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Biological characterization of *Drosophila* Rapgap1, a GTPase activating protein for Rap1

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ABSTRACT **The activity of Ras family proteins is modulated *in vivo* by the function of GTPase activating proteins, which increase their intrinsic rate of GTP hydrolysis. We have isolated cDNAs encoding a GAP for the *Drosophila* Rap1 GTPase. *Drosophila Rapgap1* encodes an 850-amino acid protein with a central region that displays substantial sequence similarity to human RapGAP. This domain, when expressed in *Escherichia coli*, potently stimulates Rap1 GTPase activity *in vitro*. Unlike *Rap1*, which is ubiquitously expressed, *Rapgap1* expression is highly restricted. *Rapgap1* is expressed at high levels in the developing photoreceptor cells and in the optic lobe. *Rapgap1* mRNA is also localized in the pole plasm in an *oskar*-dependent manner. Although mutations that completely abolish *Rapgap1* function display no obvious phenotypic abnormalities, overexpression of *Rapgap1* induces a rough eye phenotype that is exacerbated by reducing *Rap1* gene dosage. Thus, Rapgap1 can function as a negative regulator of Rap1-mediated signaling *in vivo*.**

A variety of cellular processes are regulated by low molecular weight GTPases of the ras superfamily. These include cell proliferation, differentiation, cell morphology, nuclear transport, and intracellular transport of vesicles (1). Among the best-characterized members of the ras superfamily are the Ras proteins, which regulate cell proliferation and differentiation in response to extracellular growth factors. The study of Ras proteins has provided us with significant insights into the mechanisms by which they act as signal transducers and the mechanisms by which they become activated and inactivated.

Ras proteins bind GTP, which alters their confirmation to the "active" state and enables them to bind to effector molecules. They then hydrolyze the bound GTP to GDP and return to the inactive state. In the cell, the relative levels of active and inactive Ras are determined by the activity of guanine nucleotide exchange factors and GTPase activating proteins (GAPs) (1). Guanine nucleotide exchange factors facilitate GDP release and hence activate Ras and the GAPs (2, 3) greatly increase the intrinsic GTPase activity of Ras and hence inactivate Ras. There is also evidence that RasGAP may also have some effector functions (4).

The Rap proteins are highly related to Ras and they are extremely conserved among diverse species. Indeed, the human and *Drosophila* Rap proteins are more closely related than are human and *Drosophila* Ras. However, the function of the Rap proteins are poorly understood in any organism. It was initially proposed that Rap proteins function as Ras antagonists, largely based on experiments in which the expression of high levels of wild-type Rap or of activated Rap is able to attenuate Ras-mediated signaling (5, 6). However, it has always been unclear whether Rap antagonizes Ras function under physiological circumstances. Evidence is accumulating for Ras-independent functions of Rap. Rap may function in platelet aggregation and degranulation and the production of superoxide in neutrophils (7, 8), although the pathways that mediate these processes have not been elucidated. More recently, it has been shown in PC12 cells that phosphorylation of Rap by cAMP-dependent protein kinase leads to the accumulation of GTP-bound Rap (9). This, in turn, leads to activation of B-raf (but not Raf) and mitogen-activated protein kinase, and ultimately, the transcription factor Elk1. Thus, Rap may activate mitogen-activated protein kinase signaling in response to a different set of stimuli than those that activate Ras.

The role of mammalian Rap in regulating cell proliferation and differentiation is still poorly understood, partly because of the lack of primary cells or cell lines where Rap function has been eliminated. The existence of at least four highly related *rap* genes in mammals suggests that they may serve redundant functions. In contrast, the *Rap1* gene of *Drosophila melanogaster* serves an essential function because mutations in *Rap1* are lethal at the larval stage (10). By studying animals that lack *Rap1* function, we have gained some insights into the biological role of *Rap1*. Embryos that lack maternally provided *Rap1* develop abnormally, largely because of defects in morphogenesis, suggesting a role for *Rap1* in the regulation of cell shape or cell–cell adhesion (H. Asha and I.K.H., unpublished results).

The mechanisms that regulate Rap activation and inactivation *in vivo* are still not well understood. A potentially important regulator in mammalian cells is RapGAP (11), which surprisingly displays no similarity in primary amino acid sequence to the GAPs for Ras. More recently the *tuberin* gene, which is mutated in the disease tuberous sclerosis (characterized by hamartomas and malignancies) has been shown to encode a protein with sequence similarity to RapGAP (12). The Tuberin protein has also been shown to colocalize with Rap1 in cells and to function as a GAP for Rap1 *in vitro* (13, 14). Thus, the RapGAP family of proteins is likely to play an important role in regulating cell proliferation and differentiation. To help understand the regulation of *Drosophila* Rap1 *in vivo*, we set out to clone and characterize a GAP for *Drosophila* Rap1.

In this paper we describe *Rapgap1*, a gene whose product functions as a GAP for Rap1 *in vitro* and interacts genetically with *Rap1 in vivo*. The tissue-specific expression of Rapgap1 argues that Rap1 activity in distinct cells may be regulated by specific GAPs. We demonstrate the regulation of *Rapgap1* mRNA and protein localization by posterior group genes during the formation of pole plasm and describe the generation and characterization of loss-of-function mutations in *Rapgap1*.

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Abbreviations: GAP, GTPase activating protein; GST, glutathione *S*-transferase.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. AF023478).

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MATERIALS AND METHODS

Characterization of cDNA and Genomic Clones of Rapgap1. A human RapGAP cDNA clone was used to screen a *Drosophila* cDNA library constructed by Alan Cowman from mRNA purified from eye-imaginal discs as described (15). Hybridization was under conditions of low stringency (0.9 M NaCl/25% formamide at 42°C). Approximately 600,000 plaques were screened and 30 hybridizing plaques were identified. DNA was prepared from 10 of these plaques, and these were each characterized further. Phage clones covering part of the *Rapgap1* genomic region were isolated from a *Drosophila* genomic library in the EMBL3 phage (Stratagene) using the *Rapgap1* cDNA as a probe. A P1 clone (clone no. 2-4, Berkeley *Drosophila* Genome Project) covering the 5′ region of the *Rapgap1* locus was obtained from D. Hartl (Harvard University). The λ phage were mapped using standard protocols. For mapping, DNA from the P1 phage was restricted and separated either by conventional agarose gel electrophoresis or using pulse-field gel electrophoresis (Bio-Rad CHEF-DR II) using the manufacturer's instructions. The *Rapgap1* cDNA sequence has been deposited in GenBank (accession number AF023478).

GTPase Assays. For the *in vitro* GTPase assays, *Drosophila* Rap1, Ras1, and the putative catalytic domain of Rapgap1 (amino acids 182–550) were expressed as fusions of glutathione *S*-transferase (GST) in *Escherichia coli* strain XA-90. Cultures of transformed bacteria (500 ml) were grown at 37°C to an $A_{550} = 0.5$ and then expression of the fusion protein was induced by adding 0.2 mM isopropyl β-D-thiogalactoside and growing the cells for 3 h more at 25°C. After centrifugation, the bacterial cell pellet was sonicated in PBS/1% Triton/1 mM $MgCl_2$/0.1 mM EDTA/0.1 mM EGTA/0.1 mM DTT/1 mM phenylmethylsulfonyl fluoride. Proteins were purified using 0.5 ml glutathione agarose beads (Sigma). They were eluted with 5 mM reduced glutathione and 50 mM Tris (pH 8) and then were dialyzed against a buffer containing 20 mM Tris, pH 7.2/2 mM $MgCl_2$/0.1 mM EDTA/1 mM EGTA/50 mM NaCl/50% glycerol/0.1 mM DTT.

The Ras1 GTPase was labeled with [γ-^{32}P]GTP (10 μCi, 6,000 Ci/mmol (1 Ci = 37 GB_q); DuPont/NEN) in 50 mM Hepes, pH 7.9/2 mM EDTA/100 mM NaCl/0.1 mM DTT. Because the Rap1 GTPase labeled poorly under these conditions, the Rap1 protein was labeled in 50 mM Hepes, pH 7.9/100 mM NaCl/2 mM $MgCl_2$/0.5 mM DTT. All labeling reactions were at 30°C for 10 min. Purified baculovirus-expressed human p120 RasGAP was kindly provided by Jeff Settleman (Massachusetts General Hospital Cancer Center).

GAP assays were performed as described (16, 17). GTPases (1–2 μg) and GAP proteins (50–100 ng) were diluted in assay buffer (50 mM Hepes, pH 7.9/100 mM NaCl/2 mM $MgCl_2$/0.5 mM DTT) in the presence of 10 μM of unlabeled GTP. GTPase reactions were carried out at 30°C. Reactions were stopped by adding 0.5 ml ice-cold wash buffer (25 mM Hepes, pH 7.9/1 mM $MgCl_2$) and were filtered through nitrocellulose (BA85, Schleicher and Schuell). Filters were washed twice with 5 ml of wash buffer, and radioactivity remaining on the filters was determined.

Preparation of Antibodies to Rapgap. The C-terminal 230 amino acids of Rapgap1 were fused in-frame to a His6 tag using the pQE9 vector and expressed in *E. coli*. M15 cells. Fusion proteins were isolated with Ni-NTA resin (Qiagen, Chatsworth, CA) using the manufacturer's instructions and used to immunize mice, using standard protocols (18). Subsequently mAbs were generated as described (18). The experiments described in this paper were conducted using supernatants from mAb RG4G5H3 used at a dilution of 1:4. A peptide corresponding to residues 5–19 was also used to generate rabbit antisera by Research Genetics (Huntsville, AL) and used at a dilution of 1:250.

P-Element-Mediated Transformation. A 3.4-kb Rapgap1 cDNA fragment was cloned into the pGMR vector (19). Transformant lines bearing single insertions on the second and third chromosomes were generated.

Fly Stocks. Alleles of *oskar* (*osk*^{54}, *osk*^{166}, *osk*^{Ak}, *osk-bcd3′UTR*), nanos (*nos*^{BN}), vasa (*vas*^{D1}, *vas*^{PD}), *tudor*^{WC8}, *pumilio*^{680}, *staufen*^{D3} *cappuccino*^{RK}, and *spire*^{RP} were obtained from Ruth Lehmann and described in refs. 20 and 21.

Immunohistochemistry. RNA *in situ* hybridizations were performed as described by Gavis (22). Antibody staining of embryos was as described by Patel (23). Third instar larval eye disc complexes were immunostained as described (24).

Generation of Loss-of-Function Mutations in Rapgap1. A P-element insertion, rM455, in the 28B region was obtained from Todd Laverty and Gerald Rubin (University of California, Berkeley). Its location was determined by generating probes from DNA flanking the P-element insertion site by inverse PCR (25) and then probing filters containing digests of cloned DNA from the *Rapgap1* locus. The line rM455 was found to contain a P[ry] insertion 5 kb upstream of *Rapgap1*. Insertions in the *Rapgap1* locus were subsequently generated by mobilizing this P-element and generating local hops (26). Fly crosses and methodology for generating mutants were as described (25, 27). The new hops were once again identified by generating inverse PCR probes from pools of lines and probing filters with digests of cloned *Rapgap1* DNA.

None of the new insertions disrupted *Rapgap1* gene function. However, one line, L-259-179, had a new P-element insertion 3 kb 3′ of the *Rapgap1* transcription unit while retaining an insertion 5′ to *Rapgap1*. By crossing this chromosome bearing two P-element insertions to a stable genomic source of transposase as described by Cooley *et al.* (28), we were able to generate, at high frequency (approximately 30%), lines that had deleted the whole *Rapgap1* gene and consequently expressed no *Rapgap1* protein as assessed by Western blot analysis. These lines were homozygous viable.

RESULTS

Identification of a *Drosophila* RapGAP. To identify a *Drosophila* rapGAP homologue, we screened a *Drosophila* cDNA library prepared from eye-imaginal discs under hybridization conditions of low stringency with a human RapGAP cDNA probe (see *Materials and Methods*). Ten cDNA clones were isolated and characterized. Restriction mapping indicated that they were all derived from the same locus, which we have named *Rapgap1. Rapgap1* is located at 28B on the *Drosophila* cytogenetic map (data not shown).

The longest cDNA clones were 4.5 kb in size. These clones are likely to represent nearly full-length cDNAs because they detect a single mRNA of 4.5 kb on Northern blots of RNA prepared from imaginal discs (data not shown). Sequence analysis revealed a single long ORF that encodes a protein of 850 amino acids with a predicted molecular weight of 93.5 kDa (Fig. 1*B*). A central region of 369 amino acids displays significant sequence similarity to human rapGAP (46% identity, 56% similarity). Within this region, there is a 39-amino acid region (residues 403–441) that displays significant sequence similarity (33% identity, 62% similarity) with the product of the *tuberin* or TSC2 gene that is mutated in the human childhood disease tuberous sclerosis (12). In addition, in the C-terminal portion of Rapgap1, there is a 9-amino acid stretch (DTGLESMSS) that is almost identical to a sequence found in human rapGAP (DTGLESVSS). This peptide is not found in any other protein in the database, and hence the significance of this motif is unclear. The genomic organization of Rapgap1 was determined (Fig. 1*A*). The gene consists of three exons with a large 5′ intron (≈30kb) and a small (≈200bp) 3′ intron. The main region conserved between the human and *Drosoph-*

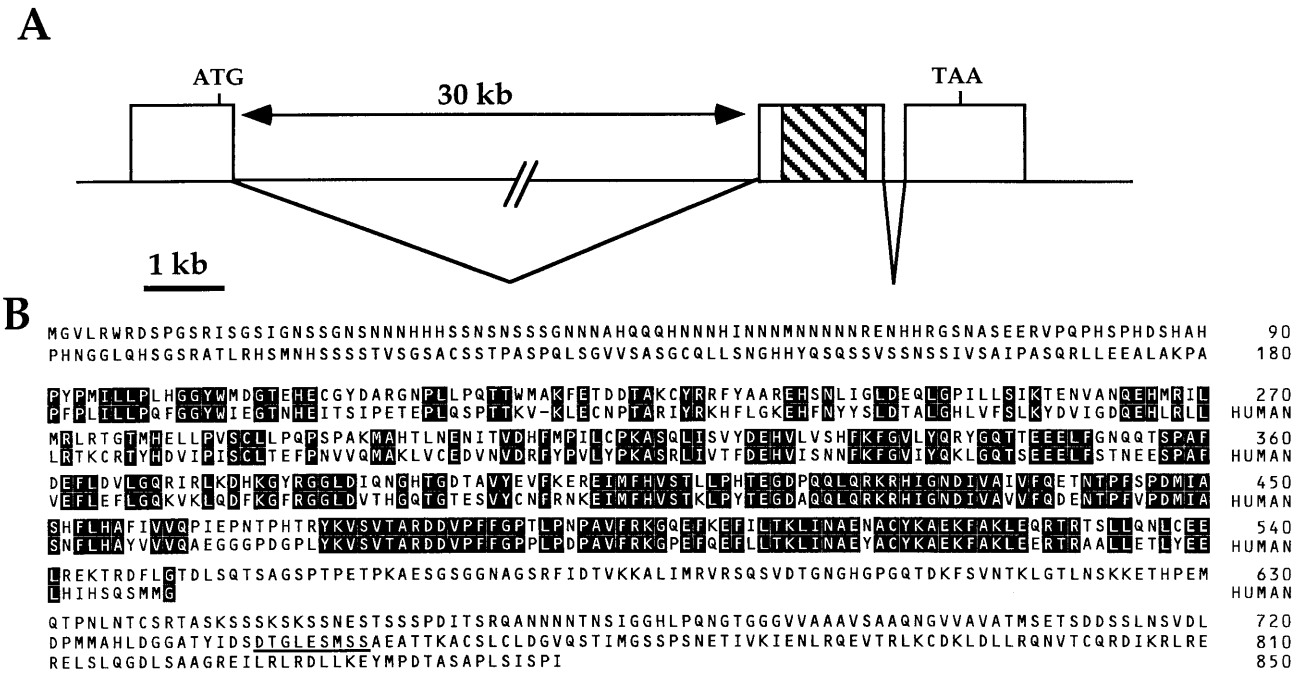

FIG. 1. Structure of the *Rapgap1* gene and the protein. (*A*) Structure of the *Rapgap1* gene. Exons are indicated as open boxes. The start codon (ATG) and the stop codon (TAA) are indicated. The region with sequence similarity to human RapGAP is ccrosshatched. (*B*) Amino acid sequence of *Drosophila* Rapgap1. Residues 181–640 of *Drosophila* Rapgap1 are aligned with residues 42–410 of human RapGAP (11). Black shading indicates identical residues. A 9-amino acid motif which is similar to a sequence in human RapGAP is underlined.

ila genes, which is likely to encode the catalytic domain, is located entirely within exon 2.

***Drosophila* Rapgap1 Is a Negative Regulator of Rap1.** To test whether the region conserved between *Drosophila* Rapgap1 and human rapGAP is sufficient for GAP activity, we expressed this region as a fusion of GST in *E. coli* and tested the fusion protein for GAP activity *in vitro* (Fig. 2*A*). In the absence of the Rapgap1 fusion protein, Rap1 hydrolyses GTP poorly. However, the addition of the bacterially expressed GST–Rapgap1 results in rapid hydrolysis of GTP by Rap1. In contrast, addition of either the hydrolysis buffer alone, GST alone, or mammalian p120 RasGAP failed to increase the rate of GTP hydrolysis. Furthermore, the GTPase activity of *Drosophila* Ras1 protein is not increased by Rapgap1 but is increased by adding mammalian p120 RasGAP (data not shown). Thus, the region conserved between the *Drosophila* and human proteins appears to be sufficient for function as a GAP for Rap1.

The ability of Rapgap1 to accelerate GTP hydrolysis by Rap1 and thereby promote the inactive GDP form of Rap1 argues that Rapgap1 is likely to antagonize the function of Rap1 *in vivo*. We tested this prediction by examining the effects of varying *Rap1* gene dosage on a *Rapgap1*-induced phenotype. We generated transgenic flies that expressed *Rapgap1* under the control of the glass multimer reporter (GMR) promoter which is expressed in all cells in the eye-imaginal disc posterior to the morphogenetic furrow (19, 29). Flies expressing three copies of *GMR–Rapgap1* display a mild rough eye characterized by missing pigment cells (Fig. 2*B*). Flies that are heterozygous for a null allele of *Rap1* have wild-type eyes in the absence of *Rapgap1* overexpression (Fig. 2*C*). However, removing one copy of *Rap1* enhances the phenotype caused by *Rapgap1* overexpression (Fig. 2*D*). Thus, the genetic interaction between *GMR–Rapgap1* and *Rap1* is consistent with the biochemical properties of the two proteins and indicates that Rapgap1 can function as a negative regulator of Rap1 *in vivo*.

Rapgap1 Expression Is Restricted to Distinct Cell Types. The Rap1 GTPase is expressed in most, if not all cells (ref. 30; H. Asha and I.K.H., unpublished observations). To analyze the expression pattern of *Rapgap1* during development, we first used *in situ* hybridization to detect *Rapgap1* mRNA. Subsequently, we raised polyclonal antisera and generated mAbs that detect Rapgap1 protein. Unlike *Rap1*, *Rapgap1* is expressed in distinct subsets of cells during development. *Rapgap1* RNA is first detected in the posterior pole of the embryo. The protein is first detected in the pole cells as they form (Fig. 3*A*) and is found in the pole cells during the early stages of their migration through the wall of the midgut. Once the pole cells migrate through the midgut wall, the protein is no longer detectable. During germ-band elongation, Rapgap1 protein is found in groups of cells in the neuroectoderm (Fig. 3*B*). Following germband retraction, Rapgap1 is detected in the cells of the peripheral nervous system (Fig. 3*C*), in the garland cells, and in the gut (Fig. 3*D*).

At later stages of development, Rapgap1 is expressed in the larval imaginal discs. In the eye-imaginal disc, Rapgap1 is expressed in most cells in the morphogenetic furrow. Immediately posterior to the furrow, expression is rapidly restricted to the developing ommatidial clusters (Fig. 3 *E* and *F*). Rapgap1 protein is also detected in the axons of the optic nerve and in the optic lobe (Fig. 3*F*).

Posterior Group Genes Regulate *Rapgap1* mRNA Localization During Pole Cell Formation. Pole cell formation at the posterior pole of the embryo is contingent on the presence of specialized cytoplasmic components referred to as pole plasm. Pole plasm includes a number of RNA and protein components, and its precise composition and function is not known. A number of mutations that disrupt pole plasm formation have been identified and have been ordered into a hierarchy by genetic epistasis experiments (reviewed in ref. 31). These experiments indicate that pole plasm is organized in a stepwise manner and that *oskar* mRNA plays a key role in the assembly of pole plasm (20, 32). Assembly of pole plasm components (e.g. *vasa* and *tudor*) is dependent on the dosage of *oskar*. Furthermore, ectopic localization of *oskar* to the anterior pole of the embryo results in the localization of other pole plasm components, anteriorly leading to the formation of pole cells at the anterior pole as well (21).

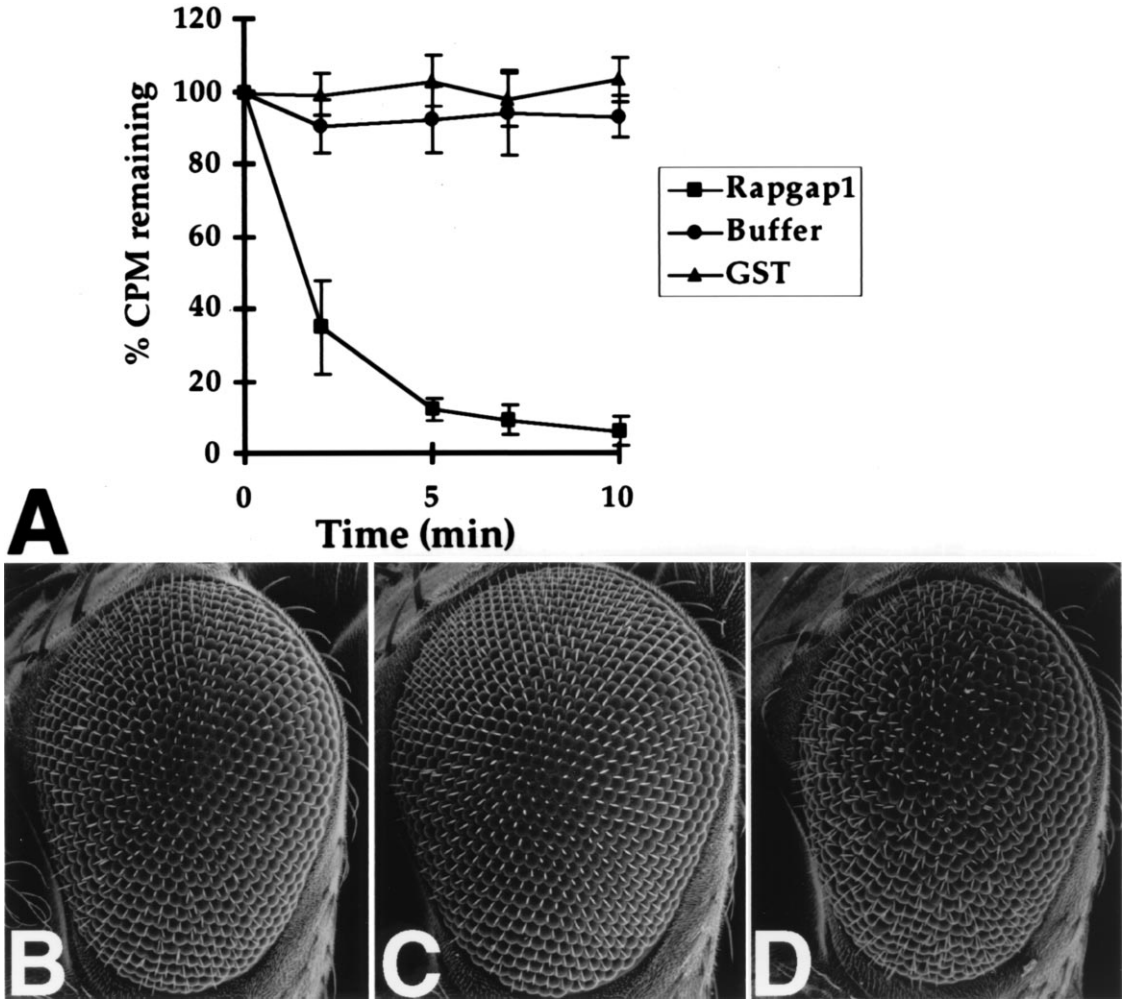

FIG. 2. Interaction of Rapgap1 with Rap1 *in vitro* and *in vivo*. (*A*) Hydrolysis of GTP by GST–Rap1 in the presence of GST–Rapgap1. Control experiments measuring hydrolysis in the presence of GST alone or in the reaction buffer are shown. (*B*) Flies expressing three copies of the *GMR–Rapgap1* transgene. (*C*) Flies heterozygous for a loss of function mutation in *Rap1*. (*D*) Flies carrying three copies of the *GMR-Rapgap1* transgene that are also heterozygous for a loss-of-function mutation in *Rap1*.

Our analysis of *Rapgap1* expression is suggestive of a role of *Rapgap1* in either pole cell formation or migration. First, *Rapgap1* mRNA is detected in the nurse cells during oogenesis and is found at the posterior pole of early embryos (Fig. 4*A*). Second, the localization of *Rapgap1* mRNA to the posterior pole is dependent on the function of a number of the posterior group genes. We examined the localization of *Rapgap1* mRNA in eggs laid by females that bear mutations in posterior group genes. These experiments indicated that, as for many other components of germ plasm (20, 32), the localization of *Rapgap1* is dependent on *oskar* localization. In embryos laid by mothers carrying four copies of the oskar gene, posterior localization of *Rapgap1* is increased (Fig. 4*B*). We also examined *Rapgap1* localization in embryos laid by mothers that also carry an *oskar* transgene with the 3′ untranslated region from the *bicoid* gene. In these embryos, where *oskar* RNA is also localized to the anterior pole (21), *Rapgap1* RNA is also localized anteriorly (Fig. 4*C*). In embryos laid by *oskar* mothers, *Rapgap1* RNA fails to localize posteriorly (data not shown). Thus, *Rapgap1* RNA localizes with other pole-plasm components in an *oskar*-dependent manner.

To place *Rapgap1* in the context of the hierarchy of posterior group genes, we examined the localization of *Rapgap1* RNA in eggs laid by females that were mutant for various posterior group genes. As expected, mutations in *cappuccino, spire,* and *staufen* which fail to localize *oskar* RNA to the posterior pole also fail to localize *Rapgap1* posteriorly (data not shown). *Rapgap1* localization also requires *vasa* (Fig. 4*D*) (33, 34) and *tudor* (Fig. 4*E*) (35) but not *nanos* (Fig. 4*F*) (36) or *pumilio* (data not shown) (37). *nanos* and *pumilio* are required for abdomen formation but not for pole cell formation. Thus, *Rapgap1* localization correlates with pole cell formation but not necessarily with abdomen formation, suggesting a specific role for *Rapgap1* in pole cell formation or migration.

Loss-of-Function Mutations in *Rapgap1* Are Viable and Fertile. To characterize the requirement of *Rapgap1 in vivo*, we generated mutations in Rapgap1 by P-element-mediated mutagenesis. We initially identified a line carrying a P-element 5 kb 5′ of the *Rapgap1* transcription unit. The P-element was mobilized to obtain "local hops" into the *Rapgap1* locus (26). Approximately 2,500 lines were screened by inverse PCR for P-element insertions in the *Rapgap1* locus. Although five independent insertions were obtained in the region of *Rapgap1*, none disrupted the transcription unit. In one instance, line 259-179, two P-element insertions were obtained that flanked the *Rapgap1* gene. By crossing flies bearing this chromosome to flies expressing a stable source of transposase, we generated, at high frequency, deletions that removed sequences between the two P-element insertion sites including the entire *Rapgap1* gene.

Flies homozygous for chromosomes that had deleted *Rapgap1* were generated. As expected, they did not express any Rapgap1 protein as detected by Western blot analysis of embryonic extracts or by antibody staining of whole-mount

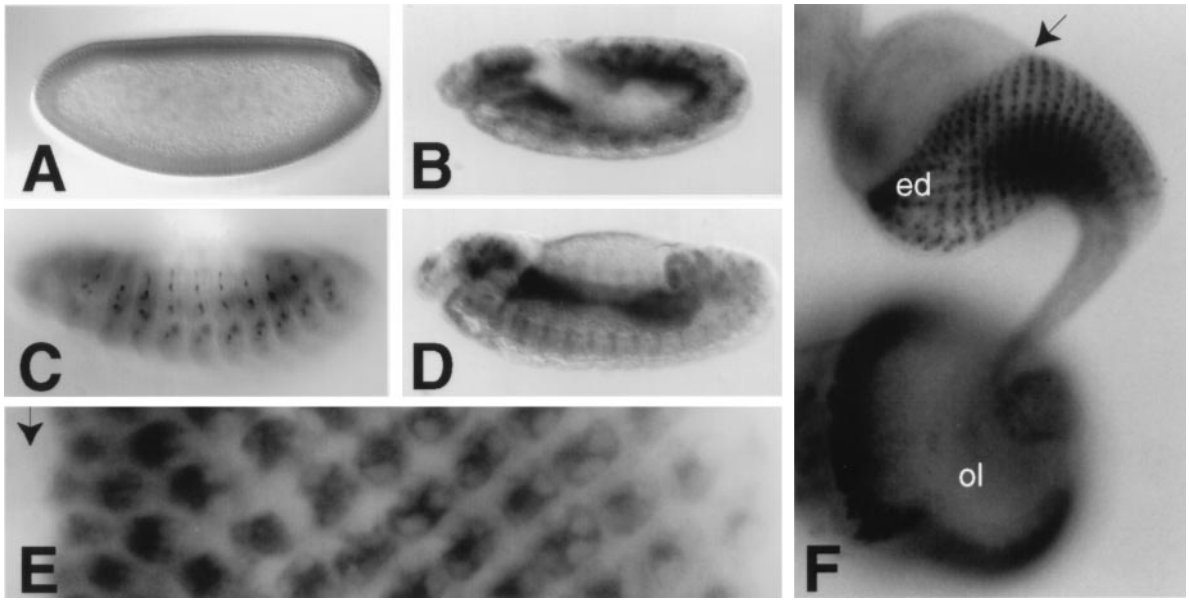

FIG. 3. Expression of Rapgap1 protein. (*A*) Cellular blastoderm embryo. (*B*) Germ-band extended embryo. (*C*) Germ-band retracted embryo; focused at the level of the peripheral nervous system. (*D*) Germ band retracted embryo; midgut primordia in focus. (*E*) Eye-imaginal disc from late third instar larva. (*F*) Eye-imaginal disc and optic lobe. The arrow in *E* and *F* indicates the morphogenetic furrow. Anterior is to the left in *A–E*. In *F*, ed refers to eye-imaginal disc and ol refers to the optic lobe.

preparations of embryos. Animals lacking *Rapgap1* appeared to be patterned normally, arguing that *Rapgap1* does not have a nonredundant function in development. Antibody staining of embryos with mAbs that recognized neurons (elav) (38), central nervous system and peripheral nervous system axons (BP104) (39), and muscle (myosin heavy chain) (40) did not reveal any obvious abnormalities. Retinal sections from flies a week after eclosion were examined for the degenerative changes that are observed in mutations which disrupt retinal innervation of the lamina. None was observed, arguing that the retinal axons had made connections with the lamina. We also examined pole cell migration in mutant animals by staining mutant embryos with anti-Vasa antibody. Pole cell formation occurs normally and pole cell numbers are not reduced. However, whereas pole cells appear to be aligned precisely in the gonad in stage 13 wild-type embryos, pole cells in mutant embryos occasionally displayed minor irregularities in their alignment. In summary, loss-of-function mutations in *Rapgap1* do not result in obvious phenotypic abnormalities.

DISCUSSION

We have identified and characterized a *Drosophila* gene, *Rapgap1*, that encodes a protein with similarity to mammalian RapGAP. The sequence similarity is confined to a 360-amino acid region in the middle of the protein, which suggested that this domain was likely to be the catalytic domain. We have shown that this domain is sufficient to function as a GAP for Rap1 *in vitro*. This domain that is conserved between human RapGAP and *Drosophila* Rapgap1 closely approximates the region defined as the catalytic domain by others using deletions in mammalian RapGAP (41). Thus, this region appears to constitute the core RapGAP domain; regions outside the core domain may specify specialized properties of individual RapGAPs. The sequences in Rapgap1 outside this domain have no significant similarity to known genes except for a short motif near the C terminus which displays similarity to human RapGAP. Thus, the function of the rest of the protein is at present unclear.

In stark contrast to *Rap1*, which is expressed widely (30), *Rapgap1* is expressed in specific groups of cells. For instance, in the eye-imaginal disc, Rapgap1 is expressed in cells of the photoreceptor clusters posterior to the morphogenetic furrow and is not expressed in the proliferating cells anterior to the morphogenetic furrow. Thus, different subpopulations of cells in the same tissue differ in terms of Rapgap1 expression. Moreover, Rapgap1 protein is found in the axons of the

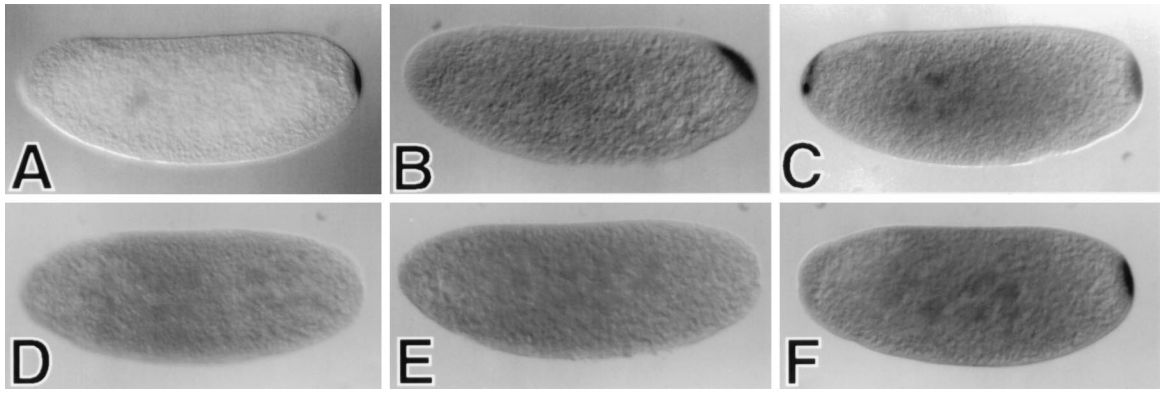

FIG. 4. *Rapgap1* mRNA localization is dependent on posterior group genes. *Rapgap1* RNA detected by *in situ* hybridization. Embryos collected from females of the following genotypes: (*A*) wild-type, (*B*) $oskar^{AK}$ (four copies *oskar*), (*C*) *oskar-bcd-3′UTR*, (*D*) $vasa^{D1}$, (*E*) $tudor^{WC8}$, and (*F*) $nanos^{BN}$. Anterior is to the left.

developing photoreceptor cells but not in central nervous system axons, thus emphasizing its strict tissue-specific expression. This suggests the existence of other GAPs for Rap1; multiple GAPs may have partially overlapping patterns of expression. This also appears to be the case for Ras1 and its GAPs. Although *Ras1* is expressed ubiquitously, at least one of the GAPs for Ras1, Gap1, is expressed in a very restricted pattern.

Most intriguingly, we have shown that Rapgap1 colocalizes with germ plasm, which suggests a role for Rap1-mediated signaling in pole-cell formation, maintenance, or migration. We have also observed that pole-cell migration is perturbed in embryos that lack maternally provided Rap1 (H. Asha and I.K.H., unpublished observation). Thus, Rapgap1 may modulate Rap1 signaling in pole cells. However, pole-cell formation and migration occurs normally in flies that completely lack *Rapgap1* function. Thus, such a function of *Rapgap1* must be largely redundant.

The apparent redundancy of Rapgap1 function is reminiscent of some of the properties of mutations in genes encoding GAPs for *Drosophila* Ras1. Mutations in *Gap1* cause patterning defects in the eye and in adult appendages (42). Mutations in NF1 result in a growth defect and in defects in synaptic transmission at the larval neuromuscular junction (27). Flies bearing mutations in both *Gap1* and *NF1* appear to be inviable, suggesting that the two GAPs may, to some extent, have a redundant function. Examples of mutations that result in no obvious phenotype except in the background of other mutations include the genes *trp* and *trpl* that encode the light-activated ion channels (43) as well as the *arrestin 1* and *arrestin 2* genes which function in inactivating metarhodopsin (44). In each of these instances, one of the two genes accounts for most of the function and as a consequence, mutations in *arrestin 1* and *trpl* have no discernible phenotype on their own and their role becomes apparent only when the other gene is mutated. Thus, the absence of an obvious phenotype in loss-of-function *Rapgap1* mutants argues for the existence of other GAPs for Rap1.

The demonstration that the phenotype induced by overexpression of *Rapgap1* is extremely sensitive to *Rap1* gene dosage argues that Rapgap1 is likely to function as a GAP for Rap1 *in vivo*. However, *Rapgap1* function may be significantly redundant and may become only apparent under extremely specific experimental conditions or in situations where mutations compromise the function of the other GAPs for Rap1. In this context, it is worth considering that in mammalian cells, the *tuberin* gene product also functions as a GAP for Rap1. Hence, a *Drosophila tuberin* homologue may functionally substitute for *Rapgap1* in some situations.

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