Simulated Cell Division Processes of the *Xenopus* Cell Cycle Pathway by Genomic Object Net

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Summary

Matsuno et al.[1] modeled and simulated that multicellular patterning by the *Drosophila* Delta-Notch signaling pathway by using the software "Genomic Object Net" which was developed based on hybrid functional Petri net (HFPN) architecture. In this model, cellular formation is fixed throughout the simulation. This paper constructs an HFPN model of the *Xenopus* cell cycle pathway, which includes the mechanism for cell division control as well as checkpoint processes. This model simulates dynamic cell division processes of the early *Xenopus* embryo, including the changes in cell division cycles from synchronous to asynchronous.

1 Introduction

We have been developing a biosimulation system, Genomic Object Net (GON), whose architecture is based on hybrid functional Petri net (HFPN) [2,3] and XML technology [4]. With GON, we have modeled and simulated many biopathways, including the gene switch mechanisms of λ phage [2,5], the gene regulation for circadian rhythm in *Drosophila* [2,5], the signal transduction pathway for apoptosis induced by the protein Fas [3,5], and the glycolytic pathway in *E.coli* with the *lac* operon gene regulatory mechanism [5].

Moreover, we tried to apply the HFPN modeling technique to multicellular development systems and succeeded in simulating *Drosophila* multicellular patterning by the Delta-Notch signaling pathway with GON [1]. As a next step for the multicellular simulation, this paper constructs an HFPN model of the *Xenopus* cell cycle pathway and presents a method for modeling the cell division mechanism in the early *Xenopus* embryo, where the changes in cell division cycles from synchronous to asynchronous take place [6,7].

The constructed HFPN model of the *Xenopus* cell cycle pathway includes the mechanism for checkpoint processes as well as the mechanism for cell division control. In the simulation, we show the influences of the checkpoint mechanism on four phases G1, S, G2 and M of the *Xenopus* cell cycle. In addition, cell dividing processes are visualized by “GON Visualizer” with CSV data exported from the simulations by GON.

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2 Required Facts for Pathway Construction

Construction of the HFPN model of the *Xenopus* cell division mechanism is based on the following facts.

(A) Although the somatic cell cycle of *Xenopus* consists of the M phase and S phase with the two intervening G1 and G2 phases, the early embryonic cell cycle of *Xenopus* rapidly alternates M phase and S phase without the G1 and G2 phases. The early embryonic cell cycle continues until completion of the 12th mitotic division. The period of this 12th mitotic division is known as the midblastula transition (MBT). After the MBT, the cycle lengthens and becomes asynchronous, and zygotic transcription begins [6,7].

(B) The cell cycle is driven by the M-phase promoting factor (MPF), which is activated at G2/M and inactivated at the end of M phase [8]. On the other hand, Strausfeld et al. [9] defined the S-phase promoting factor (SPF) as cyclin dependent kinase activity required for DNA replication, which is activated at G1/S and inactivated at the end of S phase. In summary, it can be considered that complementary oscillations MPF and SPF determine the periods of M phase, S phase, and the two gap phases G1 and G2.

(C) It is known that the cell cycle period of the early *Xenopus* embryo changes depending on cell size [10]. That is, until the size of a cell reaches approximately 37.5 micrometers after several divisions, the period of cell cycle has not changed. After this point, the cell cycle changes depending on the cell size; as size becomes smaller, the cell cycle period becomes longer.

3 Cycle Pathway Model Including Cell Division Mechanism by Hybrid Functional Petri Net

Hybrid Petri net (HPN) [11] has been introduced as an extension of the discrete Petri net [12]. In the HPN, two kinds of places and transitions are used: discrete/continuous places and discrete/continuous transitions. A discrete place and a discrete transition are the same notions used in the discrete Petri net. A continuous place holds a nonnegative real number as its content. A continuous transition fires continuously in the HPN and its firing speed is given as a function of values in the places in the model. The HFPN [3] was introduced by expanding the notion of HPN. For example, any functions can be assigned to arc/transition for controlling the speed/condition of consumption/production/firing. These expansions allow us to model various aspects in biopathways very easily [3,5].

M-phase promoting factor (MPF), which is a dimer of cyclin-dependent protein kinase (Cdc2) and cyclin B (CycB), is essential to initiate mitosis. Although the concentration of Cdc2 keeps at a constant level throughout the cell cycle, the concentration of CycB fluctuates considerably, being low in G1 and rising steadily through S, G2 and early M phases.

S-phase promoting factor (SPF) was firstly defined by Strausfeld et al. [9] as analogous to MPF. By associating with cyclin-dependent kinase 2 (Cdk2), cyclin A and cyclin E (CycE) regulate S-phase progression [9]. However, in the early *Xenopus* embryo, most Cdk2 exists as a complex with CycE [13]. In this paper, we treat the complex of Cdk2 and CycE as SPF.

By focusing on the dynamic behaviors of MPF and SPF, we can know which of four phases G1, S, G2, and M a cell is in. In the following, we construct HFPN pathways for regulation mechanisms of the MPF and the SPF. Unless otherwise noted, for modeling HFPN pathways, we used the information described in the papers Marlovits et al. [6] and Iwabuchi et al. [7].

3.1 HFPN Pathway for MPF Regulation

Figure 1 shows the constructed HFPN pathway for the MPF regulation. Refer to the literature [2,3] for the basic methods to represent simple biological reactions, such as protein production and protein degradation by using hybrid (functional) Petri net elements.

This pathway consists of four types of basic reactions: protein composition and decompositions, enzyme reaction, protein degradation, and feedback circuits. In the following, we will explain how these basic reactions are used in Figure 1. In all transitions, formulas describing reaction speeds are assigned. However, we omit to describe these formulas in Figure 1 in order to simplify the view of this figure. Readers can view and obtain the complete version of this figure from the website [14].

The catalytic activity of Cdc2 is determined not only by the availability of CycB, but also by phosphorylation at the sites of three specific amino acids: Thr-14, Tyr-15, and Thr-161. Thr-161, which is phosphorylated by Cdc2 activating kinase (CAK), is necessary for Cdc2 kinase activity. That is, the MPF can be active only when Thr-161 is phosphorylated. Thr-14 and Tyr-15 activity, which inhibits Cdc2 kinase activity, is high during S phase and early G2 phase and decreases rapidly as cells enter M phase.

3.1.1 Protein composing and decomposing

An HFPN for the reaction that forms the complex of proteins Cdc2 and CycB is described with the places CycB, Cdc2, Cdc2/CycB, the transition T1, and the arcs connecting these places and the transition. The rate of complex formation is given at the transition T1, which depends on the amounts of the places CycB and Cdc2.

Four reactions of protein decomposition are contained in Figure 1. The decomposition rates of corresponding protein complexes are assigned at the transitions connected to the arcs from the places Cdc2/CycB, Cdc-p14p15/CycB, Cdc2-3p/CycB, and Cdc2-p161/CycB,

3.1.2 Enzyme reactions

Phosphorylations (Dephosphorylations) of Thr-14 and Tyr-15 are carried out by two enzymes Wee1 and Myt1 kinases (Cdc25 kinase). However, we treat the Wee1 and Myt1 kinases as a single kinase, Wee1, since they collaborate in phosphorylating Cdc2 and are regulated similarly.

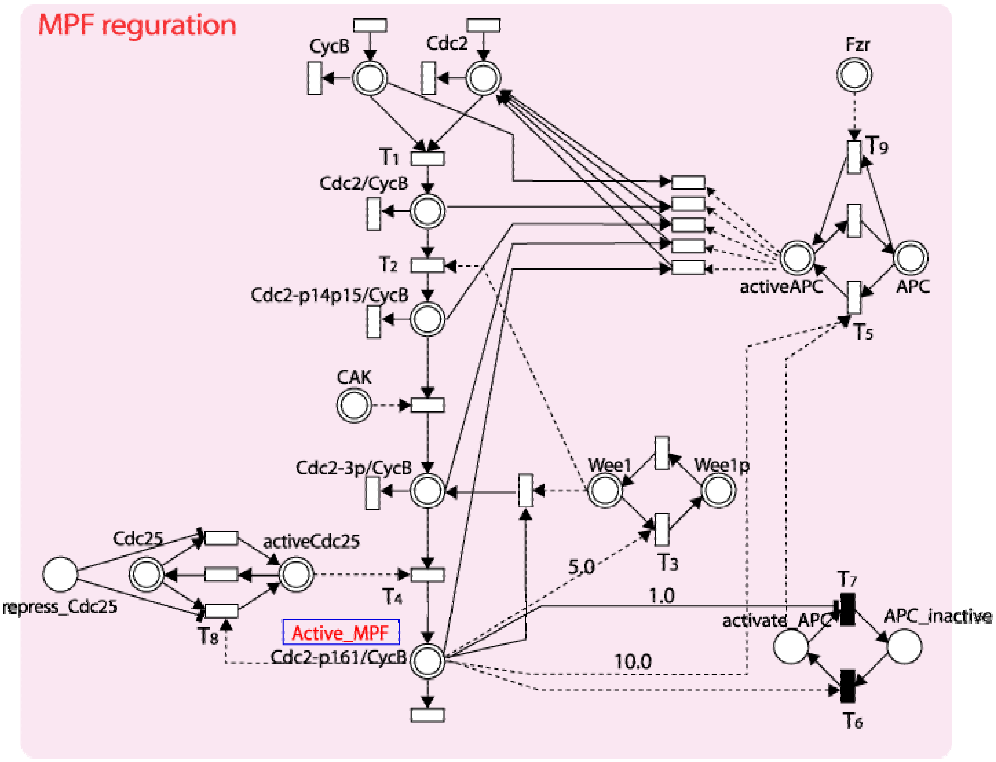

Figure 1. An HFPN model for MPF regulation. In the MPF regulation, four complex types of proteins Cdc2 and CycB play essential roles, which are characterized by the phosphorylation patterns for the three sites Thr-14, Tyr-15, and Thr-161. The places (1) Cdc2/CycB, (2) Cdc2-p14p15/CycB, (3) Cdc2-3p/CycB, and (4) Cdc2-p161/CycB correspond to the four complex types of proteins Cdc2 and CycB: (1) no phosphorylation, (2) Thr-14 and Tyr-15 are phosphorylated, (3) Thr-161 is phosphorylated, and (4) all of three sites are phosphorylated, respectively.

The places Cdc2/CycB, Cdc2-p14p15/CycB, Wee1, the transition T2, and the arcs connecting these elements constitute one enzyme reactions. To represent this enzyme reaction, we used the test arc from the place Wee1 to the transition T2 (Note that, because of the nature of enzyme, test arc is used as an outgoing arc out from the enzyme place for an enzyme). The proteins corresponding to the places CAK and activeCdc25, as well as Wee1, function as enzymes. For the places Wee1 and activeCdc25, bidirectional reactions are adopted for inactivating reactions.

3.1.3 Protein degradation

MPF activates anaphase-promoting complex (APC), which indirectly initiates the degradation of CycB. In addition, from the fact suggested in Kotani et al. [15], it can be assumed that protein Fzr plays an essential role for APC in breaking down CycB during the G1 phase.

In Figure 1, five test arcs are used from the place activeAPC to the transitions for the CycB degradation. The activation of the APC is carried out by firing the transition T5. The test arc from the place Cdc2-p161/CycB to the transition T5, explained in the above subsection, is used so that Cdc2-p161/CycB works as an enzyme activating the APC. The transition T9 is used for representing the reaction promoting APC activation via the protein Fzr.

In addition, one more test arc comes from the discrete place activate_APC to the transition T5. This place activate_APC can get a token when the discrete transition T6 fires. In order to fire this transition, the content of the place Cdc2-p161/CycB should exceed 10.0, which is the weight of the arc from this place to the transition T5; and at the same time, the discrete place APC_inactive should have at least one token. If the content of the place

Cdcp161/CycB becomes less than 1.0, which is assigned at the inhibitory arc from this place to the transition T7, the place activate_APC loses its token, transferring this token to the place APC_inactive through the transition T7.

3.1.4 Feedback circuits

Feedbacks make the mitotic control system particularly sensitive to cellular conditions and decisive in its responses. Figure 1 contains negative and positive feedback circuits for the reaction of MPF.

- Negative feedback

  When the token value of the place Cdc2-p161/CycB exceeds the level 5.0, the token in the place Wee1 is transferred to the place Wee1p according to the reaction rate at the transition T3. Since the Wee1 works as an enzyme which converts the active MPF (Cdc-p161/CycB) to inactive form, the more the amount in the place Wee1 increases, the more the amount in the place Cdc2-p161/CycB decreases. Thus, this series of reactions establishes the negative feedback for activating MPF.

- Positive feedback

  As the amount in the place Cdc2-p161/CycB grows, the reaction speed of the transition T8 becomes faster, increasing the amount in the place activeCdc25. Consequently, the reaction at the transition T4 is promoted. These series of reactions establish the positive feedback for activating MPF.

3.2 HFPN Pathway for SPF Regulation

Figure 2 shows an HFPN pathway for SPF regulation. In the following, the HFPN model for the SPF regulation pathway is explained by referring the places in the HFPN model which correspond to proteins in the SPF regulation pathway.

Cdk2 and CycE form a complex CycE/Cdk2 (SPF) [9] which is converted to active form CycE/Cdk2active by activeCdc25A or maternalMBTactiveCdc25A [16,17]. According to the suggestion by Hartley et al. [18], maternal CycE is included as well as zygotic CycE. Although the complex CycE/Cdk2 is decomposed to Cdk2 and DegradCycE, the mechanism of this reaction is unknown. Based on our assumption that there is an enzyme which works as APC in the MPF regulation mechanism, we describe the same HFPN component as the MPF regulation mechanism in Figure 2. When the place activate_CycE_degrad gets a token, CycE degradation begins. Because of the fact that the level of CycE remains constant until the MBT [13,18], there is a path from the place DegradCycE to the place CycE via one transition which is activated while the place keep_CycE_level has a token.

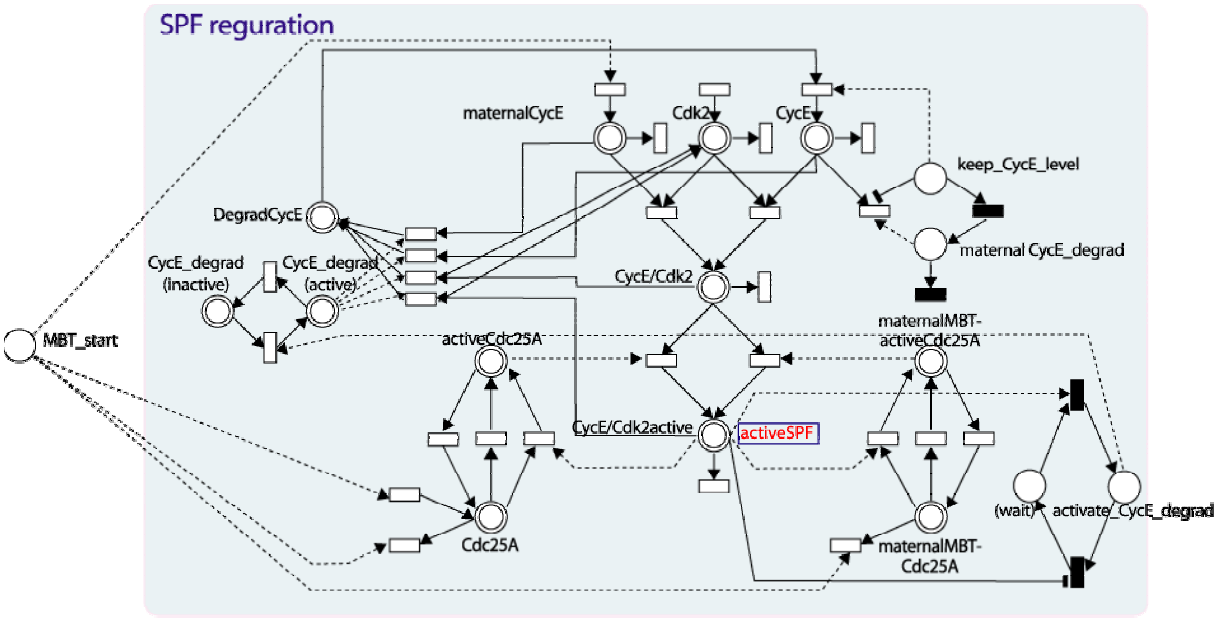

Figure 2. An HFPN model for SPF regulation. This pathway contains reactions of protein composing and decomposing, enzyme reaction, and protein degradation. Refer to the corresponding explanations in Section 3.2 for these reactions.

3.3 Mechanisms for Cell Division Control and Checkpoint Sequences

Figure 3 shows mechanisms by the HFPN which process cell division control and checkpoint sequences.

When the concentration level of Cdc2-p161/CycB becomes low, the place M_end gets a token through one discrete transition. At the same time, the number of tokens in the place mitosis time is incremented. When the number of tokens in this place reaches the weight at the test arc from this place, the place MBT_start gets a token. This means that, including G1 and G2 phases from this point, the cell cycle lengthens, becomes asynchronous, and zygotic transcription begins.

Initially, the place ensure_one_time should have one token so that each DNA damage checkpoint process, DNA replication process, and cell division occurs exactly once per cell.

- When a cell is in G1 phase (the place G1 keeps a token);
    - a token is produced at the transition T10, transferring to the place noDNAdamage(G1) unless the place damagedDNA(G1) contains no token. (By putting a token in this place, we can track the amount DNA damaged.)

In order to exit G1 phase (by firing the transition T11), the following three conditions should be fulfilled: (1) a cell is in G1 phase, (2) DNA is not damaged, (3) according to the observation that the cell cycle elongates during the G1 phase [19], the delay time of the transition T11 is set to be inversely proportional to the size of the cell.

- Check DNA damage and DNA replication after exiting S phase;
    - After the S phase is finished, DNA damage and DNA replication checks have to be carried out. When the place DNAdamage_check (DNAreplication_check) has a token, a cell is ready to check them. By putting a token in the place damagedDNA(S) (failedDNAreplication), we can represent the abnormal status of a cell.

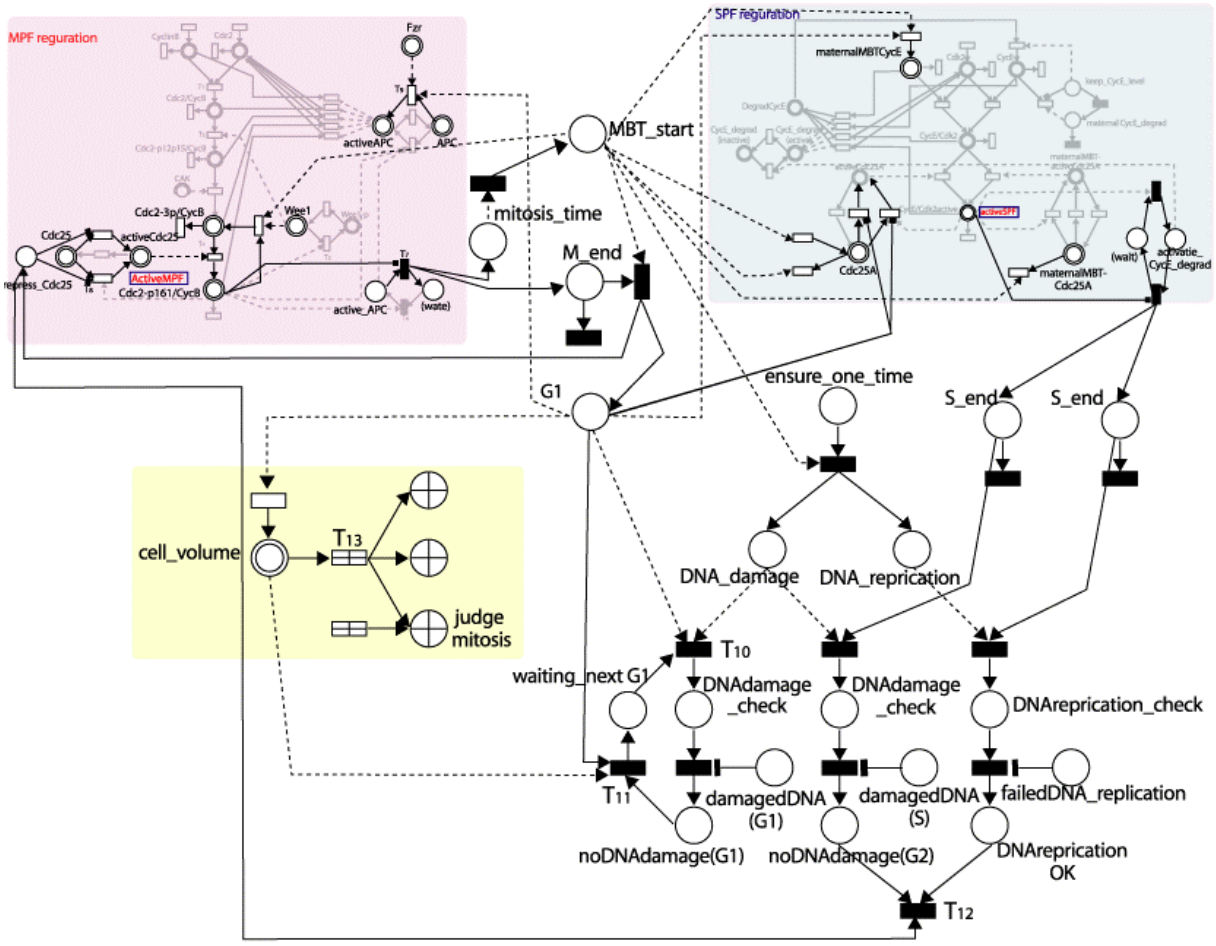

Figure 3. Mechanisms for cell division control and checkpoint sequences. The rectangular parts of pink and light blue colors correspond to Figures 1 and 2. The small circuit on the yellow rectangular is a mechanism to determine the timing of cell division. More specifically, each time the transition T13 fires, a cell divides. The elements in the circuit are special elements called "generic place and generic transition" and the functions of them have not been presented yet since concepts of these elements are quite new. The details for this circuit including the usage of these elements are described in the URL http://genome.ib.sci.yamaguchi-u.ac.jp/~fujita/CellCycle/

- The reaction activating the Cdc25 enzyme stops if the following condition holds (This condition is verified at the transition T12.);
  - DNA damage checkpoint and the DNA replication checkpoint successfully passed.

4 Simulation by Genomic Object Net

GON allows us to model target biopathways without complicated mathematical formula, and to perform simulations easily by manipulating parameters directly and smoothly using its GUI. With GON Visualizer [4,5], users can visualize simulation results of biological phenomena described by an XML document, in which CSV files produced from GON are included as basic data for visualization.

4.1 Influences of Checkpoints and Cell Volume on MPF and SPF Behaviors

As is explained in Section 3.3, influences of checkpoints on MPF and SPF behaviors can be verified.by putting tokens in the places damagedDNA(G1), damagedDNA(S), and failedDNA_replication. (See Figure 3.)

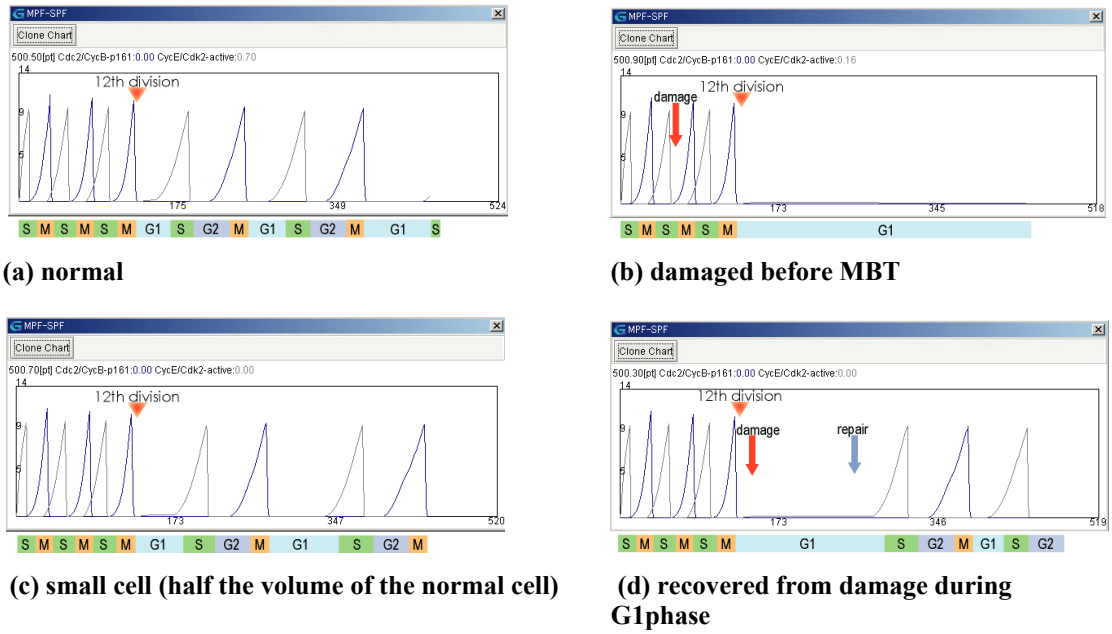

Figure 4 . Simulation results of MPF and SPF concentration behaviors. (a) Normal cell. Both oscillation cycles of MPF and SPF concentrations change after the 12th division (G1 and G2 phases are inserted). (b) DNA is damaged before the 12th division. Oscillations stop at the 12th division due to checkpoint mechanisms. (c) Small cell. The volume of the small cell is half of the normal cell. The period of oscillation is longer than the normal cell. (d) A cell is damaged soon after the 12th division, but recovers from the damage during G1 phase.

Figure 4 shows four types of MPF (blue) and SPF (gray) concentration behaviors from the 10th to 14th mitotic division. Mitotic divisions should not continue unless all checkpoints for the DNA damages during G1 and G2 phases and the DNA replication just after S phase are successfully passed. From the following observations, it can be said that our HFPN cell cycle model succeeds in simulating the influences of checkpoints and cell volume on MPF and SPF oscillations. Although a cell is damaged before the 12th division, MPF and SPF oscillate until the 12th division, since checkpoint mechanisms do not work before the 12th division ((b)). The MPF and SPF oscillation cycles of the small cell are lengthened compared to the normal sized cell ((c)). After a mechanism for damage repair is triggered, MPF and SPF begin to oscillate again ((d)).

4.2 Multicellular Simulation: Cell Dividing Process Triggered by Cell Volume

Based on the facts (A), (B), and (C) in Section 2, we made a simulation of *Xenopus* cell dividing process. For the constructed 31 HFPN pathway files, 31 CSV files are produced: X1, X1p's, X1pq's, X1pqr's, or X1pqrs's (p,q,r,s $\in$ {1, 2}) as shown in Figure 5 (a). In fact, HFPN pathway files at some division stage are copied manually from the file of the previous stage by watching the firing of the transition T13 (See the legend of Figure 3.). If the function to copy .les in response to the firing of a designated transition is installed in GON, we can obtain these HFPN files automatically. Figure 5 (b) shows the simulation results. In order to

model asynchronous cell division, we assume that a cell divides into two cells whose ratio of volume is 5.5 to 4.5.

In the simulation (see the website [14]), although the times for doubling four cells X1pq's to eight cells X1pqr's are the same, different times are required for doubling the 8 cells X1pqr's to the 16 cells X1pqr's, where p,q,r,s $\in$ {1, 2}. This means that, our *Xenopus* HFPN pathway, in which the mechanism for varying cell cycle according to the cell volume is installed, succeeds in simulating the change in the period of cell cycle from synchronous to asynchronous at the 12th cell division.

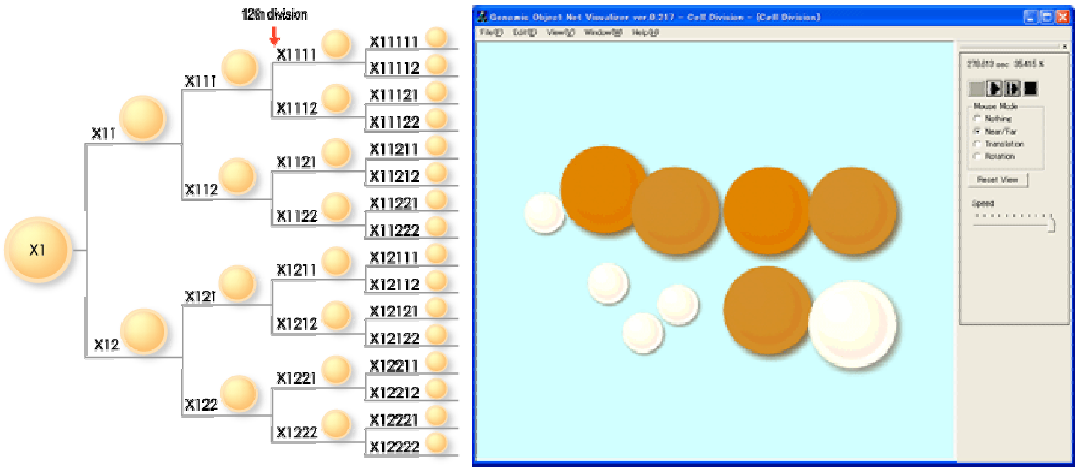

Figure 5. Cell dividing process based on data from simulation. (a) Cell dividing process from the 10th division to the 13th division. At each cell, a name such as X1122 is assigned, which is the name of the cell among eight cells just after the 12th division. Each of 31 CSV files produced from the simulation by GON has the same file name as the cell name. (b) Screenshot of GON Visualizer. The diameter and the color of each cell change according to two series of values for the cell volume and the MPF concentration in the CSV file, respectively. The simulation can be viewed through the web site http://genome.ib.sci.yamaguchi-u.ac.jp/~f ujita/CellCycle/

5 Conclusions

Establishing methods for modeling multicellular systems is the current important issue in biopathway simulations.

Our previous paper [5] proposed a method to model a pattern formation of multicellular system with the simulation of the *Drosophila* Delta-Notch signaling pathway. Although this system included cell-to-cell interactions as well as intracellular regulations, there was no change in cellular formation. In contrast with this, this paper proposed a new method for modeling cell division processes by using a famous multicellular phenomenon "the changes in cell division cycles from synchronous to asynchronous in *Xenopus*" and succeeded in simulating this phenomenon with GON.

Our next aim is to model more complex multicellular organism. As a candidate, we are now planning to use a pathway mechanism in *C.elegans* development, since both cell interaction and cell division begin from a very early stage of its development.

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