Genetic oscillators in development

Genetic oscillators in development

Koichiro Uriu

Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

Email: uriu@staff.kanazawa-u.ac.jp
Tel: +81-76-264-6246
Fax: +81-76-264-6246

Running Head: Genetic oscillators in development

Abstract

In development, morphogenetic processes are strictly coordinated in time. Cells in a developing tissue would need mechanisms for time-keeping. One such time-keeping mechanism is to use oscillations of gene expression. Oscillatory gene expression can be generated by transcriptional/translational feedback loops, usually referred to as a genetic oscillator. In this review article, we discuss genetic oscillators in the presence of developmental processes such as cell division, cell movement and cell differentiation. We first introduce the gene regulatory network for generating a rhythm of gene expression. We then discuss how developmental processes influence genetic oscillators. Examples include vertebrate somitogenesis and neural progenitor cell differentiation, as well as the circadian clock for comparison. To understand the behaviors of genetic oscillators in development, it is necessary to consider both gene expression dynamics and cellular behaviors simultaneously. Theoretical modeling combined with live imaging at single-cell resolution will be a powerful tool to analyze genetic oscillators in development.

Keywords: cell cycle, negative feedback loop, simulation, synchronization, time delays

Introduction

Many rhythms can be observed in organisms. Examples include the cell cycle, the heartbeat of animals, the 24-hour physiological rhythms, and the annual reproductive cycle of animals and plants. Some of them may be driven by periodic signals from the surrounding environment, such as light and temperature, but others are generated by organisms themselves. One of such self-sustained rhythms can be observed at a single cell level. The concentrations of mRNAs and proteins increase and decrease rhythmically with a well-defined temporal period in cells. The oscillations of mRNA and protein concentrations are often caused by transcriptional/translational feedback loops, a mechanism which is referred to as a genetic oscillator. These genetic oscillators can be seen, for example, in the cell cycle, circadian rhythms, and inflammatory response (Goldbeter *et al.*, 2012). One important role of such oscillations would be to work as a molecular clock for the time-keeping of biological processes.

In development, many morphogenetic processes are orchestrated correctly to form organs. Cells in a tissue need to proceed to their developmental fates at the right times. Time-keeping is crucial in development and one time-keeping mechanism in development is to use molecular clocks in cells. During development, the surrounding environment of a population of genetic oscillators is changing drastically over time due to morphogenesis. Recent advances in live imaging at single cell resolution reveal that cells with genetic oscillators divide to increase cell number, move around in the tissue, and differentiate into different cell types after stopping their oscillators. This is different from other molecular clocks, such as the circadian clock in the suprachiasmatic nucleus

(SCN) in an adult mammalian brain. In the adult SCN, the tissue environment would be relatively steady and the clock keeps oscillating to help the organism survive in a periodic external environment. Thus, to understand genetic oscillators in development, it will be necessary to examine both gene expression dynamics inside cells and cellular behaviors such as division and movement simultaneously.

Theoretical modeling has been playing key roles for understanding genetic oscillators. Theoretical studies put the oscillators in the context of dynamical systems, a branch of study that analyzes the existence and stability of solutions in a system of differential equations (Strogatz, 1994). Typically, the time evolution of mRNAs and proteins in a genetic oscillator is described by a set of ordinary differential equations or delay differential equations. Strong nonlinearity in gene regulation, such as cooperativity or saturation, precludes derivation of the analytical solution of the equations that represent the biochemical reactions. The system of differential equations is therefore often solved numerically with computers, but in some particular cases, approximations allow properties of the oscillations, such as period and precision, to be calculated with paper and pencil. These theoretical studies have analyzed the motifs of gene regulatory networks for robust oscillations, the factors that influence the period and amplitude, and the synchronization of a population of genetic oscillators.

In this article, we discuss genetic oscillators in the context of embryonic development by reviewing both experimental and theoretical studies. We first introduce gene regulatory networks that generate oscillations of gene expression. In several developmental situations, these genetic oscillators interact with each other by

intercellular signaling to organize their collective behaviors such as synchronization of their phase of oscillation. We then explore how developmental processes such as cell division and cell movement affect genetic oscillators. We also discuss how genetic oscillators influence cell proliferation and differentiation in tissues. Examples include genetic oscillators during vertebrate somitogenesis and neural progenitor cell differentiation in developing mammalian brains. In some cases we compare the genetic oscillators in development with the circadian clock, highlighting similarities and differences among them. In the studies introduced below, live imaging at single-cell resolution plays key roles. We emphasize the importance of theoretical modeling in the analysis of genetic oscillators in development.

Genetic oscillators composed of negative feedback loops

In this section, we introduce key aspects of genetic oscillators for better understanding of the following sections. We focus on the topics relevant to the oscillators in development. There are several good reviews on biological oscillators, for example (Novak & Tyson, 2008, Ferrell *et al.*, 2011, Goldbeter *et al.*, 2012). Expression of a gene may be controlled by several other genes, forming networks of regulatory relations. Different motifs in these networks have different biological functions, such as bistable genetic switches for decision making, noise filtering, and pulse generation as responses to input signals (Alon, 2006). Oscillations of gene expression typically arise from complex gene regulatory networks. Feedback regulation is the key element and a negative feedback loop is one such mechanism for causing oscillations. We first review

general requirements for a negative feedback loop to generate oscillation of gene expression. Next, we discuss how the same motif of a negative feedback creates different lengths of periods of oscillation, ranging from tens of minutes to tens of hours. Then, we see the mechanisms to generate reliable rhythms from noisy gene expression.

Requirements for oscillation

The simplest form of the negative feedback loop is that a protein represses the transcription of its own gene (Fig. 1A). Once a protein is formed in the cytoplasm by mRNA translation, the protein goes to the cell's nucleus and binds to its own promoter region on the DNA. The binding represses transcription, and therefore new protein molecules are not produced while the concentration of previously made proteins is high. Before this repression sets in, however, the mRNA concentration becomes high and the protein concentration tracks this mRNA level, leading to an over-shoot of protein above the minimum level required to repress. The amount of the protein decreases over time due to degradation and when the concentration of the protein drops below a certain threshold, the transcription of mRNA starts again, entering a new cycle of oscillation. A negative feedback loop may include several intermediate genes, for example, gene A activates gene B, gene B activates gene C and it represses gene A (Fig. 1B).

Negative feedback regulation can be formulated as a system of ordinary differential equations or in the form of delay differential equations (Fig. 1C). There are several key elements to generate sustained oscillations by a negative feedback loop (Fig. 1C-E). The time taken for the gene to repress its own transcription by making its mRNA

and protein products should be long enough to cause overshoots and undershoots of gene expression. These time delays in the negative feedback loop are caused by the splicing of mRNA, transport of molecules between cytoplasm and nucleus, and modifications of these molecules like phosphorylation. In addition, strong non-linear reaction steps are necessary somewhere in the regulatory processes. Cooperative binding of proteins to regulatory elements on the DNA would be a candidate for the non-linearity. Transcriptional regulation by cooperative binding is often modeled as the Hill function (Fig. 1D). It causes a sharp on-off switch of gene expression (Fig. 1D), which promotes oscillation by a negative feedback. Saturated degradation of mRNAs and proteins is also known to promote sustained oscillations (Kurosawa & Iwasa, 2002).

Period of oscillation

A negative feedback loop seems to be an universal mechanism to generate genetic oscillations in organisms (Goldbeter *et al*., 2012, Novak & Tyson, 2008, Ferrell *et al*., 2011). Examples of genetic oscillators in development are limited, but they are also composed of a negative feedback loop. However, the period of oscillation is different among genetic oscillators. Genetic oscillators in development introduced in the later sections typically have the period of tens of minutes to a few hours. In contrast, the circadian clock that is also composed of negative feedback loops has the period of nearly 24 hours. How can the same motif of a negative feedback loop create wide variety of periods?

In the formulation with delay differential equations (Fig. 1C) the period of a

genetic oscillator is mostly determined by the time delays involved in the negative feedback loop (Fig. 1F) and the half-lives of mRNA and protein molecules (Fig. 1G). Longer time delays in the feedback cause longer period of oscillation (Fig. 1F). Short half-lives of the molecules make the period shorter by speeding up the removal of repressing proteins (Fig. 1G). A negative feedback loop can also be modeled with a set of ordinary differential equations where intermediate reaction processes, such as transport and post-translational modification of proteins, are explicitly described. In this formulation, time delays do not appear in the model as single parameters. Instead, they are implemented as a sequence of biochemical reactions. In this description, the period is more sensitive to the transcription rate and the degradation rate of mRNA (Kurosawa & Iwasa, 2005, Uriu *et al*., 2009). These two parameters set the duration of repression because repressor proteins are produced until its mRNA is present. Accordingly, the period increases with the increase in the transcription rate while the period decreases with the increase in the degradation rate of mRNA.

The negative feedback loop motif can thus create different periods by changing the length of time delays. Longer time delays may be created by including several genes in a feedback loop (Fig. 1B). For example, in the circadian clock, several genes are involved in a single negative feedback loop as shown in Figs. 1B and 2A. In contrast, a single gene alone (Fig. 1A) tends to form a negative feedback loop in genetic oscillators with a shorter period. Thus, required period may limit the number of genes that can be involved in a feedback loop. Timescales of biochemical reactions, for example degradation rates of molecules, are also different among genetic oscillators. In

the circadian clock, the degradation of molecules occurs in the order of hours (Friedel *et al*., 2009). In contrast, in genetic oscillators with a shorter period in development, the degradation of molecules occurs in the order of minutes (Hirata *et al*., 2002, Ay *et al*., 2013). Thus, the same motif of a negative feedback loop can create different periods by changing the timescales of biochemical reactions involved.

Although a single negative feedback loop has the ability to generate oscillations, in some situations a gene regulatory network have both positive and negative feedback loops. This additional positive feedback loop helps to generate oscillation of gene expression by the negative feedback loop (Novak & Tyson, 2008, Tsai *et al.*, 2008) and makes oscillation more robust against noise in gene expression (Barkai & Leibler, 2000, Vilar *et al.*, 2002). A positive feedback loop can create bistability, where two stable cellular states (e.g. low and high gene activity) coexist. A repressor in the negative feedback loop is activated when the gene activity in the positive feedback loop is in the high state. When repression sets in, the gene activity switches from the high state to the low state. In the low state, the repressor is also inactivated. Then, the gene activity gradually increases and at certain point, the activity switches from the low state to the high state. Thus, the cell can periodically traverse these two states created by the positive feedback under the influence of negative feedback regulation and generate oscillation of gene activity. It has been shown that an additional positive feedback loop can provide a tunable period of oscillation (Tsai *et al.*, 2008). The period can be changed orders of magnitude while keeping the amplitude almost constant by varying a reaction parameter in the positive feedback loop. In

contrast, a negative feedback loop without any positive feedbacks cannot change the period without compromising the amplitude.

Negative feedback loops with some positive feedback regulations are found for example in the regulatory networks for the cell cycle (Pomerening *et al.*, 2005, Pomerening *et al.*, 2003). Controlling the duration of the cell cycle at each developmental stage may be crucial for embryos. In such a case, tunable period realized by positive feedbacks would be beneficial. Tunable period was also realized by a positive feedback loop in bacteria in the field of synthetic biology (Stricker *et al.*, 2008). Except for the cell cycle, involvement of intracellular positive feedbacks in genetic oscillators in development has not been identified yet. Instead, a positive feedback loop is implemented through intercellular interactions such as Delta-Notch signaling in development. This positive feedback is rather responsible for intercellular communications as described in the later sections.

Precision and collective rhythms

To be a reliable biological clock, a genetic oscillator is required to ensure precision of oscillations. How is the precision of a clock conferred? The mechanisms for robust oscillations can be found at a single cell level and at a population level.

When the number of molecules is small, the stochasticity in biochemical reactions could strongly affect the precision of a genetic oscillator. For example, binding of repressor proteins to the DNA is inherently stochastic. The stochastic binding of a repressor protein to the DNA can cause fluctuations of transcription rate.

Fluctuation of the number of molecules would result in variations in both the amplitude and period of oscillation over time (Elowitz & Leibler, 2000), which reduces the precision of oscillation. Theoretical studies have addressed the question of how a genetic oscillator becomes robust against noise in gene expression. These theoretical studies revealed that some structures of gene regulatory networks can provide the precision of oscillation. Proposed mechanisms are the inclusion of a positive feedback loop for averaging stochastic gene expression by timescale separations (Vilar *et al.*, 2002, Barkai & Leibler, 2000) and coupling between protein phosphorylation and transcription-translation cycles (Zwicker *et al.*, 2010). A recent synthetic biology study demonstrated that dual negative feedback can also increase the robustness of genetic oscillation (Chen *et al.*, 2015). Translational bursting from a single mRNA can improve the quality of genetic oscillations (Morelli & Julicher, 2007). Thus, a single cell might have intracellular mechanisms to be a precise oscillator.

Genetic oscillators may interact with other rhythms like periodically changing environment. For example, the circadian clock is influenced by a light-dark cycle and quickly becomes entrained to it. In some cases, genetic oscillators interact with each other in a tissue to generate collective behaviors. The interactions are often mediated by signaling molecules, such as diffusing molecules or membrane proteins that we will see in the later sections. It is known that interactions between oscillators increase the precision of oscillation against noise in the gene regulatory network (Kori *et al.*, 2012, Clay & DeHaan, 1979). In the presence of interactions, time intervals between two successive peaks of oscillation for a single oscillator become more regular than without

interactions. Interactions may also work to synchronize the phase of genetic oscillators. Because individual oscillators are exposed to noise in gene expression as described above, a population typically loses synchronization if they do not have any interactions. Hence, interaction is especially important when a collective rhythm of a population works as a biological clock. In addition, interactions among genetic oscillators can regulate their collective period when time delays exist in the interactions (Herrgen *et al.*, 2010).

Thus, a genetic oscillator may have mechanisms for robust oscillations at a single-cell scale to a tissue scale. Oscillators in development have started being live-imaged as we will see below. Quantification of quality of oscillation both at a single-cell level and at a population level will reveal how cells maintain precision of rhythmic gene expression in developing tissues.

Cell division

In development, cells divide. In this section we discuss the influence of cell divisions on genetic oscillators. We first introduce the example of a circadian clock in cell culture systems where interactions between the circadian clock and cell divisions have been characterized in detail. We explore how a cell division affects the phase of oscillatory gene expression. Then, we discuss the example of vertebrate somitogenesis where cells divide in an embryonic tissue. These cells keep synchronization of genetic oscillators across the tissue in the presence of phase disturbances by cell divisions.

Cell division and the cell cycle may potentially affect a genetic oscillator in

several ways. During mitosis, the transcription of mRNAs is repressed (Gottesfeld & Forbes, 1997). A nuclear membrane disappears during mitotic phase, which can affect the localization of molecules. The amount of molecules in an individual cell is considered to be halved after a cell division, because the two daughter cells typically share the molecules from their mother cell almost equally. This partition can be stochastic and unequal partitioning of molecules may occur. Halving the number of molecules may induce a large stochastic fluctuation of a reaction in the daughter cells, if the number of molecules is already low. Partitioning of molecules could cause rapid changes of molecular concentration in single cells, if, for example, the volume of a relevant cellular compartment remained constant or the timescale of cell volume growth is faster than that of a genetic oscillator. In addition, transcription and translation rates may vary across the cell cycle. When the DNA replicates in S phase, the number of target genes in a negative feedback loop doubles. An important question would then be how cell divisions and the cell cycle influence genetic oscillators.

Cell divisions in the circadian clock

Most organisms have daily 24-hour physiological rhythms. In mammals circadian rhythms are generated by the SCN in the brain. Cells in the tissue have genetic oscillators composed of transcriptional/translational feedback loops. These regulatory networks generate sustained oscillation of gene expression with a period of about 24-hours. The core circadian clock genes for mice include *Bmal1*, *Clock*, *Period1*, *2* (*Per*), *Cryptochrome1*, *2* (*Cry*), and *Rev-erbα, β*. The regulatory network of these

core-clock genes has double negative feedback loops (Fig. 2A). Clock/Bmal1 protein complex induces the expression of *Per*, *Cry,* and *Rev-erb* through binding to E-boxes in their promoter regions. Per and Cry proteins form a complex and the complex represses Clock/Bmal1 activity, forming a negative feedback loop. In addition, Rev-erb proteins repress *Bmal1* transcription, adding another negative feedback loop. These negative feedback loops generate sustained oscillation of clock gene expression. The timescales of these negative feedbacks are the order of hours as described in the previous section, resulting in the period of 24 hours. For more details, see reviews (Zhang & Kay, 2010, Lowrey & Takahashi, 2011, Sehgal, 2015). Cells in peripheral tissues like the liver also have an autonomous circadian clock (Mohawk *et al.*, 2012). These peripheral clocks receive signals from the SCN.

The circadian clock affects the progression of the cell cycle. Cell cycle in mammals is regulated by the activities of cyclins and cyclin dependent kinases (Cdk). Regulators of cyclin/Cdk activities control the duration of certain cell cycle phases. By influencing these regulators the circadian clock affects G1-S phase and G2-M phase transitions in the cell cycle (Fig. 2B). Cell cycle regulating genes such as *Wee1*, *p21*, and *p54nrb* (also known as *NONO*) are influenced by circadian clock components. Wee1 protein retards M phase progression by phosphorylating Cdk1 and Cdk2. The transcription of *Wee1* is activated by Clock/Bmal1 complex (Matsuo *et al.*, 2003). p21 protein inactivates cyclinE/Cdk2 and retards G1 phase progression. Rev-ervα protein represses the transcription of *p21* (Grechez-Cassiau *et al.*, 2008). NONO together with Per protein induces *p16-ink4A* (Kowalska *et al.*, 2013) that promotes G1 arrest.

It has been considered that cell cycle progression is allowed only when the circadian clock is at certain circadian phases, a phenomenon called circadian gating (Fig. 2B). This gating of the cell cycle by the circadian clock is also present in cyanobacteria (Yang *et al.*, 2010). A recent study proposed that the cell cycle is not gated, but phase locked to the circadian clock by entrainment or synchronization (Feillet *et al.*, 2014). The study showed multiple phase locked states of the cell cycle to the circadian clock (1:1 and 3:2) using mouse fibroblasts. A mathematical model including key molecules in the cell cycle and circadian clock showed that regulation by the core circadian clock genes entrains the cell cycle into either 24-hour or 48-hour period oscillation (Gerard & Goldbeter, 2012). The entrained period of the cell cycle depends on the strength of the regulation by the circadian clock and the difference between the period of the cell cycle and that of the circadian clock (Gerard & Goldbeter, 2012).

The converse question would be whether the cell cycle influences the progression of the circadian clock. Experimental studies demonstrated that cell divisions affect the circadian phases in a cell culture system of fibroblasts (Bieler *et al.*, 2014, Nagoshi *et al.*, 2004). These studies used a YFP reporter driven by the *Rev-erbα* promoter and monitored how cell divisions affected the time intervals between two successive peaks of the reporter at a single cell level (Fig. 2C). The average time interval was about 23.7 hours when a cell did not divide within the interval. In contrast, the average time interval between two circadian peaks became shorter (~21.9 hours) when a cell division occurred within that interval (Bieler *et al.*, 2014). This tendency is supported by a separate study (Feillet *et al.*, 2014). Moreover, changes in the length of

the time interval depends on the circadian phase at which a cell division occurs (Fig. 2C). When a division occurs in the later half of the time interval (increasing phase of Rev-erbα), the time interval are likely to become shorter (top panel of Fig. 2C), while it tends to become longer when a division occurs in the earlier half (decreasing phase of Rev-erbα; bottom panel of Fig. 2C). Bieler et al. used a coupled oscillator model that includes time evolution of the circadian clock and cell cycle to quantify the strength of interactions between these two oscillators. The model that was fitted to the experimental data demonstrated that the cell cycle influences the circadian clock more strongly than the circadian clock does the cell cycle in the fibroblasts.

Some of the factors associated with a cell division and the cell cycle listed above might cause the observed phase responses of the circadian clock (Bieler *et al.*, 2014, Nagoshi *et al.*, 2004). A question is, then, which factors are more dominant in a genetic oscillator. Theoretical models that include these factors will identify the dominant effects to the phase responses of genetic oscillators.

A theoretical study examined the robustness of genetic oscillators against changes in molecular amounts caused by cell divisions (Gonze, 2013). The model includes stochastic dynamics of gene expression, time evolution of a cell volume and cell division cycles. The study assumed that the partition of the molecules into two daughter cells after a division is a stochastic process and the number of molecules one daughter cell receives follows a binomial distribution. Numerical simulations showed that oscillations become noisy in the presence of cell divisions. When the period of the cell division cycles is shorter than that of the genetic oscillator, the genetic oscillator

severely loses precision. However, as soon as the period of cell division cycles becomes longer than that of the genetic oscillator, cell divisions hardly affect the oscillations. Interestingly, a genetic oscillator can be entrained to the cell division cycles in the formulation (Gonze, 2013). When cells divide, the amount of repressor proteins in a negative feedback is abruptly decreased due to partition. Then, the repression of mRNA transcription is relieved and a new cycle with mRNA transcription begins. A cell division causes a phase shift of a genetic oscillator, which allows the cell division cycles to entrain the genetic oscillator. Thus, theoretical modeling predicts the effects of partitioning molecules on genetic oscillators.

However, some questions remain to be answered. Can the phase responses of the circadian clock observed in fibroblast cells (Bieler *et al.*, 2014, Nagoshi *et al.*, 2004) be explained only by the partition of molecules at cell divisions? Are there other factors responsible for the observed responses? Future theoretical modeling could reveal the determining factors of the phase responses to cell divisions.

Cell divisions in the segmentation clock in vertebrate embryos

Another example of genetic oscillators undergoing cell divisions is the segmentation clock in vertebrate somitogenesis. In vertebrate embryos, somites that develop into vertebra, ribs and muscles in later stages are formed rhythmically from the anterior to the posterior of the embryonic axis. Somitogenesis period is species specific, roughly 30 minutes in zebrafish, 90 minutes in chick and 120 minutes in mouse. Each somite buds off from the unsegmented tissue, the presomitic mesoderm (PSM), one by one with the

period described above. Cells in the posterior PSM exhibit oscillation of gene expression with a period similar to the somitogenesis period. Hence, the cyclic gene expression in the PSM has been considered as a biological clock, referred to as the segmentation clock, that sets the timing of somite formation. The oscillation of gene expression is caused by negative feedback loops including bHLH proteins Hes for mice (*Hes1* and *Hes7*) and Her for zebrafish (such as *Her1* and *Her7*). These proteins bind to the regulatory elements on the DNA and repress their own transcription. The timescales of the negative feedback loops are order of minutes, which generates the ultradian rhythms.

These genetic oscillators in the PSM can interact with each other to synchronize their phase of oscillation. Cells in the tissue express membrane proteins Delta and Notch. Binding of Delta to Notch induces the cleavage of Notch intercellular domain (NICD). NICD goes to the nucleus and activates the transcription of *Hes/Her* genes. In mouse and zebrafish embryos Delta activity or the amount of Delta protein, respectively, oscillates because Delta is regulated by the oscillatory Hes/Her proteins. Thus, cells can send information of their phase to their neighbors through Delta-Notch signaling. Although Delta-Notch signaling is well known for lateral inhibition in neuronal cell differentiation, it synchronizes the phase of the genetic oscillators in vertebrate somitogenesis. Mathematical models for the genetic oscillators and for their synchronization in vertebrate somitogenesis have been proposed and helped understand gene expression dynamics. For more details of somite formation in vertebrate embryos, see reviews (Oates *et al.*, 2012, Pourquie, 2011, Hubaud & Pourquie, 2014).

To understand the dynamics of the genetic oscillators in somitogenesis, live-reporters of these cyclic proteins have been constructed in mouse (Aulehla *et al.*, 2008, Masamizu *et al.*, 2006, Takashima *et al.*, 2011) and zebrafish (Delaune *et al.*, 2012, Soroldoni *et al.*, 2014) embryos. Delaune et al. constructed a live-reporter of Her1 in zebrafish embryos and monitored the oscillation of the concentration of Her1 protein in the PSM (Delaune *et al.*, 2012). They tracked cells undergoing mitosis in the tissue and measured the time evolution of the phase of Her1 oscillation. It was reported that about 10-15% of cells undergo M phase during one oscillation cycle (Horikawa *et al.*, 2006). Delaune et al. found that two sibling cells are likely to have similar phase values after a division (Delaune *et al.*, 2012). This suggests that molecules in their mother cell are equally partitioned between these sibling cells. However, the phases of these divided cells are often delayed compared to the surrounding cells in the PSM (Fig. 2D), indicating that a cell division affects the phase of the genetic oscillators.

As introduced in the example of the circadian clock (Gonze, 2013), partitioning the amount of molecules in a mother cell into two daughter cells could cause phase shifts of the segmentation clock. In addition, duration of M phase, when transcription is repressed, is at least 15 minutes in cells in the PSM (Horikawa *et al.*, 2006), which is comparable to the period of the segmentation clock in zebrafish embryos (~ 30 minutes). Hence, the effect of the duration of M phase on the phase of oscillation could be stronger than that observed in the circadian clock where the period is much longer, about 24 hours.

How do cell divisions affect tissue level oscillations? Zhang et al. compared

phase synchronization of *Her1* gene expression in cell cycle mutant embryos where no cell divisions occur in the tissue with that in sibling embryos where cell divisions occur normally (Zhang *et al.*, 2008). The authors injected morpholinos against two Delta mRNA, *DeltaC* and *DeltaD* into these embryos to block Delta-Notch signaling. In the absence of intercellular interactions through Delta-Notch signaling, synchronization of *Her1* cyclic gene expression across cells is gradually lost due to noise. If cell divisions work as a noise source for the genetic oscillators, synchronization would remain longer in the absence of cell divisions. Zhang et al. found that the degree of phase synchronization at early somite-stage embryos was slightly better in the cell cycle mutants than their siblings, suggesting that cell division is only a modest source of noise for a population of these genetic oscillators (Zhang *et al.*, 2008). Considering the large effect that cell division causes on the phase of the circadian clock, it remains to be solved why cell divisions in the PSM do not affect synchronization so much, even without intercellular interactions.

Phase differences caused by cell divisions among recently divided cells and other surrounding cells are reduced by intercellular interaction through Delta-Notch signaling in wild type animals. Horikawa et al. showed by both experiment and numerical simulations that Delta-Notch signaling keeps dividing cells synchronized with surrounding cells (Horikawa *et al.*, 2006). Interestingly, cells tend to enter M phase when Her1 protein concentration is around a trough of oscillation (Delaune *et al.*, 2012). These cells that enter M phase at a trough of oscillation are synchronized with surrounding cells quicker compared to cells that enter M phase at a different point in the

cycle (Delaune *et al.*, 2012). In addition, the observation of Delaune et al. suggests the possibility that the segmentation clock might regulate the entry to the M phase of the cell cycle. Synchronization dynamics of genetic oscillators in vertebrate somitogenesis has been studied using theoretical models (Riedel-Kruse *et al.*, 2007, Uriu *et al.*, 2010, Ay *et al.*, 2013, Lewis, 2003). Incorporation of cell divisions in such models is desirable, but a proper mathematical description of phase shifts due to cell divisions is still lacking. Thus, understanding the mutual influences of cell divisions and the genetic oscillators would be important to know their synchronization dynamics in the tissue.

In summary, cell divisions can cause phase shifts of genetic oscillators regardless of their characteristic timescales, although phase responses to cell divisions may vary among genetic oscillators. Factors in the cell division process that cause observed phase responses remain to be revealed. Because a divided cell would have a phase value different from that of surrounding cells, intercellular interactions are required to maintain a collective rhythm of genetic oscillators.

In general cell divisions play key roles in development, for example, in tissue growth and cell fate determination. These processes are regulated by the expression of relevant genes. Cell divisions would affect the expression of multiple genes, as well as the components of genetic oscillators. Therefore, it is necessary to address the question of how a division itself affects an intracellular state during development. Theoretical models will identify the factors in the cell division that are most relevant to observed changes in gene expression. Genetic oscillators in dividing cells might be a good model system to address that question.

Cell movement

In embryonic development, massive cell rearrangements occur in tissues. Cells in a tissue can exchange their relative positions by movement. In addition, a population of cells sometimes exhibits a collective motion where they have a correlation in the direction of motions (Uriu *et al.*, 2014). A key question, but one that is poorly understood, is how cell movement affects gene expression dynamics by changing the flow of information across a tissue. In this section, we discuss the effect of cell movement on a population of genetic oscillators. Synchronization of genetic oscillators in vertebrate somitogenesis, as introduced above, would provide a model system to address this question. We will see that movement effectively extends interaction ranges of cells. In general, a longer interaction range is preferable for global synchronization of coupled genetic oscillators. A longer interaction range caused by cell movement can promote synchronization of genetic oscillators. We also discuss different mechanisms for extending intercellular interaction ranges.

Cell movement in vertebrate somitogenesis

During vertebrate somitogenesis, cells in the posterior PSM and tailbud move around (Benazeraf *et al.*, 2010, Lawton *et al.*, 2013). Fgf signaling induces cell movement in these regions and cells move spontaneously with extending cellular protrusions (Benazeraf *et al.*, 2010, Delfini *et al.*, 2005). The type of cell movement in the PSM has been quantified experimentally (Benazeraf *et al.*, 2010, Lawton *et al.*, 2013). In chick embryos cells exhibit random walk in the posterior PSM (Benazeraf *et al.*, 2010). In

contrast, collective cell movement was reported in the zebrafish tailbud (Lawton *et al.*, 2013) where there is a positive correlation of velocities among cells (Fig. 3B). The measured velocity correlation length is around 2-10 cell diameters, indicating that neighboring cells tend to move in a similar direction. Molecules that regulate the collective cell movement were identified, including Wnt, Wnt inhibitor *notum1a*, Fgf and *cadherin2* (Lawton *et al.*, 2013). It was shown that cell movement in the PSM is necessary for the axis elongation of vertebrate embryos (Benazeraf *et al.*, 2010, Lawton *et al.*, 2013). In addition, because these cells are interacting with their local neighbors by Delta-Notch signaling, as we saw in the previous section, exchanging neighboring cells may affect the synchronization dynamics of the genetic oscillators.

Cell movement promotes synchronization

How does cell movement affect synchronization of the coupled genetic oscillators? Theoretical studies revealed that random movement of interacting oscillators (Fig. 3A) promotes their synchronization (Uriu *et al.*, 2010, Uriu *et al.*, 2012). If cells do not move, they keep interacting with their local neighbors and tend to form patches of local synchronization (Fig. 3C). These local patches persist for a long time, making the attainment of global synchronization difficult. When cells move, they can meet and interact with cells initially distant from each other. This prevents the formation of local synchronization and leads to faster attainment of global synchronization across a population (Fig. 3D). Thus, cell movement is considered to effectively extend the interaction range of cells, which promotes synchronization (Uriu *et al.*, 2013).

These previous theoretical models (Uriu *et al.*, 2012, Uriu *et al.*, 2013, Uriu *et al.*, 2010) assumed random cell movement to derive some mathematical formulas. However, collective cell movement was observed in the zebrafish tailbud (Lawton *et al.*, 2013). A question would then be how observed collective cell movement in the tailbud affects synchronization of the population of genetic oscillators. If a group of cells move in a same direction (Fig. 3B), the rate of neighbor exchange could be reduced. Hence, collective cell movement might not enhance synchronization.

The effect of collective cell movement on synchronization of coupled genetic oscillators was studied by using a physical model (Uriu & Morelli, 2014). The model includes spontaneous cell movement and intercellular physical forces such as repulsion and adhesion between cells, as well as the phase dynamics of coupled genetic oscillators. The study revealed by numerical simulations that there is an optimal velocity correlation length to attain synchronization across a population of oscillators. The optimal correlation length found in the simulations is about 2~3 cell diameters (Uriu & Morelli, 2014), which is relatively close to the one observed in experiment (Lawton *et al.*, 2013).

A short-range velocity correlation enhances cell mixing in simulations (Fig. 3E). If cells move randomly and there is no velocity correlation among them in a packed tissue (Fig. 3A), the cells tend to bump into each other because they try to move in different directions. These cells cannot move smoothly, reducing cell mixing. When there is a short-range velocity correlation, neighboring cells tend to move in a same direction, so these cells can move smoothly. Because the correlation length is short, two

neighboring cells move in different directions once they are slightly separated from each other. If the velocity correlation length becomes even longer-ranged, cells can move faster but frequency of neighbor exchange decreases. Thus, there are two opposing effects of collective motions on cell mixing. The balance between these two sets the optimal velocity correlation length for cell mixing (Fig. 3E). A strong cell mixing effectively extends the interaction range of cells. This extended interaction range promotes synchronization of coupled oscillators. Thus, this theoretical study suggested that collective cell movement in the zebrafish tailbud might promote synchronization of genetic oscillators.

These theoretical results need to be tested experimentally by, for example, blocking cell movement and see whether the genetic oscillators lose synchronization. Live imaging techniques developed recently in zebrafish embryos (Delaune *et al.*, 2012, Soroldoni *et al.*, 2014) would be a key to test the theory.

In general, longer interaction ranges of coupled oscillators are favorable to their global synchronization (Uriu *et al.*, 2013). Cell movement would be one mechanism to extend intercellular interaction ranges. In addition to movement, cells in the PSM extend filopodia. It was suggested that these cells might use their filopodia to communicate with the second and third nearest neighbors (Riedel-Kruse *et al.*, 2007). If these filopodia contain Delta and Notch proteins, they could also extend the interaction range of cells and promote synchronization. Indeed, cells in *Drosophila* notum are known to use filopodia to send Delta-Notch signaling to the second and third nearest neighbors in the context of bristle pattern organization (Cohen *et al.*, 2010). Thus,

dynamic cellular behaviors such as cell movement and filopodial extensions could affect collective behaviors of genetic oscillators.

In the circadian clock, cells also generate a collective rhythm across a tissue by intercellular interactions. In the mammalian SCN where the central circadian clock locates, cells do not move. These cells use diffusing neurotransmitters for interactions such as vasoactive intestinal polypeptide (Aton *et al.*, 2005). Because these neurotransmitters diffuse more than one cell diameter, cells can know the phase of distant cells. In contrast, cells in the PSM use Delta-Notch signaling where cells need a direct contact for interactions. In this case, cell movement may serve as a substitute of the diffusing molecule identified in the circadian clock to extend interaction ranges for robust synchronization.

Cell differentiation

In development, a genetic oscillator in a cell typically stops oscillation and the cell differentiates into some other cell types. This is a difference from other molecular clocks like the circadian clock that keeps oscillating until an organism dies. To induce subsequent developmental processes the genetic oscillators need to interact with other signaling pathways. In this section, we discuss how genetic oscillators influence cell differentiation and their possible roles.

Genetic oscillator in neural progenitor cells

A remarkable example of cell differentiation with genetic oscillators is the fate

determination of neural progenitor cells in the developing mouse brain (Imayoshi & Kageyama, 2014, Kageyama *et al.*, 2008). In the early stage of the developing brain, the neural progenitor cells proliferate to increase the population size. At some developmental time points (around E11), these neural progenitor cells start to differentiate into neurons, oligodendrocytes, and astrocytes depending on their states of gene expression. Cell fate determination is conducted by proneural genes, such as *Ascl1* (also known as *Mash1*) and *Neurogenin2* (*Ngn2*). The *Olig2* gene induces oligodendrocyte differentiation. Interestingly, the expression of these genes oscillates in neural progenitor cells. The oscillatory expression of proneural genes allows neural progenitor cells to proliferate. When the expression of one of these proneural genes stops oscillating and is sustained at a higher constant level, a neural progenitor cell begins to differentiate into neurons (Imayoshi *et al.*, 2013, Shimojo *et al.*, 2008). Cells with the higher level of *Olig2* expression differentiate into oligodendrocyte. Other cells with a lower proneural gene expression level remain as neural progenitor cells. Some of them differentiate into astrocytes in later developmental stages.

A driver of the oscillatory proneural gene expression is a negative feedback loop of *Hes1* gene (Fig. 4A). Hes1 proteins repress its own transcription by binding its regulatory region on DNA (Hirata *et al.*, 2002). Hes1 oscillations were imaged live (Shimojo *et al.*, 2008, Imayoshi *et al.*, 2013, Masamizu *et al.*, 2006), and the period of Hes1 oscillation is about 2 to 3 hours. Half-life of Hes1 protein is around 20 minutes (Hirata *et al.*, 2002). Hes1 proteins repress the expression of proneural genes *Ascl1* and *Ngn2* (Fig. 4A), causing their oscillatory expression. Accordingly, the amount of Ascl1

proteins oscillates with a period of 2 to 3 hours (Imayoshi *et al.*, 2013). Oscillations of *Hes1* gene expression have also been observed in embryonic stem cells (Kobayashi *et al.*, 2009) and cells in the PSM (Hirata *et al.*, 2002, Masamizu *et al.*, 2006). The amount of Olig2 proteins oscillates in neural progenitor cells but this oscillation is independent of Hes1 oscillation.

Hes1 influences the cell cycle. In neural progenitor cells, *Hes1* is expressed in S and G2 phases while it is almost absent in G1 phase (Shimojo *et al.*, 2008). It was experimentally shown that *Hes1* retards G1 phase progression in developing mouse brains (Baek *et al.*, 2006). In the context of contact inhibition of cell proliferation in cell culture systems, *Hes1* represses the expression of *E2F-1* that triggers S phase entry (Hartman *et al.*, 2004, Noda *et al.*, 2011). *Hes1*-knockdown cells do not arrest cell cycle even at confluence (Noda *et al.*, 2011). The inhibition of *E2F-1* by *Hes1* is also observed in other cell types (Hartman *et al.*, 2004). However, it has also been known that *Hes1* represses the Cdk inhibitor $p27^{Kip1}$ that is known to retard G1 phase (Murata *et al.*, 2005). Thus, there is contradictory experimental evidence that *Hes1* both promotes and retards cell cycle progression. Given these Hes1's two opposing effects, a question would be how the cell cycle progresses when it is coupled to *Hes1* oscillation.

The neural progenitor cells interact with each other by Delta-Notch signaling. Hes1 proteins repress the production of Delta (Fig. 4A), making the concentration of Delta protein oscillate in neural progenitor cells. Therefore, cells can send information about the phase of oscillation to their neighboring cells, but unlike in vertebrate somitogenesis, the oscillatory Delta proteins seem not to synchronize oscillations.

Rather, it seems to create phase differences between two neighboring cells. When the amount of Hes1 in a neural progenitor cells becomes lower and the activity of proneural genes becomes a higher constant level, the oscillation of Delta expression stops and it also keeps a higher constant level in the cell. The higher level of Delta activity promotes *Hes1* expression in neighboring cells. These neighboring cells remain undifferentiated because higher *Hes1* expression represses the activities of their proneural genes. This process is well known as lateral inhibition. Apart from the developing mouse brain, patterning of neuronal cell differentiation by lateral inhibition with Delta-Notch signaling has been studied extensively using mathematical models (Shaya & Sprinzak, 2011). Recent theoretical studies dealt with cis-inhibition of Delta-Notch (Sprinzak *et al.*, 2011), differentiation waves in tissues (Formosa-Jordan *et al.*, 2012), and versatile functions of Delta-Notch signaling (Agrawal *et al.*, 2009). These studies mainly examined patterning of cell differentiation by the positive feedback loop through Delta-Notch signaling (Fig. 4A). A recently engineered Delta-Notch positive feedback loop demonstrated that the positive feedback is indeed sufficient to induce binary cell fate decisions (Matsuda *et al.*, 2015).

Roles of Hes1 oscillation

For lateral inhibition to work, the oscillation of *Hes1* gene expression is not required as these previous studies have indicated. Then, a question would be what are the roles of Hes1 oscillation in neural progenitor cell differentiation. Does it work as a clock for some purpose? It was demonstrated by optogenetics that the oscillatory expression of

Ascl1 that is regulated by *Hes1* enhances the proliferation of neural progenitor cells (Imayoshi *et al.*, 2013). In addition, *Hes1* itself is also related to the progression of G1 phase in the cell cycle as described above. Thus, it was proposed that *Hes1* oscillation is necessary for the proliferation of neural progenitor cells (Kageyama *et al.*, 2008, Imayoshi & Kageyama, 2014).

To address this question, a mathematical model was developed recently for the gene regulatory network including the cell cycle, *Hes1* and *Ngn* for proliferation and differentiation of neural progenitor cells (Pfeuty, 2015). The model includes the cell cycle regulation by *Hes1* and *Ngn*. In the model, *Hes1* retards G1 phase progression by inhibiting cyclinD. Hence, a high constant amount of Hes1 arrests the cell cycle and cells cannot proliferate but become quiescent. On the other hand, when the amount of Hes1 is low, Ngn activity becomes higher, leading to cell cycle arrest and the differentiation of neural progenitor cells. Thus, only intermediate or oscillatory *Hes1* expression allows the cell cycle to progress in the mathematical model (Pfeuty, 2015).

Since Hes1 oscillation can be observed in undifferentiated cells including embryonic stem cells (Kobayashi *et al.*, 2009), the role of Hes1 oscillation might also be related to differentiation. Using a mathematical model, Pfeuty and Kaneko studied the sensitivity of cell differentiation against noise in the presence of an oscillatory gene activity (Pfeuty & Kaneko, 2014). They considered two different scenarios of binary cell fate decision in the context of bifurcation theory. In the first scenario, the state of a cell changes from a constant steady state to another by an external differentiation signal (Fig. 4B). In the second scenario, an undifferentiated cell first exhibits an oscillatory

state and then changes into a constant steady state to differentiate (Fig. 4C), like the case of neural progenitor cells. The authors considered a small differentiation bias toward one cell type and examined how noise affects the sensitivity to the bias. In the first scenario, the sensitivity to the bias decreases as noise becomes large. In contrast, the sensitivity is not much affected by noise in the second scenario where cells undergo the change from an oscillatory state to the constant steady state (Fig. 4C). Thus, this study proposes another possible role of oscillatory gene expression in ensuring a robust binary fate decision in neural progenitor cells.

In neural progenitor cells the period of *Hes1* oscillation is 2~3 hours. Is this length of the period relevant to cell proliferation and differentiation? An optogenetic gene expression system demonstrated that *Ascl1* induction with a 6-hour period does not affect proliferation of neural progenitor cells (Imayoshi *et al.*, 2013). This result suggests that the period of *Hes1* that generates *Ascl1* oscillation should be shorter than 6 hours for efficient proliferation. The observation also implies that there would be another timescale setting cell proliferation, probably the cell cycle, and the relation between these two timescales is a key. It remains to be revealed that 2~3-hour period of *Hes1* is optimal for the proliferation of neural progenitor cells. Also, a question would be whether the length of the period of a genetic oscillator affects the proportion of different cell types. The engineered Delta-Notch circuits showed that modulation of Delta-Notch signaling by *Lfng* changes the ratio of two different cell types (Matsuda *et al.*, 2015). Varying the period of a genetic oscillator in a theoretical model that includes related gene regulatory network and cell proliferation might answer these questions.

Transition from an oscillatory state to a stationary state

Another key question would be what determines the change from an oscillatory mode to a stationary mode of *Hes1* and Delta-Notch signaling as seen in neural progenitor cells. There may be two possibilities to undergo this transition. One possibility is that the gene regulatory network of *Hes1* itself generates transient oscillation of gene expression followed by a steady lateral inhibition pattern. The other possibility is that some external differentiation signals trigger the transition by changing some biochemical reaction parameters in *Hes1* regulation.

The gene regulatory network in Fig. 4A has both a negative feedback loop of *Hes1* and a positive feedback loop through Delta-Notch signaling. What kinds of dynamics do these two loops generate? Mathematical models for *Hes1* and Delta-Notch regulations can have parameter regions for oscillatory solutions and steady state solutions with lateral inhibition patterns (Momiji & Monk, 2009, Pfeuty, 2015). Hence, the above question may be formalized as how the system transitions from a parameter region for oscillatory solutions to one for stationary solutions. Sensitivity analysis in mathematical models could reveal whether changes in some reaction parameters cause the transition more likely than other parameters.

Momiji and Monk modeled Delta-Notch signaling with a negative feedback loop of *Hes* gene by a system of delay differential equations (Momiji & Monk, 2009). They considered time delays in the intercellular signaling by Delta-Notch and in the negative feedback loop of a *Hes* gene. The authors analyzed the behaviors of two coupled cells mathematically. If the two cells in the model are completely synchronous,

they keep oscillations with the negative feedback. When the initial states of the two cells are similar but not exactly same, the gene regulatory network itself can generate a transient oscillation of gene activities followed by differentiation through lateral inhibition with Delta-Notch signaling. The positive feedback of Delta-Notch gradually amplifies a difference between these two cells, driving the system out from an oscillatory regime. Mathematical analysis revealed that the duration of the transient oscillation depends on the time delays involved in the intercellular signaling and those in the negative feedback loop of *Hes* gene. Thus, the study showed that even without an external signal that changes the behavior of Delta-Notch signaling, the gene regulatory network can inherently cause the change from an oscillatory state to a stationary state.

The mathematical model developed by Pfeuty shows the existence of bistability in the gene regulatory network for the cell cycle and cell differentiation (Pfeuty, 2015): a proliferation state where the cell cycle, Hes1 and Ngn keep oscillating, and a differentiated state where the cell cycle is arrested and Ngn expression is constantly high (and Hes1 is constantly low) coexist. The author considered an external differentiation signal (e.g. Fgf, Tgfβ or Wnt signal) that retards the progression of the cell cycle and represses *Hes1* expression while activating *Ngn* and promoting cell cycle arrest. A model for two coupled cells demonstrated that a pulse of the differentiation signal switches one cell from the proliferation state to the differentiated state. When one cell differentiates by the pulse, the other cell stays in the proliferation state due to the lateral inhibition by Delta-Notch signaling. A phase difference in Hes1 oscillation between the two cells is important for such an asymmetric cell differentiation to occur.

When the phase difference is larger, one cell is more likely to respond to a pulse of the differentiation signal. If Hes1 oscillations of these two cells are synchronous, they need a longer time for one cell to differentiate or sometimes even fail to differentiate. When the two cells are synchronous, they tend to respond to a pulse of the differentiation signal similarly. Then, the lateral inhibition works almost equally between them and it prevents both of them from switching their cellular states.

Could cell divisions influence the mode of *Hes1* gene expression and cell differentiation in the context of bistability? Before cell divisions the expression of *Ascl1* tends to become up-regulated, causing a bias toward neuronal differentiation (Imayoshi *et al.*, 2013). After cell division, daughter cells seem to have almost equal amount of Ascl1 protein (Imayoshi *et al.*, 2013). It remains to be revealed whether small fluctuations caused by cell divisions could switch a cell state from one stable branch to the other in the presence of bistability of gene activities.

In summary, mathematical models have demonstrated that the regulatory network of *Hes1* encompasses both an oscillatory mode and a differentiated mode of gene expression. The mechanisms for the transition between these two modes have not been identified yet. Theoretical modeling can list possible scenarios and future experimental studies will determine the factors responsible for this transition.

Conclusion

In this review, we have discussed behaviors of genetic oscillators in the presence of developmental processes. Cells need to carefully orchestrate their behaviors for

morphogenesis. They must have good time-keeping mechanisms to enable this orchestration. Since a genetic oscillator can provide a reliable clock, it may play a central role in the orchestration. To better understand the dynamics of genetic oscillators in a developing tissue, it will be necessary to examine the influences of various developmental processes on the oscillations. Timescale comparisons can help estimate their influences on the oscillator, and live imaging of both genetic oscillators and cellular behaviors simultaneously allows such timescale comparisons. Interactions between oscillators and other developmental signaling events will be another important theme. Theoretical modeling and numerical simulations are powerful tools to analyze these processes, and in combination with advances in imaging and genetic perturbation techniques, will shed light on the key issue of timing in development.

Acknowledgement

The author thanks Gen Kurosawa, Luis G. Morelli, Andrew C. Oates and Hajime Tei for helpful comments on the manuscript. This work was supported by JSPS KAKENHI Grant Number 26840085.

References

Agrawal, S., Archer, C. & Schaffer, D. V. 2009. Computational models of the Notch network elucidate mechanisms of context-dependent signaling. *PLoS Comput. Biol.,* **5,** e1000390.

Alon, U. 2006. *An Introduction to Systems Biology: Design Principles of Biological Circuit.* Chapman and Hall/CRC Press/Taylor & Francis, Boca Raton, FL.

Aton, S. J., Colwell, C. S., Harmar, A. J., Waschek, J. & Herzog, E. D. 2005.

Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nat. Neurosci.,* **8,** 476-483.

Aulehla, A., Wiegraebe, W., Baubet, V. et al. 2008. A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. *Nat. Cell Biol.,* **10,** 186-193.

Ay, A., Knierer, S., Sperlea, A., Holland, J. & Ozbudak, E. M. 2013. Short-lived Her proteins drive robust synchronized oscillations in the zebrafish segmentation clock. *Development,* **140,** 3244-3253.

Baek, J. H., Hatakeyama, J., Sakamoto, S., Ohtsuka, T. & Kageyama, R. 2006. Persistent and high levels of Hes1 expression regulate boundary formation in the developing central nervous system. *Development,* **133,** 2467-2476.

Barkai, N. & Leibler, S. 2000. Circadian clocks limited by noise. *Nature,* **403,** 267-268.

Benazeraf, B., Francois, P., Baker, R. E., Denans, N., Little, C. D. & Pourquie, O. 2010. A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature,* **466,** 248-252.

Bieler, J., Cannavo, R., Gustafson, K., Gobet, C., Gatfield, D. & Naef, F. 2014. Robust synchronization of coupled circadian and cell cycle oscillators in single mammalian cells. *Mol. Sys. Biol.,* **10,** 739.

Chen, Y., Kim, J. K., Hirning, A. J., Josic, K. & Bennett, M. R. 2015. SYNTHETIC BIOLOGY. Emergent genetic oscillations in a synthetic microbial consortium. *Science,* **349,** 986-989.

Clay, J. R. & Dehaan, R. L. 1979. Fluctuations in interbeat interval in rhythmic heart-cell clusters. Role of membrane voltage noise. *Biophys. J.,* **28,** 377-389.

Cohen, M., Georgiou, M., Stevenson, N. L., Miodownik, M. & Baum, B. 2010. Dynamic filopodia transmit intermittent Delta-Notch signaling to drive pattern refinement during lateral inhibition. *Dev. Cell,* **19,** 78-89.

Delaune, E. A., Francois, P., Shih, N. P. & Amacher, S. L. 2012. Single-cell-resolution imaging of the impact of Notch signaling and mitosis on segmentation clock dynamics. *Dev. Cell,* **23,** 995-1005.

Delfini, M. C., Dubrulle, J., Malapert, P., Chal, J. & Pourquie, O. 2005. Control of the segmentation process by graded MAPK/ERK activation in the chick embryo. *Proc. Natl. Acad. Sci. U. S. A.,* **102,** 11343-11348.

Elowitz, M. B. & Leibler, S. 2000. A synthetic oscillatory network of transcriptional

regulators. *Nature,* **403,** 335-338.

Feillet, C., Krusche, P., Tamanini, F. et al. 2014. Phase locking and multiple oscillating attractors for the coupled mammalian clock and cell cycle. *Proc. Natl. Acad. Sci. U. S. A.,* **111,** 9828-9833.

Ferrell, J. E., Jr., Tsai, T. Y. & Yang, Q. 2011. Modeling the cell cycle: why do certain circuits oscillate? *Cell,* **144,** 874-885.

Formosa-Jordan, P., Ibanes, M., Ares, S. & Frade, J. M. 2012. Regulation of neuronal differentiation at the neurogenic wavefront. *Development,* **139,** 2321-2329.

Friedel, C. C., Dolken, L., Ruzsics, Z., Koszinowski, U. H. & Zimmer, R. 2009. Conserved principles of mammalian transcriptional regulation revealed by RNA half-life. *Nucleic. Acids. Res.,* **37,** e115.

Gerard, C. & Goldbeter, A. 2012. Entrainment of the mammalian cell cycle by the circadian clock: modeling two coupled cellular rhythms. *PLoS Comput. Biol.,* **8,** e1002516.

Goldbeter, A., Gerard, C., Gonze, D., Leloup, J. C. & Dupont, G. 2012. Systems biology of cellular rhythms. *FEBS Lett.,* **586,** 2955-2965.

Gonze, D. 2013. Modeling the effect of cell division on genetic oscillators. *J. Theor. Biol.,* **325,** 22-33.

Gottesfeld, J. M. & Forbes, D. J. 1997. Mitotic repression of the transcriptional machinery. *Trends Biochem. Sci.,* **22,** 197-202.

Grechez-Cassiau, A., Rayet, B., Guillaumond, F., Teboul, M. & Delaunay, F. 2008. The circadian clock component BMAL1 is a critical regulator of p21WAF1/CIP1 expression and hepatocyte proliferation. *J. Biol Chem.,* **283,** 4535-4542.

Hartman, J., Muller, P., Foster, J. S., Wimalasena, J., Gustafsson, J. A. & Strom, A. 2004. HES-1 inhibits 17beta-estradiol and heregulin-beta1-mediated upregulation of E2F-1. *Oncogene,* **23,** 8826-8833.

Herrgen, L., Ares, S., Morelli, L. G., Schroter, C., Julicher, F. & Oates, A. C. 2010. Intercellular coupling regulates the period of the segmentation clock. *Curr. Biol.,* **20,** 1244-1253.

Hirata, H., Yoshiura, S., Ohtsuka, T. et al. 2002. Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop. *Science,* **298,** 840-843.

Horikawa, K., Ishimatsu, K., Yoshimoto, E., Kondo, S. & Takeda, H. 2006. Noise-resistant and synchronized oscillation of the segmentation clock. *Nature,*

441, 719-723.

Hubaud, A. & Pourquie, O. 2014. Signalling dynamics in vertebrate segmentation. *Nat. Rev. Mol. Cell Biol.,* **15,** 709-721.

Imayoshi, I., Isomura, A., Harima, Y. et al. 2013. Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. *Science,* **342,** 1203-1208.

Imayoshi, I. & Kageyama, R. 2014. bHLH factors in self-renewal, multipotency, and fate choice of neural progenitor cells. *Neuron,* **82,** 9-23.

Kageyama, R., Ohtsuka, T., Shimojo, H. & Imayoshi, I. 2008. Dynamic Notch signaling in neural progenitor cells and a revised view of lateral inhibition. *Nat. Neurosci.,* **11,** 1247-1251.

Kobayashi, T., Mizuno, H., Imayoshi, I., Furusawa, C., Shirahige, K. & Kageyama, R. 2009. The cyclic gene Hes1 contributes to diverse differentiation responses of embryonic stem cells. *Genes Dev.,* **23,** 1870-1875.

Kori, H., Kawamura, Y. & Masuda, N. 2012. Structure of cell networks critically determines oscillation regularity. *J. Theor. Biol.,* **297,** 61-72.

Kowalska, E., Ripperger, J. A., Hoegger, D. C. et al. 2013. NONO couples the circadian clock to the cell cycle. *Proc. Natl. Acad. Sci. U. S. A.,* **110,** 1592-1599.

Kurosawa, G. & Iwasa, Y. 2002. Saturation of enzyme kinetics in circadian clock models. *J. Biol. Rhythms,* **17,** 568-577.

Kurosawa, G. & Iwasa, Y. 2005. Temperature compensation in circadian clock models. *J. Theor. Biol.,* **233,** 453-468.

Lawton, A. K., Nandi, A., Stulberg, M. J. et al. 2013. Regulated tissue fluidity steers zebrafish body elongation. *Development,* **140,** 573-582.

Lewis, J. 2003. Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. *Curr. Biol.,* **13,** 1398-1408.

Lowrey, P. L. & Takahashi, J. S. 2011. Genetics of circadian rhythms in Mammalian model organisms. *Adv. Genet.,* **74,** 175-230.

Masamizu, Y., Ohtsuka, T., Takashima, Y. et al. 2006. 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.,* **103,** 1313-1318.

Matsuda, M., Koga, M., Woltjen, K., Nishida, E. & Ebisuya, M. 2015. Synthetic lateral inhibition governs cell-type bifurcation with robust ratios. *Nat. Commun.,* **6,**

6195.

Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F. & Okamura, H. 2003. Control mechanism of the circadian clock for timing of cell division in vivo. *Science,* **302,** 255-259.

Mohawk, J. A., Green, C. B. & Takahashi, J. S. 2012. Central and peripheral circadian clocks in mammals. *Annu. Rev. Neurosci.,* **35,** 445-462.

Momiji, H. & Monk, N. A. 2009. Oscillatory Notch-pathway activity in a delay model of neuronal differentiation. *Phys. Rev. E, Stat., Nonlin., Soft Matter Phys.,* **80,** 021930.

Morelli, L. G. & Julicher, F. 2007. Precision of genetic oscillators and clocks. *Phys. Rev. Lett.,* **98,** 228101.

Murata, K., Hattori, M., Hirai, N. et al. 2005. Hes1 directly controls cell proliferation through the transcriptional repression of p27Kip1. *Mol. Cell. Biol.,* **25,** 4262-4271.

Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naef, F. & Schibler, U. 2004. Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell,* **119,** 693-705.

Noda, N., Honma, S. & Ohmiya, Y. 2011. Hes1 is required for contact inhibition of cell proliferation in 3T3-L1 preadipocytes. *Genes Cells,* **16,** 704-713.

Novak, B. & Tyson, J. J. 2008. Design principles of biochemical oscillators. *Nat. Rev. Mol. Cell Biol.,* **9,** 981-991.

Oates, A. C., Morelli, L. G. & Ares, S. 2012. Patterning embryos with oscillations: structure, function and dynamics of the vertebrate segmentation clock. *Development,* **139,** 625-639.

Pfeuty, B. 2015. A computational model for the coordination of neural progenitor self-renewal and differentiation through Hes1 dynamics. *Development,* **142,** 477-485.

Pfeuty, B. & Kaneko, K. 2014. Reliable binary cell-fate decisions based on oscillations. *Phys. Rev. E, Stat., Nonlin., Soft Matter Phys.,* **89,** 022707.

Pomerening, J. R., Kim, S. Y. & Ferrell, J. E., Jr. 2005. Systems-level dissection of the cell-cycle oscillator: bypassing positive feedback produces damped oscillations. *Cell,* **122,** 565-578.

Pomerening, J. R., Sontag, E. D. & Ferrell, J. E., Jr. 2003. Building a cell cycle

oscillator: hysteresis and bistability in the activation of Cdc2. *Nat. Cell Biol.,* **5,** 346-351.

Pourquie, O. 2011. Vertebrate segmentation: from cyclic gene networks to scoliosis. *Cell,* **145,** 650-663.

Riedel-Kruse, I. H., Muller, C. & Oates, A. C. 2007. Synchrony dynamics during initiation, failure, and rescue of the segmentation clock. *Science,* **317,** 1911-1915.

Sehgal, A. 2015. *Circadian Rhythms and Biological Clocks, Part A.* Academic Press, Waltham.

Shaya, O. & Sprinzak, D. 2011. From Notch signaling to fine-grained patterning: Modeling meets experiments. *Curr. Opin. Genet. Dev.,* **21,** 732-739.

Shimojo, H., Ohtsuka, T. & Kageyama, R. 2008. Oscillations in notch signaling regulate maintenance of neural progenitors. *Neuron,* **58,** 52-64.

Soroldoni, D., Jorg, D. J., Morelli, L. G. et al. 2014. Genetic oscillations. A Doppler effect in embryonic pattern formation. *Science,* **345,** 222-225.

Sprinzak, D., Lakhanpal, A., Lebon, L., Garcia-Ojalvo, J. & Elowitz, M. B. 2011. Mutual inactivation of Notch receptors and ligands facilitates developmental patterning. *PLoS Comput. Biol.,* **7,** e1002069.

Stricker, J., Cookson, S., Bennett, M. R., Mather, W. H., Tsimring, L. S. & Hasty, J. 2008. A fast, robust and tunable synthetic gene oscillator. *Nature,* **456,** 516-519.

Strogatz, S. H. 1994. *Nonlinear Dynamics and Chaos: With Applications to Physics, Biology, Chemistry and Engineering.* Addison-Wesley, MA.

Takashima, Y., Ohtsuka, T., Gonzalez, A., Miyachi, H. & Kageyama, R. 2011. Intronic delay is essential for oscillatory expression in the segmentation clock. *Proc. Natl. Acad. Sci. U. S. A.,* **108,** 3300-3305.

Tsai, T. Y., Choi, Y. S., Ma, W., Pomerening, J. R., Tang, C. & Ferrell, J. E., Jr. 2008. Robust, tunable biological oscillations from interlinked positive and negative feedback loops. *Science,* **321,** 126-129.

Uriu, K., Ares, S., Oates, A. C. & Morelli, L. G. 2012. Optimal cellular mobility for synchronization arising from the gradual recovery of intercellular interactions. *Phys. Biol.,* **9,** 036006.

Uriu, K., Ares, S., Oates, A. C. & Morelli, L. G. 2013. Dynamics of mobile coupled phase oscillators. *Phys. Rev. E, Stat., Nonlin., Soft Matter Phys.,* **87,** 032911.

Uriu, K. & Morelli, L. G. 2014. Collective cell movement promotes synchronization of coupled genetic oscillators. *Biophys. J.,* **107,** 514-526.

Uriu, K., Morelli, L. G. & Oates, A. C. 2014. Interplay between intercellular signaling and cell movement in development. *Semin. Cell Dev. Biol.,* **35,** 66-72.

Uriu, K., Morishita, Y. & Iwasa, Y. 2009. Traveling wave formation in vertebrate segmentation. *J. Theor. Biol.,* **257,** 385-396.

Uriu, K., Morishita, Y. & Iwasa, Y. 2010. Random cell movement promotes synchronization of the segmentation clock. *Proc. Natl. Acad. Sci. U. S. A.,* **107,** 4979-4984.

Vilar, J. M., Kueh, H. Y., Barkai, N. & Leibler, S. 2002. Mechanisms of noise-resistance in genetic oscillators. *Proc. Natl. Acad. Sci. U. S. A.,* **99,** 5988-5992.

Yang, Q., Pando, B. F., Dong, G., Golden, S. S. & Van Oudenaarden, A. 2010. Circadian gating of the cell cycle revealed in single cyanobacterial cells. *Science,* **327,** 1522-1526.

Zhang, E. E. & Kay, S. A. 2010. Clocks not winding down: unravelling circadian networks. *Nat. Rev. Mol. Cell Biol.,* **11,** 764-776.

Zhang, L., Kendrick, C., Julich, D. & Holley, S. A. 2008. Cell cycle progression is required for zebrafish somite morphogenesis but not segmentation clock function. *Development,* **135,** 2065-2070.

Zwicker, D., Lubensky, D. K. & Ten Wolde, P. R. 2010. Robust circadian clocks from coupled protein-modification and transcription-translation cycles. *Proc. Natl. Acad. Sci. U. S. A.,* **107,** 22540-22545.

Figure legends

Figure 1. A negative feedback loop generates sustained oscillation. (A) Negative feedback loop including a single gene. The line with a perpendicular bar indicates repression. (B) Negative feedback loop including three genes. Arrows indicate activation. (C) One variable negative feedback model with time delay τ. $p(t)$ represents a protein concentration at time t, a is synthesis rate, p_0 is the threshold constant for repression, n is the Hill coefficient and b is the degradation rate. (D) Hill function. (E)

Time evolution of p in (C). (F), (G) Dependence of the period of oscillation on (F) the time delay τ and (G) the half-life of the molecule (ln2)/b.

Figure 2. A cell division influences genetic oscillators. (A) Simplified gene regulatory network of the circadian clock. Arrows indicate activation and lines with a perpendicular bar indicate repression. (B) Cell cycle gating by the circadian clock. (C) Time evolution of reporter intensity of a circadian clock gene with (red) and without (black) a cell division. Top (bottom): A division occurs at increasing (decreasing) phase of the reporter. The black and red bars indicate time intervals between two successive peaks. (D) A cell division affects the phase of a Her1 reporter. The red cell divides and two daughter cells (red and orange) emerge. Sibling cells keep synchronization after the division while their phases are delayed to surrounding cells (green).

Figure 3. Collective cell movement promotes synchronization of genetic oscillators. (A), (B) Two types of cell movement (A) random movement and (B) collective movement. (C), (D) Snapshots of spatial phase profiles of genetic oscillators. Oscillators are arranged in a two-dimensional space. The color code indicates the phase of oscillators. (C) Oscillators form patches of local synchronization and (D) they attain nearly global synchronization. (E) Cell mixing rate as a function of the velocity correlation length. The cell mixing rate has a maximum at the velocity correlation length of 2 to 3 diameters. A larger cell mixing rate enhances synchronization of oscillators.

Figure 4. A genetic oscillator regulates cell differentiation. (A) Simplified gene regulatory network of neural progenitor cells. *Hes1* both promotes and retards cell cycle progression (black dotted lines), see the text. Arrows indicate activation and lines with a perpendicular bar indicate repression. (B), (C) Binary cell fate decisions (B) from a steady state to another steady state and (C) from an oscillatory state to a steady state. The cells change their states depending on the level of a differentiation signal (not shown).

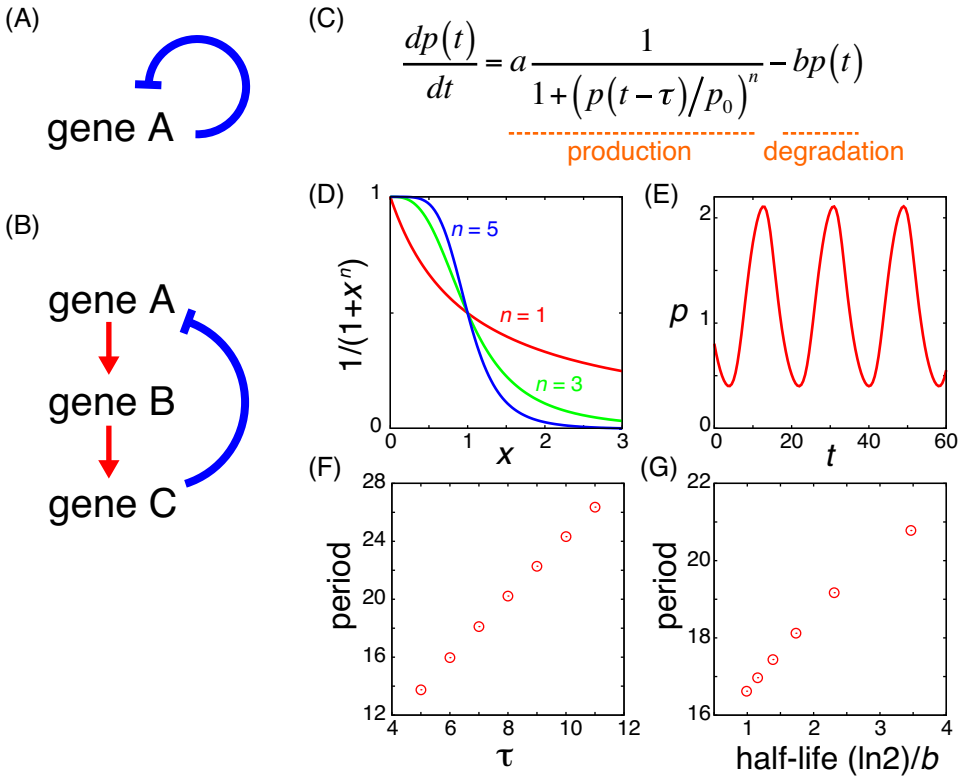

Figure 1

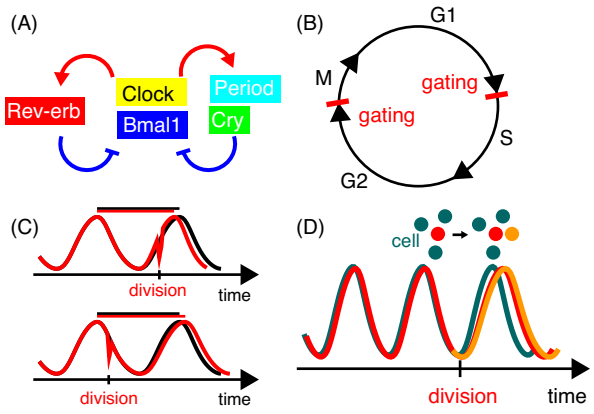

Figure 2

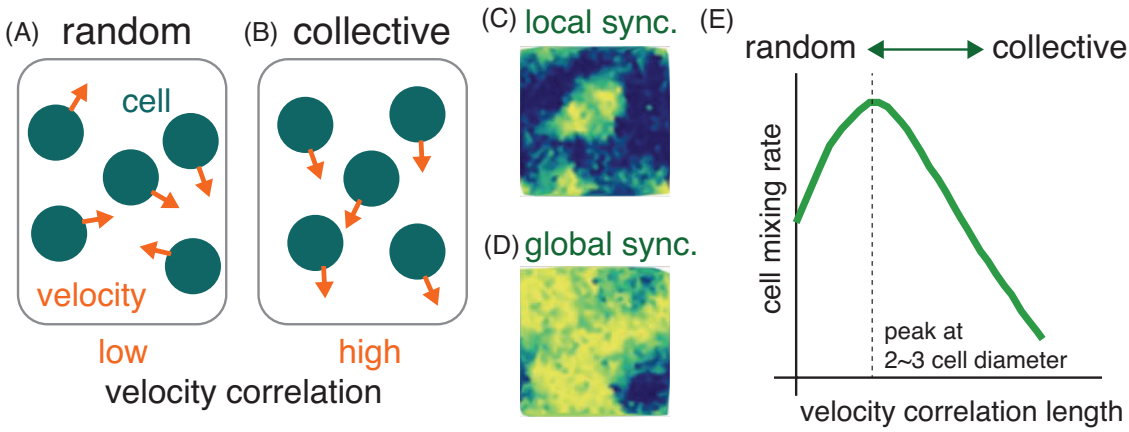

Figure3

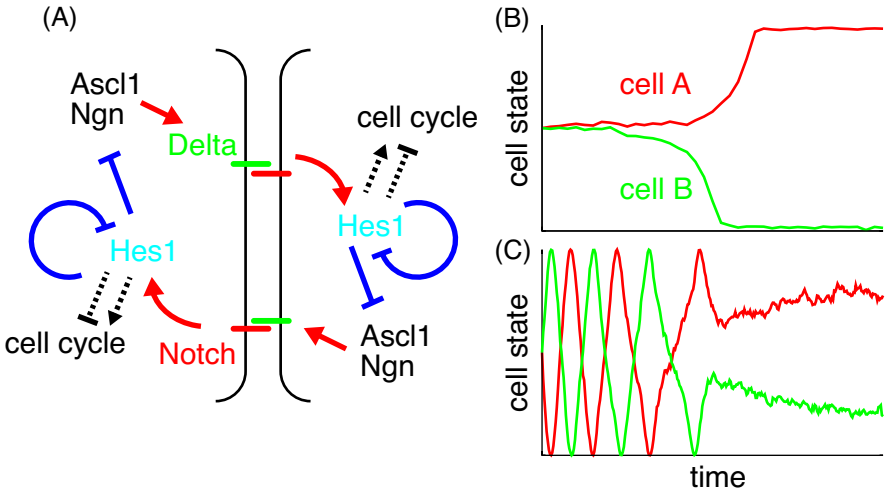

Figure4