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Segmentation Clock Networks in Vertebrate Somitogenesis*

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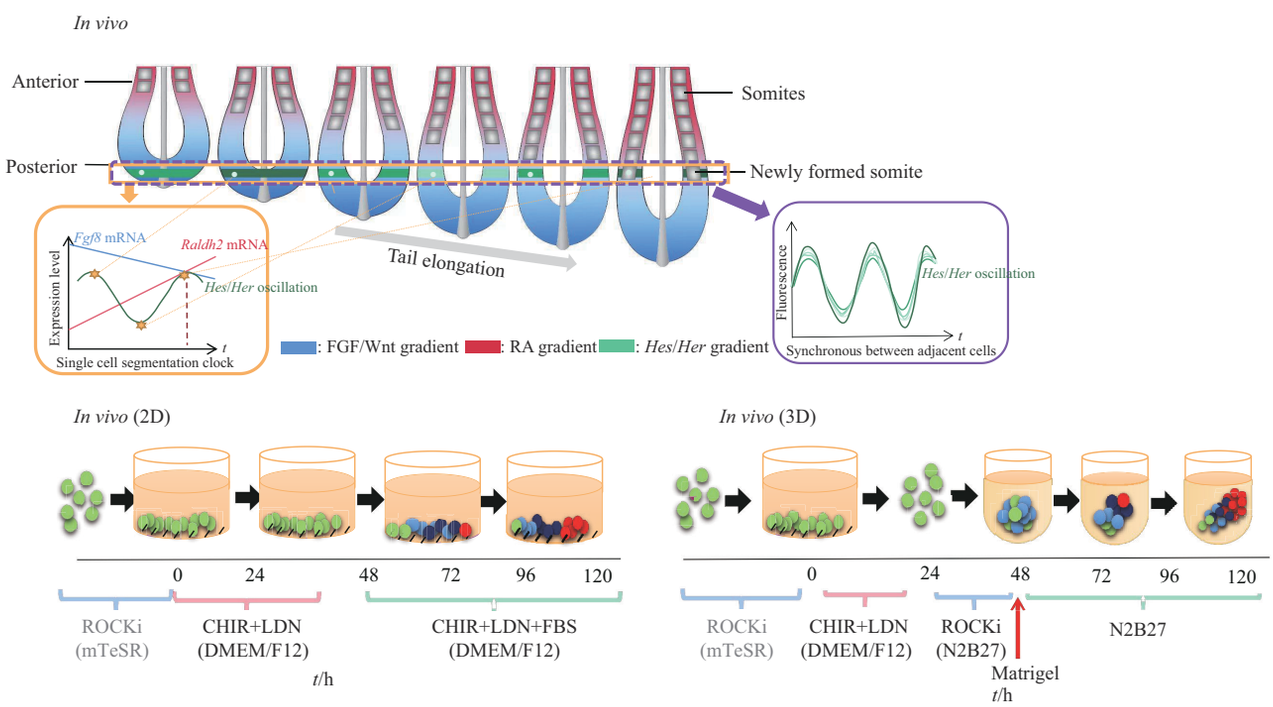
Graphical abstract



Abstract In vertebrate embryonic development, the segmentation clock controls the cyclic formation of somites through presomitic mesoderm (PSM) cells. Somites are paired segmented structures along the anterior-posterior axis that eventually develop into vertebrae and ribs. Disruptions in the segmentation clock leads to defects in somitogenesis, resulting in congenital spinal diseases. The major patterning modules that are involved in segmentation clock is the clock and wavefront, which primarily relies on

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signaling gradients and cyclic oscillation. Mesodermal differentiation is regulated by combinatorial gradient system that involves the activity of the fibroblast growth factor (FGF), the Wnt/β-catenin, and the retinoic acid (RA) signaling pathways. The antagonistic gradients of these signals set a position of the determination front. In the tail bud and posterior mesoderm, FGF and Wnt signaling prevent cell maturation and the molecular oscillators start to express. The molecular oscillators rely on negative feedback loops to maintain their oscillatory expression patterns. As the cells move anteriorly, FGF signaling gradually decays and RA signaling began to strengthen. Meanwhile, the molecular oscillators propagate anteriorly with wave pattern. At the determination front, low levels of FGF signaling and high levels of RA signaling eliminate differentiation inhibition and initiate molecular oscillators to activate cyclic genes, such as *Mesp*2, leading to the formation of repetitive structures in somites. Advancements in live reporter and 2D culture systems have revealed that coupling delays in cell communication can maintain the synchronous segmentation clock between adjacent cells. Studies have shown that these coupling delays are controlled by *Lfng* gene, it can adjust coupling delays to fit in-phase oscillations by increasing the time required for intercellular DLL1-Notch signaling. To sum up, the dual homeostasis of opposing signaling gradients determines the segment boundaries, the distance traveled by a molecular oscillator in one oscillation cycle determines the somite size, and the delayed coupling in intercellular signaling regulates the synchronization of clock oscillations. These three factors interact with each other to form a segmentation clock network coordinating somitogenesis. Recent studies have revealed that the intercellular coupling delay mechanism is a major factor influencing the maintenance of oscillation synchronization. Intercellular coupling delay errors, such as increased or decreased delay time, can desynchronizing intercellular oscillations and resulting in somite fusion. However, the mechanisms governing how intercellular communication becomes involved in oscillation synchronization remains unclear. Congenital scoliosis (CS) is a result of anomalous development of the vertebrate which associate with somitogenesis malformation. We observed that deficiency or overdose of vitamin A intake in gestation may lead to CS. While the deep mechanism of how RA signaling regulates oscillation synchronization still need to be detected. With the rapid development of 3D culture systems, researchers have successfully recapitulated the formation of somite-like structures with antero-posterior identity and indicated that the rate of metabolism is directly proportional to that of development. In summary, deconstructing the segmentation clock *in vitro* facilitates the dissection of regulation networks of the segmentation clock and offers an excellent proxy for studying the metabolic regulation of somitogenesis speed across species and the mechanisms underlying the formation of bilateral symmetry. It also creates a platform for exploring dysregulation mechanisms involved in the development of pathological somite defects.

Key words segmentation clock, somitogenesis, signal gradient, cyclic oscillation, synchronous oscillation
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In general, vertebrate embryonic development involves two types of cellular differentiation and arrangements: formation of functional organs with nonrepetitive structures, such as the heart and limbs, and formation of the external frame with repetitive structures, such as the spine and feathers. The spine is developed from somites, which is derived from presomitic mesoderm (PSM). Somites are epithelial cell clusters that are periodically and segmentally formed from the head posteriorly in the early stages of embryonic development. As the embryo develops, these somites gradually differentiate into bone-generating areas, skin-generating areas, and muscle-generating areas, subsequently forming tissues such as the bone, endothelium, dermis, muscle, and cartilage[1]. After a pair of somites are formed from the PSM, the cells in the adjacent posterior PSM begin to aggregate, forming the next pair of somites. Simultaneously, new cells enter the posterior PSM from the tail bud, while somites toward the anterior end differentiate to form the spinal structures. This development process is tightly regulated both temporally and spatially, ensuring that somites are generated at the correct locations and at regular time intervals (Figure 1). Disruption of somitogenesis leads to spinal fusion or asymmetric development, resulting in various congenital diseases. In this review, we discuss the precise network regulation mechanisms, known as the segmentation clock, that allow the somites to form orderly, symmetrical, and equal-length at exact positions, thereby leading to the development of a completely symmetrical spine.

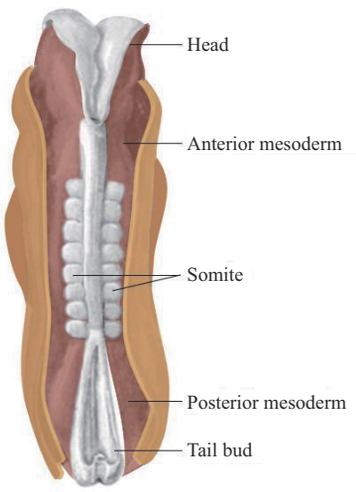

Fig. 1 Vertebrate somitogenesis

1 The clock and wavefront patterning module

1.1 Signal gradient regulation mechanism in mesoderm differentiation

PSM cells originate from the tail bud and begin differentiating when they move to the anterior PSM. This process is tightly regulated by several signaling gradient systems, including the fibroblast growth factor (FGF), Wnt/β-catenin (Wnt), and retinoic acid (RA) signaling pathways[2-6].

1.1.1 Gradually weakened FGF and Wnt signaling

FGF and Wnt signaling pathways establish gradually weakened signaling gradients in PSM through the mRNA decay mechanism. In 2004, Dubrulle and Pourquie[3] performed an *in situ* hybridization (ISH) experiment on 9.5-day-old mouse embryos, confirming that *Fgf8* mRNA was distributed in a gradient in the PSM. An immunofluorescence test also revealed that *Fgf8* proteins exhibit an identical concentration gradient (Figure 2). This was caused by continued transcription of *Fgf8* mRNA in the tail bud but cessation of transcription after the cell entered the PSM. As the cells moved anteriorly, *Fgf8* mRNA concentrations decreased, and the protein gradients reflected this change. In a similar experiment, Aulehla *et al.*[5] observed that Wnt3a, which is the ligand of the Wnt pathway, was generally expressed in the tail bud and posterior mesoderm cells. Similar to *Fgf8*, the ligand expression decreased as the cells moved anteriorly, resulting in a gradual decay of signal intensity. High FGF and Wnt signaling levels in posterior PSM keeps cells in an immature state by inhibiting the transcription of several segmentation genes. As the cells move anteriorly, FGF and Wnt signaling decays, resulting in cellular differentiation and somitogenesis [7-9].

1.1.2 Gradually strengthened retinoic acid （RA） signaling

RA signaling establishes opposite gradients to those of FGF in the PSM due to the local expression of RA metabolic enzymes. In 1991, Rossant *et al.*[10] expressed that the activity of RA can be detected in transgenic reporter mice that carry an RA signaling responsive element driving *LacZ* expression (*i. e.*, *RARE-LacZ*). They indicated that RA signals gradually increased from the tail to the anterior parts of the embryo. This was consistent with the expression of Raldh2, an enzyme supporting RA synthesis in the somites and anterior PSM, and that of Cyp26A1, an enzyme that degraded it, in the tail buds[11]. As the cells develop from the tail bud and move anteriorly, *Raldh2* accumulation gradually increases, which enhances the RA signals. In the anterior third of the PSM, RA levels are high enough to induce differentiation, leading to somitogenesis.

1.1.3 Role of antagonistic signaling gradients in the segmentation clock

The mutual inhibition FGF/Wnt and RA signaling jointly determines where the determination front occurs. In the posterior PSM, high FGF signaling restricts RA expression by activating *Cyp26* while inhibiting *Raldh2*. As cells move anteriorly, the gradually strengthening RA signals inhibit FGF signals by repressing *Fgf8* transcription or activating dual-specificity phosphatase MKP3[8, 12]. This mutually inhibiting signal coupling mechanism prevents cell maturation in the tail bud and posterior mesoderm *via* FGF and Wnt signaling. In the anterior third of the PSM, the inhibited FGF and Wnt signaling and gradually increasing RA signaling reach a balance, which initiates the segmentation clock and results in the transcription of several differentiation genes and, eventually, somite development[13-14]. When signaling gradients reach a balance where initially called the determination front, segmentation clock begins to induce cell differentiation[15-16]. During this process, abnormal increases in FGF signaling or abnormal decreases in RA signaling affect the initiation of segmentation, resulting in delays or absence of PSM cellular differentiation[8, 17-18] (Figure 3).

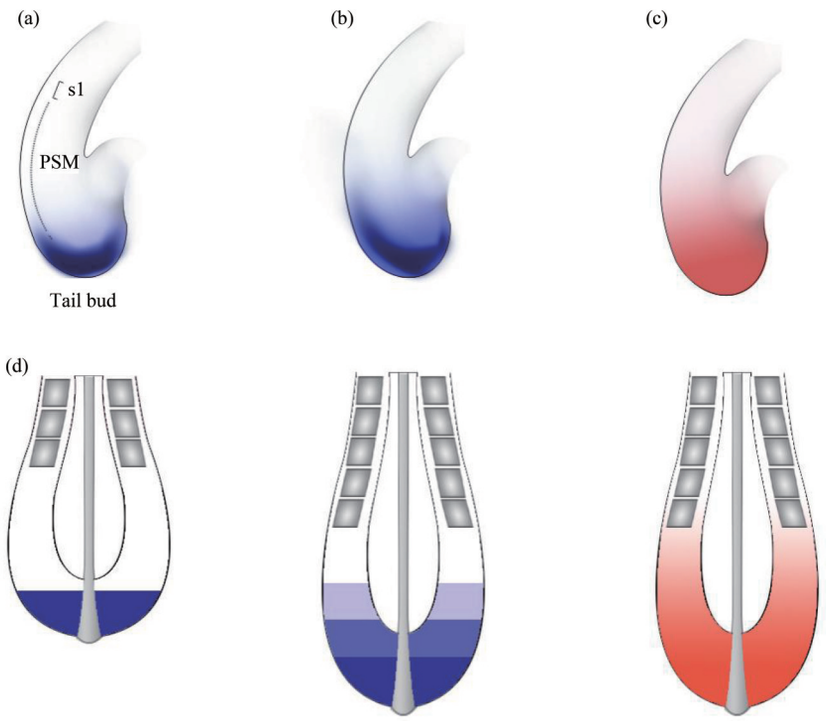

Fig. 2　An RNA decay mechanism establishes the *Fgf8* gradient within the PSM

(a) Gene expression pattern based on *in situ* hybridization (ISH) results. S1: last formed somite; PSM: presomitic mesoderm. (b) ISH for mature *Fgf8* mRNA shows a graded distribution within the PSM. (c) Immunoflfluorescent detection of *Fgf8* shows a posterior-anterior protein gradient. (d) Scheme of gradient formation using an RNA decay mechanism. In the temporal series (a−c), a constant group of cells (orange square) and its *Fgf8* RNA expression characteristics are shown. In (a), this group of cells is located in the posterior-most PSM and tail bud and hence shows *de novo Fgf8* transcription. For simplicity, only *de novo* synthesized RNA is shown in (a). At a later time point (b), the same group of cells is now located in the posterior one-third of PSM because of the posterior addition of cells during axis elongation. *Fgf8* transcription in the PSM ceased, and as a consequence, *Fgf8* mRNA decayed compared with the levels in the tail bud. At an even later time point (c), this group of cells is located in the middle PSM, and again *Fgf8* mRNA levels are further decreased. In consequence, *Fgf8* mRNA levels are graded in the PSM. This mRNA gradient is translated into a posterior-anterior *Fgf8* ligand gradient (d). In addition, (a−c) show a side view of the embryonic tail, (d) shows the corresponding dorsal view.

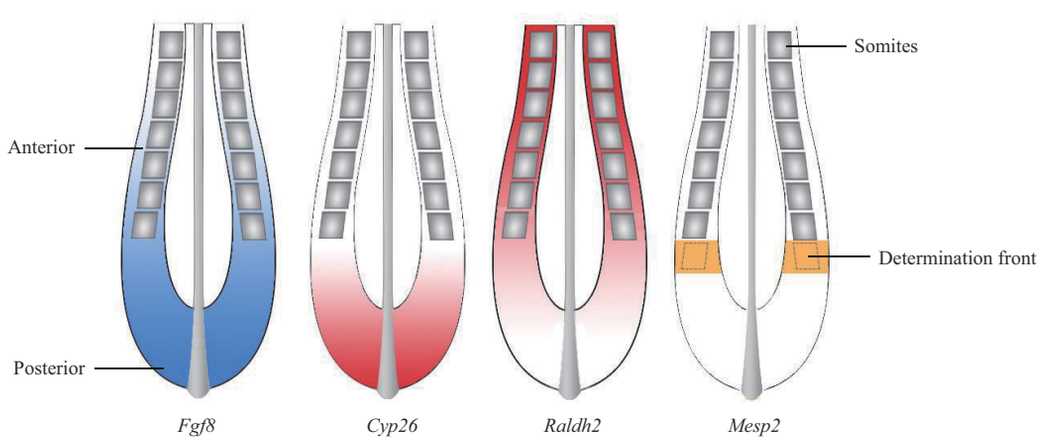

Fig. 3　Opposite gradients of FGF and RA signaling in the developing PSM

Schematic representation of the expression domains of F*gf8* and *Raldh2* and of their respective targets *Cyp26* and *Mesp2*. Gene expression domains are shown in gradient.

1.2 Oscillatory transcription mechanism of cyclic gene networks

The formation of repetitive structures in somites primarily relies on networks of cyclic genes in PSM, which exhibit oscillatory transcriptional activity[19]. After sequencing the PSM transcriptomes of mice, chicken, and zebrafish, Krol *et al*.[20-21] discovered a network with 40−100 cyclic genes, most of which were involved in Notch, Wnt and FGF signaling pathways. These cyclic genes were activated by the molecular oscillators of segmentation clock and influenced the timing of somite formation[22-23]. One of the typical molecular oscillators is the *Hes*/*Her* gene family acting in the downstream of Notch signaling pathways. *Hes*/*Her* is first expressed in the posterior PSM and then propagates anteriorly, slowing down and finally stopping at the expected somite locations. As the embryo grows posteriorly, the next oscillation is initiated, and the gene continues to propagate anteriorly in traveling waves.

Molecular oscillators rely on negative feedback loops to maintain their oscillatory expression patterns. *Hes*/*Her* family genes encode a bHLH transcription factor that can bind with their own promoter regions and repress expression[24]. In addition to self-inhibition, inhibition through a coupled negative feedback loop occurs[25]. Studies have reported that *Hes7* and *Lfng*, the most critical molecular oscillators in the *Hes/Her* family, are induced by Notch and FGF signals. Meanwhile, *Hes7* represses its own and *Lfng* expression, resulting in *Hes7* and *Lfng* oscillations in phase. Moreover, *Lfng* inhibits Notch activity, enhancing oscillation of both *Hes7* and *Lfng.* A coupling-regulated cyclic oscillation network among *Hes7*, *Lfn*g, and Notch subsequently forms[26-29].

The transcription initiation and propagation of *Hes7* and *Lfng* are regulated by the FGF and Notch signaling pathways. FGF signaling induces the initial *Hes7* oscillation in the posterior PSM. After that, Notch signaling is required for the amplification and the anterior propagation of *Hes7* oscillation[30]. Niwa *et al*.[30] observed that treating cells with the FGF signaling inhibitor SU5402 or U0126 severely affected *Hes7* gene expression in the entire mesoderm. Accordingly, if cells do not experience FGF-dependent *Hes7* oscillation in the posterior PSM, they cannot induce *Hes7* oscillation in the anterior PSM, even though Notch signaling is intact[3]. *Lfng* expression does not depend on FGF signaling, however, in the absence of FGF signaling, *Lfng* expression does not oscillate even though *Dll1* and *Notch1* are expressed, because *Hes7* expression is lost. Thus, FGF signaling is required for not only *Hes7* oscillation but also *Lfng* oscillation[30].

1.3 Synergistic regulation of the segmentation clock by signaling gradients and cyclic oscillation

FGF/Wnt and RA signaling gradients produce two homeostatic environments in the anterior-posterior mesoderm, which are coupled through mutual inhibition. Undifferentiated homeostasis, induced by FGF signaling, is observed in the posterior mesoderm, whereas mature homeostasis, induced by RA signaling, is observed in the anterior mesoderm. These opposing gradients create a bistability window, and the cells undergo the bistable transition can be triggered by the segmentation clock to switch abruptly between the two homeostatic environments at the determination front[18]. As the embryo elongates posteriorly, newly formed cells in the tail buds are exposed to high FGF signaling levels. This initiates molecular oscillation, activating cyclic Notch, FGF, and Wnt signaling. Because cells are in undifferentiated homeostasis at this time, they cannot respond to the oscillatory signals. As the cells move anteriorly, FGF signaling gradually decays and RA signaling began to strengthen. Concurrently, Notch signaling controls molecular oscillators to periodically propagate anteriorly. At the determination front, low levels of FGF signaling and high levels of RA signaling eliminate differentiation inhibition and initiate the segmentation clock. Several differentiation genes (*e.g.*, *Mesp2*) are activated in response to clock signals, initiating and regulating somitogenesis (Figure 4)[14].

To summarize, signaling gradients determine the location of somite formation, and the distance traveled by the wavefront during one oscillation cycle determines the size of a segment. The FGF gradient affects the determination front when disrupted. In the cultured mouse embryos, FGF pathway inhibition blocked the oscillations of Wnt and Notch cyclic genes with varying kinetics. This experimental step determined the front of FGF signaling, which acted upstream of the Wnt and Notch pathways, thereby

controlling molecular oscillations. Taken together, these findings indicate that FGF signaling acts on both signaling gradients and cyclic oscillations and is a key pathway affecting somitogenesis[31].

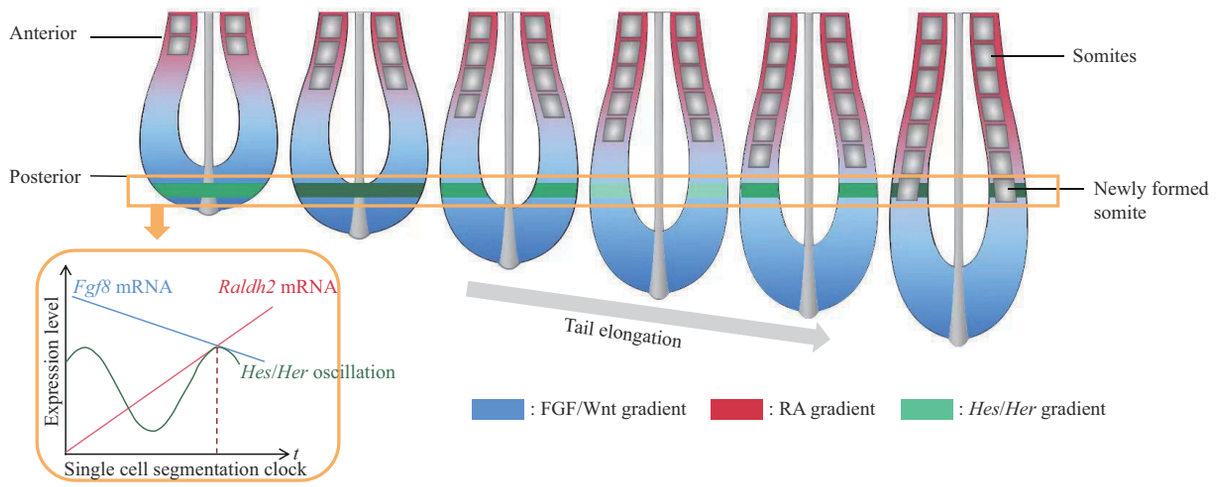

Fig. 4　Schematic view of the vertebrate segmentation clock

FGF and RA signaling arise from the posterior and anterior ends of the PSM, respectively. Meanwhile, *Hes*/*Her* gene family shows cyclic expression patterns and repeats every oscillation cycle as the embryo elongates posteriorly. Clock signal will ensure that a bistable transition occurs during each oscillation cycle and moves posteriorly owing to the posterior extension of the axis and the decay of the FGF and Wnt signals.

2　Generation mechanism of bilateral symmetry

2.1　Live reporter and 2D culture systems

To elucidate the synchronized oscillatory mechanism resulting from the bilateral symmetry of somites, Pourquié *et al*.[32] and Kageyama *et al*.[33] integrated live reporter systems (*e.g*., luciferase and GFP) and 2D culture system (differentiated using embryonic stem cells), successfully replicating the segmentation clock *in vitro*[6, 34-36]. Concerning Kageyama *et al*. were the first research team to successfully differentiate mouse embryonic stem cells, which contain the *Hes7*-luciferase reporter gene, into the mesoderm, thus realizing the 2D visualization of segmentation clock oscillation in individual cells[33]. The next obvious move was Pourquié *et al*. optimized these protocols and successfully differentiating embryonic stem cells and inducing pluripotent stem cells into mouse and human PSM cells. They achieved the abovementioned feat by using only the Wnt agonist CHIRON99021 and the bone morphogenetic protein inhibitor LDN193189 in CL medium. Thus, the oscillatory effects of human segmentation clocks were simulated, introducing new methods for investigating the mechanisms underlying the formation of the bilateral symmetry[32, 37-41].

2.2　Coupling delays control the synchronous oscillation of the segmentation clock

To ensure the bilateral symmetry of somites, the segmentation clock is synchronized between adjacent cells, manifesting as the synchronous differentiation of local tissues on the left and right sides of the embryo. Therefore, intercellular communication is crucial to maintaining coordinated oscillations, indicating the existence of a phase-coupling mechanisms between neighboring cells[42]. That is to say, individual cells carry their own clock, but are coupled to each other and generate coherent oscillatory waves to from segmentation boundaries. Furthermore, formation of a pair of somites requires a high spatiotemporal precision of coupled oscillations between adjacent cells for establishing well-defined differentiation boundaries; uncoupling leads to severe somite fusion and morphological abnormalities[43-46].

To review the cellular coupling mechanism controlling synchronous oscillations, the Kageyama research team at Kyoto University established a system that observed the dynamic changes of clock genes at a single cell level. The system successfully induced the differentiation of mouse embryonic stem cells to mesoderm *in vitro* and constructed a fluorescent reporter system in ES cells, which realized

the two-dimensional visualization of the segmentation clock oscillation of a single cell. This powerful live-cell imaging method was used to review the quantitative oscillatory dynamics of single-cell resolution[33] (Figure 5).

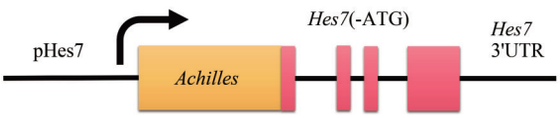

Fig. 5 Analysis of oscillations of the *Hes7−Achilles* reporter in each PSM cell

Structure of the *Achilles-Hes7* transgene. Expression of the *Achilles-HES7* fusion protein was quantified and calculated for oscillation phase mapping in each PSM cell. UTR: untranslated region.

Quantification of oscillatory dynamics with single-cell resolution indicated that the Notch signaling pathway is a critical mediator of this coupling mechanisms. Clock gene oscillations between neighboring cells are synchronized *via* the Delta-Notch intercellular signaling pathway. The time required for signals to be transmitted from one cell to another is known as coupling delay[47]. The Kageyama research team used single live-cell imaging combined with an optogenetic send-receive system to examine the role of Notch signaling in cell-cell coupling. They demonstrated that intercellular coupling delay errors, such as increased or decreased delay time, can severely suppress in-phase oscillations, thus desynchronizing intercellular oscillations and resulting in somite fusion. Studies have shown that these coupling delays are controlled by *Lfng* gene, a downstream target of Notch. It increases the response time and amplitude of signaling genes in receiver cells, thereby delaying signal sending time and increasing signal receiving amplitude. By increasing the time required for intercellular DLL1-Notch signaling, *Lfng* can adjust coupling delays to fit in-phase oscillations. Therefore, for spinal diseases with *Lfng* functional defects, small molecular compounds that correct coupling delays can be administered to regulate the synchronization[33].

Further research indicated that FGF signaling pathways can also modulate oscillatory synchronization. In a 2D visual cellular model of *Hes7* oscillations, when treated PSM cells with the MEK inhibitor PD0325901 to block the FGF signaling, the oscillator rhythms been impaired, leading to the regulation of not only oscillation arrest at the wavefront but also the dynamics of cyclic gene oscillations including period, phase, and amplitude[32].

Additionally, RA signaling pathways maintain the bilateral synchronous segmentation of the PSM. Vermot *et al*.[48] used *RARE-LacZ* to visualize RA signaling expression in *Raldh2*-knockout mice and observed that molecular oscillations were not synchronized during somitogenesis, resulting in delays on one side. The same results were also observed in chicken and zebrafish embryos[49-50]. Subsequently, Vilhais-Neto *et al*.[51] identified an RA coactivator, which forms a complex with *Wdr5* and *HDAC1/2* (WHHERE complex). This regulator maintains oscillation synchronization by regulating RA signaling. These findings indicated that RA signaling maintains the bilateral synchronous segmentation of the PSM, thereby controlling the bilateral and symmetrical formation of somites.

3 The control mechanism of the speed of somitogenesis

3.1 Reconstruction and deconstruction somitogenesis with 3D culture systems

In 2022, Pourquié *et al*.[52-55] established new 3D culture systems (somitoid and segmentoid) on the basis of the 2D differentiation system. They replicated the formation of the segment-like structures of the anteroposterior (AP) axis (Figure 6, 7). These findings indicated that the segmentation clock plays a key role in converting temporal rhythmicity into spatial regularity in AP somite intervals. In newly formed somites, the expression of the initial somite gene *MESP2* is modulated for the formation of AP compartments through an active cell-sorting mechanism. Accordingly, in the *in vitro* system, the major patterning modules involved in somitogenesis including the clock and wavefront, AP polarity patterning, and somite epithelialization. They work independently and can be individually analyzed. These 3D culture systems have introduced a new framework for understanding the symmetry-breaking process that initiates somite polarity patterning; this framework offers a valuable platform for decoding general principles of somitogenesis and advancing knowledge of human development.

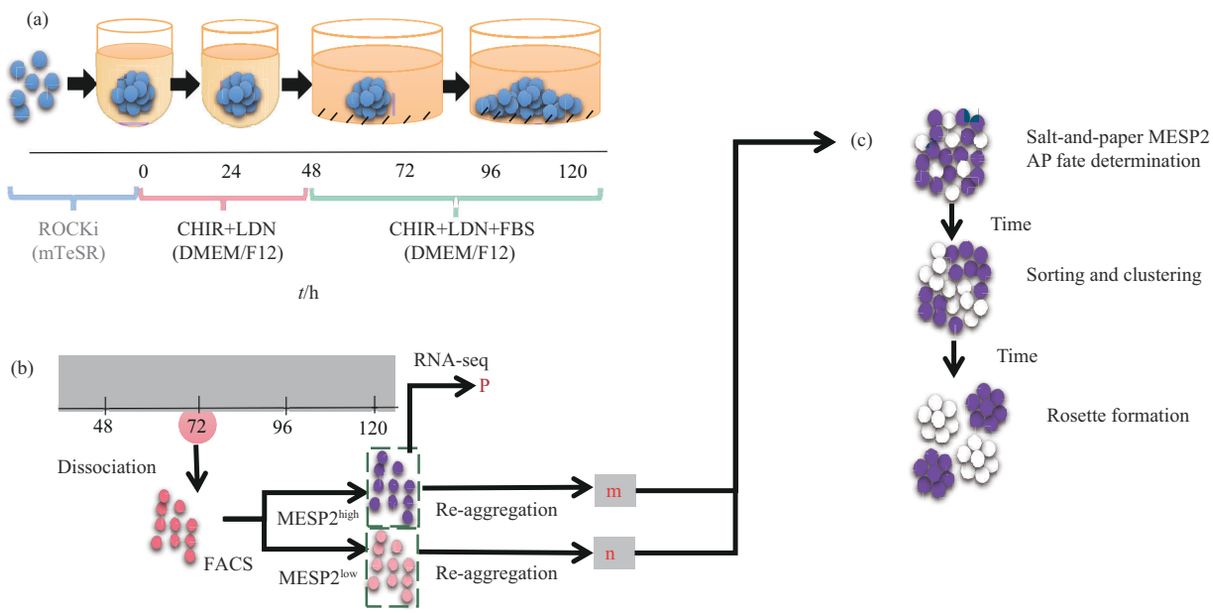

Fig. 6　AP polarity patterning in the somitoid model

(a) Generation of human induced PS (iPS) cell spheroids in suspension, and treated with the Wnt agonist CHIR and the BMP inhibitor LDN for 48 h to induce the PSM fate. (b) The *MESP2*^{high} or *MESP2*^{low} fractions from cultures were dissociated at 72 h and re-aggregated separately to investigate when AP fates in individual cells are determined. At 120 h, similar rosette morphogenesis was observed in both types of aggregates. (c) An initial heterogeneity of *MESP2* expression levels is translated into defined compartments of anterior and posterior identity through an active cell-sorting mechanism.

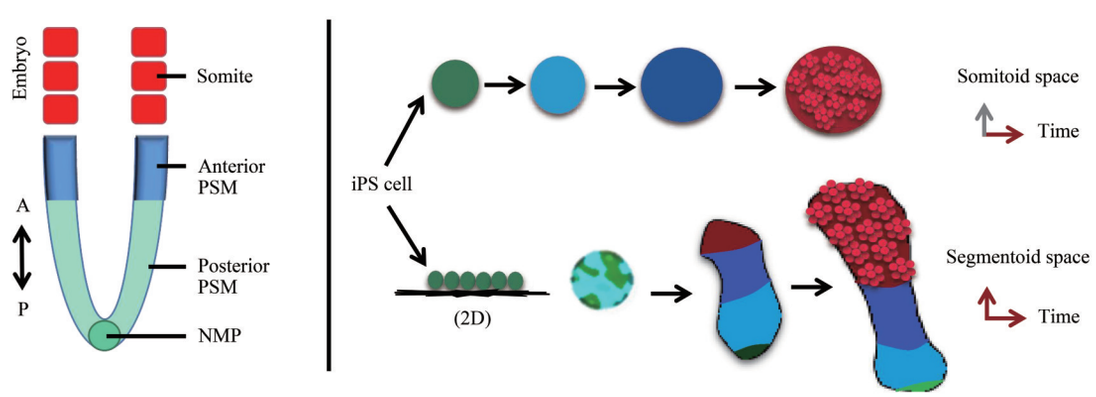

Fig. 7　Characterization of the segmentoid model

Both 2D and 3D culture systems can recapitulate somitogenesis *in vitro* with somitoids showing synchronized cell differentiation while segmentoids exhibit a spatially organized progressive maturation similar to that of the embryonic tissue.

3.2　Metabolic regulation of differential speed of somitogenesis among species

Although the process of embryonic development is relatively conserved across species, the developmental rate varies considerably. The rate of various biochemical reactions, such as protein synthesis and degradation, has long been believed to be responsible for such differences. However, the actual reasons for the differences remain to be clarified. Pourquié *et al.*[56] recapitulates the two-fold difference in the developmental rate between mouse and human embryos using the 3D culture systems; their findings indicated that the rate of metabolism is directly proportional to that of development.

Reducing these metabolic rates by inhibiting the electron transport chain may disrupt the intracellular NAD^+/NADH redox balance, thereby slowing down segmentation clock and protein synthesis and inhibiting embryonic development. The overexpression of the *Lactobacillus brevis* NADH oxidase (lbNOX) increases the rate of translation, accelerating the rate of embryonic development. These findings confirm that when the mass is constant, the respiration rate affects the protein translation rate through the regulation of the NAD(H) redox balance, thereby influencing the segmentation clock. This partially explains the difference in the rate of development between mouse and human embryos during their early stages of embryonic development. Future studies may focus on accelerating the differentiation of human pluripotent stem cells by regulating their rate of development; this approach may help advance human stem cell therapy and *in vitro* disease treatment[56].

4 Conclusion and perspectives

In summary, the cyclic genesis of vertebral somites is regulated by the segmentation clock networks. When two antagonistic signals (*i. e*., FGF and RA) reach a critical point of equilibrium in the anterior PSM, the segmentation clock initiates the differentiation process, forming a pair of somites. The dual homeostasis of opposing signaling gradients determines the segment boundaries, the distance traveled by a molecular oscillator in one oscillation cycle determines the somite size, and the delayed coupling in intercellular signaling regulates the synchronization of clock oscillations. These three factors interact with each other to form a segmentation clock network coordinating somitogenesis. The clock and wavefront patterning module unraveled mechanisms that regulate signaling gradients and cyclic oscillations in somitogenesis, however, the mechanisms underlying the formation of the bilateral symmetry remain to be elucidated. Recent studies have revealed that the intercellular coupling delay mechanism is a major factor influencing the maintenance of oscillation synchronization, the mechanisms governing how intercellular communication (*e. g*., Notch, FGF, and RA signaling) becomes involved in oscillation synchronization remains unclear.

As we know, congenital scoliosis (CS) is a result of anomalous development of the vertebrate including failure of formation or segmentation and frequently associate with somitogenesis malformation. Despite over twenty years of progress on the pathogenesis of CS, we still have a very superficial understanding of the vertebrate segmentation process. We observed that deficiency or overdose of vitamin A intake in gestation may lead to CS. RA, a metabolite of vitamin A, is synergistic with Wnt, Notch, FGF, MAPK and BMP signaling pathways involved in abnormal development of vertebrae during embryogenesis. While the deep mechanism of how RA signalling regulates oscillation synchronization still need to be detected[57]. Access to *in vitro* system and real time reporters has allowed to generate large amounts of synchronized cells can be used to apply large scale omics techniques to dissect the genetic or epigenetic regulation of the clock by direct examination of gene expression or chromatin regulation[58-61]. These new methods could help elucidating the mechanisms underlying bilateral symmetry formation to be introduced. Such information may be useful for unveiling the dysregulation mechanisms involved in the development of pathological somite defects such as CS[62]. Accordingly, a 3D culture system with a somite-like structure was developed in recent studies to elucidate of mechanisms that regulate the speed of somitogenesis[63-64]. Future studies may focus on accelerating the differentiation of human pluripotent stem cells by regulating their rate of development; this approach may help advance human stem cell therapy and *in vitro* disease treatment.

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脊椎动物体节形成的分节时钟调控机制*

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摘要　脊椎动物胚胎发育早期中胚层细胞的分节时钟控制着体节的周期性形成。体节是沿身体轴的重复结构，最终发育形成椎骨和肋骨。如果分节时钟受到干扰，体节形成就会出现缺陷，从而导致身体发育异常，最终产生脊柱先天性疾病。参与体节发育的主要模型是时钟和波前模型。中胚层分化由组合梯度系统调节，该系统涉及成纤维细胞生长因子（FGF）、Wnt/β-catenin和视黄酸（RA）信号通路。FGF信号和Wnt/β-catenin信号控制后中胚层处于未分化状态，RA信号则诱导前中胚层细胞分化导致体节成熟。因此相反的信号梯度在特定位点达到平衡。当分子振荡器从尾芽起始表达并以行波模式向前传播至信号平衡临界点时，将启动分节时钟程序，触发*Mesp2*等分化基因表达，表现为未成熟的前体节中胚层发育形成一对体节。随着细胞二维培养体系和时事报告系统的成熟，研究人员成功在体外将干细胞诱导分化至中胚层并实现了分节时钟的二维可视化振荡。研究表明，细胞通信中的耦合延迟可以保持相邻细胞之间同步振荡，因此导致体节边界和双侧对称形成。此后研究人员在体外重建了诱导多能干细胞的三维培养系统，再现了具有前-后（AP）轴特征的体节样结构的形成。这为解码分节时钟网络调控机理、探索体节双侧对称形成以及不同物种发育速率的代谢调控机制提供了一个宝贵的研究体系。同时为探索病理性体节缺陷发展中的失调机制创造了一个平台。

关键词　分节时钟，体节形成，信号梯度，周期振荡，同步振荡
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