Caenorhabditis elegans β-G Spectrin Is Dispensable for Establishment of Epithelial Polarity, but Essential for Muscular and Neuronal Function

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Abstract. The *Caenorhabditis elegans* genome encodes one α spectrin subunit, a β spectrin subunit (β-G), and a β-H spectrin subunit. Our experiments show that the phenotype resulting from the loss of the *C. elegans* α spectrin is reproduced by tandem depletion of both β-G and β-H spectrins. We propose that α spectrin combines with the β-G and β-H subunits to form α/β-G and α/β-H heteromers that perform the entire repertoire of spectrin function in the nematode. The expression patterns of nematode β-G spectrin and vertebrate β spectrins exhibit three striking parallels including: (1) β spectrins are associated with the sites of cell–cell contact in epithelial tissues; (2) the highest levels of β-G spectrin occur in the nervous system; and (3) β spectrin-G in striated muscle is associated with points of attachment of the myofilament apparatus to adjacent cells. Nematode β-G spectrin associates with plasma membranes at sites of cell–cell contact, beginning at the two-cell stage, and with a dramatic increase in intensity after gastrulation when most cell proliferation has been completed. Strikingly, depletion of nematode β-G spectrin by RNA-mediated interference to undetectable levels does not affect the establishment of structural and functional polarity in epidermis and intestine. Contrary to recent speculation, β-G spectrin is not associated with internal membranes and depletion of β-G spectrin was not associated with any detectable defects in secretion. Instead β-G spectrin-deficient nematodes arrest as early larvae with progressive defects in the musculature and nervous system. Therefore, *C. elegans* β-G spectrin is required for normal muscle and neuron function, but is dispensable for embryonic elongation and establishment of early epithelial polarity. We hypothesize that heteromeric spectrin evolved in metazoans in response to the needs of cells in the context of mechanically integrated tissues that can withstand the rigors imposed by an active organism.

Key words: membrane skeleton • unc-70 • *RNAi* • cell–cell contact

Introduction

Maintenance of tissue integrity, particularly in tissues that undergo constant stress, is critical to the survival of an organism. Components important in this process are likely to include cytoskeletal proteins. Protein localization and in vitro studies make spectrin a likely candidate.

Members of the spectrin gene family and their associated proteins have been identified in diverse tissues of vertebrates, echinoderms, and *Drosophila melanogaster*, suggesting that spectrin-based membrane skeletal structures are a ubiquitous feature of metazoan animal cells (Bennett and Gilligan, 1993; Thomas et al., 1997; De Matteis and Morrow, 1998). A specialized version of one spectrin-based structure has been resolved in molecular detail in the human erythrocyte membrane, where this assembly of proteins is essential for the survival of erythrocytes in the circulation (Lux and Palek, 1995). Since most cell types contain additional transcellular cytoskeletal elements, paradigms derived from the red cell membrane skeleton are of limited use in studying functions of nonerythroid spectrin.

Current views of physiological functions of spectrin are derived from localization of spectrin in cells and tissues and interactions of spectrin with proteins in in vitro assays. Spectrin has been implicated in the establishment and/or maintenance of epithelial polarity based on localization at sites of cell–cell contact and association in complexes with cadherins (Nelson and Veshnock, 1987; Nelson and Hammerton, 1989; Yeaman et al., 1999). Spectrin also has been proposed to participate in the establishment of membrane subdomains in striated muscle (Craig and Pardo, 1983; Bloch and Morrow, 1989), in lymphocyte capping (Black

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et al., 1988), and in the organization of synaptic vesicles at presynaptic nerve endings (Sikorski et al., 1991). Moreover, spectrin is thought to participate in axonal transport, based on localization and dynamic behavior in axons (Levine and Willard, 1981). Recently, β-spectrin has been linked to Golgi function based on colocalization with Golgi membranes (Beck et al., 1994; Devarajan et al., 1997; Fath et al., 1997; Stankewich et al., 1998). These observations and other results (Holleran et al., 1996) have led to speculation on possible roles of spectrin in maintaining Golgi structure and in intracellular vesicular transport (Beck and Nelson, 1998; De Matteis and Morrow, 1998; Holleran and Holzbaur, 1998).

The diversity of functions attributed to spectrins is explained by several factors including the following: the large number of proteins that interact with and regulate spectrin in each unique cellular environment, and the complexity of the genes and their alternatively spliced variants resulting in multiple spectrin subunits. In vertebrates, two α spectrin genes and three β spectrin genes have been characterized (Viel and Branton, 1996; Ohara et al., 1998; Stankewich et al., 1998). Additional spectrin subunits that have not yet been cloned, have been reported in various tissues (Glenney et al., 1983; Bloch and Morrow, 1989). These considerations, combined with the fact that each protein interacting with spectrin is also a member of a large gene family, lead to the impression of a daunting level of complexity.

One approach to studying the roles of spectrin is to establish its function in simpler organisms. Spectrin is not present in the yeast genome, but proteins related to α spectrin have been identified in the unicellular organisms *Acanthamoeba* and *Dictyostelium* (Pollard, 1984; Bennett and Condeelis, 1988). However, heteromeric spectrin consisting of α and β subunits has been reported only in metazoans. *Drosophila* α and β spectrin subunits are closely related to their vertebrate orthologues, and have similar biochemical and biophysical properties (Byers et al., 1989, 1992; Dubreuil et al., 1989). A study of *Drosophila* spectrin established phasing of the conformational units of spectrin into 106 amino acid repeats (Winograd et al., 1991) and provided evidence of a larger eight-repeat supramotif (Byers et al., 1992). *Drosophila* spectrin is associated with the plasma membranes of almost all cell types, and undergoes regulated changes in concentration and distribution during embryonic and adult development (Pesacreta et al., 1989). α Spectrin was shown to be an essential protein, as null mutants resulted in first instar larval lethality (Lee et al., 1993). Examination of the spectrin-deficient larvae revealed a loss of contact between epithelial cells of the gut and disruption of cell–substratum interactions (Lee et al., 1993, 1997). These results suggested that spectrin played a role in the stabilization of cell–cell interactions critical for the maintenance of cell shape and subcellular organization within tissues. Due to the maternal load of protein in these mutants, these experiments could not directly address the role of spectrin in the establishment of epithelial polarity. The role of spectrin in the structure and function of the *Drosophila* nervous system also has not been studied so far.

In addition to identifying certain roles spectrin plays, analysis of spectrin in *Drosophila* has also revealed additional spectrin subunits that have not yet been observed in vertebrates. A novel *Drosophila* βHeavy (β-H)[1] spectrin subunit of ~430 kD may have a role in establishing an apicolateral membrane domain, and it may be associated with contractile processes (Dubreuil et al., 1990; Thomas and Kiehart, 1994; Dubreuil et al., 1997; Thomas et al., 1998; Zarnescu and Thomas, 1999). α and β spectrins, the adducin-like *hts* product, and ankyrin all have been identified as molecular components of *Drosophila* fusomes (Lin et al., 1994; de Cuevas et al., 1996) and a related structure in germline stem cell termed the spectrosome. The precise function of spectrin in these structures has yet to be established.

Caenorhabditis elegans is a simple metazoan with a defined genome that is accessible to both genetics and cell biology. The *C. elegans* genome contains only three spectrin genes encoding an α subunit, a β-G subunit, and a β-H subunit (see Fig. 2). Mutations in each of these spectrin subunits have been identified genetically. A mutation in the α spectrin gene, *spc-1(ra409)*, has been isolated in a screen for lethal mutations that cause muscle defects (Norman, K., and D. Moerman, personal communications). Mutations in β-H spectrin encoded by the *sma-1* gene cause elongation and morphogenetic defects (McKeown et al., 1998). Recently, Hammarlund et al. (2000) mapped mutations in the *unc-70* gene to β-G spectrin, resulting in animals exhibiting multiple defects in the muscle and the nervous system.

This study presents an overview of spectrins and, in particular, β-G spectrin in *C. elegans*, and critically examines the proposed roles in intracellular membranes and in the establishment of epithelial polarity. The expression pattern of the single β-G spectrin in all cells throughout the life cycle of the nematode is described, and striking parallels are seen between this pattern and that previously characterized for *Drosophila* and vertebrate orthologues of spectrin. Further experiments critically examine the effects of depletion of β-G spectrin on epithelial polarity, embryogenesis, and Golgi function. A surprising conclusion is that β-G spectrin is not required for these processes. Similar results have been obtained by analysis of *unc-70* β-G spectrin–null animals (Hammarlund et al., 2000). Instead, the results support the hypothesis that heteromeric spectrin evolved in metazoans in response to the need for a multipotential adaptor that could help integrate individual cells into complex tissues and organs.

Materials and Methods

Reagents

DNA. Plasmid CEESK92 was from Y. Kohara (Mishima, Japan); *C. elegans* mixed stage λZAP cDNA library was from R. Barstead; all cosmids from the Sanger Center; *nmy-2* plasmid (Guo and Kemphues, 1996) from K. Kemphues (Cornell University, Ithaca, NY); plasmids pRF4 and pPD95.69 from A. Fire (Carnegie Institution, Wash., DC).

Immunofluorescence. Rabbit antiserum against UNC-44 (Otsuka et al., 1995) was obtained from A. Otsuka (Illinois State University, Normal, IL); rabbit antibody against LAD-1 from L. Chen; mAb MH27 recogniz-

[1]*Abbreviations used in this paper:* β-G, *C. elegans* β spectrin; β-H, βHeavy; dsRNA, double-stranded RNA; ORF, open reading frame; PH, pleckstrin homology; *RNAi*, RNA-mediated interference; ssRNA, single-stranded RNA.

ing JAM-1, mAb DM5.6 recognizing mhcA, mAb MH25 against *C. elegans* β-integrin, and mAb MH5 against a hemidesmosomal component (all from Francis and Waterston, 1985) from R.W. Waterston. Secondary antibodies conjugated with FITC, TRITC, or Cy5, and the Cy5-conjugated streptavidin were obtained from Jackson ImmunoResearch Laboratories Inc. FITC, the cationic membrane tracer dye DiI, and biotinXX-phalloidin were obtained from Molecular Probes, Inc.

Sequence Analysis

Orthologues of *C. elegans* spectrin genes were identified using BLAST (Altschul et al., 1997). Sequences accessed from GenBank for this paper are as follows: human nonerythroid α spectrin SPTAN1 (J05243), nonerythroid β-G spectrin SPTBN1 (NM_003128), βIII-spectrin SPTBN2 (AB008567); for *Drosophila*, β spectrin DROBSPEC (M92288); and for *C. elegans*, α spectrin (KN12345), β-H spectrin SMA-1 (AF053496), and β-G spectrin BGS-1 (AF166170 and AF166169). The SPC-1 open reading frame (ORF) was predicted using the Baylor College of Medicine GeneFinder programs. Dot matrix plots were generated using MacVector v6.0.1. Sequence alignments were made using Geneworks v2.5.1.

Molecular Biology

Standard cloning techniques were used (Sambrook et al., 1989). The ADD-2 polypeptide was identified because of partial homology to vertebrate adducin, and cloned by S. Moorthy. The (*add-2::GFP*) plasmid construct pSM69.57 was made using plasmid pPD95.69 as parent vector, and the cosmid F57F5 as the source of *add-2* promoter sequence. The plasmid pSM69.57 fuses 8.1 kb of upstream genomic sequence and a part of the ADD-2 ORF, in-frame with the GFP.

Worm Culture

C. elegans strains were maintained at 20°C using standard culture conditions (Sulston and Hodgkin, 1988). *sma-1(ru18)* strain (McKeown et al., 1998) was a gift from J. Austin (University of Chicago, Chicago, IL). Transgenic strains carrying the extrachromosomal arrays (cosmid T23C12; pRF4) and (pSM69.57; pRF4) (see above) were selected using the pRF4 (*rol-6(su1006)* roller phenotype as a dominant selectable marker; the latter strains express GFP only in the gut cells.

Nematode β-G Spectrin Antibody

BGS-1 COOH-terminal residues, A1851 to K2257 of the shorter splice, were cloned into pGemex-2 (Promega) in-frame with viral Gene10. Recombinant fusion protein was purified to near homogeneity from inclusion bodies (Li and Bennett, 1996), and was used to immunize male New Zealand rabbits. Serum from individual rabbits was preadsorbed over a Gene10 column to eliminate antibodies recognizing Gene10, and affinity-purified over an antigen fusion protein column. The final antibody, which recognizes both spectrin-spliced isoforms, showed a minimal cross-reaction with other *C. elegans* proteins or purified Gene10.

Immunoblots

Synchronized nematode populations (Epstein and Shakes, 1995) at different developmental stages were harvested and directly dissolved in boiling SDS-PAGE buffer. Protein lysates were separated on a gradient PAGE gel as described (Li and Bennett, 1996). Electrophoresed proteins were transferred onto nitrocellulose and blotted with affinity-purified rabbit antibodies against β-G spectrin. The blot was washed, incubated with ^{125}I-labeled protein A, washed again, and exposed on either film or a PhosphorImager screen to visualize the band that corresponds to β-G spectrin.

Immunofluorescence Microscopy

Embryos, young larvae, and adults were fixed and stained for indirect immunofluorescence using standard protocols (Epstein and Shakes, 1995). In brief, the freeze crack methanol fixation method was used for embryos and young larvae, whereas older larvae and adults were fixed using the formaldehyde-methanol freeze thaw method of Finney and Ruvkun (Epstein and Shakes, 1995). Specimens were preblocked with 10% normal goat serum in PBS containing 2% BSA and 0.1% Tween 20. Primary antibodies and sera were diluted (1:500 for β-G spectrin, 1:1,000 for MH27, 1:250 for DM5.6 and MH25, and 1:100 for MH5) and incubated with fixed specimens at 4°C overnight, followed by three PBS-Tween 20 washes at room temperature (15 min each for embryos, 1-h washes for adults). Incubation with the secondary antibody (1 μg/ml) was for 2 h at 4°C, followed by similar washes. Hoechst 33248 (Sigma Chemical Co.) was used to visualize DNA and was added along with secondary antibody. Immunofluorescent images were obtained using a laser scanning microscope. Phase-contrast images were obtained using excitation at 633 nm to minimize bleaching of GFP and FITC. For muscle, the freeze crack protocol for embryos and L1 larvae was modified by using the formaldehyde-methanol fixative of Finney and Ruvkun (Epstein and Shakes, 1995) at −20°C. BiotinXX-phalloidin (Molecular Probes, Inc.; 1 μg/ml) was added along with primary antibodies, and Cy5-conjugated streptavidin (Jackson ImmunoResearch Laboratories Inc.) at 1 μg/ml was added with secondary antibodies.

Visualization of Dye-filling Amphid Neurons. Living L1 larvae were suspended in a 1–5 μg/ml solution of the dye in M9 buffer for 1–2 h, and then washed three times for 10 min at room temperature (Hedgecock et al., 1985). Animals were allowed to recover briefly on unseeded plates before being mounted on a soft agarose pad using 10 mM NaN_3 in M9 buffer as an anesthetic. The pad was gently sealed with a No. 1.5 coverslip, using a small amount of mineral oil to prevent evaporation. Animals were observed on the confocal microscope using rhodamine optics for DiI.

Protein Depletion Using RNAi

Single-stranded RNA (ssRNA) 1–2 kb in length was synthesized using the MegaScript RNA Synthesis Kit (Ambion, Inc.). Plasmids used as templates for RNA synthesis were the following: CEESK92, pSCSM-2 and pSCSM-II for *C. elegans* β-G spectrin, yk19h9 and yk36c9 for *spc-1* α spectrin, and yk96e11 and yk148d4 for *sma-1* β-H spectrin. Sense/antisense ssRNA annealing to generate double-stranded RNA (dsRNA) was carried out in 1× STOP buffer for 30 min, at 68°C (Fire et al., 1998). Animals were microinjected in the syncytial gonad with each dsRNA at a concentration of 1 μg/μl or higher (Fire et al., 1998). To interfere with two or more genes, dsRNA preparations corresponding to each gene were concentrated and mixed to give a final concentration of 1 μg/μl with respect to each component dsRNA. Survivors of the injection were pooled on a single plate for ~8 h and placed separately on individual seeded plates. The adult hermaphrodite was transferred to a fresh plate every 12–24 h, resulting in a semi-synchronous set of embryos being left behind at each transfer. Progeny in the 24–72-h interval after egg laying either were analyzed for phenotype or were killed for immunoblots to determine the efficiency of RNAi depletion of β-G spectrin. All preparations corresponding to a given gene gave identical results. Usually >90% of the F1 progeny of surviving animals displayed the same gene-specfic phenotype. Injected adult worms did not seem affected by the *RNAi* and lived a normal life span. In all the *RNAi* experiments, the *C. elegans* N2 parental strain was used as the negative control.

Live Imaging of C. elegans Embryos and Larvae

Gravid adult hermaphrodites were killed by cutting their uteri to release embryos into egg salts buffer (Epstein and Shakes, 1995). Embryos were mounted on a soft agarose pad in the same buffer, gently covered with a coverslip, and sealed with mineral oil. Development from zygote to hatching stage was observed using a 40× dry objective with Nomarski differential interference optics at 22–23°C, and also recorded on videotape. The developmental stage (1.5-fold, 2-fold, 3-fold, pretzel, cuticle expression, and pharyngeal pumping) of each embryo was recorded at 30-min intervals and later compared with the tape. Embryos that arrested early during embryogenesis were eliminated from the analyses. Embryonic lengths were measured off the video monitor using appropriate conversion factors.

Phosphoimaging to Determine Efficiency of Protein Depletion by RNAi

Immunoblots were exposed to a phosphoimage screen to quantitate the amount of β-G spectrin in *RNAi* and control progeny using the phosphoimage program, ImageQuaNT (Molecular Dynamics). The immunoblot background was subtracted from each set of phosphoimage data, and the result was divided by the number of animals used to make the lysates (250 for both control and *bgs-1(RNAi)*). The resulting numbers were presented as arbitrary units of β-G spectrin per animal.

Electron Microscopy

Animals were fixed and embedded for electron microscopy (Epstein and

Shakes, 1995). Stained, dehydrated, and resin-infiltrated animals were cut into 90-nm sections, and the sections were placed on uncoated copper grids.

Results

Isolation of *C. elegans* β-G Spectrin (BGS-1) cDNAs

cDNA and reverse transcriptase–PCR clones corresponding to the *C. elegans* β-spectrin were isolated, the ORF was determined, and a functionally significant alternate splice isoform was identified. A BLAST search (see Materials and Methods) using the sequence of human β-G spectrin (SPTBN1) recognized a putative β-spectrin subunit distributed between adjacent cosmids K11C4 and T19F4 on *C. elegans* chromosome *V* (*C. elegans* Sequencing Consortium, 1998). The search also identified a 2.8-kb fragment (clone CEESK92) from Yuji Kohara's expressed sequence tag library. Probes derived from CEESK92 were used to screen a *C. elegans* multistaged cDNA library (see Materials and Methods; Barstead and Waterston, 1991). Multiple cDNAs covering ~5.8 kb of a predicted 7.6-kb mRNA were obtained (Fig. 1 A). Reverse transcriptase–PCR and 5′RACE were used to extend this sequence further 5′. The very 5′ region of the ORF was not reached; however, based on homology with human β-G spectrin (Hu et al., 1992), results of the BLAST search, and the consensus sequence for *C. elegans* intron/exon borders (Blumenthal and Steward, 1997), a good candidate for the first exon was identified.

The final predicted nematode β-G spectrin cDNA encodes a protein made of 2,302 amino acids that is distributed over 13 exons (Fig. 1 B). The protein contains all domains typical of β spectrin including the following: an NH_2-terminal calponin homology region, which is a domain shown to be involved in the formation of a ternary complex between β spectrin, actin, and adducin (Li and Bennett, 1996) and for lateral association between α and β spectrin subunits (Viel and Branton, 1996); a middle region with 17 106–amino acid spectrin repeats; and a COOH-terminal pleckstrin homology (PH) domain shown to bind phosphatidylinositol lipids and localize spectrin to the plasma membrane (Wang et al., 1996; Fig. 2 A).

Four independent partial cDNAs identified an mRNA in which alternate splicing of two exons introduced 135 additional base pairs before the PH domain. The introduction of the alternately spliced region before the PH domain of BGS-1 may modify the substrate specificity and subcellular localization of the protein in *C. elegans*. Interestingly, the 45 introduced amino acids (Fig. 1 C) form a hydrophilic highly basic stretch with four serines that constitute consensus sites for phosphorylation by several protein kinases.

A comparison of nematode β-G spectrin with those of *Drosophila* (Byers et al., 1992) and the three human β spectrins (Hu et al., 1992; Winkelmann et al., 1990; Ohara et al., 1998; Stankewich et al., 1998) reveals that β spectrins are highly conserved through evolution (Fig. 2 B). The level of homology is exceptionally high in the NH_2-terminal ~550 residues, and a comparison of the PH domains of human, *Drosophila*, and *C. elegans* β-G spectrins reveals that the most highly conserved residues fell within loop regions that determine substrate specificity of the PH domain (Macias et al., 1994; Fig. 2 D).

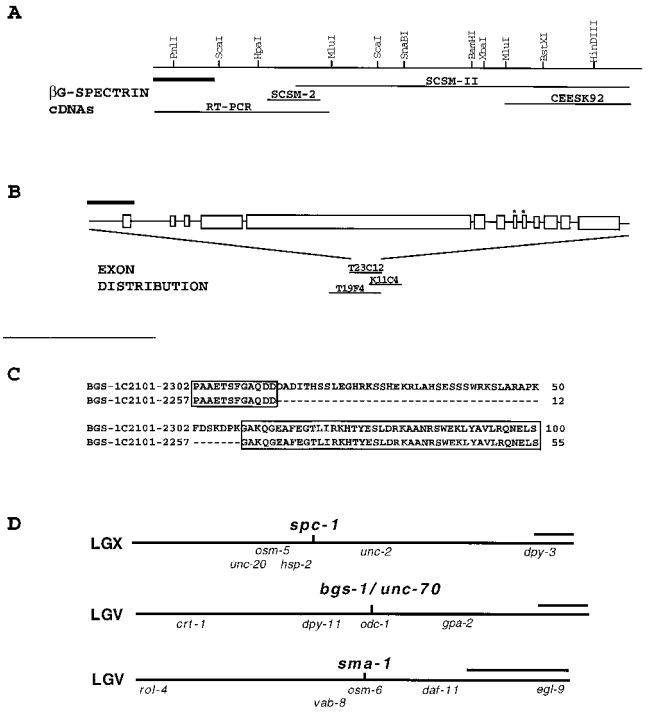

Figure 1. Cloning of *C. elegans* β-G spectrin cDNAs, including two alternatively spliced variants. (A) A partial cDNA clone, CEESK92, was used to screen a *C. elegans* mixed stage λZAP cDNA library. Positions of representative partial cDNAs (SCSM-II and SCSM-2) and the reverse transcriptase–PCR sequence corresponding to the β-G spectrin (BGS-1) mRNA are shown. (B) *Bgs-1* exon distribution. The transcriptional unit is distributed over 13 exons, of which at least two are alternately spliced (asterisks). The BGS-1–predicted ORFs code for two polypeptides of 2,302 and 2,257 amino acids (see below). The coding sequence is completely contained within the unsequenced cosmid T23C12, and distributed between sequenced cosmids K11C4 and T19F4. (C) An alternate splice. The two exons marked by asterisks in B are alternatively spliced in a small number of transcripts to introduce 45 novel amino acids before the PH domain. Only the region flanking the alternate splice (extending into the PH domain) was included in the alignment. (D) Genomic location of *C. elegans* spectrin genes. Sequencing of the genome has identified three candidate spectrin genes: an α subunit (*spc-1*), a β-G subunit (*bgs-1/unc-70)*, and a β-H (heavy) subunit (*sma-1*). The chromosome number and genes mapped to the vicinity of each spectrin subunit are indicated. Bars: (A and B) 1 kb; (D) 0.5 kb.

To date, no other β-spectrin gene except for the β-H spectrin *sma-1* has been identified. With 97% of the *C. elegans* genome already sequenced, it is unlikely that there would be another β spectrin. Hence, the nematode β spectrin is a *C. elegans* orthologue of the human β-spectrin family including SPTB (erythrocyte β spectrin), SPTBN1 (β2 or β-G), and SPTBN2 (β3; Fig. 2 C). *C. elegans* β spectrin is referred to in this paper as β-G or BGS-1 (β-G spectrin-1) to distinguish this protein from β-H, and to reflect the general expression in multiple tissues and throughout development (see below).

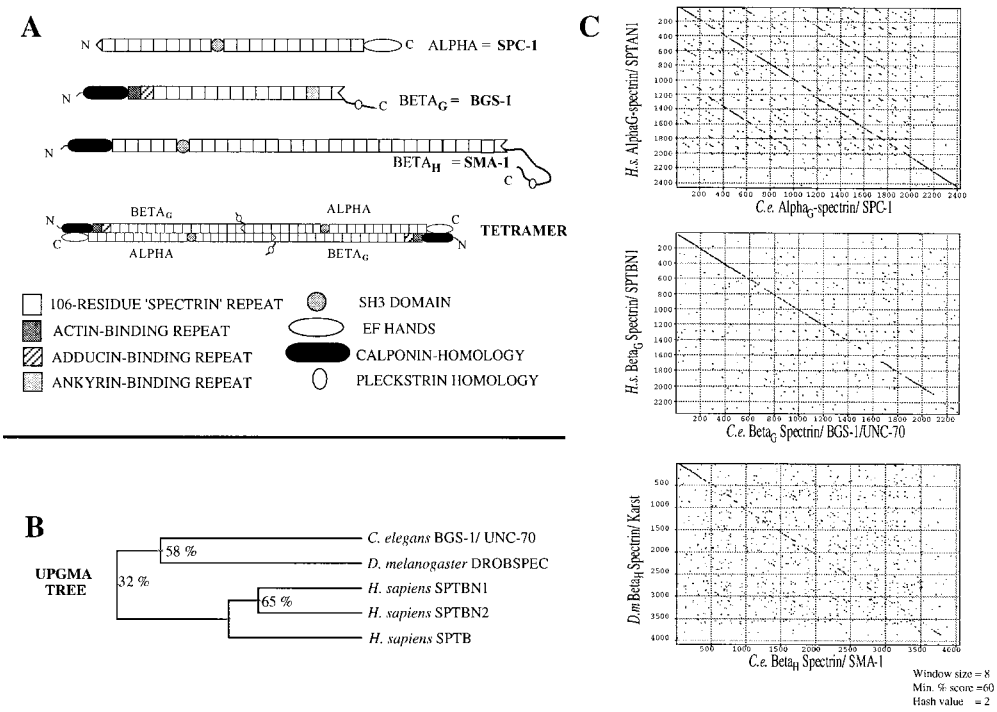

Figure 2. Three spectrin genes of *C. elegans*. (A) Domain organization of α and β spectrin polypeptides. *C. elegans* orthologues are indicated. Spectrin is an $(\alpha\text{-}\beta)_2$ tetramer; an α-β-G tetramer is shown. (B) Evolutionary relationship of β spectrin subunits. A UPGMA tree showing relative homology between five β-spectrin subunits from different species was plotted using Geneworks v2.5.1 software. Of the three identified human β-spectrin proteins (SPTB, SPTBN1, and SPTBN2), the two nonerythroid β-spectrins SPTBN1 and SPTBN2 are more closely related (65% identity). The two invertebrate β-G spectrins show ~58% identity, whereas ~32% of β-spectrin residues are conserved among all five proteins. (C) Dot matrix alignments of *C. elegans* predicted spectrin polypeptides with their human (α and β-G) or *Drosophila* (β_H) orthologues. Note the additional diagonals that arise from the multiple spectrin repeats that make up the bulk of each subunit. (D) Comparison of PH domains of β_G-spectrins from *Homo sapiens* (SPTBN1), *Drosophila* (DROBSPEC), and *C. elegans* (BGS-1, shorter spliceform). Residues used in the alignment are indicated. Note that conserved residues fall in loop regions that determine the substrate specificity of PH domains.

β-G Spectrin Associates with the Plasma Membrane at all Sites of Cell–Cell Contact throughout C. elegans Development

The expression pattern of *C. elegans* β-G spectrin during development was determined using an affinity-purified rabbit polyclonal antibody raised against the COOH-terminal 407 residues of the shorter spliceoform (Fig. 1 C). This antibody recognized a single band at ~225–235 kD (Fig. 3 A) in embryos (E), L2-L3 larvae (L), mixed populations (M), and Dauer larvae (D). The relative molecular mass of this band is lower than that predicted by the molecular mass of the BGS-1 protein (253,200 for the larger splice form), but this faster migration on SDS-PAGE gels is characteristic of other β spectrins.

Indirect immunofluorescence staining of the embryos of a single gravid hermaphrodite showed that β-G spectrin levels were low but discernible in early embryos. β-G spectrin was localized to the plasma membrane at sites of cell–cell contact throughout development, starting at the first cell division (two-cell embryo; Fig. 3 C, i). β-G spectrin expression increased significantly after the gastrulation stage, a time when most cells are undergoing differentiation into various tissue types (Fig. 3, B and C). Embryos that are undergoing morphogenesis show significantly higher levels of β-G spectrin in many cells, including those of the primordial gut (Fig. 3 C, iii), the epidermis (Fig. 3 C, iv), the myoepithelia of the pharynx (ph), and the polarizing endothelia of the gut (gt; Fig. 3 C, v). Cytoplasmic staining was observed principally in early embryos; when present, it was diffuse and did not show any distinctive pattern.

In the adult (Fig. 3, D and E), β-G spectrin staining was detected in all cells including the epidermal seam cells (s), body-wall muscles (m), and the intestine (gut). Tissues that experience mechanical stress expressed higher levels of β-G spectrin; these included the pharynx (ph), the spermatheca (sp), and the vulva (not shown). The nervous system (the nerve ring [nr], nerve cords [vnc, ventral nerve cord], and the commissural axonal bundles [ca]) had the highest levels of β-G spectrin. The only cell lacking β-G spectrin is the mature amoeboid sperm cell in which spectrin was sequestered into the residual body along with actin (data not shown).

The RNAi Phenotype of the Single C. elegans α Spectrin Can Be Reproduced by Tandem Depletion of β-G and β-H Spectrin Subunits

One α spectrin and two β spectrin subunits comprise the currently known spectrin family in *C. elegans* (Fig. 1 D). Since vertebrate spectrins are known to function as α2β2 tetramers, a hypothesis was that *C. elegans* α spectrin assembles into separate α/β-G and α/β-H complexes, which

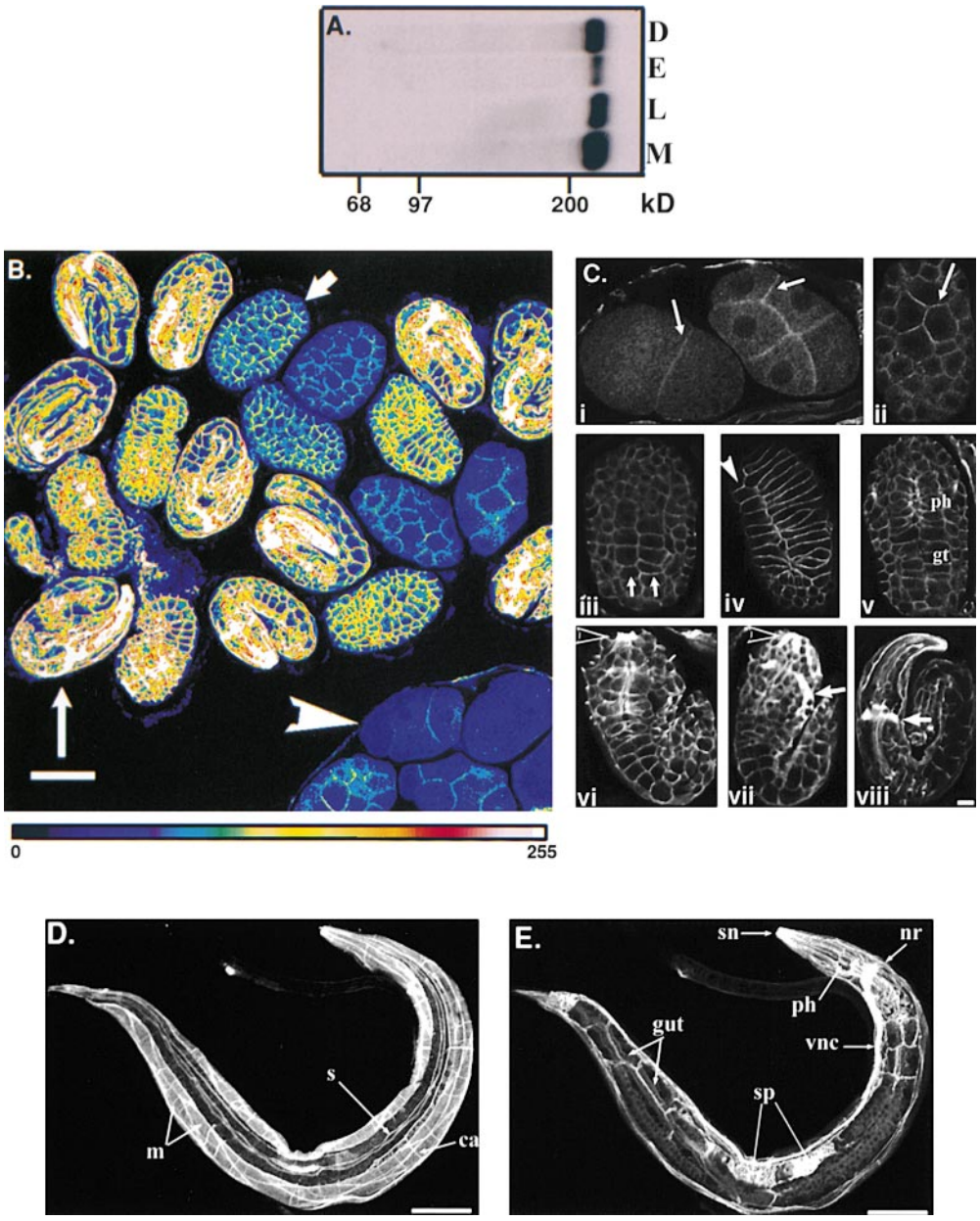

Figure 3. β-G spectrin is associated with the plasma membrane at sites of cell–cell contact throughout development in *C. elegans*. (A) Immunoblot. Protein lysates were prepared from different developmental stages of N2 wild-type worms, and a Western blot was performed using affinity-purified rabbit polyclonal β-G spectrin antibody (see Materials and Methods). A single band of M_r = 225–235 kD is seen at all stages. D = Dauer stage, E = embryos, L = L2-L3 larvae, and M = mixed stages. (B) β-G-spectrin levels during embryogenesis. All embryos in the uterus of a single adult hermaphrodite were stained by indirect immunofluorescence using β-G spectrin antibody and an FITC-coupled secondary antibody (see Materials and Methods). Levels of β-G spectrin are low, but are detectable in early embryos (arrowhead indicates four-cell stage), increase after the ~100-cell stage (short arrow), and continue to dramatically increase throughout embryogenesis. The image in B has been color coded using a heat scale (Adobe Photoshop) to indicate the sharp increase in levels of β-G spectrin after gastrulation and in several tissues such as the developing nervous system in older embryos. The heat scale ranges from 0 (black) to 255 (white) to indicate the lowest and highest amounts of spectrin, respectively. (C). β-G spectrin localization during embryogenesis. β-G spectrin is associated with plasma membranes at sites of cell–cell contact (i and ii, arrows), starting at the two-cell stage, with cell membranes in the gut (iii, arrows; v, gt), in epidermal cells (iv, arrow indicates row of lateral seam cells), and in the developing pharynx (v, ph). Levels are particularly high in the nervous system, with intense labeling at the tips of the sensillae (vi and vii, arrowhead), and in the developing nerve ring (vii and viii, arrow) and nerve cords (see E below). (D) β-G spectrin in the adult. A confocal optical section centered at the level of epidermis in an adult hermaphrodite stained for β-G spectrin. Shows labeling of the lateral domains of seam cells (s), the cell borders of the spindle-shaped body-wall muscle cells (m), and the bundles of commissural axons (ca). (E) An optical section taken through the same worm, 25 μm below D. The intestine (gut) continues to show staining, but the highest levels of β-G spectrin are associated with the pharyngeal region (ph indicates the metacorpus), the spermathecae (sp), the tips of the sensillae (sn), the nerve ring (nr), and ventral nerve cord (vnc). Bars: (B) 25 μm; (C) 10 μm; (D and E) 50 μm.

together perform spectrin activities. This idea was tested by determining the phenotypes resulting from depleting the three spectrin genes, individually and in various combinations using the RNA-mediated interference *(RNAi)* technique (Guo and Kemphues, 1996; Fire et al., 1998).

dsRNA was prepared using partial cDNAs corresponding to different regions of each of the three identified spectrin subunits and was microinjected at 1 μg/μl into the syncytial gonad of young adult hermaphrodites (Fire et al., 1998). The F1 progeny born in the 8–72-h period after injection was analyzed. In all cases, dsRNA which were prepared from different regions of each gene, gave identical phenotypes that were identical to those exhibited by genetic nulls of each gene (McKeown et al., 1998; Hammar-

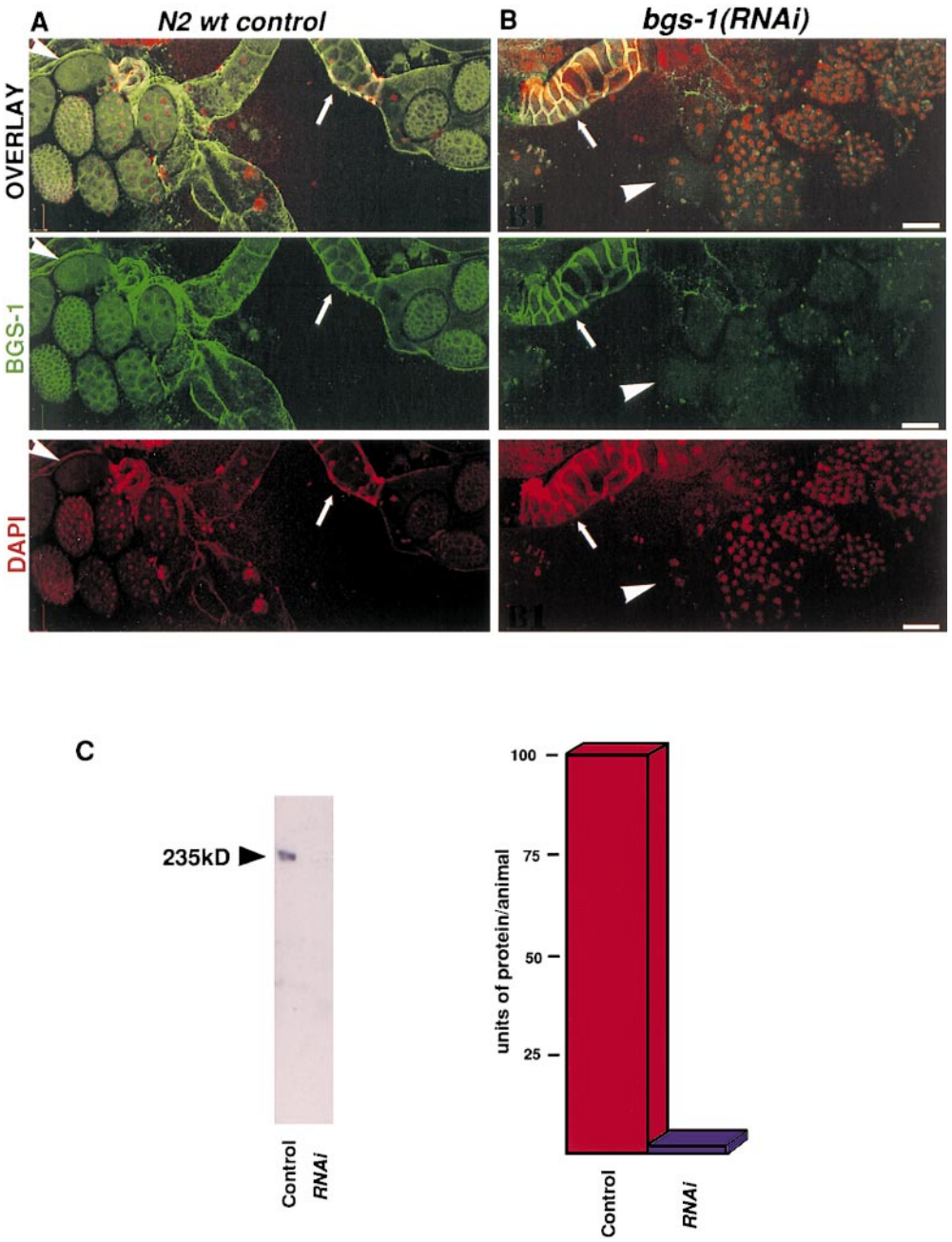

Figure 4. Depletion of β-G spectrin early in embryogenesis by *RNAi.* A noninjected adult hermaphrodite (A) and an adult (B) injected with β-G spectrin dsRNA were killed, and their embryos were processed for immunofluorescence labeling (see Materials and Methods). Double labeling with β-G spectrin antibody (green) and DNA (Hoechst, red) revealed that β-G spectrin is depleted in the F1 progeny of hermaphrodites injected with *bgs-1* dsRNA. Staining is reduced even in very early embryos (arrowhead indicates two-cell embryos in each panel), and the protein levels are demonstrably lower compared with similarly staged wild-type animals all through embryogenesis. β-G spectrin in the somatic spermatheca (arrows) serves as an internal staining control. Note that some of the intense FITC labeling of the spermatheca has bled into the Hoechst channel. (C) Measuring the effectiveness of *RNAi* in depleting animals of β-G spectrin. The amounts of β-G spectrin in the control, wild-type, and *RNAi* progeny, which are shown in an immunoblot, were quantitated using a PhosphorImager (ImageQuaNT). Taking the background signal into account, the counts were divided by the number of animals used in each lysate (250) to give an arbitrary unit of β-G spectrin per animal as shown in the graph. The numbers indicate that *RNAi* is effective in depleting β-G spectrin by at least 98%. Bars, 25 microns.

lund et al., 2000; Norman, K., and D. Moerman, personal communication).

In *bgs-1(RNAi)* embryos, β-G spectrin was reduced to undetectable levels at all stages of embryogenesis (Fig. 4 A, arrowheads indicate two-celled embryos). Immunoblots of *bgs-1(RNAi)* larvae indicated that *RNAi* was effective in reducing β-G spectrin by 98% (Fig. 4, C and D). Thus, as expected, *bgs-1(RNAi)* L1 larvae showed undetectable levels of β-G spectrin in all tissues with the exception of a low but reproducible level of staining in the nervous system (Fig. 5 G). β-G spectrin was present in normal levels in both *sma-1(RNAi) and spc-1(RNAi)* animals (Fig. 5, H and I) as predicted, which indicates that β-G spectrin depletion is specific to *bgs-1* dsRNA treatment.

The *bgs-1(RNAi)* progeny arrested as lethal L1 larvae (also known as the Let phenotype) and exhibited uncoordinated (Unc) coiling and kinking movements that severely limited motility (Fig. 5 B). *Sma-1(RNAi)* offspring were viable but had small rounded heads (also known as the Sma phenotype; Fig. 5 C). *Spc-1(RNAi)* animals displayed an Sma, Unc, Let phenotype; i.e., they arrested as uncoordinated early larvae and, in addition, had morphogenetic defects similar to those of *sma-1* L1 larvae (Fig. 5 D; McKeown et al., 1998). *bgs-1(RNAi);sma-1(RNAi)* double mutants arrest with an Sma, Unc, Let phenotype (Fig. 5 E) that was indistinguishable from that of *spc-1(RNAi)* animals. Hence, α-β-G and α-β-H complexes have separate and apparently complementary functions.

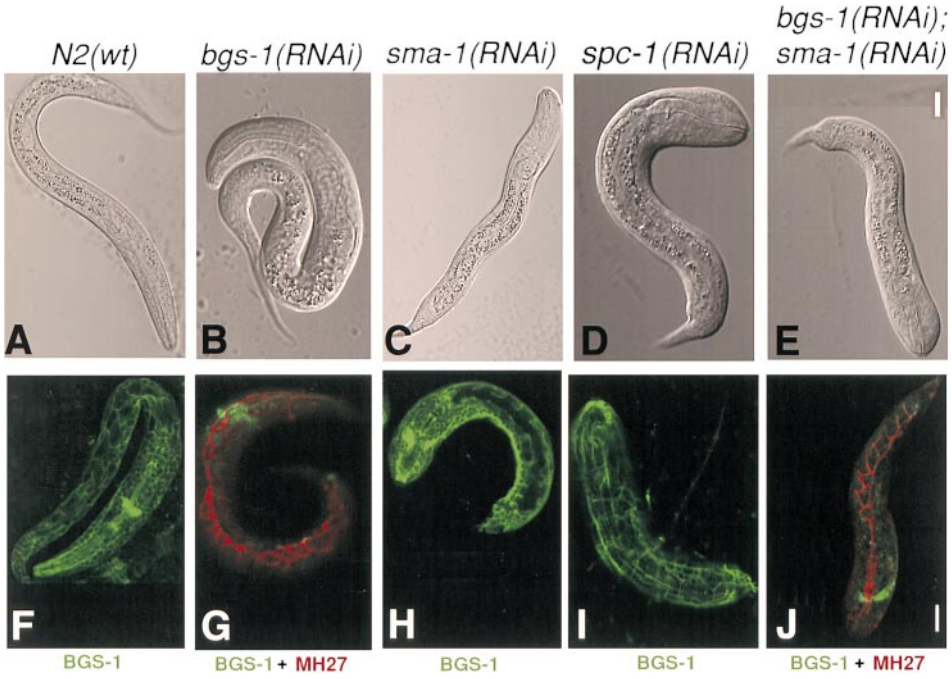

Figure 5. Phenotypic effects of α, β-G, and β-H spectrin depletion by *RNAi*. dsRNA corresponding to the three *C. elegans* spectrin genes (*spc-1*, *bgs-1*, *and sma-1* were microinjected into the syncytial gonad of young adults (see Materials and Methods). Progeny born 8 h after injection were analyzed and their phenotypes were recorded. *bgs-1(RNAi)* animals arrest as early uncoordinated L1 larvae (B), *sma-1(RNAi)* animals survive to form small (sma) viable adults (C), and both *spc-1(RNAi)* and [*bgs-1(RNAi);sma-1(RNAi)*] double mutants arrest as early small uncoordinated larvae (D and E). For each spectrin *RNAi* experiment, differential interference micrographs and indirect immunofluorescence labeling using *bgs-1* antibody (green) are shown. The mAb MH27 (red) recognizes adherens junctions, and was used as a permeabilization control in double labeling experiments for *bgs-1*, *sma-1*, and *[(bgs-1;sma-1) RNAi* larvae. Note that the low levels of residual β-G spectrin appear to stain the nerve rings in G and J. Bars, 10 μm.

Finally, since the observed *RNAi* mutant phenotypes of the single α spectrin subunit can be reproduced by depletion of the two β subunits in tandem, it is likely that *spc-1*, *bgs-1*, and *sma-1* together define the complete spectrin family in *C. elegans.*

β-G Spectrin Is Not Required for Embryonic Morphogenesis

As mentioned, morphogenetic defects were observed in *sma-1*, *spc-1*, and *sma-1*;*bgs-1* (*RNAi*) animals. To examine the role of spectrins in morphogenesis, embryonic elongation in *RNAi* progeny of various spectrin genes was followed by light microscopy (see Materials and Methods). All animals completed the initial proliferative stage of embryogenesis within the appropriate time interval (∼350 min) and, in all cases, epidermal cells completed ventral closure normally (Fig. 6 B, bean stage). The body lengths of *RNAi* animals were measured (Fig. 6 A) at the start of morphogenesis (1.5-fold stage), through elongation (Fig. 6 B, middle row of ∼2-fold embryos), and to the stage just before hatching (Fig. 6 B; Priess and Hirsh, 1986). *bgs-1 (RNAi)* embryos showed elongation kinetics close to that of the control embryos, whereas the other three *RNAi* animals (*sma-1*, *spc-1*, and *bgs-1*;*sma-1*) had slower rates of elongation, and usually failed to reach >50–80% of the length of control L1 larvae. These results indicate that β-G spectrin is not required for embryonic elongation.

β-G Spectrin Is Not Required for the Establishment of Epithelial Polarity in C. elegans Embryos

To determine if apical and basolateral domains of polarized epithelia were still physically segregated in the β-G spectrin RNAi mutants, adherens junctions were visualized using the mAb MH27. In control animals, β-G spectrin associates with only the lateral membranes of polarized epithelia, both in the epidermis (Fig. 7 A, arrowheads, left column; see seam cells in Fig. 3 D), and in the intestine (Fig. 7 A, arrows, left column, Di). Depletion of either β-G spectrin (Fig. 7 A, middle column) or both β-G and β-H spectrin subunits (Fig. 7 A, right column) does not affect localization of the MH27 antigen to the apicolateral domains of epidermal (arrowheads) or gut cells (arrows), which indicates that at least some adherens junction structures are assembled normally.

We determined the distribution of two proteins that normally distribute to the lateral domains of epidermal and intestinal epithelia: the *C. elegans* orthologue of vertebrate ankyrin, UNC-44 (Otsuka et al., 1995), and the ankyrin-binding protein, L1-related cell adhesion molecule LAD-1 (Chen, L., and V. Bennett, unpublished data). Each protein showed a normal polarized distribution in *bgs-1(RNAi)* animals to the lateral domains of epidermis (arrowheads) and intestine (Fig. 7 B, arrow in i points along the direction of the lumen visible to its left). Hence, ankyrin and LAD-1 did not require β-G spectrin for their normal assembly at the lateral domain of polarized epithelia. Further, ankyrin, and LAD-1 remained excluded from the apical domain, indicating that the signals required to recruit and retain these molecules at the lateral domains were independent of β-G spectrin.

Polarity and structural integrity of epithelia was confirmed at the ultrastructural level using electron microscopy of *bgs-1(RNAi)*-arrested larvae (Fig. 8 B; see Materials and Methods). A transverse section through the middle region showed that the L1 larvae in *bgs-1(RNAi)* animals had normal cuticle and alae (al), which are collagen-containing structures produced by the secretory activity of

seam cells. Polarized secretory functions that were associated with epidermal cells, therefore, were unaffected in these mutants. Further evidence of normal Golgi function is that storage granules (y) were clearly visible in the cytoplasm of gut cells. The intestinal microvilli (mv) had distinct terminal webs as expected, since the BGS-1 protein does not localize to the apical domain. The adherens junctions (aj) between the two intestinal cells were normal in structure and located at the apicolateral domain. Lateral membranes (lm) were distinct and intact, as was the basement membrane encasing the gut (note the coelomocyte (Cc) situated in the pseudocoelomic cavity). These results indicate that β-G spectrin is not required for the establishment of embryonic epithelial polarity in *C. elegans.*

spc-1(RNAi) larvae have normal levels of β-G spectrin (Figs. 7 C and 5 I). Because of severe morphogenetic defects in *spc-1(RNAi)* larvae, the intestinal cells, which normally twist 180° along the length of the animal (Sulston et al., 1983), instead were squashed and not properly aligned. However, it was clear that β-G spectrin continued to distribute to only the lateral domains of epithelia (portion of the gut enlarged). β-G spectrin staining is also restricted to the lateral domains in the intestinal epithelia (arrows) of *sma-1(ru18)* mutant adults (Fig. 7 D, arrows in ii). These data suggest that β-G spectrin subunits may be expressed and targeted to the membrane independently, but both α and β subunits are needed to form a functional spectrin molecule. Together, these results indicate that the establishment of embryonic epithelial polarity in *C. elegans* does not require spectrin function.

Depletion of β-G Spectrin by RNAi Leads to Progressive Defects in the Organization of the Myofilament Lattice of Body-wall Muscles

β-G spectrin is associated with the plasma membranes of the spindle-shaped body-wall muscle cells (m) from the embryonic stage (not shown) into adulthood (Fig. 3 D). Immunofluorescence of the adult muscle labeled for β-G spectrin identified two patterns of staining at two very close but distinct focal planes near the sarcolemma. When a thin optical section was centered closer to the myofilament lattice, staining adjacent to the sarcolemma appeared mainly as longitudinal striations (Fig. 9 A, L).

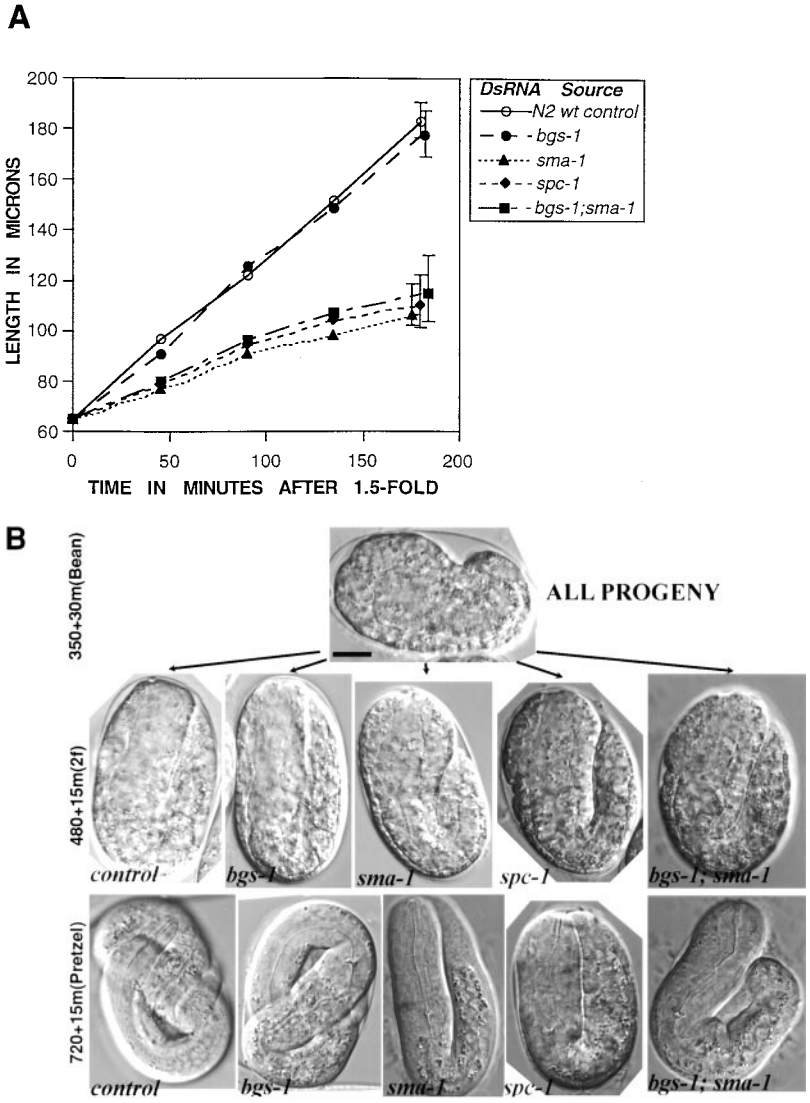

Figure 6. RNAi embryos depleted of β-G spectrin complete normal morphogenesis, unlike those α or β-H spectrin *RNAi* progeny. *RNAi* experiments were performed with various dsRNAs, and the microinjected mothers were killed on the second day. F1 progeny embryos were observed using differential interference optics and simultaneously videotaped (see Materials and Methods). Morphogenesis was considered to begin when the animal reached the 1.5-fold stage (~65 μm). The developmental stage of each animal was noted at regular intervals thereafter. (A) Elongation kinetics. Average length of each *RNAi* mutant strain was plotted against time starting at the 1.5-fold stage. *bgs-1(RNAi)* animals show kinetics of elongation close to that of control animals, unlike the remaining three strains that elongate more slowly and do not achieve normal length. Error bars indicate the range of lengths at the final time point. (B) Phase micrographs. Examples of three time points during embryonic development. Elongation is completed by 600 min, though animals were followed until they showed increased refractivity in the terminal bulb, which caused by secretion of the pharyngeal cuticle (bottom row, embryos at ~720 min).

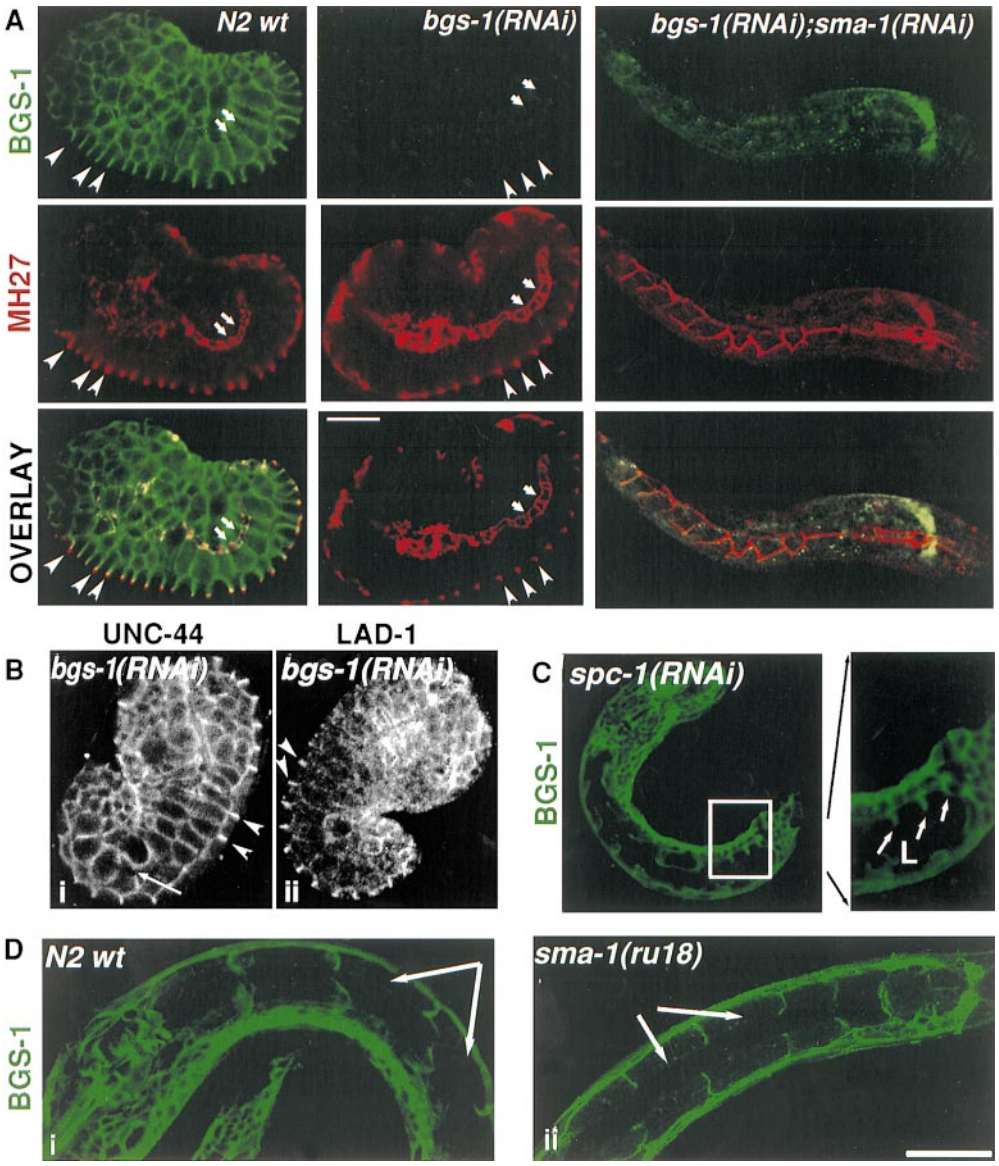

Figure 7. Depletion of each of the three *C. elegans* spectrins does not alter localization of proteins to distinct domains in the epithelia. (A) Formation of apicolateral adherens junctions. Control (left column) and *bgs-1(RNAi)* (middle) embryos at the bean stage were double labeled for the antibody against β-G spectrin (green) and the mAb MH27 (red), which recognizes an adherens junction component. Depletion of β-G spectrin (middle) did not affect localization of MH27 to the adherens junctions at the apicolateral domain of the epidermal epithelia (arrowheads) or the intestinal cells (arrows). *RNAi* using both *bgs-1* and *sma-1* β spectrin dsRNAs still did not prevent formation of properly localized adherens junctions in the gut (arrows, right column). (B) Localization of lateral domain markers. *bgs-1(RNAi)* animals at the bean stage were stained for the β-G spectrin-binding protein ankyrin/UNC-44 (i) and the ankyrin binding L1-related cell adhesion molecule LAD-1 (ii). Both localized normally to the lateral membranes of epidermal (arrowheads) and gut cells (arrows). (C) *spc-1(RNAi)* larvae were stained with the BGS-1 antibody. Though they arrest at the L1 stage with morphogenetic defects, these animals continued to express normal levels of β-G spectrin limited to the lateral membranes of gut cells. Inset shows a higher magnification of the boxed region, arrows point to the lateral domains of the gut cells. (D) In N2 adults (I), β-G spectrin clearly stains only the lateral but not the apical domains of the gut cells. In *sma-1(ru18)*–null mutants (ii), the absence of β-H spectrin does not affect the retention of β-G spectrin at the lateral membranes of the gut (arrows). Bars: (A–C) 10 μm; (D) 50 μm.

When the optical section was centered closer to the epidermal cell border, β-G spectrin staining at the sarcolemma formed a grid pattern (Fig. 9 A, S).

Double labeling experiments using antibodies against β-G spectrin and specific muscle components (Francis and Waterston, 1985; Hresko et al., 1994) revealed that β-G spectrin is associated with the sarcolemma adjacent to the I-bands (Fig. 9). When muscle sarcolemma was double labeled (Fig. 9 B) for β-G spectrin (BGS-1) and *pat-3*, which encodes β-integrin (MH25; Gettner et al., 1995), the two proteins colocalized (arrowheads) at the base of dense bodies, nematode homologues of Z discs. The two staining patterns did not completely overlap since BGS-1 staining extended more continuously along the I-bands. Conversely, at muscle–muscle cell boundaries, β-integrin staining was relatively more intense. When muscle cells were double labeled (Fig. 9 C) for BGS-1 and the myosin heavy chain A (mhcA), the broader longitudinal stripes of BGS-1 staining (arrowheads) lay between the stripes defined by mhcA (arrows), which is confined to the center of A-bands (Waterston, 1989).

It was not possible to determine whether the BGS-1 staining pattern that runs perpendicular to the long axis of the muscle cell (Fig. 9 A, S) represented β-G spectrin that was associated with the muscle sarcolemma or the epidermal membrane cytoskeleton. This was because the microscope's limit of resolution in the z axis is ~1 μm, whereas the hypodermal cell itself is only ~50 nm thick in the region overlaying the muscle cell, and the plasma membranes of the two cells are separated only by a basement

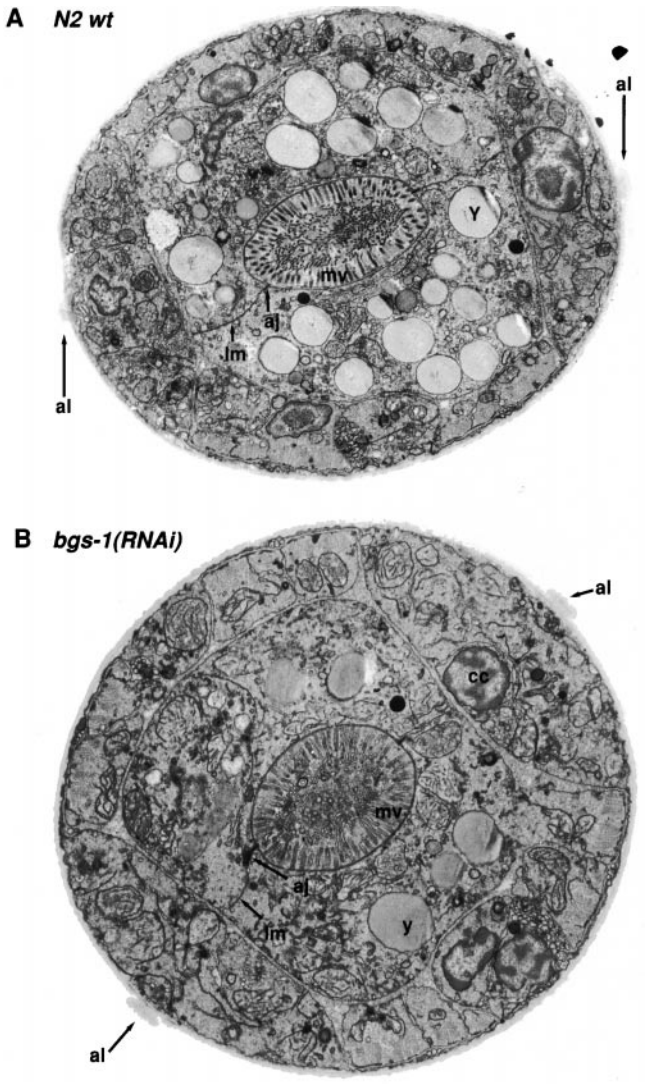

Figure 8. RNAi depletion of β-G spectrin does not affect the ultrastructural morphology of L1 larval intestinal epithelia. *bgs-1(RNAi)* and control L1 larvae were processed for electron microscopy (see Materials and Methods). Transverse sections, which were taken through the middle of the animal, show that gross morphology appears relatively normal in recently arrested L1 larvae. The seam cells polarize to deposit the distinctive cuticle of the alae (al). The adherence junction (aj) between the two intestinal cells shows appropriate localization and structure, and the intestinal microvilli (mv) and yolk granules (y) are abundant and morphologically normal. In particular, the lateral cell membranes (lm) separating the two cells appear distinct and unaltered at this stage. Note, coelomocyte (Cc) in pseudocoelom of B.

membrane. The BGS-1 staining pattern was similar (Fig. 9 D) but distinct from the pattern obtained by staining with mAb MH5, which recognizes a component of hemidesmosomes. Finally, we detected low but distinct BGS-1 staining at the base of the M-lines, in a pattern that colocalized with β-integrin (Fig. 9, small vertical arrows in B and C).

These results suggest that β-G spectrin is associated with the sarcolemma in regions where it is attached to adjacent cells. At the level of individual sarcomeres, β-G spectrin localizes to the region adjacent to the sarcolemma, adjoining I-bands, dense bodies, and to the base of the M-line; the latter two are regions of attachment of the muscle cells to the overlaying epidermal cell (Hresko et al., 1994). Spectrin further localizes in a pattern mimicking that of hemidesmosomes, components of the fibrous bodies that link together muscle sarcolemma, epidermis, and cuticle, and help transmit the force generated by muscle contraction to the cuticle during movement.

Consequences of depletion of β-G spectrin for organization of actin filaments and myosin thick filaments were evaluated in L1 larvae (Fig. 10). *bgs-1(RNAi)* L1-arrested larvae showed a perturbation in the distribution of actin microfilaments in muscle tissue, compared with wild type (Fig. 10 A). This disarray in arrangement of F-actin filaments was observed in both body-wall muscle and the myoepithelia of the pharynx. Freshly arrested larvae appeared to have fewer defects, whereas larvae that had survived on the plate for longer periods accumulated more disorganized foci. When body-wall muscles were stained for the thick filament component mhcA (Fig. 10 B), the myofilament lattice of animals depleted of β-G spectrin often displayed an unraveled appearance of A-bands. This progressive dystrophy of muscle tissue in *bgs-1(RNAi)* larvae suggests the hypothesis that β-G spectrin provides essential strength and elasticity to the sarcoplasmic membrane during activity of *C. elegans* body-wall muscles.

Depletion of β-G Spectrin in Early Larvae by RNAi Leads to Multiple Defects in the Nervous System

A subset of chemosensory neurons in the head concentrate fluorescent dye when nematodes are suspended in a solution containing the cationic membrane tracer dye DiI, allowing visualization of their neurite processes in live animals (see Materials and Methods; Hedgecock et al., 1985). When these neurons were observed in *bgs-1(RNAi)* animals, they usually displayed defects in the morphology of the neurite processes (Fig. 11). These lesions included apparent vacuolation in the dendritic processes of some amphid neurons (Fig. 11 B, white arrowhead), enlarged neuronal cell bodies (Fig. 11 B, thick arrow in ii), and occasional ectopic displacement of cell bodies (Fig. 11 B, thin arrow in ii). These data suggest that β-G spectrin plays a structural role in stabilizing neurite membranes, but do not exclude additional roles in membrane recycling and synaptic transmission.

Discussion

This paper presents a global analysis of *C. elegans* spectrins, and in particular β-G spectrin, the single orthologue of vertebrate β spectrins. This analysis includes the developmental expression, cellular localization, as well as consequences of depletion of β-G spectrin alone and in combination with other members of the *C. elegans* spectrin family via *RNAi. RNAi* has proven to be an effective approach in depleting *C. elegans* of many gene products (Fire et al., 1998). Indeed, our data show that spectrin is successfully removed with *RNAi.* Immunoblots indicate that at least 98% of spectrin is removed in *RNAi*-treated animals, and immunolabeling of *RNAi*-treated animals reveals es-

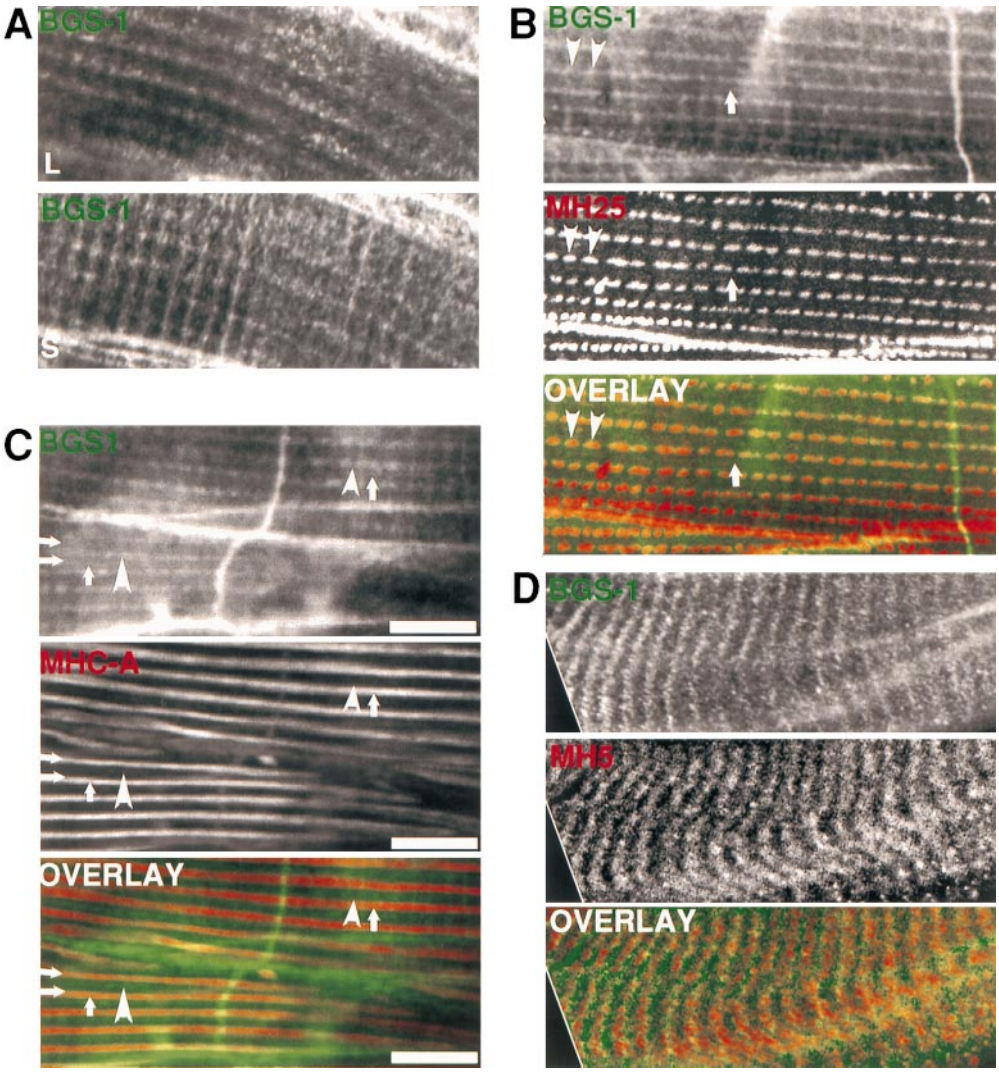

Figure 9. β-G spectrin is associated with multiple structures of the sarcolemma of body-wall muscle cells. (A) Optical sections in two focal planes through the same body-wall muscle cell stained with β-G spectrin antibody. L is centered closer to the myofilament lattice, whereas S is centered near the sarcolemma and closer to the overlaying epidermal cell. (B) Double labeling to visualize β-G spectrin and β-integrin (MH25). The two proteins colocalize at the base of dense bodies (arrowheads) and at the base of M-lines (arrow). β-G spectrin staining extends beyond the dense bodies and appears to lie close to the sarcolemma adjoining the I-bands. (C) Double labeling to visualize β-G spectrin and the myosin heavy chain A (MHC-A), the latter is restricted to the center of A-bands. The broader bands of spectrin staining (arrowheads) alternate with the MHC-A staining (arrows), while there is a faint staining of BGS-1 in the region corresponding to the M-line (vertical arrows). (D) Double labeling with antibodies against β-G spectrin and the hemidesmosomal component recognized by the mAb MH5 reveals that the patterns are very similar, but the two proteins do not colocalize. The animal was fixed with methanol, which reduces staining along the longitudinal tracts. All images are to the same scale. Bars, 10 μm.

sentially undetectable amounts of spectrin. Moreover, the *RNAi* of each spectrin gene results in phenotypes identical to those of the spectrin-null alleles (McKeown et al., 1998; Hammarlund et al., 2000; Norman, K., and D. Moerman, personal communication). The results of this study indicate that spectrin is not required for embryonic morphogenesis or the establishment of epithelial polarity, but it is important for proper body-wall muscle and nervous system development.

Several previous observations have established a role for spectrin in the maintenance of epithelial polarity, but could not directly address a possible role in its establishment. β Spectrin is recruited to the cytoplasmic surface of the basolateral plasma membranes of epithelial cells and to fibroblasts transfected with cadherins (McNeil et al., 1990). Spectrin also participates in complexes with cadherins and ankyrin (Nelson and Hammerton, 1989; Nelson et al., 1990). When overexpressed in cell lines, functional domains of human β-G spectrin disrupt membrane skeleton organization and cell polarity (Hu et al., 1995). In *Drosophila*, α spectrin is essential for larval survival and development, and spectrin plays essential roles in the maintenance of cell shape and cell–cell interactions (Lee et al., 1993, 1997). These studies support some role of spectrin in epithelial polarity, but do not address the consequences of the absence of spectrin from the onset of differentiation. In this study, the *RNAi* technique was successful in depleting β-G spectrin at a very early stage in embryogenesis (Fig. 4), before zygotic expression or tissue differentiation takes place. Surprisingly, depletion of the nematode β-G spectrin by *RNAi*, to essentially undetectable levels, does not affect establishment of structural and functional polarity in the epidermis and the intestine. In addition, the absence of the β-G spectrin does not affect enclosure of the embryo by migrating epithelia (Fig. 6 B, bean stage embryo), nor does it prevent embryonic elongation that depends on the epidermis (Fig. 6). Moreover, the spectrin-binding protein, ankyrin, and the ankyrin-

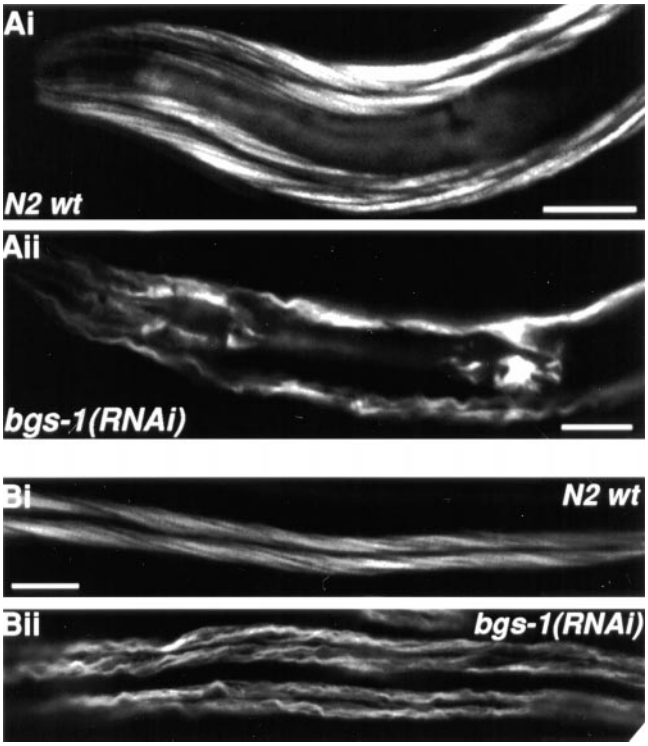

Figure 10. Depletion of β-G spectrin leads to progressive perturbation of the myofilament lattice of body-wall muscle. (A) Thin filaments. L1 larvae were fixed and stained with biotinylated phalloidin, followed by Cy5-coupled streptavidin to visualize F-actin in body-wall muscle. The F-actin–containing thin filaments in *bgs-1(RNAi)* L1 larvae (ii) are disorganized compared with controls (i). Faint staining from the pharynx is seen in both panels. Animals were costained with β-G spectrin antibody to ensure its depletion by *RNAi* (not shown). (B) Thick filaments. Labeling with the mAb DM5.6 that recognizes the myosin heavy chain A (mhcA). *bgs-1(RNAi)* larvae (ii) present an unraveled appearance of the A-bands. Bars, 5 μm.

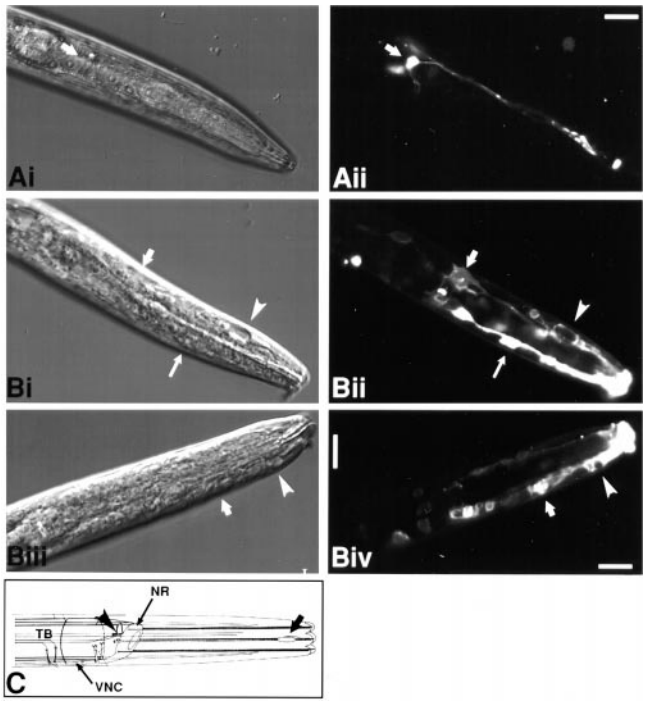

Figure 11. Depletion of β-G spectrin from the neurons of *C. elegans* larvae leads to defects in cell body size, location, and in neurite morphology. The dye-filling amphid neurons of (A) N2 and (B) *bgs-1(RNAi)* larvae were visualized using the cationic membrane tracer dye DiI (see Materials and Methods). The dye was visualized on the rhodamine channel (A ii and B ii). *bgs-1(RNAi)* larvae displayed a variety of defects, including cell bodies that are enlarged (large arrow) or ectopic (small arrow). Vacuoles (B, arrowhead in i and ii) were often observed in neurites and adjacent sheath cells, and resembled those formed by cell death in sick strains. The axonal processes leading into the nerve ring (black arrowhead) and the dendritic tree (black arrow) are marked. Bars, 5 μm.

binding cell adhesion molecule, LAD-1, continue to target to lateral membranes and are excluded from the apical domain (Fig. 7). The epithelial cells retain integrity and function, and are ultrastructurally normal (Fig. 8). These data indicate that β-G spectrin is not required for the establishment of polarity in *C. elegans* epithelia; instead, they support a model in which cell adhesion molecules direct assembly of the spectrin-based membrane skeleton to the sites of cell–cell contact (Yeaman et al., 1999), and where this cytoskeleton plays a subsequent role in the maintenance of integrity of the epithelial monolayer.

While we cannot formally rule out the possibility that the remaining 2% of β-G the spectrin, which is not depleted by *RNAi*, may play a role in establishing early epithelial polarity, it is extremely unlikely that such a small amount of a structural protein would be sufficient to carry out such a process. Moreover, the epithelial polarity in *unc-70*–null mutants is normal (Hammarlund et al., 2000). Another, albeit remote, possibility is that β-H spectrin may be performing redundant functions as β-G spectrin in the epithelia. However, β-H and β-G spectrins expression patterns do not overlap (apical [McKeown et al., 1998] versus basolateral, respectively). Moreover, the loss of both β-H and β-G spectrins does not affect epithelial polarity as indicated by the presence of adherens junctions and proper localization of basolateral markers in the epithelia (Fig. 7 A).

The *RNAi* phenotype of the single *C. elegans* α-spectrin can be reproduced by tandem depletion of both β-G and β-H spectrin subunits. This data combined with a lack of other spectrin genes in the *C. elegans* genome suggests that α spectrin associates with β-G and β-H subunits to form α/β-G and α/β-H heteromers, which perform the entire repertoire of spectrin function in the nematode. The loss of either α spectrin or both β spectrins (β-G and β-H), thus, results in animals with no functional spectrin. Removal of functional spectrin by the depletion of either the *spc-1* α spectrin or the *sma-1* β-H spectrin also did not affect epithelial polarity. Hence, spectrin is not required for the establishment of epithelial polarity.

The concept of a widespread Golgi-centric spectrin–ankyrin skeleton is exciting (Beck and Nelson, 1998; De Matteis and Morrow, 1998; Holleran and Holzbaur, 1998), but the precise Golgi spectrin isoforms have yet to be de-

fined. The newly cloned β-spectrin III, SPTBN2, (Ohara et al., 1998), which was reported as a Golgi-associated spectrin (Stankewich et al., 1998) but has a restricted tissue expression, is most highly expressed in the brain and stains dendrites more strongly than cell bodies (Ohara et al., 1998). In this study, an affinity-purified antibody against the only *C. elegans* β-G spectrin did not recognize any specific intracellular organelle. No distinctive cytoplasmic staining was evident in early founder cells (Fig. 3 C, i and ii), in differentiated gut or hypodermal cells (Fig. 3 C, iii–viii, and Fig. 7) or in any other examined cell type. *RNAi* depletion of β-G spectrin to levels below the threshold of detection did not prevent *C. elegans* embryos from developing from a one-cell zygote to a morphologically normal L1 larva of ~550 cells. Further, several developmental processes that require Golgi-dependent secretory functions proceeded normally; these included the secretion of cuticle by the epidermal cells, the formation of collagen-derived alae by the seam cells, and the generation of the various basement membranes. These data do not support a role for β spectrin in Golgi function and intracellular transport in *C. elegans*. Several caveats should be considered in extrapolating the lack of a Golgi spectrin in *C. elegans* to vertebrates. It is conceivable that Golgi spectrin did not appear till later in evolution, or that *C. elegans* lost the requirement for this proposed function of the spectrin cytoskeleton. Since ~3% of the genome remains to be sequenced, it is also formally possible but highly unlikely that a third β-spectrin and a second α spectrin gene remain to be identified in *C. elegans* that may localize to Golgi membranes.

The expression patterns of both nematode β-G spectrin and vertebrate β spectrins exhibit striking parallels. In both cases, β spectrins are associated with sites of cell–cell contact in epithelial tissues; the highest levels of β spectrin occur in the nervous system; and β spectrin in striated muscle is associated with points of attachment of the myofilament apparatus to adjacent cells.

Spectrin in a vertebrate-striated muscle is organized in a two-dimensional lattice at the sarcolemma, with longitudinal and transverse components. The transverse bands repeat in register with I-bands of the adjacent layer of myofibrils, and are part of riblike structures termed costameres (Craig and Pardo, 1983). Visualization of β-G spectrin localization *C. elegans* muscle reveals a strikingly similar lattice pattern, but the origins of the two patterns are distinct, since there is no costamere equivalent in the nematode. In addition, the I-bands run in longitudinal stripes, with the Z-disc equivalents (dense bodies) no longer in register but interspersed as discrete structures at the center of the stripes (Francis and Waterston, 1985). Thus, in the *C. elegans* β-G spectrin–staining lattice, the longitudinal bands arise from subsarcolemmal β-G spectrin adjacent to I-bands in a series of sarcomeres that are slightly offset from each other. The transverse elements in the *C. elegans* β-G spectrin lattice may actually define a different structure, one that seems to mimic the distribution of hemidesmosomes in the overlaying epidermis. One important similarity between the two muscle types is the occurrence of β-spectrin at regions where the myofilament apparatus is attached to the sarcolemmal membrane. In *C. elegans*, these sites also help to anchor the muscle to the overlaying hypodermis, and through it to the cuticle, allowing direct transmission of force from the contractile apparatus to the exoskeleton. The progressive disorganization of muscle seen in L1 larvae depleted of β-G spectrin may be due to an activity-based detachment of the myofilament apparatus from the sarcolemma, indicating a structural role for spectrin at these sites.

Spectrin and its associated proteins show robust expression in the *C. elegans* nervous system, along with the spectrin-binding protein ankyrin (UNC-44; Otsuka et al., 1995), the ankyrin-binding protein LAD-1 (Chen, L., and V. Bennett, unpublished data), and the spectrin assembly factor adducin, ADD-1 (Moorthy, S., and V. Bennett, unpublished data). Spectrin has been suggested to have a role in axonal transport (Levine and Willard, 1981), neurite extension (Sobue and Kanda, 1989; Sihag et al., 1996), and organization of synaptic vesicles (Sikorski et al., 1991; Sakaguchi et al., 1998). The defects accumulated by *C. elegans* neurons depleted of β spectrin may reflect a disruption of these activities as well as a role in stabilizing plasma membranes of axonal and dendritic processes.

Spectrin has not been detected in yeast, and while spectrin-related proteins have been reported in unicellular organisms (Pollard, 1984; Bennett and Condeelis, 1988), a spectrin α-β heteromer has been reported only in metazoans (for review see Thomas et al., 1997). The remarkable similarity of spectrins from organisms as diverse as *C. elegans*, *Drosophila melanogaster*, and *Homo sapiens* suggests that many functions can be expected to be conserved among these species. One hypothesis is that heteromeric spectrin evolved in metazoan animal cells in response to the need for formation of mechanically integrated tissues that can withstand the rigors imposed by an active organism. Such a role would preclude participation of spectrin in fundamental cellular processes, but would account for the wide range of spectrin isoforms observed in vertebrates. Consistent with this hypothesis is the expression pattern of spectrin, which is first observed at the plasma membrane of a two-cell embryo. Spectrin levels remain low but distinct through gastrulation, when most cell proliferation has been completed (Sulston et al., 1983). However, spectrin levels dramatically increase after gastrulation, when most cells are undergoing differentiation. Spectrin levels are particularly high in the nervous system, and are high in tissues that experience mechanical stress such as the body-wall muscles, the pharynx, and the spermatheca. Such a dramatic increase in spectrin levels after gastrulation similarly is seen during *Drosophila* development (Pesacreta et al., 1989). The spectrin heteromer could perform functions ranging from that of a simple plasma membrane scaffold providing elasticity and strength to the plasma membrane, as in erythrocytes, to that of a component that integrates extracellular signal transduction with cytoplasmic responses. This paper has helped define the range of cell types in which spectrin may play an essential role in a relatively simple metazoan. With genetic mutations now available in all three spectrin genes *C. elegans* promises to become an important model system for the study of the spectrin-based membrane cytoskeleton.

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References

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.

Barstead, R.J., and R.H. Waterston. 1991. Vinculin is essential for muscle function in the nematode. *J. Cell Biol.* 114:715–724.

Beck, K.A., J.A. Buchanan, V. Malhotra, and W.J. Nelson. 1994. Golgi spectrin: identification of an erythroid beta-spectrin homolog associated with the Golgi complex. *J. Cell Biol.* 127:707–723.

Beck, K.A., and W.J. Nelson. 1998. A spectrin membrane skeleton of the Golgi complex. *Biochim. Biophys. Acta.* 1404:153–160.

Bennett, V., and D. Gilligan. 1993. The spectrin-based membrane skeleton and micron-scale organization of the plasma membrane. *Annu. Rev. Cell Biol.* 9:27–66.

Bennett, H., and J. Condeelis. 1988. Isolation of an immunoreactive analogue of brain fodrin that is associated with the cell cortex of *Dictyostelium* amoebae. *Cell Motil. Cytoskeleton*. 11:303–317.

Black, J.D., S.T. Koury, R.B. Bankert, and E.A. Repasky. 1988. Heterogeneity in lymphocyte spectrin distribution: ultrastructural identification of a new spectrin-rich cytoplasmic structure. *J. Cell Biol.* 106:97–109.

Bloch, R.J., and J.S. Morrow. 1989. An unusual β-spectrin associated with clustered acetylcholine receptors. *J. Cell Biol.* 108:481–493.

Blumenthal, T., and K. Steward. 1997. RNA processing and gene structure. *In C. elegans* II. D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 117–146.

Byers, T.J., A. Husain-Chisht, R.R. Dubreuil, D. Branton, and L.S. Goldstein. 1989. Sequence similarity of the amino-terminal domain of *Drosophila* β spectrin to α-actinin and dystrophin. *J. Cell Biol.* 109:1633–1641.

Byers, T.J., E. Brandin, R. Lue, E. Winograd, and D. Branton. 1992. The complete sequence of *Drosophila* β spectrin reveals supra-motifs comprising eight 106-residue repeats. *Proc. Natl. Acad. Sci. USA*. 89:6187–6191.

C. elegans Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science.* 282:2012–2018.

Craig, S.W., and J.V. Pardo. 1983. Gamma actin, spectrin and intermediate filament proteins colocalize with vinculin at costameres, myofibril-to-sarcolemma attachment sites. *Cell Motil.* 3:449–462.

de Cuevas, M., J.K. Lee, and A.C. Spradling. 1996. Alpha-spectrin is required for germline cell division and differentiation in the *Drosophlia* ovary. *Development*. 122:3959–3968.

De Matteis, M.A., and J.S. Morrow. 1998. The role of ankyrin and spectrin in membrane transport and domain formation. *Curr. Opin. Cell Biol.* 10:542–549.

Devarajan, P., P.R. Stabach, M.A. De Matteis, and J.S. Morrow. 1997. Na,K-ATPase transport from endoplasmic reticulum to Golgi requires the Golgi spectrin-ankyrin G119 skeleton in Madin Darby canine kidney cells. *Proc. Natl. Acad. Sci. USA*. 94:10711–10716.

Dubreuil, R.R., T.J. Byers, A.L. Sillman, D. Bar-Zvi, L.S. Goldstein, and D. Branton. 1989. The complete sequence of *Drosophila* α-spectrin: conservation of structural domains between α-spectrins and α-actinin. *J. Cell Biol.* 109:2197–2205.

Dubreuil, R.R., T.J. Byers, C.T. Stewart, and D.P. Kiehart. 1990. A β-spectrin isoform from *Drosophila* (beta H) is similar in size to vertebrate dystrophin. *J. Cell Biol.* 111:1849–1858.

Dubreuil, R.R., P.B. Maddux, T.A. Grushko, and G.R. MacVicar. 1997. Segregation of two spectrin isoforms: polarized membrane-binding sites direct polarized membrane skeleton assembly. *Mol. Biol. Cell.* 8:1933–1942.

Epstein, H.F., and D.C. Shakes. 1995. *Caenorhabditis elegans*: Modern Biological Analysis of an Organism. *In* Methods in Cell Biology. Vol. 48. Academic Press Inc., USA.

Fath, K.R., G.M. Trimbur, and D.R. Burgess. 1997. Molecular motors and a spectrin matrix associate with Golgi membranes in vitro. *J. Cell Biol.* 139: 1169–1181.

Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, and C.C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans. Nature.* 391:806–811.

Francis, R., and Waterston, R.H. 1985. Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. *J. Cell Biol.* 101:1532–1549.

Gettner, S.N., C. Kenyon, and L.F. Reichardt. 1995. Characterization of β pat-3 heterodimers, a family of essential intergrin receptors in *C. elegans. J. Cell Biol.* 129:1127–1141.

Glenney, J.R., P. Glenney, and K. Weber. 1983. The spectrin-related molecule, TW-260/240, cross-links the actin bundles of the microvillus rootlets in the brush borders of intestinal epithelial cells. *J. Cell Biol.* 96:1491–1496.

Guo, S., and K.J. Kemphues. 1996. A non-muscle myosin required for embryonic polarity in *Caenorhabditis elegans. Nature.* 382:455–458.

Hammarlund, M., W.S. Davis, and E.M. Jorgensen. 2000. Mutations in β-spectrin disrupt axon outgrowth and sarcomere structure. *J. Cell Biol.* 149:931–942.

Hedgecock, E.M., J.G. Culotti, J.N. Thomson, and L.A. Perkins. 1985. Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. *Dev. Biol.* 111:158–170.

Hobert, O., D.G. Moerman, K.A. Clark, M.C. Beckerle, and G. Ruvkun. 1999. A conserved LIM protein that affects muscular adherens junction integrity and mechanosensory function in *Caenorhabditis elegans. J. Cell Biol.* 144:45–57.

Holleran, E.A., M.K. Tokito, S. Karki, and E.L.K. Holzbaur. 1996. Centractin (ARP1) associate with spectrin revealing a potential mechanism to link dynactin to intracellular organelles. *J. Cell Biol.* 135:1815–1829.

Holleran, E.A., and E.L. Holzbaur. Speculating about spectrin: new insights into the Golgi-associated cytoskeleton. 1998. *Trends Cell Biol.* 8:26–29.

Hresko, M.C., B.D. Williams, and R.H. Waterston. 1994. Assembly of body wall muscle and muscle cell attachment structures in *Caenorhabditis elegans. J. Cell Biol.* 124:491–506.

Hu, R.J., M. Watanabe, and V. Bennett. 1992. Characterization of human brain cDNA encoding the general isoform of beta-spectrin. *J. Biol. Chem*. 267: 18715–18722.

Hu, R.J., S. Moorthy, and V. Bennett. 1995. Expression of functional domains of βG-spectrin disrupts epithelial morphology in cultured cells. *J. Cell Biol.* 128:1069–1080.

Johnsen, R.C., and D.L. Baillie. 1991. Genetic analysis of a major segment [LGV(left)] of the genome of *Caenorhabditis elegans. Genetics.* 129:735–752.

Lee, J.K., R.S. Coyne, R.R. Dubreuil, L.S. Goldstein, and D. Branton. 1993. Cell shape and interaction defects in α-spectrin mutants of *Drosophila melanogaster. J. Cell Biol.* 123:1797–1809.

Lee, J.K., E. Brandin, D. Branton, and L.S. Goldstein. 1997. α-Spectrin is required for ovarian follicle monolayer integrity in *Drosophila melanogaster. Development.* 124:353–362.

Levine, J., and M. Willard. 1981. Fodrin: axonally transported polypeptides associated with the internal periphery of many cells. *J. Cell Biol.* 90:631–642.

Li, X., and V. Bennett. 1996. Identification of the spectrin subunit and domains required for formation of spectrin/adducin/actin complexes. *J. Biol. Chem*. 271:15695–15702.

Lin, H., L. Yue, and A.C. Spradling. The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. 1994. *Development*. 120:947–956.

Lux, S.E., and J. Palek. 1995. Disorders of the red cell membrane. *In* Blood: Principles and Practice of Hematology. R.I. Handin, S.E. Lux, and T.P. Stossel, editors. J.B. Lippincott Co., Philadelphia. 1701–1818.

Macias, M.J., A. Musacchio, H. Ponstingl, M. Nilges, M. Saraste, and H. Oschkinat. 1994. Structure of the pleckstrin homology domain from beta-spectrin. *Nature.* 369:675–677.

McKeown, C., V. Praitis, and A.J. Austin. 1998. *Sma-1* encodes a βH-spectrin homolog required for *Caenorhabditis elegans* morphogenesis. *Development.* 125:2087–2098.

McNeill, H., M. Ozawa, R. Kemler, and W.J. Nelson. 1990. Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. *Cell*. 62:309–316.

Nelson, W.J., and P.J. Veshnock. 1987. Modulation of fodrin (membrane skeleton) stability by cell–cell contact in Madin-Darby canine kidney epithelial cells. *J. Cell Biol.* 104:1527–1537.

Nelson, W.J., and R.W. Hammerton. 1989. A membrane–cytoskeletal complex containing Na^+,K^+-ATPase, ankyrin, and fodrin in Madin-Darby canine kidney (MDCK) cells: implications for the biogenesis of epithelial cell polarity. *J. Cell Biol.* 108:893–902.

Nelson, W.J., E.M. Shore, A.Z. Wang, and R.W. Hammerton. 1990. Identification of a membrane–cytoskeletal complex containing the cell adhesion molecule uvomorulin (E-cadherin), ankyrin, and fodrin in Madin-Darby canine kidney epithelial cells. *J. Cell Biol.* 110:349–357.

Ohara, O., R. Ohara, H. Yamakawa, D. Nakajima, and M. Nakayama. 1998. Characterization of a new beta-spectrin gene which is predominantly expressed in the brain. *Brain Res. Mol. Brain Res.* 57:181–192.

Otsuka, A.J., R. Franco, B. Yang, K.-H. Shim, L.Z. Tang, Y.Y. Zhang, P. Boontrakulpoontawee, A. Jeyaprakash, E. Hedgecock, V.I. Wheaton, and A. Sobery. 1995. An ankyrin-related gene (*unc-44*) is necessary for proper axonal guidance in *Caenorhabditis elegans. J. Cell Biol.* 129:1081–1092.

Pesacreta, T.C., T.J. Byers, R. Dubreuil, D.P. Kiehart, D. Branton. 1989. *Drosophila* spectrin: the membrane skeleton during embryogenesis. *J. Cell Biol.* 108:1697–1709.

Pollard, T.D. 1984. Purification of a high molecular weight actin filament gelation protein from *Acanthamoeba* that shares antigenic determinants with vertebrate spectrins. *J. Cell Biol.* 99:1970–1980.

Priess, J.R., and D.I. Hirsh. 1986. *C. elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* 117:156–173.

Sakaguchi, G., S. Orita, A. Naito, M. Maeda, H. Igarashi, T. Sasaki, and Y. Takai. 1998. A novel brain-specific isoform of beta spectrin: isolation and its interaction with Munc13. *Biochem. Biophys. Res. Commun.* 248:846–851.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sihag, R.K., T.B. Shea, and F.-S. Wang. 1996. Spectrin-actin interaction is required for neurite extension in NB 2a/dl neuroblastoma cells. *J. Neurosci. Res.* 44:430–437.

Sikorski, A.F., G. Terlecki, I.S. Zagon, and S.R. Goodman. 1991. Synapsin I-mediated interaction of brain spectrin with synaptic vesicles. *J. Cell Biol.* 114:313–318.

Sobue, K., and K. Kanda. 1989. Alpha-actinins, calspectin (brain spectrin or fodrin), and actin participate in adhesion and movement of growth cones. *Neuron.* 3:311–319.

Stankewich, M.C., W.T. Tse, L.L. Peters, Y. Ch'ng, K.M. John, P.R. Stabach, P. Devarajan, J.S. Morrow, and S.E. Lux. 1998. A widely expressed betaIII spectrin associated with Golgi and cytoplasmic vesicles. *Proc. Natl. Acad. Sci. USA*. 95:14158–14163.

Sulston, J., and H. Horvitz. 1977. Postembryonic cell lineages of the nematode *Caenorhabditis elegans. Dev. Biol.* 82:41–55.

Sulston, J., E. Schierenberg, J. White, and J. Thomson. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans. Dev. Biol.* 100:64–119.

Sulston, J., and J. Hodgkin. 1988. Methods. *In* The Nematode *Caenorhabditis elegans.* W.B. Wood and the Community of *C. elegans* Researchers, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 587–652.

Thomas, G.H., and D.P. Kiehart. 1994. Beta heavy-spectrin has a restricted tissue and subcellular distribution during *Drosophila* embryogenesis. *Development.* 120:2039–2050.

Thomas, G.H., E.C. Newbern, C.C. Korte, M.A. Bales, S.V. Muse, A.G. Clark, and D.P. Kiehart. 1997. Intragenic duplication and divergence in the spectrin superfamily of proteins. *Mol. Biol. Evolution.* 14:1285–1295.

Thomas, G.H., D.C. Zarnescu, A.E. Juedes, M.A. Bales, A. Londergan, C.C. Korte, and D.P. Kiehart. 1998. *Drosophila* betaHeavy-spectrin is essential for development and contributes to specific cell fates in the eye. *Development.* 125:2125–2134.

Viel, A., and D. Branton. 1996. Spectrin: on the path from structure to function. *Curr. Opin. Cell Biol.* 8:49–55.

Wang, D.S., R. Miller, R. Shaw, and G. Shaw. 1996. The pleckstrin homology domain of human beta I sigma II spectrin is targeted to the plasma membrane in vivo. *Biochem. Biophy. Res. Comm.* 225:420–426.

Waterston, R.H. 1989. The minor myosin heavy chain, mhcA, of *Caenorhabditis elegans* is necessary for the initiation of thick filament assembly. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3429–3436.

White, J.G., E. Southgate, J.N. Thomson, and S. Brenner. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans. Phil. Trans. Roy. Soc. Lond.* 314:1–340.

Winkelmann, J.C., J.G. Chang, W.T. Tse, A.L. Scarpa, V.T. Marchesi, and B.G. Forget. 1990. Full-length sequence of the cDNA for human erythroid beta-spectrin. *J. Biol. Chem.* 265:11827–11832.

Winograd, E., D. Hume, and D. Branton. 1991. Phasing the conformational unit of spectrin. *Proc. Natl. Acad. Sci. USA*. 88:10788–10791.

Yeaman, C., K.K. Grindstaff, and W.J. Nelson. 1999. New perspectives on mechanisms involved in generating epithelial cell polarity. *Phys. Rev.* 79:73–98.

Zarnescu, D.C., and G.H. Thomas. 1999. Apical spectrin is essential for epithelial morphogenesis but not picobasal polarity in *Drosophila. J. Cell Biol.* 146: 1075–1086.