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Review

Balancing segmentation and laterality during vertebrate development

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ABSTRACT

Somites are the mesodermal segments of vertebrate embryos that become the vertebral column, skeletal muscle and dermis. Somites arise within the paraxial mesoderm by the periodic, bilaterally symmetric process of somitogenesis. However, specification of left–right asymmetry occurs in close spatial and temporal proximity to somitogenesis and involves some of the same cell signaling pathways that govern segmentation. Here, we review recent evidence that identifies cross-talk between these processes and that demonstrates a role for retinoic acid in maintaining symmetrical somitogenesis by preventing impingement of left–right patterning signals upon the paraxial mesoderm.

Contents

1. Introduction

1.1. Somitogenesis—a brief overview

Somites, segmented bilateral columns of paraxial mesoderm flanking the notochord, are one of the defining characteristics of the vertebrate phylotype. The process of somitogenesis occurs sequentially in an anterior to posterior progression, as groups of cells periodically bud off from the anterior end of the unsegmented presomitic mesoderm (PSM) to form a new somite. Concomitantly, the PSM is replenished with cells added by the posterior growth of the embryo. In vertebrates, somitogenesis consistently occurs in a bilaterally symmetrical manner such that segments are aligned on the left and right sides of the embryo. The symmetric development of somites is essential to the subsequent formation of the bilaterally symmetric musculature and bones of the adult body. This relationship is particularly obvious in the axial skeleton, where the metamerism of the paraxial mesoderm prefigures the segmental organization of the vertebral column and where each vertebra is composed of cells originating from somites on both left and right sides of the embryo.

The prevailing model of the mechanism governing somitogenesis is the “clock and wavefront” [1]. According to this model, the clock causes cells to undergo repeated oscillations, with a particular phase of each oscillation defining the competency of cells in the PSM to form a somite. The wavefront controls the position of somite formation and is correlated with posterior growth of the embryo. The wavefront effectively sweeps through the embryo once in an anterior to posterior direction during the course of development. In this model, somite boundary formation only occurs when the wavefront reaches a group of cells in the appropriate phase of the clock.

The wavefront is comprised, at least in part, of gradients of Fgf and Wnt signaling that emanate from the posterior tip of the

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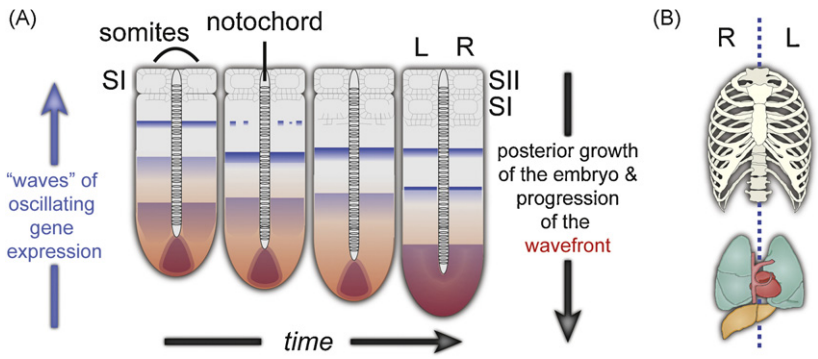

Fig. 1. Somitogenesis and laterality. (A) Somites form in pairs flanking the axial mesoderm, or notochord. Somitogenesis is bilaterally symmetric in both space and time. Four stages within a single somite cycle are diagramed. The most recently formed somite is the SI. When a new somite forms the previous SI becomes the SII. Somitogenesis occurs as the tail extends posteriorly along with the wavefront (red gradient). The segmentation clock generates oscillations in gene expression that manifest as stripes (blue) that pass through the segmenting tissue in a posterior to anterior direction. Once the cells escape the wavefront gradient, they fix their segmental position according to the pattern generated by the clock. Anterior is up. (B) The derivatives of the somites such as the axial skeleton are bilaterally symmetric. Conversely, some products of lateral plate mesoderm and endoderm are bilaterally asymmetric. For example, the heart loops to the left and the right lobe of the liver is much larger than the left lobe. The left lung as two lobes while the right lung has three.

embryo and decline toward the anterior end of the PSM (Fig. 1A) [2–6]. Fgf/Wnt signaling serves to maintain cells in an "immature" state in which they cannot initiate the morphogenetic program required for somite formation. The clock is manifest as oscillations in gene transcription in cells of the PSM. These oscillations are coordinated between neighboring cells leading to reiterated waves of expression that spread through the PSM in a posterior to anterior direction (Fig. 1A). In the anterior PSM, these waves reach cells that are beyond the influence of the Fgf/Wnt wavefront, permitting the induction of gene expression that leads to somite formation.

The Notch signaling pathway functions in the segmentation clock of all vertebrates [7–9]. Oscillation in Notch activity causes periodic transcription of genes of the *Hairy/Enhancer of Split*-related family (abbreviated as *Hes* in mice, *her* in zebrafish). The protein products of these genes act to repress their own transcription, producing a negative feedback loop that helps generate the oscillations [10]. Other important oscillating genes are *Lunatic fringe* (*Lfng*) in mouse and chick, which encodes a glycosyltransferase that modifies Notch activity [11,12], and *deltaC* in zebrafish, which encodes a Notch ligand [13]. These two genes play important roles in propagating the waves of oscillation through the PSM and in ensuring that cells maintain the same phase of oscillation as their neighbors [13–18]. In the mouse, components of the Wnt and Fgf signaling pathways also oscillate in the PSM [19,20]. Exactly how these three pathways interact within the mouse segmentation clock is not yet clear, and there is no evidence of Wnt or Fgf oscillations in the zebrafish, suggesting that the make-up of the clock may vary considerably between species.

1.2. Establishing left–right asymmetry in vertebrates

The bias in position of our own heart to the left side of our body is a familiar reminder that the external bilateral symmetry of vertebrates belies their internal asymmetry. In addition to the heart, the lungs, stomach, spleen, liver, gall bladder and gut all show asymmetry of form and/or position with respect to the left–right (LR) axis (Fig. 1B). Moreover, this asymmetry is directional, with LR differences in organ shape and distribution being identical among members of a species and conserved across vertebrates in general. Specification of the LR axis in vertebrate embryos proceeds from an initial symmetry-breaking event, through a directional flow of signaling information, to the establishment of side-specific gene expression, and thence to asymmetric organogenesis. In a little over a decade, a great deal of progress has been made in understanding the molecular basis of LR patterning [21,22]. While there are still many unanswered questions [23], two very broad themes have emerged. First, there is a common and critical role for cilia-dependent asymmetric fluid flow in all vertebrates. Second, the initial symmetry-breaking event and the timing and mechanism of early LR specification may vary between species.

In the mouse gastrula, the node is a pit-like structure at the anterior end of the primitive streak. It is lined with ciliated cells, and the characteristic rotational movement of those cilia generates a leftward flow of fluid across the node [24]. This nodal flow is required for proper LR patterning, perhaps by causing the asymmetric distribution of signaling molecules and/or by activating mechanosensory cilia [22]. Cilia-driven fluid flow associated with LR patterning has also been identified in Kupffer's vesicle in zebrafish and gastrocoel roof cells in *Xenopus* [25–27]. In chick, cells of Hensen's node are ciliated and express left–right dynein, a component of the ciliary motor necessary for LR specification in mouse and zebrafish [28,29]. However, cilia-driven fluid flow has not yet been explicitly demonstrated in chick. Nevertheless, it is clear that asymmetric signaling emanating from the node is required in all these species to initiate a highly conserved cascade of gene expression in the left lateral plate mesoderm (LPM). This process defines the left side of the embryo. The "leftness" cascade begins with expression of the TGF-β *Nodal* (mouse and chick), the *Nodal* homolog *Xnr1* in *Xenopus* or the *Nodal*-related gene *southpaw* (*spaw*) in zebrafish, and subsequently involves homologues of the Nodal inhibitor *Lefty* and the transcription factor *Pitx2* [21,22].

Prior to the onset of nodal flow, no LR asymmetry has yet been demonstrated in the mouse embryo, and it remains possible that this process may represent the symmetry-breaking step. Conversely, overtly asymmetric gene expression around Hensen's node precedes and is required for the onset of left-sided expression in the chick LPM. This feature of LR patterning is apparently unique to birds [21,22]. Early asymmetric gene expression is dependent on antecedent activity of an H^+/K^+-ATPase, which causes cells to the left of Hensen's node to be more depolarized than those on the right [30]. Regulation of ion flux seems to be a conserved feature of early LR specification, as blocking ion pump function also disrupts the process in both zebrafish and *Xenopus* [29,30]. Although the H^+/K^+-ATPase is not itself asymmetrically expressed in chick and zebrafish, LR differences in distribution of ion pumps can be detected from the 2-cell stage onwards in *Xenopus*, defining an extremely early establishment of the LR axis in frogs [30,31]. Furthermore, asymmetric phosphorylation of syndecan-2 also precedes the appearance of cilia in *Xenopus* and is required for LR determination [32]. Several other means of signaling (serotonin,