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Review

Towards understanding the roles of position and geometry on cell fate decisions during preimplantation development

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ABSTRACT

The first lineage segregation event in mouse embryos produces two separate cell populations: inner cell mass and trophectoderm. This is understood to be brought about by cells sensing their position within the embryo and differentiating accordingly. The cellular and molecular underpinnings of this process remain under investigation and have variously been considered to be completely stochastic or alternately, subject to some predisposition set up at fertilisation or before. Here, we consider these views in light of recent publications, discuss the possible role of cell geometry and mechanical forces in this process and describe how modelling could contribute in addressing this issue.

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1. Introduction: environmental influences and stochasticity in lineage segregation

The first lineage segregation event during mouse embryogenesis is the formation of the pluripotent inner cell mass (ICM) and trophectoderm (TE) at a stage when the embryo is composed of approximately 32 cells. The TE arises from cells on the outside of the embryo while the ICM arises from those inside cells enclosed by the TE. Subsequent to this, the ICM further differentiates into the pluripotent epiblast and overlying primitive endoderm. Concomitantly, the blastocyst undergoes morphogenetic changes, as the cavity expands. The molecular genetic basis for the differentiation of these early cell types has been extensively studied and several excellent reviews of the field exist [1–3].

However, in the past few years there have been exciting findings that have started to reveal the mechanisms by which blastomeres can incorporate information about their physical environment into the 'internal' genetic imperatives that drive their differentiation. In this perspective piece, we look at the potential role of geometry and mechanics on cell fate determination in the early embryo. We use *geometry* to refer to the relative positions of cells within the embryo particularly with respect to each other and *mechanics* to refer to the forces on cells, irrespective of position. We discuss ideas relating to how the actin cytoskeleton, apical polarity complex proteins and the YAP transcriptional regulator provide mechanisms by which blastomeres can incorporate cues arising from geometry or

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their mechanical environment into the decision making process determining their fate. We consider ways in which computational approaches can help us understand how these varied inputs can act together to give consistent lineage allocation outcomes.

The role of environmental influences on lineage segregation is closely linked to the question of whether some sort of lineage information is present in the embryo as early as at the time of fertilisation – whether the course of development is in some way already *determined* in the way, for example, axial information is pre-determined in the *Drosophila* embryo or, as an extreme example, cell fate is determined in *Caenorhabditis elegans* [4–6]. In these organisms, lineage segregation is in a sense 'hardwired' into the zygote at fertilisation by the localisation of specific molecular determinants and there is little need (and possibly room) for environmental influences on the course of development. Given that the highly regulative nature of mouse development is beyond debate, in the murine context such pre-determination is described rather in terms of a predisposition, in the unperturbed state, of specific cells to particular fates [7].

The opposing view is that lineage determination is a *stochastic* process [8,9]. Since this term is subject to interpretation, we note that we use stochastic to mean that the outcome of a particular process cannot be predicted with certainty given the starting conditions, though one can ascribe a probability to particular outcomes. In this view environmental input such as the position of a blastomere or the forces it is subject to could play a significant role in lineage determination. They could act as a source of stochasticity, for example through the 'chance' position of a blastomere affecting its fate. Equally, they could act conversely as a regulatory buffer against underlying sources of stochasticity such as transcriptional noise.

Geometry as a regulatory buffer sounds abstract, but is made concrete by a simple example. If you break a strand of dry spaghetti by holding the two ends and bending, it infamously typically breaks in two places along its length [10] giving three pieces which can be thought of as two 'outside' pieces and one 'inside' piece. Despite considerable stochasticity in the precise places the spaghetti breaks, in cell-fate terms the output appears deterministic (one inside and two outside pieces every time) because of the starting geometry.

In practice, the modes of embryonic development in different species fall on a spectrum. At one end lies predetermined development, where an embryonic template is laid down at or shortly after fertilisation and every healthy embryo produces the same lineage tree. At the other end lies highly stochastic development where, at early stages, there is no template and cells behave independently, leading different embryos to produce completely different lineage trees. Stochastic development *requires* regulation so that the differing lineage trees produce consistent embryos. Mammalian embryos are certainly not completely predetermined, but there is uncertainty about where along this spectrum they lie. Advocates of "predisposition" suggest that cell fates are biased by inherited characteristics such as the sperm entry point, leading to a template that results in somewhat homogenous lineage trees [11]. Advocates of stochasticity typically emphasise the role of external perturbations and chance variations in cell positions, division angles, *etc.* in driving divergences between lineage trees [9]. However, the two positions are neither polar opposites nor irreconcilable, rather the question is one of degree: what elements of stochasticity and predisposition explain the observed variation between mammalian embryos? In this context, computational modelling can make an important contribution to understanding the possible influence of geometry and mechanics on lineage segregation and the extent to which decisions made in the early embryo are stochastic, whether constrained by geometry or not.

2. Modelling stochasticity in early embryonic development

At its best theory does not just describe, it unifies, explains and predicts. By this yardstick quantitative modelling of mammalian embryos is itself embryonic, not yet even describing how and when early cell fates are specified (or even a clear sense of what fate specification means) let alone elucidating unifying design principles or providing insights into developmental tradeoffs. The difficulty has several origins, including that mammalian embryos appear to sit uncomfortably somewhere between determinism and stochasticity, and that they have rather too many cells to elegantly model each individually but too few to model as a continuum.

However, we see several avenues for progress. Firstly, we take inspiration from recent advances in the stochastic modelling of stem-cell maintained tissue [12–15]. The key to much of this success has been modelling stem-cell division and fate choices as independent random processes, a surprisingly simple assumption which allows the modelling to be done in the language of random-branching processes. In maintenance problems this leads to nice mathematical results because maintenance is quasi-static, so the branching processes are "critical". These results are surely lacking in embryos, where cell number grows exponentially, but we expect similar stochastic modelling of cell division and fate choices will nonetheless be useful [16].

The need for stochastic models is illustrated by our previous work [17]. At the 32 cell stage, mouse embryos reliably produce 11–12 ICM cells and 20–21 trophectoderm cells, but we saw substantial variation in the lineage trees that give rise to these consistent results. For example, we saw embryos where all 16-cell-stage blastomeres gave rise to daughters with matching fates, and examples where five 16-cell-stage blastomeres had one ICM daughter and one trophectoderm daughter. These consistent results from varying pathways are indicative of the balance between stochasticity and regulation that a successful model must capture. Our data sets were not large enough to meaningfully test stochastic models. For this reason, we look forward to full linage trees for much larger numbers of embryos in the future.

A good place to start is likely to be with cell division angles at the 8- to 16-cell transition. In our recent work we showed that the division angle chosen by cells at this point is not isotropically distributed, but biased towards asymmetric divisions. A simple stochastic model, inspired by the aforementioned stem cell maintenance, is that each cell "draws" a division angle from this biased distribution independently. This independence is, with a large enough data set, a statistically testable prediction, as it demands that the levels of variation within an embryo and between embryos must be consistent. If true, this isolates a significant source of stochasticity and we should next investigate how it is regulated, asking to what extent is the resultant inter-embryo variation still present before the next division round. If it is not true, we learn that cell choices of division angle are coordinated, indicating the 8-cell embryo is to some extent patterned.

Going beyond the 8- to 16-cell division, we run into the added difficulty of geometry which can, in fact, provide a degree of regulation. A random-branching stochastic model of cell fate choice would yield a non-zero chance of all cells ending up on the inside or on the outside, both of which are clearly geometrically impossible. A very pertinent question is whether geometry can provide enough regulation on its own to explain early term embryo lineage trees. A good framework to start testing this idea in would be a stochastic analogue of the traditional inside-outside model [18] in which cells divide stochastically and independently and the position of the resultant daughter cells determines their ultimate fate. In such a model geometry will indeed provide a degree of regulation, making the final numbers of ICM and trophectoderm cells

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