Embryonic handedness choice in *C. elegans* involves the Gα protein GPA-16

Dominique C. Bergmann[1], Monica Lee[1], Barbara Robertson[1], Meng-Fu B. Tsou[2], Lesilee S. Rose[2] and William B. Wood[2,*]

[1]Department of MCD Biology, University of Colorado, Boulder, CO 80309, USA
[2]Section of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA
*Author for correspondence (e-mail: wood@colorado.edu)

Accepted 29 August 2003

Development 130, 5731-5740

doi:10.1242/dev.00839

Summary

The mechanism by which polarity of the left-right (LR) axis is initially established with the correct handedness is not understood for any embryo. *C. elegans* embryos exhibit LR asymmetry with an invariant handedness that is first apparent at the six-cell stage and persists throughout development. We show here that a strong loss-of-function mutation in a gene originally designated *spn-1* affects early spindle orientations and results in near randomization of handedness choice. This mutation interacts genetically with mutations in three *par* genes that encode localized cortical components. We show that the *spn-1* gene encodes the Gα protein GPA-16, which appears to be required for centrosomal association of a Gβ protein. We will henceforth refer to this gene as *gpa-16*. These results demonstrate for the first time involvement of heterotrimeric G proteins in establishment of embryonic LR asymmetry and suggest how they might act.

Supplemental data and movies available online

Key words: *C. elegans*, Asymmetry, G protein

Introduction

Although most animals are approximately bilaterally symmetric on the exterior, all exhibit some left-right (LR) asymmetry of internal structures, and the handedness of this asymmetry is essentially invariant among individuals of the same species. It follows that all embryos at some point must establish a polarity of the LR axis, again with nearly invariant handedness. In all embryos for which there is evidence, LR polarity arises after anteroposterior (AP) and dorsoventral (DV) polarities are fixed (reviewed by Wood, 1997). The mechanism by which LR symmetry is broken to establish the unique LR polarity and handedness of embryos has been a long-standing puzzle in developmental biology (see Ludwig, 1932). Evidence from several species indicates that handedness choice is made autonomously by the embryo, rather than being dictated by external cues. Presumably, therefore, the basis for choice of one handedness over the other resides in the handedness or chirality of some internal embryonic component (reviewed in Wood, 1997).

Although much is now known about how LR asymmetry is elaborated and maintained in various embryos (for a review, see Mercola and Levin, 2001), the mechanism of the initial symmetry-breaking event has remained mysterious. Until recently, it appeared that the initial event in vertebrate embryos involved the chirality and asymmetric beating of nodal cilia (Nonaka et al., 1998; Essner et al., 2002; Nonaka et al., 2002), and must therefore be quite different from the much earlier decision made in invertebrates such as snails (Crampton, 1894) and nematodes (Wood, 1998) (see below). However, evidence has accumulated during the past year that vertebrates also exhibit LR asymmetries during cleavage stage (Kramer et al., 2002; Levin et al., 2002), raising the possibility that there are common aspects of the handedness choice mechanism in both classes of animals.

In *C. elegans* embryos, LR asymmetry first becomes apparent between the four-cell and six-cell stages. The anterior (ABa) and dorsal (ABp) blastomeres of the four-cell embryo enter mitosis in synchrony, with their spindles oriented parallel to the LR axis, orthogonal to the AP and DV axes. During telophase, just before cytokinesis begins, they skew about 20°, always in a counter-clockwise direction (as viewed ventrally), with the result that the two daughter cells formed on the left (ABal and ABpl) lie anterior to their respective sisters (ABar and ABpr) on the right (see Fig. 1B and Movie 1 at http://dev.biologists.org/supplemental/). From this time onwards, the embryo is LR asymmetric (Sulston et al., 1983), and this invariant choice of handedness dictates the polarity of all subsequent LR asymmetries during embryonic and larval development (Wood, 1991). We have arbitrarily designated this normal handedness as dextral. Under standard laboratory growth conditions (Sulston and Hodgkin, 1988), neither sinistral wild-type *C. elegans* embryos nor adults have ever been observed in our laboratory (n>15,000) or elsewhere to our knowledge. However, sinistral embryos can be obtained by micromanipulation (Wood, 1991), early removal of the egg shell (Wood and Kershaw, 1991), cold treatment (Wood et al., 1996) and as we show below, as the result of maternal-effect mutations. Sinistral embryos develop normally into healthy fertile adults with all of their normal LR asymmetries reversed (for a review, see Wood, 1998).

Spindle orientations during the first three cleavages in the embryo are rigidly controlled by mechanisms that are still incompletely understood, although several of the major players have been identified (for a review, see Gonczy, 2002; Lyczak et al., 2002). Following establishment of the posterior pole as the point of sperm entry, many proteins important for early blastomere behavior and specification become asymmetrically partitioned along the AP axis, including the cortical proteins PAR-1 and PAR-2 in the posterior half of the zygote and PAR-3 and PAR-6 in the anterior half, while the cortical protein PAR-4 is distributed uniformly in both halves. These asymmetries are retained at least during the next two cleavages. The two-cell embryo is formed by AP division of the zygote into the larger anterior AB cell and the smaller posterior P_1 cell. When the AB and P_1 spindles form in preparation for the next cleavage in the apparently radially symmetric two-cell embryo, they are oriented orthogonal to the AP axis. However, the spindle in P_1 then is rotated 90° to realign with the AP axis in an active process that involves interactions between the astral microtubules and the cortex, under control of PAR proteins (Hyman and White, 1987; Hyman, 1989; Tsou et al., 2003b). PAR-3 and PAR-6 appear necessary to suppress rotation in the AB cell. As the AB spindle lengthens, constraints of the egg shell force it to skew from its orthogonal orientation so that one of the resulting AB daughters, ABa, lies anterior to its sister ABp, the position of which defines the dorsal side of the embryo. The subsequent polarized cleavage of P_1 is constrained by the AB cell positions, so that the anterior daughter EMS lies ventrally and the posterior daughter P_2 lies posteriorly (see Fig. 1B, top panel). The handedness-determining second cleavage of ABa and ABp then occurs as described above.

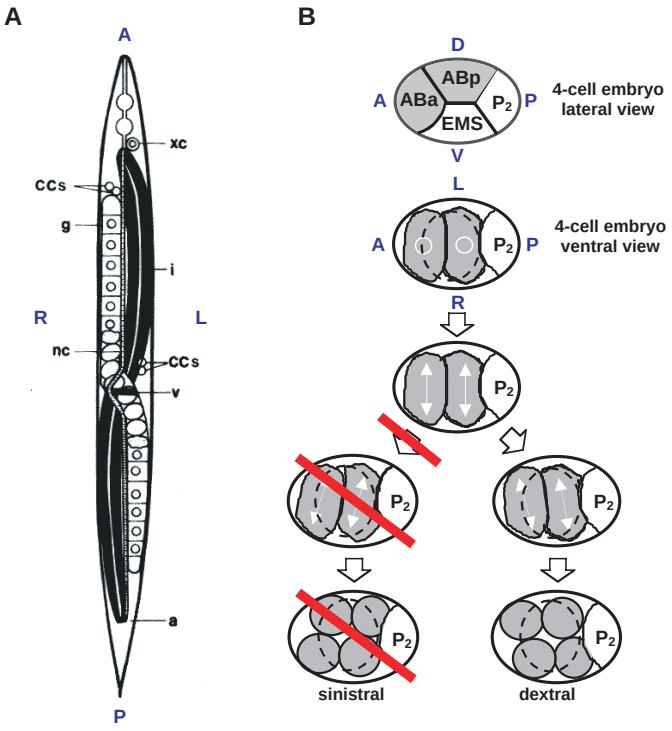

Fig. 1. Handedness of embryonic and adult asymmetries. Colored letters A, P, D, V, R and L indicate anterior, posterior, dorsal, ventral, right and left, respectively. (A) A dextral (normal) adult hermaphrodite viewed ventrally, showing asymmetric placement of the gonad (g), intestine (i), excretory cell (xc), coelomocytes (cc), and the traverse around the vulva (v) of the ventral nerve cord (nc). (B) Cell positions and spindle orientations before, during, and after the second AB cleavage. AB cells are colored gray. No LR asymmetry is apparent at the four-cell stage. In ventral view, the EMS cell (broken circle) lies above the ABa and ABp cells. As ABa and ABp enter mitosis, their spindles (arrows) are oriented parallel to the LR axis. The spindles then skew as shown, always in a counter-clockwise direction (from this vantage point), so that the resulting daughter cells ABal and ABpl are shifted anteriorly relative to their respective sister cells ABar and ABpr, giving the embryo LR asymmetry with the dextral handedness that is maintained throughout development.

Control of spindle orientations during the first two cleavages also involves heterotrimeric G proteins (Zwaal et al., 1996; Jansen et al., 1999) and the product of the *let-99* gene (Rose and Kemphues, 1998; Tsou et al., 2002). In early cleavages, the Gβ protein GPB-1 is associated with centrosomes in prophase and metaphase (Zwaal et al., 1996), and there is evidence that Gα, Gβ and Gγ proteins are all involved in controlling centrosomal behavior, in particular the plane of centrosomal migration around the interphase nucleus that determines orientation of the subsequently forming spindle (Gotta and Ahringer, 2001). Aberrant third cleavage spindle orientations are also observed in G-protein-deficient embryos, but it is not clear whether these effects are a direct consequence of G-protein defects or a secondary consequence of earlier anomalies (see Discussion).

With the knowledge that sinistral embryos are viable, we undertook screens for mutations that would increase the frequency of sinistral animals, in attempts to identify factors involved in the initial handedness choice. Such screens are cumbersome, because mutations affecting third cleavage are expected to have maternal effects, and because the animals always lie on their sides, so that viewing LR asymmetries with a dissecting microscope is impractical without manually rolling individual worms 90° to obtain a dorsal or ventral view. In a small screen of about 2000 genomes, in which we scored gonad handedness in the F3 generation following EMS mutagenesis, we found no handedness reversal mutants. As an alternative strategy, we screened known maternal-effect mutants with early cleavage defects, looking for survivors with sinistral handedness. We describe the results of this screen and characterization of a gene originally designated *spn-1*, which can mutate to cause near randomization of handedness choice at the four- to six-cell stage (Wood, 1998). We show that *spn-1* is the same as a molecularly defined gene encoding the Gα protein GPA-16 (Jansen et al., 1999), and we will subsequently refer to this gene as *gpa-16*.

Materials and methods

Origins and culture of *C. elegans* strains

Wild-type (N2) and mutant strains were from our collection or obtained from the *C. elegans* Genetic Stock Center (CGC), except for *gpa-16(it143)*, *spn-2(it149)* and *spn-3(it151)*, which were isolated by L. S. R. (Rose and Kemphues, 1998), and *gpa-16(pk481)*, which was kindly provided by G. Jansen (Jansen et al., 1999) as a homozygous-viable but semi-sterile strain carrying a *dpy-20(e1282) IV* marker.

After 10 backcrosses to a *bli-3(e767)* strain (which was first backcrossed ten times to N2), the *pk481* allele resulted in almost complete sterility (see Results) and was maintained as a quite stably balanced + *gpa-16(pk481)/bli-3(e767)* + heterozygote. [Based on RNAi phenotypes (Fraser et al., 2000), the predicted gene F56C11.1 near *mex-3* is probably *bli-3*; recombination in this region may be suppressed by the *pk481* rearrangement described below and in Results.] Presence of the *pk481* deletion was verified using appropriate primers for PCR (see Fig. S2 at http://dev.biologists.org/supplemental/). The originally obtained homozygous *pk481* strain reverted spontaneously at a very low frequency in hermaphrodite stocks (more frequently in male stocks) to give apparently wild-type animals that lacked the deletion. This was one of several indications that *pk481* is not a simple deletion, but probably a complex rearrangement that includes a wild-type copy of the gene (see Results). Worms were cultured and mated under standard conditions (Sulston and Hodgkin, 1988), with OP50 bacteria as food unless otherwise indicated.

Screen of maternal-effect lethals for handedness reversal

Alleles were selected on the basis of reported cleavage-plane-defects and where possible, incomplete penetrance so that adult animals could be obtained. Each strain was cultured at 20 or 25°C, and adults were scored for handedness reversal under a dissecting microscope by rolling 90° to give a dorsal or ventral view and observing the position of the anterior gonad relative to the gut [gonad handedness is a reliable predictor of overall handedness (Wood, 1991; Wood et al., 1996)]. For two mutations that resulted in lack of any viable embryos from homozygous mutant mothers, the embryonic spindle orientations in ABa and ABp were scored for handedness using Nomarski optics (* in the list below). For three mutations that caused low penetrance gonad abnormalities, handedness was checked by observation of ventral nerve cord and coelomocyte positions (**in the list below).

For the following strains, no reversed animals were seen at either temperature ($n \geq 50$ for each strain): N2 (n>15,000), *emb-5(g16ts)*, *emb-5(g65ts)*, *emb-8(hc69ts)*, *emb-11(g1)*, *emb-12(g5ts)*, *emb-14 (g14ts)*, *emb-18(g21ts)*, *emb-21(g31ts)*, *emb-26(g47)*, *emb-27(g48)*, *emb-34(g62)*, *gad-1(ct226)***, *glp-1(q224ts) (n=11*)*, *glp-1(e2141) (n=8*)*, *let-99(it141)*, *let-99(s1201)*, *par-2(it5)***, *par-4(it33)***, *par-4(it47)*, *spd-1(oj5)*, *spd-2(oj29)*, *spn-2(it149)*, *stu-10(oj14)*, *stu-11(oj18)*, *zyg-2(g57)*, *zyg-10(b261ts)*, *zyg-11(b273ts)*, *zyg-11(b2)* and *zyg-11(mn40)*.

A few percent of reversed animals were seen in strains of genotypes: *goa-1(n363)*, 2%; *gpb-1(pk44);Ex170*, 4%; *spn-3(it151)*, 3% (n>300); *zyg-8(b235ts)*, 1% at 25°C only (n=100); *zyg-9(b288ts)*, 2% at 25°C only; *zyg-11(b271ts)*, 2% at 25°C only. In the *gpa-16(it143)* strain, 20% of animals were reversed at 20°C (n>10,000) and 39% at 25°C (n>6000).

Genetic mapping and complementation testing

All phenotypic analysis was performed with a *gpa-16(it143)* strain that had been outcrossed to wild-type (N2) at least ten times. This mutation was located on Linkage Group (LG) I using STS mapping (Williams et al., 1992) and was further mapped by standard 2- and 3-factor crosses relative to *dpy-5*, *unc-13*, *egl-30*, *src-1* and *mex-3* (see Fig. 4A). The *it143* mutation mapped close to the left telomere of linkage group (LG) I in a region poor in known genetic markers and cosmid coverage. The free duplication *sDp2* complemented *it143*; the deletion *hDf10* deletes the *gpa-16* locus.

Failure of the *it143* mutation to complement *gpa-16(pk481)* was demonstrated in two experiments. In the first, *it143; him-5(e1490)* males were crossed to the original NL2332 *gpa-16(pk481); dpy-20(e1282)* strain of G. Jansen and non-Dpy hermaphrodite progeny were tested for semi-sterility (fewer than 20 eggs laid) or fertility (more than 100 eggs laid) in comparison with controls. Control *pk481/+* animals (obtained from a separate cross) and *pk481/it143* animals reared at 16°C were fertile, but *pk481/it143* animals reared at 25°C were semi-sterile, like *pk481/pk481* controls. In the second experiment, males of the ten times backcrossed strain of *gpa-16(pk481)/bli-3(e767)* (see Results) were crossed to an *it143 unc-13* strain, and the non-Unc progeny hermaphrodites that did not segregate Bli-3 animals were scored for sterility or fertility, with similar results that showed failure of complementation at 25°C. Handedness reversal was not scored in these experiments.

Temperature-sensitive period (TSP) of *it143*

The TSP for the *it143ts* mutant phenotypes was determined by shifting homozygous *it143* hermaphrodites or early embryos dissected from them between the permissive (16°C) and non-permissive (25°C) temperatures. Onset of the TSP was determined by rearing homozygous *i143* hermaphrodites at 25°C, then shifting them to 16°C, collecting laid eggs at 2 hour intervals and estimating the stage of oogenesis at which each batch of eggs was shifted from the known time course of oocyte maturation, fertilization, early embryogenesis and egg-laying at these two temperatures (Wood et al., 1980), as described elsewhere (Wood et al., 1996). To determine the end of the TSP, early embryos from homozygous *it143* hermaphrodites reared at 16°C were removed at the one- to two-cell stage in a 16°C constant temperature room. The initial cell divisions were observed with a dissecting microscope and embryos at various stages (up to eight cells) were transferred to pre-warmed 25°C plates to mature. In both protocols, embryos were assayed for viability (hatching within 24 hours), and the adults that developed from viable embryos were scored for dextral or sinistral handedness as described in the text. The range of embryonic arrest phenotypes for all shifted embryos was indistinguishable from that observed in embryos developing entirely at 25°C.

Microscopy

For observations of early spindle orientations by Nomarski microscopy, one- to two-cell embryos were dissected from gravid hermaphrodites reared at either 25 or 20°C. To mount embryos in a suitable orientation for observing the LR cleavages of ABa and ABp (dorsal or ventral aspect), embryos were allowed to settle through a small drop of 25% EGM/75% egg salts onto a poly-lysine coated coverslip and covered with a drop of silicone oil. The coverslip was inverted over a slide onto two pieces of double-sided tape (3M core series 2-0300) for support. Development of suitably oriented embryos was recorded using a multi-focal-plane, time-lapse video microscopy system (4D microscope) as described elsewhere (Powell-Coffman et al., 1996; Knight and Wood, 1998). Recordings were made at 20-22°C on embryos raised at 20°C except where noted. A through-focus series of optical sections in 2 μm steps was recorded every 30 seconds until embryos reached the 28-cell stage, and a final series was taken 14 hours later to record terminal phenotype. Handedness was scored in the adults developing from hatched first-stage (L1) larvae that could be recovered to NGM plates from the hanging drop mounts.

For immunofluorescence microscopy, embryos were prepared by standard procedures for *C. elegans* (Miller and Shakes, 1995), with the following modifications. All PBS washes were supplemented with 0.5% Triton X-100. The following antibodies were used: 3A5 mouse monoclonal anti-tubulin (gift of J. R. McIntosh); rabbit polyclonal anti-GPB-1 (gift of R. Plasterk), rabbit polyclonal anti-PAR-3 (gift of K. Kemphues). Secondary FITC- and TRITC-conjugated antibodies were from Molecular Bioprobes. Embryos were also stained with DAPI to visualize nuclei. Immunofluorescence was observed on the Leica microscope described for 4D recording, and images were deconvoluted using SlideBook v.2.6 software (3I, Denver, CO).

Blastomere isolations

Isolations were performed as described elsewhere (Edgar, 1995), with a few modifications. Embryos were cultured in a different medium (Shelton and Bowerman, 1996), and because at 25°C *it143* mutant

embryos lysed easily, blastomere isolations were performed at room temperature (22°C) on embryos from hermaphrodites reared at 20°C. In addition, the time of incubation in hypochlorite was reduced to 1 minute to increase survival of sensitive mutant embryos.

Laser ablations

Laser ablations were performed with a VSL-337 (Laser Science) nitrogen-pumped dye laser through a Zeiss microscope equipped with Nomarski optics. Embryos were mounted in hanging drops as described above, but in egg salts. Each target cell was irradiated until debris accumulated in the nucleus and the cell subsequently divided no more than once.

RNA interference (RNAi)

To silence *gpa-16* expression by RNAi, *gpa-16* dsRNA was prepared in vitro and administered either by injection (Fire et al., 1998) or by soaking (Tabara et al., 1998) as described elsewhere (Van Auken et al., 2002). RNAi effects on first and second cleavage and on lethality and handedness reversal were analyzed using a 425 bp fragment and full-length *gpa-16* dsRNA, respectively. In some RNAi experiments, worms were reared on a feeding vector strain of *E. coli* carrying a *gpa-16*-derived construct (kindly provided by Monica Gotta) that could be induced by IPTG to produce both *gpa-16* sense and antisense transcripts (Timmons and Fire, 1998).

Results

The *it143ts* mutation causes partially penetrant maternal-effect embryonic lethality with handedness reversal among survivors

In an attempt to identify genes that might be required for normal embryonic handedness choice, we screened for sinistral animals, generally identified by gonad reversal, among mutant strains with known defects in early cleavage plane orientations under semi-permissive conditions that allowed some embryonic survival. Results for 35 of these strains are summarized in the Materials and methods. Only the *it143ts I* mutation resulted in a substantial percentage of sinistral animals. Although mutations in a few other genes resulted in low frequencies (<5%) of reversal, it appears that loss of control over early cleavage plane orientation does not always cause randomization of handedness, suggesting that *it143* is somehow unusual among these mutations (see Discussion).

The *it143* mutation was originally isolated in a screen for maternal-effect lethal mutants with defects in early spindle orientations (Rose and Kemphues, 1998), and its effect on handedness choice has been mentioned previously (Bergmann et al., 1998; Wood, 1998). It causes temperature-sensitive, partially penetrant maternal-effect lethality (Mel phenotype) as well as LR reversal, as shown in Table 1. When *it143* homozygous hermaphrodites are reared at 25°C, about 70% of their embryos are inviable. The survivors grow to adulthood, and about 40% of these animals are sinistral. At 16°C, about 98% of the embryos survive, and only a few percent develop into sinistral animals, while at 20°C, the results are intermediate.

The following evidence indicates that the effects of *it143* on viability and handedness reversal at 25°C are strictly maternal. The *it143* homozygous progeny of *it143/+* hermaphrodites are fully viable and do not include sinistral animals. However, when *it143* hermaphrodites reared at 25°C are mated to wild-type males, the resulting *it143/+* progeny show percentages of embryonic lethality and adult sinistrality similar to those above. Homozygous *it143* males mated to marked hermaphrodites produce only normal dextral *it143/+* progeny (Table 1).

Table 1. Effects of temperature, parental genotype and gene dosage on *it143* mutant phenotypes

Maternal genotype	Temperature (°C)	Embryonic lethality (%)*	Reversed progeny (%)*
Wild type (N2)	16	<1	0
Wild type (N2)	25	3	0
it143/it143	16	2	6
it143/it143	20	30	19
it143/it143	25	70	39
it143/+	16	<1	0
it143/+	25	<1	0
it143/it143/+†	16	<1	0
it143/0‡	16	33	0
it143/0‡	25	75	23
it143/it143 × +/+ males	25	68	41
+/+ × *it143/it143* males	25	<1	0

*Among progeny of survivors. At least 200 embryos were scored for each entry.
†Genotype *it143 I; sDp2 (I;f)*.
‡Genotype *it143/hDf10 I*. Lethality figures corrected for inviability of *hDf10* homozygous progeny.

Like the LR reversed animals produced by micromanipulation of four- to six-cell embryos (Wood, 1991; Wood and Kershaw, 1991), sinistral *it143* mutant animals exhibit reversal of all LR asymmetric anatomical features examined, including positions of the gonad and intestine, positions of the coelomocytes, organization of the ventral nerve cord, migration of the Q neuroblasts, and patterning of the motor axons from the ventral cord that innervate dorsal muscles (D.C.B., unpublished). The only exception is chirality of the cuticle, which remains the same in dextral and sinistral animals (Bergmann et al., 1998).

Characterization of the *it143* phenotype

To define the temperature-sensitive period (TSP) of *it143* for embryonic lethality and handedness reversal, homozygous mutant hermaphrodites or their embryos were shifted from 16°C to 25°C or vice versa at different stages (see Fig. S1 at http://dev.biologists.org/supplemental/). Downshifting of hermaphrodites during different stages of oocyte maturation defined the start of the TSP as after oocyte maturation for reversal and mid-oogenesis for lethality. Upshifting of individual embryos isolated and staged at the two- to eight-cell stages showed that the TSP for reversal was over by the four-cell stage and for lethality by the eight-cell stage. Therefore, as *it143* is a loss-of-function mutation (see below), action of the gene it defines is required at the four-cell stage or before to dictate the handedness of all subsequent LR asymmetries. These results are consistent with the previous demonstration that reversal of handedness by micromanipulation during the ABa and ABp cleavages is sufficient to reverse all LR asymmetries during development (Wood, 1991).

Effects of the mutation on spindle orientations could be directly observed by DIC microscopy of *it143* mutant embryos. When produced and observed at 25°C, these embryos exhibited aberrant behavior of mitotic spindles in each of the first three cleavages.

In one-cell mutant embryos, nuclear rotation occurred, and

first cleavage was asymmetric (as in the wild type), producing a larger AB and a smaller P_1 cell. However, the oscillations of the posterior spindle pole during anaphase were absent or greatly reduced (9/13 embryos) compared with wild-type embryos. During second cleavage, nuclear rotation in P_1 during prophase failed in most *it143* mutant embryos (14/18), although late rotations during anaphase were sometimes observed (5/15).

Dramatic effects were observed in the third-cleavage behavior of the ABa and ABp spindles, whose orientations are critical for establishing embryonic handedness. To analyze LR asymmetries, 27 mutant embryos from hermaphrodites reared at 20°C (the temperature at which the highest percentage of LR reversals was seen among all, including inviable, embryos), were observed in dorsal or ventral view. Under these conditions, spindle misorientations in cells other than ABa and ABp were rare (4/27). At third cleavage, most of the remaining embryos initially elongated their ABa and ABp spindles correctly along the LR axis (e.g. Fig. 2H). In nine out of these 23 embryos, the ABa and ABp spindles skewed approximately normally (not shown; similar to Fig. 2C) to generate dextral embryos and adults. However, in about 40% of the embryos (10/23), the elongated spindles skewed in the opposite direction (Fig. 2I), causing the right daughters to be anterior at the six-cell stage. The result was a sinistral embryo, and for three that were recovered and allowed to mature, a sinistral adult. The remaining three animals exhibited highly abnormal A/P or D/V skewing (Fig. 2F,L), and these embryos were inviable. Lack of P_1 nuclear rotation did not appear to correlate with third-cleavage spindle behavior. The third-cleavage spindle orientations and terminal phenotypes for the 27 embryos described above are summarized in Table S1 at http://dev.biologists.org/supplemental/.

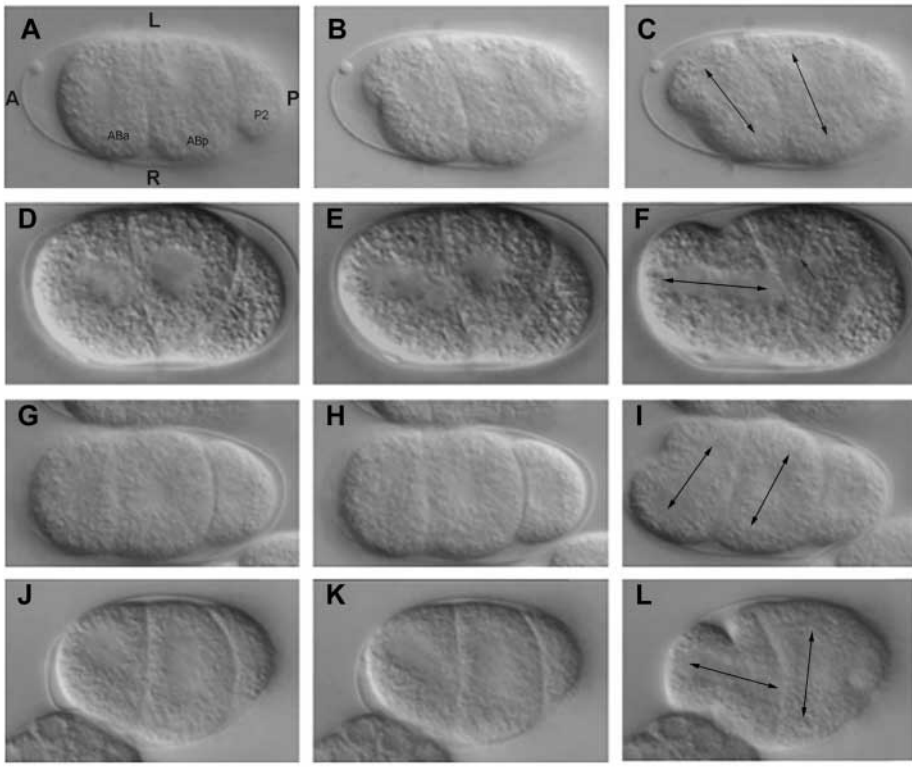

Fig. 2. Spindle orientations during cleavage of ABa and ABp in wild-type and *it143* mutant embryos. All embryos are viewed ventrally just prior to and during second AB cleavage (see Materials and methods). Double-headed arrows indicate orientations of the ABa and ABp spindles. (A-C) Normal spindle orientations in a wild-type embryo. (D-L) The aberrant spindle orientations in three abnormal *it143* mutant embryos at 20°C. The embryo in G-I developed into a sinistral adult, while the other two mutant embryos (D-F and J-L) were inviable.

To ask whether the misorientation of ABa and ABp spindles was cell-autonomous or influenced by neighboring blastomeres, the cleavage of AB cells isolated either singly (n=4) or in combination with P_2 or P_2-derived cells as an orientation marker (n=6) from *it143* mutant embryos was observed in culture at 20°C. Isolated AB cells from wild-type embryos undergo several rounds of synchronous ordered helical cleavage (Laufer et al., 1980). By contrast, AB cells from the mutant exhibited more random cleavage orientations (Fig. 3), consistent with a cell-autonomous spindle orientation defect. A reversed helical pattern with the opposite screw sense was not observed in these experiments. Under conditions more closely approximating their normal embryonic environment, AB-cell spindle orientations also appear to be cell-autonomous based on laser ablation experiments. When the EMS cell (n=10) or the P_2 cell (n=20) was ablated in four-cell wild-type embryos, no effects were seen on the subsequent LR dextral cleavage of the two AB cells (data not shown).

The gene defined by *it143* encodes a Gα heterotrimeric G-protein subunit

The *it143* mutation was mapped genetically in standard two- and three-factor crosses to the left end of LGI, near the *bli-3* marker (Fig. 4A). Attempts at fine mapping and positional cloning were hampered by the paucity of genetic and SNP markers and the poor cosmid coverage in this region, as well as the difficulty of rescue experiments using the partially penetrant *it143* phenotypes as assays. However, a candidate gene approach proved successful, following the finding that a predicted Gα-encoding gene named *gpa-16*, located in the same vicinity (Jansen et al., 1999), was involved in control of centrosomal positioning and spindle orientation during the first three embryonic cleavages (Jansen et al., 1999; Gotta and Ahringer, 2001). A strain homozygous for a reported internal deletion of this gene, *pk481* (kindly provided by G. Jansen), did not display a Mel phenotype like *it143*, but exhibited reduced hermaphrodite fertility (10-30 progeny) relative to wild type (200-300). In *trans*-heterozygotes, *it143* complemented the *pk481* phenotype at 16°C but failed to complement at 25°C (see Materials and Methods). Determination of the *gpa-16* genomic sequence in DNA from

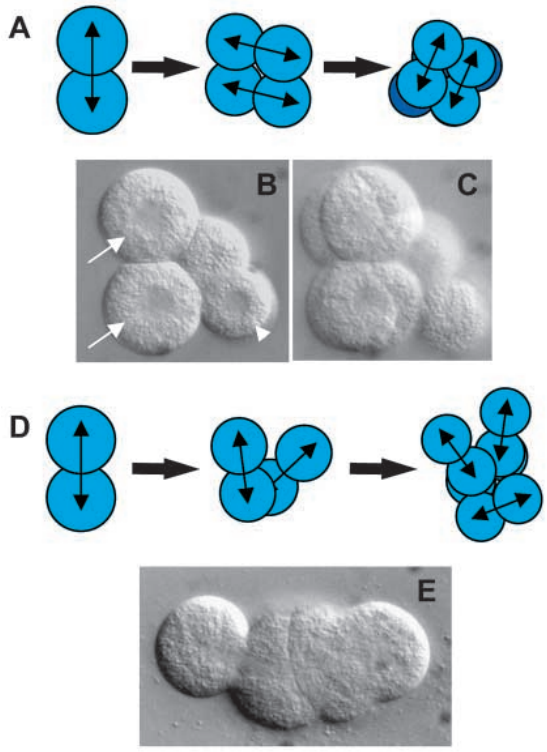

Fig. 3. Cleavage of blastomeres in culture from wild-type and *it143* mutant embryos. (A) The regular helical pattern of cleavages exhibited by AB cells separated from a cultured four-cell de-vitellinized embryo (egg shell removed) (Edgar and Wood, 1993). (B,C) DIC images of such an embryo (arrows indicate AB cells) at the four- and six-cell stages, respectively. The EMS and P_2 (arrowhead) cells have been left in place to provide positional markers. In C, images at two focal planes have been superimposed to show positions of the four AB blastomeres. (D) Example of the more random cleavage patterns seen in cultured *it143* mutant AB cells. (E) DIC image of AB cells from a mutant embryo. Two AB cells isolated from a de-vitellinized four-cell embryo have divided with their spindles oriented in approximately the same direction as those in the previous cleavage, to give an almost linear arrangement of four AB cells. Similar results were obtained in 10 independent experiments (see text).

the *it143* mutant revealed a single base substitution in exon 6 (Fig. 4B), predicted to cause a G→D substitution at the highly conserved residue 202 in the resulting Gα protein. Therefore, the gene defined by the mutation originally designated as *spn-1(it143)* appears to be *gpa-16*.

gpa-16(it143) is a loss-of-function mutation

The *gpa-16(it143)* mutation behaved genetically as a fully recessive, loss-of-function (*lf*) allele at 25° (Table 1). Homozygous *gpa-16* animals hermaphrodites carrying one copy of the wild-type gene on a free duplication exhibited no embryonic lethality or reversal. The defective phenotypes of a hemizygous strain carrying *gpa-16(it143)* in *trans* to a deficiency that uncovers the *gpa-16* locus were only slightly more severe than those of *gpa-16(it143)* homozygotes at all three temperatures tested.

To test whether *gpa-16(RNAi)* mimics the *it143* phenotype, as predicted if *it143* is a *lf* mutation, *gpa-16* double-stranded (ds) RNA was injected into wild-type N2 adult hermaphrodites. The resulting *gpa-16(RNAi)* embryos observed at 25°C exhibited spindle abnormalities similar to those described above for *gpa-16(it143)* at 25°C: reduction or absence of posterior spindle pole oscillations preceding first cleavage (9/10), and failure of P1 nuclear rotation prior to spindle elongation during second cleavage (7/9).

Embryonic lethality and handedness reversals were analyzed on larger numbers of embryos following soaking of N2 hermaphrodites in *gpa-16* dsRNA. Soaking at 16°C or 20°C had little effect, but soaking at 25°C caused up to 50% embryonic lethality, with a handedness reversal rate of about 10% among survivors (n=50). *gpa-16(RNAi)* in the *gpa-16(1t143)* background caused no increase in embryonic lethality at 25° over the 70% observed to result from *gpa-16(it143)* alone. Therefore, *gpa-16(RNAi)* and *gpa-16(it143)* cause similar Mel and reversal phenotypes, supporting the conclusion that this allele results in loss of gene function at 25°C.

The *it143* allele did not cause sterility of surviving animals at 25°C (n>100). By contrast, the *pk481* strain, after 10 backcrosses to wild type, exhibited no zygotic lethality among progeny of *pk481*/+ hermaphrodites, but nearly complete

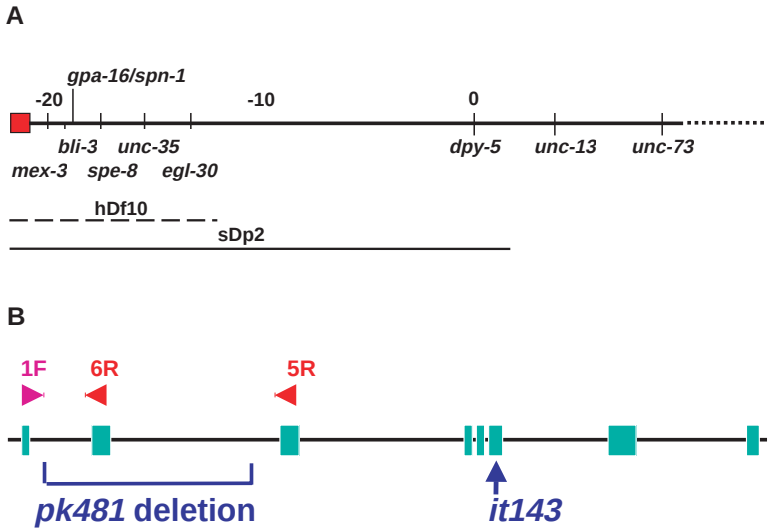

Fig. 4. Map position and predicted structure of the *gpa-16/spn-1* gene. (A) Map of the left arm of LGI, showing selected markers and the telomere (red square). Numbers above the line indicate map units. Regions deleted by the *hDf10* mutation and duplicated by the *sDp2* duplication are indicated by horizontal lines below the map. *gpa-16(it143)* was positioned close to *bli-3* by two- and three-factor mapping. (The *src-1* locus, used for mapping but not shown, is located one map unit to the left of *unc-35*.) (B) Structure of the *gpa-16* gene as determined by comparing cDNA sequence to the genomic sequence. The extent of the deletion *pk481* and the position of the point mutation *it143* are indicated in blue. PCR primers designated 1F, 5R and 6R (sequences available on request) were used to assay presence of the wild-type and the deletion sequence in single mutant animals by direct PCR sequencing (see Fig. S2 at http://dev.biologists.org/supplemental/ and text). The sequence around the G→A substitution (bold) in the mutant is TTTTCGACGTTG**A**CGGACAGCGATCC.

sterility of the progeny homozygotes, regardless of temperature. This sterility could not be overcome by mating with wild-type males. These results suggested that the *gpa-16*-null phenotype might be a gonadogenesis or oogenesis defect resulting in hermaphrodite sterility. However, further characterization of *pk481* indicated that interpretation of the resulting phenotype might be problematic, for two reasons. First, about 10% of *pk481*/+ heterozygotes were found to be sterile (see supplemental text at http://dev.biologists.org/supplemental/), suggesting that the allele is semidominant. Second, analysis by PCR using appropriate primers showed that in addition to the reported deletion mutation, *pk481* homozygotes also carried a linked, wild-type copy of the *gpa-16* gene, suggesting that *pk481* is a complex rearrangement (see Fig. S2 at http://dev.biologists.org/supplemental/). Based on these findings, plus the prediction that the *pk481* deletion should result in synthesis of a GPA-16 protein lacking the GTP binding domain but otherwise complete (Jansen et al., 1999), it seemed possible that *pk481* might be an antimorphic rather than a null allele.

To obtain a further indication of the null phenotype, we asked whether *gpa-16(RNAi)* also caused sterility. Wild-type worms were reared from hatching on an appropriate feeding vector expressing *gpa-16* dsRNA or on a control strain expressing the empty vector, at 16, 20 and 25°C. The effects on maternal-effect embryonic lethality were weaker than in the soaking experiments, and again were seen only at 25°C. The rate of maternal-effect lethality increased from 13% (*n*=597) on control plates to 23% (*n*=250) on the feeding vector, while sterility increased from 2% (*n*=60) to 98% (*n*=58). These results suggest that the *gpa-16* null phenotype is maternal-effect lethality if gene function is lacking during oogenesis, and hermaphrodite sterility if gene function is lacking during larval development. The basis for sterility has not been investigated, except that in each of four *pk481* homozygous adult hermaphrodites examined the gonad morphology appeared abnormal.

Loss of *gpa-16* function reduces normal centrosomal association of the Gβ protein GBP-1

Earlier results indicated that the Gβ protein of *C. elegans*, encoded by the *gpb-1* gene (Zwaal et al., 1996), is required for correct orientations of early blastomere cleavages and that GPB-1 associates with the centrosomes of these cells during mitosis. It was further shown that maternal silencing of the two Gα genes *goa-1* and *gpa-16* by RNAi caused loss of both cortical and centrosomal localization of GPB-1 (Gotta and Ahringer, 2001). Our screen (Materials and methods), showed that reduction of *gpb-1* function in early embryos also caused a low frequency of handedness reversals, although its effect was not as strong as that of *gpa-16(it143)*. Tests for effects of the *gpa-16(it143)* mutation on localization of GPB-1 were done using an anti-GPB-1 antibody (Zwaal et al., 1996) to stain wild-type and *gpa-16(it143)* mutant embryos of two to 28 cells. Staining was seen around the cortex of each blastomere in both genetic backgrounds at all temperatures tested. In wild-type embryos at 25°C, centrosomal staining was seen in 74% of cells in mitosis (*n*=58; see Fig. 5). In mutant embryos from hermaphrodites reared at 16°C, the centrosomal association did not appear significantly affected (69%; *n*=13). However, in mutant embryos from animals reared at 20°C or 25°C, centrosomal staining was significantly reduced to 28% (*n*=40) and 25% (*n*=36), respectively. These results suggest that functional Gα protein GPA-16 may be required for the association of Gβ protein with centrosomes in early embryos.

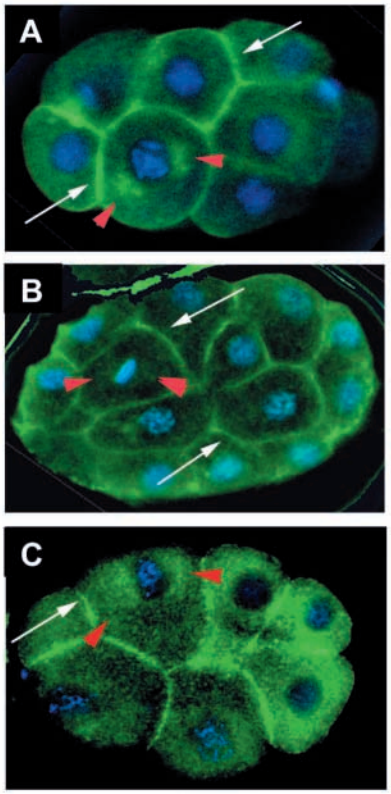

Fig. 5. The *gpa-16(it143)* mutation reduces centrosomal association of the Gβ protein GPB-1 in early cleavages at 25°C. Embryos were stained with DAPI (blue), rabbit anti-GPB-1 visualized using an FITC-conjugated secondary antibody (green), and mouse anti-tubulin visualized with a TRITC-labeled secondary antibody (not visible). Mitotic cells were identified by their chromosomal (DAPI) and spindle (anti-tubulin) morphologies (not shown), recorded prior to examination of anti-GPB-1 staining. (A) Wild-type embryo showing cortical (white arrows) and centrosomal (red arrowheads) GPB-1 staining in a prophase nucleus. (B) A *gpa-16* embryo at 25°C shows cortical but not centrosomal staining; red arrowheads indicate a metaphase and a prophase nucleus. (C) *gpa-16(it143)* embryo at 16°C showing both cortical and centrosomal staining. See text for quantitative data.

gpa-16(it143) interacts genetically with mutations in three *par* genes

The genes *par-1*, *par-2*, *par-3*, *par-4* and *par-6* encode proteins that associate in a position-specific manner with the cell cortex in early blastomeres and are required for normal spindle orientations in the first two embryonic cleavages (see Introduction). Loss-of function mutations in the *par* genes are fully recessive and in homozygotes cause strict maternal-effect embryonic lethality with aberrant early cleavages.

In *gpa-16/+; par/+* double heterozygotes, a significant frequency of embryonic lethality was observed with the *par-4(it33)* allele but not with another *par-4(lf)* allele or the alleles of other *par* genes that were tested. However, in animals of

Table 2. Interaction of *gpa-16(it143)* with Par mutations

Par allele	Temperature (°C)	Embryonic lethality in % of total embryos*				Enhanced lethality
		+/+;par	*gpa-16/+;par/+*	*gpa-16;par/+*	*gpa-16/+;par*	
par (+)	16	<1 (many)	<1 (many)	2 (>1000)†	<1 (many)	
	25	<1 (many)	<1 (many)	69 (>1000)†	<1 (many)	
par-3(it62)	16	100 (8)	<1 (5)	15 (896)†	99 (55)	Yes
	25	100 (8)	<1 (5)	92 (350)†	100 (10)	Yes
par-4(it47ts)	16	80 (618)†	<1 (5)	41 (1491)†	97 (549)†	Yes
	25	100 (911)†	<1 (5)	–	100 (16)	
par-4(it33)	25	100 (8)	10 (332)†	–	100 (12)	Yes
par-6(zu22)	16	100 (8)	<1 (20)	84 (560)†	100 (42)	Yes
	25	100 (8)	<1 (20)	92 (144)†	100 (42)	Yes

*Percent lethality was scored on broods of 100-200 embryos. *n* values (parentheses) indicate the number of broods scored except as noted. See text for handedness reversal frequencies.

†*n* values (in parentheses) indicate total embryos scored for lethality.

genotype *gpa-16; par/+*, a single copy of *par-3(it162)*, *par-4(it147)*, or *par-6(zu222)* in the homozygous *gpa-16(it143)* background caused significant enhancement of the *gpa-16* embryonic lethality (Table 2). The *par-6(zu222)* allele, but not the others tested, also enhanced the *gpa-16* handedness reversal frequency: embryos from *gpa-16(it143); par-6(zu222)/+* hermaphrodites at 16°C were 16% viable with a reversal frequency of 51% among survivors (*n*=60). Finally, using two *ts* alleles, it was possible to make the doubly homozygous strain *gpa-16(it143); par-4(it47)*. At 16°, this strain exhibited a similar handedness reversal frequency to the *gpa-16(it143)* single mutant, but enhanced embryonic lethality (from 2% to ~70%). In addition, the *gpa-16(it143)* mutation, which was never observed to affect production of differentiated intestinal cells in inviable embryos (D.C.B., unpublished) as assayed by presence of gut granules (Laufer et al., 1980), appeared to largely suppress the lack of gut granules seen in 70% of *par-4(it47)* single mutant embryos (Morton et al., 1992), to 8% (*n*=92) in the double mutant. Thus, it appears that *gpa-16(it143)* can interact genetically with mutations in *par-3*, *par-4* and *par-6*, all of which encode components of the AB-cell cortex.

Discussion

Non-redundancy of *gpa-16* function in early cleavages

We have shown that the genetically defined *spn-1* gene encodes the Gα protein GPA-16, and we suggest that this gene be referred to from now on by the more descriptive name of *gpa-16*. The loss-of-function allele *gpa-16(it143)* causes a partially penetrant Mel phenotype with defects in early spindle behavior and near randomization of handedness among survivors. *gpa-16(RNAi)* causes a similar though somewhat weaker phenotype. In contrast to our results, Gotta and Ahringer (Gotta and Ahringer, 2001) concluded that *gpa-16* and the major embryonic Gα-protein gene *goa-1* have redundant essential functions, based on the finding of only weak spindle orientation defects in single mutants and strong genetic interactions between them. Their results implied that *gpa-16(RNAi)* alone would have no early embryonic phenotype, although this experiment was not reported. This apparent difference between our results may have two explanations: (1) we found that *gpa-16(RNAi)* effects are temperature sensitive and seen only at 25°C, while the Gotta and Ahringer experiments were carried out at lower temperature; and (2) their observations of the *gpa-16* defective phenotype were carried out using *gpa-16(pk481)*, which we have shown is a complex, non-null allele. We have, however, confirmed the strong genetic interaction of *goa-1* and *gpa-16* by showing that animals of genotype *gpa-16(it143) goa-1(n363)* do not survive to adulthood even at 16°C (W.B.W., unpublished).

There are also two possible interpretations of the temperature effects we have observed. First, the *gpa-16* functions in early cleavages could be required only at higher temperatures, so that any loss-of-function condition would show temperature sensitivity. Second, the *it143* mutation could cause loss of function only at higher temperatures, and the temperature dependence of the *gpa-16(RNAi)* phenotypes could result from temperature-sensitivity of the RNAi soaking method for this gene. As temperature effects on RNAi efficiency have been noted previously (e.g. Winston et al., 2002), we cannot yet distinguish between these alternatives.

The embryonic lethality and handedness reversal resulting from loss of *gpa-16* function results from the apparent randomization of AB-cell spindle orientations during the third cleavage in *gpa-16* mutant embryos. At 25°C, *gpa-16* defects also affect spindle behavior during second cleavage as discussed further below. However, our observations at 20°C, where second cleavage is normal, suggest that the misorientation at third cleavage is a specific effect of the *gpa-16* defect rather than an indirect consequence of abnormal second cleavage.

There are some indications that GPA-16 may play a special role, particularly in controlling the third-cleavage AB-cell spindle orientations required for normal establishment of LR asymmetry and handedness choice. Although GPA-16 and GOA-1 functions are at least partially redundant for spindle orientation during first and second cleavages (Miller et al., 2000; Gotta and Ahringer, 2001) (this report), we have shown that *gpa-16* loss of function alone also causes aberrant spindle behaviors in one-cell and two-cell embryos. Moreover, while maternal *goa-1* and *gpb-1* loss of function cause only low percentages of embryonic lethality and very little handedness reversal (Materials and methods), *gpa-16* loss of function

causes up to 70% embryonic lethality and nearly complete randomization of handedness. Our results suggest that in the absence of *gpa-16* function, embryos are more often reversed but otherwise normal than when other genes affecting spindle orientation are defective. The basis for this difference is not clear; at this point we can conclude only that there is some specific requirement for GPA-16 in orientation of the AB-cell spindles during third cleavage.

Possible roles of G-proteins in spindle orientation and handedness choice

The function of the GPA-16 Gα protein in control of third-cleavage AB-cell spindle orientations remains to be determined, but possible roles can be suggested based on previous results with G-protein-defective embryos. The *C. elegans* Gβ protein GPB-1 was shown to associate with centrosomes and the cortex in early cleavages, and lack of maternal GPB-1 caused spindle orientation defects and embryonic lethality (Zwaal et al., 1996). *Gα(RNAi)*, that is, simultaneous silencing of the two embryonic Gα-protein genes *goa-1* and *gpa-16*, was reported to prevent the normal migration of centrosomes around the nucleus prior to second cleavage, leading to abnormal spindle orientations (Gotta and Ahringer, 2001). Silencing of *gpb-1* appeared to disrupt normal centrosomal steering prior to second cleavage, with similar effects. In addition, both Gα and Gβ silencing caused defects in P_1 nuclear rotation, which depends on interactions between astral microtubules and the cortex. Finally, several recent studies have implicated Gα proteins in the force-generating mechanisms that asymmetrically position the first-cleavage spindle to produce daughter cells of different sizes (Colombo et al., 2003; Gotta et al., 2003; Grill et al., 2003; Srinivasan et al., 2003; Tsou et al., 2003a), although the roles of G-proteins in these mechanisms remain unclear.

Thus, Gα and Gβ proteins appear to function together in dictating early spindle orientations, by controlling centrosomal migration as well as cortical interactions with astral microtubules in force generation. Consistent with an important role for GPA-16 in such interactions, we have presented evidence that this protein is required at high temperature for centrosomal association of the GPB-1 Gβ protein and for normal P_1 nuclear rotation, as well as the normal positioning of the ABa and ABp spindles preceding the skewing that results in handedness choice. GPA-16 could also be involved in generating the forces that cause this asymmetric skewing. Finally, a role in astral-cortical interaction is supported by the genetic interactions of *gpa-16(it143)* with *par* genes that encode cortical components of both the ABa and ABp cells.

Our results demonstrate for the first time that heterotrimeric G proteins play a role in establishment of embryonic LR asymmetry and handedness choice. However, it is too early to conclude whether the initial symmetry-breaking event occurs before, during, or after the GPA-16-mediated process of AB-cell spindle orientation parallel to the LR axis. The initial establishment of LR asymmetry could occur subsequently, as the ABa and ABp spindles skew from their initial LR orientation. Alternatively, it could already have occurred in the four-cell embryo prior to ABa and ABp cleavage, causing a so-far undetected asymmetry that becomes apparent only when these cells divide. Searches for earlier asymmetries using molecular markers and further elucidation of the G-protein requirements for spindle orientation will help to distinguish these alternatives.

We are grateful to Ricci Rivera (Boulder) and Adam Hayashi (Davis) for expert technical assistance; to J. Ahringer and M. Gotta for communication of results prior to publication; to G. Jansen and R. Plasterk for *gpb-1* and *pk481* mutant strains; to R. Plasterk for anti-GPB-1 antibody; to M. Gotta for a *gpa-16* RNAi feeding construct; to C. Malone and J. White for assistance in making movies of GFP-labeled spindles with the multiphoton microscope at University of Wisconsin (Madison); and to S. Grill, A. Hyman, J. White and C. Malone for stimulating discussions. Other mutant strains were supplied by the *Caenorhabditis* Genetics Center, which is supported by a grant from the Division of Research Resources, NIH. This research was supported by an NIH grant to W.B.W. (HD-14958) an American Cancer Society RPG to L.S.R. (00-76-01-DDC), and by NIH training grant support for D.C.B. (F32-GM-06965).

References

Bergmann, D. C., Crew, J. R., Kramer, J. M. and Wood, W. B. (1998). Cuticle chirality and body handedness in *Caenorhabditis elegans*. *Dev. Genet.* **23**, 164-174.

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.

Crampton, H. (1894). Reversal of cleavage in a sinistral gasteropod. *Ann. New York Acad. Sci.* **8**, 167-170.

Edgar, L. G. (1995). Blastomere culture and analysis. In Caenorhabditis elegans*: Modern Biological Analysis of an Organism* (ed. H. F. Epstein and D. C. Shakes), pp. 303-322. New York: Academic Press.

Essner, J. J., Vogan, K. J., Wagner, M. K., Tabin, C. J., Yost, H. J. and Brueckner, M. (2002). Conserved function for embryonic nodal cilia. *Nature* **418**, 37-38.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811.

Fraser, A. G., Kamath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M. and Ahringer, J. (2000). Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* **408**, 325-330.

Gonczy, P. (2002). Mechanisms of spindle positioning: focus on flies and worms. *Trends Cell Biol.* **12**, 332-339.

Gotta, M. and Ahringer, J. (2001). Distinct roles for Ga and Gbg in regulating spindle position and orientation in *Caenorhabditis 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 AGS3/PINS control spindle position in the early embryo. *Curr. Biol.* **13**, 1029-1037.

Grill, S. W., Howard, J., Schaffer, E., Stelzer, E. H. and Hyman, A. A. (2003). The distribution of active force generators controls mitotic spindle position. *Science* **301**, 518-521.

Hyman, A. (1989). Centrosome movement in the early divisions of *Caenorhabditis elegans*: a cortical site determining centrosome position. *J. Cell Biol.* **109**, 1185-1193.

Hyman, A. and White, J. (1987). Determination of cell division axes in the early embryogenesis of *Caenorhabditis elegans*. *J. Cell Biol.* **105**, 2123-2135.

Jansen, G., Thijssen, K. L., Werner, P., van der Horst, M., Hazendonk, E. and Plasterk, R. H. (1999). The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nat. Genet.* **21**, 414-419.

Knight, J. K. and Wood, W. B. (1998). Gastrulation initiation in *Caenorhabditis elegans* requires the function of *gad-1*, which encodes a protein with WD repeats. *Dev. Biol.* **198**, 253-265.

Kramer, K. L., Barnette, J. E. and Yost, H. J. (2002). PKCg regulates inside-out signaling by syndecan-2 during Xenopus left-right development. *Cell* **111**, 981-990.

Laufer, J., Bazzicalupo, P. and Wood, W. (1980). Segregation of developmental potential in early embryos of *Caenorhabditis elegans*. *Cell* **19**, 569-577.

Levin, M., Thorlin, T., Robinson, K., Nogi, T. and Mercola, M. (2002). Asymmetries in H(+)/K(+)-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell* **111**, 77-89.

Ludwig, W. (1932). *Das Rechts-Links Problem im Tierreich und beim Menschen*. Berlin: Springer-Verlag.

Lyczak, R., Gomes, J. E. and Bowerman, B. (2002). Heads or tails: cell polarity and axis formation in the early *Caenorhabditis elegans* embryo. *Dev. Cell* **3**, 157-166.

Mercola, M. and Levin, M. (2001). Left-right asymmetry determination in vertebrates. *Annu Rev Cell Dev Biol* **17**, 779-805.

Miller, D. M. and Shakes, D. C. (1995). Immunofluorescence microscopy. In Caenorhabditis elegans*: Modern Biological Analysis of an Organism*, Vol. 48 (ed. H. F. Epstein and D. C. Shakes), pp. 365-394. New York: Academic Press.

Miller, K. G., Emerson, M. D., McManus, J. R. and Rand, J. B. (2000). RIC-8 (Synembryn): a novel conserved protein that is required for G(q)alpha signaling in the *C. elegans* nervous system. *Neuron* **27**, 289-299.

Morton, D., Roos, M. and Kemphues, K. (1992). *Par-4*, a gene required for cytoplasmic localization and determination of specific cell types in *Caenorhabditis elegans* embryogenesis. *Genetics* **130**, 771-790.

Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M. and Hirokawa, N. (1998). Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* **95**, 829-837.

Nonaka, S., Shiratori, H., Saijoh, Y. and Hamada, H. (2002). Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature* **418**, 96-99.

Powell-Coffman, J. A., Knight, J. and Wood, W. B. (1996). Onset of *C. elegans* gastrulation is blocked by inhibition of embryonic transcription with an RNA polymerase antisense RNA. *Dev. Biol.* **178**, 472-483.

Rose, L. S. and Kemphues, K. (1998). The *let-99* gene is required for proper spindle orientation during cleavage of the *C. elegans* embryo. *Development* **125**, 1337-1346.

Shelton, C. and Bowerman, B. (1996). Time-dependent responses to *glp-1*-mediated inductions in early *C. elegans* embryos. *Development* **122**, 2043-2050.

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.

Strome, S., Powers, J., Dunn, M., Reese, K., Malone, C. J., White, J., Seydoux, G. and Saxton, W. (2001). Spindle dynamics and the role of gamma-tubulin in early *Caenorhabditis elegans* embryos. *Mol. Biol. Cell* **12**, 1751-1764.

Sulston, J. and Hodgkin, J. (1988). Methods. In *The Nematode* Caenorhabditis elegans (ed. W. B. Wood), pp. 81-122. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sulston, J., Schierenberg, E., White, J. and Thomson, J. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.

Tabara, H., Grishok, A. and Mello, C. C. (1998). RNAi in *C. elegans*: soaking in the genome sequence. *Science* **282**, 430-431.

Timmons, L. and Fire, A. (1998). Specific interference by ingested dsRNA. *Nature* **395**, 854.

Tsou, M.-F. B., Hayashi, A., DeBella, L. R., McGrath, G. and Rose, L. S. (2002). LET-99 determines spindle position and is asymmetrically enriched in response to PAR polarity cues in *C. elegans* embryos. *Development* **129**, 4469-4481.

Tsou, M.-F. B., Hayashi, A. and Rose, L. S. (2003a). LET-99 opposes Gα/GPR signaling to generate asymmetry for spindle positioning in response to PAR and MES-1/SRC-1 signaling. *Development* **130** 5717-5730.

Tsou, M.-F. B., Ku, W., Hayashi, A. and Rose, L. S. (2003b). PAR-dependent and geometry-dependent mechanisms of spindle positioning. *J. Cell Biol.* **160**, 845-855.

Van Auken, K. M., Weaver, D., Robertson, B., Sundaram, M., Saldi, T., Edgar, L., Elling, U., Lee, M., Boese, Q. and Wood, W. B. (2002). Roles of the Homothorax/Meis/Prep homolog UNC-62 and the Exd/Pbx homologs CEH-20 and CEH-40 in *C. elegans* embryogenesis. *Development* **129**, 5255-5268.

Williams, B., Schrank, B., Huynh, C., Shownkeen, R. and Waterston, R. (1992). A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* **131**, 609-624.

Winston, W. M., Molodowitch, C. and Hunter, C. P. (2002). Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. *Science* **295**, 2456-2459.

Wood, W., Hecht, R., Carr, S., Vanderslice, R., Wolf, N. and Hirsh, D. (1980). Parental effects and phenotypic characterization of mutations that affect early development in *Caenorhabditis elegans*. *Dev. Biol.* **74**, 446-469.

Wood, W. B. (1991). Evidence from reversal of handedness in *C. elegans* embryos for early cell interactions determining cell fates. *Nature* **349**, 536-538.

Wood, W. B. (1997). Left-right asymmetry in animal development. *Annu. Rev. Cell Dev. Biol.* **13**, 53-82.

Wood, W. B. (1998). Handed asymmetry in nematodes. *Semin. Cell Dev. Biol.* **9**, 53-60.

Wood, W. B. and Kershaw, D. (1991). Handed asymmetry, handedness reversal and mechanisms of cell fate determination in nematode embryos. In *Biological Asymmetry and Handedness*, Vol. 162 (ed. G. R. Bock and J. Marsh), pp. 143-164. J. Wiley, Chichester.

Wood, W. B., Bergmann, D. and Florance, A. (1996). Maternal effect of low temperature on handedness determination in *C. elegans* embryos. *Dev. Genet.* **19**, 222-230.

Zwaal, R., Ahringer, J., van Luene, H., Rushforth, A., Anderson, P. and Plasterk, R. (1996). G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos. *Cell* **86**, 619-629.

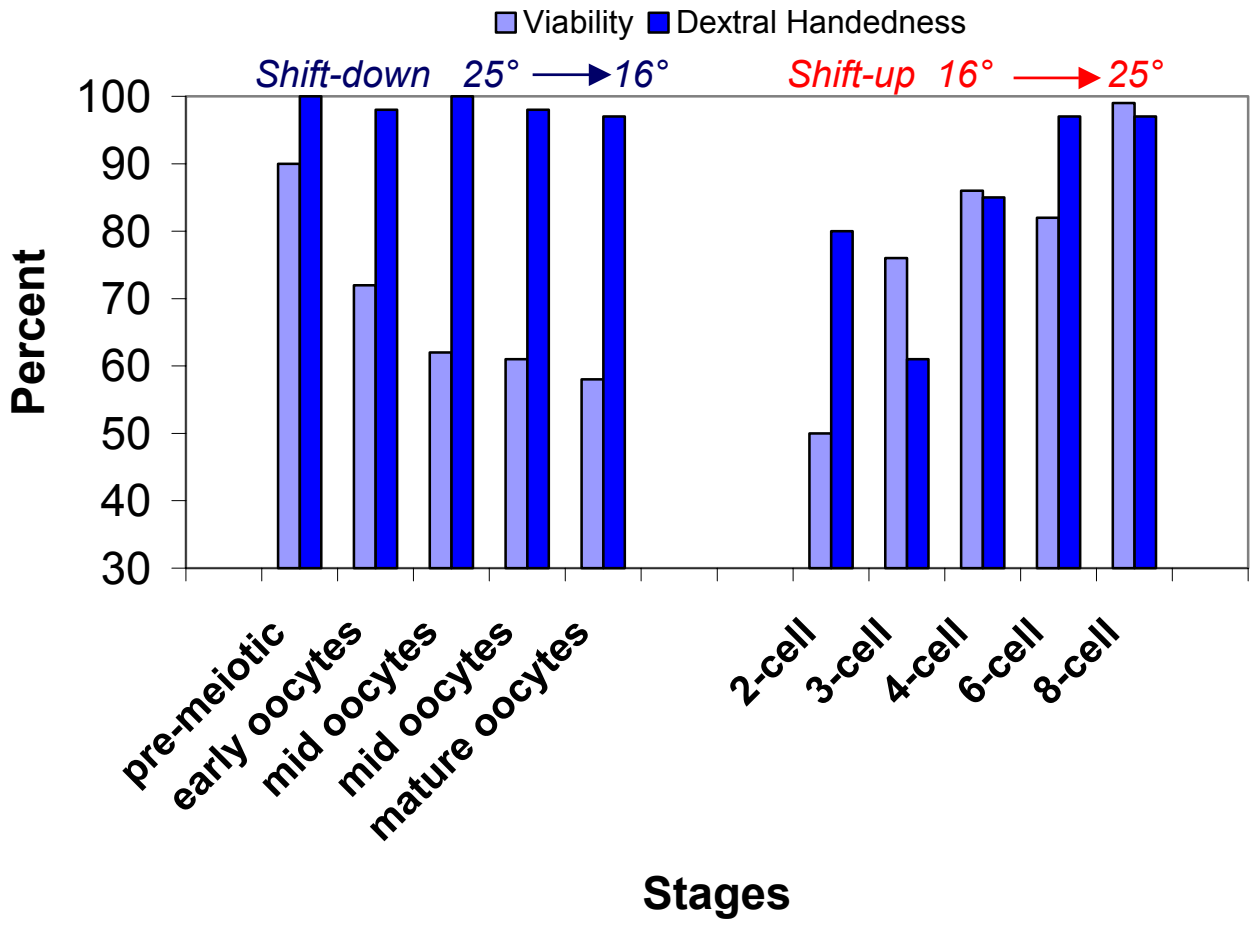
Viability
Dextral Handedness
Shift-down 25° → 16°
Shift-up 16° → 25°
Percent
100
90
80
70
60
50
40
30
pre-meiotic
early oocytes
mid oocytes
mid oocytes
mature oocytes
2-cell
3-cell
4-cell
6-cell
8-cell
Stages

Figure S1

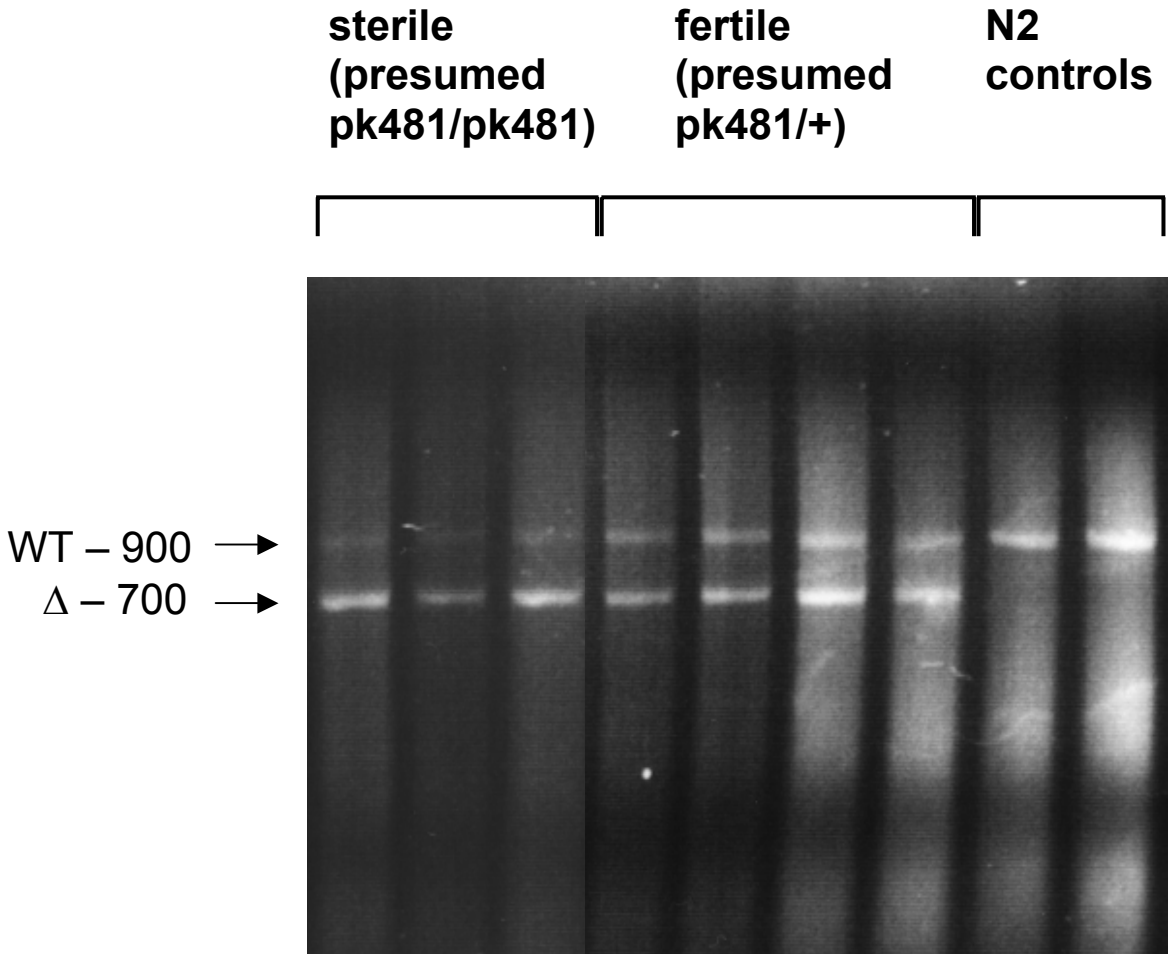
sterile (presumed pk481/pk481)
fertile (presumed pk481/+)
N2 controls
WT – 900
Δ – 700

Figure S2