Abnormal Turning Behavior in Drosophila Larvae: Identification and Molecular Analysis of *scribbler* (*sbb*)

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ABSTRACT

Our genetic dissection of behavior has isolated *scribbler* (*sbb*), a vital gene that encodes a novel protein expressed in the embryonic and larval nervous systems and in the imaginal discs. Larvae with mutations in *sbb* exhibit abnormally high amounts of turning behavior in the absence of food. *sbb* is a large gene spanning >50 kb of genomic DNA with four major developmentally regulated transcripts. Transgenic rescue of scribbler behavior was demonstrated by targeting expression of a normal *sbb* transgene (sbb^+) expressing one of the major transcripts to the nervous system. The vital function of *sbb* was restored by ubiquitous expression of this transgene throughout development.

MANY organisms perform turning behaviors to fine-tune their spatial position in response to variability in their environment. Changes in turning behavior are performed in specific environmental contexts, for example, in order to reorient in the direction of a food source. Bacteria tumble while swimming (Amsler 1996), the worm *Caenorhabditis elegans* performs pirouettes (Pierce-Shimomura *et al.* 1999), hungry insects increase their turning rates in the presence of food (Dethier 1976; Bell 1990), fish and mammals increase their turning rates in a variety of environmental contexts including foraging, predation, and exploratory behavior (Bell 1991). The mechanisms underlying changes in turning behavior are not understood, although abnormal amounts of circling behavior in rats appear to be mediated by changes in the dopaminergic system (Miwa *et al.* 1998; Hebb and Robertson 1999; Kaufman *et al.* 1999; Richter *et al.* 1999).

One proven method for identifying the mechanisms that underlie behavior is genetic dissection. This involves the mutagenesis and screening of animals for behavioral alterations. Genetic dissection proceeds without any assumptions as to the molecular bases of the behavior of interest. Consequently, novel genes and proteins that would otherwise remain undetected can be discovered using this method. These novel genes pave the road toward an understanding of the multiple pathways involved in generating behavioral phenotypes. The genetic dissection strategy, as used in model organisms such as *Drosophila melanogaster* or *C. elegans*, provides us with potential candidate genes to explore the functions of homologous genes in mammalian systems. For example, the circadian rhythm genes *period* (*per*) and *timeless* (*tim*), whose cyclic transcription forms the basis for the circadian pacemakers in all cells, were discovered in Drosophila by a genetic dissection of circadian behaviors. The function of the *per* and *tim* mammalian homologues parallels that initially unraveled in flies (see Hall 1998 for review).

Behavior-genetic analysis in Drosophila has focused on adult behaviors such as rhythms (Hall 1998), learning (Davis 1996; Dubnau and Tully 1998), olfaction (Carlson 1996), courtship (Hall 1994; Yamamoto *et al.* 1997), foraging (Pereira and Sokolowski 1993), locomotion (Strauss and Heisenberg 1993), visually guided behaviors (Heisenberg 1997), and hearing (Eberl 1999). Mutations that alter larval behavior have also been identified including *uncoordinated* (Kernan *et al.* 1994), *calmodulin* (Heiman *et al.* 1996), *yellow* (Inestrosa *et al.* 1996), *Chaser* (Pereira *et al.* 1995), *no-bridge* and *ellipsoid-body* (Varnam *et al.* 1996), *tamas* (Iyengar *et al.* 1999), and *foraging* with its naturally occurring rover (for^R) and sitter (for^s) alleles (Sokolowski 1980; Osborne *et al.* 1997; Sokolowski *et al.* 1997). Together, the molecular and neurogenetic analyses of these genes have enabled us to begin to explore the numerous and overlapping signal transduction pathways that underlie behavioral phenotypes [for example, cAMP signaling (Davis 1996; Dubnau and Tully 1998), camkinase (Griffith *et al.* 1993), and protein kinase (Kane *et al.* 1997) in learning; cGMP signaling in foraging (Osborne *et al.* 1997; Sokolowski and Riedl 1999); and IP3 signaling in olfaction and vision (Carlson 1996)].

Here we report the identification, cloning, and expression of a new vital gene called *scribbler* (*sbb*). Normal larvae exhibit straight-line movement in the absence of food (on agar). In contrast, *sbb* larvae exhibit high amounts of turning on agar such that their locomotion

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trails are indecipherable. In other words, they leave a "scribble" on the agar surface. The expression of scribbler behavior is conditional on the absence of food in the environment. Thus the scribbler larva leaves a unique signature on agar but not on yeast surfaces. We suggest that the *sbb* gene may play a role in food search behavior.

MATERIALS AND METHODS

Strains: Twenty-five pupal lethal strains from the Karpen and Spradling (1992) collection were identified from 176 second chromosome embryonic lethal strains generated by *P*-element insertional mutagenesis. These recessive lethal mutations were maintained as balanced heterozygotes with *In*(2LR), *Cy dp*^{lvl} *pr cn*^{2}, hereafter referred to as *CyO*. The standard *D. melanogaster* rover (*for*^{R}) and sitter (*for*^{s}) strains (Osborne *et al.* 1997) were used in behavioral and Northern analyses along with the wild-type Canton-S (CS) laboratory strain. The scribbler alleles *sbb*$^{l(2)04440}$ and *sbb*$^{l(2)k00702}$, the deficiency *Df(2L)Pc4/Cy0* that uncovered scribbler behavior and lethality and the Δ*2-3* transposase source strain *Sp/CyO;ry*^{506}*,Sb,[*Δ*2-3]/TM6* (Robertson *et al.* 1988) were obtained from the Bloomington Stock Center. The scribbler alleles *sbb*$^{EP(2)0328}$ [an EP line (Rorth 1996)] and *sbb*$^{l(2)03432}$ were obtained from the Berkeley Drosophila Genome Project (BDGP). The white-eyed strain *w*^{1} (described in Osborne *et al.* 1997) was used as a host for transformation of the transgene. The double balancer strain *w;Sp/Cy0;Ly/TM3* was used to find the chromosomal location of each transgene and to cross all transgenes into each of two *sbb* mutant backgrounds: *w*^{1}*;sbb*$^{l(2)03432}$*;ry*^{506} and *w*^{1}*;sbb*$^{EP(2)0328}$;ry^{506}. The C155 *GAL4* strain with a *GAL4* insertion in the *elav* gene (Lin and Goodman 1994) and a *hs-GAL4* strain were used to drive the UAS-*sbb* transgenes in larvae. Flies were housed in plastic bottles containing 45 ml of standard fly medium and were kept at 25 ± 1° on a 12L:12D light cycle with lights on at 0800 hours (standard conditions).

Larval behavior: Larval locomotion was tested by placing individual third instar larvae in the center of petri dishes (8.5 cm diameter, 1.4 cm height) coated with 10 ml of hardened nonnutritive 3.2% agar (Pereira *et al.* 1995; Shaver *et al.* 2000). On average, 25 larvae were tested per strain. Dishes were covered with petri dish lids and the trail traversed by each larva in a 5-min test interval was drawn on the lid. The number of grid squares entered or the foraging trail length traversed by the larva was analyzed using one-way analyses of variance (ANOVA). The Student-Neuman-Keuls test (SNK; $P = 0.05$) was performed as an *a posteriori* test (Zar 1984) to determine which strains differed significantly. After the behavior test each larva was placed in a small tube with 1 ml of food to undergo further development. Homozygous *sbb* mutant larvae were either those that did not survive beyond the pupal stage or those that emerged as straight-winged escapers. A similar procedure was used to test larval foraging behavior except that the larva was placed on a thin layer of yeast and water paste (2:1 yeast to water) described in Pereira *et al.* (1995).

***P*-element excision procedure:** The *sbb*$^{l(2)03432}$ mutant carried a *P{PZ} P*-element insert. To verify that this insert tagged *sbb* behavior, the *P* element was excised using standard crosses to the Δ*2-3* transposase source (Robertson *et al.* 1988). Briefly, *sbb/CyO* virgin females were mated to males with the Δ*2-3* source of transposase (*Sp/CyO; ry*^{506}*Sb[*Δ*2-3]/TM6*). Male progeny with both the *P*-element insert and the Δ*2-3* element were chosen and mated individually to *Sp/CyO;ry*^{506} virgin females. Revertants were recovered from the progeny of this cross and were identified as *ry*^{506} male progeny. These *ry*^{506} *Sb*$^{+}$ males were selected and mated to females of the *Sp/CyO;ry*^{506} balancer stock. The resulting *CyO;ry*^{506} progeny were collected and allowed to mate *en masse*. Cultures in which only *CyO* progeny emerged were kept as second chromosome lethal excision lines. Stocks with *Cy*$^{+}$*;ry*^{506} progeny were viable excision lines.

***P*-element plasmid rescue and restriction mapping of the *sbb* microregion:** Genomic DNA was isolated from *sbb*$^{l(2)03432}$ and *sbb*$^{l(2)k00702}$ larvae using standard techniques (Sambrook *et al.* 1989). DNA was digested into fragments by *Sca*I and *Eco*RI, respectively. DNA was ligated by T4 DNA ligase (New England Biolabs, Beverly, MA) and transformed into *Escherichia coli* Dh5α or XL1-blue cells. A 3.2-kb fragment (solid bar in Figure 3) was rescued from *sbb*$^{l(2)03432}$ and a 200-bp fragment of genomic DNA flanking the *P* element was rescued from *sbb*$^{l(2)k00702}$. Part of the 3.2-kb rescue product [1.2-kb *Eco*RI DNA fragment (shaded box in Figure 3) adjacent to the *sbb*$^{l(2)03432}$ *P* element] was radiolabeled with [α-^{32}P]dATP by random priming and used to screen 6×10^{6} plaques from a Drosophila CS genomic bacteriophage λDASH II library. A total of four genomic clones were recovered; restriction mapping by *Eco*RI, *Sal*I, *Hin*dIII, *Eco*RI + *Sal*I, *Eco*RI + *Hin*dIII, and *Sal*I + *Hin*dIII indicated that they covered roughly a 20-kb region of genomic DNA.

3′ RACE and 5′ RACE: Poly(A)$^{+}$ RNA was isolated from CS larvae by using TRIZOL reagent (GIBCO BRL, Gaithersburg, MD) and the PolyAT tract mRNA isolation system (Promega, Madison, WI). An adapter primer containing poly(dT) (5′-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTT TTT-3′) from the 3′ RACE system for rapid amplification of cDNA ends (GIBCO BRL) was used for cDNA synthesis by SUPERSCRIPT II RT (GIBCO BRL). cDNA was used as a template for a reverse transcription (RT)-PCR amplification by using gene-specific primer 1 (5′-TTGCTGTTGCTTTCGTT TTG-3′) and primer 2 (5′-ATTCCTTTGCTTCCTGGCTT-3′) to confirm the existence of the >35-kb intron in *sbb* (Figure 3). The 3′ RACE was performed by using the same single strand cDNA template as the RT-PCR described above. The primers used for the 3′ RACE were gene-specific primer 2 and abridged universal amplification primer (AUAP; 5′-GGCCAC GCGACTAGTAC-3′; GIBCO BRL). 5′ RACE was performed according to the manufacturer's instructions (GIBCO BRL). The first and second gene-specific primers for the 5′ RACE were 5′-AAGCCAGGAAGCAAAGGAAT-3′ (Figure 3, primer 3) and 5′-TTTGAGTAATGCGAGGGGAGG-3′ (Figure 3, primer 4).

Northern analysis: Total RNA was isolated from third instar larvae, pupae, adult body, and adult head by TRIZOL reagent (GIBCO BRL). One milligram of total RNA was used to isolate the mRNA by the PolyAT tract mRNA isolation system (Promega, Madison, WI). About 5 μg poly(A)$^{+}$ RNA from each sample was loaded on a 1% formaldehyde agarose gel and run in 1× 3-(*N*-morpholino) propane sulfonic acid (MOPS) plus 4% formaldehyde running buffer. The poly(A)$^{+}$ RNA was transferred to Zeta-GT membrane (Bio-Rad, Richmond, CA) in 20× SSC and probed with [α-P^{32}]dATP random labeled 3.0-kb LD13770 [or 0.7-kb Drosophila ribosomal protein 49 cDNA (*rp49*) used as a loading control (O'Connell and Rosbash 1984)] in 50% formamide, 7% SDS, and 0.25 mm $NaPO_4$ (PH, 7.2)at 43° overnight according to manufacturer's instructions (Bio-Rad). Band intensities were determined on a phosphoimager and were normalized within each lane using the *rp49* control.

RNA *in situ* hybridization: Embryos were collected over a 24-hr period and probed with digoxigenin UTP (Boehringer Mannheim, Indianapolis) labeled single strand antisense and sense RNA from LD13770 (*sbb*-cDNA LD13770). The antisense

RNA was used as a positive probe and sense RNA was used as a control probe. Both the antisense and sense RNA were produced by *in vitro* transcription using T7 and T3 RNA polymerases (Promega), respectively, according to manufacturer's instructions. The *in situ* procedure was based on the protocol developed by Tautz and Pfeifle (1989), with a few modifications: the hybridization buffer was 50% formamide, 4× SSC, 1× Denhardt's, 0.27 mg/ml salmon sperm DNA, 7% SDS, and 0.1 % Tween. Larvae were treated the same way as embryos except that they were dissected in Rnase-free 1× PBS and were treated by proteinase K for 5 min at room temperature before hybridization with the probe.

Transgene construction: Restriction and sequence analysis showed that the EST clone LD13770 contained an ~3.0-kb insert. This 3.0-kb insert was cloned into the transformation vector *P{UAST}* in two steps. The insert was released from pBluescript SK (Stratagene, La Jolla, CA) by *Eco*RI and *Kpn*I, which gave a 2.1-kb *Eco*RI fragment and a 0.9-kb *Eco*RI + *Kpn*I fragment. This 0.9-kb fragment was directionally cloned into the *P{UAST}* vector, which was cut by *Eco*RI and *Kpn*I. The product of the *P{UAST}* plus the 0.9-kb insert was digested by *Eco*RI and then ligated with the 2.1-kb *Eco*RI LD13770 fragment. The clones with correct cloning orientation were identified by using *Xho*I and *Not*I double digestions; the released fragment sizes were 1.96 kb (correct orientation) and 0.74 kb (wrong orientation). This transgene was designated UAS-*sbb*. The same cloning approach was used to clone the LD13770 insert that was released from the *P{UAST}* + LD13770 insert into *P{CaSpeR-hs}* by using *Eco*RI and *Xba*I polylinker sites. This transgene construct was called *hs-sbb*. Both *P{UAST}* and *P{CaSpeR-hs}* have a mini w^+ gene as a selectable marker. The *P{CaSpeR-hs}* has a hsp70 promoter, which allows both heat-induced expression and often leaky expression of the inserted open reading frame (ORF; Pirrotta 1988). P1 clones were from BDGP and EST clones were from Research Genetics (Huntsville, AL).

Fly transformation, crossing, and heat shock: *P*-element-mediated germ-line transformation was done as described by Rubin and Spradling (1982). Embryos from the w^1 flies were collected and injected with *P*-element expression constructs at 0.6 μg/μl with helper plasmid pπ25.7wcΔ*2-3* at 0.2 μg/μl (Karess and Rubin 1984). G_0 injected flies were mated individually to w^1 flies. G_1 progeny were screened for the mini w^+ marker. Flies with a transgenic insert were crossed singly to w^1 flies to establish a strain. The locations of the inserts were mapped by crossing flies to the *w;Sp/Cy0;Ly/TM3* strain. Only homozygous viable transgenic inserts on the *X* and third chromosomes were crossed into the $sbb^{l(2)03432}$ or $sbb^{EP(2)0328}$ mutant background, which carried $w^1;ry^{506}$ mutations. For behavior experiments the UAS-*sbb* strain was crossed to the C155 *GAL4* strain, which had a *GAL4* insert in the *elav* gene (Lin and Goodman 1994). This allowed us to target expression of *sbb* to all presynaptic neurons in the larva. We also crossed UAS-*sbb* to a *hs-GAL4* for Northern analysis. This resulted in ubiquitous expression of *sbb* enabling us to detect on Northerns whether or not the UAS-*sbb* transgene was functional in larvae. Heat-shocked larvae used for Northern analysis were raised at 25° and transferred with their food to a 37° incubator for 45 min prior to RNA isolation.

RESULTS

Behavior-genetic analysis of *scribbler*: The scribbler mutant was found by screening the Karpen and Spradling (1992) collection of *P*-element-tagged second chromosome pupal lethal lines for abnormal foraging and/or locomotion behavior (Shaver *et al.* 2000). One line (*l(2)03432*) carried a mutation that caused an unusual larval behavioral phenotype on nonnutritive agar. The scribbler larval trail covered a small area and looked as if the larva had scribbled on the agar surface (Figure 1, A and B). This was in direct contrast to the relatively straight trails of wild-type larvae (Figure 1D). We quantified scribbler behavior in two ways. First, we calculated the percentage of larvae in a line that exhibited scribbler behavior. Second, we counted the number of 6-mm grid squares entered by each larva during its 5-min test on the agar substrate. Scribbler behavior was 64% penetrant in homozygous $sbb^{l(2)03432}$ larvae. These larvae also entered significantly fewer grid squares than did their heterozygous sibs (*sbb/CyO*; Figure 1C) or larvae of the wild-type for^R (rover) control strain (Figure 1D). Scribbler behavior was exhibited only when $sbb^{l(2)03432}$ mutant larvae were traveling on a nonnutritive substrate (agar) and not when $sbb^{l(2)03432}$ mutant larvae were foraging on a yeast and water paste. Thus, the expression of scribbler turning behavior was conditional on the environment, specifically on the absence of food. The present study investigates the molecular basis of scribbler turning behavior on agar.

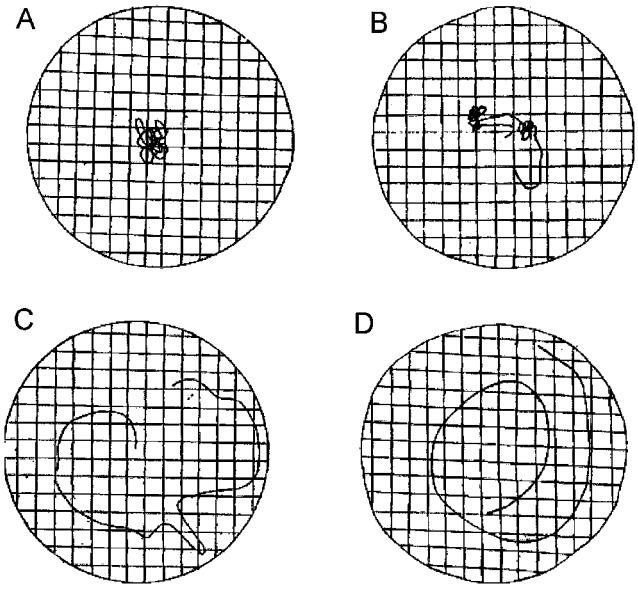

Figure 1.—Homozygous $sbb^{l(2)03432}$ scribbler larvae (A and B) exhibit significantly more turning behavior on agar than both heterozygous (C) $sbb^{l(2)03432}/CyO$ and wild-type (D) larvae, which do not differ from each other. Trails were superimposed on a 6-mm grid and the number of squares a larva entered in the 5-min test was counted (see below).

The *P*-element insertion in $sbb^{l(2)03432}$ was localized to the region 55C1-2 on the Drosophila polytene chromosome (BDGP). We co-localized scribbler behavior to this region using complementation analysis with the deficiency *Df(2R)Pc4/CyO* whose breakpoints are 55A-55F. Sixty percent of scribbler/*Df(2R)Pc4* larvae (n = 67) exhibited scribbler behavior. None of the *sbb/CyO*

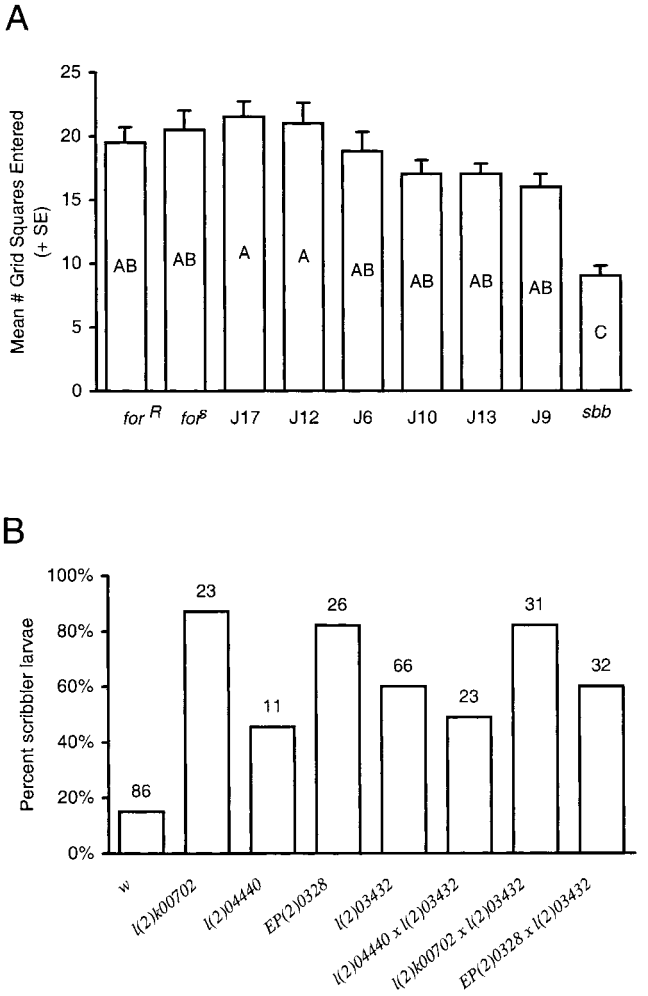

Figure 2.—(A) *sbbl*$^{(2)03432}$ *P*-element excision lines (J17, J12, J6, J10, J13, and J9) exhibit wild-type larval locomotor behavior. The behavior of the *for*^{R} and *for*^{s} wild-type control strains does not differ on agar. Larvae of all viable excision lines tested entered a significantly greater number of 6-mm grid squares than did *sbbl*$^{(2)03432}$ homozygotes (one-way ANOVA, $F(8,228) = 12.99$, $P < 0.001$). Means are presented + one standard error (SE). Sample sizes were $30 < n < 50$. Different letters represent different statistical groupings (SNK, $P = 0.05$). (B) Complementation tests of *sbb* alleles. The percentage of *sbb* larvae from the control strain (*w*^{1}), the original *sbb* strain (*sbb*$^{l(2)03432}$), three other *sbb* alleles (*sbb*$^{l(2)04440}$, *sbb*$^{l(2)k00702}$, and *sbb*$^{EP(2)0328}$), and their crossed progeny are shown. Crosses show the lack of complementation for behavior between the four different *sbb* *P*-element alleles. Sample size is shown above each histogram.

($n = 53$) or *Df(2R)Pc4/CyO* ($n = 47$) larvae exhibited this phenotype. The control strains *for*^{R} and *for*^{s} did not exhibit scribbler behavior in our mutagenesis and behavioral screen. However, two other control strains used later in this study for complementation and rescue experiments, *w*^{1} and *ry*^{506}, did exhibit a low percentage of scribbler behavior (15 and 7%, respectively).

P-element excision demonstrated that the *P*-element insertion in *sbb*$^{l(2)03432}$ was responsible for scribbler behavior and pupal lethality. Twenty-four excision lines yielded viable fertile progeny while one (*sbb*^{l2}) caused homozygous lethality during the embryonic or early (first or second instar) larval stage. Normal larval locomotion was restored in the six viable *sbb* excision lines (J6, J9, J10, J12, J13, J17) that were tested on agar (Figure 2A). The *sbb*$^{l(2)03432}$ mutation resulted in late-stage pupal lethality with 10% of the pupae emerging from the pupal case but dying within 3 days of emergence. These adult escapers lacked part of a wing vein at the end of L5.

The pupal lethal phenotypes of *sbb*$^{l(2)03432}$, *sbb*$^{l(2)04440}$, and *sbb*$^{l(2)k00702}$ did not complement, confirming the lethal complementation analysis reported by BDGP. Tests of larval locomotion showed that (1) larvae of all strains (*sbb*$^{1(2)04440}$, *sbb*$^{1(2)k00702}$, and *sbb*$^{EP(2)0328}$) exhibited scribbler behavior and (2) the scribbler behavior of larvae with the original *sbb*$^{l(2)03432}$ allele did not complement with the scribbler behavior of larvae with the other three *sbb* alleles (*sbb*$^{l(2)04440}$, *sbb*$^{l(2)k00702}$, and *sbb*$^{EP(2)0328}$; Figure 2B). These genetic data and the lack of complementation of all the pleiotropic phenotypes associated with the original *sbb*$^{l(2)03432}$ allele strongly suggested that the *P*-element insertions in these four independently generated mutant strains disrupted the same gene. This was confirmed by our molecular analysis of the *sbb* microregion described below.

Genomic organization of the *sbb* microregion and localization of *P*-element insertions: Figure 3 shows a map of the *scribbler* microregion. Our analysis of the region shows that *sbb* spans >50 kb of genomic DNA. The scribbler mutant *sbb*$^{l(2)03432}$ was generated using a *P{PZ}* transposable element. Thus we were able to clone the DNA sequence that flanked the transposon insertion using plasmid rescue (Karpen and Spradling 1992). A 3.2-kb rescue fragment (solid bar in Figure 3) was used to identify a group of overlapping λ genomic clones (λ dash) that covered an ~20-kb region around the *sbb*$^{l(2)03432}$ insert. The *Eco*RI resriction map of the region is shown in Figure 3. We found three overlapping P1 clones (DS01974, DS02561, and DS08374) from this region 55C (BDGP). The insertion sites of the *sbb* mutant (*sbb*$^{l(2)04440}$, *sbb*$^{l(2)04525}$, *sbb*$^{EP(2)0328}$, and *sbb*$^{l(2)k00702}$) alleles are shown (Figure 3). *sbb*$^{EP(2)0328}$ is a homozygous viable strain with a *P*-element insertion about 120 bp away from *sbb*$^{l(2)03432}$ (BDGP). Sequence analysis of a plasmid rescue product from *sbb*$^{l(2)k00702}$ showed that it was 1.24 kb away from *sbb*$^{l(2)03432}$. The insertions in the *sbb*$^{l(2)04440}$ and *sbb*$^{l(2)04525}$ were located more than 40 kb away from *sbb*$^{l(2)03432}$. *sbb*$^{l(2)04525}$ was inserted in the ORF; unfortunately, this mutant is no longer available. All other *sbb* alleles were found in the 5′ end of the gene. A 1.2-kb fragment (shaded box in Figure 3) plasmid rescue product from *sbb*$^{l(2)03432}$ mapped to P1 clone DS01974. Southern blot analysis showed that DS01974 overlapped with DS02561 (data not shown) and DS02561 overlapped with DS08374 (BDGP). Twelve incomplete cDNA clones were identified from larval, pupal, and

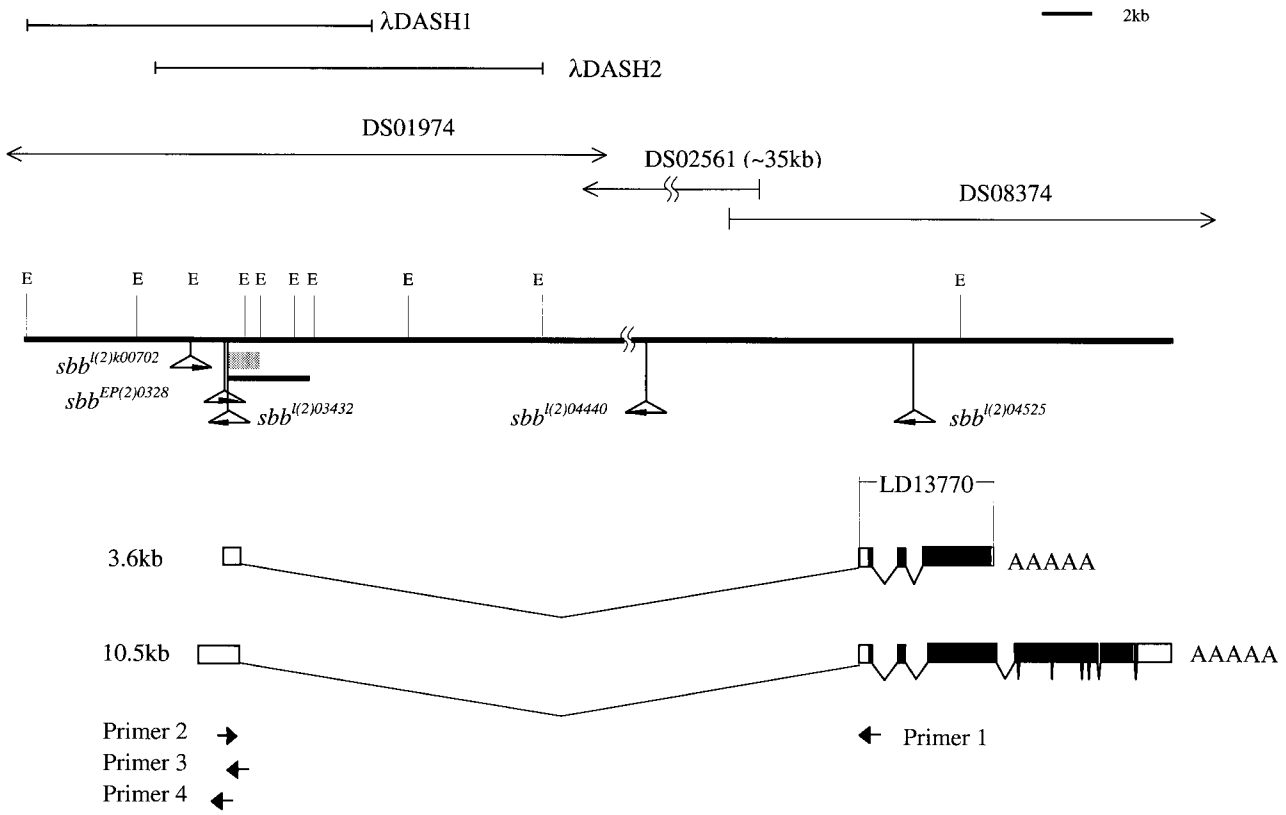

Figure 3.—The genomic organization of the *sbb* microregion. Genomic clones (λ dash) and P1 clones (designated DS) spanning the *sbb* microregion are shown at the top of this figure; 5′ is on the left and 3′ on the right. The *Eco*RI restriction map and the insertion sites of the *P* elements are shown in the middle of the figure. A break (∫∫) in the line shows genomic DNA that is not to scale. Triangles show the *P*-element insertion sites. $sbb^{l(2)03432}$ is the original *sbb P*-element insert strain isolated in our behavior screen and it carries a [*P{PZ}*] insert. $sbb^{l(2)04440}$ (*P{PZ}* insert) and $sbb^{l(2)k00702}$ (*P{lacW}* insert) do not complement for lethality and scribbler behavior. [The behavior of $sbb^{l(2)04525}$ (*P{PZ}* insert) larvae could not be tested because this strain was discarded by BDGP.] $sbb^{EP(2)0328}$ (with an EP insert) is a viable insert strain that exhibits *sbb* behavior. The 3.2-kb plasmid rescue product (from $sbb^{l(2)03432}$) is shown as a solid bar. The 1.2-kb probe used to screen genomic and cDNA libraries is represented by the shaded box. Exons are indicated as boxes, and the open boxes show the predicted noncoding regions; introns are shown as lines connecting the boxes. The full cDNA clones for the two *sbb* isoforms (3.6 and 10.5 kb) are shown near the bottom of the figure (for further details see text). The 3-kb LD13770 EST clone (also called scribbler cDNA LD13770 in the text) is shown near the bottom right and it contains the complete ORF of the 3.6-kb transcript. This clone was used in the construction of the *hs-sbb* and UAS-*sbb* transgenes. The 10.5-kb sequence that came from a combination of two EST clones (LD13770 + SD01229) is shown near the bottom of the figure. The 3′ and 5′ RACE products were achieved by using gene-specific primer 2 and primers 3 and 4, respectively (see materials and methods for primer sequences). RT-PCR, using primers 1 and 2, was used to confirm the existence of a >35-kb intron in this gene.

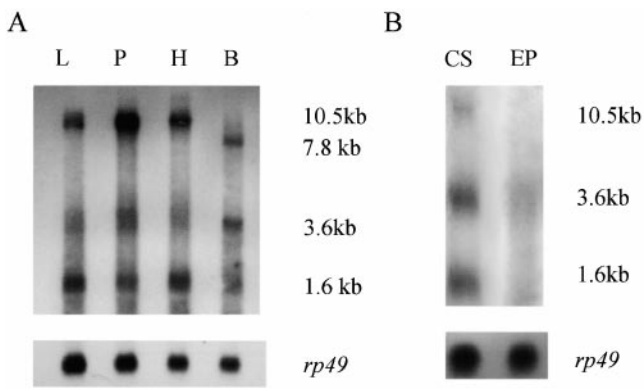

Figure 4.—(A) A developmental Northern blot was done by probing with *sbb*-cDNA LD13770 wild-type (for^R) poly(A)$^+$ RNA. The poly (A)$^+$ RNA was isolated from third instar larvae (L), pupae (P), adult heads (H), and adult bodies (B). The 10.5-kb transcript was more strongly expressed in the pupa than in the larva and adult head and was absent in the adult body. In all the stages, 3.6- and 1.6-kb transcripts were expressed. A 7.8-kb transcript was detected only in body. All lanes contain 5 μg poly(A)$^+$ RNA. This blot was reprobed with ribosomal protein 49 (*rp49*) to quantitate relative amounts of RNA loaded into each lane. Numbers on the right indicate RNA size in kilobases. (B) RNA abundance of wild-type (CS) and mutant $sbb^{EP(2)0328}$ larvae was measured by probing poly(A)$^+$ RNA with *sbb*-cDNA LD13770. The 10.5-, 3.6-, and 1.6-kb transcripts were reduced or hardly detected in the $sbb^{EP(2)0328}$ larvae (EP). All lanes contain 5 μg poly(A)$^+$ RNA. *rp49* was used as a control. Numbers on the right indicate RNA size in kilobases.

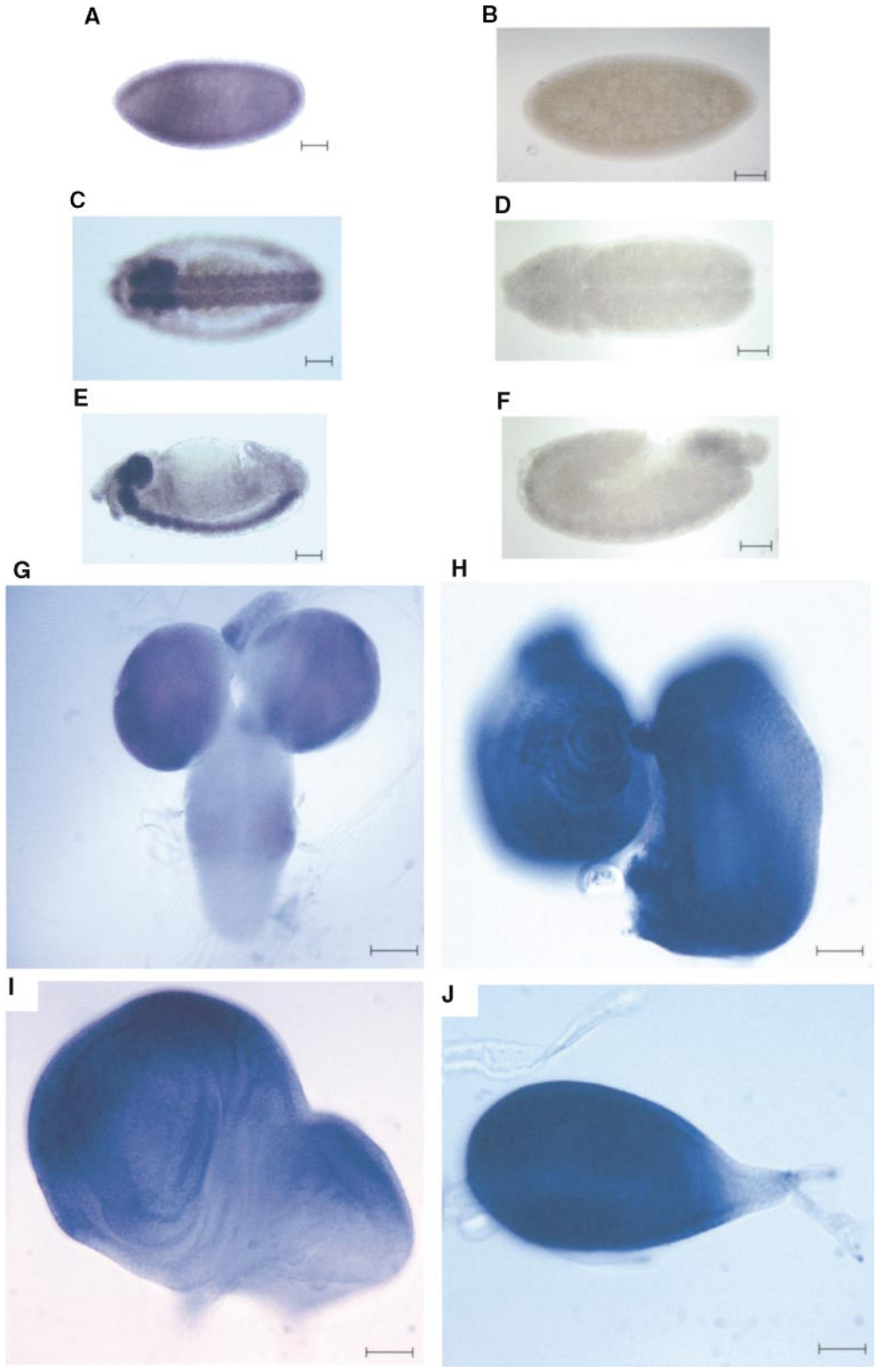

Figure 5.—The localization of *sbb* transcript by whole mount *in situ* hybridization using digoxigenin labeling. Wild-type embryos at stage 5 (A) and 13 (C and E) were probed by antisense *sbb*-RNA LD13770. *sbb* RNA was expressed in the brain and ventral ganglion. No signal was detected when wild-type embryos, stage 5 (B) and 13 (D and F) were probed with the sense *sbb*-RNA LD13770 control. The wild-type larval CNS was probed with antisense *sbb*-RNA LD13770, which detected signal in nervous system including the brain, optic lobe, the ring gland, and the medial region of ventral ganglion (G). Antisense *sbb*-RNA LD13770 detected *sbb* RNA in wild-type eye and antennal discs (H), in the posterior and ventral region of wing discs (I), and in leg discs (J). Heterozogous embryos of $sbb^{l(2)04440}$ and $sbb^{l(2)03432}$ at stage 14 and 15 probed with antisense *sbb*-RNA LD13770 showed normal expression (K and M). Homozogous *sbb* embryos from $sbb^{l(2)04440}$, $sbb^{l(2)03432}$, and $sbb^{EP(2)0328}$ probed with antisense *sbb*-RNA LD13770 showed a reduction in abundance in both $sbb^{l(2)04440}$ (L) and $sbb^{l(2)03432}$ (N) embryos. *sbb* RNA was found in $sbb^{EP(2)0328}$ embryos; however, the signal was reduced in the ventral cord (O). $sbb^{EP(2)0328}$ mutant larvae showed a reduction in *sbb* RNA in the brain and the medial region of the ventral ganglion (P). Bars, 100 μm.

ovary cDNA libraries using the 1.2-kb *Eco*RI fragment (shaded box) as a probe to screen larval, pupal, adult, and ovary cDNA libraries (Sambrook *et al.* 1989). Eight of these cDNA clones overlapped with a 5′ expressed sequence tag (EST) clone (LD13770) located more than 45 kb away from the $sbb^{l(2)03432}$ *P*-element insertion site (Figure 3). RT-PCR using primers 1 and 2 (Figure 3) confirmed the existence of >35 kb of intron(s) in this gene. The full cDNA clones for the two *sbb* isoforms (3.6 and 10.5 kb) are shown (Figure 3). The 3.6-kb tran-

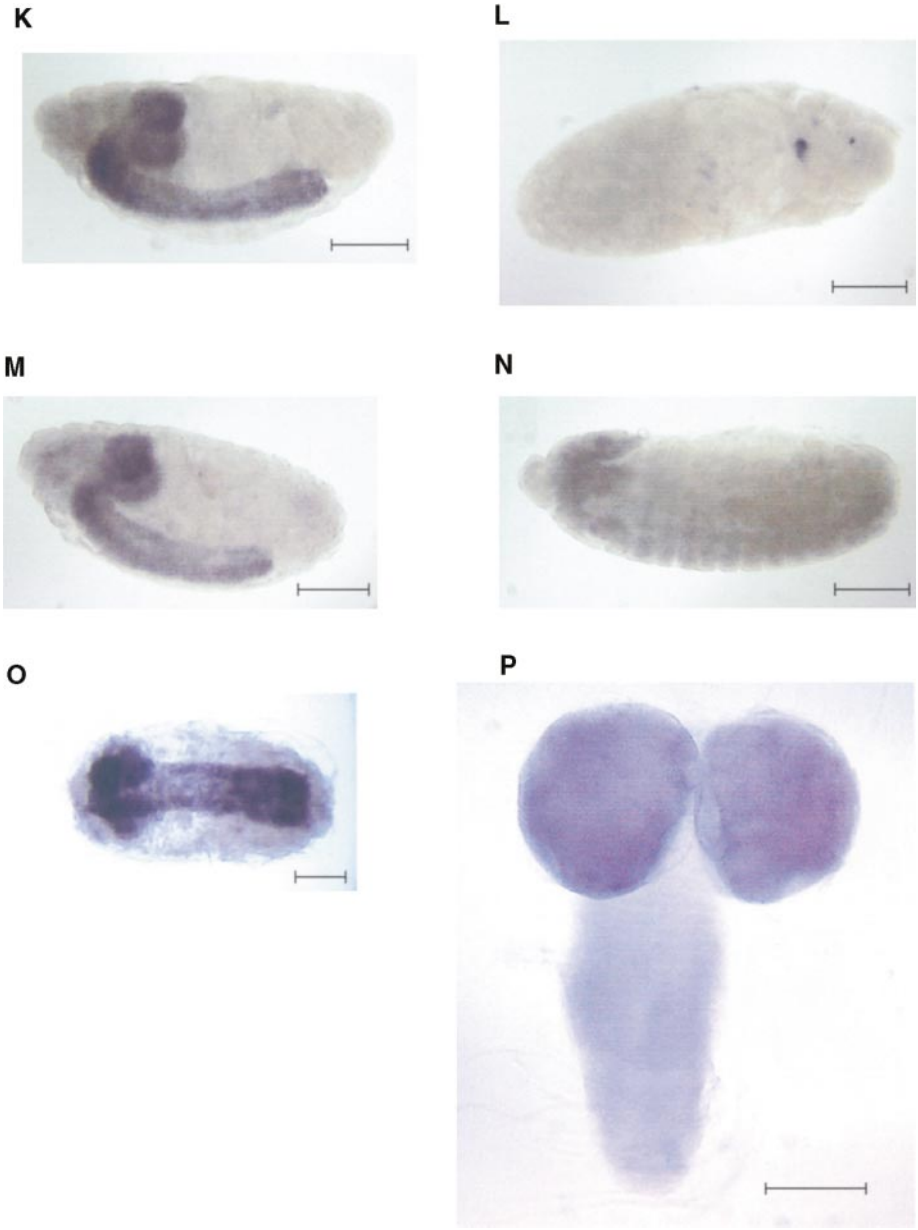

Figure 5.—*Continued.*

script differed from the 10.5-kb transcript both at the 5′ start site and the 3′ termination site. The 10.5-kb transcript has an alternative splicing site that eliminates the stop codon found in the smaller (3.6-kb transcript) whose ORF is found in LD13770. Thus the 10.5-kb transcript is produced by the addition of 5.3 kb of exon to the 3′ end of the smaller (3.6-kb) transcript (Figure 3). The 10.5-kb transcript also has an additional 2 kb of untranslated region at its 5′ end compared to the 3.6-kb transcript. We identified these full-length transcripts by 5′ RACE using specific primer 3 (Figure 3), which overlapped with an EST clone called GM04742 (BDGP). The EST clone LD13770 contained an ~3-kb cDNA with one large putative open reading frame that encoded a 929-amino-acid peptide. Thus it lacked ~0.6 kb of the 5′ nontranslated region found in the 3.6-kb RNA transcript shown in the Northern analysis (Figure 4A). We used the full 3-kb ORF (shown as LD13770) as a probe in Northern analyses, *in situ* hybridizations to tissue, and to generate sbb^{+} transgenes for transformation.

***sbb* transcripts are expressed throughout development and are reduced in *sbb* mutants:** The 3-kb EST clone LD13770 (to be called *sbb*-cDNA LD13770) was used in Northern blot analysis to probe wild-type (for^{R}) poly(A)$^{+}$ RNA isolated from larvae, pupae, adult head, and adult body (Figure 4A). *sbb* RNA is expressed at all stages of development. Three transcripts (10.5, 3.6, and 1.6 kb) were detected in larvae, pupae, and adult heads, whereas the 7.8-, 3.6-, and 1.6-kb transcripts were detected in adult body (Figure 4A). We have not yet characterized the 7.8-kb and 1.6-kb transcripts. These different-sized transcripts suggested the involvement of

alternative splicing, transcript initiation, or termination. Northern analysis using the same *sbb*-cDNA LD13770 probe showed a reduction in the abundance of mRNA in homozygous $sbb^{EP(2)0328}$ compared to larvae of the CS control strain (Figure 4B). Similar results were found for $sbb^{l(2)03432}$ and sbb^{12}, the early larval lethal excision line, which carries a >10-kb deletion (data not shown).

***sbb* transcripts are expressed in the embryonic and larval CNS and the larval imaginal discs:** *In situ* hybridization to CS embyros with antisense RNA from *sbb*-cDNA LD13770 revealed that *sbb* is expressed in the embryonic central nervous system (CNS; Figure 5, A, C, and E). This expression was not found in CS when probed with sense RNA (Figure 5, B, D, and F), demonstrating that the hybridization found in Figure 5, A, C, and E was representative of *sbb* RNA expression. Wild-type *sbb* expression was strong in early stage embryos (stage 5) before gastrulation (Figure 5A). In larvae, wild-type *sbb* RNA expression was found in the nervous system including the brain, the optic lobes, the ring gland, and the medial region of ventral ganglion (Figure 5G). We also found expression in the eye-antennal (Figure 5H), wing (Figure 5I), and leg discs (Figure 5J). RNA was expressed at normal levels in heterozygous embryos (Figure 5, K and M), but expression was reduced in homozygous mutant embryos of $sbb^{l(2)04440}$, $sbb^{l(2)03432}$, and $sbb^{EP(2)0328}$ (Figure 5, L, N, and O). $sbb^{l(2)04440}$ appeared to have a more severe reduction in transcript abundance than did $sbb^{l(2)03432}$ and $sbb^{EP(2)0328}$. Expression of *sbb* RNA in $sbb^{EP(2)0328}$ larvae was reduced in the brain and missing in the medial region of the ventral ganglion (Figure 5P).

Expression of *hs-sbb* rescues *sbb* mutant behavior, pupal lethality, and the wing vein phenotype: To correctly identify the transcript responsible for scribbler behavior and lethality we expressed the full ~3-kb ORF encoded by the EST clone LD13770 in transgenic flies. The cloning ensured that only the RNA from the 3.6-kb transcript was being overexpressed in transgenic flies (see materials and methods). Northern blot analysis confirmed that the 3-kb *hs-sbb* transgene was expressed in larvae. *sbb*-cDNA LD13770 was used to probe poly(A)$^+$ RNA from CS and *hs-sbb* transgenic larvae. Figure 6A shows the leaky expression of the *hs-sbb* transgene in two independent transgenic strains in the $sbb^{EP(2)0328}$ background at 25° (Figure 6A, lanes 3 and 5) and the expected very large increase in expression of the transgene after 37° heat shock (Figure 6A, lanes 4 and 6). We concluded that (1) the transgene cloned in the *P{CaSper-hs}* was functioning as expected, (2) the 3-kb transgene alone was overexpressed in larvae, and (3) we could rely on the leaky expression of this transgene for our behavioral experiments. Indeed, scribbler behavior was rescued by the *hs-sbb* transgene. Two independent transgenic strains carrying the *hs-sbb* transgene in the $sbb^{EP(2)0328}$ mutant background showed a reduction in the mean percentage of scribbler larvae on agar relative to the $sbb^{EP(2)0328}$ mutant strain (Figure 6B) and an increase in the number of grid squares entered on agar (Figure 6C). Thus the *hs-sbb* transgene restored *sbb* mutant behavior to wild type on agar. *hs-sbb* transgenes also fully rescued pupal lethality in the w^1; $sbb^{l(2)03432}$ / *CyO;* ry^{506} mutant background. These rescued *sbb* flies were completely fertile. The wing vein phenotype was restored to normal when four (but not two) copies of the *hs-sbb* were present in flies reared at 25°.

Rescue of *sbb* behavior by targeting expression of the 3-kb *sbb* transcript to neurons: *In situ* hybridization revealed that the *sbb* gene was expressed in the embryonic and larval CNS (Figure 5). The P[GAL4] system was used to manipulate gene expression (Brand and Perrimon 1993) to determine whether expression of the 3-kb *sbb* RNA in the nervous system alone was sufficient to rescue scribbler behavior. In this binary system the yeast transcription factor GAL4 directs the expression of any gene fused downstream of the activation sequence UAS, thus permitting ectopic expression of the fused gene (Brand and Perrimon 1993). To target expression of UAS-*sbb* to the nervous system we used an *elav-GAL4* driver (Lin and Goodman 1994). The full-length *sbb*-cDNA LD13770 (Figure 3) was cloned into a *P{UAST}* vector; we called this transgene UAS-*sbb.* UAS-*sbb* transgenes were crossed into a $sbb^{1(2)03432}$ mutant background. To verify that the UAS-*sbb* construct was functioning, we crossed two independent UAS-*sbb* transgenic strains to a *hs-GAL4* strain that was expected to produce ubiquitous expression of the transgene. The UAS-*sbb* transgene in both transgenic strains in the $sbb^{l(2)03432}$ background was highly expressed after a 37° heat shock (data not shown). We found that the expression of the UAS-*sbb* in the nervous system alone was sufficient to rescue scribbler behavior. The mean percentage of scribbler larvae on agar was reduced when the expression of *sbb* was targeted to neurons using two independent transgenic strains carrying the UAS-*sbb* transgene in the $sbb^{l(2)03432}$ background (Figure 6D). A significant increase in the number of grid squares entered on agar was also found when *sbb* was expressed in the nervous system (Figure 6E). When *sbb* was targeted to the nervous system alone, 60% of the expected number of flies emerged as fertile adults, indicating only partial rescue of the lethal phenotype. Targeted expression of the UAS-*sbb* to neurons did not rescue the L5 wing vein phenotype suggesting that this phenotype resulted from *sbb* expression outside the nervous system, perhaps in the nonneuronal cells in the imaginal discs.

***sbb* transcripts encode a novel protein:** DNA sequencing revealed that the 3.6-kb *sbb* transcript contains a long open reading frame encoding 929 amino acids (Figure 7). A ScanProsite search revealed that this 929-amino-acid protein contains 2 putative cAMP/cGMP-dependent protein kinase phosphorylation sites, 15 protein kinase C phosphorylation sites, and 7 casein kinase II phosphorylation sites. A PSORT (prediction of protein sorting signals and localization sites in amino acid

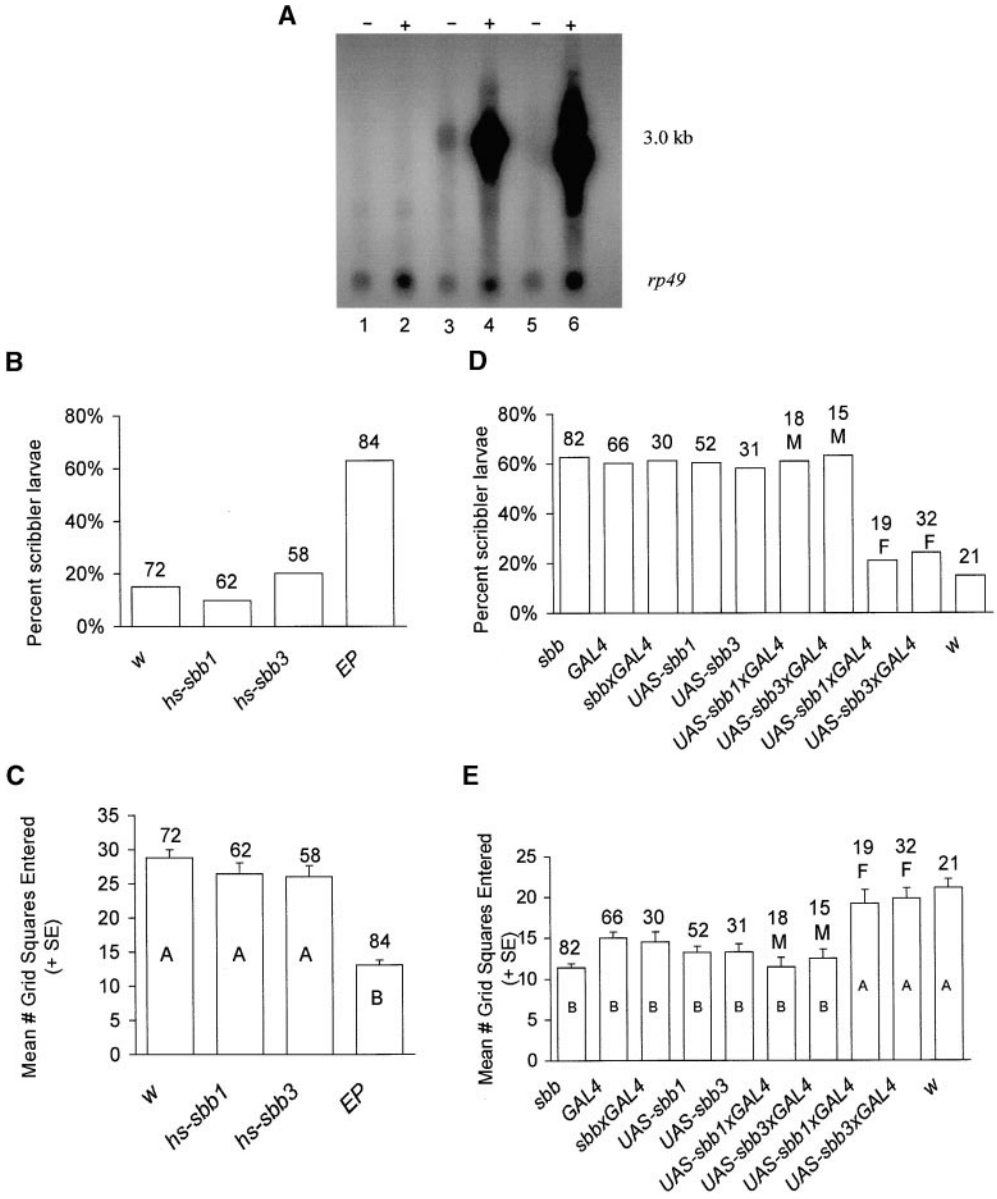

Figure 6.—Rescue of scribbler behavior by leaky expression of *hs-sbb* and targeted expression of UAS-*sbb* to neurons. (A) Lanes contain 5 μg poly(A)$^+$ RNA isolated from larvae of CS (lanes 1 and 2), *hs-sbb1* (lanes 3 and 4) and *hs-sbb3* (lanes 5 and 6). Numbers on the right indicate RNA size in kilobases. +, heat shock; −, no heat shock. As expected, the control (CS) strain did not show induction of the 3.0-kb transgene. Two independently generated transgenic lines showed leaky expression at 25° (lanes 5 and 3) in the $sbb^{EP(2)0328}$ mutant background. *hs-sbb1* showed two times (lane 5) and *hs-sbb3* showed six times (lane 3) the abundance of the 3-kb transgene compared to the control strain (CS, lane 1). After heat shock both *hs-sbb* transgenes showed a very large induction of expression of the 3.0-kb transcript (lanes 4 and 6). This blot was reprobed with *rp49* gene used as a loading control. (B) Rescue of scribbler behavior was shown in both transgenic lines (*hs-sbb1* and *hs-sbb3*) on a $sbb^{EP(2)0328}$ background. The leaky expression of both transgenes shown in Figure 6A was sufficient to decrease the percentage of scribbler larvae to the level found in the w^1 control. (C) Leaky expression of the *hs-sbb* transgene also increased the number of grid squares entered by larvae of the transgenic lines in a $sbb^{EP(2)0328}$ mutant background to the level of the w^1 control. The transgenic larvae entered a significantly greater number of 6-mm grid squares than did $sbb^{EP(2)0328}$ mutant larvae (one-way ANOVA $F(3,272) = 60.14$, $P < 0.0001$). Sample sizes are shown above each histogram. Different letters represent different statistical groupings (SNK, $P = 0.05$). Strains are labeled as in Figure 6B. (D) The mean percentage of scribbler larvae on agar was also reduced when *sbb* was expressed in the nervous system. Two independently generated UAS-*sbb* transgenic lines (UAS-*sbb1* and UAS-*sbb3*) on a $sbb^{l(2)03432}$ mutant background were crossed to a *GAL4* driver strain (*elav-GAL4*) on a $sbb^{l(2)03432}$ mutant background. The first seven histograms show the appropriate control crosses. When virgin females from the transgenic lines UAS-*sbb1* and UAS-*sbb3* were crossed to males from the *GAL4* strain only female (F) progeny received both the UAS-*sbb* and *GAL4* transgenes. Thus data from the male (M) larval progeny served as an internal sib control for the experiment. *sbb* was expressed in the nervous system of the female larvae in the last two crosses (UAS-*sbb1* × *GAL4* and UAS-*sbb3* × *GAL4*) where we found a significant reduction in the average percentage of *sbb* larvae. These data demonstrate rescue of scribbler behavior by expression of the 3-kb *sbb* transgene in the nervous system alone. (E) Targeted expression of the UAS-*sbb* transgene to neurons driven by *elav-GAL4* also significantly increased the number of grid squares entered on agar by $sbb^{l(2)03432}$ mutant larvae. The two lines of transgenic female larvae from the UAS-*sbb1* × *GAL4* and UAS-*sbb3* × *GAL4* crosses entered a significantly greater number of 6-mm grid squares than did the *sbb* mutant [controls; one-way ANOVA $F(9,385) = 11.33$, $P < 0.0001$]. Sample sizes are shown above each histogram. Different letters represent different statistical groupings (SNK, $P = 0.05$). All strain names are as in Figure 6D.

sequences) search revealed two nuclear localization signals at position 840 (PPAKRVK) and position 841 (PAKRVKH) with 94.1% reliability. This suggested that the SCRIBBLER protein is a nuclear protein. No homology was found with any known protein in the database when a BLASTP search of this deduced amino acid sequence was done. However, a BLASTN search did reveal four EST clones isolated from mouse heart

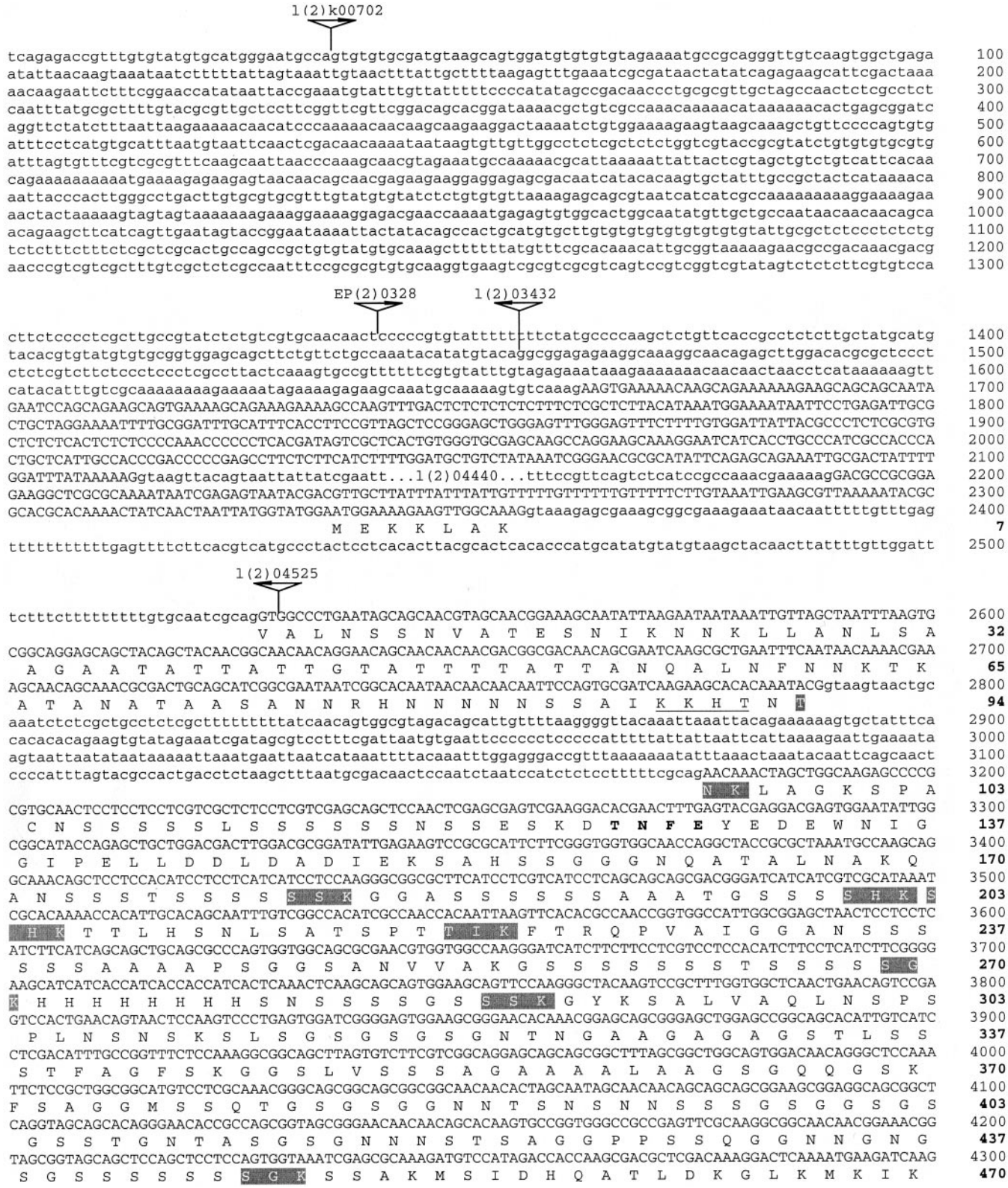

Figure 7.—Nucleotide and deduced amino acids of the 3.6-kb *sbb* transcript. Uppercase nucleotide sequence represents the cDNA; lowercase represents the introns. Dotted line indicates a >45-kb intron that contains the $sbb^{l(2)04440}$ *P*-element insertion site. The $sbb^{l(2)k00702}$, $sbb^{EP(2)0328}$, and $sbb^{l(2)03432}$ *P*-element insertion sites are shown as triangles. Arrows indicate direction, 5′ to 3′ of the *P*-element insertion. The predicted protein sequence of the longest open reading frame is shown. PSORT search showed that this deduced 929 amino acid contained two nuclear localization signals at positions 840 and 841 (boxed). ScanProsite also found 26 *N*-glycosylation sites, 2 cAMP/cGMP-dependent protein kinase phosphorylation sites (underlined), 9 protein kinase C phosphorylation sites (shaded), 7 casein kinase II phosphorylation sites (boldface), 1 phosphopantetheine attachment site (boldface and underlined) and 88 N-myristoylation sites. BLASTP search of the *sbb* protein did not show homology with any known proteins in the database. This 3.6-kb *sbb* transcript has been assigned GenBank accession no. AF247563. The 10.5-kb *sbb* transcript has been assigned GenBank accession no. AF247562.

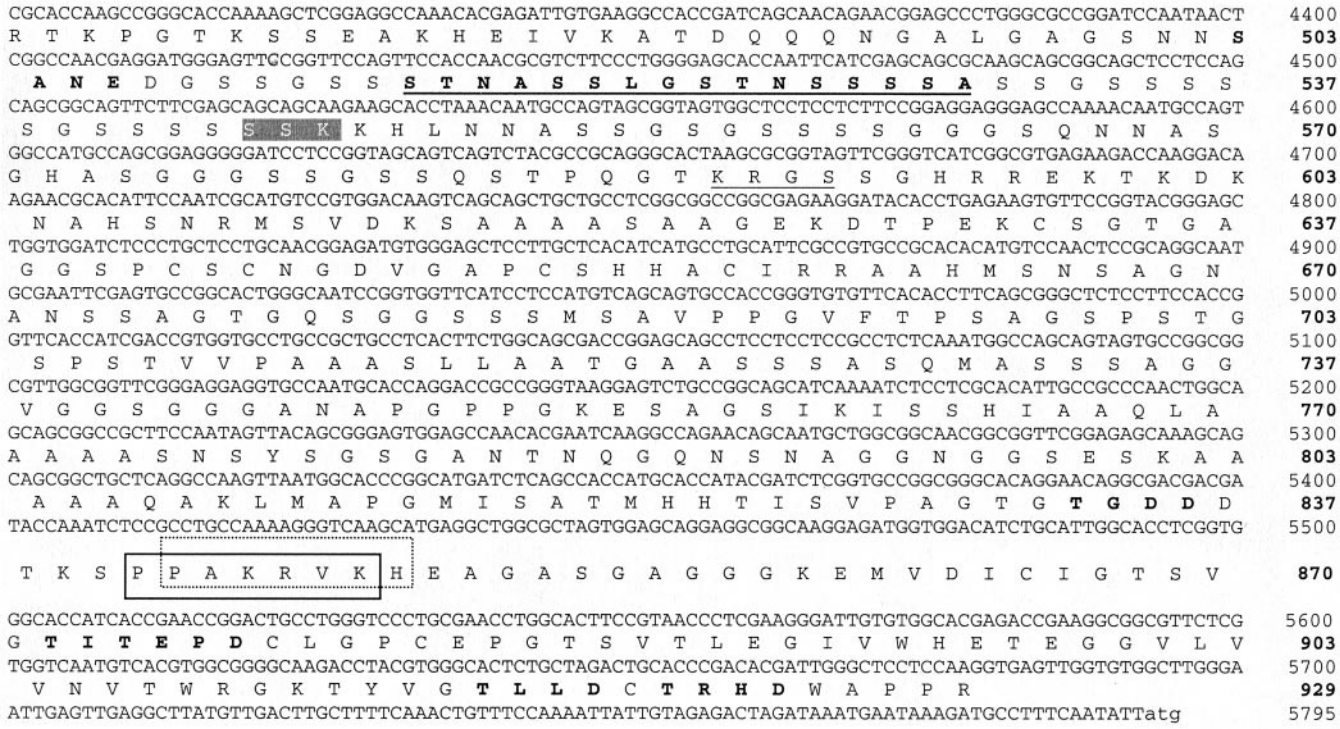

Figure 7.—*Continued.*

(AI644380, AI614389), *Xenopus neurula* (AI031376), and human (AI223051) that were highly homologous (70–90% identity) with the C-terminal region of the SCRIBBLER protein. These data suggest the existence of SCRIBBLER counterparts in vertebrates.

The 10.5-kb transcript contains a larger open reading frame (encoding 2302 amino acids) than the 3.6-kb transcript. The first 929 amino acids encoded by the 10.5-kb transcript are the same as the protein encoded by the 3.6-kb ORF. SCANPROSITE revealed that this larger protein contained one single zinc finger (C2H2 type) domain and one tyrosine kinase phosphorylation site that are not part of the first 929 amino acids shared with the 3.6-kb transcript. The PSORT search reveals five nuclear localization signals in this larger 2302-amino-acid protein. A BLASTP search of this deduced amino acid sequence showed homology with a human EST clone (AB002293) that contained a single zinc finger motif that had 70% identity within 30 amino acids in the zinc finger region. This human EST clone was originally isolated from a male brain cDNA library (Nagase *et al.* 1997). This zinc finger region of the 2302 amino acids was also highly homologous (63% identity) with a Zebrafish EST clone (AI722347) suggesting the existence of a conservative single zinc finger domain.

DISCUSSION

Our genetic dissection of larval behavior has uncovered *sbb*, a new vital gene that encodes a novel protein that is expressed in the nervous system. Behavioral and neurogenetic evidence suggests that *sbb* plays a role in larval turning behavior. It is of interest that the scribbler turning phenotype was displayed only in the absence of food, since turning rate and localized traveling are known to be important components of food search behavior (Bell 1990). Search behavior is the means by which most motile animals find essential resources and hence is a trait that can strongly influence the survival of an individual (Bell 1991). Genetic control of search behavior is not unprecedented. It has been shown that larvae carrying the rover allele of the *foraging* (*for*) gene exhibit long foraging trails in a large yeast patch and tend to move between depleted food patches, whereas homozygous sitter larvae locate the closest food patch and remain feeding on it (Sokolowski 1980; Sokolowski *et al.* 1983). Similarly, adult rover flies walk significantly farther from a recently consumed sucrose drop than sitter flies (Pereira and Sokolowski 1993) whose higher turning rate promotes revisiting and keeps the fly near the drop (Bell and Tortorici 1987). *sbb* larvae exhibit characteristic patterns of food search behavior (turning, bending, and feeding movements); however, these patterns are exhibited in the absence of food.

Many developmental genes are known to be pleiotropic; their gene products play multiple roles throughout development (for example, Bier *et al.* 1990; Hartenstein and Posakony 1990; Boulianne *et al.* 1991; Tzertzinis *et al.* 1994). Genes that affect both larval and adult behavior can play important roles in development

and sometimes have vital functions [for example, in Drosophila, learning genes such as *dunce*, *latheo*, and *linotte* (Bolwig *et al.* 1995; Dubnau and Tully 1998); courtship mutants such as *fruitless* (Ryner *et al.* 1996) and *fickle* (Baba *et al.* 1999); larval behavioral mutants such as *foraging* (de Belle *et al.* 1989) and *tamas* (Iyengar *et al.* 1999); and ion channel mutants such as *slow poke* (Atkinson *et al.* 1998)]. One well-studied example of a pleiotropic gene that affects behavior is the learning gene *dunce* (*dnc*). Mutations in *dnc* cause female sterility and abnormal learning. The *dnc* gene is widely expressed; its expression is not restricted to the mushroom bodies of the fly brain known to play an important role in learning and memory (Tully 1991; Davis 1996). Pleiotropic genes that play a role in behavior are usually expressed in multiple tissues and during more than one developmental stage. Mutations in pleiotropic genes often affect more than one aspect of neuronal structure and/or function (for review, see Hall 1993).

sbb is a pleiotropic gene with a vital function. Four out of five ($sbb^{l(2)03432}$, $sbb^{l(2)04440}$, $sbb^{l(2)k00702}$, and $sbb^{l(2)04525}$ but not $sbb^{EP(2)0328}$) *P*-element insertion alleles of *sbb* exhibited lethality, primarily in the late pupal stage. Mutations in the *sbb* gene lead to multiple phenotypic defects that include larval scribbler behavior, pupal lethality, and a defect in the pattern of the L5 wing vein in the adult escapers. *sbb* transcripts were observed in all developmental stages (Figure 4A) and in multiple tissue types (the embryonic and larval CNS and the imaginal discs; Figure 5). These expression data support the phenotypic data suggesting that *sbb* likely functions during multiple developmental stages and in more than one tissue. The four different-sized transcripts (Figure 4A) suggest the existence of at least four different SCRIBBLER isoforms that may arise from differential RNA splicing or alternative polyadenylation or initiation. Although we have not investigated this, we predict that some of these isoforms will have different functions and be found in different tissue. Some initial support for this prediction comes from the finding of a body-specific transcript (7.8 kb; Figure 4A) and from the lack of complete rescue of pupal lethality when *sbb* was only targeted to neuronal cells. Further studies are needed to address the question of how, when, and where *sbb* acts to accomplish its pleiotropic functions.

Our *in situ* hybridization showed that wild-type *sbb* RNA is expressed in the embryonic and larval CNS and larval imaginal discs (Figure 5). Two pupal lethal homozygous mutant *sbb* embryos, $sbb^{l(2)03432}$ and $sbb^{l(2)04440}$, showed little to no expression whereas the viable mutant strain $sbb^{EP(2)0328}$ may have altered expression (Figure 5). Larval *in situ* hybridization of $sbb^{EP(2)0328}$ also revealed a significant reduction in RNA abundance in the CNS, ventral ganglion (Figure 5P), and imaginal discs (data not shown). These data suggest that *sbb* mutants suffer from a disorder originating from disruptions in the nervous system. This was confirmed by the restoration of normal larval behavior with a UAS-*sbb* transgene expressed in neurons using an *elav-GAL4* driver (Figure 6, D and E). Targeted expression of *sbb* to the muscle was not needed to rescue scribbler behavior.

Sequence analysis of the 3.6-kb and 10.5-kb transcripts revealed that they differ in both their start and termination sites. Their deduced amino acid sequences are identical at the N-terminal end. The 10.5-kb transcript, when compared to the 3.6-kb transcript, encodes an additional 1373 amino acids at the C-terminal end. We found that both the 3.6-kb and 10.5-kb gene products are novel proteins that are most likely localized within the nucleus. The large form contains one zinc finger C2H2 type motif that is different from the zinc finger domains found in numerous nucleic acid-binding proteins that have two or more sets of zinc finger motifs (Evans and Hollenberg 1988). Usually the two zinc finger domains are 7–8 amino acids apart (Blumberg and Silver 1991). Two widely separated sets of C2H2 type zinc finger domains have been reported (Fan and Maniatis 1990; Blumberg and Silver 1991). In this case, each set of zinc finger motifs interacts with the same DNA sequence with similar affinities (Fan and Maniatis 1990). For example one peptide thought to function in metal transport and/or modulation of gene expression was found to contain one single zinc finger domain (Sillard *et al.* 1993). Other experimental evidence has shown that zinc finger domains can play a role in protein-protein interactions (Fox *et al.* 1998; Choi *et al.* 1999). We hypothesize that the larger SCRIBBLER protein encoded by the 10.5-kb transcript may play a role in nucleic acid-binding and/or protein-protein interactions. Future experiments will address this hypothesis.

Differential RNA splicing and post-translational cleavages can generate a large number of protein isoforms (Maeda *et al.* 1985; Byers *et al.* 1993; Austin *et al.* 1995). It is not known whether or not there is differential post-translational cleavage of the SCRIBBLER protein. We do know that two of the *sbb* RNA species (3.6-kb and 10.5-kb transcripts) have different start and termination sites. Sequence analysis indicated that both isoforms are likely found in the nucleus. We have demonstrated that expression of the smaller isoform alone is sufficient to rescue *sbb* behavior. At present it is not known whether the other isoforms play a role in larval behavior.

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Note added in proof: The *scribbler* gene reported here is synonymous with the *brakeless* gene reported in K.-A. Senti, K. Keleman, F. Eisenhaber and B. J. Dickson (2000, *brakeless* is required for lamina targeting of R1-R6 axons in the *Drosophila* visual system. Development **127:** 2291–2301).

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