Cell biology in development

Getting to know your neighbor: Cell polarization in early embryos

Jeremy Nance[1,2]

[1]Helen L. and Martin S. Kimmel Center for Biology and Medicine, the Skirball Institute of Biomolecular Medicine, and [2]Department of Cell Biology, New York University School of Medicine, New York, NY 10016

Polarization of early embryos along cell contact patterns—referred to in this paper as radial polarization—provides a foundation for the initial cell fate decisions and morphogenetic movements of embryogenesis. Although polarity can be established through distinct upstream mechanisms in *Caenorhabditis elegans*, *Xenopus laevis*, and mouse embryos, in each species, it results in the restriction of PAR polarity proteins to contact-free surfaces of blastomeres. In turn, PAR proteins influence cell fates by affecting signaling pathways, such as Hippo and Wnt, and regulate morphogenetic movements by directing cytoskeletal asymmetries.

Introduction

Early embryos in many species polarize along their radial axis when blastomeres—the cells that result from the initial cleavage divisions of the egg—develop molecularly distinct contacted and contact-free surfaces (Fig. 1 A). Conserved polarity regulators, including the PAR (Partitioning defective) proteins, localize asymmetrically and help to distinguish contacted (basolateral) and contact-free (apical) surfaces. In some species, such as *Xenopus laevis* and mouse, radial polarization leads to the formation of an external epithelial cell layer that surrounds internal nonepithelial cells with differences in developmental potential. However, in other species, such as *Caenorhabditis elegans*, radially polarized blastomeres do not become epithelial, and polarity is not required for cell fate decisions. Rather, radial polarity directs cytoskeletal asymmetries important for the outward to inward ingression movements of cells during gastrulation.

Radial polarity arises in two different ways from the cleavage of embryonic blastomeres. In one mechanism, cell–cell interactions that form as a result of cleavage trigger polarity directly, potentially through the patterning action of cell surface adhesion proteins such as E-cadherin. Alternatively, polarity can arise as a result of asymmetric trafficking, when membrane distinct in composition from that at the cell surface is deposited between cells undergoing cytokinesis. This review focuses on the mechanisms of radial polarization in three model systems—*C. elegans*, *Xenopus*, and mouse—in which the process has been studied using both embryological manipulation and molecular analysis.

Radial polarization of blastomeres and epithelial cell formation

The radial polarization of blastomeres is often, but not always, the first step in forming an epithelial cell layer on the surface of the embryo. Epithelial cells are characterized by their distinct apical and basolateral surfaces as well as by defined junctional complexes that maintain polarity, promote adhesion, and limit diffusion across the cell layer (Fig. 1 B). Much of our understanding of epithelial cell formation arises from in vitro studies of cultured mammalian cells and from in vivo genetic analyses in *Drosophila* and *C. elegans*. The molecular events that lead to epithelial cell formation have been reviewed recently in detail (St Johnston and Ahringer, 2010; Roignot et al., 2013; Rodriguez-Boulan and Macara, 2014) and are summarized briefly here for a prototypical cell. Importantly, although many aspects of epithelial cell formation are deeply conserved, the mechanisms of epithelial cell formation can differ between species and even among the various epithelial cell types within a given species.

Polarity is established and oriented by cues that arise from cell–cell or cell–matrix interactions. These cell surface cues translate polarity to the cortex by inducing the asymmetric localization of apical and basolateral polarity regulators. Polarity regulators fall into three main groups of proteins (Fig. 1 B; St Johnston and Ahringer, 2010; Roignot et al., 2013; Rodriguez-Boulan and Macara, 2014). The apical PAR group, which includes the PDZ domain scaffolding proteins PAR-3 and PAR-6, and the atypical PKC (aPKC) localize to the apical domain (PAR-6 and aPKC)

Correspondence to Jeremy Nance: Jeremy.Nance@med.nyu.edu

Abbreviations used in this paper: AJ, adherens junction; A/P, anterior–posterior; aPKC, atypical PKC; CRIB, CDC-42/Rac interactive binding; ICM, inner cell mass; MT, microtubule; PCP, planar cell polarity; RhoGAP, Rho GTPase-activating protein; RhoGEF, Rho guanine nucleotide exchange factor; TE, trophectoderm; TJ, tight junction.

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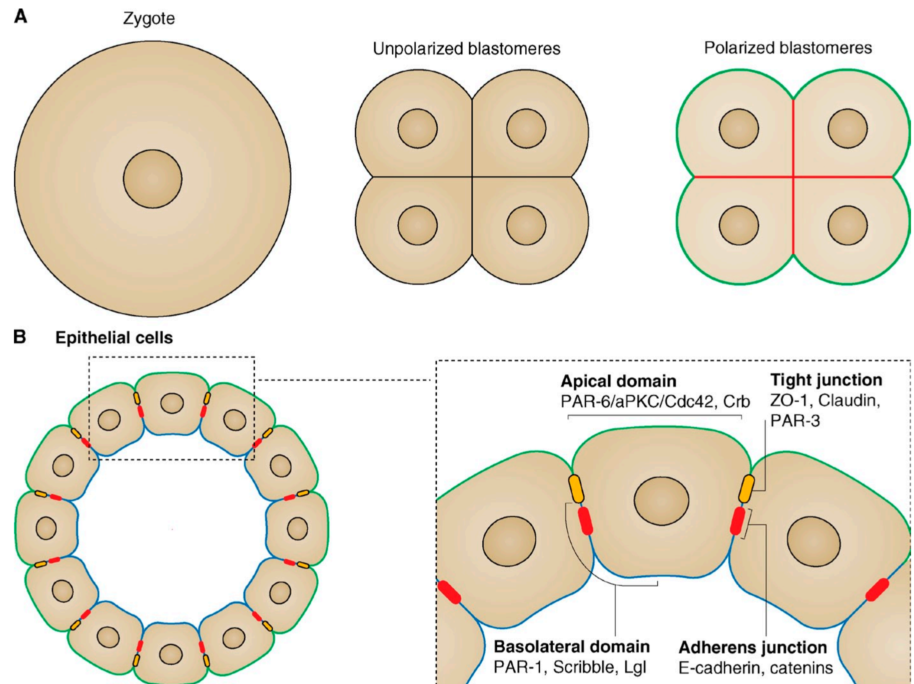


Figure 1. **Blastomeres and epithelial cells.** (A and B) A generic zygote, morula stage embryos containing unpolarized then polarized blastomeres (A), and a blastocyst stage embryo containing polarized epithelial cells (B) illustrate the concepts of blastomere polarization and subsequent epithelial cell formation. (A) The zygote undergoes cleavage to first produce unpolarized blastomeres. Blastomeres subsequently develop radial polarity by differentiating their contacted (red) and contact-free (green) surfaces. (B) Polarized blastomeres eventually develop into fully polarized epithelial cells. Cell surface domains of generic mammalian epithelial cells, along with representative polarity and junction proteins found within these domains, are shown. The developmental stage when blastomeres polarize radially and the time required for cells to transition to mature epithelia vary considerably among species, as exemplified by the three model systems that are the focus of this review. Crb, Crumbs; Lgl, Lethal giant larvae.

and to junctions (PAR-3). The Crumbs complex, which includes the transmembrane protein Crumbs and several interacting proteins, also localizes apically and is involved in maintaining this domain. Finally, the Scribble complex, which includes the leucine-rich repeat protein Scribble, the membrane-associated guanylate kinase Discs large, and Lethal giant larvae, localizes to the basolateral surface. Together with the kinase PAR-1, the Scribble complex antagonizes proteins in the apical PAR and Crumbs groups to help restrict them to the apical surface. Likewise, the apical PAR and Crumbs groups prevent PAR-1 and Scribble group proteins from localizing to the apical domain. Thus, polarity establishment occurs through a series of mutually antagonistic interactions between apical and basolateral polarity regulators. At least in *Drosophila* and *C. elegans*, PAR-3 appears to sit at or near the top of the hierarchy of polarity regulators, as it is required for the localization of proteins in all three polarity groups (Bilder et al., 2003; Tanentzapf and Tepass, 2003; Harris and Peifer, 2004; Achilleos et al., 2010).

Through mechanisms that are poorly understood, polarity regulators promote the formation of tight junctions (TJs) at the boundary between apical and basolateral surfaces (Fig. 1 B). TJs include the scaffolding protein ZO-1, which aids in TJ assembly, and proteins, such as Claudins, that help form a permeability barrier (Günzel and Fromm, 2012). Finally, adherens junctions (AJs) form between adjacent epithelial cells (AJ position varies among species but is basal to the TJ in mammals) to promote adhesion (Fig. 1 B). AJs include homophilic adhesion proteins of the classic cadherin family and catenin adaptors that couple the cadherin cytoplasmic tail to cortical F-actin and signaling proteins (Harris and Tepass, 2010b; Nelson et al., 2013).

It is important to note that the radial polarization of blastomeres is a transient state that often, but not always, leads to the formation of a mature epithelium. The models that are the focus of this review highlight this point. In *C. elegans*, blastomeres polarize radially at the four-cell stage, but epithelial cells do not form until a much later stage of embryogenesis, and the two processes can be mechanistically uncoupled (Anderson et al., 2008). In *Xenopus* embryos, radial polarity and signs of epithelial formation (the presence of TJs) are both evident at the two-cell stage. Finally, in mouse embryos, radial polarity develops at the eight-cell stage, but mature epithelia do not form until several divisions later.

C. elegans

Cell polarity is first evident in the *C. elegans* embryo soon after fertilization, when a signal from the sperm centrosome polarizes the zygote along its anterior–posterior (A/P) axis (St Johnston and Ahringer, 2010; Nance and Zallen, 2011). Similar to the apicobasal polarity of epithelial cells, A/P polarity of the zygote is mediated by PAR proteins. PAR-3, PAR-6, and PKC-3/aPKC become enriched at the anterior cortex in response to the sperm cue (Fig. 2 A). As PAR-3, PAR-6, and PKC-3 asymmetry develops, the RING domain protein PAR-2 and the serine–threonine kinase PAR-1 localize in a complementary pattern to the posterior cortex (Fig. 2 A). Although PAR-3, PAR-6, and PKC-3 are essential for establishing polarity, PAR-2 and PAR-1 help to maintain it by inhibiting the anterior PAR proteins from localizing to the posterior cortex. This is accomplished at least in part by PAR-1, which phosphorylates PAR-3 to remove it from the cortex (Motegi et al., 2011). Together, anterior and posterior PAR proteins form complementary domains that polarize other cortical and cytoplasmic components of the zygote, preparing it for asymmetric division.

During the four-cell stage, the axis of PAR asymmetry switches as the embryo begins to polarize radially. Before radial polarization, PAR-3, PAR-6, and PKC-3 are enriched symmetrically at the cortex of all somatic cells (the single germline precursor retains the A/P PAR asymmetry pattern of the zygote).

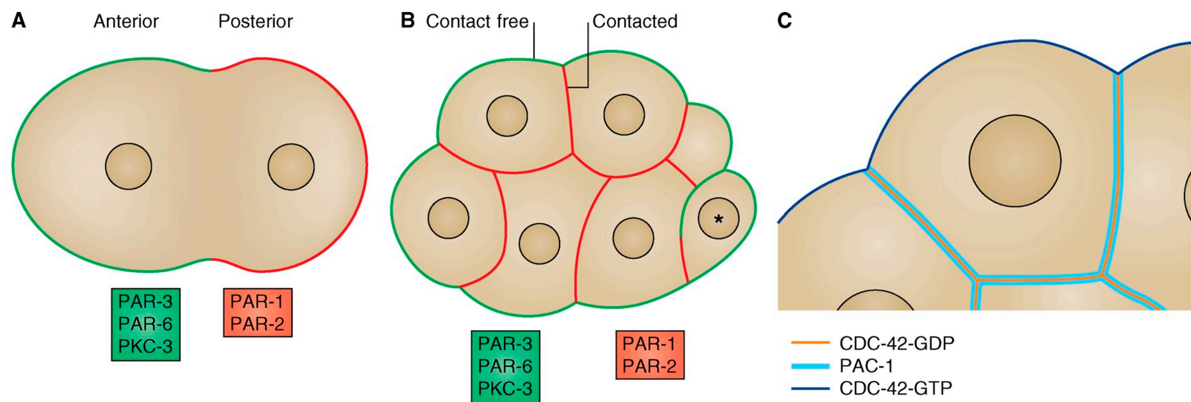


Figure 2. **A/P and radial polarity in the *C. elegans* embryo.** (A) The zygote polarizes along its A/P axis, distributing PAR proteins to distinct anterior and posterior domains. PAR-3, PAR-6, and PKC-3/aPKC enrich at the anterior cortex, whereas PAR-1 and PAR-2 concentrate at the posterior cortex. (B) During the four-cell stage (an eight-cell embryo is shown), the axis of PAR protein asymmetry switches from A/P to contacted and contact free as the embryo polarizes radially. PAR-3, PAR-6, and PKC-3 are found at contact-free surfaces of cells, whereas PAR-1 and PAR-2 are found at contacted surfaces. The single germline precursor cell (asterisk) does not polarize radially and instead retains the A/P asymmetry of PAR proteins seen in the zygote. (C) Radial polarization is initiated and maintained by a contact-induced asymmetry in Rho GTPase activity. The RhoGAP PAC-1 binds to the cortex adjacent to contact sites, where it is predicted to locally inactivate CDC-42 (CDC-42–GDP). Active CDC-42 (CDC-42–GTP) is thus restricted to contact-free surfaces where it recruits PAR-3, PAR-6, and PKC-3.

Polarization occurs quickly—within 15–20 min—and results in the disappearance of PAR-3, PAR-6, and PKC-3 from contact sites and their enrichment at contact-free surfaces (Fig. 2 B; Etemad-Moghadam et al., 1995; Hung and Kemphues, 1999; Nance and Priess, 2002; Nance et al., 2003). Creating ectopic contacts by combining embryos causes PAR-3 to redistribute based on the contact pattern, and removing cell contacts by isolating blastomeres causes PAR-3 to localize pancortically (Nance and Priess, 2002). Therefore, cell–cell contact, rather than an extraembryonic signal, such as the eggshell, provides a continuous cue needed for radial polarization.

PAR-2 and PAR-1 localize in a complementary fashion to cell contact sites, and depleting PAR-3 or PAR-6 at this stage causes PAR-2 and PAR-1 to spread to contact-free surfaces (Nance and Priess, 2002; Nance et al., 2003). The exclusion of PAR-2 and PAR-1 from contact-free surfaces is likely mediated by PKC-3, which depends on PAR-3 and PAR-6 for its cortical localization (Tabuse et al., 1998; Nance et al., 2003). PKC-3 phosphorylates PAR-2 within its localization domain to block cortical association (Hao et al., 2006), and PAR-1 depends on PAR-2 for its localization (Motegi et al., 2011). In contrast, PAR-2 is not needed to maintain PAR-3 asymmetry within blastomeres (Nance and Priess, 2002), although forcing PAR-2 to bind contact-free surfaces strips PAR-3 off of these sites (Hao et al., 2006). The lack of a role for PAR-2 in maintaining radial polarity may reflect the nature of the polarity cue: cell contact cues are read continuously (Nance and Priess, 2002; Anderson et al., 2008), allowing polarity to be adjusted dynamically as contact patterns change. Therefore, the same mechanisms that establish radial polarity likely also function to maintain it.

Polarized blastomeres in *C. elegans* remain tightly adherent but do not form junctions with one another and do not differentiate into epithelial cells (the first epithelia appear during organogenesis, several hours later; Nance and Priess, 2002). In contrast to frog or mouse embryos, polarity is not needed for cell fate specification but rather is important for the first cell movements of gastrulation (Nance et al., 2003). Gastrulation begins at the 26-cell stage when the two endodermal cells ingress by constricting their contact-free (apical) surfaces (Nance and Priess, 2002; Lee and Goldstein, 2003). Nonmuscle myosin accumulates specifically at apical surfaces and is required for ingression. In embryos depleted of PAR-3 at this stage, myosin fails to accumulate apically, and ingression movements are impaired (Nance et al., 2003). The connection between PAR polarity and the asymmetric accumulation of nonmuscle myosin in gastrulating endodermal cells is not yet known. Attractive candidates include Rho GTPases, which can interact with PAR proteins and also regulate myosin activity (Nance and Zallen, 2011).

It is not yet known how blastomeres recognize contacts outside of the cell and translate this information to the adjacent cortex. However, the factor that breaks cortical PAR protein symmetry in response to cell contact cues is known. The Rho GTPase-activating protein (RhoGAP) PAC-1/ARHGAP21 was identified in a genetic screen for embryos that fail to develop radial polarity (Anderson et al., 2008). Cell contacts recruit PAC-1 to the adjacent cortex, where it breaks symmetry within the cell by excluding PAR-3, PAR-6, and PKC-3 from contact sites. RhoGAPs are inhibitors of Rho GTPases (Cherfils and Zeghouf, 2013), and the Rho GTPase target of PAC-1 important for radial polarization is CDC-42. CDC-42 is cortically enriched in blastomeres but does not become asymmetric. However, PAC-1 locally inactivates CDC-42 at cell contact sites, leaving CDC-42 active at contact-free surfaces where it localizes PAR-3, PAR-6, and PKC-3 (Fig. 2 C). Active CDC-42 likely recruits or stabilizes PAR-6 directly because PAR-6 contains a semi–CDC-42/Rac interactive binding (CRIB) domain that specifically binds to active CDC-42 (Gotta et al., 2001), and deleting the semi-CRIB domain largely prevents PAR-6 cortical localization (Aceto et al., 2006; Anderson et al., 2008). PAR-6 binds PKC-3 and is required for its cortical association in blastomeres (Nance et al., 2003; Aceto et al., 2006), suggesting that CDC-42 recruits PKC-3 through PAR-6. However, it is not yet clear how CDC-42

controls PAR-3 localization because PAR-3 lacks a CRIB domain and can localize to contact-free surfaces even when PAR-6 is absent (Nance et al., 2003). CDC-42 has many effectors that regulate cytoskeletal organization and trafficking (Harris and Tepass, 2010a) and could direct PAR-3 and PAR-6 asymmetry through entirely different downstream effectors.

How is CDC-42 activated at contact-free surfaces? Rho guanine nucleotide exchange factors (RhoGEFs) are enzymes that activate Rho GTPases (Cherfils and Zeghouf, 2013). Of the 23 predicted *C. elegans* RhoGEFs, at least two—CGEF-1 and ECT-2—function redundantly to activate CDC-42 in blastomeres. CGEF-1 and ECT-2 were identified in an overexpression screen for their ability to recruit PAR-6 to cell contacts and therefore depolarize blastomeres (Chan and Nance, 2013). Both proteins localize pancortically, and removing them simultaneously causes a partial loss of PAR-6 from the cortex, suggesting that CDC-42 is activated throughout the cortex by CGEF-1, ECT-2, and additional RhoGEFs. Therefore, the radial polarization of blastomeres occurs as a result of competition between RhoGEFs, which activate CDC-42 at all cell surfaces, and the RhoGAP PAC-1, which inactivates CDC-42 specifically at cell contact sites. Although PAC-1 is found at the contact in two-cell embryos, blastomeres at this stage are not yet radially polarized (Anderson et al., 2008). One possibility is that the relative balance of RhoGEF and RhoGAP activity at cell contacts does not favor CDC-42 inactivation until the four-cell stage.

Xenopus

Frog embryos are large and rich in yolk and use a mode of cleavage that simultaneously divides the dense cytoplasm into blastomeres and differentiates apical and basolateral membranes. Blastomeres in the *Xenopus* embryo adopt epithelial character and begin to assemble TJs at first cleavage (Cardellini et al., 1996; Fesenko et al., 2000). Remarkably, TJs and polarity can form in the complete absence of cell adhesion, and polarity persists when blastomeres are separated from one another (Müller and Hausen, 1995; Cardellini et al., 1996; Fesenko et al., 2000; Chalmers et al., 2003). Thus, although in *C. elegans* contact is essential for forming and maintaining polarity, it is important for neither in the frog embryo. A reasonable though untested explanation is that the presence of TJs at the onset of polarization in frogs, as well as their absence in *C. elegans*, explains this difference.

During cleavage, the "new" (contacting) surfaces of each cell arise from cytoplasmic vesicles that are delivered to the ingressing furrow during cytokinesis (Bluemink and de Laat, 1973; Gawantka et al., 1992; Roberts et al., 1992). Accordingly, the egg membrane remains on the contact-free surface of each cell (Fig. 3 A). These membranes are molecularly distinct and provide blastomeres with a mechanism for distinguishing their apical and basolateral surfaces. The membrane polarity that appears during cleavage reflects a redirecting of at least some vesicular traffic. For example, the membrane protein VSV G is delivered to the oocyte surface when its RNA is injected before fertilization but accumulates only at cell contacts (basolateral surfaces) when the RNA is injected after fertilization (Roberts et al., 1992).

Membrane trafficking to the furrow is mediated in part by furrow microtubules (MTs). MTs are arranged in parallel bundles at the ingressing furrow and are required for delivery of vesicles (Danilchik et al., 1998; Takayama et al., 2002; Danilchik et al., 2003), suggesting that vesicles travel along oriented MT tracks toward the furrow (Fig. 3 A). However, injection of RNA encoding VSV G showed that this protein accumulates at cell contacts even when MTs are depolymerized (Roberts et al., 1992), indicating that there are redundant basolateral delivery systems. A similar organization of MTs has been observed in zebrafish embryos, in which there is also exocytosis from perinuclear vesicles to the ingressing furrow (Jesuthasan, 1998; Feng et al., 2002).

Despite substantial differences in the mechanisms that establish radial polarity in frog and worm embryos, the PAR proteins aPKC and Par6b develop an analogous localization to the apical cortex in *Xenopus* (Chalmers et al., 2003; Wang et al., 2013). aPKC promotes the localization of apical polarity regulator Crb3/Crumbs and antagonizes the localization of basolateral regulators Par1 and Lgl2 (Fig. 3 B; Chalmers et al., 2005; Ossipova et al., 2007). In addition to the mislocalization of Par1, Lgl2, and Crb3, blastomeres in embryos with compromised aPKC activity lose TJs and polarity (Chalmers et al., 2005). PAR proteins are not integral membrane proteins, although they can regulate trafficking (Harris and Tepass, 2010a), so it is not yet clear how targeted trafficking during cleavage leads to aPKC and Par6b asymmetry.

Beginning at the 32-cell stage, a subset of superficial (outer) cells undergoes oriented division perpendicular to the embryo surface, producing a population of deep (inner) cells (Fig. 3 B; Chalmers et al., 2003). Deep cells arise from the basolateral portion of superficial cells and adopt fates distinct from superficial cells. In contrast to mouse embryos, where cells read their position along the radial axis to adopt distinct fates (see next section), superficial and deep cells arise from an asymmetric division; their fates are determined even if their position along the radial axis is altered experimentally (Müller and Hausen, 1995; Chalmers et al., 2002). A key difference between superficial and deep cells is the presence of aPKC at the contact-free surface of superficial cells (Fig. 3 B). Par1, which aPKC restricts to the basolateral surface of superficial cells through phosphorylation (Hurov et al., 2004; Suzuki et al., 2004; Ossipova et al., 2007), helps to distinguish these two cell populations by inhibiting Notch signaling, which is high in superficial cells and is required for their fate (Ossipova et al., 2007). An important Notch pathway target of Par1 is Mib (Mind bomb), a ubiquitin ligase that promotes Notch ligand activity. Par1 phosphorylates Mib, causing it to degrade and leading to repressed Notch signaling (Ossipova et al., 2009). In addition to its role in regulating Notch signaling, Par1 helps to generate deep cells by orienting the mitotic spindle in superficial cells to favor asymmetric division (Tabler et al., 2010). Finally, aPKC appears to have an additional role in promoting superficial fates beyond restricting cortical Par1 and other proteins to basolateral surfaces: aPKC is also found in the nucleus of superficial cells, and interfering with its function there promotes deep cell fates (Sabherwal et al., 2009).

It was recently shown that Wnt–planar cell polarity (PCP) signaling also contributes to the differentiation of deep cells by polarizing the distribution of the Wnt coreceptor Lrp6 to basolateral surfaces of superficial cells (Huang and Niehrs, 2014).

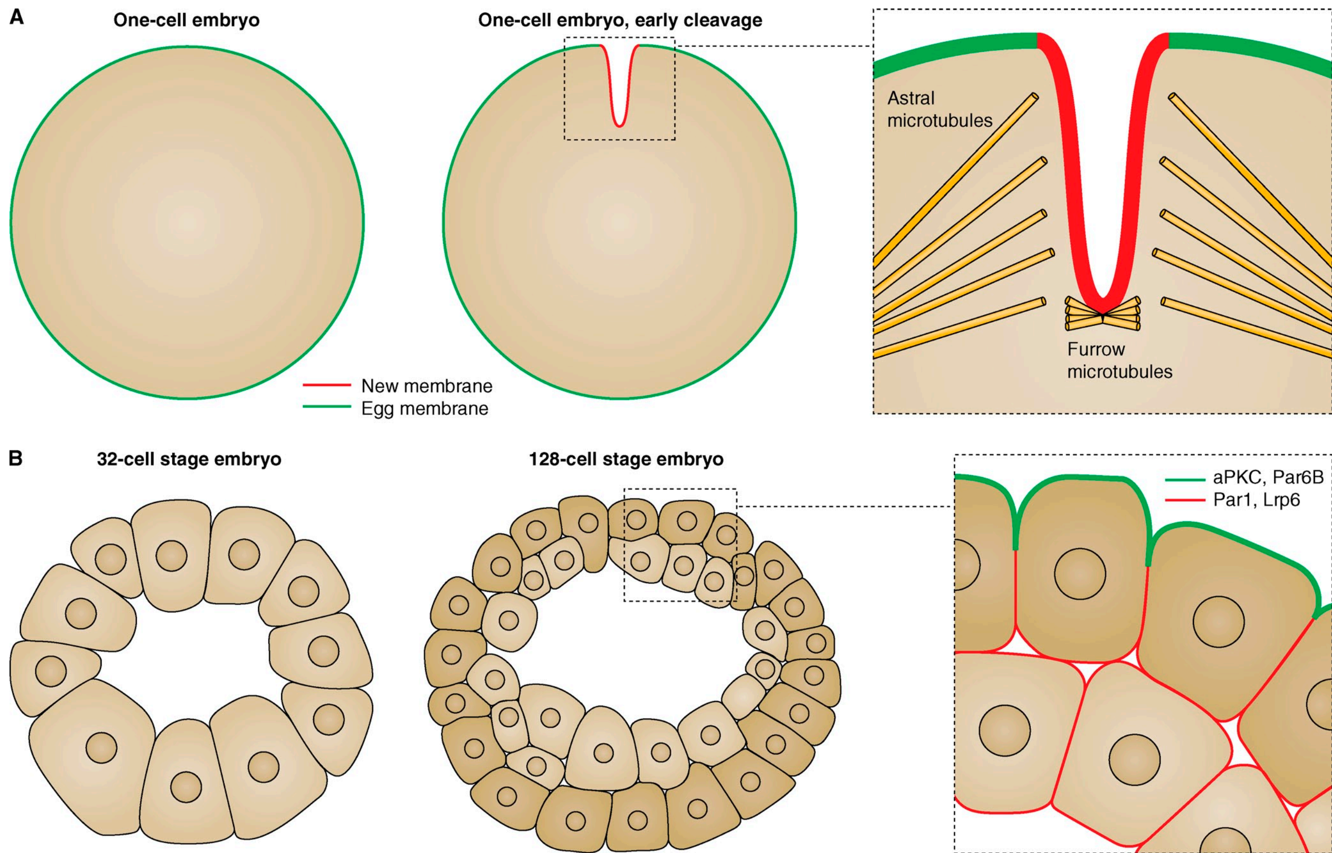


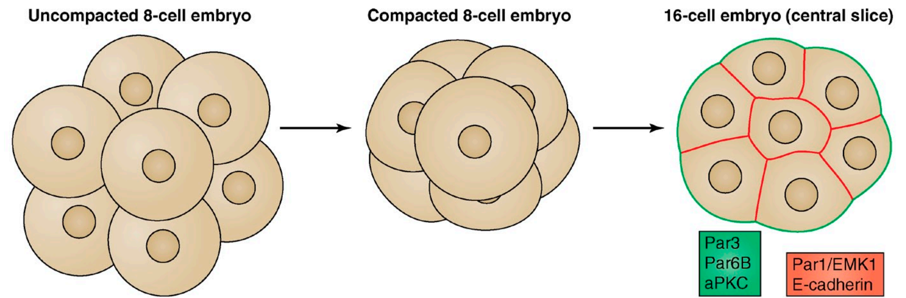
Figure 3. **Polarization of *Xenopus* embryos during cleavage.** (A) Deposition of new (basolateral) membrane during cleavage. A dividing one-cell embryo is shown, with membrane inherited from the egg and new membrane trafficked to the furrow. In the inset, concentrations of furrow MTs are seen at the base of the ingressing cleavage furrow. Separate astral MTs are also present. (B) Formation of superficial (outer) and deep (inner) cell layers. All cells have a superficial surface at the 32-cell stage. Asymmetric divisions over the next several cleavage cycles produce a population of deep cells that lie in the interior of the embryo, as shown in a 128-cell embryo. Membranes of superficial cells are polarized, with aPKC and Par6B at apical surfaces and Par1 and Lrp6 at basolateral surfaces. Notch signaling, which is inhibited by Par1, is high in superficial cells (dark shading) and low in deep cells (light shading). Lrp6 asymmetry also creates differences in Wnt signaling between superficial and deep cells (not depicted).

After division, deep cells inherit Lrp6 and show high levels of canonical Wnt signaling compared with superficial cells, promoting their differentiation (Fig. 3 B). The Wnt–PCP pathway component Dvl (Dishevelled), which is also enriched basolaterally, binds to the Lrp6 cytoplasmic tail and is required for Lrp6 asymmetry (Dollar et al., 2005; Huang and Niehrs, 2014). PAR proteins also contribute to Lrp6 localization because expanding the basolateral domain by expressing a form of Par1 that cannot be phosphorylated by aPKC causes Lrp6 to localize symmetrically (Huang and Niehrs, 2014). It will be important to resolve how PAR and Wnt–PCP pathways intersect and to determine whether Wnt–PCP signaling performs a permissive or instructive role in polarizing superficial cells. It was recently shown that β-catenin in chick neuroepithelial cells promotes the expression and apical accumulation of aPKC (Herrera et al., 2014), providing a possible mechanism linking the two pathways in the early frog embryo.

Mouse

Radial polarization in mouse shares some characteristics with both *C. elegans* and *Xenopus* but also distinct differences. During the eight-cell stage, mouse blastomeres compact with one another by increasing intercellular adhesion, causing cell surfaces to flatten (Fig. 4). Simultaneously, radial polarity develops, and the contact-free surface of each blastomere forms a distinct apical domain containing microvilli and enriched in actin and some actin-binding proteins (Ducibella et al., 1977; Lehtonen and Badley, 1980; Reeve and Ziomek, 1981; Louvet et al., 1996). Over the next several rounds of division, some cells divide asymmetrically, such that one daughter remains superficial (outer cell) and the other becomes positioned in the interior of the embryo, surrounded on all sides by cell contacts (inner cell). TJs between outer cells begin to form at the onset of radial polarization and mature by the 32-cell stage (Fleming et al., 1989), allowing the fluid-filled blastocoel cavity to form. Isolated blastomeres that lack contact with other cells cannot polarize efficiently (Ziomek and Johnson, 1980). However, polarized cells can retain at least some aspects of their polarity once isolated (Johnson and Ziomek, 1981). Thus, cell contacts are necessary and sufficient to polarize cells but are dispensable for polarity maintenance. This characteristic of mouse blastomeres is different from *C. elegans*, in which contacts are needed for establishing and maintaining polarity, and from *Xenopus*, in which contacts are required for neither.

Figure 4. **Compaction and radial polarization in the eight-cell mouse embryo.** During the eight-cell stage, rounded nonpolar blastomeres compact with one another and also polarize along their radial axis. A 16-cell embryo is shown on the right. Par3, Par6B, and aPKC are restricted to contact-free surfaces, whereas Par1/EMK1 and E-cadherin are found at cell contacts.



Before radial polarization, cells in the embryo are totipotent. After polarization, outer and inner cells begin to express distinct subsets of transcription factors and adopt different fates (Johnson, 2009; Stephenson et al., 2012). Outer cells express Cdx2 (Fig. 5 A), develop epithelial character, and differentiate into the trophectoderm (TE). Inner cells, which are collectively called the inner cell mass (ICM), lack Cdx2 and instead express Sox2, Nanog, and Oct4. The ICM remains pluripotent, producing the embryo proper and extraembryonic endoderm (primitive endoderm). At least until the 32-cell stage, forcing inner cells to return to the embryo surface causes them to express Cdx2 and differentiate into TE, whereas outer cells transplanted into the interior of a group of host blastomeres can down-regulate Cdx2 and give rise to embryonic tissues (Ziomek and Johnson, 1982; Ziomek et al., 1982; Suwińska et al., 2008; Tarkowski et al., 2010). These findings suggest that cells read their position on the radial axis to execute the TE versus ICM fate choice. The molecular events that trigger this positional difference in fate are beginning to emerge and are described next.

As in the worm and frog, radial polarization involves the asymmetric localization of PAR proteins. Par3, Par6b, and the aPKC proteins aPKCλ and aPKCζ each become restricted to contact-free surfaces as polarity develops, whereas Par1/EMK1 is found at contacted surfaces (Fig. 4; Pauken and Capco, 2000; Plusa et al., 2005; Vinot et al., 2005). Experiments with recombined cells have revealed that cell–cell contact, rather than division plane, determines the pattern of Par6b asymmetry but that its asymmetry eventually becomes fixed and resistant to new contact patterns (Vinot et al., 2005). Basolateral polarity regulators Scribble and Lgl1 are present at cell contacts, and depletion of aPKC or Par6b causes these proteins to expand to the contact-free surfaces of outer cells (Hirate et al., 2013). In chimeric embryos, cells lacking Par3 or aPKCλ preferentially adopt positions in the interior of the embryo (Plusa et al., 2005; Dard et al., 2009), although the cellular basis for this positional preference is unknown. One possibility is that removing PAR function causes differences in cell fate that result in adhesive changes, leading to the new position. Alternatively, removing PAR proteins could inhibit junction formation, resulting in inappropriate cell sorting. Consistent with both of these models, knocking down Par6b interferes with TE specification and also disrupts TJ formation (Alarcon, 2010).

An essential goal of future studies will be to identify the cell contact cues that induce radial polarization. One appealing model is that adhesion proteins, which could enrich at cell contacts through homophilic or heterophilic interactions with partners on touching cells, transduce polarity information from the cell surface to the adjacent cortex. The homophilic adhesion protein E-cadherin is an attractive candidate. In cultured epithelial cell models, E-cadherin is required for the formation of contact-induced apicobasal polarity and is one of the first proteins to accumulate at nascent contact sites (Harris and Tepass, 2010b; Nelson et al., 2013). E-cadherin (Cdh1) in the embryo becomes enriched at cell contacts as radial polarization initiates (Fig. 4; Vestweber et al., 1987). In embryos lacking both maternal and zygotic sources of E-cadherin, aPKCζ is no longer restricted to contact-free surfaces of outer cells but is instead found around the entire cell cortex of both inner and outer cells (Stephenson et al., 2010). This finding is consistent with an instructive role for E-cadherin in triggering polarity by inducing PAR protein asymmetry. However, in addition to defective polarity, mutant embryos lacking E-cadherin have poor cell–cell adhesion (Stephenson et al., 2010), making its molecular role in polarization unclear. Does homophilic binding of E-cadherin at cell contacts recruit a factor to the cortex that breaks symmetry within the cell? Or alternatively, is E-cadherin simply required for cells to make sufficient contact with one another such that a cadherin-independent polarity cue can operate? It will be difficult to resolve these potential functions unless a symmetry-breaking factor that E-cadherin recruits to the cortex is identified.

An exciting recent development is the identification of several members of the Hippo signaling pathway as a relay that translates polarity state into cell fate. The Hippo pathway is a signaling cascade that can respond to cellular interactions to regulate the nuclear localization of transcriptional coactivator Yap (Schroeder and Halder, 2012; Yu and Guan, 2013). When Hippo signaling occurs, Yap is phosphorylated and localizes to the cytoplasm; when the pathway is inactive, Yap localizes to the nucleus, where it pairs with Tead family transcription factors to regulate target genes. In the early embryo, Yap is nuclear in outer cells and cytoplasmic in inner cells, indicating that the Hippo pathway is off in outside cells and on in inside cells. Tead4 and Yap (together with the related Taz protein) induce Cdx2 expression and repress Sox2 expression in outer cells, promoting TE fate (Fig. 5 A; Yagi et al., 2007; Nishioka et al., 2008, 2009; Wicklow et al., 2014). Differences in cell position alter Hippo signaling because outer cells forced into an internal position lose nuclear Yap and fail to express Cdx2 (Nishioka et al., 2009). Recently, it was shown that the Notch signaling pathway, which is active specifically in outer cells, cooperates with Tead4 to directly induce Cdx2 expression (Rayon et al., 2014). Inappropriately activating Notch signaling within inner

cells can cause these cells to adopt superficial positions, express Cdx2, and differentiate into TE. The Notch pathway appears to function in parallel to the Hippo pathway, and it is currently unknown how Notch activity is limited to outer cells.

How does the Hippo signaling pathway interpret cell position? Several upstream components of the pathway have polarized distributions in outer cells. Lats1/2 kinases, which phosphorylate Yap to prevent its nuclear accumulation, are found at contact-free surfaces within outer cells but are mostly cytoplasmic within inner cells (Fig. 5 B; Cockburn et al., 2013). Inhibiting or knocking down Lats1/2 reduces Yap phosphorylation and results in the ectopic expression of Cdx2 within inner cells (Nishioka et al., 2009; Leung and Zernicka-Goetz, 2013; Lorthongpanich et al., 2013). The cortical protein Amot (Angiomotin) also shows asymmetries and is required (together with related Amotl2) to activate the Hippo pathway (Hirate et al., 2013; Leung and Zernicka-Goetz, 2013). In outer cells, Amot is found at contact-free surfaces, whereas in inner cells, it is phosphorylated and enriched at all cell surfaces, eventually becoming expressed at high levels specifically in inner cells (Fig. 5, A and B). Amot can interact with actin, E-cadherin, and Yap and can be phosphorylated by Lats, suggesting that it may integrate cell contact information with Hippo signaling activity. Although the mechanistic details are still emerging, the association between Amot and E-cadherin may be particularly important for asymmetric Hippo signaling (Hirate et al., 2013). E-cadherin is required to exclude Yap and Cdx2 from the nucleus of some inner cells (Nishioka et al., 2009; Stephenson et al., 2010), suggesting that it may be needed for Hippo signaling, although it is difficult to discount an indirect requirement here for proper adhesion. The interaction between Amot and E-cadherin is promoted by Nf2/Merlin, a pancortical regulator of Hippo signaling that is also required for inner cell specification (Cockburn et al., 2013; Hirate et al., 2013). Importantly, Amot only colocalizes with E-cadherin within inner cells, where its function is required, because Amot is excluded from basolateral surfaces in outer cells (Fig. 5, A and B; Hirate et al., 2013; Leung and Zernicka-Goetz, 2013).

The asymmetric localization of Amot and Lats1/2, as well as inner–outer differences in Hippo signaling, is determined by cell polarity. In embryos lacking Par6b or both aPKCλ and aPKCζ, nuclear Yap and Cdx2 expression is low or lacking in outer cells, indicating that Hippo signaling was active in both inner and outer cells (Hirate et al., 2013). This effect depends on cell adhesion, as dissociated cells lacking Par6b have nuclear Yap, like wild-type dissociated cells. Therefore, both cell polarity and cell adhesion are required for asymmetric Hippo signaling. Interestingly, the daughters of some asymmetric divisions can transiently localize to outer surfaces even though they appear apolar (Anani et al., 2014). These cells are eventually internalized but can activate Hippo signaling while still on the surface of the embryo, suggesting that cell polarity rather than position per se dictates Hippo activity. Loss of Par6b or aPKC activity causes Amot to be symmetric in outer cells and therefore to colocalize with E-cadherin at contact sites in outer cells (Hirate et al., 2013). An appealing model is that sequestration of Amot to contact-free surfaces in outer cells, through the direct or indirect action of PAR proteins, prevents Amot from associating with E-cadherin

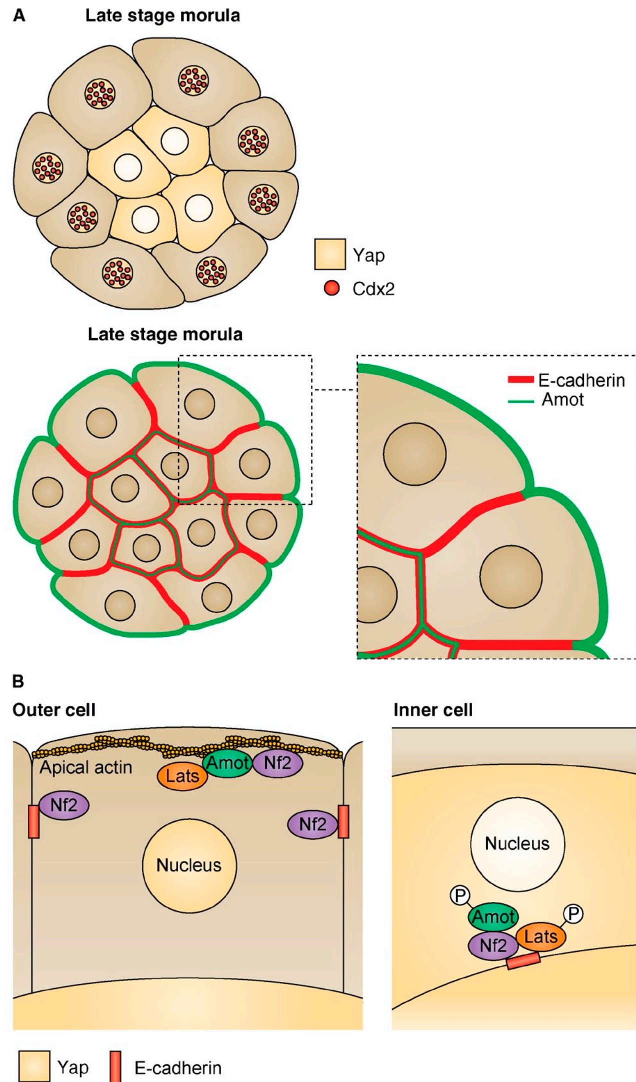


Figure 5. **Hippo signaling in outer and inner cells of the mouse embryo.** (A) Hippo signaling patterns inner and outer cell fates in the morula. Yap is found in the cytoplasm in inner cells (where Hippo signaling is active) and in the nucleus in outer cells. In outer cells, Yap together with Tead4 (not depicted) is required for the expression of Cdx2, a transcription factor that promotes TE differentiation. Yap asymmetry is directed by Amot, which is found all around the surfaces of inner cells but only at contact-free surfaces of outer cells. Amot and the interacting protein E-cadherin colocalize only in inner cells. (B) Model for polarity and adhesion-mediated Hippo pathway asymmetry in outer and inner cells. In outer cells, nonphosphorylated Amot interacts with apical actin, Nf2, and Lats, sequestering Lats and preventing Hippo pathway activity. Consequently, Yap is nuclear. In inner cells, phosphorylated Amot complexes with Nf2 and Lats at AJs, leading to Hippo pathway activity and restriction of Yap to the cytoplasm. The model is based on Hirate and Sasaki (2014). P, phosphorylation.

at contact sites, thereby inhibiting Hippo signaling (Fig. 5 B). One possible mechanism of sequestering Amot to contact-free surfaces in outer cells is through actin binding: because Amot can bind actin, actin binding is influenced by Lats-mediated phosphorylation, and actin is enriched at contact-free surfaces in these cells (Fig. 5 B; Hirate et al., 2013). Going forward, it will be important to learn how E-cadherin interaction influences Amot or other pathway members, how PAR proteins exclude Amot from contact sites in outer cells, and whether Lats1/2 asymmetry in outer cells contributes to Hippo pathway activity.

Conclusions and future directions

A comparison of blastomere polarization in worm, frog, and mouse embryos illustrates significant mechanistic variation in how polarity is established. However, in each species, polarization cues lead to the accumulation of PAR-3, PAR-6, and aPKC at contact-free surfaces. An important challenge in both worm and mouse, in which contacts initiate polarity, is to identify the cell surface cues that initiate polarity and learn how it is translated to the cortex to induce PAR asymmetry. E-cadherin is an attractive candidate, at least in mouse in which it is known to be needed for polarity. Determining whether E-cadherin performs an instructive role in polarity, separate from its contribution to adhesion, is an important goal for future studies. More is known in *C. elegans* about how cortical polarity is broken—through a PAC-1–mediated asymmetry in Rho GTPase signaling between contacted and contact-free surfaces. It has not yet been tested whether PAC-1 homologues in mouse (ARHGAP21 and ARHGAP23) or asymmetries in Rho GTPase signaling are needed for radial polarization. Although cell contacts are dispensable for radial polarity in frogs (even though PAR asymmetries follow cell contact patterns), it is possible that contact cues operate redundantly with the vesicle-trafficking pathways that polarize cells during cleavage. Conversely, asymmetric vesicle trafficking may contribute to polarity establishment in mouse or worms. When more is known about the trafficking mechanisms that polarize frog blastomeres, it will be interesting to learn whether there are similarities to the formation of the blastoderm epithelium in *Drosophila*. This initial epithelial cell layer polarizes at the same time as it forms through the inward growth of cell membranes from the egg surface (Lecuit and Wieschaus, 2000) and thus is not simply patterned by cell contact cues. Indeed, E-cadherin is not required to initiate polarity in the developing blastoderm epithelium in flies, as PAR-3 (Baz) can localize apically when E-cadherin is depleted (Harris and Peifer, 2004).

Once localized, PAR proteins are likely to have very different targets in each species. For example, PAR proteins do not regulate cell fate in worm blastomeres, as they do in frog and mouse, but instead control cytoskeletal asymmetries. Surprisingly little is known in any system about how PAR proteins interface with direct downstream effectors to elaborate polarity, and that holds true in early embryos as well. A particularly exciting area for future investigation will be to determine how PAR proteins interface with the Wnt pathway in frogs and the Hippo pathway in mouse to control cell fate decisions and to understand how the activity of these signaling pathways is spatially regulated.

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