The Segmentation Clock: Converting Embryonic Time into Spatial Pattern

Olivier Pourquié

Science **301**, 328 (2003);
DOI: 10.1126/science.1085887

tal stage 26. By stage 41, the eyes release melatonin rhythmically, indicating that *Xenopus* embryos develop functional photoresponsive circadian clocks within the first few days of life. In embryonic zebrafish, a circadian oscillator that regulates melatonin synthesis becomes functional and light-responsive between 20 and 26 hours postfertilization (*27*). However, there is little evidence yet that suggests these embryonic clocks are gating key patterning events within the spatial axes. Clocks that play that role are more likely to have periods similar to those of the somite clocks that exhibit a *Hes1* gene–dependent oscillation with a period of about 2 hours (*28*, *29*). Recent studies showing that a mouse strain that bears mutations at the *PER2* locus exhibits enhanced susceptibility to cancer (*30*) may implicate a role for circadian rhythmicity in cell proliferation, although it has not been ruled out that *PER2* may have a role independent from circadian control that is yet to be elucidated.

Circadian clocks have a well-defined role in regulating physiological and behavioral events on a 24-hour basis and have extended that role into seasonal timing and photoperiodism. It remains to be elucidated what early developmental patterning events might be gated by circadian clocks, although a major role here seems unlikely given the relatively normal morphology and lack of heterochronic events exhibited by organisms that bear strong mutations in their circadian systems.

References and Notes

1. M. J. Dowson-Day, A. J. Millar, *Plant J.* **17**, 63 (1999).
2. D. Alabadi *et al.*, *Science* **293**, 880 (2001).
3. Z. Y. Wang, E. M. Tobin, *Cell* **93**, 12077 (1998).
4. R. Schaffer *et al.*, *Cell* **93**, 1219 (1998).
5. P. Mas, D. Alabadi, M. J. Yanovsky, T. Oyama, S. A. Kay, *Plant Cell* **15**, 223 (2003).
6. D. E. Somers, A. A. Webb, M. Pearson, S. A. Kay, *Development* **125**, 485 (1998).
7. A. Millar, S. Kay, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 15491 (1996).
8. H. G. McWatters, R. M. Bastow, A. Hall, A. J. Millar, *Nature* **408**, 716 (2000).
9. X. L. Liu, M. F. Covington, C. Fankhauser, J. Chory, D. R. Wagner, *Plant Cell* **13**, 1293 (2001).
10. A. Mouradov, F. Cremer, G. Coupland, *Plant Cell* **14**, S111 (2002).
11. I. Kardailsky *et al.*, *Science* **286**, 1962 (1999).
12. A. Samach *et al.*, *Science* **288**, 1613 (2000).
13. P. Suarez-Lopez *et al.*, *Nature* **410**, 1116 (2001).
14. M. J. Yanovsky, S. A. Kay, *Nature* **419**, 308 (2002).
15. R. S. Poethig, *Science* **301**, 334 (2003).
16. M. Yano *et al.*, *Plant Cell* **12**, 2473 (2000).
17. S. Kojima *et al.*, *Plant Cell Physiol.* **43**, 1096 (2002).
18. Y. Takahashi, A. Shomura, T. Sasaki, M. Yano, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7922 (2001).
19. S. Sugano, C. Andronis, M. S. Ong, R. M. Green, E. M. Tobin, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12362 (1999).
20. B. D. Goldman, *J. Biol. Rhythms* **16**, 283 (2001).
21. P. L. Lowrey *et al.*, *Science* **288**, 483 (2000).
22. K. Shimomura, D. E. Nelson, N. L. Ihara, M. Menaker, *J. Biol. Rhythms* **12**, 423 (1997).
23. D. S. Saunders, *Invert. Neurosci.* **3**, 155 (1997).
24. E. Tauber, B. P. Kyriacou, *J. Biol. Rhythms* **16**, 381 (2001).
25. L. M. Beaver *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2134 (2002).
26. E. M. Myers, J. Yu, A. Sehgal, *Curr. Biol.* **13**, 526 (2003).
27. N. Kazimi, G. M. Cahill, *Brain Res. Dev. Brain Res.* **117**, 47 (1999).
28. O. Pourquie, *Science* **301**, 328 (2003).
29. M. N. Nitabach, J. Blau, T. C. Holmes, *Cell* **109**, 485 (2002).
30. M. N. Nitabach, J. Blau, *Nat. Genet.* **32**, 559 (2002).
31. We thank S. Panda for Fig. 2 and C. Green and T. Imaizumi for comments.

REVIEW

The Segmentation Clock: Converting Embryonic Time into Spatial Pattern

Olivier Pourquié

In most animal species, the anteroposterior body axis is generated by the formation of repeated structures called segments. In vertebrate segmentation, a specialized mesodermal structure called the somite gives rise to skeletal muscles, vertebrae, and some dermis. Formation of the somites is a rhythmic process that involves an oscillator—the segmentation clock—driven by Wnt and Notch signaling. The clock ticks in somite precursors and halts when they reach a specific maturation stage defined as the wavefront, established by fibroblast growth factor and Wnt signaling. This process converts the temporal oscillations into the periodic spatial pattern of somite boundaries. The study of somite development provides insights into the spatiotemporal integration of signaling systems in the vertebrate embryo.

In most vertebrate species, somites appear as epithelial blocks of paraxial mesoderm cells forming synchronously on both sides of the body axis from the mesenchymal presomitic mesoderm (PSM). The rhythm of somite production is characteristic of the species at a given temperature (90 min in the chick embryo at 37°C and 20 min for the zebrafish embryo at 25°C). The total number of somites is constant within a given species. It is usually about 50, although in some animals, such as snakes, it can reach up to 400. The process of embryonic segmentation is now well understood at the molecular level in *Drosophila*, in which segments form simultaneously in the syncytial embryo. In most invertebrate and vertebrate species, segmentation takes place sequentially from head to tail in a cellularized embryo and accompanies the progressive formation of the body axis. Accordingly, the genetic networks at play in fly segmentation do not appear to be conserved in vertebrates, and it remains unclear whether segmentation arose independently in invertebrates and vertebrates.

Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA. E-mail: olp@stowers-institute.org

The Segmentation Clock: A Molecular Oscillator Underlying Vertebrate Segmentation

In vertebrates, segmentation involves a molecular oscillator—the segmentation clock—which acts in the PSM (*1*). Evidence for this oscillator was first provided by the observation of the regular pulses of expression in PSM cells of the mRNA coding for the basic helix-loop-helix (b-HLH) transcription factor c-hairy1, a vertebrate homolog of the protein encoded by the fly pair-rule gene *hairy* (*1*). *c-hairy1* mRNA is expressed as a wave sweeping across the whole PSM once during each somite formation (Fig. 1A). This rhythmic expression begins during gastrulation in the paraxial mesoderm precursors of the primitive streak and their descendants and is maintained throughout somitogenesis (*2*). Existence of such an oscillator or clock, whose role is to generate a temporal periodicity that can be translated spatially into the periodic boundaries of the somites, was originally proposed in theoretical models such as the "clock and wavefront" (*3*). Several additional genes, referred to as cyclic genes, that exhibit a dynamic behavior similar to that of *c-hairy1* have now been identified in fish, frog, chick, and mouse embryos, suggesting that the segmentation clock is conserved among vertebrates (*4*–*12*).

The best characterized set of cyclic genes is involved in Notch signaling, suggesting that Notch activation lies at the heart of the oscillator. Such genes encode several transcription factors of the Hairy and Enhancer of Split (HES) family, acting downstream of Notch signaling (*4*, *7*, *8*, *10*–*12*), as well as the glycosyl transferase Lu-

natic Fringe (*5*, *6*) and the Notch ligand deltaC (*9*). Their cycling behavior in the PSM is regulated at the transcriptional level (*13*, *14*). All these genes oscillate largely in synchrony in the PSM, suggesting that they are downstream of a common cycling activator.

The mechanism driving the oscillations of the cycling genes has been actively studied in zebrafish, chick, and mouse. In fish, oscillations of *deltaC* were proposed to drive the periodic activation of Notch, leading to the cyclic expression of the genes coding for the Hairy and Enhancer of Split–Related (HER) b-HLH repressor genes *Her1* and *Her7* (*9*, *15*). HER1 and HER7 further play a central role in the oscillator by establishing a negative feedback loop regulating their own expression (*15*, *16*). In the chick embryo, *lunatic fringe* is also directly activated by Notch signaling and is involved in a negative feedback loop leading to Notch inhibition (*17*). Given the unstable nature of the Lunatic Fringe protein, which also oscillates, this inhibition is transient and could participate in the establishment of the cyclic expression pattern (*17*). In contrast, the role of Lunatic Fringe in the control of the oscillations in the mouse is currently unclear (*18*). In the mouse, *lunatic fringe* is regulated through promoter sequences termed "clock elements" (*13*, *14*), which contain CBF1 binding sites and E boxes. This suggests that the cyclic genes are directly regulated by Notch and by b-HLH proteins. Accordingly, in mouse mutant embryos, disruption of Notch signaling prevents the oscillations of the cyclic genes (*7*, *14*). The repressor HES7 appears to play a critical role in the control of the oscillations in the mouse, because its mutation prevents the oscillations of *lunatic fringe* (*8*). This gene was shown to act downstream of Notch and to bind and negatively regulate its own promoter, suggesting that like the *her* genes in zebrafish, it could establish a negative feedback loop participating in the control of the oscillations (*8*). Another mouse cyclic gene, *hes1*, exhibits the required characteristics to implement such a loop: It acts downstream of Notch and codes for an unstable protein able to negatively regulate its own promoter (*7*, *19*). However, *hes1* is not essential for cycling, because *hes1* mutants do not exhibit a segmentation phenotype (*7*).

Therefore, it seems that in all species examined thus far, Notch activation lies at the heart of the oscillator. Notch plays a critical role in the control of the oscillations by directly activating the cyclic genes, thus accounting for their synchronous expression. Notch activation drives the expression of transcriptional repressors of the HES family, which in turn negatively regulate their own expression and that of the other cyclic genes (Fig. 1B). This negative feedback loop model requires that the HER proteins be highly unstable and that their repressive effect be dominant over Notch activation. Additional regulatory loops aimed at establishing periodic Notch activation, such as the Lunatic Fringe–based loop identified in the chick, act in concert with this mechanism (*17*). A role for Notch signaling in the control of the spatiotemporal coordination of the cyclic genes' expression among PSM cells was also proposed in zebrafish (*9*).

A second group of cyclic genes linked to the Wnt signaling pathway has recently been uncovered. Thus far, only one cycling gene in this class has been identified: the inhibitor of Wnt signaling Axin2 (*20*). In the mouse, *axin2* is expressed in a dynamic sequence similar to, but out of phase with, that of the Notch-related cyclic genes (Fig. 1A). *axin2* is directly regulated by Wnt signaling and could participate in the establishment of an autoregulatory negative feedback loop involved in its periodic expression. *axin2* oscillations persist in Notch pathway mutants, whereas both *axin2* and *lunatic fringe* oscillations are disrupted in *wnt3a* mutants, indicating that Wnt signaling acts upstream of the Notch-regulated cyclic genes (Fig. 1B). Therefore, in the mouse, the segmentation clock appears to be composed of a Wnt-based regulatory loop entraining a series of Notch-based loops (Fig. 1B). The details of the interactions between these different loops are presently not understood. Also, the conservation of the Wnt-based loop across vertebrates remains to be examined.

The exact role of this oscillator in the segmentation process remains unclear. The clock might serve to implement periodic activation of Notch signaling in the anterior PSM, which was shown to be required for the establishment of somite boundaries and subcompartments (*21*, *22*). Also, the bilateral desynchronization of somitic boundaries that accompanies the loss of oscillations of the cyclic genes in Notch pathway mutant embryos suggests that the clock could play a role in coordinating the timing of boundary production during development. Whether the clock plays a role in the initial establishment of the segmental pattern remains to be demonstrated.

No other examples of oscillations involving such a period range have been reported in developmental systems. Recently, however, oscillations of the mouse *hes1* gene expression, with a periodicity similar to that seen in segmentation, were triggered in vitro by applying a serum shock to various cultured mouse cell lines

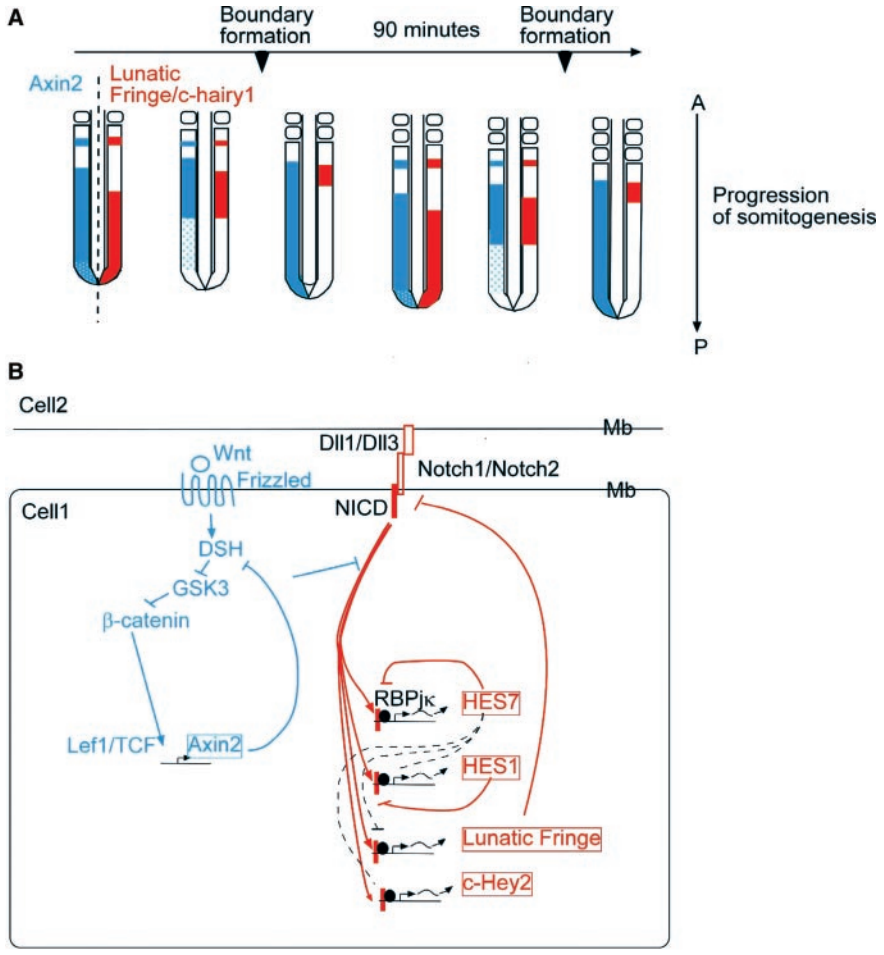

Fig. 1. (**A**) Expression sequence of *lunatic fringe/c-hairy1* (red) and *axin2* (blue) mRNAs during the formation of two somites. (**B**) Schematic representation of the segmentation clock oscillator in a PSM cell, integrating mouse and chick data. The Notch-based loop is shown in red; the Wnt-based loop is in blue. Cyclic genes are boxed. Mb, membrane.

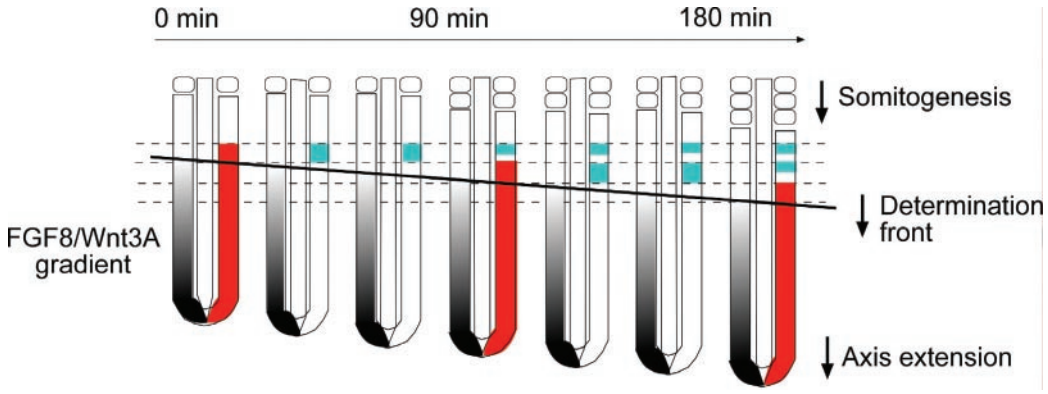

Fig. 2. Model for segment formation in vertebrates based on mouse and chick data. The FGF8/Wnt3A gradient, which regresses posteriorly during somitogenesis, is shown in black. The anterior boundary of the gradient defines the determination front, which corresponds to the position of the wavefront (thick black line). The phase I expression of Notch-related cyclic genes is shown in red (*26*). The expression of *Mesp2/c-meso1* is shown in blue.

(*19*). These oscillations appear to be mediated by a periodic repression of *hes1* on its own promoter. These observations were carried out in cells that are not related to the paraxial mesoderm, thus raising the possibility that the segmentation clock ticks in cells other than the PSM cells. This oscillatory behavior could reflect a more generic property of gene expression, perhaps related to transcription. The oscillations, which are relatively easy to see in segmentation, where they are well coordinated in all cells, might also take place in an uncoordinated manner in other cell types but would be undetectable given our current methods of analysis. However, thus far, no gene oscillations similar to those seen in PSM segmentation have been observed in other tissues.

FGF Signaling: Translation of the Clock Pulsation into Spatial Periodicity

Recent studies have shown that the secreted growth factor FGF8 (fibroblast growth factor 8) could be implicated in converting the clock pulsation into the periodic arrangement of segment boundaries (*23*, *24*). *fgf8* mRNA is strongly expressed in PSM precursors in the primitive streak and tail bud as well as in the posteriormost PSM, and its expression progressively decreases in more anterior cells, thus establishing a gradient over two-thirds of the PSM length (Fig. 2, black). Its expression domain correlates with the region of the PSM in which the segmental pattern is not yet irreversibly determined (*23*). Overexpression of FGF8 in PSM cells can maintain their posterior identity and block segmentation, suggesting that high concentrations of FGF8 are required to actively maintain newly formed posterior PSM cells in an immature state. It was proposed that, because of the progressive decrease of *fgf8* expression during maturation of the PSM, when cells become located in the anterior PSM, they reach a threshold of FGF signaling allowing them to activate their segmentation program. This threshold level, which was termed the "determination front" (Fig. 2), marks a transition in genetic regulation in PSM cells, as shown by the activation of new sets of genes such as *paraxis*, the down-regulation of posterior genes such as *Brachyury*, and the slowing down and stopping of the oscillations of the clock genes (*21*, *23*, *24*). Wnt3A was also recently proposed to assume a role similar to that of FGF8 by establishing a gradient-controlling segmentation in the PSM (*20*). However, because Wnt3A acts upstream of *fgf8* in the PSM, it could act together with or by way of the *fgf8* gradient.

At the determination-front level, gene coding for the transcription factor *Mesp2/c-meso1* becomes periodically activated in a one-somite-wide domain, providing the earliest evidence for segmentation in the PSM (Fig. 2, blue) (*21*). The transcription factors of the Mesp family were shown to act upstream of a genetic cascade involving the Notch pathway, which ultimately results in boundary positioning and formation of anterior and posterior somitic compartments (*21*, *22*). Therefore, it is tempting to speculate that the periodic activation of genes of the Mesp family, which takes place at the determination-front level, is controlled by the segmentation clock. This would provide a link between the segmentation clock, the determination front, and the boundary-formation process.

As a result of the constant posterior elongation of the body axis during early development, the *fgf8* gradient and *wnt3a* expression are continuously displaced posteriorly, and thus the absolute position of the determination front constantly recedes (Fig. 2). This movement of the determination front ensures that boundaries will be separated by a distance corresponding to the posterior displacement of the determination front during one period of the oscillation. Accordingly, altering the dynamics of the *fgf8* gradient affects the position of somite boundaries (*23*, *24*). Therefore, the determination front corresponds to the wavefront of the clock and wavefront model. In the model, when cells of the PSM reach the wavefront level, the phase of their oscillation is definitively imprinted on these cells, and the oscillation stops (*3*).

These coordinated processes will result in the production of a series of repeated somites, which subsequently differentiate into vertebrae exhibiting different morphologies depending on their position along the anteroposterior axis. The developmental program defining the particular shape of the vertebrae is controlled by the *Hox* genes in the somites. Regulation of the *Hox* genes is discussed in the review by Kmita and Duboule in this issue (*25*). The segmentation clock also controls aspects of spatiotemporal *Hox* gene activation, thus ensuring a perfect match between segment boundary position and future regional identity of the somites (*23*).

Although a number of components of the segmentation clock have been identified, their detailed interactions resulting in the generation of the oscillations of the cyclic genes remain to be elucidated. Also, investigation into the existence of such a mechanism in invertebrate species that exhibit a progressive mode of segmentation, such as annelid worms, should tell us whether this oscillator was already operating in the ancestor of vertebrates and invertebrates.

References and Notes

1. I. Palmeirim, D. Henrique, D. Ish-Horowicz, O. Pourquié, *Cell* **91**, 639 (1997).
2. C. Jouve, T. Iimura, O. Pourquié, *Development* **129**, 1107 (2002).
3. J. Cooke, E. C. Zeeman, *J. Theor. Biol.* **58**, 455 (1976).
4. Y. Li, U. Fenger, C. Niehrs, N. Pollet, *Differentiation* **71**, 83 (2003).
5. M. J. McGrew, J. K. Dale, S. Fraboulet, O. Pourquié, *Curr. Biol.* **8**, 979 (1998).
6. H. Forsberg *et al.*, *Curr. Biol.* **8**, 1027 (1998).
7. C. Jouve *et al.*, *Development* **127**, 1421 (2000).
8. Y. Bessho *et al.*, *Genes Dev.* **15**, 2642 (2001).
9. Y. J. Jiang *et al.*, *Nature* **408**, 475 (2000).
10. A. Sawada *et al.*, *Development* **127**, 1691 (2000).
11. S. A. Holley *et al.*, *Genes Dev.* **14**, 1678 (2000).
12. C. Leimeister *et al.*, *Dev. Biol.* **227**, 91 (2000).
13. S. E. Cole *et al.*, *Dev. Cell* **3**, 75 (2002).
14. A. V. Morales *et al.*, *Dev. Cell* **3**, 63 (2002).
15. A. C. Oates, R. K. Ho, *Development* **129**, 2929 (2002).
16. S. A. Holley, D. Julich, G. J. Rauch, R. Geisler, C. Nusslein-Volhard, *Development* **129**, 1175 (2002).
17. J. K. Dale *et al.*, *Nature* **421**, 275 (2003).
18. K. Serth, K. Schuster-Gossler, R. Cordes, A. Gossler, *Genes Dev.* **17**, 912 (2003).
19. H. Hirata *et al.*, *Science* **298**, 840 (2002).
20. A. Aulehla *et al.*, *Dev. Cell* **4**, 395 (2003).
21. Y. Takahashi *et al.*, *Nature Genet.* **25**, 390 (2000).
22. W. C. Jen, V. Gawantka, N. Pollet, C. Niehrs, C. Kintner, *Genes Dev.* **13**, 1486 (1999).
23. J. Dubrulle *et al.*, *Cell* **106**, 219 (2001).
24. A. Sawada *et al.*, *Development* **128**, 4873 (2001).
25. M. Kmita, D. Duboule, *Science* **301**, 331 (2003).
26. O. Pourquié, P. P. Tam, *Dev. Cell* **1**, 619 (2001).
27. The author thanks members of the Pourquié lab and P. Baumann, K. Dale, D. Duboule, J. Dubrulle, A. Gossler, B. Hermann, D. Ish-Horowicz, R. Kageyama, and P. Kulesa for helpful comments and discussions and for communicating data before their publication.