Developmentally Regulated Expression of a Balbiani Ring 1 Gene for a 180-kD Secretory Polypeptide in *Chironomus tentans* Salivary Glands before Larval/Pupal Ecdysis

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Abstract. The expression of a Balbiani ring 1 gene that codes for a salivary gland–specific 180-kD secretory polypeptide (sp180) is regulated developmentally. Immunoblots of salivary gland protein incubated with an affinity-purified nonapeptide-reactive antibody demonstrated that the salivary gland content of sp180 increases as much as 10-fold between stages 8 and 10 of the fourth larval instar. Hybridization of RNA dot-blots with an oligonucleotide probe indicated that the observed increase in sp180 was preceded by a parallel 20-fold increase in the steady state level of its mRNA beginning between stages 7 and 8. In vitro nuclear transcription experiments demonstrated that there was a 10-fold acceleration in the rate of sp180 gene transcription between stages 6 and 10. The limited period of expression of the sp180 gene contrasted dramatically with the expression of Balbiani ring genes BR1, BR2α, BR2β, and BR6, which code for the sp-I family of fibrous secretory polypeptides. The appearance of sp180 in secretion coincided with microscopically visible changes in the bundling of these fibrous polypeptides. At the same time, we noticed changes in the appearance and consistency of feeding tubes that larvae construct with this secretion. These results lead us to propose that sp180 may modify the structure or utilization of fibrous secretory polypeptides specifically for the assembly of pupation tubes necessary for larval/pupal ecdysis.

SALIVARY glands in larvae of the midge, *Chironomus tentans*, contain secretory cells that possess polytene chromosomes. Some of these chromosomes contain large tissue-specific puffs called Balbiani rings (BRs)[1] which are sites of intensive transcription (4, 17). Three rings (BR1, BR2, and BR6) contain members of a homologous multigene family. Each gene is composed of a hierarchy of tandemly repeated 180–240-bp protein coding DNA sequences (30, 37). These particular BR genes are transcribed into similar sized (6, 12, 13), ~37-kb (5) mRNAs (19, 32, 36) that code for a family of 1,000-kD secretory polypeptides (the sp-I family): a BR1 gene codes for sp-Ia, a BR2α gene codes for sp-Id, a BR2β gene codes for sp-Ib, and a BR6 gene codes for sp-Ic (3, 11, 14, 15, 21, 26, 31). The sp-I components are fibrous polypeptides (17, 18), some of which are glycosylated (16, 20) and phosphorylated (14, 26). sp-I components are often the major secretory polypeptides in salivary glands and polymerize into a silken thread that larvae spin for construction of feeding tubes (34, 35).

We recently discovered that a second gene is located in BR1. This gene is also comprised of tandemly repeated sequences that are transcribed into a 6.5-kb salivary gland–specific polyadenylated RNA (9). This 6.5-kb BR1 RNA is mRNA for a 180-kD secretory polypeptide (sp180) which, in contrast to sp-I components, is part of a gelatinous nonfibrous component of larval secretion (8).

We report here that the pattern of sp180 gene expression during the fourth larval instar is different from other BR genes. Expression of the sp180 gene is apparently regulated developmentally at the level of transcription, resulting in a maximal glandular concentration of sp180 just before larval/pupal ecdysis. The appearance of sp180 in salivary glands coincides with changes in larval secretion and the type of tubes that larvae construct from it. These observations lead us to propose that sp180 has a role in the modification and utilization of fibrous components of secretion in preparation for larval/pupal ecdysis.

Materials and Methods

Staging of Larvae

Each experiment was done with larvae from a single egg string that were grown at 16°C with a 6-h light/18-h dark cycle to induce the onset of oligopause around stage 5 of the fourth larval instar (24, 28). To obtain a

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Abbreviations used in this paper: BR, Balbiani ring; sp, secretory polypeptide; sp-I, family of 1,000-kD secretory polypeptides.

rather synchronous population of larvae proceeding through the remaining stages of the fourth instar, larvae were shifted to fresh culture medium and maintained at 20°C with a 16-h light/8-h dark cycle (28). Individual live larvae were staged based upon the morphology and orientation of imaginal disks according to Ineichen et al. (23).

Immunoblotting

Protein was extracted (26) from individual salivary glands that were removed from staged fourth instar larvae. Each extract was divided into two samples that were subjected to electrophoresis on parallel polyacrylamide gradient gels (26) and then transferred electrophoretically (8) to separate sheets of nitrocellulose. Polypeptides on one blot were visualized by staining with colloidal gold (AuroDye; Janssen Pharmaceutica, Beerse, Belgium). The duplicate blot with unstained polypeptides was incubated as described (8) with an affinity-purified nonapeptide-reactive antibody specific for sp180. Bound antibody was detected with ^{125}I-labeled *Staphylococcus aureus* protein A and autoradiography (2).

Nucleic Acid Hybridization

Total salivary gland RNA was usually extracted (5) from the second of each pair of glands used for protein extraction. The DNA sequence and hybridization specificity of BR1, BR2α, BR2β, and BR6 oligonucleotide probes and their cloned DNA templates have been described (3). Oligonucleotide TD1544A and its cDNA template have been described (9) and corresponded to a portion of sp180 mRNA (8). Salivary gland RNA and cloned DNA templates were spotted on nitrocellulose (3, 25) and hybridized (3), with end-labeled oligonucleotide probes or a nick-translated *C. tentans* genomic rDNA clone (9).

In Vitro Nuclear Transcription

For each in vitro transcription reaction, nuclei were isolated (33) from 10 salivary glands and incubated for 45 min at 20°C in 2 μl of buffer (33) containing 50 μM [α-^{32}P]UTP. ^{32}P-labeled nuclear RNA was extracted (5) and hybridized (3) to DNA dot-blots at 65°C for 72 h followed by a posthybridization incubation with pancreatic RNase.

Quantification of Data

A series of timed autoradiograms were subjected to scanning densitometry using a video densitometer (model 620; Bio-Rad Laboratories, Richmond, CA). This instrument was also used for scanning reflectance densitometry of blots containing colloidal gold-stained polypeptides. In many instances appropriate areas of a blot were punched out and radiation measured in a gamma counter (^{125}I-labeled protein A) or beta counter (^{32}P-labeled hybridization probes).

Immunofluorescence Microscopy

Individual staged larvae were contained within glass capillaries (8) and the process of secretion deposition and tube construction monitored with a stereomicroscope equipped with a video camera and television monitor. Samples of secretion were periodically removed and prepared for immunofluorescence microscopy as described (8). The primary antibody was affinity-purified nonapeptide-reactive rabbit antibody specific for sp180; the second was a fluorescein-conjugated goat anti-rabbit antibody.

Results

Salivary Gland Content of Secretory Polypeptides

We began this study by asking whether or not the same set of secretory polypeptides were present in salivary glands at various stages of the fourth larval instar. It is technically difficult to simultaneously maximize the electrophoretic resolution of secretory polypeptides ranging from 55 to 1,000 kD. Therefore, our description below of the relative glandular content of various secretory polypeptides summarizes the apparent patterns observed on dozens of stained gels that were run under a variety of conditions (i.e., different gel concentrations, voltage gradients, and times). Conclusive results, however, must await the availability of a repertoire of secretory polypeptide-specific antibodies.

The results for sp180 are described first because it is the only secretory polypeptide for which we have a specific antibody. When equivalent amounts (half a gland's worth) of protein were loaded in each lane of a gel, stainable amounts of sp180 were usually first observed at stage 8; the intensity typically increased to a maximum at stage 9.5–10 (Fig. 1 *a*). Immunoblots made with a peptide-reactive antibody specific for sp180 displayed a similar result (Fig. 1 *b*). When bound antibody was detected using ^{125}I-labeled *S. aureus* protein A, autoradiograms showed that the antibody selectively bound as a single band coincident with sp180 which was first detectable at stage 8 and increased as much as 10-fold by stage 10.

Some contrasting trends were noticed for the relative abundance of sp-I components. For example (Fig. 1 *a*), the levels of sp-Id and sp-Ib increased from stage 5.5 to a maximum at about stage 8 to 8.5. Then, the level of sp-Id decreased compared with the level of sp-Ib, which held relatively constant through stage 10. Compared with sp-Ib and sp-Id, sp-Ia was often not detected until stage 7–8 and increased to a maximum at stage 8–8.5, after which the level declined until it was barely detectable at stage 10. While similar trends were observed in other sets of extracts, the degree of variability rendered it impossible to ascribe a specific pattern of sp-I components to a particular developmental stage.

Larval salivary glands contain several other secretory polypeptides (8). We observed that sp130 was detectable as early as stage 5.5, increased abruptly at stage 8, and maintained an elevated level throughout the remainder of the fourth instar. The amount of sp170 was variable, often remaining constant (Fig. 1 *a*), though in some experiments it increased at the later stages. In comparison, stainable levels of sp150, sp115, sp60, and sp55 (the latter three are not abundant in Fig. 1 *a*) remained relatively constant throughout this instar (7).

Reflectance densitometric analysis of colloidal gold-stained blots (e.g., Fig. 1 *a*) indicated that at stage 5.5 the sp-I components accounted for 15–20% of the total gland protein, while sp130 and sp170 accounted for 5–15%. By stage 10, however, the combined levels of sp180, sp130, and sp170 increased to as much as 40% of total gland protein, while the sp-I components decreased to 10–15%. These are dramatic alterations in the dominant protein constituents of this tissue.

Steady-State Levels of BR mRNAs

Dot-blot hybridization was used to simultaneously measure the steady-state level of the five BR mRNAs using ^{32}P-labeled oligonucleotide probes specific for each BR gene. Autoradiograms indicated that while the amount of rRNA remained essentially constant during the fourth instar, the steady-state level of sp180 mRNA exhibited a stage-specific pattern of accumulation (Fig. 2). A basal level of sp180 transcripts was detectable between stages 5 and 7. Then, at stage 8, the transcript level rapidly increased so that by stages 9–10 there was at least a 20-fold increase. Hybridization of the sp180 oligonucleotide probe to Northern blots of salivary gland RNA confirmed that the stage-specific increase in dot-

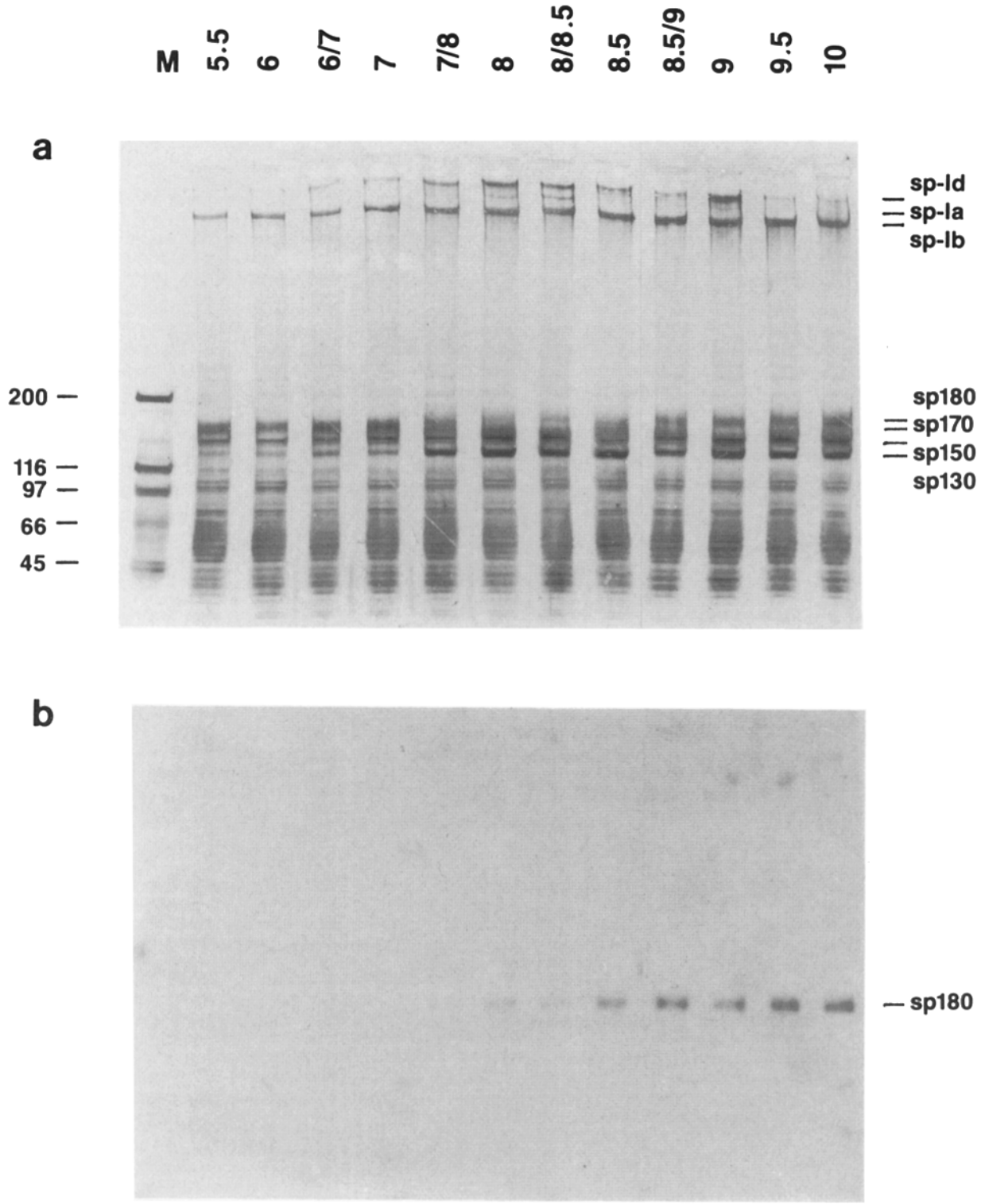

Figure 1. Developmental changes in the salivary gland content of secretory polypeptides at various stages of the fourth larval instar. Salivary glands were removed from larvae at different stages (5.5–10) of development. Equivalent amounts of protein (half a gland's worth) were separated on parallel polyacrylamide gradient gels and transferred electrophoretically to nitrocellulose. (*a*) The blot from one gel was stained with colloidal gold. The position and molecular mass (in kilodaltons) of markers (*M*) are indicated on the left and secretory polypeptides (*sp*) are indicated on the right. (*b*) An unstained duplicate blot was used for immunoblotting with an affinity-purified peptide reactive antibody that binds specifically to sp180. Bound antibody was detected with ^{125}I-labeled protein A and autoradiography.

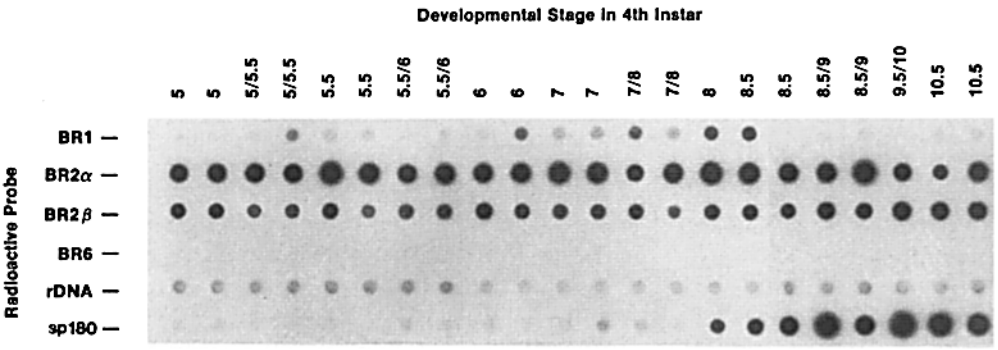

Figure 2. Steady-state levels of BR mRNAs and rRNA in salivary glands at various stages of the fourth larval instar. Vertical rows of dots contain equal amounts of RNA extracted from one salivary gland of a staged (5–10.5) larva. Each horizontal row of dots was hybridized with an end-labeled BR gene-specific oligonucleotide or nick-translated genomic rDNA clone.

blot hybridization coincided with an equivalent increase in hybridizable 6.5-kb RNA (data not shown). In addition, sequence titration dot-blot experiments were done in which the amount of ^{32}P-labeled oligonucleotide hybrids obtained with salivary gland RNA was compared with a serial dilution of a known quantity of cloned sp180 cDNA. We calculated that stage 10 larvae can attain a steady-state concentration of 1×10^7 molecules of sp180 mRNA per secretory cell. This calculation was based upon the following assumptions: (*a*) each salivary gland contains 38 secretory cells (17); (*b*) a 6.5-kb sp180 mRNA molecule is comprised entirely of 75-base repeats (9); and (*c*) each 75-b repeat in sp180 mRNA hybridizes one molecule of oligonucleotide (9).

The developmental dot-blot hybridization pattern obtained for BR mRNAs coding for sp-I components was completely different (Fig. 2). BR2α and BR2β mRNAs appeared to be most abundant, with no obvious pattern of stage-specific expression. Based upon sequence titration experiments and assumptions similar to those described for sp180 mRNA, we estimated that maximum level of hybridization obtained with either BR2α or BR2β probes corresponded to 1×10^7 mRNA molecules per cell. The steady-state level of BR1 mRNA was considerably variable (i.e., compare individuals at stages 5/5.5, 6, or 8.5), but generally highest between stages 6.5 and 8, reaching levels as high as 5×10^6 molecules per cell. In contrast, BR6 mRNA was not detectable; we calculated that its concentration during these stages of the fourth larval instar must be at least 100-fold lower than the other BR mRNAs. Note that the BR6 oligonucleotide probe readily detects BR6 mRNA in galactose-induced larvae (3).

Transcription of BR Genes in Isolated Salivary Gland Nuclei

The relative transcriptional rate of each BR gene was measured during various stages of the fourth instar by in vitro nuclear transcription. At each stage, ^{32}P-labeled nuclear RNA from 10 glands was extracted and hybridized to a set of DNA dots containing an excess of cloned BR DNA or rDNA sequences. The amount of radiolabeled, RNase-resistant hybrid in each dot was determined and two general observations were made (Fig. 3). First, nuclei obtained from larvae in oligopause were transcriptionally inactive compared with nuclei from postoligopause larvae. Second, the relative transcriptional rate of most BR genes was not constant; some exhibited maximal rates at particular developmental stages. For example, there was a transient surge in transcription of sp180 mRNA at stage 5.5, shortly after the animals were induced to continue development. This surge usually subsided by stage 6. Beginning at stage 7, however, transcription of sp180 mRNA accelerated to attain a maximum rate at stage 10 that average 10-fold higher than the rate measured at stage 6. We have performed genomic Southern blotting experiments using salivary gland DNA and found that there was no change in the sp180 gene copy number between stages 5 and 10 (data not shown).

Patterns of relative transcriptional rates for (sp-I)–coding BR genes resembled each other more than they did the pattern of the sp180 gene. BR1, BR2α, and BR2β genes all exhibited maximal rates of transcription at stage 5.5. As the fourth instar proceeded, the rate for BR1 decreased an average of 30%, BR2α decreased about 75%, and after an initial decrease of 75%, BR2β maintained a rate equal to 50% of its maximum. Since all five BR mRNAs contain a similar number of repeats and nearly identical amounts of uridine 5′-monophosphate (12.7–15.9%; see references in 9, 30, and 37), values in the ordinate of Fig. 3 provide a direct comparison of BR gene transcriptional rates. Thus, the transcriptional rates for sp180, BR1, and BR2α genes at stages 9 and 10 were nearly the same and about threefold less than BR2β. A comparatively minor amount of transcription of the BR6 gene was detected at stage 8.

The pattern of in vitro nuclear transcription of rRNA contrasted with BR mRNAs. There was a gradual increase in rate as animals proceeded through stages 5.5 and 6, and the rate accelerated between stages 7 and 8 so that by stage 9 it was 40-fold higher than it was at oligopause. The rate then

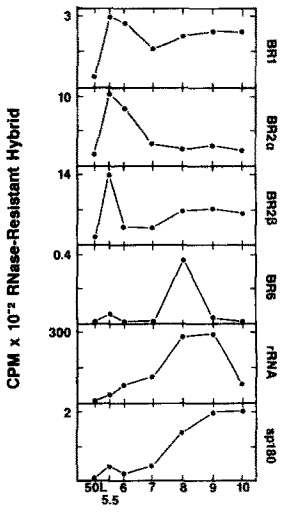

Figure 3. In vitro nuclear transcription of BR mRNAs and rRNA at various stages of the fourth larval instar. Nuclei were isolated from 10 salivary glands at each stage (5OL–10) of the fourth larval instar and incubated in a transcription medium containing [α-^{32}P]-uridine 5′-triphosphate. All the radiolabeled nuclear RNA from each stage was extracted and hybridized to a set of DNA dots containing cloned segments of BR genes (BR1, BR2α, BR2β, BR6, and sp180) or rDNA. After post-hybridization treatments, dots were punched out, and RNase-resistant radioactivity determined by liquid scintillation spectroscopy.

decreased by 60% as animals proceeded to stage 10. Since the UMP content of nucleolar pre-rRNA is 27% (10), the peak transcriptional rate measured for rRNA was ~25-fold greater than the rate of any BR mRNA at that stage.

Microscopic Changes in Larval Secretion

Since the salivary gland content of certain secretory polypeptides changed during the fourth instar (Fig. 1 *a*), we decided to observe in detail individual staged larvae during the processes of secretion deposition and tube construction. Early- and middle-stage fourth instar larvae spun secretion that looked like a silken thread. They deposited this thread on the walls of their containment vessel until they surrounded themselves with a silken tube. These tubes often ranged up to five times the length of the larva that constructed it. Late-stage larvae spun a thread that appeared macroscopically similar. Periodically, however, a larva gathered threads into a dense bundle and wrapped them around its midsection until it had covered itself with a tube that was typically shorter and more tensile than tubes made by younger larvae. These observations prompted us to examine secretion microscopically.

We recently described (8) fibrous and nonfibrous components of larval secretion, including the immunofluorescent localization of sp180 to punctate bodies within the nonfibrous component. We subsequently discovered that this composition changes during the fourth instar. Secretion spun by stage 7 larvae consisted almost entirely of fibrous material: loose networks or masses of long, thick fibers that were 2 μm in diameter (Fig. 4 *a*). These masses were easily teased apart and revealed that thick fibers consisted of several thinner fibers with diameters of 450 to 900 nm (Fig. 4 *b*). Secretion spun by stage 10 larvae contained apparently similar (8) thick fibers, but they were tightly aggregated into bundles (Fig. 4, *c* and *d*). In addition, stage 10 secretion contained a nonfibrous component that consisted of gelatinous material packed around and adhering to bundles of thick fibers. Immunofluorescence microscopy was used to demonstrate that the peptide-reactive antibody specific for sp180 (Fig. 1 *b*) selectively bound to discrete bodies within the nonfibrous component of secretion (Fig. 4, *c'* and *d'*). Control experiments verified

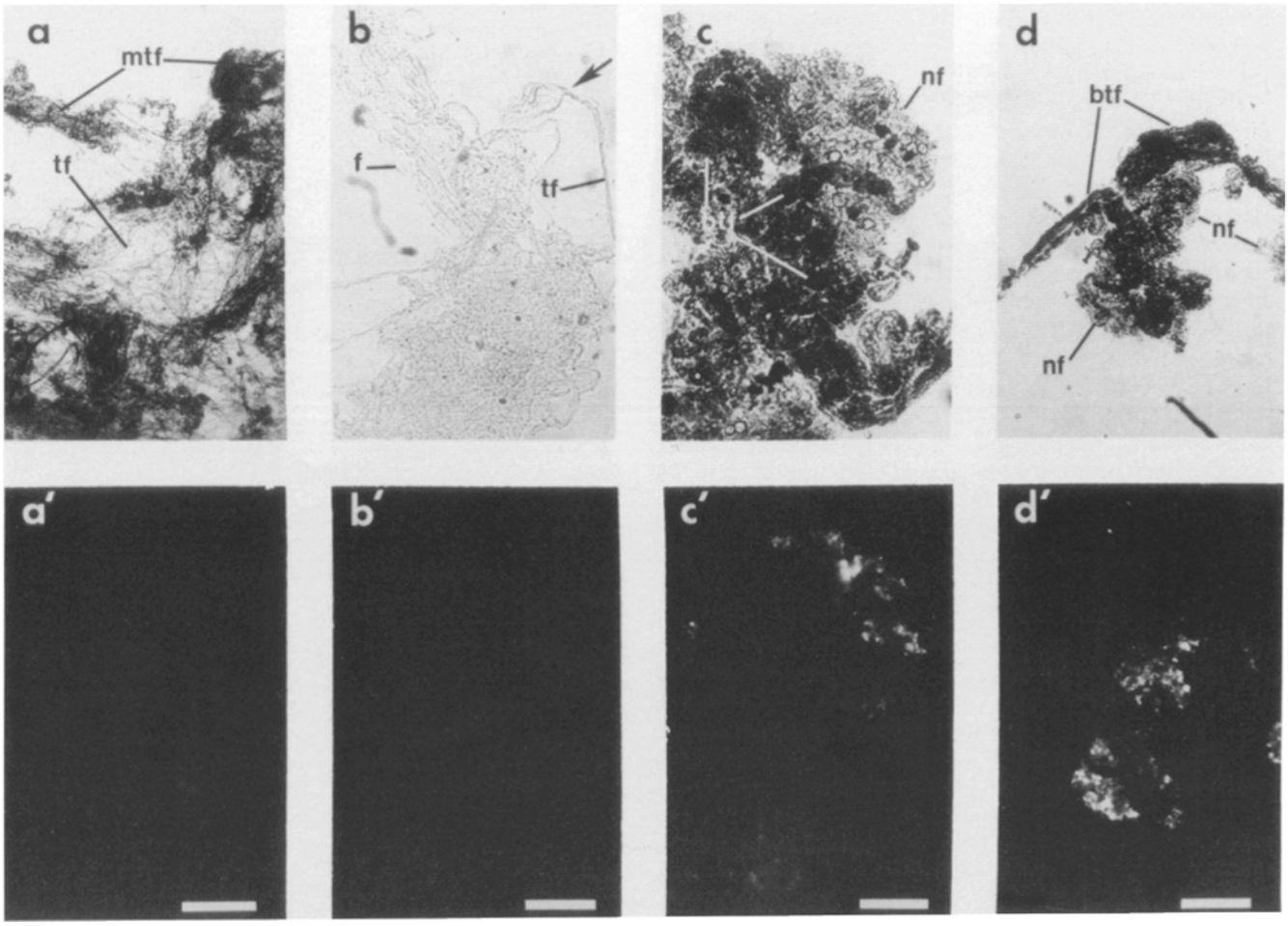

Figure 4. Microscopic comparison of salivary gland secretion spun by a middle- or late-stage fourth instar larva. Secretion spun by a stage 7 (*a* and *b*) and stage 10 (*c* and *d*) larva was gently (*a* and *c*) or extensively (*b* and *d*) teased apart and prepared for immunofluorescence microscopy. The primary antibody was an affinity purified peptide-reactive rabbit antibody specific for sp180. The second antibody was a fluorescein-conjugated goat anti-rabbit antibody. Brightfield (*a–d*) and fluorescent (*a'–d'*) images were photographed from the same fields of view. Thick fibers (*tf*) were sometimes unraveled (*arrow* in *b*) to reveal that they were comprised of thinner fibers (*f*). *mtf*, masses of thick fibers; *btf*, bundles of thick fibers; *nf*, nonfibrous component. Bars: (*a* and *d*) 250 nm; (*b* and *c*) 100 nm.

that both primary (antipeptide) and secondary (fluorescein-conjugated) antibodies were required to obtain the fluorescent images. Furthermore, immunofluorescence was not detectable in secretion spun by larvae younger than stage 8, even when both antibodies were used (Fig. 4, *a'* and *b'*).

Discussion

Developmentally Regulated Expression of the BR1 Gene for sp180

Expression of the sp180 gene in salivary glands was restricted to the latter stages of the fourth larval instar. While there was no measurable change in gene number, there was a 10-fold increase in transcriptional rate parallel to a 20-fold increase in the steady-state level of sp180 mRNA between stages 7 and 10. The accumulation of sp180 mRNA was nearly concomitant with a 10-fold increase in the glandular content of sp180. This is probably an underestimation of the increase in sp180; it resides only transiently in the glandular lumen before its exportation in secretion (8). We cannot exclude the involvement of mRNA stability in regulating the stage-specific expression of an sp180 gene. However, the parallel increase in transcription, mRNA, and protein accumulation suggest that synthesis of new transcripts is probably a key element in this regulation.

Expression of BR Genes for sp-I Components

The timing and regulation of sp-I gene expression during the fourth instar contrasted with the sp180 gene. First, BR genes for sp-I components did not exhibit reproducible stage-specific patterns of expression. Second, their levels of expression were not coupled proportionately to transcriptional rates. For example, the BR1 gene was transcribed at nearly the same rate during the fourth instar; however, the relative steady-state level of its mRNA and sp-Ia was often elevated between stages 6 and 8.5. BR2α and BR2β genes exhibited altered rates of transcription that were not reflected in changes in mRNA levels. Furthermore, whereas the relative levels of BR2β mRNA and sp-Ib were reasonably constant, the apparent level of sp-Id fluctuated more than the level of BR2α mRNA. These results suggest that variations in the salivary gland content of particular sp-I components (Fig. 1 *a*) may reflect a larva's ability to independently regulate the expression of BR1, BR2α, and BR2β genes in response to subtle changes in habitat (3, 27). Furthermore, noncoordinate levels of transcription, mRNA, and polypeptide imply that expression of these BR genes may involve regulation at the posttranscriptional and translational levels of control.

In contrast to BR1, BR2α, and BR2β genes, detectable levels of BR6 gene transcription, BR6 mRNA, and sp-Ic are only found in larvae exposed to specific inducers (1, 3, 11, 14, 21, 26). During the course of this study, we found that BR6 gene expression can be most effectively achieved before the onset of oligopause (data not shown).

Synthesis of rRNA

The rate of rRNA transcription increased 40-fold from stages 6 to 9 (Fig. 3). Remarkably, this increase was not accompanied by an increase in the steady-state level of rRNA (Fig. 2). This suggests that either newly synthesized precursors to rRNA undergo rapid intranuclear degradation or that the half-life of cytoplasmic rRNA begins to decrease.

Whereas rRNA is the most abundant cellular RNA, the autoradiographic intensity of dots hybridized with rDNA was generally less than the intensity of dots exhibiting maximum hybridization with BR probes (Fig. 2). This was most likely due to the fact that BR mRNAs can become abundant and that each molecule contains tandemly repeated sequences with up to several hundred oligonucleotide hybridization sites (see reference 3 for details).

Dynamics of the Prepupal Salivary Gland

Stage 8 of the fourth larval instar is the beginning of the prepupal period. Larval/pupal apolysis occurs, resulting in a pharate pupa that has acquired internal physiological features that are distinctively pupal, though the external larval cuticle has not been shed (22). Shedding of the larval cuticle takes place at the larval/pupal ecdysis or "molt" which occurs after stage 10.5. Chironomids undergo larval/pupal ecdysis in underwater tubes made of larval secretion (29): free-swimming species build a pupation tube especially for this purpose, whereas tube-dwelling species, such as *C. tentans*, modify existing feeding tubes. The prepupal period is also accompanied by macromolecular alterations in secretion.

We demonstrated that at least one sp-I component is present in secretion throughout the fourth instar and that microscopically visible changes occur in the fibrous component starting at stage 8. These alterations coincide with the appearance of sp180 in secretion and construction of tubes that are noticeably different in size and texture. We therefore propose that sp180, and perhaps other developmentally regulated secretory polypeptides (i.e., sp130), in some way modify the structure and/or utilization of the fibrous sp-I components to enable their assembly into a pupation tube necessary for larval/pupal ecdysis.

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References

1. Beerman, W. 1973. Directed changes in the pattern of Balbiani ring puffing in *Chironomus*: effects of a sugar treatment. *Chromosoma (Berl.)*. 41: 297–326.
2. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195–203.
3. Case, S. T. 1986. Correlated changes in steady-state levels of Balbiani ring mRNAs and secretory polypeptides in salivary glands of *Chironomus tentans*. *Chromosoma (Berl.)*. 94:483–491.
4. Case, S. T., and B. Daneholt. 1977. Cellular and molecular aspects of genetic expression in *Chironomus* salivary glands. *Int. Rev. Biochem.* 15: 45–77.
5. Case, S. T., and B. Daneholt. 1978. The size of the transcription unit in Balbiani ring 2 of *Chironomus tentans* as derived from analysis of the primary transcript and 75S RNA. *J. Mol. Biol.* 124:223–241.
6. Daneholt, B. 1972. Giant RNA transcript in a Balbiani ring. *Nature (Lond.)*. 240:229–232.

7. Dreesen, T. D. 1986. Developmental regulation of a newly discovered Balbiani ring 1 gene. Ph.D. thesis. The University of Mississippi Medical Center, Jackson, MS.
8. Dreesen, T. D., and S. T. Case. 1987. A peptide-reactive antibody to a Balbiani ring gene product: immunological evidence that a 6.5-kb RNA in *Chironomus tentans* salivary glands is mRNA for a 180-kDa nonfibrous component of larval secretion. *Gene (Amst.).* 55:55–65.
9. Dreesen, T. D., J. R. Bower, and S. T. Case. 1985. A second gene in a Balbiani ring: *Chironomus* salivary glands contain a 6.5 kb poly(A)$^+$ RNA that is transcribed from a hierarchy of tandem repeated sequences in Balbiani ring 1. *J. Biol. Chem.* 260:11824–11830.
10. Edström, J.-E., and W. Beermann. 1962. The base composition of nucleic acids in chromosomes, puffs, nucleoli and cytoplasm of *Chironomus* salivary gland cells. *J. Cell Biol.* 14:371–379.
11. Edström, J.-E., L. Rydlander, and C. Francke. 1980. Concomitant induction of a Balbiani ring and a giant secretory protein in *Chironomus* salivary glands. *Chromosoma (Berl.).* 81:115–124.
12. Edström, J.-E., H. Sierakowska, and K. Burvall. 1982. Dependence of Balbiani ring induction in *Chironomus* salivary glands on inorganic phosphate. *Dev. Biol.* 91:131–137.
13. Egyházi, E. 1975. Inhibition of Balbiani ring RNA synthesis at the initiation level. *Proc. Natl. Acad. Sci. USA.* 72:947–950.
14. Galler, R., L. Rydlander, N. Riedel, H. Kluding, and J.-E. Edström. 1984. Balbiani ring induction in phosphate metabolism. *Proc. Natl. Acad. Sci. USA.* 81:1448–1452.
15. Galler, R., H. Saiga, R. M. Widmer, M. Lezzi, and J.-E. Edström. 1985. Two genes in Balbiani ring 2 with metabolically different 75S transcripts. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2977–2982.
16. Gorman, C. M., and H. L. Hosick. 1982. Tests for carbohydrates and isopeptide bonds in larval salivary proteins of *Chironomus tentans. Insect Biochem.* 12:323–330.
17. Grossbach, U. 1977. The salivary gland of *Chironomus* (diptera): a model system for the study of cell differentiation. *In* Results and Problems in Cell Differentiation. Vol 8. W. Beermann, editor. Springer-Verlag, Berlin/Heidelberg. 147–196.
18. Hamodrakas, S., and F. C. Kafatos. 1984. Structural implications of primary sequences from a family of Balbiani ring-encoded proteins in *Chironomus. J. Mol. Evol.* 20:296–303.
19. Hardy, P. A., and C. Pelling. 1980. Cell-free synthesis and immunological characterization of salivary proteins from *Chironomus tentans. Chromosoma (Berl.).* 81:403–417.
20. Hertner, T., H. M. Eppenberger, and M. Lezzi. 1983. The giant secretory proteins of *Chironomus tentans* salivary gland: the organization of their primary structure, their amino acid and carbohydrate composition. *Chromosoma (Berl.).* 88:194–200.
21. Hertner, T., B. Meyer, H. M. Eppenberger, and R. Mahr. 1980. The secretion proteins of *Chironomus tentans* salivary glands: electrophoretic characterization and molecular weight estimation. *Wilhelm Roux's Arch. Dev. Biol.* 189:69–72.
22. Hinton, H. E. 1968. Spiracular gills. *Adv. Insect Physiol.* 5:65–162.
23. Ineichen, H., B. Meyer, and M. Lezzi. 1983. Determination of the developmental stage of living fourth instar larvae of *Chironomus tentans. Dev. Biol.* 98:278–286.
24. Ineichen, H., U. Riesen-Willi, and J. Fischer. 1979. Experimental contribution to the ecology of *Chironomus* (diptera). II. The influence of the photoperiod on the development of *Chironomus plumosus* in the 4th instar. *Oecologia (Berl.).* 39:161–183.
25. Kafatos, F. C., C. W. Jones, and A. Efstratiadis. 1979. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. *Nucleic Acids Res.* 7:1541–1552.
26. Kao, W.-Y., and S. T. Case. 1985. A novel giant secretion polypeptide in *Chironomus* salivary glands: implications for another Balbiani ring gene. *J. Cell Biol.* 101:1044–1051.
27. Kao, W.-Y., and S. T. Case. 1986. Individual variations in the content of giant secretory polypeptides in salivary glands of *Chironomus. Chromosoma (Berl.).* 94:475–482.
28. Meyer, B., R. Mahr, H. M. Eppenberger, and M. Lezzi. 1983. The activity of Balbiani rings 1 and 2 in salivary glands of *Chironomus tentans* larvae under different modes of development and after pilocarpine treatment. *Dev. Biol.* 98:265–277.
29. Oliver, D. R. 1971. Life history of the Chironomidae. *Annu. Rev. Entomol.* 16:211–230.
30. Pustell, J., F. C. Kafatos, U. Wobus, and H. Bäumlein. 1984. Balbiani ring DNA: sequence comparisons and evolutionary history of a family of hierarchically repetitive protein-encoding genes. *J. Mol. Evol.* 20:281–295.
31. Rydlander, L., and J.-E. Edström. 1980. Large size nascent protein as dominating component during protein synthesis in *Chironomus* salivary glands. *Chromosoma (Berl.).* 81:85–99.
32. Rydlander, L., A. Pigon, and J.-E. Edström. 1980. Sequences translated by Balbiani ring 75S RNA *in vitro* are present in giant secretory protein from *Chironomus tentans. Chromosoma (Berl.).* 81:101–113.
33. Sass. H. 1980. Features of *in vitro* puffing and RNA synthesis in polytene chromosomes of *Chironomus. Chromosoma (Berl.).* 78:23–78.
34. Wallace, J. B., and R. W. Merritt. 1980. Filter-feeding ecology of aquatic insects. *Annu. Rev. Entomol.* 25:103–132.
35. Walshe, B. M. 1950. The feeding habits of certain *Chironomid* larvae (subfamily tendipedinae). *Proc. Zool. Soc. Lond.* 121:63–79.
36. Weber, F., R. Mahr, B. Meyer, H. M. Eppenburger, and M. Lezzi. 1983. Cell-free translation of Balbiani ring RNA (75S) of *Chironomus tentans* salivary glands into high molecular weight products. *Wilhelm Roux's Arch. Dev. Biol.* 192:200–203.
37. Wieslander, L., C. Höög, J.-O. Höög, H. Jörnvall, U. Lendahl, and B. Daneholt. 1984. Conserved and nonconserved structures in the secretory proteins encoded in the Balbiani ring genes of *Chironomus tentans. J. Mol. Evol.* 20:304–312.