Locomotion defects, together with Pins, regulates heterotrimeric G-protein signaling during Drosophila neuroblast asymmetric divisions

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Heterotrimeric G proteins mediate asymmetric division of Drosophila neuroblasts. Free Gβγ appears to be crucial for the generation of an asymmetric mitotic spindle and consequently daughter cells of distinct size. However, how Gβγ is released from the inactive heterotrimer remains unclear. Here we show that Locomotion defects (Loco) interacts and colocalizes with Gβ1 and, through its GoLoco motif, acts as a guanine nucleotide dissociation inhibitor (GDI) for Gβ1. Simultaneous removal of the two GoLoco motif proteins, Loco and Pins, results in defects that are essentially indistinguishable from those observed in Gβ1 or Gγ1 mutants, suggesting that Loco and Pins act synergistically to release free Gβγ in neuroblasts. Furthermore, the RGS domain of Loco can also accelerate the GTPase activity of Gβ1 to regulate the equilibrium between the GDP- and the GTP-bound forms of Gβ1. Thus, Loco can potentially regulate heterotrimeric G-protein signaling via two distinct modes of action during Drosophila neuroblast asymmetric divisions.

Keywords: Neuroblast, asymmetric cell division, Loco, heterotrimeric G proteins

Received January 5, 2005; revised version accepted April 19, 2005.

Asymmetric cell division is a universal mechanism used to generate cellular diversity during development. The Drosophila embryonic central nervous system (CNS) derives largely from neural progenitors called neuroblasts (NBs). NBs delaminate from the neuroectoderm and undergo asymmetric cell division along the apical/basal axis to give rise to two daughters of distinct fate and size. The larger apical daughter cell retains a NB identity and undergoes repeated asymmetric divisions, whereas the smaller basal daughter differentiates into a ganglion mother cell (GMC) that divides only once to generate two neurons/glia (Campos-Ortega 1997). Three well-characterized features of the NB asymmetric divisions (Jan and Jan 2001; Knoblich 2001; Wodarz and Huttner 2003) are (1) asymmetric localization and segregation of cell fate determinants and their adaptor proteins Numb/Partner of Numb (Pon), Prospero (Pros)/Miranda (Mira) into the basal GMC; (2) reorientation of the mitotic spindle along the apical/basal axis at metaphase; (3) generation of an apically biased asymmetric mitotic spindle (Kaltschmidt et al. 2000; Kaltschmidt and Brand 2002) and the displacement of the spindle toward the basal cortex during ana/telophase as well as asymmetric formation of astral microtubules (MTs) (Giansanti et al. 2001), which lead to the generation of two unequal-sized daughter cells.

These features of the NB asymmetric division are controlled by an apically localized complex of proteins that include the Drosophila homologs (Doe and Bowerman 2001) of the conserved Par3 (Bazooka, Baz)/Par6 (DmPar6)/aPKC (DaPKC) protein cassette first identified in Caenorhabditis elegans (Kemphues 2000), the novel protein Inscuteable (Insc), Gβ1, a subunit of heterotrimeric G proteins (Schaefer et al. 2001; Yu et al. 2003), and an evolutionarily conserved molecule, Partner of Insc (Pins) (Parmentier et al. 2000; Schaefer et al. 2000; Yu et al. 2000) that acts as a guanine nucleotide dissociation inhibitor (GDI) for Gai. Loss of single members of the apical complex, such as baz or pins, results in defective basal protein localization and spindle misorientation in mitotic NBs up to metaphase, although these defects can be partially corrected late in mitosis, a phenomenon called telophase rescue (Schuber et al. 1999; Peng et al. 2000). However, unlike basal protein localization and

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Article and publication are at http://www.genesdev.org/cgi/doi/10.1101/gad.1295505.
spindle orientation, the generation of an asymmetric spindle and its displacement toward the basal cortex are largely unaffected, and NBs lacking one component of the apical complex usually divide like wild-type NBs to produce two unequal-sized daughter cells. Simultaneous disruption of the two redundant apical pathways, Baz/DaPKC and Pins/Gai, prevents the formation of an asymmetric spindle, and two daughter cells of similar size are produced [Cai et al. 2003].

Heterotrimeric G proteins have been shown to be involved in controlling distinct microtubule-dependent processes in one-cell embryos of C. elegans [Gotta and Ahringer 2001]. Gβγ is important for correct centrosome migration around the nucleus and spindle orientation, while Ga subunits, GOA-1 and GPA-16, are required for asymmetric spindle positioning. Recent studies have shown that the GoLoco-motif-containing proteins, GPR1/2, act as GDIs for GOA-1 and GPA-16 to translate polarity cues, mediated by the asymmetrically localized Par proteins, into asymmetric spindle positioning in the C. elegans zygote [Colombo et al. 2003; Gotta et al. 2003; Srinivasan et al. 2003]. In Drosophila NBs, heterotrimeric G proteins Gβ13F and Gγ1 are required for the asymmetric localization/stability of the apical components and, hence, the formation of an asymmetric spindle. This is likely to be achieved through the generation of free Gβγ since depletion of Gβγ function by overexpression of wild-type Gai/Gao [Schafer et al. 2001; Yu et al. 2003] or loss of Gβ13F or Gγ1 function [Fuse et al. 2003; Izumi et al. 2004] can lead to the generation of a symmetric and centrally placed mitotic spindle, and NBs frequently divide to produce daughter cells of similar size [henceforth referred to as “similar-sized divisions,” defined below]. Thus, generation of free Gβγ is crucial for NB asymmetric divisions. However, it is not clear whether Gβγ mediates spindle geometry independently of the Go subunit(s) or alternatively by controlling the localization of Go subunit(s) and/or the GoLoco proteins. Pins has previously been shown to act as a GDI to facilitate the dissociation of Gβγ from heterotrimeric binding to and stabilizing the GDP-bound form of Gai [GDP-Gai] [Schafer et al. 2001]. However, paradoxically, loss of pins function does not produce the severe spindle defects seen in the Gβ13F or Gγ1 mutant NBs, suggesting that the absence of the Pins GDI activity does not prevent the generation of free Gβγ. Similarly, loss of Gai, while causing defects in spindle orientation and the localization of the basal proteins up to metaphase, like pins loss of function, also does not cause the severe spindle asymmetry defects seen in Gβ13F or Gγ1 mutant NBs; however, it remains possible that additional Go subunits may be involved in this process.

Here we show that locomotion defects [loco], a gene previously shown to be required for glial cell differentiation and dorsal–ventral patterning [Granderath et al. 1999; Pathirana et al. 2001], encodes a novel component of the NB apical complex that exhibits both guanine nucleotide dissociation inhibitor (GDI) and GTPase-activating protein (GAP) activities for Gai. Loco interacts with GDP-Gaii through its GoLoco motif [Siderovski et al. 1999] and forms a complex with Gai in vivo. Loco colocalizes with Gai and Pins at the apical cortex of NBs throughout mitosis and is required for the asymmetric localization/stabilization of Pins/Gai. Analyses of various double-mutant NBs suggest that Loco, like Pins and Gai, functions redundantly with the Baz/DaPKC pathway in regulating spindle geometry. Interestingly, loss of both loco and pins functions leads to similar-sized divisions in the majority of NBs, similar to that seen in either Gβ13F or Gγ1 mutants, suggesting that activation of Gβγ is mediated in a redundant manner by both Loco and Pins. Our data therefore provide functional support for the idea that the activation of heterotrimeric G-protein signaling through the generation of free Gβγ, crucial for NB asymmetric divisions, can occur via a receptor-independent mechanism by using multiple GDIs that functionally overlap. Moreover, we show that Loco can, through its RGS domain [De Vries and Gist Farquhar 1999], also function as a GAP to regulate the balance between GDP-Gai and GTP-Gai. Hence, both the GDI and GAP functions of Loco are important for NBs to regulate the activities of Gai and Gβγ.

Results

Loco, a GoLoco motif protein, interacts with GDP-Gaii and can function as a GDI

In Drosophila NBs, the activation of heterotrimeric G-protein signaling can in principle occur via a receptor-independent mechanism through the release of Gβγ from the inactive heterotrimer GDP-GaiiGβγ, which is facilitated by the binding of Pins as a GDI to GDP-Gaii [Schafer et al. 2001]. The GoLoco motif of Pins should therefore play a critical role through its GDI function to complex with GDP-Gaii and generate free Gβγ. However, previous studies have shown that inactivation of Gβγ by either loss of function of Gβ13F or Gγ1 or overexpression of wild-type Gai/Gao leads to delocalization/destabilization of both apical pathway components and the generation of similar-sized daughter cells in the majority of telophase NBs, whereas loss of pins function has relatively milder effects, for example, producing similar-sized daughters [defined as telophase NBs from stage 10 embryos in which the ratio of the GMC/NB diameter is >0.8; for wild-type NBs, GMC/NB = 0.43 ± 0.08] from only a small proportion of NB divisions [15%] [Cai et al. 2003; Fuse et al. 2003; Yu et al. 2003; Izumi et al. 2004]. We reasoned that if a GDI-mediated receptor-independent mechanism were to be responsible for G-protein activation in NBs, then other unidentified GDI(s) must exist that can activate Gβγ activity even in the absence of pins function. We therefore searched the annotated Drosophila genome and identified only three GoLoco-motif-containing proteins, namely, Pins, Loco, and RapGAP2. Further analysis indicated that while RapGAP2 appears not to be expressed in NBs [R. Kaushik, unpubl.], Loco plays a key role and is asymmetrically localized in mitotic NBs.
There exist at least four alternatively spliced forms of Loco protein that all include a common core region containing a RGS domain, two Ras-like Raf-binding domains (RBDs), and a GoLoco motif [Fig. 3A, below]. Database searches further revealed that two homologs of Drosophila Loco, RGS12 and RGS14, exist in vertebrates [Kimple et al. 2001], suggesting that loco is duplicated in vertebrates during evolution. We have confirmed a previously reported [Granderath et al. 1999] interaction between Gai and the GoLoco motif of Loco in yeast two-hybrid assays. We further observed that GaiQ205L, a presumably constitutively active (GTP-bound) form, fails to interact with the GoLoco motif of Loco in yeast two-hybrid assays, suggesting that the GoLoco motif of Loco preferentially binds to GDP-Gai [Fig. 1A]. These observations were further confirmed using GST pull-down assays. 35S-labeled Gai can interact with GST-GoLoco but not with GST alone, whereas 35S-labeled GaiQ205L cannot interact with GST alone and interacts very poorly with GST-GoLoco [Fig. 1B].

To show that the physical interaction between Gai and Loco reflects an in vivo interaction, we made use of a transgenic fly strain that can be induced by heat shock to express Loco-C2 fused with two tandem Flag epitopes at its C terminus. Loco-Flag, when induced at low levels, colocalizes with Pins and Gai as apical cortical crescents in NBs [data not shown; see also Fig. 2A–D]. In coimmunoprecipitation (CoIP) experiments, when the immunocomplex was precipitated using anti-Gai antibody [Schafer et al. 2001], Loco-Flag can be detected by an anti-Flag antibody, only from HS but not non-HS embryonic extracts; endogenous Pins, detected using an anti-Flag antibody [Yu et al. 2002], CoIPs with Gai from both HS and non-HS embryonic extracts [Fig. 1C]. Although Gai can CoIP both Loco and Pins, Loco-Flag can CoIP only Gai but not Pins from HS embryonic extracts [Fig. 1D], suggesting that Loco and Pins do not simultaneously complex with the same Gai molecule. To test whether the GoLoco motifs of Loco and Pins can act as GDIs, we carried out in vitro GDI assays. The GoLoco motifs of Loco and Pins decrease the rate of exchange of GDP for GTP on Gai [Fig. 1E], indicating that both Pins and Loco can act as GDIs for Gai.

Loco colocalizes with and depends on Pins and Gai for its apical localization

To ascertain the subcellular localization of Loco, we generated anti-Loco antibodies against two regions of the core domain shared by all Loco isoforms [amino acids 357–636 and 564–731 of Loco-C1]. These two antibodies were found to be specific for Loco since identical immunofluorescence signals were seen in wild-type embryos and these signals were absent in embryos depleted for both maternal and zygotic loco [Fig. 3P]. Loco localizes as a crescent to the apical cortex as early as late interphase [Fig. 2A’]. From prophase onward, Loco forms an apical crescent and segregates into the apical daughter cell at telophase [Fig. 2B’–D’], colocalizing with Pins [Fig. 2A–D] and Gai [Fig. 2E, E’] in mitotic NBs.
which both maternal and zygotic components were removed. In insc NBs, Loco was observed as an apical crescent of reduced intensity (75%, n = 48) [Fig. 2F] or is undetectable (25%, n = 48) [data not shown]. Similar results were seen in baz NBs [data not shown]. Loco is uniformly distributed around the cortex in pins metaphase NBs [100%, n = 20] [Fig. 2G]; while in Gai NBs, Loco is unable to be localized to the cortex and shows cytosolic localization [100%, n = 29] [Fig. 2H]. Similar to that seen in Gai NBs, Loco is distributed in the cytosol with no obvious cortical signal in Gβ13F NBs [100%, n = 32] [Fig. 2I]. When wild-type Gai is overexpressed, Loco [Fig. 2J] as well as Gai [Fig. 2I] and Pins [data not shown] become uniformly distributed around the cell cortex [100%, n = 20]. When Insc is overexpressed in epithelial cells, Loco is recruited from the basolateral to the apical cortex [data not shown], similar to Pins [Yu et al. 2000].

Taken together, these data indicate that Loco is a novel component of the apical complex and its asymmetric localization/stability requires other apical components as well as Gβ13F; its cortical localization requires Gai, and its apical localization requires Pins.

Loco is required for asymmetric localization of Gai and Pins and acts in parallel with the Baz/DaPKC pathway to mediate asymmetric daughter cell size

Given that no embryos could be obtained from germline clones [GLCs] using previously described loss-of-function alleles of loco and analyses of zygotic loss-of-function embryos revealed no obvious defects in NB asymmetric division, we carried out imprecise excisions using a P-element, EY04589, which is inserted 310 bp upstream of the start point of loco-c1 transcription [Bellen et al. 2004]; three new alleles, loco
to, loco
p263, and loco
p237, were isolated that delete either partially or entirely the core region of the loco protein isoforms [Fig. 3A]. The detailed molecular lesions associated with these alleles are given in Materials and Methods. These alleles do not show zygotic loss-of-function defects for NB divisions. Both loco
p263 and loco
p237 homozygotes are viable and display severe locomotion defects, similar to homozygotes of Gai and pins null mutants, suggesting that they may share similar function. To obtain loco mutant embryos that lack both maternal and zygotic components, we crossed mutant mothers homozygous for the alleles loco
p263 or loco
p263 or trans-heterozygous for the alleles loco
p263 and loco
p237 to heterozygous loco
p263, loco
p263, or loco
p237 males. Immunofluorescence confirmed that those resultant embryos are antigen-minus [Fig. 3P], suggesting that both loco
p263 and loco
p237 are strong, possibly null alleles. Embryos derived from either loco
p263/loco
p263 or loco
p263/loco
p237 mothers display indistinguishable phenotypes in NB asymmetric divisions, suggesting that loco
p263 is an amorphic allele. We henceforth refer to loco
p263 embryos lacking both maternal and zygotic components as loco mutants. In this study all phenotypic analyses described for single- and double-mutant combinations were performed using embryos lacking both maternal and zygotic components.

In the majority of loco mutant NBs, Pins is no longer apical but rather shows uniform cortical distribution with some cytosolic signal [90%, n = 90] [Fig. 3B–E]. Occasionally, weak crescents of Pins were observed in in-
terphase/prophase NBs (12%, n = 43), where Pins colocalizes with Goi (Fig. 3Q). When detected using a specific antibody raised against full-length Goi (see Materials and Methods), Goi shows uniform cortical localization in both pins (100%, n = 19) (Schaef et al. 2001; data not shown) and loco mutant metaphase NBs (100%, n = 25) (Fig. 3G). Insc is cytoplasmic (67%, n = 45) (Fig. 3I); DaPKC (86%, n = 50) (Fig. 3K) and Baz (data not shown) remain asymmetrically localized in the majority of loco mutant NBs, although the intensity of the crescents was dramatically reduced, a phenotype also seen in NBs lacking pins, Goi, or Gβ13F function. Similar to that seen in pins or Goi mutants, in loco mutants the basal proteins Mira/Pros and Pon/Numb can be mislocated relative to the overlying ectoderm at metaphase (52%, n = 21) (Fig. 3M; data not shown). Gβ13F remains uniformly cortical, similar to that seen in wild-type NBs (data not shown). Mitotic spindle orientation is also disturbed in loco mutants; in cells of mitotic domain 9, mitotic spindle that normally rotates by 90° to align along the apical/basal axis in wild type (Fig. 3N) often fails to reorientate (Fig. 3O).

Wild-type NBs normally divide to give rise to a large apical NB and a smaller basal GMC (Fig. 4A,E). The great...
Loco acts redundantly with the Baz/DaPKC/DmPar6/Insc pathway to regulate spindle displacement and asymmetry, as well as daughter cell size difference. In wild-type telophase NBs (A,E), the mitotic spindle (deduced from positions of the centrosomes) [E] is apically biased and spindle displacement occurs toward the basal cortex to give rise to two daughter cells of unequal size. In loco mutants (B,F), 10% of telophase NBs generate two daughter cells of similar size. loco/insc double-mutant NBs (C,G) show similar-sized divisions in all telophase NBs [100%, see text]. (D,H) Similarly, removal of baz function in loco NBs increases the frequency of similar-sized divisions to full expressivity. [F-H] In similar-sized divisions, the mitotic spindle is symmetric and both centrosomes lie in close vicinity of the cell cortex. NBs were marked by Asense, which is cytosolic green in A–D, BP106, a plasma membrane marker, in red [A–H], and CNN, a centrosome marker in green in E–H. DNA is in cyan. Apical is up.

The majority of loco mutant NBs divide asymmetrically to produce daughters of different size like wild-type NBs (data not shown). However, similar to pins or Gai mutants, a small proportion of loco mutant NBs undergo similar-sized division [10%, n = 69] (Fig. 4B,F). Previous studies have suggested that two redundant pathways, the Pins/Gai and the Baz/DaPKC/[DmPar6/Insc] pathways, act redundantly to control daughter cell size difference (Cai et al. 2003). We analyzed the relative size of the two daughter cells in double mutants of loco/insc or loco/baz RNAi. In all dividing NBs, similar-sized divisions were observed in loco/insc [100%, n = 42] (Fig. 4C,G) and loco/baz RNAi [97%, n = 31] (Fig. 4D,H) double mutants. In addition, spindle displacement and asymmetry are both disrupted in these double mutants, as revealed by anti-centrosomin [CNN] staining (Fig. 4F–H).

Taken together, loco loss of function displays defects similar to those seen in pins or Gai mutants, and Loco acts redundantly with the Baz/DaPKC pathway to regulate spindle displacement and asymmetry, as well as daughter cell size difference.


ectopic expression of Loco can drive Pins off the apical cortex

To ascertain the effects of overexpressing Loco on NB asymmetric divisions, we expressed the Loco-C1 isoform under the control of a strong maternal driver, mata-gal4 VP16 V32. Under these conditions, anti-Loco immunofluorescence in NBs appears more intense than in wild type [Fig. 6C]; two types of Loco distribution were observed, uniformly cortical [25%, n = 64] (Fig. 6A) or apically enriched [75%, n = 64] (Fig. 6B). In either case, Loco colocalizes with Gai in mitotic NBs [Fig. 6A,B]. Strikingly, ectopic expression of Loco leads to cytoplasmic
To determine whether the RGS domain of Loco is able to interact with Gai and whether this interaction is nucleotide-dependent, bacterially expressed GST or GST-RGS was incubated with in vitro translated 35S-labeled Gai in the presence of GTPγS, GDP, or GDP + AlF4− to mimic the transition state of GTP hydrolysis. While GST-RGS is able to pull down Gai only to a low extent in the presence of either GDP or GTPγS, the presence of GDP + AlF4− strongly promotes the interaction between GST-RGS and Gai [Fig. 7A, upper panel]. These results suggest that the RGS domain of Loco possesses preferential affinity to the transition-state conformation of Gai during GTP hydrolysis. To ascertain that GST-RGS can interact with endogenous Gai from embryos, GST-RGS or GST alone was incubated with embryonic extracts. A significant amount of Gai could be detected by immunoblotting the protein complex bound to GST-RGS, but not in the control [Fig. 7A, lower panel], suggesting that the RGS motif of Loco is likely to interact with Gai in vivo. Since the RGS domain is able to interact with Gai, we further carried out GAP assays to test whether the RGS domain can stimulate GTP hydrolysis. In the absence of GST-RGS, Gai has only weak intrinsic GTPase activity; addition of GST-RGS fusion protein accelerates the GTPase activity of Gai significantly [Fig. 7B]. Taken together, these data indicate that Loco can also act as a GAP for Gai through its RGS domain, which may, in turn, contribute to the regulation of the balance between GTP-Gai and GDP-Gai levels in NBs.

**Figure 5.** Loco acts to activate Gαγ activity in conjunction with Pins. [A–F] Confocal images of triple-labeled telophase NBs [BP106, a membrane marker, red [A–D]; DNA, cyan [A–F]; Asense, a NB marker, cytoplasmic green [A,B]; CNN, a centrosome marker, green [C,D]; α-tubulin, red [E,F]; and Miranda, green [E,F]] showing unequal size divisions in wild-type [A,C,E] and similar-sized divisions in loco/pins double mutants [B,D,F]. NBs from pins/loco double GLC embryos show high frequencies of similar-sized divisions [B] (63%, see text) in which the mitotic spindle is symmetric, as judged from CNN staining (cf. wild-type [C] and double mutants [D]). [E] In wild-type NBs astral microtubules are associated only with the apical centrosome, they grow out robustly and form a prominent, cap-like structure (arrow). [F] However, in loco/pins NBs that undergo similar-sized divisions, two astral microtubule caps are formed, one over each centrosome (arrows). Gai, apical in wild-type NBs [G], is cortically localized in pins/loco double mutants [H]. In loco/pins double mutants, DaPKC is nearly undetectable in 71% of NBs [I] and show weak crescents in the rest of the NBs [K], compared with that in wild-type NBs [J]. Forty percent of loco/pins NBs [n = 40] show mislocalization [M] or cortical localization [N] of Mira at metaphase; however, as in wild type [O], Mira segregates to one of the daughter cells at telophase [P]. DNA is in cyan. Apical is up. [Q] Quantitation of the daughter cell sizes and their ratios in wild-type and various mutant NBs. n is the number of telophase NBs scored. The diameters of GMCs (the relatively smaller cell) and NBs (the relatively large cell) in telophase NBs of stage 10 embryos were measured for each genotype. The data are means ± SD. GMC/NB is the ratio of the diameter of GMC relative to its sibling NB.

**Loco can act as a GAP to regulate the GTPase activity of Gai through its RGS domain**

To determine whether the RGS domain of Loco is able to interact with Gai and whether this interaction is nucleotide-dependent, bacterially expressed GST or GST-RGS was incubated with in vitro translated 35S-labeled Gai in the presence of GTPγS, GDP, or GDP + AlF4− to mimic the transition state of GTP hydrolysis. While GST-RGS is able to pull down Gai only to a low extent in the presence of either GDP or GTPγS, the presence of GDP + AlF4− strongly promotes the interaction between GST-RGS and Gai [Fig. 7A, upper panel]. These results suggest that the RGS domain of Loco possesses preferential affinity to the transition-state conformation of Gai during GTP hydrolysis. To ascertain that GST-RGS can interact with endogenous Gai from embryos, GST-RGS or GST alone was incubated with embryonic extracts. A significant amount of Gai could be detected by immunoblotting the protein complex bound to GST-RGS, but not in the control [Fig. 7A, lower panel], suggesting that the RGS motif of Loco is likely to interact with Gai in vivo. Since the RGS domain is able to interact with Gai, we further carried out GAP assays to test whether the RGS domain can stimulate GTP hydrolysis. In the absence of GST-RGS, Gai has only weak intrinsic GTPase activity; addition of GST-RGS fusion protein accelerates the GTPase activity of Gai significantly [Fig. 7B]. Taken together, these data indicate that Loco can also act as a GAP for Gai through its RGS domain, which may, in turn, contribute to the regulation of the balance between GTP-Gai and GDP-Gai levels in NBs.

**The effects of disturbing the balance of GTP-Gai and GDP-Gai on NB asymmetric divisions**

To assess the effects of shifting the equilibrium of Gai toward either the GTP- or GDP-bound forms on NB
In the above situation, there should still be residual wild-type endogenous GDP-Gα. To create a more extreme situation, we overexpressed GαQ205L in a Gαi mutant background. Under these conditions, where there should be no GDP-Gα with all the Gαi in the GTP-bound form, we observed more severe defects in asymmetric protein localization; the low level of Pins that can be detected is cytosolic (Fig. 7I), while Loco (Fig. 7J) and GαQ205L [Fig. 7K] remain uniformly cortically localized. These observations suggest that, in vivo, Pins can associate only with GDP-Gαi, GTP-Gαi in the absence of GDP-Gαi cannot direct Pins to the cell cortex, in contrast to Pins, Loco can be localized to the cortex by either GTP-Gαi or GDP-Gαi [see also the next paragraph]. These observations along with the biochemical data support the view that both GTP-Gαi and GDP-Gαi (see also the next paragraph). These observations along with the biochemical data support the view that both GTP-Gαi and GDP-Gαi (see also the next paragraph)

Discussion

Previous studies have shown that heterotrimeric G-protein components play important roles in NB asymmetric divisions [Schaefer et al. 2001; Fuse et al. 2003; Yu et al. 2003; Izumi et al. 2004]. In this study we consider the
issues of how heterotrimeric G-protein activation might be mediated during NB asymmetric divisions and the roles that Gβγ, GTP-Gai, and GDP-Gai play in this process. We show that Loco is a novel asymmetrically localized component of the NB asymmetric division machinery that possesses both GDI and GAP activities for Gai. We provide evidence that indicates that the redundant GDI activities of Pins and Loco lead to the generation of free Gβγ, which plays a crucial role for the formation of an asymmetric mitotic spindle and daughter cells of distinct size. Based on loss-of-function phenotype, Gai appears to play a less important role than Gβγ in this process; however, the proper balance between the levels of GTP- and GDP-bound forms of Gai, which may be mediated, at least in part, by the GAP activity of Loco, is crucial for the asymmetric localization of Pins and Insc. It is important to note that there may exist additional Gai subunit[s] that might functionally overlap with Gai in the generation of an asymmetric spindle. Therefore the possibility that Gβγ might mediate asymmetric spindle geometry by regulating the localization Gai subunit[s] (and GoLoco proteins) cannot be excluded at this point.

Figure 7. Loco also acts as a GAP to regulate the GTPase activity of Gai through its RGS domain. [A] GST-RGS can also bind to Gai. [Upper panel] The binding assay was carried out between 35S-labeled Gai and GST alone or GST-RGS. GST-RGS has weak binding activity with Gai in the presence of GTPγS or GDP but much higher affinity to Gai in the presence of GDP and AlF_4^- [Lower panel] GST-RGS but not GST alone is capable of complexing with endogenous Gai [see text]. [B] Loco exhibits GAP activity for Gai. GST-RGS can accelerate the GTPase activity of Gai. [C–P] Overexpression of two mutant forms of Gai in wild-type or Gai mutant backgrounds. In wild-type NBs, Pins [E, red] and Insc [G, green] are localized as intense apical crescents. In wild-type NBs ectopically expressing GaiQ205L, GaiQ205L is cortically distributed [C, red] and colocalizes with Loco [D, green]. [E] Ectopic expression of GaiQ205L leads to disruption of Pins crescents [red] in 84% of NBs. [F] Similarly, Insc localization [green] is also disrupted. Note that NBs in panels E and F are identical to those in panels G and H, respectively, and those images were taken at the same gain. In Gai NBs ectopically expressing GaiQ205L [I–L], GaiQ205L is cortically localized [I, red] and Pins is cytosolic [J]. while Loco is distributed around the cell cortex [L]. In Gai NBs ectopically expressing GaiG204A [the GDP-bound form] [M–P], GaiG204A is cortically localized during mitosis [M, O, red]; both Loco [N, green] and Pins [P, green] are localized around the cell cortex. [Q] A working model for receptor-independent activation of heterotrimeric G proteins in Drosophila NBs. See Discussion.

Multiple GDIs mediate receptor-independent activation of heterotrimeric G proteins during NB asymmetric divisions

Heterotrimeric G proteins are classically known to transmit extracellular signals to targets within the cell
through seven transmembrane, G-protein coupled receptors (GPCRs). Upon ligand binding, GPCR acts as a GEF to stimulate release of GDP from the Go subunit, which, in turn, is converted to the GTP-bound form. GTP-Go and Gβγ dissociate and activate their respective effectors to initiate downstream signaling. G-protein signaling is attenuated through the hydrolysis of GTP to GDP by the GTPase activity of Go, which is accelerated by GAPs, which often contain a RGS domain. GDP-Go can reassociate with and inactivate Gβγ.

Analyses of loss of function of GB13F and Gγ1 as well as gain of function of Gai in NBs have provided compelling support for the view that free Gβγ is required for the asymmetric localization/stability of both apical pathway components as well as the generation of asymmetric spindle and daughter cell size. Gai is required primarily for the asymmetric localization of Pins and makes only a minor contribution in regulating spindle geometry and asymmetric daughter cell size. The mechanism by which heterotrimeric G-protein activation (generation of free Gβγ) is mediated in NBs has been unclear. The fact that no G-protein-coupled receptors (GPCRs) have been implicated in NB asymmetric divisions, the apparent intrinsic polarity exhibited by cultured NBs, as well as the observed GDI activity associated with Pins have raised the possibility that heterotrimeric G-protein activation may occur via a receptor-independent mechanism since GoLoco-containing molecules like Pins should be able to generate free Gβγ from the heterotrimeric complex by competing for binding to GDP-Gai [Takesono et al. 1999, Natochin et al. 2000; Schaefer et al. 2001]. However, loss of pins does not cause the majority of NBs to produce daughters of similar size and is therefore inconsistent with a failure to activate G-protein signaling.

This apparent contradiction is resolved by our observations, which indicate that receptor-independent activation of heterotrimeric G-protein signaling may be mediated through the GDI activities of both Pins and Loco. Like Pins, Loco can interact with GDP-Gai through its GoLoco motif and form an in vivo complex with Gai. In NBs, Loco colocalizes with Gai and Pins at the apical cortex throughout mitosis. Removal of maternal and zygotic loco leads to delocalization of Gai/Pins. Analysis of double mutants indicates that Loco functions redundantly with the Baz/DaPKC pathway with respect to the generation of differential daughter size. Simultaneous loss of both loco and pins results in phenotypic defects essentially indistinguishable to those seen in GB13F or Gγ1 loss-of-function NBs. These observations indicate that receptor-independent activation of heterotrimeric G proteins during Drosophila NB asymmetric division may be achieved through the actions of the two functionally redundant GDI activities of Pins and Loco [Fig. 7Q].

The GAP activity of Loco and relevance of the equilibrium between GDP-Gai and GTP-Gai

In addition to its GDI activity, Loco also possesses a RGS domain that exhibits GAP activity for Gai in vitro, suggesting that Loco can regulate Gai via two distinct modes of action, both as a GDI and as a GAP. Our studies suggest that Gβγ, activated by the GDI activity of Pins and Loco, is crucial for NBs to produce daughters of unequal size, while the equilibrium between GDP-Gai and GTP-Gai, regulated, at least in part, by the GAP activity of Loco, is required for the localization of Insc/Pins/Loco at the apical cortex in NBs. When the equilibrium is shifted toward GTP-Gai, that is, when GaiQ205L (the constitutively GTP-bound form) is expressed in the absence of endogenous wild-type Gai, Pins becomes delocalized/distabilized because it requires binding to GDP-Gai to localize to the cell cortex; however, the ability to generate an asymmetric spindle and unequal-size daughters is not compromised since Gβγ function should not be compromised. Conversely, when the equilibrium is shifted toward GDP-Gai, through the ectopic expression of GaiG204A (the constitutively GDP-bound form) in the absence of endogenous wild-type Gai, free Gβγ fails to be generated and defects similar to those seen in GB13F or Gγ1 loss of function result.

While the Loco-associated GAP activity can facilitate the conversion of GTP-Gai to GDP-Gai in NBs, how might the reverse reaction be catalyzed without invoking the involvement of a GPCR associate GEF activity? A possible nonreceptor GEF that can fulfill this role may be the Drosophila homolog of the mammalian Ric-8A (Synembrin). Mammalian Ric-8A has been shown to act as a nonreceptor GEF for GoO, Gq, and Gai subunits [Tall et al. 2003]. Ric-8A is evolutionarily conserved from worm to mammals. More recent reports on C. elegans Ric-8 suggest that it is a GEF for the Gα subunits, GOA-1 and GPA-16, to regulate asymmetric divisions in the zygote [Afshar et al. 2004; Couwenbergs et al. 2004; Hess et al. 2004]. We also found that the fly homolog, DmRic-8, is able to associate with Gai and is involved in NB asymmetric divisions [F. Yu, unpubl.]. Hence, in principle, a model along the lines schematized in Figure 7Q may explain how heterotrimeric G-protein signaling is regulated during the process of NB asymmetric divisions.

The role of heterotrimeric G proteins in Drosophila neuroblasts and nematode zygotes

While receptor-independent activation of heterotrimeric G-protein signaling appears to be a mechanism conserved between fly and nematode, there are clear differences between the two systems. In the nematode zygote, previous studies have suggested that the Gα subunits, GOA-1 and GPA-16, are required for generation of a net pulling force from the posterior cortex that leads to the displacement of the mitotic spindle toward the posterior cortex. Either (possibly both) of the GoLoco/GPR motif proteins, GPR1/2, which are enriched at the posterior pole of the zygote [Colombo et al. 2003; Gotta et al. 2003], can act as GDIs to asymmetrically activate heterotrimeric G-protein signaling. The Gα subunits and GPR1/2 both appear to act downstream of the PAR proteins and their inactivation using RNAi results in identical spindle phenotypes that resemble those seen in
pur-2 mutants for which a reduction in cortical spindle forces have been directly demonstrated (Colombo et al. 2003; Gotta et al. 2003). More recently, it has been reported that loss of ric-8 function also disrupts the movement of the posterior centrosome, suggesting that RIC-8 acts in the same pathway as GPR-1/2 to establish Gα-dependent force generation (Afshar et al. 2004; Couwenbergs et al. 2004; Hess et al. 2004), whereas loss of function of rgs-7, encoding a GAP protein for GOA-1, leads to overly vigorous posterior spindle rocking and more exaggerated size difference between two daughter cells, indicating that Go passes through the GTP-bound state during its activity cycle to regulate the force in one-cell-stage nematode embryos (Hess et al. 2004). In contrast, Gβγ does not appear to regulate spindle displacement in the worm zygote (Srinivasan et al. 2003).

For Drosophila NBs, spindle geometry and displacement appear to be regulated to a large extent through Gβγ activation by the GoLoco proteins Loco and Pins. The spindle defects associated with loco/pins double loss-of-function NBs resemble those seen in the Gβ13F and Gγ1 mutants. However, it is clear that in Gβ13F and Gγ1 mutants there is a small degree of residual asymmetry in the size of the NB daughters; this residual size difference can be removed by the additional loss of baz function [Izumi et al. 2004]. There is no evidence implicating a major role for Gai in spindle asymmetry since loss of Gai has relatively mild effects [Yu et al. 2003]. However, the possibility that multiple Go subunits redundantly regulate NB spindle geometry cannot be ruled out.

Furthermore, in contrast to the C. elegans zygote where heterotrimeric G-protein signaling acts downstream of the PAR polarity cues, the precise hierarchical relationship between the heterotrimeric G proteins and the PAR proteins in Drosophila NBs is more complex. On the one hand, some observations can be interpreted, at least formally, to suggest that free Gβγ acts upstream of the apical components, since mutations in Gβ13F and Gγ1 cause delocalization of Pins/Loco/Gao1 and affect the stability (intensity) of the Baz and DaPKC apical crescents [Yu et al. 2003]. However, reduced levels of Baz and DaPKC can nevertheless asymmetrically localize and maintain residual levels of asymmetry despite the loss of free Gβγ, suggesting that some aspects of NB asymmetry and PAR polarity cues act in parallel or upstream of heterotrimeric G proteins (Fuse et al. 2003; Yu et al. 2003; Izumi et al. 2004). This study provides evidence that in Drosophila NBs, both Loco and Pins contribute toward the generation of free Gβγ and the asymmetric localization of Pins/Loco/Gai depends not only on Gβγ but also the right balance of GDP-Gao1 and GTP-Gao1. It remains to be seen whether in NBs Gβγ mediates the formation of an asymmetric spindle by regulating Go subunits.

Materials and methods

Isolation of new loco alleles

EY04589 was mobilized using P(ry Δ2–3)(99B) as a transposase source, and 500 independent w− revertant lines were established and analyzed. Three small deletions, loco237, loco208, and loco082, that remove part or all of the loco-cl-coding region were subjected to PCR mapping and DNA sequencing to determine their precise breakpoints. The recessive lethal allele loco237 removes the entire loco-cl-c and loco-cl-c transcripts as well as the flanking gene mRpl45. The allele loco208 removes the region from nucleotide −310 to +2195 of the loco-cl transcript, while loco082 removes the region from nucleotide −310 to +1277 of the transcript (the start point of loco-cl transcription is +1). The region that is removed in the loco082 allele includes the RGS domain, two RBD domains, and the GoLoco motif, while loco082 deletes only up to and including the region encoding the RGS domain.

In loco208 mutant neuroblasts [lacking both maternal and zygotic components] overexpressing Loco-C1 [uas-loco-C1 driven with mata-Gal4 VP16 V32], Gao1 apical crescents can be restored in 89% of metaphase NBs (n = 74), and Pins crescents can be observed in 70% of metaphase NBs (n = 60), indicating that these defects in loco mutant NBs are due to loss of loco function. When we attempted to rescue using the same procedure with a truncated form of Loco-C1 lacking the GoLoco motif but including the RGS and RBD domains (Loco-C1A GoLoco, containing amino acids 1–640), Gao1 apical crescents could be restored in 64% of mitotic neuroblasts (n = 33), and Pins apical crescents could be seen in 85% of neuroblasts (n = 20). However, in the rescue experiments with a truncated form of Loco-C1 lacking the RGS domain (Loco-C1ARGS, containing amino acids 232–830), the majority of NBs exhibit uniform cortical distribution of Pins (81%, n = 26) and Gao1 (95%, n = 23). Together with the biochemical experiments, these rescue results indicate that the RGS domain of Loco, and its associated GAP activity for Gao1, is important for NB asymmetric divisions.

Plasmid constructs, fusion proteins, and anti-Loco antibodies

MBP-Gao1 was constructed by introducing the coding region of Gao1 into pMAL-c2X [NEB]. Various GST fusion proteins of Loco-C1 [amino acids 61–298, 337–502, 357–636, and 564–731] were generated using pGEX 4T-1 (Amersham). GST-C-Pins was generated according to Yu et al. (2002). Anti-Loco antibodies were generated in guinea pigs and affinity-purified as described in Yu et al. (2003). An anti-Gao1 antibody was raised against the full-length Gao1 fused to MBP in mice and guinea pigs. No Gao1 signal could be detected in Gao1 mutant embryos by Western blotting and immunofluorescent staining (data not shown), indicating that this anti-Gao1 antibody can recognize Gao1 specifically.

Yeast two-hybrid, protein binding assays, and GDI and GAP assays

Yeast two-hybrid assays were carried out as described in Yu et al. (2000). The fragments encoding amino acids 564–829 of Loco-C1 or amino acids 378–658 of Pins were inserted into pAS2-1. The full-length Gao1 and the mutant version Gao1Q205L were inserted into pACT2. Their corresponding binding activities were tested based on the ability of colonies to turn blue in an X-gal filter lift assay: +, 60 min; −, no significant staining.

Full-length Gao1 and the mutant version, Gao1Q205L, were inserted into pET15b [Novagen]. 35S-labeled Gao1 and Gao1Q205L proteins were produced by using TNT in vitro transcription and translation kit [Promega]. The GST pull-down assays were conducted as described in Yu et al. (2000). To test for the nucleotide-dependent interaction between Gao1 and the RGS domain of Loco, 10 µL of 35S-labeled Gao1 was incubated for 30 min at room temperature by adding 90 µL of buffer A [50 mM Tris-HCl at pH 8.0, 0.1 M NaCl, 1 mM MgSO4, 20 mM imidazole, 10 mM

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mercaptoethanol, 10% glycerol] supplemented with GTPγS (10 µM), GDP (10 µM) or GDP and AlF₄⁻ (10 and 30 µM), respectively. GST-RGS [1 µg] or control GST [3 µg], bound to agarose beads, was separately incubated with the Gai mixture for 30 min at 4°C. The agarose beads were washed four times with buffer containing the respective nucleotides and/or AlF₄⁻. To test whether GST-RGS can pull down endogenous Gai, 200 µg of GST-RGS or GST alone was incubated with embryo extracts, followed by three washes in the lysis buffer. Bound proteins were Western-blotted with anti-Gai antibody.

[35S]GTPγS binding experiments were essentially performed as described in Notochin et al. (2000). Reaction mixtures containing 1 µM MBP-Gai-GDP, 1 µM GST-GoLoco (amino acids 564–731), GST-C-Pins (amino acids 378–658), or control GST were mixed with 2 µM [35S]GTPγS [1000 Ci/mmoll] and incubated at 30°C for different time periods. The reactions were terminated and measured for scintillation counts.

Immunocytochemistry and confocal microscopy

Embryos were collected and fixed according to Yu et al. (2003). Immunocytochemistry and confocal microscopy were performed as described in Yu et al. (2003). Anti-Gai or anti-Flag (m2) was used for immunoprecipitation. Bound proteins were analyzed with anti-Flag, anti-Pins, and anti-Gai by Western blots (Yu et al. 2000).

**Flies, germline transformation, and RNAi experiments**

Insc<sup>202</sup>, pins<sup>p96</sup>, pins<sup>p96</sup>, baz<sup>c206</sup>, FRT<sup>–2</sup>, scabrous-gal4 (scagal4), mata-gal4 VP16 V32, and UAS-Gai were described earlier in Yu et al. (2000) and Yu et al. (2003). Gai<sup>3</sup>[P261]<sup>FRT<sup>–2</sup></sup> and Gyi<sup>1[N159]<sup>FRT<sup>0</sup>–G15</sup></sup> were kindly provided by F. Matsuzaki (Center for Developmental Biology, RIKEN, Lobe, Japan). UAS-GaiG204A was obtained by introducing the mutant GaiG204A cDNA in which Gly 204 had been replaced with alanine into pUAST [Brand and Perrimon 1993]. Overexpression of GaiQ205L and GaiG204A in either wild-type or Gai mutant embryos was driven by mata-gal4 VP16 V32 at 26°C. Full-length loco-c2 [GH08607 from BDGP], loco-c1ΔGoLoco (encoding the region amino acids 1–630 of the Loco-C1 protein), and loco-c1ΔARG3 (encoding the region amino acids 232–830) were inserted into pUAST. The coding region of loco-c2 fused to two tandem Flag epitopes was also cloned into pUAST and hs-Casper vectors and was used for germline transformation. The RNAi experiments were performed essentially as previously described in Yu et al. (2003).

**References**

Afshar, K., Willard, F.S., Colombo, K., Johnston, C.A., McCudden, C.R., Siderovski, D.P., and Gonczy, P. 2004. RIC-8 is required for GPR-1/2-dependent Go function during asymmetric division of *C. elegans* embryos. *Cell* 119: 219–230.

Bellen, H.J., Levis, R.W., Liao, G., He, Y., Carlson, J.W., Tsang, G., Evans-Holm, M., Hiesinger, P.R., Schulze, K.L., Rubin, G.M., et al. 2004. The BDGP gene disruption project: Single transposon insertions associated with 40% of *Drosophila* genes. *Genetics* 167: 761–781.

Brand, A.H. and Perrimon, N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118: 401–415.

Cai, Y., Yu, F., Lin, S., Chia, W., and Yang, X. 2003. Apical complex genes control mitotic spindle geometry and relative size of daughter cells in *Drosophila* neuroblast and pi asymmetric divisions. *Cell* 112: 51–62.

Campos-Ortega, J.A. and Hartenstein, V. 1997. *The embryonic development of Drosophila melanogaster*. Springer Verlag, Berlin.

Colombo, K., Grill, S.W., Kimple, R.J., Willard, F.S., Siderovski, D.P., and Gonczy, P. 2003. Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. *Science* 300: 1957–1961.

Couwenbergs, C., Spilker, A.C., and Gotta, M. 2004. Control of embryonic spindle positioning and Go activity by *C. elegans* RIC-8. *Curr. Biol.* 14: 1871–1876.

De Vries, L. and Gist Farquhar, M. 1999. RGS proteins: More than just GαPs for heterotrimeric G proteins. *Trends Cell Biol.* 9: 138–144.

Doe, C.Q. and Bowerman, B. 2001. Asymmetric cell division: Fly neuroblast meets worm zygote. *Curr. Opin. Cell Biol.* 13: 68–75.

Fuse, N., Hisata, K., Katzen, A.L., and Matsuizaki, F. 2003. Heterotrimeric G proteins regulate daughter cell size asymme-
try in Drosophila neuroblast divisions. Curr. Biol. 13: 947–954.
Giansanti, M.G., Gatti, M., and Bonaccorsi, S. 2001. The role of centrosomes and astral microtubules during asymmetric di-
vision of Drosophila neuroblasts. Development 128: 1137–
1145.
Gott, M. and Ahringer, J. 2001. Distinct roles for Gα and Gβγ
in regulating spindle position and orientation in Caenorhab-
ditis elegans embryos. Nat. Cell Biol. 3: 297–300.
Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S.M., and Ahringer,
J. 2003. Asymmetrically distributed C. elegans homologs of
AGSS/PINS control spindle position in the early embryo.
Curr. Biol. 13: 1029–1037.
Granderath, S., Stollewerk, A., Greig, S., Goodman, C.S.,
O’Kane, C.J., and Klambt, C. 1999. loco encodes an RGS protein
required for Drosophila glial differentiation. Development
126: 1781–1791.
Hess, H.A., Roper, J.C., Grill, S.W., and Koelle, M.R. 2004.
RGS-7 completes a receptor-independent heterotrimeric G
protein cycle to asymmetrically regulate mitotic spindle po-
sitioning in C. elegans. Cell 119: 209–218.
Izumi, Y., Ohta, N., Itoh-Furuya, A., Fuse, N., and Matsuzaki,
F. 2004. Differential functions of G protein and Baz-aPKC sig-
naling pathways in Drosophila neuroblast asymmetric divi-
sion. J. Cell Biol. 164: 729–738.
Jan, Y.N. and Jan, L.Y. 2001. Asymmetric cell division in the
Drosophila nervous system. Nat. Rev. Neurosci. 2: 772–779.
Kaltschmidt, J.A. and Brand, A.H. 2002. Asymmetric cell divi-
sion: Microtubule dynamics and spindle asymmetry. J. Cell
Sci. 115: 2257–2264.
Kaltschmidt, J.A., Davidson, C.M., Brown, N.H., and Brand,
A.H. 2000. Rotation and asymmetry of the mitotic spindle
direct asymmetric cell division in the developing central
nervous system. Nat. Cell Biol. 2: 7–12.
Kemphues, K. 2000. PARising embryonic polarity. Cell 101:
345–348.
Kimple, R.J., De Vries, L., Tronchere, H., Behe, C., Morris, R.A.,
Gist Farquhar, M., and Siderovski, D.P. 2001. RGS12 and
RGS14 GoLoco motifs are G α[i] interaction sites with gua-
nine nucleotide dissociation inhibitor Activity. J. Biol.
Chem. 276: 29275–29281.
Knoblich, J.A. 2001. Asymmetric cell division during animal
development. Nat. Rev. Mol. Cell Biol. 2: 11–20.
Natochin, M., Lester, B., Peterson, Y.K., Bernard, M.L., Lanier,
S.M., and Artemyev, N.O. 2000. AGS3 inhibits GDP disso-
ciation from Gα subunits of the Gi family and rhodopsin-
dependent activation of transducin. J. Biol. Chem. 275:
40981–40985.
Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A.,
Bryant, P., and O’Kane, C.J. 2000. Rapsynoid/Partner of
InsCuteable controls asymmetric division of larval neuro-
blasts in Drosophila. J. Neurosci. (Online) 20: RC84.
Pathirana, S., Zhao, D., and Bownes, M. 2001. The Drosophila
RGS protein Loco is required for dorsal/ventral axis forma-
tion of the egg and embryo, and nurse cell dumpling. Mech.
Dev. 109: 137–150.
Peng, C.Y., Manning, L., Albertson, R., and Doe, C.Q. 2000. The
tumour-suppressor genes lgl and dlg regulate basal protein
targeting in Drosophila neuroblasts. Nature 408: 596–600.
Schafer, M., Shevchenko, A., and Knoblich, J.A. 2000. A pro-
tein complex containing InsCuteable and the Gα-binding
protein Pins orients asymmetric cell divisions in Dro-
sophila. Curr. Biol. 10: 353–362.
Schafer, M., Petronczki, M., Dorner, D., Forte, M., and Knob-
lich, J.A. 2001. Heterotrimeric G proteins direct two modes
of asymmetric cell division in the Drosophila nervous sys-
tem. Cell 107: 183–194.
Schober, M., Schaefer, M., and Knoblich, J.A. 1999. Bazooka
recruits InsCuteable to orient asymmetric cell divisions in
Drosophila neuroblasts. Nature 402: 548–551.
Siderovski, D.P., Diverse-Pierluissi, M., and De Vries, L. 1999.
The GoLoco motif: A Gai/o binding motif and potential gua-
nine-nucleotide exchange factor. Trends Biochem. Sci. 24:
340–341.
Srinivasan, D.G., Fisk, R.M., Xu, H., and van den Heuvel, S.
2003. A complex of LIN-5 and GPR proteins regulates G
protein signaling and spindle function in C. elegans. Genes &
Dev. 17: 1225–1239.
Takesono, A., Cismowski, M.J., Ribas, C., Bernard, M., Chung,
P., Hazard, S.R., Duzic, E., and Lanier, S.M. 1999. Receptor-
dependent activators of heterotrimeric G-protein signaling
pathways. J. Biol. Chem. 274: 33202–33205.
Tall, G.G., Krumins, A.M., and Gilman, A.G. 2003. Mammalian
Ric-8A (synembryn) is a heterotrimeric Gα protein guanine
nucleotide exchange factor. J. Biol. Chem. 278: 8356–8362.
Wodarz, A. and Huttner, W.B. 2003. Asymmetric cell division
during neurogenesis in Drosophila and vertebrates. Mech.
Dev. 120: 1297–1309.
Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. 2000. Analysis
of partner of inscuteable, a novel player of Drosophila asym-
metric divisions, reveals two distinct steps in inscuteable
apical localization. Cell 100: 399–409.
Yu, F., Ong, C.T., Chia, W., and Yang, X. 2002. Membrane tar-
taging and asymmetric localization of Drosophila partner of
inscuteable are discrete steps controlled by distinct regions
of the protein. Mol. Cell. Biol. 22: 4230–4240.
Yu, F., Cai, Y., Kaushik, R., Yang, X., and Chia, W. 2003. Distin-
cent roles of Gai and Gβ13F subunits of the heterotrimeric
G protein complex in the mediation of Drosophila neuro-
blast asymmetric divisions. J. Cell Biol. 162: 623–633.
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*Genes Dev.* 2005, 19:
Access the most recent version at doi:10.1101/gad.1295505

References

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