

Clock and Induction Model for Somitogenesis

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ABSTRACT After many years of research, somitogenesis is still one of the major unresolved problems in developmental biology. Recent experimental findings show a novel type of pattern formation in which a signal sweeps along the presomitic mesoderm and narrows simultaneously as a new somite is formed. The signal then residues in the posterior half of the new somite, and another wave begins to sweep up from the caudal end. This behaviour is not easily explained by the existing theoretical models. We present a new model for somitogenesis that can account for this behaviour and is consistent with previous experimental observations. *Dev Dyn* 2000;217:415–420. © 2000 Wiley-Liss, Inc.

Key words: somite formation; somitogenesis; Notch-Delta; hairy; lunatic fringe; clock; induction; model

The body plan is an architectural challenge that higher organisms have addressed through the process of segmentation or metamerisation. During segmentation, the body axis is divided along the anterior-posterior (AP) axis into similar repetitive structures formed from the embryonic layers. In insects, such as *Drosophila melanogaster*, segments are generated by the simultaneous division of the syncytial blastoderm. In other invertebrates, such as annelids and crustaceans, and in vertebrates the mechanism of metamerisation is different; the segments are formed at the cranial end of a multicellular embryo and segmentation propagates caudally (for a review, see Weisblat et al., 1994).

In vertebrates and cephalochordates, the segments are known as somites. They form as paired epithelial spheres arranged bilaterally along the AP axis and emerge in strict craniocaudal order (for a review, see Gossler and Hrabě de Angelis, 1998). The generation of somites occurs by the successive segmentation, caudal to the most recently formed somite, of the presomitic or paraxial mesoderm. Simultaneously, new cells are incorporated into the presomitic mesoderm (PSM) from the regression of Hensen's node at the same rate as new somites are formed rostral to the PSM (for a survey in the subject, see Catala et al., 1995; Psychoyos and Stern, 1996). Somites are divided by a fissure into anterior and posterior halves that differ in their gene expression and differentiation (for a review, see Tam and Trainor, 1994; Gossler and Hrabě de Angelis, 1998).

The formation and differentiation of somites is the result of three distinct morphological events progressing in a strict temporal-spatial order: (1) the prepattern of the PSM, (2) somite and somitic boundary formation, and (3) the differentiation of a somite into anterior and posterior halves (Gossler and Hrabě de Angelis, 1998). Several experimental observations confirm these events. Scanning electron microscopy observations (for a review, see Jacobson and Meier, 1986) and transplantation experiments (for a review, see Keynes and Stern, 1988) show that PSM displays a prepattern before segmentation. Recent molecular studies have shown that the basic helix-loop-helix (bHLH) transcription factors, *her-1* and *c-hairy-1*, are expressed rhythmically and dynamically in the PSM of zebrafish and chick (Müller et al., 1996; Palmeirim et al., 1997). In addition, Notch-Delta pathway genes are involved in the second and third processes of somite formation and differentiation (for a survey, see del Barco Barrantes et al., 1999). These molecular results suggest the existence of a conserved mechanism for segmentation in protostomes and deuterostomes (McGrew and Pourquié, 1998).

Despite these experimental results, the mechanism for somitogenesis is still one of the major unsolved problems in developmental biology. A number of theoretical models have been proposed to explain somitogenesis. These include the clock and wavefront model (Cooke and Zeeman, 1976), the wave gradient model (Flint et al., 1978), the reaction-diffusion type model (Meinhardt, 1982, 1986), the cell-cycle model (Primm et al., 1988, 1989; Stern et al., 1988), and the wave-cell polarisation model (Polezhaev, 1992, 1995a, 1995b). These models are satisfactory in a number of aspects, but objections have been made to all of them. Furthermore, such models cannot explain the dynamic expression of the bHLH transcription factors *her-1* and *c-hairy-1* and some elements of the Notch-Delta pathway.

In the present article we have developed a new theoretical model for somitogenesis. After an exposition of the up-to-date experimental facts about somitogenesis, we present previous theoretical models, our new model, and a discussion that follows.

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Somitogenesis

During somitogenesis, inductive interactions with Hensen's node, notochord, neural tube, and endoderm are not necessary for somite formation (Bellairs, 1963; Bellairs and Veini, 1980; Stern and Bellairs, 1984), but the midline structures are necessary after experimental disruption of the PSM (Packard et al., 1993). Scanning electron microscopy observations indicate that the PSM is not a homogeneous tissue. Before segmentation, the PSM displays metameric arrangements of groups of cells, named somitomeres by Meier (1979), that are evidently the predecessors of somites (Jacobson and Meier, 1986; Gossler and Hrabě de Angelis, 1998). The observation of this prepattern is confirmed in microsurgical experiments (Packard and Jacobson, 1976; Chernoff and Hilfer, 1982) in which isolated parts of the PSM form somites some time after their isolation in strict craniocaudal order, differentiating into anterior and posterior halves in each somite. Furthermore, the prepattern of anterior and posterior halves is also established before the formation of the somites (Keynes and Stern, 1988). Transplantation experiments reversing the AP axis of the PSM demonstrate that the AP polarity of the resulting pattern of somites is also reversed, so somite halves developed according to their original orientation (Aoyama and Asamoto, 1988).

The total number of somites is regulated in an embryo. The *Amputated* mouse mutant, which is shorter than the wild-type mouse, has the same number of somites, but their somites are considerably smaller than those of the wild-type embryos (Flint et al., 1978). However, the number of somites can be altered with an experimental perturbation (Keynes and Stern, 1988). For example, heat shock applied to chick embryos can induce the formation of an extra somite (Veini and Bellairs, 1986; Primmitt et al., 1988).

Other interesting results have been obtained through heat shock experiments. When a single heat shock was applied to chick embryos (Primmitt et al., 1988), up to four repeated somite anomalies, confined to one or two segments, 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 precursors (Stern et al., 1988).

Recently, the study of the expression of the transcriptional factor *c-hairy-1* in the PSM of chick embryos has provided molecular evidence for the existence of a segmentation clock (Palmeirim et al., 1997; Cooke, 1998). During segmentation, the cells of the PSM go through 12 cycles of *c-hairy-1* expression before becoming part of a somite, while more cells are continuously incorporated into the posterior end of the PSM. This observation suggests that the segmentation clock controls the time duration of cells in the PSM before they will form part of a somite. The expression of *c-hairy-1* displays a very particular AP pattern, which

progressively sweeps along the PSM in an AP sequence while narrowing, once during each somite formation. This wavefront-like expression finally stops, and it is maintained in a half somite-sized domain that gives rise to the caudal half of the forming somite. The *c-hairy-1* expression is independent of cell movements and does not result from the propagation of a signal in the plane of the PSM; it is an intrinsic cell autonomous property of this tissue (McGrew and Pourquié, 1998; Pourquié, 1998).

Previous Models

During the last three decades, several models have been proposed to explain the formation of somites (Cooke and Zeeman, 1976; Flint et al., 1978; Bellairs, 1980, 1986; Meinhardt, 1982, 1986; Jacobson and Meier, 1986; Keynes and Stern, 1988; Primmitt et al., 1989; Polezhaev, 1992, 1995a, 1995b). Some of these incorporate the different aspects of somitogenesis previously mentioned and are satisfactory in many respects.

Among these models, Cooke and Zeeman (1976) were the first to propose a cellular oscillator, which interacts with a progressing wave of cell determination traveling along the AP axis of the PSM. This model, known as the clock and wavefront, is able to explain the control of somite number (Slack, 1991) but is in conflict with the results of microsurgical experiments (Packard and Jacobson, 1976), transplantation experiments reversing the AP polarity (Aoyama and Asamoto, 1988), and repetitive anomalies of the single heat shock experiments (Primmitt et al., 1988, 1989; Stern et al., 1988). In addition, it cannot explain the formation of the anterior and posterior halves of a somite.

Meinhardt (1982, 1986) proposed a reaction diffusion type model involving two autocatalytic substances that behave in a short-range activation, long-range inhibition manner. It is assumed that cells are distributed in a uniform density and that the chemicals in the reaction diffusion model generate a spatial pattern resulting in a spatially homogeneous region of cells oscillating between two states corresponding to the anterior and posterior halves of a somite. Meinhardt's model is in agreement with two observations of Palmeirim et al. (1997): one full cycle of *c-hairy-1* oscillation corresponds to the formation of one somite, and *c-hairy-1* expression seems to be reminiscent of the spatiotemporal dynamics of one of the autocatalytic substances, because its wavefront expression stops and is maintained in the posterior half of the somites. However, the model cannot explain the isolation and transplantation experiments, the heat shock effects, and seems to be contradicted by the cell autonomous nature of the *c-hairy-1* oscillations and its peculiar dynamics (Palmeirim et al., 1997; McGrew and Pourquié, 1998; Pourquié, 1998).

The cell cycle model (Keynes and Stern, 1988; Stern et al., 1988; Primmitt et al., 1989) relies on an intracellular oscillator that controls cell division and inter-

acts with a kinematic wave that produces a signal that recruits other cells in the vicinity shortly before segmentation. This model explains the periodic anomalies of the heat shock experiments and the isolation and transplantation experiments. However, it cannot explain oscillations of *c-hairy-1* and its pattern in the PSM.

Polezhaev (1992,1995a,1995b) proposed a wave of cell determination moving along the PSM. This wave causes cell differentiation in a particular phase of the cell cycle, which then results in these cells secreting an inhibitor that impedes the differentiation of other cells. The model explains the heat shock, the isolation, and transplantation experiments but cannot explain the pattern of the *c-hairy-1* waves, its cell autonomous character, and the formation of the anterior and posterior halves.

Clock and Induction Model

After decades of experimental and theoretical work, somitogenesis is still one of the major unsolved problems in developmental biology. In the previous section, we reviewed several models proposed to explain the formation and regulation of somites. Because somitogenesis is a complex process, the models proposed have been aimed at addressing specific experimental observations. However, objections have been made to all of them, and at present, no model is consistent with all experimental data.

The Cooke and Zeeman (1976) model prompted the speculation that somitogenesis might be controlled by a clock in the cells of PSM. We know now, from the results of Palmeirim et al. (1997), that PSM cells, in fact, produce synchronised oscillations of a bHLH transcription factor *c-hairy-1*, a homologue of one of the *Drosophila* pair-rule segmentation genes (McGrew and Pourquié, 1998). The time taken for one oscillation equals the time of formation of one somite, and each cell experiences the same number of oscillations before becoming a somite. At that point, the expression of *c-hairy-1* stabilises, cells in the posterior half of the newly formed somite continue expressing this factor, whereas those in the anterior half no longer do so. The crucial role of the oscillations is evident, but how are the oscillations stabilised? Does the expression cycle gradually slow down as the cell progresses along the segmental plate? Is there a connection between *c-hairy-1* expression and differentiation into a posterior half?

Over the past few years, genetic studies support the view that a periodic biochemical pattern controls the physical pattern in somitogenesis. In particular, the role of the Notch-Delta signalling pathway in somitogenesis has been demonstrated in experiments involving mice mutants: the somites, if they form at all, are irregular in size and shape, no longer symmetrical, and their AP polarity is also affected (Conlon et al., 1995; Oka et al., 1995; Habré de Angelis et al., 1997; Wong et al., 1997; Evrard et al., 1998; Kusumi et al., 1998;

Zhang and Gridley, 1998). Furthermore, the expression of some components of the Notch-Delta pathway in the PSM suggests that the segmentation clock could control somitogenesis by modulating their signalling during segmentation.

Recent studies by McGrew et al. (1998) and Forsberg et al. (1998) have shown that *lunatic fringe* (*l-fng*) gene expression resembles the expression of *c-hairy-1* in PSM. In fact, they show that both expressions are coincident and are responding to the same segmentation clock. Experiments blocking protein synthesis in the PSM block the progression of the *l-fng* wavefront but not that of *c-hairy-1*. These experiments suggest that both genes are downstream of the segmentation clock but that *l-fng* only requires protein synthesis for its expression.

In *Drosophila*, Fringe is a secreted protein that acts to potentiate Notch activation by Delta and to inhibit Notch activation by the alternative ligand Serrate (Painin et al., 1997; Yuan et al., 1997) controlling the formation of the wing margin. In *l-fng* mutant mice, the formation of somites is disrupted, and if a somite forms, its AP patterning is disturbed (Evrard et al., 1998; Zhang and Gridley, 1998). Therefore, *l-fng* seems to be the Notch-Delta component coupled to the segmentation clock. However, other components could also be involved. The key questions here are how are the segmentation clock and Notch-Delta pathway linked? How are the oscillations stabilised in the PSM?

We propose that as a group of cells destined to form a somite traverse the PSM, they will undergo a series of *l-fng* expression pulses, followed by a longer final pulse that will remain at the posterior half of the newly forming somite. If we assume that *l-fng* expression in PSM synthesises a l-fng protein associate with the cell membrane, and this protein is stable, then *l-fng* mRNA pulses would sequentially increase, in ratchet fashion, the membrane levels of l-fng protein, which become proportional to the number of cycles experienced. The formation of a new intersomite boundary could then be triggered at a threshold level of l-fng protein. Then, once the somite is formed, a switch of Notch activation by Delta and inhibition of Notch activation by Serrate to Delta signalling, would allow the formation of a boundary and AP pattern, through an induction mechanism (similar to that proposed by Lewis, 1998). That is, groups of neighbouring cells, having a locally uniform level of Delta expression, behave cooperatively.

In addition, the increasing AP production of l-fng protein would indirectly arrest the segmentation clock via Notch-Delta signalling. In *Drosophila* and other invertebrates, members of the bHLH gene family, such as *hairy-1*, are the target of notch signalling (Greenwald, 1998). The rhythmical expression of *c-hairy-1* and *l-fng* would be arrested by a regulatory factor downstream to the effect of l-fng protein, explaining the narrowing of the wave front expression of *c-hairy-1* and *l-fng* in PSM.

From the descriptive model that we have given above, it is difficult to fully understand how certain types of experimental perturbation to various parts of the system would affect the outcome. To this end, a mathematical formalisation of the full model would be useful, enabling one to make experimentally testable predictions. Below, we show how one of the elements of the model can be expressed in mathematical form. We are presently in the process of incorporating this sub-model into a larger model for the whole process of somitogenesis.

We consider the PSM as a one-dimensional growing domain of presomitic clusters that are added caudal to Hensen's node at a rate of one cluster per segmentation clock cycle. We denote by m_i the maturity of the cluster added in the i th cycle and assume that it depends on the total number of cycles experienced by that cluster. At cycle n , we define m_i by

$$m_i = n - i \quad (0 \leq m_i \leq M) \quad (1)$$

where M is the number of cycles that a cluster must experience before becoming mature. Hence, the initial maturity of a cluster is 0, and after another M cycles, its maturity is M , which we take to be the somitic state. We further assume that as a cluster matures, its ability to synthesise the signal (*l-fng* or *c-hairy-1*, for example) decreases monotonically. Therefore the level, λ_i , of the signal synthesised by the cluster c_i has the form

$$\lambda_i = f_i w_i \quad (2)$$

where w_i is a monotonic decreasing function of m_i , and f_i is a function of m_i that represents the rhythmical expression of the signal. We assume that f_i is zero until m_i reaches a critical value, at which point it becomes 1 (after suitable scaling). Figure 1 illustrates how such a model results in the narrowing of the signalling wave as it propagates along the clusters.

Note that this model incorporates the domain growth that occurs during somitogenesis and assumes that it is the signal that is arrested rather than the clock itself. Thus, it is different, and in fact simpler, than the scenario envisaged in the model proposed by Lewis (1997).

DISCUSSION

In this article, we have proposed a global model for somitogenesis, which can explain the formation of somites, their boundaries, and AP patterning. The key elements of our model are conserved across protostomes and deuterostomes, suggesting that segmentation in the animal kingdom has some common features.

Previous theoretical models have been proposed to address specific experimental observations. These models were proposed before the first molecular evidence for a segmentation clock and for the participa-

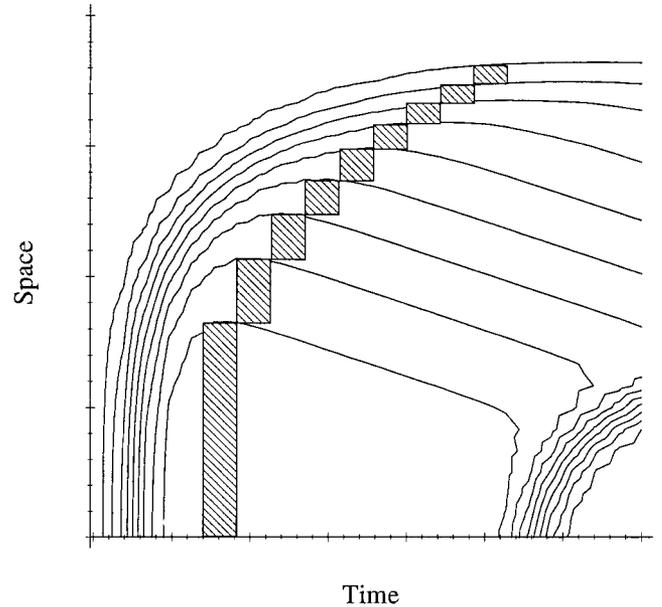


Fig. 1. A contour plot showing how the signal λ changes in space and time, with maturity decreasing from top to bottom. The shading shows the narrowing of the signal expression as it propagates cranially. For illustrative purposes, we have taken $w = 1/(1 + am^h)$, $f = 1/(1 + b \exp \Theta)$, where $\Theta = \cos(2\pi wt)$ and t is time. Parameter values used are $a = 0.05$, $b = 10$, and $h = 4$.

tion of the Notch-Delta signalling in somitogenesis. Although this molecular evidence contradicts many of these models, the models present certain key ideas that can be modified and/or expanded to take into account the new experimental evidence.

The clock and induction model we have proposed is consistent with the experimental facts. The observed rhythmical expression of *l-fng* in PSM results in the synthesis of a stable protein associated with the membrane. As new cells are incorporated, older cells at the posterior part of the PSM have more *l-fng* protein than those in the anterior part of the PSM. This creates a cell autonomous prepatterning along the AP axis, which is consistent with the observation of Meier (1979) and the isolation and transplantation experiments (Packard and Jacobson, 1976; Chernoff and Hilfer, 1982; Aoyama and Asamoto, 1988). The AP polarity of the somites is formed by an induction mechanism in the Notch-Delta signalling pathway (Lewis, 1998), which also would modulate the signal generated by the clock. The total number of somites is regulated by the rate at which cells are incorporated in the PSM and/or by the rate of the segmentation clock. Heat shock (Pimm et al., 1988) would perturb the segmentation clock, affecting the formation of somites.

Our model has focussed on how the cell autonomous signals generated by the clock propagate forward into the PSM to create somites and assumes that the rate of regression of Hensen's node is correlated with the speed at which somites are formed. There are two pos-

sible mechanisms to account for this correlation: the regression of Hensen's node may be controlled by the same clock that controls segmentation. Alternatively, cells in newly forming somites may signal back to Hensen's node, perhaps via the midline structures, thus making the model consistent with the results of Packard et al. (1993).

The great challenge of this model is to understand the function of *l-fng* or any other possible key component of the molecular mechanisms that link the segmentation clock with the formation of the somites in the PSM. Further experimental research must be conducted to study how *c-hairy-1* and *l-fng* expressions are modulated by the segmentation clock. At the same time, it is essential to understand the structure of the segmentation clock and how it is modulated. The experimental observations presented in this manuscript are the consequence of the segmentation clock, but we do not yet know the molecular origin of the clock.

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