Available online at www.sciencedirect.com
SCIENCE DIRECT®
Nonlinear Analysis 62 (2005) 817–831
Nonlinear Analysis
www.elsevier.com/locate/na

Mathematical analysis of the Tyson model of the regulation of the cell division cycle

Alberto d'Onofrio*

Division of Epidemiology and Biostatistics, European Institute of Oncology, Via Ripamonti 435, I-20141 Milano, Italy

Received 20 December 2004; accepted 18 March 2005

Abstract

In this paper, we study the mathematical properties of a family of models of Eukaryotic cell cycle, which extend the qualitative model proposed by Tyson [Proc. Natl. Acad. Sci. 88 (1991) 7328–7332]. By means of some recent results in the theory of Lienard's systems, conditions for the uniqueness of the limit cycle and on the global asymptotic stability of the unique equilibrium (idest of the arrest of the cell division) are given.

Keywords: Cell cycle; Biochemical reactions; Limit cycles; Lienard's equation; Stability theory

1. Introduction and main results

"As highly organized units in a universe favoring disorder, cells are subject to wear and tear as well as to accidents. Any individual cell is therefore bound to die. If an organism is to continue to live, it must create new cells at a rate as faster than the rate at which its cells die. For this reason, cell division is central to the life of all organisms" [1].

"The periodic repetition of certain events—DNA synthesis, mitosis and cytokinesis—that transform a single cell into two daughter cells" [2] is called cell cycle (CC) [3].

"Major events of the cell cycle... are regulated by a complex network of protein interactions that control the activities of cyclin-dependent kinases" [4,1]. This and other cell-related

* Tel.: +39 02574 89819.
E-mail address: z20263@ieo.it.

0362-546X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.na.2005.03.094

biochemical dynamical interplays [3] may be modeled well by means of nonlinear ordinary or impulsive differential equations [5–7].

Since the early 1990s, many interesting models of the control of cell cycle have been proposed (see [2,4,8–23] and references therein). Some of these models are low dimensional and qualitative, other (mainly the more recent) are more realistic by far but they are high dimensional and are difficult or approximately analytical analysis. Note that the increasing complexity of recent models reflects the progressive discovery of proteins involved in the regulation of the CC [21].

Following Okubo [24], we may classify all bio-mathematical models in general (and the models of the CC, in particular) as educational qualitative or practical realistic. The former are simple and formulated by isolating and idealizing some essential features of the biological phenomenon in study, but their output *may* be divorced from the reality. However, their "real virtue... lies rather in the fact that they provide a process for gaining insight, expressing ideas, and eventually extending to more complex models" [24].

In this paper, we will study the mathematical properties of a pioneering qualitative model of the regulation mechanism of the mitotic cycle proposed by Tyson [9]. This model has had and has a great role in the above said process of gaining insight into the phenomenon of cell division cycle.

The model in [9] is biologically based on the fact that the mitotic cycle appears to be controlled by the activity of an enzyme [3] called maturation promotion factor (MPF), which is an heterodimer composed of Cyclin and the protein kynase Cdc2: P-Cyclin-Cdc2. By means of some drastic biological simplifications interplay between Cyclin and Cdc2 may be described as follows: Cyclin and pre-existing phosphorylated Cdc2 (Cdc2-P) form inactivated MPF: P-Cdc2-cyclin-P (referred as preMPF). PreMPF is activated autocatalytically by MPF itself by dephosphorylation. At the transition Metaphase–Anaphase [3] MPF breaks down releasing Cyclin-P and Cdc2, which, in turn, is then phosphorylated giving Cdc2-P, etc. This simplified biochemical network, and the related model, may be considered as "a reasonable 'first approximation' to the cell-cycle regulatory network" [9]. We will refer the reader to that excellent work for further biological details and numerical simulations, but we stress here that a global mathematical analysis of the above model seems to us not only a mathematical exercise of some interest, but also and mainly a useful biological complement to the inferences done in [9], which are based mainly on numerical simulations.

Recently in [25], an interesting mathematical analysis of the transient state local variational stability of Tyson's model has been done by means of the KCC theory [26]. However, in the present work, by means of the qualitative theory of planar dynamical systems and by means of some novel results in the theory of Lienard's equation, we shall try to find global conditions such that the CC stops, conditions such that the cell may cycle and conditions such that the cycle is unique and globally stable. Since we will use extensively the theory of limit cycles and, more in particular, Lienard's equation, two excellent sources of information on these topics are the classical books by Farkas [28] and Zhang et al. [27].

This paper is organized as follows: in Section 2, we extend the model [9] by allowing a more general function for the autocatalysis. In Section 3, we study the stability of the CC arrest. Furthermore, we will show that the solutions are bounded and that, under simple conditions, there is at least a limit cycle. We also state conditions for the uniqueness and global stability of the CC. In Section 4, we return to the standard Tyson's model and we

apply to it the general theorems of Section 3. In particular, we find numerically a region, in the space of the parameters, such that to each point of it there corresponds a unique globally stable periodic solution. The final section is devoted to a short biological discussion of the mathematical analysis done.

2. Definition of the family of models

In [9], Tyson proposed the following family of models of CC:

$$\begin{aligned} \frac{\mathrm{d}}{\mathrm{d}t}u(t) &= K_4 \times (v-u)K(u) - K_6 u, \\ \frac{\mathrm{d}}{\mathrm{d}t}v(t) &= \hat{K}_1 - K_6 u, \end{aligned} \tag{1}$$

where

- $u(t) = [\mathrm{MPF}]/[\text{Total Cdc2}] \geqslant 0$;
- $v(t) = ([\mathrm{MPF}] + [\mathrm{preMPF}] + [\mathrm{Cyclin}])/[\text{Total Cdc2}]$. This implies, of course, that we must have

$$v(t) \geqslant u(t). \tag{2}$$

- All the parameters are positive. Biologically, K_6 is the rate of dissociation of the active MPF complex.
- $K(u) > 0$ and $K'(u) > 0$ for $u > 0$.

It is easy to see that constraints $u \geqslant 0$ and $v \geqslant u$ are fulfilled naturally by (1) since the set

$$\Gamma = \{(u, v) | u \geqslant 0, v \geqslant u\} \tag{3}$$

is positively invariant for it. In [9], the particular case

$$K(u) = a + u^2 \tag{4}$$

(where $a = \hat{K}_4/K_4$) is examined, but it is explicitly remarked that formula (4) is "only one of many possible ways to describe the autocatalytic feedback of the active MPF on its own production" [9]. Therefore, here we examine the general family and we return to the functional form (4) in the Section 4. We shortly note here that, when $K(u) = a + u^2$, K_4 is biologically the rate of autocatalytic activation of MPF.

Noting that $K(u) > 0$, instead of (1) we will study the following topologically equivalent system:

$$\begin{aligned} u'(\tau) &= v - v_1(u), \\ v'(\tau) &= \frac{c-u}{\phi K(u)}, \end{aligned} \tag{5}$$

(in short notation $(u, v)' = \mathbf{A}(u, v)$) where $c = \hat{K}_1/K_6$, $\phi = K_4/K_6$ and

$$\frac{\mathrm{d}\tau}{\mathrm{d}t} = K_4 \times K(u(t)). \tag{6}$$

Furthermore, the nullcline $v' = 0$ is the vertical straight line $u = c$, whereas the nullcline $u' = 0$ is given by

$$v_1(u) = u + \frac{u}{\phi K(u)}. \tag{7}$$

The function $v_1(u)$ has the following properties:

- $u \leqslant v_1(u) \leqslant (1 + 1/\phi K(0))u$;
- For all $m \in (1, 1 + (\phi K(0))^{-1})$, the curve $v = v_1(u)$ intersects the curve $v = mu$ in the origin and in one another point.

We add the following reasonable constraint on $v_1(u)$: equation $v_1'(u) = 0$ has no solutions or two, which we will call u_M and u_m, with $u_M < u_m$. In the light of the second property of $v_1(u)$, this constraint is not excessively sharp. This may be seen as follows: let us define the following function $\sigma(w) = E^{-w} v_1(E^w)$. It is easy to see that the equation $v_1'(u) = 0$, after the transformation $u = E^w$, becomes $\sigma'(w) + \sigma(w) = 0$. If $\sigma(w)$ has only one inflection point, as it happens when $K(u)$ has no inflection points and for many functions $K(u)$ having only one inflection point, the equation has zero or two solutions. We stress here that also in some cases in which $\sigma(w)$ has more than a single inflection points the equation has 0 or 2 solutions.

Now that the family is totally defined, we may pass to its analysis.

3. Qualitative properties of the solutions of family (5)

Family (5) has the unique equilibrium $E = (c, v_1(c))$, to which corresponds the following characteristic equation:

$$\lambda^2 + v_1'(c)\lambda + \frac{1}{\phi K(c)} = 0. \tag{8}$$

Note that the equilibrium value of u is $u_E = c$, which is inversely proportional to the dissociation rate K_6. It is easy to verify that

Proposition 3.1. *If $v_1'(c) > 0$ then E is LAS. If u_M and u_m exist and $u_M < c < u_m$ then $v_1'(c) < 0$ and E is unstable. Finally, if $c < u_M$ or $c > u_m$ then E is LAS.*

Remark. Note that when E is unstable, the characteristic equation has two solutions real and positive or having positive real part. So E is never a saddle and, as a consequence, homoclinic orbits are ruled out.

Proposition 3.2. *If $v_1'(u) > 0$ in $\mathbb{R}_+$ then E is GAS.*

Proof. Since $\mathrm{Div}(u', v') = -v_1'(u)$, if $v_1'(u)$ has constant sign there are no closed orbits and E is also LAS, so E must be GAS for the Poincare' Bendixon's trichotomy. □

However important, the instability criterion $u_M < c < u_m$ of Proposition 3.1 does not guarantee the existence of limit cycles, which is the main aim of a model of a periodical phenomenon. In order to demonstrate the existence of such solutions we should find a bounded invariant set.

Proposition 3.3. *The set*

$$\Omega = \left\{ (u, v) | u \geqslant 0, v \geqslant u, v \leqslant u + \frac{c}{\phi K(0)}, v \leqslant c + \frac{c}{\phi K(0)} \right\} \tag{9}$$

is positively invariant and attractive for family (5).

Proof. Let us consider for $q \geqslant (c/\phi K(0))$, the following points: $O = (0, 0)$, $P_q = (0, q)$, $Q_q = (c, c + q)$, $R_q = (c + q, c + q)$. The segments $\overline{OP_q}$, $\overline{P_q Q_q}$, $\overline{Q_q R_q}$, and $\overline{Q_q O}$ form the border of a family of bounded sets, whose members are as follows:

$$\Omega_q = \left\{ (u, v) | u \geqslant 0, v \geqslant u, v \leqslant u + q, v \leqslant c + q, q \geqslant \frac{c}{\phi K(0)} \right\}. \tag{10}$$

Thus $\Omega = \Omega_{(c/\phi K(0))}$. Studying the flux of (5) on

$$\partial\Omega_q = \overline{OP_q} \cup \overline{P_q Q_q} \cup \overline{Q_q R_q} \cup \overline{Q_q O},$$

it is easy to see, via direct calculations, that in all points of $\partial\Omega_q$ the flux points towards the interior of Ω_q, which implies that each Ω_q is positively invariant. For example, the flux on $\overline{P_q Q_q}$, whose external normal vector is $\mathbf{n} = 2^{-0.5}(-1, 1)$, points towards the interior of Ω_q for all $q \geqslant (c/\phi K(0))$ because we have $\mathbf{A}(u, v) \cdot \mathbf{n} = -q + (c/\phi K(u))$. Furthermore, since the sets $\{\Omega_q\}_{q \geqslant (c/\phi K(0))}$ cover all $\mathbb{R}^2_+$ and are such that $\partial\Omega_{q_1} \cap \partial\Omega_{q_2} = \{\}$ if $q_1 \neq q_2$, and since each set Ω_q contains the equilibrium point, it follows that each Ω_q is attractive. As a consequence, Ω is also attractive as we claimed. □

From Propositions 3.1 and 3.3 it follows that

Proposition 3.4. *If $u_M < c < u_m$ then in Ω there is at least one periodic solution.*

Furthermore:

Proposition 3.5. *If u_M, u_m exist, then at $c = u_M$ and at $c = u_m$ there are Hopf's bifurcation.*

Proof. Let $\lambda(c)$ and $\lambda^*(c)$ be the two complex conjugate eigenvalues at E, expressed in function of the bifurcation parameter c. From Hopf's bifurcation theory if for $c = c_h$ (in our case $c_h = u_M$ or $c_h = u_m$) $\Re(\lambda)|_{c=c_h} = 0$ and $\Re(\lambda')|_{c=c_h} \neq 0$ then at $c = c_h$ there is an Hopf's bifurcation. In our case $\Re(\lambda') = -v_1''(c)$ which is not zero ($u_M \neq u_m$). □

We will study later the uniqueness and stability of the limit cycle.

We note that (5) is in non-translated Lienard's Canonical form (non-translated since we did not translate the state variables in order to have the origin as equilibrium point) and that

eliminating v one obtains the Lienard's equation:

$$u'' + v_1'(u)u' + \frac{u-c}{\phi K(u)} = 0. \tag{11}$$

The Lienard's functions f, g and F, and the Lienard's potential G are defined as follows:

$$f(u) = v_1'(u) \Rightarrow F(u) = v_1(u) - v_1(c), \tag{12}$$

$$g(u) = \frac{u-c}{\phi K(u)} \Rightarrow G(u) = \int_c^u g(s)\,\mathrm{d}s \tag{13}$$

(note that when $v_1'(u) = 0$ has two solutions, F is N-shaped).

Let us now call u_l the nontrivial solution of the equation $v_1(u) = v_1(u_m)$ and u_r that of $v_1(u) = v_1(u_M)$. Using these notations we have the following proportions.

Proposition 3.6. *If $c < u_l < u_M$ or $c > u_r > u_m$ then E is GAS.*

Proof. If $c < u_l$ then $v_1(u_m) > v_1(c) \Rightarrow F(u_m) > 0$ and $\operatorname{sign}(F(u)) = \operatorname{sign}(u-c)$. Similar considerations hold for the case $c > u_r$. So, as in Corollary 2.3 of [31], using the Liapunov function $W = G(u) + (v - v_1(c))^2/2$ and applying the LaSalle's Theorem [33] it follows easily that E is GAS in Ω. □

This result may be further improved.

Proposition 3.7. *Let $u_m < c < u_r$. If $G(u_r) < G(u_l)$ and $\forall s \in (c, u_r)$ $F(s) \neq F(\Psi_+(s))$ (where $\Psi_+(s) = G^{-1}(G(s))$, $s > c$) then E is GAS.*

Proof. Note firstly that when $G(u_r) \geqslant G(u_l)$, equation $F(s) = F(\Psi_+(s))$ has solutions. In fact, we have $s_x < s_\Delta < u_r$, and also $F(\Psi_+(s_x)) = 0$ and $F(\Psi_+(s_\Delta)) = F(u_M)$. So because of the continuity of $F(s)$ and of $F(\Psi_+(s))$ there is a $s_a \in (s_x, s_\Delta)$ such that $F(s_a) \neq F(\Psi_+(s_a))$. On the contrary, when $G(u_r) < G(u_l)$ the equation $F(s) = F(\Psi_+(s))$ may have no solutions. In such a case, Lemma 9 of [30] holds (see also the equivalent Theorem 2.4 of [31]) and there are no closed orbits. As a consequence, E is GAS in Ω. □

A similar proposition holds for $c \in (u_l, u_M)$. Summarizing:

Corollary 3.8. *There is a $\hat{u_l} \in [u_m, u_r]$ ($\hat{u_l} \in [u_l, u_M]$) such that if $c < \hat{u_l}$ ($c > \hat{u_r}$) then E is GAS.*

Coming to the study of the uniqueness and stability of the limit cycle, currently to the best of our knowledge there are essentially two possibilities of mathematical investigation. (1) for $u \neq c$ the function $(f(u)/g(u))'$ is positive so that the Zhang's theorem [29] can be applied. (2) Another feasible alternate is Theorem 2.6 of [31], which summarizes Theorems 1 and 4 of [30], and which-in our case-reads as follows:

Proposition 3.9 (*Zeng et al. [30], Xiao and Zhang [31]*). *If*

$$G(u_M) \leqslant G(u_1) \leqslant G(u_2) \tag{14}$$

(*where* $u_2 < c < u_1$ *are such that* $F(u_1) = F(u_2) = 0$) *and the function* $Q(u) := F(u)f(u)/g(u)$ (*or* $Q(u) := f(u)/g(u)$) *is such that*

$$Q'(u) \geqslant 0 \quad \text{for } u \geqslant u_1, \tag{15}$$

then (5) *has at most one limit cycle and it is stable.*

If follows that

Corollary 3.10. *If by means of some* (*present or future*) *theorem one can establish the uniqueness and local stability of a limit cycle of* (5), *then from Proposition* 3.4 *it follows that this unique limit cycle is also GAS.*

Remark. We stress that the authors of [30] studied the Lienard's system $x' = y - F(x)$, $y' = -g(x)$ in a rectangular domain $[x_1, x_2] \times [y_1, y_2]$. However, the constraint $-\infty \leqslant y_1 < y < y_2 \leqslant +\infty$ is never used, directly or indirectly, in the demonstrations of the theorems we used here (and in the related lemmas, of course). In other and rough words, the "rectangularity" does not play an essential role. Thus theorems and lemmas of [30] may be as well applied—as we did—in case of domains of the following type: $\{(x, y) | x_1 \leqslant x \leqslant x_2 \text{ AND } \alpha(x) \leqslant y \leqslant \omega(x)\}$, where $\alpha(x)$ and $\omega(x)$ are continuous functions. Another more trivial difference is that we used an non-translated form of the Lienard's system.

4. Applications to Tyson's specific model

In this section, we will apply the generic propositions of the previous section to Tyson's model.

For this model it is:

$$v_1(u) = u + \frac{u}{\phi(a + u^2)}, \tag{16}$$

$$\begin{aligned} f(u) = v_1'(u) &= \frac{\phi u^4 + (2a\phi - 1)u^2 + a(a\phi + 1)}{\phi(a + u^2)^2} \Rightarrow F(u) = v_1(u) - v_1(c) \\ &= u + \frac{u}{\phi(a + u^2)} - c - \frac{c}{\phi(a + c^2)}, \end{aligned} \tag{17}$$

$$\begin{aligned} g(u) &= \frac{u - c}{\phi(a + u^2)} \Rightarrow G(u) \\ &= \frac{1}{2\phi} \log\left(\frac{a + u^2}{a + c^2}\right) + \frac{c}{\phi\sqrt{a}} \left(\arctan\left(\frac{c}{\sqrt{a}}\right) - \arctan\left(\frac{u}{\sqrt{a}}\right)\right), \end{aligned} \tag{18}$$

where, as we have mentioned $a = \hat{K}_4/K_4$, $c = \hat{K}_1/K_6$ and $\phi = K_4/K_6$.

Remark. In [9], the following estimates are given: $K_4 \in (10, 1000)$, $K_6 \in (0.1, 10)$, $\hat{K}_1 = 0.015$ and $\hat{K}_4 = 0.018$. The measure unit is $\min^{-1}$.

Proposition 3.2 is now:

Proposition 4.1. *If* $1 - 8a\phi < 0$ (*i.e., if* $K_6 < 8\hat{K}_4$) *then E is GAS.*

Proof. The numerator of $f(u)$ is the bi-quadratic polynomial $\phi u^4 + (2a\phi - 1)u^2 + a(a\phi + 1)$ whose discriminant is $1 - 8a\phi$. Thus, if $1 - 8a\phi < 0$ then $f(u) > 0$. □

By using the above estimates, if $K_6 < 0.144$ there is globally stable cell arrest.
For $1 - 8a\phi > 0$, the equation $f(u) = 0$ has 4 roots (of which only two are positive):

$$u_M(a, \phi) = \sqrt{\frac{1 - 2a\phi - \sqrt{1 - 8a\phi}}{2\phi}}, \quad \hat{u}_M = -u_M, \tag{19}$$

$$u_m(a, \phi) = \sqrt{\frac{1 - 2a\phi + \sqrt{1 - 8a\phi}}{2\phi}}, \quad \hat{u}_m = -u_m. \tag{20}$$

Proposition 3.4 on the existence of the limit cycle is now:

Proposition 4.2. *If* $u_M(a, \phi) < c < u_m(a, \phi)$, *i.e., if*

$$\frac{K_6^3}{2\hat{K}_1^{\,2}}\left(1 - 2\frac{\hat{K}_4}{K_6} - \sqrt{1 - 8\frac{\hat{K}_4}{K_6}}\right) = L(K_6) < K_4 < H(K_6) = \frac{K_6^3}{2\hat{K}_1^{\,2}}\left(1 - 2\frac{\hat{K}_4}{K_6} + \sqrt{1 - 8\frac{\hat{K}_4}{K_6}}\right), \tag{21}$$

then there is at least a limit cycle in Ω.

When using the values in [9], the region $R = \{(K_6, K_4) | L(K_6) \leqslant K_4 \leqslant H(K_6)\})$ such that there is at least a periodic solution is showed in Fig. 1. Since the maximum value estimated of K_4 is 1000 and the minimum is 10, it follows that for $K_6 \gtrsim 3.5$ and for $K_6 \lesssim 0.16$ there is no stable limit cycle (in theory, there might be a limit cycle surrounded by two unstable cycles) and E is (at least) LAS.

For which regards the Hopf's bifurcations, the following holds:

Proposition 4.3. *At* $K_4 = L(K_6)$ *and at* $K_4 = H(K_6)$ *the characteristic equation has two imaginary roots. For constant* K_6, *assuming* K_4 *as bifurcation parameter, if* $c^3 \neq 3a$ *then there is an Hopf's bifurcation with stable arising cycle. For constant* K_4, *assuming* K_6 *as bifurcation parameter, if* $c^2 \neq (1 + 2/\sqrt{3})\phi$ *there is an Hopf's bifurcation with stable arising cycle.*

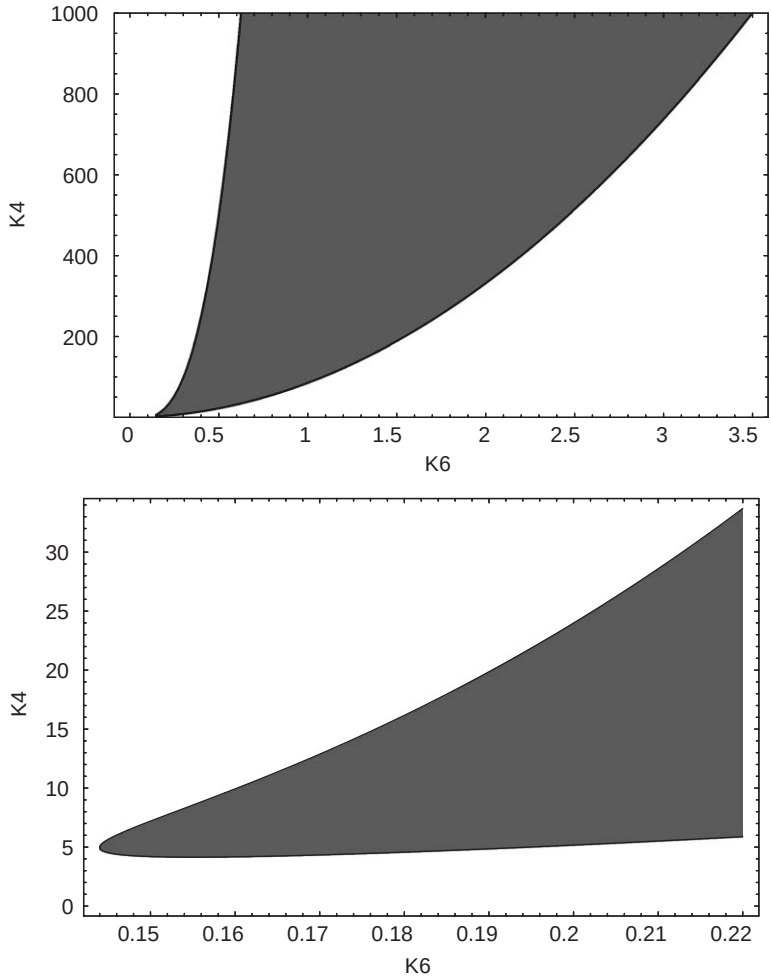

Fig. 1. Plots of $L(K_6)$ and $H(K_6)$. High: plot of both curves. In gray is plotted the Region R such that there is at least one limit cycle (and E is unstable). Low: zoom for low values of K_6. Since [9] $K_4 \in (10, 1000)$, for $K_6 \gtrsim 3.50$ and for $K_6 \lesssim 0.16$ there is no unique limit cycle and E is (at least) locally stable.

Proof. The relation $c^3 \neq 3a$ ($c^2 \neq (1 + 2/\sqrt{3})\phi$) is obtained by imposing the non nullity of $(\partial f/\partial K_4)(c)$ ($(\partial f/\partial K_6)(c)$). Following Guckenheimer and Holmes [32], we reduced for $K_4 = L(K_6)$ system (1) (with $K(u) = a + u^2$) to the standard form (3.4.10) of [32]. Hence, we calculated symbolically the formula (3.4.11) of [32]. Then, we repeated the same calculations for $K_4 = H(K_6)$. We obtained, of course, two different functions for the two different cases. However, the sign of both of them was ruled by the same expression:

$$v = -8(a\phi)^2 - (1 - 8a\phi) - (1 - 4a\phi)\sqrt{1 - 8a\phi}. \tag{22}$$

When $(a\phi) \in (0, \frac{1}{8}]$, v is negative, being the sum of three negative quantities. Thus the cycles arising are stable in both cases. □

For which regards the uniqueness and GAS of the limit cycle, the region Θ in which Proposition 3.9 holds must be a subset of the set $R = \{(K_6, K_4) | L(K_6) \leqslant K_4 \leqslant H(K_6)\}$. However, inequalities (14) cannot be solved analytically, so the set Θ has been calculated numerically by using Mathematica 4.0 (TM). This software has also been used to assess if given a point $p \in \Theta$ it is $Q(u)$ is non-decreasing for $u > u_1$ (condition (15)). This task has been quite easy since $Q'(u) = P_7(u)/(\phi(a+c^2)(a+u^2)^3)$, where $P_7(u)$ is a seventh degree polynomial with positive coefficient for u^7. So, for a given pair (K_6, K_4), if $\tilde{u}$ denotes the maximum positive root of $P_7(u)$ and $\tilde{u} < u_1$, then $Q(u)$ is non-decreasing for $u > u_1$. It follows that in Θ $Q'(u) > 0$ for $u > u_1$. So both conditions of Theorem 3.9 are fulfilled. The result of our calculation is that the region Θ is considerably smaller than R (see Fig. 2), which is a consequence of the nature of the sharp condition (14). However, Θ is not neglectable, since it contains a significant part of the set $[0.1, 10] \times [10, 1000]$.

Finally, since after some algebras it turns out that

$$u_l = u_M(a, \phi)\frac{1-\sqrt{1-8a\phi}}{1+\sqrt{1-8a\phi}} = u_M\left(\frac{\hat{K}_4}{K_4}, \frac{K_4}{K_6}\right)\frac{1-\sqrt{1-8\frac{\hat{K}_4}{K_6}}}{1+\sqrt{1-8\frac{\hat{K}_4}{K_6}}} \tag{23}$$

and

$$u_r = u_m(a, \phi)\frac{1+\sqrt{1-8a\phi}}{1-\sqrt{1-8a\phi}} = u_m\left(\frac{\hat{K}_4}{K_4}, \frac{K_4}{K_6}\right)\frac{1+\sqrt{1-8\frac{\hat{K}_4}{K_6}}}{1-\sqrt{1-8\frac{\hat{K}_4}{K_6}}}, \tag{24}$$

it is easy to see that Proposition 3.6 becomes

Proposition 4.4. *If*

$$K_4 < A(K_6) = L(K_6)\frac{1-\sqrt{1-8a\phi}}{1+\sqrt{1-8a\phi}} \text{ or } K_4 > B(K_6) = H(K_6)\frac{1+\sqrt{1-8a\phi}}{1-\sqrt{1-8a\phi}}, \tag{25}$$

then E is GAS.

By using Tyson's values for $\hat{K}_1$ and $\hat{K}_4$, we obtained that $A(K_6)$ decreases very quickly also for small values of K_6, whereas $B(K_6)$ for small K_6 has biologically meaningful values, as depicted in Fig. 3. In reality, by applying Theorem 3.7, it is possible to find a larger region to which it corresponds a GAS equilibrium. For example, for $K_6 \in (0.3, 3.50)$ and $K_4 < L(K_6)$, the GAS region found is equal to the LAS region, whereas for $K_4 > H(K_6)$ the GAS zone is considerable, but smaller than the LAS region as in Fig. 4

5. Summary and biological discussion

For the general family (1), a positively invariant set has been detected, surrounding the unique equilibrium point. As a consequence, when the equilibrium is unstable there is at

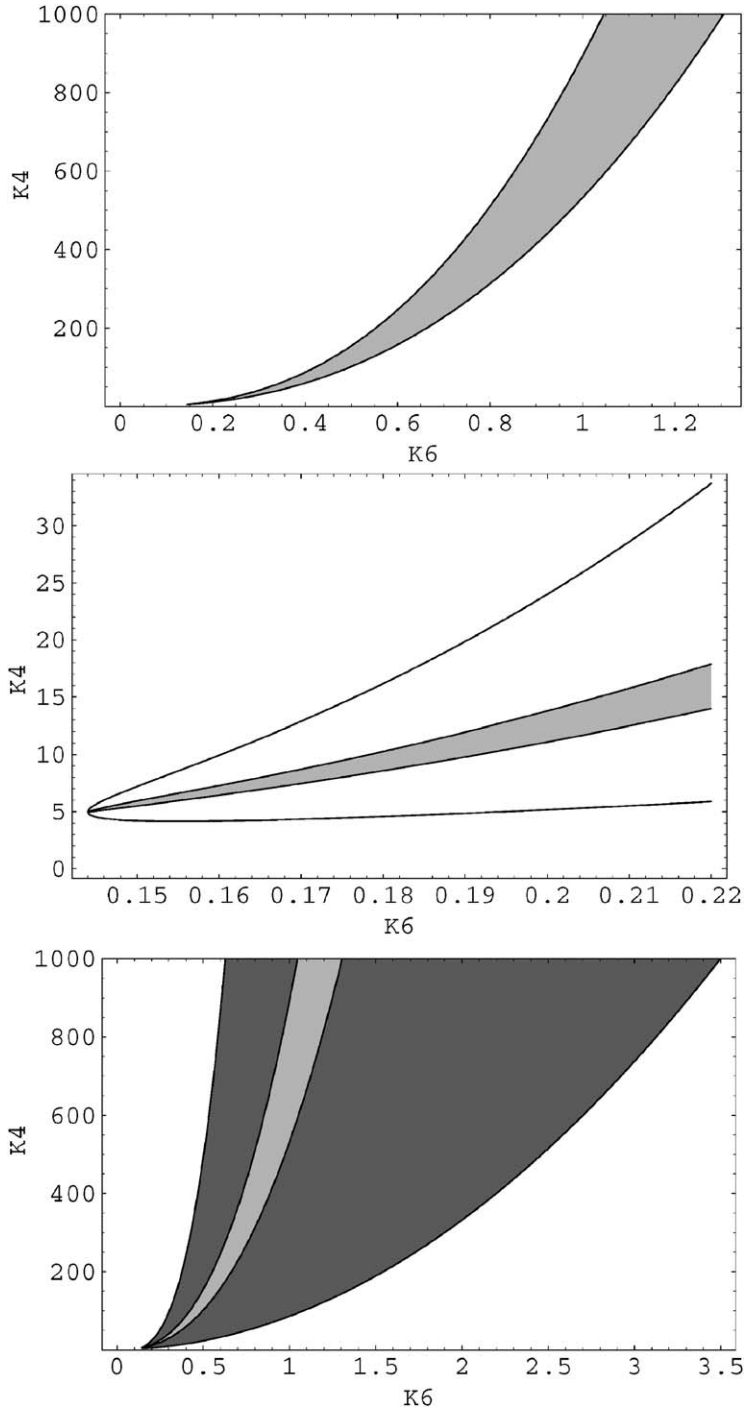

Fig. 2. High: Region Θ which guarantees the existence and uniqueness of a GAS limit cycle. Low: comparisons between the above figure and the region R.

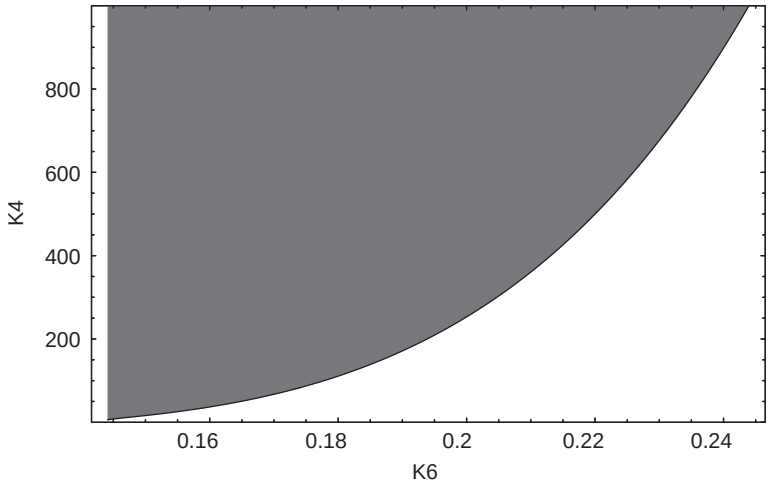

Fig. 3. Plot of $B(K_6)$, in gray the region $B(K_6) < K_4 < 1000$ in which the criterion 3.6 is fulfilled and E is GAS.

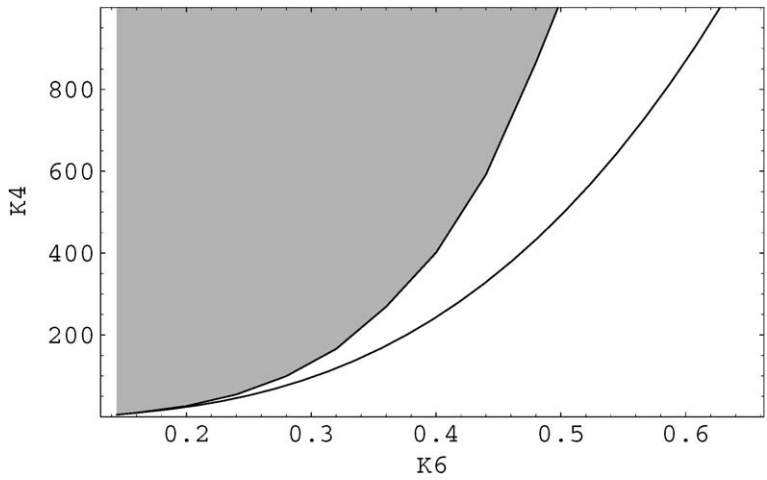

Fig. 4. In the region in gray, obtained by applying the Theorem 3.7, the equilibrium point E is GAS. In figure also the curve $K_4 = H(K_6)$ is plotted.

least one stable limit cycle. Biologically this means that in this case the cell cycles. We may think the following take the three main configurations:

- There is an unique limit cycle, which must be GAS;
- There is one stable limit cycle L_o and one or two unstable LCs, which, because of their instability, are not physically observable: in the "real world" all the orbits will tend to L_o. Roughly speaking, it is "as if" the system had an unique GAS LC;
- There may be two locally stable cycles (birhytmicity), or even more than two.

The third case physically means that the periodic behavior of the cell division and, in particular, the period may depend not only on the kinetic features (such as the parameters ϕ, c and the shape of $K(u)$), but also on the initial abundance of the MPF and of the total cyclin. Therefore, we gave also some general conditions which guarantee the uniqueness of the cycle.

For the general family, we studied also the phenomenon of the cell cycle arrest. We obtained two mathematical conditions which may be roughly summarized as follows:

- If $v_1'(u) > 0$ then the equilibrium point is globally stable.
- If $v_1'(u)$ has variable sign and if $c < u_M$ or $c > u_m$ then the cell cycle stops.

Biologically, remembering that the equilibrium value for the relative concentration of MPF is $u_{eq} = c \propto K_6^{-1}$, we may *read* the above conditions as follows:

- The possibility of cycling depends ultimately on the shape of autocatalytic function $K(u)$, since some classes of $K(u)$ resulting in positive $v_1'(u)$ are not compatible with the existence of periodic solutions.
- There is no cycle if the equilibrium value of the relative MPF concentration is excessively low or high, i.e. if the dissociation rate K_6 is excessively large or low.

Then we studied Tyson's model.

The space of the positive parameters K_4 and K_6 is divided into two regions. Let us call them R and $Q = \mathbb{R}_+^2 - R$. In the set Q, E is at least LAS (but in large portions of Q we showed that E is GAS). In all R, E is unstable and at least a stable LC exists. At the border of R, Hopf's bifurcations occur.

Biologically, and with reference to Fig. 1, we may see that very low levels of autocatalyctic activation rate does not allow the possibility of cycling, independent the value of K_6. Symmetrically, excessively low dissociation rates K_6 are not compatible with cycling, independent the value of the activation rate K_4. For intermediate values of K_6, there are two threshold values for K_4: $L(K_6)$ and $H(K_6)$. There is cell cycle only when $L(K_6) \leqslant K_4 \leqslant H(K_6)$. Numerically, however, we showed that the higher threshold increases very quickly, so that only the lower threshold for the activation rate is biologically meaningful.

Finally, we assessed the uniqueness and stability of the limit cycle, by using some recent sufficient theorem and the numerical values given in [9].

We found a set $\Theta \subset R$ such that for all $p \in \Theta$ there corresponds a GAS limit cycle. Since Θ was located by applying a strong sufficient condition, Θ is quite smaller than R. However, we obtained that Θ lies in a biologically meaningful zone of the space of parameters. Now less restrictive theorems on uniqueness of limit cycles could allow to demonstrate the following conjecture: for all $p \in R$ there corresponds a GAS limit cycle.

The assessment of the global stability of the cycle has an interesting biological meaning: independent the initial concentrations of the chemicals, two cells having the same kinetic parameters oscillate not only with the same period, but also with the same "law". Furthermore, when there is a GAS limit cycle, in a cell also large random perturbations of the concentrations do not destroy its normal behavior, since the orbit will tend to the same limit cycle. On the contrary, if there are multiple coexisting limit cycles, their period and

general dynamics would be a function of the initial concentrations. Starting point belonging to different basins of attraction of different solutions would result in a different behavior. As a consequence, a perturbation of the values of MPF, for example, might result in a different cycle.

Summarizing, our analysis of the family and of the specific Tyson's model seems to indicate that

- Only perturbations in kinetic parameters may cause the arrest of the cell cycle.
- In the most complex case (i.e. coexistence of multiple LAS periodic orbits), variations in the concentrations of the involved proteins may cause dramatic dynamical variations to the cell cycle (e.g. a non-small variation of its period), but cannot stop it.

Acknowledgements

I would like to thank very much many people: an anonymous referee, who pointed out to me some typing errors and some unclear demonstrations in the first version of the manuscript, and suggested to extend and deepen the biological discussion; the editors of the journal for their excellent and rapid work; Dr. Giorgio Donnini, for his precious bibliographical help.

References

[1] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson, Molecular Biology of the Cell, Garland, New York, London, 1994.
[2] B. Novak, J.J. Tyson, Modeling the cell division cycle: M-phase trigger, Oscillations and size control, J. Th. Biol. 165 (1993) 101–134.
[3] W.K. Purves, D. Sadava, G.H. Orians, H.C. Heller, Life: The Science of Biology, fifth ed., W. H. Freeman, New York, 1998.
[4] J.J. Tyson, A. Csikasz-Nagy, B. Novak, The dynamics of cell cycle regulation, BioEssays 24 (2002) 1095–1109.
[5] J. Keener, J. Sneyd, Mathematical Physiology, Springer, Heidelberg, New York, 1998.
[6] J.D. Murray, Mathematical Biology, vol. I, third ed., Springer, Heidelberg, New York, 2003.
[7] J.W. Zwolak, J.J. Tyson, L.T. Watson, Finding all steady state solutions of chemical kinetic models, Nonlinear Anal. Real World Appl. 5 (2004) 801–804.
[8] R. Norel, Z. Agur, A model for the adjustment of the mitotic clock by cyclin and MPF levels, Science 251 (1991) 1076–1078.
[9] J.J. Tyson, Modeling the Cell division cycle: cdc2 and cyclin interaction, Proc. Natl. Acad. Sci. USA 88 (1991) 7328–7332.
[10] A. Goldbeter, A minimal cascade model for the mitotic oscillator involving Cyclin and cdc2 kinase, Proc. Natl. Acad. Sci. 88 (1991) 9107–9111.
[11] S. Busenberg, B. Tang, Mathematical models of the early embryonic cell cycle: the role of MPF activation and cyclin degradation, J. Math. Biol. 32 (1994) 573–596.
[12] B. Novak, J.J. Tyson, Quantitative analysis of a molecular model of mitotic control in fission yeast, JTB 173 (1995) 283–305.
[13] R.A. Weinberg, The retinoblastoma protein and cell-cycle control, Cell 81 (1995) 323–328.
[14] M.N. Obeyesekere, J.R. Herbert, S.O. Zimmerman, A model of the G1 phase of the cell cycle incorporating cyclin E = cdk2 complex and retinoblastoma protein, Oncogene 11 (1995) 1199–1205.

[15] B. Novak, J.J. Tyson, Modeling the control of DNA replication in fission yeast, Proc. Natl. Acad. Sci. USA 94 (1997) 9147–9152.
[16] Z. Agur, Lessons on models of cell division cycle, Lecture notes of the Termoli summer school on biomathematics, handouts, 1999.
[17] M.N. Obeyesekere, S.O. Zimmerman, E.S. Tecarro, et al., A model of cell cycle behavior dominated by kinetics of a pathway stimulated by growth factors, Bull. Math. Biol. 61 (5) (1999) 917–934.
[18] Zuohuan Zheng, Tiansou Zhou, Suochun Zhang, Dynamical behavior in the modeling of cell division cycle, Chaos Soliton Fractals 11 (2000) 2371–2378.
[19] B. Novak, Z. Pataki, A. Ciliberto, J.J. Tyson, Mathematical model of the cell division cycle of fission yeast, Chaos 11 (1) (2001) 277–286.
[20] Zhilin Qu, W.R. MacLellan, J.N. Weiss, Dynamics of the cell cycle: checkpoints, sizers, and timers, Biophys. J. 85 (2003) 3600–3611.
[21] E.S. Tecarro, M.N. Obeyesekere, G. Auchmuty, Mathematical analysis of a 3-variable cell cycle model, Nonlinear Anal. RWA 4 (1) (2003) 87–107.
[22] B. Novak, J.J. Tyson, Modeling the controls of the eukaryotic cell cycle, Biochem. Soc. Trans. 31 (2003) 1526–1529.
[23] A d'Onofrio, On a family of models of the cell division cycle, Chaos Solitons Fractals, in press, 2005.
[24] A. Okubo, Diffusion and Ecological Models Problems: Mathematical Models, Springer, Heidelberg, New York, 1978.
[25] P.L. Antonelli, S.F. Rutz, S.V. Sabau, A transient-state analysis of Tyson's model for the cell division cycle by means of KCC-theory, Open Syst. Inf. Dyn. 9 (3) (2002) 223–228.
[26] P.L. Antonelli, L. Bevilacqua, S.F. Rutz, Theories and models in symbiogenesis, Nonlinear Anal. Real World Appl. 4 (2003) 743–753.
[27] Z. Zhang, T. Ding, W. Huang, Z. Dong, Qualitative Theory of Differential Equations, American Mathematical Society, Providence, RI, 1997.
[28] M. Farkas, Periodic motions, Springer, Heidelberg, 1994.
[29] Z. Zhang, Proof of the uniqueness theorem of limit cycles of generalized Lienard equations, Appl. Anal. 23 (1986) 63–76.
[30] X. Zeng, Z. Zhang, S. Gao, On the uniqueness of the limit cycles of the generalized Lienard equation, Bull. London Math. Soc. 26 (1994) 213–247.
[31] D. Xiao, Z. Zhang, On the uniqueness and nonexistence of limit cycles for predator-prey systems, Nonlinearity 16 (2003) 1185–1201.
[32] J. Guckenheimer, P. Holmes, Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields, Springer, Heidelberg, 1983.
[33] J.K. Hale, Ordinary Differential Equations, Wiley-Interscience, New York, 1969.