Genetic localization of a regulatory site necessary for the production of the glue protein P5 in *Drosophila melanogaster*

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Summary

The glue proteins are products of a developmentally regulated gene family. These genes are transcriptionally active during the third larval instar and code for the major protein products of salivary glands. The activity of several of the genes can be visualized as intermoult puffs in the polytene salivary gland chromosomes. The amount of one of these proteins, P5, varies widely among wild-type strains. We have used biochemical and genetic methods to investigate the source of this variation. The results of *in vitro* translation of salivary gland RNA suggest that the variation occurs pretranslationally. Genetic mapping experiments showed that sites on several chromosomes can modulate the amount of P5, but that one site on the third chromosome determines the absence and presence of this protein. We have mapped this glue protein gene, called *GP5*, to the interval between *bx* (3–58·8) and *sr* (3–62·0) which also includes the intermoult puff at 90BC. We discuss the relationship between P5 and the glue protein gene *Sgs-5* which is also located at 90BC.

1. Introduction

The major synthetic activity of the salivary gland cells during the last larval stage of *Drosophila melanogaster* is the production of the glue polypeptides. In a screen of glue proteins from 7 wild-type stocks by SDS polyacrylamide gel electrophoresis Beckendorf & Kafatos (1976) noted the existence of both electrophoretic mobility variation and quantitative changes in some of the glue proteins. They found that the glue protein which they named P5 shows the most extensive quantitative variation although these differences were not measured. Here we have examined the glue proteins from a total of 24 stocks and quantified the differences in P5 production.

We have investigated the genetic basis of the quantitative variation in P5 production by constructing isogenic lines and by genetic mapping. Since individuals must be killed as larvae to characterize their salivary gland proteins, previous studies have often relied on the use of larval markers (or deficiencies) (Velissariou & Ashburner, 1980, 1981; Akam *et al.* 1978) to locate specific glue protein genes. However the paucity of larval markers and the availability of chromosomes carrying several adult markers encouraged us to use balancer chromosomes to establish stable stocks of recombinant chromosomes from each mapping cross. We thus could first score the adult traits and then assess the larval phenotype by sacrificing a later generation. We have used this method to map the glue protein gene *GP5* to within a region which includes the intermoult puff 90BC.

2. Materials and methods

(i) *Fly stocks*

Wild-type and marker stocks used in the survey for P5 polymorphism were obtained from the following stock centres: Caltech (Pasadena, California), Bowling Green (Ohio) and Umea (Sweden). Third chromosome balancers and the multiply marked stocks used for mapping (except for *cu kar bx sr* e^{s}) were from Caltech. The multiply marked third chromosome *cu kar bx sr* e^{s} was produced by recombination be-

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tween a *cu kar ry* and a p^p *bx sr* e^s chromosome. For further details and a description of mutations used see Lindsley & Grell (1968). All flies were grown either at 25 or 22 °C on a corn meal and yeast medium described by Beckendorf & Kafatos (1976).

(ii) *Construction of co-isogenic stocks*

To identify linkage groups co-isogenic stocks were constructed using the two wild-type stocks, Hikone-AS (HAS, $P5^+$) which produces P5 and Stromsvreten-10 (S10, $P5^-$) which does not product P5, and a stock which had balancer chromosomes for both the second and third chromosomes: *In(2L) Pm, Pm dp b/ In(2LR)CyO, Cy* dp^{1vI} *pr* cn^2; *In(3LR) Ubx, Sb ri sr* e^s*/ In(3R)CxD, D*, which will be abbreviated Pm/Cy; Sb/D, respectively. HAS and S10 were simultaneously crossed to the balancer-chromosome containing stock (Fig. 1 step 1a and 1b). Ten heterozygous HAS/Pm; HAS/Sb males were separately mated to individual S10/Cy; S10/D females. The progeny from each pair mating fell into four classes as described in step 3. In each pair-mating only a single HAS and S10 homologue for the second and third chromosomes was isolated.

(iii) *Genetic mapping of* GP5

To map *GP5* we needed both to identify recombinants by scoring adult markers and to analyse the corresponding larval glue. Since the larvae are sacrificed when the glue is isolated their adult phenotype cannot be scored. Therefore stocks homozygous for each recombinant chromosome were established before the glue it specified was analysed. Each mapping cross was based on the same rationale, which will be described in detail for one mapping experiment.

The wild-type S10 ($P5^-$) was crossed to the third chromosome mapping stock *ru h th st cu sr* e^s *ca*

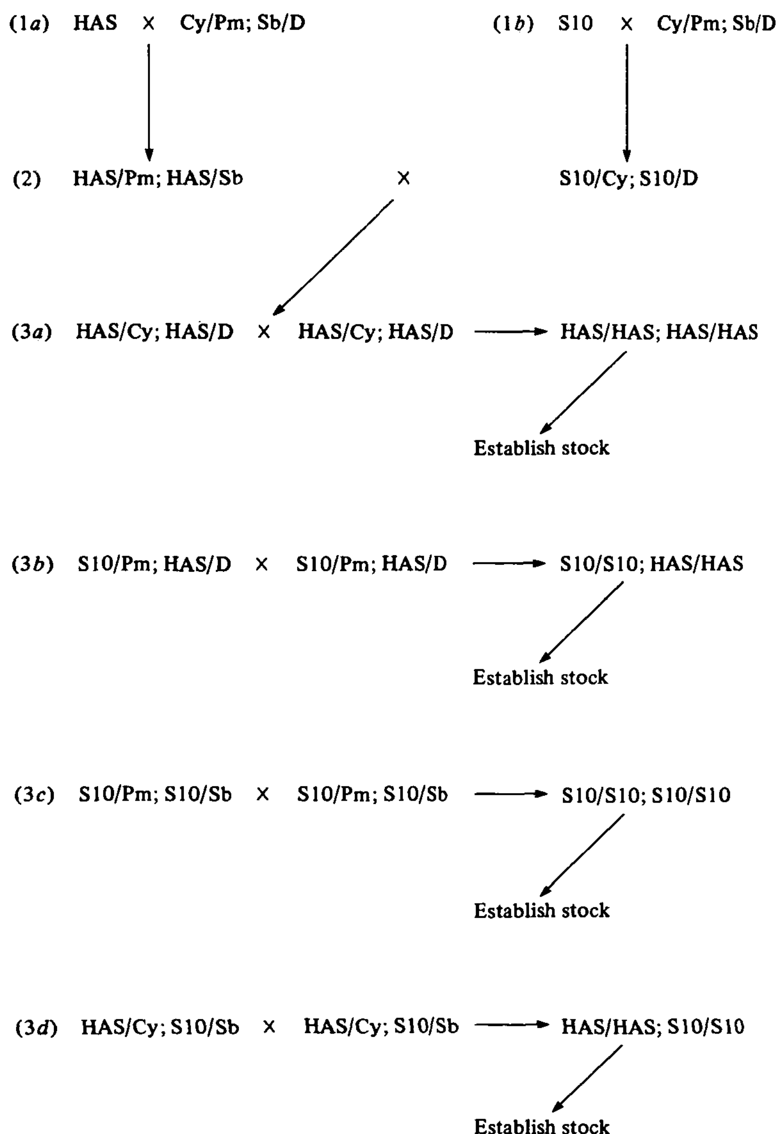

Fig. 1. Construction of isogenic stocks.

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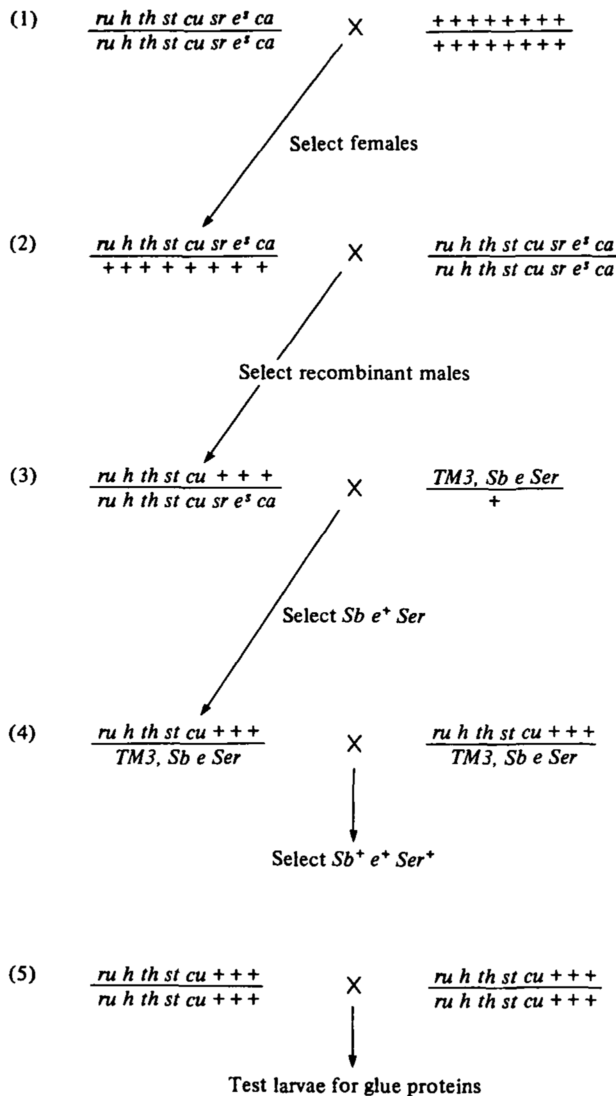

Fig. 2. Crosses to map *GP5*.

($P5^+$) (Fig. 2, line 1). Heterozygous females were backcrossed to *ru h th st cu sr* e^s *ca* males (line 2) and recombinants were recovered in the following generation (line 3). Each recombinant chromosome was amplified using the balancer chromosome *TM3, Sb e Ser*. Only phenotypically e^+ recombinants were chosen (Fig. 2 line 3) so that the recombinant chromosome could be distinguished from the tester chromosome. The e^+ heterozygous progeny (e.g. *TM3, Sb e Ser/ ru h th st* in Fig. 2) derived from each recombinant male were collected and sib mated (line 4) to establish a homozygous stock before analysis of the glue proteins.

(iv) *Mass isolation of salivary glands*

Salivary glands used in preparing RNA were mass isolated from 300–500 ml of mid-third instar larvae by a procedure modified from Zweidler & Cohen (1971). Preparations containing 95% pure glands were obtained and stored in organ medium (Cohen & Gotchel, 1971) at −80 °C until used.

(v) *RNA preparation*

Approximately 300 mg of mass isolated salivary glands (95% pure) were used in each RNA preparation. All solutions were pretreated with 50 μl/100 ml of diethyl pyrocarbonate and all work was carried out at 0–4 °C unless otherwise indicated. The salivary glands were gently homogenized in 6 ml of buffer A (25 mM-NaCl, 5 mM-$MgCl_2$, 25 mM Tris-HCl, pH 7·5, 2% Triton-X, 1 mg/ml heparin) using a Dounce homogenizer with the loose fitting (A) pestle. Nuclei and cellular debris were removed by centrifugation (16000 g × 5 min) at 0 °C. An equal volume of buffer B (25 mM-NaCl, 200 mM-$MgCl_2$, 25 mM Tris-HCl, pH 7·5, 2% Triton X-100, 1 mg/ml heparin) was added to the supernatant and refrigerated at 4 °C overnight to precipitate the polysomes (Palmiter, 1974).

Polysomes were collected by centrifugation (16000 g × 15 min) at 0 °C through a 1 M sucrose pad made up in 25 mM-NaCl; 5 mM-$MgCl_2$; 25 mM Tris-HCl, pH 7·5 and dissolved in 4 ml of 0·01 M HEPES pH 7·5; 0·01 M-Na acetate pH 5·0; 0·5% SDS at room temperature. RNA was prepared by repeated phenol and chloroform : isoamyl alcohol (24 : 1) extractions and precipitated at −20 °C by the addition of 2 volumes of 100% ethanol. Approximately 1·5 mg of RNA were recovered. Poly(A)-containing RNA was approximately 3·6% of total RNA loaded after a single pass through an oligo-dT cellulose column as described by Efstradiatis & Kafatos (1976). Electrophoretic analysis of the poly(A)-containing fraction revealed that 20% of the material was nuclear and mitochondrial ribosomal RNA.

(vi) In vitro *translation*

Wheatgerm lysate was prepared according to Roberts & Patterson (1973) except that 1 mM dithiothreitol was substituted for 2-mercaptoethanol. The standard protein synthesis assay contained the following: 10 μl of lysate, 20 mM HEPES pH 7·6, 100 mM-KCl, 2·2 mM-Mg acetate, 1·4 mM dithiothreitol, 1 mM ATP, 0·1 mM GTP, 2·4 μl/ml creatine phosphate, 1·8 μg/μl creatine phosphokinase, and 0·28 mM of each unlabelled amino acid except for proline. 18 μCi of [^{3}H]proline (specific activity 4·2 Ci/mM, Schwarz-Mann), 2 μg of poly(A)-containing RNA and water were added to give a final volume of 25 μl.

Reactions were incubated at room temperature for 1 h. Protein synthesis was monitored by the incorporation of [^{3}H]proline into TCA precipitated material. One μg of poly(A)-containing RNA caused a 60-fold stimulation of protein synthesis. The *in vitro* translation products were precipitated with acetone and dissolved in electrophoresis sample buffer (Laemmli, 1970).

(vii) *Isolation of secretion from glands*

To collect only the secreted glue, fully bloated glands were transferred to 95% ethanol. The secretion then

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contracts and becomes a solid mass which can then be dissected free of the salivary gland cells (Kodani, 1948). Isolated secretion masses were dissolved in electrophoresis sample buffer.

(viii) *Polyacrylamide gels*

Two sizes of SDS polyacrylamide gels containing either a single polyacrylamide concentration or an exponential gradient of polyacrylamide were made as described by Beckendorf & Kafatos (1976). The larger gels (14 cm × 14 cm × 0·1 cm) were used to screen stocks and the smaller gels (12 cm × 10 cm × 0·1 cm) were used to analyze the glue from individual larvae. Acid urea gels were made as in previous studies (Korge, 1975). We detected P5 as a faint and diffuse band; however resolution was improved using modifications as described by Williams & Reisfeld (1964).

For quantitative protein detection small gels were fixed and stained in 150 ml of 0·1% Coomassie Brilliant Blue R, 50% methanol, 10% acetic acid for 1 h and destained in two 250 ml washes of 10% methanol, 7·5% acetic acid in a 47-hour period.

Gels were scanned densitometrically using a double beam recording microdensitometer (Joyce–Loebl) and the bands quantitated by planimetry.

(ix) *Autofluorography*

Gels containing ^{3}H-labelled proteins were subjected to autofluorography according to the method of Bonner & Laskey (1974). The Kodak RP Royal film used was pre-exposed to 0·1–0·2 A_{360} according to Laskey & Mills (1975).

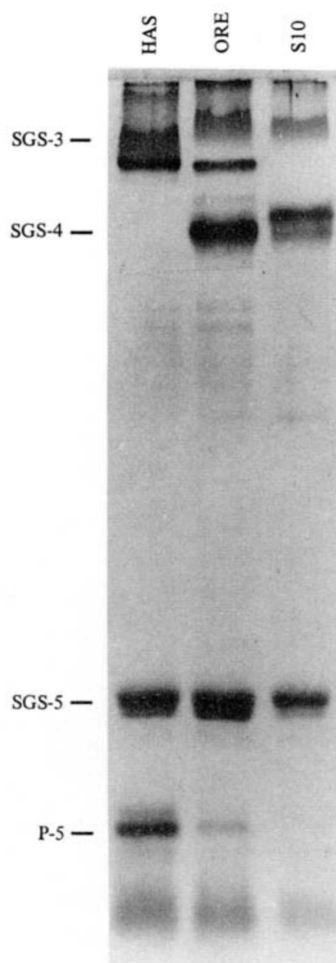

Fig. 3. SDS polyacrylamide gel of glue isolated from three different wild-type stocks. Hikone-AS (HAS); Oregon-R (ORE); Stromsvreten-10 (S10).

3. Results

(i) *Quantitative variation of P5*

The glue protein P5 is one of the smaller proteins found in glue (apparent molecular weight 15 kDa) and unlike most glue proteins is not highly glycosylated (Beckendorf & Kafatos, 1976). The P5 protein is easily detected on SDS polyacrylamide gels (Fig. 3). We have determined the extent of P5 variation by isolating the glue proteins from 24 wild-type and marker stocks. While electrophoretic variants were detected for several other glue proteins (e.g. SGS-3, SGS-4), no electrophoretic variation for P5 was seen. The amount of P5 in each stock was measured relative to total glue; P5 represented 0 to greater than 20% of the glue. These stocks were divided into five classes based on the amount of P5 they produced (Table 1). Representatives from three of these classes, Hikone-AS (HAS) from the high producing class, Oregon-R (ORE) from the low intermediate class and Stromsvreten-10 (S10) from the non-producing class, were chosen for further analysis.

(ii) In vitro *translation of salivary gland RNA*

Since glue protein P5 is not highly glycosylated nor otherwise modified as determined by pulse chase experiments (Beckendorf & Kafatos, 1976), it was anticipated that P5 produced *in vitro* would co-migrate with its *in vivo* produced polypeptide during SDS polyacrylamide gel electrophoresis. Salivary gland poly(A)-containing RNA was isolated from three stocks, HAS, ORE and S10 and translated *in vitro*. The electrophoretic pattern produced by the *in vitro* translation products was compared to that obtained from secreted glue. In contrast to Guild & Shore (1984) we detected a 15 kDa translation product which co-migrated with P5. When the *in vitro* translation products of RNA from the three stocks were compared, we found that the amount of this 15 kDa band paralleled the amounts of P5 in the three strains. The HAS preparation produced large amounts of this band, ORE RNA produced small amounts, and no detectable amount was produced from S10 RNA (Fig. 4). These results suggest that differences in P5 mRNA concentrations are the major cause of variation in P5 production.

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Table 1. *Classification of wild-type and marker stocks according to their P5 production*

P5(%)[a]	Stocks	
20	Hikone-AS Hikone-AW	
10–20	Seto Florida	
3–10	Amherst Hikone-R Oregon-R *ru h th st cu sr e^s ca*	Daek Lausanne Wageningen *y w*
2	*ca* Hopkins Nyassa Lake Samarkand Urbana	Chieti-V Karnas-60 Roma *st* Varese
0	Stromsvreten-10 *th*	

[a] As percentage of the total glue.

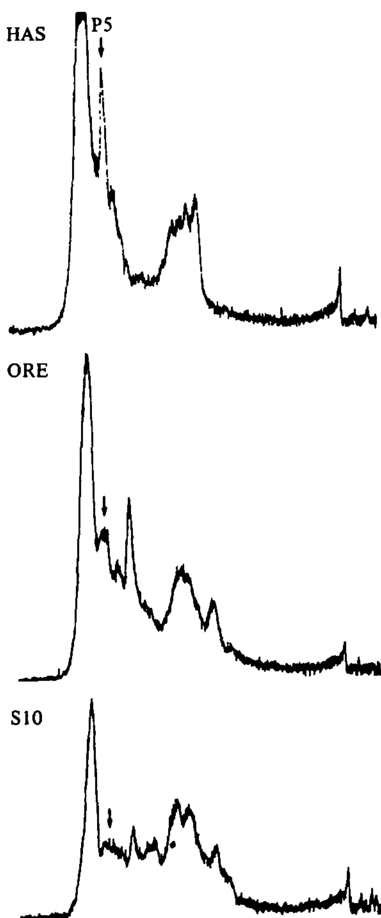

Fig. 4. Densitometer tracings of *in vitro* translation products from poly(A)-containing salivary gland RNAs. Poly(A)-containing RNAs from HAS, ORE, and S10 were translated *in vitro* and the translation products analysed by gel electrophoresis. Translation products were detected by fluorography and compared to the HAS secreted (*in vivo*) glue proteins stained with Coomassie Brilliant Blue R.

(iii) *Linkage analysis for* GP5

The wild-type stocks HAS (P5$^+$) and S10 (P5$^-$) were used to determine the chromosomal location of the gene(s) involved in the production of P5. HAS and S10 do not carry any visible markers but their chromosomes can be conveniently identified by the glue protein variants produced by previously mapped glue protein genes. We could therefore directly test by gel electrophoresis whether the absence/presence of P5 protein was linked to these glue protein genes.

HAS/S10 hybrids were made using either HAS females (Table 2, Cross A) or S10 females (Table 2, Cross B). In Cross A the F_1 hybrid males carry only the maternally derived HAS X chromosome and in Cross B the F_1 males carry only the S10 X chromosome. However, males from both crosses produced P5 (Table 2). Since the F_1 hybrid males from Cross B carry the S10 X chromosome the absence or presence of P5 is not linked to the X chromosome.

The hybrid males from crosses A and B were backcrossed to S10 and HAS females, respectively, to determine autosomal linkage. The third chromosome is marked by electrophoretically distinct forms of the glue protein SGS-3 (Korge, 1975). HAS possesses a fast-migrating SGS-3 (*Sgs-3F*) and S10 a slow-migrating SGS-3 (*Sgs-3S*). Individuals from the backcross can be divided into two classes according to their third chromosome constitution. Individuals which are homozygous for the third chromosome will produce only a single form of SGS-3 while the individuals heterozygous for the thrd chromosome will produce both electrophoretic forms of SGS-3. In Cross A, individuals homozygous for the S10 third chromosome lacked P5, while heterozygous individuals produced P5 (Table 2).

These results show that a gene (*GP5*) required for production of P5 is linked to the third chromosome but that sites which modulate the relative amounts of P5 are not located solely on the third chromosome. Third chromosome heterozygotes, whether from Cross A or B males, make less P5 than the amount expected if the two third chromosomes were additive in P5 expression (8 *vs.* 27/2%). Furthermore, individuals homozygous for the HAS third chromosomes do not make the same amount of P5 (*t* test, 95% confidence level) as the parental HAS stock.

(iv) *Co-isogenic lines for HAS and S10 chromosomes*

To test for additional genes which might contribute to P5 production, co-isogenic lines were constructed using HAS and S10 chromosomes. The X chromosome was not tested in this analysis for two reasons. First, the results from the F_1 hybrids described in Table 2 rule out the possibility that the S10 and HAS X chromosomes are contributing differentially towards P5 production. Second, the males in backcross B which are homozygous for the HAS third chromosome and carry the HAS X chromosome still

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Table 2. *The production of P5 and SGS-3 in F_1 hybrids and in F_2 backcrosses*

Stocks	SGS-3[a]	P5(%)[b]	*n*
Parental stock			
HAS	F/F	27	
S10	S/S	0	
F_1 Hybrid males			
Cross A			
HAS males × S10 females	S/F	8	16
Cross B			
S10 males × HAS females	S/F	8	16
F_2 Backcross males from			
F_1 males from Cross A	S/S	0	8
× S10 females	S/F	9	12
F_1 males from Cross B	F/F	17	16
× HAS females	F/S	11	10

[a] F and S represent the fast and slow alleles of SGS-3, respectively.
[b] As percentage of total glue; *n* = number of individuals examined.

produce only 63% of the parental amounts of P5. We inferred that the second (and/or fourth) chromosome was influencing P5 production. This was tested by constructing stocks which were co-isogenic for the HAS or S10 second and third chromosomes (Fig. 1).

Four kinds of stocks were isolated. Each stock contained identical homologues for the second and third chromosomes, respectively, and were derived from either the S10 or HAS stock. The stocks co-isogenic for the S10 third chromosome, regardless of the origin of the second chromosomes, did not produce P5, while stocks composed of third chromosomes derived from HAS did produce P5. (Table 3). These results confirm the linkage of a gene(s) on the third chromosome which is necessary for P5 production.

Two of the stocks co-isogenic for the HAS second and third chromosomes produced 60% of the P5 found in the HAS parental stock. One stock co-isogenic for both HAS second and third chromosomes produced only 40% of the parental amounts of P5. The second chromosomes in the stock producing only 40% of the HAS amount of P5 were replaced with co-isogenic S10 second chromosomes. These newly constructed stocks produced 60% of the P5 found in the HAS parental stock. Based on these results there are at least two types of HAS second chromosomes. Both the third and second chromosomes contribute to the production of P5, although the contribution from these chromosomes alone is not sufficient to restore the parental HAS levels.

Table 3. *P5 production in co-isogenic lines from HAS and S10*

Stock genotype: Second chromosome	Stock genotype: Third chromosome	Number of lines isolated	P5(%)
S10	S10	2	0
HAS	S10	4	0
HAS	HAS	3	60; 60; 40
S10	HAS	4	All 60

(v) *Electrophoretic variants*

The site which is responsible for the absence of P5 in the S10 strain is clearly linked to the third chromosome. To test whether this site contains the structural gene for P5, we attempted to identify P5 electrophoretic variants. However, in a survey of the 22 P5 producing strains using SDS gels or of the 7 highest P5 producing strains using acid urea gels, no P5 variants were found. In light of these results only the site responsible for the absence or presence of P5 (*GP5*) was mapped.

(vi) *Mapping the presence or absence of P5*

The gene necessary for P5 production was located using a wild-type third chromosome from S10 ($P5^-$) and a *ru h th st cu sr e^s ca* chromosome ($P5^+$). Five single and five double recombinant classes were recovered (Table 4, Cross A). Glue was isolated from larvae homozygous for each line (see Materials and methods) and analysed by SDS polyacrylamide gel electrophoresis. As shown in Table 4 (Cross A) *GP5* must be located between *cu* and *sr*. The interval between *cu* and *sr* spans 12 map units and approximately 25 band regions. To localize *GP5* within this interval we constructed a ($P5^+$) *cu kar bx sr e^s* chromosome. S10 was crossed to the *cu kar bx sr e^s* stock (Cross B) and recombinants recovered as above. SDS polyacrylamide gel electrophoretic analysis of these recombinants further local-

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Table 4. *Classes of recombinants analysed from the mapping crosses between S10 and several multiply marked chromosomes*

Parental stocks		P5
All crosses	S10	−
Cross A[a]	*ru h th st cu sr e^s ca*	+
Cross B[b]	*red cvc cu kar bx sr e^s*	+
Cross C	*jvl bx sr e^s*	+
Cross D	*sbd² ss bx sr gl*	+

Recombinants	P5	Number of recombinants
Cross A		
(1) *h th st cu sr* + +	+	4
(2) *h th st cu* + + +	−	3
(3) *h th st* + + + +	−	5
(4) *h* + + + + + +	−	8
(5) + + + + + + *ca*	−	5
(6) + *th st* + + + +	−	1
(7) + *th st cu sr* + +	+	2
(8) + *th st cu* + + +	−	1
(9) + *th st cu* + + *ca*	+ and −	1 and 1
(10) *h* + + + + + *ca*	−	1
Cross B		
(1) *cu* + + + +	−	8
(2) *cu kar* + + +	−	27
(3) *cu kar bx* + +	+	5
Cross C		
(1) *jvl* + + +	−	2
(2) *jvl bx* + +	−	1
(3) *jvl bx sr* +	+	1
(4) + + *sr e^s*	+	2
Cross D		
(1) + + + *gl*	−	1
(2) + + *sr gl*	− and +	2 and 3
(3) + *bx sr gl*	+	2
(4) *ss bx sr* +	+	3

[a] *ru* not scored; [b] *red cvc* not scored.

Based on the recombinants between *kar* and *sr* (Cross B, class 2 and 3) *GP5* can be mapped to position 60·4 (59·9–60·8,Chi-squared; 95% confidence limit). Gene markers flanking *bx* and *sr* (*jvl* and *e^s* in Cross C and *ss* and *gl* in Cross D) were used to confirm the position of *GP5* to the *bx sr* interval. Eight recombinants were recovered between *bx* and *sr* (Cross C and Cross D). In six cases recombination took place between *bx* and *GP5* (Cross C line 2 and line 4; Cross D line 2) and in two cases recombination took place between *GP5* and *sr* (Cross D line 2). *GP5* therefore is distal to *bx* at position 60·8 (60·2–61·4 Chi-squared, 95% confidence limit).

ized *GP5* to the *kar–sr* interval and suggested that *GP5* is near *bx*.

To determine more closely the position of *GP5* relative to *bx*, two further crosses were carried out. P5 producing stocks *red cvc jvl bx sr e^s* (Cross C) and *sbd² ss bx sr gl* (Cross D) were each crossed to the null stock S10. The heterozygous females were backcrossed to their multiply marked parent stock and recombinant males carrying either e^+ (for the *red cvc jvl bx sr e^s* cross) or sbd^+ (for the *sbd² ss bx sr gl* cross) were collected. The e^+ recombinant males were crossed to TM3 and the *sbd²* recombinant males to TM1 to establish homozygous stocks in a manner analogous to previously described crosses. *GP5* was found to map distal to *bx* (Table 4, crosses C and D) at position 60·8.

4. Discussion

The glue protein genes are responsible for the synthesis of at least 10 developmentally regulated polypeptides. These polypeptides serve as a pupal glue at the end of larval development and are the major protein products of the salivary glands during mid-third

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instar. The glue protein genes are dispersed throughout the genome and their transcriptional activity is correlated with the intermoult puffs.

Electrophoretic variants of many glue proteins have been identified and have been used along with chromosome deficiencies to map the corresponding glue protein genes (Velissariou & Ashburner, 1980 for *Sgs-1*; Korge, 1975; Akam *et al.* 1978 for *Sgs-3*; Korge, 1975, 1977 for *Sgs-4*; Velissariou & Ashburner, 1981 for *Sgs-6*). The amount of glue protein produced by these loci shows a strong dosage dependency (*Sgs-1*, Velissariou & Ashburner, 1980; *Sgs-3*, Akam *et al.* 1978; *Sgs-4*, Korge, 1975; *Sgs-6*, Velissariou & Ashburner, 1981) suggesting that the structural gene has been identified. The association of each gene with an intermoult puff lends support to this assertion. Molecular cloning of individual genes has confirmed this conclusion (Wolfner, 1980).

Previous examples of quantitative variation in the expression of glue proteins have resulted from changes at or near the structural gene. For example, several strains have been identified which produce reduced but detectable amounts of SGS-4 (Beckendorf & Kafatos, 1976; Korge, 1977, 1981). Several of these result from DNA sequence changes just upstream from the gene (Muskavitch & Hogness, 1982; McGinnis *et al.* 1983*a*, *b*). A single strain was found to produce about half as much SGS-6 protein as other SGS-6 plus strains (Velissariou & Ashburner, 1981). The glue protein P5 shows the most extensive quantitative variation (Beckendorf & Kafatos, 1976 and this work). We have shown here that factors on the second and other chromosomes unlinked to *GP5* cause much of this variation.

Null variants have been identified for four glue proteins, P5 (this work), SGS-5 (Guild & Shore, 1984), SGS-4 (Beckendorf & Kafatos, 1976; Korge, 1975) and SGS-6 (Velissariou & Ashburner, 1981). We have mapped the null variant for P5. The locus we identified has not been given an SGS designation but named *GP5* since it is not clear whether this is the structural gene for P5 or strictly a regulatory gene. We have performed a search for P5 mobility variants without success. Whereas changes in electrophoretic mobility can confidently be attributed to changes in the structural gene, quantitative variation alone, such as the *GP5* null we have mapped here, may be due to inactivation of either the structural gene or a separate regulatory gene.

We have mapped *GP5* to the right arm of the third chromosome, between *bx* (3–58·8; 89E) and *sr* (3–62·0, 90DE) at map position 3–60·8. Since this interval includes the 90BC puff, *GP5* like all previously mapped glue protein genes may be associated with an intermoult puff. Since the *Sgs-5* structural gene has already been localized to this puff (Guild & Shore, 1984), there may be two glue protein genes within the same puff. Clustering of genes within polytene chromosome puffs is a recurring motif that has been seen for heat shock genes (Ish-Horowicz *et al.* 1979; Corces *et al.* 1980), late ecdysone responding genes at 71E (Restifo & Guild, 1986), glue protein genes at 68C (Meyerowitz & Hogness, 1982) and a combination of glue protein and preintermoult genes at 3C11-12 (Malone & Beckendorf, unpublished data).

We have measured the translation products of salivary gland mRNA's *in vitro*. The amount of P5 produced *in vitro* from RNA isolated from the wild-type stocks HAS, ORE, and S10 is correlated with its production *in vivo*. Thus the quantitative variation of P5 in these wild-type stocks appears to be due to differences in the concentration of P5 mRNA. We can not however eliminate the possibility that the P5 null stock S10 produces P5 mRNA which is not translated *in vitro* or *in vivo*. The correlation of glue protein mRNA abundance and the amount of protein produced has been reported as the cause of the quantitative variation in SGS-4 (Muskavitch & Hogness, 1980). It is likely though that the mechanisms by which the amounts of SGS-4 and P5 RNA are controlled will differ. Unlike *Sgs-4*, where quantitative variants and electrophoretic variants map to the same site (1–3·5), here we show that there is a single site controlling the absence or presence of P5 and that additional quantitative differences are linked to at least two other chromosomes. The quantitative regulation of P5 is *trans*-acting and appears to be more complex than the regulation of SGS-4. It should therefore provide an intriguing system to further investigate the quantitative regulation of gene expression.

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