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Modeling the segmentation clock as a network of coupled oscillations in the Notch, Wnt and FGF signaling pathways

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Abstract

The formation of somites in the course of vertebrate segmentation is governed by an oscillator known as the segmentation clock, which is characterized by a period ranging from 30 min to a few hours depending on the organism. This oscillator permits the synchronized activation of segmentation genes in successive cohorts of cells in the presomitic mesoderm in response to a periodic signal emitted by the segmentation clock, thereby defining the future segments. Recent microarray experiments [Dequeant, M.L., Glynn, E., Gaudenz, K., Wahl, M., Chen, J., Mushegian, A., Pourquie, O., 2006. A complex oscillating network of signaling genes underlies the mouse segmentation clock. Science 314, 1595–1598] indicate that the Notch, Wnt and Fibroblast Growth Factor (FGF) signaling pathways are involved in the mechanism of the segmentation clock. By means of computational modeling, we investigate the conditions in which sustained oscillations occur in these three signaling pathways. First we show that negative feedback mediated by the Lunatic Fringe protein on intracellular Notch activation can give rise to periodic behavior in the Notch pathway. We then show that negative feedback exerted by Axin2 on the degradation of β-catenin through formation of the Axin2 destruction complex can produce oscillations in the Wnt pathway. Likewise, negative feedback on FGF signaling mediated by the phosphatase product of the gene *MKP3*/*Dusp6* can produce oscillatory gene expression in the FGF pathway. Coupling the Wnt, Notch and FGF oscillators through common intermediates can lead to synchronized oscillations in the three signaling pathways or to complex periodic behavior, depending on the relative periods of oscillations in the three pathways. The phase relationships between cycling genes in the three pathways depend on the nature of the coupling between the pathways and on their relative autonomous periods. The model provides a framework for analyzing the dynamics of the segmentation clock in terms of a network of oscillating modules involving the Wnt, Notch and FGF signaling pathways.

Keywords: Model; Somitogenesis; Segmentation; Oscillation; FGF; Wnt; Notch

1. Introduction

The segmented or metameric aspect of the body axis is a basic characteristic of many animal species ranging from invertebrates to human. The vertebrate body is built on a metameric organization, which consists of a repetition along the antero-posterior (AP) axis of functionally equivalent units, each comprising a vertebra, its associated muscles, peripheral nerves and blood vessels. The segmented distribution of vertebrae derives from the earlier metameric pattern of the embryonic somites which are epithelial spheres generated in a rhythmic fashion from the mesenchymal presomitic mesoderm (PSM). The segmental pattern was proposed to be established in the PSM by a mechanism involving an oscillator (the segmentation clock) which is thought to set the periodicity of the process (see scheme in Fig. 1), and a traveling wavefront defined by antagonistic gradients of the signaling molecules Fibroblast Growth Factor (FGF) and retinoic acid (RA), which controls the spacing mechanism of somite boundaries (Pourquié, 2003; Dubrulle and Pourquié, 2004; Delfini et al., 2005). We recently proposed a model in which the mutual antagonism of FGF and RA gradients generates a sharp threshold associated with a phenomenon of bistability (Goldbeter et al., 2007). This phenomenon, which

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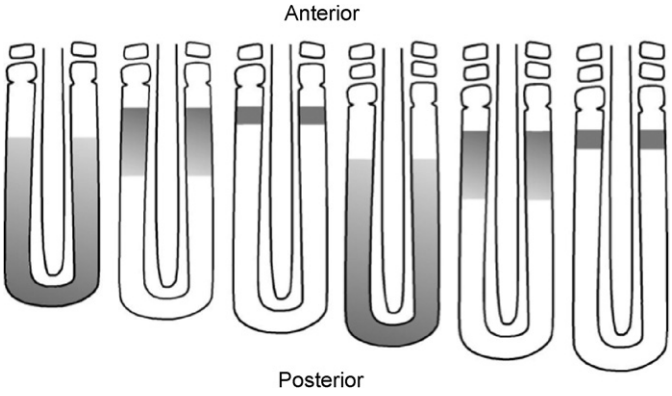

Fig. 1. Scheme illustrating the operation of the segmentation clock in the presomitic mesoderm (PSM) in chick embryos. Oscillations originate at the posterior end of the PSM and propagate as a transcription wave (in gray) toward the anterior end where the periodic signal emitted by the clock triggers the expression of genes, such as *Mesp2*, specific for somite formation (not shown). Each cycle of the segmentation clock oscillation corresponds to the formation of a new pair of somites. Growth of the PSM at the posterior end occurs continuously until the final number of somites is reached. The period of the segmentation clock in chick embryos is close to 90 min.

involves the coexistence between two stable steady states, is restricted to a defined AP level within the PSM. We suggested that the abrupt bistable steady-state switch that can occur in this window can explain the coordinated segmental gene activation which takes place in presumptive segments in response to the periodic signal of the segmentation clock.

The existence of the segmentation clock has been substantiated by numerous experimental observations, which first showed that genes in the Notch signaling pathway are expressed in a periodic manner, with a period of the order of 90 min in the chick embryo (Fig. 1) (Palmeirim et al., 1997; Pourquié, 2003; Giudicelli and Lewis, 2004). Negative feedback on gene expression appears to underlie the periodic transcription of the gene *Lunatic fringe* (*lfng*) (Dale et al., 2003). The segmentation clock also involves oscillations in the Wnt signaling pathway, which appear to govern the periodic operation of the Notch pathway (Aulehla et al., 2003). Microarray studies of the mouse PSM transcriptome recently revealed (Dequeant et al., 2006) that the oscillator associated with the segmentation clock drives the periodic expression of a large network of cyclic genes involved in the Notch, Wnt and also the FGF signaling pathways. The cyclic genes identified in the Notch and FGF pathways oscillate synchronously, but in antiphase with respect to the cyclic genes in the Wnt pathway. By this microarray approach, six of the eight known mouse cyclic genes—*Hes1*, *Hes5*, *Hey1*, *Lfng*, *Axin2* and *Nkd1*—were identified with periods of 94, 102, 112, 81, 102 and 112 min, respectively (Dequeant et al., 2006). The observations of Dequeant et al. (2006) throw light on the molecular nature of the segmentation clock and suggest that it relies on coupled oscillations in the Wnt, FGF and Notch signaling pathways. The antiphase relationship between the genes cyclically expressed in the FGF and Notch pathways on one hand, and the Wnt pathway on the other hand, further points to the existence of cross-talk between the FGF/Notch and Wnt pathways possibly in the form of mutual inhibition (Dequeant et al., 2006).

Here, turning to the nature of the oscillatory process involved in the clock and wavefront mechanism (Cooke and Zeeman, 1976), we propose a model for the segmentation clock in amniotes like chick and mouse. The model is based on a network of coupled oscillators in the Wnt, FGF and Notch signaling pathways. We first show how oscillations can occur in each of the three pathways, as a result of negative feedback regulation, in the presence of a constant level of an external triggering signal. We then study the oscillatory behavior of the coupled Notch–Wnt–FGF signaling network and discuss possible mechanisms capable of accounting for the tight temporal coordination of cycling genes oscillations in the mouse PSM. Our approach at this stage is qualitative rather than quantitative, as we wish to explore the dynamical consequences of negative feedback regulation within each pathway and of the coupling between the Wnt, Notch and FGF pathways without trying to use experimentally established parameter values, many of which have yet to be determined. By including the interactions between the FGF, Wnt and Notch signaling pathways the model differs from previously proposed models which show how autonomous oscillations can arise due to Notch signaling in zebrafish (Lewis, 2003; Monk, 2003) or to the interaction between Notch and Wnt signaling in mice (Rodriguez-Gonzalez et al., 2007).

The modeling approach allows us to address a series of questions: (i) can the coupling of the Notch, Wnt and FGF oscillators result in their synchronization? (ii) Does synchronization require that the autonomous periods of the three oscillating pathways, i.e. their period in the absence of coupling, be sufficiently close to each other? (iii) Can quasiperiodic, complex periodic or aperiodic (i.e. chaotic) oscillations result from the coupling of the three oscillating pathways when their autonomous periods are too far apart from each other? (iv) Is mutual inhibition required to explain the antiphase oscillations in the Wnt and in the FGF/Notch pathways, or can this phase relationship be obtained in other conditions?

Together with Marc Kirschner and co-workers, Reinhart Heinrich modeled in great detail signal transduction in the Wnt signaling pathway (Lee et al., 2003; Krüger and Heinrich, 2004), without focusing, however, on the possibility of oscillatory behavior. Here we examine the conditions in which oscillations in the segmentation clock might arise in the Wnt pathway, and also in the Notch and FGF pathways, as a result of negative feedback regulation. The model supports the view (Dequeant et al., 2006) that the network formed by these three signaling pathways