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Cell-based simulation of dynamic expression patterns in the presomitic mesoderm

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Abstract

To model dynamic expression patterns in somitogenesis we developed a Java-application for simulating gene regulatory networks in many cells in parallel and visualising the results using the Java3D API, thus simulating the collective behaviour of many thousand cells. According to the 'clock-and-wave-front' model mesodermal segmentation of vertebrate embryos is regulated by a 'segmentation clock', which oscillates with a period of about 2 h in mice, and a 'wave front' moving back with the growing caudal end of the presomitic mesoderm. The clock is realised through cycling expression of genes such as *Hes1* and *Hes7*, whose gene products repress the transcription of their encoding genes in a negative feedback loop. By coupling the decay of the *Hes1* mRNA to a gradient with the same features and mechanism of formation as the mesodermal *Fgf8* gradient we can simulate typical features of the dynamic expression pattern of *Hes1* in the presomitic mesoderm. Furthermore, our program is able to synchronise *Hes1* oscillations in thousands of cells through simulated Delta–Notch signalling interactions.

Keywords: Somitogenesis; Object-oriented modelling; *Hes1*; *Fgf8* gradient; Delta–Notch signalling

1. Introduction

The segmentation of the adult vertebrate body is evident for example in the reiterated structures of the vertebrate axial skeleton, spinal nervous system and body muscles. It is established in embryogenesis after gastrulation when at the rostral end of the presomitic mesoderm (PSM) on both sides of the neural tube segments termed somites separate from the PSM. In these somites the outer cells change their tissue type from mesodermal to epithelial. Later the somitic cells change their adhesive and migratory properties, and finally contribute to the adult structures mentioned above. Somites are generated successively, one pair after the other, from the PSM. Waves of gene expression starting at the posterior tip of the PSM and running in anterior direction are leading to the formation of one somite at the anterior end of the PSM at both sides of the neural tube for each upcoming 'wave' (Dale and Pourquie, 2000; Saga and Takeda, 2001).

The first of this 'cycling genes' was discovered in the development of the chick. It was shown (Palmeirim et al., 1997) by in situ hybridisation that the *Hairy1* gene shows a periodically repeating expression pattern. It starts with a strong expression at the caudal end, extends then rostrally, while weakening at the tail, and finally contracts into a narrow stripe. This anterior stripe of expression marks the region where the next somite will form. Interestingly, experimental manipulation in chick showed that this 'wave' of gene expression could not even be stopped by cutting out a wedge of the middle PSM (Palmeirim et al., 1997), which gives rise to the question how the 'wave' can bridge this gap in mesodermal tissue. This experiment argues against a mechanism based on the diffusion of signalling

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molecules. A similar expression pattern for the orthologous gene *Hes1* was found in mice (Jouve et al., 2000). Later, it was discovered that *Hes1* is not only expressed in the PSM but in many other tissues as well. Another member of the Hes-family of bHLH-proteins, Hes7, was identified (Bessho et al., 2001a, b). It is restricted to the PSM and the corresponding mRNA displays a similar expression pattern as *Hes1*. However, only *Hes1* could be studied in cell culture. Cultured fibroblast cells were induced to express the gene in an oscillatory manner (Hirata et al., 2002). It was shown that Hes1 represses its own transcription by binding as homo-dimers to three so-called N-boxes in its promoter (Takebayashi et al., 1994). The half-life times of the *Hes1* mRNA and protein were measured. These data allowed a differential equation model to be built for mRNA, protein and a postulated factor, termed *Z*, which exhibited oscillatory behaviour with the expected period of roughly 2 h (Hirata et al., 2002).

Later, Monk could describe these oscillations without the *Z*-factor by using delay differential equations (Monk, 2003). The same was accomplished in the zebrafish system for the her1 and her7 genes (Lewis, 2003). Lower bounds for the delays were estimated from the length of the genes and proteins by using the known polymerisation rates of RNA-polymerase II and ribosome, respectively (Lewis, 2003). This model was also used to describe the *Hes7* oscillations in mouse and its abolishment observed in mouse embryos expressing mutant Hes7 protein with a longer half-life (Hirata et al., 2004).

It is now generally believed that the cycling genes represent the clock part of the 'clock- and wave-front' model formulated by Cooke and Zeeman (1976). The 'wave-front', which moves backward with the caudal end and determines where new somites are formed in the PSM, could possibly be explained by the *Fgf8* gradient discovered recently in the PSM of mice (Dubrulle and Pourquie, 2004a, b). This gradient is not generated by diffusion, but by the constant growth of the PSM and the continuous transcription of *Fgf8* in the growing tail-bud, while transcription ceases in the rest of the PSM. The *Fgf8* mRNA decays with a comparatively long half-life of the order of hours (Dubrulle and Pourquie, 2004a). It is translated into protein in the entire PSM—not only the growth zone—leading to a graded distribution of mRNA and protein along the rostro-caudal axis of the PSM.

Here we present a computer model to explain the dynamic gene expression patterns in somitogenesis and the collective behaviour of many cells. The model is cell based with a gene regulatory network inside each cell described by differential equations. The cells can proliferate and display the concentration of a user-selected mRNA or protein by the intensity of their colouration. As a first step we try to understand the dynamics of *Hes1* expression in the tail-bud phase when a group of stem cells provides for a roughly constant length of the PSM while new somites separate from the anterior end of the PSM (Brown et al., 2006; Dale and Pourquie, 2000; Deschamps and van Nes, 2005). When we incorporated the Fgf8 gradient described above in our computer model of the growing PSM and coupled the gradient linearly to various models of the *Hes1* oscillator in each cell, we observed the characteristic 'wave' progressing from posterior to anterior PSM, narrowing while moving forward, and coming to a stop finally. This process repeats itself as long as the PSM is growing, forming the characteristic stripe pattern for the *Hes1* mRNA expression.

The cycling genes are mostly part or effectors of the Delta–Notch signalling pathway. Disturbing this pathway leads to a disruption of somitogenesis (Hrabe de Angelis et al., 1997), probably because the direct cell to cell signalling synchronises the oscillations in neighbouring cells and stabilises the expression patterns against fluctuations (Jiang et al., 2000). Therefore, as a next step, we gave our virtual cells the ability to recognise nearest neighbours and synchronise their oscillations by Delta–Notch signalling.

2. Methods

To simulate the dynamics of the mRNA expression during somitogenesis we developed a program written in the Java language using the Java3D API.

As somitogenesis is a dynamic phenomenon which involves cell proliferation, waves of gene expression, cell polarisation, etc., a data structure was needed to model this collective behaviour of many cells. In addition, visualisation of cell behaviour and gene expression had to be integrated. Therefore object-oriented modelling was employed: A cell is described as an instance of a Java object with methods for cell division, cell death, propagating in time the concentration variables of the gene regulatory network inside the cell, and displaying concentration of protein or mRNA as intensity of colouration of each cell (virtual in situ staining). To shorten rendering time each cell is displayed as a sphere or symmetrical polyhedron. Furthermore, the simulated cells are able to recognise their nearest neighbours. Internal variables describing Delta–Notch pairs are created accordingly and integrated into the reaction network. Cell division and death are modelled phenomenologically, i.e. purely descriptive, and provide the 'boundary conditions' for processes that are simulated by more detailed models (e.g. reaction networks). For example, cell proliferation is modelled by a process which creates a copy of the 'mother cell' furnished with the equivalent variables for the gene regulatory network. The 'daughter cell ''grows' out of the 'mother cell' along a fixed direction. This growth stops when both cells are separated. The 'daughter cell' then develops as an autonomous unit.

Of course, it is currently not possible to describe all the thousands of polymerisation reactions (for the generation of the mRNA and its translation into protein) and the numerous processing steps of the mRNAs in detail. To simulate the biochemical reactions we employed a kinetic equation framework with differential equations for the temporal development of the variables describing mRNA