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Expression and Function of Scalloped During *Drosophila* Development

Kirsten A. Guss[1], **Michael Benson**[1], **Nicholas Gubitosi**[1], **Karrie Brondell**[1], **Kendal Broadie**[2], and **James B. Skeath**[3]

[1]Department of Biology, Dickinson College, Carlisle, Pennsylvania

[2]Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee

[3]Department of Genetics, Washington University School of Medicine, St. Louis, Missouri

Abstract

Background—The *scalloped (sd)* and *vestigial (vg)* genes function together in *Drosophila* wing development. Little is known about *sd* protein (SD) expression during development, or whether *sd* and *vg* interact in other developing tissues. To begin to address these questions, we generated an anti-SD antibody.

Results—During embryogenesis, SD is expressed in both central and peripheral nervous systems, and the musculature. SD is also expressed in developing flight appendages. Despite SD expression herein, the peripheral nervous system, musculature, and dorsal limb primordia appeared generally normal in the absence of *sd* function. SD is also expressed in subsets of ventral nerve cord cells, including neuroblast 1–2 descendants and ventral unpaired median motor neurons (mVUMs). While *sd* function is not required to specify these neurons, it is necessary for the correct innervation of somatic muscles by the mVUMs. We also show that SD and VG are co-expressed in overlapping and distinctive subsets of cells in embryonic and larval tissues.

Conclusions—We describe the full breadth of SD expression during *Drosophila* embryogenesis, and identify a requirement for *sd* function in a subset of motor neurons. This work provides the necessary foundation for functional studies regarding the roles of *sd* during *Drosophila* development.

Keywords

transcription factor; *vestigial*; imaginal disc; central nervous system; peripheral nervous system; muscle

INTRODUCTION

The classical *Drosophila scalloped (sd)* mutant wing phenotype was first described in 1929 (Gruneberg, 1929), and the gene was subsequently cloned over 60 years later (Campbell et al., 1991). It was established that *sd* encodes a protein with a TEA family DNA-binding domain (Campbell et al., 1992; Jacquemin and Davidson, 1997; Anbanandam et al., 2006) and binds DNA in a sequence-specific manner (Halder et al., 1998b; Guss et al., 2001). SD acts as a transcription factor with two coactivators, *vestigial* (VG) and *yorkie* (YKI), functioning in wing development and the control of cell proliferation. With VG (Williams et al., 1991), SD functions as the selector (Mann and Carroll, 2002) for wing identity, forming

Correspondence and requests for reagents to: Kirsten A. Guss, Department of Biology, Dickinson College, Carlisle, PA 17013 gussk@dickinson.edu.

a complex that is necessary and sufficient to direct wing development (Kim et al., 1996; Halder et al., 1998b; Simmonds et al., 1998; Halder and Carroll, 2001). SD binds to and directly regulates the wing-specific *cis*-regulatory elements for genes controlling many aspects of wing morphogenesis (Halder et al., 1998b; Guss et al., 2001; Lunde et al., 2003). With YKI, SD functions as an activator of growth and proliferation, in a complex regulated by the Hippo signaling pathway (reviewed in Bandura and Edgar, 2008).

Despite these extensive investigations of *sd* function, it has not before been possible to examine the expression of the SD protein. To address this challenge, we made an anti-SD antibody, and used it to characterize the full breadth of SD expression during *Drosophila* development. In addition to the expected distribution of SD in the wing and haltere imaginal discs, we show that SD is strongly expressed in subsets of neuronal and muscle cells, where it colocalizes with VG. Based on this characterization, we present data that SD functions in neuronal pathfinding to muscle innervation of the ventral unpaired median motor neurons. This work lays the foundation for the systematic study of SD function in the development of the neuromusculature.

RESULTS AND DISCUSSION

Synthesis and specificity of the SD antibody

To define the cellular expression of SD during *Drosophila* development, we generated an anti-SD antibody. To test the specificity of this probe, its immunoreactivity was assessed in embryos showing loss of expression, or overexpression, of *sd* (Figure 1). The SD antibody failed to label any tissues in Df(1)Exel6251/Y embryos, which carry an X chromosome deficiency that uncovers the *sd* locus (Parks et al., 2004; Fig. 1B). Expression was also assessed in embryos carrying the recessive lethal sd^{3L} allele (data not shown). The molecular lesion associated with this allele is a nonsense mutation in the first leucine codon within the Vestigial Binding Domain (Campbell et al., 1991; Srivastava et al., 2004), Very low, diffuse signal was observed in the central nervous systems of sd^{3L} embryos suggesting it is a strong hypomorphic mutation. Conversely, the antibody labeled additional cells in embryos in which *patched-GAL4* drove *UAS-sd* in many cells in every segment (Fig. 1D). Taken together, these data show that our antibody recognizes specifically the SD protein.

SD expression during embryonic development

We observe close correlation between the patterns of expression the *sd* RNA and SD protein, which further supports the specificity of our antibody (Fig. 2). The *sd* RNA is detectable in the central nervous system (CNS; Fig. 2A, D, E; see also Campbell et al., 1992) and the peripheral nervous system (PNS; Fig. 2B, C). SD protein is expressed in the ventral nerve cord (VNC) detectable beginning at stage 10 (Fig. 2F). The expression in the CNS continues through stages 11 (Fig. 2G), 12 (Fig. 2H), 14 (Fig. 2I), and 16 (Fig. 2J). Expression in the PNS is also observed at stage 14 (Fig. 2I), while expression in the muscles is discernible at stage 16 (Fig 2J). We also observe strong SD expression in adepithelial cells (Bate et al., 1991) located in the between the dorsal and ventral limb primordia (arrows, Fig. 2H; see also Fig. 3P–R).

We examined more closely the expression of SD during development (Fig. 3). In the VNC, SD appears to be initially expressed in many cells (Fig. 3A, B), although the expression resolves to higher levels in a subset of cells as embryogenesis proceeds (Fig. 3C–F). To more thoroughly characterize the SD expression in other tissues during embryogenesis, we performed colocalization studies with tissue or cell type specific molecular markers. In the PNS, SD is expressed in most sensory neurons, based on close coexpression with Embryonic Lethal Abnormal Vision (ELAV; (ELAV; Robinow and White, 1991; Fig. 3G–I). This

coexpression continues through embryogenesis, although the level of SD expression in cells in the PNS is reduced. SD is also expressed in the somatic bodywall muscles during embryogenesis. At stage 12, SD is detectable in the ventral lateral muscles 1–4 (VLs 1–4, muscle nomenclature per Bate, 1993). This expression continues through stage 16, with higher expression in the muscle nuclei (Fig. 3J–L). By this stage SD is also detectable in ventral acute muscles 1 and 2 (VA1 and 2) and lateral longitudinal muscle 1 (LL1). In late stage 16 embryos, additional dorsal muscles also express SD. Therefore, expression of SD occurs in the most ventral muscles first, then expands to more dorsal muscles as development proceeds. Deng et al. (2009) observed broader *sd* expression, earlier in development, by employing *sd* RNA and enhancer trap detection methods, with reduced expression by late stage 16, whereas we observed the most extensive expression this late embryonic stage.

SD is also expressed at low levels in the embryonic wing and haltere primordia, based on colocalization with Esgargot (ESG; Fig. 3M–O). The cells expressing the higher level of SD in the lateral regions of the three thoracic segments are progenitors of the adult flight muscles (Fig. 3P–R). We make this conclusion based on the coexpression of SD and βGAL driven by *twist-GAL4*, a marker for these adepithelial cells (Bate et al., 1991). Our observation correlates well with those made by Bernard et al. (2003), who showed that VG and *sd-lacZ* are coexpressed in these cells later in development.

Scalloped expression in the larval imaginal discs

We also examined SD expression in tissues of the wandering third instar larva (Fig. 4). In the wing imaginal disc, SD is expressed in the wing pouch and margin (Fig. 4A), with expression extending into the hinge and notum (Cohen, 1993). SD is expressed in the corresponding structures in the haltere disc (Fig. 4B). The strong expression of SD in the wing disc correlates well with its documented role in wing development. In comparison, SD is expressed at much lower levels in the leg (Fig. 4C) and eye antennal (Fig. 4D) imaginal discs. We also examined the expression of *sd* RNA in the imaginal discs (Fig. 5). Concordant with the high levels of the protein in the wing and haltere, strong RNA expression is observed in these imaginal discs (Fig. 5A, B). We also observe expression, at lower levels, in the leg and eye antennal discs (Fig. 5C, D). In the leg and antenna, *sd* appears to be expressed in rings that may correspond to different locations along the proximal-distal axes of these tissues.

Our observations generally agree with the expression pattern of *sd* that has been reported using the *sd* enhancer trap line *ETX4* (Campbell et al., 1992), although we do see some differences. For example, we report here what appear to be higher levels of expression of *sd* and SD in cells in the embryo using direct detection of these gene products versus those levels reflected by *ETX4* (Campbell et al., 1992 and our own observations). In addition, Campbell et al. (1992) show that *ETX4* drives high expression posterior to the morphogenetic furrow in eye imaginal disc, whereas we observe both RNA and protein at higher levels to the anterior of this landmark. Such discrepancies may be due to subtle differences in regulation, and/or perdurance, of the reporter protein in the enhancer trap line.

Coexpression of Scalloped and Vestigial in developing tissues

Scalloped (SD) and Vestigial (VG) have a well-established role working together in wing development (Halder et al., 1998b; Simmonds et al., 1998). Based on a characterized VG antibody, the identity of many VG-expressing cells have been determined in developing tissues (Williams et al., 1991; Landgraf et al., 2003; Guss et al., 2008). Therefore, using this tool, we next examined the coexpression of SD and VG in both embryos and larvae. This approach allowed us to determine the identity of SD-expressing cells, and provides the

necessary foundation to identify tissues in which SD and VG may function together, as well as tissues in which they may function independently. A summary of these data is shown in Figure 6.

SD is coexpressed with VG in subsets of cells in both the embryo and in the wing and haltere imaginal discs (Fig. 6). As discussed above, SD expression appears to be initially broad in the ventral nerve cord, and to resolve to high expression in a clear subset of cells. Interestingly, these same identified neurons also express VG (Fig. 6A–F). These cells include neuroblast 1–2 (NB1-2) descendants (Fig. 6A–C; referred to as mVg in Landgraf et al., 2003; Guss et al., 2008) and the ventral unpaired median motor neurons (mVUMs; Fig. 6D–F). With respect to the somatic muscles, both SD and VG are expressed at elevated levels in the ventral muscles VLs 1–4 and LL1 (Fig. 6G–I) At this stage, VG is additionally expressed in dorsal acute muscles (DA)1–3 (Landgraf et al., 1999). In the larval imaginal discs, consistent with their shared role as selectors for wing development, both SD and VG are expressed in the wing and haltere imaginal discs (Fig. 6J–K).

Functional requirements for Scalloped in the neuromusculature

To begin to investigate the function of Scalloped in embryonic tissues, we surveyed general morphological markers the nervous systems and muscle in sd^{3L} (strong hypomorph) and Df(1)Exel6251 (null) embryos (Fig. 7). It appears that *sd* function is not required for cell fate specification in the CNS, including in the SD-expressing NB1-2 descendants and mVUMs. Based on labeling for DVSX1 (Erclik et al., 2008), which is expressed in the NB1-2 descendants and mVUMs, we found no consistent difference between control and Df(1)Exel6251 null embryos (Fig. 7A–H). Although SD is broadly expressed in the PNS, we did not observe a detectable change in the number/position of neuronal cell bodies or the gross morphology of the sensory neuron axons. This conclusion is based on similar expression of microtubule-associated Futsch/22C10 (Hummel et al., 2000) in control and *sd* mutant embryos (Fig. 7I, J). Similarly, we observed no detectable change in embryonic muscle patterning or differentiation in the absence of SD function. Based on labeling for Myosin Heavy Chain (MHC), we observed a subtle change in the distribution of muscle nuclei in *sd* mutant versus control embryos (Fig. 7K, L). We focused on VLs 1–4 and LL1, because we observed these muscles to consistently express SD from stage 12 through later embryogenesis. For this reason, we did not examine the ventral oblique muscles and cardiac cells, reported by Deng et al. (2009) to show defects in *sd* mutants.

Finally, we observe that *sd* function is not required for the establishment of the flight appendage primordia. This conclusion is based on the same distribution of ESG expressing cells in the dorsal and ventral flight appendage primordia in the thoracic segments of *sd* mutant and control embryos (Fig. 7M, N), and is consistent with the observation that the wing disc forms in *sd* mutants (Williams et al., 1993). We conclude that SD is either not required for the specification of these tissues, or that its effects are subtler or address different aspects of differentiation than are accessible with the markers we used.

The fact that *sd* is not required for the cell fate specification in the neuromusculature led us to ask whether it may function in a later differentiation stage. Because the mVUM gene expression profile (Wheeler et al., 2006) and target muscles (Landgraf et al., 1997) have been characterized, we tested for *sd* phenotypes in this select cell population. Collectively, the three mVUM neurons innervate dorsal oblique muscles 3–5 (DO 3–5) via the intersegemental nerve (ISN), lateral transverse muscles 1 and 2 (LT1 and 2), via segmental nerve a (SNa), and ventral oblique muscles 1–6 (VO 1–6) via SNb and SNd (Landgraf et al., 1997). More recent accounts (e.g., Ruiz-Canada and Budnik, 2006) refer to only the ventral muscles VO 1–5 as being innervated by mVUMs, so we have focused on these muscles, and the structures of SNb and SNd. The monoclonal antibody ID4 for Fasciclin II (FasII; Van

Vactor et al., 1993) was used to label all the motorneuron axons in control and *sd* mutant embryos. We observed disruptions of the segmental nerve branches in the absence of *sd* (Fig. 8). Because SNd could not consistently be identified and it was difficult to identify the stereotyped branches of SNb (Halpern et al., 1991), we focused on SNb length, from its projection from ISN to its distal tip. The length of SNb is shorter in *sd* mutant embryos than in controls (mean=43.52, s.e.m.=4.38 versus mean=68.73, s.e.m.=5.38, p=0.0034). This result indicates that *sd* function is required for the normal innervation of the ventral muscles.

SUMMARY

Here we use a new antibody probe to characterize the expression of the TEA domain transcription factor SD during Drosophila development. Although this protein is known for its role in wing development and growth control, SD is expressed in many tissue types during embryogenesis, including the central and peripheral nervous systems, and the muscles. We report a new role for *sd*, in the innervation of the ventral muscles. We also examined the coexpression of SD with VG, its cofactor in wing development. These proteins are distributed in similar but not identical expression patterns, presenting the possibilities that they may fine tune each other's expression, function together outside the wing, and/or function independently.

EXPERIMENTAL PROCEDURES

Drosophila stocks

The genotype w^{1118} was used as wild type. Df(1)Exel6251 is a deficiency that uncovers the *sd* locus (FlyBase), and sd^{3L} is a recessive lethal allele (Campbell et al., 1991; Srivastava et al., 2004). Both mutations were maintained over FM7c *act-lacZ* (Bloomington Drosophila Stock Center) and male embryos carrying the mutation were identified by the absence of beta-galatosidase (βGAL) expression. Additional stocks used were *patched-GAL4*, *twist-GAL4*, *UAS-lacZ*.nls (all from Bloomington Drosophila Stock Center) and *UAS-sd* (Sean Carroll).

SD antibody generation

Both known SD isoforms span from N-terminal MVDSK through C-terminal RLIKE (FlyBase R5.8, 2008). This 375 amino acid SD sequence was subjected to the Jameson-Wolf antigenic index (Lasergene, Madison, WI). PCR primers were designed to generate a form of SD containing 369 amino acids, and had the following sequences, including restriction enzyme recognition sequences (bold) and end sequences to facilitate enzyme activity (underline):

SD_7_28: 5′ CTA**CATATG**GATAGCAAAAACCTGGATGTCG 3′

SD_1113_1092: 5′ CTA**AAGCTT**ACGGTATATGTGATGGGTGGTG 3′

Samples containing as template the *sd* open reading frame and the thermostable polymerase PfuTurbo were subjected to 30 cycles of amplification using an annealing temperature of 65°C. The PCR products of triplicate reactions were processed using the QIAQuick PCR purification protocol. The products were then digested with NdeI and HindIII for cloning. Following digestion, the products were again processed using the QIAQuick PCR purification protocol. The insert was cloned in-frame via NdeI and HindIII sites in the pET29a expression vector. The construct was transformed into competent BL21 cells, with expression induced by addition of IPTG to 1 mM to bacterial culture with OD600 approximately 0.5, followed by an additional 2.5 hours growth. The 46 kDa protein was recovered as described by Williams et al. (1995). Inclusion bodies recovered by treatment with lysozyme followed by recovery with a French press were solubilized in 10% SDS, then

dialyzed with phosphate buffered saline (PBS). The protein at ~2 mg/ml concentration was injected by Pocono Rabbit Farm and Laboratory, Inc. (Canadensis, PA) into 3 rats and 3 rabbits. The final exsanguinations were affinity purified against the SD protein.

Whole mount in situ hybridization

Detection of RNA in Drosophila embryos was performed as described by Small (2000), and in third instar larval imaginal discs as described by Mark Sturtevant and Ethan Bier (1997). A DNA construct containing the *sd* open reading frame cloned into pBluescript (Anj Hudson and Sean Carroll) was used as template to synthesize T3-primed sense or T7-primed antisense RNA probes using digoxigenin-labeled RNA synthesis materials from Roche. No signal was observed in embryos or imaginal tissues processed with the sense RNA probes (data not shown).

Immunocytochemistry

Embryos from overnight collections were washed with 0.4% NaCl/0.1% Triton X-100, dechorionated by incubating in bleach for 2.5 minutes, and rinsed with dH_20. Embryos were then fixed by agitating for 20 minutes in equal volumes heptane and fix buffer comprised of 0.1 M PIPES, 2 mM EGTA, 1 mM $MgSO_4$, and 4% formaldehyde. Following fixation, embryos were devitellinized and washed with methanol. For immunolabeling, embryos were rehydrated into PBS with 0.1% Triton X-100 and 1% bovine serum albumin, which was used for all antibody incubations and washes. Wandering third instar larval imaginal discs were dissected, fixed, blocked, and processed with antibodies as described by Halder et al (1998a).

Antibodies

SD was detected using either guinea pig anti-SD (1:500 or 1:1000 in embryos, 1:1000 in larvae) or rat anti-SD (1:5 in embryos). Guinea pig anti-DVSX1 (1:750; Erclik et al., 2008) was provided by Howard Lipshitz. Rabbit anti-VG (1:25 in embryos, 1:100 in larvae; Williams et al., 1991) was the gift of Sean Carroll and in embryos was detected with biotinyl tyramide amplification as described in Guss et al (2008). Rat anti-ESG (1:500; Fuse et al., 1994) was provided by Shigeo Hayashi. Rabbit anti MHC (1:500) was provided by Dan Kiehart via Emma Rushton. Rat anti-ELAV (7E8A10, 1:200), mouse anti FUTSCH (22C10; 1:100), mouse anti-FASII (ID4, 1:20) were developed by Gerald Rubin, Seymour Benzer, and Corey Goodman, respectively, and obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242. βGAL was detected using antibodies raised in either rabbit (Invitrogen) or mouse (Promega) and diluted 1:1000. Primary antibodies were detected by either Alexa Fluor-conjugated secondary antibodies (1:300, Molecular Probes) or biotin-conjugated secondary antibodies (1:400, Jackson) using the ABC kit (Vector Laboratories) and DAB Substrate Kit (Pierce). All antibodies were routinely preadsorbed against fixed embryos before use. Samples were incubated overnight in primary antibodies at 4°C, and two hours at room temperature in secondary antibodies. For imaging, embryos were suspended and mounted in VECTASHIELD® (Vector Laboratories) or 70% glycerol.

Image collection and presentation

Fluorescent specimens were imaged using Zeiss LSM 510 META and Olympus Fluoview 500 inverted laser scanning confocal microscopes. Data were collected using sequential scans to avoid artifacts from overlapping emission spectra, with image slices of 0.5 μM. Except as noted, each figure panel shows a single confocal section. Non-fluorescent specimens were collected using Zeiss Axioplan and Nikon E800 compound microscopes

equipped for differential interference contrast illumination. Images were merged, cropped and adjusted for brightness, contrast and levels using Adobe Photoshop versions 10.0.1 and 13.0.1. Except as noted, images of specimens being directly compared (e.g., wild type versus mutant, wing versus leg imaginal discs) were collected using the same microscope settings, and subjected to the same image adjustments.

Characterization of SNb length

To assess the length of segmental nerve projections, maximum projections of Z-series were collected of FASII expression in posterior abdominal segments of late stage 16 embryos. The length of SNb was measured from the branch from the ISN to the most distal tip of SNb using MetaMorph Software. In sd^{3L} embryos, eight segments in three animals were measured, while in controls, seven segments in three animals were measured. The data was analyzed using the Student's t-test with a two-tailed distribution assuming unequal variance. The standard error of the mean (s.e.m) is reported.

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Bullet points

- Scalloped (SD) protein expression is characterized for the first time using an anti-SD antibody.
- SD is expressed in the central and peripheral nervous systems, the musculature and the flight appendage primordia of the Drosophila embryo, and in the larval imaginal discs.
- *sd* function is required for the correct innervation of the somatic muscles by the ventral unpaired median motor neurons.

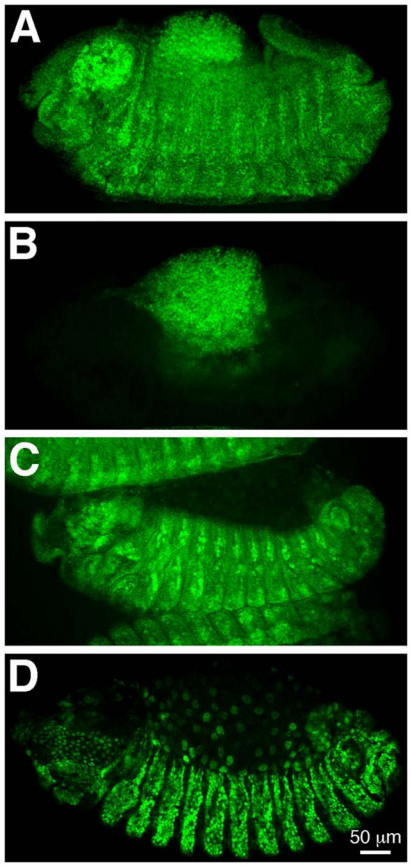

Figure 1. Specificity of the anti-SD antibody

(A, B) Detection of the guinea pig anti-SD antibody in stage 14 embryos of genotypes Df(1)Exel6251 or FM7c *act-lacZ*/FM7c *act-lacZ* (A) and Df(1)Exel6251/Y (B). While the anti-SD antibody is detectable in embryos carrying the balancer chromosome (A; βGAL expression not shown), no detection is observed in embryos missing the *sd* locus (B). (C, D) Detection of the guinea pig anti-SD antibody in stage 12 embryos in which *sd* is expressed at normal levels (C) or overexpressed (D). The pattern of anti-SD antibody detection in embryos carrying *patched-GAL4* is comparable to that observed in wild type embryos (C; compare to Figure 2I), while ectopic expression is detected in embryos that additionally carry *UAS-sd* (D; note that this image was collected using lower laser settings than panel C). Our rat anti-SD antibody showed the same results in *sd* loss of expression and overexpression experiments (data not shown). Anterior is to the left in all panels.

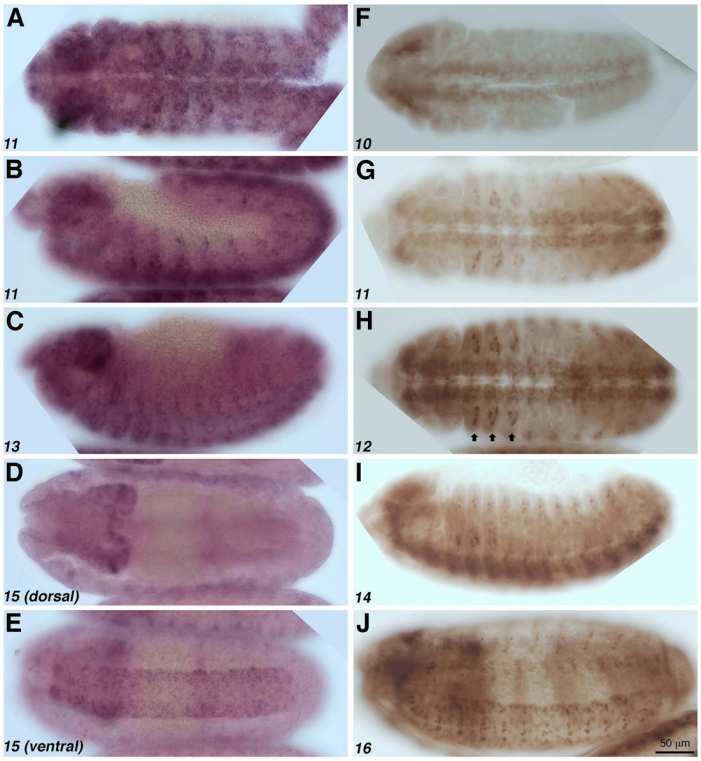

Figure 2. SD expression during embryogenesis

(A–E) Whole mount in situ hybridization showing *sd* RNA distribution (purple) in embryos at stages 11 (A, B), 13 (C), and 15 (D, E). *sd* is expressed in the central nervous system at stages 11 (A) and 15 (D, E) and is also detectable in the peripheral nervous system of embryos at stages 11 (B) and 13 (C). (F–J). SD protein expression (brown). SD is detectable in the ventral nerve cord at stages 10 (F), 11 (G), 12 (H) and becomes localized to subsets of cells by stage 16 (J). SD is also expressed in the peripheral nervous system at stage 14 (I), the developing somatic mesoderm and cells between the dorsal and ventral limb primordia (G–I; arrows, H). Anterior is to the left in all panels, and stages of development are in the lower left.

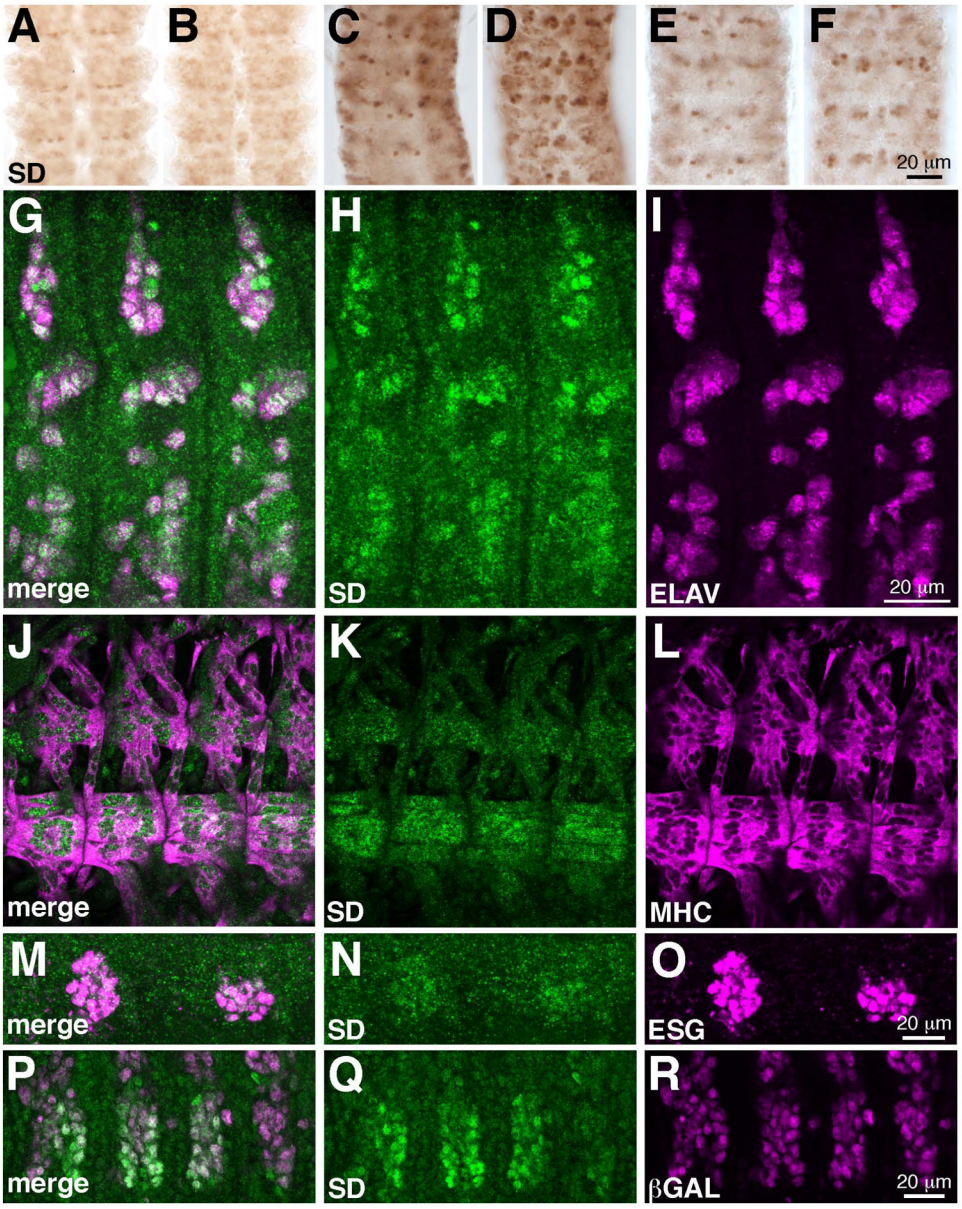

Figure 3. SD expression in the embryonic CNS, PNS, limb primordia, and somatic muscles

(A–F) Expression of SD expression VNCs dissected from stage 12 (A, B), stage 15 (C, D) and stage 16 (E, F) embryos. For each stage, dorsal (A, C, E) and ventral (B, D, F) focal planes are shown. SD is initially broadly expressed in the VNC, and expression resolves to subsets of cells as development proceeds. (G–I) SD (green) and ELAV (magenta) in three abdominal segments of a stage 12 embryo, showing that most SD expressing cells are sensory neurons. These images are maximum projections of multiple confocal slices. (J–L) Four abdominal segments of a late stage 16 embryo showing SD (green) expression in muscles VL1–4 and LL1, which are marked by MHC (magenta). (M–O) SD (green) is coexpressed with ESG (magenta) in the dorsal limb primordia of the second and third thoracic segments of a stage 17 embryo. These images are maximum projections of multiple confocal slices. (P–O) Three thoracic segments and first abdominal segment of a stage 12

embryo. SD (green) is coexpressed with βGAL driven by *twist-GAL4* in the three thoracic segments. Specimens are oriented with anterior up (A–F) or to the left (G–R).

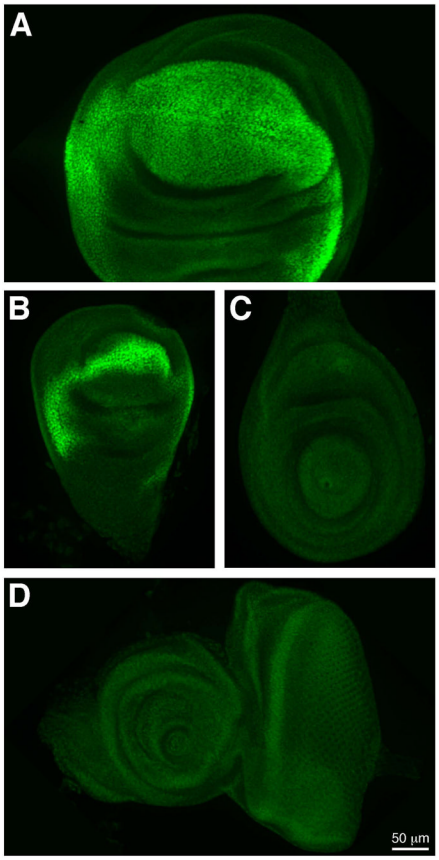

Figure 4. SD expression in larval imaginal discs

SD (green) is expressed in the wing (A) and haltere (B) imaginal discs, and at low levels in the leg (C) and eye antennal (D) discs.

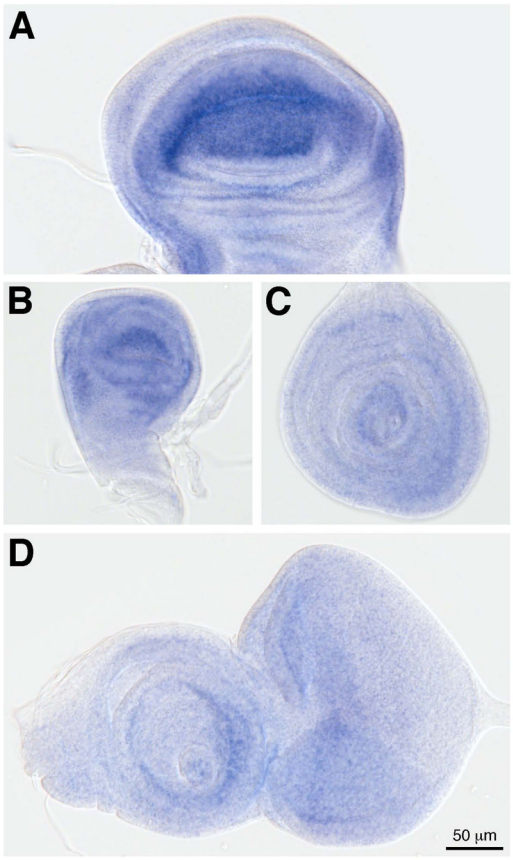

Figure 5. *sd* expression in larval imaginal discs

sd (blue) is expressed in the wing (A) and haltere (B) imaginal discs. It is also expressed at lower levels in the leg (C) and eye antennal (D) discs.

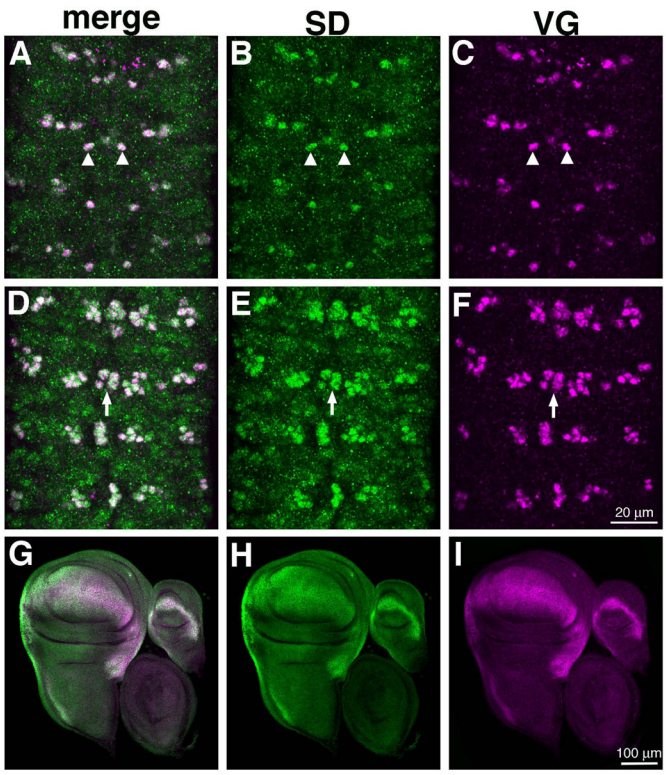

Figure 6. SD and VG coexpression in the ventral nerve cord and imaginal discs

Dorsal (A–C) and ventral (D–F) focal planes of a stage 16 embryonic VNC showing expression of SD (green) and VG (magenta). These images are maximum projections of multiple confocal slices. SD is expressed at high levels in cells that also express VG, including descendants of NB1-2 (arrowheads, A–C) and the mVUMS (arrows, D–E). (G–I) SD (green) and VG (magenta) expression in the somatic muscles of abdominal segments of a stage 15 embryo. At this stage, both proteins are expressed in VLs 1–4 (arrowheads, G–I), but VG is expressed in dorsal muscles as well (arrowheads, G–I). Specimens are oriented with anterior up (A–F) or to the left (G–I). (J–L) Wing, haltere and leg imaginal discs from a wandering third instar larva. SD (green) and VG (magenta) are coexpressed at high levels in the wing and haltere imaginal discs.

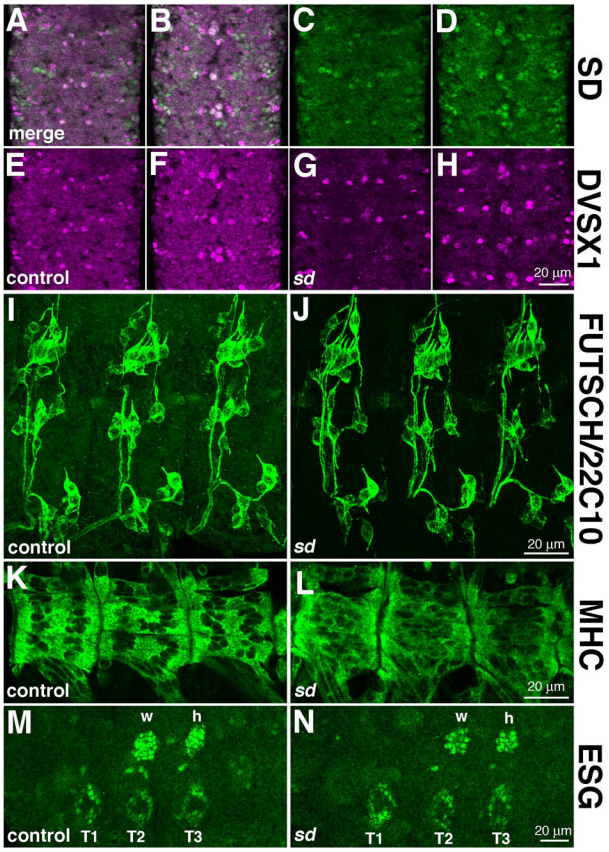

Figure 7. *sd* function is not required for global patterning of the PNS, CNS, muscles, and limb primordia

(A–H) *sd* is not required for cells fate specification of the mVUMs and NB1-2 descendants in the ventral nerve cord. SD (green, A–D) is coexpressed with DVSX1 (magenta, A, B, E–H) in cells in the ventral nerve cord, including the mVUMs and NB1-2 descendants. The distribution of these cells is normal in Df(1)Exel6251 embryos, which are null for *sd*. (I, J) Antibody 22C10 was used to assess the distribution of cell bodies and axons in control (I) and sd^{3L} embryos (J); these images are maximum projections of multiple confocal slices. We observed no reproducible difference between the wild type and mutant, showing that *sd* is not required for general PNS patterning. (K, L) Muscle structure was assessed using myosin heavy chain (MHC) in control (K) and Df(1)Exel6251 (L) embryos. Muscle structure is generally normally in *sd* mutant embryos, although altered distribution of nuclei was observed. (M, N) Distribution of ESG expressing cells in the dorsal and ventral limb primordia of control (M) and Df(1)Exel6251 (N) embryos. The normal distribution of these cells in *sd* mutant embryos confirms the observation that *sd* function is not required for the establishment of the limb primordia or the dorsal migration of the flight appendage cells (marked w, h) from the leg primordia (marked T1, T2, T3). Specimens are oriented with anterior up (A–H) or to the left (I–N).

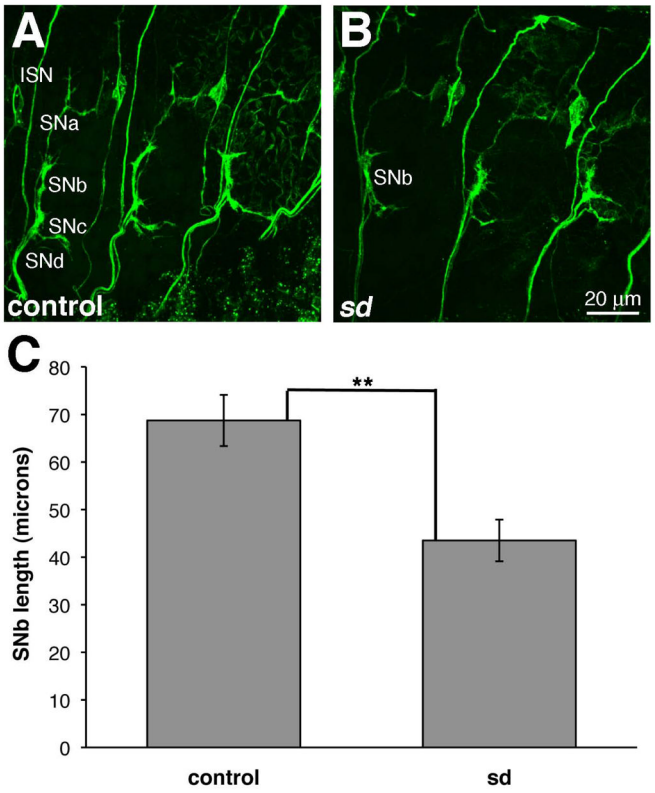

Figure 8. *sd* is required for the innervation ventral muscles

Embryos of genotypes (A) w^{1118} and (B) sd^{3L} showing expression of FASII in three posterior abdominal segments of late stage 16 embryos. ISN and SNa-d are labeled in a representative control segment (A) and SNb is labeled in a segment in the *sd* mutant (B). These images, which are maximum projections of multiple confocal sections, are oriented anterior to the left. (C) SNb length is shorter in the *sd* mutant than the control. Error bars represent the s.e.m, and $p<0.01$.