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Modelling asymmetric somitogenesis: Deciphering the mechanisms behind species differences

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ABSTRACT

Somitogenesis is one of the major hallmarks of bilateral symmetry in vertebrates. This symmetry is lost when retinoic acid (RA) signalling is inhibited, allowing the left-right determination pathway to influence somitogenesis. In all three studied vertebrate model species, zebrafish, chicken and mouse, the frequency of somite formation becomes asymmetric, with slower gene expression oscillations driving somitogenesis on the right side. Still, intriguingly, the resulting left-right asymmetric phenotypes differ significantly between these model species.

While somitogenesis is generally considered as functionally equivalent among different vertebrates, substantial differences exist in the subset of oscillating genes between different vertebrate species. Variation also appears to exist in the way oscillations cease and somite boundaries become patterned. In addition, in absence of RA, the FGF8 gradient thought to constitute the determination wavefront becomes asymmetric in zebrafish and mouse, extending more anteriorly to the right, while remaining symmetric in chicken. Here we use a computational modelling approach to decipher the causes underlying species differences in asymmetric somitogenesis. Specifically, we investigate to what extent differences can be explained from observed differences in FGF asymmetry and whether differences in somite determination dynamics may also be involved.

We demonstrate that a simple clock-and-wavefront model incorporating the observed left-right differences in somitogenesis frequency readily reproduces asymmetric somitogenesis in chicken. However, incorporating asymmetry in FGF signalling was insufficient to robustly reproduce mouse or zebrafish asymmetry phenotypes. In order to explain these phenoptypes we needed to extend the basic model, incorporating species-specific details of the somitogenesis determination mechanism. Our results thus demonstrate that a combination of differences in FGF dynamics and somite determination cause species differences in asymmetric somitogenesis. In addition,they highlight the power of using computational models as well as studying left-right asymmetry to obtain more insight in somitogenesis.

1. Introduction

The vertebrate body plan displays bilateral symmetry, for instance in the placement of limbs and cranial features; somitogenesis is one of the major hallmarks of this symmetry. The regular blocks of tissue patterned during somitogenesis later on give rise to the vertebrae, ribs and skeletal axial muscles. Somite pairs are generated periodically in an anterior to posterior direction from the presomitic mesoderm (PSM). The use of mathematical modeling has a long and rich tradition in the somitogenesis research field and has played a critical role in our understanding of the mechanisms underlying somite formation (Cooke and Zeeman, 1976; Hubaud and Pourquié, 2014). It is now generally accepted (but see Cotterell et al. (2015)) that periodic somite patterning arises from a so-called clock and wavefront mechanism (Cooke and Zeeman, 1976; Hubaud and Pourquié, 2014). In the posterior part of the PSM, a complex regulatory network with multiple negative feedbacks generates regular gene expression oscillations, called the somitogenesis clock (Palmeirim et al., 1997; Resende et al., 2014). The transition from temporal oscillations to spatial stripes is thought to be governed by the so-called determination wavefront, a morphogen gradient that extends from the posterior to the anterior (Aulehla and Pourquié, 2010). In the posterior, where morphogen levels are high, cells are maintained in an undifferentiated state and gene expression oscillations are supported. As cells progress towards the anterior, they experience lower and lower morphogen levels, which eventually allows them to differentiate and cease to oscillate. This process results in the periodic generation of pairs of somites flanking the notochord, with left and right somites being generated with identical timing and spacing.

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This symmetry becomes essential during later developmental stages when parts of the left and right somites fuse to form the vertebrae, and disturbances of somite symmetry can have severely disabling consequences such as scoliosis (Pourquié, 2011).

The somitogenesis clock, like all biological processes, is inherently noisy (Jiang et al., 2000; Herrgen et al., 2010). Therefore, additional levels of control are necessary to coordinate the behaviour of individual cells to ensure sharply delineated, coherent boundary formation and generate precise left-right symmetry. The processes synchronising cells along one side of the notochord have been studied extensively. Experimental data demonstrate that Delta-Notch mediated cell-cell signalling synchronises directly neighbouring cells (Özbudak and Lewis, 2008; Soza-Ried et al., 2014), an effect well known from modelling studies on coupled oscillators (Morelli et al., 2009; Herrgen et al., 2010). In addition, modelling studies have elucidated the importance of cell-mixing for synchronised oscillations (Uriu et al., 2009) and of cell-sorting for coherent somite patterning (Hester et al., 2011). In contrast, the precise mechanism underlying left-right coordination has only been partly elucidated experimentally and have thusfar not been investigated using a computational approach.

During part of the somitogenesis process, the left-right signalling pathway is active to confer left- or right-handed identity to the distal lateral plate mesoderm from which internal organs such as the heart and liver are generated (Brent, 2005). This left-right signalling not only passes through Hensen's node (Kupffer's vesicle in zebrafish) and the posterior PSM, but als leads to a transient asymmetrical distribution of signalling molecules such as FGF, Delta-Notch and Wnt that are also involved in somitogenesis (Boettger et al., 1999; Raya et al., 2003, 2004; Krebs et al., 2003; Kawakami et al., 2005; Tanaka et al., 2005; Nakaya et al., 2005; Jacobs-McDaniels and Albertson, 2011; Huang et al., 2011; Kato, 2011). Given the symmetry of somitogenesis this implies that under normal conditions compensatory mechanisms act to counteract the effects of left-right signalling on somitogenesis.

Experiments indicate that retinoic acid (RA) normally buffers the effects of the left-right pathway on somitogenesis, as somite symmetry is perturbed when RA is inhibited while left-right signalling remains unaltered (Kawakami et al., 2005). Interestingly, in absence of RA the left side becomes delayed in chick, while the right side becomes delayed in zebrafish and mouse (Kawakami et al., 2005; Vermot and Pourquié, 2005; Vermot et al., 2005; Sirbu and Duester, 2006; Brent, 2005). A potential cause for this difference could be the observed difference in FGF8 dynamics in absence of RA. While in chick the FGF8 gradient remains symmetric, in zebrafish and mouse the gradient of FGF8 extends more anteriorly on the right. Since FGF8 is an important component of the determination front, this may explain the different observed delays. However, there also exist additional species differences in the genes taking part in the somitogenesis oscillator (Krol et al., 2011), and in the precise dynamics of somite determination (Akiyama et al., 2014; Niwa et al., 2011). In this study, we use a modelling approach to investigate the mechanisms underlying the different asymmetry phenotypes observed in zebrafish, chick and mouse. We build on well-established clock-and-wavefront models of somitogenesis that were previously applied to study the influence of noise, delays in cell-cell signalling and mixing on synchronised somitogenesis (Morelli et al., 2009; Ares et al., 2012), by incorporating the asymmetric slowing of oscillator frequency in absence of RA. We subsequently extend this model in a stepwise fashion with the experimentally observed species differences in wavefront dynamics and somite determination to investigate their importance for the different asymmetry phenotypes.

We demonstrate that a simple clock and wavefront mechanism combined with asymmetric oscillator frequency is sufficient to explain the chick asymmetry phenotype. However, incorporating the additional asymmetry in FGF8 wavefront observed in zebrafish and mouse is insufficient to robustly reproduce zebrafish and mouse asymmetric somitogenesis. We show that the additional incorporation of species specific differences in somite boundary patterning mechanism is necessary to robustly simulate zebrafish and mouse asymmetry phenotypes. An additional advantage of these model extensions is that they pattern somites in a block-like fashion and well before gene expression oscillations cease which more closely resembles experimental observations (Shih et al., 2015; Niwa et al., 2011) than the cell-by-cell fashion concurrent with ceasing of oscillations that is typical of most clock-and-wavefront models. With our model, we can explain the paradoxical delay of chick somitogenesis on the left, while oscillator frequency is slowest on the right, from the ensuing differences in somite size. Finally, our models suggest that rostro-caudal somite polarity may arise from the temporal sequence of within somite patterning that is dictated by the frequency profile, a prediction that can be experimentally tested.

2. Methods

2.1. Clock and wavefront model

We model the presomitic mesoderm (PSM) as a 2D strip of cells. In the posterior the cells form a single coherent tissue representing the posterior zone where cells are added to the PSM (Posterior Addition Zone, or PAZ), more anteriorly the cells form two strips of tissue flanking the notochord (Fig. 2A). Each individual cell is endowed with an internal oscillation clock that is represented by a simple sinusoidal phase oscillator, as described in Jaeger and Goodwin (2001), Morelli et al. (2009), Murray et al. (2011), Ares et al. (2012) (Fig. 2A). We ignore the influence of noise or cell-cell signalling that have been extensively investigated in previous studies (Morelli et al., 2009; Herrgen et al., 2010; Murray et al., 2011; Ares et al., 2012). We assume that at the tissue level, a spatial frequency profile dictates oscillation frequency as a function of position in the PSM. Following work from Morelli et al. (2009) we described the frequency profile as:

$$\omega(x) = \omega_{max} * \left(1 - \frac{1}{\sigma^n} * x^n\right) \quad (1)$$

where $\omega(x)$ is the frequency at a certain distance x away from the posterior end of the PSM (Fig. 2B). ω_{max} is the oscillation frequency of cells at the posterior end of the PSM, and σ is the length over which the frequency will drop to 0. Usually σ is taken to be the PSM length, unless otherwise indicated. Finally, n is the exponent that determines the nonlinearity of the frequency profile: the higher the exponent, the further anterior in the PSM the frequency will start decreasing and the steeper the slope will be. When cells stop oscillating (at position σ, the anterior end of the PSM), they memorize their phase and become incorporated into a (pre)somite. Morelli et al. (2009) demonstrated that a frequency gradient of this shape reproduces the experimentally observed narrowing of waves of gene expression as these move anteriorly.

Cells are continuously added at the posterior end of the PSM, and the oscillators of these new cells are assumed to obtain the phase and frequency of the cells already present there (Fig. 2A). The anterior wavefront of somite determination travels toward the posterior at the same speed as cells are added, so that the PSM maintains a constant size (Morelli et al., 2009). The frequency profile shifts along, so that cells experience a progressively lower oscillation frequency, until the wavefront passes and their phase becomes frozen (Fig. 2B). We adapt this model of somite formation as we go on to account for differences between animals in the next section.

2.2. Left-right differences

When we implement left and right differences, we change the frequency ω_0, and/or the extent of the frequency profile σ differently in the left and right PSM, which results in different behaviour for the left