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Acto-myosin reorganization and PAR polarity in *C. elegans*

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The symmetry-breaking event during polarization of *C. elegans* embryos is an asymmetric rearrangement of the acto-myosin network, which dictates cell polarity through the differential recruitment of PAR proteins. The sperm-supplied centrosomes are required to initiate this cortical reorganization. Several questions about this event remain unanswered: how is the acto-myosin network regulated during polarization and how does acto-myosin reorganization lead to asymmetric PAR protein distribution? As we discuss, recent studies show that *C. elegans* embryos use two GTPases, RHO-1 and CDC-42, to regulate these two steps in polarity establishment. Although RHO-1 and CDC-42 control distinct aspects of polarization, they function interdependently to regulate polarity establishment in *C. elegans* embryos.

Introduction

The signals that induce cell polarization are as varied as the functions of polarized cells themselves. Epithelial cells polarize in response to their neighbors, whereas migrating neutrophils respond to long-range chemoattractants. Mating yeast cells respond to each other, and alga zygotes polarize according to light. Despite the variety of signals, the polarizing signal leads to a seemingly conserved outcome: modulation of the cell cortex to generate distinct cortical domains (Gonczy and Hyman, 1996; Nelson, 2003). The cell cortex, an acto-myosin-rich layer underlying the plasma membrane, provides both mechanical stability and the capacity for force generation. Spatial differences in the properties of the acto-myosin cortex allow the asymmetric localization of effector molecules and thus provide polarity to a cell. Cortical acto-myosin reorganization is central to generating polarized domains, but it remains largely unclear how asymmetry in an acto-myosin cortex is generated in response to a signal, and how changes in the cortex can be translated into asymmetric function in a polarized cell.

Although the cortex is a fundamental part of cell polarity, we have only a basic idea of its composition and mechanical properties. The cortex is rich in actin and myosin, which interact to generate contractility. Actin assembles into a linear polar polymer, which can provide structure or force for movement (Stossel, 1984). Myosin is an actin-dependent ATPase; it uses energy to undergo a conformational change that allows movement along actin filaments (Warrick and Spudich, 1987). The myosin found in the cortex (type II myosin) can form tail-to-tail multimers, called myosin filaments, which cross-link actin (Niederman and Pollard, 1975; Pollard et al., 1978; Yumura and Fukui, 1985). The classical view of acto-myosin contraction is based largely on the mechanics of muscle sarcomeres, which contain highly ordered and polarized arrays of actin filaments cross-linked by myosin filaments (Fig. 1A). The acto-myosin arrays interact to generate anti-parallel sliding of actin, and thus either uniform contraction or uniform relaxation (Huxley, 2000).

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In the cell cortex, by contrast, actin filaments do not seem to have an ordered arrangement and thus the mechanics of cortical contraction are less clear. However, a simple model of the cortex has emerged (Fig. 1B). The cortical acto-myosin network consists of actin filaments, myosin filaments and additional cross-linking proteins (Adelman and Taylor, 1969; Hatano and Oosawa, 1966; Hatano and Tazawa, 1968; Pollard, 1981). The actin filaments are arranged isotropically within the cortex (Kane, 1983; Pollard and Korn, 1973; Stossel, 1984), but without any particular orientation, and are connected to each other by myosin and other cross-linking proteins. Myosin-driven motility slides the filaments against each other (Condeelis and Taylor, 1977; Taylor et al., 1976), as in sarcomeres, but because of the random filament orientation, the cortex undergoes local contraction complemented by local relaxation (Fig. 1B). The result of cortical contraction is regions of high acto-myosin density surrounded by holes in the network (Kane, 1983). Modifications of cortical contractility can result in both reorganization of the cell and remodeling of the cortex (Hellewell and Taylor, 1979; Janson et al., 1991; Janson and Taylor, 1993; Stendahl and Stossel, 1980; Taylor and Fechheimer, 1982), both of which are essential aspects of domain formation.

Several studies in 2006 revealed important insights into the mechanisms that generate an asymmetric acto-myosin network in *C. elegans* embryos and how the acto-myosin network, in turn, contributes to the asymmetric distribution of PAR proteins. The mechanics of cortical polarization in *C. elegans* embryos appear to be conserved in a range of biological contexts. These recent studies suggest that the molecules that regulate cortical polarization in *C. elegans* embryos are also conserved. In this review, we summarize current ideas on how the activity of the acto-myosin network is related to the establishment of polarity in *C. elegans* embryos.

Polarity establishment in *C. elegans*

The *C. elegans* embryo is an excellent model system in which to address polarity establishment. The anterior-posterior (AP) axis in one-cell *C. elegans* embryos is defined by two coordinated cortical polarities: PAR polarity and contractile polarity (Cowan and Hyman, 2004a; Munro, 2006). PAR polarity involves the formation of two complementary cortical domains, each occupying half of the one-cell embryo (Fig. 2B). The conserved anterior PAR complex (PAR-3–PAR-6–PKC-3) localizes to one half; PAR-1 and PAR-2 localize to the other half, defining the posterior. PAR polarity correlates with contractile polarity. The anterior cortex of one-cell embryos is highly dynamic, undergoing cycles of cortical contraction and regression, whereas the posterior cortex is quiescent (Fig. 2). How are these polarities established? *C. elegans* oocytes lack developmental polarity, and fertilization itself does not induce polarization of the zygote. Following fertilization, the oocyte nucleus undergoes two rounds of meiotic divisions, and upon entry into the first mitotic cell cycle the embryo cortex remains unpolarized. Cortical ingressions/regressions occur throughout the surface, corresponding to contractility generated by a cortical acto-myosin network (Fig. 2C). The anterior PAR complex localizes uniformly around the cortex (Fig. 2B). Within the first two minutes of the cell cycle, polarity is initiated in the embryo. Polarity establishment requires

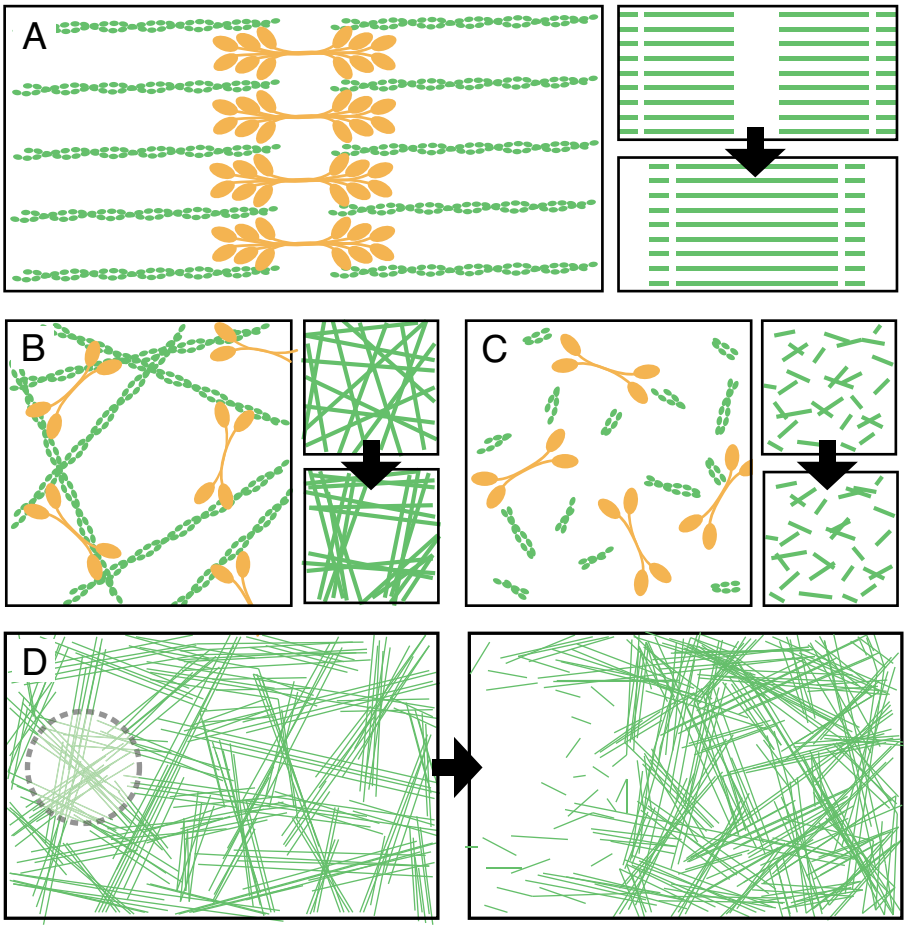

Fig. 1. Contraction of acto-myosin networks. Actin filaments (green) and myosin filaments (orange) interact to generate contractility. (**A-C**) Large boxes illustrate the structure of the acto-myosin network; small boxes illustrate the overall network before (top) and after (bottom) contraction. The black arrows indicate a contractile stimulus, for instance, ATP addition. Muscle sarcomeres (A) are highly ordered and contract uniformly. (B) Cortical networks are randomly organized; contraction results in local densities of contractility and local breaks in the network. (C) Contractile interactions do not exist in a solated network. (**D**) A uniform acto-myosin network (as depicted in B) exhibits local contractions throughout the network (left box), but the local downregulation of myosin activity or the severing of actin filaments (circle, left box) can lead to a global asymmetric contraction of the entire network (right box).

centrosomes, which lie near the cortex at the time of polarization (Fig. 2A) (Cowan and Hyman, 2004b; Cuenca et al., 2003; Hamill et al., 2002; O'Connell et al., 2000). The earliest visual indication of polarity establishment is that contractions cease in the small region of the cortex overlying the centrosomes, where the acto-myosin network disassembles (Fig. 2C) (Munro et al., 2004). This region correlates with the site of sperm entry (Goldstein and Hird, 1996), although whether this coincidence is an active or passive consequence of fertilization remains unknown. The local change in contractility breaks the symmetry of the egg. The acto-myosin network, contractility, and the anterior PAR complex proceed to shrink in a coordinated manner until they occupy half of the embryo (Fig. 2B,C) (Cuenca et al., 2003; Munro et al., 2004; Strome, 1986). This establishes the anterior domain. The complementary cortical domain is non-contractile and accumulates posterior PAR proteins (PAR-1 and PAR-2) (Cuenca et al., 2003). Thus, roughly eight minutes after the initiation of polarity, the embryo consists of anterior and posterior cortical domains that exhibit different properties of acto-myosin contractility: the anterior domain is contractile and the posterior domain is non-contractile.

Although the polarity of the embryo depends on the presence of PAR proteins, the establishment of contractile polarity apparently dictates the establishment of PAR polarity. Contractility, in general, is required for PAR polarity: acto-myosin mutants prevent PAR polarity establishment (Fig. 3D) (Guo and Kemphues, 1996; Hill and Strome, 1988; Hill and Strome, 1990; Severson et al., 2002; Severson and Bowerman, 2003; Shelton et al., 1999). The initiation of contractile polarity is independent of PAR polarity: acto-myosin contractility becomes asymmetrically localized in Par mutant embryos (Fig. 3B,C) (Kirby et al., 1990; Munro et al., 2004). PAR-3, however, contributes to cortical contractility, and thus *par-3* mutants fail to completely segregate the acto-myosin network (Fig. 3C). Contractile polarity appears to be sufficient for some aspects of PAR polarity: PAR-6 is asymmetrically distributed during polarity establishment in *par-2* mutants (Fig. 3B) (Cuenca et al., 2003). It has been proposed that the anterior PAR complex could be physically linked to the contractile acto-myosin cortex, such that segregation of acto-myosin contractility pulls the anterior PAR proteins with it (Munro et al., 2004). How posterior PAR proteins recognize the non-contractile cortex is less clear. The absence of the anterior PAR

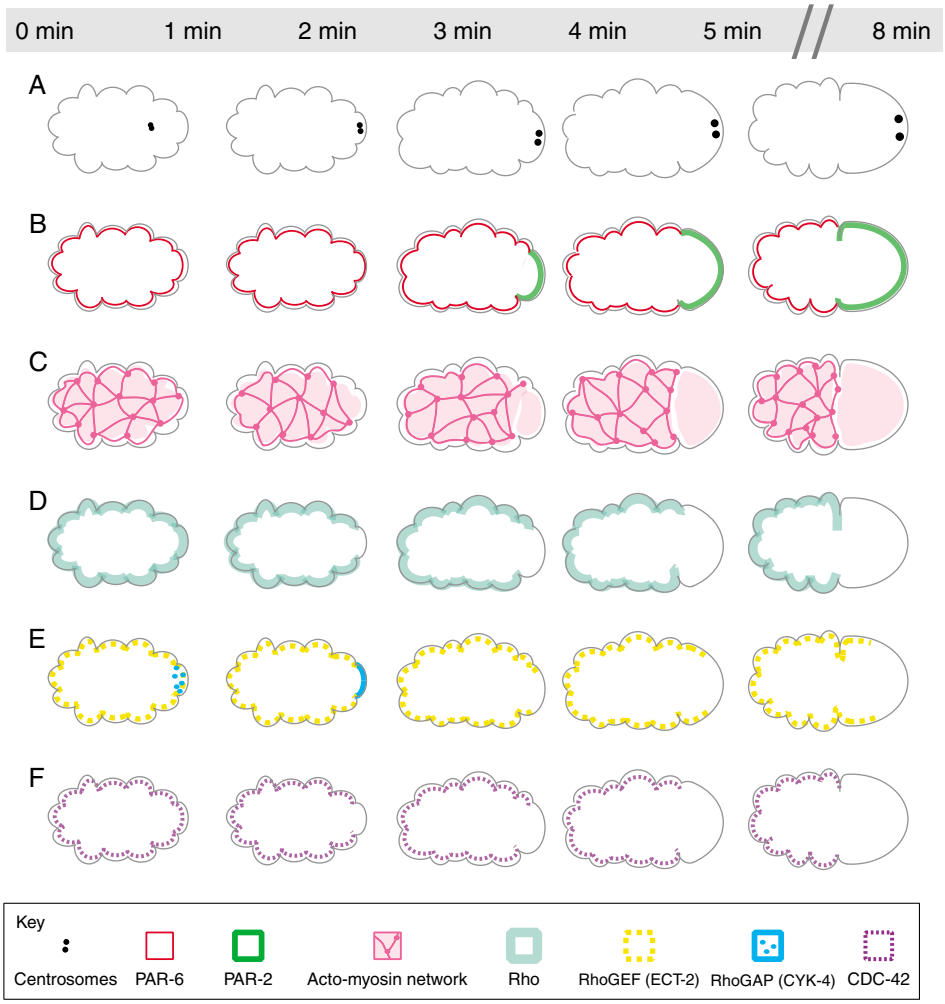

Fig. 2. Cortical polarity in one-cell *C. elegans* embryos. (**A-F**) Illustrations of aspects (see key) of *C. elegans* embryos as they change from entry into the first cell cycle (left), to the completion of polarity establishment (right), a period of about eight minutes. The approximate timing of events relative to entry into the first cell cycle is shown in the upper bar. Cortical activity is indicated by the gray outline of the embryos; contractility is indicated by the cortical ingressions. The identity of the proteins or complexes depicted is indicated in the key. The acto-myosin network in C is indicated from a surface view of the embryo cortex; other proteins and centrosomes are shown in the embryo mid-plane. Embryo anterior is to the left. The complete kinetics of RhoGAP CYK-4 localization (E) have not been investigated and thus are not depicted following the initiation of polarization.

complex from the non-contractile cortex may allow PAR-2 to localize to the cortex (Hao et al., 2006), although the precise mechanism is likely to be more complex.

Regulation of acto-myosin contractility

Actin and myosin provide the structural basis of contractility, and changes in the acto-myosin network change the contractile properties of the cortex. The modulation of contractility appears to play a central role in polarity establishment in *C. elegans* embryos. Prior to polarization, the entire cortex undergoes contractions. After polarization, half the cortex is contractile and the other half is non-contractile. Thus, polarity establishment requires the inhibition of contractility at the posterior cortex. How is contractility regulated?

A crucial feature of an acto-myosin network is the ability to transition between a ‘gel’ and a ‘sol’. Gels consist of highly cross-linked actin filaments (Fig. 1B). The sol (solation) state comprises short, non-cross-linked actin filaments (Fig. 1C). The spatial regulation of these two states can create distinct domains (Hellewell and Taylor, 1979; Janson et al., 1991; Janson and Taylor, 1993; Stendahl and Stossel, 1980; Taylor and Fechheimer, 1982). For instance, localized solation of an acto-myosin gel in vitro causes the remaining network to contract away from the weakened point, resulting in a contractile and a non-contractile domain (Fig. 1D) (Janson and Taylor, 1993). Classically, it was proposed that such an asymmetric contraction could polarize one-cell *C. elegans* embryos (Hird and White, 1993; White, 1990; White and Borisy, 1983). Solation can occur in two ways: reducing actin filament length, and eliminating myosin activity (Taylor and Fechheimer, 1982). Actin filament length can be influenced by the number of nucleators, actin-severing proteins or actin-capping proteins (Pollard and Cooper, 1986; Stossel et al., 1985). Currently, there is no evidence that such molecules are required to modulate contractility in *C. elegans* embryos, although redundancy may complicate the analysis of phenotypes. Myosin appears to be a more likely control point in *C.*

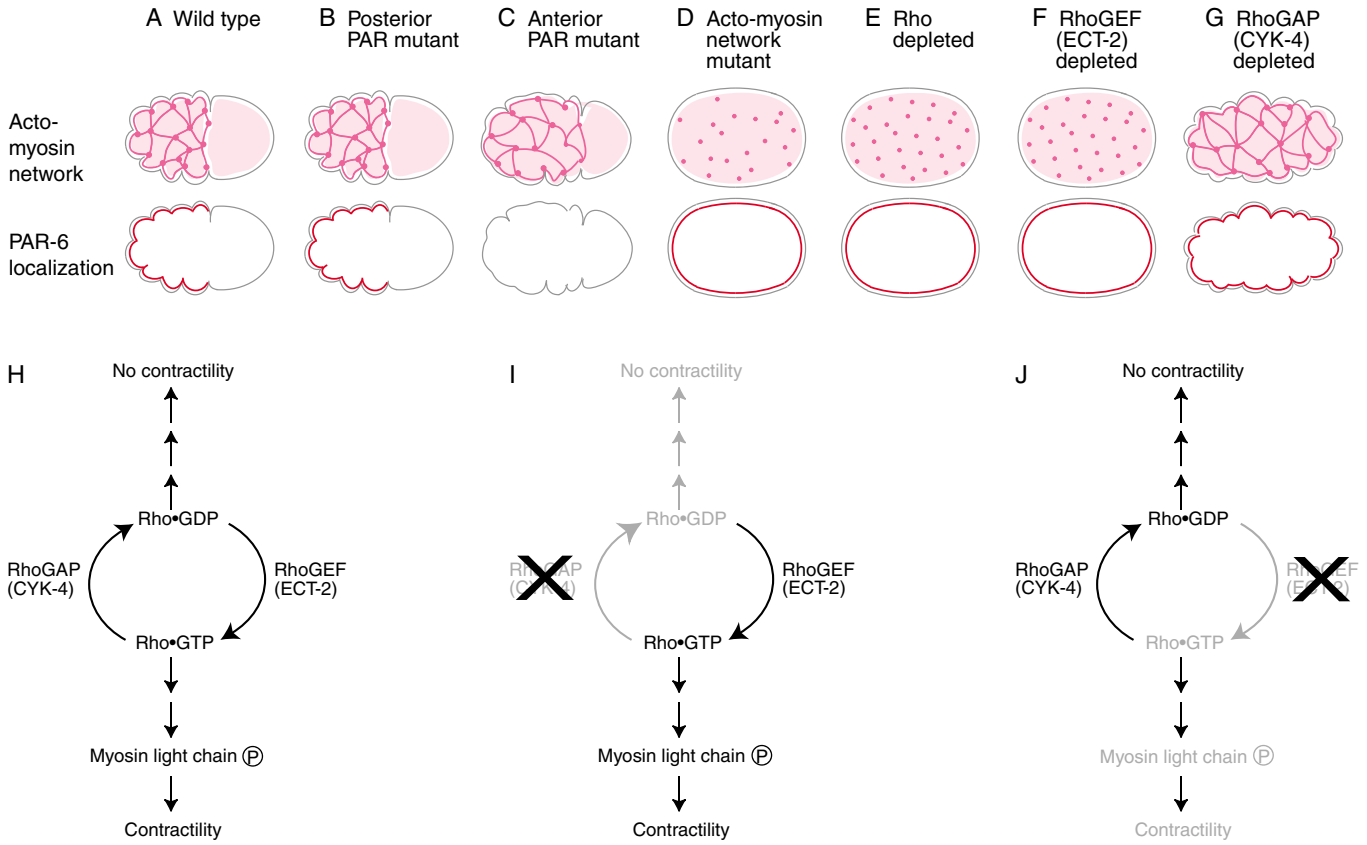

Fig. 3. Establishing contractile polarity: Rho. (**A-G**) Illustrations of *C. elegans* embryos (E) depleted of Rho (RHO-1), (F) depleted of RhoGEF ECT-2, and (G) depleted of RhoGAP CYK-4, in comparison with (D) embryos lacking a contractile acto-myosin network and mutants of (B) posterior and (C) anterior PAR proteins. Embryo anterior, or the meiotic pole in D-G, is to the left. Rho and the acto-myosin network (pink) are required for contractile polarity. PAR polarity does not determine contractile polarity, although anterior PAR proteins such as PAR-6 modify contractility, and thus the respective sizes of the anterior and posterior domains are altered in *par-3* mutants (C). (B) Contractile polarity can dictate anterior PAR polarity during polarity establishment in *par-2* mutants, but thereafter PAR-2 is essential for maintaining anterior PAR polarity (not depicted). The acto-myosin network (pink) is indicated from a surface view of the embryo cortex; PAR-6 (red) is shown in the embryo mid-plane. (**H-J**) Diagrams of acto-myosin contractility regulation by Rho signaling. Arrows indicate positive regulation, although the precise molecular components involved have not been determined. (H) Without spatial or temporal regulation of Rho activity, 'contractility' and 'no contractility' compete. (I) In the anterior cortical domain, only RhoGEF ECT-2 is present, promoting contractility. (J) In the posterior cortical domain, only RhoGAP CYK-4 is present, eliminating contractility.

elegans embryos. Myosin activity is regulated positively by phosphorylation (Adelstein and Conti, 1975; Craig et al., 1983; Umemoto et al., 1989), and the phosphorylation state is determined by the balance of kinase and phosphatase activities (Amano et al., 1996; Frearson and Perry, 1975; Kureishi et al., 1997; Morgan et al., 1976; Pires and Perry, 1977). Myosin regulation is highly conserved, and the small GTPase Rho provides the central point of control (Kimura et al., 1996). Recent work has uncovered that Rho-dependent signaling is central to the regulation of contractility in *C. elegans* embryos.

Establishing contractile asymmetry: Rho

Three recently published papers have shown that the activity of Rho is required for contractility and PAR polarity in *C. elegans* embryos (Jenkins et al., 2006; Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006). The studies show that if Rho (RHO-1) is uniformly active, contractility occurs over the entire surface (Fig. 3G); if Rho is uniformly inactive, contractility is inhibited throughout (Fig. 3E,F). In both cases, the anterior PAR complex does not shrink to the anterior, remaining uniformly distributed over the entire embryo surface. The activity of Rho during polarity establishment is controlled by two factors, which also regulate Rho during cytokinesis (Glotzer, 2005): the RhoGEF ECT-2 and the RhoGAP CYK-4. GTPase-activating proteins (GAPs) promote GTP hydrolysis, leading to inactivation of signaling. Guanidine nucleotide exchange factors (GEFs) accelerate the loading of GTP, leading to activation of signaling. Therefore, CYK-4 and ECT-2 are antagonistic with respect to contractility: RhoGAP CYK-4 inhibits Rho and thus inhibits contractility, whereas RhoGEF ECT-2 activates Rho and thus activates contractility.

Polarity requires the local regulation of contractility – specifically, suppression of contractility at the posterior. This change in contractility could be mediated by local inactivation of Rho, in principle provided either by downregulation of the GEF or upregulation of the GAP. Indeed, Motegi and Sugimoto (Motegi and Sugimoto, 2006) show that RhoGEF ECT-2 is absent from the non-contractile region of the cortex (Fig. 2E). On the other hand, Jenkins et al. (Jenkins et al., 2006) show that RhoGAP CYK-4 localizes to a limited cortical region adjacent to the sperm-supplied centrosome-pronucleus complex (Fig. 2E). RhoGAP CYK-4 and RhoGEF ECT-

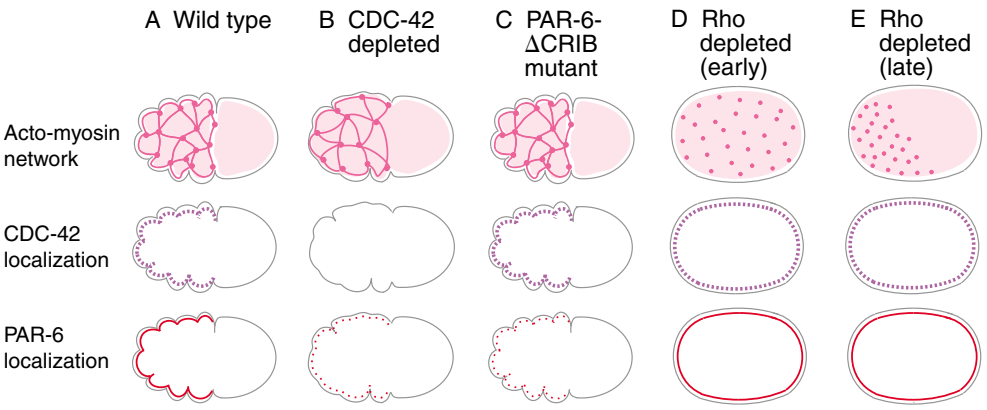

Fig. 4. Linking PAR polarity to contractile polarity: CDC-42. Illustrations of *C. elegans* embryos: (**A**) wild type; (**B**) depleted of CDC-42; (**C**) expressing a PAR-6-ΔCRIB mutant; (**D**,**E**) depleted of Rho early (D) and late (E) in the first cell cycle. The acto-myosin network (pink) is indicated from a surface view of the embryo cortex; CDC-42 (purple dashed line) and PAR-6 (red) are shown in the embryo mid-plane. Embryo anterior, or the meiotic pole in C, is to the left. Embryos lacking CDC-42 or the CDC-42-binding CRIB domain of PAR-6 have reduced cortical localization of PAR-6, indicated by the dotted red line in B and C. Both PAR-6 and CDC-42 remain uniformly distributed around the cortex despite asymmetry of the acto-myosin network in *rho-1(RNAi)* embryos late in the cell cycle (E), suggesting that CDC-42 cannot respond to the acto-myosin network when Rho signaling is disrupted.

2 might show reciprocal localizations when polarization is initiated, although this has not been demonstrated. Thus, the initial contractile asymmetry of *C. elegans* embryos could be generated, at least in part, as follows: a local region of the cortex becomes non-contractile through local downregulation of Rho signaling, mediated by the presence of RhoGAP CYK-4 and exclusion of RhoGEF ECT-2 from this region.

After the initial contractile asymmetry is established, the contractile domain shrinks until it occupies half the embryo; the non-contractile domain occupies the other half. Based on experiments in isolated acto-myosin gels (Janson et al., 1991; Janson and Taylor, 1993), as discussed above, the initial break in contractility could cause the remaining contractile network to contract away from the break (Fig. 1D). In theory, however, in the context of the cell, contractility could be re-established in the nascent posterior. Thus, there should be a mechanism that ensures that contractility remains suppressed in the posterior but continues in the anterior. Motegi and Sugimoto (Motegi and Sugimoto, 2006) provide evidence for such a mechanism. Rho, and to a lesser extent its activator RhoGEF ECT-2, shrink with the contractile acto-myosin network (Fig. 2D,E), leaving the non-contractile domain devoid of the regulatory molecules that support contractility. Thus, the asymmetric segregation of both contractility and the capacity for contractility ensure that only the anterior domain is contractile. Furthermore, the continued contractility of the anterior domain seems to support continued shrinking of the domain, resulting in a feedback loop. The contractile domain may stop shrinking either when further collapse is limited by physical constraints of the contractile network, or when contractility is eliminated, for instance by the downregulation of Rho, which could be mediated by the disappearance of RhoGEF ECT-2.

Spatial and temporal regulation of Rho activity

The initial event in polarity establishment comes in the form of a signal that dictates where and when the cell should polarize. In many polarized embryos, there is a general consensus regarding the signal: the site of fertilization and the sperm-contributed centrosomes establish the initial axis (Astrow et al., 1989; Carre and Sardet, 1984; Dondua et al., 1997; Eckberg, 1981; Fernandez et al., 1998; Freeman, 1978; Hable and Kropf, 2000; Hasegawa et al., 2004; Luetjens and Dorresteijn, 1998; Roegiers et al., 1995; Ubbels et al., 1983). Likewise, in *C. elegans* embryos, centrosomes are essential for polarity establishment (Cowan and Hyman, 2004b). How does the centrosome relate to the regulation of Rho activity? The initial asymmetric regulation of Rho activity may require centrosomes. RhoGEF ECT-2 exclusion from the presumptive posterior depends on centrosomes (Motegi and Sugimoto, 2006); the centrosome-dependency of asymmetric RhoGAP CYK-4 has not been investigated. Two populations of RhoGAP CYK-4 appear to exist in one-cell *C. elegans* embryos: sperm- and oocyte-derived. Whereas sperm-derived CYK-4 is required for polarity establishment, oocyte-derived CYK-4 is not (Jenkins et al., 2006). This difference between paternally- and maternally-contributed RhoGAP CYK-4 suggests that the two populations may be differentially regulated. One significant difference between the two CYK-4 pools may be their proximity to centrosomes. The sperm delivers both paternal CYK-4 and centrosomes, and thus they are likely to be near each other within the egg. Centrosomes, however, can travel significant distances in the cytoplasm prior to the establishment of polarity (Cowan and Hyman, 2004b), and whether the ultimate position of centrosomes at the time of polarity establishment is random or predetermined is not known.

A second level of control during polarity establishment comes in the form of temporal regulation. About thirty minutes pass from fertilization until entry into the first mitotic cell cycle, a time during which the polarizing signal is kept inactive. Upon entry into the cell cycle, the entire embryo cortex is contractile, suggesting that the polarity establishment mechanism is not yet active. After approximately one and a half minutes, however, contractions in the presumptive posterior cease, indicative of polarity establishment and the activation of RhoGAP CYK-4. During this initial one and a half minutes of the first cell cycle, the centrosome recruits a number of centrosomal proteins that are required for polarity (Cowan and Hyman, 2004b). A delay in centrosomal protein recruitment – for instance, in embryos depleted of cyclin E–CDK-2 – leads to a failure to establish polarity (Cowan and Hyman, 2006). Thus, one

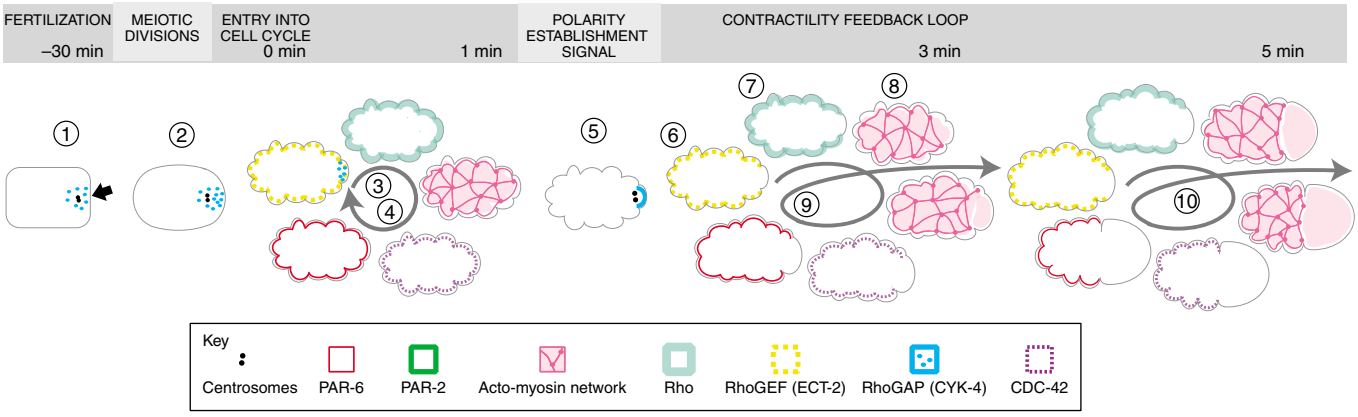

Fig. 5. A model of polarity establishment in one-cell *C. elegans* embryos. Illustrations of *C. elegans* embryos from fertilization (left) through to the initial phase of polarity establishment (right). Thin arrows indicate the temporal hierarchy of events. The identity of the proteins or complexes depicted is indicated in the key. The approximate timing of events relative to entry into the first cell cycle is shown in the upper bar. The acto-myosin network is indicated from a surface view of the embryo cortex; centrosome localization and the distribution of other proteins is shown in the mid-plane of embryos. Anterior, or the meiotic pole, is to the left. The events indicated are as follows, with numbers corresponding to those in the figure. (1) Fertilization, indicated by the thick black arrow, contributes centrosomes and RhoGAP CYK-4 to the oocyte. (2) CYK-4 localizes around the centrosomes and to a local cortical region. (3) Following entry into the cell cycle, RhoGEF ECT-2 becomes active throughout the cortex, leading to myosin activation via Rho•GTP and thus uniform contractility. (4) CDC-42 helps link the anterior PAR complex to the contractile cortex. (5) Centrosome assembly and RhoGAP CYK-4 activation provide a polarity establishment signal. (6) RhoGEF ECT-2 is eliminated from the cortex overlying the centrosomes. (7) Rho activity is downregulated in the local region, leading to downregulation of myosin activity and cessation of contractility. (8) The local region of non-contractility breaks the symmetry of the cortex and promotes a partial collapse of the acto-myosin network. (9) CDC-42 shrinks with the contractile domain, causing the anterior PAR complex to follow; RhoGEF ECT-2 and Rho shrink with the contractile domain. (10) RhoGEF ECT-2, RHO-1, CDC-42 and PAR-6 in the anterior domain promote contractility of the acto-myosin network, allowing for continued contraction of the anterior domain through a feedback loop.

possibility is that the recruitment of the centrosomal proteins may be a prerequisite for activation of RhoGAP CYK-4. A second possibility is that centrosome assembly and Rho activity may be regulated in parallel by a common upstream pathway. A common regulator could depend on global signals, such as the 'egg-to-embryo' transition pathways (Pellettieri et al., 2003).

Connecting contractility to PAR polarity: CDC-42

In order for polarity to be established in the embryo, downstream polarity effectors – in this case the PAR proteins – must recognize contractile polarity. A parallel analysis of the shrinking myosin network and the anterior PAR domain during polarization has shown that these two cortical structures move with similar kinetics (Munro et al., 2004). Thus, it was suggested that the anterior PAR complex is physically connected to the acto-myosin cortex. When the contractile cortex moves, so too would the PAR complex. This simple idea could account for how anterior PAR proteins localize specifically to the contractile cortex, i.e. they are attached. But how are they attached? Two recent studies implicate the small GTPase CDC-42 in helping to mediate the linkage of anterior PAR proteins to the acto-myosin network (Aceto et al., 2006; Schonegg and Hyman, 2006). CDC-42 moves away from the non-contractile posterior during polarity establishment (Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006), similar to the acto-myosin network and the anterior PAR complex (Fig. 4A), and the cortical localization of PAR-6 depends, in part, on CDC-42 (Aceto et al., 2006; Gotta et al., 2001; Schonegg and Hyman, 2006).

The extent to which CDC-42 is required for cortical localization of the anterior PAR proteins remains a matter of debate. Complete CDC-42 loss-of-function embryos have not been examined; RNAi-mediated CDC-42 depletion most likely represents a partial loss-of-function phenotype, perhaps explaining the varying interpretations of the role of CDC-42 in anterior PAR protein localization. CDC-42 binds to the anterior PAR complex in many polarized cell types (Gotta et al., 2001; Hutterer et al., 2004; Joberty et al., 2000; Johansson et al., 2000; Lin et al., 2000; Qiu et al., 2000), and it is well established that CDC-42 interacts with the CRIB domain of its binding partners. Likewise, in *C. elegans*, PAR-6 contains a CRIB domain, which indeed is required for CDC-42–PAR-6 interactions in vitro (Aceto et al., 2006). Aceto et al. (Aceto et al., 2006) have shown that PAR-6 that lacks the CRIB domain (PAR-6-ΔCRIB) shows reduced cortical localization as compared with wild-type PAR-6; the reduction in cortical PAR-6-ΔCRIB localization is enhanced if endogenous PAR-6 is not present (Fig. 4C). These results demonstrate that the CRIB domain of PAR-6, which interacts with CDC-42, is required for efficient PAR-6 cortical localization, but that PAR-6–PAR-6 interactions may contribute to cortical localization independently of CDC-42–PAR-6-CRIB binding. However, the data do not exclude the possibility that PAR-6 and CDC-42 can interact at the cortex through PAR-6-CRIB-independent mechanisms. Finally, a recent report (Beers and Kemphues, 2006) suggests that PAR-3 is required for the efficient localization of PAR-6 to the cortex independently of CDC-42. This study supports the idea that multiple pathways contribute to the cortical localization of the anterior PAR complex, but the relative contributions of these pathways or the existence of additional pathways remain unknown.

In embryos depleted of Rho or RhoGEF ECT-2, myosin can shrink asymmetrically despite the apparent lack of contractility; neither the anterior PAR complex nor CDC-42, however, follows the acto-myosin network and PAR polarity is not established (Fig. 4E) (Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006). Why don't CDC-42 and the anterior PAR proteins segregate with the acto-myosin network? One possibility is that the opportunity for

segregation of CDC-42 and the anterior PAR complex is temporally regulated. The asymmetric segregation of myosin in *rho-1(RNAi)* or *ect-2(RNAi)* embryos occurs approximately ten minutes after the normal time of polarity establishment. Perhaps CDC-42, and with it the anterior PAR complex, can only follow the acto-myosin network during the polarity establishment phase (Cuenca et al., 2003). A second possibility is that the segregation of CDC-42 and the anterior PAR complex requires a specific acto-myosin network property. The myosin network in *rho-1(RNAi)* or *ect-2(RNAi)* embryos appears much less contractile and exhibits fewer interconnected foci than in wild-type embryos (Fig. 4A,D,E) (Jenkins et al., 2006; Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006). Perhaps CDC-42 can only respond to a highly contractile, interlinked acto-myosin network. A third possibility is that CDC-42 is directly regulated by Rho signaling. PAR-6 and CDC-42 localize to the cortex in *rho-1(RNAi)* and *ect-2(RNAi)* embryos (Fig. 4D), suggesting overall CDC-42 function is not disrupted when Rho signaling is inhibited. However, Rho signaling may control a CDC-42 activity that is required specifically for its segregation with the acto-myosin cortex. Thus, CDC-42 appears to function in two aspects of polarization: CDC-42 helps link PAR-6 to the cortex, and CDC-42 coordinates segregation of the anterior PAR complex with the acto-myosin cortex.

Supporting continued contractility: the anterior domain

Following the initial symmetry-breaking event that establishes contractile and non-contractile cortical regions, a feedback loop appears to take over, mediated in part by the anterior segregation of Rho and its activator RhoGEF ECT-2. Further promotion of contractility in the anterior comes from two additional sources, which also segregate exclusively with the anterior: the anterior PAR complex and CDC-42 (Kirby et al., 1990; Motegi and Sugimoto, 2006; Munro et al., 2004; Schonegg and Hyman, 2006). Several lines of evidence suggest that acto-myosin contractility is aberrant in embryos lacking PAR-3 or CDC-42 function. *par-3* mutants exhibit altered anterior and posterior domain sizes, such that the anterior domain does not segregate completely (Fig. 3C) (Kirby et al., 1990; Munro et al., 2004). This phenotype may be consistent with reduced contractility. *par-3(RNAi)* embryos also exhibit inefficient CDC-42 segregation toward the anterior (Motegi and Sugimoto, 2006), a likely correlative to the reduced acto-myosin network movement. Embryos depleted of CDC-42 have altered contractility dynamics, such that the cortical ingressions that result from acto-myosin contraction in the anterior are shallower and more static than in wild-type embryos (Fig. 4B) (Schonegg and Hyman, 2006). Finally, in both *par-3(RNAi)* and *cdc-42(RNAi)* embryos, RhoGEF ECT-2 movement out of the non-contractile region is also reduced, a phenotype similar to that in embryos depleted of myosin activity, suggesting again that PAR-3 and CDC-42 regulate contraction (Motegi and Sugimoto, 2006). It remains unresolved whether the *cdc-42(RNAi)* contractility defects result from reduced cortical localization of the anterior PAR complex, or reflect a direct role of CDC-42 in contractility. In both cases, contractility is not abolished, consistent with the ability of *par-3* and *cdc-42* mutant embryos to initiate contractile polarity; the dynamics of contractility, however, appear to be altered. Thus, several molecules associated with the anterior cortex, including RHO-1, ECT-2, PAR-3 and CDC-42, promote cortical contractility and help generate the necessary mechanical force for continued anterior-directed segregation of the contractile domain during polarity establishment.

Polarity establishment and the cortex: a model

A possible model of polarity establishment in *C. elegans* embryos emerges from the insight offered by these recent studies, although many of the steps remain speculative (Fig. 5). RHO-1, RhoGEF ECT-2, the acto-myosin network, CDC-42 and the anterior PAR complex behave as a unit that promotes contractility. RhoGEF ECT-2 activates RHO-1, RHO-1 activates acto-myosin network contractility, CDC-42 helps link the anterior PAR complex to the cortex, and CDC-42 and the anterior PAR complex promote acto-myosin network contractility. Sperm-supplied RhoGAP CYK-4 and centrosomes cause this contractile unit to be displaced from a small region of the cortex, resulting in a break in the contractile network (Fig. 1D). This break in the network results in a progressive collapse of the remaining contractile unit in a sort of 'snow ball' effect: once contractile asymmetry is initiated, it cannot be reversed, as the positive regulators of contractility are found only within the contractile unit.

Conclusion

Testing this model and filling in both its molecular and mechanical gaps will be the next challenges in understanding the relationship between cortical activity and cell polarization. Is downregulation of myosin activity sufficient for the symmetry-breaking event during polarization? GTPases can modulate multiple cellular pathways; perhaps RHO-1 (or another GTPase regulated by RhoGAP CYK-4) controls additional polarity establishment pathways. How is RhoGAP CYK-4 temporally and spatially regulated? Phosphorylation states modulate the activity of many GEFs and GAPs, and several kinases are required for polarization. How do centrosomes integrate into the Rho signaling pathway? Centrosomes are essential for polarity establishment but their precise role remains unknown. How is the anterior PAR complex linked to the cortex? CDC-42 has an important role, but alternative pathways also exist. How does Rho ensure that CDC-42 follows the acto-myosin network as it shrinks? Different properties mediate segregation of CDC-42 compared with acto-myosin. How do CDC-42 and the anterior PAR complex regulate contractility? Downstream targets of these molecules may modulate the cortex through Rho or through alternative pathways, such as actin polymerization. As more details of the molecular control of polarity establishment are uncovered, it will become possible to understand which of the activities in *C. elegans* represent general principles in polarity establishment, and which are specialized to the particular case of embryonic polarity establishment.

The recent *C. elegans* papers referred to in this review are not summarized here in full. All of the studies offer significant insights that we have not covered, and we therefore encourage interested readers to consult the original publications. We thank Ed Munro (University of Washington) for insightful discussions and to three reviewers for helpful suggestions and for pointing out omissions.

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