Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Cell Biology

Oscillating signaling pathways during embryonic development

Alexander Aulehla[1] and Olivier Pourquié[1,2]

Oscillatory signaling pathway activity during embryonic development was first identified in the process of vertebrate somite formation. In mouse, this process is thought to be largely controlled by a cyclic signaling network involving the Notch, FGF, and Wnt pathways. Surprisingly, several recent genetic studies reveal that the core oscillation pacemaker is unlikely to involve periodic activation by these pathways. The mechanism(s) responsible for the production of oscillatory gene activity during somite formation remains, therefore, to be discovered. Oscillatory signaling activity has recently been identified in developmental processes distinct from somite formation. Both the processes of limb development in chick embryos and the maintenance of neural progenitors in mouse embryos involve oscillatory gene activity related to the Notch pathway. These discoveries indicate that oscillatory signaling activities during embryonic development might serve a more general function than previously thought.

Addresses
[1] Stowers Institute for Medical Research, 1000 E. 50th Street, Kansas City, MO 64110, USA
[2] Howard Hughes Medical Institute, 1000 E. 50th Street, Kansas City, MO 64110, USA

Corresponding author: Aulehla, Alexander (ala@stowers-institute.org) and Pourquié, Olivier (olp@stowers-institute.org)

Current Opinion in Cell Biology 2008, **20**:632–637

This review comes from a themed issue on
Cell differentiation
Edited by Vann Bennett

Available online 23rd October 2008

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2008.09.002

Introduction

Embryonic development is intrinsically dynamic. After fertilization, the embryo relentlessly changes its shape and new patterns emerge constantly, while countless developmental programs are activated simultaneously. It is breathtaking to observe the precision and beauty of development and to consider the underlying complexity of signals that regulate this process. This complexity is not only derived from the large number of involved gene products and their numerous and multifaceted interactions, but also from their precise spatial and temporal control during embryogenesis. In this review, we will discuss the recent findings that address the phenomenon of oscillating gene regulation in three distinct developmental processes: somitogenesis, limb development, and neural progenitor maintenance.

Oscillating gene regulation during somitogenesis

Somitogenesis — the periodic formation of the vertebrae precursors — is a striking example of a dynamic embryonic process that relies on precise spatial and temporal control of gene expression. In 1997, the oscillatory expression of *c-hairy 1*, a homolog of the drosophila pair-rule gene *hairy*, was identified in the paraxial presomitic mesoderm (PSM) of chick embryos [1]. Strikingly, the period of oscillatory gene expression matches the period of somite formation. Therefore, these transcriptional oscillations were proposed to reveal the activity of an underlying clock, called the segmentation clock. While the role of the segmentation clock during the process of somitogenesis has not as yet been conclusively established, it is believed that the clock generates a rhythmic signal which controls the periodic production of somites from the PSM. Since this initial discovery, it has been shown that target genes of the Notch-signaling and Wnt-signaling pathways undergo oscillatory expression in the PSM of mouse embryos [2]. In addition, periodic fibroblast growth factor (Fgf) signaling has been recently observed in the PSM [3•,4••,5••]. Thus, several interconnected signaling pathways appear to constitute a complex, oscillating signaling network involved in somitogenesis [6]. The scale of this network was analyzed in a genome-wide search for cyclic genes in the PSM of mouse embryos [4••]. Approximately 50 genes, whose transcripts show an oscillatory expression in the PSM with a periodicity that correlates with somite production, have been identified through this approach. With the identification of more oscillating players and pathways, the current challenge is to understand how these multiple pathways interact. A key question in this field, however, is to understand how oscillations are generated in the first place, or in other words, what mechanism underlies the core oscillator? On the basis of experimental [4••,5••,7–9] and theoretical evidence [10,11], three distinct circuits have been proposed to act as the clock pacemaker in mouse: first, Notch/hairy and enhancer of split 7 (Hes7)/Lunatic fringe (Lfng), second, Wnt/β-catenin, and third, Fgf/dual specificity phosphatase [Dusp]. Here, we will discuss the recent results which challenge the role of these three circuits within the core oscillator.

Notch signaling does not appear to be part of the core oscillator mechanism

Strikingly, all the oscillating pathways identified thus far — Notch, Wnt, and Fgf — generate various negative

feedback loops that, in theory, could generate oscillations of their activity. In fact, several downstream targets with oscillatory expression are known inhibitors of the pathways that induce their expression. Such simple feedback mechanisms can generate oscillations, provided the inclusion of a delay in the process [10], and for the Notch pathway, experimental evidence exists that supports this proposal [12–14]. For instance, in mouse embryos, the cyclic Notch target gene *Hes7* acts as a repressor of its own transcription [15]. Its inactivation in mutant mice results in an arrest of *Lfng* oscillations (which act downstream of Notch signaling) and in an upregulation of the *Hes7* promoter because of the lack of the *Hes7* repressive activity [8]. Furthermore, stabilization of Hes7 protein *in vivo* disrupts Notch-signaling oscillations as predicted by a mathematical model based on delayed feedback [16]. However, in the absence of RBP-Jk, which results in blocking Notch signaling, *Hes7* oscillations are still observed in the posterior PSM [5••]. Thus, while a *Hes7*-based feedback mechanism is in place in the PSM, Notch signaling does not appear to be required to activate this circuit. Furthermore, the Hes7-feedback mechanism is not essential to generate oscillations of Wnt signaling. Thus, mice mutant for *Hes7* show clear evidence of ongoing *Axin2* oscillations, a *bona fide* cyclic Wnt target in the PSM [16]. In conclusion, it appears that the Hes7-dependent feedback loop does not act as the pacemaker of the core oscillator in mouse embryos.

A second Notch-based mechanism involving a negative feedback loop based on the glycosyl-transferase Lfng has been proposed based on the gain-of-function experiments in chick embryo [7]. In mouse and chick embryos, *Lfng* is a downstream target of the Notch pathway and its overexpression in the chick embryo by *in ovo* electroporation results in an inhibition of Notch signaling. This led to the suggestion that Lfng is involved in a negative feedback loop controlling Notch periodic activation in the PSM. These data were further supported by genetic evidence which indicates that Notch1 intracellular domain (NICD) expression loses its oscillatory pattern and becomes steadily expressed throughout the PSM when *Lfng* is mutated in mice [17], a condition that was previously shown to cause defects in somite formation [18,19]. Additionally, by selectively deleting an enhancer domain shown to control *Lfng* oscillatory expression [20,21], Shifley *et al.* generated mutant mouse embryos in which only the oscillatory domain of *Lfng* expression was absent, while the anterior stripes remained unaffected [22•]. As observed in the *Lfng*-null embryos [17], this mutation abolished NICD oscillations in the PSM [22•], supporting the existence of a negative feedback mechanism in the PSM. However, this selective deletion of only the oscillatory expression resulted in a very mild defect in somite formation compared to the null mutation [18,19] and was limited to the cervical and thoracic axial regions [22•]. In addition, both the loss of *Lfng* [5••], and the overexpression of *Lfng* in transgenic mice using a PSM-specific promoter fail to inhibit *Hes7* cyclic transcription [23]. Together, these results indicate that the Lfng-based negative feedback loop is not required to drive segmentation clock oscillations.

A recent report reveals that in mouse embryos, even the constitutive activation of Notch signaling does not prevent Wnt-signaling oscillations [24••]. In these experiments, NICD, which mediates Notch-signaling activity [25] and which shows oscillatory protein levels in the PSM of wild-type embryos [17,26], was constitutively expressed under the control of a PSM-specific promoter in transgenic mice. This led to a steady expression of the Notch targets *Lfng* and *Hes7*, but under these conditions the Wnt target *Axin2* still showed dynamic expression [24••].

Together, these data argue that Notch signaling is not part of the segmentation clock pacemaker. This view is also supported by recent data obtained in zebrafish embryos, which argue against a role for Notch signaling in the generation of the segmentation clock oscillations [27–29]. These data indicate that a key function of Notch signaling is to synchronize oscillations among PSM cells (reviewed in [30]), as suggested previously [31].

A β-catenin-based feedback circuit is not essential for oscillator activity in the PSM

Wnt-signaling pathway oscillations are maintained in Notch mouse mutants indicating that they are not dependent on Notch signaling. Furthermore, Wnt signaling has been shown to act upstream of Notch oscillations [9,32,33] since, for instance, both Notch and Wnt oscillations are disrupted in the Wnt3a mutant *vestigial tail* (*vt*) [9]. What role does Wnt signaling play in the core oscillator? As proposed for the Notch-signaling pathway, it has been hypothesized that a negative feedback mechanism underlies the oscillatory output of the Wnt pathway [9,11]. Several Wnt inhibitors with oscillating expression have been identified in the PSM. Some of these, including Axin2, dickkopf homolog 1 (Dkk1), or dapper homolog 1 (Dact1), function upstream of β-catenin, the key mediator of canonical Wnt signaling [34]. Thus, the cyclic expression of these inhibitors should result in the periodic destabilization of β-catenin. However, no evidence of such oscillations in β-catenin protein levels in the PSM could be obtained. In contrast, β-catenin is expressed as a clear posterior-to-anterior protein gradient in the PSM during all phases of the segmentation clock cycle [35••]. A dynamic output of Wnt signaling is still observed even when a stabilized form of β-catenin is conditionally expressed in the PSM [35••]. Most strikingly, under these experimental conditions, ongoing oscillations of the Notch pathway are clearly observed [35••,36••]. Real-time imaging experiments using a fluorescent reporter system demonstrate the occurrence of

transcriptional oscillations controlled by the *Lfng* promoter in mutant mouse embryos with constitutive expression of β-catenin [35••]. Therefore, this argues against a role of a β-catenin-based negative feedback loop in the pacemaker of the segmentation clock.

A Fgf-based feedback mechanism is unlikely to be the core oscillator mechanism

Finally, what is the status of knowledge about Fgf signaling in this context? The observation that several Fgf inhibitors, such as Sprouty2 or Dusp6 and Dusp4, are expressed in an oscillatory fashion in phase with Notch cyclic genes led to the suggestion that FGF signaling is periodically regulated in the PSM. In addition, FGF signaling has been shown to be required in order for segmentation clock activity to occur, since all oscillatory activities of Fgf, Wnt, and Notch pathways cease when Fgf signaling is abolished in the PSM [5••,37••]. In this respect, FGF behaves similarly to Wnt signaling, which has also been shown to be absolutely required for segmentation clock activity [9,32,36••]. Genetic evidence in mice, however, suggests that the absence of Fgf signaling can be compensated by elevated canonical Wnt signaling. Accordingly, while in mice mutant for Fgfr1 oscillations of *Lfng* are absent [5••,37••], dynamic expression of *Lfng* appears to be restored if stabilized β-catenin is overexpressed in Fgfr1 mutants [35••].

A feedback mechanism involving the Dusp4 or Dusp6 inhibitors of extracellular-regulated kinase (ERK) activity [38,39] has been proposed to generate FGF-signaling oscillations in the PSM [4••,5••]. In *Hes7* mutants, the constitutive expression of Dusp4 is observed [5••]; yet, Wnt signaling still oscillates in these mutants [16], indicating that a Dusp4-based feedback mechanism is unlikely to be part of the core oscillator, as well. Recent cell culture experiments have led to propose yet another potential mechanism for the generation of periodic FGF signaling, involving negative feedback via ERK-dependent son of sevenless homolog 1 (SOS1) inactivation [40]. It will be important to evaluate the physiological role of this feedback *in vivo*.

In summary, the results of these genetic experiments indicate that Notch, Wnt/β-catenin, or Fgf negative feedback mechanisms are unlikely to constitute the core pacemaker of the segmentation clock. Whether the identified network of cyclic genes is actually entrained by a yet unknown pacemaker remains to be investigated.

Oscillatory gene activity during development is not restricted to the paraxial mesoderm

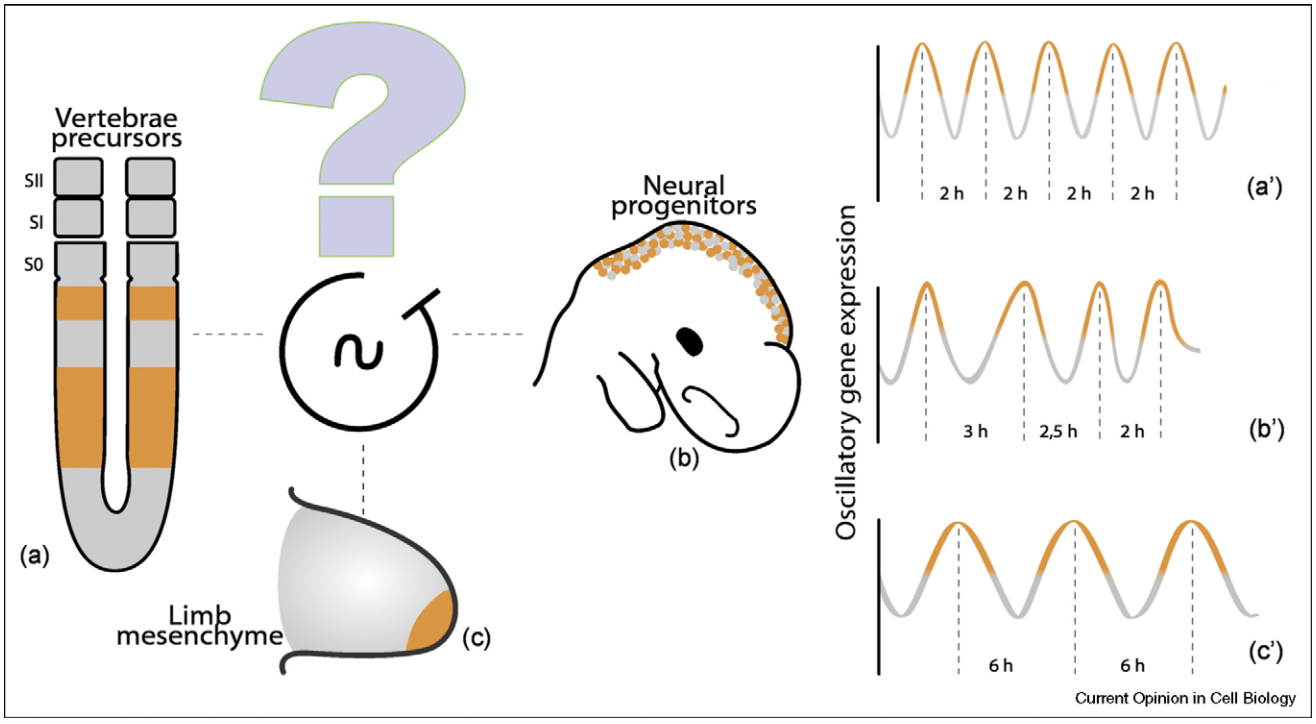
Autopod limb outgrowth

Clear oscillatory gene expression during development was first identified within the PSM and associated with the process of embryonic segmentation. Other segmented structures are found in the embryo, but whether similar oscillatory processes are involved in their patterning has been only recently addressed. For example, the vertebrate limb has often been considered a segmented structure along the proximo-distal axis because it is composed of repeated elements including the stylopod, the zeugopod, and periodic elements in the autopod (e.g. the phalanxes). In chick embryos, Pascoal *et al.* have identified the periodic expression of *hairy 2* during limb outgrowth [41••]. The period of these transcriptional oscillations has been determined to be 6 hours, while a new autopod limb element is formed every 12 hours. Accordingly, the authors postulate that a new limb element forms every two oscillations [41••]. Thus, as in somitogenesis, the observed oscillations relate to the production of limb elements, although the correlation between the formation of one segment and an oscillation cycle is not as clear as that observed during somitogenesis. The role of this limb clock in the periodic production of limb elements remains to be established.

Hes1 oscillations in neural progenitors in the mouse brain

The observation of oscillations of the Hairy and enhancer of split homolog 1 (*Hes1*) in cultured fibroblasts [42] subjected to a serum shock led to the realization that such transcriptional oscillations might occur more frequently *in vivo* than initially thought. A recent report which reveals oscillatory mRNA and protein expression of *Hes1* in neural progenitor cells located in the telencephalon of mouse embryos further argues in this direction [43••]. In contrast to oscillations during somitogenesis and limb outgrowth, oscillations of *Hes1* in neural progenitors are not synchronized between cells and show variable periods (Figure 1). Thus, these oscillations remained undetected by classical methods (e.g. *in situ* hybridization). Shimojo and colleagues discovered these oscillations with the help of real-time imaging technology, allowing them to track Hes1 promoter activity using a bioluminescent approach in transgenic embryos [43,44•]. Interestingly, while *Hes1* expression is required to maintain the neural progenitor population and prevent differentiation, the presence of Hes1 also inhibits progression through the cell cycle. Thus, the authors propose that the function of *Hes1* oscillatory expression is to provide enough *Hes1* to prevent neuronal differentiation, while simultaneously, to periodically downregulate *Hes1* in cells that are in the G1-phase, hereby allowing these cells to progress through the cell cycle. The authors demonstrate that *Hes1* expression depends on the Jak-Stat pathway, which, in turn, has been proposed previously to be involved in neural progenitor maintenance [45]. Interestingly, the Jak-Stat pathway has been shown *in vitro* to activate *Hes1* oscillations in cultured fibroblasts involving a feedback mechanism [46]. Again, it will be exciting to test *in vivo* the significance of this feedback.

Figure 1



Schematic representation of oscillatory gene expression during embryonic development. Currently, the processes shown to involve oscillatory expression include somite formation **(a**, **a′)**, neural progenitor maintenance in mouse embryo brain **(b**, **b′)**, and limb development **(c**, **c′)**. The core oscillator(s) which generates cyclic gene activity has yet to be identified. Oscillations in gene activity are regular and are synchronized between neighboring cells during somite formation (a′, period approximately two hours) and limb development (c′, period six hours). In contrast, oscillations in neural progenitors in the mouse embryo brain are not synchronized between neighboring cells and show varying periodicities (b′, average period approximately three hours).

Conclusion

Ten years after the discovery of oscillatory gene activity during somitogenesis, the underlying core oscillator has yet to be identified. In addition, a future challenge is to comprehensively characterize the network of oscillating signaling pathways and to precisely characterize the role of this network during somite formation. The discovery of oscillatory gene activity during several developmental processes — somitogenesis, limb outgrowth, and neural progenitor maintenance — suggests that oscillatory gene expression might be more widespread during embryonic development than initially thought. Since this oscillatory activity does not necessarily need to be synchronized within a cell population, it is a challenge to identify these phenomena using traditional visualization techniques. Thus, the technical advance in revealing gene activity in real-time will be instrumental in approaching these dynamic phenomena during embryonic development.

Acknowledgements

The authors thank Silvia Esteban for artwork and Joanne Chatfield for editorial assistance. AA was funded by the Swiss foundation for medical–biological grants/Swiss National Science Foundation. This research was supported by Stowers Institute for Medical Research. OP is a Howard Hughes Medical Institute Investigator.

References and recommended reading

Papers of particular interest published within the period of review have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Palmeirim I, Henrique D, Ish-Horowicz D, Pourquié O: **Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis**. *Cell* 1997, **91**:639-648.

2. Aulehla A, Herrmann BG: **Segmentation in vertebrates: clock and gradient finally joined**. *Genes Dev* 2004, **18**:2060-2067.

3. • Dale JK, Malapert P, Chal J, Vilhais-Neto G, Maroto M, Johnson T, Jayasinghe S, Trainor P, Herrmann B, Pourquie O: **Oscillations of the snail genes in the presomitic mesoderm coordinate segmental patterning and morphogenesis in vertebrate somitogenesis**. *Dev Cell* 2006, **10**:355-366.
The identification of oscillatory expression of snail genes in the PSM of chick and mouse embryos. This oscillatory transcription is independent of Notch signaling, but requires Fgf and Wnt signaling.

4. •• Dequeant ML, Glynn E, Gaudenz K, Wahl M, Chen J, Mushegian A, Pourquie O: **A complex oscillating network of signaling genes underlies the mouse segmentation clock**. *Science* 2006, **314**:1595-1598.
This microarray study represents the first genome-wide search for cyclic genes during somite formation and identified a large number of oscillating genes in three clusters: Wnt, Notch, and Fgf signaling.

5. •• Niwa Y, Masamizu Y, Liu T, Nakayama R, Deng CX, Kageyama R: **The initiation and propagation of Hes7 oscillation are**

cooperatively regulated by Fgf and notch signaling in the somite segmentation clock. *Dev Cell* 2007, **13**:298-304.
Report of oscillations in Fgf/Erk activity, which are proposed to be mediated via a Dusp4-dependent feedback mechanism. In addition, this study provides evidence that Fgf signaling activates Hes7 in the posterior PSM.

6. Dequeant ML, Pourquie O: **Segmental patterning of the vertebrate embryonic axis**. *Nat Rev Genet* 2008, **9**:370-382.

7. Dale JK, Maroto M, Dequeant ML, Malapert P, McGrew M, Pourquie O: **Periodic Notch inhibition by Lunatic Fringe underlies the chick segmentation clock**. *Nature* 2003, **421**:275-278.

8. Bessho Y, Hirata H, Masamizu Y, Kageyama R: **Periodic repression by the bHLH factor Hes7 is an essential mechanism for the somite segmentation clock**. *Genes Dev* 2003, **17**:1451-1456.

9. Aulehla A, Wehrle C, Brand-Saberi B, Kemler R, Gossler A, Kanzler B, Herrmann BG: **Wnt3a plays a major role in the segmentation clock controlling somitogenesis**. *Dev Cell* 2003, **4**:395-406.

10. Lewis J: **Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator**. *Curr Biol* 2003, **3**:1398-1408.

11. Goldbeter A, Pourquie O: **Modeling the segmentation clock as a network of coupled oscillations in the Notch-, Wnt- and FGF-signaling pathways**. *J Theor Biol* 2008, **252**:574-585.

12. Holley SA, Julich D, Rauch GJ, Geisler R, Nusslein-Volhard C: **her1 and the notch pathway function within the oscillator mechanism that regulates zebrafish somitogenesis**. *Development* 2002, **129**:1175-1183.

13. Oates AC, Ho RK: **Hairy/E(spl)-related (Her) genes are central components of the segmentation oscillator and display redundancy with the Delta/Notch signaling pathway in the formation of anterior segmental boundaries in the zebrafish**. *Development* 2002, **129**:2929-2946.

14. Henry CA, Urban MK, Dill KK, Merlie JP, Page MF, Kimmel CB, Amacher SL: **Two linked hairy/enhancer of split-related zebrafish genes, her1 and her7, function together to refine alternating somite boundaries**. *Development* 2002, **129**:3693-3704.

15. Bessho Y, Miyoshi G, Sakata R, Kageyama R: **Hes7: a bHLH-type repressor gene regulated by Notch and expressed in the presomitic mesoderm**. *Genes Cells* 2001, **6**:175-185.

16. Hirata H, Bessho Y, Kokubu H, Masamizu Y, Yamada S, Lewis J, Kageyama R: **Instability of Hes7 protein is crucial for the somite segmentation clock**. *Nat Genet* 2004, **36**:750-754.

17. Morimoto M, Takahashi Y, Endo M, Saga Y: **The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity**. *Nature* 2005, **435**:354-359.

18. Evrard YA, Lun Y, Aulehla A, Gan L, Johnson RL: **lunatic fringe is an essential mediator of somite segmentation and patterning**. *Nature* 1998, **394**:377-381.

19. Zhang N, Gridley T: **Defects in somite formation in lunatic fringe-deficient mice**. *Nature* 1998, **394**:374-377.

20. Cole SE, Levorse JM, Tilghman SM, Vogt TF: **Clock regulatory elements control cyclic expression of lunatic fringe during somitogenesis**. *Dev Cell* 2002, **3**:75-84.

21. Morales AV, Yasuda Y, Ish-Horowicz D: **Periodic lunatic fringe expression is controlled during segmentation by a cyclic transcriptional enhancer responsive to Notch signaling**. *Dev Cell* 2002, **3**:63-74.

22. • Shifley ET, Vanhorn KM, Perez-Balaguer A, Franklin JD, Weinstein M, Cole SE: **Oscillatory lunatic fringe activity is crucial for segmentation of the anterior but not posterior skeleton**. *Development* 2008, **135**:899-908.
The selective deletion of the clock enhancer element for oscillatory Lfng expression in mouse embryos is reported. The phenotype differs substantially from the conventional deletion of Lfng (reported in [18,19]).

23. Serth K, Schuster-Gossler K, Cordes R, Gossler A: **Transcriptional oscillation of Lunatic fringe is essential for somitogenesis**. *Genes Dev* 2003, **17**:912-925.

24. •• Feller J, Schneider A, Schuster-Gossler K, Gossler A: **Noncyclic Notch activity in the presomitic mesoderm demonstrates uncoupling of somite compartmentalization and boundary formation**. *Genes Dev* 2008, **22**:2166-2171.
This study reports the effect of constitutive expression of Nicd, and thus Notch activity, in respect to somite formation. Although Notch targets are expressed in a noncyclic, constant fashion, somite borders form, and Wnt-signaling oscillations are ongoing.

25. Mumm JS, Kopan R: **Notch signaling: from the outside in**. *Dev Biol* 2000, **228**:151-165.

26. Huppert SS, Ilagan MX, De Strooper B, Kopan R: **Analysis of Notch function in presomitic mesoderm suggests a gamma-secretase-independent role for presenilins in somite differentiation**. *Dev Cell* 2005, **8**:677-688.

27. Ozbudak EM, Lewis J: **Notch signalling synchronizes the zebrafish segmentation clock but is not needed to create somite boundaries**. *PLoS Genet* 2008, **4**:e15.

28. Riedel-Kruse IH, Muller C, Oates AC: **Synchrony dynamics during initiation, failure, and rescue of the segmentation clock**. *Science* 2007, **317**:1911-1915.

29. Mara A, Schroeder J, Chalouni C, Holley SA: **Priming, initiation and synchronization of the segmentation clock by deltaD and deltaC**. *Nat Cell Biol* 2007, **9**:523-530.

30. Ozbudak EM, Pourquie O: **The vertebrate segmentation clock: the tip of the iceberg**. *Curr Opin Genet Dev* 2008. Aug 14 [Epub ahead of print].

31. Jiang YJ, Aerne BL, Smithers L, Haddon C, Ish-Horowicz D, Lewis J: **Notch signalling and the synchronization of the somite segmentation clock**. *Nature* 2000, **408**:475-479.

32. Nakaya MA, Biris K, Tsukiyama T, Jaime S, Rawls JA, Yamaguchi TP: **Wnt3alinks left–right determination with segmentation and anteroposterior axis elongation**. *Development* 2005, **132**:5425-5436.

33. Satoh W, Gotoh T, Tsunematsu Y, Aizawa S, Shimono A: **Sfrp1 and Sfrp2 regulate anteroposterior axis elongation and somite segmentation during mouse embryogenesis**. *Development* 2006, **133**:989-999.

34. Clevers H: **Wnt/beta-catenin signaling in development and disease**. *Cell* 2006, **127**:469-480.

35. •• Aulehla A, Wiegraebe W, Baubet V, Wahl MB, Deng C, Taketo M, Lewandoski M, Pourquie O: **A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation**. *Nat Cell Biol* 2008, **10**:186-193.
Demonstration of a β-catenin protein gradient in the PSM of mouse embryos, which is present irrespective of the oscillation cycle. While this gradient critically controls PSM maturation, real-time imaging experiments in mutant embryos expressing a stabilized form of β-catenin protein the PSM demonstrate ongoing oscillations of Notch signaling.

36. •• Dunty WC Jr, Biris KK, Chalamalasetty RB, Taketo MM, Lewandoski M, Yamaguchi TP: **Wnt3a/beta-catenin signaling controls posterior body development by coordinating mesoderm formation and segmentation**. *Development* 2008, **135**:85-94.
Conditional gain-of-function and loss-of-function experiments with β-catenin demonstrate its critical role in mesoderm development and somite formation. The stable expression of β-catenin does not abolish Notch-signaling oscillations.

37. •• Wahl MB, Deng C, Lewandoski M, Pourquie O: **FGF signaling acts upstream of the NOTCH and WNT s ignaling pathways to control segmentation clock oscillations in mouse somitogenesis**. *Development* 2007, **134**:4033-4041.
Conditional deletion of Fgfr1 in mouse embryos demonstrates the requirement for Fgf signaling for PSM maturation and for ongoing oscillations of Fgf, Notch, and Wnt signaling.

38. Chu Y, Solski PA, Khosravi-Far R, Der CJ, Kelly K: **The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation**. *J Biol Chem* 1996, **271**:6497-6501.

39. Li C, Scott DA, Hatch E, Tian X, Mansour SL: **Dusp6 (Mkp3) is a negative feedback regulator of FGF-stimulated ERK signaling during mouse development**. *Development* 2007, **134**:167-176.

40. Nakayama K, Satoh T, Igari A, Kageyama R, Nishida E: **FGF induces oscillations of Hes1 expression and Ras/ERK activation**. *Curr Biol* 2008, **18**:R332-R334.

41. •• Pascoal S, Carvalho CR, Rodriguez-Leon J, Delfini MC, Duprez D, Thorsteinsdottir S, Palmeirim I: **A molecular clock operates during chick autopod proximal–distal outgrowth**. *J Mol Biol* 2007, **368**:303-309.
First demonstration that oscillations of *hairy2* mRNA are present during limb development and patterning.

42. Hirata H, Yoshiura S, Ohtsuka T, Bessho Y, Harada T, Yoshikawa K, Kageyama R: **Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop**. *Science* 2002, **298**:840-843.

43. •• Shimojo H, Ohtsuka T, Kageyama R: **Oscillations in notch signaling regulate maintenance of neural progenitors**. *Neuron* 2008, **58**:52-64.
First discovery of oscillatory gene activity in neural progenitors using real-time imaging of Hes1 activity. Hes1 oscillations are controlled by Jak-Stat signaling and are suggested to allow the progression through the cell cycle, while simultaneously preventing the differentiation of neural progenitors.

44. • Masamizu Y, Ohtsuka T, Takashima Y, Nagahara H, Takenaka Y, Yoshikawa K, Okamura H, Kageyama R: **Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells**. *Proc Natl Acad Sci U S A* 2006, **103**:1313-1318.
First description of a real-time imaging technology, based on bioluminescence, that can monitor transcriptional oscillations in transgenic mouse embryos.

45. Kamakura S, Oishi K, Yoshimatsu T, Nakafuku M, Masuyama N, Gotoh Y: **Hes binding to STAT3 mediates crosstalk between Notch and JAK-STAT signalling**. *Nat Cell Biol* 2004, **6**:547-554.

46. Yoshiura S, Ohtsuka T, Takenaka Y, Nagahara H, Yoshikawa K, Kageyama R: **Ultradian oscillations of Stat, Smad, and Hes1 expression in response to serum**. *Proc Natl Acad Sci U S A* 2007, **104**:11292-11297.