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Setting the stage for embryo segmentation

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https://doi.org/10.1016/j.stem.2024.06.014

Morphogen gradients are critical regulators of embryonic development. In this issue, Liu et al.[1] introduce a microfluidic system that externally applies morphogen gradients to an *in vitro* model of human embryo segmentation. It enables the investigation of signaling gradients during this developmental process at unprecedented levels of precision.

Morphogen gradients establish the spatial framework of multicellular systems and determine the major embryonic axes. One key process, somitogenesis, involves periodic formation of tissue blocks from the presomitic mesoderm (PSM), giving rise to vertebrae, axial muscles, and skin. Signaling gradients and oscillations regulate this process. Our knowledge largely stems from constitutive perturbations of signaling or locally restricted perturbations using morphogen-soaked beads, yet with little control over gradient parameters and dynamics. To experimentally test signaling function, it is necessary to modulate signaling with high spatial or temporal precision. Microfluidics can provide such functionalities (reviewed in Sonnen and Merten[2]), such as the temporal perturbation of signaling oscillations in cultured mouse embryos.[3] However, the role of signaling gradients in somitogenesis[4] remained difficult to address.

In the past, microfluidics has been used to apply external gradients to two-dimensional (2D) models of gastrulation[5] or neural development.[6] However, in three-dimensional (3D) models, it is more challenging to create stable, reproducible gradients without interfering with cell viability in microfluidics. Liu et al.[1] overcame these challenges by culturing a human *in vitro* somitogenesis model[7] on a microfluidic chip, which allows for the formation of spatial gradients in a central chamber (Figure 1). Importantly, a gel-coated "micro-trench" in the central chamber enabled the self-organization of 3D structures inside. Induced PSM cells were loaded on-chip and exposed to antagonistic gradients recapitulating the endogenous system (including fibroblast growth factor [FGF] inhibition on one side and activation on the other). As a result, a gradient of differentiation was established by day three with Pax3-positive somitic tissue on one side and Tbx6-positive PSM on the other side. Moreover, cell motility was graded, there was sequential Hox gene expression, and—importantly—somite formation occurred in a rostral (front) to caudal (back) direction. Thus, the externally applied gradients determined the axis of self-organization in the *in vitro* somitogenesis model.

How external gradients influence intracellular signaling has direct implications for the resulting developmental process. Interestingly, this study highlighted that external perturbation of morphogen gradients did not immediately change endogenous signaling. Using signaling reporters, Liu et al. found that Wnt and FGF signaling activity gradients only became apparent after three days of incubation in external gradients. In contrast, effects on graded differentiation were visible a day earlier, indicating that an imbalance in signaling along the rostrocaudal axis was present before it could be resolved using reporters. Further analysis is required to understand how external gradients impact endogenous signaling and morphogen gradient establishment at subcellular levels in the 3D volume of the micro-trench. Future studies might also provide more insights into endogenous morphogen gradients in the developing embryo.

During somitogenesis signaling, waves travel through the PSM from the caudal to rostral side, where a new pair of somites forms with every wave. The relationship between signaling gradients and waves has not been clearly resolved. Here, Liu et al. found that initially, the whole tissue oscillated uniformly. From day three onwards, oscillations persisted only in the undifferentiated part, and waves traveled in a caudal to rostral direction, slowing down rostrally. Future studies should investigate whether the direction of signaling waves is determined by the gradient or as a result of differentiation into caudal versus rostral PSM cells. Importantly, the microfluidic chip allows the dynamic modulation of external gradients. In future work, gradients could be altered in their composition, shape, or amplitude or entirely inverted after the establishment of signaling waves to reveal the immediate effect on wave dynamics.

Somite size is thought to be regulated by signaling gradients and oscillations, with modulations of FGF levels affecting somite size in embryos.[4] Liu et al. experimentally lowered the external FGF gradient amplitude, indeed shifting the point of differentiation toward the caudal side. Yet, the size of forming somites remained unchanged. Furthermore, the authors did not identify a direct link between signaling waves and somite formation. Given the inherent propensity of somitic tissue to epithelialize into tissue blocks,[8] it is plausible that external gradients create a differentiation gradient, leading to self-organized formation of somites in the "rostral" region of the chip, independent of wave dynamics. The discrepancy between the present results and previous *in vivo* findings implies that a regulatory component of somitogenesis might be missing in the *in vitro* system. This could be explored using microfluidics by varying the composition and shapes of the antagonistic gradients over time and analyzing the effect on segmentation and differentiation of somites into rostral and caudal halves.

Scaling of somite to PSM size is an integral part of somitogenesis, with larger PSM leading to larger somites (Figure 1). In this study, the authors indeed observed scaling, even though the link from neither signaling gradients nor signaling waves to somite formation was apparent, as discussed above. However, the relative somite size was higher compared to *in vivo* tissue, potentially due to missing

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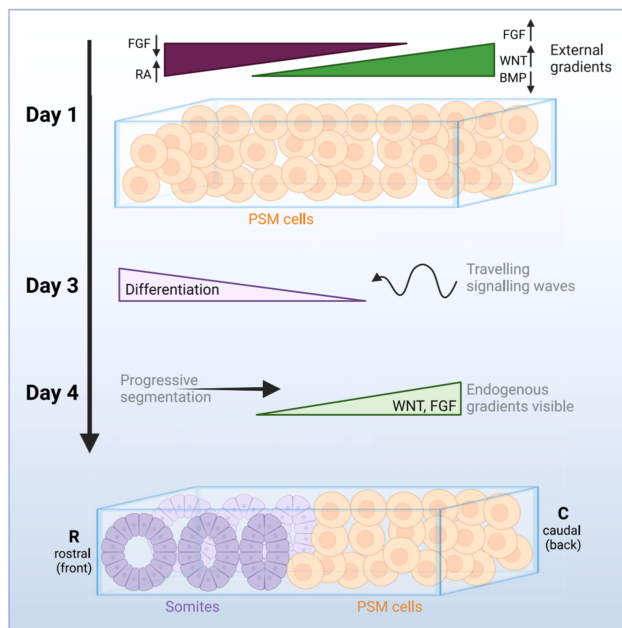

Figure 1. External gradients guide somitogenesis in an *in vitro* model of human embryo segmentation using microfluidics
Stem cells are differentiated into presomitic mesoderm (PSM) cells and then loaded onto a microfluidic chip. Application of external gradients from day one onwards leads to progressive differentiation and somite formation along the external gradients, recapitulating a rostral to caudal direction *in vivo*. (Figure generated using BioRender).

factors, missing surrounding tissues, or incorrect signaling (levels) in the *in vitro* system. The authors developed a theoretical scaling model based on tissue mechanics that recapitulates the experimental data. Experiments to test the model indicated that perturbing components essential for mesenchymal-to-epithelial transition prevented somite formation entirely. Conversely, two experiments subtly altered somite sizes. In the first, a vacuum system was integrated into the microfluidic system, allowing reversible stretching of the cell-containing micro-trench, which led to a decrease in somite size. Whether this change occurred because of shifting endogenous gradients after moving the poles apart or due to altered cellular mechanics remains to be addressed. In the second experiment, N-cadherin inhibition also led to smaller somites. Future research should explore how tissue mechanics and cadherin levels influence somite formation. Since levels of cadherins themselves are graded along the PSM-to-somite axis, the microfluidic system could be applied to simultaneously modulate biochemical and mechanical gradients to disentangle their reciprocal interactions and contributions to segment formation.

In summary, the microfluidic system by Liu et al. provides an experimental approach to modulating signaling gradients in somitogenesis, allowing detailed studies of the function of gradients. In the future, this setup could be applied to study not only stem cell-based systems of somitogenesis but also other *in vitro* models, as well as developing embryonic tissue. Further adaptations could also provide the opportunity to manipulate somite formation in more complex ways, enabling tissue growth or integrating orthogonal gradients, as recently published by the same lab,[9] to model dorsoventral or mediolateral patterning. Although such biofabrication approaches offer unprecedented precision in studying developmental processes, implementing them in biology labs remains challenging. Detailed protocols for non-experts[10] can lower the bar, yet technical requirements persist. In the longer term, dedicated core facilities in research institutes and integration of biofabrication in university curricula can promote microfluidics as a standard procedure.

ACKNOWLEDGMENTS

This work was supported by the Hubrecht Institute and received funding from the European Research Council under an ERC starting grant agreement no. 850554 to K.F.S.

DECLARATION OF INTERESTS

The author declares no competing interests.

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