The EMBO Journal (2007) 26, 5083–5092 | © 2007 European Molecular Biology Organization | All Rights Reserved 0261-4189/07
www.embojournal.org

THE EMBO JOURNAL

RAP-1 and the RAL-1/exocyst pathway coordinate hypodermal cell organization in *Caenorhabditis elegans*

Ester W Frische[1], Wendy Pellis-van Berkel[1], Gijs van Haaften[2], Edwin Cuppen[2], Ronald HA Plasterk[2], Marcel Tijsterman[2], Johannes L Bos[1] and Fried JT Zwartkruis[1,*]

[1]Department of Physiological Chemistry, Centre for Biomedical Genetics, UMC Utrecht, Utrecht, The Netherlands and [2]Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands

The small Ras-like GTPase Rap1 has been identified as a regulator of integrin activation and cadherin-mediated cell–cell contacts. Surprisingly, null mutants of RAP-1 in *Caenorhabditis elegans* are viable and fertile. In a synthetic lethal RNAi screen with *C. elegans rap-1* mutants, the Ras-like GTPase *ral-1* emerged as one of seven genes specifically required for viability. Depletion of *exoc-8* and *sec-5*, encoding two putative RAL-1 effectors and members of the exocyst complex, also caused lethality of *rap-1* mutants, but did not affect wild-type worms. The RAP-1 and the RAL-1/exocyst pathway appear to coordinate hypodermal cell movement and elongation during embryonic development. They mediate their effect in part through targeting the α-catenin homologue HMP-1 to the lateral membrane. Genetic interactions show that the RAP-1 and RAL-1/exocyst pathway also act in parallel during larval stages. Together these data provide *in vivo* evidence for the exocyst complex as a downstream RAL-1 effector in cell migration.

The EMBO Journal (2007) **26,** 5083–5092. doi:10.1038/sj.emboj.7601922; Published online 8 November 2007
Subject Categories: cell & tissue architecture; development
Keywords: adherens junction; embryogenesis; Ral; Rap1; synthetic lethality

Introduction

Cell migration is an important aspect of various biological processes like development and immune responses. Several members of the Ras-like family of GTPases are involved in the control of cell migration. For example, Rap1 affects migration by enhancing integrin-mediated cell–matrix attachment in many different cell types *in vitro* (Caron *et al*, 2000; Katagiri *et al*, 2000). In addition, Rap1 may inhibit migration of cells by stimulating the formation of cell–cell contacts via E-cadherin in adherens junctions (Hogan *et al*, 2004; Price *et al*, 2004). Also VE-cadherin, present in endothelial cells, is under the control of Rap1 (Fukuhara *et al*, 2006). The current view is that Rap1 acts locally via different effectors to mediate its effect on integrins and cadherins. For example, the Rap1-binding proteins RIAM, RAPL, TIAM and VAV have all been invoked as Rap1 effectors functioning in enhancement of cell–matrix contacts, whereas AF-6 and Cdc42 are prime candidates for regulators of cadherin in adherens junctions (for a review, see Bos, 2005; Kooistra *et al*, 2007)). However, Rap1 can induce cell polarity in suspension cells (Shimonaka *et al*, 2003) and this polarizing activity of Rap1 may also contribute to the effects seen on integrins and cadherins. A second Ras-like GTPase involved in cell migration is Ral (Suzuki *et al*, 2000; Rosse *et al*, 2006). In its active, GTP-bound form, Ral binds to various effectors that play specific roles in migration. For example, Ral can induce filopodia formation by interacting with the actin-bundling protein filamin (Ohta *et al*, 1999). Filopodia are often found at the leading edge of migrating cells and outgrowing neurites. Furthermore, active Ral can bind to Sec5 (Brymora *et al*, 2001) and Exo84 (Moskalenko *et al*, 2003; Zhang *et al*, 2005), two proteins present in the exocyst complex that mediates targeting of E-cadherin to the basolateral plasma membrane. Binding of Ral to Sec5 and Exo84, which are most likely present in distinct subcomplexes of the exocyst, is mutually exclusive. Thus, Ral may enhance the formation of a functional exocyst complex at a specific subcellular site by bringing together different subcomplexes of the exocyst, containing either Sec5 or Exo84 (Jin *et al*, 2005). The exocyst complex was originally discovered in yeast as a protein complex required for polarized secretion at the tip of newly formed buds (TerBush *et al*, 1996). In vertebrate cells, the exocyst complex mediates polarized delivery of membranes from the Golgi apparatus and recycling endosomes to the basolateral membrane, but not to the apical membrane (Lipschutz and Mostov, 2002; Prigent *et al*, 2003; Yeaman *et al*, 2004). Among proteins targeted to the basolateral membranes by the exocyst complex are the LDL receptor and E-cadherin (Grindstaff *et al*, 1998; Langevin *et al*, 2005; Shipitsin and Feig, 2004). Targeting of E-cadherin to the basolateral membrane is of special importance in epithelial cells that are about to establish cell–cell contacts with other epithelial cells. Once E-cadherin is stabilized at the membrane by homotypic interactions with E-cadherin on neighboring cells, it may help in targeting more exocyst to the lateral membrane, thereby enhancing the formation of cell–cell contacts and elaborating cell polarity.

In vivo, the requirement for Ras-like GTPases in cell migration is clearly illustrated by the mutant phenotype of Rap1 mutant flies. Embryos, devoid of Rap1 protein, display defects in ventral furrow closure, head involution and

*Corresponding author. Department of Physiological Chemistry, Centre for Biomedical Genetics, UMC Utrecht, Universiteitsweg 100, Utrecht 3584 CG, The Netherlands. Tel.: +31 30 2533992; Fax +31 30 2539035; E-mail: G.J.T.zwartkruis@umcutrecht.nl

Received: 27 April 2007; accepted: 19 October 2007; published online: 8 November 2007

migration of mesodermal and presumptive primordial germ cells (Asha *et al*, 1999). Thus, both morphogenetic events of complete cell layers as well as individual cell migration are disturbed. Also the migration of a group of specialized follicle cells, called border cells, is under the control of Ras-like GTPases in *Drosophila*. Migration of border cells represents a cadherin-dependent mode of cell movement and requires signaling via Ras and via Ral (Lee *et al*, 1996). It is presently unclear which Ral effector is required in this process.

A major migration event in *Caenorhabditis elegans* is that of hypodermal cells during embryonic development. Hypodermal cells are the epidermal cells in *C. elegans*. They arise as a series of six rows at the dorsal side of the embryo. The two dorsal-most arrays of cells undergo a process called dorsal intercalation, which organizes them into a single dorsal row. After this process has begun, the ventral-most cells on each side start to migrate ventrally to envelope the embryo. In this process of ventral enclosure, the two most anteriorly located cells, named leading cells, are crucial and the first to establish cell–cell contacts with their contralateral neighbors (Williams-Masson *et al*, 1998; for a review Simske and Hardin, 2001). In between the dorsal and ventral cells, a row of lateral epidermal cells, called seam cells, is present. After ventral enclosure is completed, the ovoid embryo elongates about fourfold. This process relies on circumferentially oriented actin bundles that are linked at the apical site of adherens junctions in hypodermal cells. In embryos mutant for the α-catenin homologue HMP-1 or the β-catenin homologue HMP-2, the circumferential actin bundles in dorsal hypodermal cells detach from adherens junctions, which probably explains the elongation phenotype seen in these animals (Costa *et al*, 1998).

So far, relatively few gene products have been identified, which play a crucial role in ventral migration of hypodermal cells. Among them are HMP-1, HMP-2 and the cadherin HMR-1, which are physically interacting in the cadherin–catenin complex (CCC). In HMR-1 homozygous embryos, or embryos devoid of maternal HMP-1 or -2 protein, the leading cells do not migrate completely to the ventral side (Costa *et al*, 1998). Other proteins involved are the APC-related protein APR-1 that may also be present in the CCC, the inositol 1,4,5-triphosphate receptor ITR-1 and ephrin receptors and ligands (George *et al*, 1998; Hoier *et al*, 2000; Thomas-Virnig *et al*, 2004). These latter proteins are required in the neuroblast cells, over which the hypodermal cells migrate. Also the cytoskeletal regulatory WAVE/WASP proteins are required for normal hypodermal cell migration (Withee *et al*, 2004). The interconnectivity of these different genes has not been established. Remarkably, no role for any of the *C. elegans* integrins has been established in hypodermal cell migration (Cox and Hardin, 2004).

In the present study, we have performed a synthetic lethal RNAi screen with a *C. elegans rap-1* mutant, which revealed that *rap-1* mutants are highly sensitive to diminished signaling via the RAL-1/exocyst pathway. During embryogenesis, the Ras-like GTPases RAP-1 and RAL-1 act in concert to orchestrate hypodermal cell migration and sorting. Interfering in the Ral-1/exocyst pathway in a *rap-1* mutant background leads to loss of the CCC at adherens junctions. Interestingly, the observed phenotype is more severe than that of CCC mutants alone, indicating additional roles of the Ral-1/exocyst pathway. Furthermore, our screen has identified various other genes that may be involved in the RAL-1/exocyst pathway or otherwise are required for viability of *rap-1* mutants.

Results

RAP-1 and RAP-2 are not required during C. elegans embryonic development

In addition to our previously described *rap-1(pk2082)* allele, a second *rap-1* allele (*tm861*) has been isolated, in which a 549 bp deletion removes exon 3 and part of exon 4, leaving only the first 42 amino acids of RAP-1 intact (Figure 1). Both homozygous mutant strains are viable and fertile, although progression through larval stages is delayed (Pellis-van Berkel *et al*, 2005; data not shown). Like most *rap-1*(*pk2082*);*rap-2(gk11)* animals, the majority of *rap-1*(*tm861*);*rap-2(gk11)* animals die during late larval stages. The small fraction of double homozygous adults, derived from either *rap-1*(*pk2082*/+); *rap-2(gk11)* or *rap-1*(*tm861*/+);*rap-2(gk11)* animals, is egg-laying defective. Surprisingly, inside such animals viable offspring is present. We, therefore, isolated embryos from N2 or *rap-1(tm861);rap-2(gk11)* animals and determined the percentage of non-hatched embryos. Although the percentage of non-hatched *rap-1(tm861);rap-2(gk11)* embryos was higher (19%, $n=340$) as compared with wild-type embryos (3%, $n=365$), the majority of double mutants hatched normally. Of the embryos that did not hatch, the majority (73%) had elongated more than twofold. This showed that whereas RAP-1 or RAP-2 is required for normal viability in late larval stages, there is no strict requirement for RAP-1 and RAP-2 during embryogenesis.

Synthetic lethal screen with rap-1 mutants

The normal development of *rap-1* mutants in *C. elegans* contrasts the situation in *Drosophila* and this suggests that other signaling pathways functionally compensate for the

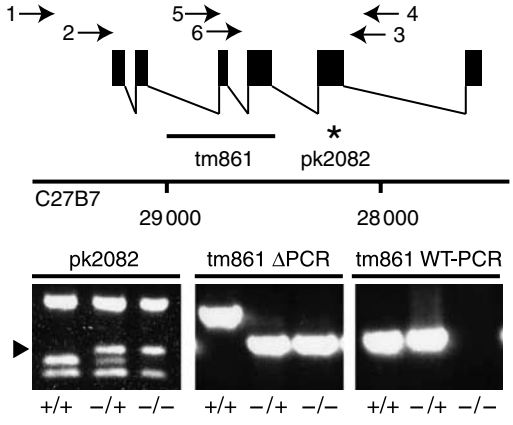

Figure 1 Schematic representation of the genomic organization of the *rap-1* locus and detection of mutant alleles. Filled boxes indicate exons and the line underneath the graphic representation indicates the position of the deletion in *rap-1*(*tm861*) animals and the star indicates the amber codon (amino acid 130) in the *pk2082* allele. Numbers indicate the position of the *rap-1* locus on the C27B7 cosmid. Below are PCR reactions shown, performed on single-worm lysates that demonstrate the presence of the wild-type allele in +/+ and +/− worms and the absence of the wild-type allele in −/− worms. An arrowhead indicates a 170 bp *Sau*3A product that is characteristic for the *pk2082* allele. Arrows indicate the position of the PCR primers used for detection of the deletion and wild-type alleles.

rap-1 pathway. To investigate this option, a genome-wide synthetic lethal RNAi screen was performed using the Ahringer library (Kamath *et al*, 2003; van Haaften *et al*, 2004). Apart from wild type and *rap-1*(*pk2082*) mutants, *rap-2*(*gk11*) and *epac-1*(*pk1313*) mutants were included in this screen. *epac-1*(*pk1313*) mutants carry a deletion in the homologue of the cAMP-dependent Rap-specific guanine nucleotide exchange factor (GEF) Epac (T20G5.5; unpublished results) (de Rooij *et al*, 1998). Foods that caused specific lethality with one of the mutant strains, were re-screened using the same mutants in the case of *rap-2* and *epac-1* or with both *rap-1*(*pk2082*) and *rap-1*(*tm861*) (see Supplementary data). In this rescreen, no genes emerged that caused clear and reproducible synthetic lethality with *rap-2* or *epac-1*. In contrast, seven genes caused specific synthetic lethality of *rap-1* mutants (Table I). These include the MKP7 homologue *vhp-1* (F08B1.1)(Mizuno *et al*, 2004) and a gene with unknown function C01B7.1. Interestingly, *ral-1* and *exoc*-8 were found that encode homologues of vertebrate exocyst complex members. Finally, the genes encoding the phosphatase *sur-6* (Sieburth *et al*, 1999), the vesicle-sorting protein *phi-24* (Howard *et al*, 2001) and *him-3*, known to be involved in meiosis, gave synthetic lethality, but with variable results for *rap-1(tm861)*. To exclude the possibility that an additional mutation present in both *rap-1* strains was responsible for the observed synthetic lethality, two independent *rap-1(tm861)* strains, with *rap-1* under control of the general *let-858* promoter, were generated. Although the level of RAP-1 was too low for detection on blot, we observed a partial rescue of the phenotype when these worms were subjected to *exoc-8(RNAi)* food as L1-stage animals (Table II).

We focused on RAL-1 and the exocyst member EXOC-8, as their vertebrate homologues RalA and Exo84 have previously been reported to directly interact: in its active GTP-bound form, RalA binds to Exo84. Therefore, we retested the effects of RNAi of other exocyst complex members on the viability of both *rap-1* mutants (Supplementary Table I). No or only very limited effects were scored for *sec-6*, *sec-8*, *sec-15*, *sec-3* and *exoc-7*. Although not found to be synthetic lethal in the screen, quantification showed less progeny on *sec-10(RNAi)*. Finally, also *sec-5(RNAi)* resulted in specific synthetic lethality with both *rap-1* alleles.

In vertebrates, Sec5 is identified as a member of the exocyst complex and also as a direct downstream effector of Ral. Other Ral effectors include RalBP, involved in endo- and exocytosis, and the actin-bundling protein filamin. For each of these effectors, a single homologue was found in the *C. elegans* genome (*rlbp-1* (T23G11.5), *flna-1* (C23F12.1)). In addition, a single homologue for the family of RALGEFs is found, RGL-1 (F28B4.2). We tested the effect of RNAi feeding of L1 larvae on plate for each of these genes. *ral-1* and *sec-5(RNAi)* prevented *rap-1*(*pk2082*) from producing viable offspring, whereas *rlbp-1(RNAi)* had no effect. *flna-1(RNAi)* resulted in a high number of sick animals, but this was also seen with wild-type animals (Supplementary Figure 1). Together, these results suggest that full functionality of the *ral-1/sec-5/exoc-8* pathway is required in *rap-1* mutants, but not in wild-type worms. Further evidence comes from the observation that also *rgl-1(RNAi)* reduces the number

Table II *rap-1(tm861); dpy-20* animals carrying transgenic *rap-1* (rescue 1 and 2) are less sensitive to *exoc-8(RNAi)* than *rap-1(tm861); dpy*-20 animals

	Control RNAi	*exoc-8 (RNAi)-1*	Control RNAi (%)
Wild type	62	70	113
rap-1(tm861); dpy-20	57	10	17
Rescue 1	61	36	59
Rescue 2	65	86	132

Numbers in the first and second column are absolute numbers of viable progeny after a 16-h egg lay. Numbers are the mean value of progeny of hermaphrodites ($n = 15$ for rescue strain 1 and 2, $n = 10$ for wild-type and *rap-1(tm861); dpy-20*). The third column shows the percentage of the amount of progeny on *exoc-8(RNAi)* in comparison with animals subjected to control RNAi.

Table I Overview of genes found in the synthetic lethal screen with *rap-1(pk2082)*

Localization	Gene	Mammalian homologue	N2	*rap-1 (pk2082)*	*rap-2 (gk11)*	*epac-1 (pk1313)*
90G7	*ral-1*	RalA, RalB	21 (549)	0 (0)	36 (702)	19 (344)
	Y53G8AR.3		45 (1068)	0 (0)	23 (408)	36 (640)
25G8	*exoc-8*	EXOC8	38 (984)	2 (26)	55 (1087)	21 (377)
	Y105E8B.2		55 (1314)	14 (189)	69 (1204)	97 (1721)
2C2	*phi-24*	CHMP1B	146 (2198)	22 (433)	93 (2300)	139 (2699)
	F23C8.6		88 (1038)	30 (256)	83 (1141)	50 (813)
			26 (826)	9 (158)	68 (1200)	—
16E8	*sur-6*	PR55/B	59 (892)	4 (75)	44 (1101)	47 (912)
	F26E4.1		47 (550)	0 (0)	51 (696)	53 (614)
			47 (1533)	1 (13)	55 (973)	46 (740)
44D3	*vhp-1*	MKP7	91 (2165)	0 (0)	38 (935)	163 (2350)
	F08B1.1		166 (3150)	8 (72)	24 (327)	95 (2282)
101G7	*him-3*	HORMAD1	54 (819)	14 (276)	58 (1424)	51 (984)
	ZK381.1		108 (1259)	35 (296)	68 (1203)	126 (1472)
			107 (3479)	7 (121)	—	55 (884)
104B8		ARP10	137 (2068)	27 (529)	79 (1934)	109 (2129)
	C49H3.8		74 (2393)	80 (681)	100 (1378)	123 (1437)
			—	35 (583)	71 (1257)	111 (1785)
146B2		Zn-finger	117 (1760)	9 (168)	62 (1545)	99 (1928)
	C01B7.1	Protein	102 (1208)	7 (64)	96 (1319)	90 (1054)
			43 (1373)	—	17 (299)	20 (330)

Numbers represent the percentage of progeny relative to those found on control RNAi for each strain as determined in rescreening experiments. Numbers in brackets are total numbers of worms per well at day 7. Successive lines represent independent experiments.

of *rap-1* offspring, whereas no effect was seen on wild-type worms (Supplementary Figure 1A; data not shown). A drawback of RNAi studies is that the efficacy of RNAi can be variable. However, when the same set of RNAi constructs was tested in a more RNAi-sensitive background using the *rrf*-3 strain, similar results were observed (Supplementary Figure 1B, C). Strikingly, a further reduction was seen in the number of *rap-1;rrf-3* mutants on *rgl-1(RNAi)*.

Comparative analysis of ral-1/sec-5/exoc-8(RNAi) in rap-1 mutants

To obtain more definitive proof that *ral-1*, *sec-5* and *exoc-8* operate in the same pathway in *C. elegans*, we compared the phenotypes of *rap-1* mutants after RNAi feeding on plate. When *rap-1* L1 larvae are subjected to RNAi for any of these genes, most animals make it to adulthood but are sluggish and largely sterile. In contrast, when L4 larvae were used at the start of RNAi, the resulting adults appeared healthy. Initially, they produce viable offspring but then start to shed many embryos that do not hatch. Inspection of these embryos showed a phenotypic series that can be roughly classified into two types (Figure 2). In class I embryos, the hypodermis still covers the entire embryo, but little morphogenesis is seen following the comma stage. Class II embryos appeared more disorganized and hypodermal cells are clustered and do not envelope the embryo. Hypodermal cell junctions have been characterized in much detail and consist of a single electron-dense junction, in which an apical and basal domain can be discerned by immunofluorescence. The apical domain of the junction harbors the CCC, whereas the basal domain contains the membrane-associated protein DLG-1 and its binding partner AJM-1. We used DLG-1 and AJM-1 localization to visualize adherens junctions in the hypodermis and developing gut, which allows the simultaneous evaluation of the development of both tissues relative to each other (Segbert *et al*, 2004). The pattern and organization of hypodermal cells in *rap-1* mutant worms on control RNAi is identical to that of wild-type worms and the dorsal, seam and ventral cells form regular arrays of cells (Figure 2A; reviewed by Simske and Hardin, 2001). In contrast, many of the class I embryos obtained with *ral-1*, *sec-5* or *exoc-8(RNAi)* are characterized by a failure of cells to align in the stereotyped pattern of dorsal, seam and ventral cells (Figure 2B–D; Supplementary Figure 2). 3D-animation indicates that this results from defects in both dorsal intercalation and ventral migration (data not shown). In other class I embryos, migration appears to have stopped before reaching the ventral midline. Analysis of pharyngeal and intestinal cells of these embryos showed that these tissues are not affected (Figure 2F, G), although in rare cases abnormalities were seen (Figure 2H). Overall, the phenotypes of *ral-1*, *sec-5* or *exoc-8 (RNAi)* are very similar. In class II embryos, DLG-1 and

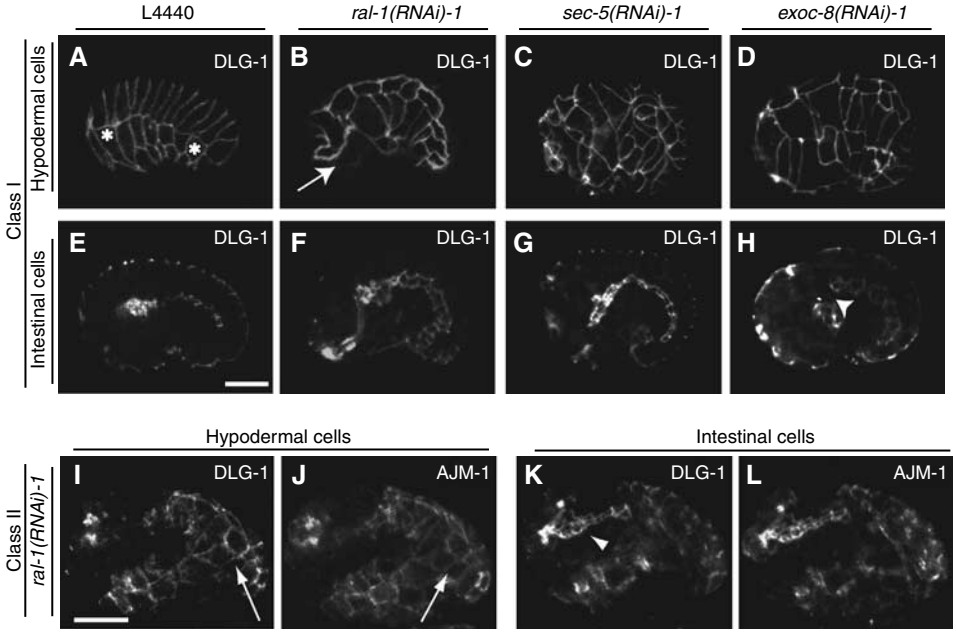

Figure 2 Phenotype of *rap-1* mutant embryos carrying the *dlg-1*::GFP gene subjected to control, *ral-1*, *sec-5* or *exoc-8(RNAi)*. Phenotypes of *rap-1* mutant embryos carrying the *dlg-1*::GFP marker (FZ271) that were derived from animals subjected to RNAi for *ral-1*, *sec-5* or *exoc-8*. DLG-1 indicates *dlg-1*::GFP expression, AJM-1 indicates immunofluorescence staining using the MH27 antibody. Genes used for RNAi are indicated above each panel. L4440 is the empty vector control RNAi. (**A**–**H**) show class I embryos, (**I**–**L**) class II embryos. (A–D) and (I–J) show focal planes to visualize hypodermal cells, (E–H) and (K–L) are focal planes at the level of the gut. The most anterior and posterior visible seam cells are marked with *. In all cases, AJM staining was performed (only shown for class II, subjected to *ral-1 (RNAi)*), which demonstrated clear colocalization with DLG-1::GFP). Class I embryos are characterized by halted migration of hypodermal cells as examplified by a *ral-1(RNAi)* embryo (arrow in B) or misalignment of hypodermal cells as shown here for a *sec-5* and *exoc-8(RNAi)* embryo (C, D). Halted migration can be discerned from early stages of migration by comparison of the developmental stage of the gut. Note that during normal hypodermal cell migration (A, E), the gut has not yet extended along the entire length of the embryos, in contrast to the gut of the *ral-1(RNAi)* embryo, which has established clear adherens junctions and shows a more mature pharynx (B, F). In some cases, a gap between intestinal and pharyngeal cells is observed (arrowhead H). In class II embryos, hypodermal cells do not envelope the entire embryo (arrow I, J) and a clearly recognizable gut structure is found on the outside of the embryo (arrowhead K). All embryos are oriented such that their pharynx is on the left side. Scale bar: 10 μm.

AJM-1 staining showed the presence of disorganized patches of hypodermal cells at various sites, but both proteins still colocalized (Figure 2I–L, *sec-5* and *exoc-8(RNAi)*; data not shown). The intestine of these embryos was frequently recognizable on the exterior, indicating that despite the severe morphogenetic defect, cell specification of this tissue had occurred normally (arrowhead Figure 2K). To study cell specification of hypodermal cells in class II embryos, we used the seam cell marker SCM::GFP, which is specifically expressed in 10 hypodermal seam cells on each lateral side from the 1.5-fold stage onwards (Koh and Rothman, 2001). GFP-positive cells were detected in the aberrantly localized hypodermal cell clusters following *ral-1*, *sec-5* or *exoc-8(RNAi)* feeding of *rap-1*(*tm861*) animals, showing that specification of at least a subset of hypodermal cell fates had occurred in these embryos (Figure 3, showing a single focal plane). However, the number of SCM::GFP-positive cells was reduced in some cases. Whether this is the consequence of altered cell division, defects in specification or loss to cell death is presently not clear. In conclusion, the phenotype of *rap-1* mutants on *ral-1*, *sec-5* and *exoc-8(RNAi)* is consistent with the fact that these latter three genes operate in a single pathway involved in hypodermal cell organization.

Localization of HMP-1

Certain aspects of the RNAi phenotypes like the arrested migration of hypodermal cells seemed consistent with a defect in CCC function. We, therefore, investigated whether HMP-1 was normally located at adherens junctions. HMP-1 colocalized with DLG-1 in wild type on control, *ral-1*, *sec-5* or *exoc-8 (RNAi)* and *rap-1* mutants on control RNAi. In contrast, in almost all *rap-1* mutant embryos on *ral-1*, *sec-5* or *exoc-8(RNAi)*, a diffuse distribution of HMP-1 was observed (Figure 4). So these gene products are required for targeting HMP-1 to adherens junctions or for stabilizing it there. This diffuse staining was clearly distinct from that of

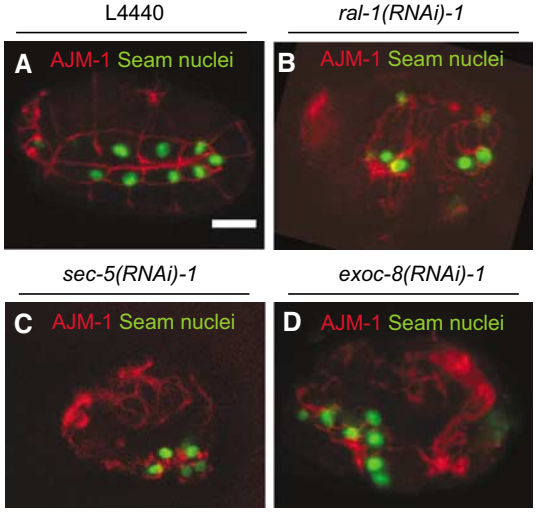

Figure 3 Phenotype of *rap-1* mutant embryos carrying the SCM::GFP gene subjected to control, *ral-1, sec-5* or *exoc-8(RNAi)* (**A–D** respectively). Expression of the seam cell marker SCM::GFP in nuclei of the disorganized hypodermis of class II embryos. Hypodermal cell borders are visualized using the AJM-1 antibody (red). Only one focal plane is shown per condition, seam nuclei are also present in other slices (data not shown). Scale bar: 10 μm.

EEA-1 (early endosome) and RAB-11 (recycling endosome) staining (Andrews and Ahringer, 2007; Poteryaev *et al*, 2007; Supplementary Figure 3). Genetic disruption of CCC members has been shown to result in elongation defects. We, therefore, performed a limited time lapse analysis of *rap-1* mutants on *ral-1(RNAi)*. Elongation in such embryos is almost completely blocked, whereas the development of the intestine and pharynx is not disturbed (Figure 5). Loss of HMR-1 or maternal and embryonic HMP-1 or -2 causes migration defects of hypodermal cells, but has not been reported to cause dorsal intercalation defects or to result in an abnormal alignment of dorsal, lateral or ventral cells (Costa *et al*, 1998). In this respect, the defects seen in *rap-1* mutants after *ral-1*, *sec-5* or *exoc-8(RNAi)* appear more severe. Possibly, deletion of *rap-1* enhances the phenotype of *hmr-1* mutants. Alternatively, the RAL-1/exocyst pathway targets more proteins than those of the CCC to the membrane, which are also involved in cell migration. To investigate this, we constructed *hmr-1;rap-1* double mutants. Double homozygous embryos, derived from *rap-1(tm861);hmr-1(zu389/+)* hermaphrodites did not differ from those derived from *hmr-1(zu389/+)* hermaphrodites (Supplementary Figure 4). Due to the fragility of *hmr-1* embryos, we were not able to stain sufficient numbers of embryos for adherens junction markers. As loss of maternal HMP-1 protein from *hmp-1* homozygous embryos strongly enhances their phenotype to resemble that of *hmr-1* embryos, we also investigated the effect of loss of RAP-1 in *hmp-1(zu278)* embryos. Again, double homozygous embryos could not be discerned from *hmp-1* mutants (Supplementary Figure 4). Together, these results indicate that the aberrant organization of hypodermal cells in *rap-1* embryos seen after *ral-1*, *exoc-8* or *sec-5(RNAi)* is not a simple compound phenotype of *rap-1* and members of the CCC.

Genetic interactions between the RAP-1 and RAL-1/exocyst pathway

To obtain additional proof for genetic interactions between the RAP-1 and RAL-1 pathways in *C. elegans*, we studied three independent mutants. *sec-5*(*pk2357*) and *sec-5*(*pk2358*) carry the same amber mutation at amino acid position 389 and were obtained with target selected mutagenesis (Cuppen *et al*, 2007; Figure 6). Animals homozygous for *sec-5*(*pk2357*) or *sec-5*(*pk2358*) become adult but produce only few embryos that elongate normally but never progress to the L2 stage. These *sec-5* mutations are hypomorphic based on the fact that when placed over a deficiency (*mnDf67*) most adults do not produce elongated embryos. The *sec-5*(*tm1443*) allele is most likely a null, as it has a 385 bp deletion removing the first two exons and part of the third (Figure 6). Indeed, *sec-5(tm1443)* mutants have a more severe phenotype: homozygotes never produce progeny and die as late L4-stage larvae.

When introduced into a *rap-1*(*tm861*) mutant background, the phenotype of all *sec-5* alleles was clearly enhanced, showing RAP-1 and SEC-5 act in different pathways. *sec-5(pk2357);rap-1(tm861)* double homozygotes arrested as late L3 early L4 larvae ($n = 23$, confirmed by PCR). Importantly, *sec-5(tm1443);rap-1(tm861)* die at the L2 (25%) or L3 (72%) stage. This phenotype is less severe than that of *rap-1(tm861)* animals on *sec-5(RNAi)*. This is most likely due to maternal input of SEC-5, as *sec-5(tm1443)* homozygous embryos shed

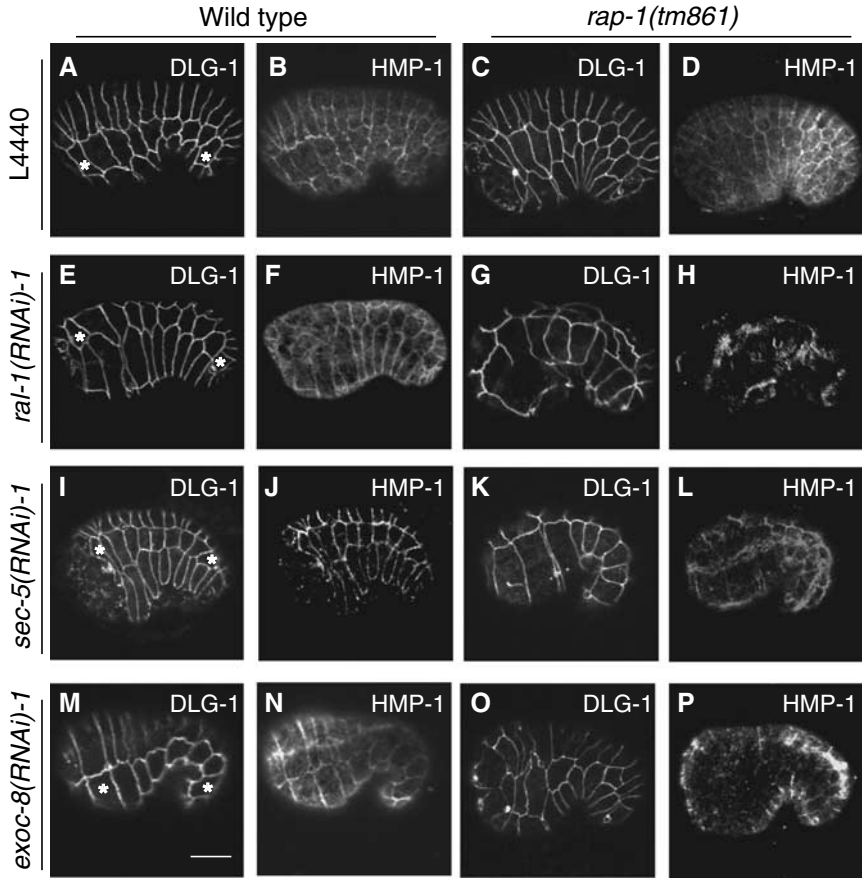

Figure 4 Localization of HMP-1 in wild-type or *rap-1* mutant embryos subjected to control, *ral-1*, *sec-5* or *exoc-8(RNAi)*. *dlg-1*::GFP and HMP-1 staining in a lateral view of wild type carrying *dlg-1*::GFP (FZ224) (**A, B, E, F, I, J, M, N**) and *rap-1* mutant embryos (FZ271) (**C, D, G, H, K, L, O, P**), derived from animals subjected to either control L4440 (A–D), *ral-1(RNAi)* (E–H), *sec-5(RNAi)* (I–L) and *exoc-8(RNAi)* (M–P). Pictures show hypodermal cells in which DLG-1 indicates *dlg-1*::GFP expression and HMP-1 indicates immunofluorescence staining using the P1E11 antibody. The most anterior and posterior visible seam cells are marked with *. Scale bar: 10 μm.

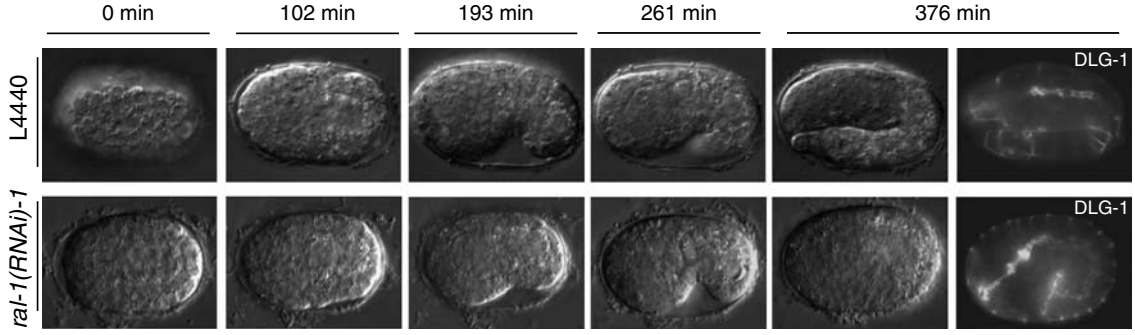

Figure 5 Time-lapse analysis of *rap-1* mutant embryos subjected to control and *ral-1(RNAi)*. Time lapse recordings of embryos derived from *dlg-1*::GFP;*rap-1(tm861)* mutant (FZ271) embryos on control L4440 (upper panels) or *ral-1(RNAi)* (lower panels). Last timepoint also shows DLG-1::GFP expression in the gut of the same embryo.

by heterozygous animals on *sec-5(RNAi)* die as embryo (data not shown).

For *ral-1*, we did not identify mutants with a stop codon. As an alternative, we generated transgenic animals, in which the dominant negative RAL-1(S31N) or the constitutively GTP-bound RAL-1(G26V) are under the control of a heat-shock promoter. Despite being constitutively GTP-bound, mutations corresponding to RAL-1(G26V) (like Ral G23V in mammals), have been described as interfering mutants (Moskalenko *et al*, 2002; Feig, 2003). Expression of either mutant protein (Figure 7A) did not severely affect these transgenic animals, although it took slightly longer to reach adulthood as compared to heat shocked, wild-type worms. In contrast, expression of RAL-1 mutant proteins in a *rap-1* mutant background induced a clear larval arrest, which was strongest for RAL-1(G26V) (Figure 7B, C). Notably, whereas expression of HA-RAL-1(G26V) only delays development in wild-type worms, *rap-1* worms expressing the same protein

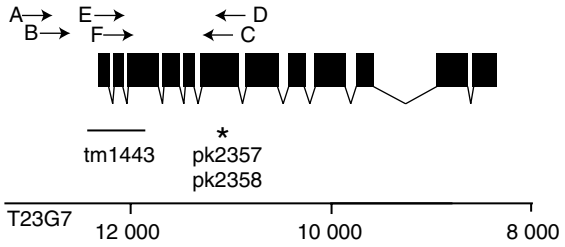

Figure 6 Schematic representation of the genomic organization of the *sec-5* locus. Filled boxes indicate exons and the line underneath the graphic representation indicates the position of the deletion in *sec-5(tm1443)* animals and the star indicates the stop codon (amino acid 389) in the *pk2357* and *pk2358* allele. Numbers indicate the position of the *rap-1* locus on the T23G7 cosmid. Arrows indicate the position of the PCR primers used for detection of the deletion and wild-type alleles.

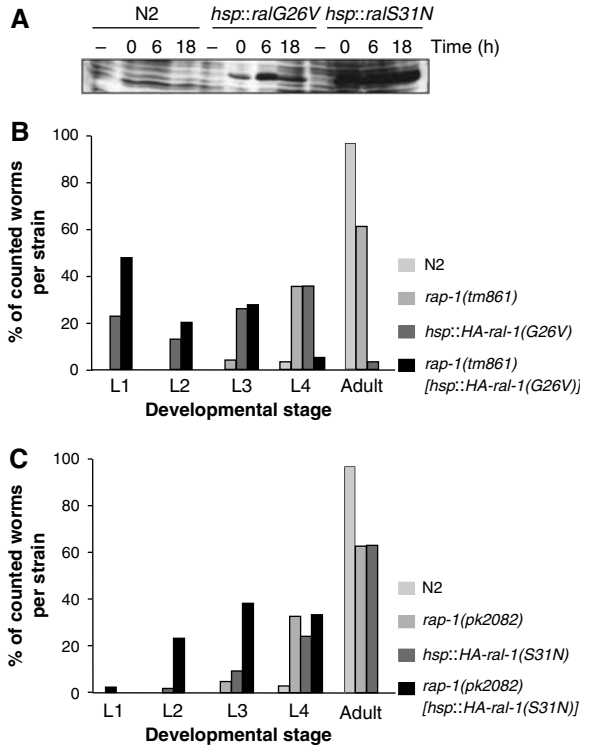

Figure 7 Effect of heat-shock-driven expression of RAL-1 proteins in wild-type and *rap-1* mutant backgrounds. (**A**) Western blot of total lysates from L1 larvae. Larvae were lysed without heat shock (−) or at 0 h (0), 6 h (6) or 18 h (18) after a 2-h heat shock. Probing was done with the 12CA5 antibody, which recognizes the HA-tag at the N-terminus of overexpressed proteins. (**B, C**) Embryos subjected to heat shock were allowed to develop 5 days before determination of developmental stage. Percentage of animals at the different developmental stages of the total number of hatched animals is shown for overexpression RAL-1(G26V) and RAL-1(S31N) in (B) and (C), respectively.

never reach adulthood. The effects of expression of Ral-1(G26V) in a *rap-2*(*gk11*) background were not significantly different from those observed in wild-type worms, showing the specificity of the effect of RAL-1(G26V) on *rap-1* animals (data not shown). Together these data show that the RAP-1 and RAL-1/exocyst pathway also interact during other stages of the life cycle.

Discussion

Synthetic lethal screens are an efficient approach towards identification of signaling pathways that function in a redundant fashion to mediate critical functions during development or any other stage of an organism's life cycle (Tong *et al*, 2001; van Haaften *et al*, 2004; Withee *et al*, 2004). Here, we have used an RNAi feeding-based method to screen for signaling routes that function in conjunction with the RAP-1 pathway in *C. elegans*. Seven genes were identified, whose full function is required for two distinct *rap-1* mutants to produce viable offspring. These genes are synthetic lethal with two independent *rap-1* mutations, strongly suggesting that mutations in *rap-1* rather than a closely linked mutation is responsible for the observed effect. Indeed, we obtained a partial rescue of the phenotype on *exoc-8(RNAi)* with lines expressing *rap-1* from a heterologous promoter. Synthetic lethality on *ral-1(RNAi)* that is generally more robust, was not rescued, which we contributed to the low levels of RAP-1 expression from this transgene (data not shown). High levels of RAP-1 expression appear not to be tolerated in *C. elegans* as is also seen for Rap1 in tissue culture cells (data not shown).

Not all genes may function in the same pathway (see below), but based on literature, it seems likely that *ral-1*, *sec-5* and *exoc-8* do so; in vertebrate cells, active Ral directly binds to the exocyst members Sec5 or Exo84 (Brymora *et al*, 2001; Moskalenko *et al*, 2003), which results in targeting of proteins, including E-cadherin, to the basolateral membrane (Moskalenko *et al*, 2002; Shipitsin and Feig, 2004). The fact that Ral binds to Sec5 or Exo84 in a mutually exclusive manner may hint to two distinct pathways. In addition, Ral collaborates with Sec5 in the formation of filopodia (Sugihara *et al*, 2002). Analysis of embryos derived from *rap-1* mutant worms, grown on *ral-1*, *sec-5* or *exoc-8(RNAi)*, showed that they displayed virtually identical phenotypes. This strongly suggests that also in *C. elegans* these genes function together in hypodermal cell migration.

Rap1 has been claimed to activate the Ral pathway in *Drosophila* by direct interaction with the RalGEF RGL (Mirey *et al*, 2003). Also *C. elegans* RAP-1 and RGL-1 interact in a yeast two-hybrid assay (J Riedl and F Zwartkruis, unpublished results). Although our studies did not exclude the option that RAP-1 contributes to activation of RAL-1 in *C. elegans*, our current hypothesis is that RAP-1 has a distinct function in parallel to that of RAL-1. This is based on the fact that RAP-1 null mutants have only a very mild phenotype under normal conditions, indicating that RAP-1 is not a major activator of the RAL-1/exocyst pathway. Furthermore, interfering at the level of RAL-1 by expression of mutant RAL-1 proteins has a strong effect in *rap-1* mutants but not in wild-type animals. It is possible that RAP-1 converges with RAL-1 pathway at the level of the exocyst complex or below. As no interactions of RAP-1 with the exocyst have been documented, we favor the latter option. In agreement with this, we find that RAP-1 clearly enhances the phenotype of *sec-5* mutations.

The defects observed in *rap-1* embryos following *ral-1*, *sec-5* or *exoc-8(RNAi)* are most prominent in hypodermal cell migration during ventral enclosure. It may be that in the most severely affected class II embryos also gastrulation movements are abnormal, but this has not been investigated in any

detail. Various causes may underlie the improper alignment and defective migration of hypodermal cells. For example, hypodermal cells need to be specified correctly to accommodate to their correct position. Abnormal cell specification, leading to defects in dorsal intercalation, ventral enclosure and elongation, has been proposed to underlie the phenotype of *apr-1* mutants (Hoier *et al*, 2000). However, cell specification is unlikely to cause the migration defects found in *ral-1(RNAi)*, *rap-1* embryos. First, even in class II embryos, expression of a seam cell marker could be detected, which demonstrates that at least some specification within the hypodermis has occurred. Second, we did not observe any abnormal patterning of hypodermal cells before the onset of migration, in contrast to what was reported for *apr-1* mutants (data not shown). Finally, the Ral-1/exocyst pathway was found to regulate migration in tissue culture cells, which is unlikely to depend on cell fate specification (Rosse *et al*, 2006).

A more likely explanation for the observed phenotype is the inability of hypodermal cells to change their adhesive properties that can support the extensive reorganization of cell–cell contacts required for dorsal intercalation and ventral migration. Cell–cell adhesion is of fundamental importance in cell sorting, migration and cell shape changes. This is exemplified in *Drosophila*, where loss of DE-cadherin in border cells blocks migration and in follicle cells has dramatic effects on cell sorting (Lee *et al*, 1996; Niewiadomska *et al*, 1999; Pacquelet and Rorth, 2005). Even loss of DE-cadherin from part of the cell circumference, as seen in clones of Rap1 mutant cells following cell division in the wing, changes their sorting behavior (Knox and Brown, 2002). Indeed, our analysis of proteins present in adherens junctions revealed the absence of HMP-1 and thus most likely the complete CCC (Costa *et al*, 1998). This indicates that cell adhesive properties have changed. Loss of HMP-1 from adherens junctions upon interfering in the RAL-1/exocyst route is in line with recent studies in vertebrate tissue culture cells and *Drosophila* (Shipitsin and Feig, 2004; Classen *et al*, 2005; Langevin *et al*, 2005). It should be noted, however, that in *C. elegans*, loss of HMP-1 from adherens junctions upon *ral-1(RNAi)* was only seen in *rap-1* worms and not in wild-type worms. Thus, upon *ral-1* depletion, RAP-1 is crucial for targeting the CCC to the lateral membrane or stabilizing it. In mammalian cells, Rap1 has been shown to promote E-cadherin interaction at newly forming adherens junctions in tissue culture cells, whereas it seems not required for the maintenance of mature junctions (Hogan *et al*, 2004; Price *et al*, 2004). Possibly, Rap1 acts by inhibiting endocytosis of E-cadherin proteins, which are not yet ligated to E-cadherin on other cells. To do so, Rap1 is suggested to interact via the actin binding protein AF-6 with p120 catenin, an established stabilizer of adherens junctions (Hoshino *et al*, 2005). Although it is attractive to explain the lack of HMP-1 in adherens junctions in terms of the combined effect of diminished targeting via the RAL-1/exocyst complex and enhanced internalization, due to lack of RAP-1, the situation may be more complex. It should be kept in mind that during reorganization of cell–cell contacts, cadherin is recycled via endosomes (Bryant and Stow, 2004). Indeed, disturbing the function of these endosomes in the *Drosophila* wing during the period when cells organize themselves into hexagonal arrays, results in a loss of DE-cadherin at selective cell contact sites (Classen *et al*, 2005). Consequently, loss of HMP-1 may not result from effects of RAP-1 on endocytosis, but on sorting events in recycling endosomes or exit from such endosomes. The situation is further complicated by the observation that the exocyst member Sec5 functions not only in exocytosis, but also in endocytosis (Sommer *et al*, 2005; Sonnichsen *et al*, 2005). Indeed, it is currently unclear if and how the exocyst complex interacts with the recycling endosomes. In this respect, *phi-24* may be an interesting hit from our screen, as this protein is homologous to the endosomal protein CHMP1 (Howard *et al*, 2001), which is involved in vesicular sorting.

In contrast to HMP-1, DLG-1 and AJM-1 localized normally to adherens junctions and this is consistent with the finding that the localization of these latter proteins occurs independently of the CCC (Costa *et al*, 1998; Koppen *et al*, 2001). In addition, it shows that the RAL-1 and RAP-1 pathways affect only the transport of a subclass of proteins present at the basolateral membrane. Differential effects of inhibition of the exocyst complex on transport to the basolateral membrane have previously been shown in *Drosophila*, where DE-cadherin transport is blocked but delivery of the septate junction protein Coracle is normal (Langevin *et al*, 2005). Importantly, class I embryos differ from *hmr-1* embryos in that the shape and alignment of hypodermal cells is clearly abnormal. Therefore, the synthetic lethal phenotype observed cannot be simply attributed to loss of the CCC at the adherens junction. Double mutant analysis demonstrates that the phenotype of *hmr-1* or *hmp-1* embryos does not become more severe if *rap-1* is simultaneously absent. Consequently, the RAL-1/exocyst pathway is not only required for targeting the CCC, but has also additional functions.

It will be interesting to learn if and how the other genes uncovered in the synthetic lethal screen fit in the RAL-1/exocyst pathway. At least one gene, *vhp-1*, has been indirectly linked to Ral signaling. VHP-1 is a phosphatase for the stress-induced kinases KGB-1 and PMK-1, which are homologous to Jnk and p38 MAPK, respectively (Mizuno *et al*, 2004). Previously, Ral has been found to activate the Jnk-pathway in tissue culture cells (de Ruiter *et al*, 2000), whereas in *Drosophila* it was found to act as a negative regulator of Jnk (Sawamoto *et al*, 1999). Therefore, *vhp-1* may act together with RAL-1 in other processes, possibly with different RAL-1 effectors.

In conclusion, a synthetic lethal screen has been used as a starting point to learn more about genes, that function in a redundant fashion with RAP-1. Our data demonstrate a functional overlap for the Ras-like GTPases RAP-1 and RAL-1 during various phases of the life cycle. Using RNAi to time the interference in the RAL-1/exocyst pathway, we identify both GTPases as novel elements in *C elegans* hypodermal cell migration.

Materials and methods

Worms

General methods for culturing and manipulating worms used were as described previously (Lewis and Fleming, 1995). Worms were cultured on NGM plates at 20°C. Strains, constructs and detection of mutants are described in Supplementary data.

Transgenic animals were obtained by injection of plasmid DNA into the gonads of *dpy-20(e1362)* animals, or *rap-1(tm861);dpy-20(e1362)* (Mello *et al*, 1991). FZ311 and FZ312 were made with pPD103.5 containing untagged cDNA sequence of *rap*-1 (C27B7.8).

Transgenic arrays were integrated by irradiating animals with 40 Gy of gamma radiation from a 137 Cs source (Way *et al*, 1991). Target-selected mutagenesis for obtaining *sec-5* or *ral-1* mutants was performed as described previously (Cuppen *et al*, 2007). Mutant animals were outcrossed at least four times to N2 animals before phenotypic analysis. Determination of viability of N2 and *rap-1(tm861);rap-2(gk11)* was performed by cutting gravid adults in water and collecting the embryos with a drawn-out pipette. Embryos were counted and non-hatched embryos were scored after 24 h.

The synthetic lethal screen was performed essentially as described previously (van Haaften *et al*, 2004). However, we used 20–25 L1 larvae per well in 100 µl of $M9^+$ buffer, to which 50 µl of induced bacterial suspension was added. Scoring was done after 7 days either by eye for genome scale screen or by counting the total amount of progeny per well and comparing it to the amount of progeny on control RNAi (L4440).

Antibody staining and time-lapse recording

Antibody staining of embryos was performed as described previously (Bossinger *et al*, 2001). Antibodies used are: α-HMP-1 (clone P1E11, Chemicon), α-AJM-1 (provided by O Bossinger), mabMH27, mouse, hybridoma supernatant), EEA-1 (provided by B Grant), RAB-11 (provided by A Spang) and the Alexa anti-mouse 568 secondary antibody (Jackson ImmRes Lab). Embryos were visualized under a Zeiss Axiokop 2. For time-lapse recordings, L4 animals were put on an RNAi feeding plate on day 1. On day 3, adults were moved to a fresh plate and embryos were collected after a 2-h egg lay. Embryos were mounted on a 3% agarose pad in $M9^+$ buffer, covered with a coverslip and sealed with Vaseline.

Heat shock protocol and Western blotting

For heat shock experiments, hermaphrodites were allowed to lay eggs for 2 h (day 0). At 1 h after removal of the adults, the plates were incubated at 33°C for 30 min. On days 1, 2 and 3 a 2-h heat shock was given. Detection of HA-tagged RAL-1 in lysates of heat-shocked animals was done by immunoblotting using the 12CA5mAb against HA (Pellis-van Berkel *et al*, 2005).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

Acknowledgements

We thank R Vulders for technical assistance, Dr S Mitani for the *rap-1(tm861)* and *sec-5(tm1443)* mutant, Dr M Labouesse for the DLG-1::GFP strain, Dr J Ahringer, Dr A Spang and Dr B Grant for antibodies. Some nematode strains used were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR) and we thank T Stiernagle for sending these. We thank Dr O Bossinger for valuable discussions and teaching us antibody labeling of embryos, Dr A Fire for making available heat shock constructs and J Mudde for technical assistance in target-selected mutagenesis. This work was supported by the CBG (EF, WP-vB, FZ).

References

Andrews R, Ahringer J (2007) Asymmetry of early endosome distribution in *C. elegans* embryos. *PLoS ONE* **2:** e493

Asha H, de Ruiter ND, Wang MG, Hariharan IK (1999) The Rap1 GTPase functions as a regulator of morphogenesis *in vivo*. *EMBO J* **18:** 605–615

Bos JL (2005) Linking Rap to cell adhesion. *Curr Opin Cell Biol* **17:** 123–128

Bossinger O, Klebes A, Segbert C, Theres C, Knust E (2001) Zonula adherens formation in *Caenorhabditis elegans* requires dlg-1, the homologue of the *Drosophila* gene discs large. *Dev Biol* **230:** 29–42

Bryant DM, Stow JL (2004) The ins and outs of E-cadherin trafficking. *Trends Cell Biol* **14:** 427–434

Brymora A, Valova VA, Larsen MR, Roufogalis BD, Robinson PJ (2001) The brain exocyst complex interacts with RalA in a GTP-dependent manner: identification of a novel mammalian Sec3 gene and a second Sec15 gene. *J Biol Chem* **276:** 29792–29797

Caron E, Self AJ, Hall A (2000) The GTPase Rap1 controls functional activation of macrophage integrin alphaMbeta2 by LPS and other inflammatory mediators. *Curr Biol* **10:** 974–978

Classen AK, Anderson KI, Marois E, Eaton S (2005) Hexagonal packing of *Drosophila* wing epithelial cells by the planar cell polarity pathway. *Dev Cell* **9:** 805–817

Costa M, Raich W, Agbunag C, Leung B, Hardin J, Priess JR (1998) A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J Cell Biol* **141:** 297–308

Cox EA, Hardin J (2004) Sticky worms: adhesion complexes in *C. elegans*. *J Cell Sci* **117:** 1885–1897

Cuppen E, Gort E, Hazendonk E, Mudde J, van de Belt J, Nijman IJ, Guryev V, Plasterk RH (2007) Efficient target-selected mutagenesis in *Caenorhabditis elegans*: toward a knockout for every gene. *Genome Res* **17:** 649–658

de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* **396:** 474–477

de Ruiter ND, Wolthuis RM, van Dam H, Burgering BM, Bos JL (2000) Ras-dependent regulation of c-Jun phosphorylation is mediated by the Ral guanine nucleotide exchange factor-Ral pathway. *Mol Cell Biol* **20:** 8480–8488

Feig LA (2003) Ral-GTPases: approaching their 15 minutes of fame. *Trends Cell Biol* **13:** 419–425

Fukuhara S, Sakurai A, Yamagishi A, Sako K, Mochizuki N (2006) Vascular endothelial cadherin-mediated cell–cell adhesion regulated by a small GTPase, Rap1. *J Biochem Mol Biol* **39:** 132–139

George SE, Simokat K, Hardin J, Chisholm AD (1998) The VAB-1 Eph receptor tyrosine kinase functions in neural and epithelial morphogenesis in *C. elegans*. *Cell* **92:** 633–643

Grindstaff KK, Yeaman C, Anandasabapathy N, Hsu SC, Rodriguez-Boulan E, Scheller RH, Nelson WJ (1998) Sec6/8 complex is recruited to cell–cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* **93:** 731–740

Hogan C, Serpente N, Cogram P, Hosking CR, Bialucha CU, Feller SM, Braga VM, Birchmeier W, Fujita Y (2004) Rap1 regulates the formation of E-cadherin-based cell–cell contacts. *Mol Cell Biol* **24:** 6690–6700

Hoier EF, Mohler WA, Kim SK, Hajnal A (2000) The *Caenorhabditis elegans* APC-related gene apr-1 is required for epithelial cell migration and Hox gene expression. *Genes Dev* **14:** 874–886

Hoshino T, Sakisaka T, Baba T, Yamada T, Kimura T, Takai Y (2005) Regulation of E-cadherin endocytosis by nectin through afadin, Rap1, and p120ctn. *J Biol Chem* **280:** 24095–24103

Howard TL, Stauffer DR, Degnin CR, Hollenberg SM (2001) CHMP1 functions as a member of a newly defined family of vesicle trafficking proteins. *J Cell Sci* **114:** 2395–2404

Jin R, Junutula JR, Matern HT, Ervin KE, Scheller RH, Brunger AT (2005) Exo84 and Sec5 are competitive regulatory Sec6/8 effectors to the RalA GTPase. *EMBO J* **24:** 2064–2074

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421:** 231–237

Katagiri K, Hattori M, Minato N, Irie S, Takatsu K, Kinashi T (2000) Rap1 is a potent activation signal for leukocyte function-associated antigen 1 distinct from protein kinase C and phosphatidylinositol-3-OH kinase. *Mol Cell Biol* **20:** 1956–1969

Knox AL, Brown NH (2002) Rap1 GTPase regulation of adherens junction positioning and cell adhesion. *Science* **295:** 1285–1288

Koh K, Rothman JH (2001) ELT-5 and ELT-6 are required continuously to regulate epidermal seam cell differentiation and cell fusion in *C. elegans*. *Development* **128:** 2867–2880

Kooistra MR, Dube N, Bos JL (2007) Rap1: a key regulator in cell–cell junction formation. *J Cell Sci* **120:** 17–22

Koppen M, Simske JS, Sims PA, Firestein BL, Hall DH, Radice AD, Rongo C, Hardin JD (2001) Cooperative regulation of AJM-1 controls junctional integrity in *Caenorhabditis elegans* epithelia. *Nat Cell Biol* **3:** 983–991

Langevin J, Morgan MJ, Sibarita JB, Aresta S, Murthy M, Schwarz T, Camonis J, Bellaiche Y (2005) *Drosophila* exocyst components Sec5, Sec6, and Sec15 regulate DE-Cadherin trafficking from recycling endosomes to the plasma membrane. *Dev Cell* **9:** 355–376

Lee T, Feig L, Montell DJ (1996) Two distinct roles for Ras in a developmentally regulated cell migration. *Development* **122:** 409–418

Lewis JA, Fleming JT (1995) Basic culture methods. *Methods Cell Biol* **48:** 3–29

Lipschutz JH, Mostov KE (2002) Exocytosis: the many masters of the exocyst. *Curr Biol* **12:** R212–R214

Mello CC, Kramer JM, Stinchcomb D, Ambros V (1991) Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *Embo J* **10:** 3959–3970

Mirey G, Balakireva M, L'Hoste S, Rosse C, Voegeling S, Camonis J (2003) A Ral guanine exchange factor-Ral pathway is conserved in *Drosophila melanogaster* and sheds new light on the connectivity of the Ral, Ras, and Rap pathways. *Mol Cell Biol* **23:** 1112–1124

Mizuno T, Hisamoto N, Terada T, Kondo T, Adachi M, Nishida E, Kim DH, Ausubel FM, Matsumoto K (2004) The *Caenorhabditis elegans* MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. *EMBO J* **23:** 2226–2234

Moskalenko S, Henry DO, Rosse C, Mirey G, Camonis JH, White MA (2002) The exocyst is a Ral effector complex. *Nat Cell Biol* **4:** 66–72

Moskalenko S, Tong C, Rosse C, Mirey G, Formstecher E, Daviet L, Camonis J, White MA (2003) Ral GTPases regulate exocyst assembly through dual subunit interactions. *J Biol Chem* **278:** 51743–51748

Niewiadomska P, Godt D, Tepass U (1999) DE-Cadherin is required for intercellular motility during *Drosophila* oogenesis. *J Cell Biol* **144:** 533–547

Ohta Y, Suzuki N, Nakamura S, Hartwig JH, Stossel TP (1999) The small GTPase RalA targets filamin to induce filopodia. *Proc Natl Acad Sci USA* **96:** 2122–2128

Pacquelet A, Rorth P (2005) Regulatory mechanisms required for DE-cadherin function in cell migration and other types of adhesion. *J Cell Biol* **170:** 803–812

Pellis-van Berkel W, Verheijen MH, Cuppen E, Asahina M, de Rooij J, Jansen G, Plasterk RH, Bos JL, Zwartkruis FJ (2005) Requirement of the *Caenorhabditis elegans* RapGEF pxf-1 and rap-1 for epithelial integrity. *Mol Biol Cell* **16:** 106–116

Poteryaev D, Fares H, Bowerman B, Spang A (2007) *Caenorhabditis elegans* SAND-1 is essential for RAB-7 function in endosomal traffic. *EMBO J* **26:** 301–312

Price LS, Hajdo-Milasinovic A, Zhao J, Zwartkruis FJ, Collard JG, Bos JL (2004) Rap1 regulates E-cadherin-mediated cell–cell adhesion. *J Biol Chem* **279:** 35127–35132

Prigent M, Dubois T, Raposo G, Derrien V, Tenza D, Rosse C, Camonis J, Chavrier P (2003) ARF6 controls post-endocytic recycling through its downstream exocyst complex effector. *J Cell Biol* **163:** 1111–1121

Rosse C, Hatzoglou A, Parrini MC, White MA, Chavrier P, Camonis J (2006) RalB mobilizes the exocyst to drive cell migration. *Mol Cell Biol* **26:** 727–734

Sawamoto K, Winge P, Koyama S, Hirota Y, Yamada C, Miyao S, Yoshikawa S, Jin MH, Kikuchi A, Okano H (1999) The *Drosophila* Ral GTPase regulates developmental cell shape changes through the Jun NH(2)-terminal kinase pathway. *J Cell Biol* **146:** 361–372

Segbert C, Johnson K, Theres C, van Furden D, Bossinger O (2004) Molecular and functional analysis of apical junction formation in the gut epithelium of *Caenorhabditis elegans*. *Dev Biol* **266:** 17–26

Shimonaka M, Katagiri K, Nakayama T, Fujita N, Tsuruo T, Yoshie O, Kinashi T (2003) Rap1 translates chemokine signals to integrin activation, cell polarization, and motility across vascular endothelium under flow. *J Cell Biol* **161:** 417–427

Shipitsin M, Feig LA (2004) RalA but not RalB enhances polarized delivery of membrane proteins to the basolateral surface of epithelial cells. *Mol Cell Biol* **24:** 5746–5756

Sieburth DS, Sundaram M, Howard RM, Han M (1999) A PP2A regulatory subunit positively regulates Ras-mediated signaling during *Caenorhabditis elegans* vulval induction. *Genes Dev* **13:** 2562–2569

Simske JS, Hardin J (2001) Getting into shape: epidermal morphogenesis in *Caenorhabditis elegans* embryos. *BioEssays* **23:** 12–23

Sommer B, Oprins A, Rabouille C, Munro S (2005) The exocyst component Sec5 is present on endocytic vesicles in the oocyte of *Drosophila melanogaster*. *J Cell Biol* **169:** 953–963

Sonnichsen B, Koski LB, Walsh A, Marschall P, Neumann B, Brehm M, Alleaume AM, Artelt J, Bettencourt P, Cassin E, Hewitson M, Holz C, Khan M, Lazik S, Martin C, Nitzsche B, Ruer M, Stamford J, Winzi M, Heinkel R *et al* (2005) Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. *Nature* **434:** 462–469

Sugihara K, Asano S, Tanaka K, Iwamatsu A, Okawa K, Ohta Y (2002) The exocyst complex binds the small GTPase RalA to mediate filopodia formation. *Nat Cell Biol* **4:** 73–78

Suzuki J, Yamazaki Y, Li G, Kaziro Y, Koide H (2000) Involvement of Ras and Ral in chemotactic migration of skeletal myoblasts. *Mol Cell Biol* **20:** 4658–4665

TerBush DR, Maurice T, Roth D, Novick P (1996) The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J* **15:** 6483–6494

Thomas-Virnig CL, Sims PA, Simske JS, Hardin J (2004) The inositol 1,4,5-trisphosphate receptor regulates epidermal cell migration in *Caenorhabditis elegans*. *Curr Biol* **14:** 1882–1887

Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Page N, Robinson M, Raghibizadeh S, Hogue CW, Bussey H, Andrews B, Tyers M, Boone C (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* **294:** 2364–2368

van Haaften G, Vastenhouw NL, Nollen EA, Plasterk RH, Tijsterman M (2004) Gene interactions in the DNA damage-response pathway identified by genome-wide RNA-interference analysis of synthetic lethality. *Proc Natl Acad Sci USA* **101:** 12992–12996

Way JC, Wang L, Run JQ, Wang A (1991) The mec-3 gene contains cis-acting elements mediating positive and negative regulation in cells produced by asymmetric cell division in *Caenorhabditis elegans*. *Genes Dev* **5:** 2199–2211

Williams-Masson EM, Heid PJ, Lavin CA, Hardin J (1998) The cellular mechanism of epithelial rearrangement during morphogenesis of the *Caenorhabditis elegans* dorsal hypodermis. *Dev Biol* **204:** 263–276

Withee J, Galligan B, Hawkins N, Garriga G (2004) *Caenorhabditis elegans* WASP and Ena/VASP proteins play compensatory roles in morphogenesis and neuronal cell migration. *Genetics* **167:** 1165–1176

Yeaman C, Grindstaff KK, Nelson WJ (2004) Mechanism of recruiting Sec6/8 (exocyst) complex to the apical junctional complex during polarization of epithelial cells. *J Cell Sci* **117:** 559–570

Zhang X, Zajac A, Zhang J, Wang P, Li M, Murray J, TerBush D, Guo W (2005) The critical role of Exo84p in the organization and polarized localization of the exocyst complex. *J Biol Chem* **280:** 20356–20364

