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PERSPECTIVE

In preprints: tick, tick, somite – an intrinsic timer regulates segmentation

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Our vertebrae and peripheral nerves are periodically arranged along the body axis, representing a fundamental feature of our body plan – segmentation. This feature is first established in the early embryo by the formation of repeated epithelial blocks called somites on either side of the neural tube. The process of somite formation is fascinating because it occurs sequentially from head to tail in coordination with body axis elongation. Each somite periodically buds off from the anterior-most part of the unsegmented tissue termed presomitic mesoderm (PSM). This process is precisely orchestrated by an oscillating gene regulatory network famously known as the segmentation clock, which is conserved from fish to humans. In each cycle of somite formation, activity of the clock is initiated in the posterior PSM and travels anteriorly as a kinematic wave. It has long been observed that the traveling wave progressively slows down along the PSM. That is, the oscillation period in the anterior PSM is longer than that of the posterior PSM. This dynamic feature has been proposed to regulate somite morphogenesis and patterning (Lauschke et al., 2013; Shih et al., 2015; Sonnen et al., 2018), yet the mechanisms underlying the slowing oscillations along the PSM remain unclear. In Rohde et al. (2023 preprint), the authors combined *in vitro* and *in vivo* quantifications to provide new insights. Their elegant work suggests that the slowing of clock oscillations is a cell-autonomous property. By concurrently analyzing cell differentiation, they further proposed a model of segmentation governed by an intrinsic timer that can be tuned by extrinsic factors.

A series of *in vitro* studies have revealed that the clock oscillation itself is autonomous and does not require extrinsic factors such as cell-cell contact (Diaz-Cuadros et al., 2020; Hubaud et al., 2017; Webb et al., 2016; Yoshioka-Kobayashi et al., 2020). In this preprint, the authors went a step further to demonstrate that the temporal evolution of the oscillation profile, which underlies the wave slowing on the tissue scale, is also cell intrinsic (Rohde et al., 2023 preprint). They dissociated the posterior PSM of zebrafish embryos and cultured isolated cells in the absence of signaling molecules. By following the expression of a fluorescently tagged clock component, Her1, they observed that clock oscillations autonomously slow down before abruptly arresting. When single cells in the embryo from the same PSM region were analyzed, similar key features of progressive period slowing were found. This remarkable mirroring between *in vitro* and *in vivo* suggests that oscillation slowing is cell autonomous independent of tissue environment. As many models explaining wave slowing focus on extrinsic regulation such as coupling delay between cells (Takagi et al., 2020), this finding brings new perspectives for understanding the spatial dynamics of oscillations along the PSM.

In concert with oscillation arrest, the onset of a segmental differentiation marker Mesp-ba was detected in isolated single cells, consistent with tissue-level expression patterns in the embryo. Overall, more noise regarding oscillation dynamics, such as cycle number and amplitude, as well as the coordination between clock arrest and Mesp-ba expression, was observed among cells *in vitro*, suggesting that extrinsic factors normally present in the embryo might tune the precision of oscillations and differentiation. Consistent with previous findings (Diaz-Cuadros et al., 2020; Miao et al., 2023), the Mesp-ba onset is independent of clock dynamics because similar patterns of expression were observed in isolated cells lacking a functional clock. Nevertheless, the temporal association between clock dynamics and Mesp-ba onset was maintained in the presence of exogenous FGF8, which prolonged the duration of oscillations *in vitro* and accordingly delayed the onset of differentiation. Altogether, these suggest that a cell intrinsic timer governs both clock slowing and differentiation onset, and this can be regulated by extrinsic factors. The nature of the intrinsic timer, as well as its regulation, will be exciting areas of study in the future.

What does this mean for segmentation? As cells are progressively deposited into the PSM from the tailbud, the position of a given PSM cell becomes more anterior due to the posterior elongation of the body axis. Concurrently, its intrinsic timer keeps running before eventual cell differentiation. Thus, the authors proposed that the intrinsic timer could be used to provide positional information. They observed that cells isolated from the more anterior region of the PSM displayed fewer cycles of clock oscillations and earlier onset of Mesp-ba expression. Cells dissociated from the tailbud showed similar oscillation profiles and differentiation timing as cells from the very posterior part of the PSM, suggesting that exiting from the tailbud triggers the start of the timer. This is further supported *in vivo*, where cells with a later exit from the tailbud exhibited delayed initiation of oscillation slowing. In sum, the authors proposed that the elapsed time following tailbud exit could serve as positional information to instruct somite formation.

The classical ‘clock and wavefront’ model proposes that a clock controls the oscillation between a somite forming and non-forming state and a wavefront of maturation provides positional information. The two independent entities together instruct the spatiotemporal dynamics of segmentation. Although the segmentation clock is generally considered as the oscillating clock, many factors are proposed to serve as the wavefront. The positional information was initially thought to be a simple threshold of absolute FGF and/or Wnt signaling (Aulehla et al., 2003; Dubrulle et al., 2001; Sawada et al., 2001). Recent quantitative investigations propose that the crucial parameter is the spatial fold change of the phosphorylated Erk (pErk) gradient (Simsek and Özbudak, 2018) – the ratio

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between the local slope of the pErk gradient and the absolute pErk level. Other studies break away from the concept of independent wavefront by proposing that the positional information is encoded solely by clock oscillations, e.g. a crucial phase difference between neighboring cells (Lauschke et al., 2013) or the phase relationship between different sub-oscillators in the same cell (Sonnen et al., 2018). How the intrinsic timer model proposed here relates with the existing models remains to be elucidated. Given the close mutual influence among various players from FGF signaling to Her1 oscillations, it is possible that the key parameters used in each model to define positional information might be correlated. The experimental platform in this preprint, which allows quantitative characterization both *in vitro* and *in vivo* at the same time, can be combined further with live reporters of FGF signaling and Wnt sub-oscillators. It represents an exciting opportunity to discern or unify different models of segmentation and advance our knowledge of developmental patterning.

Competing interests
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