primary spermatocytes during spermatogenesis. During embryonic development, we find expression in the early blastoderm embryo, in the developing central nervous system, and in various organs such as the amnioserosa, the salivary glands, the muscles, the tracheal system, and the foregut. A functional analysis of the 5′ region in vivo, using P-element-mediated germ line transformation of Fex-*lacZ* reporter gene constructs, identifies a *cis*-acting control region that lies within ORF 1 and could account for most of the aspects of temporally and spatially controlled Fex expression in *D. melanogaster*.

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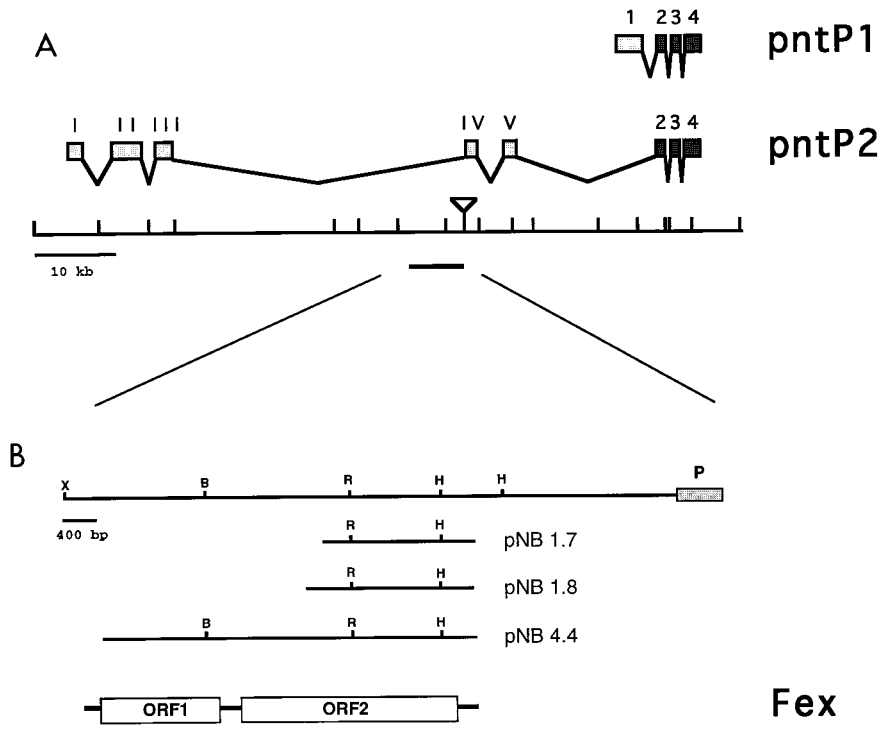

FIG. 1. Isolation of the Fex element from the *pointed* locus. (A) Genomic organization of the *pointed* gene at chromosomal location 94F with its two differentially spliced transcripts, *pointed*^{P1} and *pointed*^{P2} (pntP1 and pntP2) (27) (genomic *Eco*RI sites are indicated). Both *pointed* transcripts code for DNA-binding proteins with an ETS-binding domain (common exons 2, 3, and 4; black boxes). Exons 1 of *pointed*^{P1} and I to V of *pointed*^{P2} are transcript specific (shaded boxes). The P element of the enhancer trap line l(3) 07825 has inserted at the border region of exon IV of the *pointed*^{P2} transcript (triangle), thereby causing a homozygous lethal mutation (23, 39). The 6.5-kb genomic region next to the P element which was isolated by plasmid rescue (Materials and Methods) is shown below as a bar. (B) Restriction map of the 6.5-kb genomic plasmid rescue fragment. H, *Hin*dIII; E, *Eco*RI; X, *Xba*I. The 4-kb *Xba*I-*Hin*dIII fragment was used to screen an embryonic cDNA library (see text). Four cDNA clones were isolated: a 1.7-kb species (pNB1.7), a 1.8-kb species (pNB1.8), and two 4.4-kb species (one of them, pNB4.4, is shown). Sequence determination of the genomic plasmid rescue fragment and of cDNAs led to the identification of a novel member of the F-element family, which was termed Fex. Fex is 4,690 bp in length and codes for a putative untranslated leader region and two complete ORFs.

MATERIALS AND METHODS

***Drosophila* strains and fly care.** We used the *D. melanogaster* wild-type strain Canton S (provided by the Tübingen stock center). The enhancer trap line l(3) 07825 was selected from the Spradling collection (23) and obtained from the Bloomington stock center. The flies were maintained and embryo collections were made according to standard procedures.

Molecular biology. The plasmid rescue reaction with genomic DNA of the enhancer trap line was performed as described previously (34) and led to the isolation of a 6.5-kb genomic fragment next to the P-element insertion site (Fig. 1A and B). A subfragment of this region, the genomic 4-kb *Xba*I-*Hin*dIII fragment (Fig. 1B), was used to isolate four cDNA clones from an embryonic 4- to 8-h plasmid cDNA library (5). From about 300,000 clones screened, we isolated four cDNA clones, one 1.7 kb, one 1.8 kb, and two 4.4 kb in size (Fig. 1B). Handling of the library, preparation and radioactive labeling of DNA, restriction analysis, and subcloning were done according to standard procedures (44). Restriction analysis and cross-hybridization experiments showed that the 1.7- and the 1.8-kb cDNAs were part of the longer 4.4-kb cDNA species. In addition, restriction analysis and sequencing data showed that the cDNAs were completely contained within the genomic plasmid rescue region (Fig. 1B). For DNA sequencing, overlapping subfragments of the longest 4.4-kb cDNA (Fig. 1B) and the genomic plasmid rescue region were cloned into pBluescript (Stratagene), and most of both strands were sequenced by the dideoxynucleotide method (44) with an automatic sequencer (Applied Biosystems, Inc.). Sequence comparisons were performed with the EMBL and GenBank databases.

In situ hybridization experiments with DNA probes and riboprobes. For the chromosome in situ hybridizations, polytene chromosomes of third-instar Canton S wild-type larvae were prepared and hybridized to digoxigenin-labeled DNA fragments according to standard procedures (3). As DNA probes, we used the genomic 4-kb *Xba*I-*Hin*dIII fragment and the complete 4.4-kb cDNA (Fig. 1B). The hybridization signals were detected by means of antidigoxigenin antibodies coupled to alkaline phosphatase (Boehringer Mannheim). To detect transcripts, RNA in situ hybridizations to embryos, ovaries, and testes were performed. Labeling reactions were done according to standard procedures (46). To obtain strand-specific probes of the Fex element, the 4.4-kb cDNA (Fig. 1B) was cut with *Sca*I to linearize the plasmid; 1 μg of this template was then transcribed with T7 RNA polymerase to synthesize the antisense product and with Sp6 RNA polymerase to synthesize the sense product. The *lacZ* probe was generated as described previously (46). Fixing of ovaries and testes and the hybridization reaction were done according to standard procedures (30, 48). After staining, the ovaries and testes were mounted in 70% glycerol–phosphate-buffered saline.

X-Gal staining. 5-Bromo-4-chloro-3-indolylphosphate-β-D-galactopyranoside (X-Gal) staining of ovaries and testes of transgenic whole-mount embryos was performed according to standard procedures (3).

Generation of reporter gene constructs. The segment from positions +295 to +512 of the 5′ region of the Fex element (see Fig. 7A) was subcloned as an *Nhe*I fragment of the 4.4-kb cDNA into pBst (Stratagene) and subsequently cloned as an *Eco*RI-*Bam*HI fragment into the polylinker of the P-element vector pCaSpeR hs43 (49) (construct N). This vector contains the basal *hs43* promoter cloned in front of the *lacZ* gene as the reporter gene. The region from +295 to +762 was cloned as a *Hin*dIII-*Bam*HI subfragment of the 4.4-kb cDNA (Fig. 1B) into the *Hin*dIII and *Bam*HI sites of pBst and subsequently as an *Eco*RI-*Bam*HI fragment into the polylinker of pCaSpeR hs43 (construct B). To confirm the results obtained with the 0.47-kb *Hin*dIII-*Bam*HI element, we generated an additional construct containing the same *cis*-regulatory region by cloning the 0.7-kb *Eco*RI-*Bam*HI fragment of the 4.4-kb cDNA into pCaSpeR hs43 (construct RB). This fragment contains, in addition to the 0.47-kb *Hin*dIII-*Bam*HI sequence, 300 bp of cDNA vector sequences.

Germ line transformation. The reporter gene constructs were integrated into the *Drosophila* genome by P-element-mediated germ line transformation (43), using the helper plasmid Δ2,3 (46). For this purpose, the DNA constructs were injected into *w snw* homozygous mutant embryos (20). Transformant lines were established, and their embryonic progeny were analyzed by RNA in situ hybridization with a *lacZ* probe (46). For each experiment, three independent transformant lines were analyzed.

RESULTS

Isolation of a novel F-element family member. In the course of analyzing the function of the *Drosophila* gene *pointed* for

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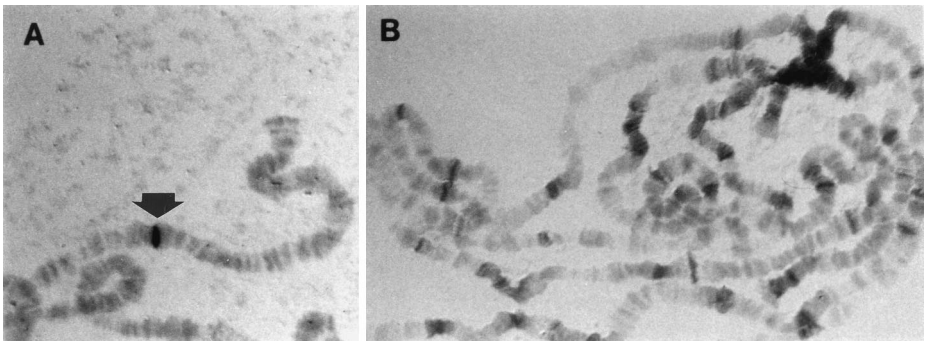

FIG. 2. In situ hybridization to polytene chromosomes (see Materials and Methods) with a *lacZ* probe (A) showing the location of the P element at chromosomal location 94F (arrow) and with the genomic 4-kb *Xba*I-*Hin*dIII fragment (see Fig. 1B) as a probe (B). The multiple banding pattern in the heterochromatin and at about 60 euchromatic sites is consistent with the Fex element belonging to the F-element family of retrotransposable elements.

development of the Malpighian tubules, the excretory organs of the fly (50), we characterized a P-element enhancer trap line which was mapped to the *pointed* locus (Fig. 1A). We isolated a 6.5-kb genomic region next to the P element (Fig. 1A) by plasmid rescue (34) and performed in situ hybridization to *D. melanogaster* embryos with the genomic region as a probe. This experiment revealed complex expression patterns during embryonic development predominantly in ectodermally derived tissues. The patterns were, however, largely different from the known expression patterns of the *pointed* gene in the embryo, suggesting that the genomic DNA contained transcribed sequences belonging to another transcription unit. Further in situ hybridization experiments to whole-mount embryos with subfragments of the genomic rescue region as probes allowed us to delimit the potential coding regions to the 4-kb *Xba*I-*Hin*dIII and the 0.7-kb *Hin*dIII fragments at the 5′ end of the genomic sequence (Fig. 1B); fragments adjacent to the P element gave no expression pattern. Chromosome in situ hybridizations with the *Xba*I-*Hin*dIII and the *Hin*dIII subfragments as probes revealed a multiple banding pattern, with the most intense labeling in the heterochromatin (Fig. 2). In the euchromatin, about 60 sites were labeled. This finding suggested that the 4- and the 0.7-kb fragments were part of a repetitive element which was strongly transcribed during embryonic development of *D. melanogaster.*

To further investigate the nature of this putative repetitive element, we screened a cDNA plasmid library prepared from poly(A)$^+$ RNA of 4- to 8-h-old embryos (5) with the genomic 4-kb *Xba*I-*Hin*dIII fragment as a probe. We isolated four related cDNAs of different lengths, the longest being 4.4 kb in size (Fig. 1B). Restriction analysis and sequencing data suggested that the cDNA sequences were completely contained within the genomic plasmid rescue fragment (Fig. 1B). The sequence of the 4.4-kb cDNA was determined (see Materials and Methods) and showed a strong homology in its 3′ portion to Fw, a previously characterized F-element family member about 3,500 bp in size which was found to be inserted in the *white* locus of *D. melanogaster* (11). The family member Fw is truncated at its 5′ end compared with full-size elements, which are about 4,700 bp in length (11, 12) (Fig. 3A). However, the Fw element is the only F-element family member whose sequence has been determined completely. Our results provided strong evidence that the 4.4-kb cDNA coded for a previously uncharacterized member of the F-element family of retrotransposable elements (12). We named this member Fex, for F expressed (see below). Sequence comparison between the Fex cDNA and the genomic region encoding the Fex element revealed identical sequences. The genomic Fex sequence, however, extended another 300 bp more 5′, yielding a total length of the Fex element of 4,690 bp.

Structure of the Fex element. The Fex element contained two complete ORFs that are derived from two different reading frames separated from each other by 344 bp (Fig. 3A); ORF 1 codes for a polypeptide of 447 amino acids, and ORF 2 codes for one of 859 amino acids (Fig. 3A). Both of the putative polypeptides showed the predicted homologies that are characteristic of LINEs. ORF 2 was found to encode a putative protein homologous to reverse transcriptases; 98% of the ORF 2 sequence was found to be identical to the sequence of the previously characterized ORF 2 of Fw at the nucleotide level (Fig. 3B) (11). ORF 1 encoded a putative polypeptide with closest similarity to the nucleic acid-binding proteins of the *Drosophila* Doc (38) and the *Culex pipiens* (1) LINEs (Fig. 3B). These putative nucleic acid-binding proteins have characteristic cysteine-rich stretches in the carboxy-terminal region that most likely interact with nucleic acids. Apart from the cysteine-stretches, several regions of homology, especially between the Doc and the Fex elements, could be identified in the 5′ region of ORF 1 (Fig. 3B). The functions of these regions are unknown (see Discussion). In addition to the two ORFs, the Fex element contained a putative 5′ untranslated leader region about 190 bp in length (Fig. 3A; see Fig. 7A).

Spatial and temporal control of Fex transcription during *Drosophila* embryogenesis. To monitor Fex element expression in the organism, we used strand-specific RNA probes of the Fex 4.4-kb cDNA and probes covering the genomic 5′ untranslated leader region which contains Fex-specific sequences. All probes used gave the same expression patterns. In the syncytial blastoderm embryo, Fex transcription occurred in the posterior region of the embryo (stages are described in reference 8) (Fig. 4A). This expression was maintained during cellular blastoderm stage and gastrulation but was found to become restricted during germ band extension to the neuroectodermal region from which the nervous system develops (Fig. 4B). Expression could be detected in the amnioserosa, which is the extraembryonic membrane covering the embryo dorsally (Fig. 4B). During germ band retraction stage, expression in the amnioserosa was weakened but still persisted strongly in the developing central nerve cord (Fig. 4C). In addition, expression in the foregut region and low-level expression in muscle cells and salivary gland precursor cells were found (Fig. 4C and D). Expression in the foregut region was observed in a narrow belt in the posterior region of the esophagus, not reaching into the midgut portion of the proventriculus, which is a valve-like structure regulating food passage from the foregut into the midgut (40). At late stages of embryogenesis, Fex transcripts

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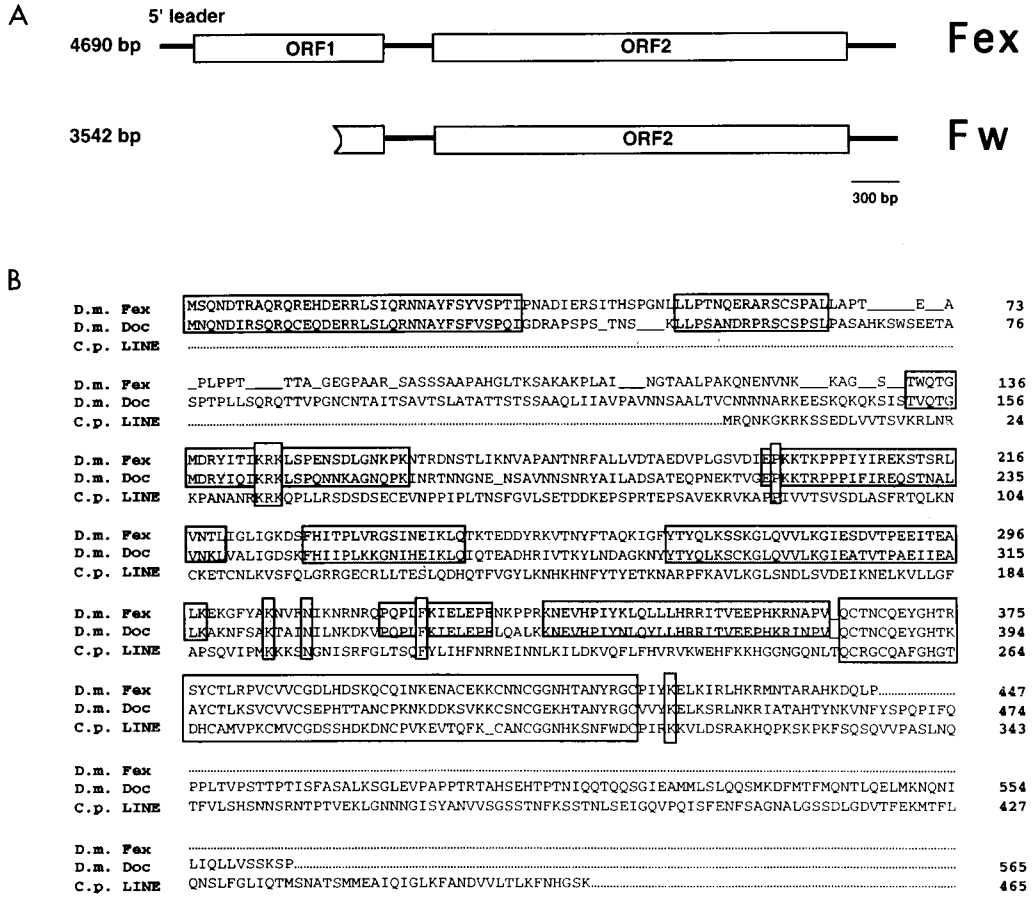

FIG. 3. Schematic structures of the Fex and Fw elements and sequence comparison of the putative proteins encoded by ORFs 1 of different LINEs. (A) The Fex element codes for two complete ORFs and can be considered a complete element. The exact 5′ terminus is derived from comparison with other F elements (10, 33). The Fw element whose sequence is known codes for a complete ORF 2 but is truncated at its 5′ end and thus contains only part of ORF 1. (B) Sequence comparison among the putative proteins encoded by ORFs 1 of the *D. melanogaster* Fex and Doc elements and the *C. pipiens* LINEs. ORF 1 is thought to code for a nucleic acid-binding protein. All three putative proteins are homologous in the 3′ region where the Cys motifs thought to be required for nucleic acid binding reside (open box) (11). In this domain, the putative Doc protein shows 64% and the LINE protein shows 28% similarity to the Fex protein. However, there are additional regions of similarity in the more 5′ regions of the proteins whose functions are unknown (see text). The Fex and Doc ORF1 proteins are highly homologous to each other (filled boxes).

were very strongly transcribed in the tracheal system (Fig. 4E) and in the central nervous system (Fig. 4F), but only low-level expression was maintained in the other expression domains. In the ventral nerve cord, stronger Fex expression could be detected in the midline cells and in two rows of cells on each side of the midline (Fig. 4F). More or less homogeneous expression was found in the two brain lobes (Fig. 4F; see Fig. 7B for a summary of the expression domains).

Cell-type-specific Fex expression in the male and female germ lines of *D. melanogaster*. During oogenesis, Fex transcription occurred very early at the tip of each ovariole in the germarium, within which the stem cells of the germ line and the somatic follicle cell precursors reside (Fig. 5B) (25). The germ line stem cells and follicle cells go through a defined set of division cycles and become organized into egg chambers which progressively leave the germarium and continue developing as they move posteriorly within the ovariole. In the mature egg chamber, which consists of the oocyte and 15 nurse cells that are both surrounded by a monolayer of somatic follicle cells, we detected Fex transcripts in the nuclei of the nurse cells and in the somatic follicle cells (Fig. 5C and D). The nurse cells provide the oocyte with RNA and other cytoplasmic components and have an important role in the determination of the anterior-posterior polarity in the oocyte (37). The somatically derived follicle cells are required for the determination of the dorsal-ventral polarity in the early embryo (37). In addition, the follicle cells secrete the vitelline membrane and the chorion. Fex transcription persisted until the late stages of oogenesis and became progressively restricted to a patch of follicle cells around the micropile (Fig. 5D).

Unlike that in the female germ line, Fex transcription in the male testis tube did not occur in the germinal proliferation center, which is at the tip of each testis tube and contains the germ line stem cells and the cyst progenitor cells that enclose the germ line stem cells (16). However, Fex transcripts could be detected during later stages of spermatogenesis in the nuclei of primary spermatocytes (Fig. 5A). It is noteworthy that only a subset of primary spermatocytes were marked (Fig. 5A). The primary spermatocytes are derived from the primary spermatogonial cell that produces via four mitotic divisions 16 primary spermatocytes in the cyst (16). As male germ cells enter the primary spermatocyte stage, they switch from a program of cell division to one of growth and gene expression. This switching correlated with the start of Fex expression.

Internal *cis*-acting elements control Fex transcription in the embryo and in the germ line. To determine whether the Fex

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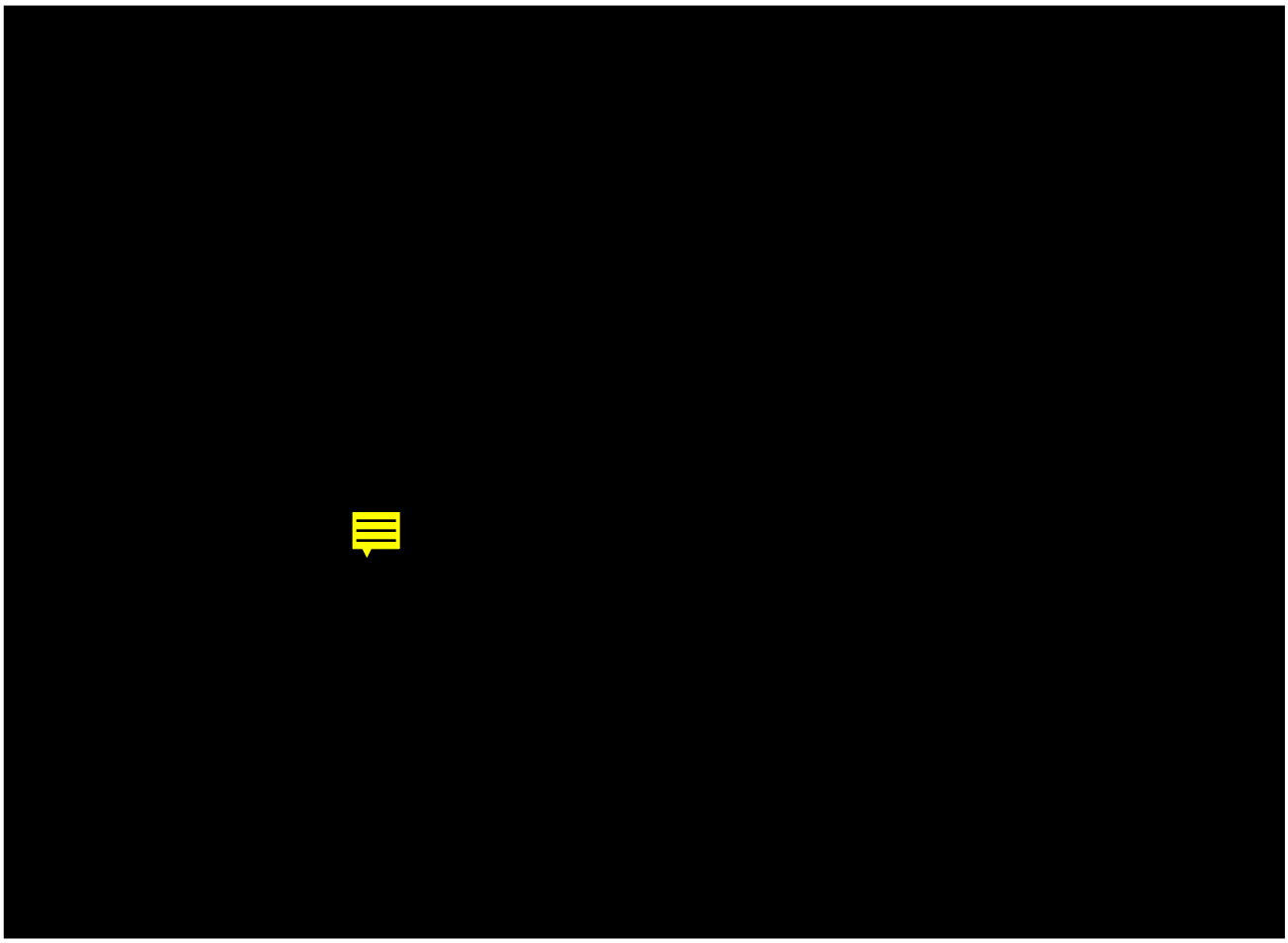

FIG. 4. Expression pattern of the Fex element during *Drosophila* embryogenesis monitored by in situ hybridization with Fex RNA probes to whole-mount embryos (Materials and Methods). (A) Fex expression is initiated at the blastoderm stage (8) in a posterior expression domain. (B) During the germ band elongation stage, Fex transcripts can be localized in amnioserosa precursors (upper arrow), in the segmentally repeated primordia of the central nervous system (middle arrow), and in muscle precursor cells (lower arrow). (C) During the germ band retraction stage, Fex expression occurs in single cells of the amnioserosa (upper arrow), in the foregut region (middle arrow), and strongly in the central nervous system (lower arrow). (D) Dorsal view of a stage 15 embryo. Fex expression occurs in sense organs in the head region (upper arrow) and in the developing salivary glands (lower arrow). (E) Dorsal view of a stage 17 embryo. Fex expression can be detected in muscles (upper arrow) and in the tracheae (lower arrows). (F) In late embryos, Fex transcripts are highly abundant in the muscles (upper arrow) and in the brain lobes (middle arrow) and in distinct rows of cells in the ventral nerve cord (lower arrow). The orientation of the embryos is anterior left and dorsal up.

element contained intrinsic *cis*-acting elements or whether extrinsic enhancers of neighboring genes were controlling Fex expression during germ line and embryonic development, we tested the ability of Fex 5′ sequences derived from the longest Fex cDNA to drive *lacZ* reporter gene expression in transgenic animals (Fig. 6 and 7). The fusion constructs were integrated into the *Drosophila* genome by P-element-mediated germ line transformation (43). We analyzed the reporter gene expression patterns of transgenic flies by in situ hybridization to whole-mount embryos with a digoxigenin-labeled *lacZ* RNA probe and by X-Gal stainings of ovaries and testis tubes (see Materials and Methods) (Fig. 6 and 7).

This analysis revealed that the region between +295 and +762 of the Fex element, which lies within ORF 1 (Fig. 7A), is capable of controlling most aspects of the complex Fex transcription pattern in the germ line and in the embryo. This region mediates reporter gene expression during the segmentation period in the blastoderm embryo, during neurogenesis in the central nervous system, and during organogenesis in the amnioserosa, the foregut, the muscles, the trachea, and the salivary glands (constructs B and RB) (Fig. 6A to D and 7). In addition, this region controls reporter gene expression in the follicle cells during oogenesis (Fig. 6E and 7B). A subfragment of this region, containing sequences from position +295 to +512 (construct N) (Fig. 7A), was solely capable of mediating reporter gene expression in the amnioserosa (Fig. 6F), providing evidence that the distal part of the region from +295 to +762 is essential for controlling major aspects of the Fex transcription pattern in vivo. We have not found *cis*-acting elements for gene expression in the nurse cells or in the testis tubes (see Discussion). In summary, a region of about 470 bp that lies within ORF 1 at the 5′ end of the Fex element was able to control gene expression in 8 of 10 expression domains in which the Fex element is transcribed during germ line and embryonic development. This region therefore most likely represents a major control region of Fex transcription in *D. melanogaster*. Furthermore, the fact that this internal control region lies within an ORF suggests that the transcription of other full-length F elements in the genome is probably controlled similarly to Fex, thereby resulting in similar expression patterns.

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FIG. 5. Expression pattern of the Fex element during male (A) and female (B to D) germ line development of *D. melanogaster* monitored by in situ hybridization with Fex RNA probes (Materials and Methods). (A) During spermatogenesis, Fex transcripts are found below the germinal proliferation center at the tip of the testis tube (upper arrow) in a subpopulation of primary spermatocytes (lower arrow). (B) Fex transcripts can be detected during the initial stages of oogenesis in the germinal proliferation center (upper arrow). In later stages, Fex transcripts are localized in nurse cell nuclei (lower arrows) and (C) in nuclei of follicle cells (arrows on the right; note also the punctuate staining in the nurse cell nuclei [arrow on the left]). (D) At late stages of oogenesis, Fex expression is maintained in the follicle cells that have moved to the anterior of the egg below the micropiles (arrow).

DISCUSSION

We have isolated and characterized a previously uncharacterized member of the *Drosophila* F-element retrotransposon family, Fex. This full-size element contains the two complete ORFs characteristic of LINEs. We showed that the Fex element is transcribed in dynamic expression patterns in various tissues during embryonic development and in specific cell types in the female and male germ lines. The complex expression patterns of Fex are mediated by internal *cis*-acting control regions in the 5′ region within ORF 1 of this element.

Isolation of the Fex element. We found the Fex element to be integrated into the *pointed* locus at chromosomal location 94 F (Fig. 1A). The *pointed* gene has been shown to be required during embryogenesis in glial cells for proper central nervous system development and also in the adult fly during eye development (26, 27, 31, 39). In the enhancer trap line studied, the P element had integrated into the border region of exon 4 of the *pointed*^{P2} transcript (Fig. 1A) (39). It is known that a variety of LINEs can cause mutations upon integration into genes. Well-known examples are the Fw element and Doc elements, which were found to be integrated into the *white* locus of *D. melanogaster* (11, 38). The homozygous lethal phenotype of the enhancer trap line that we have used in our studies is, however, most likely due to the integration of the P element, not the F element. Consistent with this conclusion, we could revert the lethal phenotype of the enhancer trap line to wild type by excising the P element in a jump-out experiment (34).

Sequence analysis revealed that the Fex element is a full-size element with two complete ORFs. The distal region of Fex (ORF 2 and 366 bp of ORF 1) was found to be highly homologous to that of the previously characterized family member Fw, a truncated family member whose sequence is known (11). Determination of the Fex sequence allowed us to obtain complete sequence information for the two ORFs of an F element. Sequence comparison among ORFs 1 of Fex, the *C. pipiens* LINE, and the *D. melanogaster* Doc elements revealed several regions of homology outside the previously characterized region encoding the putative nucleic acid-binding domain (Fig. 3B) (11). The functions of these regions are, however, unknown. Since the ORFs 1 are thought to encode element-specific nucleic acid-binding proteins, these regions of high homology might be responsible for conserved protein-protein interactions during the processes of reverse transcription or transposition instead of providing element-dependent specificity.

Since it was known from analyses of other LINEs that they contain internal promoters (2), F-element family members were investigated to obtain clues as to how F-element transcription is controlled (10, 33). These studies were performed in tissue culture cells and focused mainly on the 5′ untranslated leader region of specific F-element family members. F12 is the family member which has been characterized extensively in this respect. These tissue culture experiments revealed the existence of two internal promoters that control low-level F element transcription: F_{in} and F_{out} (10, 33). With transient expression assays, sequence elements which seem to be important for F-element transcription, among them an octamer sequence that is also conserved in other LINE elements, were identified (2, 10, 33). The 5′ untranslated leader sequence of

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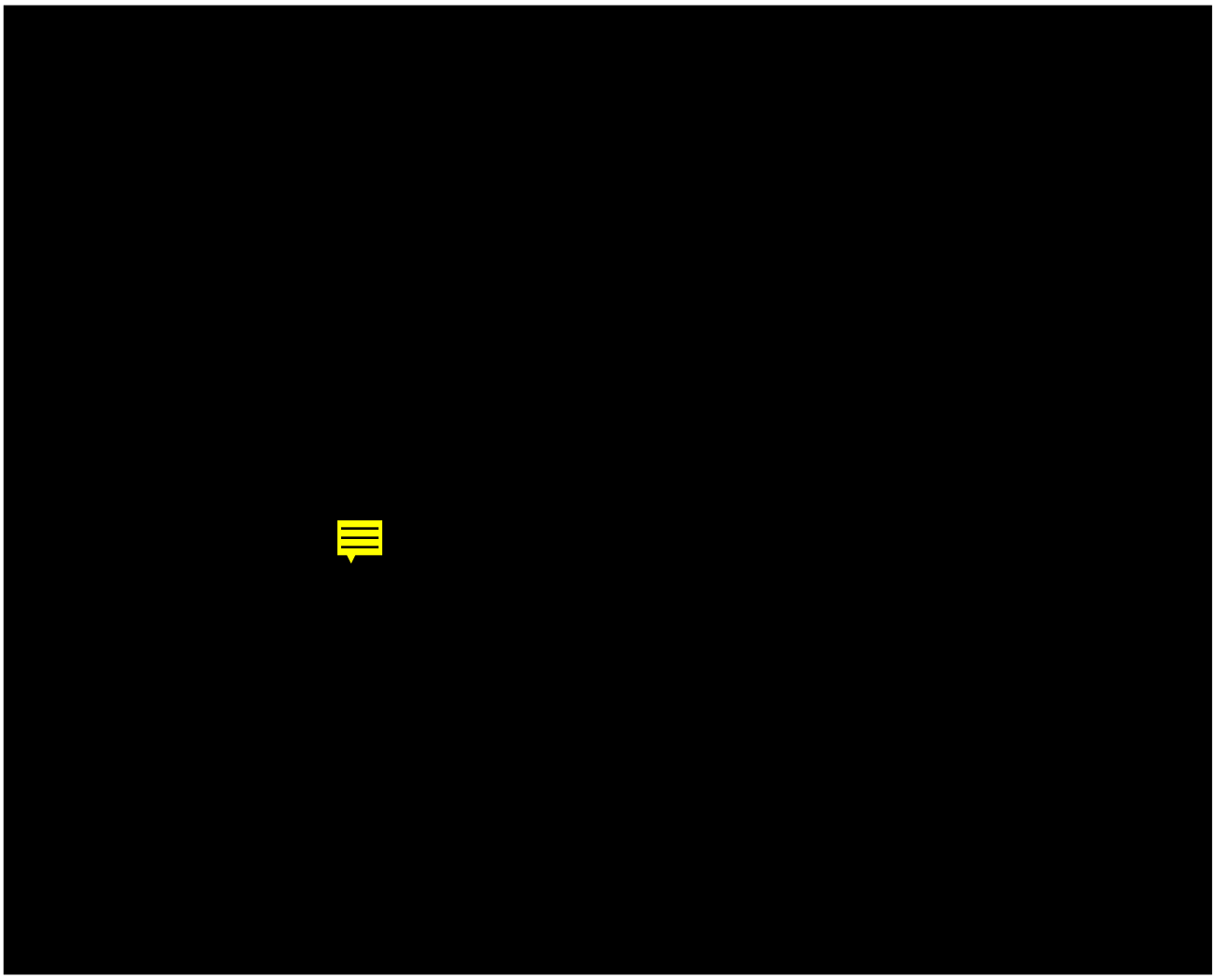

FIG. 6. Expression patterns of transgenic animals carrying reporter genes driven by sequences of the 5′ region of the Fex element. Reporter gene expression was monitored by in situ hybridization with a *lacZ* RNA probe (Materials and Methods; see Fig. 7 for a summary of the patterns and for details of the constructs). (A to E) HB construct carrying a 470-bp fragment from +295 to +762 of the Fex 5′ region (see Fig. 7A for sequence). This fragment is contained within the 5′ portion of ORF 1. (F) N construct carrying a region of +295 to +512 of the Fex 5′ region. (A) Reporter gene expression in the posterior domain of the blastoderm embryo. (B) During germ band extension, reporter gene expression can be detected in the amnioserosa primordium (upper arrow), in the developing central nervous system and muscle precursor cells (middle arrow), and in the foregut region (lower arrow). (C) During germ band retraction, the reporter gene is expressed (arrows from top to bottom) in the amnioserosa, the brain lobes, the foregut, and distinct cells in the ventral nerve cord. (D) Dorsal view of a stage 15 embryo. Reporter gene expression occurs in the amnioserosa, the developing salivary gland, and the brain lobes (arrows). (E) Reporter gene expression during oogenesis in the follicle cells (arrow). (F) Transgenic embryos carrying the N construct show expression in the amnioserosa only (arrow). Orientation of the embryos is anterior left and dorsal up.

Fex is highly homologous to that of F12 and therefore also contains most of the sequence elements shown to be important for F12 transcription in tissue culture cells (Fig. 7A). However, major control elements for tissue-specific Fex transcription in vivo reside in a neighboring 470-bp region within ORF 1 (Fig. 7A). This region is sufficient to control most aspects of tissue-specific Fex transcription (Fig. 6 and 7; see below).

Dynamic expression patterns in the germ line and in the embryo. During oogenesis, Fex transcripts are present in follicle cells and nurse cells (Fig. 5B to D). Both of these cell types have an essential role in determining the anterior-posterior and the dorsal-ventral axes of *D. melanogaster* during oogenesis. These polarities arise as a result of inductive interactions between the oocyte and the nurse cells and the surrounding layer of somatic follicle cells (37). In the final stages of oogenesis, the nurse cells empty their contents into the oocyte to provide it with cytoplasmic components. Fex RNAs might also enter the oocyte by this means. Transposition events would then be inherited by the next generation. During spermatogenesis, Fex transcription occurs in primary spermatocytes (Fig. 5A). The onset of Fex expression correlates with the switching of these cells from a program of cell division to one of growth and gene expression. Interestingly, Fex transcription occurs in only a subset of the primary spermatocytes. Cell-specific transcriptional repressors might inhibit Fex transcription (and thereby mobilization) in some spermatocytes but not in others.

During embryonic development, we find Fex transcripts in a variety of tissues during early determination as well as late differentiation processes (Fig. 4). In the early blastoderm embryo, Fex transcripts are localized in the posterior domain of the embryo, overlapping the expression domains of the segmentation genes *knirps* and *giant*, which code for DNA-binding proteins (19). Whether Fex transcription is regulated by these transcription factors is not known. In the developing central

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A

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1   GACCACTGAA GCATTTCGAT CGCCGACGTG TGAAGACGTT TTTATCGAGT CCGCACAAAT CGGTTGTTTT
71  GAGTGAAGGT GACCGCCAAT AAGTAACCTA ATTAAAAATT CCGAAAGCGA AAGAGACGCT CTATGCGATG
                                                      ORF1
141 CAAGTTCGCT TAAATATATA GTGATTTGTT ATCTTAAATA ATAAAACTAT GAGTCAGAAC GATACTCGCG
211 CACAGCGTCA GCGCGAGCAT GACGAACGCC GGCTCTCAAT TCAACGCAAC AACGCGTACT TCTCCTACGT
281 CTCACCGACA ATCCCAAACG CAGACATCGA GCGGTCAATA ACCCATAGCC CAGGAAACCT TCTTCTACCA
351 ACAAATCAAG AAAGAGCGCG CTCCTGCTCT CCCGCTCTAT TGGCTCCGAC AGAAGCCCCG CTACCTCCAA
421 CAACAACAGC TGGAGAGGGA CCGGCAGCCC GCTCTGCCTC GTCATCGGCT GCACCCGCTC ACGGTCTGAC
                                  N
491 TAAGTCAGCG AAAGCAAAAC CGCTAGCAAT AAACGGTACT GCTGCACTGC CAGCAAAACA AAACGAAAAC
561 GTAAACAAAA AAGCTGGGTC GACCTGGCAG ACTGGAATGG ACCGCTACAT TACAATAAAG CGAAAGCTCA
631 GCCCGGAAAA TTCAGATTTG GGAAACAAGC CGAAAAATAC ACGCGATAAC TCTACCTTGA TCAAAAATGT
                                                                  B
701 AGCCCCTGCA AATACCAACA GATTTGCCTT GCTGGTAGAT ACCGCTGAGG ACGTGCCGCT GGGATCCGTT
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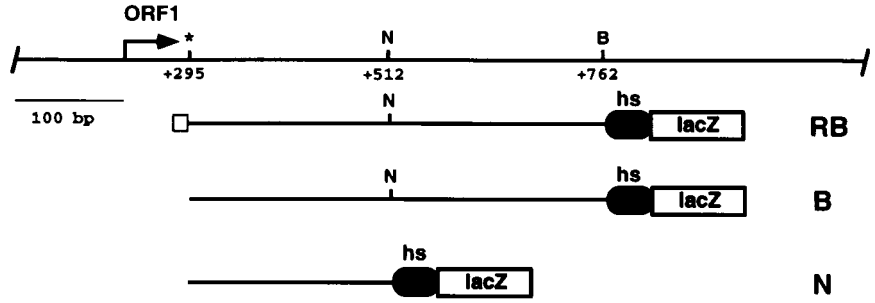

B

	B	CNS	AS	FG	SG	M	TS
RNA WT	+	+	+	+	+	+	+
B - 1	+	-	+	+	+	+	-
B - 2	+	+	+	+	+	+	-
B - 3	+	+	+	-	-	+	-
RB - 1	(+)	(+)	+	+	+	+	+
RB - 2	-	-	+	+	+	+	-
RB - 3	-	+	+	+	+	+	-
N - 1	-	-	+	-	-	-	+
N - 2	-	-	+	-	-	+	-
N - 3	-	-	+	+	+	+	-

	Follicle cells	Nurse cells	Testes
RNA WT	+	+	+
B	+	-	-
RB	+	-	-
N	-	-	-

FIG. 7. Generation of reporter gene constructs driven by sequences of the Fex element (A) and summary of the reporter gene expression domains (B) compared with the wild-type expression patterns of the Fex transcripts. (A) The sequence of the 5′ region of the Fex element is shown at the top. The arrow marks the beginning of ORF 1. The restriction sites N (*Nhe*I) and B (*Bam*HI) and position +295 (asterisk) used to generate the reporter gene constructs (bottom) are indicated. Below are sequences of the reporter gene constructs. The arrow marks the beginning of the putative ORF 1, and N (*Nhe*I; position +512) and B (*Bam*HI; position +762) designate restriction sites (see the top portion for sequence information). Three Fex-*lacZ* fusion constructs (Materials and Methods) were generated to identify *cis*-acting elements within ORF 1; the B and RB constructs both contain Fex sequences from bp +295 to +762 (up to the B site). The results obtained with the B construct were confirmed by generating the RB construct, which was cloned differently and contains in addition to the sequence in B a small piece of vector sequence (square) at its 5′ end (see Materials and Methods); the N construct contains Fex sequences from bp +295 to +512. The region from bp +295 to +762 of the Fex element is able to drive expression in 8 of 10 expression domains of the Fex element and therefore represents a major control region conferring tissue-specific expression in vivo. (B) Summary of Fex expression patterns in the wild-type embryo (top) and in the male and female germ lines (bottom) and of the reporter gene expression patterns of the transgenic embryos carrying the Fex-*lacZ* fusion genes (see panel A). Abbreviations: B, posterior domain in the blastoderm; CNS, central nervous system; AS, amnioserosa; FG, foregut; SG, salivary glands; M, muscles; TS, tracheal system. Three independent transgenic lines (numbers) were analyzed for each construct. +, strong expression; (+), weak expression; −, no expression. Note that variations in the expression patterns between independent transgenic lines are known to occur as a result of position effects of the chromosomal integration sites (20).

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nervous system and in various organs such as the amnioserosa, the salivary glands, the muscles, the tracheal system, and the foregut, Fex expression occurs early in the primordia and then becomes downregulated as differentiation proceeds. Strong Fex transcription persists only in the central nervous system (Fig. 4F). The dynamics of the Fex expression patterns suggest that its transcription is under the control of factors that initiate the processes of neurogenesis and organogenesis. In all expression domains, we find predominantly nuclear transcripts of Fex, which are most clearly visible in the nuclei of the nurse cells since these cells are relatively large (Fig. 5C). This finding is consistent with the current view that transcripts of non-LTR retrotransposons are found mainly within the nucleus (2, 15).

Fex *cis*-acting control elements for in vivo transcription reside within its 5′ region. Transcription of a retroelement is required for its transposition. Therefore, a functional retroelement has to possess an active promoter to transpose in the genome. The existence of strain-independent expression patterns for a variety of retrotransposon families indicated the existence of element-specific regulatory sequences which mediate these expression patterns. Both in vivo approaches and transient expression assays in *Drosophila* tissue culture cells have been used to dissect putative promoter regions with reporter gene fusion constructs (2, 6). Specific regulatory elements have been found in a number of LTR-containing retrotransposons; examples are a testis-specific element within the *micropia* transposon (29), an element in the 3′ end of the *17.6* retrotransposon mediating lamina-specific expression (36), and regulatory elements mediating mesoderm-specific expression of the *B104* element (4). For LINEs, only a few cases in which regulatory elements mediate tissue-specific expression in vivo are known. The I factor is one example: a specific regulatory element directing ovary-specific expression could be isolated in the 5′ untranslated leader region (28, 32).

Our in vivo analysis of the 5′ region of the Fex element showed that the *cis*-acting region of the Fex element mediates expression patterns which are much more complex than those mediated by the *cis*-regulatory regions of other retrotransposable elements: a 470-bp region within ORF 1 in the 5′ region of the Fex element mediates dynamic gene expression patterns in eight distinct domains during germ line and embryonic development of *D. melanogaster* (Fig. 7). In this respect, the control region of the Fex element very much resembles the complex *cis*-regulatory regions of many developmental control genes. In the case of the *Drosophila* segmentation gene *Krüppel*, for example, the *cis*-acting region contains more than 12 distinct control elements that are required for *Krüppel* expression during the segmentation period and during organogenesis and neurogenesis later in development (17, 20). The *Krüppel* segmentation enhancer was shown to interact with multiple maternal and zygotic transcription factors that bind mostly to overlapping sites, thereby controlling spatial *Krüppel* expression (18, 21). The Fex element most likely also contains a complex array of binding sites for *trans*-acting factors that drive the complex dynamic expression patterns during *Drosophila* development. However, whereas the *cis*-acting elements for *Krüppel* expression are distributed over a region of 18 kb (20), binding sites for *trans*-acting factors mediating the complex Fex expression pattern seem to be clustered in the region from +295 and +762, as the analysis of the Fex 5′ region suggests (Fig. 7A). This reveals an extraordinary compactness of the *cis*-regulatory region of Fex. As opposed to most developmental control genes, whose *cis*-acting regions are normally situated in the upstream or downstream regions of the genes, the *cis*-acting elements for tissue-specific Fex transcription reside internally overlapping with ORF 1. We have not been able to identify *cis*-acting elements for Fex expression in the nurse cells and in male testes. These elements might be localized elsewhere in the retrotransposon. Also, we cannot exclude the existence of redundant control elements as frequently found in the regulatory regions of developmental control genes (20). Finally, we should point out that we cannot exclude the possibility that the Fex element at the *pointed* locus is not transcribed and that the transcription patterns that we monitored are due to Fex elements located elsewhere in the genome or to other F-element family members. However, since the *cis*-acting control region of Fex is located in ORF 1, all F-element family members that are expressed will probably be transcriptionally regulated similarly, resulting in similar expression patterns.

How is Fex transcription regulated? Since transcription of a retrotransposon is considered to be the first step in its transposition, transcription in many tissues would mean a high rate of de novo integration, potentially leading to mutations in genes and consequently to lethality in somatic cells and also in the germ cells. This is, however, obviously not the case, since wild-type flies expressing the Fex elements are viable. We speculate that this finding might be due to two main reasons. (i) It is known that the level of RNA synthesis does not necessarily reflect the frequency of transposition, since the transcribed RNAs could be inactive in terms of not being properly processed or translated (2, 6, 15). In fact, it is not known whether ORFs 1 and 2 of F elements are translated into proteins. What function these proteins might have in the transposition cycle is only guessed from sequence homology to more extensively analyzed transposable elements. (ii) We have found that Fex elements also transcribe antisense transcripts which are coexpressed with the sense transcripts in all embryonic and germ line expression domains (24a). It has been speculated for the *micropia* element that a balance between the amounts of sense and antisense transcripts, which could in principle form hybrids so that translation would be blocked, might determine transposition (29). It is possible that a similar type of regulation occurs in the case of Fex transcription such that transposition is kept at a minimal rate.

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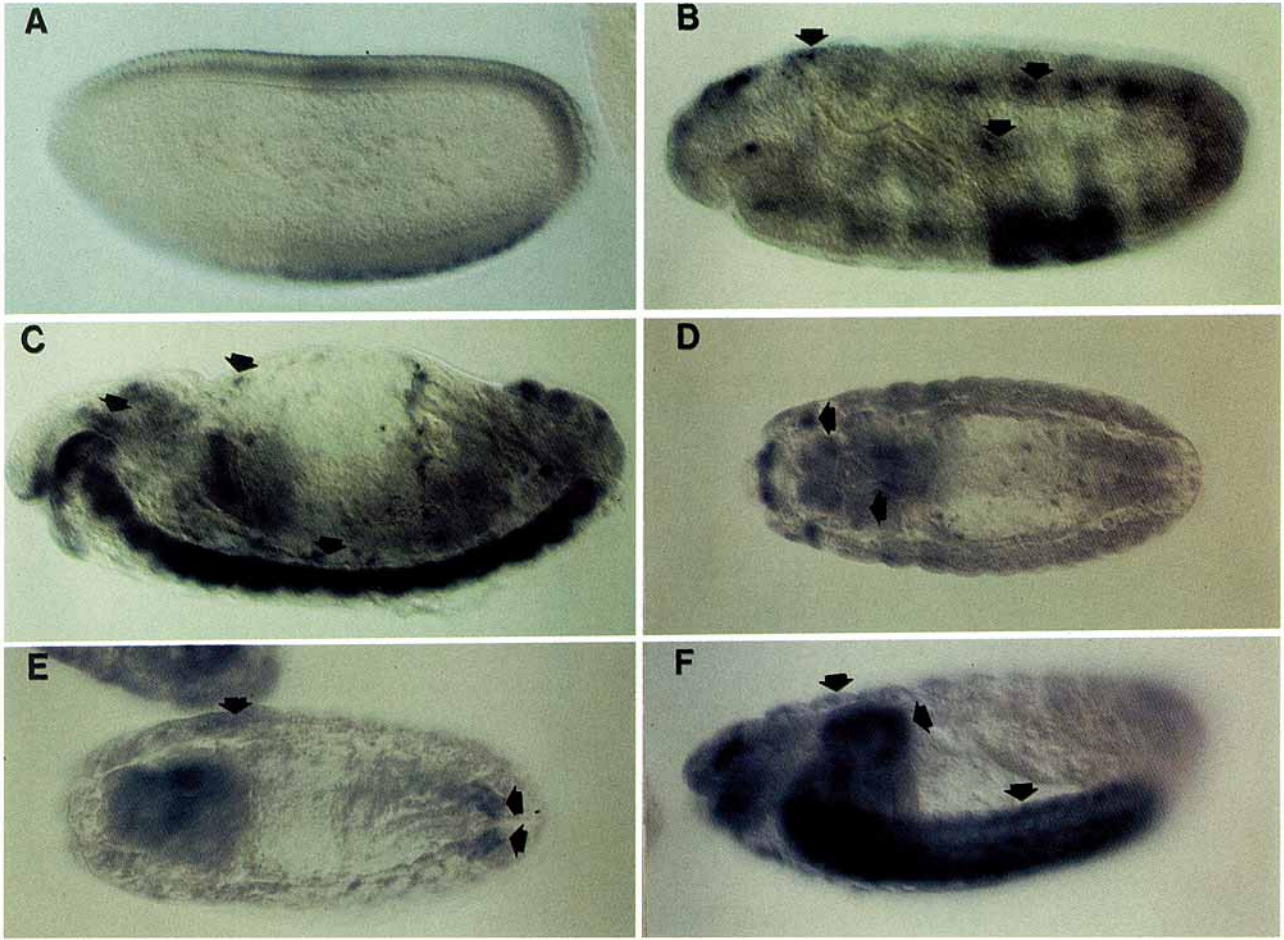
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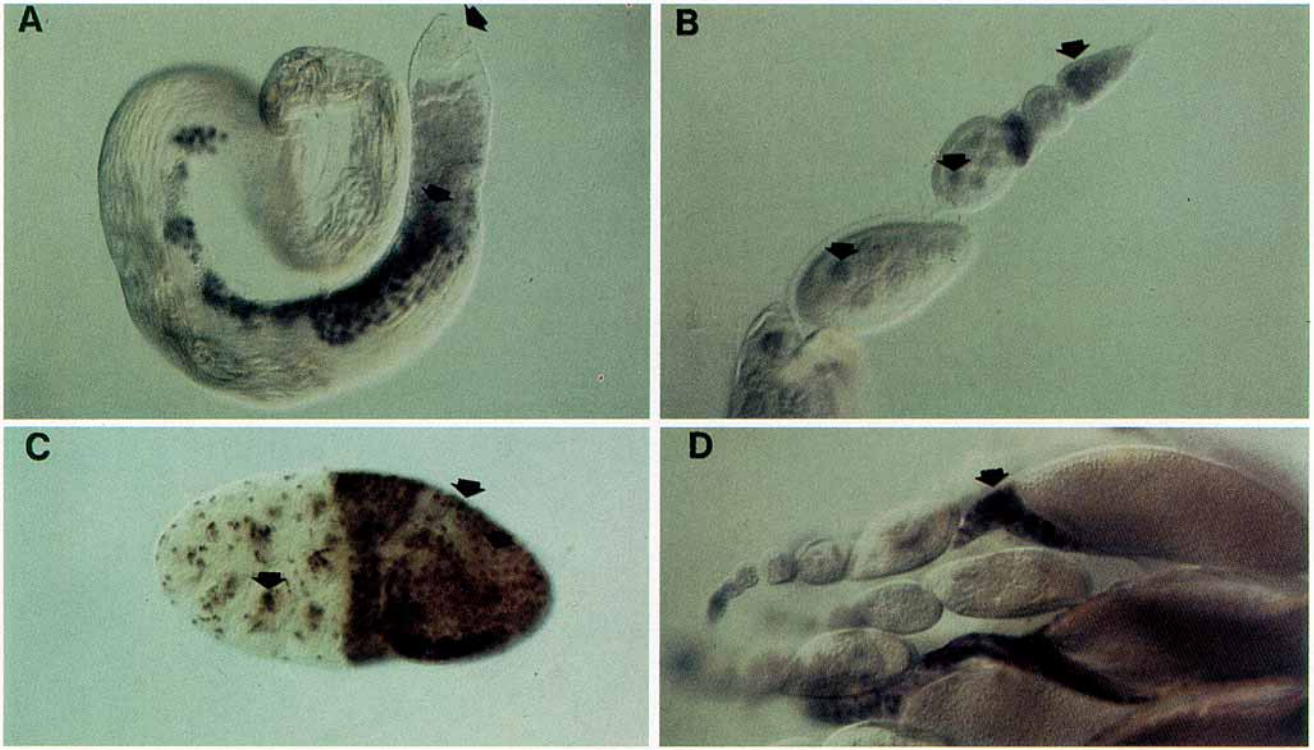
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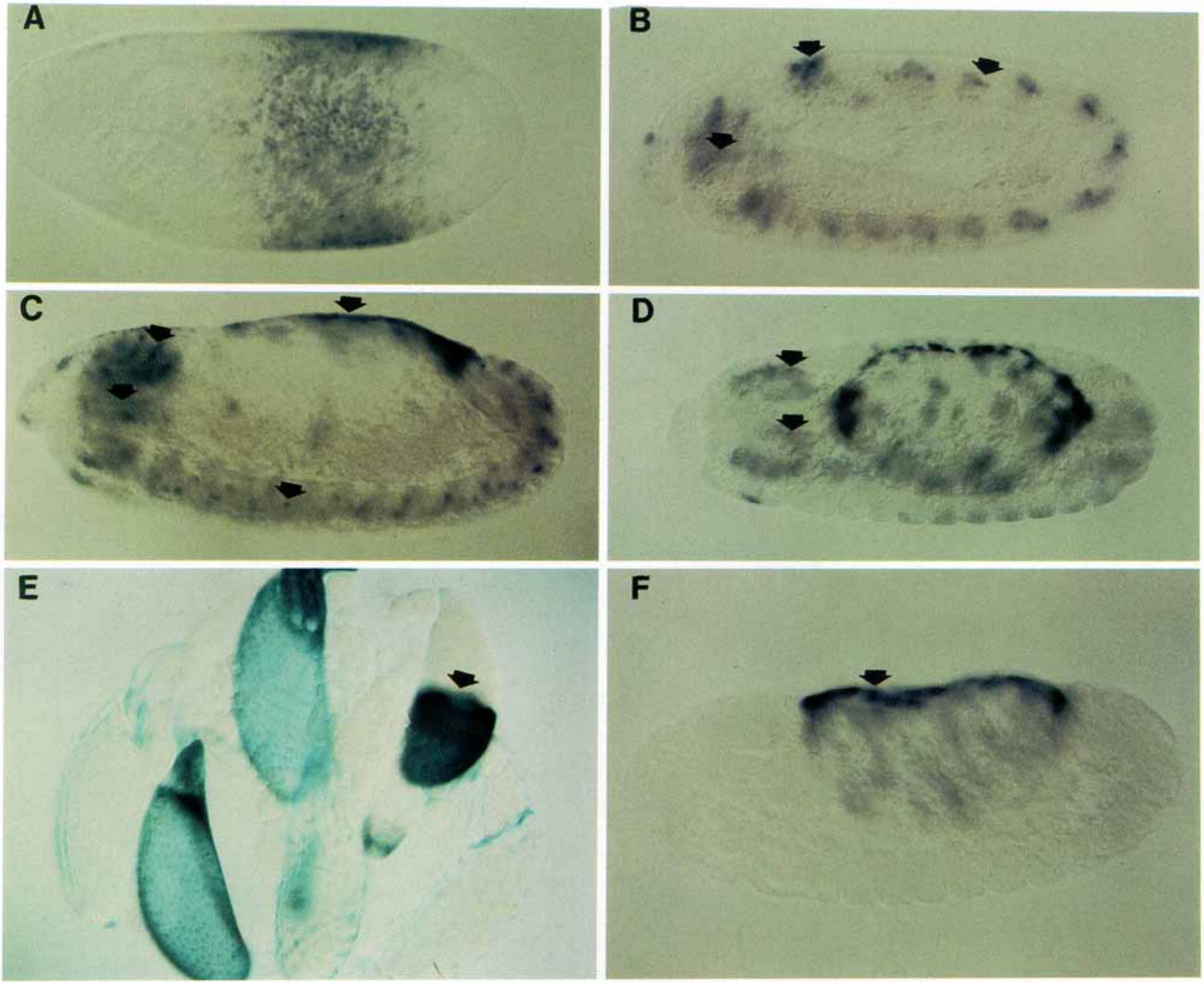
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