Axis determination in *C. elegans*: initiating and transducing polarity

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The anterior–posterior axis in *Caenorhabditis elegans* is determined by the sperm and leads to the asymmetric localisation of PAR (partitioning-defective) proteins, which are critical for polarity. New findings demonstrate that sperm asters play a critical role and suggest models for how PAR asymmetry is established. In addition, studies of blastomere fate determination and heterotrimeric G proteins have started to uncover how initial polarity may be translated into the asymmetric distribution of maternal proteins and the control of spindle position.

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Abbreviations

AB	anterior blastomere at the two-cell stage
a–p	anterior–posterior
MF	microfilament
MT	microtubule
P1	posterior blastomere at the two-cell stage
PAR	partitioning-defective protein
PKC	protein kinase C
MAPK	mitogen-activated protein kinase

Introduction

Caenorhabditis elegans embryogenesis begins when a mature oocyte, arrested in prophase of meiosis I, is fertilised by sperm in the spermatheca. Just before fertilisation, the oocyte pronucleus moves away from the side contacting the spermatheca and the nuclear envelope breaks down; sperm normally enters at the opposite end, which will become posterior. After fertilisation, meiosis is completed with the extrusion of two polar bodies and a protective chitin eggshell is made (Figure 1a). The eggshell is rigid and constrains the developing embryo to an ovoid shape [1].

After completion of meiosis, the centrosomes, brought in by the sperm, organise large microtubule (MT) asters (Figure 1a) and the oocyte pronucleus migrates to meet the sperm pronucleus at the posterior. During this time, a prominent pseudocleavage furrow is formed, which later regresses and cytoplasmic flows occur: internal cytoplasm flows towards the posterior whereas cortical cytoplasm flows towards the anterior (Figure 1b). MTs are required for oocyte pronuclear migration whereas microfilaments (MFs) are required for the flows and for overall embryonic polarity [2–5].

The two pronuclei meet in the posterior and then migrate to the centre of the embryo. During this migration the pronuclear–centrosomal complex rotates 90° to orient the spindle along the anterior–posterior (a–p) axis (Figures 1c and d). The initially central mitotic spindle becomes asymmetrically placed during anaphase, when the anterior aster remains fairly stationary but the posterior aster undergoes a rocking movement and moves towards the posterior (Figure 1e). At telophase, the asymmetrically placed spindle also has asymmetric morphology, with a round anterior aster and a flattened posterior aster (Figure 1f; [6]). The first division gives rise to two daughter cells of different size: a larger anterior cell, AB, and a smaller posterior cell, P1 (Figure 1g). AB and P1 also have different spindle orientations (Figures 1h–j), different cell-cycle lengths and different cell fates. In addition, a number of components important for the fates of AB and P1 are differentially partitioned at the first division. For example, P granules, which are thought to play a role in determining the germline fate, are segregated to germline precursors (P1, P2, P3 and P4) [1,7].

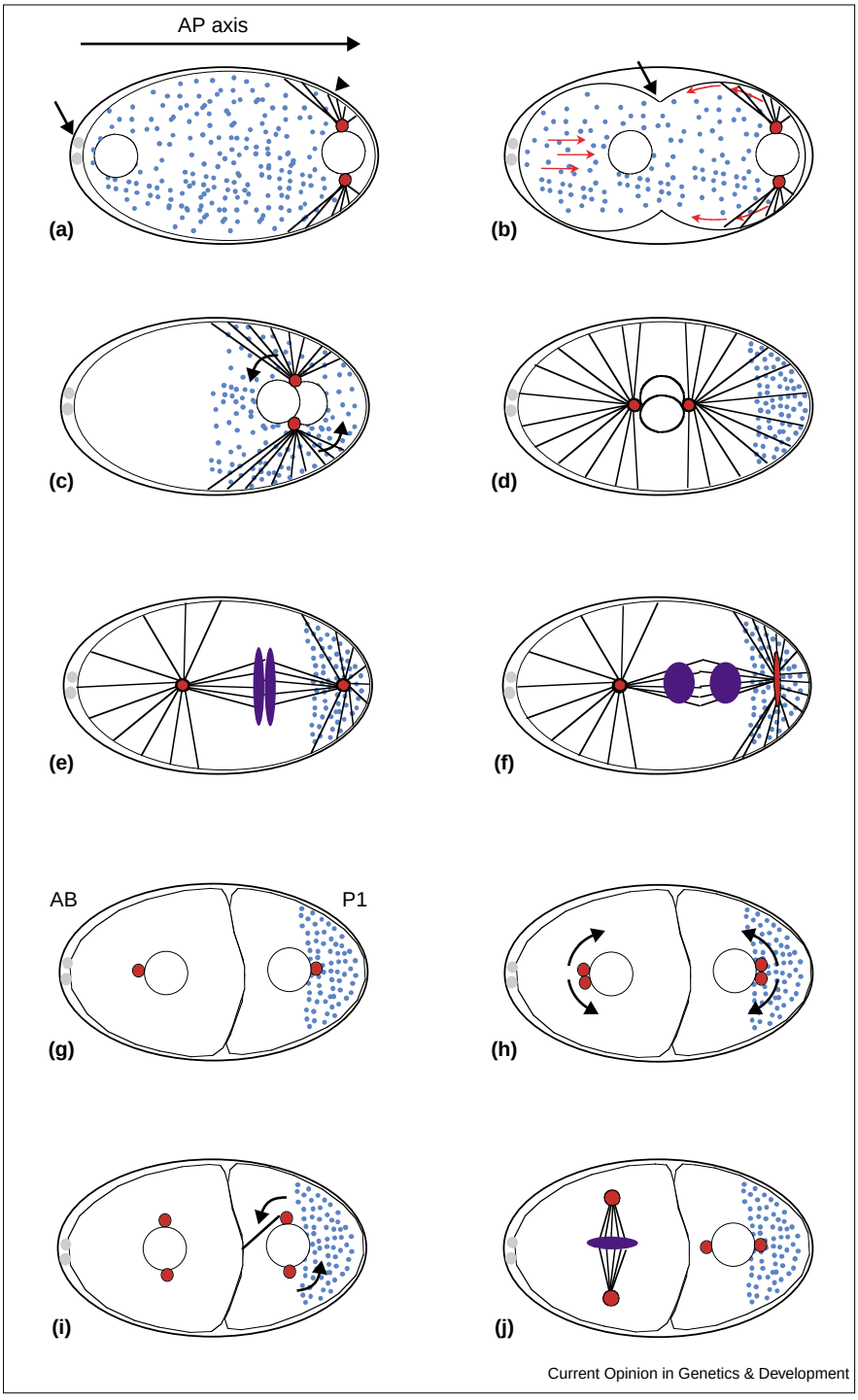
During the first cell-cycle, a group of proteins that are critical for overall polarity become localised asymmetrically. Partitioning-defective-1, a serine–threonine kinase, and PAR-2, a RING finger protein, localise at the posterior cortex [8–10], whereas PAR-3, PAR-6 and PKC-3 (an atypical protein kinase C) localise in a reciprocal pattern at the anterior cortex [11–13]. PAR-3 and PAR-6 contain PSD-95/Discs large/ZO-1 (PDZ) domains, important for protein–protein interactions [11–13]. Mutations in PAR genes and PKC-3 disrupt many early asymmetries, such as asymmetric spindle position and morphology, P granule localisation and the different fates of the AB and P1 cells [14].

In this review, we discuss new data that shed light on the initial events of primary axis determination, how the initial asymmetry is translated into PAR asymmetry and how PAR polarity is transduced into downstream events of asymmetric protein localisation and regulated spindle orientation.

Initial establishment of anterior–posterior polarity

C. elegans oocytes do not appear to have predetermined polarity. Goldstein and Hird [15] showed that the sperm defines the posterior end and, therefore, the a–p axis, which is the long axis of the egg. The sperm polarises the cytoskeleton and organises the cytoplasmic flows, which are directed towards its position [2]. Sperm that lack DNA are capable of fertilisation and establishment of polarity, suggesting that a component of the centrosome or MT asters might be active in polarity generation [16]. Although MT play a major role in polarity establishment in other

Figure 1



Early events in *C. elegans* embryogenesis. **(a)** Newly fertilised embryo. Anterior is left and posterior is right. Meiosis has completed with the extrusion of two polar bodies at the anterior (grey circles indicated by the arrow). The arrowhead points to the rigid eggshell. The oocyte and sperm pronucleus have reformed (empty circles) and sperm centrosomes (red circle) organise MT asters (black lines). P granules (blue dots) are randomly distributed at this stage. **(b)** The oocyte pronucleus migrates toward the sperm pronucleus. During this migration, internal cytoplasm flows towards the posterior whereas cortical cytoplasm flows towards the anterior (red arrows) and a prominent pseudocleavage furrow forms (indicated by the black arrow). P granules start migrating toward the posterior. **(c)** The two pronuclei meet at the posterior and migrate toward the centre. During this migration the pronuclear–centrosome complex rotated 90° occurs to orient the spindle along the a–p axis. **(d)** The pronucleus–centrosomal complex is central and the centrosomes are oriented along the a–p axis. P granules are now localised to the posterior. **(e)** During anaphase (dark blue oval represents DNA) the spindle is displaced towards the posterior. **(f)** At telophase (dark blue circle represent telophase DNA) the spindle has asymmetric morphology, with a round anterior aster and a flat posterior aster. **(g)** The first division gives rise to two daughter cells of different size: a larger anterior cell, AB, and a smaller posterior cell, P1. P granules are exclusively segregated to the P1 cell. **(h)** The centrosomes in AB and P1 duplicate and start migrating around the nucleus until they are opposed. **(i)** The position of the centrosomes at the end of migration will determine the orientation of the mitotic spindle in AB, which is orthogonal to the first division. This division determines the dorsoventral axis. In P1 the nuclear–centrosomal complex rotates 90° (illustrated by the arrows) to orient the spindle along the a–p axis. This probably occurs through capture of astral MTs by a cortical site [6]. **(j)** AB divides before P1. The nuclear–centrosomal complex in P1 has rotated so that the spindle will form along the a–p axis.

systems, previous studies using MT-depolymerising drugs such as nocodazole had suggested that MTs were not involved in these early processes in *C. elegans*; however, a caveat to these studies is that a small number of MTs are still present after treatment with depolymerising drugs [6]. Now, recent work from two different groups indicate that a MT-organising centre is necessary and sufficient for at least some aspects of a–p polarity [17••,18••].

A paper from O'Connell *et al.* [17••] describes the lack of polarity in the *spd-2* mutant, which has very delayed and attenuated sperm aster formation. In *spd-2* mutants, cytoplasmic flows are absent and neither PAR proteins nor P granules are localised. These results suggest that sperm asters may be the sperm component required for polarity establishment, and that sperm asters are necessary for cytoplasmic flows; however, lack of polarity could be

because of the defect in aster formation or because of a distinct function of *spd-2* in establishing a–p polarity [17••].

In an elegant series of experiments, Wallenfang and Seydoux [18••] showed that a MT-organising centre is sufficient to drive at least some aspects of a–p polarity. The authors took advantage of mutants arrested in meiosis I (*mat* mutants) [19]: in these mutants, sperm asters are not formed and a persistent meiotic spindle is present at the presumptive anterior end (opposite to sperm entry). In contrast, in wild-type embryos, the sperm asters constitute a MT-organising centre at the opposite (posterior) end. In the *mat* mutants, a–p polarity is established; however, it is reversed compared to wild type. For example, PAR-2, normally found at the posterior (where the sperm pronucleus is located), is localised at the cortex where the meiotic spindle is found and PAR-3 is at the opposite cortex. Disruption of the meiotic spindle in *mat* mutants leads to loss of a–p polarity. These two studies demonstrate for the first time the importance of MTs in a–p polarisation of the *C. elegans* embryo [17••,18••]. MTs are also involved in a–p polarity in *Drosophila* [14]. This, together with the finding that a homologue of *C. elegans* PAR-1 is required for a–p polarity in the *Drosophila* oocyte [20,21], suggests that some aspects of primary axis generation may be evolutionarily conserved.

Although a reversed PAR distribution is established in *mat* mutant embryos, the meiotic apparatus is not sufficient to drive cytoplasmic flows or to localise P granules. In addition, the established polarity is only transient. This indicates that although a MT-organising centre is sufficient to drive some aspects of polarity, it is not sufficient to maintain it. Further, absence of cytoplasmic flows indicates that MTs are not sufficient to induce actin-dependent events.

The next challenge is to understand how MTs drive polarisation of the embryo. For example, do the MTs themselves induce polarity or is a MT-associated protein the active species? An interesting observation is that the domain occupied by PAR-2 seems to be defined by the MTs [18••]. Perhaps PAR-2 is transported along MTs or the interaction of MTs with the cortex somehow modifies the cortex in a way that allows PAR-2 localisation. Consistent with this idea, inhibition of the myosin light chain gene, *mlc-4*, causes loss of cytoplasmic flow and loss of many aspects of polarity, but a small patch of PAR-2 is often found adjacent to the sperm asters (Shelton *et al.* [22] and Shelton, personal communication). As PAR localisation is uncoupled from cytoplasmic flow in *mat* and *mlc-4* mutants, it suggests that these two events driven by sperm entry are separable. These results suggest that cytoplasmic flow is not necessary for initial PAR localisation and may be required to maintain polarity rather than to establish it.

Downstream of sperm entry

A MT-organising centre might be the initial polarity cue, but it is not sufficient for all aspects of polarity — what other processes are involved? Two groups found that the conserved small G protein, CDC-42, has an important role [23•,24•]. Embryos where CDC-42 was inhibited by RNA interference closely resemble those lacking PAR-3, PAR-6 or PKC-3; three proteins that form a complex in mammalian and *C. elegans* cells ([13,25–27], and Hung and Kemphues, personal communication). In CDC-42(RNAi) embryos, PAR proteins are initially asymmetrically localised as in wild type, but become uniformly distributed during the first mitosis so that they are present around both AB and P1 at the two-cell stage [23•,24•]. This suggests that CDC-42 is necessary to maintain but not to initiate polarity. Localisation of PAR-3–PAR-6–PKC-3 in wild-type AB cells is thought to prevent nucleocentrosomal rotation [11,28]; however, rotation occurs in CDC-42(RNAi) embryos, suggesting that CDC-42 might activate the PAR-3–PAR-6–PKC-3 complex [23•,24•]. Interestingly, both mammalian and *C. elegans* CDC-42 bind PAR-6 [23,25–27], and results from the mammalian system are consistent with the possibility that CDC-42 activates the complex by promoting the kinase activity of PKC-3. CDC-42 and PAR-6 have been implicated in controlling epithelial polarity in mammalian and *Drosophila* cells [29–31], suggesting that the interaction between CDC-42 and a PAR-3–PAR-6–PKC-3 complex and its function in generating polarity have been conserved as a functional unit.

A study of another class of genes suggests that intracellular trafficking may play an important role in early polarity in *C. elegans*. For example, *pod-1* and *pod-2* (polarity and osmotic defective) mutants have abnormal PAR protein distributions and show osmosensitivity and eggshell defects [32•,33•]. POD-1 is linked in some way to intracellular trafficking, as *pod-1* mutants accumulate abnormal endocytic vesicles. POD-1 is a coronin-like protein that was isolated biochemically as an actin-binding protein and is asymmetrically localised to the anterior in a cell-cycle-dependent manner [33•,34]. This suggests that it may play a role in cell polarity by targeting transport of proteins to the anterior. Further support for the idea that protein trafficking is important for polarity comes from the analysis of *ooc-3* and *ooc-5* mutants, in which polarity is lost at the two-cell stage [35,36•]. OOC-3 is localised to the endoplasmic reticulum, suggesting that proteins involved in polarity might be modified or targeted though the endoplasmic reticulum [36•]. Mutations in another gene, *ooc-5*, share many similarities with *ooc-3* mutants, implying that it may also be involved in the same process [35]; however, the molecular nature of *ooc-5* is not yet known. Finally, there may be a link between genes involved in trafficking and CDC-42, as CDC-42(RNAi) embryos also show osmosensitivity and eggshell defects [23•,24•].

Transducing anterior–posterior polarity to the asymmetric localisation of maternal proteins

How does PAR-induced one-cell polarity lead to the proper asymmetric localisation of proteins that determine cell fates? Two recent papers have revealed the nature of this

linkage. Schubert *et al.* [37••] found that two closely related CCCH finger proteins, MEX-5 and MEX-6, appear to act downstream of the PAR proteins in establishing protein asymmetries, but do not affect spindle asymmetry in one-cell embryos. MEX-5 is localised towards the anterior in a PAR-dependent manner. Anterior localisation of MEX-5 is complementary to that of a group of posterior-localised germline proteins, and ectopic expression of MEX-5 is sufficient to inhibit their expression. The results suggest that MEX-5 and MEX-6 transduce polarity information from the PAR proteins resulting in the inhibition of germline proteins in the anterior. In an exciting addition to these findings, Page *et al.* [38•] have found normal protein asymmetries appear to depend on a balance between MEX-5–MEX-6 function and the level of Ras–MAP kinase activity. For example, reduction of function mutants in the Ras pathway partially suppresses *mex-5* mutants. In order to understand how the Ras pathway influences protein asymmetries, an important future goal will be to find MAPK targets.

Although *mex-5* and *mex-6* mutants have normal P granule distribution and spindle orientation at the one-cell stage, *mex-5 mex-6* double mutants are defective in these processes at later stages [37••], suggesting that these genes might have a more general role in early polarity than just affecting maternal protein distribution. Alternatively, the abnormal distribution of particular proteins might cause the later P granule distribution and spindle orientation defects.

Interestingly, a number of other proteins asymmetrically localised in the *C. elegans* embryo contain two CCCH domains, including the PIE-1, POS-1 and MEX-1 [39–41]. The CCCH domains play a role in the posterior localisation of these three proteins. The first CCCH domain directs degradation in somatic blastomeres and the second targets the proteins to P granules [42•]. Although the localisation of MEX-5 is complementary to these proteins, it contains CCCH domains; therefore, its anterior localisation might be achieved through protein degradation. If so, then a mechanism must exist for distinguishing proteins that should be degraded in the anterior from those degraded in the posterior. In the case of PIE-1, PAR-1 is required for the correct localisation of somatic degradation activity, suggesting a way that PAR polarity could be transduced into the localisation of maternal proteins [42•].

Polarity and spindle orientation: the G-protein connection

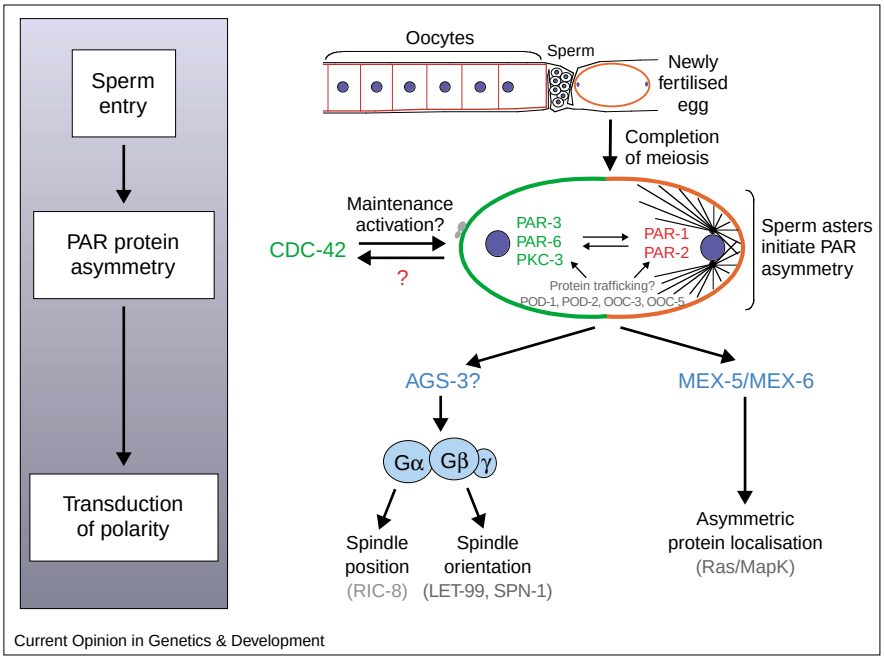
PAR mutants have defects in polarity that lead to defects in the position and polarity of mitotic spindles. Insight into regulation of spindle position by PAR proteins has come from beautiful studies from Grill *et al.* [43••]. Through a series of spindle-cutting experiments, they showed that the pulling force at the flat posterior aster is greater than that at the round anterior aster, which could explain the posterior displacement of the spindle. The force difference depends on PAR-2 and PAR-3. In *par-2* mutants, both asters are round and the pulling force at each pole is low; in *par-3* mutants both asters are flat and the pulling force at each pole is high.

How is polarity information transduced to spindle behaviour? It has recently become clear that heterotrimeric G proteins are one important key. It was previously shown that GPB-1, the Gβ-subunit of a heterotrimeric G protein, is required for orientation of early cell division axes [44]. Recent work has shown that two Gα-subunits, GOA-1 and GPA-16, redundantly function with GPB-1 in the early embryo and that Gα and Gβγ control distinct MT-dependent processes [45•]. Gβγ is necessary for the correct centrosome migration path around the nucleus and, hence, in orienting the mitotic spindle. Interestingly, mammalian Gβγ dimers have been shown to promote MTs polymerisation *in vitro* [46] suggesting the possibility that *C. elegans* Gβγ may control centrosome migration by directly regulating MTs dynamics. Gα is required for the asymmetric placement and morphology of the first mitotic spindle. Gα only affects spindle asymmetry and not overall embryonic polarity because the PAR proteins and P granules are correctly localised in Gα(–) embryos [45•]. This raises the possibility that Gα specifically translates polarity information from the PAR proteins into mitotic spindle behaviour, and suggests that it may be involved in regulating spindle forces in a yet unknown fashion.

Three other genes that may be involved in regulating centrosome position in concert with Gα and Gβγ have been identified: *spn-1*, *let-99* and *ric-8*. Both *spn-1* and *let-99* mutants have spindle orientation defects similar to those seen in embryos lacking Gβ (Rose and Kemphues [47]; Bergmann, Rose and Wood, personal communication). The nature of the *let-99* link is not yet know, but Gβ localisation is abnormal in *spn-1* mutants, so SPN-1 could directly affect G-protein activity (Bergmann, Rose and Wood, personal communication). The *ric-8* mutant embryos have defects in spindle morphology and position similar to those of Gα(–) embryos [48•]. These phenotypes are greatly enhanced by a 50% reduction in maternal *goa-1* gene dosage, suggesting that *ric-8* and *goa-1* function in the same pathway [48•].

Although PAR proteins and heterotrimeric G proteins play a role in spindle position, it is not yet known how they are linked. AGS-3, a *C. elegans* homologue of mammalian Ags3 (a receptor independent activator of G protein signalling; [49]) may be part of the answer. Inhibition of two *ags-3* homologues results in a phenotype identical to that of Gα(–) embryos suggesting that it might be a regulator (M Gotta, J Ahringer, unpublished data; [50•]). In an exciting parallel, the *Drosophila* homologue of Ags3, PINS, is essential for polarity and spindle orientation in the neuroblast and is found in a complex with Inscuteable, Bazooka (the *Drosophila* homologue of PAR-3) and a Gα [51,52]. Thus, spindle regulation by heterotrimeric G proteins may be a conserved process.

Figure 2



Model for initiation and transduction of polarity in the *C. elegans* zygote. The top part of the figure shows a portion of an adult gonad. Oocytes are arranged in a line, with the oldest adjacent to the spermatheca holding the sperm. When mature, this oocyte moves into the spermatheca and a sperm enters, usually at the end that first enters the spermatheca. The newly fertilised egg then exits the spermatheca and moves into the uterus. At this point, the oocyte and sperm pronuclei are condensed (blue dots at ends of newly fertilised egg) and PAR-1 and PAR-2 proteins are uniformly cortical (red) as they are in oocytes [8,10]. Meiosis is completed with the extrusion of two polar bodies (grey anterior circles in middle panel) and the oocyte and sperm pronuclei decondense (blue circles). At this time, the sperm centrosomes nucleate MTs (black lines), which have a role in the asymmetric localisation of PAR proteins, perhaps by determining the domain of PAR-2 [18]. PAR proteins are also dependent on each other for their localisation pattern (arrows). CDC-42 has a role in maintaining PAR asymmetry and possibly in activating the PAR-3–PAR-6–PKC-3 complex; this complex might also regulate CDC-42. Intracellular protein trafficking plays an unknown role in PAR asymmetry. Once established, polarity information is transduced to downstream events such as spindle position and orientation (through heterotrimeric G proteins) and the asymmetric localisation of maternal proteins (through MEX-5 and MEX-6). Proteins and pathways in grey at the bottom of the figure are involved in the indicated processes but their roles are not yet clear.

Conclusions

We can now begin to see an outline of how the a–p axis is determined in *C. elegans* and how this information is transduced into downstream events such as spindle position and asymmetric protein localisation (Figure 2); however, many pieces of the puzzle are still missing. For example, what are the targets of the PAR proteins? Although MEX-5–MEX-6 and heterotrimeric G proteins act downstream of the PARs, a direct link remains to be found. Once Gα is activated, how does it control spindle position? For example, does it regulate interaction between the astral MTs and the cortex, or MT stability? Again, targets need to be identified, and genes implicated in the process, such as *ric-8* and *ags-3*, need to be placed into the pathway.

It is also still unclear how MFs are regulated by sperm entry. NMY-2, a non-muscle myosin that binds to PAR-1 is required for polarity [53], as is the myosin light chain MLC-4 [22]. These proteins, POD-1 and some PAR proteins are also required for cytoplasmic flow [22,33•,53,54]. In addition, CDC-42(RNAi) embryos and some PAR mutants have an altered MF distribution in very early one-cell embryos [23•,54]. Identification of new cytoskeletal regulators through genetic and RNAi screens should help to establish the connection between MFs and polarity ([50•,56•–58•]; P Zipperlen *et al.*, unpublished data.

Although we have highlighted the areas where there are still large gaps, the field is moving very rapidly. Part of the rapid pace and excitement is coming from the realisation that work in different systems (e.g. the *C. elegans* zygote, *Drosophila* oocytes and neuroblasts, mammalian epithelial cells and *Xenopus* oocytes [14,20,29,59,60]) is showing that mechanisms and molecules involved in cell polarisation and spindle orientation are similar. Going back and forth between these should be very fruitful for generating answers to many of the open questions.

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