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gutfeeling, a Drosophila Gene Encoding an Antizyme-Like Protein, Is Required for Late Differentiation of Neurons and Muscles

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ABSTRACT

The *gutfeeling* (*guf*) gene was uncovered in a genetic screen for genes that are required for proper development of the embryonic peripheral nervous system. Mutations in *guf* cause defects in growth cone guidance and fasciculation and loss of expression of several neuronal markers in the embryonic peripheral and central nervous systems. *guf* is required for terminal differentiation of neuronal cells. Mutations in *guf* also affect the development of muscles in the embryo. In the absence of *guf* activity, myoblasts are formed properly, but myoblast fusion and further differentiation of muscle fibers is severely impaired. The *guf* gene was cloned and found to encode a 21-kD protein with a significant sequence similarity to the mammalian ornithine decarboxylase antizyme (OAZ). In mammals, OAZ plays a key regulatory role in the polyamine biosynthetic pathway through its binding to, and inhibition of, ornithine decarboxylase (ODC), the first enzyme in the pathway. The elaborate regulation of ODC activity in mammals still lacks a defined developmental role and little is known about the involvement of polyamines in cellular differentiation. GUF is the first antizyme-like protein identified in invertebrates. We discuss its possible developmental roles in light of this homology.

THE development of the peripheral nervous system (PNS) is a complex process that requires the sequential activity of a large number of genes (for review see JAN and JAN 1993; for additional genes see SALZBERG *et al.* 1994; KANIA *et al.* 1995). The PNS of Drosophila embryos serves as an excellent model system to study the genetic and molecular basis underlying these processes due to its simple structure, the availability of many markers, and the amenability of the fly to genetic manipulations. During early stages of embryonic development, a small number of ectodermal cells are selected and determined as sensory organ precursors (SOP) (reviewed by CAMPOS-ORTEGA 1988). These precursors then divide and their progeny differentiate to give rise to neurons and associated support cells that constitute various types of sensory organs.

Whereas the role of genes involved in early determinative events that regulate SOP recruitment has been partially elucidated (reviewed by CAMPUZANO and MODOLELL 1992; GHYSEN *et al.* 1993), little is known about genes that play a role in terminal stages of neuronal differentiation. Several genes that are expressed in all the SOPs following their selection have been identified (*e.g.*, BELLEN *et al.* 1992; BIER *et al.* 1992; KANIA *et al.* 1993). These genes may be required for early aspects of neural differentiation. However, their function in sensory organ formation is still unclear. One exception is the *prospero* gene that was shown to affect differentiation, but not identity of sensory neurons. In the absence of *prospero*, the axonal trajectories in the periphery are aberrant. The *prospero* gene is thought to be involved in regulation of gene expression as in its absence the expression of other neuronal precursor genes persists longer than normal in the developing PNS (VAESSIN *et al.* 1991).

Many lines of similarity can be drawn between the developing PNS and the developing muscles. Like the specification of SOPs in the PNS, muscle development is thought to begin with the specification of a single founder cell for each muscle (BATE 1990). The founder cells then fuse with neighboring fusion-competent cells to form syncytial muscle precursors with specific identities. These precursors then differentiate to form a specific muscle fiber following the developmental program presumably dictated by the founder cells. One difference in the developmental strategies used in these two systems is that PNS development is a strictly lineage dependent process, whereas muscle development involves the recruitment of neighboring nonlineage-related cells. However, despite this difference, common mechanisms seem to be used for the specification of primary precursors through the activation of helix-loop-helix proteins (for review see LASSAR and WEINTRAUB 1992; JAN and JAN 1995). In addition, the neurogenic genes have been shown to play a similar role in both systems where they function to restrict the number of

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sensory organ and muscle precursors that are singled out from a population of ectodermal or mesodermal cells respectively (CORBIN *et al.* 1991; BATE *et al.* 1993; NYE *et al.* 1994). Interestingly, mutations in genes that have been shown to participate in PNS development, such as *numb*, *extramacrochaetae*, and *tramtrack*, also affect muscle development (UEMURA *et al.* 1989; CUBAS *et al.* 1994; GUO *et al.* 1995). Hence, muscle and nervous system development clearly share some molecular pathways required for determination and differentiation.

In recent years we have initiated systematic screens for genes that play a role in embryonic PNS development. We have carried out mutagenesis screens on the second and third chromosome of Drosophila to identify mutations that disrupt normal PNS development in the embryo (SALZBERG *et al.* 1994; KANIA *et al.* 1995; A. SALZBERG and H. J. BELLEN, unpublished data). Here we report the phenotypic analysis and cloning of the *gutfeeling (guf)* gene that was uncovered in one of these screens (KANIA *et al.* 1995). In its absence, neural precursors are determined and divide properly to give rise to neurons and associated support cells. However, these neurons fail to complete terminal neural differentiation and express very reduced amounts of many late neuronal markers. Interestingly, mutations in *guf* also cause defects in the embryonic musculature. In the absence of *guf*, myoblasts are formed properly and the identity of different muscles is specified correctly. However, myoblast fusion and further differentiation of muscle fibers is impaired.

The *guf* gene was cloned and found to encode a homologue of the vertebrate ornithine decarboxylase antizyme (OAZ). Ornithine decarboxylase (ODC) is the first enzyme in the polyamine biosynthetic pathway, and its activity is regulated by an elaborate array of mechanisms that respond to various cellular signals for growth or differentiation (reviewed by HAYASHI 1989). OAZ binds to ODC, inhibits its function and induces its rapid degradation (HAYASHI and CANELLAKIS 1989; MURAKAMI *et al.* 1992). In addition, OAZ represses polyamine uptake into the cell (MITCHELL *et al.* 1994; SUZUKI *et al.* 1994). Polyamines are essential for most living cells and have been implicated in multiple cellular processes such as the control of DNA, RNA and protein synthesis, post-translational modifications and protein cross linking (reviewed by HEBY 1981; TABOR and TABOR 1984). However, despite the wealth of biochemical data, the complex regulation of ODC activity and polyamine levels still lack defined developmental roles. The data presented here suggest that GUF, a putative antizyme in Drosophila, is required for cellular differentiation in neuronal and myogenic lineages during embryonic development.

MATERIALS AND METHODS

Molecular techniques: Sequencing of genomic and cDNA clones was performed using the dideoxy nucleotide chain termination method (SANGER *et al.* 1977) with the automated fluorescence procedure (Applied Biosystems) in the Department of Molecular and Human Genetics sequencing core. Poly(A)$^+$ RNA was prepared from different developmental stages of Canton-S embryos and larvae as described by SAMBROOK *et al.* (1989). Library screening, Southern and Northern analyses were conducted as described in SAMBROOK *et al.* (1989). Sequence analysis and data base searches were carried out using the Genetic Computer Group (GCG) sequence analysis software package.

Fly strains and genetic protocols: All stocks were maintained on standard Drosophila medium at room temperature (ASHBURNER 1989). Genetic nomenclature is as outlined by LINDSLEY and ZIMM (1992). Canton-S was used as the wild-type strain in this study. The *P*-element insertion strains 118/3, 95/12, and 95/40 were generated by TÖRÖK *et al.* (1993) and are of the *y w; P{lacZ,w$^+$}/CyO* genotype. The *P{lacZ,w$^+$}* is an enhancer detector described in BIER *et al.* (1989).

Excision mutagenesis was conducted as follows: 118/3 virgins were crossed to *Sp/CyO; ry^{506}Sb P{ry$^+$, Δ2-3}99B/TM6* males. Individual *y w; P{lacZ, w$^+$}/CyO; ry^{506}Sb P{ry$^+$, Δ2-3}99B/+* male progeny with mottled eyes were crossed to *w; Alp/CyO* virgins and the progeny was scored for loss of eye color. Fifty-seven w^- excision strains (50 lethal and seven viable) were established. Genomic DNA of the excision strains was analyzed by PCR to determine the molecular nature of the excision event. The primers used were the 31 bp of the *P*-element inverted repeat 5′CGACGGGACCACCTTATGTTATTTCATCATG3′ and two genomic primers located 154 bp upstream and 242 bp downstream of the 118/3 insertion: 5′GCCGGTGATGGTTTCGCAAGTGATTATG3′ and 5′AACGAAAGGCTTATCAGCGAAGCGGCGA3′, respectively. Lethal excision strains that did not contain *P*-element sequences (based on the PCR data) were further characterized by Southern analysis to identify deletions caused by imprecise excision events.

RNA *in situ* hybridization to whole mount embryos: Digoxigenin-labeled RNA probes were prepared as described in the RNA labeling and detection kit (nonradioactive) from Boehringer Mannheim. *In situ* hybridization to whole mount embryos was performed as described by INGHAM *et al.* (1991). The full length cDNA was used as a template to synthesize RNA probes for studying the distribution of *guf* transcripts in wild-type embryos. To assess the presence of *guf* transcripts in the deletion allele *guf^{lex47}*, a shorter probe corresponding to the ORF and 3′ untranslated region was generated by using the 900-bp *Sal*I to 3′ *Eco*RI fragment as a template. For unambiguously identifying the homozygous *guf^{lex47}* embryos, a *CyO, wg-lacZ* balancer was used and a *lacZ* RNA probe was added to the hybridization.

Immunocytochemistry and β-galactosidase activity staining: For β-galactosidase (β-gal) activity staining, embryos were dechorionated in bleach washed with water and fixed for 20 min in a 1:1 mixture of heptane and fixative (0.1 M phosphate buffer pH 7.6 plus 4% formaldehyde). After fixation embryos were washed thoroughly with 0.1 M phosphate buffer containing 0.5% Triton X-100 and 0.3% deoxycholate (PBT) and incubated in staining solution (10 mM phosphate buffer pH 7.2, 150 mM NaCl, 1 mM MgCl2, 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide, and 0.2% X-gal). To remove vitelline membrane following X-gal staining, the embryos were placed in equal volumes of methanol and heptane and shaken vigorously. Embryos were then washed twice with methanol and twice with PBT and immunocytochemically stained using standard techniques (BIER *et al.* 1989).

For the characterization of embryonic phenotypes, the antibodies listed below were used. MAbs 22C10 and 1D4 (GOODMAN *et al.* 1984; VAN VACTOR *et al.* 1993) were used in a 1:50

dilution. Polyclonal anti-sera against Pros (VAESSIN *et al.* 1991), Cut (CLP2, BLOCHLINGER *et al.* 1990), RK2 (CAMPBELL *et al.* 1994), Synaptotagmin (LITTLETON *et al.* 1993), Drosophila Muscle Myosin (KIEHART and FEGHALI 1986), D-MEF2 (LILLY *et al.* 1995) and Neurotactin (DE LA ESCALERA *et al.* 1990; HORTSCH *et al.* 1990) were used in a 1:1000 dilution. Anti-MSP-300 anti-serum (VOLK 1992) was used in a 1:300 dilution. Anti-sera against CPO (BELLEN *et al.* 1992), Atonal (JARMAN *et al.* 1994) and EVE (FRASCH *et al.* 1987) were used in a 1:5000 dilution.

RESULTS

Isolation of mutations in the *guf* gene: Mutations in the *gutfeeling* (*guf*) gene were uncovered in a genetic screen designed to identify genes that play a role in the development of the embryonic PNS (KANIA *et al.* 1995). Embryos from 2000 strains carrying homozygous lethal *P*-element insertions on the second chromosome (TÖRÖK *et al.* 1993) were stained with monoclonal antibody (MAb) 22C10 (FUJITA *et al.* 1982; GOODMAN *et al.* 1984) and examined for defects in the PNS. The *guf* gene was identified by three insertional mutations that caused similar phenotypes and were mapped to cytological position 48E5-12 (strains 118/3, 95/12 and 95/40; KANIA *et al.* 1995). Precise or near precise excision of the *P*{118/3} insertion in *guf* reverts both the lethality and the PNS phenotype, demonstrating that the insertion causes the phenotype.

Additional *guf* alleles were generated by imprecise excision of *P*{118/3}. One allele, guf^{lex47}, corresponds to a small deficiency that removes most of the *guf* transcription unit and the entire open reading frame (see below). This deletion was found to abolish zygotic expression of *guf* (see below) and hereafter is referred to as a null allele. The insertional alleles, $guf^{P95\text{-}12}$, $guf^{P95\text{-}40}$ and $guf^{P118\text{-}3}$ cause phenotypes that are very similar to those observed in guf^{lex47} embryos (see below), suggesting that they are severe loss of function or null alleles.

***guf* is required for terminal differentiation in the PNS:** Mutations in *guf* are embryonic lethal and cause multiple defects in the embryonic PNS. First, a smaller number of PNS neurons express 22C10 in homozygous *guf* embryos than in wild-type embryos and the overall intensity of 22C10 immunoreactivity is much decreased when compared to wild-type embryos (KANIA *et al.* 1995; Figure 1, A–C). The lateral and ventral PNS clusters are more severely affected than the dorsal and ventral clusters. On average, six to seven neurons are labeled with MAb 22C10 in lateral clusters of *guf* embryos as compared to 10–11 in wild-type (open arrows in Figure 1, A–C), and only three to four neurons label in the ventral cluster of mutant embryos as compared to seven in wild-type (not shown). The lateral chordotonal neurons seem to be most severely affected by mutations in *guf* and only one to three chordotonal neurons are evident in each lateral cluster as compared to five in wild-type embryos. However, the loss of 22C10 immunoreactivity is not restricted to chordotonal neurons and is evident in all types of sensory neurons.

In addition to the lack of 22C10 expression, PNS axons of *guf* embryos display severe defects in growth cone guidance and fasciculation (Figure 1, A–C). One of the most noticeable defects that is observed in all *guf* embryos is splitting of the intersegmental nerve (ISN) into two fascicles (closed arrows in Figure 1, B and C). The ISN "loop" phenotype was observed in 57% of abdominal segments in $guf^{P118\text{-}3}$ homozygous embryos ($n = 168$ segments) and 62% of abdominal segments in guf^{lex47} embryos ($n = 121$ segments). Crossing of the ISN into neighboring segments (see Figure 1C) is much less frequent and was observed in single segments of ~40% of the examined guf^{lex47} embryos. Defects in growth cone guidance and fasciculation are not restricted to ISNs but are also evident in segmental nerves.

MAb 22C10 is a relatively late marker for PNS development as neuronal cells express the 22C10 antigen only after they exit the cell cycle and start differentiating as neurons. Thus, lack of 22C10 expression may indicate a true loss of neurons stemming from a failure of precursor formation, failure of precursor division, transformation of neurons into lineage-related support cells, or cell death. Alternatively, this phenotype may reflect a failure of neuronal cells to fully differentiate and express late neuronal markers. To distinguish among these possibilities, we used a variety of cell-type-specific markers to assess number and identity of PNS cells in *guf* embryos of various developmental stages. Since chordotonal neurons are among the most severely affected neurons (Figure 1, B and C), we mostly focused our analysis on the formation of the conspicuous cluster of lateral chordotonal organs.

To determine whether the precursors of chordotonal organs were affected in *guf* embryos, we immunocytochemically stained them with antibodies against the Atonal protein (JARMAN *et al.* 1994). The pattern of chordotonal SOPs in the mutant was found to be indistinguishable from that of wild-type embryos (data not shown). To determine whether SOP division is impaired, we examined the PNS of mutant embryos with anti-Couch potato (CPO) antibodies (BELLEN *et al.* 1992). The CPO protein is present in nuclei of all the cells that constitute each sensory organ. Thus, the number of CPO positive cells reflects the number of cell divisions each precursor went through. As shown in Figure 1E, the level of CPO protein is reduced in *guf* mutant embryos when compared to wild-type (compare Figure 1, E and D), but a wild-type number of CPO positive nuclei is evident in each chordotonal organ. These observations indicate that zygotic *guf* is not required for the determination of SOPs or their division but is required during later stages of PNS development.

Loss of 22C10 immunoreactivity may reflect a transformation of chordotonal neurons into another type of neurons that normally express lower levels of this

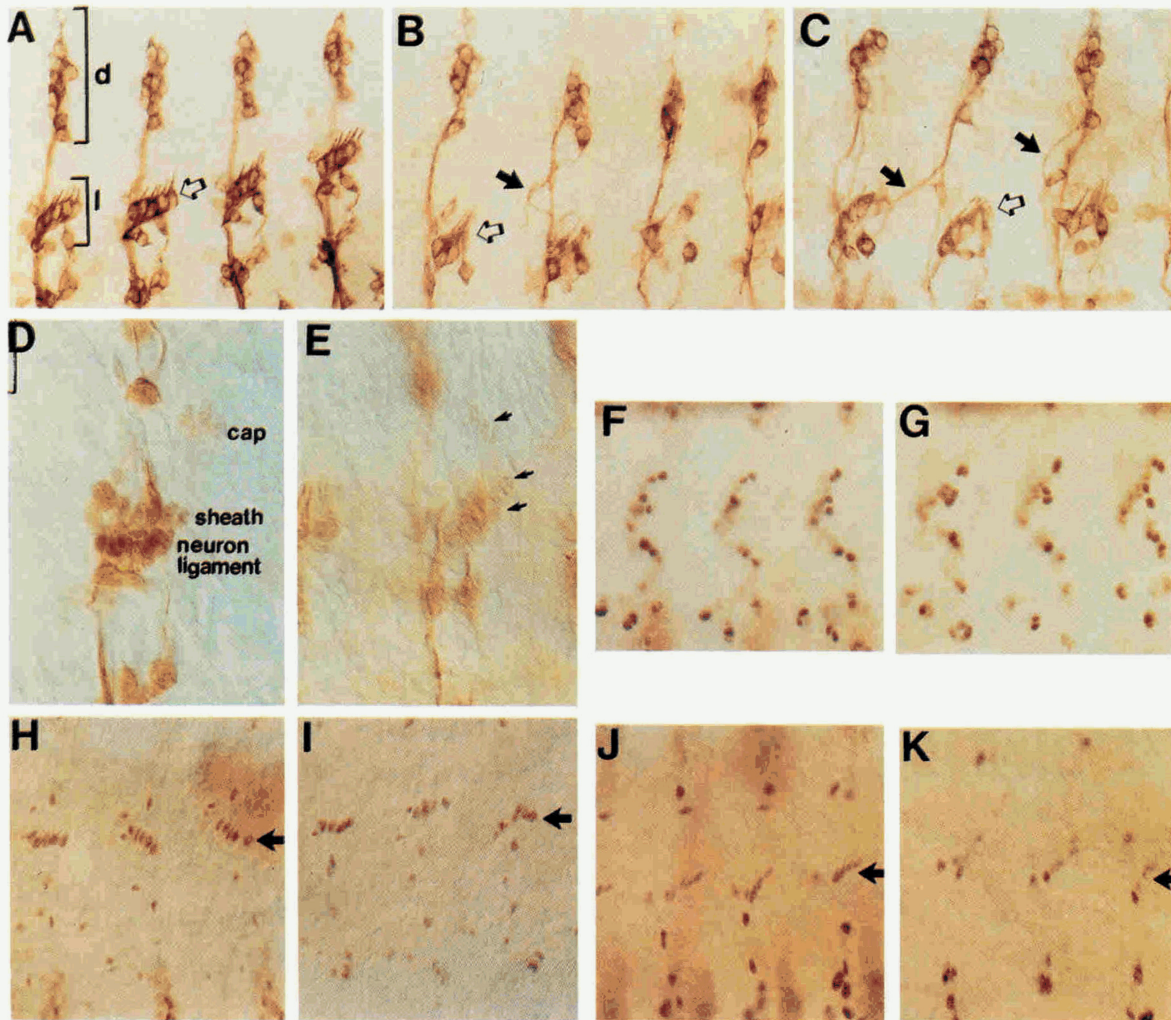


FIGURE 1.—Loss of *guf* activity alters differentiation but not cell identity in the PNS. All the micrographs in Figure 1 are oriented such that anterior is to the left and dorsal is up. (A–C) Abdominal segments of stage 16 wild-type (A), *guf*$^{P118\text{-}3}$ homozygous (B), and *guf*^{lex47} homozygous (C) embryos stained with MAb 22C10. The dorsal (d) and lateral (l) PNS clusters are indicated in A, and the lateral chordotonal organs are marked with open arrows. Note the obvious reduction in the level of 22C10 expression, the defasciculated appearance of the intersegmental nerve and the misrouting of axons (closed arrows) in mutant embryos. (D and E) Detailed view of lateral PNS clusters of wild-type (D) and *guf*^{lex47} (E) embryos doubly labeled with MAb22C10 (labels neuronal membranes, darker brown) and anti-CPO (nuclear staining in all PNS cells). The cap cells, sheath cells, neurons and ligament cells are indicated in D. The intensity of anti-CPO and 22C10 staining is severely reduced in mutant embryos. However, cap cells, sheath cells and neurons are still identifiable. The ligament cells that normally stain faintly with anti-CPO cannot be seen in this micrograph. (F and G) Stage 15 *guf*^{lex47}*/CyO, P{wg-lacZ}* (F) and homozygous *guf*^{lex47} (G) embryos doubly labeled with anti β-gal (brown stripes in F) and anti-Cut antibody (clp2, BLOCHLINGER *et al.* 1990). No obvious defects are observed in the PNS of mutant embryos when stained with this antibody. (H and I) Stage 15 *guf*^{lex47}*/CyO, P{wg-lacZ}* (H) and homozygous *guf*^{lex47} (I) embryos doubly labeled with anti β-gal (brown stripes) and anti-Pros antibody (VAESSIN *et al.* 1991). Nuclei of chordotonal sheath cells are indicated by arrows. (J and K) Similar view of *guf*^{lex47}*/CyO, P{wg-lacZ}* (J) and homozygous *guf*^{lex47} (K) embryos doubly labeled with anti β-gal (brown stripes) and anti-Repo antibody (RK2; CAMPBELL *et al.* 1994) that reveals peripheral glia and ligament cells of the lateral chordotonal organs (arrows). Note that ligament cells in *guf* mutants express decreased levels of the Repo protein and they appear less organized than in wild-type embryos.

antigen. Hence, we stained embryos with anti-Cut antibody (BLOCHLINGER *et al.* 1990) that labels only external sensory organs and a subset of multiple dendritic neurons allowing us to establish whether the identity of chordotonal neurons is altered in *guf* mutants. As shown in Figure 1, F and G, the pattern of Cut expression appears normal in *guf* embryos, indicating no change in the identity of sensory organs. To determine whether the identity of cells within each chordotonal organ is altered, we stained embryos with anti-Prospero (VAESSIN *et al.* 1991) to reveal sheath cells, and the anti-Repo/RK2 antibody (CAMPBELL *et al.* 1994; HALTER *et al.* 1995) to reveal ligament cells. As shown in Figure 1, H–K, normal numbers of sheath and ligament cells are evident in most lateral chordotonal organs of *guf* embryos. The results of these experiments suggest that loss of *guf* activity does not alter the identity of sensory organs, nor does it alter the identity of cells within each

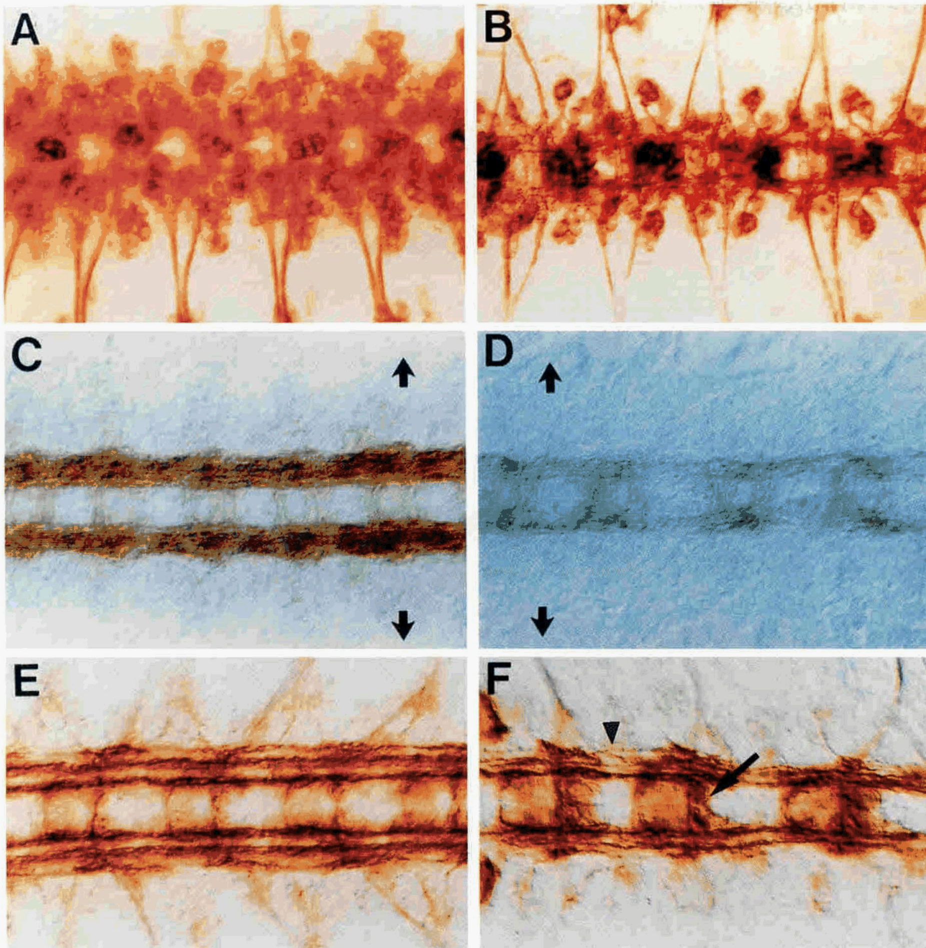


FIGURE 2.—CNS phenotype of *guf* mutant embryos. (A and B) Ventral view of the ventral nerve cord of stage 16 wild-type (A) and guf^{lex47} (B) embryos immunocytochemically stained with MAb 22C10 (anterior is to the left). Severe reduction in the number of 22C10-expressing cells is evident in mutant embryos. (C and D) Dissected nerve cords of wild-type (C) and guf^{lex47} (D) embryos stained with an anti-Synaptotagmin antibody (LITTLETON *et al.* 1993). The level of Synaptotagmin protein is greatly reduced in the mutant and the nerve tracts appear narrower than in wild-type embryos. Note that the overall width of the CNS is similar in mutant and wild-type embryos (indicated with arrows). (E and F) Anti-Fas II staining reveals defects in axonal pathways of CNS neurons in the ventral nerve cord. Wild-type (E), guf^{lex47} (F) embryos stained with MAb 1D4 and filleted. Whereas three distinct fascicles can be seen on either side of the midline in wild-type embryos (E), only two fascicles are evident in most segments of *guf* embryos (F). In addition, the nerve fascicles appear defasiculated in the mutant (arrowhead in F) and the commissures appear thicker than normal (arrow).

organ. We conclude that *guf* is not required for cell fate determination but rather for terminal differentiation of the developing PNS cells.

Whereas cell type-specific antigens are expressed in a normal spatial pattern in the PNS of *guf* mutants, the level of many markers is greatly reduced. Reduced expression levels were observed for the 22C10 antigen (Figure 1, B and C) and CPO protein in mature embryos (Figure 1E). In addition, the level of the glial homeo protein Repo in ligament cells of the lateral chordotonal organs, but not in exit and peripheral glia, is severely decreased when compared to wild-type embryos (arrow, Figure 1, K and J). However, the levels of Prospero and Cut proteins in the PNS seem to remain unaffected by mutations in *guf* (Figure 1, G and I). These observations indicate that mutations in *guf* may exert their effect on late PNS differentiation by altering protein levels.

Central nervous system (CNS) neurons of *guf* embryos exhibit similar defects to those observed in the PNS: As mutations in *guf* seem to affect most or all types of sensory neurons, we examined the CNS of mutant embryos to determine whether CNS neurons are affected in a similar fashion. *guf* embryos exhibited a severe reduction in the number of 22C10-expressing cells in the ventral nerve cord when compared to wild-type embryos (Figure 2, A and B). However, the number of neuroblasts in the CNS of stage 10–11 *guf* embryos appeared normal when visualized with antibodies against Prospero (data not shown). The overall width of the ventral nerve cord appeared normal when examined with Nomarski optics (demarcated by arrows in Figure 2, C and D). This suggests that like in the PNS, the lack of 22C10 expression in the CNS does not reflect a true loss of neuronal cells but a failure of these cells to fully differentiate as neurons.

To further assess late neuronal differentiation in the CNS, we stained embryos with antibodies against the synaptic vesicle protein Synaptotagmin (LITTLETON *et al.* 1993). This protein serves as a terminal differentiation marker, as expression and synaptic localization of Synaptotagmin just precedes synaptic activity (LITTLETON *et al.* 1993). As shown in Figure 2, C and D, Synaptotagmin is barely detectable in *guf* embryos when compared to wild type. This observation again supports the view that mutations in *guf* impair late neuronal differentiation by altering protein levels.

To examine the axonal pathways in the CNS of *guf* mutants, we immunohistochemically stained embryos with MAb 1D4 that recognizes the Fasciclin-II (Fas II) protein (VAN VACTOR *et al.* 1993). In wild-type embryos, anti-FAS-II staining reveals three distinct longitudinal

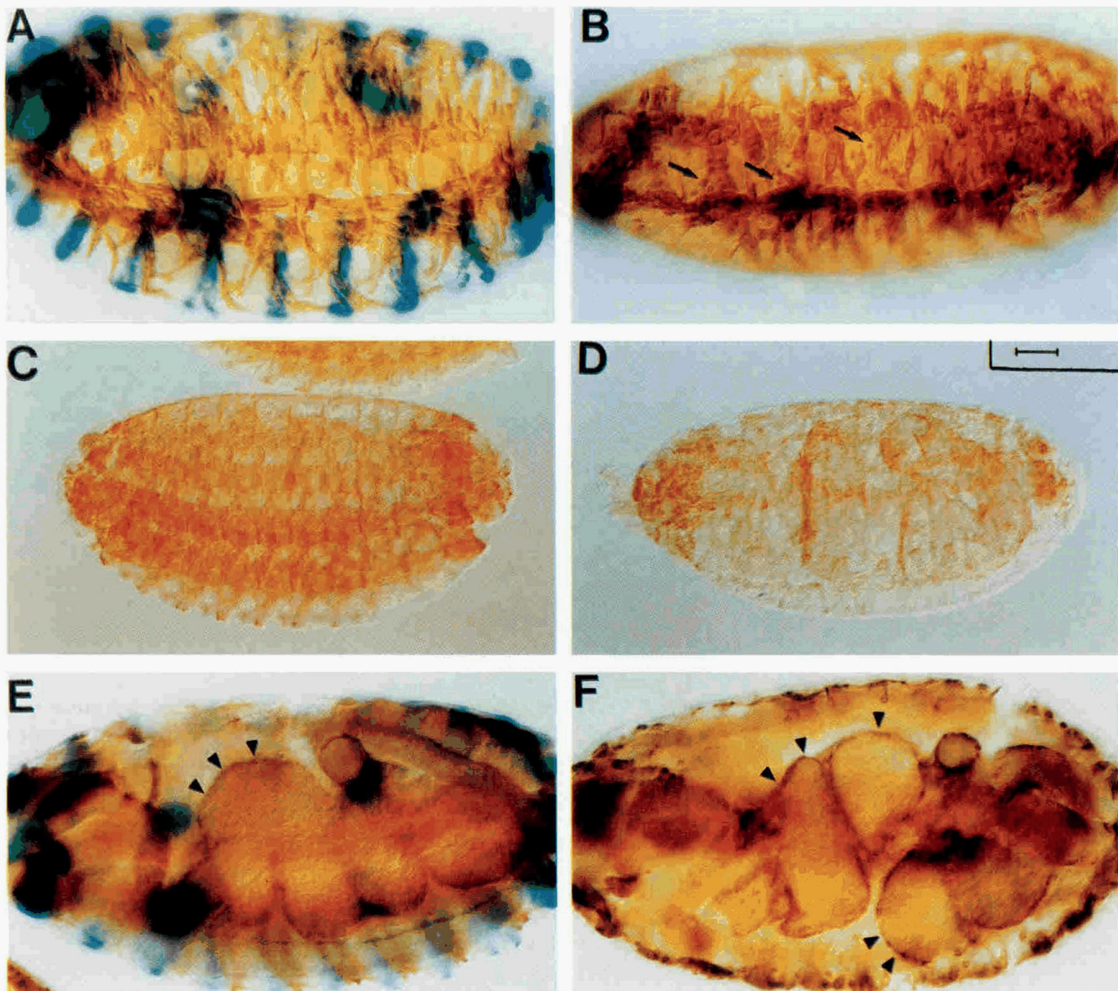


FIGURE 3.—*guf* is required for muscle differentiation. The embryo in micrograph (C) is oriented such that anterior is to the right and dorsal is up. All other embryos in this figure are oriented such that anterior is to the left. (A) Lateral view of a stage 16 $guf^{P118\text{-}3}$/CyO, *P*{wg-lacZ} embryo stained for β-gal activity (blue stripes) and immunocytochemically stained with antibodies against Drosophila Muscle Myosin (DMM, KIEHART and FEGHALI 1986). Note the highly organized array of bodywall muscles in every segment. (B) A similar view of homozygous $guf^{P118\text{-}3}$ embryo reveals many muscle abnormalities. Myoblasts often fail to fuse and remain mononuclear (small arrows) and many muscle fibers display abnormal shapes. The degree of abnormalities is quite variable from embryo to embryo in $guf^{P118\text{-}3}$ as well as in the deletion mutant guf^{lex47}. (C and D) Lateral view of stage 16 embryos stained with antibodies against MSP-300 (VOLK 1992). Note the severe reduction in the level of MSP-300 protein in guf^{lex47} embryo (D) when compared to wild-type embryo (C). (E and F) Anti-Myosin staining reveals defects in visceral muscle formation in the absence of *guf*. (E) Lateral view of a $guf^{P118\text{-}3}$/CyO, *P*{wg-lacZ} embryo stained with X-gal (blue stripes) and anti-Myosin. The visceral muscles form a thin smooth layer around the gut (arrows). (F) A similar view of $guf^{P118\text{-}3}$ homozygous embryo reveals the rippled appearance of the visceral muscles.

tracts on either side of the midline (Figure 2E). The organization of these tracts in *guf* embryos is aberrant (Figure 2F). Although three distinct fascicles can be identified in some segments, most often only two fascicles are evident and these fascicles appear less tight than normal. The most medial fascicle, MP1, is the least severely affected, whereas the most lateral fascicle is the most severely affected. In addition, the posterior commissure appears thicker than normal (arrow in Figure 2F).

In summary, the defects observed in the CNS of *guf* embryos are very similar to those observed in the PNS and consist of alterations in the protein levels of neuronal-specific markers coupled to misrouting of axons and defects in fasciculation. These data suggest that *guf* plays a similar role in PNS and CNS development. However, further analysis of cell identities in the CNS of *guf* mutants is required to exclude the possible involvement of *guf* in cell fate determination in the CNS.

Muscle differentiation is impaired in the absence of *guf*: Examination of *guf* embryos with Nomarski optics indicated that mutant embryos also display defects in muscle development. To study the fully developed muscle pattern, we stained embryos with antibody against Drosophila Muscle Myosin (KIEHART and FEGHALI 1986). This antibody labels all somatic muscle fibers, the cardial cells and the visceral muscles. As shown in Figure 3A, somatic muscles of the body wall are organized in a highly stereotyped pattern in wild-type embryos. This

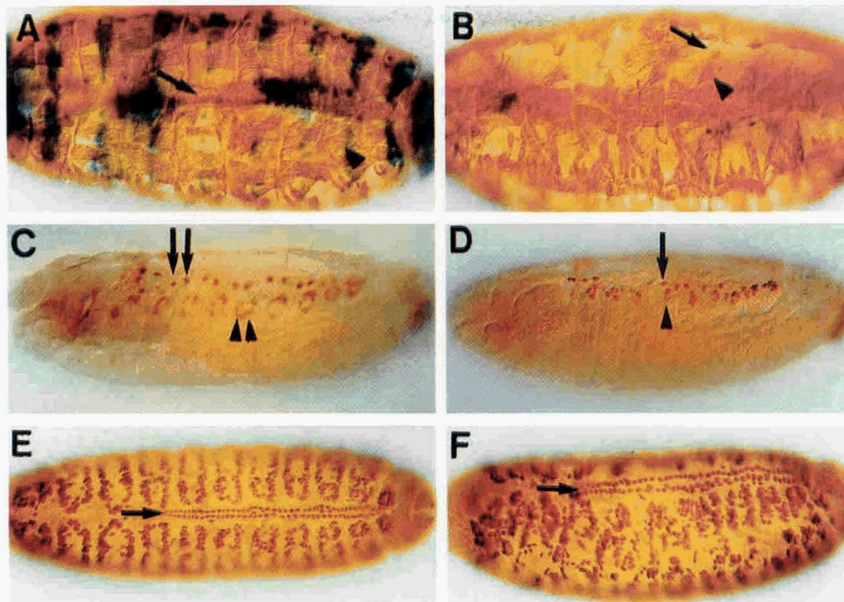


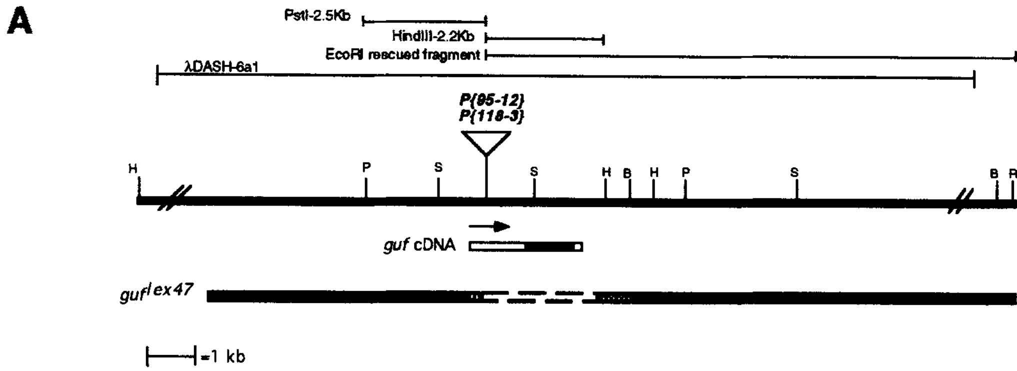
FIGURE 4.—Heart formation in *guf* mutant embryos. (A) The two rows of cardial cells that form the contractile part of the heart express Muscle Myosin in wild-type embryos (arrow). (B) In $guf^{P118\text{-}3}$ homozygous embryos, Myosin expression in the cardial cells is largely absent and only occasionally Myosin expression is detected in these cells (arrow). Arrowheads point toward dorsal-most muscles. (C and D) Dorsal view of stage 15 wild-type and $guf^{P118\text{-}3}$ homozygous embryos immunocytochemically stained for the EVE protein (FRASCH *et al.* 1987). In wild-type embryos, EVE is expressed in two rows of nuclei of the dorsal-most muscles in each abdominal segment (arrowheads) and pairs of pericardial cells (arrows). (C). No obvious reduction in the number of EVE positive nuclei is seen in the mutant, but the nuclei remain clumped together and are not distributed normally within muscle fibers (D). (E and F) Dorsal view of wild-type (E) and $guf^{P118\text{-}3}$ homozygous (F) embryos stained with an antibody against D-MEF2 (LILLY *et al.* 1995). Similar number of D-MEF2 positive nuclei is evident in mutant embryos when compared to wild type, but the distribution of nuclei in the bodywall muscles is clearly aberrant (F). Note that the assembly of the heart into a linear tube appears relatively normal in the mutant (arrow).

pattern is considerably disrupted in $guf^{P118\text{-}3}$ or guf^{lex47} homozygous embryos where numerous muscles are missing, displaced or exhibit aberrant shapes (Figure 3B). In addition, these embryos contain many mononucleate myoblasts that failed to fuse into muscle syncytia (Figure 3B). Similar defects were observed in *guf* mutant embryos stained with antibodies against the spectrin-like MSP-300 protein that is expressed in muscles (VOLK 1992) and chordotonal neurons. As shown in Figure 3D the level of MSP-300 protein is greatly reduced in muscle fibers and chordotonal neurons of mutant embryos when compared to wild type (Figure 3C).

Anti-Myosin staining also revealed defects in the visceral and cardiac musculature (Figure 3, E and F and Figure 4). In wild-type embryos, a thin layer of evenly spread visceral muscles is detected around the gut (Figure 3E). In *guf* embryos the visceral muscles seem to spread properly around the developing gut, however the thickness of this muscle layer appears less uniform than that of wild-type embryos leading to its rippled appearance (arrowheads in Figure 3F). The constrictions of the gut form properly in *guf* mutants. The contractile part of the heart normally consists of a double row of Myosin-expressing cardial cells that form a linear tube underneath the dorsal midline (arrow in Figure 4A). Although the cardial cells form properly and the assembly of the heart into a linear tube appears relatively normal in *guf* mutants (arrow in Figure 4F), Myosin expression in the cardial cells is essentially absent (compare Figure 4, A and B).

To determine whether the defects observed in the somatic musculature of *guf* embryos stem from aberrant specification of these muscles or from later differentiation defects, we stained *guf* embryos for *nautilus* RNA (MICHELSON *et al.* 1990) and for the Evenskipped (EVE) protein (FRASCH *et al.* 1987). *nautilus* is normally expressed in a subset of developing muscles in stage 12/13 embryos. This expression pattern does not appear to be altered in *guf* embryos (data not shown). The EVE protein is also present in the nuclei of the dorsal-most muscle (arrowhead in Figure 4C) and pairs of pericardial cells in each abdominal segment (arrows in Figure 4C). In *guf* mutants, the number of EVE positive cells appears similar to wild type, but the distribution of nuclei in the dorsal somatic muscle is abnormal. Instead of segregating into two crescent rows of nuclei on either side of the segment, these nuclei remain clumped together (arrowheads in Figure 4D). Similarly, these dorsal muscles display abnormal morphology when visualized with anti-Myosin antibody and do not appear as broad and spread out as in wild type (compare arrowheads in Figure 4, A and B). The aberrant distribution of nuclei within muscle fibers is not restricted to the dorsal-most muscles and is evident in all muscle fibers

A



B

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   1 CGTGGGCCAAAGTGCCCAACTCACCCTGTGCGCGCAGAATGGCCGCCTTGCCCCGTCCCG    60
  61 CCGTCCCACCAAGTCCCTTGCCCGTCTGCTTCTTGAACATCGGGGCATTTTTCAGCATGT   120
 121 CGGGCAGTATGAGGAATCGGATCTTGGAGCCGCGAATGTAGACGTTCTCCAGGTTGGCGG   180
 181 TGCGTCCGTCTCGGTAGGTCACCGTGATCTGGGTCATTTGGCAGTTCATGTTGTCCTCCG   240
 241 CCTCGATGAGTTTGCCGCGGTACACCTCGCCGGTGATGGTTTCGCAAGTGATTATGTGGC   300
 301 CCTCGGCCTCGTGCAGAACTTTAATGGGCACTCCGATAGACATGTTCGCTAGCTTGGTAT   360
 361 GTTGCTTGTCTGTTCTTGTTCGTTCAACTATATATTTTTCACTGTTTTTTTTTCCACCGA   420
 421 AACCGCTAAANTTTTTCTCCGTTTTTCTTTGTGCAAGCGTTTATGCCGAGTGTTTGCGAG   480
 481 TGTATGTGTTTGCGTGCTCGCTCCCGCTTTGACAGCTGCCGGCGGCCATCTTGTGGTGAA   540
 541 ATTGGTGGTGCGTGAGCGCAGGGCTGCATGCGCTGCGTGACTATCGGAAACAGCTTATCG   600
 601 GTTTGCCGATAAGGCGCGCGGGCGTATCTTATCGCTTTAAAATAAACAACTGCAATTTGT   660
 661 TAGCGTCATAAAAGCGAAATAATTAAATTGTCGCCGCTTCGCTGATAAGCCTTTCGTTCT   720
 721 GCCTTCGTGGCCATCAGTTTGTTAGCAGCTTTTGCGACGTTCAGTTACCAATTGAGTTTT   780
 781 ACTCTACACCATGTTGTGGTAAAGATTTCGGGTTTATAAATTCAATTCATAATTCACATA   840
 841 AGTAATTTGTAAATGGGAAATAATCTTTATAAAAACATGGGAATATTCGCTATATGCTTG   900
 901 CCATTAAAAAGGGGATATAAACTTTCCAACAGATGGAGTTGCGCTACAAAATCGAACAGG   960
 961 CACATCGCAGTTCGATATGGGGTTTTTTGACTAAATACCCAATTTTTTTCCACAGCGACC  1020
1021 CAGTGTTTAGTAGTGGCTTCGTTAGAAGAGAATTTAATGACAGCGGAATTGCCGACGGCA  1080
1081 AGCTCCGCACCATCTCGACTTCGTCTTGCGCCACGACCATGTCCTCGGAGTCCTACCGCA  1140
1141 TCTCTCTCTGCGTAGGGCCTCTGTGGTGGTCCTGATGTCCCTGTCCACCACAGAACAGAT  1200
1201 CACGATAGGGCATCGCTCTTAACGGGTGAGTAATAATAACTAACCATTTAAGTACTTATT  1260
1261 ATGCTAAAATGTGTTAATTCCTTCCGTGTAGGCTACAGCCGTAAGTCTTCGGTCGACTCG  1320
   1  M  L  K  C  V  N  S  F  R  V  G  Y  S  R  K  S  S  V  D  S    19
1321 GCTGGTGGCAGCCTGTACGAGGCCAGCTCCCGGGCGTCGTCGTTGTCGTCCTCCCAGTCG  1380
  20  A  G  G  S  L  Y  E  A  S  S  R  A  S  S  L  S  S  S  Q  S    39
1381 GATTGCTCCGACTTGGAGTCGCAGCCCGACATCCACTCGCTGTGCTCGGACGATGACTGC  1440
  40  D  C  S  D  L  E  S  Q  P  D  I  H  S  L  C  S  D  D  D  C    59
1441 CAGGAAGTGCTGCGCCAGATCTTGCAGCACGACCAGCCGGTGCAGATCACCATCAAGCTG  1500
  60  Q  E  V  L  R  Q  I  L  Q  H  D  Q  P  V  Q  I  T  I  K  L    79
1501 CATGTCACCGAGGACCAGTACACCAATTGGAATACGATTCTGAACCCAGTCAACAACTTG  1560
  80  H  V  T  E  D  Q  Y  T  N  W  N  T  I  L  N  P  V  N  N  L    99
1561 TTGTATGTCGCTCTACCGAAGGACCTTCCTCCGGCCGGCTCCAAGCAGACCTTCATCTCG  1620
 100  L  Y  V  A  L  P  K  D  L  P  P  A  G  S  K  Q  T  F  I  S   119
1621 CTTCTGGAGTTCGCCGAGGAGAAGCTGGAGGTGGACGGCATCGTTATGGTAATGCCTAAA  1680
 120  L  L  E  F  A  E  E  K  L  E  V  D  G  I  V  M  V  M  P  K   139
1681 GATCAGCCGGATCGAGCTCGCCTCATCGAGGCGTTCCTCTTCATGGGCTTCGAGCCGCTG  1740
 140  D  Q  P  D  R  A  R  L  I  E  A  F  L  F  M  G  F  E  P  L   159
1741 TCCAGGAAGGCACCGCAGGCACCGCCGGCTGCTATTAACGACAACGAGAACTACTACTTC  1800
 160  S  R  K  A  P  Q  A  P  P  A  A  I  N  D  N  E  N  Y  Y  F   179
1801 CTCTACAGCATCGAGGAGTAGTTCGTCGGCAAGGGGACGATTTTCGCCACAGAAACGAAA  1860
 180  L  Y  S  I  E  E                                              186
1861 CAAAGAAGAAGTAATATCAGTAGCAATCCCAATATTCTGTTTCGTAGTTTCCGCAAATAA  1920
1921 CTTTTATTAGCCAGCCGTAGATACAATGACATATTGAGCTCAATATGTGTTCAGTGAAAA  1980
1981 TCAGCAAATCAATCCTTTTTGAATCTTCGCGTTGTTTATGTAGTCGAAAATCGTTTTGAA  2040
2041 AATTGTCTTCGGAGCGGCTTCATTTTTGGTAGGCTTTCAATTATGGCCAGCCACGTTAAG  2100
2101 AGCCTGATCGCAAATTACTATATTAAACTATATCCAACAACAACAAAAATCGAACGAAAA  2160
2161 CG  2164
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C

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                     .          ◊              ***  *                   ***
Dro:   MLKCVNSFRV GYSRKSSVDS AGGSLYEASS RASSLSSSQS DCSDLESQPD IHSLCSDDDC 60
Xen:   ---------- ---------- --DVPHPPLK IPGGRGNSQR DHNLSANL.. ...FYSDNR. 90
Rat:   ---------- ---------- --DVPHPPLK IPGGRGNSQR DHSLSASI.. ...LYSDER. 100/67
Hum:   ---------- ---------- --DAPHPPLK IPGGRGNSQR DHNLSANL.. ...FYSDDR. 98/63

        **  *  •   **    * **    ****    *    * *       *** **      * *** **
Dro:   QEVLRQILQH DQPVQITIKL HVTEDQYTNW NTILNPVNNL LYVALPKDLP PAGSKQTFIS 120
Xen:   LNITEELTSN NRTRILNVQS SLTDGKQVSW RAVLNNNNN.. LYIEIPSGTL PEGSKDSFAI 148
Rat:   LNVTEEPTSN DKTRVLSIQC TLTEAKQVTW RAVWNGGG.. LYIELPAGPL PEGSKDSFAA 158/125
Hum:   LNVTEELTSN DKTRILNVQS RLTDAKRINW RTVLSGGS.. LYIEIPGGAL PEGSKDSFAV 156/121

       *********     *  *  *    * ** *** **    * ***** *       *   *         *   *
Dro:   LLEFAEEKLE VDGIVMVMPK DQPDRARLIE AFLFMGFEPL SRKAPQAPPA AINDNENYYF 180
Xen:   LLEYAEEQLQ VDHVFICFHK NRDDRAMLLR TFRFLGFEIV IPGHPLVP.. ..KRPDACFM 204
Rat:   LLEFAEEQLR ADHVFICFPK NREDRAALLR TFSFLGFEIV RPGHPLVP.. ..KRPDACFM 214/181
Hum:   LLEFAEEQLR ADHVFICFHK NREDRAALLR TFSFLGFEIV RPGHPLVP.. ..KRPDACFM 212/177

       *** *
Dro:   LYSIEE.... .... 186
Xen:   AYTFER.DSS DED. 216
Rat:   VYTLEREDPG EED. 227/199
Hum:   AYTFERESSG EEEE 226/191
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when stained with antibodies against D-MEF2 (compare Figure 4, E and F). These results indicate that the initial specification of individual muscles occurs properly in the absence of *guf* activity, but myoblast fusion and distribution of the syncytial nuclei within the developing muscle fibers is aberrant. Hence, we conclude that in the myogenic lineages, as in the PNS, *guf* is not required for cell type specification but for terminal differentiation of muscle cells. We propose that mutations in *guf* affect muscle development by reducing the level of various muscle-specific proteins.

The *guf* gene encodes an antizyme-like protein: To clone the *guf* gene, ~16 kb of genomic sequences flanking the *P*{118-3}, *P*{95-40} and *P*{95-12} insertions were isolated by plasmid rescue (WILSON *et al.* 1989). Additional genomic clones that span the *guf* locus were subsequently isolated from a λDASH genomic library and a restriction map was constructed (Figure 5A). The *P*-element insertion sites were mapped by restriction analysis and found to lie within a 50-bp region. The precise location of *P*{118/3} and *P*{95/12} insertions was subsequently determined by sequence analysis (Figure 5B).

To identify transcribed regions, individual genomic fragments were labeled and hybridized to whole-mount embryos. Two fragments rescued from either side of the *P*{118/3} and *P*{95/12} insertions (a 2.5-kb *Pst*I and a 2.2-kb *Hin*dIII fragments, see Figure 5A) hybridized to embryos in a pattern consistent with the β-gal expression observed in the *guf* insertional strains (see below). The 2.5-kb *Pst*I fragment was therefore used as a probe to screen a 9–12-hr-old embryonic cDNA library (ZINN *et al.* 1988) and two identical 2.1-kb cDNA clones were isolated. These cDNAs correspond probably to a full length, or nearly full length, *guf* mRNA since a predominant 2.1-kb band is detected on Northern blots probed with *guf* cDNA or genomic probes (Figure 6). Southern analysis and partial sequencing of genomic clones indicate that the cDNA clones are most likely colinear with the genomic DNA and hence, *guf* is probably an intronless gene.

As shown in Figure 5B, *guf* cDNA contains a single long open reading frame that is preceded by an uncommonly long (MARONI 1993) untranslated leader (1260 bp). The predicted 20.8×10^3 *Mr* GUF protein is hydrophilic and acidic (predicted pI = 4.39) containing no

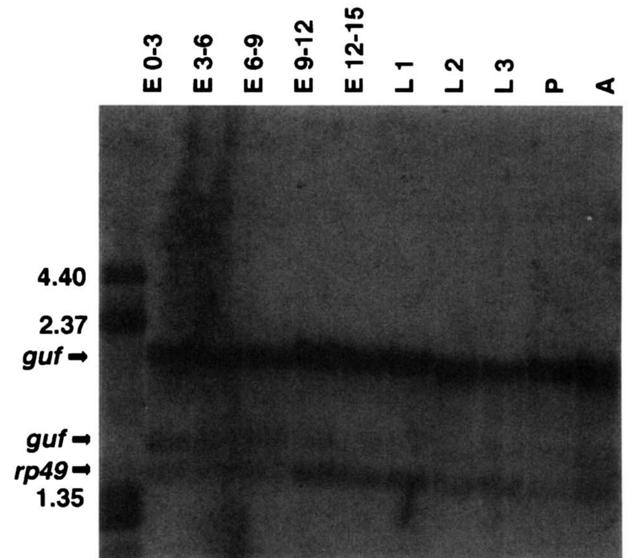

FIGURE 6.—Developmental Northern analysis. Northern blot of embryonic (E; the numbers denote age of embryos in hours), larval (L), pupal (P), and adult (A) Poly(A)$^+$ RNA probed with a 6.0-kb *Pst*I genomic fragment that spans the *guf* locus (Figure 5). Similar results were obtained when *guf* cDNA clones were used as probes (not shown). Poly(A)$^+$ RNA (2.5 μg) was loaded in each lane. The *rp49* probe (ribosomal protein) was used as a standard to evaluate the amount of RNA loaded in each lane.

hydrophobic regions that may correspond to a transmembrane domain or a signal sequence. Search of the PROSITE dictionary of protein sites and patterns did not identify any potential sites for posttranslational modifications such as phosphorylation. BLAST searches (ALTSCHUL *et al.* 1990) revealed a significant sequence similarity between GUF and the vertebrate ODC antizyme (OAZ) (MIYAZAKI *et al.* 1992; TEWARI *et al.* 1994; ICHIBA *et al.* 1995). The sequence similarity between GUF and OAZ spans almost the entire length of the GUF protein excluding the first 30 amino acids (Figure 5C). The overall homology is 24.4% identity and 42% similarity. The highest degree of conservation, 43% identity and 61% similarity, is found in the region of amino acids 100–157. Interestingly, a 22-amino acid stretch that was shown by ICHIBA *et al.* (1994) to be essential for the binding of OAZ to ODC is found in the core of this domain (amino acids 116–137, under-

FIGURE 5.—Structure and sequence of the *guf* gene. (A) Structural map of the *guf* genomic region and transcription unit. The *P*-element insertions, *P*{95/12} and *P*{118/3}, are inserted within the transcribed region at positions +467 and +448, respectively. The orientation of the *lacZ* gene in both insertions is the same as the *guf* gene. Deletion of genomic DNA associated with the guf^{lex47} excision allele is represented by dashed lines in the lower bar. The breakpoints of this deletion were estimated by Southern analysis. H, *Hin*dIII; P, *Pst*I; S, *Sal*I; B, *Bam*HI; R, *Eco*RI; black box in the cDNA represents the putative coding region. Clones and probes mentioned in the text are depicted at the top. (B) Nucleotide sequence and predicted amino acid sequence of *guf* transcript. The 5′ region of the cDNA contains two copies of the most frequently occurring downstream promoter elements in the +20/+30 region (bold) (ARKHIPOVA 1995). The PCR primers used for the analysis of P{118/3} excisions are underlined. This sequence has been submitted to the GenBank database under accession number U29529. (C) Sequence alignment between GUF and OAZ from frog rat and human. Two forms of OAZ (29 and 23 kD) that differ from each other in their amino-terminal are produced in mammalian. Both products contain the sequence shown in Figure 5. Bold, identities between Drosophila and vertebrate antizymes; asterisks, similar residues in all four proteins. The region that was defined by ICHIBA *et al.* (1994) as necessary for binding to ODC is underlined.

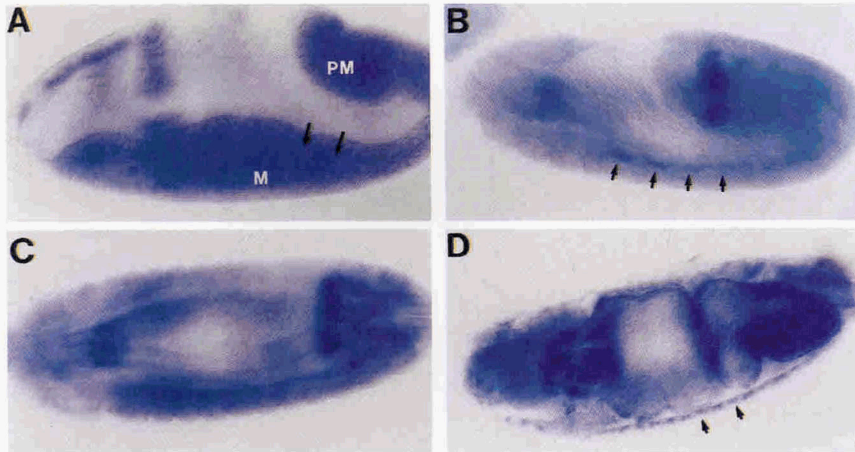

FIGURE 7.—Distribution of *guf* mRNA in the embryo. *In situ* hybridization to whole embryos using *guf* antisense RNA as a probe. (A) Lateral view of stage 7 embryo. *guf* expression is mainly detected in the mesoderm and the invaginating posterior midgut. Arrows, ventral furrow; M, mesoderm; PM, posterior midgut invagination. (B) Dorso-lateral view of a stage 12 embryo. Fat body precursors are indicated with arrows. (C) Dorsal view of stage 13 embryo reveals *guf* expression in the developing fat body, Malpighian tubules, foregut and midgut. (D) Lateral view of stage 16 embryo. The expression of *guf* in body-wall muscles is indicated by arrows.

lined in Figure 5C). In contrast, the short stretch that was found by ICHIBA *et al.* to be most important for the ODC-destabilizing activity of OAZ (amino acids 74–79) is not conserved.

***guf* mRNA distribution:** Northern analysis revealed that the *guf* gene is expressed in all stages of embryonic and larval development as well as in pupae and adults (Figure 6). An abundant 2.1-kb transcript is detected in all developmental stages. An additional transcript (~1.6 kb) that labels faintly with *guf* probes (Figure 6) may correspond to a different splicing variant of *guf*. However, this band could not be detected in other RNA samples prepared from similar developmental stages, suggesting that it may be a degradation product of the 2.1-kb transcript.

The expression pattern of the *guf* gene was determined with *guf* anti-sense RNA probes using *in situ* hybridization to whole mount embryos. Low levels of uniformly distributed *guf* RNA is detected in precellularized embryos, suggesting a maternal contribution of *guf* transcripts. During gastrulation and germband extension *guf* transcripts are mostly abundant in the mesoderm and the invaginating posterior midgut (Figure 7A). At stage 11, patches of mesodermal cells that, based on their position, probably correspond to fat body precursors (HOSHIZAKI *et al.* 1994) begin to express higher levels of *guf* RNA (Figure 7B). A strong signal is also detected at this stage in the Malpighian tubule rudiments and the foregut. As germband retraction takes place, *guf* expression is maintained in the developing fat body, Malpighian tubules and the posterior and anterior midgut. In addition, at late stage 12 and stages 13–14 *guf* is expressed at low levels throughout most of the embryo (Figure 7C) including the subectodermal layer that contains most of the PNS cells. At stage 14, *guf* transcripts are first detected in the developing body wall muscles. Expression of *guf* in body wall muscles, fat body and the midgut is maintained throughout the rest of embryonic development (Figure 7D).

The *P*-element enhancer detectors inserted in the *guf* gene confer β-gal expression in a pattern similar to the distribution of *guf* mRNA. Homozygous *P*{118/3}, *P*{95/12} and *P*{95/40} embryos exhibit very high levels of β-gal activity in the midgut (KANIA *et al.* 1995 and data not shown). (Due to the strong expression in the gut and the defects in the sensory nervous system caused by these insertions we named the gene *gutfeeling*). Very weak *lacZ* expression was detected in the developing somatic muscles starting at stage 14 of embryonic development. Embryos heterozygous for these insertions display weak β-gal expression in developing muscles, whereas expression in other tissues mentioned above is undetectable.

Since *guf* is expressed in Malpighian tubules and fat bodies, we examined the Malpighian tubules and fat bodies of guf^{lex47} embryos to determine whether they exhibit morphological defects. Malpighian tubules were visualized by anti-Cut staining and found to be morphologically normal in mutant embryos (Figure 1G). Fat bodies were visualized with antibodies against Neurotactin (DE LA ESCALERA *et al.* 1990; HORTSCH *et al.* 1990) and no obvious morphological defects were observed (data not shown).

To assess the extent of maternal contribution of *guf* mRNA and to determine whether zygotic *guf* expression is eliminated in the deletion allele guf^{lex47}, mutant embryos were examined by *in situ* hybridization with *guf* antisense RNA probe. This probe did not contain sequences from the 5′ terminus that are not removed by this deletion (the probe corresponds to nucleotides 1310–2164 of the cDNA, see Figure 5). These experiments revealed that *guf* mRNA is present in guf^{lex47} homozygous embryos until cellular blastoderm at levels similar to those found in wild-type embryos (data not shown). The store of maternal *guf* mRNA is depleted rapidly during gastrulation, and by the end of stage 7 only low levels of residual *guf* transcripts are detected in the invaginating posterior midgut. No *guf* expression was detected in guf^{lex47} embryos at later stages of embryogenesis. These data suggest that zygotic *guf* expression is eliminated in guf^{lex47} embryos.

DISCUSSION

The *guf* gene is required for differentiation of neurons and muscles in Drosophila embryos: The network of gene interactions that takes place in determined cells leading to their terminal differentiation as neurons remains largely unknown. Through systematic genetic screens we have identifed many genes that are required for normal PNS development (SALZBERG *et al.* 1994; KANIA *et al.* 1995). The phenotypes associated with some of these genes suggest that they play a role in neuronal differentiation rather than determination (*e.g., senseless, depleted;* SALZBERG *et al.* 1994). Here we describe the phenotype associated with mutations in one such gene, *guf*, that was uncovered in a second chromosome screen (KANIA *et al.* 1995). Phenotypic analysis of *guf* suggests that this gene plays a role in terminal differentiation of PNS cells.

Loss of function mutations in *guf* are embryonic lethal and cause defects in the embryonic peripheral and central nervous system. The PNS of *guf* embryos exhibit frequent defects in growth cone guidance and fasciculation of peripheral axons. In addition, the level of the neuronal specific antigen 22C10 is greatly reduced when compared to wild-type embryos. In spite of the defects observed in the PNS at late stages of development, early markers revealed no alterations in the number or identity of SOPs that are formed in *guf* embryos as compared to wild type. In addition, cell fate determination within the PNS lineages seems not to be affected in the absence of *guf*, such that the number and identity of cells that constitute sensory organs appear normal. These data suggest that *guf* plays a role in terminal differentiation of PNS cells following their specification as neurons or support cells but is not required for the specification of cell identities. Based on the observation that the levels of several neuronal-specific proteins are abnormally low in mutant embryos, we propose that *guf* is required for the regulation or maintenance of gene expression in the differentiating PNS. The failure of PNS cells to express late neuronal markers may explain the abnormal morphology of neuronal cells and the defects in growth cone guidance and fasciculation.

The pattern of body wall muscles is also disrupted in *guf* mutant embryos as many muscle fibers are missing or misplaced. In addition, the layer of visceral muscles surrounding the gut appears rippled and less regular than normal. Early subdivision of the mesoderm to somatic, visceral and cardiac mesoderm was found to occur normally in the absence of *guf* (data not shown), and the initial specification of individual muscles does not seem to be affected. However, myoblast fusion and segregation of the syncytial nuclei within the developing muscles is aberrant leading to abnormal morphology or complete loss of specific muscle fibers. Based on these data we propose that *guf* is required for differentiation but not cell fate determination of all types of embryonic muscles.

Possible function of *guf*: Phenotypic analyses of PNS and muscle development in *guf* embryos suggest that the requirement for *guf* function is very similar in these two systems. First, different types of sensory organs and different types of muscles are affected similarly by mutations in *guf*. Second, in both systems, the specification of primary precursors and their descendants occurs normally in terms of number and identity, but terminal differentiation is impaired. Finally, in both systems *guf* seems to affect the level of proteins normally expressed in terminally differentiated cells.

The predicted GUF protein exhibits a significant sequence similarity to ODC-antizyme that plays a key role in the regulation of polyamine levels in mammalian cells (reviewed by HAYASHI and CANELLAKIS 1989). The highest degree of similarity is found in the region found to be important for binding of the antizyme to ODC (ICHIBA *et al.* 1994; LI and COFFINO 1994; MAMROUD-KIDRON *et al.* 1994; see Figure 5C). However, the domain that was defined as necessary for the destabilization of ODC is not conserved in GUF. These data suggest that GUF binds to ODC and inhibits its activity but does not induce its destabilization and rapid degradation. This prediction is in agreement with the assumption that Drosophila ODC is a stable protein, as was suggested by ROM and KAHANA (1993) based on sequence comparisons between Drosophila ODC and ODC from other species. However, experimental data to support our hypothesis are still lacking.

The possibility that mutations in *guf* affect embryonic development through an alteration of polyamine levels is very intriguing. Polyamines were implicated in many cellular processes including protein biosynthesis and post-translational modifications (reviewed by TABOR and TABOR 1984). Inhibition of polyamine synthesis in the early chick embryo by ODC-specific inhibitor was shown to inhibit general transcription and block gastrulation (LÖWKVIST *et al.* 1986). Targeted disruption of the ODC-1 gene in nematodes revealed no apparent phenotypes except for a reduction in brood size of homozygous mutants (MACRAE *et al.* 1995). Most of the knowledge in this field comes from *in vitro* studies and cell culture systems in which the tight regulation of ODC activity seems to be dispensable (COFFINO and POZNANSKI 1991). The role of polyamines in embryonic development remains largely unknown.

Spermidine is the predominant polyamine in Drosophila embryos, and its concentration fluctuates throughout embryonic development (CALLAERTS *et al.* 1992). It has been shown that superphysiologic concentrations of spermidine can inhibit protein translation *in vitro* (MATSUFUJI *et al.* 1995). This invites the speculation that lack of GUF activity may affect protein translation by generating an excess of spermidine at certain stages of embryogenesis. However, to this end, it is not known whether the abnormal patterns of protein expression observed in *guf* mutants reflect alteration in the tran-

scription or translation levels of the corresponding genes or defects in the maintenance of the produced proteins. In addition, there is no evidence that an excess of spermidine can inhibit translation *in vivo*. Another possibility is that an excess of polyamines causes a cytotoxic effect as was proposed by COFFINO and POZNANSKI (1991).

When and where is *guf* required?: Several questions arise when one compares the expression pattern of the *guf* gene and the phenotypes associated with its loss. The early expression of *guf* in the invaginating mesoderm of gastrulating embryos suggest that this gene may play an early role in mesoderm differentiation. Our data indicate that early mesoderm development occurs properly in the absence of zygotic *guf*. However, maternal contribution of *guf* transcripts could circumvent the need for zygotic expression during early embryogenesis and mask early phenotypes. This question should therefore be addressed by phenotypic analysis of embryos that lack both zygotic and maternal *guf*.

Expression of *guf* in bodywall muscles is first detected by *in situ* hybridization at stage 14 when myoblast fusion and migration takes place. The timing of expression correlates well with the proposed role of *guf* in muscle differentiation. In the PNS however, the picture is less clear. Low levels of *guf* mRNA are distributed uniformly throughout most of the embryo during stages 12–14. Therefore, we could not determine unambiguously by *in situ* hybridization whether *guf* is expressed in the developing PNS. Hence, a nonautonomous role for GUF in the nervous system cannot be excluded at this point. By examining CNS development in *snail* and *twist* mutant embryos, which lack mesoderm all together, RAO *et al.* (1991) have concluded that the early mesoderm is not essential for the initiation of neural development. However, examination of late aspects of nervous system development in *snail⁻ twist⁻* background is not feasible. To assess whether the somatic musculature itself is required for normal PNS development, we examined the PNS of embryos that lack the *D-mef2* gene (*Df(2R)P544*, LILLY *et al.* 1995). In *Df(2R)P544* embryos, myoblasts are specified properly, but myoblast fusion does not take place and the somatic and visceral muscles do not form (BOUR *et al.* 1995; LILLY *et al.* 1995). When visualized with MAb 22C10, the PNS of *Df(2R)P544* embryos exhibited defects in the organization of neuronal clusters and misrouting of peripheral axons (data not shown). However, the morphology of neuronal cell bodies and axonal fascicles, as well as the level of 22C10 antigen, appear normal. These data suggest that muscle formation is not required for induction or maintenance of neuronal properties in the PNS and we therefore conclude that the reduced protein levels in the PNS of *guf* mutants are not secondary to defects in muscle formation.

Previous work on ODC-antizyme in vertebrates has shown that it is not only involved in the regulation of ODC activity but that it is also involved in the regulation of polyamine uptake by cells (MITCHELL *et al.* 1994). Thus, it is possible that in *guf* mutants some cells overproduce polyamines and secrete them, leading other cells, that also lack GUF activity, to take up too many polyamines. The lack of GUF could thus produce defects in specific sensitive tissues, even though the *guf* gene is normally not expressed in these tissues or is expressed at very low levels. This may explain the defects in the PNS and CNS where we observe very low levels of *guf* mRNA.

Finally, it is interesting to note that polyamines are potent blockers of inward rectifying K^+ channels (FICKER *et al.* 1994; LOPATIN *et al.* 1994), and that these channels are found in muscles (SAKMANN and TRUBE 1984) and neurons (CONSTANTINI and GALVAN 1983). Recently, mutations in an inward rectifying K^+ channel have been shown to underlie the neuronal defect in the mice *weaver* mutants that exhibit developmental cerebellar defects (SLESINGER *et al.* 1996). Hence elevated levels of polyamines in Drosophila may severely affect the resting membrane potentials of muscles and neurons by blocking the inward rectifying K^+ channel, possibly causing many late developmental defects, such as growth cone guidance defects.

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