# c a · -d·, · d· c· a a · a d **position mitotic spinon mitotic spin-** *Cae o* abd ee a

#### **Aaron F. Severson and Bruce Bowerman**

 $\mathbf{F}_{\mathcal{O}}$  is the Molecular Biology,  $\mathbf{F}_{\mathcal{O}}$  of  $\mathbf{F}_{\mathcal{O}}$  or  $\mathbf{F}_{\mathcal{O}}$  or  $\mathbf{F}_{\mathcal{O}}$  and  $\mathbf{F}_{\mathcal{O}}$  is the  $\mathbf{F}_{\mathcal{O}}$ 

*Caenorhabditis elegans,* the partition proteins proteins proteins proteins proteins proteins proteins proteins proteins and protei  $(\mathcal{A}, \mathbf{A})$ , microfilaments (MFs), dynactin, and an non localize to the cortex of early the cortex of early embryonic cells. Both the PARs and the actomyonic cells.  $A$ eton are required to polarize the anterior-posterior-posterior-posterior-posterior (anterior-posterior) (a-p)  $\epsilon$  by axis in order cyclic but it remains unknown how  $\mathcal{J} = \mathcal{J}$ MFs in the control of the MFs in t  $a^2$  are required for the corresponding of PAR-2 and  $A$  $\mathcal{A}-3.$  Furthermore, we show that  $\mathcal{A}=\frac{1}{2}$  polarity regulates  $\mathcal{A}=\frac{1}{2}$ dependent corresponding to assume that  $\mathbf{r} = \mathbf{r} \cdot \mathbf{r}$ 

(MTs). These forces, which appears to be mediated by dynamics of the mediated by dynamics of  $\mathcal{F}_1$ and dynamics in the shape and orientation in the shape and orientation of the shape and orientation or  $\mathbf{r}$ of mitotic spindles. Unlike MFs, dynein, and dynactin, myosin II is not required for the production of these forces. Instead, my operation in flux influences embryonic polarity by limiting  $\mathcal{L}$  $P$ Ar-3 to the anterior cortex. This in turn produces as  $P$ in the forces applied to  $\mathcal{A}-2$  $t_0 = t_0$  and  $t_1 = t_0$  in the posterior corresponding  $t_0$  on  $t_0$  and  $t_1$  and  $t_2$  $\overline{a}$ 

## **I** d c

First identified in the nematode *Caenorhabditis elegans*  $\mathcal{A}(\mathbf{r}) = \mathcal{A}(\mathbf{r}, \mathbf{r}, \mathbf{r})$  and conserved partitioning partitions proteins proteins proteins proteins proteins  $\mathcal{A}(\mathbf{r})$  $\mathcal{P}(P_{\mathcal{A}})$  are required for cell polarity in many animal cell polarity in  $\mathcal{P}(P_{\mathcal{A}})$  $\frac{1}{2}$  types (for review see Doe and Bowerman, 2001; Wodarz, Wodar 2002). In the one-cell stage *C. elegans* embryo, the PDZ domain protein PAR-3 and the Ring finger protein PAR-2 concentrate in complementary anterior and posterior cortical domains, respectively. Both are required to specify the anteriorposterior (a-p) body axis and to orient and position mitotic spindles relative to the a-p axis (Kemphues et al., 1988; Cheng et al., 1995; Etemad-Moghadam et al., 1995; Boyd et al., 1996) (Fig. 1996)

One a-p asymmetry regulated by PAR-2 and PAR-3 appears during telophase of the first mitosis when the initially spherical posterior centrosome changes shape to form a disc, whereas

the anterior centrosome remains spherical (Hill and Strome, 1988; Cheng et al., 1995; Keating and White, 1998). Previous observations of early embryos made using Nomarski DIC microscopy have suggested that PAR-3 inhibits flattening of the anterior spin dependence pole, whereas  $P_{\rm eff}$ bition from occurring at the posterior pole (Cheng et al., 1995). Centrosome flattening may reflect an asymmetry in forces applied to the centrosomes through astral microtubules  $(\sqrt{X})$  that contact the contact the contact the contact the corresponding mitosis. This is expected with  $\sigma$ 

 $1995$  ). Conversely, Para-2 accumulated throughout throughout throughout throughout throughout the cor- $\frac{1}{\sqrt{2}}$  *par-3(it71)* mutants, and both centros to resemble a wild-type posterior centrosome (Fig. 1, g–i)  $\mu$  $($  et al., 1995; Boyd et al., 1995).

### Centrosome flattening requires many control and the ma

 $D_{\rm tot}$  assembly results in a-p polarity  $D_{\rm tot}$  a-p polarity defects in asimilar to those caused by mutations in *par-2*. In wild-type  $\epsilon$ embryos treated with  $\frac{1}{2}$   $\frac{1}{2}$  (Hill and Strome,  $\frac{1}{2}$  and  $\frac{1}{2}$ 1988) or later  $\mathbf{r}_{\mathbf{a}}$  (Latrice  $\mathbf{U}$  or lattice empirically expressed on  $\mathbf{U}$ bryos), neither centrosome flattened. The failure of either  $p$ ole to flatten could result from mislocalized  $P$  in the flatten mislocalized  $P$ ing flattening at both poles as in *par-2* mutants (Cheng et al.,  $1995$ ). Moreover, MFs might be required for cortical localization of the PAR proteins, with such localization being important for their function. Therefore, we examined the local ization of PAR-2 and PAR-3 in embryos exposed to LatA.  $\mathbf{C}$  which is presented that  $\mathbf{P}_{\mathbf{A}}$  and  $\mathbf{C}$  and  $\mathbf{C}$  and  $\mathbf{P}_{\mathbf{A}}$  both requires into to localize to the corresponding to the corresponding  $\alpha$  $\phi^{\rm st}$  , or present at severely reduced levels, in the presence of  $\phi$  $\mathcal{F}_j$  (fig.  $\mathbf{r}$ ,  $n \geq 0$  and  $\mathbf{r}$  and  $\mathbf{r}$  $\sqrt{n}$  accumulated around the centrosomes of  $\sqrt{n}$  and  $\sqrt{n}$  accumulated around the centrosomes of  $\sqrt{n}$ LatA-treated embryos as was observed recently in *pod* mutants with defects in a-p polarity (Rappleye et al., 2002). We can expect the  $\mathbf{r}_i$ also examined centrosome flattening and  $P$ in embryos with reduced levels of the profiles  $\mathcal{F}_\text{A}$ we have recently shown is required for the assembly of cortical for the assembly of cortical for cortical for  $\alpha$  $\mathcal{C}_\mathbf{z}$  ,  $\mathcal{C}_\mathbf{z}$  and  $\mathcal{C}_\mathbf{z}$  ,  $\mathcal{C}_\mathbf{z}$  our findings in LatA-treated embryos, the posterior centrosome failed to flatten in embryos depleted of  $P$ N-1 users depleted ing dsRNA-mediated gene silencing, or RNAi (Fig. 2 g), and PAR-2 was undetectable at the cortex but instead localized around centrosomes (Fig. 2 f; 10 out of 12 embryos).  $\Delta$ though PAR-3 was always detected at the cortex in PFN-N-3 was always detected at the cortex in  $\Delta$ 

1–depleted embryos, it was present at much reduced levels compared with wild-type embryos fixed on the same slides on the same slides on the same slides on the same slides of  $\alpha$  $\mathbf{F}(\mathbf{F}, \mathbf{F}) = \mathbf{F}_{\mathbf{F}}(\mathbf{F}, \mathbf{F})$ . The remaining corrigion  $\mathcal{P}_{\mathcal{A}}$  may simply reflect residual MF assembly because lower lo levels of corresponding to corresponding in embryos with re-  $\mathbf{r}_i$ duced levels of profilin (Severson et al., 2002). We conclude that centrosome flattening and the cortical localization of PAR-2 and PAR-3 all require an intact MF cytoskeleton. PAR-3 prevents flattening of the anterior centrosome in wild-type embryos and of both the anterior and posterior and posterior and posterior and posterior and posterior and posterior and positive and centrosomes in *par-2* mutants. In LatA-treated embryos, PAR-3 is not present at the cortex, but cytoplasmic PAR-3  $\mathbf{r}^{(k)}$ could still function to prevent centrosome flattening. There-<br>could be prevent centrosome flattening. Therefor each  $f^{\mu\nu}$  , we examined centrosome shapes  $\mu$  *par-3* mutants exposed to LatA. We found that both centrosomes, which are flattened in *par-3* single mutants and in *par-2 par-3* double mutants, were spin-type as in Lata  $T$  as in Lata  $t$  as in Lata  $t$  $\mathbf{b}$  and  $\mathbf{c}$  (Fig. 2 d;  $\mathbf{a}$  6). Both centrosomes were also spherically were also spherical spheri cal in *pfn-1; par-3* double mutation of  $\alpha$  double mutation  $\alpha$ 

**Microphants, myosingle in AR polarity in AR** 

 $224$  The Journal of Cell Biology Volume 161, Number 1, 2003

# $M$   $M \cdot C$  PAR-3 ta to the anterior cortexts **PAR-3** to the antenna cortext of the

 $\begin{bmatrix} 1 \\ 1 \end{bmatrix}$  of  $\begin{bmatrix} 1 \\ 0 \end{bmatrix}$  of  $\begin{bmatrix} 1 \\ 0 \end{bmatrix}$  of  $\begin{bmatrix} 1 \\ 0 \end{bmatrix}$ 

As described above, PAR-3 accumulates around the cortex of myosin-depleted embryos, whereas PAR-2 and PAR-3 local-particle ize in mutually exclusive corresponding to the corresponding of  $\sigma$ gotes. Myosin II could influence the localization of  $P$  $\alpha$  by facilitating expansion of the PAR-2 domain,  $\alpha$ thereby restricting  $P$  is the anterior cortex. Alterna- $3$  $t_{\rm eff}$  , and the anterior might limit limit limit  $T_{\rm eff}$  localization to the anterior to the anterior  $t_{\rm eff}$ hemisphere, thus permitting expansion of the PAR-2 domain. To distinguish between these two models, we examine ined the localization of PAR-2 in NMY-2–depleted and in  $M_c$  – dependent par-3 mutant embryos. In both cases, we can found that  $P_{\rm eff}$  is present throughout throughout throughout throughout throughout throughout the corresponding to  $\sim$  $g_{\rm tot}$ gesting that neither myosin II subunit is required for cortical for co

localization or expansion of PAR-2 (Fig. 3, c and g; *n* -

 $S_{\rm c}$  milarly, we observe denote between spherical centrosomes in some  $D_{\rm c}$ 1–depleted embryos (Fig. 4 c; 4 out of 20 embryos; 4 embryos exhibited defects in chromosome segregation and in centrosome flattening, whereas 16 embryos appeared wild  $\sqrt{\epsilon}$  type during the first mitotic division). Exposure of embryos to low doses of notice that shorten but do not eliminate  $\sigma$ ,  $\mathcal{T}_\text{c}$  also disrupted centrosome flattening (Fig. 4 d; fig. 4 d; fig. 4 d; of seven embryos). We conclude that both dynein function and contact between  $\mathcal{R}$  and the cortex are required as for centrosome flattening.

 $\begin{bmatrix} 1 \\ 1 \end{bmatrix}$  of  $\begin{bmatrix} 1 \\ 0 \end{bmatrix}$  of  $\begin{bmatrix} 1 \\ 0 \end{bmatrix}$  of  $\begin{bmatrix} 1 \\ 0 \end{bmatrix}$ 

 $\frac{C}{\sqrt{1 + \frac{C}{c}}}\frac{C}{c}$  $\mathcal{O}(n)$  data suggest that the nonmuscle myosin II subunits in II sub  $N_{\rm eff}=2$  and  $N_{\rm eff}=4$  mediate only a subset of  $\Gamma_{\rm eff}$ dependent processes during polarization of the a-p axis in a *C. elegans* zygot<sub>e.</sub> F-actin is required for at least for at least for at least  $\epsilon$ functions in the one-cell stage embryo: the correct stage embryo: the correct stage embryo: the cortical localizations of  $\mu$ tions of  $P_{\rm{A}}$  and  $P_{\rm{A}}$  and  $P_{\rm{A}}$  and  $P_{\rm{A}}$  and centros tening (Fig. 5 and Muslim  $\mathcal{F}_{\mathbf{a}}$  and  $\mathcal{F}_{\mathbf{a}}$  are distinct and  $\mathcal{F}_{\mathbf{a}}$  are distinct and  $\mathcal{F}_{\mathbf{a}}$ pensable for cortical PAR localization and for centrosome  $f_{\rm eff}$  instead restricts  $\mathcal{F}_{\rm eff}$  instead restricts  $\mathcal{F}_{\rm eff}$  to the anterior restricts  $\mathcal{F}_{\rm eff}$  $c_{\rm c}$ cortex, which permits expansion of the PAR-2 domain. As  $\sigma_{\rm c}$  $e^{i\phi}$  and  $e^{i\phi}$  accumulates in the par-2 single of  $p$  $m_{\text{max}} = m_{\text{max}}$  is not sufficient to restrict  $\mathbf{P}$  as  $\mathbf{P}$  as  $\mathbf{P}$ 

Mir ofilaments -Centro some Cori Flattening/ $\vdash$ **PANSY SPITCIE FOSTIA SALE** Dynein/Dynact<sup>\*</sup> Netivity

Figure 5. **Models of the polarization of the** *C. elegans* **zygote.**   $\left( \bullet -_{\bullet} \circ \bullet \right)$ addition, MFs or MF-associated proteins act on the mitotic spin dependence on the mitotic spin dependence on t<br>Associated proteins act of the mitotic spin dependence on the mitotic spin dependence on the mitotic spin depe flattening the posterior centrosome. Myosin II and Parties is a second the posterior centrosome. Myosin II and PAR-<br>A restriction in the posterior centrosome. Myosin II and Parties is a second the posterior centrosome. My  $\mathcal{P}_\mathcal{A}$  to the anti-stock where  $\mathcal{A}$  in  $\mathcal{A}$  in  $\mathcal{A}$  in the PAR-3 inhibits centros flattening. (b)  $A_n = \frac{1}{n}$  in the extension of corresponding  $A_n$  $M_{\rm{max}}$  recruit dynamics records the dynamics of the dynamics of the cortex. Dynamics  $\alpha$ pulls on astral MTs nucleated by the centrosomes. Parameters on  $\mathcal{A}$ inhibits dynein localization or function or function  $\mathcal{F}_{\mathbf{z}}$ in the anterior hemisphere than in the posterior (blue triangles). The posterior (blue triangles) is a set of  $C$  is applied to the anterior is applied to the anterior centrosome than  $C$ the posterior centrosome (arrows), and the spinolar posteriorly becomes posteriorly posteriorly posteriorly posteriorly  $\mathcal{F}_{\mathbf{r}}$ displaced. The high lateral forces in the posterior hemisphere in the second term in the second term in the s stretch the posterior centrosome, flattening it is into a disc shape. For a disc shape  $\mathcal{F}_1$ and alternative model see Tsou et al.  $(2002)$ .

 $\mathbf{F}_{\mathcal{S}} = \mathbf{F}_{\mathcal{S}} \mathbf{F}_{\mathcal{S}} = \mathbf{H}_{\mathcal{S}} \mathbf{H}_{\mathcal{S}} \mathbf{F}_{\mathcal{S}} \mathbf{F}_{\mathcal{S}}$  to limit  $\mathcal{S}_{\mathcal{S}}$ the anterior cortex.

The flattening of the posterior centrosome along the transverse axis may occur as a result of cortical forces that are surface that are surface that are surface th applied to astral MTS and displace the first mitotic spin displace the first mitotic spin displace the first m toward the posterior pole (Grill et al.,  $T_{\rm c}$  ),  $\tau_{\rm c}$  ,  $T_{\rm c}$  ),  $\tau_{\rm c}$  $200$  2002). The second of these forces is greater in the second theorem posterior hemisphere, and the posterior pole of the first mitotic spin de rocks from side totics from side to side  $\sigma$  ,  $\mathcal{F}$  to sind lateral forces act on astronomic MTs that contact the posterior  $\mathbf{F}_\text{c}$ cortex late in mitosis when centrosome flattening is observed. Because both spindle poles exhibit rocking motions in *par-3* mutant embryos, whereas neither pole shows  $\mathbf{r}$ ing in *par-2* mutants, normal PAR polarity appears necessary  $\tau_{\rm c}$  restrict lateral forces to the posterior pole (Cheng et al.,  $\tau_{\rm c}$ 

 $1995$ . Our data suggest that  $\mathcal{M}_{\rm eff}$  recruit that  $\mathcal{M}_{\rm eff}$  records that  $\mathcal{M}_{\rm eff}$ ting time to the complete to  $\mathcal{F}$  and apply the corresponding to astral MTs. NMY-2 and MLC-4 are required for a polarized distribution of the PAR proteins, which in turn regulate the  $P_{\rm eff}$ localization or the function of the dynein–dynactin motor  $\omega$  complex, thus influencing both the position and shape of position and shape of position and shape of position  $\mathbf{t}_\mathrm{t}$  the first mitotic spin definition  $\mathbf{t}_\mathrm{t}$ 

Recently, two models have been proposed to explain the establishment of asymmetry in the forces that position minor minor minor  $\mathbf{r}_i$ totic spindles in *C. elegans* zygotes. First, a DEP domain pro tein called LET-99 accumulates in a cortical stripe that is distributed in a cortical stripe that is distributed in  $\frac{dL}{dt}$ placed toward the posterior pole, and high levels of  $\mathcal{T}_\text{c}$ have been proposed to attenuate dynein-dependent forces ap $p$  as the contact to astral contact the contact the contact the cortex  $\mathcal{T}_\alpha$  $200$ , Properly position would lower between  $\overline{P}$ forces that normally oppose those applied to the spindle pole from the posterior-most cortex, producing a greater net force toward the posterior (see Fig. 7 in Tsou et al., 2002).

 $\mathcal{A}$ lternatively, it has been suggested that  $\mathcal{A}$  are unlikely are unlikely are unlikely are unlikely are unlikely are unlikely associated that  $\mathcal{A}$ to be involved in generating the cortical forces that act on spindle poles (Hill and Strome, 1988; Grill et al., 2001).  $\overline{\mathcal{T}}$  is a based on experiment on experiments in which brief  $\overline{\mathcal{T}}$ pulses of cytochalasin D, applied and washed out before anaphase, were sufficient to prevent posterior displacement positive  $\mathbf{v}$ of the first mitotic spinoles of the first mitotic spinoles and  $\mathbf{m}$ cytochalasin D pulses applied during anaphase did not pre- $\varphi$ ent posterior displacement (Hill and Strome, 1988). These  $\mathfrak{so}_N$  findings suggest that  $\mathfrak{so}_N$  are not directly required for posterrior displacement of the first mitotic spindle. Grill et al.  $\mathcal{L}_\mathbf{z}$  therefore suggested that increases that increases of  $\mathcal{L}_\mathbf{z}$  instability of  $\mathcal{L}_\mathbf{z}$ ity associated with the posterior cortex might account for the  $\mathbf{g}(\mathbf{u}, \mathbf{u})$  and  $\mathbf{g}(\mathbf{v}, \mathbf{v})$  for example, such instability instability  $\mathbf{p}_{\text{max}} = \mathbf{p}_{\text{max}}$  facilitate pulling of the spin distribution toward the pole toward rior cortex as astral MTs shorten.

Our findings support a role for MFs and the dynein–<br>Application of MFs and the dynein–dynein–dynein–dynein–dynein–dynein–dynein– dynactin motor complex in applying forces to spindle poles via astral MTs that contact the contact the cell contact the cell cortex. It is possible that  $\mathcal{T}_\text{tr}$ the pulses of cytochalasin D used by  $\mathcal{S}_{\text{th}}$  and  $(1988)$ were sufficient to dispute some as polarity but not to the polarity but not to the  $\mu$ disrupt dynein–dynactin-mediated application of forces to astral  $\overline{\mathcal{M}}$  and  $\overline{\mathcal{M}}$  are fully cytochalasin D pulses may fully may disrupt MF function, but two different force mechanisms of  $\mathbf{F}_{\text{tot}}$ could operate during spin during spin of  $\mathcal{T}$  instability spin during. might account for posterior displacement, with dynein– dynactin forces generating only lateral rocking and flatten-



 $2003$ 

ing of the posterior spin dependence of the pole of this pole. In support of this pole. In support of this pos we sometimes observed an absence of spindle pole flattening even though the spindle was displaced normally toward the posterior pole (Fig. 4). MF function is not limited to spindle flattening and rocking though, because MFs, dynein, and dynactin also are required for spindle rotation at the two-cell stage in wild-type and *par-3* mutant embryos. Finally, MT asters undergo abnormal lateral rocking movements early in mitosis in one-cell *let-99*