The Ecdysone-Induced Puffing Cascade in Drosophila Salivary Glands: A *Broad-Complex* Early Gene Regulates Intermolt and Late Gene Transcription

Pamela S. Guay and Gregory M. Guild

Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6018

Manuscript received March 4, 1991
Accepted for publication May 24, 1991

ABSTRACT

The steroid hormone 20-OH ecdysone triggers a classic and well-defined program of chromosome puffing that is assumed to reflect changes in transcriptional activity in Drosophila salivary glands. Mutations in each of four *Broad-Complex* locus (*BR-C*) complementation groups were analyzed for their effects on the expression of other genes that reside in several major salivary gland puffs. RNA blot analysis showed that the *rbp* function of the *BR-C* is required for the transcription of six genes in the 71E late puff and is the first demonstration that an ecdysone-induced early gene controls the transcription of late genes within the puffing cascade. In addition, the *rbp* function is required for the transcription of four intermolt genes (*Sgs-3*, *Sgs-4*, *Sgs-5* and 71E gene *VII*). Mutations in the *broad*, *l(1)2Bc* and *l(1)2Bd* functions of the *BR-C* had no effect on the expression of the genes examined. We propose that the *BR-C* functions to control transcription at many other salivary gland loci at the beginning of metamorphosis.

METAMORPHOSIS in Drosophila is signaled by a pulse of the steroid molting hormone 20-OH ecdysone (hereafter referred to as ecdysone) at the end of the third larval instar. The ensuing dramatic changes in gene expression guide the morphogenetic events that shape post-larval development. These ecdysone-induced alterations in gene expression can be visualized in the third instar salivary gland as a cascade of three temporally distinct puff sets on the polytene genome (reviewed in ASHBURNER and BERENDES 1978). The intermolt puffs, responsible for salivary gland glue production, become active in the mid-third instar and rapidly regress in response to the rise in ecdysone titer. A small number of early puffs are induced rapidly in response to ecdysone, remain active for several hours in the continued presence of hormone, and then regress. Protein synthesis inhibitors do not affect the induction of the early puffs (ASHBURNER 1973) indicating that their hormonal activation is a primary event. Coincident with early puff regression, is the induction of a large number of late puffs (>100) which remain active for 9–12 hr. Their induction is dependent on protein synthesis and early gene function (ASHBURNER 1973; BELYAEVA *et al.* 1981; WALKER and ASHBURNER 1981; BELYAEVA and ZHIMULEV 1982; ZHIMULEV, VLASSOVA and BELYAEVA 1982).

ASHBURNER *et al.* (1974) proposed that early puff genes are the primary targets for ecdysone induction, and that their products are essential for late gene activation and for their own negative autoregulation. This model suggests a hierarchical mechanism to explain the hormone-induced but temporally distinct expression of the early and late gene sets. The *Broad-Complex* (*BR-C*) locus, located in the 2B5 early puff, is one of the regulatory early genes and is vital to the normal progression of the ecdysone-induced puffing cascade. *BR-C* null mutations result in abnormalities in each of the puff sets. For example, in *BR-C* null mutants the 68C intermolt puff fails to regress, the puffing response of the 74EF and 75B early puffs is inhibited by 60–70%, and the 63F early puff remains uninduced. More strikingly, the absence of *BR-C* function prevents the induction of the entire late puff set (BELYAEVA *et al.* 1981; ZHIMULEV, VLASSOVA and BELYAEVA 1982).

The *BR-C* is a large genetically complex locus, encompassing approximately 100 kb of the genome (CHAO and GUILD, 1986; BELYAEVA *et al.* 1987). As many as four complementation groups have been described at the locus [*br*, *broad*; *rbp*, *reduced bristle pairs on the palpus;* *l(1)2Bc;* and *l(1)2Bd*], as well as a set of mutations (*npr1*, *nonpupariating*) that fail to complement mutations in each of these complementation groups. Lethal alleles have been described for each complementation group, with the exception of the single viable *l(1)2Bd* allele (BELYAEVA *et al.* 1981, 1982; KISS *et al.* 1988). In addition, several ecdysone-inducible *BR-C* RNA products have been described by cDNA cloning and RNA blotting analyses (CHAO and GUILD 1986; GALCERAN *et al.* 1990; DIBELLO *et al.* 1991). Sequence analysis indicates that the *BR-C*

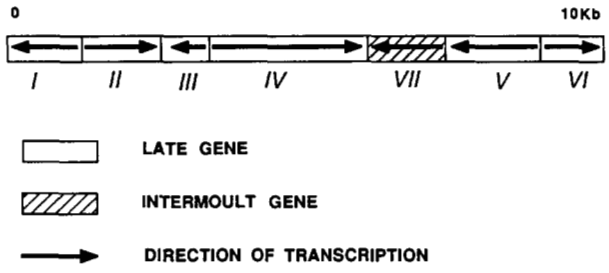

FIGURE 1.—The 71E gene cluster. The boxes within the map represent transcribed regions that are defined as those restriction fragments with homology to cDNA clones and/or poly(A)$^+$ RNA from late third instar larval or prepupal salivary glands (RESTIFO and GUILD 1986a). The transcriptional orientation of each region is denoted with arrows (5′ → 3′). Those regions expressed in prepupal salivary glands (*I–VI*) represent "late" genes and are denoted by open boxes. That region expressed in larval salivary glands (*VII*) represents an "intermolt" gene and is denoted by a cross-hatched box.

encodes at least three classes of related zinc finger-containing proteins that may function as DNA-binding transcription factors (DIBELLO *et al.* 1991). The existence of multiple *BR-C* products is consistent with the observations that this locus provides several different functions and that distinct and apparently tissue-limited phenotypes are exhibited by mutations at this locus (BELYAEVA *et al.* 1980, 1981; ZHIMULEV, VLASSOVA and BELYAEVA 1982; KISS *et al.* 1988).

To determine whether the *BR-C* is involved in the transcriptional regulation of genes in the ecdysone-induced puffing cascade, we examined the effects of mutations in each of the *BR-C* complementation groups on the expression of intermolt and late genes. We investigated the *Sgs-3*, *Sgs-4* and *Sgs-5* intermolt glue protein genes, and seven other salivary gland-specific genes located in a closely linked cluster within the 71E puff. This cluster contains one intermolt gene and six temporally coregulated late genes, the latter being arranged as three divergently transcribed gene pairs (Figure 1). We provide evidence for the ASHBURNER hierarchical model by demonstrating that downstream late genes are dependent on *BR-C* function for proper transcriptional expression and identify one of the *BR-C* complementation groups (*rbp*) as being responsible for this. In addition, we extend results obtained by others (CROWLEY, MATHERS and MEYEROWITZ 1984; GALCERAN *et al.* 1990; GEORGEL *et al.* 1991) by showing that the *rbp* function is also responsible for regulating intermolt gene transcription.

MATERIALS AND METHODS

Mutant stocks and crosses: The *BR-C* mutant alleles (and their former designations, see KISS *et al.* 1988) and other chromosomes used in this study are listed in Table 1 or described in LINDSLEY and GRELL (1968). The t-series *BR-C* mutations were generated on *X* chromosomes marked with the *yellow* mutation and balanced with the *Binsn X* chromosome, with the exception of the viable *l(1)2Bd^1* allele, which is marked with *yellow* and *white* and is not balanced. The *Df(1)S39* deficiency was maintained as *C(1)RM y shi^{ts}/y^2 Dp(1;Y)67g19.1/Df(1)S39*. The *Dp(1;Y)67g19.1* duplication contains a small portion of the *X* chromosome (cytogenetic regions 1A-2B17,18) translocated to the *Y* chromosome (BELYAEVA *et al.* 1982) and was maintained as *C(1)Dx y f/ Dp(1;Y)67g19.1 /y w*. Flies were maintained on standard corn flour medium at 25° except for the *Df(1)S39* stock, which was maintained at 18°.

TABLE 1

Mutant symbols and abbreviations

Mutant class[a]	Allele[b]	Former designation[b]	Lethal period[c]	Reference[d]
broad	*br^5*	*t35*	Early prepupa	1
rbp	*rbp^1*	*t99*	Pharate adult	1
	rbp^4	*t358*	Late pupa	1
	rbp^5	*t376*	Late pupa	1
l(1)2Bc	*2Bc^1*	*t10*	Late prepupa	1
	2Bc^2	*t76*	Late prepupa	1
l(1)2Bd	*2Bd^1*	*t252*	Viable	1
Other stocks	*Df(1)S39*	None	Embryonic	1, 2
	Dp(1;Y)67g19.1	*Dp(1;Y)67g*	Viable	1, 2

[a] LINDSLEY and ZIMM (1986).
[b] KISS *et al.* (1988); BEATON *et al.* (1988).
[c] As determined at either at 25° or 29° by BELYAEVA *et al.* (1980, 1982); KISS *et al.* (1988).
[d] 1, BELYAEVA *et al.* (1980); 2, CRAYMER and ROY (1980).

Collection of larvae and prepupae: Animals were collected and staged using the morphological criteria of BAINBRIDGE and BOWNES (1981) essentially as described previously (RESTIFO and GUILD 1986a). Late third instar larvae were at the wandering stage. The beginning of the prepupal period was taken to be at white puparium formation. Animals were collected at timed intervals (0–24 hr at ~25°) after this distinct morphological event. The *yellow* phenotype of mouth hooks and denticle belts was used to distinguish hemizygous mutant animals from their wild-type siblings.

RNA isolation and hybridization analysis: RNA was extracted from whole animals using phenol (GUILD 1984; ASHBURNER 1989). The polyadenylated fraction was purified by oligo(dT)-cellulose chromatography and fractionated on denaturing formaldehyde-agarose gels as described previously (RESTIFO and GUILD 1986a). RNA was transferred to GeneScreen nylon membrane (NEN/Du Pont) or Immobilon PVDF membrane (Millipore) using 10 × or 20 × SSPE, respectively.

Radiolabeled nick translated DNA or single-stranded RNA probes (initiated from bacteriophage SP6, T7 or T3 promoters) were prepared (SHORE and GUILD 1986, 1987) from genomic DNAs cloned into plasmid vectors. Most cloned DNA segments were transferred from their original vector into pBluescript II KS$^+$ (Stratagene). The genes used in this study along with their original (parentheses) and new clone designations are listed below. *Sgs-3* (paDm2023; GARFINKEL, PRUITT and MEYEROWITZ 1983) paaDm798; *actin5C* (Hd-19; VIGOREAUX and TOBIN 1987) paaDm809; 71E gene *I* (meDm131; RESTIFO and GUILD 1986a) paaDm801; 71E gene *II* (meDm134) paaDm802; 71E gene *III* (meDm108) paaDm803; 71E gene *IV* (meDm114) paaDm804; 71E gene *V* (mhDm275) paaDm805; 71E gene *VI* (meDm123) paaDm806; 71E gene *VII* (mhDm282) paaDm807. The *Sgs-4*-containing genomic region (MUSKAV-

ITCH and HOGNESS 1982) corresponding to nucleotides −2 (*Eco*RI) to +796 (*Hin*dIII) was cloned into the pGEM-1 vector (Promega) and generously supplied by C. GATES and S. BECKENDORF (Uuniversity of California at Berkeley). The *Sgs-5*-containing genomic clone pnDm384 has been described (SHORE and GUILD 1986). All intermolt and late genes analyzed in this study exhibit salivary gland-specific expression (MEYEROWITZ and HOGNESS 1982; GUILD 1984; RESTIFO and GUILD 1986a; MCNABB and BECKENDORF 1986). Hybridizations were carried out as previously described (GUILD 1984; SHORE and GUILD 1987). If nylon membranes were reused, previously hybridized probe was stripped from the blot in a solution of 25% formamide, 0.1% sodium dodecyl sulfate (SDS), 0.1 × SSPE at 70° (2 × 15 min). These blots were subsequently rehybridized as described above.

RESULTS

***BR-C* mutations affect gene expression in developing salivary glands:** RNA blot analysis was used to examine the effects of *BR-C* mutations on gene expression during the late third instar to prepupal interval. RNA from hemizygous rbp^1 larvae and prepupae was probed for intermolt transcripts. The transcripts from three glue protein genes were less abundant in rbp^1 animals than in wild-type controls (Figure 2a). Expression of *Sgs-3* was less than 3% and expression of *Sgs-4* and *Sgs-5* was between 10 and 33% of wild-type levels. Although the third instar expression level of the multiple 71E gene *VII* transcripts (RESTIFO and GUILD 1986a) was moderately reduced compared to that of the wild-type control, the ensuing temporal pattern of expression was strikingly different. In rbp^1 animals, transcripts from this gene were detected for at least 6 hr after puparium formation. This was in contrast to wild-type animals, where these transcripts were greatly reduced or absent at puparium formation (the 0 hr time point).

The expression of the six late genes of the 71E cluster was analyzed using similar methods. Each of the late genes tested showed a reduced level of expression in rbp^1 animals when compared to wild-type controls (Figure 2b). Expression was less than 3% of that in wild type for genes *I* and *II* and genes *V* and *VI*, and was approximately 10% for genes *III* and *IV*. It is interesting to note the correlation between the molecular organization of the 71E cluster (Figure 1) and the pairwise nature of these late gene responses. This suggests that the divergently transcribed gene pairs share *BR-C* responsive, *cis*-acting sequences.

To verify that these transcriptional phenotypes were a result of a mutation in the *BR-C* region, a small duplication of the *X* chromosome that includes a wild-type copy of the *BR-C* was used to complement the rbp^1 mutation. This duplication, when added to hemizygous rbp^1 animals, restored wild-type expression to each of the genes tested (Figure 3).

These results demonstrate that *BR-C* activity, and in particular the rbp^1 function, is necessary for several

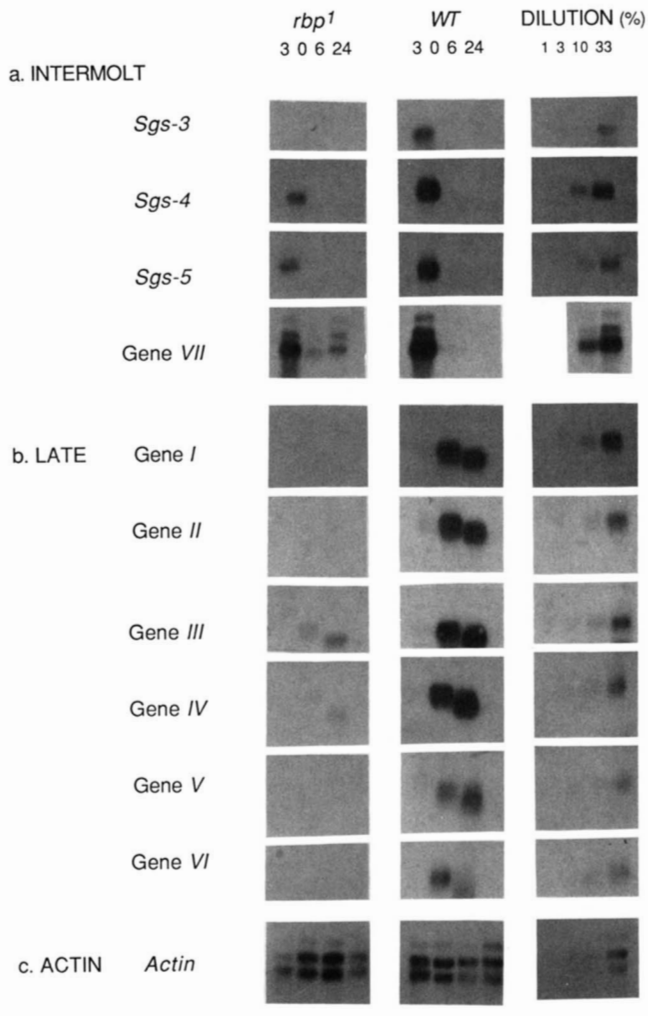

FIGURE 2.—Intermolt and late gene expression in rbp^1 animals. (a) Polyadenylated RNA (0.5 μg) from the indicated sources was probed for the presence of intermolt gene transcripts. RNA was isolated from animals of the following genotypes: *y* rbp^1 */Y* (designated rbp^1) or a mixture of +/+ and *y* rbp^1/+ (WT). RNA was collected from late third instar animals (3); white prepupae (0); white prepupae aged 6 hr (6); and white prepupae aged 24 hr (24). Dilutions of wild-type late third instar RNA equivalent to 33, 10, 3 and 1% of that loaded in the other lanes were also loaded for comparison. Only those portions of the autoradiograms that yielded signals are shown. The three species of RNA complementary to the gene *VII* probe have been described previously (RESTIFO and GUILD 1986a). (b) RNAs identical to those described above were probed for the presence of late gene transcripts. In this case, dilutions of wild-type white prepupal RNA were loaded for comparison. The developmental decrease in late transcript size is due to the shortening of their poly(A) tracts (RESTIFO and GUILD 1986b). (c) RNA populations were probed for the presence of actin transcripts. Blots previously probed with *Sgs-4* and 71E gene *VI* (above) were hybridized with a Drosophila *actin5C* probe to estimate the amount of RNA loaded in each lane. Multiple RNA species are detected with this probe as expected (VIGOREAUX and TOBIN 1987). Equivalent results (not shown) were obtained with all other blots shown in (a) and (b). RNA populations from wild-type males (+/*Y*) were also used and yielded results equivalent to those shown in the WT lanes (data not shown).

aspects of transcriptional regulation in the ecdysone-regulated puffing cascade in developing salivary

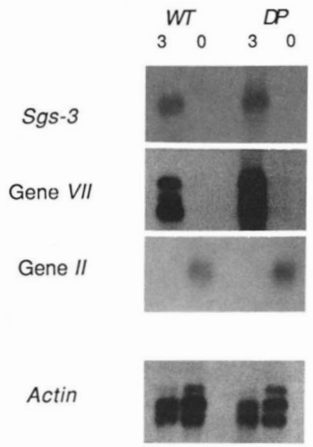

FIGURE 3.—The *Dp(1;Y)67g19.1* duplication complements the *rbp*^{1} mutation. Polyadenylated RNA (0.25 μg) from the indicated sources was probed for the presence of the indicated transcripts. RNA was isolated from animals of the following genotypes: *C(1)Dx y f/y*^{2} *Dp(1;Y)67g19.1* (designated WT) or *y rbp*^{1}*/y*^{2}*Dp(1;Y)67g19.1* (DP). These RNAs were collected from late third instar animals (3) or white prepupae (0). Only those portions of the autoradiograms that yielded signals are shown. The stock [*C(1)Dx y f/y*^{2}*Dp(1;Y)67g19.1/y w*] was used to construct the indicated genotypes. Note that the expression of gene *VII* appears greater in the duplication than in the wild type. This difference could reflect differences in genetic background since these animals contain *BR-C*$^+$ alleles from different sources.

glands. Subsequent experiments were undertaken to determine whether any other *BR-C* function is involved in the transcriptional regulation of this cascade.

Only the *rbp* function of the *BR-C* is necessary for intermolt and late transcription in salivary glands: Mutations representing each of the *BR-C* complementation groups were examined for their effects on intermolt and late gene expression as described above. Interestingly, only mutations in the *rbp* complementation group had any effect on the expression of the genes tested.

Intermolt gene expression was affected by all of the *rbp* mutations tested. For example, *rbp*^{4} and *rbp*^{1} mutant animals exhibited similar intermolt gene transcriptional phenotypes. However, the consequences of the *rbp*^{5} mutation were more severe than those of the other *rbp* mutations (Figure 4). In this case, expression of each of the glue genes and of 71E gene *VII* was reduced to less than 3% of wild-type levels. Animals carrying the *br*^{5}, *l(1)2Bc*^{1} (data not shown), *l(1)2Bc*^{2}, and *l(1)2Bd*^{1} mutations did not show these effects, exhibiting instead, levels of intermolt gene expression similar to wild-type controls (Figure 4).

Late gene expression in *rbp*^{4} and *rbp*^{5} mutants was greatly reduced and similar to the levels observed in *rbp*^{1} mutants, with the exception of 71E genes *III* and *IV*. In this case, transcript levels in *rbp*^{4} (data not shown) and in *rbp*^{5} animals (Figure 5) were reduced to even lower levels when compared to *rbp*^{1} animals. There was no detectable difference in late gene expression in *br*^{5}, *l(1)2Bc*^{1} (data not shown), *l(1)2Bc*^{2}, and *l(1)2Bd*^{1} mutants when compared to wild-type controls (Figure 5).

As in the case of the *rbp*^{1} mutation, a small chromosomal duplication was used to complement the intermolt and late gene transcriptional defects exhibited by the *rbp*^{4} and *rbp*^{5} mutations. This duplication restored wild-type regulation to each of the genes tested in both mutants (data not shown). We conclude that the transcription of genes in the ecdysone-induced puffing cascade is dependent on the *rbp* function of the *BR-C*.

DISCUSSION

ASHBURNER *et al.* (1974) proposed a hierarchical model to account for the regulation of the chromosomal puffing cascade in developing salivary glands. We provide evidence for this model at the transcriptional level by demonstrating that the expression of at least six downstream genes located in the 71E late puff is dependent on an activation function supplied by the *BR-C*, an early puff gene (summarized in Table 2).

Since transcription from the 71E late genes is drastically reduced in *rbp* mutants, one might predict a similar reduction in puffing at the 71E locus. Interestingly, CROWLEY, MATHERS and MEYEROWITZ (1984) showed that expression of *Sgs-3*, *Sgs-7* and *Sgs-8* was virtually eliminated in *BR-C npr1* null mutants even though the corresponding cytogenetic locus was characteristically puffed. It is possible that 71E puffing is also unaffected by *BR-C* mutations. Although we have no information in this regard, study of 71E puffing would be problematical since this late gene cluster represents only a portion of a larger and more complex puff (SEMESHIN *et al.* 1985) that exhibits intermolt, early, and late temporal components [see RESTIFO and GUILD (1986a) for discussion].

CROWLEY, MATHERS and MEYEROWITZ (1984), GALCERAN *et al.* (1990) and GEORGEL *et al.* (1991) described reduced levels of intermolt gene expression in *BR-C npr1* null mutations. We extend these results by showing that intermolt gene expression depends on the *rbp* function of the *BR-C* (Table 2). Thus, even though the *BR-C* is a member of the early puff set and is instrumental in the regulation of downstream late genes, it is also active prior to the late third instar rise in ecdysone titer since it plays a role in the regulation of the intermolt genes. This is consistent with the observation that the major species of *BR-C* RNA in salivary glands is present prior to, and is further stimulated by, this ecdysone pulse (CHAO and GUILD 1986) and supports a feature of the cascade model added by ZHIMULEV, VLASSOVA and BELYAEVA (1982), which holds that the *BR-C* is also involved in intermolt gene regulation.

The *rbp* complementation group represents the only *BR-C* function involved in the transcriptional

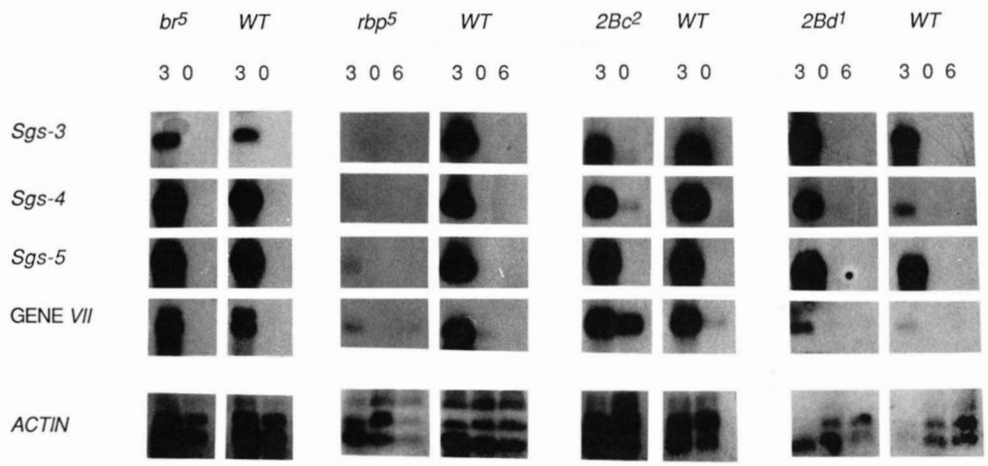

FIGURE 4.—Intermolt gene expression in *BR-C* mutants. Polyadenylated RNA (0.25 μg) from the indicated sources was probed for the presence of intermolt transcripts. RNA was isolated from animals of the following genotypes: br^5: *y br^5/Y* (br5), *y br^5/Binsn* and *Binsn/Binsn* siblings (WT); rbp^5: *y rbp^5/Y* (rbp^5), *y rbp^5/Binsn* and *Binsn/Binsn* siblings (WT); 2Bc^2: *y 2Bc^2/Y* (2Bc^2), *y 2Bc^2/Binsn* and *Binsn /Binsn* siblings (WT); 2Bd^1: *y 2Bd^{1}w/Df(1)S39* (2Bd^1), *y 2Bd^{1}w/y^{2}Dp(1;Y)67g19.1* (WT). Genes examined and time points for RNA collection are as in Figure 2. Only those portions of the autoradiograms that yielded signals are shown. Note that the *2Bc^2* panel for gene *VII* and the *2Bd^1* panels for *Sgs-4* and gene *VII* show higher expression than the corresponding wild-type controls. Examination of the actin signals from these blots indicate that the wild-type lanes were underloaded. Comparison of the *br^5* and corresponding WT panels to a wild-type RNA dilution series (not shown) indicates that gene expression in both types of white prepupae is less than 3% of that in observed in third instar animals.

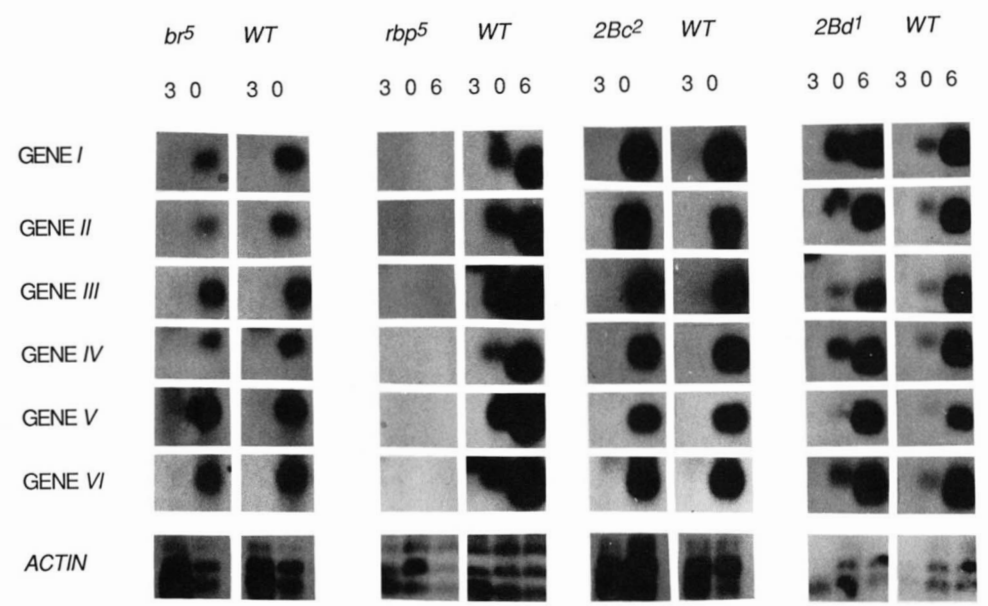

FIGURE 5.—Late gene expression in *BR-C* mutants. Polyadenylated RNA (0.25 μg) from the indicated sources was probed for the presence of late transcripts. RNA sources are as in Figure 4. Genes examined and time points for RNA collection are as in Figure 2. Only those portions of the autoradiograms that yielded signals are shown. Note that blots were probed multiple times and as such, remnants from earlier signals appear in subsequent autoradiograms. This is the case for the following panels: *br^5* probed with gene *V*, L3 lane; *rbp^5* and WT probed with genes *III* and *VI*, L3 lanes; and *2Bd^1* probed with gene *III*, L3 lane.

regulation of the four intermolt and six late genes that we tested. This regulation has both positive and negative components. Positive regulation is indicated by the observation that the most severe mutant allele (*rbp^5*) fails to activate all intermolt and late genes in our analysis. Negative regulation is indicated by the observation that every *rbp* mutant tested failed to repress fully the 71E gene *VII* intermolt gene after puparium formation. In these mutants the level of the gene *VII* transcripts appears to drop at puparium formation and rise again after several hours. This phenomenon could be due to the location of gene *VII* within a late gene cluster which is transcriptionally active at this time. Since the transcription of all ten genes that we examined was affected by the *rbp* mutations, we suggest that the *rbp* function is involved in the regulation of many more, and perhaps all, genes in this cascade.

The structure of the *BR-C* proteins suggests that transcriptional regulation is mediated through direct

TABLE 2

Gene expression in *BR-C* mutants

Gene	*BR-C* allele							
	WT	*rbp¹*	*rbp⁴*	*rbp⁵*	*br⁵*	*2Bc¹*	*2Bc²*	*2Bd¹*
Intermolt								
Sgs-3	+++	−	−	−	+++	+++	+++	+++
Sgs-4	+++	++	++	−	+++	+++	+++	+++
Sgs-5	+++	+++	+++	−	+++	+++	+++	+++
VII	+++	+++[a]	+++[a]	−[a]	+++	+++	+++	+++
71E Late								
I	+++	−	−	−	+++	+++	+++	+++
II	+++	−	−	−	+++	+++	+++	+++
III	+++	+	−	−	+++	+++	+++	+++
IV	+++	+	−	−	+++	+++	+++	+++
V	+++	−	−	−	+++	+++	+++	+++
VI	+++	−	−	−	+++	+++	+++	+++

RNA was analyzed as described in Figures 2, 4, and 5. Each entry represents data from multiple independent experiments (N = 2–4) with the exception of *rbp⁴* and *rbp⁵* (N = 1). Qualitative estimates of RNA levels were made by comparing hybridization signals to those obtained from dilutions of wild-type RNA: (−) <3% WT expression; (+) between 3 and 10%; (++) between 10 and 33%; (+++) between 33 and 100%. Data included for the intermolt genes (late third instar) and the late genes (white prepupae and white prepupae aged 6 hr) were obtained from animals of the indicated developmental age.

[a] Gene *VII* transcripts were detected in these animals for at least 6 hr after puparium formation (*e.g.*, Figure 1). In the case of *rbp⁵* (Figure 4), transcripts were detectable but at levels less than 3% of wild type (at 0 hr) and were therefore scored as (−).

DNA interactions. Every *BR-C* protein characterized to date contains a pair of zinc fingers and other motifs common to DNA-binding transcriptional regulators (DIBELLO *et al.* 1991). In addition, the *BR-C* proteins share homology with the Drosophila *tramtrack* protein, which has been shown to bind enhancer domains at the *fushi-tarazu* (*ftz*) locus (HARRISON and TRAVERS 1990). We suggest that the *BR-C* represents a family of DNA-binding transcription factors that have both positive and negative consequences.

In addition to its role in salivary gland gene expression, the *BR-C* is also involved globally in the development and metamorphosis of Drosophila. Most *BR-C* mutations are lethal at some point during metamorphosis and exhibit abnormalities in several tissues. For example, broad wings, reduced palpus size and bristle number, and shortened, thickened legs can each result from mutations in the *BR-C* (BELYAEVA *et al.* 1981; ZHIMULEV, VLASSOVA and BELYAEVA 1982; KISS *et al.* 1988). We therefore expect to find *BR-C*-mediated transcriptional regulation in other tissues. This is consistent with observations by LEPESANT *et al.* (1986) who describe reduced fat body gene expression in *npr1* and *l(1)2Bc* mutants. It seems unlikely that the salivary gland defects seen in *rbp* mutants could completely explain the larval lethality associated with these mutations. It is interesting to note that the differences in severity among the *rbp* mutations, as judged by the response of intermolt and late genes, mirror their differences in severity in terms of lethality. For example, *rbp⁴* and *rbp⁵* mutants show more transcriptional defects (Table 2) and an earlier lethal period (Table 1) than the *rbp¹* mutant. We therefore suspect that the *rbp* function is also not limited to salivary glands.

Two other early genes (*E74*, *E75*) that participate in the salivary gland puffing cascade (WALKER and ASHBURNER 1981) have been studied extensively, and a regulatory role is consistent for each. Both genes encode products that exhibit characteristics of DNA-binding proteins, and are related to known transcriptional regulators (SEGRAVES and HOGNESS 1990; URNESS and THUMMEL 1990; BURTIS *et al.* 1990). Taken together, this information suggests that the early gene products function as mediators of the hormonal stimulus through the induction and repression of gene expression. Since the *BR-C*, *E74* and *E75* each encode multiple products (SEGRAVES and HOGNESS 1990; BURTIS *et al.* 1990; DIBELLO *et al.* 1991.) and the *BR-C* and *E74* are expressed in many larval and imaginal tissues (THUMMEL, BURTIS and HOGNESS 1990; G. M. GUILD, unpublished observations), it seems possible that tissue-specific late gene expression could be orchestrated by the specific combination of these and perhaps other early gene products (BURTIS *et al.* 1990).

We would like to thank I. ZHIMULEV, J. FRISTROM and L. RESTIFO for fly stocks; C. GATES, E. MEYEROWITZ, M. MUSKAVITCH and S. TOBIN for cloned DNAs; and S. POETHIG, S. ROTH, W. TELFER, K. CROSSGROVE, P. DIBELLO, J. EMERY and I. FREIXAS for helpful discussions and comments on the manuscript. This work was supported by a grant from the National Science Foundation (DCB 8905031). P.S.G was supported by the U.S. Public Health Service Training Program in Cell and Molecular Biology (5 T32 GM07729).

LITERATURE CITED

ASHBURNER, M., 1973 Sequential gene activation by ecdysone in polytene chromosomes of *Drosophila melanogaster*. II. The effects of inhibitors of protein synthesis. Dev. Biol. **39:** 141–157.

ASHBURNER, M., 1989 *Drosophila: A Laboratory Manual*, pp. 325–326. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

ASHBURNER, M., and H. D. BERENDES, 1978 Puffing in polytene chromosomes, pp. 316–395 in *The Genetics and Biology of Drosophila*, Vol. 2B, edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, New York.

ASHBURNER, M., C. CHIHARA, P. MELTZER and G. RICHARDS, 1974 On the temporal control of puffing activity in polytene chromosomes. Cold Spring Harbor Symp. Quant. Biol. **38:** 655–662.

BAINBRIDGE, P., and M. BOWNES, 1981 Staging the metamorphosis of *Drosophila melanogaster*. J. Exp. Embryol. Morphol. **66:** 57–80.

BEATON, A. H., I. KISS, D. FRISTROM and J. W. FRISTROM, 1988 Interaction of the *Stubble-stubbloid* locus and the *Broad-Complex* of *Drosophila melanogaster*. Genetics **120:** 453–464.

BELYAEVA, E. S., and I. F. ZHIMULEV, 1982 Cytogenetic analysis of the X chromosome region 2B3–4 to 2B11 of *Drosophila melanogaster*. IV. Mutation at the *swi* (singed wing) locus interfering

with the late 20-OH ecdysone puff system. Chromosoma **86:** 251–263.

BELYAEVA, E. S., M. G. AIZENON, V. F. SEMESHIN, I. KISS, K. KOEZKA, E. M. BARITCHEVA, T. D. GORELOVA and I. F. ZHIMULEV, 1980 Cytogenetic analysis of the 2B3-4 to 2B11 region of the X-chromosome of *Drosophila melanogaster*. I. Cytology of the region and mutant complementation groups. Chromosoma **81:** 281–306.

BELYAEVA, E. S., I. E. VLASSOVA, Z. M. BIYASHEVA, V. T. KAKPAKOV, G. RICHARDS and I. F. ZHIMULEV, 1981 Cytogenetic analysis of the 2B3-4 to 2B11 region of the X-chromosome of *Drosophila melanogaster*. II. Changes in 20-OH ecdysone puffing caused by genetic defects of puff 2B5. Chromosoma **84:** 207–219.

BELYAEVA, E. B., M. AIZENON, I. KISS, T. GORELOVA, S. PAK, G. UMBETOVA, P. KRAMERS and I. F. ZHIMULEV, 1982 New mutants. Drosophila Inform. Serv. **58:** 184–190.

BELYAEVA, E. S., M. O. PROTOPOPOV, E. M. BARICHEVA, V. F. SEMESHIN, M. L. IZQUIERDO and I. F. ZHIMULEV, 1987 Cytogenetic analysis of the 2B3-4 to 2B11 region of the X-chromosome of *Drosophila melanogaster*. VI. Molecular and cytological mapping of the *ecs* locus of the 2B puff. Chromosoma **95:** 295–310.

BURTIS, K. C., C. S. THUMMEL, C. W. JONES, F. D. KARIM and D. S. HOGNESS, 1990 The Drosophila 74EF early puff contains *E74*, a complex ecdysone-inducible gene that encodes two *ets*-related proteins. Cell **61:** 85–99.

CHAO, A. T., and G. M. GUILD, 1986 Molecular analysis of the ecdysterone-inducible 2B5 "early" puff in *Drosophila melanogaster*. EMBO J. **5:** 143–150

CRAYMER, L., and E. ROY, 1980 New mutants. Drosophila Inform. Serv. **55:** 200.

CROWLEY, T. E., P. H. MATHERS and E. M. MEYEROWITZ, 1984 A *trans*-acting regulatory product necessary for expression of the *Drosophila melanogaster* 68C glue gene cluster. Cell **39:** 149–156.

DIBELLO, P. R., D. A. WITHERS, C. A. BAYER, J. W. FRISTROM and G. M. GUILD, 1991 The Drosophila *Broad-Complex* encodes a family of related proteins containing zinc fingers. Genetics (in press).

GALCERAN, J., J. LLANOS, J. SAMPEDRO, O. PONGS and M. IZQUIERDO, 1990 Transcription at the ecdysterone-inducible locus 2B5 in Drosophila. Nucleic Acids Res. **18:** 539–545.

GARFINKEL, M. D., R. E. PRUITT and E. M. MEYEROWITZ, 1983 DNA sequences, gene regulation and modular protein evolution in the Drosophila 68C gene cluster. J. Mol. Biol. **168:** 765–789.

GEORGEL,P., P. RAMAIN, A. GIANGRANDE, G. DRETZEN, G. RICHARDS and M. BELLARD, 1991 *Sgs-3* chromatin structure and *trans*-activators: Developmental and ecdysone induction of a glue enhancer-binding factor, GEBF-I, in *Drosophila* larvae. Mol. Cell. Biol. **11:** 523–532.

GUILD, G. M., 1984 Molecular analysis of a developmentally regulated gene which is expressed in the larval salivary gland of Drosophila. Dev. Biol. **102:** 462–470.

HARRISON, S. D., and A. A. TRAVERS, 1990 The *tramtrack* gene encodes a Drosophila finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. EMBO J. **9:** 207–216.

KISS, I., A. H. BEATON, J. TARDIFF, D. FRISTROM and J. W. FRISTROM, 1988 Interactions and developmental effects of mutations in the *Broad-Complex* of *Drosophila melanogaster*. Genetics **118:** 247–259.

LEPESANT, J. A., F. MASCHAT, J. KEJZLAROVA-LEPESANT, H. BENES and C. YANICOSTAS, 1986 Developmental and ecdysteroid regulation of gene expression in the larval fat body of *Drosophila melanogaster*. Arch. Insect. Biochem. Physiol. **s1:** 133–141.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Drosophila Inform. Serv. **64:** 7–8.

MCNABB, S. L., and S. K. BECKENDORF, 1986 Cis-acting sequences which regulate expression of the *Sgs-4* glue protein gene of Drosophila. EMBO J. **5:** 2331–2340.

MEYEROWITZ, E. M., and D. S. HOGNESS, 1982 Molecular Organization of a Drosophila puff site that responds to ecdysone. Cell **28:** 165–176.

MUSKAVITCH, M. A. T., and D. S. HOGNESS, 1982 Molecular analysis of a gene in a developmentally regulated puff of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **77:** 7362–7366.

RESTIFO, L. L., and G. M. GUILD, 1986a An ecdysterone-responsive puff site in Drosophila contains a cluster of seven differentially regulated genes. J. Mol. Biol. **188:** 517–528.

RESTIFO, L. L., and G. M. GUILD, 1986b Poly(A) shortening of coregulated transcripts in Drosophila. Dev. Biol. **115:** 507–510.

SEGRAVES, W. A., and D. S. HOGNESS, 1990 The *E75* ecdysone-inducible gene responsible for the 75B early puff in Drosophila encodes two new members of the steroid receptor superfamily. Genes Dev. **4:** 204–219.

SEMESHIN, V. F., E. M. BARICHEVA, E. S. BELYAEVA and I. F. ZHIMULEV, 1985 Electron microscopic analysis of Drosophila polytene chromosomes. II. Development of complex puffs. Chromosoma **91:** 210–233.

SHORE, E. M., and G. M. GUILD, 1986 Larval salivary gland secretion proteins in Drosophila: Structural analysis of the *Sgs-5* gene. J. Mol. Biol. **190:** 149–158.

SHORE, E. M., and G. M. GUILD, 1987 Closely linked DNA elements control the expression of the Sgs-5 glue protein gene of Drosophila. Genes Dev. **1:** 829–839.

THUMMEL, C. S., K. C. BURTIS and D. S. HOGNESS, 1990 Spatial and temporal patterns of *E74* transcription during Drosophila development. Cell **61:** 101–111.

URNESS, L. D., and C. S. THUMMEL, 1990 Molecular interactions within the ecdysone regulatory hierarchy: DNA binding properties of the Drosophila early ecdysone-inducible E74A protein. Cell **63:** 47–61.

VIGOREAUX, J., and S. TOBIN, 1987 Stage specific selection of alternative transcriptional initiation sites from the 5C actin gene of *Drosophila melanogaster*. Genes Dev. **1:** 1161–1171.

WALKER, V. K., and M. ASHBURNER, 1981 The control of ecdysterone-regulated puffs in Drosophila salivary glands. Cell **26:** 269–277.

ZHIMULEV,I. F., I. E. VLASSOVA and E. S. BELYAEVA, 1982 Cytogenetic analysis of the 2B3-4 to 2B11 region of the X-chromosome of *Drosophila melanogaster*. III. Puffing disturbance in salivary gland chromosomes of homozygotes for mutation *l(1)pp1t10*. Chromosoma **85:** 659–672.

Communicating editor: P. CHERBAS