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Genetic Analysis of the Larval Secretion Gene *Sgs-4* and Its Regulatory Chromosome Sites in *Drosophila melanogaster**

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Abstract. Larval salivary gland secretion from seven wild-type stocks of *Drosophila melanogaster* was electrophoretically analyzed. Considerable variability occurs in the X-chromosomally coded secretion protein 4, both qualitatively, as expressed by differences in electrophoretic mobilities, and quantitatively as seen by its relative amount in the secretion. *Drosophila* stocks with "normal" amounts of protein 4 show approximately 80–90% dosage compensation in the males, whereas in two stocks with lower amounts of protein 4 there is no indication of dosage compensation. – Genetic analysis showed that the properties of secretion protein 4 and the level of expression of the *Sgs-4* gene are controlled by the X-chromosome. Recombination experiments indicate that the stock-specific characteristics of protein 4 are properties of the structural gene *Sgs-4* itself or of a chromosome region immediately adjacent to *Sgs-4*. One recombinant (R+79), manifesting an intermediate level of dosage compensation, indicates that a chromosome segment closely distal to *Sg-4* is responsible for the regulation of the gene and for dosage compensation in particular. Accordingly, *Sgs-4* must be transcribed from distal to proximal. Its position on the genetic map is 3.6. Two stocks, Hikone-R and Kochi-R, which were originally described as 0-mutants produce very low amounts of a specific secretion protein, 4 h, as revealed by a transvection effect and also by fluorography of overloaded gels.

Introduction

The larval salivary gland of *Drosophila melanogaster* is composed of highly specialized cells which produce a limited number of secretion proteins in relative huge amounts. The structural genes coding for the secretion proteins have been localized and the corresponding m-RNAs have been found to be abundant during the secretion synthesis phase (Korge, 1980).

* Dedicated to Professor W. Beermann on the occasion of his 60th birthday

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Out of six different genes for secretion proteins the structural gene *Sgs-4* which lies on the X-chromosome is of particular interest. Among various tested wild-type stocks of *D. melanogaster* variants were observed in which gene activity in female larvae differs significantly and in which different levels of dosage compensation are attained in male larvae.

We were interested in finding out whether the variantions in activity of the gene *Sgs-4*, and its ability to perform dosage compensation are due to the structural gene itself or can be separated from the latter. In the present paper an attempt is made to genetically localize chromosomal sites involved in the regulation of the gene *Sgs-4*.

Some preliminary results have been published elsewhere (Korge, 1980).

Material and Methods

The wild-type stocks of *Drosophila melanogaster* the secretions of which were analyzed electrophoretically are indicated in Table 1. For the recombination analysis the genetic markers: *y* and y^2, *yellow* (0.0), *w*, *white* (1.5), *spl*, *split* (3.0), *dm*, *diminutiv* (4.6), *cho*, *chocolate* (5.4), *cv*, *crossveinless* (13.7), *ct*, *cut* (20.0), and *v*, *vermilion* (33.0), localized on the X-chromosome, were used in various combinations and are given at the appropriate points. The *Sgs-4* gene for secretion protein 4 lies between the markers *spl* and *dm* (Fig. 7). The secretions of the recombinants resulting from a crossing-over between *w* or *spl* and *cho* or *dm* (cf. Figs. 5 and 6) were analyzed. Males with recombinant phenotype were crossed with virginal *C(1)RM, y* females which possess attached X-chromosomes and are marked *y*.

To test for a transvection effect the *In(1)FM1* stock was used which is marked with $y^{31d}sc^8w^a lz^s B$. For more information on the genetic markers see Lindsley and Grell (1968).

Secretion plugs were prepared from fixed salivary glands of larvae which were just about to form prepupae. Secretion proteins were reduced, alkylated, and electrophoretically separated in 7% acrylamide slab gels or cylindrical gels with 4M urea, respectively (Grossbach, 1969; Korge, 1977a). The gels were scanned at 590 nm with a Gilford gel scanner and the optical density profiles were planimetrically analyzed. In most cases the maximum of the absorbance of the protein peaks ranged between 0.5 and 1.5 O.D. In test series the linearity between the amounts of protein and the optical density up to 2.5 was ascertained. For a good reproducibility of the values standardized conditions of the procedures are an important requisite.

Secretion proteins were labelled in vivo by feeding larvae a yeast suspension in a solution of ^{14}C-proline (228 mCi/mmol) from the middle of the third larval stage. Shortly before prepupa formation the secretions from the glands were isolated for electrophoresis and after electrophoresis the rate of incorporation was determined by fluorography.

Further details on methods will be given at the appropriate places.

Results

Variability in Secretion Proteins

Analyses of the larval secretions from roughly 80 *D. melanogaster* wild-type stocks showed that the electrophoretic mobilities of the secretion fractions vary strongly in urea-acrylamide gels (Korge, 1975, 1977a; Beckendorf and Kafatos, 1976). At present, so far, nine different protein types of fraction 1 (Velissariou and Ashburner, 1980), five of fraction 3 (Korge, 1977a; Velissariou and Ashburner, 1980) and five of fraction 4 (Korge, 1977a, b) could be distinguished. The variability is especially noticeable in the X-chromosomally coded protein 4.

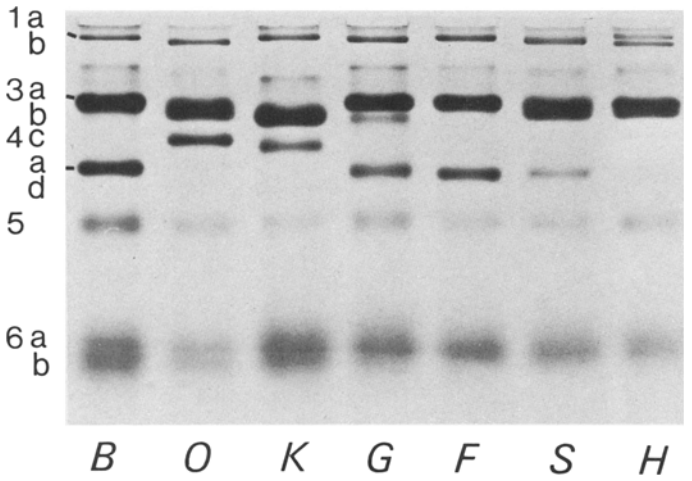

Fig. 1. Variability in larval secretion proteins from *Drosophila melanogaster*. Secretion proteins from the *Drosophila* stocks Berlin B, Oregon O, Karsnäs K, Gruta G, Falsterbo F, Samarkand S, and Hikone-R H were separated in urea containing 5 to 10% gradient acrylamide slab gel. Prior to electrophoresis secretions were treated with 0.1 M dithiothreitol. For each sample ten saliva plugs were used. *1a–6b*, secretion proteins. Coomassie blue staining

In Fig. 1 the protein patterns of the secretions from seven different wild-type stocks are shown. In contrast to earlier results obtained with cylindrical gels (Korge, 1975, 1977a, b), the slab gels used in the present study revealed the existence of six instead of five different fractions. Separations in gradient gels demonstrated that fraction six consists of two bands (Fig. 1). No variability was found for fractions 5 and 6. Therefore, their structural genes could not be localized. The fractions 1–4 are represented in Figure 1 by different variants. In the stocks examined here fraction 4 is represented by variants a, c, d (Fig. 1) and h (Figs. 3 and 4). The identity of protein 4 in the Karsnäs stock with the c-type variant is not completely certain. In this stock proteins 4 and 3 moved slightly faster than the homologous proteins from other stocks (Fig. 1). This behaviour was observed in all gels.

In addition to the variability of the electrophoretic mobility of secretion protein 4, a variability in the quantity of this protein can also be observed in Figure 1. The secretions from the Karsnäs stock and especially from the Samarkand stock contain significantly less protein 4 relative to fraction 3 than those from the Berlin, Oregon, Gruta and Falsterbo stocks. In the secretion from the Hikone-R stock protein 4 apparently is completely absent (Fig. 1). This variability becomes particularly apparent from the optical density profiles of the gels (Fig. 2). The relative amounts of protein 4 found in the wild-type stocks indicated in Figure 1 are compiled in Table 1.

The values for female larvae of the Berlin, Oregon, Gruta, and Falsterbo stocks range between 0.28 (Table 1, Falsterbo) and 0.34 (Table 1, Oregon) and do not significantly differ from each other. By contrast to this the quotient 4/3 of Karsnäs-♀♀ is only 0.19 and that of Samarkand even is only 0.14. Still

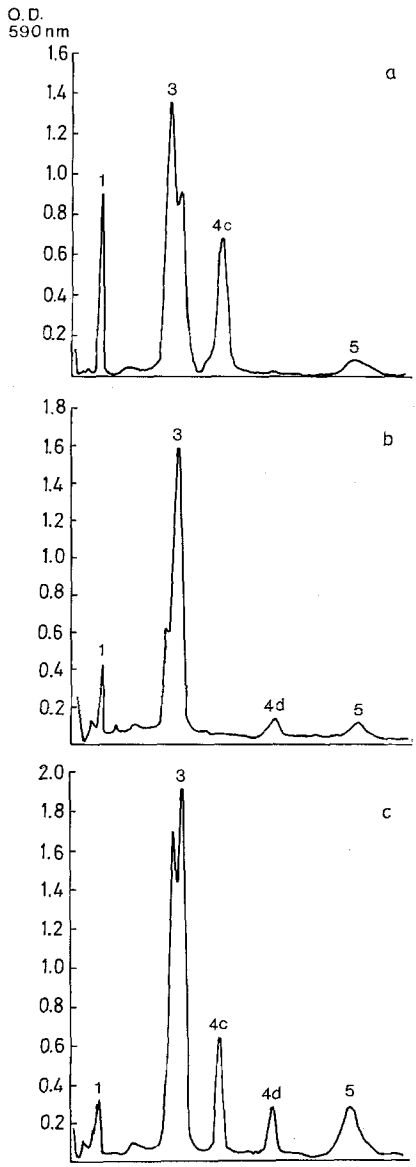

Fig. 2a–c. Optical density profiles of reduced and alkylated secretion proteins from female larvae of the Oregon (**a**) and of the Samarkand (**b**) stock and from heterozygous Oregon/Samarkand female larvae (**c**). *1–5* secretion proteins

more drastic differences in the share of protein 4 in the secretion can be observed in the secretions from male larvae (Table 1). In the Karsnäs and Samarkand stocks the values are only 0.11 or 0.06, respectively, and thus are even lower than those of Berlin, Oregon, Gruta, and Falsterbo stocks (Table 1). The latter lie between 0.23 (Table 1, Falsterbo-♂♂) and 0.30 (Table 1, Oregon-♂♂). The especially low content of protein 4 in the saliva from the Karsnäs and Samarkand stocks is not a characteristic of the particular type of protein 4 produced. Thus, in the case of Karsnäs it is protein 4c and in the case of Samarkand protein 4d which appears suppressed.

Table 1. Relative amounts of secretion protein 4 in different wild-type stocks of *Drosophila melanogaster*. These were determined by forming the quotients 4/3 (see Material and Methods). Values are given with their standard deviations. Number of gels in parentheses

Wild-type stock	Type of protein 4	4/3	Relative amount in ♂ (4/3-♀=100)
Berlin – ♀	4a	0.29 ± 0.06 (30)	
Berlin – ♂		0.27 ± 0.05 (22)	93
Oregon – ♀	4c	0.34 ± 0.04 (18)	
Oregon – ♂		0.30 ± 0.04 (20)	88
Karsnäs – ♀	4c	0.19 ± 0.03 (16)	
Karsnäs – ♂		0.11 ± 0.02 (26)	58
Gruta – ♀	4d	0.29 ± 0.08 (19)	
Gruta – ♂		0.24 ± 0.06 (18)	83
Falsterbo – ♀	4d	0.28 ± 0.05 (11)	
Falsterbo – ♂		0.23 ± 0.04 (19)	82
Samarkand – ♀	4d	0.14 ± 0.03 (15)	
Samarkand – ♂		0.06 ± 0.02 (17)	43
Hikone-R – ♀+♂	4h	lower than 0.01	–

Dosage Compensation and Dosage Effect

Genes localized on the X-chromosome in *D. melanogaster* are present in double dose in the female and in single dose in the male. Nevertheless, as a rule, the phenotypical expression of X-chromosomal genes is identical in both sexes. This phenomenon is known as dosage compensation (Stern, 1929, 1960; Muller et al., 1931; Muller, 1950). Apparently genes on the single X-chromosome in the male are nearly twice as active in transcription as those on one of the two X-chromosomes in the females (Mukherjee and Beermann, 1965).

Since *Sgs-4*, the gene coding for the secretion protein 4, is localized on the X-chromosome (Korge, 1975) we were interested in its status as regards dosage compensation. A direct comparison of absolute amounts of protein 4 is not feasible, because on the average, male and female salivary glands differ in size. When glands from both sexes were selected for equal size, the amounts of the autosomally coded secretion proteins are the same. Therefore,

one of these, protein 3, was used as an internal standard to which the X-chromosomal coded protein 4 was related in the same gel (Table 1, Q 4/3).

The results for the stocks examined are listed in Table 1, column 3. The Berlin, Oregon, Gruta and Falsterbo stocks which possess considerably more protein 4 than the stocks Karsnäs and Samarkand (Table 1, column 3) display dosage compensation with an efficiency of 82–93% in the male larvae. This level of dosage compensation, though not complete, can be regarded as normal. On the other hand, there is no appreciable compensation in the Karsnäs and Samarkand strains: in both stocks the male larvae possess only half as much protein 4 (58% and 43%), respectively, as the females (Table 1, column 4).

The Inheritance of Protein Quantities

The described differences in activity of the *Sgs-4* gene could originate (1) from a mutation in the structural gene itself, (2) from a mutation in flanking sequences with regulative function, or (3) from alterations in regulatory functions located in other parts of the genome. (4) On the other hand variations in the quantity of protein 4 could also be due to post-transcriptional events. To distinguish

Table 2. Relative amounts of secretion protein 4 in hybrids of reciprocal crosses between Berlin and Oregon, and Oregon and Samarkand, respectively. For the determination of these relative amounts see Table 1. Values are given with their standard deviations. Number of gels in parentheses. B Berlin, O Oregon, Sa Samarkand

Genotype	4a/3	4c/3	4d/3	4/3	Relative amount in ♂ (4/3-♀=100)
B×O					
F_1 – ♀	0.21±0.06 (14)	0.22±0.05		0.43	
F_1 – ♂	0.32±0.09 (11)	–		0.32	74
O×B					
F_1 – ♀	0.22±0.06 (13)	0.22±0.05		0.44	
F_1 – ♂	–	0.32±0.09 (13)		0.32	73
O×Sa					
F_1 – ♀	–	0.19±0.04 (20)	0.12±0.03	0.31	
F_1 – ♂	–	0.32±0.07 (17)	–	0.32	103
Sa×O					
F_1 – ♀	–	0.19±0.04 (19)	0.10±0.03	0.29	
F_1 – ♂	–	–	0.08±0.02 (20)	0.08	28

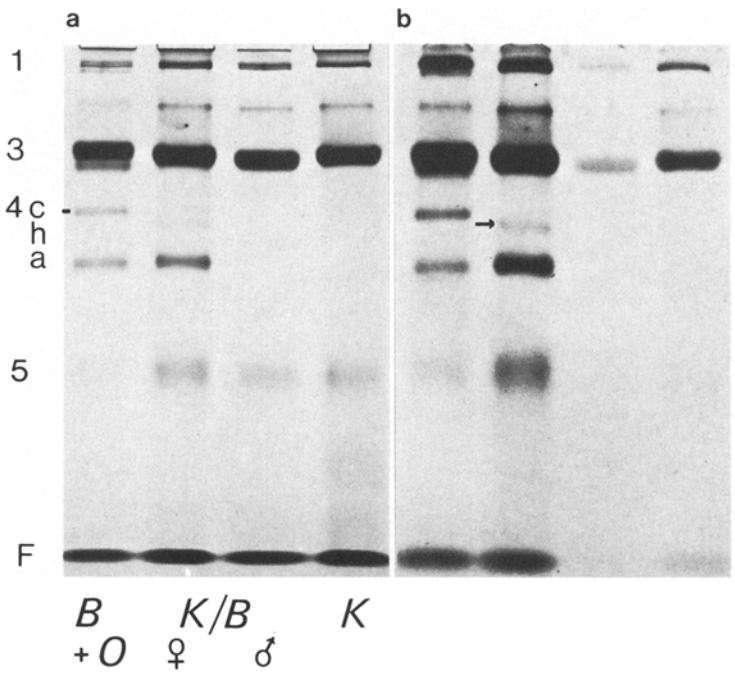

Fig. 3. Transvection effect for protein 4h. Reduced and alkylated secretion proteins from the stocks Berlin *B* plus Oregon O (*left*) and Kochi-R *K* (*right*) and from heterozygous female and male F_1-larvae *K/B* (*center*) of the cross Kochi-R-♀♀ × Berlin-♂♂ were separated in 7% urea-acrylamide slab gel. Larvae were fed a yeast suspension containing ^{14}C-proline. For each sample eight saliva plugs were used. *1 a-5*, secretion proteins; *F*, front. **a** Coomassie blue staining. **b** Fluorography; 90 days exposure. Arrow indicates protein 4h which can only be observed in *K/B*-♀♀ and is caused by transvection effects

between these possibilities, reciprocal crosses were carried out between the Berlin and Oregon and Oregon and Samarkand stocks, and the secretions of the F_1-larvae examined by electrophoresis. The results are shown in Table 2.

In all female F_1-larvae both the paternal and maternal types of protein 4 are represented. Corresponding to their amounts in the pure wild-type stocks, the amounts of proteins 4a and 4c in the F_1-females of Berlin and Oregon were equal. Both proteins, however, are more strongly represented than in the original stocks (Table 1, Berlin 0.15, Oregon 0.17). An increase is also observed in the male heterozygous larvae but the level of dosage compensation is reduced in comparison to the original stocks (Table 1).

Similar results were obtained with the reciprocal crosses between Oregon and Samarkand, two of which are of particular interest. 1) In heterozygous F_1-females, the concentration of protein 4d in the saliva is considerably less than that of protein 4c (Fig. 2c; Table 2). 2) The F_1-males of the cross Sa × O produce considerably less protein 4d as compared to 4c in the F_1-males of the reciprocal cross. Together with the differences in the electrophoretic mobility, the F_1-larvae apparently have also inherited the characteristic quantities of

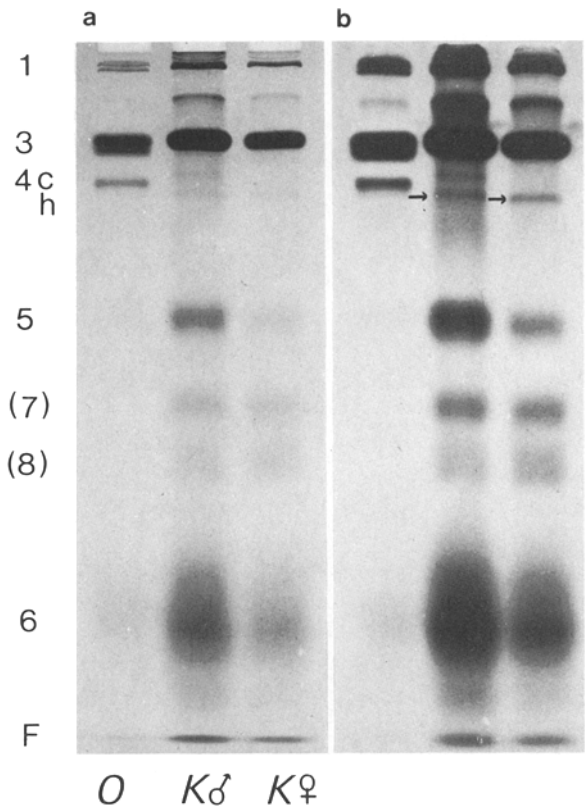

Fig. 4a and b. Reduced and alkylated secretion proteins from the stocks Oregon-*O* and Kochi-RK were separated in 7% urea-acrylamide slab gel. Larvae were fed a yeast suspension containing ^{14}C-proline. In slot *O* ten saliva plugs from Oregon female larvae and in slots *K*♂ and *K*♀ 40 saliva plugs from Kochi-R larvae, respectively, were applied. *1–6*, secretion proteins; *F*, front. (*7*) and (*8*), proteins the secretion specificity of which has not been tested so far. Arrows indicate protein 4h in the gel overloaded with Kochi-R secretion proteins. **a** Coomassie blue staining. **b** Fluorography; 20 days exposure

the protein variants involved. In addition, those F_1-males having protein 4c show dosage compensation. The high level of compensation (103%), however, is only apparent. It results from the fact that in the heterozygous females fraction 4d is only barely present. If the amount of protein 4c in the F_1-males (Table 2, 0.32) is compared with twice the amount of 4c in the F_1-females (Table 2, 0.19), an estimated dosage compensation level of 84% results. In the F_1-males of the reciprocal cross on the other hand, complete dosage effect can be observed for protein 4d. The capacity for dosage compensation is thus passed on with the X-chromosome carrying the *Sgs-4* gene from Oregon, while the chromosome carrying the *Sgs-4* gene from Samarkand fails to elicit dosage compensation. Post-transcriptional factors and genes possibly involved in the regulation of the activity of gene *Sgs-4* on chromosomes other than the X-chromosome have therefore no recognizable influence either on the amount

of synthesized protein 4 in the heterozygous females and males, or on the dosage compensation with *Sgs-4*^{c}, or the dosage effect in the case of *Sgs-4*^{d}.

Transvection Effect

The increased amounts of protein 4 in the heterozygous females from Berlin and Oregon and from Samarkand and Oregon (Table 2) may be attributed to a transvection effect (Lewis, 1954), so that the *Sgs-4* gene of one X-chromosome influences the activity of its homologue. In the case of the heterozygous Sa/O-females, the activity of the *Sgs-4*^{d} allele from Samarkand has apparently been raised under the influence of the *Sgs-4*^{c} gene from Oregon. Strict pairing of homologous chromosomes is the precondition for the transvection effect (Ashburner, 1967; Korge, 1977b). In order to test this, the secretion of heterozygous females of the cross Samarkand $\times$*In(1)FM1* was examined. The *FM1*-chromosome has two inversions. One of the breakpoints lies near 3C, the region that includes the *Sgs-4* gene. In heterozygous females region 3C remains unpaired in approximately 95% of the cases. The *FM1*-chromosome bears the *Sgs-4*^{d} gene coding for secretion protein 4d. Thus, the protein cannot be distinguished from that of the Samarkand stock by electrophoresis. The average value of ten scanned gels, each with six secretions of Sa/*FM1*-females was Q4/3=0.21. In order to determine the share of the *FM1*-chromosome in the 4d protein of the secretion, the *FM1* stock was crossed with the Hikone-R stock. In this stock no protein 4 can be detected under the chosen conditions. From 11 gels of female Hi/*FM1*-larvae each with six secretions, a quotient Q4/3 of 0.15 was obtained. Supposing that the same value is valid for the *FM1*-chromosome of the heterozygote Sa/*FM1*-females a difference of 0.06 is left to account for the contribution from the *Sgs-4*^{d} gene on the Sa-chromosome. This value corresponds to that obtained for the Samarkand wild-type stock (Table 1). It follows that in heterozygous females in which the X-chromosomes are unpaired in the region of the *Sgs-4* gene, there is no increase in *Sgs-4* activity in Samarkand chromosomes.

Using the transvection effect it has already been possible to prove that the apparent 0-mutant Hikone-R still bears the *Sgs-4* gene, because in heterozygous females with an X-chromosome from Hikone-R and one from Oregon or Berlin, an additional, although weak protein band appeared (Korge, 1977b). This was referred to as 4 h because it was obviously Hikone-R specific. Another 0-mutant in protein 4 from the wild-type stock Kochi-R has now been tested. Females of Kochi-R were crossed with Oregon females and ten secretions per gel were analyzed. The heterozygotes showed a new faint band of protein in the same position as 4 h (Fig. 3). Since the protein 4 h of the Hikone-R and Kochi-R stocks differs in its electrophoretic mobility from all proteins 4 found so far, it may be assumed that the two wild-type stocks are identical at least with respect to their *Sgs-4* genes.

Only recently small amounts of m-RNA originating from gene *Sgs-4* were detected in the salivary glands from Hikone-R by hybridization to cloned DNA fragments of this gene (Muskavitch and Hogness, 1980). In gels which were overloaded with secretion proteins, it could be shown in the present work

that, corresponding to the small amounts of m-RNA, small amounts of the secretion protein 4 h do in fact appear in the salivary glands of these supposed 0-mutants (Fig. 4a). This fraction becomes even more evident after labelling with ^{14}C-proline (Fig. 4b).

Localization of Sgs-4 and of Chromosome Loci Involved in Regulation of Sgs-4

The results of the reciprocal crosses suggest that the stock-specific level of protein 4, as well as its dosage compensation and the transvection effect are inherited with the X-chromosome. Whether these phenomena result from properties of the *Sgs-4* structural gene itself or whether they are due to activity of adjacent chromosome regions can be determined by linkage studies. For this purpose, the following crosses were carried out:

1. Hikone-R, *Sgs-4^h* × *y spl Sgs-4^a cho*
2. *Sgs-4^h cho* × *y spl Sgs-4^a dm*
3. *y spl Sgs-4^h* × *y w Sgs-4^a dm.*

From the F_2-generations of these crosses, those males were selected the X-chromosomes of which resulted from a crossing-over between *spl* and *cho* (experiment 1) or between *spl* and *dm* (experiments 2 and 3). These are due to recombination between *spl* and *Sgs-4* or between *Sgs-4* and *cho* or *dm* (Fig. 5).

The recombinant males were individually crossed with *C(1)RM, y*-females, and the secretion of the male larvae analyzed with respect to fraction 4. The results are shown in Table 3.

Out of these recombinants in which no protein 4 could be detected under the given conditions, 38 were crossed with Oregon females and the secretion of female larvae were analyzed. In addition to the secretion fraction 4c, protein 4h could be observed in all cases. In comparison to this, heterozygotes resulting

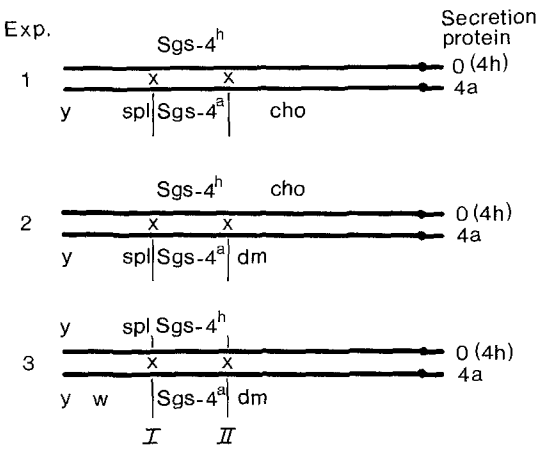

Fig. 5. Schematic diagram of the sites of meiotic recombination between *split (spl)* and secretion protein genes *Sgs-4^h* and *Sgs-4^a* (position I), and between *Sgs-4^h* and *Sgs-4^a* and *chocolate* (*cho*), Exp. 1, or *diminutive* (*dm*), Exps. 2 and 3 (position II), respectively. *Sgs-4^h* originated from the Hikone-R stock, *Sgs-4^a* from the Berlin stock. Only exchange chromatids are shown

Table 3. Recombinats from crosses shown in Figure 5

Experiment	Crossing-over in position	Phenotype of recombinants	Number
1.	I	4a-protein *cho*	6
		y spl 0-protein	10
	II	0-protein *cho*	48
		y spl 4a-protein	83
2.	I	*y spl* 0-protein *cho*	56
		4a-protein *dm*	39
	II	*y spl* 4a-protein *cho*	156
		0-protein *dm*	74
3.	I	*y spl* 4a-protein *dm*	22
		y w 0-protein	–[a]
	II	*y spl* 0-protein *dm*	37
		y w 4a-protein	–[a]
		Sum	531

4a-protein of Berlin type, both in electrophoretic mobility and in quantity. O-protein of Hikone-R type, i.e. no recognizable protein 4 under the given conditions, but 4h protein in heterozygot females (see text)

[a] Not tested

from the cross between recombinants with protein 4a and Oregon, displayed no protein 4h.

The recombination experiments show that the *Sgs-4^h* gene must lie at the same chromosome position as the *Sgs-4^a* gene, i.e., they are mutually both exclusive. Also no recombinant was found in which the amount of the secretion protein 4h was visibly increased or in which the amount of protein 4a was significantly decreased. This suggests that the weak expression of the gene *Sgs-4^h* is due to a mutation which occurred very close to or within the structural gene *Sgs-4^h*.

The locus of the alleles *Sgs-4^a* and *Sgs-4^h* can be delimited by the recombination data as follows. In experiments 2 and 3 (Table 3) 384 cross-overs occurred between *spl* (3.0) and *dm* (4.6) in a chromosome section of 1.6 Morgan units. Within this segment, recombination between *spl* and *Sgs-4* occurred 117 times and between *Sgs-4* and *dm* 267 times. This corresponds to a genetic distance of $1.6/384 \times 117 = 0.5$ Morgan units between *spl* and *Sgs-4*. Thus the position of *Sgs-4^a* and *Sgs-4^h* in the genetic map is 3.5.

In a similiar analysis the Samarkand stock was examined in which protein 4d is only weakly produced. The following crosses were carried out:

1. *y spl Sgs-4^d* × *Sgs-4^c dm cho*
2. *y spl Sgs-4^d cv ct v* × *y^2 Sgs-4^c dm cho.*

From the second generation those males were selected, whose X-chromosome were recombinant between *spl* and *dm* (Fig. 6). The recombinant males were crossed with attached-X females and the secretions of the male larvae tested by electrophoresis.

Table 4. Recombinants from the crosses shown in Fig. 6

Experiment	Crossing-over in position	Phenotype of recombinants	Number
1.	I	*y spl* $4c^{st}$ *dm cho*	7
		$4d^{w}$	53
		$4d^{i}$	1
	II	*y spl* $4d^{w}$ *dm cho*	12
		$4c^{st}$	69
2.	I	*y spl* $4c^{st}$ *dm cho*	48
		y^{2} $4d^{w}$ *cv ct v*	83
	II	*y spl* $4d^{w}$ *dm cho*	91
		y^{2} $4c^{st}$ *cv ct v*	104
		Sum	468

$4c^{st}$ protein 4 with electrophoretic mobility and in quantity as in Oregon (Table 1). $4d^{w}$ protein 4 with electrophoretic mobility and in quantity as in Samarkand. $4d^{i}$ electrophoretic mobility as in Samarkand but quantity intermediate between Oregon and Samarkand (Table 5)

Table 5. Relative amounts of secretion protein 4 in recombinants R+79 and in hybrids of reciprocal crosses between Oregon (O) and R+79. Calculation of rel. amounts see Table 1. Values are given with their standard deviations. Number of gels in parentheses

Genotype	4d/3	4c/3	4/3	Relative amount in ♂ (4/3-♀=100)
R+79 – ♀	0.26±0.04 (10)	–	0.26	
R+79 – ♂	0.20±0.02 (10)	–	0.20	77
O×R+79				
F_1 – ♀	0.15±0.02 (7)	0.15±0.02	0.30	
F_1 – ♂	–	0.26±0.04 (7)	0.26	87
R+79×O				
F_1 – ♀	0.16±0.03 (13)	0.16±0.02	0.32	
F_1 – ♂	0.22±0.04 (13)	–	0.22	69

The results of these recombination experiments are shown in Table 4. Out of a total of 468 recombinants there was only one in which the electrophoretic mobilities and the relative amounts of protein 4 did not correspond to one or the other of the two parental types. The recombinant referred to as *R+79* obviously resulted from a recombination between *spl* and *Sgs-4* in position I (Fig. 6). With regard to the genetic markers *y*, *spl*, *dm*, and *cho*, the recombinant

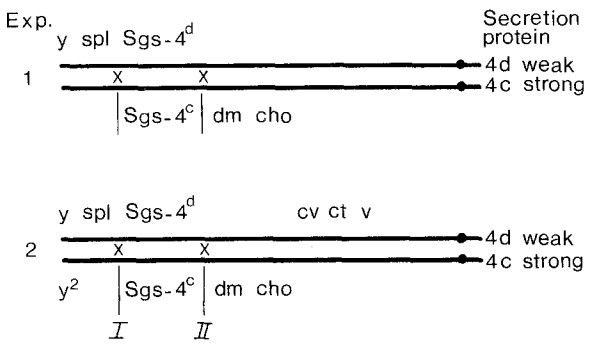

Fig. 6. Schematic diagram of the sites of meiotic recombination between *split* (*spl*) and secretion protein genes *Sgs-4*^{c} and *Sgs-4*^{d} at position I and between *Sgs-4*^{c} and *Sgs-4*^{d} and *diminutiv* (*dm*) at position II. *Sgs-4*^{c} originated from the Oregon stock, *Sgs-4*^{d} from the Samarkand stock. Only exchange chromatids are shown

was phenotypically wild-type. The secretion protein 4 was of the 4d type in its electrophoretical mobility. However, its amount (Table 5, Q4/3=0.20) was intermediate between that of the Oregon stock (Table 1, 4/3=0.30) and that of the Samarkand stock (Table 1, Q4/3=0.06).

The correspondence of the electrophoretic mobility of protein 4 from the recombinant *R+79* with that of the Samarkand parent stock suggests that the structural gene *Sgs-4* is only very negligibly altered if at all. Apparently in the recombinant *R+79* crossing-over had occurred at a site in immediate proximity of the structural gene and had caused the alteration in its activity.

With the aid of the balancer-chromosome *FM1* a stock was built up in which males and females bear the recombinant (*R+79*) X-chromosome. The quotient 4/3 is 0.26 (Table 5) in the saliva of female larvae for stock *R+79*. The level of dosage compensation of the male larvae thus amounts to 77% (Table 5) and lies between the values of both original stocks, Samarkand and Oregon (Table 1).

In order to test whether the intermediate content of protein 4d in *R+79* is determined by the X-chromosome alone and whether it is enhanced in heterozygotes, reciprocal crosses between *R+79* and Oregon were carried out and the secretions of the F_1-larvae examined. The results are shown in Table 5.

The protein fractions 4c and 4d are equally expressed in the heterozygous female larvae from both crosses (Table 5). Probably, the *Sgs-4* gene in the *R+79*-chromosome is activated as a result of transvection effect. On the other hand, in the male F_1-larvae protein 4c is more strongly represented than the 4d protein. This is also manifest in the different levels of dosage compensation in the F_1-males of the reciprocal crosses (Table 5).

Discussion

There is a great variability in the larval secretion proteins from various wild-type stocks of *Drosophila melanogaster*. This variability is expressed particularly by

differences in electrophoretical mobilities in urea-acrylamide-gels. Such variations are most probably caused by differences in the structural genes.

Using variants of the structural genes *Sgs-1* to *Sgs-4* for secretion proteins 1 to 4 these genes could be genetically and cytogenetically localized (Korge, 1975, 1977b; Akam et al., 1978; Velissariou and Ashburner, 1980). – The terminology follows that used by Korge (1975) with the one exception that secretion protein 6 (Akam et al., 1978) was renamed secretion protein 2 in place of the non-secretion protein 2 (Korge, 1975).

The variability is most pronounced in secretion protein 4 which is coded for by a gene in the region 3C11-12 on the X-chromosome. Polymorphism is expressed both qualitatively in various electrophoretic mobilities and quantitatively in terms of relative amounts in the saliva from various stocks (Fig. 1; Table 1). The level of this protein can vary between nearly 0% (Hi) and 60% (Korge, 1975, *Dp (1;f)z*^{9}) without recognizable deleterious effects on the viability of larvae.

The results of reciprocal crosses between the Samarkand stock with relatively little protein 4d and Oregon with a relatively large amount of protein 4c prove that the stock-specific quantities of protein 4 are autonomously determined by the X-chromosome (Table 2). Posttranscriptional regulation plays no recognizable role.

The various stock-specific quantities of protein 4 are interpreted as the phenotypical manifestation of different transcriptional activities of the structural gene *Sgs-4*. Only very small amounts of m-RNA from the *Sgs-4* gene could be observed in the larval salivary glands of Hikone-R and Kochi-R (Muskavitch and Hogness, 1980). These small amounts of m-RNA correspond to the very small amounts of protein 4h in these stocks.

In the males of the stocks Berlin, Oregon, Gruta and Falsterbo the production of secretion protein 4 exhibits dosage compensation in the range of 82–93% (Table 1). These values are very close to those for the incorporation of ^{3}H-uridine into the X-chromosomes of male and female larvae of *Drosophila*. Holmquist (1972) reported a value of 71% for the rate of RNA synthesis in the male X in comparison to that of the two female X-chromosomes. By contrast to this X-chromosomally coded enzymes display the same activities in males and females (reviewed by Stewart and Merriam, 1980). This complete compensation observed at the level of enzyme activity, despite incomplete compensation on the RNA level, could result from an additional direct control of enzyme activities. This possibility does not pertain to structural genes as examplified by the secretion protein 4 which is characterized by its amount and not by some kind of biological activity.

Of particular interest are the two *Drosophila* stocks, Karsnäs and Samarkand. Besides the fact that the secretion of their female larvae contains less protein 4 than females of the other tested stocks, the male larvae show no dosage compensation (Table 1). Lack of dosage compensation had already been observed in two other cases in *Drosophila*. Females which are homozygous for the X-linked mutation *white-eosin* (*w*^{e}) have twice as much pigment in their eyes as have the hemizygous males (Smith and Lucchesi, 1969). In the haemolymph of female larvae of the Oregon and Samarkand stocks the quantity

of the α-subunit of the larval serum protein 1 (LSP-1) is twice as large as in the haemolymph of male larvae (Roberts and Evans-Roberts, 1979). To explain this fact, these authors propose: "that the α-chain gene has only "recently" arrived on the X-chromosome, and that it has not acquired the regulation for dosage compensation". The lack of dosage compensation is tolerated because of the existence of the autosomally coded β- and γ-chains with the same function which reduce the selection pressure for the α-chain gene. The great qualitative and quantitative differences between the secretion proteins 4 of various *Drosophila* stocks and particularly the almost complete lack of this protein in Hikone-R and Kochi-R would indicate that there is also only low selection pressure on the secretion gene *Sgs-4*. This is understandable since, due to the presence of several other secretion proteins, the larval secretion can apparently completely fulfill its function of attaching the pupa case to the substrate in the absence of protein 4. Low selection pressure on the *Sgs-4* gene would also explain the mechanism of dosage compensation is not always complete or, in the case of Karsnäs and Samarkand, does not function at all.

Since the discovery of the phenomenon of dosage compensation (Bridges, 1922; Stern, 1929; Muller et al., 1931) data have been compiled and models have been constructed in order to explain the mechanism of dosage compensation (Muller, 1950; Stern, 1960; Smith and Lucchesi, 1969; Lucchesi, 1973, 1977, 1978; Belote and Lucchesi, 1980; Stewart and Merriam, 1980). All authors agree that characteristic features of the X-chromosomal genes themselves are responsible for their functional behavior apart from the relation between the number of X-chromosomes (Maroni and Lucchesi, 1980) and the number of sets of autosomes.

These characteristic features are causes and preconditions for the success of the dosage compensation. They could be based on molecular modifications of the X-chromosomal material which results during the embryogenesis.

That most genes on the X-chromosome must contain special regulatory sequences to respond to signals for the compensation of various gene dosages is shown by the existence of mutant alleles which fail to do so (Table 1). The clearest indication is given by the recombinant *R+79* (Table 5) which differs from its parents by an intermediate level of dosage compensation (Table 1). In addition, *R+79* females exhibit a change in the amount of protein 4d. Both effects are produced by a recombination event which could be localized by genetic markers (Fig. 6). The following conclusions are drawn: (1) In the immediate proximity distal to the structural gene *Sgs-4* a chromosome section is located which is responsible for the regulation of the activity of the gene in general and also for the dosage compensation in particular. (2) The *Sgs-4* gene is transcribed from distal to proximal on the chromosome (Fig. 7). (3) The genetic position of the structural genes $Sgs\text{-}4^c$ and $Sgs\text{-}4^d$ in the chromosome map is 3.6 (Fig. 7).

The genetic data allow no conclusion as to the size of the chromosome region which has a regulatory function for the *Sgs-4* gene. Since the identification of recombinants for *Sgs-4* is rather tedious, a fine structural analysis similar to that for the *rosy*-locus (Chovnik et al., 1976; Hilliker et al., 1980) seems out of the question on a genetic basis. The search for further recombinants

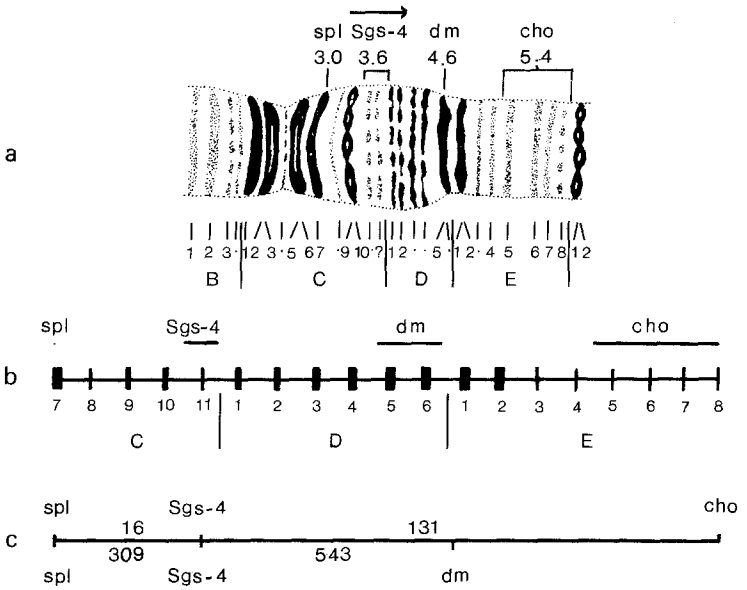

Fig. 7a–c. Cytologic and genetic localization of the gene *Sgs-4* for secretion protein 4. The genetic markers *spl split, dm diminutiv, cho chocolate* which are adjacent to *Sgs-4* were used for localizing the gene *Sgs-4*. **a** Cytological localization of the genetic markers with their linkage data. Arrow indicates transcription direction concluded from the recombination result. Modified chromosome map of Bridges. **b** Schematic illustration of the chromosome map and positions of the genetic markers used. **c** Numbers of recombinants between *spl, Sgs-4* and *cho* (Table 3, Exp. 1) and between *spl, Sgs-4* and *dm* (Table 3, Exps. 2 and 3; Table 4, Exps. 1 and 2), respectively

will, however, be continued. Recombinants which are complementary to $R+79$ and which possess the structural gene *Sgs-4* in less than normal activity would be particularly interesting and desirable.

As in the case of dosage compensation the basis of the transvection effect in *D. melanogaster* remains obscure. The results of the secretion protein experiments presented here have shown that in heterozygous females the X-chromosomally coded secretion fraction 4 is apparently produced in larger amount than in homozygous females (Tables 2 and 5). The transvection effect is especially obvious in the case of Oregon/Kochi-R heterozygous females (Fig. 3). As in earlier experiments for the putative 0-mutant Hikone-R (Korge, 1977b) it has been established for the Samarkand stock that strict pairing of the homologous chromosomes is a prerequisite for transvection effect.

A somewhat analogous situation has been described for the *white* locus (Judd, 1976) which is said to consist of a proximal, regulative region and the structural gene(s) in the distal portions. A mutation in the proximal part has been found to alter the activity of the distal part and impair the capability of performing dosage compensation. In addition, the proximal part of the *white* locus interacts with the *zeste* locus in such a way as to require somatic pairing at the *white* locus for the *zeste-white* interaction to occur, indicating a typical transvection effect (Jack and Judd, 1979).

Data have been published recently concerning the size of the DNA region required for expression of *Sgs-4* (McGinnis et al., 1980; Muskavitch and Hogness, 1980). With the help of deletions the size of the *Sgs-4* structural gene and essential flanking sequences were found to be 16 to 19 kilobases (McGinnis et al., 1980). The size of the structural gene *Sgs-4* was determined to be 0.9 kilobases and tandemly repeated sequences were observed within the *Sgs-4* gene (Muskavitch and Hogness, 1980): "They provide an explanation for the variation in length of m-RNAs in different strains" which was also noticed in this work. Different lengths of m-RNAs in various strains could phenotypically result in different sized *Sgs-4* proteins. This would explain the great variability observed in protein 4 from various *Drosophila* stocks. Furthermore, no evident correspondence between the length and the amount of the m-RNAs in the different strains could be revealed. This agrees with the present result that different types of protein 4 are produced in different but stock-specific amounts (Table 1). The very low production of m-RNA in the "0-mutants" Hikone-R and Kochi-R (Muskavitch and Hogness, 1980) could be caused by a small deletion of 50–110 base pairs found upstream from the structural gene *Sgs-4* (Muskavitch and Hogness, 1980). Our conclusion that *Sgs-4* must be transcribed in the distal-to-proximal-direction on the chromosome (Fig. 7) also agrees with the findings at the molecular level (Muskavitch and Hogness, 1980).

The genetic data presented in this work will provide a basis for the molecular analysis of the functional units of secretion genes. Secretion genes and their adjacent sequences will be cloned and analyzed in the hope of obtaining insight into control mechanisms of gene activities, and also those concerned with dosage compensation and transvection effects.

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