Membrane-targeted WAVE mediates photoreceptor axon targeting in the absence of the WAVE complex in Drosophila

Raiko Stephan*,†, Christina Gohl*, Astrid Fleige, Christian Klämbt, and Sven Bogdan
Institut für Neurobiologie, Universität Münster, 48149 Münster, Germany

ABSTRACT A tight spatial-temporal coordination of F-actin dynamics is crucial for a large variety of cellular processes that shape cells. The Abelson interactor (Abi) has a conserved role in Arp2/3-dependent actin polymerization, regulating Wiskott-Aldrich syndrome protein (WASP) and WASP family verprolin-homologous protein (WAVE). In this paper, we report that Abi exerts nonautonomous control of photoreceptor axon targeting in the Drosophila visual system through WAVE. In abi mutants, WAVE is unstable but restored by reexpression of Abi, confirming that Abi controls the integrity of the WAVE complex in vivo. Remarkably, expression of a membrane-tethered WAVE protein rescues the axonal projection defects of abi mutants in the absence of the other subunits of the WAVE complex, whereas cytoplasmic WAVE only slightly affects the abi mutant phenotype. Thus complex formation not only stabilizes WAVE, but also provides further membrane-recruiting signals, resulting in an activation of WAVE.

INTRODUCTION

Growth cone motility and axon guidance require a number of signaling pathways to transmit extracellular signals to intracellular signal transduction cascades converging on a dynamic cytoskeleton (Pak et al., 2008; Lowery and Van Vactor, 2009; O’Donnell et al., 2009). The dynamics of the actin cytoskeleton are controlled by a number of conserved proteins, such as formins, Spire-like proteins, and the Arp2/3 complex (Kerkhoff, 2006; Pollard, 2007). The Arp2/3 complex represents an efficient actin nucleation machine activated by members of the Wiskott-Aldrich syndrome protein/WASP family verprolin-homologous protein (WASP/WAVE) protein family (Goley and Welch, 2006). Given the low intrinsic nucleating activity of the Arp2/3 complex, WASP and WAVE proteins play a central role as nucleation-promoting factors (NPF) to drive actin polymerization in space and time (Stradal and Scita, 2006; Insall and Machesky, 2009; Pollitt and Insall, 2009). Despite their similar biochemical properties, WAVE and WASP proteins fulfill distinct cellular functions. WAVE function is essential for proper formation and protrusion of lamellipodia, whereas WASP is primarily required for membrane internalization through endocytosis and vesicle movement. Given the essential function for membrane protrusions in nonneuronal cells, it has been assumed that WAVE would play a similar role in protruding growth cones. However, the role of Arp2/3-dependent actin polymerization in neurons is still controversial. In hippocampal neurons, a dominant-negative approach shows that the Arp2/3 complex is dispensable for lamellipodia formation but acts as a negative regulator of growth cone translocation (Strasser et al., 2004). In contrast, knockdown of the Arp2/3 complex in hippocampal neurons, as well as in neuroblastoma cells, impairs lamellipodia and filopodia formation in growth cones (Korobova and Svitkina, 2008). Recent studies using primary Drosophila mutant neurons confirmed an essential role of the Arp2/3 complex in regulating growth cone motility (Goncalves-Pimentel et al., 2011). Likewise, loss-of-function studies in different model systems document an important role of WAVE proteins in regulating Arp2/3-dependent actin polymerization.
during nervous system development. In mice, all three WAVE isoforms differentially localize at the leading edges of growth cones and knockout of both WAVE1 and WAVE2 causes several neuroanatomical defects (Dahl et al., 2003; Nozumi et al., 2003; Soderling et al., 2003; Yan et al., 2003; Kim et al., 2006). In particular, disruption of the brain-enriched WAVE1 isoform results in abnormal growth cone morphology, decreased neurite outgrowth, and a reduced number of dendritic spines, which are important structures for the formation of excitatory synaptic connections (Dahl et al., 2003; Soderling et al., 2003, 2007; Kim et al., 2006)). Defects in nervous system development were also observed for the knockdown of wee-1 in Caenorhabditis elegans causing defects in both axon outgrowth and guidance (Shakir et al., 2008). In Drosophila, WAVE acts as the main Arp2/3 regulator during axonal growth, whereas WASP is required for cell fate decisions during sensory organ development (Ben-Yaacov et al., 2001; Zallen et al., 2002). Mutants lacking wave show severe CNS defects, including ectopic midline crossing and motor neuron branching (Zallen et al., 2002; Schenck et al., 2003). Thus, the requirement for WAVE proteins in the nervous system is conserved.

Over the past few years, insight into molecular regulation of WAVE/WASP proteins has been achieved. WASP and WAVE proteins are regulated by similar molecular principles (Derivery and Gautreau, 2010; Padrick and Rosen, 2010). Both, WASP and WAVE exist in multi-protein complexes, are primarily inactive, and become activated by different signals. WASP proteins are predominantly found in an autoinhibited conformation within a stable complex with the WASP-interacting protein (Anton et al., 2007; Ramesh and Geha, 2009). This autoinhibition is released by the cooperative binding of the small GTPase Cdc42, the phospholipid phosphatidylinositol 4,5-biphosphate, and the F-BAR protein Cip4/Toca-1 (Ho et al., 2004). Pure WAVE proteins are basally active but in vivo, trans-inhibited in a pentameric protein complex with the Abelson interactor (Abi), Nap1/Kette, hematopoietic stem progenitor cell 300 (HSPC300), and, specifically, Rac-1 associated protein 1 (Sra-1; Eden et al., 2002; Gautreau et al., 2004; Derivery et al., 2009; Lebensohn and Kirschner, 2009).

WAVE complex stability depends on its integrity and coinciding signals, such as activated Rac, phosphorylation, and binding to phospholipids, lead to the full activation of WAVE in vitro (Kunda et al., 2002; Lebensohn and Kirschner, 2009). High-resolution crystal structure of a recombinant WAVE1 complex recently confirmed that the catalytic verprolin-cofilin-acidic (VCA) motif of WAVE is sequestered by a combination of intramolecular and intermolecular contacts within the WAVE complex (Chen et al., 2010). The crystal structure also provides a plausible mechanism for how Rac1 and phospholipids could cooperatively recruit the complex to membranes and how they might release the trans-inhibition of WAVE. Contrary to earlier, simplified models, the Rac1 effector Sra-1 is not a peripheral subunit but forms a heterodimer with Nap1/Kette, which creates an oppositely charged platform for the WAVE-HSPC300-Abi trimer. The catalytic VCA domain of WAVE is sequestered by Sra-1. On Rac1 binding to Sra-1, the VCA domain is released, and WAVE becomes active. This model also implies that acidic phospholipids cooperate with Rac1 to recruit the complex at the membrane by binding to the positively charged faces of the Sra-1/Nap1/Kette platform and the polybasic region of WAVE.

Despite this significant progress in our understanding of the WAVE complex biochemistry, less is known about how processes driven by actin dynamics are coordinated by WAVE and its regulatory complex, including Abi in vivo. Genetic studies have demonstrated that Abi family proteins orchestrate cell migration, cell adhesion, and cell differentiation during neuronal and cardiovascular development (Ring et al., 2011; Grove et al., 2004; Proepper et al., 2007; Pollitt and Insall, 2008; Stephan et al., 2008; Lin et al., 2009; Schmidt et al., 2009; Dubielecka et al., 2011). These differential functions are reflected by the modular domain structure of the Abi protein. Abi represents a multi-domain adaptor protein that is not only an integral component of the WAVE complex but also directly interacts with WASP, Diaphanous (Dia1), and the nonreceptor tyrosine kinase abl (Dai and Pendergast, 1995; Shi et al., 1995; Juang and Hoffmann, 1999; Bogdan et al., 2005; Innocenti et al., 2005; Ryu et al., 2009; Liebau et al., 2011). Abi contains an amino-terminal WAVE-binding domain (WAB), which is followed by the Kette/NAP1-interacting homeodomain homologous region (HHR), and a carboxy-terminal SRC homology 3 domain (SH3), which directly binds and activates Abi and WASP (Bogdan et al., 2005; Stephan et al., 2008; Ryu et al., 2009). Unlike vertebrates, Drosophila has only a single gene each for wave, wasp, and abi, and in vivo analyses are not complicated by redundancy (Ben-Yaacov et al., 2001; Zallen et al., 2002; Lin et al., 2009). Loss of both maternal and zygotic abi functions severely disrupts CNS development, resulting in embryonic lethality (Lin et al., 2009).

In this study, we analyzed the zygotic function of abi in the context of the developing fly visual system, and dissected the molecular regulation of WAVE activity by Abi in vivo. The developing Drosophila visual system has served as a good model to identify and study factors, including cytoskeletal regulators that control axonal growth and axonal targeting (Martin et al., 1995; Berger et al., 2008). The adult fly eye is a compound eye comprising 750 individual eye units called ommatidia (Ting and Lee, 2007). Each ommatidium harbors eight photoreceptor neurons (R-cells) that are specialized for light sensitivity (Choe and Clandinin, 2005; Ting and Lee, 2007). The so-called outer photoreceptor cells R1 to R6 terminate in the first optic ganglion, which is called the lamina. The inner photoreceptor cells (R7 and R8) in turn pass through the lamina and terminate in the next optic ganglion, the medulla (Clandinin and Zipursky, 2002; Taylor and Garrity, 2003; Yamaguchi et al., 2006; Morante and Desplan, 2008). The exact termination of the growth cones of the photoreceptor axons at the lamina also requires the presence of a set of specialized glial cells and neurons highlighting the importance of cellular interactions for proper axonal targeting (Poecck et al., 2001; Dearborn and Kunes, 2004; Chotard et al., 2005; Chotard and Salecker, 2007).

Genetic analysis and cell-specific rescue experiments demonstrate that Abi and WAVE, but not WASP, are required in neurons of the target area to regulate non-cell-autonomous photoreceptor axon targeting. We show that, in Drosophila eye, Abi is mostly found in 400- to 500-kDa protein complexes, cofractionating with WAVE and Kette, and that Abi is required for the integrity of the WAVE complex in vivo. Structure–function analysis shows that WAVE activity requires interaction with Abi but also membrane interactions mediated by the phosphatidylinositol 3,4,5-trisphosphate–binding domain. Moreover, we were able to suppress the abi mutant phenotype by reexpression of a membrane-tethered WAVE protein in the absence of the WAVE complex. Furthermore, we show that the rescue ability of membrane-recruited WAVE is due to an activation of Arp2/3 in the absence of Abi. This demonstrates that membrane recruitment of WAVE is sufficient for its activation in vivo.

RESULTS
Neuronal abi controls photoreceptor axon targeting
To analyze abi function during development, we generated an abi mutant by imprecise excision of the EY20423 transposon located in the abi gene (Figure 1A). In the allele abi<sup>22c</sup>, most of the
FIGURE 1: abi is required for photoreceptor targeting. (A) Schematic overview of the abi locus with the neighboring twf gene. The position of the transposon used to generate the abi mutant is indicated. A dashed line indicates the extension of the genomic deletion in the abi mutant. (B and C) Western blot analysis of larval brain extracts. (B) The abiΔ20 mutation results in a loss of Abi protein, whereas (C) the expression of the adjacent Twf protein is unaffected. (D–P) Analysis of photoreceptor projection pattern in the fly larval visual system in abi mutants. (D) Schematic overview of the projection pattern of photoreceptor axons (adapted from Tayler and Garrity, 2003). (E) In wild type, Abi (gray) is expressed in photoreceptor axons, as well as in the lamina (la, arrows) and medulla (me, asterisk). (F–H) R-cell axons (green, R1–R8; antibody 24B10) show a stereotyped projection to the lamina and medulla with R2–R5 (red, marked by rough-τ lacZ; α-β-galactosidase) terminating in the lamina (note a few axons overshooting the lamina, asterisk in G). (J–M) The loss of abi leads to a highly abnormal targeting of R-cell axons with axonal bundling and uneven appearance of the lamina with gaps and clumps (K). The vast majority of the R2–R5 axons fail to terminate in the lamina and terminate in deeper layer medulla (L). (I and N) Rescue of the abi-dependent projection defects of R-cell axons. Neural resupply (N) of Abi (elavGal4 > Abi) rescues abi-mediated targeting abnormalities of R-cell axon whereas (I) glial reexpression of Abi (repoGal4 > Abi). (O–P) Quantification of the photoreceptor targeting defects. (O) The number of axonal bundles in the medulla per optic lobe was quantified for the indicated genotypes. abi, elav > Abi: neural reexpression of Abi in abi mutant animals. abi, repo > Abi: glial reexpression of Abi in abi mutant animals. Error bars represent SEM. ***, p < 0.001 (analysis of variance [ANOVA]); n.s., not significant. (P) Severity of R2–R5 overshooting defects in the indicated genotypes. Animals used for the quantification of axonal bundles were grouped according to the numbers of R2–R5 axons overshooting the lamina. The numbers represent percent of optic lobes with R2–R5 axons in the medulla. abi, elav > Abi: neural reexpression of Abi in abi mutant animals.
protein-coding region, including the start codon, is removed, resulting in a complete loss of Abi protein expression (Figure 1B) but not affecting the expression of the neighboring twinfilin gene (Figure 1C). abiΔ20 mutants show early pupal lethality in homozygosity, as previously reported for an abi mutation generated by gene targeting (Lin et al., 2009). Ubiquitous reexpression (daGal4) of Abi in the mutant background fully rescues the lethality of the abiΔ20 allele (Supplemental Table S1), confirming this mutant is an abi loss-of-function mutation.

Since the loss of the Abi interaction partner Kette results in axon targeting defects in the larval visual system (Hummel et al., 2000), we determined the projection pattern of retinal axons in abiΔ20 mutants. To monitor the projection pattern of R-cells in wild type and abi mutants, we visualized the axons of all photoreceptors R1–R8 and, concomitantly, the axons of the outer R-cell axons R2–R5, which terminate in the lamina (Figure 1D).

In wild type, Abi is expressed in R-cell axons (Figure 1E, arrowheads), as well as in the target area (Figure 1E, asterisk). The axons of all R-cells target in a highly stereotyped manner to the lamina and medulla (Figure 1F), whereas the R-cells R2–R5 project to the lamina with only few axons overshooting (Figure 1, G, asterisk, and P). In abi mutants, axonal targeting is strongly affected (Figure 1, J–M). R-cell axons terminate in the brain, but the projection pattern appears highly irregular. Individual axons fasciculate and form abnormal bundles and gaps in the lamina (Figure 1K, arrows). In the medulla, axons do not appear as individual axons anymore, but form bundles (7.7 axonal bundles per optic lobe vs. 0.1 in wild type; see Figure 1O). Furthermore, large numbers of R2–R5 axons overshoot the lamina and misproject into the medulla (in 94% of the brains >15 axons vs. 0% of the brains in wild type; Figure 1, L and P). The differentiation of abi mutant R-cells appears normal, as the numbers of different subsets of R-cell nuclei in abi mutant eye imaginal disks are indistinguishable from wild type (Supplemental Figure S1). We conclude that the abnormal projection pattern and lamina overshooting is not due to abnormal development of R-cells.

It has been reported that the correct projection pattern of R-cell axons not only depends on the R-cells themselves and neurons in the target area, but also on the presence of specialized glia cells in the lamina (Poeck et al., 2001; Ting and Lee, 2005). Therefore, the abi-dependent targeting defects might either be due to compromised neuronal or glial abi function. To differentiate between these possibilities we conducted differential rescue experiments and quantified the number of axonal bundles in medulla, as well as the overshooting of R2–R5 axons. Expression of Abi in all glia cells (repoGal4) in abi mutants does not modulate the targeting defects, whereas Abi expression in all neurons (elavGal4) completely restores the axonal projection pattern (Figure 1, I, N, O, and P). Taken together, the mutant analysis and the rescue experiments show that abi is required in neurons to ensure proper R-cell projections.

**abi function is required in the target area**

Arp2/3-mediated actin polymerization has an essential function for membrane protrusions in nonneuronal cells (Goley and Welch, 2006). Thus Abi might play a similar cell-autonomous role in protruding growth cones of photoreceptor axons. We also assumed that Abi plays a cell-autonomous role in axonal outgrowth and axon targeting of R-cells. To verify this assumption, we performed a mosaic analysis with a repressible cell marker (MARCM) analysis (Lee and Luo, 2001) that allowed us to induce and simultaneously visualize abi mutant cell clones in the visual system (Figure 2). We generated abi mutant photoreceptor cells using two different eyeless (ey) promotor-driven flippases (FLP), called ey3.5-FLP (Bazigou et al., 2007) and ey-FLP (Newsome et al., 2000). ey3.5-FLP is an eye disk-specific FLP excluding the brain target area (Figure 2, A–C), whereas ey-FLP drives FLP expression in the eye disk and in the optic lobe (Figure 2, D–F). Interestingly, the projections of ey3.5-FLP–induced abi mutant R-cells into a wild-type target were largely indistinguishable from control clones (Figure 2, J–L, vs. 2, G–I). This suggests that abi is not required in the projecting R-cell axons, but rather acts in neurons in the target area. To test this, we used the ey-FLP driver to induce large abi mutant cell clones in the target area, as well as in the eye disk (Figure 2, M–R). Compared with control clones (Figure 2, M–O), strong axonal targeting defects can be observed in brains within large abi mutant clones (Figure 2, P–R, arrow in Q).

Recruitment experiments further support this nonautonomous function of Abi. Reexpression of Abi in mutant animals only in the eye disk, but including the R-cells (using the eye disk–specific GMRGal4 driver), did not rescue axonal targeting defects (Figure S2, A–C). The nonautonomous requirement of Abi was further confirmed by analyzing mutant clones induced by the heat shock–driven expression of FLP (Figure S2, D–F). These data show that neurons in the target area require abi function to regulate targeting of R-cell axons.

**An axonal scaffold in the target area is disorganized in abi mutants**

Analysis of the organization of the optic lobe revealed that certain neuron–neuron and neuron–glia interactions mediate the precise formation of the R-cell projection pattern (Dearborn and Kunes, 2004; Yoshida et al., 2005; Sugie et al., 2010). For example, optic lobe neurons provide an axonal scaffold controlling the correct migration of glia cells, which is essential for retinal axon targeting. R-cell axons misproject if the glia cells fail to migrate due to the absence or malformation of the axonal tracts of optic lobe neurons (Dearborn and Kunes, 2004; Yoshida et al., 2005).

To test whether the loss of abi influences the organization of the target area in the optic lobe, we visualized the axonal scaffold mediating correct glia migration, using cytoplasmic expression of β-galactosidase from a lacZ gene under the control of a wingless (wg) promoter (Dearborn and Kunes, 2004; Figure 3). In wild type, several wg-lacZ–labeled foci emerge from each of the dorsal and ventral wg domains and extend to the lamina region (Figure 3, A, C, E, and G). By contrast, in abi mutant brains, scaffold axons project aberrantly and some glia cells line these abnormal trajectories and accumulate in abnormal destinations (Figure 3, B, D, F, and H). However, reexpression of Abi in abi mutants in the wg pattern does not rescue the photoreceptor projection defects (data not shown), suggesting an additional requirement of Abi in additional neurons of the target area. This indicates that the organization of the target area, including the establishment of an axon scaffold, is affected by the loss of abi.

**Abi acts through WAVE during R-cell axon targeting**

Abi represents a multi-domain adaptor protein that is not only able to regulate WAVE but also WASP activity (Bogdan et al., 2005; Innocenti et al., 2005; Liebau et al., 2011). Distinct interaction domains within the Abi protein mediate the functional relationship between Abi and both Arp2/3 activators (Figure 4A). Abi binds to regulate WAVE but also WASP activity (Bogdan et al., 2007) and acts through WAVE during R-cell axon targeting.

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neuronal reexpression of distinct Abi deletion transgenes in the abi mutant background. Rescue activity of each transgene was quantified by measuring the numbers of abnormal axon bundles in the medulla and overshooting R2–R5 axons observed in abi mutants.
FIGURE 4: The N-terminus of Abi is required during photoreceptor targeting. (A) Schematic overview of the domain structure of Abi and Abi variants used in the analysis. Numbers indicate the amino acid position of an indicated domain. WAB (red); HHR (gray) required for Kette binding; P (gray): proline-rich regions; SH3 (magenta) required for WASP rescue

| Domain Structure | R1- R8 | R2- R5 | Merge |
|-----------------|--------|--------|-------|
| Abi             | +      |        |       |
| Abi ΔN          |        | -      |       |
| Abi ΔC          |        | +      |       |
| Abi-WAB-HHR     |        | +      |       |

F

Axon bundles in the medulla per optic lobe

G

% of optic lobes with R2-R5 axons overshooting

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wave and arp2/3 but not wasp function are required for R-cell axon targeting

Zygotic wave mutants mostly die at early larval stages, with some rare wandering third instar larvae escapers (Zallen et al., 2002). Thus we next analyzed those wave escapers to test the function of WAVE during targeting of R-cell axons. Loss of wave results in severe photoreceptor targeting defects, similar to those seen in abis mutants (Figure 5B). In contrast, wasp mutant brains show no axonal targeting phenotype in the visual system, confirming that the Abi-WASP interaction is not essential in photoreceptor targeting (Figures 4C and 5C). Similarly, reexpression of WAVE in neurons, but not in glia cells, completely restores a wild-type axonal projection in Abis deficient brains (Figure 5, D and E). Further rescue experiments with distinct truncated WAVE variants revealed the domain requirements of WAVE activity in vivo. Expression of WAVE lacking the Abi-interacting domain (WHD; Echarri et al., 2004) does not restore the targeting defects in wave mutants (WAVEΔN, Figure 5, F and H). WAVE variants lacking the basic region mediating membrane lipid binding (WAVEΔN) or both the basic and the proline-rich regions (WAVEΔB+ΔP), exhibit strongly reduced rescue activities (Figures 5, G and H). On the other hand, deletion of the Arp2/3-activating VCA domain completely abolishes WAVE activity, suggesting that WAVE-induced, Arp2/3-mediated actin polymerization is required in photoreceptor targeting. We consistently found similar targeting defects in mutant cell clones for the gene sop2, which encodes one of the Arp2/3 regulatory subunits p40/Arpc1 (Figure S3).

We next analyzed whether WAVE has a similar nonautonomous function in regulating photoreceptor targeting. And indeed, we found that wave function is also required in the target area neurons and not in R-cells. Eye disk–specific reexpression of WAVE in mutant animals (using the eye disk–specific GMRGal4 driver) does not rescue axonal targeting defects (Figure 5A). Conversely, eye disk–specific suppression of wave function by RNA interference (RNAi) does not affect axonal targeting (Figure 5B), whereas pan-neural RNAi knockdown in the elav or scabrous (sca) pattern results in axonal targeting defects (Figure S4, C and D). Clonal analysis confirmed a nonautonomous function of WAVE in regulating photoreceptor axon targeting. ey-FLP–induced wave mutant cell clones in the target area, but not ey3.5-FLP–induced mutant R-cells, show strong axonal targeting defects (Figure S5, A–F). The nonautonomous requirement of WAVE was further shown by analyzing mutant clones induced by the heat shock–driven expression of FLP (Figure S5, G–L). Thus we propose that Abi acts through WAVE to regulate Arp2/3-mediated actin polymerization in the target area neurons.

Integrity of the WAVE complex in vivo

The dependence of proper R-cell axon targeting on the interaction of Abi and WAVE and the independence from wasp function suggests that WAVE is the major activator of Arp2/3 during photoreceptor targeting. Thus R-cell axon targeting seems to differ in this respect from Drosophila myoblast fusion and wing epithelium polarization, where both wasp and wave functions are required (Fricke et al., 2009; Gildor et al., 2009). The distribution of protein complexes containing Abi, WASP, and WAVE might be different in the nervous system compared with epithelia or muscle tissue. We have previously shown that Abi is present in wing disk epithelia in 200- to 300-kDa complexes with WASP, but also together with WAVE in complexes at 400–500 kDa (Fricke et al., 2009). Gel filtration analysis from adult heads containing mainly nervous system tissue revealed that a large fraction of Abi cofractionates with WAVE and the WAVE complex subunit Kette in 400- to 500-kDa protein complexes. A smaller fraction of Abi cofractionates with WASP in 200- to 300-kDa complexes that hardly contain WAVE and the WAVE complex member Kette (Figure 6A). This suggests that the composition of WAVE and WASP complexes might depend on the tissue or cell type, and that Abi mainly associates with WAVE in the nervous system. Consistent with previous cell culture studies Abi is required for the stability of WAVE in vivo (Kunda et al., 2003; Figure 6). In the eye disk, WAVE levels are clearly reduced in abis mutant cell clones (Figure 6, B and C). Additionally,
FIGURE 5: Abi acts through WAVE during photoreceptor axon targeting. (A–G) Representative images of R-cell projection patterns of the indicated genotypes. R-cell axons (green) are visualized with 24B10. (A–C) Compared with wild type, the loss of wave, but not wasp, leads to an axonal targeting phenotype similar to abi mutants with axonal bundling and uneven appearance of the lamina with gaps and clumps. (D–G) Rescue of the wave-dependent projection defect. Resupply of full-length Wave in glial cells (repoGal4 > WAVE) does not improve the R-cell targeting in the wave mutant background (D). Neural reexpression of full-length Wave (elavGal4 > WAVE) in wave mutants restores a wild-type appearance of the projection pattern of R-cell axons (E). In contrast, a Wave variant lacking the N-terminal Abi-binding region (WAVEΔN) is unable to rescue the targeting defects neurally expressed in wave mutants (F). Expression of a Wave protein missing the basic region (WAVEΔB) mediating membrane association only partially restores the wave-dependent targeting defects upon neural expression (G). (H) Schematic overview of the domain structure of WAVE and WAVE variants used in the rescue experiments. WHD (green): WAVE homology domain mediating Abi binding; B (gray): basic region required for lipid binding; PolyPro (blue): proline-rich regions; VCA (red) region required for Arp2/3 binding and activation. rescue: summary of ability of WAVE variants to rescue R-cell targeting defect upon neural reexpression (+ indicates full rescue; − indicates no rescue; −/+ indicates partial rescue).

WAVE levels in brain lysates are strongly down-regulated in abi and kette mutants, whereas WASP levels appear unaffected (Figure 6D, lanes 2 and 4; see also Figure 6E, quantifications for lanes 2 and 4). More importantly, neuronal reexpression (elavGal4) of Abi (Figure 6D, lane 5; Figure 6E, quantification for lane 5) or Kette (Figure 6D, lane 3; Figure 6E, quantification for lane 3) restores the level of endogenous WAVE, depending on the presence of the WAVE-binding domain in Abi (Figure 6, D and E, lane 6 vs. lane 1 and lane 4). Similarly, the reduced Kette and Sra-1 levels in abi mutants are restored by neuronal resupply of Abi (Figure 6, D and E). Further expression analysis suggests that increased reexpression improves complex stabilization. Fivefold overexpression of Kette in kette mutants (Figure 6D, lane 3; see also Figure 6E, quantifications for lane 5) more efficiently restores endogenous proteins above wild-type levels. It is worth mentioning at this point that we cannot fully exclude Abi also affecting WAVE protein levels due to an effect at the transcriptional or translational level, in addition to the effect on WAVE stability. Taken together, whenever Abi or other WAVE complex members, such as Kette, are missing, the stability of WAVE is compromised, resulting in similar axonal projection defects.
Membrane recruitment of WAVE regulates its biological activity

Previous in vitro studies suggested the WAVE complex is not only required for the stability of WAVE, but also directly or indirectly regulates WAVE activity (Innocenti et al., 2004; Steffen et al., 2004). To examine WAVE activity in the absence of the WAVE complex in vivo, we reexpressed WAVE in abi mutants and monitored R-cell axon targeting (Figure 7A). Since previous in vitro studies indicated Abi might activate WAVE by relocalizing WAVE to the leading edge, we also expressed a membrane-tethered WAVE (Myr-WAVE) in abi mutants (Figure 7B). A cytoplasmic WAVE exerts little biological activity upon neuronal expression. WAVE expression weakly rescues the abi-dependent deficits in axonal targeting (Figure 7A; quantification in Figure 7, E and F; 5.0 ± 2.0 axonal bundles in the medulla vs. 7.7 ± 2.1 in the abi mutant). A WAVE variant (WAVEΔB+ΔP) lacking the basic and the proline-rich regions (WAVEΔB+ΔP, Figure 7, E and F) also weakly restores axonal targeting to an extent similar to that of a cytosplasmic full-length WAVE protein. In contrast, expression of Myr-WAVE substantially rescues the frequency and severity of the abi mutant defects (Figure 7B; quantification in Figure 7, E and F). Membrane-recruited WAVE significantly suppresses the appearance of axonal bundles in the medulla (1.8 ± 1.8 vs. 7.7 ± 2.1 in the abi mutant; see Figure 7E). We found that 51% of abi mutant brains expressing Myr-WAVE show from zero to five R2–R5 axons.
FIGURE 7: WAVE activity at the membrane in the absence of the WAVE complex (A–F) Analysis of ability of WAVE variants to rescue the abi-dependent projection defects. (A–D′′′): Representative images of projection patterns of all R-cell axons and R2–R5 axons of the indicated genotypes. R-cell axons (R1–R8, red) are visualized by anti-24B10 and
overshooting the lamina compared with 0% of abi mutant brains and 68% of wild-type brains (Figure 7F). Thus we conclude membrane recruitment is important for the activity of WAVE in vivo.

These findings prompted us to test whether membrane-tethered WAVE might promote actin polymerization. In contrast to cytoplasmic WAVE (Figure 5A), overexpression of Myr-WAVE in wing imaginal disks results in a significant elevation in the level of F-actin in hetero- and homozygous abi mutant background (Figure 5B and E). Thus membrane recruitment results in an increased actin nucleation–promoting activity of WAVE. Conversely, the overexpression of a membrane-tethered WAVE variant lacking the VCA domain (Myr-WAVEΔVCA) does not induce actin polymerization, but results in a reduction of F-actin (Figure 5C). To finally test whether the increased rescue efficiency of the membrane-tethered WAVE corresponds to an enhanced actin-nucleating activity, we compared the rescue activities of Myr-WAVE and Myr-WAVEΔVCA in abi mutants. To ensure an equal expression rate, we integrated both transgenes into the same landing site (68E) using the FLP-mediated transgenesis strategy (Bischof et al., 2007). Expression of Myr-WAVEΔE results in a similar rescue of axonal bundles in medulla in abi mutants as observed for Myr-WAVE (Figure 7, C, E, and F; 2.3 ± 1.6 for Myr-WAVEΔE vs. 1.8 ± 1.8 for Myr-WAVE). The rescue activity of Myr-WAVEΔE with respect to the overshooting of R2–R5 axons is weaker than Myr-WAVE (19% of optic lobes with from zero to five R2–R5 axons vs. 51%, respectively). However, expression of Myr-WAVEΔVCAΔE fails to rescue the phenotypic defects in abi mutant brains (Figure 7, D–F). Expression of Myr-WAVEΔVCAΔE in abi mutant brains does not significantly change the axonal bundling defect in the medulla compared with abi mutants (Figure 7E; 6.6 ± 1.5 vs. 7.7 ± 2.1, respectively). Furthermore, expression of Myr-WAVEΔVCAΔE leads only to a very weak improvement of R2–R5 mistargeting compared with abi mutants (9% of brains with 5–10 R2–R5 axons overshooting the lamina vs. 0 and 60% of brains with >15 R2–R5 axons overshooting vs. 94%, respectively). As Myr-WAVEΔE and the Myr-WAVEΔVCAΔE are expressed at equal levels, we conclude that the rescue of activity of membrane-tethered WAVE is due to an activation of Arp2/3. These findings strongly indicate that WAVE activation in vivo can be achieved upon membrane targeting of WAVE in the absence of the WAVE complex.

DISCUSSION

In summary, we have shown that abi and wave functions are required for early targeting of R-cell axons but are not needed in the R-cells themselves. Our observations strongly suggest a nonautonomous role for the Arp2/3 activator WAVE and its regulator Abi in the brain target area, indicating that in their absence proper cellular communications between projecting R-cell axons and neurons in the target area might be disrupted. It is well established that WAVE and its regulatory complex are effectors of the activated GTPase Rac (Yan et al., 2003; Innocenti et al., 2004) and one might also assume a similar nonautonomous role for Rac, as for WAVE and Abi. Previous analysis of genetic mosaics in the Drosophila brain lacking rac function indeed revealed an unexpected degree of nonautonomous effects in axon guidance and branching (Ng et al., 2002).

How might Abi/WAVE control the targeting of retinal axons into the optic lobe? The formation of the photoreceptor projection pattern depends on complex bidirectional interactions between R-cell axons and different populations of glia, as well as neurons in the lamina target field (Mast et al., 2006). In wild type, incoming photoreceptor axons induce the outgrowth of scaffold axons, which in turn act as substrates for glia migration (Dearborn and Kunes, 2004). Conversely, lamina glia cells provide an essential stop signal for photoreceptor axons to terminate their outgrowth in the lamina (Poeck et al., 2001). These findings highlight the importance of the correct organization of the target area in the establishment of the R-cell projection pattern. The abnormal projections of the wg-positive scaffold axons indicate that neuronal Abi function might be required for the correct organization of the target area. The precise organization of the optic lobe by Abi could control axonal targeting directly (neuron–neuron; Sugie et al., 2010) or indirectly (neuron–glia; Dearborn and Kunes, 2004; Yoshida et al., 2005). The failure of the Abi reexpression in the wingless domain to rescue suggests that Abi/WAVE function is needed in additional neurons in the target area.

Loss-of-function studies in different model organisms clearly revealed a conserved function of Abi/WAVE in regulating axon guidance and axonal outgrowth in developing nervous systems. However, the precise role of WAVE-induced, Arp2/3-mediated actin polymerization in neuronal development is still controversial. Inhibition of Arp2/3 activity in cultured hippocampal neurons resulted in decreased axon length but no significant effects on growth cone morphology (Strasser et al., 2004; Pinyol et al., 2007), whereas it has been recently reported that the knockdown of the Arp2/3 complex impairs lamellipodia and filopodia formation in growth cones of hippocampal neurons and neuroblastoma cells (Korobova and Svitkina, 2008). Recent studies using primary Drosophila mutant neurons confirmed an essential role of the Arp2/3 complex in regulating growth cone motility (Goncalves-Pimentel et al., 2011).

Analyzing early retinal axon targeting in abi mutants represents a good experimental paradigm to measure WAVE activity in vivo.
The targeting process does not require wasp function, but only wave function, in contrast to other developmental processes (Fricke et al., 2009; Gildor et al., 2009). The functional rescue assay in abi mutants allows examination of the activity of WAVE and WAVE variants in the absence of other wave complex subunits. Membrane recruitment of WAVE in abi mutants results in a partial but clear rescue of R-cell projection defects. Several conclusions can be made based on these data. Membrane localization is sufficient to confer partial activity to WAVE without regulation by the WAVE complex. Members of the WAVE complex are not only required to control the integrity of WAVE but also provide means for the membrane recruitment of WAVE. We conclude from our rescue experiments that WAVE activated by artificial membrane targeting induces activation of the Arp2/3 complex. It would be interesting to see whether and to what extent an artificial activation of Arp2/3 will rescue the phenotypic traits associated with a loss of wave. As cytoplasmic, full-length WAVE exerts only slight rescue activity, we propose that the Abi/WAVE complex might not only control membrane relocation but also might be required for full activation of WAVE. The finding that membrane recruitment of WAVE leads to a partial activation might also be true for mammalian neurons. It has recently been shown that artificial membrane recruitment of WAVE partially rescues axonal growth defects in rac-deficient cerebellar granule neurons (Tahirovic et al., 2010).

Taken together, recruitment of WAVE to the membrane leads to activation of the Arp2/3 complex and is an important step during its activation but not the only one. Other important signals might include a specific state of phosphorylation and interaction with activated Rac (Lebensohn and Kirschner, 2009). We propose that the analysis of Drosophila photoreceptor axon targeting in abi mutants will facilitate investigation of WAVE activity and regulation by the WAVE complex, as well as other signals independent of the WAVE complex in the context of a developmental process in vivo.

MATERIALS AND METHODS

Drosophila genetics

w118, Df(3R)Exel1589, FRT40A sca-front, elav-Gal4, tubGal4, FRT82B, FRT82B GMR-GFP[per], FRT82B tubP-Gal80, FRT40A tubP-Gal80, repoGal4, daGal4, scaGal4, gcmGal4, GMRGal4, UAS-mCD8-GFP, hs-FLP, elav-Gal4, UAS-mCD8-GFP, FRT7301 (Bloomington Stock Center, Bloomington, IN), P[EPgy2]EY20423 (Drosophila Gene Disruption Project at Berkeley); wasp1, wasp2 (Ben-Yaacov et al., 2001); rough-tlazC (Garrity et al., 1999); Δ2-3, k (Robertson et al., 1988); LGMRGal4 (Wernet and Desplan, 2004); lamaGal4 (Chotard et al., 2005); c855aGal4 (Hrdlicka et al., 2002); ey-FLP (Newsense et al., 2000); ey-3.5-FLP (Bazigou et al., 2007); UAS-WaveGFP (NIG-FLY), UAS-Wave (Zallen et al., 2002). The transgenes UAS-Abi, UAS-AbiΔC, UAS-WaveAN, UAS-WaveΔB, UAS-Wave[per] were generated as described previously (Bogdan et al., 2005). All crosses were performed at 25°C. See specific references and Supplemental Information for details regarding all mutant alleles and constructs described throughout this study.

Western blot analysis and immunohistochemistry

For Western blot analysis, brains from third instar wandering larvae of the different genotypes were collected and homogenized in lysis buffer (2 μl/brain; 50 mM Tris-HCl, pH 7.5, 150 mM KCl, 5 mM MgCl2, 0.25M sucrose, 0.5% Triton100 + protease inhibitor cocktail [Roche, Indianapolis, IN]). Lysates were centrifuged at 13,000 rpm for 20 min at 4°C. The lipid phase was removed, and the centrifugation step was repeated. Equal amounts of protein lysates were separated by SDS–PAGE (10%) and analyzed by Western blot. Antibodies were used as follows: rabbit α-Abi (1:1000, peptide antibody raised against the C-terminal part of Abi; CS9HGMHTLRNRINRN; affinity-purified, gift from A. Gautreau; recognizes strongly endogenous Abi but not AbiΔC); α-Kette (1:1000); α-Sra-1 (1:1000); mouse α-WAVE (1:5000); mouse α-tubulin (1:400 E7; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); and rabbit α-talin (1:2000 [Wahlstrom et al., 2001]).

Third instar wandering larvae were dissected and stained as previously described (Hummel et al., 2000). Primary antibodies were used at the following dilutions: mouse 24B10: 1:40 (α-Chaoptin); rat α-Elav: 1:10 (7E810); mouse α-Prospero: 1:100 (MR1A); mouse α-Rough: 1:100 (ro-62C2A8; Developmental Studies Hybridoma Bank); guinea pig α-Abi (1:700, this work); rabbit α-β-galactosidase: 1:1000 (Cappel); rabbit α-GFP: 1:1000 (MP Biomedicals, Solon, OH); goat α-HRP-Cy3: 1:300 (Dianova, Hamburg, Germany); goat α-HRP-Cy5 1:200 (Dianova); guinea pig α-Senseless: 1:1000 (Nolo et al., 2000); mouse anti-Seven up: 1:1300 and guinea pig α-Wave: 1:5000 (Bogdan et al., 2005).

Fluorescent images were collected on a Zeiss (Jena, Germany) LSM510 confocal system. Images were processed with Adobe Photoshop (San Jose, CA).

Gel filtration

Gel filtration experiments were carried out as described previously (Fricke et al., 2009).

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REFERENCES

Anton IM, Jones GE, Wandosell F, Geha R, Ramesh N (2007). WASP-interacting protein (WIP): working in polymerisation and much more. Trends Cell Biol 17, 555–562.

Bazigou E, Apitz H, Johansson J, Loren CE, Hirst EMA, Chen PL, Palmer RH, Salecker I (2007). Anterograde jelly belly and Alk receptor tyrosine kinase signaling mediates retinal axon targeting in Drosophila. Cell 128, 961–975.

Ben-Yaacov S, Le Borgne R, Abramson I, Schweisguth F, Schejter ED (2001). UAS-Wave partially rescues axonal growth defects in rcd-deficient cerebellar granule neurons. J. Cell Biol 152, 1–13.

Berge J, Sent KA, Sent G, Newsome TP, Apling B, Dickson BJ, Suzuki T (2008). Systematic identification of genes that regulate neuronal wiring in the Drosophila visual system. PLoS Genet 4, e1000085.

Bischof J, Maeda RK, Hediger M, Karch F, Basler K (2007). An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. Proc Natl Acad Sci USA 104, 3312–3317.

Bogdan S, Stephan R, Lobke C, Mertens A, Klambt C (2005). Abi acts autonomously to regulate both glial and neuronal development in the visual system of Drosophila. Nat Cell Biol 7, 961–975.

Boger DD, Otwinowski Z, Rosen MK (2010). Structure and control of the actin regulatory WAVE complex. Nature 468, 533–538.

Choe KM, Clandinin TR (2005). Thinking about visual behavior; three fundamental issues. Trends Neurosci 28, 195–200.

Chotard C, Leung W, Salecker I (2005). Glial cells missing and gcm2 cells autonomously regulate both glial and neuronal development in the visual system of Drosophila. Neuron 48, 237–251.

Chotard C, Salecker I (2007). Glial cell development and function in the Drosophila visual system. Neuron Glia Biol 3, 17–25.
Kunda P, Craig G, Dominguez V, Baum B (2003). Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. Curr Biol 13, 1867–1875.

Lebensohn AM, Kirshner MW (2009). Activation of the WAVE complex by coincident signals controls actin assembly Mol Cell. 36, 512–524.

Lee T, Luo L (2001). Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci 24, 251–254.

Liebau S et al. (2011). An SK3 Channel/nWASP/Abi-1 complex is involved in early neurogenesis. Plos One 6, e18118.

Lin TY, Huang CH, Kao HH, Liou GG, Yeh SR, Cheng CM, Chen MH, Pan RL, Juang JL (2009). Abi plays an opposing role to Abi in Drosophila axonogenesis and synaptogenesis. Development 136, 3099–3107.

Lowery LA, Van Vactor D (2009). The trip of the tip: understanding the growth cone machinery. Nat Rev Mol Cell Biol 10, 332–343.

Martin KA, Poeck B, Roth H, Ebens AJ, Ballard LC, Zipursky SL (1995). Mutations disrupting neuronal connectivity in the Drosophila visual system. Proc Natl Acad Sci USA 102, 229–240.

Mast JD, Prakash S, Chen PL, Clandinin TR (2006). The mechanisms and molecules that connect photoreceptor axons to their targets in Drosophila. Seminars Cell Dev Biol 17, 42–49.

Morante J, Desplan C (2008). The color-vision circuit in the medulla of Drosophila. Curr Biol 18, 535–563.

Newcombe TP, Asling B, Dickson BJ (2000). Analysis of Drosophila photoreceptor axon guidance in eye-specific mosaics. Development 127, 851–860.

Nolito J, Nardine T, Harms M, Tzu J, Goldstein A, Sun Y, Dietzl G, Dickson BJ, Luo L (2002). Rac GTPases control axon growth, guidance and branching. Nature 416, 442–447.

Nolo R, Abbott LA, Bellen HJ (2000). Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in Drosophila. Cell 102, 349–362.

Nozumi M, Nakagawa H, Miki H, Takenawa T, Miyamoto S (2003). Differential localization of WASH subunits in Drosophila and lamellipodia of the neuronal growth cone. J Cell Sci 116, 239–246.

O’Donnell M, Chance RK, Bashaw GJ (2009). Axon growth and guidance: receptor regulation and signal transduction. Annu Rev Neurosci 32, 383–412.

Paddick SB, Rosen MK (2010). Physical mechanisms of signal integration by WASP family proteins. Annu Rev Biochem 79, 707–735.

Pak CW, Flynn KC, Bamburg JR (2008). Actin-binding proteins take the reins in growth cones. Nat Rev Neurosci 9, 136–147.

Pinol R, Haakecl A, Ritter A, Qualmann B, Kessels M (2007). Regulation of N-WASP and the Arp2/3 complex by Abp1 controls neuronal morphology. PLoS One 2, e400.

Poeck B, Fischer S, Gunnin D, Zipursky SL, Salecker I (2001). Glial cells regulate target layer selection of retinal axons in the developing visual system of Drosophila. Neurosci 112, 99–113.

Pollard TD (2007). Regulation of actin filament assembly by Arp2/3 complex and forminis. Annu Rev Biophys Biomol Struct 36, 451–477.

Pollitt AF, Inshall RH (2008). Abi mutants in Dicystostelium reveal specific roles for the SCAR/WAVE complex in cytokinesis. Curr Biol 18, 203–210.

Pollitt AF, Inshall RH (2009). WASP and SCAR/WAVE proteins: the drivers of actin assembly. J Cell Sci 122, 2575–2578.

Proepper C, Johannsen S, Liebau S, Dahl J, Vaida B, Bockmann J, Kreutz MR, Gundelfinger ED, Boeckers TM (2007). Abelson interacting protein 1 (Abi-1) is essential for dendrite morphogenesis and synapse formation. EMBO J 26, 1397–1409.

Ramesh N, Geha R (2009). Recent advances in the biology of WASP and WAVE family members. Immunol Rev 224, 99–111.

Ring C, Ginsberg MH, Haling J, Pendergast AMAbi-1 interacts with a role in cardiovascular and placental development and is a binding partner of the alpha4 integrin. Proc Natl Acad Sci USA 108, 149–154.

Robertson HM, Preston CR, Phillips RW, Johnsonsclitz DM, Benz WK, Engels WR (1988). A stable genomic source of P element transposase in Drosophila melanogaster. Genetics 118, 461–470.

Ryu JR, Echarri A, Li R, Pendergast AM (2009). Regulation of cell-cell adhesion by Abi/Diaphanous complexes. Mol Cell Biol 29, 1735–1748.

Schnek A, Bardoni B, Langmann C, Harden N, Mandel JL, Giangrande A (2003). CYFIP/Sra-1 controls neuronal connectivity in Drosophila and links the Rac1 GTPase pathway to the fragile X protein. Neuron 38, 877–889.

Schmitz KL, Marcus-Gueret N, Adeleye A, Webber J, Baillie D, Stringham EG (2009). The cell migration molecule UNC-53/NAV2 is linked to the Arp2/3 complex by ABI-1. Development 136, 563–574.
Shakir MA, Jiang K, Struckhoff EC, Demarco RS, Patel FB, Soto MC, Lundquist EA (2008). The Arp2/3 activators WAVE and WASP have distinct genetic interactions with Rac GTPases in Caenorhabditis elegans axon guidance. Genetics 179, 1957–1971.

Shi Y, Alin K, Goff SP (1995). Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. Genes Dev 9, 2583–2597.

Soderling SH, Guire ES, Kaech S, White J, Zhang F, Schutz K, Langeberg DK, Banker G, Raber J, Scott JD (2007). A WAVE-1 and WRP signaling complex regulates neuronal polarization through the WAVE complex. J Neurosci 27, 355–365.

Soderling SH, Langeberg DK, Soderling JA, Davee SM, Simerly R, Raber J, Scott JD (2003). Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. Proc Natl Acad Sci USA 100, 1723–1728.

Steffen A, Rottner K, Ehinger J, Innocenti M, Scita G, Wehland J, Stradal TE (2004). Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. EMBO J 23, 749–759.

Stephan R, Greuelhorster A, Wenderdel S, Klambt C, Bogdan S (2008). Abi induces ectopic sensory organ formation by stimulating EGFR signaling. Mech Dev 125, 183–195.

Stradal TE, Scita G (2006). Protein complexes regulating Arp2/3-mediated actin assembly. Curr Opin Cell Biol 18, 4–10.

Strasser GA, Rahim NA, VanderWaal KE, Gertler FB, Lanier LM (2004). Arp2/3 is a negative regulator of growth cone translocation. Neuron 43, 81–94.

Sugie A, Umetsu D, Yasugi T, Fischbach KF, Tabata T (2010). Recognition of pre- and postsynaptic neurons via nephrin/NEPH1 homologs is a basis for the formation of the Drosophila retinotopic map. Development 137, 3303–3313.

Tahirovic S, Hellal F, Neukirchen D, Hindges R, Garvalov BK, Flynn KC, Stradal TE, Chrostek-Grashoff A, Brakebusch C, Bradke F (2010). Rac1 regulates neuronal polarization through the WAVE complex. J Neurosci 30, 6930–6943.

Tayler TD, Garrity PA (2003). Axon targeting in the Drosophila visual system. Curr Opin Neurobiol 13, 90–95.

Ting CY, Lee CH (2007). Visual circuit development in Drosophila. Curr Opin Neurobiol 17, 65–72.

Wahlstrom G, Vartiainen M, Yamamoto L, Mattila PK, Lappalainen P, Heino TI (2001). Twinfilin is required for actin-dependent developmental processes in Drosophila. J Cell Biol 155, 787–796.

Wernet MF, Desplan C (2004). Building a retinal mosaic: cell-fate decision in the fly eye. Trends Cell Biol 14, 576–584.

Yamaguchi S, Wolf R, Heisenberg M, Desplan C (2008). Motion vision is independent of color in Drosophila. Proc Natl Acad Sci USA 105, 4910–4915.

Yan C et al. (2003). WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. EMBO J 22, 3602–3612.

Yoshioka S, Soustelle L, Giangrandi A, Umetsu D, Murakami S, Yasugi T, Awasaki T, Ito K, Sato M, Tabata T (2005). DPP signaling controls development of the lamina glia required for retinal axon targeting in the visual system of Drosophila. Development 132, 4587–4598.

Zallen JA, Cohen Y, Hudson AM, Cooley L, Wieschaus E, Schejter ED (2002). SCAR is a primary regulator of Arp2/3-dependent morphological events in Drosophila. J Cell Biol 156, 689–701.