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Synthesis and secretion of salivary gland proteins in *Drosophila gibberosa* during larval and prepupal development

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Summary. The late larvae of *Drosophila gibberosa* Patterson and Mainland choose different pupariation sites than the larvae of *Drosophila melanogaster* Meigen. Since the larvae of *D. gibberosa* do not attach themselves to the substratum, the salivary glands contain only a small amount of the "glue" proteins before pupariation. Proteins comprising the salivary gland secretions of late larvae of these two species were compared and found to be qualitatively quite different. Only five polypeptides with the same molecular masses were identified in both species. The rate of protein synthesis in the salivary glands of *D. gibberosa* continued to increase through the late larval stage and pupariation. As a consequence, the total amount of protein contained in the salivary glands also continued to increase after pupariation. To demonstrate temporal changes in protein synthesis from 48 h before pupariation to 28 h after pupariation, newly synthesized polypeptides were pulse labeled by culturing salivary glands in vitro. The patterns of polypeptide synthesis fell into four major groups depending upon whether the synthesis of a protein stopped shortly after pupariation, stopped during late pupariation, increased at pupariation, or was initiated after pupariation. Changing patterns of protein synthesis are correlated with the known changes in gene puffing during this developmental period.

Key words: Salivary glands – Protein synthesis – Larval development – *Drosophila gibberosa, Drosophila melanogaster*

Introduction

The major role of the salivary gland tissues of the late third instar larva of *Drosophila melanogaster* Meigen is the biosynthesis of a group of proteins that comprise the "glue" that the larva uses to attach itself to a substrate before pupariation (Fraenkel and Brookes 1953). A total of ten glue proteins have been identified in the material of the glue plug contained within the lumen of the salivary glands (Korge 1975; Beckendorf and Kafatos 1976; Crowley et al. 1983). Synthesis of the glue proteins begins about 14 h before puparium formation (Beckendorf and Kafatos 1976), and the proteins are stored within the gland cells in large secretory granules until a few hours before pupariation (Lane et al. 1972). The glue proteins are produced in very large quantities during the synthetic period, and they account for 30% of the protein of the salivary glands (Zhimulev and Kolesnilov 1975). After the glue has been discharged from the salivary glands, the gland tissues continue biosynthetic activity until autolysis of the glands is induced after pupal formation. However, the pattern of protein synthesis again changes during this period and can be correlated with new polytene puffing patterns (Zhimulev et al. 1981).

The appearance of the glue proteins has been correlated with puffing activity on the polytene chromosomes regulated by ecdysteroid hormone titers (c.f. Ashburner and Richards 1976; Ashburner and Berendes 1978). Loci for the glue protein genes (*sgs*) have been established (Korge 1975, 1977a, b; Akam et al. 1978; Velissariou and Ashburner 1980, 1981; Crowley et al. 1983), and *sgs3, sgs4, sgs7* and *sgs8* have been cloned (Muskavitch and Hogness 1980; McGinnis et al. 1980; Crowley et al. 1983). Analysis of 5′ flanking DNA sequences has led to the identification of *cis*- and *trans*-acting regulatory elements that have a role in the control of the ecdysteroid hormone response (Meyerowitz and Hogness 1982; Muskavitch and Hogness 1982; Shermoen and Beckendorf 1982; McGinnis et al. 1983; Richards et al. 1983; Crowley and Meyerowitz 1984; Crowley et al. 1984; Bourouis and Richards 1985; Mettling et al. 1985).

Although the regulation and function of the glue proteins have been well documented for *D. melanogaster,* little is known of the homologous salivary gland products in other, more distantly related species of *Drosophila* that have divergent life histories. The fly *Drosophila gibberosa* Patterson and Mainland, which pupariates in the ground, was found to produce salivary gland secretions at a relatively low rate prior to pupariation (Roberts and MacPhail 1985). However, after pupariation the salivary glands began producing large amounts of secretory proteins that were accumulated in the gland lumen. In addition to the differences in the developmental production of "glue," the chromosomes from the salivary glands, fat body, Malpighian tubules, and midgut are of sufficient polyteny that cytological correlation of chromomeric structure can be made between the various tissues throughout development. In order to establish a basis for correlating puff activity between the various tissues, the structure and activity of the salivary gland chromosomes of *D. gibberosa* were characterized (Roberts and MacPhail 1985). The work reported here describes the developmental changes in protein synthesis that occur in the salivary glands during the late larval and prepupal

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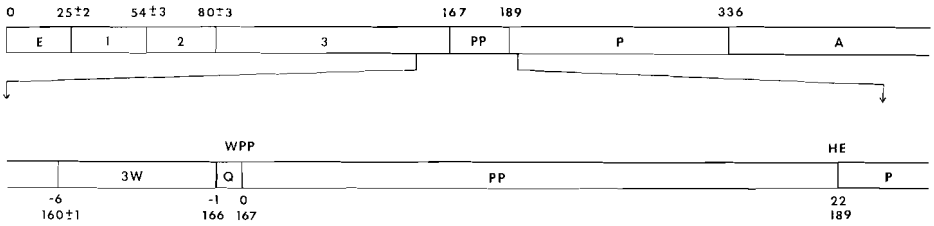

Fig. 1. Time course for the development of *D. gibberosa* from egg deposition to adult. Times in full scale are presented in hours from egg deposition. Times on expanded scale are presented in hours from pupariation as well as from egg deposition. *E:* embryo; 1, 2 and 3: first, second and third larval instars; *3W:* wandering late third instar larva; *Q:* quiescent larva; *WPP:* white prepupa; *PP:* prepupa; *HE:* head eversion; *A:* adult

period of *D. gibberosa* to establish a temporal correlation between protein production and puffing patterns.

Materials and methods

Culture conditions and timing of developmental stages

Both *D. gibberosa* and *D. melanogaster* were grown at 25° C on Lewis standard *Drosophila* medium (Lewis 1960). To reduce the variation of the developmental stages within a group of cohort flies, egg laying by *D. gibberosa* adults was restricted to 1 h, which resulted in 15 to 20 eggs being laid per 1/2-pt. bottle. Under these conditions, the larvae began wandering at about 160 h after egg deposition, became white prepupae at 167 h, and completed head eversion, which marked the beginning of the pupal stage, 22 h after the formation of the white prepupae (Fig. 1). The total time from egg deposition to pupation was 188–189 h, which represented a shorter developmental period for this fly than was reported previously (Wheeler 1947; Kambysellis 1968). The difference in the length of the developmental period may be due to a difference in culture conditions, especially crowding.

To obtain larvae that were at a synchronized developmental stage, wandering larvae were transferred to fresh medium. The animals were further synchronized as white prepupae (for the purpose of focusing on the events around pupariation, the white prepupae will be referred to as 0 h prepupae), which were identified by tracheal eversion. The 0 h prepupae were again transferred to new medium in a 15 mm × 100 mm petri dish.

Isolation of salivary gland secretions

Salivary glands of various developmental stages from the late third instar larvae and prepupae were removed from the animals in *Drosophila* saline (Chan and Gehring 1971). The secretory plug was removed according to Kodani (1948) by fixing the glands in a mixture of 0.5 N HCl and 100% ethanol (1:3) for 1 min, and then transferring the glands to 100% ethanol for 5 min. This treatment precipitated the secretory material in the lumen of the salivary glands; the precipitated secretory plug could then be separated from the gland tissue with forceps. The dissected secretory plugs were transferred to 40 µl of sodium dodecyl sulfate (SDS) sample buffer (0.05 M Tris [pH 6.8], 0.2% SDS, 10% glycerol, 1% 2-mercaptoethanol, and 0.001% bromphenol blue) (Laemmli 1970). The proteins were denatured by boiling the samples for 5 min, and the samples were stored at −20° C.

Table 1. Preferred pupariation sites of *D. gibberosa* and *D. melanogaster*

	Frequency (%) of pupae attaching to			Total number of pupae examined
	Glass surface	Cotton plug	On or in medium	
D. gibberosa	10.1	56.9	33.0	2502
D. melanogaster	81.7	0.0	18.3	2317

Incorporation of ^{35}S-methionine into salivary gland proteins

Three pairs of salivary glands from each developmental stage were dissected in *Drosophila* saline (saturated with O_2 for 1 h at 25° C). The salivary glands were transferred to 5 µl O_2 saturated incubation medium (*Drosophila* saline plus 2 µCi/µl ^{35}S-methionine (^{35}S-met) [New England Nuclear; >1000 Ci/mmol]). The cultures were placed in a modular incubation chamber (Billups/Rothberg), O_2 was flushed through the chamber for 5 min, and the chamber was sealed. The cultures were maintained at 25° C for 20 min. The glands were removed from the medium, homogenized in 40 µl of SDS sample buffer, boiled, and stored at −20° C. The amount of radioactive material incorporated in each sample was measured by spotting an aliquot on a 1 cm^2 piece of Whatman # 1 filter paper, immersing the paper in 10% trichloroacetic acid (TCA) (0° C) for 10 min, boiling the paper in 5% TCA 10 min, and washing the paper in dionized water, 95% ethanol and acetone. The amount of labeled protein precipitated on the paper was measured by scintillation counting in a Minaxi B Tricarb 4000® (Packard).

SDS gel electrophoresis and autoradiography

Salivary gland proteins were resolved by gradient SDS polyacrylamide gel electrophoresis (SDS-PAGE) (8–15%, 0.75 mm thick, 15 cm × 20 cm) (O'Farrell 1975). Molecular mass markers (Sigma, myosin [205 kDa], β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), chicken egg albumin (43 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20.1 kDa), and lactalbumin (14.2 kDa) were co-electrophoresed on each gel for molecular mass estimation. Equal amounts of radioactive material were layered on each lane. After electrophoresis, the proteins were stained with Coomassie brilliant blue, and the dried gel was autoradiographed with Kodak X-AR X-ray film. Quantitative estimation of the relative propor-

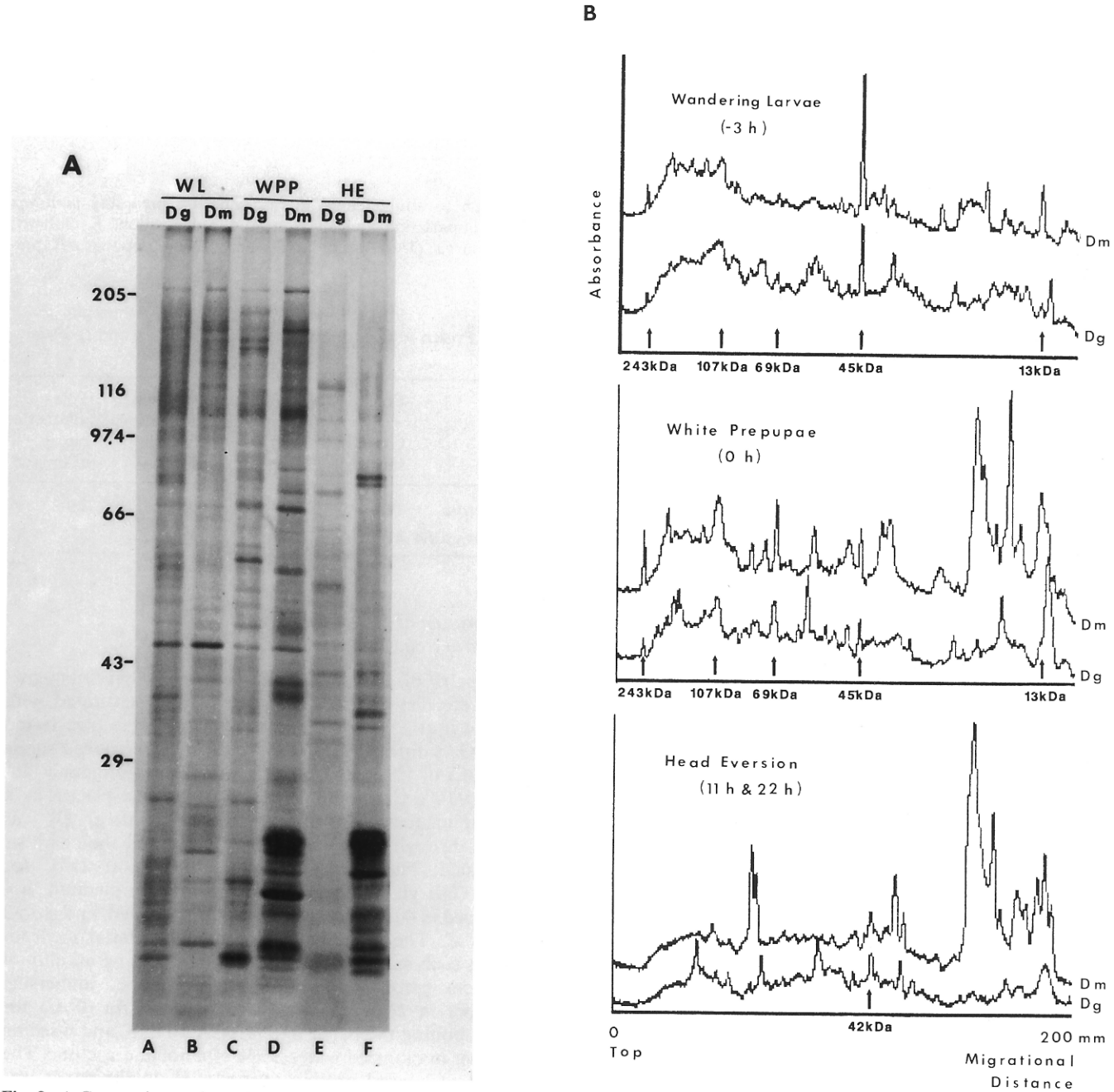

Fig. 2. **A** Comparison of newly synthesized proteins from salivary glands of *D. gibberosa* and *D. melanogaster*. The autoradiogram shows the newly synthesized polypeptides labeled by incubation of the salivary glands in vitro. The positions of the molecular mass markers (kDa) are shown on the left. **B** Comparative densitometric scans of salivary gland proteins from the three developmental periods. Arrows designate polypeptides of matching molecular masses. Lane designations for **A**: (*A*) −3 h wandering larvae of *D. gibberosa*, (*B*) −3 h wandering larvae of *D. melanogaster*, (*C*) 0 h white prepupae of *D. gibberosa*, (*D*) 0 h white prepupae of *D. melanogaster*, (*E*) 22 h head everting pupae of *D. gibberosa*, (*F*) 11 h head everting pupae of *D. melanogaster*. Abbreviations: *WL*, wandering larvae; *WPP*, white prepupae; *HE*, head eversion; *Dg*, *D. gibberosa*; *Dm*, *D. melanogaster*

tion of individual proteins was made by scanning the autoradiograms with a 2222 Ultroscan XL Laser densitometer® and analyzing the scans with 2400 GelScan XL® software (LKB Instruments).

Determination of protein concentration

Protein concentrations were determined essentially as described by Kahn (1983). The proteins from five pairs of salivary glands were precipitated in 0.1 M Tris-HCl (pH 7.5), 0.2% SDS, and 15% trichloroacetic acid. The precipitated proteins were collected on nitrocellulose (BA85, Schleicher and Schuell), and the proteins were stained with 0.25% amido black in 50% methanol and 10% acetic acid. The stained proteins were eluted from the nitrocellulose with 50% ethanol, 25 mM NaOH, and 0.5 mM EDTA and the absorbance read at 630 nm. Bovine serum albumin was used as a standard.

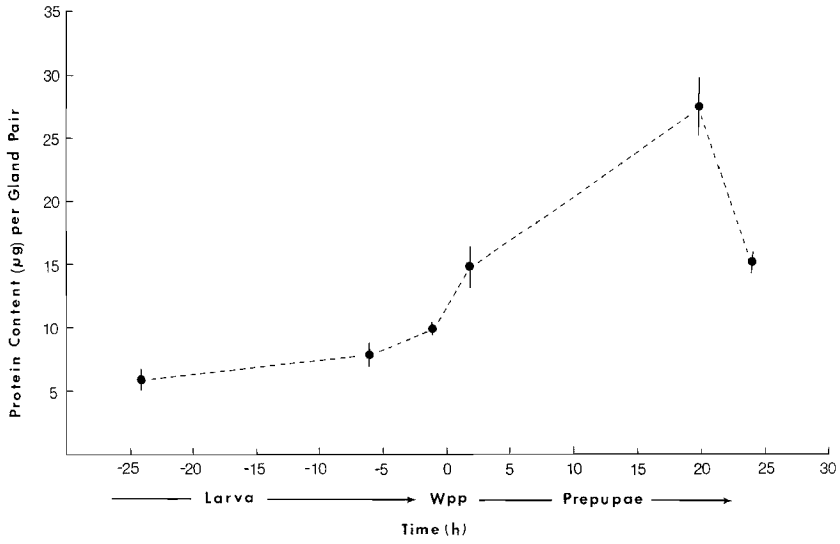

Fig. 3. Total protein content of the salivary glands from the late larval period to the beginning of pupation. Each point represents the mean and standard error of three replicates of the protein determination for five gland pairs adjusted to the protein content per gland pair

Results

Pupariation site preference of Drosophila gibberosa

As larval development is completed, the larvae change behavioral patterns from feeding behavior to wandering behavior, and the larva begins searching for a pupariation site. The larvae of *D. gibberosa* were found to prefer a considerably different pupariation site than the larvae of *D. melanogaster*. The frequency of a pupariation site choice was determined by counting the number of pupae attached to the various surfaces of the culture bottles. The pupae of *D. gibberosa* were found predominantly on the cotton plug (57%) or on the surface of the medium (33%), while the pupae of *D. melanogaster* were found predominantly on the glass surface of the bottle above the medium (Table 1). None of the *D. melanogaster* larvae chose the cotton plug as a pupariation site. Since the natural pupation site of *D. gibberosa* most probably is underground (Roberts and MacPhail 1985), the difference in pupariation site preference in the laboratory reflects the different natural histories of these two insects. The very low production of proteins by larval salivary glands of *D. gibberosa* correlates with its natural history.

Comparison of salivary gland proteins from Drosophila gibberosa and D. melanogaster

Salivary glands were removed from wandering larvae (−3 h), white prepupae (0 h), and head everting pupae of both *D. gibberosa* and *D. melanogaster* (22 and 11 h, respectively) and placed in radioactive incubation medium containing ^{35}S-met to label the newly synthesized proteins. An autoradiogram of the labeled proteins resolved by SDS-PAGE showed few similarities between the proteins synthesized by the salivary glands from the two species (Fig. 2a). There were only five major polypeptides appearing in the −3 h larvae and 0 h prepupae that had the same migration distances and corresponding molecular masses between the two species (Fig. 2b). The five polypeptides had molecular masses of 243 kDa, 107 kDa, 69 kDa, 45 kDa, and 13 kDa as shown by the arrows in Fig. 2b. Additionally, the relative quantitative profiles for these polypeptides were different between the species. At the time of head eversion (22 h *D. gibberosa* prepupae and 11 h *D. melanogaster* prepupae), only one polypeptide was found between the two species that had the same molecular mass of 42 kDa. Even though these various polypeptides have the same corresponding apparent molecular masses, this type of analysis does not offer any evidence of homology between these polypeptides. However, from this comparison we conclude that there is little qualitative or quantitative similarity and most probably little functional similarity between the salivary gland proteins of *D. gibberosa* and *D. melanogaster*.

Identification of secretory proteins in salivary glands

Secretory materials were observed within the lumen of the salivary glands of wandering *D. gibberosa* larvae from 8 h before pupariation. Since the secretory material did not bloat the glands before the wandering phase, it was not possible to isolate the secretory plugs before 8 h prior to pupariation. The amount of total protein contained within the salivary glands was measured from 24 h before pupariation until 24 h after. The total protein of the glands increased only two-fold between −25 and −1 h larvae (Fig. 3). The secretory plugs were removed from salivary glands of larvae 5 h before pupariation of both *D. gibberosa* and *D. melanogaster* and resolved by SDS-PAGE. The pattern of polypeptides comprising the secretory plug of *D. gibberosa* was considerably different from that of *D. melanogaster*. Whereas the secretory plug of *D. melanogaster* larvae contained five major polypeptide bands (the analysis of glue proteins reported previously used different gel electrophoresis system (Beckendorf and Kafatos 1976) and is most likely the reason for the difference in the observed number of glue proteins), *D. gibberosa* was found to have 17 proteins in the lumen of the larval salivary glands (Fig. 4, lanes a, b). Although there were a large number of proteins, the total amount of material was small when compared with *D. melanogaster*. These observations reflect the physiology of the secretions in *D. gibberosa* which does not use the secretory plug as a glue. Between 2–3 h before pupariation, the secretory plugs were discharged from the glands. However, the total amount of protein in the glands contin-

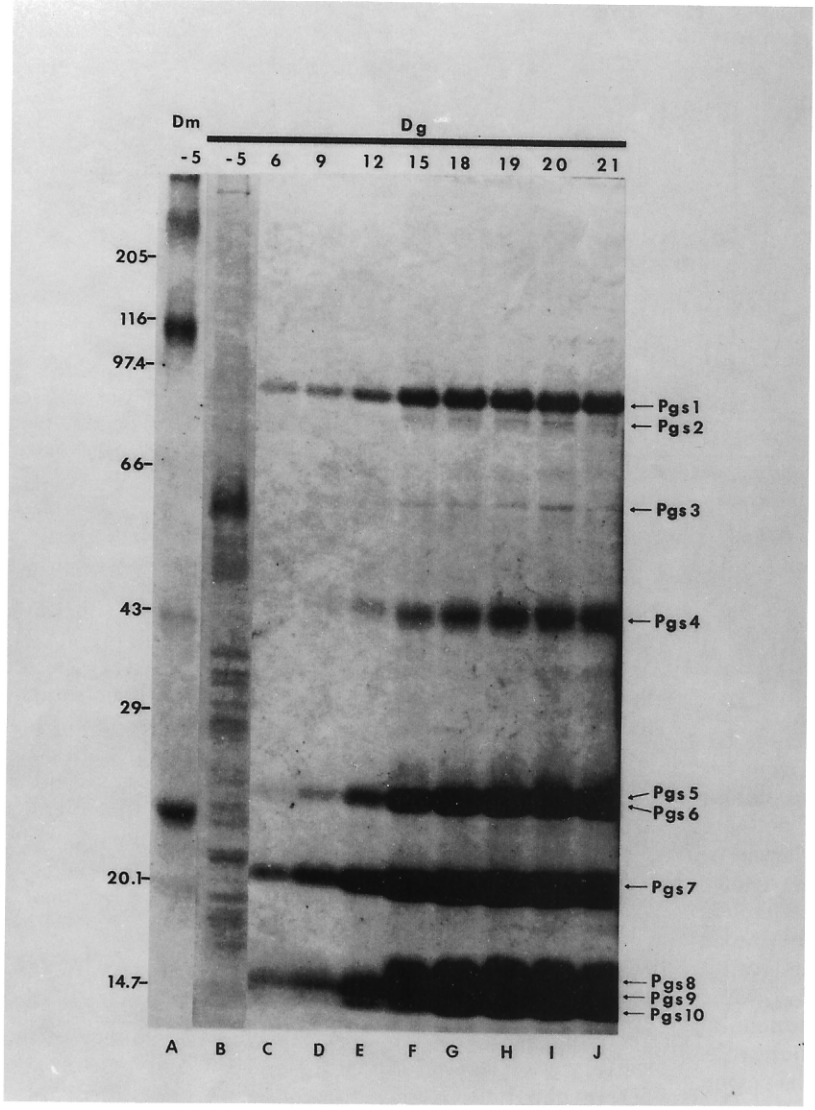

Fig. 4. Secretory plug proteins from the prepupal salivary glands of *D. gibberosa*. The secretory plug was removed from the salivary glands at various times through the prepupal period. The proteins were resolved by SDS-PAGE and the proteins were stained with Coomassie brilliant blue. The positions of the molecular mass markers (kDa) are shown on the left. Lane designations: (*A*) −5 h kinetic larvae of *D. melanogaster*; (*B*) −5 h wandering larvae; (*C*) 6 h prepupae; (*D*) 9 h prepupae; (*E*) 12 h prepupae; (*F*) 15 h prepupae; (*G*) 18 h prepupae; (*H*) 19 h prepupae; (*I*) 20 h prepupae; (*J*) 21 h prepupae. Abbreviations: *Dm*, *D. melanogaster*; *Dg*, *D. gibberosa*; *Pgs*, prepupal gland saliva

ued to increase, and the glands from quiescent phase larvae (−1 h) were found to contain more protein than −6 h wandering larvae that had not discharged the secretory plug (Fig. 3). Following pupariation, the rate of protein accumulation increased dramatically and continued until 20 h prepupae where the glands had a maximum protein content of 27.4 (±2.3) μg. After 20 h when the prepupae initiate head eversion, the amount of protein in the glands decreased rapidly. The fate of the secretory proteins from the salivary glands has not been determined, but the loss is probably due to expulsion of the secretory plug from the gland.

The secretory plugs were removed from the salivary glands of 6–21 h prepupae and resolved by SDS-PAGE. The profile of the time course shows that throughout the prepupal period the majority of the secretory plug protein was accounted for by ten polypeptides (Fig. 4). The proteins within the glands were designated prepupal gland saliva (*Pgs*), and the polypeptides were given an additional numerical designation according to descending molecular mass. Although there was a five-fold increase in the amount of protein present in the plug between 6 and 21 h, the proportions of the major polypeptides (*Pgs1* (89.5 kDa), *Pgs4* (45.6 kDa), *Pgs5/6* combined (24.9 kDa), *Pgs7* (20.3 kDa), *Pgs8* (14.9 kDa), and *Pgs9* (13.9 kDa)) remained fairly constant with a ratio of 0.2:0.2:0.6:1.0:1.0:0.3, respectively, between the polypeptides throughout the developmental period.

Developmental changes in salivary gland protein synthesis

The developmental changes of the protein synthesis patterns in the salivary glands were visualized by pulse labeling the newly synthesized proteins of glands from approximately 48 h before to 28 h after pupariation. Fifty-six polypeptide bands were resolved in the autoradiograms (Fig. 5). Of the major polypeptide bands, 27 were found to undergo discernable changes in the rate of synthesis with the majority of the changes taking place around the time of pupariation (0 h) and again at the time of head eversion (22 h). The most radical change in the pattern of protein synthesis was observed at the time of head eversion where almost all protein synthesis was terminated. To simplify analysis

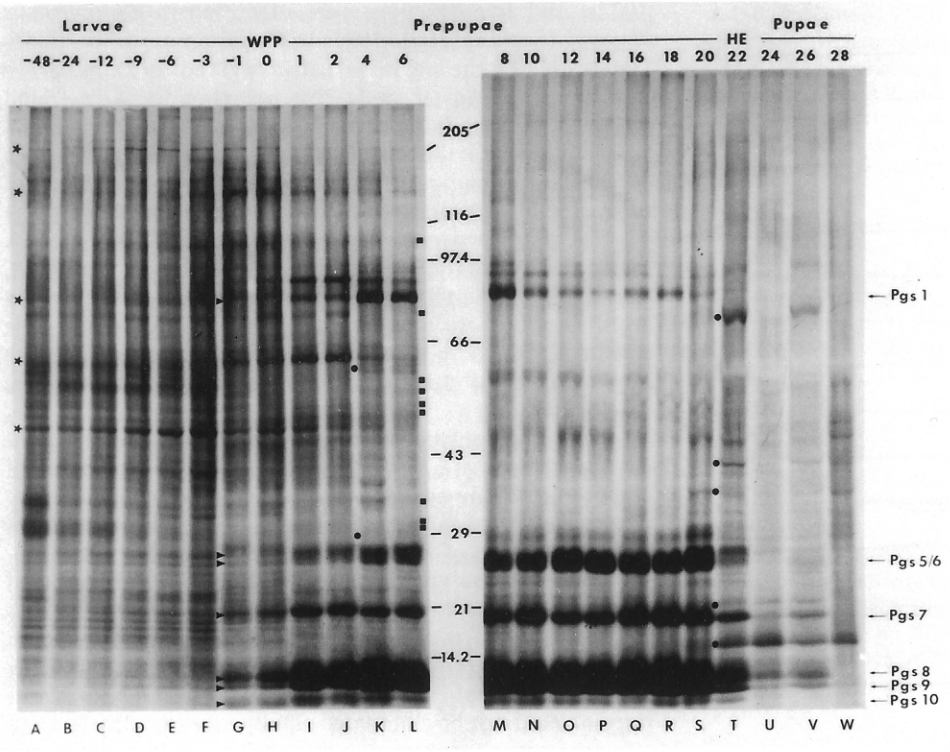

Fig. 5. Developmental profile of protein synthesis by salivary glands from the late third instar larvae to the pupal stage. The autoradiogram shows the newly labeled polypeptides from salivary glands incubated in vitro resolved by SDS-PAGE. The positions of the molecular mass markers (kDa) are shown in the center of the two autoradiograms. Those polypeptides marked with □ terminated synthesis at 6 h (group A). Those polypeptides marked with * were present at −48 h but synthesis ended between 12 and 22 h (group B). Those polypeptides marked with → were present from −48 h but began an increase at 0 h (group C). Those polypeptides marked with 0 started synthesis from that time point (group D). Lane designations: (*A*) −48 h larvae; (*B*) −24 h larvae; (*C*) −12 h larvae; (*D*) −9 h larvae; (*E*) −6 h larvae; (*F*) −3 h larvae; (*G*) −1 h larvae; (*H*) 0 h white prepupae; (*I*) 1 h prepupae; (*J*) 2 h prepupae; (*K*) 4 h prepupae; (*L*) 6 h prepupae; (*M*) 8 h prepupae; (*N*) 10 h prepupae; (*O*) 12 h prepupae; (*P*) 14 h prepupae; (*Q*) 16 h prepupae; (*R*) 18 h prepupae; (*S*) 20 h prepupae; (*T*) 22 h head everting pupae; (*U*) 24 h pupae; (*V*) 26 h pupae; (*W*) 28 h pupae. Abbreviations: *WPP*, white prepupae; *HE*, head everting pupae; *Pgs*, prepupal gland saliva

of the temporal changes, polypeptides were segregated on the basis of the timing of their synthesis, and the patterns were divided into four major categories: A) the polypeptide was present at −48 h but the synthesis ended by 6 h (those marked at 6 h with a □ in Fig. 5); B) the polypeptide was present at −48 h but the synthesis ended between 12 and 22 h (those marked at −48 h with an * in Fig. 5); C) the polypeptide was present at −48 h and the synthetic rate began to increase in quiescent larvae (−1 h) (those marked at −1 h with a → in Fig. 5); and D) the synthesis of the polypeptide started after pupariation (those marked at either 4 or 22 h with a 0 in Fig. 5). The molecular mass for each of the polypeptides is marked beside the axis for the synthetic rate profile in Fig. 6. Only one of the polypeptides, 77.8 kDa, showed changes characteristic of two categories; 77.8 kDa had an increased synthetic rate beginning at −1 h (group C), but its synthesis ended at 22 h (group D). All but six of the polypeptides (those of group D) were apparent in the −48 h salivary glands. However, not all of those polypeptides present at −48 h were found to be at a maximal synthetic rate. Ten of the polypeptides were found to have maximal synthesis 2 h before pupariation, 4 polypeptides had a maximal rate around pupariation, and 12 polypeptides had a maximal synthetic rate that occurred after 4 h pupariation (Fig. 6).

Discussion

The larvae of *D. gibberosa* generally do not attach themselves to a substrate for pupariation (Table 1). Correlated with this, we found that the salivary glands produce and accumulate very small amounts of secretory proteins just before pupariation (Fig. 3). It seems unlikely, therefore, that the primary function of the salivary glands of late larval *D. gibberosa* is the production of a "glue" as is the case in *D. melanogaster*. The large number of proteins in the salivary glands of *D. gibberosa* may reflect the lack of a critical "glue" function for the larval salivary secretions. The *D. gibberosa* larval secretory plug (if in fact is even merits this description) apparently is diminished from that of *D. melanogaster* (which perhaps more closely represents the ancestral condition – Throckmortin 1962).

Although the secretory plug proteins may not function as a glue, the 17 secretory proteins identified in the late larval salivary glands of *D. gibberosa* represents the largest number of proteins from the salivary gland secretory plug reported in a fly. Ten glue proteins have been identified in the salivary glands of *D. melanogaster* (Korge 1975; Beckendorf and Kafatos 1976; Crowley et al. 1983), six proteins were identified in *Sciara coprophila* (Been and Rasch

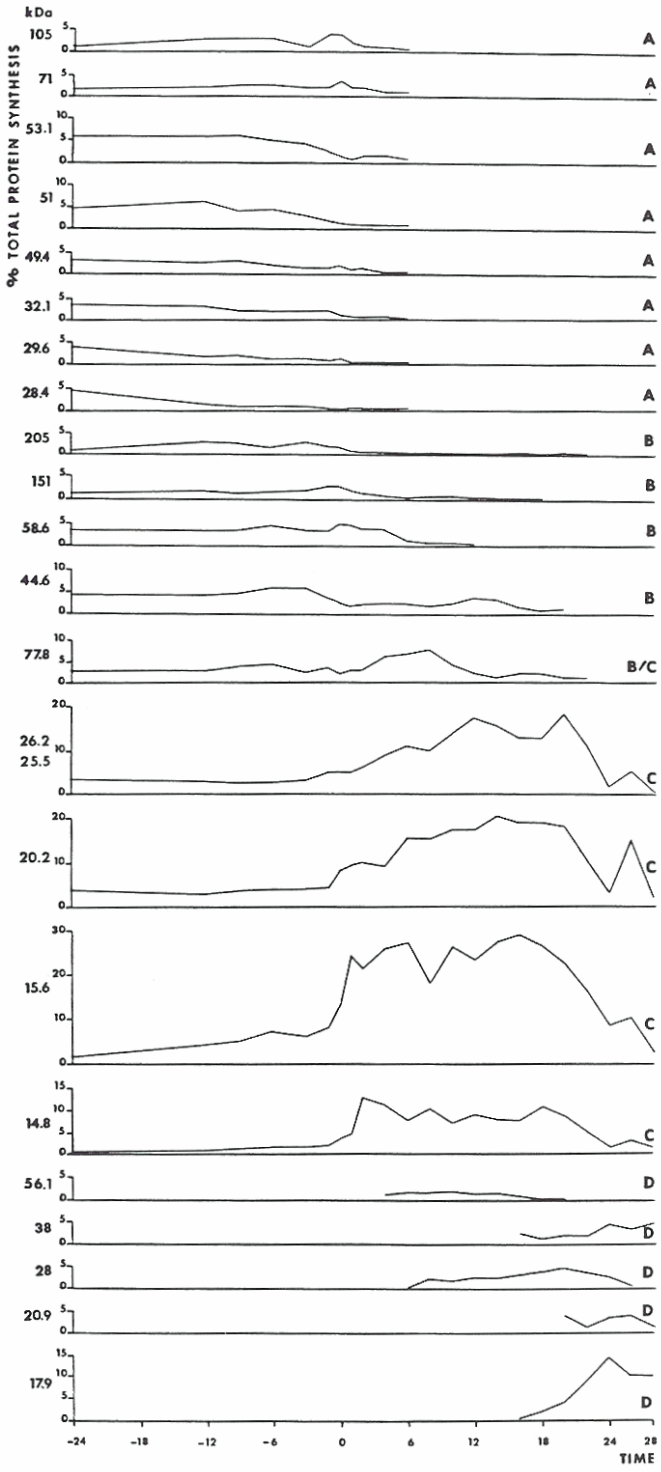

Fig. 6. Densitometric scans of proteins synthesized by the salivary glands from late larvae and prepupae of *D. gibberosa*. The relative molecular mass for each polypeptide is given on the left for each profile. The level of synthetic rate for each polypeptide is given as a percent of the total protein synthesis. Each point for each polypeptide represents the mean of four trials to determine the percent of total labeled protein from three pairs of incubated salivary glands. The capital letters on the right of each lane designate (*A*) the polypeptide was present at −48 h, but synthesis ended by 6 h; (*B*) the polypeptide was present at −48 h, but synthesis ended before 22 h; (*C*) the polypeptide was present at −48 h, and the rate of synthesis began to increase in quiescent larvae (−1 h); (*D*) the synthesis of the polypeptide started after pupariation

1972), and two proteins were identified in *Chironomous thummi* (Serfling et al. 1983). Of the major polypeptides synthesized by the late larval salivary glands of *D. gibberosa* and *D. melanogaster*, only five polypeptides were found to have the same molecular masses, while only the 42 kDa polypeptide had the same corresponding molecular mass in the late prepupae of both species (Fig. 2B). Although a reasonable amount of variation in the pattern of glue proteins has been observed between various strains of *D. melanogaster* (Beckendorf and Kafatos 1974; Korge 1980), the small amount of similarity between these secretions from *D. gibberosa* and *D. melanogaster* was striking. We presume that these differences have developed due to the apparent lack of glue function for salivary gland secretions in *D. gibberosa*.

Ten major polypeptides, designated prepupal gland saliva proteins (*Pgs*), were found in the secretory plugs of the prepupal salivary glands of *D. gibberosa* (Fig. 4). The *Pgs* accumulated rapidly after pupariation and were the apparent products of a coordinately regulated increased synthetic rate for polypeptides that were being synthesized at lower rates prior to pupariation (Figs. 5–6, group C). The putative labeled precursors were identified for only seven of the ten *Pgs* polypeptides. As *Pgs2* and *Pgs3* are only minor components and the possibility of proteolytic degradation could not be eliminated totally, our inability to identify precursors for these two minor *Pgs* bands was not surprising. However, notably absent from the labeling pattern was the precursor for *Pgs4* (45.6 kDa), one of the major polypeptides appearing in the secretory plug of the prepupal salivary glands. The putative labeled precursors of the *Pgs* proteins were identified for seven of the ten. The only major *Pgs* precursor that was not observed in the proteins synthesized by the prepupal salivary glands was the precursor for *Pgs4* (45.6 kDa). The absence of *Pgs4* could have been due to a number of factors varying from inadequate culturing conditions (unlikely, because protein synthesis appeared to be continuing normally for the other proteins) *Pgs4* arising from proteolytic cleavage of a larger precursor such as *Pgs1* (89.5 kDa), or *Pgs4* being taken up from the hemolymph. However, the second alternative was unlikely also since the rate of *Pgs1* synthesis was maximal around 6 h after pupariation and decreased steadily afterwards. A greater synthetic rate of *Pgs1* would have been required to support the accumulation of *Pgs1* and *Pgs4* in the secretory plug than was apparent in Fig. 5.

Although the prepupal salivary glands of *D. melanogaster* show a similar high level of protein biosynthetic activity (Zhimulev et al. 1981), the proteins comprising the prepupal secretory plug have not been identified. The physiological function of the *Pgs* has not been determined for either species. However, an 80 kDa prepupal secretory protein was found to migrate from the salivary glands to the developing thoracic epidermal cells in *D. melanogaster* (Mitchell et al. 1977). This suggests that these proteins have various other functions outside of the salivary glands (as proposed by Ashburner 1967). We propose, too, that the secretory proteins of the prepupal salivary glands have a functional role early in pupation of *D. gibberosa*. Larval salivary gland proteins of the two species apparently have different synthetic patterns and functions. However, comparison of the prepupal gland secretions from both species may show greater similarities since these proteins may be more critical to the ensuing developmental processes, and

consequently, more likely to retain homologies by selection following divergence of the two species from a common ancestor.

The relative constancy of protein synthesis throughout the late third instar larva of *D. gibberosa* shown here is matched by the relative constancy of the cytologically visible pattern of gene activity described previously (Roberts and MacPhail 1985). What few puffs appear at this time are of relatively low grade. Pulse labeling with ^{3}H-uridine during this period reveals 50–60 loci that have transcription rates above background, which corresponds roughly to the number of protein bands visible in gels.

By injecting 20-hydroxyecdysone into late third instar larvae of *D. gibberosa*, Roberts and MacPhail (1985) demonstrated that an ecdysteroid pulse at −6 h was necessary to initiate a complex cycle of gene activity which led to many more loci (60–70) being significantly puffed over the next 6–8 h. As shown here, the synthetic rate of ten proteins (of group A) begins to decline, while several proteins of groups B and C (Fig. 6) begin to increase their synthetic rate during this developmental period. The few proteins of group C with relatively rapid rates of synthesis are matched, perhaps, by a very few large puffs during the prepupal period. Certainly a prime candidate for a group C locus is the largest and most prolonged prepupal puff, 23 C–E which is hyperactive for the first 12 h of prepupal development and also is less active at earlier and later stages of development (matching group C protein synthesis). Other loci that are possible candidates for pupal proteins are 107c, which is very active in the early puparium then declines, 44B–E (in the late puparium) and possibly, 61B–E, 64A–C, 70A–B, B–D, 88C–F, 94A–E, 110B–E as well as several minor puffs.

The initiation of head eversion (pupation) and, apparently, programs leading to the termination of the salivary glands (which disappear 8–12 h later) most likely require another pulse of ecdysteroids just prior to the onset of head eversion (Roberts and MacPhail 1985). The rapid decrease in protein synthesis in the salivary glands that also occurs during this time period may reflect a response dependent on the endocrine changes regulating the initiation of pupation. Many of the puffs active between −6 and 0 h are again prominent (but at lower levels of activity) and a small number of proteins (within group D) appear at this time. One locus, 92A, is at a maximum at this time, but other closely linked loci on chromosome 5 also are active at this time, as is region 67 in chromosome 4. Whether these late puffing loci code for proteins concerned with autolysis of the glands remains to be determined.

Although there is little apparent conservation of the proteins in the salivary gland secretions of the late larvae from *D. gibberosa* and *D. melanogaster*, the salivary gland secretions from the prepupal stage are similar. What may be more important is the similarity in the dynamics of the developmental processes occurring in the salivary glands during this period. The coordinately controlled expression of gene groups progresses in the same manner in both species. The protein synthesis flows from turning off one set of genes to increasing the synthesis of another set, and finally turning on another set of genes that leads to the autolysis of the glands themselves. The genetic features intrinsic to each gene of a related group that permit coordinated global control of the genes during the prepupal stage of *D. gibberosa* are being examined.

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