Patterning embryos with oscillations: structure, function and dynamics of the vertebrate segmentation clock

Andrew C. Oates1,2, Luis G. Morelli1,2 and Saúl Ares3,4,*

Summary
The segmentation clock is an oscillating genetic network thought to govern the rhythmic and sequential subdivision of the elongating body axis of the vertebrate embryo into somites: the precursors of the segmented vertebral column. Understanding how the rhythmic signal arises, how it achieves precision and how it patterns the embryo remain challenging issues. Recent work has provided evidence of how the period of the segmentation clock is regulated and how this affects the anatomy of the embryo. The ongoing development of real-time clock reporters and mathematical models promise novel insight into the dynamic behavior of the clock.

Key words: Gradient, Modeling, Negative feedback, Oscillator, Signaling, Somitogenesis

Introduction
The segmented anatomy of the vertebrate embryo is evident in the two bilaterally symmetrical rows of somites that flank the notochord along the body axis. These blocks of mesodermal cells give rise primarily to bone, muscle and skin of the adult body, which is correspondingly segmented. Somitogenesis is a rhythmic and sequential process in which each successive bilateral somite pair segregates at a regular time interval from the anterior end of the pre-somatic mesoderm (PSM, see Glossary, Box 1) as the body axis elongates (Fig. 1A,B). Somitogenesis has long been of interest to developmental biologists because it involves the coordination of patterning and growth of a tissue by a regularly repeated morphogenetic process. The topic of this review is the molecular segmentation clock that underlies this periodicity. The segmentation clock has attracted the attention of those interested in biological clocks and the molecular mechanisms of developmental timing, as well as those studying the function and stability of rapidly acting genetic circuits, and the interplay between the properties of single cells and their collective behavior at the tissue level. Finally, the rhythmic nature of the process is the seed for a theoretical interest in somitogenesis that is decades old and now promises a powerful synthesis of experiment and theory that is emblematic of modern embryology.

This review first provides an overview of somitogenesis, then describes the prevailing dynamic model for somitogenesis, the Clock and Wavefront mechanism, its molecular phenomenology.

Box 1. Glossary

Biological oscillator. A system that generates a periodic variation in the state of a cell, tissue or organism. The vibrating stereocilia bundles of inner ear hair cells, the contraction cycle of cardiac muscle cells, circadian clocks and rhythmic neuronal circuits are all biological oscillators.

Coupling. Communication between neighboring oscillators that leads to mutual adjustment of their frequencies. For example, activation of Notch receptors in a presomitic mesoderm cell by Delta from neighboring cells can affect the dynamics of Notch pathway components in the target cell.

Frequency profile. Dependence of the frequency of the oscillators in an array on their position. In the segmentation clock, oscillators are proposed to be faster in the posterior and slower in the anterior, gradually slowing down across the tissue and so creating a spatial profile of frequencies.

Genetic oscillation. A type of biological oscillation in which regulation of gene expression generates oscillations in the levels of gene products.

Kinematic wave. A wave in which transport is absent or negligible. Examples include news ticker displays, in which the change of the state of the lamps, and not their movement, produces the moving pattern of words.

Negative feedback. An inhibitory control loop in which a downstream product inhibits an upstream step of a pathway, for example the inhibition of gene expression by the products of the gene.

Phase oscillator. A simplified representation of an oscillator where all physical variables, e.g. concentrations of oscillating species, are discarded and the dynamics are reduced to only one variable: the phase in the cycle. The phase is an abstract variable that measures the degree of progress of an oscillator through an oscillation cycle.

Pre-somatic mesoderm. The posterior-most and unsegmented paraxial mesoderm that will give rise to the somites and is directly continuous with the tailbud.

Segment. A repeated unit of subdivision in a tissue, typically along the anterior-posterior axis. A somite is a type of segment.

Somitogenesis period. The regular time interval between the formation of successive somite boundaries.

Steady state. A situation in which the dynamics of a given system does not change with time, as opposed to transient dynamics. An oscillator is in steady state when the change of its variables is identical in each cycle, implying that its period is constant.

Synchronization. Emergence of coherent dynamics among a population of oscillators. Noisy and incoherent oscillators can synchronize if the coupling between them overcomes noise and variability in individual oscillators.

Time delay. A time interval used to describe the completion of a process or series of processes that are not instantaneous, e.g. the time delay in producing an mRNA molecule is the time elapsed in its transcription and splicing.

1Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany. 2CONICET, Departamento de Fisica, FCyN, UBA, Pabellón I, Ciudad Universitaria, Buenos Aires, Argentina. 3Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Strasse 38, 01187 Dresden, Germany. 4Grupo Interdisciplinar de Sistemas Complejos (GIISC), Spain.

*Present address: Logic of Genomic Systems Laboratory, Centro Nacional de Biotecnología – CSIC, Calle Darwin 3, 28049 Madrid, Spain

†Author for correspondence (oates@mpi-cbg.de)
and components, and recent findings on the regulation of the period of the segmentation clock. We then organize what is known about the underlying molecular and cellular mechanisms of the segmentation clock into a three-tiered model, which begins with single-cell genetic oscillations (see Glossary, Box 1), moves on to the synchronization (see Glossary, Box 1) of these oscillators and ends with the controlled arrest of the oscillating cells. We also discuss the contributions of mathematical modeling to our understanding of this complex process and the many unanswered questions that remain.

**An overview of somite development**

Together with the tailbud, the PSM forms the posterior-most part of the vertebrate embryo. As the embryo elongates, driven by cell division and cell rearrangement, cells are added to the posterior of the PSM from the tailbud and are removed from the anterior into forming somites. It is often useful to describe this tissue in a co-moving reference frame in which the observer ‘sits’ atop the PSM and travels with it (Pourquié and Tam, 2001). In this perspective, cells appear to flow through the PSM. The PSM is thus a polarized tissue with immature mesodermal progenitors in the posterior transitioning to increasingly mature cells in the anterior. This polarity is set up by gradients of signaling factors that span the transitioning to increasingly mature cells in the anterior. This polarity is set up by gradients of signaling factors that span the anterior where the somite boundaries will form (Aulehla and Pourquié, 2010). In most vertebrates, this forms a three-dimensional epithelial block that has a basal surface on the outside, which forms the somite boundaries, and a cluster of mesenchymal cells in the interior that contribute to the differentiated somite derivatives. The formation and stability of these morphological boundaries involves a mesenchymal-to-epithelial transition of the PSM tissue via coordinated changes in cellular shape and adhesion, and a deposition of extracellular matrix into the resulting gap or furrow (reviewed by Watanabe and Takahashi, 2010). These complex morphogenetic processes involve Eph-ephrin and integrin-fibronectin signaling, but lie outside of the scope of this review.

This review focuses on the mechanism by which the spatial pattern of somites is first generated. For this purpose, the key features of the somites are their anterior-posterior (AP) lengths and the temporal periodicity with which they are formed. Although both somite length and somitogenesis period (see Glossary, Box 1) change slowly and reproducibly along the AP axis during embryogenesis, these changes occur much more slowly than the rhythm of somitogenesis itself (Schröter et al., 2008). Thus it is both valid and useful to describe the fundamentals of somitogenesis in steady state (see Glossary, Box 1). Somite length and somitogenesis period also vary according to species: somitogenesis period is ~30 minutes in zebrafish, 90 minutes in chick and *Xenopus*, 2 hours in mouse, and 6 hours in human (Gomez et al., 2008). In externally developing species, the periodicity, like the overall rate of development, is temperature sensitive (Pearson and Elsdale, 1979; Schröter et al., 2008). Thus, somitogenesis appears broadly conserved at anatomical, morphogenetic and genetic levels, but when viewed in greater detail, every vertebrate segmentation clock performs its own elegant rhythmic dance. Before describing the molecular genetics of the segmentation clock that are responsible for these rhythms, we first discuss the unusual idea of using a biological oscillator (see Glossary, Box 1) to pattern an embryo.
The Clock and Wavefront mechanism

The rhythmic nature of somitogenesis suggests that a biological oscillator plays a role in this process. How is the rhythm of a biological oscillator used to segment an elongating body axis? Currently, the favored conceptual scheme for the regulation of somitogenesis is the Clock and Wavefront mechanism (Cooke and Zeeman, 1976), the fundamental components of which (Cooke, 1981) have significant experimental support. The Clock and Wavefront mechanism is a very general scheme that does not specify cellular or molecular details. The Clock consists of undefined cellular oscillators in the cells of the PSM that are coordinated to generate coherent tissue-level oscillations. In its simplest form, the Wavefront moves posteriorly across the PSM from the anterior, freezing the cellular oscillators as it passes and thereby leaving a permanent record of their activity at the time of arrest (Fig. 1C). In this way, the Clock and Wavefront mechanism translates the temporal information of an oscillator into a fixed periodic pattern in space (see supplementary material Movie 1).

Regardless of the molecular components involved, the length of each somitic segment \( S \) can be mathematically described at steady state as being the product of the velocity of the wavefront \( v \) and the period of the clock \( T \), \( S = vT \) (see supplementary material Movies 1, 2).

During somitogenesis, cells are continuously added to the posterior of the PSM as somites are formed from the anterior, so the passage of the wavefront does not simply consume the PSM. In principle, segmentation could continue as long as the wavefront keeps moving, and posterior cells keep oscillating. Regardless of how segmentation is finally terminated, at steady state the total number of segments \( n \) will be given by the total duration of segmentation \( d \) divided by the period of the clock \( T \), \( n = d/T \). Thus, the hallmark of the Clock and Wavefront mechanism is that the period of the clock plays a causal role in determining the size and number of somites, and thus the anatomy of the vertebrate embryo.

With this general dynamic model in hand, we now turn to the evidence for a biological oscillator at work during segmentation.

The molecular segmentation clock

A molecular counterpart to the morphological rhythm of somitogenesis, termed the segmentation clock (see Box 2), was first discovered in the chick embryo (Palmeirim et al., 1997) and has since been observed in other vertebrate species, including the mouse, snake, frog and fish (Table 1). Here, we introduce the striking oscillating patterns of gene expression that reveal the molecular segmentation clock.

Traveling waves of cyclic gene expression

Oscillating patterns of gene expression are present in the PSM and tailbud of all examined vertebrate embryos, which indicates that these oscillations are an evolutionarily conserved mechanism (see Box 3). mRNA in situ hybridization experiments have defined a small group of so-called cyclic genes that display similar oscillating mRNA patterns (Fig. 2A, Table 1). Proteins encoded by some of these cyclic genes have oscillating levels, as shown by antibody staining (Bessho et al., 2003; Dale et al., 2003), indicating that unstable mRNA and proteins of cyclic genes are expressed and degraded in every cycle of the segmentation clock. Nevertheless, protein levels from very few cyclic genes have been measured, and it remains to be seen how many exhibit meaningful protein oscillations (Wright et al., 2009).

The key observable features of these oscillating patterns are: (1) a traveling wave of gene expression moving anteriorly through the PSM; (2) the slowing and shortening of the wave in the AP direction, and its arrest in the anterior of the PSM at a position that prefigures prospective somite boundaries; and (3) the repetition of the pattern with the formation of each new somite pair. These properties of the traveling wave patterns have been confirmed by time-lapse microscopy in living mouse embryos using luminescent

---

**Box 1. A segmentation clock or an oscillating ruler?**
The circadian clock is probably the best-understood biological clock (Hogenesch and Ueda, 2011). An important feature of the circadian clock, and indeed any clock, is temperature compensation of the period, which allows time to be measured accurately by the organism, regardless of short-term fluctuations in environmental conditions, such as the weather. By contrast, the period of somitogenesis is not temperature compensated. In those species that develop externally to the mother, the somitogenesis period becomes shorter at higher temperatures (Pearson and Eldsdale, 1979; Schröter et al., 2008). Growth rate also increases with elevated temperature, and the period of somitogenesis exactly matches this increase so that the somite length is temperature compensated (Schröter et al., 2008). This preserves the proportions of the body plan, and thus its biomechanical properties. Thus, the segmentation clock that underlies somitogenesis is actually a poor clock, because its period changes dramatically with temperature, but it appears to be a good ruler, because the length of body segments and the final proportions of the body plan do not vary with temperature.

**Box 2. A segmentation clock or an oscillating ruler?**
The circadian clock is probably the best-understood biological clock (Hogenesch and Ueda, 2011). An important feature of the circadian clock, and indeed any clock, is temperature compensation of the period, which allows time to be measured accurately by the organism, regardless of short-term fluctuations in environmental conditions, such as the weather. By contrast, the period of somitogenesis is not temperature compensated. In those species that develop externally to the mother, the somitogenesis period becomes shorter at higher temperatures (Pearson and Eldsdale, 1979; Schröter et al., 2008). Growth rate also increases with elevated temperature, and the period of somitogenesis exactly matches this increase so that the somite length is temperature compensated (Schröter et al., 2008). This preserves the proportions of the body plan, and thus its biomechanical properties. Thus, the segmentation clock that underlies somitogenesis is actually a poor clock, because its period changes dramatically with temperature, but it appears to be a good ruler, because the length of body segments and the final proportions of the body plan do not vary with temperature.

**Box 3. Evolution of segmentation**
Most metazoans are segmented along their anterior-posterior axis. Although some arthropods show wave-like Notch pathway gene expression patterns in their posterior growth zones (Chipman and Akam, 2008), definitive evidence for a segmentation clock in non-vertebrate species is still lacking (Damen, 2007). For example, Drosophila, certainly the best-understood system, does not use oscillations to segment its body. How is the diversity of the observed vertebrate body forms generated and how is somitogenesis regulated in these different bodies (Richardson et al., 1998)? Transitions between segment numbers in evolution could be brought about by alterations in the ratio of the period of the clock to the duration of somitogenesis (Gomez et al., 2008); indeed, a change in vertebral number in a viable fertile zebrafish mutant that is due to a change in the period of the clock has been recently reported (Schröter and Oates, 2010). Oscillating signal transduction pathways are also conserved but different individual genes are cyclic in different species (Krol et al., 2011) (see also Table 1). For example, Delta expression is cyclic in mouse and zebrafish but not in chick, and lunatic fringe expression is cyclic in mouse and chick but not in zebrafish. Only orthologs of Hes1 and Hes7 are cyclic in all species so far examined (Krol et al., 2011), implying that these genes are part of an ancestral vertebrate segmentation clock mechanism.

Was the last common ancestor of bilateria segmented or did segmentation evolve independently in the extant lineages? As the Clock and Wavefront mechanism is generic, any oscillatory circuit could in principle provide the necessary activity for the Clock. Transcriptional repressors are the basic building blocks of genetic oscillators, and the genomes of metazoans are replete with these genes, suggesting that it might be common to evolve oscillatory circuits. Thus, what evidence would be necessary to demonstrate conclusively a shared common ancestry for a segmentation clock is not clear.
and fluorescent transgenic reporters of oscillating segmentation clock genes (Fig. 2B, Table 1) (Aulehla et al., 2008; Masamizu et al., 2006; Takashima et al., 2011).

As these traveling waves of gene expression are the most obvious manifestation of the molecular segmentation clock, yet did not appear in the formulation of the Clock and Wavefront mechanism above, it is worth discussing their origin and implications. The waves of gene expression sweep across the field of PSM cells, indicating that they are not tied to a segment-specific cell lineage (Masamizu et al., 2006; Palmeirim et al., 1997). Furthermore, the maintenance of the oscillating gene expression patterns for some time after the PSM has been surgically transected or explanted indicates that these patterns do not require the bulk transport of material or the propagation of signals (Masamizu et al., 2006; Palmeirim et al., 1997). This implies that the patterns are the result of a wave of gene expression timing, also called a kinematic wave (see Glossary, Box 1). Individual cells in different positions along the AP axis of the PSM are in different phases of the oscillating gene expression cycle. This AP phase profile manifests as a visible pattern of cyclic gene expression waves. Given a kinematic wave with the properties (1-3) described above, the observed phase profile implies a gradual slowing of the frequency of oscillators across the PSM (Palmeirim et al., 1997) (Fig. 2C, supplementary material Movie 4). Regardless of the underlying mechanism through which this frequency profile (see Glossary, Box 1) is established, alterations to the profile change the number and length of traveling waves within the PSM (Fig. 2C,D). The observed differences between species in the number of traveling waves of gene expression (e.g. three in zebrafish and one in mouse) imply differences in the frequency profile.

Importantly, the traveling wave pattern in the PSM repeats itself with every newly forming somite. This fact indicates that, regardless of the details of the waves or the arrest mechanism, the entire multicellular system of PSM and tailbud oscillates with a regular rhythm corresponding to the rate of somite formation. Thus, the molecular segmentation clock observed in vivo is consistent

As these traveling waves of gene expression are the most obvious manifestation of the molecular segmentation clock, yet did not appear in the formulation of the Clock and Wavefront mechanism above, it is worth discussing their origin and implications. The waves of gene expression sweep across the field of PSM cells, indicating that they are not tied to a segment-specific cell lineage (Masamizu et al., 2006; Palmeirim et al., 1997). Furthermore, the maintenance of the oscillating gene expression patterns for some time after the PSM has been surgically transected or explanted indicates that these patterns do not require the bulk transport of material or the propagation of signals (Masamizu et al., 2006; Palmeirim et al., 1997). This implies that the patterns are the result of a wave of gene expression timing, also called a kinematic wave (see Glossary, Box 1). Individual cells in different positions along the AP axis of the PSM are in different phases of the oscillating gene expression cycle. This AP phase profile manifests as a visible pattern of cyclic gene expression waves. Given a kinematic wave with the properties (1-3) described above, the observed phase profile implies a gradual slowing of the frequency of oscillators across the PSM (Palmeirim et al., 1997) (Fig. 2C, supplementary material Movie 4). Regardless of the underlying mechanism through which this frequency profile (see Glossary, Box 1) is established, alterations to the profile change the number and length of traveling waves within the PSM (Fig. 2C,D). The observed differences between species in the number of traveling waves of gene expression (e.g. three in zebrafish and one in mouse) imply differences in the frequency profile.

Importantly, the traveling wave pattern in the PSM repeats itself with every newly forming somite. This fact indicates that, regardless of the details of the waves or the arrest mechanism, the entire multicellular system of PSM and tailbud oscillates with a regular rhythm corresponding to the rate of somite formation. Thus, the molecular segmentation clock observed in vivo is consistent

### Table 1. Expression and genetics of cyclic (oscillating expression) segmentation clock genes

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Species</th>
<th>Cyclic expression</th>
<th>Segmentation phenotype</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairy1</td>
<td>Transcriptional repressor</td>
<td>Chick</td>
<td>mRNA in situ</td>
<td>Not determined</td>
<td>(Palmeirim et al., 1997)</td>
</tr>
<tr>
<td>Lfng</td>
<td>Notch glycosylase</td>
<td>Chick</td>
<td>mRNA in situ</td>
<td>Western blot</td>
<td>(Aulehla and Johnson, 1999; Dale et al., 2003; McGrew et al., 1998)</td>
</tr>
<tr>
<td>Hes7</td>
<td>Transcriptional repressor</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>Yes</td>
<td>(Bessho et al., 2003; Bessho et al., 2001; Ferjentsik et al., 2009; Takashima et al., 2011)</td>
</tr>
<tr>
<td>Hes1</td>
<td>Transcriptional repressor</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>No</td>
<td>(Masamizu et al., 2006; Ohtsuka et al., 1999)</td>
</tr>
<tr>
<td>Hes5</td>
<td>Transcriptional repressor</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>No</td>
<td>(Masamizu et al., 2006; Ohtsuka et al., 1999)</td>
</tr>
<tr>
<td>Dll1</td>
<td>Notch ligand</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>Yes</td>
<td>(Hrabé de Angelis et al., 1997; Maruhashi et al., 2005)</td>
</tr>
<tr>
<td>Lfng</td>
<td>Notch glycosylase</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>YFP transgene</td>
<td>(Aulehla and Johnson, 1999; Aulehla et al., 2008; Evrard et al., 1998; Forsberg et al., 1998; Zhang and Gridley, 1998)</td>
</tr>
<tr>
<td>Nrarp</td>
<td>Destabilizes Notch ICD</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>Yes</td>
<td>(Kim et al., 2011; Wright et al., 2009)</td>
</tr>
<tr>
<td>Dkk1</td>
<td>Extracellular regulator of Wnt signaling</td>
<td>Mouse</td>
<td>mRNA in situ (intron?)</td>
<td>Yes</td>
<td>(Dequeant et al., 2006; MacDonald et al., 2004)</td>
</tr>
<tr>
<td>Dact</td>
<td>Intracellular Wnt signal transduction</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>Yes*</td>
<td>(Suriben et al., 2006; Suriben et al., 2009)</td>
</tr>
<tr>
<td>Axin2</td>
<td>Cytoplasmic inhibitor of Wnt signaling</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>No</td>
<td>(Aulehla et al., 2003; Yu et al., 2005)</td>
</tr>
<tr>
<td>Snail</td>
<td>Transcriptional repressor</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>Yes*</td>
<td>(Carver et al., 2001; Dale et al., 2006)</td>
</tr>
<tr>
<td>Dusp4</td>
<td>ERK Phosphatase in FGF signaling</td>
<td>Mouse</td>
<td>mRNA in situ</td>
<td>Western blot Protein (WMIC)</td>
<td>No</td>
</tr>
<tr>
<td>Lfng</td>
<td>Notch glycosylase</td>
<td>Corn snake</td>
<td>mRNA in situ</td>
<td>Not determined</td>
<td>(Gomez et al., 2008)</td>
</tr>
<tr>
<td>Esr9</td>
<td>Transcriptional repressor</td>
<td>Xenopus</td>
<td>mRNA in situ</td>
<td>Not determined</td>
<td>(Li et al., 2003)</td>
</tr>
<tr>
<td>her1</td>
<td>Transcriptional repressor</td>
<td>Zebrafish</td>
<td>mRNA in situ</td>
<td>Yes</td>
<td>(Holley et al., 2000; Sawada et al., 2000)</td>
</tr>
<tr>
<td>her7</td>
<td>Transcriptional repressor</td>
<td>Zebrafish</td>
<td>mRNA in situ</td>
<td>Yes</td>
<td>(Henry et al., 2002; Oates and Ho, 2002)</td>
</tr>
<tr>
<td>deltaC</td>
<td>Notch ligand</td>
<td>Zebrafish</td>
<td>mRNA in situ</td>
<td>Protein (WMIC)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Severe axial deformations in these embryos preclude assessment of the specificity of the segmentation defects.

Dact, dapper homolog, antagonist of β-catenin; Dkk, dikkopf homolog; Dll, delta like; Dusp, dual specificity phosphatase; Esr9, enhancer of split related 9; her, hairy related; Hes, hairy and enhancer of split; Lfng, lunatic fringe; Nrarp, Notch-regulated ankyrin repeat protein; WMIC, whole-mount immunohistochemistry.
with the basic relationship that exists between segment length, wavefront velocity and clock period, as is predicted by the Clock and Wavefront mechanism. The correlation of cyclic gene expression patterns with somite formation suggests that the molecular segmentation clock may be causal in this process and we next examine the evidence that supports this idea.

**Molecular genetics of the segmentation clock**

The segmentation clock appears to be a complex oscillating genetic network (see also Box 4) (Dequeant et al., 2006; Krol et al., 2011). Members of this network include the cyclic genes, which encode a range of proteins that are primarily linked to three major signaling pathways: Delta-Notch, Wnt and fibroblast growth factor (FGF). In addition, members of the Hes/Her (hairy and enhancer of split/hairy related) family of bHLH transcriptional repressors are cyclic in somitogenesis in all species examined (Krol et al., 2011) (Table 1). The role of most cyclic genes in the segmentation clock is not known, but here we attempt to categorize these cyclic genes based on the evidence from loss-of-function phenotypes.

Loss of function of several mouse and zebrafish cyclic genes yields specific somitogenesis phenotypes that are characterized by defective somite boundaries and disrupted traveling wave patterns (Table 1) (Bessho et al., 2001; Evrard et al., 1998; Henry et al., 2002; Hrabe de Angelis et al., 1997; Julich et al., 2005; MacDonald et al., 2004; Oates and Ho, 2002; Zhang and Gridley, 1998). In

---

**Fig. 2. Traveling waves of gene expression in the PSM.**

(A) In situ hybridization of deltaC mRNA in the pre-somatic mesoderm (PSM) of six individual six-somite zebrafish embryos (dorsal view). Embryos are staged sequentially, showing evolution of the gene expression pattern during one cycle of the segmentation clock. Waves of gene expression appear in the posterior PSM and move anteriorly (asterisk), becoming thinner as they do. Three traveling waves are seen in zebrafish at this developmental stage. (B) Bioluminescence imaging of the PSM of a mouse embryo at E10.5 expressing a Hes1 promoter-driven luciferase reporter, showing a single traveling wave of expression (asterisk) from posterior to anterior. (C) Frequency profiles (green) of slowing oscillators (blue) across the PSM (gray). The phase of the oscillators is represented by the angle of the hand of a clock. The slowing of oscillators from posterior to anterior as they approach the arrest front is described by a spatial profile of their frequency. (D) Simulation of a model of the segmentation clock (Morelli et al., 2009), showing traveling waves of gene expression (blue) in three scenarios: sudden arrest (no profile), rapid slowing (mouse) and gradual slowing (zebrafish). The waves appear owing to the frequency profile of oscillating cells across the PSM. In profile 1, oscillators are stopped abruptly at the wavefront, which corresponds to the original proposal of the Clock and Wavefront mechanism (Cooke and Zeeman, 1976). In profiles 2 and 3, the oscillators slow down gradually with increasingly shallow frequency profiles, illustrating the typical traveling wave patterns found in mouse and zebrafish, respectively. Despite the differences in traveling waves, the simulations in all three panels have an identical segmentation period, wavefront velocity and resulting segment length (supplementary material Movie 4). (A) Reproduced, with permission, from Oates et al. (Oates et al., 2009). (B) Reproduced, with permission, from Masamizu et al. (Masamizu et al., 2006).
Box 4. Starting and finishing segmentation

Although the material in the review focuses on the notion of steady-state segmentation, the segmentation of a vertebrate embryo has a beginning and end. Oscillation of the segmentation clock begins during gastrulation, long before the first somite forms (Ishimatsu et al., 2010; Jouve et al., 2002; Riedel-Kruse et al., 2007). The spatial patterns of the first oscillations are distinct from the later traveling waves, and the emergence of the waves may be tied to spatial changes in fibroblast growth factor (FGF) signaling that emerge as the mesoderm elongates (Ishimatsu et al., 2010). A function for these ‘warm up’ oscillations remains to be established, but it appears to have no connection to the potential segmentation of the head mesoderm (Jouve et al., 2002).

How segmentation ceases is both an embryological and an evolutionary question, as changing when segmentation stops could lead to changes in segment number and body proportions. What cues determine when segmentation stops? The period of somitogenesis lengthens in the tail (Schröter et al., 2008). Cyclic gene expression changes as the tailbud and presomitic mesoderm (PSM) shrink in size at the end of somitogenesis, and the clock stops even though the PSM is still present (Tenin et al., 2010). One recent hypothesis to explain the cessation of the segmentation clock is that as the PSM becomes smaller, the level of somite-derived retinoic acid rises in the tail bud, eventually repressing the high level FGF signaling required for ongoing oscillations (Gomez et al., 2008; Tenin et al., 2010); however, functional evidence for this is lacking. Alternatively, it is possible that the embryo has a separate timing mechanism to shut off the segmentation clock in coordination with the end of elongation.

some mutants of cyclic genes from the FGF and Wnt pathways, such as mouse Snail, which encodes a FGF-inducible transcriptional repressor, and Dact1, which encodes a Wnt pathway signal transduction component, axial deformations and truncations potentially mask the specific functions of these genes in segmentation (Carver et al., 2001; Suriben et al., 2009). Some cyclic gene mutants, such as the mouse bHLH transcriptional repressors Hes1 and Hes5, and the Wnt-pathway signal transduction component Axin2, have no observable segmentation phenotype (Ohtsuka et al., 1999; Yu et al., 2005). For others, the severity of the phenotype is increased in double knock-down combinations, for example zebrafish bHLH transcriptional repressors her1;her7 and her1;hes6 (Henry et al., 2002; Oates and Ho, 2002; Sieger et al., 2006). This indicates that the architecture of the segmentation clock is partially redundant, and its function is robust to perturbation.

Many genes from these pathways are expressed in the PSM but without a traveling wave pattern, and mutations in some of these genes, such as those encoding the cell-cell signaling receptor Notch1, its ligand Delta-like 3 and the activated Notch protease presenilin, also disrupt segmentation (Conlon et al., 1995; Dunwoodie et al., 2002; Shen et al., 1997; Wong et al., 1997) (Table 2). In some cases, the encoded protein is modified or localized in a periodic manner. For example, the cleaved Notch intracellular domain, which is indicative of active Notch signaling, is visible in a traveling wave pattern in mouse PSM (Huppert et al., 2005; Morimoto et al., 2005). From enhancer dissection studies of the mouse cyclic lunatic fringe (Ltfng) gene, which activates Notch signaling in the PSM via glycosyltransferase activity, it has been proposed that Notch signaling is primarily required in the anterior PSM downstream of the segmentation clock (Shifley et al., 2008; Stauber et al., 2009). However, the existence of weak residual posterior PSM oscillations driven by the tested enhancer constructs (Stauber et al., 2009) and the ability of the posterior-specific cyclic regulatory region of Hes7 to rescue completely the loss of function Ltfng phenotype (Oginuma et al., 2010) suggest that oscillating Notch signaling in the segmentation clock itself is essential for mouse somitogenesis.

Another example of post-translational oscillation is the periodic phosphorylation in the mouse PSM of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) serine/threonine kinase, a key component of FGF signal transduction (Niwa et al., 2011). Cyclic expression in the PSM of the ERK phosphatase dual specificity phosphatase 4 (Dusp4), itself an FGF target gene, may be responsible for the observed rhythm in ERK phosphorylation (Niwa et al., 2007; Niwa et al., 2011). Interestingly, sustained oscillations in the ERK signaling network have been observed in other contexts (Shankaran and Wiley, 2010), but segmental defects have not been reported in Dusp4 mutant mice (Al-Mutairi et al., 2010), and the role of these intriguing phosphorylation cycles in the mouse segmentation clock remains unclear.

In combination, these results indicate that cyclic genes are the transcriptionally oscillating component of a larger molecular segmentation clock. Whenever cyclic traveling wave patterns are disrupted, normal somitogenesis is also disrupted. Thus, these phenotypes are consistent with the idea that cyclic genes and their linked signaling pathways function in a molecular segmentation clock that regulates somitogenesis.

Although these mutations identify some of the components of the segmentation clock, their disruption does not address the role of timing in somite patterning. To determine whether the embryo uses a Clock and Wavefront mechanism to time the regular and sequential formation of somites, mutations or other perturbations that change the period of the clock but do not concomitantly disrupt somite boundaries or axial extension need to be investigated.

Regulation of period in the molecular segmentation clock

Recent experimental and theoretical advances have explored the control of the period of the clock, as well as how the embryo uses this timing to control the size, number and identity of the resulting segmented structures. Through the use of a sensitive and precise multiple-embryo time-lapse protocol that measures the period of somitogenesis in freely developing zebrafish embryos, a series of mutations in genes that encode components of the Delta-Notch signaling pathway were recently discovered to alter the period of somitogenesis (Herrgen et al., 2010; Schröter and Oates, 2010). These mutations cause posterior segmentation defects, yet leave the somite boundaries of the anterior trunk largely intact (Holley et al., 2000; Holley et al., 2002; Itoh et al., 2003; Julich et al., 2005). Although the mutant embryos extend normally with a wild-type wavefront velocity, these anterior somites form with a longer period and have a correspondingly increased length (Herrgen et al., 2010). This observation is consistent with the first expectation of the Clock and Wavefront mechanism, that a slower clock will cause longer segments, $S=vT$. A longer segmentation clock period is also predicted to change the length of the traveling waves of gene expression in the PSM (Morelli et al., 2009), a prediction that was confirmed by measurements of this length distribution in mutant embryos (Herrgen et al., 2010). Thus, the changes in Delta-Notch mutant embryos are consistent with a change in the period of the molecular segmentation clock.

A mutation in the gene that encodes the Hes6 bHLH transcriptional repressor protein gives rise to a highly informative period phenotype. hes6 mutant zebrafish embryos extend their axis...
normally with a wild-type wavefront velocity, but their somites form more slowly and are correspondingly longer, again as would be predicted by the Clock and Wavefront mechanism (Schröter and Oates, 2010). Furthermore, because hes6 mutants can complete segmentation without boundary defects in the same total time as wild-type siblings do, it is possible to determine how many segments they form altogether. The striking result is a reduction in segmentation without boundary defects in the same total time as would be expected according to the Clock and Wavefront mechanism (Gibb et al., 2009). This apparent contradiction may be explained by concomitant alterations in the velocity of the wavefront, perhaps owing to changes in embryonic extension (Wilson et al., 2009). A role for Wnt signaling in regulating the period of the segmentation clock has been previously discussed (Aulehla et al., 2003; Aulehla et al., 2008), and this new work will spur further investigation.

Surgical separation of the notochord from the paraxial mesoderm in chick results in a dramatic slowing of somite formation accompanied by altered cyclic gene expression patterns on the separated side (Resende et al., 2010). Inhibition of sonic hedgehog (Shh) signaling mimics these phenotypes and addition of exogenous Shh can rescue the effect, as can exogenous retinoic acid (RA), implicating these pathways in the timing of somite formation in chick. The Shh pathway had not of period the underlying molecular segmentation clock; these do sufficiently shorter than the period, in order to allow the detection of small changes or longer-term trends in the period. Finally, care must be taken when equating altered cyclic expression patterns with altered segmentation clock period. Furthermore, measurement or estimation of period, wavefront velocity and segment length in the same experiment. Theoretical studies suggest that alterations to the length of the PSM or in the frequency profile may cause changes in cyclic gene expression patterns independently of the period of the segmentation clock (Giudicelli et al., 2007; Gomez et al., 2008; Morelli et al., 2009). Thus, care must be taken when equating altered cyclic expression patterns with altered segmentation clock period. Further, measurement of period must be carried out with a sampling interval that is sufficiently shorter than the period, in order to allow the detection of small changes or longer-term trends in the period. Finally, care must be taken to distinguish between somitogenesis period and the period of the underlying molecular segmentation clock; these do not have to be the same. Use of theory can guide the measurement and interpretation of static images (Giudicelli et al., 2007; Herrgen et al., 2010), but integrating imaging of live reporters of the segmentation clock will play a crucial role in future studies (Aulehla et al., 2008; Masamizu et al., 2006; Takashima et al., 2011).

Investigation of somitogenesis in mice with a mutation in the Notch target gene Notch-regulated ankyrin repeat protein (Nrrap) reveals a slight lengthening of the somitogenesis period and a corresponding decrease in somite and vertebral body number (Kim et al., 2011). Nrrap encodes a negative regulator of Notch signaling, and molecular markers of Notch signaling are upregulated in the Nrrap mutant PSM. Strikingly, pharmacological inhibition of Notch signaling results in an increase in somite number over wild type, suggesting a reduced somitogenesis period. Without measurements of wavefront velocity, somite length or total duration of somitogenesis, these data are inconclusive with regards to a Clock and Wavefront mechanism, but are nevertheless consistent with a role for Notch signaling in regulating the period of the segmentation clock in mouse. Why does loss of Notch signaling increase the period of somitogenesis in zebrafish (Herrgen et al., 2010), but decrease it in mouse (Kim et al., 2011)? The answer is not known, but this difference between zebrafish and mouse was previously predicted to be due to different relative delays in the coupling between oscillating cells (Morelli et al., 2009).

Combined, these recent discoveries identify genes that appear to control the period of the segmentation clock and also describe more generally how this temporal period is translated into the periodic segmentation of the vertebrate anatomy (see also Box 5). Although direct imaging of the molecular segmentation clock in somitogenesis period mutants has yet to be reported, the mutant studies described above are consistent with a Clock and Wavefront mechanism controlling vertebrate segmentation. However, testing the Clock and Wavefront mechanism is fraught with experimental and conceptual difficulties. In general, testing requires some measurement or estimation of period, wavefront velocity and segment length in the same experiment. Theoretical studies suggest that alterations to the length of the PSM or in the frequency profile may cause changes in cyclic gene expression patterns independently of the period of the segmentation clock (Giudicelli et al., 2007; Gomez et al., 2008; Morelli et al., 2009). Thus, care must be taken when equating altered cyclic expression patterns with altered segmentation clock period. Further, measurement of period must be carried out with a sampling interval that is sufficiently shorter than the period, in order to allow the detection of small changes or longer-term trends in the period. Finally, care must be taken to distinguish between somitogenesis period and the period of the underlying molecular segmentation clock; these do not have to be the same. Use of theory can guide the measurement and interpretation of static images (Giudicelli et al., 2007; Herrgen et al., 2010), but integrating imaging of live reporters of the segmentation clock will play a crucial role in future studies (Aulehla et al., 2008; Masamizu et al., 2006; Takashima et al., 2011).

Table 2. Expression and genetics of non-cyclic segmentation clock genes

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Species</th>
<th>Oscillatory activity</th>
<th>Segmentation phenotype</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notch1</td>
<td>Delta receptor</td>
<td>Mouse</td>
<td>Proteolytic cleavage of intracellular domain</td>
<td>Yes</td>
<td>(Conlon et al., 1995; Huppert et al., 2005; Morimoto et al., 2005)</td>
</tr>
<tr>
<td>Dll3</td>
<td>Notch ligand</td>
<td>Mouse</td>
<td>ND</td>
<td>Yes</td>
<td>(Dunwoodie et al., 2002)</td>
</tr>
<tr>
<td>Psen1</td>
<td>Gamma secretase activity, cleaves NIDC signaling</td>
<td>Mouse</td>
<td>ND</td>
<td>Yes</td>
<td>(Shen et al., 1997; Wong et al., 1997)</td>
</tr>
<tr>
<td>Mapk1</td>
<td>A kinase involved in FGF signaling</td>
<td>Mouse</td>
<td>ERK2 phosphorylation</td>
<td>No*</td>
<td>(Niwa et al., 2011; Yao et al., 2003)</td>
</tr>
<tr>
<td>hes6</td>
<td>Transcriptional repressor</td>
<td>Zebrafish</td>
<td>ND</td>
<td>Yes</td>
<td>(Schröter and Oates, 2010)</td>
</tr>
<tr>
<td>DeltaD</td>
<td>Notch ligand</td>
<td>Zebrafish</td>
<td>ND</td>
<td>Yes</td>
<td>(Herrgen et al., 2010; Holley et al., 2002)</td>
</tr>
<tr>
<td>mindbomb</td>
<td>Delta ubiquitin ligase</td>
<td>Zebrafish</td>
<td>ND</td>
<td>Yes</td>
<td>(Herrgen et al., 2010; Itoh et al., 2003)</td>
</tr>
<tr>
<td>notch1a</td>
<td>Delta receptor</td>
<td>Zebrafish</td>
<td>ND</td>
<td>Yes</td>
<td>(Herrgen et al., 2010; Holley et al., 2002)</td>
</tr>
</tbody>
</table>

*Mutant mouse does not form mesoderm, precluding assessment of a specific role in segmentation.

Dll3, delta-like 3; hes6, hairy and enhancer of split 6; Mapk1, mitogen-activated protein kinase 1; Psen1, presenilin 1.
Box 5. Can the embryo count segments?

How does the embryo make the correct number of segments with the correct anterior-posterior identities of the body plan? There are two basic hypotheses for the observed very tight regulation of segment number and identity. First, the embryo could ‘count’ the number of oscillations and finally stop segmenting when the correct number was reached. AP identities would be assigned according to the increasing number (Dubrulle et al., 2001; Jouve et al., 2002). In this scheme, the period of segmentation would not directly impact either segment number or AP identity. Alternatively, the embryo might allocate a defined time window during development for segmenting, which in combination with a tight control of the period of the segmentation clock would yield the same number of segments every time (Cooke and Zeeman, 1976). Segmental AP identities would be instructed by some independent form of positional information along the axis. In this scheme, segment number and identity would be affected by changes to the period of segmentation.

The zebrafish hes6 mutant has a slower somitogenesis period and makes fewer longer segments in the same total time as wild-type siblings (Schröter and Oates, 2010), in agreement with the latter hypothesis. Furthermore, in this normally elongating mutant, anatomical landmarks of AP regional identity are not positioned at the same segment number as in wild type, but rather shift segment number to remain in the same absolute position in the body plan. From this, it would appear that vertebrate embryos do not count their segments, either to determine their total number or their regional identity. The segmentation clock thus appears to be acting in parallel to some higher-level control of body extension and regional identity.

The picture of the molecular segmentation clock that emerges from the different experiments in various species appears somewhat fragmented and, in places, apparently contradictory. We will argue below that it is nevertheless possible to place these findings within a framework that allows general properties to be highlighted and so make fertile avenues of inquiry apparent.

A three-tier model of the segmentation clock

In order to synthesize the Clock and Wavefront mechanism with experimental data on the molecular segmentation clock and with mathematical models of segmentation, we outline below a preliminary framework for organizing this information, called the three-tier model, in which the clock is defined as a multi-scale rhythmic pattern generator whose output is at the tissue level (Fig. 3). Although each of the tiers in this model corresponds to distinct experimental and theoretical investigations, the processes within each tier must integrate seamlessly within the embryo for the segmentation clock to function.

In the bottom tier is the single cell oscillator (Fig. 3A). This is the idea that each of the cells in the PSM is capable of oscillating, at least for a time, in isolation. The genetic circuits that underlie these cell-autonomous oscillations are therefore central to our understanding of the period of the clock, and regulation of the period by various factors at other levels must at some point interact with these intrinsic pace-making circuits.

The middle tier is the local synchronization of oscillating cells (Fig. 3B). This is the idea that the observed coherent oscillation of many PSM cells requires an active synchronization mechanism. Synchronization implies that individual cells can measure the phase of their neighbors, and speed up or slow down accordingly to match them. Thus, the genetic circuits involved are cell-cell communication pathways that must interact with the cell-autonomous pace-making circuits.

The upper tier is the global control of the arrest of the oscillating cells (Fig. 3C). This is the idea that the position in the anterior PSM where the oscillating cells convert their temporal phase information into a stable periodic spatial pattern must be precisely regulated. The traveling waves of gene expression emerge in this tier. Control of positional information over hundreds of micrometers involves gradients of signaling molecules, and these signals must be able to regulate the pace-making circuit of the oscillators by some means.

We believe that this three-tier model provides a useful and explicit framework with which we can organize the diverse pieces of information about the segmentation clock. New data can be interpreted with respect to the tiers, experiments can be designed to test a tier, and if a new tier of description is discovered, it can be integrated into the framework. It is possible that the importance of a given tier will vary depending on the species. Of course, our organization of data and models within these categories does not itself explain the various molecular or dynamic mechanisms at work. Below, we discuss the evidence for these tiers and how they might interact. Mathematical models that have been influential in motivating experiments or tested against data are described. Unanswered questions are framed and listed in Box 6.

Single cell oscillations

What is the smallest oscillating unit in the PSM? Could it be an individual PSM cell, or do oscillations require intercellular communication, as is the case in reaction-diffusion models (Meinhardt, 1982)? PSM tissue dissected from the embryo exhibits traveling waves of endogenous cyclic genes, indicating that the information for the oscillations is contained within the PSM (Palmeirim et al., 1997). When chick PSM is further dissected into small pieces along the AP axis, each piece continues to oscillate approximately on schedule (Maroto et al., 2005). When single cells are dissociated from PSM and fixed in a time-series, variable expression of endogenous cyclic genes is observed, consistent with persistent oscillations in single cells (Maroto et al., 2005).

The most direct evidence for single-cell oscillations comes from time-lapse imaging of mouse cells that express a luciferase reporter driven by the Hes1 cyclic gene promoter (Masamizu et al., 2006). Cultured fibroblasts carrying this transgene show a range of expression dynamics: some dampened the bioluminescent signal quickly, whereas others displayed persistent periodic fluctuations in luminescence over 12 hours. PSM cells isolated from a transgenic mouse carrying the same reporter showed a similar activity. The period and amplitude of these oscillations was variable, with an average period longer than expected from the intact mouse embryo. The noise of the individual oscillating cells appears much higher than the tissue-level variability (Masamizu et al., 2006; Riedel-Kruse et al., 2007; Schröter et al., 2008).

Thus, there is significant evidence to support the existence of single cell oscillators that underlie the rhythm of the segmentation clock. How are these oscillations generated at the molecular level?

Hes/Her auto-regulatory loop: a candidate pace-making circuit

From the list of known cyclic genes (Table 1), candidates for the generation of such single cell oscillations are the Hes/Her bHLH transcriptional repressors, as a transcription factor able to repress its own expression could build the simplest genetic auto-regulatory negative-feedback loop (Novak and Tyson, 2008) (Fig. 4). The mutation or knockdown of cyclic Hes/Her genes in
mouse and zebrafish leads to defective somite formation and to the disruption of cyclic gene expression, indicating that they are essential parts of the segmentation clock (Bessho et al., 2001; Henry et al., 2002; Oates and Ho, 2002; Sieger et al., 2006). The persistent oscillation of several Wnt and FGF pathway genes in the Hes7-null mutant mouse suggests that sub-circuits of the segmentation clock network are still active (Ferjentsik et al., 2009; Hirata et al., 2004), but the profound segmentation defects observed in these embryos indicate that this remaining activity is insufficient to pattern the PSM.

A role for Hes/Her-mediated negative feedback (see Glossary, Box 1) at the single-cell level is supported by observations and perturbations carried out in vitro and in vivo. Mouse Hes1 mRNA and protein abundance oscillates in a variety of cell lines with a period approximately equal to mouse somitogenesis (Hirata et al., 2002). Biochemical data from manipulating Hes1 production, half-life and activity in these cells indicate that unstable Hes1 protein periodically represses its own expression. This regulatory logic was corroborated in vivo for Hes7 by similar manipulations in wild-type embryos and in Hes7 mutant mouse embryos, in which segmentation is severely defective (Bessho et al., 2003). Importantly, Hes7-binding N-box regulatory elements are found in the Hes7 promoter, and Hes7 protein is detected on its own promoter in vivo by chromatin immunoprecipitation, indicating that the auto-regulatory action is direct (Bessho et al., 2003; Chen et al., 2005).

Models of transcriptional oscillations (Lewis, 2003; Monk, 2003) require a negative-feedback loop, a time delay (see Glossary, Box 1) in the feedback, sufficient non-linearity and a balancing of the timescales of the reactions involved (Novak and Tyson, 2008). When oscillating, the period of the single-cell oscillator is set by delays that arise from a combination of the steps that make up one cycle: transcription and translation, the transport of molecules in and out of the nucleus, additional biochemical events, such as splicing and post-translational modifications, and the decay of the products (Fig. 4). Elegant studies have changed the stability of Hes7 protein or the delays associated with transcribing the Hes7 gene, and these alterations disrupt oscillations in vivo in agreement with modeling predictions (Hirata et al., 2004; Takashima et al., 2011). However, there are potentially many ways to disrupt an oscillator, and disruption is not a definitive outcome for testing theories of an oscillating circuit (Oswald and Oates, 2011). Although the Hes6 mutant appears to have an altered segmentation clock period (Schröter and Oates, 2010), directly connecting this gene family to control of the period, there are currently no models that explain this change.

Thus, although many questions remain unanswered (see Box 6), it seems likely that individual PSM cells possess a noisy single-cell oscillatory circuit that depends on the auto-regulatory activity of Hes/Her genes. In the next section, we discuss the transition from single cells to the multicellular organization of the segmentation clock.

Synchronization of neighboring oscillating cells

Depending on developmental stage and species, a forming somite can span tens to hundreds of cells. For these cells to be coordinated, gene expression must be coherent across a similarly sized domain. How can gene expression from many noisy,
fluctuating individual oscillators be so well coordinated? How is the observed precision in the timing of somitogenesis achieved if the cellular timekeepers are so noisy, as described in the previous section? The answer is not unique to the PSM: noisy oscillators can be synchronized by coupling (see Glossary, Box 1) (Kuramoto, 1984; Winfree, 1967).

Delta-Notch signaling synchronizes neighboring PSM cells

Candiates for a local cell coupling mechanism can be found among the list of cyclic genes (Table 1) (Jiang et al., 2000). The Delta-Notch signaling system is a well-studied cell-cell communication pathway, with many functions in development and disease (Lai, 2004). Traveling waves of Notch pathway gene expression and Notch receptor activation (Huppert et al., 2005; Maroto et al., 2005) suggest that PSM cells signal to each other with every cycle of the clock (Fig. 5).

As mentioned above, mutations in zebrafish Delta-Notch signaling components cause posterior segmentation defects. The transition from normal to disrupted boundaries is sharp and reliably positioned along the AP axis at a mutant-specific somite number (Holley et al., 2000; Holley et al., 2002; Itoh et al., 2003; Julich et al., 2005). Underlying this morphological phenotype, the traveling waves of gene expression in the PSM slowly lose their coherence, decaying to a ‘salt and pepper’ uncorrelated pattern of juxtaposed high- and low-expressing cells (Jiang et al., 2000; Oates and Ho, 2002). This loss of coherence can be quantitatively compared with wild-type patterns using spatial autocorrelation functions that measure the organization in the traveling wave patterns (Herrgen et al., 2010). Together, these data suggest that Delta-Notch signaling is a coupling mechanism that keeps the oscillations of the PSM cells locally synchronized (Jiang et al., 2000) (supplementary material Movies 5, 6).

Cyclic gene expression initiates simultaneously throughout the future mesoderm at the onset of gastrulation in zebrafish, even in the absence of Notch signaling (Riedel-Kruse et al., 2007). The transition point from ordered to disordered segments can be positioned along the axis by experimentally manipulating when Notch signaling is inhibited, as well as the level of Notch inhibition (Mara et al., 2007; Ozbudak and Lewis, 2008; Riedel-Kruse et al., 2007). After re-activating Delta-Notch coupling in the fully disrupted state, the PSM resynchronizes, coherent traveling waves are formed and normal segments are made once again. In combination with a theoretical description of the clock as a population of coupled phase oscillators (see Glossary, Box 1) (Kuramoto, 1984), these manipulations and observations can be related to the loss of synchrony in the clock (Riedel-Kruse et al., 2007). Using this theory, the time elapsed from Notch inhibition to the loss of synchrony in the PSM can be related to the noise level in the oscillations of individual cells and the strength of the coupling that tries to keep them synchronized.

The main function of PSM cell coupling is to prevent their desynchronization and the subsequent loss of a coherent genetic segmental pre-pattern. However, cells require time to make and deploy the large proteins of the Delta-Notch system, and this delay in coupling appears to introduce an additional dynamic effect in the period of the synchronized population of cells (Morelli et al., 2009). As mentioned above, when Delta-Notch signaling is compromised in zebrafish, somitogenesis period, segment length and the traveling wave pattern of cyclic gene expression show a coordinated change that is consistent with a change in the period of the segmentation clock (Herrgen et al., 2010). Furthermore, by gradually altering Notch signaling strength, the somitogenesis period can be gradually tuned. Thus, even though each cell has its own rhythm, the period of the segmentation clock of the embryo appears to be a fundamentally multicellular property, self-organized from the dynamic interactions between synchronized PSM cells. Whether delays in the coupling cause the population to speed up, as is observed in zebrafish, or slow down depends on the ratio of the delay to the period of oscillations (Morelli et al., 2009). Because of the longer period in mouse, it was predicted that the ratio of coupling to period in this species might cause a coupling-mediated slowing of the oscillations (Morelli et al., 2009), which has recently been experimentally reported (Kim et al., 2011).

How does coupling work in molecular terms? How does Notch signaling change the period of the single-cell pacemaker circuit? Zebrafish Her genes are transcriptional targets of Notch signaling, although not dependent on Notch, and local overexpression of Delta alters cyclic Her gene expression in neighboring cells (Horikawa et al., 2006). This local phase shift can be reproduced using a mathematical model of the Her feedback loop (Lewis, 2003) that describes a chain of cells coupled through Delta-Notch signaling (Horikawa et al., 2006). In addition to becoming desynchronized, the level of Her gene expression gradually decreases in the absence of Delta-Notch coupling (Oates and Ho, 2002; Oates et al., 2005; Ozbudak and Lewis, 2008), as might be expected from the loss of a weak positive transcriptional regulatory input. In turn, gain- or loss-of-function of Her genes perturbs Delta expression (Oates and Ho, 2002; Shankaran et al., 2007; Sieger et al., 2006). The current hypothesis is that, during a cycle of the
segmentation clock, the timing of the Notch signal changes the timing of transcriptional initiation of Her genes in the receiving cell, and, reciprocally, this change is communicated to neighboring cells by the change in timing of Delta gene repression (Fig. 5). Combined, these changes in timing comprise the regulation of the period of the cells via coupling.

Thus, although it seems clear that cell-cell communication brings coherence to the oscillators of the PSM, unanswered questions about the synchronization of neighboring oscillating cells remain (see Box 6). In the next section, we discuss the slowing and arrest of oscillations at the tissue level.

**Arresting cell oscillations: global control**

Somite progenitor cells have access to several gradients of positional information across the PSM that arise from release and propagation of signaling molecules from the somites and tailbud (Fig. 6). How do the PSM cells read these gradients to regulate their oscillations? In the Clock and Wavefront mechanism, the position in the PSM where cells arrest is of fundamental importance, because it is the movement of this point with the elongating tissue that corresponds to the velocity of the wavefront. Following the relationship \(S = vT\), alterations to wavefront velocity \(v\) should cause changes in segment length \(S\).

In all cases, oscillating gene expression arrests in the anterior PSM. Although the expression of some cyclic genes persists in formed segments, it does not oscillate (Jiang et al., 2000; McGrew et al., 1998; Palmeirim et al., 1997). The position where the oscillations arrest can be inferred by fitting the traveling wave patterns of cyclic genes to models of the clock that describe the gradual slowing down and its relation to the observed phase pattern (Giudicelli et al., 2007; Herrgen et al., 2010; Morelli et al., 2009). The most anterior observable traveling wave of gene expression appears to overlap with the first permanent patterns of segmental gene expression and the emergence of a defined rostrocaudal polarity within the future somite territory (Sawada et al., 2000). This observation is consistent with the expectation from the Clock and Wavefront mechanism that the segmental pattern is established where the oscillations arrest. In mouse, this transition occurs between S–I and S0 (Morimoto et al., 2005), whereas in zebrafish it appears more posteriorly with respect to the most recently formed somite, between S–II and S–I (Sawada et al., 2000). Surgical rotation of PSM tissue in chick suggests that segments may be ‘determined’ more posteriorly around S–IV (Dubrulle et al., 2001). These segments may appear as determined because oscillations are already too slow at this position in the PSM to allow for a correction of the pattern in the remaining time before the cells become part of a segment.

The arrest of oscillations is the most extreme change possible to the behavior of a single cell in the segmentation clock, and understanding this arrest should reveal key details about the oscillatory mechanism.

**FGF, RA and Wnt gradients position the arrest front**

In all vertebrates, various members of the FGF and Wnt families are expressed in the tailbud and posterior PSM. A correspondingly graded distribution of FGF protein across the PSM has been observed, as has graded distribution of FGF and Wnt downstream signal transduction components and target gene expression (Aulehla et al., 2008; Dubrulle and Pourqué, 2004). Conversely, RA is synthesized in formed somites and anterior PSM, and correspondingly graded expression of an RA response element transgene has been seen in mouse (Sirbu and Duester, 2006; Vermot et al., 2005). RA signaling appears mutually antagonistic to both FGF and Wnt signaling in the posterior body, and thus the shapes of their opposing gradients are interdependent (Diez del Corral et al., 2003; Moreno and Kintner, 2004; Olivera-Martinez and Storey, 2007; Sirbu and Duester, 2006; Zhao and Duester, 2009).

Disruption of these signaling pathways early in development can lead to axis truncations. Transient perturbations of these pathways have thus yielded the clearest evidence of their roles during
An elongating vertebrate axis showing cyclic gene expression patterns in blue/white and arrested segments (dashed boundaries) and somites (solid boundaries) in red/white. Gradients of fibroblast growth factor (FGF) and Wnt (brown) span the tissue from the posterior. An opposing shorter-range retinoic acid (RA) gradient (gray) expands from the recently formed somites. The actual shape of these gradients is not known. Sustained high-frequency oscillations are observed in the posterior region of the tissue. Oscillators gradually slow down as they approach the wavefront of arrest (horizontal line). The panel on the left shows the different stages that single-cell oscillators undergo as they traverse the tissue. The right panel highlights the maturation program of pre-somatic mesoderm cells, running in parallel to segment length specification controlled by the clock.

Fig. 6. Global gradients control slowing and arrest of oscillating cells. An elongating vertebrate axis showing cyclic gene expression patterns in blue/white and arrested segments (dashed boundaries) and somites (solid boundaries) in red/white. Gradients of fibroblast growth factor (FGF) and Wnt (brown) span the tissue from the posterior. An opposing shorter-range retinoic acid (RA) gradient (gray) expands from the recently formed somites. The actual shape of these gradients is not known. Sustained high-frequency oscillations are observed in the posterior region of the tissue. Oscillators gradually slow down as they approach the wavefront of arrest (horizontal line). The panel on the left shows the different stages that single-cell oscillators undergo as they traverse the tissue. The right panel highlights the maturation program of pre-somatic mesoderm cells, running in parallel to segment length specification controlled by the clock.

Conclusions

The Clock and Wavefront mechanism accounts well for current dynamic observations of somitogenesis ($S=mT$ and $n=dT$). The three-tier model of the segmentation clock introduced here attempts to describe the interactions of the cellular and molecular processes in the tailbud and PSM that generate the segmental pre-pattern observed in vivo. The concept of the Clock seems to map cleanly onto the single-cell oscillator and local synchronization tiers, and the Wavefront appears to correspond to the global control tier. However, the existence of traveling waves of cyclic gene expression reveals a more complex and subtle relationship. A gradual slowing of the oscillators before arrest implies that the Wavefront is not simply freezing the oscillations of the Clock at a specific position. Rather it must be in continuous communication with the Clock. Thus, the consequence of a gradual arrest is that the Clock and Wavefront cannot be neatly separated as independent processes. Despite progress in our understanding of the regulation of the arrest position by thresholds of gradients of signaling molecules that span the PSM, how these signaling systems act on the pacemaker circuits of individual cells to control their oscillation is not known. Understanding how the oscillators are slowed and switched off in greater molecular detail will also provide better insight into the basic oscillatory mechanism.

The causal role of the period of the segmentation clock in determining the anatomy of the vertebrate embryo highlights how little we know about how the period is set. Period appears to be regulated at the single-cell oscillator tier through a pace-making circuit, although we do not understand how. The period is also regulated at the local synchronization tier, and here we have a greater understanding of how this modulation of the single cell
rhythm arises from delays in the coupling. Whether, in addition to their role in slowing and stopping oscillations, the signaling gradients at the global arrest tier affect the period of the segmentation clock in the posterior PSM and tailbud has not been investigated experimentally.

How will progress be made in understanding this challenging system? To obtain an accurate picture of the dynamic behavior of the system at cell and tissue levels, the power of real-time reporters of the segmentation clock must be fully exploited to observe the dynamics of cyclic gene expression in real time. This new information will allow existing models of the segmentation clock to be directly tested, and will spur the development of detailed well-constrained genetic and molecular models. In addition, methods to count the number of molecules in oscillating cells and in spatial gradients will be essential for understanding the sources of noise in the system and, in turn, how precision is achieved despite this noise. The integration of these experimental and theoretical approaches is likely to find application in a wider domain, establishing the segmentation clock as a key model system for understanding dynamic, fluctuating genetic systems in development and disease.

Acknowledgements

We thank Lola Bajard, Adrian Jacobo, David Richardson, Laurel Rohde, Yumiko Saga, Christian Schröter, Koichiro Uriu and Alexis Webb for critical reading of the manuscript and/or discussion. We sincerely apologize to all whose work could not be cited due to space constraints.

References

A.C.O. is supported by the Max Planck Society and by the European Research Council under the European Communities Seventh Framework Programme. S.A. acknowledges funding from MOSAICO grant from the Ministerio de Ciencia e Innovación, Spain.

Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material available online at http://dev.biologists.orglookup/submit/doi:10.1242/dev.063735f-DC1


