From Segment to Somite: Segmentation to Epithelialization Analyzed Within Quantitative Frameworks

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One of the most visually striking patterns in the early developing embryo is somite segmentation. Somites form as repeated, periodic structures in pairs along nearly the entire caudal vertebrate axis. The morphological process involves short- and long-range signals that drive cell rearrangements and cell shaping to create discrete, epithelialized segments. Key to developing novel strategies to prevent somite birth defects that involve axial bone and skeletal muscle development is understanding how the molecular choreography is coordinated across multiple spatial scales and in a repeating temporal manner. Mathematical models have emerged as useful tools to integrate spatiotemporal data and simulate model mechanisms to provide unique insights into somite pattern formation. In this short review, we present two quantitative frameworks that address the morphogenesis from segment to somite and discuss recent data of segmentation and epithelialization. *Developmental Dynamics 236:1392–1402, 2007.* © 2007 Wiley-Liss, Inc.

INTRODUCTION

Somite formation in the vertebrate embryo is a classic paradigm of segmentation (Fig. 1A–C). The complex assembly of a spherical somite structure consists of sculpting a rectangular block of homogeneous tissue into a discrete group of cells that form an epithelial layer to surround loosely packed mesenchymal cells (Fig. 1F,G; Fig. 1G shows the same tissue visualized after ~100 min). Each somite segment must be created in a timely manner so that the pattern propagates in the direction in which cells arise to produce a repeating pattern. The early spherical structure of somites

is transformed to give rise to muscle, skeletal and skin derivatives and provides a scaffold for assembly of the peripheral nervous system (Christ and Ordahl, 1995; Holloway and Currie, 2005; Buckingham, 2006). The concept of relating somite segmentation to quantitative frameworks has recently gained popularity (Cooke, 1998; Pourquie and Goldbeter, 2003; Baker et al., 2006a), primarily due to the correlation of a theoretical clock mechanism with periodic gene expression patterns that travel through the presomitic mesoderm (PSM) (Dubrulle and Pourquie, 2004). However, there is still a great

deal to learn about the link between the mechanisms that ensure a repeating periodic pattern and local cell rearrangements that result in normal or aberrant muscle and skeletal morphogenesis (Maisenbacher et al., 2005, Huh et al., 2005).

Technical advances in computation, microscopy and molecular biology have allowed scientists to extend observations made by pioneers working at the intersection of developmental biology and mathematical biology. Recent investigations of somite formation have revealed unique insights into the dynamics of cell rearrange-
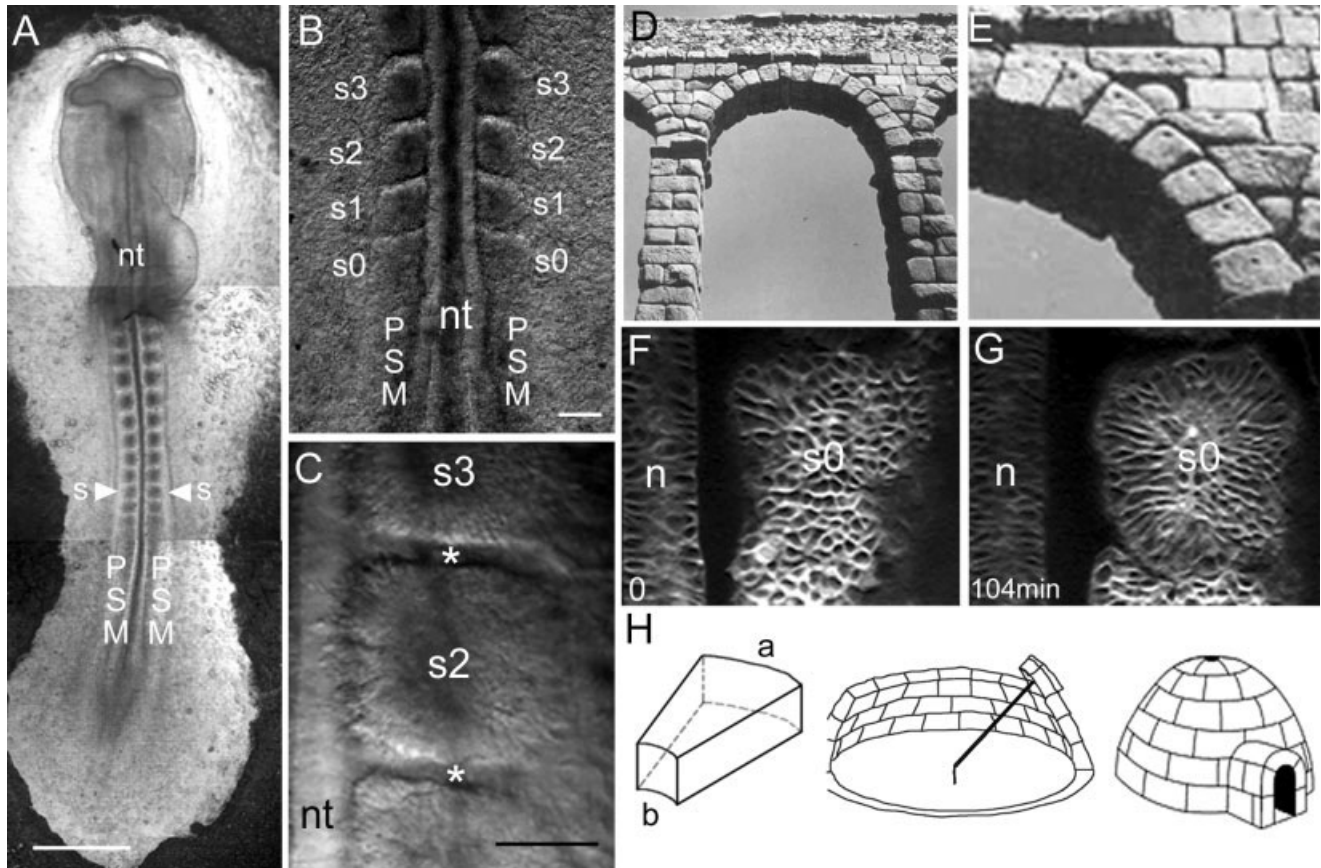
Fig. 1. Somite segmentation and analogy to other physical structures. **A:** A typical chick embryo reveals the somite (s) pattern in pairs along the vertebrate axis and neural tube (nt) with the unsegmented presomitic mesoderm (PSM) in the posterior direction. **B,C:** Higher magnification with oblique light illumination shows the somites labeled as s0 (newly forming somite) and higher in the anterior direction (B), with somite borders separating each segment (asterisk, in C). **D,E:** A typical ancient roman arch structure showing the different shapes of stones assembled (E) to create a semicircular structure. **F,G:** In a vertebrate embryo, cells within the PSM change shape (F) and alignment (G) to form a circular structure as shown in confocal sections of bodipy-ceramide–labeled cells in chick taken 104 min apart. n, notochord. **H:** An example of the three-dimensional set of blocks with a different apical (a) to basal (b) length ratio assembled a fixed radial distance from a center, resulting in a common igloo form. Scale bars = 500 μm in A, 100 μm in B, 50 μm in C.

ments and molecular function analyzed in vivo in a wide variety of animal model systems. This explosion of both cellular and molecular data has inspired both excitement and anxiety in experimentalists and theoreticians posing the question of how to best integrate all the data. In this review, we highlight recent advances in our understanding of somite border formation and epithelialization discussed with advances in the design of quantitative frameworks.

CONVERGENCE OF DEVELOPMENTAL AND MATHEMATICAL BIOLOGY TO STUDY SOMITOGENESIS

At the level of an individual somite, the cell rearrangements and cell shape changes present wonderful questions of how to properly fit cells together from a rectangular block into a curved epithelialized structure (Fig. 1F,G). It is interesting to note that several ancient cultures worked to develop, but, the Romans refined how to integrate shapes of stones to produce a semicircular arch (Wheeler, 2001; Fig. 1D,E). The arch provides a means to resolve the structural forces such that vertical loads become axial forces that transfer the load from stone-to-stone to the foundation (Wheeler, 2001). To construct a semicircular arch from polygonal blocks of stone, the Romans refined how to vary the apical and basal lengths of a block to construct an arch of a given radius and curvature (Fig. 1D,E). The larger the apical-to-basal ratio, the higher the curvature and smaller the radius of the arch. The principles of a two-dimensional (2D) arch may be expanded to form 3D structures, such as an igloo (Fig. 1H). The construction of an igloo consists of using an open block, with distinct apical and basal lengths, that when filled with snow and placed at a set distance and angle from a center forms a 3D structure (Huesers, 2004). Visual inspection with 3D confocal microscopy and analysis in 2D suggests that cells within somites may perhaps resemble the Roman blueprint (Fig. 1). But, curiously, how does the embryo sculpt cells within a rectangular block into a spherical structure (Fig. 1E–G)? Equally fascinating is how the somite segmentation pattern is propagated in a repeating periodic manner. Thus, a major goal of somite biologists is to understand how molecular mechanisms that control the production of repeating structures within the PSM
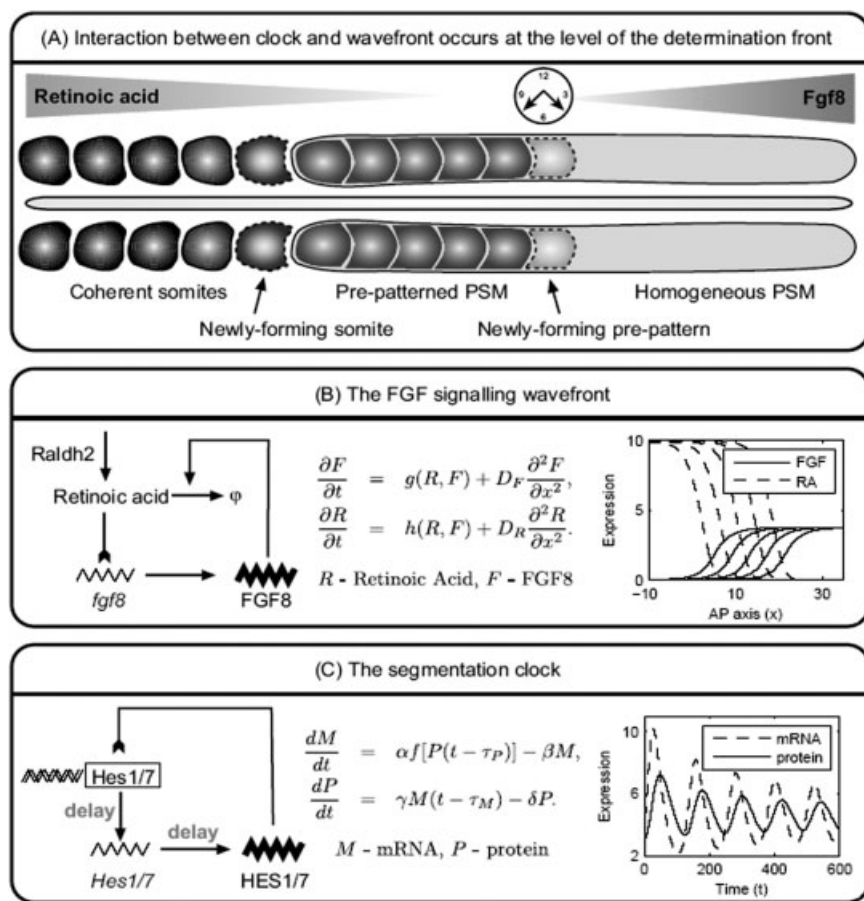
Fig. 2. The model mechanisms underlying somite segmentation. **A:** An illustration of the antero-posterior (AP) axis and the various stages of somite formation according to the clock and wavefront model. The posterior presomitic mesoderm (PSM; shown in light gray) is homogeneous and cells are undetermined with respect to their developmental pathway. At the level of the determination front (depicted by the posterior-most dotted presumptive segment), the interaction of the clock and gradient specifies the chemical prepattern. Cells of the PSM that lie anterior to the determination front will follow a specific developmental pathway, which cannot be altered by subsequent perturbation of the clock or wavefront. At the anterior end of the PSM, cells undergo changes in their morphological properties and condense to form coherent somites (dark gray segments). **B:** A schematic of the network underpinning the fibroblast growth factor (FGF) signaling gradient. FGF8 (F) acts in a negative feedback loop with retinoic acid (R): FGF8 accelerates the decay of retinoic acid (fi), whereas retinoic acid down-regulates fgf8 transcription (tail-headed arrow). The interactions between FGF8 and retinoic acid are represented in the mathematical formulation by the interaction terms g(R,F) and h(R,F), and diffusion of FGF8 and retinoic acid takes place at rates D_F and D_R, respectively. Numerical solution of the mathematical model is shown on the right hand side—with the gradient of FGF signaling receding along the AP axis as time proceeds (i.e., moving in a positive x direction). For more details of the mathematical model, see Baker and Maini (2007). **C:** A schematic of the network underpinning the segmentation clock. The protein product (bold symbol) negatively regulates mRNA (fine symbol and tail-headed arrow) transcription, and delays τ_P and τ_M are assumed to take place during transcription and translation, respectively. Numerical solution of the mathematical model is shown on the RHS—repeated oscillations in mRNA and protein expression levels occur, with the peak in mRNA expression slightly preceding that of its protein product. For more details of the mathematical model, see Monk (2003).

interface with mechanisms that control local cell rearrangements and cell shape changes.

One of the first people to mathematically analyze visually striking patterns in nature was D'Arcy Thompson (1860–1948). He was a contemporary of Charles Darwin and sought to suggest to naturalists how mathematical concepts and quantitative frameworks may help guide the study of morphogenesis. He studied a wide variety of patterns in nature, from rates of growth, to forms of cells and tissues during early cleavage cycles, and shapes of structures, including teeth and horns (Thompson, 1992). His methods of analysis, developed within a biological setting, would evolve further with the help of innovative mathematicians who constructed hypothetical model mechanisms to reproduce biological patterns. Early mathematical models suggested chemical and mechanical bases for morphogenesis (Turing, 1952; Murray et al., 1983) and helped to set the stage for a wide range of pattern formation model mechanisms applied to segmentation (Meinhardt, 1977; Kauffman et al., 1978), and repeating, periodic structures, such as somites (Cooke and Zeeman, 1976; Flint et al., 1978) and skin (Cruywagen et al., 1993).

Discrete modeling approaches, based on the physical properties of cells and their interactions, have also emerged to study developmental systems and provide a foundation to model somitogenesis. These approaches use a cellular Potts model (Graner and Glazier, 1992), stochastic Monte Carlo simulations (Drasdo and Forgacs, 2000), or cellular automata models (Deutsch and Dormann, 2005) and have been applied successfully to several biological phenomena, including convergent extension (Zajac et al., 2003), early cleavage and blastula formation (Drasdo and Forgacs, 2000), and somite border formation (Ying Zhang, personal communication). Together, these modeling efforts have created hypothetical mechanisms that, when constructed in close collaboration with biologists and simulated with biological parameters have produced unique insights and helped direct new experiments (Murray, 1993; Maini et al., 2006).

SOMITE SEGMENTATION STUDIED WITHIN QUANTITATIVE FRAMEWORKS

There has been a long history of modeling the processes underlying somite formation (Fig. 2; Table 1): from Cooke and Zeeman's *clock and wavefront* model (Cooke and Zeeman, 1976; Zeeman, 1974) to Meinhardt's *reaction–diffusion* model (Meinhardt, 1982, 1986) and the *cell cycle* model (Primmett et al., 1988, 1989; Collier et al., 2000; McInerney et al., 2004), to name but a few. Each model can account for several aspects of somite formation—but with the discovery of recent exper-

TABLE 1. Evolution of Models Related to Somite Segmentation[a]

Year	Type	Function	Key references
1917	Model	Rates of growth and shapes of cells and tissue	Thompson
1952	Chemical model	Proposed model for the chemical basis of morphogenesis	Turing
1976	Clock and wavefront model	Coupled a biochemical intracellular oscillator (clock) in the PSM with a wavefront of maturation that arrests the oscillation and initiates somite formation in a sequential manner	Cooke and Zeeman
1977	Chemical model	Mutual interactions of activator and inhibitor expressed as a set of differential equations act as a morphogen gradient from which cells read out positional information; applied to early insect segmentation	Meinhardt
1978	Chemical	Reaction–diffusion system generates a sequence of differently shaped chemical patterns that produce a sequence and geometry of compartmental boundaries; applied to *Drosophila* segmentation	Kauffman et al.
1978	Chemical	Cells entering PSM synthesize a morphogen and continue to do so throughout their travel in the anterior direction; when cells reach the anterior PSM, the morphogen reach a threshold and begin to decay, creating a local sink and neighboring subthreshold peak that generates a somite border	Flint et al.
1983	Mechanical model	Mechanical model for mesenchymal morphogenesis	Murray et al.
1986	Chemical + oscillator of 2-cell state model	Periodic pattern of A and P states in PSM coupled to a morphogen gradient produce repeating segmental units	Meinhardt
1992	Kinematic wave and cell cycle model	Proposes an interaction between contact cell polarization and kinematic wave of cell determination	Polezhaev
1993	Mechanochemical model	Mechanochemical model to produce sequential pattern; applied to chick feather primordial initiation	Cruywagen et al.
2000	Clock and induction model	Proposes that PSM cells undergo a series of lunatic fringe expression pulses that synthesize a lunatic fringe protein that sequentially increases in ratchet fashion; a new somite border is formed when lunatic fringe protein levels cross a threshold and interact with Delta–Notch signaling. This model is similar to that proposed by Lewis (1998).	Schnell and Maini
2000	Cell cycle model	Proposes there exists a time window within the cell cycle that gates emerging PSM cells such that the cells make a discrete number of somites per cycle	Collier et al.
2000	Clock and trail model	Proposes that, as cells leave a caudal progress zone and enter the PSM, a permanent record is made of the state of the oscillator; cells traverse through the PSM and record the number of subsequent oscillations and coalesce into a somite after a set number of cycles	Kerszberg and Wolpert
2002	Cell oscillator	Dynamics of gene expression patterns in PSM modeled by a series of cell oscillators that freeze into a spatial order as the clocks slow or stop at different phases in their cycles	Jaeger and Goodwin
2002	Cell cycle and cell adhesion model	An extension of the cell cycle model that incorporates cell movement and cell aggregation in response to an increase in cell adhesion	Schnell et al.
2003	Oscillator model	Proposes a model that direct autorepression of a gene by its own product can generate oscillations with a period determined by transcriptional and translational delays; the model is directly applied to the zebrafish oscillating genes, her1 and her7	Lewis
2003	Oscillator model	Proposes a transcriptional and translational delay mechanism that drives oscillations in feedback inhibition loops.	Monk
2004	Cell cycle model	In-depth analysis of the Collier et al. model that reveals a better understanding of what parameters influence somite size	McInerney et al.
2006	Cell–cell adhesion	Considers the movement of cells (sorting) in response to adhesive forces generated through binding, using a continuum model approach	Armstrong et al.
2006	Clock and wavefront model revisited	Develops and analyzes a mathematical formulation of a version of the clock and wavefront model and shows a simplification of the model predicts anomalies that result from perturbation of the wavefront	Baker et al.
2007	Somite border formation model	Considers the physical properties of cells in the anterior PSM using a cellular Potts model to model somite border formation	Zhang et al.
2007	Clock and wavefront model	Models the FGF signaling gradient in further detail	Baker and Maini

[a]PSM, presomitic mesoderm; FGF, fibroblast growth factor.

imental evidence, one model in particular has been favored. Cooke and Zeeman's original model postulated the existence of a longitudinal *positional information* gradient along the anteroposterior (AP) axis of vertebrate embryos, which interacts with a smooth cellular oscillator (the clock) to set the time at which cells undergo a *catastrophe* (Cooke and Zeeman, 1976; Zeeman, 1974). This represents a rapid change of state, such as the changes in adhesive and migratory behavior of PSM cells as they form somites.

Support for the existence of a segmentation clock came from the discovery that several genes exhibit periodic expression along the PSM, with cycling times equal to the time taken to form one new pair of somites (Palmeirim et al., 1997; Pourquie, 2001, 2003). For example, during the formation of one somite, bands of *c-hairy 1* and *lunatic fringe* expression sweep along the PSM, coming to rest within the newly forming somite. On the other hand, support for the positional information gradient came from the observation that a gradient of fibroblast growth factor (FGF) signaling exists along the PSM, with cells moving anteriorly through the gradient as the AP axis extends. Cells are initially part of a region where FGF signaling prevails and are undetermined with respect to their developmental pathway (Dubrulle et al., 2001). As the gradient recedes, a threshold level of FGF signaling, known as the *determination front*, is reached and cells become committed to a specific developmental fate (Dubrulle et al., 2001; Saga and Takeda, 2001; Dubrulle and Pourquie, 2004). With the discovery of such experimental evidence, Pourquie and coworkers proposed a revised version of the model which is now widely accepted (Dubrulle et al., 2001). In line with the original model, they suppose that the clock controls *when* the boundaries of the somites form, and the wavefront of FGF signalling controls *where* they form. Figure 2A illustrates the process of somite formation, as predicted by the clock and wavefront model.

Maini and coworkers proposed a mathematical formulation of the clock and wavefront model (Baker et al., 2003, 2006a,b), using the signaling basis of the cell cycle model (Collier et al., 2000; McInerney et al., 2004). The mathematical formulation concentrates on modeling the role of the FGF signaling gradient, and the resulting developmental anomalies that are observed when the gradient is perturbed. There are emerging models that concentrate on modeling the FGF signaling gradient in more detail (Baker and Maini, 2007; Pourquié [R.E.B., personal communication]): these models consider the negatively regulating interactions of retinoic acid and FGF8, which have been shown to be present along the PSM (Diez del Corral et al., 2002, 2003; Diez del Corral and Storey, 2004). Figure 2B illustrates the possible interactions between retinoic acid and FGF8 that lead to gradient formation. The mathematical model of Baker and Maini is outlined, and results of numerical simulation of the model are also shown (further details in Fig. 2 legend and Baker and Maini, 2007).

In contrast, other models for somitogenesis have concentrated on understanding the mechanisms underlying the segmentation clock. For example, Monk and Lewis have each proposed models in which transcriptional and translational delay drive oscillations in simple feedback inhibition loops where the protein product down-regulates its own gene expression (Monk, 2003; Lewis, 2003; Giudicelli and Lewis, 2004). Figure 2C illustrates the possible interactions between mRNA and protein that lead to clock oscillations. The mathematical models of Monk and Lewis are outlined, and results of numerical simulation of the models are also shown (further details in Fig. 2 legend and Monk, 2003; Lewis, 2003; Giudicelli and Lewis, 2004). More recently, Horikawa and colleagues investigated the coupling of segmentation clocks within individual PSM cells by means of Notch-dependent intercellular communication regulated by the internal *hairy* oscillator. They showed, using a combination of experimental methods and modeling, that this could lead to synchronized oscillations robust to the presence of noise (Horikawa et al., 2006).

The current models have been largely successful in replicating experimentally observed results; however, there are three main areas in which further focus is required. The first is that none of the current models fully integrate the mechanisms underlying the clock and the wavefront: the models of Maini and coworkers assume a simple readout from the clock in the form of a switch, whereas the wavefront plays no part in the oscillator models proposed by Monk (2003) and Lewis (2003). Second, experimental evidence has shown that the clock and wavefront may be intricately linked by an underlying gradient of Wnt3a along the PSM (Aulehla et al., 2003; Aulehla and Herrmann, 2004): a gradient of Wnt signaling acts upstream of the FGF8 gradient, and Wnt signaling also acts in a negative feedback loop with the cycling gene Axin2 in the mouse PSM. Future models should take this factor into account.

The third way in which these models do not completely capture the phenomena of somite formation is that they only account for the formation of the prepattern of gene expression visualized before any morphological changes take place. The increase in cell adhesion molecules and subsequent cell rearrangements have not been extensively investigated. Schnell et al. (2002) were the first to develop a mathematical model to describe the bulk movements of somitic cells to form a somite in response to an increase in cell adhesion. However, the drawbacks of this model are that it is an extension of the cell cycle model for somitogenesis and it does not take into account the intercellular mechanical forces involved in somite formation. It would be possible to incorporate the mechanism of cell adhesion into the mathematical formulation of the clock and wavefront model by assuming that cells produce adhesion molecules, and/or preferentially direct their migration according to the genetic prepattern predicted by the model; this is the subject of current work (Armstrong et al., 2006; Armstrong et al., [R.E.B., personal communication]; Ying Zhang et al., personal communication).

Recently, oscillations of the snail genes, independent of Notch signaling, have been shown in the PSM (Dale et al., 2006). Cyclic mRNA expression of *Snail1* (mouse) and *Snail2* (chick) in the PSM appear to require
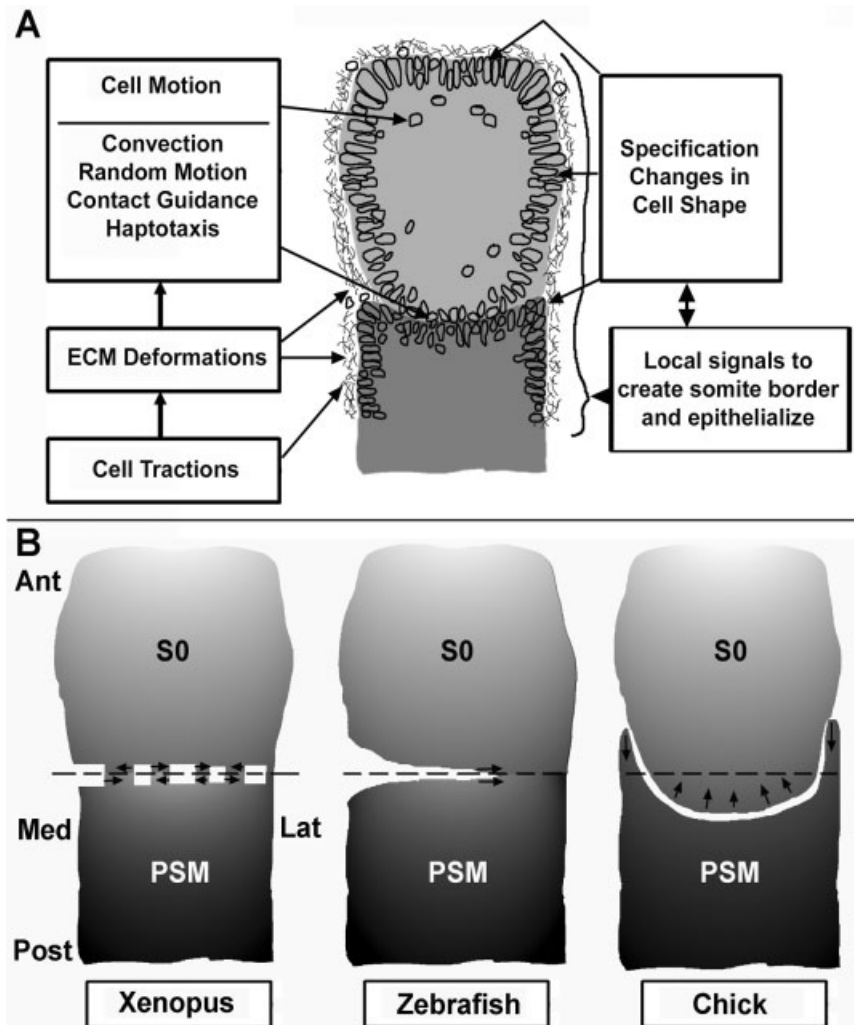
Fig. 3. Local framework of the somite microenvironment to study border formation and epithelialization. **A:** A general schematic of the components of a framework that may be converted to quantitative forms in a mathematical model mechanism. **B:** Two-dimensional representation of the cell movements that sculpt the somite border formation in *Xenopus* as discrete fissures, in zebrafish as a medial-to-lateral expanding fissure, and in chick as a ball and socket separation of tissue. The arrows depict the direction of the separation of tissue and the dotted lines represent the presumptive somite border. S0, forming somite; Ant, anterior; Post, posterior; Med, medial; Lat, lateral; PSM, presomitic mesoderm.

Xenopus, a recent very detailed 3D confocal analysis of embryos confirms earlier explant observations (Wilson et al., 1989) that the expansion of short discrete fissures (Fig. 3B) acts to gently bisect the PSM to form a stable somite border (Afonin et al., 2006). Similarly, in zebrafish, cell rearrangements within a forming somite are minimal and a somite border forms when cells appear to gently detach from neighbors along a medio-to-lateral direction (Fig. 3B) and do an about face toward the respective somite center or PSM (Wood and Thorogood, 1994; Henry et al., 2000; Jiang et al., 2000).

In chick embryos, the cell rearrangements during somite border formation are more dramatic. Somitogenesis occurs in a precise spatiotemporal order and is not a simple, straightforward slicing of the PSM (Kulesa and Fraser, 2002). Time-lapse analyses reveal that a somite pulls apart from the PSM in a ball-and-socket tissue separation (Fig. 3B); a series of complex movements in which cells move across the presumptive somite boundary and violate gene expression boundaries thought to correlate with the site of the somite boundary (Kulesa and Fraser, 2002). Careful tissue transplantation studies have shown that cells in the chick PSM, in the region near the presumptive posterior border or caudal portion of the ball (and socket), possess border forming signals that are mediated by Notch and confined by Lunatic fringe (Sato et al., 2002). Further analysis of the presumptive posterior somite border cells has revealed that the ventral-most cells are sufficient to induce an ectopic border in a unidirectional manner (Sato and Takahashi, 2005). Thus, in chick, the initial separation of a somite from the PSM appears to occur in a ventral-to-dorsal direction that involves complex cell rearrangements.

How do we reconcile the differences in cell rearrangements between animal model systems with gene expression boundaries that appear similar during somite border formation? If the cell rearrangements are different between animal models, is the underlying molecular choreography different or is the variation due to aspects of cell adhesion and/or local microenvi-

Wnt and Fgf signaling. Results suggest that one role of the snail genes is to integrate the Notch, Wnt, and Fgf pathways (Dale et al., 2006). Furthermore, new microarray studies of the mouse PSM transcriptome reveal that the oscillator associated with this process drives the periodic expression of a large network of cyclic genes involved in cell signaling (Dequeant et al., 2006); however, it seems that the oscillator largely relies on the three pathways, Notch, Wnt, and Fgf. How multiple components of the oscillator are functionally coordinated with the wavefront to produce a precise assembly line of structures will be a future

challenge to the mathematical modeling of somite segmentation.

VARIATIONS IN SOMITE BORDER FORMATION

The formation of a new somite is accomplished by the physical separation of tissue, and the coalescing of cells, leading to the establishment of a stable border within the PSM. Exploration of the cellular rearrangements underlying the formation of the somite border shows that there are differences between animal model systems and how aggressively the new somite pulls apart from the PSM. In

ronmental conditions? Our understanding of the coordination of these factors studied within a quantitative framework could help to identify the crucial mechanisms by which the somite separates from the PSM.

LOCAL QUANTITATIVE FRAMEWORKS OF THE SOMITE MICROENVIRONMENT TO STUDY BORDER FORMATION AND EPITHELIALIZATION

We have previously mentioned that the prevailing model of somitogenesis supposes that the PSM is segmented into somites by a clock and wavefront mechanism. During segmentation, mesenchymal cells undergo compaction, followed by a detachment of the presumptive somite from the rest of the PSM and the subsequent morphological changes leading to rounded somites. Thus, at the level of an individual somite, cell adhesion, cell contact-dependent repulsion and cell rearrangements compete within the somite microenvironment. However, the actual process of somite formation, that is how a somite separates or pulls apart from the PSM and the ensuing morphological changes, are not well understood and have not been the subject of mathematical modeling to date.

Recent investigations of the somite border have focused on integrin–fibronectin interactions (reviewed in detail in Chong and Jiang, 2005). Fibronectin is found at chick (Duband et al., 1987) and zebrafish (Crawford et al., 2003) somite boundaries; in the latter case their accumulation is dependent on integrinalpha5 (Julich et al., 2005; Koshida et al., 2005). In zebrafish embryos deficient for both fibronectin and integrinalpha5, somite boundaries are initially formed but are not maintained, suggesting their role in boundary maintenance (Koshida et al., 2005).

Recent experimental evidence shows that segmentation requires a complex, large-scale coordinated movement of cells and extracellular matrix (ECM). The ECM filaments have a reproducible morphogenic destiny that is characterized by directed transport (Czirok et al., 2004). Fibrillin 2 particles initially deposited in the PSM are translocated along an unexpected trajectory where they eventually polymerize into an intricate scaffold of cables parallel to the AP axis (Czirok et al., 2004). The cables coalesce near the midline before the appearance of the next-formed somite. This experimental observation suggests that cells during segmentation migrate within the ECM, and on other cells, generating long-range mechanical traction forces that will lead to the formation of somites.

Somite formation is also regulated by members of the Ena/VASP family of actin regulatory proteins and FAK, as shown during *Xenopus* development. Kragtorp and Miller (2006) have found that inhibition of Ena/VASP or FAK leads to abnormal somite rotation and failure to form intersomitic boundaries. Ena/VASP proteins appear to act as a molecular link between cell-surface integrins and the actin cytoskeleton to orchestrate changes in adhesive strength and cell motility (Schlaepfer and Mitra, 2004). Another potential mechanism by which these proteins might regulate cell motility is through activation of integrin adhesion and fibronectin matrix assembly (Kragtorp and Miller, 2006).

In avian and murine embryos, somite formation is preceded by compaction of the newly formed somite region, s0 (that is, a reduction of the intercellular spaces between cells), and the simultaneous heightened expression of two types of cadherin molecules, N-CAM and N-cadherin (Duband et al., 1987; Kimura et al., 1995). These two molecules are also expressed in the remainder of the PSM but at reduced levels. Of interest, *cadherin-11* expression is strictly correlated with s0 and is not expressed in other parts of the mouse PSM (Kimura et al., 1995). Such could also be the case in avians and zebrafish, perhaps through the expression of a different cellular adhesion molecule. Thus, it may be hypothesized that the expression of different adhesion molecules and the modulation of cadherin quantity may be linked to the morphogenetic changes in somite formation. Similar conjectures have previously been put forward (Newman, 1993) and more recently (Murakami et al., 2006)

for somitogenesis in zebrafish. The regulation of cell adhesion molecules has been linked to the protocadherin, papc, shown to play an important role in the formation of the epithelium along the segmental border (Rhee et al., 2003).

Despite all the experimental evidence, there is no model mechanism that takes into account the subcellular processes mentioned above that may be pivotal to somite border formation and epithelialization. We illustrate a model mechanism framework that includes cell shape changes, cell–cell interactions, and ECM deformations that lead to and respond to cell arrangements (Fig. 3A). The experiments of Czirok and colleagues (2004) illustrate that the ECM is a fibrous material, capable of transmitting stresses over several hundred cell diameters. Curiously, previous mechanochemical models developed to explain mesenchymal morphogenesis (Murray et al., 1983; Oster et al., 1983) may serve as a basic framework for new mathematical models of somite formation. Models of this form can help to explain the interactions between cells and the ECM, and the resulting forces that are generated as cells extend filopodia and adhere to the ECM. In this model mechanism, cell rearrangement and pattern specification are coupled, but the prominent mechanisms driving cell movement are force-generated by the regulation of cell adhesion and shape. Thus, improved insights into the mechanisms that govern somite border formation and epithelialization, studied within this type of local model framework, might be relevant for the identification of interactions of cell adhesion regulation, cell rearrangements and the ECM.

TARGETING SOMITE EPITHELIALIZATION

The subsequent step of somite segmentation involves a morphological process that includes cell shape changes and cell alignment to give each somite an initial characteristic structure (Fig. 1F,G). In most vertebrates, subgroups of mesenchymal cells dynamically alter their shape to form an outer epithelial layer enclosing a mesenchymal core (Duband et

al., 1987). This mesenchymal-to-epithelial transition (MET) of cells within a somite is distinct from the segment border formation, as was shown by the knockdown of *Paraxis* in mouse (Burgess et al., 1996). *Paraxis* is likely regulated in part by ectodermal and neural tube derived signals (Sosic et al., 1997; Schmitt et al., 2004), and its expression is a consistent marker for the onset of the epithelialization process.

New studies of somite epithelialization have focused on events in the anterior PSM. *Mesp1* and *Mesp2*, homologous basic helix–loop–helix (bHLH) transcription factors, are expressed in the anterior PSM just before somite formation (Saga and Takeda, 2001). In chimeric experiments, where mutant cells are aggregated with wild-type cells at the eight-cell stage and transplanted into a wild-type host, Mesp1/Mesp2 double-null cells do not contribute to an epithelial somite, suggesting a cell-autonomous behavior (Takahashi et al., 2005a).

However, the epithelial pattern of wild-type cells is disrupted, suggesting a non–cell-autonomous affect mediated by cellular interactions (Takahashi et al., 2005a). Interestingly, overexpression of *Snail2* in the chick PSM prevents the expression of the *Mesp2* homolog, *Meso1*, and is accompanied by a down-regulation of *Paraxis* (Dale et al., 2006). Thus, *Snail2* appears to have a second role in controlling the morphogenetic processes associated with epithelialization (Dale et al., 2006). Transcriptional regulation of Mesp2 can also occur by means of the T-box transcription factor, Tbx6 (Yasuhiko et al., 2006).

At the individual cell level, the transition of cell shape that is crucial for epithelialization involves the rearrangement of the cytoskeleton. The Rho family of small GTPases, including Cdc42 and Rac1, mediate the necessary forces for changes of polarity, motility, and structural stability of the cell (Etienne-Manneville, 2004). It has been shown recently in chick that when Cdc42 is overexpressed in the PSM, cells remain mesenchymal (Nakaya et al., 2004). In contrast, when inhibition of endogenous Cdc42 is performed by overexpression of the CRIB domain of N-WASP, cells in the PSM are hyperepithelialized (Nakaya et al., 2004). From this, Takahashi and colleagues proposed a binary switch mechanism based on Cdc42 activity levels such that low levels produce epithelialized cells (Takahashi et al., 2005b). Further insights may come from new imaging technology to visualize the dynamics of Cdc42 activity in living cells (Nalbant et al., 2004) and mathematical models of Cdc42 activity regulation (Ozbudak et al., 2005) applied to cell shaping during epithelialization.

Do the complex molecular signals that sculpt an epithelial somite propagate in a precise spatiotemporal manner from a specific location or occur uniformly throughout the somite at a precise time? There are at least two possibilities. First, signaling molecules may act on the somite and establish a secondary epithelialization signal source located near the center of each somite. This would act as an organizer to radially align the cells toward the somite center and stimulate the cells to elongate toward the center. Second, signals may be localized and influence a subset of cells that, in turn, through cell–cell crosstalk and mechanical re-arrangement propagate to form an epithelial somite.

Some preliminary evidence exists to address the epithelialization order. Interestingly, although *Xenopus* somites undergo a 90-degree rotation but do not epithelialize, time-lapse analyses showed that individual cells bend in a precise spatiotemporal manner that propagates in a dorsal-to-ventral direction (Afonin et al., 2006). In a similar manner, in analysis of histological sections from the chick PSM (Duband et al., 1987; Sato et al., 2002; Nakaya et al., 2004), the presumptive posterior border, including the dorsal, ventral, medial, and lateral sleeves of the new somite, appear epithelialized. Sato and Takahashi (2005) later revealed that the ventral-to-dorsal propagation of tissue separation is accompanied by an epithelial morphology of cells along the somite border. Together, these static studies offer the temptation to suggest that epithelialization propagates from discrete regions within the somite.

To test the spatiotemporal manner of epithelialization, we used an embryonic chick model. Confocal time-lapse imaging revealed two significant observations. When fluorescently labeled chick embryos were monitored in time-lapse, cell shape changes in the newly formed somite propagate from cell-to-cell around the somite circumference to give rise to an epithelialized structure (P.M.K. and S.R., unpublished observations). In current studies, when the PSM is longitudinally divided on one side of the embryo into two equally sized portions and the tissue gently spread apart so the cut sides do not contact, somite segments continue to form in each split half of the PSM in a ball and socket manner and cells align radially to epithelialize the somite, including mesenchymal cells in the mid-PSM that normally remain mesenchymal (P.M.K. and S.R., unpublished observations). Of interest, two half-length somites formed when an ectopic somite border was induced in the chick PSM by transplantation of quail posterior border cells (Sato et al., 2002). In addition, Freitas and colleagues (2001) observed half-width epithelialized somites in the medial PSM in static experiments, but differently, half-width somites did not form in the lateral PSM when the medial PSM was ablated. These results suggest the normal epithelialization process is propagated (Sato et al., 2002; Sato and Takahashi, 2005), occurs independently of PSM width and mesenchymal cells within the PSM are not predetermined to a particular phenotype (Sato et al., 2002; Sato and Takahashi, 2005). Thus, improved insights into the mechanisms that govern epithelialization, studied within the somite microenvironment, may yield clues to the spatiotemporal program of epithelialization and cell specification.

PERSPECTIVES

There is a need to elucidate the intricacies of the signaling pathways in somite segmentation, which holds promise for yielding new targets to prevent muscle and skeletal birth defects. It is equally important to appreciate the complexity of the oscillator mechanism and the intersection with other signaling pathways underlying local cell rearrangements and shape changes during somite border forma-

tion and epithelialization. The latter process holds promise for yielding new insights into cell specification during mesenchymal-to-epithelial transitions. In this review, we have described recent biological work within the context of the history of mathematical modeling and novel quantitative frameworks to handle the emerging complexity of cellular and molecular data on somite segmentation and epithelialization. Current qualitative models for somitogenesis can place hypotheses and concepts into a rigorous framework to test the effects of multiple, competing nonlinear effects. However, a major challenge is to refine these models from a qualitative to quantitative form that includes appropriate parameter values and close integration between modeling and experiment. For example, significant headway could be made by a better understanding of the mechanisms that tie together the clock and the somite determination front. Second, the complexity of 3D cell rearrangements, cell shape changes and cell alignment during somite border formation and epithelialization insists we combine more sophisticated two-photon time-lapse imaging and real-time molecular readout. This strategy will require quantitative frameworks to include the multitude of data coming from microarray analyses and time-lapse imaging, without compromising the strengths of existing mathematical simulations that have identified key signaling component interactions. Lastly, the rapid specification of cells from mesenchymal to epithelial in normal and re-specification in operated embryos suggests that cells within a forming somite are not prepatterned to a specific phenotype and offer a paradigm to study cell–cell interactions and cell reprogramming.

In summary, we have gained new insights into the potential of two quantitative frameworks to integrate data across multiple spatial scales and the ability of cells in the PSM to rapidly re-specify between an epithelial and mesenchymal state to assemble a complete somite structure. The discovery of key signaling pathways underlying the precise spatiotemporal program of somite border formation and epithelialization coupled with mathematical modeling will likely yield novel strategies to suppress aberrant somite patterning. In ancient Rome, architects who designed and sculpted the roman arches were required to also be mathematicians (Salingaros, 1999). Perhaps at the crossroads of work by developmental biologists and researchers that build and simulate mathematical models we will find equal success at unraveling the biology of somite patterning.

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