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Genetics

Molecular analysis of a gene in a developmentally regulated puff of *Drosophila melanogaster*

(steroid hormone/ecdysone/salivary gland/transcription/glue proteins)

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ABSTRACT An increase in the concentration of the steroid hormone ecdysone in late larval life triggers a profound change in the pattern of polytene chromosome puffs in the *Drosophila melanogaster* salivary gland. One of the preexisting puffs that regress as the ecdysone concentration increases is located at the 3C11-12 bands, the site of the *Sgs-4* gene, which codes for the sgs-4 protein, one of the proteins in the salivary glue secretion. We have isolated cloned segments of chromosomal DNA that define a 60-kilobase region containing the 0.9-kilobase *Sgs-4* gene, and we have determined its position and orientation within this region. Fine structure restriction endonuclease mapping shows that approximately 45% of this gene consists of tandemly repeated sequences of 21 base pairs that occupy most of its 5′ half, indicating that most of the amino-terminal half of the sgs-4 protein consists of tandemly repeated amino acid sequences of seven residues. We also report on the amount of the *Sgs-4* mRNA as a function of developmental stage and in nine different strains, four of which produce little or no sgs-4 protein. Three of the null strains produce minute amounts of the mRNA and one yields none, whereas the five sgs-4 producing strains yield abundant amounts. The mRNAs from these strains exhibit different lengths, which correlate with different gene lengths that appear to result from different numbers of the repeated sequences in their tandem arrays.

A rapid increase in the concentration of the steroid hormone ecdysone near the end of the larval life of *Drosophila melanogaster* (1) signals the beginning of metamorphosis to the adult fly. Imaginal tissues initiate their transformation to adult structures and larval tissues commence their downward path to histolysis. These transitions presumably result from changes in gene expression that are, at least in part, triggered by ecdysone. That ecdysone can indeed trigger ordered changes of genetic activity is strikingly demonstrated by its effects on the puffs of transcription in the polytene chromosomes of the larval salivary gland (reviewed in ref. 2). The increase in ecdysone concentration is accompanied by the regression of certain of the preexisting or "intermolt" puffs, the concurrent induction of a small class of "early" puffs, and the subsequent formation of a complex class of "late" puffs—*in vivo* transitions that are mimicked *in vitro* by exposure of explanted glands to ecdysone.

We have initiated an investigation of ecdysone-triggered regulatory hierarchies by isolating and analyzing the genes that are transcribed in these puffs. A procedure for isolating cloned cDNAs derived from the transcripts of these genes has been developed and will be described in detail elsewhere.‡ In brief, it consists of constructing libraries of cDNA clones from the total poly(A)$^+$ RNA of glands at different stages of development, and of assigning individual clones to developmental classes by screening these libraries with a set of stage-specific hybridization probes, each probe representing the sequences in the poly(A)$^+$ RNA population of glands at a given stage. For example, clones that exhibit strong hybridization with probes from glands obtained prior to the increase in ecdysone concentration, but reduced or no hybridization with probes from glands exposed to ecdysone, were assigned to the intermolt class. Such a class can be divided into homologous sequence groups by cross-hybridization tests among its members, and such a sequence group can be mapped on the polytene chromosomes by *in situ* hybridization of one or more of its members to these chromosomes.

In this paper, we consider a gene represented by one of five nonoverlapping sequence groups that belong to the intermolt class. It is called group I and maps, by *in situ* hybridization, within the 3C7-3D1 region of the X chromosome,‡ a region that contains an intermolt puff (2). This region also contains the *Sgs-4* gene. Deletion mapping has placed this gene at bands 3C11-12 (refs. 3 and 4), the site of the intermolt puff (5, 6). Genetic analysis of electrophoretic variants and gene dosage studies, as well as the deletion mapping, indicate that *Sgs-4* codes for one of the proteins that are synthesized in large amounts during the period of intermolt puffing and that are subsequently secreted from the salivary gland to form the glue by which pupae attach to solid surfaces (3, 4, 7–9). These correlations not only indicate that the mRNA for this sgs-4 protein [protein 4 of Korge (3, 7, 9) and the P3 protein of Beckendorf and Kafatos (8)] is transcribed within the 3C11-12 puff but also suggest that it is the source of the group I cDNA clones, which provide the starting point for our studies on the group I gene.

MATERIALS AND METHODS

***Drosophila* Strains.** Oregon-R was obtained from M. Green (University of California, Davis, CA); Canton-S was from the Caltech Stock Center; Kochi-R was from the University of Umea (Sweden) stock collection. BER-1, D323, Hikone-R, Seto, Chieti-V, and Urbana-S were obtained from S. K. Beckendorf (University of California, Berkeley, CA).

Recombinant DNA. The group I cDNA clones, pkdm6B3 and pkdm4B1, were isolated by Wolfner *et al.*‡ They consist of cDNA segments inserted by $(dA)_n \cdot (dT)_n$ joints (10) into the pSC105 plasmid (11) at its *Bam*HI site. The isolation of cloned segments of chromosomal DNA from Oregon-R and Canton-S is described in the legend to Fig. 1, and in *Results*. These hybrid

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Abbreviations: kb, 1000 bases or base pairs in single- and double-stranded nucleic acids, respectively; Dm, a cloned segment of chromosomal DNA from *D. melanogaster*; DBM, diazobenzyloxymethyl.

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‡ M. F. Wolfner, D. J. Kemp, M. A. T. Muskavitch, G. H. Guild, and D. S. Hogness, unpublished data.

plasmids or phage were propagated in EK1 host-vector systems under P2 containment, as specified by the National Institutes of Health guidelines.

Enzymes. Restriction endonucleases were purchased from New England BioLabs or Bethesda Research Labs (Rockville, MD), except that *Eco*RI was the gift of J. R. Carlson and *Sal* I was the gift of J. T. Lis. Reverse transcriptase (RNA-dependent DNA polymerase) from avian myeloblastosis virus was obtained from J. Beard (12), and phage T4 DNA ligase and *Escherichia coli* DNA polymerase I were the gifts of S. Scherer and of S. Scherer and J. Widom, respectively.

Isolation and Labeling of Nucleic Acids. *D. melanogaster* embryonic DNA (13), λ phage DNA (14), and plasmid DNA (10) were isolated as described in the indicated references, with minor modifications. The procedure for isolation of third instar larval salivary glands and the RNA from these glands will be described elsewhere;‡ isolation of RNA from whole organisms was carried out by this procedure, except that the organisms were disrupted in a Dounce homogenizer before treatment with phenol. Mouse mitochondrial RNA was the gift of M. Walberg. Unless otherwise indicated, DNAs were radioactively labeled by nick translation (15).

Hybridization Conditions. Hybridizations were performed in 50% (vol/vol) formamide/0.75 M NaCl/0.075 M sodium citrate/500 μg of salmon sperm DNA per ml/0.2% sodium dodecyl sulfate/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin/0.02% Ficoll at 42°C for 48 to 72 hr. The nitrocellulose or diazobenzyloxymethyl (DBM) paper containing the hybridized nucleic acids was washed four times for 30 min in 0.15 M NaCl/0.015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C and exposed for autoradiography at −70°C to Kodak XR-5 film with a Du Pont Lightning Plus intensifying screen.

RESULTS

Mapping the Group I Gene Within the Chromosomal DNA. The group I gene is defined by the chromosomal DNA sequences that are homologous to the poly(A)$^+$ RNA from which the group I cDNA clones were isolated. This group contains eight independently cloned cDNAs derived from the poly(A)$^+$ RNA of intermolt salivary glands ("puff stage 1"; ref. 2) from the Oregon-R wild-type strain. The pkdm6B3 clone is a member of this group and provides the starting material for the isolation of the cloned segments of *Drosophila* chromosomal DNA (Dm segments) that contain the group I gene and its surrounding DNA.

Fig. 1 shows a set of Dm segments that define a 60-kilobase (kb) region containing the gene and a 31-kb restriction map for a part of that region. Isolation of these segments was initiated by using labeled pkdm6B3 as a plaque-hybridization probe (17) to screen a library of λ hybrid phage clones containing segments obtained by shear breakage of embryonic DNA from the Canton-S wild-type strain (16); Dm2151–2154 were isolated in this manner. Dm1568–1572 were isolated from this Canton-S library by a "walking" method described in the legend to Fig. 1, which also describes the isolation of a set of cloned Oregon-R embryonic DNA segments. These consist of the large *Sac* I fragment, Dm1528, and the *Hin*dIII fragments A–E, G, and H, indicated on the restriction map (Fig. 1). The *Hin*dIII F fragment was obtained as a subclone of Dm2153, and hence derives from Canton-S.

When these cloned *Hin*dIII fragments were labeled and hybridized to Southern blots (23) of gels containing electrophoretically separated fragments from a *Hin*dIII digest of total Canton-S embryonic DNA, each revealed a single band of hybridization consisting of fragments the same length as the

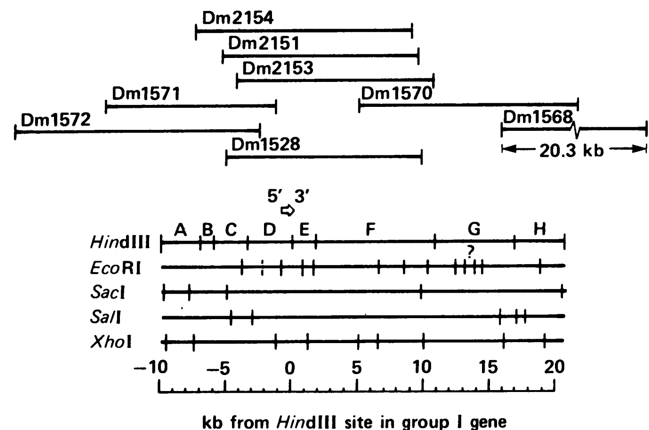

FIG. 1. Organization of the chromosomal DNA in a region containing the group I gene. Segments Dm2151–2154 were isolated as described in the text from a library of Canton-S segments cloned in the λ Charon 4 vector (16). Labeled restriction fragments at or near the right and left ends, respectively, of Dm2153 and Dm2154 were used as plaque-hybridization probes (17) to screen this same library to obtain Dm1570 and Dm1571, whose near-terminal fragments were similarly used to isolate Dm1568 and Dm1572. The *Hin*dIII F fragment from Dm2153 was cloned in λ590 (18) by previously described procedures (19) and is termed Dm1551. The other cloned *Hin*dIII fragments (A–E, G, and H) were isolated from a library of *Hin*dIII fragments from Oregon-R embryonic DNA cloned in λ590 by a previously described procedure (20), using appropriate fragments from the cloned Canton-S Dm segments as probes. The Dm numbers for the A–E fragments are 1526–1522, respectively, and for G and H are 1520 and 1519, respectively. Dm1528 was isolated from a library of Oregon-R *Sac* I fragments obtained from embryonic DNA and cloned in λSep6 by the above method (20), using Dm1551 as the probe. Dm hybrids with λSep6, λ Charon 4 and λ590 are referred to as λbDm, λcDm, and λdDm, respectively, followed by the number of the Dm segment. The restriction map was determined from lengths of fragments in single, double, and partial digests of cloned segments, as determined by agarose gel electrophoresis, using the lengths of restriction fragments in λ (21) or pBR322 (22) as standards. The *Eco*RI site indicated by the vertical broken line is present in Canton-S but not in Oregon-R. The question mark over the *Eco*RI map of fragment G indicates the site is present at one of the two flanking locations shown. The open arrow indicates the group I gene.

fragment used as the probe. These results indicate that the 31-kb region defined by these fragments consists of single-copy sequences, with the possible exception of sequences that are too small to be detected or that are internally repeated within a fragment. The same conclusion applies to the equivalent region in Oregon-R and is consistent with our observation that the *in situ* hybridization of Dm1528 and Dm2151 to polytene chromosomes is confined to the 3C7-3D1 region, with one qualification that is peculiar to our stock. Thus, when the above experiment was repeated with a *Hin*dIII digest of total Oregon-R embryonic DNA, the same result was obtained with each of the probes except that formed from the cloned F fragment. In this case, three length classes of hybridizing fragments were observed: one exhibiting the same length as the 9.0-kb F fragment, and two others of 6.3 kb and 12.0 kb. However, the Oregon-R stock used in the accompanying paper (4) is like our Canton-S population in that it carries only the 9.0-kb *Hin*dIII fragment. We have not identified the origins of the additional 6.3-kb and 12.0-kb fragments in our stock, but we suspect they represent a polymorphism at this locus.

The cloned group I cDNAs contain a single *Hin*dIII site. We demonstrated that this site is equivalent to that joining the D and E *Hin*dIII fragments by hybridizing labeled pkdm6B3 to a Southern gel blot of a *Hin*dIII digest of λcDm2151 DNA (Fig. 1); hybridization was restricted to the D and E fragments. Be-

cause the cloned cDNAs obtained by Wolfner *et al.* are often shorter than the poly(A)$^+$ RNAs from which they derive, this result does not eliminate the possibility that the group I gene extends to other *Hin*dIII fragments. This possibility was tested by hybridizing each of the cloned *Hin*dIII fragments defining the 31-kb region to DBM paper blots (24) of agarose gels containing electrophoretically fractionated total RNA from Oregon-R larvae in the intermolt puff stage. Channel Or of Fig. 4 shows that the D fragment hybridized only to 0.95-kb RNA molecules. Longer RNAs that might serve as precursors to this RNA were not detected, even after very long exposures. The E fragment probe yielded the same result, as did probes obtained from the group I cDNA clones, pkdm6B3 and pkdm4B1. By contrast, probes obtained from the other *Hin*dIII fragments did not yield any detectable hybridization. We conclude that the group I gene is confined to the right and left ends of the D and E fragments, respectively. Furthermore, this is the only gene within the 31-kb region that yields an abundant RNA in larvae at this stage of development.

Fig. 2 shows a fine structure restriction map for a 1.3-kb region of Oregon-R DNA that includes the *Hin*dIII site of the group I gene. The left end of the gene, which corresponds to the 5′ end of its transcript (see below), was located within this region by hybridization of restriction fragments to electrophoretically fractionated total RNA from Oregon-R larvae in the intermolt puff stage, according to the procedure used above to localize the gene to the D and E *Hin*dIII fragments. Among the different fragments assayed, the results from only the following four need be noted. The 0.63-kb *Fnu*4HI–*Hin*dIII fragment hybridized strongly to the 0.95-kb RNA, whereas the *Xho* I–*Fnu*4HI fragment immediately to its left exhibited a weak but definite hybridization to this RNA; hence, a small fraction of the gene lies to the left of this *Fnu*4HI site. The 0.69-kb *Eco*RI–*Hin*dIII fragment also hybridized strongly to the group I RNA, but the *Xho* I–*Eco*RI fragment to its left exhibited no detectable hybridization to the RNA. Given that hybridization to regions of homology less than 50 base pairs in length might not be detected, these data indicate that the 5′ end of the group I gene is 0.70 ± 0.04 kb to the left of its *Hin*dIII site. Results consistent with this location have been obtained from S1 nuclease protection experiments (25) that will be reported in a subsequent publication concerned with a nucleotide sequence analysis of the group I gene in Oregon-R and other strains. Neither fine structure restriction mapping nor S1 protection experiments have yet been employed to locate the right or 3′ end of the gene. However, the nucleotide sequence analysis noted above suggests that it is located 0.20 kb to the right of the *Hin*dIII site, which is consistent with the 0.95-kb length of the RNA, given that it is polyadenylylated to some degree.

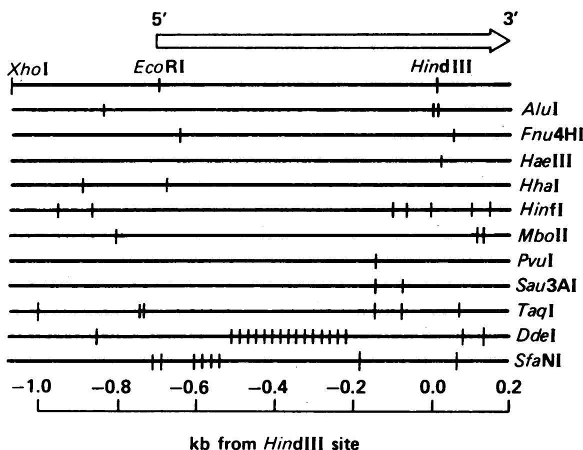

FIG. 2. Fine structure restriction map of the Oregon-R group I gene. This map was constructed in the same manner as that in Fig. 1, with the addition that nucleotide sequence analysis (unpublished) was used to identify certain sites. The number of *Dde* I sites shown in the tandem array is accurate to ±1. The 5′ and 3′ ends of the group I gene indicated by the open arrow are approximate (see text).

Group I Gene Is Transcribed from Left to Right. The direction of transcription of the group I gene was determined by the experiment shown in Fig. 3. The gene used in this experiment is that cloned in λbDm1528, the hybrid λ phage carrying the long *Sac* I fragment, Dm1528, that is shown in Fig. 1. Restriction analysis of this hybrid DNA demonstrated that the left and right ends of Dm1528, oriented as in Fig. 1, have been joined, respectively, to the left and right fragments of the λ phage vector, as is indicated in Fig. 3. The two strands of this hybrid phage were separated by gel electrophoresis and the 5′-to-3′ direction of each was determined by hybridizing each of two strand-specific probes to Southern blots of the gel. The 5′-to-3′ direction of the RNA transcribed from the group I gene, and hence the direction of transcription, was simply determined by identifying the strand of the λbDm1528 DNA that could hybridize with a labeled cDNA probe prepared by reverse transcription of the poly(A)$^+$ RNA from Oregon-R salivary glands in the intermolt puff stage. Fig. 3 shows that this cDNA probe hybridized to the strand whose 5′-to-3′ direction is from left to right, which must therefore be the direction in which the group I gene is transcribed.

Interstrain Correlations Between the Group I and *Sgs-4* Gene Products. A large number of *D. melanogaster* strains have been examined for the amounts of the sgs-4 protein they produce (refs. 3 and 7–9; S. K. Beckendorf, personal communication). Some strains produce no detectable sgs-4 protein, and we examined the group I RNA level in four of these null strains (BER-1, Hikone-R, Kochi-R, and Seto), as well as five sgs-4-producing strains (Canton-S, Chieti-V, D323, Oregon-R and Urbana-S).

Fig. 4 shows the results of this examination. The total RNA isolated from the intermolt larvae of each strain was tested for the presence of group I RNAs by hybridization with labeled *Hin*dIII fragment D as described in the first section of *Results*.

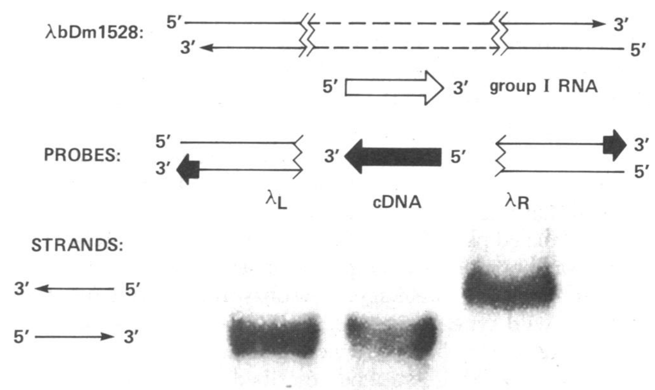

FIG. 3. Orientation of transcription. Strands of λbDm1528 were separated on a neutral 0.5% agarose gel (26) and transferred to nitrocellulose (23). Strips containing single tracks were then hybridized with one of three probes, washed, and autoradiographed. The cDNA probe was synthesized from poly(A)$^+$ RNA of Oregon-R larval salivary glands in the intermolt puff stage under conditions described previously (12). The λ_L and λ_R probes are the left-terminal 5.5-kb *Bam*HI fragment and the right-terminal 11.5-kb *Eco*RI fragment, respectively, of λSep6 DNA (legend, Fig. 1) isolated after 3′-end labeling by filling in the cohesive sites with reverse transcriptase under the conditions for cDNA synthesis (12). The thick, filled portion of the arrows indicates the ^{32}P-labeled region in each probe.

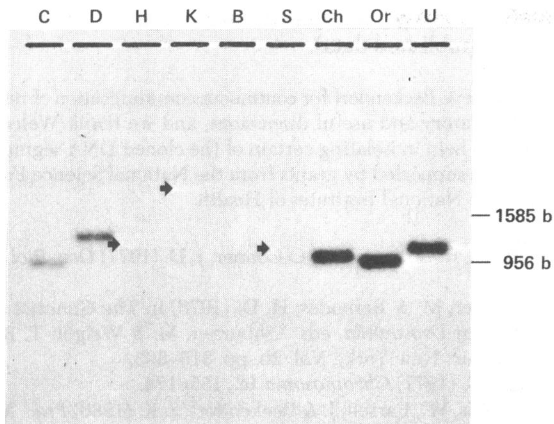

FIG. 4. Interstrain variation in the group I RNA. Equal amounts (5 μg) of total RNA from larvae in the intermolt puff stage were electrophoretically fractionated in 1.5% agarose gels, transferred to DBM paper (24), and hybridized with ^{32}P-labeled Dm1551 (legend, Fig. 1). C, Canton-S; D, D323; H, Hikone-R; K, Kochi-R; B, BER-1; S, Seto; Ch, Chieti-V; Or, Oregon-R; U, Urbana-S. The 956- and 1585-base length standards are mouse mitochondrial rRNAs (27). Exposure, 24 hr. The filled black arrows indicate the positions of the RNAs detected from the Hikone-R, Kochi-R, and Seto strains after 10-fold longer exposure. No RNA from BER-1 was detected at this or longer exposures.

No hybridization could be detected with the RNA from one of the null strains (BER-1) even under conditions in which 0.2% of the amount in Oregon-R can be detected, and the RNAs from the other three null strains exhibit only weak hybridization bands representing less than 3% of the Oregon-R amount. By contrast, each of the five sgs-4-producing strains yields a strong hybridization band. Consideration of the different lengths of the RNAs produced by the different strains is reserved to *Discussion*.

Rise and Fall of the Group I RNA During Development. Fig. 5 shows a low-resolution developmental profile for the group I RNA in the Oregon-R strain. The RNA was assayed by hybridization of a labeled restriction fragment from within the group I gene to DBM paper blots (24) of gels containing equal amounts of electrophoretically fractionated total RNA from whole organisms at different stages of development. The group I RNA was first detected in larvae that had completed approximately 40% of the third-instar period. Its frequency in the total RNA population increases to yield a strong hybridization band by the time 60% of this period is completed, and similarly strong bands were observed with samples taken at 75% and 85% completion (not shown in Fig. 5). However, by the time the white prepupae are formed immediately upon termination of the third-instar period, a precipitous drop in this frequency occurs, and no group I RNA could be detected a few hours later in pigmented prepupae.

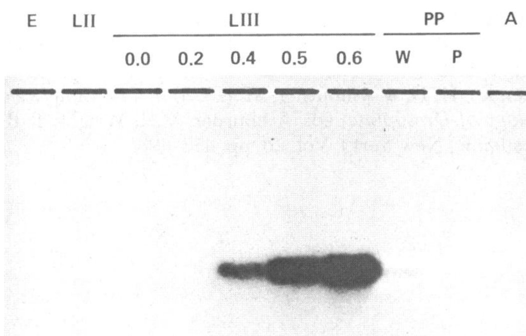

FIG. 5. Developmental profile for the group I RNA. Equal amounts (5 μg) of total RNA isolated from Oregon-R embryos (E), second-instar larvae (LII), third-instar larvae (LIII), prepupae (PP), and adults (A) grown at 24°C were analyzed as described in the legend of Fig. 4. Fractional times of the third-instar period (84–140 hr) are given for the LIII samples. Prepupae were divided into white (W) and pigmented (P) populations. The ^{32}P-labeled hybridization probe consisted of the 0.63-kb *Fnu*4HI–*Hin*dIII fragment located within the group I gene (Fig. 2).

This developmental profile is strikingly like that observed for the salivary glue proteins. Cytochemical analysis of the glands indicates that the glue proteins appear after 40–50% completion of the third-instar period (reviewed in ref. 28). More specific analyses by Korge (9) indicate that the sgs-4 protein, along with other glue proteins, appears after 45–50% completion of third instar, although Beckendorf and Kafatos (8) first detected these proteins at a somewhat later stage. Pulse-label experiments by these authors indicate that the rate of synthesis of sgs-4, and of most other glue proteins, drops precipitously to zero during the transition to white prepupae.

DISCUSSION

The following three lines of evidence sustain the conclusion that the group I gene that we have isolated is equivalent to the *Sgs-4* gene, which codes for the sgs-4 protein.

(i) *Position in the chromosomal DNA*. At the cytogenetic level, the *Sgs-4* gene maps (3, 4) within the region to which the group I gene has been mapped by *in situ* hybridization. At the molecular level, the *Sgs-4* gene has been mapped to a 16- to 19-kb region that includes the group I gene; this is shown in the accompanying paper (4), in which the cloned DNA segments shown in Fig. 1 have been used to locate deletion breakpoints that define a region containing *Sgs-4*. Given that sgs-4 protein is abundant (9), and that the abundant group I RNA is the only transcript from this region that is detectable at a time of sgs-4 synthesis, this positional correlation of the genes provides a strong argument for their equivalence.

(ii) *Time and place of expression*. The developmental period during which the group I RNA is detectable (Fig. 5) corresponds to the period during which the sgs-4 protein is synthesized (8, 9). Similarly, the methods by which the group I cDNA clones and the sgs-4 protein are isolated and identified indicate that both genes are expressed in the third-instar salivary glands. That the expression of the group I gene is restricted to the salivary glands, as appears to be the case for the *Sgs-4* gene (9), has recently been demonstrated by *in situ* hybridization of labeled group I DNA sequences to the RNA in thin sections of third-instar larvae (M. E. Akam and D. S. Hogness, unpublished data).

(iii) *Variant strains*. Four out of four strains that did not contain detectable amounts of the sgs-4 protein yield little or no group I RNA, whereas this RNA is abundant in five out of five sgs-4-producing strains (Fig. 4). Furthermore, S. K. Beckendorf (personal communication) has noted that the amount of the sgs-4 protein produced by the Canton-S strain is significantly less than that produced by Oregon-R, and we have observed a similar ratio for the amounts of the group I RNA.

The conclusion that the group I gene and the *Sgs-4* structural gene are equivalent eliminates the utility of maintaining independent definitions, and hereafter we encompass both under the *Sgs-4* title. Because only one length class of RNA homologous to this gene was detected in a given strain (Figs. 4 and 5), we presume that it represents the messenger RNA coding for the sgs-4 protein. The 0.95-kb mRNA in Oregon-R has a maximal coding capacity of 35,000 daltons of polypeptide. While the molecular weight of the sgs-4 polypeptide must be somewhat less than this value, it provides the best estimate of that molecular weight. Attempts to evaluate the molecular weight

of the sgs-4 protein by electrophoresis in sodium dodecyl sulfate/acrylamide gels have not been successful (8, 9). This is because the protein is glycosylated and behaves anomalously in these gels to yield inflated "apparent molecular weights" that vary with the acrylamide concentration (8).

The structure of the Oregon-R *Sgs-4* gene provides further information about the sgs-4 protein. Inspection of the *Dde* I restriction map given in Fig. 2 reveals a region that is cut by this enzyme into 14 fragments, each of 21 base pairs. The tandemly repeated sequence implied by this result has been confirmed by nucleotide sequence analysis, which also revealed another tandem array of 21-base-pair repeat units located immediately upstream from the first (unpublished data). The sequence of this second unit is similar to that of the first but contains a *Sfa*NI site instead of a *Dde* I site, as indicated in Fig. 2. The two arrays define a region representing ≈45% of the gene that is located ≈0.1 kb downstream from its 5′ end, and is not cut by any of the 30 other restriction endonucleases tested. Evidently the amino-terminal half of the sgs-4 protein consists, for the most part, of a tandem array of very similar seven-residue amino acid sequences.

The tandemly repeated sequences within the *Sgs-4* gene are also of interest because they provide an explanation for the variation in length of the mRNA in different strains (Fig. 4). Examination of the structure of *Sgs-4* in each of these strains reveals a variation in the length of the *Eco*RI-*Hin*dIII fragment encompassing the tandem arrays (Fig. 2) that directly correlates with the variation in length of the corresponding mRNAs; furthermore, comparison of the nucleotide sequences for this region in three of these strains indicates that variation in the fragment length results from variation in the number of tandem repeats (unpublished). Thus, it appears that the tandem repeats provide a means whereby the *Sgs-4* gene can expand or contract, yielding mRNAs, and presumably sgs-4 proteins, that contain different numbers of repeat units.

Inspection of Fig. 4 reveals no evident correlation between the length and the amount of the mRNAs in the different strains. This suggests that the decrease in the amount of mRNA in the sgs-4 null strains is not due to a decrease in their stability resulting from differences in structure—i.e., from a difference in the number of repeat units. Given that we could not detect longer RNAs that might be processed to yield the mRNAs, we have adopted the working hypothesis that differences in the amounts of mRNA obtained from sgs-4 null and producing strains are primarily determined by differences in rates of transcription. Korge's observation that the Hikone-R null strain is also deficient in puffing, and apparently in [^{3}H]uridine incorporation at the 3C11-12 site, is consistent with this hypothesis (3). Our approach has been to examine the DNA sequence organization at and upstream from the 5′ end of the *Sgs-4* genes in both sgs-4-null and sgs-4-producing strains; *mirabile dictu*, our results indicate that all four null strains suffer a small deletion of 50–110 base pairs upstream from the *Eco*RI site shown in Fig. 2 (unpublished data).

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