14. Issa, E.B., Cadieu, C.F., and DiCarlo, J.J. (2018). Neural dynamics at successive stages of the ventral visual stream are consistent with hierarchical error signals. eLife *7*, e42870.

15. Yamins, D.L., and DiCarlo, J.J. (2016). Using goal-driven deep learning models to understand sensory cortex. Nat. Neurosci. *19*, 356–365.

16. Pasupathy, A., and Connor, C.E. (2001). Shape representation in area V4: position-specific tuning for boundary conformation. J. Neurophysiol. *86*, 2505–2519.

17. Pasupathy, A., and Connor, C.E. (1999). Responses to contour features in macaque area V4. J. Neurophysiol. *82*, 2490–2502.

18. Pasupathy, A., and Connor, C.E. (2002). Population coding of shape in area V4. Nat. Neurosci. *5*, 1332–1338.

19. Gallant, J.L., Braun, J., and Van Essen, D.C. (1993). Selectivity for polar, hyperbolic, and Cartesian gratings in macaque visual cortex. Science *259*, 100–103.

Cell Polarity: Getting the PARty Started

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Polarity establishment is a key developmental process, but what determines its timing is poorly understood. New research in *Caenorhabditis elegans* demonstrates that the PAR polarity system extensively reconfigures before becoming competent to polarize. By inhibiting membrane localization of anterior PAR proteins, AIR-1 (aurora A) and PLK-1 (polo kinase) prevent premature polarization.

Polarity, the asymmetric organization of cellular components, is a near universal aspect of animal cells. Yet, we all started life as a single cell zygote, without a defined front or back and lacking predetermined structures. This poses a clear question: when and how does polarity arise during development? Much insight has come from studies in *Caenorhabditis elegans,* which revealed a self-organizing molecular network that polarizes the zygote by creating two opposing PAR (partitioning defective) protein domains [1,2]. In mammals, PAR protein asymmetry arises when apical and basolateral cellular domains are formed, following compaction of the 8-cell embryo [3]. In contrast, the *C. elegans* oocyte polarizes shortly after fertilization, triggered by a symmetry-breaking cue from the mature sperm-derived centrosome pair. This determines the anterior–posterior body axis however, *C. elegans* mutants that arrest in meiosis without centrosome maturation eventually polarize with a reverse orientation [4–7]. What, then, controls the correct developmental timing and cue for polarization? A new study from Reich *et al.,* recently published in *Current Biology,* shows that part of the answer lies in the PAR system itself, which has to go through a maturation phase before becoming competent to polarize the embryo [8].

PAR proteins and many aspects of their functional interactions have been discovered through groundbreaking genetic studies of early *C. elegans* embryos by Ken Kemphues and colleagues [9]. This work revealed two classes of PAR proteins — one needed for anterior identity and one for posterior identity. The localization of PAR proteins in the one-cell embryo corresponds with these functions, but only after polarity establishment. Following fertilization, the oocyte pronucleus first needs to complete meiosis I and II. At the end of this process, the anterior PAR (aPAR) proteins PAR-3, PAR-6 and PKC-3 occupy the entire cell membrane or underlying cortex, while the posterior PAR (pPAR) proteins PAR-1 and PAR-2 are spread through the cytoplasm. Shortly after completion of meiosis, this configuration suddenly changes (symmetry breaking) to one where the aPAR and pPAR proteins form two opposing domains at the cell cortex (Figure 1).

How the PAR proteins achieve their polarized localization has been intensively studied. Reorganization of the actomyosin cytoskeleton beneath the cell membrane contributes an important mechanism [2]. The proximity of the paternal centrosome pair locally inhibits actomyosin contractility, which triggers an anterior-directed cortical actomyosin flow and removes the aPAR proteins from the posterior pole through advective transport. This allows the pPAR proteins to load onto the cortex. Mutually inhibitory interactions between the two groups of PAR proteins then establish and maintain a stable polarized state [2]. In the absence of cortical flows, a secondary mechanism for symmetry breaking becomes apparent, in which centrosomal microtubules locally protect PAR-2 from inhibitory phosphorylation by PKC-3 [10]. While not essential, this mechanism does serve to accelerate the process of polarization in wild-type embryos. The timing of symmetry breaking depends on maturation of the centrosome, and an experimentally induced delay or block in centrosome maturation causes a delay or failure in polarization [5,11,12]. These observations led to a model in which maturation of the paternal centrosome provides a temporal cue in the formation of a single axis of polarity upon completion of meiosis. However, the reverse polarity in meiotically-arrested

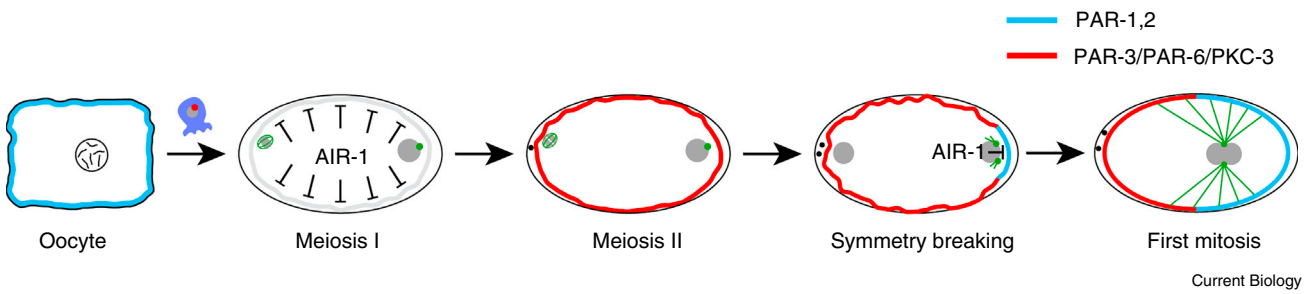

Figure 1. Schematic representation of polarity establishment in the *C. elegans* zygote.
Localization of the PAR proteins at the cell cortex is indicated in blue (posterior PAR proteins) and red (anterior PAR proteins). AIR-1 plays a critical role in preventing premature cortex association of the anterior PAR proteins in meiosis I. Following meiosis, centrosomal AIR-1 serves as the symmetry breaking cue by locally inhibiting cortical actomyosin contractility.

embryos, and why wild-type embryos do not respond to cues from the meiotic spindle, remained unresolved.

Previous studies had noted that PAR-1 and PAR-2 initially localize to the plasma membrane of immature oocytes, and become cytoplasmic during oocyte maturation [13–15]. Whether this behavior reflects some functionality of the PAR system had not been explored. In the new study, Reich *et al.* hypothesize that the PAR system itself may be regulated in its ability to mediate polarization, and that this regulation helps to ensure that embryos do not polarize early in response to non-centrosomal cues. The authors started their investigation with a careful determination of the dynamics of the PAR system. *C. elegans* oocytes are arrested in prophase of meiosis I until the major sperm protein (MSP) triggers meiotic maturation, which in turn causes ovulation into the spermatheca and fertilization. Before maturation, oocytes display a PAR localization that is the inverse of the situation before symmetry breaking of the embryo: PAR-1 and PAR-2 are localized to the cortex, while PAR-6 and PKC-3 are in the cytoplasm (Figure 1). The onset of maturation then triggers a reconfiguration in which PAR-1,2 relocate to the cytoplasm. After a period in which neither anterior nor posterior PAR proteins are enriched at the cortex, the anterior PAR proteins begin to accumulate at the cortex at the end of meiosis I. Importantly, the pre-maturation state and initial steps of PAR network reconfiguration are not determined by mutual inhibition. For example, RNA interference of *par-2* does not cause a precocious cortical accumulation of PAR-6, and PAR-2 was still cleared from the oocyte membrane in the absence of *par-6*. By the end of meiosis I, however, the PAR proteins show mutual antagonism.

Even during the antagonism-independent phase, the aPAR proteins are in principle competent to antagonize the pPAR proteins. Elegant experiments showed that induced targeting of PKC-3 to the membrane causes both a recruitment of PAR-6 and a loss of PAR-2 from the membrane. This indicates the presence of a mechanism that actively keeps the aPAR proteins from the membrane until meiosis I completes. To identify this mechanism, the authors investigated candidate kinases and found that the Aurora A kinase AIR-1 and POLO kinase PLK-1 are required to prevent premature accumulation of the aPAR proteins at the oocyte membrane. This is an interesting finding since AIR-1 and PLK-1 are ideally positioned, as cell-cycle related kinases, to temporally couple PAR network reorganization to development of the embryo. Two observations support that AIR-1–PLK-1 indeed control the polarization ability of the PAR system. First, embryos arrested in meiosis I polarized prematurely when *air-1* was depleted. Second, in wild-type embryos, depletion of *air-1* resulted in polarity defects, including reversed polarity and bi-polarity. Thus, a prerequisite maturation step appears to prevent the PAR network responding to cryptic symmetry breaking cues, such as the meiotic spindle, and ensures that polarization occurs only in response to a signal from the sperm-derived centrosomes.

The identification of *air-1* as a regulator of maturation of the PAR system comes hot on the heels of two other studies that identified key roles for AIR-1 in early embryonic polarity [16,17]. Zhao *et al.* identified AIR-1 as the long sought-after centrosome-associated symmetry-breaking cue that locally downregulates actomyosin contractility at the future posterior pole (Figure 1) [16]. In addition, this study uncovered an important role for a non-centrosomal pool of AIR-1 in suppressing cortical contractility during late prophase. In the other recent study, Klinkert *et al.* found that AIR-1 exerts a dual role in polarity: a cytoplasmic AIR-1 pool preventing symmetry breaking early in the cell cycle, and a centrosomal AIR-1 pool mediating symmetry breaking [17]. Triggered by the observation that PAR-2 domains arise at both narrow ends of the embryo, Klinkert *et al.* investigated whether membrane curvature plays a role in the spontaneous symmetry breaking observed in *air-1(RNAi)* embryos. By placing embryos in microfabricated chambers, they demonstrate that PAR-2 domains indeed form preferentially in curved regions, and postulate that high membrane curvature contributes to PAR-2 loading and symmetry breaking in the absence of a centrosomal cue [17].

Together, these three studies place AIR-1 at the center of key events in *C. elegans* embryo polarization (Figure 1). First, AIR-1 controls the timing of PAR maturation. Then, centrosomal AIR-1 acts as the symmetry breaking cue by locally downregulating actomyosin contractility — possibly through accumulation at the mature centrosome followed by diffusion. Finally, AIR-1 globally inhibits actomyosin contractility after the cessation of cortical flows. These novel insights also immediately raise new questions. Most importantly, what are the

targets of AIR-1 and PLK-1? PLK-1 was recently shown to phosphorylate PAR-3, controlling its oligomerization and formation of the aPAR complex [18]. Reich *et al.* favor a model in which AIR-1 acts via PLK-1 to regulate membrane association of PAR-3, but were unable to test this as mutating the relevant phosphorylation sites resulted in sterility. Of equal interest is the substrate of AIR-1 in the symmetry breaking event, which may be a component of the RHO-1 pathway [16]. Another open question is the mechanism that causes loss of the pPARs from the oocyte membrane, which requires initiation of maturation but not ovulation or fertilization [8]. Future studies will undoubtedly endeavor to shed light on these questions, further completing the complex picture of embryonic polarity establishment and increasing our understanding of self-organizing PAR polarity in various developmental contexts.

REFERENCES

1. Rose, L., and Gönczy, P. (2014). Polarity establishment, asymmetric division and segregation of fate determinants in early *C. elegans* embryos. WormBook Online Rev. *C. elegans* Biol. 1–43.
2. Lang, C.F., and Munro, E. (2017). The PAR proteins: from molecular circuits to dynamic self-stabilizing cell polarity. Development *144*, 3405–3416.
3. Graham, S.J.L., and Zernicka-Goetz, M. (2016). The acquisition of cell fate in mouse development: how do cells first become heterogeneous? Curr. Top. Dev. Biol. *117*, 671–695.
4. Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of *C. elegans* is a microtubule-directed process. Nature *408*, 89–92.
5. O'Connell, K.F., Maxwell, K.N., and White, J.G. (2000). The *spd-2* gene is required for polarization of the anteroposterior axis and formation of the sperm asters in the *Caenorhabditis elegans* zygote. Dev. Biol. *222*, 55–70.
6. Liu, J., Vasudevan, S., and Kipreos, E.T. (2004). CUL-2 and ZYG-11 promote meiotic anaphase II and the proper placement of the anterior-posterior axis in *C. elegans*. Development *131*, 3513–3525.
7. Sonneville, R., and Gönczy, P. (2004). *Zyg-11* and *cul-2* regulate progression through meiosis II and polarity establishment in *C. elegans*. Development *131*, 3527–3543.
8. Reich, J.D., Hubatsch, L., Illukkumbura, R., Peglion, F., Bland, T., Hirani, N., and Goehring, N.W. (2019). Regulated activation of the PAR polarity network ensures a timely and specific response to spatial cues. Curr. Biol. *29*, 1911–1923.e5.
9. Kemphues, K.J., Priess, J.R., Morton, D.G., and Cheng, N.S. (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. Cell *52*, 311–320.
10. Motegi, F., Zonies, S., Hao, Y., Cuenca, A.A., Griffin, E., and Seydoux, G. (2011). Microtubules induce self-organization of polarized PAR domains in *Caenorhabditis elegans* zygotes. Nat. Cell Biol. *13*, 1361–1367.
11. Hamill, D.R., Severson, A.F., Carter, J.C., and Bowerman, B. (2002). Centrosome maturation and mitotic spindle assembly in *C. elegans* require SPD-5, a protein with multiple coiled-coil domains. Dev. Cell *3*, 673–684.
12. Cowan, C.R., and Hyman, A.A. (2006). Cyclin E-Cdk2 temporally regulates centrosome assembly and establishment of polarity in *Caenorhabditis elegans* embryos. Nat. Cell Biol. *8*, 1441–1447.
13. Guo, S., and Kemphues, K.J. (1995). *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell *81*, 611–620.
14. Hung, T.J., and Kemphues, K.J. (1999). PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in *Caenorhabditis elegans* embryos. Development *126*, 127–135.
15. Boyd, L., Guo, S., Levitan, D., Stinchcomb, D.T., and Kemphues, K.J. (1996). PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in *C. elegans* embryos. Development *122*, 3075–3084.
16. Zhao, P., Teng, X., Tantirimudalige, S.N., Nishikawa, M., Wohland, T., Toyama, Y., and Motegi, F. (2019). Aurora-A breaks symmetry in contractile actomyosin networks independently of its role in centrosome maturation. Dev. Cell *48*, 631–645.e6.
17. Klinkert, K., Levernier, N., Gross, P., Gentili, C., von Tobel, L., Pierron, M., Busso, C., Herrman, S., Grill, S.W., Kruse, K., *et al*. (2019). Aurora A depletion reveals centrosome-independent polarization mechanism in *Caenorhabditis elegans*. eLife *8*, e44552.
18. Dickinson, D.J., Schwager, F., Pintard, L., Gotta, M., and Goldstein, B. (2017). A single-cell biochemistry approach reveals PAR complex dynamics during cell polarization. Dev. Cell *42*, 416–434.e11.

Spatial Cognition: Allowing Natural Behaviours to Flourish in the Lab

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Understanding the computational basis of spatial cognition requires observations of natural behaviour and the underlying neural circuits, which are difficult to do simultaneously: however, recent studies show how we might achieve this, combining rich virtual reality set-ups and the use of optogenetics in freely moving animals.

All animals have to process spatial information in order to control their behaviour and similarities can be seen between animals in the strategies they use to control orientation and navigation [1,2]. These similarities suggest that the