Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5047–5051, June 1993
Genetics

The interaction of bride of sevenless with sevenless is conserved between *Drosophila virilis* and *Drosophila melanogaster*

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Contributed by G. M. Rubin, March 12, 1993

ABSTRACT **An inductive interaction between the sevenless (sev) transmembrane tyrosine kinase receptor and the bride of sevenless (boss) transmembrane ligand is required for the development of the R7 photoreceptor neuron in the compound eye of *Drosophila melanogaster*. The boss protein is proposed to contain a large N-terminal extracellular domain, seven transmembrane segments, and a C-terminal cytoplasmic tail. The boss protein from *Drosophila virilis* ($boss^{vir}$) retains strong amino acid identity with boss from *D. melanogaster* ($boss^{mel}$): 73% identity in the N-terminal extracellular domain and 91% identity in the seven-transmembrane domain, including the cytoplasmic tail. By using *P*-element-mediated DNA transformation, the $boss^{mel}$ and $boss^{vir}$ genes were shown to rescue the *D. melanogaster* $boss^{1}$ mutation. The expression of $boss^{vir}$ protein in *D. melanogaster* is indistinguishable from that of $boss^{mel}$ protein. Noncoding sequences which may regulate *boss* expression were identified based on their conservation during evolution. The predicted sev protein from *D. virilis* (sev^{vir}) was previously shown to be 63% identical to sev from *D. melanogaster* (sev^{mel}). A chimeric gene, ($sev^{vir/mel}$), encoding the extracellular domain of sev^{vir} and the cytoplasmic domain of sev^{mel} rescues the *D. melanogaster* sev^{d2} mutation through interaction with either $boss^{vir}$ or $boss^{mel}$.**

The development of the R7 photoreceptor cell in the *Drosophila melanogaster* compound eye requires a specific inductive cue from the adjacent R8 photoreceptor (1). This interaction is mediated by the sev tyrosine kinase receptor (2–8) and by its transmembrane ligand, boss (9–11). The sev receptor is expressed by the R7 precursor cell, as well as other cells in the developing eye imaginal disc (7, 8), whereas the boss protein is expressed by only one cell type, the R8 cell (10). Mutations in either the *sev* or the *boss* gene result in the transformation of the R7 precursor into a nonneuronal cone cell (12, 13).

As a step toward a structural and biochemical analysis of the interaction between the boss and sev proteins, we examined the predicted amino acid sequences of boss and sev homologs from *Drosophila virilis*, a species thought to have diverged from *D. melanogaster* some 60 million years ago (14). The sequence of sev from *D. virilis* was previously reported (15). Using *P*-element-mediated DNA transformation of *D. melanogaster*, we assessed the ability of *boss* and *sev* isolated from *D. virilis* to function with their *D. melanogaster* partners. In addition, we have identified genomic sequences which are potentially important for regulating the precise pattern of *boss* gene expression during development.¶

MATERIALS AND METHODS

Materials. The *D. virilis* genomic library was obtained from J. Tamkun (University of California, Santa Cruz). The cloning vectors were previously described (9). Chloramphenicol was obtained from Sigma. The sev^{mel} germ-line transformation construct contains 17.5 kb of wild-type *D. melanogaster* DNA (16). *Taq* I DNA polymerase was obtained from Perkin–Elmer/Cetus.

DNA Cloning and Sequencing. DNA cloning, RNA blots, and sequencing techniques were previously described (9). Hybridization of DNA blots and plaque lifts of *D. virilis* DNA was performed at reduced stringency levels using 35% formamide (17). Washes were for 20 min at 52°C. Twelve λ clones from the *D. virilis* genomic library were identified and restriction-mapped. Both *boss* rescue constructs were sequenced as described (9). Nucleic acid sequences and predicted protein sequences were analyzed with the Genetics Computer Group package programs (18) using default definitions of amino acid similarity.

Transformation. A 7.5-kb *Xho* I–*Sal* I *D. melanogaster* genomic fragment (9) and a 9-kb *Sal* I *D. virilis* genomic fragment containing the entire *boss* gene were subcloned into pDM23 (19) for *P*-element-mediated transformation of a *h ry* $boss^{1}$ line as previously described (20). $sev^{vir/mel}$ contains a fusion of *D. virilis sev* cDNA and genomic sequences with *D. melanogaster* genomic sequences. A *D. virilis sev* cDNA fragment was generated from *D. virilis* poly(A)$^+$ RNA [prepared as described (17, 21)] by reverse transcription and amplification (22) with the polymerase chain reaction (23). The reverse transcription reaction was primed with the oligonucleotide 5′-GTCCGCCAGATTGTGCCA-3′, which anneals to nucleotides 4436–4453 in the *D. virilis* genomic sequence (GenBank accession no. M34544). The cDNA fragment was amplified by using the oligonucleotide above and the oligonucleotide 5′-GCTGTCGACGCCGCCATCGATGA-3′, which anneals to nucleotides 3559–3581 in the *D. virilis* genomic sequence (GenBank accession no. M34543). The cDNA product was cleaved with *Cla* I and *Sal* I.

The order of the $sev^{vir/mel}$ fusion is (*i*) *Aha* III–*Cla* I *D. virilis* genomic DNA including sequence 60 bp upstream of the putative initiator ATG; (*ii*) *Cla* I–*Sal* I *D. virilis* cDNA fragment (above); (*iii*) *Sal* I–*Hin*dIII *D. virilis* genomic fragment terminating within intron 7; (*iv*) *Eco*RI–*Cla* I *D. virilis* genomic fragment spanning exon 8, including the sequence encoding the transmembrane domain; (*v*) *Hin*dIII–*Eco*RI *D. melanogaster* genomic fragment encoding all required sequences 3′ to intron 8. The resulting chimeric protein fuses the N-terminal amino acids 1–2165 from the *D. virilis* sev protein extracellular and transmembrane domains to the C-terminal amino acids 2149–2552 from *D. melanogaster*, containing the cytoplasmic tail. This fusion gene was sub-

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Abbreviation: CAT, chloramphenicol acetyltransferase.

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L08132 (*D. virilis boss*) and L08133 (*D. melanogastor boss*).

cloned 3′ to the 800-bp *sev* promoter and 8-kb enhancer (24, 25) for transformation of sev^{d2};ry^{506} flies, as described (19).

Analysis of Rescue Constructs. Staining of eye imaginal discs with anti-sev monoclonal antibody CT(114) (26) and anti-boss polyclonal antiserum CT2 was done as described (11). The Rh4/CAT reporter construct (27) and chloramphenicol acetyltransferase (CAT) assay (28) were previously described, as was light-microscopy analysis of plastic sections from adult eyes (20, 29). Rescue levels for *D. melanogaster boss* rescue constructs were determined in flies carrying two copies of homozygous viable insertions or one copy of lethal insertions. CAT activity levels were each determined from 10 adult heads and averaged for at least two independent trials.

RESULTS AND DISCUSSION

The boss Proteins from *D. virilis* and *D. melanogaster* Are Highly Conserved. The *D. virilis boss* gene ($boss^{vir}$) was initially detected as a 9-kb *Sal* I fragment on a genomic DNA blot probed at reduced stringency with a *D. melanogaster boss* ($boss^{mel}$) cDNA clone which contains the entire open reading frame. The same conditions were used to identify and isolate the $boss^{vir}$ gene from a genomic library. The *Sal* I fragment was shown to contain the entire $boss^{vir}$ gene by genomic rescue of $boss^{1}$ mutant flies (see below) and was sequenced. The intron/exon boundaries were determined by comparison with *D. melanogaster boss* splice sites and by using splice-site consensus rules. Both $boss^{mel}$ and $boss^{vir}$ have six exons; the length of the exons is highly conserved, whereas variation in the size of introns was observed. A 2.7-kb *Hin*dIII–*Eco*RI fragment from the $boss^{vir}$ genomic clone contains the bulk of the open reading frame. This fragment hybridized to a single message of about 3 kb on an RNA blot of *D. virilis* head poly(A)$^+$ RNA (data not shown). This is similar in size to the $boss^{mel}$ mRNA.

The $boss^{vir}$ and $boss^{mel}$ proteins are 80% identical and 89% similar over their entire length (Fig. 1*A*). This is comparable to the degree of conservation of the engrailed and hunchback gene products (80% identical), and greater than that of other eye-specific gene products sequenced from *D. virilis*, including rough (60% identity) and *sev* (63% identity). There are two potential initiator ATG sequences only 9 bp apart in $boss^{mel}$. The $boss^{vir}$ sequence contains only one potential initiator ATG, corresponding to the second in-frame ATG of the $boss^{mel}$ gene. Should the second ATG codon be utilized, then the predicted translation products of $boss^{vir}$ and $boss^{mel}$ would be identical in length. Signal-peptide cleavage of $boss^{mel}$ and $boss^{vir}$ is proposed to occur at amino acid positions 29 and 30, respectively (Fig. 1*A*).

The deduced protein sequences of $boss^{mel}$ and $boss^{vir}$ were compared by using the Genetics Computer Group's LIMGAP program (see *Materials and Methods*). A graphical representation of the similarities between the two boss proteins (Fig. 1*B*) highlights the areas of greatest conservation. In addition to regions of identity in the extracellular domain, the trans-

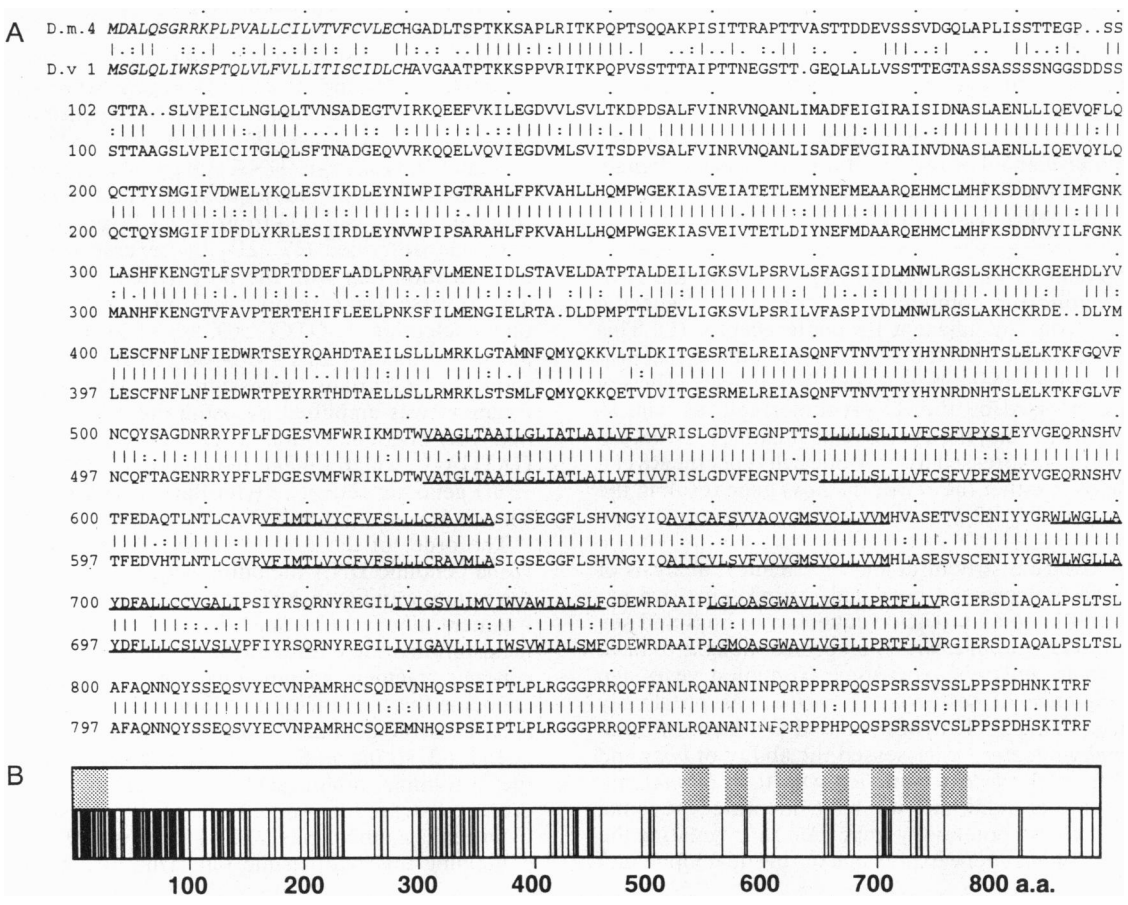

FIG. 1. Comparison of predicted boss proteins from *D. melanogaster* and *D. virilis*. (*A*) Sequence comparison. The predicted amino acid sequences of $boss^{mel}$ (D.m.) and $boss^{vir}$ (D.v.) were aligned by the Genetics Computer Group's LIMGAP program to maximize identity between the proteins and minimize gaps in alignment. Vertical lines between the sequences correspond to identities between the two proteins, colons represent similarities, and single dots represent less similar amino acids. A period in the protein sequence indicates a gap inserted in the sequence by the alignment program. Predicted signal peptides are italicized and transmembrane domains are underlined. (*B*) Schematic representation of divergence between $boss^{mel}$ and $boss^{vir}$ proteins. Differences in amino acids (a.a.) are indicated along the length of the protein by vertical lines in the lower bar. The upper bar represents the boss protein. The shaded regions indicate the signal peptide (far left) and the seven transmembrane domains (right half of the bar). Gaps in protein alignment listed in *A* are not shown. The N terminus is at the left end of the bar.

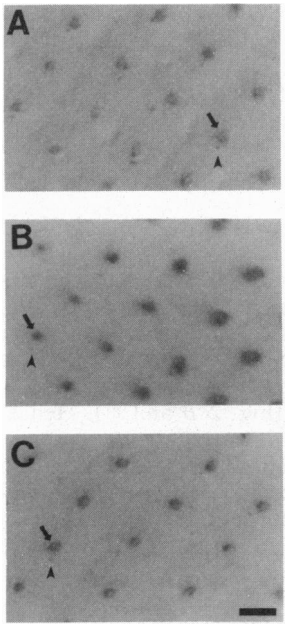

FIG. 2. The boss^{vir} protein is expressed in the R8 cell in *D. melanogaster*. (*A*) boss^{mel} protein in the *D. melanogaster* eye imaginal disc. (*B*) boss^{vir} in the *D. virilis* eye imaginal disc. (*C*) boss^{vir} expression in a *D. melanogaster* eye imaginal disc from a *boss^{1}* mutant carrying two copies of the *boss^{vir}* genomic rescue construct. The boss protein is expressed in R8 (arrow) and accumulates in a multivesicular body in R7 (arrowhead). (Bar = 6.4 μm.)

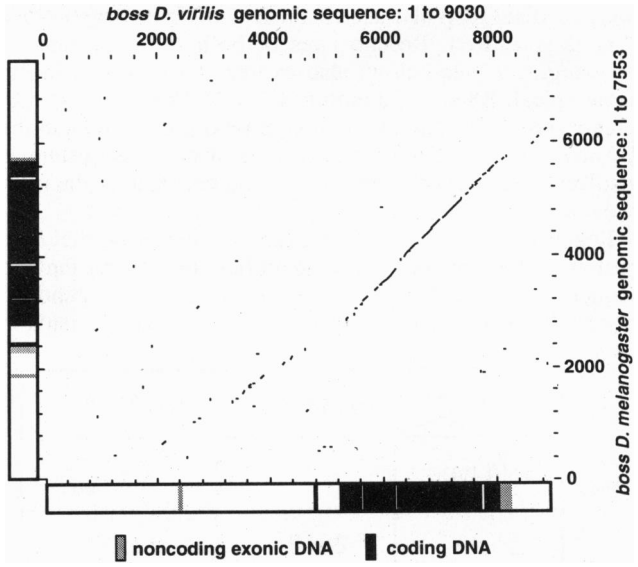

FIG. 3. Dot-matrix homology comparison of the *boss* genomic rescue constructs. The DNA sequences of the *boss* rescue constructs from *D. melanogaster* and *D. virilis* were compared by the Genetics Computer Group's COMPARE and DOTPLOT programs. Each dot represents 15 out of 20 identical nucleotides. Graphical representations of *boss^{vir}* and *boss^{mel}* genes are shown on the horizontal and vertical axes, respectively. Shaded regions correspond to exons, and black regions to protein-coding sequences.

membrane domains and cytoplasmic tail are particularly well conserved. The extracellular domain of the mature boss^{mel} protein is 73% identical and 93% similar to the corresponding domain of the boss^{vir} protein. Strikingly, the transmembrane domains and cytoplasmic tails of the two boss proteins are 91% identical and 99% similar. The highly conserved nature of the transmembrane domain suggests that it plays an important role in boss function.

Conservation of boss Expression and Putative Regulatory Elements. The apical, R8-specific expression pattern of the boss protein previously described in the *D. melanogaster* eye

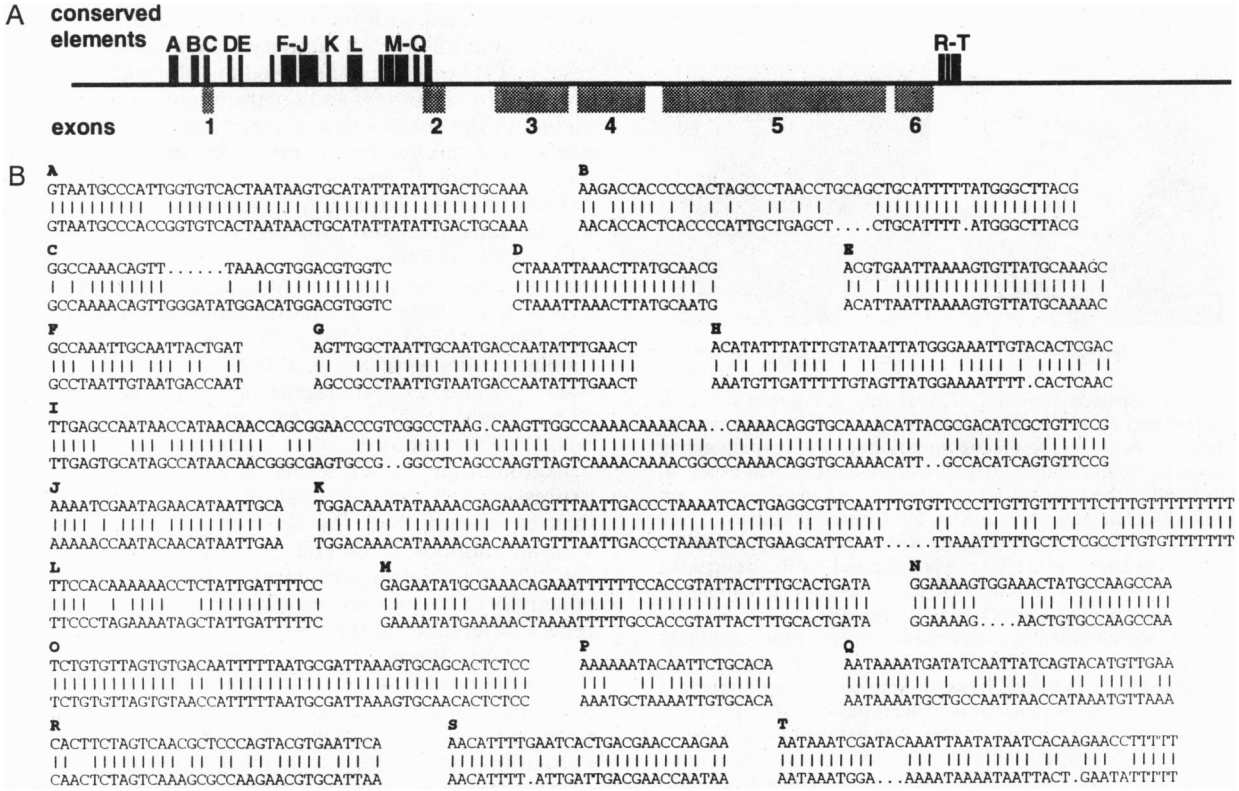

FIG. 4. Putative regulatory elements of the *boss* gene. (*A*) The conserved sequence elements within noncoding genomic segments are lettered and indicated as black rectangles above the line. The exons are numbered and indicated as gray boxes below the line. (*B*) Putative regulatory sequences were identified by their conservation in the *boss* rescue constructs, which exceeds 75% for 20 or more identical nucleotides with one or no gaps in alignment for each sequence. Only conserved elements whose linear order is conserved in the *boss* gene are listed. DNA sequences which are within another transcription unit mapping 5′ to *boss^{mel}* (9) are not included. Sequences were identified and aligned by using COMPARE, DOTPLOT, and GAP programs from the Genetics Computer Group.

imaginal disc (10) is also seen in the *D. virilis* eye imaginal disc (Fig. 2 *A* and *B*). Rescue lines of both *D. virilis* and *D. melanogaster* (see below) also express boss protein in the same apical, R8-specific pattern [Fig. 2*C* (boss^{vir}); data not shown (boss^{mel})]. The expression of boss protein seen in the *D. melanogaster* embryonic peripheral nervous system is also restored by both genomic rescue constructs (data not shown).

Regions of sequence conservation outside of the *boss* protein-coding regions were identified by comparing the sequences of the *D. melanogaster* and *D. virilis* genomic rescue constructs (Fig. 3). Conserved sequence elements in other genes have been shown to play a role in the regulation of gene expression (27). Putative regulatory elements in the two *boss* rescue constructs were arbitrarily defined as sequences which are >75% identical in the two species for ≥20 nucleotides (allowing for no more than 1 gap per sequence). These sequences are listed in Fig. 4. We propose a role for some of these conserved noncoding genomic sequences in the regulation of *boss* expression.

A

boss \ sev	wild type	sev$^{vir/mel}$
wild type	37±7	37±3
boss^{vir}	28±2	28±3

B

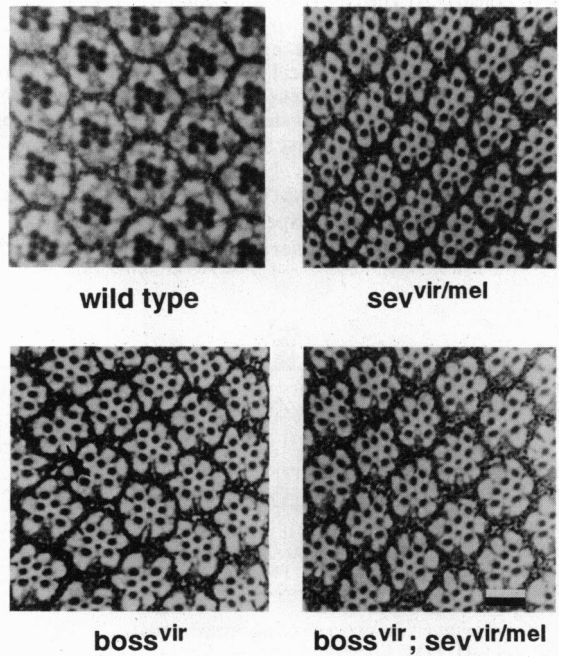

FIG. 5. Interspecies function of *boss* and *sev* genes from *D. melanogaster* and *D. virilis*. The ability of the *boss^{vir}* and *sev$^{vir/mel}$* transformation constructs to rescue mutations in the corresponding *D. melanogaster* genes and to interact with each other was assayed. (*A*) The R7 cell is the only source of Rhodopsin 4 (*Rh4*) expression in the adult (30). The activity of a *Rh4*/CAT reporter construct (see *Materials and Methods*) containing the *Rh4* promoter regulating the expression of the bacterial CAT gene is indicated for the designated genotypes. *sev$^{vir/mel}$* is a chimeric *sev* rescue construct containing the extracellular domain of *D. virilis* (see text). *boss^{vir}* is a *D. virilis boss* rescue line. R7 development was assessed in the appropriate mutant flies carrying one copy each of the *Rh4*/CAT reporter construct and the rescue constructs as listed. Flies designated *boss* wild type are *boss^{1}/boss^{+}*, and those designated *sev* wild type are *sev^{d2}/sev^{+}*. The ability of *sev$^{vir/mel}$* and *boss^{vir}* to interact was assayed in *sev^{d2};boss^{1}* flies. Similar results were obtained with one and two additional *sev$^{vir/mel}$* and *boss^{vir}* independent insertion lines, respectively. (*B*) Light microscopy of sections through adult eyes of the genotypes designated. The wild-type eye shown is *sev^{+}/sev^{+};boss^{+}/boss^{+}*. An ommatidium which is missing the R7 cell due to partial rescue by the *boss^{vir}* construct is shown in the *boss^{vir}* panel (arrowhead). Anterior is to the left in the wild-type and *boss^{vir}* panels and to the right for the panels showing sections of *sev$^{vir/mel}$* and *boss^{vir};sev$^{vir/mel}$*. (Bar = 2.5 μm.)

boss^{vir} Functions in *D. melanogaster*. *P*-element-mediated transformation with a 7.5-kb *D. melanogaster* genomic DNA fragment containing the *boss* gene rescues the *boss^{1}* phenotype. Similarly the 9-kb *Sal* I fragment containing *boss^{vir}* was shown to rescue *boss^{1}* (Fig. 5). As previously shown (10) for boss^{mel}, the boss^{vir} protein is transferred from R8 to a multivesicular body in R7 in both *D. virilis* and transgenic *D. melanogaster* expressing boss^{vir} (Fig. 2 *B* and *C*). Hence, boss^{vir} can successfully interact with sev^{mel}.

A Chimeric sev Protein Containing the Extracellular Domain from *D. virilis* and the Cytoplasmic Domain from *D. melanogaster* Interacts with boss^{vir} and boss^{mel}. The sev proteins from *D. melanogaster* (sev^{mel}) and *D. virilis* (sev^{vir}) share only 60% identity in their extracellular domains. To address the significance of these changes in the boss/sev interaction, the function of a chimeric sev protein containing the extracellular and transmembrane domains from *D. virilis* and the intracellular domain from *D. melanogaster* (sev$^{vir/mel}$) was compared with sev^{mel}. The *sev$^{vir/mel}$* construct was introduced by *P*-element-mediated DNA transformation into *D. melanogaster sev^{d2}* flies. Three independent lines were generated which rescued 100% of R7 cells as determined histologically (Fig. 5*B*). Additionally, as the R7 cell is the only adult source of Rhodopsin 4 (*Rh4*) gene expression (30), a reporter construct with the *Rh4* promoter driving the expression of the bacterial CAT gene (27) was also used to determine the amount of rescue obtained with the various *boss* and *sev* rescue constructs (Fig. 5*A*). CAT activity levels correlated with the rescue of R7 cell fate as assessed histologically. The relative ability of *D. virilis boss* and *sev* transgenes to interact with *D. melanogaster* genes was addressed by comparing the level of phenotypic rescue. The intraspecies and interspecies combinations showed similar levels of R7 rescue (Fig. 5).

To assess the relative ability of these proteins to interact in a more sensitive assay, the amount of R7 rescue obtained with the *sev* rescue constructs was compared by using a *D. melanogaster boss* rescue line, *boss^{melG69}*, in which the expression of boss^{mel} is limiting. One copy of *boss^{melG69}* in a *boss^{1}* background yielded a CAT activity level of 17 ± 4. Similar results were obtained in flies with one copy each of *boss^{melG69}* and *sev^{mel}* transgenes in a *sev^{d2}; boss^{1}* background (CAT levels of 24 ± 3). In contrast, one copy each of *boss^{melG69}* and the *sev$^{vir/mel}$* rescue construct in a *sev^{d2}; boss^{1}* background gave CAT levels of 0.8 ± 0.7. However, the expression from the *sev^{mel}* transgene was significantly greater than expression from the *sev$^{vir/mel}$* transgene, as assessed with an antibody to an epitope shared between sev^{mel} and sev$^{vir/mel}$ (data not shown). Hence, the difference in the level of activity of the two sev proteins with boss^{melG69} may simply reflect differences in the level of expression.

Concluding Remarks. The boss proteins from *D. virilis* and *D. melanogaster* share considerable homology. Not only does the strong conservation of the region containing the seven transmembrane domains suggest that it plays an important functional role, biochemical experiments using truncated forms of boss indicate that this domain is essential for boss function (31). Given the high degree of conservation between boss^{vir} and boss^{mel} proteins, it is not surprising that both interact with the sev^{mel} and sev$^{vir/mel}$ proteins. Evidence for interaction between the boss and sev proteins is also provided by the sev-dependent internalization of both boss^{vir} and boss^{mel} mediated by sev^{mel}. In addition, the extracellular

domains of sev^{vir} and sev^{mel}, though only 63% identical, function in a similar fashion with $boss^{vir}$ and $boss^{mel}$. Since we can only roughly quantify the relative levels of sev^{mel} and $sev^{vir/mel}$ expression in the developing eye disc, it is not possible to rigorously compare the relative efficiency of R7 induction in the interspecies combinations.

The expression of the $boss^{vir}$ gene in *D. melanogaster* is indistinguishable from the expression of $boss^{mel}$; it is expressed in the R8 cell in developing eye disc and in sensory structures in the embryo. DNA sequence analysis of $boss^{vir}$ and $boss^{mel}$ genes revealed 20 stretches of nucleotide sequence which share >75% identity over ≥20 nucleotides. We propose that some of these conserved elements regulate the spatial and temporal pattern of *boss* expression.

We thank the members of the Zipursky and Rubin laboratories for helpful discussions. This research was supported by the Howard Hughes Medical Institute, a United States Public Health Service National Research Service Award (A.C.H.), a European Molecular Biology Organization Long-Term Fellowship (S.D.H.), a McKnight Foundation Development Award (S.L.Z.), and a National Institutes of Health grant (S.L.Z.). S.L.Z. and G.M.R. are Investigators of the Howard Hughes Medical Institute.

1. Reinke, R. & Zipursky, S. L. (1988) *Cell* **55**, 321–330.
2. Hafen, E., Basler, K., Edstroem, J.-E. & Rubin, G. M. (1987) *Science* **236**, 55–63.
3. Simon, M. A., Bowtell, D. D. L. & Rubin, G. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8333–8337.
4. Basler, K. & Hafen, E. (1988) *Cell* **54**, 299–311.
5. Harris, W. A., Stark, W. S. & Walker, J. A. (1976) *J. Physiol. (London)* **256**, 415–439.
6. Campos-Ortega, J. A., Jürgens, G. & Hofbauer, A. (1979) *Roux's Arch. Dev. Biol.* **186**, 27–50.
7. Banerjee, U., Renfranz, P. J., Hinton, D. R., Rabin, B. A. & Benzer, S. (1987) *Cell* **51**, 151–158.
8. Tomlinson, A., Bowtell, D. D. L., Hafen, E. & Rubin, G. M. (1987) *Cell* **51**, 143–150.
9. Hart, A. C., Krämer, H., Van Vactor, D. L., Jr., Paidhungat, M. & Zipursky, S. L. (1990) *Genes Dev.* **4**, 1835–1847.
10. Krämer, H., Cagan, R. C. & Zipursky, S. L. (1991) *Nature (London)* **352**, 207–212.
11. Cagan, R. C., Krämer, H., Hart, A. C. & Zipursky, S. L. (1992) *Cell* **69**, 393–399.
12. Tomlinson, A. & Ready, D. F. (1986) *Science* **231**, 400–402.
13. Cagan, R. L., Thomas, B. & Zipursky, S. L. (1992) in *Molecular Basis of Morphogenesis*, 51st Annual Symposium of the Society for Developmental Biology, ed. Bernstein, M. (Wiley–Liss, New York), in press.
14. Beverly, S. M. & Wilson, A. C. (1984) *J. Mol. Evol.* **21**, 1–13.
15. Michael, W. M., Bowtell, D. D. L. & Rubin, G. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5351–5353.
16. Simon, M. A., Bowtell, D. D. I., Dodson, G. S., Laverty, T. R. & Rubin, G. M. (1991) *Cell* **67**, 701–716.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Plainview, NY).
18. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
19. Mismer, D. & Rubin, G. M. (1987) *Genetics* **116**, 565–578.
20. Van Vactor, D. L., Jr., Cagan, R. C., Krämer, H. & Zipursky, S. L. (1991) *Cell* **67**, 1145–1155.
21. Ashburner, M. (1992) *Drosophila: A Laboratory Manual* (Cold Spring Harbor Lab., Plainview, NY).
22. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Ehrlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
23. Orlandi, R., Güssow, D. T., Jones, P. T. & Winter, G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3833–3837.
24. Kimmel, B. E., Heberlein, U. & Rubin, G. M. (1990) *Genes Dev.* **4**, 712–727.
25. Bowtell, D. D. L., Lila, T., Michael, W. M., Hackett, D. & Rubin, G. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6853–6857.
26. Simon, M. A., Bowtell, D. D. L. & Rubin, G. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8333–8337.
27. Fortini, M. E. & Rubin, G. M. (1990) *Genes Dev.* **4**, 444–463.
28. Neumann, J. R., Morency, C. A. & Russian, K. O. (1987) *Biotechniques* **5**, 444–447.
29. Tomlinson, A. & Ready, D. (1987) *Dev. Biol.* **123**, 264–275.
30. Montell, C., Jones, K., Zuker, C. & Rubin, G. M. (1987) *J. Neurosci.* **7**, 1558–1566.
31. Hart, A. C., Krämer, H. & Zipursky, S. L. (1993) *Nature (London)* **361**, 732–735.