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Neurally expressed *Drosophila* genes encoding homologs of the NSF and SNAP secretory proteins

(neurotransmitter release/neurogenetics/molecular neurobiology/synaptic transmission)

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ABSTRACT **Several lines of investigation have now converged to indicate that the neurotransmitter release apparatus is formed by assembly of cytosolic proteins with proteins of the synaptic vesicle and presynaptic terminal membranes. We are undertaking a genetic approach in *Drosophila melanogaster* to investigate the functions of two types of cytosolic proteins thought to function in this complex: *N*-ethylmaleimide-sensitive fusion protein (NSF) and the soluble NSF attachment proteins (SNAPs). We have identified *Drosophila* homologs of the vertebrate and yeast NSF and SNAP genes. Both *Drosophila* genes encode polypeptides that closely resemble their vertebrate counterparts and are expressed in the nervous system; neither appears to be in a family of closely related *Drosophila* genes. These results indicate that the *Drosophila* NSF and SNAP genes are excellent candidates for mutational analysis of neurotransmitter release.**

Chemical synaptic transmission is the primary form of signaling between neurons. A critical part of this process is the exocytotic release of neurotransmitter by regulated fusion of synaptic vesicles with the membrane of the presynaptic nerve terminal. Molecular analysis of the apparatus responsible for neurotransmitter release has recently been advanced by the remarkable convergence of studies on regulated and constitutive secretory mechanisms (reviewed in refs. 1–7). This work has indicated that a neurosecretory complex is formed by assembly of cytosolic proteins with proteins of the synaptic vesicle and presynaptic terminal membranes and has revealed key functional components of this complex.

N-ethylmaleimide-sensitive fusion protein (NSF) and the soluble NSF attachment proteins α-, β-, and γ-SNAP are cytosolic proteins now thought to be components of the neurotransmitter release apparatus. Although NSF and the SNAPs were originally identified as proteins required for constitutive secretion in a mammalian cell-free Golgi transport assay (8) and in yeast (6), their assembly with proteins known to function in neurotransmitter release has strongly implicated them in this process as well (5, 9). It is now important to confirm directly that NSF and the SNAPs function in neurotransmitter release, to further define their specific roles, and to investigate their functional interactions with other proteins involved in this process. One powerful approach to addressing these issues is by mutational analysis of NSF and SNAP function. *Drosophila* is an ideal model system for accomplishing this because of the combination of experimental approaches possible in this organism, including classical genetics, molecular genetics, and synaptic electrophysiology (for example see ref. 10). To initiate such an analysis, we have identified *Drosophila* NSF and SNAP homologs expressed in the nervous system.*

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MATERIALS AND METHODS

Isolation and Sequencing of cDNA Clones. ^{32}P-labeled NSF and SNAP probes were used to screen 6×10^5 recombinant phage from a λ ZAP cDNA library (Stratagene) prepared from *Drosophila melanogaster* head mRNA (kindly provided by Tom Schwarz, Stanford University). The NSF probe was generated by PCR amplification of a subfragment of the *SEC18* gene (11) from *Saccharomyces cerevisiae* genomic DNA (strain LRB228; kindly provided by Ching Kung, University of Wisconsin–Madison). Degenerate oligodeoxynucleotide primers (Operon Technologies, Alameda, CA) for PCR corresponded to SEC18 peptide sequences YGPPG [5′-CTCGAATTCTA(C,T)GG(A,C,G,T)CC(A,C,G,T)C-C(A,C,G,T)GG-3′] and PDEKG [5′-CAGGGTACC(C,T)T-T(C,T)TC(A,G)TC(A,C,G,T)GG-3′]. The PCR product was cloned and its identity was confirmed by dideoxynucleotide sequencing from double-stranded templates (Sequenase version 2.0 DNA sequencing kit; United States Biochemical). The SNAP probe was a 0.74-kb *Pst* I/*Sph* I restriction fragment from the mouse β-SNAP cDNA, I47 (12) (kindly provided by Kikuya Kato, Research Development Corporation of Japan, Kyoto).

Library screening was carried out under conditions of low stringency (34% mismatch). Hybridizations were performed for 12–24 hr at 42°C in solutions containing 20% formamide, 10× Denhardt's solution, 5× standard saline citrate phosphate (SSCP), and 250 μg of autoclaved and denatured salmon sperm DNA per ml. Washes were carried out at 42°C in 2× SSC/0.1% SDS. Following purification of phage, cDNA clones in pBluescript SK− plasmids were generated by autoexcision using the Exassist helper phage system (Stratagene). cDNAs were mapped with commercially available restriction enzymes in the buffers supplied (New England Biolabs).

Sequencing of cDNAs was performed using the dideoxynucleotide chain-termination method from single-stranded templates with dITP and pyrophosphatase substituting for dGTP (Sequenase version 2.0 DNA sequencing kit; United States Biochemical). The open reading frames (ORFs) were sequenced completely on both strands. Sequences were analyzed using the PILEUP and DISTANCES programs of the Genetics Computer Group (Madison, WI) software package (13).

Southern Blot Analysis. Southern analysis of cDNAs was performed at high stringency using enhanced chemiluminescence detection (ECL; Amersham). Clones were digested with *Hin*dIII and *Pst* I, separated on a 1.0% agarose gel, and transferred to nylon membrane (Hybond-N; Amersham). Probe labeling, hybridization, washes, and detection of hybridizing bands were performed as described (ECL; Amersham). The

Abbreviation: ORF, open reading frame.
*The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U09373 (dNSF) and U09374 (dSNAP)].

blot of putative NSF clones was probed with the largest putative NSF cDNA, dN20. Similarly, cDNA clone dS2 was used to probe the blot of putative SNAP cDNA clones.

Southern blots of Canton S genomic DNA were hybridized at high and low stringency with probes derived from the *Drosophila* NSF and SNAP cDNAs to search for closely related sequences (those hybridizing only at low stringency). Genomic DNA was separated on an agarose gel and blotted after digestion with the following enzymes: *Ban* I, *Cla* I, *Eco*RI, *Hin*cII, *Pst* I, *Sna*BI, and *Xba* I (New England Biolabs). The NSF probe was a 2.3-kb *Spe* I/*Eco*RV restriction fragment containing most of the ORF of cDNA dN20 (the *Spe* I site derives from pBluescript and is at the 5′ end of dN20). Similarly, a 1.0-kb *Eco*RI/*Bam*HI ORF-containing fragment of the dS2 cDNA was used to probe the SNAP Southern blot. High-stringency hybridizations were carried out for 12–24 hr at 65°C in 10× Denhardt's solution/2× SSCP/250 μg of autoclaved and denatured salmon sperm DNA per ml, followed by several 20- to 30-min washes at 65°C in 0.2× SSC/0.1% SDS. Conditions for low stringency (27% mismatch) consisted of a 12- to 24-hr hybridization at 42°C in 25% formamide/10× Denhardt's solution/5× SSCP/250 μg of autoclaved and denatured salmon sperm DNA per ml, and washes at 42°C in 2× SSC/0.1% SDS.

Northern Blot Analysis. Total RNA was isolated from Canton S adults and third-instar larvae and subjected to poly(A)$^+$ RNA selection as described (14) except that tissue homogenization was carried out with a Brinkman homogenizer. Approximately 5 μg of mRNA per lane was separated on a 0.65 M formaldehyde-agarose gel as described (15) and transferred to nylon membrane (Hybond-N; Amersham). Blots were hybridized with ^{32}P-labeled probes identical to the *Drosophila* NSF and SNAP probes used in the genomic Southern blots (see above) and washed according to the manufacturer's instructions (Hybond-N; Amersham).

***In Situ* Hybridization.** Unstaged Canton S embryos were prepared for tissue *in situ* hybridization as described (16). Following preparation, embryos were fixed with equal parts of 4% formaldehyde in phosphate-buffered saline/50 mM EGTA and heptane by shaking gently for 20 min. The aqueous phase was then removed and embryos were washed once in methanol and three times in ethanol and stored at −20°C. Subsequent fixation, hybridization, and staining of embryos were performed essentially as described for tissue *in situ* hybridization to whole-mount imaginal disks (17). Digoxygenin-labeled antisense RNA probes were generated from the dN20 and dS2 cDNA clones as described (Boehringer Mannheim). Stained embryos were dehydrated in ethanol and then mounted in methyl salicylate for photography. *In situ* hybridization to polytene chromosomes was carried out as described (18) with digoxygenin-labeled DNA probes derived from the same DNA fragments used as probes in the genomic Southern and Northern analyses.

RESULTS

Cloning and Characterization of cDNAs Encoding a *Drosophila* Homolog of NSF. To identify *Drosophila* homologs of NSF, a head cDNA library was screened at low stringency with a probe from the *SEC18* gene, which encodes the *S. cerevisiae* counterpart of NSF (11). From ≈70 positive clones, 11 were characterized and found to be derived from a single gene by hybridization and restriction mapping. Sequencing of the largest clone, a 3.2-kb cDNA termed dN20, revealed that it contains a 2.2-kb ORF (Fig. 1*A*) and terminates in a run of adenylate residues. The polypeptide encoded by dN20 aligns well with NSF from Chinese hamster ovary (CHO) cells and the *SEC18* gene product (Fig. 2). On the basis of this alignment, the dN20 polypeptide shares 62% identities with CHO NSF and 42% with SEC18. These findings indicate that dN20 derives from a *Drosophila* NSF gene, which we designate dNSF.

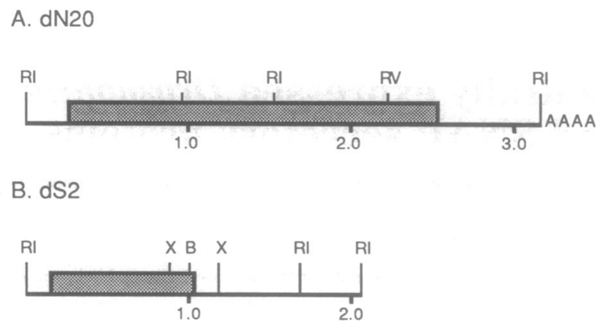

FIG. 1. Restriction maps of cDNAs dN20 (*A*) and dS2 (*B*). Shaded boxes designate protein coding sequences. RI, *Eco*RI; RV, *Eco*RV; X, *Xba* I; B, *Bam*HI.

The dNSF gene maps to position 11D9-E4 on the X chromosome by *in situ* hybridization to polytene chromosomes.

A Single Transcript from the dNSF Gene. A probe from the dNSF cDNA, dN20, detects a single 3.2-kb transcript on a Northern blot of mRNA from adult flies (Fig. 3*A*). A similar result was obtained with mRNA from larvae. The similarity in size of the dNSF transcript and the dN20 clone indicates that this cDNA is full length, or nearly so.

Cloning and Characterization of *Drosophila* SNAP cDNAs. To identify *Drosophila* homologs of the SNAP genes, a head cDNA library was screened at low stringency with a mouse β-SNAP cDNA probe. Among ≈100 positive clones, 9 were purified and were found to derive from a single gene by hybridization, restriction mapping, and sequence analysis.† The largest clone, a 2.0-kb cDNA designated dS2, was sequenced and found to contain a 1.0-kb ORF (Fig. 1*B*). This cDNA is probably incomplete because it does not terminate in a run of adenylate residues.

Sequence alignments show that the polypeptide encoded by dS2 is most closely related to the bovine α- and β-SNAP polypeptides with 62% and 61% amino acid identities, respectively (Fig. 4). The dS2 polypeptide is more distantly related to the *S. cerevisiae SEC17* and bovine γ-SNAP polypeptides with 31% and 20% identities, respectively. In addition, 25 amino acids present in the dS2-encoded polypeptide are identical in all five sequences. These findings indicate that the dS2 cDNA derives from a *Drosophila* SNAP gene, which we designate dSNAP.

The dSNAP gene maps to position 77B1-4 on chromosome 3 by *in situ* hybridization to polytene chromosomes.

Multiple Transcripts from the dSNAP Gene. Northern blot analysis of mRNA from adult flies using a probe derived from the dS2 cDNA detected multiple transcripts ranging in size from 1.5 to 2.3 kb (Fig. 3*B*). Among these were two abundant species of 1.5 and 1.9 kb, as well as a poorly resolved group of larger transcripts. As with dNSF, similar results were obtained using larval mRNA. The size of the dS2 cDNA and the likelihood that it is incomplete at its 3′ end indicate that it derives from the larger class of transcripts. Although the origins of the dSNAP transcript heterogeneity have not been determined, it is of interest to note that a similar complexity of transcripts has been observed for the bovine α- and γ-SNAP genes (19).

†A polymorphism was evident in *Xba* I digests of the nine dSNAP cDNAs. Six of the clones (including dS2) yielded a 0.3-kb *Xba* I fragment, while a 0.33-kb fragment was obtained from the other three clones. Partial sequence analysis of four clones showed them to be identical except for a 26-nucleotide sequence present in the 3′ untranslated region of the two clones containing the 0.33-kb *Xba* I fragment but absent from the remaining two clones. The functional significance of this polymorphism, if any, remains unclear.

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CHONSF                  SEPDV SAKMAGRSMQAARCPTDELS LSNCAVVSEKDYQSG.QHVI VRTSPNHKYIFTLRTHPSVV  64
  dNSF                            MAYILKATKCPTDELS LTNRAIVNVGDFPEEIKYAD ISPAPGQHFIFALEKTVEVP  56
 SEC18 MFKIPGFGKAAANHTPPDMT NMDTRTRHLKVSNCPNNSYA LANVAAVSPNDFPNNIYIII .....DNLFVFTTRHSNDIP  75

CHONSF PGSVAFSLPQRKWAGLSIGQ EIEVA...LYSFDKAKQCIG TMTIEIDFLQKKNIDSNPYD TDKMAAEFIQQFNNQAFSVG 141
  dNSF SGYVGFSLVQRKWAMVSINQ ELEVR...PYRFDASSDVIT CVSFETDFLQKKTVSQEPYD SDQMAKEFIMQFAGMALTVG 133
 SEC18 PGTIGFNGNQRTWGGWSLNQ DVQAKAFDLFKYSGKQSYLG SIDIDISFRARGKAVSTVFD QDELAKQFVRCYESQIFSPT 155

CHONSF QQLVFSFND.KLFGLLVKDI EAMDPSILKGEPASGKRQKI EVGLVVGNSQVAFEKAENSS LNLIGKAKTKENRQSIINPD 220
  dNSF QSLVFNFKDKKLLGLAVKSL EAIDPKSL.GEGKDTAMRNV RFGRILGNTVVQFEKAENSS LNLQGKSKGKVVRQSIINPD 212
 SEC18 QYLIMEFQG.HFFDLKIRNV QAIDLGDIEPTSAVATGIET K.GILTKQTQINFFKGRDGL VNLKSSNSLRPRSNAVIRPD 233

CHONSF WNFEKMGIGGLDKEFSDIFR RAFASRVFPPEIVEQMGCKH VKGILLYGPPGCGKTLLARQ IGKMLNAREPKVVNGPEILN 300
  dNSF WDFGKMGIGGLDKEFNSIFR RAFASRVFPPELVEQLGCKH VKGILLYGPPGTGKTLMARQ IGTMLNAREPKIVNGPQILD 292
 SEC18 FKFEDLGVGGLDKEFTKIFR RAFASRIFPPSVIEKLGISH VKGLLLYGPPGTGKTLIARK IGTMLNAKEPKIVNGPEILS 313

CHONSF KYVGESEANIRKLFADAEEE QRRLGANSGLHIIIFDEIDA ICKQRGSMAGSTGVHDTVVN QLLSKIDGVEQLNNILVIGM 380
  dNSF KYVGESEANVRRLFAEAEEE EKRLGPNSGLHIIIFDEIDA ICKQRGSVAGNSGVHDTVVN QLLTKIDGVDQLNNILVIGM 372
 SEC18 KYVGSSEENIRNLFKDAEAE YRAKGEESSLHIIIFDELDS VFKQRGSRGDGTGVGDNVVN QLLAKMD.VDQLNNILVIGM 392

CHONSF TNRPDLIDEALLRPGRLEVK MEIGLPDEKGRLQILHIHTA RMRGHQLLSADVDIKELAVE TKNFSGAELEGLVRAAQSTA 460
  dNSF TNRRDMIDEALLRPGRLEVQ MEISLPNEQGRVQILNIHTK RMREFNKINDDVDNKEIAAL TKNFSGAELEGLVRAAQSSA 452
 SEC18 TNRKDLIDSALLRPGRFEVQ VEIHLPDEKGRLQIFDIQTK KMRENNMMSDDVNLAELAAL TKNFSGAEIEGLVKSASSFA 472

CHONSF MNRHIK.ASTKVEVDMEKAE SLQVTRGDFLASLENDIKPA FGTNQEDYASYIMNGIIKWG DPVTRVLDDGELLVQQTKNS 539
  dNSF MNRLIK.ADAKVTVDPEAME KLKVNRDDFLHSLEHDIKPA FGTAQEILDNMLARGVINWG APVSNLLEDGMLYVQQAKAP 531
 SEC18 INKTVNIGKGATKLNTKDIA KLKVTREDFLNAL.NDVTPA FGISEEDLKTCVEGGMMLYS ERVNSILKNGARYVRQVRES 551

CHONSF DRTPLVSVLLEGPPHSGKTA LAAKIAEESNFPFIKICSPD KMIGFSETAKCQAMKKIFDD AYKSQLSCVVVDDIERLLDY 619
  dNSF ESSGLVSVLVAGAPNSGKTA LAAQLAKMSDFPFVKVCSPE DMVGYTESAKCLHIRKIFDD AYRSMLSCIVVDNVERLLDY 611
 SEC18 DKSRLVSLLIHGPAGSGKTA LAAEIALKSGFPFIRLISPN ELSGMSESAKIAYIDNTFRD AYKSPLNILVIDSLETLVDW 631

CHONSF VPIGPRFSNLVLQALLVLLK KAPPQGRKLLIIGTTSRKDV LQEMEMLNAFSTTIHVPNIA TGEQLLEALELLGNFKDKER 699
  dNSF GSIGPRYSNMTLQALLVLLK KQPPKGRKLLILCTSSRREV LEEMEMLTAFTSVLHVPNLS KPDHVLAVLENTDIFSKGEI 691
 SEC18 VPIGPRFSNNILQMLKVALK RKPPQDRRLLIMTTTSAYSV LQQMDILSCFDNEIAVPNMT NLDELNNVMIESNFLDDAGR 711

CHONSF TTIAQQV..KGKKVWIGIKK LLMLIEMSLQMDPEYRVRKF LALLREEGASPLDFD      752
  dNSF QAIGKKM..AGKRVFIGIKK LLGLIDMARQTEQSQRAIKF LSKMEEEGGLDMVARQ     745
 SEC18 VKVINELSRSCPNFNVGIKK TLTNIETARHDED..PVNEL VELMTQSA             757
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FIG. 2. Alignment of the deduced amino acid sequence of *Drosophila* NSF (dNSF) with those of CHO NSF and *S. cerevisiae* SEC18. Identities with dNSF are highlighted.

The dNSF and dSNAP Genes Are Expressed in the Embryonic Central Nervous System. To investigate whether dNSF and dSNAP are expressed in the nervous system and thus might be components of the neurotransmitter release apparatus, the expression pattern of these genes was determined by *in situ* hybridization to whole embryos. dNSF transcripts were detected throughout the embryo, with dark staining in the central nervous system (Fig. 5*A*). Similar central nervous system staining was observed for dSNAP, with little expression detected elsewhere in the embryo (Fig. 5*B*). Although the expression of dNSF appears to be more widespread than that of dSNAP, both are clearly expressed in the embryonic central nervous system.

DISCUSSION

We have identified and characterized *Drosophila* homologs of the vertebrate and yeast NSF and SNAP genes encoding proteins that have been implicated in neurotransmitter release. *Drosophila* NSF and SNAP cDNAs were isolated from a head library and their neural expression was confirmed by *in situ* hybridization to whole embryos. Although all of the bovine SNAPs have been shown to be expressed in brain, with β-SNAP expression largely limited to this tissue, neural expression of NSF has not previously been demonstrated. The expression of dNSF and dSNAP in the nervous system indicates that the encoded proteins may be components of the neurotransmitter release apparatus in *Drosophila*.

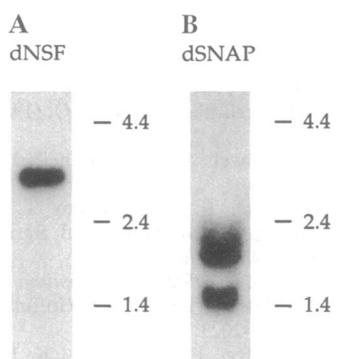

FIG. 3. Northern analysis of poly(A)$^+$ RNA obtained from Canton S adults and probed with a dNSF probe (*A*) and a dSNAP probe (*B*). Numbers on right are kb.

To lay the groundwork for a genetic analysis of dNSF and dSNAP, it is important to consider whether either gene might be part of a family of closely related genes with redundant functions in *Drosophila*. This does not appear to be the case because all of the NSF and SNAP cDNAs isolated in low-stringency screens of the *Drosophila* head library were derived from a single dNSF or dSNAP gene. Furthermore, low-stringency genomic Southern analysis failed to reveal genes closely related to dNSF or dSNAP (see *Materials and Methods*). This apparent lack of redundancy indicates that these genes are promising candidates for mutational analysis.

Although dSNAP does not appear to be in a family of closely related *Drosophila* genes, it is most similar to a pair of bovine genes, α- and β-SNAP, that are closely related to each other (19). Thus, these bovine genes appear to have a single counterpart in *Drosophila*. Consistent with this, dSNAP is equally similar to α-SNAP and β-SNAP, suggesting that a duplication event gave rise to the two bovine genes after the evolutionary divergence of arthropods and vertebrates. Whether there is also a *Drosophila* counterpart to the bovine γ-SNAP gene is unknown. By analogy to the bovine SNAP family, we would expect a *Drosophila* γ-SNAP to be distantly related to dSNAP; thus, it probably would not have been detected in our analyses.

Genetic analysis of neurotransmitter release has been facilitated by identification of several *Drosophila* genes encoding homologs of mammalian proteins involved in this process (20–24). One of these, synaptotagmin, has been

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α-SNAP                          MDNSG KEAEAMALLA EAERKVKNSQ SFFSGLFGGS S.KIEEACEI YARAANMFKM  54
β-SNAP                          MDNAG KEREAVQLMA EAEKRVKASH SFLRGLFGGN T.RIEEACEM YTRAANMFKM  54
dSNAP                             MGD NEQKALQLMA EAEKKLTQQK GFLGSLFGGS N.KVEDAIEC YQRAGNMFKM  52
SEC17                                 MSDPVELLK RAEKKGVPSS GFMKLFSGSD SYKFEEAADL CVQAATIYRL  49
γ-SNAP     MDGAGASAST SGPRREMAAQ KINEGLEHLA KAEK...... .YLKTGFLKW KPDYDSAASE YGKAAVAFKN  63

α-SNAP     AKNWSAAGSA FCQAAHVHLQ LQSKHDAATC FVDAGNAFKK .ADPQEAINC LMRAIEIYTD MGRFTIAAKH 123
β-SNAP     AKNWSAAGNA FCQAAKLHMQ LQSKHDSATS FVDAGNAYKK .ADPQEAINC LNAAIDIYTD MGRFTIAAKH 123
dSNAP      SKNWTKAGEC FCEAATLHAR AGSRHDAGTC YVDASNCYKK .VDVESAVNC LMKSIDIYTD MGRFTMAAKH 121
SEC17      RKELNLAGDS FLKAADYQKK AGNEDEAGNT YVEAYKCFKS GGNSVNAVDS LENPIQIFTH RGQFRRGANF 119
γ-SNAP     AKQFEQAKDA CLKEAVAHEN NRALFHAAKA YEQAGMMLKE MQKLPEAVQL IEKASMMYLE NGTPDTAAMA 133

α-SNAP     HISIAEIYET ELVDIEKAIA HYEQSADYYK GEESNSSANK CLLKVAGYAA QLEQYQKAID IYEQVGTNAM 193
β-SNAP     HITIAEIYET ELVDIEKAIA HYEQSADYYK GEESNSSANK CLLKVAAYAA QLEQYQKAIE IFEQIGANTM 193
dSNAP      HQSIAEMYES DPNNLAKSIQ HYEQAADYFK GEESVSSANK CMLKVAQYAA QLEDYEKAIS IYEQVAASSL 191
SEC17      KFELGEILEN DLHDYAKAID CYELAGEWYA QDQSVALSNK CFIKCADLKA LDGQYIEASD IYSKLIKSSM 189
γ-SNAP     LERAGKLIEN ..VDPEKAVQ LYQQTANVFE NEERLRQAVE LLGKASRLLV RGRRFDEAAI SIQKEKNIYK 201

α-SNAP     DSPLLKYSAK DYFFKAALCH FCIDMLNAKL AVQKYEELFP AFSDSRECKR IKKLLEAHEE QNVDSYTEAV 263
β-SNAP     DNPLLKYSAK DYFFKAALCH FIVDELNAKL ALEKYEEMFP AFTDSRECKL LKKLLEAHEE QNSEAYTEAV 263
dSNAP      ESSLLKYSAK EYFFRAALCH LSVDLLNAQH AIEKYAQQYP AFQDSREFKL IKVLCENLEE QNIEGFTEAV 261
SEC17      GNRLSQWSLK DYFLKKGLCQ LAATDAVAAR TLQEGQSEDP NFADSRESNF LKSLIDAVNE GDSEQLSEHC 259
γ-SNAP     EIENYPTCYK K.TIAQVLVH LHRNDYVAAR RCVRESYSIP GFNGSEDCAA LEQLLEGYDQ QDQDQVAEVC 270

α-SNAP     KE......YD SISRLDQWLT TMLLRIKKTI QGDEE...DL R  295
β-SNAP     KE......FD SISRLDQWLT TMLLRIKKSI QGDGEGDGDL K  298
dSNAP      KD......YD SISRLDQWYT TILLRIKKAA DEDP....DL R  292
SEC17      KE......FD NFMRLDKWKI TILNKIKESI QQQEDDLL      291
γ-SNAP     NSPLFKYMDN DYAKLGLSLV VPGGGVKKKA AAPPQAKPEG TAAPAAEEEE DEYAGGLC  328
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FIG. 4. Alignment of deduced amino acid sequence of *Drosophila* SNAP (dSNAP) with those of the bovine α-, β-, and γ-SNAPs and the *S. cerevisiae SEC17* gene product. Identities with dSNAP sequence are highlighted.

subjected to mutational analysis. Synaptotagmin null mutants have shown that the absence of this protein impairs, but does not abolish, regulated release of neurotransmitter in *Drosophila* (25, 26). These results, together with work in other systems (27, 28), have led to new models of synaptotagmin function (29, 30). Other *Drosophila* homologs include those of the neurosecretory proteins synaptobrevin, Rab 3a, and SNAP-25. All of these homologs, like dNSF and dSNAP, are closely related to their vertebrate counterparts, suggesting that their functional roles in neurotransmitter release have also been conserved. Because of this conservation, and because of the powerful combination of experimental approaches available, *Drosophila* should serve as an excellent model system for the genetic analysis of neurotransmitter release. Such an analysis of dNSF and dSNAP function promises to further define their roles in neurotransmitter release and to reveal their functional interactions with other proteins involved in this process.

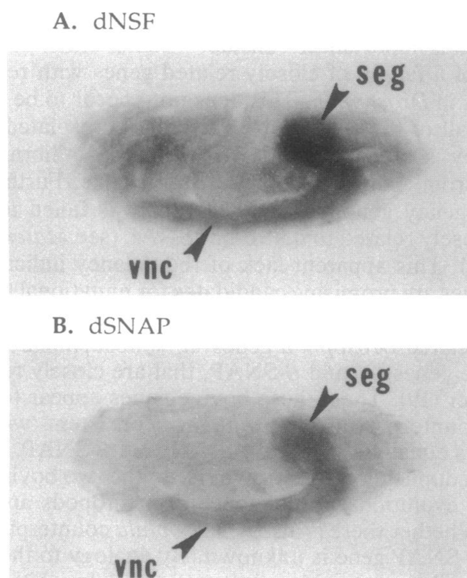

FIG. 5. Embryonic expression patterns of dNSF (*A*) and dSNAP (*B*) determined by *in situ* hybridization. Embryos are oriented with the anterior end to the right and the dorsal surface up. Note that for both dNSF and dSNAP, prominent staining is observed throughout the central nervous system including the ventral nerve cord (vnc) and supraesophageal ganglia (seg).

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