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Unifying synthetic embryology

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During embryonic development, a fertilized egg transitions into a complex organism, whereby diverse cell types are spatially organized into functional tissues. This sequential unfurling of complexity is exemplified perhaps best at gastrulation, where the major axes of the adult are laid down and the principal germ layers are defined in tandem with a dramatic change in morphology[1,2]. Understanding the mechanisms by which this highly choreographed process plays out demands investigation at different spatial scales[3]: from the cell-intrinsic gene-regulatory networks that govern cell fate decisions; to the signaling interactions that coordinate divergent fate trajectories across tissues. It requires understanding of how these regulatory processes are coupled to global characteristics including embryonic geometry, patterning, and inter-tissue interactions. Dissecting mechanisms across such scales — and crucially understanding their interactions — requires the capacity to manipulate genes, signals and morphology, a task that has proved challenging particularly for mammalian experimental embryology given the inaccessibility of the conceptus at implantation, a time when many of these important events occur[4,5]. Synthetic embryology has risen to this challenge, engineering embryo-like structures — "stembryos" — using stem cells derived from embryos (~~notably Embryonic Stem Cells, ESCs~~), situating cells in both near-native and more foreign contexts, and probing the consequences for patterning and morphogenesis. Given the recent explosion of diversity in stembryo models[6-12 13], this Special Issue reflects on the successes made and the challenges that remain in using *in vitro* culture platforms to study early mammalian embryogenesis. Here, we offer a perspective on the purpose and utility of synthetic embryology, proposing that focusing efforts on probing fundamental mechanisms of self-organization, spurred via novel bioengineering strategies, can help unify the field towards its shared motivations of understanding natural development and building reliable, translatable experimental models.

The objectives of the field of synthetic embryology can be broadly clustered into two opposing classes: **reconstitution** and **reconstruction**. Firstly, synthetic embryology aims to build stembryo models that **reconstitute** the processes of early embryogenesis given challenges in experimenting on mammalian — particularly human — embryos. In particular, the potential of a reliable model that can be grown *en masse* holds huge promise in drug discovery and personalized medicine[14,15], avenues that remain impractical or impossible using natural embryos. Conversely, the field's second aim is to **reconstruct** developmental processes by culturing cells in contexts or combinations that diverge from natural embryogenesis in order to probe underlying mechanisms.

Just as the discipline of synthetic biology is uncovering design principles of genetic circuits by combining components in alternative combinations[16,17], the culturing of stem cells in alternative tissue geometries (e.g. 2D gastruloids made using micropatterned colonies[12]) or in the absence of principal extra-embryonic tissues (e.g. 3D gastruloids[10,18]) is helping unveil the design principles of multi-cellular development, across the three key scales. Indeed, we envision these opposing objectives to bring the reciprocal benefits of recapitulation and of abstraction: in probing the necessary conditions of embryogenesis by removing or reconstituting them, it will be possible to distinguish processes that are robust from those that are sensitive.

New insights into early mammalian development are starting to be gained both by comparing models to the natural embryo, but also by comparing them one to another. Across models, stem-cells show the remarkable capacity for self-organization, coordinating differential cellular activities at a global scale, often undergoing both cell-fate patterning and morphogenetic transitions. Yet the space of morphological possibilities is surprisingly broad across the various stembryo models, differing in culture conditions but all centered on pluripotent stem cells or their derivatives. Different stembryo models thus explore alternative modes of self-organization and so the cross-comparison of models one to another can help inform how these underlying mechanisms operate. For example, unlike the natural mammalian embryo where the anterior visceral endoderm (mice) or hypoblast (humans) is thought to provide positional information[2,19], spatially organized germ-layer patterning can occur autonomously to varying degrees in stembryo models that lack a synthetic analogue of this structure. However, the nature of this patterning varies dramatically: 2D gastruloids show concentric rings of differential gene expression[12], but are unable to break radial symmetry without the provision of external gradients[20,21]; whereas 3D gastruloids[10] and ETS stembryos[6] break symmetry to specify nascent mesoderm. Hence, we can see these models as exploring alternative paths in a common developmental landscape, meaning comparing differences in self-organization provides insight into the basic principles of natural development **(Figure 1A-B)**. The job of the synthetic embryologist is thus both to map out the topography of this landscape by reconstruction, and to reconstitute development by channeling stem-cells towards the embryo-like region of this broader space. In this Issue, Veenvliet and Herrmann provide such a perspective on synergies between reconstitution and reconstruction in the context of trunk development[22].

Ambitions to reconstitute and reconstruct embryonic development have prompted important technological advances both in controlling culture conditions necessary for embryo-like self-organization, and in comparing models with natural embryos. Stem cells cultured *in vitro* experience a drastically different environment to their embryonic counterparts, with both chemical and mechanical factors known to alter cellular fate decisions and morphogenesis[23,24]. In spite of the striking capacity of stem-cells to self-organize their own micro-environment[9,25-27], studies have revealed that cells need additional environment conditions to channel fate-decisions and

morphogenesis towards the embryo-like region of the developmental landscape. For example, given growing knowledge on the impact of the local mechanical environment on fate allocation and tissue morphogenesis[28,29], there has been a rising emphasis on culture platforms that recapitulate key aspects of this microenvironment, seen in the successes of growing stem-cells in biomimetic gels[30]. Likewise, the importance of morphogen gradients in canalizing embryonic pattern formation, established by interactions between the embryo and its surrounding extra-embryonic tissues, is being tested *in vitro* through the use of microfluidic devices[21,31]. The article by Brivanlou and Ali reviews how novel bioengineered culture platforms help provide newfound control over tissue self-organization[32]. Additionally, embryonic development proceeds sequentially, ensuring that at each stage cell types are spatially organized in the appropriate configurations to facilitate the necessary intercellular signaling.[2] This is in stark contrast to synthetic embryos, where multiple cell types are often mixed at random. 3D tissue printing strategies[33-35] and "tissue origami"[36] hold promise in ensuring the starting conditions that push stem-cells along the embryo-like path of this self-organization landscape. Here, Little provides perspective on tissue engineering strategies that bring the right cells together in the right orientations and combinations in the context of making synthetic kidneys[37]. Reciprocally, novel approaches in assaying stembryo development are helping map out alternative modes of self-organization, within and between models. At the cellular level, transcriptomic profiling strategies (e.g. single-cell RNAseq and seqFISH) are helping chart the multiple cell types that emerge during stembryo development, as well as the dynamics of their allocation, providing an indispensable tool to compare fate allocation of models to the natural embryo[38,39] and to one another[11]. In this Issue, Boonekamp et al. utilise RNAseq on organoid models derived from five adult endodermal tissues to identify novel targets of the Wnt pathway[40]. At the tissue-level, advances in high-throughput culture, imaging and data-analysis[12,41,42] hold promise in defining "phenotypic landscapes" of stembryo development. Investigating these alternative modes of self-organization by comparing phenotypic variations can help tease out underlying mechanisms. Here, Gritti, Oriola and Trivedi reflect on the promise of such data-driven strategies in unravelling the subtleties of developmental mechanisms[43].

Beyond a shared technological toolkit, we believe a refocusing on underpinning theories of development is essential to unify synthetic embryology. Multicellular organization can be seen as being governed by the combined activities of two types of control strategy, with stembryo models testing theories under both classes **(Figure 1C)**. Under **extrinsic control**, external sources of information instruct tissue organization, stressing roles for inter-tissue boundaries in instructing fate decisions and morphogenesis. For example, boundary conditions are shown to be crucial in germ-layer patterning in 2D gastruloids, where the periphery shows enhanced BMP signaling due to influx from the medium, and increased Wnt signaling via local mechanotransduction[26-28]. Similarly, 3D gastruloids and trunk-like structures recapitulate somite morphogenesis only when embedded within Matrigel, an ECM surrogate[11,39]. In this Issue, Gartner and Martyn review the importance of

engineered and self-organized tissue boundaries in stembryos and natural embryos[44]. Conversely, **intrinsic control** involves the *emergence* of organization via local cellular interactions, without the need for an external supply of information. Cell sorting represents a paradigm in intrinsic control, wherein mixtures of multiple cell types re-organize to form functional tissues in reproducible configurations, driven by differences in adhesion and/or tension among the different cell types[45]. Sorting is exemplified in mixtures of embryonic, trophoblast and extraembryonic endoderm stem-cells which sort-out to establish analogues of the three principal embryonic tissues in an embryo-like orientation in ETX stembryos[9], and similarly in the sorting of germ-layers in 2D and 3D gastruloids[38,46]. Another paradigm is self-organized patterning, where signaling interactions among initially identical cells can establish divergent cell fates in a spatially organized manner, characterized best in Turing-like systems, where the combined contributions of local signaling activation and long-range signaling inhibition can promote spontaneous symmetry breaking[47,48]. Such theories stress the importance of inducing the expression of signaling inhibitors, an emerging theme in anterior-posterior axis establishment in both 2D and 3D stembryo models[27,49]. This Issue contains a piece by Schauer and Heisenberg that reviews the relative contributions of intrinsic and extrinsic control in symmetry breaking [50], and Sozen, Cornwall-Scoones and Zernicka-Goetz provide a perspective on how stembryo models are shedding new light on both self-organized axis establishment and the roles of embryonic/extra-embryonic interactions in regulating this pattern formation[51]. Beyond these paradigms, stembryo models may provide the ideal testbed to investigate new mechanisms of intrinsic control. For example, trunk-like structures exposed to elevated Wnt or reduced BMP signaling display a sporadic arrangement of somite-like structures[11], equivalent to the “bunch-of-grapes” phenotype seen first in chick embryos[52]. Here, individual somites are approximately embryo-like in morphology, but do not show the serial organization characteristic of normal development as expected under the canonical clock-and-wavefront model[53], potentially suggesting an intrinsic program of somite morphogenesis.

Stembryos hold particular promise in unveiling mechanisms of human development. In spite of substantial advances in the *in vitro* culture and molecular analysis of human embryos[54-56], essential ethical considerations (notably in the 14 day rule[57]) and a limited number of specimens have prevented a thorough interrogation of developmental mechanisms in our own species. Headway has been made using human ESCs to uncover cell signaling interactions that may govern axis establishment and germ-layer patterning[12,18,49], yet to date we lack a comprehensive stembryo model that can reconstitute patterning, morphogenesis and inter-tissue interactions *in vitro*. Two reviews, one from Weatherbee, Cui and Zernicka-Goetz[58] and the other from Ghimire, Mantziou, Moris, and Martinez Arias[59], summarize our limited knowledge of human development and provide perspective on the successes and challenges of modelling developmental mechanisms of our own species using stembryos. With increasingly faithful models likely to emerge in the coming years, we anticipate a newfound appreciation of the key mechanistic divergences between mouse and

human embryogenesis. Further, given a substantial proportion of embryonic fatalities and developmental defects can be traced to the embryonic timepoint that stembryo models aim to recapitulate[60-63], such *in vitro* strategies will likely become a mainstay of translational research in embryonic pathology, especially given the promise of high-throughput analysis in drug-screening and personalized medicine[15].

By culturing stem-cells in alternative combinations and environments, studies in synthetic embryology collectively chart a common landscape of early embryonic development. The topography of this landscape is shaped by intrinsic control mechanisms that govern self-organization (e.g. signaling feedback driving symmetry breaking), whereas extrinsic control mechanisms direct the trajectories of (st)embryo development (e.g. external gradients that orient embryonic axes). Indeed, these influences are mirrored in technological breakthroughs in the generation and analysis of stembryos, where high-throughput assays are starting to capture alternative modes of self-organization and hence investigate landscape topography, whereas biomimetic culture platforms are helping push stembryo development along increasingly embryo-like trajectories. The two interpretations of what makes synthetic embryology "synthetic" chart different regions of this landscape: synthetic embryology for reconstitution identifies conditions that allow stembryos to mimic natural development as closely as possible; and synthetic embryology for reconstruction explores new regions of this landscape by analyzing the consequences of culturing stem-cells in alternative combinations and configurations. We anticipate future strides to be made at the interface between these two objectives, requiring synergies between bioengineering and data-driven strategies, orienting research via a set of fundamental developmental principles. While no stembryo single model perfectly recapitulates all aspects of mammalian embryogenesis, imperfections provide a wealth of information, both in improving the models of the future, but also in uncovering sensitive features of natural embryonic development. Moreover, challenging stem-cells with novel culture conditions and analyzing consequences on fate allocation and morphogenesis may help identify previously undefined regulative mechanisms of development, where the remarkable capacity of stem-cells to self-organize reflects pathways of embryonic plasticity. This Special Issue showcases the astonishing progress made in this nascent field of synthetic embryology, a field we believe will provide unprecedented opportunities in the research of the future, both in understanding basic mechanisms of embryogenesis, and in building tractable experimental models for translational science.

Figure 1: The developmental landscape of early embryonic self-organization

(A) Schematic representation of the developmental landscape of self-organization underpinning mammalian embryogenesis (illustrated for mouse), where embryo-like architecture occupies one of multiple valleys. Overlayed are the two objectives of synthetic embryology, represented as arrows: recapitulation, considering paths towards the embryo-like valley; and reconstruction, exploring other valleys in the landscape. **(B)** The same developmental landscape, where various stembryo models occupy alternative valleys in the broader space (shown for mouse models). **(C)** Development, conceptualized as movement within this landscape, is regulated by two modes of control: intrinsic control, shaping the topography of the landscape itself; and extrinsic control, biasing (st)embryo developmental trajectories.

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