Computational multiscale modeling of embryo development

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Recent advances in live imaging and genetics of mammalian development which integrate observations of biochemical activity, cell–cell signaling and mechanical interactions between cells pave the way for predictive mathematical multi-scale modeling. In early mammalian embryo development, two of the most critical events which lead to tissue patterning involve changes in gene expression as well as mechanical interactions between cells. We discuss the relevance of mathematical modeling of multi-cellular systems and in particular in simulating these patterns and describe some of the technical challenges one encounters. Many of these issues are not unique for the embryonic system but are shared by other multi-cellular modeling areas.

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Introduction

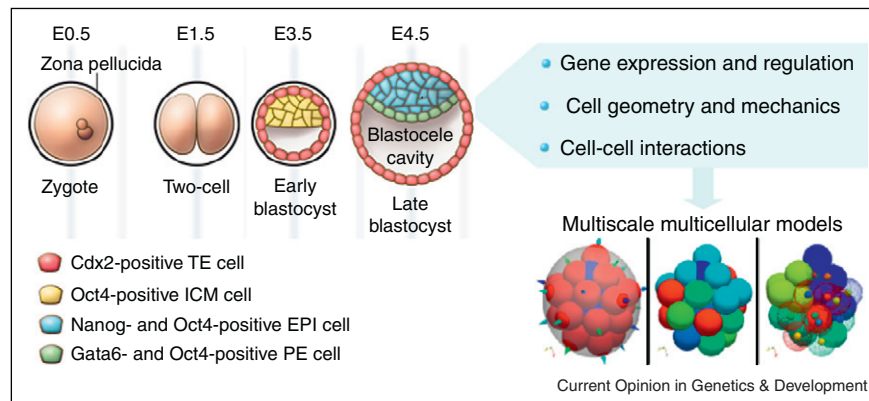The early mammalian embryo development is characterized by cell differentiation events leading to the formation of two extraembryonic lineages: trophectoderm (TE) and primitive endoderm (PE). Valuable insights into this process have been gained with single cell expression studies and live imaging techniques [1–4,5●,6]. Most efforts have focused on determining the gene expression patterns that characterize the different lineages. Identified transcription factors for specific cell fates are, for example, *Cdx2* and *Gata6*, accompanied by pluripotency genes: *Nanog, Oct4* and *Sox2* (Figure 1). The dynamics of the genetic network involving these genes has been explored in several deterministic [7,8] as well as stochastic models [9,10]. Single cell experiments [11] could provide the impetus to understand what drives cell commitment, that is, permissive versus instructive cell fate decision [12,13]. While genetic network approaches offer invaluable insight into tissue differentiation at the genetic level, embryogenesis involves also co-ordination of cell

division, movement and cell differentiation leading to tissue formation [14●●]. The high plasticity of single cell developmental fate and adaptivity to changing conditions within early embryo [6,15●] suggests that robust formation of precisely localized specialized tissue precursors involves mechanisms going beyond single cells. Events like cell polarization during specification of the TE, directional cell migration and selective apoptosis during PE formation, require cell–cell signaling and interactions which convey relative positional information to cells [16●●]. Hence, a complete understanding of early embryogenesis regulation must consider different scales of multicellular interactions, including intracellular and intercellular biochemical signaling. In addition to these effects, the importance of mechanical properties of cells in the processes of morphogenesis has been recognized [17]. Therefore, successful models of embryogenetic events should integrate genetic, biochemical and mechanical interactions at the cellular level. Recent research in the plant sciences has shown the success of the tight integration of theory and experiment in understanding how bio-chemistry and mechanics leads to the development of organs such as the shoot, roots and leaves [18]. Here we discuss computational multicellular, multiscale modeling techniques [19,20] and their implementations in early developmental events of mammalian embryogenesis. In addition to the current state-of-the-art in modeling, we describe future challenges that must be met to successfully integrate multicellular models of different scales. We also suggest how these models could be useful in other areas such as models of tumor evolution in cancer and stem cell regeneration.

Models of embryo development

Despite the wealth of information gained from experiments [21–24], our understanding of early mammalian embryogenesis is far from complete [25]. One reason is the complexity of biological systems in general where interactions between even a few components can lead to complicated and unpredictable behavior making it difficult to deduce interaction rules from observations of the entire system. Also, some important interactions might not yet be identified. In both cases computational modeling offers valuable contribution. It allows for hypotheses testing, quantifying observations, complementing missing elements and isolating crucial components during iterative validation of models with experimental results. From a modeling perspective, the early mammalian embryo is both interesting and challenging system to study. Starting from a single cell, it develops over 4.5 days, through the cell cleavages forming a blastocyst which consists of more than 120 cells (Figure 1). At this

Figure 1

Developmental events in early mouse embryogenesis (left). During the first 4.5 days the embryo develops from a single cell to the late blastocyst. Processes of trophectoderm and primitive endoderm formation involve specific gene expression (bottom) as well as cell and tissue mechanics and cell–cell signaling (right). Taking into account interactions between all these processes requires multiscale and multicellular models integrating biochemical and mechanical aspects of morphogenesis. The lower right shows results from such model [37] which correspond to **(a)** cell polarity, **(b)** gene expression levels and **(c)** lineage information.
A part of the figure was adapted from Figure 1 in Cockburn and Rossant [6].

stage there are three well-specified differentiated cell types — the trophectoderm cells, PE cells and the inner cell mass (ICM). This limited number of cells constrained by the pellucid zone, and well-defined morphological events make this a tractable system to model. Availability of good spatio-temporal resolution confocal microscopy data renders this system a perfect target for studying interactions of genetic and mechanical signals in a 3D modeling context.

Systems biology has proven to be a powerful approach for elucidating stem cell lineage decisions [26]. It facilitates construction of theoretical models of gene interactions regulating this process. An early model of the genetic network describing cell fate [7] suggested that mutual inhibition between pluripotency and differentiation genes drive the switching between different developmental states. In this model interplay between Oct4 and Cdx2 governs the trophectoderm fate. The core of this model is mutual antagonism between Cdx2 and Oct4 as well as the self-regulation of each individual gene. These key interactions lead to a bistable switch-like behavior, in which either Oct4 is on, Cdx2 is off or vice versa. This aspect of the model has been used in Krupinski *et al.* [37•] with an input from the polarity network to model the spatial patterning of the trophectoderm. Interactions between Gata6 and Nanog determine endoderm formation. These two modules for the two distinct developmental states interact through a network of connections with other genes. This core network was recently extended with Tead4 placed upstream of Cdx2 and Eomes and Elf5, which form a positive feedback loop with Cdx2 stabilizing the trophectoderm fate [25,27]. Interestingly, a plausible conceptual model of trophectoderm differentiation [28•] involving Tead4, its

co-activator Yap and the Hippo signaling pathway, connected gene expression to a relative position of a cell in the embryo mass (inside–outside) and the cell polarity.

Embryogenesis is essentially a multicellular process. The mechanics of cells and tissues has been studied less extensively and only recently has this important aspect of morphogenesis gained some attention in different organisms [29–32]. The simplest description of multicellular systems is by population models in which individual cell interactions are abstracted into average behavior of cell classes. The observed time evolution of cell numbers in each class can be then compared to experimental data. This model was used for analysis of different mechanisms of cell organization during PE and epiblast separation [16••], concluding that the separation is the most robust when gene expression induced cell sorting is accompanied by the cell position influenced gene expression induction.

In Honda *et al.* [33•], cell–cell mechanical interactions are introduced leading to positioning of ICM and blastocoel within the pellucid zone. Cells are represented by polyhedral compartments identified by their vertex positions. This model successfully demonstrated the emergence and positioning of the blastocoel cavity within the blastocyst; however, it does not include genetic interactions coupled to the spatial degrees of freedom and cell divisions. Another frequently used modeling technique for describing mechanical interactions, especially cell sorting, is the Cellular Potts Model [34], in which space is divided into a grid with sites which consist of a set of variables. Each biological cell is defined as a collection of connected sites with identical index variables. Interactions between sites contribute to the total free energy

of the system. The configuration of the entire multi-cellular system is evolved in time by attempting to minimize this energy through a Monte Carlo procedure. This technique was used for modeling of the mechanics of the early embryo evolution including the compaction process [35].

While both of the above models were able to describe the mechanical aspects of the cell dynamics in embryogenesis, recent experiments suggest that feedback between cell mechanics and genetic networks could be important [36]. In particular, cell polarization and segregation during trophectoderm and PE formation involves such a feedback. Recently a model connecting mechanical and genetic interactions was developed to analyze these different scenarios of cell fate specification during these processes of embryogenesis [37•]. Here each cell, which hosts a simplified genetic network with *Cdx2/Oct4* and *Gata6/Nanog*, is represented by an ellipsoidal incompressible elastic cell interacting with other cells and the pellucid zone boundary through elastic, drag and adhesion forces. In addition to a set of mechanical properties each cell contains parameters which describe cell cycle, polarization, molecule concentration, etc. These intrinsic cell properties are accompanied by division rules which orient the direction of a cell division plane depending on the state of the cell. With this model, spatio-temporal patterns were analyzed with emphasis on the lineage specification in the pre-implantation embryo — the trophectoderm and endoderm layer formations. The coupling of gene expression with the mechanics is important for both cases. Two hypotheses have been suggested for trophectoderm formation: the position determines gene expression or the gene expression determines the position [25,38]. In the model implementations of each scenario the Cdx2/Oct4 mutual inhibition and self-interactions which lead to a bistable switch mechanism played a pivotal role of switching between pluripotent and trophectoderm states with the differences how the transition between states is triggered. In the first case, the outside position of the cell promotes the Cdx2 expression and hence determines trophectoderm fate. In the second case, asymmetric cell divisions biased by high Cdx2 levels together with apical polarization of Cdx2 mRNA provides a feedback loop between gene expression and cell position and lead to the emergence of spatially segregated pattern of Cdx2 expression. The simulations suggested that the requirement of robust emergence of Cdx2 expression in outer cells favors the first case (Figure 2a). During endoderm formation, the tissue is patterned by mechanical properties which depend on gene expression. Cells express *Nanog* and *Gata6* in a non-overlapping way (which is assumed to arise from the mutual antagonism between Nanog and Gata6) and experience differential adhesion between different cell types (*Nanog* cells have higher self-adhesion than *Gata6* expressing cells, with the cross-adhesion being the

lowest). In addition to this mechanism (Figure 2b), two different models of mechanical interaction of cells with blastocoel were tested: static boundary and dynamic interactions via pressure forces. Both of these models could lead to formation of the endoderm; however, the former required additional assumption of directional signal from blastocoel attracting *Gata6* cells.

The computational model was instrumental in comparing different hypothesis for robust mechanisms in tissue patterning.
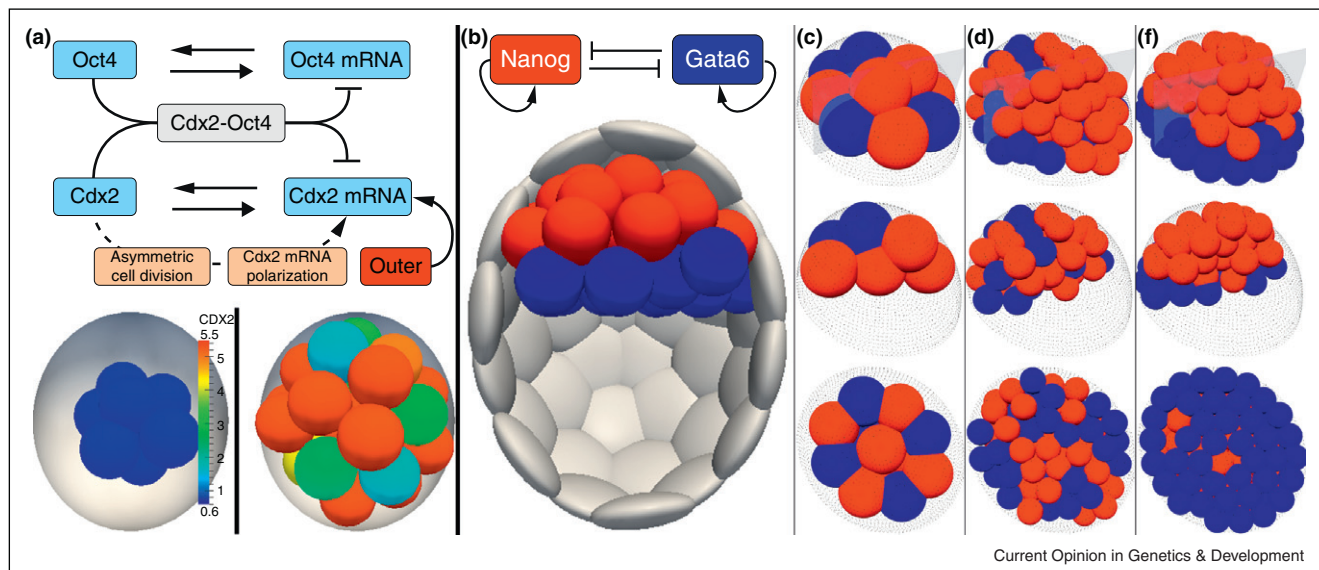
Challenges in genetic-mechanical multiscale modeling

There are several challenges in multi-scale modeling of multi-cellular systems, for example, bridging different spatio-temporal scales, handling of discrete events and interactions, effective use of computational resources.

For example, the time scales at which mechanical and biochemical interactions occur can be very different, as in Krupinski *et al.* [37•]. To make the simulations efficient, numerical solvers should treat these processes with separate time steps appropriately. To take care of interactions between these processes or events such as cell division, cell death, which could involve feedback between the mechanical and chemical degrees of freedom, algorithms for scheduling the updates of variables operating at different time scales need to be implemented.

An important issue is how the computational demand scales with system size. If the number of operations required to perform the simulation grows too rapidly with number of cells (N) the model might be unsuited to treat some processes. With most cellular models, the interactions are limited to direct neighbors and since the number of neighbors (order of 10) does not grow with system size, the complexity of those models only grows like N, which is not too prohibitive. In models like the cellular Potts, biological cells are defined by many computational elements (sites m), and the computational demand grows like $N * m$. If the model is to describe intracellular processes we have to retain high cellular resolution of the lattice (large m), which might lead to excessive computational demands. In general high computational cost of multiscale simulations will require development of optimized algorithms or parallel computations. A major future challenge is to integrate the dynamics of processes at different spatial scales, for example, intercellular processes within multicellular models. This requires the direct representation of the intercellular structure, for example, including description of the cell membrane, nucleus and the transport of molecules between them, or careful approximate treatment of the dynamics of the components of this structure. One example relevant to mammalian embryogenesis is the spatio-temporal dynamics of cell polarity genes

Figure 2

Examples of multicellular models connecting genetic and mechanical interactions in early embryogenesis from [37]. **(a)** Trophectoderm specification is governed by a simplified gene network based on mutual repression between Oct4 promoting pluripotency and Cdx2 deciding the trophectoderm fate. Expression of Cdx2 can, for example, be affected by simple positional signal in outer cells as in the 'inside–outside' model. Alternatively, it can be regulated by a more intricate mechanism in which the Cdx2 mRNA polarization and its asymmetric distribution during division form a positive feedback loop enhancing Cdx2 expression in outer cells indirectly. The bottom pane presents results of simulation of a polarity-based model showing differentiation of outer cells expressing higher levels of Cdx2. **(b)** A schematic view of primitive endoderm formation with a genetic switch based on mutual inhibition and self-activation of Nanog and Gata6. The Gata6 expressing cells, marking endoderm fate, locate themselves closer to ab-embryonic part of the blastocyst, separating epiblast (expressing Nanog) from blastocoel (void space in the picture). (c, d, f) Results from a cell sorting simulation illustrating the principles of differential adhesion. Top pane shows a perspective view, middle pane presents the cross section and the bottom pane displays a view from blastocoel side. **(c)** Initial setup of the simulation with salt-and-pepper distribution of Nanog and Gata6 expressing cells. **(d)** Distribution of Nanog and Gata6 cells after two rounds of cleavages when there is no difference in adhesion properties between cells. **(f)** If Nanog expression is assumed to promote higher self-adhesion and lower cross-adhesion to Gata6 cells one observes a full separation of both cell populations.

ultimately involved in the trophectoderm tissue patterning [38].

Related areas of research

We have focused on the development of the early embryo. The interplay between mechanics and gene regulation is, however, a common feature of most developmental problems, such as limb formation [39]. Development of multiscale models is crucial for understanding of interactions also in such systems.

Some parallels can be drawn from modeling of stem cell niches in plant meristems. There the genetic networks are fairly well known [40] and their connection to spatial and mechanical aspects of morphogenesis has begun to emerge [30], suggesting some intricate interaction between biochemical processes within cell and physical signals like mechanical stress [41].

The evolution of cancer cells share many properties with embryonic development as biochemical reactions couple to cell growth and mechanical properties that are important for migration. Furthermore, the recent concept of

cancer stem cells brings the two problems even further together. Most cancer cell modeling so far has focused on mechanical properties [42,43]. Recently, also multiscale modeling approaches with both biochemical and mechanical interactions have been pursued [44•].

Future applications

The varied repertoire of final differentiated roles that stem cells play makes them crucial in studies of tissue regeneration. In their natural environment, stem cells are harbored in niches which provide the appropriate growth factors, cellular signals and mechanical cues. These maintain homeostasis as well as provide differentiation signals upon request by the organism. Elucidating these mechanisms could pave the way for artificial generation of differentiated cells, by mimicking the *in vivo* environment with an artificial niche [45–47]. Cells can be exposed to a combination of diffusing signals, external shear stresses due to fluid as well as the surrounding niche surface. The latter can itself be subjected to external stresses. The multiscale computational framework described above can be gainfully used to study some of these processes — simulating an *in vitro* stem cell niche.

The aim would be to simulate cell growth, division and differentiation of cells which are subject to spatial and temporal signals and mechanical perturbations. Some of the challenges would be to explicitly describe cell–surface interactions with the underlying niche which could exert variable stress and adhesive forces; fluid interactions with individual cells; cell–cell interactions and differentiation events. The ultimate goal would be to engineer a specific tissue starting with stem cells within a simulated niche [48].

Conclusion

The future is ripe for computational multi-scale modeling as available data have matured with regard to appropriate detail and resolution. Models provide hypothesis for further testing and could significantly advance our understanding both for fundamental biology and the more applied clinical setting of tissue regeneration. There are several technical advancements required at multiple scales to move these models forward.

There is now growing evidence from single cell experiments that stochasticity plays an important role [9,11,13,49] in cell fate specification, which suggests that an important advance would be to include stochastic simulations into intercellular and intracellular network models. The role of cell shape and cellular adhesion, critical in determining cell–cell interactions [29], needs to be addressed in the next generation of models in which each cell maintains the integrity of its own cellular membrane. Furthermore, models should include mechanisms in which cells sense signals at their membranes and relay them to the organelles, thereby simulating the cell–cell interactions. A subject for the future is how these varied complex processes could lead to predictable and useful computational models of development.

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