Collective epithelial cell migration requires the maintenance of cell–cell junctions while enabling the generation of actin-rich protrusions at the leading edge of migrating cells. Ventral enclosure of Caenorhabditis elegans embryos depends on the collective migration of anterior-positioned leading hypodermal cells towards the ventral midline where they form new junctions with their contralateral neighbours. In this study, we characterized the zygotic function of RGA-7/SPV-1, a CDC-42/Cdc42 and RHO-1/RhoA-specific Rho GTPase-activating protein, which controls the formation of actin-rich protrusions at the leading edge of leading hypodermal cells and the formation of new junctions between contralateral cells. We show that RGA-7 controls these processes in an antagonistic manner with the CDC-42’s effector WSP-1/N-WASP and the CDC-42-binding proteins TOCA-1/2/TOCA1. RGA-7 is recruited to spatially distinct locations at junctions between adjacent leading cells, where it promotes the accumulation of clusters of activated CDC-42. It also inhibits the spreading of these clusters towards the leading edge of the junctions and regulates their accumulation and distribution at new junctions formed between contralateral leading cells. Our study suggests that RGA-7 controls collective migration and junction formation between epithelial cells by spatially restricting active CDC-42 within cell–cell junctions.

Keywords: Caenorhabditis elegans, Cdc42, Rho GAP, collective migration, ventral enclosure, epithelial, morphogenesis

Introduction

Collective migration of epithelial cells is characterized by maintaining cell–cell adhesion whilst creating an antero-posterior polarity essential for directional migration (Khursheed and Bashyam, 2014). It plays an important role in organ morphogenesis, tissue regeneration, and tumour dissemination (Friedl and Gilmour, 2009). Collective migration is also required for dorsal and ventral enclosure in Drosophila melanogaster (Bastock and Strutt, 2007) and Caenorhabditis elegans, respectively (Chisholm and Hardin, 2005).

The late phase of C. elegans embryonic development includes epidermal morphogenetic events that enable the embryo to acquire its final tubular shape (Chisholm and Hardin, 2005). One of these events, termed ventral enclosure, involves the migration of ventral hypodermal cells towards the ventral midline to cover the embryo in an epidermal layer. This event occurs in two phases. In the first phase, the anterior ventral hypodermal cells, referred to as the leading cells, migrate towards the ventral midline using large actin-rich protrusions, where they form junctions with their contralateral neighbours (Chisholm and Hardin, 2005). Afterwards, the posterior ventral hypodermal cells, called the pocket cells, migrate towards the ventral midline using a contraction-dependent, purse-string mechanism, which is still poorly described (Williams-Masson et al., 1997). These migratory mechanisms are supported by signals from underlying neuroblasts (Chisholm and Hardin, 2005).

During ventral enclosure, Rho GTPases control hypodermal cell migration in a cell-autonomous manner. Rho GTPases are molecular switches controlling a wide range of cellular functions including shape changes and cell migration (Takai et al., 2005). They cycle between an ‘ON’ GTP-bound form, during which they interact with specific effectors, and an ‘OFF’ GDP-bound form. They are regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The Rho GTPase CED-10/Rac1 regulates early migration of leading and pocket cells during ventral enclosure through the activation of effectors, including GEX-1/WVE-1/Scar and the ARP2/3 complex, to promote remodeling of the actin cytoskeleton (Lundquist et al., 2001; Withee et al., 2004). An additional pathway involving another potential effector of CED-10, the ENA/VASP UNC-34, was shown to specifically control the protrusive activity of leading cells in parallel with the CDC-42’s effector WSP-1/N-WASP/WASP (Withee et al., 2004; Sheffield et al., 2007). In mammals, regulation of actin cytoskeleton
remodelling and membrane trafficking by N-WASP/WASp and Cdc42 has been shown to depend on the F-BAR proteins TOCA1/FBP17 (Pichot et al., 2010). While CDC-42 has not been directly studied in C. elegans ventral enclosure, the two redundant homologues of TOCA1, TOCA-1 and TOCA-2, were shown to control endocytosis of junctional proteins at that stage together with WSP-1 (Giuliani et al., 2009). Another Rho GTPase, RHO-1/RhoA, and its effector LET-502/ROCK may also control myosin-dependent contraction events during ventral enclosure (Fotopoulos et al., 2013).

Coordinating the different Rho GTPases and generating spatially distinct active zones at the leading edge of migrating fibroblasts is required for the generation of actin-rich protrusions (Pertz, 2010). Spatially controlling the activity of the different Rho GTPases at cell–cell junctions is also important for the transmission of forces from the leading cells to those that follow (Friedl et al., 2014) and for the maintenance of cell–cell junction integrity (Hidalgo-Carcedo et al., 2011).抗性质 between Cdc42 and RhoA at cell–cell junctions reduces actomyosin contractility between collectively migrating cells, which enables a better coordination of their movement (Hidalgo-Carcedo et al., 2011). In collectively migrating MDCK cells, active RhoA is found at the front of the leading cell(s), while active Cdc42 is restricted to more posterior/back locations (Reffay et al., 2014). Similarly, spatially distinct concentric zones of active RhoA and Cdc42 form in Xenopus oocytes during wound healing (Benink and Bement, 2005). This suggests that the spatial restriction of active Rho GTPases is evolutionary conserved and important for collective migration.

Signalling mechanisms spatially controlling active Cdc42 have been described in the yeast Saccharomyces cerevisiae (Park and Bi, 2007). The clustering of active Cdc42 at the bud site involves exocytic mechanisms targeting Cdc42 from intracellular compartments to the plasma membrane. It also involves feed-forward loops in which active Cdc42 controls exocytic mechanisms promoting its own accumulation and activation by GEFs at forming clusters (Harris and Tepass, 2010). The spatial restriction of active Cdc42 also involves actin-dependent endocytosis, and the recruitment of Rho GAPs to the bud site (Park and Bi, 2007; Wu and Lew, 2013). These studies highlight the interdependence of the spatial regulation of active Cdc42 and membrane trafficking, and the critical role for Cdc42-specific GAPs and GEFs in these processes in yeast.

In the epithelia of multicellular organisms, the mechanisms controlling the spatial distribution of active Cdc42 are less well understood. Membrane trafficking was however shown to spatially control the targeting of active Cdc42 at the leading edge of migrating primary rat astrocytes (Osmani et al., 2010). The function of Cdc42 in regulating actin-dependent endocytosis together with the F-BAR protein TOCA1/FBP17 and N-WASP/WSP-1 and endocytic recycling events at cell junctions is also well established in multicellular organisms (Leibfried et al., 2008; Giuliani et al., 2009; Bu et al., 2010; Harris and Tepass, 2010). Whether regulation of membrane trafficking by Cdc42 contributes to the spatial regulation of its active zone at the junctions between epithelial cells is, to date, unknown. Feed-forward loops involving N-WASP/WASp and Cdc-42-specific GEFs (Hussain et al., 2001; Kovacs et al., 2011; Humphries et al., 2014), as well as feedback mechanisms involving recruitment of Rho GAPs to prevent excessive activation of Cdc42 at cell–cell junctions were also described in the epithelia of multicellular organisms (Elbediwy et al., 2012). However, whether these mechanisms spatially control active Cdc42 at cell junctions and collective migration of epithelial cells is still unknown.

In this study, we characterized the function of the Rho GTPase-activating protein 7 (rga-7) during C. elegans ventral enclosure. This gene, called also spv-1 (Spermaphetica variant 1), encodes a protein containing a RhoGAP domain, an F-BAR domain that interacts with curved membranes, and a C1 domain that may interact with diacylglycerol (DAG) (Tan and Zaidel-Bar, 2015). RGA-7/SPV-1 was previously shown to regulate the activity of RHO-1/RhoA in the spermaphetica during expulsion of embryos towards the uterus, and has three close human homologues: GMIP, ARHGAP29/PARG1, and HMHA1 (Tan and Zaidel-Bar, 2015). We show here that RGA-7 displays GAP activity towards CDC-42 in addition to RHO-1/RhoA. RGA-7 is required for migration of leading cells towards the ventral midline and regulation of the dynamics of junction formation between contralateral leading cells. We also show that RGA-7 functions in parallel with LET-502/ROCK and antagonistically with WSP-1/N-WASP and TOCA-1/TOCA1/FBP17. RGA-7 regulates the accumulation and distribution of active CDC-42 clusters at the junctions between adjacent leading cells and between contralateral leading cells. Our data suggest that spatial regulation of active CDC-42 at the cell–cell junctions by RGA-7 plays an important role in the ability of leading cells to migrate collectively towards the ventral midline and also regulates junction expansion between contralateral cells.

**Results**

**RGA-7 is expressed in the hypodermis and controls ventral enclosure in a cell-autonomous manner**

To gain a better understanding of the regulation of Rho GTPases during ventral enclosure, we searched for available strains carrying mutations in genes encoding for Rho GTPase regulators and displaying ventral enclosure defects. We obtained a strain carrying a 577-bp deletion within **rga-7** from the C. elegans genetic center (CGC), which was previously described as a loss-of-function mutation (Tan and Zaidel-Bar, 2015) (Supplementary Figure S1A). We observed that **rga-7(ok1498)** hermaphrodites laid 27.9% ± 3.5% SEM dead embryos (n = 227; Figure 1A). Imaging **rga-7(ok1498)** embryonic development revealed that 62.9% (34 out of 54) of embryos displayed morphogenetic problems during late embryogenesis: 9.3% (5 out of 54) of the embryos arrested their development during ventral enclosure (Figure 1B, D and Supplementary Video S1); 11.1% (6 out of 54) arrested during elongation (arrow; Figure 1B, E and Supplementary Video S2); and 37% (20 out of 54) displayed slow ventral enclosure—defined as ventral enclosure that lasted for > 1 h at 25°C but hatched and developed into adults (Figure 1B). A small proportion of embryos arrested before morphogenesis (7.4%; 4 out of 54; Figure 1B) and 3.7% (2 out of 54) displayed spherical eggshells and died at various stages of embryonic development (eggshells defects; Figure 1B). Finally, 31.5% (17 out of 54) developed normally into adults and did not display any of the above phenotypes.
We crossed *rga-7(ok1498)* hermaphrodites with wild-type (wt) males and found that the ventral enclosure defects observed in *rga-7* mutant embryos are due to zygotic requirements for the gene (see Supplementary data and Figure S1B). We also tested whether *rga-7* loss-of-function was responsible for the embryonic lethality (Emb) phenotype observed in *ok1498*-carrying animals using rescue experiments. To do so, we characterized the molecular structure and expression of *rga-7* in embryos (see Supplementary data and Figure S1A, C, and D). This revealed that *rga-7* codes for three transcripts with the two larger ones, *rga-7l* and *rga-7m*, detected in embryos (see Supplementary data and Figure S1A, C, and D). We then generated transgenic animals carrying *sajls22[rga-7p::rga-7::GFP; unc-119R]*, driving the expression of a fusion protein between *RGA-7* and the green fluorescent protein (GFP) under the control of the *rga-7p* endogenous promoter (Supplementary Figure S2A, see Materials and methods). RGA-7::GFP was detectable in several cells of the embryo during ventral enclosure where it accumulated as perinuclear punctate structures (Figure 1H). RGA-7::GFP was more tubular during early elongation (arrow; Figure 1I). During late elongation, it was mainly expressed in the dorsal and ventral hypodermal cells where it organized itself into stripes reminiscent of filamentous actin bundles (Chisholm and Hardin, 2005) (Figure 1J). RGA-7::GFP was also expressed in a wide range of cells in larvae and adults including the hypodermis, head and tail neurons, and spermatheca (Supplementary Figure S2B–D, respectively). Embryos expressing *sajls22* did not present any significant Emb or significant delay during ventral enclosure compared with wild-type (wt) embryos (Figure 1A and B). When expressed in *rga-7(ok1498)* hermaphrodites, this transgene fully rescued *rga-7(ok1498)*-associated Emb and ventral enclosure defects (Figure 1A, B, and F). Our results suggest that the mutation in *rga-7*(ok1498) is responsible for the ventral enclosure defects observed in the mutant strain and that *sajls22* expresses functional RGA-7 protein.

Ventral enclosure defects arise in embryos carrying mutations in genes required either in the hypodermis for their migration or in the underlying neuroblasts (Chisholm and Hardin, 2005). To assess whether *rga-7* is required in the hypodermis during ventral enclosure, we generated transgenic animals expressing RGA-7::GFP under the control of the hypodermal-specific promoter *lin-26p* (*sajls20[lin-26p::rga-7::GFP; unc-119R]; Supplementary Figure S2A). *rga-7*(ok1498) mutant embryos expressing this transgene did not display any Emb phenotypes or ventral enclosure defects (Figure 1A, B, G and Supplementary Video S3), suggesting that the function of *rga-7* is required in the hypodermal cells during ventral enclosure.

![Figure 1](http://jmcb.oxfordjournals.org/)

**Figure 1** *rga-7* controls ventral enclosure. (A) Percentage of embryonic lethality (Emb) is measured for each indicated genotype. (B) Percentage of embryonic defects is indicated for each genotype. (C–G) Embryonic development progress for *wt* (C), *rga-7(ok1498)* (D and E), *rga-7(ok1498);sajls22[rga-7p::rga-7::GFP] (F), and *rga-7(ok1498);sajls22[lin-26p::rga-7::GFP] (G). Time = 0 min corresponds to the time when leading cells start to be observable ventrally (arrows). Expression pattern of RGA-7::GFP in *sajls22[rga-7p::rga-7::GFP] animals during ventral enclosure (H); early elongation (I) and late elongation (J). Arrow in I indicates tubular structures enriched in RGA-7::GFP. Scale bar, 10 μm.
**RGA-7 is a GAP for CDC-42 and RHO-1 and controls ventral enclosure in an antagonistic manner with WSP-1 and in parallel with LET-502.**

(A) The rate of GTP remaining bound on recombinant GTPases after 5 min incubation in the presence of 10 or 100 nM of recombinant GAP domain of RGA-7 compared with control (0 nM RGA-7) is indicated. (B) Percentages of embryonic defects for indicated genotypes. Phenotypes indicated by different shades of grey are associated to embryonic lethality. 

**C–I** Embryonic development progress from time = 0 min corresponding to the time when leading cells start to be observable ventrally (arrows) for wt (C), wsp-1(gm324) (D and E), let-502(sb118ts) at 25.5°C (F), rga-7(ok1498);wsp-1(gm324) (G), and rga-7(ok1498);let-502(sb118ts) at 25.5°C (H, I). Scale bar, 10 μm. *t-test P-value < 0.05.
let-502 during ventral enclosure. These data also support the hypothesis that rga-7 may function in the cdc-42/wsp-1 pathway and in parallel with rho-1/let-502 during ventral enclosure. The fact that rga-7 and wsp-1 mutants display similar ventral enclosure defects and mutually rescue each other is intriguing.

N-WASP/WASP was shown in several systems to be involved in feed-forward loops promoting Cdc42 activation by GEFs (Hussain et al., 2001; Kovacs et al., 2011; Humphries et al., 2014). In light of this, we hypothesize that during ventral enclosure, WSP-1 and RGA-7 control CDC-42 activation and inactivation, respectively. Following this hypothesis, the balance of CDC-42 cycling and consequently its function may be restored in the rga-7;wsp-1 double mutant, although it would be reduced compared with wt.

RGA-7 controls the formation of actin-rich protrusions at the leading edge of leading cells

To gain a better understanding of the cellular function of rga-7, we assessed whether it controls the formation of actin-rich protrusions at the leading edge of leading cells during ventral enclosure, as demonstrated for wsp-1 (Sawa et al., 2003). To do so, we expressed the filamentous actin-binding probe lin-26p::VAB-10(ABD)::GFP in hypodermal cells of wt (Supplementary Video S8) or mutant animals (Supplementary Videos S9 and S10) and measured the size of protrusions in leading (LCs; anterior – Figure 3I) and pocket (PCs; posterior – Figure 3J) cells. Both wsp-1(gm324) and rga-7(ok1498) embryos displayed a significant reduction in the LC protrusion rate compared with wt embryos ($t$-test $P$-values = $10^{-11}$ and $10^{-14}$, respectively; compare Figure 3A with B and C; Figure 3I), but did not display any change in their PC protrusion rates (Figure 3K). rga-7(ok1498); wsp-1(gm324) double-mutant embryos displayed a significantly

![Figure 3](image-url)
increased protrusion rate for the LCs compared with rga-7(ok1498) and wsp-1(gm324) animals (t-test P-values = 10^{-8} and 10^{-12}, respectively; Figure 3J), though still significantly lower compared with wt embryos (t-test P-value = 0.002; Figure 3J). In addition, while rga-7(ok1498); wsp-1(gm324) embryos enclosed faster than single mutants (compare Figure 3D with B and C), they were still delayed when compared with wt embryos (compare Figure 3D with A). These data suggest that rga-7 and wsp-1 control the formation of actin-rich protrusions in the LCs in an antagonistic manner. In addition, the loss of both rga-7 and wsp-1 functions only partially restored the ability of the LCs to form actin-rich protrusions and to migrate towards the ventral midline.

Since RGA-7 may negatively regulate CDC-42 activity during ventral enclosure, and WSP-1 activity may depend on active CDC-42 and/or promote its activation, we propose that the misregulation of CDC-42 activity should cause ventral enclosure defects similar to those seen in rga-7(ok1498) and wsp-1(gm324) embryos. Overexpressing CFP::CDC-42 in hypodermal cells using the lin-26p promoter triggered a significant reduction in the protrusion rate of LCs (t-test P-value = 10^{-6}; Figure 3I), while causing a significant increase in the PC protrusion rate when compared with wt embryos (t-test P-value = 10^{-6}; Figure 3K). Moreover, CFP::CDC-42-expressing embryos had delayed ventral enclosure compared with wt embryos (compare Figure 3E and A), and 72.2% of them (13 out of 15 embryos) failed to complete ventral enclosure. Interestingly, overexpression of CFP::RHO-1 in hypodermal cells did not significantly alter the LC protrusion rate (Figure 3F and J), but significantly increased the PC protrusion rate (t-test P-value = 10^{-11}; Figure 3K). Moreover, the LCs of the CFP::RHO-1 embryos migrated in a similar manner as the LCs of wt embryos, while the PCs were delayed (compare Figure 3F and A). Supporting the predominant role of CDC-42 regulation over that of RHO-1 during LC migration, the average intensity of overexpressed CFP::RHO-1 was ~1.67-fold as high as CFP::CDC-42 (Supplementary Figure S3A). These data suggest that misregulation of CDC-42, but not of RHO-1, may be responsible for the reduced protrusion rate of LCs in rga-7(ok1498) embryos.

The F-BAR proteins TOCA1/FBP17 and their orthologs in C. elegans, TOCA-1 and TOCA-2, control actin cytoskeleton remodeling and membrane trafficking together with WSP-1/N-WASP/WASP and CDC-42 (Pichot et al., 2010). We then assessed whether rga-7 antagonizes toca-1/2 functions during ventral enclosure, similar to wsp-1. To do so, we submitted rga-7(ok1498) and wt animals expressing VAB-10(ABD)::GFP to RNAi against toca-1 and toca-2 and measured the protrusion rate of LCs and PCs during ventral enclosure. toca-1/2(RNAi) embryos did not display any change in their LC protrusion rate relative to wt embryos (Figure 3I), but the protrusion rate of PCs was significantly higher (t-test P-value = 0.0009; Figure 3K). However, the protrusion rate of LCs was significantly increased in rga-7(ok1498); toca-1/2(RNAi) compared with rga-7(ok1498) animals (t-test P-value = 0.006; Figure 3I). These data suggest that rga-7 controls the formation of actin-rich protrusions in the leading cells in an antagonistic manner with wsp-1 and toca-1/2, possibly by regulating CDC-42 activity. In addition, in the absence of both rga-7 and wsp-1/toca-1/2, a parallel pathway drives the formation of actin-rich protrusions in LCs, which is slightly less efficient than the rga-7/wsp-1 pathway(s).

**RGA-7::GFP is recruited to cell–cell junctions**

To better understand the molecular function of RGA-7 in leading cells during ventral enclosure, we assessed its subcellular location in these cells. To do so, we used embryos expressing RGA-7::GFP and the actin-binding probe VAB-10(ABD)::mCherry under the control of the hypodermal promoter lin-26p (Figure 4). As observed in transgenic animals expressing RGA-7::GFP under the control of its own promoter (Figure 1H), the fusion protein was found mainly at punctate and tubular structures within the cytosol of ventral hypodermal cells during ventral enclosure (Figure 4). It was also excluded from the actin-rich protrusions observed at the leading edges of both leading and pocket cells (orange line; Figure 4A and B). We measured RGA-7::GFP intensity along the junction located between adjacent leading cells (dashed arrow; Figure 4C), and identified two subdomains: the distal junction located within 4 μm from the edge of the junction (light blue line; Figure 4A) and the proximal junction located between 4 and 7 μm from this edge (dark blue line; Figure 4A). RGA-7::GFP was excluded from distal junctions (Figure 4C) and was found to form clusters with variable intensities along the proximal junction (Figure 4C). During the later stages of ventral enclosure, we measured the levels of RGA-7::GFP at new junctions that form and expand between contralateral neighbouring cells (red line; Figure 4A). RGA-7::GFP was excluded from expanding junctions immediately after collision of contralateral cells (Figure 4D and F) and was found to form clusters with variable intensities along the junction in fully enclosed embryos (Figure 4E and G). These data suggest that RGA-7::GFP is recruited to proximal junctions between adjacent leading cells and at a late stage of junction formation between contralateral cells.

**RGA-7 promotes the accumulation of active CDC-42 to proximal junctions between adjacent leading cells**

In light of the subcellular localization of RGA-7::GFP at proximal junctions and the known function of WSP-1 in actin cytoskeleton remodelling, we measured the accumulation of F-actin along the junctions between adjacent leading cells. We found that rga-7, wsp-1, and toca-1/2 control the accumulation of F-actin at the distal junctions (see Supplementary data and Figure S4). These data also reveal that the parallel pathway driving the formation of actin-rich protrusions in LCs in rga-7(ok1498); wsp-1 and rga-7(ok1498); toca-1/2(RNAi) double-mutants does not drive F-actin accumulation at distal junctions as efficiently as rga-7/wsp-1/toca-1/2 pathway(s) (see Supplementary data and Figure S4).

We then determined whether RGA-7 controls the activation levels of CDC-42 at proximal and distal junctions. To do this, we first assessed the distribution of hypodermally expressed CFP::CDC-42 at junctions between adjacent LCs (Figure 5A and B; arrow head indicates distance = 0 μm). We observed that CFP::CDC-42 accumulates at proximal junctions rather than distal junctions (Figure 5B and C). We then expressed a probe in hypodermal cells that contains the Cdc42/Rac interactive binding (CRIB) domain of WSP-1 fused
Figure 4  RGA-7::GFP is located at intracellular compartments and recruited to cell–cell junctions. (A) Schematic representation of embryos during ventral enclosure. The leading edge, distal junctions, and proximal junctions between adjacent cells (left panel) as well as expanding junctions between contralateral cells (right panel) are indicated. (B–G) Z-projection confocal images for sa/i[lin-26p::rga-7::GFP]; mcIs140[lin-26p::VAB-10(ABD)::mCherry + myo-2p::GFP] embryos during ventral enclosure. RGA-7::GFP (left panel) and F-actin-binding probe (middle panel) are shown at leading edge of pocket cells (B), distal and proximal junctions between adjacent leading cells (C), embryo and expanding junctions between colliding contralateral leading cells (D and F), fully enclosed embryo and fully expanded junctions between contralateral cells (F and G). Plain arrow in G represents RGA-7::GFP at intracellular tubular structures. Scale bar, 10 μm (D and E) and 2 μm (B, C, F, and G). Dashed arrows indicate the line-scans used to generate the intensity plots associated to each panel (B, C, F, and G). n = number of embryos submitted to this analysis; 3–10 measurements were done per embryo.

with mCherry, which was previously shown to specifically detect the active form of CDC-42 (Kumfer et al., 2010). We expressed this probe together with VAB-10(ABD)::GFP in wild type and rga-7(ok1498) animals. WSP-1(CRIB)::mCherry accumulated at junctions between adjacent LCs and PCs (Figure 5D). Interestingly, this probe was enriched at the proximal junctions between adjacent LCs (arrow; Figure 5D and E) and was significantly reduced in rga-7(ok1498) embryos (one-way ANOVA; genotype effect, distance between 4 and 6 μm; P-value = 10^-14; Figure 5D and E). These data suggest that RGA-7 is required for the accumulation of active CDC-42 at proximal junctions between adjacent leading cells.

The accumulation of CDC-42 at subdomains of the plasma membrane is regulated by CDC-42-specific GAPs and GEFS (Park and Bi, 2007; Harris and Tepass, 2010). Although counter-intuitive, since loss-of-function of a CDC-42 line, Figure 5D). Considering the genetic interaction of rga-7 and wsp-1 during ventral enclosure (Figures 2 and 3) and the recruitment of RGA-7::GFP to expanding junctions between contralateral cells upon enclosure (Figure 3A, F, and G), we determined the function of rga-7, wsp-1, and toca-1/2 during the expansion of these junctions.

We monitored the accumulation of filamentous actin (F-actin) at expanding junctions between contralateral leading cells (red line, Figure 4A) in embryos expressing the actin-binding probe VAB-10(ABD)::mCherry together with the junctional marker AJM-1::GFP (Figure 6A). F-actin began accumulating at the site of collision between contralateral leading cells before AJM-1::GFP junctions, presumably by regulating the targeting of CDC-42 at this specific location.

RGA-7, WSP-1, and TOCA-1/2 control junction expansion between contralateral cells

In the initial phase of epithelial cell–cell junction formation, Rac1 and Cdc42 are activated and control the expansion of the junctional surface through N-WASP/WAVE2/Arp2/3-mediated actin polymerization (Citi et al., 2014). They also promote directed targeting of membrane vesicles to cell junctions and the accumulation of junctional proteins (Citi et al., 2014). Considering the genetic interaction of rga-7 and wsp-1 during ventral enclosure (Figures 2 and 3) and the recruitment of RGA-7::GFP to expanding junctions between contralateral cells upon enclosure (Figure 3A, F, and G), we determined the function of rga-7, wsp-1, and toca-1/2 during the expansion of these junctions.

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(time = 0 min; Figure 6A). AJM-1::GFP started to accumulate at the expanding junctions between 6 and 8 min post-collision (Figure 6A). By following F-actin, we measured changes in the length of the expanding junctions over time in wt and mutant embryos expressing the actin-binding probe VAB-10(ABD)::GFP (arrows; Figure 6A). The arrows in Figure 6A indicate the extremities of the line-scans used to perform these measurements. We also used these values to calculate the expansion rate of mutants vs. wt embryos (Figure 6C; empty cells indicate an expansion rate not significantly different than 1; two-way ANOVA without interaction; genotype effect P-value > 0.05). This analysis revealed that in wsp-1(gm324) embryos, junction expansion was significantly reduced between 2 and 6 min after collision compared with wt embryos (Figure 6B and C; one-way ANOVA; P-value = 0.0006) and accelerated between 8 and 16 min (Figure 6B and C; one-way ANOVA; P-value = 0.0004). From 8 min after collision, junctions expanded 1.5 to 1.7-fold faster in rga-7(ok1498) mutants and 1.3 to 1.6-fold faster in toca-1/2(RNAi) than in wt embryos (one-way ANOVA; P-value = 1.92 × 10^{-8} and 5.7 × 10^{-8}, respectively; Figure 6A–O). rga-7(ok1498); wsp-1(gm423) and rga-7(ok1498); toca-1/2(RNAi) embryos displayed expansion rates similar to wt embryos (Figure 6B and C).

These data suggest that wsp-1 promotes junction expansion at an early stage of junction formation (up to 8 min after cell collision) as shown in mammals (Citi et al., 2014), and subsequently displays an anti-expansion function together with rga-7 and toca-1/2. In addition, rga-7 antagonizes wsp-1 and toca-1 anti-expansion functions.

We also measured F-actin accumulation during junction expansion between contralateral LCs in wt and mutant animals (see Supplementary data and Figure S5A). This analysis reveal that (i) rga-7, wsp-1, and toca-1/2 control the accumulation of F-actin at

**Figure 5** wsp-1 controls the accumulation of activated CDC-42 at the proximal junctions between adjacent leading cells. (A) Schematic representation of embryos indicating the location of the distal and proximal junctions between adjacent leading cells. (B) Transgenic embryo expressing CFP::CDC-42 in the hypodermis. Arrowhead indicates the reference point of intensity plots (distance = 0 μm in C). (C) Intensity plot showing the accumulation of CFP::CDC-42 along the distal and proximal junctions between adjacent leading cells. (D) Spatial distribution of activated CDC-42-binding probe (WSP-1(CRIB)::mCherry respectively) in wt and rga-7(ok1498) embryos during ventral enclosure (left panel) and in fully enclosed embryo (right panel). Scale bar, 10 μm. Arrows indicate the proximal junctions between adjacent leading cells. (E) Quantification of WSP-1(CRIB)::mCherry accumulation along the junctions between adjacent leading cells in wt and rga-7(ok1498) animals (distance = 0 is located at the leading edge of the distal junction). Student’s t-test P-values are indicated. n = number of embryos quantified.
these junctions in a synergistic manner; and (ii) F-actin accumulation is regulated differently by rga-7, wsp-1, and toca-1/2 at distal junctions between adjacent LCs and at expanding junctions between contralateral cells.

RGA-7 controls the accumulation and distribution of active CDC-42 at cell junctions

Given that RGA-7 promotes the accumulation of active CDC-42 at proximal junctions between adjacent leading cells (Figure 5D), we measured this accumulation at expanding junctions between contralateral leading cells (red line; Figure 4A) in wt and rga-7(ok1498) embryos. To do so, we measured the accumulation of the WSP-1(CRIB)::mCherry probe across the expanding junctions identified with the VAB-10(ABD)::GFP marker (dashed yellow line; Figure 7A). This analysis revealed that WSP-1(CRIB)::mCherry accumulated significantly less at expanding junctions in rga-7(ok1498) embryos than in wt (Figure 7B; t-test \(P\)-value = 0.005). This suggests that RGA-7 promotes the accumulation of active CDC-42 at expanding junctions between contralateral leading cells.

We then assessed the distribution of RGA-7::GFP fusion protein at a late stage of junction expansion (>8 min after collision). This analysis revealed that RGA-7::GFP was centrally positioned within the expanding junctions (Figure 7C)—RGA-7::GFP was excluded from the domains of the junctions located on average 1.94 ± 0.323 SEM from the anterior and the posterior extremities of the expanding junctions (measured on 6.26 to 9.8 μm-wide expanding junctions; \(n = 6\) embryos; Figure 7C).

Our study reveals that F-actin, RGA-7::GFP, and active CDC-42 form clusters of variable intensities along cell–cell junctions (Figures 4C, G and 7A, C). We then investigated the distribution of these clusters at cell–cell junctions in wt and rga-7 mutant animals (see Materials and methods). Measurement of the average distance between RGA-7::GFP and F-actin clusters (A-G; Figure 7E) revealed that this distance was significantly lower than the distance observed between F-actin clusters (A-A; Figure 7E; t-test \(P\)-value < 0.0001) and between RGA-7::GFP clusters (G-G; Figure 7E; t-test \(P\)-value < 6.9 × 10^{-5}) at expanding, distal, and proximal junctions (Figure 7D and E). This suggests that RGA-7::GFP accumulates close to F-actin clusters at cell–cell junctions.

Similarly, analysis of the distribution of WSP-1(CRIB)::mCherry clusters revealed that they were also distributed in close proximity to F-actin clusters in wt animals (A-C; Figure 7F; t-test between A-C and C-C \(P\)-value < 0.003). Moreover, the average distance between WSP-1(CRIB)::mCherry and F-actin clusters did not significantly vary between wt and rga-7(ok1498) mutant animals (Figure 7G; t-test \(P\)-value > 0.05). This suggests that active CDC-42 accumulates close to F-actin clusters and that rga-7 function is not required to ensure this relative positioning.

Interestingly, while the average density of WSP-1(CRIB)::mCherry (defined as the number of clusters per micrometer of junction, clusters/μm; Figure 7H) was similar at all junctions in wt
animals, this density was significantly higher at distal junctions in *rga-7(ok1498)* animals (Figure 7H; *t*-test *P*-value = 0.012). This suggests that RGA-7 inhibits the formation/maintenance of active CDC-42 clusters at distal junctions between adjacent LCs. In addition, the average distance between WSP-1(CRIB)::mCherry clusters was significantly higher at the expanding junctions in *rga-7(ok1498)* mutants compared with wt (*C-C*; *t*-test *P*-value = 0.004; Figure 7H). This suggests that *rga-7* is required to maintain the relative distribution of active CDC-42 clusters at the expanding junctions.

All together, these data suggest that both RGA-7 and active CDC-42 are targeted to the vicinity of F-actin clusters at the proximal, distal, and expanding junctions. The distribution of these proteins relative to F-actin clusters may not specifically involve RGA-7 function. However, RGA-7 may negatively regulate the spreading of active CDC-42 clusters through inhibiting the generation/maintenance of these clusters at distal junctions between adjacent leading cells and maintaining the relative distance between clusters at new junctions expanding between contralateral cells. We hypothesize that RGA-7 functions in restricting active CDC-42 to specific...
domains within both the junctions between leading cells undergoing collective migration, as well as newly formed and expanding junctions.

Discussion

Here, we show that rga-7 gene codes for a RhoGAP with a GAP activity that is specific to CDC-42 and RHO-1/RhoA. We also propose that RGA-7 controls the spatial distribution of active CDC-42 at the junctions between leading cells during their collective migration towards the ventral midline and at the junctions forming and expanding between contralateral leading cells.

In this function, RGA-7 is required in hypodermal cells, in parallel with the RHO-1’s effector LET-502/ROCK. More specifically, RGA-7 controls the formation of actin-rich protrusions at the front of migrating leading cells in a manner that is antagonistic with the CDC-42’s effector WSP-1/N-WASP and the CDC-42-interacting proteins TOCA-1/2/Toca1/FBP17. It also functions in parallel with a pathway that is only functional in the absence of the combination of RGA-7 and WSP-1 or RGA-7 and TOCA-1/2 (Figure 8A). RGA-7, WSP-1, and TOCA-1/2 modulate the accumulation of F-actin at the junctions between adjacent leading cells during their migration—an accumulation inefficiently promoted by the parallel pathway identified above (Figure 8A). Importantly, our study reveals that RGA-7 is recruited to proximal junctions between adjacent leading cells where it promotes the accumulation of active CDC-42. It also inhibits the accumulation of activated CDC-42 at distal junctions located closer to the leading edge of migrating cells (Figure 8A).

The characterization of the functional roles of RGA-7, WSP-1, and TOCA-1/2 during the formation of new junctions between contralateral leading cells reveals that: (i) WSP-1 promotes the expansion of these junctions at an early stage of the junction formation independently of TOCA-1/2 or RGA-7 (<8 min after collision; Figure 8B); (ii) when junctional proteins start to accumulate at the junctions (≥8 min after collision; Figure 8B), the functions of RGA-7, WSP-1, and TOCA-1/2 tend to reduce the rate of expansion of the junctions in an antagonistic manner and in parallel with a pathway that remains to be identified. At this specific stage, RGA-7 promotes the accumulation of active CDC-42 clusters at the junctions and negatively regulates their spreading during the expansion process.

RGA-7 was recently shown to regulate embryo expulsion from spermatheca through the regulation of RHO-1/RhoA activity (Tan and Zaidel-Bar, 2015). This function is supported by the alleviating/epistatic interaction observed between rga-7(ok1498) and let-502 hypomorphic allele in this system (Tan and Zaidel-Bar, 2015). We showed that rga-7 controls ventral enclosure synergistically with let-502 and antagonistically with wsp-1 and toca-1/2, suggesting that RGA-7 regulates the GTPase activity of CDC-42 in the hypodermis during ventral enclosure. This hypothesis is also supported by the observation that overexpression of CDC-42 but not RHO-1 displays similar migratory defects of leading cells as rga-7 loss-of-function.

Most RhoGAPs present GAP specificity towards several GTPases (Jenna and Lamarche-Vane, 2003). They usually display a more stringent specificity in vivo than in vitro (Jenna and Lamarche-vane, 2003), demonstrating a clear preference for a particular GTPase. However, few RhoGAPs display a specific GAP activity towards several GTPases in vivo. For instance, Rga6 controls cell polarity and morphogenesis of Schizosaccharomyces pombe through regulation of Cdc42 GTPase activity and cell integrity and cell separation through regulation of Rho2 (Cansado et al., 2010). Recent data, including ours, suggest that RGA-7 regulates the GTPase activity of RHO-1 in spermatheca during embryos expulsion to the uterus (Tan and Zaidel-Bar, 2015) and CDC-42 during ventral enclosure. Interestingly, RGA-7 exhibits almost 10-times more GAP activity towards CDC-42 than RHO-1 in vitro, and is more expressed in the spermatheca of adults than in the hypodermis of embryos during ventral enclosure (Supplementary Figure S2D). This suggests that RGA-7 GAP specificity may be, at least partially, regulated by its expression level. While genetic interaction with let-502 in the two systems suggests that rga-7 may exhibit GAP specificity predominantly towards either RHO-1 or CDC-42, the possibility that RGA-7 may regulate the GTPase activity of both CDC-42 and RHO-1 in both systems in a spatially restricted manner cannot be excluded.

As detailed in the introduction, restriction of the Cdc42 activation zone depends on exocytosis, endocytosis, feed-forward loops promoting Cdc42 accumulation and activation, as well as recruitment of RhoGAPs to limit the spatial expansion of activated Cdc42 clusters in yeast (Park and Bi, 2007; Harris and Tepass, 2010). Interestingly, Cdc42 together with the F-BAR protein TOCA1/TOCA-1 and N-WASP/WSP-1 were shown to control actin-dependent endocytosis of junctional proteins in several systems including C. elegans (Giuliani et al., 2009). N-WASP was also shown to participate in feed-forward mechanisms promoting Cdc42 activation during endocytosis (Hussain et al., 2001; Humphries et al., 2014).

The efficient cycling of Cdc42 between its active (GTP-bound) and inactive (GDP-bond) forms is essential for Cdc42 biological function across multiple systems (Fidyk et al., 2006). This is supported by experiments with mutants that either block Cdc42 activation (guanine-nucleotide exchange activity) or inactivate it (GTP hydrolysis activity) but still display similar phenotype(s) (Fidyk et al., 2006). An appealing hypothesis that explains RGA-7 antagonism with both WSP-1 and TOCA-1/2 during ventral enclosure is that the Cdc42-specific GAP activity of RGA-7 may antagonize WSP-1 and TOCA-1/2 function during endocytosis at cell junctions as well as WSP-1 potential feed-forward function on Cdc42 activation through GEFs. This later hypothesis is strongly supported by the observation that rga-7; wsp-1 embryos complete ventral enclosure significantly more efficiently than single mutants but not quite as efficiently as wildtype embryos. Inhibition of Cdc42-dependent endocytosis at proximal and expanding cell junctions by RGA-7 may lead to the accumulation of Cdc42 at the junctions and explain the observed reduction of the accumulation of active Cdc42 at these junctions in rga-7 mutants.

Studies using drugs disrupting actin structures in budding yeast revealed that once Cdc42 clusters at the bud site is established, exocytic delivery of Cdc42 is required to counteract the dispersal of Cdc42 clusters at the plasma membrane mediated by actin-dependent endocytosis (Park and Bi, 2007). Inhibition of endocytosis by RGA-7 may then reduce the dispersal of
active CDC-42 clusters within junctions. This hypothesis also accounts for the increased spreading of these clusters from proximal to distal junctions between adjacent leading cells and within expanding junctions between contralateral cells in rga-7(ok1498) mutants.

Given the RGA-7::GFP accumulation at punctate and tubular endomembranes and its recruitment to proximal junctions, we cannot exclude that RGA-7 may regulate exocytic and/or endocytic recycling processes. Negative regulation of both CDC-42-dependent exocytosis and endocytosis by RGA-7 is also an attractive hypothesis that may explain the accumulation of activated CDC-42 clusters at distal junctions observed in rga-7(ok1498) embryos. Dissection of mechanisms that regulate CDC-42-dependent exocytosis and endocytosis/endocytic recycling at the junctions between hypodermal cells during ventral enclosure will be required to confirm these hypotheses.

During cell migration, coordination of Rho GTPases involves spatial segregation of active RhoA and Cdc42 as shown in migrating fibroblasts (Machacek et al., 2009) and during wound healing in Xenopus oocytes (Benink and Bement, 2005). This distribution of active RhoA at the distal/front of leading cells of collectively migrating cell groups and of Cdc42 at a proximal/back location compared with RhoA may be evolutionary conserved (Benink and Bement, 2005; Reffay et al., 2014). In this context, the function of RGA-7s in spatially controlling active CDC-42 accumulation at proximal junctions may contribute to the spatial segregation of active RHO-1/RhoA and CDC-42/Cdc42 at cell junctions between leading cells and to their ability to drive collective migration towards the ventral midline.

In conclusion, we show that the CDC-42 and RHO-1-specific GAP, RGA-7, controls the accumulation and distribution of the active CDC-42 at restricted sites of the junctions between leading cells migrating collectively and during the expansion of new junctions forming between contralateral cells. We show that RGA-7 functions antagonistically with WSP-1/N-WASP and TOCA-1/2, two proteins controlling actin- and CDC-42-dependent endocytosis. Our data suggest that spatial restriction of CDC-42 active zone by RGA-7 is critical for collective migration of hypodermal cells and important in regulating the dynamics of cell–cell junction formation and expansion.

**Materials and methods**

**Strains and culture methods**

Nematodes were maintained under standard conditions at 20°C (Brenner, 1974). Worm strains carrying the following mutations

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**Figure 8** RGA-7 spatially controls active CDC-42 at cell—cell junctions during collective migration and expansion of newly formed junctions. (A) During early phase of ventral enclosure, RGA-7 is recruited to the proximal junctions between adjacent leading cells. It promotes the accumulation of active CDC-42 clusters at proximal junctions and inhibits their spreading towards the distal junctions. RGA-7 controls the formation of actin-rich protrusions at the leading edge of leading cells in an antagonistic manner with WSP-1 and TOCA-1/2 and in parallel of a pathway that remains to be identified. The function of RGA-7, WSP-1, and TOCA-1/2 is essential for the accumulation of F-actin at the distal junctions between adjacent leading cells. (B) Once leading cells meet at the ventral midline, the junctions between contralateral cells accumulate F-actin, and the expansion is promoted by WSP-1. Eight minutes after collision, junctional proteins start to accumulate at the expanding junctions, and RGA-7, WSP-1, and TOCA-1/2 tend to reduce the expansion rate of these junctions. At that specific stage, RGA-7 promotes the accumulation of activated CDC-42 at the expanding junctions and inhibits the dispersion of the clusters they form.
and markers were obtained from the Caenorhabditis Genetic Center (CGC): rga-7(ok1498) II, wsp-1(gm324) IV, mcls50 [lin-26::VAB-10(ABD)::mCherry + myo-2::GFP], mcls50 [lin-26::VAB-10(ABD)::GFP + myo-2::GFP], and jcs1[ajm-1::GFP, pRF4[rol-6 (su1006)]. The strain carrying let-502(sb118ts) I was kindly provided by Dr Paul Mains (University of Calgary, Calgary, Canada). Mutant strains were backcrossed at least 4 times against wild-type (wt) animals prior to analysis.

**Generation of transgenic animals**

All transgenic animals were generated through biolistic bombardment of unc-119(ed3) animals, using a PDS-1000/He system with the Hepta adaptor (Bio-Rad) as previously reported (Berezikov et al., 2004). At least three independent lines were isolated and characterized per construct. sajIs22[rga-7::rga-7::GFP; unc-119] was generated through recombination of pDONR4P1R-rga-7p, containing 5 kb of the genomic sequence upstream of RGA-7 initiation codon, with pDONR201-rga-7 and pMB14. pDONR201-rga-7 contains genomic sequence from RGA-7 initiation codon in exon 1 up to exon 8 fused to CDNA sequence up to the stop codon of the gene located at exon 20. Similarly, sjIs32[lin-26::rga-7::GFP; unc-119] was generated through the recombination of pDONR4P1R-lin-26p containing 5 kb of the lin-26 promoter (Martin et al., 2014), pDONR201-rga-7, and pMB14. pDONR201-lin-26 contains genomic sequence from RGA-7 initiation codon in exon 1 up to exon 8 fused to CDNA sequence up to the stop codon of the gene located at exon 20. Similarly, sjIs32[lin-26::GFP::rho-1::3'UTR; unc-119] and sjIs30[lin-26::CFP::rho-1::3'UTR; unc-119] were obtained through recombination of pDONR4P1R-lin-26p, pDONR201-CFP-rho-1 or pDONR201-CFP-cdc-42, pCM5.37 containing the unc-54 3'UTR (Addgene), and pCG1505 destination vector (Addgene). wsp-1 CRIB domain (cDNA coding for 78 amino-acids from position 230 to 308 of WSP-1) was cloned in pDONR201 and recombined together with pDONR4P1R-lin-26p in PULSRG1 (containing attB4-ccdB-attB2 followed by mCherry-pie-1 3' UTR; kindly provided by Dr Ian Hope, Univ. Leeds, UK). The resulting vector was used to generate sjIs31[lin-26::wsp-1(CRIB)::mCherry; unc-119] transgenic animals. See Supplementary Methods for details.

Isolation of strains carrying several mutant alleles and transgenes

rga-7(ok1498) II; wsp-1(gm324) IV was generated by crossing wsp-1(gm324) IV hermaphrodites with rga-7(ok1498) II males. Mutant animals expressing lin-26::VAB-10(ABD)::GFP were obtained by crossing mutant hermaphrodites with mcls50 [lin-26::VAB-10(ABD)::GFP + myo-2::GFP] males. Double-mutant homozygotes and animals carrying two copies of transgenes were isolated from the F2 progeny as animals segregating only fluorescent progeny and mutant alleles in genomic DNA were identified using PCR. let-502(sb118ts) I was maintained at 20°C, and rga-7(ok1498) II; let-502(sb118ts) I was generated by crossing let-502(sb118ts) I hermaphrodites with rga-7(ok1498) II males. let-502(sb118ts) I homozygotes were identified through scoring of embryonic lethality (Emb) and larval arrest (Lva) phenotypes at 18°C and 25.5°C and identification of thermosensitive behaviour of isolated populations. pl4440 constructs containing toca-1 and toca-2 sequences were retrieved from the genome-wide RNA interference (RNAi) library (Kamath et al., 2003) and confirmed by sequencing. RNAi treatment was done using feeding protocol as detailed in Supplementary Methods.

**Phenotyping mutant animals and 4-dimensional microscopy**

Emb phenotype was scored after isolation of 10–12 worms on NGM agar with OP50 as a source of food. Worms were allowed to lay eggs at 20°C for 5–6 h and were removed from the plate, and laid embryos were counted. After 24 h, dead embryos were counted to assess Emb. The stage of embryonic arrest was confirmed in mutant animals using time-lapse DIC microscopy as previously described (Martin et al., 2014). Embryos dissected from adult hermaphrodites were mounted on 3% agarose pads in M9 buffer, and coverslips were sealed with drawing gum (Pebéo). Elongation was recorded using 4-dimensional microscopy (3D and time), which recorded a Z-stack every 2 min during 10 h at room temperature or 25.5°C using a Leica DMR5000 microscope equipped with a 63× oil immersion objective upon differential interference contrast illumination (DIC). Images were captured using Leica LAS AF imaging software. These recordings were used to assess morphological defects during embryonic development. Slow ventral enclosure was identified for embryos completing their ventral enclosure in >1 h from the time leading cells could be observed migrating at the embryo periphery.

**In vitro GAP activity assay**

To assess the specificity of RGA-7 GAP domain as a recombinant protein in bacteria using pTRChis gateway converted vector as previously reported (Jenna et al., 2005). We purified the recombinant protein and measured its ability to catalyse the hydrolysis of GTP by the six recombinant Rho GTPases identified in the C. elegans genome, the Racs CED-10 and RAC-2, the RhôG MIG-2, the Cdc42 CDC-42, the RhoA RHO-1, and the atypical Rho GTPase CRP-1, which were expressed as recombinant Glutathione S Transferase (GST)-fusion proteins. To do so, we used a filtration assay as previously described (Jenna et al., 2005). We measured the amount of GTP–γS remaining bound to the GTPase after 5 min incubation at 25°C in the absence (control condition) or presence of 10 or 100 nM of GAP and computed the ratio of GAP–remaining bound on the GTPase over the control condition at each concentration of GAP.

**Confocal fluorescence microscopy**

The expression pattern of RGA-7::GFP and its subcellular localization in living animals was observed using a Nikon A1R confocal microscope using 100× oil CFI NA 1.45 Plan Apochromat λ objective. All images were captured using NIS-Elements software (Nikon) with a pinhole size of 85.6 μm, a calibration of 0.10 μm/pixel (radial resolution of 0.20 μm), and a Z-step of 0.200 μm. Deconvolution was done using Autoquant3X, 3D deconvolution software. Orthogonal views were generated using ImageJ software. Dynamic time-lapses were respectively done on sajIs29- and sajIs30-carrying embryos by capturing 19–21 Z-planes of 0.400 μm each every 2 min during 40 min. Acquisitions for ruffle ratios were obtained on embryos of other transgenic animals described in the Results by capturing 21 Z-planes of 0.400 μm each every 62 sec (no delay) during 1 h. Acquisition on unc-119(ed3);sajIs31[lin-26::wsp-1(CRIB)::...
mCherry];mcs50[lin-26p::VAB-10(ABD)::GFP + myo-2p::GFP] and rga+7(ok1498);unc-119(ed3); sajs31[lin-26p::wsp-1(CRIB)::mCherry];
mcs50[lin-26p::VAB-10(ABD)::GFP + myo-2p::GFP] were done using a swept-field confocal microscope with a 100× oil CFI NA 1.45 Plan Apochromat λ objective. Image and statistical analysis were done as detailed in Supplementary Methods.

Supplementary material
Supplementary material is available at Journal of Molecular Cell Biology online.

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