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THE ROLE OF TIME DELAYS, SLOW PROCESSES, AND CHAOS IN MODULATING THE CELL-CYCLE CLOCK

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Abstract. The regulation of the cell cycle clock is examined using a theoretical model for the embryonic cell cycle, where the clock is described as a single-limit cycle [1]. By taking the coefficient of the autocatalytic reaction as proportional to the deviation of the system from its equilibrium state, we show how such clocks can be adjusted to function on several time scales. This feedback control, causing a periodic change in the sign of the autocatalytic reaction, may be interpreted as a periodic change in the ratio of cdc25/wee1 activity. Its introduction results in the appearance of a double limit cycle, signifying the acquisition of the G1 phase and the G2 phase, during embryonic development. Following the loss of stability of the double cycle, through a period-doubling bifurcation, another limit set—a strange attractor—is born. The complicated geometry of this strange attractor can be viewed as an unlimited reservoir of periods in the phase space.

We hypothesize that the existence of such a reservoir is advantageous in morphogenetic tissues, such as the bone marrow, as it enables time- and site-specific selection of the optimal cell-cycle period for any specific microenvironment. This can be obtained by the addition of a time delay in the autocatalytic reaction, reflecting, for example, the influence of external molecular signals on cell-cycle progression.

Dedicated to the memory of Lee A. Segel (1932–2005), a pioneer in mathematical biology.

1. **Introduction.** "Human cancer is a collection of phenomena with one common denominator: in all the different manifestation of human cancer the cell-cycle clock is deranged" [2]. What is this clock, which activities does it regulate and where is its master modulator situated? Two general approaches exist with respect to modelling the cell-cycle clock. The more common approach refers to its mechanism, to the specific activity of molecules that are involved in the biochemical cascade underlying the cell cycle (e.g., [3]—[6]). In contrast, our own interest lies in the control of the cell-cycle clock. For this reason, we have focused our analysis on the general dynamic properties of the cell cycle and the laws that govern these dynamics, rather than on the details of the biochemistry. In the present work,

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the dynamic behavior of this biochemical system is visualized by a limit cycle, a double limit cycle, or a strange attractor, traced out by its trajectory in phase space [7, 8]. Within this overall framework, the simple clock, representing the embryonic cell cycle, is described as a limit cycle. A doubling of the limit-cycle period takes place during embryonic development. Subsequently, a breakdown of the double cycle occurs, and a strange attractor is born. Below we discussed the significance of these patterns for the cell-cycle clock in developmental processes.

2. **The simple clock–a single time scale.** Evidence that, at least in certain kinds of eggs, the cell cycle is regulated by an autonomous oscillator can be provided by a series of self-perpetuating chemical reactions in the cytoplasm. Initiation and completion of mitosis in cells involve several gene products. The key element is a CDK called $p34^{cdc2}$, which is encoded by the gene *cdc*2. The amount of the *cdc*2 gene product does not vary during cell division, but its kinase activity is positively regulated by other gene products that appear and disappear during specific stages of the cell cycle.

It has been shown that $p34^{cdc2}$ and cyclin combine to form a heterodimer, a maturation promotion factor (MPF), which when activated, triggers all the major events of mitosis and cell division. A striking feature of MPF is its ability to autoactivate so that injection of a small portion of an egg with high MPF activity into another egg with low activity stimulates an increase in MPF activity in the latter [9].

For describing this simple clock, we have employed the model for the embryonic cell cycle introduced by Norel and Agur [1]. In [1], C and M denote cyclin and active MPF concentrations at any given moment, respectively, whereas $\dot{C}$ and $\dot{M}$ denote the rates of change in these concentrations. For formally describing MPF activation, they use the assumptions that (i) in the early embryos, cyclin synthesis is sufficient for the activation of MPF and for the induction of mitosis [10] and that (ii) MPF activity is autocatalytic [9, 12]. These assumptions are taken into account in the first two terms in Equation 1. The third term in this equation describes the Michaelis-Menten deactivation of MPF [9, 10]. In Equation 2, the rate of change in cyclin concentration, $\dot{C}$ is given by the difference between its constant rate of accumulation [13] and its rate of degradation. Because cyclin is known to be an essential component of active MPF [14], and its rapid degradation occurs immediately after the maximum in MPF activity, it is assumed that the rate of cyclin degradation depends on the cellular concentration of active MPF. For simplicity, it is also assumed that no constraints exist with respect to space and nutrients. Using the above assumptions, the following dimensionless equations are obtained [1]:

$$\dot{M} = eC + fCM^2 - g\frac{M}{M+1}, \tag{1}$$

$$\dot{C} = i - CM, \tag{2}$$

where e, f, g, and i are coefficients standing for the respective reaction rates.

To simplify the analysis, let us introduce a new scale for the variables M and C, so that the system of differential equations (1) and (2) is replaced by the following system:

$$\begin{cases} \dot{x} = ay + bx^2y - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \end{cases} \tag{3}$$

where $x = M$, $y = C/i$, $a = ei$, $b = fi$, and $d = g$. There are no analytic methods that permit us to investigate the integral behavior of the solution of such nonlinear differential equations. Therefore, we need some arguments to choose the parameter values for which periodic solutions of these equations (3) exist (appendix 1); this we check by computer simulation. In our case, the condition for the stability of the limit cycle can be satisfied: the system (3) is similar to (1) and (2) in yielding a limit-cycle behavior, that is, an oscillatory change in MPF and cyclin concentrations.

3. **Increasing the complexity of the clock–multiple time scales.** Primitive clocks can be characterized by a single, unmodulable time scale. For example, the common hourglass operates on a scale of minutes. More sophisticated clocks use a basic ticking mechanism for measuring time on several scales, for example, seconds, minutes, or hours. By a similar reasoning it is conceivable that in multicellular organisms the ticking of the basic cell-cycle clock may be employed for measuring different biological processes on different time scales, ranging from the scale of a single cell cycle to that of the entire organism's lifetime. Note that the more evolved the multicellular organism, the richer its time-hierarchical clock is expected to be.

How can such a control be effectuated? In fission yeast, the changes in active MPF and cyclin concentrations are accelerated by the activity of the gene *cdc*25 (positive regulation - activation) and retarded by that of the gene *wee*1 (negative regulation - inhibition). These genes control the entry into mitosis, and it seems likely that the ratio of *cdc*2 and *wee*1 activity is altered by signals that influence the entry into mitosis [10]. To allow for this control, Norel and Agur used a function that reflects the change in the activity ratio *cdc*25/*wee*1 [1]. This function replaces the parameter of the autocatalytic MPF reaction, and, by assuming that in aging tissues its value decreases by a constant fraction in each cell cycle, one can study the sensitivity of the period and amplitude of the oscillations in these tissues to the *cdc*25/*wee*1 ratio control. Thus, it is shown that such a simple modulation can account for the progressive increase in cell-cycle length and for the finite, roughly constant, number of cell divisions, characterizing senescent cell lineage.

However, a constant slow decline in one of the reaction rates requires that a larger time-scale clock (yet to be accounted for) is part of the system. In the present work, we show how the latter clock may be embedded by using relatively simple biochemical controls in the simple cell-cycle clock.

The dynamical system (3) has three basic components: synthesis, autocatalysis, and deactivation. We attempt to check the mathematical description for them by extending the basic system (3). If the parameters a, b, and d in system (3) represent biochemical controls, whose values may vary by many orders of magnitude, the system will possess a time hierarchy. For simplicity, let us first consider a system where the parameters a, d and b are constants and is the biochemical control variable. Considering that any system tends to return to equilibrium with a force that is proportional to its deviation from its equilibrium state, we take $\dot{b}$ as proportional to (in suitable units equal to) the deviation of system. (3) from its equilibrium state[1]. More precisely, the "coefficient" b depends on the deviation of the autocatalytic term, x^2y, from its equilibrium value in the system (3). Taking the equilibrium solution of the fast system (3) as $x_{eq} = 1$, $y_{eq} = 1$ (see Appendix

[1]This assumption is our expression of Le Chatelier's principle: parametric deviations away from the equilibrium values induce spontaneous processes tending to restore the system to equilibrium; in modern language we will say that the system must have a control.

A1), we obtain the following time-hierarchical system:

$$\begin{cases} \dot{x} = ay + bx^2y - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \\ \dot{b} = \varepsilon(1 - x^2y,) \end{cases} \tag{4}$$

Here we suggest an autocatalytic "sway" of the system (3). The constant b becomes a new phase variable like x and y and can become negative (see Section 5). The new dynamical system (4) contains the fast subsystem (3), and if $\varepsilon \ll 1$ it is a fast-slow system. The fast-slow system (4) can have regular relaxation oscillations; that is, the trajectories can form an attracting cycle (see Fig. 1). We speculate that it is the appearance of the double cycle during the variation of initial conditions (see Fig. 2(a)) that enables further specialization of the simple cell-cycle clock, or, in biochemical terms, the separation of a single cyclin-CDK pair into different S-phase and M-phase cyclin-CDK pairs. If we assume that in (4) $\varepsilon = 1$–so that (4) is no longer a fast-slow system–a new attractor in the system (4) is born (Fig. 2). This attractor is a strange attractor, has a complicated geometry, and can be viewed as virtually an unlimited reservoir of periods . Note, that the strange attractor has an "away" shape of double cycle in the three-dimensional-phase space (see Figs. 2 and 3).

REMARK 1. The transition to a system with strange attractor (deterministic chaos) means that complicated nonperiodic oscillations, whose details are very sensitive to small changes in the initial conditions, can be observed. In other words, phase trajectories on the strange attractor are unstable. Note, however, that the average characteristics of this behavior are stable and do not depend on the initial conditions (they vary within a given domain) [15]. From a general point of view, and using computer simulations, one can see that the system of a single or a double limit cycle, as well as that of a strange attractor are structurally stable (robust) systems. The property of structural stability is absent only for the bifurcation values of the system's parameters.

A more complex model assumes that the biochemical control of the system also includes the coefficients a and d. In formalizing this model too we can rely on Le Chatelier's principle. However, other meaningful changes to system (3) can be considered. Applying similar considerations (see system (4)) for synthesis and deactivation, and we get another dynamical system (5). Now $\dot{a}$, $\dot{b}$, $\dot{d}$ are proportional to (in suitable units equal to) the deviation of system (3) from its equilibrium state (see above), and we obtain the following time hierarchical system with the :

$$\begin{cases} \dot{x} = ay + bx^2y - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \\ \dot{a} = \varepsilon(1 - y), \\ \dot{b} = \varepsilon(1 - x^2y), \\ \dot{d} = \varepsilon\left(0.5 - \frac{x}{x+1}\right). \end{cases} \tag{5}$$

Here x, y are the fast variables and a, b, d are the slow variables, if ε is small ($\varepsilon \ll 1$). The slower response of the equations for $\dot{a}$, $\dot{b}$, $\dot{d}$ is said to give the feedbacks. Some typical behaviors of the system (5) are shown in Figs. 4 - 6, where we checked many different values of ε on the interval $[10^{-5}, 1]$. One can note in these figures that the stability of the oscillations is very sensitive to the rate of reactions of the control variables a, b, d. This sensitivity implies that, in general, the system

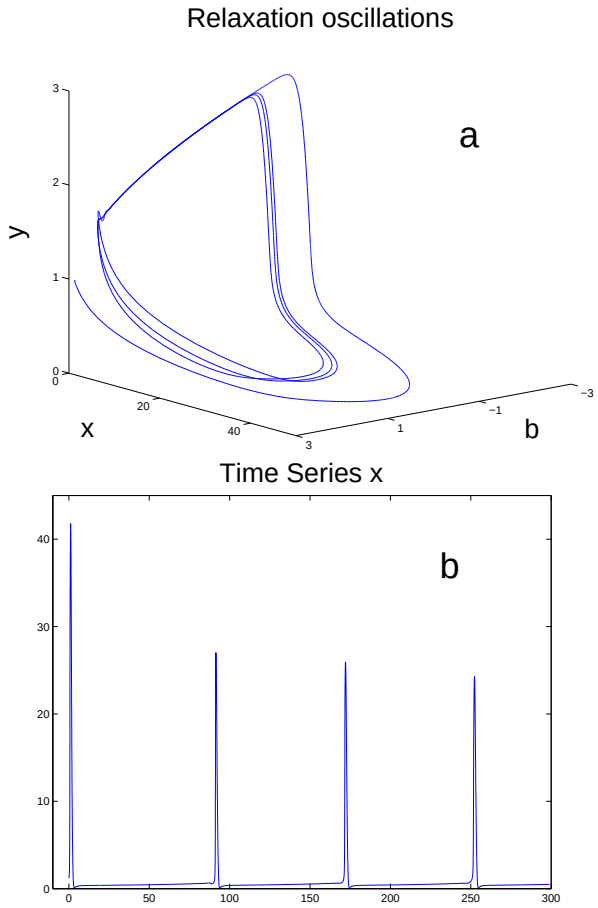

FIGURE 1. The relaxation oscillations of the fast-slow system, with initial conditions $x_0 = 1.2$, $y_0 = 1$, $b_0 = 3$, $a = 1$, $d = 8$, and $\varepsilon = 0.1$: (a) depicts phase trajectory in the phase space (x, y, b) – limit cycle; (b) shows time plots for the x, y and b – bursting. Note that the variable b can become negative.

(5) is unstable. For this reason the possibility that a and d vary during the cell cycle is not very likely, and system (4) seems to be more relevant.

The results presented above suggest that a reservoir of periods can be created naturally by a relatively simple, single control of the major cell-cycle reactions. Next we show that only some initial conditions in this phase space can be accessed when an appropriate time-delay argument is applied to one of the system parameters.

4. **Controlling the multiscale clock by introducing time-delay arguments.**

Deterministic chaos is characterized by long-term unpredictability arising from an extreme sensitivity to initial conditions. Therefore, a priori it may be assumed that such a behavior is undesirable, particularly for processes that are dependent on temporal regulation, such as the one discussed here. However, we show that the

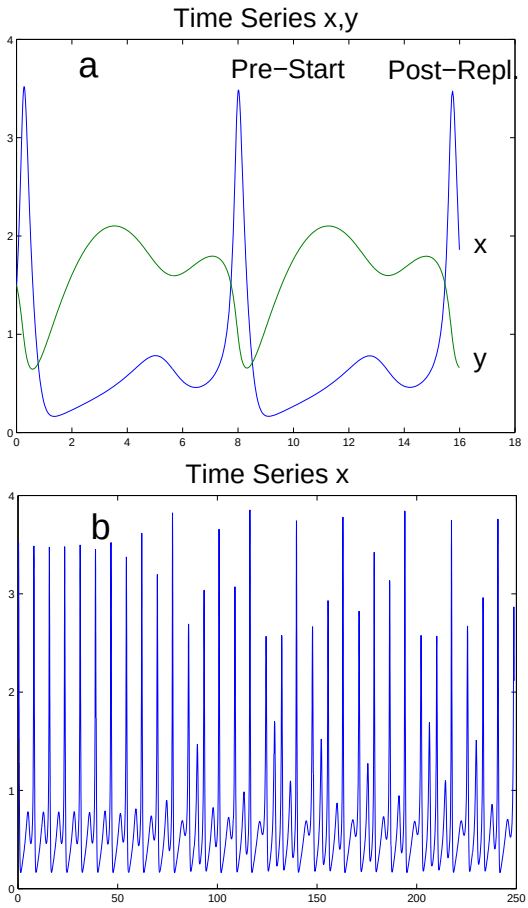

FIGURE 2. Time plots of the variable x for the autonomous system (3) depicting breakdown of the double-cycle symmetry for initial conditions, $x_0 = 1.5$, $y_0 = 1.5$, $a = 1$, $d = 8$, $\varepsilon = 1$: (a) shows the double cycle for $b_0 = 3$; (b) depicts the strange attractor for $b_0 = 2.4$ (see Fig. 3). The amplitudes and periods of oscillations for x have disordered behavior.

chaotic system can be stabilized, and the desired specific initial conditions can be selected, when an appropriate delay argument is applied to one of the variables.

Let us assume the following delay in the system (4):

$$\begin{cases} \dot{x} = ay(t-\tau) + bx^2y - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \\ \dot{b} = \varepsilon(1 - x^2y). \end{cases} \tag{6}$$

System (6) can have a double cycle if $\varepsilon \cong 1$ (Fig. 7a-c). When $\varepsilon \ll 1$, (6) is a fast-slow system that can have bursting like that of the initial nondelayed fast-slow system (4) (Fig. 7d)). This bursting is reminiscent of the theoretical time plot of abrupt activation in the cell cycle [16]. Here, too, the slow variable b can become negative (see Section 5 for discussion).

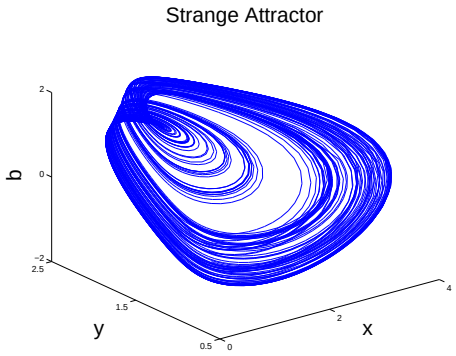

FIGURE 3. A strange attractor. The behavior of the autonomous system (4) for $x_0 = 1.5$, $y_0 = 1.5$, $b_0 = 2.4$, $a = 1$, $d = 8$, and $\varepsilon = 1$ in the phase space (x, y, b). Although this strange attractor appears to have the shape of Möbius band, a more detailed examination shows that this band is not a manifold, however, but is instead, folded like a baker transformation [31]. This transformation allows the mixing of trajectories; only one trajectory is shown.

A similar pattern is observed when the delay argument is in the autocatalytic term:

$$\begin{cases} \dot{x} = ay + bx^2y(t-\tau) - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \\ \dot{b} = \varepsilon(1 - x^2y). \end{cases} \tag{7}$$

Here, too, a double cycle is observed for $\varepsilon = 1$ (Fig. 8a), bursting under initial conditions the same as those for the fast-slow system (6) if $\varepsilon \ll 1$.

The delay system

$$\begin{cases} \dot{x} = ay(t-\tau) + bx^2y(t-\tau) - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \\ \dot{b} = \varepsilon(1 - x^2y) \end{cases} \tag{8}$$

has a single cycle if $\varepsilon = 1$ (Fig. 8b), and bursting if $\varepsilon \ll 1$, with initial conditions as for the system (6) (Fig. 3).

In appendix B1 we checked the isolated effect of time-delay arguments in cyclin activation of $p34^{cdc2}$, where the coefficient b is constant. We did so by introducing time-delay arguments in various terms of (3), and by analyzing their effects on the oscillatory behavior of the system. Our results show that only a weak time-delayed effect of cyclin on the activation of $p34^{cdc2}$ does not destabilize the oscillations of the system; a strong time-delay effect destabilizes the otherwise stable cell-cycle, as long as the coefficient of the autocatalytic reaction is constant in time.

REMARK 2. Delay Differential Equations (DDEs) and their respective Ordinary Differential Equations (ODEs) differ in mathematical structure. For example, for ODE there is a smooth vector field in the phase space, but for DDEs this object is absent. For this reason, our knowledge about ODEs cannot aid in the analysis of the respective DDEs, and vice versa. In particular, the "delay" for DDEs is not a parameter for the strange attractor, as the strange attractor is an object of ODE and not an object of DDEs. In the context of the present analysis, it means

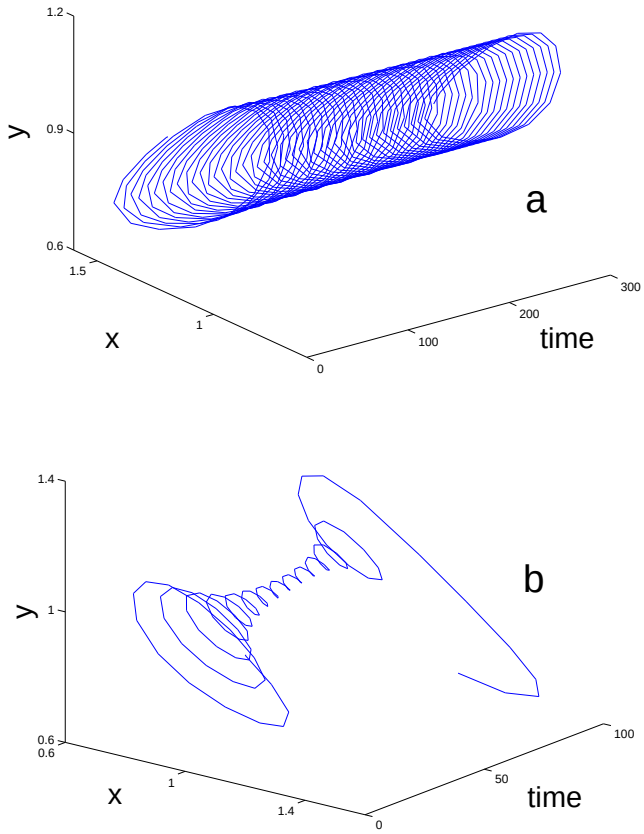

FIGURE 4. The behavior of trajectories of the fast-slow system (5) in the space (x, y, t), $x_0 = 1.2$, $y_0 = 1$, $b_0 = 1.006$, $a_0 = 1$, and $d_0 = 4$: (a) shows limit cycle, $\varepsilon = 0.00001$; (b) shows delayed bifurcation, $\varepsilon = 0.1$. This structure is brought out even more spectacularly in Figure 6.

that our ODE results about the strange attractor and the bifurcation delay become irrelevant after replacing the ODE with the DDE.

5. **Discussion.** In this work, we investigated different controls in the cyclin-$p34^{cdc2}$ double oscillator system, and their effect on the function of the cell-cycle clock.

The single stable cycle, generated by the simple uncontrolled model (Eqs. (3)) may represent a "virtual" beginning of the embryonic cell cycle, where there exist only two functionally different levels of cyclin-CDK complex activity: high and low: mitosis and interphase [10]. The addition of the slow feedback control in the production of this active complex (Eqs. (4)) results in the transition of the system from a single stable cycle to a chaotic regime, through an intermediate period-doubling stage. We assumed that this feedback control is achieved through sensitivity of the autocatalytic reaction to the deviation of the system from its

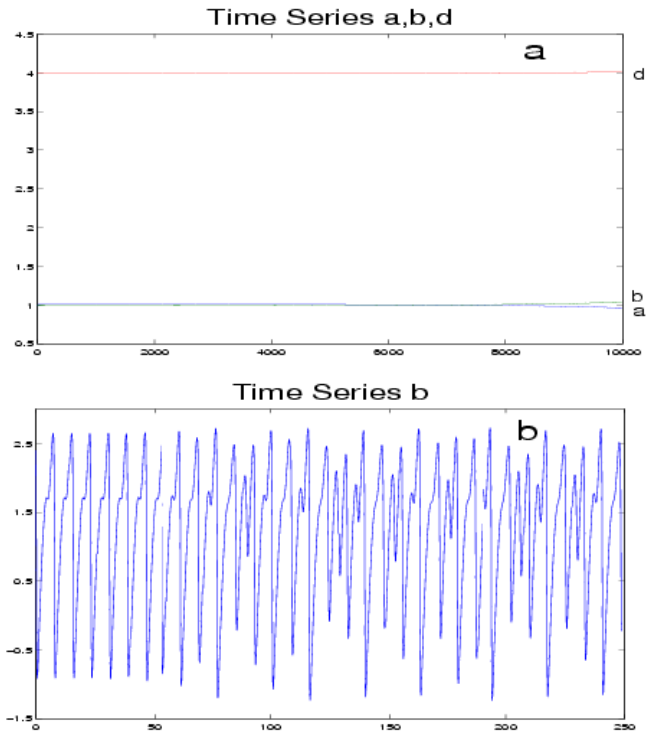

FIGURE 5. Dynamics of the control variables: (a) depicts a weak feedback control, with time plots of the variables a, b, and d for the fast-slow system (5), $\varepsilon = 0.04$. (see Fig. 7a); (b) depicts a strong feedback control with a time plot of the variable b for the system (4), $\varepsilon = 1$ (see Fig. 4). In (b) the amplitudes and the periods of oscillations for b show disordered behaviors; note that the variable b can be negative.

equilibrium. This feedback, changing the sign of the autocatalysis, may be related to a periodic change in the ratio of *cdc*25/*wee*1 activity during the cell cycle.

The double cycle, defining cell-cycle oscillations for system (4), is the mathematical reflection of the natural transition from the embryonic (simple) to the somatic (complex) cell cycle. It has been shown in *Drosophila* and *Xenopus* that maternally provided regulators are removed at defined developmental stages during embryogenesis, resulting in the acquisition of a G1 phase and a G2 phase [17]. Such specialization of the somatic cell cycle enables further subdivision of interphase into two sequential states by the restriction point ("start") that allows the coordination of the stepwise events of the cell cycle with cell growth and external signals. Three characteristic states can be defined for the somatic cell cycle: metaphase, prestart interphase (the part of the G1 phase before commitment to cell replication), and postreplication interphase (G2 phase) [10]. In Fig. 2a we can see a model situation for the above suggestions. Namely, we have for x two sequential characteristic positions (local minima - states with minimum values of the variable) that are prestart and postreplication states. From Fig. 2b we can see that the beginning of the time

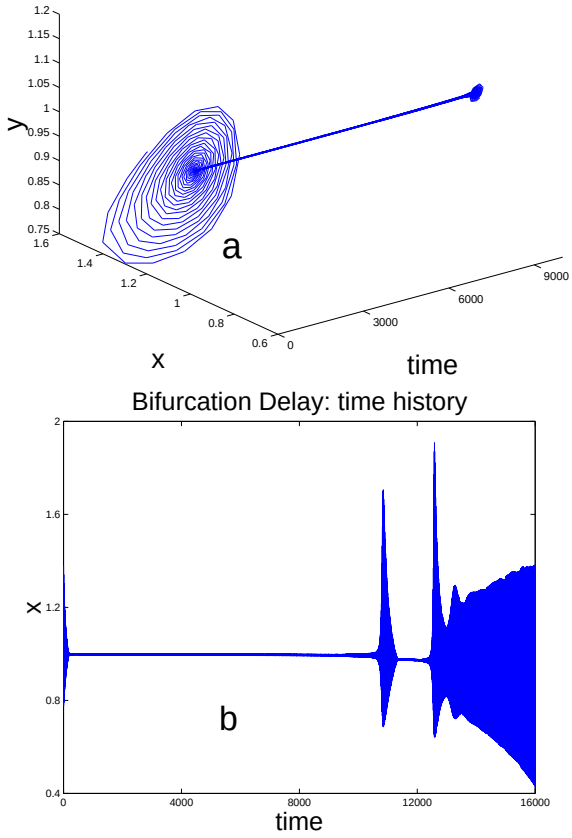

FIGURE 6. Bifurcation delay: (a) shows the behavior of the one trajectory in the space-time (x, y, t) for $b_0 = 1.006$, $\varepsilon = 0.004$. (b) shows time plot of x for $b_0 = 1$, $\varepsilon = 0.001$. System (5) with complex slow and fast dynamics for $x_0 = 1.2$, $y_0 = 1$, $a_0 = 1$, and $d_0 = 4$. Here we can see the rapid onset of oscillatory behavior, first decreasing in amplitude but then increasing. The oscillatory behavior diminishes to almost zero amplitude before increasing again (see also [32] – [37]). A pair of conjugate eigenvalues of operator L leaves the half-plane without passing through 0 ($b = 1$): $\lambda_{1,2} = \pm i$. The solution of the fast-slow system (5) rapidly approaches the focus at the distance of order ε. Phase points remain near the unstable equilibrium position during a time period of length of order $1/\varepsilon$.

plots for the strange attractor looks more or less like the right double cycle (see Fig. 2a). And only later do we observe a disorder in the double oscillations pattern–a birth of a strange attractor (Fig. 2b).

That period-doubling bifurcation represents early developmental and evolutionary cell-cycle state transitions may be manifested in the following observations: in

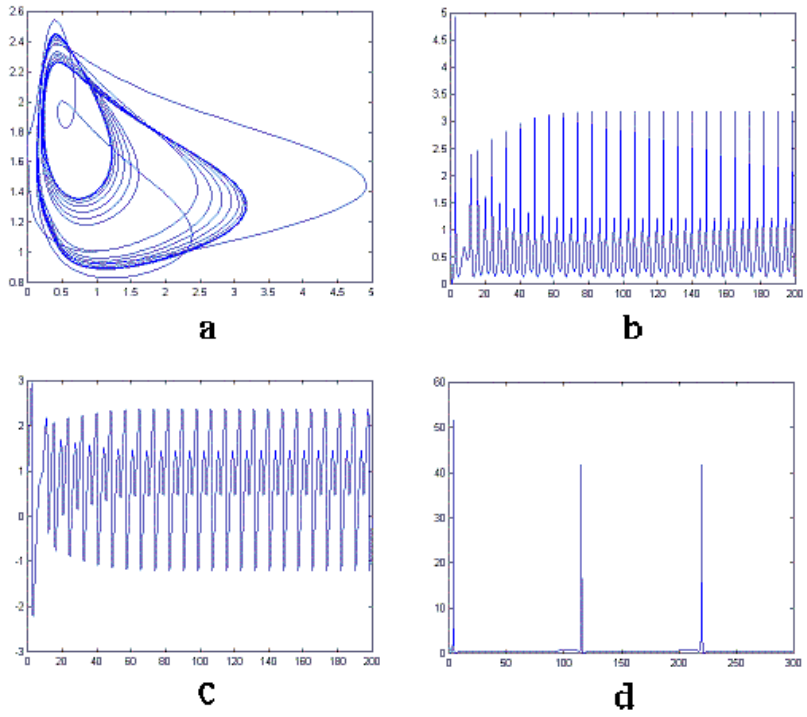

FIGURE 7. Computer simulations of the delay system (6) with $a = 1$, $d = 8$, and the delay condition: $\tau = 1$, $y(t) = 1$, and $t \in [-1, 0]$. For $b(0) = 1$, $\varepsilon = 1$, and $x(0) = 1$, the system has irregular behavior with a double cycle on the phase plane (a), with a time plot of x (b) and a time plot of b (c). For: $b(0) = 3$, $\varepsilon = 0.1$, $x(0) = 1.2$, the system is bursting (d).

animal cells, S phase is induced by CDK2 complexed with S phase cyclins (types E or A) and M phase by CDK1 complexed with M phase cyclins (types A and B), whereas in both budding and fission yeast, S and M phases are induced by CDK1 associated with B-type cyclins, which are S phase- and M phase-specific . Moreover, M phase CDK's can assume the function of S phase and trigger chromosome duplication in G1 cells [18].

The feedback mechanism we described here generates a strange attractor, whose attraction domain is sufficiently large enough to stabilize the process of cell divisions. Note that in this model the variability of cell-cycle times is a consequence of a chaotic trajectory with a purely deterministic basis. A cell-cycle oscillator with a strange attractor has previously been considered
(cite7), cite(18)– (20), but here we obtained it by a simple control of the coefficients. In the fast-slow system (5), the slow variables a, b, and d are almost constant, but in the system (4) with the strange attractor ($\varepsilon = 1$) and with the burst ($\varepsilon \ll 1$), the variable b can become negative (Figs. 5, and 7). We showed how the fast control of the autocatalytic reaction creates a reservoir of cell-cycle periods in the phase space.

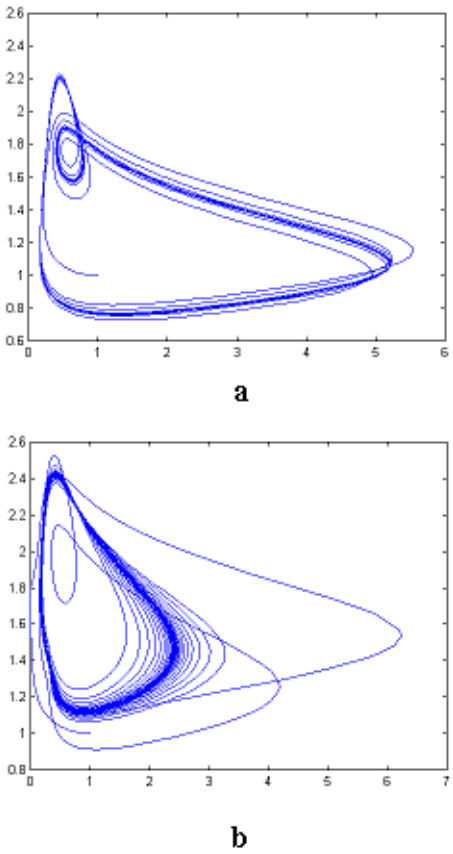

FIGURE 8. The examples of stable oscillations in the delay systems (7) and (8) observed by computer simulation for: $a = 1$, $d = 8$, $b(0) = 1$, $\varepsilon = 1$, and $x(0) = 1$ and the delay condition: $\tau = 1$, $y(t) = 1$, and $t \in [-1, 0]$: (a) shows the double cycle for (7); (b) shows the single cycle for (8).

The existence of a strange attractor with an unlimited reservoir of periods may be an important property of multicellular organisms, where the proper structure and function of the adult organism is depends strongly on intricate developmental processes as well as on sophisticated homeostatic mechanisms. A striking example of the need for such a spatio-temporal regulation in a multicellular organism is the ongoing, highly homeostatic, developmental process of blood production in the bone marrow (BM). Human blood contains a remarkable variety of cells, each precisely tailored to its own vital functions. All these cells develop from a kind of master cell, *the totipotent stem cell*, which resides in the BM; a few totipotent stem cells can reconstitute the entire blood. The process of blood-cell production, initiated by the totipotent stem cells, has a treelike developmental structure: different cell progenitors are located at different branch nodes, according to their degree of differentiation. Injury to blood, from chemotherapy, radiation, or disease, creates

a cascade of feedback signals that are received at different nodes of the tree. Such feedback loops may change the balance between the rates of self-renewal and maturation of stem cells, and essentially may result in accelerated production of the cells that are essential for repairing the specific damage [22]. Indeed, it has been observed that stem cells under such hemopoietic stress are capable of exceedingly rapid cell cycles [23].

Given the size of the hemopoietic tree and the very complex interactions between its constituents, it seems reasonable to assume that optimal production of blood cells crucially depends on optimal timing of replication and differentiation of cells in many different microenvironments. This, in turn, may depend on local fine tuning of cell-cycle time [24]. We hypothesize that such fine tuning is feasible by an ad-hoc process, operating on the reservoir of periods by the adjustments of the proportional feedback.

In the model presented here, each individual set of initial conditions can be selected by the superposition of an additional control argument in the form of a time delay in the involved reactions. We show that, generally, the introduction of discrete time-delay arguments alone destabilizes the two oscillators in the cyclin-$p34^{cdc2}$ reactions. It means that these oscillators are very sensitive to initial conditions. However, by the coupling of these two relatively simple controls of the double oscillator, the cell-cycle clock can be modulated so as to control cellular events occurring on different time scales. The additional control, representing, for example, the effect of local molecular signals, may reflect the increase in the complexity of the cell cycle during development [10].

Our suggestion that the strange attractor guarantees homeostasis in a developmental system, such as the BM, is supported by another mathematical model, specifically constructed for describing BM hemopoiesis. In this model, BM stem cells, postmitotic cells, and empty space are represented as valued sites of a two-dimensional lattice. Every cell is equipped with a type-specific internal counter, representing its cell cycle, while proliferation or maturation of a cell is determined according to its internal state and its immediate neighborhood. The model thus constructed can be rigorously proven to possess the following property: apart from a few inevitable pathological cases, starting from any initial state the system never reaches a fixed state, and there are arbitrarily large times for which the resulting state contains a sufficient number of postmitotic (mature) cells. It can be shown that this inherent robustness is achieved by the, theoretically, unlimited reservoir of cell cycles in the system and its resulting chaotic dynamics. In particular, under the assumption of a fixed cell-cycle duration, the system either collapses or oscillates rapidly with large amplitudes. The general conclusion of this work is that the existence of multiple cell-cycle time scales in the BM is a necessary condition for normal hemopoiesis [11].

It has been observed that unlike untransformed cells, SV-40 transformed tumor cells do not respond with an intermitotic delay upon exposure to serum-free media or low doses of protein synthesis inhibitor cyclohexamide [25]. Moreover, recently it has been reported that the overexpression of cyclin-D mRNA (which is effectuated in shortening the characteristic time delay in the cyclin D/CDK4 reaction) is an early event of mammary carcinogenesis [26]. In general, unscheduled expressions of cyclins can be detected in several tumor-transformed cell lines [27]. These observations, in conjunction with our results, allow us to conjecture that the main

difference between normal and neoplastic cells may be crystallized in a loss of capacity to keep the appropriate time-delay arguments in the cyclin-CDK's reactions in cancer.

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Appendix A. Analyzing the existence of limit cycle and its stability (the autonomous system).

A.1. **The small perturbations.** In contrast to the global Poincaré-Bendixson theory a local bifurcation theory, applies locally, hence allowing us to investigate the existence of limit cycles and their stability for small perturbations of the Hamiltonian systems [28].

The physically realistic system described in this paper has an equilibrium state for positive values of variables $\{x, y\}$. So we consider a nondegenerate singular point (an equilibrium solution) of system (3). We assume that this singular point is at the point $S1 = (1, 1)$. Therefore, we have the additional condition $d = 2a+2b$. The operator of the linear part of the field (3) at the point S1 is

$$L = \begin{pmatrix} 2b - \frac{1}{4}d & a+b \\ -1 & -1 \end{pmatrix}, \tag{9}$$

The conditions of bifurcation of the center at singularity S1 are expressed by the following relations for the operator L at the point S1:

$$\begin{cases} \mathrm{tr}L = 0, \\ \mathrm{dis}(|L - \lambda E| = 0) < 0. \end{cases} \tag{10}$$

The conditions in that a pair of conjugate eigenvalues of the operator at the point S1 must be purely imaginary (10) mean[2]

The left part of the second condition in 10 means the discriminant of the characteristic equation $\det(L - \lambda E) < 0$. As a consequence, we have the following conditions for the parameters: $d = 2a + 2b$; $a = 3b - 2$; $b > 0.75$ and $d > 0$ thereafter. We choose these conditions so that the system (3) can be transformed into the following system:

$$\begin{cases} \dot{u} = \omega v + P(u, v), \\ \dot{v} = -\omega u + Q(u, v), \end{cases} \tag{11}$$

with a singular point S1=(0, 0) in new coordinates (u, v), where $x = 1 + u - \frac{v}{\omega}$; $y = 1 + \frac{v}{\omega}$ and $\omega = \sqrt{4b - 3}$. In this system, P and Q contain terms of degree 2 and greater.[3] The linear part of system (11) is a harmonic oscillator (Hamiltonian system) with the first integral $H = \frac{1}{2}(u^2 + v^2)$. The phase trajectories of the harmonic oscillator are concentric cycles around the point $(0, 0)$.

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[2]It is clear that if $\mathrm{dis}(|L-\lambda E| = 0) = 0$, then $\det L > 0$. D.C. Thron [38] investigates a similar system and defines a simple singular point of unstable focal or unstable nodal type, which satisfies this condition. In this way, Thron successfully obtains limit cycles.

[3]$P(u, v) = (2b - 1)(2 + u) + bu^2 + (1 - 2b - u + bu^2)\frac{v}{\omega} + (1 - b - 2bu)\frac{v^2}{\omega^2} + b\frac{v^3}{\omega^3} + \frac{4(2b-1)\omega}{\omega u - v + 2\omega}$ and $Q(u, v) = \frac{v^2}{\omega} - uv$.

Let us consider the perturbated system

$$\begin{cases} \dot{u} = \omega v + \varepsilon P(u,v), \\ \dot{v} = -\omega u + \varepsilon Q(u,v), \end{cases} \tag{12}$$

where $\varepsilon \ll 1$ is a small parameter. In other words, we have a one-parameter family of system (12). Obviously the singularity $(0,0)$ is a fixed, simple (nondegenerate), singular point of perturbation equations too. We are interested in the metamorphosis of the configuration of phase curves in the neighborhood of the point $(0,0)$ under a small change in these equations, so we use (below) the standard analysis from Arnold's textbook [29].

In contrast to the conservative case ($\varepsilon = 0$) for $\varepsilon \neq 0$, the phase curve of the system (12) is not necessarily closed: it may have the form of a spiral in which the distance between adjacent coils is small (of order ε). To determine whether the phase curve approaches the origin or recedes from it, we consider the increment of in the energy H over one revolution about the origin. Let δH be the increment of the Hamiltonian H under one revolution along the closed phase curve $H = constant$. Then

$$\delta H \approx \oint \dot{H}\, dt = \oint u(\omega v + \varepsilon P) + v(-\omega u + \varepsilon Q)dt = \varepsilon G(r). \tag{13}$$

If the increment δH is positive (for small positive ε), the phase curve is an expanding spiral; the system undergoes increasing oscillations, but if δH is negative the phase spiral contracts and the oscillations die out. If the function $G(r)$ changes sign, then for the small ε, the equation $\delta H = 0$, is satisfied by a closed curve on the phase plane, which is close to a circle. This closed curve is a limit cycle of our system. Consequently, to first approximation, the condition of the birth of a cycle of radius r_0 is $G(r_0) = 0$. Following a detailed consideration (13), we obtain

$$G(r) = -\frac{b}{2\omega^3}\pi r^4 + \frac{2b-1}{\omega}\pi r^2 + 4\pi\omega \tag{14}$$

and $r_0 = 2\omega$. The condition for stability of the limit cycle of radius r_0 is $\varepsilon G'(r_0) < 0$ and in our case (14) is true if $\varepsilon > 0$.

A.2. **Hopf bifurcation.** This is another way to find a limit behavior in our system (3). The parameter values from conditions (10) allow us to locate the limit cycle of system (3).

Let the point $S1 = (1,1)$ be again a simple singular point of the system (3). There is a plane $2a + 2b - d = 0$ in the parameter space $Z = (a,b,d)$, which corresponds to the systems with a singularity at the point S1. This plane is not a hypersurface of singular cases in the parameter space Z. However, this plane in our parameter space may intersect some of these hypersurfaces, and therefore we must consider a domain of generic cases on this plane:

$$\begin{cases} \mathrm{tr}L > 0, \\ \det L > 0. \end{cases} \tag{15}$$

For a closer investigation of the behavior of the system (3), let us consider a one-parameter family of the system, for example, $a = b$. In this case, the second singular unstable point $S2 = (k + \sqrt{k^2 + k}, -1 + \sqrt{1 + l})$, where $k = 1/l = a/b$ (see (15)) is fixed, too. Let us investigate a neighborhood of the bifurcation value of parameters $b = 1$, that is, a case where a pair of conjugate eigenvalues of the operator (A1.0) at the equilibrium S1 crosses the imaginary axis from the left to the right. As the pair passes through 1, the focus at the point S1 loses stability. In this case,

the corresponding pair of conjugate eigenvalues of operator (9) is equal to $\pm i$. For $b = 1$, at the point S1 the focus is also stable but not robust: the phase curves approach S1 non-exponentially. For $b = 1 + \varepsilon$ where $\varepsilon > 0$, moving from the focus to a distance proportional to $\sqrt{\varepsilon}$, the phase curves wind onto a stable limit cycle. Consequently the loss of stability in the passage of b through 1 takes place with the birth of a stable cycle whose radius increases with $\sqrt{\varepsilon}$.

In other words, the stationary state S1 loses stability, and a stable periodic regime arises whose amplitude is proportional to the square root of the deviation of the parameter from the critical value. This form of loss of stability is called a mild loss of stability, since the oscillating behavior for small criticality differs little from the equilibrium state. It is a Hopf bifurcation, or a soft generation of self-sustained oscillations (see [28]).

Appendix B. **Analyzing the existence of limit cycle and its stability (The delay system).** A similar analytical investigation of the delay system gives a different result.

Let τ be a small delay ($\tau <<\ll 1$). Consequently, $y(t-\tau) \approx y(t) - \tau\dot{y}(t)$ in some neighborhood of the point t. This implies that the system (3) will have the following form:

$$\begin{cases} \dot{x} = ay + bx^2y - d\frac{x}{x+1} + \tau bx^2(xy - 1), \\ \dot{y} = 1 - xy. \end{cases} \tag{16}$$

The bifurcation conditions of the system (16), for $\tau = 0$, are similar to the bifurcation conditions for the system (3) and after the transformations of the system (16) we get the following perturbated system:

$$\begin{cases} \dot{u} = \omega v + \varepsilon P(u, v) + \tau R(u, v), \\ \dot{v} = -\omega u + \varepsilon Q(u, v), \end{cases} \tag{17}$$

where ε, τ are two small independent parameters. Assuming now that $\varepsilon = 0$, we obtain

$$V(r) = \oint uRdt = -\frac{3b^2}{4\omega^3}\pi r^4 + \frac{b}{\omega}\pi r^2. \tag{18}$$

For the limit cycle of radius r_1 we have $V'(r_1) < 0$ and therefore if $\tau > 0$, the cycle of radius r_1 is stable. However, this analysis of the delay equations is true only in some neighborhood of the point t and not true elsewhere. Generally, for infinite t, a limit cycle does not exist.

B.1. **Introducing time delays.** We introduced a time delay, τ, in the activation terms in (3) as follows:

$$\begin{cases} \dot{x} = ay(t-\tau) + bx^2y(t-\tau) - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy. \end{cases} \tag{19}$$

Alternatively, we can assume that only the autocatalytic process is delayed

$$\begin{cases} \dot{x} = ay + bx^2y(t-\tau) - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy, \end{cases} . \tag{20}$$

A weaker assumption about the delay can be made, as follows:

$$\begin{cases} \dot{x} = ay(t-\tau) + bx^2y - d\frac{x}{x+1}, \\ \dot{y} = 1 - xy. \end{cases} \tag{21}$$

By methods of numerical integration we showed that of the three delay systems, (19)-(21), only (19) can have stable periodic oscillations. However, even this delay system has a certain sensitivity to variation in the coefficient b. A critical case is $b \approx 0.6$, where the limit cycle attractor of delay system (21) does not have an oval shape but rather a beak-shape singularity.[4]

REMARK 3. The shape of the invariant attracting curve that was obtained in the phase plane $(y(t-1), y(t))$ is formally analogous to that obtained for the time-delay differential equation $x_{n+1} = ax_n(1 - x_{n-1})$[30].

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