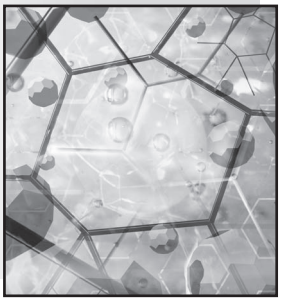

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A Step Toward Optimization of Cancer Therapeutics

Physiologically Based Modeling of Circadian Control on Cell Proliferation

BY JEAN CLAIRAMBAULT

Cancer growth and response to therapy by anticancer drugs have been shown to be dependent on circadian clock inputs [15], [27]. Indeed, tumor growth is enhanced by perturbations of the central hypothalamic clock [11]–[13] and this is most likely related to cell cycle control disruption [14], [15]. 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, which takes into account the 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.

Cell Proliferation: Populations of Cells

In tissues subject to renewal, in particular in tumors, 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 cancer growth and healthy tissue-controlled proliferation are a matter of cell population dynamics. This leads us 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, G_0, phase (where no growth occurs), 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.

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An Age-Structured PDE Model of the Cell Cycle

The model chosen [6]–[8] is linear and of the Von Foerster-McKendrick type. For each phase i ($1 \le i \le I$) 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 either due 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 $I+1=1$):

$$\begin{cases} \dfrac{\partial}{\partial t} n_i + \dfrac{\partial}{\partial a}[v_i(a) n_i] + [d_i + K_{i\to i+1}] n_i = 0, \\ v_i(0) n_i(t, a=0) = \displaystyle\int_{\alpha\ge 0} K_{i-1\to i}(t,\alpha)\; n_{i-1}(t,\alpha)\; d\alpha, \\ \quad 2 \le i \le I \\ n_1(t, a=0) = 2 \displaystyle\int_{\alpha\ge 0} K_{I\to 1}(t,\alpha)\; n_I(t,\alpha)\; d\alpha, \end{cases}$$

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

The transition rates are chosen as $\psi_i(t)\mathbf{1}_{\{a\ge a_i\}}(a)$: a minimum age a_i must be spent in phase i, and a dynamic control $t \mapsto \psi_i(t)$ is exerted on the transition from phase i to phase $i+1$. The functions ψ_i integrate physiological control (hormonal or circadian) as well as pharmacological influences. Another possibility to take circadian control into account would be by an action on the death rates d_i, an apparently natural representation since anticancer drugs increase death rates in cell populations. But it can be shown that at least by itself such control representation is not compatible with observations from laboratory experiments on tumor growth involving circadian clock disruption [7], [8]. The main output of this model is a (positive) growth exponent, λ, the first eigenvalue of an underlying differential operator, which determines its asymptotic behavior; its existence is granted by the Krein-Rutman theorem for compact positive operators [10].

Tumor Cells: Unlimited Growth

As it is, with positive functions as parameters, this linear model is bound to show exponential growth. The first eigenvalue, or

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Cancer growth and response to therapy by anticancer drugs have been shown to be dependent on circadian clock inputs.

Malthus exponent λ, of the underlying differential operator is positive and governs the asymptotic behavior (in time t) of the solutions n_i. This means that for all i, the normalized solutions $t \mapsto e^{-\lambda t} n_i(t)$ are bounded, asymptotically constant if ψ is constant, and asymptotically periodic if the ψ_i are periodic, with the same common period. This Malthus exponent λ may be assessed in the case of solid tumors by in vivo tissue growth measurements (only approximately, if one neglects the presence of necrotic material) by $\lambda = (\ln 2)/T_{\mathrm{d}}$, if T_{d} is the apparent doubling time of tumor size.

Healthy Cells: Tissue Homeostasis

In the case of healthy cells, 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 modeled 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. This has been done in an extended version of this model including cyclin concentration as a structure variable [12]. In this case, the Malthus exponent λ is zero since the asymptotic growth behavior of the total population of cells is evolution to stationarity or periodicity, and there is no exponential growth, except when the total cell population is small, but on the contrary convergence toward a steady state when the total cell population grows.

An interest in simultaneously representing a tumor and a healthy cell population is, in the perspective of therapeutic optimization, 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 transitions by the circadian clock, but possibly with phase differences, by comparison with tumors: a phase delay of 12 h between circadian peaks of anti-tumor therapeutic efficacy and circadian peaks of unwanted toxicity to healthy tissues is usually observed, whatever the drug [27]. The therapeutic control objective may be seen as to obtain a negative Malthus exponent for tumor cells and zero growth for healthy cells.

Cell Proliferation Control: Cyclins and Cyclin-Dependent Kinases

Control of Cell Cycle Phase Transitions by Cyclins and Cyclin-Dependent Kinases

Cyclins and their activating kinases (cyclin-dependent kinases [CDKs]) are the proteins that are the most important determinants for the processing through cell cycle phases and transitions between phases. Cyclin E associated with CDK2 controls G_1/S transition, and its dimerization is dependent on protein $p21$, and Cyclin B associated with CDK1 is essential for G_2/M transition, and the dimerization is controlled by the clock-controlled kinase Wee1 [15], [24], [30].

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, blocking cells in G_2. It has been modeled by Goldbeter in [16] with an ODE system in which Wee1 is a parameter. This CDK system oscillates transiently with an intrinsic frequency that depends on its parameters, but it may be entrained at a 24-h period by Wee1 if Wee1 is an output of the molecular circadian clock (in fact, the clock protein *Bmal*1 plays this role whereas the control on G_1/S through $p21$ is exerted by *PER*). The entrainment of the normalized M-cell population $t \mapsto e^{-\lambda t} \int_0^{+\infty} n_2(t,a)\, da$, in a two-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 as function ψ_1 in shown in Figure 1.

This means that for $t \longrightarrow +\infty$ the total population in phase i, $N_i(t) = \int_0^{+\infty} n_i(t,a)\, da$ is of the form $N_i(t) = e^{\lambda t} \Psi_i(t)$, where Ψ_i is a periodic function with the same period as ψ_i, λ being the same for all phases $1 \leq i \leq I$.

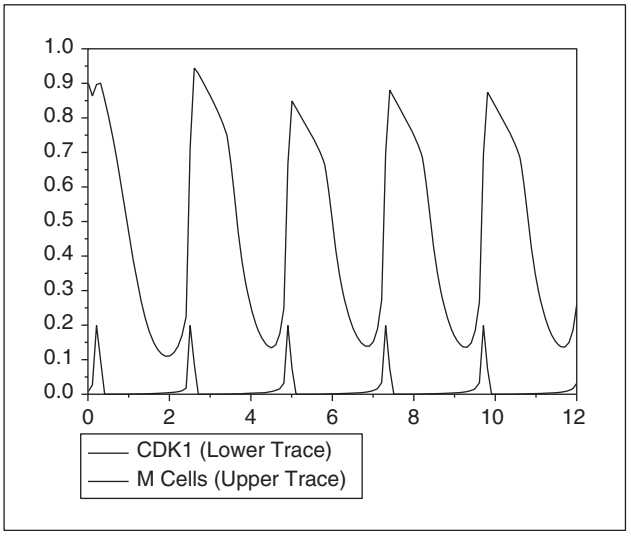

Fig. 1. Entrainment of G_2/M transition in a two-phase cell cycle population model ($n_1 : G_1 - S - G_2$ cells; n_2: M cells) by the circadian clock: CDK1 kinase (lower trace), taken as function ψ_1 in the cell cycle model and entrained by Wee1 (here a square wave, not shown, of 4 h duration with 24 h period) in Goldbeter's mitotic oscillator model, and normalized population of cells in M phase (upper trace) (abscissae: tens of hours; ordinates: arbitrary units).

In the case of healthy cells, there can be no exponential growth and tissue homeostasis must be ensured.

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 ([30]). 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 ([4]). Hence, the molecular circadian clock exerts its control on cell cycle transitions both physiologically and when an external pharmacological control is applied. In this modeling frame, the target of both controls will be on the time-dependent transition functions $t \mapsto \psi_i(t)$ introduced earlier.

The Circadian System: A Network of Oscillators

This circadian clock control on the cell division cycle is not independent of individual or environmental factors. Although this clock is endowed with an intrinsic circa 24-h (circa diem) period given by a hypothalamic pacemaker, the suprachiasmatic nuclei (SCN), 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 do patients with preserved circadian rhythmicity [27], [29].

The Network and Its Constituents

The body circadian clock [18], [28], 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, not communicating together, to the central pacemaker located in the SCN (the conductor), located in the hypothalamus.

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 behavior [16], [17], [21]. The simplest such physiological model of circadian clock is the three-dimensional ODE model of FRQ (or PER) protein regulation in *Neurospora Crassa* [21], and it was chosen for the individual cell clock model. In this model

$$\frac{d\mathrm{RNA_m}}{dt} = V_\mathrm{s}\frac{K^n}{K^n + Z^n} - V_\mathrm{m}\frac{\mathrm{RNA_m}}{K_\mathrm{m} + \mathrm{RNA_m}}$$

$$\frac{d\mathrm{PER}}{dt} = k_\mathrm{s}\mathrm{RNA_m} - V_\mathrm{d}\frac{\mathrm{PER}}{K_\mathrm{d} + \mathrm{PER}} - k_1\mathrm{PER} + k_2 Z$$

$$\frac{dZ}{dt} = k_1\mathrm{PER} - k_2 Z,$$

where 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.

The Conductor: The SCN

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 through gap junction connections between suprachiasmatic neurons, through local release in the intercellular space of neurotransmitters such as vasointestinal peptide, abundant in the ventrolateral part of these nuclei, where the main pacemaker is supposed to be located, and binding to $\mathrm{VPAC_2}$ membrane receptors on these same neurons, or even through electrical signaling with glial participation [26]. 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 h, relevant for cell division cycle timing. The coupling is formulated as

$$\frac{d\mathrm{mRNA}(i)}{dt} = V_\mathrm{s}\frac{K^n}{K^n + Z(i)^n} - V_\mathrm{m}(i)\frac{\mathrm{mRNA}(i)}{K_\mathrm{m} + \mathrm{mRNA}(i)}$$

$$\frac{d\mathrm{PER}(i)}{dt} = k_\mathrm{s}\mathrm{mRNA}(i) - V_\mathrm{d}\frac{\mathrm{PER}(i)}{K_\mathrm{d} + \mathrm{PER}(i)} - k_1\mathrm{PER}(i) + k_2 Z(i) + K_\mathrm{e}\sum_{j\neq i}[\mathrm{PER}(j) - \mathrm{PER}(i)]$$

$$\frac{dZ(i)}{dt} = k_1\mathrm{PER}(i) - k_2 Z(i),$$

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* period. The output of this pacemaker network, transmitted to the periphery, is an

average $\frac{1}{N}\sum_{i=1}^{N} \mathrm{PER}(i)$ between neurons, which shows as higher amplitude in its periodic variations (i.e., good synchronization) as the coupling strength K_e is stronger.

Synchronizing Photic Inputs

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 $V_s = V_{s_0}[1 + L\cos(2\pi t)/24]$. This modulation actually entrains the suprachiasmatic pacemaker, initially endowed with a period dependent on the parameters, e.g., 21 h 30 min 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 resulting from the coupling between neurons inside the pacemaker. A higher risk of cancer is related with circadian disruption induced by light/dark rhythm perturbations in shiftwork (see review in [25], and the present model aims at taking such perturbations into account.

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 [19], and elevated levels of cytokines have been shown to be correlated with fatigue [29], a symptom constantly found in cancer, 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 SCN. It may be taken into account in the model by an additive effect on variable Z, which represents transcriptional inhibition, in the central clocks.

Transmission Pathways from the Center to Peripheral Cells

These pathways are not completely known but the autonomic nervous system, and neurohormonal ways using the hypothalamocorticosurrenal axis, is a likely candidate to this role [15]. The messengers may be represented by supplementary evolution equations describing in a simple way a chain between messengers: intercentral, hormonal, such as adrenocorticotropic hormone (ACTH), and peripheral tissue terminal (such as cortisol)

$$\frac{dU}{dt} = k_3\overline{PER(SCN)} - k_4U$$

$$\frac{dV}{dt} = k_4U - k_5V$$

$$\frac{dW}{dt} = \frac{aV}{b+V} - cW.$$

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 peripheral cell clocks. The common terminal messenger (W, e.g., cortisol) will act itself on each peripheral circadian clock by additively modifying to $Z + rW$ the feedback variable Z, where r is a parameter. If the central pacemaker rhythm is strong enough, i.e., constituted of enough synchronized neuronal oscillators, it will entrain each peripheral individual clock at a 24 h-rhythm; but there is no intercellular communication between peripheral cells unlike between neurons in the central pacemaker. In this setting, cell cycle phase transition functions ψ_i in peripheral tissues will be supposed to be tissue-averaged versions of local *PER* proteins., i.e., $\psi_i = k_i\overline{PER}$, where k_i are constants and $\overline{PER}$ is a time-dependent function, averaged of $PER(j)$ over the cells ($1 \le j \le N$, each j cell having its own period, distributed around a central value) of a peripheral tissue assembly. They can also be CDKs, as in Figure 1, controlled by a protein such as Wee1, itself resulting from an averaging of *PER* (or rather *Bmal*1 in the case of *Wee*1, but *PER* and *Bmal*1 are in antiphase, i.e., phase delayed by 12 h in their circadian rhythm). 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.

Discussion

The behavior of the cell cycle model, which is presented here in simple form, completely linear, limits its applicability to the early stages of tumor growth. They may be considered as the most important because even when a tumor is discovered at an advanced stage of its growth, the first administered anticancer treatments, when they do not eradicate it, may shrink it to cell population conditions putting it in such early stages. Besides, an extended version of this model with nonlinear feedback by cyclin concentration and total cell population number [2], [3] opens it to the representation of different types of tumor behavior, namely, polynomial in time, as has been experimentally observed [5].

The circadian system representation, with its central and peripheral components, is meant to be able to account for disrupting inputs to it from tumor-produced cytokines or anticancer drugs, and their possible overcoming by shielding molecules such as epidermal growth factor receptor (EGFR) antagonists. An analysis of its effects onto cell cycle phase transitions (by functions ψ_i in this model) remains to be done. More detailed representations of the molecular circadian clock, as recent ones by Leloup and Goldbeter [20], with

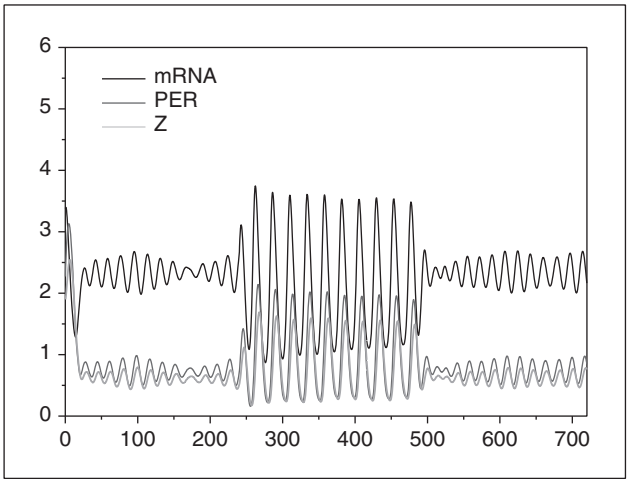

Fig. 2. Three epochs of 240 h for variables *mRNA, PER* and *Z* of a peripheral circadian clock (average of local circadian clocks): a) without entrainment by light (L = 0); b) with entrainment (L = 1); c) without (L = 0) (abscissae: hours; ordinates: arbitrary units).

Bmal1, Clock, Per, Cry, and other genes, rather than the simplified model used here may also be used.

Conclusion

This article presents a modeling frame for the circadian control of cell and tissue proliferation. It aims at providing a rationale for therapeutic optimization of cancer chemotherapies, that is, maximization of tumor cell kill while shielding healthy renewing tissues from unwanted toxic side effects, by using synchronization of the drug delivery schedule and its cell processing with intrinsic cell cycle timing. Such synchronization naturally relies on the circadian system and it has been in use in clinical [22], [27] and in theoretical [1], [9] settings in oncology with macroscopic modeling 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 modeling framework will provide clinicians 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 synchronizing control of both cell proliferation and cell drug processing mechanisms by the circadian system, which may be routinely assessed by noninvasive measurements in the clinic [29]. Future modeling developments will aim at designing practical clinical rules for dynamic drug delivery schedule optimization based on molecular pharmacokinetic-pharmacodynamic data for the drugs in use and drug enzymatic metabolism and circadian profiles for patients under anticancer treatment, as recently suggested [23].

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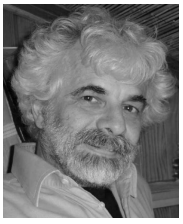

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