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Multiscale morphogenesis of the mouse blastocyst by actomyosin contractility

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Abstract

During preimplantation development, the mouse embryo forms the blastocyst, which consists of a squamous epithelium enveloping a fluid-filled lumen and a cluster of pluripotent cells. The shaping of the blastocyst into its specific architecture is a prerequisite to implantation and further development of the embryo. Recent studies identified the central role of the actomyosin cortex in generating the forces driving the successive steps of blastocyst morphogenesis. As seen in other developing animals, actomyosin functions across spatial scales from the subcellular to the tissue levels. In addition, the slow development of the mouse embryo reveals that actomyosin contractility operates at multiple timescales with periodic cortical waves of contraction every ~80 s and tissue remodeling over hours.

1- Mouse preimplantation development

Within 4 days after fertilization, the mouse embryo forms the blastocyst, a structure that mediates implantation in the uterus [1,2]. From the zygote stage, blastomeres undergo cleavage divisions, producing smaller cells after each successive round. Blastomeres remain spherical and of equivalent size until they reach the 8-cell stage on the 3rd day. Then, the mouse embryo undergoes compaction,

a morphogenetic process during which blastomeres draw themselves into closer contact (Fig 1A). Concomitantly, blastomeres gather their apical material into an apical domain [3–5]. This domain affects the divisions from the 8- to 16-cell stage by tethering one pole of the mitotic spindle, which can result both in the asymmetric partitioning of the apical domain among sister cells and in divisions oriented perpendicularly to the embryo surface [4,6–8]. During the 16-cell stage (Fig 1B), cells adopt inner or outer positions causing them to initiate distinct differentiation processes: inner cell mass (ICM) and trophectoderm (TE) [4,8–12]. TE cells progressively epithelialize and, during the 32-cell stage (Fig 1C), pump fluid into the intercellular space to create the first mammalian lumen called the blastocoel [13–15]. The formation of this lumen is accompanied with further differentiation of the ICM, turning either into epiblast (Epi) or primitive endoderm (PrE). Eventually, Epi cells become sandwiched between the TE and PrE cells, which line the lumen [16]. When the blastocyst reaches its full expansion, it consists of nearly a hundred cells and is ready to implant.

Shaping the blastocyst relies on the deformation of cells and the rearrangement of their contacts (Fig 1). These result from the generation of forces and changes in the

mechanical properties of cells and tissues. In animals, cell shape and mechanics are controlled in large part by the actomyosin cortex. As reported in several recent studies, the early mammalian embryo is no exception.

2- Contractility of the actomyosin cortex

The actomyosin cortex consists of a cross-linked meshwork of dynamic actin filaments that are tethered to the plasma membrane [19]. The contractility of the actomyosin cortex is controlled by the dynamics and cross-linking of actin filaments as well as the activity of the myosin motor non-muscle myosin II [20–

22]. The spatio-temporal regulation of contractility patterns the stresses at the surface of animal cells and drives several key cellular processes. For example, stronger contractility at the cleavage furrow cleaves cells into two during cytokinesis [23]; contractility at the back of migrating cells help them retract their cell body [24]; weaker contractility at cell-cell contacts relaxes them and promotes their spreading [25,26]. Importantly, surface stresses generated by contractility can be controlled across multiple scales: from the subcellular [27] to the tissue levels [28] and from seconds [29] to hours [17].

Figure 1: Morphogenetic events during mouse preimplantation development.

Mouse morphogenesis begins on the 3rd day after fertilization with compaction and polarisation (A) driven by increased contractility (green) and accumulation of apical material in a restricted domain (red), respectively [4,5,17]. (B) Some 16-cell stage blastomeres inherit the apical domain from symmetric or asymmetric divisions and remain at the embryo surface [4,7,8]. Cells without apical material further increase their contractility until they pull themselves inside the embryo. As this cell sorting takes place, cells initiate lineage specification into either TE (blue) or ICM (red) [8,9]. (C) When tight junctions of the TE seal, microlumens filled with intercellular fluid appear at cell-cell contacts [15]. Contractility repairs these fractures [18] and pushes the fluid into a single large lumen [15].

The first description of actomyosin during preimplantation development dates to 1983 [30]. Since then, actomyosin was found to be involved in all of the morphogenetic processes forming the blastocyst (Fig 1). Here, we elaborate on how actomyosin contractility acts at multiple spatial and temporal scales to shape the early mouse embryo.

3- *Tempos of the beating blastocyst*

Unlike other model organisms typically used to study animal morphogenesis, the mouse embryo develops slowly (Fig 1). However, actomyosin keeps beating with the same tempo in the mouse as it does in fast-developing organisms. In this section, we discuss how contractility acts at short and long timescales.

3.1- *Seconds to minutes*

Morphogenetic processes such as compaction, cell internalization, or lumen

formation take ~ 10 hours each, while actomyosin contractility typically acts within seconds to minutes. On such short timescale, mouse blastomeres show rapid and reversible deformations. Initially spotted in 1980 [31], these movements were later identified as Periodic Cortical Waves of Contraction - in short, “PeCoWaCo” [17]. PeCoWaCo consists of a surface deformation that propagates around the cell with a speed of $\sim 0.8 \mu\text{m.s}^{-1}$ (Fig 2). They require intact actin and myosin function and, as such, resemble waves of compression and dilations of the actomyosin cortex [32,33] rather than pure actin waves [34], which were both described in cell lines. The ~ 80 s oscillation period of PeCoWaCo is similar to the ones of periodic contractions observed in other early embryos such as in the fly [35,36], frog or starfish [37]. In fly embryos, actomyosin turnover, the architecture of the meshwork and a mechanical feedback

Figure 2: Spatio-temporal control of contractility during the 8- and 16-cell stages.

PeCoWaCo (black arrows) is most visible on the most contractile regions of the cortex (dark green) [8,17]. In cells isolated from the embryo, PeCoWaCo can be observed circulating freely [17]. When in contact, PeCoWaCo is locally weakened by adhesion molecules (purple) [8,17]. The apical domain (red), also shows reduced actomyosin levels and PeCoWaCo becomes further confined [8]. As polarized cells enlarge their apical domains [4], the most contractile part of the cortex disappears when eventually the apical domain reaches tight junctions (yellow) [13].

on Rho GTPases are thought to regulate the duration of pulsed contractions [36,38,39]. However, what sets the travelling motion of contractions observed in mouse, frog and starfish embryos remain unknown [17,37]. In *C. elegans* embryos, pulsed contractions move towards the anterior pole of the zygotes as a result of tension anisotropies triggered by local downregulation of contractility ensuing signals mediated by the sperm entry [40,41]. This cortical flow advects polarity proteins as it couples to the cytoplasm and induces its streaming (Fig 3) [42,43]. However, such cytoplasmic streaming is not observed in mouse blastomeres. This could be explained by weak coupling of the cortex with the cytoplasm and/or by the absence of cortical flow altogether. Indeed, it remains to be established whether PeCoWaCo involves any movement of material or if they simply consist of a local deformation that would occur successively in adjacent parts of the cortex.

3.2- Hours to days

In mouse embryos, PeCoWaCo can be detected most often from the 8-cell stage onwards [8,17], when actomyosin begins a slower, longer-term reorganisation. This reorganisation is mirrored by changes in surface tension and leads to the compaction of the embryo over a timescale of ~10 h [5,17]. As in other species [25,44–46], compaction of the mouse embryo is set by the ratio of surface tensions between cell-cell contacts and the embryo surface [17,47]. During mouse compaction, the tension ratio gradually changes primarily thanks to the elevation of contractility throughout the 8-cell stage (Fig 1A) [17]. What controls contractility increase and, therefore, the timing of compaction? As noted in the 1980s, protein synthesis inhibitors applied from the late 2-cell stage on do not block compaction. Moreover, this treatment could induce

compaction as early as at the 2- or 4-cell stage, suggesting the presence of an inhibitor of compaction that would be continuously produced [48,49]. The timing of compaction could be controlled by PKC signalling since PKC activators trigger premature compaction at the 4-cell stage [50,51]. Recently, PKC activation was proposed to activate actomyosin contractility via the Rho GTPase [5], which was earlier found to be required for compaction [52]. What would occur downstream of Rho remains unclear since Rho kinase (Rock), the usual suspect for controlling actomyosin contractility, does not seem to be involved in compaction as neither Rock inhibitors [13,53,54], nor Rock knockout [55] show a compaction phenotype (or other signs of contractility inhibition). What controls the onset of contractility and kick-starts preimplantation morphogenesis remains unclear.

4- Spatial control of actomyosin contractility

While contractility rises during the 8-cell stage, it becomes spatially patterned at the cellular and tissue levels, definitively transforming a loosely-organised cluster of cells into an elaborate cyst.

4.1- Sub-cellular scale

During the 8-cell stage, blastomeres gather their apical material into the apical domain, a master regulator of preimplantation development. Indeed, the apical domain provides positional information that guides cell differentiation by sequestering regulators of the TE fate promoting Yap co-transcriptional regulator [9]. It also determines cell positioning by orienting the spindle during the 4th and 5th cleavages [4,7,56] and by controlling contractility [8]. While enriched in microvilli [3] and apical proteins [4,7,13,56], the apical domain is depleted in actomyosin (Fig 2) [8]. The absence of actomyosin at

the apical domain seems to depend on the apical kinase aPKC, as double mutants for the zeta and iota isoforms of aPKC show ectopic actomyosin enrichments at what remains of their apical domain [8]. Since the apical domain can be asymmetrically distributed during the 8- to 16-cell stage division, sister cells inherit distinct contractility [8]. This translates into distinct amplitudes of PeCoWaCo on the short timescale and into the sorting of cells on the long timescale with the most contractile cells being internalised [8,54]. After their spatial allocation, outer cells initiate their differentiation in TE, while inner cells opt for the ICM fate (Fig 1B). This differentiation relies in part on the Yap co-transcriptional activator, which responds to mechanical stimuli [57,58], including the ones generated by actomyosin contractility [59]. During the internalization of the ICM, surface cells become stretched, and Yap

becomes nuclear while it remains cytoplasmic in the rounder inner cells [8]. Interestingly, myosin function is essential for both the positioning of cells and the correct localization of Yap [8]. Whether this phenomenon is controlled by mechanosensing requires further experiments.

What controls the formation of the apical domain at the 8-cell stage? Interestingly, *de novo* polarization in *C. elegans* zygotes relies on actomyosin contractility (Fig 3) [40–43]. Inhibiting actomyosin contractility in the mouse embryo leads to conflicting results when it comes to apical domain establishment. Blebbistatin, a direct inhibitor of non-muscle myosin II [60], efficiently blocks cell division, PeCoWaCo, and lowers surface tension [17] but does not affect apical domain formation [5]. Similarly, heterozygous knockout of the myosin

Figure 3: Contractility and *de novo* polarity establishment in early embryos of *C. elegans* and mouse

Early *C. elegans* (top) and mouse (bottom) embryos undergo *de novo* polarisation at the zygote and 8-cell stage respectively. In *C. elegans*, anisotropic pulsed contractions (green) produce cortical flows that entrain the cytoplasm, which starts streaming (middle) [40,41]. These help segregating polarity proteins into distinct domains (red and blue) [42]. In the 8-cell stage mouse embryo (bottom), contractility gradually rises and generates PeCoWaCo (left, green) [8,17]. Concomitantly, cells undergo cell-autonomous *de novo* polarisation but, contrary to *C. elegans*, no cytoplasmic streaming is observed (middle) and contractility seems unnecessary to form the apical domain (right) [5,8].

heavy chain isoform Myh9 or homozygous knockout of another isoform Myh10 does not seem to affect polarity since these mutants successfully go through processes requiring polarity (they form a blastocyst with TE, ICM and a blastocoel) [8]. Nevertheless, Zhu and colleagues conclude that contractility is required for apical domain establishment [5,61] based on the effect of ML7, an inhibitor of the myosin regulatory light chain kinase [62], which prevents the formation of the apical domain. From these conflicting observations, we suspect that ML7 affects mouse blastomeres beyond their contractility and we do not think that contractility is required for apical polarity establishment in the early mouse embryo.

4.2- Tissue scale

Additionally to the apical domain, cell-cell contacts also downregulate contractions (Fig 2). Originally discovered in the compacting mouse embryo [63], the cell-cell adhesion molecule E-cadherin (or Cdh1) signals to reduce contractility locally at contacts between cells [25,64,65] as well as globally [23]. The control of contractility by cadherins can be mediated via regulators of the small GTPase Rho, which are recruited by p120-catenin, a core component of adherens junctions [66,67]. During compaction, actomyosin levels decrease at cell-cell contacts as compared to the cell-medium interface in a Cdh1-dependent manner [17]. On the long timescale, the local downregulation of contractility could account for up to 1/4 of the entire compaction of the mouse embryo [17].

As actomyosin is reduced at cell-cell contacts and at the apical domain, PeCoWaCo becomes confined to the non-apical part of the non-contact interface (Fig 2). This contractile interface disappears when embryos develop further at 16-cell stage, as cells which do not inherit the

apical domain from their mother cell internalize [8], and polarized cells enlarge their apical domain [4,5] until the most contractile part of the exposed surface disappears [13]. This is concomitant with the sealing of the tight junctions when the apical domains of contacting cells zip together and seal the embryo (Fig 2) [13]. Zenker and colleagues conclude that the expansion of the apical domain does not result from the difference in contractility between the apical and non-apical part of the cell. This is based on experiments with drugs that were thought to inhibit contractility. However, as stated by the authors [13], these drugs did not affect cell shape, which is inconsistent with a number of other studies in which contractility is inhibited [5,8,14,15,17]. At that point, it is worth highlighting a technical note on Blebbistatin, a potent contractility inhibitor, which is, as many other drugs, hydrophobic and not compatible with usual mouse embryo culture conditions under mineral oil [8,15,17]. Therefore, it remains conceivable that the expansion of the apical domain results from the different contractility between the apical and non-apical part of the exposed cell surface.

Once tight junctions are sealed [68], intercellular fluid becomes trapped within the intercellular space [13] and accumulates to form the blastocoel. This occurs after the fracturing of cell-cell contacts by pressurized fluid leading to the formation of micron-size water pockets (Fig 1C) [15]. As observed in vitro [18], contractility mediates the healing of these fractures and can redistribute the extra-cellular fluid. The higher contractility of the ICM, as compared to the TE, squishes the fluid away from the ICM and progressively coarsens the many smaller water pockets into a larger one until there is only one lumen remaining [15]. Interestingly, on short time scales, water pockets seem to

pulse at a rhythm close to the one of PeCoWaCo [15,17]. After a few hours of expansion, the blastocoel undergoes cycles of collapse and re-growth reaching a long term steady state [69]. Collapses seem to result from transient seal rupture due to cell division within the TE [14,70]. Once again, tight junction repair is aided by contractility [14], which could be recruited by Rho, as observed in the surface epithelium of *Xenopus* gastrula [71].

5- Concluding Remarks

After the many discoveries on actomyosin contractility during worm, fly or fish development, the mammalian embryo finally joined the club. With its comparably slow development, the mammalian embryo can now provide unique information on the different timescales at which actomyosin functions. This will become possible thanks to the advancements in imaging technologies, which will require preserving long-term viability while enabling high temporal resolution. In this regard, light-sheet microscopy will be key to put the multiple scales of contractility under the spotlight [72,73].

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