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Perspective

Multiple Roles of a Trimeric G Protein in *Drosophila* Cell Polarization

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KEY WORDS

cell polarization, trimeric G proteins, *Drosophila*, planar cell polarity, asymmetric cell divisions, cytoskeleton, microtubules, Frizzled receptors

ABBREVIATIONS

Dgo	Diego
Dsh	Dishevelled
Fmi	Flamingo
Fz	Frizzled
GEF	guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
PCP	planar cell polarity
Pk	Prickle
PTX	pertussis toxin
SOP	sensory organ precursor cell
Vang	Strabismus/Vang Gogh

ABSTRACT

Polarization of the cellular cytoskeleton underlies many cellular processes including axon growth cone guidance, chemotaxis and yeast mating. Planar cell polarity (PCP) is a similar phenomenon in which cells in an epithelium become uniformly polarized to generate a field of aligned structures such as the hair cells of the cochlea. In Drosophila PCP is under the hierarchical control of Frizzled (Fz)—a serpentine receptor (that also functions in the Wnt signaling pathway). Serpentine receptors are routinely transduced by trimeric G-proteins, but until recently the general consensus was that Fzs were not G-protein linked. In *Drosophila* a G-protein ($G\alpha_o$) has now been identified that functions in both the Wnt and PCP pathways. Here we review the cell polarity phenotypes of $G\alpha_o$ mutants and discuss the evidence that it plays multifarious roles in PCP and the organization of the cytoskeleton.

INTRODUCTION

Epithelial structures in many organisms are highly polarized: bird feathers, mammalian hairs or fish scales are all uniformly oriented with respect to body axes. All these are manifestations of a phenomenon known as planar cell polarity (PCP) in which cells are polarized—not only in the standard apico-basal axis—but also perpendicularly in the plane of the epithelium. PCP has been extensively investigated in *Drosophila*, where examples include the distally projecting wing hairs, posteriorly pointing thoracic and abdominal bristles, and the uniform shapes and orientations of ommatidia in the eyes (Fig. 1A–H). A number of proteins have been identified that cause aberrant PCP when mutated in Drosophila, including Dishevelled (Dsh), Prickle (Pk), Strabismus/Vang Gogh (Vang), Flamingo (Fmi), Diego (Dgo);[1,2] a number of these proteins were later found necessary for PCP in other organisms.[3-5]

The signaling cascade responsible for the initiation of the Drosophila PCP program is under the hierarchical control of the transmembrane receptor Frizzled (Fz).[6-9] Fz proteins are conserved throughout metazoans, and are better known as receptors for the Wnt family of secreted glycoproteins, and regulate many aspects of development.[10] The Wnt and PCP signaling pathways have distinctly different cellular outputs: Wnt transduction directs gene expression, while PCP signaling reorganizes the cytoskeleton.

FZ SIGNALING AND TRIMERIC G PROTEINS

Fz receptors contain seven transmembrane helices, with an extracellular N-terminus and an intracellular C-terminus.[8] Receptors of this structure are known as G protein-coupled receptors (GPCRs) because they usually use trimeric G protein complexes as their immediate transducers. Trimeric G proteins are composed of α-, β- and γ-subunits, and in the resting state the α-subunit is GDP-bound. Upon ligand binding, the receptor acts as a nucleotide exchange factor, replacing GTP for GDP on the α-subunit. This triggers the dissociation of the complex into the GTP-α-subunit and the βγ heterodimer, each of which may then engage downstream effectors.[11]

Despite the homology to the GPCR superfamily,[12] until recently Fz receptors were not considered G protein-linked because extensive screens for Wnt and PCP mutants had not identified a trimeric G protein. However, the fly genome encodes six Gα subunits (Fig. 2C), and several hundred GPCRs. Thus any given trimeric G protein is likely to transduce a large number of GPCRs (in addition to any receptor-independent function this G protein might have) which would cause extensive pleiotropy, and prevent easy genetic identification.

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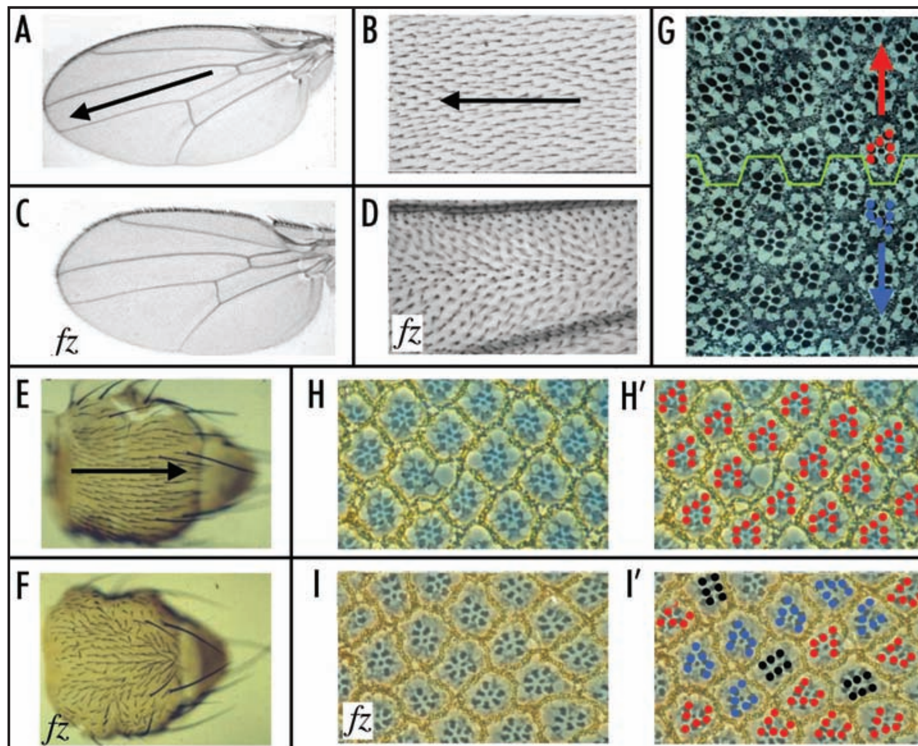

Figure 1. PCP and its mutant phenotypes. (A and B) A *Drosophila* wing is decorated with hundreds of hairs all pointing to the distal end (arrow) of the wing. (C and D) In *fz* mutants the orientations of the hairs is severely disrupted. (E) The small and large bristles of the thorax point posteriorly (arrow). (F) A *fz* thorax shows disorganized orientation of bristles. (G) The ommatidia of the eye occur in two different shapes (color-coded red and blue) and in any one half of the eye they are all of the same shape and point away (arrows) from the midline (green line). (H and H′) In the dorsal half of a right eye all ommatidia are of the red shape and are coordinately aligned. (I, I′) In *fz* eyes ommatidia of the two types are intermixed with non chiral forms (black) and orientation is disrupted.

There was however encouraging evidence for the action of trimeric G proteins in Fz signaling,[13] and pertussis toxin (PTX) was shown to suppress Fz overexpression phenotypes in *Drosophila* eyes (Fig. 2A and B), suggesting that a Ptx-sensitive Gα subunit was active in Fz signaling. $G\alpha_o$ (Go) is the only potential substrate of Ptx in *Drosophila* (Fig. 2C), and testing of other Gα subunits suggested that in *Drosophila* Go is probably the only trimeric G protein involved in Fz signal transduction.[14] This contrasts with the results obtained more recently in a mammalian system, where both Gq and Go appear active downstream from Fz.[15]

THE EVIDENCE THAT GO TRANSDUCES FZ

Loss- and gain-of-function *Go* mutations disturb both Wnt and PCP signaling[14] confirming a role for Go in *Drosophila* Fz signaling. These results however suggest only that Go is active in Fz signaling, they do not necessarily argue for a role as an immediate transducer. There are three pieces of evidence that suggest that Go may indeed be the immediate transducer of Fz. First, epistatis experiments place it high in the signaling cascade—as would be expected for an immediate transducer.[14] Second, Go is active in both types of Fz signaling and proximity to the receptor is expected for such an activity—Dishevelled (Dsh)[6,16] is the only other protein known to function in both pathways, and is generally considered an immediate transducer. Third, if Fz acts as the

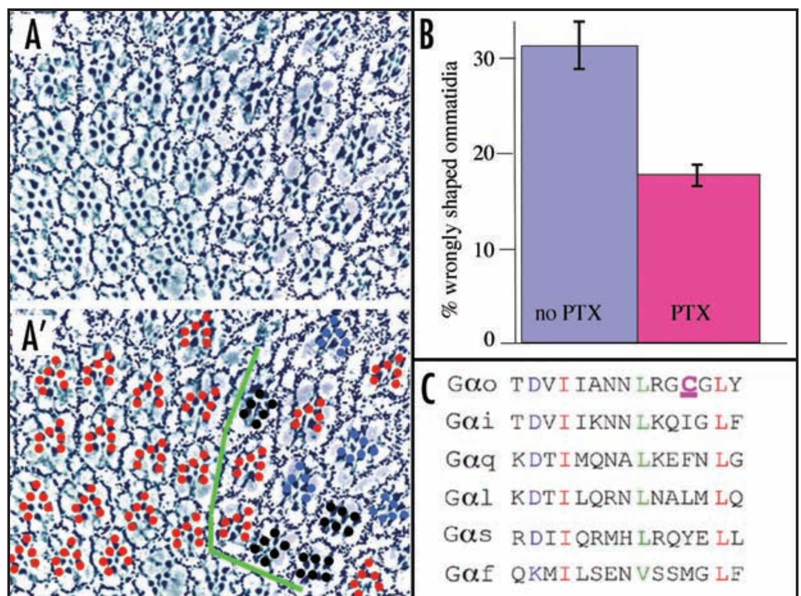

Figure 2. The effects of Ptx on Fz signaling. (A, A′) To the right: *sev-Gal4; UAS-fz* induces disruption of ommatidia shapes, but when *UAS-Ptx* is also expressed (to the left of the green line) there is a clear suppression of the effects. Fly genotypes and methods are described in ref. 14. (B) Graph depicting the numbers of incorrectly shaped ommatidia caused by overexpression of fz when Ptx is present and not. Data is shown as mean ± sem. (C) Ptx ADP-ribosylates a cysteine 4 amino acids from the terminus of Gα subunits. The terminal amino acid sequences of the 6 fly Gα-subunits are listed; only Go has the requisite cysteine (underlined).

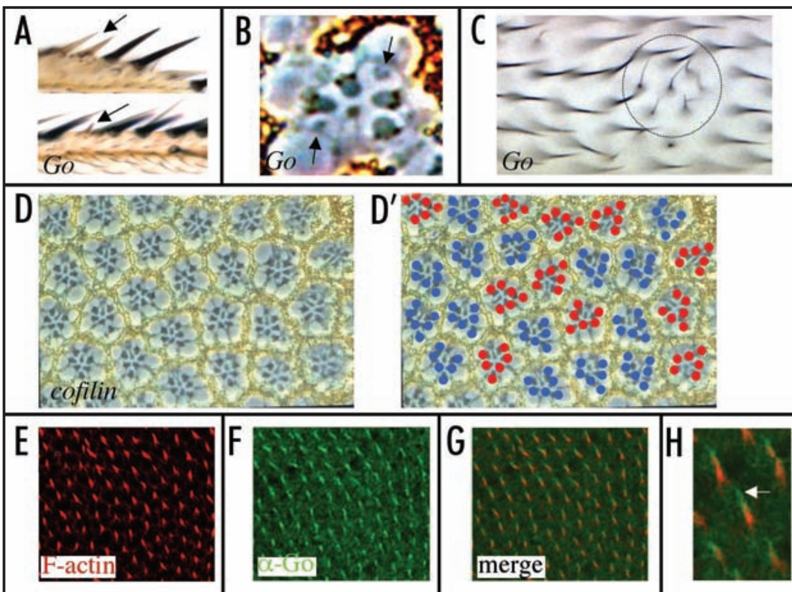

Figure 3. Actin-associated phenotypes of Go and PCP. (A-C) Actin rich structures are degenerate in Go mutant cells. (A) Go wing margin bristles (arrows) are stunted. (B) Go photoreceptors (arrows) have degenerate rhabdomeres. (C) Go wing hairs (demarked by the circle) are delicate. (D, D') A weak mutation in the gene encoding cofilin randomizes ommatidial shapes and disorders orientation. (E-H) Go (green) sits atop of the phallodin stained actin bundles (red) of the growing wing hairs. (H) shows a higher magnification of (G). 36h APF pupal wings are stained with rhodamine phalloidin and anti-Go as described in ref. 14.

exchange factor for Go then the wild-type form of Go would require Fz's presence to get into the GTP-bound active condition, whereas the form of Go constitutively locked in the GTP-bound state would be Fz-independent. In this regard overexpression phenotypes of wild-type Go required the presence of Fz, whereas the Go-GTP mutant form did not.[14,17] This genetic evidence argues for a role for Go in both forms of Fz signaling, and many data are consistent with it acting as an immediate transducer. However, without biochemical confirmation of the relationship, the argument that Go is an immediate transducer of Fz remains speculative.

WHAT IS THE PCP LIGAND FOR FZ?

Convergent extension is a process that occurs in gastrulating vertebrates and shares many mechanistic and molecular features with fly PCP,[3,18] and here the ligands for the Fz receptors are Wnts. However, in insect PCP the ligand(s) remain unclear. It does not appear to be a Wnt,[19,20] and although important extracellular roles are played by the transmembrane proteins Fat and Dachsous[21,22] neither of these has been shown to be a Fz ligand. It is not even clear that Fz binds a graded ligand. What is clear is that there is some form of graded extracellular information (including, and possibly restricted to Fat and Dachsous), and that Fz is required for the cells to appropriately respond to this information.

THE MECHANISTIC AND MOLECULAR SIMILARITIES OF PCP AND CHEMOTAXIS

The standard view of PCP is that graded extracellular information is presented to the epithelium, and the cells "read" this gradient to direct the coordinated asymmetrical organizations of their cytoskeletons. This is inherently similar to chemotaxis (directed cell movement) or chemotropism (directed cell growth) in which cells migrate (or grow) along extracellular gradients. Chemotaxis of leukocytes and slime mold amoebae, as well as chemotropism in mating yeast cells have been extensively studied,[23-25] and the basic principles of how these phenomena occur are understood. Furthermore, trimeric G proteins play major roles in these processes.

Following receptor activation, trimeric G proteins dissociate into two potential transducing moieties: Gα-GTP and the βγ heterodimer. In leukocyte chemotaxis, free βγ plays a major role.[23] It is anchored to the plasma membrane through lipid modification, and directs the translocation of PI3-kinase γ (a key regulator of chemotaxis[26-28]) to the membrane.[29,30] This lipid kinase becomes locally concentrated in excess of its substrate which effectively renders it constitutive.[31] In yeast chemotropism, freed βγ acts to attract a complex containing the scaffolding protein Far1, the guanine nucleotide exchange factor Cdc24, and the small GTPase Cdc42 to the tip of the mating projection.[32] Since a key aspect of leukocyte chemotaxis signaling is the role of receptor-freed βγ in localizing crucial downstream targets, it is interesting that in *Drosophila* Dsh translocates to the plasma membrane upon Fz activation,[33,34] and furthermore, Dsh can bind βγ.[35] Thus βγ may function to bring Dsh to the membrane in PCP in a similar manner to which it recruits PI3-kinase γ in leukocytes and Cdc42 in yeast cells. The plasma membrane localization of Dsh correlates with its hyperphosphorylation[36] and it can be phosphorylated by a number of protein kinases, including casein kinase 1 and 2,[37,38] PAR-1[39] and protein kinase C isoforms.[40,41] This hyperphoshorylation is thought to direct a conformational change in Dsh allowing its domains to interact with several proteins involved in PCP signaling, such as Vang, Pk, Dgo, and Fz itself.[42-44]

In contrast to βγ, the role played by leukocyte $G\alpha^{GTP}$ is less instructive.[45] In yeast cells, $G\alpha^{GTP}$ was recently shown to relocalize to endosomes, where it controlled more downstream events in the mating response.[46]

A key difference between chemotaxis and PCP is the relocalization of the receptor (or not) in response to the gradient. As will be described below, Fz is initially generally distributed in the apical plasma membrane of the cells but then redistributes to one side of the cell, establishing a pronounced asymmetric localization. This is in contrast to chemotaxis where receptors usually remain uniformly distributed across the plasma membrane of migrating cells[47-49] (although some slowly migrating lymphocytes show enrichment of the GPCR on their leading edge[50]). Thus although there are intriguing molecular similarities between PCP and chemotaxis, there are also clear differences that tempers enthusiasm for drawing strict analogies.

GO AND THE REGULATION OF THE ACTIN CYTOSKELETON

In other systems Gα activates guanine nucleotide exchange factors of the Rho family small G proteins.[51] Rho family proteins are critical regulators of the actin cytoskeleton[52] and a number of observations implicate Go with the actin cytoskeleton. First, actin-rich structures are severely compromised in cells with reduced Go function. These include photoreceptor rhabdomeres, epithelial trichomes,[14] and shafts of the sensory bristles. These structures show partial or complete degeneration in *Go* mutant cells (Fig. 3A–C). Second,

expression of the constitutively active form of Go induces multiple hair phenotypes (Fig. 4I): formation of multiple (instead of single) outgrowths by each cell.[14] This phenotype mimics those described for RhoA and Rho-kinase (an effector of RhoA signaling).[53,54] Third, the LIM-kinase/cofilin pathway acts downstream of Rho proteins in mammalian systems,[55] and (weak) mutations of it have recently been shown to produce *fz*-like phenotypes such as randomized ommatidial shapes (Fig. 3D) or the formation of single, but incorrectly oriented hairs.[56]

The three points above relate to at least two distinct roles for Go and actin polymerization in PCP. Take for example the formation of a wing hair. Here it is thought that Fz signaling defines a unique position in the cell, and from that position the hair grows. The LIM-kinase/cofilin manipulations and weak alleles of *Go* show that a normal and singular hair can be produced, but in the wrong position —suggesting that Go and actin polymerization are required to define the correct position of the outgrowth. But stronger effects from Go or LIM-kinase/cofilin manipulations cause the degeneration/aborted growth of the hair (the actin-rich structure). Thus it appears that PCP uses Go and the actin polymerization pathway in at least two steps: first, to specify where in the cell the actin-rich entity should be positioned, and second to organize the outgrowth and the elaboration of its structure. This prompted us to examine Go localization in the growing hairs to see whether it could be detected in association with the emerging actin cytoskeleton of the nascent hair. Figures 3E-H shows a ca. 36h APF pupal wing stained with rhodamine phalloidin (to visualize F-actin) and anti-Go. In these pupal hairs, anti-Go staining "sits" strikingly on the tip of the growing F-actin bundles, suggesting that Go might instruct actin polymerization and hair growth.

GO AND THE REGULATION OF THE TUBULIN CYTOSKELETON

Fz PCP signaling is important for correct orientation of sensory bristles of the adult fly cuticle. Each sensory bristle contains four cells, two external and two internal, which are derived from organized asymmetric divisions of the sensory organ precursor cell (SOP)[57] (Fig. 4A). The axes of these divisions are controlled by Fz signaling, and become random in *fz* or weak *Go* mutants (Fig. 4H).[17,58] Contrasting with the organization of the wing hair described above, the actin cytoskeleton seems not to play a role in the orientation of SOP divisions. Instead, the microtuble cytoskeleton appears as the target here (see below) and thus, it appears in *Drosophila* that Go can direct polarizing information through differing cytoskeletal components.

Tubulins are constituents of microtubules—the omnipresent eukaryotic cytoskeleton.[59] Tubulins occur as dimers of α and β monomers, and dimeric structure of the building blocks imposes polarity on a microtubule into which they polymerize: one end is terminated with a β-subunit [plus (+) end], and the other with an α-subunit [minus (-) end]. Addition of new tubulin dimers occurs preferentially at the plus end. Tubulins can be considered G proteins: both α- and β-tubulin bind GTP, and only in this form can the heterodimer polymerize; β-tubulin can also hydrolyze GTP to GDP. The hydrolysis rate is lower than the polymerization rate; as a result, the main body of the microtubule may contain β-tubulin-GDP, but towards the plus end, the freshly added β-tubulins are still GTP-bound and form a "GTP cap". Loss of this cap (through accelerated hydrolysis of GTP or removal of GTP-containing dimers) leads to "catastrophic" rapid depolymerization of the microtubule from its plus end.[60]

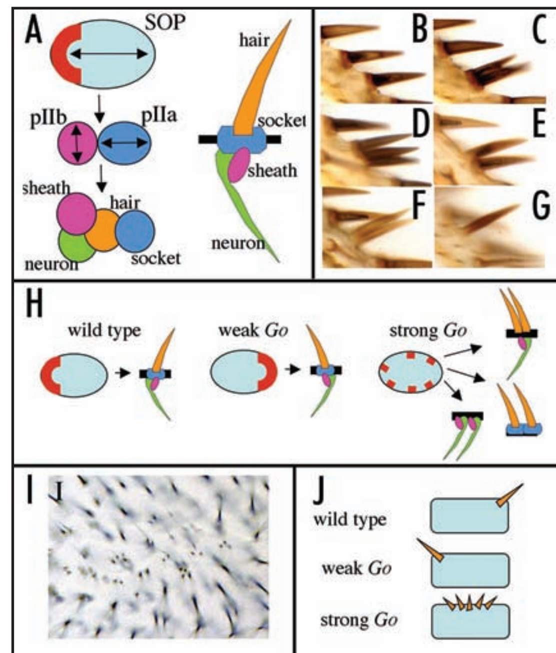

Figure 4. Asymmetric cell division and the effects of strong and weak mutations of Go. (A) An SOP polarizes and segregates Numb (red crescent) to one end of the cell which directs the fates of the daughters pIIa and pIIb. Further asymmetric divisions lead to the generation of all the cells of the bristle including the hair (brown), the socket (blue) the sheath (pink) and the neuron (green). (B) Wild type wing margin shows a single hair and socket for each bristle. Loss or overexpression of Go can change the fates of the cells and many different outcomes occur. For example there can be: (C) 2 hairs and 2 sockets; (D) 3 hairs and 1 socket; (E) 2 hairs and 1 socket; (F) 2 hairs and 0 sockets; (G) 1 hair and 0 sockets. (H, to the left) shows a wild type SOP and its resultant bristle. All the cells are present in correct number and correctly oriented. The middle shows the effects of weak *Go* mutants - the orientation of the polarization is disturbed, the Numb crescent is incorrectly localized which leads to perfectly formed but inappropriately oriented bristles. The same phenotype is observed in *fz* mutants. The right shows the effects of strong *Go* mutants. Now the Numb crescent is fragmented into small foci which can lead to a variety of cell specification defects. (I) Manipulation of *Go* can lead to wing cells secreting multiple little hairs. (J) Wild-type wing cells secrete single correctly oriented hairs. When *Go* is weakly disturbed a single hair is still produced but its orientation is defective (as in *fz*). Strong *Go* mutants lead to the formation of many small and incorrectly oriented hairs.

Asymmetric cell divisions have been extensively characterized in *Drosophila* and *C. elegans*, and regulation of the microtubule cytoskeleton is a key conserved mechanism. In *C. elegans*, trimeric G proteins GAO and GPA16 (homologs of $G\alpha_o$ and $G\alpha_i$, respectively) are redundantly used to generate the centrosome pulling forces.[61,62] Similar functions of $G\alpha$ subunits probably operate in *Drosophila*[17,63,64] and mammalian cells.[65] In all cases, the Pins (GPR-1,2 in *C. elegans*) protein plays a crucial role, linking $G\alpha$ with the spindle pole-organizing protein NuMA (LIN-5 in C.elegans, Mud in *Drosophila*).[62,65-70] NuMA binds both microtubules and the microtubule motor proteins dynein and dynactin.[71,72] These complex interactions likely control the motor-induced pulling forces on astral microtubules, relocating the centrosomes.[73,74] In addition to the motor-induced pulling force, GTP hydrolysis on microtubule (+) ends and resulting depolymerization of microtubules can generate a pulling force on astral microtubules.[75]

In addition to regulating the microtubule cytoskeleton indirectly (e.g., through Pins) several mammalian $G\alpha$ subunits directly bind

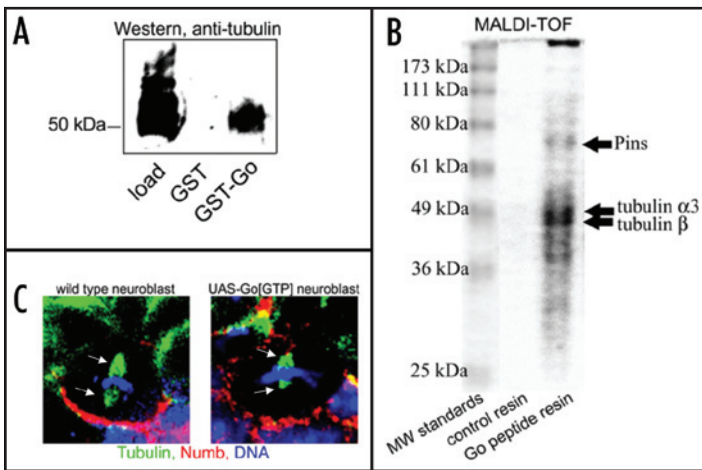

Figure 5. Interaction of Go with the microtubule cytoskeleton. (A and B) full-length GTP-loaded Go (A) or a C-terminal decapeptide of Go (B) immobilized on a resin can retain tubulin from *Drosophila* extracts; control resins are ineffective. Tubulins were identified using anti-tubulin antibodies (A) or by peptide mass-spectrometry (B). (C) A wild-type metaphase neuroblast (left panel) shows a characteristic apico-basal mitotic spindle [stained with anti-tubulin, green (arrows)], which is drastically reduced upon overexpression of Go-GTP (right panel). Methods: GST and GST-Go were produced in *E. coli*, immobilized on glutathione sepharose and treated with *Drosophila* extracts as described in ref. 17. Concentrated mouse anti-β-tubulin antibodies (clone E7, Developmental Studies Hybridoma Bank) were used at 1:500 in Western blots. The C-teminal decapeptide of Go was synthesized by Protein Chemistry Core Facility, Columbia University, and immobilized on the CNBr-activated Sepharose 4B (Amersham Biosciences). Proteins from *Drosophila* extracts specifically retained on the Go peptide resin were identified by in-gel trypsin digestion and MALDI peptide mapping on the QSTAR XL mass spectrometer performed at the Proteomics/Mass Spectrometry Facility at Columbia University. Immunostaining of embryonic neuroblasts was performed as in ref. 17; mouse anti-α-tubulin antibodies (clone DM1A, Sigma) were used at 1:1000.

tubulin polymers and dimers.[76,77] In vitro, $G\alpha_s$ and $G\alpha_i$ were shown to stimulate GTP hydrolysis of β-tubulin, removing the "GTP cap" and triggering microtubule depolymerization.[78] On the other hand, tubulins can act as non-GPCR activators of Gα subunits. This is achieved through transactivation—exchanging GDP on Gα with GTP from tubulin.[79-81]

The binding of mammalian Gα subunits (including $G\alpha_o$) to tubulin is well established.[76,77] We therefore tested whether *Drosophila* Go and tubulins would interact. For this, recombinantly-expressed Go was loaded with GTP or GDP and bound to a matrix. *Drosophila* extracts were passed through the matrix, and the retained fraction was probed with anti-tubulin antibodies. Both forms of Go showed interactions with tubulins (Fig. 5A shows binding of tubulin to Go-GTP). Interestingly, we could reproduce this binding using the C-terminal decapeptide of Go alone (Fig. 5B). The C-terminus of Gα subunits is important for their coupling to, and activation by GPCRs.[82-84] The fact that the Go interaction with tubulin also involves this region suggests an interesting parallel between GPCR and tubulin in their actions as exchange factors for Gα subunits.

To test whether Go affects microtubules in vivo, we expressed the activated form of Go in *Drosophila* embryonic neuroblasts. At metaphase, wild type neuroblasts show a characteristic apico-basal orientation of the mitotic spindle, with the apical spindle moiety being larger than the basal part[85] (Fig. 5C, left panel). Overexpression of Go-GTP led to a drastic reduction in the size of both spindle halves (Fig. 5C, right panel). Effects on spindle structure were also obtained by overexpression of βγ,[86] but βγ induces formation of small symmetric spindles, while Go-GTP reduces the size of the spindle but does not affect the asymmetry (Fig. 5C). The reduction in the spindle size by Go-GTP agrees with the results obtained in vitro with other Gα-subunits[78] and suggests that activated Go can lead to microtubule depolymerization.

GO AND THE RELOCALIZATION OF PCP PROTEINS

During the execution of PCP, key proteins involved undergo dramatic cellular relocalizations.[87] For example, Fz receptors, originally ubiquitously distributed on the apical plasma membrane of wing epithelial cells, gradually redistribute distally. This distal accumulation of Fz reaches its peak shortly before hair growth initiates from the same region.[88] Similarly, Dsh first translocates to the plasma membrane but later accumulates with Fz on distal apical membranes.[33,34] In contrast, Pk and Vang redistribute to the opposite, proximal apical membrane, while Fmi becomes depleted in lateral membranes and accumulates at the proximal and distal sites.[43,89,90] The accumulations described above are mutually dependent: genetic ablation of any one protein leads to the incorrect localization of the others, and defective PCP organization ensues.[87,88]

What is the function of the protein localizations? One explanation is that it serves to amplify the original polarizing signal,[33,89] another is that the relocalizations are merely a read-out of the polarization[91] and are only important for the PCP execution step (e.g., secreting the hair). A third explanation is that these proteins take part in communication between adjacent cells as a quality control to ensure that all cells are similarly polarized and ready to secrete their hairs in a uniform manner.[92] None of these explanations precludes the others, and all functions may occur to some extent.

Go is also asymmetrically localized and is required for the appropriate localization of the other PCP proteins. Initially Go segregates to both ends of the cells but then becomes enriched (along with Pk and Vang) at the side opposite to Fz, and this asymmetric distribution occurs in a Fz-dependent manner.[14] The fact that Go, the inferred immediate transducer of Fz, accumulates away from its receptor, might seem counterintuitive. This becomes less surprising if these localizations are indeed just a manifestation of PCP signaling that occurred previously when both Fz and Go were ubiquitously localized across the cells perimeter. It should also be stressed that while Fz by 32 h APF shows an apparently exclusive distal accumulation,[88] Go is merely enriched proximally,[14] implying that a certain amount of Go is also present at the distal side. Indeed the Go cap that sits above the actin bundles of the hair (above, and Fig. 3E–H) is clear evidence for Go at the distal side of the cells.

Relocalization of Fz depends on endocytosis. From ca. 24 h APF, vesicles containing Fz can be seen inside wing epithelial cells.[93] GPCRs often become internalized following their activation.[94] In most cases, the internalization occurs through the action of G protein coupled receptor kinases (GRKs) and β-arrestins.[95] GRKs phosphorylate the receptors in response to their activation by βγ or Gα-GTP, and the phosphorylated receptors then recruit β-arrestin which triggers GPCR endocytosis.[94,95] Fz receptors show a variation of this theme—for example in human cells, PKC-phosphorylated Dsh2 recruits β-arrestin2 to Fz4 to engender its endocytosis.[41] Fz receptors generally have short C-tails, and may have dispensed with the GRK phosphorylation in favor of using Dsh to recruit β-arrestin.

In accordance with this, Dsh is found to colocalize with Fz vesicles in wing epithelial cells.[93]

Following endocytosis, the relocalization of PCP proteins appears to be mediated by microtubule-based trafficking. Vesicles containing Fz and Fmi localize to the apical microtubule web in wing epithelial cells and become transported along the microtubules in the proximo-distal direction.[93] Microtubule polarity may thus be a prime factor in mediating the PCP protein relocalizations. The apical microtubule web of epithelial cells is predominantly aligned along the proximo-distal axis,[93,96] and surprisingly, this alignment is Fz-independent.[93] Thus, epithelial cells appear partially prepolarized before Fz PCP signaling occurs. How this prepolarization is established is unknown, but it may be a molecular memory of the axis of cell division, which in the fly wing occurs predominantly in the proximo-distal axis.[97]

Transportation of Fz and other PCP proteins along microtubules is achieved by motor proteins,[93] which can be either (+) end or (-) end-directed.[98] It is crucial that in wild type wing epithelial cells, the distal membrane contains more microtubule (+) ends than (-) ends, while the proximal membrane has the opposite ratio.[93] In other words, microtubules are not only aligned in the proximo-distal orientation in epithelial cells, but there exists also a general microtubule polarization, such that most microtubules have their (-) ends proximally, and their (+) ends distally. This microtubule polarity can explain opposite localization of PCP proteins by the end of PCP read-out. Some PCP proteins, such as Fz and Dsh, are likely transported by the (+) end-directed motors,[93,99] while others, such as Pk, Vang, and Go, may be transported by the (-) end-directed motors.

While the general proximo-distal alignment of the microtubules is Fz signaling-independent, we predict that the (+)/(-) polarization of the microtubule web is a consequence of the early steps of Fz signaling. Furthermore, given the genetic effects of Go and it molecular propensity to interact with tubulin, we predict that Go plays a key role in this reorganization of the microtubule architecture.

GO PLAYS AT LEAST 2 ROLES IN POLARIZING CELLS

A key fact to be considered in regard to the role of PCP in organizing cell polarization is that Fz signaling only instructs the direction of cell polarization—not the polarization itself. For example *fz* mutant wings still produce (usually) single hairs, and *fz* mutant SOPs still divide asymmetrically to produce the correct structure of sensory bristles—it is only the orientation of these hairs and bristles that is aberrant. Thus, the cells without active Fz signaling still possess the intrinsic property of polarization, they only lose the ability to align this polarization to extracellular cues. We will call this intrinsic ability the 'spontaneous polarization', and we will call the Fz-directed form the 'guided polarization'. Above we compared the Fz-directed guided polarization to chemotaxis (directed cell migration), and the spontaneous polarization can be compared to chemokinesis (random cell migration). In both chemokinesis and spontaneous polarization cells polarize their cytoskeletons without reference to extra-cellular information, and examples are legion.[100] Guided polarizations likely work by directing the orientation of the spontaneous mechanism, and thus molecular interactions between the two are predicted.

Mutations in the PCP genes (*fz, dsh, Pk, fmi*, etc) selectively affect the guided polarization—the cells still polarize, but not in the correct direction. Go mutants in contrast affect both the guided and spontaneous polarizations; weak effects show randomization of the guided polarizations, but stronger effects prevent the spontaneous polarization. For example epithelial cells with strongly compromised Go can produce up to 5 hairs[14] indicating that the cells failed to produce a single focus from which a hair could grow, and instead generated many foci (Fig. 4I). Similarly SOPs strongly mutant for Go generate a number of foci (rather than one) at which the Numb protein (a cell fate determinant) accumulates (Fig. 4H). The subsequent division inappropriately distributes Numb between the daughters and defective sensory structures form (Fig. 4B–G).[17] Thus, Go is required not only for the transduction of the extracellular signal perceived by Fz receptors (guided polarization), but also for the cell's intrinsic ability to polarize (spontaneous polarization). Go is thus ideally placed to serve the function of integrating these two semi-independent pathways.[17]

WHAT ELSE (BESIDES FZ) ACTIVATES GO?

If Go functions in both the spontaneous and guided polarizations, and since the spontaneous mechanism occurs normally in the absence of Fz, what then would be the activator of Go here? A number of candidates exist. One is the cytoplasmic protein Ric-8 (Synembryn), which catalizes the GDP-GTP exchange of mammalian $G\alpha_i$ and $G\alpha_o$ in vitro.[102] In *C. elegans*, Ric-8 plays a crucial role in the asymmetric division of the zygote,[103-105] where it acts as a guanine nucleotide exchange factor (GEF) for GAO (the fly Go homologue) but not for GPA16 (the fly Gi) Gα subunits.[103,104,106] Furthermore Ric-8 activity is required for the membrane localization of GPA16 but not GAO. Thus, it regulates the nucleotide bound state on one, and the plasma membrane localization of the other. Ric-8 also plays a crucial role in the asymmetric cell divisions of *Drosophila* neuroblasts and SOP cells[107-109] where it regulates plasma membrane association of Gi,[107-109] but not Go.[107] In a parallel to *C. elegans*, will Ric-8 serve as a GEF for *Drosophila* Go and not Gi?

Ric-8 activity regulates only free (not βγ-bound) Gα subunits.[102] Free Gα subunits can be generated by the actions of a GPCR, or another type of trimeric complex-dissociating proteins. Thus Ric-8 is likely an amplifier of previously liberated Gα subunits (maintaining them in the GTP-bound state), rather than their primary activator.

Drosophila and mammalian Pins have been shown to dissociate trimeric $G\alpha_i$-βγ complexes in vitro.[63,110] Pins, as described above, interacts with Go in the SOP asymmetric divisions.[17] However, Pins has no role in PCP, as Pins loss-of-function or overexpression failed to produce any PCP phenotypes (our unpublished observations). Pins thus is unlikely to be an activator of Go and is more likely a transducer of Go in asymmetric cell divisions.[17] Other proteins, collectively called AGS (Activators of *G*-protein Signaling), might play an important role in activation of trimeric G proteins.[111] Of the AGS proteins, a ras-like small GTPase AGS1 was shown to stimulate GTP incorporation into isolated Gα-subunits as well as heterotrimeric G protein complexes.[112] It remains to be investigated whether such proteins act in cell polarizations in *Drosophila*.

Tubulin is another potential activator of Go. As described above, both tubulin dimers and polymers can transactivate Gα subunits, whether isolated or in the trimeric complexes.[79-81,113] Trimeric complexes can interact with microtubules as effectively as free Gα subunits.[76] However, the physiological ability of tubulin to activate trimeric G protein complexes is probably limited, which might explain data in some cell types, where tubulin only potentiated GPCR-induced cellular responses.[114,115]

The potential exchange factors listed above are likely to have low efficiency in dissociating trimeric complexes, and yet in *fz* mutants

spontaneous polarization is largely functional, and thus, if Go works in this process it is by inference effectively activated. One possible explanation here is that in spontaneous polarizations a simple feedback mechanism may obviate the need for extensive dissociations of the Go trimeric complexes. This feedback mechanism is discussed in the model below.

A MODEL OF GO REGULATION OF POLARIZATION

A major clue as to how Go may function in spontaneous polarizations comes from experiments using Cdc42 (a small GTPase) in yeast. Here expression of an activated form (Cdc42-GTP) was sufficient to trigger polarization in the absence of any external polarity cue.[116] Furthermore, the activated Cdc42, originally distributed uniformly in the plasma membrane, with time concentrated into a single (occasionally two) polar membrane cap. Thus the Cdc42-GTP not only triggered polarization, it also engineered its own focal accumulation. Cdc42 controls actin polymerization,[117] and Cdc42 is also transported in vesicles along actin cables towards the cap.[116] Thus a positive feedback loop is the core of this cellular self-polarization: a spot on plasma membrane with a stochastic increase in Cdc42 induces higher local actin polymerization which leads to increased transport of Cdc42 to this spot, triggering further actin polymerization.

Using the yeast Cdc42/actin polymerization polarization mechanism as a guide we can propose a Go/microtubule-based positive feedback mechanism for PCP in *Drosophila* epidermal cells. First, a single focal site is established in the cell. In wild type cells, higher Fz activation at one pole of the cell leads to higher release of Go-GTP and this defines the focus. In *fz* mutant cells, a stochastic local increase in Go activation (triggered by one of the many possible exchange factors discussed above) creates a less robust but equivalent focus. A positive feedback mechanism now acts: Go-GTP induces the GTP hydrolysis of microtubule (+) ends causing the “catastrophic” depolymerization from the denuded (+) ends. This leads to a local increase in the relative concentration of microtubule (-) ends at the focus, and a decrease elsewhere. If Go-GTP is transported along the microtubules by a (-) end-directed motor protein, the focus will be progressively strengthened. Upon GTP hydrolysis on Go, the monomeric Go-GDP will be effectively exchanged back into Go-GTP by e.g., Ric-8. Thus, even a small local production of Go-GTP may result in accumulation and maintenance of activated Go and microtubule (-) ends in this spot through the proposed feedback mechanism.

In the wild type wing epithelia, the initial Fz-activating signal likely comes from the proximal direction—opposite to the future accumulation of Fz.[2,118] Such early proximal Fz activation creates the local burst in Go-GTP production. Through the proposed Go/microtubule-based positive feedback, the proximal increase in Go-GTP would translate into the (-)/(+) microtubule polarization in the proximo-distal direction, which, in turn, would help enrich Fz receptors distally before hair growth is initiated.

CONCLUSIONS

Go appears to play many different roles in the organization of polarization in *Drosophila* epidermal cells. First, Go seems to organize the actin cytoskeleton, as the actin-rich structures are deficient in *Go*$^-$ cells, and Go localizes to the tips of growing actin-rich wing hairs. Second, Go appears important in organization of the microtubules. The cytoskeleton activities of Go likely underlie the crucial role of this G protein in spontaneous cell polarization. On the other hand, Go appears as a transducer of the Fz receptors which instruct the guided cell polarization in PCP. Thus, Go may link these two semi-independent cell polarization mechanisms. Specific functional interplay between Go and microtubules is proposed to serve as the feedback amplification which is the core of Go participation in both spontaneous and guided cell polarizations. Similar G protein mechanisms might operate in cell polarization in other cell types.

References

1. Adler PN. Planar signaling and morphogenesis in *Drosophila*. Dev Cell 2002; 2:525-35.
2. Klein TJ, Mlodzik M. Planar cell polarization: An emerging model points in the right direction. Annu Rev Cell Dev Biol 2005; 21:155-76.
3. Wallingford JB, Fraser SE, Harland RM. Convergent extension: The molecular control of polarized cell movement during embryonic development. Dev Cell 2002; 2:695-706.
4. Dabdoub A, Kelley MW. Planar cell polarity and a potential role for a Wnt morphogen gradient in stereociliary bundle orientation in the mammalian inner ear. J Neurobiol 2005; 64:446-57.
5. Doudney K, Stanier P. Epithelial cell polarity genes are required for neural tube closure. Am J Med Genet C Semin Med Genet 2005; 135:42-7.
6. Gubb D, Garcia-Bellido A. A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*. J Embryol Exp Morphol 1982; 68:37-57.
7. Vinson CR, Adler PN. Directional noncell autonomy and the transmission of polarity information by the frizzled gene of *Drosophila*. Nature 1987; 329:549-51.
8. Vinson CR, Conover S, Adler PN. A *Drosophila* tissue polarity locus encodes a protein containing seven potential transmembrane domains. Nature 1989; 338:263-4.
9. Wong LL, Adler PN. Tissue polarity genes of *Drosophila* regulate the subcellular location for prehair initiation in pupal wing cells. J Cell Biol 1993; 123:209-21.
10. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20:781-810.
11. Gilman AG. G proteins: Transducers of receptor-generated signals. Annu Rev Biochem 1987; 56:615-49.
12. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 2003; 63:1256-72.
13. Malbon CC, Wang H, Moon RT. Wnt signaling and heterotrimeric G-proteins: Strange bedfellows or a classic romance? Biochem Biophys Res Commun 2001; 287:589-93.
14. Katanaev VL, Ponzielli R, Semeriva M, Tomlinson A. Trimeric G protein-dependent frizzled signaling in *Drosophila*. Cell 2005; 120:111-22.
15. Liu X, Rubin JS, Kimmel AR. Rapid, Wnt-induced changes in GSK3beta associations that regulate beta-catenin stabilization are mediated by Galpha proteins. Curr Biol 2005; 15:1989-97.
16. Theisen H, Purcell J, Bennett M, Kansagara D, Syed A, Marsh JL. Dishevelled is required during wingless signaling to establish both cell polarity and cell identity. Development 1994; 120:347-60.
17. Katanaev VL, Tomlinson A. Dual roles for the trimeric G protein Go in asymmetric cell division in *Drosophila*. Proc Natl Acad Sci USA 2006; 103:6524-9.
18. Heisenberg CP, Tada M, Rauch GJ, Saude L, Concha ML, Geisler R, Stemple DL, Smith JC, Wilson SW. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. Nature 2000; 405:76-81.
19. Wehrli M, Tomlinson A. Independent regulation of anterior/posterior and equatorial/polar polarity in the *Drosophila* eye; evidence for the involvement of Wnt signaling in the equatorial/polar axis. Development 1998; 125:1421-32.
20. Lawrence PA, Casal J, Struhl G. Towards a model of the organisation of planar polarity and pattern in the *Drosophila* abdomen. Development 2002; 129:2749-60.
21. Yang CH, Axelrod JD, Simon MA. Regulation of Frizzled by fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. Cell 2002; 108:675-88.
22. Ma D, Yang CH, McNeill H, Simon MA, Axelrod JD. Fidelity in planar cell polarity signalling. Nature 2003; 421:543-7.
23. Van Haastert PJ, Devreotes PN. Chemotaxis: Signalling the way forward. Nat Rev Mol Cell Biol 2004; 5:626-34.
24. Katanaev VL. Signal transduction in neutrophil chemotaxis. Biochemistry (Mosc) 2001; 66:351-68.
25. Chant J. Cell polarity in yeast. Annu Rev Cell Dev Biol 1999; 15:365-91.
26. Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, Sozzani S, Mantovani A, Altruda F, Wymann MP. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. Science 2000; 287:1049-53.
27. Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I, Joza N, Mak TW, Ohashi PS, Suzuki A, Penninger JM. Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. Science 2000; 287:1040-6.
28. Li Z, Jiang H, Xie W, Zhang Z, Smrcka AV, Wu D. Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. Science 2000; 287:1046-9.
29. Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nurnberg B, et al. Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science 1995; 269:690-3.

30. Stephens LR, Eguinoa A, Erdjument-Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT. The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. Cell 1997; 89:105-14.
31. Bondeva T, Pirola L, Bulgarelli-Leva G, Rubio I, Wetzker R, Wymann MP. Bifurcation of lipid and protein kinase signals of PI3Kgamma to the protein kinases PKB and MAPK. Science 1998; 282:293-6.
32. Dohlman HG, Thorner JW. Regulation of G protein-initiated signal transduction in yeast: Paradigms and principles. Annu Rev Biochem 2001; 70:703-54.
33. Axelrod JD. Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. Genes Dev 2001; 15:1182-7.
34. Shimada Y, Usui T, Yanagawa S, Takeichi M, Uemura T. Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. Curr Biol 2001; 11:859-63.
35. Angers S, Thorpe CJ, Biechele TL, Goldenberg SJ, Zheng N, MacCoss MJ, Moon RT. The KLHL12-Cullin-3 ubiquitin ligase negatively regulates the Wnt-beta-catenin pathway by targeting Dishevelled for degradation. Nat Cell Biol 2006; 8:348-57.
36. Yanagawa S, van Leeuwen F, Wodarz A, Klingensmith J, Nusse R. The dishevelled protein is modified by wingless signaling in *Drosophila*. Genes Dev 1995; 9:1087-97.
37. Willert K, Brink M, Wodarz A, Varmus H, Nusse R. Casein kinase 2 associates with and phosphorylates dishevelled. Embo J 1997; 16:3089-96.
38. Peters JM, McKay RM, McKay JP, Graff JM. Casein kinase I transduces Wnt signals. Nature 1999; 401:345-50.
39. Sun TQ, Lu B, Feng JJ, Reinhard C, Jan YN, Fantl WJ, Williams LT. PAR-1 is a Dishevelled-associated kinase and a positive regulator of Wnt signalling. Nat Cell Biol 2001; 3:628-36.
40. Kinoshita N, Iioka H, Miyakoshi A, Ueno N. PKC delta is essential for Dishevelled function in a noncanonical Wnt pathway that regulates *Xenopus* convergent extension movements. Genes Dev 2003; 17:1663-76.
41. Chen W, ten Berge D, Brown J, Ahn S, Hu LA, Miller WE, Caron MG, Barak LS, Nusse R, Lefkowitz RJ. Dishevelled 2 recruits beta-arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. Science 2003; 301:1391-4.
42. Wong HC, Bourdelas A, Krauss A, Lee HJ, Shao Y, Wu D, Mlodzik M, Shi DL, Zheng J. Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. Mol Cell 2003; 12:1251-60.
43. Bastock R, Strutt H, Strutt D. Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. Development 2003; 130:3007-14.
44. Jenny A, Reynolds-Kenneally J, Das G, Burnett M, Mlodzik M. Diego and Prickle regulate Frizzled planar cell polarity signalling by competing for Dishevelled binding. Nat Cell Biol 2005; 7:691-7.
45. Neptune ER, Iiri T, Bourne HR. Galphai is not required for chemotaxis mediated by Gi-coupled receptors. J Biol Chem 1999; 274:2824-8.
46. Slessareva JE, Routt SM, Temple B, Bankaitis VA, Dohlman HG. Activation of the phosphatidylinositol 3-kinase Vps34 by a G protein alpha subunit at the endosome. Cell 2006; 126:191-203.
47. Xiao Z, Zhang N, Murphy DB, Devreotes PN. Dynamic distribution of chemoattractant receptors in living cells during chemotaxis and persistent stimulation. J Cell Biol 1997; 139:365-74.
48. Servant G, Weiner OD, Neptune ER, Sedat JW, Bourne HR. Dynamics of a chemoattractant receptor in living neutrophils during chemotaxis. Mol Biol Cell 1999; 10:1163-78.
49. Jin T, Zhang N, Long Y, Parent CA, Devreotes PN. Localization of the G protein betagamma complex in living cells during chemotaxis. Science 2000; 287:1034-6.
50. Gomez-Mouton C, Lacalle RA, Mira E, Jimenez-Baranda S, Barber DF, Carrera AC, Martinez AC, Manes S. Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. J Cell Biol 2004; 164:759-68.
51. Sah VP, Seasholtz TM, Sagi SA, Brown JH. The role of Rho in G protein-coupled receptor signal transduction. Annu Rev Pharmacol Toxicol 2000; 40:459-89.
52. Jaffe AB, Hall A. Rho GTPases: Biochemistry and biology. Annu Rev Cell Dev Biol 2005; 21:247-69.
53. Strutt DI, Weber U, Mlodzik M. The role of RhoA in tissue polarity and Frizzled signalling. Nature 1997; 387:292-5.
54. Winter CG, Wang B, Ballew A, Royou A, Karess R, Axelrod JD, Luo L. *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. Cell 2001; 105:81-91.
55. Bamburg JR. Proteins of the ADF/cofilin family: Essential regulators of actin dynamics. Annu Rev Cell Dev Biol 1999; 15:185-230.
56. Blair A, Tomlinson A, Pham H, Gunsalus KC, Goldberg ML, Laski FA. Twinstar, the *Drosophila* homolog of cofilin/ADF, is required for planar cell polarity patterning. Development 2006; 133:1789-97.
57. Bardin AJ, Le Borgne R, Schweisguth F. Asymmetric localization and function of cell-fate determinants: A fly's view. Curr Opin Neurobiol 2004; 14:6-14.
58. Gho M, Schweisguth F. Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature 1998; 393:178-81.
59. Nogales E. Structural insight into microtubule function. Annu Rev Biophys Biomol Struct 2001; 30:397-420.
60. Desai A, Mitchison TJ. Microtubule polymerization dynamics. Annu Rev Cell Dev Biol 1997; 13:83-117.
61. Gotta M, Ahringer J. Distinct roles for Galpha and Gbetagamma in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. Nat Cell Biol 2001; 3:297-300.
62. Colombo K, Grill SW, Kimple RJ, Willard FS, Siderovski DP, Gonczy P. Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. Science 2003; 300:1957-61.
63. Schaefer M, Petronczki M, Dorner D, Forte M, Knoblich JA. Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system. Cell 2001; 107:183-94.
64. Yu F, Cai Y, Kaushik R, Yang X, Chia W. Distinct roles of Galphai and Gbeta13F subunits of the heterotrimeric G protein complex in the mediation of *Drosophila* neuroblast asymmetric divisions. J Cell Biol 2003; 162:623-33.
65. Du Q, Macara IG. Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell 2004; 119:503-16.
66. Gotta M, Dong Y, Peterson YK, Lanier SM, Ahringer J. Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. Curr Biol 2003; 13:1029-37.
67. Srinivasan DG, Fisk RM, Xu H, van den Heuvel S. A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in *C elegans*. Genes Dev 2003; 17:1225-39.
68. Izumi Y, Ohta N, Hisata K, Raabe T, Matsuzaki F. *Drosophila* Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. Nat Cell Biol 2006.
69. Siller KH, Cabernard C, Doe CQ. The NuMA-related Mud protein binds Pins and regulates spindle orientation in *Drosophila* neuroblasts. Nat Cell Biol 2006.
70. Bowman SK, Neumuller RA, Novatchkova M, Du Q, Knoblich JA. The *Drosophila* NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. Dev Cell 2006; 10:731-42.
71. Merdes A, Ramyar K, Vechio JD, Cleveland DW. A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. Cell 1996; 87:447-58.
72. Merdes A, Heald R, Samejima K, Earnshaw WC, Cleveland DW. Formation of spindle poles by dynein/dynactin-dependent transport of NuMA. J Cell Biol 2000; 149:851-62.
73. Grill SW, Gonczy P, Stelzer EH, Hyman AA. Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. Nature 2001; 409:630-3.
74. Grill SW, Howard J, Schaffer E, Stelzer EH, Hyman AA. The distribution of active force generators controls mitotic spindle position. Science 2003; 301:518-21.
75. Dogterom M, Kerssemakers JW, Romet-Lemonne G, Janson ME. Force generation by dynamic microtubules. Curr Opin Cell Biol 2005; 17:67-74.
76. Wang N, Yan K, Rasenick MM. Tubulin binds specifically to the signal-transducing proteins, Gs alpha and Gi alpha 1. J Biol Chem 1990; 265:1239-42.
77. Wu HC, Chiu CY, Huang PH, Lin CT. The association of heterotrimeric GTP-binding protein (Go) with microtubules. J Biomed Sci 2001; 8:349-58.
78. Roychowdhury S, Panda D, Wilson L, Rasenick MM. G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. J Biol Chem 1999; 274:13485-90.
79. Rasenick MM, Wang N. Exchange of guanine nucleotides between tubulin and GTP-binding proteins that regulate adenylate cyclase: Cytoskeletal modification of neuronal signal transduction. J Neurochem 1988; 51:300-11.
80. Roychowdhury S, Wang N, Rasenick MM. G protein binding and G protein activation by nucleotide transfer involve distinct domains on tubulin: Regulation of signal transduction by cytoskeletal elements. Biochemistry 1993; 32:4955-61.
81. Rasenick MM, Donati RJ, Popova JS, Yu JZ. Tubulin as a regulator of G-protein signaling. Methods Enzymol 2004; 390:389-403.
82. Hamm HE, Deretic D, Arendt A, Hargrave PA, Koenig B, Hofmann KP. Site of G protein binding to rhodopsin mapped with synthetic peptides from the alpha subunit. Science 1988; 241:832-5.
83. Sullivan KA, Miller RT, Masters SB, Beiderman B, Heideman W, Bourne HR. Identification of receptor contact site involved in receptor-G protein coupling. Nature 1987; 330:758-60.
84. Conklin BR, Farfel Z, Lustig KD, Julius D, Bourne HR. Substitution of three amino acids switches receptor specificity of Gq alpha to that of Gi alpha. Nature 1993; 363:274-6.
85. Kaltschmidt JA, Davidson CM, Brown NH, Brand AH. Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. Nat Cell Biol 2000; 2:7-12.
86. Fuse N, Hisata K, Katzen AL, Matsuzaki F. Heterotrimeric G proteins regulate daughter cell size asymmetry in *Drosophila* neuroblast divisions. Curr Biol 2003; 13:947-54.
87. Strutt DI. The asymmetric subcellular localisation of components of the planar polarity pathway. Semin Cell Dev Biol 2002; 13:225-31.
88. Strutt DI. Asymmetric localization of frizzled and the establishment of cell polarity in the *Drosophila* wing. Mol Cell 2001; 7:367-75.
89. Tree DR, Shulman JM, Rousset R, Scott MP, Gubb D, Axelrod JD. Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. Cell 2002; 109:371-81.
90. Usui T, Shima Y, Shimada Y, Hirano S, Burgess RW, Schwarz TL, Takeichi M, Uemura T. Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. Cell 1999; 98:585-95.
91. Lawrence PA, Casal J, Struhl G. Cell interactions and planar polarity in the abdominal epidermis of *Drosophila*. Development 2004; 131:4651-64.
92. Strutt H, Strutt D. Long-range coordination of planar polarity in *Drosophila*. Bioessays 2005; 27:1218-27.
93. Shimada Y, Yonemura S, Ohkura H, Strutt D, Uemura T. Polarized transport of Frizzled along the planar microtubule arrays in *Drosophila* wing epithelium. Dev Cell 2006; 10:209-22.

94. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: The role in receptor desensitization and signaling. Pharmacol Rev 2001; 53:1-24.
95. Kohout TA, Lefkowitz RJ. Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. Mol Pharmacol 2003; 63:9-18.
96. Turner CM, Adler PN. Distinct roles for the actin and microtubule cytoskeletons in the morphogenesis of epidermal hairs during wing development in *Drosophila*. Mech Dev 1998; 70:181-92.
97. Baena-Lopez LA, Baonza A, Garcia-Bellido A. The orientation of cell divisions determines the shape of *Drosophila* organs. Curr Biol 2005; 15:1640-4.
98. Goldstein LS, Yang Z. Microtubule-based transport systems in neurons: The roles of kinesins and dyneins. Annu Rev Neurosci 2000; 23:39-71.
99. Miller JR, Rowning BA, Larabell CA, Yang-Snyder JA, Bates RL, Moon RT. Establishment of the dorsal-ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of dishevelled that is dependent on cortical rotation. J Cell Biol 1999; 146:427-37.
100. Wedlich-Soldner R, Li R. Spontaneous cell polarization: Undermining determinism. Nat Cell Biol 2003; 5:267-70.
101. Katanaev VL, Tomlinson A. Dual roles for the trimeric G protein Go in asymmetric cell division in *Drosophila*. Proc Natl Acad Sci USA 2006.
102. Tall GG, Krumins AM, Gilman AG. Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor. J Biol Chem 2003; 278:8356-62.
103. Hess HA, Roper JC, Grill SW, Koelle MR. RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in *C. elegans*. Cell 2004; 119:209-18.
104. Afshar K, Willard FS, Colombo K, Johnston CA, McCudden CR, Siderovski DP, Gonczy P. RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of *C. elegans* embryos. Cell 2004; 119:219-30.
105. Couwenbergs C, Spilker AC, Gotta M. Control of embryonic spindle positioning and Galpha activity by *C. elegans* RIC-8. Curr Biol 2004; 14:1871-6.
106. Afshar K, Willard FS, Colombo K, Siderovski DP, Gonczy P. Cortical localization of the Galpha protein GPA-16 requires RIC-8 function during *C. elegans* asymmetric cell division. Development 2005; 132:4449-59.
107. David NB, Martin CA, Segalen M, Rosenfeld F, Schweisguth F, Bellaiche Y. *Drosophila* Ric-8 regulates Galphai cortical localization to promote Galphai-dependent planar orientation of the mitotic spindle during asymmetric cell division. Nat Cell Biol 2005; 7:1083-90.
108. Hampoelz B, Hoeller O, Bowman SK, Dunican D, Knoblich JA. *Drosophila* Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins. Nat Cell Biol 2005; 7:1099-105.
109. Wang H, Ng KH, Qian H, Siderovski DP, Chia W, Yu F. Ric-8 controls *Drosophila* neural progenitor asymmetric division by regulating heterotrimeric G proteins. Nat Cell Biol 2005; 7:1091-8.
110. Ghosh M, Peterson YK, Lanier SM, Smrcka AV. Receptor- and nucleotide exchange-independent mechanisms for promoting G protein subunit dissociation. J Biol Chem 2003; 278:34747-50.
111. Blumer JB, Cismowski MJ, Sato M, Lanier SM. AGS proteins: Receptor-independent activators of G-protein signaling. Trends Pharmacol Sci 2005; 26:470-6.
112. Cismowski MJ, Ma C, Ribas C, Xie X, Spruyt M, Lizano JS, Lanier SM, Duzic E. Activation of heterotrimeric G-protein signaling by a ras-related protein. Implications for signal integration. J Biol Chem 2000; 275:23421-4.
113. Roychowdhury S, Rasenick MM. Tubulin-G protein association stabilizes GTP binding and activates GTPase: Cytoskeletal participation in neuronal signal transduction. Biochemistry 1994; 33:9800-5.
114. Popova JS, Garrison JC, Rhee SG, Rasenick MM. Tubulin, Gq, and phosphatidylinositol 4,5-bisphosphate interact to regulate phospholipase Cbeta1 signaling. J Biol Chem 1997; 272:6760-5.
115. Popova JS, Rasenick MM. Muscarinic receptor activation promotes the membrane association of tubulin for the regulation of Gq-mediated phospholipase Cbeta(1) signaling. J Neurosci 2000; 20:2774-82.
116. Wedlich-Soldner R, Altschuler S, Wu L, Li R. Spontaneous cell polarization through actomyosin-based delivery of the Cdc42 GTPase. Science 2003; 299:1231-5.
117. Etienne-Manneville S. Cdc42-the centre of polarity. J Cell Sci 2004; 117:1291-300.
118. Le Garrec JF, Lopez P, Kerszberg M. Establishment and maintenance of planar epithelial cell polarity by asymmetric cadherin bridges: A computer model. Dev Dyn 2006; 235:235-46.