Spring Forward and Fall Back: Dynamics in Formation of Somite Boundaries

Oscillating signaling systems mediate the progressive division of mesoderm into segmental units, termed somites. A recent study using time-lapse analysis in living chick embryos has revealed that the process of somite boundary formation relies on a carefully choreographed series of cell movements, which are both unexpected and surprisingly intricate.

In vertebrates, somites are segmental units that subdivide the mesoderm into repeated building blocks that go on to generate the axial skeleton and its associated musculature. The process of somitogenesis forms these segments as the embryo grows and elongates, and entails the progressive budding off of groups of cells into epithelial balls at regular timed intervals (Maroto and Pourquie, 2001; Pourquie, 2001; Saga and Takeda, 2001). Many models have been put forward to explain how the timing and iterative process of somite formation is coordinated. This is a classic problem in developmental biology, but recently progress has been made with the discovery that the expression of many components of the Notch pathway display oscillating waves of gene expression in the unsegmented mesoderm or segmental plate (Maroto and Pourquie, 2001; Pourquie, 2001; Saga and Takeda, 2001). The timing of these expression patterns correlates with somite formation, and functional analyses in many vertebrates have shown convincingly that the Notch pathway is a critical player in modulating multiple aspects of somitogenesis. Current data support a “clock and wavefront” model, in which a timed oscillator (the clock) sets the periodicity of somite formation. Coupled to this, a signaling wave spreads transiently through the tissue to position the anteroposterior (A-P) border of the forming somites (Maroto and Pourquie, 2001; Pourquie, 2001; Saga and Takeda, 2001). Recent regulatory analyses have identified control elements in the Lunatic fringe gene responsible for modulating its cyclic expression at the transcriptional level, opening up new approaches for investigating the molecular components of the clock (Cole et al., 2002; Morales et al., 2002). However, very little is known about the actual cellular events that result in the placement and formation of somite boundaries, or how they are coupled to regulation by the “clock and wavefront”.

A new study by Kulesa and Fraser (2002) provides important insight into the cellular behaviors that subdivide mesoderm in the segmental plate and result in the sequential delineation of epithelialized somites. By developing methods to mark and visualize individual cell movements over time in living chick embryos, in concert with analysis of gene expression, these authors have generated a detailed picture of the intricate and carefully choreographed events that lead to the formation of boundaries in newly forming somites. Dil lineage tracing indicates that, as cells enter the segmental plate in the posterior region near the node, they freely mix with their neighbors and disperse over broad areas. Hence, as closely grouped cells leave the node and enter the segmental plate, they do not comprise a prespecified cohort that is ultimately fated to form a particular somite. Over time, as the node regresses and the A-P axis elongates, labeled cells now in the anterior region of the segmental plate undergo progressively less mixing. Cells within close proximity (4–5 somite distance) to the border with the most newly forming somite undergo minimal short-range movements and maintains their relative A-P registration.

By examining the dynamic movements and morphology of cells in the prospective location of the next somite boundary, Kulesa and Fraser (2002) have demonstrated that the process that generates a border and buds off the new somite does not occur via a simple periodic slicing or segregation mechanism. Initially, cells positioned closest to the midline and anterior to the prospective border organize into a small group that separates slightly from its neighbors (see Figure). This creates a cleft that spreads posteriorly, forming a wedge of cells anterior to the eventual position of the border. This same process happens at the lateral edges of the segmental plate, resulting in a cup or socket shape filled by the cells that will form the new somite (s0). In a similar manner, cells in the middle of the segmental plate (at the bottom of the cup) and posterior to the border coalesce at approximately the same time into another distinct group. In the next set of orchestrated movements, the anterior wedge of cells falls back in a posterior direction, while the posterior cohort moves forward anteriorly (see Figure). These reciprocal movements result in the two distinct cell populations exchanging positions on the A and P sides of the presumptive somite boundary. This shift leaves the clefts oriented in a mediolateral direction. In the final phase, the gap in the clefts propagates across the entire population leading to a complete separation between s0 and the segmental plate. This series of steps is faithfully reiterated each time a new boundary is formed.

These complex and dynamic cell movements would not have been predicted based on the previous analysis of gene expression patterns, which showed relatively sharp, restricted, and dynamic boundaries in presomitic mesoderm and newly formed somites. Most of the expression patterns that oscillate or cycle in waves sweeping through the segmental plate are associated with genes related to the Notch signaling pathway. Some components of the FGF and Eph/Ephrin pathways also have domains of expression that are restricted to the anterior or posterior region of presumptive and newly forming somites. Furthermore, experiments in a number of vertebrates have demonstrated that these pathways have functional roles in the molecular mechanisms that regulate the positioning and formation of somite boundaries (Dubrulle et al., 2001; Saga and Takeda, 2001; Sato
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Schematic of Results on Cell Movements through Lineage Tracing of Opposing Cell Populations during Somite Boundary Formation in Living Chick Embryos

(A) The far left indicates the earliest stage where the last formed somite (s1) at the top has just separated from the newly forming somite (s0). The red dot indicates a lineage tracer placed anterior to the prospective boundary and the black dot a tracer placed posterior to the boundary. The light blue shading indicates EphA4 expression in the anterior half of the s1, s0, and the segmental plate (sp).

(B) A cleft begins to form anterior to the border of EphA4 expression in the segmental plate on both the medial and lateral sides.

(C) This cleft spreads posteriorly extending centrally into the domain of EphA4 expression. This creates a ball and socket or cup like configuration.

(D) The anterior and posterior cell populations marked by the lineage tracers move in opposite directions. This results in the two markers having now switched their orientations with respect to the order of EphA4 expression. The cleft is oriented in a mediolateral direction.

(E) In the final phase of separation the cleft is propagated across the somite separating sp from s0. Increasing time is to the right. A, anterior; P, posterior; M, medial; L, lateral; sp, segmental plate; s0, newly forming somite; s1, last fully formed somite.

et al., 2002; Sawada et al., 2001). Based on their roles in other contexts, it seems likely that these signaling pathways collaborate to cyclically subdivide the segmental plate into groups of adjoining cells with different regional characteristics. This leads to the periodic formation of a segment boundary and subsequent separation of tissues by changes in attractive, repulsive, or adhesive interactions.

While defining the series of cell movements and morphologies that mark the presumptive somite border over time, Kulesa and Fraser (2002) directly analyzed the degree to which the segmental gene expression of EphA4 correlates with somite boundaries at the cellular level. EphA4 is part of a bidirectional signaling system that modulates repulsive interactions between cells and is expressed in the anterior portion of the presumptive and newly formed somites (Xu et al., 1999). Across most of the somite boundary there was a good correlation between EphA4 expression and the future position of cells at the somite border. The notable exceptions were the two early groups of coalescing cells that undergo reciprocal shifts in their relative positions to the somite boundary (see Figure). The anterior wedge of cells that initiate cleft formation do not express EphA4 until they move into the area already strongly expressing the gene. Conversely, the posterior group of cells that spring forward rapidly downregulate the levels of EphA4 (see Figure). These results indicate that the pattern of EphA4 expression is not simply related to the precise cellular positioning of the somite boundary and instead responds to the cellular movements. This suggests that there is dynamic signaling across the border between the posterior and anterior territories that rapidly modulates EphA4 expression following the reorganization of cellular relationships. This may be related to the kinds of instructive signals at boundaries seen by manipulating Notch signaling (Sato et al., 2002). Plasticity or refinement of gene expression patterns at somite borders is reminiscent of the kinds of sharpening seen at rhombomere boundaries in the hindbrain (Trainor and Krumlauf, 2000) and may be part of the mechanism used to generate precise compartments following gene activation in more diffuse domains. This work by Kulesa and Fraser provides an exciting basis for examining the molecular mechanisms and tissue interactions that regulate dynamic cell behaviors during somitogenesis and segmentation in vertebrates. It’s an elegant example of the old adage that “seeing is believing,” and one can hardly wait to see what additional surprises are uncovered as more scientists apply time-lapse imaging in vivo.

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