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A mathematical model of the cell cycle and its circadian control

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Summary. 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 population 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 abovementioned 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 the abovementioned observation, control of death rates inside phases is not sufficient, and that transition rates between phases are a key target in proliferation control.

Key words: cell cycle, age-structured population, population growth, circadian rhythm

1.1 Cell cycle and circadian rhythm

The goal of this paper is to address by means of mathematical and numerical models the following idea: circadian rhythms regulate cell proliferation and

their disruption favours the growth of ill-controlled proliferative cell populations. In particular, tumour growth has been showed to be favoured in mice by disruptions of the normal circadian rhythm, as assessed e.g. by central body temperature or rest-activity recordings [9, 10], both by surgical resection of suprachiasmatic nuclei and by jet-lag like perturbations of the light-dark cycle. Also several epidemiological studies have shown that workers exposed to light-dark rhythm perturbations due to prolonged shift work are significantly more exposed to the risk of developing breast or colorectal cancer than others with regular work time schedules [8, 23]. It is thus suspected that a loss of circadian control on cell cycle dynamics may account for an acceleration in tumour progression. This is also supported by clinical observations according to which patients with cancer *and* disrupted circadian rhythms are less responsive to chemotherapy and have poorer prognosis with shorter life expectancy than others with the same diseases but strong circadian rhythmicity[15, 20]. This idea is now sustained by a better understanding of the mechanisms underlying apoptosis and cell cycle phase transitions through proteins such as cyclins and p53. Indeed, the expression of cyclin dependent kinases Cdk1 and Cdk2 and their dimerisation with Cyclins B and E, respectively, control the cell cycle phase transitions G2/M and G1/S, while protein p53 favours cell cycle arrest in G2/M or G1/S, by acting on these cyclins, and secondary apoptosis induction. And for instance, phosphorylation of the dimer CycB-Cdk1 by the kinase Wee1 is directly controlled by the circadian gene Bmal1, and p53 expression by the circadian gene Per1, see [16, 21, 24, 3, 26].

In this work (an abridged version of which has appeared in [6]) our approach relies on mathematical equations for the cell cycle which are well established nowadays. We introduce circadian control through periodic coefficients standing for phase transitions and apoptosis regulation by clock genes. We assess the hypothesis according to which periodicity diminishes the population growth as compared to constant coefficients (with the same average), i.e., we want to decide if a loss of circadian control theoretically favours tumour growth.

General references and experimental validations of the topic of age-structured population dynamics and cell cycle can be found in [1, 2, 4, 14, 17, 22]. For a more recent approach based on entropy properties, we refer to [18, 19]. Here and following earlier work [5], 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 $i = 1, ...I$, at time t.

$$\begin{cases} \frac{\partial}{\partial t} n_i(t,x) + \frac{\partial}{\partial x} n_i(t,x) + [d_i(t,x) + K_{i\to i+1}(t,x)] n_i(t,x) = 0, \\ \\ n_i(t,x=0) = \displaystyle\int_{x'\geq 0} K_{i-1\to i}(t,x')\; n_{i-1}(t,x')\; dx', \quad 2 \leq i \leq I, \\ \\ n_1(t,x=0) = 2 \displaystyle\int_{x'\geq 0} K_{I\to 1}(t,x')\; n_I(t,x')\; dx'. \end{cases} \tag{1.1}$$

Here and below we identify $I+1$ to 1. We denote by $d_i(t,x) \geq 0$ the apoptosis rate, $K_{i\to i+1}$ the transition rates from one phase to the next, and the last one ($i = I$) is mitosis where the two cells separate. These coefficients can be constant in time (no circadian control) or time T-periodic in order to take into account the circadian rhythm . Our assumptions are

$$K_{i\to i+1}(t,x) \geq 0,\ d_i(t,x) \geq 0 \quad \text{are bounded,} \tag{1.2}$$

and, setting $\min\limits_{0\leq t\leq T} K_{i\to i+1}(t,x) := k_{i\to i+1}(x)$, $\max\limits_{0\leq t\leq T}[d_i + K_{i\to i+1}] := \mu_i(x)$ (lower bound for the transition kernels and upper bound for the cell loss terms), $M_i(x) = \displaystyle\int_0^x \mu_i(y)dy$,

$$\prod_{i=1}^{I} \int_0^\infty k_{i\to i+1}(y) e^{-M_i(y)} dy > 1/2, \tag{1.3}$$

(thus ensuring positive population growth). 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

$$\begin{cases} \frac{\partial}{\partial t}N_i(t,x) + \frac{\partial}{\partial x}N_i(t,x) + [d_i(t,x) + \lambda_{per} + K_{i\to i+1}(t,x)]N_i(t,x) = 0, \\ \\ N_i(t,x=0) = \displaystyle\int_{x'\geq 0} K_{i-1\to i}(t,x')\ N_{i-1}(t,x')\ dx', \quad 2 \leq i \leq I, \\ \\ N_1(t,x=0) = 2\displaystyle\int_{x'\geq 0} K_{I\to 1}(t,x')\ N_I(t,x')\ dx', \\ \\ \displaystyle\sum_{i=1}^{I} \int N_i(t,x)dx = 1. \end{cases} \tag{1.4}$$

Under our assumptions (1.2)–(1.3), the existence of a solution to (1.4), with $\lambda_{per} > 0$, follows from an infinite dimensional version of Floquet theory and one has (see for instance [18])

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

where $\varphi_i(t,x)$ denotes the periodic positive solution to the adjoint problem to (1.4) normalised by $\displaystyle\sum_i \int N_i(t,x)\varphi_i(t,x)dx = 1$, and $\rho = \displaystyle\sum_{i=1}^{N} \int n_i(t=0,x)\varphi_i(t=0,x)dx$. In other words, the periodic solution is the observed stable state after renormalisation by the rate λ_{per}.
One can also introduce the coefficients averaged in time

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

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

$$\begin{cases} \frac{\partial}{\partial x}\bar{N}_i(x) + [\langle d_i(x)\rangle + \lambda_s + \langle K_{i\to i+1}(x)\rangle]\bar{N}_i(x) = 0, \\ \bar{N}_i(x=0) = \displaystyle\int_{x'\geq 0} \langle K_{i-1\to i}(x')\rangle\ \bar{N}_{i-1}(x')\ dx', \quad 2\leq i\leq I, \\ \bar{N}_1(x=0) = 2\displaystyle\int_{x'\geq 0} \langle K_{I\to 1}(x')\rangle\ \bar{N}_I(x')\ dx', \\ \displaystyle\sum_{i=1}^{I}\int \bar{N}_i(x)dx = 1. \end{cases} \tag{1.5}$$

For these problems, we address the hypothesis that circadian control reduces the population growth, i.e., $\lambda_{per} \leq \lambda_s$ (index *per* standing for "periodic" and s for "stationary"). In section 1.2, we firstly study the effect of small variations, with respect to a circadian control, from constant transition and apoptosis rates, on the resulting eigenvalue λ_{per}. Then in section 1.3, we prove that, surprisingly enough, a result opposite to our experimental conjecture is true, i.e., $\lambda_{per} \geq \lambda_s$, when the circadian control acts only on the apoptosis rate. In section 1.4, we show by numerical experiments that no hierarchy exists between the two eigenvalues when the circadian control acts on the transition rate $K_{1\to 2}$ in a reduced 2-phase model. These results give hints toward designing physiologically based models of the cell cycle for cancer therapeutics which are discussed in section 1.5. The results are summarised in section 1.6.

1.2 Analysis of local variation by small oscillations

In this section, we study the small variations, with respect to a circadian control, of the growth rate λ and we show that its effect is only of the second order.

To do so, we consider that the transition kernels and the death rates show small variation of order $\varepsilon > 0$ from their averages. Therefore we set

$$\tilde{K}^{\varepsilon}_{i-1\to i}(t,x) := \varepsilon\tilde{K}_{i-1\to i}(t,x) + \langle K_{i-1\to i}(x)\rangle,$$

$$\tilde{K}^{\varepsilon}_{I\to 1}(t,x) := \varepsilon\tilde{K}_{I\to 1}(t,x) + \langle K_{I\to 1}(x)\rangle,$$

$$d^{\varepsilon}_i(t,x) := \varepsilon\tilde{d}_i(t,x) + \langle d_i(x)\rangle,$$

where the quantities $\tilde{d}_i$, $\tilde{K}_{i\to j}$ have vanishing averages:

$$\langle \tilde{K}_{i-1\to i}(x)\rangle = \langle \tilde{K}_{I\to 1}(x)\rangle = \langle \tilde{d}_i(x)\rangle = 0.$$

Then we define the solution $n_i^\varepsilon(t,y)$ to the cell cycle system

$$\begin{cases} \frac{\partial}{\partial t} n_i^\varepsilon(t,x) + \frac{\partial}{\partial x} n_i^\varepsilon(t,x) + [d_i^\varepsilon(t,x) + \tilde{K}_{i\to i+1}(t,x)] n_i^\varepsilon(t,x) = 0, \\ n_i^\varepsilon(t,0) = \displaystyle\int_{x'\geq 0} \tilde{K}^\varepsilon_{i-1\to i}(t,x') n^\varepsilon_{i-1}(t,x')dx', \quad 2\leq i\leq I, \\ n_1^\varepsilon(t,x=0) = 2\displaystyle\int_{x'\geq 0} K^\varepsilon_{I\to 1}(t,x')\; n_I(t,x')\; dx'. \end{cases} \tag{1.6}$$

Now, using the results recalled in section 1.1, we know that, for all $\varepsilon \in [0,1]$, there exist eigenelements associated with this problem, $(N_i^\varepsilon, \lambda_\varepsilon, \varphi_i^\varepsilon)$. They are solution to

$$\begin{cases} \frac{\partial}{\partial t} N_i^\varepsilon(t,x) + \frac{\partial}{\partial x} N_i^\varepsilon(t,x) + [d_i^\varepsilon(t,x) + \lambda_\varepsilon + K_{i\to i+1}^\varepsilon(t,x)] N_i^\varepsilon(t,x) = 0, \\ N_i^\varepsilon(t,x=0) = \displaystyle\int_{x'\geq 0} K^\varepsilon_{i-1\to i}(t,x')\; N^\varepsilon_{i-1}(t,x')\; dx', \quad 2\leq i\leq I, \\ N_1^\varepsilon(t,x=0) = 2\displaystyle\int_{x'\geq 0} K^\varepsilon_{I\to 1}(t,x')\; N_I^\varepsilon(t,x')\; dx' \qquad \displaystyle\sum_{i=1}^I \int N_i^\varepsilon(t,x)dx = 1, \end{cases} \tag{1.7}$$

$$\begin{cases} -\frac{\partial}{\partial t}\varphi_i^\varepsilon(t,x) - \frac{\partial}{\partial x}\varphi_i^\varepsilon(t,x) + [d_i^\varepsilon(t,x) + \lambda_\varepsilon + K_{i\to i+1}^\varepsilon(t,x)]\varphi_i^\varepsilon(t,x) = \\ \varphi^\varepsilon_{i+1}(t,0) K^\varepsilon_{i\to i+1}(t,x), \quad 1\leq i\leq I-1, \\ -\frac{\partial}{\partial t}\varphi_I^\varepsilon(t,x) - \frac{\partial}{\partial x}\varphi_I^\varepsilon(t,x) + [d_I^\varepsilon(t,x) + \lambda_\varepsilon + K_{I\to 1}^\varepsilon(t,x)]\varphi_I^\varepsilon(t,x) = \\ 2\varphi^\varepsilon_1(t,0) K^\varepsilon_{I\to 1}(t,x), \end{cases} \tag{1.8}$$

with

$$\int_0^\infty \sum_{i=1}^I N_i^\varepsilon(t,x)\varphi_i^\varepsilon(t,x)dx = 1, \quad \forall t\geq 0. \tag{1.9}$$

With these notations, we clearly have $\lambda_0 = \lambda_s$. As a first step towards our main result, we gather some formulae that are used to prove the

Theorem 1. *The function $\lambda \mapsto \lambda_\varepsilon$ is differentiable for all $\varepsilon \in]0,1[$, and*

$$\begin{aligned} \frac{d\lambda_\varepsilon}{d\varepsilon} &= \frac{1}{T}\int_0^T\int_0^\infty N_I^\varepsilon(t,x)\Big[2\tilde{K}_{I\to 1}(t,x)\varphi_1^\varepsilon(t,0) - \big(\tilde{d}_I(t,x) + \tilde{K}_{I\to 1}(t,x)\big)\varphi_I^\varepsilon(t,x)\Big]dxdt \\ &+ \frac{1}{T}\int_0^T\int_0^\infty \sum_{i=1}^{I-1} N_i^\varepsilon(t,x)\Big[\tilde{K}_{i\to i+1}(t,x)\varphi_{i+1}^\varepsilon(t,0) - \big(\tilde{d}_i(t,x) + \tilde{K}_{i\to i+1}(t,x)\big)\varphi_i^\varepsilon(t,x)\Big]dxdt. \end{aligned} \tag{1.10}$$

The proof of the theorem is somewhat lengthy and will be detailed in an appendix, see section 1.7.

Corollary 1. *For small circadian effect ε, the variations of λ_ε are of order ε^2, in other words*

$$\left.\frac{d\lambda_\varepsilon}{d\varepsilon}\right|_{\varepsilon=0} = 0.$$

This Corollary follows from the fact that for $\varepsilon = 0$ the functions $N_i^\varepsilon(t,x)$ and $\varphi_i^\varepsilon(t,x)$ are independent of time. Therefore, in (1.10), we are left only with the time averages of $\tilde{K}_{i\to i+1}(t,x)$ and $\tilde{d}_i(t,x)$, which vanish.

We can also deduce from this Theorem that, in the particular case when $K_{i\to i+1}$ is independent of time and d_i is independent of age, we cannot control locally the growth rate λ (see also section 1.3 for a direct proof and a derivation of a global variation in a more general case). Indeed, we have

Corollary 2. *Assume $\tilde{d}_i(t,x) = \rho_i(t)$, $\tilde{K}_{i\to i+1}(t,x) = 0$ then*

$$\frac{d\lambda_\varepsilon}{d\varepsilon} = 0, \tag{1.11}$$

and $\lambda_s = \lambda_{per}$.

Proof. Using (1.10), we find

$$\frac{d\lambda_\varepsilon}{d\varepsilon} = -\frac{1}{T}\int_0^T \int_0^\infty \sum_{i=1}^I N_i^\varepsilon(t,x)\varphi_i^\varepsilon(t,x)dx\rho_i(t)dt,$$

but we have $\displaystyle\int_0^T \rho_i(t)dt = 0$ and (1.9), thus we find (1.11) and

$$\lambda_{per} - \lambda_s = \int_0^1 \frac{d\lambda_\varepsilon}{d\varepsilon} = 0. \quad \square$$

As a conclusion of this section, we see that a direct computation in the most general case, when $K_{i\to i+1}$ and d_i are time dependent leads to hardly tractable formulae; the local variation of the first eigenvalue cannot be found directly because it is of the second order in ε. For this reason it is natural to turn to numerical computations as we do it in section 1.4.

1.3 Control exerted on apoptosis

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

Theorem 2. *Assume that $d_i(t,x) \geq 0$, $K_{i\to i+1}(x) \geq 0$ are bounded and that (1.3) holds, then the eigenvalue problems (1.4), (1.5) have unique solutions $(\lambda_{per}, N(t,x))$, $(\lambda_s, \bar{N}(x))$, and*

$$\lambda_{per} \geq \lambda_s. \tag{1.12}$$

Proof. The existence part for the two problems is standard and we do not prove it again (see [5, 18]). For the ordering of eigenvalues, consider the function $q_i(x) =< \log\left(\frac{N_i(t,x)}{\bar{N}_i(x)}\right) >$. It satisfies

$$\frac{\partial}{\partial x} q_i + \lambda_{per} - \lambda_s = 0,$$

$$q_i(x=0) =< \log\left[\int K_{i-1\to i}(x) \frac{\bar{N}_{i-1}(x)}{\bar{N}_i(0)} \, \frac{N_{i-1}(t,x)}{\bar{N}_{i-1}(x)} \, dx\right] > .$$

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

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

Therefore, summing over i from 1 to I,

$$0 \geq (\lambda_s - \lambda_{per}) \sum_{i=1}^{I} \int_{x=0}^{\infty} x \, d\mu_i(x),$$

and the result follows. □

Notice that the same question has been addressed for positive matrices, in [7]. Of course a discrete version of equations (1.4), (1.5) based, say on an upwind scheme, leads to study the same inequalities for matrices with positive coefficients and our method applies to matrices with periodic diagonal terms.

1.4 Control exerted on phase transitions

We have performed numerical tests for the cell cycle systems (1.4), (1.5) based on a classical upwind scheme with $CFL = 1$ which gives the exact transport

solver (see [5] for details). We have taken a simplified version of the cell cycle with two phases ($I = 2$): G1-S-G2 and M. In other words, in the full cell-cycle (G1, synthesis, G2, mitosis) we only retain as a major event the transition from G2 to M. The apoptosis rate has been taken constant and the transition rates are

$$K_{1\to 2}(t,x) = \psi(t)\mathbb{1}_{\{x\geq x_*\}}, \qquad K_{2\to 1}(t,x) = \mathbb{1}_{\{x\geq x_{**}\}}.$$

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 G1-S-G2 and M, from 1 to 20. The reason for this is that although the G2/M transition is known to be a circadian control target with an identified mechanism (Bmal1→Wee1→cdc2 -the cyclin dependent kinase cdc2 being rather known as cdk1 in mammals), another control target, with as yet unidentified mechanism (though the genes *per* and *cMyc* have been shown to be involved [11, 12]), could take place at the G1/S transition, and the G1 phase may have a 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 cdc2 (cdk1)-like factor. The function $\psi(t)$ has 24h period. We have tested for ψ several shapes (*cosine* and square wave functions), but eventually kept only 2 square waves, a brief one with 4 hours at value 1 and the remaining 20 hours at 0, the other one with 12 hours at 1 and 12 hours at 0. The first one mimics the shape of the cdc2 kinase behaviour, with entrainment by 24 h-rhythmic Wee1, according to A. Goldbeter's model of the mitotic oscillator [13], the other a version of the same cdc2 model, with no entrainment, but fixed coefficients yielding also a 24 h period. In Table 1.1 we show a comparison between the two eigenvalues (periodic and stationary), for the two tested ψ periodic transition functions.

It is apparent from this table that no clear hierarchy can be seen between the two eigenvalues, even if some regularity may be suspected, and these simulations show cases favorable to our initial hypothesis in the interval 2≤G1-S-G2/M≤7.

1.5 Discussion

Circadian control in general. Theorem 1 in section 1.2 shows that in the general case one cannot drive any conclusion on the initial question: is the growth of the population hampered by a periodic control on phase transitions and death rates? This negative result invited us to examine particular cases.

Circadian control of population growth by targeting apoptosis. The rather surprising result, given previous experimental observations, that periodic control of apoptosis in one phase resulted in enhanced proliferation, as compared to constant control (Theorem 2 in section 1.3), may indicate that apoptosis is

Table 1.1. *The periodic and stationary eigenvalues for different duration G1-S-G2/M ratios and for 2 periodic phase transition functions: ψ_1 is a brief square wave (4h / 24 h), ψ_2 a longer one (12 h / 12 h). For better reading convenience, the greater of the two eigenvalues is underlined.*

time ratio, ψ_1	λ_{per}	λ_s
1	<u>0.2385</u>	0.2350
2	0.2260	<u>0.2923</u>
3	0.2395	<u>0.3189</u>
4	0.2722	<u>0.3331</u>
5	0.3065	<u>0.3427</u>
6	0.3305	<u>0.3479</u>
7	0.3472	<u>0.3517</u>
8	<u>0.3622</u>	0.3546
10	<u>0.3808</u>	0.3588
20	<u>0.4125</u>	0.3675

time ratio, ψ_2	λ_{per}	λ_s
1	0.2623	<u>0.2821</u>
2	0.3265	<u>0.3448</u>
3	...	...
4	...	...
5	...	...
6	...	...
7	0.4500	<u>0.4529</u>
8	<u>0.4588</u>	0.4575
10	<u>0.4713</u>	0.4641
20	<u>0.5006</u>	0.4818

no physiological target for population growth control bt the circadian clock. As regards p53, its circadian expression is thus likely to be linked more to its cell cycle arrest than to its apoptosis inducing capacities.

Circadian control of population growth by targeting phase transitions. It was simulated in a simplified way, with two different sorts of square waves, in a reduced 2-phase cell cycle model, and even in these simple settings, we obtained contrasted results, which did not enable us to answer the initial question. It is likely that 2 phases only in the model may not be sufficient to account for the physiopathological observation which guided us for this modelling work, and that, as it is, this model aggregates in an inaccurate way physiological effects of the G1/S and G2/M transition controls. Future work on the basis of this experimental observation should encompass 3 phases: G1, S-G2 and M, better knowledge of circadian control both at the G1/S and G2/M transitions, and synchronisation between these transitions.

Possible medical implications. Circadian rhythms are nonnegligible regulating factors of cell population growth, as results from experimental and clinical observations. Cancer chronotherapy [15, 20] for fifteen years has been taking advantage of circadian variations in cytotoxic drug therapeutic efficacy and unwanted toxicity. These variations are likely due to the simultaneous regulation by the circadian clock of cell cycle progression on the one hand, and of drug detoxification mechanisms on the other hand. Elucidating in a theoretical way both these control processes might give better rationale to anticancer treatment optimisation. But other *adjuvant* therapies may be used, aiming at resynchronisation of individual proliferating cells in growing populations by strengthening circadian control, possibly using hormones as melatonin and cortisol, or even feeding schedule [25, 10]. In this prospect, the present work may offer new tracks to design such therapeutic control processes.

1.6 Conclusion

To summarise these results:

1/ This model allows us to study the interactions in proliferating tissues between the cell cycle and physiological control systems such as the circadian clock. We have shown with theorem 1 and its corollaries that classical perturbation analysis is unlikely to provide an answer to the initial question of the effect of circadian control on population growth.

2/ More than 2 phases and better knowledge of other mechanisms (cortisol, Cyclin E on G1/S) might be necessary to account for the physiopathological facts reported from animal experimentation and human clinical observations which guided us in this investigation of the first eigenvalues of the periodic and stationary problems.

3/ The unexpected result $\lambda_{per} \geq \lambda_s$ for apoptosis controlshown in theorem 2 suggests that the sole control of death rate inside cell cycle phases is unable to describe control of proliferation by cytotoxic drugs in cancer treatment. Transition rates should be considered in a therapeutic perspective.

1.7 Appendix: proof of Theorem 1

First we introduce more condensed defininitions: $(N^\varepsilon, \lambda^\varepsilon, \varphi^\varepsilon)$ by

$$\forall (t,y) \in [0,\infty[^2, \quad N^\varepsilon(t,y) \in [0,\infty[^I, \quad N^\varepsilon(t,y)|_i := N_i^\varepsilon(t,y),$$

$$\forall (t,y) \in [0,\infty[^2, \quad \varphi^\varepsilon(t,y) \in [0,\infty[^I, \quad \varphi^\varepsilon(t,y)|_i := \varphi_i^\varepsilon(t,y),$$

$$\lambda^\varepsilon := \lambda_\varepsilon.$$

Then, we define the operator $\mathcal{L}_\varepsilon^*$ such that $\mathcal{L}_\varepsilon^* \varphi^\varepsilon = \lambda^\varepsilon \varphi^\varepsilon$,

$$\mathcal{L}_\varepsilon^*(g)|_i := \frac{\partial}{\partial t} g_i(t,x) + \frac{\partial}{\partial x} g_i(t,x) - [d_i^\varepsilon(t,x) + K_{i\to i+1}^\varepsilon(t,x)] g_i(t,x) + g_{i+1}(t,0) K_{i\to i+1}^\varepsilon(t,x), \quad 1 \leq i \leq I-1,$$

$$\mathcal{L}_\varepsilon^*(g)|_I := \frac{\partial}{\partial t} g_I(t,x) + \frac{\partial}{\partial x} g_I(t,x) - [d_I^\varepsilon(t,x) + K_{I\to 1}^\varepsilon(t,x)] g_I(t,x) + 2 g_1(t,0) K_{I\to 1}^\varepsilon(t,x),$$

and its dual satisfying $\mathcal{L}^\varepsilon N^\varepsilon = \lambda^\varepsilon N^\varepsilon$. Thus, for all ε and ε' such that ε and $\varepsilon - \varepsilon' \in]0,1[$, we have :

$$\lambda^\varepsilon = \int_0^\infty \mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) N^\varepsilon(y) dy.$$

Therefore, we find

$$\lambda^\varepsilon - \lambda^{\varepsilon-\varepsilon'} = \int_0^\infty \mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) N^\varepsilon(y) dy - \int_0^\infty \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^{\varepsilon-\varepsilon'})(y) N^{\varepsilon-\varepsilon'}(y) dy.$$

But, the normalisation gives

$$\int_0^\infty \varphi^\varepsilon(y)N^\varepsilon(y)dy = \int_0^\infty \varphi^{\varepsilon-\varepsilon'}(y)N^{\varepsilon-\varepsilon'}(y)dy = 1, \tag{1.13}$$

and so, we can write

$$\begin{aligned}\lambda^\varepsilon - \lambda^{\varepsilon-\varepsilon'} = &\int_0^\infty \Big(\mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) - \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^\varepsilon)(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy\\ &+ \int_0^\infty \mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y)\Big(N^\varepsilon(y) - N^{\varepsilon-\varepsilon'}(y)\Big)dy\\ &- \int_0^\infty \Big(\mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^{\varepsilon-\varepsilon'})(y) - \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^\varepsilon)(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy.\end{aligned}$$

Using the definition of $\mathcal{L}^*$, $\mathcal{L}$ and their duality, we find :

$$\begin{aligned}\lambda^\varepsilon - \lambda^{\varepsilon-\varepsilon'} = &\int_0^\infty \Big(\mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) - \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^\varepsilon)(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy\\ &+ \lambda^\varepsilon\int_0^\infty \varphi^\varepsilon(y)\Big(N^\varepsilon(y) - N^{\varepsilon-\varepsilon'}(y)\Big)dy\\ &- \lambda^{\varepsilon-\varepsilon'}\int_0^\infty \Big(\varphi^{\varepsilon-\varepsilon'}(y) - \varphi^\varepsilon(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy.\end{aligned}$$

Thus, using the normalisation (1.13), we deduce from the above idendity

$$\begin{aligned}\lambda^\varepsilon - \lambda^{\varepsilon-\varepsilon'} = &\int_0^\infty \Big(\mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) - \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^\varepsilon)(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy\\ &+ \lambda^\varepsilon\int_0^\infty \varphi^\varepsilon(y)\Big(N^\varepsilon(y) - N^{\varepsilon-\varepsilon'}(y)\Big)dy\\ &- \lambda^{\varepsilon-\varepsilon'}\int_0^\infty \varphi^\varepsilon(y)\Big(N^\varepsilon(y) - N^{\varepsilon-\varepsilon'}(y)\Big)dy,\end{aligned}$$

and

$$\begin{aligned}\lambda^\varepsilon - \lambda^{\varepsilon-\varepsilon'} = &\int_0^\infty \Big(\mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) - \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^\varepsilon)(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy\\ &+ \lambda^\varepsilon\int_0^\infty \Big(\varphi^{\varepsilon-\varepsilon'}(y) - \varphi^\varepsilon(y)\Big)N^{\varepsilon-\varepsilon'}(y)\\ &- \lambda^{\varepsilon-\varepsilon'}\int_0^\infty \Big(\varphi^{\varepsilon-\varepsilon'}(y) - \varphi^\varepsilon(y)\Big)N^{\varepsilon-\varepsilon'}(y).\end{aligned}$$

And so, we arrive at

$$\begin{aligned}(\lambda^\varepsilon - \lambda^{\varepsilon-\varepsilon'})\left(1 - \int_0^\infty \Big(\varphi^{\varepsilon-\varepsilon'}(y) - \varphi^\varepsilon(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy\right) =\\ \int_0^\infty \Big(\mathcal{L}_\varepsilon^*(\varphi^\varepsilon)(y) - \mathcal{L}_{\varepsilon-\varepsilon'}^*(\varphi^\varepsilon)(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy.\end{aligned}$$

Using the equality

$$(\mathcal{L}_*^{\varepsilon} - \mathcal{L}_*^{\varepsilon-\varepsilon'})(g) = \varepsilon'\big(- [\tilde{d}_i + \tilde{K}_{i\to i+1}]g_i + g_{i+1}(0)\tilde{K}_{i\to i+1}\big),$$

we deduce

$$(\lambda^{\varepsilon} - \lambda^{\varepsilon-\varepsilon'})\left(1 - \int_0^{\infty}\Big(\varphi^{\varepsilon-\varepsilon'}(y) - \varphi^{\varepsilon}(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy\right) = \varepsilon'\int_0^{\infty}\Big(- [\tilde{d}_i + \tilde{K}_{i\to i+1}]\varphi_i^{\varepsilon} + \varphi_{i+1}^{\varepsilon}(0)\tilde{K}_{i\to i+1}\Big)N^{\varepsilon-\varepsilon'}(y)dy,$$

And finally, we obtain

$$\frac{\lambda^{\varepsilon} - \lambda^{\varepsilon-\varepsilon'}}{\varepsilon'} = \frac{\displaystyle\int_0^{\infty}\Big(- [\tilde{d}_i + \tilde{K}_{i\to i+1}]\varphi_i^{\varepsilon} + \varphi_{i+1}^{\varepsilon}(0)\tilde{K}_{i\to i+1}\Big)N^{\varepsilon-\varepsilon'}(y)dy}{\displaystyle 1 - \int_0^{\infty}\Big(\varphi^{\varepsilon-\varepsilon'}(y) - \varphi^{\varepsilon}(y)\Big)N^{\varepsilon-\varepsilon'}(y)dy}. \tag{1.14}$$

Using the Lebesgue dominated convergence theorem, we can pass to the limit and find that the function $\varepsilon \mapsto \lambda^{\varepsilon}$ (i.e., λ_{ε}) is differentiable and (1.10) is satisfied. □

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