Models for pattern formation in somitogenesis: a marriage of cellular and molecular biology

S. Schnell †‡[1], P. K. Maini ‡[2], D. McInerney ‡, D. J. Gavaghan | and P. Houston ||

† Christ Church, Oxford OX1 1DP, UK, ‡ Centre for Mathematical Biology Mathematical Institute, 24–29 St Giles', Oxford OX1 3LB, UK, | Computing Laboratory, Wolfson Building, Parks Road, Oxford, OX1 3QD, UK, || Department of Mathematics and Computer Science, University of Leicester, University Road, Leicester, LE1 7RH, UK

1. E-mail: schnell@maths.ox.ac.uk

2. E-mail: maini@maths.ox.ac.uk

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- Address for proofs: S. Schnell c/o Prof P. K. Maini, Centre for Mathematical Biology, Mathematical Institute, 24–29 St Giles', Oxford OX1 3LB, UK
- Telephone: +44-(0)1865-280614
- Fax: +44-(0)1865-270515
- The authors wish to submit the final manuscript electronically in LaTeX

Abstract

Somitogenesis, the process by which a bilaterally symmetric pattern of cell aggregations is laid down in a cranio-caudal sequence in early vertebrate development, provides an excellent model study for the coupling of interactions at the molecular and cellular level. We extend a recent chemical pre-pattern model based on the cell cycle *Journal of theoretical Biology* **207** (2000), 305–316, by including cell movement and show that the resultant model exhibits the correct spatio-temporal dynamics of cell aggregation. We also postulate a model to account for the recently observed spatio-temporal dynamics at the molecular level.

1 Introduction

In recent years, dramatic advances made in genetic and molecular biology have led to detailed descriptions of a number of events in early embryological development. This unprecedented flood of experimental data may allow us to understand how genes and proteins work collectively in cells to develop an individual. This is one of the great challenges for modern science, but in the absence of specific mechanistic details, it is an impossible task. To understand the mechanisms underlying developmental events it is necessary to bridge the gap between experimental observations at the biochemical and genetic levels and those at the cellular level. In other areas of biology, such as neurophysiology, mathematical modelling has led to fundamental insights and discoveries through a process of synthesis and integration of experimental observations. In such cases, mathematical modelling techniques are used as a research tool, comparable with any powerful laboratory technique, for hypotheses testing and for making experimentally verifiable predictions.

Somitogenesis is a fascinating developmental process for studying such issues, because it has a long history of experimentation at the cellular level and, recently, a number of key molecular components have been identified. In addition, somitogenesis serves as a model process for studying pattern formation and a number of different aspects of embryogenesis, such as the role of biological clocks during development, gene expression and cell differentiation, cell-cell signalling and signalling cascades, cell migration and adhesion [54].

In vertebrates and cephalochordates, the body axis is divided along the cranio-caudal axis into similar repetitive structures known as somites. These structures are formed during the regression of the primitive streak, when the neural tube folds to gather at the centre of the embryo and the segmental plates, often referred to as the paraxial mesoderm or presomitic mesoderm (PSM), are periodically segmented to produce somites as spheres of cells formed on each side of the anterior-posterior (AP) axis in a cranio-caudal sequence (see Figure 1). A somite is formed by the condensation of groups of mesenchymal cells, which become polarised and form a three-dimensional epithelium. Somites are divided by a fissure into anterior and posterior halves that differ in their gene expression and differentiation. Later in development, somites govern the segmental organisation of peripheral spinal nerves, vertebrae, axial muscles, and the metameric distribution of early blood vessels [58, 59].

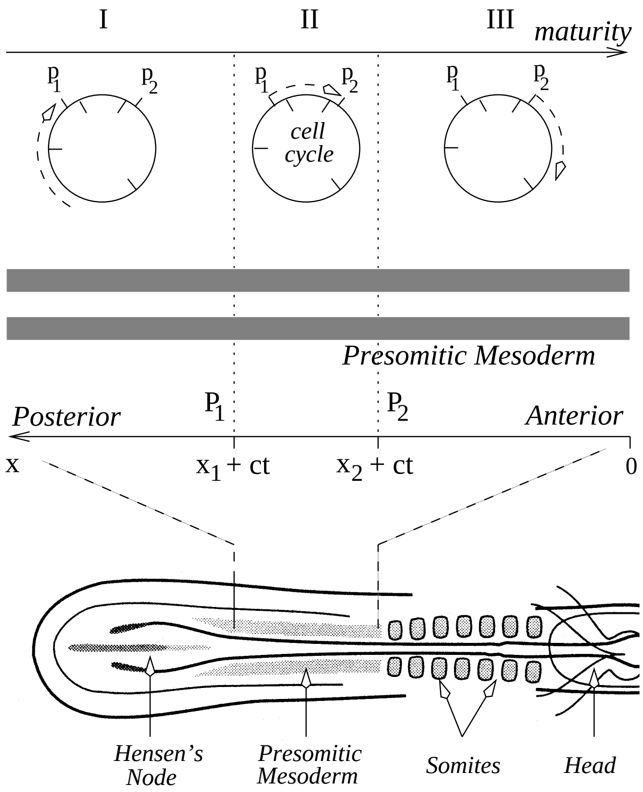

Figure 1: Diagrammatic representation of the cell cycle model illustrating the two time points P_1 and P_2 and the three key stages proposed by the cell cycle model. Cells at the posterior-most margin of the PSM (I) are at a younger developmental age than those undergoing somitogenesis. As these cells mature, they become capable (II) of responding to the signalling molecule which is secreted by cells at P_2. As they mature to the next stage (III) they begin to form somites. At stage (III) they are no longer able to respond to or emit a signal. (Redrawn with modifications from [9]).

Cells condense into somites by undergoing changes in their adhesive and migratory properties until they have arrived at their destination, and later differentiate according to the developmental program [23, 15]. The condensation of mesenchymal cells and the formation of somites is most likely triggered by the interaction of cell adhesion molecules (CAMs), such as N-cadherins, on the surface membrane of somitic cells. At least one type of Ca^{2+}-independent CAM, known as NCAM, and two Ca^{2+}-dependent CAMs, namely cadherin 11 and N-cadherin, are expressed in cells of the PSM during somitogenesis. Expression of NCAM, N-cadherin and cadherin 11 are high in the anterior part of the PSM, coinciding with the condensation of mesenchymal cells and the formation of somites. NCAM and N-cadherin are expressed in the entire somite, whereas cadherin 11 is restricted to the posterior half of the somite [12, 48, 26]. The importance of N-cadherin in somite formation is further supported by phenotypes of mouse embryo mutant for N-cadherin [49] and also by antibody-blocking experiments [31]. Somites in N-cadherin mice mutants are smaller and irregular. Mutations in NCAM and cadherin 11 show no structural anomaly on somite formation [61, 17]. This requires spatial and temporal control of the expression of CAMs and other components like integrins. The regulation of these components is unknown: it might be an intrinsic activity of the PSM or require an inductive signal (presently unknown).

Genetic or environmental factors can disturb somitogenesis, and there are many clinical conditions which occur as a result. The total number of somites is regulated in an embryo. The variation in somite number among vertebrates is very small. Somite numbers are usually around 50-70, except in the case of snakes which can have several hundred [52]. The variability of somite number within a species is less than 5% [10]. However, the number of somites can be altered by environmental disturbances [23]. For example, heat shock applied to chick embryos can induce the formation of an extra somite [62] and in cold-blooded vertebrates the duration of somite formation is temperature-dependent [14].

There is very strong evidence that somite and somitic boundary formation is controlled by one or two segmentation clocks. The first experimental evidence to support the segmentation clock was obtained after heat shock was applied to embryos. When a single heat shock is applied to chick embryos [47] several somitic anomalies, which may be uni- or bilateral, appear separated by relatively constant distances of six to seven normal somites. The repeated anomalies suggest that heat shock affects an oscillatory process within the somite precursor cells [47, 46, 56]. In addition, there appears to be some degree of cell-cycle synchrony between the cells in the PSM which are destined to segment together to form a somite [55]. Similar periodic anomalies in somite formation can also be caused by drugs inhibiting cell-cycle progression [46]. These experimental observations link the segmentation clock with the cell-cycle.

Recently, the study of the expression of *c-hairy-1* and *lunatic fringe* (*l-fng*) in the PSM of chick embryos has provided molecular evidence for the existence of a clock [44, 33]. During segmentation, the cells of the PSM go through at least 12 cycles of *c-hairy-1* and *l-fng* expression before becoming part of a somite, while more cells are continuously incorporated into the posterior end of the PSM. During the time taken for one somite to form, the expression of *c-hairy-1* and *l-fng* appears to sweep along the PSM in the posterior-anterior direction, narrowing as it moves along. This wavefront-like expression finally stops and is maintained only within the caudal half of each forming somite [44, 33] activating several genes of the Notch/Delta pathway [11]. However, it is not yet known whether the major role of *c-hairy-1* and *l-fng* is in the allocation of cells to individual somites or rather in the subdivision of somites into rostral and caudal compartments [9].

Although the principle differentiation pattern of all somites is very similar, subsequent differentiation causes groups of somites to form unique anatomical structures, depending on their position along the AP axis. There is now a large body of experimental work showing that positional specification of the PSM requires members of the *Hox* gene family [28, 22, 21]. Interestingly, the five to seven somite interval corresponding to the duration of the cell-cycle in the chick embryo correlates with the length of somite derivatives sharing the same regional identity at the vertebral level [46].

Despite considerable experimental work, many questions still remain. We can divide these into three categories:

i. *Segmentation:* What regulates the segmentation of the PSM? What drives the segmentation clock? Is there a relation between the cell-cycle and the *c-hairy-1* and *l-fng* oscillations? What regulates the refinement of these oscillations in the PSM? What is the interplay between *l-fng* and the Notch-Delta pathway? What determines differentiation into anterior and posterior halves within a somite? How can the heat shock experiments be explained?

ii. *Shape of segments:* What are the mechanical forces involved in the formation of a somite? How do cell aggregation, migration, alignment and cell-shape changes contribute to somite formation? What regulates the number and size of somites?

iii. *Positional information:* What determines the regional specification of somites? What is the role of cell division in this process? What is the precise role of the *Hox* gene family in this process and how is it controlled?

During the past three decades, several mathematical models have been proposed to address some of these questions. These models, in the main, consist of coupled systems of non-linear partial differential equations proposed to describe the spatio-temporal dynamics of cells in the PSM. Recently, we have reviewed a large number of theoretical models for somitogenesis and have found that although some of these successfully account for certain aspects of somitogenesis, they fail to explain, or even contradict, other observations [53, 54]. In a previous paper, we have claimed that in the light of recent molecular evidence the cell cycle model of Stern and co-workers [56, 46] is still a realistic model that incorporates several known aspects of somitogenesis better than most models. Furthermore, we developed a mathematical formulation which enables us to test the cell cycle as the segmentation clock, the effects of heat shock in somite formation and make experimentally verifiable predictions [9]. However, the previous study did not incorporate other aspects such as boundary formation, the affect of cell movement and adhesion, and positional information effects in somite differentiation. In the present article we extend our mathematical formulation of the cell cycle model for somitogenesis by adding a third equation to account for the cell density. In Section 2, after introducing the mathematical assumptions and formulation under which the model must operate, we present a qualitative description of the behaviour of the model solving numerically the governing equations. Some conclusions of the model are discussed with special emphasis on the nature of the segmentation clock, followed by the presentation of a model for the regionalisation of somites (Section 3).

2 The cell cycle model

The cell cycle model of Stern and collaborators [56, 46] links the cell cycle with somite segmentation. This hypothesis is supported by the following observations: (1) the existence of discrete regions of cell synchrony in the PSM, (2) a cell cycle duration of 9 hours, which corresponds to the development of six to seven somites in the chick embryo, and (3) periodic anomalies caused by heat shock experiments are mimicked by drugs inhibiting the progression of the cell cycle.

The formation of a somite is explained by assuming that there are two successive time points, P_1 and P_2, a time interval of 1/7 of the cell cycle time apart. Somite cells recognise the P_1-P_2 time window. According to the model, cells destined to form somites leave Hensen's node strictly in the order in which they were derived from founder cells in the node, and they remain in that order. Hence there is some degree of cell cycle synchrony of somite cells at the same level, and these cells will be arranged along the embryonic axis in the same order as they are positioned in their cell cycles; older cells further anterior than younger ones [57]. We assume that a fraction of the cohort of cells destined to segment together reach P_2 before others and produce a signal to which cells situated between P_1 and P_2 would respond by later increasing their adhesion to each other shortly before segmentation, regardless of their position within the PSM. The signal produces a somitic factor, which according to our hypothesis is the precursor of a CAM. It has been demonstrated that CAMs can direct cell differentiation, regulate gene expression and tissue formation [30, 1]. Thus, the resulting group of cells would actually undergo segmentation one cell cycle after the P_1-P_2 time window. As they move out of the P_1-P_2 window, cells become refractory to the signal and/or unable to signal (see Figure 1).

The model proposes that heat shock temporarily blocks the cell cycle, so altering the number of cells that segment together. Such an alteration would occur once in each cell cycle in the segmental plate posterior to the P_1-P_2 time window, accounting for the repetitive anomalies resulting from heat shock.

We have recently formulated these ideas mathematically as a coupled system of nonlinear partial differential equations and have shown that the resultant mathematical model can account for the periodic abnormalities observed after a single heat shock, as well as the cell autonomous character of somite formation in the PSM explants and the transplantation experiments reversing the AP axis [9].

The model equations are developed in two spatial dimensions but many of the key properties of the model can be understood by considering the one-dimensional version. In this case, the model equations in non-dimensional form are:

$$\frac{\partial u}{\partial t} = \frac{(u+\mu v)^2}{\gamma+\kappa u^2}\chi_u(x,t) - \frac{u}{\kappa}, \tag{1}$$

$$\frac{\partial v}{\partial t} = \frac{\chi_v(x,t)}{\epsilon+u} - v + D\frac{\partial^2 v}{\partial x^2} \tag{2}$$

$$\frac{\partial \rho}{\partial t} = \frac{\partial}{\partial x}\left[-\tau\rho\frac{\partial v}{\partial x} + D_\rho\frac{\partial \rho}{\partial x}\right], \tag{3}$$

where

$$\chi_u(x,t) = H(ct - x + x_1) \qquad \tau = k_1 H(v-\xi)H(x-ct) \tag{4}$$

$$\chi_v(x,t) = H(ct - x + x_2) \qquad D_\rho = k_2 H(v-\xi)H(x-ct)\ , \tag{5}$$

and μ, γ, κ, ϵ, D, x_1, x_2, c, k_1, k_2 and ξ are positive constants, with $x_2 < x_1$ and $x_1 - x_2 = 1$. The Heaviside function, H, is of the general form $H(\alpha - x)$, where

$$H(\alpha - x) = \begin{cases} 1 & \text{if } x \le \alpha, \\ 0 & \text{if } x > \alpha, \end{cases} \tag{6}$$

for any α.

One of the crucial assumptions in the cell cycle model is the signalling switch-behaviour occurring in the kinetics of the somitic factor u and the signal v. This is captured in the above mathematical formulation by equations (1)–(2). In the right-hand side of (1), the switch-behaviour kinetics arises from linear degradation of u (the $-u/\kappa$ term) and production of u via the term $(u + \mu v)^2/(\gamma + \kappa u^2)$ which represents autocatalysis of u and enhanced u production by v, with saturation for large u. The term $1/(\epsilon + u)$ in the v equation (2) assumes that v is inhibited by u, so that cells which have already been specified as somitic (and hence have a high level of u) cannot signal. We assume that v also degrades linearly (the $-v$ term) and diffuses rapidly. Determination of a somite occurs one cell cycle before the somite actually forms, so the equation (3) for the cell movement can be decoupled from (1)–(2). The somitic cell density ρ is assumed to depend on the haptotactic sensitivity function τ and the diffusion function D_ρ. By choosing τ and D_ρ to be a function of v [see, (4)–(5)], changes in cell density are related to the levels of signalling molecules, restricting aggregation to certain regions of the spatio-temporal domain. Therefore, the functional form of τ and D_ρ has a crucial effect on the spatio-temporal behaviour of the cell density.

The embryonic axis is taken fixed with respect to the cells, and the node and segmental plates assumed to move down it at a constant rate c. We consider

$$0 \leq x \leq d(t), \ t \geq 0,$$

where $d(t)$ is the position of a point which moves down the embryonic axis at constant speed c. For points on the axis posterior to $x_2 + ct$, u and v tend to zero, while for points anterior to $x_2 + ct$, u and v tend to some fixed value. This leads to the following boundary conditions:

$$\begin{aligned} u, v \to 0 \qquad & \text{as } x - (x_2 + ct) \to +\infty \\ u, v \text{ are bounded} \qquad & \text{as } x - (x_2 + ct) \to -\infty \ . \end{aligned}$$

We have derived [9] constraints on the model parameter values to ensure that the cells of a potential somite adhere together resulting in the segmentation of a discrete somite. Setting $\kappa \gg 1$, $\gamma \ll 1$, $\mu \ll 1$, $\epsilon \ll 1$ and $D \gg 1$, we ensure that the constraints on the model parameter values are satisfied. The speed of the pattern is assumed to be $c = 5 \times 10^{-3}$ in non-dimensional terms. This is reasonable for the trunk region of the chick.

In our numerical simulations, the equations are solved on a closed bounded interval of $\mathbb{R}$, which we denote by Ω, typically $\Omega = [-10, 10]$. This domain is large enough to ensure that conditions at the boundary do not effect the patterning process. As an approximation to these infinite domain boundary conditions we impose zero flux boundary conditions at $x = -10$ and $x = 10$ (diffusion of v out of the domain is not considered).

The signalling behaviour of the model

In equations (1)–(2), we have presented a mathematical formulation of the cell cycle model proposed by Stern and collaborators [56, 46]. In this mathematical model, at time $t = 0$, $u \approx 0$ for all points $x > x_2$ and $u \approx 1$ for points $x < x_2$, while the level of signalling molecule is $O(1)$ throughout the domain. As time increases the heaviside functions χ_u and χ_v (see Figure 1) move caudally at speed c. Points where $u \approx 0$, which were initially posterior to χ_v, quickly become part of the region where χ_v is active. These low values of u activate v production leading to a pulse in v centred at $x_2 + ct$. Shortly after $t = 0$, at some time $t = \hat{t}$, the increase in v activates u between points $x = x_2$ and $x = x_1 + c\hat{t}$. As u rises it inhibits v production, and the pulse-like distribution of v quickly falls returning to $v \sim 1$. As v falls the rise in u slows. A short time after $t = \hat{t}$, $u \approx 1$ for all points between x_2 and $x_1 + c\hat{t}$. In this way, the region $x_2 < x < x_1 + c\hat{t}$ is specified as somitic. The process begins again when the jump in the heaviside function χ_v reaches point $x_1 + c\hat{t}$. Points where $u \approx 0$ become part of the region in which χ_v is active. These low values of u activate v production leading to a second pulse in v. From this description, it is clear that there is a small delay between signalling and specification. Hence, cells gain the potential to form a somite a short time after the signal is emitted. Although cells passing point P_2 at time $t = 0$ start producing v almost immediately, the pulse-like signal does not rise and fall in an instant. It is not until $t = \hat{t}$ that v is sufficiently high to activate u.

A typical numerical solution to (1)–(2) is shown in Figure 2. The results show successive pulses of v, occurring about 200 time units apart. A pulse in v is emitted at $x \approx 0$ leading to a jump in u between points $x \approx 0$ and $x \approx 1$. This jump in u corresponds to a collection of cells that will later form a somite. The first pulse at $x \approx 0$ is followed by a second at $x \approx 1$ and a third at $x \approx 2$, causing a series of jumps in u along the x-axis.

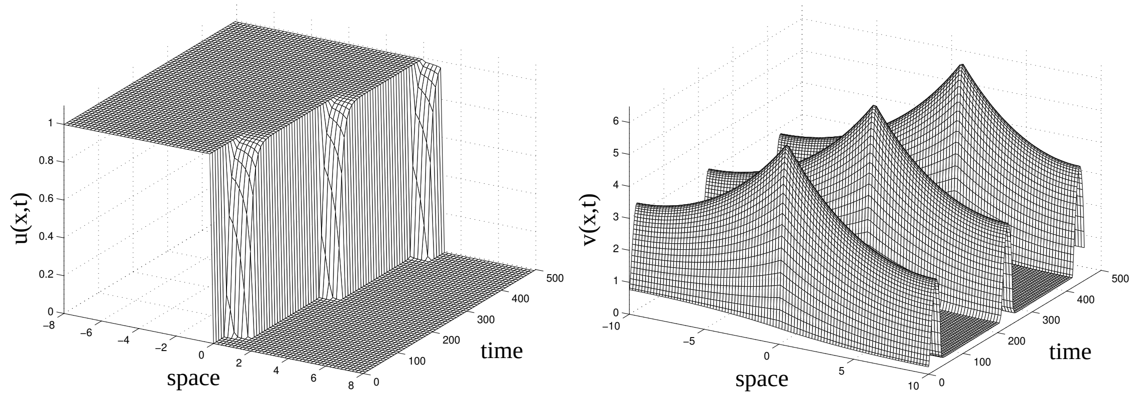

Figure 2: Numerical solution for the model equations (1)–(2) showing the spatio-temporal dynamics of $u(x,t)$ and $v(x,t)$. Each peak of v results in an abrupt increase in u which, in turn, triggers somite specification. Three "somites" are shown. Parameter values are $\mu = 10^{-4}$, $c = 5 \times 10^{-2}$, $\kappa = 10$, $\epsilon = 10^{-3}$, $D = 50$, $\gamma = 0.01$.

The process begins with the formation of the first peak in v (see Figure 2) at the position of P_2, quickly followed by a surge of v throughout the spatial domain. The level of v then decreases rapidly to a constant level. In response to this pulse-like signal, a fairly rapid increase in u occurs between P_1 and P_2. Thus we have a wavefront of the somitic factor (u) moving down the axis in jumps, as successive groups of cells are triggered to become somitic. Note that the level of u rises at approximately the same time in all cells between P_1 and P_2 when a signal is emitted. According to the cell cycle model, this results in the coordinated segmentation of a somite.

The effects of heat shock in somite formation are also considered in our mathematical formulation. Single heat shock experiments of Primmett and collaborators [47] in chick embryos resulted in multiple but discrete segmental anomalies. Up to 4 segmental anomalies could be observed in an embryo, including one small somite, one large somite or two fused somites. The experimental evidence [47] suggests that the pattern of anomalies depends on the relation between two parameters: i) the number of somites formed per cell cycle (ψ), which is approximately 6 to 7 somites, and ii) the length of the block caused by heat shock relative to the time between P_1 and P_2 (η); we estimate $\eta \approx 0.6$ (equal to the time duration of heat shock divided by the time to form a somite).

Within the framework of the cell cycle model heat shock treatment of an embryo has the effect of increasing the time between the points P_1 and P_2, since it blocks M phase cells in the cell cycle. In the mathematical formulation this leads to a delay in the production of u and v. The heaviside terms can be carefully modified to mimic the required delay. Note that in (1)–(2), for a given point x, the production of u and v begins at $t = (x-1)/c$ and $t = x/c$, respectively. To simulate the effect of heat shock, we partition the x-axis into intervals $I_i = (\alpha_i, \beta_i)$, for $i = 0, 1, 2, \dots,$ where

$$\alpha_i = \delta + i\psi \qquad \beta_i = \delta + \eta + i\psi \tag{7}$$

with $\delta \geq 0$. Then, once for each cell cycle, the condition

$$\{x : x \in I_i\} \mapsto \beta_i \tag{8}$$

is imposed, so that for any point of a given cell cycle within an interval, the production of u and v does not begin until $t = (\beta_i - 1)/c$ and $t = \beta_i/c$, respectively.

The effect of condition (8) on the signalling process produces a discontinuity in the heaviside function χ_u at the point $x = \alpha_i$ when $t = \hat{t}_1 = (\alpha_i - 1)/c$ and at the point

$x = \beta_i$ when $t = \hat{t}_2 = (\beta_i - 1)/c$. When $t \geq \hat{t}_1$, the discontinuity in the heaviside function remains fixed at $x = \alpha_i$ until $t = \hat{t}_2$. Therefore, points in the interval (α_i, β_i) are unable to respond to changes in the concentration of v until time $\hat{t}_2$. If at $t \leq \hat{t}_1$, there is a pulse in v, then for $t > \hat{t}_1$, only cells allocated in points where $x \leq \alpha_i$ will respond to the signal. The cells which lie in the interval (α_i, β_i) are unable to respond until $t = \hat{t}_2$, at which time the level of v may be too low to activate production of u. Condition (8) also produces a discontinuity in the heaviside function χ_v at the point $x = \alpha_i$ when $t = \alpha_i/c$ and at the point $x = \beta_i$ when $t = \beta_i/c$. When $t \geq \alpha_i/c$, the discontinuity in the heaviside function remains fixed at $x = \alpha_i$ until time $t = \beta_i/c$. Hence, there is no pulse in v until $t = \beta_i/c$, although u may be low for $x \geq \alpha_i$. The pulse in v emitted at $t = \beta_i/c$ is much larger than normal as all points in the interval (α_i, β_i) can begin production of v.

In Figure 3, we present the results of a heat shock on the pulse of signalling molecule u. In this figure, a schematic representation of the first ten somites shows a sequence of one normal, two abnormal, four normal, two abnormal and one normal somite. This shows that the model can exhibit periodic anomalies due to heat shock.

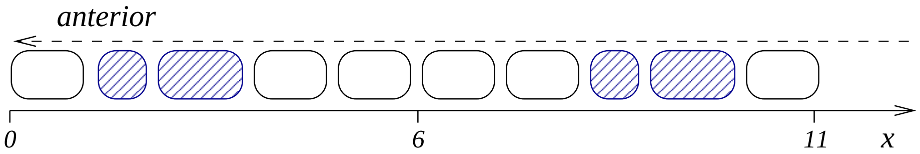

Figure 3: Schematic representation of the spatio-temporal pattern in u obtained by solving (1)–(2) subject to the condition (8) with parameter values $\mu = 10^{-4}$, $c = 5 \times 10^{-2}$, $\kappa = 10$, $\epsilon = 10^{-3}$, $D = 25$, $\gamma = 0.01$. The effect of the heat shock over one cell cycle was simulated by choosing $i = 0$, $\delta = 2$, $\psi = 6.333$ and $\eta = 0.6$. The delay results in a sequence of one normal, two abnormal, four normal, two abnormal and one normal somite. Each somite of the diagram corresponds to one pulse in u as shown in Figure 2.

The mathematical formulation of the cell cycle model enables us to predict correctly the anomalies caused by exposing the embryo to a single heat shock. We can also use the model to make experimentally testable predictions on the outcome of heat shock treatments occurring at different times and on the effects of multiple heat shock treatments. For example, we predict that applying two heat shocks during one cell cycle would produce a sequence of abnormal somites separated by one normal somite. This is presently being tested experimentally.

The inclusion of cell density

We incorporate the effect of cell adhesion and movement by including equation (3) and assuming that somitogenesis follows the mechano-chemical theory framework of biological pattern formation [42, 43, 41, 40]. Note that somite formation occurs one cell cycle after cells have been specified as somitic, decoupling the signalling from the compaction process.

As mentioned in the Introduction (Section 1), the condensation of mesenchymal cells and the formation of somites is most likely triggered by the interaction of CAMs, such as N-cadherins, on the surface membrane of somitic cells. This requires spatial and temporal control of expression of CAMs and other components like integrins. Our key biological assumption is that changes in the concentration of the signalling molecule v are related to the density of adhesive sites to which cells in the PSM can attach. Therefore, our model incorporating cell density can be viewed as a chemical pre-pattern model.

We assume that somitic cells [density $\rho(x,t)$] satisfy a cell conservation equation in which two main factors, haptotaxis $\mathbf{J}_h$ and diffusion $\mathbf{J}_d$, contribute to the cellular flux in the PSM

$$\frac{\partial \rho}{\partial t} = -\frac{\partial}{\partial x}\left[\mathbf{J}_h + \mathbf{J}_d\right]$$

on a domain Ω subject to zero flux boundary conditions. The flux of the adhesive gradient is given in terms of the haptotactic sensitivity function τ and the cell density ρ at position x,

$$\mathbf{J}_h = \tau \rho \frac{\partial v}{\partial x}$$

and the random motion is given in terms of the diffusion function D_ρ and a diffusive flux

$$\mathbf{J}_d = -D_\rho \frac{\partial \rho}{\partial x}\ .$$

These two processes are highly nonlinear because τ and D_ρ need to be chosen as functions of v for biological realism. The details leading to these results will be reported elsewhere. We have chosen τ and D_ρ [see, (4)–(5)] based on two assumptions. Firstly, cells aggregate after experiencing a level of signalling molecule above a certain threshold. Secondly, changes in cell density are limited to regions in the spatio-temporal domain where $x > ct$. Our numerical simulations yield results consistent with those observed *in vivo*.