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Profilin and the Abl Tyrosine Kinase Are Required for Motor Axon Outgrowth in the *Drosophila* Embryo

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Summary

The ability of neuronal growth cones to be guided by extracellular cues requires intimate communication between signal transduction systems and the dynamic actin-based cytoskeleton at the leading edge. Profilin, a small, actin-binding protein, has been proposed to be a regulator of the cell motility machinery at leading edge membranes. However, its requirement in the developing nervous system has been unknown. Profilin associates with members of the Enabled family of proteins, suggesting that Profilin might link Abl function to the cytoskeleton. Here, genetic analysis in *Drosophila* is used to demonstrate that mutations in Profilin (*chickadee*) and Abl (*abl*) display an identical growth cone arrest phenotype for axons of intersegmental nerve b (ISNb). Moreover, the phenotype of a double mutant suggests that these components function together to control axonal outgrowth.

Introduction

As neuronal growth cones navigate through complex environments toward their appropriate targets, they encounter extracellular cues that are translated into directional locomotion. At the leading edge of the growth cone, where the interpretation of guidance information is initiated, dynamic and specialized membrane structures extend and explore the extracellular environment. These membrane structures (filopodia and lamellipodia) are supported by a complex cytoskeletal architecture composed largely of actin and a host of associated proteins. Rearrangements in this actin-based scaffold are responsible for both the motility of the leading edge (reviewed by Mitchison and Kirschner, 1988; Smith, 1988; Theriot and Mitchison, 1991; Stossel, 1993) and the directional specificity of the neuronal growth cone (e.g., Letourneau and Marsh, 1984; Bentley and Toroian-Raymond, 1986).

Actin-based structures at the leading edge are organized and regulated by multiple actin-binding proteins. Some of these proteins function to bundle and cross-link actin filaments into large cables or networks, whereas others are involved in regulating the ongoing cycle of actin assembly and disassembly (reviewed by Pollard and Cooper, 1986). Several actin-associated proteins also interact with intermediates of signal transduction pathways and are thus candidates for mediating a link between actin dynamics and the cellular communication that controls motility (reviewed by Forscher, 1989). For example, the small, actin-binding protein Profilin binds and may regulate the cleavage of phosphoinositide 4,5 bisphosphate (e.g., Lassing and Lindberg, 1985; Goldschmidt-Clermont et al., 1990; Machesky et al., 1990). Numerous biochemical studies suggest that Profilin controls actin assembly (e.g., Pollard and Cooper, 1984; Goldschmidt-Clermont et al., 1991). In vitro, Profilin can act as a G-actin-sequestering factor (e.g., Cao et al., 1992); however, Profilin can also promote polymerization by catalyzing actin nucleotide exchange (Goldschmidt-Clermont et al., 1992) or by reducing the critical concentration of actin monomer in the presence of thymosin-β_4 (Pantaloni and Carlier, 1993). However, the precise mechanism by which Profilin regulates actin in vivo is a controversial topic.

In motile cells, Profilin is recruited to sites of active cytoskeletal assembly, presumably through associations with other intracellular proteins. Profilin binds to N-WASP, a signaling protein that links multiple signaling components to direct, actin-dependent events (Suetsugu et al., 1998). In addition, Profilin associates with the Arp2–3 complex (Mullins et al., 1998), which is present in lamellipodia and other dynamic, actin-based structures (Machesky et al., 1997) and appears to initiate actin polymerization during the intracellular motility of the bacterial pathogen Listeria monocytogenes (Reinhard et al., 1995; Welch et al., 1997). Profilin also associates with members of the Enabled (Ena) protein family, including vasodilator-stimulated phosphoprotein (VASP; Reinhard et al., 1995) and mammalian Enabled (Mena; Gertler et al., 1996). Like the Arp2–3 complex, VASP and Mena are recruited by the Listeria protein ActA to sites of actin polymerization, where they colocalize with Profilin (Theriot and Mitchison, 1993; Gertler et al., 1996; Niebuhr et al., 1997). Blockade of either VASP binding to ActA (Smith et al., 1996) or Profilin binding to VASP (Kang et al., 1997) impairs Listeria motility, implying a role for Profilin in accelerating the actin-dependent motility. The fact that Enabled was originally identified as a component in the *Drosophila* Abl tyrosine kinase pathway (Gertler et al., 1990, 1995) has raised the additional possibility that Profilin functions to link Abl activity to actin assembly in some way.

Genetic analysis of Profilin function in different systems supports the notion that this protein regulates the assembly of actin cytoskeleton. For example, yeast lacking Profilin show defects in cell shape and actin localization (Magdolin et al., 1988; Haarer et al., 1990). Furthermore, in mutant *Drosophila* that lack Profilin during oogenesis, specific classes of actin cytoskeletal structures fail to assemble properly at key stages of oocyte development (Cooley et al., 1992; Manseau et al., 1996).

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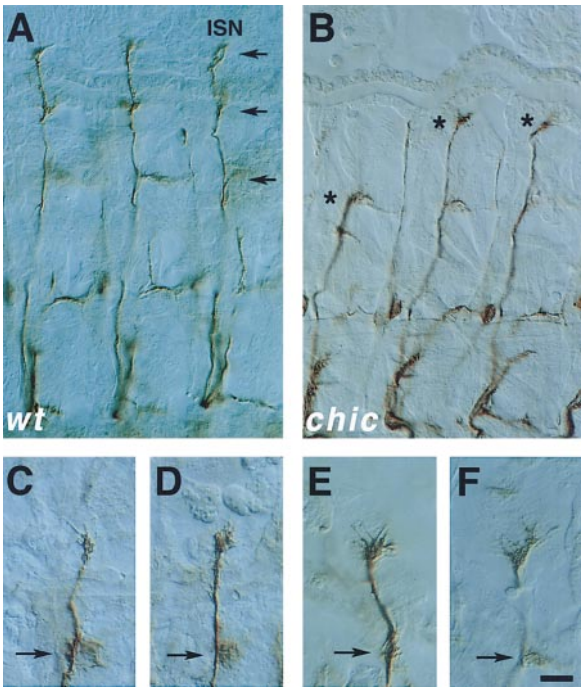

Figure 1. Embryonic Motoneuron Projections in Wild-Type and *stranded* Mutants

(A) The periphery of three abdominal hemisegments is shown in a stage 16 wild-type embryo fillet stained with mAb 1D4. The ISN extends to contact dorsal muscles, making three branches (arrows) at contacts with the major targets. Segmental nerve a (SNa) innervates lateral muscles, and intersegmental nerve b (ISNb, out of focus) innervates the ventral–longitudinal muscles. Ventral is at the bottom and anterior is to the left.

(B) The same view shown in (A) is seen in a *chic*^{sand1}*/Df(2L)GpdhA* mutant embryo. ISN growth cones arrest at branching choice points (asterisks). SNa branches fail to innnervate lateral muscles 21–24. ISNb branches stop short of final targets (arrowheads; see also Figure 5).

(C and D) Wild-type ISN growth cones located in the vicinity of the second branch choice point, at early embryonic stage 16, just beyond the first motor branchpoint in contact with PT3 (arrow).

(E and F) Arrested ISN growth cones in *chic*^{sand1}*/Df(2L)GpdhA* embryos, as seen at embryonic stage 17. Although these growth cones have stopped short of potential targets, they display normal morphology; many filopodia can be seen.

Scale bar, 15 μm for (A) and (B) and 3 μm for (C) through (F).

Drosophila Profilin is also required for correct bristle morphogenesis, a process dependent on coordinated actin assembly (Verheyen and Cooley, 1994). Indeed, immunolocalization studies in a variety of motile cells show Profilin to be associated with regions of dynamic cytoskeletal rearrangements and leading edge structures (Tseng et al., 1984; Bub et al., 1992). In neuronal growth cones, Profilin appears to be associated with lamellipodia structures (Neely and Macaluso, 1997). In cultured N1E–115 cells, a dominant-negative mutant form of Profilin has been shown to block the formation of neurites in vitro (Suetsugu et al., 1998); however, the role of Profilin during axonogenesis in vivo has not been addressed.

We previously reported on a genetic screen in *Drosophila* to identify genes required for the correct navigation and outgrowth of motoneuron growth cones (Van Vactor et al., 1993). In this screen, we recovered two alleles of *stranded* (*sand*) in which motor growth cones arrest before reaching their final targets. The molecular genetic analysis shown here reveals that *stranded* alleles are zygotic lethal mutations in Profilin (*chickadee*). In vitro experiments confirm that axon extension is impaired in Profilin mutants. Moreover, phenotypic comparisons and genetic interactions between *chickadee* (*chic*) and *abl* mutants support the notion that Profilin and Abl cooperate to promote axon extension.

Results

stranded Mutations Are Alleles of *chickadee*

Using available collections of deficiency chromosomes, we mapped the lethal and nervous system phenotypes of *stranded* (*sand1* and *sand2*) to region 26A on chromosome 2R. Four ethyl methanesulfonate– (EMS-) induced lethal complementation groups (Kotarski et al., 1983) and several lethal P element insertional mutations (Karpen and Spradling, 1992) have also been mapped to the region containing *stranded*. Genetic crosses with a number of these lethal mutations show that *sand1*, *sand2*, *gdh-5*, and the P element insertion *l(2)*^{5205} fall into the same complementation group. This complementation group is uncovered by *Df(2L)cl*^{h3} and *Df(2L)GpdhA* but not by *Df(2L)cl*^{7}, positioning some or all of the *stranded* gene within a roughly 50 kb interval, as previously mapped (Knipple et al., 1991). We used the bacterial origin of replication in the P element insertion of *l(2)*^{5205} to isolate genomic DNA adjacent to the insertion site. To identify the *stranded* coding region, genomic fragments obtained from *l(2)*^{5205} were hybridized to genomic phage clones from a chromosome walk spanning the proximal breakpoints of *Df(2L)cl*^{7} and *Df(2L)GpdhA* (Knipple et al., 1991). Positive fragments were contained within clones λ5.1 and λ4A.1, spanning sequences that overlap the coding region of *Drosophila* Profilin (*chickadee*) (Cooley et al., 1992). Using polymerase chain reaction (PCR) with a pair of oligonucleotides complementary to the 5′ end of *chickadee* exon 1 and to the 3′ P element inverted repeat, we mapped the *l(2)*^{5205} P element insertion to a position 3.2 kb downstream of the start of exon 1. This places the P element within *chickadee* intron 3.

Alleles of *chickadee* were originally identified as viable, female sterile mutations. To confirm that our new EMS-induced lethal mutations are indeed *chickadee* alleles, we crossed our strong lethal allele *sand1* to *ms,fs(2L) chic*^{1320} and assayed the egg-laying capacity of female heterozygotes; these females failed to lay eggs, whereas their siblings were fertile. Furthermore, both independent *sand* alleles were lethal in combination with *Df(2)chic*^{221}, a null intragenic deletion in the Profilin gene (Verheyen and Cooley, 1994). The failure to complement both female sterile and lethal phenotypes, in addition to the molecular mapping of the *l(2)*^{5205} insertion, indicates that the nervous system phenotypes of *sand* alleles are caused by a deficit in *Drosophila* Profilin. Henceforth, we refer to the new alleles as *chic*^{sand1}, *chic*^{sand2}, *chic*$^{l(2)5205}$, and *chic*$^{gdh-5}$.

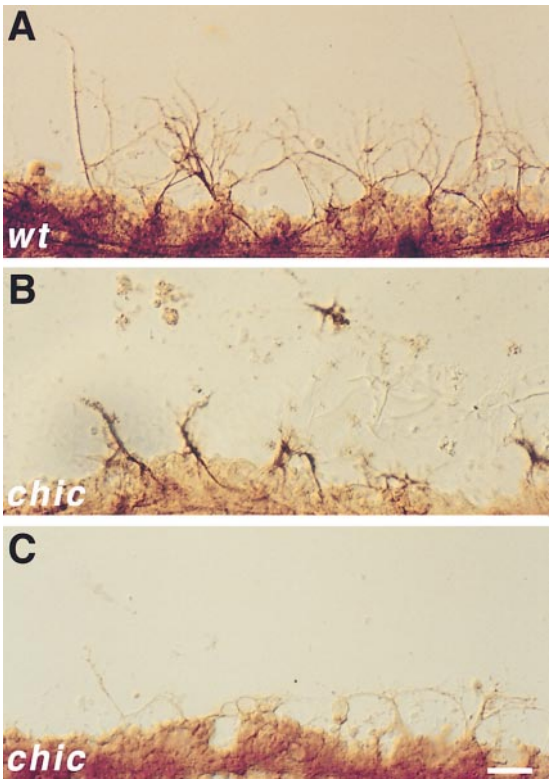

Figure 2. In Vitro Axon Outgrowth Is Reduced in Profilin Mutants

(A) Regenerating axon fascicles are seen extending in vitro from a wild-type stage 16 ventral nerve cord on a substrate of poly-L-lysine. Motor axon bundles are visualized after 8 hr in culture with mAb 1D4. (B and C) Axon growth from *chic*^{sand1} homozygous nerve cords can be seen but is reduced by comparison with wild type.
Scale bar, 10 μm.

Profilin Is Required for Axon Extension In Vivo

We originally isolated lethal Profilin mutants based on embryonic defects in the pattern of motor axon projections (Van Vactor et al., 1993). In the *Drosophila* embryo, each hemisegment (A2–A7) contains an invariant array of 30 muscle fibers that are innervated by at least 34 motoneurons in a highly stereotyped and specific pattern (Johansen et al., 1989; Bate, 1990; Sink and Whitington, 1991a, 1991b). For example, the intersegmental nerve (ISN) projects to dorsal muscles, whereas an ISN derivative, intersegmental nerve b (ISNb), projects to ventral muscles (Figure 1A). In *chic*^{sand1}, *chic*^{sand2}, *chic*$^{l(2)5205}$, and *chic*^{221}, embryonic motoneurons extend out into the periphery, correctly following their initial trajectories. However, these mutant growth cones stop later in the pathfinding process before reaching their final muscle targets (Figure 1B). Premature arrest is seen in all motor branches. Interestingly, stalled ISN mutant motor growth cones display numerous filopodia and are as large if not larger than wild-type counterparts (Figures 1E and 1F). This suggests that the actin cytoskeleton that supports these leading edge structures does not completely collapse as Profilin function becomes limiting. Although we suspect that the phenotypes of *chic* and *abl* mutants (see below) result from some type of abnormality in growth cone cytoskeleton, we have been unable to detect alterations in F-actin structure in mutant *Drosophila* growth cones at the light level (Z. W. et al., unpublished data).

Profilin Plays a General Role in Axon Extension

Although axon outgrowth defects are observed in all motor pathways in *chic* alleles, supporting a general role for Profilin in all aspects of axon outgrowth, these defects arise only late in development, when motor growth cones must navigate certain key "choice points" (Van Vactor et al., 1993). Since Profilin is expressed abundantly during oogenesis, it is possible that a maternal supply of Profilin protein can function in the absence of zygotic Profilin for the initial stages of embryogenesis, thus masking a more general role for Profilin. To test whether maternally supplied Profilin function might modulate the zygotic phenotype of *chic*^{sand} mutants, we compared the phenotypes of embryos from mothers with one or two copies of the wild-type Profilin gene. Comparing embryos with the same zygotic genotype (*chic*^{sand1}/*Df*), we found that those derived from mothers carrying a duplication at the Profilin locus (*Dp(2L)C619*) showed substantial rescue of the "stranded" axonal phenotype. One hundred percent of *chic*^{sand1}/*Df* embryos from fathers carrying *Dp(2L)C619* displayed a clear ISN stall in the dorsal periphery, whereas only 12% of *chic*^{sand1}/*Df* embryos from mothers carrying the duplication showed any phenotype (only slightly exceeding background levels of subtle ISN phenotypes in wild-type embryo collections). Thus, doubling the supply of maternally expressed Profilin rescues the *chic* mutant zygote through embryonic stage 17. This is consistent with the residual Profilin immunoreactivity seen in late stage *chic* null embryos (D. V. V. et al., unpublished data). The failure of motor growth cones at particular locations in the periphery of *chic*^{sand} embryos is likely to reflect regions with the highest requirement for Profilin function.

The choice point regions where *chic* mutant growth cones frequently arrest correspond to locations where growth cones typically slow down, become more complex in shape, and probe their environment (Sink and Whitington, 1991b; Broadie et al., 1993; Van Vactor et al., 1993). Although we believe that the failure of these mutant growth cones reflects an intrinsic deficit in forward locomotion, it is also possible that *chic* mutant growth cones are unable to respond to specific extrinsic guidance cues. Therefore, we examined the context dependence of *chic* mutant axon outgrowth by measuring outgrowth from dissected nerve cords in vitro. In these experiments, mutant and wild-type nerve cords were removed from embryos at embryonic stage 16 and were cultured on poly-L-lysine-coated coverslips. Under these in vitro conditions, regenerating axons begin extending from transected motor nerve roots and central nervous system (CNS) longitudinal connectives after 3 hr and continue to extend for the next 8–9 hr, long after growth cones would have arrested in mutant embryos. In this assay system, homozygous *chic*^{sand1} and *chic*$^{l(2)5205}$ nerve cords produced Fasciclin II– (Fas II–) positive neurite fascicles, but the mutant axons did not extend as far as wild-type controls. Neurite fascicles extend 50 μm on average from wild-type nerve cords (n = 53 fascicles

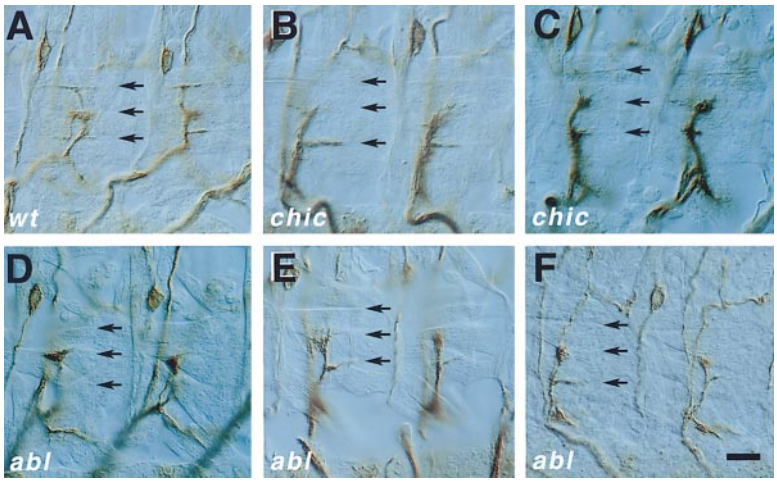

Figure 3. Abl Mutations Block Motor Axon Outgrowth In Vivo

(A) In abdominal segments A2–A7 of stage 17 wild-type embryos, ISNb axons make stereotyped neuromuscular junctions at the clefts between ventral longitudinal muscles 7, 6, 13, and 12 (arrows).

(B) In *l(2)P^{5205}/Df(2)GpdhA* embryos, ISNb axons innervate proximal targets (muscles 6 and 7) and extend to contact muscle 13 (second arrow) but fail to reach distal target muscle 12 (middle arrow marks point of premature ISNb arrest).

(C) The ISNb phenotype observed in *chic^{sand1}/Df(2)GpdhA* shown in this panel is identical to the phenotypes seen in *l(2)P^{5205}/Df(2)cl^{7}* or in *chic^{221}/Df(2)cl^{7}* (data not shown).

(D and E) Two examples of the stage 17 ISNb arrest phenotype observed in *abl^{1}/Df(3)st^{34C}* embryos are shown. Just as in *chic* mutants, ISNb growth cones fail to contact distal target muscle 12; however, contacts with muscles 6, 7, and 13 appear grossly normal.

(F) The same phenotype is seen in an *abl^{2}/Df(3)st^{34C}* genetic background.

Scale bar, 8 μm.

measured), while *chic* mutant fascicles extended 32 μm on average (n = 44 fascicles), only 64% of wild-type growth (Figures 2B and 2C). These results support the conclusion that Profilin plays a role in general axonal extension, regardless of the environment, and suggest that the axonal defects in *chic* embryos result from loss of Profilin function in the mutant axons and not in the surrounding tissue (i.e., a cell autonomous function for Profilin in motoneurons).

The ISNb Phenotypes of *abl* and *chic* Are Indistinguishable

The discovery that Profilin interacts biochemically with members of the Enabled family (Reinhard et al., 1995; Gertler et al., 1996) suggests that Profilin might provide a link between the Abl tyrosine kinase pathway and the actin cytoskeleton. Although axonal phenotypes have been observed in *ena* mutants (Gertler et al., 1995; Wills et al., 1999 [this issue of *Neuron*]), previous studies employing general axon markers had identified defects only when *abl* mutations were combined with mutations in other axon guidance genes (Gertler et al., 1989, 1993; Elkins et al., 1990; Hill et al., 1995; Giniger, 1998; Loureiro and Peifer, 1998). However, using the mAb 1D4 antibody to examine motor pathways during late embryonic development (stage 17), we observed a previously unappreciated growth cone arrest phenotype in the ISNb projection of *abl* homozygous mutant embryos that is essentially identical to the ISNb phenotype of *chic* mutants (Figure 3). In *abl* mutants, ISNb axons frequently stop at contact with muscle 13 and/or the adjacent muscle 30, failing to reach the distal target muscle 12 (quantitated in Table 1). Less frequently, *abl* mutant ISNb axons stop earlier at contacts with muscles 14 or 28; such defects are rare in wild-type controls but less penetrant in *abl* mutants than in strong *chic* backgrounds (Van Vactor et al., 1993). Other peripheral motor axon pathways appear normal in *abl* mutants, as assessed with mAb 1D4.

The Role of *abl* in ISNb Axons Requires an Intact Kinase Domain

Since some Abl functions are independent of kinase activity (Henkemeyer et al., 1987, 1990), it was important to reexamine this question in the context of ISNb development. Previously, an Abl transgene (under the control of the endogenous promotor) was constructed that contains a K-to-N point mutation which completely abolishes kinase activity yet rescues *abl* lethality P[*abl$^{(K–N)}$*]; Henkemeyer et al., 1990). Therefore, to address the requirement for the Abl kinase domain in ISNb outgrowth, we introduced the same P[*abl$^{(K–N)}$*] into an *abl^{1}* genetic background and compared its ability to rescue the motor axon phenotype to that of a P[*abl^{+}*] transgene. Embryos of the genotype P[*abl$^{(K–N)}$*];*abl^{1}* display no rescue of the ISNb arrest phenotype, whereas P[*abl^{+}*] attenuated the *abl^{1}* phenotype to 20% of the original penetrance (Table 1). Thus, Abl requires an active kinase domain to function normally in ISNb development.

Genetic Interaction between *abl* and *chic* in the CNS

In addition to the requirement for Profilin and Abl in ISNb development, both components are also necessary for the accurate formation of axon pathways within the CNS. Staining of *chic* or *abl* mutant embryos with mAb

Table 1. Abl Is Required for ISNb Axon Outgrowth

Genotype	ISNb Arrest[a]	(n)[b]
abl^{2}/Df(3)st^{34C}	24%	n = 58
abl^{2}/abl^{4}	35%	n = 200
abl^{1}/abl^{1}	53%	n = 200
abl^{1}/Df(3)st^{34C}	63%	n = 43
abl^{1}/abl^{1};P[abl^{+}]	13%	n = 170
abl^{1}/abl^{1};P[abl$^{(K–N)}$]	66%	n = 214

[a] ISNb growth cones terminate before reaching muscle 12.
[b] Number of embryonic stage 17 abdominal (A2–A7) hemisegments.

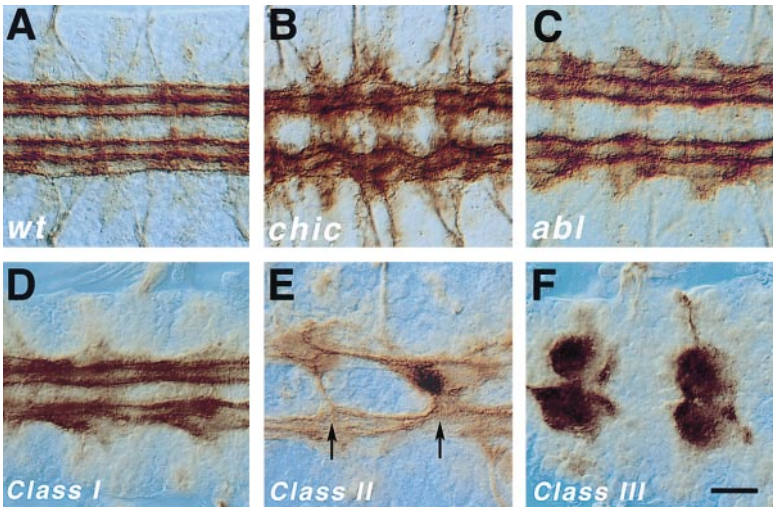

Figure 4. CNS Pathways in Profilin Mutant Embryos

(A) Several CNS segments in a wild-type embryo are shown stained with mAb 1D4 at stage 17, revealing three large parallel fascicles of longitudinal axons on each side of the midline.

(B) The CNS morphology observed in *l(2)P*^{5205}/*Df(2L)GpdhA* is shown at the same stage as in (A). Note that although three fascicles can be seen in each hemisegment, they are disorganized, often fusing together or extending in abnormal directions (see arrowheads).

(C) Longitudinal axon pathways are disorganized in *abl*^{1}/*Df(3)st*^{34C} mutant embryos. Breaks in the most lateral longitudinal Fas II–positive fascicle are common in this background.

(D–F) Three photomicrographs show the classes of embryonic CNS phenotypes observed in the *chic*$^{l(2)5205}$/*chic*^{221};*abl*^{2}/+ genetic background. Class I = mild, Class II = intermediate, and Class III = extreme. Although only a few embryonic segments are shown, each mutant embryo tends to display a consistent phenotype from segment to segment. Scale bar, 5 μm.

1D4 reveals similar disorganization in the parallel longitudinal fascicles of Fas II–positive axons on either side of the CNS midline. Although the prevalent phenotype observed in both single mutant genotypes is mild (Figures 4B and 4C; quantitated in Figure 5), a range of defects can be seen, from mild (Class I; Figure 4D), to intermediate (Class II; Figure 4E), to extreme (Class III; Figure 4F). The prevalent defects are not likely to be a product of alterations in CNS cell fates, since patterning in strong *chic*^{sand} mutants was previously assessed with several different antibody probes (Van Vactor et al., 1993). In Class I embryos, longitudinal pathways are often diverted, causing fusions and/or breaks in these fascicles; occasionally, inappropriate midline crossing can be seen. In Class II embryos, Fas II–positive axons often cross the midline barrier, in addition to the collapse of longitudinal fascicles. In Class III embryos, axonal connections between segments along the anterio–posterior axis are often absent, consistent with a major failure in axonal extension; this extreme phenotype is very rare in *chic* or *abl* single mutants (Figure 5). In Class III embryos, we also observe defects in muscle patterning; such defects have also been reported previously in *abl* mutants (Bennett and Hoffmann, 1992).

The similarity between the *chic* and *abl* phenotypes, both in the CNS and periphery, raised the question of whether these genes cooperate in axonal development. To determine if the function of Profilin is sensitive to the amount of Abl, as expected for components in the same pathway, we examined *chic* homozygous embryos that lacked one allele of *abl* (*chic*$^{l(2)5205}$/*chic*^{221};*abl*^{2}/+). Twofold reduction of Abl function in the *chic* background resulted in a dramatic shift in the distribution of CNS axon phenotypes (Figure 5); in these embryos, the extreme Class III phenotype increases 10-fold in comparison to *chic*$^{l(2)5205}$/*chic*^{221} alone. This dose-sensitive genetic interaction suggests that Profilin and Abl cooperate in the same overall process. In double homozygous embryos (*chic*$^{l(2)5205}$/*chic*^{221};*abl*^{2}/*abl*^{4}), the distribution shifts further, making Class III the prevalent phenotype (Figure 5). Because peripheral axon pathways are highly disorganized in Class III (and some Class II) embryos, we did not compare ISNb phenotypes in the double mutant backgrounds.

Discussion

Ultimately, to understand how cell signaling is translated into directional cell motility in vivo, the relationships between signaling pathways and the constituents of the cytoskeletal motility apparatus must be explored. Here, we provide the first in vivo evidence that Profilin is required for axon outgrowth. We also show for the first time that loss of *Drosophila* Abl function alone results in embryonic axon defects that are dependent on an intact tyrosine kinase domain. Previous demonstrations of the association between Profilin and Enabled family members have raised the possibility that Profilin function is regulated in some way by the Abl pathway (Reinhard et al., 1995; Gertler et al., 1996). Our discovery of similar motor and CNS axonal phenotypes in *chic* and *abl* mutants, in addition to dramatic dose-sensitive interactions between these genes, supports a general model whereby Abl and Profilin work cooperatively to promote general axon outgrowth.

Previous genetic analyses have provided convincing evidence that Profilin is necessary for a variety of actin-dependent processes; however, the precise nature of this control has been difficult to establish. In vitro, Profilin can act to promote or to antagonize actin assembly, depending upon the conditions (e.g., Tseng et al., 1984; Goldschmidt-Clermont et al., 1992; Pantaloni and Carlier, 1993). In vivo, loss of Profilin alters the distribution and structure of the actin cytoskeleton but does not prevent microfilaments from forming (Haarer et al., 1990; Cooley et al., 1992; Verheyen and Cooley, 1994). In fact, actin appears to hyperassemble in *chic* mutants during *Drosophila* bristle formation (Verheyen and Cooley,

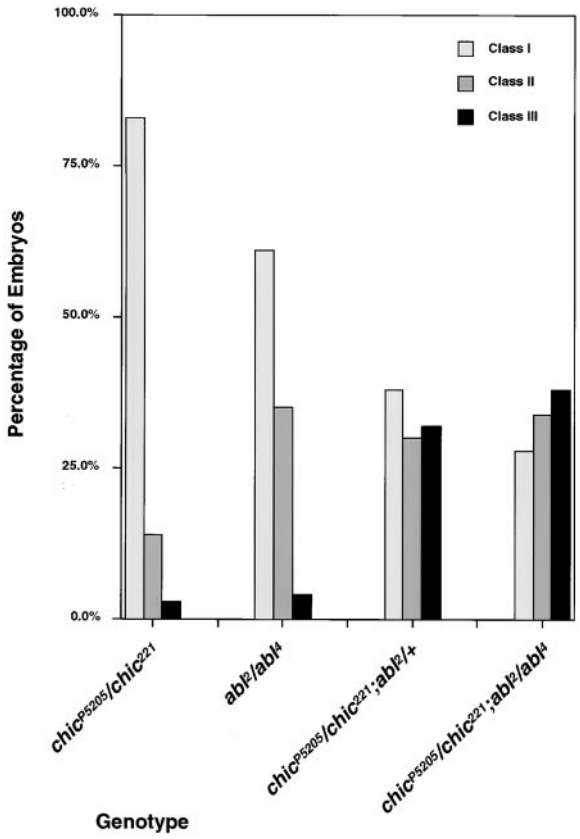

Figure 5. Abl Is a Dose-Sensitive Enhancer of Profilin

The relative penetrance of CNS phenotypes in different *chic* and *abl* mutant backgrounds is shown, quantitated in units of whole embryos. Mutant embryos tended to display consistent severity of defects throughout most segments. The total number of mutant embryos in each collection was n = 29 for $chic^{l(2)5205}/chic^{221}$, n = 46 for abl^2/abl^4, n = 57 for $chic^{l(2)5205}/chic^{221};abl^2/+$, and n = 77 for $chic^{l(2)5205}/chic^{221};abl^2/abl^4$.

1994). Conversely, microinjection of Profilin into living cells shows that high levels of Profilin can inhibit actin assembly (Cao et al., 1992). A negative regulatory model for Profilin function could explain why *chic* mutant growth cones display complex leading edge morphology even after they manifest delays or arrest in outgrowth. However, this model contrasts the conclusions of Suetsugu and colleagues (1998), who propose that Profilin I is necessary for both Cdc42- and N-WASP-induced formation of filopodia. Indeed, neural specific expression of dominant-negative Dcdc42(N17) results in an ISNb phenotype very similar to *chic* and *abl* (Kaufmann et al., 1998). Perhaps the answer lies in the fact that Profilin has several jobs in promoting actin dynamics, each consummated through interactions with a different set of partners. Thus, as maternal stores of Profilin slowly become limiting in *chic* mutant motor growth cones, the first effect in vivo (growth arrest as opposed to loss of filopodia) is determined by the process most sensitive to Profilin concentration.

Precisely how Abl and Profilin work cooperatively to foster axon outgrowth is unclear; however, Ena is an excellent candidate for linking these components together. In mouse, mutations in Mena and Profilin I show dramatic, dose-sensitive genetic interactions (Lanier et al., 1999 [this issue of *Neuron*]). Unlike Profilin, *Drosophila* Ena antagonizes Abl in a dose-dependent fashion (Gertler et al., 1990). Ena family members bind both Profilin and the Abl SH3 domain with the same proline-rich motif (Gertler et al., 1995, 1996). Several sites of Abl-dependent tyrosine phosphorylation have been mapped in this region of Ena (Comer et al., 1998). Although phosphorylation of these sites alters the binding of Abl SH3 (Comer et al., 1998), it is not known how this affects Profilin binding or whether Profilin and Abl compete for access to Ena. Interestingly, *ena* mutants display an ISNb axon phenotype whereby motor axons bypass and extend far beyond their normal targets (Wills et al., 1999). The fact that mild perturbation of actin assembly results in an ISNb phenotype identical to *ena* loss of function (Kaufmann et al., 1998) may mean that Ena works to promote actin assembly. The observation that Mena overexpression in fibroblasts induces the formation of actin-rich protrusions is consistent with this idea (Gertler et al., 1996), as are the roles of VASP, Mena, and Evl in Listeria motility (Gertler et al., 1996; Niebuhr et al., 1997; F. Gertler, personal communication). This model is also consistent with the notion that Abl, an antagonist of Ena, acts to inhibit actin assembly. In fact, loss of murine Abl and Arg (an Abl-related gene) function results in the accumulation of F-actin in the developing neuroepithelium (Koleske et al., 1998). However, it is likely that the mechanism is far more complex than a simple linear pathway from Abl through Ena to Profilin. The fact that *abl;chic* double mutants display phenotypes far more severe than either single mutant supports the idea that the pathway involves additional inputs or outputs. It is already known that Ena family members bind to other partners, such as Zyxin and Vinculin (e.g., Gertler et al., 1996) and that Abl interacts with other genes, such as *disabled* and *fax* (Gertler et al., 1993; Hill et al., 1995). The challenge will be to find any remaining players and to understand how these components fit together in a coherent mechanism.

One key question remains: are Abl and Profilin important in executing axon guidance decisions, or are they simply part of the engine that drives forward motility? In addition to growth cone arrest phenotypes, we observe inappropriate crossing of axons at the CNS midline in *abl* and *chic* mutants; this may implicate these genes in some aspect of guidance at the midline choice point (reviewed by Flanagan and Van Vactor, 1998). In the past, *Drosophila* Abl function has been associated with axonal development through genetic interactions with putative guidance molecules at the cell surface, although it has been difficult to show that these interactions are direct (e.g., Fasciclin I; Elkins et al., 1990). In a companion paper, we show that Abl appears to function as an antagonist of the receptor protein tyrosine phosphatase Dlar during ISNb choice point navigation (Wills et al., 1999). At the same choice point, Ena appears to be necessary for ISNb guidance, since the *ena* phenotype is very similar to Dlar loss of function (Wills et al., 1999). Interestingly, both Abl and Ena bind to the Dlar cytoplasmic domain (Wills et al., 1999), suggesting that these proteins play a direct role in the guidance machinery. Our data showing that tyrosine kinase activity is a

central feature of Abl function in ISNb guidance and outgrowth (this paper and Wills et al., 1999) suggest that phosphorylation regulates the key players in an emerging pathway that links cell surface to cytoskeletal dynamics and motility.

Experimental Procedures

Classical and Molecular Genetics

Deficiency stocks used to map the *stranded* locus are previously described (Lindsley and Zimm, 1992); additional *chic* alleles were obtained from Dr. L. Cooley. *abl* and *ena* alleles were obtained from Dr. F. M. Hoffmann. Plasmid rescue of the $chic^{l(2)5205}$ insert was performed as described (Cooley et al., 1992; Karpen and Spradling, 1992) using XbaI to cleave the genomic DNA. DNA flanking the P element insertion was mapped onto genomic phage clones covering the *chickadee* region (O'Kane and Gehring, 1987). Southern blots and hybridizations were performed with GeneScreen nylon membrane as instructed by the manufacturer (Dupont).

When crossed to $chic^{gdh\text{-}5}$, EMS-induced alleles $chic^{sand1}$ and $chic^{sand2}$ display 65% and 68% viability to adulthood, respectively, at 25°C and 14% viability at 18°C; surviving adults display a held up or held down wing phenotype. However, $chic^{gdh\text{-}5}/chic^{l(2)5205}$ displays a level of survival (29% at 25°C and 7% at 18°C) much closer to $chic^{l(2)5205}/Df(2L)GpdhA$ (40% at 25°C and 9% at 18°C). *l(2)gdh-5* carried over $fasIII^{E25}$, a background equivalent to $chic^{sand1}$ and $chic^{sand2}$, but wild type at the Profilin locus showed normal viability (100%) and no wing phenotype.

Histology

Embryos were stained and dissected as previously described; to evaluate axonal pathways, mAb 1D4, which recognizes transmembrane forms of Fasciclin II (Van Vactor et al., 1993), was used at a dilution of 1:5. In most cases, we utilized anti-*lacZ* antibodies to distinguish homozygous mutant embryos from heterozygotes. In some cases (with TM6B-*lacZ* balancers), the *lacZ* pattern was too weak to score this accurately; however, we found that visual inspection of CNS morphology allowed us to separate homozygotes accurately from other Mendelian classes (e.g., when embryos from abl^{2}/*TM6B-lacZ* × abl^{4}/*TM6B-lacZ* were selected for CNS defects ranging from Class I to Class III, 54 were found to be abnormal from a total collection of 213 stage 16–17 embryos). Embryos were staged according to Campos-Ortega and Hartenstein (1985). Nerve cords cultured in vitro were stained on open coverslips with mAb 1D4 after formaldehyde fixation with the same solutions used for whole-mount staining; histological activity staining for *lacZ* activity was employed to distinguish homozygous mutant embryos from their phenotypically normal siblings that carry a balancer chromosome with a P[$lacZ^{+}$] insertion.

Drosophila CNS Cultures

Staged, dechorionated embryos were dissected in saline solution (135 mM NaCl/5 mM KCl/4 mM $MgCl_2$/2 mM $CaCl_2$/5 mM TES/36 mM sucrose, pH 7.2) under sterile conditions, and nerve cords were placed in ordered arrays on poly-L-lysine-coated coverslips. Coverslips were transferred to 35 mm culture plates containing Schneider's Medium, preconditioned with primary cultures of dissociated embryos (plus 17% fetal calf serum and antibiotics). These tissue cultures were incubated for 12 hr at 25°C. Axon fascicles extending onto the coverslip surface from lateral exit points on mutant and wild-type nerve cords were measured for comparison. The rate of growth failure from mutant explants was comparable to controls (20% of mutant explants failed to extend any axons, whereas 26% of wild-type explants failed).

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