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Genetics

Transformation of salivary gland secretion protein gene *Sgs-4* in *Drosophila*: Stage- and tissue-specific regulation, dosage compensation, and position effect

(*P* element transformation/transcription/protein quantification/compensation effect)

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ABSTRACT **The *Sgs-4* gene of *Drosophila melanogaster* encodes one of the larval secretion proteins and is active only in salivary glands at the end of larval development. This gene lies in the X chromosome and is controlled by dosage compensation—i.e., the gene is hyperexpressed in males. Therefore, males with one X chromosome produce nearly as much *Sgs-4* products as females with two X chromosomes. We used a 4.9-kilobase-pair (kb) DNA fragment containing the $Sgs\text{-}4^d$ coding region embedded in 2.6 kb of upstream sequences and 1.3 kb of downstream sequences for *P*-element-mediated transformation of the $Sgs\text{-}4^h$ underproducer strain Kochi-R. $Sgs\text{-}4^d$ gene expression was found in all 15 transformed lines analyzed, varying with the site of chromosomal integration. The transposed gene was subject to tissue- and stage-specific regulation. At X-chromosomal sites, the levels of gene expression were similar in both sexes, signifying dosage compensation. At autosomal sites, it was on average 1.5 times higher in males than in females. The results indicate that the transforming DNA fragment contains all sequences necessary for tissue- and stage-specific regulation and for hyperexpression in males.**

Genes for larval secretion proteins, including *Sgs-4* of *Drosophila melanogaster*, are regulated in a tissue- and stage-specific manner during development. They produce abundant amounts of RNAs that are translated into a few saliva proteins (1–4). How the genes are activated is unknown, but there is evidence that they are inactivated with the increase of the ecdysone level approximately 6 hr prior to prepuparium formation (3, 5, 6).

The structural gene *Sgs-4* is simply constructed. Its size varies among different stocks due to repetitive sequences. It does not contain introns (4, 7). Among wild-type stocks there is a great variability in the activity of *Sgs-4* (2–4, 7, 8). Stocks that produce little or no sgs-4 protein carry mutations in the 5′ upstream region (7).

Sgs-4 lies in the X chromosome. Though male larvae possess only one gene, they produce in most stocks nearly as much sgs-4 protein as females, which have two genes ("dosage compensation"; see refs. 8 and 9). In some underproducer stocks males produce half as much sgs-4 protein as females ("dosage effect"; see ref. 8). Recombination analyses have shown that 5′ sequences adjacent to the structural gene *Sgs-4* are responsible for dosage compensation (8).

We want to answer the question, are the properties of stage, tissue, and sex specificity of the *Sgs-4* activity properties of its coding or adjacent DNA sequences or both? Using the elegant method of *P*-element-mediated transformation (10–12), we show that the activity of a 1.0-kilobase-pair (kb) *Sgs-4* gene contained in a 4.9-kb DNA fragment is normally regulated after transposition.

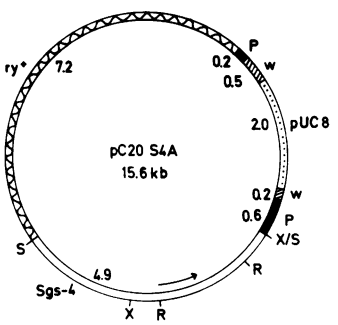

FIG. 1. Transforming plasmid pC20S4A, which contains the 4.9-kb *Sal* I/*Xho* I fragment (open bar) that includes the $Sgs\text{-}4^d$ gene from an Oregon-R (Stanford) stock in A orientation. The arrow indicates the position of the $Sgs\text{-}4^d$ structural gene. In the B orientation the 4.9-kb *Sal* I/*Xho* I fragment is reversed. Lengths of the fragments are given in kb within the circle. Bar with wavy line, DNA fragment containing the ry^+ gene; solid bars, *P* element sequences; hatched bars, *Drosophila* DNA from the white (*w*) region; dotted bar, pUC8 DNA. Restriction endonuclease sites: R, *Eco*RI; X, *Xho* I; S, *Sal* I.

MATERIALS AND METHODS

Recombinant DNA. λdDm 1523, which contains 2.9 kb of 5′ sequences and 0.75 kb of the coding sequences of the $Sgs\text{-}4^d$ gene of *D. melanogaster* Oregon-R (Stanford) strain, and λdDm 1522, which contains the remaining 0.25 kb of the gene and 1.7 kb of 3′ DNA sequences, were provided by D. Hogness and M. A. T. Muskavitch. The inserts were subcloned in pBR322 and the $Sgs\text{-}4^d$ gene was reconstructed according to the published map of the gene (4). To construct the $Sgs\text{-}4^d$-$rosy^+$-*P* element hybrid plasmid, the 4.9-kb *Sal* I/*Xho* I fragment was inserted into the *Sal* I site of a *P* element containing transposon pC20 [provided by G. Rubin and A. Spradling (12)] to yield pC20S4A and pC20S4B (Fig. 1). These plasmids differ in the orientation of the $Sgs\text{-}4^d$ fragment. Standard procedures were used for ligations and cloning in *Escherichia coli* HB101 (13).

***Drosophila* Strains and Injections.** The *D. melanogaster* strain ry^{506} (obtained from A. Spradling) was crossed with the $Sgs\text{-}4^h$ underproducer Kochi-R strain (3, 8) to obtain the homozygous recipient strain $Ko(Sgs\text{-}4^h); ry^{506}$. Underproduction of sgs-4^h was verified by analysis of the protein (Fig. 2). Injections into embryos were performed as described by Rubin and Spradling (10–12). A mixture containing equal amounts of pC20S4A and pC20S4B (each at 150 μg/ml) was

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Abbreviation: kb, kilobase(s) or kilobase pairs(s).

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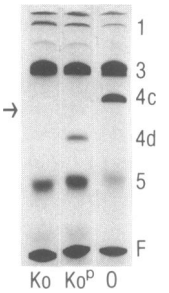

FIG. 2. Electrophoretically separated reduced and alkylated secretion proteins from larvae of the recipient strain *Ko(Sgs-4^h); ry^{506}*, (Ko), the *P*-element-mediated transformed line 6-1 (Ko^P), and the wild-type strain Oregon-R(ORN) (O). Acrylamide/urea gel electrophoresis (1); Coomassie blue staining. Bands 1–5 are saliva proteins; F, front; 4d refers to the transposed *Sgs-4^d* gene. The arrow indicates the faint 4^h protein band in secretions of the recipient stock Kochi-R.

injected with the plasmid pπ25.1 (50 μg/ml) containing the full-length transposon (10).

Analysis of DNA and RNA. DNA from adult flies was isolated as described (14). DNA from salivary glands was isolated by NaDodSO$_4$ lysis and simultaneous proteinase K treatment at 50°C, followed by extractions with phenol/chloroform. DNAs were digested with restriction endonucleases, subjected to electrophoresis on agarose gels, and blotted onto nitrocellulose filters (15).

For RNA isolation, larvae were homogenized in phenol/cresol, and an equal volume of 0.08 M sodium phosphate buffer, pH 6.8/0.75 M NaCl/1% NaDodSO$_4$ was added. The aqueous phase was collected after centrifugation and the RNA was precipitated with ethanol. RNAs were electrophoresed in 1.5% formaldehyde/agarose gels and blotted onto nitrocellulose filters (13).

Hybridization probes were prepared by nick-translation with ^{32}P-labeled nucleotides and hybridized to DNA or RNA on filters in 50% (vol/vol) formamide, 5× Denhardt solution (1× = 0.02% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone), and 100 μg of sonicated and denatured salmon sperm DNA per ml at 42°C for 40 hr. Filters were washed twice in 0.3 M NaCl/0.03 M sodium citrate/0.1% NaDodSO$_4$ at room temperature for 30 min and then twice in 15 mM NaCl/1.5 mM sodium citrate/0.1% NaDodSO$_4$ at 50°C for 30 min. For quantification of RNA, preflashed X-ray film was exposed to the filters at −70°C and scanned in a microdensitometer (16) or bands were cut out from the filters and assayed by scintillation counting.

Protein Analysis. Secretion proteins were analyzed as described in ref. 1.

Hybridization *in Situ*. Hybridization *in situ* was performed essentially by the procedure of Pardue and Gall (17) with modifications described in refs. 18 and 19. ^{3}H-labeled pC20-S4A (Fig. 1) or pOW3, the latter containing 2.9 kb of 5′ upstream and 1.0 kb of *Sgs-4^c* coding sequences from the strain Oregon-R(ORN), were used for hybridization (20).

RESULTS

Protocol of Transformation. The underproducer strain *Ko(Sgs-4^h); ry^{506}* was used as a recipient for *P* element transformation for two reasons: (*i*) The rosy phenotype allows the selection of transformants with rosy$^+$ eyes. (*ii*)

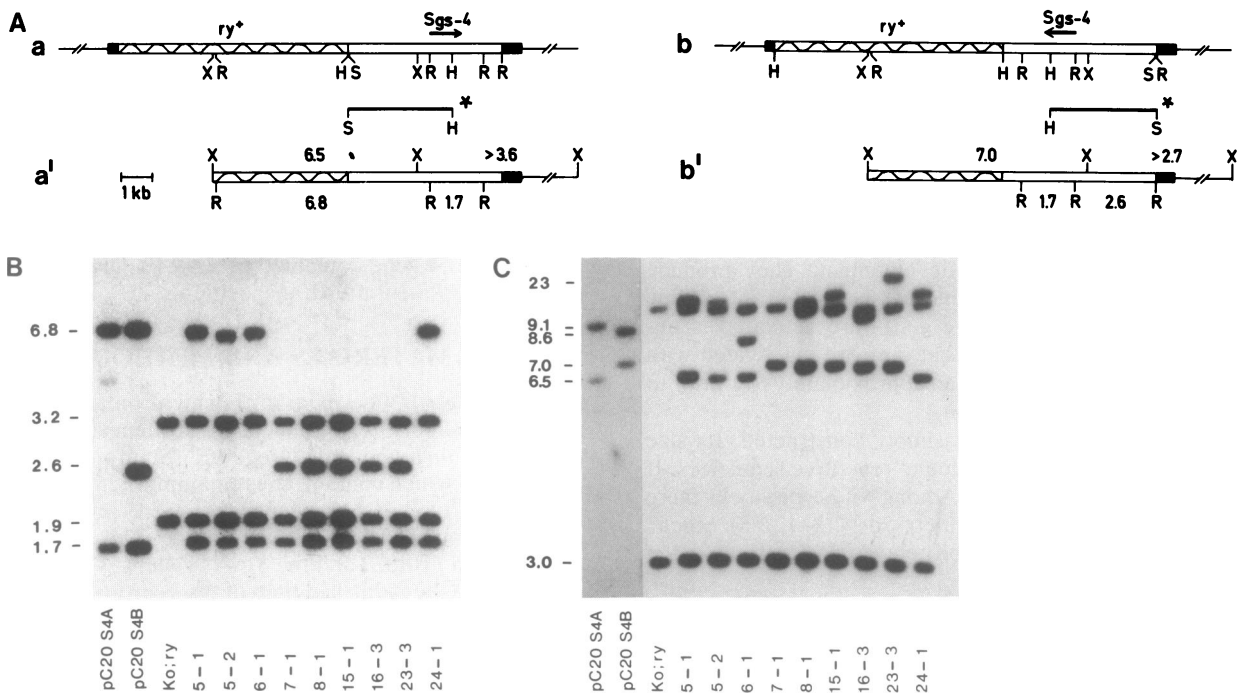

FIG. 3. Southern blot analysis of genomic DNA from transformed strains, *Ko(Sgs-4^h); ry^{506}* recipient strain (Ko; ry), and plasmids pC20S4A and pC20S4B as controls. (*A*) Predicted integration and hybridization fragments of the transforming plasmids pC20S4A (*a*) and pC20S4B (*b*). The *Hin*dIII/*Sal* I fragment containing 2.6-kb upstream and 0.75-kb *Sgs-4* coding sequences subcloned in pBR322 was used for hybridization probe (*). Fragment lengths produced by *Xho* I or *Eco*RI are given in kb (*a′*, *b′*). Open bars, *Sgs-4* DNA; bars with wavy line, DNA fragment containing the *ry*$^+$ gene; solid bars, *P* element DNA; thin lines, flanking genomic DNA. Restriction sites: H, *Hin*dIII; S, *Sal* I; R, *Eco*RI; X, *Xho* I. (*B* and *C*) *Eco*RI-digested (*B*) and *Xho* I-digested (*C*) DNA from adult flies (2 μg of DNA per lane). In *B* the 6.8-kb band in the pC20S4A control is a doublet, containing the *Sgs-4-ry*$^+$ fragment as indicated in *a′* and a fragment spanning pUC8 sequences hybridizing to pBR322 DNA of the probe. The latter also produced the 6.8-kb hybridizing band in pC20S4B. However, the pUC8 fragment lies outside of the integration fragments of pC20S4A and pC20S4B (*A a* and *b*) and is not transposed. Therefore, in *Eco*RI digests (*B*) only *Sgs-4^d* DNA of A orientation shows a hybridizing 6.8-kb fragment (lines 5-1, 5-2, 6-1, and 24-1), whereas only transposed *Sgs-4^d* DNA of B orientation gives hybridizing 2.6-kb fragments (lines 7-1, 8-1, 15-1, 16-3, and 23-3). Fragments of 6.8 kb (A orientation) and 2.6 kb (B orientation) indicate full-length integration of upstream sequences, and 1.7-kb fragments (both orientations) indicate integrations of complete coding and 0.7-kb downstream sequences. (*C*) *Xho* I-digested DNA also demonstrates orientation of *Sgs-4* fragments in the transposed DNA (6.5 kb in the case of A orientations, 7.0 kb in B orientations). In addition, the junction fragments of >3.6 kb and of >2.7 kb, respectively, inform about numbers and completeness of the integrations. The apparently missing junction fragment in line 7-1 could be contained in the 3.0-kb fragment that shows strong hybridization efficiency in relation to the other bands of the same lane.

RNA and protein products of the *Sgs-4^h* gene are almost undetectable in our analyses (Fig. 2). Moreover, compared to the injected *Sgs-4^d* gene, *Sgs-4^h* contains more copies of a repeated sequence within the structural gene (data not shown). Therefore, DNAs, RNAs, and proteins of *Sgs-4^h* and *Sgs-4^d* are separable on gels.

For transformation, *P* element plasmids containing 2.6-kb upstream, 1.0-kb *Sgs-4^d*-coding, and 1.3-kb downstream DNA sequences (Fig. 1) were injected with the transposon pπ25.1 into embryos of *Ko(Sgs-4^h); ry^{506}*. G_0 adults were mated in single crosses with *Ko(Sgs-4^h); ry^{506}*. Single G_1 *ry$^+$* flies were backcrossed with *Ko(Sgs-4^h); ry^{506}* and the G_2 *ry$^+$* offspring were inbred. The *ry$^+$* flies from G_3 were also inbred to establish homozygous lines. Out of 132 injected embryos 34 fertile adults developed, which gave rise to 14 stable transformed *ry$^+$* lines. From some injected embryos we obtained and characterized several sublines (5-1,2; 7-1,2; 15-1,2,3; and 16-3,8). Those of lines 5, 7, and 15 were identical as shown by Southern analysis and hybridization *in situ* (Fig. 3; Table 2). Fifteen lines were characterized in detail (see Table 2).

Test of Integration: Southern Analysis and Hybridization *in Situ*. Integration of *ry$^+$* and *Sgs-4^d* DNA into the *Ko(Sgs-4^h); ry^{506}* genome was confirmed by Southern blot analysis (Fig. 3). DNA from the transformed lines was digested by either *Eco*RI or *Xho* I to determine full-length integration of the *Sgs-4^d* DNA fragment, its orientation, and the number of integration sites by use of a *Sgs-4^d* gene probe (Fig. 3). As judged from the lengths of the hybridizing DNA fragments, from the signal intensities of the new bands as compared to those of the endogenous Kochi-R gene, and from the number of junction fragments of the *Xho* I-digested DNA (Fig. 3*C*), 12 of the *ry$^+$* lines contain a single copy of the transformed DNA (Table 2). Line 16-8 contains at least four copies (data not shown). In line 7-1 we could not detect the *Xho* I junction fragment; however, the *Eco*RI digest shows the presence of the *Sgs-4^d* DNA (Fig. 3*B*).

The integration sites of the *Sgs-4^d* genes were localized on polytene chromosomes by *in situ* hybridization using both

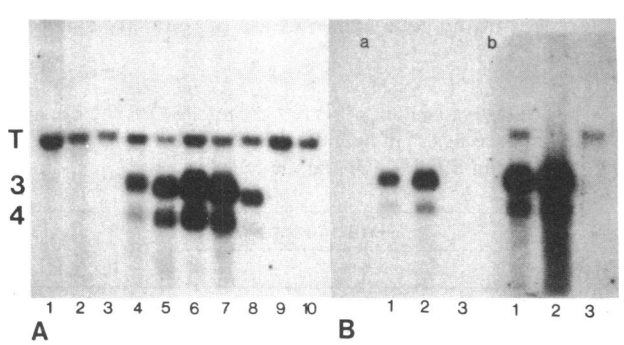

FIG. 4. Stage- and tissue-specific expression of the transposed *Sgs-4^d* gene in line 24-1 demonstrated by blot hybridization analysis of total RNA. Filters were first hybridized to pS34 containing the structural genes *Sgs-3* and *Sgs-4^d* (*Sgs-3* obtained from E. Meyerowitz) and then rehybridized to a plasmid containing the β1-tubulin gene for a control (pTU 56, obtained from R. Renkawitz-Pohl). (*A*) RNA isolated from different developmental stages. Lanes: 1, 0–40 hr; 2, 48–57 hr; 3, 55–72 hr; 4, 73–83 hr; 5, 87–97 hr; 6, 95–105 hr; 7, 100–110 hr; 8, prepupae; 9, pupae; 10, adults (0 hr is time of egg deposition). Simultaneous appearance and disappearance of hybridizing *Sgs-3* and *Sgs-4^d* RNA indicates normal stage-specific gene expression. (*B*) RNA isolated from late third instar larvae (lanes 1), salivary glands (lanes 2), and late third instar larvae with salivary glands removed (lanes 3). Exposure time in *a*, 2 hr; *b*, 16 hr. T, 3, and 4 indicate RNA species hybridizing to the tubulin, *Sgs-3*, and *Sgs-4* genes, respectively. Neither *Sgs-3* RNA nor *Sgs-4^d* RNA was found in tissues other than salivary glands. This indicates tissue-specific gene expression of the transposed *Sgs-4^d* gene.

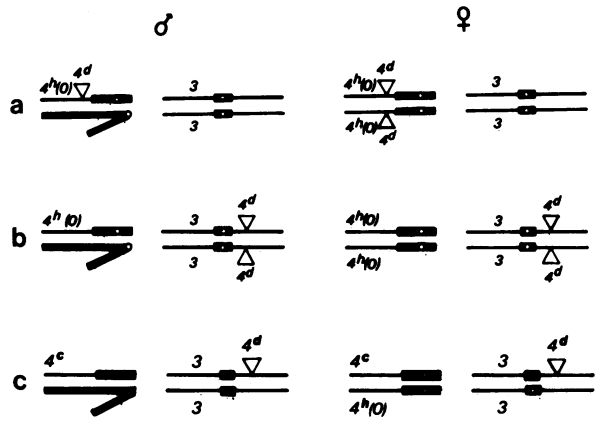

FIG. 5. Cytological positions and doses of *Sgs-4^d* genes in transformants (*a*, *b*) and in F_1 hybrids of Oregon-R(ORN) females crossed with transformed males (*c*) in relation to the endogenous X-chromosomally located alleles *Sgs-4^h* from the Kochi-R strain (underproducer) and *Sgs-4^c* from Oregon-R(ORN) (normal producer) and the gene *Sgs-3* in autosome III.

pC20S4A (Fig. 1) and pOW3 as probes (Table 2). Except for three lines, the number of integration sites as judged from Southern analysis was confirmed. In lines 21-1, 23-3, and 28-2 we detected second sites of integration. These sites, however, were found only in a few larvae and sometimes on one homolog only.

Transposed *Sgs-4^d* Genes Are Expressed at the Correct Stage and in the Correct Tissue. Blot hybridization analyses of RNA demonstrate that the *Sgs-4^d* transcripts from transformed lines have the expected size of 1.0 kb (Fig. 4). The 1.0-kb RNA was first detected in 73- to 83-hr-old larvae and disappeared in pupae and adults (Fig. 4*A*). This indicates that the *Sgs-4^d* gene at the new chromosomal site is expressed at the correct stage of development in synchrony with the endogenous secretion gene *Sgs-3* (Fig. 4*A*). *Sgs-4^d* RNA was detected in RNA of whole larvae and salivary glands but not in RNA of carcasses from which salivary glands had been removed, indicating tissue-specific expression of the transposed gene (Fig. 4*B*).

Dosage Compensation and Compensation Effect of Transposed *Sgs-4^d* Genes. To quantify the activity of the transposed genes, both RNA and protein were compared to internal standards. First, the gene products were compared to the *Sgs-3* products. *Sgs-3* lies in the third chromosome; therefore, males and females have the same gene dosage (Fig. 5) and produce the same amount of sgs-3 protein (8). Second, some of the transformed lines were crossed with the wild-type strain Oregon-R(ORN). *Sgs-4^d* RNA and protein of the

Table 1. Relative amounts of RNA in transformed lines and in hybrids of wild-type stock Oregon-R(ORN) females (O) and line 8-1 males, densitometrically determined

Line	RNA ratios* ♂♂	RNA ratios* ♀♀	♂/♀ RNA ratio†
	$4^d/3$		
16-3	0.09, 0.07 (1)	0.12, 0.10 (2)	0.72
24-1	0.36, 0.42 (2)	0.18, 0.22 (2)	1.95
	$4^d/4^c$		
O × 8-1 F_1	0.15, 0.21 (1)	0.11, 0.19 (1)	1.20

*4^d, RNA encoded by the transposed gene *Sgs-4^d*; 3, RNA encoded by the endogenous autosomal gene *Sgs-3*; 4^c, RNA produced by the endogenous X-chromosomally located gene *Sgs-4^c*. See Fig. 5. Data from two independent experiments are presented; *Sgs-4^d* gene dosage is given in parentheses.

†Mean values were used for calculation.

Table 2. Relative amounts of sgs-4^d protein in transformed lines

Transformed line	Integrated transposon: Orientation of *Sgs-4^d**	Integrated transposon: Cytological position	4^d/3 protein ratios (*n*): ♂♂	4^d/3 protein ratios (*n*): ♀♀	♂/♀ 4^d/3 protein ratio
7-1	B	X-3A	0.04 ± 0.01 (21)	0.06 ± 0.02 (16)	0.67
7-2	B	X-3A	0.04 ± 0.01 (10)	0.06 ± 0.02 (11)	0.67
16-3	B	X-18D	0.07 ± 0.01 (10)	0.09 ± 0.02 (17)	0.78
21-1	B	X-14A	0.09 ± 0.04 (8)	0.08 ± 0.02 (9)	1.13
5-1	A	3R-82F	0.05 ± 0.01 (15)	0.03 ± 0.01 (13)	1.67
6-1	A	2L-25A	0.10 ± 0.03 (8)	0.08 ± 0.03 (11)	1.25
8-1	B	3L-79F	0.09 ± 0.02 (13)	0.05 ± 0.01 (13)	1.80
15-1	B	2L-35A	0.06 ± 0.02 (13)	0.04 ± 0.02 (12)	1.50
15-2	B	2L-35A	0.06 ± 0.01 (6)	0.04 ± 0.01 (13)	1.50
15-3	B	ND	0.07 ± 0.03 (11)	0.05 ± 0.01 (11)	1.40
20-1	B	3R-86A	0.08 ± 0.01 (10)	0.06 ± 0.01 (5)	1.33
23-3	B	3L-63E	0.02 ± 0.01 (6)	0.01 ± 0.00 (4)	—
24-1	A	3R-85BC	0.15 ± 0.04 (21)	0.08 ± 0.03 (20)	1.88
28-2	B	3R-89B	0.03 ± 0.01 (13)	0.02 ± 0.01 (16)	1.50
33-4	B	2L-27C	0.04 ± 0.01 (10)	0.03 ± 0.01 (8)	1.33

Ratios of proteins are given with their standard errors; number of determinations is given in parentheses. Line 21-1 sometimes contains an additional integration at 2L-25DE, line 23-3 sometimes has one at X-3A, and line 28-2 sometimes has one at 3L-65AB. ND, not determined.
*See Fig. 3.

F_1 hybrids were compared to those of the endogenous X-chromosomally *Sgs-4^c* of Oregon-R(ORN) and the transformed sgs-4^d protein was also compared with sgs-3 protein (Fig. 5*c*).

On the level of RNA, three lines were analyzed (Table 1). Line 16-3 carries *Sgs-4^d* on the X chromosome. Consequently, males have the gene dosage 1, females the dosage 2 (Fig. 5*a*). The amount of *Sgs-4^d* RNA, relative to *Sgs-3* RNA, is in males 72% of that in females, indicating hyperexpression, i.e., dosage compensation, though incomplete.

The second line (24-1) carries *Sgs-4^d* in chromosome 3. Both sexes have, therefore, the gene dosage 2 (Fig. 5*b*). Males produce almost twice the amount of *Sgs-4^d* RNA of females. Also in this case the *Sgs-4^d* gene is hyperexpressed in males. Although located in an autosome, the gene shows a *compensation effect* (Table 1).

The third line was analyzed in F_1 hybrid progeny of Oregon-R(ORN) females and transformed males of line 8-1. Both F_1 males and females carry one *Sgs-4^c* gene from Oregon-R(ORN) on the X chromosome at the original site and one *Sgs-4^d* transposed to chromosome 3 (Table 2, Fig. 5*c*). The ratio of their RNAs is about the same in males and females (Table 1). Since the *Sgs-4^c* gene shows almost complete dosage compensation in the Oregon-R(ORN) strain (8) and also in hybrids (see 4c/3 ratios in Table 3) a $4^d/4^c$ male-to-female ratio of about 1.0 indicates hyperexpression and, therefore, compensation effect for the autosomally located *Sgs-4^d* gene.

The analysis of the secretory proteins (Table 2) corroborates the result obtained for RNA. In all lines that carry the transposed *Sgs-4^d* gene on an X chromosome the males show distinct dosage compensation.

In the case of autosomal integration, male larvae always produce more sgs-4^d protein than females. The male-to-female protein ratios vary from 1.25 to 1.88 (Table 2). Furthermore, the protein data of F_1 hybrids between transformed lines and Oregon-R(ORN) clearly demonstrate hyperexpression of the sgs-4^d protein in males carrying the *Sgs-4^d* gene on an autosome (Table 3). The activity of the endogenous *Sgs-4^c* of Oregon-R(ORN), measured at the protein level relative to sgs-3, was unchanged in these hybrids as compared to earlier results obtained from Oregon-R(ORN) (8). The male-to-female protein ratios are in good agreement with those obtained for RNA of hybrids (Table 1) and those for RNA and protein of the pure lines (Tables 1 and 2).

In all transformed lines analyzed, the activity of the transposed *Sgs-4^d* gene is low compared to wild-type Oregon strains. Measuring protein, line 24-1 with the strongest activity produced only about 25% of the Oregon value [4^c/3 for males = 0.29 (8)]. For this line, however, we reproducibly find about 2-fold higher values for RNA as compared to the protein value. On the other hand, for lines 8-1 and 16-3 RNA and protein data are in reasonable agreement (Tables 1 and 2).

In addition to the weak expression, great differences in the activity of the transposed *Sgs-4^d* genes can be seen in different lines. The 4^d/3 ratios in pure lines range from 0.04 in line 33 males to 0.15 in line 24-1 males (Table 2). These differences in the gene activities are presumably caused by

Table 3. Relative amount of secretion protein 4^d coded by the transposed gene *Sgs-4^d* in hybrids of reciprocal crosses between Oregon-R(ORN) (O) and the transformed lines 8-1 and 24-1

Hybrid*	Protein ratios†: 4^c/3	Protein ratios†: 4^d/3	Protein ratios†: $4^d/4^c$	♂/♀ $4^d/4^c$ protein ratio‡
O × 8-1				
F_1 ♂ (4)	0.23	0.03	0.11	
F_1 ♀ (5)	0.16	0.02	0.13	0.92
8-1 × O				
F_1 ♂ (5)	—	0.03	—	
F_1 ♀ (3)	0.14	0.02	0.10	
O × 24-1				
F_1 ♂ (9)	0.32 ± 0.11	0.07 ± 0.02	0.23	
F_1 ♀ (13)	0.15 ± 0.04	0.04 ± 0.01	0.29	0.82
24-1 × O				
F_1 ♂ (4)	—	0.07 ± 0.03	—	
F_1 ♀ (8)	0.18 ± 0.06	0.05 ± 0.02	0.26	

*Parental females are given first. Number of determinations in parentheses.
†4^c, protein encoded by the *Sgs-4^c* gene of the Oregon-R(ORN) X chromosome; 4^d, protein encoded by the transposed gene *Sgs-4^d*; 3, protein encoded by the endogenous autosomal gene *Sgs-3*. Some ratios are given with their standard errors.
‡Data from reciprocal crosses were used for calculation.

position effects. No integration was found in heterochromatin or in close proximity to it.

Transposed Genes Are Not Underreplicated. The weak expression of the transposed *Sgs-4^d* genes could be caused by their underreplication in the polytene chromosomes (21). Therefore salivary gland DNA from both male and female larvae from the lines 5-1, 7-1, and 15-1 with very weak *Sgs-4^d* activity and line 24-1 with higher activity (Table 2) was analyzed on Southern blots by comparing the signal strengths of the sequences of the transposed *Sgs-4^d* and the endogenous *Sgs-4^h* gene. The relative intensities showed no sign of underreplication of the *Sgs-4^d* gene (data not shown). Therefore, there must be reasons other than underreplication accounting for the weak activity.

DISCUSSION

Among wild-type stocks of *D. melanogaster Sgs-4* genes of different activities had been found. Sequence analysis of DNA flanking the 5′ end of *Sgs-4* had shown that under- and nonproducer genes carry small deletions 100–300 base pairs (bp) upstream of the mRNA start site (7). During the secretion synthesis phase DNase I-hypersensitive sites have been observed within the region from −70 to −480 bp upstream of the gene (14). Those findings may describe the DNA region responsible for the level of gene activity, but they do not define DNA sequences responsible for stage- or tissue-specific expression or for dosage compensation.

Injections of the plasmids pC20S4A/B carrying both the *ry$^+$* and the *Sgs-4^d* locus DNA into *Ko(Sgs-4^h); ry^{506}* embryos gave rise to stable transformed *Drosophila* lines. In all lines analyzed, both genes were stably functioning for more than 1 year and *Sgs-4^d* transcription and translation appear to be correct. The orientation of the *Sgs-4^d* fragment in the transforming plasmid relative to *ry$^+$* sequences seems to be without influence on the expression of the transposed gene (Table 2).

The activity of the transposed genes is only 3–25% of that of the *Sgs-4* gene in the donor strain Oregon. It is most unlikely that the weak gene expression is caused by position effect only, because none of the 12 lines with different integration sites in euchromatic regions showed normal gene activity (Table 2). In our transformants there was apparently no effect on *Sgs-4^d* expression whether the gene was integrated close to condensed bands or puffed regions. For example, in line 24-1 the gene is integrated into the close proximity of highly condensed bands, but the activity is 3-fold higher than in line 5-1, where the gene is integrated into the puffed region 82F.

The *Sgs-4^d* genes are expressed stage and tissue specifically (Fig. 3). Therefore, the injected 4.9-kb *Sgs-4^d* fragment includes all sequences necessary for its specific regulation. Correct stage- and tissue-specific expression had been demonstrated for other transposed *Drosophila* genes as well (22–27).

Four transformants carried *Sgs-4^d* on the X chromosome, 11 on an autosome. In males of all these lines the gene is hyperexpressed and shows dosage compensation. In pure lines with X-chromosomal integration (7-1; 16-3) the ratio of expression between males and females is 0.7–0.8, i.e., a 1.5-fold hyperexpression in the males (Table 2). This dosage compensation is not complete; it is, however, in reasonable agreement with data obtained earlier for wild-type strains and for hybrids with "normal" *Sgs-4* genes (8). In male larvae with an autosomal integration the degree of hyperexpression is also about 1.5. This value corresponds well to that obtained for the X-chromosomal duplication *Dp(1;f)z^9*, which carries *Sgs-4* (1). Similar values for hyperexpression have been found for the eye-pigment gene white in transformants that carried the gene on autosomes (22).

Earlier observations on *Drosophila* wild-type stocks had implied that *Sgs-4* underproduction might be linked to dosage effect (8). However, comparison of our transformed lines reveals no clear correlation between the level of gene activity and that of hyperexpression. Considering the results of a recombination analysis between strains with compensating normal active and noncompensating weakly active *Sgs-4* genes (8), we conclude that the capability for compensation lies within the 2.6 kb of upstream sequences. Obviously this DNA acts in *cis* on X-chromosomal genes. On the other hand it seems to act over greater distances on autosomal genes that have been transposed to X chromosomes (23, 24). In these cases the degree of hyperexpression corresponds to that of dosage compensation of X-chromosomal genes.

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