Review

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Going with the flow: insights from *Caenorhabditis elegans* zygote polarization

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Cell polarity is the asymmetric distribution of cellular components along a defined axis. Polarity relies on complex signalling networks between conserved patterning proteins, including the PAR (*partitioning defective*) proteins, which become segregated in response to upstream symmetry breaking cues. Although the mechanisms that drive the asymmetric localization of these proteins are dependent upon cell type and context, in many cases the regulation of actomyosin cytoskeleton dynamics is central to the transport, recruitment and/or stabilization of these polarity effectors into defined subcellular domains. The transport or advection of PAR proteins by an actomyosin flow was first observed in the *Caenorhabditis elegans* zygote more than a decade ago. Since then a multifaceted approach, using molecular methods, high-throughput screens, and biophysical and computational models, has revealed further aspects of this flow and how polarity regulators respond to and modulate it. Here, we review recent findings on the interplay between actomyosin flow and the PAR patterning networks in the polarization of the *C. elegans* zygote. We also discuss how these discoveries and developed methods are shaping our understanding of other flow-dependent polarizing systems.

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1. Introduction

Most metazoan cells are polarized along a defined axis, presenting molecular and structural asymmetries that are key to their function. Cell polarity can be triggered in response to a wide variety of cell-intrinsic or extrinsic cues that lead to different cellular responses, such as activation of signalling cascades, changes in membrane composition and cytoskeletal rearrangements. Through these processes, polarity cues promote the asymmetric cortical distribution of patterning proteins, the localization of which also depends on complex signalling networks, typically involving cooperative and mutually antagonistic interactions among these patterning proteins (figure 1*a*). From their established localizations, these proteins ultimately define distinct subcellular domains by controlling the activity of downstream targets that deliver the polarized phenotype. The coordination of these polarization events permits a multitude of higher biological outcomes ranging from cell fate determination and differentiation to tissue patterning and morphogenesis [1,2]. Among the polarity effectors, the *partitioning defective* (or PAR) proteins constitute a highly conserved network that includes the scaffolding proteins, Par3 and Par6, the small GTPase Cdc42 and the atypical protein kinase aPKC. PARs were initially identified in screens for mutations affecting the asymmetric cell division of the one-cell embryo (zygote) of the nematode *Caenorhabditis elegans* [3–5]; since then they have been implicated in patterning distinct regions in most polarized cells. For example, the same set of PARs defines the 'anterior' domain not only in the *C. elegans* zygote (figure 1*a,f*) but also

Figure 1. PAR patterning depends on actomyosin flow in the *C. elegans* zygote. (a) PAR protein network in the *C. elegans* zygote. In the anterior domain, CDC-42 and PAR-3 are required for stable membrane association of the PAR-6/aPKC heterodimer. PAR-3 can form clusters that recruit PAR-6/aPKC, which can switch between PAR-3- and CDC-42-dependent states. At the posterior, the accumulation of PAR-3 clusters is inhibited by PAR-1, which is stabilized at the posterior domain through PAR-2-binding. PAR-1, PAR-2 and the other posterior PAR, LGL-1, can be phosphorylated by aPKC and displaced from the membrane. Exclusion of the CDC-42-GAP CHIN-1 clusters from the anterior is dependent on aPKC, but whether it is a direct target of aPKC is unknown. In return, CHIN-1 at the posterior inactivates CDC-42. (b) The actomyosin cortex is a thin meshwork below the membrane that flows towards the zygote anterior during polarity establishment. At the membrane, PAR-6/aPKC follows advective actomyosin flow when bound to PAR-3 clusters (diffusion coefficient: D_c approx. $0.001 \mu\text{m}^2 \text{s}^{-1}$). In association with CDC-42, PAR-6/aPKC diffuses freely in the plane of the membrane (lateral diffusion: D_c approx. $0.1 \mu\text{m}^2 \text{s}^{-1}$). PAR proteins at the membrane exchange with a cytoplasmic pool, where they can freely diffuse with high mobility dissipating any source of asymmetry in the cytoplasm (D_c approx. $1 \mu\text{m}^2 \text{s}^{-1}$). (c) Midplane view of the zygote during polarity establishment or symmetry breaking (SB), with the initiation of cortical actomyosin and concomitant cytoplasmic flow (zygote's length approx. $45 \mu\text{m}$). The inset graph shows anterior (orange) and posterior (blue) PAR cortical levels (y -axis) along the length of the zygote (x -axis). The PARs exclude each other from their respective membrane domains. During polarity establishment, the boundary of anterior and posterior PARs shifts with the anterior-directed cortical flow (approx. $7 \mu\text{m min}^{-1}$), which transports PAR-6/aPKC in cluster with PAR-3. (d) Temporal snapshots during SB: (i) after fertilization, aPARs initially occupy the entire membrane, while pPARs are mostly cytoplasmic. The sperm-derived centrosomes (purple spheres) are positioned close to the cortex at the future posterior pole. (ii) A diffusive cue of Aurora A from the centrosomes inhibits actomyosin contractility in the posterior pole, resulting in a cortical flow towards the anterior domain. Microtubules emanating from the centrosomes are thought to aid the deposition of PAR-2 at the membrane. (iii) The actomyosin flow also generates a cytosolic backflow, which contributes to placing the posterior male pronucleus (PN) closer to the membrane. This holds the cue in place to promote further cortical flow and, synergizing with cortically-attached microtubules, facilitates the separation of the centrosomes around the male PN. (e) In the cortex, the microtubules associate with the $G\alpha$ /GPR-1/2/LIN-5 complex via dynein and are pulled towards the anterior thanks to the actomyosin flow. This coupling of microtubules to flow and the positioning of the male PN within the zygote posterior pole drives the separation of the centrosomes. (f) Midplane view of the embryo during polarity maintenance phase. The male and female pronuclei meet, and the actomyosin network disassembles. aPAR and pPARs each occupy 50% of the embryo cortex and exclude each other from the membrane. In the posterior, CHIN-1 clusters follow the weaker actomyosin flow (approx. $2 \mu\text{m}^2 \text{s}^{-1}$) towards the anterior where they disassemble, presumably owing to aPKC activity.

in *Drosophila melanogaster* (*Drosophila* from here onwards) oocytes, the 'front' in migrating cells, and the 'apical' domain in epithelial cells and mouse oocyte blastomeres [6–8]. The adaptability of this core PAR module resides in its dynamism. Variant combinations of PAR components and/or modulation by other cell type-specific patterning regulators allow these core polarity effectors to respond to different cues and mediate distinct polarity outputs [9,10].

The asymmetric localization of polarity effectors is produced by a variety of different mechanisms. In some instances, their polarization does not depend on a symmetry breaking cue. In budding yeast, a positive feedback loop, whereby active Cdc42 GTPase at the membrane promotes the recruitment of its own activator (the guanine nucleotide exchange factor, Cdc24), leads to several clusters of Cdc42-GTP (active, GTP-bound). The later specification of a

unique cluster does not seem to rely on a prior polarity cue but on a competition event where the largest cluster becomes the bud by outcompeting the others in the recruitment of polarity factors from the cytoplasm [11].

Conversely, PAR polarity is generally driven by directional cues. For example, PARs can be assembled at a specific site in response to the activation of a signalling cascade. In migrating cells, integrin signalling leads to the local activation of Cdc42 and subsequent enlisting of the aPKC–Par6–Par3 complex to the leading edge of the cell [12]. Similarly, in epithelial cells, PTEN phosphatase activation leads to PIP₂ (phosphatidylinositol-4,5-bisphosphate) enrichment and consequent recruitment of Cdc42, aPKC and Par6 at the developing apical domain [13]. Other means to dock PARs to specific cellular domains involve rearrangements in the cytoskeleton. Par-1 kinase is trapped at the *Drosophila* oocyte posterior in an actin-dependent manner [14], and Par3 is thought to be recruited to the apical domain of outer cells in the eight-cell stage mouse embryo by an apical contractile actomyosin network [15].

The enrichment of PARs to specific cellular domains can also be attained by their active directional transport via motor proteins walking on polarized cytoskeleton structures, such as the localization of Bazooka (Baz, *Drosophila* Par3 homologue) to epithelial adherens junctions by the microtubule (MT) minus-end-directed motor dynein [16] or the transport of Cdc42-enriched vacuoles to the nascent apical domain of endothelial cells during tubule formation [17,18]. Indirect transport or advection that does not involve direct contact of PARs with the underlying cytoskeleton has also been reported. In this review, we will focus on this alternative means of generating PAR asymmetry, which typically involves cortical actomyosin reorganization and bulk cytosolic movements, referred to as cytoplasmic flow or streaming.

Cytoplasmic flows are present in many cell types and occur by a variety of mechanisms, including cell shape changes, osmotic gradients and most frequently by cytoskeletal dynamics at the cell cortex [19–21]. These cytoskeletal dynamics entail the motor-dependent transport of organelles/vesicles along tracks and the long-range movement of cytoskeletal networks, typically of the actomyosin network underlying the membrane (actomyosin cortex) [20]. These movements are transmitted to the cortical cytosol that permeates the cytoskeletal structures, powering bulk movements of the cytoplasm [22]. Cytoplasmic flow was first observed in Characean algae [23] and is a common feature of large eukaryotic cells (greater than 100 µm) such as plant cells [24] and oocytes [21,25,26]. In this context, streaming of the cytoplasm is proposed to aid the transport of metabolites, proteins and other cellular components over distances that would be impossible by simple diffusion, at required timescales [20,27].

However, over the past decade, there has been an appreciation that flow can lead to the generation of organelle and molecular asymmetries within cells [21]. For example, the posterior localization of *oskar* mRNA, key to the patterning of the *Drosophila* oocyte, is achieved by the combined action of directional transport and advection. Kinesin 1-dependent transport on a weakly polarized MT network [28,29] together with cytoplasmic streaming, which promotes the encounter and trapping of cytoplasmic *oskar* mRNAs by the posterior actomyosin cortex [26,30], drive *oskar* mRNA posterior localization. This cytoplasmic streaming is generated by kinesins

and their cargoes dragging the cytoplasm as they walk on cortical MTs attached by their minus-end to the cortex, ultimately leading to the coupling of MT movement and the formation of MT parallel arrays responsible for the long-range cytoplasmic flow [26,31]. A similar mechanism of MT-dependent cytoplasmic streaming exists in the *C. elegans* zygote, but here the alignment of MTs relies on the endoplasmic reticulum [32–34]. This meiotic cytoplasmic streaming (MeiCS) influences the position of the sperm-derived pronucleus (PN) and associated centrosomes (PN/centrosome complex), which will determine the antero-posterior axis (AP axis) of the *C. elegans* embryo [35–38].

In many cases, however, flows are driven by the activity of the actomyosin cortex. Retrograde actin flow in migrating cells is involved in cell movement and leads to the asymmetric accumulation of myosin and polarity regulators along the migration axis [39–45]. The asymmetric position of the meiotic spindle in the mouse oocyte and the male PN in the *C. elegans* zygote (this occurs at a later stage than MeiCS) depends on reverse-fountain-like cytoplasmic streaming. These bulk movements of cytosol arise from the dynamics of the actin cortex underlying the membrane, which lead to a cortical cytoplasmic flow followed by a countercurrent central flow that pushes these nuclear structures close to the cortical site from where the flow originated (figure 1*c,d*, shown for *C. elegans*) [46–50]. Different actomyosin dynamics are responsible for the above-indicated flows; in the mouse oocyte, it is the actin treadmilling along the inner cell surface, in the *C. elegans* zygote it is the contractility of the actomyosin network and in migrating cells it appears that a combination of both may drive cytoplasmic flow (figure 2*a,b*, shown for *C. elegans*). In *C. elegans* zygote asymmetric division, the described cortical cytoplasmic flow also establishes the AP axis of the embryo by driving the anterior localization of PAR-3, PAR-6, aPKC and CDC-42 (Par3, Par6, aPKC and Cdc42 *C. elegans* homologues, also referred to as anterior PARs or aPARs) by advection [36,37,51–57] (figure 1*b,c*). This is arguably one of the best-studied systems, where a comprehensive description of the interplay between flow and PAR polarity has emerged.

Caenorhabditis elegans cytoplasmic flow was first observed in 1960 by Nigon *et al.* [58]. Contractions in the *C. elegans* zygote produced movements in the cytoplasm that appeared necessary to mediate the asymmetric cell division of the zygote. Further investigation revealed that the actomyosin cortex became asymmetric during this first cell division [59–61] and that the observed cytoplasmic flow could be a product of this reorganization of cortical actomyosin [35,60]. Cortical and central cytoplasmic flow speeds were first directly measured by Hird & White, who also proposed that these movements may arise as a result of a gradient of cortical tension within the actomyosin cortex [35,62]. With the advent of live-cell imaging and GFP reporters, Munro and co-workers supported this hypothesis and revealed the cytoskeletal dynamics responsible for the cortical cytoplasmic flow [36,37]. Since its initial description, we have come a long way in our understanding of the biophysical and molecular properties of this actomyosin-dependent cortical flow. Here, we will review the most current work in the *C. elegans* zygote.

Advection of PAR proteins by an actomyosin-dependent cytoplasmic flow (figure 1*b*) was first observed by Munro and co-workers in the *C. elegans* zygote [36,37]. Since initial observation, the establishment of PAR polarity by flow has been proposed in other systems. These include the fly

Figure 2. Regulation of actomyosin-dependent flow. (a) Cortical view of the embryo just after symmetry breaking. The actomyosin network (actin filaments and NMY-2 foci) retracts towards the anterior domain and carries clusters of anterior PARs (orange dots) with it. This retraction to anterior allows posterior PARs (in blue) to load to the posterior domain. (b) Actomyosin flow is associated with anisotropies in cortical tension, which is higher orthogonal to the AP axis in anterior (blue arrows). NMY-2 foci move towards the anterior, along the AP axis (x-axis), but also present chiral displacement—a directional component orthogonal to the AP axis (y-axis). (c) Cortical image of PAR-3 and PAR-2, showing PAR-3 clusters retracted. (d) Myosin contraction can result in the accumulation of components in the actomyosin foci via local recruitment or advective flow (green arrows). (e) Non-muscle myosin II is composed of two heavy chains (NMY-2 in *C. elegans*), two essential light chains (ELC, MLC-5 in *C. elegans*) and two regulatory light chains (RLC, MLC-4 in *C. elegans*). (f) The small GTPase RHO-1 is the main regulator of actomyosin contractility during polarity establishment. RHO-1 can cycle between its active (RHO-1/GTP) and inactive (RHO-1/GDP) states through the action of GAPs (RGA-3/4) and GEFs (ECT-2). In addition, RHO-1 can promote its own activity by an unknown mechanism (looped arrow). RHO-1/GTP can activate its downstream kinase ROCK, which phosphorylates the myosin RLC, increasing its motor activity and promoting the formation of myosin minifilaments, all leading to actomyosin contraction. In addition, RHO-1 can promote F-actin assembly, possibly via formin (CYK-1). These parallel outputs of RHO-1 lead to foci assembly. During foci assembly, enrichment of actin can lead to a delayed recruitment of RGA-3/4 causing foci disassembly, from where the cycle can start again. Therefore, pulses in RHO-1 activity coordinate the cyclical assembly and disassembly of NMY-2 foci.

neuroblast, where it aids the generation of an apical cap of aPKC and Bazooka [63–65], and in mammalian epithelia, where actomyosin contractility promotes apical localization of aPKC–Par6 [66]. In these systems, PARs could couple to the actomyosin flow by advection, as described in *C. elegans*, given that they are embedded in the cytosol influenced by the motion of the contractile actomyosin cortex [10,21,67] (figure 1b, shown for the *C. elegans* zygote). Here, we will review the molecular mechanisms underlying PAR advection by flow as they have been the subject of recent work in *C. elegans*.

The possibility that the PARs could, in turn, affect actomyosin network organization and dynamics was discussed from their very discovery in *C. elegans* [3,68]. It has subsequently become apparent that, as PARs are asymmetrically localized in response to actomyosin dynamics, these polarity effectors can, in turn, modulate properties of the cytoskeleton. For example, in *Drosophila* amnioserosa cells, Bazooka and Par-6/aPKC are recruited to the apical domain by a highly contractile medio-apical actomyosin network, where they concomitantly regulate actomyosin contractility leading to dorsal closure [69–73]. A similar positive feedback loop exists in the *C. elegans* zygote,

whereby anterior PARs become asymmetrically localized through the actomyosin flow that they promote [9,67,74,75].

Recent publications have greatly enhanced our understanding of the interplay that exists between cortical actomyosin flow and patterning mechanisms in the *C. elegans* zygote. In §2, we give an overview of the polarization of the zygote, indicating stages and factors that are critical in the creation of PAR-based asymmetry. In §3, we focus upon aspects of cortical flow generation and regulation. We discuss the identification of regulators of emergent physical behaviours of the actomyosin cortex and the origins and biological relevance of one of these physical behaviours, pulsatility. Section 4 reviews the molecular and physical properties membrane PAR proteins must exhibit to be transported by flow, and the positive feedback PARs have on the flow that transports them. In §5, we discuss the generation of cytoplasmic asymmetries for which the original dogma of flow as the source of these asymmetries has been replaced by reaction–diffusion and phase separation models. In §6, we review the contribution of flow to other key aspects of *C. elegans* zygote asymmetric cell division, in particular centrosome separation leading to a bipolar spindle, and

polarity domain correction during cytokinesis. Given the prevalence of actomyosin flow in the polarization of cells, throughout the review we indicate how research in the *C. elegans* zygote compares with or can inform studies in other systems.

2. Overview of *Caenorhabditis elegans* zygote polarization

PAR-dependent patterning of the one-cell embryo of *C. elegans* defines the AP axis and drives its asymmetric division, critical for the initiation of distinct cell lineages. Prior to polarization, a highly contractile cortex, involving a dynamic network of actin filaments and non-muscle myosin II foci, occupies the entire zygote [35–37,59–61]. Upon fertilization and completion of maternal meiosis, the sperm-donated centrosome induces a local inhibition of actomyosin contractility at the posterior pole of the zygote. This leads to flow of the actomyosin network towards the anterior, leaving a smoothened posterior cortex (akin to placing an elastic band under tension and then cutting one end) [35–37,76,77] (figures 1c,d(ii) and 2a,b). As a result of this actomyosin retraction, cytoplasmic flow is generated; an anterior-directed cortical flow, which subsequently produces countercurrent posterior-directed central flow owing to the fluid-like properties of the cytoplasm and geometry of the zygote [35–37,78] (figure 1c, zygote). Posterior-directed cytoplasmic flow and centrosomal MT asters synergize to position the PN/centrosome complex close to the cortex at the posterior pole, ensuring robust polarization of the zygote [37,46,48,78–82] (figure 1d(ii)). This initiates a mechanochemical-positive feedback loop whereby the signal emanating from the centrosomes promotes actomyosin flow, which maintains centrosome–cortex proximity and strengthens the polarity signal [37,46,48,49,79,82–88].

The anterior-directed cortical flow also transports membrane-associated PAR-3, PAR-6, aPKC and CDC-42 into the anterior [36,37,51–57] (figures 1b,c and 2a–c). aPARs stimulate cortical contractility, further promoting flow and, hence, their own asymmetric localization [36,37,52,54,55,82,89–93]. In this establishment phase of polarity, actomyosin contractility is primarily dependent on the small GTPase RhoA homologue, RHO-1 [77,94,95], the activity of which is regulated by the GEF ECT-2 (guanine nucleotide exchange factor) [77,90–92] and the GAPs (GTPase-activating proteins), RGA-3 and RGA-4 [94–97] (figure 2f). The precise molecular mechanism(s) of how PARs regulate actomyosin flow are still under investigation; however, aPARs have been described to stabilize myosin II at the anterior domain [82] and might regulate RHO-1 via the TAO kinase KIN-18 [98].

Concomitantly to the anterior PAR protein transport, PAR-2, PAR-1 and LGL-1 load to the posterior membrane domain (hence also referred to as posterior PARs or pPARs) (figures 1c and 2a,c). Their loading is reported to be aided by MT asters from the centrosome that protect PAR-2 from aPKC-mediated membrane expulsion [99] (figure 1d(ii)). Anterior and posterior PAR domains are further established through kinase-dependent mutual antagonism. The posterior PARs can be phosphorylated by aPKC, which drives their dissociation from the membrane [93,99–103], and similarly, the PAR-1 kinase, recruited by membrane-associated PAR-2, removes cortical aPARs via phosphorylation of PAR-3 [99,101,104] (figure 1a). Following this ‘establishment phase’ of polarity, the zygote enters a ‘maintenance phase’ during

which large-scale flow ceases and control of actomyosin contractility switches to CDC-42 [90,91]. This small GTPase moves to the anterior alongside the other aPARs and is inhibited at the posterior by its cognate GAP, CHIN-1. In the absence of high-speed flow, mutual antagonism between anterior and posterior PARs plays a major role in ensuring opposing gradients of aPARs and pPARs at the membrane [9,37,51,52,54,89,93,99,101,102,105] (figure 1a,f).

Cortical flow and asymmetrically localized PARs drive downstream events, such as the segregation of cytoplasmic cell fate determinants and mitotic spindle organization and positioning [67,106]. All of this will lead to an essential asymmetric division that generates two daughter cells, the somatic AB cell and the germline P1 precursor [107] (figure 4).

3. The anterior-directed cortical flow that polarizes the *Caenorhabditis elegans* zygote

The transport of PAR proteins by an actomyosin-directed cortical flow was first described in the *C. elegans* zygote by Munro and co-workers in 2004 [36,37,51] (figures 1b,c and 2a–c). In this seminal work, the authors found that the high contractility of the actomyosin cortex, leading to strong membrane ruffling (including a pseudocleavage furrow) and the generation of flow, is dependent on the motor ability of non-muscle myosin II (*C. elegans* non-muscle myosin heavy chain, NMY-2), with cortical flow being abrogated upon depletion of the regulatory myosin light chain (RLC, MLC-4 in *C. elegans*) (figure 2a,d,e). The authors also supported a previous hypothesis, indicating that the cortex is under tension [19,35,62], as they observed that contractions between neighbouring NMY-2 foci were mechanically coupled. In this scenario, after fertilization, the local reduction of NMY-2 foci at the posterior pole would produce an imbalance in cortical tension and cause the actomyosin network to retract towards the opposite pole [35,37]. The actomyosin flow presented as a collective movement of actin filaments and NMY-2 foci towards the anterior of the zygote, with foci speed of approximately $7 \mu\text{m min}^{-1}$ (figure 2a,b). Since initial description, cortical flow in the zygote has been extensively studied and we now know that flow is indeed associated with anisotropies in cortical tension in the anterior domain, where tension is higher orthogonal to the AP axis [76] (figure 2b, blue arrows). In addition, the gradient in actomyosin contractility, caused by the reduction of NMY-2 at the posterior, supports a long-range flow thanks to the viscosity of the cortex, which presents fluid-like properties constantly contracting, rearranging and turning over (hydrodynamic properties). These physical qualities allow the force of a single contraction to influence the network over a significant distance (approx. $14 \mu\text{m}$) within the zygote (total length approx. $45 \mu\text{m}$) [76]. Flow also presents an orthogonal component to the AP axis, leading to chiral counter-rotatory flow (figure 2b) that reflects the molecular torque properties of actomyosin (counter-rotation of cross-linked actin filaments). There is no role described for this chiral flow in the zygote; however, similar chiral flows drive embryo left–right asymmetry at a later stage (four- to six-cell stage) [108,109]. Another emergent large-scale mechanical property of this actomyosin flow is its pulsatile nature, which might emerge from the regular cyclic assembly and disassembly

of NMY-2 foci during flow. This property has been the focus of recent studies reviewed here [94,95].

From a molecular point of view, the organization and contractility of the actomyosin cortex is known to be regulated by the small GTPases RHO-1 in polarity establishment [77,94,95] and CDC-42 in maintenance, although a role in establishment has also been reported [37,52,90,91]. These GTPases cycle between active GTP-bound and inactive GDP-bound states, modulated by their GEFs and GAPs [52,77,90–92,94–97]. The GTP-bound state of the RHO-1 homologue, RhoA, is known to exert its effects through the recruitment and/or activation of formins, anillin and Rho-associated protein kinase (ROCK) [110,111]. Active ROCK stimulates myosin II motor activity and facilitates minifilament assembly and activity by phosphorylating the myosin regulatory light chain and inhibiting myosin phosphatase [112] (figure 2f). In the *C. elegans* zygote, depletion of the ROCK homologue, LET-502, leads to defects in actomyosin organization and flow [94]. During maintenance, CDC-42-dependent activation of non-muscle myosin II is driven by the kinase MRCK-1 (myotonic dystrophy-related CDC-42-binding kinase homologue) [52,54]. In addition, several actin-binding proteins (ABPs) have been implicated in the organization of this cortex [113], including F-actin-polymerizing (profilin PFN-1, formin CYK-1, ARX-2/3) [114–116], -cross-linking (anillin, ANI-1/2) [117–119] and -bundling (plastin, PLST-1) [120,121] proteins.

Here, we will review recent studies that have begun to illuminate how known and newly identified regulators contribute to large-scale biophysical properties of the actomyosin flow, showing a molecular degeneracy where multiple molecular activities modulate each property [120,122]. Therefore, the gradient of actomyosin contractility responsible for this patterning flow could be shaped by various factors, making it difficult to identify the polarity cue(s) that trigger this cortical flow. Recent publications debate the exact molecular nature of the polarity cue and mechanisms underlying the polarization of the zygote.

(a) Aurora A regulates actomyosin flow and ensures a monopolar antero-posterior axis

For over 20 years, evidence has supported that the sperm-derived centrosomes produce a cue that inhibits local contractility of the actomyosin cortex at the presumptive posterior pole, leading to the actomyosin flow that polarizes the zygote [36,37,46,48,51,79,123] (figure 1d(ii)). However, the nature of this polarity cue has remained elusive until recently [83,84,88] in part owing to polarity being dependent on centrosomal maturation [124–129] and on MTs organized by the centrosomes [46,48,80,92,99,130,131]. *Caenorhabditis elegans* zygote polarization also relies on several feedback loops, so it is difficult to pin-point where the polarity cue is acting. For example, both sets of PARs mutually antagonize each other to occupy and define their respective domains [9,67,74] (figure 1a,c), and the anterior PARs promote the actomyosin flow that transports them [9,67,74,75] (figures 1c and 2a).

Recent theory observations indicate that, in the early zygote, polarization feedback loops remain subcritical to avoid spontaneous pattern formation [82]. Hence, the system will only polarize in response to a guidance cue that will strongly promote instability in the patterning feedback loops. For example, the symmetry breaking cue initiates a flow that is stronger than required to transport the aPARs to an opposing domain from which they can stably antagonize their posterior

counterparts [82]. In agreement with a strong guidance cue being needed to start PAR asymmetry, it has been observed that centrosomes can signal from a distance (greater than 10 μm), but polarization is faster and more robust if centrosomes are closer to the cell cortex [48,85–87] (figure 1d(ii)). This led to the idea of a diffusive polarization cue that emanates from the centrosomes and promotes the downregulation of NMY-2 at the posterior pole, initiating the contractile imbalance that will drive flow and subsequent asymmetric localization of PARs.

Recent publications have reactivated discussion on the cue's definitive molecular nature and function, each proposing that the Aurora A kinase homologue, AIR-1, ensures a monopolar AP axis independent of its role in both centrosome maturation and MT organization [83,84,88,132].

The kinase's spatio-temporal regulation appears to be tightly controlled before and during zygote polarization. Prior to symmetry breaking, AIR-1 is associated with MTs at the cortex and diffusely distributed in the cytoplasm [83], from where it suppresses cortical actomyosin contractility globally [83,88]. AIR-1 is subsequently recruited to the pericentriolar material, where it is required for full MT organizing activity of centrosomes [83,125,133,134] and for zygote polarity (figure 1d(ii)). From this location, it is proposed that AIR-1 forms a diffusive gradient reaching the posterior pole of the zygote, where it robustly targets polarization via local inhibition of actomyosin contractility and/or loading of PAR-2 to the membrane [83,84,88] (figure 1d(ii)). During polarity maintenance, AIR-1 becomes cytoplasmic once more and aids global disassembly of the actomyosin network, suppressing later centrosome-independent polarization mechanisms [84]. Interestingly, for all these functions, AIR-1 localization to MTs is not required, unlike at a later stage of mitosis where TPXL-1 recruits AIR-1 to astral MTs, from where the kinase inhibits polar actomyosin contractility [135].

Intriguingly, knockdown of *air-1* does not lead to loss of polarity as would be expected from the symmetry breaking cue. Instead, it causes a previously reported bipolar phenotype, where PAR-2 is aberrantly localized at both poles of the zygote [129,133], and weak cortical flows are directed towards the embryo centre from the poles [83]. This bipolar phenotype could derive from events in the oocyte, before fertilization has even occurred. During ovulation, AIR-1 and PLK-1 (Polo-like kinase) prevent premature polarization, by regulating the timing of aPAR membrane loading [132]. This is presumably due to PAR-3 phosphorylation by PLK-1, which could prevent PAR-3 membrane loading, as observed at a later stage in the zygote [55]. Binding of PAR-3 to the oocyte membrane therefore initiates a PAR maturation cycle that takes 20–30 min to complete [132]. Upon maturation, the PAR system becomes sensitive to symmetry breaking cues, which, in wild-type embryos, permits a timely response to the correct guidance cue of the posterior centrosomes. The removal of AIR-1 or PLK-1 causes the PAR system to mature early and become sensitive to spontaneous, cryptic or maternal symmetry breaking cues, producing the reverse and bipolar phenotypes observed in AIR-1 depletion [83,84,88,132].

The penetrance of the PAR-2 bipolar phenotype versus a complete reverse polarity, where PAR-2 is only in the anterior pole, varies between each study (bipolarity favoured in [83,88,132], whereas reverse polarity in [84]), possibly owing to different knockdown strengths or reporter strains used, leading to some discrepancies. The reverse anterior polarity

observed by Zhao *et al.*, upon *air-1* RNAi, led the authors to further support the requirement of centrosomal AIR-1 to mediate local inhibition of actomyosin contractility at the posterior pole of the zygote [84]. Upon AIR-1 depletion, Klinkert *et al.* mostly observed bipolar recruitment of PAR-2 that does not require cortical flow or MTs [83]. In addition, PAR-2 always associates with the membrane at the poles, independent of where the centrosomes contact the cortex, possibly owing to increased membrane curvature permitting geometry-dependent PAR-2 recruitment/stabilization (as observed in zygotes grown in micro-patterns [83]). The authors postulate that the cortical and cytoplasmic pools of AIR-1, prior to the kinase's recruitment to the centrosome, protect the zygote from spontaneous symmetry breaking through PAR-2 membrane recruitment at high-curvature sites [83]. This is different from what was proposed by Kapoor & Kotak [88], who observed that PAR-2 bipolarity depends on actomyosin contractility, and hence postulated that AIR-1 global suppression of actomyosin contractility prevents early ectopic polarization (anterior PAR-2 localization) by flow.

The multipolarity observed in AIR-1 loss of function is reminiscent of the capacity of certain cells to spontaneously polarize in random orientations in the absence of tight regulation from a specific guidance cue(s) [136]. Overall, AIR-1 could protect the zygote from inappropriate symmetry breaking by promoting the subcritical state of polarization feedback loops proposed by Gross *et al.* [82]. Furthermore, by supporting symmetry breaking in response to the sperm centrosome, AIR-1 helps establish the correct monopolar axis. These studies and the molecular degeneracy of the actomyosin cortex [120,122] (discussed below) indicate that the polarization of the zygote most likely relies on the timely and spatial coordination of multiple molecular processes. This is further exemplified by a recent finding from De Henau *et al.* [137], who have reported a novel mechanism/requirement for symmetry breaking. Here, high levels of H₂O₂, released from a polarized mitochondrial network, can drive posteriorization of the zygote. All the above indicate that a single guidance cue being responsible for the polarization of the zygote is unlikely; instead, several cues may regulate the different molecular functions required for timely symmetry breaking, as indicated in these AIR-1 studies.

It is still unclear how AIR-1 exerts its regulatory capacity. Given the tight feedback loops that exist between PARs and the actomyosin cytoskeleton in the polarization of the zygote [9,67,74,75], it is currently hard to determine if AIR-1 is directly targeting PARs (for example, by influencing aPAR membrane loading) or cortical contractility, or both. AIR-1 phosphorylates PARs and actomyosin regulators in other systems. In *Drosophila*, Aurora A inhibits ROCK [138], activates the aPAR complex by phosphorylation of Par-6 at a conserved Aurora site [139], and releases Lgl from the membrane through phosphorylation [140,141]. It would therefore be interesting to determine whether Aurora A plays a universal role in controlling the timing of response of the PAR network and/or influencing the anisotropies in cortical tension to ensure monopolarity in other systems.

(b) Large-scale actomyosin behaviours can be attained by modulating diverse molecular functions

Over a hundred proteins are proposed to modulate cortical dynamics in other systems [142,143], yet for most it is unknown

how they influence large-scale biophysical properties of actomyosin flow. Recently, unbiased methods have been used to assign known and novel cortical regulators to these properties of flow in the *C. elegans* zygote [120,122]. Candidates were selected from high-throughput genetic screens where knock-down altered the dynamic properties of the actomyosin cortex [120,122]. This identified enhancers and suppressors of NMY-2, LET-502 (ROCK), MEL-11 (myosin phosphatase) and ACT-2 (actin) function [120]. Sixty-five putative regulators were found, among them genes encoding known actomyosin modulators (e.g. myosin chaperone UNC-45, WASP-interacting protein WIP-1 and myosin light chain MLC-5) and ABPs (plastin PLST-1, ezrin ERM-1) from other systems, indicating that the identified proteins are valid candidates for cortical regulation. Eleven selected hits were studied in more detail to assess their involvement in actomyosin organization and dynamics. Flow velocities (both along the AP axis—AP flow, and orthogonal to the AP axis—chiral flow, figure 2b), flow pulsatility, hydrodynamic length (as a proxy of cortex viscosity) and density/size of NMY-2 foci in the zygote were measured following knockdown. This analysis was done in parallel with 33 ABPs [120,122].

Altogether, this implicated 14 new regulators (eight identified in these screens) of cortical actomyosin dynamics during AP axis establishment. These proteins clustered into functional groups, each containing effectors modulating diverse molecular mechanisms [122]. For example, chiral counter-rotatory flow (figure 2b) was found to be regulated by two upstream regulators of RHO-1 (the GAP, RGA-3, and the casein kinase 1, CSNK-1) and two ABPs, an actin membrane cross-linker (annexin, NEX-1) and an inhibitor of myosin ATPase activity (calponin, CPN-1). This suggests that this diverse set of proteins regulates the molecular torque properties of actomyosin, leading to cell-scale changes where the cortex of the zygote twists along the AP axis [108]. As another example, pulsatility of cortical flow is modulated by regulators of actin filament turnover, such as F-actin-polymerizing (PFN-1, CYK-1 and ARX-2), -severing (UNC-60 and FLI-1) and -capping (CAP-1) proteins. This requirement of cortical turnover for pulsation is also supported in more detailed studies [94,95], reviewed in the later section on pulsatility. For the zygote to have such a diverse set of proteins, each with distinct molecular functions, regulating the same large-scale physical property (degenerate functioning) is extremely advantageous, as it provides robustness to the underlying morphogenetic process.

Recent single gene studies of actomyosin regulators identified in these screens [120,122] have subsequently deepened understanding of cortical regulatory processes. The cross-linker PLST-1, which regulates AP flow velocity, has been proposed to increase the connectivity of the actomyosin network facilitating coordinated and persistent cortical flow [121]. Optimal levels of cross-linkers might provide the correct connectivity to propagate tension and allow long-range flow [121,143]. A similar effect has been described during cytokinesis, where above or below optimal levels of anillin and NMY-2 can be detrimental to ring closure [144]. Another AP flow velocity regulator, the septin UNC-59, is asymmetrically localized in the zygote (downstream of PAR signalling) and can also regulate cytokinesis [145,146]. The chiral flow regulator casein kinase, CSNK-1, is also necessary for NMY-2 foci disassembly in meiosis through inhibition of the RHO-1 pathway [147] and for the

rotational cortical flow required for cytokinesis under mechanical stress [148].

Unbiased screens combined with detailed phenotypic analyses of actomyosin dynamics have proved to be a successful strategy to identify proteins underlying long-range flow and properties of the cortex such as pulsatility. The future challenge will be to mechanistically decipher how the combined function of these proteins leads to cellular-scale dynamic behaviours of the cortex, bridging the gap between molecular- and cellular-scale processes. Synthetic biology approaches, studying minimal systems, are beginning to address these challenges [75,149,150]. Approaches using super-resolution imaging in combination with methods that manipulate the function and location of the proteins *in vivo* (i.e. optogenetics, temperature-sensitive mutants and molecule-trapping techniques) will greatly assist progress in this field [151–153].

(c) Organized actomyosin pulsatility leads to an efficient flow

Pulsatility is an intrinsic feature of highly contractile actomyosin networks, documented in a wide variety of developmental processes [37,70,154–159]. In the *C. elegans* zygote, NMY-2 foci pulse with an intensity that increases and decreases cyclically over a period of approximately 30 s [94,95,122]. The network is responsive to these pulses, and flow velocity exhibits temporal fluctuations accordingly [94,122] (figure 2*a,d,f*). Currently, there are two major streams of thought on how actomyosin pulsatility is achieved in single cells, with RhoA playing a central role in both models [160–162]. However, the proposed requirement of RhoA is different for the two models. In the first, RhoA is recruited by NMY-2 contraction as part of a mechanochemical feedback, whereas in the second, cyclic activity of RhoA orchestrates the actomyosin pulses [163].

The mechanochemical feedback model is described in *Drosophila* embryonic cells [160,162]. Here, increased apical non-muscle myosin II contractility produces an advective enrichment of both non-muscle myosin II and upstream positive regulators (RhoA pathway), which promote further contraction (figure 2*d*). Parallel accrual of actin filaments and negative regulators of myosin II activity (myosin phosphatase), by the advective flow, decreases contractile ability and thus advection. This delayed negative feedback promotes dissipation of the foci, allowing the cycle to start again.

Contrary to this mechanochemical system that is driven by the stochastic upregulation of myosin II contractility, in the *C. elegans* zygote the cyclical activity of the RhoA homologue, RHO-1, sits upstream and is responsible for the coordination of NMY-2 pulses [94,95] (figure 2*f*). RHO-1 promotes its own activity, leading to a rapid accumulation of active RHO-1 that is necessary for pulsation [95]. These fluctuations in RHO-1 activity temporally precede those of NMY-2 and are not dependent on contractility of the motor protein [94,95]. Furthermore, single-molecule tracking shows that the enrichment of NMY-2 and actin to contractile foci is mediated by the modulation of local assembly/disassembly, with little contribution from advection [95]. The pulse is then terminated through the delayed actin-dependent recruitment of the GAPs, RGA-3 and RGA-4 [95] (figure 2*f*). Interestingly, zygotes with aberrant spatio-temporal oscillation of RHO-1 activity still have anterior-directed cortical flow, yet strong fluctuation and irregular behaviours in the actomyosin disrupt the normal

establishment of polarity along the AP axis. Thereby, the pulsatile behaviour of RHO-1 activity acts as a pacemaker of contractile instabilities of the actomyosin cortex, defining the spatio-temporal oscillation pattern of cortical actomyosin foci assembly–disassembly [94,95].

We are beginning to understand the mechanisms underlying this mode of contraction and its biological relevance beyond the context of a single cell [164]. The *Drosophila* embryo represents the best-studied system for tissue-scale pulsatility. In this model, distinct tissue folding events such as dorsal closure [69–73], ventral furrow formation [154,160,165–167] and germband extension [155,162,168,169] all use the pulsatile nature of actomyosin networks to coordinate morphogenesis. For example, in formation of the ventral furrow the combination of pulsatility, which leads to stimulation of contractions in neighbouring cells [165], and a tissue-scale actomyosin contractility gradient [166] facilitates correct tissue folding. It has recently been proposed that actomyosin pulses may also contribute to tissue integrity, accommodating rapid cell and tissue deformations in different systems [159,160,164,170,171]. It would be interesting to determine if RhoA also holds a pacemaker role in other systems and at a tissue scale.

4. PAR protein advection by actomyosin-dependent flow

Cortical actomyosin flow is able to transport certain membrane-bound proteins to the anterior of the *C. elegans* zygote, facilitating their asymmetric distribution (figures 1*c* and 2*a*). Given that not all membrane proteins are segregated by retraction of the actomyosin network, how then do certain proteins couple to flow?

Membrane proteins may be physically bound to the underlying cortex and are then ‘dragged’ through the membrane. An example of this in *C. elegans* is the E-cadherin homologue HMR-1 [172]. Cadherins are canonically known for their role in cell–cell contacts, yet during polarity establishment HMR-1 forms clusters that are dragged to the anterior domain by actin filaments [37,172]. These clusters decrease actomyosin flow velocity as they create a frictional component, with HMR-1 acting as a ‘picket fence’ against the movement of the cortex [172].

Anterior PAR proteins, on the other hand, show a similar directed movement yet in the absence of physical interactions with the actomyosin cytoskeleton [36,37,51–57] (figure 1*b*). PAR proteins move freely on the membrane by lateral diffusion (diffusion coefficient, D_c , in the order of $0.1 \mu\text{m}^2 \text{s}^{-1}$) [53,105,173]. Here, they exchange at different rates of binding and unbinding with the cytoplasmic pool, in which their diffusion (random movement) is an order of magnitude faster [36,105,173,174] (figure 1*b*). Once aPARs start to become segregated, there are several mechanisms in place that maintain their asymmetry: the antagonism of pPARs, which begin to accumulate in the posterior membrane as aPARs are cleared [9,67,74] (see §2, figure 1*a,c,f*), and the spatially differential binding and unbinding rates of PARs from the newly defined anterior and posterior membrane domains [173,174]. For example, PAR-6 is recruited to the membrane approximately seven times faster at the anterior in agreement with the enrichment of several binding partners in this domain, while dissociation rates of PAR-6 are uniform [173]. But

how are aPARs initially segregated? Their movement needs to be somehow coupled to the actomyosin flow triggered by the polarity cue [36,37,53] (figure 1*c,d*). The physical mechanism proposed is that shear stress transmits the bulk movement of the actomyosin cortex to the nearby cytoplasm, creating an advective cytoplasmic flow. This is possible given the hydrodynamic properties (liquid-like behaviour, viscosity) of both elements (actomyosin cortex and cytoplasm) [21,22]. PAR proteins attached to the membrane and embedded in this cytoplasm will be advected by this flow (approx. $7 \mu\text{m min}^{-1}$) as long as their exchange rate with the cytoplasm and lateral diffusion are slow enough (membrane lifetime approx. 100 s and D_c in the order of $0.001 \mu\text{m}^2 \text{s}^{-1}$) [53,173]. In this way, PARs will remain on the membrane long enough to be transported by the flow and their directional movement will surpass their free membrane diffusion, producing their anterior asymmetric localization [21,53]. However, the molecular determinants that allow advection by flow have, until recently, been largely unclear.

(a) PAR proteins sense flow by clustering

Recent work has proposed that the mechanism which allows the anterior scaffolding protein PAR-3 to couple to cortical flow is its ability to form clusters [54–57] (figures 1*b,c* and 2*a,c*). PAR-3 oligomerization, through its N-terminal CR1 domain, has been reported to mediate PAR-3 membrane localization and clustering in a wide range of polarizing systems [55–57,175–181]. In *C. elegans*, disruption of PAR-3 oligomerization strongly decreases PAR-3 protein levels at the membrane [55–57,178]. Forcing a PAR-3 mutant that cannot oligomerize to the membrane is insufficient to restore PAR-3 asymmetric localization by flow [56]. This indicates that clustering not only stabilizes PAR-3 at the membrane (increased residency time), but also reduces its capability to move freely on the membrane (reduced lateral diffusion). These two outcomes of PAR-3 clustering are in agreement with single particle tracking observations, where larger clusters remain associated with the membrane longer and are more efficiently transported by cortical flow to the anterior of the zygote [55]. PAR-3 clusters have a total diffusive length of less than $3 \mu\text{m}$ in the plane of the membrane [55] and a low diffusion coefficient of approximately $0.008 \mu\text{m}^2 \text{s}^{-1}$ (inferred from subdiffusive PAR-6), which fulfils advection requirements [53,173]. It is not known exactly how clustering stabilizes PAR-3 at the membrane, but we can speculate that the coalescence of multiple membrane-binding domains could synergize to increase avidity for the membrane [182], and in a similar way, multiple membrane contact sites might increase resistance and prevent the lateral diffusion of the cluster. In addition, cluster size alone may also restrict diffusion or alter the ability of clusters to associate with or be corralled by features in the membrane or the cortex. More work is needed to dissect the precise mechanistic basis of flow-sensing by clusters.

PAR-3 clustering also promotes the recruitment of PAR-6, allowing the coupling of the PAR-6/aPKC heterodimer to the advective flow sensed by PAR-3 during establishment [55] (figures 1*b,c* and 2*a*). At the membrane, the PAR-6/aPKC heterodimer exists in two states: a clustered state bound to PAR-3 that follows advective flow and a diffuse state that is associated with CDC-42 [54,56,57,173,183] (figure 1*b*). Both of these PAR-6/aPKC membrane states

become asymmetrically localized to the anterior of the zygote during polarity establishment. The model proposed for total PAR-6/aPKC to tap into flow is dynamic switching between these two membrane states. Transient interaction between flow-sensitive PAR-3 and flow-insensitive CDC-42 during PAR-6/aPKC transfer may facilitate the asymmetric localization of all parties even though only the PAR-3-associated state can couple to flow [56] (figure 1*b*). In addition, both clustered and diffusive populations are required for correct spatio-temporal regulation of kinase activity. aPKC phosphorylates downstream targets when in complex with PAR-6 and CDC-42 [56,91,183–186]. Therefore, the ability to dynamically transfer between PAR-3 clusters, which sense flow, and the diffusive CDC-42 assembly, which permits kinase activity, produces the anterior enrichment and specific spatial activation of aPKC [56] (figure 1*a–c*).

The clustering ability of PAR-3 also exhibits tight spatio-temporal modulation. During polarity establishment increased cortical tension, generated by actomyosin contractility, stimulates PAR-3 oligomerization, therefore promoting PAR-3 advection to the anterior [57]. Furthermore, cluster formation is linked to the cell cycle, as active PLK-1 can phosphorylate PAR-3 at its N-terminus, inhibiting oligomerization. The periodic activity of PLK-1 throughout the cell cycle can therefore regulate membrane loading, clustering and thus advective transport of PAR-3 [55,132]. In establishment, lack of PLK-1 activity allows PAR-3 clustering and hence anterior advection, whereas in mitosis (during polarity maintenance) temporally activated PLK-1 inhibits oligomerization, preventing the disruption of PAR-3 asymmetry by later flows [55].

Posterior PAR proteins also form clusters in the zygote. PAR-2 forms an oligomeric gradient across the AP axis, with larger oligomers (up to tetramers) enriched at the posterior cortex [174]. However, tetrameric PAR-2 stoichiometry is much smaller than the stoichiometry reported for PAR-3 clusters (up to 15mers in establishment) [55]. This could explain why PAR-2 motion at the membrane does not present a directional movement that could alter its asymmetric localization. Instead, cortical PAR-2 asymmetry is mostly mediated by local exchange reactions dependent on aPKC, which promote higher dissociation from the anterior and recruitment at the posterior membrane domains [105,174].

The CDC-42 GAP, CHIN-1 [52], forms clusters at the cortex during polarity maintenance phase [54] and is presumed to be restricted to the posterior by aPKC [52,54,56] (figure 1*a,f*). CHIN-1 clusters can be tracked on the cortex for longer periods than PAR-3 clusters (greater than 100 s), suggesting that its oligomerization results in very stable association with the membrane [54]. This agrees with the strong coupling observed for CHIN-1 clusters to the weaker anterior-directed flow that occurs in maintenance (approx. $2 \mu\text{m min}^{-1}$) compared with establishment (approx. $7 \mu\text{m min}^{-1}$). Clusters switch from growth to decay as they approach the anterior domain of the zygote. Here, at a threshold density of PAR-6/aPKC, they uncouple from flow and drop from the membrane [54]. More importantly, modelling supports that anterior PAR-3 and posterior CHIN-1 clusters, when coupled to flow and with a cluster growth ultrasensitive to antagonism by PAR-1 and PAR-6/aPKC, respectively, provide the anterior–posterior cross-inhibitory circuits (figure 1*a*) with bistable dynamics (two stable equilibrium states). This allows robust formation of PAR cortical asymmetries [54] (figure 1*c,f*, graphs).

The above are examples of mechanochemical events, where the regulation of protein clustering can determine whether a protein couples to flow. Given the existence of similar cortical flows [63,65,66] and clusters of polarity determinants [181,187–190] in other contexts, the described mechanisms here in *C. elegans* could account for an emerging patterning system, where cells can modulate their polarity programmes through the spatio-temporal regulation of dynamic clusters.

(b) Central cytoplasmic flow can contribute to the generation of PAR asymmetries

Concomitantly to the anterior-directed cortical actomyosin flow, a central cytoplasmic flow arises, both driving recirculation of particles [78,79,81] (figure 1c). This central cytoplasmic flow (approx. $5 \mu\text{m min}^{-1}$) also depends on the contractility of the cortex. The shear forces generated by NMY-2 at the cortex could be transmitted towards the central cytoplasmic region owing to the hydrodynamic properties of the cytoplasm [78], which behaves as an incompressible fluid; hence, anterior-directed cortical flow in the confines of the zygote leads to a countercurrent posterior-directed central flow [78]. Cytoplasmic flow in the zygote was thought to drive the posterior localization of certain cytoplasmic components [35,36,191,192]. However, as for cortical flow, proteins in the cytoplasm will need to present certain dynamic properties that will grant them sensitivity to cytoplasmic flow (further discussed in §5).

A novel non-invasive technique has permitted the study of cytoplasmic flow alterations, giving opportunity to further explore flow properties and their effects in the zygote. Mittasch *et al.* [81] employed focused-light-induced cytoplasmic streaming (FLUCS), where an infrared laser beam induces temperature-travelling fields in the cytoplasm that can lead to flows [193]. Laser scan patterns permit the modulation of both the speed and direction of the intracellular flows [81]. Surprisingly, increasing cytoplasmic flow speed perpendicular to the posterior PAR-2 domain to twice the velocity observed in wild-type zygotes resulted in a threefold increase of PAR-2 loading at the membrane [81]. Flows could increase the volume of cytoplasm scanned by a domain with docking properties, and in this way increase loading/trapping of a highly diffusive molecule. This scenario has been proposed for the enrichment of mRNA- and RNA-binding proteins at the posterior pole of the *Drosophila* oocyte [30,194,195]. Furthermore, generating clockwise rotational cortical flow, by FLUCS, can rotate both the PAR-2 domain and the actomyosin cortex [81]. The complete reverse polarity of PAR-2 can also be generated, but only if the domain is rotated over 90° past its usual posterior alignment, highlighting the robustness of cortical polarization against abnormal rotational forces. This work has activated research in a previously underappreciated process. However, further studies will need to elucidate the degree of contribution of central cytoplasmic flow to the polarization of the wild-type zygote.

(c) PAR proteins regulate the actomyosin flow that transports them

PAR proteins have been observed to regulate the actomyosin cytoskeleton in varied cellular contexts. For example, Bazooka is required for actin cortex integrity in the *Drosophila* oocyte [196], aPKC promotes RhoA degradation in migrating cells

[197] and Par3 suppresses Rac GTPase activity and controls the organization of the actin cytoskeleton to stabilize nascent tight junctions in epithelial cells [198]. In the *C. elegans* zygote, a tight mechanochemical feedback exists between PAR proteins and the actomyosin cortex [9,67,74,75].

Anterior PARs are transported by the cortical flow [36,37,51–57] yet also contribute to the flow by modulation of actomyosin [36,37,52,54,82,89–93]. Depletion of either PAR-3 or CDC-42 severely reduces flow and the clearance of the RHO-1/GEF, ECT-2, from the posterior at symmetry break [37,90]. Here, the observed effects are not due to an expansion of the posterior PAR domain, as simultaneous knockdown of PAR-2 in PAR-3-depleted embryos does not change the PAR-3 phenotype [37]. In agreement with pPARs not having an early effect on flow, zygotes depleted of PAR-2 show a normal ECT-2 clearance from the posterior pole [90].

A recent publication indicates more specifically how anterior PARs can regulate actomyosin flow [82]. FRAP (fluorescence recovery after photobleaching) and fluorescence-based image quantification revealed that the NMY-2 association rate to the cortex is similar in the anterior and posterior PAR domains (anterior $k_{\text{on}} = 0.19 \mu\text{m s}^{-1}$, posterior $k_{\text{on}} = 0.21 \mu\text{m s}^{-1}$). However, the dissociation rate in the anterior is two times slower than in the posterior (anterior $k_{\text{diss}} = 0.072 \text{ s}^{-1}$, posterior $k_{\text{diss}} = 0.14 \text{ s}^{-1}$) [82]. aPARs seem to stabilize cortical NMY-2 and mediate their effect independently of pPARs, as knockdown of *par-6* and *par-2* produces the same dissociation rate as that observed in the wild-type posterior domain.

The posterior PARs, PAR-2 and LGL-1, which show no direct role in early establishment, might prevent the recruitment and/or stabilization of NMY-2 at the posterior cortex during polarity maintenance [37,89,93]. In agreement with this, cortical NMY-2 levels are downregulated in *cdc-42* RNAi-treated zygotes, where PAR-2 becomes strongly localized over the whole cortex [90,91]. However, it is likely that CDC-42 could also be regulating NMY-2 levels/function independently of pPARs, via the kinase MRCK-1, at this later stage of zygote polarization [52,54]. Supporting this idea, loss of posterior PARs leads to ectopic posterior-directed cortical flow during the polarity maintenance phase that can be rescued by inhibiting non-muscle myosin II contractility through depletion of MRCK-1 [37,54,93].

Mechanochemical-positive feedback between PARs and actomyosin has been described in other systems and, as in the zygote, it promotes robustness of the patterning process [69–73]. The identification of the molecular mechanisms involved will greatly impact developmental research, most immediately of polarizing systems where cooperative regulations between flow and cortical polarity are emerging, such as in the *Drosophila* neuroblast [65,199,200].

5. PAR polarity leads to cytoplasmic asymmetries through phase separation and reaction–diffusion mechanisms

Concomitantly to the establishment of the zygote cortical polarization, the asymmetric localization of cytoplasmic determinants contributes to cell fate specification. As described above, the anterior-directed cortical flow is critical for the production of cortical PAR asymmetry and generates a countercurrent

posterior-directed cytoplasmic flow [78,79,81] (figure 1c) that was initially thought to drive the posterior localization of certain cytoplasmic components [35,36,191,192]. However, recent studies indicate that gradients of several proteins and RNA/protein assemblies are not reliant on this backflow. Instead, they depend more directly on PAR asymmetry and on intrinsic physical properties of the cytoplasmic proteins themselves, such as their diffusion coefficients or capacity to phase separate from the cytoplasm [192,201–207]. Below we will discuss these mechanisms, focusing on the segregation of germ granules and RNA/protein assemblies in the zygote.

(a) Spatial regulation of phase separation drives P granule asymmetry supporting germline specification

The zygote divides asymmetrically along the AP axis into an anterior daughter cell, which will give rise to the worm's somatic tissues, and a posterior germline blastomere [107]. This cell stems the immortal germ lineage that will generate sperm and oocytes [208]. Embryonic germ cell fate specification is supported by inheritance of germ granules, known as P granules in *C. elegans*, that contain RNA and RNA-binding proteins [209,210]. P granules are non-membrane-bound cellular compartments that share similar properties with the P bodies and stress granules of somatic cells [211]. They were among the first cytoplasmic components described to de-mix from their environment (the cytoplasm) through liquid–liquid phase separation (LLPS) [192,201]. Individual components of P granules can dynamically exchange between condensed (granule) and dilute (cytoplasm) liquid phases. Interestingly, it is through the regulation of P granule condensation/dissolution and not through advective transport by cytoplasmic flow that P granules accumulate at the posterior pole of the zygote after polarity is established [201].

Individual granule components diffuse freely and become incorporated into P granules preferentially at the posterior (figure 3a). The separation into the condensed granule phase by LLPS causes the concentration of each individual component to become lower in the surrounding posterior cytoplasm. P granules act as a sink, promoting the posterior-directed flux of P granule constituents from the anterior. Through this AP flux, P granule components continue to condense and become incorporated into growing granules. These larger assemblies diffuse slowly and stabilize at the posterior. This strategy could allow rapid and efficient sorting of complex mixtures in cells without requiring active transport [67,201].

As granule formation reduces the entropy state of the cytoplasm, phase separation is likely driven by energetically favoured interactions between condensate components [214]. Out of the dozens of proteins present in P granules [215], MEG-3, PGL-1, its paralogue PGL-3 and the DDX3 RNA helicase LAF-1 are considered to be the main drivers of phase separation and act as scaffolding proteins [201–203,207,216,217]. All contain RNA-binding and intrinsically disordered domains, a combination of motifs canonically implicated in phase separation of RNA/protein assemblies [214]. By replicating physiological conditions *in vitro*, PGL-3, LAF-1 and MEG-3 were observed to phase separate into droplets. RNA enhances the phase separation of these proteins, both *in vitro* and *in vivo* [202,203,217] (figure 3a).

Upstream polarity signalling controls the LLPS of granules by dynamically regulating accessibility of RNAs to the P granule scaffolding proteins [202,203]. The PAR-1 kinase promotes an anteriorly enriched cytoplasmic gradient of the MEX-5 RNA-binding protein that, in turn, enforces P granule asymmetry [192,201,212,218–221] (figure 3a,c). MEX-5 outcompetes PGL-3 and MEG-3 for RNA-binding *in vitro*, decreasing the ability of the P granule proteins to undergo RNA-stimulated phase separation. *In vivo* experiments and kinetic modelling support that a gradient of MEX-5-dependent RNA competition is responsible for the observed posterior segregation of P granules [202,203] (figure 3a,c).

Interestingly, despite the similar requirements and regulations for PGL-3 and MEG-3 in P granule formation, they occupy different sites in the condensates. MEG-3 can surround the granule, while the majority of PGL-3 is localized to the interior of the structure (figure 3a phase separation inset) [216]. This variant structural distribution of MEG-3 in the P granule reflects a recently identified role. MEG-3 can phase separate into more stable gel-like condensates, which further enhances granule stability at the posterior [207]. The ability of condensates to present subdomains with different properties (liquid phase–dynamic growth versus gel phase–stabilization) that can be subjected to independent regulation highlights further fine-tuning mechanisms that cells can utilize in the spatio-temporal partitioning of proteins.

(b) Spatial regulation of diffusion-state switching produces the gradient of the cell fate determinant MEX-5

P granule asymmetry is reliant upon the inverse gradient of MEX-5 in the *C. elegans* zygote. A recent study on MEX-5 mobility has revealed a physical mechanism that can generate the MEX-5 gradient. Diffusion-state switching can produce intracellular gradients of highly mobile proteins [205], which do not respond to cytoplasmic flow and would typically dissipate any source of asymmetry by rapid diffusion [222]. Tracking of single MEX-5 particles in the cytoplasm identified that the protein exists in two different diffusive states, fast (FD, D_c approx. $5 \mu\text{m}^2 \text{s}^{-1}$) and slow (SD, $D_c = 0.1 \mu\text{m}^2 \text{s}^{-1}$) [205] (figure 3b). These particles show isotropic displacement with no bias towards anterior-directed movement. However, MEX-5 dynamically switches between fast and slow states, with the rate of this switching varying along the AP axis (figure 3b). MEX-5 switches more frequently from FD to SD in the anterior (approx. 3.1-fold higher), where it persists in the SD state longer than at the posterior (SD to FD in the zygote posterior is approx. 1.8-fold higher). In addition, mathematical modelling demonstrates that the spatially regulated kinetics of the MEX-5 state switch are optimal for rapid gradient formation and enrichment in the zygote anterior [205].

The origin of the hierarchical cytoplasmic gradients in the *C. elegans* zygote is the effect of the posterior PAR-1 kinase on MEX-5 mobility (figure 3c). It has been proposed that PAR-1 phosphorylation promotes MEX-5 release from a slow-diffusing substrate, increasing its mobility in the posterior cytoplasm. Fast-diffusing MEX-5 could then rapidly access the anterior domain, where the action of the PP2A phosphatase would lead to MEX-5 incorporation into a slow diffusion substrate and the stabilization of MEX-5 at the anterior [212,220]. By being able to track individual molecules, it has become

Figure 3. Generation of cytoplasmic cell fate determinant asymmetries. (a) In the anterior domain, MEX-5 outcompetes MEG-3 and PGL-3 for RNA-binding, whereas in the posterior, MEG-3 and PGL-3 can bind RNA and undergo phase separation, leading to P granule formation. MEG-3 phase separates into more stable gel-like condensates at the surface of P granules (see phase separation inset); hence, MEG-3 gradient can lead to granule stability at the posterior. (b) An inferred posterior-enriched activity gradient of the kinase PAR-1 [212,213] in combination with the opposing PP2A phosphatase (homogeneously distributed) is proposed to generate a phosphorylated MEX-5 gradient that controls MEX-5 kinetics along the AP axis. The protein MEX-5 locally switches between a fast and slow diffusive state (FD D_c approx. $5 \mu\text{m}^2 \text{s}^{-1}$ and SD D_c approx. $0.1 \mu\text{m}^2 \text{s}^{-1}$) through its binding to/dissociation from a slow diffusive substrate (cytoplasmic RNAs). FD to SD is enhanced at the anterior, whereas the opposite switch is promoted at the posterior, leading to the accumulation of SD MEX-5 state at the anterior and hence the formation of the stable protein gradient of MEX-5 in the zygote. (c) Hierarchical interactions between PAR-1, MEX-5 and the P granule scaffolding proteins MEG-3, PGL-3. A gradient of PAR-1 activity controls the dynamic switch of MEX-5 between FD and SD states along the AP axis, favouring the accumulation of slow-diffusing MEX-5 state towards the anterior. This MEX-5 protein gradient in turn leads to the formation of an opposite gradient of slow-diffusing MEG-3 by regulating MEG-3's spatial kinetics. Furthermore, MEX-5 outcompetes MEG-3 and PGL-3 for RNA-binding in the anterior, promoting P granule formation at the posterior.

more apparent that the action of posterior-enriched PAR-1 and uniformly distributed PP2A could lead to a gradient of MEX-5 phosphorylation along the AP axis that translates into local differences in the kinetics of binding/dissociation of MEX-5 to/from its slow-diffusing substrate [205] (figure 3b). As mentioned above, the frequency at which FD MEX-5 transitions to the SD state is approximately three times higher in the anterior than in the posterior of wild-type embryos. Upon PAR-1 depletion, the rate of fast to slow switching becomes uniform along the AP axis. PAR-1 is therefore postulated to create the MEX-5 gradient by inhibiting the transition from FD to SD

[205]. The physical mechanism and nature of the slow-diffusing substrate that leads to the MEX-5 SD state is not clear; however, SD MEX-5 depends on RNA-binding. Therefore, the simplest model is that MEX-5 binding to RNA can slow MEX-5 down [205,212].

Similar studies have characterized the dynamics of fellow RNA-binding proteins, such as POS-1, PIE-1 and MEG-3, which become segregated to the posterior in response to the MEX-5 gradient [203,218,223–225]. MEX-5 gradient locally controls the dynamic switch between fast- and slow-diffusing particles such that the SD state accumulates at the posterior

Figure 4. Actomyosin-dependent flows and PAR domains in cytokinesis. (a) Midplane view of zygote prior to cytokinesis. Astral microtubules cause a relaxation of polar cortical tension, while equatorial active RHO-1 (*C. elegans* RhoA homologue) increases myosin II activity. Anisotropy in cortical tension produces cortical flows directed towards the equatorial region of the dividing cell. These flows may transport PARs, making the PAR domain boundary match with the position of the cleavage furrow. (b) Cortical view of the zygote prior to cytokinesis. The flows initiated have been predicted to aid the equatorial alignment of actin filaments in the RHO-1 zone. However, recent work indicates that actin filament turnover is too fast to support alignment by compressing flows.

[204–206] (figure 3c). It is not yet clear how MEX-5 controls these kinetics. MEX-5 can recruit PLK-1, which in turn phosphorylates POS-1, inhibiting its retention in slow-diffusing particles [204]. Also, the POS-1 and PIE-1 switch to the SD state seems to depend on their ability to bind RNA [204]; hence, a MEX-5 RNA competition could be a possible additional mechanism. Whether analogous processes regulate MEG-3 kinetics is not known; however, genetic data support a role for PLK-1 in MEG-3 segregation [206]. In the future, it will be key to determine and characterize the slow-diffusing substrate of these different RNA-binding proteins as similar mechanisms might underlie the asymmetries of RNA and RNA-binding proteins in other cell types.

Overall, the molecular mechanisms responsible for the cytoplasmic asymmetries described here nicely exemplify how modulation in space of association to/dissociation from slow-diffusing structures, either P granules or unknown substrates, can generate patterns of asymmetry. These mechanisms could help overcome the stirring effect that a cytoplasmic ‘whirlpool’ type of flow might have and ensure stable cytoplasmic gradients within the spatio-temporal scales required (AP axis approx. 45 μm ; polarization time approx. 10 min) [205]. In this regard, it would be interesting to determine how robust these patterning mechanisms are to different types of flows (i.e. creating artificial flows by FLUCS [81]).

6. Cortical flow powers other key aspects of the zygote asymmetric division

(a) Forces generated by the anterior-directed actomyosin flow can be used to separate centrosomes

Forces generated between the cortex and astral MTs have been extensively studied during mitotic spindle positioning. Initial work in the *C. elegans* zygote revealed that pulling forces exerted at the cortex on astral MTs are primarily generated by an evolutionarily conserved ternary complex: $\text{G}\alpha/\text{GPR}/\text{LIN}-5$ ($\text{G}\alpha/\text{LGN}/\text{NuMA}$ in mammals) (recently

reviewed in Kotak [106]). This complex anchors the dynein motor to the membrane, where it can capture and pull on astral MTs by virtue of its minus-end-directed motility [226–228]. PAR proteins drive the posterior displacement of the mitotic spindle by regulating the activity and localization of this ternary complex, leading to stronger MT pulling forces at the posterior [229–235]. Recently, it has also been proposed that, during polarity establishment, this molecular machinery can transmit the forces generated by the anterior-directed actomyosin flow to the MT asters at the sperm centrosomes [236,237]. The spatial configuration of the duplicated centrosomes positioned between the male PN and the posterior cortex (figure 1d(ii)) allows these forces to separate the centrosomes, a pre-requisite to bipolar spindle assembly [237] (figures 1d(iii) and 4a).

During polarity establishment, cortical depletion of dynein or reduction of actomyosin contractility severely reduces centrosome separation [236]. Given the high degree of movement correlation between flow versus cortical MT anchors (GPR-1/2) and local cortical flow versus the closest centrosome to the flow, the proposed model is that MTs emanating from each of the sperm centrosomes are anchored by dynein, via the $\text{G}\alpha/\text{GPR}/\text{LIN}-5$ complex, to the cortical region closest to their position (figure 1d(ii,iii),e). Given that the anterior-directed flow is induced at either side of the male PN, opposing forces ensure correct centrosome separation. As the actomyosin network retracts, each centrosome is pulled towards the anterior around the male PN, which is simultaneously pushed towards the posterior of the zygote by the concomitant cytoplasmic backflow [236,237] (figure 1d(ii,iii)). The interplay of all these forces ensures correct centrosome separation and bipolar spindle assembly prior to the zygote’s asymmetric cell division.

The question remains of how the membrane-bound $\text{G}\alpha/\text{GPR}/\text{LIN}-5$ complex senses the actomyosin flow. Computational modelling indicates that the motor activity of dynein is not required for the transmission of actomyosin-derived forces, it may simply act as a linker together with the $\text{G}\alpha/\text{GPR}/\text{LIN}-5$ complex [236]. GPR-1 particles seem to form small aggregates that persist over time, similar to aPAR clusters, which could be captured by cortical flow [236]. Another

possibility is that complex members could interact with or be regulated by ABPs. For example, in HeLa cells, MISF regulates the cortical distribution of the LIN-5 homologue (NuMA) and the dynein–dynactin complex [238,239], while the membrane–actin linkers ezrin/radixin/moesins can promote the cortical recruitment of GPR/LIN-5 homologues (LGN/NuMA) during metaphase [239,240].

Alternatively, actomyosin has been described to regulate MT dynamics [241], and loss of actomyosin contractility can affect MT pulling forces owing to a softened cortex [242]. Moreover, anisotropic actomyosin contractility, leading to flow, could also support centrosome orientation and spindle positioning independently of the Gα/LGN/NuMA complex, as recently described in *C. elegans*, *Drosophila* and mouse embryos [243,244]. This variant mechanism is dependent upon cell–cell contacts that reduce actomyosin contractility at the contact area. Cell divisions are subsequently oriented relative to cell contacts in the developing embryo [244]. Given that actomyosin contractility/flow affects centrosome separation/orientation in a wide range of biological contexts [108,243–248], it is key to understand how these seemingly divergent processes are coupled.

(b) Crosstalk between cortical flows and PAR proteins during cytokinesis

Once organized and properly positioned, the mitotic spindle separates the duplicated chromosomes, which are then partitioned into each of the daughter cells during cytokinesis. Cell viability depends on the correct position of the cleavage furrow in between the segregating chromosomes. Animal cells have resorted to using the mitotic spindle as a primary source of cleavage positional information. Briefly, a combination of positive and negative regulatory signals from the central spindle and astral MTs promotes the activation of RhoA signalling at the cell equator [249] (figure 4a). This leads to the subsequent assembly of an actomyosin-based contractile ring that constricts to divide the cell. In addition, the assembly of the contractile ring can be aided by cortical flows [250,251]. Suppression of cortical contractility at the poles (polar relaxation) by astral MTs and/or equatorial contractility activation has been proposed to lead to cortical flows that move towards the furrow [252–262] (figure 4a). The molecular mechanism leading to polar relaxation is still unclear. However, recent work in the *C. elegans* zygote proposes that cortical non-muscle myosin II is removed by dynein-mediated transport on astral MTs [263]. Interestingly, coordination of polar myosin II clearance with the cell cycle could be mediated by AIR-1 signalling. AIR-1 activation and localization to astral MTs via TPXL-1 promotes the removal of contractile ring components at the poles, leading to cortical relaxation [135].

White & Borisy [62] proposed in the early 80s that cortical flows could aid the assembly of the contractile ring, owing to compression of the actomyosin network and alignment of actin filaments at the cell equator (figure 4b). Spatio-temporal quantification of compression and filament orientation in the *C. elegans* zygote in combination with theory prediction support that compression by flow can drive ring formation during cytokinesis [264]. Interestingly, it was proposed that this mechanism can lead to actin alignment with minimal contribution from equatorial RHO-1 activity and the corresponding local increase in non-muscle myosin II [264]. This

phenomenon is particularly apparent in pseudocleavage formation, which occurs during polarity establishment as a result of anisotropies in cortical tension, in the absence of an active RHO-1 band [37,76,264]. However, an interplay between active ring myosin II and flows has been observed during ring constriction. In the proposed model, ring myosin-mediated compression of the cortical surface at the equator coupled with cortical pole relaxation drives cortical flows. Myosin-driven compression pulls the adjacent cortex into the equatorial RHO-1 active zone, leading to more myosin II recruitment and hence more pull. The model predicts that this positive feedback loop can maintain a high constriction rate during ring closure [262,265]. Contrary to the above-described work, a very recent publication indicates that flows cannot mediate actin filament alignment, owing to the rapid turnover of these filaments. Alternatively, the mechanism proposed is that equatorially aligned filaments serve as templates to guide the growth of new ones [266]. The prevalence of equatorial alignment might be supported by other mechanisms. For example, myosin minifilaments turn over more slowly than single actin filaments [267] and hence may be aligned by flow [148]. Local membrane curvature induced by ring constriction can also promote local filament alignment [268].

In asymmetrically dividing cells, crosstalk between PAR proteins and the division machinery ensures correct segregation of polarity effectors and cell fate determinants into daughter cells. Many studies have focused on the alignment of the mitotic spindle relative to the existing PAR polarity [269,270]. However, spindle-independent mechanisms can also ensure correct positioning of the division plane relative to a polarity axis. In the *C. elegans* zygote, cortical PAR asymmetry promotes cytokinesis by restricting anillin and septin to the anterior cortex. This facilitates F-actin accumulation and ring constriction at the furrow site, in a manner that appears independent of PARs' role in the organization of the mitotic spindle [146]. Instead, PARs, by specifying the localization of these actin cross-linkers, may control ring formation and/or global properties of the cortex that could alter the described cortical flows [262,264]. The non-asymmetrically localized PAR-4 and PAR-5 [271,272] also regulate cytokinetic furrow position by modulating cortical dynamics. The kinase PAR-4 (LKB1 homologue) restricts cortical levels of non-muscle myosin II, inhibiting the formation of an anterior myosin cap that can lead to the anterior displacement of the furrow. PAR-4 and its potential target PIG-1/MELK may synergize with anillin to control furrow position [117,119]. PAR-5 limits RhoA activation by inhibiting cortical localization of centralspindlin complex [273]. Moreover, cortical localization of centralspindlin at the equatorial region is mediated through the local inhibition of PAR-5 by AIR-2/Aurora B [273]. Further demonstration that a polarized actomyosin cortex can drive furrow positioning independently of the spindle occurs in the Q neuroblast lineage of *C. elegans*, where the anterior enrichment of myosin II leads to an asymmetric division even when the cell presents a centred spindle [274]. Surprisingly, this asymmetric enrichment of myosin II is promoted rather than inhibited by the PAR-4 pathway described above [119,274,275]. Spindle-independent furrow positioning cues have also been reported in other cell types [276,277]. In *Drosophila* neuroblasts, a basally polarized cap of myosin II downstream of the LGN/Dlg polarity pathway controls furrow formation [199,276], and in human cell

cultures, perturbation of the cortex at the poles of dividing cells leads to furrow displacement [278].

Processes leading to cell division can also reposition the PAR domain boundary to ensure correct segregation and inheritance of these patterning proteins. In the *C. elegans* zygote, errors in the relative sizes of aPAR and pPAR cortical domains and thus the position of the boundary between them can be corrected during anaphase to match the cleavage furrow [96,279,280]. This polarity domain correction is regulated by G α , which facilitates cortical flows via astral MT–cortex interactions [255,280–282]. These flows are proposed to transport PARs towards the cleavage furrow [280]. In this context, PARs could be advected by flows, as observed during polarity establishment [36,37,51–57]. In the *Drosophila* neuroblast, a polarity domain correction mechanism, known as telophase rescue, repositions the cortical G α , LGN, Dlg domain with respect to the spindle orientation. This correction is mediated by the binding of kinesin Khc-73 to the adaptor protein Dlg [283,284]. In agreement with MTs influencing cortical domains in neuroblasts, during interphase, MT–cortex signalling acts as a source of polarity memory, maintaining the constant orientation of cortical polarity and subsequent asymmetric division over successive divisions [285]. In the neuroblast, it is likely that actomyosin-dependent flows influence polarity domain formation [65,200] and correction; however, these flows may not originate from MT–cortex interaction, as in the *C. elegans* zygote, but from the site of contact with its own daughter cell [286].

Studies in the *C. elegans* zygote have greatly advanced our understanding of the signalling pathways that specify the site of cleavage in asymmetrically dividing cells [249]. As summarized here, the process is tightly regulated by crosstalk between cell polarity, spindle positioning and cytokinesis furrow. Moreover, recent research suggests the existence of mechanochemical feedback between cortical polarity and actomyosin flows, similar to that observed in the zygote during polarity establishment [146,280,37,53]. In cytokinesis, PARs can regulate actomyosin cortical dynamics (directly or via the mitotic spindle), possibly influencing the flows that transport them and ensure their correct segregation. In this way, coordination between these processes might lend robustness to patterning of asymmetric divisions [270].

7. Concluding remarks

Actomyosin-dependent cytoplasmic flow has emerged as a mechanism to drive cell patterning in a wide range of organisms, from plants to mammals [21]. Different actomyosin organization/dynamics can transmit forces to the cytoplasm by a variety of mechanisms. These include the treadmilling effect of actin filaments [44,45,47,50], the transport of cargoes along actin bundles [20] and bulk cortical movements or reorganizations, such as those described for the *C. elegans* zygote [37,76]. Polarity effectors can acquire their asymmetric localization through direct contact with cortical constituents such as the actin cytoskeleton, motor proteins or ABPs, or by physical entanglement with the network itself [2,10]. An indirect transport of membrane proteins, through advection, is also observed as a mechanism by which polarity effectors can tap into long-range cytoplasmic flow generated by actomyosin dynamics [20,21].

Patterning by advection relies on the transfer of movement between the actomyosin cortex, permeating cytoplasm and

embedded patterning proteins. To operate during restricted time frames and to capture specific proteins, physical and molecular mechanisms regulate the dynamic behaviour of each of the above patterning elements and coordinate their functional coupling. The biophysical basis underlying these processes is an area of active research, where both physical studies and mathematical modelling are greatly aiding our understanding of how cytoskeleton dynamics and cytoplasmic movements are impacting patterning systems [22]. Evolving technologies are supporting investigation of these spatio-temporally-dependent processes *in vivo*, such as in studies examining cortical dynamics upon laser ablation [76], the creation of artificial flows by FLUCS [81], live super-resolution imaging in combination with methods that manipulate protein function and localization [54–57,173,205,266] and synthetic biology approaches, studying minimal systems [75,149,150].

Concerning the molecular mechanisms involved in flow-dependent patterning, a great challenge is to understand how the molecular players contribute to cellular-scale events [143]. For example, how do actomyosin regulators (GTPases, motors, ABP and kinases/phosphatases) influence the dynamic behaviours of the cortex that generate flow? Recent efforts have identified actomyosin regulators involved in the patterning flow of the *C. elegans* zygote. Through high-throughput phenotypic profiling, this work has identified the contribution of regulators to different large-scale biophysical properties of actomyosin flow, indicating that molecular degeneracy (multiple molecular activities that regulate one biophysical property) underlies the actomyosin dynamic behaviours that polarize the zygote [120,122]. While conferring robustness to this patterning system, as loss of a single effector is likely to alter the dynamic behaviour but not to abolish it, this research also leaves open the question of how different molecular functions that influence a large-scale behaviour are spatially and temporally coordinated.

Spatio-temporal coordination of molecular activities could be driven either by a master regulator or through self-organization. We have discussed examples for both scenarios, suggesting that, in principle, a combination of master regulators and self-organizing systems could operate in cells to direct flow-dependent patterning. In the *C. elegans* zygote, Aurora A fulfils the role of a key polarity regulator as it restricts actomyosin contractility, flow and the activation of the patterning PAR network [83,84,88,132]. Whether Aurora A is a master regulator in this regard across polarizing systems remains to be seen. The presence of self-organizing systems, on the other hand, is being increasingly discussed across developmental contexts [287]. Here, we have described self-organizing systems that rely on biochemical networks and mechanochemical feedback loops. These drive pulsatility of the actomyosin cortex, key to the patterning of the *C. elegans* zygote as well as to morphogenetic processes such as those involved in the *Drosophila* embryo and in mammalian blastocyst compaction [70,94,95,154,155,159,165–167,169]. Similar mechanochemical feedbacks may also operate between actomyosin flows and patterning domains in asymmetrically dividing cells to ensure correct segregation and inheritance of polarity effectors and cell fate determinants [65,82,96,117,119,146,199,200,262,264,273,276,279,280].

Spatio-temporal regulation of protein clustering at the membrane is an emerging mechanism that can regulate the sensitivity of proteins to patterning flows. Clustering of PAR proteins may reduce their diffusion in the plane of the

membrane and their dissociation rates from the membrane, granting PARs the ability to tap into cortical flows [54–57]. In addition, PAR clustering and advection by flow may provide cross-inhibitory PAR networks with bistable dynamics [54]. Clustering is also exploited in a mechanical positive feedback, whereby cortical flows that advect anterior PARs also promote their clustering, further ensuring PAR asymmetric localization in the zygote [57]. Furthermore, Polo-like kinase inhibits PAR clustering, providing a biochemical link between the cell cycle and PAR advection by flow [55,132]. If phase separation is involved in PAR clustering, as suggested by a recent report [288], this could provide another means to regulate PAR advection. Clustering is also observed for other polarity determinants and cytoskeleton regulators [181,187–190], indicating that they might be able to sense flow. We have discussed this possibility in relation to flow-dependent mitotic spindle organization and position [235,237], which is emerging as a means to generate the diversity of division plane orientations needed during embryonic development [243,244].

Cytoplasmic flow also influences the transport of cytoplasmic proteins, allowing rapid polarization of large cells. Flows can increase the exposure of a cytoplasmic factor to a polarized docking site [30] or speed the generation of a stable protein gradient in the cytoplasm [195]. However, this stirring effect of cytoplasmic flow can also be detrimental to patterning maintenance. Here, we have reviewed how phase separation and diffusion-state switching may provide mechanisms by which proteins can become insensitive to flow. P granule

posterior localization thus relies upon the spatial control of phase transition to overcome advection by flow. Nevertheless, cytoplasmic streaming might aid this process by ensuring the homogeneous distribution of P granule components [201]. Similarly, patterning of cytoplasmic proteins that diffuse too fast to be advected by flow relies on spatial regulation of their association/dissociation from slow-diffusing structures/anchors [204–207]. The anchoring mechanism is still under study, yet the biophysical implications will no doubt extend to other polarized contexts with observed cytoplasmic asymmetries.

In conclusion, we are experiencing an exponential discovery phase, where the *C. elegans* zygote is helping bridge theory and live observations, yielding insights into the molecular- to cellular-scale processes that create polarity through flow.

Data accessibility. This article has no additional data.

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