



Partial Differential Equations

Circadian rhythm and tumour growth

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Abstract

We address the following question: can one sustain, on the basis of mathematical models, that for cancer cells, the loss of control by circadian rhythm favours a faster growth? This question, which comes from the observation that tumour growth in mice is enhanced by experimental disruption of the circadian rhythm, may be tackled by mathematical modelling of the cell cycle. For this purpose we consider an age-structured population model with control of death (apoptosis) rates and phase transitions, and two eigenvalues: one for periodic control coefficients (via a variant of Floquet theory in infinite dimension) and one for constant coefficients (taken as the time average of the periodic case). We show by a direct proof that, surprisingly enough considering the above-mentioned observation, the periodic eigenvalue is always greater than the steady state eigenvalue when the sole apoptosis rate is concerned. We also show by numerical simulations when transition rates between the phases of the cell cycle are concerned, that, without further hypotheses, no natural hierarchy between the two eigenvalues exists. This at least shows that, if such models are to take account of the above-mentioned observation, control of death rates inside phases is not sufficient, and that transition rates between phases are a key target in proliferation control. *To cite this article: J. Clairambault et al., C. R. Acad. Sci. Paris, Ser. I 342 (2006).*

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Résumé

Rythme circadien et croissance tumorale. L'objet de cette Note est de questionner, sur des bases mathématiques, le fait expérimental que les populations de cellules de souris cancéreuses échappant au contrôle circadien, ont tendance à se développer plus vite. Pour cela nous considérons un modèle du cycle cellulaire avec contrôle des taux de mort (apoptose) et de transition entre phases, et deux valeurs propres. L'une est associée aux coefficients périodiques via la théorie de Floquet (dans une version de dimension infinie), l'autre est associée au problème stationnaire avec des coefficients moyens. Nous montrons par une preuve directe que, de façon inattendue si l'on considère l'observation expérimentale évoquée plus haut, la valeur propre périodique est plus grande que la valeur propre stationnaire dans le cas où le contrôle périodique est effectué sur l'apoptose. Nous montrons aussi, par des tests numériques dans le cas où le contrôle périodique est effectué sur le taux de transition d'une phase à l'autre du cycle cellulaire, qu'il n'existe alors aucune hiérarchie naturelle entre les deux types de valeurs propres. Ceci montre au moins que, pour que de tels modèles puissent rendre compte des observations expérimentales ci-dessus, le seul contrôle des taux de mort dans

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chaque phase est insuffisant, et que les taux de transition entre phases sont une cible clef pour le contrôle de la prolifération. **Pour citer cet article :** J. Clairambault et al., C. R. Acad. Sci. Paris, Ser. I 342 (2006).

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Version française abrégée

Nous étudions dans cette Note au moyen de modèles mathématiques et de simulations numériques l'idée suivante, qui est à l'origine de la chronothérapie des cancers : les rythmes circadiens influencent la prolifération cellulaire. En particulier, on a pu observer que la croissance tumorale est favorisée par une perturbation de ces rythmes, mesurés notamment par l'enregistrement de la température corporelle ou du cycle repos/activité. Plusieurs études épidémiologiques ont ainsi montré que des travailleurs soumis à des variations prolongées de leurs rythmes de travail sont plus exposés au risque de cancer colorectal que ceux ayant un rythme de travail régulier. On peut donc se demander si la perte du contrôle circadien sur le cycle cellulaire peut entraîner une accélération de la progression tumorale. Cette hypothèse s'appuie sur une bonne compréhension des mécanismes moléculaires contrôlant l'apoptose et les transitions entre phases du cycle cellulaire, sous l'influence de protéines telles que p53, mais aussi des cyclines et cycline-kinases (cdk). En effet certains de ces mécanismes, comme la phosphorylation du dimère CycB-cdk1 par la kinase Wee1, sont directement contrôlés par des gènes circadiens, comme Bmal1.

Notre approche repose sur l'analyse mathématique de modèles du cycle cellulaire qui sont maintenant bien établis. Nous introduisons le rythme circadien comme une périodicité de certains des coefficients de ces modèles, et examinons si cette périodicité diminue ou non la croissance du système par rapport au modèle à coefficients constants (même moyenne). Notre but est de déterminer si ce niveau de modélisation peut rendre compte du fait expérimentalement observé qu'une diminution du contrôle circadien favorise la croissance tumorale.

Il existe de nombreuses références classiques, s'appuyant éventuellement sur des comparaisons expérimentales, sur le sujet des populations structurées et, comme cas particulier, du cycle cellulaire, voir [1–3,8,11,15]. Pour une analyse récente de ces systèmes s'appuyant sur des méthodes d'entropie on peut consulter [12,13]. Nous retenons, suivant en cela une étude précédente [4], le système d'équations aux dérivées partielles (1) pour décrire la densité de cellules $n_i(t, x) \geq 0$ d'âge x dans la phase $i = 1, \dots, I$ à l'instant t . Ici nous identifions la phase 1 à la phase $I + 1$. Le coefficient $d_i(t, x)$ représente le taux d'apoptose et $K_{i \rightarrow i+1}$ les taux de transition d'une phase à la suivante (on a choisi pour la transition $I \rightarrow 1$ la mitose terminée par le doublement du nombre des cellules). Ces coefficients peuvent être constants en temps (pas de contrôle circadien) ou avoir une période T lorsqu'on prend en compte le rythme circadien. Des hypothèses sur ces coefficients sont données en (2) et (3), qui permettent de prouver que l'opérateur différentiel sous-jacent admet un premier vecteur propre positif non seulement dans le cas des coefficients constants (théorie de Krein–Rutman), associé à une valeur propre notée λ_s (s pour « stationnaire »), classiquement appelée valeur propre de Perron dans le cas de la dimension finie, mais aussi dans le cas des coefficients périodiques (théorie de Floquet, généralisée ici au cas de la dimension infinie), vecteur propre associé à une valeur propre notée λ_{per} (per pour « périodique »).

En ce qui concerne le contrôle périodique par l'apoptose (les $K_{i \rightarrow i+1}$ sont alors pris constants), nous démontrons que, de façon surprenante au regard des résultats des expérimentations animales, on a toujours $\lambda_{\text{per}} > \lambda_s$. Pour le contrôle par les taux de transition, les simulations numériques montrent qu'il n'y a pas d'ordre général entre ces deux valeurs propres, alors que les données physiopathologiques sur la transition de G1-S-G2 à M obtenues à partir de courbes expérimentales de croissance tumorale sur des animaux de laboratoire vont toutes dans le sens $\lambda_s > \lambda_{\text{per}}$.

Il n'est pas surprenant que l'apoptose ne suffise pas à expliquer dans le cadre de ce modèle le phénomène observé expérimentalement, puisque l'influence attendue du rythme circadien se situe, du moins en ce qui concerne le mécanisme bien identifié du contrôle circadien de la kinase cdk1, au niveau des transitions de phases et non du taux de mort dans chaque phase.

1. Cell cycle and circadian rhythm

The goal of this Note is to address by means of mathematical and numerical models the following fact which underlies chronotherapy [9,14]: circadian rhythms influence cell proliferation. In particular, tumour growth has been shown to be favoured by disruptions of the circadian rhythm, as assessed e.g. by body temperature or rest-activity

recordings [5]. This is supported by clinical observations according to which patients with cancer *and* disrupted circadian rhythms are less responsive to chemotherapy and have poorer prognosis than others with the same diseases but strong circadian rhythmicity [9,14]. Furthermore, molecular mechanisms underlying circadian control on apoptosis and cell cycle phases through proteins such as p53 and cyclins are currently being unveiled [6,10,16].

Our approach relies on equations for the cell cycle which are well settled nowadays, see [1–3,8,11,15] for references on structured population dynamics and the cell cycle, and [12,13] for a more recent approach based on entropy properties. We introduce circadian control through periodic coefficients and assess the hypothesis according to which periodicity diminishes the system growth as compared to constant coefficients (same average), in order to decide if a loss of circadian control theoretically favours tumour growth. Here and following earlier work [4], we model our population of cells by a Partial Differential Equation for the density $n_i(t, x) \geq 0$ of cells with age x in the phase $i = 1, \dots, I$, at time t .

$$\begin{cases} \frac{\partial}{\partial t} n_i(t, x) + \frac{\partial}{\partial x} n_i(t, x) + [d_i(t, x) + K_{i \rightarrow i+1}(t, x)] n_i(t, x) = 0, \\ n_i(t, x = 0) = \int_{x' \geq 0} K_{i-1 \rightarrow i}(t, x') n_{i-1}(t, x') dx', \quad 2 \leq i \leq I, \\ n_1(t, x = 0) = 2 \int_{x' \geq 0} K_{I \rightarrow 1}(t, x') n_I(t, x') dx'. \end{cases} \quad (1)$$

Here and below we identify $I + 1$ to 1. We denote by $d_i(t, x) \geq 0$ the apoptosis rate, $K_{i \rightarrow i+1}$ the transition rates from one phase to the next, the last one ($i = I$) being mitosis. These coefficients can be constant or T -periodic in time in order to take into account presence or absence of circadian control. Our assumptions are

$$K_{i \rightarrow i+1}(t, x) \geq 0, \quad d_i(t, x) \geq 0 \quad \text{are bounded}, \quad (2)$$

and, setting $\min_{0 \leq t \leq T} K_{i \rightarrow i+1}(t, x) := k_{i \rightarrow i+1}(x)$, $\max_{0 \leq t \leq T} [d_i + K_{i \rightarrow i+1}] := \mu_i(x)$, $M_i(x) = \int_0^x \mu_i(y) dy$,

$$\prod_{i=1}^I \int_0^{\infty} k_{i \rightarrow i+1}(y) e^{-M_i(y)} dy > \frac{1}{2}. \quad (3)$$

Classically, one can introduce the growth rate of the system λ_{per} (Malthus parameter, first eigenvalue) such that there is a unique T -periodic *positive* solution to

$$\begin{cases} \frac{\partial}{\partial t} N_i(t, x) + \frac{\partial}{\partial x} N_i(t, x) + [d_i(t, x) + \lambda_{\text{per}} + K_{i \rightarrow i+1}(t, x)] N_i(t, x) = 0, \\ N_i(t, x = 0) = \int_{x' \geq 0} K_{i-1 \rightarrow i}(t, x') N_{i-1}(t, x') dx', \quad 2 \leq i \leq I, \\ N_1(t, x = 0) = 2 \int_{x' \geq 0} K_{I \rightarrow 1}(t, x') N_I(t, x') dx', \quad \sum_{i=1}^I \int N_i(t, x) dx = 1. \end{cases} \quad (4)$$

Under our assumptions (2) and (3), the existence of a solution to (4), with $\lambda_{\text{per}} > 0$, follows from an infinite dimensional version of Floquet theory and one has (see for instance [12])

$$\sum_i \int |n_i(t, x) e^{-\lambda_{\text{per}} t} - \rho N_i(t, x)| \varphi_i(t, x) dx \rightarrow 0 \quad \text{as } t \rightarrow \infty,$$

where $\varphi_i(t, x)$ is the periodic positive solution of the adjoint problem to (4) normalised by $\sum_i \int N_i(t, x) \varphi_i(t, x) dx = 1$, and $\rho = \sum_{i=1}^I \int n_i(t = 0, x) \varphi_i(t = 0, x) dx$. In other words, the periodic solution is the observed stable state after renormalisation by the growth rate λ_{per} .

One can also introduce the coefficients averaged in time

$$\langle K_{i \rightarrow i+1}(x) \rangle := \frac{1}{T} \int_0^T K_{i \rightarrow i+1}(t, x) dt, \quad \langle d_i(t, x) \rangle := \frac{1}{T} \int_0^T d_i(t, x) dt,$$

and consider the associated steady state solution. This allows us to define another growth rate λ_s , and a steady state solution \bar{N}_i to

$$\begin{cases} \frac{\partial}{\partial x} \bar{N}_i(x) + [(d_i(x)) + \lambda_s + \langle K_{i \rightarrow i+1}(x) \rangle] \bar{N}_i(x) = 0, \\ \bar{N}_i(x=0) = \int_{x' \geq 0} \langle K_{i-1 \rightarrow i}(x') \rangle \bar{N}_{i-1}(x') dx', \quad 2 \leq i \leq I, \\ \bar{N}_1(x=0) = 2 \int_{x' \geq 0} \langle K_{I \rightarrow 1}(x') \rangle \bar{N}_I(x') dx', \quad \sum_{i=1}^I \int \bar{N}_i(x) dx = 1. \end{cases} \quad (5)$$

For these problems, we address the hypothesis that circadian control reduces growth, i.e., that $\lambda_{\text{per}} \leq \lambda_s$. In Section 2, we prove that, surprisingly enough, the opposite is true, i.e., $\lambda_{\text{per}} \geq \lambda_s$ when the control acts only on the apoptosis rate. In Section 3, we show by numerical experiments that no hierarchy exists between the two eigenvalues when the control acts on the transition rate $K_{1 \rightarrow 2}$ in a reduced 2-phase model. Section 4 sums up these results and gives hints toward designing physiologically based models of the cell cycle for cancer therapeutics.

2. Control by apoptosis

In this section, we consider the case when circadian control only acts on apoptosis, i.e., $K_{i \rightarrow i+1}$ depends only upon x .

Theorem 2.1. *Assume that $d_i(t, x) \geq 0$, $K_{i \rightarrow i+1}(x) \geq 0$ are bounded and that (3) holds, then the eigenvalue problems (4), (5) have unique solutions $(\lambda_{\text{per}}, N(t, x))$, $(\lambda_s, \bar{N}(x))$, and*

$$\lambda_{\text{per}} \geq \lambda_s. \quad (6)$$

Proof. The existence part for the two problems is standard and we do not prove it again (see [4,12]). Now consider the function $q_i(x) = \langle \log(N_i(t, x)/\bar{N}_i(x)) \rangle$. It satisfies

$$\frac{\partial}{\partial x} q_i + \lambda_{\text{per}} - \lambda_s = 0, \quad \text{and} \quad q_i(x=0) = \left\langle \log \left[\int K_{i-1 \rightarrow i}(x) \frac{\bar{N}_{i-1}(x)}{\bar{N}_i(0)} \frac{N_{i-1}(t, x)}{\bar{N}_{i-1}(x)} dx \right] \right\rangle.$$

Since $d\mu_i(x) = K_{i-1 \rightarrow i}(x) (\bar{N}_{i-1}(x)/\bar{N}_i(0)) dx$ is a probability measure thanks to the condition $\bar{N}_i(0)$ (a factor 2 should be included for $i = 1$), we also have

$$\begin{aligned} q_i(x=0) &\geq \left\langle \int \log \frac{N_{i-1}(t, x)}{\bar{N}_{i-1}(x)} d\mu_i(x) \right\rangle \quad (\text{by Jensen's inequality}) \\ &= \int q_{i-1}(x) d\mu_i(x) = \int [q_{i-1}(0) + (\lambda_s - \lambda_{\text{per}})x] d\mu_i(x). \end{aligned}$$

Therefore, summing over i , $0 \geq (\lambda_s - \lambda_{\text{per}}) \sum_{i=1}^I \int_{x=0}^{\infty} x d\mu_i(x)$, and the result follows. \square

3. Control by phase transition

We have performed numerical tests for the cell cycle systems (4), (5) based on a classical upwind scheme with $CFL = 1$ which gives the exact transport solver (see [4] for details). We have taken a simplified version of the cell cycle with two phases ($I = 2$): G1-S-G2 and M. The apoptosis rate has been taken constant and the transition rates are: $K_{1 \rightarrow 2}(t, x) = \psi(t) \mathbb{1}_{\{x \geq x_*\}}$, and $K_{2 \rightarrow 1}(t, x) = \mathbb{1}_{\{x \geq x_{**}\}}$. We have in mind the following order of magnitudes for several animal tumour cells: total cycle duration is 21 h, 8 h for G1, 8 h for S, 4h for G2, 1 h for M (therefore in this case $x_* = 20$ h and $x_{**} = 1$ h). But we will also consider different duration ratios x_*/x_{**} between the 2 phases, from 1 to 20. In fact, although the G2/M transition is known to be a circadian control target with a well identified mechanism ($Bmal1 \rightarrow Wee1 \rightarrow cdk$), another control target, with another molecular mechanism in which genes *per* and *cMyc* have been shown to be involved [6], also takes place at the G1/S transition, and the G1 phase may have a

Table 1

The periodic and stationary eigenvalues for 2 periodic phase transition functions and different duration ratios between the first and second phases. See text for details

Tableau 1

Les valeurs propres stationnaires et périodiques pour un modèle à deux phases avec transitions périodiques et différentes durées relatives de phases

G1-S-G2/M, brief sq. w.	λ_{per}	λ_{s}	G1-S-G2/M, 12-12 sq. w.	λ_{per}	λ_{s}
1	<u>0.2385</u>	0.2350	1	0.2623	<u>0.2821</u>
2	0.2260	<u>0.2923</u>	2	0.3265	<u>0.3448</u>
3	0.2395	<u>0.3189</u>	3	–	–
4	0.2722	<u>0.3331</u>	4	–	–
5	0.3065	<u>0.3427</u>	5	–	–
7	0.3472	<u>0.3517</u>	7	0.4500	<u>0.4529</u>
8	<u>0.3622</u>	0.3546	8	<u>0.4588</u>	0.4575
10	<u>0.3808</u>	0.3588	10	<u>0.4713</u>	0.4641
20	<u>0.4125</u>	0.3675	20	<u>0.5006</u>	0.4818

very variable duration. So that while in principle testing here the G2/M transition, we may also be testing the G1/S gate control by an unknown 24 h-rhythmic factor. The function $\psi(t)$ has 24 h period. We have tested for ψ 2 square waves, a brief one with 4 hours at value 1 and the remaining 20 hours at 0, mimicking the shape of the cdk1 kinase behaviour, with entrainment by 24 h-rhythmic Wee1, according to Goldbeter's model of the mitotic oscillator [7], the other one with 12 hours at 1 and 12 hours at 0, a version of the same cdk1 model, with no entrainment, but fixed coefficients yielding also a 24 h period. In Table 1, we show a comparison between the two eigenvalues (periodic and stationary), for the tested ψ periodic transition functions.

Thus no clear hierarchy can be seen between the two eigenvalues (even though some regularity may be suspected, and these simulations show cases favourable to our initial hypothesis in the interval $2 \leq \text{G1-S-G2/M} \leq 7$). It is likely that 2 phases only are not sufficient to account for the experimental observation which guided us in this modelling work, and that, as it is, this model aggregates in a too simplistic way circadian effects on the G1/S and G2/M transitions.

4. Concluding remarks

- (1) This model allows to study the interactions in proliferating tissues between the cell cycle and physiological control systems such as the circadian clock.
- (2) More than 2 phases and better knowledge of other mechanisms (e.g. control of Cyclin E-cdk4 at G1/S transition) might be necessary to further investigate the first eigenvalues of the periodic and stationary problems.
- (3) The result $\lambda_{\text{per}} \geq \lambda_{\text{s}}$ for apoptosis control suggests that the sole control of death rate inside cell cycle phases might be unable to describe control of proliferation by cytotoxic drugs in cancer treatment. Transition rates should be considered in a therapeutic perspective.

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