Cells & Development xxx (xxxx) xxx

Contents lists available at ScienceDirect

Cells & Development

journal homepage: www.journals.elsevier.com/cells-and-development

Review

A theoretical understanding of mammalian preimplantation development

Edgar Herrera-Delgado, Jean-Léon Maître *

Institut Curie, PSL Research University, CNRS UMR3215, INSERM U934, Paris, France

ARTICLE INFO

Keywords:
Modelling
Blastocyst
Gene regulatory network
Patterning
Contractility
Lumen

ABSTRACT

The blastocyst has long been a hallmark system of study in developmental biology due to its importance in mammalian development and clinical relevance for assisted reproductive technologies. In recent years, the blastocyst is emerging as a system of study for mathematical modelling. In this review, we compile, to our knowledge, all models describing preimplantation development. Coupled with experiments, these models have provided insight regarding the morphogenesis and cell-fate specification throughout preimplantation development. In the case of cell-fate specification, theoretical models have provided mechanisms explaining how proportion of cell types are established and maintained when confronted to perturbations. For cell-shape based models, they have described quantitatively how mechanical forces sculpt the blastocyst and even predicted how morphogenesis could be manipulated. As theoretical biology develops, we believe the next critical stage in modelling involves an integration of cell fate and mechanics to provide integrative models of development at distinct spatiotemporal scales. We discuss how, building on a balanced base of mechanical and chemical models, the preimplantation embryo will play a key role in integrating these two faces of the same coin.

1. Introduction

Preimplantation development of mammalian embryos has been a mainstay subject of study for developmental biology (Rossant, 2016). We refer to preimplantation development as the time from fertilization to the formation of the blastocyst, which, in mammalian species such as human and mouse, is when the embryo implants into the uterus (Fig. 1). The entire developmental process has been studied extensively due to its great importance for embryogenesis, as well as for medical sciences in the contexts of assisted reproduction technology (Shahbazi, 2020; Wamaitha and Niakan, 2018).

Preimplantation development is best studied in the mouse, where the blastocyst implants on the 4th day after fertilization (Chazaud and Yamanaka, 2016; Maître, 2017; Tosenberger et al., 2019; White et al., 2018; Zhang and Hiiragi, 2018). The early blastocyst consists of a squamous epithelium, the trophectoderm (TE), enveloping a fluid-filled lumen, the blastocoel, and a cluster of pluripotent stem cells, the inner cell mass (ICM) (Fig. 1). In the late blastocyst stage, the ICM further differentiates into primitive endoderm (PrE) and epiblast (Epi), while the TE separates into polar and mural TE (pTE and mTE). The PrE and mTE line the lumen and the Epi is sandwiched between the PrE and pTE (Fig. 2A). Until that point, the embryo develops inside a glycoprotein shell, the zona pellucida (ZP). Once the ZP is removed, the mouse embryo is ready to implant.

The specific architecture of the blastocyst results from a series of morphogenetic movements (Maître, 2017; White et al., 2018). At the 8-cell stage, compaction transforms the loosely contacting cells of the embryo into a tight cluster (Maître et al., 2015). Then, at the 16-cell stage, some cells internalise and do not contact the outside medium any longer (Korotkevich et al., 2017; Maître et al., 2016). At the 32-cell stage, fluid accumulates within the embryo to form a lumen, which expands and pushes the inner cells into one quadrant (Chan et al., 2019; Dumortier et al., 2019; Zenker et al., 2018). Finally, at the 64-cell stage, within the ICM, PrE and Epi cells sort into their respective positions (Plusa et al., 2008; Yanagida et al., 2020).

Simultaneously to the morphogenesis of the blastocyst, cells differentiate into the first mammalian lineages. At the end of the 16-cell stage, the transcription factor Cdx2 is found only in surface cells, whereas the transcription factor Sox2 is specifically expressed in inner cells (Ralston and Rossant, 2008; Wicklow et al., 2014). Often before finding their final positions, PrE cells specifically express the transcription factor Gata6 and lose Sox2 while Epi cells maintain Sox2 and go on to express the transcription factor Nanog (Chazaud et al., 2006; Plusa et al., 2008). In the late blastocyst, mTE cells lose Cdx2, which is maintained in pTE cells (Christodoulou et al., 2019; Nakamura et al., 2015).

Morphogenesis and fate specification are tightly linked (Collinet and

* Corresponding author.
E-mail address: Jean-Leon.Maitre@curie.fr (J.-L. Maître).

https://doi.org/10.1016/j.cdev.2021.203752
Received 24 April 2021; Received in revised form 30 September 2021; Accepted 30 September 2021
Available online 8 October 2021

Lecuit, 2021; Gilmour et al., 2017; Hannezo and Heisenberg, 2019). In the case of TE and ICM lineages, morphogenesis is upstream of specification while the opposite occurs for the PrE and Epi lineages. The formation of the apical domain at the 8-cell stage guides cell positioning through oriented cell division and contractility-mediated cell sorting at the 16-cell stage (Dard et al., 2009; Korotkevich et al., 2017; Maître et al., 2016; Niwayama et al., 2019). This results in only outer cells containing the apical domain, which then activates Cdx2 expression by promoting the nuclear localisation of the co-transcriptional activator Yap (Hirate et al., 2013). This is prevented in inner cells by signals from cell-cell contacts, which cover the entire cell surface (Stephenson et al., 2010; Wicklow et al., 2014). Therefore, the position of cells, encoded in apicobasal signals, governs the specification of TE and ICM lineages. This is not the case for PrE and Epi fates, which appear in a salt and pepper pattern within the ICM as a result of the morphogen Fgf4 being secreted by a subset of ICM cells (Kang et al., 2013). Fgf4 steers receiving cells towards the PrE fate, which then sort out next to the lumen (Plusa et al., 2008; Yanagida et al., 2020). In the case of pTE and mTE, on which much less research can be found, the position of TE cells relative to the Fgf4-secreting ICM is thought to be at play (Simon et al., 2020).

Importantly for this review, the blastocyst is a very convenient model system as it consists of few accessible large cells developing slowly. Despite its simplicity, the blastocyst manifests a variety of phenomena that are of great importance across biology in general such as tissue compaction, cell sorting, lumen formation, apicobasal polarisation, epithelialisation, programmed apoptosis, periodic contractions, metabolic switch or tissue patterning (Chazaud and Yamanaka, 2016; Maître, 2017; Shahbazi, 2020; White et al., 2018; Zhang and Hiiragi, 2018). We think that in being a system capable of developing such complex processes yet being relatively simple, it is perfectly tailored to the minimalistic approach of modelling (Toolbox). Models developed for and tested on the preimplantation embryo can then be used in more complex contexts in which modelling from scratch would have been more arduous.

In this review, we discuss the insight and results that have emerged from modelling preimplantation development, as illustrated in a diagram of some of this work covering different developmental stages (Fig. 1). These models involve a variety of modelling formalisms and tackle different questions and developmental stages, yet demonstrate a collective effort from the community to incorporate mathematical modelling further into the field. We then discuss the advantages of this system and why we believe the blastocyst will become a hallmark system for mathematical modelling of developmental systems.

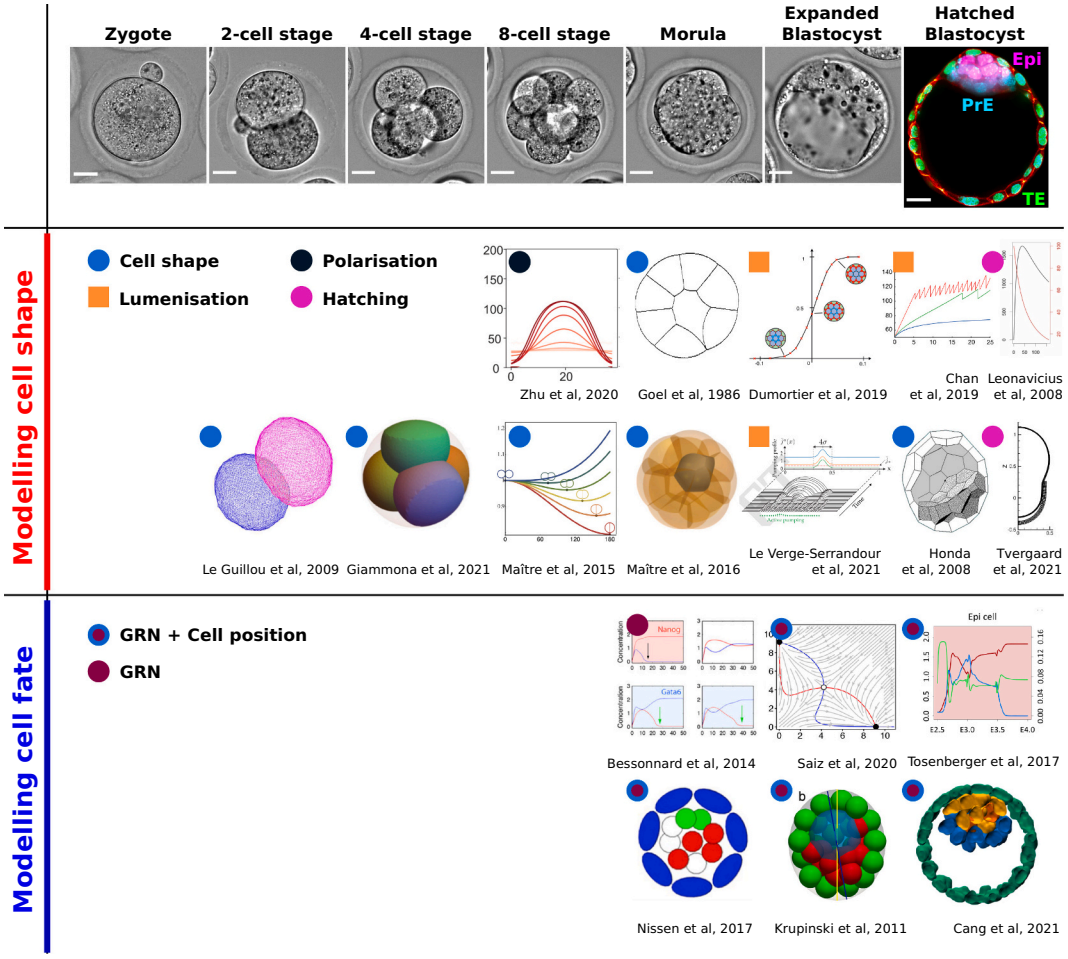

Fig. 1. Modelling preimplantation development. During preimplantation development, the zygote undergoes cleavage divisions reaching the 2-, 4- and 8-cell stage before starting morphogenesis with the formation of the compacted morula and then the expanded and hatched blastocyst. The corresponding brightfield snapshots from mouse preimplantation development were provided by Özge Özgüç and Francesca Tortorelli. The hatched embryo shows trophectoderm (TE, green), epiblast (Epi, magenta) and primitive endoderm (PrE, cyan). The scale bar in each snapshot corresponds to 20 μm. Theoretical models considering the morphogenesis of the blastocyst are noted below the corresponding stage. Theoretical models describing the differentiation of the first lineages are mentioned further below. Furthermore, each model is labeled with the specific process it is modelling. It is clear from the dates of publication that this is a growing field, as the number of publications in the last 5 years is almost twice that of all the years that came before.

2. Modelling lineage specification in the blastocyst

One of the key questions of preimplantation development is that of when and how are the first cell fate decisions made (Rossant, 2016). This initially constitutes the choice between ICM and TE (Fig. 1). Then a further decision within the ICM takes place with the choice between Epi and PrE. It is understood that gene regulatory networks (GRN) play a major role in making these initial decisions (Chazaud and Yamanaka, 2016; Tosenberger et al., 2019) (Fig. 2B). A specificity that modelling GRNs should consider at these stages is that cells can often revert to different lineages for a variety of reasons: change in position, compensate for a lack of a given cell type or, mechanical stress (Chazaud and Yamanaka, 2016; Collinet and Lecuit, 2021; Hannezo and Heisenberg, 2019). It is in answering these questions that mathematical modelling can play an important role, and has done so already in some contexts.

In the early blastocyst, a long-standing question had been that of how TE and ICM are specified. Competing models had been put forward: "position-based" in which the position of cells determines TE fate; the other model being "polarity-based" in which cells are assumed to inherit a polarity which would ultimately determine their fate, regardless of their position (Wennekamp et al., 2013). This question was tackled through the use of a GRN based model that also accounts for position of cells through modelling each cells simply as spheres with attraction and repulsion terms (Krupinski et al., 2011). In this study, the authors conclude that the position-based model is more robust and likely to drive TE-ICM patterning. While the components in the model were limited by the biological knowledge at that time, the mechanism the model proposes for cells sensing their apical position matches with the now well-described molecular mechanism of Yap-mediated TE specification based on positional cues (Fig. 2B) (Hirate et al., 2013; Wicklow et al., 2014). This is a clear example of modelling providing a correct mechanistic answer to a biological question, even when the specific components are unknown (Toolbox).

With this knowledge, subsequent models have fully embraced the position-based model to describe the TE-ICM differentiation (Nissen et al., 2017). One such model explores a mechanism that provides robustness to noise at a stage where low cell count means noise plays an important role (Holmes et al., 2017). This model proposes that through noise driven-transitions between a TE and ICM fate, cells can also "correct" their fate in a manner akin to that observed in other systems that form precise boundaries (Exelby et al., 2021; Zhang et al., 2012). Recently, a model explicitly modelling the Yap pathway captured the mechanism through which apical signals drive Cdx2 expression, thereby better reflecting our current knowledge of the molecular mechanisms underlying TE-ICM differentiation (De Caluwé et al., 2019). Finally, in an effort to incorporate multiple scales of preimplantation development, such as cell position, the GRN, constraint from the ZP and lumen, as well as intrinsic noise, an integrative model was recently constructed (Cang et al., 2021). This model, with many parameters (Toolbox), steers cells towards TE or ICM fates depending on their cell-cell contacts, which are set *ab initio*. It furthermore predicts that noise-induced transitions between cell fates must be suppressed after the lineages are established. However, this model considers stochasticity as proportional to TF levels instead of coupled to reaction parameters as is more often the convention (Gillespie, 2000). This assumption may influence the results, as it has been shown that anisotropic distributions in stochastic fluctuations play an important role in stabilising stochastic systems and could explain how transitions are suppressed without need of additional components (Exelby et al., 2021).

Unlike TE-ICM differentiation, the second lineage commitment of the mammalian embryo into PrE and Epi does not seem to be determined by cell positions. Instead, the two lineages appear within the ICM in a salt and pepper pattern while the proportions of PrE and Epi adjust fairly robustly. To fine tune the proportions of Gata6 positive PrE and Nanog positive Epi, cells can transiently show high levels of both Gata6 and Nanog before committing to a single marker and/or undergoing apoptosis (Chazaud et al., 2006; Plusa et al., 2008). The expression of Gata6 is promoted by Fgf4 that is secreted by Nanog positive cells, while apoptosis of PrE cells occurs when they fail to relocate near the lumen. An early model of PrE-Epi differentiation captured the mutual exclusivity between Nanog and Gata6 (Bessonnard et al., 2014). Fgf4 and downstream Erk signalling were included as mutually antagonistic with Nanog and mutually agonistic with Gata6 (Fig. 2B), In addition, the model includes signalling between cells to account for Fgf4's diffusive

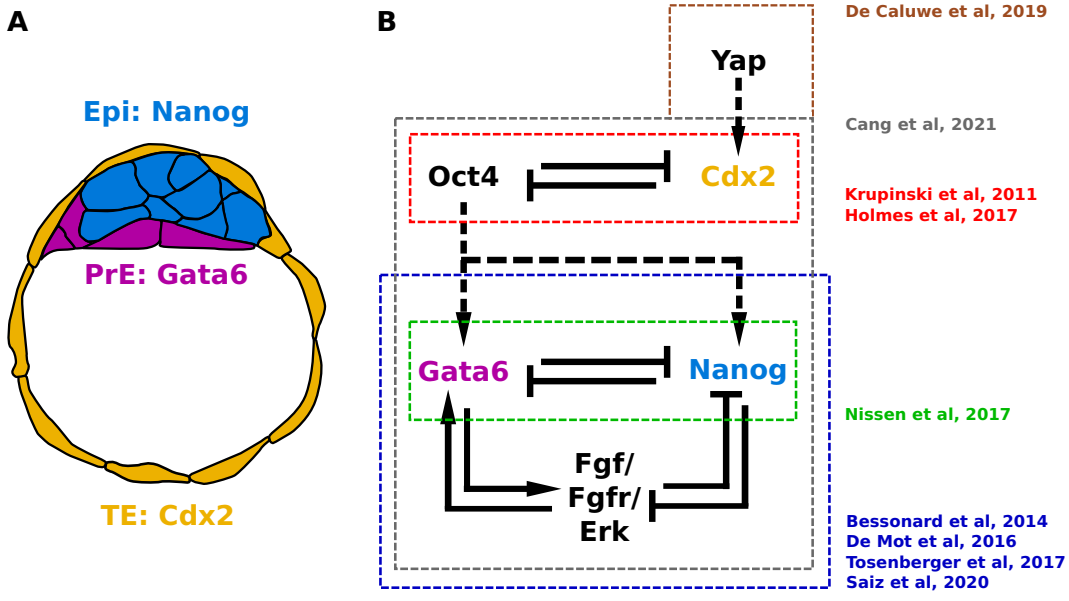

Fig. 2. Modelling lineage specification during preimplantation development. A. Schematic diagram of an expanded blastocyst with TE in yellow, Epi in blue and PrE in purple. B. Gene Regulatory Networks encompassing models of cell-fate specification in the blastocyst, with blunt and normal arrows indicating repression and activation respectively. The dashed boxes with corresponding references detail which part of the network has been explicitly modelled in each study. In this diagram, we only include a component in each model if it has been explicitly accounted for with a dynamical variable. The activation from Oct4 into Gata6 and Nanog is dashed because this interaction is not modelled explicitly. Some of these studies use multiple variables to model each component, such as Fgf/Fgfr/Erk or Yap/Taz.

Toolbox
On the good use of modelling to study biological processes

or better insight into this review we provide a brief discussion on why it is useful to perform modelling in the first place and on the challenges associated with modelling (Goldstein, 2018; Paluch, 2015).

A model is a mathematical formalisation of the logic we use to understand a system. Through capturing the parts we find relevant, we are able to reproduce specific behaviour of interest. While the predictive capabilities of models have been promoted in biology, the benefits of modelling extend further than providing predictions, a model can: help find gaps in our logic, generalise results to other phenomena, and aid with mechanistic understanding (Brodland, 2015).

Models are best put to use when capturing emergent behaviour *i.e.* a behaviour that manifests as something greater than the sum of its parts. Broad examples of such behaviour in biology include self-organisation, temporal oscillations, phase transitions or tissue scaling to name but a few. A classical example in development is somitogenesis, where the intuition of a simple negative feedback results in temporal oscillations that drive the patterning of the somites (Lewis, 2003). A non-embryological example involves the behaviour of individual migrating cells, by accounting simply for their local non-reciprocal interactions, capturing a phase transition from disordered to ordered (Szabó et al., 2006). A further recent example describes how meta-synchronic cell divisions change cells connectivity and trigger a phase transition of the tissue, which becomes more fluid within a few minutes and allows zebrafish doming (Petridou et al., 2021). In each of these examples, relatively simple models with minimal assumptions provide an intuitive explanation for complex behaviours that would be otherwise difficult to obtain.

When constructing a model, the standard approach is to construct it as simple as possible, distilling a process to its simplest form. A well-thought model focuses directly on a question and does not have extra components that serve only for decoration. Unnecessary parameters will make the model too permissive when comparing with data. In capturing only the necessary components of a system a model can then provide an interpretation of how they function. All models by definition will have shortcomings, one needs to make sure these limitations don't affect the phenomena of interest. Indeed, distinguishing which aspects to keep and which to discard is one of the great challenges of modelling (Gunawardena, 2010).

This takes us to our next point, making the model biologically relevant. Some examples of questions to ask are: is our model capturing the data correctly? Do we want quantitative or qualitative results? Is there a justification for the assumptions the model makes? Is the modelling formalism suitable for the phenomena? Concrete examples of tests are: it captures perturbations to the system, it does not "explode" resulting in absurd predictions, the model is not overfit making it too sensitive to parameter changes. These are classical tests for physicists and mathematicians that should be explained simply and concretely when building a model to provide confidence regarding its construction to the reader.

Finally, it is always important to consider whether a model is required for the study at hand. It is not a necessity that every project must have a model associated with them, it must be very clear what results the model are contributing to the study and not to construct trivial or non-rigorous models that may obscure the interpretation of results.

properties. Through parametrising this system, the model produced three steady states: high Nanog, high Gata6 and dual expression as seen *in vivo*. To ensure the validity of the model (Toolbox), it is confronted to WT and Gata6 homozygous and heterozygous mutants. It predicts that heterogeneities in Fgf4 levels reduce the number of dual expressing cells forcing cells to decide for one state or the other as suggested by an Fgf4 mutant. In addition, communication through Fgf4 leads to a correct specification of the proportion of PrE to Epi in the WT system (Bessonnard et al., 2014). Although this early model captured the essence of PrE-Epi patterning, further efforts were made to directly account for noise, pointing Fgf4 as an important source of variation for consistent, yet not excessive, cell-fate heterogeneity (De Mot et al., 2016). The authors then further extend this theory by explicitly modelling the rough position of cells and validate their previous results while predicting the additional importance of noise induced by cell divisions (Tosenberger et al., 2017). To provide an explanation to the robustness of lineage proportions after adding and removing cells, the same sphere-based model was used in conjunction with a simple GRN (Saiz et al., 2020). The model was able to capture the blastocyst's capacity to compensate for both the decrease and increase of both PrE and Epi cells as observed *in vivo*. As had been suggested in previous work (Bessonnard et al., 2014), Fgf4 provides a form of communication between ICM cells and allows the system to sense the proportion of cells and adjust accordingly. In summary, the initial model from (Bessonnard et al., 2014) was rigorously constrained by multiple mutant phenotypes, ensuring the model captured the essential parameters, which were robustly tested. This then enabled additional components to be added to a reliable baseline model (Toolbox).

As cells of the ICM commit to distinct lineages, prospective PrE cells sort themselves out of the Epi cells and position themselves near the lumen. To explain this process, the authors in (Krupinski et al., 2011) model the requisites for cell sorting given the simple sphere-based formalism mentioned above. They predict that differential adhesion is necessary for cell sorting while suggesting that some unknown signal is required to keep Epi cells near the pTE, or direct PrE cells towards the lumen (Toolbox). A similar 2D model was developed that also emphasises the role of adhesion while suggesting that cell-fate transitions between PrE and Epi fates are an important component for robustness (Nissen et al., 2017). Recently, Eph-Ephrin-based adhesion/repulsion was proposed to drive Epi-PrE sorting, as suggested by differential expression of EphA4 and EphB2 in these cells (Cang et al., 2021). However, the involvement of Eph-Ephrin in Epi-PrE sorting has not been experimentally tested and remains hypothetical. Together, the sorting process has been included in several models of PrE-Epi differentiation without formally considering cell shape and cell mechanical properties, which are likely to affect the sorting process (Krens and Heisenberg, 2011). Further modelling may for example include the role of membrane fluctuations that were recently described in PrE cells as a potential driver of the cell sorting (Yanagida et al., 2020).

3. Modelling the mechanical forces shaping the blastocyst

Since the position and shape of cells is an important determinant of cell fate (Hirate et al., 2013; Maître et al., 2016; Wicklow et al., 2014), understanding how the blastocyst sculpts its specific architecture is key. Recent studies have identified the forces generated by the cells to shape the blastocyst. Importantly, biophysical methods such as micropipette aspiration, atomic force microscope indentation or pressure gauge needle allow measuring mechanical properties in absolute values (Chan et al., 2019; Dumortier et al., 2019; Lenne et al., 2021; Maître et al., 2015, 2016). This provides quantitative data with a scale, which further constrains theoretical modelling and makes predictions more specific

(Toolbox). As a result, mechanical models explicitly consider physical properties, with corresponding parameters rather than proxies for these forces. The elements of cells that generate and/or experience physical forces, such as cell membranes or the underlying cytoskeletal cortex, are also considered, which provide a mechanistic explanation to observed emergent behaviour.

Enclosing the embryo, the ZP is dispensable for preimplantation development, since removing it does not prevent blastocyst formation (Mintz, 1962; Tsunoda et al., 1986). Most of the time, the embryo has enough space not to be constrained by the ZP. Nevertheless, the constrain of an elastic shell could affect how blastomeres are arranged, especially when cytokinetic movements push dividing cells against the egg shell (Pierre et al., 2016). At the 4-cell stage, mouse blastomeres most often form a tetrahedron, which minimizes the space taken by cells (Giammona and Campàs, 2021). In addition, the ZP can influence the arrangement of the mouse embryo when it inflates to form the blastocoel. The ZP can be slightly oblong, which is sufficient to guide the orientation of the axis formed by the ICM and blastocoel in a vertex model of the blastocyst (Honda et al., 2008).

Compaction, at the 8-cell stage, defines a key change in shape from spherical cells to stretched out cells that together form a sphere. In essence, as captured in the early model from (Goel et al., 1986), compaction is a surface area minimisation process, which can be driven by changing the ratio of tension between the cell-cell contact and contact-free interfaces (Fig. 3A). In fact, the ratio of tension directly relates to the external contact angle formed by the cells and can be referred to as the compaction parameter (Goel et al., 1986; Maître et al., 2015). Similarly, an agent based model could reproduce the dynamics of compaction by reducing the tension at the cell-cell contact, *i.e.* by increasing adhesion (Le Guillou et al., 2009). Eventually, direct measurements of surface tensions revealed that tensions at the contact-free interface double, while cell-cell contacts relax by one third (Maître et al., 2015). Using these measurements, an analytical model could capture the relative contributions of these tension changes to compaction: ¾ for increased tension at the contact-free interface and ¼ for decreased tension at cell-cell contacts.

To model compaction, the tension of cells is considered to be similar, since variations in tensions among cells could lead to their sorting into distinct layers (Krieg et al., 2008; Maître et al., 2012). This is precisely what happens during the 16-cell stage when cells adopt inner positions (Fig. 3B). Prospective inner cells display higher tensions than cells remaining at the surface of the embryo. This stems from differences in contractility inherited from the asymmetric divisions of the apical domain, which shows little contractility compared to the rest of the contact-free surface of the cell. Theoretical modelling of the internalisation process reveals that above a defined threshold of tension asymmetry between contacting cells, the cell with highest tension becomes fully internalised (Maître et al., 2016). The threshold value depends on the compaction parameter of the embryo. For normally compacting mouse embryos, cells internalise when their tension becomes 1.5 times the one of their neighbours. This prediction from an analytical model was tested experimentally using mutant embryos with reduced contractility. Interestingly, this prediction implies that mouse mutants with defective compaction or other species with different compaction parameter than the mouse could have distinct internalisation thresholds, which remains to be tested. The mechanism unveiled in this study further highlights the essential function of the apical domain in controlling the position of cells within the blastocyst (Dard et al., 2009; Korotkevich et al., 2017; Maître et al., 2016; Niwayama et al., 2019). Interestingly, the apical domain initially appears as a small cap in the centre of the contact-free surface of cells during the 8-cell stage before expanding until it reaches tight junctions during the 16-cell stage (Korotkevich et al., 2017; Zenker et al., 2018). Recently, a model of apical domain centring and expansion, inspired by a model of polarity domain formation in *C. elegans* zygotes (Goehring et al., 2011), proposed that Ezrin competitive binding for a limited pool of PIP2 could drive the centring and expansion (Zhu et al., 2020). This model was fed with measurements of Ezrin dynamics using mRNA overexpression, which could affect the proposed competitive binding. Since the apical domain is a master organiser of the lineages and morphogenesis of the blastocyst, accurately capturing its appearance and regulation could be key to model preimplantation development.

Once outer and inner cells are sorted, surface cells seal the intercellular space of the embryo with tight junctions (Zenker et al., 2018). As surface cells start pumping osmolytes through the cell, into the intercellular space, water is drawn into the embryo to form the blastocoel (Schliffka et al., 2021). Fluid does not immediately accumulate into one waterbody. Instead, pressurised fluid breaks open cell-cell contacts into

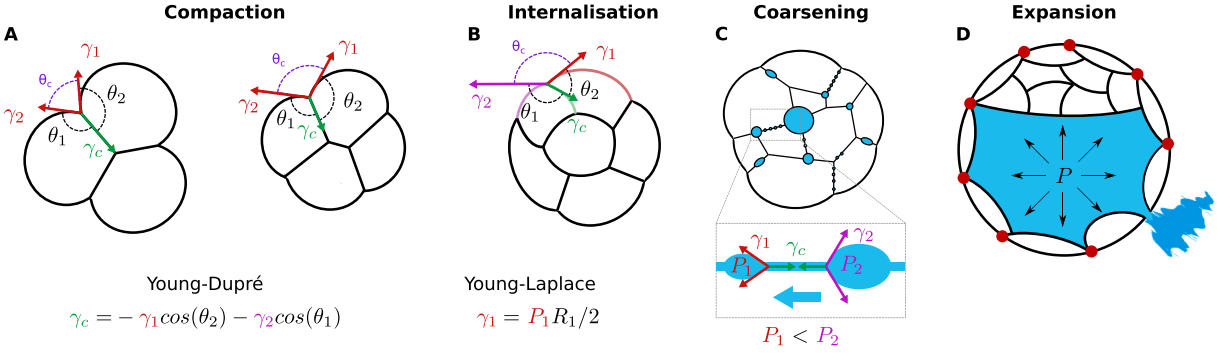

Fig. 3. Representation of modelling of mechanical phenomena at different stages. (A. Compaction) Up to the 4-cell stage, the cells of the embryo are round with small external contact angles (θe). As embryos develop from the 8-cell stage, the contractility of cells increases raising the tension at the embryo surface while cell-cell contacts relax their tension. This tension change causes contact angles to increase. This shape change can be captured directly by the Young-Dupré equation, which relates the contact angles and tensions of the contacting interfaces (Maître et al., 2015). (B. Internalisation) At this stage, some cells present higher contractility than others, leading to asymmetry in surface tension of adjacent cells. This difference in contractility results in cells with higher contractility being internalised, leading to the formation of the ICM. This process has been observed experimentally and captured theoretically with a 2D analytical model and 3D simulations (Maître et al., 2016). (C. Coarsening) The embryo forms its first lumen through active fluid pumping that results in numerous microlumens, which exchange fluid dependent on their respective pressures. This process is captured by the Young-Laplace equation, which relates the pressure, tension and radius of curvature of microlumens. Microlumens with lower tension or higher radii of curvature will display lower pressure and therefore draw fluid from contacting microlumens (Dumortier et al., 2019). With the aid of modelling, it has been detailed how this process leads to coarsening of the microlumens and the eventual formation of the lumen (Le Verge-Serandour and Turlier, 2021). (D. Expansion) During lumen growth, a transient breakage of tight junctions can result when pressure overcomes the resistance of the epithelium (which can fluctuate during cell division). The size of the lumen can be predicted from the balance between the hydrostatic pressure of the lumen and the tightness of the epithelium, as captured theoretically (Chan et al., 2019; Ruiz-Herrero et al., 2017).

hundreds of micron-size intercellular pockets (Dumortier et al., 2019). To coarsen into a single lumen, these water pockets do not seem to coalesce but exchange fluid in a process akin to Ostwald ripening (Fig. 3C). Modelling the coarsening of microlumens reveals the influence of connectivity and surface tension (Dumortier et al., 2019). Further theoretical analysis describes how differential pumping between surface cells could also steer the coarsening of the blastocoel (Le Verge-Serandour and Turlier, 2021). However, this remains to be experimentally explored. The positioning of the blastocoel is an important step of mammalian development as it sets the first axis of symmetry of the mammalian embryo, which determines where the polar and mural TE, PrE and Epi are located (Honda et al., 2008; Dumortier et al., 2019). When considering symmetry breaking, reaction-diffusion mechanisms are often at play (Kondo and Miura, 2010), yet here we find a mechanism based on fluid mechanics. This was already proposed in a 3D vertex model considering the shape of the ZP as a mechanical determinant for aligning the blastocyst axis of symmetry (Honda et al., 2008). It will be interesting to investigate whether other biological processes result from mechanical symmetry breaking.

After blastocoel formation, abrupt leakage caused by divisions of the TE lead to repeated collapses of the blastocyst (Leonavicius et al., 2018). This occurs when the hydrostatic pressure within the blastocoel overcomes the sealing of the surface epithelium, which is challenged during cytokinesis (Fig. 3D). Modelling this process of hydraulic gating predicts the size of the blastocyst, which has been proposed to influence the proportions of TE and ICM lineages (Chan et al., 2019; Ruiz-Herrero et al., 2017).

Finally, when cultured *in vitro*, blastocyst hatch from the ZP. It is unclear whether this event takes place *in vivo* since the ZP may simply get digested within the uterus. Nonetheless, this process is essential for the implantation of embryos grown *in vitro*, such as during assisted reproductive technologies procedures. Through modelling the blastocyst as a pressurised "balloon", researchers highlight the importance of hydrostatic pressure in the range of kPa to drive hatching (Leonavicius et al., 2018). Interestingly, they find that without ZP, the hydrostatic pressure remains in the range of a few hundreds of Pa, indicating the importance of mechanical resistance to build hydrostatic pressure. Further modelling of hatching considering the blastocyst and ZP as elastic shells highlight the influence of the relative mechanics of the embryo and ZP as well as of the opening of the hatching hole (Tvergaard et al., 2021).

4. Perspectives

The ease of access of the preimplantation embryo has provided an ideal platform for modelling lineage specification where multiple perturbations and live imaging are required for well-constrained models (Toolbox). As highlighted above, this system has also quickly become a useful tool for exploring the role of mechanics in mammalian development. As both fields move forward, we think that incorporating mechanics with cell-fate decisions will become a central aspect of theoretical modelling in biology (Collinet and Lecuit, 2021; Hannezo and Heisenberg, 2019). In such a scenario, we believe that explicitly modelling mechanical forces, alongside chemical reactions, such as from GRNs, is the next step for an integrated understanding of development. Important advances have recently been made in this direction (Boocock et al., 2020; Dye et al., 2021; Erzberger et al., 2020), yet this is a nascent field and there are many aspects to be understood.

The approach to modelling mechanochemical systems has been very quantitative since its inception (Oates et al., 2009), leading to concrete physical parameters being quantified, particularly from the mechanical side (Paluch and Heisenberg, 2009; Sugimura et al., 2016). While this is extremely useful for comparability across different systems, it also allows tackling an interesting question, present in lineage specification as well: How are such parameters interpreted in a biological system? While a membrane may possess a particular tension, or a tissue a specific stiffness, it is at present unclear how this is incorporated quantitatively in the biological system. Do cells interpret these forces linearly, logistically, through a threshold mechanism, is there a "saturation" point for integrating them? Our choice of observables will be a trade-off between those forces we can most easily measure and those that prove to be most relevant in biology (Sugimura et al., 2016). As a quantitatively oriented field, unit-based parameters are emerging, and it will be of great importance to understand how cells interpret magnitudes of signals to progress as a truly quantitative field (Sugimura et al., 2016; Lenne et al., 2021).

Some mechanochemical modelling approaches so far have focused on observables such as: cell length, cell position, cell shape, membrane tension, tissue stress, TF concentration and polarity. As mentioned in this review, the choice of observable should not be universal to all publications, and will depend on the question at hand. Nonetheless, we believe that as the field progresses, tools and methods will be developed, and specific observables will become standard for mechanochemical modelling, akin to how cell fate specification is often modelled through ODEs capturing TF dynamics (Davidson, 2010).

Such type of models will become ever more frequent, it is likely that underlying mechanisms of emergent phenomena such as scaling, symmetry breaking or cell sorting will be shown to rely on mechanical and chemical feedbacks. Through studying the early embryo, we as a community will help to unravel many such mechanisms that are likely prevalent throughout biology.

Acknowledgements

We thank Özge Özgüç and Diane Pelzer for critical reading of the manuscript. We also thank Özge Özgüç and Francesca Tortorelli for sharing microscopy images. E.H.D. is funded by the Fondation pour la Recherche Médicale. Research in the lab of J.-L.M. is supported by the Institut Curie, the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé Et de la Recherche Médicale (INSERM), and is funded by grants from the ATIP-Avenir program, the Fondation Schlumberger pour l'Éducation et la Recherche, the European Research Council Starting Grant ERC-2017-StG 757557, the European Molecular Biology Organization Young Investigator program (EMBO YIP), the INSERM transversal program Human Development Cell Atlas (HuDeCA), Paris Sciences et Lettres (PSL) "nouvelle équipe" and QLife (17-CONV-0005) grants and Labex DEEP (ANR-11-LABX-0044) which are part of the IDEX PSL (ANR-10-IDEX-0001-02).

References

Bessonnard, S., Mot, L.De, Gonze, D., Barriol, M., Dennis, C., Goldbeter, A., Dupont, G., Chazaud, C., 2014. Gata6, nanog and erk signaling control cell fate in the inner cell mass through a tristable regulatory network. Development (Cambridge) 141 (19), 3637–3648. https://doi.org/10.1242/dev.109678.

Boocock, D., Hino, N., Ruzickova, N., Hirashima, T., Hannezo, E., 2020. Theory of mechanochemical patterning and optimal migration in cell monolayers. Nat. Phys. https://doi.org/10.1038/s41567-020-01037-7.

Brodland, G.W., 2015. How computational models can help unlock biological systems. Semin. Cell Dev. Biol. 47–48, 62–73. https://doi.org/10.1016/j.semcdb.2015.07.001.

Cang, Z., Wang, Y., Wang, Q., Cho, K.W.Y., Holmes, W., Nie, Q., 2021. A multiscale model via single-cell transcriptomics reveals robust patterning mechanisms during early mammalian embryo development. PLoS Comput. Biol. 17 (3), e1008571 https://doi.org/10.1371/journal.pcbi.1008571.

Chan, C.J., Costanzo, M., Ruiz-Herrero, T., Mönke, G., Petrie, R.J., Bergert, M., Diz-Muñoz, A., Mahadevan, L., Hiiragi, T., 2019. Hydraulic control of mammalian embryo size and cell fate. Nature 571 (7763), 112–116. https://doi.org/10.1038/s41586-019-1309-x.

Chazaud, C., Yamanaka, Y., 2016. Lineage specification in the mouse preimplantation embryo. Development (Cambridge) 143 (7), 1063–1074. https://doi.org/10.1242/dev.128314.

Chazaud, C., Yamanaka, Y., Pawson, T., Rossant, J., 2006. Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. Dev. Cell 10 (5), 615–624. https://doi.org/10.1016/j.devcel.2006.02.020.

Christodoulou, N., Weberling, A., Strathdee, D., Anderson, K.I., Timpson, P., Zernicka-Goetz, M., 2019. Morphogenesis of extra-embryonic tissues directs the remodelling

of the mouse embryo at implantation. Nat. Commun. 10 (1), 3557. https://doi.org/10.1038/s41467-019-11482-5.

Collinet, C., Lecuit, T., 2021. Programmed and self-organized flow of information during morphogenesis. Nat. Rev. Mol. Cell Biol. 22 (4), 245–265. https://doi.org/10.1038/s41580-020-00318-6.

Dard, N., Le, T., Maro, B., Louvet-Vallée, S., 2009. Inactivation of aPKCλ reveals a context dependent allocation of cell lineages in preimplantation mouse embryos. PLoS One 4 (9), e7117. https://doi.org/10.1371/journal.pone.0007117.

Davidson, E.H., 2010. Emerging properties of animal gene regulatory networks. Nature 468 (7326), 911–920. https://doi.org/10.1038/nature09645.

De Caluwé, J., Tosenberger, A., Gonze, D., Dupont, G., 2019. Signalling-modulated gene regulatory networks in early mammalian development. J. Theor. Biol. 463 (3), 56–66. https://doi.org/10.1016/j.jtbi.2018.12.008.

De Mot, L., Gonze, D., Bessonnard, S., Chazaud, C., Goldbeter, A., Dupont, G., 2016. Cell fate specification based on tristability in the inner cell mass of mouse blastocysts. Biophys. J. 110 (3), 710–722. https://doi.org/10.1016/j.bpj.2015.12.020.

Dumortier, J.G., Le Verge-Serandour, M., Tortorelli, A.F., Mielke, A., De Plater, L., Turlier, H., Maître, J.L., 2019. Hydraulic fracturing and active coarsening position the lumen of the mouse blastocyst. Science 365 (6452), 465–468. https://doi.org/10.1126/science.aaw7709.

Dye, N.A., Popovic, M., Iyer, K.V., Fuhrmann, J., Piscitello-Gómez, R., Eaton, S., Jülicher, F., 2021. Self-organized patterning of cell morphology via mechanosensitive feedback. elife 10. https://doi.org/10.7554/eLife.57964.

Erzberger, A., Jacobo, A., Dasgupta, A., Hudspeth, A.J., 2020. Mechanochemical symmetry breaking during morphogenesis of lateral-line sensory organs. Nat. Phys. https://doi.org/10.1038/s41567-020-0894-9.

Exelby, K., Herrera-Delgado, E., Perez, L.G., Perez-Carrasco, R., Sagner, A., Metzis, V., Sollich, P., Briscoe, J., 2021. Precision of tissue patterning is controlled by dynamical properties of gene regulatory networks. Development (Cambridge, England) 148 (4). https://doi.org/10.1242/dev.197566.

Giammona, J., Campàs, O., 2021. Physical constraints on early blastomere packings. PLoS Comput. Biol. 17 (1), e1007994 https://doi.org/10.1371/journal.pcbi.1007994.

Gillespie, D.T., 2000. Chemical langevin equation. J. Chem. Phys. 113 (1), 297–306. https://doi.org/10.1063/1.481811.

Gilmour, D., Rembold, M., Leptin, M., 2017. From morphogen to morphogenesis and back. Nature 541 (7637), 311–320. https://doi.org/10.1038/nature21348.

Goehring, N.W., Trong, P.K., Bois, J.S., Chowdhury, D., Nicola, E.M., Hyman, A.A., Grill, S.W., 2011. Polarization of PAR proteins by advective triggering of a pattern-forming system. Science 334 (6059), 1137–1141. https://doi.org/10.1126/science.1208619.

Goel, N.S., Doggenweiler, C.F., Thompson, R.L., 1986. Simulation of cellular compaction and internalization in mammalian embryo development as driven by minimization of surface energy. Bull. Math. Biol. 48 (2), 167–187. https://doi.org/10.1007/BF02460021.

Goldstein, R.E., 2018. Are theoretical results 'results'? elife 7, 1–9. https://doi.org/10.7554/eLife.40018.

Gunawardena, J., 2010. Models in systems biology: the parameter problem and the meanings of robustness. Elem. Comput. Syst.Biol. 21–47 https://doi.org/10.1002/9780470556757.ch2.

Hannezo, E., Heisenberg, C.P., 2019. Mechanochemical feedback loops in development and disease. Cell 178 (1), 12–25. https://doi.org/10.1016/j.cell.2019.05.052.

Hirate, Y., Hirahara, S., Inoue, K., Suzuki, A., Alarcon, V.B., Akimoto, K., Hirai, T., Hara, T., Adachi, M., Chida, K., Ohno, S., Marikawa, Y., Nakao, K., Shimono, A., Sasaki, H., 2013. Polarity-dependent distribution of angiomotin localizes hippo signaling in preimplantation embryos. Curr. Biol. 23 (13), 1181–1194. https://doi.org/10.1016/J.CUB.2013.05.014.

Holmes, W.R., Reyes de Mochel, N.S., Wang, Q., Du, H., Peng, T., Chiang, M., Cinquin, O., Cho, K., Nie, Q., 2017. Gene expression noise enhances robust Organization of the Early Mammalian Blastocyst. PLoS Comput. Biol. 13 (1), 1–23. https://doi.org/10.1371/journal.pcbi.1005320.

Honda, H., Motosugi, N., Nagai, T., Tanemura, M., Hiiragi, T., 2008. Computer simulation of emerging asymmetry in the mouse blastocyst. Development 135 (8), 1407–1414. https://doi.org/10.1242/dev.014555.

Kang, M., Piliszek, A., Artus, J., Hadjantonakis, A.-K., 2013. FGF4 is required for lineage restriction and salt-and-pepper distribution of primitive endoderm factors but not their initial expression in the mouse. Development 140 (2), 267–279. https://doi.org/10.1242/dev.084996.

Kondo, S., Miura, T., 2010. Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329 (5999), 1616–1620. https://doi.org/10.1126/science.1179047.

Korotkevich, E., Niwayama, R., Courtois, A., Friese, S., Berger, N., Buchholz, F., Hiiragi, T., 2017. The apical domain is required and sufficient for the first lineage segregation in the mouse embryo. Dev. Cell 40 (3), 235–247.e7. https://doi.org/10.1016/j.devcel.2017.01.006.

Krens, S.F.G., Heisenberg, C.-P., 2011. Cell sorting in development. Curr. Top. Dev. Biol. 95, 189–213. https://doi.org/10.1016/B978-0-12-385065-2.00006-2.

Krieg, M., Arboleda-Estudillo, Y., Puech, P.H., Käfer, J., Graner, F., Müller, D.J., Heisenberg, C.P., 2008. Tensile forces govern germ-layer organization in zebrafish. Nat. Cell Biol. 10 (4), 429–436. https://doi.org/10.1038/ncb1705.

Krupinski, P., Chickarmane, V., Peterson, C., 2011. Simulating the mammalian blastocyst - molecular and mechanical interactions pattern the embryo. PLoS Comput. Biol. 7 (5) https://doi.org/10.1371/journal.pcbi.1001128.

Le Guillou, L., Dard, N., Jérôme, G., Bernard, M., Sophie, L.-V., Bertrand, L., 2009. A 3D mechanical model of the early mammalian embryo. J. Biol. Phys. Chem. 9 (4), 11–16. https://doi.org/10.4024/10gu09a.jbpc.09.01.

Lenne, P.-F., Munro, E., Heemskerk, I., Warmflash, A., Bocanegra-Moreno, L., Kishi, K., Tlili, S., 2021. Roadmap for the multiscale coupling of biochemical and mechanical signals during development. Phys. Biol. 18 (4), 041501 https://doi.org/10.1088/1478-3975/abd0db.

Le Verge-Serandour, M., Turlier, H., 2021. A hydro-osmotic coarsening theory of biological cavity formation. PLoS Comput. Biol. 17 (9), e1009333 https://doi.org/10.1371/journal.pcbi.1009333.

Leonavicius, K., Royer, C., Preece, C., Davies, B., Biggins, J.S., Srinivas, S., 2018. Mechanics of mouse blastocyst hatching revealed by a hydrogel-based microdeformation assay. Proc. Natl. Acad. Sci. U. S. A. 115 (41), 10375–10380. https://doi.org/10.1073/pnas.1719930115.

Lewis, J., 2003. Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. Curr. Biol. 13 (16), 1398–1408. https://doi.org/10.1016/S0960-9822(03)00534-7.

Maître, J.-L., 2017. Mechanics of blastocyst morphogenesis. Biol. Cell. 109 (9), 323–338. https://doi.org/10.1111/boc.201700029.

Maître, J.-L., Niwayama, R., Turlier, H., Nedelec, F., Hiiragi, T., 2015. Pulsatile cell-autonomous contractility drives compaction in the mouse embryo. Nat. Cell Biol. 17 (7), 849–855. https://doi.org/10.1038/ncb3185.

Maître, J.-L., Turlier, H., Illukkumbura, R., Eismann, B., Niwayama, R., Nédélec, F., Hiiragi, T., 2016. Asymmetric division of contractile domains couples cell positioning and fate specification. Nature 536 (7616), 344–348. https://doi.org/10.1038/nature18958.

Maître, J.L., Berthoumieux, H., Krens, S.F.G., Salbreux, G., Jülicher, F., Paluch, E., Heisenberg, C.P., 2012. Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells. Science 338 (6104), 253–256. https://doi.org/10.1126/science.1225399.

Mintz, B., 1962. Experimental study of the developing mammalian egg: removal of the zona pellucida. Science 138 (3540), 594–595. https://doi.org/10.1126/science.138.3540.594.

Nakamura, T., Yabuta, Y., Okamoto, I., Aramaki, S., Yokobayashi, S., Kurimoto, K., Sekiguchi, K., Nakagawa, M., Yamamoto, T., Saitou, M., 2015. SC3-seq: a method for highly parallel and quantitative measurement of single-cell gene expression. Nucleic Acids Res. 43 (9) https://doi.org/10.1093/nar/gkv134.

Nissen, S.B., Perera, M., Gonzalez, J.M., Morgani, S.M., Jensen, M.H., Sneppen, K., Brickman, J.M., Trusina, A., 2017. Four simple rules that are sufficient to generate the mammalian blastocyst. PLoS Biol. 15 (7), e2000737 https://doi.org/10.1371/journal.pbio.2000737.

Niwayama, R., Moghe, P., Liu, Y., Fabrèges, D., Buchholz, F., Piel, M., Hiiragi, T., 2019. A tug-of-war between cell shape and polarity controls division orientation to ensure robust patterning in the mouse blastocyst. Dev. Cell 1–11. https://doi.org/10.1016/j.devcel.2019.10.012.

Oates, A.C., Gorfinkiel, N., González-Gaitán, M., Heisenberg, C.-P., 2009. Quantitative approaches in developmental biology. Nat. Rev. Genet. 10 (8), 517–530. https://doi.org/10.1038/nrg2548.

Paluch, E.K., 2015. After the greeting: realizing the potential of physical models in cell biology. Trends Cell Biol. 25 (12), 711–713. https://doi.org/10.1016/j.tcb.2015.08.001.

Paluch, E.K., Heisenberg, C.-P., 2009. Biology and physics of cell shape changes in development. Curr. Biol. 19 (17), R790–R799. https://doi.org/10.1016/j.cub.2009.07.029.

Petridou, N.I., Corominas-Murtra, B., Heisenberg, C.P., Hannezo, E., 2021. Rigidity percolation uncovers a structural basis for embryonic tissue phase transitions. Cell 184 (7), 1914–1928.e19. https://doi.org/10.1016/j.cell.2021.02.017.

Pierre, A., Sallé, J., Wühr, M., Minc, N., 2016. Generic theoretical models to predict division patterns of cleaving embryos. Dev. Cell 39 (6), 667–682. https://doi.org/10.1016/j.devcel.2016.11.018.

Plusa, B., Piliszek, A., Frankenberg, S., Artus, J., Hadjantonakis, A.K., 2008. Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. Development 135 (18), 3081–3091. https://doi.org/10.1242/dev.021519.

Ralston, A., Rossant, J., 2008. Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. Dev. Biol. 313 (2), 614–629. https://doi.org/10.1016/j.ydbio.2007.10.054.

Rossant, J., 2016. Making the mouse blastocyst. In: Current Topics in Developmental Biology, , 1st ed.Vol. 117. Elsevier Inc, pp. 275–288. https://doi.org/10.1016/bs.ctdb.2015.11.015.

Ruiz-Herrero, T., Alessandri, K., Gurchenkov, B.V., Nassoy, P., Mahadevan, L., 2017. Organ size control via hydraulically gated oscillations. Development 144 (23), 4422–4427. https://doi.org/10.1242/dev.153056.

Saiz, N., Mora-Bitrià, L., Rahman, S., George, H., Herder, J.P., García-Ojalvo, J., Hadjantonakis, A.K., 2020. Growth factor-mediated coupling between lineage size and cell fate choice underlies robustness of mammalian development. elife 9, 1–38. https://doi.org/10.7554/eLife.56079.

Schliffka, M.F., Tortorelli, A.-F., Özgüç, Ö., de Plater, L., Polzer, O., Pelzer, D., Maître, J.-L., 2021. Multiscale analysis of single and double maternal-zygotic Myh9 and Myh10 mutants during mouse preimplantation development. elife 10, e68536. https://doi.org/10.7554/eLife.68536.

Shahbazi, M.N., 2020. Mechanisms of human embryo development: from cell fate to tissue shape and back. Development (Cambridge) 147 (14). https://doi.org/10.1242/dev.190629.

Simon, C.S., Rahman, S., Raina, D., Schröter, C., Hadjantonakis, A.-K., 2020. Live visualization of ERK activity in the mouse blastocyst reveals lineage-specific signaling dynamics. Dev. Cell 1–13. https://doi.org/10.1016/j.devcel.2020.09.030.

Stephenson, R.O., Yamanaka, Y., Rossant, J., 2010. Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin. Development 137 (20), 3383–3391. https://doi.org/10.1242/dev.050195.

Sugimura, K., Lenne, P.-F., Granner, F., 2016. Measuring forces and stresses in situ in living tissues. Development 1432, 186–196. https://doi.org/10.1242/dev.119776.

Szabó, B., Szöllösi, G.J., Gönci, B., Jurányi, Z., Selmeczi, D., Vicsek, T., 2006. Phase transition in the collective migration of tissue cells: experiment and model. Phys. Rev. E 74 (6), 061908. https://doi.org/10.1103/PhysRevE.74.061908.

Tosenberger, A., Gonze, D., Bessonnard, S., Cohen-Tannoudji, M., Chazaud, C., Dupont, G., 2017. A multiscale model of early cell lineage specification including cell division. NPJ Syst.Biol. Appl. 3 (1), 1–10. https://doi.org/10.1038/s41540-017-0017-0.

Tosenberger, A., Gonze, D., Chazaud, C., Dupont, G., 2019. Computational models for the dynamics of early mouse embryogenesis. Int. J. Dev. Biol. 63 (3–5), 131–142. https://doi.org/10.1387/ijdb.180418gd.

Tsunoda, Y., Yasui, T., Nakamura, K., Uchida, T., Sugie, T., 1986. Effect of cutting the zona pellucida on the pronuclear transplantation in the mouse. J. Exp. Zool. 240 (1), 119–125. https://doi.org/10.1002/jez.1402400115.

Tvergaard, V., Needleman, D., Needleman, A., 2021. A mechanical model of blastocyst hatching. Extreme Mechanics Letters 42, 101132. https://doi.org/10.1016/j.eml.2020.101132.

Wamaitha, S.E., Niakan, K.K., 2018. Human pre-gastrulation development. In: Current Topics in Developmental Biology, , 1st ed.Vol. 128. Elsevier Inc, pp. 295–338. https://doi.org/10.1016/bs.ctdb.2017.11.004.

Wennekamp, S., Mesecke, S., Nédélec, F., Hiiragi, T., 2013. A self-organization framework for symmetry breaking in the mammalian embryo. Nat. Rev. Mol. Cell Biol. 14 (7), 454–461. https://doi.org/10.1038/nrm3602.

White, M.D., Zenker, J., Bissiere, S., Plachta, N., 2018. Instructions for assembling the early mammalian embryo. Dev. Cell 45 (6), 667–679. https://doi.org/10.1016/j.devcel.2018.05.013.

Wicklow, E., Blij, S., Frum, T., Hirate, Y., Lang, R.A., Sasaki, H., Ralston, A., 2014. HIPPO pathway members restrict SOX2 to the inner cell mass where it promotes ICM fates in the mouse blastocyst. PLoS Genet. 10 (10), e1004618 https://doi.org/10.1371/journal.pgen.1004618.

Yanagida, A., Revell, C., Stirparo, G.G., Corujo-Simon, E., Aspalter, I.M., Peters, R., de Belly, H., Cassani, D.A.D., Achouri, S., Blumenfeld, R., Franze, K., Paluch, E.K., Nichols, J., Chalut, K.J., 2020. In: Cell Surface Fluctuations Regulate Early Embryonic Lineage Sorting. BioRxiv, pp. 1–9. https://doi.org/10.1101/2020.08.16.250084.

Zenker, J., White, M.D., Gasnier, M., Alvarez, Y.D., Lim, H.Y.G., Bissiere, S., Biro, M., Plachta, N., 2018. Expanding actin rings zipper the mouse embryo for blastocyst formation. Cell 173 (3), 776–791.e17. https://doi.org/10.1016/j.cell.2018.02.035.

Zhang, H.T., Hiiragi, T., 2018. Symmetry breaking in the mammalian embryo. Annu. Rev. Cell Dev. Biol. 34, 405–426. https://doi.org/10.1146/annurev-cellbio-100617-062616.

Zhang, L., Radtke, K., Zheng, L., Cai, A.Q., Schilling, T.F., Nie, Q., 2012. Noise drives sharpening of gene expression boundaries in the zebrafish hindbrain. Mol. Syst. Biol. 8 (613), 613. https://doi.org/10.1038/msb.2012.45.

Zhu, M., Cornwall-Scoones, J., Wang, P., Handford, C.E., Na, J., Thomson, M., Zernicka-Goetz, M., 2020. Developmental clock and mechanism of de novo polarization of the mouse embryo. Science (New York, N.Y.) 370 (6522). https://doi.org/10.1126/science.abd2703.