Physiologically based modelling of circadian control on cell proliferation

Jean Clairambault

INRIA, Domaine de Voluceau, BP 105, F78153 Rocquencourt, France
Email: jean.clairambault@inria.fr

Abstract— The molecular circadian clock which is present in almost all cells of animal organisms exerts a control on the cell division cycle in proliferating tissues by modulating the activity of cyclins and cyclin dependent kinases (CDKs), the proteins which determine transitions from one phase of the cell cycle to the following one, until effective division. Each peripheral cell circadian clock is under the synchronising control of a central hypothalamic pacemaker which itself receives inputs, synchronising or disruptive, from external light and from circulating molecules such as cytokines.

Principles for modelling these interacting systems are exposed. They rely on age-structured partial differential equations for cell proliferation in a population of cells and ordinary differential equations for the control of cell cycle phase transtions and for the circadian system presented as a network of oscillators with synchronisation and desynchronisation. These physiological cellular systems are coupled together and subject to pharmacological inputs, e.g. from anticancer therapies, which may be synchronised with cell cycle timing by the knowledge of the body circadian clock status, investigated by noninvasive measurements.

The output of the controlled cell proliferation is a population growth exponent identifiable by in vivo tissue measurements; it allows to assess the proliferative status of the tissues under investigation, as a function of the circadian clock status, well fit or disrupted, and of pharmacological inputs such as used in anticancer treatments.

I. INTRODUCTION

Cancer growth and response to therapy by anticancer drugs have been shown to be dependent on circadian clock inputs ([9], [15]). Indeed, tumour growth is enhanced by perturbations of the central hypothalamic clock ([6], [7]) and this is most likely related to cell cycle control disruption ([8], [9]). What are the relationships between molecular circadian clock and cell cycle timings? How should anticancer therapeutics be designed so as to induce efficient recovering of such control mechanisms? To answer these questions, an integrative physiology model has been designed, taking into account cell proliferation at the level of a population of cells by age-structured partial differential equations (PDEs), its control by cell cycle proteins (variables in ordinary differential equations, ODEs), and the control of these molecular mechanisms by the circadian system, designed as a network of coupled oscillators also described by ODEs.

II. CELL PROLIFERATION: POPULATIONS OF CELLS

In tissues subject to renewal, in particular in tumours, but also in fast renewing healthy tissues such as gut, skin and bone marrow, the individual cell is the fundamental level of description for the interacting molecular mechanisms at stake in cell cycle progression and circadian clock timing systems. But it is tissue proliferation which is at stake in cancer growth, and also in healthy tissue homeostasis, and that is a matter of cell population dynamics. This leads to consider age-structured cell populations, which are described, for each phase of the cell division cycle, classically divided in phases G_1, $S-G_2$ and M, by evolution equations for cell densities dependent on time and age spent in each phase. Cells in renewing tissues are either in the quiescent, or G_0, phase (where nothing happens), or in the proliferating phases G_1, S (for DNA synthesis, i.e., genome duplication), G_2 and M (for mitosis, or actual cell division). Exchanges of cells between phases G_0 and G_1 may occur at any time in phase G_1 as long as the so-called *restriction point* has not been reached; afterwards, a proliferating cell is irreversibly committed to proceed until actual division -unless it is stopped, e.g. by anticancer drugs, in its progression at cell cycle phase transition *checkpoints*, the most important of which occur at the G_1/S and G_2/M transitions. The transition rates from one phase to the following one (hereafter noted $K_{i \to i+1}$ in the model) are the main targets of the circadian (and pharmacological) control on cell cycle progression.

A. An age-structured partial differential equations (PDE) model of the cell cycle

The model chosen ([4]) is linear and of the Von Foerster-McKendrick type. For each phase i ($1 \leq i \leq I$) of the cell division cycle, $n_i = n_i(t,a)$ denotes the density of cells of age a in phase i at time t. Inside each phase, the variation of the density of cells is due either to a spontaneous death rate $d_i = d_i(t,a)$ or to a transition rate $K_{i \to i+1} = K_{i \to i+1}(t,a)$ from phase i to phase $i+1$ (with the convention $I+1 = 1$):

$$\begin{cases} \frac{\partial}{\partial t}n_i + \frac{\partial}{\partial a}\left[v_i(a)n_i\right] + [d_i + K_{i \to i+1}]n_i = 0, \\[2mm] v_i(0)n_i(t,a=0) = \int_{\alpha \geq 0} K_{i-1 \to i}(t,\alpha)\, n_{i-1}(t,\alpha)\, d\alpha, \\[2mm] \quad 2 \leq i \leq I \\[2mm] n_1(t,a=0) = 2\int_{\alpha \geq 0} K_{I \to 1}(t,\alpha)\, n_I(t,\alpha)\, d\alpha, \end{cases}$$

where $\sum_{i=1}^{I} \int_{\alpha \geq 0} n_i(t,\alpha)d\alpha = 1$, $v_i(a)$ denotes a "speed" function of age a in phase i with respect to time t, and $K_{i \to i+1}(t,a) = \psi_i(t)\mathbf{1}_{\{a \geq a_i\}}(a)$.

The transition rates are chosen as $\psi_i(t)\mathbf{1}_{\{a \geq a_i\}}(a)$, which means that a minimum age, from 0 to a_i, must be spent in phase i, and that a dynamic control $t \mapsto \psi_i(t)$ is exerted on the transition. The control function ψ_i integrates physiological control, such as hormonal or circadian, as well as external environmental and pharmacological influences. Another possibility to take circadian control into account would be by action on the death rates d_i, but it can be shown that at least by itself such control is not compatible with observations from laboratory experiments on tumour growth with modifications of the circadian clock ([4]).

B. Tumoral cells: unlimited growth

As it is, with positive functions as parameters, this linear model is bound to show exponential growth. It can be shown indeed that it yields a so-called *Malthus exponent* λ which governs the asymptotic behaviour of the solutions n_i, in the sense that for all i, the normalised solutions $t \mapsto e^{-\lambda t} n_i(t)$ are bounded, asymptotically constant if ψ_i is constant, and asymptotically periodic if ψ_i is periodic, with the same period. This Malthus exponent λ may be assessed in the case of solid tumours by in vivo tissue growth measurements (only approximately, if one neglects the presence of necrotic material) by $\lambda = \dfrac{\ln 2}{T_d}$, if T_d is the apparent doubling time of tumour size.

C. Healthy cells: tissue homeostasis

In the case of healthy cells, of course there can be no exponential growth, and tissue homeostasis must be ensured, in the sense that for healthy renewing tissues, cell loss must be made up for, and with no excess, by influx from newly formed cells. This can be modelled by the introduction of a G_0 (or quiescent) phase exchanging cells with the G_1 phase, taking into account in the control of these exchange mechanisms a limitation by cell density dependent inhibition, see [2]. In this case, the Malthus exponent λ is zero since the asymptotic growth behaviour of the total population of cells is evolution to stationarity or periodicity, and there is no exponential growth.

An interest in simultaneously representing a tumoral and a healthy cell population is, in the perspective of therapeutic optimisation, to control unwanted toxicity on healthy tissues, a constant side effect in anticancer therapies. These unwanted effects on the healthy cell division cycle are also dependent on the control of cell cycle phase transition by the circadian clock, but possibly with phase differences, as compared with tumours, which may explain observed differences between worst toxicity and best efficacy times for drug infusion ([15]). In this respect, the therapeutic control objective may be seen as to obtain a negative Malthus exponent for tumour cells, and zero growth for healthy cells.

III. CELL PROLIFERATION CONTROL: CYCLINS AND CDKS

A. Control of cell cycle phase transitions by cyclins and CDKs

Cyclins and their activating kinases (Cyclin dependent kinases, or CDKs) are the proteins which are the most important determinants for the processing through cell cyle phases and transitions between phases. In particular, Cyclin E associated with CDK2 is essential for the G_1/S transition, and Cyclin B associated with CDK1 must be at a level high enough to allow G_2/M transition.

B. Circadian control on cyclins and CDKs

Of these two mechanisms, the most known is the inhibition of G_2/M transition by the circadian clock-controlled kinase Wee1, which deactivates CDK1, thus blocking cells in G_2. It has been described by A. Goldbeter in [10] by an ODE system in which Wee1 is a mere parameter. This system oscillates with an intrinsic frequency which depends on its parameters, but which may be entrained at a 24 h period by Wee1 if Wee1 becomes an output of the molecular circadian clock. A simulation of the entrainment of the normalised M-cell population $t \mapsto e^{-\lambda t} \int_0^{+\infty} n_2(t,a)\, da$, in a simplified 2-phase model (n_1 = cells in $G_1 - S - G_2$, n_2 = cells in M) of the population, by a 24 h-entrained CDK1 is shown on Figure 1.
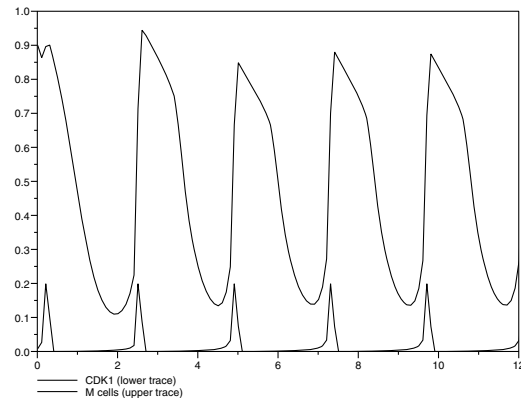

Fig. 1. (abscissae: tens of hours; ordinates: arbitrary units) Entrainment of G2/M transition in a simplified 2-phase cell cycle population model (n_1: G1-S-G2 cells; n_2: M cells) by the circadian clock: CDK1 kinase (lower trace), taken as function ψ in the cell cycle model and entrained by Wee1 (square wave, not shown, of 4 h duration with 24 h period) in A. Goldbeter's mitotic oscillator model, and normalised population of cells in M phase (upper trace).

C. Pharmacological control on cyclins and CDKs

Cell cycle control proteins such as $p53$ act both at the G_1/S and G_2/M checkpoints as sensors of DNA damage and effectors of cell cycle arrest by inhibiting the formation of Cyc E/CDK2 and Cyc B/CDK1 complexes. Anticancer drugs such as alkylating agents act by damaging DNA, and thus provoke cell cycle arrest by triggering p53 and its effects on phase transitions at G_1/S and G_2/M ([18]). And it has been shown that $p53$ on the one hand, and many cellular enzymatic drug detoxification mechanisms (such as reduced glutathione) on the other hand, are dependent on the cell circadian clock, showing 24 h periodicity in their gene expression ([3]). Hence the molecular circadian clock exerts its control on cell cycle transitions both physiologically and when an external pharmacological control is applied. In this modelling frame, the

target of both controls will be on the time-dependent transition functions $t \mapsto \psi_i(t)$ introduced above.

IV. THE CIRCADIAN SYSTEM: A NETWORK OF OSCILLATORS

This circadian clock control on the cell division cycle is not a constant independent of individual or environmental factors. Though it is endowed with an intrinsic *circa 24 h (circa diem)* period given by a hypothalamic pacemaker - the suprachiasmatic nuclei-, it is dependent on photic inputs (entrainment by the light/dark cycle through the retinohypothalamic tract), and also on the disruptive input of circulating molecules such as cytokines, often elevated in cancers, which may perturb its amplitude so deeply that any physiological body circadian rhythm (temperature, cortisol, rest/activity, etc.) becomes undetectable. Indeed, it has been shown that patients with cancer showing disrupted circadian rhythms are less responsive to chemotherapy and have poorer prognosis than patients with preserved circadian rhythmicity ([15], [17]).

A. The network and its constituents

The body circadian clock ([12], [16]), as far as its control on peripheral cell proliferation is concerned, may be seen as an orchestra consisting in the same basic individual oscillators: cell molecular circadian clocks, since the molecular mechanisms are the same whatever the cells, its peripheral constituents ("the musicians") being slaves, hardly or not communicating together, to the central pacemaker located in the suprachiasmatic nucleus ("the conductor").

Various physiological ODE models of individual circadian clocks have been published in the last ten years. They rely on transcriptional regulation, a mechanism possibly yielding limit cycles, which is a natural mathematical way to represent robust periodic behaviour ([10], [11], [13]). The simplest such physiological model of circadian clock is the 3-dimensional ODE model of FRQ (or PER) protein regulation in *Neurospora Crassa* ([13]), and it was chosen for the individual cell clock model. In this model (see next paragraph for detailed equations), PER and $mRNA$ stand for the PER cytoplasmic protein and its messenger RNA concentrations, and Z for a transcriptional retroinhibition factor linked to PER synthesis.

B. The conductor: the suprachiasmatic nuclei (SCN)

1) Diffusive neuronal coupling in the SCN: From this individual clock model, a network of circadian oscillators results by diffusive coupling of PER. Such coupling may be physiologically achieved either through gap junction connections between suprachiasmatic neurons, or through local release in the intercellular space of neurotransmitters such as vasointestinal peptide (VIP), abundant in the ventrolateral part of these nuclei, where the main pacemaker is supposed to be located, and binding to $VPAC_2$ membrane receptors on these same neurons, or even through electrical signalling with glial participation ([14]). This coupling is thus not necessarily instantaneous, possibly relying mainly on slow tissue diffusion through gap junctions or ligand-receptor connections, but

nevertheless may be considered as fast at a time scale of 24 hours, relevant for cell division cycle timing. The coupling is formulated as:

$$
\begin{cases}
\dfrac{dmRNA(i)}{dt} = V_s \dfrac{K^n}{K^n + Z(i)^n} - V_m(i)\dfrac{mRNA(i)}{K_m + mRNA(i)} \\
\dfrac{dPER(i)}{dt} = k_s mRNA(i) - V_d \dfrac{PER(i)}{K_d + PER(i)} - k_1 PER(i) \\
\qquad\qquad + k_2 Z(i) + K_e \displaystyle\sum_{j \neq i} [PER(j) - PER(i)] \\
\dfrac{dZ(i)}{dt} = k_1 PER(i) - k_2 Z(i)
\end{cases}
$$

where $1 \le i, j \le N$, N being the number of neurons connected in the pacemaker network. The degradation rate V_m of the messenger RNA is supposed to differ from one neuron to the other (with random distribution around a central value) and thus holds variability in individual PER level period. The output of this pacemaker network, to be transmitted to the periphery, is an average $\dfrac{1}{N}\displaystyle\sum_{i=1}^{N} PER(i)$ between neurons, which shows as higher amplitude in its periodic variations (i.e., good synchronisation) as the coupling strength K_e is stronger.

2) Synchronising photic inputs: But it is also known that light, through the retinohypothalamic tract, modulates, equally for all neurons, the transcription rate V_s, which is thus supposed to be $V_s = V_{s_0}\left[1 + L\cos\dfrac{2\pi t}{24}\right]$, and this modulation actually entrains the suprachiasmatic pacemaker, initially endowed with a period dependent on the parameters, e.g. 21 h 30 with our parameter set, to a forced 24 h period, provided that the entrainment by light L is strong enough to overcome the spontaneous period yielded by diffusive coupling between neurons inside the pacemaker.

3) Disruptive inputs from cytokines and drugs: Circulating molecules such as cytokines (interferon, interleukins), either secreted by the immune system in the presence of cancer cells, or delivered by therapy, are known to have a disruptive effect on the circadian clock, most likely at the central pacemaker level, and elevated levels of cytokines have been shown to be correlated with *fatigue* ([17]), a symptom constantly found in cancer, and which presents close relationships with the "jet-lag" of transmeridian flights. This has also been found with anticancer drugs, and is supposed to be a result of drug toxicity on the suprachiasmatic nuclei. It may be taken into account in the model by a positive effect on variable Z, which represents transcriptional inhibition, in the central neuronal clocks.

C. Transmission pathways from the centre to peripheral cells

These pathways are not completely known, but the autonomic nervous system, and neurohormonal ways using the hypothalamo-corticosurrenal axis, are likely candidates to this role. The messengers may be represented by supplementary evolution equations (not shown) describing a chain between messengers: intercentral, hormonal (such as ACTH) and tissular (such as cortisol), the terminal control being exerted on peripheral cells by acting on the transcription of cell clock

variables, acting themselves on cell cycle control proteins in the case of proliferating cells, in the model through the target functions ψ_i introduced above.

D. The musicians: peripheral cell circadian clocks

This terminal control is supposed, as in the case of cytokines in the pacemaker, to be exerted at the transcriptional level through a modification of variable Z in individual cell clocks. Clocks in peripheral cells will be entrained by the central pacemaker at a 24 h rhythm if the transmitted rhythm is strong enough. To illustrate this, Figure 2 shows variables $mRNA$, PER and Z of the model circadian clock averaged in a population of peripheral cells subject to entrainment by the central pacemaker when it is itself entrained or not by light.
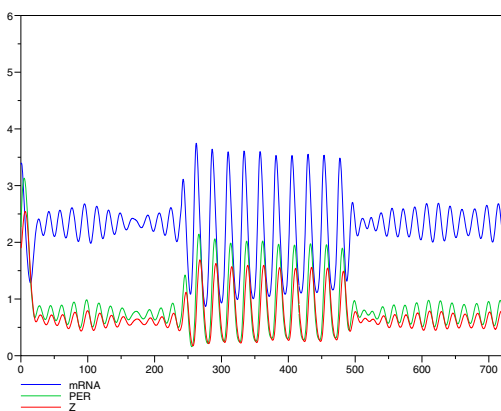

Fig. 2. (abscissae: hours; ordinates: arbitrary units) 3 epochs of 240 h for variables $mRNA$, PER and Z of a peripheral circadian clock: a) without entrainment by light (L=0); b) with entrainment (L=1); c) without (L=0).

V. Conclusion

This paper presents a modelling frame for the circadian control of cell and tissue proliferation. It aims at providing a rationale for therapeutic optimisation of cancer chemotherapies, that is, maximisation of tumour cell kill with shielding of healthy renewing tissues from unwanted toxic side effects, by using synchronisation of the drug delivery schedule and its cell processing with intrinsic cell cycle timing. Such synchronisation naturally relies on the circadian system and it has been in use in clinical ([15]) and in theoretical ([1], [5]) settings in oncology with macroscopic modelling for drug delivery regimen and therapeutic efficacy and unwanted toxicity representation. Anticancer drugs are delivered at the whole organism level, but act at the cell and tissue level on cell cycle control mechanisms. This multiscale modelling framework provides oncologists with a theoretical tool -still under construction- to bridge the gap between the pharmaceutical clinical control level and the molecular pharmacological hidden level of drug action; optimal design of pharmacological control on the cell cycle thus may rely on the knowledge of the natural synchronising control of both cell proliferation and cell drug processing mechanisms by the circadian system, which may be routinely assessed by noninvasive measurements in clinics. Future modelling developments will aim at designing practical clinical rules for dynamic drug delivery schedule optimisation based on molecular pharmacokinetic-pharmacodynamic data for the drugs in use and drug enzymatic metabolism and circadian profiles for patients under anticancer treatment.

References

[1] C. Basdevant, J. Clairambault and F. Lévi, *Optimisation of time-scheduled regimen for anti-cancer drug infusion*, Mathematical Modelling and Numerical Analysis, 39(6):1069-1086, 2005.

[2] F. Bekkal Brikci, J. Clairambault, B. Ribba and B. Perthame, *A cell population model with proliferation and quiescence for healthy and tumoral tissues*, submitted, 2006.

[3] G.A. Bjarnason, R.C.K. Jordan and R.B. Sothern, *Circadian variation in the expression of cell-cycle proteins in the human oral epithelium*, Am.J. Pathol., 154:613-622, 1999.

[4] J. Clairambault, Ph. Michel and B. Perthame, *Circadian rhythm and tumour growth*, C. R. Acad. Sci. Paris 342(1):17-22, 2006.

[5] J. Clairambault, *Modelling oxaliplatin drug delivery to circadian rhythms in drug metabolism and host tolerance*, Advanced Drug Delivery Reviews, invited paper (submitted), 2006.

[6] E. Filipski, F. Delaunay, V.M. King, M.W. Wu, B. Claustrat, A. Gréchez-Chassiau, C. Guettier, M.H. Hastings and F. Lévi, *Effects of Chronic Jet Lag on Tumor Progression in Mice*, Cancer Res., 64(21):7879-7885, 2004.

[7] E. Filipski, P. Innominato, M.X. Wu, X.M. Li, S. Iacobelli, L.J. Xian and F. Lévi, *Effects of Light and Food Schedules on Liver and Tumor Molecular Clocks in Mice.*, J. Natl. Cancer Inst., 97(7):507-517, 2005.

[8] L.N. Fu, H. Pelicano, J. Liu, P. Huang and C.C. Lee, *The circadian gene Per2 plays an important role in tumor suppression and DNA damage response in vivo* Cell, 111:41-50, 2002.

[9] L.N. Fu and C.C. Lee, *The circadian clock: pacemaker and tumor suppressor*, Nature reviews / Cancer, 3:350-361, 2003.

[10] A. Goldbeter, *Biochemical oscillations and cellular rhythms*, Cambridge University Press, 1996.

[11] D. Gonze, S. Bernard, C. Wassermann, A. Kramer and H. Herzel, *Spontaneous synchronization of coupled circadian oscillators*, Biophys. J. 89(1):120-129, 2005.

[12] M.H. Hastings, A.B. Reddy and E.S. Maywood, *A clockwork web: circadian timing in in brain and periphery, in health and disease*, Nature Reviews / Neuroscience, 4:649-661, 2003.

[13] J.-C. Leloup, D. Gonze and A. Goldbeter, *Limit cycle models for circadian rhythms based on transcriptional regulation in* Drosophila *and* Neurospora, J. Biol. Rhythms, 14(6):433-448, 1999.

[14] S. Montanes, *Study of the biological clock within the Suprachiasmatic Nucleus (SCN) in mammals' brain*, Internal report, Benjamin Franklin Klinikum für medizinische Informatik, Charité-Universitätsmedizin, Berlin, 2006.

[15] M.-C. Mormont and F. Lévi, *Cancer chronotherapy: principles, applications and perspectives*, Cancer, 97(1):155-169, 2003.

[16] S.M. Reppert and D.R. Weaver, *Coordination of circadian timing in mammals*, Nature, 418:935-941, 2002.

[17] T. Rich, P.F. Innominato, J. Boerner, M.-C. Mormont, S. Iacobelli, B. Baron, C. Jasmin and F. Lévi, *Elevated serum cytokines correlated with altered behavior, serum cortisol rhythm, and dampened 24-hour rest-activity patterns in patients with metastatic colorectal cancer*, Clin. Cancer Res. 11(5):1757-64, 2005.

[18] S. You, P.A. Wood, Y. Xiong, M. Kobayashi, J. Du-Quiton and W.J.M. Hrushesky, *Daily coordination of cancer growth and circadian clock gene expression*, Breast Canc. Res. Treatment, 91:47-60, 2005.