The Rap GTPase Activator Drosophila PDZ-GEF Regulates Cell Shape in Epithelial Migration and Morphogenesis

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Epithelial morphogenesis is characterized by an exquisite control of cell shape and position. Progression through dorsal closure in Drosophila gastrulation depends on the ability of Rap1 GTPase to signal through the adherens junctional multidomain protein Canoe. Here, we provide genetic evidence that epithelial Rap activation and Canoe effector usage are conferred by the Drosophila PDZ-GEF (dPDZ-GEF) exchange factor. We demonstrate that dPDZ-GEF/Rap/Canoe signaling modulates cell shape and apicolateral cell constriction in embryonic and wing disc epithelia. In dPDZ-GEF mutant embryos with strong dorsal closure defects, cells in the lateral ectoderm fail to properly elongate. Postembryonic dPDZ-GEF mutant cells generated in mosaic tissue display a striking extension of lateral cell perimeters in the proximity of junctional complexes, suggesting a loss of normal cell contractility. Furthermore, our data indicate that dPDZ-GEF signaling is linked to myosin II function. Both dPDZ-GEF and cno show strong genetic interactions with the myosin II-encoding gene, and myosin II distribution is severely perturbed in epithelia of both mutants. These findings provide the first insight into the molecular machinery targeted by Rap signaling to modulate epithelial plasticity. We propose that dPDZ-GEF-dependent signaling functions as a rheostat linking Rap activity to the regulation of cell shape in epithelial morphogenesis at different developmental stages.

Epithelial morphogenetic processes constitute genetically programmed cell movement or tissue growth that shapes the developing embryo or organ. Such processes entail alterations in tissue shape that are brought about by a variety of different cellular mechanisms, including cell adhesion, cell contractility, cell intercalation, and polarized cell division (3, 27, 56). Concurrently, the integrity of the epithelium has to be maintained at all times. The dorsal closure (DC) process late in Drosophila gastrulation has proven to be a superb system with which to study the signaling pathways and mechanical alterations involved in cell shape changes. DC is initiated after the germ band has retracted and the transient amnioserosa is dorsally exposed. Following initiation, the bilateral ectodermal sweeps over the amnioserosa in the dorsal direction to finally engage in zipperimg at the dorsal midline. In this manner the embryo is tightly sealed. This process, both in terms of genetic contributions and its physiological aspects, is highly reminiscent of the closure of an epithelial wound after a mechanical insult (reviewed in reference 39). The sequence of events that comprises DC is accompanied by dramatic cell shape changes in the contributing tissues. Ectodermal cells elongate along the dorsal-ventral axis, and while gradually spreading over the space occupied by the shrinking amnioserosa, they morph substantially from a thick- to a thin-layered epithelium (29, 39). In contrast, the differentiation of imaginal disc epithelia is driven largely by the orchestration of the growth and orientation of cell division. Newly born cells have to adopt and maintain a well-defined position in the epithelium and adjust their shape to the requirements of their environment. The shape of individual cells critically depends on the polarity, adhesion, and tension parameters that govern their neighbors (8, 16, 23).

Cell shape changes in DC and other epithelial migration events are driven largely by nonmuscle myosin, also referred to as myosin II (MyoII), which generates mechanical force at the leading edge, the lateral ectoderm, and the amnioserosa by modulating contractile events proximal to cell-cell adhesion complexes (13). In proliferating and differentiating epithelia, MyoII provides general cell tension and is involved in the establishment of compartment boundaries (38). MyoII assembly and activity are tightly controlled by signal transduction pathways, among which the Rho GTPase/Rho-kinase pathway is the best studied (11, 43, 55). However, recent studies have documented the existence of Rho-independent pathways that regulate MyoII assembly and contractility. For instance, in the slime mold Dictyostelium, multiple MyoII heavy-chain kinases have been identified that affect MyoII assembly but function without a regulatory input from the Rho pathway (49, 62). In Xenopus gastrulation, a pathway involving the Shroom protein and the small GTPase Rap1 likely engages MyoII in the closure of the neural tube (17, 20, 21). The precise operational mechanisms of these pathways largely remain to be deciphered, but they underscore that epithelial cell shape can be regulated by both Rho-dependent and Rho-independent means.

The Rap GTPases have emerged as key regulators not only of integrin-mediated adhesion to extracellular substrates but also of intercellular adhesion and cell motility (reviewed in references 5, 6, and 32). In Drosophila, Rap1 mutations are associated with failures in several morphogenetic processes. Rap1 mutant embryos are defective in ventral and dorsal closure, and mesodermal precursors as well as primordial germ cells are impaired in their stereotypical migration patterns (2, 4). Thus, Rap1 is vital for various morphogenetic processes

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that require cell migration and associated cell shape changes. We previously demonstrated that Rap1 relies to a large extent on its effector protein AF-6/Canoe (Cno) to mediate its action in the DC process (4). Here, we report that the exchange factor Drosophila PDZ-GEF (dPDZ-GEF) serves as a Rap1 activator to confer cell shape changes and to modulate cell plasticity in epithelial cells. In analyzing DC in the embryo and wing disc morphogenesis at later developmental stages, we find that dPDZ-GEF links via Rap1 to the effector protein Cno to regulate MyoII function and to generate and adjust lateral cell contractility. Thus, our data reveal a novel Rap1-dependent pathway that is critical for the maintenance and modulation of cell shape and plasticity in epithelial tissues.

MATERIALS AND METHODS

Drosophila strains and genetics. Canton S flies were used for germ line transformation and control experiments. dPDZ-GEF alleles were the following: Gef261 (Bloomington 6299); Gef26B1-2;26D1-2 (FlyBase). Other alleles used in this study were Df(2L)BSC5 (obtained from D. Kiehart). pUAST-EN-IR, Gef26C1, Gef26C2, Gef263, Gef26C1-26B1-2;26D1-2 (FlyBase). Other alleles used in this study were DRap1WT, DRap1N17, and DRap1V12, which were generated in this study. For the isolation of the dPDZ-GEF promoter fragment starting 2,975 bp upstream of the ATG start codon was inserted into pBTM116 (54). For RNA interference (RNAi) purposes, the dPDZ-GEF coding sequence from bp 277 to 1031 was amplified with AvrII- or Nhel-linked complementary primer sequence 5′ AGCTTACCGTCGGCGATGAC and XbaI-linked primer sequence 5′ TCCGTGGTCTTGCGCATCC and inserted stepwise as inverted repeats in a tail-to-tail-orientation into pWIZ (36).

Immohistochemistry. Embryos were fixed with methanol (53) or 5% paraformaldehyde (25), stained with the indicated antibodies, and mounted in Vectashield medium (Vector Laboratories). Third-instar larval imaginal discs were fixed in 4% paraformaldehyde–phosphate-buffered saline. Antibodies used were the following: rat anti-Cno (1:50; (4); mouse anti-Arm (N2 7A1; 1:25) (Developmental Studies Hybridoma Bank [DSHB]); mouse anti-Dlg (4F3; 1:50) (DSHB); mouse anti-Fas (7G10; 1:20) (DHSB); rabbit anti-Zip/Myoi (1:800) (a gift of R. Kares); mouse anti-β-galactosidase (1:2,000) (Sigma); rabbit anti-phospho-Cno (rabbit anti-Cno; 1:1,000) (Oxford); rabbit anti-GFP (RPN1095) (MBL). Alexa flouro-phore-conjugated secondary antibodies were from Molecular Probes and were used at 1:250. Confocal images were captured on an LSM 510 Zeiss laser-scanning microscope.

For DC survival analysis, embryos not carrying GFP-expressing balancers were selected, transferred to fresh grape plates, and aged for 36 h at 25°C. Cuticle preparations were as described in Takahashi et al. (50), and differential interference contrast images of cuticles were collected with an Axioplan 2 Zeiss microscope. Adult wings were dehydrated in isopropanol, mounted in Canada Balsam (Sigma), and digitally photographed with bright-field illumination using a Nikon SMZ microscope. For quantification, tricomb regions from five independent wings were photographed at ×40 magnification with bright-field illumination. Files were imported into the MetaLab 7.1 program (Mathworks), and tricomb numbers per fixed area were counted and compiled.

RESULTS
dPDZ-GEF is expressed in the gastrulating embryonic ectoderm and colocalizes with Cno. In a previous study, we demonstrated an essential role for the Rap1/Cno GTPase-effector complex in the process of epithelial migration driving DC (4). It remained, however, unknown how Rap1 becomes activated in DC and which cellular processes Rap1/Cno signal- ing impinges upon. To further elucidate the signaling networks to which Rap1 is integral, we performed a YTH screen using Rap1 as bait. This resulted in the isolation of a cDNA encoding a splice variant of the Rap1 exchange factor dPDZ-GEF or dizzy, which we termed dPDZ-GEF2. Computational sequence analysis revealed six coding exons, of which the last exon de- viates from the previously described dPDZ-GEF/dizzy. dPDZ-GEF2 harbors a cyclic nucleotide monophosphate-binding domain, a GefN domain, a PDZ domain, and a Ras- associating (RA) domain, as well as a consensus catalytic gua-nine nucleotide exchange factor (GEF) domain (Fig. 1B), all of which are encoded by the third coding exon. We observed that dPDZ-GEF2 binds to the wild-type form of Rap1 as well as to both a constitutively active (CA) and a dominant-negative
(DN) mutant form of Rap1 (Fig. 1C). The interaction with CA Rap1 is mediated by the RÅ domain, whereas binding to DN Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown). No interaction was detected with analogous mutants of the Drosophila Ras1 protein (Fig. 1C). The domain composition and Rap-binding and activation specificity are conserved among the mammalian and Caenorhabditis elegans homologues of the protein (12, 46). Of note, the mammalian homologue was found to reside in cell-cell junctional complexes of epithelial cells (26, 40). So far, dPDZ-GEF isoforms have been implicated in macrophage and male stem cell biology as well as in eye differentiation (22, 35, 57); however, a potential role in epithelial migration and homeostasis has not been reported.

To explore whether dPDZ-GEF plays a role in epithelial processes, we began by investigating its embryonic localization pattern. We developed a fully functional fluorescent genomic transgene, which we termed dPDZ-GEFEGFP. Importantly, dPDZ-GEFEGFP reverted all of the defects associated with a dPDZ-GEF loss-of-function (LOF) allele to wild type (Fig. 1A; also see below). When monitoring dPDZ-GEFEGFP expression in a dPDZ-GEF mutant background during embryogenesis, we found that the fluorescent protein is strongly expressed in the ectoderm during cellularization and subsequently in the cellular blastoderm (data not shown). The strong presence of dPDZ-GEFEGFP protein prior to the onset of zygotic transcription indicates that a large maternal component contributes to gene expression in the early embryo and may partially persist into later stages. At subsequent stages, we detected high protein levels in primordial germ cells, in the ectoderm during ventral invagination, in internalized cells that will differentiate into mesodermal and neuroectodermal structures, and in the ectodermal tissue performing the germ band elongation and retraction movements (data not shown). Of particular interest, at DC stages, during initiation, sweeping, and zipper, all epithelial tissues involved in the closure process displayed large amounts of dPDZ-GEFEGFP protein (Fig. 1D to D').

Both Rap1 and Cno, the putative downstream effectors of dPDZ-GEF, are expressed at near-ubiquitous levels throughout gastrulation. Both proteins overlap in their subcellular localization patterns at the adherens junction (AJ) of cells that undergo DC (4). To trace the subcellular localization of dPDZ-GEF, we again employed dPDZ-GEFEGFP in a mutant background. In embryos at DC stages, we observed dPDZ-GEF protein to be present at AJs of leading-edge and lateral ectodermal cells, where it colocalizes with the β-catenin homologue Armadillo (Arm) (Fig. 1E). Moreover, we found that dPDZ-GEF co-distributes with Cno at the AJ at early (stage 13) and late (stage 14) DC stages during ectodermal sweeping and suture (Fig. 1G to I). Notably, the dPDZ-GEF protein also is detectable in more basal sections of the membrane, as demonstrated by staining with anti-fascin 3 (anti-Fas3) antibodies, which decorate the lateral membrane (Fig. 1F). Overall, the distribution of dPDZ-GEF is highly reminiscent of Rap1’s localization in the embryonic ectoderm (4). Thus, dPDZ-GEF, Rap1, and Cno colocalize at the AJ in the epithelial cells involved in DC, supporting the notion that they participate in a common signaling pathway.

dPDZ-GEF signals through Rap1 and Cno during DC. Our binding and protein expression data are suggestive of a scenario in which the dPDZ-GEF gene product functions as a relevant Rap1 activator during DC. We next sought genetic evidence of a functional pathway that links dPDZ-GEF with Rap1 signaling during DC. We made use of a P element (Gef26′) that is inserted in the first untranslated exon of the dPDZ-GEF gene. Gef26′, also termed l(2)k13270, represents a recessive lethal allele, and it has been associated with undetectable dPDZ-GEF transcript levels (22, 35, 57). To determine a role for dPDZ-GEF in DC, we examined the Gef26′ allele for phenotypic effects in the embryonic ectoderm. Thorough analysis of a Gef26′ mutant population (n = 277) revealed a significant percentage of embryos that display late gastrulation defects, ranging from a complete failure in DC (31%) to milder closure phenotypes exemplified by smaller anterior holes (29%) or the inability to invaginate the head cuticle with the adhering head skeleton (18 out of 23% of embryos with a closed cuticle) (Fig. 2C and D; Table 1). A small portion of anterior-open and -closed cuticles (5% of the total population) displayed a tail-up phenotype (Fig. 2C), indicative of a not-entirely-completed germ band retraction at the onset of DC. Seventeen percent of Gef26′ mutants completed embryogenesis and survived into larval stages. We found this phenotypic distribution to be closely mirrored by the Gef26′ allele in conjunction with the Df(2L)BSC5 deficiency that covers the locus (Fig. 2B and Table 1). Of note, the observed phenotypic variability also has been found in mutant situations for other genes impacting DC (4, 33). One likely reason for this variability is the presence of a maternal component that varies in strength. Our analyses of dPDZ-GEF GLCs (see Materials and Methods) that lack maternal and

FIG. 1. dPDZ-GEF gene and protein structure and expression in the embryonic ectoderm. (A) Genomic organization of the dPDZ-GEF locus. dPDZ-GEF2 protein sequence encompasses 1,569 amino acids (aa) and encodes cNMP (cyclic nucleotide monophosphate; yellow)-binding, GefN and Ras1 protein (Fig. 1C). The domain composition and nucleotide exchange reaction on Rap1 (data not shown). No Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown). No Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown). No Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown). No Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown). No Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown). No Rap1 occurs through the GEF domain, which carries out the nucleotide exchange reaction on Rap1 (data not shown).
zygotic expression support this notion. We found that the majority of embryos derived from these GLCs exhibit a dorsal-open phenotype (Fig. 2E and Table 1). To ascertain that the observed defects are due to the Gef261 insertion and a specific obliteration of the dPDZ-GEF gene function, we performed two additional experiments. First, we precisely excised the transposon, which resulted in a complete reversion of the DC phenotypes described above, and conferred viability to the chromosome (Table 1). Second, when a genomic rescue construct encompassing the complete dPDZ-GEF gene, dPDZ-GEFEGFP, was introduced into the Gef261 background, the ectodermal defects were abolished (Table 1). Together, these data indicate that dPDZ-GEF plays an important role in the DC process.

In subsequent experiments, we examined whether the dPDZ-GEF gene is indeed linked to Rap1/Cno signaling in DC. Our previous study, in which we removed zygotic Cno from a heteroallelic cno background, demonstrated a strong genetic interaction between Rap1 and cno (4). Both genes provided sufficient dose sensitivity to shift the phenotypic spectrum to stronger DC defects. To assess a genetic interaction between dPDZ-GEF and Rap1 and between cno and dPDZ-GEF, we generated two new hypomorphic alleles (Gef261C7 and Gef261C2, respectively) of the dPDZ-GEF gene (see Materials and Methods). Both hypomorphs, when homozygous, survived into late larval stages, and embryogenesis was completed without noticeable defects. When combined with the strong Gef261 allele, neither of the two hypomorphic mutations exhibited gastrulation defects, and only a small percentage of the embryos displayed embryonic lethality: 9 (n = 278) and 13% (n = 264) for Gef261C7 and Gef261C2, respectively (see Table 1). Due to a strong maternal contribution, Rap1 zygotic mutants are not compromised during embryogenesis and survive into larval stages (Table 1) (2, 4). However, eliminating zygotic Rap1 expression by combining the recessive null alleles Rap1CD3 and Rap1B3 in the Gef261C7/Gef261C2 background significantly exacerbated the severity of defects. Eighteen percent of Gef261C7/Gef261C2 embryos (n = 212) showed severe dorsal-open phenotypes (Table 1). Sixty-seven percent of the mutant embryos are mildly closure defective, with smaller anterior holes (Fig. 2G), and 14% have a closed cuticle. Within the latter group, 4% of the embryos were unable to involute their head skeletons. The high survival rates of Gef261C7/Gef261C2 and Rap1CD3/Rap1B3 embryos (91 and 100%, respectively) were reduced to 1% in the double transheterozygotic embryos. Similar results were obtained with Gef261C2 (Fig. 2F and Table 1). We next analyzed double transheterozygotic combinations of dPDZ-GEF and cno alleles and found an even more severe distribution of late gastrulation phenotypes (Fig. 2H and I and Table 1). Sixty-nine percent and 75% of double mutant embryos (Gef261C7/Gef261C2; cno1/m2/cno2 and Gef261C7/Gef261C2; cno1/m2/cno2, respectively) remained open, with strong ectodermal migration defects, and about a quarter of the mutant embryos exhibited weaker anterior-open defects (Table 1). Thus, allelic combinations between dPDZ-GEF and Rap1 or cno almost entirely blocked progression into larval stages. These data indicate a remarkable degree of cooperation between all three genes in the final stages of embryonic gastrulation and support the notion that dPDZ-GEF acts in a common pathway with Rap1 and cno.

To explore the epistatic relationships of dPDZ-GEF to Rap1 and cno, we made use of the GAL4/UAS system (9). In order to perform a dPDZ-GEF tissue-specific rescue analysis, we developed a driver line by fusing a GAL4VP16 transcriptional activator sequence to a functional dPDZ-GEF promoter fragment (henceforth referred to as dPDZ-GEF GAL4; see Materials and Methods) and generated transgenic lines. We validated the functionality of dPDZ-GEF GAL4. First, GAL4 activity was strong in the lateral ectoderm throughout DC, as determined by EGFP expression (Fig. 2M and N). Second, dPDZ-GEF GAL4 did not noticeably alter the phenotypic spectrum of Gef261 mutant embryos (Table 1). Third, driving expression of a dPDZ-GEF2 cDNA with dPDZ-GEF GAL4 significantly alleviated the ectodermal phenotypes of dPDZ-GEF mutant animals (Table 1). Lastly, forced expression of DN Rap1N17 and CA Rap1T22 cDNAs with dPDZ-GEF GAL4 gave
rise to a consistent inability to proceed through DC (Fig. 2K and L; Table 1). The fact that both DN and CA versions of Rap1 are able to halt the DC process may reflect the requirement for active cycling between the active and inactive GTPase states. Such oscillation would allow for a more flexible modulation of effector pathway activities during the different stages of ectodermal migration. Interferences by both types of dominant mutants of small GTPases have been observed in other migratory processes as well (37, 44). In sum, these observations prove the gene-specific deficiency.

### Table 1. Quantitative analysis of cuticular phenotypes of genetic interaction and rescue experiments

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Larvae hatched&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cuticle closed (but with head involution defect)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Cuticle partially open&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Dorsal open&lt;sup&gt;d&lt;/sup&gt;</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Gef26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>23 (18)</td>
<td>29</td>
<td>31</td>
<td>277</td>
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<tr>
<td>Gef26&lt;sup&gt;b&lt;/sup&gt;/Df(2L)BSC5</td>
<td>15</td>
<td>21 (14)</td>
<td>30</td>
<td>34</td>
<td>234</td>
</tr>
<tr>
<td>Gef26&lt;sup&gt;b&lt;/sup&gt;/Df(2L)BSC5 p&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1 (1)</td>
<td>48</td>
<td>51</td>
<td>138</td>
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<td>0</td>
<td>0</td>
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<td>3 (3)</td>
<td>0</td>
<td>0</td>
<td>152</td>
</tr>
<tr>
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<td>9 (7)</td>
<td>2</td>
<td>0</td>
<td>170</td>
</tr>
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<td>0</td>
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<td>cno&lt;sup&gt;a&lt;/sup&gt;; cno&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58</td>
<td>21 (12)</td>
<td>19</td>
<td>2</td>
<td>239</td>
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<tr>
<td>Gef26&lt;sup&gt;c&lt;/sup&gt;/Gef26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91</td>
<td>9 (8)</td>
<td>0</td>
<td>0</td>
<td>278</td>
</tr>
<tr>
<td>Gef26&lt;sup&gt;c&lt;/sup&gt;/Gef26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87</td>
<td>13 (8)</td>
<td>0</td>
<td>0</td>
<td>264</td>
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<tr>
<td>Gef26&lt;sup&gt;c&lt;/sup&gt;/Gef26&lt;sup&gt;b&lt;/sup&gt;; Rap1&lt;sup&gt;c&lt;/sup&gt;; Rap1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>14 (4)</td>
<td>67</td>
<td>18</td>
<td>212</td>
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<td>Gef26&lt;sup&gt;c&lt;/sup&gt;/Gef26&lt;sup&gt;b&lt;/sup&gt;; cno&lt;sup&gt;a&lt;/sup&gt;; cno&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>31</td>
<td>37</td>
<td>335</td>
</tr>
<tr>
<td>Gef26&lt;sup&gt;c&lt;/sup&gt;; dPDZ-GEF GAL4:UAS-dPDZ-GEF2</td>
<td>34</td>
<td>51 (32)</td>
<td>15</td>
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<td>282</td>
</tr>
<tr>
<td>Gef26&lt;sup&gt;c&lt;/sup&gt;; dPDZ-GEF GAL4:UAS-Rap1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44</td>
<td>36 (24)</td>
<td>20</td>
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<td>194</td>
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<tr>
<td>dPDZ-GEF GAL4:UAS-Rap1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31</td>
<td>43 (22)</td>
<td>21</td>
<td>5</td>
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<tr>
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<td>166</td>
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<td>256</td>
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<td>dPDZ-GEF GAL4:UAS-Rap1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>1 (1)</td>
<td>63</td>
<td>36</td>
<td>120</td>
</tr>
</tbody>
</table>

<sup>a</sup> The larvae hatched category did not exhibit any ectodermal defects during embryogenesis, and first-instar larvae emerged.

<sup>b</sup> Embryos with closed cuticles did not show apparent dorsal closure defects but, as indicated in parentheses, were head involution defective.

<sup>c</sup> Cuticles partially open were scored as anterior closure defects that left less than a third of the embryo open.

<sup>d</sup> Dorsal-open phenotypes were characterized by a prominent hole that encompassed more than one-third of the ectodermal surface.

<sup>e</sup> Germ line mutant clones in which the maternal contribution was removed with the Gef26 allele and any paternal contribution was eliminated with the Df(2L)BSC5.
expression in the entire cephalic complex, which represents the developing epithelium in larval life (data not shown). Analysis of the wing imaginal disc showed that dPDZ-GEF<sub>EGFP</sub> is present in all developing wing compartments, including the pouch, margin, and hinge regions (Fig. 4A). Furthermore, confocal cross-sectioning showed a marked dPDZ-GEF accumulation at the lateral membrane that strongly resembles the distribution of Rap1 (Fig. 4B) (31) and a colocalization with Arm at the apical border of the lateral membrane (Fig. 4C to C'). The conservation of dPDZ-GEF's subcellular localization between the embryonic ectoderm and the wing disc epithelium prompted us to perform a mosaic analysis employing the FLP/FRT system, which enables the generation of mitotic mutant clones in an otherwise wild-type epithelium (60). The clones we obtained were, however, relatively small, which impeded a detailed analysis of larger contiguous areas of mutant cells. To generate larger clones, we exploited the Minute technique (42). Minute mutations impose a growth disadvantage on wild-type cells, which often allows for clonal expansion and survival of mitotic clones into adult stages. This technique previously has been employed to define the roles of adhesion molecules and cytoskeletal regulators in wing disc morphogenesis (15, 58).

We analyzed the distribution of cell-cell junctional markers and the shape of cells in clonal and surrounding wild-type tissues. Consistent with our findings for the embryo, we found that the AJ components, Arm and DE-cadherin, were evenly distributed around the circumference of dPDZ-GEF mutant cells (Fig. 4D to D'' and data not shown), indicating that AJ integrity is not perturbed by the loss of dPDZ-GEF function. However, we noticed a frequent widening of mutant cell perimeters in zones in which mutant cells contacted surrounding wild-type cells (Fig. 4D''). In contrast, wild-type cells that border mutant clones often displayed diminished circumferences (Fig. 4D''). When examining the more basally located septate junction (SJ), using Discs large (Dlg) as a marker, we also found that the Dlg protein is present evenly around the entire circumference of mutant cells. However, here as well we observed that mutant cell perimeters were widened to an even larger extent than that at the AJ of dPDZ-GEF cells bordering on wild-type tissue (Fig. 4E' and E''). Cell circumferences were vastly extended not only at the borders of mutant clones but also in interiorly residing mutant cells (Fig. 4E''). Wild-type cells close to the border of mutant clones, in contrast, were even more shrunken than those at the more apical AJ (Fig. 4E''). Thus, a diminished circumference in wild-type cells contrasts with a circumferential expansion in mutant cells, which suggests a loss of contractile force around the apicolateral membrane domain in dPDZ-GEF mutant cells. Importantly, these effects are not due to the Minute background, since wild-type control clones within the same Minute background did not exhibit cell shape abnormalities. Neither Arm nor Dlg staining revealed any alterations in cell constriction at the clonal boundaries of wild-type control clones (Fig. 4F to G'). These findings suggest that dPDZ-GEF regulates lateral contractility and that its function is important for proper wing morphogenesis.

Due to the growth disadvantage of Minute tissue, features of mutant clones originating in the larval wing disc epithelium often survive through pupal development into the adult (42). Hence, we next surveyed adult wings for the phenotypic ex-
FIG. 4. Loss of dPDZ-GEF is associated with a reduction of apicolateral cell contractility. (A) x-y section through a third-instar larval Gef26' dPDZ-GEFEGFP wing disc. (B) x-z section through a Gef26' dPDZ-GEFEGFP wing disc. dPDZ-GEF is ubiquitously expressed throughout the epithelium. (C to C') x-z sections through a Gef26' dPDZ-GEFEGFP wing disc immunostained for Arm (C). dPDZ-GEF (C) localizes to the lateral membrane and displays overlap with Arm staining at the AJ (C'). (D to D') Gef26' Minute' mutant clones (marked by a lack of β-galactosidase expression, which labels wild-type tissue in green) immunostained for Arm (red in panels D and D' or white in panels D' and D''). (D' and D'') High magnification of the region of Gef26' mosaic tissue. Mutant cells bordering on wild-type areas (encircled with a dashed yellow line) often are stretched (white arrowheads in panel D''), whereas more distal wild-type cells are slightly contracted (yellow arrowhead). (E to E') Gef26' Minute' mutant clones lacking β-galactosidase expression (green) and immunostained with anti-Dlg as an SJ marker (red in panels E and E' or white in panels E' and E''). (E and E') Clonal areas are less tightly packed (white arrowheads) than adjacent wild-type areas (yellow arrowheads). (E' and E'') High magnification of Gef26' mosaic tissue stained for Dlg. Clonal areas (white arrowheads) display markedly widened cell perimeters compared to those of wild-type cells (yellow arrowhead). (F and F') High magnification of a Minute control mosaic region labeled with an anti-Arm antibody (red in panel F and white in panel F') and β-galactosidase expression (green) present in Minute mutant cells. (G and G') High magnification of the Minute control region immunostained with anti-Dlg (red in panel G and white in panel G'). Yellow lines in panels F' and G' demarcate clone boundaries. (H to M) Representative pictures of wild-type and Gef26' Minute' wings. (H) Wild-type control wing. (I) The Gef26' Minute' wing is characterized by a rounded geometry. (J to M) Magnified view of the anterior margin in wild-type (J and L) and Gef26' Minute' (K and M) wings. Images in panels L and M represent enlarged views of the boxed areas in panels J and K, respectively. Margin bristles in mutant areas display wider, uneven spacing or are not formed. (N and O) Tricomb fields in wild-type (N) and Gef26' Minute' mutant backgrounds. Mutant tricomb patterns exhibit widened spacing and lack the parallel organization visible in a wild-type wing. Scale bars in panels A, B, D, and E are 50 μm; those in panels C, D', E', F, and G are 10 μm.
pression of dPDZ-GEF mutant clones. We found that wings harboring multiple dPDZ-GEF mutant clones are more rounded and broadened than wild-type wings or wings harboring wild-type control clones (Fig. 4H and I). Each cell in the adult wing epithelium has an apically protruding hair-like structure, which in cells at the margin is a large bristle and in interiorly located cells is a smaller-sized tricomb. In wild-type wing blades, bristles and tricombs are regularly organized and spaced, the former lining the wing margin and the latter running in parallel rows through intervein regions (Fig. 4J, L, and N). dPDZ-GEF mutant clones display vastly disorganized bristle and tricomb patterns, with an uneven and markedly increased spacing between them (Fig. 4K, M, and O). Since the spacing between bristles and tricombs is a direct reflection of the circumferential sizes of the cells that produce them, this phenotype is consistent with the extension of lateral cell perimeters at earlier stages during the morphogenesis of dPDZ-GEF mutant clones in imaginal discs and suggests difficulties in maintaining proper tissue architecture.

We reasoned that if the loss of dPDZ-GEF function affects cell shape and constriction, then excess amounts of dPDZ-GEF should impact the same parameters. To address this, we ectopically expressed a UAS-dPDZ-GEFEGFP transgene (Fig. 1A) in a defined wing territory. In particular, enGAL4 was used as a driver, which results in high levels of dPDZ-GEF expression in the posterior compartment of the wing (Fig. 5D and E). Interestingly, when staining wing imaginal discs with an antibody against Arm, we observed that dPDZ-GEF-overex-
FIG. 6. dPDZ-GEF gain of function and LOF alter wing geometry and are modified by Rap and cno. (A) ptcGAL4>UAS-EGFP expression (green) along the A/P compartment boundary in a wing disc stained with an anti-Arm antibody (red). The scale bar is 50 μm. (B and B’) Expression of UAS-dPDZ-GEF-IR with ptcGAL4 in a wing disc immunostained for Arm (red in panel B) eliminates the dPDZ-GEFEGFP signal (green in panels B and B’). (C to K) Effects of modulated dPDZ-GEF levels in the posterior compartment of the wing. (C) Wild-type control wing. A and P indicate the anterior and posterior sides of the wing, respectively. (D and E) enGAL4>UAS-dPDZ-GEF-IR-expressing wings that are curled downwards with the margin folding back (D) or dorsally ruptured due to enhanced tension (E). (F) enGAL4>UAS-dPDZ-GEFEGFP-expressing wing that shows a rounded geometry, particularly at the anterior margin and the longitudinal L2 and L3 veins. The posterior cross vein is forked at its anterior attachment site. (G) enGAL4>UAS-dPDZ-GEF-IR/UAS-dPDZ-GEFEGFP wing. RNAi and overexpression effects ameliorate each other. (H) enGAL4>UAS-Rap1WT wing with venation defects but intact margins and overall geometry. (I) enGAL4>UAS-dPDZ-GEF-IR/UAS-Rap1WT wing. Ectopic levels of Rap largely rescue the dPDZ-GEF RNAi-specific tension defects. (J) enGAL4>UAS-dPDZ-GEFEGFP/cno2 wing. Reduction of the cno gene dosage partially suppresses the dPDZ-GEF overexpression phenotypes. (K) Heterozygous cno2 wing with a wild-type morphology. (L to N) Posterior margins of a control enGAL4>UAS-EGFP wing (L), a defective enGAL4>UAS-dPDZ-GEF-IR wing (M), and an enGAL4>UAS-dPDZ-GEF-IR/ UAS-Rap1WT wing in which the RNAi-induced defects are rescued (N).

pressing posterior cells that directly face the A/P compartment boundary are aberrantly stretched in the A/P direction (Fig. 5E and F). Importantly, overexpression of EGFP in control discs did not yield any appreciable stretching effects in posterior cells lining the A/P boundary (compare Fig. 5C to F). The dPDZ-GEF overexpression effect also is represented in emerging adult enGAL4>UAS-dPDZ-GEFEGFP wings (Fig. 6F). Adult wings posteriorly overexpressing dPDZ-GEF assumed a more rounded geometry, with the anterior cells pulling at the posterior margin (Fig. 6F). The longitudinal veins L2 and L3 were shifted towards the anterior direction, describing a curve instead of the relatively straight line typically observed in a wild-type wing. Furthermore, posterior tricoms close to the A/P compartment boundary displayed increased distances between each other (Fig. 5G and H). Conversely, tricoms at the opposite side of the compartment boundary were positioned much closer to each other than in a wild-type control wing (Fig. 5G and H; for quantifications, see Fig. 5I). Of note, these phenotypes differ from the ones observed for flies overexpressing growth regulators (for example, Rheb) in posterior cells. Growth-regulatory genes primarily alter the size of targeted wing territories without drastically affecting cell shape in adjoining territories (45). Interestingly, our data reveal that both the loss and gain of dPDZ-GEF function produce similar phenotypes, supporting the notion that tightly controlled cycling between active and inactive forms of Rap GTPase is important to establish tensile strength around lateral cellular circumferences.

To assess whether dPDZ-GEF regulates tissue organization through the Rap/Cno complex, we established a dPDZ-GEF-directed RNAi transgene, UAS-dPDZ-GEF-IR (see Materials and Methods). Before examining the effect of the RNAi transgene on wing architecture, we confirmed that it (i) was able to significantly decrease the fluorescence signal produced by dPDZ-GEFEGFP (Fig. 6B and 6B’; and (ii) could counteract the overexpression phenotype (compare Fig. 6F to G). Thus, dPDZ-GEF-IR was effective in knocking down dPDZ-GEF expression levels. We noted that expressing dPDZ-GEF-IR posteriorly using enGAL4 forced the wing to round up and curl downwards (Fig. 6D), most likely as a result of a loss of lateral contractility. Two to 3 days after eclosion, wings frequently (8/32 wings) ruptured at the posterior margin (Fig. 6E). This differs somewhat from the aberrations observed in wings overexpressing enGAL4>UAS-dPDZ-GEFEGFP, in which rupture never occurred (Fig. 6F). Bristles of enGAL4>UAS-dPDZ-GEF-IR wings were wider and unevenly spaced at the posterior wing margin (Fig. 6M), and in rare cases the anterior cross vein (9/32 wings) and posterior cross vein (1/32 wings) failed to form. To determine whether dPDZ-GEF functions in a Rap1- and Cno-dependent manner in wing development, we conducted two types of experiments. First, we tested whether concomitant overexpression of wild-type Rap1 can rescue the enGAL4>UAS-dPDZ-GEF-IR-associated posterior wing shape defect. While ectopic levels of Rap1 interfered with the correct establishment of posterior cross veins and occasionally interfered with that of longitudinal veins, it had no influence on the overall wing geometry, and the wing blades were extended in a straight orientation (Fig. 6H). We found, however, that Rap1 overexpression could rescue dPDZ-GEF-IR-induced wing bending substantially. Downward curling of the wing blades and the irregular spacing of margin bristles triggered by dPDZ-GEF-IR expression were largely suppressed, suggesting that these phenotypes are caused by insufficient Rap activation (Fig. 6I and N). Overexpression of Cno with enGAL4 had lethal consequences, and no viable adults emerged. Hence, we performed an alternative interaction study in which we reduced the endogenous cno gene dose in an enGAL4>UAS-dPDZ-GEFEGFP overexpression background. Removing one copy of cno, which by itself has no effect on wing morphogenesis (Fig. 6K), weakened the effects of dPDZ-GEF overexpression (compare Fig. 6F to J). Posterior cell stretching along the A/P boundary was ameliorated, and the positions of
longitudinal veins L2 and L3 shifted toward a wild-type pattern (Fig. 6J). These modifications indicate that Rap and Cno participate in the regulation of apicolateral cell constriction downstream of dPDZ-GEF. Together, our data imply that a dPDZ-GEF/Rap/Cno pathway is reiteratively used in epithelial morphogenesis to adjust apicolateral cell circumferences and cell shape in morphogenetic processes.

**MyoII regulation by dPDZ-GEF signaling.** Our observation that the levels and distribution of functional components appear to be unperturbed in dPDZ-GEF LOF situations during DC and wing disc morphogenesis raises the question of which other cellular elements might be dysregulated and causative for the observed cell shape phenotypes. Increasing evidence supports the idea that contractile events in the epithelium help drive morphogenesis and that myosin proteins, particularly the nonmuscle myosin/MyoII, generate force to modulate cell shape in morphogenetic movements and in developing epithelia (13, 23). The *Drosophila* MyoII heavy chain is encoded by the zipper gene (*zip*) (28). It forms a dimer and, together with two essential and two regulatory light chains, constitutes a functional hexamer. In light of our observation that lateral contractility is weakened in dPDZ-GEF LOF clones, we decided to assess whether this effect is dependent on MyoII. We began by investigating the localization of MyoII in dPDZ-GEF LOF clones in wing imaginal discs by immunostaining with a MyoII-specific antibody. Strikingly, we observed that mutant clones display an abnormal, less compact organization of the MyoII network, whereas wild-type tissues surrounding mutant clones frequently exhibit foci with excessively high MyoII accumulation (Fig. 7A to B’). These differences in MyoII distribution are paralleled by the loss and gain of apicolateral constriction that we previously described (Fig. 4D” and E”). Thus, a failure of dPDZ-GEF mutant cells to sufficiently assemble MyoII in order to establish homeostatic contraction levels provides a likely explanation for the expansion of mutant cell perimeters and constriction in the surrounding wild-type tissue.

We then asked whether a dysregulation of MyoII also could be one of the reasons why dPDZ-GEF pathway deficiencies display DC defects. MyoII in wild-type embryos appears in a typical bars-on-a-string-type distribution on the leading edge (Fig. 7C and C’). Immunostaining of dPDZ-GEF mutant embryos, however, revealed a striking failure to assemble MyoII at the leading edge (Fig. 7D and D’). This is particularly apparent in ectodermal segments for which the leading-edge cells are stretched along the dorsoventral axis (Fig. 7E and E’). Analysis of *cno* mutant embryos showed a similar mislocalization of MyoII in leading-edge cells. *cno* mutant embryos that failed to proceed through DC often show MyoII localizing discontinuously in their leading edges (Fig. 7F and F’). These data are in agreement with earlier studies showing that compromising MyoII function prevents the proper generation of contractile forces in the leading edge and DC (13).

To obtain genetic evidence for a role of dPDZ-GEF signaling in MyoII regulation, we asked whether a concomitant reduction of gene dosages at the *zip* and dPDZ-GEF or *cno* loci would reveal genetic interdependencies. To this end, we made use of a transheteroallelic zipper combination (*zip<sup>ms</sup>/*zip<sup>ms</sup>) that does not provoke significant embryonic lethality (Table 2) (38). Reduced dosages of the *zip* and dPDZ-GEF (Gef26<sup>c2</sup>/Gef26<sup>c2</sup>) genes together yielded a significant number of dead embryos (52%; *n* = 248). Six percent of the double transheterozygotic animals remained dorsal open and 25% retained anterior-open defects, while the remaining 21% proceeded through DC but did not involute their head cuticles (Fig. 7H and Table 2). Viability into the first-instar larval stage was reduced to 48%, whereas it was 93 and 87% for *zip<sup>ms</sup>/*zip<sup>ms</sup> and Gef26<sup>c2</sup>/Gef26<sup>c2</sup> embryos, respectively (Table 2). Thus, these findings indicate that the dPDZ-GEF and *zip* genes cooperate in DC. We next...
lowered gene dosages concomitantly at the zip (zip^{vbr}/zip^2) and cno (cno^{mis1}/cno^2) loci and found an even more profound effect. Embryonic lethality increased to 95%. Among them, 29% of embryos remained dorsal open and 57% remained anterior open, while 9% of embryos completed DC but did not hatch into larvae (n = 93) (Fig. 6I and Table 2). To analyze the interaction we observed between the cno and zip genes during DC later in wing morphogenesis, we modified the zip gene dose by introducing zip^{vbr} into the viable hypomorphic cno^{mis1}/cno^2 background. Flies carrying these genetic modifications developed malformed wings at a significant frequency (7/25 wings), while heterozygous zip^{vbr} and hypomorphic cno wings were not affected (Fig. 7J to L). Together, these data demonstrate a genetic interaction of the zip gene with dPDZ-GEF and cno and corroborate our immunocytochemical observations. This finding, in conjunction with the decline in lateral constriction we observed in mitotic clones and mutant embryos, compels us to propose a model in which dPDZ-GEF signaling through Rap GTPases regulates MyoII to modulate cell contractility in epithelial migration and homeostasis.

**DISCUSSION**

In developing tissues, Rap has been found to promote various morphogenetic processes, ranging from epithelial migration and invagination in embryogenesis to the maintenance of epithelial integrity in proliferating tissues at later stages (2, 4, 17, 31, 46). However, the mechanisms by which Rap is regulated and mediates its effects in morphogenetic episodes remain poorly understood. In this report, we delineate a pathway in which the *Drosophila* GEF dPDZ-GEF links Rap activity to MyoII in the regulation of lateral contractility and cell shape in different epithelial morphogenetic episodes.

We identified dPDZ-GEF as a putative activator of Rap GTPases in a YTH screen and subsequently demonstrated that it specifically associates with Rap, but not Ras, GTPases. PDZ-GEF is highly conserved among metazoans (12, 26, 40, 46), suggesting that it might serve common physiological roles. We found that dPDZ-GEF is highly expressed in epithelial tissues involved in embryonic DC, and, importantly, our data revealed that it functions as an activator of Rap1/Cno signaling in this process. First, as in the case of Rap1 and cno, loss of zygotic dPDZ-GEF function is associated with an ectodermal failure, which is manifested by dorsal-open phenotypes. Eliminating both zygotic and maternal dPDZ-GEF elevates the frequency of late gastrulation defects. Second, our genetic analysis places dPDZ-GEF upstream of the Rap/Cno GTPase/effector complex, as both Rap1 and Cno were able to rescue the dPDZ-GEF LOF phenotype to a large extent. Third, all three proteins show an overlapping localization at AJs in ectodermal cells involved in DC. Thus, our findings demonstrate that dPDZ-GEF serves as a Rap1/Cno activator to promote late epithelial gastrulation movements. In support of a conserved role of dPDZ-GEF in epithelial morphogenesis, studies with *C. elegans* demonstrated that pxf-1, the dPDZ-GEF homolog, is vital for epithelial integrity (46). pxf-1 mutant animals often are confronted with hypodermal malfunctions; the underlying cellular basis of these defects, however, remains to be elucidated.

Epithelial migration processes often entail striking alterations in cell shape, and much effort has been devoted to unraveling the underlying cellular and molecular mechanisms. Our study highlights that dPDZ-GEF as a Rap activator adjusts cell shape to the demands of morphogenetic movements and imaginal disc morphogenesis. We observed that dPDZ-GEF mutant embryos involved in DC often exhibit bunched regions in their leading edge and an incompetence of ectodermal cells to elongate dorsally. These phenotypes also characterize embryos that either overexpress DN Rap1 or are mutant for cno (4). Thus, signaling through dPDZ-GEF, Rap, and Cno (i) is vital for the organization of a coherently moving leading edge and (ii) enables the typical dorsoventral stretching of lateral ectodermal cell sheets. Our studies also unveiled a requirement for dPDZ-GEF for the adjustment of epithelial cell shape in the differentiation program of the wing imaginal disc. We found that a dPDZ-GEF LOF situation generated in a clonal analysis of mosaic wing discs is associated with a decline in apicolateral contractility in the vicinity of junctional complexes. Loss of contractility, as visualized by a widening of apicolateral circumferences, is coupled to a partially compensating gain of contractility in adjacent wild-type tissue. Wild-type cells in close proximity to mutant clones display smaller apicolateral circumferences. Interestingly, overexpression of dPDZ-GEF in restricted areas of the wing disc causes contractile aberrations. When ectopically expressed in the posterior compartment, dPDZ-GEF leads to a loss of apicolateral contractility in cells lining the A/P boundary. Thus, both gain and loss of dPDZ-GEF function compromise normal contractile strength and result in aberrant adult tissue formation. These observations suggest that a finely tuned level of Rap activation

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**TABLE 2. Quantitative analysis of cuticular phenotypes for genetic interactions of zip and dPDZ-GEF or cno**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Larvae hatched</th>
<th>Cuticle closed (but with head involution defect)</th>
<th>Cuticle partially open</th>
<th>Dorsal open</th>
<th>n</th>
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<td>cno^{mis1}/cno^2</td>
<td>58</td>
<td>21 (12)</td>
<td>19</td>
<td>2</td>
<td>239</td>
</tr>
<tr>
<td>Gef26(-)/Gef26^vbr</td>
<td>87</td>
<td>13 (8)</td>
<td>0</td>
<td>0</td>
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<td>zip^vbr/zip^2</td>
<td>93</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Gef26(-)/zip^vbr; Gef26^vbr, zip^vbr</td>
<td>48</td>
<td>21 (21)</td>
<td>25</td>
<td>6</td>
<td>248</td>
</tr>
<tr>
<td>zip^vbr/zip^2; cno^{mis1}/cno^2</td>
<td>5</td>
<td>9 (6)</td>
<td>57</td>
<td>29</td>
<td>93</td>
</tr>
</tbody>
</table>

*Cuticle defects were categorized as described in the footnotes to Table 1.*

*b* Weak heteroallelic combination at the cno locus.

*c* Weak heteroallelic combination at the dPDZ-GEF locus.

*d* Weak heteroallelic combination at the zip locus.

*e* In the case of the dPDZ-GEF interaction with zip, recombinant chromosomes were generated.
is crucial for normal cellular and organismal development to occur. Tight requirements for activation of small GTPases in vivo have been documented previously, e.g., for Rho GTPases and their function in axon guidance (37). Importantly, we found in genetic modification experiments that reduced or enhanced dPDZ-GEF activity in the developing wing can be rescued by ectopic Rap1 or lowered cno doses, respectively, suggesting that signaling through the dPDZ-GEF/Rap/Cno module at least partially controls disc morphogenesis. This, together with the vital cooperative roles of all three genes in embryonic cell sheet migration, corroborates the reiterated function of dPDZ-GEF/Rap/Cno signaling during epithelial development.

What are the mechanisms that translate Rap signaling downstream of dPDZ-GEF into the modulation of cell shape? Our analysis of dPDZ-GEF LOF situations during gastrulation and wing disc morphogenesis showed that junctional integrity is not corrupted. Both AJ and SJ belts around the apicolateral circumference are seamlessly maintained in dPDZ-GEF LOF tissue. However, our data support a role for the MyoII heavy chain, the product of the zip gene, as an effector. MyoII assembly and disassembly in migrating cells and tissue homeostasis are tightly balanced processes. In epithelial cells, MyoII localizes to cell-cell junctional complexes and is essential for establishing and maintaining intercellular adhesion and tension (16). We found that the decline in apicolateral constriction associated with dPDZ-GEF LOF in mitotic clones in the wing disc epithelium is accompanied by a less compact MyoII localization and that adjacent constricted wild-type cells display overassembled MyoII, which concentrates in ectopic focal structures. Also, in the DC paradigm, dPDZ-GEF and cno mutant embryos that are involved in DC exhibited failures of leading-edge cells to properly assemble MyoII. The abundant MyoII localization at the leading edge that characterizes wild-type embryos during DC is significantly diminished, and the bars-on-a-string-like MyoII distribution is lost in these mutants. In particular, regions of the leading edge adjacent to the bunched segments retain only minimal amounts of assembled MyoII. These observations strongly suggest that loss of MyoII control at the leading edge is contributing to the bunching phenotype observed in dPDZ-GEF and cno mutants. Consistent with a spatiotemporal regulation of MyoII in distinct regions of the leading edge are elegant life-imaging studies undertaken with embryos undergoing DC (13). The latter study visualized dynamic cycles of MyoII-dependent contraction and relaxation that are limited to smaller regions within the leading edge during the migration process.

In further support of the notion that dPDZ-GEF signaling acts on MyoII, we obtained evidence that dPDZ-GEF and cno genetically interact with zip in late gastrulation and, moreover, that cno is genetically linked to zip in wing morphogenesis. In particular, our data show a strong enhancement of dorsal-open frequencies in embryos that are double transheterozygous for hypomorphic combinations of zip and either dPDZ-GEF or cno. We also found that combined mutations at the zip and cno loci cause a malformation of wings. Together, our findings imply that signaling through the dPDZ-GEF/Rap/Cno module is required for MyoII function at different stages of epithelial development. Future experimentation will be required to determine the precise biochemical link between this module and MyoII regulation. Of note, a recent study demonstrated that the mammalian Cno homolog, AF-6/Afadin, in a two-dimensional tissue culture system moves together with MyoII at the edge of wounds induced by laser ablation (51). At the onset of wound closure, a subpopulation of MyoII resides apically in the lateral membranes of cells lining the wound. However, when closure progresses into advanced stages, MyoII, together with AF-6/Afadin, migrates basally to constrict both the wound perimeter and the apicobasal membranes facing the wound.

Our data do not rule out additional Rap effector pathways acting in synergy with Cno to bring about appropriate lateral cell contractility and MyoII regulation. The most extensively investigated signaling pathway that regulates MyoII activity in diverse systems is the Rho/Rho kinase pathway. Several studies have demonstrated that the zip gene exhibits strong interactions with genes encoding Rho signaling components (18, 43, 55, 59). Abundant evidence from mammalian cells indicates that Rho-stimulated Rho kinase activity leads to the phosphorylation of the regulatory myosin light chain (MLC) as well as an inhibition of MLC phosphatase, both of which in concert are stimulating MyoII assembly and contractility (1, 30). In light of recently uncovered molecular links between Rap and Rho involving Rap-associating RhoGAPs, it is an intriguing possibility that Rap is connected to Rho signaling in certain aspects of epithelial morphogenesis (34, 61). Alternatively, however, Rap could signal directly to an enzyme modifying the MyoII complex, such as kinases and phosphatases targeting MLC. Interestingly, the heavy chains of MyoII also have been reported to be subject to phosphorylation (62). Studies with chemotactic Dictyostelium have identified MyoII heavy-chain kinases that regulate the subcellular distribution of assembled MyoII complexes (7, 62). More recently, the Ser/Thr kinase Phg2, which acts as a potential Rap1 effector in Dictyostelium, also has been reported to induce MyoII heavy-chain phosphorylation (24). However, we failed to identify an obvious Drosophila homolog of Phg2 by extensively searching numerous databases. Future studies will be important to delineate how far Rap and Rho signaling activities are intertwined in epithelial morphogenetic events.

Our data support a model in which dPDZ-GEF, through Rap activation and MyoII regulation, contributes to the adjustment of lateral cell contractility in epithelial cells of the embryo and the developing wing. In a previous study, the analysis of Rap1 mutant clones in the wing imaginal disc revealed a direct effect of Rap1 on the reorganization of AJs at the end of cytokinesis, where resealing of their belts has to occur between daughter cells (31). Since our data showed that AJ integrity is unperturbed in clones comprised of dPDZ-GEF LOF cells, we surmise that dPDZ-GEF either is not relevant for Rap1 activation in the reconstitution of a seamless AJ belt during cytokinesis or is compensated for by a still-unknown factor conferring the necessary exchange activity. On the other hand, the apicolateral constriction defects we detected as a consequence of clonal loss of dPDZ-GEF function so far have not been described for Rap1 mutant clones in the same scenario. We presume either that they have escaped scrutiny or, more likely, that Rap1 acts redundantly with its close homolog Rap2l in adjusting apicolateral constriction, while the reorganization of AJs during cytokinesis relies solely on Rap1. In fact,
**Rapl and Rap2l** have been shown to compensate for each other in the male stem cell niche (57). In this context, both Rap proteins cooperate downstream of dPDZ-GEF to anchor germ line stem cells to their niche. In future experiments, we plan to generate Rap1 and Rap2l mitotic clones in parallel and to examine and compare their effects on cell shape and contractility.

A picture is emerging in which specialized GEFs activate Rap GTPases and selective effectors in different morphogenetic scenarios and cellular processes. For example, Rap1 signaling has been implicated in cell/extracellular matrix-dependent force transduction at focal adhesion sites of cultured cells (52). In this scenario, Rap1 is regulated by an Src/p130Cas/C3G-triggered mechanism (47). Also, apical constriction during neural tube closure in the Xenopus blastula has been demonstrated to depend on Rap1 function downstream of the Shroom protein (17); however, the relevant GEF in this scenario remains to be identified. The notion that Rap activation in distinct developmental processes is specified by dedicated GEFs also suggests that Rap effectors are selected in order to fulfill pathway requirements. In light of this, dPDZ-GEF and Rap1 have been implicated in the regulation of mitogen-activated protein kinase activity during differentiation of the Drosophila compound eye (19, 35), and a more recent study reported that D-Raf relays a signal from Rap to mitogen-activated protein kinase in Torsos receptor-dependent terminal differentiation of the early Drosophila embryo (41). Together, these findings suggest the possibility that dPDZ-GEF could trigger the activation of the Rap/D-Raf pathway to regulate certain differentiation processes. Our data reveal a novel function for dPDZ-GEF as an activator of Rap in the implementation of epithelial cell shape changes required for sheet migration and homeostatic cell shape maintenance in the genesis of the wing imaginal disc epithelium. We further provide evidence that Cno functions as a relevant effector of Rap downstream of dPDZ-GEF and Rap1, Cno module is connected to the regulation of MyoII and the generation and modulation of appropriate lateral cell contractility. Thus, our findings have unveiled a pathway linking the Rap activator dPDZ-GEF to MyoII and the regulation of lateral contractility and cell shape in epithelium migration and homeostasis. Further elucidation of dPDZ-GEF-interacting proteins and the molecular underpinnings of MyoII regulation downstream of this module in epithelial cells will be key to our understanding of these aspects of tissue morphogenesis.

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