COMMENTARY

Self-organization principles in stem-cell-derived synthetic embryo models

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As embryos, we were extremely lucky to survive implantation, when we transitioned from a simple ball of undifferentiated cells to a complex structure of distinct lineages. During development, fertilization generates a totipotent zygote that ultimately gives rise to the myriad cell types of the whole organism. In mouse embryos, the first cell fate decision leads to trophectoderm (TE) progenitors on the outside of the early embryo, surrounding the inner cell mass. Epiblast (EPI) and primitive endoderm (PE) cells then arise from the inner cell mass and are initially intermingled (embryonic day 3.5). Thus, the mammalian pre-implantation embryo comprises three lineage compartments: TE, destined to become the placenta; PE, destined to become the yolk sac; and EPI, pluripotent cells that will generate the entire body.

Just before mouse embryo implantation, PE cells sort "below" the EPI, and the TE organizes into two regions. The polar TE forms "above" the EPI and will become extra-embryonic ectoderm (ExE), whereas mural TE surrounds the blastocyst cavity and will invade the uterus. After implantation, the ExE and EPI become enveloped by the PE-derived visceral endoderm, forming the "egg cylinder" (Figure 1A). These dramatic reorganization and transition events are essential for life and viability but are frequently unsuccessful, and how they occur has remained a mystery. Although the self-assembly instructions are intrinsic to the embryo, they have been impossible to uncover—because during this process, the embryo is embedded within the mother and, hence, is inaccessible.

Stem-cell-derived embryo models have given us the unprecedented opportunity to uncover the biophysical mechanisms underpinning self-organization in early embryo development.[1] TE stem (TS) cells, extra-embryonic endoderm (XEN) cells, and embryonic stem cells (ESCs) are derived from the polar TE, PE, and EPI of the pre-implantation embryo, respectively. Remarkably, these stem cells can be used to build "synthetic" embryos *in vitro* that recapitulate different aspects of early mouse embryo development, termed ETX synthetic embryo models.[2] ETX embryos resemble the post-implantation mouse embryo and recapitulate both the gene expression patterns and the cell movements typical of

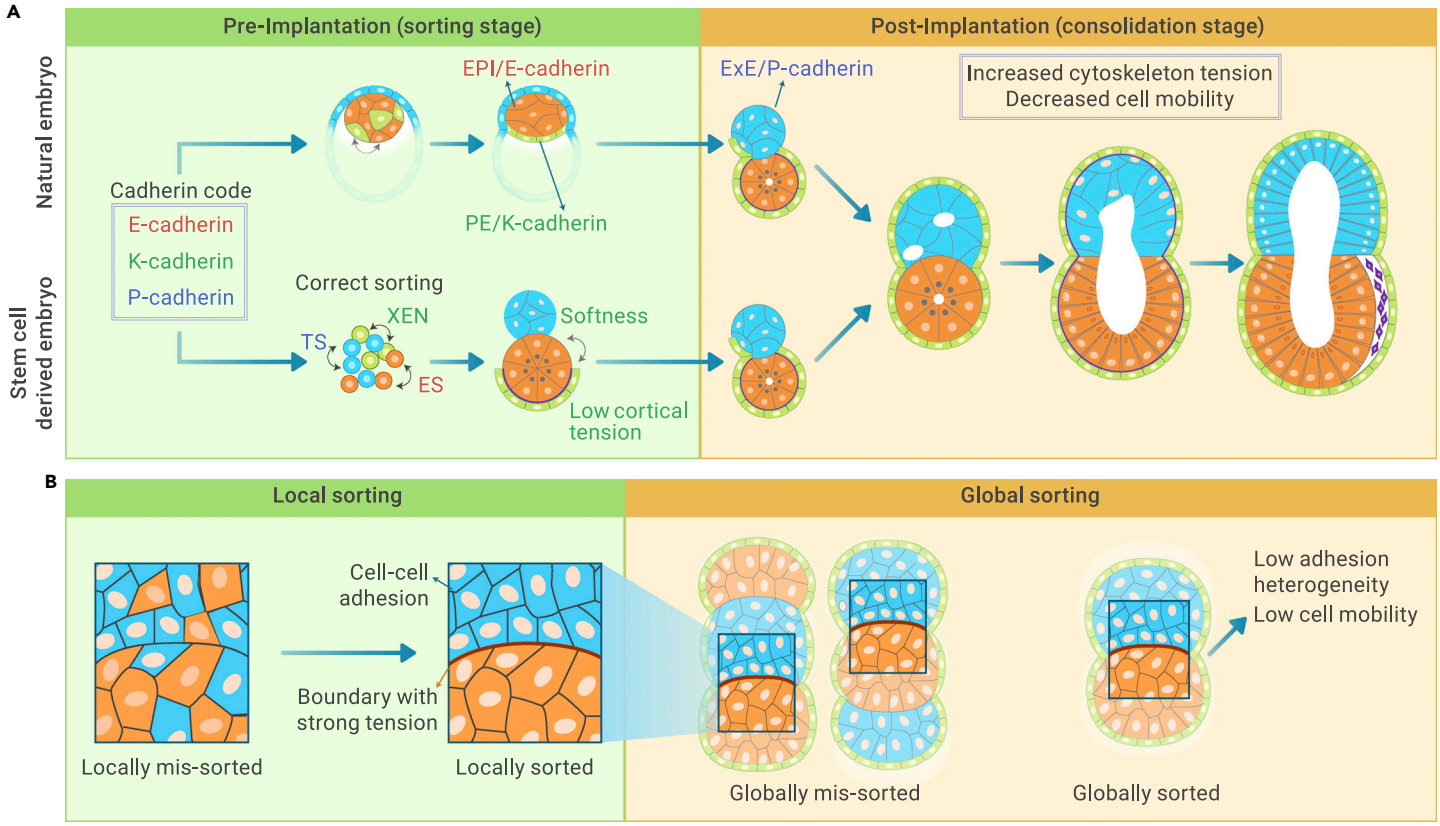

Figure 1. Self-organization principles in stem-cell-derived and natural embryos (A) Self-organization principles in stem-cell-derived ETX synthetic embryo models. During the cell-sorting stage, differential expression of E- and P-cadherins enable ESCs and TS cells to be specified and sorted from each other through differentiation adhesion. The low cortical stiffness of XEN cells enables robust cell externalization and formation of an outside XEN monolayer through K-cadherin adhesion. From cell sorting to tissue consolidation stage, increased tension and adhesion, together with decreased cell mobility, can contribute to the stabilization of sorting outcome. Importantly, cells require low adhesion heterogeneity during cell sorting and the tissue consolidation stage to achieve well-sorted ETX embryos at the global level. The proper morphogenesis, including cavity formation and basement membrane formation as well as symmetry breaking, can be observed in well-sorted structures. (B) Distinction between sorting on a "local" scale and sorting on a "global" scale. Differential adhesion hypothesis and differential interfacial tension hypothesis can initiate cells to be sorted from each other on a local scale. On a global scale, sorting can be determined by cell mobility and cell adhesion heterogeneity. Locally sorting can be necessary for both globally mis-sorting and globally well sorting. In the global scale, cells are maintained in position as a result of strengthened heterotypic tension and reduced mobility. Consequently, if cells remain in "local minima" before cell sorting is complete, structures will remain mis-sorted.

gastrulation. ETX embryos can also develop into an egg cylinder from a random assortment of ESCs, TS cells, and XEN cells. Strikingly, ESCs generate an EPI compartment, TS cells generate an ExE compartment, and XEN cells form the enveloping visceral-endoderm-like layer (Figure 1A). How self-assembly of ETX synthetic embryos occurs and to what extent this process recapitulates the essential lineage reorganization events in the natural embryo are not known.

The self-organization of many cellular systems relies on the formation of distinct cell–cell contacts, which in turn depend on differential adhesion, via cadherin molecules and/or differential cortical tension, due to reorganization of the actin cortical network.[3] Indeed, synthetic genetic programs controlling the expression of distinct cadherins can direct the formation of custom multicellular structures that have illuminated fundamental principles of self-organization.[4] However, the principles that operate in the self-assembly of *in vitro* embryo structures have never been examined.

Recently, in a work published in *Nature Cell Biology*,[5] we combine biophysical measurements, mathematical modeling, and functional perturbations to uncover the mechanisms of self-organization in ETX synthetic embryos. We discover that self-organization progresses through two sequential stages. A cell-type-specific cadherin code drives reversible lineage sorting, followed by differential tension that stabilizes irreversible tissue compartments. The first stage requires a cell-sorting process that allows mixtures of the three cell types to reproducibly self-assemble into a complex 3D architecture, with the three distinct tissues arranged in an embryo-like conformation. Sorting is facilitated by differential adhesion among the three cell types, conferred by a unique cadherin code: *Cdh1* in ESCs, *Cdh3* in TS cells, and *Cdh6* in XEN cells. Additionally, cell cortical tension modulates the sorting capacity: XEN cells are softer and therefore envelope the stiffer ESCs and TS cells. As ETX embryos develop, progressively accumulated tension decreased cell mobility, locking cells into position at the onset of the second stage: tissue consolidation. This stage represents a point of no return: both correctly and incorrectly consolidated groups of cells become fixed in position (Figure 1A). Importantly, by generating chimeras with different cadherin-overexpressed ESCs, we show that the rules governing the self-assembly of these three stem cell types into ETX synthetic embryos also govern lineage reorganization during natural embryogenesis.

We also exploit natural variation in ETX self-organization to unpack mechanisms of incomplete sorting—which might yield clues as to why this process frequently goes awry *in vivo*. We found that low cadherin expression within a heterogeneous population of TS cells and ESCs underlies sorting failure. Strikingly, overexpressing cadherin in these cells increases the efficiency of self-organization three-fold—from ∼15% to ∼42% of structures now showing complete sorting and perfect organization. This finding not only gives us insight into the mechanisms and vulnerabilities of sorting but also dramatically improves the utility and feasibility of the ETX embryo model.

The many instances of incomplete ES-TS sorting emphasize an underappreciated distinction between "local" order and complete sorting on a "global" scale (Figure 1B). Strictly speaking, differential adhesion hypothesis and differential interfacial tension hypothesis can only explain sorting on a local scale, consistent with the formation of large homotypic clusters of ESCs and TS cells even in mis-sorted structures. For complete sorting, ETX embryos must bypass these "local minima," defined as locally correct neighborhoods within globally incorrect patterns (Figure 1B). Robust self-organization must thus enable escape from unfavorable local minima to explore many possible conformations and yet remain in a global minimum when this state is reached. We propose that the capacity of aggregates to escape these "local minima" is shaped by other physical cellular properties and that these properties are dynamic during self-organization. Cell sorting is most effective in a "fluid" tissue state where local minima are shallow, with abundant swapping events being driven by cell mobility and lower-affinity interactions. Tissue/lineage consolidation requires "solidification," whereby cells are maintained in position as a result of strengthened heterotypic tension and reduced mobility (Figure 1B). Consequently, if cells remain in "local minima" before cell sorting is complete, structures will remain mis-sorted. Our study defines a stage-dependent requirement for tension in which tissue consolidation critically depends on strong heterotypic tension to prevent cells from mis-localizing after sorting is complete. Together, our results point toward a broader integrative view of self-organization where differential adhesion hypothesis/differential interfacial tension hypothesis provide the necessary conditions for local order, and other physical properties ensure that the trade-off between exploring different conformations and consolidating compartments is balanced optimally.

The critical balance in cell sorting and tissue consolidation that we show reveals for the first time the biophysical mechanisms that underlie the fundamental and initial self-organization event in mammalian development. From this perspective, stem-cell-derived embryo models provide powerful tools for unveiling the causes and consequences of aberrant self-organization in natural development and for identifying the genetic and pharmacological modulators of these regulative mechanisms. Our findings will also have general relevance in understanding mechanisms that establish correct tissue architecture and tissue pattern.

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DECLARATION OF INTERESTS

The authors declare no competing interests.