Cloning and analysis of the *dec-1* female-sterile locus, a gene required for proper assembly of the *Drosophila* eggshell

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Female-sterile mutations at the *dec-1* (defective chorion-1) locus of *Drosophila* severely disrupt the organization of the eggshell late in oogenesis. Previous characterization of *dec-1* mutations has correlated the defects with failure to accumulate an early eggshell protein that undergoes proteolytic cleavage during choriogenesis. To enable further study of the regulation and processing of *dec-1* products, we have molecularly cloned the locus and characterized its transcripts. Chromosome jumping was used to isolate a deficiency breakpoint within the locus. Overlapping genomic clones from a wild-type library were then obtained, and a region including the *dec-1* locus was identified by hybridization to cDNA probes complementary to RNA from stage 9–10 egg chambers. Analysis of genomic rearrangements associated with the locus verified its identity. Two transcripts from the locus have been identified and characterized using cDNA clones, RNase protection, and primer extension analyses. A 4.0-kb transcript accumulates maximally in stages 9–10, when the primary follicle cell protein associated with *dec-1* mutations is synthesized. A second transcript of 5.8 kb, generated by alternative splicing, accumulates during stages 11–12. These results are discussed in light of previous analysis of *dec-1* mutations.

[Key Words: Eggshell assembly; *Drosophila*; *dec-1*; oogenesis; 7C female-sterile mutants]

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Synthesis of the *Drosophila* eggshell provides an excellent opportunity to integrate morphological, biochemical, genetic, and molecular studies toward the understanding of a complex macromolecular assembly process under developmental regulation. In a relatively brief period near the end of oogenesis, somatically derived follicle cells sequentially produce and secrete proteins assembled into the vitelline membrane, innermost chorionic layer, endochorion, and exochorion (King and Koch 1963; Quattrocchi and Anderson 1969; Margaritis et al. 1980). An extensive review of eggshell structure and morphogenesis has been published recently by Margaritis (1985). Major protein components of the chorion and vitelline membrane have been identified (Petri et al. 1976; Waring and Mahowald 1979; Fargnoli and Waring 1982), but little is known about their individual roles in eggshell structure.

The potential of genetic analysis in *Drosophila* can be exploited to identify loci essential to proper eggshell morphogenesis. Two female-sterile mutants that produce eggs with abnormal chorions, oc and cor36, have been found defective in the synthesis of proteins encoded by the 7F chorion gene cluster (Digan et al. 1979; Spradling et al. 1979). Screens for X-linked female-sterile mutants have recovered additional mutations affecting eggshell morphology (Gans et al. 1975; Komitopoulos et al. 1983; Mohler 1977; Mohler and Carrol 1984; L. Engstrom, unpubl.). Among these are alleles of a large complementation group [*dec-1; fs(1)384; fs(1)410*] mapping at 7C on the X chromosome.

*dec-1* mutants produce eggs with gross eggshell deformities that become apparent late in oogenesis, during stages 13–14. Ultrastructural analysis of mutant stage-14 egg chambers revealed a general lack of endochorion organization and accumulation of chorion material within the vitelline membrane (Bauer and Waring 1987). Biochemical analysis correlated the mutations with a failure to accumulate a 130-kD stage-10 follicle cell protein that is cleaved and incorporated into wild-type stage-10 eggshells as an 85-kD product. s85 is subsequently processed to a 67-kD eggshell component, primarily during stages 13–14, when mutant eggshell morphological abnormalities first appear. Genetic and biochemical analyses, using *dec-1* alleles and a wild-type strain producing electrophoretic variants of these proteins, strongly suggest that the mutant phenotype derives from mutations at the locus encoding the 130-kD product (Lineruth and Lambertsson 1986; Bauer and Waring 1987).

The multiple, temporally regulated cleavages that
dec-1 proteins undergo make them unique among eggshell proteins analyzed to date. Furthermore, the temporal coincidence of the s85–s67 cleavage in wild-type eggshells and the extreme loss of endochorion organization in dec-1 mutant eggshells suggest that dec-1 products may provide an essential framework for assembly of eggshell proteins synthesized and secreted later in oogenesis. In addition, the dec-1 locus appears to be exceptionally mutable, as dec-1 mutations have been recovered at a frequency far greater than female-sterile alleles with eggshell defects at other known eggshell loci (Komitopoulou et al. 1983). As a first step in further analyzing the role of these products and their processing in eggshell morphogenesis, we have cloned and characterized the dec-1 locus. Previous cloning of chorion (Griffin-Shea et al. 1980; Spradling et al. 1980) and putative vitelline membrane (Higgins et al. 1984; Mindrinos et al. 1985; Burke et al. 1987) genes has relied upon the relatively great abundance of transcripts from these loci. In this study, chromosome jumping and walking techniques were used to isolate DNA from the region cytogenetically determined to include the dec-1 locus. Genomic rearrangements of the locus have been identified in a dec-1 mutant and a geographic strain producing variant dec-1 proteins. Two alternatively spliced transcripts with different temporal accumulation profiles have been mapped in the locus. Our characterization of the locus is discussed in connection with previous genetic and biochemical analyses.

Results

Molecular cloning of the dec-1 locus

Previous cytogenetic mapping of dec-1 alleles placed the locus between the 7C1 breakpoint of Df(1) ct^{508-22}, which fails to uncover the recessive sterility of the locus, and the 7C3 breakpoint of Df(1) ct^{6b1}, which succeeds (Komitopoulou et al. 1983; Bauer and Waring 1987). Additionally, the fs(1)1501 mutation characterized by Komitopoulou et al. (1983) fails to complement the sterility of all dec-1 alleles tested, yet is fully complemented by Df(1) ct^{6b1}. This unusual complementation pattern suggested to us that fs(1)1501 may be an allele of the locus and that the ct^{6b1} deficiency breaks in or near the locus without totally eliminating genetic function. Our strategy in cloning the locus, therefore, was to isolate DNA surrounding the 7C3 breakpoint of the ct^{6b1} deficiency by chromosome jumping (Bender et al. 1983) and identify transcription units capable of encoding the 130-kD primary follicle cell protein that fails to accumulate in dec-1 mutants. Using in situ hybridization to the Df(1) ct^{6b1} chromosome, a genomic clone with sequences crossing the 7B1,2 breakpoint of the deficiency was identified among clones isolated previously from the cut region (Jack 1985). A restriction fragment from this clone, distal to the breakpoint, was used to isolate a breakpoint containing clone from a genomic library of the deficiency stock [Fig. 1]. Using 7C sequences from this clone, approximately 20 kb of DNA on both sides of the 7C3 breakpoint was isolated from a wild-type Canton S genomic library [Maniatis et al. 1978].

To identify potential dec-1 transcription units, Southern blots of genomic DNA from the 7C region were probed with labeled cDNA reverse transcribed from stage-9 to stage-10 egg chamber poly(A)^{+} RNA. Message encoding the 130-kD follicle cell protein is expected to accumulate maximally during these stages. Figure 2 shows that a single 9.5-kb region (thick line), which includes the Df(1) ct^{6b1} breakpoint, was found complementary to abundant stage-9 to stage-10 egg chamber poly(A)^{+} RNA. A second region, detected only in long exposures, is also indicated (thin line). Probes from this latter region detect a low abundance 2.7-kb stage-10 transcript that has not been characterized further.

Stage-specific accumulation of overlapping egg chamber RNAs

To assess the size and orientation of the transcript(s) encoded by the 9.5-kb region indicated above, single-stranded RNA probes covering both strands of the entire region were hybridized to Northern blots of total RNA from stage-9 to stage-14 egg chambers. Transcripts of approximately 4.0 and 5.8 kb, both transcribed from the same strand, were detected [data not shown]. Probes covering the 3’-most 2.9 kb of the region fail to detect any egg-chamber transcripts, further defining the transcription unit boundaries between coordinates 0 and 6.6 of Figure 2. Both transcripts are complementary to probes from several subcloned genomic fragments, indicating that they overlap extensively. The temporal accumulation pattern of these transcripts [Fig. 3] was determined

![Figure 1. Isolation 7C3 breakpoint containing clone from Df(1) ct^{6b1}. Sequences distal to the 7B1,2 breakpoint of Df(1) ct^{6b1} were used to select the breakpoint containing clone λoctptg-13 from a genomic library of the deficiency stock. In situ hybridization of biotin-labeled DNA from the clone detects sequences at 7B and 7C on X chromosomes from Oregon R salivary glands.](image-url)
Cloning of the Drosophila dec-1 locus

Figure 2. 7C3 DNA homologous to poly(A)+ RNA from stage-9 to stage-10 egg chambers. A Southern blot of EcoRI-digested DNA from three overlapping Canton S genomic clones covering 40 kb of the 7C3 region was hybridized with 32P-labeled cDNA reverse transcribed from stage-9 to stage-10 poly(A)+ RNA. The thick line above the EcoRI restriction map indicates the region homologous to abundant stage-9 to stage-10 egg chamber RNA. The region is defined by the EcoRI site at coordinate 0 and the beginning of λ-15 sequences at coordinate 9.5, which did not hybridize to the probe. The thin line indicates a second region of homology, detected only in long exposures. 7C3 sequences deleted by Df(1) ct 461 are indicated by the dashed line.

by hybridization of a single-stranded probe covering the 5'-most 1.9 kb of the 6.6-kb region (coordinates 0–1.9 of Fig. 2) to total RNA from egg chambers of different developmental stages. The 4.0-kb transcript accumulates maximally in stage 9, diminishes greatly thereafter, and is undetected by stage 13. The profile is compatible with a message encoding the 130-kD follicle cell protein. fcl30 synthesis peaks in stage 10A, is reduced slightly in stage 10B, and is undetected in stage 11 (Lineruth and Lambertsson 1985; Bauer and Waring 1987). Maximal accumulation of the 5.8-kb transcript is less than that for the smaller transcript and occurs later in oogenesis, during stage 11. Potential function of the large transcript will be addressed in the Discussion.

Deletion of DNA in a strain producing variant 7C eggshell proteins

A codominant locus responsible for electrophoretic variations in a set of follicle cell proteins in the wild-type Shahrinau strain of Drosophila melanogaster has been mapped previously to the 7C region of the X chromosome (Lineruth et al. 1985). In vitro translation of poly(A)+ egg chamber RNA traced the variation to RNA encoding the fcl30 protein (Bauer and Waring 1987). These observations placed the structural locus for the dec-1 related proteins near or at the dec-1 mutant locus. To determine whether a deletion of DNA within the 6.6-kb region identified above could account for the increased electrophoretic mobility of the Shahrinau proteins, genomic Southern blots of Shahrinau and Canton S DNAs were hybridized to a nick-translated probe covering the region. Figure 4 shows that a deletion of Shahrinau DNA approximately 200 bp in length is detected with a HincII fragment from the 6.6-kb region. The size of the deletion is consistent with the approximately 7.5-kD difference in apparent molecular weight between proteins of the different strains (G.L. Waring, unpubl.).

Genomic rearrangement of the 6.6-kb region in a dec-1 mutant

Analysis of electrophoretically variant 7C proteins placed the structural locus for the proteins that fail to accumulate in dec-1 mutants near or at the dec-1 locus. Further analysis by Lineruth and Lambertsson (1986) found that females heterozygous for the Shahrinau variant chromosome and either of two dec-1 mutant chromosomes failed to accumulate standard-size dec-1-related proteins. Together, these results strongly suggest that the dec-1-related proteins are encoded by the dec-1 locus. To determine whether dec-1 mutations could be correlated with defective transcript accumulation from

Figure 3. Temporal profile of transcripts from the 7C3 region. Northern blot of total RNA from 30 egg chambers of the indicated stages, 30 previtellogenic ovaries from newly eclosed flies (PV), and 300 0–2-hr embryos (E) was probed with a T7 transcript probe covering coordinates 0–1.9 (Figs. 2 and 6).
Three clones, which together encompass all the dependent clones from the 6.6-kb region and genomic rearrangements from the 7C3 region covering coordinates 0.3-6.6 (Fig. 6). A deletion of approximately 200 bp is detected within the 1.9-kb HindIII fragment (between coordinates 1.9 and 3.8), whereas flanking fragments of 1.6 and 2.8 kb are unaffected. Additional blots (not shown) place the deletion between HindIII and BamHI sites at coordinates 1.9 and 2.7.

The 6.6-kb region under investigation, Northern blots of egg chamber RNA from dec-1 alleles in our collection [Bauer and Waring 1987] were hybridized with an RNA probe complementary to the 4.0- and 5.8-kb transcripts. Although none of the six dec-1 alleles examined are RNA null mutations, one allele, fs(1)764, accumulates a single 4.4-kb transcript (Fig. 5A).

Genomic Southern blots of fs(1)764 DNA [Fig. 5B] revealed that the aberrantly sized transcript from this mutant was associated with a 1.6-kb insertion within the 6.6-kb region. Although rare in ethyl methanesulfonate (EMS) generated mutants such as those in our collection, translocations have been noted at low frequency [Vogel and Natarajan 1979]. The origin of this inserted DNA has not yet been determined, but genomic Southern blots of restriction fragments with sites within the insertion (not shown) indicate that it is not a duplication of sequences from the 6.6-kb region analyzed.

Figure 4. Deletion of 7C3 sequences in the Shahrinau strain. Genomic Southern blots of HindIII/Smal double digests of DNA from Shahrinau [S] and Canton S [C] strains probed with a nick-translated Smal fragment from the 7C3 region covering coordinates 0.3-6.6 (Fig. 6). A deletion of approximately 200 bp is detected within the 1.9-kb HindIII fragment (between coordinates 1.9 and 3.8), whereas flanking fragments of 1.6 and 2.8 kb are unaffected. Additional blots (not shown) place the deletion between HindIII and BamHI sites at coordinates 1.9 and 2.7.

Mapping of the dec-1 locus transcripts

The size and temporal specificity of the 4.0-kb transcript from the 6.6-kb region and genomic rearrangements within the region in a dec-1 mutant and a strain producing electrophoretically variant dec-1 proteins provide strong evidence that the region under investigation is the dec-1 locus. In addition, the proximal breakpoint of Df(1) ct*10 falls within this region (Figs. 2 and 6), consistent with earlier speculation. RNase protection, cDNA clone, and additional Northern blot analyses were used in order to define the dec-1 transcription unit more precisely and determine the relationship between the two overlapping dec-1 transcripts. The results are summarized in Figure 6.

A library of cDNA clones greater than 2 kb in length was prepared from ovarian poly(A)+ RNA, and 18 independent clones from the dec-1 locus were isolated. Three clones, which together encompass all the sequences recovered, are shown in Figure 6C. The transcription unit defined by hybridization of these clones to Southern blots of cloned genomic DNA is bounded by the EcoRI site at coordinate 0 and the Smal site at coordinate 6.6, consistent with boundaries indicated by Northern blot hybridization and the cDNA probe experiment shown in Figure 2. The total length of unique sequence in clones c23 and c27 is approximately 4.0 kb, indicating that nearly all of the smaller transcript is represented in these two clones. A single clone, c16, contained sequences hybridizing to the 1.4-kb XbaI-SstI genomic fragment (coordinates 4.0-5.4). Probes from this region detect only the 5.8-kb transcript on Northern blots of egg chamber RNA (not shown). This result suggested that failure to splice these sequences from the later stage transcript may account for much of the size difference between the two transcripts.

Figure 5. Molecular defects of a dec-1 mutation. (A) Northern blots of total RNA from 30 stage-9 to stage-12 egg chambers of Oregon R [wt] and fs(1)764 [fs] flies were hybridized with an antisense RNA probe covering coordinates 0-1.9 (Fig. 6). Note the accumulation of a single, aberrantly sized transcript in阶段9 RNA is 146 nucleotides in length, whereas stage-12 RNA protects a predominant fragment that is...
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Figure 6. Molecular map of the dec-1 locus. [A] Heavy lines indicate positions of exons comprising the two poly[A]+ RNAs. Approximate exon sizes are indicated below the lines. The larger message differs from the smaller by the absence of a splice between coordinates 3.9 and 5.6 (broken line). The exact placement of the 272-nucleotide third exon between coordinates 3.2 and 3.6 has not been determined. The Df(1) ct4b1 breakpoint lies between coordinates 3.6 (AccI) and 4.0 (XbaI) and deletes sequences, indicated by the dashed line. A 200-bp deletion in the Shahrinau strain is between coordinates 1.9 (HincII) and 2.7 (BamHI). A 1.6-kb insertion in fs(1)764 maps between coordinates 3.2 (AvalI) and 3.6 (AccI). [B] Restriction map of the Canton S dec-1 locus. Coordinates are identical to those in Fig. 2. EcoRI (R), Smal (M), PstI (P), XhoI (X), HindII (C), AccI (A), XbaI (Z), HindIII (H), SstI (S). The starred BamHI site is not present in the Oregon R strain. [C] Maps for 3 of 18 independent cDNA clones isolated from an ovarian kgtl0 library. C16 is the only clone with sequences unique to the larger message. Gaps in the lines indicate regions where cDNAs diverge from genomic sequences. [D] Antisense RNA transcripts from genomic subclones used for RNase protection mapping.

488 nucleotides long. Additional protection assays with shorter probes extending from the HindIII site suggest that the two protected fragments share a splice acceptor site but diverge at a splice donor site utilized in the 4.0-kb transcript. Protection assays with probes downstream of this region indicate that the large transcript continues without interruption to a polyadenylation site common to both transcripts. No differences upstream of the alternative splice were detected in the two transcripts, suggesting that they differ only by the additional splice in the 4.0-kb message. Small introns near the ends of some probes shown in Figure 6D may have gone undetected; precise mapping will depend upon DNA sequence analysis (G.L. Waring, in prep.).

To determine precisely the transcription initiation site for the two dec-1 transcripts, a combination of RNase protection and primer extension assays was used. Results of 5'-end mapping by RNase protection of our antisense probes are shown in Figure 7B. No difference in fragments protected by stage-9 and stage-12 RNA were detected, indicating the transcripts utilize the same transcription start site. Probes 1 and 3 terminate at the EcoRI site at coordinate 0 and share a 229-nucleotide fragment protected by the first exon. Probes 2 and 4 terminate at an SstI site near coordinate 0.3 and share a 208-nucleotide fragment protected by the first exon. This analysis indicates that the 5' end of the gene lies 21 nucleotides upstream of the last nucleotide in the SstI site. Upstream of the XhoI site at coordinate 0.8, 219 nucleotides of probes 1 and 2 are protected by exon 2 sequences, indicating that the first intron is approximately 60 nucleotides long. Exon 2 also protects fragments of probes 3 and 4 greater than 1 kb in length, not shown in Figure 7B.

To ensure that the 229-bp exon marked the 5' end of the gene, the position of the RNA cap site was also de-
Figure 7. (A) Differential RNase protection of probe by stage-9 and stage-12 RNA. RNA from 50 stage-9 or stage-12 egg chambers was hybridized to an antisense probe covering coordinates 3.6–4.2, where the two dec-1 transcripts diverge (see Fig. 6). The predominant fragments protected by stage-9 and stage-12 egg chamber RNA (146 and 488 nucleotides, respectively) are clearly different. (B) 5’-end mapping by RNase protection. Total RNA from 50 stage-9 or stage-12 egg chambers was hybridized to the indicated probes (designated by arrows) from coordinate region 0–1.9 and digested with RNase T1 and A, as described in Materials and methods. Below the restriction map of the region (R = EcoRI; S = SstI; X = XhoI; c = HincII) is the proposed intron–exon structure of the 5’ end of the gene. The first exon protects 229 nucleotides of probes 1 and 3 and 208 nucleotides of probes 2 and 4. This places the 5’ end approximately 21 nucleotides upstream of the last nucleotide in the SstI site. The second exon protects 219 nucleotides of probes 1 and 2, placing the end of the first intron approximately that distance form the XhoI site. The second exon also protects long (>1000 nucleotide) fragments of probes 3 and 4, not shown in this figure. No difference in fragments protected by stage-9 and stage-12 RNA is detected. A Klenow-labeled HinfI digest of x-174 RF DNA was used as size markers.

determined by primer extension analysis. Subcloned genomic restriction fragments including the putative 5’ end were sequenced in both directions, and a 19-mer from the 229-bp exon was synthesized. The end-labeled primer was hybridized to ovarian poly(A) + RNA and extended with reverse transcriptase. Extension products of this primer are shown in Figure 8, alongside 121 nucleotides of Canton S genomic subclone sequence generated with the same primer by dideoxy chain termination. This analysis placed the RNA cap site within the nucleotide of that indicated by RNase protection. The sequence around the start site matches 12 of 13 positions of the consensus sequence ANNYRTCAGTYNR, compiled by Cherbas et al. (1986). Less well-matched stretches overlap this sequence; however, the primer extension products suggest that they are used as start sites rarely, if ever. The sequence TATTT is found at −30 and may function as a TATA-type element. The first ATG triplet occurs at +76 and heads an open reading frame that encodes a potential signal peptide, as might be expected for a secreted eggshell protein (G.L. Waring, in prep.).
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Figure 8. Primer extension mapping of the 5' end. (A) An end-labeled 19-mer, 5'-GTTACTTCACTTTGTCCGG-3' complementary to nucleotides +125–143 was annealed to poly(A)+ RNA from 100 ovaries and extended with reverse transcriptase, as described in Materials and methods. The same primer was used to generate a sequence ladder from a XhoI-EcoRI subclone of Canton S genomic DNA (coordinates 0–0.8 of Fig. 6) by dideoxy chain termination. [B] Sense strand sequence of the region shown in A. Sequencing of both strands of Canton S genomic subclones was used to obtain the sequence shown. Arrow indicates start of transcription, which is 20 nucleotides upstream of the first ATG triplet begins at +76.

Discussion

Previous analysis has correlated the extreme loss of endochorion organization in *Drosophila dec-1* mutant eggshells with failure to accumulate a *dec-1* locus-encoded follicle cell protein produced during early eggshell formation. In wild-type flies, the follicle cell protein is precursor to at least two eggshell proteins produced by temporally regulated proteolytic cleavages. Cloning and characterization of the *dec-1* locus reported here will enable us to further study the role of these proteins and their processing with the aid of protein sequence information, production of specific immunological reagents, and analysis of extant and in vitro–generated mutations. Further discussion of molecular genetic analysis is appropriate in light of the characterization of the *dec-1* locus presented here.

Prior to cloning the locus, we postulated that the proximal breakpoint of *Df(1)ct 461* may lie in or near the locus based upon the unusual complementation pattern of *fs(1)1501* with the deficiency and many *dec-1* alleles [Komitopoulou et al. 1983; R.J. Hawley, unpubl.]. *fs(1)1501* females are sterile but lay eggs with no gross eggshell defects. All trans combinations of *fs(1)1501* and *dec-1* alleles tested are sterile and lay eggs characteristic of *fs(1)1501* homozygotes, suggesting that *fs(1)1501* may be an allele of the *dec-1* locus with less severe effects on eggshell morphogenesis. Since this work began, Lineruth and Lambertsson [1986] have reported the synthesis of high-molecular-weight ovarian proteins believed to be related to wild-type *dec-1* proteins by females homozygous and heterozygous for the 1501 mutation. In addition, normal *dec-1* proteins are absent in 1501 homozygotes. Similar high-molecular-weight proteins, approximately 25 kD larger than their wild-type *dec-1* proteins, have been found in 1501 follicle cell fractions with the same temporal profile as wild-type *dec-1* proteins [G.L. Waring, unpubl.]. It is conceivable that such proteins could provide sufficient wild-type function to account for the absence of gross eggshell defects in 1501 egg chambers.

More problematic is the complementation of *fs(1)1501* sterility by *Df(1)ct 461*. The deficiency breaks within the *dec-1* locus, deleting DNA encoding a significant portion of the 3' ends of the two transcripts [Fig. 6]. Although it is possible that the *fs(1)1501* chromosome could carry a female-sterile lesion not related to the *dec-1* locus and lying outside the deficiency region, the failure to observe complementation of 1501 sterility by at least 15 *dec-1* alleles makes this an unlikely explanation. More likely is the possibility that both the deficiency and 1501 chromosomes can provide some *dec-1* function. Consistent with this hypothesis is our recent finding that the deficiency chromosome retains a sufficient portion of the *dec-1* locus to encode fc130. Females heterozygous for the *Df(1)ct 461* and Shahrinav variant chromosomes produce standard- and variant-sized versions of fc130 and its cleavage products in equimolar quantities [G.L. Waring, unpubl.]. Standard-sized fc130 is apparently encoded by a stable 3.8-kb transcript of the deficiency chromosome [R.J. Hawley, unpubl.].

The failure of the deficiency to complement most *dec-1* alleles suggests that most alleles lack a second function at the locus, which can be provided by *fs(1)1501*. A likely candidate for this function is a product encoded by the 5.8-kb transcript. Although the finding of the 5.8-kb *dec-1* transcript is consistent with a multifunctional locus, we have yet to link a follicle cell or eggshell protein larger than 130 kD to this locus. The potential size of such a protein, however, along with the stage specificity and abundance of the alternative transcript, suggests that a wild-type translation product...
could have gone undetected in previous analysis. DNA sequencing now in progress will allow us to predict the nature of the potential translation product from this transcript. It may be useful for further biochemical analysis to determine the spatial accumulation pattern of the alternative transcript. Recent in situ hybridization studies by Parks and Spradling [1987] have found that minor transcripts from the 7F chorion gene region accumulate in follicle cell subpopulations that give rise to specialized eggshell structures. Functional tests of the role of the large transcript are also possible by determining to what extent constructs bearing mutations specific to the large transcript are capable of rescuing eggshell defects and sterility of extant dec-1 mutants.

Materials and methods

General nucleic acid techniques

Procedures for screening phage libraries, DNA labeling, subcloning, Southern transfers, and RNA fractionation on formaldehyde–agarose gels are described by Maniatis et al. [1982]. Drosophila RNA and DNA were isolated from egg chambers, ovaries, and whole flies, as described by Spradling and Mahowald [1979, 1980]. RNA transcript probes were prepared according to Melton et al. [1984] from DNA subcloned into pGEM-3 and pGEM-4 vector [Promega Biotec]. In situ hybridization to polytene chromosomes was as described previously [Waring and Pollack 1987].

Chromosome jumping

DNA (7B1,2) distal to the 7B1,2 breakpoint of Df(1) ct 461 [a 4-kb Sall fragment of phage MR 3.0; Jack 1985] was identified by in situ hybridization of 7B genomic clones to deficiency chromosomes and by genomic Southern blotting. A genomic library of DNA from Df(1) ct 461, ct 461 oc ptg/Y, Dp(1;3) sn 461/1Ubx flies was constructed in the λ phage vector EMBL-3, as described by Frischauf et al. [1983]. The Sall fragment was used to screen the unamplified library plated on NM539, and the breakpoint containing clone loctp-g 13 was isolated. Then, 7C3 sequences from loctp-g13 were used to initiate a short walk in a wild-type Canton S library [Maniatis et al. 1978].

cDNA library

A cDNA library was constructed from poly(A)+ RNA of 120 ovaries in the vector agt10 [Huyhn et al. 1985], essentially as described by Watson and Jackson [1985], with the following modifications. Following digestion of EcoRI linkers, the double-stranded cDNA was treated with calf intestinal phosphatase [5 units in 80 μl of 50 mM Tris–HCl at pH 9.5, 1 mM spermidine, and 100 mM EDTA for 30 min at 37°C] to prevent formation of insert chimeras during ligation into the vector. The phosphatase and some excess linkers were then removed by extraction and spin-column chromatography [Maniatis et al. 1982], and the product ethanol precipitated twice from 2 mM ammonium acetate. The cDNA was then fractionated on a 0.8% low melting point agarose gel, and products in the 500- to 2000-bp and >2000-bp size ranges were extracted, ligated to vector, and packaged separately. The large size library was plated on C600 Hfl and screened without amplification using a nick-translated EcoRI–HindIII fragment [coordinates 0–42] of subcloned genomic DNA. Approximately 6 × 108 phage were obtained from an estimated 22 ng of large fraction dscDNA.

RNase protection analysis

Total RNA from 30 to 50 egg chambers was hybridized to antisense RNA probes [typically 106 cpm synthesized using [α-35S]UTP at 100 Ci/mmmole in 10 μl of 80% formamide buffer [80% formamide, 40 mM PIPES (pH 6.6), 400 mM NaCl, 1 mM EDTA]]. Samples were heated at 85°C for 10 min and incubated overnight at 45°C. Then, 100 μl of RNase digestion mixture (1000 μm/μl boiled RNase T1 and 5 μg/μl boiled RNase A in 10 mM Tris–HCl at pH 7.5, 300 mM NaCl, 5 mM EDTA) was added, and digestion was allowed to proceed for 30 min at room temperature. Following digestion, SDS was added to 0.1% and proteinase K to 150 μg/ml, and the samples were incubated for 35°C for 15 min. The hybrids were then extracted with phenol/chloroform and precipitated with 10 μg tRNA as carrier. Hybrids were heated for 10 min at 90°C in 90% formamide, 0.1% bromophenol blue and 0.1% xylene cyanol and analyzed on 4–6% acrylamide–urea sequencing gels. In some instances, heteroduplexes of antisense transcripts from genomic DNA and sense transcripts from subcloned cDNAs were analyzed as above to confirm results of egg chamber RNA analyses.

Primer extension

A 19-mer, 5′-GGTTAACCTCATTGTCCCG-3′, complementary to nucleotides +125–143 was synthesized on an Applied Biosystems Model 380A DNA synthesizer and end-labeled with T4 polynucleotide kinase [sp. act. >5000 Ci/mmmole]. Approximately 0.3 pmole of gel-purified primer was combined with poly(A)+ RNA from 100 ovaries in 6.2 μl of H2O. One microliter of 0.5 M NaCl, 0.5 M Tris–HCl (pH 8.3) was added, the sample was heated to 90°C for 15 min and slowly cooled to 37°C. After 15 min incubation at 37°C, the following was added: 1 μl of 100 mM MgCl2, 100 mM DTT, 1 μl of a 10 mM mixture of all dNTPs, 0.3 μl [12 units] RNasin, and 0.5 μl [5 units] Avian myeloblastosis virus [AMV] reverse transcriptase. The sample was heated at 37°C, and again for 15 min at 43°C. The reaction was terminated by the addition of 3.3 μl of 50 mM EDTA, 8 mM ammonium acetate, and products were precipitated with ethanol on dry ice. Extension products were analyzed on an 8% acrylamide–urea sequencing gel alongside sequence of a subclone of Canton S genomic DNA generated with the same primer by dieoxy chain termination [Sanger et al. 1977].

Drosophila strains

Strains and culture conditions have been described previously [Bauer and Waring 1987].

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Cloning and analysis of the dec-1 female-sterile locus, a gene required for proper assembly of the Drosophila eggshell.

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