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The Role of *su(f)* Gene Function and Ecdysterone in Transcription of Glue Polypeptide mRNAs in *Drosophila melanogaster*

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Summary. The effect of the $l(1)su(f)^{ts67g}$ mutation and ecdysterone on the expression of glue polypeptide genes in *Drosophila melanogaster* has been studied. Using cloned DNA harboring glue protein genes Sgs3, Sgs4, Sgs7, and Sgs8, and Northern blot hybridization we show that no or extremely reduced accumulation of RNAs from these genes occurs in mutant larvae shifted to 30° C before and through 70 h after oviposition. On the other hand, a shift-up at 75 h or later results in the production of nearly normal amounts of these RNAs 48 h after shift-up. The results indicate functions of the *su(f)* gene other than suppression of forked bristles, namely that it is essential early in the third instar for the later transcription of the glue polypeptide genes. We also show that administration of exogenous ecdysterone to hormone deficient larvae induces transcription of Sgs3, Sgs4, Sgs7, and Sgs8 RNAs.

Introduction

The salivary glands of *Drosophila melanogaster* larvae synthesize large amounts of a limited number of secretion proteins, the glue proteins. As a consequence of the increase in ecdysterone titer before puparium formation they are secreted into the glands' lumen, and, as puparium formation occurs, released through the mouth (Korge 1975, 1977a; Beckendorf and Kafatos 1976; Zhimulev and Kolesnikov 1975). The secretion functions to affix the puparium to its substrate.

The genes encoding several of the glue polypeptides have been genetically mapped to intermolt puffs that are active in the salivary gland polytene chromosomes during glue protein synthesis (Korge 1975, 1977b; Akam et al. 1978; Velissariou and Ashburner 1980, 1981). It has recently been confirmed at the molecular level that puff 3C11–12 contains the structural gene for glue protein sgs4 (Muskavitch and Hogness 1980, 1982; McGinnis et al. 1980), and that 68C3–5 harbors the coding sequences for glue proteins sgs3, sgs7 and sgs8 (Meyerowitz and Hogness 1982; Meyerowitz 1982; McGinnis, personal communication). The intermolt puffs regress just before puparium formation in response to the dramatic increase in the concentration of ecdysterone that occurs at that time (reviewed by Ashburner and Berendes 1978). It has recently been shown by Gronemeyer and Pongs (1980) that ecdysterone binds to the 68C intermolt puff at the time of its regression, suggesting that transcription at this chromosomal site is directly affected by a hormone-receptor complex.

We have previously reported that the $l(1)su(f)^{ts67g}$ mutation, subsequently referred to as *ts67*, suppresses the developmentally regulated expression of the glue protein genes at 30° C (Hansson et al. 1981). These larvae become ecdysterone deficient in the sense that the hormone titer fails to increase when shifted to 30° C (Hansson et al. 1981). Two results from this study and two other observations provide the basis for the experiments presented here. First, no or extremely reduced synthesis of glue polypeptides could be detected at 30° C, general protein synthesis being unaffected (Hansson et al. 1981). Second, the chromosomal regions known to code for glue proteins at the same time showed prominent puffs (Hansson et al. 1981). We speculated then that the puffs observed at 30°C were active in RNA transcription, and that the lesion caused by the *ts67* mutation was at the translational level. We could not, however, rule out the possibility that the puffs were inactive or that the structural gene within the puff was not being transcribed. Third, a small ecdysterone peak was observed at about 84 h after oviposition (Berreur et al. 1979), that is, just before the Sgs3, Sgs4, and Sgs7/Sgs8 RNAs can first be detected. Finally, *ts67* larvae form pseudopupae when ecdysterone is added to the food at 30° C (Hansson and Lambertsson 1983). These observations implicate *su(f)* gene function in the biosynthesis or regulatory system of ecdysterone, and suggested to us that transcription of glue polypeptide genes may be ecdysterone-inducible.

Using cloned probes of glue polypeptide genes Sgs3, Sgs4, Sgs7, and Sgs8 (Muskavitch and Hogness 1980; Meyerowitz and Hogness 1982; generously provided by Dr. S. Beckendorf, Berkeley), we analyzed whether these genes produce any transcripts at the nonpermissive temperature, with or without exogenous ecdysterone. We report here that no or extremely reduced amounts of mRNA from these genes are transcribed in *ts67* larvae shifted to 30° C before and up to 70 h after oviposition. However, a shift-up at 75 h or later results in the production of nearly normal amounts of these transcripts. We also show that administration of ecdysterone to hormone deficient larvae induces transcription of Sgs3, Sgs4, Sgs7, and Sgs8 RNAs.

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Materials and Methods

Materials. Restriction endonucleases were purchased from Boehringer Mannheim, FRG or Bethesda Research Labs, USA. Nick translation components (including isotope) were obtained from New England Nuclear, USA. *l(1)su(f)^{ts67g}* (*ts67*) was that kept at the Drosophila Stock Center, Umeå, Sweden. The BER-1 wild-type strain was obtained from Dr. S. Beckendorf, Berkeley, USA. Cloned segments of DNA from the Sgs4 region of the X-chromosome are represented in λ*c*Dm2151 and in the plasmid pRHO.75, which contains the *Eco*RI – *Hin*dIII 750 bp fragment (cloned in pBR322), and DNA from the Sgs3, Sgs7, and Sgs8 region of chromosome 3 is represented in λ*c*Dm2007 (Muskavitch and Hogness 1980; Meyerowitz and Hogness 1982). They were generously made available to us by Dr. S. Beckendorf, Berkeley.

Culture of Flies, Synchronization, and Shift-Up. Flies and larvae were raised on standard yeast-cornmeal-molasses medium at 25° C. Eggs were collected over 2 h intervals, and larvae were shifted to the restricted temperature 60 h after oviposition unless otherwise stated.

Ecdysterone Feeding. At 30 and 48 h after shift-up the larvae were divided into two groups. One group was fed a 5% ethanol solution containing ecdysterone (Sigma, USA) at a concentration of 1 mg/ml and baker's yeast (Garen et al. 1977). The other group was fed the same media without the hormone. Both groups were kept at 30° C during treatment.

Nucleic Acid Isolation. For total RNA isolation salivary glands or whole larvae were briefly homogenized in 7 M urea, 2% SDS, 10 mM Tris-HCl, pH 8.0, 0.35 M NaCl, 1 mM Na_2EDTA, deproteinized by extractions with phenol/chloroform/isoamylalcohol (50:49:1), and chloroform/isoamylalcohol (24:1) (Thireos et al. 1980). The nucleic acids were ethanol precipitated and used for electrophoresis either directly or after selection of poly(A^+) with oligo-dT cellulose chromatography.

Phage DNA was isolated by the method described by Schleif and Wensink (1981). Plasmid DNA was prepared according to the alkali method described by Maniatis et al. (1982).

Electrophoresis, Blotting, and Hybridization. The RNA was separated on 1% agarose-formaldehyde gels. The gel contained 2.2 M formaldehyde and 1 × running buffer (20 mM MOPS, 1 mM EDTA, and 5 mM NaAc, pH 7.0). The samples were dissolved in 50% deionized formamide, 2.2 M formaldehyde, and 1 × running buffer, treated at 60° C for 5 min, and loaded onto the gel.

RNA transfer to nitrocellulose filters, and hybridization were carried out as described by Thomas (1980), except that Dextran sulfate was used in the prehybridization mixture as well, and the first four washes at room temperature were for 20 min in 500 ml of washing solution.

Preparation of Nick-Translated Probes. Specific restriction fragments containing DNA from the Sgs4 region were isolated from λ*c*Dm2151 (Muskavitch and Hogness 1980). The λ*c*Dm2007 (Meyerowitz and Hogness 1982) was used undigested. After appropriate restriction the cloned DNAs were fractionated by electrophoresis on agarose gels containing ethidium-bromide. The desired fragment(s) was identified and recovered as described by Yang et al. (1979). The fragments were then nick-translated according to the supplier's instructions (New England Nuclear). Detailed restriction maps of the 3C11-12 (Sgs4) and 68C3-5 (Sgs3, Sgs7, and Sgs8) chromosomal regions can be found in Muskavitch and Hogness (1980, 1982), and Meyerowitz and Hogness (1982), respectively.

Results

Developmental Profile of the Expression of Sgs3, Sgs4, and Sgs7/Sgs8 RNAs in ts67

We were first interested to see whether the *ts67* mutation had any effect(s) on the transcription of glue protein genes at 25° C. As shown previously (Muskavitch and Hogness 1980; Meyerowitz 1982; Meyerowitz and Hogness 1982) for several wild-type strains, RNAs from puff sites 3C11-12 (Sgs4) and 68C3-5 (Sgs3, Sgs7 and Sgs8) are first detected in larvae that have completed more than 20% and less than 40% of the third instar, that is, between 82–90 h after oviposition. These transcripts are undetected by the time of the larval-to-prepupal transition. The Sgs7 and Sgs8 transcripts are not separated on the 1% agarose gels used here, and they are therefore subsequently referred to as Sgs7/Sgs8.

Hybridization with a ^{32}P-labeled *Xho*I 2.6 kb fragment, harboring the Sgs4 gene, and λ*c*Dm2007 DNA, containing the Sgs3, Sgs7, and Sgs8 genes, respectively, shows that the four RNAs in *ts67* exhibit the same developmental profile (Fig. 1 A, b) as shown for the wild-type strains studied by Muskavitch and Hogness (1980) and Meyerowitz and Hogness (1982). The results show that the *ts67* mutation has no detectable effect on transcription of glue polypeptide genes Sgs3, Sgs4, and Sgs7/Sgs8 at 25° C. Since the other glue proteins are normally expressed at the protein level their transcription is probably also unaffected.

Using the λ*c*Dm2007 DNA as probe also revealed the presence of a hybridizing band of approximately 0.7 kb (Fig. 1 B, lanes 3, 4; denoted by an arrowhead). Having a mobility very close to that of the Sgs4 transcript it was initially classified as a cross-hybridization to this RNA. We found, however, that, apart from being salivary gland specific (result not shown), this 0.7 kb transcript is absent in the wild-type strains BER-1, the Sgs4 nonproducer strain (Fig. 1 B, lane 6) and Karsnäs (result not shown). Also, Meyerowitz and Hogness (1982) did not detect RNAs other than those from genes Sgs3 and Sgs7/Sgs8 in the wild-type strains Canton-S and Oregon-R. The level of hybridization of the 0.7 kb band in independent RNA preparations also appears to be higher with RNA from larvae 100 h after oviposition than with RNA from earlier or later stages (Fig. 1 B, lane 3). Furthermore, including 1 μg/ml of the 2.6 kb *Xho*I fragment in the hybridization mixtures did not alter the relative amount of hybridization. These observations suggest that the 0.7 kb band may be a *ts67* specific glue polypeptide mRNA rather than a cross-hybridization to the Sgs4 RNA. Obviously further studies are needed to establish the origin of the 0.7 kb hybridizing band.

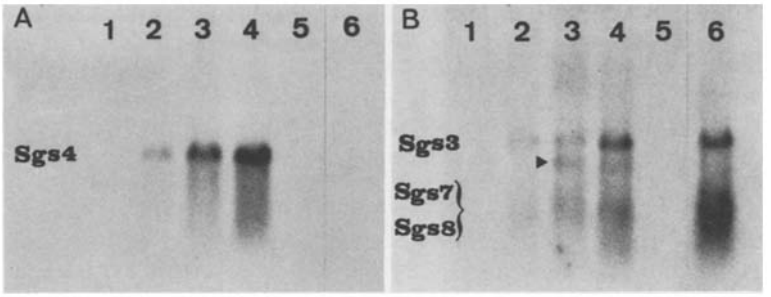

Fig. 1A, B. Accumulation profile of the amount of RNAs at 25° C. RNA was prepared from *ts67* larvae 75 (lane 1), 90 (lane 2), 100 (lane 3), 110 (lane 4), and 120 h (lane 5) after oviposition. RNA from BER-1 larvae 110 h (Sgs4 nonproducer) is included as control (lane 6). After electrophoresis and blotting the filter was hybridized with ^{32}P-labeled *Xho*I 2.6 kb DNA (**A**). Eliminating the previous probe, the filter was subsequently hybridized with labeled λ*c*Dm2007 DNA (**B**). 8 µg total RNA was loaded into each slot

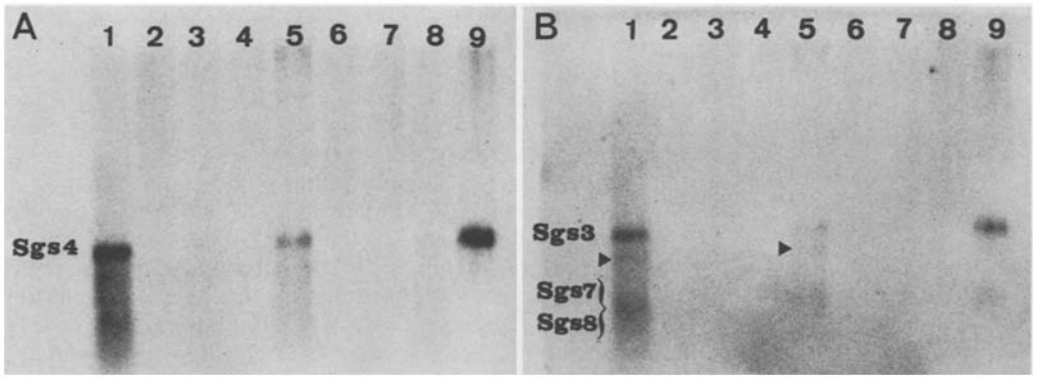

Fig. 2A, B. Expression of genes Sgs3, Sgs4, and Sgs7/Sgs8 at 30° C. RNA from *ts67* larvae at 110 h (lane 1) at 25° C. *ts67* larvae were shifted to 30° C 60, 65, 70, and 75 h after oviposition, and the RNA was prepared 30 h after shift-up (lanes 2–5) and 48 h after shift-up (lanes 6–9). **A** shows hybridization with the labeled *Xho*I 2.6 kb fragment. **B** the same filter after elimination of the previous probe and rehybridization with ^{32}P-labeld λ*c*Dm2007. 8 µg of total RNA was analyzed per slot

The Expression of Genes Sgs3, Sgs4, and Sgs7/Sgs8 at 30° C

It should be recalled that *ts67* larvae become ecdysterone deficient in the sense that the hormone titer fails to increase when shifted to 30° C before or early in the third instar (Hansson et al. 1981). Larvae shifted up later in the third instar pupate and develop to the stage of red eye pigment formation but they fail to eclose (Dudick et al. 1974).

Thoroughly synchronized larvae were shifted to the restrictive temperature 60, 65, 70, and 75 h after oviposition. The larvae were allowed to develop at this temperature for 30 or 48 h before isolating the RNA. The RNA was fractionated on agarose-formaldehyde gels, transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled probes.

First, we used the *Xho*I 2.6 kb fragment as probe, and Fig. 2A shows that there is no synthesis of Sgs4 RNA in larvae shifted up at 60, 65, or 70 h after egg-laying, and kept there for 30 or 48 h (lanes 2–4, 6–8). Trace amounts of this transcript may be detected after 48 h at 30° C in larvae shifted up at 70 h (Fig. 2A, lane 8). This may be due to one or more larvae being somewhat older than 70 h, despite the thorough synchronization, or that the mutation is leaky. A shift-up at 75 h after oviposition results in the production of Sgs4 RNA both after 30 and 48 h (105 and 123 h after oviposition, respectively) (Fig. 2A, lanes 5, 9). The accumulation of the Sgs4 transcript is virtually the same after 48 h as that at 25° C, whereas the amount after 30 h is considerably lower (Fig. 2A). This should be compared with the developmental profile of the amount of mRNA at 25° C, which shows that the accumulation of Sgs4 RNA is considerable already 100 h after oviposition, that is, about 12 h after transcription is first detected (Muskavitch and Hogness 1980; Fig. 1A). The maximum amount of transcript is found 110 h after egg-laying, which agrees with earlier observations (Muskavitch and Hogness 1980). That the maximum amount of transcript at 30° C is found 48 h after shift-up (123 h after oviposition) suggests that the rate of transcription is reduced at 30° C, or that the turnover time of the RNAs is shorter. This observation also indicates that once active at 30° C the Sgs4 gene continues to function for a considerable period of time.

The Sgs4 transcript produced at 30° C is translated into glue polypeptide sgs4 as revealed by ^{3}H-proline labeling of the salivary gland proteins, and analysis of the protein synthetic pattern by gel electrophoresis (results not shown). This shows that the *ts67* mutation acts at the level of transcription, and not at that of translation as earlier suggested (Hansson et al. 1981).

Washing away the *Xho*I 2.6 kb probe, and rehybridizing the filter with ^{32}P-labeled λ*c*Dm2007 DNA revealed virtually the same transcription profile for genes Sgs3 and Sgs7/Sgs8 within the 68C3-5 puff as that described for Sgs4 (Fig. 2B). However, the accumulation of Sgs3 transcript after 30 h in larvae shifted up at 75 h is significantly lower in comparison with that of Sgs4 at the same time (Fig. 2B, lane 5; cf. Fig. 2A, lane 5). After 48 h at 30° C the amount of the Sgs3 RNA approximately equals that at 25° C 110 h after oviposition (Fig. 2B, lanes 1 and 9). Accumulation of the Sgs7/Sgs8 RNA is considerably reduced in comparison with that observed after 110 h at 25° C (Fig. 1B; Fig. 2B, lanes 1, 5, and 9). These observations may further support the view that the rate of transcription of these genes

is reduced at 30° C, and also the notion that genes Sgs7/Sgs8 are differently regulated from Sgs3 (Meyerowitz and Hogness 1982). As in the case of Sgs4, once active at 30° C genes Sgs3 and Sgs7/Sgs8 continue to produce functional mRNAs (results not shown).

It is interesting to note that the 0.7 kb hybridizing band discussed above is present, admittedly in low amounts, at 25° C and after 30 h at the restrictive temperature (Fig. 2B, lanes 1 and 5, denoted by an arrowhead). It is not, however, detected after 48 h (Fig. 2B, lane 9), at which time the amount of Sgs4 RNA is prominent. Thus, if the 0.7 kb hybridizing band be due to crosshybridization with the Sgs4 transcript it should certainly be detected after 48 h. The 0.7 kb band is not detected in larvae shifted to 30° C before and up to 70 h after oviposition (Fig. 2B).

The Effect of Exogenous Ecdysterone at 30° C

As a first step in investigating the possible role of ecdysterone in transcription induction of glue polypeptide genes, *ts67* larvae were shifted to 30° C 60 h after oviposition, and exogenous hormone was administered 30 or 48 h after shift-up. Total RNA was isolated at 2 or 3 h intervals, fractionated on 1% agarose-formaldehyde gels, and transferred to nitrocellulose filters.

As shown in Fig. 3A, the pRH0.75 probe reveals that ecdysterone administered after 30 h at 30° C induces a notable accumulation of Sgs4 RNA within 2 h of stimulation (Fig. 3A, lane 2). Virtually the same amount of Sgs4 RNA is also detected 5 and 8 h after ecdysterone administration (Fig. 3A, lanes 3 and 4), indicating that very little or no additional accumulation occurs after the first 2 h. There are several likely explanations of this result. First, a short pulse of RNA synthesis which finishes by 2 h and the RNA is stable. Second, a low continuous rate of synthesis with normal RNA half life so that the plateau is low. Finally, a normal rate of RNA synthesis with a shortened half-life. Our data do not exclude any of the three. By 10 h after hormone stimulation no band corresponding to the Sgs4 RNA could be seen in the *ts67* larvae (Fig. 3A, lane 5).

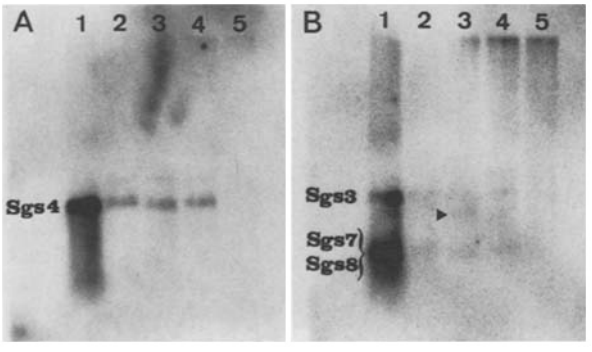

Fig. 3A, B. Induction of Sgs3, Sgs4, and Sgs7/Sgs8 by ecdysterone. RNA from larvae 110 h after oviposition at 25° C (lane 1). *ts67* larvae were shifted to 30° C 60 h after oviposition, ecdysterone was provided after 30 h, and RNA was prepared after 2 (lane 2), 5 (lane 3), 8 (lane 4), and 10 h (lane 5). **A** Hybridization with ^{32}P-labeled pRH0.75 (the *Eco*RI-*Hin*dIII 0.75 kb fragment cloned in pBR322); **B** the same filter rehybridized with labeled λ*c*Dm2007 DNA. The arrowhead denotes a transcript of about 0.7 kb sometimes detected when using λ*c*Dm2007 as probe. 8 μg total RNA was electrophorized in each lane

This observation suggests that there is an abrupt termination of transcription as well as a rapid degradation of the Sgs4 transcript between 8 and 10 h after ecdysterone administration. It should be mentioned that these larvae form pseudopupae after 10–12 h but there is no further differentiation (Hansson and Lambertsson 1983).

After washing away the pRH0.75 probe the filter was rehybridized with λ*c*Dm2007 DNA, which contains the coding sequence for the Sgs3, Sgs7, and Sgs8 genes (Meyerowitz and Hogness 1982). Figure 3B reveals virtually the same hybridization profile for Sgs3 and Sgs7/Sgs8 RNAs as that for Sgs4. However, the accumulation of Sgs3 and Sgs7/Sgs8 transcripts, relative to the 25° C samples, is much lower compared to that of Sgs4 (compare Figs. 3A and B). Again, there appears to be no increase in the accumulation of RNAs from 2 h after ecdysterone administration. No hybridizing bands corresponding to Sgs3, Sgs4, and Sgs7/Sgs8 could be detected before the hormone was administered.

Giving exogenous ecdysterone to *ts67* larvae after 48 h at 30° C results in the formation of pseudopupae after about 12 h (Hansson and Lambertsson 1983). This shows that the larvae retain the ability to respond to the hormone even after a prolonged period at the restrictive temperature. Figure 4A represents a study of the accumulation profile of the Sgs4 RNA in these larvae after ecdysterone administration at 48 h. At 2 h after the addition of hormone no band corresponding to the Sgs4 RNA could be detected (Fig. 4A, lane 3). A low but fully detectable accumulation of Sgs4 transcript is found after 4 h (Fig. 4A, lane 4), and an increasing accumulation can be seen up to 10 h after ecdysterone administration (lanes 5, 6, and 7). After the apparent accumulation maximum of Sgs4 RNA at 10 h, the amount appears to have decreased by 12 h poststimulation (Fig. 4A, lane 8).

It is interesting to note that the accumulation profile of the Sgs4 RNA after the addition of ecdysterone to *ts67* larvae after 48 h at 30° C is very similar to that observed at 15° C (cf. Fig. 1A). However, the accumulation maximum is reached much faster in the ecdysterone-treated larvae at 30° C compared to the profile at 25° C, where it occurs after about 24 h (Fig. 1A; Muskavitch and Hogness 1980). It is also obvious that the amount of Sgs4 RNA transcribed after exogenous hormone stimulation is lower than that observed under normal conditions.

There are marked differences in the responses of the Sgs4 gene to ecdysterone administered 30 and 48 h after shift-up (compare Figs. 3A and 4A). After 30 h at 30° C Sgs4 RNA is detected already after 2 h, accumulation of the transcript does not increase, and by 10 h no synthesis of the RNA could be seen. After 48 h, on the other hand, Sgs4 transcription is first detected after 4 h, and there is an accumulation maximum 10 h after hormone administration (Fig. 4A).

The pRH0.75 probe was washed away, and the filter was rehybridized with ^{32}P-labeled λ*c*Dm2007 DNA. Figure 4B shows a weak but detectable band corresponding to the Sgs7/Sgs8 RNAs after 4 h of ecdysterone stimulation (lane 4). The amount appears to remain at the same level up to 12 h after the hormone was added. Furthermore, the accumulation of the Sgs7/Sgs8 transcripts is considerably lower compared to that of Sgs4 (compare Figs. 4A and B).

Admittedly, the Sgs3 band is weak in the 25° C sample shown in Fig. 4B. This could be due to somewhat older larvae in which the synthesis of Sgs3 RNA has been re-

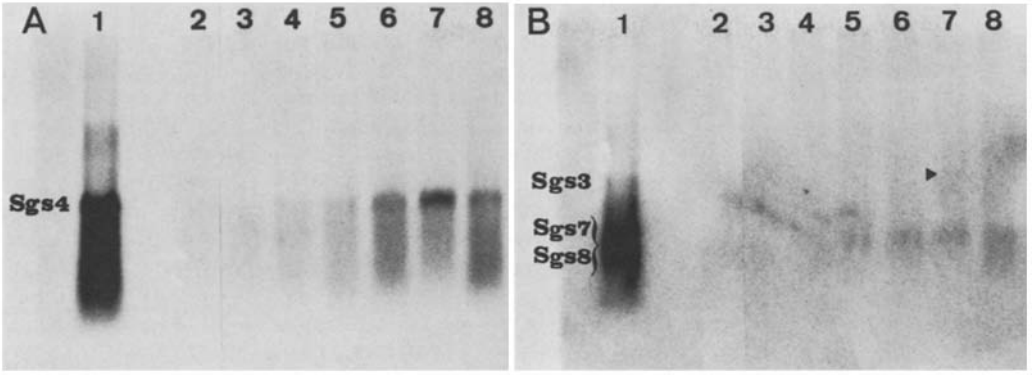

Fig. 4A, B. Ecdysterone induced accumulation of Sgs3, Sgs4, and Sgs7/Sgs8 RNAs 48 h after shift-up. RNA from larvae 110 h after oviposition at 25° C (lane 1). *ts67* larvae were treated as in Fig. 3 except that ecdysterone was administered 48 h after shift-up. RNA was prepared 0 (lane 2), 2 (lane 3), 4 (lane 4), 6 (lane 5), 8 (lane 6), 10 (lane 7), and 12 h (lane 8) after shift-up. **A** Hybridization with ^{32}P-labeled *Xho*I 2.6 kb fragment; **B** the same filter after elimination of the previously hybridized probe and subsequent hybridization with labeled λ*c*Dm2007 DNA. 6 µg total RNA was electrophorized in each lane

pressed, and that its degradation is under way. In the 30° C samples Sgs3 RNA is just detectable 8 h after ecdysterone administration. In no other RNA samples analyzed by blotting-hybridization was the Sgs3 RNA detected. Thus, with the exception of the late appearance of Sgs3 RNA at 48 h, it appears as if the response of genes Sgs3 and Sgs7/Sgs8 is the same regardless of whether ecdysterone was added after 30 or 48 h at 30° C – a weak but detectable synthesis of the corresponding RNAs (compare Figs. 3B and 4B). However, as with the Sgs4 RNA, the Sgs3 and Sgs7/Sgs8 transcripts are detected earlier when the ecdysterone is administered 30 h after shift-up than after 48 h.

Discussion

The results described above show that there is no or extremely reduced transcription of genes Sgs3, Sgs4, and Sgs7/Sgs8 in *ts67* larvae shifted to 30° C before and up to 70 h after oviposition, whereas larvae shifted up at 75 h or later produce transcripts from all four genes. With the exception of Sgs7/Sgs8 the profile of the amount of mRNAs at various times after shift-up shows that the accumulation after 48 h virtually equals that at 25° C, the amount after 30 h being low but fully detectable. We have found considerable accumulation of glue protein mRNAs even after 55–60 h at 30° C (results not shown). The fact that in *ts67* larvae the ecdysterone level fails to increase when shifted to 30° C (Hansson et al. 1981) may explain the prolonged transcription period at the restrictive temperature since it is postulated that the dramatic increase in the ecdysterone concentration regresses the intermolt puffs, several of which harbor glue polypeptide genes (Ashburner and Berendes 1978).

These results allow the following tentative conclusions. First, functioning *su(f)* gene product or accumulation of active gene product must occur very early in the third instar for transcription of glue polypeptide genes later in development. This does not preclude functioning gene product before or after this stage. It only establishes that activity or accumulation of active gene product must occur very early in the third instar. We know that the temperature sensitive period for suppression of forked bristles (Dudick et al. 1974), and enhancement of white apricot (L. Hansson, unpublished results) occurs postpupation. Second, both initiation of and transcription itself are unaffected by the high temperature; this is shown by the requirement for functioning *su(f)* gene product or accumulation of active gene product 8–12 h before glue polypeptide transcripts are first detected at 25° C, and that *ts67* larvae shifted to 30° C at 75 h do synthesize functional glue protein mRNAs. These two observations also suggest that the involvement of *su(f)* is secondary. Third, the rate of accumulation is reduced at 30° C as demonstrated by the shift-up profile of the amounts of Sgs3, Sgs4, and Sgs7/Sgs8 transcripts. We speculate that the reduced rate of synthesis of glue protein mRNAs at 30° C (when initiated) may be due to reduced availability or activity of the *ts67* gene product at, or before, the time of the shift-up. As revealed by ^{3}H-proline labeling of the salivary gland proteins, and analysis of the protein synthetic pattern by gel electrophoresis the glue polypeptide mRNAs produced at 30° C are translated into glue proteins (results not shown). This clearly rejects our earlier suggestion that the *ts67* mutation acts at the level of translation (Hansson et al. 1981).

The results described in this paper also show that accumulation of Sgs3, Sgs4, and Sgs7/Sgs8 RNAs is induced when exogenous ecdysterone is administered to hormone deficient *ts67* larvae at 30° C, implicating ecdysterone in transcription induction of these genes. One striking observation is that, under the conditions used, ecdysterone is a potent inducer of Sgs4 RNA accumulation, whereas its effect on Sgs3 and Sgs7/Sgs8 appears to be less pronounced. Several explanations could account for this difference: differential regulation of the genes, differences in the response of the genes to the hormone or high hormonal stimulation may either decrease the efficiency of processing or the stability of the mature Sgs3, Sgs7, and Sgs8 mRNAs relative to that of the Sgs4 RNA.

Another notable observation is that all four genes respond more rapidly when ecdysterone is added 30 h after shift-up than after 48 h and that accumulation does not increase from 2 h after hormone administration. Admittedly, larvae between 5 and 8 h have not been analyzed, but the present results do not indicate an accumulation maximum during this period. Also, between 8 and 10 h poststimulation the RNAs appear to be rapidly degraded or their stability drastically lowered.

The varied effects induced by a steroid hormone during the development of an organism, as exemplified by ecdyster-

one in *Drosophila*, depend on the coordinated occurrence of both quantitative changes in the amount of hormone and qualitative changes in the capacity of tissues to respond to the hormonal signal (Ashburner 1973; Richards 1976; Zhimulev et al. 1981). The differences in the responses to the hormone 30 and 48 h after shift-up, respectively, may reflect changes both at the structural and molecular level due to the heat treatment. These changes may, in turn, alter the genes' ability to respond rapidly to the hormone-receptor complex. It should be noted, however, that the response of Sgs4, and to some extent also Sgs7/Sgs8, to administration of ecdysterone after 48 h at 30° C is very similar to the 25° C accumulation profile.

Glue protein genes Sgs4, and Sgs3, Sgs7, and Sgs8 are located within the intermolt puffs 3C11-12 and 68C3-5, respectively (Korge 1977a, b; Muskavitch and Hogness 1980; McGinnis et al. 1980; Meyerowitz and Hogness 1982). The transcription of these genes is thought to be responsible for the puffs, which regress toward the end of the third instar when the dramatic increase in the ecdysterone concentration occurs (reviewed by Ashburner and Berendes 1978). Furthermore, it has recently been shown by Gronemeyer and Pongs (1980) that ecdysterone binds to 68C at the time of its regression indicating that the regression is mediated, directly or indirectly, by binding of the hormone. However, the regression of the glue protein correlated puffs 3C and 25B appears not to be hormone influenced to the same extent as puff 68C (Ashburner 1973; Bonner and Pardue 1977; Velissariou and Ashburner 1980). On the other hand, our results indicate the reversed situation with respect to transcription induction by exogenous ecdysterone. The fact that the glue protein genes appear to be induced as well as suppressed by ecdysterone is puzzling, of course, but a hormonally induced differentiation may also include a change in the cells' responsiveness to the hormone that precipitated their change, e.g., from production to suppression of mRNA.

What, then, of wild-type *su(f)* gene function? Taken together, the above observations provide a basis for the hypothesis that the *su(f)* gene function mediates the induction of glue protein gene transcription. Whether the involvement is primary or secondary is not known at the present time. However, the fact that *su(f)* gene function is necessary at least 8–12 h before the glue polypeptide transcripts are first detected makes it quite reasonable to suppose that *su(f)* acts by means of an intermediary agent. In the light of the present findings we want to propose that this agent is the steroid hormone ecdysterone. We do not know, however, whether the *ts67* mutation affects the biosynthetic or regulatory system for this hormone or whether the *ts67* effects may result from the temperature-sensitivity of a more general cellular function than ecdysterone synthesis. This has been discussed by Redfern and Bownes (1983) regarding the *ecdysoneless-1* (*1(3)ecdysone-1^{ts}*; Garen et al. 1977), which in many respects resembles *ts67*.

A small ecdysterone peak, preceding the major release of hormone accompanying moulting, has been described in *D. melanogaster* (Berreur et al. 1979), and in other insects (Bollenbacher et al. 1975; Bouthier et al. 1975; Delbecque et al. 1975; Nijhout 1976) but its role is not fully understood. The possibility that this first pulse may be responsible for the genetic switch-over from larval to pupal development has been discussed (Nijhout 1976). Since this peak just precedes the time at which glue polypeptide mRNAs are first detected we speculate that this programming may also include the induction of the glue protein genes.

It was suggested by Muskavitch and Hogness (1982) that some part of the 95 bp between −392 and −486 is required for the developmentally specific regulation of Sgs4. This region is AT-rich, and is deleted in the wild-type strain BER-1, which lacks Sgs4 mRNA as well as the 3C puff (Muskavitch and Hogness 1982). Could it be that this AT-rich region is homologous to the recognition sites for the hormone-receptor complex found in higher organisms (Tata 1982)? It is also intriguing to note that the AT-rich region deleted in BER-1 contains an 18 bp sequence, AATAAATAAATAATAAAC, at −463 to −481 (Muskavitch and Hogness 1982) that shares homology with an 18 bp AT-rich sequence, AATTAAAAACTAATATTT, localized between −135 and −247 upstream from the hen ovalbumin gene (Compton et al. 1983). The hen 18 bp AT-rich region is a likely candidate for the binding of hen oviduct progesterone receptor A subunit (Compton et al. 1983).

It was shown by Berendes (1968) that in *Drosophila* the initiation of puffing is *not* dependent on active RNA synthesis. This is supported by our observations that the intermolt puffs are prominent in ecdysterone deficient *ts67* larvae at 30° C (Hansson et al. 1981) but no glue polypeptide mRNAs can be detected. On the basis of these results, we want to propose that the intermolt puffs may be induced by a developmentally regulated stimulus which opens up the chromatin. This stimulus may be dependent on DNA regions surrounding the structural gene (Muskavitch and Hogness 1982), and/or tissue-specific co-factors. Once the chromatin is opened up (puffed) transcription induction can be affected by the ecdysterone-receptor complex. If, on the one hand, specific DNA sequences are missing, as is the case in BER-1 – closed chromatin but normal ecdysterone titer, or, on the other hand, the hormone concentration is insufficient to form ecdysterone-receptor complex, as in *ts67* at 30° C – open chromatin but low ecdysterone titer, then the Sgs4 will not be transcribed.

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