Dynamic actin polymerization drives a variety of morphogenetic events during metazoan development. Members of the WASP/WAVE protein family are central nucleation-promoting factors. They are embedded within regulatory networks of macromolecular complexes controlling Arp2/3-mediated actin nucleation in time and space. WAVE (Wiskott-Aldrich syndrome protein family verprolin-homologous protein) proteins are found in a conserved pentameric heterocomplex that contains Abi, Kette/Nap1, Sra-1/CYFIP, and HSPC300. Formation of the WAVE complex contributes to the localization, activity, and stability of the various WAVE proteins. Here, we established the Bimolecular Fluorescence Complementation (BiFC) technique in *Drosophila* to determine the subcellular localization of the WAVE complex in living flies. Using different split-YFP combinations, we are able to visualize the formation of the WAVE-Abi complex in *in vivo*. We found that WAVE also forms dimers that are capable of forming higher order clusters with endogenous WAVE complex components. The N-terminal WAVE homology domain (WHD) of the WAVE protein mediates both WAVE-Abi and WAVE-WAVE interactions. Detailed localization analyses show that formation of WAVE complexes specifically takes place at basal cell compartments promoting actin polymerization. In the wing epithelium, hetero- and homooligomeric WAVE complexes co-localize with Integrin and Talin suggesting a role in integrin-mediated cell adhesion. RNAl mediated suppression of single components of the WAVE and the Arp2/3 complex in the wing further suggests that WAVE-dependent Arp2/3-mediated actin nucleation is important for the maintenance of stable integrin junctions.

Many biological processes are controlled by networks of interacting proteins organized in macromolecular complexes. Members of the WASP/WAVE family are found to be part of such macromolecular complexes coordinating Arp2/3-mediated actin polymerization in time and space (1). Purification of these multiprotein complexes and studies of the underlying protein interactions *in vitro* have led to significant advances in our understanding of how these molecular machines control actin nucleation. WAVE proteins are found in a pentameric heterocomplex that contains Abi, Kette/ Nap1, Sra-1/CYFIP, and HSPC300 (2). The interactions within the complex are mediated by direct protein-protein interactions (3–6). The central subunit of the WAVE complex represents the *Abelson interactor* Abi, which directly binds WAVE, HSPC300, and Kette/Nap1 through different domains. Sra-1 is a peripheral subunit recruited by Kette/ Nap1 and links the complex to Rac1 signaling. The WAVE complex is essential for the localization, activity, and stability of the various WAVE proteins (5, 7–11).

However, purification and reconstitution experiments are based on the removal of the interacting proteins from their endogenous cellular context. To visualize WAVE complex formation in living flies we established the bimolecular fluorescence complementation (BiFC) technique in *Drosophila*. The BiFC technique is based on the fusion of two non-fluorescent segments from a split fluorescent protein (e.g. YFP) to two interacting proteins (12, 13). Once the proteins bind to each other, the interaction brings the two non-fluorescent fragments into close proximity resulting in the reconstitution of a functional fluorescent protein (Fig. 1A). We engineered *Drosophila* BiFC vectors utilizing the advantages of the GATEWAY technique (14, 15) for fast recombinational cloning and the ΦC31-integrate system for site-specific genome integration (16, 17). We visualize WAVE-Abi complexes in the wing epithelium and in the visual system. We show that the N-terminal WAVE homology domain (WHD) of the WAVE protein mediates not only WAVE-Abi but also WAVE-WAVE interactions *in vivo*. In contrast to free WAVE and Abi, WAVE complexes specifically co-localize with Integrin and Talin to the basal wing epithelial surface, where stable connections between the extracellular matrix (ECM) and the actin cytoskeleton are made to hold the dorsal and ventral wing epithelia together (19). Formation of BiFC-stabilized WAVE complexes specifically promotes F-actin formation at the basal surface of the wing epithelium suggesting a possible function of WAVE in Arp2/3-mediated actin polymerization in integrin-mediated cell adhesion in the wing. Expression of double-stranded RNAs (dsRNAs) directed against Arp2/3 and WAVE complex components causes a mild cell adhesion phenotype. Thus, our data suggest that...
WAVE dependent Arp2/3-mediated actin nucleation is important for the maintenance of stable integrin junctions.

**EXPERIMENTAL PROCEDURES**

**Molecular Cloning**—The HA-CYFP fragment (YFP aa 156–239) for C-terminal fusions was amplified per PCR from pSPYCE(M) (29) with the following primers: 5'-HA-YFP_C156 (CGG AAT TCT ATG TAC CCA TAC GAT GTT CC) and 3'-HA+YFP_C156 (GCT CTA GAT TAC TTG TAC AGC TCG TCC ATG). The CYFP-HA fragment (YFP aa 156–239) for N-terminal fusions was amplified per PCR from pSPYCE(MR) (29) with the following primers: 5'-YFP_C156-HA (CGG AAT TCA TGG ACA AGC AGA AGC GC) and 3'-YFP_C156-HA (GCT CTA GAA GCG TAA TCT GGA ACA TCG). The myc-NYFP fragment (YFP aa 1–173) for C-terminal fusions was amplified per PCR from pSPYNE173 (29) with the following primers: 5'-myc+YFP_N173 (CGG AAT TCT ATG GAG CAA AAG TTG ATT TC) and 3'-myc+YFP_N173 (GCT CTA GAC TAC TCG ATG TTG TGG CGG). The NYFP-myc fragment (YFP aa 1–173) for N-terminal fusions was amplified per PCR from pSPYNE(R)173 (29) with the following primers: 5'-YFP_N173+myc (CGG AAT TCA TGG TGA GCA AGG GCG AGG AGC) and 3'-YFP_N173+myc (GCT CTA GAA AGA AGC TCC TCC TCA GAA ATC AAC). All PCR products were cloned into the MCS of the pUAST vector via EcoRI and XbaI.

pUAST-BiFC vectors were converted into Gateway cloning vectors by introducing the Gateway cassette frame B (RfB, Invitrogen) into the XbaI site for N-terminal fusions or the EcoRI site for C-terminal fusion constructs. The recognition site for the pUAST-BiFC vectors via Gateway-cloning (Invitrogen). The Gateway cassette frame B (RfB, Invitrogen) into the XbaI site for N-terminal fusion constructs. The Gateway cassette frame B (RfB, Invitrogen) into the MCS of the pUAST vector via EcoRI and XbaI.

 Fly Genetics—All strains and crosses were grown on Drosophila standard medium. All crosses were performed at 25 °C. The following strains were used: w^{1118}, sdGal4, daGal4, tubGal4, enGal4, GMRGal4, Ubx-flp, and sop2^{225S;FRT40A} (Bloomington Stock Center), scar^{A37} (20), abi^{220} 3, mysRNAi, sra-IRNAi, ketterRNAi, waveRNAi, p20RNAi, abiRNAi (VDRC). The following transgenes were generated by Fc31 integrase-mediated integration into the landing site M{3xP3-RFP.attB}ZH-68E (chromosome 3L (14)): UAS-myc-NYFP, UAS-HA-CYFP, UAS-Abi-myc-NYFP, UAS-Abi-HA-CYFP, UAS-WAVE-myc-NYFP, UAS-WAVE-HA-CYFP, UAS-WAVE\Delta N-myc-NYFP, UASP-Myc-WAVE.

**Immunostaining and Antibodies**—Immunohistochemistry was performed as described (27). Primary antibodies were used at the following dilutions: α-WAVE 1:5000 (23), mouse α-HA 1:1000 (clone 16B12, Covance), rabbit α-Myc 1:1000 (A-14, Santa Cruz Biotechnology), α-Chaoptin (24B10) 1:40 (Developmental Studies Hybridoma Bank). F-actin was labeled with Phalloidin-Alexa568 (Molecular Probes). Alexa568- or Alexa647-conjugated anti-guinea pig, anti-mouse and anti-rabbit antibodies were used as secondary antibodies at a dilution of 1:1000 (Molecular Probes). The epitope-tagged proximity ligation assay (PLA) was performed according to the manufacturer’s instructions (Olink; (21).

**Imaging**—Images were obtained using a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss AG, Jena, Germany). Images were processed using the Zeiss LSM software and Adobe® Photoshop® CS2.

**Gel Filtration**—Fly heads were lysed in 25 mM Tris-HCl at pH 7.6, 100 mM NaCl, 2 mM MgCl2, 0.5 mM EDTA, 5% glycerol, and protease inhibitor mixture (Roche). Lysates were centrifuged 2 × 15 min at 16,000 × g to yield the cytoplasmic supernatant. The cytoplasmic supernatant was applied to a Sephadex 200 10/300G column (GE Healthcare). Collected fractions were precipitated with trichloroacetic acid, and equal volumes of fractions were separated on standard SDS-PAGE. Proteins were analyzed by Western blots using following antibodies: α-WAVE 1:2000 (23), mouse α-HA 1:1000 (clone 16B12, Covance).

**RESULTS**

**BiFC Is a Versatile Tool to Validate and Identify New Protein-Protein Interactions in Drosophila**—Previous work has identified different combinations of fluorescent protein fragments that can be used for bimolecular fluorescence complementation (22, 23). The combination of the N-terminal 173 amino acids of the yellow fluorescent protein (YFP) with a C-terminal fragment containing amino acids 156–239 enhances the reconstitution of YFP (Fig. 1B and Ref. 23). Similar results were recently obtained by in planta BiFC analysis (24). We constructed the analogous Drosophila expression plasmids for targeted expression in the fly. We amplified the N-terminal 1–173 aa (NYFP) and the C-terminal 156–239 aa (CYFP) split-YFP fragments containing a Myc- and a HA tag, respectively by PCR and cloned them into the pUAST vector (Fig. 1C and Ref. 18). For fast and efficient recombinational Gateway cloning of cDNAs we inserted a Gateway cassette up- or downstream of the NYFP and CYFP fragments enabling the construction of both N-terminal and C-terminal NYFP and CYFP fusions. Finally, we introduced an attB site for Fc31-mediated transgenesis into all vectors (16). The site-specific recombination mediates the integration of genes at a high frequency into defined genomic sites, thereby eliminating position effects that can cause strong differences in the expression level. Consequently, this BiFC vector system efficiently allows not only to validate interactions between candidate proteins in vivo but also facilitates large-scale approaches to identify new protein-protein interactions.

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3 R. Stephan, C. Gohl, C. Klämbt, and S. Bogdan, unpublished data.
Visualization of WAVE and Abi Interactions in the Visual System and in the Wing—We next used these BiFC vectors to visualize the interaction between WAVE and the Abelson interactor Abi, a member of the highly conserved pentameric WAVE complex. We designed different Abi and WAVE vector combinations fused with C-terminal NYFP and CYFP fragments and generated transgenic flies by P element-mediated integration into the same genomic landing site. To ensure that all split-YFP fusion proteins were functional we first performed rescue experiments. Loss of abi as well as wave function results in early pupal lethality (20, 25). Ubiquitous re-expression of split-YFP fusion proteins in abi and wave mutants completely rescued lethality. Thus, the split YFP moiety does not interfere with Abi or WAVE function.

Because abi and wave functions are required to control axonal targeting in the visual system through the Arp2/3 complex, we first visualized the interaction between WAVE and Abi in photoreceptor neurons. Each ommatidium of the fly compound eye harbors eight photoreceptor neurons (R-cells) that exhibit a highly polarized morphology (Fig. 2A). The region of the photoreceptor cell bodies lies in the apical region of the developing eye (eye imaginal disc) whereas the axons extend basally into two distinct neuropile areas of the brain (the lamina and the medulla; for review, see Ref. 26).

Co-expression of WAVE-NYFP and Abi-CYFP in photoreceptor cells using the GMR-Gal4 driver (Fig. 2B, R-cells in red; stained with 24B10) results in strong YFP signals in the lamina plexus, along the axons and in the central part of the growth cones in the medulla. Thus, the formation of WAVE-Abi BiFC complexes preferentially takes place at the most basal compartment of the neuron, the growth cone where axon navigation in response to extracellular signals is regulated by actin dynamics.

To further validate the BiFC approach in Drosophila we next visualized WAVE-Abi interaction in wing imaginal discs. We used the en-Gal4 driver, which induces expression in the posterior compartment of wing imaginal discs, whereas the anterior compartment serves as a negative control. All Abi/WAVE fusion proteins were expressed at comparable levels as determined by Western blot analysis using monoclonal anti-HA and anti-Myc antibodies (data not shown). To rule out unspecific fluorescence complementation in Drosophila, we first tested fluorescence complementation between Abi/WAVE split-YFP fusion proteins and the corresponding YFP fragments alone. The expression of split-YFP constructs was verified by antibody staining. In all these control combinations no or only weak background fluorescence was observed (Fig. 3A). In contrast, strong fluorescence was observed in the posterior compartment of wing discs co-expressing either WAVE-NYFP and Abi-CYFP or Abi-NYFP and WAVE-CYFP combinations (Fig. 3A). We next validated the specificity of Abi-WAVE-mediated fluorescence complementation. Because previous studies had shown that Abi directly binds to the N-terminal WHD domain of WAVE (8), we co-expressed the Abi-CYFP fusion with a truncated WAVE protein lacking the first 118 amino acids (ΔN) fused to NYFP. Co-expression of WAVEΔN-NYFP and Abi-CYFP fusion proteins did not produce a fluorescent signal under the same experimental conditions (Fig. 3B). Thus, the formation of BiFC complexes depends on the direct protein interaction between Abi and WAVE.
The N-terminal WHD Mediates WAVE Homodimerization—Previous in vitro studies revealed that oligomerization can increase the affinity of active WASP proteins for the Arp2/3 complex by up to 180-fold (27). However, whether WAVE is able to dimerize in vivo is not known yet. To test this hypothesis we co-expressed WAVE-NYFP and WAVE-CYFP. Upon co-expression of WAVE-NYFP and WAVE-CYFP, strong YFP fluorescence was observed. Thus, WAVE is capable of forming dimers in vivo (Fig. 3B). WAVE homodimerization also depends on the WHD domain. Deletion of the WHD domain abolished the BiFC signal (Fig. 3B). To exclude that the WAVE-WAVE interaction is indirect and mediated by endogenous Abi protein, we co-expressed WAVE-NYFP and WAVE-CYFP in abi mutant background. However, loss of endogenous Abi did not affect the formation of WAVE-WAVE BiFC complexes (supplemental Fig. S1).

To finally exclude that BiFC complex formation artificially triggers WAVE dimerization we co-expressed HA- and Myc-tagged WAVE proteins without any YFP fragments in Drosophila S2R+ cells and performed co-immunoprecipitation experiments. Myc-tagged WAVE clearly co-precipitates full-length HA-WAVE but not HA-WAVEΔN lacking the WHD domain (Fig. 3C) confirming that WAVE is capable to form dimers.

To further analyze whether WAVE forms free dimers, we performed gel filtration chromatography analysis from lysates of wild-type adult heads versus adult heads co-expressing WAVE-NYFP and WAVE-CYFP fusions and analyzed the distribution of tagged as well as of endogenous WAVE protein (Fig. 3D). In wild type, endogenous WAVE protein was mainly found in 400–500 kDa complexes as previously shown (28). WAVE-WAVE BiFC complexes were exclusively co-fractionated with high molecular mass complexes at 500–700 kDa sizes (Fig. 3D). Importantly, co-expression of WAVE-NYFP and WAVE-CYFP resulted in a shift of endogenous WAVE protein from complexes at 400–500 kDa to 500–700 kDa sizes (Fig. 3D). Thus, WAVE does not exist as a free dimer but it rather becomes incorporated into the endogenous Abi-WAVE complex.

Abi-WAVE and WAVE-WAVE BiFC Complexes Specifically Localize at the Basal Side of the Wing Epithelium—We next analyzed the subcellular localization of Abi-WAVE and WAVE-WAVE in wing imaginal discs at high resolution by confocal laser microscopy. The Drosophila wing disc is a bi-
layered epithelial tissue consisting of a columnar monolayer epithelium covered by a squamous peripodial epithelium (Fig. 4A). The columnar epithelial cells are polarized along the apico-basal axis and represent the proper disc that differentiates into the wing, the hinge and the notum (Fig. 4A and Ref. 29). The primordium of the wing blade is in the center of the disc, the so-called wing pouch surrounded by the wing hinge and the notum (Fig. 4A). The localization of bimolecular Abi-WAVE complexes is different from endogenous WAVE protein (compare Fig. 4, B with C). Upon co-expression of WAVE-NYFP and Abi-CYFP, the strongest YFP fluorescence was detected in bright loci at the basal side of late third instar wing pouches, whereas weaker YFP signals were found at the apical surface (Fig. 4C). However, the immunological detection of both split-YFP fragments, WAVE-NYFP or Abi-CYFP, revealed a strong localization at both sides, basally as much as apically (Fig. 4C). Thus, the Abi-WAVE complex specifically localizes at the basal side. Interestingly, a similar subcellular localization was found for WAVE-WAVE split YFP complexes (Fig. 4C).

The localization of bimolecular Abi-WAVE complexes is no artifact of the BiFC system we examined the subcellular localization of WAVE-Abi complexes by using the in situ proximity ligation method (31, 32). We used an
adapted epitope-tagged proximity ligation assay (PLA), which depends on the dual binding of primary antibodies followed by species selected PLA probe binding (21). For visualization of WAVE/Abi complexes by PLA we co-expressed a Myc-tagged WAVE and HA-tagged Abi protein only in the posterior compartment of wing imaginal discs (Fig. 4D). Similar to the BiFC system we observed a strong PLA signal in bright puncta at the basal side of late third instar wing pouches (Fig. 4D). As development proceeds Abi-WAVE and WAVE-WAVE complexes persist at the basal side in pupal wings where integrin forms stable connections between the ECM and the actin cytoskeleton (Fig. 5, A and B and Ref. 19).

**FIGURE 4. Subcellular localization of WAVE complexes in wing imaginal discs.** A, schematic representation of third instar larvae wing imaginal disc. A: anterior, P: posterior. Cross-section reveals the bilayered structure consisting of a columnar monolayer epithelium covered by a squamous peripodial epithelium B; top: detail of a wing imaginal disc (wing pouch) expressing waveRNAi in the en-Gal4 pattern, stained for endogenous WAVE protein (green) and F-actin (red). Note the strong reduction of WAVE protein in the posterior compartment of the wing disc. Bottom: orthogonal section of a wild type wing imaginal discs, stained for endogenous WAVE protein (green) and F-actin (red). C, detail of (wing pouch) co-expressing WAVE-NYFP and Abi-CYFP (left) and WAVE-NYFP and WAVE-CYFP (right) stained for Myc (red) and HA (blue). The strongest YFP signal is observed at the basal side of the wing disc, whereas the strongest antibody staining for the transgenes is at the apical side (orthogonal view). D, detail of wing imaginal discs (wing pouch) co-expressing Myc-WAVE and Abi-HA-CYFP in the posterior compartment detected by an adapted epitope-tagged PLA; PLA signal in red; F-actin (phalloidin) in green. The strongest PLA signal is observed at the basal side of the wing disc. Scale bar, 50 μm.
Despite this strong apical concentration of WAVE-NYFP and WAVE-CYFP proteins we only found slightly increased F-actin formation at the apical surface suggesting that WAVE proteins are less active at apical sides.

Increased actin polymerization was particularly detected at the basal surface of wing imaginal disc and pupal wings where BiFC complexes are mainly formed (Fig. 5, B and C and supplemental Fig. S2 and supplemental Movie M1). Interestingly, we found slightly increased levels of Talin at the posterior wing compartment (Fig. 5C). Because Talin directly links integrins to the actin cytoskeleton the increased actin polymerization induced by BiFC-stabilized WAVE complexes might either recruit additional Talin protein or might affect the dynamic turnover of Talin at integrin junctions. Thus, clustering of stabilized BiFC complexes might cause a prolonged activation of WAVE resulting in an increased actin polymerization.

**DISCUSSION**

The observed co-localization of hetero-oligomeric WAVE-complexes with Integrin and Talin in the wing epithelium further suggests a role of Arp2/3-mediated actin polymerization in regulating integrin-mediated cell adhesion in vivo. To test this, we induced wing clones mutant for the regulatory subunit of the Arp2/3 complex, arpc1(sopQ25st) and asked if arpc1 mutant wings show wing blisters, a characteristic loss of integrin function phenotype caused by a detachment of both wing epithelia. And indeed, arpc1 mutant wings show frequently wing blisters (Fig. 6B). However, the morphology of these mutant wings was grossly affected suggesting that Arp2/3 function is not only important for cell adhesion but also required for proliferation and differentiation of the wing epithelium.

Therefore, we only partially suppressed single components of the Arp2/3 complex and WAVE complex by RNA interference (RNAi). In contrast to silencing β-integrin myospheroid (mys) expression knockdown of the Arp2/3 subunit p20, wave, kette, and sra-1 did not cause penetrant wing blisters (Fig. 6, <3%, n = 30 flies). However, suppression of p20, wave and single components of the WAVE-complex showed fully penetrant wrinkled wings (80–90%, n = 30 flies), a mild cell adhesion phenotype observed in αPS1 integrin mutant wing clones (35) or after overexpression of dominant-negative Ten- sin (36). Thus, we conclude that the WAVE complex and the Arp2/3 complex are important for maintenance of stable integrin junctions in the wing epithelium.
been adapted to different model systems, including mammalian cells (22), yeast (37, 38), Caenorhabditis elegans (39), plants (40, 41), and Xenopus (42). In this work, we established for the first time the BiFC system in Drosophila to validate and identify protein interactions within the WAVE complex in living flies. We used a pUAST-derived vector, which allows the targeted expression of the tagged split-YFP fusion proteins in a temporally and spatially controlled fashion (18). We combined this unique expression tool with the Gateway cloning technique and the ΦC31-mediated transgenesis strategy. These features allow not only fast and efficient cloning of cDNAs but also a highly efficient site-specific integration of large expression vectors into the genome. Site-specific transgenesis also ensures a comparable expression rate of both split-YFP fusion proteins, an important prerequisite for efficient reconstitution of split-YFP. The availability of a large collection of different landing sites offers great flexibility regarding the choice of integration sites and the expression levels of transgenes (17). To avoid high background fluorescence we used a genomic landing site ensuring a low expression of the split-YFP fragments as determined by Western blot analysis. The integration into distinct landing sites also allows to perform a detailed comparative analysis between mutant proteins, different interaction partners of the same protein or the influence of post-translational modifications like phosphorylation on complex formation.

Clustering of Hetero- and Homodimeric WAVE Complexes Promotes Actin Polymerization—Previous reconstitution studies have shown that WAVE proteins are part of a pentameric 440-kDa complex with a 1:1:1:1 stoichiometry of the complex members WAVE, Abi, Kette/Nap1, Sra-1/CYFIP, and HSPC300 (2–4). The existence of the WAVE complex has also been demonstrated in Drosophila by fractionation of the crude extracts by gel filtration (28). Drosophila WAVE protein mainly co-fractionates with Abi in complexes ranging from 400 to 500 kDa. However, depending on the cell type or tissue even larger complexes have been observed suggesting either additional components or different stoichiometries by dimerization (28). Whether WAVE can dimerize or whether dimerization of WAVE plays a role in vivo has not been determined so far. In this BiFC study, we validated not only the central interaction between WAVE and Abi but also identified a dimeric interaction of WAVE in vivo. However, WAVE does not exist as a free dimer but rather forms higher-order clusters with endogenous WAVE complex components. Interestingly, a recent study revealed that forcing the dimerization of WASP proteins increases their affinity for the Arp2/3 complex and enhances its nucleation activity (27). In line with this notion, we observed a strong induction of F-actin at sites of WAVE-Abi and WAVE-WAVE BiFC complex formation. However, the stabilization of protein complexes by BiFC can potentially interfere with the function or activity of complexes (4, 36). The irreversible formation of such WAVE BiFC complexes might also contribute to the increased formation of F-actin in the developing wing.

WAVE Complex Specifically Localizes at the Basal Wing Epithelium Regulating Integrin-dependent Cell Adhesion—Both interactions, WAVE-Abi and WAVE-WAVE require the N-terminal WHD domain of WAVE and both BiFC complexes are enriched at the basal surface of the wing epithelium. The high sensitivity of BiFC analysis allows the visualization of transient protein interactions. The formation of basal WAVE complexes might be such a transient event, which is normally subject to a rapid turnover. Remarkably, the endogenous WAVE protein or free WAVE split-YFP proteins accumulate apically. This striking difference in the subcellular localization indicates that complex formation preferentially takes place at the basal side of the wing epithelium suggesting a possible function of WAVE in formation of stable connections between the ECM and the actin cytoskeleton. Loss of cell adhesion in the wing leads to a detachment of both epithelia causing the formation of liquid-filled blisters (19). Similar wing blistering was also observed in sop2/arpc1 (encoding a subunit of the Arp2/3 complex) mutant clones. Partial suppression of arpc2/3 as well as wave function by RNAi results in mild cell adhesion defects.

In summary, our data revealed not only a localization of WAVE complexes at integrin junction but also suggest a functional requirement of WAVE-dependent Arp2/3-mediated actin polymerization in the formation or maintenance of integrin-mediated cell adhesion in the wing.

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REFERENCES

1. Pollitt, A. Y., and Insall, R. H. (2009) J. Cell Sci. 122, 2575–2578
2. Eden, S., Rohatgi, R., Podtelejnikov, A. V., Mann, M., and Kirschner, M. W. (2002) Nature 418, 790–793
3. Gautreau, A., Ho, H. Y., Li, J., Steen, H., Gyggi, S. P., and Kirschner, M. W. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 4379–4383
4. Innocenti, M., Zucconi, A., Disanza, A., Frittoli, E., Areces, L. B., Steffen, A., Stradal, T. E., Di Fiore, P. P., Carlier, M. F., and Scita, G. (2004) Nat. Cell Biol. 6, 319–327
5. Bogdan, S., Grewe, O., Strunk, M., Mertens, A., and Klambt, C. (2004) Development 131, 3981–3989
6. Bogdan, S., and Klambt, C. (2005) Development 130, 4427–4437
7. Kunda, P., Craig, G., Dominguez, V., and Baum, B. (2003)Curr. Biol. 13, 1867–1875
8. Bogdan, S., Stephan, R., Löbke, C., Mertens, A., and Klambt, C. (2005)Nat. Cell Biol. 7, 977–984
9. Steffen, A., Rottner, K., Ehinger, J., Innocenti, M., Scita, G., Wehland, J., and Stradal, T. E. (2004) EMBO J. 23, 749–759
10. Ismail, A. M., Padrick, S. B., Chen, B., Umetani, J., and Rosen, M. K. (2009)Nat. Struct. Mol. Biol. 16, 561–563
11. Derivery, E., Lombard, B., Loew, D., and Gautreau, A. (2009)Cell Motil. Cytoskeleton 66, 777–790
12. Kerppola, T. K. (2009)Chem. Soc. Rev. 38, 2876–2886
13. Kerppola, T. K. (2008)Annu. Rev. Biophys. 37, 465–487
14. Walhout, A. J., Temple, G. F., Brasch, M. A., Hartley, J. L., Lorson, M. A., van den Heuvel, S., and Vidal, M. (2000)Methods Enzymol. 328, 575–592
15. Hartley, J. L. (2003)Curr. Protoc. Protein Sci., Chapter 5, Unit 5.17
16. Groth, A. C., Fish, M., Nusse, R., and Calos, M. P. (2004)Genetics 166, 1775–1782
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17. Bischof, J., Maeda, R. K., Hediger, M., Karch, F., and Basler, K. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 3312–3317
18. Brand, A. H., and Perrimon, N. (1993) Development 118, 401–415
19. Bükel, C., and Brown, N. H. (2002) Dev. Cell 3, 311–321
20. Zallen, J. A., Cohen, Y., Hudson, A. M., Cooley, L., Wieschaus, E., and Schejter, E. D. (2002) J. Cell Biol. 156, 689–701
21. Gajadhar, A., and Guha, A. (2010) BioTechniques 48, 145–152
22. Hu, C. D., Chinenov, Y., and Kerppola, T. K. (2002) Mol. Cell 9, 789–798
23. Hu, C. D., and Kerppola, T. K. (2003) Nat. Biotechnol. 21, 539–545
24. Waadt, R., Schmidt, L. K., Lohse, M., Hashimoto, K., Bock, R., and Kudla, J. (2008) Plant J. 56, 505–516
25. Lin, T. Y., Huang, C. H., Kao, H. H., Liou, G. G., Yeh, S. R., Cheng, C. M., Chen, M. H., Pan, R. L., and Juang, J. L. (2009) Development 136, 3099–3107
26. Tayler, T. D., and Garrity, P. A. (2003) Curr. Opin. Neurobiol. 13, 90–95
27. Padrick, S. B., Cheng, H. C., Ismail, A. M., Panchal, S. C., Doolittle, L. K., Kim, S., Skehan, B. M., Umetani, J., Brautigam, C. A., Leong, J. M., and Rosen, M. K. (2008) Mol. Cell 32, 426–438
28. Fricke, R., Gohl, C., Dharmalingam, E., Grevelhorster, A., Zahedi, B., Harden, N., Kessels, M., Qualmann, B., and Bogdan, S. (2009) Curr. Biol. 19, 1429–1437
29. Fristrom, D., Wilcox, M., and Fristrom, J. (1993) Development 117, 509–523
30. Brown, N. H., Gregory, S. L., Rickoll, W. L., Fessler, L. I., Prout, M., White, R. A., and Fristrom, J. W. (2002) Dev. Cell 3, 569–579
31. Fredriksson, S., Gullberg, M., Jarvius, J., Olsson, C., Pietras, K., Gustafsdottir, S. M., Ostman, A., and Landegren, U. (2002) Nat. Biotechnol. 20, 473–477
32. Söderberg, O., Gullberg, M., Jarvius, M., Ridderstråle, K., Leuchowius, K. I., Jarvius, J., Wester, K., Hydbring, P., Bahram, F., Larsson, L. G., and Landegren, U. (2006) Nat. Methods 3, 995–1000
33. Robida, A. M., and Kerppola, T. K. (2009) J. Mol. Biol. 394, 391–409
34. Butler, B., Gao, C., Mersich, A. T., and Blystone, S. D. (2006) Curr. Biol. 16, 242–251
35. Brown, D. L. (2003) Curr. Opin. Cell Biol. 15, 607–613
36. Torgler, C. N., Narasimha, M., Knox, A. L., Zervas, C. G., Vernon, M. C., and Brown, N. H. (2004) Dev. Cell 6, 357–369
37. Sung, M. K., and Huh, W. K. (2007) Yeast 24, 767–775
38. Cole, K. C., McLaughlin, H. W., and Johnson, D. I. (2007) Eukaryot. Cell 6, 378–387
39. Hiatt, S. M., Shyu, Y. J., Duren, H. M., and Hu, C. D. (2008) Methods 45, 185–191
40. Bracha-Drori, K., Shichrur, K., Katz, A., Oliva, M., Angelovici, R., Yalovsky, S., and Ohad, N. (2004) Plant J. 40, 419–427
41. Walter, M., Chaban, C., Schütze, K., Batistic, O., Weckermann, K., Näke, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., Harter, K., and Kudla, J. (2004) Plant J. 40, 428–438
42. Saka, Y., Hagemann, A. I., and Smith, J. C. (2008) Methods 45, 192–195